UNIVERSITE LOUIS PASTEUR
UFR DES SCIENCES DE LA VIE ET DE LA SANTE

THESE

Présentée en vue de l'obtention du titre de

DOCTEUR DE L'UNIVERSITE LOUIS PASTEUR DE STRASBOURG

Spécialité : Chimie et Immunologie Thérapeutiques

Par

Julien MARIN

Utilisation de *δ*-lactames monohydroxylés énantiopurs comme précurseurs d'analogues glycosylés des 4- et 5-hydroxylysines. Application à la synthèse de glycopeptides dérivés du collagène de type II.

Soutenue publiquement le 21 Novembre 2003 devant la commission d'examen :

Pr. Dr. William D. Lubell	**Rapporteur externe**
Pr. Dr. Jean-Charles Quirion	**Rapporteur externe**
Pr. Dr. Maurice Goeldner	**Rapporteur interne**
Pr. Dr. Horst Kunz	**Examinateur**
Dr. Catherine Fournier	**Examinateur**
Dr. Gilles Guichard	**Directeur de thèse**

This research work was realised in the *laboratoire d'Immunologie et Chimie Thérapeutique (IBMC, Strasbourg)* under the supervision of **Dr. G. Guichard** and was financed with a grant (Bourse de Docteur Ingénieur) from *NEOSYSTEM* and the *CNRS*.

I am deeply indebted to **Dr. S. Muller** and **Dr. J.-P. Briand** for having me welcomed in the Unit. I would also like to express my gratitude to **Dr. S. Plaué** (C.E.O. of *NEOSYSTEM*) for the financial support given in partnership with the *CNRS*.

I am also grateful to **Pr. W. D. Lubell** (*Université de Montréal, Canada*), **Pr. J.-C. Quirion** (*Université de Rouen*), **Pr. M. Goeldner** (*Université Louis-Pasteur*), **Pr. H. Kunz** (*Universität Mainz, Deutschland*) and **Dr. C. Fournier** (*Hôpital Cochin, Paris*) for having accepted to judge my research work.

My very special thanks go to **Dr. G. Guichard** whose infectious passion for research in organic and medicinal chemistry made my work interesting and exciting. Working with **Dr. G. Guichard** was undoubtedly a unique and extremely enriching experience. Additionally, **Dr. G. Guichard** has the gift to see new synthetic routes when others would have only seen undesired side-reactions.

I also would like to express my gratitude to **Dr. A. Aubry** & **Dr. C. Didierjean** (*LCM3B, Nancy*) who transformed the crystals into beautiful 3D structures, **Dr. C. Fournier** who initiate the immunological studies of this project and **Dr. R. Graff** & **Dr. J.-D. Sauer** (*Department of NMR, Strasbourg*) who are accomplishing everyday a fantastic job for all the chemists of the university.

A special thank goes to all my dear colleagues from *ICT* and more specifically my lab-mates (**Weimin** and **Davide**) and the two students who worked with me on this project during the course of their DEA, namely **Aude** and **Nathalie**.

Finally, as you turn this page of three years of research, I would like to dedicate this manuscript to my wife **Fériel** who shared with me the successes (and the deceptions) of research.

Merci …,

Abbreviations :

$[\alpha]_D$	optical rotation
μL	microliter
Ac	acetyl
AcOEt	ethyl acetate
AcOH	acetic acid
All	allyl
APC	antigen presenting cell
APL	altered peptide ligand
bCII	bovine type II collagen
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP	benzotriazol-1-yl-oxy-tris(dimethylamino)hexafluorophosphate
cCII	chick type II collagen
CII	type II collagen
CFA	complete Freund adjuvant
CIA	type II collagen induced arthritis
CSO	10-(camphorsulfonyl)oxaziridine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide

de	diastereomeric excess
Dhn	5,6-dihydroxynorleucine
DHQ	dihydroquinine
DHQD	dihydroquinidine
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIC	1,3-diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMARD	disease-modifying anti-rheumatic drug
DMF	*N*,*N*-dimethylformamide
DPD	dibenzylperoxydicarbonate
DTT	dithiotreitol
EDC	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide
ee	enantiomeric excess
Et	ethyl
EtOH	ethanol
Fmoc	9*H*-fluoren-9-ylcarbonyl
FmocOSu	*N*-(9*H*-fluoren-9-ylcarbonyloxy)succinimide
g	gram
Gal	galactose
Glc	glucose
h	hour
hCII	human type II collagen
Hnl	6-hydroxynorleucine
Hnv	5-hydroxynorvaline
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
Hyl	(2*S*,5*R*)-5-hydroxylysine
Hz	herz
iBu	isobutyl
IFA	incomplete Freund adjuvant
IgG	immunoglobulin G

IL-1	interleukin-1
iPr	isopropyl
J	coupling constant
KHMDS	potassium hexamethyldisilazide
LiHMDS	lithium hexamethyldisilazide
M	mole / liter
MALDI-TOF	matrix-assisted laser desorption ionization – time of flight
mCII	mouse type II collagen
mCPBA	3-chloroperoxybenzoic acid
Me	methyl
MeOH	methanol
MHC	major histocompatibility complex
min	minute
mL	milliliter
mmol	millimole
MoOPH	MoO_5•pyridine•HMPA
Mpm	4-methoxybenzyl
MS	molecular sieve
Ms	methanesulfonyl
MTT	*O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxine
NaHMDS	sodium hexamethyldisilazide
NIS	*N*-iodosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
p	*para*
PNB	*para*-nitrobenzylcarbonyl
Ph	phenyl
PHAL	phtalazine
Piv	pivaloyl
PPO	trans-2-(phenylsulfonyl)-3-phenyloxaziridine
PTSA	*para*-toluenesulfonic acid
RA	rheumatoid arthritis
RP-HPLC	reverse phase - high performance liquid chromatography
RT	room temperature

SPPS	solid phase peptide synthesis
TBAF	tetrabutylammonium fluoride
TBDMS	*tertio*-butyldimethylsilyl
TBDPS	*tertio*-butyldiphenylsilyl
^{t}Bu	*tertio*-butyl
TCR	T-cell receptor
tert	*tertio*
TESOTf	triethylsilyl trifluoromethanesulfonate
TFA	trifluoroacetic acid
TFMSA	trifluoromethanesulfonic acid
Th-cell	helper T-cell
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TNF	tumor necrosis factor
t_R	retention time
Z	benzyloxycarbonyl

Résumé :

Introduction

La polyarthrite rhumatoïde (PR) est l'une des maladies auto-immunes systémiques les plus fréquentes avec une prévalence d'environ 0.8%.[1] Elle se caractérise essentiellement par l'inflammation chronique des articulations qui peut mener à long terme à des déformations gravement invalidantes.

La prédilection de l'attaque inflammatoire pour les articulations et la présence de taux élevés d'auto-anticorps anti-collagène de type II dans le sérum et les articulations de patients atteints de PR indique un rôle du collagène de type II (CII) comme source possible de peptide(s) antigénique(s). Par ailleurs, la forte association entre la susceptibilité à la PR et l'expression de certains gènes montre le rôle du complexe majeur d'histocompatibilité de classe II (CMH II) comme récepteur de ce(s) peptide(s). Enfin, les infiltrats massifs de cellules T $CD4^+$ dans les articulations de patients atteints de PR suggère un rôle central des cellules T. Ces trois éléments joueraient donc un rôle crucial dans la PR sous forme d'une interaction ternaire **« cellule T $CD4^+$ / peptide dérivé de CII / molécule de CMH »**.

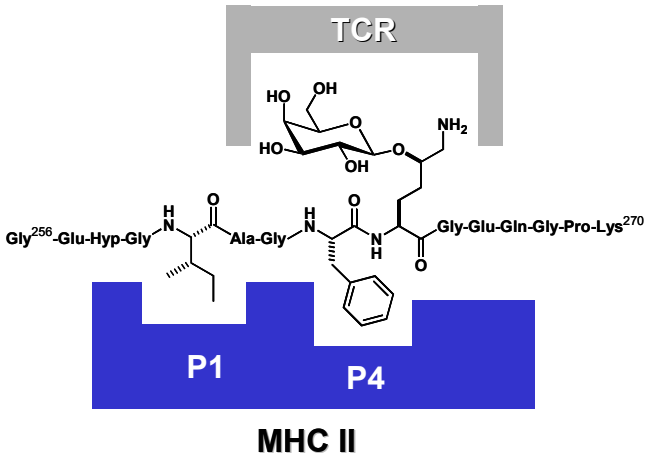
Il existe un certain nombre de modèles animaux de PR, le plus utilisé étant l'arthrite expérimentale au collagène (AEC). Dans ce modèle, plusieurs groupes ont mis en évidence la présence d'un épitope T immunodominant ayant comme séquence minimum CII(260-267). De la même manière, les résultats obtenus plus récemment dans des modèles de souris « humanisées » montrent que la séquence comprenant les résidus 263-270 correspond à la séquence minimale immunodominante présentée par des molécules CMH humaines.

L'une des caractéristiques importantes du CII réside dans les différentes modifications post-traductionelles qui génèrent à partir d'une même séquence une multitude de variants possibles. Dans le cas de l'épitope CII(256-270), les possibilités d'hydroxylation des résidus proline ou lysine suivie de la glycosylation des résidus hydroxylysine sous forme de β-D-galactopyranosyle ou α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyle génère un groupe de 64 peptides ou glycopeptides naturels différents.

[1] statistics from the WHO Statistical Information System (WHOSIS), http://www3.who.int/whosis/menu.cfm

En utilisant la (2*S*,5*R*)-5-hydroxylysine disponible commercialement,[2] le groupe de recherche dirigé par le Pr. *J. Kihlberg* (Université de Umea, Suède) a préparé un certain nombre de ces glycopeptides. Leur collaboration avec l'équipe du Pr. *R. Holmdahl* (Université de Lund, Suède) a permis (i) d'identifier les deux points d'ancrage principaux du peptide CII(256-270) au CMH II permettant la présentation au récepteur T et (ii) de mettre en évidence l'importance de l'hydroxylation et de la galactosylation en position 264 sur la pathogénicité et l'immunogénicité de l'épitope CII(256-270). Ainsi, ils ont pu élaborer un modèle de l'interaction ternaire mise en jeu (Figure A).[3,4]

Figure A. Description schématique de l'interaction ternaire



Ils ont également étudié l'impact de modifications de la partie saccharidique des glycopeptides sur la spécificité des cellules T.[5,6] Ces différents travaux ont permis de montrer que l'amine primaire en position ε et le groupement HO-4 de la partie saccharidique du glycopeptide sont les éléments importants pour la reconnaissance de l'épitope immunodominant.

[2] (5*R*)-5-Hydroxy-L-lysine dihydrochloride monohydrate: Fluka 55501, € 169.50 pour 1 g.

[3] Kjellén, P.; Brunsberg, U.; Broddefalk, J.; Hansen, B.; Vestberg, M.; Ivarsson, I.; Engström, A.; Svejgaard , A.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 755.

[4] Corthay, A.; Bäcklund, J.; Broddefalk, J.; Michaëlsson, E.; Goldschmidt, T. J.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 2580.

[5] Holm, B.; Bäcklund, J.; Recio, M. A. F.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2002**, *3*, 1209.

[6] Holm, B.; Baquer, S. M.; Holm, L.; Holmdahl, R.; Kihlberg, J. *Bioorg. Med. Chem.* **2003**, *11*, 3981.

