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Présentée par

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DIVERSITY-ORIENTED SYNTHESIS OF POCHONINS A PRIVILEGE SCAFFOLD FOR ATPASE AND KINASE INHIBITION

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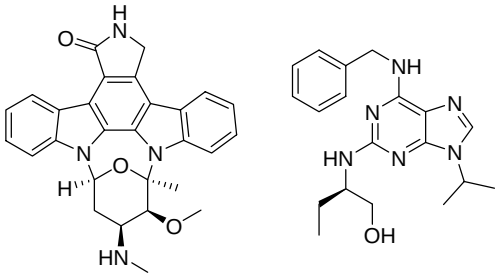
Résumé

Introduction

Les kinases et les phosphatases jouent un rôle essentiel dans les mécanismes de transduction du signal par phosphorylation/déphosphorylation des protéines et subséquente modulation de leur activité.[1] L'utilisation d'inhibiteurs tels que la staurosporine (Figure I) a permis de mieux comprendre leurs fonctions biologiques:[2] les kinases se lient à l'ATP et transfèrent le groupe γ-phosphate aux résidus sérine, tyrosine ou thréonine d'une protéine spécifique. D'un point de vue thérapeutique, la déstabilisation de l'équilibre phosphorylation/déphosphorylation est à la base de procédés oncogéniques, les kinases se révélant ainsi être d'importantes cibles en chimiothérapie.[3] Par ailleurs, la mise au point d'inhibiteurs sélectifs est primordiale afin de déterminer la fonction de ces enzymes au sein des voies de signalisation. Dans cette optique, et bien que les sites de reconnaissance de l'ATP soient similaires pour l'ensemble des kinases,[4] des inhibiteurs sélectifs comme la (*R*)-roscovitine (Figure I) ont pu être synthétisés par différents groupes de recherche à partir des bases puriques.[5]



Staurosporine **Roscovitine**

Figure I: Inhibiteurs de kinases

En raison de l'intérêt biologique et thérapeutique afférant à l'inhibition de ces enzymes, nous avons recherché de nouvelles classes de molécules présentant une structure très différente du squelette adénosine afin d'améliorer la sélectivité. Parmi les produits naturels, la famille des macrolides résorcyliques a particulièrement retenu notre attention. Le radicicol (Figure II) est un inhibiteur compétitif et hautement sélectif du site de liaison de l'ATP au sein de la protéine

[1] Hunter, T. Signaling--2000 and beyond. *Cell* **100**, 113-27 (2000).
[2] Hong, S. K., Matsumoto, A., Horinouchi, S. & Beppu, T. Effects of protein kinase inhibitors on in vitro protein phosphorylation and cellular differentiation of Streptomyces griseus. *Mol Gen Genet* **236**, 347-54 (1993).
[3] Cohen, P. Protein kinases--the major drug targets of the twenty-first century? *Nat Rev Drug Discov* **1**, 309-15 (2002).
[4] Manning, G., Whyte, D. B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298**, 1912-34 (2002).
[5] Meijer, L. & Raymond, E. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. *Acc Chem Res* **36**, 417-25 (2003).

de choc thermique HSP90 (Heat Shock Protein 90).[6] Cette protéine est un chaperon moléculaire, c'est-à-dire une protéine qui protège d'autres protéines des altérations structurales pouvant être provoquées par un choc thermique ou autre stress,[7] et qui joue un rôle majeur dans la maturation de protéines impliquées dans la régulation des voies de signalisation des cellules cancéreuses. Depuis quelques années, de nombreux groupes de recherche se sont intéressés à HSP90 à des fins chimiothérapeutiques, son inhibition conduisant à la destruction des protéines oncogéniques par le protéasome. Par ailleurs, parmi les macrolides résorcyliques, le composé LL-Z1640-2 (Figure II) est un inhibiteur sélectif de la kinase TAK-1 pour laquelle le radicicol ne présente aucune activité.[8] Son analogue partiellement hydrogéné, le L-783,277 (Figure II) est sélectif de la kinase MEK.[9] De plus, de nouveaux macrolides résorcyliques ont été isolés de *Pochonia chlamydosporia* var. *catenulata* (pochonines A-F, Figure II), parmi lesquelles la pochonine C s'est montrée inhibitrice de l'hélicase du virus de l'herpès, structurellement proche d'une ATPase.[10]

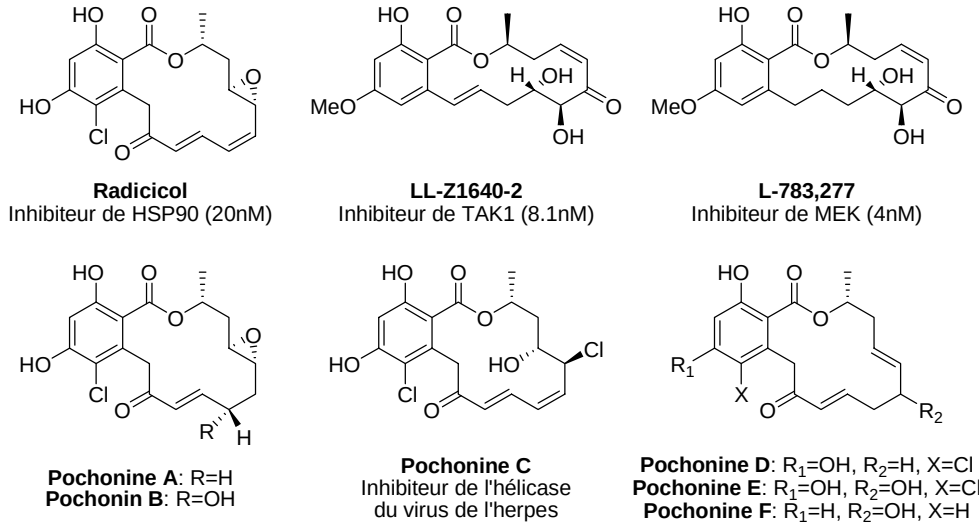


Figure II: Structures de macrolides résorcyliques d'intérêt biologique

Compte tenu des similarités structurales entre les différents composés résorcyliques et de leurs cibles variées (HSP90, hélicase, kinases MEK et TAK), nous avons souhaité développer

[6] Roe, S. M. et al. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. *J Med Chem* **42**, 260-6 (1999).
[7] Whitesell, L. & Lindquist, S. L. HSP90 and the chaperoning of cancer. *Nat Rev Cancer* **5**, 761-72 (2005).
[8] Ninomiya-Tsuji, J. et al. A resorcylic acid lactone, 5Z-7-oxozeaenol, prevents inflammation by inhibiting the catalytic activity of TAK1 MAPK kinase kinase. *J Biol Chem* **278**, 18485-90 (2003).
[9] Zhao, A. et al. Resorcylic acid lactones: naturally occurring potent and selective inhibitors of MEK. *J Antibiot (Tokyo)* **52**, 1086-94 (1999).
[10] Hellwig, V. et al. Pochonins A-F, new antiviral and antiparasitic resorcylic acid lactones from Pochonia chlamydosporia var. catenulata. *J Nat Prod* **66**, 829-37 (2003).

de nouvelles méthodes de synthèse suffisamment flexibles, et permettant d'accéder aussi bien aux produits naturels qu'à des analogues synthétiques afin d'élargir le spectre et la sélectivité des molécules inhibitrices de kinases et d'ATPases.

Résultats

A partir de travaux préliminaires effectués au sein du laboratoire, des synthèses en solution et sur phase solide du radicicol et de la pochonine C ont été réalisées selon un schéma rétrosynthétique (Figure III) basé sur trois étapes principales : une fermeture de cycle par métathèse, une réaction d'acylation et une estérification de Mitsunobu.

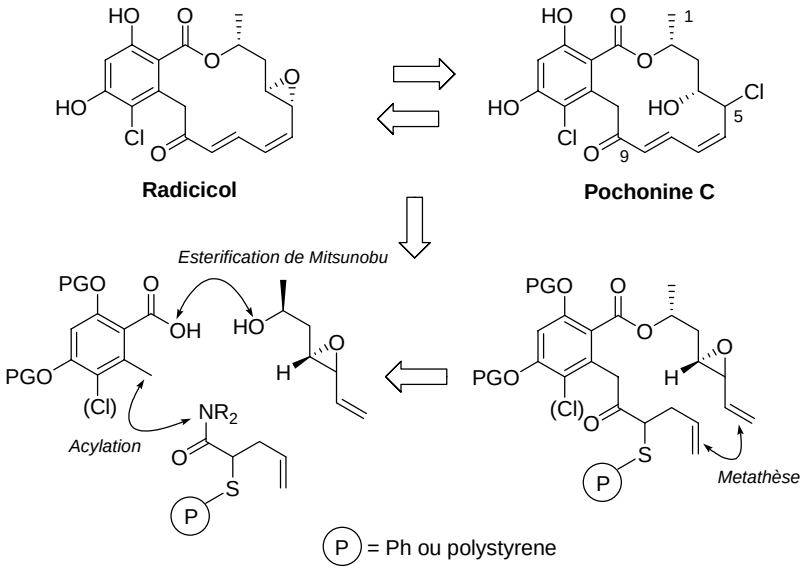


Figure III: Schéma rétrosynthétique pour le radicicol et la pochonine C

Le radicicol et la pochonine C ont ainsi pu être synthétisés respectivement en huit (22 % de rendement) et sept (25 % de rendement) étapes et la méthodologie développée a également permis la synthèse d'analogues présentant une fonction cyclopropyle au lieu de l'époxyde. Les tests biologiques effectués en compétition avec la geldanamycine[11] (inhibiteur connu de HSP90) ont révélé des inhibitions très différentes pour le radicicol (IC_{50} (HSP90) = 20nM) et la pochonine C (IC_{50} (HSP90) = 1200nM). De plus, des études de spectroscopie NOE pour ces deux molécules ont montré des conformations très différentes pouvant ainsi expliquer les profils biologiques différents. Nous nous sommes donc intéressés plus précisément au profil

[11] Zhou, V. et al. A time-resolved fluorescence resonance energy transfer-based HTS assay and a surface plasmon resonance-based binding assay for heat shock protein 90 inhibitors. *Anal Biochem* **331**, 349-57 (2004).

conformationnel de certains macrolides résorcyliques aussi bien naturels que synthétiques. Une étude en collaboration avec le Pr. Martin KARPLUS a établi une corrélation entre l'activité biologique du radicicol pour HSP90 et l'énergie libre de sa conformation bioactive. Cette modélisation moléculaire a également révélé que la pochonine D présente une conformation similaire au radicicol et de ce fait, peut être un inhibiteur potentiel de la protéine HSP90. La synthèse de cette molécule a été élaborée en six étapes à partir de produits commerciaux avec un rendement global de 20% (Figure IV), en utilisant des réactifs supportés et permettant la synthèse de banques de molécules.

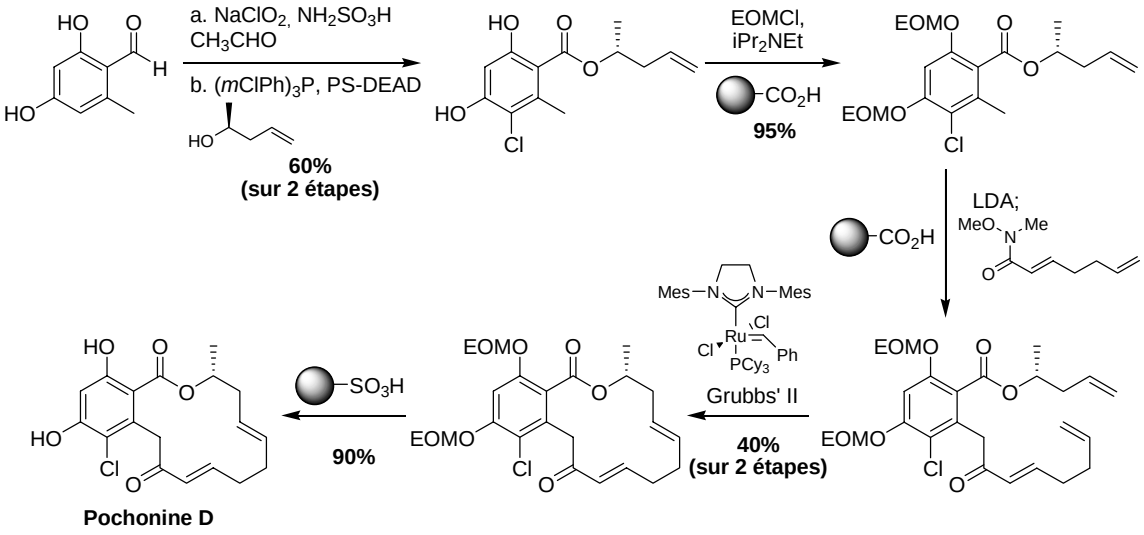


Figure IV: Synthèse de la pochonine D avec des réactifs supportés

Il est intéressant de noter que cette synthèse peut être réalisée en solution et permet d'obtenir de larges quantités de produit (jusqu'à 1g). Des tests biologiques (suivant le protocole utilisé pour l'évaluation biologique de la pochonine C)[10] ont montré que la pochonine D (IC_{50} = 80nM) présente une efficacité comparable au radicicol (IC_{50} = 20nM) pour l'inhibition de la protéine HSP90. Par ailleurs, compte tenu de la similarité entre les pochonines D et A, il nous apparaissait intéressant de synthétiser la pochonine A, non seulement pour confirmer sa structure mais également afin de comparer son activité biologique à celle de la pochonine D. La pochonine A a ainsi pu être obtenue en une étape (époxidation en utilisant le dimethyldioxirane) à partir de la pochonine D. Cependant, des problèmes de solubilité lors de cette réaction nous ont conduits à proposer une synthèse de cette molécule en utilisant de groupements silylés (Figure V) pouvant être déprotégés sans ouverture de la fonction époxyde. L'évaluation biologique de cette molécule contre HSP90 a également établi que la pochonine A est un inhibiteur avec une IC_{50} de 90nM. L'analyse de ces résultats pour les

quatre produits naturels précédemment synthétisés révèle que la fonction époxyde et la double liaison γ,δ-insaturée ne sont pas primordiales pour l'inhibition de la protéine HSP90. Cette même étude sur des autres analogues synthétiques a par ailleurs montré l'importance de l'atome de chlore et des phénols déprotégés.

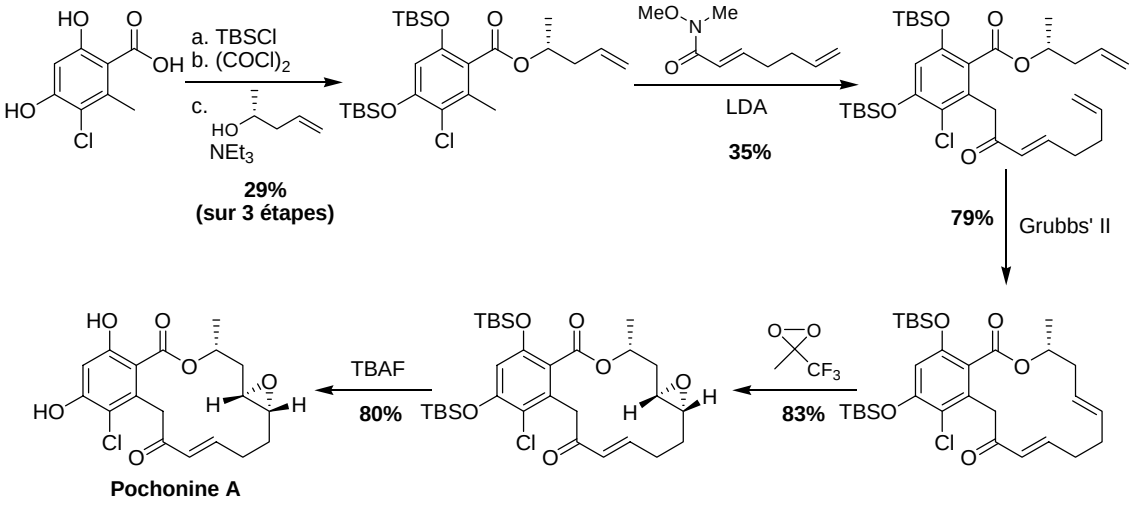


Figure V: Synthèse de la pochonine A avec des groupements protecteurs silylés

Compte tenu du potentiel du radicicol pour l'inhibition de la protéine HSP90 et de l'importance des macrolides résorcyliques pour l'inhibition de kinases, une banque de molécules présentant cinq points de diversité (Figure VI) par rapport à la pochonine D a été établie afin d'évaluer voire de confirmer la capacité du squelette pochonine pour ces deux cibles.

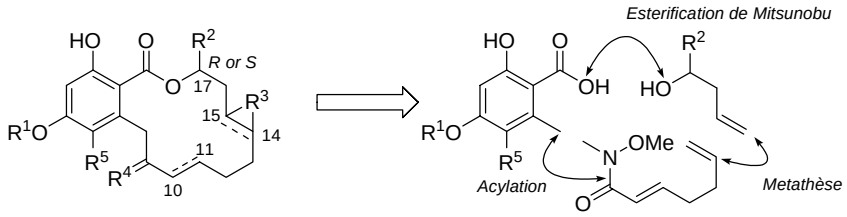


Figure VI: Structure générale de la banque de molécules et son analyse rétrosynthétique

Ainsi, des modifications ont été introduites sur le phénol en *para* de l'ester (R^1), au niveau du groupement présent sur le macrocycle (R^2), sur la double liaison conjuguée (R^3), sur le système α,β-conjugué (R^4) et en position *méta* sur le cycle aromatique (R^5). A partir de la synthèse proposée pour la pochonine D, une banque d'une centaine de molécules (Figure VII) a été synthétisée en utilisant majoritairement des réactifs supportés, facilitant ainsi l'isolation des divers analogues.

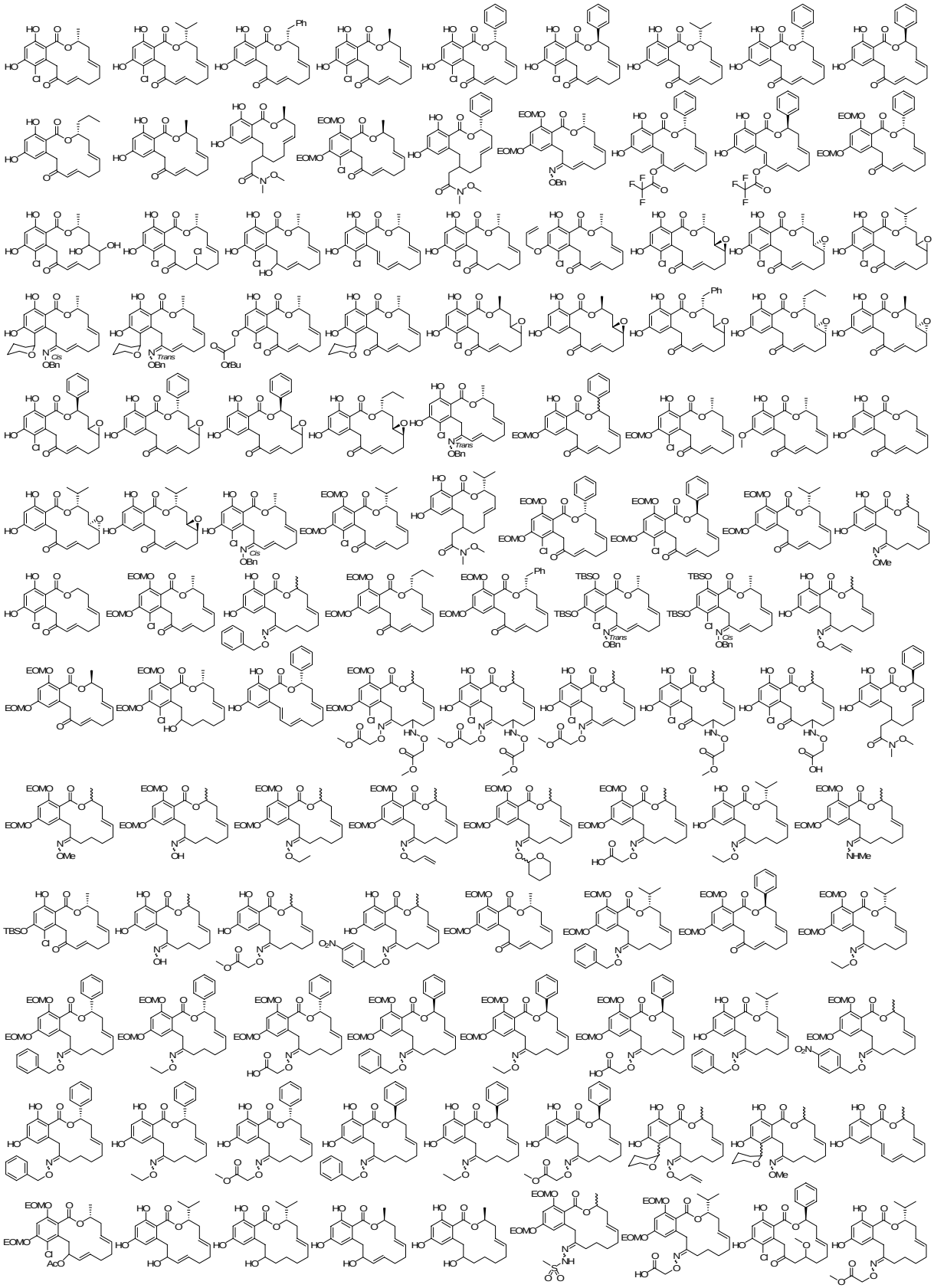
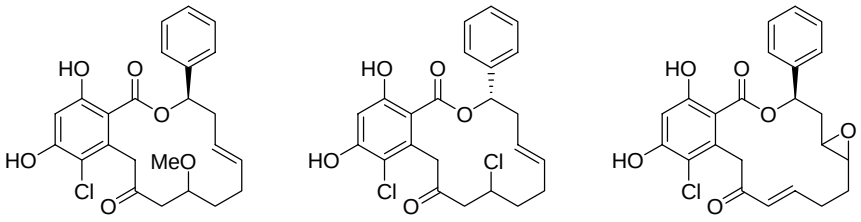


Figure VII : Structure des analogues synthétiques de la pochonine D

Parmi la centaine de molécules synthétisées, les composés présentant une fonction oxime au lieu de la cétone inhibent la protéine HSP90. De plus, le criblage de 24 kinases a révélé plusieurs inhibiteurs sélectifs à 10 µM. Ainsi, les analogues présentant un groupement phényl en R^2 et des substitutions en R^3 ou R^4 (Figure VIII) inhibent des enzymes thérapeutiquement importantes (src, Aurora A, IGF1-R).



Inhibiteur de src (8 µM) | Inhibiteur de Aurora A (12 µM) | Inhibiteur de IGF1-R (11 µM)

Figure VIII : Structure d'analogues de la pochonine D inhibiteurs de kinases

Finalement, une preuve de principe permettant la synthèse sur phase solide d'analogues du squelette pochonine a été établie. Cette méthode mettrait en jeu conjointement la synthèse développée pour la pochonine D et celle d'oligomères d'acides peptido-nucléiques (APN) développée au laboratoire (Figure IX). [12]

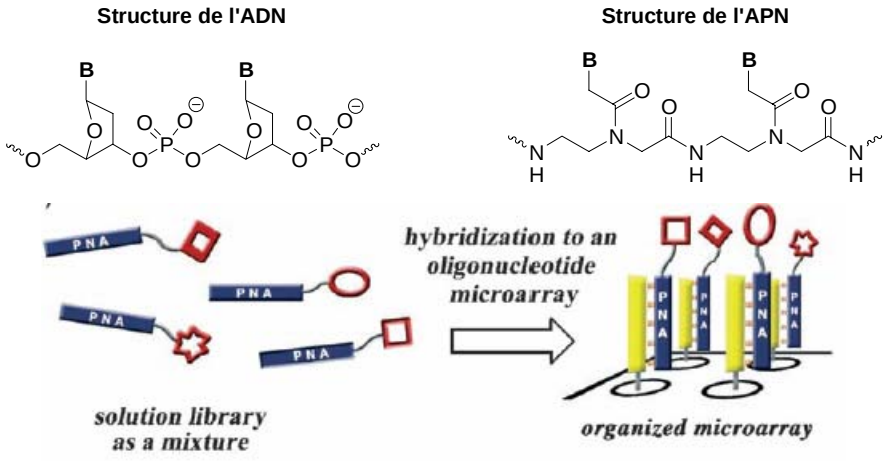


Figure IX: Structures comparées de l'ADN et de l'APN et représentation schématique d'une banque de molécules encodée avec une séquence d'APN et son hybridation organisée sur une puce à ADN

Considérant une banque présentant quatre points de diversité, plus de 600 analogues encodés avec une séquence d'APN pourraient être synthétisés et criblés directement avant hybridation

[12] Harris, J. L. & Winssinger, N. PNA encoding (PNA=peptide nucleic acid): from solution-based libraries to organized microarrays. *Chemistry* **11**, 6792-801 (2005).

organisée sur des puces à ADN, permettant ainsi l'évaluation rapide d'inhibiteurs potentiels contre une large palette de cibles.

Conclusions

Une méthode générale permettant la synthèse de produits naturels mais aussi d'analogues synthétiques a été développée permettant ainsi l'accès au radicicol, aux pochonines A, C et D (premières synthèses totales décrites dans la littérature) et à une banque d'une centaine d'analogues.

Les tests biologiques contre la protéine de choc thermique HSP90 ont corroboré les résultats déjà décrits pour le radicicol et mis en évidence l'activité des pochonines D et A. La banque de molécules a également révélé des inhibiteurs de kinases à des concentrations micromolaires. Il est aussi intéressant de noter que les molécules actives contre HSP90 ne présentent aucune activité significative contre les kinases étudiées.

Le travail réalisé au cours de cette thèse a donné lieu à cinq publications dans des journaux à comité de lecture :

Barluenga, S.; Lopez, P.; Moulin, E.; Winssinger, N., ***Modular Asymmetric Synthesis of Pochonin C.*** *Angew. Chem.* **2004**, *116*, 3549-3552; *Angew. Chem. Int. Ed.* **2004**, *43*, 3467-3470.

Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N., ***Design, Synthesis and Biological Evaluation of HSP90 Inhibitors Based on Conformational Analysis of Radicicol.*** *J. Am. Chem. Soc.* **2005**, *127*, 6999-7004.

Barluenga, S.; Moulin, E.; Lopez, P.; Winssinger, N., ***Solution and Solid-Phase Synthesis of Radicicol (Monorden) and Pochonin C.*** *Chem., Eur. J.* **2005**, *11*, 4935-4952.

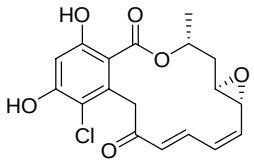
Moulin, E.; Barluenga, S.; Winssinger, N., ***Concise Synthesis of Pochonin A, an HSP90 inhibitor.*** *Org. Lett.* **2005**, *7*, 5637-5639.

Moulin, E.; Barluenga, S.; Totzke, F.; Winssinger, N., ***Diversity-Oriented Synthesis of Pochonins and biological evaluation against a panel of kinases.*** *Chem., Eur. J.* **2006**, *12*, 8819-8834.

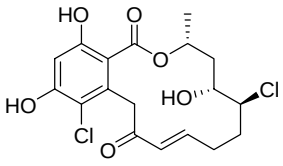
Table of Contents

Abstract

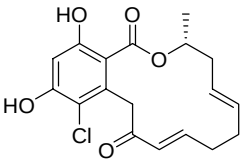
Pochonins A − F are six new members of the 14-membered resorcylic acid lactones (RALs) family which were identified in a Herpes Simplex Virus replication assay. Our interest in the pochonins stems from the observation that several other RALs are known to inhibit ATPases or kinases. Among them, radicicol was shown to be a potent inhibitor of HSP90 (Heat Shock Protein 90) whose activity is required for the functional maturation of a number of oncogenes, thus making this protein an attractive target for chemotherapy.



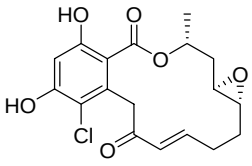
Radicicol



Pochonin C



Pochonin D

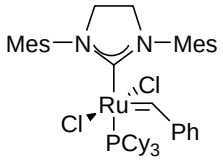


Pochonin A

A modular synthesis of pochonin C and its conversion to radicicol is presented. Both natural products are prepared in seven and eight steps, respectively, from three readily available fragments. Alternative syntheses of these compounds were also achieved using a combination of polymer-bound reagents and reactions on solid phase. Based on a molecular dynamics/minimization of radicicol and several known analogs, a correlation between the HSP90-inhibitory activity and the free energy of the bioactive conformer was established, leading to the identification of pochonin D as a potential inhibitor of HSP90. Its synthesis using polymer-bound reagents allowed us to confirm this finding and pochonin D was shown to be nearly as potent an HSP90 inhibitor as radicicol. Considering their closely-related structure, pochonin A was also synthesized and tested for HSP90 inhibition. The polymer-assisted chemistry developed for the pochonins syntheses enabled us to prepare a library based on the pochonin scaffold bearing five points of diversity, allowing us to extend beyond the modifications of the natural resorcylic acid lactones. Testing the library for its inhibition against a panel of 24 kinases at 10 μM and against HSP90 reveals several hits, thereby demonstrating the potential of the resorcylides towards the inhibition of therapeutically relevant kinases and ATPases. These syntheses of the pochonins were also the first reported in the literature allowing us to confirm structural assignments and to provide optical rotations as well as to define the stereochemistry of pochonin C's carbon bearing the chlorine atom.

Ac	Acetyl ($CH_3C=O$)
ADP	Adenosine diphosphate
AIBN	Azobis(isobutyronitrile)
All	Allyl
ATP	Adenosine triphosphate
BER	Borohydride exchange resin
BBN	Borabicyclononane
Bn	Benzyl
Bz	Benzoyl
CAN	Ceric ammonium nitrate
CSA	Camphorsulfonic acid
δ	Chemical shift (NMR)
dba	Dibenzylideneacetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
d.e.	Diastereoisomeric excess
DET	Diethyl tartrate
DHP	Dihydropyran
DIBAL or Dibal-H	Diisobutylaluminum hydride
DIC	*N,N'*-diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMDO	Dimethyldioxirane
DMF	Dimethylformamide
DMPI	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
DNA	Desoxyribo nucleic acid
dppe	1,2-Bis(diphenylphosphino)ethane
EC_{50}	Plasma concentration required for obtaining 50% of a maximum effect *in vivo*
EDC	1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
e.e.	Enantiomeric excess

EOM	Ethoxymethyl ($CH_3CH_2OCH_2$-)
FDA	Food and Drug Administration
Fmoc	9-Fluorenylmethoxycarbonyl
GI_{50}	Concentration required for 50% inhibition of cell growth
Grubbs'II	Grubb's second generation catalyst: (ruthenium[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene) (tricyclohexylphosphane)
HFIP	Hexafluoroisopropanol
HMDS	Hexamethyldisilazide
HMPA	Hexamethylphosphorictriamide
HOBt	*N*-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSP90	Heat shock protein 90
Hünig's Base	Diisopropylethylamine
IC_{50}	Concentration of a drug that is required for 50% inhibition *in vitro*
imid.	Imidazole
Ipc_2BH	Bis-isopinocampheylborane
J	Coupling constant
KHMDS	Potassium hexamethyldisilylazide
L.C.	Liquid chromatography
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilylazide ($LiN(SiMe_3)_2$)
MAP	Mitogen-activated protein
*m*CPBA	*meta*-Chloroperoxybenzoic acid
MOM	Methoxymethyl (CH_3OCH_2-)
mRNA	Messenger ribonucleic acid
M.S.	Mass spectrum
NaHMDS	Sodium hexamethyldisilylazide
NMR	Nuclear magnetic resonance
NMM	*N*-Methylmorpholine
NMO	*N*-Methylmorpholine-*N*-oxide
NOE(SY)	Nuclear overhauser effect
PCC	Pyridinium chlorochromate



Grubbs' II

PDC	Pyridinium dichromate
PG	Protecting group
PMB	*para*-Methoxybenzyl
PNA	Peptide nucleic acid
Piv	Pivaloyl
PS-	Polymer supported
PS-TBD	(1,5,7)-Triaza-bicyclo[4.4.0]dodeca-5-ene-7-methyl polystyrene
Pyr or Py	Pyridine
rac	Racemic
RAL	Resorcylic acid lactone
RCM	Ring-closing metathesis
RedAl	Sodium bis(methoxyethoxy) aluminium hydride
R_f	Retention factor
RNA	Ribonucleic acid
RT	Room temperature
SAE	Sharpless asymmetric epoxidation
SAR	Structure-activity relationship
SEM	2-Trimethylsilylethoxymethoxy
TBAF	Tetra-*n*-butylammonium fluoride
TBAI	Tetra-*n*-butylammonium iodide
TBDPS	*t*-Butyldiphenylsilyl
TBHP	*t*-Butylhydroperoxide
TBS	*t*-Butyldimethylsilyl
Teoc	2-(Trimethylsilyl)ethoxycarbonyl
Tf	Triflate (CF_3SO_3)
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Ts	Tosyl (p-$CH_3C_6H_4SO_2$)
p-TSOH	*para*-Toluenesulfonic acid

Introduction

Protein kinases are essential components of cellular signal pathways and are directly involved in numerous diseases including cancer, diabetes and inflammation. Intensive research programs have led to the identification of several specific kinase inhibitors that show encouraging results as a new class of therapeutics. The vast majority of these compounds target the ATP-binding site of the kinase. Similarly, HSP90 (Heat Shock Protein 90) inhibitors target the ATP-binding pocket of the protein and several of them have been identified as potential anticancer agents. Among them, radicicol, a 14-membered resorcylic acid lactone (RAL), stands out as the most potent one. RALs have been known for decades but, the original bioactivity ascribed to these compounds did not solicit much interest from the organic chemistry community. In the early 90's, radicicol's inhibition of a kinase revived the interest in this molecule and, a few years later, several reports regarding selective kinase inhibitors amongst other RALs were published. Total syntheses of these natural products were then reported and new RALs such as the aigialomycins or the pochonins continue to be isolated.

At the beginning of my PhD, the discovery of the pochonins was particularly attractive. Having in mind the potential of radicicol and its derivatives as HSP90 inhibitors, we thought about developing efficient synthetic strategies towards these molecules. A molecular minimization done in collaboration with Pr. KARPLUS revealed a potential lead for HSP90 inhibition among this new class of RALs. Biological evaluation against HSP90 established its inhibitory activity to be in the same order of magnitude as for radicicol. Considering the suitability of the RALs for kinase inhibition, libraries of pochonins were then envisioned to extend beyond the diversity of this important class of natural products. Screening of these molecules against both HSP90 and a panel of kinases revealed several hits. This work has been done during my PhD in the Organic and Bioorganic Chemistry Laboratory headed by Pr. Nicolas WINSSINGER in close collaboration with Dr. Sofia BARLUENGA and the help of Dr. Maria Pilar LOPEZ-DEBER, Pierre-Yves DAKAS and Lise BRETHOUS.

Part I

Bibliographical Introduction

Chapter I − Kinase Inhibition: A Short Overview

In the mid-50's, it was discovered that phosphorylation can reversibly alter the function of enzymes by the mean of protein kinases which catalyze phosphorylation, or by protein phosphatases which are involved in the dephosphorylation step (Figure 1).[1]

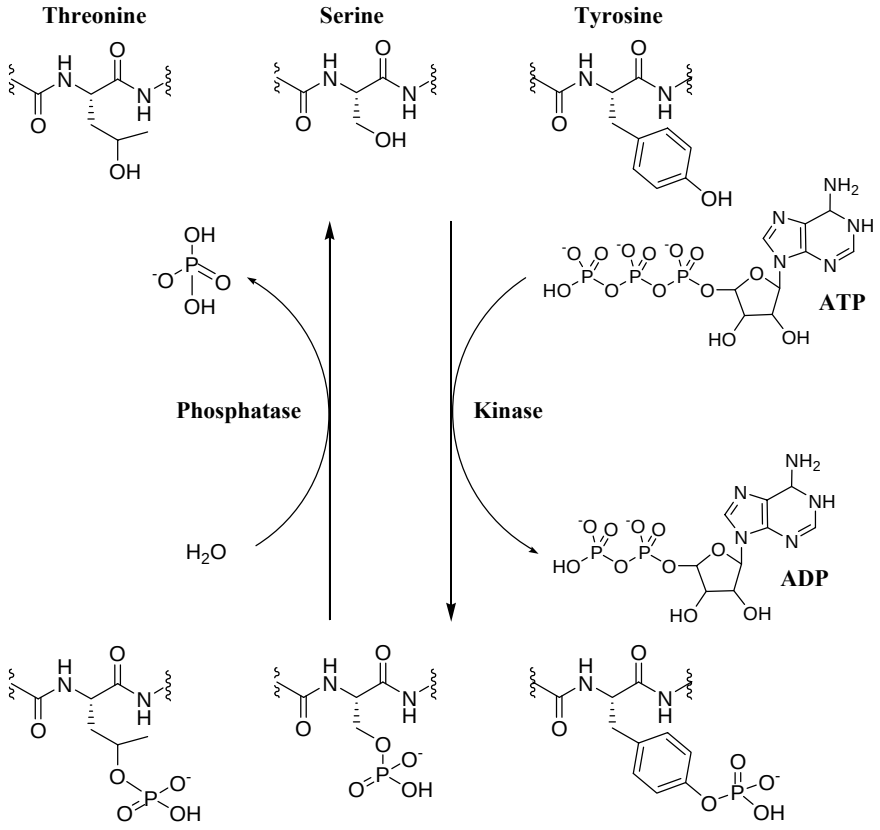


Figure 1: Phosphorylation and dephosphorylation reactions mediated by kinases and phosphatases

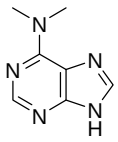
These reactions play a crucial role in living organisms for the regulation of a large number of cellular processes (signaling transduction pathways). At the end of the 70's, the discoveries that the transforming factor of the Rous sarcoma virus (v-Src) is a protein kinase[2] and that tumor-promoting phorbol esters are potent activators of protein kinase C[3] were of relevant significance. These two major observations emphasized the importance of protein phosphorylation at the cellular level, shedding the light on the first connections between

[1] Krebs, E. G. & Beavo, J. A. Phosphorylation-dephosphorylation of enzymes. *Annu Rev Biochem* **48**, 923-59 (1979).
[2] Collett, M. S. & Erikson, R. L. Protein kinase activity associated with the avian sarcoma virus src gene product. *Proc Natl Acad Sci U S A* **75**, 2021-4 (1978).
[3] Castagna, M. et al. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J Biol Chem* **257**, 7847-51 (1982).

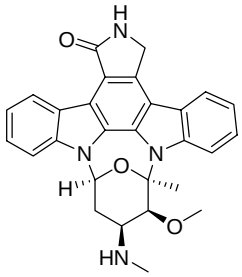
abnormal protein phosphorylation and disease. Intensive research over the last thirty years has shown that defects in transduction mechanisms are at the basis of cancer and a number of other human diseases (diabetes, inflammatory disorders, cardiovascular diseases, *etc.*).[4] Thus, the interest in finding selective kinase and phosphatase inhibitors has emerged not only for their use as new anti-cancer agents with an innovative mode of action but also for investigating signaling processes.

1. The first kinase inhibitors

In the 80's, 6-dimethylaminopurine (**1-1**, Figure 2) was frequently used as a kinase inhibitor albeit with no selectivity. Isolated by Rebhun and co-workers in 1973 as a puromycin analog, it was shown to inhibit mitosis of the sea urchin embryo.[5] Further studies revealed that this molecule **1-1** blocked M-phase specific phosphorylation and histone H1 kinase, albeit with an IC_{50} of 120µM.[6]



1-1



Staurosporine
1-2

Figure 2: First kinase inhibitors

In 1986, staurosporine (**1-2**, Figure 2) was discovered as a nanomolar inhibitor of protein kinase C[7] leading to an increase interest of the pharmaceutical community for such a scaffold. However, it was later shown that this compound lacked selectivity by inhibiting a significant portion of protein kinases *in vitro*.[8] Nevertheless, it is noteworthy that some of its

[4] Hunter, T. Signaling--2000 and beyond. *Cell* **100**, 113-27 (2000).
[5] Rebhun, L. I., White, D., Sander, G. & Ivy, N. Cleavage inhibition in marine eggs by puromycin and 6-dimethylaminopurine. *Exp Cell Res* **77**, 312-8 (1973).
[6] (a) Meijer, L. & Pondaven, P. Cyclic activation of histone H1 kinase during sea urchin egg mitotic divisions. *Ibid.* **174**, 116-29 (1988), (b) Neant, I. & Guerrier, P. 6-Dimethylaminopurine blocks starfish oocyte maturation by inhibiting a relevant protein kinase activity. *Exp Cell Res* **176**, 68-79 (1988).
[7] Tamaoki, T. et al. Staurosporine, a potent inhibitor of phospholipid/Ca++dependent protein kinase. *Biochem Biophys Res Commun* **135**, 397-402 (1986).
[8] (a) Davies, S. P., Reddy, H., Caivano, M. & Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* **351**, 95-105 (2000), (b) Alessi, D. R. The protein kinase C inhibitors Ro 318220 and GF 109203X are equally potent inhibitors of MAPKAP kinase-1beta (Rsk-2) and p70 S6 kinase. *FEBS Lett* **402**, 121-3 (1997).

analogs are currently undergoing clinical trials for the treatment of several diseases (cancer, diabetes, *etc.*).[9]

By the end of the 80's, no kinase inhibitor had been found selective. It was clear that the aforementioned protein-kinase inhibitors had to compete with ATP present at high concentrations (2 to 10mM) in the intracellular medium. Furthermore, the first X-ray structures of different kinases showed that residues involved in ATP binding were highly conserved[10] from one protein to another throughout the kinome,[11] which led to an increasing scepticism for finding selective small molecules that target protein kinases specifically.

Considering that protein tyrosine kinases represent a major portion of all the oncoproteins, some tyrphostins (tyrosine phosphorylation inhibitors resembling tyrosine with low molecular weight, **1-3**, Figure 3) started to be designed as prospective antiproliferative agents.[12]

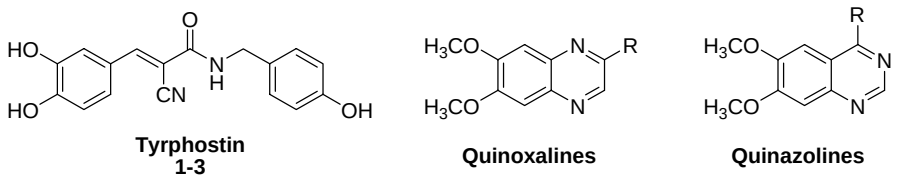


Figure 3: First specific kinase inhibitors

After several disappointments, Levitzki and co-workers finally showed that these molecules could distinguish between two different kinases, EGFR/Her-1 and Her-2/neu, which show more than 80% homology in their ATP-binding pocket and used them as powerful tools for dissecting signaling transduction pathways.[13] Within a short period of time, quinazolines and quinoxalines (Figure 3) were shown to be highly selective for epidermal growth factor receptor (EGFR)[14] and for platelet-derived growth factor receptor (PDGFR) respectively,[15]

[9] Cohen, P. Protein kinases--the major drug targets of the twenty-first century? *Nat Rev Drug Discov* **1**, 309-15 (2002).

[10] (a) Knighton, D. R. et al. Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* **253**, 407-14 (1991), (b) Knighton, D. R. et al. Structure of a peptide inhibitor bound to the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* **253**, 414-20 (1991).

[11] Manning, G., Whyte, D. B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298**, 1912-34 (2002).

[12] (a) Gazit, A., Yaish, P., Gilon, C. & Levitzki, A. Tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. *J Med Chem* **32**, 2344-52 (1989), (b) Levitzki, A. Tyrphostins: tyrosine kinase blockers as novel antiproliferative agents and dissectors of signal transduction. *Faseb J* **6**, 3275-82 (1992).

[13] Osherov, N., Gazit, A., Gilon, C. & Levitzki, A. Selective inhibition of the epidermal growth factor and HER2/neu receptors by tyrphostins. *J Biol Chem* **268**, 11134-42 (1993).

[14] Gazit, A. et al. Tyrphostins IV--highly potent inhibitors of EGF receptor kinase. Structure-activity relationship study of 4-anilidoquinazolines. *Bioorg Med Chem* **4**, 1203-7 (1996).

two factors of particular importance in the proliferation and/or differentiation of some cells, making them attractive targets for chemotherapy. Indeed, several molecules bearing the quinazoline motif and targeting EGFR and PDGFR are currently ongoing clinical trials.[16] One of them called ZD1839/gefinitib marketed as Iressa® (**1-4**, Figure 4) shows great efficiency against several cancers in humans and was approved by the FDA in 2003 for the treatment of non-small cell lung cancer (NSCLC).

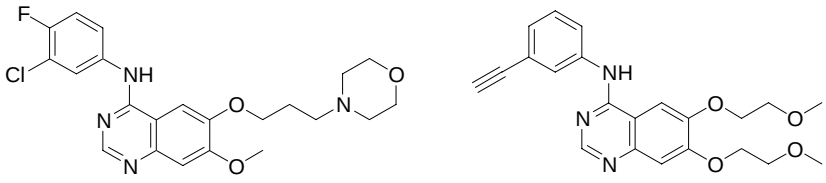


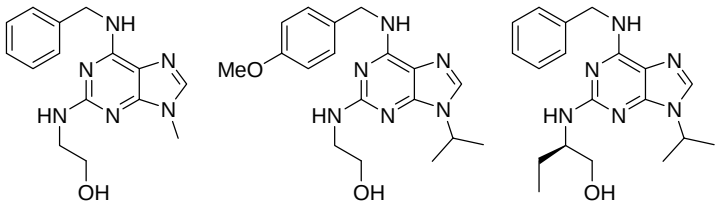
Figure 4: FDA-approved quinazoline-based kinase inhibitors

However, in 2004, a Phase III survival trial proved disappointing with no improved survival in the trial population, forcing the FDA to modify its approval.[17] At the same time, another EGFR inhibitor called erlotinib marketed as Tacerva® (**1-5**, Figure 4) was approved in combination with gemcitabine for treatment of locally advanced, unresectable, or metastatic pancreatic cancer.

2. Purine: an attractive scaffold for kinase inhibition

In parallel to these protein tyrosin kinase inhibitors, in the mid 90's, Meijer and co-workers began working on the purine scaffold to find inhibitors of cyclin-dependant kinases (CDKs), a family of enzymes directly involved in cell cycle control. Although the first results in targeting these kinases specifically were very disappointing, light came from olomoucine (**1-6**, Figure 5) which showed a good efficacy (IC_{50} = 7µM) and an unexpected selectivity for CDKs.[18] Indeed, olomoucine was the first small molecule to be found to inhibit CDK1 by competing with ATP in its active site.

[15] Gazit, A. et al. Tyrphostins. 5. Potent inhibitors of platelet-derived growth factor receptor tyrosine kinase: structure-activity relationships in quinoxalines, quinolines, and indole tyrphostins. *J Med Chem* **39**, 2170-7 (1996).
[16] Morin, M. J. From oncogene to drug: development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents. *Oncogene* **19**, 6574-83 (2000).
[17] http://www.fda.gov/bbs/topics/news/2004/new01145.html
[18] Vesely, J. et al. Inhibition of cyclin-dependent kinases by purine analogues. *Eur J Biochem* **224**, 771-86 (1994).



1-6
Olomoucine

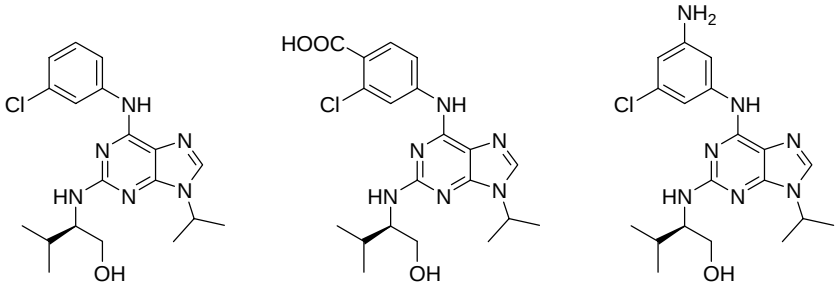
1-7

1-8
Roscovitine

Figure 5: Kinase inhibitors based on the purine scaffold

The first crystal structure of CDK2[19] and its co-crystal structure with olomoucine[20] revealed that the molecule actually binds in the ATP-binding pocket of the kinase however with a completely different orientation than ATP. Inspired by the selectivity of olomoucine, Schultz and co-workers developed solid-phase synthetic methods to access C-2, C-6 and N-9 modified purines as a scaffold for kinase inhibition leading to the discovery of the first synthetic CDK2/cyclin A inhibitor (**1-7**, Figure 5) that shows an IC_{50} of 600nM.[21] In parallel, using a medicinal chemistry approach on olomoucine, Meijer and co-workers discovered that roscovitine (**1-8**, Figure 5) was a quite selective and potent CDK1/cyclin B inhibitor (IC_{50}: 450nM), the (*R*) form showing a slightly better activity.[22,23] These results were the starting point of important combinatorial chemistry efforts for finding even more efficient analogs. Based on a combinatorial synthesis approach both on solid- and solution-phase, Schultz and co-workers rapidly found that the purvalanols analogs (**1-9** to **1-11**, Figure 6) could display very interesting potencies *in vitro* (IC_{50} from 0.004 to 0.04µM for cdc2/cyclin B kinase) along with high selectivities.[24]

[19] De Bondt, H. L. et al. Crystal structure of cyclin-dependent kinase 2. *Nature* **363**, 595-602 (1993).
[20] Schulze-Gahmen, U. et al. Multiple modes of ligand recognition: crystal structures of cyclin-dependent protein kinase 2 in complex with ATP and two inhibitors, olomoucine and isopentenyladenine. *Proteins* **22**, 378-91 (1995).
[21] Norman, T. C., Gray, N., Koh, J. T. & Schultz, P. G. A Structure-Based Library Approach to Kinase Inhibitors. *J Am Chem Soc* **118**, 7430-7431 (1996).
[22] De Azevedo, W. F. et al. Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine. *Eur J Biochem* **243**, 518-26 (1997).
[23] Meijer, L. et al. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Ibid*., 527-36.
[24] (a) Gray, N. S. et al. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* **281**, 533-8 (1998), (b) Rosania, G. R. et al. A cyclin-dependent kinase inhibitor inducing cancer cell differentiation: biochemical identification using Xenopus egg extracts. *Proc Natl Acad Sci U S A* **96**, 4797-802 (1999), (c) Chang, Y. T. et al. Synthesis and application of functionally diverse 2,6,9-trisubstituted purine libraries as CDK inhibitors. *Chem Biol* **6**, 361-75 (1999).



1-9
Purvalanol A
IC_{50}: 0.004µM

1-10
Purvalanol B
IC_{50}: 0.006µM

1-11
Aminopurvalanol
IC_{50}: 0.033µM

Figure 6: Purvulanols, highly potent and selective CDKs inhibitors

These promising preliminary results led to their evaluation as inhibitors of the growth of U937 human leukemic cells. Purvalanol A (**1-9**) and aminopurvalanol (**1-11**) were found to be active albeit with a higher IC_{50} than *in vitro* (7.5 and 5 µM respectively), whereas purvalanol B (**1-10**) was inactive. These data can be rationalized as a result of a competition with higher concentrations of ATP *in vivo* (~ mM) and as a problem of cell permeability for the carboxylic acid analog (**1-10**). The two active compounds (**1-9** and **1-11**) also proved useful to investigate the role of CDK in cell cycle progression. Other major actions such as induction of apoptosis or differentiation of cells have also been reported for these molecules depending on the model studied or on the conditions tested.[24c] In addition, it is noteworthy to say that some of these purine-derived inhibitors are of considerable importance as potent anti-cancer agents. Purvalanol A (**1-9**) and its aminated analog **1-11** have shown significant selectivity to KM12 colon cancer cells (GI_{50} of nanomolar range). The first one (**1-9**) has been tested in combination with taxol in a mouse xenograft model leading to interesting results: tumor growth suppression, improved animal survival or even indefinite survival if the treatment was continuous.[25] In addition, (*R*)-roscovitine (**1-8**, also known as CYC202 for clinical trials) has shown cytostatic effects when given at low dose for a long time and could drive cancer cells into apoptosis when given at high concentrations on animal models.[26] After an extensive Phase I program revealing its good tolerability, CYC202 has entered recently Phase IIa clinical trials with two major programs: the treatment of stage IIIB/IV Non-Small Cell Lung Cancer (NSCLC) in combination with gemcitabine and cisplatinum and the treatment of advanced breast cancer in combination with capecitabine, an oral prodrug of 5-fluorouracil.[27]

[25] O'Connor, D. S., Wall, N. R., Porter, A. C. & Altieri, D. C. A p34(cdc2) survival checkpoint in cancer. *Cancer Cell* **2**, 43-54 (2002).
[26] McClue, S. J. et al. In vitro and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine). *Int J Cancer* **102**, 463-8 (2002).
[27] http://www.cyclacel.com/company_profile/press2003/2003_1_20.htm

In parallel to all these purine derivatives, some molecules bearing either an anthraquinone, a tetrahydroxystilbene or a benzopyranone moiety have been reported in the literature to be kinase inhibitors but did not yield high selectivity among the tested kinases or were proven ineffective *in vivo*.[28]

Besides aforementioned molecules such as gefitinib and erlotinib, twenty three other protein-kinase inhibitors were undergoing human clinical trials and many more were still at the preclinical stage in 2002.[10] Moreover, two inhibitors had been approved for clinical use: Fasudil (**1-12**, Figure 7) as a RHO-dependant protein kinase inhibitor used for cerebral vasospasm and Gleevec® as an anti-cancer agent (**1-13**, Figure 7). More recently, two other drugs called sunitinib marketed as Sutent® (**1-14**, Figure 7)[29] and dasatinib marketed as Sprycel® (**1-15**, Figure 7)[30] have been approved as multiply-targeted tyrosine kinase inhibitors for the treatment of kidney cancer/gastrointestinal stromal tumors and of chronic myeloid leukemia respectively.

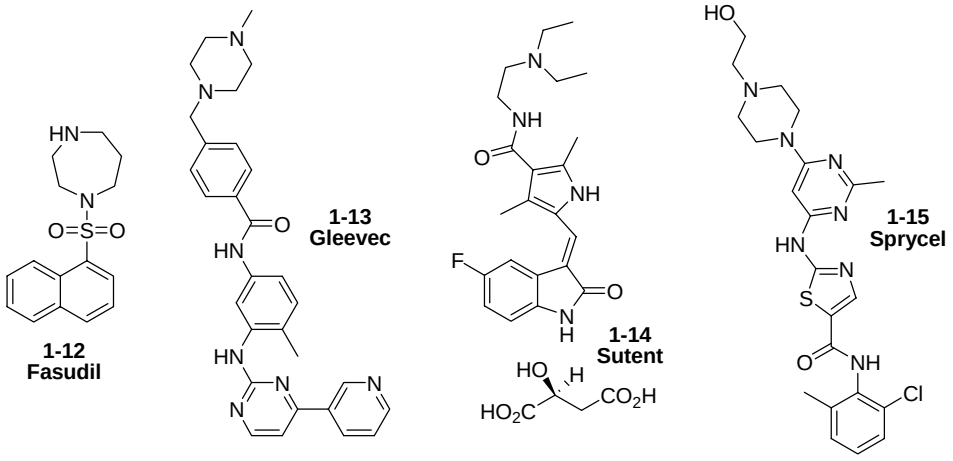


Figure 7: FDA-approved kinase inhibitors

Gleevec® is the first important drug developed as a kinase inhibitor and approved in 2001 for clinical use against chronic myelogenous leukaemia (CML).[31] It is an Abelson tyrosine kinase (ABL) inhibitor that stabilizes the kinase in an inactive conformation.[32] Due to its spectacular

[28] Lawrence, D. S. & Niu, J. Protein kinase inhibitors: the tyrosine-specific protein kinases. *Pharmacol Ther* **77**, 81-114 (1998).
[29] http://www.centerwatch.com/patient/drugs/dru893.html
[30] http://www.centerwatch.com/patient/drugs/dru903.html
[31] (a) Buchdunger, E. et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Res* **56**, 100-4 (1996), (b) Druker, B. J. et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* **2**, 561-6 (1996).
[32] (a) Schindler, T. et al. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **289**, 1938-42 (2000)., (b) Levinson, N. M. et al. A Src-like inactive conformation in the abl tyrosine kinase domain. *PLoS Biol* **4**, e144 (2006).

efficacy and minimal side effects,[33] it was rapidly approved. Further studies have later shown that Gleevec® inhibits also the c-KIT and PDGF-receptor tyrosine kinases,[34] leading to its approval for treatment of gastrointestinal stromal tumors.[35,9] Considering the multiply-targeted activity of Gleevec® and also the recent FDA-approval of two multiple receptor tyrosine kinase inhibitors, achieving high selectivity may not be essential for therapeutic purpose as long as the multiply-inhibited kinases have coherent functions.

3. Methods developed to screen inhibitor selectivity within the kinome

There has also been an increasing interest in developing efficient and innovative methods that allow the evaluation of the selectivity of kinase inhibitors. The most effective approach for the development of kinase-directed drugs seems to be by targeting the catalytic site of the enzymes with ATP competitive inhibitors.[36] Selectivity is a major issue of this strategy as many of the important residues present in the ATP-binding pocket are highly conserved. Therefore, general methods are required for the assessment of this specificity.

In a collaboration between the groups of Schultz and Meijer, it was reported that a simple affinity chromatography approach, using purvalanol B (**1-10**) immobilized to an agarose matrix,[37] allows the screening of several cellular extracts coming from diverse sources and facilitates the isolation and identification of intracellular targets relevant to kinases. Using this methodology, they confirmed that CDK is the intracellular target for purvalanols (Figure 6) but, they also assessed that these compounds target several other kinases (Erk1, Erk2, *etc.*) with a higher IC_{50} than *in vitro* probably due to a higher cellular abundance. This approach could be considered as a general method for identifying intracellular targets of a given ligand.

In 2004, a method, based on a three-hybrid approach that allows the scan of the entire proteome, was developed to identify targets of small molecule kinase inhibitors.[38] This methodology differs from the previous ones in the fact that the interaction between the

[33] Druker, B. J. et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* **344**, 1031-7 (2001).
[34] Buchdunger, E. et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J Pharmacol Exp Ther* **295**, 139-45 (2000).
[35] Joensuu, H. et al. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* **344**, 1052-6 (2001).
[36] Fabbro, D. et al. Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacol Ther* **93**, 79-98 (2002).
[37] Knockaert, M. et al. Intracellular targets of cyclin-dependent kinase inhibitors: identification by affinity chromatography using immobilised inhibitors. *Chem Biol* **7**, 411-22 (2000).
[38] Becker, F. et al. A three-hybrid approach to scanning the proteome for targets of small molecule kinase inhibitors. *Chem Biol* **11**, 211-23 (2004).

enzyme and the prospective lead occurs in the intact cell. By this mean, it was observed that purvalanol B (**1-10**) interacts with 35 different kinases inside the cell, 31 being novel targets (results confirmed by regular *in vitro* assays or affinity chromatography). This methodology might be proved useful for the discovery of candidate drug targets but also for the study of mechanism of actions. However, these two methods have the drawback that the studied kinase inhibitors need to be derivatized (by chemical linking, labelling or immobilization on a matrix) before starting the assay, leading to some tedious preparations.

More recently, Lockhart and co-workers proposed a novel method involving directly the small molecule in a competition assay with immobilized probe ligands that show affinity for a wide range of kinases (Figure 8).[39] Although this methodology does not allow the screening of the full kinome, it has been used for a panel of 119 protein kinases (~1/4 of the kinome) and the profile of twenty known inhibitors has been displayed.

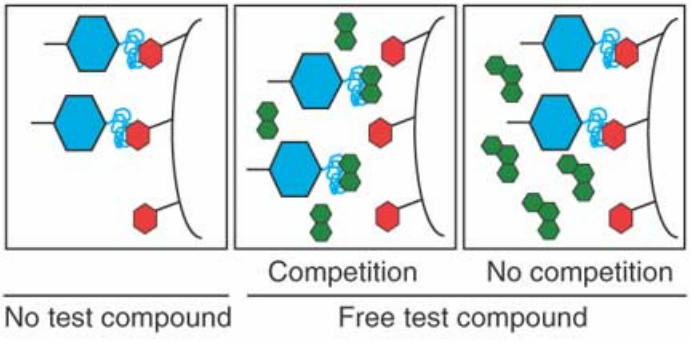


Figure 8: Schematic approach of the competition assay by Lockard and co-workers
(Tagged kinase in blue, test compound in green, immobilized probe ligands in red)

By this mean, binding affinities can be detected as low as 1-10pM but are not dependent on ATP concentrations. In addition to cell-based assays or even clinical observations, this method can be considered a systematic small molecule-protein interaction map for clinical compounds in order to evaluate properly the biological consequences of inhibiting a particular kinase.

The methods described here are only some selected examples of binding assays used to determine the selectivity of given kinase inhibitors.[40] These approaches might be of considerable value in finding new selective inhibitors but also in trying to understand the role

[39] Fabian, M. A. et al. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol* **23**, 329-36 (2005).
[40] von Ahsen, O. & Bomer, U. High-throughput screening for kinase inhibitors. *Chembiochem* **6**, 481-90 (2005).

of the kinases in signaling transduction pathways. There is no doubt that some more methods dealing with the selectivity purpose are going to be developed in the following years.

4. Conclusions

This succinct overview about kinase inhibition clearly shows that designing selective kinase inhibitors remains challenging. Several SAR studies have been done to design combinatorial libraries around the purine scaffold with some noteworthy successes. From a chemical biology perspective, deconvoluting the function of individual kinases within complex networks by using a cell-permeable small molecule can be considered as an attractive approach. The biological function of kinases is often regulated by their conformation, and small molecule inhibitors have the potential to discriminate between the activated or inactivated forms of a given kinase thus providing a mean to dissect their respective functions. From a therapeutic point of view, misregulation of kinases is at the basis of numerous oncogenic processes and of several diseases such as neurodegenerative disorders, cardiovascular diseases, and viral infections. Over the last ten years, enormous efforts have been made in developing kinase inhibitors for targeted chemotherapies. In the near future, there is no doubt that some more powerful and selective kinase inhibitors will be found.

Chapter II − Heat Shock Protein 90: A Promising Target

Rapidly after exposure to environmentally stressful conditions such as heat, hypoxia or acidosis, cells in most tissues dramatically increase the production of a class of proteins that are generally known as heat shock or stress proteins. Extensive researches over the last 30 years[41] have led to the consideration that heat shock proteins (HSPs) are actually molecular chaperones, namely a class of proteins that prevent improper associations and assist in the correct folding of other cellular proteins collectively termed clients and substrates.[42] Their accumulation is not only observed in normal cells disturbed by a stress but also in pathophysiological conditions and tumors. In fact, chaperone proteins facilitate the survival of tumor cells in a stressful environment but also allow tumor cells to tolerate alterations from inside the cell.[43] HSPs are ubiquitous, highly conserved proteins among the species and are usually classified considering their molecular weight to the following major families: HSP100, HSP90, HSP70, HSP60 and the family of small HSPs.[44] Besides their structural and functional differences, all of them work cooperatively at different stages of the protein folding. Among the different families, HSP90 has attracted a lot of attention from the scientific community over the last ten years as being associated with a large number of signaling molecules such as v-Src, Raf, *etc.* that play a crucial role in malignant transformation and metastasis development.[45] Thus, its inhibition would open a new era for chemotherapeutic treatment and help in understanding its implication in complex signaling networks.

[41] Selected reviews : (a) Csermely, P., Schnaider, T., Soti, C., Prohaszka, Z. & Nardai, G. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacol Ther* **79**, 129-68 (1998), (b) Bagatell, R. & Whitesell, L. Altered Hsp90 function in cancer: a unique therapeutic opportunity. *Mol Cancer Ther* **3**, 1021-30 (2004), (c) Sreedhar, A. S., Soti, C. & Csermely, P. Inhibition of Hsp90: a new strategy for inhibiting protein kinases. *Biochim Biophys Acta* **1697**, 233-42 (2004), (d) Whitesell, L. & Lindquist, S. L. HSP90 and the chaperoning of cancer. *Nat Rev Cancer* **5**, 761-72 (2005).

[42] Hartl, F. U. Molecular chaperones in cellular protein folding. *Nature* **381**, 571-9 (1996).

[43] Takayama, S., Reed, J. C. & Homma, S. Heat-shock proteins as regulators of apoptosis. *Oncogene* **22**, 9041-7 (2003).

[44] (a) Craig, E. A., Weissman, J. S. & Horwich, A. L. Heat shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell. *Cell* **78**, 365-72 (1994), (b) Johnson, J. L. & Craig, E. A. Protein folding in vivo: unraveling complex pathways. *Cell* **90**, 201-4 (1997).

[45] (a) Pratt, W. B. & Toft, D. O. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med (Maywood)* **228**, 111-33 (2003), (b) Nollen, E. A. & Morimoto, R. I. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci* **115**, 2809-16 (2002).

1. HSP90: A molecular chaperone

As mentioned above and despite their name, heat shock proteins are expressed under normal conditions in almost all cells. Under non-stressed conditions, HSP90 is one of the most abundant proteins present in the eukaryotic cells, representing between 1-2% of the total cellular protein content and increasing about only two-fold when stressed. Upon binding with the native client, HSP90 operates as an essential housekeeper in the cell assuming functions such as protein folding of nascent polypeptides, translocation of proteins across membranes or normal protein turnover.[41a] Moreover, it has been shown to play a crucial role in the post-translational regulation of signaling molecules, leading to their activation. To achieve all these functions, HSP90 rarely functions alone but displays its activity along with another chaperone HSP70, several other co-chaperones (HSP40, CDC37/p50, AHA1, p23) and accessory proteins, forming the HSP90-based chaperone machinery.

HSP90 presents two isoforms namely HSP90α (major form) and HSP90β (minor form) and two homologues GRP94 (in the endoplasmic reticulum) and TRAP1 (in the mitochondria). HSP90 is located mostly in the cytoplasm where it exists predominantly as a homodimer, each monomer consisting of three main domains: the N-terminal, the middle and the C-terminal domains (Figure 9).

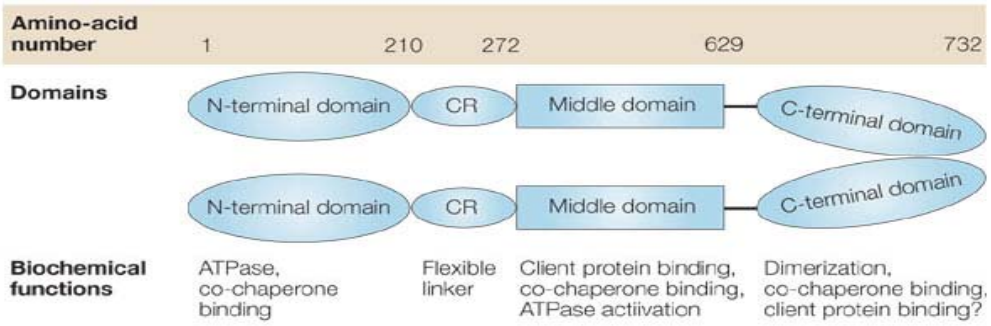


Figure 9: Structure of the HSP90 dimer (Figure reproduced from reference 41d)

Crystal structures of the N-terminal[46] and middle domains[47] have been reported for eukaryotes, as well as structures of the three domains for the bacterial HSP90 homologue

[46] (a) Stebbins, C. E. et al. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* **89**, 239-50 (1997), (b) Prodromou, C. et al. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* **90**, 65-75 (1997).

[47] Meyer, P. et al. Structural and functional analysis of the middle segment of hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. *Mol Cell* **11**, 647-58 (2003).

(htpG).[48] The N-terminal domain contains a rather unique ATP-binding site known as the Bergerat fold,[49] which is characteristic of the GHKL superfamily (bacterial gyrase, HSP90, histidine kinase, and MutL).[50] In eukaryotes, a charged domain serves as a flexible linker between the N-terminal and the middle domains (CR on Figure 9). The middle domain seems to be crucial in modulating ATP hydrolysis as it interacts with the γ-phosphate of ATP molecules bound to the N-terminal pocket of the chaperone,[47] and also in binding many client proteins of HSP90. Finally, a second flexible linker connects the middle domain to the C-terminal one that is responsible for the dimerization. Removal of this C-terminal domain dramatically decreases the ATPase activity of HSP90 that is necessary for its chaperone activity.[51] Furthermore, this region contains a particular motif (MEEVD) that is essential for recruiting co-chaperones which help for the formation of the chaperone complex.

In addition to the components involved in the chaperone machinery, HSP90 binds many cellular proteins that play a crucial role in growth control, cell survival and development processes.[52] The number of these recognised HSP90 clients, that require interactions with the chaperone to function properly, is increasing rapidly.[53] Receptor tyrosine kinases,[54] serine/threonine kinases,[55] steroid hormone receptors,[56] transcription factors[57] and telomerase[58] are common substrates of HSP90. Their oncogenic mutants are also clients but lead to higher requirements for HSP90 function probably because of the conformationally destabilizing nature of the mutation. One convincing example is related to the SRC tyrosine

[48] (a) Huai, Q. et al. Structures of the N-terminal and middle domains of E. coli Hsp90 and conformation changes upon ADP binding. *Structure* **13**, 579-90 (2005), (b) Harris, S. F., Shiau, A. K. & Agard, D. A. The crystal structure of the carboxy-terminal dimerization domain of htpG, the Escherichia coli Hsp90, reveals a potential substrate binding site. *Structure* **12**, 1087-97 (2004).

[49] Bergerat, A. et al. An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* **386**, 414-7 (1997).

[50] Dutta, R. & Inouye, M. GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem Sci* **25**, 24-8 (2000).

[51] Pearl, L. H. & Prodromou, C. Structure, function, and mechanism of the Hsp90 molecular chaperone. *Adv Protein Chem* **59**, 157-86 (2001).

[52] Pratt, W. B. The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. *Proc Soc Exp Biol Med* **217**, 420-34 (1998).

[53] For a regular update of known HSP90 client proteins, http://www.picard.ch/downloads/Hsp90interactors.pdf

[54] Munster, P. N., Marchion, D. C., Basso, A. D. & Rosen, N. Degradation of HER2 by ansamycins induces growth arrest and apoptosis in cells with HER2 overexpression via a HER3, phosphatidylinositol 3'-kinase-AKT-dependent pathway. *Cancer Res* **62**, 3132-7 (2002).

[55] Schulte, T. W. et al. Destabilization of Raf-1 by geldanamycin leads to disruption of the Raf-1-MEK-mitogen-activated protein kinase signaling pathway. *Mol Cell Biol* **16**, 5839-45 (1996).

[56] Beliakoff, J. et al. Hormone-refractory breast cancer remains sensitive to the antitumor activity of heat shock protein 90 inhibitors. *Clin Cancer Res* **9**, 4961-71 (2003).

[57] (a) Muller, L., Schaupp, A., Walerych, D., Wegele, H. & Buchner, J. Hsp90 regulates the activity of wild type p53 under physiological and elevated temperatures. *J Biol Chem* **279**, 48846-54 (2004), (b) Walerych, D. et al. Hsp90 chaperones wild-type p53 tumor suppressor protein. *J Biol Chem* **279**, 48836-45 (2004).

[58] Holt, S. E. et al. Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes Dev* **13**, 817-26 (1999).

kinase. While the normal c-SRC requires only low assistance from the HSP90 machinery for its maturation and activation,[59] v-SRC shows an important association with the chaperone[60] which is required for its activity.[61] Likewise, mutations of the tumor-suppressor protein p53 lead to the most common molecular genetic defect found in human cancers and most p53 mutants show extended interactions with HSP90 (probably because of aberrant conformations), preventing their usual ubiquitylation and subsequent degradation by the proteasome.[62]

Despite a fairly ubiquitous role, HSP90 has mostly pro-growth signaling proteins as clients and its chaperoning function is subverted during oncogenesis thus leading to the development of malignant transformation and the maintenance of transformed phenotype. Since the mid-90's, important efforts have been done in understanding the implication of HSP90 in signaling pathways and targeting the chaperone has appeared as a much broader and effective strategy for anti-cancer therapies than focusing on a single signaling network.

2. Inhibition of HSP90

Based on the role of HSP90 in regulating several proteins that are responsible for malignant transformations and having in mind that it is constitutively expressed at two- to ten-fold higher levels in tumor cells than in their normal counterparts,[63] this chaperone has emerged as a powerful target for anti-cancer research.[64] Inhibition of HSP90 results in the degradation of its clients via ubiquitination of the unfolded protein followed by proteasome-mediated hydrolysis. Based on the molecular structure of HSP90, its inhibitors can be divided in two categories depending on their binding site: the N-terminal domain inhibitors and the C-terminal domain inhibitors. Although most of the inhibitors reported so far bind to the N-

[59] Xu, Y., Singer, M. A. & Lindquist, S. Maturation of the tyrosine kinase c-src as a kinase and as a substrate depends on the molecular chaperone Hsp90. *Proc Natl Acad Sci U S A* **96**, 109-14 (1999).
[60] Oppermann, H., Levinson, W. & Bishop, J. M. A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat-shock protein. *Ibid.* **78**, 1067-71 (1981).
[61] Whitesell, L., Mimnaugh, E. G., De Costa, B., Myers, C. E. & Neckers, L. M. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Ibid.* **91**, 8324-8 (1994).
[62] Blagosklonny, M. V., Toretsky, J., Bohen, S. & Neckers, L. Mutant conformation of p53 translated in vitro or in vivo requires functional HSP90. *Ibid.* **93**, 8379-83 (1996).
[63] Ferrarini, M., Heltai, S., Zocchi, M. R. & Rugarli, C. Unusual expression and localization of heat-shock proteins in human tumor cells. *Int J Cancer* **51**, 613-9 (1992).
[64] Selected reviews: (a) Neckers, L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol Med* **8**, S55-61 (2002), (b) Chiosis, G., Vilenchik, M., Kim, J. & Solit, D. Hsp90: the vulnerable chaperone. *Drug Discov Today* **9**, 881-8 (2004), (c) Janin, Y. L. Heat shock protein 90 inhibitors. A text book example of medicinal chemistry? *J Med Chem* **48**, 7503-12 (2005).

terminal domain (*vide infra*), some molecules have been reported as interacting with the C-terminal one.

In a target-oriented screening, novobiocin (**1-16**, Figure 10), a well-known antibiotic, was discovered as the first weak inhibitor of the C-terminal domain of HSP90.[65]

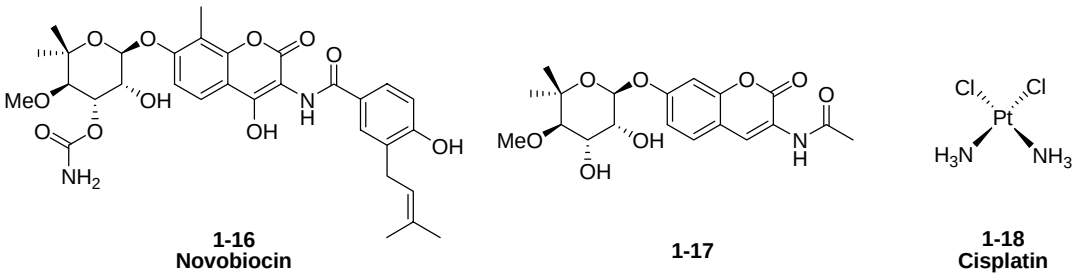


Figure 10: HSP90 inhibitors binding to the C-terminal domain

Under high concentrations (more than 500 μM), novobiocin destabilizes a number of important oncogenic clients such Raf-1, Her2, v-src and mutant p53 and *in vivo* testings showed reduction in Raf-1 levels in human PMB cells. This inhibitor led also to the discovery that HSP90 possesses a second binding-site for ATP in the C-terminal domain.[66] More recently, a library of analogs of novobiocin has revealed compound **1-17** (Figure 10) as a more potent inhibitor (~1 μM) with significant decrease of clients' expression in prostate cancer cell lines.[67] In 2002, cisplatin (**1-18**, Figure 10), a well-known chemotherapeutic drug, was also reported to interact with the C-terminal region of HSP90 and to interfere with nucleotide binding in this domain.[68] More recently, Rein and co-workers have shown that cisplatin can inhibit the transcriptional activity of the androgen and glucocorticoid receptors by disrupting their binding to HSP90, whereas other HSP90 clients were not affected.[69] Besides its probable implication in the ATPase activity and in the cycling of the machinery, the function of the C-terminal part of HSP90 remains unclear but, compounds interacting in this domain clearly impair HSP90 function leading to anti-cancer effects.

[65] Marcu, M. G., Schulte, T. W. & Neckers, L. Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. *J Natl Cancer Inst* **92**, 242-8 (2000).

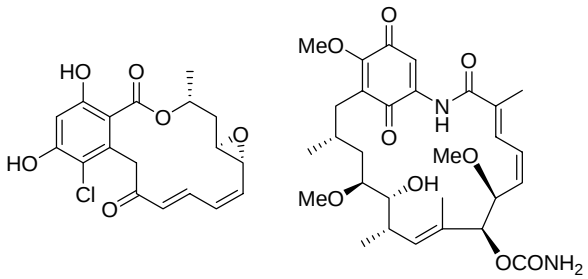
[66] (a) Marcu, M. G., Chadli, A., Bouhouche, I., Catelli, M. & Neckers, L. M. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. *J Biol Chem* **275**, 37181-6 (2000), (b) Garnier, C. et al. Binding of ATP to heat shock protein 90: evidence for an ATP-binding site in the C-terminal domain. *J Biol Chem* **277**, 12208-14 (2002).

[67] Yu, X. M. et al. Hsp90 inhibitors identified from a library of novobiocin analogues. *J Am Chem Soc* **127**, 12778-9 (2005).

[68] Soti, C., Racz, A. & Csermely, P. A Nucleotide-dependent molecular switch controls ATP binding at the C-terminal domain of Hsp90. N-terminal nucleotide binding unmasks a C-terminal binding pocket. *J Biol Chem* **277**, 7066-75 (2002).

[69] Rosenhagen, M. C. et al. The heat shock protein 90-targeting drug cisplatin selectively inhibits steroid receptor activation. *Mol Endocrinol* **17**, 1991-2001 (2003).

As mentionned above, most of the HSP90 inhibitors reported so far act by binding to the N-terminal domain of the chaperone. Although nothing was known about their mechanism of action, two natural products radicicol (**1-19**, Figure 11) and geldanamycin (**1-20**, Figure 11) isolated respectively in 1953[70] and 1970,[71] were shown to suppress the transformed phenotype of cell expressing activated Src.[72,73]



1-19
Radicicol

1-20
Geldanamycin

Figure 11: First HSP90 inhibitors

Affinity chromatography demonstrated that these two compounds target specifically Heat Shock Protein 90.[74,75] Further investigations and especially, co-crystal structures showed that they were both bound in the N-terminal ATP-binding pocket of HSP90[76] by competing with ATP.

Geldanamycin (**1-20**, Figure 11) and herbimycin[77] (**1-21**, Figure 12) were the first compounds reported as targeting HSP90.

[70] Delmotte, P. & Delmotte-Plaquee, J. A new antifungal substance of fungal origin. *Nature (London, United Kingdom)* **171**, 344 (1953).
[71] DeBoer, C., Meulman, P. A., Wnuk, R. J. & Peterson, D. H. Geldanamycin, a new antibiotic. *J Antibiot (Tokyo)* **23**, 442-7 (1970).
[72] Kwon, H. J., Yoshida, M., Fukui, Y., Horinouchi, S. & Beppu, T. Potent and specific inhibition of p60v-src protein kinase both in vivo and in vitro by radicicol. *Cancer Research* **52**, 6926-30 (1992).
[73] Uehara, Y., Murakami, Y., Mizuno, S. & Kawai, S. Inhibition of transforming activity of tyrosine kinase oncogenes by herbimycin A. *Virology* **164**, 294-8 (1988).
[74] Sharma, S. V., Agatsuma, T. & Nakano, H. Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Oncogene* **16**, 2639-45 (1998).
[75] Whitesell, L., Mimnaugh, E. G., De Costa, B., Myers, C. E. & Neckers, L. M. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. *Proc Natl Acad Sci U S A* **91**, 8324-8 (1994).
[76] (a) Stebbins, C. E. et al. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* **89**, 239-50 (1997), (b) Roe, S. M. et al. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. *J Med Chem* **42**, 260-6 (1999).
[77] Omura, S. et al. Herbimycin, a new antibiotic produced by a strain of Streptomyces. *J Antibiot (Tokyo)* **32**, 255-61 (1979).

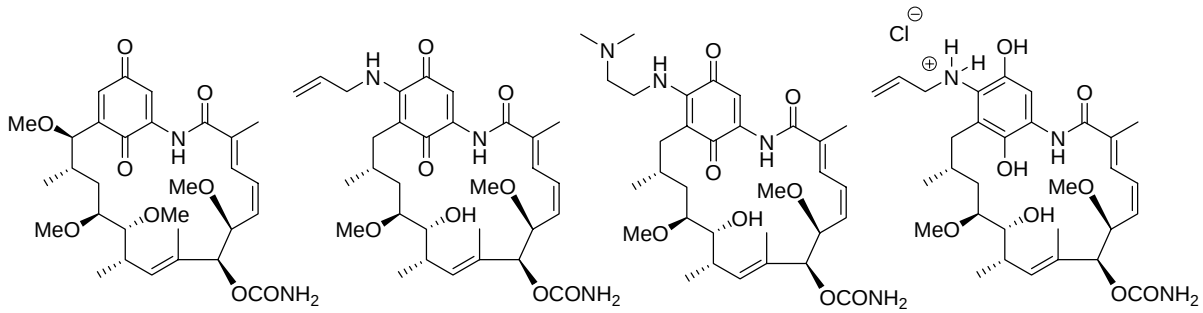


Figure 12: Ansamycin-based HSP90 inhibitors

HSP90 inhibitors alter the chaperone function by preventing the dissociation of HSP90 clients from the chaperone machinery. As the client proteins do not reach their conformational maturation, they are degraded by the proteasome. Although geldanamycin and herbimycin induce growth arrest followed by differentiation and/or apoptosis,[78] their use as clinical agents has been limited particularly because of their hepatotoxicity[79] (that could be attributed to the benzoquinone functionality) and due to their low solubility in aqueous media. Accordingly, two analogs so called 17AAG[80] (**1-22**, Figure 12) and 17DMAG[81] (**1-23**, Figure 12) were selected based on their similar cellular efficacy and their lower hepatotoxicity compared to geldanamycin. 17AAG is highly potent *in vitro* and its efficacy has been shown in several animal models presenting breast, prostate or colon cancers.[82] Consequently, it has entered clinical trials in cancer patients. Phase I has been successfully achieved for some cancers[83] and Phase II is on going with patients presenting melanoma, metastatic breast cancer, *etc*.[84] The first results are promising with prolonged stable disease in patients with melanoma or

[78] Munster, P. N., Srethapakdi, M., Moasser, M. M. & Rosen, N. Inhibition of heat shock protein 90 function by ansamycins causes the morphological and functional differentiation of breast cancer cells. *Cancer Res* **61**, 2945-52 (2001).
[79] Supko, J. G., Hickman, R. L., Grever, M. R. & Malspeis, L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother Pharmacol* **36**, 305-15 (1995).
[80] Schulte, T. W. & Neckers, L. M. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Ibid.* **42**, 273-9 (1998).
[81] Kaur, G. et al. Antiangiogenic properties of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. *Clin Cancer Res* **10**, 4813-21 (2004).
[82] (a) Solit, D. B. et al. 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. *Ibid.* **8**, 986-93 (2002), (b) Kelland, L. R., Sharp, S. Y., Rogers, P. M., Myers, T. G. & Workman, P. DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J Natl Cancer Inst* **91**, 1940-9 (1999).
[83] http://www.clinicaltrials.gov/ct/gui/show/NCT00004241?order)13
[84] http://www.clinicaltrials.gov/ct/gui/search?term=17-N-Allylamino-17-Demethoxygeldanamycin&submit=Search

renal cancer.[85] However, some problems of solubility and of formulation have been reported and for unknown reasons, 17AAG (**1-22**) is not active against some tumor cell lines. As a consequence, a second-generation inhibitor 17DMAG (**1-23**) has been developed. Compound **1-23** present the same *in vivo* and *in vitro* activities than 17AAG along with water soluble properties and potentially oral availability.[81] After promising preclinical studies,[86] it has currently entered Phase I clinical trials in patients with advanced cancers.[87] However, the problem concerning the hepatic toxicity of these geldanamycin analogs may remain as the benzoquinone functionality is essential for the activity of the ansamycins and that may be a limiting factor for clinical uses. This year, Infinity Pharmaceuticals has proposed a highly soluble and potent inhibitor of HSP90 known as IPI-504 (**1-24**, Figure 12).[88] Specific responses in SKBR3 and SKOV3 cell lines are consistent with HSP90 inhibition. Considering its water-solubility and its interesting pharmacological properties, this compound is under evaluation in Phase I clinical trials in multiple myeloma and gastrointestinal stromal tumors.

Another well-explored class of HSP90 inhibitors is based on the radicicol scaffold.[74] As previously discussed, radicicol (**1-19**, Figure 11) binds to the ATP-binding pocket of HSP90 and presents cellular effects similar to those of geldanamycin.[89] However, despite its impressive *in vitro* activity (IC_{50} = 19nM against 12µM for geldanamycin),[76b] it is inactive *in vivo*[90] probably due to its metabolic instabilities (Michael acceptor, lability of the epoxide).[91] To circumvent the first of these problems, oxime derivatives such as KF25706 (**1-25**, Figure 13)[92] and KF55823 (**1-26**, Figure 13)[93] have been synthesized to decrease the electrophilicity of the Michael acceptor.

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[85] Sausville, E. A., Tomaszewski, J. E. & Ivy, P. Clinical development of 17-allylamino, 17-demethoxygeldanamycin. *Curr Cancer Drug Targets* **3**, 377-83 (2003).

[86] Eiseman, J. L. et al. Pharmacokinetics and pharmacodynamics of 17-demethoxy 17-[[(2-dimethylamino)ethyl]amino]geldanamycin (17DMAG, NSC 707545) in C.B-17 SCID mice bearing MDA-MB-231 human breast cancer xenografts. *Cancer Chemother Pharmacol* **55**, 21-32 (2005).

[87] http://www.clinicaltrials.gov/ct/search;jsessionid=5B464DE9874B877FD46DE0522E6CF974?term=17DMAG&submit=Search

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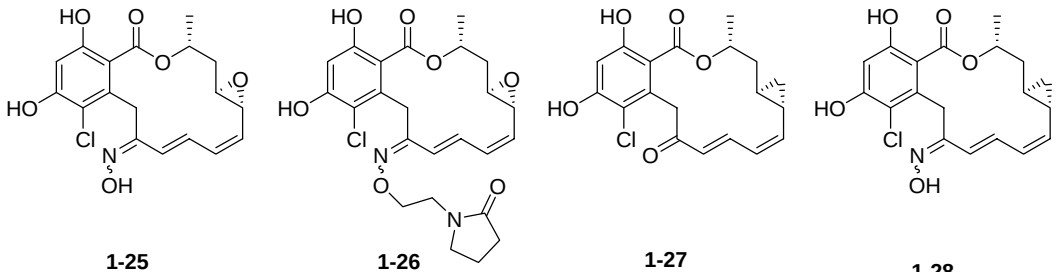
[89] Schulte, T. W. et al. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. *Cell Stress Chaperones* **3**, 100-8 (1998).

[90] Kwon, H. J. et al. Suppression of morphological transformation by radicicol is accompanied by enhanced gelsolin expression. *Oncogene* **15**, 2625-2631 (1997).

[91] Agatsuma, T. et al. Halohydrin and oxime derivatives of radicicol: synthesis and antitumor activities. *Bioorg Med Chem* **10**, 3445-54 (2002).

[92] Soga, S. et al. KF25706, a novel oxime derivative of radicicol, exhibits in vivo antitumor activity via selective depletion of Hsp90 binding signaling molecules. *Cancer Res* **59**, 2931-8 (1999).

[93] Soga, S. et al. Stereospecific antitumor activity of radicicol oxime derivatives. *Cancer Chemotherapy and Pharmacology* **48**, 435-445 (2001).



1-25
KF25706

1-26
KF55823

1-27
Cycloproparadicicol

1-28

Figure 13: Radicicol-based HSP90 inhibitors

In vitro, compound **1-25** has shown antiproliferative activities against various cultured rat and human cell lines (IC_{50} from 26 to 210nM, relatively closed or even better than the IC_{50} of radicicol). Interestingly, this molecule showed significant growth-inhibitory activity in different xenograft models and was able to deplete several HSP90-associated signaling molecules such as Raf-1 or Cdk4 *in vivo*. Similarly, both isomers of compound **1-26** have been tested separately in KPL-4 cells (*in vitro*) and KPL-4 human breast cancer xenografts (*in vivo*). The results demonstrated that both molecules were active *in vitro* but, only the *E* form of the oxime showed *in vivo* antitumor activity. In fact, in a tumor specimen recovered from nude mice, only the *E* isomer of KF55823 (**1-26**) led to the depletion of HSP90 client proteins such as erbB2, Raf-1 and Akt showing that, in this specific case, the stereochemistry of the oxime moiety is crucial for the biological activity. Several other examples of these radicicol derivatives have been reported in the literature, most of them showing better efficacy *in vivo* than radicicol.[94] Considering their biological potential, there is no doubt that they could be used for clinical cancer treatment. At the beginning of our work on radicicol and pochonin C, Danishefsky and co-workers developed the cyclopropane analog of radicicol commonly named cycloproparadicicol (**1-27**, Figure 13).[95] Indeed, during their work on epothilone, they demonstrated that the epoxide moiety, which could induce non-specific toxicity and *in vivo* instability, could be replaced by a cyclopropane functionnality.[96] Biological evaluation in a binding-competition assay for HSP90 revealed that cycloproparadicicol (**1-28**) still retains activity although with a fourfold decrease in comparison to radicicol (IC_{50} (**1-27**) = 160nM, IC_{50} (radicicol) = 45nM)). Further evaluation of its growth-inhibition activity in MCF-7 breast cancer cells corroborated the results found *in vitro* with an IC_{50} in the same order of

[94] Ikuina, Y. et al. Synthesis and antitumor activity of novel O-carbamoylmethyloxime derivatives of radicicol. *J Med Chem* **46**, 2534-41 (2003).
[95] Yamamoto, K. et al. Total synthesis as a resource in the discovery of potentially valuable antitumor agents: cycloproparadicicol. *Angew Chem Int Ed Engl* **42**, 1280-4 (2003).
[96] Rivkin, A., Chou, T. C. & Danishefsky, S. J. On the Remarkable Antitumor Properties of Fludelone: How We Got There. *Ibid.* **44**, 2838-2850 (2005).

magnitude (IC_{50} (**1-27**) = 43nM, IC_{50} (radicicol) = 23nM)). Considering the promising results of this cyclopropane analog and the encouraging ones of the oxime derivatives, the same group developed compound **1-28** (Figure 13) bearing both functionalities.[97] In the same assay against MCF-7 breast cancer cells, the oxime (*Z*)-**1-28** was found to be half as active as cycloproparadicicol, while the *E* oxime was even less active (three times less than (*Z*)-**1-28**). Evaluation of their abilities to degrade Her2, one of the most sensitive clients of the chaperone, showed that both **1-27** and (*Z*)-**1-28** were able to deplete the client protein at 0.3µM.

In an interesting approach to combine the pharmacophores of radicicol and geldanamycin, Blagg and co-workers focused their efforts on the synthesis of chimeric inhibitors bearing both the carboxyl resorcinol part of radicicol and the benzoquinone moiety of geldanamycin.[98] Based on the analysis of the co-crystal structures reported for HSP90 with radicicol and geldanamycin respectively, the hypothesis was made that the binding of these two functionalities in the ATP binding pocket was critical for the biological activity of both geldanamycin and radicicol. So, the first chimera called radamide was developed (**1-29**, Figure 14).

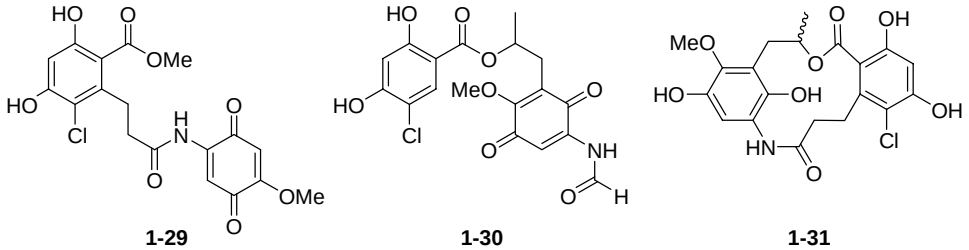


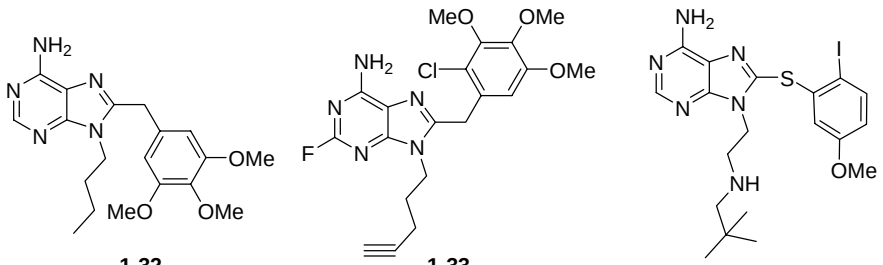
Figure 14: Chimeric inhibitors of HSP90

In a biological assay for inhibition of HSP90's ATPase activity, compound **1-29** showed an IC_{50} of 5.9µM (IC_{50} (geldanamycin) = 2.5µM). In MCF-7 breast cancer cells, a decrease of Her2 was observed proving that **1-29** clearly inhibits HSP90 in cells albeit at 100µM. Later studies proved radester (**1-30**, Figure 14) to share the biological properties of radamide with almost the same potency.[99] Further efforts in designing more chimeric inhibitors led to the

[97] Yang, Z. Q. et al. New efficient synthesis of resorcinylic macrolides via ynolides: establishment of cycloproparadicicol as synthetically feasible preclinical anticancer agent based on Hsp90 as the target. *J Am Chem Soc* **126**, 7881-9 (2004).
[98] Clevenger, R. C. & Blagg, B. S. Design, synthesis, and evaluation of a radicicol and geldanamycin chimera, radamide. *Org Lett* **6**, 4459-62 (2004).
[99] Shen, G. & Blagg, B. S. Radester, a novel inhibitor of the Hsp90 protein folding machinery. *Ibid.* **7**, 2157-60 (2005).

discovery of radanamycin (**1-31**, Figure 14) that showed improved potency both *in vitro* and *in vivo*.[100] In fact, depletion of Her2 and Akt could be observed with 1-5μM of compound **1-31** and an antiproliferative activity with an IC_{50} of 1.2μM was reported, both liking HSP90 inhibition and cell growth arrest. More recently, the same group reported a series of chimeras with their biological evaluation in antiproliferative and client degradation assays[101] corroborating the previous results and paving the road for the development of these compounds as possible useful alternatives to geldanamycin in clinical trials. It is worth noticing that in the last generation of chimeras (**1-31**) the benzoquinone moiety of geldanamycin, that is thought to induce hepatotoxicity, has been replaced by a dihydroquinone thus, a lower toxicity may be expected from these compounds.

In an effort to address the limitations of both geldanamycin and radicicol, Chiosis and co-workers designed new small molecules that could fit in the ATP binding site of HSP90. Co-cystal structures of radicicol, geldanamycin and ADP in the N-terminal domain of HSP90 revealed several important factors necessary to achieve high selectivity. A folded shape even in the free state and a higher affinity than ADP for HSP90 were two other requirements for their design. With all these prerequisites in mind, the first designed inhibitor of HSP90 based on a purine scaffold was developed and called PU3 (**1-32**, Figure 15).[102]



1-32 **PU3** **1-33** **PU24FCl** **1-34**

Figure 15: Purine-based designed HSP90 inhibitors

This molecule (**1-32**) was shown to compete with geldanamycin for HSP90 binding with an IC_{50} of 15-20μM whereas 17AAG only required 1μM. Nevertheless, degradation of HSP90 clients was shown in different cancer cell lines (in a similar manner to geldanamycin) and PU3 inhibited the growth of breast cancer cells causing G1 arrest and cell differentiation at

[100] Wang, M., Shen, G. & Blagg, B. S. Radanamycin, a macrocyclic chimera of radicicol and geldanamycin. *Bioorg Med Chem Lett* **16**, 2459-62 (2006).
[101] Shen, G., Wang, M., Welch, T. R. & Blagg, B. S. Design, synthesis, and structure--activity relationships for chimeric inhibitors of Hsp90. *J Org Chem* **71**, 7618-31 (2006).
[102] Chiosis, G. et al. A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. *Chem Biol* **8**, 289-299 (2001).

100µM. Based on these promising results and in order to achieve a higher potency, a first generation library of 70 compounds was synthesized leading to the identification of PU24FCl (**1-33**, Figure 15) as a potent inhibitor of the chaperone.[103] PU24FCl bound to HSP90 with an affinity comparable to 17AAG (EC_{50} (**1-33**) = 0.45µM, thirty-times more than PU3) and primary *in vivo* assays were very encouraging. Further studies showed that PU24FCl presents ten- to fifty-times higher affinity for HSP90 in cancer cells than in normal ones.[104] In contrast to geldanamycin and radicicol, this compound was shown to be active at similar doses (3-6µM) in almost all tested tumors, even those resistant to 17AAG. Consequently to HSP90 inhibition, PU24FCl leads to several anti-tumor specific effects such as inhibition of cancer cell growth, delay of cell cycle progression, induction of morphological and functional changes, and apoptosis. In order to increase the potency of their HSP90 inhibitors, Chiosis and co-workers focused their attention on developing 8-arylsulfanyl, 8-arylsulfoxyl and 8-arylsulfonyl adenine derivatives. Although these analogs showed an improved potency both *in vitro* and *in vivo* (EC_{50} = 30nM for the best one) in comparison to **1-33**,[105] they were poorly water soluble and therefore unacceptable for oral bioavailability. To circumvent these problems, other derivatives were synthesized[106] leading to a new purine analog (**1-34**, Figure 15).[107] This compound shows an IC_{50} of 90nM in a Her2 degradation assay and 200nM in *in vitro* growth inhibition assays. The amino side-chain proved to be essential for its aqueous solubility and greatly facilitated its formulation for oral delivery.