Objectifs et stratégie

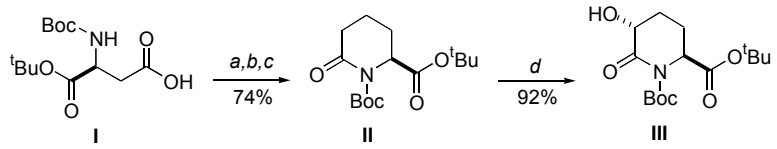
Au cours de ce travail de thèse, nous nous sommes proposés de déterminer la spécificité fines des cellules T auto-réactives en effectuant des modifications au niveau de la chaîne latérale de la 5-hydroxylysine. De cette manière, les fonctions indispensables à la reconnaissance de l'épitope, ou au contraire, celles sans aucune influence peuvent être identifiées précisément. En couplant nos résultats à ceux obtenus par le groupe de *Kihlberg*, nous pourrons alors établir une « carte » précise des caractéristiques de l'interaction ternaire.

Pour ce faire, nous avons développé deux nouvelles stratégies de synthèse asymétrique de la 4- et la 5-hydroxylysine à partir d'un précurseur commun issu du pool chiral.

Préparation de dérivés glycosylés d'hydroxylysine

Notre première stratégie, nous a permis de préparer le *δ*-lactame **III** par une réaction d'oxydation stéréosélective avec un rendement global de 68% à partir de l'acide aspartique **I** (Schéma A).

Schéma A. Preparation du *δ*-lactame *α*-hydroxylé III



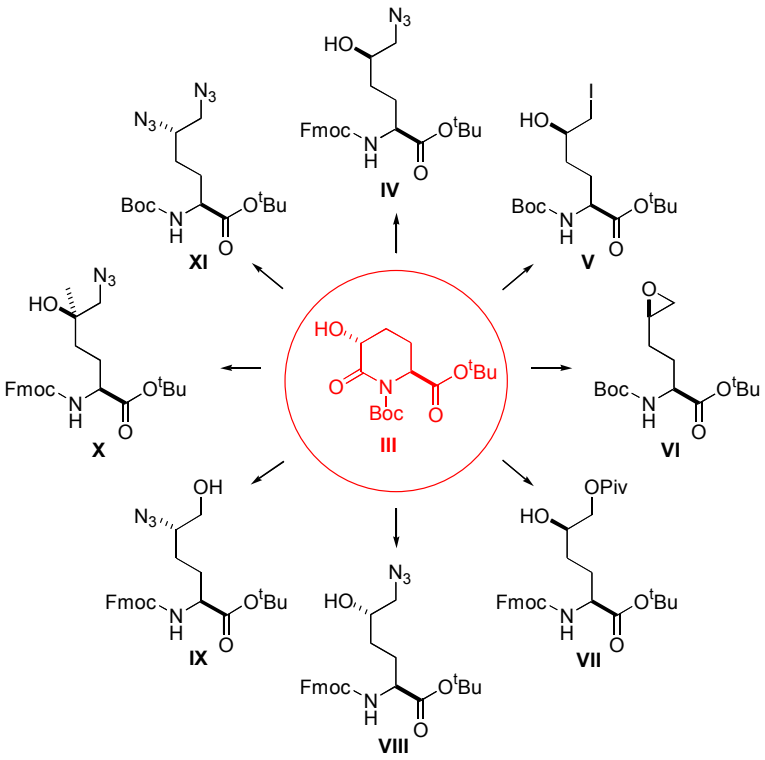
I II III

(a) acide de *Meldrum*, EDC, DMAP, CH_2Cl_2; (b) $NaBH_4$, CH_2Cl_2/AcOH (10:1); (c) toluene, reflux; (d) LiHMDS, (+)-CSO, THF, -78°C.

Le *δ*-lactame **III** est une molécule clé puisqu'elle permet la préparation des dérivés naturels et non-naturels de la 5-hydroxylysine (présentés dans la Figure B) nécessaires à notre étude (composés **IV** et **VII**-**X**) ou intermédiaires de synthèse d'autres produits à intérêt biologique[7] (composés **V** et **VI**).

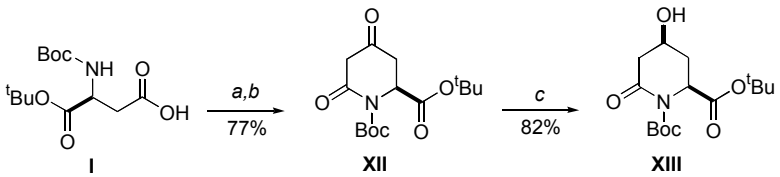
[7] (a) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron* **1999**, *55*, 63. (b) Waelchli, R.; Beerli, C.; Meigel, H.; Révész, L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2831.

Figure B. Analogues de la 5-hydroxylysine



La voie de synthèse dédiée à la formation d'analogues de la 4-hydroxylysine a également été développée à partir de l'acide aspartique **I**. Cette fois, le composé clé **XIII** est obtenu par réduction stéréosélective avec un rendement global de 63% (Schéma B).

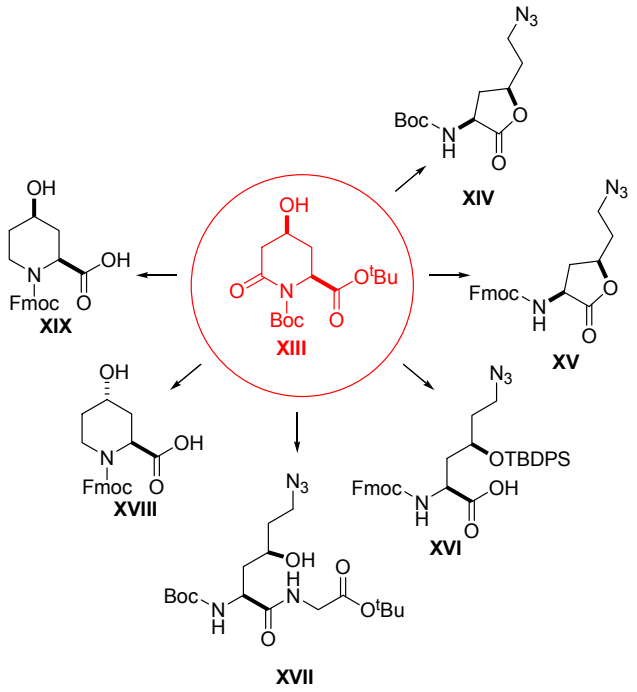
Schéma B. Préparation du *δ*-lactame *β*-hydroxylé XIII



(a) acide de *Meldrum*, EDC, DMAP, CH_2Cl_2; (b) ethyl acetate, reflux; (c) $NaBH_4$, CH_2Cl_2/AcOH (10:1).

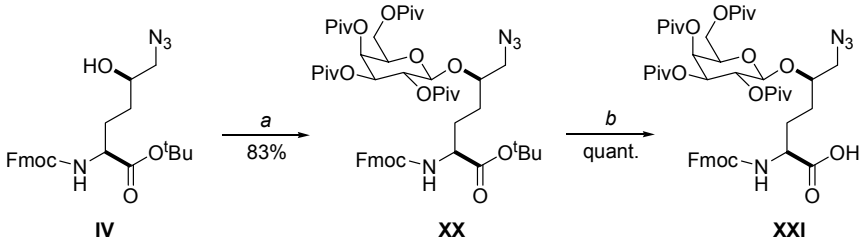
Ce *δ*-lactame *β*-hydroxylé est un précurseur de dérivés de la 4-hydroxylysine **XVI** mais également des 4-hydroxy pipécolates **XVIII** et **XIX**, produits à fort intérêt pharmacologique. De plus, cette voie de synthèse nous a permis d'obtenir les lactones **XIV** et **XV** en peu d'étapes ; **XIV** a été utilisé comme précurseur du dipeptide **XVII**.

Figure C. Analogues de la 4-hydroxylysine



Pour effectuer la glycosylation de ces dérivés, nous avons développé une stratégie générale mise au point à partir du dérivé de la (2*S*,5*R*)-5-hydroxylysine « naturelle » **IV**. Les meilleures conditions donnent la (2*S*,5*R*)-5-hydroxylysine galactosylée **XXI**, prête à l'utilisation en synthèse peptidique sur support solide (SPPS), avec un rendement de 83% (Schéma C).

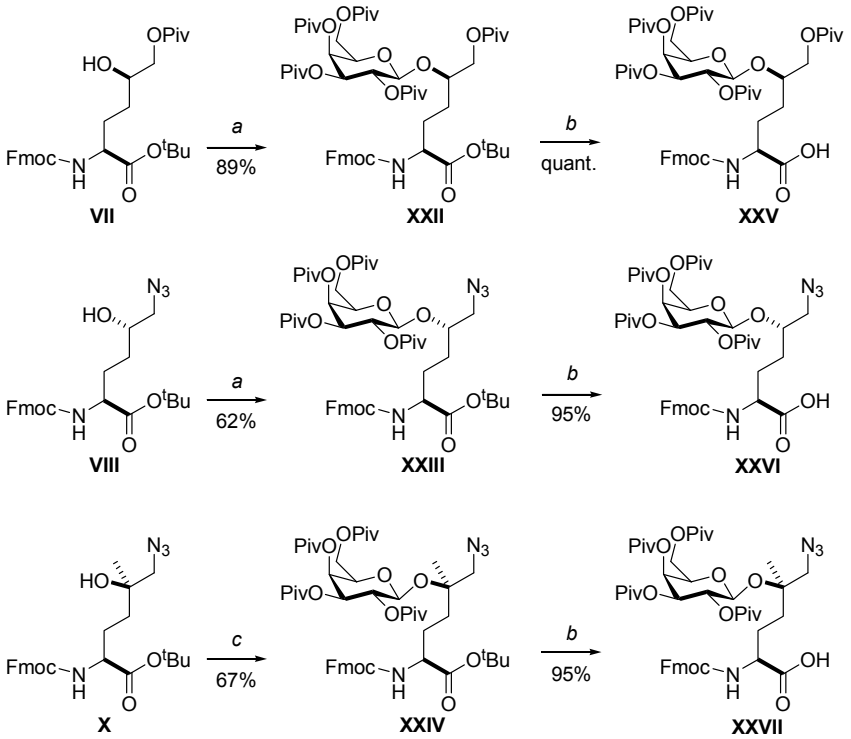
Schéma C. Galactosylation de la (2*S*,5*R*)-5-hydroxylysine



(a) $Gal(Piv)_4Br$, $AgSiO_4$, tamis moléculaire 4Å, CH_2Cl_2 ; (b) CH_2Cl_2/TFA (1:1).

De la même manière, nous avons préparé les dérivés galactosylés des analogues de la 5-hydroxylysine **XXV**-**XXVII** (Schéma D).

Schéma D. Préparation d'analogues de XXI

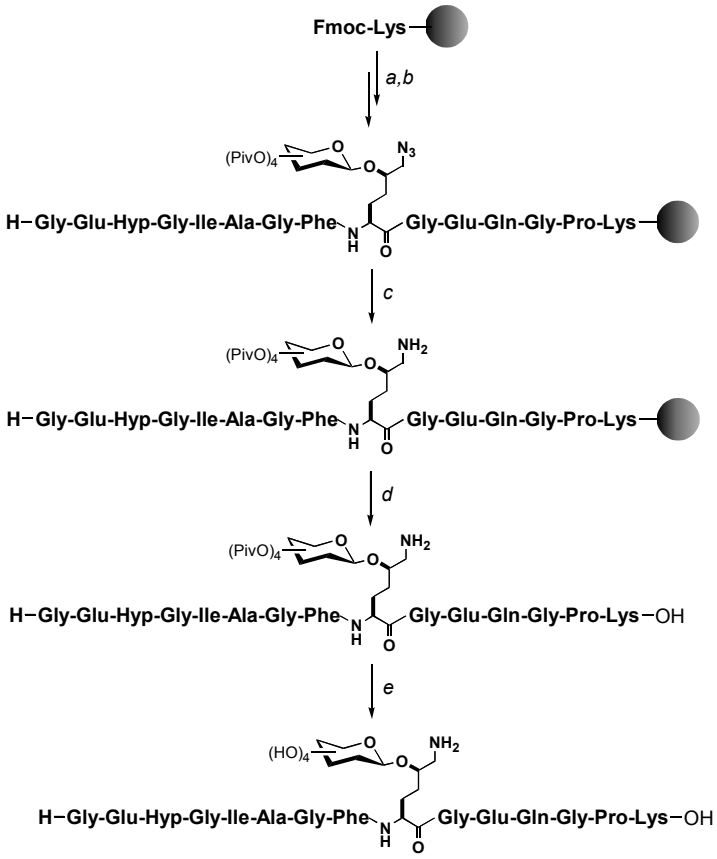


(a) $Gal(Piv)_4Br$, $AgSiO_4$, tamis moléculaire 4Å, CH_2Cl_2 ; (b) CH_2Cl_2/TFA (1:1).