Other compounds with different structures such as pyrazoles (**1-35**, Figure 16)[108] or benzothiazolothio-purines (**1-36**, Figure 16)[109] have been reported recently in the literature.

[103] Chiosis, G., Lucas, B., Shtil, A., Huezo, H. & Rosen, N. Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. *Bioorg Med Chem* **10**, 3555-64 (2002).

[104] Vilenchik, M. et al. Targeting wide-range oncogenic transformation via PU24FCl, a specific inhibitor of tumor Hsp90. *Chem Biol* **11**, 787-97 (2004).

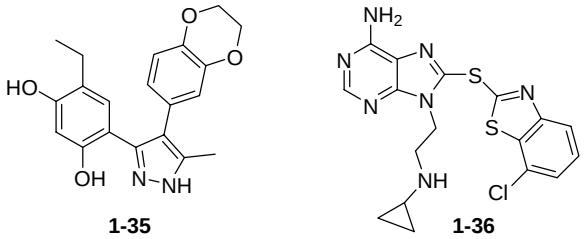
[105] Llauger, L. et al. Evaluation of 8-arylsulfanyl, 8-arylsulfoxyl, and 8-arylsulfonyl adenine derivatives as inhibitors of the heat shock protein 90. *J Med Chem* **48**, 2892-905 (2005).

[106] He, H. et al. Identification of potent water soluble purine-scaffold inhibitors of the heat shock protein 90. *Ibid.* **49**, 381-90 (2006).

[107] Biamonte, M. A. et al. Orally active purine-based inhibitors of the heat shock protein 90. *Ibid.* **49**, 817-28 (2006).

[108] Rowlands, M. G. et al. High-throughput screening assay for inhibitors of heat-shock protein 90 ATPase activity. *Anal Biochem* **327**, 176-83 (2004).

[109] Zhang, L. et al. 7'-substituted benzothiazolothio- and pyridinothiazolothio-purines as potent heat shock protein 90 inhibitors. *J Med Chem* **49**, 5352-62 (2006).



1-35 **1-36**

Figure 16: Other classes of HSP90 inhibitors

The pyrazole derivative **1-35** was identified in a high throughput screening of 60 000 compounds for ATPase activity inhibition of yeast HSP90 and shown to inhibit human colon, ovarian and melanoma tumor cells at around 8µM.[108] Some closely-related analogs have shown inhibition of HCT116 human colon tumor cells in a similar range as 17-AAG.[110] However, further studies concerning their *in vivo* efficacy and toxicity still remain to be published. Recently, compound **1-36** has been shown to exhibit low nanomolar inhibition in a Her2 degradation assay (28nM) and to inhibit tumor growth in a N87 human colon cancer xenograft model after oral administration (due to good aqueous solubility).[109] As HSP90 has been shown to play a key role in central nervous system (CNS) disorder and inflammation, the authors suggest that this novel class of compound may be of utility beyond oncology.

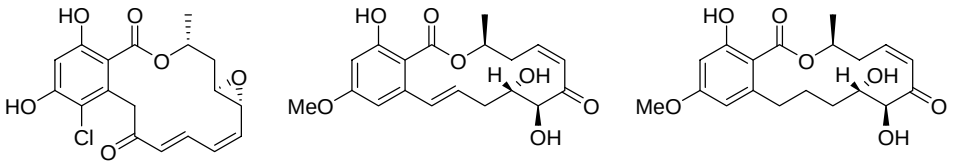
3. Conclusions

Heat shock protein 90 and its molecular complex are essential for the survival of cells with regards to their "housekeeping" ability. Inhibition of HSP90 leads to the degradation of its clients by the proteasome through ubiquitination. From a therapeutic point of view, important efforts have been devoted to developing new inhibitors with a variety of scaffolds to find more potent and "drugable" agents. The fact that 17AAG is currently in Phase II clinical trials is an encouraging validation of HSP90 as a chemotherapeutic target. Combined treatments with radiation therapy or conventional cytotoxic agents are also underway. Furthermore, several new compounds are expected to enter clinical trials upon validation of pharmacological studies. From a biological perspective, inhibiting HSP90 can be interesting in defining its complex role in various biological processes. Besides advances done in finding efficient anti-cancer agents, the large panel of HSP90 inhibitors reported so far might also represent a useful tool to understand HSP90-related processes.

[110] Dymock, B. W. et al. Novel, potent small-molecule inhibitors of the molecular chaperone Hsp90 discovered through structure-based design. *Ibid.* **48**, 4212-5 (2005).

Chapter III − Resorcylic Acid Lactones

While resorcylic acid lactones (RALs) have been known for a long time, the more recent discoveries that radicicol (**1-19**, Figure 17) is a potent and selective HSP90 inhibitor while other members such as LL-Z1640-2 (**1-37**, Figure 17) and L-783,277 (**1-38**, Figure 17) are potent kinase inhibitors (*vide infra*) have stimulated a renewed interest in this family of natural products.[111]



1-19
Radicicol
HSP90 Inhibitor (20nM)

1-37
LL-Z1640-2
TAK1 inhibitor (8.1nM)

1-38
L-783,277
MEK inhibitor (4nM)

Figure 17: Selected potent kinase or ATPase macrocyclic inhibitors

1. From isolation to biological properties

Monorden (**1-19**, Figure 17) was the first isolated RAL in 1953 from *Monocillium nordinii*.[70] Ten years later the same molecule was independently isolated from *Nectria radicicola*[112] and called radicicol (**1-19**). With the assignment of its three chiral centers by X-ray crystallography in the late-80's[113] it was shown that the initial structure proposed for monorden was incorrect leading to the common acceptance of radicicol as the name of this molecule. In 1964, mild sedative and moderate antibiotic properties were reported for radicicol.[114] While radicicol was reported to inhibit Src in cellular assays, it was later shown that this observation comes from the fact that radicicol is a potent and selective HSP90 inhibitor. On the other hand, several RALs bearing a *cis*-enone on the 14-membered macrocycle such as LL-Z1640-2 (**1-37**, Figure 17), L-783,277 (**1-38**, Figure 17),

[111] Winssinger, N. & Barluenga, S. Chemistry and biology of resorcylic acid lactones. *J. Chem Soc., Chem. Commun.*, DOI: 10.1039/b610344h (2007).
[112] Mirrington, R. N., Ritchie, E., Shoppee, C. W., Taylor, W. C. & Sternhell, S. Constitution of radicicol. *Tetrahedron Letters* **6**, 365-70 (1964).
[113] Cutler, H. G. et al. Monorden from a novel source, Neocosmospora tenuicristata: stereochemistry and plant growth regulatory properties. *Agricultural and Biological Chemistry* **51**, 3331-8 (1987).
[114] McCapra, F., Scott, A. I., Delmotte, P., Delmotte-Plaquee, J. & Bhacca, N. S. Constitution of monorden, an antibiotic with tranquilizing action. *Tetrahedron Letters* **6**, 869-75 (1964).

hypothemycin (**1-39**, Figure 18) and radicicol A (**1-40**, Figure 18) have all been reported to be kinase inhibitors.

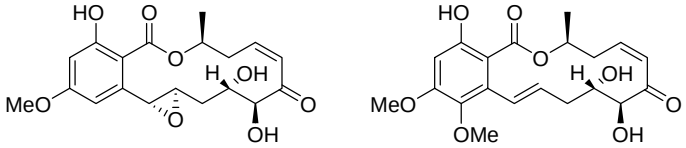


Figure 18: Hypothemycin and Radicicol A

Radicicol A (**1-40**, also known as F87-250904) was isolated in 1987 from the fungus strain F/87-2509.04 while looking for interleukin 1 beta (IL1β) inhibitory activity, an important mediator of inflammation.[115] Several years later, Traber and co-workers corroborated this finding along with inhibition of the tumor necrosis factor alpha (TNF-α) secretion, another major factor of inflammation.[116] Its mode of action is related to the degradation of specific mRNA sequences containing AU-rich elements (AREs).[117] Radicicol A (**1-40**) was found to inhibit tyrosine phosphorylation of several proteins differentially expressed or modified by a tyrosine kinase. Its effect is not directly on protein/RNA interaction or on the state of phosphorylation of a protein/RNA complex at the ARE but, it modifies directly proteins that bind mRNA. Several other analogs were also found to inhibit IL1β secretion but, without destabilizing mRNA, leading to the speculation that small structural differences are able to change the mode of action and presumably the target of inhibition. This hypothesis was even later corroborated by other members of this family such as LL-Z1640-2 (**1-37**) isolated in 1978[118], hypothemycin (**1-39**) in 1980[119] from *Hypomyces tricothecoides* and L-783,277 (**1-48**). In 1999, researchers at Merck isolated L-783,277 from organic extracts of a *Phoma* sp. (ATCC 74403) and showed L-783,277 (**1-38**) to be a potent, ATP-competitive and

[115] Schnyder, J., Payne, T. & Dinarello, C. A. Human monocyte or recombinant interleukin 1's are specific for the secretion of a metalloproteinase from chondrocytes. *J Immunol* **138**, 496-503 (1987).
[116] Kastelic, T. et al. Induction of rapid IL-1 beta mRNA degradation in THP-1 cells mediated through the AU-rich region in the 3'UTR by a radicicol analogue. *Cytokine* **8**, 751-61 (1996).
[117] Cheneval, D. et al. Increased mature interleukin-1beta (IL-1beta) secretion from THP-1 cells induced by nigericin is a result of activation of p45 IL-1beta-converting enzyme processing. *J Biol Chem* **273**, 17846-51 (1998).
[118] Ellestad, G. A., Lovell, F. M., Perkinson, N. A., Hargreaves, R. T. & McGahren, W. J. New zearalenone related macrolides and isocoumarins from an unidentified fungus. *J Org Chem* **43**, 2339-43 (1978).
[119] Nair, M. S. R. & Carey, S. T. Metabolites of pyrenomycetes XIII: Structure of (+) hypothemycin, an antibiotic macrolide from Hypomyces trichothecoides. *Tetrahedron Letters* **21**, 2011-2012 (1980).

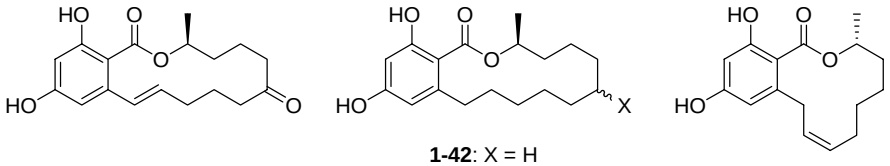
irreversible inhibitor of MEK1 with an IC_{50} of 4nM.[120] At the same time, hypothemycin (**1-39**) was shown to be a slightly less active MEK1 inhibitor with an IC_{50} of 15nM, providing an explanation to previously reported effects such as inhibition of the *ras*-signaling pathway.[121] In 2003, Matsumoto and co-workers showed that LL-Z1640-2 (**1-37**) inhibited irreversibly the kinase activity of TAK1 (IC_{50} = 8.1nM) by competing with ATP, whereas structurally related molecules were inactive.[122] MEK1 and TAK1 are two serine/threonine-specific protein kinases involved in the MAP kinase cascade.[123,124] The importance of these kinases in regulating cellular response to stimuli and translating them into gene expression, cell growth and apoptosis has made MEK1, TAK1 and more generally the kinases involved in the MAP cascade primary targets in drug discovery.

Santi and co-workers have recently shown that the irreversible inhibition of MEK by hypothemycin (**1-39**) can be explained by a Michael addition of a cysteine residue, present in the ATP-binding pocket of the kinase, onto the *cis*-enone.[125] In the same report, hypothemycin (**1-39**) was shown to be able to discriminate with some efficiency (clearly showing differences in the corresponding K_i) amongst 16 tested kinases, despite the fact that their ATP-binding pockets are known to be highly conserved and that most of them present the same residue (13 of them containing this cysteine).[126] Likewise, it can be rationalized that the irreversible inhibiton of MEK and TAK by LL-Z1640-2 and L-783,277 can occur through the same mechanism. Concerning radicicol A (**1-40**), although no mode of action has been

[120] Zhao, A. et al. Resorcylic acid lactones: naturally occurring potent and selective inhibitors of MEK. *J Antibiot (Tokyo)* **52**, 1086-94 (1999).
[121] Tanaka, H., Nishida, K., Sugita, K. & Yoshioka, T. Antitumor efficacy of hypothemycin, A new ras-signaling inhibitor. *Jap J Cancer Res* **90**, 1139-1145 (1999).
[122] Ninomiya-Tsuji, J. et al. A resorcylic acid lactone, 5Z-7-oxozeaenol, prevents inflammation by inhibiting the catalytic activity of TAK1 MAPK kinase kinase. *J Biol Chem* **278**, 18485-90 (2003).
[123] The MAP kinases relay, amplify and integrate signals from a variety of extracellular stimuli thereby regulating a cell's response to its environment. The fidelity and amplitude of the signal is controlled by a phosphorelay system composed of three sequentially activated kinases. In a generic fashion, a stimulus turns on the activator which phosphorylates the first kinase (MAPKKK) which then phosphorylates the second kinase (MAPKK), which in turn phosphorylates the third kinase (MAPK) that finally phosphorylates a cytosolic protein or transcription factor. In mammalian organisms, at least three subfamillies of MAP kinases have been identified which include the extracellular signal-regulated kinases (ERK); the c-JUN NH2-terminal kinases (JNK); and the p38 enzymes. There are at least seventeen MAPKKKs, seven MAPKKs and twelve MAPKs. The specificity of these cascades is also regulated by scaffolding proteins which specifically organize and localize these kinase cascades to provide a unique combinatorial arrangement and down stream signal to a given stimuli.
[124] (a) Chang, L. & Karin, M. Mammalian MAP kinase signaling cascades. *Nature* **410**, 37-40 (2001), (b) Johnson, G. L. & Lapadat, R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* **298**, 1911-2 (2002).
[125] Schirmer, A., Kennedy, J., Murli, S., Reid, R. & Santi, D. V. Targeted covalent inactivation of protein kinases by resorcylic acid lactone polyketides. *Proc Natl Acad Sci U S A* **103**, 4234-9 (2006).
[126] Based on the human kinome sequence database, some 46 of 510 identified kinases contained this cysteine residue.

reported to explain its inhibitory activity, its closely related structure to the other *cis*-enone macrolides **1-37** to **1-39** led us to speculate that it may inhibit a MAP kinase.

In addition to the aforementioned macrolides, several other RALs have been reported so far in the literature.[127] Representatives of the zearalenone family are zearalenone itself (**1-41**, Figure 19), zearalane (**1-42**, Figure 19), zeranol (**1-43**, Figure 19) and lasiodiplodin (**1-44**, Figure 19).



1-41
Zearalenone

1-42: X = H
Zearalane
1-43: X = OH
Zeranol

1-44
Lasiodiplodin

Figure 19: Resorcylic acid lactones derived from zearalenone

Zearalenone (**1-41**) was first isolated in 1962 from the fungus *Gibberella zeae* and reported as exhibiting anabolic, estrogenic and antibacterial properties.[128] Further studies allowed to identify the structure of this molecule.[129] Compound **1-41** was shown to adopt a conformation that mimics the one of 17-estradiol which explained its agonistic estrogenic properties.[130] During these different studies, some reduced derivatives such as compounds **1-42** and **1-43** were also discovered and their biological evaluation showed that they both share the properties of zearalenone.[131] Zearalane (**1-42**) has also shown anthelminthic and immunomodulating properties whereas α-zearanol (**1-43**, also commercially available as Ralgro® or Ralabol®) has been used to promote growth in cattle and to relieve post-menopausal stress in women.[130] Among these molecules, lasiodiplodin (**1-44**) bears a 12-membered macrocycle (unusual for the RALs) and have been isolated several times[132] since

[127] More than 340 compounds found on Sci Finder when similarity search for the structure of zearalane on the 22nd of September.

[128] Stob, M., Baldwin, R. S., Tuite, J., Andrews, F. N. & Gillette, K. G. Isolation of an anabolic, uterotrophic compound from corn infected with Gibberella zeae. *Nature (London, United Kingdom)* **196**, 1318 (1962).

[129] (a) Urry, W. H., Wehrmeister, H. L., Hodge, E. B. & Hidy, P. H. The structure of zearalenone. *Tetrahedron Letters* **7**, 3109-3114 (1966), (b) Cordier, C., Gruselle, M., Jaouen, G., Hughes, D. W. & McGlinchey, M. Structures of zearalenone and zearalanone in solution: A high-field NMR and molecular modelling study. *Magnetic Resonance in Chemistry* **28**, 835-845 (1990).

[130] Miksicek, R. J. Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. *J Steroid Biochem Mol Biol* **49**, 153-60 (1994).

[131] Reviews: (a) Betina, V. *Zearalenone and its Derivatives* in *Mycotoxins: Chemical, Biological and Environmental Aspects* (Elsevier, Amsterdam, 1989), (b) Shipchandler, M. T. Chemistry of Zearalenone and Some of its Derivatives. *Heterocycles* **3**, 471-520 (1975).

[132] (a) Cambie, R. C., Lal, A. R., Rutledge, P. S. & Woodgate, P. D. Ent-14[S],16β,17-trihydroxyatisan-3-one and further constituents from Euphorbia fidjiana. *Phytochemistry* **30**, 287 (1991), (b) Lee, K.-H. et al. Lasiodiplodin, a potent antileukemic macrolide from Euphorbia splendens. *Phytochemistry* **21**, 1119 (1982), (c) Xin-Seng, Y. et al. Structure of arnebinol, a new ANSA-type monoterpenylbenzenoid with inhibitory effect to

its first isolation in 1991 from the fungus *Botrysdiplodia theobromae* (formerly *Lasiodiplodia theobromae*).[133] Further investigations proved that this compound inhibits prostaglandin biosynthesis[132c] and exhibits antileukemic activities.[132b]

Besides the biological properties, zearalenone (**1-41**) and its derivatives have been extensively studied by chemists in order to find the most efficient synthetic route (*vide infra*).

More recently, new RALs were discovered in a screen for anti-malarial activity.[134] In 2002, along with hypothemycin (**1-39**, Figure 18) as the major secondary metabolite, five new analogs were isolated from the marine mangrove fungus *Aigialus parvus*.

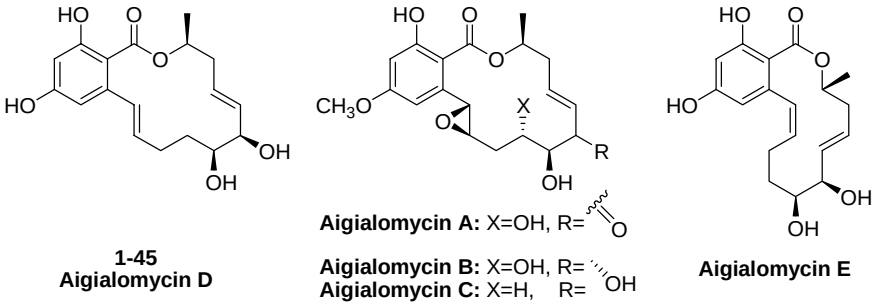


Figure 20: Aigialomycin D and its four analogs

Aigialomycin D (**1-45**, Figure 20) exhibited moderate antimalarial activity against *Plasmodium falciparum K1* and showed cytotoxicity in two cancer cell lines (KB and BC-1) in the same range as hypothemycin, whereas their analogs were completely inactive. Further studies have shown that aigialomycin D is able to inhibit some kinases namely CDK1/cyclinB, CDK5/p25 and GSK3 in the micromolar range (IC_{50} from 5 to 14µM).[135] Interestingly, as it could be expected, the antimalarial activity was not related to the inhibition of the *Plasmodium falciparum* homologue of GSK3.

prostaglandin biosynthesis. *Tetrahedron Letters* **24**, 2407 (1983), (d) Yang, R. Y. et al. Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activities. *Bioorg Med Chem Lett* **16**, 4205-8 (2006).

[133] Aldridge, D. C., Galt, S., Giles, D. & Turner, W. B. Metabolites of Lasiodiplodia theobromae. *J. Chem. Soc. (C)*, 1623 (1971).

[134] Isaka, M., Suyarnsestakorn, C., Tanticharoen, M., Kongsaeree, P. & Thebtaranonth, Y. Aigialomycins A-E, new resorcylic macrolides from the marine mangrove fungus Aigialus parvus. *J Org Chem* **67**, 1561-1566 (2002).

[135] Barluenga, S., Dakas, P. Y., Ferandin, Y., Meijer, L. & Winssinger, N. Modular Asymmetric Synthesis of Aigialomycin D, a Kinase-Inhibitory Scaffold. *Angew Chem Int Ed Engl* **45**, 3951-3954 (2006).

In 2003, new members of the RAL family, pochonin A-F (**1-46** to **1-51** respectively, Figure 21) isolated from *Pochonia chlamydosporia* var. *catenulata* along with radicicol, were reported.[136]

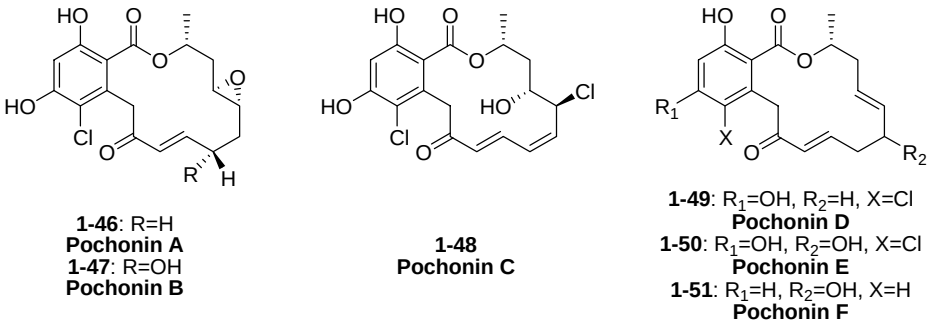


Figure 21: The pochonins, some newly isolated RALs

These molecules were identified in a Herpes Simplex Virus 1 (HSV1) replication assay. While monorden (**1-19**) showed activity in the nanomolar range, all the pochonins except pochonin D exhibited bioactivities in the low micromolar range and pochonin D (**1-49**) only showed cytostatic effects. In this assay, HSV inhibition was always accompanied by weak cytostatic effects (pochonin C (**1-48**) being the least cytotoxic (90µM)), therefore proving the low tolerability of these molecules *in vitro*.

2. Synthetic strategies towards RALs

Besides the biological properties described above, the RALs have been an interesting scaffold for organic chemists to elaborate innovative total syntheses and to develop new methodologies. The first molecule targeted by synthetic chemists was zearalenone (**1-41**) with its first total synthesis reported in 1968 (probably because of its anabolic properties).[137] Since then, several other research groups have shown interests in developing total syntheses of this natural product[138] and it has also served as a testing ground for new cyclisation methodologies

[136] Hellwig, V. et al. Pochonins A-F, new antiviral and antiparasitic resorcylic acid lactones from Pochonia chlamydosporia var. catenulata. *J Nat Prod* **66**, 829-837 (2003).
[137] Taub, D. et al. Total synthesis of the macrolide, zearalenone. *Tetrahedron* **24**, 2443-61 (1968).
[138] (a) Hitchcock, S. A. & Pattenden, G. Total synthesis of the mycotoxin (-)-zearalenone based on macrocyclisation using a cinnamyl radical intermediate. *J. Chem. Soc., Perkin Trans. 1*, 1323-1328 (1992), (b) Keinan, E., Sinha, S. C. & Sinha-Bagchi, A. Thermostable enzymes in organic synthesis, Part 6. Total synthesis of (S)-(-)-zearalenone using a TBADH-generated trifunctional chiron. *J. Chem. Soc., Perkin Trans. 1*, 3333-3339 (1991), (c) Takahashi, T., Nagashima, T., Ikeda, H. & Tsuji, J. An important role played dianiones in the highly efficient macrocycle formation by an intramolecular alkyltion method: application to the synthesis of zearalenone. *Tetrahedron Letters* **23**, 4361-4364 (1982), references 145 to 149 and references cited therein.

such as the Corey-Nicolaou macrolactonization,[139] Masamune's thioester-lactonization,[140] and more recently the ring-closing metathesis (RCM).[141]

In 1992, Lett and Lampilas reported the first total synthesis of radicicol, confirming the absolute configuration of its three stereocenters.[142] Having in mind the sensitivity of the epoxide along with the readily enolisable ketone at the benzylic position, a retrosynthetic analysis based on a Stille coupling and a Mitsunobu macrocyclization as key steps was proposed (Figure 22).

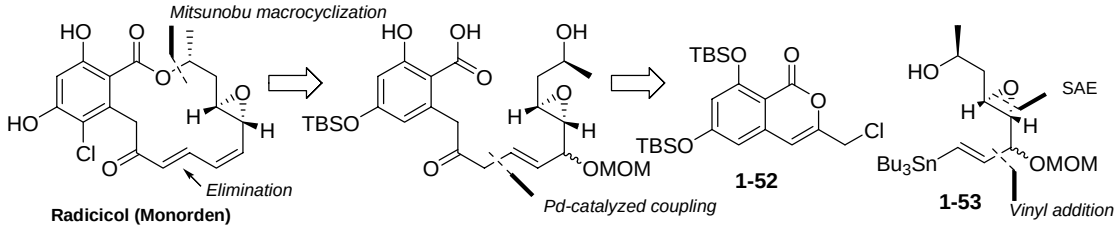
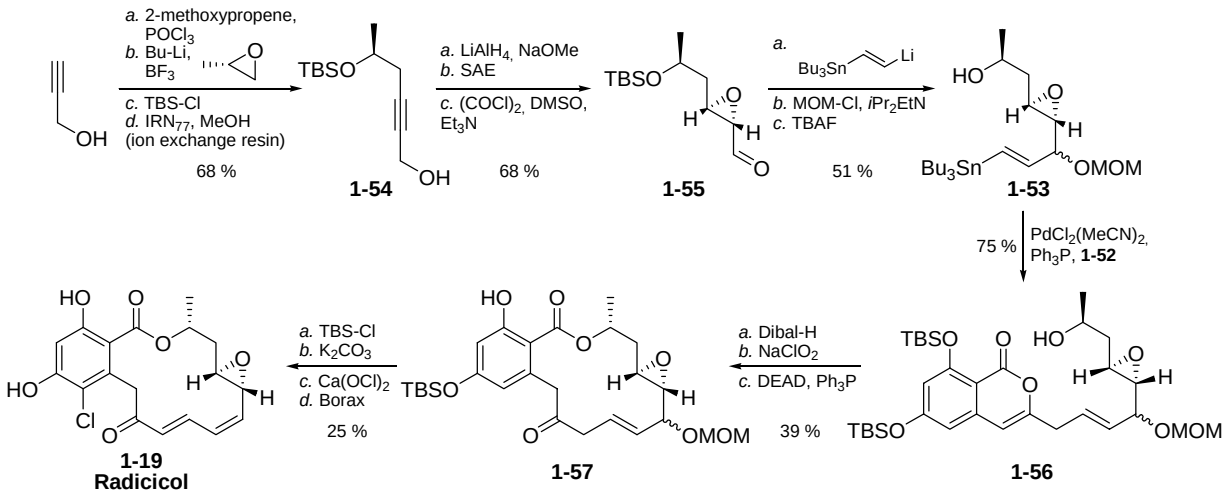


Figure 22: Retrosynthetic analysis for radicicol by Lett and co-workers

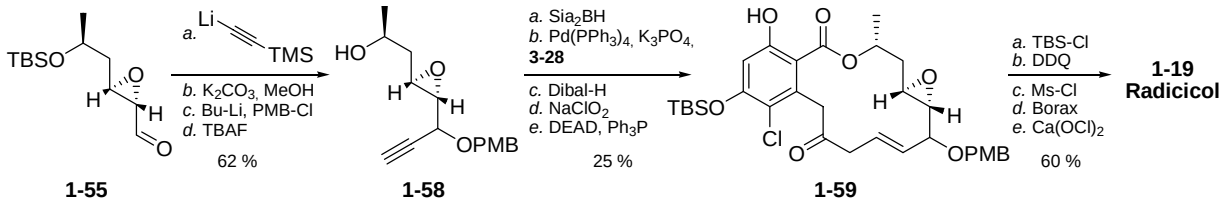
The enolisable ketone was masked as an isocoumarin (**1-52**) in order to reveal the α,β,γ,δ-conjugated system only at a late stage in the synthesis. Starting with commercially available propargyl alcohol, the stannane derivative **1-53** was obtained in 24 % yield (10 steps) *via* a sequence which includes a condensation with a lithio-derivative, reduction of alkyne and Sharpless asymmetric epoxidation as main steps (Scheme 1).

[139] Corey, E. J. & Nicolaou, K. C. Efficient and mild lactonization method for the synthesis of macrolides. *J Am Chem Soc* **96**, 5614-16 (1974).
[140] Masamune, S., Kamata, S. & Schilling, W. Syntheses of macrolide antibiotics. III. Direct ester and lactone synthesis from S-tert-butyl thioate (thiol ester). *Ibid.* **97**, 3515-6 (1975).
[141] Fürstner, A., Thiel, O. R., Kindler, N. & Bartkowska, B. Total syntheses of (S)-(-)-zearalenone and lasiodiplodin reveal superior metathesis activity of ruthenium carbene complexes with imidazol-2-ylidene ligands. *J Org Chem* **65**, 7990-5 (2000).
[142] (a) Lampilas, M. & Lett, R. Convergent stereospecific total synthesis of monochiral monocillin I related macrolides. *Tetrahedron Letters* **33**, 773-6 (1992), (b) Lampilas, M. & Lett, R. Convergent stereospecific total synthesis of monocillin I and monorden (or radicicol). *Tetrahedron Letters* **33**, 777-80 (1992).



Scheme 1: First generation synthesis of radicicol by Lett and co-workers

The fragment **1-53** was then engaged in a Stille coupling with the chlorinated isocoumarin **1-52** (obtained in 32 % yield from orcinol hydrate) to yield the acyclic precursor **1-56** in good yield (75 %). Further isocoumarin opening, oxidation of the resulting aldehyde, Mitsunobu macrolactonization, release of the conjugated dienone and chlorination as the major steps allowed the isolation of radicicol albeit in only 10 % yield over 7 steps. It is also important to mention the low yield obtained in the release of the conjugated dienone (25-30 %) although this transformation was efficient in a model system (quantitative yield on non-chlorinated dimethylradicicol). Another important feature of this synthesis is the Mitsunobu macrocyclisation in which the unprotected *ortho*-phenol was found necessary for high efficiency (71 % yield). This way, Lett and co-workers achieved the first total synthesis of radicicol albeit in less than 2 % overall yield (18 steps, longest linear sequence) and confirmed the absolute *R* configuration of the three chiral centers of radicicol.[113] A decade later, the same group reported improvements and simplifications of their first generation synthesis as depicted on scheme 2.[143]



Scheme 2: Second generation synthesis of radicicol by Lett and co-workers

[143] (a) Tichkowsky, I. & Lett, R. Convergent stereospecific total synthesis of monocillin I and radicicol: some simplifications and improvements. *Tetrahedron Letters* **43**, 3997-4001 (2002), (b) Tichkowsky, I. & Lett, R. Improvements of the total synthesis of monocillin I and radicicol via Miyaura-Suzuki couplings. *Tetrahedron Letters* **43**, 4003-4007 (2002).

The first significant improvement involved unmasking the dienone. An orthogonal protecting group on the alcohol in α to the epoxide was proved necessary for the success of the Mitsunobu macrocyclisation and a PMB protecting group was found to work the best. It was easily removed after the macrolactonization and replaced by a mesolate that, under basic conditions, allowed release of the dienone in 76 % yield over two steps.[143a] The second one is related to the Stille coupling. Although this step was very efficient (75 % yield), the use of stannane had major drawbacks such as high costs, purification problems and tin contamination. To circumvent the problem, Lett and co-workers proposed an alternative Suzuki-Miyaura coupling achieved in 58 % yield (over two steps) from compound **1-58**. Several attempts to improve this yield were reported but, none of the procedures were competitive with the Stille coupling in terms of efficiency.[143b] Several months before these improvements were published, another total synthesis of radicicol was proposed by Danishefsky and co-workers.[144] Inspired by their asymmetric synthesis of radicicol dimethyl ester disclosed a year earlier,[145] its retrosynthetic analysis is based on three main disconnections: a ring-closing metathesis, a dithiane addition and a Mitsunobu esterification (Figure 23).

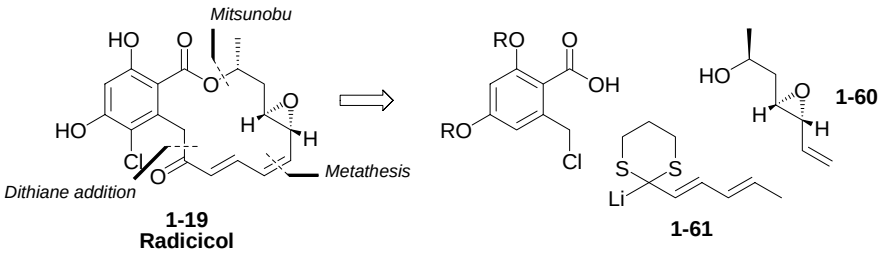
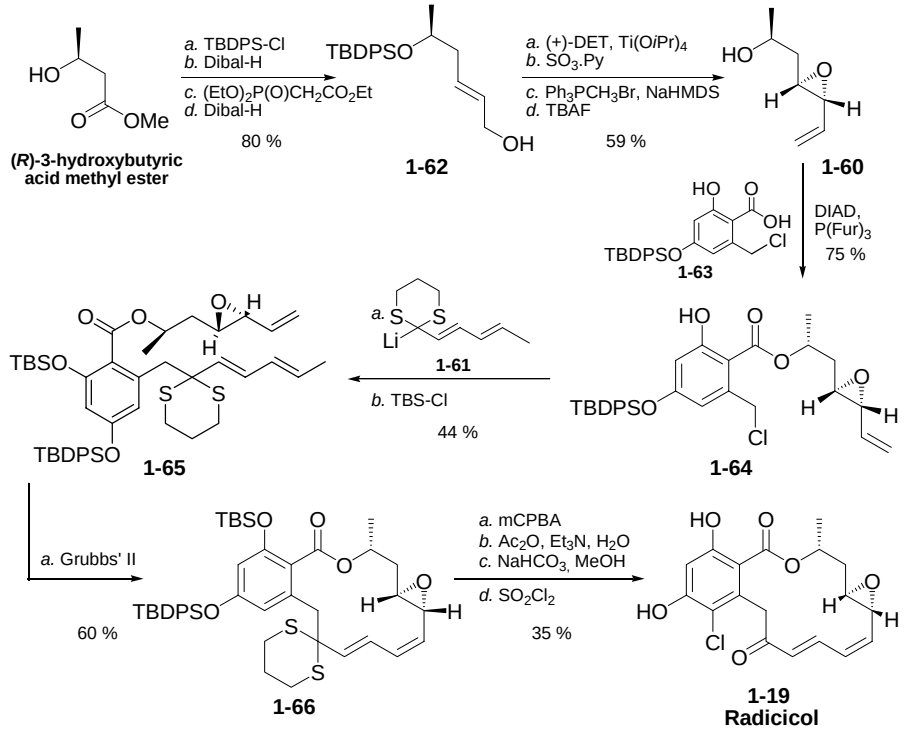


Figure 23: Retrosynthetic analysis of radicicol by Danishefsky and co-workers

The diathiane moiety **1-61** was used as a masked acyl anion equivalent, in order to prevent any isocoumarin formation. In the synthesis of the methylated analog, esterification with the benzoic acid bearing methyl protecting groups occurred without any problem *via* the acid chloride. However, when changing to more labile protecting groups, this step proved challenging because of a phtalide formation under diverse conditions (standard Mitsunobu, carbodiimide or acid chloride). Once more, the presence of the free *ortho*-phenol was essential for the success of this reaction. Modified Mitsunobu esterification conditions using a

[144] Garbaccio, R. M., Stachel, S. J., Baeschlin, D. K. & Danishefsky, S. J. Concise Asymmetric Syntheses of Radicicol and Monocillin I. *J Am Chem Soc* **123**, 10903-10908 (2001).
[145] Garbaccio, R. M. & Danishefsky, S. J. Efficient Asymmetric Synthesis of Radicicol Dimethyl Ether: A Novel Application of Ring-Forming Olefin Metathesis. *Org Lett* **2**, 3127-3129 (2000).

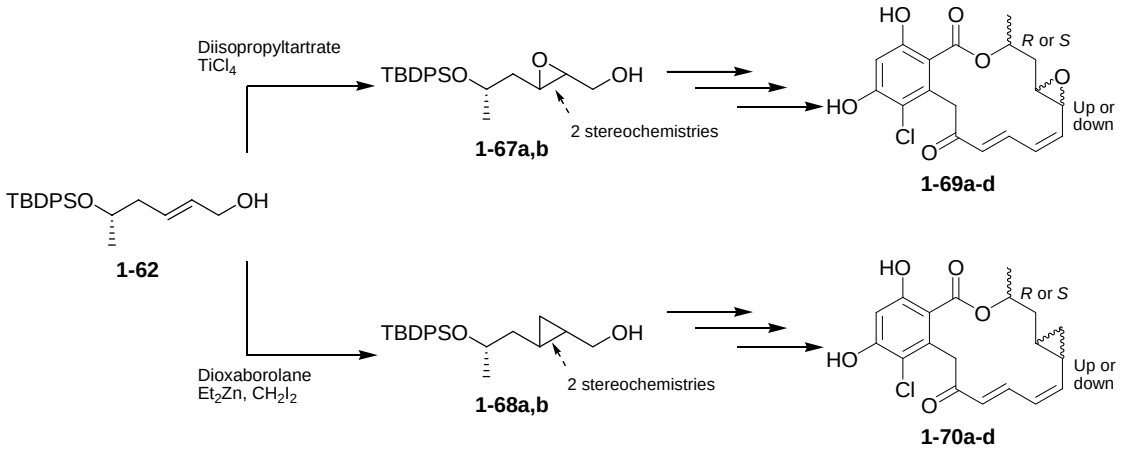
less polar solvent (benzene) along with a less nucleophilic phosphine ($P(Fur)_3$) allowed the formation of the desired ester **1-64** in 75 % yield (Scheme 3).



Scheme 3: Synthesis of radicicol by Danishefsky and co-workers

Starting with alcohol **1-60** bearing the three chiral centers of radicicol (obtained in 47 % yield after 8 steps from the commercially available (*R*)-3-hydroxybutyric acid methyl ester), further Mitsunobu reaction with compound **1-63** under optimised conditions and alkylation with the dithiane adduct **1-61** allowed the formation of the acyclic intermediate **1-65**. Importantly, alkylation conditions had to be tuned to avoid any γ-addition on the diene. With metathesis precursor **1-65** in hands, the road was paved to yield satisfyingly radicicol. Ring-closing metathesis was achieved using Grubbs' second generation catalyst in good yield and excellent selectivity. However, the presence of the TBS-protected *ortho*-phenol was crucial for the success of this reaction otherwise leading to low rate of closure and low yield. Further release of the masked conjugated ketone followed by desilylation and chlorination afforded the desired natural product. Although the chlorination method was claimed by the authors to represent significant improvements for this transformation,[144] it is important to note that the yield was moderate probably because of the addition of a second chlorine atom on the molecule. This synthetic sequence allowed the formation of radicicol in a 3 % overall yield (longest linear sequence). Following this methodology, using either the Sharpless asymmetric

epoxidation or the cyclopropanation chemistry developed by Charette and co-workers,[146] four alcohols (**1-67a,b** and **1-68a,b**, Scheme 4) were synthesized leading after to eight different analogs (**1-69a-d**, **1-70a-d**) bearing all possible stereochemistries on the epoxide/cyclopropane moiety and on the methyl group.[95] All these compounds were then evaluated for HSP90 inhibition. A related SAR (structure-activity relationship) study was established showing that the epoxide was not crucial for HSP90 inhibition and that the configuration of the methyl ester was very important for high activity.



Scheme 4: Divergent synthesis of radicicol and cyclopropanation analogs

However, the methodology developed for radicicol itself showed some limitations for the synthesis of the cyclopropane analog **1-70**:[147] the Mitsunobu esterification was achieved in only 48 % yield, the selectivity for the dithiane addition was lower than the one for radicicol and the ring-closing metathesis needed strong optimised conditions[148] (110 °C during 10min) to afford the desired macrocycle (55 % yield). In order to prepare larger amount of cyclopropanation **1-70**, a second generation synthesis was proposed.[149] The key step of the retrosynthetic analysis is the formation of the aromatic ring through a Diels-Alder

[146] (a) Charette, A. B. & Juteau, H. Design of Amphoteric Bifunctional Ligands: Application to the Enantioselective Simmons-Smith Cyclopropanation of Allylic Alcohols. *J Am Chem Soc* **116**, 2651-2652 (1994), (b) Charette, A. B., Juteau, H., Lebel, H. & Molinaro, C. Enantioselective Cyclopropanation of Allylic Alcohols with Dioxaborolane Ligands: Scope and Synthetic Applications. *J Am Chem Soc* **120**, 11943-11952 (1998), (c) Charette, A. B., Prescott, S. & Brochu, C. Improved Procedure for the Synthesis of Enantiomerically Enriched Cyclopropylmethanol Derivatives. *J. Org. Chem.* **60**, 1081-1083 (1995), (d) Charette, A. B. & Lebel, H. (2S,3S)-(+)-(3-PHENYLCYCLOPROPYL)METHANOL. *Org. Synth.* **76**, 86-100 (1999).

[147] Geng, X., Yang, Z.-Q. & Danishefsky, S. J. Synthetic development of radicicol and cyclopropanation: Highly promising anticancer agents targeting Hsp90. *Synlett*, 1325-1333 (2004).

[148] Yamamoto, K., Biswas, K., Gaul, C. & Danishefsky, S. J. Effects of temperature and concentration in some ring-closing metathesis reactions. *Tetrahedron Letters* **44**, 3297-3299 (2003).

[149] Yang, Z. Q. & Danishefsky, S. J. A concise route to benzofused macrolactones via ynolides: cyclopropanation. *J Am Chem Soc* **125**, 9602-3 (2003).

cycloaddition between the ynolide moiety **1-72** as the dienophile and a dimedone-derived diene **1-71** (Figure 24).

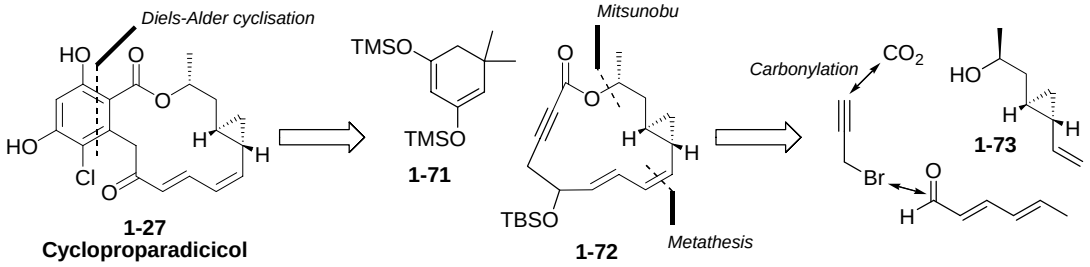
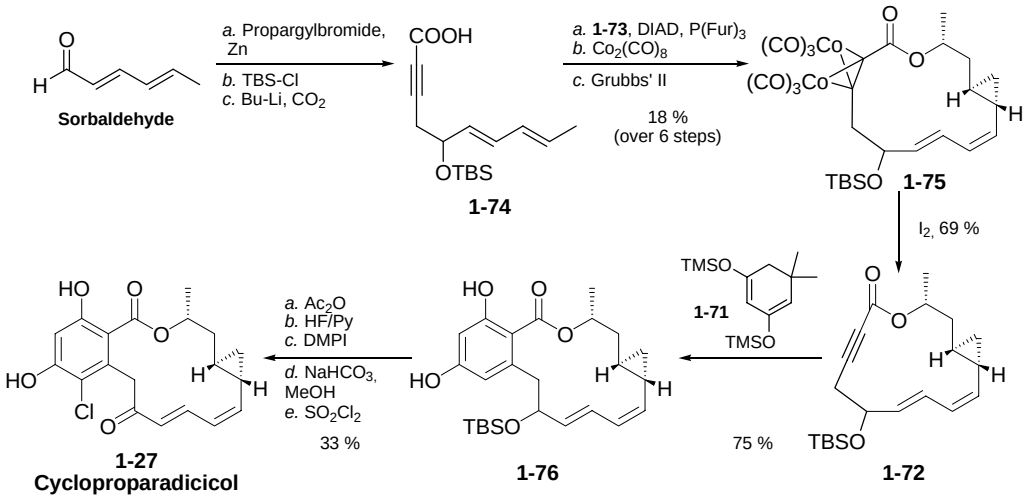


Figure 24: Retrosynthetic analysis for cyclopropradicicol by Danishefsky and co-workers

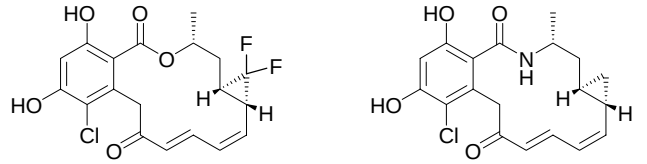
One innovative feature of this strategy is the formation of the aromatic moiety at a late stage of the synthesis whereas most of the known protocols started with a modified benzoic acid substrate. Thus, sorbaldehyde was used in a Reformatsky-like condensation with propargyl bromide followed by a silyl protection and carbonylation to yield the conjugated carboxylic acid **1-74** (Scheme 5).



Scheme 5: Second generation synthesis of cyclopropradicicol by Danishefsky and co-workers

Esterification of acid **1-74** with alcohol **1-73** under the previously optimised Mitsunobu conditions followed by alkyne complexation with cobalt and ring-closing metathesis under mild conditions (45 °C in CH_2Cl_2) afforded the macrocyclic precursor **1-75**. It is important to note that attempts to achieve the metathesis reaction with the free alkyne only led to recovery of the starting material, probably due to the rigidity of the scaffold in the presence of the alkyne. After decomplexation of the alkyne, intermediate **1-72** was ready to undergo the Diels-Alder cycloaddition with the diene **1-71** and the resorcylic acid lactone intermediate **1-**

76 was obtained after concomitant retro-Diels-Alder loss of isobutene and hydrolysis of the TMS protecting group during flash chromatography. Futher protecting group manipulation, oxidation and chlorination afforded cycloproparadicicol (**1-27**) in 13 steps and 5 % overall yield. Although the yield achieved by this new strategy was not far superior to the first generation one, the methodology was suitable to provide the final compound in gram quantities, therefore permitting the synthesis of simple analogs such as alcohols (instead of the conjugated ketone) and oximes.[97] Nevertheless, Diels-Alder attempts to reach radicicol, difluorocycloproparadicicol and cycloproparadicicol lactam (Figure 25) resulted in low yield mostly because of thermal decomposition.



Difluorocycloproparadicicol **Cycloproparadicicol Lactam**

Figure 25: Cycloproparadicicol analogs

When these analogs were described, our team was already involved in the synthesis of radicicol and our methodology has already been disclosed in the literature (*vide infra*).[150]
Using the same protocol as developed for cycloproparadicicol, Danishefsky and co-workers proposed the first total synthesis of Aigialomycin D less than two years after its isolation (Figure 26).[151,97,147]

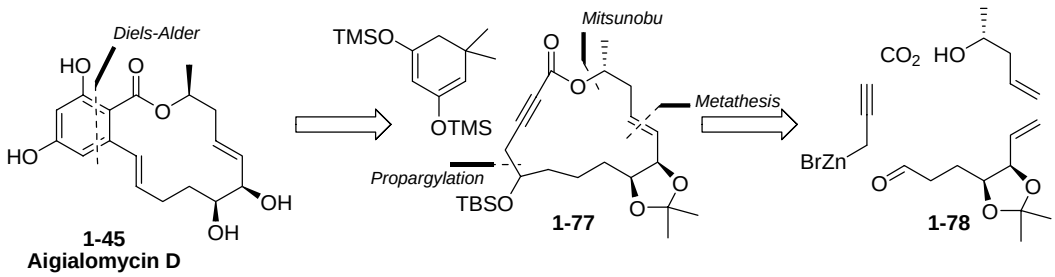
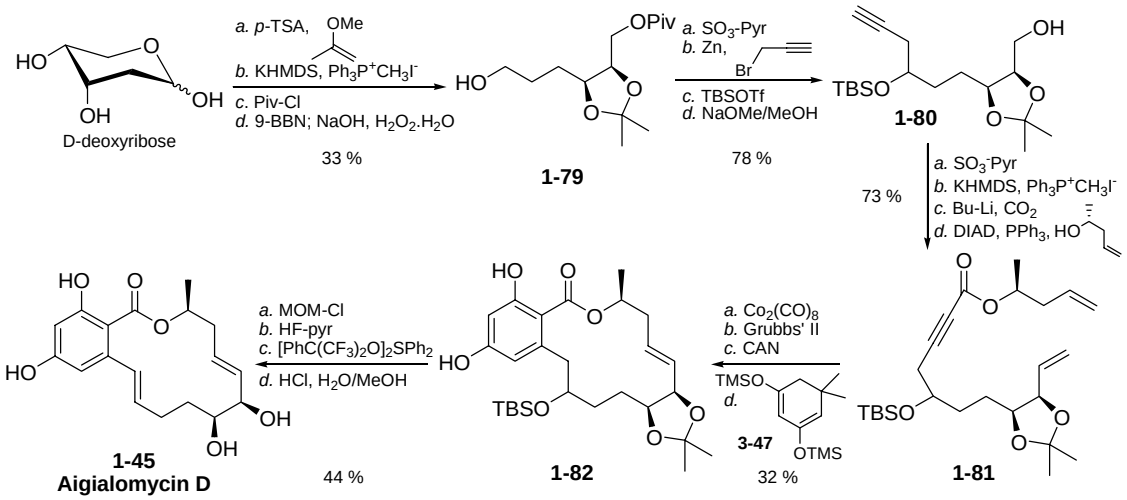


Figure 26: Retrosynthetic analysis of Aigialomycin D by Danishefsky and co-workers

Starting from D-deoxyribose that is presenting the right stereochemistry for the diol moiety **1-78**, the chiral alcohol **1-79** was obtained in four steps (Scheme 6). Subsequent oxidation

[150] (a) Barluenga, S., Lopez, P., Moulin, E. & Winssinger, N. Modular asymmetric synthesis of pochonin C. *Angew Chem Int Ed Engl* **43**, 3467-70 (2004), (b) Barluenga, S., Moulin, E., Lopez, P. & Winssinger, N. Solution- and solid-phase synthesis of radicicol (monorden) and pochonin C. *Chemistry* **11**, 4935-52 (2005).
[151] Geng, X. & Danishefsky, S. J. Total synthesis of aigialomycin D. *Org Lett* **6**, 413-6 (2004).

followed by propargylation and protecting groups' manipulation afforded alcohol **1-80** as a mixture of diastereoisomers. Conversion of the alcohol to an alkene, carboxylation of the alkyne and Mitsunobu esterification led to the acyclic precursor **1-81** of aigialomycin D.

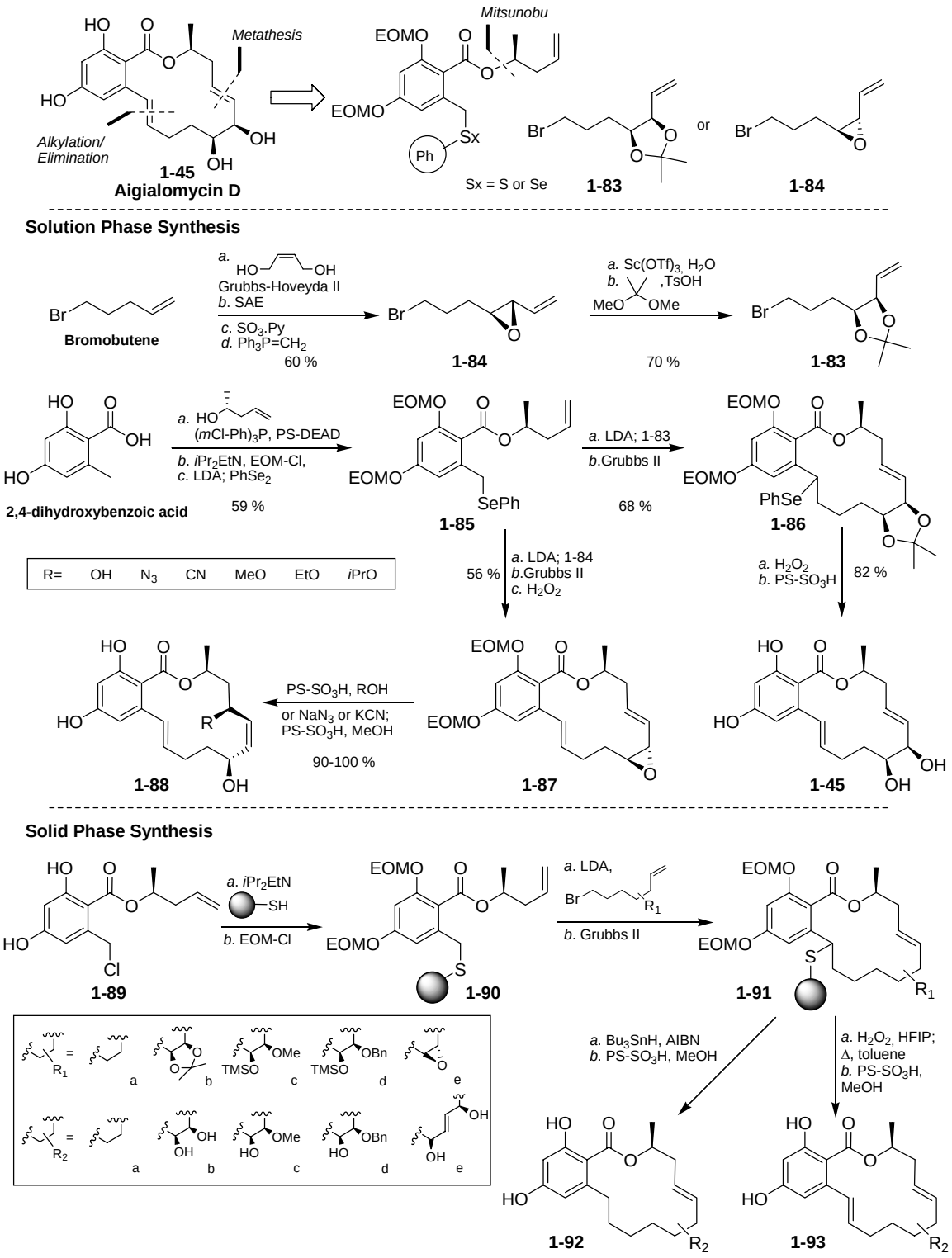


Scheme 6: First total synthesis of Aigialomycin D by Danishefsky and co-workers

Following the procedure described for cycloproparadicicol, cobalt complexation, ring-closing metathesis, alkyne release and Diels-Alder cycloaddition provided the resorcyclic acid lactone intermediate **1-82** in 60 % yield over four steps. It is worthy to mention that both the metathesis and the Diels-Alder reactions were highly reliable for this molecule (80 % and 84 % yield respectively). Finally, standard protection/deprotection steps and dehydration allowed access to aigialomycin D (**1-45**) in 5 % overall yield (longest linear sequence, 20 steps). By completing this synthesis, Danishefsky and co-workers proved the general feature of their methodology albeit in poor yields. This year, two new synthesis of Aigialomycin D have been disclosed in the literature.[135,152] One of them, reported by our group, gave access to the molecule either in solution or on solid support, allowing the efficient synthesis of librairies of compounds. Using a Mitsunobu esterification, an alkylation/elimination step and a ring-closing metathesis as key steps, aigialomycin D (**1-45**) was synthesized in gram quantities from bromobutene with 23 % overall yield over ten steps (Scheme 7). In solution, the chemistry developed was amenable to diversity-oriented synthesis as opening of the epoxide **1-87** failed to give the expected diol **1-45** but let in all cases to SN_2' addition compounds **1-88**. By replacing the phenyl selenide with the polymer-bound thioether, the chemistry was carried out on solid phase extending again the scope of aigialomycin D analogs (**1-92a-e**, **1-93a-e**).

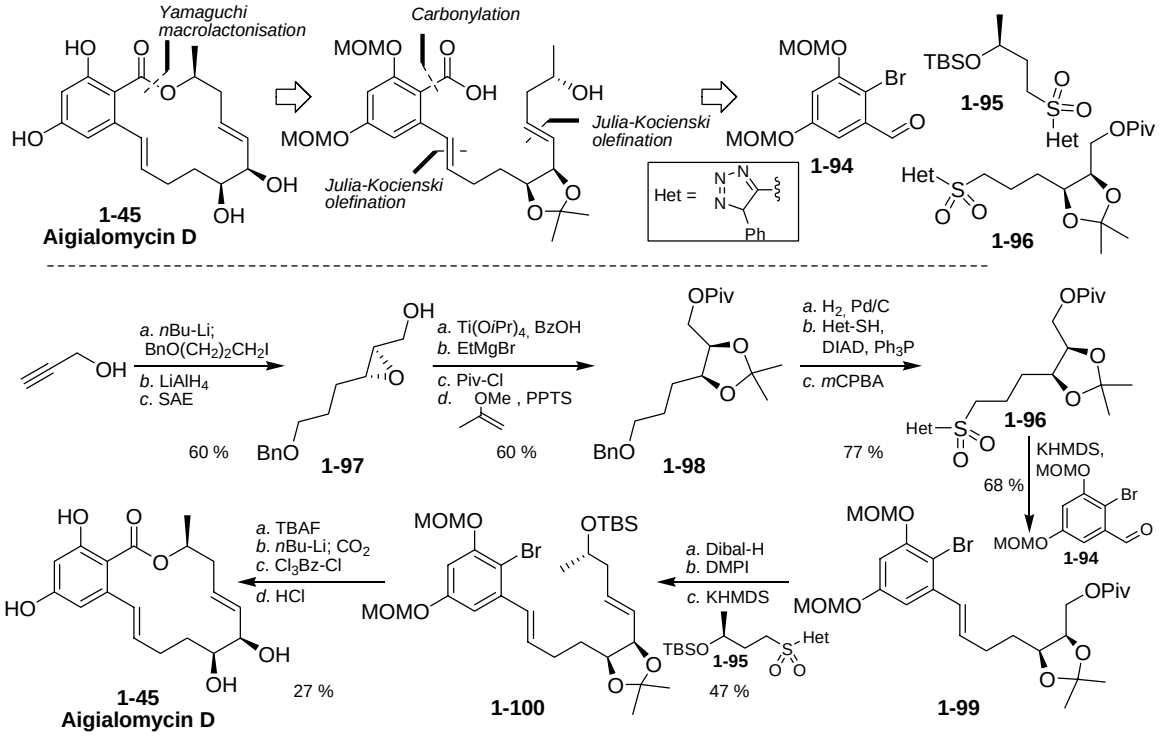
[152] Lu, J. et al. Enantioselective total synthesis of aigialomycin D. *Tetrahedron Asymmetry* **17**, 1066-1073 (2006).

Interestingly, the ring-closing metathesis conditions used in solution were unsuccessful on the support leading to the development of metathesis conditions using activation by microwaves irradiation (Grubbs' II, CH_2Cl_2, 120°C). The high efficiency of this method will probably lead to the development of structural analogs to achieve better potency.



Scheme 7: Solution- and solid-phase synthesis of Aigialomycin D by Winssinger and co-workers

Simultaneously, Pan and co-workers proposed another enantioselective synthesis of aigialomycin D[152] with three key steps: a Yamaguchi macrocyclisation and two Julia-Kociensky couplings to establish the *E* geometry of both olefins (Scheme 8).



Scheme 8: Aigialomycin D total synthesis by Pan and co-workers

The key sulfone **1-96** bearing the chiral diol masked as an acetonide was obtained in ten steps for propargyl alcohol using a large variety of chemical reactions (alkylation, oxidation/reduction, protecting groups' manipulation, *etc.*). A Julia-Kociensky condensation between fragment **1-96** and the functionnalized benzaldehyde **1-94** led to the acyclic precursor **1-99**. Deprotection of the pivaloyl protecting group followed by oxidation of the resulting alcohol and a second Julia-Kociensky olefination afforded compound **1-100**. Finally, silyl deprotection, carbonylation, Yamaguchi macrolactonization and acetonide/MOM deprotection allowed the isolation of aigialomycin D (**1-45**) in 18 steps (longest linear sequence) and more than 3 % overall yield.

In 2001, the group of Tatsuta described the first total synthesis of LL-Z1640-2 in a straightforward strategy starting from D-ribose.[153] The key features of the protocol are a

[153] Tatsuta, K., Takano, S., Sato, T. & Nakano, S. The first total synthesis of a macrocyclic anti-protozoan, LL-Z1640-2. *Chem Lett*, 172-173 (2001).

Mukaiyama macrocyclisation, a Corey-Fuchs protocol to yield the *E* olefin and a Sonogashira coupling to yield the *Z* one (Figure 27).

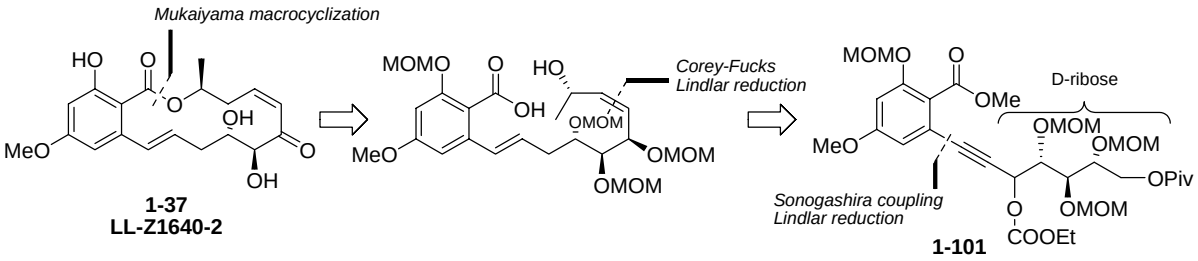
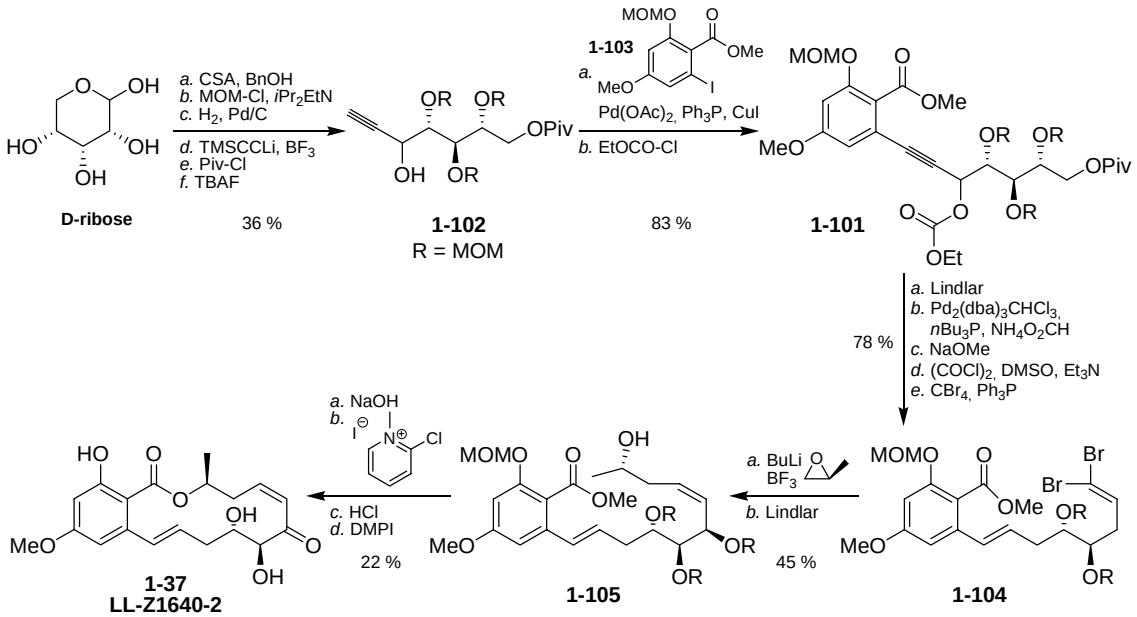


Figure 27: Retrosynthetic analysis for LL-Z1640-2 by Tatsuta and co-workers

Manipulation of protecting groups and alkyne addition on D-ribose afforded the key alkynyl intermediate **1-102** in six steps (Scheme 9).

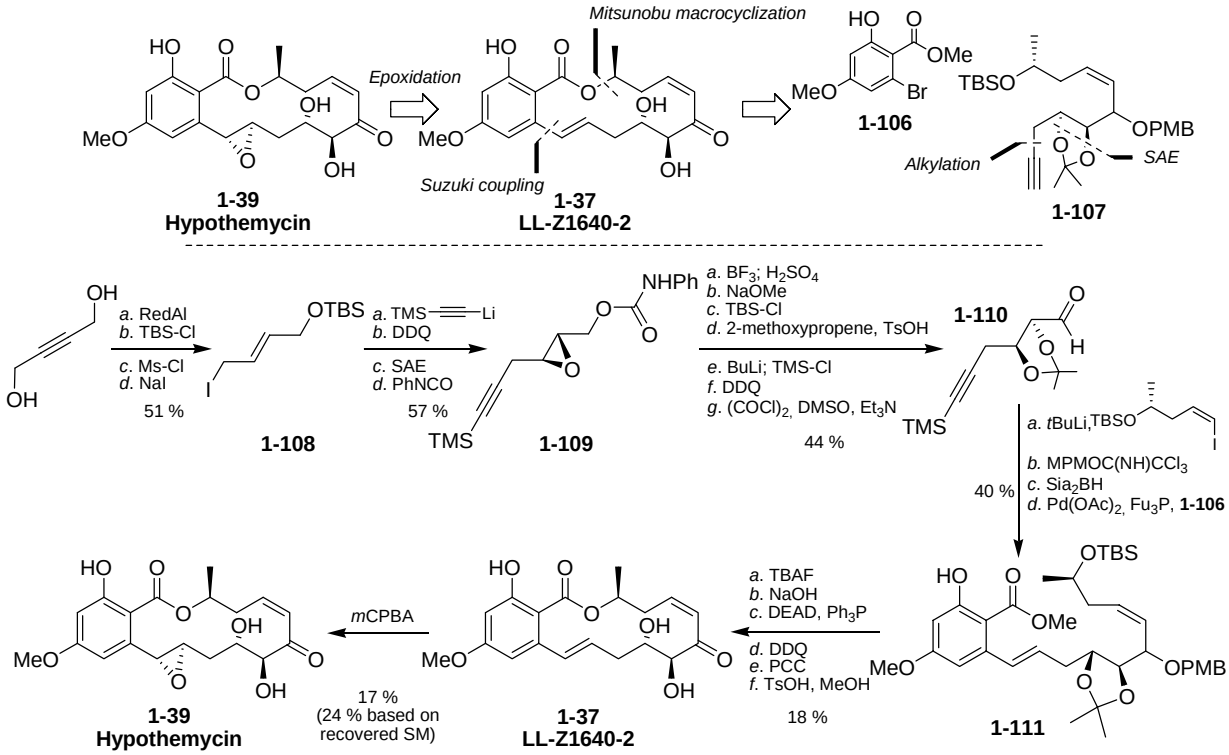


Scheme 9: First total synthesis of LL-Z1640-2 by Tatsuta and co-workers

Further Sonogashira coupling with the iodobenzoate **1-103** and ethoxycarbonate protection of the alcohol furnished the acyclic precursor **1-101**. Reduction of the triple bond and of the allylic carbonate under Tsuji's conditions, deprotection of the pivaloyl, oxidation of the resulting alcohol and modified Wittig olefination gave access to the bis-brominated compound **1-104**. Further exposure to buthyllithium afforded the alkynyl intermediate which upon reaction with the *S*-propylene oxide followed by a Lindlar reduction was transformed in the alcohol intermediate **1-105** presenting both *E* and *Z* olefins. Finally, saponification,

Mukaiyama macrocyclisation, MOM deprotection and selective oxidation of the alllylic alcohol provided the desired natural product LL-Z1640-2 (**1-37**).

A year later, in 2002, based on their previous work on radicicol and monocillin, Lett and co-workers reported another total synthesis of LL-Z1640-2 along with the first total synthesis of hypothemycin.[154] Similarly to their second generation synthesis of radicicol,[143] key steps of their protocol are a Mitsunobu macrocyclisation and a Suzuki coupling (Scheme 10). 1,4-butynediol led to the synthesis of the epoxidized intermediate **1-109** in eight steps, which was further converted seven steps after into the advanced aldehyde **1-110**.



Scheme 10: From LL-Z1640-2 to hypothemycin: a new total synthesis by Lett and co-workers

Transmetallation of the vinyl iodide and reaction with the aldehyde **1-110** afforded the alcohol intermediate that was then protected with a PMB. Hydroboration of the triple bond and Suzuki coupling with the aryl bromide furnished the desired acyclic compound **1-111**. Further silyl deprotection, saponification, Mitsunobu macrocyclisation, PMB deprotection, allylic alcohol oxidation and acetonide deprotection allowed the synthesis of the desired LL-Z1640-2 (**1-37**). It is interesting to note that the Mitsunobu reaction was as effective as for radicicol (67

[154] (a) Selles, P. & Lett, R. Convergent stereospecific synthesis of C292 (or LL-Z1640-2), and hypothemycin. Part 1. *Tetrahedron Letters* **43**, 4621-4625 (2002), (b) Selles, P. & Lett, R. Convergent stereospecific synthesis of LL-Z1640-2 (or C292), hypothemycin and related macrolides. Part 2. *Tetrahedron Letters* **43**, 4627-4631 (2002).

% yield) but all attempts to invert the sequence (Mitsunobu esterification and then Suzuki coupling) failed. Furthermore, the oxidation with PCC worked only with one diastereoisomer, the other needed Jones oxidation conditions to afford the desired α,β-conjugated ketone. The last step to reach hypothemycin (**1-39**) was done enantioselectively (as predicted by the authors based on the crystal structure of the molecule)[155] using *m*CPBA albeit in poor yield, probably because of the poor reactivity of the benzylic olefin and due to the lability of the final product.

To date, no total synthesis of radicicol A has been published in the literature. However, since it can be produced in gram quantities from a fungal strain,[156] some chemists at Novartis reported the synthesis of a library starting directly from the natural product (Figure 28).[157]

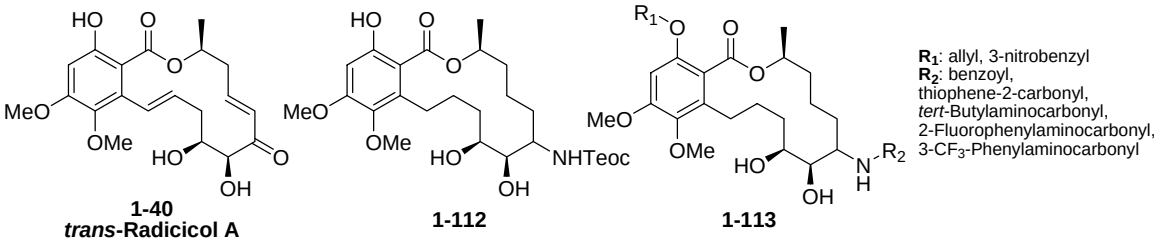


Figure 28: Trans-radicicol A and some analogs

After linking the *seco*-radicicol A with a protected amine **1-112** (in place of the ketone) to a resin through the diol, two points of diversity were introduced on the scaffold: one by alkylation of the unprotected phenol and the other by acid chloride or isocyanate reaction with the amine. Although only five compounds **1-113** are reported using this chemistry, there is no doubt that larger librairies can be produced using this protocol.

3. Conclusions

The more recent discoveries that RALs can mimic ATP and be potent inhibitors of ATPases such as HSP90 or kinases such as MEK, TAK and VGFR have raised interest in this family of natural products. From a chemical biology perspective, the fact that some RALs have been shown to be irreversible inhibitors may prove to be an asset as they can be used to selectively

[155] Agatsuma, T., Takahashi, A., Kabuto, C. & Nozoe, S. Revised structure and stereochemistry of hypothemycin. *Chem Pharm Bull* **41**, 373-5 (1993).
[156] Dreyfuss, M. M.; Leutwiler, A. ; Mas Kenzie, A. R.; Schnyder, J.; Traber, R.; Mattes, H. Eur. Pat. Appl. 606044, **1994**; Chem. Abstr. **2004**, *122*, 81004s.
[157] Grosche, P., Akyel, K. & Marzinzik, A. Synthesis of macrocyclic scaffolds from natural products and their utilization for solid-phase chemistry. *Synthesis*, 2015-2021 (2005).

label given kinases or as probes for activity based profiling. To which extend the selectivity of given inhibitors can be modulated with changes in the functionalities around the macrocycle remains to be defined. From a therapeutic perspective, the targets of many RALs (HSP90 and MAP kinases) are considered amongst the most promising targets for chemotherapy as well as inflammation treatment and several RALs have already been shown to be effective in animal models (hypothemycin and radicicol derivatives). Over the last five years, important efforts including innovative strategies have been done for the total synthesis of RALs mostly concerning radicicol and its analogs. More recently, aigialomycin D was the focus of several research teams but, only few syntheses have been reported concerning LL-Z1640-2 (**1-37**) and hypothemycin (**1-39**) and our contribution to the pochonins (**1-46** to **1-51**) that will be discussed in the following part of this manuscript.[158]

[158] (a) Moulin, E., Zoete, V., Barluenga, S., Karplus, M. & Winssinger, N. Design, synthesis, and biological evaluation of HSP90 inhibitors based on conformational analysis of radicicol and its analogues. *J Am Chem Soc* **127**, 6999-7004 (2005), (b) Moulin, E., Barluenga, S. & Winssinger, N. Concise synthesis of pochonin A, an HSP90 inhibitor. *Org Lett* **7**, 5637-9 (2005), (c) Moulin, E., Barluenga, S., Totzke, F. & Winssinger, N. Diversity-Oriented Synthesis of Pochonins and Biological Evaluation against a Panel of Kinases. *Chemistry*, **12**, 8819-8834 (2006).

PART II

Total Syntheses of Pochonins and their Derivatives

I. Total Syntheses of Radicicol and Pochonin C

In 2003, different pochonins were isolated from *Pochonia chlamydosporia* var. *catenulata* and pochonin C was reported to have the highest selectivity index (Tox_{50}/IC_{50}) against Herpes Simplex Virus (*vide supra*).[136] When we directed our first efforts towards radicicol and pochonin C based on their therapeutic potentials, the stereochemistry of C5 bearing the chlorine atom on pochonin C was undefined.[159] Considering the closely related structures of radicicol and pochonin C, we engaged ourselves in developing a total synthesis allowing the formation of both molecules. A major aspect in the development of the synthesis was its amenability to combinatorial synthesis and suitability of the chemistry to prepare analogs extending beyond the diversity of natural resorcylides. The commercial availability or the ease of preparation of fragments was an important criterion for the design of the retrosynthesis. Thus, our strategy was developed following a retrosynthetic analysis that disconnects the macrocycle into three fragments **2-4** to **2-6** (Figure 29).

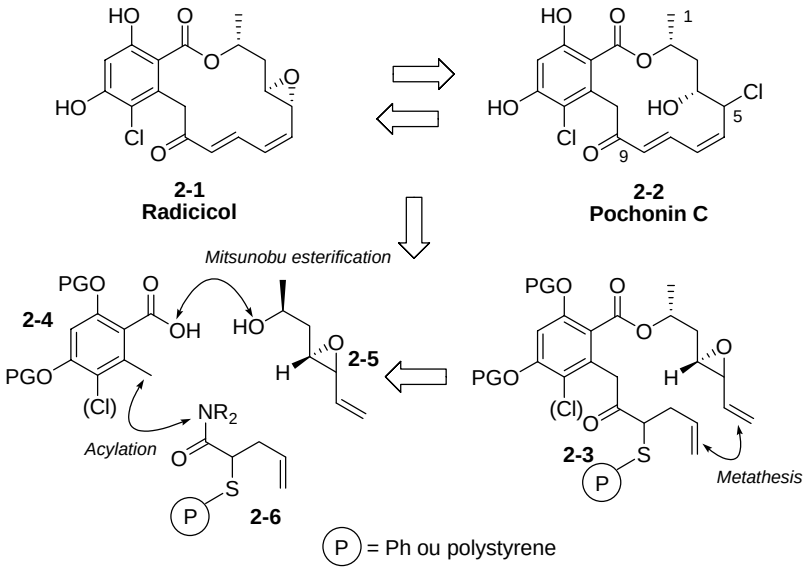


Figure 29: Retrosynthetic analysis of radicicol and pochonin C

Considering the importance of ring-closing metathesis (RCM) in the total synthesis of natural products[160] and more importantly in the one of resorcylic acid lactones,[161] we envisaged that

[159] Although an HCl-promoted ring opening of radicicol was already described in the literature, the two compounds had not been compared.

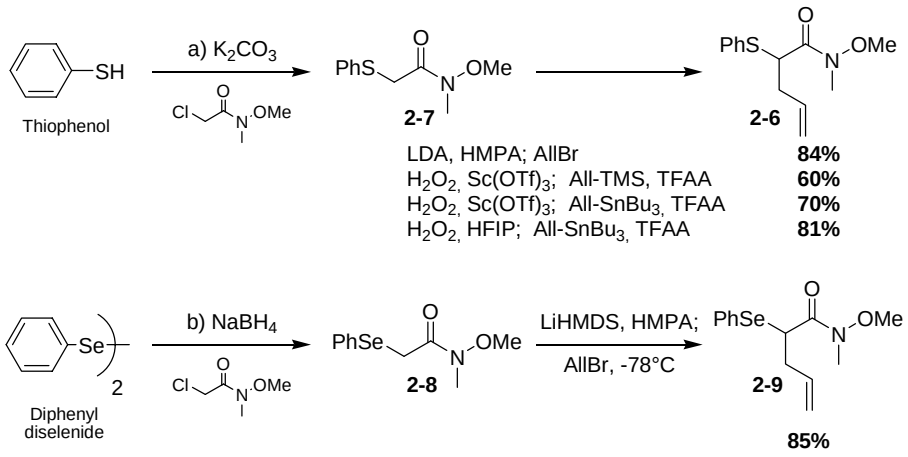
[160] (a) Nicolaou, K. C., Bulger, P. G. & Sarlah, D. Metathesis reactions in total synthesis. *Angew Chem Int Ed Engl* **44**, 4490-527 (2005), (b) Mulzer, J. & Öhler, E. in *Topics in Organometallic Chemistry* 269-366 (Springer Berlin / Heidelberg, 2004).

the α,β,γ,δ-conjugated diene could arise from this reaction. For both radicicol and pochonin C, a *cis* olefin would be required as the product of the metathesis reaction. Having in mind the possibility that RCM of the conjugated diene could lead to four different products (12- and 14-membered rings and *cis*/*trans* isomers), the diene was masked as a thioether which could prevent undesired cyclisation. We anticipated that the use of this thioether could not only be important for the ring-closing metathesis but also in protecting the α,β-conjugated system (**6**). The timing of the epoxide opening and thioether elimination could also be used to alter the conformational strain and relative equilibrium of the *cis* and *trans* products. Furthermore, this thioether linkage could serve as an attachment point to a polystyrene resin for solid phase synthesis, the corresponding selenide being also considered as an alternative. Being aware of the unknown stereochemistry of C5 of pochonin C, a divergent way of synthesis was developed toward the alcohol (**2-5**) to access both diastereoisomers. In the following section, results regarding the development of the synthetic strategy will be reported, particularly the synthesis of the three building blocks (**2-4** to **2-6**) and a proof of principle establishing the methodology to access both natural products.

A. Preliminary studies

As aforementioned, the Weinreb amide moiety (**2-6**) was proposed to serve as an attachment point to a resin through a thio- or a selenoether linkage. Thus, Weinreb amide fragments **2-6** (thio-) and **2-9** (seleno-) were prepared in two steps. First, alkylation of commercially available 2-chloro-*N*-methoxy-*N*-methylacetamide with thiophenoxide or selenophenoxide allowed us to obtain **2-7** and **2-8** respectively (Scheme 11).

[161] Gradillas, A. & Perez-Castells, J. Macrocyclization by Ring-Closing Metathesis in the Total Synthesis of Natural Products: Reaction Conditions and Limitations. *Angew Chem Int Ed Engl* **45**, 6086-6101 (2006).



Scheme 11: Synthesis of selenidyl and sulfuryl Weinreb amide

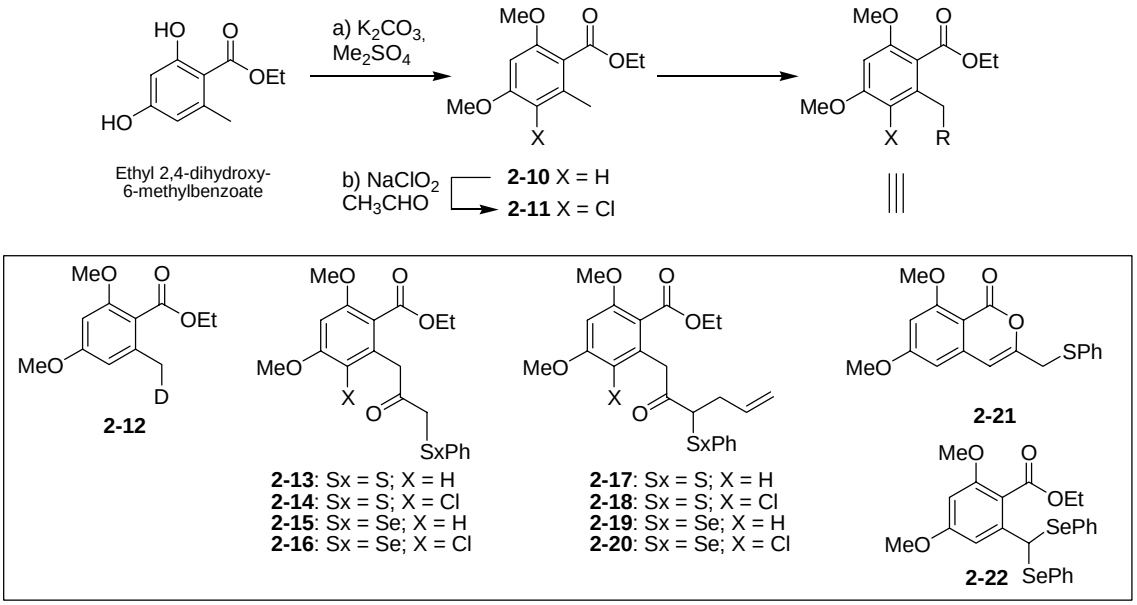
a) thiophenol (1.0 equiv.), K_2CO_3 (1.0 equiv.), DMF, 23 °C; 2-chloro-*N*-methoxy-*N*-methylacetamide (1.0 equiv.), 23 °C, 4 h, 95 %; b) diphenyl diselenide (1.0 equiv.), $NaBH_4$ (2.0 equiv.), 23 °C, 1 h; 2-chloro-*N*-methoxy-*N*-methylacetamide (1.0 equiv.), 23 °C, 4 h, 90 %.

Both of these products could be allylated smoothly under alkylation condition (LDA or LiHMDS, HMPA; All-Br) to afford product **2-6** and **2-9** in good yields. The thioether **2-7** was also allylated using a Pummerer reaction, the first step consisting in the oxidation of compound **2-7**. The use of *m*CPBA as oxidizing agent at – 78 ˚C gave poor results with substantial amount of over-oxidation to the sulfone despite incomplete reaction. Oxidation with H_2O_2 in the presence of Lewis acids such as $Sc(OTf)_3$[162] or protic acid such as hexafluoroisopropanol[163] was found more reliable. Further treatment of the sulfoxide derived from **2-7** with trifluoroacetic anhydride in the presence of nucleophiles such as All-$SiMe_3$ or All-$SnBu_3$ afforded the desired product **2-6** in comparable yield to the alkylation procedure. The Pummerer allylation of selenide **2-8** was found to be less efficient using the same conditions as for **2-7**.

Coupling of fragment **2-4** and **2-6** was then investigated (Scheme 12). In the 70's, Cleger reported the alkylation of different toluic acids with 1-bromobutane.[164] Using LDA, alkylated compounds were obtained in ~70 % yield with *o*-toluic acid. Based on this result, we explored the alkylation of a "model" toluic ester with previously synthesized Weinreb amides. To this end, commercially available ethyl 2,4-dihydroxy-6-methylbenzoate was protected with methyl groups to obtain **2-10** which was subsequently chlorinated (**2-11**). The method used

[162] Matteucci, M., Bhalay, G. & Bradley, M. Mild and Highly Chemoselective Oxidation of Thioethers Mediated by $Sc(OTf)_3$. *Org Lett* **5**, 235-237 (2003).
[163] Ravikumar, K. S., Begue, J.-P. & Bonnet-Delpon, D. A selective conversion of sulfide to sulfoxide in hexafluoro-2-propanol. *Tetrahedron Letters* **39**, 3141-3144 (1998).
[164] Creger, P. L. Metalated carboxylic acids. II. Monoalkylation of metalated toluic acids and dimethylbenzoic acids. *J Am Chem Soc* **92**, 1396-7 (1970).

for the chlorination of the aromatic ring is based on the oxidation of acetaldehyde with sodium chlorite ($NaClO_2$) in acidic media (NH_2SO_3H) and the subsequent formation of hypochlorous acid HOCl.[165,166]



Scheme 12: Formation and reactions of toluates **2-10** and **2-11** with various electrophiles

a) K_2CO_3 (4.0 equiv.), Me_2SO_4 (4.0 equiv.), acetone, reflux, 4 h, 98 %; b) NH_2SO_3H (3.5 equiv.), CH_3CHO (1.0 equiv.), $NaClO_2$ (3.25 equiv.), THF/H_2O 1:2, 0 → 23 °C, 20h, 78 %.

Interestingly, although the acid functionality was protected as an ester, the alkylation of **2-10** required two equivalents of LDA despite the fact that the characteristic deep red color of the toluate anion was obtained with the first equivalent. Deuterium quenching experiments using one equivalent of LDA showed no incorporation of deuterium at the benzylic position (Table 1, entry 1) whereas the use of two equivalents afforded 70 % deuterium incorporation (entry 2). This observation could be attributed to the fact that although the toluate anion is formed with one equivalent of LDA, it forms a strong hydrogen bond to the diisopropylamine and is neither accessible to the D_2O nor to the Weinreb amide **2-6** as was observed by Seebach and co-workers for enolates.[167] The use of HMPA only afforded a marginal improvement (entry

[165] (a) Swain, C. G. & Crist, D. R. Mechanism of chlorination by hypochlorous acid. The last of chloronium ion, Cl+. *J Am Chem Soc* **94**, 3195-3200 (1972), (b) Dalcanale, E. & Montanari, F. Selective oxidation of aldehydes to carboxylic acids with sodium chlorite-hydrogen peroxide. *J Org Chem* **51**, 567-569 (1986).

[166] As reported by Swain (ref. 165), electrophiles might be ClO_2, Cl_2 or H_2OCl^+ that are more reactive than HOCl itself but, it is extremely doubtful that Cl^+ acts as the electrophile.

[167] Laube, T., Dunitz, J. D. & Seebach, D. Über die Wechselwirkung zwischen Lithium-enolaten und sekundären Aminen in Lösung und im Kristall. *Helv. Chim. Acta* **68**, 1373-1393 (1985).

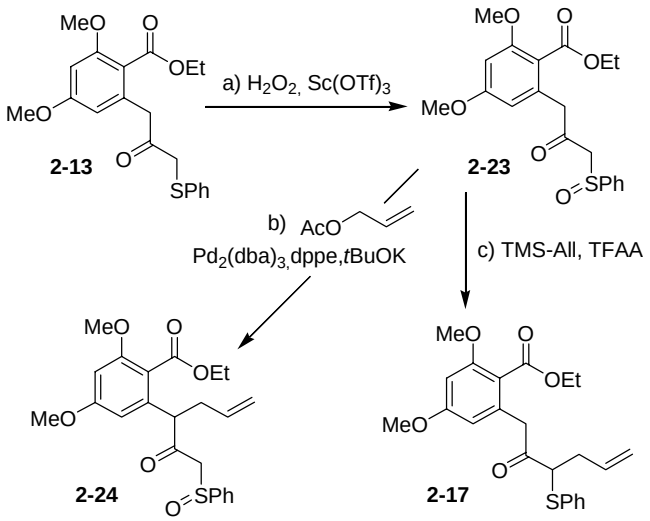
3). The alkylation of **2-10** with two equivalents of LDA and Weinreb amide **2-6** or **2-7** afforded the desired product in good yield (entries 4 and 7).

Table 1: Reaction conditions for acylation/alkylation of toluates **2-10** and **2-11**

	Toluate	***Base***	***Electrophile***	***Product***	***Yield [%]***
1	**2-10**	1 equiv. LDA, -78°C	D_2O	**2-12**	0
2	**2-10**	2 equiv. LDA, -78°C	D_2O	**2-12**	70
3	**2-10**	1 equiv. LDA, HMPA, -78°C	D_2O	**2-12**	5
4	**2-10**	2 equiv. LDA, -78°C	**2-7 (S)**	**2-13**	88
5	**2-10**	2 equiv. LDA, -78 → 0°C	**2-7 (S)**	**2-21**	84
6	**2-10**	2 equiv. LDA, -78°C	**2-8 (Se)**	**2-15**	90
7	**2-10**	2 equiv. LDA, -78°C	**2-6 (AllS)**	**2-17**	85
8	**2-10**	2 equiv. LDA, -78°C	**2-9 (AllSe)**	**2-22**	45
9	**2-11 (Cl)**	2 equiv. LDA, -78°C	**2-7 (S)**	**2-14**	75
10	**2-11 (Cl)**	2 equiv. LDA, -78°C	**2-6 (AllS)**	**2-18**	0

The alkylation could also be carried out with the more congested chlorinated toluate **2-11** (entry 9), however only the non-allylated Weinreb amide **2-7** was reactive toward the anion (entry 9 *vs* entry 10). Further investigations concerning these alkylation reactions showed the necessity to keep the reaction at -78 ˚C to avoid any formation of isocoumarin **2-21** (entry 5). Furthermore, while the reaction of the anion of toluate **2-10** with 2-seleno-Weinreb amide **2-8** proceeded as well as with 2-thio-Weinreb amide **2-7** (entry 4 *vs* entry 6), its reaction with the allylated 2-seleno-Weinreb amide **2-9** unexpectedly afforded compound **2-22** as the only isolable reaction product (entry 8). Thus, the Weinreb amide bearing a sulfur atom (**2-6** or **2-7**) proved more efficient than the seleno-derivatives (particularly **2-9**) and further work was carried out with Weinreb amides **2-6** and **2-7**.

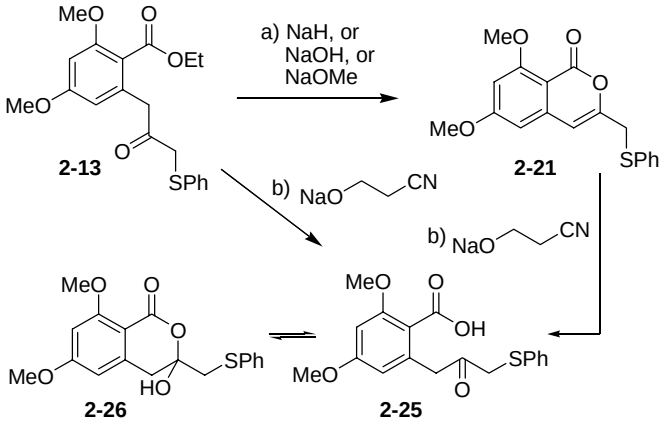
According to our previous success concerning the allylation of compound **2-7** under Pummerer conditions, the introduction of the allyl group could potentially be carried out after the coupling between the toluate fragment and the Weinreb amide using this method. As shown on Scheme 13, oxidation of **2-13** with H_2O_2 and catalytic scandium triflate afforded the sulfoxide **2-23** which was further allylated under Pummerer conditions (trifluoroacetic anhydride, All-$SiMe_3$) to reach compound **2-17** in good yield.



Scheme 13: Allylation trials of ketosulfoxide **2-23**

a) H_2O_2 (5.0 equiv.), $Sc(OTf)_3$ (0.2 equiv.), CH_2Cl_2/EtOH 10:1, 23 °C, 3 h, 91 %; b) *t*BuOK (1.0 equiv.), $Pd_2(dba)_3CHCl_3$ (0.1 equiv.), dppe (0.1 equiv.), allylacetate (5.0 equiv.), THF, 0 → 23 °C, 10 min, 84 %; c) AllylTMS (5.0 equiv.), CH_2Cl_2, -78 °C; TFAA (3.0 equiv.), -78 → 23 °C, 80 %.

Interestingly, alkylation of the ketosulfoxide **2-23** using Tsuji-Trost conditions with a palladium π-allyl complex afforded product **2-24** suggesting that the benzylic position is more nucleophilic than the ketosulfoxide one. Nevertheless, cyclization to isocoumarin **2-21** was not observed during the reaction. The propensity of compound **2-13** to form the isocoumarin **2-21** is well known and, indeed, treatment of **2-13** with alkoxide or hydroxide led to the rapid formation of isocoumarin **2-21**(Scheme 14).

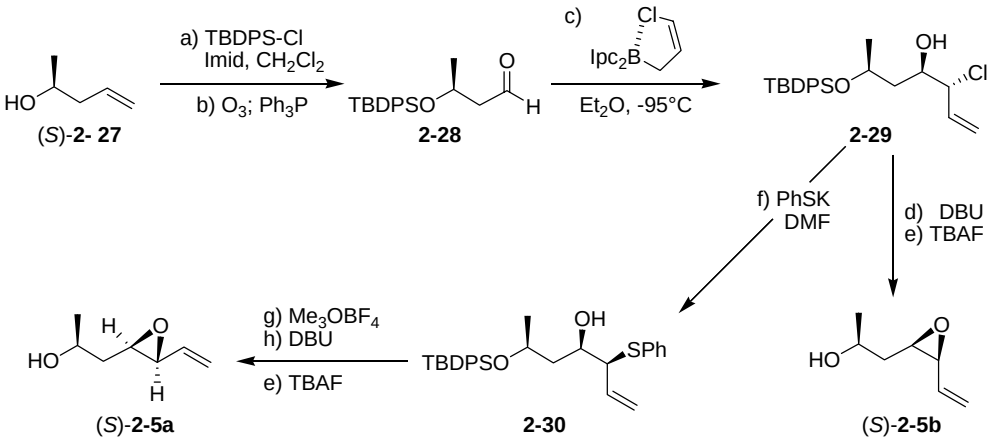


Scheme 14: Hydrolysis of ester **2-13** and isocoumarin **2-21**

a) NaH (2.0 equiv.), THF, 0 → 23 °C, 10 min, quant.; b) 3-hydroxypropionitrile (10.0 equiv.), NaH (4.0 equiv.), THF, 0 → 23 °C, 10 min, 93 %.

We were gratified to find that hydrolysis of ester **2-13** was nevertheless possible using the alkoxide of 3-hydroxypropionitrile which presumably first participates in a transesterification followed by an elimination thus affording the hydrolyzed product as a mixture of hemiketals **2-25** and **2-26**. It is noteworthy that this procedure was also effective to hydrolyze the isocoumarin **2-21**. Esterification of the acid **2-25** through activation of the carbonyl led to significant formation of isocoumarin **2-21** while Mitsunobu conditions were not effective presumably due to the fact that the *ortho*-phenol is protected.[142] These observations led us to envision the esterification reaction with the alcohol **2-5** before the alkylation with Weinreb amide **2-6** or **2-7**. Considering the nucleophilic addition that takes place during the Pummerer reaction and having in mind the sensivity of the epoxide present on alcohol **2-5**, the allylated Weinreb amide **2-7** appeared as the most suitable one for the completion of the synthesis.

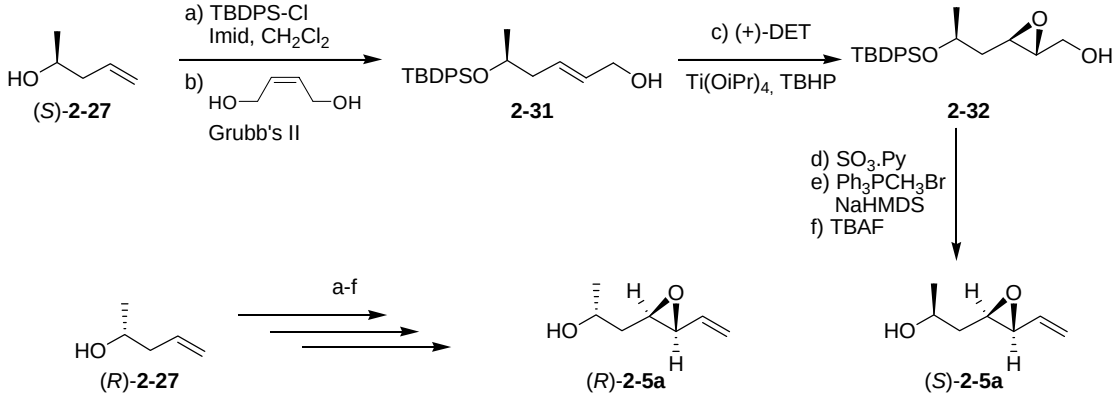
Our next effort concerned the synthesis of the alcohol **2-5**. As the stereochemistry on the chlorine atom on pochonin C was not known, access to both the *cis* and *trans* epoxides ((*S*)-**2-5a** and (*S*)-**2-5b**) was necessary and a divergent strategy starting from commercially available alcohol (*S*)-**2-27** was established (Scheme 15). Protection of the hydroxyl functionnality with a silyl group (TBDPS) and a subsequent ozonolysis furnished aldehyde **28** in excellent yield.