Synthèse des glycopeptides et évaluation de leur reconnaissance par les cellules T auto-réactives

A partir de ces synthons, les glycopeptides dérivés de CII(256-270) ont été préparés par chimie peptidique classique (Figure D). A la fin de l'élongation de la chaîne peptidique, la réduction des fonctions azide, suivie de la coupure peptide / support solide et de la déprotection des fonctions hydroxyle de la partie glycosidique, nous a permis d'isoler les glycopeptides présentés en Figure E avec des rendements voisins de 55%.

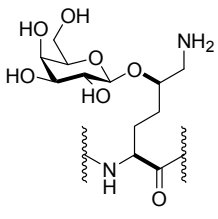
Figure D. Procédure générale de préparation des glycopeptides



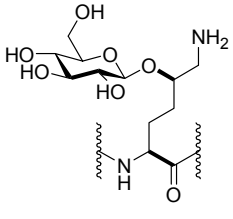
(a) 20% piperidine in DMF ; (b) Fmoc-Xaa-OH, BOP, HOBt, DIEA, DMF ; (c) PPh_3, THF/H_2O ; (d) TFA/H_2O/TIPS/DTT (8.8:0.5:0.2:0.5) ; (e) NaOMe, MeOH.

Figure E. Liste des glycopeptides préparés lors de cette étude

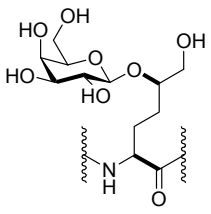
H-Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-**Xaa^{264}**-Gly-Glu-Gln-Gly-Pro-Lys^{270}-OH



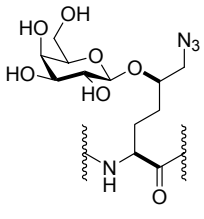
GalHyl



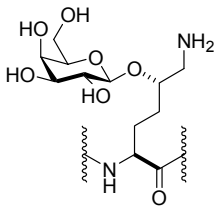
GlcHyl



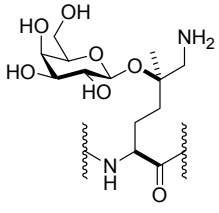
GalDhn



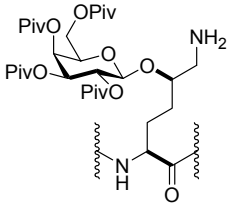
GalHyl(N_3)



Gal(5*S*)Hyl



GalHyl(Me)



Gal(Piv_4)Hyl

Peptides	Xaa^{264}
XXVIII	GalHyl
XXIX	GlcHyl
XXX	GalHyl(N_3)
XXXI	GalDhn
XXXII	Gal(5*S*)Hyl
XXXIII	GalHyl(Me)
XXXIV	Gal(Piv_4)Hyl

Les sept glycopeptides ont ensuite été purifiés par CLHP semi-préparative pour donner des puretés supérieures à 99% avant leur utilisation lors de tests biologiques.

L'évaluation de la reconnaissance de l'ensemble des glycopeptides synthétisés a été effectuée par l'équipe du Dr. *C. Fournier* (Hôpital Cochin, Paris) avec trois hybridomes (A2G10, A8E2 et A9E5) et un clone (A9.2) de cellules T spécifiques du collagène de type II. Les résultats obtenus nous ont permis de caractériser de manière plus précise le pharmacophore de l'épitope T immunodominant (**XXVIII**).

Les analogues modifiés en position ε de la chaîne latérale du résidu GalHyl^{264} (**XXX** et **XXXI**) ne sont pas du tout reconnus par les hybridomes et le clone testés. La fonction amine, vraisemblablement protonée (position ε de la chaîne latérale du résidu GalHyl^{264}), qui est indispensable à la reconnaissance des glycopeptides, est donc probablement engagée dans des interactions électrostatiques avec des résidus chargés négativement du récepteur des cellules T. L'interaction ternaire « complexe majeur d'histocompatibilité / épitope T / récepteur de cellule T » permet un certain degré de liberté au niveau du centre asymétrique C-5 (atome portant la partie saccharidique de la molécule). Les glycopeptides incorporant des analogues d'hydroxylysine modifiés en cette position clé (**XXXII** et **XXXIII**) sont toujours reconnus par les cellules T, mais à des concentrations supérieures.

Conclusion

Au cours de mon travail de thèse, nous avons développé de nouvelles voies de synthèse de dérivés d'hydroxylysine énantiopurs. Notre stratégie, à la fois rapide et versatile, nous a permis de préparer plusieurs analogues glycosylés d'hydroxylysine, qui ont été incorporés dans des glycopeptides dérivés de CII(256-270). L'évaluation de la reconnaissance de ces glycopeptides par des cellules T spécifiques de CII nous a permis de mieux définir la spécificité fine des cellules T auto-réactives impliquées dans l'arthrite expérimentale au collagène. Ces glycopeptides dont le pharmacophore a été légèrement modifié, communément appelés « peptide-ligands modifiés », pourraient également être utilisés à des fins thérapeutiques en induisant de la tolérance comme cela a déjà été démontré dans le cas d'autres maladies autoimmunes.

I. Glycopeptides & Autoimmunity in Rheumatoid Arthritis

Proper functioning of the immune system of higher vertebrates requires the processing of protein antigens by antigen presenting cells (APCs). The resulting peptides are bound by major histocompatibility complex (MHC) molecules, and then transported to the cell surface where they are displayed to T-cells. Recognition of the antigenic peptide-MHC complexes by receptors on circulating T-cells triggers responses that depend on the type of APC and T-cell involved. Recognition of peptide-class I MHC complexes by receptors on cytotoxic ($CD8^+$) T-cells triggers release of cytotoxic chemicals which selectively kill the diseased cell. Recognition of peptide-class II MHC complexes by helper ($CD4^+$) T-cells elicits T-cell activation and therefore release of immunomodulating cytokines that are essential for inducing production of high-affinity IgG antibodies in B-cells, as well as for production of memory B-cells and activation of phagocytes.

Endogenous proteins are processed by APCs in the same manner as « foreign » proteins. Large numbers of self-peptides are therefore presented by MHC molecules on the surface of APCs. Normally, T-cells stimulated by self antigens are eliminated by apoptosis during maturation in the thymus (negative selection) or inactivated in the periphery by tolerance mechanisms. However the tolerance to self peptides can be disrupted and thus in some autoimmune diseases, a pool of T-cells that recognize the self antigens, but which fail to be deleted or anergized, might exist. Rheumatoid Arthritis (RA) is believed to be such a disease.

I.1. Rheumatoid arthritis

I.1.1. The disease and its symptoms

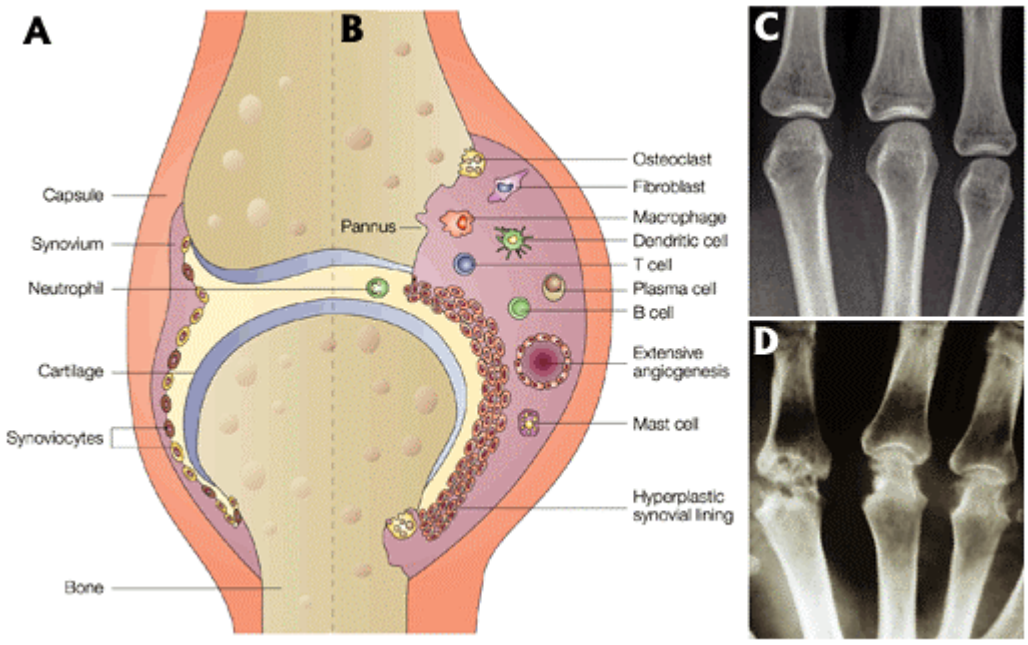
RA is a chronic inflammatory disease affecting the peripheral joints (more precisely the hands, the feet and the wrists) that leads to destruction of cartilage and bone erosion. RA is one of the most frequent systemic autoimmune diseases and affects a large part of the population worldwide (prevalence between 0.3 and 1% in industrialized countries and an overall prevalence of 0.8% adults).[1] Women, for whom the disease starts the most frequently at the menopause, are three times more concerned by RA than men. However, in about one

[1] statistics from the WHO Statistical Information System (WHOSIS), http://www3.who.int/whosis/menu.cfm

quarter of the cases, the disease appears before the age of 40 and even sometimes during the childhood (juvenile chronic arthritis).

Both cellular and humoral autoimmune mechanisms have been involved in the poorly understood pathogenesis of RA. This articular pathology is related to a massive infiltration of leukocytes (ie, T-cells and other immune cells including B-cells, macrophages and mast cells), which together with activated synoviocytes form the pannus of proliferative tissue that overgrows the articular cartilage. This leads to a progressive degradation of the cartilage and subsequent destruction of the underlying bone. Over-expression of pro-inflammatory cytokines such as TNF-α and IL-1 is considered to drive the destruction processes, but the causes for this deregulated cytokine production are unknown. The ultimate results of the inflammatory process are joint deformity and loss of joint function. The physiological modifications due to RA are illustrated in Figure I.1, showing the schematic view of a normal joint (picture A) and its changes in RA (picture B).

Figure I.1 View of a normal joint and its changes in RA (from *Nature* 2003, ref. 2)



The joint spaces, clearly visible in a normal hand (picture C), have narrowed or totally disappeared in the hand radiograph of a patient with established RA (picture D). This change in the joints of a RA patient is a sign of destruction of cartilage and erosion of the adjacent bone illustrated in the schematic view B.