Scheme 15: Stereodivergent synthesis of diastereoisomers (*S*)-**2-5a** and (*S*)-**2-5b**

a) *t*BuPh$_2$SiCl (1.1 equiv.), imid. (1.7 equiv.), CH_2Cl_2, 23 °C, 4 h, 98 %; b) O_3, CH_2Cl_2, -78 °C, 5 min; Ph_3P (1.5 equiv.), 23 °C, 2 h, 94 %; c) allyl chloride (2.0 equiv.), LiN*c*Hex (2.0 equiv.), Ipc$_2$BOMe (1.5 equiv.), BF_3OEt_2 (2.5 equiv.), -95 °C, 4 h, 68 % (85 % d.e.); d) DBU (3.0 equiv.), CH_2Cl_2, 0 °C, 8 h, 97 %; e) TBAF (1.2 equiv.), THF, 23 °C, 6 h, 98 %; f) thiophenol (4.4 equiv.), *t*BuOK (3.3 equiv.), 23 °C, 1 h and then, **2-29** (1.0 equiv.), DMF, 0 → 23 °C, 86 %; g) Me_3OBF_4 (2.0 equiv.), CH_2Cl_2, 0 → 23 °C, 4 h; h) DBU (3.0 equiv.), CH_2Cl_2, 0 °C, 4 h, 80 % (two steps).

Following a modified Brown allylation[168] procedure, treatment of aldehyde **2-28** with (*Z*)-(γ-chloroallyl)diisopinocampheylborane[169] yielded the halohydrin **2-29** with good diastereoselectivity. Direct oxirane formation with DBU followed by TBAF deprotection of the silyl group afforded the *cis*-epoxide (*S*)-**2-5b**. Importantly, it was found that the epoxide could be converted back to the chlorohydrin functionality present in pochonin C *stereospecifically*. The corresponding *trans*-epoxide (*S*)-**2-5a** was reached by SN_2 displacement of the chloride atom of alcohol **2-29** with thiophenoxide to obtain compound **2-30** which was further activated for oxirane formation by methylation of the sulfur.[170] Treatment with DBU followed by removal of the TBDPS protecting group afforded the *trans* epoxide (*S*)-**2-5a** in 42 % yield from commercially available alcohol (*S*)-**2-27**. Alternatively, alcohol (*R*)-**2-5a** could also be reached via a six step sequence (Scheme 16) involving silyl protection of alcohol (*S*)-**2-27** followed by cross metathesis[171] with an excess of 1,4-butanediol to afford allylic alcohol **2-31** which was subjected to a Sharpless asymmetric epoxidation as previously reported.[172,144]



Scheme 16: Second method for *trans* epoxide (*S*)-**2-5a** and (*R*)-**2-5a** synthesis

a) *t*$BuPh_2SiCl$ (1.1 equiv.), imid. (1.7 equiv.), CH_2Cl_2, 23 °C, 4 h, 98 %; b) 2-butene-1,4-diol (4 equiv.), Grubbs' II (10 %), toluene, 80 °C, 12 h, 56 % c) (+)-DET, Ti(O*i*Pr)$_4$, TBHP, Et_2O, -30 °C, 12 h, 90 %, > 93 % e.e.; d) SO_3·Py (1.5 equiv.), Et_3N (1.6 equiv.), DMSO, 23 °C, 1 h, 90 %, e) Ph_3PCH_3Br, NaHMDS, 0 °C, 80 % f) TBAF (1.2 equiv.), THF, 23 °C, 6 h, 98 %.

[168] Brown, H. C. & Jadhav, P. K. Asymmetric carbon-carbon bond formation via b-allyldiisopinocamphenylborane. Simple synthesis of secondary homoallylic alcohols with excellent enantiomeric purities. *J Am Chem Soc* **105**, 2092-3 (1983).

[169] (a) Jayaraman, S., Hu, S. & Oehlschlager, A. C. New access to chiral syn-a-chlorohydrins and cis-vinyloxiranes through chloroallylboration. *Tetrahedron Letters* **36**, 4765-8 (1995)., (b) Hu, S., Jayaraman, S. & Oehlschlager, A. C. Diastereo- and Enantioselective Synthesis of syn-a-Vinylchlorohydrins and cis-Vinylepoxides. *J Org Chem* **61**, 7513-7520 (1996).

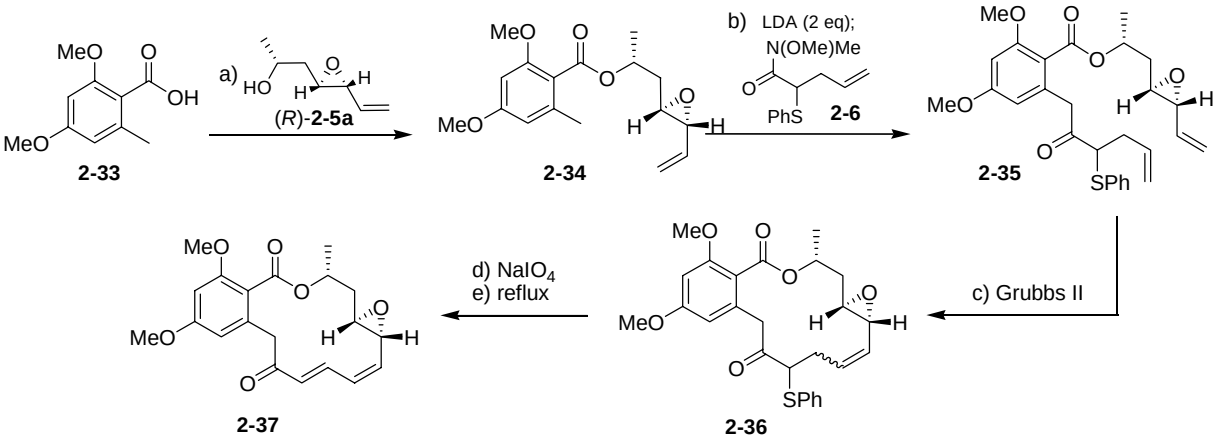
[170] Hertweck, C. & Boland, W. Reductive chloro- and thioallylations. Stereoselective two-step transformations of esters and lactones into functionalized cis- and trans-vinyloxiranes. *Eur J Org Chem*, 2143-2148 (1998).

[171] For an elegant approach of cross-metathesis, see: BouzBouz, S. & Cossy, J. Tetrafibricin: synthesis of the C1-C13, C15-C25, and C27-C40 fragments. *Org Lett* **6**, 3469-72 (2004).

[172] Schlede, U., Nazare, M. & Waldmann, H. Efficient enantioselective synthesis of α,β.-hydroxyepoxide building block for the construction of macrocyclic natural products. *Tetrahedron Letters* **39**, 1143-1144 (1998).

It is important to note that the epoxidation reaction required a bulky protecting group on the homoallylic alcohol to achieve high enantiomeric excess, the use of TBS instead of TBDPS led to disappointing results. Oxidation of alcohol **2-32** followed by a Wittig olefination and deprotection of the silyl group afforded alcohol (*S*)-**2-5a** in 35 % yield over six steps. Although the overall yield obtained by this strategy is slightly lower than with the first method, the enantiomeric excess is higher.

Having the three building blocks in hand, the efficacy of the ring-closing metathesis had to be tested, especially concerning the selectivity of this reaction (*cis*/*trans* ratio) and the compatibility of the catalyst with the thioether moiety. Thus, we prepared the open diene **2-3** (Figure 29) containing all the functional groups present in the natural product. Esterification of alcohol (*R*)-**2-5a** (Scheme 17) with 2,4-dimethoxy-toluic acid (**2-33**) using an acid chloride intermediate (methodology previously reported by Garbaccio and co-workers)[145] afforded toluate **2-34** which was further acylated with Weinreb amide **2-6** to yield the desired acyclic precursor **2-35** in 75 % yield.



Scheme 17: Synthesis of dimethyl monocillin I (**2-37**)

a) Oxalyl chloride (1.0 equiv.), DMF (cat.), CH_2Cl_2, 0 → 23 °C, 1 h, and then at 0 °C, Et_3N (2.0 equiv.), (*R*)-**2-5a** (0.8 equiv.), DMAP, 0 → 23 °C, 3 h, 80 % b) LDA (2.0 equiv.), THF, -78 °C, 5 min; **2-6** (1.0 equiv.), THF, -78 °C, 5 min, 75 %; c) Grubbs' II (5 % mol), toluene (2 mM),reflux, 10 min, 87 %; d) $NaIO_4$ (1.5 equiv.), MeOH, 23 °C, 8 h, 40 %; e) toluene, reflux, 1 h, 80 %.

Importantly, the synthesis of intermediate **2-35** showed that the acylation reaction was compatible with more complex substrate (**2-34** *vs* **2-10**). Moreover, the potential side reaction of the toluate anion with the epoxide moiety appeared relatively slow in comparison to the desired acylation with the Weinreb amide. However, it was found that leaving the toluate anion of **2-34** at -78 °C for more than 20 min led to significant decomposition of the starting

material presumably due to this type of side reactions. Treatment of this open chain diene with Grubbs' second generation catalyst[173] in toluene at reflux for 10 minutes (kinetic ring-closure conditions) [174,148] gave the cyclization product **2-36** in 85 % isolated yield as a mixture of olefin geometry. Importantly, the use of the first generation catalyst led to the recovery of the starting material. Moreover, it is noteworthy that the presence of a thioether in β-position to the carbene reactive center did not prevent the efficiency of the metathesis. In fact, Fürstner and co-workers reported the inefficacy of RCM for macrocyclization of a 14-membered resorcylic acid lactone (zeranol) in presence of a dithioacetal that poisoned the catalytic cycle.[175] Oxidation of **2-36** proved to be challenging as *m*CPBA gave unsatisfactory results (oxidation to the sulfone) as well as H_2O_2 in the presence of scandium triflate[162] which led to the epoxide opening (at the time of this experiment, we were not aware of the HFIP condition[163] which ultimately proved very effective). An acceptable oxidation was achieved using $NaIO_4$ although the reaction was sluggish and never reached completion despite alarge excess of oxidant. Isolation of the major oxidation product (there are eight possible products corresponding to a mixture of olefin geometry, stereochemistry of the α-ketosulfoxide center and of the sulfoxide itself) and heating to reflux in toluene afforded dimethyl monocillin I[176] (**2-37**) very cleanly and in good yield. It was clear from previous attempts[142,145] that the methyl groups on the phenols could not be removed in the presence of the sensitive functionalities present on radicicol and pochonin C. Nevertheless, the completion of this synthesis established a proof of principle that the proposed strategy was efficient enough to yield both natural products. The careful choice of phenol protecting groups appeared very important for the success of the synthetic strategy and MOM groups were selected based on their stability to the enolate chemistry.

[173] (a) Chatterjee, A. K., Morgan, J. P., Scholl, M. & Grubbs, R. H. Synthesis of Functionalized Olefins by Cross and Ring-Closing Metatheses. *J Am Chem Soc* **122**, 3783-3784 (2000), (b) Scholl, M., Ding, S., Lee, C. W. & Grubbs, R. H. Synthesis and Activity of a New Generation of Ruthenium-Based Olefin Metathesis Catalysts Coordinated with 1,3-Dimesityl-4,5-dihydroimidazol-2-ylidene Ligands. *Org Lett* **1**, 953-956 (1999).

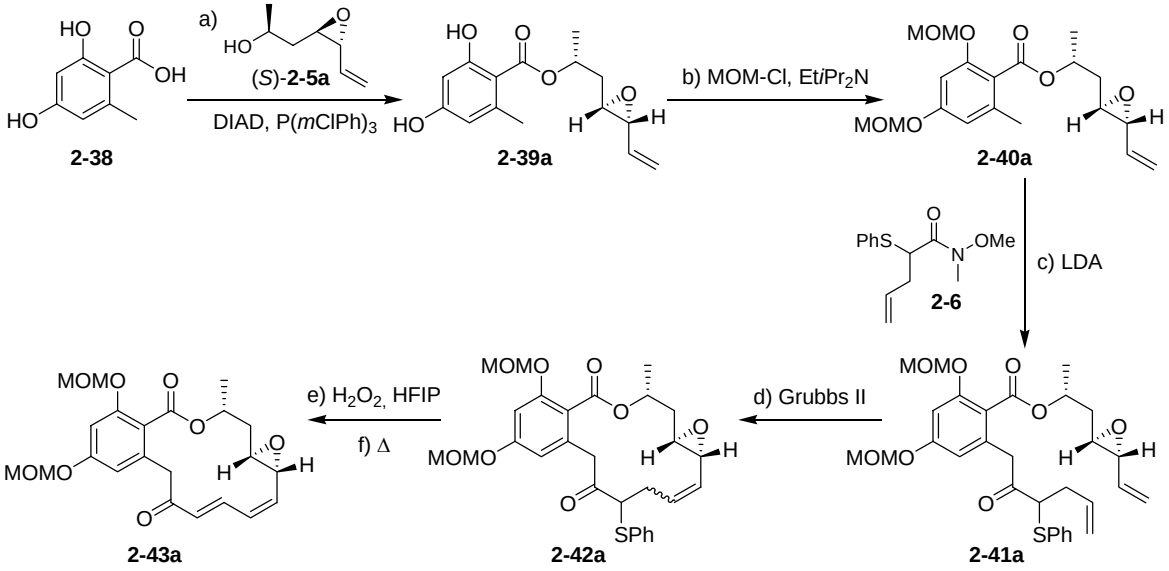
[174] Lee, C. W. & Grubbs, R. H. Stereoselectivity of Macrocyclic Ring-Closing Olefin Metathesis. *Org Lett* **2**, 2145-2147 (2000).

[175] Furstner, A., Seidel, G. & Kindler, N. Macrocycles by ring-closing-metathesis, XI: syntheses of (R)-(+)-lasiodiplodin, zeranol and truncated salicylihalamides. *Tetrahedron* **55**, 8215-8230 (1999) and articles cited therein for similar problems.

[176] Mirrington, R. N., Ritchie, E., Shoppee, C. W., Sternhell, S. & Taylor, W. C. Some metabolites of Cylindrocarpon radicicola: the structure of radicicola (monorden). *Australian J Chem* **19**, 1265-84 (1966).

B. Total syntheses

The most convergent sequence of assembly of fragments **2-4**, **2-5** and **2-6** (Figure 29) requires alcohol (*S*)-**2-5a** to be coupled to benzoic acid **2-38** prior to coupling with Weinreb amide **2-6** to avoid protecting group manipulation. As previously reported by Lett,[142] esterification of the 2-hydroxy toluic acid derivatives under Mitsunobu or carbodiimide condition was found to work best with the free *ortho*-phenol. By establishing a hydrogen bond between the free phenol and the acid, the pKa of the benzoic acid is lowered thus leading to an acceleration of the reaction. In order to avoid any protecting group manipulation, we decided to investigate a selective Mitsunobu esterification[177] on the commercially available unprotected acid **2-38** (Scheme 18). Although no selectivity between the *para*-phenol and the acid functionalities was observed using classical conditions (DIAD, PPh_3, toluene, CH_2Cl_2 or THF), the use of tris(3-chlorophenyl)phosphine[178] afforded **2-39a** with more than 95:5 selectivity and in 84 % yield.

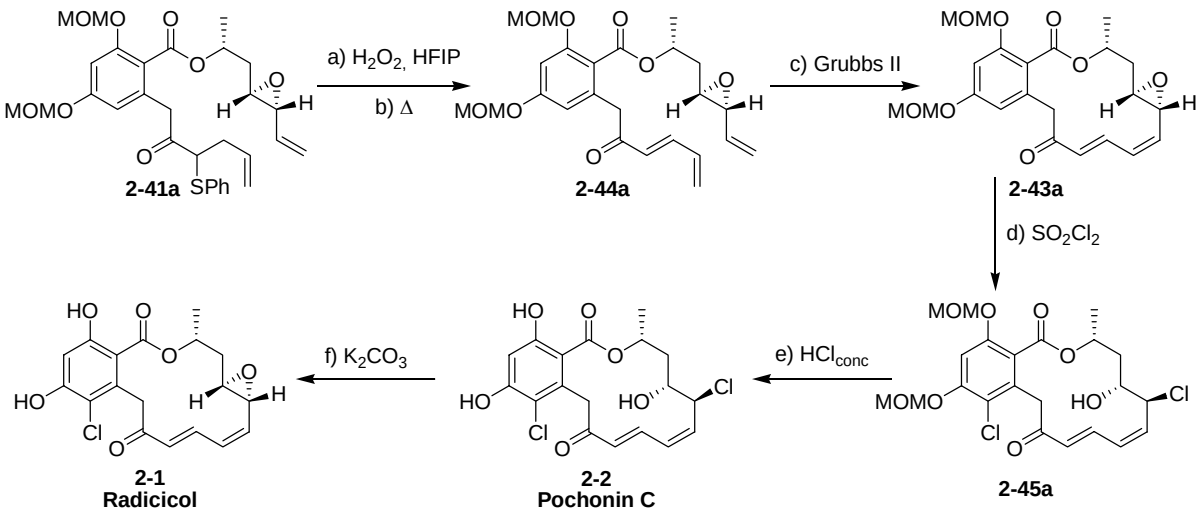


Scheme 18: Synthesis of the cyclic precursor **2-43a** of pochonin C and radicicol

a) (*S*)-**2-5a** (1.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 3 h, 84 %; b) MOMCl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), TBAI (cat.), DMF, 80 °C, 3 h, 91 %; c) LDA (2.0 equiv.), THF, -78 °C; **2-6** (1.0 equiv.), 10 min, 81 %; d) Grubbs' II (5 % mol), toluene (2 mM), reflux, 10 min, 94 %; e) H_2O_2 (2.0 equiv.), HFIP, 23 °C, 3 h; f) toluene, 80 °C, 1 h, 22 % two steps (85 % based on recovered sulfoxide).

[177] Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis*, 1-28 (1981).
[178] Hughes, D. L., Reamer, R. A., Bergan, J. J. & Grabowski, E. J. J. A mechanistic study of the Mitsunobu esterification reaction. *J Am Chem Soc* **110**, 6487-91 (1988).

The electrophilicity of the chlorine atom on tris(3-chlorophenyl)phosphine presumably generates a less basic complex with DIAD which is more apt at discriminating between the *para*-phenol (pKa ~ 10) and the acid (pKa ~ 4.5). Compound **2-39a** was then protected with MOM groups to afford ester **2-40a** which was further acylated with Weinreb amide **2-6** using two equivalents of LDA to yield acyclic precursor **2-41a** in good yield. Treatment of this intermediate with the Grubbs' second generation catalyst in toluene at reflux led to rapid ring closure and recovery of the macrocycle **2-42a** as an inseparable mixture of *cis*/*trans* olefins (1:1), in accordance with the previous results observed for **2-36**. With the low yield of the $NaIO_4$ oxidation and the inadequacy of *m*CPBA or H_2O_2/$ScOTf_3$, we were gratified to find that thioether **2-42a** could be chemoselectively oxidized with H_2O_2 in hexafluoroisopropanol[163] without affecting the epoxide moiety or observing over-oxidation to the sulfone. The over-oxidation is inhibited by a strong hydrogen bond between the oxygen atom of the sulfoxide and the alcohol functionality of HFIP and, HFIP is not acidic enough (pKa ~10 in H_2O) to open the epoxide. The following elimination was carried out on the crude sulfoxide as a mixture of eight isomers. Interestingly, only the desired *trans/cis* conjugated diene **2-43a** was obtained. Presumably, elimination of the sulfoxide *only* proceed for the compound leading to the desired *trans*/*cis* diene **2-43a**, the *trans* olefin obtained after metathesis not being able to adopt the adequate conformation to participate in a 1,2-*syn* elimination of the sulfoxide. Although this way was convenient in avoiding any 12-membered ring formation and purification of the *cis*/*trans* mixture, the overall yield was poor. Nevertheless, the selectivity observed in the elimination reflected the rigidity of the macrocycle and suggested that carrying out the metathesis on the triene **2-44a** should lead to the desired *trans*/*cis* diene (Scheme 19). Oxidation/elimination of the thioether **2-41a** followed by RCM in refluxing toluene led indeed exclusively to the desired *trans*/*cis* conjugated diene **2-43a** within 10 min in excellent yield (80 % over 3 steps).



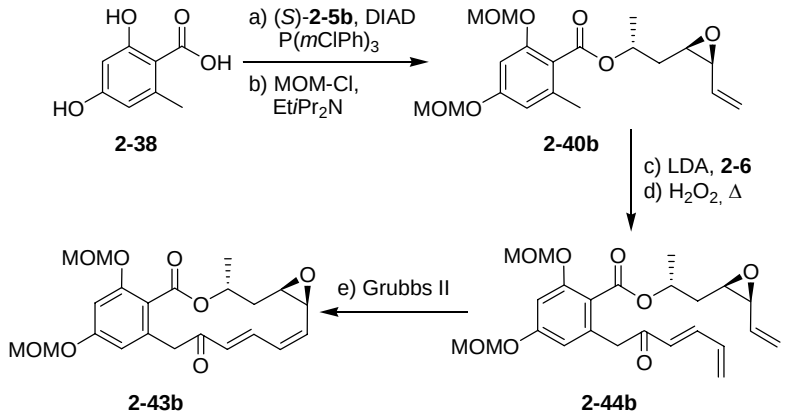
Scheme 19: Completion of pochonin C and radicicol total syntheses

a) H_2O_2 (2.0 equiv.), HFIP, 23 °C, 3 h; b) toluene, 80 °C, 1 h, 92 %; c) Grubbs' II (5 % mol), toluene (2 mM), reflux, 10 min, 87 %; d) SO_2Cl_2 (3.0 equiv.), Et_2O, 0 °C, 68 %; e) $HCl_{conc.}$ (2.5 % in dioxane), 0 → 23 °C, 1 h, 74 %; f) K_2CO_3 (2.0 equiv.), DMF, 23 °C, 1 h, 86 %.

In 2000, Lee and Grubbs reported that the *cis*/*trans* selectivity of the RCM in unsubstituted 14-membered macrocycles is kinetically controlled leading preferentially to the *cis* isomer even at low conversion.[174] In our case, the high degree of selectivity in the ring closure of **2-44a** can not be only attributed to short reaction time considering the lack of selectivity for the closure of **2-41a** under the same conditions. A first insight in a conformational rigidity of the macrocycle was obvious from this result (*vide infra*). At this stage, few steps remained to reach pochonin C. The epoxide could be stereospecifically opened using several equivalents of HCl in dioxane while the chlorine atom could be efficiently introduced using calcium hypochlorite ($Ca(OCl)_2$)). To our delight, it was found that both chlorination reactions leading to intermediate **2-45a** could be carried out in a single step by using an excess of the SO_2Cl_2 which generates an equivalent of HCl *in situ* (no overchlorination of the aromatic ring was observed).[179] Final deprotection of the MOM groups led to compound **2-2** which was found to have an identical NMR spectra to the natural pochonin C.[180] Importantly, treatment of this molecule with K_2CO_3 led to rapid and clean oxirane formation thus yielding compound **2-1** which was identical to radicicol.

A similar reaction sequence starting from alcohol (*S*)-**2-5b** (Scheme 20) and toluic acid **2-38** led to the triene **2-44b**.

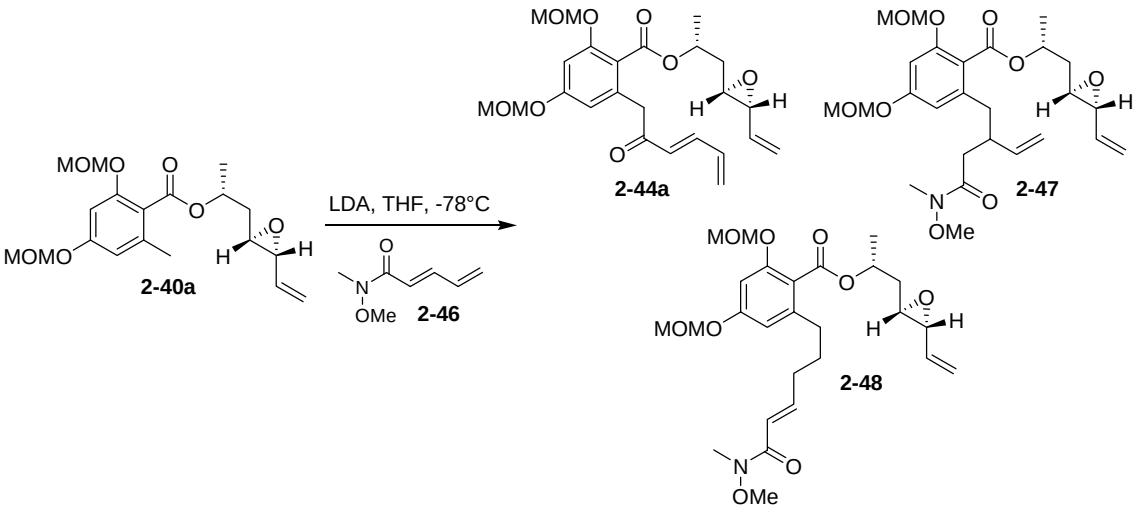
[179] Elix, J. A. & Norfolk, S. Synthesis of p-b-orcinol depsides. *Australian J Chem* **28**, 1113-24 (1975).
[180] A spectra of natural pochonin C was kindly provided by Marc Stadler, Bayer Health Care, Wuppertal, Germany.



Scheme 20: Synthesis of cyclic precursor **2-43** with the *cis*-epoxide

a) (*S*)-**2-5b** (1.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 3 h; b) MOMCl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), TBAI (cat.), DMF, 80 °C, 3 h, 76 % (over 2 steps); c) LDA (2.0 equiv.), THF, -78 °C; 2-**6** (1.0 equiv.), 10 min, 81 %; d) H_2O_2 (2.0 equiv.), HFIP, 23 °C, 3 h and then, toluene, 80 °C, 1 h, 92 %; e) Grubbs' II (5 % mol), toluene (2 mM), reflux, 10 min, 21 %.

Treatment of this compound with the successful metathesis conditions used for **2-44a** afforded the cyclic product **2-43b** as the conjugated *trans*/*cis* diene albeit in poor yield (Scheme 20). The outcome of this reaction clearly established that the configuration of the epoxide is playing a role in the conformation of the macrocycle. Considering the fact that the metathesis gives better selectivity on triene **2-44a** rather than on diene **2-41a**, we explored the possibility of acylating toluate **2-40a** directly with the α,β,γ,δ-conjugated Weinreb amide **2-46**. As depicted in Scheme 21, this reaction led to a mixture of desired 1,2-addition **2-44a** as well as the 1,4-addition and 1,6-addition products, **2-47** and **2-48** respectively.



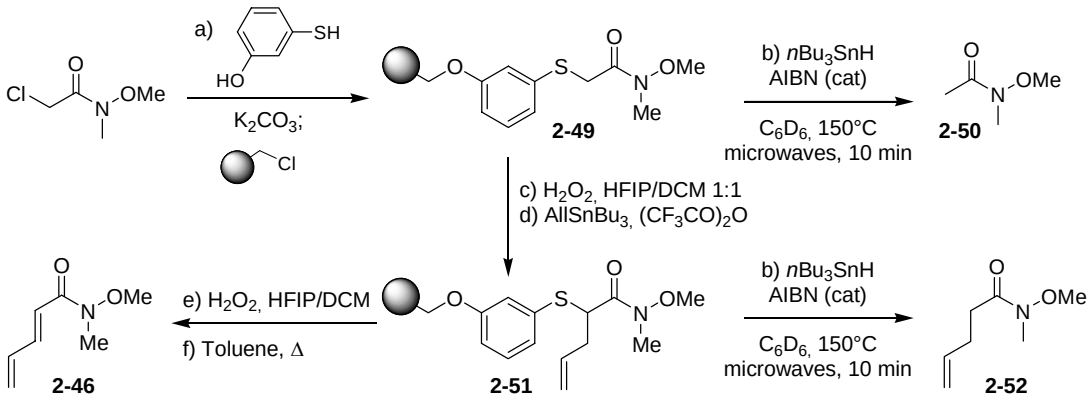
Scheme 21: Acylation trials with the α,β,γ,δ-conjugated Weinreb amide

Despite several attempts to favor the 1,2-addition, we could not obtain more than 30 % yield of compound **2-44a** with this reaction. It is interesting to note that similar reactions with simple α,β-conjugated systems rather than with conjugated dienes have been more productive.[181,158a] A concise and modular synthesis of pochonin C and radicicol has been proposed in 7 and 8 steps and 22 % and 25 % yield respectively from benzoic acid **2-38**, alcohol (*S*)-**2-5a** and Weinreb amide **2-6**. The longest linear sequence is 13 steps for pochonin C and 14 steps for radicicol. The stereoselective synthesis of pochonin C and its conversion to radicicol has been demonstrated thereby assigning the stereochemistry of C5 on pochonin C as *S*.

[181] Lewis, C. N., Spargo, P. L. & Staunton, J. A convenient synthesis of 3-substituted 8-methoxy- and 6,8-dimethoxyisocoumarins. *Synthesis*, 944-6 (1986).

II. Solid-Phase Syntheses of Radicicol and Pochonin C

As previously mentioned, aside from masking the α,β-conjugated system thus providing a protection during the acylation step, the thioether was foreseen as an attachment point to a solid phase. As shown in Scheme 22, the polymer-bound equivalent of compound **2-7** (**2-49**) was prepared in one pot by a selective *S*-alkylation of 3-mercaptophenol with 2-chloro-*N*-methoxy-*N*-methylacetamide using one equivalent of base followed by the addition of Merrifield resin and a second equivalent of base. The success of thist reaction could be assessed by taking an aliquot of the resin (10 mg) and cleaving the thioether under reductive conditions (*n*Bu_3SnH, AIBN) in deuterated benzene so that the product (**2-50**) was analyzed directly by NMR after filtration of the resin. For convenience and speed, the reactions were carried out with microwave irradiation and found to go to completion in 10 min. By this method, resin **49** was obtained in a calculated 90 % yield from Merrifield resin.



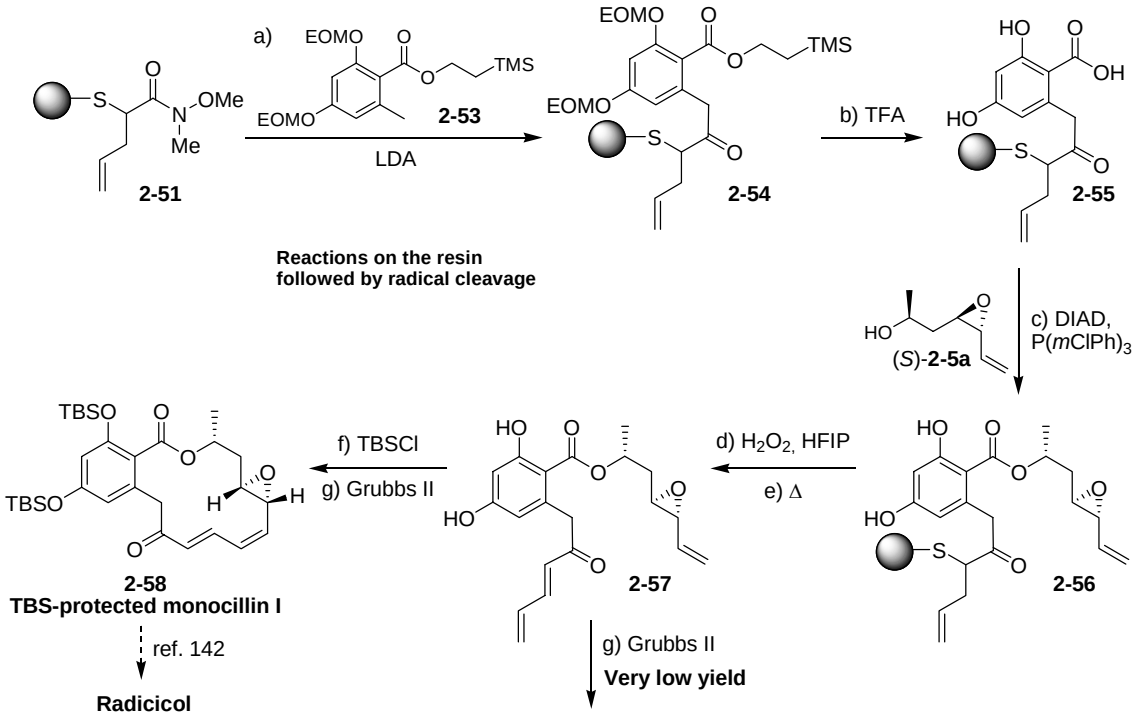
Scheme 22: Solid-phase synthesis of Weinreb amide building blocks

a) 3-mercaptophenol (1.0 equiv.), K_2CO_3 (1.0 equiv.), 2-chloro-*N*-methoxy-*N*-methylacetamide (1.0 equiv.), DMF, 23 °C, 2 h; Merrifield resin (0.6 equiv.), K_2CO_3 (2.0 equiv.), TBAI, 50 °C, 2 h, 90 %; b) *n*Bu_3SnH (5.0 equiv.), AIBN (cat.), C_6D_6, microwaves (150 °C, 300 W), 10 min; c) H_2O_2 (4.0 equiv.), HFIP/CH_2Cl_2 1:1, 23 °C, 12 h; d) AllylSnBu_3 (5.0 equiv.), -78 °C, CH_2Cl_2; then TFAA (3.0 equiv.), -78 → 23 °C; e) H_2O_2 (4.0 equiv.), HFIP/CH_2Cl_2 1:1, 23 °C, 12 h; f) toluene, 80 °C, 30 min, 80 % isolated yield from Merrifield resin.

Following aforementioned procedures, the thioether linker of resin **2-49** was then oxidized with H_2O_2 using hexafluoroisopropanol as a protic acid. The resulting sulfoxide was further activated with trifluoroacetic anhydride in the presence of All-SnBu_3 for an allylation under Pummerer conditions to obtain resin **2-51**. The efficiency of this transformation could also be assessed by using a *n*Bu_3SnH/AIBN cleavage. More importantly, oxidation of resin **2-51** with H_2O_2 followed by a brief heating afforded compound **2-46** in 80 % yield based on the loading

of the Merrifield resin and > 95 % purity as judged by ^{1}H NMR. Furthermore, the sulfoxide did not eliminate at room temperature allowing effective washing of the resin before the elimination in a solvent free of reagents.

As depicted in Scheme 23, the polymer-bound Weinreb amide **2-51** was used in an acylation step with suitably protected toluic ester **2-53**[186] to afford the polymer bound product **2-54**. Based on the free radical cleavage procedure described above, the calculated yield of this reaction was 60 %. However, using the oxidation/elimination procedure and drying the product *in vacuo* overnight showed only the expected eliminated product of resin **2-54** as the α,β,γ,δ-conjugated Weinreib amide **2-46** is volatile enough to be removed. Trying to drive the reaction to completion with more equivalents of toluic anion did not improve the yield.



Scheme 23: Solid-phase synthesis of TBS-protected monocillin I

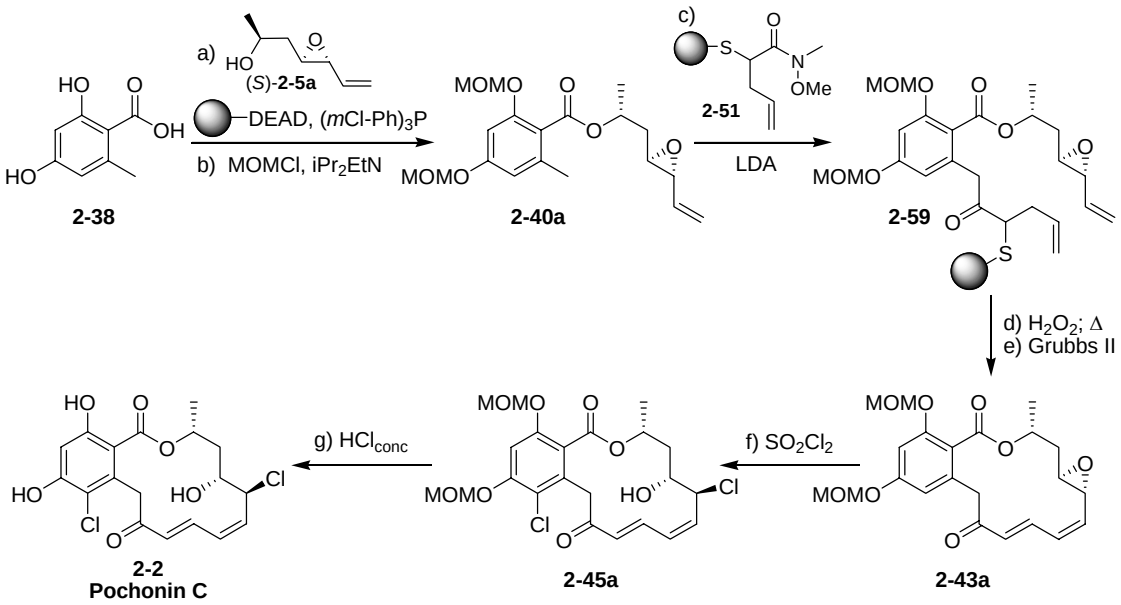
a) **2-53** (4.0 equiv.), LDA (8.0 equiv.), THF, -78 °C, 10 min, and then **2-51** (1.0 equiv.), -78 °C, 4 h, 60 % from resin **2-51**; b) TFA (20 %), CH_2Cl_2, 1 h, > 95 %; c) (*S*)-**2-5a** (4.0 equiv.), (*m*Cl-Ph)$_3$P (4.0 equiv.), DIAD (4.0 equiv.), CH_2Cl_2, 23 °C, 4 h; d) H_2O_2 (4.0 equiv.), HFIP/CH_2Cl_2 1:1, 23 °C, 12 h; e) toluene, 80 °C, 12 h, 70 % from resin **2-54**; f) TBSCl (10.0 equiv.), imid. (10.0 equiv.), DMF, 12 h, 90 %; g) Grubbs' II (5 % mol), toluene (2 mM), reflux, 83 %.

Interestingly, the use of polymer bound Weinreb amide **2-49** did not yield any acylated intermediate even though the corresponding reaction in solution proved very effective. This

[186] The synthesis of compound **2-53** is described in the experimental part.

was rationalized by the fact that the polystyrene environment altered the relative rates of the enolization of the Weinreb amide relatively to the acylation reaction and the less hindered Weinreb amide was enolized rather than alkylated. Likewise, the use of larger excess of reagents did not improve the reaction yields since the enolized Weinreb amide is simply not reactive. Nevertheless, considering that the elimination product of the unreacted Weinreb amide **2-51** was volatile, clean product could be obtained anyhow. Selective removal of the silyl ester using TBAF followed by selective deprotection of the *ortho*-MOM with 5 % TFA could be achieved easily and afford the *para*-MOM protected resin **2-55** that could then be esterified. However, based on our previous observation that a selective Mitsunobu esterification can be effective in the presence of both free phenols, we opted to remove the three protecting groups in one single step using 20 % TFA, thus yielding the fully deprotected intermediate **2-55** (Scheme 23). Esterification of this product according to the same reaction conditions as previously used in solution afforded the polymer-bound cyclization precursor **2-56** which was released from the resin by the two step oxidation/elimination procedure. Disappointingly, compound **2-57** did not give good yields in the ring-closing metathesis. In a similar system, the presence or absence of a free *ortho*-phenol was shown to affect the outcome of the RCM by virtue of the hydrogen bound to the carbonyl and its impact on the pre-organization of the open chain system.[187] However, in our case, the incompatibility stemmed from the unprotected *para*-phenol as the metathesis was very effective when only the *para*-phenol was protected (not shown). Reprotection of the phenols with TBS groups followed by RCM yielded macrocyle **2-58** in 75 % over 2 steps. Compound **2-58** was converted to radicicol according to known procedures.[142] In a different approach with supported reagents, acid **2-38** was esterified with alcohol (*S*)-**2-5a** using polymer-bound DEAD (Scheme 24) followed by MOM protection to obtain toluate **2-40a** which could be used in the subsequent acylation reaction without any further purification.

[187] Couladouros, E. A., Mihou, A. P. & Bouzas, E. A. First total synthesis of trans- and cis-resorcylide: remarkable hydrogen-bond-controlled, stereospecific ring-closing metathesis. *Org Lett* **6**, 977-80 (2004).



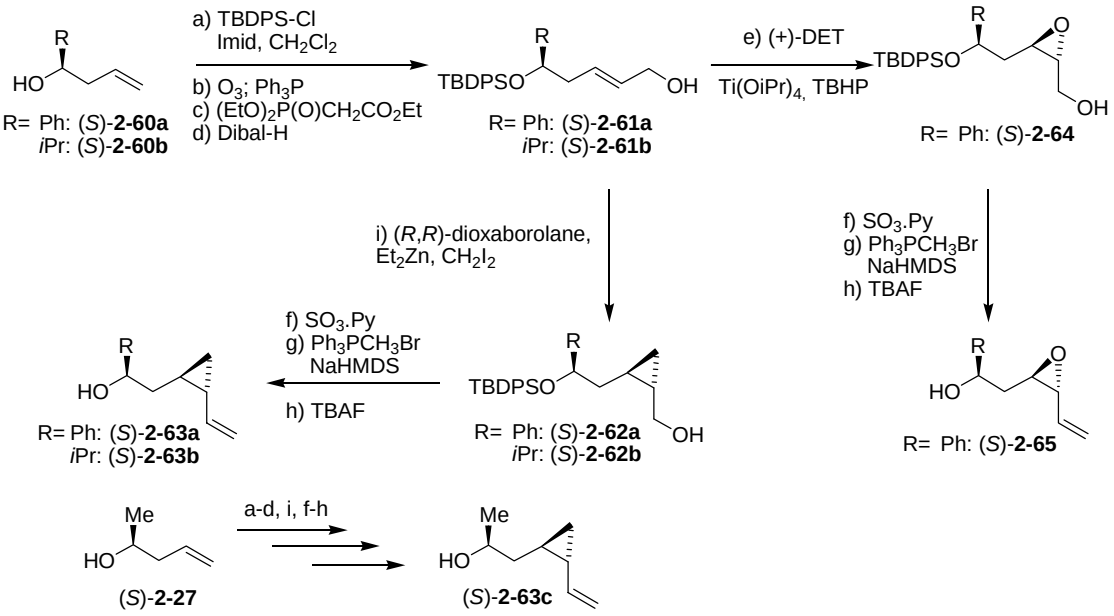
Scheme 24: Polymer-assisted synthesis of pochonin C

a) (*S*)-**2-5a** (1.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), PS-DEAD (2.0 equiv., 1.3 mmol.g^{-1}), toluene, 23 °C, 3 h, 83 %; b) MOMCl (4.0 equiv.), *i*Pr$_2$NEt (4.0 equiv.), TBAI (cat.), DMF, 80 °C, 3 h, 91 %; c) **2-40a** (2.0 equiv.), LDA (4.0 equiv.), THF, -78 °C, 10 min, and then **2-51** (1.0 equiv.) -78 °C, 4 h; d) H_2O_2 (4.0 equiv.), HFIP/CH_2Cl_2 1:1, 23 °C, 12 h and then, toluene, 80 °C, 12 h, 53 %; e) Grubbs' II (5 % mol), toluene (2 mM), 120 °C, 10 min, 87 %; f) SO_2Cl_2 (3.0 equiv.), Et_2O, 0 °C, 68 %; g) $HCl_{conc.}$ (2.5 % in dioxane), 0 → 23 °C, 1 h, 74 %.

Deprotonation of the crude toluate **2-40a** with LDA and reaction with polymer bound Weinreb amide **2-51** afforded the polymer bound diene **2-59**. Further oxidation/elimination followed by a ring-closing metathesis afforded compound **2-43a** which was the same as the one previously obtained in solution. SO_2Cl_2 mediated chlorination/halohydrin formation followed by MOM deprotection afforded pochonin C. The combination of polymer supported reagents and solid phase reactions facilitated the chemistry developed for pochonin C and radicicol as only 2 traditional chromatographic purifications were required over the 7 steps. However, it was also clear that it would be challenging to apply this chemistry to the synthesis of large libraries.

III. Cyclopropane Analogs Syntheses

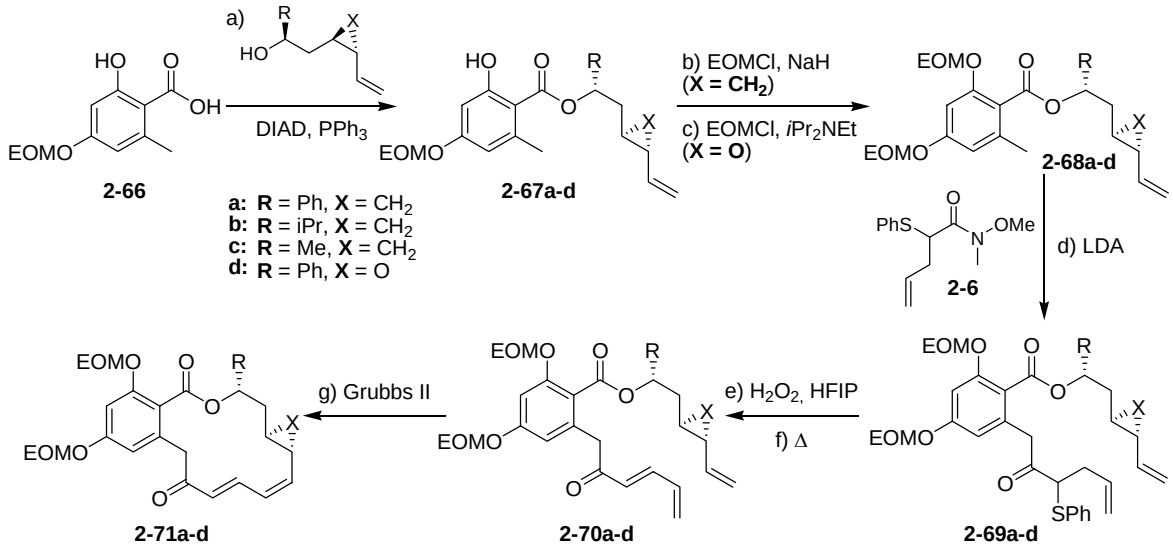
Considering the liability of the epoxide moiety, the synthesis of fragments where the epoxide is replaced by a cyclopropyl moiety was undertaken to provide the building blocks to be ultimately incorporated in the synthesis of a radicicol library. In fact, in the context of radicicol/HSP90, this substitution had already proven effective.[95] Thus, in parallel to the development of solid phase chemistry, various analogs of (*S*)-**2-27** bearing a phenyl or an isopropyl group at C1 were synthesized leading to alcohols (*S*)-**2-60a** and (*S*)-**2-60b** (for detailed preparation, see Part II Chapter VII). Starting from these alcohols, further TBDPS protection, ozonolysis, Wadsworth-Horner-Emmons homologation and reduction allowed the formation of allylic alcohol (*S*)-**2-61a-b**, which were the common building block for both cyclopropyl and epoxide derivatives. At this stage, we were not aware of the efficiency of cross metathesis with 1,4-butanediol using Grubbs Hoveyda's catalyst. In a first sequence, alcohols (*S*)-**2-61a-b** were used in a cyclopropanation reaction following the methodology developed by Charette and co-workers[146] to yield alcohols (*S*)-**2-62a-b**.



Scheme 25: Synthesis of cyclopropyl and epoxide analogs of alcohol **2-5a**

a) *t*BuPh$_2$SiCl (1.2 equiv.), imid. (1.7 equiv.), CH_2Cl_2, 23 °C, 8 h, 60-98 %; b) O_3, CH_2Cl_2, -78 °C, 2-3 h; Ph_3P (2.0 equiv.), 23 °C, 2-4 h, 67-98 %; c) LiCl (1.2 equiv.), *i*Pr$_2$NEt (1.0 equiv.), $(EtO)_2P(O)CH_2CO_2Et$ (1.2 equiv.), 23 °C, 12 h, 81-86 %; d) DIBAL (2.1 equiv.), CH_2Cl_2, -50 °C, 2 h, then, 0 °C, 1 h and then, 23 °C, 2 h, 94-98 %; e) (+)-DET, Ti(O*i*Pr)$_4$, TBHP, Et_2O, -30 °C, 12 h, 76 %; f) SO_3·Py (1.5 equiv.), Et_3N (1.6 equiv.), DMSO, 1 h, 50-90 %; g) Ph_3PCH_3Br, NaHMDS, 0 °C, 62-99 % h) TBAF (1.2 equiv.), THF, 23 °C, 6 h, 83-98 %; i) (*R*,*R*)-dioxaborolane (1.2 equiv.), Et_2Zn, (2.2 equiv), CH_2I_2 (4.0 equiv.), -10 → 25 °C, 1 h, 69-90 %.

Further oxidation, Wittig olefination and TBDPS deprotection afforded cyclopropyl alcohols (*S*)-**2-63a-b** (Scheme 25, syntheses done by Dr. LOPEZ-DEBER). Interestingly, following the same procedure, alcohol (*S*)-**2-63c** (R = Me) was synthesized from alcohol (*S*)-**2-27**.In a similar sequence, allylic alcohol (*S*)-**2-61a** was engaged in a Sharpless epoxidation reaction to afford alcohol (*S*)-**2-64**, which was further converted in alcohol (*S*)-**2-65** after oxidation, Wittig olefination and silyl group deprotection. The suitability of these alcohols was tested using the chemistry developed for radicicol and pochonin C. Using standard Mitsunobu conditions (DIAD, PPh_3), mono-EOM[188] protected acid **2-66** (obtained in three steps from orcinol, *vide infra*) was esterified with alcohols (*S*)-**2-63a-c**, (*S*)-**2-65** to yield intermediates **2-67a-d** which were further protected with a second EOM following commonly used procedures (Scheme 26).



Scheme 26: Synthesis of cyclic precursors **2-71a-d** of radicicol analogs

a) **2-63** or **2-65** (1.0 equiv.), PPh_3 (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 3 h, 60-80 %; b) EOMCl (2.0 equiv.), NaH (2.0 equiv.), THF, 0°C, 2 h, 50-70 % c) EOMCl (2.0 equiv.), Et*i*Pr_2N (2.0 equiv.), TBAI (cat.), DMF, 80 °C, 3 h, 50-70 %; d) LDA (2.0 equiv.), THF, -78 °C; **2-6** (1.0 equiv.), 50-70 % (based on recovered starting material); e) H_2O_2 (2.0 equiv.), HFIP, 23 °C, 3 h; f) toluene, 80 °C, 1.5 h, 25-35 % over two steps; d) Grubbs' II (5 % mol), toluene (2 mM), reflux, 5 min for **X** = O and 80°C, 12 h for **X** = CH_2, ~20 %.

It is important to note that no yield optimization has been done for the synthesis of these compounds and that only preliminary results are reported in this manuscript. Further acylation reactions with Weinreb amide **6** allowed synthesis of acyclic intermediate **2-69a-d** along with some unreacted starting material. Further oxidation/elimination steps proved more

[188] EOMCl was used instead of MOMCl for availability reasons.

challenging than expected. In fact, during the oxidation, although no epoxide opening was observed, deprotection of the *ortho*-EOM group occurred particularly for cyclopropyl analogs and all attempts to circumvent this problem failed. Isolation of the metathesis precursors **2-70a-d** and independently of their mono-EOM analogs was however possible albeit in poor yield. Finally, although only the *cis*-olefin and the 14-membered macrocycle were observed in the metathesis reaction for radicicol and pochonin C, this sequence proved more difficult for their analogs. Some tunings of the reaction conditions were necessary to isolate intermediates **2-71a-d**. In fact, although Danishefsky reported isolation of dimers with cycloproparadicicol,[148] we observed formation of the 12-membered ring when having the cyclopropyl chain. Optimisation trials (reaction at 40°C, at 80°C) did not avoid formation of this small macrocycle. However, the use of thermodynamic conditions for this metathesis reaction (overnight at 80°C) allowed mostly the isolation of desired compounds **2-71a-d** along with some small macrocycle (except for the *i*Pr analog that led mostly to the small macrocycle). Attempts to invert the sequence (metathesis and then, oxidation/elimination) for **2-69b** led to the isolation of only one diastereoisomer **2-71b** as previously reported for radicicol with low yield albeit without any 12-membered ring analog. Furthermore, attempts to cyclize mono-EOM **2-70c** mostly led to the isolation of the small macrocycle. Having the five intermediates **2-71a-d** in hand, further EOM deprotection followed by chlorination following the known procedure[150] should afford cyclopropyl and epoxide analogs of radicicol. At this stage of our work, interesting results from a collaboration with Pr. KARPLUS led us to shift our efforts towards other pochonins (*vide infra*).

IV. Conformational analysis

From the aforementioned synthesis, substituents on the macrocycle, particularly the configuration of the epoxide moiety, seems to play an important role in the conformation of the molecule. Indeed, an important difference in selectivities in the ring-closing metathesis reactions between compounds **2-44a** and **2-41a** has been observed (only *cis*-olefin vs 1:1 mixture of olefins respectively) and the selectivity obtained by changing the stereochemistry of the epoxide (**2-44a** and **2-44b**) clearly reflects the rigidity of the macrocycle. In order to try to understand how this rigidity may affect biological activities, we considered the conformations of pochonin C and radicicol. NMR analysis of radicicol's conformation in a CD_3OD solution showed a very well defined conformation with strong NOE correlations between the proton of the methyl group (C1) and the proton of C8 and C11 similar to its bound conformation into the active site of HSP90 (Figure 30).[76b]

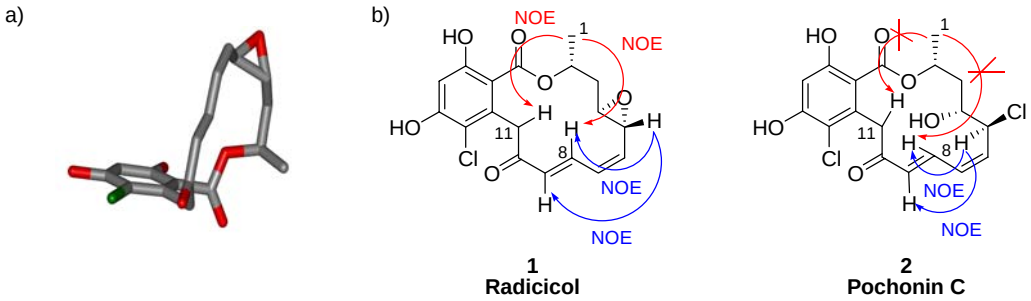


Figure 30: Conformational aspects of radicicol and pochonin C. a) Conformation of radicicol bound to HSP90 (PDB structure ID 1BGQ); b) NOE correlations observed for radicicol and pochonin C in CD_3OD.

In comparison, a very different pattern of NOE was observed for pochonin C; the NOE signal between C1 and C8/C11 was absent suggesting a more planar and less pre-organized conformation. The difference in biological activity reported by Hellwig and co-workers[136] for pochonin C and radicicol may be in part rationalized by the fact that despite the structural similarity of these compounds, they have very different conformations. Furthermore, biological assays for HSP90 inhibition in competition with geldanamycin support this idea (IC_{50} of 20nM for radicicol *vs* 1200nM for pochonin C).[189]

Suspecting that the difference in conformation may be at the origin of the different biological activities, a collaboration with Pr. KARPLUS was initiated to investigate the free energy of

[189] Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N. Design, Synthesis and Biological Evaluation of Pochonin D Analogs, Poster presentation, 6th Tetrahedron Symposium, Bordeaux, June 2005.

the different molecules. All the work presented below, and related to molecular minimization, has been done by Dr. ZOETE and Pr. KARPLUS. Each molecule was simulated by molecular dynamics with the Merck Molecular Force Field (MMFF94)[190] in the CHARMM[191] program. The simulations were carried out at 1000 K during 1 ns and 500 frames were extracted from the trajectory at 20 ps intervals. The high temperature was used to insure that conformational energy barriers were crossed. Each frame was minimized by 750 steps of steepest descent (SD) algorithm in CHARM and the MMFF energy was calculated. The resulting 500 conformations were clustered to determine the main conformations. Starting from the lowest energy conformation as representative of the first cluster, all conformations having a root mean square deviation (RMSD) lower than 1 Å were grouped into that cluster. The lowest energy conformer of the remaining conformations was taken as the starting point for the second cluster and the process was repeated until all compounds had been clustered. The RMSD between the lowest energy representative of each cluster and the bioactive conformation of radicicol in the co-crystal structure was then calculated for all heavy atoms. Based on radicicol structure, this analysis led to the identification of three main conformations: an L-shape conformation, which corresponds to the conformation of radicicol in solution as well as in HSP90,[76b] an essentially planar (P-shape) conformation and an L'-shape conformation that mainly differs from the L-shape one by the fact that the macrocycle is positioned on the opposite side of the aromatic cycle (Figure 31).

[190] (a) Halgren, T. A. & Nachbar, R. B. Merck molecular force field. IV. conformational energies and geometries for MMFF94. *J. Comput. Chem.* **17**, 587-615 (1996), (b) Halgren, T. A. Merck molecular force field. V. Extension of MMFF94 using experimental data, additional computational data, and empirical rules. *J. Comput. Chem.* **17**, 616-641 (1996), (c) Halgren, T. A. Merck molecular force field. III. Molecular geometries and vibrational frequencies for MMFF94. *J. Comput. Chem.* **17**, 553-586 (1996), (d) Halgren, T. A. Merck molecular force field. II. MMFF94 van der Waals and electrostatic parameters for intermolecular interactions. *J. Comput. Chem.* **17**, 520-552 (1996), (e) Halgren, T. A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **17**, 490-519 (1996).

[191] Brooks, B. R. et al. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **4**, 187-217 (1983).

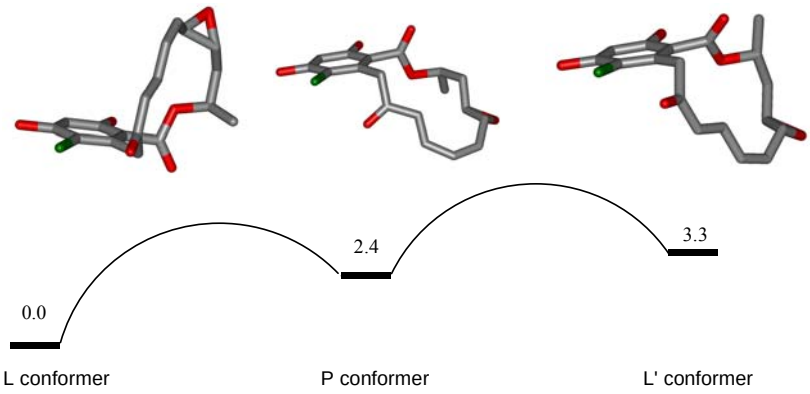


Figure 31: Wire-frame representation of radicicol's three main conformers and their relative energies (kcal/mol)

The calculated L-shape, P-shape and L'-shape conformations of the isolated molecules have an average RMSD from the bioactive radicicol conformation of about 0.7, 1.8 and 2.1 Å, respectively (the bioactive radicicol conformation was taken from the PDB structure ID 1BGQ).[76b] Importantly, for radicicol, the favored conformation isolated from the molecular dynamics analysis is the L-shape that also corresponds to its biologically active conformation. Its energy is 3.3 and 2.4 kcal/mol lower than that of the L'-shape and P-shape conformers, respectively. This result suggested that if the minimized conformation of analogs corresponds to the L-shape, the molecule might be active. On the contrary, if molecular modeling assigns one of the other conformers as the more stable one, a lack of activity might be expected for this compound. We tried to rationalize this hypothesis by analyzing the conformation of known analogs of radicicol that are potent or inactive as HSP90 inhibitors (Figure 32).

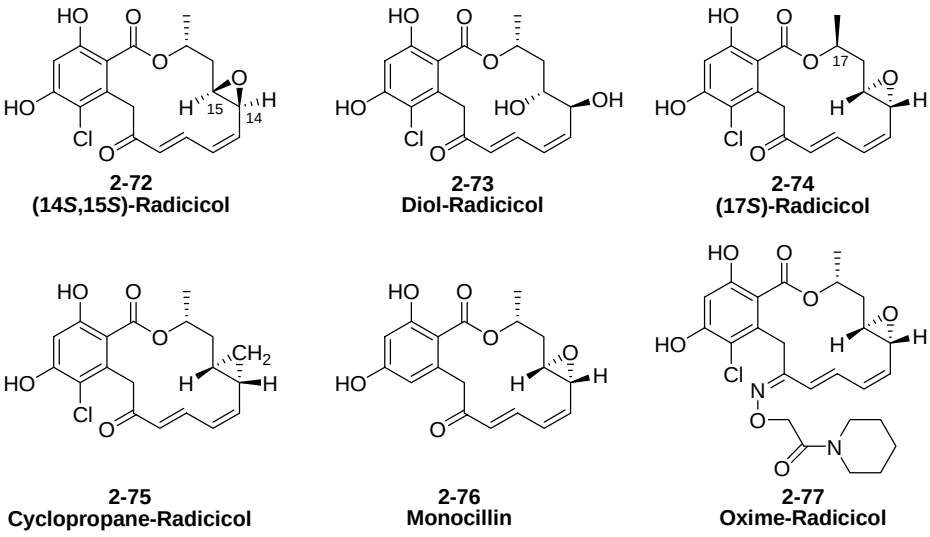


Figure 32: Selected analogs of radicicol for molecular minimization

A conformational analysis of compound **2-72**[95] in which the stereochemistry of the epoxide was inverted showed that, while the macrocycle could be clustered in the same three shapes as radicicol, the lowest energy conformation was no longer the L-shape but the L'-one (Table 2). Indeed, this molecule did not show any activity against HSP90.

Table 2: Relative energies (kcal/mol) of radicicol and its analogs main conformers

Compound	L-shape	P-shape	L'-shape	RMSD[a]	Simil.[b]
Radicicol (**2-1**)	0.0	2.4	3.3	0.60	0.024
(14*S*,15*S*)-radicicol (**2-72**)	2.7	1.7	0.0	0.60	0.029
Diol-radicicol (**2-73**)	2.7	1.6	0.0	0.61	0.025
Monocillin (**2-76**)	0.0	2.1	0.7	0.56	0.023
(17*S*)-radicicol (**2-74**)	0.0	2.3	3.0	0.62	0.027
Cyclopropane (**2-75**)	0.0	1.6	1.7	0.56	0.023
Oxime (**2-77**)	0.0	4.2	2.1	0.54	0.025
E-isomer of oxime **2-77**	0.3	3.6	0.0	0.62	0.026

[a]RMSD between the L-shape conformation and the bio-active conformation of radicicol, calculated for common heavy atoms. [b]Similarity index defined as the RMSD divided by the number of atoms included in the RMSD calculation; the lower is the similarity index, the greater is the similarity.

Likewise, opening of the epoxide to the corresponding inactive diol (**2-73**,[192] Figure 31) favored the L' conformation supporting the idea that the activity of a given molecule might be related to its conformation. Compound **2-75**[95] wherein the epoxide is replaced by a cyclopropane showed a comparable activity to radicicol. Indeed, its conformational profile was closely related to the one of radicicol with the L-shape being the lowest energetic conformer. Similarly, compound **2-77**,[94] that was reported as one of the most powerful analog (10 to 100-times more active than radicicol in selected cancer cell lines), showed a very similar profile to radicicol. The enhanced activity of compound **2-77** as compared to radicicol can be rationalized by the fact that the oxime substituent fills up a hydrophobic pocket (Figure 33). Interestingly, the *E*-isomer of oxime **2-77** had a less favorable conformational bias for the L-shape than the *Z*-isomer. This observation is consistent with experimental data from related oximes wherein the *Z*-isomer had a cytotoxicity of 98 nM *vs* 282 nM for the *E*-isomer.[97]

[192] (a) Shibata, T., Oikawa, T., Kobayashi, T. & Shimazaki, N. in Jpn. Kokai Tokkyo Koho 5 pp. ((Sankyo Co., Ltd., Japan). Jp, 1997), (b) Ikeda, A., Shinonaga, H., Fujimoto, N. & Kasai, Y. in PCT Int. Appl. 126 pp. ((Taisho Pharmaceutical Co., Ltd., Japan). Wo, 2003).

These results strongly supports the fact that the right conformation bias is necessary for activity.

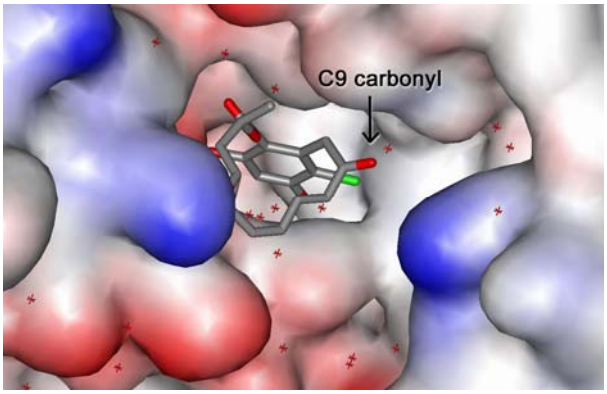


Figure 33: Representation of radicicol (wire-frame structure) bound to HSP90 (represented as a surface) Blue indicates positive regions, red indicates negative regions and + indicates water molecules [Image generated with Weblab from PDB data].

However, removal of the chlorine atom (monocillin, **2-76**)[193] still favored the L-shape but lowered the energetic bias for this conformation; and, inverting the stereochemistry at the C17 position (**2-74**,[95] Figure 32) did not have a significant impact on the conformation. In these cases, the lower activity of monocillin (**2-76**) might be explained by the loss of the chlorine atom that was filling up a hydrophobic pocket whereas the inactivity of (17*S*)-radicicol remains unclear. These investigations showed that the epoxide function (or a closely related one) influences the relative stability of the main conformation of radicicol derivatives; in particular, the stereochemistry of this functionality seems to be essential to achieve efficiency. Considering that molecular modeling may give a first insight in the activity of radicicol analogs, we analyzed the conformation profile of a series of compounds with modifications at the epoxide and/or the olefin regions (Figure 34).

[193] (a) Ayer, W. A. & Pena-Rodriguez, L. Minor metabolites of Monocillium nordinii. *Phytochemistry* **26**, 1353-5 (1987), (b) Danishefsky, S. J. et al. in PCT Int. Appl. 135 pp. ((Sloan-Kettering Institute for Cancer Research, USA). Wo, 2002).

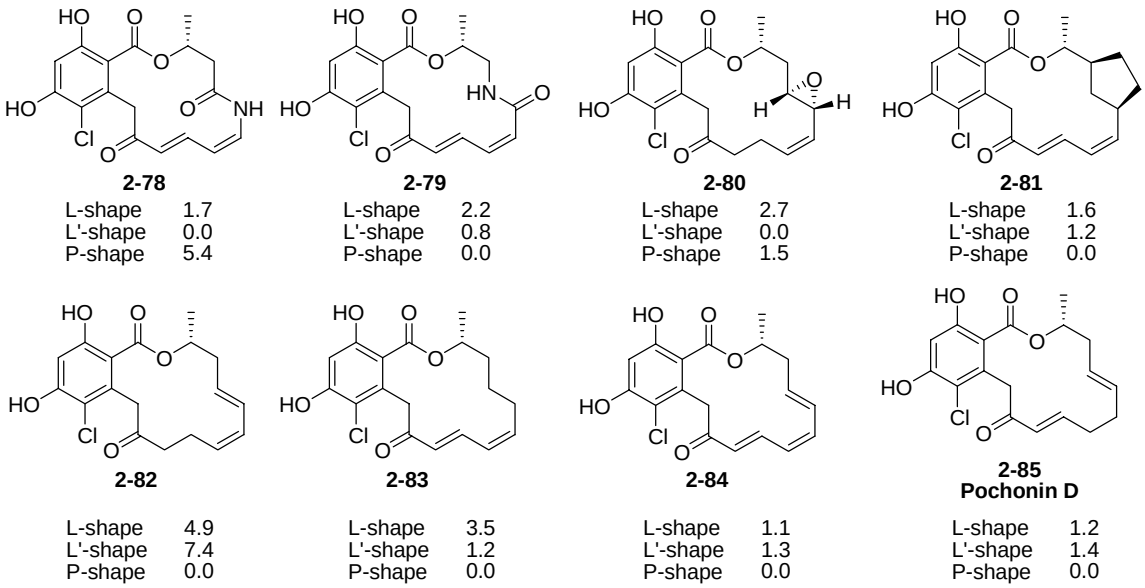


Figure 34: Structure and conformational analysis of designed radicicol analogs

Replacement of the epoxide by an amide bond (**2-78** and **2-79**) or a bridging cyclopentane (**2-81**) led to conformations that disfavored the L-shape. Removal of the epoxide (**2-83**) led to a compound that very strongly disfavored the L-shape. However, replacement of the epoxide by an olefin (**2-84** and **2-85**) only moderately disfavored the L-shape conformation. Interestingly, compound **2-80** and **2-82** lacking the α,β-conjugated olefin had a very unfavorable bias for the L-shape. As compound **2-85** represented a significant simplification in terms of chemical synthesis relatively to radicicol while only moderately disfavoring the active L-shape conformation (1.2 kcal), it particularly caught our attention. Furthermore, compound **2-85** was recently isolated from the fermentation broth of *Pochonia chlamydosporia* var. *catenulata* and named pochonin D.[136] In the isolation report, the authors found pochonin D to be cytotoxic at μM concentration which could be rationalized by its inhibition of HSP90. Moreover, a representation of pochonin D docked in HSP90 showed a good overlap with radicicol (Figure 35).

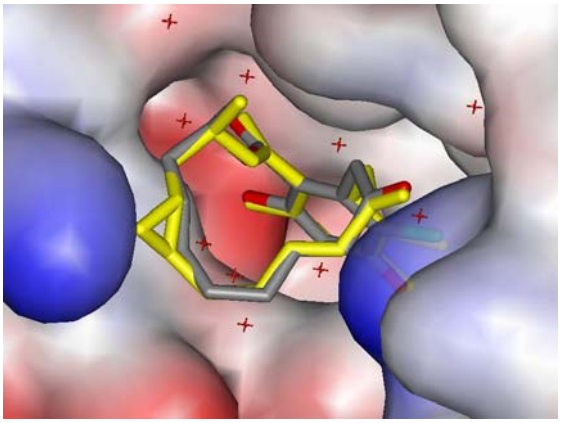


Figure 35: Representation of pochonin D (wire-frame structure) docked in HSP90 superimposed over the structure of radicicol (yellow wire-frame structure), HSP90 is represented as a surface.
[Image generated with Weblab from PDB data]

Based on molecular modeling and on the relative "simplicity" of the molecule in comparison to radicicol, we turned our attention to the development of a practical and modular synthesis of pochonin D. As for radicicol, our goal was to develop a chemistry that was sufficiently flexible to be used in libraries extending the diversity beyond the pochonin D/HSP90 context.

Chapter V – Total Synthesis of Pochonin D

A. Preliminary Work

Based on our previous experience, we envisioned similar disconnections for pochonin D (**2-85**) as for radicicol, namely a Mitsunobu esterification, an acylation and a ring-closing metathesis as the main disconnections using three building blocks: acid **2-87**, alcohol (*S*)-**2-27** and Weinreb amide **2-88** (Figure 36).

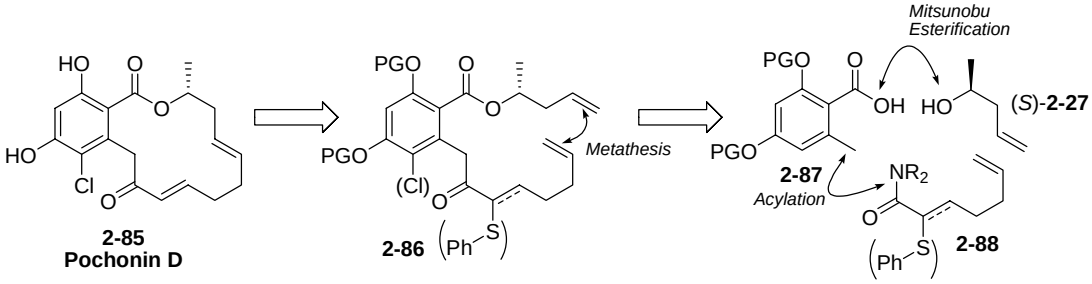
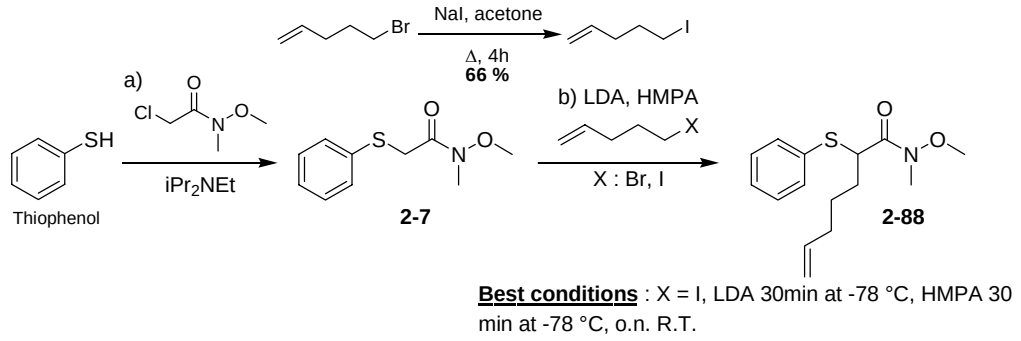


Figure 36: Retrosynthetic analysis for pochonin D based on radicicol and pochonin C syntheses

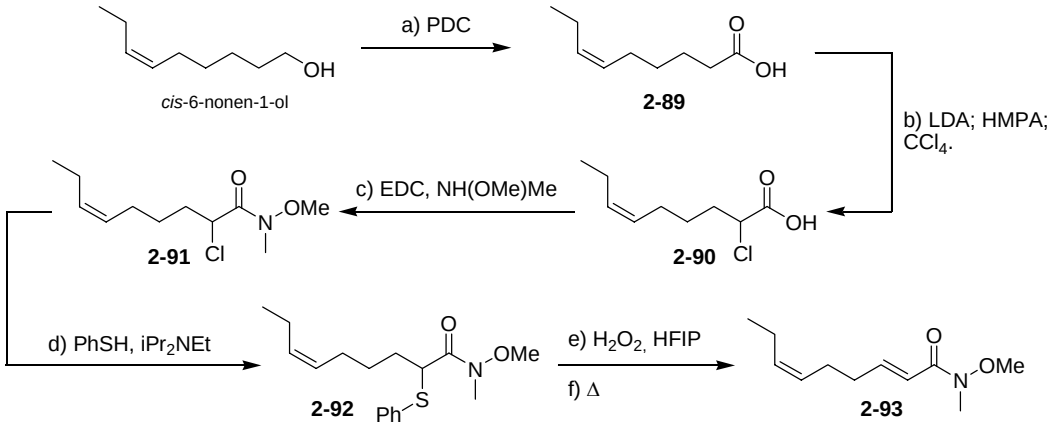
As depicted on Scheme 27, the Weinreb amide moiety **2-88** was synthesized following the same procedure as described for compound **2-6** (*vide supra*). Thus, alkylation of intermediate **2-7** with 5-iodo-1-pentene yielded Weinreb amide **2-88** in two steps from thiophenol.



Scheme 27: First generation of Weinreb amide synthesis

a) thiophenol (1.0 equiv.), K_2CO_3 (1.0 equiv.), DMF, 23 °C; 2-chloro-*N*-methoxy-*N*-methylacetamide (1.0 equiv.), 23 °C, 4 h, 95 %; b) LDA (2.0 equiv.), HMPA (2.0 equiv.), 5-iodo-1-pentene (2.0 equiv.), -78 → 23 °C, 13 h, 30 %.

In parallel, an alternative synthetic pathway was developed, starting with commercially available *cis*-6-nonen-1-ol (Scheme 28).



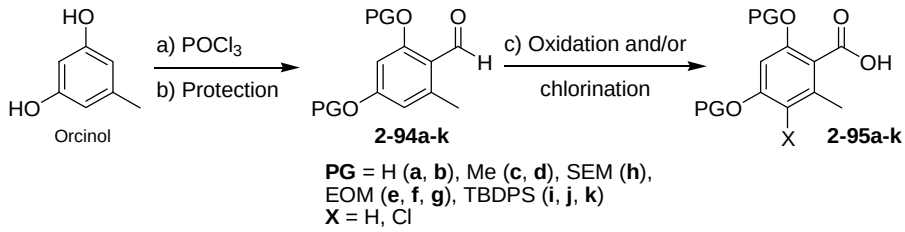
Scheme 28: Second generation of Weinreb amide synthesis

a) PDC (2.0 equiv.), DMF, 23 °C, 12 h, quant.; b) *i*Pr_2NH (2.6 equiv.), *n*BuLi (2.2 equiv.), HMPA, CCl_4 (5.0 equiv.), THF, -78 → 0 °C, 3 h, c) *N*,*O*-dimethylhydroxylamine hydrochloride (2.0 equiv.), DMAP (cat.), EDC (2.0 equiv.), CH_2Cl_2, 23 °C, 4 h, 88 % (2 steps); d) *i*Pr_2NEt (0.9 equiv.), thiophenol (0.9 equiv.), DMF, 80 °C, 12 h, 84 %; e) H_2O_2 (2.0 equiv.), HFIP, 23 °C, 3 h; f) toluene, 80 °C, 8 h, 75 % over 2 steps.

Following a classical oxidation procedure (PDC in DMF), acid **2-89** was then α-chlorinated by formation of the enolate using LDA and subsequent chlorine addition using carbon tetrachloride.[194] After work-up, compound **2-90** was obtained as a black oil although as a pure compound by ^{1}H NMR. Efforts to purify this acid proved disappointed and it was used directly in the following step. Further amide formation using *N*,*O*-dimethylhydroxylamine and EDC and displacement of the chlorine atom with thiophenol afforded compound **2-92** in 74 % overall yield from *cis*-6-nonen-1-ol. Via the oxidation/elimination reaction, the thioether Weinreb amide **2-92** could be converted in its closely related derivative **2-93**.

Due to the high cost of commercially available 2,4-dihydroxy-6-methylbenzoic acid, we developed a protocol that allows the synthesis of 2,4-dihydroxy-6-methylbenzaldehyde which can further be derivatized in the corresponding acid using various protecting groups (Scheme 29). Starting from orcinol and following a Vilsmeier-Haack procedure, aldehyde **2-94a** was obtained in 45 % yield (72 % based on recovered S.M.). As described in the experimental section, this aldehyde precipitates at pH = 7 and is recovered with good purity (> 95 % as judged by ^{1}H NMR).

[194] Snider, B. B. & Kulkarni, Y. S. Preparation of Unsaturated α-Chloro Acids and Intramolecular [2 + 2] Cycloadditions of the Chloroketenes Derived from Them. *J Org Chem* **52**, 307-310 (1987).



Scheme 29: Resorcylic acid synthesis from orcinol

a) $POCl_3$ (4.0 equiv.), DMF, 0 → 23 → 80 °C, 3 h, 45 %; b) *General procedure:* PGCl, *i*Pr_2NEt, CH_2Cl_2, 23 °C, 1 h, see experimental sections for each PG ; c) See the following table.

Compound **2-94a** was then protected with different groups to generate aldehydes **2-94c-k** which were subsequently oxidized to afford the corresponding acids **2-95a-k** in good yields (Scheme 29, Table 3). By varying the oxidation conditions, it was found possible to chlorinate the ring in a one pot sequence (oxidation/chlorination) in the required position for the synthesis of pochonin D.

Table 3: Oxidation conditions to yield various protected resorcylic acid

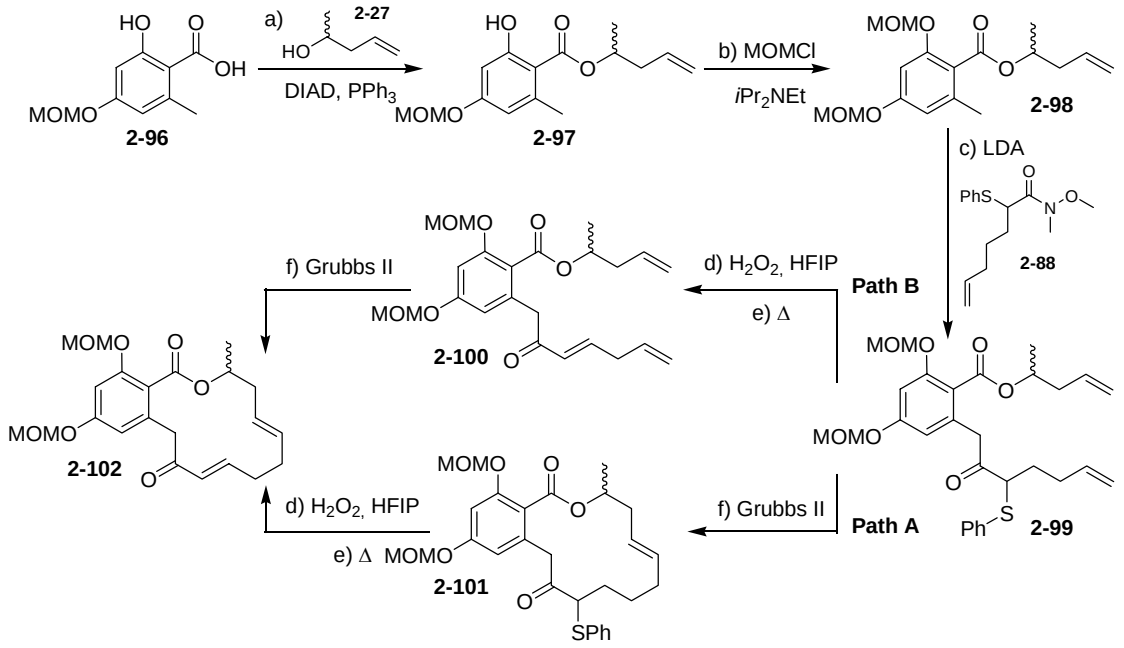
2-95	PG_1	PG_2	X	Acid	$NaClO_2$	Solvent sytem[a]	Time[b]	Yield [%]
a	H	H	H	NH_2SO_3H	3.25 equiv.	**A**	1 h	86
b	H	H	Cl	NH_2SO_3H[c]	2.0 equiv.	**B**	12 h	90
c	Me	Me	H	NH_2SO_3H	3.25 equiv.	**A**	1 h	82
d	Me	Me	Cl	NH_2SO_3H	3.25 equiv.	**B**	12 h	89
e	EOM	EOM	H	NaH_2PO_4	5.0 equiv.	**C**	12 h	68
f	EOM	EOM	Cl	NaH_2PO_4	5.0 equiv.	**B**	12 h	89
g[d]	EOM	H	H	NaH_2PO_4	5.0 equiv.	**C**	12 h	87
h	SEM	H	Cl	NH_2SO_3H	3.25 equiv.	**B**	12 h	70
i	TBDPS	H	Cl	NH_2SO_3H	3.25 equiv.	**B**	30 min	93
j	TBDPS	TBDPS	H	NH_2SO_3H	3.25 equiv.	**A**	30 min	92
k	TBDPS	TBDPS	Cl	NH_2SO_3H	3.25 equiv.	**B**	30 min	95

[a] **A**: H_2O/THF/DMSO (20:10:1) or **B**: H_2O/THF (2:1) or **C**: DMSO, [b] When run for more than 1 h, the reaction was heated up slowly to room temperature, [c] In this case, only 2 equiv. of acid were used to avoid over-chlorination, [d] This compound was previously assigned as compound **2-66**.

Depending on the protecting group, two different acidic buffers (NH_2SO_3H or NaH_2PO_4) and various solvent systems were used (Table 3). To avoid any chlorination reaction, a small percentage of DMSO as a mixture in THF/H_2O along with sulfamic acid[195] proved to be essential and very effective to quench HOCl (entries a, c, j).[165b] The one pot oxidation/chlorination sequence was done in absence of DMSO and required longer time to reach completion (entries b, d, f, h). Due to the acidic liability of the EOM protecting groups, NaH_2PO_4 was used as instead of sulfamic acid and the oxidation was carried out in pure DMSO. Importantly, trials to oxidize TBDPS protected aldehydes **2-94i-k** in the presence of NaH_2PO_4 failed and oxidation of the mono-EOM protected aldehyde **2-94g** led to uncompleted reaction. This forced us to work with the bis-protected compound which could be selectively deprotected to afford the chlorinated analog of compound **2-95g** in 77 % yield over the two steps (*vide infra*). Due to restrictive access to the commercially available starting material **2-95a** used for pochonin C synthesis, alternative strategies were thought. Therefore, access to these diverse acids **2-95a-k** was extremely important to develop the synthesis of pochonin D. In the first sequence used for the synthesis of this pochonin, we started with mono-MOM[196] protected acid **2-96**. Mitsunobu esterification under standard conditions (DIAD, PPh_3) between this acid **2-96** and racemic alcohol **2-27** afforded the desired ester **2-97** which was further converted to the bis-protected ester **2-98** (Scheme 30).

[195] (a) Lindgren, B. O. & Nilsson, T. Preparation of carboxylic acids from aldehydes (including hydroxylated benzaldehydes) by oxidation with chlorite. *Acta Chem. Scand.* **27**, 888-890 (1973), (b) Colombo, L., Gennari, C., Santandrea, M., Narisano, E. & Scolastico, C. Acetogenin Synthesis. Organocopper reagents, anions of 1,3-dithians and of protected cyanohydrins as intermediates in ketide side-chain synthesis. *J. Chem. Soc., Perkin Trans. 1*, 136-140 (1980).

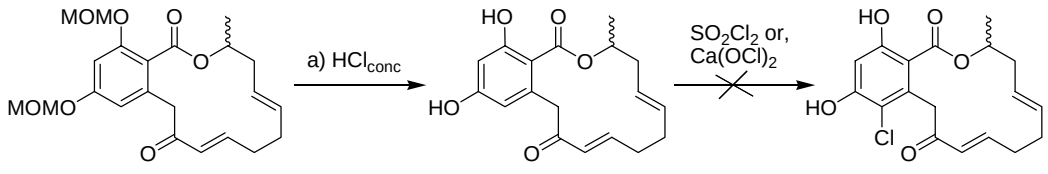
[196] At the beginning of this project, there was no problem of availability for MOMCl. The method used to access acid **2-94** is the same as the one reported with EOM protecting group.



Scheme 30: Synthesis of MOM protected Monocillin II (**2-102**)

a) **2-27** (1.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 3 h, 59 %; b) MOMCl (4.0 equiv.), Et*i*Pr$_2$N (4.0 equiv.), TBAI (cat.), DMF, 80 °C, 3 h, 78 %; c) LDA (2.0 equiv.), THF, -78 °C; **2-88** (1.0 equiv.), 10 min, 50 %; d) H_2O_2 (2.0 equiv.), HFIP, 23 °C, 3 h; e) toluene, 80 °C, 4 h, 68 % two steps (Path **A**), 48 % two steps (Path **B**); f) Grubbs' II (5 % mol), toluene (2 mM), reflux, 15 min, 63 % (path **A**, *trans/cis* 4:1), quant. (path **B**, *trans/cis* 7:1).

Acylation using previously optimised conditions (2 equiv. of LDA at -78°C) allowed the formation of the acyclic precursor **2-99** along with some unreacted starting material. At this stage, based on our previous results on pochonin C, we envisioned two different sequences (oxidation/elimination followed by ring-closing metathesis (path **A**) or *vice-versa* (path **B**)) to yield compound **2-102**. For radicicol, path **A** led to low selectivity in the metathesis (*cis/trans* ratio 1:1) reaction and low yield in the oxidation/elimination sequence. For pochonin D, both pathways proved similar (same overall yield) albeit with a higher selectivity in the metathesis reaction when following path **B** (*cis/trans* ratio 7:1 *vs.* 4:1 for path **A**). Having compound **2-102**, MOM deprotection and chlorination of the aromatic ring should have allowed the formation of pochonin D (**2-85**, Scheme 31).

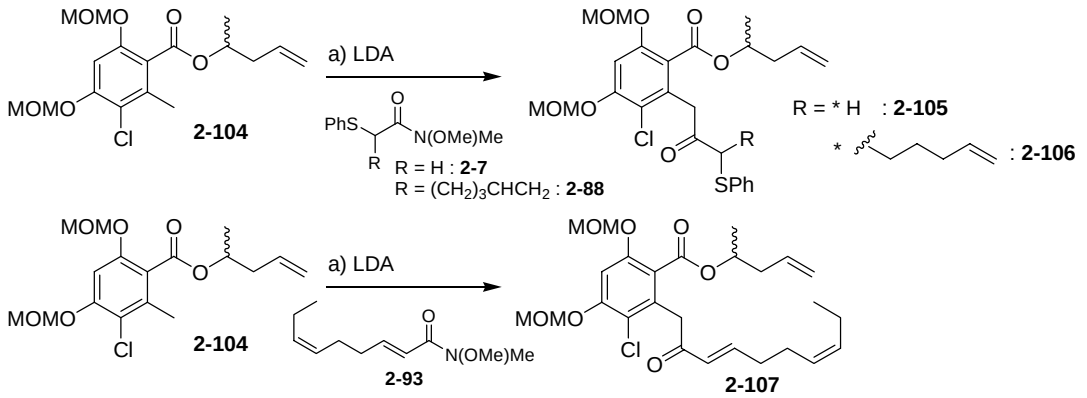


2-102 **2-103** ***rac*-Monocillin II** ***rac*-2-85**

Scheme 31: Synthesis of racemic Monocillin II (**2-103**)

a) HCl_{conc} (2.5 % in dioxane), 0 → 23 °C, 1 h, 79 %; b) SO_2Cl_2, Et_2O or CH_2Cl_2, 0 °C, or $Ca(OCl)_2$, Acetone, H_2O/AcOH, 0 °C.

Deprotection of the MOM groups following a well-known procedure allowed easily the generation of racemic monocillin II (**2-103**) but the chlorination proved much more challenging than expected. All attempts to chlorinate compound **2-103** with either SO_2Cl_2 or $Ca(OCl)_2$ failed, leading to several by-products and only traces of desired compound **2-85** as determined by L.C./M.S. Trials of chlorination on macrocycle **2-102** led to disappointment and on compound **2-101** led mostly to the exclusive oxidation of the thioether moiety. Attempts on compounds **2-98** and **2-97** led to the isolation of some chlorinated compound albeit in low yield and along with chlorination of the terminal olefin. At this stage, it was clear that the chlorine atom should be introduced prior to the olefins and the thiol moiety. From the oxidation methodology, we knew we could access to a large variety of chlorinated toluic acid in good yields (Table 3). Starting with the chlorinated analog of acid **2-96** and following the two steps sequence developed for MOM-protected Monocillin II (Scheme 30), compound **2-104** was obtained in good quantities (Scheme 32). Although acylation reaction using Weinreb amide **2-7** led to the isolation of compound **2-103** in 37 % yield, no reaction was observed when using Weinreb amide **2-88**. We thought that the failure of this last reaction might be due to the thioether linkage present on the Weinreb amide moiety and decided to use directly its α,β-conjugated analog **2-93**.



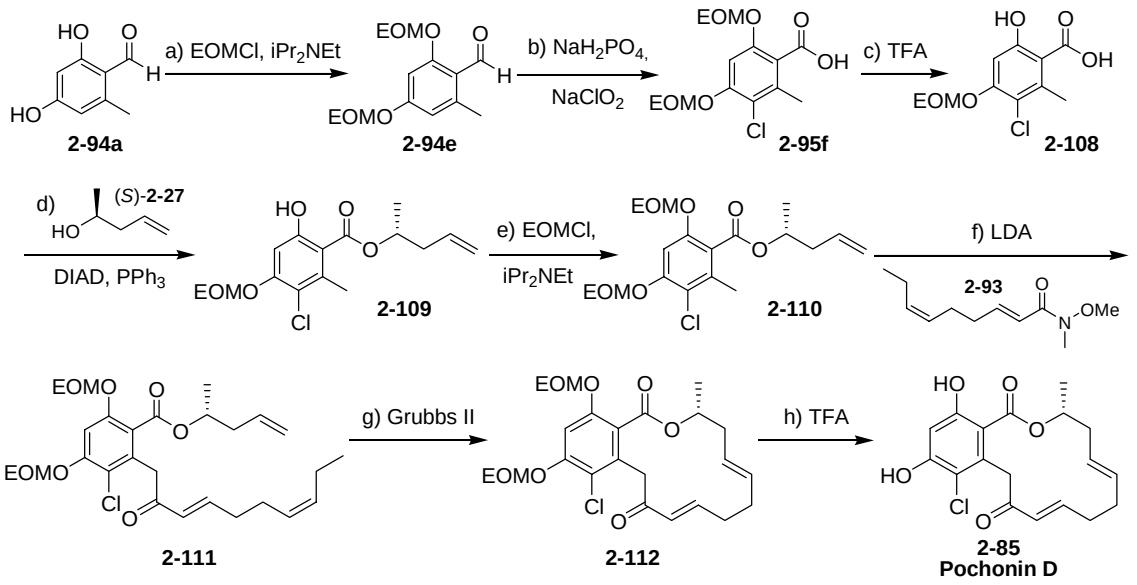
Scheme 32: Acylation trials with various Weinreb amide

a) LDA (2.0 equiv.), THF, -78 °C; **2-7** or **2-88** or **2-93** (1.0 equiv.), 10 min, 37 % (**2-105**), 40 % (**2-107**).

Indeed, acylation reaction between the toluic anion of ester **2-104** and Weinreb amide **2-93** led to the isolation of acyclic compound **2-107** in 40 % yield along with some unreacted starting material. Attempts to improve this yield were disappointed probably because of steric effects of the chlorine atom. However, the possibility of achieving the acylation reaction on the chlorinated toluic ester opened the door for the completion of pochonin D synthesis.

B.Total Synthesis

Following the same considerations as for the synthesis of pochonin C (enolate stability, acid liability), EOM protecting groups were chosen for phenolic protection. Using the chemistry described above to synthesize toluic acids, mono-EOM chlorinated acid **2-108** was synthesized in three steps from formylated orcinol **2-94a** (Scheme 33). The selective deprotection of the *ortho*-phenol was achieved using a specific concentration of TFA in a THF/MeOH mixture (THF/TFA/MeOH 7:1.5:1 (vol.)) without any bis-deprotection. Further Mitsunobu esterification using standard protocol (DIAD, PPh_3, toluene) and reprotection of the *ortho*-phenol allowed the formation of compound **2-110** in 51 % yield from acid **2-108**.



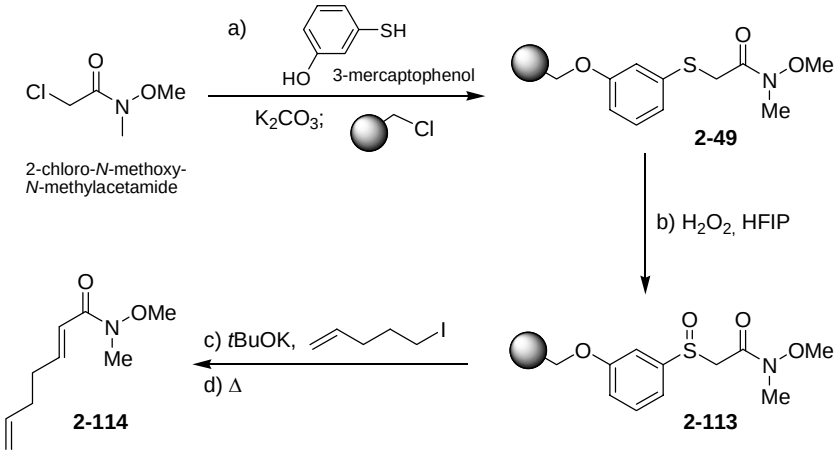
Scheme 33: First total synthesis of pochonin D

a) EOMCl (4.0 equiv.), *i*Pr_2NEt (4.0 equiv.), CH_2Cl_2, 23 °C, 1 h, 81 %; b) NaH_2PO_4 (5.0 equiv.), $NaClO_2$ (5.0 equiv.), H_2O/THF 2:1, 0 → 23°C, 12 h, 89 %; c) THF/TFA/MeOH 7:1.5:1, 23 °C, 45 min, 80 %; d) (*S*)-**2-27** (1.0 equiv.), PPh_3 (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 3 h, 72 %; e) EOMCl (2.0 equiv.), Et*i*Pr_2N (2.0 equiv.), TBAI (cat.), DMF, 80 °C, 3 h, 70 %; f) LDA (2.0 equiv.), THF, -78 °C; **2-93** (1.0 equiv.), 10 min, 52 %; g) Grubbs' II (10 % mol), toluene (2 mM), 80 °C, 12 h, 94 %; h) TFA (20 %), CH_2Cl_2, 23 °C, 2 h, 72 %.

Deprotonation of the toluic ester **2-110** followed by addition of Weinreb amide **2-93** afforded the desired metathesis precursor **2-111** in 52 % yield along with some unreacted starting material. Treatment of triene **2-111** with the Grubbs' second generation catalyst[173] at 120 °C for 15 min afforded the desired cyclization product **2-112** in an excellent yield albeit as an unseparable mixture of *cis*/*trans* olefins 1:4. Metathesis reaction under thermodynamic control at 80°C overnight[174] shifted the equilibrium to the *trans* intermediate **2-112** with > 95 % selectivity (as judged by ^{1}H NMR) and 94 % yield. It should be noted that this reaction could be performed at millimolar concentration without any detectable amount of dimerization or oligomerization. Importantly, the 10-membered ring macrocycle was not observed. Further EOM deprotection using TFA in dichloromethane allowed the first total synthesis of pochonin D which was found to have an identical NMR spectrum to the natural product.[197]

[197] A ^{1}H NMR spectrum of natural pochonin D was kindly provided by Dr. Marc Stadler, Bayer Health Care, Wuppertal, Germany.

For the purpose of diversity-oriented synthesis and as the presence of a thioether linkage was not possible having the chlorine atom on the aromatic ring, we proposed the development of a more concise synthesis of pochonin D with polymer-supported reagents.[198] The Mitsunobu reaction using directly 2,4-dihydroxy-6-methylbenzoic acid was envisaged leading to an even more concise pathway to pochonin D. As (*S*)-4-penten-2-ol ((*S*)-**2-27**) is commercially available, our first effort concerned the improvement of the sequence leading to the aliphatic Weinreb amide **2-93**. Indeed, although gram quantities could be obtained from the second generation synthesis (Scheme 28), several purifications were necessary. To circumvent this problem, a protocol using solid-support was developed. Commercially available 2-chloro-*N*-methoxy-*N*-methylacetamide (Scheme 34) was selectively S-alkylated with 3-mercaptophenol using one equivalent of base, and then loaded onto Merrifield resin in the same pot reaction by the successive addition of a second equivalent of K_2CO_3, the resin, and raising the temperature to 50 °C, as previously described in Chapter II (Part II).



Scheme 34: Solid-phase synthesis of Weinreb amide **2-114**

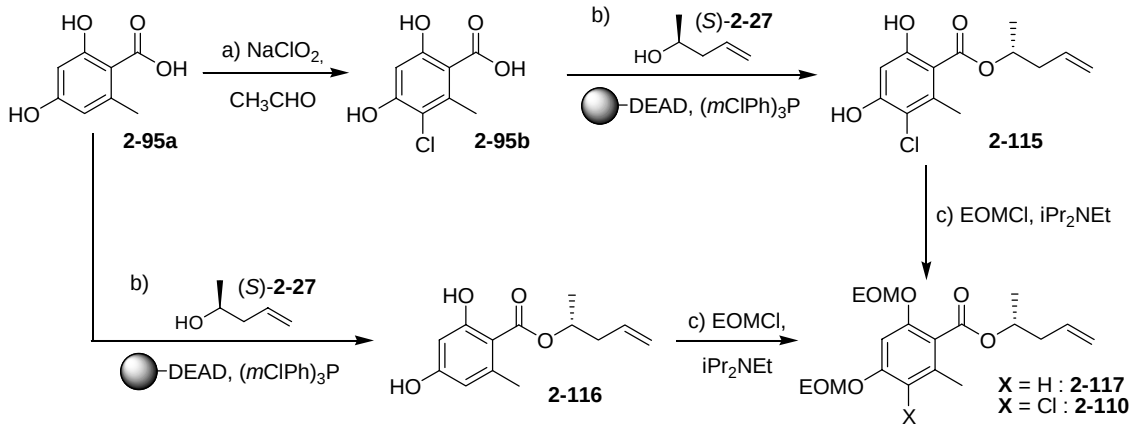
a) 3-mercaptophenol (1.0 equiv.), K_2CO_3 (1.0 equiv.), DMF, 23 °C; after 8 hours, K_2CO_3 (1.7 equiv.), Merrifield resin, TBAI (cat.), 12 h, 50 °C, 98 %; b) H_2O_2 (2.0 equiv.), HFIP/CH_2Cl_2 1:1, 12 h; c) *t*BuOK (1.0 equiv.), 5-iodo-1-pentene (1.0 equiv.), DMSO, 23 °C, 3 h; d) toluene, 80 °C, 8 h, 77 %.

[198] (a) Ley, S. V. & Baxendale, I. R. New tools and concepts for modern organic synthesis. *Nat Rev Drug Discov* **1**, 573-86 (2002), (b) Ley, S. V. et al. Multi-step organic synthesis using solid-supported reagents and scavengers: a new paradigm in chemical library generation. *J. Chem. Soc., Perkin Trans. 1* **23**, 3815-4195 (2000).

While preparations of thiol resins have been reported by direct lithiation of the resin followed by a quench with elemental sulfur,[199] this method was found more practical as it affords the polymer-bound Weinreb amide **2-49** in one step. Oxidation of the thioether **2-49** to the corresponding sulfoxide **2-113** was carried out using the aforementioned procedure involving H_2O_2 in HFIP/CH_2Cl_2.[163] This oxidation procedure was found practical and reliable with no over-oxidation to the sulfone and easy recycling of the fluorinated solvent as the reaction was carried out on solid phase. The amidosulfoxide **2-113** was then deprotonated with *t*BuOK, and the resulting enolate was quenched with 5-iodo-1-pentene. The use of DMSO was found crucial for the success of this reaction, DMF, *t*BuOH, and 1,4-dioxane giving only poor results. However, the polar nature of DMSO was unfavorable for the sulfoxide elimination, and the reaction could be heated up to 60 °C without any elimination. The use of solid-support was again valuable in cleaning the compound from DMSO. Resuspension of the resin in toluene and heating up to 80 °C released, after elimination, the desired fragment **2-114** with 77 % yield and 95 % purity (judged by ^{1}H NMR). This methodology on solid support was found very practical in terms of yield and purity of the building block **2-114** as no column chromatography was needed.

Based on the chemistry developed in solution (Scheme 33), a selective Mitsunobu esterification of 2,4-dihydroxy-6-methylbenzoic acid (**2-95a**) with (*S*)-4-penten-2-ol ((*S*)-**2-27**) using polymer-bound DEAD afforded ester **2-116** (Scheme 35). As previously mentioned, the use of (*m*ClPh)$_3$P was found essential to suppress any competing ether formation with the *para*-phenol.[182] Protection of both phenols with EOM groups afforded non-chlorinated ester **2-117**, which could be used in the subsequent alkylation without further purification.

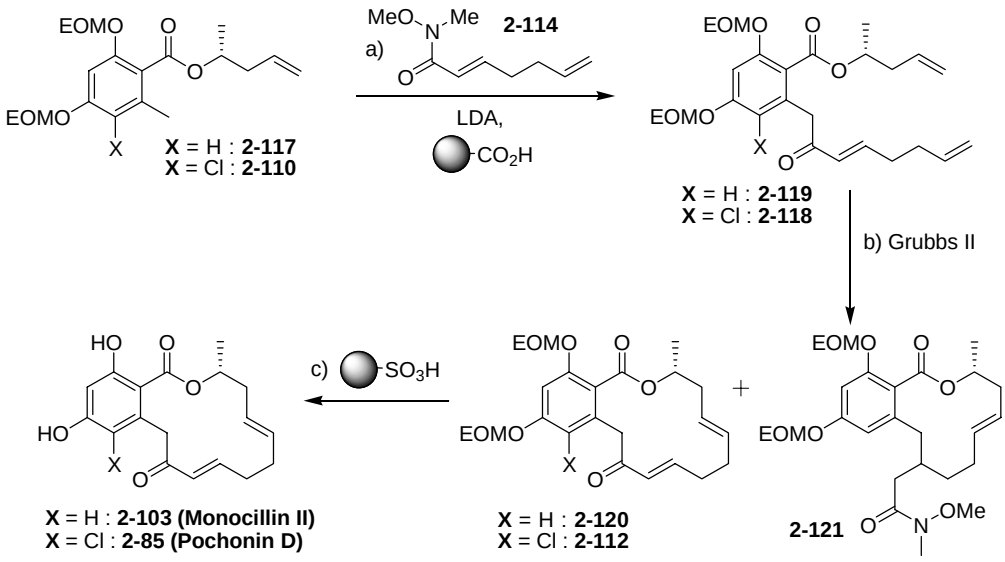
[199] Farrall, M. J. & Frechet, J. M. J. Bromination and lithiation: two important steps in the functionalization of polystyrene resins. *J Org Chem* **41**, 3877-82 (1976).



Scheme 35: Synthesis of intermediates **2-110** and **2-117** using polymer-bound reagents

a) $NaClO_2$ (5.0 equiv.), NH_2SO_3H (5.0 equiv.), CH_3CHO (1.0 equiv.), THF/H_2O 5:1, 0 °C, 0.5 h, 92 %; b) PS-DEAD (2.5 equiv., 1.3 mmol.g^{-1}), (*S*)-4-penten-2-ol (1.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), CH_2Cl_2, 23 °C, 0.5 h, 68 % for **2-116** and 65 % for **2-115**; c) iPr_2EtN (4.0 equiv.), EOMCl (4.0 equiv.), TBAI (cat.), DMF, 80 °C, 5 h, 95 %.

In parallel and as the chlorine atom could not be introduced at a later stage (*vide supra*), the halogen was thus introduced prior to esterification using HClO generated in situ by the oxidation of acetaldehyde with $NaClO_2$/sulfamic acid. Acid **2-95b** was obtained from its non-chlorinated parent **2-95a** in 92 % yield without seeing any over-chlorination. Esterification of this product under the same conditions as for **2-95a** afforded compound **2-115**. Further bis-protection with EOM groups led to ester **2-110** which could also be used in subsequent reactions without any purification. Deprotonation of the toluic esters **2-110** and **2-117** followed by addition of Weinreb amide **2-114** afforded the desired metathesis precursors **2-118** and **2-119** which could be used directly in the following step (Scheme 36). It is important to note that this acylation reaction, when performed on the non-chlorinated ester **2-117**, led to the formation of 20 % of undesired product stemming from a 1,4-conjugated addition on the Weinreb amide.



Scheme 36: Synthesis of pochonin D (**2-85**) and monocillin II (**2-103**) using supported reagents

a) LDA (2.0 equiv.), THF, -78 °C; **2-114** (1.0 equiv.), 10 min, Amberlite IRC-50 (20.0 eq, 10.0 mmol.g^{-1}); b) Grubbs' II (10 % mol), toluene (2 mM), 80 °C, 12 h, 40 % for **2-120** and 44 % for **2-112** after two steps; c) PS-TsOH (10 equiv., 3.2 mmol.g^{-1}), MeOH, 40 °C, 4 h, 90 % for **2-85** and 92 % for **2-103**.

Treatment of crude trienes **2-118** and **2-119** with the Grubbs' second generation catalyst under thermodynamic control at 80°C overnight led exclusively (more than 95 % selectivity) to the *trans* macrocycles **2-112** and **2-120**. A simple filtration through a path of silica was then used to remove all of the catalyst and its by-products, affording compound **2-112** in 44 % yield over two steps. While the metathesis reaction carried out on purified triene **2-118** was nearly quantitative, it was found more practical to carry out the whole synthetic sequence from compound **2-95a** without any purification, thus affording the protected pochonin D **2-112** in 25 % yield over five steps. For compound **2-120**, a column chromatography was necessary to remove the 12-membered macrocycle **2-121** coming from the side reaction (1,4-addition) occurring during the acylation reaction. Removal of EOM groups from both macrocycles **2-112** and **2-120** using sulfonic acid resin in MeOH allowed the synthesis of both pochonin D (**2-85**) and monocillin II (**2-103**) in 90 % and 92 % yield, respectively. As shown for the acylation reaction, the presence or absence of the chlorine atom on the aromatic ring seems to influence the reactivity of the conjugated olefin. Indeed, deprotection of compound **2-120** with HCl (2.5 % in dioxane) led to the conjugated addition of the chlorine ion, whereas compound **2-112** could be deprotected with HCl to obtain pochonin D (**2-85**). The difference in reactivity of the conjugated olefins may be attributed to the different conformations of the two compounds, which could be explained by a 1,3-allylic strain conferred by the bulky chlorine atom. It is conceivable that the differences in conformation affect the level of

conjugation between the olefin and the adjacent carbonyl group and thus its susceptibility to Michael addition. This synthesis using polymer-supported reagents allowed the achievement of pochonin D and monocillin II in six (23 % yield) and five (24 % yield) steps respectively. Starting from commercially available building blocks, only one chromatographic purification of the final compound is required for all the synthetic pathway and this methodology could be used for the synthesis of libraries. Evaluation for HSP90 affinity in a competition assay with geldanamycin revealed that pochonin D is a good ligand for HSP90 with an IC_{50} of 80nM as compared to 20nM for radicicol (*vide infra*).

Chapter VI – Total Synthesis of Pochonin A

Having access to pochonin D (**2-85**), it seemed necessary to make pochonin A (**2-122**) not only to confirm its structure but also to compare its biological activity to pochonin D and radicicol (**2-1**, Figure 37).

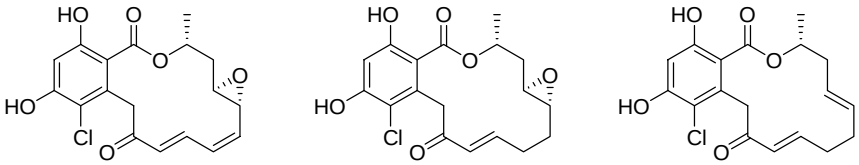
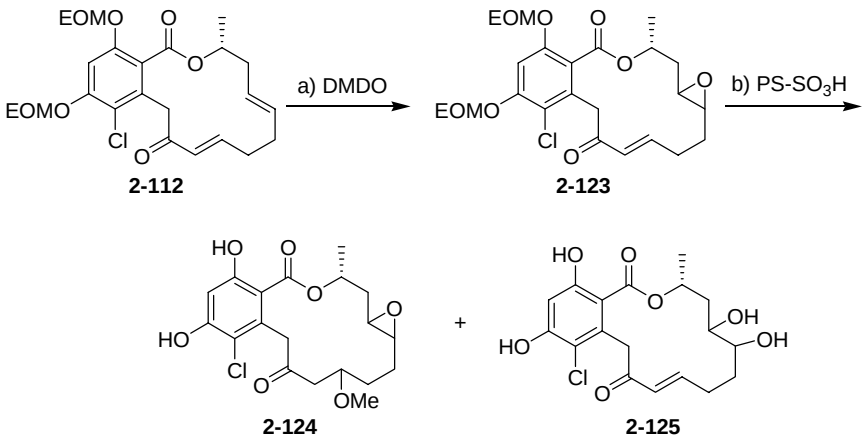


Figure 37: Stuctures of radicicol, pochonin A and pochonin D

Epoxidation of pochonin D using DMDO allowed the formation of pochonin A as a 1:1 mixture of diastereoisomers that could be separated by column chromatography. Attempts to improve the selectivity of this reaction using epoxone[200] as a chiral auxiliary failed completely with no conversion at 0 °C or at room temperature. The lack of solubility of pochonin D at low temperature did not allow us to improve this selectivity and encouraged us to investigate different synthetic routes. As depicted in Scheme 37, the epoxidation of the bis-EOM protected pochonin D (**2-112**) did not improve the selectivity and deprotection of the EOM in the presence of the epoxide did not give satisfactory results under a variety of conditions.

[200] (a) Tu, Y., Wang, Z.-X. & Shi, Y. An Efficient Asymmetric Epoxidation Method for trans-Olefins Mediated by a Fructose-Derived Ketone. *J Am Chem Soc* **118**, 9806-9807 (1996), (b) Wang, Z.-X., Tu, Y., Frohn, M. & Shi, Y. A Dramatic pH Effect Leads to a Catalytic Asymmetric Epoxidation. *J Org Chem* **62**, 2328-2329 (1997).

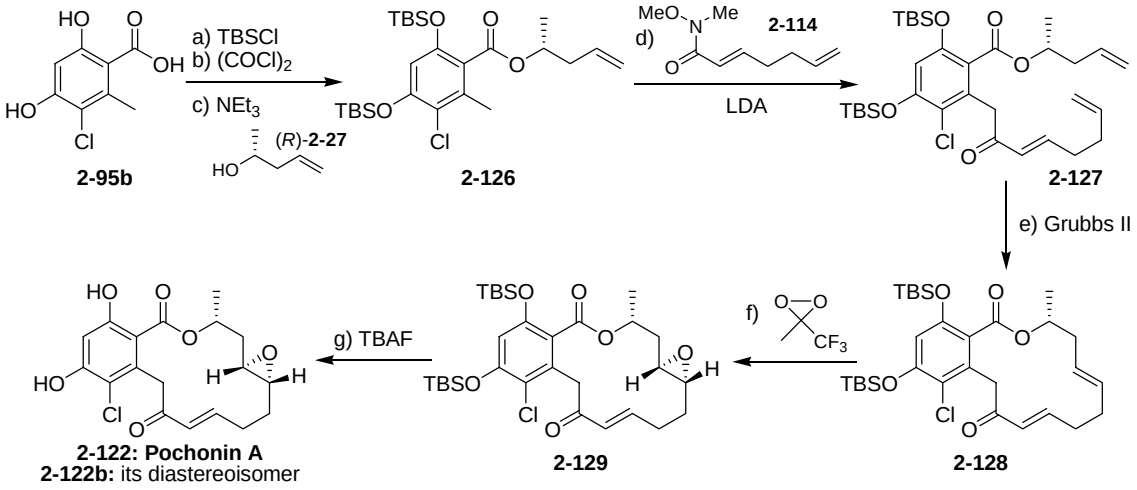


Scheme 37: Direct conversion of bis-EOM pochonin D (**2-112**)

a) DMDO (1.0 equiv.), CH_3CN, 0 °C → 23 °C, 1.5 h, 79 %; b) PS-TsOH (10.0 equiv., 3.2 $mmol.g^{-1}$), MeOH, 40 °C, 1 h.

We thus became interested in the suitability of silyl based protecting groups to access this type of molecules. This was also motivated by the fact that it was known that a silylated carboxylic acid could be converted to an acid chloride under acid-free conditions which are compatible with silyl protected phenols.[201] Thus, persilylation of benzoic acid **2-95b** (Scheme 38), followed by "acid-free" conversion of the silyl ester to the corresponding acyl chloride yielded key intermediate **2-126** upon esterification with alcohol (*R*)-**2-27**. The low yield reported for this three steps sequence was attributed to the liability of the *ortho*-TBS group. Attempts to improve the efficiency of this protocol either by purifying the persilylated intermediate (column on neutralized silica, filtration on neutral alumina, distillation or azeotrope to evaporate the excess of TBSCl) or by isolating the acid chloride only led to disappointing results.

[201] Wisnner, A. & Grudzinskas, C. V. Reaction of tert-butyldimethylsilyl esters with oxalyl chloride-dimethylformamide: preparation of carboxylic acid chlorides under neutral conditions. *J Org Chem* **43**, 3972-3974 (1978).



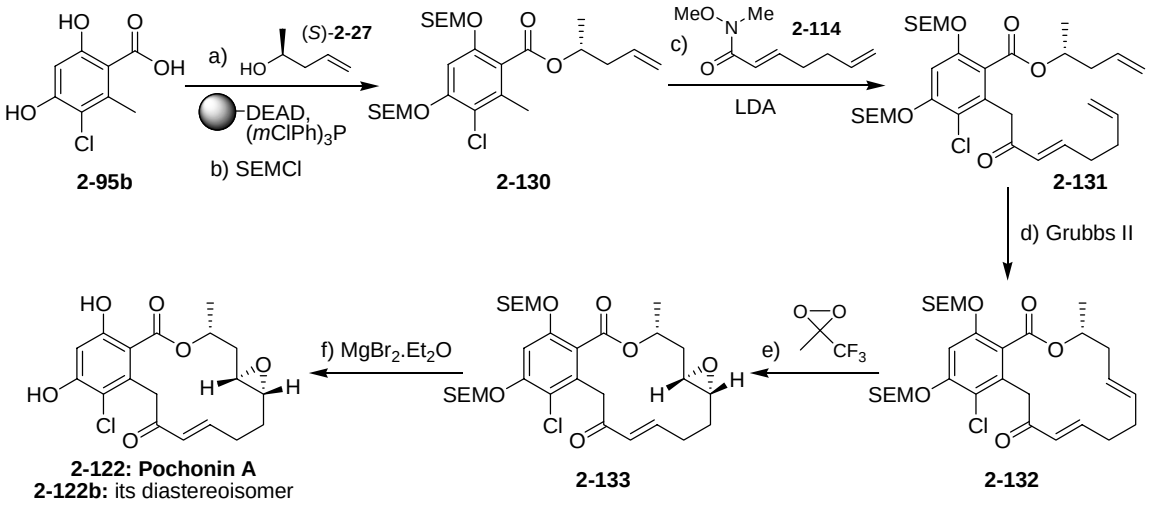
Scheme 38: Pochonin A synthesis using TBS-protecting groups

a) iPr_2EtN (6.0 equiv.), TBSCl (3.0 equiv.), CH_2Cl_2, 23 °C, 3 h; b) Oxalyl chloride (1.0 equiv.), DMF (cat.), CH_2Cl_2, 0 → 23 °C, 1 h; c) Et_3N (2.26 equiv.), *R*-(-)-penten-2-ol (3.0 equiv.), DMAP, 0 → 23 °C, 12 h, 29 % over 3 steps; d) LDA (2.0 equiv.), THF, -78 °C, Weinreib amide **2-114** (1.0 equiv.), 10 min, 35 %; e) Grubbs' II (10 % mol), toluene (2 mM), 80 °C, 12 h, 79 %; f) CF_3COCH_3, $NaHCO_3$ (7.0 equiv.), Oxone (4.7 equiv.), Na_2•EDTA (4 x 10^{-4}M), CH_3CN/Dimethoxymethane, 0 °C, 2 h, 83 %; g) TBAF (2.2 equiv.), THF, 23 °C, 20 min, 80 %, 3:1 mixture of diastereoisomers.

Deprotonation of the toluic ester **2-126** followed by reaction with Weinreb amide **2-114** afforded metathesis precursor **2-127** in modest yield. Ring-closing metathesis using Grubbs' second generation catalyst[173] under aforementioned thermodynamic conditions[174] (80 °C, overnight) afforded macrocycle **2-128** in good yield and excellent *cis*/*trans* ratio (< 5 % *cis*). Epoxidation of the non-conjugated olefin was optimal when carried out at 0 °C with methyl(trifluoromethyl)-dioxirane generated in situ,[202] affording TBS-protected pochonin A (**2-129**) in excellent yield as an inseparable 3:1 diastereoisomeric mixture. It is interesting to note that epoxidation with *m*CPBA or DMDO only proceeded at room temperature and did not give any diastereomeric excess. Attempts to further improve the stereoselectivity of the epoxidation by reducing the temperature (-10 °C) or using epoxone were not productive. Deprotection of compound **2-129** using classical silyl deprotection conditions (TBAF in THF) afforded a separable diastereomeric mixture and confirmed that the major product was indeed the desired pochonin A (**2-122**).[203] Although the overall synthetic sequence was short and could be carried out in only a few days, the poor yield in both the esterification sequence and the acylation reaction led us to consider alternative protecting groups. Based on their stability

[202] Yang, D., Wong, M.-K. & Yip, Y.-C. Epoxidation of Olefins Using Methyl(trifluoromethyl)dioxirane Generated in Situ. *Ibid.* **60**, 3887-3889 (1995).
[203] A ^{1}H NMR spectrum of natural pochonin A was kindly provided by Dr. Marc Stadler, Bayer Health Care, Wuppertal, Germany.

toward basic conditions but also on their liability towards TBAF, SEM protecting groups were considered. Following the procedure described for the polymer-assisted synthesis of pochonin D, selective Mitsunobu reaction between benzoic acid **2-95b** and chiral alcohol (*S*)-**2-27** using polymer-bound DEAD and subsequent protection with SEM-Cl afforded ester **2-130** in 72 % yield (Scheme 39).



Scheme 39: Pochonin A synthesis using SEM-protecting groups

a) PS-DEAD (2.5 equiv., 1.3 mmol.g^{-1}), *S*-(-)-4-penten-2-ol (2.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), toluene, 23 °C, 10 min; b) NaH 60 % (4.0 equiv.), SEMCl (4.0 equiv.), THF, 0 °C, 2 h, 72 % over 2 steps; c) LDA (2.0 equiv.), THF, -78 °C, Weinreib amide **2-114** (1.0 equiv.), 10 min, 60 %; d) Grubbs' II (10 % mol), toluene (2 mM), 80 °C, 12 h, 87 %; e) DMDO (1.0 equiv.), CH_3CN, 0 → 23 °C, 1.5 h, 83 %, 1:1 mixture of diastereoisomers; f) $MgBr_2$•Et_2O (8.0 equiv.), CH_2Cl_2, 23 °C, 1 h, 70 %.

Acylation of toluate ester **2-130** using Weinreb amide **2-114** led to the isolation of the acyclic precursor **2-131** in 60 % yield. Treatment of the triene **2-131** with the Grubbs' second generation catalyst under thermodynamic conditions (80 °C, overnight) afforded the corresponding macrocycle **2-132** in 87 % yield (< 5 % *cis* olefin), which was epoxidized under the same conditions as for the TBS-protected compound **2-128** [methyl(trifluoromethyl)-dioxirane], yielding compound **2-133** in 83 % yield albeit in a 1:1 diastereomeric ratio (inseparable). All attempts to deprotect the SEM groups using TBAF were unsuccessful (even when performed on macrocycle **2-132**), leading mostly to the formation of isocoumarin. Other methodologies were then examined such as sulfonic acid resin in MeOH at 40 °C, Montmorillonite K10 in toluene at 50 °C and $MgBr_2$•Et_2O in dichloromethane at room temperature. The use of either sulfonic acid resin or Montmorillonite K10 led to the opening of the epoxide. To our great delight, we found that 8.0 equiv. of $MgBr_2$•Et_2O afforded the desired pochonin A (**2-122**) along with its

diastereoisomer (**2-122b**) as a separable mixture. The yield was limited by reaction conversion, since longer times led to the formation of the corresponding bromohydrine. It is important to note that in the case of EOM-protected product **2-123**, treatment with $MgBr_2$ was found to open the epoxide faster than to deprotect the EOM. The difference of selectivity in the epoxidation reaction depending on the phenol protecting groups may be ascribed to the conformational impact of the bulky silyl group present on the *ortho*-phenol. Interestingly, in the same biological assay as used for pochonin D, pochonin A was also found to be a good ligand of HSP90 with an IC_{50} of 90nM (*vide infra*).

Chapter VII – Diversity-Oriented Synthesis of Pochonins

With the objective to extend the diversity of the RAL in the hope of finding new ATPase and kinase inhibitors, we turned our attention to the synthesis of a library. This library was envisioned to stem from five points of diversity around the resorcylic macrolide scaffold (Figure 38): modifications of the *para*-phenol (R^1, a number of natural resorcylides bearing a methyl group at this position), the group on C17 (R^2, both stereochemistries are present in natural resorcylides; however, only with a methyl substituent),

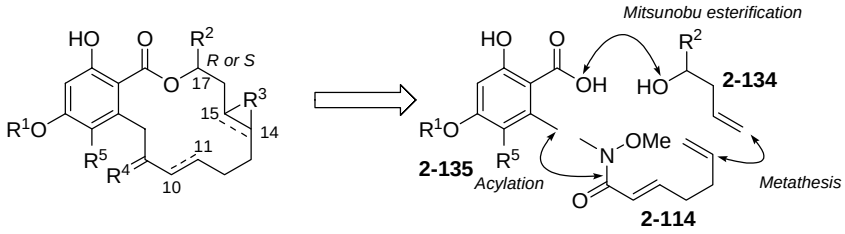
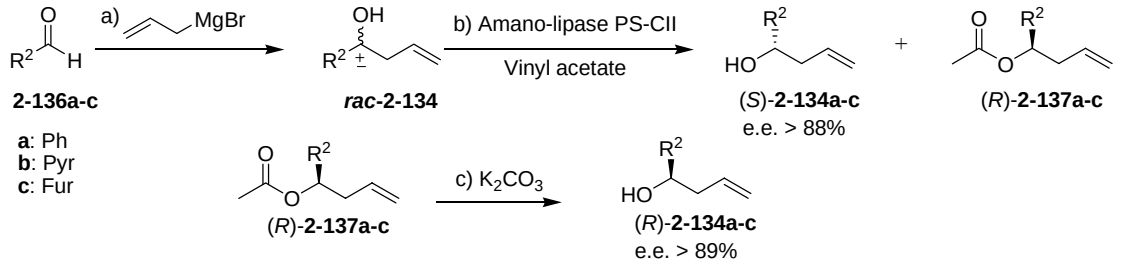


Figure 38: General structure and retrosynthetic analysis of the library

the C14–C15 olefin (R^3), the C9 carbonyl (R^4), the olefin C10–C11, and the meta position on the aryl ring (R^5, a number of natural resorcylides bear a chlorine at this position). To minimize traditional chromatography, a prerequisite in designing the chemistry used to elaborate the molecular diversity of this scaffold was its ability to be carried out by using polymer-bound reagents. Therefore, the assembly of the macrocycle was thought to rely on the chemistry developed for the synthesis of pochonin D using polymer-bound reagents (Schemes 35 and 36). The synthesis of homoallylic alcohols **2-134** bearing various substituent at R^2 such as phenyl, furyl, isopropyl, *etc.* was envisioned. All the work related to the synthesis of these chiral alcohols was carried out by Dr. LOPEZ DEBER in collaboration with Pierre-Yves DAKAS. Indeed, the homoallylic alcohols **2-134** were obtained in their highest enantiomeric form either by enzymatic resolution[204] of the racemic alcohol or by means of Brown allylation[168] of the corresponding aldehyde. Only the phenyl (**2-134a**), the pyridinyl (**2-134b**) and the furyl (**2-134c**) alcohols could be prepared by enzymatic resolution

[204] (a) Master, H. E., Newadkar, R. V., Rane, R. A. & Kumar, A. Highly efficient enzymatic resolution of homoallyl alcohols leading to a simple synthesis of optically pure fluoxetine and related compounds. *Tetrahedron Letters* **37**, 9253 (1996), (b) Singh, S., Kumar, S. & Chimni, S. S. Chemoenzymatic synthesis of optically active heterocyclic homoallylic and homopropargylic alcohols. *Tetrahedron Asymmetry* **13**, 2679 (2002).

(Scheme 40). Racemic alcohols **2-134a-c** were obtained after Grignard addition of commercially available allylmagnesium bromide on their corresponding aldehyde **2-134a-c**.



Scheme 40: Synthesis of chiral alcohols **2-134a-c** using enzymatic resolution

a) AllylMgBr (1.5 equiv.), THF, 0.5 h, 0 °C, 71 % (**2-134a**), 41 % (**2-134b**), 74 % (**2-134c**); b) $\underline{R^2}$=Ph: vinyl acetate (32.5 equiv.), Amano Lipase PS-C II (50mg/mmol of **2-134**), 23 °C, 30 h (monitored by ^{1}H NMR), $\underline{R^2}$=Pyr, Fur: vinyl acetate (10.0 equiv.), Amano Lipase PS-C II (50mg/mmol of **2-134**), THF, 23 °C, 5-30 h (monitored by ^{1}H NMR); c) K_2CO_3 (0.8 equiv.), MeOH, 23 °C, 98 % ((*R*)- **2-134a**), 92 % ((*R*)- **2-134b**), 84 % ((*R*)- **2-134c**).

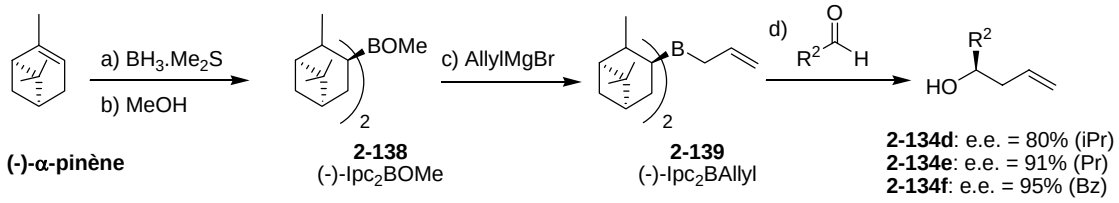
Kinetic enzymatic resolution of racemic alcohols **2-134a-c** was realized using the highly efficient Amano lipase (an immobilized version of *Pseudomonas cepacia*). This enzyme catalyzed a selective transesterification of alcohols (*R*)-**2-134a-c** with vinyl acetate as an acyl donor, the (*S*) alcohols **2-134a-c** being isolated in excellent yields and good enantiomeric excesses (Table 4).

Table 4: Enantioselective acylation of alcohols *rac*-**2-134a-c** by transesterification with lipase

Entry	***Substrate***	***Time*** (h)	***Conv. Ratio*** (%)	***Yield*** (%)	***e.e.*** (%)	***Yield*** (%)	***e.e.*** (%)
			(OH/OAc)	(*S*)- **2-134**	(*S*)-**2-134**	(*R*)-**2-134**	(*R*)-**2-134**
1	*rac*-**2-134a**	30	50:50	45	98	49	93
2	*rac*-**2-134b**	30	52:48	50	89	39	94
3	*rac*-**2-134c**	5	49:51	44	88	49	89

The enantioselectivity of this resolution is based on the difference in reaction rates of the two enantiomers with the lipase. The reaction of each enantiomer within the asymmetric catalytic pocket of the enzyme leads to diastereoisomeric transition states with different energies. Amano lipase is known to have a hydrophobic pocket in its active site which orients the alcohol into a specific fit, leading to a high degree of selection between the two enantiomers. Thus, the greater the difference in the two reaction rates is, the higher the enantiomeric purity will be. Enantiomeric excess obtained with this methodology are all above 88 % and crucially depends on the conversion of the reaction. Acetylated alcohols (*R*)-**2-137** were then

hydrolysed to the corresponding alcohols (*R*)-**2-134a-c** in excellent yields. Although this method is highly reproducible and allows multi-gram quantities of the alcohols, it is not general to all homoallylic alcohols and a more general protocol based on the Brown allylation was developed for the synthesis of the isopropyl (**2-134d**), the propyl (**2-134e**) and the benzyl (**2-134f**) alcohols (Scheme 41).

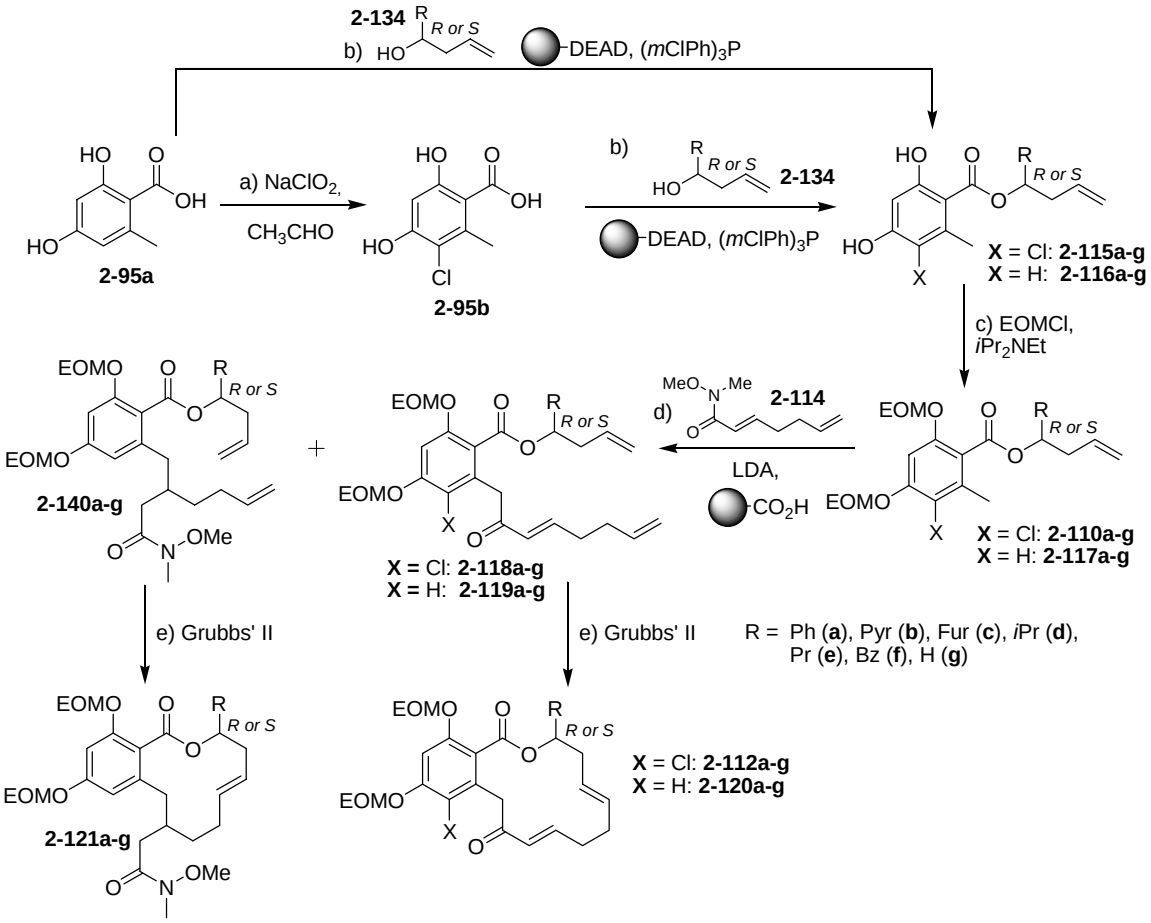


Scheme 41: Synthesis of chiral alcohols **120d-f** using Brown allylation

a) (-)-α-pinene (2.4 equiv.), BH_3•Me_2S (1.0 equiv.), THF, 23 °C for 1 h and then 4 °C for 12 h, 76 %; b) MeOH (1.2 equiv.), Et_2O, 0 °C, 2 h, 94 %; c) AllylMgBr (0.95 equiv.), Et_2O, 0 → 23 °C, 1 h, 92 %; d) **2-136d-f** (1.05 equiv.), Et_2O, -100 °C, 0.5 h; 3N NaOH, H_2O_2 35 %, reflux, 3 h, 77-93 %. Enantiomeric excesses of alcohols were determined by chiral HPLC analysis after acylation with 3,5-dinitrobenzoyl chloride.

(-)-B-Allyldiisopinocampheylborane (**2-139**, (-)-Ipc_2BAllyl) was synthesized in a three steps sequence from (-)-α-pinene involving an hydroboration, the formation of the corresponding MeO-borinic ester **2-138** and its treatment with a Grignard reagent. Further condensation on aldehydes **2-136d-f** followed by oxidation of the resulting borinates with alkaline hydrogen peroxide allowed the formation of the chiral homoallylic alcohols **2-134d-f** in good enantiomeric excess. These six alcohols (*R*)-**2-134a-f** and their *S* enantiomers along with 3-buten-1-ol (**2-134g**) will serve as the first diversification for the synthesis of the pochonin D library.

Following the chemistry developed for pochonin D, commercially available benzoic acid **2-95a** (Scheme 42) and its chlorinated analog **2-95b** (the chlorine atom was introduced using the aforementioned procedure (Scheme 35)) were esterified with thirteen different homoallylic alcohols ((*R*)-**2-134a-f**, (*S*)-**2-134a-f**, **2-134g**) by using polymer-supported DEAD to yield esters **2-115a-g** and **2-116a-g** in excellent purity.

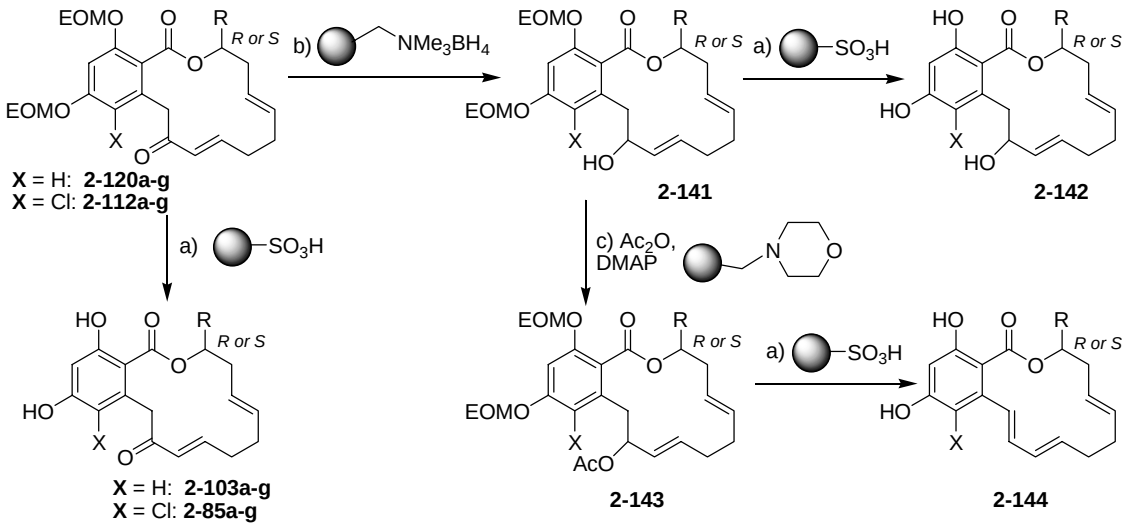


Scheme 42: Synthesis of macrocylic precursors **2-112a-g**, **2-120a-g** and **2-121a-g**

a) $NaClO_2$ (5.0 equiv.), NH_2SO_3H (5.0 equiv.), CH_3CHO (1.0 equiv.), THF/H_2O 5:1, 0 °C, 0.5 h, 92 %; b) PS-DEAD (2.5 equiv., 1.3 mmol.g^{-1}), (*R*)-**2-134a-g** or (*S*)-**2-134a-g** (1.0 equiv.), P(*m*ClPh)$_3$ (2.0 equiv.), CH_2Cl_2, 23 °C, 0.5 h, 60-80 %; c) iPr_2EtN (4.0 equiv.), EOMCl (4.0 equiv.), TBAI (cat.), DMF, 80 °C, 5 h, 80-90 %; d) LDA (2.0 equiv.), THF, -78 °C, **2-114** (1.0 equiv.), 10 min, Amberlite IRC-50 (20.0 eq, 10.0 mmol.g^{-1}); e) Grubbs' II (10 % mol), toluene (2 mM), 80 °C, 12 h, 38-70 % after two steps.

Products **2-115a-g** and **2-116a-g** were then protected with ethoxymethylene chloride (EOMCl) in the presence of Hunig's base to obtain the corresponding protected toluic esters **2-110a-g** and **2-117a-g** respectively, which could be used in the subsequent carbon-acylation reaction without any further purification. It is important that a slightly different procedure had to be followed to yield compounds **2-110b** and **2-117b** as attempts to use to polymer-bound DEAD failed. Therefore, starting from *para*-EOM-protected acids (**2-95g** and **2-108**), using a modified Mitsunobu esterification (1.0 equiv. of DIAD, 1.0 equiv. of alcohol, and 1.6 equiv. of PPh_3 in THF), compounds **2-110b** and **2-117b** were obtained after reprotection of the *ortho*-phenol in 44 % and 48 % yield (2 steps) respectively. Deprotonation of the toluic esters **2-110a-g** and **2-117a-g** by using two equivalents of LDA, followed by addition of the α,β-unsaturated Weinreb amide **2-114** afforded acylation products **2-118a-g** and **2-119a-g**. The

reaction was quenched with a polymer-bound acid which also sequestered all the diisopropylamine. As previously mentioned, the acylation reaction could lead to some level of 1,4-conjugated addition rather than the desired 1,2-addition. Indeed, while the chlorine atom suppresses this reaction (*vide supra*), compounds lacking this substitution at the *meta*-position led to 20 % of the 1,4-conjugated addition products **2-140a-g**. Unfortunately, compounds **2-110b-c** and **2-117b-c** bearing a furyl or pyridinyl side chain did not afford clean products in the acylation reaction. Attempts to reach these analogs of pochonin D using other methods did not yield any desired compounds. Importantly, the crude mixtures of this acylation reaction were used directly in the following ring-closing metathesis step. Therefore, using Grubbs' second-generation catalyst under thermodynamic conditions (80 °C for 12 h), the desired 14-membered macrocycles **2-112a-g** and **2-120a-g** were obtained in good yields. When metathesis reactions were carried out with a mixture of **2-118a-g** and **2-140a-g**, the corresponding 12-membered-ring product **2-121a-g** was obtained in addition to **2-120a-g** as a separable mixture. At this stage, all mixtures were purified by standard chromatography yielding macrocycles **2-112a-g** and **2-120a-g** in 30–60 % yield and **2-140a-g** in 8–10 % overall yield from **2-95a**. Fourteen-membered macrocycles **2-112a-g** and **2-120a-g** were then used as the starting point for further diversifications. Deprotection of their EOM groups by using sulfonic acid resin afforded compounds **2-85a-g** and **2-103a-g** in pure form and excellent yields after simple filtration of the resin and evaporation of the solvents (Scheme 43). The 12-membered-ring products **2-121a-g** were also deprotected easily using the same method (not shown). As expected, EOM deprotection under acid catalysis was slower for the chlorinated analogs and had to be monitored as prolonging the reaction times could lead to conjugated additions (*vide infra*).



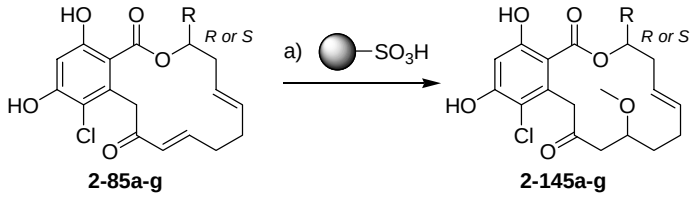
Scheme 43: Deprotection and synthesis of reduced ketone analogs

a) PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}), MeOH, 40 °C, 4 h, > 90 %; b) BER resin (1.0 equiv., 2.5 mmol.g^{-1}), MeOH, 0 °C, 12 h, ~60 %; c) Ac_2O (1.2 equiv.), PS-NMM (1.2 equiv., 3.20 mmol.g^{-1}), DMAP (0.05 equiv.), DMF, 23 °C, 0.5 h, ~80 %.

Treatment of **2-120a-g** and **2-112a-g** with reducing agents led to either carbonyl reduction with DIBAL or mixtures of carbonyl and/or conjugated olefin reduction with $NaBH_4$. It is known that using a noncoordinating counter ion for borohydride can favor the carbonyl reduction and this was most conveniently achieved by using a polymer-supported quaternary ammonium borohydride known as borohydride exchange resin (BER).[205] Thus, α,β-unsaturated ketones of **2-120a-g** and **2-112a-g** could be reduced with BER-resin to obtain both diastereoisomers of **2-141** in ~60 % yield. The EOM protecting groups were then removed with sulfonic acid resin under regular conditions to afford compounds **2-142**. Acetylation of the reduced intermediates **2-141** using PS-NMM/Ac_2O yielded compounds **2-143**. Further elimination upon deprotection with the acidic resin afforded trienes **2-144** as a mixture of olefin geometries. In addition to these variations on the conjugated ketone, pochonin D and monocillin II analogs were further diversified on the conjugated olefin (**2-145** (Scheme 44), **2-146** (Scheme 45)), on the para-phenol (**2-147**, **2-148** (Scheme 45)) and on the non-conjugated olefin (**2-149**, **2-150** (Scheme 45)). As we observed that prolonged exposure of macrocycles **2-112a-g** to methanol in the presence of sulfonic acid resin led to conjugated addition, we asked ourselves whether this reaction could be driven to completion cleanly.

[205] (a) Gibson, H. W. & Baily, F. C. Chemical modification of polymers. Borohydride reducing agents derived from anion exchange resins. *J. Chem. Soc. Chem. Commun.* **22**, 815a-815a (1977), (b) Kirschning, A. Borohydride Exchange Resrins (BER) - a Group of Versatile and Powerful Polymer-Supported Reductants. *J. Prakt. Chem.*, 342 (2000).

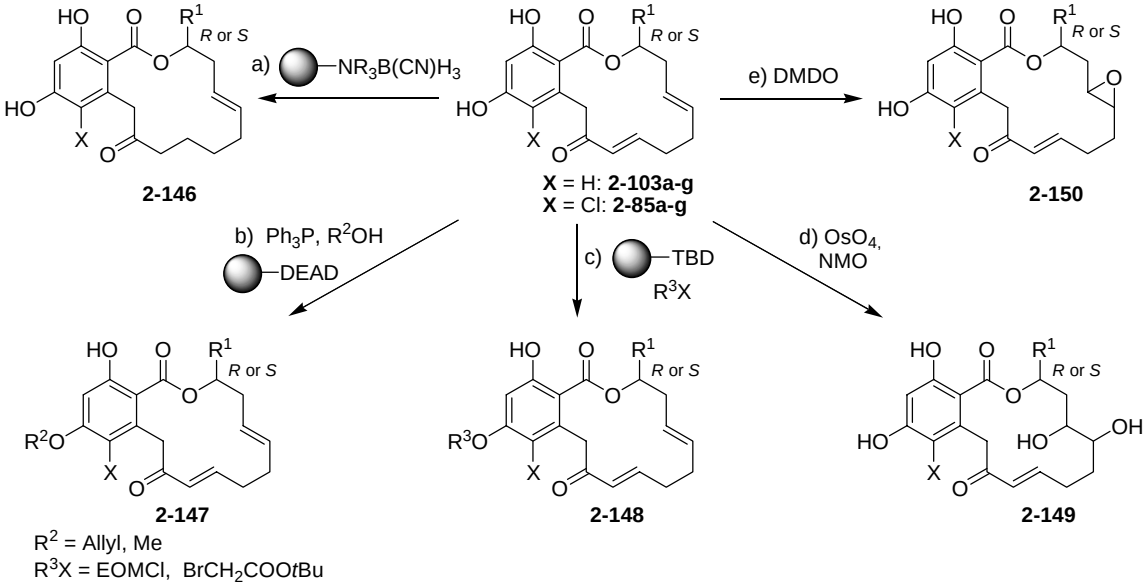
Indeed, we found that deprotected macrolides **2-85a-g** (Scheme 44) could be quantitatively converted into products **2-145a-g** after prolonged exposure to the acidic resin.



Scheme 44: Synthesis of analogs **2-145** by conjugated addition

a) PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}), MeOH, 40 °C, 15 h, 80 %.

This product could obviously be obtained directly from **2-112a-g** (EOM-protected macrocycles) under the same conditions. Further diversifications on the pochonin scaffold using polymer-bound cyanoborohydride afforded the reduction of the conjugated olefin leading to macrocycles **2-146** in moderate yields.

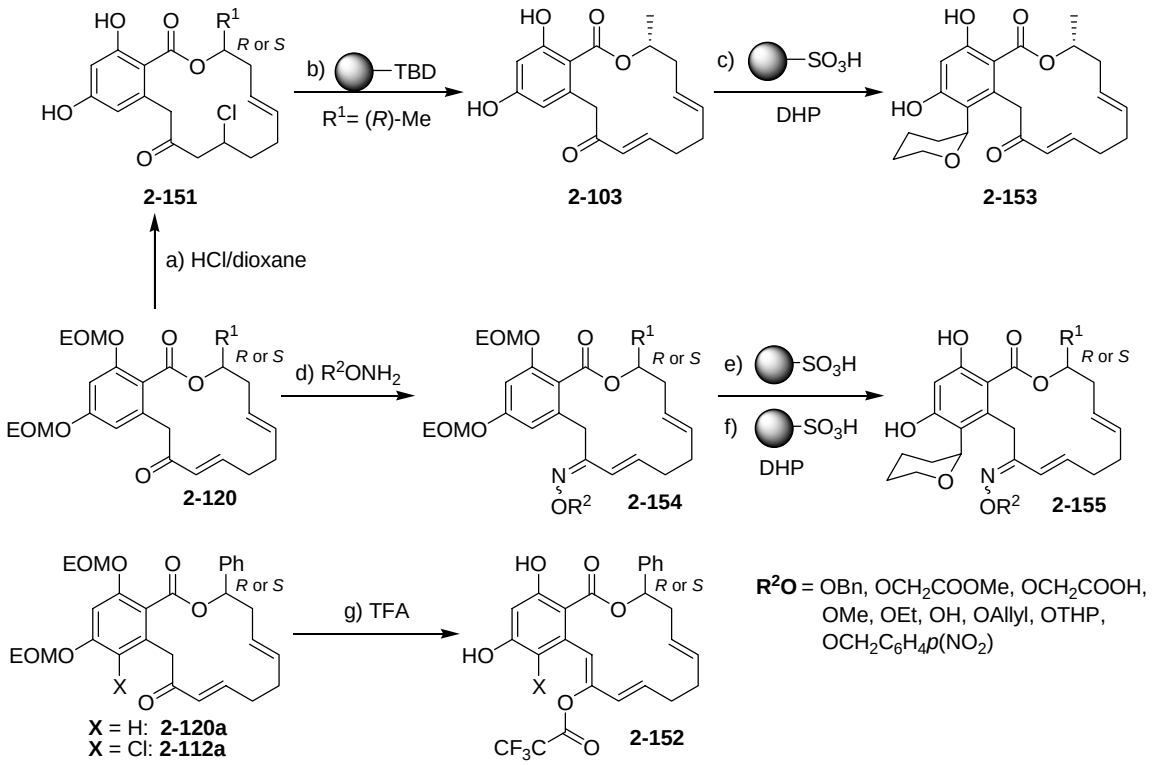


Scheme 45: Derivatization of compounds **2-85a-g** and **2-103a-g**

a) PS-TMABH$_3$CN (2.0 equiv., 3.5 mmol.g^{-1}), CH_2Cl_2/AcOH 10 :1, 23 °C, 4 h, ~50 %; b) PPh_3 (2.0 equiv.), R^2OH (2.0 equiv.), PS-DEAD (2.0 equiv., 1.3 mmol.g^{-1}), CH_2Cl_2, 23 °C, 8 h, ~60 %; c) R^3X (0.9 equiv.), PS-TBD (2.0 equiv., 2.9 mmol.g^{-1}), CH_2Cl_2, 23 °C, 3 h, ~90 %; d) OsO_4 (0.1 equiv.), NMO (1.0 equiv.), Acetone/H_2O 10 :1, 23 °C, 1 h, > 70 %; e) DMDO (1.2 equiv., 0.04 M in acetone), CH_3CN, 0 °C, 30 min, > 90 %.

The more acidic *para*-hydroxyl groups of compounds **2-85a-g** and **2-103a-g** were substituted either by Mitsunobu reaction using polymer bound DEAD or by alkylation using a polymer-bound base leading to compounds with general structure **2-147** and **2-148**, respectively.

Oxidation with OsO_4 afforded the dihydroxylation products **2-149** as a mixture of isomers along with products corresponding to the dihydroxylation of the conjugated olefin (product not shown) albeit as separable mixture. Treatment of **2-85a-g** and **2-103a-g** with freshly prepared dimethyldioxirane led to the selective epoxidation of the non-conjugated olefin as a mixture of diastereoisomers **2-150** of pochonin A analogs. Although higher selectivities can be obtained for this reaction using TBS protecting groups (*vide supra*), it was not the purpose of this work. Additionally, it is interesting to note that the conjugated olefin proved to have different reactivities depending on the presence or absence of the chlorine atom on the aryl ring. Indeed, while the EOM deprotection of compound **2-112** (X = Cl) could be carried out with HCl in dioxane, treatment of the corresponding compound **2-120** (X = H) with the same conditions led to the conjugated addition of the chlorine atom affording compound **2-151** (Scheme 46). Following this method, compounds **2-120a-g** were also converted into their bis-chlorinated analogs **2-151a-g**. The difference in reactivity may be attributed to the different conformation of these non-chlorinated compounds that may affect the level of conjugation between the olefin and the adjacent carbonyl group. Similarly, after treatment with TFA (20 %) in CH_2Cl_2, compounds bearing a phenyl substituent (**2-120a** and **2-112a**) led to the formation of the enolate and further addition of the trifluoroacetate on the conjugated alcohol (**2-152**). It seems that the phenyl substituent clearly impacts the conformation of the molecule (shown also by ^{1}H NMR where proton signals shift differently) leading to a different reactivity.



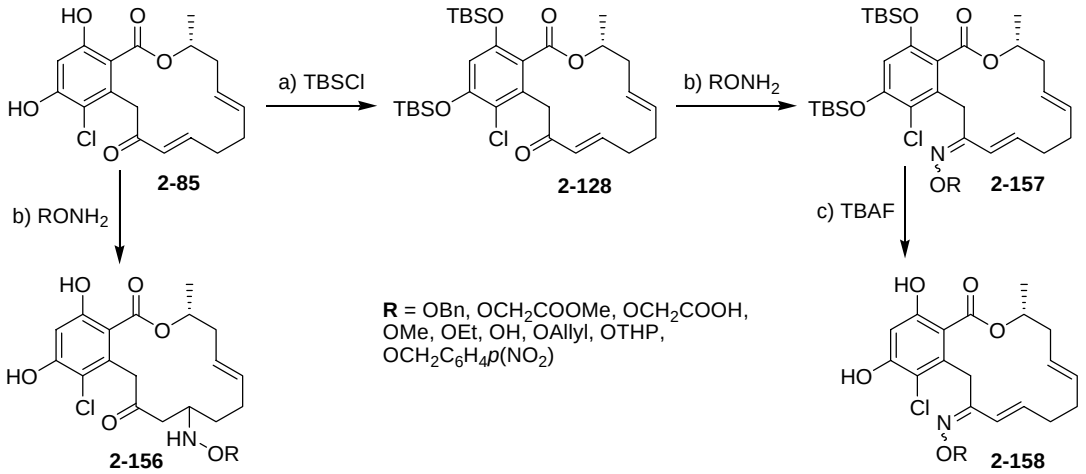
Scheme 46: Derivatization of macrocycles **2-120** and **2-120a-g**

a) HCl (2.5 % in dioxane), 23 °C, 3 h, > 75 %; b) PS-TBD (0.5 equiv., 2.6 mmol.g^{-1}), CH_2Cl_2, 23 °C, 8 h, ~90 %; c) PS-TsOH (1.0 equiv., 3.2 mmol.g^{-1}), DHP (1.0 equiv.), CH_2Cl_2, 23 °C, 5 h, ~80 %; d) R^2ONH_2.HCl (5.0 equiv.), Pyr/AcOH 5:1, 40 °C, 12 h, ~90 %; e) PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}), MeOH, 40 °C, 4 h, ~80 % f) PS-TsOH (cat., 3.2 mmol.g^{-1}), DHP (1.0 equiv.), CH_2Cl_2, 23 °C, 5 h, ~70 %; g) TFA (20 %), CH_2Cl_2, 23 °C, 2 h.

The β-chlorine on compound **2-151** could be cleanly eliminated in the presence of a polymer-bound base, thus recovering the conjugated compound **2-103**. While evaluating protecting groups for the phenols, we noticed that dihydropyran, in the presence of a strong acid, such as sulfonic acid, led to electrophilic aromatic substitution rather than phenol protection.[206] Applying these conditions to compounds **2-103** (Scheme 46) afforded **2-153** as a separable mixture of diastereoisomers. The formation of oxime proved to be sluggish on compounds with unprotected phenols. However, compounds **2-120** (Scheme 46) protected with EOM groups underwent smooth oxime formation with nine different hydroxyl amines to yield compounds **2-154** as E/Z mixtures with variable ratios. EOM deprotection of these oxime derivatives with sulfonic acid resin in methanol followed by treatment with sulfonic acid resin and dihydropyran in dichloromethane afforded a mixture of diasteroisomeric oximes **2-155** bearing a tetrahydropyran substituent on the aromatic ring. Interestingly, when the side chain

[206] Kometani, T., Kondo, H. & Fujimori, Y. Boron trifluoride-catalyzed rearrangement of 2 (aryloxy)tetrahydropyrans: a new entry to C-arylglycosidation. *Synthesis*, 1005-7 (1988).

of the oxime bore a carboxylic acid moiety (R^2: OCH_2COOH), deprotection of the EOMs with the sulfonic acid resin in methanol was simultaneously accompanied by esterification of the carboxylate. Finally, at the time of the synthesis of this library, all attempts to form the oxime on chlorinated analogs with or without EOM-protecting groups (**2-112a-g** or **2-85a-g** respectively) generated mostly the corresponding 1,4-addition of the hydroxylamines (**2-156**, Scheme 47). To our surprise, when pochonin D (**2-85**) was protected with TBS groups (**2-128**, Scheme 47), the formation of the desired oxime **2-156** was the only product observed under the same reaction conditions.

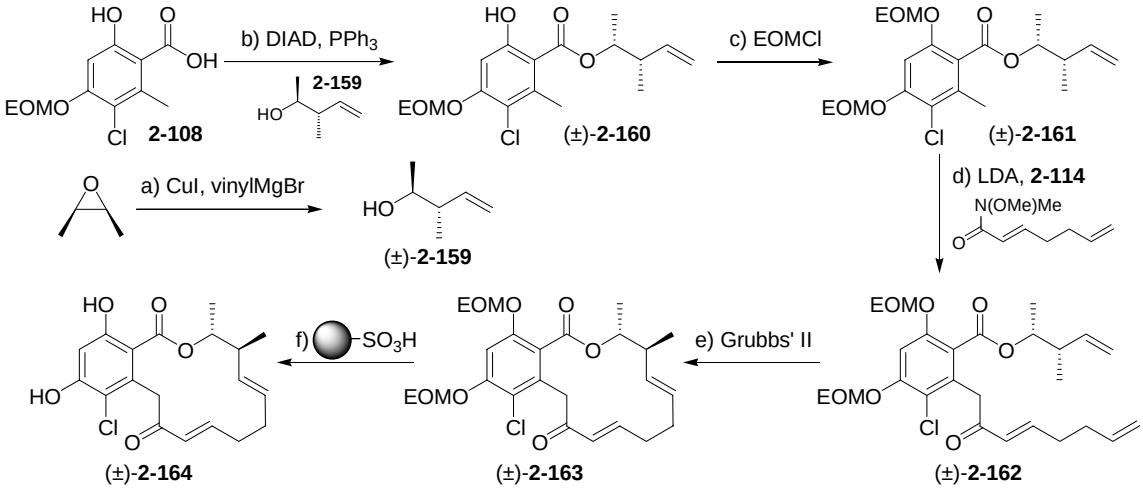


Scheme 47: Oxime formation from compound **2-85**

a) TBSCl (5.0 equiv.), imid. (5.0 equiv.), DMF, 23 °C, 3 h, ~90 %; b) $RONH_2$.HCl (5.0 equiv.), Pyr/AcOH 5:1, 40 °C, 12 h, ~90 %; c) TBAF (2.5 equiv.), THF, 23 °C, 2 h, ~80 %.

Further deprotection of the TBS groups using TBAF led to the obtention of nine oximes **2-158**. Similarly to the chlorine addition on the conjugated system (Scheme 46), this difference of reactivity is presumably due to the level of conjugation between the olefin and the carboxylate which is indeed related to the conformation of the macrocycle. In fact, the presence of a bulky-silyl protecting group on the *ortho*-phenol may have an impact on the conformation of the macrocycle when compared to the free phenol which forms a hydrogen bond with the carbonyl. While not all permutations of the five points of diversity (Figure 34) were pursued, a total of 113 compounds were prepared. Shortly after the synthesis of this library, a molecular modelling study led us to envision the synthesis of a bis-methyl substituted analog **2-164**. In order to evaluate rapidly the potency of this molecule, 3-methyl-4-penten-2-ol **2-159** was prepared in a racemic form from *cis*-2,3-dimethyloxirane using

copper iodide and vinyl magnesium bromide (Scheme 48).[207] Due to the high volatility of this alcohol, the yield of this reaction never exceeded 65 %.

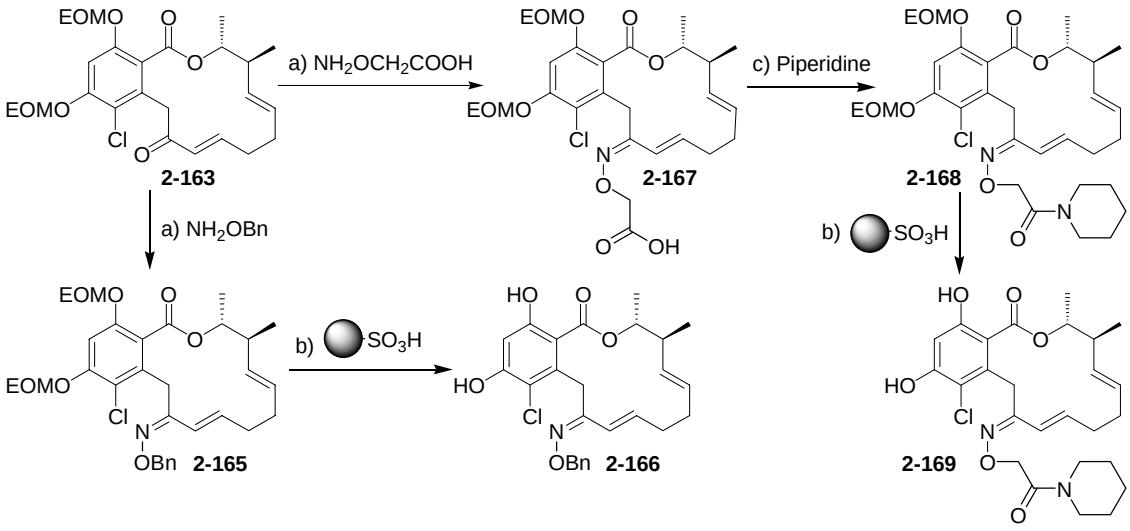


Scheme 48: Synthesis of bis-methyl substituted analog (**2-164**) of pochonin D

a) VinylMgBr (2.0 equiv.), CuI (0.3 equiv.), Et_2O, -30 → 23 °C, 12 h, 65 %; b) **2-159** (1.0 equiv.), PPh_3 (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 3 h, 23 %; c) EOMCl (2.0 equiv.), NaH 60 % (2.0 equiv.), THF, 0 °C, 2 h, 66 %; d) LDA (2.0 equiv.), THF, -78 °C; **2-114** (1.0 equiv.), 10 min, 57 %; e) Grubbs' II (10 % mol), toluene (2 mM), 80 °C, 12 h, 57 %; f) PS-TsOH (10 equiv., 3.2 $mmol.g^{-1}$), MeOH, 40 °C, 2.5 h, 40 %.

Following the procedure developed in solution for pochonin D (Scheme 33), acid **2-108** was used in a standard Mitsunobu esterification with alcohol **2-159**. The outcome of this reaction was very disappointed in terms of yield (20 %) probably due to a higher steric hindrance from the second methyl group. Attempts to improve the yield of this esterification by using a carbodiimide coupling led also to unsatisfying results. Nevertheless, compound **2-160** could be isolated in sufficient quantities and further reprotection of the *ortho*-phenol followed by acylation reaction with Weinreb amide **2-114** allowed the formation of the acyclic precursor **2-162**. Ring-closing metathesis also proved challenging with this substrate as the reaction never went to completion and led sometimes to the isolation of the corresponding dimer. However, removal of the EOM protecting groups on compound **2-163** using sulfonic acid resin furnished the bis-methylated analog **2-164**. Additionally, some oxime derivatives of this molecule were synthesized. Gratifyingly, the aforementioned methodology developed for the formation of oxime on the chlorinated scaffold (Scheme 47) allowed the formation of two different oximes **2-165** and **2-167** from macrocycle **2-163** as a separable mixture with the 1,4-addition product (Scheme 49).

[207] Driver, T. G., Franz, A. K. & Woerpel, K. A. Diastereoselective silacyclopropanations of functionalized chiral alkenes. *J Am Chem Soc* **124**, 6524-5 (2002).



Scheme 49: Synthesis of oxime derivatives of compound **2-164**

a) $BnONH_2.HCl$ or $NH_2OCH_2CO_2H$ (5.0 equiv.), Pyr/AcOH 5:1, 40 °C, 24 h, 20-35 %; b) PS-TsOH (10.0 equiv., 3.2 $mmol.g^{-1}$), MeOH, 40 °C, 2.5 h, 77-80 %; c) Piperidine (1.1 equiv.), EDC (1.1 equiv.), HOBt (1.1 equiv.), DMF, 23 °C, 2 h, 75-80 %.

The carboxylic acid moiety of oxime **2-167** was then esterified to form the corresponding piperidine amide oxime **2-168**.[94] Removal of the EOM groups using sulfonic acid resin allowed the isolation of both oximes **2-166** and **2-167** from **2-165** and **2-168** respectively. These two compounds along with their parent compound **2-164** and the 113 compounds from the library (Figure 39) were then tested in biological assays for kinase and HSP90 inhibition.

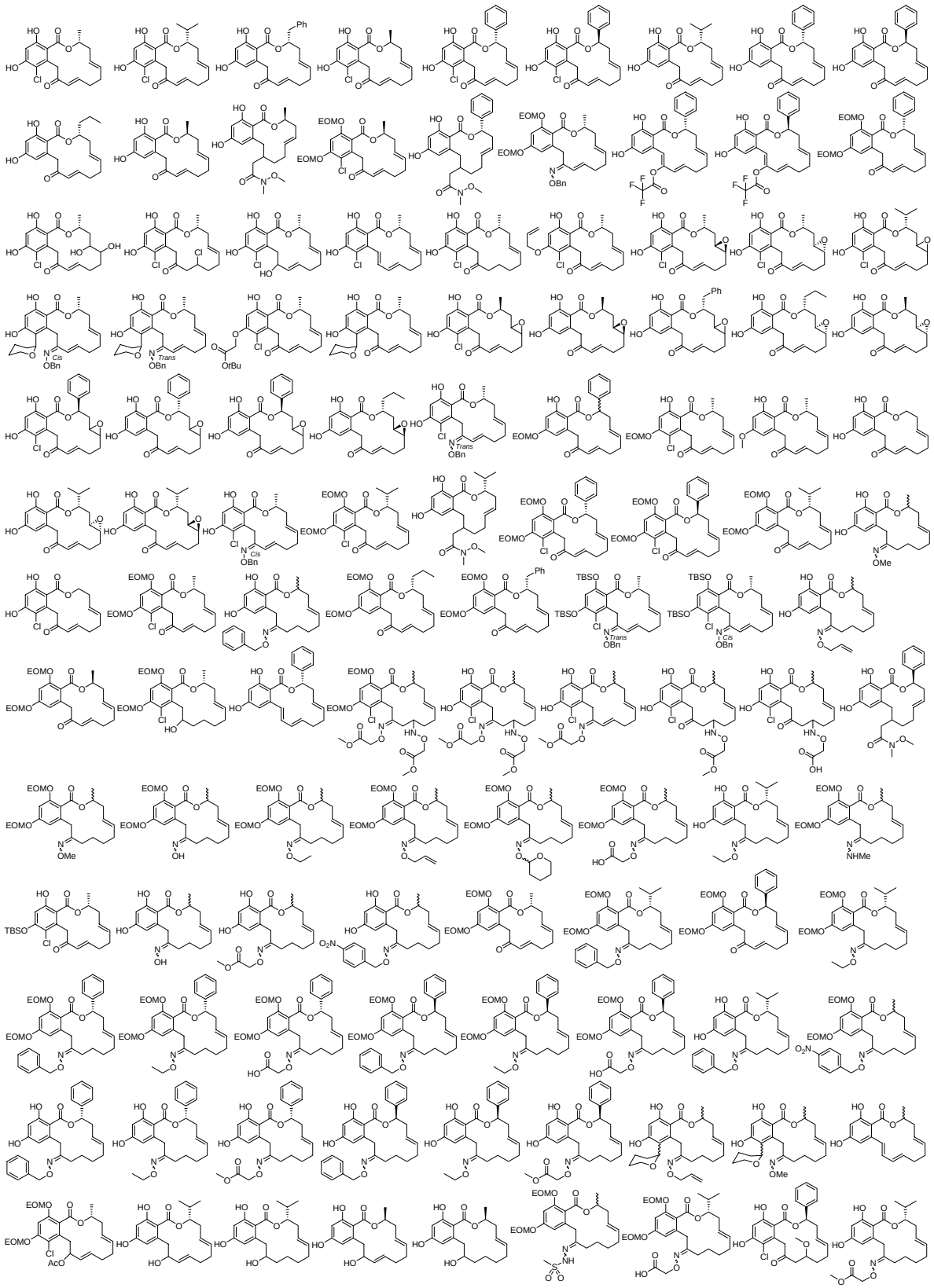
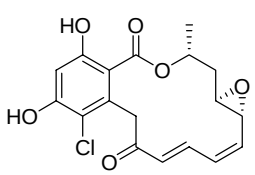


Figure 39: Structures of the library of pochonin D analogs

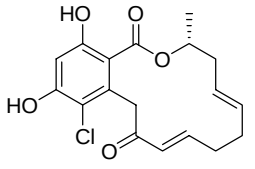
Chapter VIII – Biological Evaluation of the Pochonin D Analogs

Two biological assays (HSP90 and kinase inhibition) have been performed on the previously synthesized natural products (pochonin D and A) and all the pochonin D analogs. Radicicol (**2-1**), pochonin D (**2-85**) and pochonin A (**2-122**), along with some closely related analogs such as monocillin II (**2-103**) or the diol analog of pochonin A (**2-125**) were first evaluated for HSP90 affinity in a competition assay with geldanamycin using a previously described method.[208] The results are shown in Figure 40.

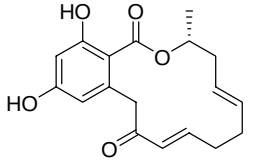
Figure 40: Structure and affinity of radicicol analogs for HSP90



2-1

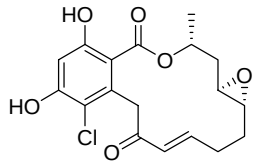


2-85

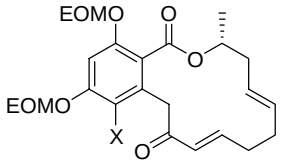


2-103

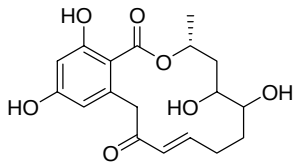
Compound	*IC_{50} (µM)*
Radicicol (**2-1**)	0.02
Pochonin D (**2-85**)	0.08
Monocillin II (**2-103**)	> 50
Pochonin A (**2-122**)	0.09
2-112	> 50
2-120	> 50
2-125	> 50



2-122



X = H: **2-120**
X = Cl: **2-112**



2-125

As aforementioned, pochonin D (**2-85**) was found to be a good ligand for HSP90 with an IC_{50} of 80nM as compared to 20nM for radicicol. Although this difference of activity constituted less than an order of magnitude, it might be rationalized by the molecular modelling study on pochonin D (Figure 34). The co-crystal structure of HSP90-radicicol reported by Pearl and co-workers showed a tightly bound water molecule making a hydrogen-bond bridge between the *ortho*-phenol, the ester, and Asp79 and a second water molecule making a bridge between the *para*-phenol and Leu34.[76b] Indeed, compounds **2-112** and **2-120** with protected phenols

[208] Zhou, V. et al. A time-resolved fluorescence resonance energy transfer-based HTS assay and a surface plasmon resonance-based binding assay for heat shock protein 90 inhibitors. *Anal Biochem* **331**, 349-357 (2004).

showed no affinity for HSP90. The importance of the chlorine atom is also evident from the comparison of pochonin D (**2-85**, 80nM) and monocillin II (**2-103**, > 50μM). The bulky chlorine not only comes within van der Waals contact of Phe124 but fills a hydrophobic pocket. Interestingly, pochonin A (**2-122**) was a good ligand (90nM), while analog **2-111** was inactive. Based on these biological data, it appeared that the presence of the unprotected phenols and of the chlorine atom is crucial to achieve efficiency for HSP90 inhibition. The comparison between radicicol, pochonin D and pochonin A confirmed that the epoxide moiety is not essential for HSP90 inhibition and established that the γ,δ-conjugated olefin is not a prerequisite for potency. The ability of these natural products (**2-1**, **2-85**, **2-122**) to antagonize ATP in the N-terminal domain of HSP90 and to inhibit ATPase activity may be related to their inhibition of HSV (Herpes Simplex Virus) helicase. Similarly, compounds **2-164**, **2-166** and **2-169** corresponding to bis-methylated analogs were evaluated for HSP90 inhibition. Further testings against HSP90 for twenty selected compounds of the library are currently underway with some preliminary interesting results.

Towards kinases, a representative subset of the synthetic library (84 compounds) was evaluated for its inhibition of a panel of 24 kinases (AKT1, ARK5, Aurora-A, Aurora-B, B-RAF-VE, CDK2/ CycA, CDK4/CycD1, CK2-a1, FAK, EPHB4, ERB2, EGFR, IGF1-R, SRC, VEGF-R2, VEGF-R3, FLT3, INS-R, MET, PDGFR-b, PLK1, SAK, TIE2, COT) at 10 μM. Significantly, twelve compounds showed more than 50 % inhibition for a kinase, thus representing a > 14 % hit rate among the library. Interestingly, pochonin D (**2-85**), pochonin A (**2-122**), and radicicol (**2-1**) which have been shown to be powerful inhibitors of HSP90 did not show significant activity against this panel of kinases. Nine compounds were selected to calculate IC_{50} against each of the 24 kinases as depicted on Table 5 and Figure 41.

Table 5: Inhibitory activity (IC_{50}: µM) of selected pochonin analogs in a panel of 24 kinase assays (blank represent an IC_{50} > 50µM)

	AKT1	ARK5	Aurora-A	Aurora-B	B-RAF-VE	CDK2 /CycA	CDK4/ CycD1	CK2-α1	EPHB4	ERBB2	EGF-R
A0											
A1											33
A2			14	16			50		16	16	10
A3			12	14			30		40	24	14
A4			30	36			48				22
A5			47		50		45		49		16
A6				16			37		40		32
A7											
A8											

	IGF1-R	SRC	VEGF-R2	VEGF-R3	FLT3	INS-R	MET	PDGFR-β	PLK1	SAK	TIE2	FAK	COT
A0			49										
A1	23	11											
A2	16	8	19	40	23	36	32			17	16	14	
A3	19	14	20	19	23	44	29			25	15	9	
A4	13	12	30	31	45					19		37	
A5	11	12	19	31						20		38	
A6	21	20	25	34	44		36			17	25	34	
A7													
A8													

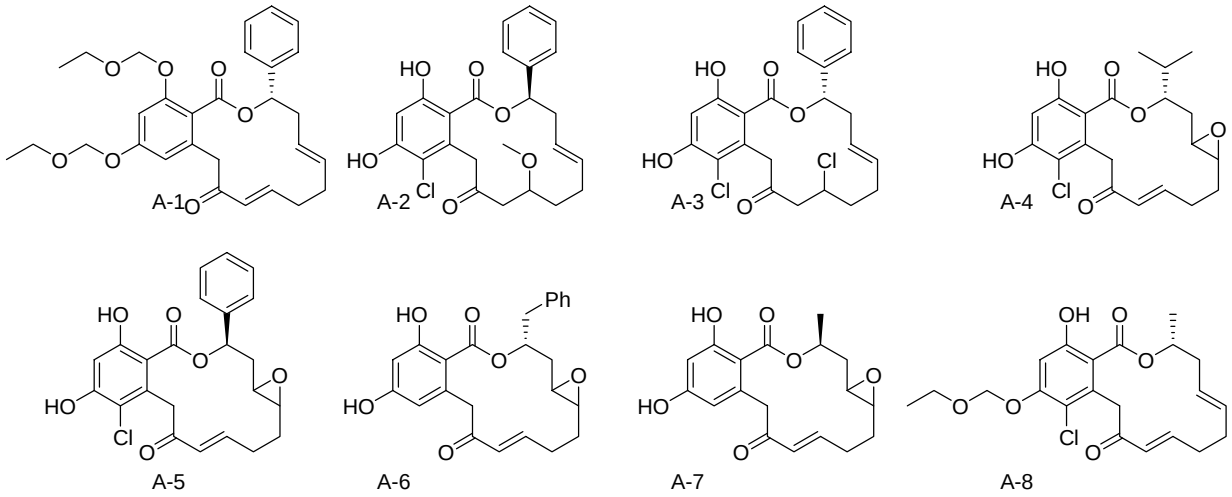


Figure 41: Structure of selected pochonin analogs

In this more detailed analysis, radicicol showed only very mild activity against VGFR-R2 with no inhibition for the twenty three other kinases. Several pochonin analogs showed a well-defined pattern of activity against therapeutically relevant enzymes, such as Src (8 µM for A2), Aurora A (12 µM for A3), and IGF1-R (11 µM for A5). Importantly, the compounds that were found to be kinase inhibitors were not inhibitors of HSP90 (data not shown) and are

not indiscriminate ATP-surrogates. The ATP-binding pocket of HSP90 targeted by radicicol and pochonin D has a specific fold that is present in a superfamily which includes functionally diverse proteins, such as DNA topoisomerase II, helicase, MutL, and histidine kinases.[50] In fact, it has been shown that radicicol does inhibit other members of this family albeit with lower affinity.[209] While the pochonin library described above will certainly contain some compounds that are good inhibitors of enzymes bearing a Bergerat fold, we wished to evaluate whether modification around the pochonin scaffold could retune the selectivity of these compounds from HSP90 inhibitors to kinase inhibitors. The fact that more than fourteen percent of the compounds showed a kinase inhibition of greater than 50 % at 10 mM clearly supports the hypothesis that RAL is a good scaffold for kinase inhibition.

[209] (a) Gadelle, D., Bocs, C., Graille, M. & Forterre, P. Inhibition of archaeal growth and DNA topoisomerase VI activities by the Hsp90 inhibitor radicicol. *Nucleic Acids Res* **33**, 2310-7 (2005), (b) Besant, P. G., Lasker, M. V., Bui, C. D. & Turck, C. W. Inhibition of branched-chain alpha-keto acid dehydrogenase kinase and Sln1 yeast histidine kinase by the antifungal antibiotic radicicol. *Mol Pharmacol* **62**, 289-96 (2002).

Chapter IX – Future Prospects Towards RALs Microarrays

With the success of microarray in screening mRNA expression profiles of thousands of genes simultaneously,[210] bioorganic chemists have been interested in adapting this format for screening small molecules. Over the past six years, a large number of chemistries have been developed to immobilize proteins[211] and small molecules[212] on the glass surface of a microarray. Our group is developing a supramolecular approach to microarray libraries of small molecules. This approach is based on the hybridization of PNA (peptide nucleic acid)-tagged libraries to readily available DNA chips (Figure 42).

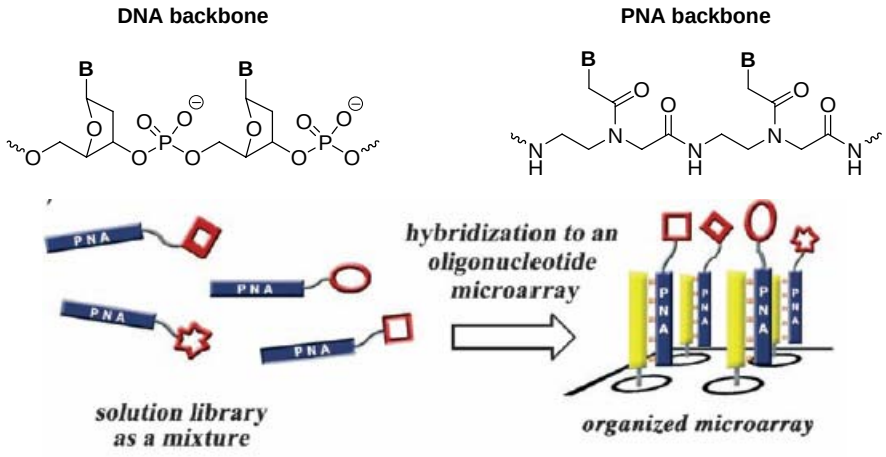


Figure 42: PNA structure compared to DNA and schematic representation of a PNA-encoded library and its conversion to an organised microarray

The motivation for using PNA as the encoding oligonucleotide is based on its chemical and biological robustness. Our group and others[213] have developed a series of protecting group strategies for the co-synthesis of PNA with peptides and other small molecules. Based on the

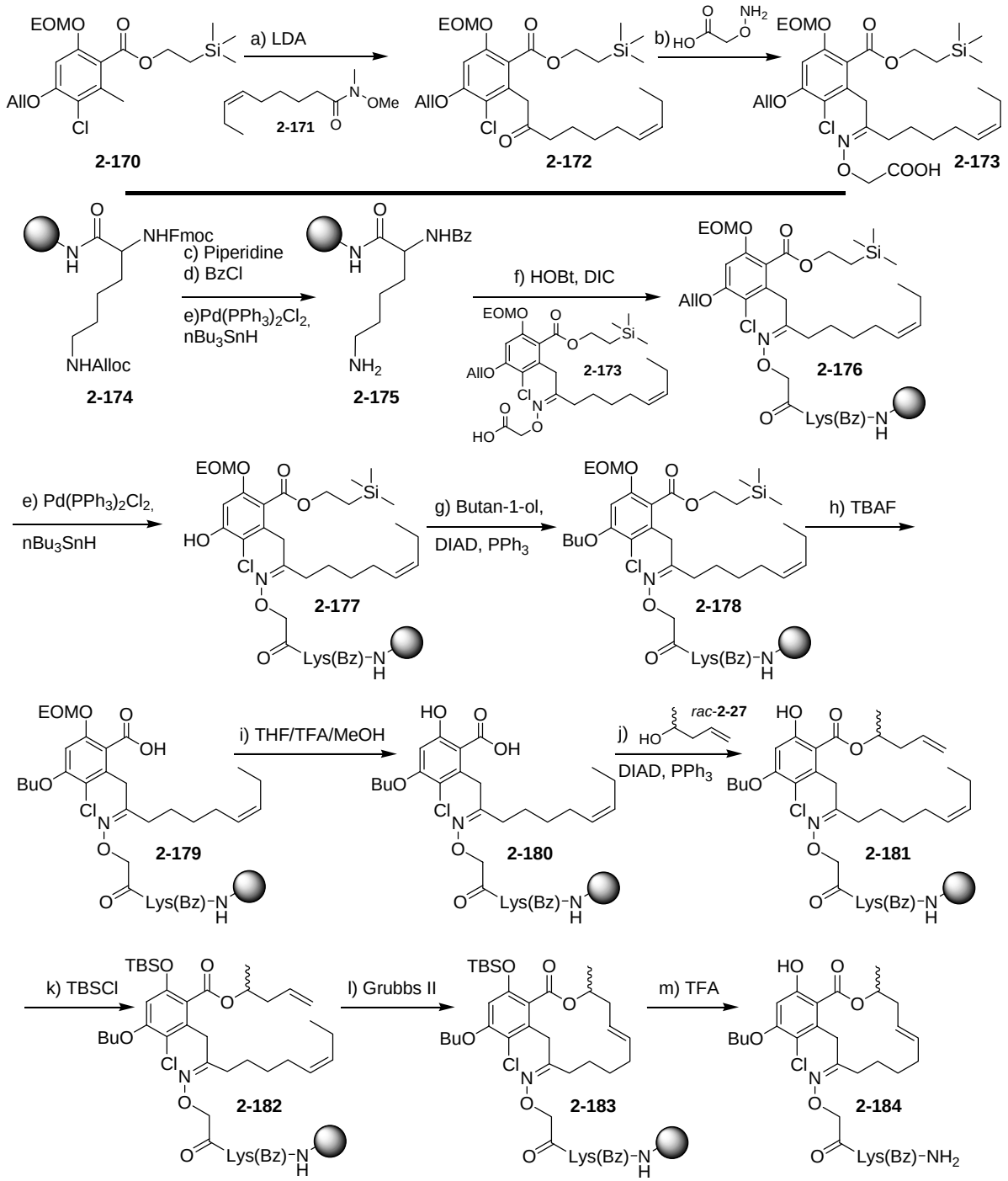
[210] (a) DeRisi, J. et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* **14**, 457-60 (1996), (b) Lockhart, D. J. et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* **14**, 1675-80 (1996), (c) Lockhart, D. J. & Winzeler, E. A. Genomics, gene expression and DNA arrays. *Nature* **405**, 827-36 (2000).

[211] (a) Zhu, H. & Snyder, M. Protein chip technology. *Curr Opin Chem Biol* **7**, 55-63 (2003), (b) Camarero, J. A., Kwon, Y. & Coleman, M. A. Chemoselective attachment of biologically active proteins to surfaces by expressed protein ligation and its application for "protein chip" fabrication. *J Am Chem Soc* **126**, 14730-1 (2004), (c) Hultschig, C. et al. Recent advances of protein microarrays. *Curr Opin Chem Biol* **10**, 4-10 (2006) and references cited therein.

[212] (a) Pellois, J. P. et al. Individually addressable parallel peptide synthesis on microchips. *Nat Biotechnol* **20**, 922-6 (2002), (b) Uttamchandani, M., Walsh, D. P., Yao, S. Q. & Chang, Y. T. Small molecule microarrays: recent advances and applications. *Curr Opin Chem Biol* **9**, 4-13 (2005) and references cited therein

[213] (a) Debaene, F. & Winssinger, N. Azidopeptide nucleic acid. An alternative strategy for solid-phase peptide nucleic acid (PNA) synthesis. *Org Lett* **5**, 4445-7 (2003), (b) Debaene, F., Meijas, L., Harris, J. L. & Winssinger, N. Synthesis of a PNA-encoded cysteine protease inhibitor library. *Tetrahedron* **60**, 8677-8690 (2004), (c) Diaz-Mochon, J. J., Bialy, L. & Bradley, M. Full orthogonality between Dde and Fmoc: the direct synthesis of PNA--peptide conjugates. *Org Lett* **6**, 1127-9 (2004).

diverse biological activities obtained with a pilot library of pochonins (*vide supra*), we became interested in adopting this chemistry to prepare PNA-encoded RAL libraries. Previous works in the laboratory have shown that several reactions such as metathesis, Mitsunobu reactions and of course, amide and ester formation are compatible with the PNA chemistry whereas reactions that requires strong basic conditions such as enolate chemistry are unlikely to work due to the presence of the amide proton on the PNA. Considering these prerequisites, the sequence of assembly needed to be modified. Based on the fact that oxime substitution was tolerated and points toward the solvent (co-crystal structure of radicicol in HSP90), it seemed to be a logical place to connect the pochonin to the PNA. Following the chemistry developed for pochonin D, compound **2-173** was prepared to investigate the solid phase chemistry (Scheme 50). Rink amide resin was derivatized with a lysine following a well-known procedure to afford resin **2-174** which was the starting point of the synthesis. Protecting groups manipulation followed by attachment of the acyclic precursor **2-173** on the solid support afforded clean resin **2-176**. Reaction on solid support were followed by L.C./M.S. after cleavage with a 1:1 mixture of TFA/DCM. Allyl deprotection of the *para*-phenol followed by Mitsunobu reaction yielded compound **2-178**. Further silyl deprotection using TBAF, EOM deprotection and Mitsunobu esterification with an excess of alcohol *rac*-**2-27** afforded compound **2-181** in good purity. The *ortho*-phenol was then protected with a TBS group to yield compound **2-182**. Further metathesis reaction using conditions developed in the laboratory for the synthesis of Aigialomycin D[135] (microwaves irradiation, Grubbs' II, CH_2Cl_2, 120°C) afforded compound **2-184** after cleavage from the resin. With the isolation of compound **2-184**, the chemistry was validated on solid support and further work will consist in combining this synthetic sequence with the PNA chemistry instead of the benzoyl group.



Scheme 50: Synthesis of compound **2-184** on solid support

a) LDA (2.0 equiv.), THF, -78 °C; **2-171** (1.0 equiv.), 10 min, 20%; b) $NH_2OCH_2CO_2H$ (5.0 equiv.), Py/AcOH 5:1, 40 °C, 24 h, 40%; c) Piperidine (20%), DMF, 23 °C, 1 h; d) BzCl (5.0 equiv.), Pyridine (5.0 equiv.), DMF, 23 °C, 12 h; e) $Pd(PPh_3)_2Cl_2$ (0.2 equiv.), PPh_3 (0.8 equiv.), $TMSN_3$ (10.0 equiv.), nBu_3SnH (5.0 equiv.), CH_2Cl_2, 1 h; f) **2-173** (2.0 equiv.), HOBt (6.0 equiv.), DIC (9.0 equiv.), DMF, 4 h; g) Butan-1-ol (5.0 equiv.), PPh_3 (2.0 equiv.), DIAD (2.0 equiv.), THF, 23 °C, 12 h; h) TBAF (2.0 equiv.), THF, 23°C, 4 h; i) THF/TFA/MeOH 7:1.5:1, 23 °C, 12 h; j) *rac*-**2-27** (1.0 equiv.), PPh_3 (2.0 equiv.), DIAD (2.0 equiv.), toluene, 23 °C, 12 h; k) TBSCl (5.0 equiv.), imid. (5.0 equiv.), DMF, 12 h; l) Grubbs' II (0.06 equiv.), CH_2Cl_2, 120°C (microwaves irradiation), 3 x 0.75h; m) TFA/CH_2Cl_2 1:1, 15 min. Reaction were followed by L.C./M.S. after cleavage from the resin with a 1:1 mixture of TFA/CH_2Cl_2.

Conclusions

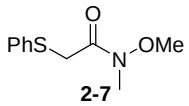
A concise and modular synthesis of pochonin C and radicicol has been achieved in seven and eight steps, respectively, from three readily available fragments. The synthetic studies add a precedent for the feasibility of the *cis*-selective ring-closing metathesis from a triene (four possible RCM products). The combination of polymer-bound reagents and solid-phase reactions also allowed the synthesis of pochonin C, requiring only two traditional chromatographic purifications. The broad scope of this synthetic strategy was shown with the synthesis of cyclopropyl analogs of radicicol. Conformational analysis of radicicol and pochonin C established that closely related resorcylides may have different biological activities due to topological diversity. Molecular minimization revealed that pochonin D could be a potential HSP90 inhibitor. A polymer-assisted synthesis afforded this natural product in six steps (31% overall yield) with only one chromatographic purification of the final compound. Importantly, despite its simple structure, pochonin D is a good ligand for HSP90 with a 80 nM affinity. A synthesis of pochonin A using silyl-protecting groups was also achieved and this product was found to be a good HSP90 ligand although the sensitive epoxide may present a liability as a therapeutic. Based on the high efficiency of pochonin D synthesis using polymer-bound reagents, a library of 113 compounds was prepared. Screening of this library for kinase inhibition yielded a number of leads for therapeutically relevant kinases including Src, EGFR and Aurora A and B. Importantly, the identified promising kinase leads were not HSP90 inhibitors and showed diverse selectivity profiles for kinase inhibition amongst 24 tested kinases. These results suggested the potential of RALs for selective kinase inhibition. Finally, a proof of principle has been established for the synthesis of pochonin D analogs on solid support. Further work towards the development of this synthetic strategy for a “split and mix” library of pochonins using PNA encoding may allow the preparation of pochonin microarrays.

The work carried out during my PhD has led to five publications in peer-reviewed journals (Angewandte Chemie, Journal of the American Chemical Society, Chemistry: A European Journal and Organic Letters).

Part III

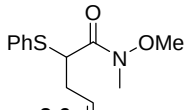
Experimental Section

General Procedures. All substituted polystyrene resins (100−200 mesh, 1 % DVB) and Merrifield resin were obtained from Novabiochem or Aldrich. The Grubbs' II catalyst was purchased from Materia Inc. Solid phase reactions were carried on a Quest 210 or round bottom flasks and filtered in fritted funnels. All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through commercially available alumina column (Innovative technology, VA). Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) by using UV light as visualizing agent and 10 % ethanolic phosphomolybdic acid or vanillin solution and heat as developing agents. E. Merck silica gel (60, particle size 0.040−0.063 mm) was used for flash-column chromatography. Preparative thin layer chromatography (preparative TLC) was carried out on 0.25 mm E. Merck silica gel plate. NMR spectra were recorded on Brucker Advance-400 instruments and calibrated byusing residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and b = broad. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. L.C./M.S. were recorded by using an Agilent 1100 HPLC with a Bruker micro-TOF instrument (ESI). Unless otherwise stated, a Supelco C8 (5 cm x 4.6 mm, 5 μm particules) column was used with a linear elution gradient from 100 % H_2O (0.5 % HCO_2H) to 100 % MeCN in 13 min at a flow rate of 0.5 mL/min. Unless otherwise stated, LDA was prepared at a concentration of 0.566 M by treating a solution of diisopropylamine (1.0 equiv.) in THF at −78 °C with *n*-butyllithium (1.0 equiv.) and stirred for 30 min at this temperature before used.



2-7

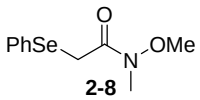
Weinreb amide 2-7: 2-chloro-*N*-methoxy-*N*-methylacetamide (10.3 g, 74.5 mmol) was added at 23 °C to a solution of thiophenol (7.6 mL, 74.5 mmol) and K_2CO_3 (10.3 g, 74.5 mmol) in dry DMF (40 mL). After 4 h stirring at room temperature, the mixture was quenched by addition of a solution of saturated $NH_4Cl_{aq.}$ (50 mL), diluted with Et_2O (50 mL), washed several times with saturated $NH_4Cl_{aq.}$ (30 mL) and brine (30 mL) and dried over $MgSO_4$. Concentration under reduced pressure afforded compound **2-7** (13.4 g, 95 %). R_f = 0.22 (silica gel, EtOAc/hexane 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.46 (d, *J* = 7.6 Hz, 2H), 7.32-7.28 (m, 2H), 7.23-7.19 (m, 1H), 3.84 (s, 2H), 3.70 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 169.9, 135.8, 129.6, 128.9 (x 3), 126.5, 61.4, 35.3, 32.3; IR (film): ν_{max} = 3059, 2925, 1654, 1584, 1438, 1380, 999, 741, 690 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{10}H_{13}O_2NSNa$: 234.0559, found 234.0533 [*M*+Na^+].



2-6

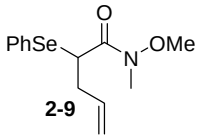
Weinreb amide 2-6: $Sc(OTf)_3$ (49 mg, 0.1 mmol) and H_2O_2 (255 µL, 5.0 mmol) were sequentially added to a solution of compound **2-7** (211 mg, 1.0 mmol) in CH_2Cl_2/EtOH (10:1, 5.5 mL). After 3 h stirring at room temperature, the reaction was diluted with EtOAc (10 mL), washed with a saturated aqueous solution of $NaHCO_3/Na_2S_2O_3$ (10:1, 10.1 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture (206 mg, 0.9 mmol) was then diluted in CH_2Cl_2 (10 mL) and treated with allyltrimethylsilane (723 µL, 4.6 mmol). The resulting solution was cooled down to –78 °C and, trifluoroacetic anhydride (384 µL, 2.7 mmol) was added. After 10 min at –78 °C, the reaction was allowed to warm up to room temperature for 1 h. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-50 % EtOAc/hexane), afforded compound **2-6** (151 mg, 60 %). R_f = 0.34 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.53-7.51 (m, 2H), 7.35-7.32 (m, 3H), 5.88-5.77 (m, 1H), 5.12 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.09 (dd, *J* = 11.2, 1.8 Hz, 1H), 4.21 (bs, 1H), 3.62 (s, 3H), 3.20 (s, 3H), 2.73-2.66 (m, 1H), 2.55-2.48 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 172.0, 134.6, 133.7 (x 2), 132.9, 128.9 (x 2), 128.1, 117.7,

61.4, 46.2, 36.4, 32.4; IR (film): ν_{max} = 2923, 1660, 1654, 1439, 1379, 990, 739, 697 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{13}H_{17}O_2SNa$: 274.0872, found 274.0812 [*M*+Na^+].