I.1.2. Recent therapeutic strategies

Drug therapies for RA are based on two principal approaches : (i) symptomatic treatment with anti-inflammatory drugs and (ii) alteration of the disease-process with anti-rheumatic drugs. Disease-modifying anti-rheumatic drugs (DMARDs) have been developed for the treatment of RA since the 1920's. These synthetic drugs are still largely used, but all of them present limited efficacy and / or toxicity problems. An initial understanding of the nature of the inflammatory attack in the joints has led to the development of biological DMARDs preventing pro-inflammatory cytokines (in particular TNF-α and IL-1) from interacting with their receptors.[2] However, these non-specific treatments most likely affect patients' capacity to resist infections and development of tumors. More recently, new therapeutic approaches have been investigated among the numerous potential therapeutic targets from the inflammatory cascade of RA.[2] The newly developed drug-candidates act by more specific intervention (ie, targeting cytokines, receptors, the signal-transduction pathway or specific cells), which should reduce the possible side effects. Interestingly, if effective, most of these newer anti-RA drugs furnish similar degrees of efficacy and the inflammatory process collapses at least partly, but rarely completely, and never in all patients.

DMARDs, which impede both the inflammatory and destructive processes of RA, offer a relative control of the disease in many patients. However, even the best available therapies at present do not cure RA or do not achieve remission. A causative therapy should allow curative treatment of the disease with no or very limited side-effects. But due to the complexity of the pathogenesis of RA, the cause(s) of the disease still remain enigmatic.

I.1.3. Antigens ? CII as a candidate

The first autoantibody described in RA[3] was rheumatoid factor which is directed against IgG.[4] Since then, an increasing number of autoimmune responses for RA have been detected. The targeted autoantigens include citrullinated proteins (ie, filaggrin),[5] collagen,[6] the

[2] for a recent review covering the therapeutic strategies for RA, see: Smolen, J. S.; Steiner, G. *Nature Reviews: Drug Discoveries* **2003**, *2*, 473.

[3] Waaler, E. *Acta Pathol. Microbiol. Scand.* **1940**, *17*, 172.

[4] Osterland, C. K.; Harboe, M.; Kunkel, H. G. *Vox. Sang.* **1963**, *8*, 133.

[5] Schellekens, G. A.; de Jong, B. A. W.; van den Hoogen, F. H. J.; van de Putte L. B. A.; van Venrooij, W. J. *J. Clin. Invest.* **1998**, *101*, 273.

heterogeneous nuclear ribonucleoprotein A2 (RA33)[7] as well as the BiP glycoprotein.[8] Most of the antibodies related to these antigens do not seem to play a major role in the pathogenicity of the disease, but a discussion is ongoing regarding the possible role of cartilage-directed autoimmunity. Indeed, early studies quickly established that autoimmunity to type II collagen (CII) occurs in patients with RA although it remained uncertain whether the autoimmunity to CII is a cause or a consequence of arthritis. Anti-CII antibodies have been detected in the serum and synovial fluids from RA patients[9] and T-cells reactive to CII have been isolated from the synovial membranes of RA patients.[10] Collectively, these observations have led to the concept that autoimmune responses to autologous human CII (hCII) may be a significant factor in the pathogenesis of RA.[11] But for many reasons inherent to human studies, much of the advances in CII autoimmunity related to RA have been made through the study of murine models. These results will be presented and developed in section I.2.

I.1.4. Susceptibility to rheumatoid arthritis

Genetic predisposition to RA has been associated with genes of the class II MHC (in particular HLA-DR4 and HLA-DR1). Approximately 80% of Caucasian patients with RA express DR4 or DR1 allotypes.[12]

Interestingly, these HLA-DR molecules share a common stretch of amino acids in their peptide-binding domain corresponding to positions 67-74 of the DRβ chain, the so-called

---

[6] for a review on the role of cartilage collagens in the pathogenesis of experimental arthritis, see: Cremer, M. A.; Rosloniec, E. F.; Kang, A. H. *J. Mol. Med.* **1998**, *76*, 275.

[7] Steiner, G.; Hartmuth, K.; Skriner, K.; Maurer-Fogy, I.; Sibski, A.; Thalmann, E.; Hassfeld, W.; Barta, A.; Smolen, J. S. *J. Clin. Invest.* **1992**, *90*, 1061.

[8] Bläss, S.; Union, A.; Raymackers, J.; Schumann, F.; Ungethüm, U.; Müller-Steinbach, S.; De Keyser, P.; Engel, J. M.; Burmester, G. R. *Arthritis Rheum.* **2001**, *44*, 761.

[9] (a) Clague, R. B.; Firth, S. A.; Holt, P. J. L.; Skingle, J.; Greenbury, C. L.; Webly, M. *Ann. Rheum. Dis.* **1983**, *42*, 537. (b) Trentham, D. E.; Kammer, G. M.; McCune, W. J.; David, J. R. *Arthritis Rheum.* **1981**, *24*, 1363. (c) Stuart, J. M.; Huffstutter, A. S.; Townes, A. S.; Kang, A. H. *Arthritis Rheum.* **1983**, *26*, 832.

[10] (a) Eklayam, O.; Zinger, H.; Zisman, E.; Segal, R.; Taron, M.; Brautbar, C.; Moses, E. *J. Rheumatol.* **1991**, *18*, 516. (b) Londei, M.; Savill, C. M.; Averhoef, A.; Brennan, F.; Leech, Z. A.; Duance, V.; Maini, R. N.; Feldman, M. *Proc. Natl. Acad. Sci.* **1989**, *86*, 636.

[11] Trentham, D. E.; Townes, A. S.; Kang, A. H. *J. Exp. Med.* **1977**, *146*, 857.

[12] Feldman, M.; Brennan, F. M.; Maini, R. V. *Cell* **1996**, *85*, 307.

shared epitope.[13] This motif is different from that of DR molecules not associated with RA. Sequence differences in this region, especially in residue DRβ71, could profoundly influence T-cell recognition and immune response. This residue is positively charged (Lys or Arg) in RA-associated allotypes and is negatively charged in the non-associated DRB1*0402 molecule.[14] Indeed, only peptides carrying a negatively charged residue (Asp and Glu) at position 4 bind to DR molecules with associated increased susceptibility to RA. This selective binding of pathogenic peptides may be a major part of the association of class II MHC particular alleles to RA.

I.1.5. T-cells in rheumatoid arthritis

Several studies that have characterized massive infiltrates of $CD4^+$ T-cells in RA[15] and a diminution or abrogation of joint inflammation by immunosuppressive drug therapies[16] suggest that this disease is mediated by T-cells. However, it has been very difficult to clone cartilage-specific T-cells from the joints or blood of RA patients. These difficulties could at least partly reflect that these putatively pathogenic T-cells are very few, are short-lived and play a role only in the initiation of an inflammatory attack. Another potential reason, which will be extensively developed further, is that these studies did not directly address that CII can become post-translationally modified.

In conclusion, the predilection of the inflammatory attack for the joint suggests a role for cartilageneous CII as a source of the antigenic peptide(s). The association of RA with DR molecules expressing the shared-epitope reflects an important role for class II MHC molecules as peptides receptors and the infiltrates of activated $CD4^+$ T-cells in the arthritic joints suggest a central role for T-cells in the pathogenesis of RA. Considering these findings, an autoimmune model has been proposed : MHC class II-restricted, specific $CD4^+$ T-cells

[13] Gregersen, P. K.; Silver, J.; Winchester, R. J. *Arthritis Rheum.* **1987**, *30*, 1205.

[14] Hammer, J.; Galozzi, F.; Bono, E.; Karr, R. W.; Guenot, J.; Valsasnini, P.; Nagy, Z. A. *J. Exp. Med.* **1995**, *181*, 1847.

[15] Gay, S.; Gay, R. E.; Koopman, W. J. *Ann. Rheum. Dis.* **1993**, *52*, S39.

[16] (a) Yocum, D. E.; Klippel, J. H.; Wilder, R. L.; Gerver, N. L.; Austin, H. A.; Wahl, S. M.; Lesko, L.; Minor, J. R.; Preuss, H. G.; Yarboro, C.; Berkebile, C.; Dougherty, S. *Ann. Intern. Med.* **1988**, *109*, 863. (b) Fehlauer, C. S.; Carson, C. W.; Cannon, G. W.; Ward, J. R.; Samuelson, C. O.; Williams, H. J.; Clegg D. O. *J. Rheumatol.* **1989**, *16*, 307.

and peptide(s) from CII are believed to play a crucial role in the disease within a ternary interaction « $CD4^+$ T-cell / autoantigen / MHC molecule ».

I.2 Collagen induced arthritis in mice, a model for rheumatoid arthritis

A detailed insight into how RA develops requires studies that range from animal models to the molecular level. The first evidence that a cartilage-specific molecule could cause autoimmune arthritis was provided by *Trentham* and colleagues.[11] Subsequently, it was found that certain strains of mice[17] are susceptible to CII-induced arthritis (CIA), which is the most widely used model for RA. Mice immunized with heterologous CII in the presence of CFA develop arthritis at a high incidence. In the same conditions, immunization with homologous CII induces a disease with a lower incidence and severity.[11,18]

I.2.1. Description of collagen induced arthritis

Mice immunized with CII present most of the clinical, histological and immunological characteristics of RA (Figure I.2).[17b,18b] The development of CIA reveals a hypertrophic inflamed synovium which spreads across the cartilage surface, degrading proteoglycan and later eroding underlying cartilage and bone.

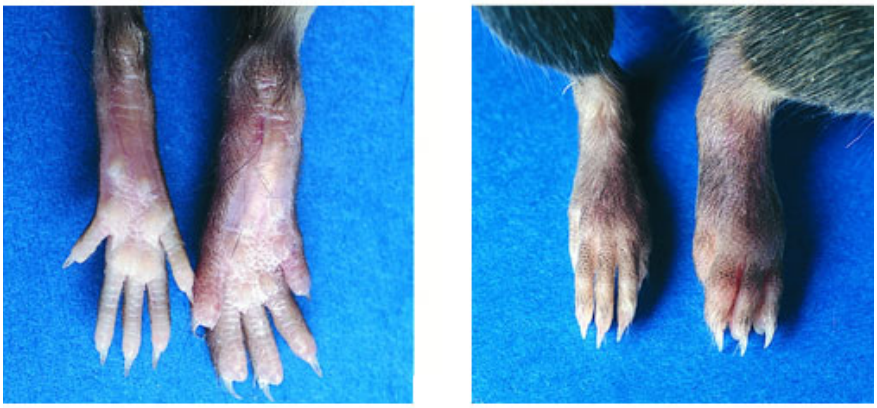
The role of antibodies in initiating arthritis and the arthritogenic properties of the anti-CII autoantibodies have been fully demonstrated.[19] Subsequent studies have defined the features needed to make autoantibodies arthritogenic (ie, present in sufficient quantities, fix complement, bind homologous CII and recognize at least three unique epitopes appropriately spaced so that antibodies do not compete for binding sites).

[17] (a) Courtenay, J.; Dallman, M.; Dayan, A.; Martin, A.; Mosedale, B. *Nature* **1980**, *283*, 666. (b) Wooley, P.; Luthra, H.; Stuart, J.; David, C. *J. Exp. Med.* **1981**, *154*, 688.

[18] (a) Boissier, M.; Feng, X.; Carlioz, A.; Roudier, R.; Fournier, C. *Ann. Rheum. Dis.* **1987**, *46*, 691. (b) Holmdahl, R.; Andersson, M.; Goldschmidt, T. J.; Gustafsson, K.; Jansson, L.; Mo, J. A. *Immunol. Rev.* **1990**, *118*, 193.

[19] Johnston, S.; Runge, L.; Johnson, J.; Phillips, P. *Clin. Exp. Rheum.* **1985**, *3*, 221.