2-8

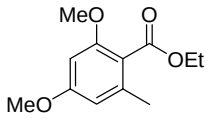
Weinreb amide 2-8: $NaBH_4$ (237 mg, 6.4 mmol) was added to a solution of diphenyl diselenide (1 g, 3.2 mmol) in EtOH/THF (4:1, 32 mL). The reaction was stirred at room temperature until the mixture turned color from orange to light yellow (~1.5 h). At this time, 2-chloro-*N*-methoxy-*N*-methylacetamide (882 mg, 6.4 mmol) was added to the mixture. The reaction was stirred for 30 min and then quenched with brine (50 mL), extracted with EtOAc (50 mL) and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane), afforded compound **2-8** (744 mg, 90 %). R_f = 0.17 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.66–7.64 (m, 2H), 7.31-7.30 (m, 3H), 3.76 (s, 2H), 3.70 (s, 3H), 3.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.2, 133.0, 129.9, 129.1 (x 3), 127.5, 61.3, 32.4, 26.6; IR (film): ν_{max} = 2935, 1661, 1578, 1478, 1438, 1379, 999, 739, 691 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{10}H_{13}O_2NSeNa$: 282.0004, found 282.9980 [*M*+Na^+].



2-9

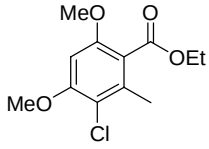
Weinreb amide 2-9: A solution of compound **2-8** (130 mg, 0.5 mmol) in anhydrous THF (5 mL) was treated at –78 °C with LiHMDS (500 µL, 1M solution, 0.5 mmol). After 10 min at this temperature, HMPA (87 µL, 0.5 mmol) and allyl bromide (43 µL, 0.5 mmol) were sequentially added. The resulting solution was then allowed to warm up to 0 °C and followed by TLC until completion (~1 to 3 h). The reaction mixture was diluted with EtOAc and washed several times with 1N HCl and brine. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-30 % EtOAc/hexane gradient) to yield compound **2-9** (127 mg, 85 %). R_f = 0.44 (silica gel, hexane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.61 (d, *J* = 7.0 Hz, 2H), 7.33-7.26 (m, 3H), 5.82-5.71 (m, 1H), 5.08 (d, *J* = 18.1 Hz, 1H), 5.04 (d, *J* = 10.5 Hz, 1H), 4.14 (bs, 1H), 3.59 (s, 3H), 3.15 (s, 3H), 2.77-2.69 (m, 1H), 2.54-2.47 (m, 1H); ^{13}C NMR (100

MHz, $CDCl_3$, 25 °C): δ = 172.6, 135.9 (x 2), 135.3, 128.9 (x 2), 128.4, 127.6, 117.3, 61.3, 38.8, 36.4, 32.5; IR (film): ν_{max} = 3073, 2973, 2936, 1658, 1578, 1477, 1438, 1382, 1175, 992, 919, 740, 692 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{13}H_{17}O_2NSeNa$: 322.0317, found 322.0266 [M+Na^+].



2-10

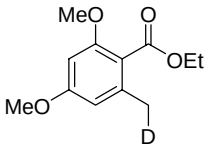
2,4-dimethoxy ester 2-10: A solution of ethyl 2,4-dihydroxy-6-mehtylbenzoate (980 mg, 5 mmol) and potassium carbonate (2.76 g, 20 mmol) in acetone (20 mL) was treated with dimethyl sulfate (1.89 mL, 20 mmol) and heated for 4 h at reflux. The mixture was then concentrated under vacuum, dissolved in EtOAc (20 mL) and washed with 1N HCl (2 x 20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to provide compound **2-10** (1.10 g, 98 %). R_f = 0.43 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.33 (s, 2H), 4.38 (q, J = 7.0 Hz, 2H), 3.81 (s, 6H), 2.32 (s, 3H), 1.38 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 168.2, 161.2, 158.1, 138.0, 116.8, 106.6, 96.2, 60.9, 55.9, 55.3, 19.8, 14.3; IR (film): ν_{max} = 2978, 1724, 1606, 1459, 1267, 1203, 1160, 1098, 1052 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{12}H_{16}O_4Na$: 247.0941, found 247.0872 [M+Na^+].



2-11

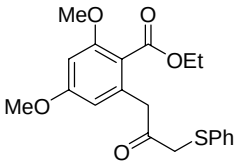
Chlorinated ester 2-11: Sulfamic acid (300 mg, 3.12 mmol) and acetaldehyde (50 µL, 0.89 mmol) were added to a solution of compound **2-10** (200 mg, 0.89 mmol) in THF/H_2O (1:2, 15 mL). After cooling down to 0 °C, sodium chlorite (260 mg, 2.90 mmol) was added and the solution was stirred for 20 h at room temperature. The reation was diluted with saturated $NH_4Cl_{aq.}$ (20 mL) and extracted with EtOAc (15 mL). The organic layer was then dried over $MgSO_4$ and concentrated under vacuum. Crude ^{1}H NMR indicated 60 % conversion. Flash chromatography (silica gel, 0-30 % EtOAc/hexane) afforded compound **2-11** (108 mg, 78 % based on recovery of the starting material). R_f = 0.49 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR

(400 MHz, $CDCl_3$, 25 °C): δ = 6.43 (s, 1H), 4.41 (q, *J* = 7.0 Hz, 2H), 3.95 (s, 3H), 3.87 (s, 3H), 2.35 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.5, 156.4, 155.7, 135.4, 117.9, 114.9, 94.3, 61.3, 56.3, 56.2, 17.4, 14.2; IR (film): ν_{max} = 2982, 1724, 1594, 1459, 1259, 1211, 1081 cm^{-1}; HRMS (ESI-TOF): m/z: calculated for $C_{12}H_{15}O_4ClNa$: 281.0551, found 281.0528 [*M*+Na^+].



2-12

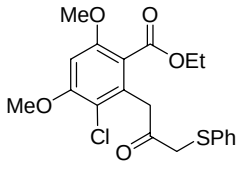
Toluate 2-12: A solution of compound **2-10** (50 mg, 0.22 mmol) in anhydrous THF (500 µL) was treated at –78 °C with freshly prepared LDA (777 µL, 0.44 mmol). The resulting mixture was then stirred for 5 min and quenched by addition of a mixture of D_2O/THF (1:1, 1 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (5 mL), washed several times with saturated $NH_4Cl_{aq.}$ (4 mL) and brine (4 mL), and dried over $MgSO_4$. Concentration under reduced pressure afforded compound **2-12**. Crude ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.34 (s, 2H), 4.38 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 6H), 2.30 (t, *J* = 2.2 Hz, 2H), 1.39 (t, *J* = 7.0 Hz, 3H).



2-13

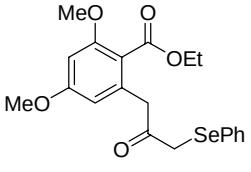
Sulfide 2-13: A solution of compound **2-10** (100 mg, 0.45 mmol) in anhydrous THF (2 mL) was treated at –78 °C with freshly prepared LDA (1.6 mL, 0.90 mmol). After 5 min stirring, a solution of compound **2-7** (95 mg, 0.45 mmol) in THF (0.5 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched at this temperature by addition of saturated $NH_4Cl_{aq.}$ (5 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (8 mL) and brine (8 mL), and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-33 % EtOAc/hexane gradient) afforded compound **2-13** (147 mg, 88 %). R_f = 0.34 (silica gel, EtOAc/hexane 1:3); ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.36-7.33 (m, 2H), 7.31-7.26 (m, 2H), 7.23-7.19 (m, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 6.31 (d,

J = 2.2 Hz, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 3.89 (s, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.78 (s, 2H), 1.34 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 201.8, 167.5, 161.7, 159.1, 134.9, 129.8, 129.4 (x 2), 129.0 (x 2), 126.7, 116.4, 107.6, 98.0, 61.2, 56.0, 55.4, 46.0, 43.1, 14.2; IR (film): ν_{max} = 2980, 1718, 1604, 1459, 1204, 1161, 1092, 741, 691 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{22}O_5SNa$: 397.1080, found 397.1021 [*M*+Na^+].



2-14

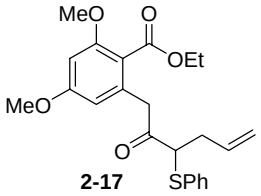
Sulfide 2-14: A solution of compound **2-11** (100 mg, 0.39 mmol) in anhydrous THF (1 mL) was treated at –78 °C with freshly prepared LDA (1.37 mL, 0.77 mmol). After 5 min stirring, a solution of compound **2-7** (82 mg, 0.39 mmol) in THF (0.5 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched at this temperature by addition of saturated $NH_4Cl_{aq.}$ (5 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (8 mL) and brine (8 mL), and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane gradient) afforded compound **2-14** (119 mg, 75 %). R_f = 0.25 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.37 (d, *J* = 7.6 Hz, 2H), 7.32-7.27 (m, 3H), 6.48 (s, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 4.10 (s, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 (s, 2H), 1.28 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.4, 166.8, 156.9, 156.7, 135.0, 132.7, 129.4 (x 2), 129.1 (x 2), 126.7, 117.6, 115.3, 95.8, 61.4, 56.3, 56.3, 43.6, 43.6, 14.1; IR (film): ν_{max} = 2936, 1719, 1592, 1257, 1212, 1083, 741, 691 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{21}O_5ClSNa$: 431.0690, found 431.0624 [*M*+Na^+].



2-15

Selenide 2-15: A solution of compound **2-10** (95 mg, 0.42 mmol) in anhydrous THF (2 mL) was treated at –78 °C with freshly prepared LDA (1.5 mL, 0.85 mmol). After 5 min stirring, a

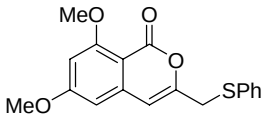
solution of compound **2-8** (109 mg, 0.42 mmol) in THF (0.5 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched at this temperature by addition of saturated $NH_4Cl_{aq.}$ (5 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (8 mL) and brine (8 mL), and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane gradient) afforded compound **2-15** (161 mg, 90 %). R_f = 0.32 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.53-7.52 (m, 2H), 7.29 (m, 3H), 6.41 (s, 1H), 6.33 (s, 1H), 4.33 (q, *J* = 7.0 Hz, 2H), 3.90 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.68 (s, 2H), 1.35 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.0, 167.5, 161.7, 159.0, 135.0, 133.1 (x 2), 129.3 (x 2), 129.1, 127.6, 116.6, 107.4, 97.9, 61.2, 56.0, 55.4, 45.9, 35.1, 14.2; IR (film): ν_{max} = 2937, 1719, 1604, 1272, 1204, 1161, 1097, 1047, 739, 691 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{22}O_5SeNa$: 445.0526, found 445.0683 [*M*+Na^+].



2-17

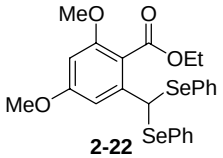
Sulfide 2-17: A solution of compound **2-10** (100 mg, 0.45 mmol) in anhydrous THF (2 mL) was treated at –78 °C with freshly prepared LDA (1.58 mL, 0.89 mmol). After 5 min stirring, a solution of compound **2-6** (112 mg, 0.45 mmol) in THF (0.5 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched at this temperature by addition of saturated $NH_4Cl_{aq.}$ (5 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (8 mL) and brine (8 mL), and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane gradient) afforded compound **2-17** (157 mg, 85 %). R_f = 0.34 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.40-7.39 (m, 2H), 7.32-7.29 (m, 3H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.31 (d, *J* = 2.3 Hz, 1H), 5.85-5.75 (m, 1H), 5.12-5.07 (m, 2H), 4.33 (q, *J* = 7.0 Hz, 2H), 4.08 (d, *J* = 16.1 Hz, 1H), 3.92 (d, *J* = 16.1 Hz, 1H), 3.82 (s + m, 4H), 3.78 (s, 3H), 2.58 (ddd, *J* = 14.9, 7.2, 7.2 Hz, 1H), 2.43 (ddd, *J* = 14.6, 7.2, 7.2 Hz, 1H), 1.35 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.1, 167.6, 161.4, 158.7, 134.6, 134.3, 133.4 (x 2), 131.8, 129.0 (x 2), 128.2, 117.6, 116.8, 107.2, 97.9, 61.1, 55.9, 55.3, 54.5, 45.1, 34.0, 14.2; IR (film): ν_{max} = 2935, 1717,

1604, 1459, 1161, 1096, 1050, 744, 691 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{26}O_5SNa$: 437.1393, found 437.1269 [M+Na^+].



2-21

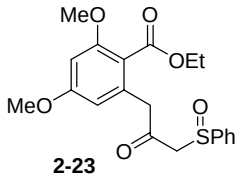
Isocoumarin 2-21: A solution of compound **2-10** (43 mg, 0.19 mmol) in anhydrous THF (1 mL) was treated at –78 °C with freshly prepared LDA (671 µL, 0.38 mmol). After 5 min stirring, a solution of compound **2-7** (40 mg, 0.19 mmol) in THF (0.5 mL) was added dropwise. The resulting mixture was then stirred for 10 min at –78 °C and heated up to 0 °C. After 30 min at this temperature, the red color of the solution had disappeared and TLC revealed the formation of isocoumarin **2-21**. The reaction mixture was warmed up to room temperature, quenched by addition of saturated $NH_4Cl_{aq.}$ (5 mL), diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (8 mL) and brine (8 mL) and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane gradient) afforded compound **2-21** (54 mg, 84 %). R_f = 0.31 (silica gel, EtOAc/hexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.40 (d, J = 7.0 Hz, 2H), 7.33-7.23 (m, 3H), 6.47 (s, 1H), 6.31 (s, 1H), 6.25 (s, 1H), 3.99 (s, 3H), 3.89 (s + s, 3H + 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 165.4, 163.3, 158.9, 154.1, 141.7, 134.8, 130.6 (x 2), 129.1 (x 2), 127.1, 104.6, 103.1, 100.2, 98.7, 56.3, 55.6, 36.3; IR (film): ν_{max} = 2933, 1718, 1663, 1598, 1570, 1458, 1370, 1214, 1164, 742, 690 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{17}O_4S$: 329.0842, found 329.0755 [M+H^+].



2-22

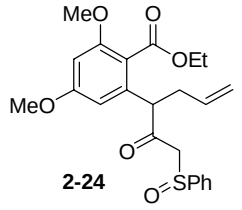
Bis-Selenide 2-22: A solution of compound **2-10** (196 mg, 1 mmol) in anhydrous THF (4 mL) was treated at –78 °C with freshly prepared LDA (2 mL, 1M in THF, 2 mmol). After 5 min stirring, a solution of compound **2-9** (109 mg, 1 mmol) in THF (1 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched at this temperature by addition of saturated $NH_4Cl_{aq.}$ (5 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$

(8 mL) and brine (8 mL), and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane gradient) afforded compound **2-22** (240 mg, 45 %). R_f = 0.45 (silica gel, hexane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.49 (d, *J* = 6.5 Hz, 4H), 7.33-7.22 (m, 6H), 6.74 (d, *J* = 1.6 Hz, 1H), 6.31 (d, *J* = 1.6 Hz, 1H), 5.82 (s, 1H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.1, 161.4, 158.0, 141.4, 136.1, 134.3 (x 4), 131.1, 128.9 (x 4), 128.0 (x 2), 114.8, 105.3, 98.4, 61.2, 56.0, 55.4, 40.1, 14.2; IR (film): ν_{max} = 2977, 2932, 1731, 1704, 1602, 1267, 1159, 1100, 1045, 737, 688 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{24}O_4Se_2Na$: 558.9902, found 558.9874 [*M*+Na$^+$].



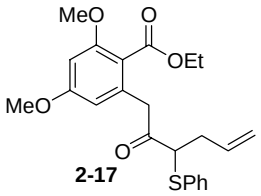
2-23

Sulfoxide 2-23: $Sc(OTf)_3$ (99 mg, 0.2 mmol) and H_2O_2 (257 µL, 5.0 mmol) were added sequentially to a solution of compound **2-13** (380 mg, 1.0 mmol) in CH_2Cl_2/EtOH (10:1, 5.5 mL). After 3 h stirring at room temperature, the reaction was diluted with EtOAc (10 mL), washed with a saturated solution of $NaHCO_3/Na_2S_2O_3$ (10:1, 10.1 mL), dried over $MgSO_4$ and concentrated under reduced pressure to yield compound **2-23** (355 mg, 91 %). R_f = 0.42 (silica gel, EtOAc/hexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.68-7.66 (m, 2H), 7.53-7.52 (m, 3H), 6.42 (d, *J* = 1.2 Hz, 1H), 6.28 (d, *J* = 1.8 Hz, 1H), 4.30 (q, *J* = 7.0 Hz, 2H), 3.92 (d, *J* = 7.0 Hz, 2H), 3.81 (s, 6H), 3.75 (d, *J* = 5.3 Hz, 2H), 1.32 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 198.6, 167.4, 162.0, 159.3, 143.3, 134.2, 131.5, 129.4 (x 2), 124.2 (x 2), 116.1, 107.7, 98.3, 67.4, 61.2, 56.0, 55.5, 49.9, 14.2; IR (film): ν_{max} = 2944, 1720, 1605, 1275, 1162, 1090, 1047, 750, 691 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{12}H_{15}O_4ClNa$: 281.0551, found 281.0528 [*M*+Na$^+$].



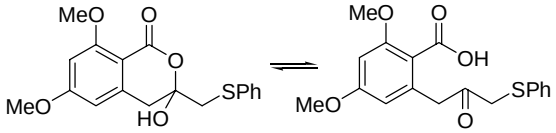
2-24

Sulfoxide 2-24: In a first flask, a mixture of allylacetate (59.8 µL, 0.55 mmol), $[Pd_2(dba)_3]$•$CHCl_3$ (11.4 mg, 0.011 mmol) and dppe (4.4 mg, 0.011 mmol) in THF (1 mL) was stirred for 30 min until the reaction color change from deep purple to orange. In another flask, a solution of compound **2-23** (46 mg, 0.11 mmol) in THF (1 mL) was sequentially treated at room temperature with *t*BuOK (13.2 mg, 0.11 mmol) and the palladium solution. After completion of the reaction, as judged by TLC, the solution was diluted with EtOAc (5 mL), washed several times with saturated $NH_4Cl_{aq.}$ and brine, and dried over $MgSO_4$. Evaporation of solvents, followed by flash chromatography (silica gel, 0-33 % EtOAc/hexane gradient) afforded compound **2-24** (43 mg, 84 %) as a mixture of diastereoisomers 1:1. R_f = 0.45 (silica gel, EtOAc/hexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.68-7.64 (m, 4H), 7.53-7.49 (m, 6H), 6.42 (s, 1H), 6.38 (s, 1H), 6.21 (s, 1H), 6.14 (s, 1H), 5.63-5.55 (m, 2H), 5.03-4.94 (m, 4H), 4.44-4.35 (m, 4H), 4.08 (d, *J* = 14.5 Hz, 1H), 4.03 (d, *J* = 14.0 Hz, 1H), 3.84-3.76 (m, 14H), 3.70 (d, *J* = 14.0 Hz, 1H), 3.65 (d, *J* = 14.5 Hz, 1H), 2.80-2.70 (m, 2H), 2.47-2.38 (m, 2H), 1.40-1.36 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 200.2, 199.7, 167.7, 167.6, 161.9, 161.8, 158.5, 158.4, 143.5, 143.2, 136.3, 136.0, 134.8, 134.6, 131.4, 131.1, 129.3 (x 2), 129.2 (x 2), 124.3 (x 2), 124.2 (x 2), 117.8, 117.7, 117.2, 117.1, 104.0, 98.2, 98.1, 67.6, 66.9, 61.6, 61.5, 56.9, 56.2, 55.9 (x 2), 55.5 (x 2), 35.4 (x 2), 14.3, (2 quaternary carbons are not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{26}O_6SNa$: 453.1342, found 453.1363 [M+Na^+].



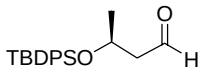
2-17

Sulfide 2-17 from sulfoxide 2-23: A solution of compound **2-23** (500 mg, 1.28 mmol) in CH_2Cl_2 (12.8 mL) was treated at –78 °C with allyltrimethylsilane (1.02 mL, 6.40 mmol). After 30 min stirring, trifluoroacetic anhydride (543 µL, 3.84 mmol) was added to the mixture. After 10 min at this temperature, the reaction was warmed up to room temperature. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-30 % EtOAc/hexane), afforded compound **2-17** (425 mg, 80 %).



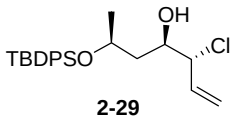
2-26 **2-25**

Compounds 2-25 and 2-26: A preformed solution of 3-hydroxypropionitrile (77 µL, 1.13 mmol) and sodium hydride (18 mg, 0.45 mmol) in THF (1.5 mL) was added to a solution of compound **2-13** (37 mg, 0.11 mmol) in THF (1.5 mL) at 0 °C. The reaction was warmed up to room temperature and after stirring for 10 min, was quenched with 1N HCl (5 mL), diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (2 x 5 mL) and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-100 % EtOAc/hexane gradient) afforded a mixture of compounds **2-25** and **2-26** (36 mg, 93 %). R_f = 0.51 (silica gel, EtOAc); ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 7.50-7.48 (d, *J* = 7.6 Hz, 2H), 7.39-7.29 (m, 6H), 7.24-7.20 (m, 2H), 6.63 (d, *J* = 1.8 Hz, 1H), 6.54 (s, 1H), 6.49 (d, *J* = 1.8 Hz, 1H), 6.45 (s, 1H), 4.07 (s, 2H), 4.02 (s, 2H), 3.94 (s, 3H), 3.90-3.86 (m, 10H), 3.85 (s, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{18}O_5SNa$: 369.0767, found 369.0662 [*M*+Na^+].



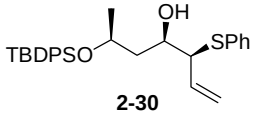
2-28

Aldehyde 2-28: Ozone was bubbled into the cooled solution (–78 °C) of (*S*)-2-(*tert*-butyldiphenylsilyloxy)pent-4-ene (820 mg, 2.52 mmol) in CH_2Cl_2 (40 mL) until it turned blue (~5 min). The reaction was purged with argon and triphenylphosphine (990 mg, 3.78 mmol) was added. The solution was removed from the cold bath and stirred at 23 °C for 2 h. Concentration under reduced pressure and purification by flash chromatography (silica gel, 0-2 % Et_2O/hexane gradient) furnished aldehyde **2-28** (750 mg, 94 %). R_f = 0.40 (silica gel, hexane/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.79 (s, 1H), 7.73-7.68 (m, 4H), 7.50-7.38 (m, 6H), 4.43-4.33 (m, 1H), 2.56 (ddd, *J* = 15.8, 5.9, 2.9 Hz, 1H), 2.49 (ddd, *J* = 15.8, 5.9, 2.3 Hz, 1H), 1.21 (d, *J* = 6.4 Hz, 3H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.0, 135.8 (x 4), 134.0, 133.6, 129.8, 129.7, 127.7 (x 2), 127.6 (x 2), 65.7, 52.7, 26.9 (x 3), 23.8, 19.2; IR (film) ν_{max} = 2959, 2930, 2857, 1709, 1428, 1113, 998 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{26}O_2SiNa$: 349.1594, found 349.1596 [*M*+Na^+]. (-)-(2*S*): $[\alpha]^{20}_D = -4.0$ (*c* 1.00, $CHCl_3$).



2-29

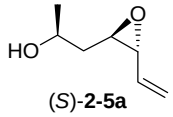
Chlorohydrin 2-29: To a stirred and cooled (−95 °C; Et_2O; dry ice) mixture of (-)-$^{d}Ipc_2BOMe$ (5.12 g, 16.2 mmol) and allyl chloride (1.76 mL, 21.6 mmol) in anhydrous Et_2O (78 mL) was added a solution of LiN(*c*-Hex)$_2$ (21.6 mmol) [prepared from dicyclohexylamine (4.3 mL, 21.6 mmol) in THF (20 mL) by deprotonation with *n*-BuLi (13.5 mL, 1.6 M in hexane, 21.6 mmol) and stirring at 0 °C for 0.5 h]. The mixture was stirred at −95 °C and after 1 hour, BF_3•OEt_2 (3.19 mL, 27.0 mmol) was added slowly. After stirring 0.5 h, a cooled (−95 °C) solution of **2-28** (3.52 g, 10.8 mmol) in anhydrous Et_2O (10 mL) was carefully added dropwise. The mixture was maintained at −95 °C for an additional 2.5 h and quenched by the addition of MeOH (11 mL). Then, 3M NaOAc (11 mL) and 35 % H_2O_2 (6.7 mL) were sequentially added and the reaction was allowed to slowly warm up to room temperature for ~10 h. Then, water was added and the reaction mixture was extracted with Et_2O (3 x 50 mL). The combined organic layers were sequentially washed with saturated $NH_4Cl_{aq.}$, brine and finally dried over $MgSO_4$. Concentration under reduced pressure and purification by flash chromatography (silica gel, 0-10 % Et_2O/hexane gradient) yield chlorohydrin **2-29** (2.96 g, 68 %). R_f = 0.39 (silica gel, hexane/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.77-7.73 (m, 4H), 7.50-7.41 (m, 6H), 6.01-5.92 (ddd, *J* = 17.0, 10.6, 8.1 Hz, 1H), 5.38 (d, *J* = 16.7 Hz, 1H), 5.29 (d, *J* = 10.6 Hz, 1H), 4.31 (dd, *J* = 8.3, 5.6 Hz, 1H), 4.27-4.22 (m, 1H), 4.11-4.06 (m, 1H), 3.02 (d, *J* = 3.8 Hz, 1H), 1.73-1.69 (m, 2H), 1.17 (d, *J* = 6.5 Hz, 3H), 1.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 135.9 (x 2), 135.9 (x 2), 135.2, 134.0, 133.5, 129.9, 129.8, 127.7 (x 2), 127.6 (x 2), 118.7, 71.2, 68.0, 67.8, 41.7, 27.0 (x 3), 23.2, 19.2; IR (film): ν_{max} = 3475, 2962, 2951, 2857, 1472, 1428, 1378, 1112 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{31}O_2SiClNa$: 425.1674, found 425.1603 [*M*+Na^+]. (-)-(2*S*, 4*R*, 5*R*): $[\alpha]^{25}_D$ = − 11.8 (*c* 1.00, $CHCl_3$).



2-30

Sulfide 2-30: A solution of thiophenol (2.3 mL, 22.0 mmol) in anhydrous DMF (100 mL) at room temperature was treated with potassium *tert*-butoxide (1.8 g, 16.4 mmol) and stirred at this temperature for 1 h. The mixture was filtered and added dropwise to a 0 °C solution of halohydrin **2-29** (2.0 g, 5.0 mmol) in anhydrous DMF (100 mL). The resulting mixture was

allowed to warm up to room temperature and stirred until comsumption of the starting material (reaction monitored by TLC). The reaction mixture was diluted with Et_2O and washed several times with water and brine. The organic phase was then dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 % Et_2O/hexane gradient) to provide compound **2-30** (1.98 g, 86 %). R_f = 0.39 (silica gel, hexane/EtOAc 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.76-7.72 (m, 4H), 7.51-7.42 (m, 8H), 7.36-7.29 (m, 3H), 5.97-5.88 (m, 1H), 5.16 (d, *J* = 10.2 Hz, 1H), 5.06 (d, *J* = 17.2 Hz, 1H), 4.27-4.21 (m, 2H), 3.66 (dd, *J* = 8.5, 4.0 Hz, 1H), 3.07 (d, *J* = 2.7 Hz, 1H), 1.80 (ddd, *J* = 14.0, 10.2, 3.8 Hz, 1H), 1.68 (ddd, *J* = 14.5, 6.4, 2.1 Hz, 1H), 1.67 (d, *J* = 6.4 Hz, 3H), 1.09 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 135.9, 134.4, 134.2, 134.1, 133.5, 132.7, 129.8, 129.7, 128.8, 127.7, 127.5, 127.3, 118.1, 69.1, 67.9, 59.3, 42.7, 26.9, 23.1, 19.2; IR (film): ν_{max} = 3408, 2929, 1472, 1427, 1378, 1111 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{36}O_2SiSNa$: 499.2098, found 499.2007 [*M*+Na$^+$]. (-)-(2*S*, 4*R*, 5*S*): $[\alpha]^{25}_D$ = − 14.2 (*c* 1.00, $CHCl_3$).

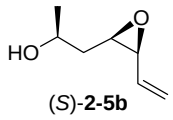


(*S*)-**2-5a**

Alcohol (*S*)-**2-5a:** A solution of the β-hydroxyphenylsulfide **2-30** (1.30 g, 2.7 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a cooled (0 °C) and well-stirred suspension of trimethyloxonium tetrafluoroborate (800 mg, 5.5 mmol) in dry CH_2Cl_2 (20 mL). The resulting mixture was stirred at 0 °C for 2 h, warmed to room temperature and stirred until TLC analysis indicated quantitative formation of the sulfonium salt by expense of the starting material (ca. 2-3 h). After re-cooling to 0 °C, the reaction was diluted with CH_2Cl_2 (10 mL) and a solution of DBU (1.2 mL, 8.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise. After stirring at 0 °C for 4 h, water was added and the mixture was quickly partitioned between water and Et_2O. After washing with brine and drying with $MgSO_4$, the organic phase was concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-5 % Et_2O/hexane gradient) to yield (*S*)-TBDPS-protected-**2-5a** (7.8 g, 80 % over 2 steps). R_f = 0.6 (silica gel, hexane/EtOAc 5:1)).

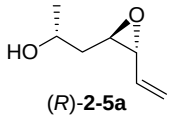
nBu_4NF (10.74 mL, 1.0 M solution in THF, 10.74 mmol) was added dropwise at 23 °C to a solution of (*S*)-TBDPS-protected-**2-5a** (3.28 g, 8.95 mmol) in anhydrous THF (85 mL). The reaction was stirred for 6 h and then, concentrated under reduced pressure. Flash

chromatography (silica gel, 0-25 % Et_2O/hexane gradient) provided alcohol (*S*)-**2-5a** (1.1 g, 98 %). R_f = 0.14 (silica gel, Et_2O/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.50 (ddd, *J* = 17.1, 9.7, 7.5 Hz, 1H), 5.44 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.25 (dd, *J* = 9.7, 1.6 Hz, 1H), 3.98 (m, 1H), 3.18 (dd, *J* = 7.5, 2.2 Hz, 1H), 3.03-3.00 (m, 1H), 1.82 (ddd, *J* = 13.9, 8.1, 4.3 Hz, 1H), 1.58 (ddd, *J* = 14.5, 7.0, 4.3 Hz, 1H), 1.20 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 135.3, 119.5, 65.5, 58.3, 58.0, 40.1, 23.4; IR (film): ν_{max} = 3413, 2969, 1458, 1408, 1137 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_7H_{13}O_2$: 129.0910, found 129.0716 [*M*+H$^+$]. (+)-(2*S*, 4*R*, 5*R*): $[\alpha]^{25}_D$ = + 35.4 (*c* 1.00, $CHCl_3$).



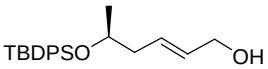
(*S*)-**2-5b**

Alcohol (*S*)-**2-5b:** Halohydrin **2-29** (2.96 g, 7.3 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and treated with a solution of DBU (3.3 mL, 22.0 mmol) in CH_2Cl_2 (15 mL) at 0 °C. Stirring was continued at 0 °C until TLC showed quantitative conversion of the halohydrin (~8 h). The mixture was poured into a saturated $NaHCO_{3aq.}$ solution (20 mL), the organic layer separated, and the aqueous phase was extracted with Et_2O (3 x 20 mL). The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatography (silica gel, 0-5 % Et_2O/hexane gradient) to yield compound (*S*)-TBDPS-**2-5b** (2.6 g, 97 %). R_f = 0.58 (silica gel, hexane/EtOAc 5:1). *n*Bu_4NF (10.74 mL, 1.0 M solution in THF, 10.74 mmol) was added dropwise at 23 °C to a solution of compound (*S*)-TBDPS-**2-5b** (3.28 g, 8.95 mmol) in anhydrous THF (85 mL). The reaction was stirred for 6 h and then, concentrated under reduced pressure. Flash chromatography (silica gel, 0-25 % Et_2O/hexane gradient) provided alcohol (*S*)-**2-5b** (1.1 g, 98 %). R_f = 0.15 (silica gel, Et_2O/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.76 (ddd, *J* = 17.2, 10.2, 3.5 Hz, 1H), 5.49 (d, *J* = 16.7 Hz, 1H), 5.40 (d, *J* = 10.5 Hz, 1H), 4.15-4.05 (m, 1H), 3.48 (dd, *J* = 7.0, 2.6 Hz, 1H), 3.34-3.29 (m, 1H), 1.76 (ddd, *J* = 14.3, 7.6, 4.7 Hz, 1H), 1.68 (ddd, *J* = 14.0, 7.6, 4.4 Hz, 1H), 1.31 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 132.4, 120.6, 66.2, 56.9, 55.9, 36.7, 23.9; IR (film): ν_{max} = 3408, 2966, 1452, 1140 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_7H_{12}O_2Na$: 151.0729, found 151.0426 [*M*+Na$^+$]. (+)-(2*S*, 4*R*, 5*S*): $[\alpha]^{25}_D$ = + 3.8 (*c* 0.16, $CHCl_3$).



(*R*)-**2-5a**

Alcohol (*R*)-**2-5a:** R_f = 0.16 (silica gel, Et_2O/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.53 (ddd, *J* = 17.0, 8.8, 8.6 Hz, 1H), 5.43 (d, *J* = 16.9 Hz, 1H), 5.24 (d, *J* = 9.4 Hz, 1H), 4.03-3.97 (m, 1H), 3.10 (d, *J* = 7.6 Hz, 1H), 2.99 (m, 1H), 1.80-1.75 (m, 1H), 1.68 (ddd, *J* = 14.1, 10.7, 7.0 Hz, 1H), 1.20 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 135.3, 119.4, 66.0, 58.3, 58.2, 40.8, 23.3; IR (film): ν_{max} = 3408, 2967, 1452, 1144 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_7H_{12}O_2Na$: 151.0729, found 151.0449 [*M*+Na^+]. (+)-(2*R*, 4*R*, 5*R*): $[\alpha]^{25}_D$ = +13.1 (*c* 1.00, $CHCl_3$).

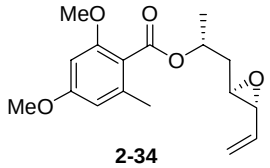


2-31

Alcohol 2-31: A solution of (*S*)-4-penten-2-ol **2-27** (228 mg, 2.65 mmol) in anhydrous CH_2Cl_2 (9 mL) at 0 °C was treated with imidazole (306 mg, 4.50 mmol) in one portion. After 10 min stirring, *tert*-butyldiphenylchlorosilane (760 µL, 2.91 mmol) was added dropwise and the reaction was allowed to warm to 23 °C and stirred for 4 h. Then, the reaction mixture was diluted with Et_2O and washed successively with 5 % $NH_4Cl_{aq.}$ and brine. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, hexane) to provide (*S*)-2-(*tert*-butyldiphenylsilyloxy)pent-4-ene (0.85 g, 98 %). R_f = 0.44 (silica gel, hexane/Et_2O 10:1)).

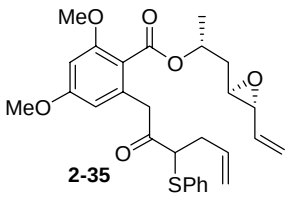
A solution of this compound (287 mg, 1.0 mmol) in toluene (5 mL) was treated at room temperature with but-2-ene-1,4-diol (329 µL, 4.0 mmol). The mixture of both compounds was heated up to 80 °C and Grubbs' II catalyst (80 mg, 0.1 mmol) was added once the solution was hot. The reaction was then stirred for 12 h at this temperature. Evaporation of the solvents, followed by flash chromatography (silica gel, 0-33 % EtO_2/hexane), afforded compound **2-31** (199 mg, 56 %) and found to be identical to previously reported compound **2-31**.[144] R_f = 0.50 (silica gel, hexane/Et_2O 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.72-7.69 (m, 4H), 7.47-7.39 (m, 6H), 5.63-5.59 (m, 2H), 4.05 (d, *J* = 3.8 Hz, 2H), 3.93 (m, 1H), 2.26-2.17 (m, 2H), 1.12 (d, *J* = 6.1 Hz, 3H), 1.09 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 135.9 (x 4), 134.6, 134.5, 131.5, 129.6, 129.5, 129.1, 127.6 (x 2), 127.5 (x 2), 69.4, 63.6, 42.4, 27.1 (x 3), 23.1, 19.3; IR (film): ν_{max} = 3332, 2930, 2857, 1427, 1111, 997 cm^{-1};

HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{30}O_2SiNa$: 377.1907, found 377.1932 [*M*+Na^+]. (-)-(2*E*, 5*S*): $[\alpha]^{25}_D = -20.6$ (*c* 1.00, $CHCl_3$).



2-34

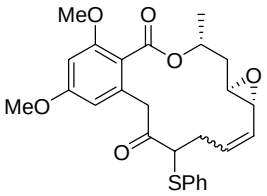
Ester 2-34: Oxalyl chloride (219 µL, 2.6 mmol) was added at 0 °C to a solution of 2,4-methoxy-6-methylbenzoic acid (500 mg, 2.6 mmol) in anhydrous CH_2Cl_2 (10 mL) and catalytic DMF (10 µL). The solution was then stirred at 25 °C. After 1 h, the reaction was recooled at 0 °C, and treated sequentially with Et_3N (798 µL, 5.8 mmol), alcohol (*R*)-**2-5a** (260 mg, 2.0 mmol) and a solution of DMAP (catalytic amount) in anhydrous CH_2Cl_2 (3 mL). The reaction was followed by TLC until consumption of the starting material (~3 h). It was then diluted with CH_2Cl_2 (50 mL), washed with saturated $NH_4Cl_{aq.}$ (50 mL) and dried over $MgSO_4$. Evaporation of the solvents followed by flash chromatography (silica gel**,** 0-10 % EtOAc/hexane gradient) yielded ester **2-34** (625 mg, 80 %). R_f = 0.33 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.34(s, 2H), 5.64-5.57 (m, 1H), 5.49 (d, *J* = 17.6 Hz, 1H), 5.39-5.34 (m, 1H), 5.30 (d, *J* = 10.5 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.18 (d, *J* = 7.3 Hz, 1H), 3.06-3.03 (m, 1H), 2.32 (s, 3H), 2.09-2.02 (m, 1H), 1.93-1.87 (m, 1H), 1.44 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.5, 161.2, 158.0, 137.7, 135.4, 119.0, 116.7, 106.6, 96.1, 69.0, 57.9, 56.9, 55.7, 55.2, 38.0, 19.9, 19.7; IR (film): ν_{max} = 2977, 1720, 1605, 1459, 1327, 1266, 1202, 1151, 1096, 1051 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{17}H_{22}O_5Na$: 329.1359, found 329.1273 [*M*+Na^+]. (-)-(2*R*, 4*R*, 5*R*): $[\alpha]^{25}_D = -22.2$ (*c* 0.46, $CHCl_3$).



2-35

Sulfide 2-35: A solution of compound **2-34** (150 mg, 0.49 mmol) in anhydrous THF (1 mL) was treated at –78 °C with freshly prepared LDA (1.78 mL, 0.98 mmol). After 5 min stirring, a solution of compound **2-6** (123 mg, 0.49 mmol) in THF (0.7 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched by addition of saturated

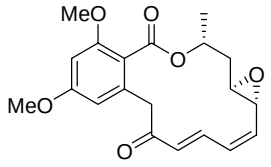
$NH_4Cl_{aq.}$. Upon warming to room temperature, the reaction was diluted with EtOAc, washed several times with saturated $NH_4Cl_{aq.}$, brine and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-50 % EtOAc/cyclohexane gradient) afforded sulfide **2-35** as a mixture of two diasteroisomers 1:1 (182 mg, 75 %). R_f = 0.28 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.39 (bs, 2H), 7.31-7.30 (m, 3H), 6.40 (d, *J* = 1.2 Hz, 1H), 6.30 (s, 1H), 5.86-5.76 (m, 1H), 5.65-5.58 (m, 1H), 5.48 (d, *J* = 16.9 Hz, 1H), 5.32-5.27 (m, 2H), 5.12-5.07 (m, 2H), 4.16-3.90 (m, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 3.85-3.77 (m, 1H), 3.14 (bd, *J* = 7.0 Hz, 1H), 3.06-3.05 (m, 1H), 2.62-2.55 (m, 1H), 2.47-2.39 (m, 1H), 2.02-1.88 (m, 2H), 1.39 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.2, 167.2, 161.6, 158.8, 135.5, 134.9, 134.4, 133.4, 129.0 (x 3), 128.3, 128.2, 119.2, 117.7, 116.8, 107.3, 97.9, 69.4, 58.1, 57.0, 55.8, 55.4, 54.8, 45.0, 38.1, 34.1, 19.9; IR (film): ν_{max} = 2973, 2939, 1713, 1604, 1579, 1459, 1272, 1205, 1161, 1094, 1048, 922, 750, 692 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{28}H_{32}O_6SNa$: 519.1812, found 519.1662 [M+Na$^+$].



2-36

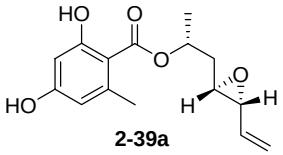
Macrocycle 2-36: A 2 mM solution of compound **2-35** (150 mg, 0.3 mmol) in anhydrous toluene was heated at reflux and treated with 5 % mol of Grubbs' II catalyst (12 mg, 0.015 mmol). The reaction mixture was stirred for 10 min at this temperature and quenched quickly by cooling down to –78 °C. The reaction mixture was then filtered through a pad of silica gel, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/cyclohexane gradient) afforded **2-36** (123 mg, 87 %) as a mixture of diastereoisomers. R_f = 0.22 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.57-7.55 (m, 4H), 7.39-7.37 (m, 6H), 6.39 (s, 2H), 6.05 (s, 2H), 5.79-5.72 (m, 2H), 5.26-5.20 (m, 2H), 5.02 (dd, *J* = 15.8, 8.8 Hz, 2H), 4.01 (d, *J* = 19.3 Hz, 2H), 3.84-3.77 (m, 2H), 3.82 (s, 6H), 3.79 (s, 6H), 3.65 (d, *J* = 19.3 Hz, 2H), 3.13 (d, *J* = 8.8 Hz, 2H), 2.80-2.77 (m, 2H), 2.53-2.30 (m, 8H), 1.36 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 203.0, 167.8, 161.5, 159.1, 135.0, 134.5 (x 2), 129.2 (x 2), 129.1, 128.9, 128.3, 107.9, 98.0, 70.5, 68.5, 56.8, 55.9, 55.8, 55.4, 54.1, 53.2, 48.4, 37.1, 34.7, 20.4;

IR (film): ν_{max} = 2924, 2854, 1712, 1604, 1459, 1272, 1204, 1162, 1094, 1047, 747, 692 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{28}O_6SNa$: 491.1499, found 491.1364 [M+Na^+].



2-37

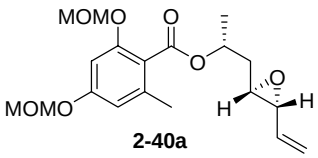
Bismethyl-monocillin 2-37: A solution of compound **2-36** (100 mg, 0.21 mmol) in MeOH (20 mL) was treated with an aqueous solution of $NaIO_4$ (68.5 mg in 200 µL of water, 0.32 mmol). The reaction was stirred for 8 h at room temperature. After extraction with EtOAc (20 mL), the organic phase was washed with saturated $NH_4Cl_{aq.}$ (20 mL), dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The major product was isolated by flash chromatography (silica gel, 0-33 % EtOAc/hexane) to recover 40 mg (40 %) of sulfoxide-**2-36**. The resulting compound was then dissolved in toluene (10 mL) and heated up to 80 °C for 1 h. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-33 % EtOAc/hexane), afforded bismethyl-monocillin **2-37** (24 mg, 32 % after two steps), which was found to have identical proton NMR to previously reported compound **2-37**.[145] R_f = 0.45 (silica gel, hexane/ EtOAc 1:1); ^{1}H NMR (400 MHz, CD_3OH , 25 °C): δ = 7.33-7.26 (dd, *J* = 16.0, 11.1 Hz, 1H), 6.53 (d, *J* = 2.0 Hz, 1H), 6.34 (s, 1H), 6.23 (dd, *J* = 11.0, 11.0 Hz, 1H), 6.01 (d, *J* = 16.1 Hz, 1H), 5.90 (d, *J* = 11.2 Hz, 1H), 5.34-5.30 (m, 1H), 4.27 (d, *J* = 13.9 Hz, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.51 (d, *J* = 13.7 Hz, 1H), 3.22 (d, 1H, obscured by CD_3OD), 3.15-3.11 (m, 1H), 2.46 (d, *J* = 14.4 Hz, 1H), 1.56-1.52 (m, 1H), 1.51 (d, *J* = 6.1 Hz, 3H).



2-39a

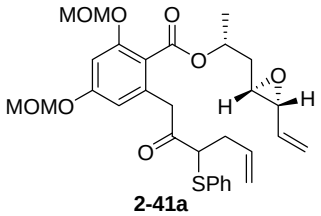
Compound 2-39a: A solution of compound **2-38** (336 mg, 2.0 mmol), compound (*S*)-**2-5a** (256 mg, 2.0 mmol) and tris(3-chlorophenyl)phosphine (1.46 g, 4.0 mmol) in anhydrous toluene (5 mL) was treated at room temperature with DIAD (788 µL, 4.0 mmol). After stirring for 3 h, the reaction mixture was diluted with EtOAc and washed several times with saturated $NH_4Cl_{aq.}$ and brine. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 %

EtOAc/cyclohexane gradient) to yield compound **2-39a** (467 mg, 84 %). R_f = 0.31 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.84 (s, 1H), 6.29 (d, *J* = 2.2 Hz, 1H), 6.26 (s, 1H), 6.22 (d, *J* = 2.2 Hz, 1H), 5.63-5.51 (m, 1H), 5.49 (d, *J* = 16.1 Hz, 1H), 5.47-5.41 (m, 1H), 5.32 (d, *J* = 10.2 Hz, 1H), 3.18 (dd, *J* = 7.0, 1.6 Hz, 1H), 3.02 (m, 1H), 2.52 (s, 3H), 2.10-1.96 (m, 2H), 1.45 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.1, 165.4, 160.7, 143.9, 134.8, 120.1, 111.6, 105.4, 101.3, 69.9, 58.4, 57.3, 38.2, 24.6, 20.1; IR (film): ν_{max} = 2928, 1646, 1448, 1383, 1313, 1260, 1199, 1159, 1106 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{15}H_{18}O_5Na$: 301.1046, found 301.1067 [*M*+Na$^+$]. (-)-(2*R*, 4*S*, 5*R*): $[\alpha]^{25}_D$ = − 15.0 (*c* 0.48, $CHCl_3$);



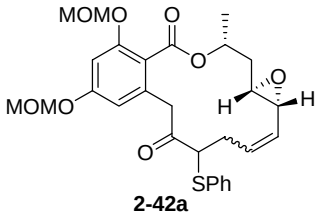
2-40a

Compound 2-40a: To a stirred solution of compound **2-39a** (467 mg, 1.6 mmol) in anhydrous DMF (5.5 mL) at room temperature were added in sequential fashion: diisopropylethylamine (1.06 mL, 6.4 mmol), tetrabutylammonium iodide (catalytic amount) and chloromethylmethyl ether (486 µL, 6.4 mmol). The resulting solution was heated up to 80 °C and stirred for 4 h at this temperature. The reaction was then allowed to cool down to room temperature, diluted with EtOAc and washed several times with saturated $NH_4Cl_{aq.}$. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-50 % EtOAc/cyclohexane gradient) to yield compound **2-40a** (719 mg, 91 %). R_f = 0.40 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.69 (d, *J* = 1.6 Hz, 1H), 6.57 (d, *J* = 1.6 Hz, 1H), 5.6 (ddd, *J* = 17.2, 10.2, 7.5 Hz, 1H), 5.49 (dd, *J* = 17.2, 1.1 Hz, 1H), 5.42-5.36 (m, 1H), 5.30 (dd, *J* = 10.2, 1.1 Hz, 1H), 5.18 (s, 2H), 5.16 (d, *J* = 1.1 Hz, 2H), 3.49 (s, 3H), 3.48 (s, 3H), 3.18 (dd, *J* = 7.0, 2.2 Hz, 1H), 3.03 (td, *J* = 5.6, 2.2 Hz, 1H), 2.32 (s, 3H), 2.00 (ddd, *J* = 14.5, 5.9, 5.9 Hz, 1H), 1.90 (ddd, *J* = 14.5, 5.4, 5.4 Hz, 1H), 1.45 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.4, 158.6, 155.2, 137.7, 135.3, 119.2, 118.6, 110.6, 101.0, 94.6, 94.2, 69.2, 57.9, 56.9, 56.1, 56.0, 38.1, 19.9, 19.6; IR (film): ν_{max} = 2933, 1724, 1607, 1452, 1320, 1268, 1214, 1268, 1148, 1095, 1051, 1025, 926 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{26}O_7Na$: 389.1571, found 389.1601 [*M*+Na$^+$]. (-)-(2*R*, 4*S*, 5*R*): $[\alpha]^{25}_D$ = − 15.3 (*c* 0.51, $CHCl_3$).



2-41a

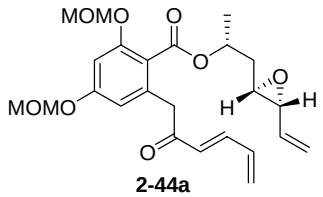
Compound 2-41a: A solution of compound **2-40a** (366 mg, 1.0 mmol) in anhydrous THF (1 mL) was treated at –78 °C with freshly prepared LDA (3.8 mL, 2.0 mmol). After 5 min stirring, a solution of compound **2-6** (251 mg, 1.0 mmol) in THF (0.7 mL) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched by addition of a solution of saturated NH_4Cl_{aq}. Upon warming to room temperature, the reaction was diluted with EtOAc, washed several times with saturated NH_4Cl_{aq}, brine and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-10 % EtOAc/cyclohexane gradient) afforded compound **2-41a** (450 mg, 81 %) as a mixture of diastereoisomers. R_f = 0.35 (silica gel, hexane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.40-7.35 (m, 4H), 7.33-7.30 (m, 6H), 6.69 (d, *J* = 2.2 Hz, 2H), 6.57 (d, *J* = 2.2 Hz, 2H), 5.83-5.77 (m, 2H), 5.63-5.44 (m, 4H), 5.35-5.26 (m, 4H), 5.16 (s, 4H), 5.14 (s, 4H), 5.18-5.08 (m, 4H), 4.10 (d, *J* = 16.1 Hz, 1H), 4.07 (d, *J* = 16.6 Hz, 1H), 4.02 (d, *J* = 16.5 Hz, 1H), 3.97 (d, *J* = 16.1 Hz, 1H), 3.85-3.77 (m, 2H), 3.48 (s, 6H), 3.46 (s, 6H), 3.13 (dd, *J* = 7.3, 2.2 Hz, 2H), 3.08-3.04 (m, 2H), 2.66-2.55 (m, 2H), 2.47-2.39 (m, 2H), 1.96-1.92 (m, 4H), 1.41 (d, *J* = 6.4 Hz, 6H).



2-42a

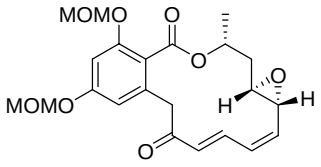
Compound 2-42a: A 2 mM solution of compound **2-41a** (100 mg, 0.18 mmol) in anhydrous toluene was heated at 120 °C and treated with 5 % mol of Grubbs' II catalyst (8 mg, 0.009 mmol). The reaction mixture was stirred for 10 min at this temperature and quenched quickly by cooling down to –78 °C. The reaction mixture was then filtered through a pad of silica gel, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded compound **2-42a** (89 mg, 94 %) as a mixture of diastereoisomers (epimers and mixture of *cis/trans* olefin 1:1). By preparative TLC (silica gel, 50 % EtOAc/hexane), it was possible to separate the two epimers.

R_f = 0.10 (silica gel, EtOAc/hexane 1:3); *Less polar epimer:* ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.43 (d, *J* = 7.0 Hz, 2H), 7.35-7.26 (m, 3H), 6.73 (d, *J* = 2.4 Hz, 1H), 6.19 (d, *J* = 2.1 Hz, 1H), 5.88-5.81 (m, 1H), 5.53-5.46 (m, 1H), 5.17-5.13 (m, 1H), 5.15 (d, *J* = 2.4 Hz, 2H), 5.07 (d, *J* = 1.2 Hz, 2H), 4.23 (d, *J* = 18.7 Hz, 1H), 4.00-3.93 (m, 1H), 3.91 (d, *J* = 18.7 Hz, 1H), 3.50 (s, 3H), 3.48 (s, 3H), 3.25 (bd, *J* = 4.1 Hz, 1H), 2.82-2.77 (m, 1H), 2.57-2.47 (m, 2H), 2.40-2.34 (m, 2H), 1.38 (d, *J* = 6.2 Hz, 3H); *More polar epimer:* ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.57–7.54 (m, 2H), 7.40-7.35 (m, 3H), 6.73 (d, *J* = 2.0 Hz, 1H), 6.35 (d, *J* = 2.0 Hz, 1H), 5.80-5.71 (m, 1H), 5.25-5.13 (m, 5H), 5.01 (dd, *J* = 15.5, 8.9 Hz, 1H), 3.98 (d, *J* = 19.3 Hz, 1H), 3.81 (dd, *J* = 12.1, 3.6 Hz, 1H), 3.64 (d, *J* = 19.3 Hz, 1H), 3.52 (s, 3H), 3.48 (s, 3H), 3.25 (dd, *J* = 8.8, 2.3 Hz, 1H), 2.79-2.76 (m, 1H), 2.65-2.50 (m, 2H), 2.44-2.29 (m, 2H), 1.37 (d, *J* = 6.2 Hz, 3H).



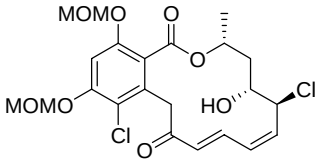
2-44a

Compound 2-44a: A solution of compound **2-41a** (333 mg, 0.6 mmol) in hexafluoroisopropanol (3 mL) was treated with hydrogene peroxide (117 µL, 1.2 mmol) and stirred for 3 h at room temperature. The reaction mixture was diluted in EtOAc, washed with a saturated aqueous solution of $NaHCO_3/Na_2S_2O_3$ (10:1, 12 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was then dissolved in toluene (5 mL) and stirred for 1 h at 80 °C. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-50 % EtOAc/cyclohexane gradient) afforded compound **2-44a** (249 mg, 92 %). R_f = 0.20 (silica gel, hexane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.22 (dd, *J* = 15.6, 10.7 Hz, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 6.56 (d, *J* = 2.2 Hz, 1H), 6.51-6.41 (m, 1H), 6.27 (d, *J* = 15.6 Hz, 1H), 5.69 (d, *J* = 17.2 Hz, 1H), 5.63-5.55 (m, 2H), 5.48 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.34-5.26 (m, 2H), 5.17 (s, 4H), 3.94 (m, 2H), 3.49 (s, 3H), 3.48 (s, 3H), 3.14 (dd, *J* = 7.5, 2.1 Hz, 1H), 3.04 (td, *J* = 5.9, 2.1 Hz, 1H), 1.99-1.87 (m, 2H), 1.41 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.3, 167.0, 159.1, 156.2, 143.2, 135.4, 135.2, 135.1, 129.2, 126.7, 119.3, 118.5, 111.4, 102.5, 94.8, 94.3, 69.5, 58.1, 56.9, 56.3, 56.2, 45.9, 38.1, 19.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{30}O_8Na$: 469.1833, found 469.1952 [M+Na^+].



2-43a

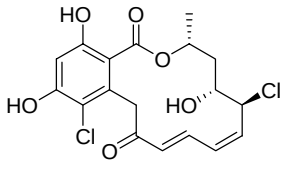
Compound 2-43a: A 2 mM solution of compound **2-44a** (150 mg, 0.34 mmol) in anhydrous toluene was heated at reflux and treated with 5 % mol of Grubbs' II catalyst (14 mg, 0.017 mmol). The reaction mixture was stirred for 10 min at this temperature and quenched by cooling down to –78 °C. The reaction mixture was then filtered through a pad of silica gel, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/cyclohexane gradient) afforded **2-43a** (122 mg, 87 %). R_f = 0.19 (silica gel, EtOAc/cyclohexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.68 (dd, *J* = 16.1, 11.3 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.59 (d, *J* = 2.1 Hz, 1H), 6.26 (dd, *J* = 11.3, 11.2 Hz, 1H), 6.05 (d, *J* = 16.1 Hz, 1H), 5.84 (dd, *J* = 10.8, 4.4 Hz, 1H), 5.42-5.33 (m, 1H), 5.21-5.17 (m, 2H), 5.14 (s, 2H), 3.97 (d, *J* = 14.0 Hz, 1H), 3.86 (d, *J* = 13.4 Hz, 1H), 3.57 (m, 1H), 3.48 (s, 3H), 3.47 (s, 3H), 3.13-3.10 (ddd, *J* = 7.5, 3.7, 2.2 Hz, 1H), 2.47 (dt, *J* = 14.5, 4.8 Hz, 1H), 1.73 (ddd, *J* = 15.0, 7.5, 3.2 Hz, 1H), 1.59 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.9, 166.8, 159.2, 156.0, 140.2, 136.6, 134.5, 131.8, 130.2, 117.8, 108.7, 102.1, 94.6, 94.3, 69.8, 56.3, 55.8, 54.9, 42.4, 37.1, 29.7, 18.9; IR (film): ν_{max} = 2923, 2849, 1719, 1664, 1602, 1461, 1286, 1149, 1027, 920 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{26}O_8Na$: 441.1520, found 441.1595 [*M*+Na$^+$]. (-)-(2*R*, 4*S*, 5*R*): $[\alpha]^{25}_D$ = − 50.0 (*c* 0.11, $CHCl_3$).



2-45a

Compound 2-45a: Neat sulfuryl chloride (7.5 µL, 0.09 mmol) was added to a cooled (0 °C) solution of compound **2-43a** (13 mg, 0.03 mmol) in Et_2O (1 mL). The reaction was stirred for 1.5 h at 0 °C and then diluted with CH_2Cl_2 (5 mL). The mixture was washed several times with saturated $NH_4Cl_{aq.}$ (8 mL) and dried over $MgSO_4$. Removal of the solvents under reduced pressure, followed by flash chromatography (silica gel, 0-33 % EtOAc/cyclohexane), afforded compound **2-45a** (10 mg, 68 %). R_f = 0.25 (silica gel, EtOAc/cyclohexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.18 (dd, *J* = 16.2, 11.2 Hz, 1H), 7.04 (s, 1H), 6.26 (dd,

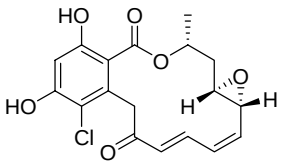
J = 10.9, 10.9 Hz, 1H), 6.10 (d, *J* = 16.4 Hz, 1H), 5.81 (dd, *J* = 10.2, 10.2 Hz, 1H), 5.47 (m, 1H), 5.35-5.12 (m, 5H), 4.11 (d, *J* = 15.8 Hz, 1H), 3.92 (d, *J* = 15.8 Hz, 1H), 3.55 (s, 3H), 3.53 (s, 3H), 3.55-3.48 (m, 1H), 2.08-2.04 (m, 2H), 1.57 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.3, 166.7, 159.3, 156.0, 140.8, 137.9, 134.7, 133.2, 131.2, 117.8, 102.0, 95.2, 94.6, 71.9, 69.7, 60.4, 56.7, 56.4, 45.1, 21.0, 18.8, (1 quaternary carbon is not detected); IR (film): ν_{max} = 3075, 2930, 1685, 1647, 1618, 1571, 1439, 1235, 1147, 1112, 1080, 1017, 942 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{26}Cl_2O_8Na$: 510.9994, found 511.0002 [*M*+Na$^+$].



2-2
Pochonin C

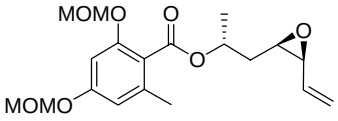
Pochonin C (2-2): A solution of compound **2-45a** (10 mg, 0.02 mmol) in dioxane (5 mL) was treated at 0 °C with 2.5 % $HCl_{conc.}$ (125 µL, to reach a 2.5 % solution). The reaction was then allowed to warm up to room temperature and stirred for 1 h. The resulting solution was filtered on a pad of silica gel and washed several times with Et_2O. The solvents were removed under reduced pressure and preparative TLC (silica gel, 50 % EtOAc/cyclohexane) provided synthetic pochonin C (**2-2**) (6.3 mg, 74 %). Synthetic pochonin C was found to have identical ^{1}H NMR as natural pochonin C. R_f = 0.11 (silica gel, EtOAc/cyclohexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.28 (s, 1H), 8.59 (s, 1H), 7.11 (dd, *J* = 14.7, 12.3 Hz, 1H), 6.66 (s, 1H), 6.31 (t, *J* = 10.4 Hz, 1H), 6.10 (d, *J* = 16.1 Hz, 1H), 5.91 (m, 1H), 5.78 (t, *J* = 9.9 Hz, 1H), 5.22 (d, *J* = 15.8 Hz, 1H), 4.92 (t, *J* = 9.1 Hz, 1H), 3.96 (d, *J* = 15.8 Hz, 1H), 3.95 (m, 1H), 2.48 (dd, *J* = 15.8, 10.7 Hz, 1H), 2.13 (m, 1H), 1.54 (d, *J* = 6.4 Hz, 3H); ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 10.58 (s, 1H), 10.13 (s, 1H), 7.12 (dd, *J* = 16.1, 11.3 Hz, 1H), 6.56 (s, 1H), 6.27 (t, *J* = 10.8 Hz, 1H), 6.04 (d, *J* = 16.1 Hz, 1H), 5.78 (t, *J* = 10.8 Hz, 1H), 5.46 (d, *J* = 5.4 Hz, 1H), 5.28 (m, 1H), 5.10 (dd, *J* = 9.9, 4.8 Hz, 1H), 4.05 (d, *J* = 16.1 Hz, 1H), 3.99 (m, 1H), 3.60 (d, *J* = 16.1 Hz, 1H), 1.87 (m, 2H), 1.37 (d, *J* = 6.2 Hz, 3H); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.28 (dd, *J* = 16.0, 11.1 Hz, 1H, H_8), 6.53 (s, 1H, H_{15}), 6.25 (dd, *J* = 10.7, 10.7 Hz, 1H, H_7), 6.02 (d, *J* = 16.6 Hz, 1H, H_9), 5.82 (dd, *J* = 10.7, 10.7 Hz, 1H, H_6), 5.47-5.43 (m, 1H, H_2), 5.20 (dd, *J* = 9.7, 5.9 Hz, 1H, H_5), 4.27 (d, *J* = 16.1 Hz, 1H, H_{11}), 4.03-4.00 (m, 1H, H_4), 3.73 (d, *J* = 16.1 Hz, 1H, H_{11}), 2.11 (dd, *J* = 14.0, 6.9 Hz, 1H, H_3),

2.00-1.92 (m, 1H, H_3), 1.48 (d, J = 6.4 Hz, 3H, H_1); ^{13}C NMR (100 MHz, [D_6]DMSO, 25 °C): δ = 197.0, 166.5, 155.5, 139.1, 137.0, 133.2, 132.1, 129.9, 115.1, 112.2, 102.9, 71.1, 69.5, 60.8, 44.9, 37.8, 19.0; HMQC 2D (125.77 MHz, CD_3OD, 25 °C): δ = 199.7, 157.1, 141.0, 138.1, 134.6, 132.4, 130.8, 116.2, 114.3, 103.7, 72.6, 70.8, 60.6, 45.7, 38.1, 19.2, (2 quaternary carbons are not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{18}Cl_2O_6Na$: 422.9995, found 423.0337 [M+Na^+]. (-)-(2*R*, 4*S*, 5*R*): $[\alpha]^{25}_D$ = − 68.3 (*c* 0.06, $CHCl_3$).



2-1
Radicicol

Radicicol (2-1): A solution of compound **2-2** (5 mg, 0.01 mmol) in DMF (500 µL) was treated with K_2CO_3 (4 mg, 0.02 mmol) and stirred for 1 h at room temperature. The reaction was then diluted with EtOAc (5 mL) and washed several times with saturated $NH_4Cl_{aq.}$ (10 mL). Removal of the solvents and preparative TLC (silica gel, 50 % EtOAc/cyclohexane) provided radicicol (**2-1**) (3.9 mg, 86 %). Analyses of synthetic radicicol were found to be identical to the natural ones. R_f = 0.32 (silica gel, EtOAc/cyclohexane 1:1); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.62 (dd, J = 16.0, 9.9 Hz, 1H, H_8), 6.48 (s, 1H, H_{15}), 6.26 (dd, J = 10.6, 10.6 Hz, 1H, H_7), 6.12 (d, J = 16.0 Hz, 1H, H_9), 5.80 (dd, J = 10.6, 3.8 Hz, 1H, H_6), 5.42-5.39 (m, 1H, H_2), 4.19 (d, J = 16.0 Hz, 1H, H_{11}), 3.95 (d, J = 16.4 Hz, 1H, H_{11}), 3.33 (m, 1H, H_5, obscured by CD_3OD), 3.08 (dt, J = 7.8, 3.1 Hz, 1H, H_4), 2.44 (dt, J = 14.7, 3.4 Hz, 1H, H_3), 1.74 (ddd, J = 18.4, 8.5, 3.7 Hz, 1H, H_3), 1.54 (d, J = 6.5 Hz, 3H, H_1); ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 198.2, 167.7, 159.0, 158.0, 139.2, 135.5, 133.6, 130.1, 129.4, 102.4, 70.6, 55.4, 55.1, 45.1, 36.3, 17.4, (2 quaternary carbons are not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{17}ClO_6Na$: 387.0611, found 387.0795 [M+Na^+]. (+)-(2*R*, 4*R*, 5*R*): $[\alpha]^{25}_D$ = +95.3 (*c* 0.06, $CHCl_3$).

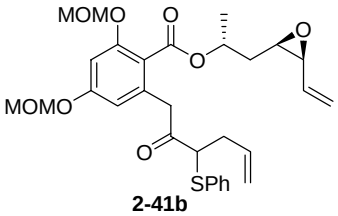


2-40b

Compound 2-40b: In a similar manner as that described for compound **2-40a**, compound **2-40b** was prepared with a 76 % yield in two steps from **2-38**.

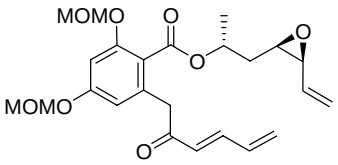
2-39b: R_f = 0.30 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.81 (s, 1H), 7.29 (s, 1H), 6.30 (d, *J* = 2.2 Hz, 1H), 6.24 (d, *J* = 2.3 Hz, 1H), 5.79-5.69 (m, 1H), 5.52 (d, *J* = 16.3 Hz, 1H), 5.45-5.36 (m + s, 3H), 3.47 (m, 1H), 3.27 (m, 1H), 2.55 (s, 3H), 2.02-1.98 (m, 2H), 1.48 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.1, 165.5, 160.3, 143.9, 131.8, 120.9, 111.3, 105.8, 101.3, 70.3, 56.3, 55.3, 34.0, 24.6, 20.1; IR (film): ν_{max} = 2928, 1646, 1458, 1378, 1314, 1262, 1173, 1098 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{15}H_{18}O_5Na$: 301.1046, found 301.1054 [*M*+Na$^+$]. (-)-(2*R*, 4*S*, 5*S*): $[\alpha]^{25}_D$ = − 16.9 (*c* 0.55, $CHCl_3$).

***2-40b*: R_f** = 0.36 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.69 (d, *J* = 1.8 Hz, 1H), 6.57 (d, *J* = 2.4 Hz, 1H), 5.80-5.72 (ddd, *J* = 16.9, 10.5, 7.0 Hz, 1H), 5.51 (d, *J* = 16.9 Hz, 1H), 5.40 (dd, *J* = 10.5, 1.8 Hz, 1H), 5.39-5.33 (m, 1H), 5.17 (s, 2H), 5.16 (s, 2H), 3.49 (s, 3H), 3.48 (s, 3H), 3.48-3.44 (dd, *J* = 7.0, 2.3 Hz, 1H), 3.33-3.29 (m, 1H), 2.32 (s, 3H), 2.02 (ddd, *J* = 14.6, 6.5, 6.4 Hz, 1H), 1.90 (ddd, *J* = 14.6, 5.4, 5.3 Hz, 1H), 1.43 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.5, 158.7, 155.3, 137.7, 132.1, 120.7, 118.7, 110.6, 101.1, 94.6, 94.3, 69.5, 56.4, 56.2, 56.1, 55.3, 34.0, 20.1, 19.7; IR (film): ν_{max} = 2957, 1724, 1606, 1451, 1267, 1147, 1050, 925 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{26}O_7Na$: 389.1571, found 389.1605 [*M*+Na$^+$]. (-)-(2*R*, 4*S*, 5*S*): $[\alpha]^{25}_D$ = − 7.9 (*c* 0.33, $CHCl_3$).



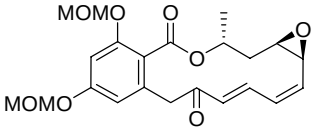
2-41b

Compound 2-41b: In a similar manner as that described for compound **2-41a**, compound **2-41b** was prepared in 81 % yield. R_f = 0.36 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.45-7.35 (m, 4H), 7.33−7.28 (m, 6H), 6.78 (d, *J* = 2.2 Hz, 2H), 6.55 (s, 2H), 5.87-5.70 (m, 4H), 5.50 (d, *J* = 17.2 Hz, 2H), 5.38 (d, *J* = 10.2 Hz, 2H), 5.33-5.27 (m, 2H), 5.17 (s, 4H), 5.15 (s, 4H), 5.20-5.09 (m, 4H), 4.12 (d, *J* = 16.6 Hz, 1H), 4.06 (d, *J* = 16.3 Hz, 1H), 4.02 (d, *J* = 14.3 Hz, 1H), 3.98 (d, *J* = 16.6 Hz, 1H), 3.82 (t, *J* = 7.5 Hz, 1H), 3.81 (t, *J* = 7.5 Hz, 1H), 3.49 (s, 6H), 3.48 (s, 6H), 3.45-3.42 (m, 2H), 3.34-3.32 (m, 2H), 2.61-2.55 (m, 2H), 2.47-2.41 (m, 2H), 2.02-1.95 (m, 2H), 1.8 (m, 2H), 1.40 (d, *J* = 6.4 Hz, 6H).



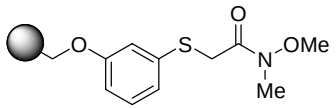
2-44b

Compound 2-44b: In a similar manner as that described for compound **2-44a**, compound **2-44b** was prepared in 92 % yield. R_f = 0.22 (silica gel, hexane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.23 (dd, *J* = 15.8, 11.1 Hz, 1H), 6.80 (d, *J* = 1.8 Hz, 1H), 6.56 (d, *J* = 2.3 Hz, 1H), 6.45 (m, 1H), 6.27 (d, *J* = 15.8 Hz, 1H), 5.77-5.67 (m, 1H), 5.69 (d, *J* = 16.4 Hz, 1H), 5.57 (d, *J* = 9.4 Hz, 1H), 5.50 (d, *J* = 17.0 Hz, 1H), 5.39 (d, *J* = 9.9 Hz, 1H), 5.33-5.27 (m, 1H), 5.19 (s, 2H), 5.18 (s, 2H), 3.95 (m, 2H), 3.50 (s, 3H), 3.48 (s, 3H), 3.44 (dd, *J* = 6.4, 4.7 Hz, 1H), 3.31 (td, *J* = 10.5, 5.8 Hz, 1H), 2.00-1.94 (m, 1H), 1.84 (ddd, *J* = 14.7, 5.3, 5.3 Hz, 1H), 1.39 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.3, 167.1, 159.1, 156.3, 143.2, 135.3, 135.2, 132.2, 129.2, 126.7, 120.6, 118.5, 111.4, 102.5, 94.8, 94.3, 69.7, 56.3, 56.3, 56.2, 55.3, 46.0, 34.0, 20.0; IR (film): ν_{max} = 2932, 1718, 1605, 1438, 1275, 1149, 1020, 924 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{30}O_8Na$: 469.1833, found 469.1913 [*M*+Na$^+$]. (-)-(2*R*, 4*S*, 5*S*): $[\alpha]^{25}_D$ = − 2.8 (*c* 0.18, $CHCl_3$).



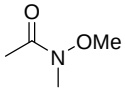
2-43b

Compound 2-43b: In a similar manner as that described for compound **2-43a**, compound **2-43b** was prepared in 21 % yield. R_f = 0.19 (silica gel, EtOAc/cyclohexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.66 (dd, *J* = 15.8, 10.8 Hz, 1H), 6.75 (d, *J* = 2.1 Hz, 1H), 6.58 (d, *J* = 2.1 Hz, 1H), 6.25 (dd, *J* = 9.7, 9.7 Hz, 1H), 6.04 (d, *J* = 15.8 Hz, 1H), 5.83 (dd, *J* = 10.8, 4.3 Hz, 1H), 5.37 (m, 1H), 5.20-5.16 (m, 2H), 5.13 (s, 2H), 3.96 (d, *J* = 13.7 Hz, 1H), 3.85 (d, *J* = 13.7 Hz, 1H), 3.56 (m, 1H), 3.48 (s, 3H), 3.46 (s, 3H), 3.11-3.09 (m, 1H), 2.47 (ddd, *J* = 15.2, 5.1, 4.3 Hz, 1H), 1.71 (ddd, *J* = 15.2, 7.2, 3.0 Hz, 1H), 1.58 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.8, 166.8, 159.2, 156.1, 140.1, 136.6, 132.8, 131.8, 130.2, 114.2, 108.7, 102.1, 94.6, 94.3, 69.8, 56.3, 55.7, 54.9, 42.4, 37.1, 29.7, 18.8; HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{26}O_8Na$: 441.1520, found 441.1599 [*M*+Na$^+$].



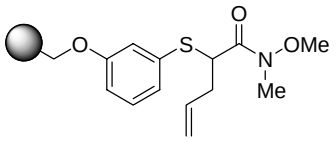
2-49

Resin 2-49: 3-mercaptophenol (1.63 mL, 16.0 mmol) and K_2CO_3 (2.2 g, 16.0 mmol) were added at 23 °C to a solution of 2-chloro-*N*-methoxy-*N*-methylacetamide (2.2 g, 16.0 mmol) in dry DMF (5 mL). The resulting suspension was stirred at 23 °C for 2 h. After this period of time, the mixture was added to Merrifield resin (10 g, 1.0 mmol.g^{-1}) swollen in dry DMF (100 mL), followed by K_2CO_3 (4.4 g, 32.0 mmol) as well as TBAI (catalytic amount), and the suspension was heated up to 50 °C. After 2 h at this temperature, the resin was filtered and washed several times: $HCl_{aq.}$ (50 mL), MeOH (50 mL), CH_2Cl_2 (50 mL) and Et_2O (50 mL). The resin was dried to constant mass under reduced pressure before use. The final mass gain of 1.72 g indicates an estimated loading of 0.77 mmol.g^{-1}. A yield of 90 % was calculated based on this mass gain.



2-50

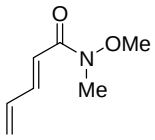
Weinreb amide 2-50: A catalytic amount of AIBN and *n*Bu_3SnH (104 µL, 0.385 mmol) were added to a suspension of resin **2-49** (100 mg, 0.77 mmol.g^{-1}) in C_6D_6 (1 mL). The resulting mixture was heated up in the microwave (150 °C, 300 Watts) for 10 min. After this period of time, the solution was directly subjected to a ^{1}H NMR. Crude ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 3.07 (s, 3H), 2.95 (s, 3H), 1.99 (s, 3H).



2-51

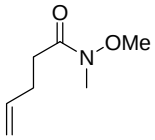
Resin 2-51: Resin **2-49** (10 g, 0.77 mmol.g^{-1}) was suspended in a 1:1 mixture of HFIP/CH_2Cl_2 (100 mL). To this suspension, H_2O_2 (3 mL, 31 mmol) was added at 23 °C and the resulting mixture was shaken overnight at room temperature. The resulting resin was then filtered, washed using MeOH (100 mL), CH_2Cl_2 (100 mL) and Et_2O (100 mL) and dried to constant mass. This resin was then suspended in dry CH_2Cl_2 (100 mL), cooled down to –78 °C and treated sequentially with allyltributyltin (11.9 mL, 38.4 mmol) and trifluoroacetic

anhydride (3.27 mL, 23.1 mmol). After 10 min at this temperature, the reaction was allowed to warm up to room temperature for 1 h and then filtered and washed several times: $HCl_{aq.}$ (100 mL), MeOH (100 mL), CH_2Cl_2 (100 mL) and Et_2O (100 mL) to obtain resin **2-51**. The resin was dried to constant mass under reduced pressure before use. A yield of 85 % was estimated based on the NMR interpretation of **2-52** relatively to **2-50** after cleavage under free radical conditions.



2-46

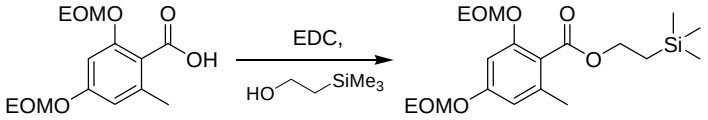
α,β,γ,δ-Unsaturated Weinreb amide 2-46: Resin **2-51** (1 g, estimated loading of 0.67 $mmol.g^{-1}$) was suspended in a 1:1 mixture of HFIP/CH_2Cl_2 (10 mL). To this suspension H_2O_2 (300 µL, 3 mmol) was added at 23 °C and the resulting mixture was shaken for 12 h. The resulting resin was then filtered, washed with MeOH (100 mL), CH_2Cl_2 (100 mL) and Et_2O (100 mL) followed by toluene. This resin was resuspended in toluene (10 mL) and heated up to 80 °C for 30 min. The resulting mixture was filtered and washed several times with more toluene. The combined toluene solutions were evaporated giving pure compound **2-46** (113 mg 80 % yield from Merriefield resin). Compound **2-46** is volatile and cannot be dried at 0.1mmHg for extended time. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.33 (dd, *J* = 15.3, 11.0 Hz, 1H), 6.52 (m, 2H), 5.61 (d, *J* = 16.6 Hz, 1H), 5.48 (d, *J* = 10.2 Hz, 1H), 3.73 (s, 3H), 3.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.8, 143.4, 135.1, 124.7, 119.7, 61.7, 32.3; IR (film): ν_{max} = 2936, 1658, 1598, 1427, 1382, 1181, 1095, 1005 cm^{-1}.



2-52

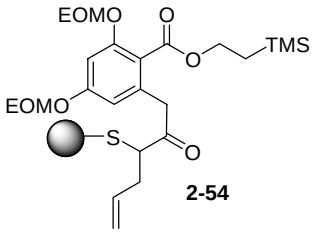
γ,δ-Unsaturated Weinreb amide 2-52: A catalytic amount of AIBN and *n*Bu_3SnH (90 µL, 0.335 mmol) were added to a solution of resin **2-51** (100 mg, estimated loading of 0.67 $mmol.g^{-1}$) in C_6D_6 (1 mL). The resulting mixture was heated up in the microwave (150 °C, 300 Watts) for 10 min. After this period of time, the solution was directly subjected to a ^{1}H

NMR. Crude ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 5.97-5.90 (m, 1H), 5.15 (d, *J* = 17.2 Hz, 1H), 5.06 (d, *J* = 10.2 Hz, 1H), 3.09 (s, 3H), 2.93 (s, 3H), 2.53 (m, 2H), 2.44-2.42 (m, 2H).



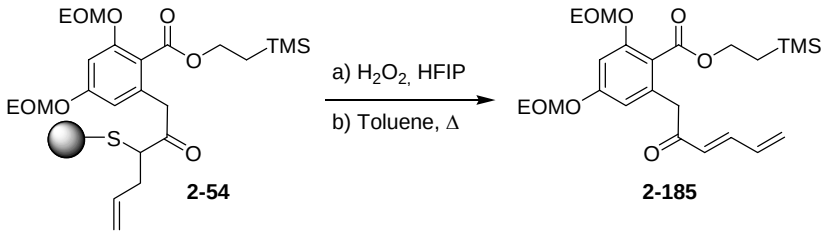
2-95e **2-53**

Compound 2-53: To a solution of acid **2-95e** (3.9 g, 13.2 mmol, *vide infra* for preparation from orcinol) dissolved in anhydrous CH_2Cl_2 (40 mL), were added in a sequential fashion: trimethylsilylethanol (3.8 mL, 26.4 mmol), EDC (2.5 g, 13.2 mmol) and DMAP (catalytic amount). After stirring at room temperature for 30 min, the reaction was diluted with EtOAc (50 mL), washed with saturated $NH_4Cl_{aq.}$ (50 mL) and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-10 % EtOAc/cyclohexane) afforded compound **2-53** (2.6 g, 52 % over 3 steps). R_f = 0.60 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.72 (s, 1H), 6.55 (s, 1H), 5.21 (s, 4H), 4.40 (t, *J* = 9.2 Hz, 2H), 3.75-3.72 (m, 4H), 2.31 (s, 3H), 1.27-1.22 (m, 6H), 1.15-1.10 (m, 2H), 0.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.5, 161.5, 158.1, 139.4, 119.7, 111.1, 101.4, 93.2, 92.6, 62.6, 61.5, 15.8 (x 2), 13.5, 11.0, 6.4 (x 3); IR (film): ν_{max} = 2954, 2897, 1724, 1607, 1266 cm^{-1}; ESI-TOF: *m/z*: calculated for $C_{19}H_{32}NaO_6Si$: 407, found 407 [*M*+Na$^+$].

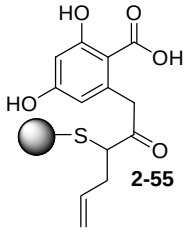


2-54

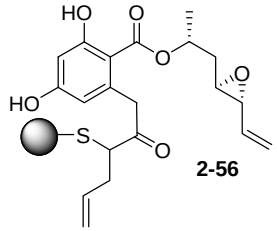
Resin 2-54: A solution of compound **2-53** (1.3 g, 5.4 mmol) in anhydrous THF (20 mL) was treated at –78 °C with freshly prepared LDA (19.1 mL, 10.8 mmol). In a separated flask, resin **2-51** (2 g, estimated loading of 0.67 mmol.g^{-1}) was swollen and cooled down to –78 °C in anhydrous THF (10 mL) and then treated with the solution of compound **2-53**. The resulting mixture was then stirred for 4 h at –78 °C. After this time, the resin was filtered and washed several times with saturated $NH_4Cl_{aq.}$ (30 mL), MeOH (30 mL), CH_2Cl_2 (30 mL) and Et_2O (30 mL). The resin was dried to constant mass under reduced pressure before use.



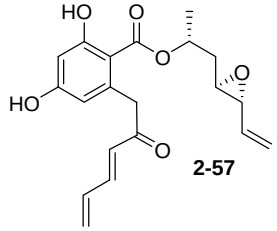
Compound 2-185: Resin **2-54** (250 mg) was suspended in a 1:1 mixture of HFIP/CH_2Cl_2 (1.2 mL). To this suspension, H_2O_2 (50 μL, 0.5 mmol) was added at 23 °C and the resulting suspension was shaken for 12 h. The resulting resin was then filtered, washed using MeOH (20 mL), CH_2Cl_2 (20 mL), Et_2O (20 mL) and toluene. The resin was resuspended in toluene (2.5 mL) and heated up to 80 °C for 30 min. The resulting mixture was then filtered and washed several times with more toluene. The combined toluene solutions were evaporated and dried overnight at 0.1 mmHg to afford pure compound **2-185** (40 mg, 60 % yield from resin **2-51**). R_f = 0.49 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.23 (dd, *J* = 15.5, 10.8 Hz, 1H), 6.85 (d, *J* = 1.8 Hz, 1H), 6.56 (d, *J* = 1.8 Hz, 1H), 6.50-6.41 (m, 1H), 6.27 (d, *J* = 15.2 Hz, 1H), 5.68 (d, *J* = 17.0 Hz, 1H), 5.56 (m, 1H), 5.24 (s, 2H), 5.22 (s, 2H), 4.37-4.32 (m, 2H), 3.89 (s, 1H), 3.79-3.70 (m, 4H), 1.27 (t, *J* = 7.0 Hz, 3H), 1.25 (d, *J* = 7.0 Hz, 3H), 1.10-1.05 (m, 2H), 0.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.4, 167.7, 159.1, 156.4, 143.6, 143.1, 135.2, 129.2, 126.6, 124.1, 111.4, 102.9, 93.8, 93.0, 64.4 (x 2), 63.4, 46.1, 17.4, 15.1, 15.0, -1.5 (x 3).



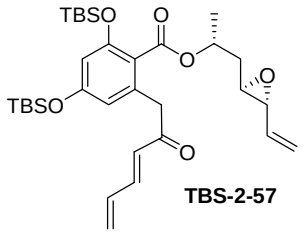
Resin 2-55: Resin **2-54** (1.5 g, estimated loading of 0.34 mmol.g^{-1}) was stirred in a mixture CH_2Cl_2/TFA (5:1, 10 mL) for 1 h at room temperature. After this time, the resin was filtered and washed several times: 1N $HCl_{aq.}$ (50 mL), MeOH (50 mL), CH_2Cl_2 (50 mL) and Et_2O (50 mL). The resin was dried to constant mass under reduced pressure before use. L.C./M.S. analysis of the oxidation/elimination product showed a complete conversion, therefore giving an estimated yield of > 95 %.



Resin 2-56: A solution of resin **2-55** (1 g, estimated loading of 0.33 mmol.g^{-1}), compound (*S*)-**2-5a** (168 mg, 1.32 mmol) and tris(3-chlorophenyl)phosphine (478 mg, 1.32 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated at room temperature with DIAD (260 µL, 1.32 mmol). After stirring for 4 h, the resin was filtered and washed several times: saturated $NH_4Cl_{aq.}$ (60 mL), MeOH (60 mL), CH_2Cl_2 (60 mL) and Et_2O (60 mL). The resin was dried to constant mass under reduced pressure before use.

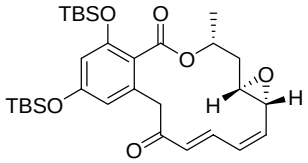


Triene 2-57: Resin **2-56** (~1 g, estimated loading of 0.34 mmol.g^{-1}) was suspended in a 1:1 mixture of HFIP/CH_2Cl_2 (10 mL). To this suspension, H_2O_2 (48 µL, 0.5 mmol) was added at 23 °C and the resulting mixture was shaken for 12 h. The resulting resin was then filtered, washed using MeOH (40 mL), CH_2Cl_2 (40 mL), Et_2O (40 mL) and toluene. This resin was resuspended in toluene (10 mL) and heated up to 80 °C for 12 h. The resulting mixture was filtered and washed several times with more toluene. The combined toluene solutions were evaporated giving pure triene **2-57** (34 mg, 70 % from resin **2-54**). R_f = 0.22 (silica gel, hexane/EtOAc 3:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.8 (s, 1H), 7.52 (s, 1H), 7.30 (dd, *J* = 15.3, 11.5 Hz, 1H), 6.56-6.46 (m, 1H), 6.30-6.28 (m, 1H), 6.25 (s, 1H), 6.11 (d, *J* = 2.1 Hz, 1H), 5.74 (d, *J* = 17.2 Hz, 1H), 5.62 (d, *J* = 10.2 Hz, 1H), 5.59-5.44 (m, 2H), 5.41-5.28 (m, 2H), 4.43 (d, *J* = 17.7 Hz, 2H), 3.95 (d, *J* = 17.7 Hz, 2H), 3.10 (dd, *J* = 7.3, 1.9 Hz, 1H), 2.92-2.88 (m, 1H), 1.97 (dt, *J* = 14.2, 4.5 Hz, 1H), 1.76 (ddd, *J* = 14.5, 7.5, 7.5 Hz, 1H), 1.29 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 198.8, 170.2, 165.6, 161.5, 143.9, 138.7, 135.1, 134.8, 129.1, 127.4, 120.0, 113.4, 105.3, 103.0, 70.4, 58.0, 57.4, 49.4, 38.1, 20.0.



TBS-2-57

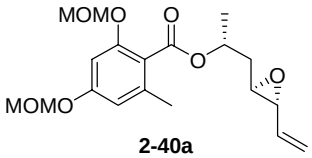
Triene TBS-2-57: A solution of compound **2-57** (30 mg, 0.08 mmol) in DMF (1 mL) was treated sequentially at room temperature with imidazole (55 mg, 0.8 mmol) and TBSCl (121 mg, 0.8 mmol). After 12 h stirring at this temperature, the reaction was diluted in Et_2O (5 mL) and washed several times with saturated $NH_4Cl_{aq.}$ and brine. Evaporation of the solvents, followed by flash chromatography (silica gel, 0-17 % EtOAc/hexane), afforded triene **TBS-2-57** (42.2 mg, 90 %). R_f = 0.15 (silica gel, hexane/EtOAc 6:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.20 (dd, *J* = 15.6, 10.7 Hz, 1H), 6.42 (dt, *J* = 16.6, 10.2 Hz, 1H), 6.30-6.29 (m, 2H), 6.23 (d, *J* = 15.1 Hz, 1H), 5.67 (d, *J* = 16.1 Hz, 1H), 5.57-5.53 (m, 2H), 5.47 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.30-5.24 (m, 2H), 3.80 (d, *J* = 4.3 Hz, 2H), 3.10 (dd, *J* = 7.5, 2.2 Hz, 1H), 2.97 (td, *J* = 5.6, 2.1 Hz, 1H), 1.93 (t, *J* = 5.6 Hz, 2H), 1.43 (d, *J* = 6.4 Hz, 3H), 0.99 (s, 9H), 0.98 (s, 9H), 0.26 (s, 6H), 0.25 (s, 6H).



2-58

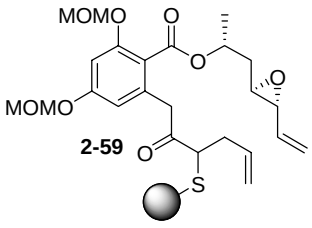
Macrocycle 2-58: A 2 mM solution of triene **TBS-2-57** (40 mg, 0.068 mmol) in anhydrous toluene was heated at 120 °C and treated with 5 % mol of Grubbs' II catalyst (3 mg, 0.0034 mmol). The reaction mixture was stirred for 10 min at this temperature and quenched quickly by cooling down to –78 °C. The reaction mixture was then filtered through a pad of silica gel, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/cyclohexane gradient) afforded **2-58** (32 mg, 83 %). R_f = 0.10 (silica gel, hexane/EtOAc 6:1); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.76 (dd, *J* = 16.4, 11.5 Hz, 1H), 6.30 (d, *J* = 1.6 Hz, 1H), 6.25 (d, *J* = 1.6 Hz, 1H), 6.30-6.20 (m, 1H), 6.03 (d, *J* = 16.1 Hz, 1H), 5.89 (dd, *J* = 10.7, 3.2 Hz, 1H), 5.41-5.33 (m, 1H), 3.93 (d, *J* = 14.0 Hz, 1H), 3.67 (d, *J* = 14.0 Hz, 1H), 3.48 (m, 1H), 3.15-3.11 (m, 1H), 2.51 (dt, *J* = 14.5, 3.5 Hz, 1H), 1.78-1.72 (m, 1H), 1.63 (d, *J* = 6.4 Hz, 3H), 0.98 (s, 9H), 0.97 (s, 9H), 0.27

(s, 6H), 0.25 (s, 6H); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{47}O_6Si_2$: 559.2906, found 559.2878 [M+H^+].



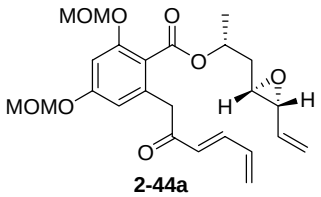
2-40a

Compound 2-40a: *Polymer assisted synthesis*: A solution of 2,4-dihydroxy-6-methyl-benzoic acid (336 mg, 2.0 mmol), compound (*S*)-**2-5a** (256 mg, 2.0 mmol) and tris(3-chlorophenyl)phosphine (1.46 g, 4.0 mmol) in anhydrous CH_2Cl_2 (32 mL) was treated at room temperature with PS-DEAD (3.2 g, 1.3 mmol.g^{-1}). After stirring for 3 h, the reaction mixture was filtered on silica and washed with hexane/EtOAc (10:1, 50 mL) and hexane/EtOAc (3:1, 50 mL). The 3:1 mixture was concentrated under reduced pressure to yield **2-39a** (460 mg, 83 %). To a stirred solution of **2-39a** (460 mg, 1.6 mmol) in anhydrous DMF (10 mL) at room temperature were added in sequential fashion: diisopropylethylamine (1.1 mL, 6.4 mmol), TBAI (catalytic amount) and chloromethylmethyl ether (500 µL, 6.4 mmol). The resulting solution was heated up to 80 °C and stirred for 3 h at this temperature. The reaction was then allowed to cool down to room temperature, diluted with EtOAc and washed several times with saturated $NH_4Cl_{aq.}$. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to provide compound **2-40a** as a crude (600 mg, > 95 % yield).



2-59

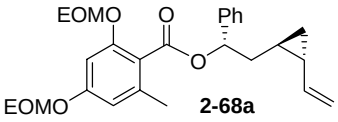
Resin 2-59: A solution of crude compound **2-40a** (500 mg, 1.36 mmol) in anhydrous THF (3 mL) was treated at –78 °C with freshly prepared LDA (4.8 mL, 2.72 mmol). After 10 min stirring at this temperature, the solution was added over resin **2-51** (380 mg, estimated loading of 0.74 mmol.g^{-1}) pre-swollen and cooled to −78°C in THF (4 mL). The resulting mixture was then stirred for 4 h at –78 °C, and then, the reaction was filtered and washed several times: saturated $NH_4Cl_{aq.}$ (30 mL), MeOH (30 mL), CH_2Cl_2 (30 mL) and Et_2O (30 mL). The resin was then dried to constant mass under reduced pressure before use.



2-44a

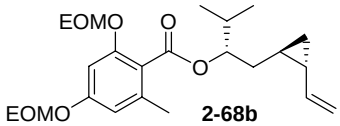
Triene 2-44a from resin 2-59: Resin **2-59** (~380 mg) was suspended in a 1:1 mixture of HFIP/CH_2Cl_2 (4 mL). To this suspension, H_2O_2 (110 µL, 1.1 mmol) was added at 23 °C and the resulting mixture was shaken for 12 h. The resulting resin was then filtered, washed using MeOH (40 mL), CH_2Cl_2 (40 mL), Et_2O (40 mL) and toluene. The resin was then resuspended in toluene (4 mL) and heated up to 80 °C for 12 h. The resulting mixture was filtered and washed several times with more toluene. The combined toluene solutions were evaporated giving pure compound **2-44a** (66 mg, 53 % after 3 steps).

General procedure for the synthesis of compounds 2-68: A solution of acid **66** (1.0 equiv.), alcohol (*S*)-**2-63** or (*S*)-**2-65** (1.0 equiv.) and triphenylphosphine (2.0 equiv.) in anhydrous toluene (0.05 M) was treated at room temperature with DIAD (2.0 equiv.). After stirring for 10 min, the reaction mixture was diluted with EtOAc and washed several times with saturated $NH_4Cl_{aq.}$ and brine. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 % EtOAc/hexane gradient) to yield compound **2-67** (60-80 %). A solution of compound **2-67** (1.0 equiv.) in THF (0.25 M) was then treated at 0 °C with sodium hydride (2.0 equiv.) and chloromethylethyl ether (2.0 equiv.). After stirring for 2 h at this temperature, the reaction mixture was diluted with EtOAc (20 mL) and washed several times with saturated $NH_4Cl_{aq.}$ (30 mL). The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 % EtOAc/hexane gradient) to yield ester **2-68** (50-70 %). Compound **2-67d** (1.0 equiv.) and TBAI (catalytic amount) was dissolved in DMF (0.15 M) and treated with diisopropylethylamine (2.0 equiv.) and chloromethylethyl ether (2.0 equiv.). After stirring for 3 h at 80 °C, the reaction mixture was diluted with EtOAc and washed several times with a saturated $NH_4Cl_{aq.}$. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 % EtOAc/hexane gradient) to yield ester **2-68d** (60 %).



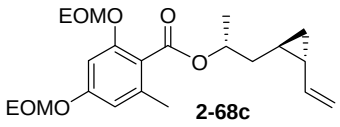
2-68a

Compound 2-68a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.47-7.45 (m, 2H), 7.39-7.29 (m, 3H), 6.74 (d, *J* = 1.8 Hz, 1H), 6.56 (d, *J* = 1.8 Hz, 1H), 6.07 (t, *J* = 7.0 Hz, 1H), 5.35 (ddd, *J* = 17.6, 8.9, 8.8 Hz, 1H), 5.22 (s, 2H), 5.13 (s, 2H), 4.95 (d, *J* = 17.0 Hz, 1H), 4.82 (d, J = 9.9 Hz, 1H), 3.73 (d, *J* = 7.0 Hz, 2H), 3.62 (d, *J* = 7.0 Hz, 2H), 2.22 (s, 3H), 2.05 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1H), 1.85 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.19-1.12 (m, 4H), 0.85-0.81 (m, 1H), 0.66-0.62 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.4, 158.8, 155.5, 141.1, 140.4, 137.8, 128.2 (x 2), 127.8, 126.9 (x 2), 118.5, 111.8, 110.5, 101.2, 93.2, 93.0, 76.9, 64.3, 64.2, 40.4, 22.3, 19.7, 17.2, 15.1, 15.0, 13.5; HRMS (ESI-TOF): *m/z*: calculated for $C_{27}H_{35}O_6$: 455.2428, found 455.2431 [M+H^+].