Figure I.2 Symptoms of CIA in susceptible strain of mice (from *PNAS* 1998, ref. 32)



The observed susceptibility to CIA is strongly linked to the MHC class II region ; in particular with the H-2^q or H-2^r molecules (eg, DBA/1 or B10RIII mice, respectively).[17b,20] Remarkably, it was found that the identified MHC class II molecule in the CIA model H-2^q has a peptide binding pocket structurally similar to that of the shared-epitope (ie, expressing DR4 molecules).[18b] The exact role that the MHC plays in determining the arthritogenicity of antibodies produced by CII-immunized mice of different allotypes is not well understood. However, it is clear that Th-cells, and subsequently the determinants they recognize, play a prominent role in the induction of CIA in genetically susceptible strains of rodents.[21]

The mapping of B-cell epitopes supports the concept that the B-cell epitope system on CII is complex, and that synovitis is initiated by a sufficient number of non-competiting, complement fixing antibodies capable of reacting with epitopes on autologous CII. This concept suggests the existence of multiple B-cell epitopes on CII.

Clearly T-cells are critically involved in the genesis of arthritis because CII is a MHC-restricted, T-dependent antigen and, as anticipated, athymic animals are totally resistant to

[20] (a) Wooley, P. H. and Chapedelaine, J. M. *CRC Crit. Rev. Immunol.* **1987**, *8*, 1. (b) Wooley, P. H.; Dillon, A. M.; Luthra, H. S.; Stuart, J. M.; David, C. S. *Trans. Proc.* **1983**, *15*, 180. (c) Brunsberg, U.; Gustafsson, K.; Jansson, L.; Michaelsson, E.; Ahrlund-Richter, L.; Pettersson, S.; Mattsson, R.; Holmdahl, R. *Eur. J. Immunol.* **1994**, *24*, 1698.

[21] (a) Myers, L. K.; Stuart, J. M.; Seyer, J. M.; Kang, A. H. *J. Exp. Med.* **1989**, *170*, 1999. (b) Myers, L. K.; Terato, K.; Seyer, J. M.; Stuart, J. M.; Kang, A. H. *J. Immunol.* **1992**, *149*, 1439. (c) Myers, L. K.; Stuart, J. M.; Seyer, J. M.; David, C. S.; Kang, A. H. *J. Immunol.* **1993**, *151*, 500. (d) Myers, L. K.; Rosloniec, E.; Seyer, J. M.; Stuart, J. M.; Kang, A. H. *J. Immunol.* **1993**, *150*, 4652. (e) Michaëlsson, E.; Andersson, M.; Engström, A.; Holmdahl, R. *Eur. J. Immunol.* **1992**, *22*, 1819.

CIA. Moreover, clonal deletion of T-cells provokes a milder arthritis[22] whereas mice transgenic for a collagen-reactive TCR develop a more severe disease.[23]

I.2.2. Characterization of tolerogenic T-cell epitopes

Cyanogen bromide (CB) fragments of heterologous CII (ie, chick CII (cCII), bovine CII (bCII) and rat CII) contain the requisite epitopes critical for inducing CIA in H-2^q mice (ie, DBA/1 and B10.Q).[24] CB11 fragment of cCII (CB11 = CII(124-402)) contains four epitopes recognized by T-cells from these disease susceptible strains (H-2^q) ; the determinants CII(181-209) and CII(245-270) being recognized more prominently. The T-cell response to bovine CB11 is mediated predominantly by a single immunodominant T-cell determinant CII(259-267). Peptides CII(136-150) and CII(184-198) may also contain T-cell determinants, although their response was far less than of that generated by the dominant epitope. On the other hand, immunization with homologous mouse CII (mCII) or mCII-derived peptides induces arthritis with lower incidence and severity.[25,18a]

Michaëlsson and colleagues explained the lack of response in this last case by the poorer MHC binding of the mouse peptide compared to that of the corresponding heterologous peptides.[21e] They suggest that the exchange from glutamic acid to aspartic acid in position 266 (see Figure I.3) and the subsequent difference in MHC binding converts the peptide from being immunodominant in heterologous CII to become cryptic in mCII. Even at low level, T-cell recognition of self CII is not totally absent and autoreactive T-cells may certainly play an essential role in the development of CIA.

[22] Anderson, G. D.; Banerjee, S.; Luthra, H. S.; David, C. S. *J. Immunol.* **1991**, *147*, 1189.

[23] Terato, K.; Hasty, K. A.; Cremer, M. A.; Stuart, J. M.; Townes, A. S.; Kang, A. H. *J. Exp. Med.* **1985**, *162*, 637.

[24] for induction of CIA with chick CII, see ref. 21c. for induction of CIA with bovine CII, see: (b) Brand, D. D.; Myers, L. K.; Terato, K.; Whittington, K.; Stuart, J. M.; Kang, A. H.; Rosloniec, E. F. *J. Immunol.* **1994**, *152*, 3088. for induction of CIA with rat CII, see: (c) Andersson, M. and Holmdahl, R. *Eur. J. Immunol.* **1990**, *20*, 1061.

[25] (a) Holmdahl, R.; Jansson, L.; Gullberg, D.; Rubin, K.; Forsberg, P. O.; Klareskog, L. *Clin. Exp. Immunol.* **1985**, *62*, 639. (b) Holmdahl, R.; Jansson, L.; Andersson, M.; Larsson, E. *Immunology* **1988**, *65*, 305.

Figure I.3 Amino acids sequences of the CII region containing the major T-cell determinant in CIA

Rat CII(245-270):	**A**^{245} T G P L G^{250} P K G Q **T** G E **P** G I^{260} A G F K G **E** Q G P K^{270}
Chick CII(245-270):	**P**^{245} T G P L G^{250} P K G Q **T** G E **L** G I^{260} A G F K G **E** Q G P K^{270}
Bovine CII(245-270):	**A**^{245} T G P L G^{250} P K G Q **T** G E **P** G I^{260} A G F K G **E** Q G P K^{270}
Human CII(245-270):	**A**^{245} T G P L G^{250} P K G Q **T** G E **P** G I^{260} A G F K G **E** Q G P K^{270}
Mouse CII(245-270):	**A**^{245} T G P L G^{250} P K G Q **A** G E **P** G I^{260} A G F K G **D** Q G P K^{270}

If administrated to neonatal DBA/1 mice (H-2^{q} strain) as a tolerogen (ie, intraperitonal injection of antigen emulsified with IFA), before CII-immunization, CB11 prevents the onset of the disease. In addition, the heterologous peptides CII(245-270) isolated as T-cell epitopes are also capable of inducing tolerance and subsequently regulating CIA. In fact, peptides generating strong T-cell responses were the most potent tolerogens : the same sequence both induces a strong T-cell response and functions as an effective tolerogen. These data support the concept that tolerance is specific for the peptides derived from this sequence and more precisely, a small active site within this sequence. *Myers* and colleagues have used this information to design a synthetic analogue of CII(245-270) containing three amino acid substitutions (Ile^{260}→Ala, Ala^{261}→Hyp, Phe^{263}→Arg) and capable of preventing the onset of CIA.[26]

hCII and mCII are approximately 97% homologous.[27] In 1996, *Krco* and colleagues determined immunodominant epitopes on hCII by immunization of H-2^{q} mice with a series of hCII-derived overlapping peptides.[28] Three antigenic peptides were isolated : hCII(82-93), hCII(254-273) and hCII(928-939). The antigenic region hCII(254-273) corresponds to residues 250-270 of cCII and 260-270 of bCII, which also elicit T-cell responses in H-2^{q} mice, as previously discussed.

[26] Myers, L. K.; Tang, B.; Rosloniec, E. F.; Stuart, J. M.; Chiang, T. M.; Kang, A. H. *J. Immunol.* **1998**, *161*, 3589.

[27] (a) Baldwin, C. T.; Reginato, A. M.; Smith, C.; Jimenez, S. A.; Prockop, D. J. *Biochem. J.* **1989**, *262*, 521. (b) Su, M. W.; Lee, B.; Ramirez, F.; Machado, M.; Horton, W. *Nucleic Acids Res.* **1989**, *17*, 9473.

[28] Krco, C. J.; Pawelski, J.; Harders, J.; McCormick, D.; Griffiths, M.; Luthra, H. S.; David, C. S. *J. Immunol.* **1996**, *156*, 2761.

I.2.3. Collagen induced arthritis in « humanized » mice

One step further in the understanding of the pathogenesis of RA was the development of HLA-DR1, HLA-DR4, and CD4 transgenic mice. Using DR1[29] and DR4[30] models, *Rosloniec* and colleagues demonstrated that immunization of these transgenic mice with hCII or bCII resulted in the development of an inflammatory autoimmune arthritis similar to CIA. Apparently, both DR molecules bind and present the same hCII peptide CII(259-273), but the DR-restricted T-cells fully discriminate DR1-hCII and DR4-hCII complexes.

Using HLA-DR4 and human CD4 transgenic mice, *Fugger* and colleagues identified an immunodominant T-cell epitope in CII corresponding to residues 261-273.[31] Interestingly, this determinant is closely related to that found in CIA. Following this work, they demonstrate that these transgenic mice are highly susceptible to CIA[32] ; the pathology and histology of the disease being seemingly similar to those observed by *Rosloniec* and colleagues.

Both groups showed a significant amount of T-cell cross-reactivity (ie, among bCII, cCII and mCII) due to similar or identical sequences in the immunodominant determinants. Thus HLA-DR1 and HLA-DR4 are capable of binding peptides derived from hCII and therefore probably play a crucial role in the autoimmune response to hCII observed in RA patients.

In addition, both groups proposed a detailed characterization of MHC and T-cell receptor contacts in « their » epitope. For *Rosloniec* and colleagues, the binding of CII(259-273) to DR1 appears to be exclusively controlled by the Phe^{263} (P1) and the Lys^{264} (P2) residues. The CII amino acids Glu^{266}, Gln^{267}, Gly^{268} and Lys^{270} (P4-P6, P8) appear to be primarily involved in TCR contact. Similarly, Phe^{263} and Lys^{264} are clearly involved in DR4 binding, but Gly^{265} (P3) and Pro^{269} (P7) also seem to play a substantial role. All the remaining core residues (Glu^{266}-Gly^{268} and Lys^{270}) appear to interact with the TCR. Again, these results confirm than

[29] Rosloniec, E. F.; Brand, D. D.; Myers, L. K.; Whittington, K. B.; Gumanovskaya, M.; Zaller, D. M.; Woods, A.; Altmann, D. M.; Stuart, J. M.; Kang, A. H. *J. Exp. Med.* **1997**, *185*, 1113.

[30] Rosloniec, E. F.; Brand, D. D.; Myers, L. K.; Esaki, Y.; Whittington, K. B.; Zaller, D. M.; Woods, A.; Stuart, J. M.; Kang, A. H. *J. Immunol.* **1998**, *160*, 2573.

[31] Fugger, L.; Rothbard, J.; McDevitt, G. S. *Eur. J. Immunol.* **1996**, *26*, 928.

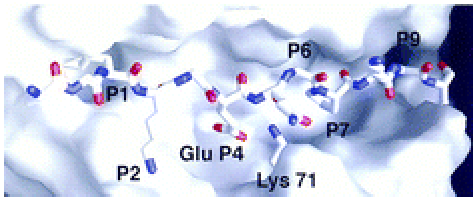
[32] Andersson, E. C.; Hansen, B. E.; Jacobsen, H.; Madsen, L. S.; Andersen, C. B.; Engberg, J.; Rothbard, J. B.; McDevitt, G. S.; Malström, V.; Holmdahl, R.; Svejgaard, A.; Fugger, L. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 7574.

despite the apparent similarities in DR1 and DR4 presentation of the epitope, there are clear differences in how these DR-peptide complexes interact with their CII-specific TCR.

The data for DR4 binding of the CII peptide described by *Andersson* and colleagues differs somewhat from those discussed above. The Phe^{263} residue is still proposed to occupy the large hydrophobic P1 pocket, but the negatively charged Glu^{266} is believed to occupy the positively charged P4 pocket in the DR4-binding site.[31]

Finally, *Dessen* and colleagues proposed an hypothetical model of CII(261-273) in the HLA-DR4 binding cleft (Figure I.4) based on the crystal structure of HLA-DR4 complexed with another CII-derived peptide.[33] This model supports the suggestion made by *Andersson* and colleagues for the anchorage residues (Phe^{263} and Glu^{266} occupying P1 and P4 pockets, respectively) ; Ala^{261}, Gly^{262}, Lys^{264}, Gly^{265}, Gln^{267}, Lys^{270}, Glu^{272} and Pro^{273} being considered as possible contacts with the TCR. Gln^{267} and Lys^{270} are particularly expected, by analogy to previous peptides, to extend prominently into solution and to be contacted by TCRs.

Figure I.4 Model of CII(261-273) in the HLA-DR4 binding cleft (from *Immunity* 1997, ref. 33)



Although the DR4-CII(261-273) complex is only a hypothetical model, it gives insight into the molecular basis of the binding of CII-derived peptides to class II MHC molecule and reveals the residues involved in T-cell recognition.

I.2.4. A glycopeptide as a T-cell epitope

In the course of the determination of T-cell epitopes in CIA, *Holmdahl* and colleagues identified several T-cell hybridomas, which responded to CB11, but did not respond to the synthetic peptide CII(256-270).[34] This observation was associated to the post-translational modifications of collagen. Indeed, CII is subjected to extensive post-translational modifications. Pro and Lys residues can undergo hydroxylation to give (4*R*)-4-hydroxy-L-

[33] Dessen, A.; Lawrence, C. M.; Cupo, S.; Zaller, D. M.; Wiley, D. C. *Immunity* **1997**, *7*, 473.

[34] Michäelsson, E.; Andersson, M.; Engström, A.; Holmdahl, R. *Eur. J. Immunol.* **1992**, *22*, 1819.

proline and (5*R*)-5-hydroxy-L-lysine, respectively. The minimum sequence requirements for these hydroxylations are fulfilled if Pro or Lys residues are located in the triplet Gly-Xaa-Pro or Gly-Xaa-Lys, respectively, which makes the Pro and the two Lys residues in CII(256-270) susceptible to this modification. The hydroxylysine residues can subsequently become glycosylated with either a *β*-D-galactopyranosyl or an *α*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranosyl moiety.[35]

In a second study, they also proved that biochemical removal of carbohydrates (ie, treatment with either sodium periodate or TFMSA) from CII resulted in loss of recognition by most of the T-cell hybridomas which did not respond to the synthetic peptide CII(256-270). These results suggest that carbohydrates are involved in the trimolecular complex « T-cell receptor / glycopeptide / MHC molecule ».[36] In addition, elimination of the carbohydrates from CII makes the synthetic peptide less arthritogenic.

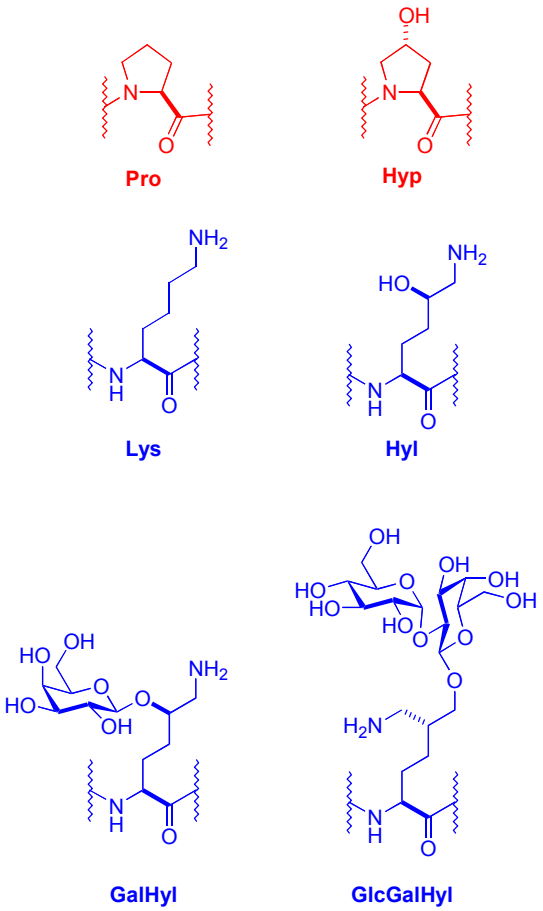
Overall, these two studies (i) demonstrate that post-translational glycosylation of the immunodominant peptide CII(256-270) generate a new distinct structural determinant and (ii) highlight the critical role of the different post-translational modifications of CII in the development of CIA. From this, the challenge was to determine precisely the T-cell determinant(s) within CII since thirty two naturally occurring peptides (Figure I.5) can be generated by post-translational modifications from CII(256-270).

[35] (a) Butler, W. T. and Cunningham, L. W. *J. Biol. Chem.* **1966**, *241*, 3882. (b) Spiro, R. G. *J. Biol. Chem.* **1969**, *244*, 602.

[36] Michaëlsson, E.; Malmström, V.; Reis, S.; Engström, A.; Burkhardt, H.; Holmdahl, R. *J. Exp. Med.* **1994**, *180*, 745.

Figure I.5 Possible post-translational modifications of CII(256-270)

H-Gly^{256}-Glu-Xaa^{258}-Gly-Ile-Ala-Gly-Phe-Xaa^{264}-Gly-Glu-Gln-Gly-Pro-Xaa^{270}-OH



Entry	Xaa^{258}	Xaa^{264}	Xaa^{270}
1	Pro	Lys	Lys
2	Pro	Lys	Hyl
3	Pro	Lys	GalHyl
4	Pro	Lys	GlcGalHyl
5	Pro	Hyl	Lys
6	Pro	Hyl	Hyl
7	Pro	Hyl	GalHyl
8	Pro	Hyl	GlcGalHyl
9	Pro	GalHyl	Lys
10	Pro	GalHyl	Hyl
11	Pro	GalHyl	GalHyl
12	Pro	GalHyl	GlcGalHyl
13	Pro	GlcGalHyl	Lys
14	Pro	GlcGalHyl	Hyl
15	Pro	GlcGalHyl	GalHyl
16	Pro	GlcGalHyl	GlcGalHyl
17	Hyp	Lys	Lys
18	Hyp	Lys	Hyl
19	Hyp	Lys	GalHyl
20	Hyp	Lys	GlcGalHyl
21	Hyp	Hyl	Lys
22	Hyp	Hyl	Hyl
23	Hyp	Hyl	GalHyl
24	Hyp	Hyl	GlcGalHyl
25	Hyp	GalHyl	Lys
26	Hyp	GalHyl	Hyl
27	Hyp	GalHyl	GalHyl
28	Hyp	GalHyl	GlcGalHyl
29	Hyp	GlcGalHyl	Lys
30	Hyp	GlcGalHyl	Hyl
31	Hyp	GlcGalHyl	GalHyl
32	Hyp	GlcGalHyl	GlcGalHyl

I.3 Preparation of naturally occurring glycosylated CII derivatives

As I just started my thesis, *Kihlberg* and colleagues (University of Umea, Sweden) published a series of paper on the synthesis of natural and non-natural glycopeptides derived from the T-cell epitope CII(256-270) with the aim to elucidate how RA develops. The synthetic challenge was to prepare the glycosylated 5-hydroxylysine building blocks required for use in solid phase synthesis of CII-derived glycopeptides.

Following initial studies on hydroxynorvaline model, they developed a route to the conveniently protected hydroxylysine building blocks starting from the commercially available (2*S*,5*R*)-5-hydroxylysine.[37] Their first strategy, tailored for the preparation of diglycosylated derivatives, involved glycosyl moieties carrying acid labile carbohydrate

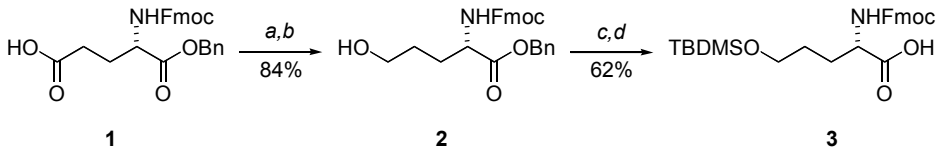
[37] (5*R*)-5-Hydroxy-L-lysine dihydrochloride monohydrate: Fluka 55501, € 169.50 for 1 gram.

protective groups which are removable during cleavage of the glycopeptide from the resin. Additionally, they developed an improved synthesis for the preparation of monoglycosylated derivatives, which will also be discussed in this section.

I.3.1. Synthesis of protected hydroxylysine and hydroxynorvaline

Hydroxynorvaline (Hnv): the simplified hydroxylysine analogue (2*S*)-5-hydroxynorvaline locates the hydroxyl group at the same distance from the α-position but lacks the ε-aminomethylene group. Protected hydroxynorvaline **3** was prepared from N^{α}-Fmoc-glutamic acid benzyl ester (**1**) (Scheme I.1).[38]

Scheme I.1 Preparation of protected hydroxynorvaline derivatives[38]



1 **2** **3**

(a) iBuOCOCl, NMM, THF, -10°C ; (b) $NaBH_4$, MeOH, 0°C ; (c) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0°C ; (d) H_2, Pd/C, AcOEt.

Conversion of **1** to a mixed carbonic anhydride by treatment with isobutyl chloroformate and subsequent reduction with sodium borohydride in methanol, gave the alcohol **2** ready for further glycosylation. Protection of the primary alcohol with a *tert*-butyldimethylsilyl (TBDMS) protecting group and further selective removal of the benzyl ester by hydrogenolysis gave the building block **3** ready for incorporation into a peptide using the Fmoc strategy.

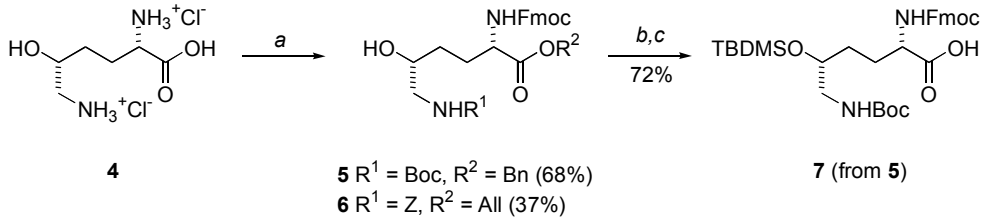
Hydroxylysine (Hyl): *Broddefalk* and colleagues have developed an optimized procedure for the preparation of conveniently *N*-Fmoc protected hydroxylysine derivatives starting from commercial (2*S*,5*R*)-5-hydroxylysine dihydrochloride.[39] The traditional method used to protect the ε-amino function of lysine was applied. Hydroxylysine was protected by transformation into a cupric chelate which allowed regioselective protection of the ε-amino

[38] (a) Broddefalk, J.; Bergquist, K. E.; Kihlberg, J. *Tetrahedron Lett.* **1996**, *37*, 3011. (b) Broddefalk, J.; Bergqvist, K. E.; Kihlberg, J. *Tetrahedron* **1998**, *54*, 12047.

[39] (a) Broddefalk, J.; Bäcklund, J.; Almqvist, F.; Johansson, M.; Holmdahl, R.; Kihlberg, J. *J. Am. Chem. Soc.* **1998**, *120*, 7676. (b) Broddefalk, J.; Forsgren, M.; Sethson, I.; Kihlberg, J. *J. Org. Chem.* **1999**, *64*, 8948.

group using di-*tert*-butyl dicarbonate or benzyl chloroformate. After dissociation of the chelate, the *α*-amino functionality was protected with a Fmoc group and the carboxyl functionality with either a benzyl or an allyl group. The desired intermediates **5** and **6** were obtained together with the corresponding lactones. The lower overall yield for the preparation of **6**, as compared to **5**, is due to a greater tendency for lactonization. Silylation of the secondary alcohol and subsequent removal of the benzyl ester gave hydroxylysine building block **7**.