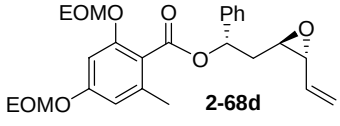
2-68b

Compound 2-68b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.77 (d, *J* = 1.7 Hz, 1H), 6.58 (s, 1H), 5.41 (ddd, *J* = 17.7, 8.9, 8.9 Hz, 1H), 5.22 (s, 2H), 5.20 (s, 2H), 5.16-5.10 (m, 1H), 5.08-5.02 (m, 1H), 4.88-4.84 (m, 1H), 3.76-3.70 (m, 4H), 2.33 (s, 3H), 2.12-2.02 (m, 1H), 1.97-1.89 (m; 0.5H), 1.72 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 0.5H), 1.55 (ddd, *J* = 14.6, 7.2, 7.2 Hz, 0.5H), 1.40-1.32 (m, 0.5H), 1.24 (q, *J* = 7.0 Hz, 6H), 1.04-0.90 (m, 8H), 0.68-0.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 168.1, 168.0, 158.6, 158.6, 155.4 (x 2), 141.5, 141.3, 137.5, 129.5, 119.0, 119.0, 111.7, 111.6, 110.5 (x 2), 101.0 (x 2), 93.2, 93.1, 93.0 (x 2), 79.4, 79.3, 64.3 (x 4), 35.1, 35.0, 30.9, 30.8, 26.6, 26.3, 19.8, 18.6, 17.4, 17.3, 15.1, 15.0, 13.8; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{37}O_6$: 421.2585, found 421.2663 [*M*+H$^+$].



2-68c

Compound 2-68c: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.73 (d, *J* = 1.7 Hz, 1H), 6.56 (d, *J* = 1.8 Hz, 1H), 5.42 (ddd, *J* = 17.5, 9.0, 8.9 Hz, 1H), 5.29-5.24 (m, 1H), 5.22 (s, 2H), 5.21 (s, 2H), 5.06 (d, *J* = 17.0 Hz, 1H), 4.87 (d, *J* = 10.2 Hz, 1H), 3.76-3.70 (m, 4H), 2.31 (s, 3H), 1.76 (ddd, *J* = 13.7, 6.8, 6.7 Hz, 1H), 1.53 (ddd, *J* = 13.9, 6.9, 6.9 Hz, 1H), 1.40 (d, *J* = 6.4 Hz, 3H), 1.26-1.22 (m, 6H), 0.93-0.86 (m, 2H), 0.68-0.63 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.6, 158.7, 155.3, 141.3, 137.4, 119.0, 111.7, 110.5, 101.2, 93.3, 93.0, 71.8, 64.3, 64.3, 39.8, 22.4, 19.8, 19.6, 17.2, 15.1, 15.0, 13.6; HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{33}O_6$: 393.2272, found 393.2334 [*M*+H$^+$].

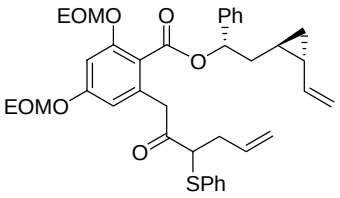


2-68d

Compound 2-68d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.49-7.47 (m, 2H), 7.41-7.34 (m, 3H), 6.75 (d, *J* = 1.8 Hz, 1H), 6.56 (d, *J* = 1.8 Hz, 1H), 6.19 (t, *J* = 7.0 Hz, 1H), 5.55-5.48 (m, 1H), 5.40-5.30 (m, 2H), 5.23 (s, 2H), 5.14 (s, 2H), 3.73 (d, *J* = 7.0 Hz, 2H), 3.63 (d, *J* = 7.0 Hz, 2H), 3.07 (dd, *J* = 7.6, 1.8 Hz, 1H), 2.94-2.91 (m, 1H), 2.44 (ddd, *J* = 14.0, 6.8, 6.8 Hz, 1H), 2.22 (s, 3H), 2.09 (ddd, *J* = 14.0, 6.8, 6.7 Hz, 1H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.2, 158.9, 155.7, 139.6, 138.0, 135.2, 128.8 (x 2), 128.2, 126.8 (x 2), 119.3, 118.1, 110.6, 101.1, 93.3, 93.0, 74.1, 64.3, 64.3,

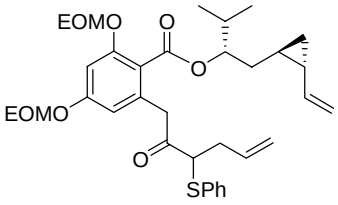
58.5, 56.9, 39.0, 19.7, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{32}O_7Na$: 479.2040, found 479.2109 [M+Na^+].

General procedure for the synthesis of compounds 2-69: A solution of compound **2-68** (1.0 equiv.) in anhydrous THF (1 M) was treated at –78 °C with freshly prepared LDA (2.0 equiv.). After 5 min stirring, a solution of compound **2-6** (1.0 equiv.) in THF (0.7 M) was added dropwise. The resulting mixture was then stirred for 5 min at –78 °C and quenched by addition of a solution of saturated NH_4Cl_{aq}. Upon warming to room temperature, the reaction was diluted with EtOAc, washed several times with saturated NH_4Cl_{aq}, brine and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded compound **2-69** (50-70 % based on recovered starting material) as a mixture of diastereoisomers.



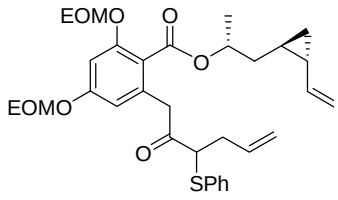
2-69a

Compound 2-69a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.47-7.45 (m, 2H), 7.39-7.29 (m, 8H), 6.85 (s, 1H), 6.53 (d, J = 2.3 Hz, 1H), 6.00 (ddd, J = 11.7, 7.0, 6.4 Hz, 1H), 5.82-5.71 (m, 1H), 5.37-5.28 (m, 1H), 5.22-5.15 (m, 4H), 5.10-5.05 (m, 2H), 4.93 (d, J = 17.0 Hz, 1H), 4.81 (d, J = 10.0 Hz, 1H), 3.97-3.81 (m, 2H), 3.75-3.57 (m, 5H), 2.50-2.42 (m, 1H), 2.38-2.30 (m, 1H), 2.02 (ddd, J = 14.0, 6.9, 6.9 Hz, 1H), 1.91-1.82 (m, 1H), 1.26-1.19 (m, 6H), 1.14-1.08 (m, 1H), 0.83-0.77 (m, 1H), 0.64-0.58 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.2, 202.1, 167.0, 167.0, 159.1, 159.1, 156.4 (x 2), 141.2, 141.2, 140.4, 140.3, 134.9, 134.8, 134.5, 134.4, 133.5 (x 2), 133.3 (x 2), 129.0 (x 2), 129.0 (x 2), 128.3 (x 2), 128.3 (x 2), 128.2, 128.2, 127.9 (x 2), 127.9 (x 2), 127.1 (x 2), 127.1 (x 2), 118.3, 118.2, 117.7, 117.6, 111.8, 111.8, 111.5, 111.4, 102.6, 102.6, 93.4, 93.4, 93.0 (x 2), 77.2, 77.1, 64.4 (x 2), 64.3 (x 2), 55.3, 55.0, 45.0, 44.7, 40.3, 40.3, 34.0, 33.9, 22.5, 22.4, 17.1, 17.1, 15.1 (x 2), 15.0 (x 2), 13.4, 13.4; HRMS (ESI-TOF): *m/z*: calculated for $C_{38}H_{45}O_7S$: 645.2881, found 645.2905 [M+H^+].



2-69b

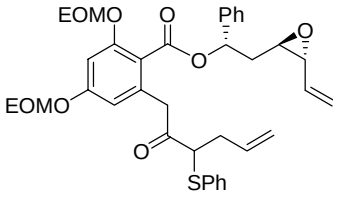
Compound 2-69b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.43-7.40 (m, 2H), 7.33-7.29 (m, 3H), 6.86 (d, *J* = 2.4 Hz, 1H), 6.55 (s, 1H), 5.88-5.79 (m, 1H), 5.44-5.36 (m, 1H), 5.23-5.00 (m, 8H), 4.88-4.83 (m, 1H), 4.09-4.03 (m, 1H), 3.97-3.92 (m, 1H), 3.84 (t, *J* = 7.6 Hz, 1H), 3.72 (q, *J* = 7.0 Hz, 4H), 2.61 (ddd, *J* = 14.6, 7.2, 7.2 Hz, 1H), 2.44 (ddd, *J* = 14.6, 7.3, 7.3 Hz, 1H), 2.07-1.99 (m, 1H), 1.88-1.82 (m, 1H), 1.66-1.55 (m, 1H), 1.24 (t, *J* = 7.0 Hz, 6H), 1.00-0.85 (m, 8H), 0.66-0.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.3, 202.2, 167.6, 167.5, 159.0, 159.0, 156.4, 156.3, 141.5, 141.4, 134.9, 134.9, 134.5, 134.5, 133.5, 133.4, 133.4, 133.4, 132.2, 129.0 (x 2), 128.9, 128.2, 128.2, 128.2, 128.2, 118.6, 118.5, 117.7, 117.7, 111.7, 111.6, 111.4, 111.3, 102.5, 102.5, 93.3, 93.2, 93.0 (x 2), 79.6, 79.5, 64.4 (x 2), 64.3 (x 2), 55.2, 55.1, 45.2, 45.1, 35.0, 35.0, 34.1, 34.1, 30.9, 30.6, 22.5, 22.4, 18.7, 18.6, 17.5, 17.4, 17.3, 17.2, 15.1 (x 2), 15.0 (x 2), 13.9, 13.8; HRMS (ESI-TOF): *m/z*: calculated for $C_{35}H_{47}O_7S$: 611.3037, found 611.3109 [M+H^+].



2-69c

Compound 2-69c: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.42-7.40 (m, 2H), 7.32-7.29 (m, 3H), 6.82 (d, *J* = 2.3 Hz, 1H), 6.55 (s, 1H), 5.86-5.77 (m, 1H), 5.41 (ddd, *J* = 17.0, 8.9, 8.8 Hz, 1H), 5.23-5.16 (m, 5H), 5.12-5.02 (m, 3H), 4.86 (d, *J* = 10.5 Hz, 1H), 4.10-4.02 (d + d, *J* = 15.8, 15.2 Hz,1H), 3.98-3.91 (d + d, *J* = 16.4, 16.4 Hz,1H), 3.82-3.76 (m, 1H), 3.74-3.69 (m, 4H), 2.58 (ddd, *J* = 14.6, 7.2, 7.2 Hz, 1H), 2.43 (ddd, *J* = 14.6, 7.2, 7.2 Hz, 1H), 1.76-1.69 (m, 1H), 1.55-1.46 (m, 1H), 1.37 (d, *J* = 6.4 Hz, 3H), 1.26-1.21 (m, 6H), 0.93-0.85 (m, 2H), 0.67-0.58 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.1, 202.0, 167.2, 167.2, 159.0, 158.9, 156.2, 156.2, 141.3, 141.3, 134.4, 134.4, 133.8, 133.6 (x 2), 133.6 (x 2), 133.5, 132.0, 131.9, 129.0 (x 2), 128.9 (x 2), 128.3, 128.2, 118.9, 117.7, 111.8, 111.7, 111.4, 111.4, 102.8, 93.5, 93.0, 72.1, 72.1, 64.4, 64.4, 55.0, 54.9, 45.0, 45.0, 39.8, 34.1, 34.1, 22.5, 22.5,

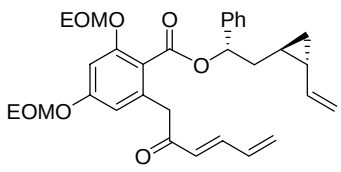
19.7, 19.7, 17.1, 17.1, 15.1, 15.0, 13.5, 13.5; HRMS (ESI-TOF): *m/z*: calculated for $C_{33}H_{43}O_7S$: 583.2724, found 583.2813 [M+H^+].



2-69d

Compound 2-69d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.49-7.45 (m, 2H), 7.39-7.29 (m, 8H), 6.84 (s, 1H), 6.52 (s, 1H), 6.11 (ddd, J = 14.6, 7.0, 6.9 Hz, 1H), 5.82-5.70 (m, 1H), 5.55-5.34 (m, 3H), 5.29-5.14 (m, 4H), 5.09-5.05 (m, 2H), 4.00-3.86 (m, 2H), 3.72 (d, J = 7.0 Hz, 2H), 3.67-3.58 (m, 3H), 3.02 (d, J = 7.0 Hz, 1H), 2.94-2.90 (m, 1H), 2.52-2.30 (m, 3H), 2.13-2.02 (m, 1H), 1.25-1.18 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 202.1, 166.9, 166.8, 159.3, 159.3, 156.6, 139.7, 139.6, 135.3, 135.2, 134.4, 134.4, 133.5 (x 2), 133.3 (x 2), 132.1, 132.0, 129.0 (x 2), 129.0 (x 2), 129.9, 128.6 (x 2), 128.5 (x 2), 128.2, 128.2 (x 2), 128.2 (x 2), 127.8, 126.9 (x 2), 126.8 (x 2), 125.8, 119.5, 119.3, 117.8, 117.7, 111.6, 111.5, 102.6, 93.4, 93.0 (x 2), 74.4, 74.4, 64.4 (x 2), 64.4 (x 2), 58.5, 58.4, 56.9, 56.9, 55.4, 55.1, 44.9, 44.8, 39.0, 38.9, 34.0, 33.9, 15.1 (x 2), 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{37}H_{43}O_8S$: 647.2673, found 647.2701 [M+H^+].

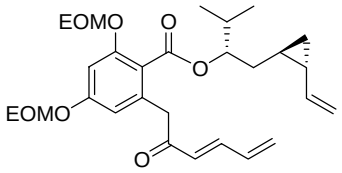
General procedure for the synthesis of compounds 2-70: A solution of compound **2-69** (1.0 equiv.) in HFIP (0.2 M) was treated with hydrogene peroxide (2.0 equiv.) and stirred for 3 h at room temperature. The reaction mixture was diluted in EtOAc, washed with a saturated aqueous solution of $NaHCO_3$/$Na_2S_2O_3$ (10:1), dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was then dissolved in toluene (0.12 M) and stirred for 1.5 h at 80 °C. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-50 % EtOAc/hexane gradient) afforded compound **2-70** (25-35 %).



2-70a

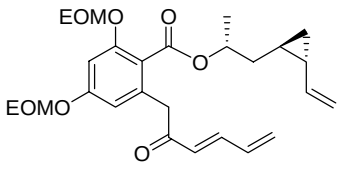
Compound 2-70a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.43-7.41 (m, 2H), 7.35-7.28 (m, 3H), 7.09 (dd, J = 15.4, 10.9 Hz, 1H), 6.84 (d, J = 2.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 6.38

(ddd, *J* = 16.9, 10.5, 10.5 Hz, 1H), 6.11 (d, *J* = 15.6 Hz, 1H), 5.99 (t, *J* = 6.8 Hz, 1H), 5.63 (d, *J* = 16.9 Hz, 1H), 5.53 (d, *J* = 9.9 Hz, 1H), 5.30 (ddd, *J* = 17.2, 8.9, 8.8 Hz, 1H), 5.20 (s, 2H), 5.14 (s, 2H), 4.92 (dd, *J* = 17.2, 1.4 Hz, 1H), 4.79 (dd, *J* = 10.2, 1.6 Hz, 1H), 3.80 (d, *J* = 16.4 Hz, 1H), 3.76-3.69 (m, 3H), 3.63 (q, *J* = 7.0 Hz, 2H), 2.00 (ddd, *J* = 14.0, 7.0, 6.9 Hz, 1H), 1.81 (ddd, *J* = 13.7, 7.1, 7.0 Hz, 1H), 1.26-1.18 (m, 6H), 1.14-1.07 (m, 1H), 0.80-0.75 (m, 1H), 0.62-0.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.3, 166.9, 159.2, 156.4, 142.9, 141.2, 140.2, 135.2, 134.9, 129.2, 128.2 (x 2), 127.9, 127.1 (x 2), 126.5, 118.4, 111.8, 111.1, 93.4, 93.0, 77.7, 64.4, 64.3, 45.7, 40.3, 22.4, 17.1, 15.0, 15.0, 13.4; HRMS (ESI-TOF): *m/z*: calculated for $C_{32}H_{38}O_7Na$: 557.2510, found 557.2545 [*M*+Na^+].



2-70b

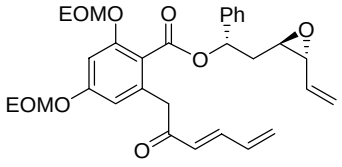
Compound 2-70b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.21 (dd, *J* = 15.5, 10.8 Hz, 1H), 6.87 (s, 1H), 6.54 (s, 1H), 6.44 (ddd, *J* = 17.0, 10.2, 10.2 Hz, 1H), 6.26 (d, *J* = 15.2 Hz, 1H), 5.66 (d, *J* = 17.0 Hz, 1H), 5.54 (d, *J* = 9.9 Hz, 1H), 5.42-5.32 (m, 1H), 5.21 (s, 4H), 5.11-4.99 (m, 2H), 4.83 (d, *J* = 10.5 Hz, 1H), 3.91-3.88 (m, 1H), 3.71 (q, *J* = 7.0 Hz, 4H), 2.05-1.96 (m, 1H), 1.85 (ddd, *J* = 14.6, 7.0, 6.9 Hz, 0.5H), 1.67 (ddd, *J* = 14.6, 7.2, 7.2 Hz, 0.5H), 1.52-1.45 (m, 0.5H), 1.33-1.28 (m, 0.5H), 1.27-1.18 (m, 6H), 1.00-0.85 (m, 8H), 0.64-0.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.4, 167.6, 159.0, 156.3, 143.1, 141.4, 135.2, 134.9, 129.2, 126.5, 118.7, 111.6, 111.1, 102.4, 93.3, 93.0, 79.5, 64.4 (x 2), 45.9, 35.0, 30.8, 22.5, 18.6, 17.4, 17.2, 15.0, 15.0, 13.8; HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{41}O_7$: 501.2847, found 501.2874 [*M*+H^+].



2-70c

Compound 2-70c: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.22 (dd, *J* = 15.5, 10.8 Hz, 1H), 6.84 (d, *J* = 2.4 Hz, 1H), 6.54 (d, *J* = 2.3 Hz, 1H), 6.45 (ddd, *J* = 17.0, 10.4, 10.4 Hz, 1H), 6.26 (d, *J* = 15.8 Hz, 1H), 5.67 (d, *J* = 17.0 Hz, 1H), 5.55 (d, *J* = 9.9 Hz, 1H), 5.44-5.32 (m, 1H), 5.24-5.18 (s + m, 4H + 1H), 5.04 (dd, *J* = 17.0, 1.2 Hz, 1H), 4.85 (dd, *J* = 10.2, 1.4 Hz,

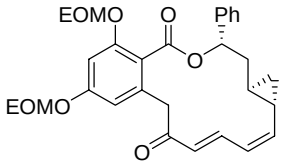
1H), 3.91 (d, *J* = 16.4 Hz, 1H), 3.85 (d, *J* = 16.4 Hz, 1H), 3.73 (2 x q, *J* = 7.0 Hz, 4H), 1.75-1.69 (m, 1H), 1.49 (ddd, *J* = 13.4, 6.9, 6.8 Hz, 1H), 1.35 (d, *J* = 6.4 Hz, 3H), 1.27-1.18 (q, *J* = 6.6 Hz, 6H), 0.92-0.84 (m, 2H), 0.66-0.59 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.3, 167.2, 159.1, 156.2, 143.1, 141.3, 135.2, 134.6, 129.1, 126.6, 118.9, 111.7, 111.1, 102.7, 93.5, 93.0, 72.1, 64.4, 64.4, 46.0, 39.7, 22.4, 19.6, 17.1, 15.0, 15.0, 13.5; HRMS (ESI-TOF): *m/z*: calculated for $C_{27}H_{37}O_7$: 473.2534, found 473.2782 [M+H^+].



2-70d

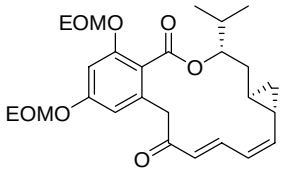
Compound 2-70d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.46-7.32 (m, 5H), 7.10 (dd, *J* = 15.2, 11.1 Hz, 1H), 6.86 (d, *J* = 1.8 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 6.40 (ddd, *J* = 16.9, 10.4, 10.4 Hz, 1H), 6.14-6.10 (t + d, *J* = 7.6 (d), 15.2 (t) Hz, 2H), 5.65 (d, *J* = 17.0 Hz, 1H), 5.55 (d, *J* = 10.5 Hz, 1H), 5.50-5.46 (m, 1H), 5.37 (d, *J* = 15.8 Hz, 1H), 5.24-5.16 (m, 5H), 3.86 (d, *J* = 16.4 Hz, 1H), 3.78 (d, *J* = 16.9 Hz, 1H), 3.72 (q, *J* = 7.0 Hz, 2H), 3.64 (q, *J* = 7.2 Hz, 2H), 3.03 (d, *J* = 7.6 Hz, 1H), 2.93-2.89 (m, 1H), 2.37 (ddd, *J* = 14.0, 6.8, 6.7 Hz, 1H), 2.08 (ddd, *J* = 14.0, 6.8, 6.7 Hz, 1H), 1.25-1.19 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.2, 166.8, 159.3, 156.5, 142.9, 139.5, 135.2, 135.2, 129.2, 128.6, 128.5 (x 2), 128.2, 126.8 (x 2), 126.5, 119.2, 117.9, 111.3, 102.6, 93.5, 93.0, 74.3, 64.4, 64.4, 58.5, 56.9, 45.7, 38.9, 15.0, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{31}H_{36}O_8Na$: 559.2302, found 559.2297 [M+Na^+].

General procedure for the synthesis of compounds 2-71: A 2 mM solution of compound **2-70** (1.0 equiv.) in anhydrous toluene was heated at 80 °C and treated with 5 % mol of Grubbs' II catalyst. The reaction mixture was stirred for 12h at this temperature. The reaction mixture was then filtered through a pad of silica gel, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded **2-71** (~20 %).



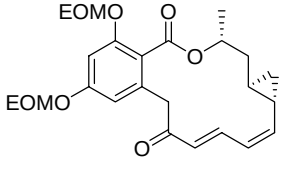
2-71a

Compound 2-71a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.06 (dd, *J* = 16.1, 111.4 Hz, 1H), 7.47-7.44 (m, 2H), 7.38-7.31 (m, 3H), 6.82 (d, *J* = 1.8 Hz, 1H), 6.63 (d, *J* = 1.7 Hz, 1H), 6.20 (dd, *J* = 10.5, 10.5 Hz, 1H), 6.13 (t, *J* = 4.1 Hz, 1H), 6.06 (d, *J* = 16.4 Hz, 1H), 5.58 (dd, *J* = 9.9, 7.6 Hz, 1H), 5.25-5.18 (m, 4H), 3.90 (d, *J* = 13.4 Hz, 1H), 3.78 (d, *J* = 14.0 Hz, 1H), 3.74-3.66 (m, 4H), 2.61-2.56 (m, 1H), 1.85-1.82 (m, 1H), 1.59-1.51 (m, 1H), 1.23 (t, *J* = 7.0 Hz, 6H), 0.97-0.90 (m, 1H), 0.72-0.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 198.5, 167.0, 159.2, 156.2, 144.3, 142.4, 139.4, 134.8, 130.1, 128.7, 128.4 (x 2), 127.9, 126.6 (x 2), 117.9, 108.7, 102.1, 93.3, 93.1, 77.6, 64.5, 64.4, 43.3, 40.3, 17.3, 16.5, 15.4, 15.0, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{34}O_7Na$: 529.2197, found 529.2273 [*M*+Na^+].



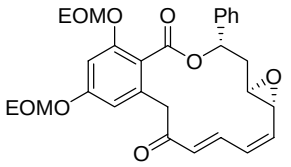
2-71b

Compound 2-71b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.02 (dd, *J* = 15.8, 11.1 Hz, 1H), 6.82 (d, *J* = 1.8 Hz, 1H), 6.64 (d, *J* = 1.8 Hz, 1H), 6.13 (dd, *J* = 10.8, 10.8 Hz, 1H), 5.97 (d, *J* = 15.8 Hz, 1H), 5.29-5.16 (m, 4H), 5.06-5.03 (m, 1H), 4.04 (d, *J* = 13.4 Hz, 1H), 3.99 (d, *J* = 14.0 Hz, 1H), 3.74-3.69 (m, 4H), 2.35-2.25 (m, 1H), 2.20-2.02 (m, 2H), 1.26-1.21 (m, 6H), 1.13 (d, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 6.4 Hz, 3H), 0.99-0.90 (m, 2H), 0.78-0.60 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 198.9, 166.9, 159.1, 156.3, 144.2, 142.9, 135.0, 132.5, 129.7, 128.7, 108.7, 102.2, 93.3, 93.0, 80.5, 64.4, 64.3, 43.4, 35.0, 30.2, 20.3, 18.6, 17.0, 16.2, 15.0, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{27}H_{36}O_7Na$: 495.2353, found 495.2423 [*M*+Na^+].



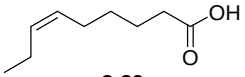
2-71c

Compound 2-71c: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.01 (dd, *J* = 15.8, 11.1 Hz, 1H), 6.79 (d, *J* = 1.8 Hz, 1H), 6.60 (s, 1H), 6.13 (dd, *J* = 10.8, 10.8 Hz, 1H), 5.99 (d, *J* = 16.4 Hz, 1H), 5.57 (dd, *J* = 8.8, 8.8 Hz, 1H), 5.39 (bs, 1H), 5.27-5.19 (m, 4H), 3.95 (d, *J* = 13.4 Hz, 1H), 3.77-3.69 (m, 5H), 2.41-2.35 (m, 1H), 1.75 (bs, 1H), 1.54 (d, *J* = 6.4 Hz, 3H), 1.31-1.21 (m, 6H), 1.06-1.01 (m, 1H), 0.91-0.86 (m, 1H), 0.79-0.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 198.5, 167.1, 159.0, 155.5, 144.6, 142.7, 134.3, 130.0, 128.9, 118.5, 108.4, 102.1, 93.2, 93.1, 72.2, 64.5, 64.4, 43.1, 38.6, 17.9, 16.7, 16.5, 15.7, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{33}O_7$: 445.221, found 445.2495 [*M*+H^+].



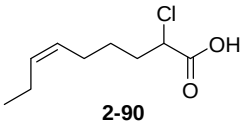
2-71d

Compound 2-71d: A 2 mM solution of compound **2-70d** (1.0 equiv.) in anhydrous toluene was heated at reflux and treated with 5 % mol of Grubbs' II catalyst. The reaction mixture was stirred for 10 min at this temperature and quenched by cooling down to –78 °C. The reaction mixture was then filtered through a pad of silica gel, washed with CH_2Cl_2 and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded **2-71d**. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.67 (dd, *J* = 16.1, 11.4 Hz, 1H), 7.51-7.49 (m, 2H), 7.42-7.35 (m, 3H), 6.83 (d, *J* = 1.8 Hz, 1H), 6.63 (d, *J* = 2.4 Hz, 1H), 6.33 (dd, *J* = 10.8, 10.8 Hz, 1H), 6.13-6.07 (m, 2H), 5.81 (dd, *J* = 10.5, 5.2 Hz, 1H), 5.24-5.16 (m, 4H), 4.19 (d, *J* = 14.0 Hz, 1H), 3.76-3.61 (m, 6H), 3.09 (bs, 1H), 2.80-2.75 (m, 1H), 2.01-1.95 (m, 1H), 1.31-1.21 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.9, 166.8, 159.6, 157.0, 139.6, 139.1, 136.8, 135.3, 131.9, 130.3, 128.6 (x 2), 128.3, 126.5 (x 2), 117.0, 109.1, 102.2, 93.3, 93.0, 75.0, 64.5, 64.5, 55.7, 54.6, 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{32}O_8Na$: 531.1989, found 531.2071 [*M*+Na^+].



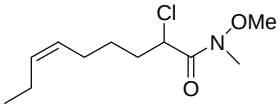
2-89

(*Z*)-6-nonenoic acid (2-89): PDC (75 g, 199 mmol) was added to a solution of *cis*-6-nonen-1-ol (16.7 mL, 100 mmol) in anhydrous DMF (500 mL). After stirring at room temperature for 12 h, the reaction was diluted in Et_2O (200 mL), washed with saturated $NH_4Cl_{aq.}$ (300 mL) and dried over $MgSO_4$. The solvents were removed under reduced pressure to afford **2-89** in a quantitative yield**.** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.42-5.32 (m, 2H), 2.39 (t, *J* = 7.5 Hz, 2H), 2.09-2.05 (m, 4 H), 1.70-1.65 (m, 2H), 1.46-1.40 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 179.5, 132.1, 128.5, 34.0, 29.1, 26.9, 25.5, 20.5, 14.3; IR (film) ν_{max} = 3444, 1636 cm^{-1}.



2-90

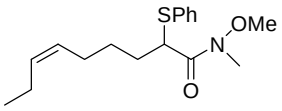
(*Z*)-2-chloro-6-nonenoic acid (2-90): Buthyllithium (13.8 mL, 1.6 M in hexane, 22 mmol) was added at 0 °C to a solution of diisopropylamine (3.2 mL, 26 mmol) in anhydrous THF (20 mL). After stirring at this temperature for 40 min, the reaction was cooled down to –20 °C and a solution of acid **2-89** (1.6 g, 10 mmol) in anhydrous THF (20 mL) and HMPA (5 mL) were added. The solution was stirred for 2 h at −20 °C and cooled to −78 °C. Carbon tetrachloride (4.82 mL, 50 mmol) in 10 mL of ahydrous THF was added rapidly. The solution turned black. After stirring at –78 °C for 2 h, the reaction was stirred at 0 °C for 1 h. The reaction was then quenched with a solution of 1N HCl, diluted with Et_2O, washed with brine and dried over $MgSO_4$. Concentration under reduced pressure afforded crude compound **2-90** used without any purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.44-5.29 (m, 2H), 4.37-4.33 (m, 1H), 2.13-2.06 (m, 6H), 1.62-1.54 (m, 2H), 1.46-1.29 (m, 2H), 0.98 (t, *J* = 7.6 Hz, 3H).



2-91

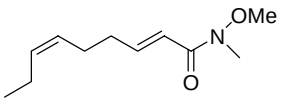
Weinreb amide 2-91: *N*,*O*-dimethylhydroxylamine hydrochloride (1.78 g, 18.2 mmol), DMAP (catalytic amount) and EDC (3.49 g, 18.2 mmol) were sequentially added to a solution of **2-90** (1.73 g, 9.1 mmol) in anhydrous CH_2Cl_2 (30 mL). After stirring at room temperature for 4 h, the reaction was diluted with EtOAc (30 mL), washed with saturated $NH_4Cl_{aq.}$ (40

mL) and dried over $MgSO_4$. Concentration under reduced pressure afforded compound **2-91** (1.86 g, 7.98 mmol) in 88 % yield over 2 steps. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.44-5.29 (m, 2H), 4.37-4.33 (m, 1H), 3.81 (s, 3H), 3.27 (s, 3H), 2.08 (m, 5H), 1.50 (m, 4H), 0.99 (t, *J* = 7.6 Hz, 3H).



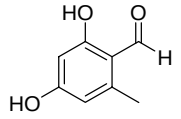
2-92

Thioether Weinreb amide 2-92: Diisopropylethylamine (500 µL, 2.8 mmol) and thiophenol (310 µL, 2.8 mmol) were sequentially added to a solution of **2-91** (700 mg, 3.0 mmol) in anhydrous DMF (6.5 mL). After stirring at 80 °C for 12 h, the reaction was diluted with Et_2O, washed with saturated $NH_4Cl_{aq.}$ and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (silica gel, 0-10 % EtOAc/cyclohexane) afforded compound **2-92** (700 mg, 2.5 mmol) in 84 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.60-7.50 (m, 2H), 7.35-7.25 (m, 3H), 5.40-5.28 (m, 2H), 4.20-4.10 (m, 1H), 3.59 (s, 3H), 3.20 (s, 3H), 2.10-1.95 (m, 4H), 1.95-1.90 (m, 2H), 1.84-1.75 (m, 1H), 1.49-1.41 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 183.5, 133.7, 132.2 (x 2), 128.8, 128.3 (x 2), 127.9, 61.4, 31.7, 27.5, 26.7, 20.5, 14.3 (3 quaternary carbons are not detected); ESI-TOF: *m/z*: calculated for $C_{17}H_{25}NSNaO_2$: 330, found 330 [M+Na^+].



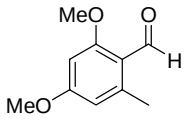
2-93

Weinreb amide 2-93: A solution of compound **2-92** (707 mg, 2.3 mmol) in HFIP (10 mL) was treated with hydrogene peroxide (447 µL, 4.6 mmol) and stirred for 3 h at room temperature. The reaction mixture was then diluted in EtOAc, washed with a saturated aqueous solution of $NaHCO_3/Na_2S_2O_3$ (10:1), dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was then dissolved in toluene (6 mL) and stirred for 8 h at 80 °C. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-33 % EtOAc/hexane gradient) afforded compound **2-93** (75 % over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.00 (dt, *J* = 15.8, 7.0 Hz, 1H), 6.44 (dt, *J* = 15.2 Hz, 1H), 5.47-5.30 (m, 2H), 3.73 (s, 3H), 3.27 (s, 3H), 2.34-2.20 (m, 4H), 2.11-2.01 (m, 2H), 0.99 (t, *J* = 7.3 Hz, 3H).



2-94a

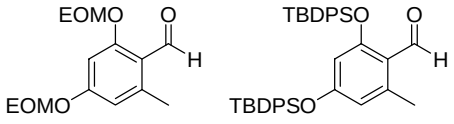
Aldehyde 2-94a: $POCl_3$ (65.8 mL, 706 mmol) was dropped slowly into anhydrous DMF (106 mL) at 0 °C. The resulting solution was then stirred at room temperature for 20 min. A solution of orcinol (21.9 g, 176 mmol) in anhydrous DMF (20 mL) was then added slowly and the reaction mixture was warmed up to 75 °C for 2 h. After cooling down to room temperature, the solution was poured into ice water (500 mL) and neutralized slowly with NaOH pellets. Crude ^{1}H NMR indicated 50 % conversion. Aldehyde **2-94a** precipitated and was filtered before being dried under vacuum (9.6 g, 72 % based on recovery of the starting material). R_f = 0.29 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.41 (s, 1H), 10.13 (s, 1H), 6.25 (s, 2H), 2.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 193.4, 166.3, 165.3, 145.1, 112.9, 110.6, 100.5, 17.3; IR (KBr): ν_{max} = 3126, 2345, 1621, 1481, 1276, 1232 cm^{-1}.



2-94c

Aldehyde 2-94c: K_2CO_3 (72.5 g, 525 mmol) and $(CH_3)_2SO_4$ (35 mL, 368 mmol) were added in a sequential fashion to a solution of orcinol (25 g, 176 mmol) in acetone (500 mL),. The reaction was refluxed for 4 h, K_2CO_3 was then filtered and the acetone removed under reduced pressure. The remaining solid was diluted in EtOAc (100 mL), washed with saturated $NH_4Cl_{aq.}$ (100 ml) and dried over $MgSO_4$. Concentration under reduced pressure afforded bis-methylated orcinol in a quantitative yield. $POCl_3$ (60 mL, 644 mmol) was dropped slowly into anhydrous DMF (100 mL) at 0 °C; the resulting solution was then stirred at room temperature for 20 min. A solution of bis-methylated orcinol (24.5 g, 161 mmol) in anhydrous DMF (20 mL) was then added slowly and the reaction mixture was warmed up to 75 °C for 2 h. After cooling down to room temperature, the solution was poured into ice water (300 mL) and neutralized slowly with NaOH pellets. Aldehyde **2-94c** precipitated and was filtered before being dried under vacuum (24 g, 81 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 10.52 (s, 1H), 6.36 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25

°C): δ = 190.5, 165.2, 164.8, 164.4, 108.7, 95.8, 55.7, 55.4, 22.3, (1 quaternary carbon is not detected); ESI-TOF: *m/z*: calculated for $C_{10}H_{12}NaO_3$: 203, found 203 [*M*+Na^+].

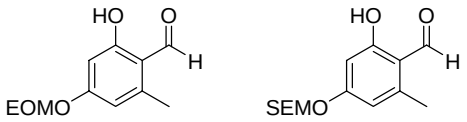


2-94e **2-94j**

General procedure for aldehydes 2-94e and 2-94j: Diisopropylethylamine (4.0 equiv.) and EOMCl/TBDPSCl (4.0 equiv.) were added to a solution of aldehyde **2-94a** (1.0 equiv.) in CH_2Cl_2 (0.05 M). The reaction was stirred for 1 h at room temperature and then, the mixture was washed several times with saturated $NH_4Cl_{aq.}$. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-10 % EtOAc/hexane gradient) afforded aldehydes **2-94e** and **2-94j** in 81 % and 87 % yield respectively.

2-94e: R_f = 0.49 (silica gel, EtOAc/hexane 1:3); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 10.54 (s, 1H), 6.76 (d, *J* = 1.8 Hz, 1H), 6.55 (d, *J* = 1.8 Hz, 1H), 5.32 (s, 2H), 5.28 (s, 2H), 3.81-3.73 (m, 4H), 2.60 (s, 3H), 1.28-1.24 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 190.6, 163.0, 162.0, 144.2, 118.6, 112.3, 100.5, 93.5, 92.7, 64.8, 64.7, 22.1, 15.0; IR (KBr): ν_{max} = 2976, 1676, 1600, 1150 cm^{-1}; ESI-TOF: *m/z*: calculated for $C_{14}H_{20}NaO_5$: 291, found 291 [*M*+Na^+].

2-94j: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 10.86 (s, 1H), 7.56-7.38 (m, 12H), 7.33-7.24 (m, 8H), 6.31 (s, 1H), 5.91 (s, 1H), 2.54 (s, 3H), 1.07 (s, 9H), 0.96 (s, 9H).



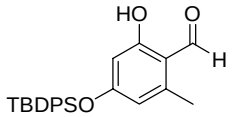
2-94g **2-94h**

General procedure for aldehydes 2-94g and 2-94h: Diisopropylethylamine (0.9 equiv.) and EOMCl/SEMCl (0.9 equiv.) were added to a solution of aldehyde **2-94a** (1.0 equiv.) in CH_2Cl_2 (0.05 M). The reaction was stirred for 1 h at room temperature and then, the mixture was washed several times with saturated $NH_4Cl_{aq.}$. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-

10 % EtOAc/hexane) afforded aldehydes **2-94g** and **2-94h** in 85 % and 75 % yield respectively.

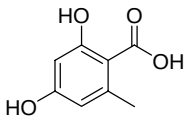
2-94g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.32 (s, 1H), 10.07 (s, 1H), 6.40 (s, 1H), 6.34 (s, 1H), 5.22 (s, 2H), 3.70 (q, *J* = 7.0 Hz, 2H), 2.51 (s, 3H), 1.21 (t, *J* = 7.0 Hz, 3H).

2-94h: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.36 (s, 1H), 10.15 (s, 1H), 6.46 (d, *J* = 1.8 Hz, 1H), 6.40 (s, 1H), 5.27 (s, 2H), 3.77 (t, *J* = 8.2 Hz, 2H), 2.57 (s, 3H), 0.99 (t, *J* = 8.2 Hz, 2H), 0.03 (s, 9H).

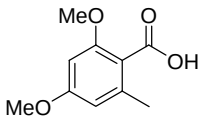


2-94i

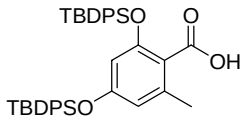
Aldehyde 2-94i: TBSCl (1.5 mL, 5.92 mmol) and imidazole (492 mg, 7.23 mmol) were sequentially added at 0 °C to a solution of aldehyde **2-94a** (1.0 g, 6.57 mmol) in a 1:1 mixture of CH_2Cl_2/THF (30 mL). The reaction was stirred for 12 h at room temperature and then, the mixture was washed several times with saturated $NH_4Cl_{aq.}$. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-5 % Et_2O/hexane) afforded aldehyde **2-94i** (1.67 g, 4.27 mmol) in 65 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.29 (s, 1H), 10.09 (s, 1H), 7.79 (d, *J* = 7.0 Hz, 4H), 7.51-7.44 (m, 6H), 6.27 (s, 1H), 6.22 (s, 1H), 2.44 (s, 3H), 1.19 (s, 9H).



2-95a



2-95c

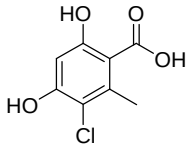


2-95j

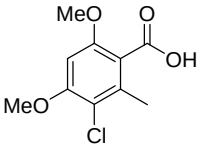
General procedure for oxidation using solvent system A: A solution of aldehyde **2-94** (1.0 equiv.) in H_2O/THF/DMSO (20:10:1, 0.03 M) was sequentially treated at 0 °C with sulfamic acid (3.5 equiv.) and a solution of sodium chlorite (3.25 equiv.) in H_2O. After 0.5-1 h stirring at this temperature, the reaction mixture was diluted with Et_2O, washed with saturated $NH_4Cl_{aq.}$ and dried over $MgSO_4$. Concentration under reduced pressure afforded the corresponding acid **2-95** which was used without any further purification in the next step.

2-95c: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 6.48 (d, *J* = 2.2 Hz, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 2.33 (s, 3H).

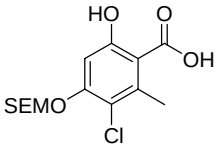
2-94j: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 7.58 (d, *J* = 7.0 Hz, 4H), 7.47-7.35 (m, 10H), 7.31-7.21 (m, 6H), 6.31 (s, 1H), 5.89 (s, 1H), 2.35 (s, 3H), 1.05 (s, 9H), 0.93 (s, 9H).



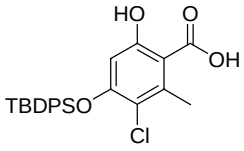
2-95b



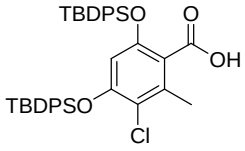
2-95d



2-95h



2-95i



2-95k

General procedure for oxidation using solvent system B: A solution of aldehyde **2-94** (1.0 equiv.) in H_2O/THF (20:10, 0.03 M) was sequentially treated at 0 °C with sulfamic acid (3.5 equiv.) and a solution of sodium chlorite (3.25 equiv.) in H_2O. After 12 h stirring at room temperature, the reaction mixture was diluted with Et_2O, washed with saturated $NH_4Cl_{aq.}$ and dried over $MgSO_4$. Concentration under reduced pressure afforded the corresponding acid **2-95** which was used without any further purification in the next step. For **2-95b**, 2.0 equiv. of sulfamic acid were necessary to avoid over-chlorination. For **2-95i** and **2-95k**, the reaction was complete after 30 min at 0 °C.

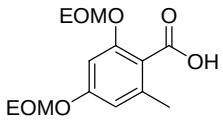
2-95b: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 6.48 (s, 1H), 2.70 (s, 3H).

2-95d: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 6.77 (s, 1H), 3.97 (s, 3H), 3.89 (s, 3H), 2.35 (s, 3H).

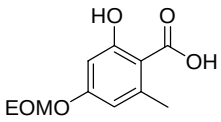
2-95h: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 6.71 (s, 1H), 5.43 (s, 2H), 3.85 (t, *J* = 8.2 Hz, 2H), 2.71 (s, 3H), 1.00 (t, *J* = 8.2 Hz, 2H), 0.04 (s, 9H).

2-95i: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 7.82-7.80 (m, 4H), 7.55-7.48 (m, 6H), 6.02 (s, 1H), 2.74 (s, 3H), 1.17 (s, 9H).

2-95k: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 7.48-7.34 (m, 12H), 7.23-7.16 (m, 8H), 5.93 (s, 1H), 2.54 (s, 3H), 1.05 (s, 9H), 1.02 (s, 9H).



2-95e



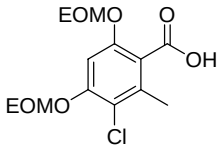
2-95g

General procedure for oxidation using solvent system C: To a solution of aldehyde **2-94** (1.0 equiv.) in DMSO (0.4 M) at 0 °C, were added slowly in a sequential fashion, $NaH_2PO_4 \bullet H_2O$ (5.0 equiv.) dissolved in H_2O (3 M) and $NaClO_2$ (5.0 equiv.) dissolved in H_2O

(3 M). After stirring for 12 h, the reaction was diluted with Et_2O, washed with saturated $NH_4Cl_{aq.}$ and dried over $MgSO_4$. Concentration under reduced pressure resulted into the corresponding acid **2-95** used without further purification in the next step.

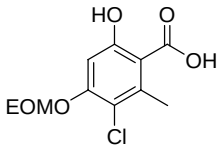
2-95e: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 6.76 (d, *J* = 1.9 Hz, 1H), 6.60 (d, *J* = 1.6 Hz, 1H), 5.25 (s, 4H), 3.75-3.69 (m, 4H), 2.31 (s, 3H), 1.18 (t, *J* = 7.0 Hz, 6H).

2-95g: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 12.13 (bs, 1H), 6.46 (s, 2H), 5.30 (s, 2H), 3.73 (q, *J* = 7.0 Hz, 4H), 2.58 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 3H).



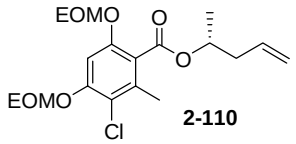
2-95f

Acid 2-95f: To a solution of aldehyde **2-94e** (19.5 g, 72.7 mmol) in a 2:1 mixture of H_2O/THF (240 mL) was added at 0°C a solution of NaH_2PO_4 (56.7 g, 363.3 mmol) in water (90 mL) and a solution of $NaClO_2$ (32.9 g, 363.3 mmol) in water (90 mL). After overnight stirring, the reaction mixture was extracted with Et_2O (400 mL) and washed several times with saturated $NH_4Cl_{aq.}$ (500 mL). Concentration under reduced pressure afforded acid **2-95f** (20.6 g, 64.7 mmol) in 89 % yield. ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 7.10 (s, 1H), 5.36 (s, 2H), 5.27 (s, 2H), 3.80-3.71 (m, 4H), 2.37 (s, 3H), 1.23-1.18 (m, 6H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$, 25 °C): δ = 167.3, 154.0, 153.0, 134.2, 120.9, 116.4, 101.9, 93.8, 93.7, 64.3, 64.1, 16.7, 14.5 (x 2).



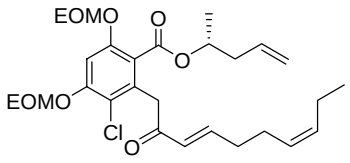
2-108

Acid 2-108: Acid **2-95f** (18.3 g, 57.4 mmol) was stirred for 45 min at room temperature in a 7:1.5:1 mixture of THF/TFA/MeOH (95 mL). Concentration under reduced pressure afforded acid **2-108** (12.0 g, 80 % yield) used without further purification in the next step. ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ = 6.71 (s, 1H), 5.42 (s, 2H), 3.78 (q, *J* = 7.0Hz, 2H), 2.71 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H).



2-110

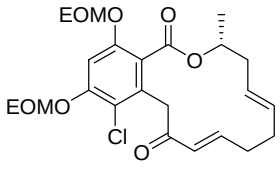
3-Chloro-4,6-bis-ethoxymethoxy-2-methyl-benzoic acid 1-methyl-but-3-enyl ester (2-110): A solution of acid **2-108** (1.04 g, 4.0 mmol), (*S*)-4-penten-2-ol (411 µL, 4.0 mmol) and triphenylphosphine (2.10 g, 8.0 mmol) in anhydrous toluene (40 mL) was treated at room temperature with DIAD (1.58 mL, 8.0 mmol). After stirring for 3 h, the reaction mixture was diluted with EtOAc and washed several times with saturated $NH_4Cl_{aq.}$ and brine. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 % EtOAc/cyclohexane gradient) to yield compound **2-109** (947 mg, 72 %). A solution of compound **2-109** (947 mg, 2.88 mmol) and TBAI (catalytic amount) in DMF (10 mL) was treated with diisopropylethylamine (962 µL, 5.76 mmol) and chloromethylethyl ether (532 µL, 5.76 mmol). After 3 h stirring at 80°C, the reaction mixture was diluted with Et_2O (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (10 mL) and the organic phase was dried on $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (silica gel, 0-20 % EtOAc/hexane) afforded compound **2-110** (780 mg, 70 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.01 (s, 1H), 5.91-5.81 (m, 1H), 5.31 (s, 2H), 5.30-5.22 (m, 2H), 5.22 (s, 2H), 5.18-5.11 (m, 1H), 3.78 (q, *J* = 7.0 Hz, 2H), 3.73 (q, *J* = 7.0 Hz, 2H), 2.53-2.35 (m, 2H), 2.35 (s, 3H), 1.37 (d, *J* = 6.4 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.0, 154.1, 152.9, 134.9, 133.6, 117.8, 107.8, 101.7, 93.9, 93.5, 71.4, 64.6, 64.4, 40.2, 19.5, 17.4, 15.0, 15.0, (1 quaternary carbon is not detected); I.R. (film): ν_{max} = 2916, 1724, 1588, 1320, 1258, 1109, 1039 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{27}O_6ClNa$: 409.1388, found 409.1452 [*M*+Na$^+$]. (-)-(2*R*): $[\alpha]^{25}_D$ = − 1.7 (*c* 1.00, $CHCl_3$).



2-111

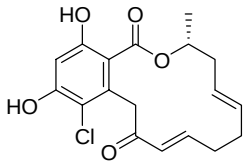
Compound 2-111: A solution of compound **2-110** (500 mg, 1.3 mmol) in anhydrous THF (6.0 mL) cooled at –78 °C was treated with freshly prepared LDA (4.6 mL, 2.6 mmol) followed by immediate addition of compound **2-93** (256 mg, 1.3 mmol) as a THF solution (3.0 mL). The resulting mixture was then stirred for 10 min at –78 °C and quenched by

addition of a solution of saturated NH_4Cl_{aq}. Upon warming to room temperature, the reaction was diluted with EtOAc (15 mL), washed several times with saturated NH_4Cl_{aq}, brine and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-33 % EtOAc/hexane gradient) afforded compound **2-111** (354 mg, 52 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.13 (s, 1H), 6.94 (dt, *J* = 15.8, 6.6 Hz, 1H), 6.20 (d, *J* = 15.8 Hz, 1H), 5.86-5.76 (m, 1H), 5.50-5.37 (m, 1H), 5.35-5.31 (s + m, 2H + 1H), 5.26-5.15 (s + m, 2H + 1H), 5.13-5.07 (m, 2H), 4.06 (s, 2H), 3.75 (2 x q, *J* = 7.2 Hz, 4H), 2.42 (ddd, *J* = 14.6, 7.0, 6.9 Hz, 1H), 2.36-2.15 (m, 5H), 2.10-2.00 (m, 2H), 1.29 (d, *J* = 6.4 Hz, 3H), 1.24 (2 x t, *J* = 7.0 Hz, 3H), 0.98 (t, *J* = 7.6 Hz, 3H).



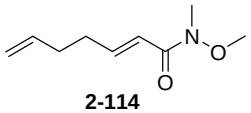
2-112

1-Chloro-2,4-bis-ethoxymethoxy-7-methyl-7,8,11,12-tetrahydro-16H-6-oxa-benzocyclotetradecene-5,15-dione (2-112): A 2 mM solution of compound **2-111** (200 mg, 0.38 mmol) in anhydrous toluene (190 mL) was treated with 10 % mol of catalyst Grubbs' II (30 mg, 0.038 mmol) and heated at 80 °C overnight. The reaction mixture was then passed through a pad of silica, which was washed with CH_2Cl_2. The combined filtrates were concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded pure macrocycle **2-112** (167 mg, 94 %). ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 7.27 (s, 1H), 6.85 (dt, *J* = 15.2, 7.6 Hz, 1H), 6.15 (d, *J* = 15.8 Hz, 1H), 5.16-4.94 (m, 7H), 4.41 (d, *J* = 17.0 Hz, 1H), 4.13 (d, *J* = 17.0 Hz, 1H), 3.61-3.45 (m, 4H), 2.18-2.07 (m, 2H), 1.86-1.62 (m, 4H), 1.38 (d, *J* = 5.8 Hz, 3H), 1.11 (t, *J* = 7.0 Hz, 3H), 1.04 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 193.5, 166.4, 155.0, 154.1, 146.0, 133.7, 131.5, 128.8, 127.7, 121.2, 118.1, 102.9, 93.7, 93.6, 71.6, 64.4, 64.4, 45.0, 39.2, 30.7, 30.4, 19.3, 14.9, 14.8; I.R. (film): ν_{max} = 2917, 1720, 1690, 1622, 1591, 1320, 1255, 1120, 1037 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{31}O_7ClNa$: 489.1651, found 489.1737 [*M*+Na^+]. (-)-(2*R*): $[\alpha]^{25}_D$ = − 24.0 (*c* 0.59, $CHCl_3$).



2-85
Pochonin D

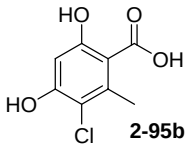
Pochonin D (2-85). Compound **2-112** (50 mg, 0.1 mmol) was stirred for 2 h in a 5:1 mixture of CH_2Cl_2/TFA (3 mL). Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-33 % EtOAc/hexane gradient) afforded synthetic pochonin D **2-85** (25 mg, 72 %). Synthetic pochonin D was found to have identical ^{1}H NMR as natural pochonin D. ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.42 (s, 1H), 6.89 (s, 1H), 6.67-6.62 (m, 1H), 5.82 (d, *J* = 15.6 Hz, 1H), 5.17-5.12 (m, 1H), 5.00-4.92 (m, 1H), 4.76-4.69 (m, 1H), 4.28 (d, *J* = 17.2 Hz, 1H), 4.18 (d, *J* = 17.7 Hz, 1H), 2.54-2.47 (m, 1H), 1.93-1.77 (m, 5H), 0.98 (d, *J* = 6.4 Hz, 3H); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.73 (s, 1H), 6.76-6.69 (m + s, 2H), 6.19 (s, 1H), 5.82 (d, *J* = 15.2 Hz, 1H), 5.46-5.40 (m, 1H), 5.31-5.15 (m, 2H), 4.37 (d, *J* = 17.6 Hz, 1H), 4.09 (d, *J* = 17.0 Hz, 1H), 2.68-2.61 (m, 1H), 2.39-2.03 (m, 5H), 1.34 (d, *J* = 7.0 Hz, 3H); ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 6.74 (dt, *J* = 15.5, 7.6 Hz, 1H), 6.51 (s, 1H), 5.83 (d, *J* = 15.5 Hz, 1H), 5.36-5.22 (m, 3H), 4.25 (d, *J* = 17.7 Hz, 1H), 4.13 (d, *J* = 17.7 Hz, 1H), 2.54-2.47 (ddd, *J* = 14.5, 8.0, 4.0 Hz, 1H), 2.31-2.15 (m, 5H), 1.31 (d, *J* = 6.6 Hz, 3H);^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 194.2, 169.9, 164.3, 157.3, 146.1, 137.1, 131.9, 128.1, 126.2, 115.5, 107.5, 103.6, 72.4, 45.0, 36.4, 31.0, 30.8, 17.2; I.R. (KBr): ν_{max} = 2936, 1654, 1603, 1347, 1313, 1239 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{19}O_5ClNa$: 373.0813, found 373.0903 [*M*+Na$^+$]. (+)-(2*R*): $[\alpha]^{25}_D$ = +11.1 (*c* 0.72, $CHCl_3$).



2-114

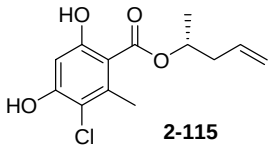
Hepta-2,6-dienoic acid methoxy-methyl-amide (2-114). To a solution of 2-chloro-*N*-methoxy-*N*-methylacetamide (6.0 g, 48.8 mmol) in dry DMF (20 mL) at 23 °C was added 3-mercaptophenol (4.44 mL, 48.8 mmol) and K_2CO_3 (6.7 g, 48.8 mmol). The resulting suspension was stirred at 23 °C overnight. After this period of time, Merrifield resin (24 g, < 2 mmol.g^{-1}, < 48.8 mmol) was added to the mixture followed by K_2CO_3 (11.4 g, 83.0 mmol) as well as TBAI (catalytic amount), and the suspension was heated up to 50 °C. After 12 hours at this temperature, the resin was filtered and washed several times: $HCl_{aq.}$ (50 mL), MeOH (50 mL), CH_2Cl_2 (50 mL) and Et_2O (50 mL). The resin was dried under reduced pressure to constant mass of 29.2 g. The final mass gain (5.2 g, 27.3 mmol) indicated an estimate loading

of 0.81 mmol.g^{-1}. Resin **2-49** (10 g, 0.81 mmol.g^{-1}) was suspended in a 1:1 mixture of HFIP/CH_2Cl_2 (50 mL). To this suspension, H_2O_2 (3 mL, 16.0 mmol) was added at 23 °C and the resulting mixture was shaken for 12 h. Resin **2-113** was then filtered, washed using MeOH (50 mL), CH_2Cl_2 (50 mL) and Et_2O (50 mL) and dried under reduced pressure to constant mass before subsequent use. Resin **2-113** (4.0 g, < 0.81 mmol.g^{-1}) was suspended in DMSO (40 mL) followed by the addition of *t*BuOK (336 mg, 3.0 mmol). After shaking the reaction for 1 h at room temperature, 5-iodo-1-pentene (588 mg, 3.0 mmol) was added to the suspension and the mixture was shaken for 3 h. The resin was filtered, washed and dried as before. Then, it was suspended in toluene and heated at 80 °C. After 8 h at this temperature, the resin was filtered and washed several times with more toluene. The combined toluene solutions were evaporated giving pure compound **2-114** as a colourless oil (321 mg, 77 %) of 95 % purity judged by NMR. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.94 (dt, *J* = 15.7, 6.7 Hz, 1H), 6.39 (d, *J* = 15.2 Hz, 1H), 5.84-5.74 (m, 1H), 5.02 (dd, *J* = 17.4, 1.7 Hz, 1H), 4.97 (d, *J* = 10.1 Hz, 1H), 3.67 (s, 3H), 3.21 (s, 3H), 2.34-2.29 (m, 2H), 2.23-2.18 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.8, 146.7, 137.3, 119.1, 115.3, 61.6, 32.3, 31.7, (one carbon is not detected); I.R. (film): ν_{max} = 2934, 1681, 1638, 1378, 1179 cm^{-1}.



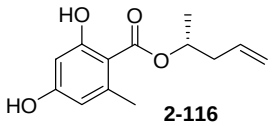
2-95b

Acid 2-95b from commercially available acid 2-95a: Sulfamic acid (582 mg, 6 mmol) and acetaldehyde (700 µL, 12 mmol) were added to a solution of 2,4-dihydroxy-6-methylbenzoic acid **2-95a** (2 g, 12 mmol) in H_2O/THF (54/28 mL). The reaction mixture was then cooled down to 0 °C and treated with a solution of sodium chlorite (540 mg, 6 mmol) in water (2 mL). After stirring for 20 min at 0 °C, the reaction was quenched with saturated $NH_4Cl_{aq.}$ (20 mL) and extracted with EtOAc (50 mL). The organic layer was dried on $MgSO_4$ and concentrated under reduced pressure to afford compound **2-95b** (2.2 g, 92 %) as a single product.



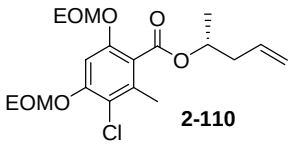
2-115

3-Chloro-4,6-dihydroxy-2-methyl-benzoic acid 1-methyl-but-3-enyl ester (2-115). A solution of acid **2-95b** (808 mg, 4.0 mmol), (*S*)-4-penten-2-ol (411 µL, 4.0 mmol) and tris-(3-chlorophenyl)phosphine (2.91 g, 8.0 mmol) in anhydrous toluene (130 mL) was treated at room temperature with PS-DEAD (13.0 g, 10.0 mmol). After stirring for 10 min, the reaction mixture was filtered on silica and washed with hexane/EtOAc (10:1, 176 mL) and hexane/EtOAc (3:1, 160 mL). The 3:1 mixture was concentrated under reduced pressure to yield compound **2-115** as a single product free of phosphines (703 mg, 65 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.55 (s, 1H), 6.56 (s, 1H), 6.16 (s, 1H), 5.89-5.79 (m, 1H), 5.36-5.29 (m, 1H), 5.19-5.15 (m, 2H), 2.66 (s, 3H), 2.57-2.44 (m, 2H), 1.42 (d, *J* = 6.4 Hz, 3H).



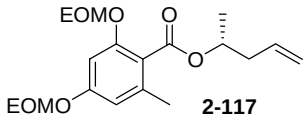
2-116

2,4-Dihydroxy-6-methyl-benzoic acid 1-methyl-but-3-enyl ester (2-116). A solution of acid **2-95a** (840 mg, 5.0 mmol), (*S*)-4-penten-2-ol (514 µL, 5.0 mmol) and tris-(3-chlorophenyl)phosphine (3.64 g, 10.0 mmol) in anhydrous toluene (190 mL) was treated at room temperature with PS-DEAD (19.5 mg, 12.5 mmol). After stirring for 10 min, the reaction mixture was filtered on silica and washed with hexane/EtOAc (10:1, 100 mL) and hexane/EtOAc (3:1, 100 mL). The 3:1 mixture was concentrated under reduced pressure to yield compound **2-116** as a single product free of phosphines (802 mg, 68 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.00 (s, 1H), 6.31 (s, 1H), 6.26 (s, 1H), 6.06 (s, 1H) 5.90-5.79 (m, 1H), 5.30 (q, *J* = 6.4 Hz, 1H), 5.18-5.13 (m, 2H), 2.52 (s, 3H), 2.55-2.43 (m, 2H), 1.40 (d, *J* = 6.4 Hz, 3H).



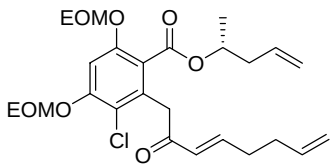
2-110

Ester 2-110 from compound 2-115: A solution of compound **2-115** (700 mg, 2.59 mmol) and TBAI (catalytic amount) in DMF (20 mL) was treated with diisopropylethylamine (1.73 mL, 10.36 mmol) and chloromethylethyl ether (957 µL, 10.36 mmol). After stirring overnight at 80°C, the reaction mixture was diluted with Et_2O (100 mL) and washed several times with saturated $NH_4Cl_{aq.}$ (100 mL). The organic phase was dried on $MgSO_4$, concentrated under reduce pressure to yield compound **2-110** as a single product (950 mg, 95 %).



2-117

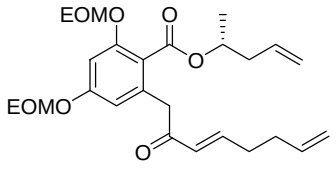
2,4-Bis-ethoxymethoxy-6-methyl-benzoic acid 1-methyl-but-3-enyl ester (2-117). A solution of compound **2-116** (800 mg, 3.3 mmol) and TBAI (catalytic amount) in DMF (20 mL) was treated with diisopropylethylamine (2.18 mL, 13.2 mmol) and chloromethylethyl ether (1.22 mL, 13.2 mmol). After stirring overnight at 80 °C, the reaction mixture was diluted with Et_2O (100 mL) and washed several times with saturated $NH_4Cl_{aq.}$ (100 mL). The organic phase was dried on $MgSO_4$, concentrated under reduced pressure to yield compound **2-117** as a single product (1 g, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.73 (d, *J* = 1.6 Hz, 1H), 6.56 (d, *J* = 1.6 Hz, 1H), 5.92-5.82 (m, 1H), 5.28-5.10 (m, 7H), 3.73 (q, *J* = 7.0 Hz, 4H), 2.53-2.35 (m, 2H), 2.31 (s, 3H), 1.36 (d, *J* = 5.9 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.6, 158.7, 155.4, 137.5, 133.8, 118.8, 117.6, 110.5, 101.2, 93.3, 93.0, 70.8, 64.3, 64.3, 40.2, 19.6, 19.5, 15.1, 15.0; I.R. (film): ν_{max} = 2975, 1719, 1604, 1263, 1149, 1103, 1047, 1018 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{27}O_6Na$: 375.1778, found 375.1832 [*M*+Na$^+$]. (-)-(2*R*): $[\alpha]^{25}_D$ = − 1.5 (*c* 1.00, $CHCl_3$).



2-118

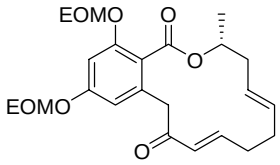
3-Chloro-4,6-bis-ethoxymethoxy-2-(2-oxo-octa-3,7-dienyl)-benzoic acid 1-methyl-but-3-enyl ester (2-118). A solution of compound **2-110** (452 mg, 1.2 mmol) in anhydrous THF (6 mL) cooled at –78 °C was treated with freshly prepared LDA (4.1 mL, 2.3 mmol) followed by immediate addition of compound **2-114** (198 mg, 1.2 mmol). The resulting mixture was then stirred for 10 min at –78 °C and quenched by addition of Amberlite (2.4 g, 24 mmol) suspended in THF (2.5 mL). Upon further warming to room temperature, the reaction was filtered on a pad of silica and washed with EtOAc. Concentration under reduced pressure afforded compound **2-118**, which was then used directly in the metathesis reaction without any further purification. A portion of the product **2-118** was purified by chromatography (silica gel, 0-20 % EtOAc/cyclohexane gradient) for characterization. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.13 (s, 1H), 6.95 (dt, *J* = 16.1, 7.0 Hz, 1H), 6.21 (d, *J* = 16.1 Hz, 1H), 5.87-5.76 (m, 2H), 5.32 (s, 2H), 5.23 (s, 2H), 5.21-5.02 (m, 5H), 4.06 (s, 2H), 3.78 (q, *J* = 7.0

Hz, 2H), 3.74 (q, *J* = 7.0 Hz, 2H), 2.46-2.30 (m, 4H), 2.28-2.22 (m, 2H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 194.5, 166.5, 154.5, 153.8, 149.9, 137.0, 133.7, 132.7, 129.3, 117.7, 115.6, 102.9, 93.9, 93.7, 71.5, 64.7, 64.4, 43.0, 40.1, 32.0, 31.7, 19.4, 15.0, 15.0, (one carbon is not detected); I.R. (film): ν_{max} = 2914, 1718, 1627, 1588, 1257, 1117, 1037 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{35}O_7ClNa$: 517.1964, found 517.2064 [*M*+Na$^+$]. (+)-(2*R*): $[\alpha]^{25}_D$ = + 1.4 (*c* 1.00, $CHCl_3$).



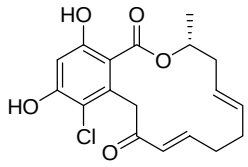
2-119

2,4-Bis-ethoxymethoxy-6-(2-oxo-octa-3,7-dienyl)-benzoic acid 1-methyl-but-3-enyl ester (2-119). A solution of compound **2-117** (529 mg, 1.5 mmol) in anhydrous THF (8.0 mL) cooled to –78 °C was treated with freshly prepared LDA (5.3 mL, 3.0 mmol), followed by immediate addition of compound **2-114** (254 mg, 1.5 mmol) as a solution in THF (1.0 mL). The resulting mixture was then stirred for 10 min at –78 °C and quenched by addition of Amberlite (3.0 g, 30 mmol) pre-swollen in THF (3 mL). Upon warming to room temperature, the reaction was filtered on a pad of silica and washed with EtOAc. Concentration of the filtrates under reduced pressure afforded compound **2-119** with 20 % of the corresponding 1,4-addition compound **2-121**. This mixture was then used directly in the metathesis reaction without any further purification. A portion of the product **2-119** was purified by flash chromatography (silica gel, 0-20 % EtOAc/cyclohexane gradient) for characterization. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.90 (dt, *J* = 15.8, 6.7 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.18 (dt, *J* = 15.8, 1.6 Hz, 1H), 5.89-5.74 (m, 2H), 5.21-4.99 (m, 9H), 3.90 (d, *J* = 16.4 Hz, 1H), 3.83 (d, *J* = 16.4 Hz, 1H), 3.73 (q, *J* = 7.0 Hz, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 2.49-2.19 (m, 6H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.1, 167.2, 159.0, 156.2, 147.1, 137.0, 134.9, 133.9, 129.5, 118.7, 117.6, 115.5, 111.2, 102.6, 93.5, 93.0, 71.1, 64.4, 64.4, 45.4, 40.2, 32.0, 31.7, 19.4, 15.0, 15.0; I.R. (film): ν_{max} = 2976, 1720, 1605, 1442, 1270, 1155, 1109, 1017 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{36}O_7Na$: 483.2353, found 483.2445 [*M*+Na$^+$]. (+)-(2*R*): $[\alpha]^{25}_D$ = + 0.7 (*c* 1.00, $CHCl_3$).



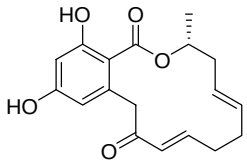
2-120

2,4-Bis-ethoxymethoxy-7-methyl-7,8,11,12-tetrahydro-16H-6-oxa-benzocyclotetradecene-5,15-dione (2-120). A 2 mM solution of crude **2-119** (1.5 mmol) in anhydrous toluene (750 mL) was treated with 10 % mol of catalyst Grubbs' II (139 mg, 0.15 mmol), and heated at 80 °C overnight. The crude reaction mixture was then passed through a pad of silica, which was washed with CH_2Cl_2. The combined filtrates were concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/cyclohexane gradient) afforded pure **2-120** (260 mg, 40 % over two steps). ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 7.08 (d, *J* = 2.2 Hz, 1H), 7.01 (d, *J* = 2.2 Hz, 1H), 6.93-6.86 (m, 1H), 6.17 (d, *J* = 16.1 Hz, 1H), 5.34-4.90 (m, 7H), 4.41 (d, *J* = 14.5 Hz, 1H), 3.75 (d, *J* = 14.5 Hz, 1H), 3.59-3.45 (m, 4H), 2.27-2.12 (m, 2H), 1.95-1.61 (m, 4H), 1.45 (d, *J* = 6.2 Hz, 3H), 1.07 (t, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 196.0, 167.2, 159.5, 156.6, 147.6, 135.8, 131.5, 130.2, 128.5, 119.1, 109.7, 102.3, 93.3, 92.9, 71.0, 64.2, 64.0, 44.5, 39.6, 30.9, 30.2, 20.1, 14.8, 14.8; I.R. (film): ν_{max} = 2976, 1717, 1602, 1438, 1284, 1155, 1110, 1036, 1018 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{32}O_7Na$: 455.2040, found 455.2135 [*M*+Na$^+$]. (-)-(2*R*): $[\alpha]^{25}_D = -50.3$ (*c* 1.00, $CHCl_3$).



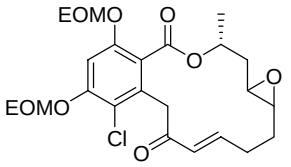
2-85
Pochonin D

Pochonin D (2-85) using polymer-bound reagents: PS-TsOH (300 mg, 3.2 mmol.g^{-1}) was added to a solution of compound **2-112** (50 mg, 0.1 mmol) in MeOH (3 mL) of and the suspension was shaken at 40 ^{o}C for 4 h. The reaction mixture was then filtered and the methanolic solution concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-20 % EtOAc/cyclohexane gradient) afforded synthetic pochonin D **2-85** (32 mg, 90 %).



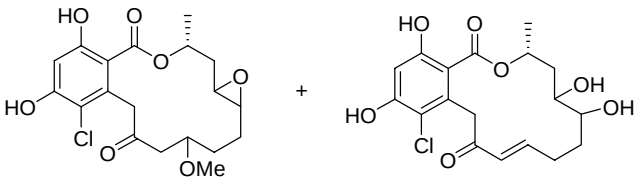
2-103
Monocillin II

Monocillin II (2-103) using polymer-bound reagents: PS-TsOH (145 mg, 3.2 mmol.g^{-1}) was added to a solution of compound **2-120** (20 mg, 0.05 mmol) in MeOH (1.5 mL) and the suspension was shaken at 40 ^{o}C for 4 h. The reaction mixture was then filtered and the methanolic solution concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-20 % EtOAc/cyclohexane gradient) afforded synthetic monocillin II **2-103** (14 mg, 92 %). ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.49 (s, 1H), 6.70-6.62 (m, 1H), 6.49 (d, *J* = 2.9 Hz, 1H), 6.09 (d, *J* = 2.4 Hz, 1H), 5.86 (d, *J* = 15.2 Hz, 1H), 5.08-4.95 (m, 2H), 4.82-4.75 (m, 1H), 4.14 (d, *J* = 16.8 Hz, 1H), 3.71 (d, *J* = 17.0 Hz, 1H), 2.64-2.57 (m, 1H), 1.83-1.76 (m, 3H), 1.74-1.66 (m, 2H), 0.97 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 195.7, 170.3, 166.5, 161.3, 146.1, 140.5, 131.7, 129.9, 126.5, 112.4, 102.8, 72.2, 49.1, 36.6, 31.0, 30.6, 17.5, (one carbon is not detected); I.R. (KBr): ν_{max} = 2936, 1654, 1603, 1347, 1313, 1239 cm^{-1}; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{21}O_5$ requires 317.3980, found 317.3978 [*M*+H^{+}]. (+)-(2*R*): $[\alpha]^{25}_{D}$ = + 40.6 (*c* 0.18, $CHCl_3$).



2-123

Macrocycle 2-123: To a solution of compound **2-112** (50 mg, 0.11 mmol) in CH_3CN (5 mL) at 0 °C was added freshly made DMDO (275 µL, 0.11 mmol, 0.04 M in acetone) and the mixture was stirred for 1.5 h. After evaporation of the solvents under reduced pressure, purification by flash chromatography (silica gel, 0-70 % Et_2O/hexane gradient) afforded compound **2-123** (41 mg, 79 %) as a 1:1 mixture of two diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.12 (s, 1H), 7.11 (s, 1H), 6.90-6.76 (m, 2H), 6.11 (d, *J* = 15.6 Hz, 1H), 6.05 (d, *J* = 15.8 Hz, 1H), 5.37-5.27 (m, 6H), 5.26-5.21 (m, 4H), 4.14 (d, *J* = 16.9 Hz, 1H), 4.12 (d, *J* = 17.4 Hz, 1H), 4.04 (d, *J* = 17.2 Hz, 1H), 3.82-3.70 (m, 9H), 2.81-2.78 (m, 1H), 2.74-2.72 (m, 1H), 2.67-2.62 (m, 2H), 2.38-2.11 (m, 8H), 2.05-2.03 (m, 1H), 2.03-2.00 (m, 1H), 1.74-1.60 (m, 2H), 1.41 (d, *J* = 7.2 Hz, 3H), 1.39 (d, *J* = 6.2 Hz, 3H), 1.27-1.22 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.1 (x 2), 166.7, 166.3, 154.9, 154.8, 154.0, 153.5, 147.8, 147.4, 132.9, 132.4, 129.1, 129.0, 119.7 (x 2), 118.0, 117.9, 102.9, 102.8, 93.9 (x 2), 93.6 (x 2), 71.1, 70.4, 64.8 (x 2), 64.6 (x 2), 58.4, 57.6, 56.9, 55.1, 43.4 (x 2), 39.0, 37.9, 29.9, 29.7, 27.9 (x 2), 18.4, 18.0, 15.0 (x 4); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{32}ClO_8$: 483.1780, found 483.1814 [M+H^+].



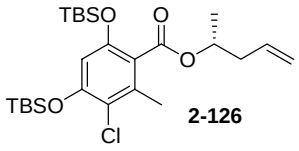
2-124 **2-125**

Macrocycles 2-124 and 2-125: PS-TsOH (264 mg, 3.2 mmol.g^{-1}) was added to a solution of compound **2-123** (41 mg, 85 µmol) in MeOH (3 mL) and the suspension was shaken at 40 °C until consumption of all starting material (~1 h). The reaction mixture was filtered and the methanolic solution concentrated under reduced pressure. L.C./M.S. analysis of the crude mixture showed clearly 2 peaks corresponding to methanol addition on the conjugated olefin (**2-124**) and opening of the epoxide as a diol (**2-125**).

***2-125*:** ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.19 (m, 1H), 6.89-6.81 (m, 1H), 6.52 (s, 1H), 6.47 (s, 1H), 6.20 (d, *J* = 16.1 Hz, 1H), 6.04 (d, *J* = 15.6 Hz, 1H), 5.54-5.49 (m, 1H), 5.43-5.36 (m, 1H), 4.50 (d, *J* = 17.7 Hz, 1H), 4.46 (d, *J* = 17.7 Hz, 1H), 4.39 (d, *J* = 17.2 Hz,

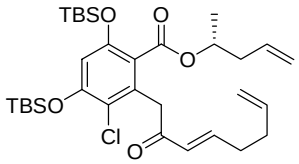
1H), 4.07 (d, *J* = 17.2 Hz, 1H), 3.80-3.64 (m, 2H), 3.51-3.46 (m, 2H), 2.62-2.58 (m, 1H), 2.39-2.30 (m, 2H), 2.27-2.18 (m, 1H), 2.08-2.00 (m, 2H), 2.00-1.85 (m, 4H), 1.44 (d, *J* = 6.4 Hz, 6H); HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{22}ClO_7$: 385.1054, found 385.0944 [*M*+H^+].

***2-124*:** compound **2-124** which was characterized as the MeOH-addition on the α,β-conjugated system based on the loose of olefinic protons in the NMR (a detailed assignment is not possible as product **2-124** represents a mixture of 4 compounds); HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{24}ClO_7$: 399.1211, found 399.1030 [*M*+H^+].



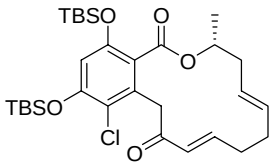
2-126

Ester 2-126: A solution of benzoic acid **2-95b** (500 mg, 2.5 mmol) in CH_2Cl_2 (10 mL) was treated sequentially with diisopropylethylamine (2.4 mL, 14.8 mmol) and TBSCl (1.1 g, 7.4 mmol). After 3 h stirring at room temperature, the reaction mixture was washed several times with brine (30 mL) and dried on Na_2SO_4. Evaporation of the solvent under reduced pressure afforded tris-TBS compound which was used directly without any further purification in the next step. Oxalyl chloride (192 µL, 2.2 mmol) and DMF (catalytic: 20 µL) was added at 0°C to a solution of the tris-TBS compound in anhydrous CH_2Cl_2 (12.5 mL) and the reaction mixture was stirred for 1 h at room temperature. The reaction was then cooled down to 0°C, and treated sequentially with Et_3N (703 µL, 5.1 mmol), (*R*)-4-penten-2-ol (700 µL, 6.7 mmol) and DMAP (catalytic amount) in anhydrous CH_2Cl_2 (2 mL). The reaction was then stirred for 12 h at room temperature and thereafter diluted with EtOAc (20 mL), washed with saturated $NH_4Cl_{aq.}$ (30 mL) and dried over Na_2SO_4. Evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 0-10 % Et_2O/hexane gradient) afforded desired ester **2-126** (357 mg, 0.72 mmol) in 29 % overall yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.33 (s, 1H), 5.84 (ddt, *J* = 16.9, 9.9, 7.0 Hz, 1H), 5.20-5.11 (m, 3H), 2.56-2.49 (m, 1H), 2.42-2.34 (m, 1H), 2.31 (s, 3H), 1.37 (d, *J* = 6.4 Hz, 3H), 1.06 (s, 9H), 1.00 (s, 9H), 0.25 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.3, 152.4, 150.9, 134.9, 133.5, 121.9, 118.8, 118.0, 109.0, 71.4, 40.2, 25.6 (x 6), 19.4, 18.3, 18.2, 17.7, -4.2, -4.3, -4.4 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{43}ClO_4Si_2Na$: 522.2281, found 522.2304 [*M*+Na^+].



2-127

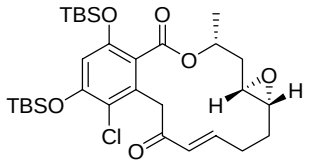
Metathesis precursor 2-127: A solution of ester **2-126** (340 mg, 0.68 mmol) in anhydrous THF (3.5 mL) cooled at –78 °C was treated with freshly prepared LDA (2.4 mL, 1.36 mmol) followed by an immediate addition of Weinreib amide **2-114** (120 mg, 0.68 mmol) as a THF solution (1.7 mL). The resulting mixture was then stirred for 10 min at –78 °C and quenched by addition of a solution of saturated $NH_4Cl_{aq.}$ (10 mL). Upon further warming to room temperature, the reaction was diluted with EtOAc, washed several times with saturated $NH_4Cl_{aq.}$ and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography (silica gel, 0-20 % Et_2O/hexane gradient) afforded compound **2-127** (145 mg) in a 35 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.91 (dt, *J* = 15.8, 6.7 Hz, 1H), 6.41 (s, 1H), 6.17 (d, *J* = 15.5 Hz, 1H), 5.86-5.72 (m, 2H), 5.13-5.01 (m, 5H), 3.95 (s, 2H), 2.48-2.41 (m, 1H), 2.41-2.20 (m, 5H), 1.30 (d, *J* = 6.4 Hz, 3H), 1.04 (s, 9H), 0.99 (s, 9H), 0.26 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 194.6, 166.6, 152.8, 151.6, 146.7, 137.0, 133.5, 132.6, 128.9, 122.2, 119.5, 118.0, 115.6, 110.3, 71.7, 43.9, 40.1, 32.0, 31.7, 25.6 (x 6), 19.3, 18.3, 18.2, -4.2 (x 2), -4.4 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{32}H_{51}O_5ClSi_2Na$: 629.2856, found 629.2881 [M+Na^+].



2-128

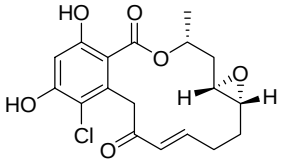
Macrocycle 2-128: A 2 mM solution of compound **2-127** (140 mg, 0.23 mmol) in anhydrous toluene (115 mL) was treated with 10 % mol of catalyst Grubbs' II (18.4 mg, 0.023 mmol) and heated up to 80 °C for 12 h. The reaction mixture was then filtered through a pad of silica, which was washed with CH_2Cl_2. The combined filtrates were concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded macrocycle **2-128** (116 mg, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.71 (dt, *J* = 15.3, 7.3 Hz, 1H), 6.45 (s, 1H), 5.81 (d, *J* = 15.3 Hz, 1H), 5.25 (s, 2H), 5.04-5.03 (m, 1H), 3.89 (d, *J* = 17.4 Hz, 1H), 3.57 (d, *J* = 17.4 Hz, 1H), 2.31-2.04 (m, 6H), 1.35 (d, *J* = 6.4 Hz, 3H), 1.03 (s, 9H), 0.99 (s, 9H), 0.28-0.24 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.8, 166.8, 152.9, 151.7, 146.5, 132.7, 131.9, 128.6, 126.8, 122.8, 119.7, 110.7,

71.9, 45.6, 38.5, 30.9, 25.7 (x 4), 25.6 (x 4), 18.7, 18.3, -4.1 (x 2), -4.4 (x 2) ; HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{47}ClO_5Si_2Na$: 601.2543, found 601.2568 [M+Na^+].



2-129

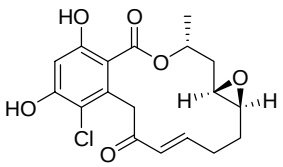
Macrocycle 2-129: An aqueous Na_2•EDTA solution (700 µL, 4 x 10^{-4}M) was added to a solution of compound **2-128** (80 mg, 0.14 mmol) in a 2:1 mixture of dimethoxymethane/acetonitrile (2.1 mL). The resulting mixture was cooled to 0 °C and treated with trifluoroacetone (150 µL) added *via* a precooled syringe. A mixture of sodium bicarbonate (88 mg, 1.05 mmol) and Oxone (430 mg, 0.70 mmol) was added in portions over a period of ~1 h to this homogeneous solution. The reaction was followed by TLC and found to be complete in 2 h. The reaction mixture was then poured into water (10 mL), extracted with CH_2Cl_2 (20 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded pure compound **2-129** (66 mg, 93 %) as a mixture of 2 diastereoisomers in a 3:1 ratio. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.90-6.77 (m, 1.33H), 6.45 (s, 1.33H), 6.06 (d, J = 15.8 Hz, 1.33H), 5.31-5.29 (m, 1H), 5.29-5.21 (m, 0.33H), 4.03 (d, J = 18.1 Hz, 1.33H), 3.63 (d, J = 17.6 Hz, 1H), 2.82-2.80 (m, 1.33H), 2.74-2.71 (m, 1.33H), 2.62-2.60 (m, 1.33H), 2.41-2.11 (m, 4.6H), 2.02-1.95 (m, 0.33H),1.80-1.78 (m; 1H), 1.78-1.68 (m, 0.7H), 1.41 (d, J = 6.4 Hz, 3.9H), 1.05 (s, 12H), 0.97 (s, 12H), 0.26 (s, 16H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.4, 195.1, 167.0, 166.2, 153.1 (x 2), 152.1, 151.5, 147.8, 146.9, 132.9, 132.2, 129.0, 128.3, 122.2, 121.4, 120.0, 119.7, 110.6, 110.3, 71.3, 70.1, 58.2, 57.9, 56.6, 55.2, 44.7, 43.9, 38.7, 37.6, 30.1, 29.4, 28.3, 27.5, 25.7 (x 4), 25.6 (x 4), 25.5 (x 4), 25.4 (x 4), 20.8, 17.8, -3.9, -4.0, -4.3 (x 3), -4.4 (x 3); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{48}O_6ClSi_2$: 595.2672, found 595. 2698 [M+H^+].



2-122
Pochonin A

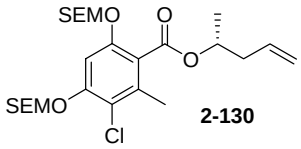
Pochonin A (2-122): TBAF (244 µL, 1M solution in hexane, 0.24 mmol) was added to a solution of compound **2-129** (66 mg, 0.11 mmol) in THF (2 mL) and the mixture was stirred

at room temperature for 20 min. The reaction was then quenched with saturated $NH_4Cl_{aq.}$ (8 mL), extracted several times with EtOAc (10 mL) and dried over Na_2SO_4. Concentration under reduced pressure followed by purification by flash chromatography (silica gel, 0-70 % Et_2O/hexane) afforded two different diastereoisomers pochonin A (**2-122**) and its diastereoisomer **2-122b** as a 3:1 mixture (80 % yield) The isomers were separated by preparative TLC with a 3:1 mixture of Et_2O/hexane. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 10.81 (s, 1H), 10.74 (s, 1H), 6.97-6.89 (m, 1H), 6.53 (s, 1H), 6.08 (d, *J* = 15.8 Hz, 1H), 5.15-5.13 (m, 1H), 4.19 (d, *J* = 17.5 Hz, 1H), 4.09 (d, *J* = 17.5 Hz, 1H), 2.81 (s, 1H), 2.60 (m, 1H), 2.44-2.40 (m, 2H), 2.30-2.22 (m, 2H), 1.80-1.78 (m, 2H), 1.32 (d, *J* = 6.4 Hz, 3H); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.85 (s, 1H), 6.94-6.87 (m, 1H), 6.70 (s, 1H), 6.14 (s, 1H), 6.12 (d, *J* = 16.4 Hz, 1H), 5.32-5.31 (m, 1H), 4.53 (d, *J* = 18.1 Hz, 1H), 4.27 (d, *J* = 18.1 Hz, 1H), 2.77 (s, 1H), 2.58-2.56 (m, 2H), 2.47-2.43 (m, 1H), 2.35-2.28 (m, 1H), 2.11-2.07 (m, 1H), 1.93-1.86 (m, 1H), 1.51 (d, *J* = 6.4 Hz, 3H), 0.94-0.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.0, 170.0, 164.1, 156.4, 147.5, 135.7, 129.9, 115.0, 107.3, 103.8, 72.2, 57.0, 55.5, 45.1, 36.3, 30.9, 29.1, 17.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{19}ClO_6Na$: 389.0762, found 389.0724 [*M*+Na^+]. (-)-(2*R*, 4*R*, 5*R*): $[\alpha]^{25}_D$ = − 7.0 (*c* 0.11, $CHCl_3$).



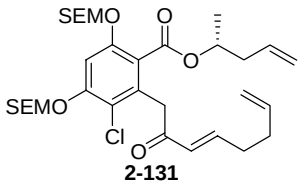
2-122b

Compound 2-122b: ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 10.74 (s, 1H), 10.39 (s, 1H), 6.94-6.89 (m, 1H), 6.52 (s, 1H), 6.02 (d, *J* = 16.4 Hz, 1H), 5.18 (m, 1H), 4.32 (d, *J* = 17.5 Hz, 1H), 3.96 (d, *J* = 17.5 Hz, 1H), 2.82 (s, 1H), 2.68 (s, 1H), 2.34-2.26 (m, 3H), 1.86-1.83 (m, 1H), 1.70-1.63 (m, 1H), 1.22 (d, *J* = 5.8 Hz, 3H), 1H masked by the solvent peak; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.37 (s, 1H), 6.90-6.83 (m, 1H), 6.67 (s, 1H), 6.24 (d, *J* = 16.4 Hz, 1H), 6.08 (s, 1H), 5.39-5.37 (m, 1H), 4.52-4.36 (m, 2H), 2.72-2.62 (m, 2H), 2.56-2.52 (m, 1H), 2.45-2.40 (m, 1H), 2.40-2.37 (m, 1H), 2.08-2.04 (m, 1H), 1.91-1.86 (m, 1H), 1.35 (d, *J* = 6.4 Hz, 3H), 1H masked by the solvent peak; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{19}ClO_6Na$: 389.0762; found 389.0796 [*M*+Na^+]. (+)-(2*R*, 4*S*, 5*S*): $[\alpha]^{25}_D$ = + 13.8 (*c* 0.13, $CHCl_3$).



2-130

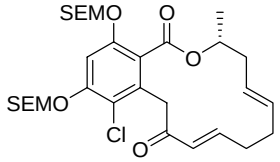
Ester 2-130: A solution of acid **2-95b** (500 mg, 2.5 mmol), (*S*)-4-penten-2-ol (508 µL, 4.9 mmol) and tris-(3-chlorophenyl)phosphine (1.8 g, 4.9 mmol) in anhydrous toluene (50 mL) was treated at room temperature with PS-DEAD (4.7 g, 6.2 mmol). After stirring for 10 min, the reaction mixture was filtered on silica and washed with hexane/EtOAc (10:1, 150 mL) and hexane/EtOAc (3:1, 150 mL). The 3:1 fraction was concentrated under reduced pressure and the resulting crude ester was directly used in the next step. A solution of this ester (2.5 mmol) in THF (10 mL) was treated at 0 °C with sodium hydride (395 mg, 9.9 mmol) and SEMCl (1.75 mL, 9.9 mmol). After stirring for 2 h at this temperature, the reaction mixture was diluted with EtOAc (20 mL) and washed several times with saturated $NH_4Cl_{aq.}$ (30 mL). The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography (silica gel, 0-10 % Et_2O/hexane gradient) to yield ester **2-130** (944 mg, 72 % over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.99 (s, 1H), 5.85 (ddt, *J* = 17.1, 10.2, 7.0 Hz, 1H), 5.29 (s, 2H), 5.28-5.23 (m, 1H), 5.19 (s, 2H), 5.17-5.10 (m, 2H), 3.82-3.73 (m, 4H), 2.48 (ddd, *J* = 7.0, 7.0, 7.0 Hz, 1H), 2.42-2.30 (s + m, 4H), 1.35 (d, *J* = 6.4 Hz, 3H), 0.99-0.94 (m, 4H), 0.03 (s, 9H), 0.02 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.0, 154.2, 153.0, 134.8, 133.6, 120.2, 117.8, 117.0, 101.5, 93.7, 93.3, 71.2, 66.6, 66.4, 40.2, 19.5, 18.0 (x 2), 17.4, -1.42 (x 6); HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{43}O_6ClSi_2Na$: 553.2179, found 553.2022 [*M*+Na^+]. (-)-(2*R*): $[\alpha]^{25}_D = -1.4$ (*c* 1.00, $CHCl_3$).



2-131

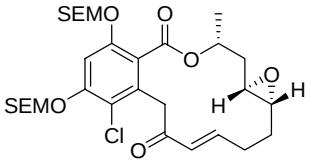
Metathesis precursor 2-131: A solution of ester **2-130** (228 mg, 0.43 mmol) in anhydrous THF (2.5 mL) cooled at –78 °C was treated with freshly prepared LDA (1.52 mL, 0.86 mmol) followed by an immediate addition of Weinreib amide **2-114** (72.6 mg, 0.43 mmol) as a THF solution (1.2 mL). The resulting mixture was then stirred for 10 min at –78 °C and quenched by addition of a solution of saturated $NH_4Cl_{aq.}$ (5 mL). Upon further warming to room temperature, the reaction was diluted with EtOAc (10 mL), washed several times with saturated $NH_4Cl_{aq.}$ (10 mL) and dried over Na_2SO_4. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-20 % Et_2O/hexane gradient) afforded

compound **2-131** (164 mg) in 60 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.12 (s, 1H), 6.94 (dt, *J* = 15.8, 6.7 Hz, 1H), 6.20 (d, *J* = 15.8 Hz, 1H), 5.86-5.76 (m, 2H), 5.31 (s, 2H), 5.21 (s, 2H), 5.20-5.01 (m, 5H), 4.06 (s, 2H), 3.83-3.74 (m, 4H), 2.44-2.30 (m, 4H), 2.27-2.22 (m, 2H), 1.29 (d, *J* = 6.4 Hz, 3H), 1.00-0.96 (m, 4H), 0.03 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 194.5, 166.5, 154.6, 153.9, 146.9, 137.0, 133.7, 132.6, 129.3, 120.3, 117.7, 117.5, 115.6, 102.8, 93.7, 93.5, 71.4, 66.7, 66.5, 43.0, 40.1, 32.0, 31.7, 19.4, 18.0 (x 2), -1.40 (x 6); HRMS (ESI-TOF): *m/z*: calculated for $C_{32}H_{51}O_7ClSi_2H_2O$: 656.2962, found 656.3188 [*M*+H_2O].



2-132

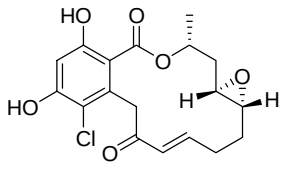
Macrocycle 2-132: A 2 mM solution of compound **2-131** (166 mg, 0.26 mmol) in anhydrous toluene (130 mL) was treated with 10 % mol of Grubbs' II (20.8 mg, 0.026 mmol) and heated up to 80 °C for 12 h. The reaction mixture was then filtered through a pad of silica, which was washed with CH_2Cl_2. The combined filtrates were concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/hexane gradient) afforded macrocycle **2-132** (136 mg, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.08 (s, 1H), 6.77-6.71 (m, 1H), 5.89 (d, *J* = 15.2 Hz, 1H), 5.35-5.24 (m, 6H), 5.09-5.05 (m, 1H), 4.02 (d, *J* = 17.0 Hz, 1H), 3.85-3.78 (m, 5H), 2.37-2.08 (m, 6H), 1.39 (d, *J* = 5.8 Hz, 3H), 1.02-0.97 (m, 4H), 0.04 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.7, 166.8, 154.6, 153.9, 147.0, 132.8, 131.7, 128.6, 127.5, 120.6, 117.8, 102.7, 93.7, 93.3, 71.9, 66.9, 66.6, 44.7, 39.2, 30.8 (x 2), 19.5, 18.0, 17.9, -1.4 (x 6); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{47}O_7ClSi_2H_2O$: 628.2649, found 628.2870 [*M*+H_2O]. (-)-(2*R*): $[\alpha]^{25}_{D}$ = − 16.3 (*c* 0.85, $CHCl_3$).



2-133

Macrocycle 2-133: An aqueous Na_2•EDTA solution (350 µL, 4 x 10^{-4}M) was added to a solution of compound **2-132** (40 mg, 65 µmol) in a 2:1 mixture of

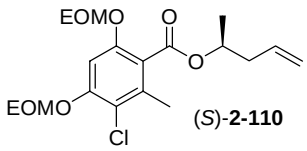
dimethoxymethane/acetonitrile (1.1 mL). The resulting solution was cooled to 0 °C and trifluoroacetone (75 µL) was added *via* a precooled syringe. A mixture of sodium bicarbonate (44 mg, 0.5 mmol) and Oxone (215 mg, 0.35 mmol) was then added in portions over a period of ~1 h to this homogeneous solution. The reaction was followed by TLC and found to be complete in 2 h. The reaction mixture was then poured into water (5 mL), extracted with CH_2Cl_2 (10 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded pure compound **2-133** (34 mg, 82 %) as a 1:1 mixture of two diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.11 (s, 1H), 7.09 (s, 1H), 6.91-6.76 (m, 2H), 6.12 (d, *J* = 15.8 Hz, 1H), 6.06 (d, *J* = 15.8 Hz, 1H), 5.36-5.31 (m, 6H), 5.23-5.22 (m, 4H), 4.15 (d, *J* = 16.9 Hz, 1H), 4.13 (d, *J* = 16.0 Hz, 1H), 4.05 (d, *J* = 17.5 Hz, 1H), 3.85-3.75 (m, 9H), 2.81-2.79 (m, 1H), 2.75-2.73 (m, 1H), 2.68-2.62 (m, 2H), 2.42-2.29 (m, 10H), 1.72-1.60 (m, 2H), 1.41 (d, *J* = 7.6 Hz, 3H), 1.39 (d, *J* = 6.4 Hz, 3H), 1.02-0.96 (m, 8H), 0.03 (s, 36H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.1 (x 2), 166.8, 166.3, 154.9 (x 2), 154.2, 153.7, 147.8, 147.4, 132.9, 132.4, 129.1, 129.0, 119.8, 119.6, 117.9, 117.8, 102.8, 102.7, 93.7 (x 2), 93.4 (x 2), 71.1, 70.3, 66.9, 66.8, 66.6, 66.5, 58.4, 57.6, 56.9, 55.1, 43.5 (x 2), 38.9, 37.9, 29.9, 29.7, 27.9 (x 2), 20.7 (x 2), 18.4, 18.0, 17.9 (x 2), -1.40 (x 12); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{48}O_8ClSi_2$: 627.2571, found 627.2551 [M+H^+].



2-122
Pochonin A

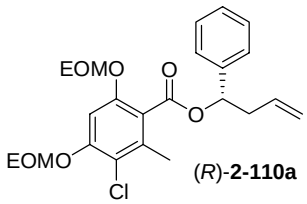
Pochonin A (2-122) from macrocycle 2-133: A solution of compound **2-133** (21 mg, 33 µmol) in CH_2Cl_2 (2.5 mL) was treated at room temperature with $MgBr_2$•Et_2O (69 mg, 0.27 mmol). The reaction was followed by L.C./M.S. until bromohydrine started appearing (~1 h). The reaction was then diluted with EtOAc (5 mL), washed with saturated $NH_4Cl_{aq.}$ (5 mL) and dried over $MgSO_4$. After concentration under reduced pressure, purification by flash chromatography (silica gel, 0-70 % Et_2O/hexane gradient) afforded pochonin A (**2-122**) (8.6 mg, 70 %) as a 1:1 mixture of diastereoisomers.