Scheme I.2 Preparation of protected hydroxylysine derivatives[39]



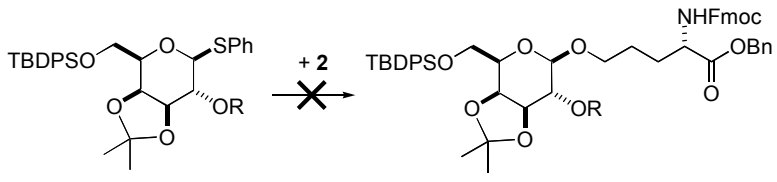
4 **5** R^1 = Boc, R^2 = Bn (68%); **6** R^1 = Z, R^2 = All (37%); **7** (from **5**)

(a) (i) $CuCO_3$•$Cu(OH)_2$, Boc_2O or ZCl, H_2O/dioxane, then Chelex 100 (H^+ form), (ii) FmocCl, Na_2CO_3, H_2O/dioxane, (iii) Cs_2CO_3, benzyl bromide or allyl bromide, DMF ; (b) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0°C ; (c) H_2, Pd/C, AcOEt.

I.3.2. Synthesis of galactosylated building blocks

Galactosylated hydroxynorvaline (GalHnv): first, galactosyl donors that have participating and orthogonally cleavable protecting groups at O-2 were investigated in attempts to link a *β*-D-galactose moiety to acceptor **2**.[38b] However, using the galactosyl donors **8**-**10** in glycosylation of **2** gave poor results with formation of the desired glycosides in less than 10% yields (Scheme I.3).

Scheme I.3 Attempted preparation of a galactosylated hydroxynorvaline building block[38b]

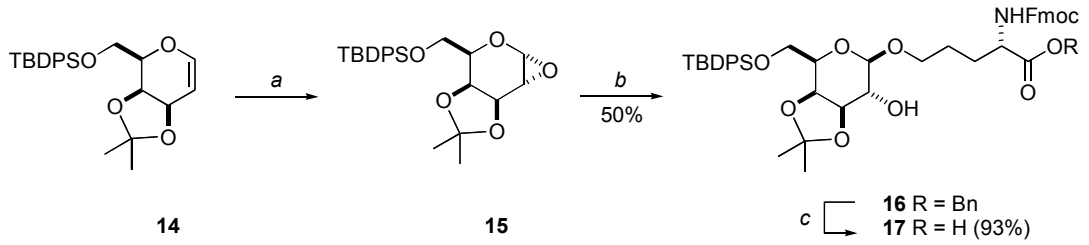


8 R = Lev
9 R = Aloc
10 R = ClAc

11 R = Lev
12 R = Aloc
13 R = ClAc

Alternatively, α-1,2-anhydrosugar **15**, formed by epoxidation of galactal **14**, was used for galactosylation of **2** using zinc chloride as promoter (Scheme I.4).[38] The desired β-glycoside was obtained in 50% yield together with the α-glycoside (6%).

Scheme I.4 Preparation of galactosylated hydroxynorvaline from the α-1,2-anhydrosugar 15[38]

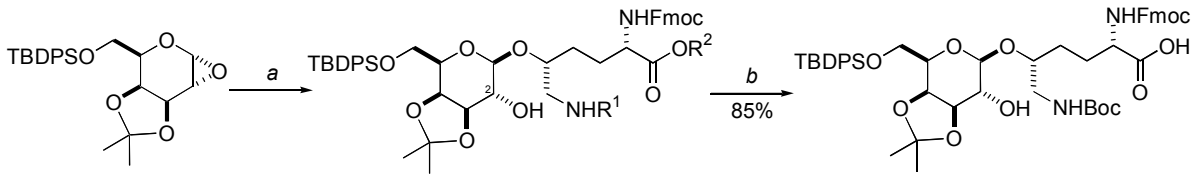


14 **15**

(a) dimethyldioxirane, acetone, CH_2Cl_2, 0°C ; (b) $ZnCl_2$, THF, MS, -50°C, then **2** ; (c) H_2, Pd/C, AcOEt.

Galactosylated hydroxylysine (GalHyl): the same procedure than the one developed for hydroxynorvaline was applied to attach a galactosyl moiety to the two hydroxylysine derivatives **5** and **6** (Scheme I.5).[39] The desired β-glycosides **18** and **19** were obtained in 37% and 30% yield, respectively. The low yields were explained by self-condensation (ie, ring opening of an anhydrosugar by the hydroxy function at C-2 of **18** or **19**). Unfortunately, using two equivalents of hydroxylysine in order to circumvent this side reaction led to the formation of the corresponding lactone and thus to the release of nucleophilic benzyl or allyl alcohols that competed with hydroxylysine as galactosyl acceptors.

Scheme I.5 Preparation of galactosylated hydroxylysine building blocks[39]



15

18 R^1 = Boc, R^2 = Bn (37%)
19 R^1 = Z, R^2 = All (30%)

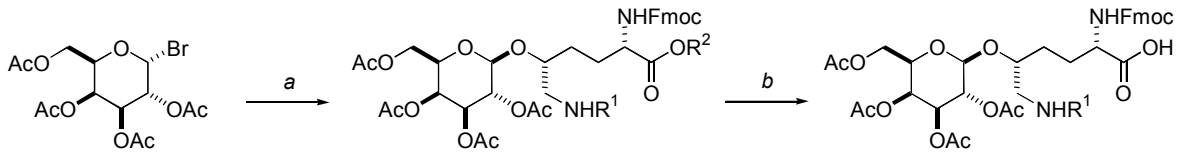
20 (from **18**)

(a) $ZnCl_2$, THF, MS, -50°C, then **5** or **6** ; (b) H_2, Pd/C, AcOEt.

This method used for the preparation of galactosylated hydroxynorvaline or hydroxylysine building blocks was developed not only to provide a 1,2-*trans*-glycosidic linkage, but also a hydroxy group at C-2 on the galactose residue for further attachment of an α-D-glucosyl

moiety. As we can see from the two previous examples, this procedure is not really suitable when a building block carrying only a single galactose moiety is required.

Scheme I.6 Improved preparation of galactosylated hydroxylysine building blocks[40]



21

22 R^1 = Boc, R^2 = Bn (19%)
23 R^1 = Z, R^2 = All (82%)

24 R^1 = Boc (72%)
25 R^1 = Z (86%)

(a) silver silicate, CH_2Cl_2, MS, 0°C, then **5** or **6** ; (b) H_2, Pd/C, AcOEt or $(PPh_3)_4Pd(0)$, *N*-methylaniline, THF.

An improved route for the preparation of the *β*-galactosylated hydroxylysine was developed by drastically changing the carbohydrate protecting group strategy (Scheme I.6).[40] Glycosylation of **5** with peracetylated galactosyl bromide **21** under promotion by silver silicate did improve the stereoselectivity (no *α*-anomer detected) but not the yield (Scheme I.6). In this case, the low yield was explained by the inadequate stability of the N^{ε}-Boc protective group under the conditions of glycosylation. In contrast, silver silicate promoted glycosylation of **6** with **21** gave *β*-glycoside **23** in 82% yield. Hydrogenolysis of **22** in AcOEt or deallylation of **23** gave the target glycosylated building blocks **24** and **25**, respectively.

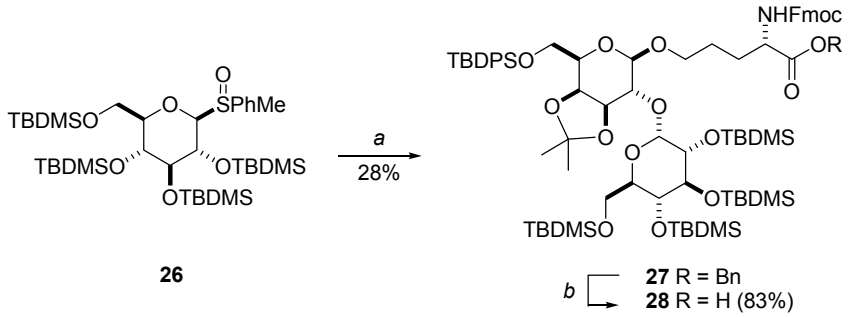
I.3.3. Synthesis of diglycosylated building blocks

Diglycosylated hydroxynorvaline (GlcGalHnv): the *α*-D-glucose moiety was attached to the acceptor **16** using the TBDMS protected thioglucoside **26**[41] as a glycosyl donor (Scheme I.7).[38] Treatment of the sulfoxide **26** with triflic anhydride and then acceptor **16** gave an anomeric mixture from which the desired *α*-glycoside **27** could be isolated in 28% yield. The corresponding *β*-glycoside (28%) and the acceptor having a TBDMS group at O-2 (10%) were also isolated.

[40] Holm, B.; Broddefalk, J.; Flodell, S.; Wellner, E.; Kihlberg, J. *Tetrahedron* **2000**, *56*, 1579.

[41] Montgomery, E. M.; Richtmyer, N. K.; Hudson, C. S. *J. Org. Chem.* **1946**, *11*, 301.

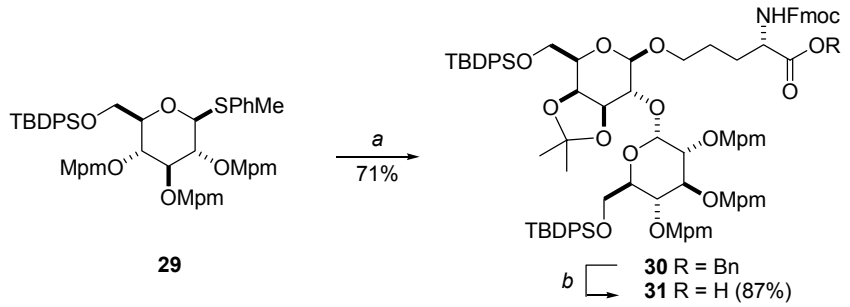
Scheme I.7 Preparation of a diglycosylated hydroxynorvaline building block[38]



(a) Tf_2O, 2,6-di-tert-butyl-4-methylpyridine, **16**, toluene, -78°C ; (b) H_2, Pd/C, AcOEt.

The *α*-selectivity was improved by changing the protective group patterns for the glucosyl donor (Scheme I.8).[38b] Coupling of donor **29** with acceptor **16** gave the *α*-linked derivative **30** (71%) and a small amount of the corresponding *β*-glycoside (9%).

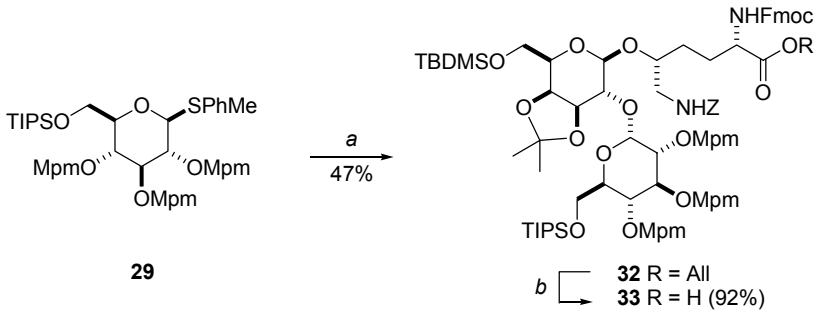
Scheme I.8 Improved *α*-selectivity to diglycosylated hydroxynorvaline building blocks[38b]



(a) NIS, AgOTf, **16**, CH_2Cl_2, MS, -45 → -15°C ; (b) H_2, Pd/C, AcOEt.