General procedure for the synthesis of compounds 2-110a-g and 2-117a-g: A solution of acid **2-95a** or **2-95b** (1.0 equiv.), homoallylic alcohol (*R*)-**120a-g** or (*S*)-**120a-g** (1.0 equiv.) and tris-(3-chlorophenyl)phosphine (2.0 equiv.) in anhydrous toluene (0.05 M) was treated at room temperature with PS-DEAD (2.5 equiv., 1.3 $mmol.g^{-1}$). After stirring for 30 min, the reaction mixture was filtered on silica and washed with hexane/EtOAc (10:1, 100 mL) and hexane/EtOAc (3:1, 100 mL). The 3:1 mixture was concentrated under reduced pressure to yield compound **2-115a-g** or **2-116a-g** (60-80 %). Without further purification, compound **2-115a-g** or **2-116a-g** (1.0 equiv.) and TBAI (catalytic amount) were dissolved in DMF (0.15 M) and treated with diisopropylethylamine (4.0 equiv.) and chloromethylethyl ether (4.0 equiv.). After stirring overnight at 80 °C, the reaction mixture was diluted with EtOAc and washed several times with a saturated $NH_4Cl_{aq.}$ solution. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to yield compounds **2-110a-g** and **2-117a-g** (80-90 %).



(*S*)-**2-110**

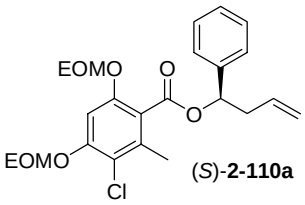
Compound (*S*)-2-110: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.01 (s, 1H), 5.85 (ddt, *J* = 17.2, 9.9, 7.0 Hz, 1H), 5.31-5.19 (m, 1H), 5.30 (s, 2H), 5.21 (s, 2H), 5.18-5.11 (m, 2H), 3.78 (q, *J* = 7.0 Hz, 2H), 3.73 (q, *J* = 7.0 Hz, 2H), 2.48 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1H), 2.42-2.31 (m, 1H), 2.35 (s, 3H), 1.38 (d, *J* = 6.4 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.0, 154.1, 152.9, 134.8, 133.6, 120.2, 117.8, 117.1, 101.7, 93.9, 93.5, 71.3, 64.6, 64.4, 40.2, 19.5, 17.4, 15.0, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{27}O_6ClNa$: 409.1388, found 409.1283 [M+Na^+].



(*R*)-**2-110a**

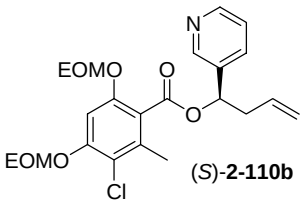
Compound (*R*)-2-110a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.46-7.44 (m, 2H), 7.40-7.33 (m, 3H), 7.00 (s, 1H), 6.09 (dd, *J* = 8.2, 5.8 Hz, 1H), 5.83 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H), 5.31 (s, 2H), 5.18-5.09 (m, 2H), 5.11 (s, 2H), 3.79 (q, *J* = 7.0 Hz, 2H), 3.60 (q, *J* = 7.0 Hz, 2H), 2.81 (ddd, *J* = 14.0, 7.3, 7.3 Hz, 1H), 2.68 (ddd, *J* = 13.7, 7.3, 7.0 Hz, 1H), 2.25 (s,

3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 154.2, 153.0, 139.7, 135.1, 133.3, 128.3 (x 2), 128.0, 126.8 (x 2), 118.0, 101.6, 93.9, 93.5, 76.3, 64.6, 64.3, 40.5, 17.4, 15.0, 14.9, (2 quaternary carbons are not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{29}O_6ClNa$: 471.1545, found 471.1438 [*M*+Na^+].



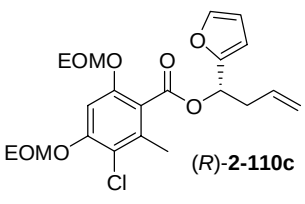
(*S*)-**2-110a**

Compound (*S*)-2-110a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.46-7.44 (m, 2H), 7.40-7.33 (m, 3H), 7.01 (s, 1H), 6.09 (dd, *J* = 7.9, 5.6 Hz, 1H), 5.83 (ddt, *J* = 17.3, 10.2, 7.0 Hz, 1H), 5.31 (s, 2H), 5.18-5.10 (m, 2H), 5.11 (s, 2H), 3.79 (q, *J* = 7.0 Hz, 2H), 3.61 (q, *J* = 7.0 Hz, 2H), 2.79 (ddd, *J* = 15.0, 7.3, 7.3 Hz, 1H), 2.67 (ddd, *J* = 15.1, 7.2, 7.2 Hz, 1H), 2.25 (s, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.7, 154.2, 153.1, 139.7, 135.1, 133.3, 128.3 (x 2), 128.0, 126.8, 126.8 (x 2), 119.9, 118.1, 101.6, 93.9, 93.5, 76.3, 64.6, 64.3, 40.5, 17.4, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{29}O_6ClNa$: 471.1545, found 471.1421 [*M*+Na^+].



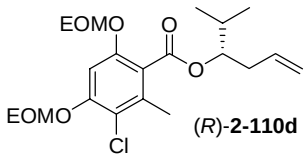
(*S*)-**2-110b**

Compound (*S*)-2-110b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.73 (s, 1H), 8.60 (d, *J* = 4.1 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.32 (dd, *J* = 7.9, 5.0 Hz, 1H), 7.02 (s, 1H), 6.10 (t, *J* = 5.8 Hz, 1H), 5.88-5.75 (m, 1H), 5.31 (s, 2H), 5.23-5.10 (m + s, 2H + 2H), 3.78 (q, *J* = 7.0 Hz, 2H), 3.61 (q, *J* = 7.0 Hz, 2H), 2.82 (ddd, *J* = 14.6, 7.3, 7.3 Hz, 1H), 2.82 (ddd, *J* = 14.6, 7.3, 6.7 Hz, 1H), 2.26 (s, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.18 (t, *J* = 7.0 Hz, 3H); ESI: *m/z*: calculated for $C_{23}H_{29}O_6ClN$: 450.16, found 450.22 [*M*+H^+].



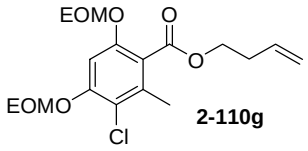
(*R*)-**2-110c**

Compound (*R*)-2-110c: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.43 (d, *J* = 1.8 Hz, 1H), 6.98 (s, 1H), 6.45 (d, *J* = 2.9 Hz, 1H), 6.38 (dd, *J* = 3.5, 1.8 Hz, 1H), 6.18 (t, *J* = 7.0 Hz, 1H), 5.83 (ddt, *J* = 17.3, 10.5, 7.0 Hz, 1H), 5.30 (s, 2H), 5.20 (dd, *J* = 17.3, 1.6 Hz, 1H), 5.14 (s, 2H), 5.11 (dd, *J* = 10.5, 1.6 Hz, 1H), 3.77 (q, *J* = 7.0 Hz, 2H), 3.65 (q, *J* = 7.0 Hz, 2H), 2.88-2.82 (m, 2H), 2.26 (s, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.6, 154.2, 153.0, 151.9, 142.5, 135.1, 132.7, 118.3, 110.2, 109.0, 101.6, 101.1, 93.8, 93.4, 68.9, 64.6, 64.3, 36.7, 17.2, 15.0 (x 2), (1 quaternary carbon is not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{27}O_7ClNa$: 461.1338, found 461.1215 [*M*+Na^+].



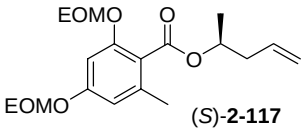
(*R*)-**2-110d**

Compound (*R*)-2-110d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.04 (s, 1H), 5.89 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H), 5.31 (s, 2H), 5.21 (s, 2H), 5.22-5.06 (m, 3H), 3.79 (q, *J* = 7.0 Hz, 2H), 3.72 (q, *J* = 7.0 Hz, 2H), 2.48-2.44 (m, 2H), 2.36 (s, 3H), 2.01 (qd, *J* = 12.4, 7.0 Hz, 1H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.02 (d, *J* = 6.4 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.3, 154.0, 152.9, 134.8, 134.1, 120.4, 117.5, 117.1, 101.5, 93.9, 93.4, 79.0, 64.6, 64.3, 35.6, 30.8, 18.4, 17.6, 17.5, 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{21}H_{31}O_6ClNa$: 437.1701, found 437.1574 [*M*+Na^+].



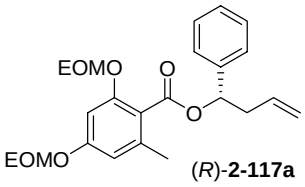
2-110g

Compound 2-110g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.99 (s, 1H), 5.88-5.78 (m, 1H), 5.27 (s, 2H), 5.18 (s, 2H), 5.17-5.07 (m, 2H), 4.36 (t, *J* = 6.7 Hz, 2H), 3.74 (q, *J* = 7.0 Hz, 2H), 3.69 (q, *J* = 7.0 Hz, 2H), 2.51-2.46 (m, 2H), 2.31 (s, 3H), 1.22-1.18 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.3, 154.2, 153.0, 135.0, 133.9, 119.9, 117.2, 117.1, 101.7, 93.8, 93.6, 64.5, 64.3, 64.3, 33.0, 17.4, 14.9 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{26}O_6Cl$: 373.1412, found 373.1364 [*M*+H^+].



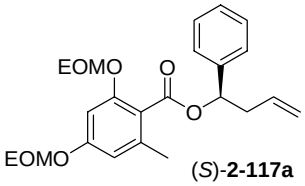
(*S*)-**2-117**

Compound (*S*)-2-117: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.73 (d, *J* = 1.7.0 Hz, 1H), 6.56 (d, *J* = 1.2 Hz, 1H), 5.86 (ddt, *J* = 17.3, 10.3, 7.0 Hz, 1H), 5.31-5.23 (m, 1H), 5.22 (s, 2H), 5.21 (s, 2H), 5.20-5.10 (m, 2H), 3.74 (q, *J* = 7.0 Hz, 4H), 2.54-2.32 (m, 2H), 2.31 (s, 3H), 1.36 (d, *J* = 6.4 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.6, 158.7, 155.4, 137.5, 133.8, 118.8, 117.6, 110.5, 101.2, 93.3, 93.0, 70.8, 64.3, 64.3, 40.2, 19.7, 19.5, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{28}O_6Na$: 375.1778, found 375.1673 [*M*+Na^+].



(*R*)-**2-117a**

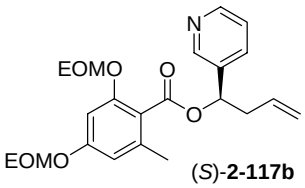
Compound (*R*)-2-117a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.48-7.45 (m, 2H), 7.40-7.32 (m, 3H), 6.75 (s, 1H), 6.57 (s, 1H), 6.11 (dd, *J* = 7.6, 5.8 Hz, 1H), 5.90-5.80 (m, 1H), 5.23 (s, 2H), 5.18-5.10 (m, 2H), 5.14 (s, 2H), 3.74 (q, *J* = 7.0 Hz, 2H), 3.63 (q, *J* = 7.3 Hz, 2H), 2.82 (ddd, *J* = 14.6, 7.3, 7.3 Hz, 1H), 2.69 (ddd, *J* = 13.8, 6.7, 6.7.0 Hz, 1H), 2.24 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.4, 158.8, 155.6, 140.1, 137.9, 133.5, 128.4, 128.3, 127.9, 126.9 (x 2), 118.5, 117.9, 110.5, 101.1, 93.3, 93.0, 75.9, 64.3, 64.2, 40.6, 19.7, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{30}O_6Na$: 437.1935, found 437.1835 [*M*+Na^+].



(*S*)-**2-117a**

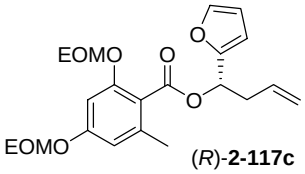
Compound (*S*)-2-117a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.47-7.45 (m, 2H), 7.40-7.29 (m, 3H), 6.74 (d, *J* = 2.0 Hz, 1H), 6.55 (d, *J* = 2.0 Hz, 1H), 6.09 (dd, *J* = 7.9, 5.8 Hz, 1H), 5.84 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H), 5.22 (s, 2H), 5.17-5.08 (m, 2H), 5.13 (s, 2H), 3.74 (q, *J* = 7.0 Hz, 2H), 3.62 (q, *J* = 7.0 Hz, 2H), 2.82 (ddd, *J* = 14.5, 7.3, 7.0 Hz, 1H), 2.68 (ddd, *J* = 13.8, 6.7, 6.7 Hz, 1H), 2.22 (s, 3H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.4, 158.8, 155.6, 140.0, 137.9, 133.5, 128.3 (x 2),

127.9, 126.8 (x 2), 117.9, 110.5, 101.1, 93.2, 93.0, 75.9, 64.3, 64.2, 40.6, 19.7, 15.1, 15.0, (1 quaternary carbon is not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{30}O_6Na$: 437.1935, found 437.1828 [M+Na^+].



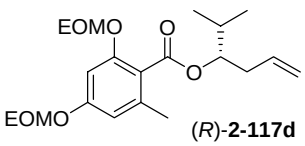
(*S*)-**2-117b**

Compound (*S*)-2-117b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.72 (s, 1H), 8.58 (d, J = 3.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.30 (dd, J = 7.6, 4.8 Hz, 1H), 6.73 (d, J = 1.8 Hz, 1H), 6.55 (d, J = 1.4 Hz, 1H), 6.09 (t, J = 6.8 Hz, 1H), 5.84 (ddt, J = 17.1, 10.2, 6.9 Hz, 1H), 5.21 (s, 2H), 5.15-5.10 (m + s, 2H + 2H), 3.72 (q, J = 7.0 Hz, 2H), 3.61 (q, J = 7.0 Hz, 2H), 2.81 (ddd, J = 14.4, 7.3, 7.2 Hz, 1H), 2.68 (ddd, J = 14.4, 6.9, 6.8 Hz, 1H), 2.22 (s, 3H), 1.24 (t, J = 7.1 Hz, 3H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.3, 159.0, 155.7, 149.3, 148.6, 137.9, 134.5, 132.6, 123.2, 118.7, 117.8, 110.5, 108.1, 101.1, 93.3, 93.0, 73.7, 64.3, 64.3, 40.3, 19.7, 15.1, 15.0; ESI: *m/z*: calculated for $C_{23}H_{30}O_6N$: 416.21, found 416.28 [M+H^+].



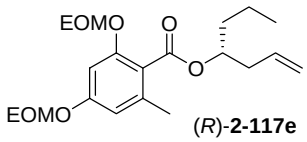
(*R*)-**2-117c**

Compound (*R*)-2-117c:^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.42 (s, 1H), 6.71 (s, 1H), 6.54 (d, J = 1.2 Hz, 1H), 6.44 (d, J = 3.5 Hz, 1H), 6.37 (s, 1H), 6.17 (t, J = 7.0 Hz, 1H), 5.83 (ddt, J = 17.2, 10.2, 7.0 Hz, 1H), 5.21 (s, 2H), 5.17-5.10 (m, 2H), 5.15 (s, 2H), 3.72 (q, J = 7.0 Hz, 2H), 3.66 (q, J = 7.0 Hz, 2H), 2.87-2.83 (m, 1H), 2.23 (s, 3H), 1.24 (t, J = 7.0 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.2, 158.9, 155.5, 152.2, 142.4, 137.9, 132.9, 118.1, 110.6, 110.2, 108.8, 101.1, 93.2, 93.0, 68.5, 64.3, 64.2, 36.8, 36.7, 19.6, 15.1, 15.0.



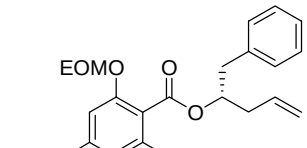
(*R*)-**2-117d**

Compound (*R*)-2-117d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.75 (d, *J* = 1.8 Hz, 1H), 6.55 (d, *J* = 1.8 Hz, 1H), 5.89 (ddt, *J* = 17.1, 10.1, 7.0 Hz, 1H), 5.21 (s, 2H), 5.20 (s, 2H), 5.16-5.04 (m, 3H), 3.73 (q, *J* = 7.0 Hz, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 2.44 (m, 2H), 2.30 (s, 3H), 2.03-1.94 (m, 1H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.7.0 Hz, 3H), 1.00 (d, *J* = 6.7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 168.0, 158.6, 155.4, 137.5, 134.3, 119.0, 117.3, 110.4, 101.0, 93.2, 93.0, 78.5, 64.3 (x 2), 35.7, 30.9, 19.8, 18.5, 17.5, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{21}H_{32}O_6Na$: 403.2091, found 403.1987 [*M*+Na^+].



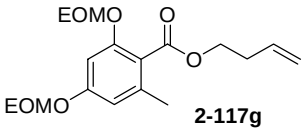
(*R*)-**2-117e**

Compound (*R*)-2-117e: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.74 (d, *J* = 1.2 Hz, 1H), 6.56 (s, 1H), 5.93-5.83 (m, 1H), 5.20 (ddt, *J* = 16.9, 9.9, 7.0 Hz, 1H), 5.21 (s, 2H), 5.20 (s, 2H), 5.16-5.08 (m, 2H), 3.72 (q, *J* = 7.0 Hz, 4H), 2.45 (t, *J* = 6.4 Hz, 2H) 2.31 (s, 3H), 1.68-1.39 (m, 4H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 0.96 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.9, 158.6, 155.3, 137.4, 133.9, 118.9, 117.5, 110.4, 101.0, 93.2, 93.0, 73.9, 64.2 (x 2), 38.7, 35.7, 19.7, 18.5, 15.0, 15.0, 13.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{21}H_{32}O_6Na$: 403.2096, found 403.2017 [*M*+Na^+].



(*R*)-**2-117f**

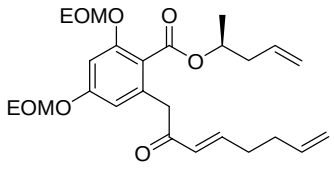
Compound (*R*)-2-117f: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.34-7.25 (m, 5H), 6.75 (s, 1H), 6.54 (s, 1H), 5.94 (ddt, *J* = 17.0, 9.9, 7.0 Hz, 1H), 5.48 (tt, *J* = 6.4, 6.4 Hz, 1H), 5.22 (s, 2H), 5.18-5.14 (m, 4H), 3.74 (q, *J* = 7.0 Hz, 2H), 3.70 (q, *J* = 7.0 Hz, 2H), 3.05 (dd, *J* = 14.0, 7.0 Hz, 1H), 2.95 (dd, *J* = 14.0, 6.4 Hz, 1H), 2.48-2.45 (m, 2H), 2.11 (s, 3H), 1.25 (t, *J* = 7.3 Hz, 3H), 1.23 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.8, 158.7, 155.4, 137.6, 137.5, 133.7, 129.5 (x 2), 128.4 (x 2), 126.5, 118.7, 117.9, 110.5, 101.1, 93.3, 93.0, 74.6, 64.3, 64.3, 39.8, 37.8, 19.4, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{32}O_6ClNa$: 451.2130, found 451.2084 [*M*+Na^+].



2-117g

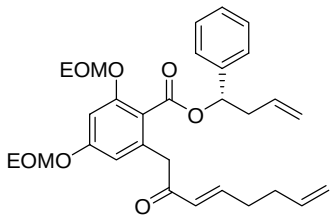
Compound 2-117g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.71 (d, *J* = 1.8 Hz, 1H), 6.53 (d, *J* = 1.7 Hz, 1H), 5.87-5.80 (m, 1H), 5.17 (s, 4H), 5.16-5.05 (m, 2H), 4.34 (t, *J* = 6.7 Hz, 2H), 3.72-3.66 (m, 4H), 2.50-2.45 (m, 2H), 2.27 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.9, 158.8, 155.6, 137.7, 134.1, 118.4, 117.1, 110.6, 101.2, 93.4, 92.9, 64.3, 64.2, 64.0, 33.0, 19.7, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{27}O_6$: 339.1802, found 339.1745 [*M*+H^+].

General procedure for the synthesis of compounds 2-119a-g and 2-140a-g: A solution of compound **2-110a-g** or **2-117a-g** (1.0 equiv.) in anhydrous THF (0.2 M) cooled at –78 °C was treated with freshly prepared LDA (2.0 equiv.). Immediately after, the α,β-unsaturated Weinreb amide **2-114** was added to the cooled solution (1.0 equiv.). The resulting mixture was then stirred for 10 min at –78 °C and quenched by addition of Amberlite resin (20 equiv.). Upon warming up to room temperature, the reaction was filtered on a pad of silica and washed with EtOAc. Concentration under reduced pressure afforded the desired compound **2-118a-g** or **2-119a-g.** This compound was used directly in the metathesis reaction without any further purification. When X = H, 20 % of the corresponding 1,4-addition compound was observed and a fraction of the mixture was purified for characterization of compounds **2-119a-g** and **2-140a-g** (silica gel, 0-20 % EtOAc/hexane gradient).



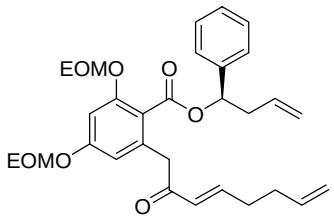
(*S*)-**2-119**

Compound (*S*)-2-119: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.90 (dt, *J* = 15.8, 6.7 Hz, 1H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.18 (dt, *J* = 15.8, 1.6 Hz, 1H), 5.89-5.74 (m, 2H), 5.21-4.99 (m, 9H), 3.90 (d, *J* = 16.4 Hz, 1H), 3.83 (d, *J* = 16.4 Hz, 1H), 3.73 (q, *J* = 7.0 Hz, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 2.49-2.19 (m, 6H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.22 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.1, 167.2, 159.0, 156.2, 147.1, 137.0, 134.9, 133.9, 129.5, 118.7, 117.6, 115.5, 111.2, 102.6, 93.5, 93.0, 71.1, 64.4, 64.4, 45.4, 40.2, 32.0, 31.7, 19.4, 15.0, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{36}O_7Na$: 483.2353, found 483.2235 [*M*+Na^+].



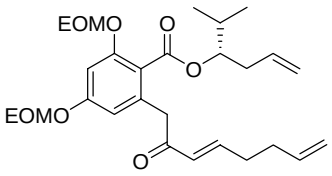
(*R*)-**2-119a**

Compound (*R*)-2-119a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.43 (d, *J* = 7.0 Hz, 2H), 7.37-7.30 (m, 3H), 6.85 (s, 1H), 6.78 (dt, *J* = 14.8, 7.1 Hz, 1H), 6.53 (s, 1H), 6.06 (d, *J* = 16.9 Hz, 1H), 6.01 (d, *J* = 5.8 Hz, 1H), 5.84-5.76 (m, 2H), 5.21 (s, 2H), 5.15 (s, 2H), 5.14-5.00 (m, 4H), 3.81 (d, *J* = 17.0 Hz, 2H), 3.72 (q, *J* = 7.0 Hz, 2H), 3.64 (q, *J* = 7.0 Hz, 2H), 2.78 (ddd, *J* = 14.5, 7.2, 7.1 Hz, 2H), 2.78 (ddd, *J* = 13.6, 6.6, 6.6 Hz, 2H), 2.27-2.18 (m, 4H), 1.25-1.19 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.1, 167.0, 159.2, 156.3, 147.0, 139.9, 137.0, 135.1, 133.5, 129.5, 128.3 (x 2), 127.9, 126.9 (x 2), 118.3, 117.9, 115.5, 111.2, 102.5, 93.4, 93.0, 76.2, 64.4, 64.3, 45.1, 40.5, 32.0, 31.7, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{31}H_{38}O_7Na$: 545.2510, found 545.2347 [*M*+Na^+].



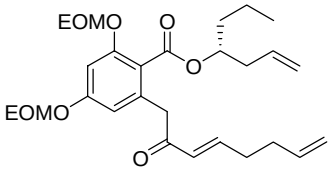
(*S*)-**2-119a**

Compound (*S*)-2-119a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.44-7.42 (m, 2H), 7.39-7.29 (m, 3H), 6.85 (d, *J* = 1.8 Hz, 1H), 6.78 (dt, *J* = 15.8, 6.4 Hz, 1H), 6.53 (s, 1H), 6.06 (d, *J* = 16.4 Hz, 1H), 6.01 (d, *J* = 7.0 Hz, 1H), 5.86-5.74 (m, 2H), 5.21 (s, 2H), 5.15 (s, 2H), 5.13-5.01 (m, 4H), 3.80 (d, *J* = 16.4 Hz, 1H), 3.76 (d, *J* = 16.4 Hz, 1H), 3.72 (q, *J* = 7.0 Hz, 2H), 3.65 (q, *J* = 7.0 Hz, 2H), 2.79 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1H), 2.65 (ddd, *J* = 14.6, 7.0, 7.0 Hz, 1H), 2.29-2.17 (m, 4H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.20 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.1, 167.2, 159.0, 156.2, 147.1, 137.0, 134.9, 133.9, 129.5, 128.2 (x 2), 127.8, 126.9 (x 2), 118.7, 117.6, 115.5, 111.2, 102.6, 93.4, 93.0, 71.1, 64.4, 64.3, 45.4, 40.2, 32.0, 31.7, 19.4, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{31}H_{38}O_7Na$: 545.2510, found 545.2346 [*M*+Na^+].



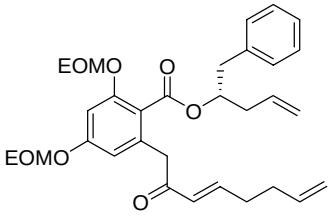
(*R*)-**2-119d**

Compound (*R*)-2-119d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.91 (dt, *J* = 15.8, 6.7 Hz, 1H), 6.87 (d, *J* = 1.8 Hz, 1H), 6.53 (d, *J* = 1.8 Hz, 1H), 6.19 (d, *J* = 15.8 Hz, 1H), 5.91-5.77 (m, 2H), 5.23 (s, 2H), 5.22 (s, 2H), 5.15-5.00 (m, 5H), 3.87 (s, 2H), 3.73 (q, *J* = 7.0 Hz, 4H), 2.45-2.40 (m, 2H), 2.35-2.29 (m, 2H), 2.25-2.20 (m, 2H), 2.00-1.92 (m, 1H), 1.24 (t, *J* = 7.0 Hz, 6H), 1.00 (d, *J* = 2.3 Hz, 3H), 0.98 (d, *J* = 3.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.3, 167.6, 159.0, 156.2, 147.1, 137.0, 135.0, 134.4, 129.5, 118.7, 117.2, 115.5, 111.0, 102.3, 93.3, 93.0, 78.8, 64.4, 64.3, 45.4, 35.8, 32.0, 31.7, 30.8, 18.5, 17.4, 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{28}H_{40}O_7Na$: 511.2666, found 511.2521 [*M*+Na^+].



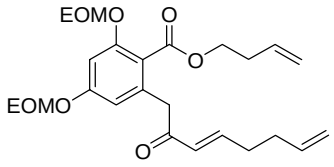
(*R*)-**2-119e**

Compound (*R*)-2-119e: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.89 (dt, *J* = 15.4, 6.7 Hz, 1H), 6.83 (d, *J* = 1.2 Hz, 1H), 6.51 (d, *J* = 1.8 Hz, 1H), 6.17 (d, *J* = 15.8 Hz, 1H), 5.90-5.73 (m, 2H), 5.20 (s, 2H), 5.19 (s, 2H), 5.16-5.12 (m, 2H), 5.06 (2 x d, *J* = 11.1 Hz, 2H), 4.99 (d, *J* = 11.1 Hz, 1H), 3.85 (s, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 3.69 (q, *J* = 7.0 Hz, 2H), 2.40 (dd, *J* = 6.4, 6.4 Hz, 2H), 2.30 (ddd, *J* = 7.2, 7.2, 7.0 Hz, 2H), 2.21 (ddd, *J* = 6.9, 6.8, 6.6 Hz, 2H), 1.66-1.53 (m, 2H), 1.52-1.34 (m, 2H), 1.22 (t, *J* = 6.4 Hz, 3H), 1.20 (t, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.1, 167.4, 159.0, 156.1, 147.1, 137.0, 134.9, 134.0, 129.5, 118.7, 117.5, 115.5, 111.0, 102.3, 93.3, 93.0, 74.2, 64.3, 64.3, 45.4, 38.6, 35.6, 32.0, 31.7, 18.4, 15.0, 15.0, 13.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{28}H_{40}O_7Na$: 511.2672, found 511.2714 [*M*+Na^+].



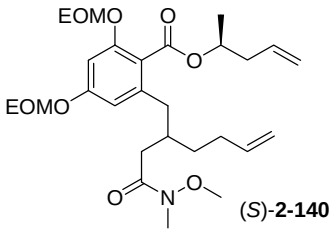
(*R*)-**2-119f**

Compound (*R*)-2-119f: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.30-7.24 (m, 5H), 6.88-6.81 (m, 1H), 6.84 (s, 1H), 6.50 (d, *J* = 1.8 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 5.95-5.86 (m, 1H), 5.84-5.74 (m, 1H), 5.44 (m, 1H), 5.21 (s, 2H), 5.17-5.11 (m, 4H), 5.10-5.00 (m, 2H), 3.79-3.66 (m, 5H), 3.61 (d, *J* = 16.4 Hz, 1H), 3.01 (dd, *J* = 13.4, 7.0 Hz, 1H), 2.93 (dd, *J* = 14.0, 6.4 Hz, 1H), 2.44-2.41 (m, 2H), 2.33-2.19 (m, 4H), 1.27-1.21 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.2, 167.3, 159.1, 156.2, 147.1, 137.6, 137.0, 134.9, 133.8, 129.5, 129.4 (x 2), 128.4 (x 2), 126.5, 118.5, 117.9, 115.5, 111.0, 102.5, 93.4, 93.0, 74.8, 64.4, 64.3, 45.2, 39.7, 37.7, 32.0, 31.7, 15.1, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{32}H_{40}O_7Na$: 559.2666, found 559.2771 [*M*+Na^+].



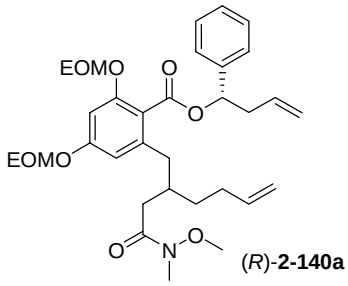
2-119g

Compound 2-119g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.90 (dt, *J* = 15.6, 6.4 Hz, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 6.53 (d, *J* = 2.1 Hz, 1H), 6.17 (d, *J* = 16.1 Hz, 1H), 5.90-5.74 (m, 2H), 5.21 (m, 4H), 5.17-4.99 (m, 4H), 4.31 (t, *J* = 6.7 Hz, 2H), 3.87 (s, 2H), 3.76-3.68 (m, 4H), 2.49-2.44 (m, 2H), 2.34-2.29 (m, 2H), 2.24-2.21 (m, 2H), 1.25-1.20 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 196.1, 167.5, 159.2, 156.5, 147.1, 137.0, 135.2, 134.1, 129.5, 118.2, 117.1, 115.6, 111.3, 102.7, 93.6, 93.0, 64.4, 64.4, 64.2, 45.6, 33.0, 32.0, 31.7, 15.0, 15.0.



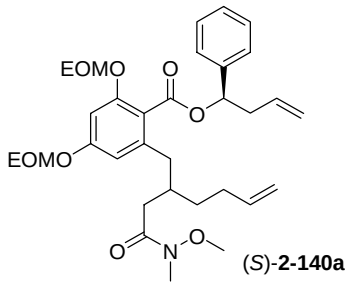
(*S*)-**2-140**

Compound (*S*)-2-140: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.73 (s, 1H), 6.59 (s, 1H), 5.91-5.73 (m, 2H), 5.24-5.16 (m, 5H), 5.11-4.91 (m, 4H), 3.70 (q, *J* = 7.0 Hz, 4H), 3.59 (s, 3H), 3.15 (s, 3H), 2.72-2.67 (m, 1H), 2.54-2.45 (m, 2H), 2.40-2.28 (m, 4H), 2.11-2.02 (m, 2H), 1.34 (d, *J* = 6.4 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 6H), 1.03 (t, *J* = 6.4 Hz, 2H); HRMS (ESI-TOF): *m/z*: calculated for $C_{28}H_{44}O_8N$: 522.2969, found 522.3061 [*M*+H^+].



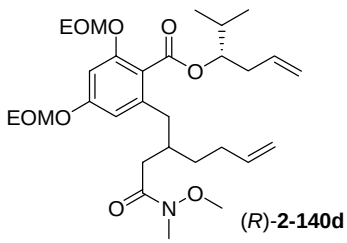
(*R*)-**2-140a**

Compound (*R*)-2-140a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.47-7.44 (m, 2H), 7.37-7.29 (m, 3H), 6.74 (s, 1H), 6.59 (s, 1H), 6.05 (t, *J* = 6.7 Hz, 1H), 5.85-5.69 (m, 2H), 5.22-5.18 (m, 2H), 5.14-5.06 (m, 4H), 4.99-4.90 (m, 2H), 3.72-3.67 (m, 2H), 3.62-3.58 (m, 2H), 3.56 (s, 3H), 3.13 (s, 3H), 2.85-2.78 (m, 1H), 2.70-2.59 (m, 2H), 2.26-2.23 (m, 3H), 2.04-1.90 (m, 3H), 1.24-1.15 (m, 6H), 1.04 (t, *J* = 6.4 Hz, 2H); HRMS (ESI-TOF): *m/z*: calculated for $C_{33}H_{46}O_8N$: 584.3218, found 584.3137 [M+H^+].



(*S*)-**2-140a**

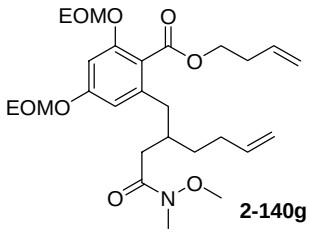
Compound (*S*)-2-140a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.48-7.44 (m, 2H), 7.37-7.28 (m, 3H), 6.74 (s, 1H), 6.59 (s, 1H), 6.05 (t, *J* = 7.0 Hz, 1H), 5.87-5.69 (m, 2H), 5.22-5.18 (m, 2H), 5.14-5.06 (m, 4H), 4.99-4.90 (m, 2H), 3.74-3.68 (m, 2H), 3.62-3.58 (m, 2H), 3.56 (s, 3H), 3.13 (s, 3H), 2.84-2.79 (m, 1H), 2.70-2.59 (m, 2H), 2.26-2.23 (m, 3H), 2.05-1.90 (m, 3H), 1.28-1.17 (m, 6H), 1.05 (t, *J* = 6.4 Hz, 2H); HRMS (ESI-TOF): *m/z*: calculated for $C_{33}H_{46}O_8N$: 584.3218, found 584.3220 [M+H^+].



(*R*)-**2-140d**

Compound (*R*)-2-140d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.75 (s, 1H), 6.60 (s, 1H), 5.91-5.72 (m, 2H), 5.21-5.15 (m, 5H), 5.10-4.90 (m, 4H), 3.73-3.68 (m, 4H), 3.58 (s, 3H), 3.16 (s, 3H), 2.72-2.66 (m, 1H), 2.60-2.41 (m, 4H), 2.24-2.16 (m, 1H), 2.10-1.95 (m, 4H),

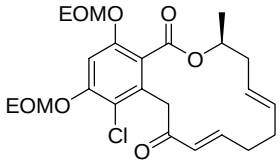
1.22-1.18 (m, 6H), 1.04-0.97 (m, 8H); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{48}O_8N$: 550.3374, found 550.3317 [M+H^+].



2-140g

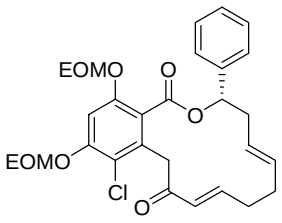
Compound 2-140g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.72 (s, 1H), 6.57 (s, 1H), 5.89-5.71 (m, 2H), 5.20-5.16 (m, 4H), 5.12-4.90 (m, 4H), 4.33 (t, J = 6.8 Hz, 2H), 3.69 (2 x q, J = 7.0 Hz, 4H), 3.57 (s, 3H), 3.13 (s, 3H), 2.69-2.64 (m, 1H), 2.53-2.45 (m, 4H), 2.32 (m, 2H), 2.08-2.03 (m, 2H), 1.19 (t, J = 6.8 Hz, 6H), 1.01 (t, J = 6.5 Hz, 2H); HRMS (ESI-TOF): *m/z*: calculated for $C_{27}H_{42}O_8N$: 508.2905, found 508.2873 [M+H^+].

General procedure for the metathesis reaction. A solution of crude **2-118a-g** or **2-119a-g** (or mixture **2-119a-g** or **2-140a-g** when X = H) in anhydrous toluene (2 mM) was treated with Grubbs' II (0.10 equiv.) and heated at 80 °C for 12 h. The reaction was cooled down to room temperature and the mixture was filtered through a pad of silica gel, washed with CH_2Cl_2 followed by a mixture EtOAc/cyclohexane 1:1, and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-25 % EtOAc/cyclohexane gradient) afforded compound **2-112a-g** or **2-120a-g** (and **2-121a-g**) (38-70 % over two steps).



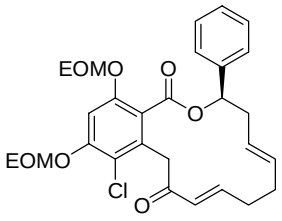
(*S*)-**2-112**

Compound (*S*)-2-112: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.10 (s, 1H), 6.75-6.71 (m, 1H), 5.88 (d, J = 15.8 Hz, 1H), 5.32 (s, 2H), 5.27-5.20 (m, 2H), 5.25 (s, 2H), 5.08-5.04 (m, 1H), 4.01 (d, J = 17.0 Hz, 1H), 3.82-3.73 (m, 5H), 2.36-2.32 (m, 2H), 2.26-2.20 (m, 3H), 2.12-2.05 (m, 2H), 1.38 (d, J = 5.8 Hz, 3H), 1.25 (t, J = 7.0 Hz, 3H), 1.24 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.5, 166.7, 154.6, 153.8, 147.1, 132.8, 131.6, 128.6, 127.5, 120.7, 117.8, 103.0, 94.0, 93.7, 72.0, 64.8, 64.6, 44.7, 39.1, 30.8, 19.4, 15.0, 2C missing; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{31}O_7ClNa$: 489.1551, found 489.1651 [M+Na^+]. (+)-(2*S*): $[\alpha]^{25}_D$ = + 25.0 (*c* 1.00, $CHCl_3$).



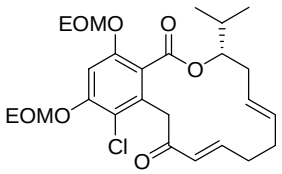
(*R*)-**2-112a**

Compound (*R*)-2-112a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.49-7.47 (m, 2H), 7.40-7.29 (m, 3H), 7.10 (s, 1H), 6.84-6.77 (m, 1H), 5.98 (d, *J* = 15.2 Hz, 1H), 5.78 (d, *J* = 8.8 Hz, 1H), 5.44-5.30 (m, 4H), 5.15 (d, *J* = 7.0 Hz, 1H), 5.05 (d, *J* = 6.8 Hz, 1H), 4.07 (d, *J* = 17.0 Hz, 1H), 3.90 (d, *J* = 17.0 Hz, 1H), 3.80 (d, *J* = 7.0 Hz, 2H), 3.60-3.51 (m, 2H), 2.68-2.62 (m, 1H), 2.50-2.47 (m, 1H), 2.38-2.29 (m, 2H), 2.14-2.02 (m, 2H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.17 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.7, 166.7, 154.8, 154.2, 147.3, 140.7, 133.3, 132.1, 128.5, 128.3 (x 2), 128.2, 127.9, 127.7, 126.7 (x 2), 120.1, 118.1, 102.9, 93.9, 93.4, 77.4, 64.8, 64.4, 44.5, 40.5, 30.7, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{33}O_7ClNa$: 551.1680, found 551.1807 [*M*+Na^+]. $[\alpha]^{25}_D$ = − 40.4 (*c* 0.79, $CHCl_3$).



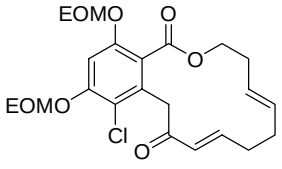
(*S*)-**2-112a**

Compound (*S*)-2-112a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.53-7.51 (m, 2H), 7.44-7.33 (m, 3H), 7.14 (s, 1H), 6.88-6.81 (m, 1H), 6.02 (d, *J* = 15.2 Hz, 1H), 5.78 (dd, *J* = 10.5, 1.8 Hz, 1H), 5.46-5.33 (m, 4H), 5.19 (d, *J* = 7.0 Hz, 1H), 5.09 (d, *J* = 7.6 Hz, 1H), 4.10 (d, *J* = 17.0 Hz, 1H), 3.94 (d, *J* = 17.5 Hz, 1H), 3.83 (d, *J* = 7.0 Hz, 2H), 3.64-3.55 (m, 2H), 2.70-2.64 (m, 1H), 2.54-2.50 (m, 1H), 2.37-2.33 (m, 2H), 2.15-2.08 (m, 2H), 1.29 (t, *J* = 7.3 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.7, 166.7, 154.8, 154.2, 147.3, 140.7, 133.2, 132.1, 128.5, 128.2, 127.9, 127.7, 126.7, 120.1, 118.0, 102.8, 93.9, 93.5, 77.4, 64.8, 64.4, 44.5, 40.5, 30.7, 15.0, 14.9, (one carbon is not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{33}O_7ClNa$: 551.1680, found 551.1704 [*M*+Na^+]. $[\alpha]^{25}_D$ = + 48.8 (*c* 1.00, $CHCl_3$).



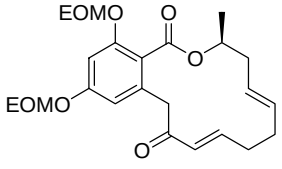
(*R*)-**2-112d**

Compound (*R*)-2-112d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.14 (s, 1H), 6.72-6.66 (m, 1H), 5.88 (d, *J* = 15.2 Hz, 1H), 5.33-5.17 (m, 6H), 4.92-4.88 (m, 1H), 4.21 (d, *J* = 17.0 Hz, 1H), 3.92 (d, *J* = 17.0 Hz, 1H), 3.79-3.67 (m, 4H), 2.33-2.17 (m, 5H), 2.07-1.96 (m, 2H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 5.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.7, 167.1, 154.7, 154.4, 147.4, 133.7, 131.2, 128.8, 128.4, 119.7, 118.0, 102.7, 93.9, 93.5, 80.0, 64.8, 64.5, 44.1, 32.3, 31.2, 30.7, 30.6, 18.3, 17.2, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{35}O_7ClNa$: 517.1964, found 517.1844 [*M*+Na^+]. $[\alpha]^{25}_D$ = + 21.3 (*c* 1.00, $CHCl_3$).



2-112g

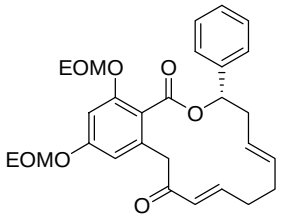
Compound 2-112g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.12 (s, 1H), 6.76-6.70 (m, 1H), 5.87 (d, *J* = 15.0 Hz, 1H), 5.33 (s, 2H), 5.26 (s, 2H), 5.24-5.16 (m, 2H), 4.25 (t, *J* = 5.1 Hz, 2H), 3.82-3.73 (m, 6H), 2.40-2.36 (m, 2H), 2.16-2.13 (m, 4H), 1.27-1.23 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.2, 167.2, 154.6, 153.4, 146.8, 132.3, 131.3, 128.9, 128.5, 121.0, 117.8, 103.0, 94.0, 93.7, 64.8, 64.7, 64.6, 45.4, 31.9, 31.0, 30.7, 15.0, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{30}O_7Cl$: 453.1675, found 453.1672 [*M*+H^+].



(*S*)-**2-120**

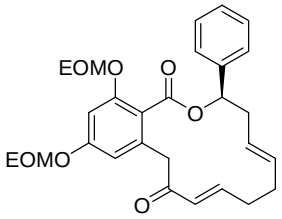
Compound (*S*)-2-120: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.81-6.72 (m, 1H), 6.74 (d, *J* = 1.8 Hz, 1H), 6.56 (d, *J* = 1.8 Hz, 1H), 5.98 (d, *J* = 15.8 Hz, 1H), 5.38-5.33 (m, 2H), 5.22-5.13 (m, 5H), 4.06 (d, *J* = 14.6 Hz, 1H), 3.75-3.65 (m, 4H), 3.46 (d, *J* = 14.6 Hz, 1H), 2.37-2.22 (m, 4H), 2.18-2.02 (m, 2H), 1.39 (d, *J* = 5.8 Hz, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.18 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.4, 167.7, 159.0, 156.1, 148.9,

135.0, 131.7, 129.8, 128.5, 118.5, 109.7, 102.2, 93.4, 93.0, 71.5, 64.5, 64.4, 44.3, 39.5, 30.9, 30.6, 20.2, 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{32}O_7Na$: 455.2040, found 455.1901 [*M*+Na^+]. (+)-(2*S*): $[\alpha]^{25}_D$ = + 59.5 (*c* 1.00, $CHCl_3$).



(*R*)-**2-120a**

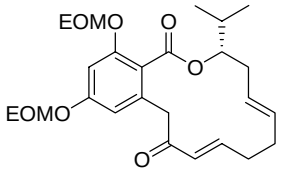
Compound (*R*)-2-120a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.56-7.54 (m, 2H), 7.41-7.29 (m, 3H), 6.89-6.82 (m, 1H), 6.78 (d, *J* = 2.3 Hz, 1H), 6.61 (d, *J* = 1.8 Hz, 1H), 6.06 (d, *J* = 16.4 Hz, 1H), 5.98 (dd, *J* = 11.7, 2.4 Hz, 1H), 5.53-5.51 (m, 2H), 5.20 (d, *J* = 7.0 Hz, 1H), 5.17 (d, *J* = 6.4 Hz, 1H), 5.07 (d, *J* = 7.0 Hz, 1H), 4.96 (d, *J* = 7.0 Hz, 1H), 4.20 (d, *J* = 14.6 Hz, 1H), 3.73-3.68 (m, 2H), 3.54-3.45 (m, 3H), 2.71-2.66 (m, 1H), 2.55-2.51 (m, 1H), 2.38-2.32 (m, 2H), 2.23-2.06 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.14 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.6, 167.4, 159.3, 156.6, 149.0, 140.8, 135.6, 132.2, 129.9, 128.5, 128.2 (x 2), 127.9, 126.9 (x 2), 117.9, 109.9, 102.3, 93.2, 93.0, 76.6, 64.4, 64.3, 44.4, 40.5, 31.0, 30.6, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{34}O_7Na$: 517.2197, found 517.2062 [*M*+Na^+]. $[\alpha]^{25}_D$ = − 108.3 (*c* 1.00, $CHCl_3$).



(*S*)-**2-120a**

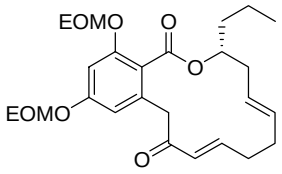
Compound (*S*)-2-120a: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.56-7.54 (m, 2H), 7.41-7.29 (m, 3H), 6.90-6.82 (m, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 6.61 (d, *J* = 1.8 Hz, 1H), 6.07 (d, *J* = 16.4 Hz, 1H), 5.98 (dd, *J* = 11.4, 2.0 Hz, 1H), 5.53-5.51 (m, 2H), 5.20 (d, *J* = 7.0 Hz, 1H), 5.18 (d, *J* = 7.0 Hz, 1H), 5.07 (d, *J* = 7.0 Hz, 1H), 4.97 (d, *J* = 7.0 Hz, 1H), 4.20 (d, *J* = 14.6 Hz, 1H), 3.74-3.69 (m, 2H), 3.55-3.46 (m, 3H), 2.71-2.66 (m, 1H), 2.55-2.52 (m, 1H), 2.38-2.33 (m, 2H), 2.23-2.09 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.15 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.6, 167.5, 159.3, 156.6, 149.0, 140.8, 135.6, 132.2, 129.9, 128.5, 128.2 (x 2), 127.9, 126.9 (x 2), 117.9, 110.0, 102.3, 93.2, 93.0, 76.6, 64.4, 64.3, 44.4,

40.5, 31.0, 30.6, 15.0, 14.9; HRMS (ESI-TOF): *m/z*: calculated for $C_{29}H_{34}O_7Na$: 517.2197, found 517.2049 [*M*+Na^+]. $[\alpha]^{25}_D$ = + 81.6 (*c* 1.00, $CHCl_3$).



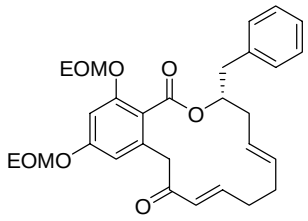
(*R*)-**2-120d**

Compound (*R*)-2-120d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.83 (d, *J* = 1.7 Hz, 1H), 6.79-6.72 (m, 1H), 6.61 (d, *J* = 1.2 Hz, 1H), 6.00 (d, *J* = 16.4 Hz, 1H), 5.38-5.36 (m, 2H), 5.26-5.09 (m, 5H), 4.30 (d, *J* = 14.6 Hz, 1H), 3.74-3.67 (m, 4H), 3.46 (d, *J* = 14.6 Hz, 1H), 2.33-2.26 (m, 4H), 2.18-2.14 (m, 2H), 2.06-2.01 (m, 1H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.06 (d, *J* = 6.6 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.9, 167.6, 159.2, 156.9, 149.2, 136.4, 131.5, 129.9, 129.2, 117.4, 109.8, 102.0, 93.3, 93.0, 78.9, 64.4 (x 2), 44.2, 33.0, 32.0, 31.0, 30.4, 18.3, 17.2, 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{36}O_7Na$: 483.2353, found 483.2215 [*M*+Na^+]. $[\alpha]^{25}_D$ = + 52.8 (*c* 1.00, $CHCl_3$).



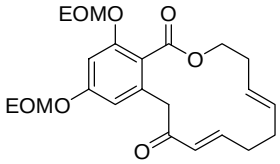
(*R*)-**2-120e**

Compound (*R*)-2-120e: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.77 (d, *J* = 1.8 Hz, 1H), 6.77-6.70 (m, 1H), 6.57 (d, *J* = 1.7 Hz, 1H), 5.97 (d, *J* = 16.4 Hz, 1H), 5.37-5.32 (m, 2H), 5.21-5.14 (m, 5H), 4.17 (d, *J* = 14.6 Hz, 1H), 3.73-3.65 (m, 4H), 3.45 (d, *J* = 14.6 Hz, 1H), 2.39-2.20 (m, 4H), 2.17-2.00 (m, 2H), 1.78-1.72 (m, 1H), 1.69-1.60 (m, 1H), 1.54-1.44 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.18 (t, *J* = 7.0 Hz, 3H), 0.97 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.7, 167.6, 159.1, 156.6, 149.0, 135.8, 131.5, 129.9, 128.7, 117.9, 109.8, 102.1, 93.3, 93.0, 74.5, 64.4, 64.3, 44.2, 37.3, 37.0, 31.0, 30.5, 18.2, 15.0, 14.9, 14.2. $[\alpha]^{25}_D$ = − 1.3 (*c* 1.00, $CHCl_3$).



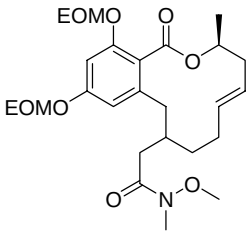
(*R*)-**2-120f**

Compound (*R*)-2-120f: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.39-7.33 (m, 4H), 7.31-7.27 (m, 1H), 6.82 (s, 1H), 6.82-6.75 (m, 1H), 6.63 (s, 1H), 6.02 (d, *J* = 16.4 Hz, 1H), 5.35-5.29 (m, 2H), 5.27-5.20 (m, 5H), 4.16 (d, *J* = 14.6 Hz, 1H), 3.79-3.70 (m, 4H), 3.52 (d, *J* = 14.6 Hz, 1H), 3.37 (dd, *J* = 13.4, 4.1 Hz, 1H), 2.78 (dd, *J* = 13.5, 9.4 Hz, 1H), 2.37-2.12 (m, 5H), 2.06-2.02 (m, 1H), 1.26 (t, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.6, 167.8, 159.2, 156.5, 149.0, 137.3, 135.5, 131.8, 129.9, 129.5 (x 2), 128.6 (x 2), 128.4, 126.7, 118.1, 109.9, 102.3, 93.5, 93.1, 75.8, 64.6, 64.4, 44.4, 41.0, 36.2, 31.0, 30.6, 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{36}O_7Na$: 531.2359, found 531.2350 [*M*+Na^+]. $[\alpha]^{25}_D$ = − 24.1 (*c* 0.33, $CHCl_3$).



2-120g

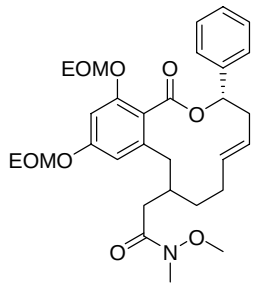
Compound 2-120g: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.85-6.78 (m + s, 2H), 6.56 (d, *J* = 2.1 Hz, 1H), 6.00 (d, *J* = 16.1 Hz, 1H), 5.38-5.34 (m, 2H), 5.23 (s, 2H), 5.19 (s, 2H), 4.33 (t, *J* = 5.4 Hz, 2H), 3.75-3.70 (m, 6H), 2.45-2.41 (m, 2H), 2.19 (bs, 4H), 1.27-1.20 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.2, 168.1, 159.1, 155.8, 148.6, 134.5, 131.7, 129.8, 129.1, 118.9, 109.9, 102.4, 93.6, 93.1, 64.5, 64.5, 64.4, 45.4, 31.9, 31.1, 30.8, 15.0, 15.0; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{30}O_7Na$: 441.1884, found 441.1888 [*M*+Na^+].



(*S*)-**2-121**

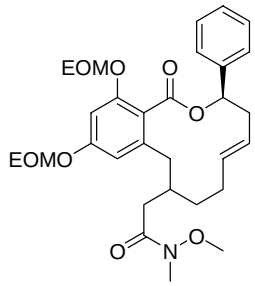
Compound (*S*)-2-121: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.77-6.72 (m, 1H), 6.57-6.47 (m, 1H), 5.62-5.35 (m, 3H), 5.24-5.16 (m, 4H), 3.77-3.70

(m, 4H), 3.62-3.60 (m, 1.5H), 3.53-3.49 (m, 1.5H), 3.17-3.11 (m, 3H), 3.04-2.97 (m, 1H), 2.57-2.44 (m, 2H), 2.36-1.99 (m, 6H), 1.37-1.33 (m, 3H), 1.28-1.21 (m, 8H); HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{39}O_8NNa$: 516.2568, found 516.2596 [*M*+Na^+].



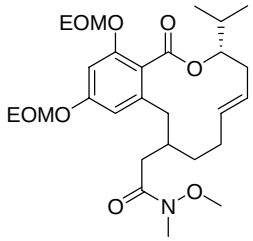
(*R*)-**2-121a**

Compound (*R*)-2-121a: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.51-7.42 (m, 2H), 7.38-7.31 (m, 3H), 6.73-6.70 (m, 1H), 6.60-6.49 (m, 1H), 6.45-6.31 (m, 1H), 5.73-5.39 (m, 2H), 5.23-5.00 (m, 4H), 3.75-3.69 (m, 2H), 3.56-3.34 (m, 6H), 3.19-3.09 (m, 3H), 2.66-2.08 (m, 8H), 1.31-1.19 (m, 5H), 1.10-1.04 (m, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{31}H_{41}O_8NNa$: 578.2724, found 578.2715 [*M*+Na^+].



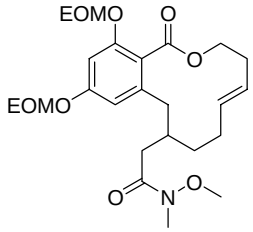
(*S*)-**2-121a**

Compound (*S*)-2-121a: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.51-7.42 (m, 2H), 7.38-7.29 (m, 3H), 6.73-6.70 (m, 1H), 6.60-6.49 (m, 1H), 6.45-6.31 (m, 1H), 5.73-5.42 (m, 2H), 5.25-5.01 (m, 4H), 3.76-3.69 (m, 2H), 3.62-3.34 (m, 6H), 3.20-3.09 (m, 3H), 2.66-2.50 (m, 2H), 2.22-2.12 (m, 4H), 1.72-1.66 (m, 2H), 1.31-1.19 (m, 5H), 1.10-1.04 (m, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{31}H_{41}O_8NNa$: 578.2724, found 578.2720 [*M*+Na^+].



(*R*)-**2-121d**

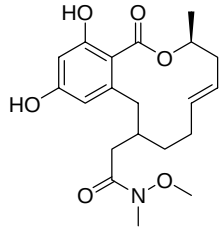
Compound (*R*)-2-121d: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.77 (s, 1H), 6.52 (s, 0.5H), 6.46 (s, 0.5H), 5.59-5.37 (m, 2H), 5.21-5.18 (m, 4H), 5.09-4.92 (m, 1H), 3.75-3.70 (m, 4H), 3.53-3.48 (m, 3H), 3.38-3.34 (m, 1H), 3.19-3.10 (m, 3H), 2.65-2.47 (m, 3H), 2.29-2.04 (m, 6H), 1.89-1.72 (m, 2H), 1.31-1.20 (m, 6H), 1.06-0.96 (m, 6H); HRMS (ESI-TOF): *m/z*: calculated for $C_{28}H_{43}O_8NNa$: 544.2881, found 544.2907 [*M*+Na^+].



2-121g

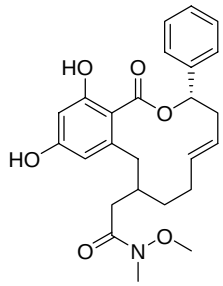
Compound 2-121g: Mixture of two diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.74-6.72 (m, 1H), 6.54 (d, *J* = 1.8 Hz, 0.6H), 6.50 (d, *J* = 1.7 Hz, 0.4H), 5.52-5.41 (m, 2H), 5.21 (s, 2H), 5.19 (s, 2H), 4.69-4.65 (m, 1H), 4.58-4.47 (m, 1H), 3.74-3.68 (m, 4H), 3.55 (s, 3H), 3.13 (s, 3H), 2.97-2.94 (m, 1H), 2.52-2.40 (m, 2H), 2.26-1.98 (m, 6H), 1.72-1.58 (m, 2H), 1.24-1.20 (m, 6H); HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{38}O_8N$: 480.2567, found 480.2592 [*M*+H^+].

General procedure for the EOM deprotection to generate compounds deprotected-2-121a-g, 2-85a-g and 2-103a-g: PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}) was added to a solution of the corresponding compound **2-121a-g** or **2-112a-g** or **2-120a-g** (1.0 equiv.) in MeOH (0.03 M) and the resulting suspension was shaken at 40 °C for 1 to 4 h. After this time, the reaction mixture was filtered and the methanolic solution concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-33 % EtOAc/cyclohexane gradient) afforded the corresponding compound **deprotected-2-121a-g** or **2-85a-g** or **2-103a-g** (> 90 %).



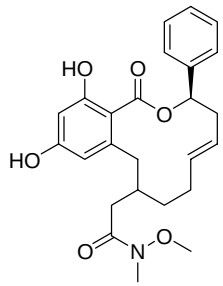
Deprotected (*S*)-**2-121**

Deprotected compound (*S*)-2-121: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.90 (s, 0.5H), 12.83 (s, 0.5H), 12.12 (s, 0.5H), 12.02 (s, 0.5H), 6.92 (s, 0.5H), 6.84 (s, 0.5H), 6.83 (s, 1H), 6.79 (s, 0.5H), 6.60 (s, 1H), 6.54 (s, 0.5H), 5.60-5.29 (m, 4H), 5.17-4.99 (m, 2H), 4.14-3.99 (m, 2H), 2.98-2.72 (m, 12H), 2.60-1.92 (m, 16H), 1.31 (d, *J* = 6.4 Hz, 1.5H), 1.22 (d, *J* = 8.7 Hz, 1.5H), 1.13-1.02 (m, 7H); HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{27}O_6NNa$: 400.1851, found 400.1731 [*M*+Na^+].



Deprotected (*R*)-**2-121a**

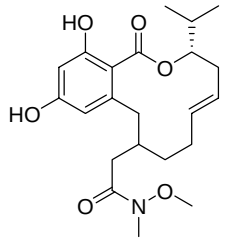
Deprotected compound (*R*)-2-121a: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.90 (s, 0.25H), 11.08 (s, 0.5H), 10.98 (s, 0.25H), 7.38-7.29 (m, 5H), 6.39 (s, 0.25H), 6.33 (s, 0.25H), 6.29 (s, 1.25H), 6.26 (s, 0.25H), 6.05-5.95 (m, 1H), 5.70-5.52 (m, 2H), 4.18-4.03 (m, 1H), 3.51-3.49 (m, 3H), 3.16-3.14 (m, 3H), 2.75-2.62 (m, 2H), 2.36-2.29 (m, 2H), 2.12-1.96 (m, 4H), 1.81-1.73 (m, 2H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{30}O_6N$: 440.2068, found 440.2103 [*M*+H^+].



Deprotected (*S*)-**2-121a**

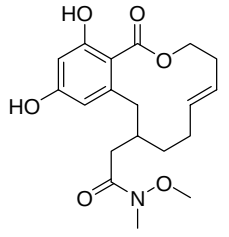
Deprotected compound (*S*)-2-121a: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.90 (s, 0.25H), 11.08 (s, 0.5H), 10.98 (s, 0.25H), 7.38-7.31 (m, 5H),

6.38 (s, 0.25H), 6.33 (s, 0.25H), 6.29 (s, 1.25H), 6.26 (s, 0.25H), 6.05-5.95 (m, 1H), 5.71-5.54 (m, 2H), 4.13-4.04 (m, 1H), 3.53-3.50 (m, 3H), 3.19-3.14 (m, 3H), 2.78-2.63 (m, 2H), 2.33-2.29 (m, 2H), 2.16-2.04 (m, 4H), 1.81-1.68 (m, 2H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{29}O_6NNa$: 462.1887, found 462.2080 [M+Na^+].



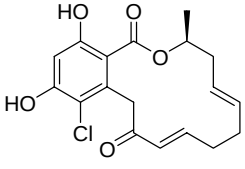
Deprotected (*R*)-**2-121d**

Deprotected compound (*R*)-2-121d: Mixture of four diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.54 (s, 1H), 6.33 (d, J = 2.3 Hz, 1H), 6.25 (s, 1H), 5.53-5.51 (m, 1H), 5.44-5.41 (m, 1H), 5.11-5.08 (m, 1H), 4.01 (d, J = 11.7 Hz, 2H), 3.45 (s, 3H), 3.11 (s, 3H), 2.83-2.73 (m, 1H), 2.68-2.59 (m, 1H), 2.27-2.20 (m, 1H), 2.10-1.87 (m, 6H), 1.82-1.72 (m, 1H), 1.01-0.94 (m, 6H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{22}H_{31}O_6NNa$: 428.2044, found 428.2109 [M+Na^+].



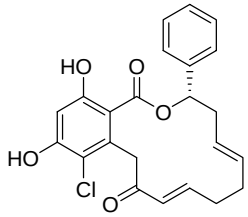
Deprotected **2-121g**

Deprotected compound 2-121g: Mixture of two diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.33 (s, 0.5H), 11.85 (s, 0.5H), 6.34-6.32 (m, 1H), 6.25-6.22 (m, 1H), 5.62-5.45 (m, 2H), 4.54-4.37 (m, 1H), 4.29-4.21 (m, 1H), 3.53-3.49 (m, 3H), 3.15-3.12 (m, 3.5H), 2.95-2.86 (m, 0.5H), 2.67-2.52 (m, 2H), 2.39-1.96 (m, 8H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{19}H_{26}O_6N$: 364.1755, found 364.1715 [M+H^+].



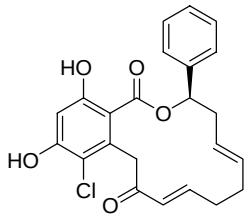
(*S*)-**2-85**

Compound (*S*)-2-85: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.40 (s, 1H), 6.83 (s, 1H), 6.66-6.61 (m, 1H), 5.96 (bs, 1H), 5.80 (d, *J* = 15.2 Hz, 1H), 5.16-5.12 (m, 1H), 4.99-4.91 (m, 1H), 4.75-4.68 (m, 1H), 4.26 (d, *J* = 17.5 Hz, 1H), 4.13 (d, *J* = 17.5 Hz, 1H), 2.52-2.45 (m, 1H), 1.86-1.79 (m, 3H), 1.75-1.67 (m, 1H), 1.54-1.49 (m, 1H), 0.97 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 193.8, 169.8, 164.3, 156.8, 146.0, 137.1, 131.9, 128.1, 126.2, 115.2, 107.6, 103.6, 72.4, 46.2, 36.3, 31.0, 30.8, 17.2. (-)-(2*S*): $[\alpha]^{25}_{D} = -21.9$ (*c* 0.62, $CHCl_3$).



(*R*)-**2-85a**

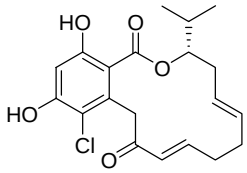
Compound (*R*)-2-85a: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.25 (bs, 1H), 7.32-7.29 (m, 2H), 7.19-7.15 (m, 3H), 6.80 (s, 1H), 6.81-6.75 (m, 1H), 6.29-6.26 (m, 1H), 5.92 (d, *J* = 15.8 Hz, 1H), 5.80 (s, 1H), 5.05-4.99 (m, 1H), 4.81-4.75 (m, 1H), 4.56 (d, *J* = 17.6 Hz, 1H), 4.12 (d, *J* = 17.5 Hz, 1H), 2.73-2.66 (m, 1H), 2.39-2.35 (m, 1H), 1.86-1.76 (m, 2H), 1.64-1.49 (m, 2H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 194.2, 169.8, 164.4, 156.8, 145.5, 138.3, 137.0, 132.9, 129.9 (x 2), 128.6, 127.3, 126.6 (x 2), 125.8, 115.2, 107.5, 103.6, 77.6, 46.6, 38.3, 31.0, 30.6; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{21}O_5ClNa$: 435.0970, found 435.0914 [*M*+Na^+]. $[\alpha]^{25}_{D} = -12.0$ (*c* 0.55, $CHCl_3$).



(*S*)-**2-85a**

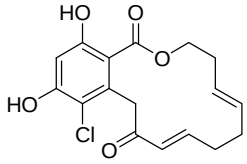
Compound (*S*)-2-85a: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.20 (bs, 1H), 7.15-7.09 (m, 3H), 6.75 (s, 1H), 6.77-6.71 (m, 1H), 6.25-6.22 (m, 1H), 5.88 (d, *J* = 15.2 Hz, 1H), 5.72 (s, 1H), 5.00-4.95 (m, 1H), 4.77-4.73 (m, 1H), 4.52 (d, *J* = 17.6 Hz, 1H), 4.08 (d, *J* = 17.5 Hz, 1H), 2.68-2.62 (m, 1H), 2.35-2.31 (m, 1H), 1.81-1.78 (m, 2H), 1.56-1.49 (m, 2H), 2H masked by the solvent peak; ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 194.2, 169.8, 164.4, 156.8, 145.5, 138.3, 137.0, 132.2, 129.9 (x 2), 128.5, 127.3, 126.5 (x 2), 125.8, 115.2, 107.5, 103.6,

77.5, 46.6, 38.2, 31.0, 30.6; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{21}O_5ClNa$: 435.0970, found 435.0885 [*M*+Na^+]. $[\alpha]^{25}_D$ = + 11.6 (*c* 0.51, $CHCl_3$).



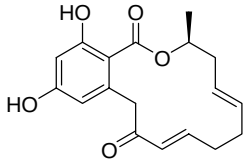
(*R*)-**2-85d**

Compound (*R*)-2-85d: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.31 (s, 1H), 6.83 (s, 1H), 6.74-6.67 (m, 1H), 5.84 (bs, 1H), 5.82 (d, *J* = 15.8 Hz, 1H), 5.03-4.95 (m, 1H), 4.88-4.86 (m, 1H), 4.76-4.70 (m, 1H), 4.40 (d, *J* = 17.6 Hz, 1H), 4.15 (d, *J* = 17.5 Hz, 1H), 2.40-2.34 (m, 1H), 2.22-2.18 (m, 1H), 1.87-1.65 (m, 4H), 1.53-1.48 (m, 1H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.66 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 193.7, 164.2, 156.8, 145.8, 137.2, 131.8, 129.3, 126.3, 115.3, 107.9, 103.6, 82.1, 46.4, 33.3, 30.9, 30.7, 28.8, 20.1, 18.5, 18.3; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{23}ClO_5Na$: 401.1126, found 401.1170 [*M*+Na^+]. $[\alpha]^{25}_D$ = − 35.6 (*c* 0.52, $CHCl_3$).



2-85g

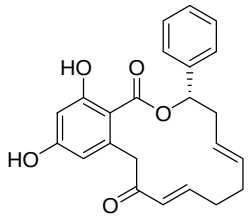
Compound 2-85g: ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 6.74-6.68 (m, 1H), 6.48 (s, 1H), 5.86 (d, *J* = 15.2 Hz, 1H), 5.31-5.25 (m, 2H), 4.39 (t, *J* = 5.3 Hz, 2H), 4.27 (s, 2H), 2.43-2.40 (m, 2H), 2.25 (m, 4H), phenols not detected; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 196.9, 170.1, 161.9, 158.1, 147.8, 135.9, 130.9, 130.2, 129.9, 115.2, 107.3, 102.4, 65.9, 46.2, 31.3, 30.9, 30.5; HRMS (ESI-TOF): *m/z*: calculated for $C_{17}H_{18}O_5Cl$: 337.0837, found 337.0797 [*M*+H^+].



(*S*)-**2-103**

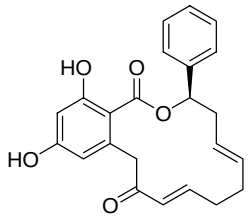
Compound (*S*)-2-103: ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 6.78-6.71 (m, 1H), 6.29 (d, *J* = 2.4 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 5.87 (d, *J* = 15.5 Hz, 1H), 5.37-5.23 (m, 3H), 4.01

(d, *J* = 17.2 Hz, 1H), 3.92 (d, *J* = 17.0 Hz, 1H), 2.67-2.61 (m, 1H), 2.29-2.15 (m, 5H), 1.31 (d, *J* = 6.4 Hz, 3H), phenols not detected; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 198.5, 169.8, 164.2, 162.3, 148.4, 139.1, 131.6, 129.6, 127.3, 111.7, 101.7, 72.0, 47.7, 36.8, 30.8, 30.7, 17.4, (one quartenary carbon is not detected); HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{20}O_5Na$: 339.1203, found 339.1141 [*M*+Na^+]. (-)-(2*S*): $[\alpha]^{25}_D$ = − 45.1 (*c* 0.27, $CHCl_3$).



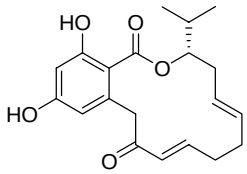
(*R*)-**2-103a**

Compound (*R*)-2-103a: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.0 (bs, 1H), 7.32-7.29 (m, 3H), 7.19-7.15 (m, 2H), 6.86-6.79 (m, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.27-6.25 (m, 1H), 6.11 (d, *J* = 2.4 Hz, 1H), 6.02 (d, *J* = 15.8 Hz, 1H), 5.49 (s, 1H), 5.17-5.10 (m, 1H), 4.97-4.90 (m, 1H), 4.40 (d, *J* = 16.4 Hz, 1H), 3.97 (d, *J* = 17.2 Hz, 1H), 2.83-2.76 (m, 1H), 2.45-2.38 (m, 1H), 1.89-1.78 (m, 2H), 1.67-1.58 (m, 2H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 196.5, 169.6, 166.1, 161.3, 146.0, 140.5, 138.8, 132.1, 130.0, 128.6 (x 2), 127.3, 126.6 (x 2), 126.3, 112.2, 105.9, 103.0, 77.1, 48.6, 38.4, 30.9, 30.3; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{22}O_5Na$: 401.1359, found 401.1271 [*M*+Na^+]. $[\alpha]^{25}_D$ = − 10.3 (*c* 0.25, $CHCl_3$).



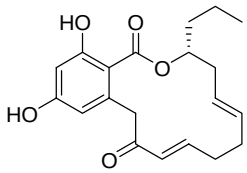
(*S*)-**2-103a**

Compound (*S*)-2-103a: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.0 (bs, 1H), 7.27-7.21 (m, 3H), 7.17-7.13 (m, 2H), 6.87-6.79 (m, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 6.29-6.26 (m, 1H), 6.16 (d, *J* = 2.3 Hz, 1H), 6.03 (d, *J* = 15.8 Hz, 1H), 5.74 (s, 1H), 5.18-5.12 (m, 1H), 4.98-4.91 (m, 1H), 4.41 (d, *J* = 15.8 Hz, 1H), 3.99 (d, *J* = 16.9 Hz, 1H), 2.84-2.77 (m, 1H), 2.46-2.43 (m, 1H), 1.85-1.79 (m, 2H), 1.70-1.58 (m, 2H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 196.9, 169.6, 166.2, 161.5, 146.3, 140.5, 138.9, 132.1, 130.0, 128.6 (x 2), 127.3, 126.6 (x 2), 126.3, 112.2, 105.8, 103.0, 77.1, 48.6, 38.4, 30.9, 30.4; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{22}O_5Na$: 401.1359, found 401.1264 [*M*+Na^+]. $[\alpha]^{25}_D$ = + 11.9 (*c* 0.51, $CHCl_3$).



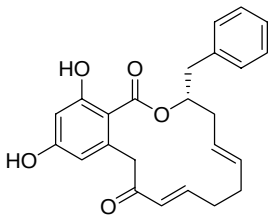
(*R*)-**2-103d**

Compound (*R*)-2-103d: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.10 (s, 1H), 6.79 (dt, *J* = 15.2, 7.6 Hz, 1H), 6.59 (d, *J* = 2.4 Hz, 1H), 6.22 (d, *J* = 2.3 Hz, 1H), 5.96 (d, *J* = 15.8 Hz, 1H), 5.88 (bs, 1H), 5.13-5.05 (m, 1H), 4.92-4.85 (m, 2H), 4.27 (d, *J* = 15.8 Hz, 1H), 4.03 (d, *J* = 15.8 Hz, 1H), 2.50-2.44 (m, 1H), 2.24-2.20 (m, 1H), 1.96-1.71 (m, 4H), 1.63-1.56 (m, 1H), 0.91 (d, *J* = 6.3 Hz, 3H), 0.71 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 196.5, 169.7, 166.0, 161.4, 146.8, 140.8, 131.7, 129.5, 126.9, 112.3, 106.0, 103.0, 81.3, 48.5, 33.7, 30.9, 30.4, 29.6, 19.7, 18.4; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{24}O_5Na$: 367.1516, found 367.1424 [*M*+Na^+]. $[\alpha]^{25}_D$ = − 31.9 (*c* 0.50, $CHCl_3$).



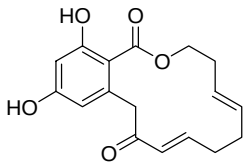
(*R*)-**2-103e**

Compound (*R*)-2-103e: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.43 (s, 1H), 6.74 (d, *J* = 1.7 Hz, 1H), 6.73-6.65 (m, 1H), 6.48 (d, *J* = 1.7 Hz, 1H), 5.92 (d, *J* = 15.8 Hz, 1H), 5.12-5.00 (m, 2H), 4.91-4.80 (m, 1H), 4.19 (d, *J* = 17.0 Hz, 1H), 3.84 (d, *J* = 16.4 Hz, 1H), 2.77 (m, 1H), 2.64-2.57 (m, 1H), 2.01-1.97 (m, 1H), 1.89-1.70 (m, 3H), 1.61-1.56 (m, 2H), 1.30-1.21 (m, 2H), 0.90 (t, *J* = 6.7 Hz, 3H), *para*-phenol not detected; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.5, 169.9, 165.6, 160.6, 147.5, 140.2, 131.9, 129.5, 127.0, 112.8, 106.1, 102.9, 76.2, 48.7, 35.7, 34.3, 31.1, 29.7, 19.4, 13.8; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{25}O_5$: 345.1697, found 345.1739 [*M*+H^+]. $[\alpha]^{25}_D$ = + 21.6 (*c* 0.36, $CHCl_3$).



(*R*)-**2-103f**

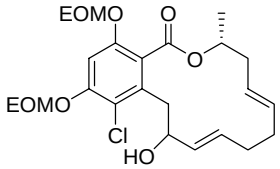
Compound (*R*)-2-103f: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.31 (s, 1H), 7.19-7.13 (m, 5H), 6.80-6.72 (m, 1H), 6.53 (d, *J* = 1.8 Hz, 1H), 6.05 (s, 1H), 5.89 (d, *J* = 15.8 Hz, 1H), 5.47-5.44 (m, 1H), 5.11-5.05 (m, 1H), 4.85-4.81 (m, 1H), 4.10 (d, *J* = 17.0 Hz, 1H), 3.62 (d, *J* = 17.0 Hz, 1H), 2.89-2.84 (m, 1H), 2.67-2.60 (m, 2H), 2.08-2.04 (m, 1H), 1.90-1.69 (m, 3H), 1.52-1.44 (m, 1H), *para*-phenol not detected; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 197.7, 169.9, 165.7, 160.7, 147.4, 140.2, 137.2, 132.2, 129.5, 128.8 (x 2), 128.7 (x 3), 126.8, 112.4, 105.9, 102.9, 48.9, 38.5, 35.3, 31.1 (x 2), 29.7; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{25}O_5$: 393.1697, found 393.1765 [*M*+H^+]. $[\alpha]^{25}_D$ = + 25.4 (*c* 0.41, $CHCl_3$).



2-103g

Compound 2-103g: ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 6.75-6.69 (m, 1H), 6.29 (s, 1H), 6.29 (d, *J* = 2.3 Hz, 1H), 5.90 (d, *J* = 15.8 Hz, 1H), 5.30-5.28 (m, 2H), 4.39 (t, *J* = 5.2 Hz, 2H), 4.02 (s, 2H), 2.45-2.41 (m, 2H), 2.28-2.24 (m, 4H), phenols not detected; ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 198.4, 170.6, 164.9, 162.5, 148.1, 139.2, 130.7, 130.6, 130.2, 112.2, 105.0, 101.6, 65.7, 47.7, 31.4, 30.9, 30.5; HRMS (ESI-TOF): *m/z*: calculated for $C_{17}H_{19}O_5$: 303.1227, found 303.1179 [*M*+H^+].

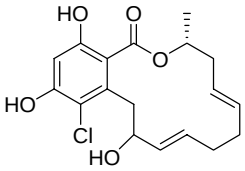
General procedure for the synthesis of compounds 2-141: BER-resin (Borohydride on Amberlite, 1.0 equiv., 2.5 mmol.g^{-1}) was added to a solution of corresponding compound **2-112a-g** or **2-120a-g** (1.0 equiv.) in MeOH (0.03 M) at 0 °C and the reaction was stirred for 12 h. The reaction mixture was then filtered and concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-20 % EtOAc/cyclohexane gradient) afforded **2-141** (~60 % yield) as a mixture of two diastereoisomers 1:1.



Selected example of compounds 2-141: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.05 (s, 1H), 6.99 (s, 1H), 5.64-5.57 (m, 2H), 5.54-5.53 (m, 2H), 5.49-5.35 (m, 7H), 5.31-5.28 (m, 4H), 5.24-5.16 (m, 4H), 5.13-5.08 (m, 1H), 4.68 (m, 1H), 4.56 (m, 1H), 3.81-3.69 (m, 8H),

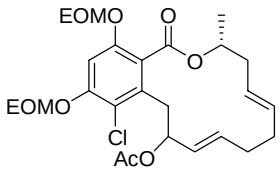
3.25 (dd, *J* = 13.9, 8.0 Hz, 1H), 3.19 (dd, *J* = 13.7, 4.8 Hz, 1H), 3.11 (dd, *J* = 13.5, 10.1 Hz, 1H), 2.90 (dd, *J* = 13.9, 5.1 Hz, 1H), 2.35 (m, 9H), 2.09-1.95 (m, 1H), 1.80-1.70 (m, 2H), 1.39 (d, *J* = 2.9 Hz, 3H), 1.37 (d, *J* = 3.2 Hz, 3H,), 1.24 (2 x q, *J* = 6.9, 5.0 Hz, 12H); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{33}ClO_7Na$: 491.1807, found 491.1729 [*M*+Na^+].

General procedure for the synthesis of compounds 2-142: PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}) was added to a solution of the corresponding compound **2-141** (1.0 equiv.) in MeOH (0.02 M) and the suspension was shaken at 40 °C for 4 h. The reaction mixture was then filtered and the methanolic solution concentrated under reduced pressure. Purification by preparative TLC (silica gel, 25 % EtOAc/cyclohexane) afforded **2-142** (~90 % yield) as a mixture of two diastereoisomers 1:1.



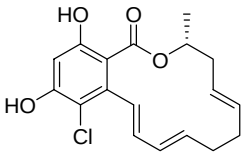
Selected example of compounds 2-142: ^{1}H NMR (400 MHz, $(CD_3)_2CO$, 25 °C): δ =12.30 (s, 2H), 11.43 (s, 2H), 6.75 (s, 2H), 6.00 (bdd, *J* = 6.4, 6.2 Hz, 1H), 5.97 (bdd, *J* = 6.4, 6.2 Hz, 1H), 5.97 (bd, *J* = 6.7 Hz, 1H), 5.77 (bd, *J* = 6.7 Hz, 1H), 5.57-5.48 (m, 4H), 5.18-5.14 (m, 2H), 3.38-3.28 (m, 3H), 3.02 (dd, *J* = 16.1, 10.5 Hz, 1H), 2.41-2.09 (m, 12H), 1.11 (d, *J* = 6.2 Hz, 6H), alcohols not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{21}ClO_5Na$: 375.0970, found 375.1029 [*M*+Na^+].

General procedure for the synthesis of compounds 2-143: Ac_2O (1.2 equiv.), morpholinomethyl polystyrene (1.2 equiv., 3.2 mmol.g^{-1}) and DMAP (0.05 equiv.) were added to a solution of the corresponding compound **2-141** (1.0 equiv.) in DMF (0.02 M) at 23 °C and the mixture was stirred for 30 min, followed by TLC until consumption of the starting material. The resin was then filtered and the organic phase was concentrated under reduced pressure. Purification by preparative TLC (silica gel, 20 % EtOAc/cyclohexane) afforded corresponding compound **2-143** (~80 % yield) as a mixture of two diastereoisomers 1:1.



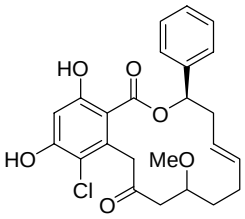
Selected example of compounds 2-143: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.04 (s, 1H), 7.01 (s, 1H), 5.86 (dd, *J* = 15.0, 6.9 Hz, 1H), 5.67 (dd, *J* = 12.4, 6.2 Hz, 1H), 5.60-5.54 (m, 4H), 5.48 (dd, *J* = 7.2, 7.2 Hz, 1H), 5.41-5.34 (m, 3H), 5.32-5.30 (m, 4H), 5.28-5.23 (m, 2H), 5.21 (dd, *J* = 11.0, 6.7 Hz, 2H), 5.17 (dd, *J* = 11.8, 6.9 Hz, 2H), 3.81-3.69 (m, 8H), 3.43 (dd, *J* = 14.2, 7.5 Hz, 1H), 3.23-3.15 (m, 2H), 2.85 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.30-2.17 (m, 8H), 2.12 (s, 3H), 2.06 (s, 3H), 2.00-1.95 (m, 4H), 1.39 (2 x d, *J* = 5.6 Hz, 6H), 1.24 (m, 12H); HRMS (ESI-TOF): *m/z*: calculated for $C_{26}H_{35}ClO_8Na$: 533.1913, found 533.1864 [*M*+Na^+].

General procedure for the synthesis of compounds 2-144: PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}) was added to a solution of corresponding compound **2-143** (1.0 equiv.) in MeOH (0.02 M) and the suspension was shaken at 40 °C for 4 h. After this time, the reaction mixture was filtered and the methanolic solution concentrated under reduced pressure. Purification by preparative TLC (silica gel, 20 % EtOAc/cyclohexane) afforded compound **2-144** (~60 % yield).



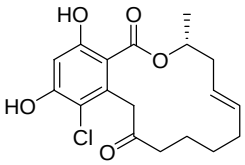
Selected example of compounds 2-144: Mixture of diastereoisomers 2:1; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.60 (s, 1H), 12.12 (s, 0.5H), 6.93 (d, *J* = 8.7 Hz, 0.5H), 6.66 (s, 1H), 6.64 (s, 0.5H), 6.62-6.60 (m, 1H), 6.10-6.05 (m, 3H), 5.47-5.33 (m, 6H), 2.60-2.53 (m, 1.5H), 2.26-2.02 (m, 7.5H), 1.44 (d, *J* = 6.2 Hz, 1.5H), 1.43 (d, *J* = 6.4 Hz, 3H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{19}ClO_4Na$: 357.0864, found 357.0898 [*M*+Na^+].

General procedure for the preparation of compounds 2-145: PS-TsOH (10.0 equiv.) was added to a solution of corresponding compound **2-85a-g** (1.0 equiv.) in methanol (0.03 M) and the suspension was stirred for 15 h at 40°C. The reaction was then filtered and the resin washed several times with CH_2Cl_2. Concentration under reduced pressure followed by purification on preparative TLC (silica gel, 50 % hexane/EtOAc) afforded desired compound **2-145** as a mixture of diastereoisomers 2:1.



Selected example of compounds 2-145: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 12.28 (s, 0.4H), 11.91 (s, 0.6H), 7.21-7.11 (m, 5H), 6.62 (s, 1H), 6.03-6.01 (m, 1H), 5.58 (bs, 1H), 5.38-5.33 (m, 1H), 5.27-5.20 (m, 1H), 4.76 (d, *J* = 17.5 Hz, 0.6H), 4.02 (d, *J* = 17.0 Hz, 0.4H), 4.18 (d, *J* = 18.1 Hz, 0.6H), 4.09 (d, *J* = 17.0 Hz, 0.4H), 3.87 (bs, 0.4H), 3.81 (bs, 0.6H), 3.15 (s, 1.8H), 3.12 (s, 1.2H), 2.83-2.78 (m, 1H), 2.45-2.30 (m, 2H), 2.18-2.16 (m, 1H), 2.02-1.97 (m, 2H), 1.79-1.72 (m, 2H); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{25}O_6ClNa$: 467.1232, found 467.1366 [*M*+Na^+].

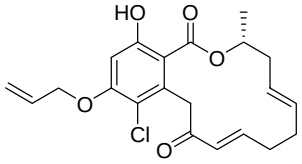
General procedure for the synthesis of compounds 2-146: (Polystyrylmethyl)trimethyl-ammonium cyanoborohydride (2.0 equiv., 3.5 mmol.g^{-1}) was added to a solution of corresponding compound **2-85a-g** or **2-103a-g** (1.0 equiv.) in CH_2Cl_2/AcOH 10:1 (0.08 M) at 23 °C and the reaction was monitored by TLC until consumption of the starting material (4 h). The resin was then filtered and the organic phase was concentrated under reduced pressure. Purification by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) afforded compound **2-146** (50-60 % yield).



Selected example of compounds 2-146: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.75 (s, 1H), 6.65 (s, 1H), 5.48 (m, 2H), 5.49 (ddt, *J* = 6.1, 3.5, 2.9 Hz, 1H), 4.53 (d, *J* = 17.5 Hz, 1H), 4.04 (d, *J* = 17.7 Hz, 1H), 2.61-2.54 (m, 2H), 2.48-2.28 (m, 3H), 2.19-2.14 (m, 1H), 2.08-1.99 (m, 1H), 1.72-1.61 (m, 3H), 1.41 (d, *J* = 6.4 Hz, 3H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{21}ClO_5Na$: 375.0970, found 375.1050 [*M*+Na^+].

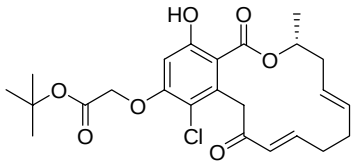
General procedure for the synthesis of compounds 2-147: The corresponding alcohol R^2OH (2.0 equiv.), triphenylphosphine (2.0 equiv.) and PS-DEAD (2.0 equiv., 1.3 mmol.g^{-1}) were added to a solution of corresponding compound **2-85a-g** or **2-103a-g** (1.0 equiv.) in THF (0.05 M) in a sequential manner. The reaction mixture was shaken at room temperature for 8

h, and then the resin was filtered and the filtrates were directly purified by preparative TLC (silica gel, 10 % EtOAc/cyclohexane) to afford a mixture of compound **2-147** along with the bis-alkylated product (78 % yield).



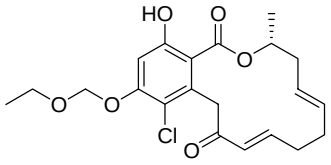
Selected example of compounds 2-147: Mixture with the corresponding bis-allylated compound 1:1; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.83 (s, 1H), 6.82 (ddd, *J* = 15.7, 8.2, 4.6 Hz, 1H), 6.72-6.65 (m, 1H), 6.46 (s, 1H), 6.41 (s, 1H), 6.09-5.98 (m, 3H), 5.82 (d, *J* = 15.7 Hz, 1H), 5.46-5.16 (m, 8H), 4.57-4.54 (m, 3H), 4.51-4.49 (m, 3H), 4.19 (d, *J* = 17.5 Hz, 1H), 4.11 (d, *J* = 14.6 Hz, 1H), 3.78 (d, *J* = 17.0 Hz, 1H), 3.51 (d, *J* = 14.2 Hz, 1H), 2.76-2.69 (m, 1H), 2.38-2.05 (m, 11H), 1.42 (d, *J* = 6.2 Hz, 3H), 1.35 (d, *J* = 6.3 Hz, 3H); *mono-allylated compound*: HRMS (ESI-TOF): *m/z*: calculated for $C_{21}H_{23}ClO_5Na$: 413.1132, found 413.1103 [*M*+Na^+]; *bis-allylated compound*: HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{27}ClO_5Na$: 453.1449, found 453.1422 [*M*+Na^+].

General procedure for the synthesis of compounds 2-148: TBD-methyl polystyrene (2.0 equiv., 2.9 mmol.g^{-1}) and the corresponding alkyl bromide or chloride ($BrCH_2COO^tBu$, EOMCl, 0.9 equiv.) were added to a solution of the corresponding compound **2-85a-g** or **2-103a-g** (1.0 equiv.) in CH_2Cl_2 (0.05 M) at 23 °C and the mixture was shaken for 3 h. The resin was then filtered and the filtrates were concentrated under reduced pressure. Purification by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) afforded corresponding compound **2-148** (> 90 % yield).



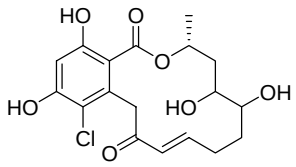
Selected examples of compounds 2-148: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.84 (s, 1H), 6.69 (m, 1H), 6.41 (s, 1H), 5.76 (d, *J* = 15.0 Hz, 1H), 5.43 (m, 1H), 5.26 (ddd, *J* = 15.0, 9.1, 4.8 Hz, 1H), 5.18-5.11 (m, 1H), 4.65 (s, 2H), 4.33 (d, *J* = 17.7 Hz, 1H), 4.16 (d, *J* = 17.5 Hz, 1H), 2.65-2.58 (m, 1H), 2.37-2.34 (m, 2H), 2.25-2.21 (m, 1H), 2.12-2.01 (m, 2H), 1.53 (s,

9H), 1.34 (d, *J* = 6.5 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{29}ClO_7Na$: 487.1494, found 487.1498 [*M*+Na^+].



^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.76 (s, 1H), 6.86 (s, 1H), 6.70 (dt, *J* = 14.9, 7.3 Hz, 1H), 5.77 (d, *J* = 15.8 Hz, 1H), 5.46-5.42 (m, 1H), 5.37 (s, 2H), 5.30-5.19 (m, 2H), 4.34 (d, *J* = 17.6 Hz, 1H), 4.16 (d, *J* = 18.1 Hz, 1H), 3.80 (q, *J* = 7.0 Hz, 2H), 2.66-2.59 (m, 1H), 2.37-2.34 (m, 2H), 2.26-2.21 (m, 1H), 2.13-2.06 (m, 2H), 1.34 (d, *J* = 6.4 Hz, 3H), 1.27 (t, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{21}H_{25}O_6ClNa$: 431.1237, found 431.1257 [*M*+Na^+].

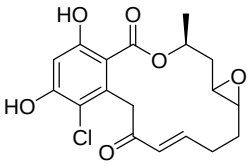
General procedure for the synthesis of compounds 2-149: OsO_4 (0.1 equiv.) and NMO (1.0 equiv.) were added to a solution of compound **2-85a-g** or **2-103a-g** (1.0 equiv.) in acetone/H_2O 10:1 (0.05 M) at 23 ^oC and the mixture was stirred for 1 h. The crude mixture was filtered through a pad of silica, concentrated and purified by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) to afford **2-149** as a mixture of two diastereoisomers 1:1 (> 70 % yield).



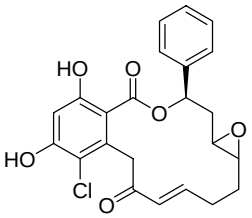
Selected example of compounds 2-149: ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.19 (m, 1H), 6.89-6.81 (m, 1H), 6.52 (s, 1H), 6.47 (s, 1H), 6.20 (d, *J* = 16.1 Hz, 1H), 6.04 (d, *J* = 15.6 Hz, 1H), 5.54-5.49 (m, 1H), 5.43-5.36 (m, 1H), 4.50 (d, *J* = 17.7 Hz, 1H), 4.46 (d, *J* = 17.7 Hz, 1H), 4.39 (d, *J* = 17.2 Hz, 1H), 4.07 (d, *J* = 17.2 Hz, 1H), 3.80-3.64 (m, 2H), 3.51-3.46 (m, 2H), 2.62-2.58 (m, 2H), 2.39-2.30 (m, 2H), 2.27-2.18 (m, 2H), 2.08-1.98 (m, 2H), 2.00-1.85 (m, 4H), 1.44 (d, *J* = 6.4 Hz, 6H), phenols and alcohols not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{21}ClO_7Na$: 407.0868, found 407.1031 [*M*+Na^+].

General procedure for the synthesis of compounds 2-150: Freshly prepared DMDO (1.2 equiv., 0.04 M in acetone) was added to a solution of compound **2-85a-g** or **2-103a-g** (1.0

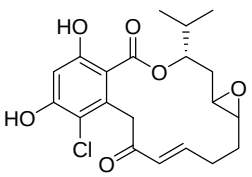
equiv.) in CH_3CN (0.03 M) at 0 °C and the mixture was stirred for 30 min. After evaporation of the solvents under reduced pressure, purification by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) afforded epoxides **2-150** (> 90 % yield) as a mixture of two diastereoisomers (1:1 to 3:1).



Selected examples of compounds 2-150: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.85 (s, 1H), 6.94-6.87 (m, 2H), 6.69 (s, 1H), 6.65 (s, 1H), 6.24 (bd, *J* = 15.2 Hz, 2H), 6.12 (d, *J* = 15.8 Hz, 1H), 5.41-5.37 (m, 1H), 5.33-5.30 (m, 1H), 4.54 (bd, *J* = 18.1 Hz, 2H), 4.52-4.48 (m, 1H), 4.40-4.34 (m, 1H), 4.27 (d, *J* = 17.5 Hz, 1H), 2.78-2.72 (m, 2H), 2.58-2.55 (m, 4H), 2.47-2.28 (m, 5H), 2.07 (m, 2H), 1.92-1.86 (m, 3H), 1.51 (d, *J* = 6.4 Hz, 3H), 1.35 (d, *J* = 6.4 Hz, 3H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{19}O_6ClNa$: 389.0762, found 389.0844 [M+Na^+].

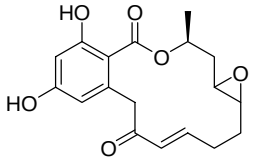


^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.80 (2 x s, 2H), 7.43-7.18 (m, 10H), 7.03-6.95 (m, 2H), 6.69 (s, 1H), 6.61 (s, 1H), 6.30 (d, *J* = 16.4 Hz, 1H), 6.21 (d, *J* = 15.8 Hz, 1H), 6.15-6.10 (m, 1H), 6.03 (d, *J* = 11.1 Hz, 1H), 4.84 (2 x d, *J* = 18.1 Hz, 2H), 4.41 (2 x d, *J* = 17.6 Hz, 2H), 2.68-2.60 (m, 4H), 2.41-2.27 (m, 8H), 1.83-1.76 (m, 4H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{21}O_6ClNa$: 451.0919, found 451.1028 [M+Na^+].

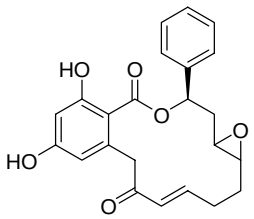


^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.56 (2 x s, 2H), 6.92-6.82 (m, 2H), 6.71 (s, 1H), 6.67 (s, 1H), 6.20 (m, 3H), 6.06 (d, *J* = 15.8 Hz, 1H), 5.11 (bs, 1H), 5.94 (m, 1H), 4.46 (2 x d, *J* = 18.1 Hz, 2H), 4.20 (2 x d, *J* = 18.1 Hz, 2H), 2.72-2.70 (m, 2H), 2.53-2.48 (m, 4H), 2.38-

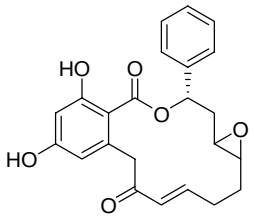
2.35 (m, 3H), 2.25-2.13 (m, 5H), 1.84-1.77 (m, 2H), 1.05-1.01 (m, 6H), 0.91-0.88 (m, 3H), 0.86-0.84 (m, 3H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{23}O_6ClNa$: 417.1075, found 417.1128 [*M*+Na^+].



^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.98 (s, 1H), 6.91-6.83 (m, 1H), 6.43 (d, *J* = 2.3 Hz, 1H), 6.24 (d, *J* = 2.4 Hz, 1H), 6.11 (d, *J* = 15.8 Hz, 1H), 5.35 (bs, 1H), 5.29 (m, 1H), 4.52 (d, *J* = 17.5 Hz, 1H), 3.63 (d, *J* = 17.5 Hz, 1H), 2.77 (m, 2H), 2.57-2.52 (m, 2H), 2.46-2.27 (m, 2H), 2.14-2.10 (m, 1H), 1.93-1.88 (m, 1H), 1.48 (d, *J* = 6.4 Hz, 3H); *other isomer:* ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.67 (s, 1H), 6.89-6.83 (m, 1H), 6.40 (d, *J* = 2.4 Hz, 1H), 6.24 (d, *J* = 2.9 Hz, 1H), 6.21 (d, *J* = 16.4 Hz, 1H), 5.37 (bs, 1H), 5.22 (m, 1H), 4.20 (d, *J* = 17.0 Hz, 1H), 4.06 (d, *J* = 17.0 Hz, 1H), 2.74 (m, 2H), 2.57-2.20 (m, 4H), 1.80-1.76 (m, 1H), 1.68-1.60 (m, 1H), 1.37 (d, *J* = 6.4 Hz, 3H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{20}O_6Na$: 355.1152, found 355.1249 [*M*+Na^+].

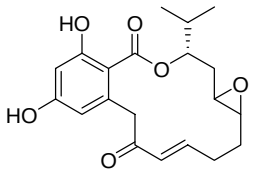


^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.98 (s, 1H), 6.91-6.83 (m, 1H), 6.43 (d, *J* = 2.3 Hz, 1H), 6.24 (d, *J* = 2.4 Hz, 1H), 6.11 (d, *J* = 15.8 Hz, 1H), 5.35 (bs, 1H), 5.29 (m, 1H), 4.52 (d, *J* = 17.5 Hz, 1H), 3.63 (d, *J* = 17.5 Hz, 1H), 2.77 (bs, 1H), 2.57-2.52 (m, 2H), 2.46-2.27 (m, 2H), 2.14-2.10 (m, 1H), 1.93-1.88 (m, 1H), 1.48 (d, *J* = 6.4 Hz, 3H).

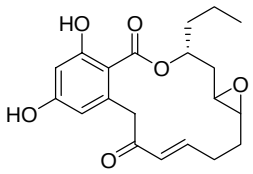


Major isomer: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.90 (s, 1H), 7.41-7.23 (m, 5H), 6.95-6.89 (m, 1H), 6.42 (d, *J* = 2.8 Hz, 1H), 6.27 (d, *J* = 2.9 Hz, 1H), 6.20 (d, *J* = 15.8 Hz, 1H), 6.13 (d, *J* = 4.1 Hz, 1H), 5.51 (m, 1H), 4.79 (d, *J* = 17.5 Hz, 1H), 3.79 (d, *J* = 17.0 Hz, 1H),

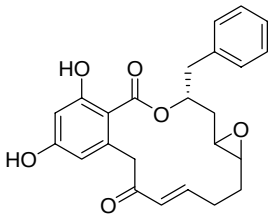
2.68-2.55 (m, 3H), 2.44-2.25 (m, 4H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{22}O_6Na$: 417.1309, found 417.1399 [M+Na^+].



^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.77 (s, 1H), 6.92-6.82 (m, 1H), 6.44 (d, J = 2.3 Hz, 1H), 6.28 (d, J = 2.4 Hz, 1H), 6.09 (d, J = 15.8 Hz, 1H), 5.40 (bs, 1H), 4.92 (m, 1H), 4.50 (d, J = 17.5 Hz, 1H), 3.61 (d, J = 17.5 Hz, 1H), 2.72-2.70 (m, 1H), 2.56-2.45 (m, 2H), 2.38-2.15 (m, 4H), 1.91-1.85 (m, 1H), 1.05-1.01 (m, 6H), *para*-phenol not detected; *other isomer:* ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.55 (s, 1H), 6.86-6.79 (m, 1H), 6.42 (s, 1H), 6.29 (s, 1H), 6.20 (d, J = 15.8 Hz, 1H), 5.40 (m, 1H), 5.16 (m, 1H), 4.14 (s, 1H), 4.12 (s, 1H), 2.72-2.70 (m, 1H), 2.53-2.37 (m, 4H), 2.18-2.10 (m, 2H), 1.92-1.86 (m, 1H), 0.91-0.85 (m, 6H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{24}O_6Na$: 383.1465, found 383.1574 [M+Na^+].

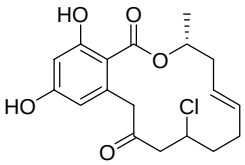


^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.87 (s, 1H), 6.90-6.82 (m, 1H), 6.43 (s, 1H), 6.26 (s, 1H), 6.10 (d, J = 15.2 Hz, 1H), 5.31 (bs, 1H), 5.18 (bs, 1H), 4.46 (d, J = 17.5 Hz, 1H), 3.60 (d, J = 17.6 Hz, 1H), 2.74 (bs, 1H), 2.57-2.38 (m, 3H), 2.32-2.22 (m, 1H), 2.08-1.82 (m, 2H), 1.73-1.67 (m, 1H), 1.42-1.37 (m, 2H), 1.33-1.28 (m, 2H), 1.01 (t, J = 7.3 Hz, 3H); *other isomer:* ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.72 (s, 1H), 6.86-6.80 (m, 1H), 6.41 (s, 1H), 6.27 (s, 1H), 6.21 (d, J = 16.4 Hz, 1H), 5.37 (m, 1H), 5.22 (m, 1H), 4.25 (d, J = 16.4 Hz, 1H), 3.96 (d, J = 16.4 Hz, 1H), 2.74 (bs, 1H), 2.60-2.37 (m, 4H), 1.87-1.78 (m, 2H), 1.70-1.58 (m, 3H), 1.38-1.22 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{20}H_{24}O_6Na$: 383.1465, found 383.1492 [M+Na^+].

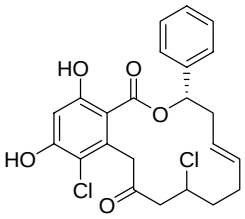


Major isomer: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.94 (s, 1H), 7.36-7.28 (m, 5H), 6.95-6.88 (m, 1H), 6.42 (s, 1H), 6.22 (s, 1H), 6.11 (d, *J* = 15.8 Hz, 1H), 5.47 (m, 1H), 5.41 (bs, 1H), 4.43 (d, *J* = 17.5 Hz, 1H), 3.56 (d, *J* = 17.6 Hz, 1H), 3.19 (dd, *J* = 13.7, 6.0 Hz, 1H), 3.03 (dd, *J* = 13.7, 7.9 Hz, 1H), 2.87 (bs, 1H), 2.70-2.28 (m, 4H), 2.03-1.93 (m, 2H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{24}H_{24}O_6Na$: 431.1465, found 431.1578 [*M*+Na^+].

General procedure for the preparation of macrocycles 2-151: $HCl_{conc.}$ (20.0 equiv.) was added to a solution of compound **2-120** (1.0 equiv.) in dioxane (0.05 M) at 23 °C, and the mixture was stirred for 3 h. After this time, the reaction was filtered through a pad of silica, the solvents evaporated under reduced pressure, and purification by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) afforded compound **2-151** (> 75 % yield) as a mixture of two diastereoisomers 1:1.



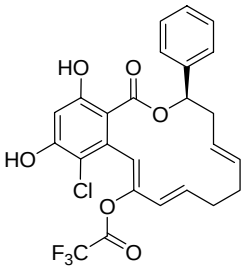
Selected examples of compounds 2-151: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.11 (s, 1H), 11.78 (s, 1H), 6.51 (s, 1H), 6.43 (s, 1H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.37 (d, *J* = 2.7 Hz, 1H), 6.21 (d, *J* = 2.4 Hz, 1H), 6.11 (d, *J* = 2.4 Hz, 1H), 5.59-5.51 (m, 3H), 5.40-5.32 (m, 3H), 4.54 (d, *J* = 17.2 Hz, 1H), 4.42 (d, *J* = 17.2 Hz, 1H), 3.60 (d, *J* = 17.2 Hz, 1H), 3.45 (d, *J* = 17.0 Hz, 1H), 3.28 (dd, *J* = 18.5, 9.4 Hz, 1H), 3.11 (dd, *J* = 13.7, 6.2 Hz, 1H), 3.07 (dd, *J* = 13.4, 4.6 Hz, 1H), 2.76 (dd, *J* = 19.0, 6.2 Hz, 1H), 2.62 (ddd, *J* = 15.5, 8.8, 4.0 Hz, 1H), 2.54 (ddd, *J* = 15.3, 6.2, 3.2 Hz, 1H), 2.40-2.26 (m, 4H), 2.25-2.13 (m, 4H), 2.03-1.91 (m, 2H), 1.42 (d, *J* = 6.4 Hz, 3H), 1.40 (d, *J* = 6.4 Hz, 3H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{18}H_{21}ClO_5Na$: 375.0970, found 375.0928 [*M*+Na^+].



^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.76 (s, 0.5H), 11.36 (s, 0.5H), 7.40-7.29 (m, 5H), 6.65 (s, 0.5H), 6.62 (s, 0.5H), 6.18 (t, *J* = 5.8 Hz, 1H), 6.14 (s, 0.5H), 6.12 (s, 0.5H), 5.67-5.62 (m, 1H), 5.55-5.49 (m, 1H), 4.93 (d, *J* = 18.1 Hz, 0.5H), 4.80 (d, *J* = 17.1 Hz, 0.5H), 4.58-4.56 (m, 1H), 4.38 (d, *J* = 18.1 Hz, 0.5H), 4.18 (d, *J* = 17.1 Hz, 0.5H), 3.33-3.27 (m, 1H), 3.10 (dd, *J* = 18.4, 3.8 Hz, 0.5H), 2.84-2.68 (m, 2.5H), 2.42-2.32 (m, 2H), 2.23-2.17 (m, 1H), 2.13-2.04 (m, 1H), *para*-phenol not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{22}O_5Cl_2Na$: 471.0737, found 471.0754 [M+Na^+].

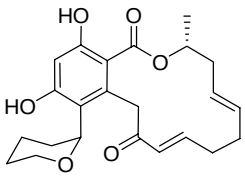
Elimination of β-Cl from compound 2-151: PS-TBD (51 mg, 2.6 mmol.g^{-1}) was added to a solution of compound **2-151** (95 mg, 270 μmol) in CH_2Cl_2 (5 mL) at 23 ^oC, and the mixture was stirred for 8 h. After this time, the reaction was filtered, the solvents were evaporated under reduced pressure, and the remaining residue was purified by flash chromatography (silica gel, 0-30 % EtOAc/cyclohexane gradient) to afford **2-103** (84 mg, 98 %).

General procedure for the synthesis of compounds 2-152: Compound **2-120a** or **2-112a** (1.0 equiv.) was dissolved in a 1:5 mixture of TFA/CH_2Cl_2 and stirred for 2 h at room temperature. Evaporation of the solvents; followed by flash chromatography (silica gel, 0-50 % Et_2O/hexane), afforded compound **2-152** (~70 % yield).



Selected example of compounds 2-152: ^{1}H NMR (400 MHz, C_6D_6, 25 °C): δ = 11.74 (s, 1H), 7.22-7.14 (m, 5H), 6.52 (s, 1H), 6.46 (dt, *J* = 15.2, 7.3 Hz, 1H), 6.28 (s, 1H), 5.89 (t, *J* = 7.0 Hz, 1H), 5.76 (s, 1H), 5.59 (d, *J* = 15.2 Hz, 1H), 5.37 (ddd, *J* = 15.2, 6.9, 6.9 Hz, 1H), 5.24 (ddd, *J* = 15.2, 7.3, 7.0 Hz, 1H), 2.56-2.49 (m, 1H), 2.44-2.39 (m, 1H), 2.01-1.92 (m, 4H); ^{13}C NMR (100 MHz, C_6D_6, 25 °C): δ = 164.8, 162.8, 158.7, 153.1, 137.6, 136.9, 136.1,

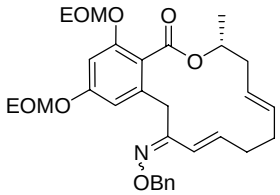
133.9, 128.7 (x 2), 126.5 (x 2), 124.2, 122.0, 102.4, 100.8, 79.9, 39.0, 32.3, 31.4, four quaternary carbons are not detected; HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{20}ClF_3O_6Na$: 531.0793, found 531.0992 [M+Na^+].



2-153

Macrocycle 2-153: DHP (3.7 µL, 40.8 µmol) and PS-TsOH (12.7 mg, 40.8 µmol, 3.2 $mmol.g^{-1}$) were added to a solution of compound **2-103** (12.9 mg, 40.8 µmol) in CH_2Cl_2 (1 mL) at 23 °C, and the mixture was stirred for 5 h. After this time, the reaction was filtered and the solvents were evaporated under reduced pressure. Purification by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) afforded **2-153** (13.8 mg, 85 %) as a mixture of two diastereoisomers. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 12.33 (s, 1H), 12.11 (s, 1H), 9.45 (s, 1H), 9.40 (s, 1H), 6.67, (m, 2H), 6.28 (2 x s, 2H), 5.83 (d, *J* = 13.2 Hz, 1H), 5.79 (d, *J* = 12.9 Hz, 1H), 5.35-5.30 (m, 3H), 5.27-5.22 (m, 3H), 5.06 (bd, *J* = 8.2 Hz, 2H), 4.10 (d, *J* = 17.5 Hz, 2H), 3.90-3.85 (m, 1H), 3.80-3.76 (m, 1H), 3.65 (d, *J* = 17.7 Hz, 2H), 3.57-3.52 (m, 2H), 3.46-3.41 (m, 2H), 2.77-2.71 (m, 3H), 2.53-2.49 (m, 3H), 2.36-2.29 (m, 4H), 2.24-1.56 (m, 12H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.28 (d, *J* = 6.4 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{23}H_{28}O_6Na$: 423.1778, found 423.1778 [M+Na^+].

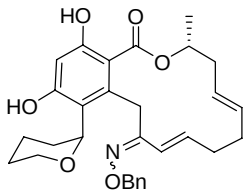
General procedure for the synthesis of compounds 2-154: The hydroxylamine R^2ONH_2 (5.0 equiv.) was added to a solution of compound **2-120** (1.0 equiv.) in pyridine/AcOH (5:1, 0.03 M) and the mixture was heated up to 40 °C. After stirring overnight, the solvents were evaporated under reduced pressure with silica gel. Purification over a short pad of silica gel with a mixture of 30 % EtOAc/cyclohexane afforded compound **2-154** (~99 %) as a mixture of two diastereoisomers *cis*/*trans*.



Selected example of compounds 2-154: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.50-7.25 (m, 10H), 6.82 (s, 1H), 6.75 (s, 1H), 6.66 (s, 1H), 6.48 (s, 1H), 6.24-6.11 (m, 2H), 6.11-6.05

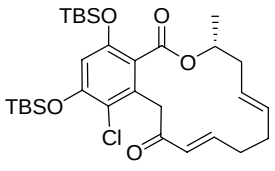
(m, 2H), 5.45-5.38 (m, 4H), 5.34-5.31 (m, 14 H), 4.50 (d, *J* = 17.2 Hz, 1H), 3.65-3.38 (m, 8H), 3.60 (d, *J* = 17.1 Hz, 1H), 3.54 (d, *J* = 17.1 Hz, 1H), 3.24 (d, *J* = 17.2 Hz, 1H), 2.48-2.36 (m, 4H), 2.21-2.17 (m, 2H), 2.11-2.04 (m, 2H), 1.95-1.83 (m, 2H), 1.62-1.51 (m, 2H), 1.49 (d, *J* = 6.4 Hz, 6H), 1.32-1.20 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 168.1, 167.9, 159.1, 158.8, 157.2, 155.6, 155.4, 154.2, 140.8, 138.2, 138.2, 137.8, 136.9, 136.7, 132.3, 132.3, 128.3 (x 2), 128.3 (x 2), 128.2, 128.1 (x 2), 128.0 (x 2), 127.7, 127.6, 125.5, 118.8, 118.6, 118.3, 108.8, 108.5, 101.7, 101.7, 93.5, 93.4, 93.1 (x 2), 77.2, 76.0, 75.9, 71.2, 71.0, 64.5, 64.5, 64.3, 64.3, 40.0, 40.0, 34.9, 32.4, 32.3, 31.6, 31.1, 28.9, 20.3, 20.2, 15.0 (x 2), 15.0 (x 2); HRMS (ESI-TOF): *m/z*: calculated for $C_{31}H_{39}NO_7Na$: 560.2619, found 560.2627 [*M*+Na^+].

General procedure for the synthesis of compounds 2-155: PS-TsOH (10.0 equiv., 3.2 mmol.g^{-1}) was added to a solution of compound **2-154** (1.0 equiv.) in MeOH (0.02 M) and the suspension was shaken at 40 ^{o}C for 4 h. After this time, the reaction mixture was filtered and the methanolic solution concentrated under reduced pressure. The crude product obtained was submitted without further purification to the next step. Thus, DHP (1.0 equiv.) and PS-TsOH (catalytic amount, 3.2 mmol.g^{-1}) were added to a solution of this crude in CH_2Cl_2 (0.02 M) at 23 ^{o}C, and the mixture was stirred for 5 h. After this time, the mixture was filtered, the solvents were evaporated under reduced pressure, and the remaining residue was purified by preparative TLC (silica gel, 30 % EtOAc/cyclohexane) to afford two different diastereoisomers 1:1 of **2-155** (~65 % yield).



Selected example of compounds 2-155: *Less polar diastereoisomer:* ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.25 (s, 1H), 9.24 (s, 1H), 7.46-7.33 (m, 10H), 6.29 (s, 1H), 6.26 (s, 1H), 6.07-6.02 (m, 2H), 5.75 (d, *J* = 15.8 Hz, 1H), 5.69 (d, *J* = 15.8 Hz, 1H), 5.44-5.38 (m, 6H), 5.23 (s, 4H), 5.03 (d, *J* = 8.8 Hz, 2H), 4.34-4.13 (m, 6H), 3.69-3.63 (m, 2H), 2.70-2.67 (m, 2H), 2.30-2.16 (m, 6H), 2.08-1.94 (m, 8H), 1.73-1.65 (m, 8H), 1.42 (t, *J* = 6.4 Hz, 3H), 1.39 (t, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{35}NO_6Na$: 528.2357, found 528.2562 [*M*+Na^+].

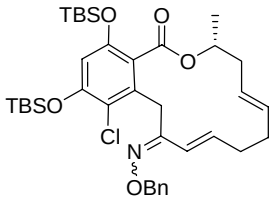
More polar diastereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.61 (s, 1H), 9.27 (s, 1H), 7.41-7.33 (m, 5H), 6.62 (d, *J* = 16.4 Hz, 1H), 6.47 (s, 1H), 6.15-6.07 (m, 1H), 5.50-5.38 (m, 3H), 5.16 (s, 2H), 5.04 (d, *J* = 10.5 Hz, 1H), 4.30 (d, *J* = 15.2 Hz, 1H), 4.24 (d, *J* = 10.5 Hz, 1H), 3.84 (d, *J* = 15.2 Hz, 1H), 3.66 (t, *J* = 11.4 Hz, 1H), 2.71-2.65 (m, 1H), 2.28-2.08 (m, 6H), 1.73-1.64 (m, 5H), 1.38 (t, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{30}H_{35}NO_6Na$: 528.2357, found 528.2494 [*M*+Na^+].



2-128

Macrocycle 2-128 from pochonin D (2-85): TBSCl (53.6 mg, 356 µmol) and imidazole (23.6 mg, 356 µmol) were added to a solution of pochonin D (**2-85**, 25 mg, 71.2 µmol) in DMF (5 mL) and the mixture was stirred for 3 h at room temperature. Purification by column chromatography (silica gel, 0-30 % EtOAc/cyclohexane gradient) afforded compound **2-128** (40 mg, 98 %).

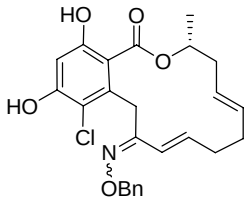
General procedure for compounds 2-157: The hydroxylamine $RONH_2$ (5.0 equiv.) was added to a solution of compound **2-128** (1.0 equiv.) in pyridine/AcOH (5:1, 250 µL) and the mixture was heated up to 40 °C. After stirring overnight, the solvents were evaporated under reduced pressure, and filtration on silica gel with a mixture of 30 % EtOAc/cyclohexane afforded two isomers of **2-157** (~90 % yield).



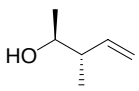
Selected example of compounds 2-157: *cis* oxime: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.42 (bd, *J* = 6.4 Hz, 2H), 7.36 (bdd, *J* = 7.5, 6.9 Hz, 2H), 7.34-7.32 (m, 1H), 6.52 (d, *J* = 16.1 Hz, 1H), 6.38 (s, 1H), 6.18-6.10 (m, 1H), 5.36-5.32 (m, 2H), 5.16 (bs, 2H), 4.99-4.95 (m, 1H), 3.79-3.76 (m, 2H), 2.40-1.99 (m, 6H), 1.45 (d, *J* = 6.2 Hz, 3H), 1.03 (s, 9H), 0.99 (s, 9H), 0.28 (s, 3H), 0.26 (s, 3H), 0.20 (s, 6H); *trans* oxime: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.44 (bd, *J* = 6.5 Hz, 2H), 7.37 (bdd, *J* = 7.6, 6.9 Hz, 2H), 7.33-7.31 (m, 1H), 6.41 (s, 1H), 6.04-5.97 (m, 1H), 5.48 (bd, *J* = 15.0 Hz, 1H), 5.29-5.27 (m, 1H), 5.22 (bs, 2H), 5.00-4.95 (m,

1H), 3.98-3.89 (m, 2H), 2.39-2.02 (m, 6H), 1.37 (d, *J* = 5.9 Hz, 3H), 1.04 (s, 9H), 0.99 (s, 9H), 0.28 (s, 3H), 0.27 (s, 3H), 0.23 (s, 3H), 0.22 (s, 3H).

General procedure for compounds 2-158: To a solution of corresponding compound **2-157** (1.0 equiv) in THF was added TBAF (2.5 equiv, 1M solution in THF) and the mixture was stirred at room temperature for 2 h. The solvents were then evaporated under reduced pressure, and filtration on silica gel with a mixture of 30 % EtOAc/cyclohexane afforded compound **2-158** in > 85 % yield.



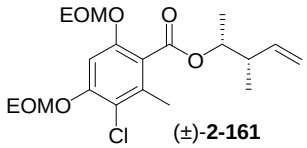
Selected example of compounds 2-158: *cis* oxime: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.52 (s, 1H), 7.45-7.34 (m, 5H), 6.64 (s, 1H), 6.09-6.02 (m, 2H), 5.34-5.25 (m, 4H), 5.18-5.08 (m, 2H), 4.33 (d, *J* = 17.0 Hz, 1H), 4.15 (d, *J* = 17.6 Hz, 1H), 2.65-2.59 (m, 1H), 2.27-2.14 (m, 3H), 2.04-2.00 (m, 1H), 1.88-1.83 (m, 1H), 1.30 (t, *J* = 6.4 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{26}ClNO_5Na$: 478.1392, found 478.1372 [*M*+Na^+].
trans oxime: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.73 (s, 1H), 7.32-7.26 (m, 5H), 6.64 (s, 1H), 6.50 (d, *J* = 16.4 Hz, 1H), 6.06-5.98 (m, 2H), 5.43-5.24 (m, 3H), 4.91 (s, 2H), 4.22 (s, 2H), 2.61-2.55 (m, 1H), 2.46-2.33 (m, 2H), 2.20-2.02 (m, 3H), 0.98 (t, *J* = 6.4 Hz, 3H); HRMS (ESI-TOF): *m/z*: calculated for $C_{25}H_{26}ClNO_5Na$: 478.1392, found 478.1522 [*M*+Na^+].



(±)-**2-159**

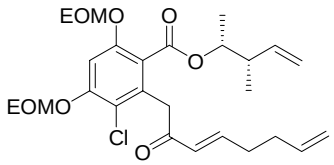
Compound (±)-2-159: A solution of *cis*-butene oxide (1.75 mL, 20 mmol) in Et_2O (10 mL) was cooled to −30 °C. Copper iodide (1.14 g, 6 mmol) was added to this solution and then, vinyl magnesium bromide (40 mL, 1M solution in THF, 40 mmol) was added dropwise over a period of 1 h. The reaction mixture was then warmed up to room temperature over 12 h and the reaction turned black. The reaction mixture was quenched slowly with saturated $NH_4Cl_{aq.}$ (20 mL), stirred for 2 h, extracted with Et_2O (20 mL) and dried over $MgSO_4$. Concentration under reduced pressure afforded compound (±)-**2-159** (1.3 g, 65 %). ^{1}H NMR (400 MHz,

$CDCl_3$, 25 °C): δ = 5.81-5.72 (m, 1H), 5.15 (d, *J* = 13.2 Hz, 2H), 3.59 (m, 1H), 2.23-2.10 (m, 1H), 1.21 (d, *J* = 6.4 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 3H).



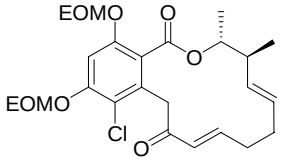
(±)-**2-161**

Compound (±)-2-161: In a similar manner as that described for compound **2-110** in solution, compound (±)-**2-161** was prepared with a 16 % yield in two steps from **2-108**. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.02 (s, 1H), 5.87-5.78 (m, 1H), 5.31 (s, 2H), 5.21-5.15 (s + m, 3H), 5.13-8.07 (m, 2H), 3.79 (q, *J* = 7.0 Hz, 2H), 3.73 (q, *J* = 7.0 Hz, 2H), 2.53-2.44 (m, 1H), 2.35 (s, 3H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.24 (q, *J* = 7.0 Hz, 6H), 1.11 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 161.1, 154.0, 152.9, 139.4, 134.8, 120.3, 117.0, 115.6, 101.6, 93.9, 93.5, 74.7, 64.6, 64.3, 42.4, 17.4, 17.0, 15.6, 15.0, 15.0.



(±)-**2-162**

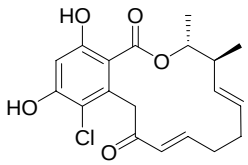
Compound (±)-2-162: In a similar manner as that described for compound **2-111** in solution, compound (±)-**2-162** was prepared with a 57 % yield from (±)-**2-161** and **2-114**. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 7.13 (s, 1H), 6.92 (dt, *J* = 15.8, 6.7 Hz, 1H), 6.18 (d, *J* = 15.8 Hz, 1H), 5.84-5.72 (m, 2H), 5.29 (s, 2H), 5.20 (s, 2H), 5.11-4.99 (m, 5H), 4.04 (s, 2H), 3.76 (q, *J* = 7.0 Hz, 2H), 3.71 (q, *J* = 7.0 Hz, 2H), 2.43-2.38 (m, 1H), 2.35-2.29 (m, 2H), 2.25-2.20 (m, 2H), 1.27-1.20 (m, 9H), 1.04 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 194.4, 166.7, 154.5, 153.7, 146.8, 139.4, 137.0, 132.7, 129.3, 120.4, 117.5, 115.6, 115.5, 102.8, 93.9, 93.6, 74.8, 64.6, 64.4, 43.0, 42.3, 32.0, 31.7, 16.9, 15.4, 15.0, 14.9.



(±)-**2-163**

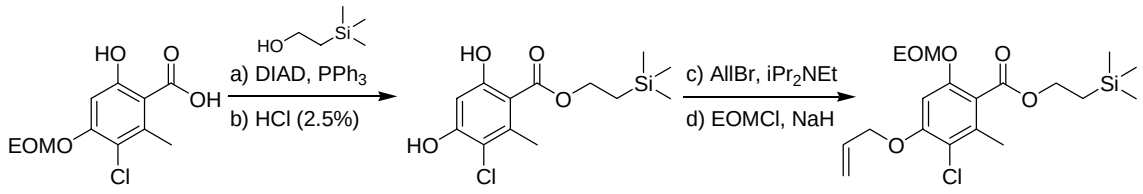
Compound (±)-2-163: In a similar manner as that described for compound **2-112**, compound (±)-**2-163** was prepared with a 57 % yield from (±)-**2-162**. ^{1}H NMR (400 MHz, $CDCl_3$, 25

°C): δ = 7.10 (s, 1H), 6.76-6.71 (m, 1H), 5.87 (d, *J* = 15.8 Hz, 1H), 5.32 (s, 2H), 5.25-5.18 (s + m, 4H), 4.87-4.80 (m, 1H), 3.99 (d, *J* = 16.9 Hz, 1H), 3.80-3.71 (m, 5H), 2.32-2.26 (m, 2H), 2.20-2.11 (m, 3H), 1.36 (d, *J* = 6.4 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 6H), 1.04 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 195.7, 166.6, 154.6, 153.8, 147.1, 133.3, 132.8, 120.7, 117.7, 102.9, 93.9, 93.6, 76.2, 64.8, 64.6, 45.1, 41.6, 30.7, 30.7, 18.2, 16.9, 15.0, 15.0.



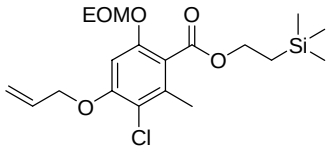
(±)-**2-164**

Compound (±)-2-164: In a similar manner as that described for compound **2-85** using PS-TsOH, compound (±)-**2-164** was prepared with a 40 % yield from (±)-**2-163**. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.52 (s, 1H), 6.78-6.69 (m, 1H), 6.67 (s, 1H), 6.05 (s, 1H), 5.93 (d, *J* = 16.4 Hz, 1H), 5.47-5.45 (m, 1H), 5.37-5.33 (m, 1H), 5.16 (dd, *J* = 15.8, 7.0 Hz, 1H), 4.48 (bs, 1H), 4.35 (d, *J* = 17.6 Hz, 1H), 2.45-2.26 (m, 5H), 1.27 (d, *J* = 6.4 Hz, 3H), 1.10 (d, *J* = 7.0 Hz, 3H); ESI: *m/z*: calculated for $C_{18}H_{21}ClO_5$: 365.12, found 365.22 [M+H^+].



2-108 **2-186** **2-170**

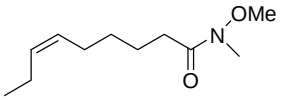
Compound 2-186: A solution of acid **2-108** (5.2 g, 20.0 mmol), trimethylsilylethanol (3.44 mL, 24.0 mmol) and triphenylphosphine (10.5 g, 40.0 mmol) in anhydrous toluene (50 mL) was treated at 0°C with DIAD (7.88 mL, 40.0 mmol). After stirring for 30 min, the reaction mixture was concentrated under reduced pressure. Purification by flash chromatography (silica gel, 0-10 % EtOAc/hexane gradient) afforded **mono-EOM ester 2-186** (3.0 g, 42 %). A solution of this ester (3.0 g, 8.3 mmol) in dioxane (110 mL) was treated with $HCl_{conc.}$ (2.75 mL, 2.5 % vol.). After stirring for 4 h, the reaction mixture was diluted in EtOAc, washed several times with saturated $NH_4Cl_{aq.}$ and dried over $MgSO_4$. Concentration under reduced pressure afforded crude ester **2-186** (2.67 g, quantitative yield) used without purification in the next step. ***Mono-EOM ester 2-186:*** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.5 (s, 1H), 6.72 (s, 1H), 5.34 (s, 2H), 4.48 (t, *J* = 8.8 Hz, 2H), 3.78 (q, *J* = 7.0 Hz, 4H), 2.68 (s, 3H), 1.27-1.18 (m, 5H), 0.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.2, 162.4, 157.2, 139.7, 116.1, 107.8, 101.6, 93.4, 65.0, 64.2, 19.7, 17.6, 15.0, -1.58 (x 3). *Ester **2-186*:* ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.58 (s, 1H), 6.52 (s, 1H), 6.36 (s, 1H), 4.46 (t, *J* = 9.0 Hz, 2H), 2.63 (s, 3H), 1.19 (t, *J* = 8.8 Hz, 2H), 0.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.1, 162.7, 156.0, 139.5, 113.9, 107.3, 101.9, 64.3, 19.9, 17.5, -1.60 (x 3).



2-170

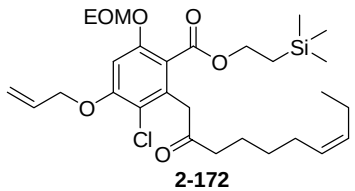
Compound 2-170: Diisopropylethylamine (2.47 mL, 14.9 mmol) and allyl bromide (1.29 mL, 14.9 mmol) were added to a solution of ester **2-186** (2.51 g, 8.3 mmol) and TBAI (catalytic amount) in DMF (20 mL). After stirring overnight at 80 °C, the reaction mixture was extracted with Et_2O (100 mL), washed extensively with saturated $NH_4Cl_{aq.}$ (150 mL) and dried over $MgSO_4$. Concentration under reduced pressure afforded mono-allylated ester **2-186**. Sodium hydride (664 mg, 16.6 mmol) and chloromethylethyl ether (1.54 mL, 16.6 mmol) were then added at 0 °C to a solution of this ester (8.3 mmol) in THF (10 mL). The

reaction was stirred for 1.5 h at 0 °C and quenched by adding saturated $NH_4Cl_{aq.}$ (25 mL). The mixture was then extracted with Et_2O (75 mL), washed with saturated $NH_4Cl_{aq.}$ (50 mL) and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography (silica gel, 0-15 % EtOAc/hexane) afforded compound **2-170** (2.0 g, 0.34 mmol) in 60 % yield over 2 steps. ***Mono-allylated ester 2-186:*** ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 11.65 (s, 1H), 6.41 (s, 1H), 6.11-6.04 (m, 1H), 5.51 (d, *J* = 17.5 Hz, 1H), 5.36 (d, *J* = 10.5 Hz, 1H), 4.63 (d, *J* = 3.5 Hz, 2H), 4.47 (t, *J* = 8.8 Hz, 2H), 2.67 (s, 3H), 1.20 (t, *J* = 8.8 Hz, 2H), 0.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 171.3, 162.7, 158.3, 139.7, 131.8, 118.2, 115.8, 106.8, 99.4, 69.5, 64.1, 19.7, 17.6, -1.57 (x 3). *Compound **2-170***: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.74 (s, 1H), 6.09-6.00 (m, 1H), 5.47 (d, *J* = 17.0 Hz, 1H), 5.31 (d, *J* = 10.5 Hz, 1H), 5.20 (s, 2H), 4.61 (d, *J* = 5.2 Hz, 2H), 4.41 (t, *J* = 8.5 Hz, 2H), 3.72 (q, *J* = 7.0 Hz, 2H), 2.34 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.12 (t, *J* = 8.5 Hz, 2H), 0.09 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.6, 155.2, 153.2, 135.0, 132.3, 119.2, 118.0, 116.5, 99.3, 93.8, 69.8, 64.3, 63.6, 17.4, 15.0, -1.55 (x 3), one carbon is not detected.



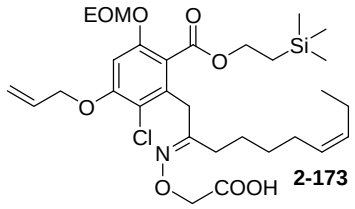
2-171

Weinreb amide 2-171: *N*,*O*-dimethylhydroxylamine hydrochloride (976 mg, 10.0 mmol), DMAP (catalytic amount) and EDC (1.92 g, 10.0 mmol) were sequentially added to a solution of **2-89** (1.90 g, 10.0 mmol) in anhydrous CH_2Cl_2 (30 mL). After stirring at room temperature for 4 h, the reaction was diluted with EtOAc (30 mL), washed with saturated $NH_4Cl_{aq.}$ (40 mL) and dried over $MgSO_4$. Concentration under reduced pressure afforded compound **2-171** (1.69 g, 8.5 mmol) in 85 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 5.35-5.27 (m, 2H), 3.65 (s, 3H), 3.15 (s, 3H), 2.41-2.37 (m, 2H), 2.05-1.97 (m, 4H), 1.65-1.58 (m, 2H), 1.40-1.34 (m, 4H), 0.92 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 131.8, 128.7, 61.1, 29.4, 26.8, 24.3, 20.4, 14.3, one carbon is not detected.



2-172

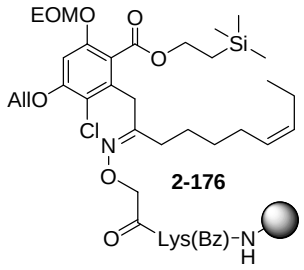
Compound 2-172: A solution of compound **2-170** (802 mg, 2.0 mmol) in anhydrous THF (10 mL) cooled at –78 °C was treated with freshly prepared LDA (7.1 mL, 4.0 mmol), followed by an immediate addition of compound **2-171** (399 mg, 2.0 mmol) as a solution in THF (5 mL). The resulting mixture was then stirred for 10 min at –78 °C and quenched by addition of saturated $NH_4Cl_{aq.}$ (30 mL). Upon warming to room temperature, the reaction was diluted with EtOAc (50 mL), washed with saturated $NH_4Cl_{aq.}$ (70 mL) and dried over $MgSO_4$. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-20 % EtOAc/hexane gradient) afforded compound **2-172** (200 mg, 0.37 mmol) in 20 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.85 (s, 1H), 6.11-6.01 (m, 1H), 5.50 (dd, *J* = 17.5, 1.2 Hz, 1H), 5.38-5.31 (m, 1H), 5.22 (s, 2H), 4.64 (d, *J* = 5.3 Hz, 2H), 4.34 (t, *J* = 8.8 Hz, 2H), 3.88 (s, 2H), 3.74 (q, *J* = 7.0 Hz, 2H), 2.46 (t, *J* = 7.3 Hz, 2H), 2.06-2.02 (m, 4H), 1.64-1.57 (m, 2H), 1.37-1.24 (m, 5H), 1.06 (t, *J* = 8.8 Hz, 3H), 0.96 (t, *J* = 7.6 Hz, 3H), 0.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 205.8, 167.1, 155.6, 154.2, 133.0, 132.1, 131.9, 128.6, 119.1, 118.3, 117.0, 100.6, 94.0, 69.8, 64.5, 63.7, 45.4, 41.6, 29.2, 26.9, 23.1, 20.5, 17.4, 15.0, 14.3, -1.58 (x 3); ESI: *m/z*: calculated for $C_{28}H_{43}ClO_6SiNa$: 561.24, found 561.39 [M+Na^+].



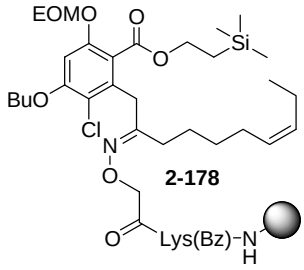
2-173

Compound 2-173: Carboxymethoxylamine hemihydrochloride (325 mg, 1.48 mmol) was added to a solution of compound **2-172** (200 mg, 0.37 mmol) in a 5:1 mixture of pyridine/AcOH (1.68 mL) at room temperature. The resulting mixture was then stirred overnight at 40 °C. Concentration under reduced pressure, followed by flash chromatography (silica gel, 0-3 % MeOH/$CHCl_3$ gradient) afforded compound **2-173** (76.5 mg, 0.12 mmol) in 34 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 6.84-6.82 (m, 1H), 6.11-6.04 (m, 1H), 5.50 (dd, *J* = 17.3, 3.2 Hz, 1H), 5.39-5.28 (m, 3H), 5.25-5.21 (m, 2H), 4.67-4.64 (m, 3H), 4.53 (s, 1H), 4.36 (t, *J* = 8.8 Hz, 2H), 3.98 (s, 1H), 3.77-3.74 (m, 3H), 2.40-2.32 (m, 1H), 2.09-1.94 (m, 5H), 1.52-1.34 (m, 3H), 1.31-1.22 (m, 4H), 1.07-1.05 (m, 2H), 1.00-0.91 (m, 3H), 0.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 173.1, 167.3, 167.0, 162.0, 160.0, 155.6, 155.5, 153.9, 153.8, 134.0, 133.2, 132.1, 132.0, 131.9, 131.7, 128.8, 128.6, 119.4, 119.2, 118.4, 118.3, 117.1, 117.0, 100.6, 100.4, 94.0, 70.3, 70.0, 69.9, 64.6, 64.5, 64.2, 63.8, 32.1, 30.8, 29.7, 29.2, 29.1, 28.4, 26.7 (x 2), 26.6, 25.3, 25.1, 20.5, 20.4, 17.3, 17.2, 15.0, 14.3

(x 2), -1.56 (x 6), three carbon are not detected; ESI: *m/z*: calculated for $C_{30}H_{47}ClO_8SiN$: 612.28, found 612.47 [M+H^+].

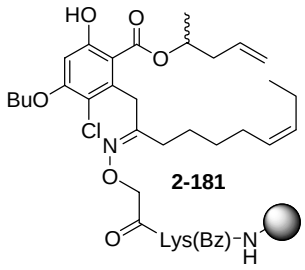


Resin 2-176: Resin **2-174** (50 mg, 0.2 mmol.g^{-1}) was treated with a 1:5 mixture of piperidine/DMF (500 µL) for 1 h. After this time, the resulting Fmoc-deprotected resin was washed several times with DMF (50 mL) and CH_2Cl_2 (50 mL) and treated with benzoyl chloride (5.8 µL, 0.05 mmol) and pyridine (4.0 µL, 0.05 mL) in DMF (500 µL). After shaking for 12 h, the Bz-protected resin was washed several times with DMF (50 mL) and CH_2Cl_2 (50 mL). This resin was then treated with a mixture of $Pd(PPh_3)_2Cl_2$ (1.4 mg, 0.002 mmol), PPh_3 (2.1 mg, 0.008 mmol) and $TMSN_3$ (13.3 µL, 0.1 mmol) in DCM (500 µL), followed by an immediate addition of nBu$_3$SnH (13.4 µL, 0.05 mmol) as a solution in DCM (500 µL) and shaken for 20 min (sequence repeated 3 times). Futher washing with DMF (50 mL) and CH_2Cl_2 (50 mL) afforded clean resin **2-175** as judged by L.C./M.S. (after cleavage with TFA/DCM 1:1). This resin was then treated with a preformed solution of compound **2-173** (12.2 mg, 0.02 mmol), HOBt (8.1 mg, 0.06 mmol) and DIC (13.9 µL, 0.09 mmol) in DMF (500 µL) and shaken for 4 h. Further washing with DMF (50 mL) and CH_2Cl_2 (50 mL) afforded resin **2-176** as determined by L.C./M.S.

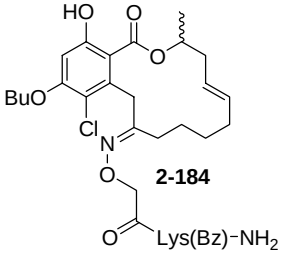


Resin 2-178: Resin **2-176** was treated with a mixture of $Pd(PPh_3)_2Cl_2$ (1.4 mg, 0.002 mmol), PPh_3 (2.1 mg, 0.008 mmol) and $TMSN_3$ (13.3 µL, 0.1 mmol) in DCM (500 µL), followed by an immediate addition of nBu$_3$SnH (13.4 µL, 0.05 mmol) as a solution in DCM (500 µL) and shaken for 20 min (sequence repeated 3 times). Further washing with DMF (50 mL) and CH_2Cl_2 (50 mL) afforded clean resin **2-177** as judged by L.C./M.S. This resin was then

treated with a premixed solution of butan-1-ol (4.6 µL, 0.05 mmol) and triphenyphosphine (26.2 mg, 0.1 mmol) in THF (300 µL), followed by an immediate addition of DIAD (19.7 µL, 0.1 mmol) as a solution in THF (300 µL) and shaken for 12 h. Further washing with DMF (50 mL) and CH_2Cl_2 (50 mL) afforded resin **2-178** as determined by L.C./M.S.

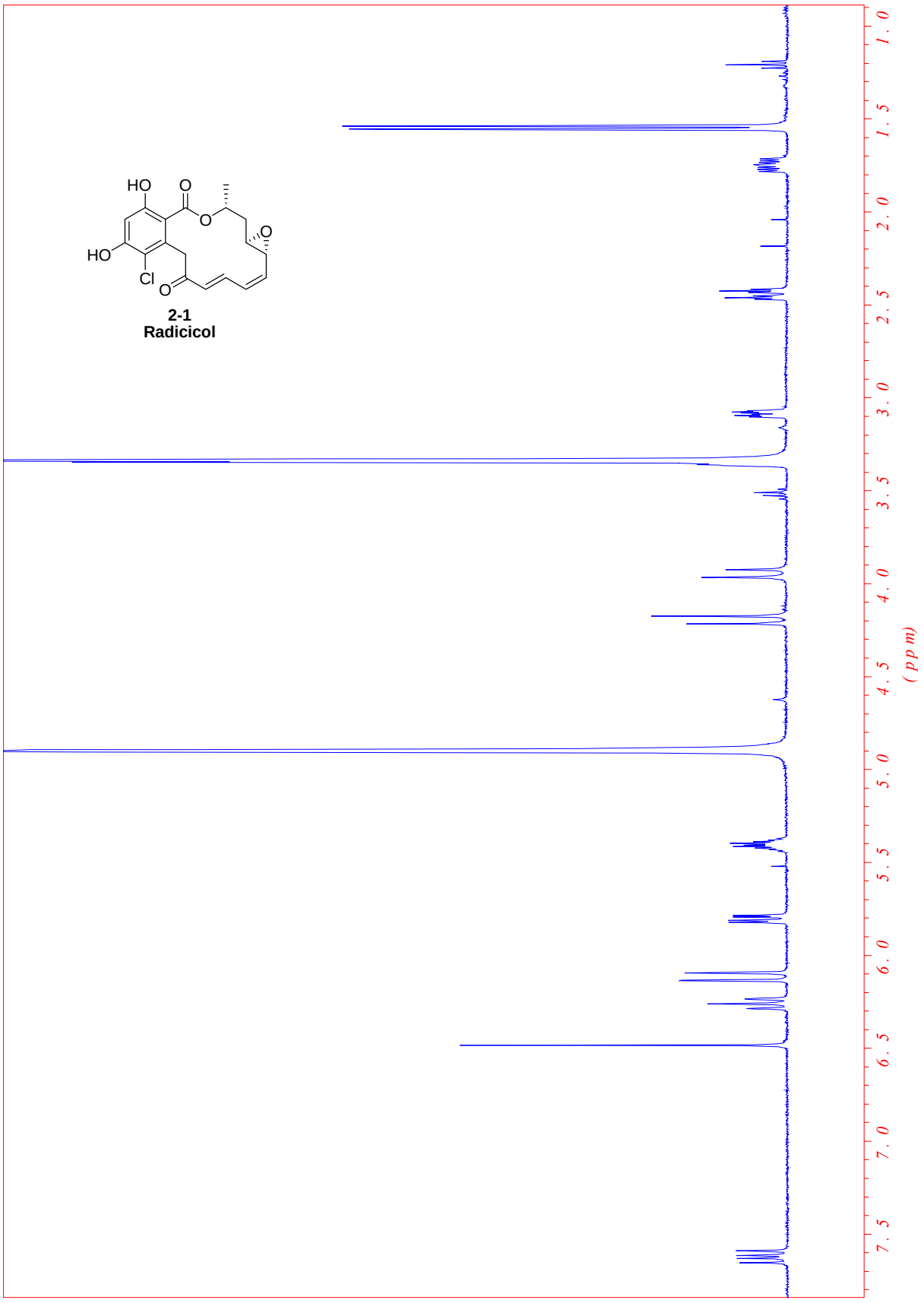


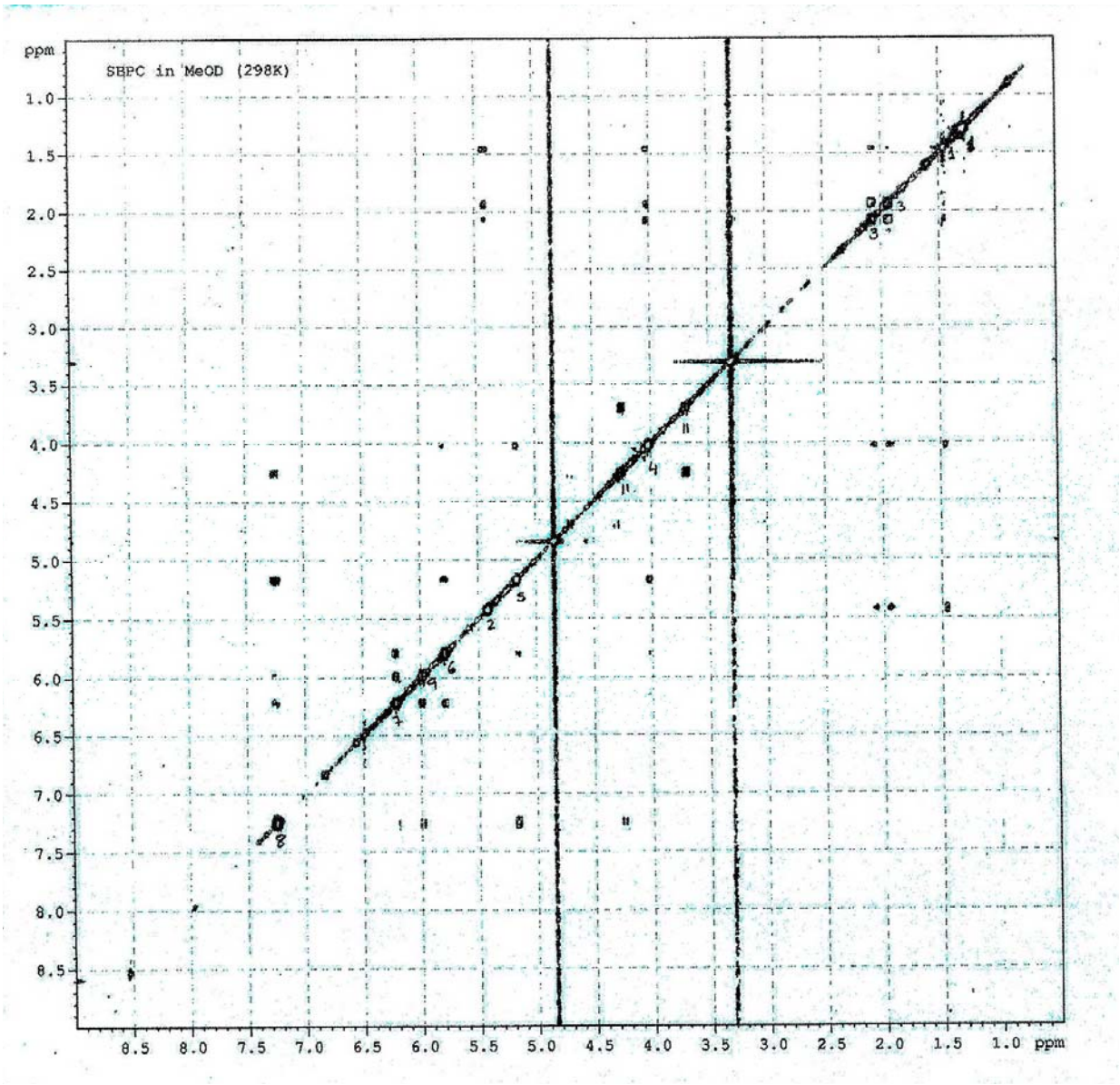
Resin 2-181: Resin **2-178** was swollen with THF (600 µL) and shaken for 4 h with TBAF (40 µL, 1M solution in THF, 0.04 mmol). The resulting resin **2-179** was washed with DMF (50 mL) and CH_2Cl_2 (50 mL) and shaken for 12 h with a 7:1.5:1 mixture of THF/TFA/MeOH (950 µL). After washing, resin **2-180** was treated with a premixed solution of 4-penten-2-ol (2.1 µL, 0.02 mmol) and triphenyphosphine (13.1 mg, 0.05 mmol) in toluene (300 µL), followed by an immediate addition of DIAD (9.8 µL, 0.05 mmol) as a solution in toluene (300 µL) and shaken for 12 h. Further washing with DMF (50 mL) and CH_2Cl_2 (50 mL) afforded resin **2-181** along with some *para*-phenol protected resin as determined by L.C./M.S.

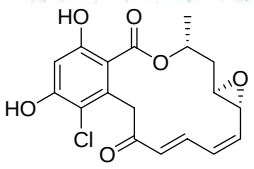


Compound 2-184: Resin **2-181** was swollen in DMF (300 µL), treated with TBSCl (5.0 equiv.) and imidazole (5.0 equiv.) as a solution in DMF (300 µL) and shaken for 12 h. Removal of the reagents followed by washing with DMF (50 mL) and CH_2Cl_2 (50 mL) afforded resin **2-182**, which was further swollen with CH_2Cl_2 (1 mL). Treatment with Grubbs'II (0.06 equiv.) and heating to 120 °C under microwaves irradiation for 45 min (sequence repeated 3 times) afforded resin **2-183** which was cleaved using a 1:1 mixture of TFA/ CH_2Cl_2 to yield decently clean compound **2-184** after 12 steps from resin **2-174**.

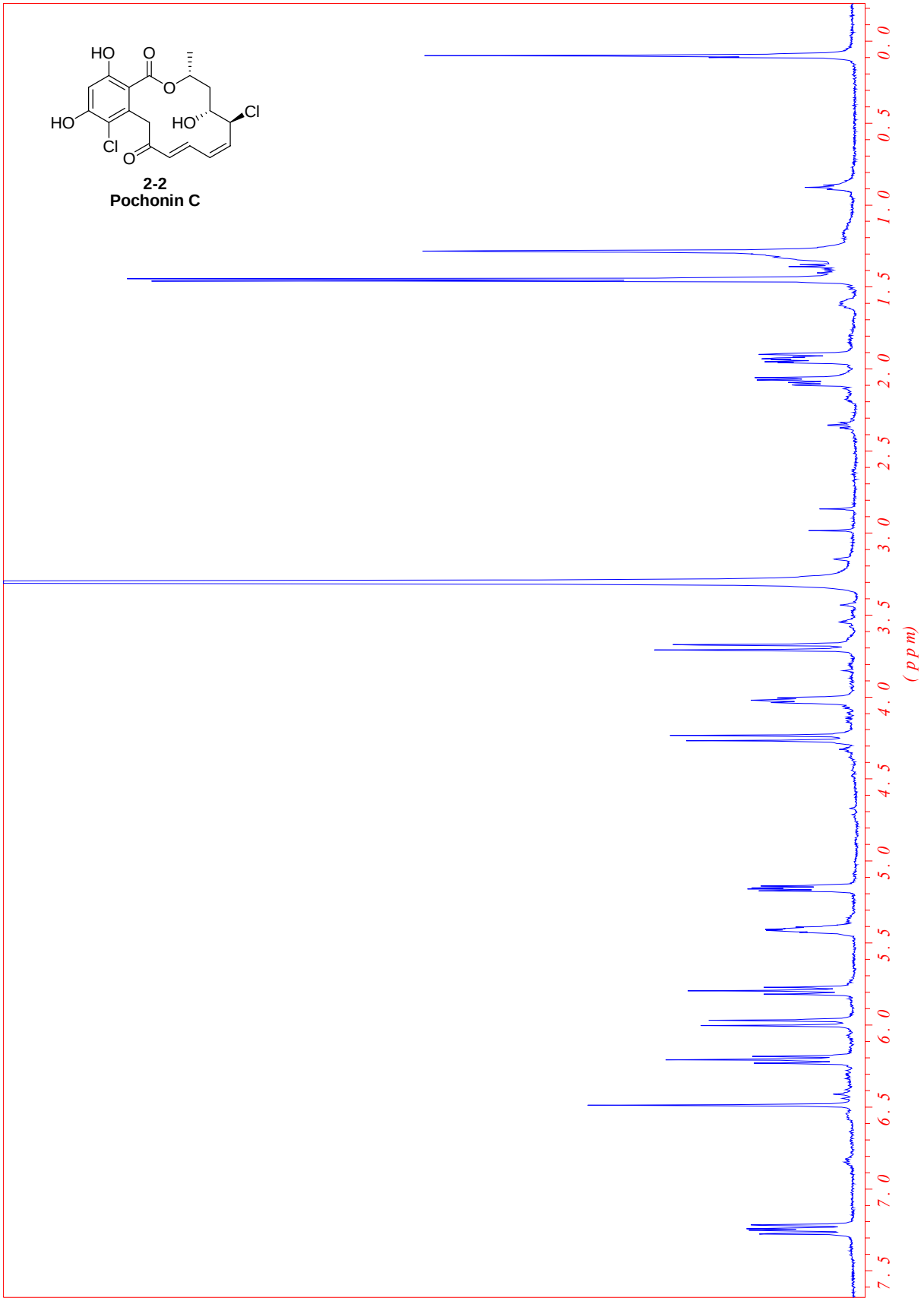
Annexes

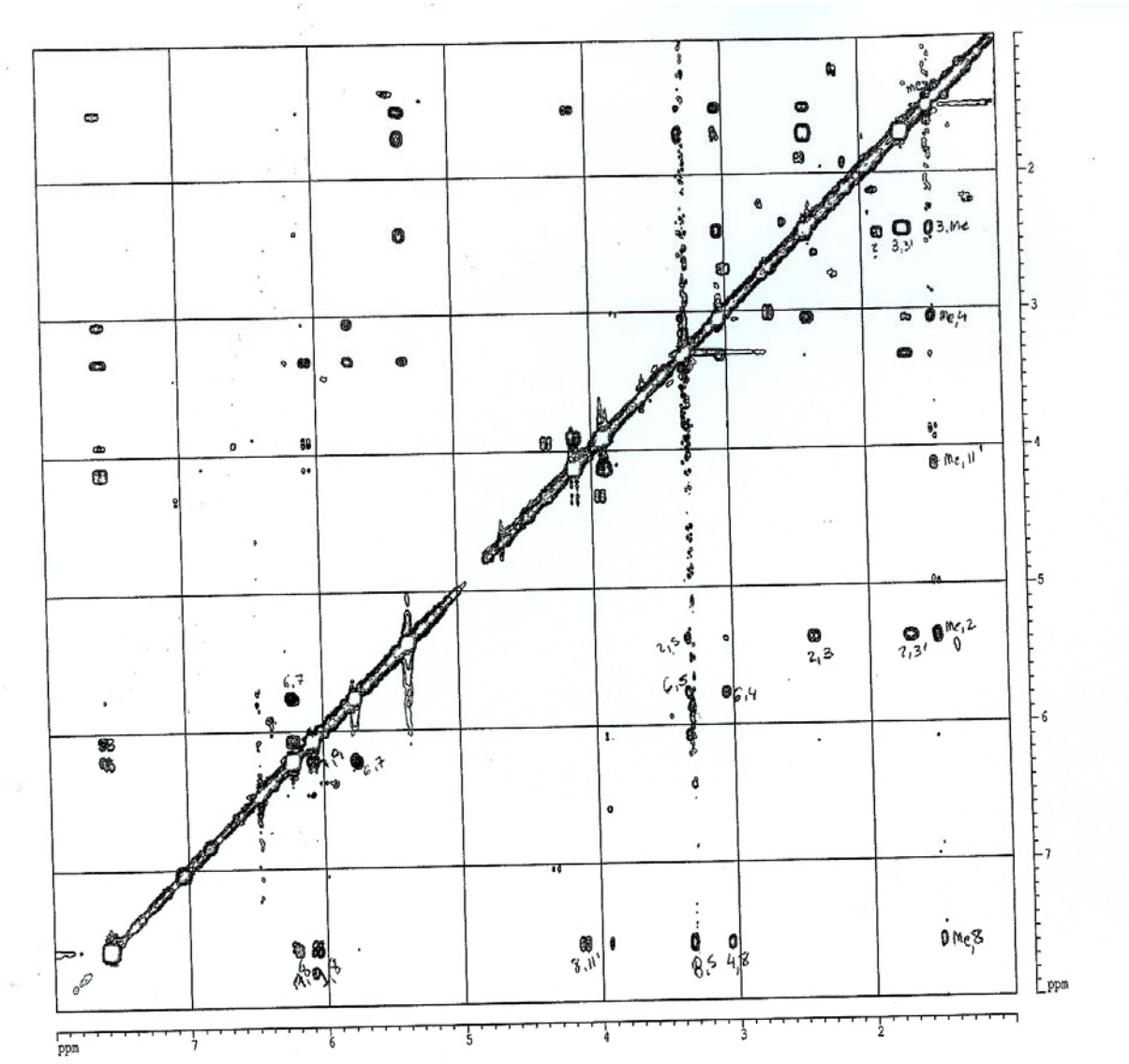

HO
O
O
O
HO
Cl
O
2-1
Radicicol
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
(ppm)

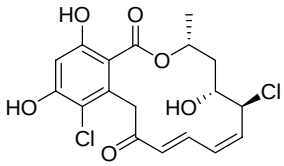




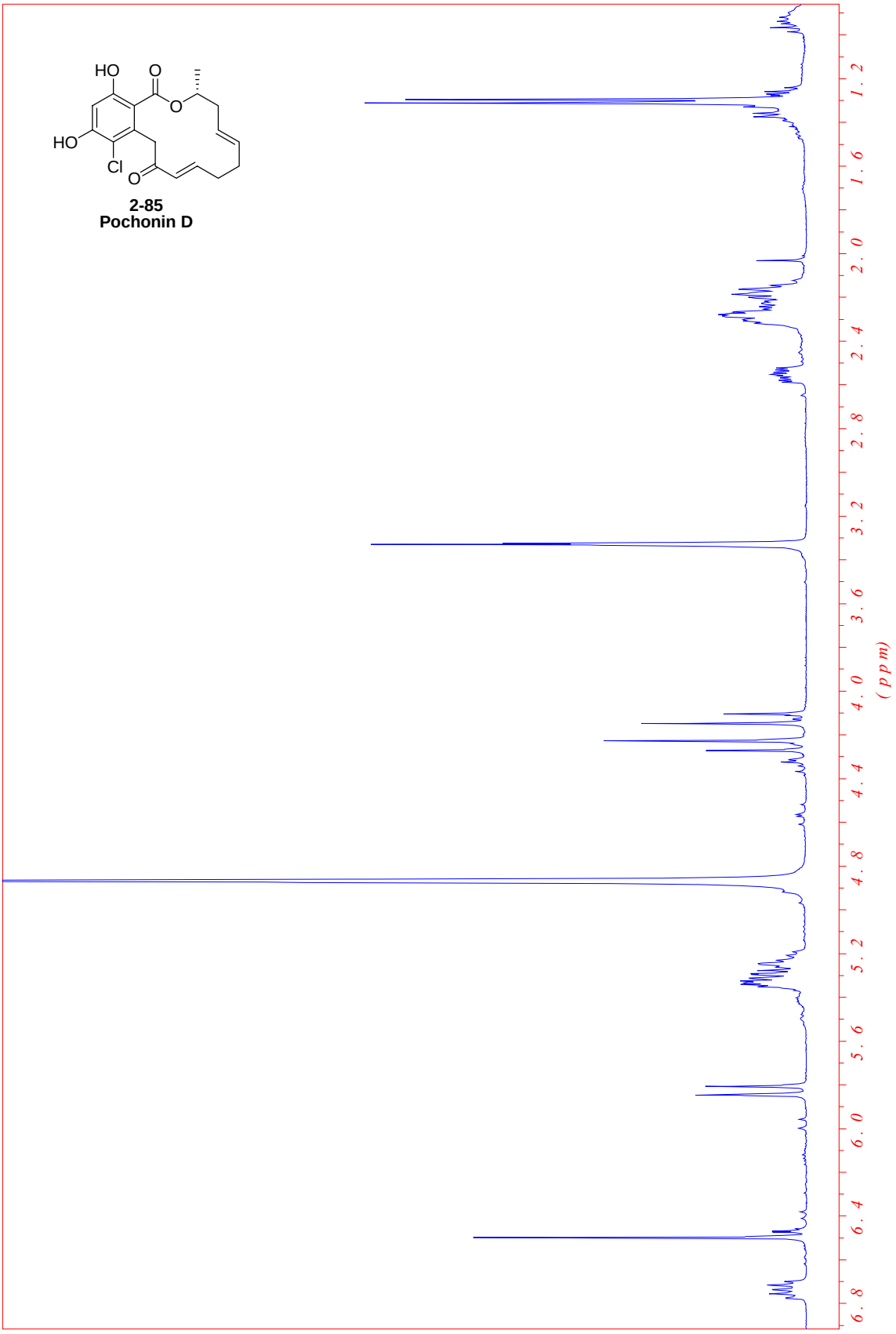
2-1
Radicicol

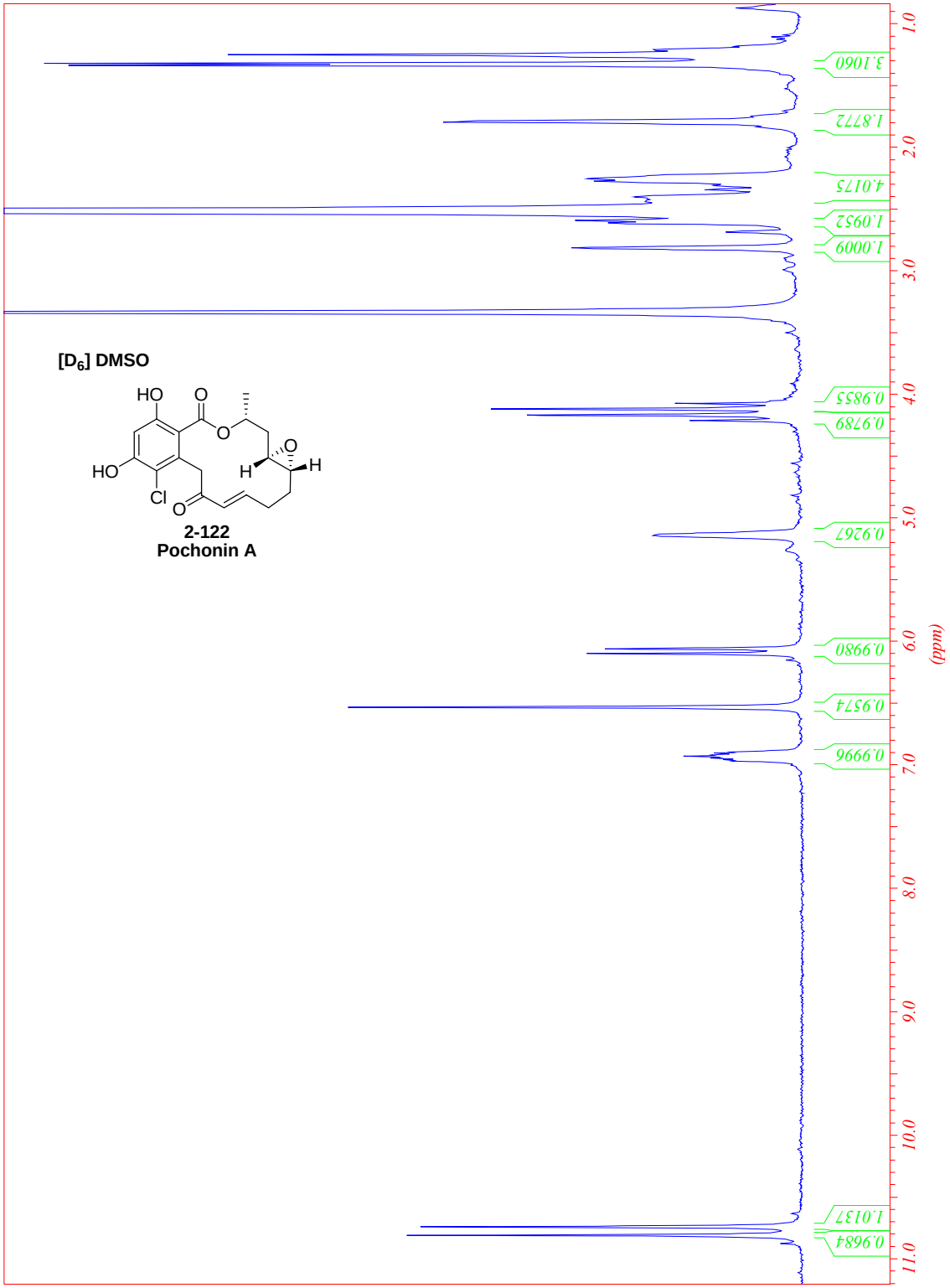

HO
O
O
HO
HO
Cl
Cl
O
2-2
Pochonin C
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
(ppm)

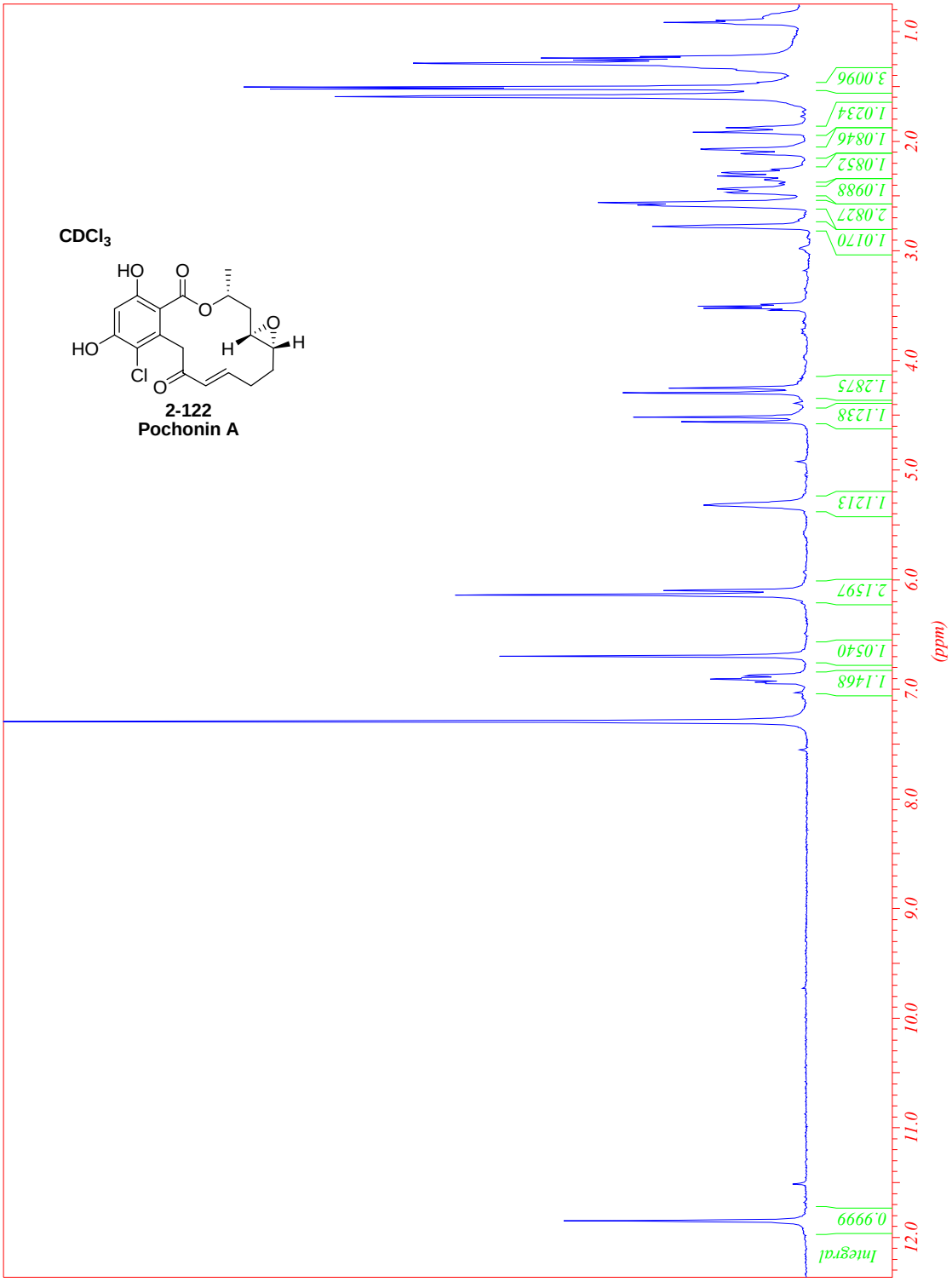


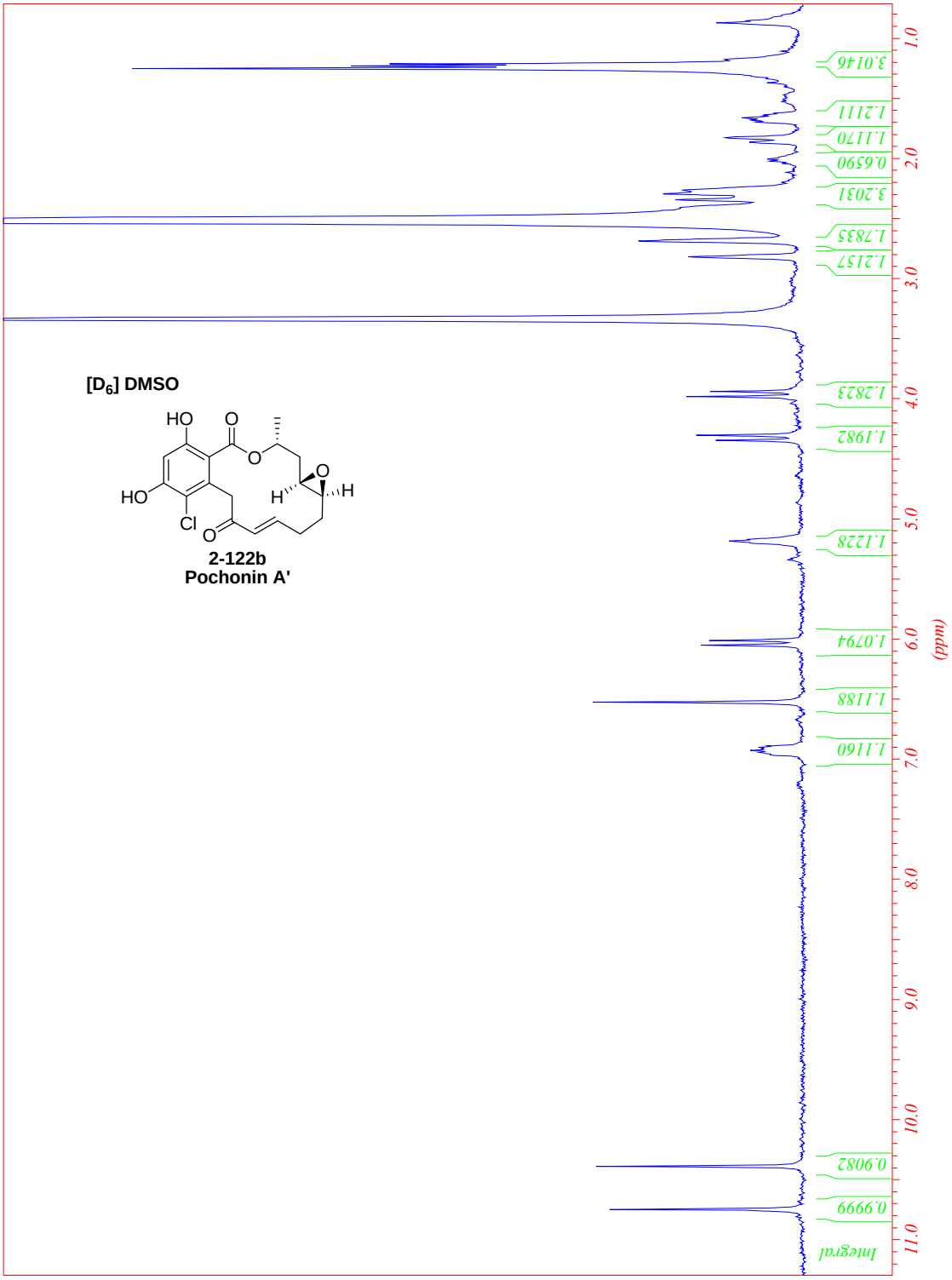


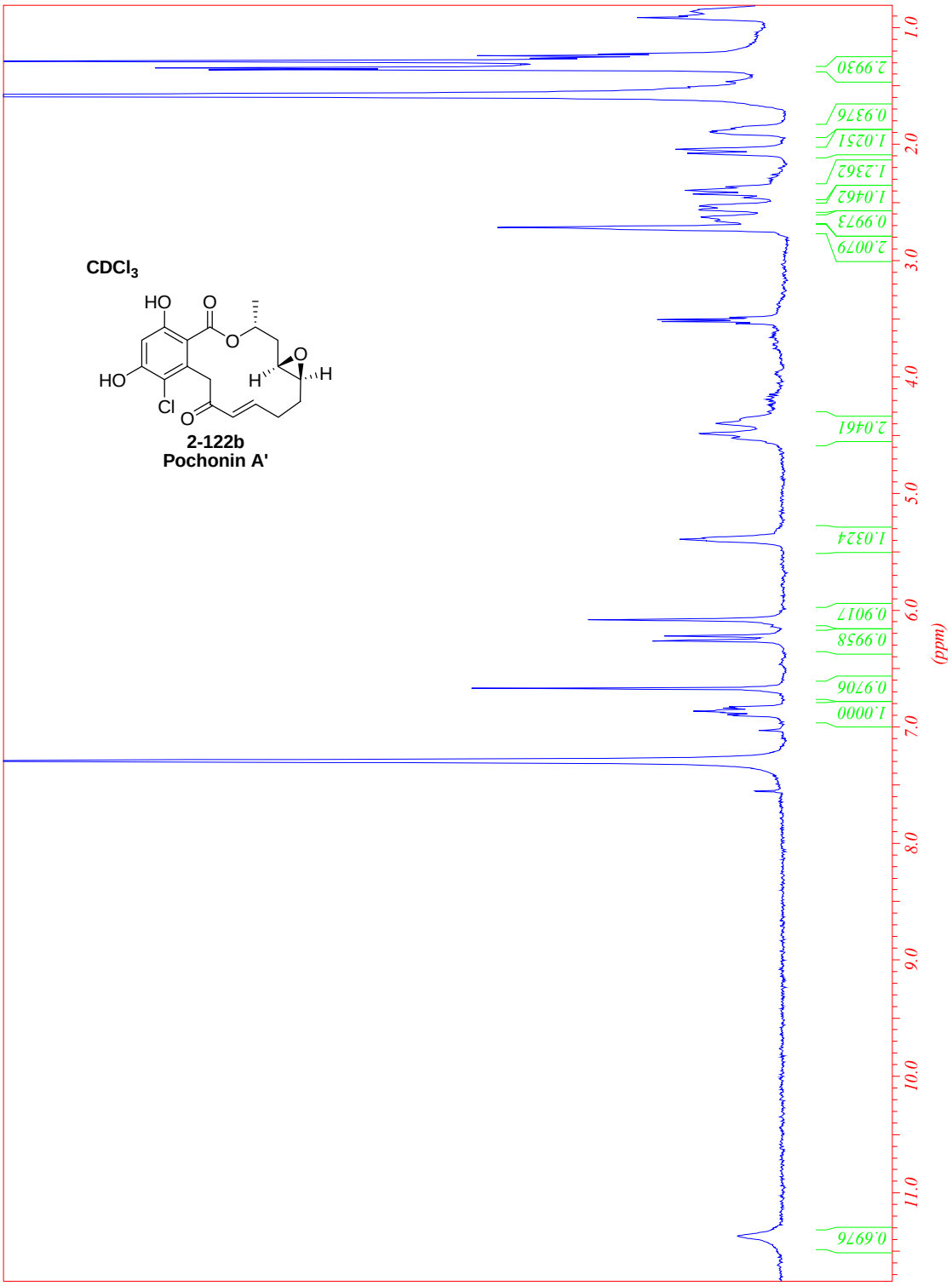
2-2
Pochonin C

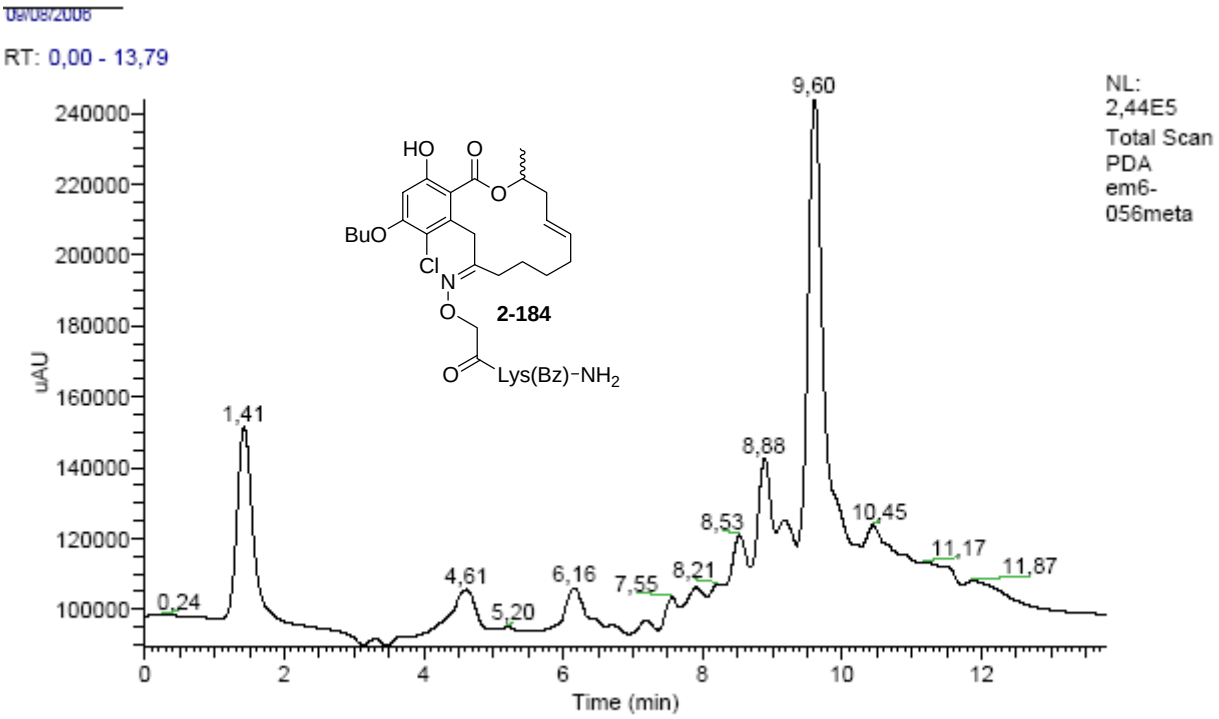

HO
O
O
HO
Cl
O
2-85
Pochonin D
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
(ppm)

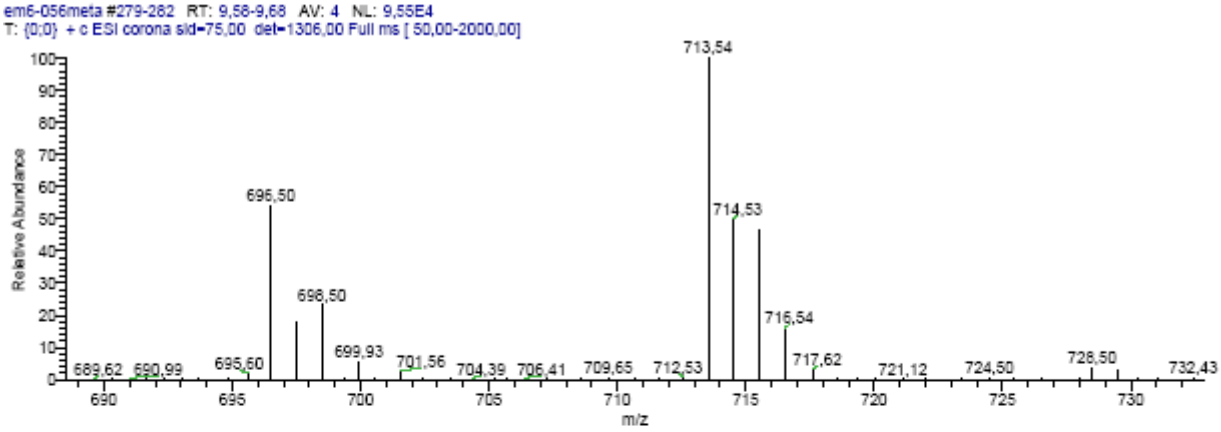

[D6] DMSO
HO
O
O
HO
O
H
H
Cl
O
2-122
Pochonin A
3.1060
1.8772
4.0175
1.0952
1.0009
0.9855
0.9789
0.9267
0.9980
0.9574
0.9996
1.0137
0.9684
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
(ppm)

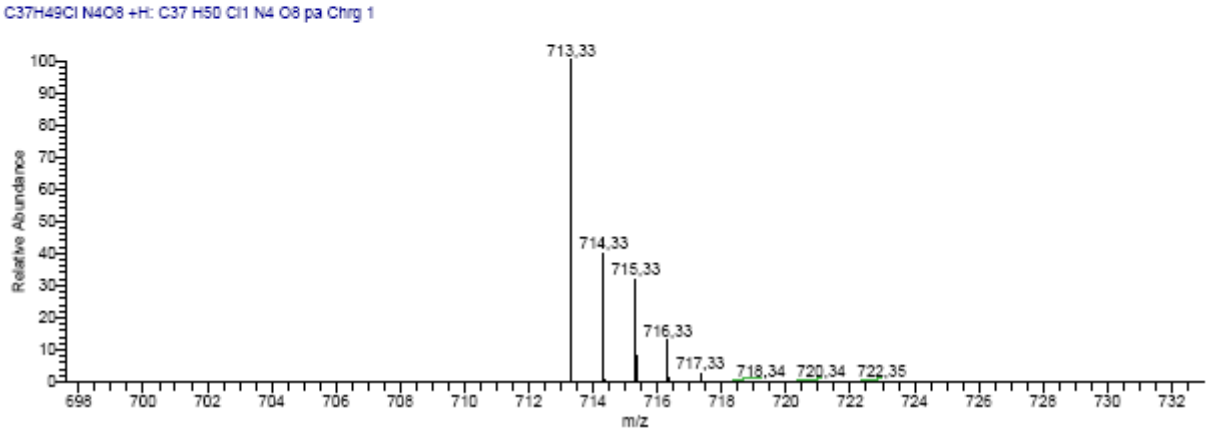

CDCl3
HO
O
O
HO
O
H
H
Cl
O
2-122
Pochonin A
3.0096
1.0234
1.0846
1.0852
1.0988
2.0827
1.0170
1.2875
1.1238
1.1213
2.1597
1.0540
1.1468
0.9999
Integral
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
(ppm)


[D6] DMSO
HO
O
O
HO
H
O
H
Cl
O
2-122b
Pochonin A'
3.0146
1.2111
1.1170
0.6590
3.2031
1.7835
1.2157
1.2823
1.1982
1.1228
1.0794
1.1188
1.1160
0.9082
0.9999
Integral
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
(ppm)


CDCl3
HO
O
O
HO
O
H
H
Cl
O
2-122b
Pochonin A'
2.9930
0.9376
1.0251
1.2362
1.0462
0.9973
2.0079
2.0461
1.0324
0.9017
0.9958
0.9706
1.0000
0.6976
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
(ppm)

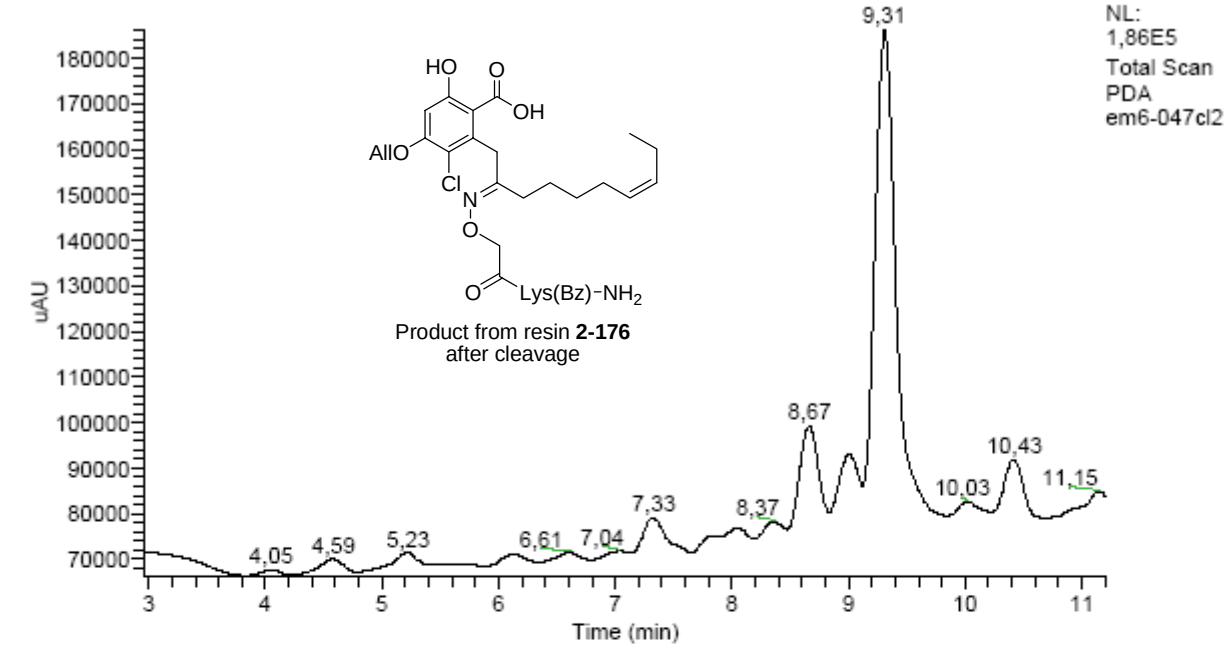

06/08/2006
RT: 0,00 - 13,79
NL:
2,44E5
Total Scan
PDA
em6-
056meta
uAU
Time (min)
HO
O
O
BuO
Cl
N
O
O
Lys(Bz)-NH2
2-184
0,24
1,41
4,61
5,20
6,16
7,55
8,21
8,53
8,88
9,60
10,45
11,17
11,87


em6-056meta #279-282 RT: 9,58-9,68 AV: 4 NL: 9,55E4
T: {0;0} + c ESI corona sid=75,00 det=1306,00 Full ms [50,00-2000,00]
Relative Abundance
m/z
713,54
696,50
714,53
698,50
716,54
699,93
701,56
717,62
689,62
690,99
695,60
704,39
706,41
709,65
712,53
721,12
724,50
728,50
732,43


C37H49Cl N4O8 +H: C37 H50 Cl1 N4 O8 pa Chrg 1
Relative Abundance
m/z
713,33
714,33
715,33
716,33
717,33
718,34
720,34
722,35

C:\Documents and Settings\...\em6-047cl2 05/08/2006 17:34:12

RT: 2,97 - 11,21



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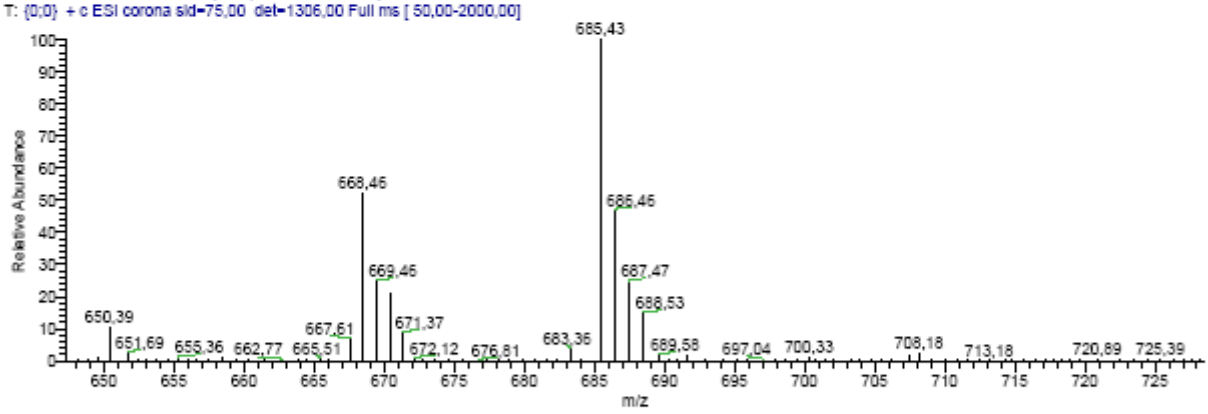
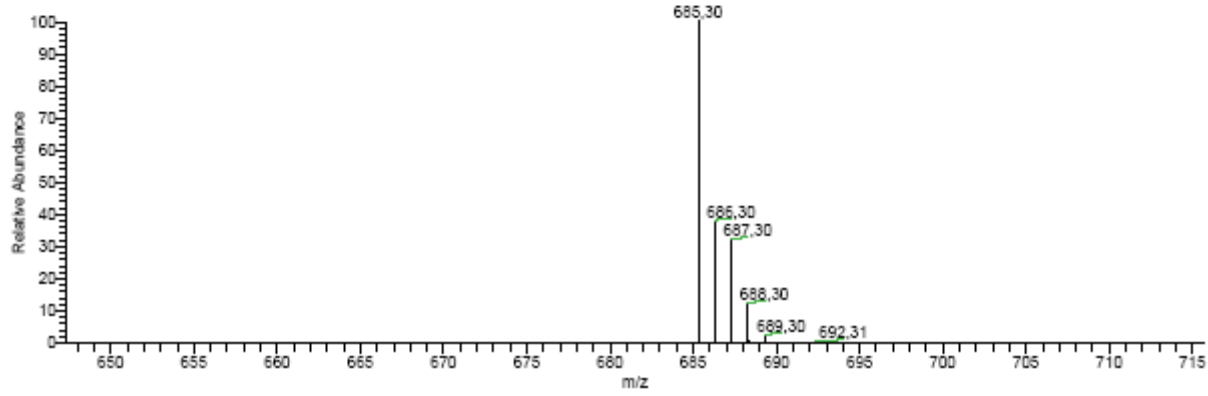


C35H45ClN4O8 +H: C35 H46 Cl1 N4 O8 pa Chrg 1



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Personnal Datas

Date of Birth: December 26, 1979
Nationality: French

Education

Oct. 2003 – Present: **Ph.D. in Organic Chemistry**
ISIS - Laboratoire de Chimie Organique et Bioorganique
Supervisors: Pr. N. Winssinger
Research project: Synthesis of resorcyclic macrolides, including radicicol, pochonin A, C, D, and libraries of those molecules. Evaluation of all those products in biological assays for HSP90 inhibition and more generally, for kinases inhibition

Oct. 2002 – July 2003: **D.E.A. (M.Sc.) in Organic Chemistry**
Université Pierre et Marie Curie (Paris VI)
Supervisors: Pr. J.-P. Genêt, Dr. A. Marinetti
Research Project: Synthesis of unsymmetric atropoisomeric ligands, application in asymmetric hydrogenation

Sept. 1999 – Sept. 2002: **Engineering studies in Chemistry** at both the E.N.S.C.T. (Ecole Nationale Supérieure de Chimie de Toulouse) and the E.N.S.C.P. (Ecole Nationale Supérieure de Chimie de Paris)

Work Experience

Feb. 2002 – July 2002: **Engineering Training** (Chemical Development Team)
Aventis Pharma, Vitry sur Seine, France
Team Manager: Dr S. Mutti, *Supervisor*: J. Malpart
Project: Synthesis of an active pharmaceutical compound, perfecting the most feasible synthetic route

June 2001 – July 2001: **Engineering Training**
I.N.S.E.R.M., Clermont-Ferrand, France
*Supervisor*s: Pr. J.-C. Madelmont, Dr. B. Bouchon
Project: Peptides and proteins studies by mass spectroscopy

Publications

Barluenga, S.; Lopez, P.; Moulin, E.; Winssinger, N., ***Modular Asymmetric Synthesis of Pochonin C*** *Angew. Chem.* **2004**, *116*, 3549-3552; *Angew. Chem. Int. Ed.* **2004**, *43*, 3467-3470.

Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N., ***Design, Synthesis and Biological Evaluation of HSP90 Inhibitors Based on Conformational Analysis of Radicicol***
J. Am. Chem. Soc. **2005**, *127*, 6999-7004.

Barluenga, S.; Moulin, E.; Lopez, P.; Winssinger, N., ***Solution and Solid-Phase Synthesis of Radicicol (Monorden) and Pochonin C*** *Chem., Eur. J.* **2005**, *11*, 4935-4952.

Moulin, E.; Barluenga, S.; Winssinger, N., ***Concise Synthesis of Pochonin A, an HSP90 inhibitor*** *Org. Lett.* **2005**, *7*, 5637-5639.

Moulin, E.; Barluenga, S.; Totzke, F.; Winssinger, N., ***Diversity-Oriented Synthesis of Pochonins and biological evaluation against a panel of kinases*** *Chem., Eur. J.* **2006**, *12*, 8819-8834.

Posters

Moulin, E.; Barluenga, S.; Winssinger, N.; Zoete, V.; Karplus, M.; ***Design, Synthesis and Biological Evaluation of Pochonin D Analogs,*** Sixth Tetrahedron Symposium, Bordeaux, 2005

Moulin, E.; Barluenga, S.; Totzke, F.; Winssinger, N., ***Diversity-Oriented Synthesis of Pochonins and biological evaluation against a panel of kinases,*** First European Chemistry Congress, Budapest, 2006

Oral Communication

Moulin, E., ***Synthèse de composés résorcycliques inhibiteurs de la protéine HSP90***
Midi des Jeunes Chercheurs, Strasbourg, France, January 24, **2006**

Moulin, E., ***Synthèse de composés résorcycliques inhibiteurs de la protéine HSP90***
Journée de communication des doctorants – SFC Section Alsace, Strasbourg, France, May 4, **2006**

Languages

French: mother tongue
English: Fluent, Scored 583 on TOEFL test in January 2001, 860 on TOEIC test in November 2001 (English spoken in the lab)
German and Spanish: beginner

References

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