Diglycosylated hydroxylysine (GlcGalHyl): diglycosylated hydroxylylsine building blocks were prepared using the improved route developed for diglycosylated hydroxynorvaline (Scheme I.9). Attempted coupling to the galactosylated and N^{ε}-Boc protected hydroxylysine derivative **18** resulted in the formation of several byproducts, and the yield of diglycosylated hydroxylysine never exceeded 10%. The problems in the coupling were mainly due to the lability of the Boc group under acidic conditions of glycosylation. Its replacement by a Z group led to significant improvement.[39b] Using **19** as the acceptor gave an anomeric mixture of glycosides (α/β = 3.3:1, 80% yield) from which pure **32** could be isolated in 47% yield. The lower *α*-selectivity obtained in formation of hydroxylysine derivative **32**, as compared to hydroxynorvaline derivative **30** (α/β = 8:1, 89% yield), suggests a steric influence from the Z-protected *ε*-amino group. Final deprotection of the allyl ester gave the diglycosylated hydroxylysine building block **33**.

Scheme I.9 Preparation of a diglycosylated hydroxylysine building block[39b]



(a) NIS, AgOTf, **19**, CH_2Cl_2, MS, -45 → -15°C ; (b) $(PPh_3)_4Pd(0)$, *N*-methylaniline, THF.

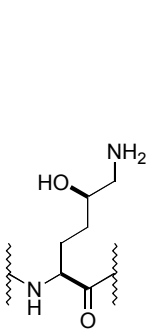
I.3.4 Synthesis of the CII-derived glycopeptides

Using the building blocks described previously (**3**, **7**, **17**, **25**, **31** and **33**), *Kihlberg* and colleagues prepared a series of eight different peptides or glycopeptides derived from CII. They were synthesized using an automatic peptide synthesizer on a polystyrene resin grafted with polyethylene glycol spacers using the Fmoc strategy (ie, coupling in presence of DIC / HOBt or DIC / HOAt and N^{α}-Fmoc deprotection by piperidine). Following the assembly of the peptidic chain, the resin was treated with trifluoroacetic acid (TFA) containing water and scavengers during 3 h to liberate the glycopeptides from the solid support. These conditions were found to be suitable for quantitative removal of the N^{ε}-protecting group of hydroxylysine (Boc or Z), as well as for the different protective groups introduced along the syntheses (ie, protections of the mono or disaccharide moieties and TBDMS protecting groups of non-glycosylated Hyl and Hnv residues), except for the glycopeptides prepared from the building block **25**. In this last case, deacetylation was performed using a 20mM solution of NaOMe in methanol.[40]

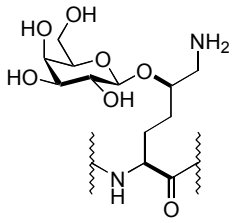
Among all the synthetic CII-derived analogues, four (**GP1**-**GP4**) corresponded directly to potential determinants from the naturally occurring pool of CII epitopes generated by post-translational modifications (Figure I.6).[39]

Figure I.6 Synthetic CII-derived peptides and glycopeptides prepared[39]

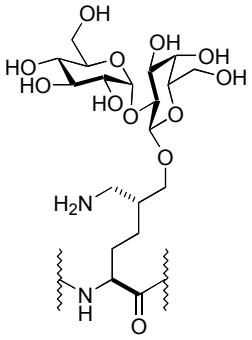
H-Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-**Xaa^{264}**-Gly-Glu-Gln-Gly-Pro-**Xaa^{270}**-Gly-Glu-Thr-Gly-Pro-Ala-Gly-Pro^{278}-OH



Hyl



GalHyl



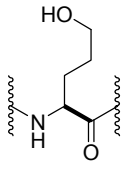
GlcGalHyl

Peptides	Xaa^{264}	Xaa^{270}	Sequence
GP1	Hyl	Lys	CII(256-270)
GP2	GalHyl	Lys	CII(256-270)
GP3	GlcGalHyl	Lys	CII(256-270)
GP4	GalHyl	GalHyl	CII(259-278)

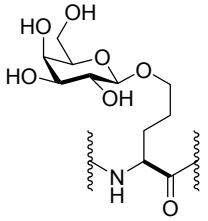
The four remaining derivatives (**GP5-GP8**) are unnatural derivatives. They were prepared using Hnv, GalHnv and GlcGalHnv instead of Hyl, GalHyl and GlcGalHyl, respectively (Figure I.7).[38,39a]

Figure I.7 Unnatural synthetic peptides and glycopeptides related to CII(256-270)[38,39a]

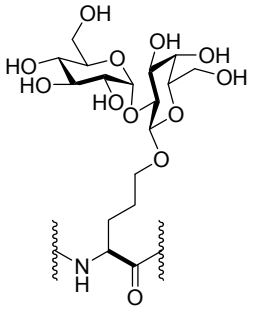
H-Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-**Xaa^{264}**-Gly-Glu-Gln-Gly-Pro-**Xaa^{270}**-Gly^{271}-OH



Hnv



GalHnv



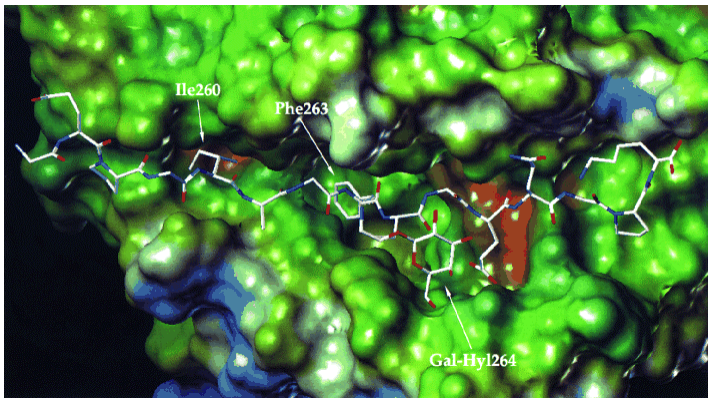
GlcGalHnv

Peptides	Xaa^{264}	Xaa^{270}	Sequence
GP5	Hnv	Lys	CII(256-270)
GP6	GalHnv	Lys	CII(256-270)
GP7	GlcGalHnv	Lys	CII(256-270)
GP8	GlcGalHnv	GlcGalHnv	CII(256-271)

I.3.5 Binding of CII-derived peptides to MHC molecules

The non-natural glycopeptides **GP6-GP8** were used to investigate the binding of glycopeptides to MHC molecules.[42] Data obtained revealed that peptides and glycopeptides bind to H-2^q molecule in the same manner. This binding involves two crucial anchoring residues : Ile^{260} and Phe^{263}. Furthermore, glycosylation at position 264 and / or 270 of CII(256-270) does not affect binding to the MHC molecule. On the basis of these observations, molecular modeling was used to gather information on the binding mode of the glycopeptide. *Kihlberg* research group proposed a model of CII(256-270) / MHC complex (Figure I.8) in which Ile^{260} and Phe^{263} occupy the P1 and P4 pockets of the H-2^q MHC molecule, respectively. Thus, the residue 264 (P5), which is located in the center of the complex, appeared to be a major TCR recognition site.

Figure I.8 Molecular modeling of the H-2^q binding site in complex with glycopeptide GP2 (from *ChemBioChem*, ref. 45)



The carbohydrate moieties carried by Hyl^{264} in **GP2** is thus optimally positioned for putative interaction with the T-cell receptor.

I.3.6 Evaluation of T-cell response to CII-derived peptides

Earlier attempts to characterize the reactivity of T-cells to CII performed with non-modified peptides essentially resulted in the identification of non-modified T-cell determinants. *Corthay* and colleagues evaluated the fine specificity of twenty nine T-cell

hybridomas obtained in CIA by testing their reactivity towards the synthetic peptides and glycopeptides (**GP1-GP4** and **GP6-GP8**) described previously.[43]

The hybridomas were divided into six different groups according to their fine specificity (Table I.1). Six out of the twenty nine hybridomas (group I) were known to recognize the non-modified peptide. They also showed a response, albeit weaker to the peptide **GP1**, having a hydroxylysine residue at position 264. One hybridoma (group II) was found to specifically recognize **GP1**. Seventeen out of the twenty two remaining hybridomas (group III) responded equally to **GP2** (GalHyl at position 264) and **GP4** (GalHyl at position 264 and 270). Reactivity was abolished when Hyl was replaced by Hnv (**GP2** vs **GP6**). The three hybridomas of group IV recognized **GP2** and **GP4**, but also glycopeptides with Gal attached to Hnv^{264} (**GP6**). Finally, group V (one hybridoma) specifically reacted with GlcGal attached to either Hyl or Hnv (**GP3**, **GP7** and **GP8**) and group VI (one hybridoma) recognized only peptide **GP3** with GlcGalHyl at position 264.

Table I.1 Summary of the response of the twenty nine different T-cell hybridomas

	Group I	Group II	Group III	Group IV	Group V	Group VI
nb of hybridomas	6	1	17	3	1	1
CII	++	+++	+++	+++	+++	+++
unmodified CII(256-270)	+++	-	-	-	-	-
GP1	++	+++	-	-	-	-
GP6	n.d.	n.d.	-	+++	-	-
GP2	-	-	+++	+++	-	-
GP4	-	n.d.	+++	+++	n.d.	-
GP7	-	-	-	+	++	-
GP3	-	-	-	-	++	++
GP8	-	-	-	+	++	-

The sensitivity of the T-cell hybridomas is given with an arbitrary scale: -, no reactivity; +, low reactivity; ++, medium reactivity; +++, high reactivity.

The TCR repertoire generated by this study is highly diverse, but the response of the T-cell hybridomas was found to be specific for the post-translational hydroxylation and then

[42] Kjellén, P.; Brunsberg, U.; Broddefalk, J.; Hansen, B.; Vestberg, M.; Ivarsson, I.; Engström, A.; Svejgaard , A.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 755.

[43] Corthay, A.; Bäcklund, J.; Broddefalk, J.; Michaëlsson, E.; Goldschmidt, T. J.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 2580.

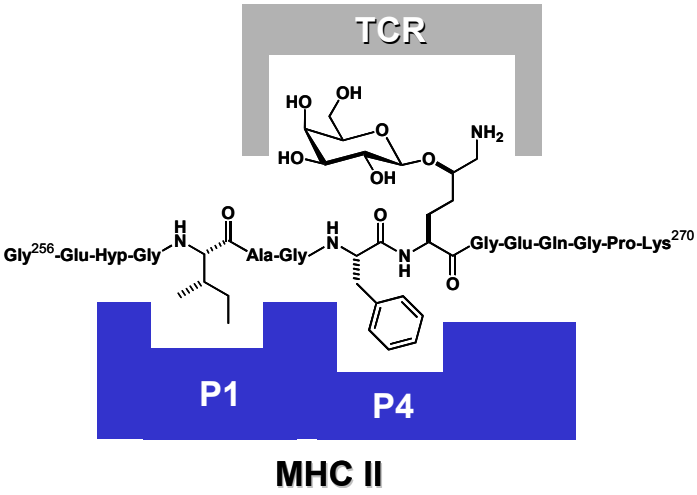
glycosylation with a β-D-galactopyranosyl residue of Lys^{264} (**GP2**). This is not surprising since the previous study shown that the residue at position 264 is a major T-cell contact.[42] In contrast, cross-reactivity towards peptides carrying different modifications at Lys^{270} was found to have no or very little influence on T-cell recognition. This suggests that residue 270 is outside the region of the peptide-MHC complex involved in the interaction with the T-cell receptor. This study also clearly indicates a preference for glycopeptides carrying small glycosides. A possible explanation for this observation could be that small carbohydrate moieties are more easily accommodated within the T-cell receptor combining site than larger diglycosides.[44] Also, enzymatic degradation of the glycan probably occurs during antigen processing thus preventing large carbohydrates to be presented in glycopeptide-MHC complexes.

I.3.7 Schematic description of the ternary complex

Results obtained by *Kihlberg* and colleagues with peptides **GP1-GP8** have been summarized in the schematic description of the ternary complex in Figure I.9. According to the model of the peptide-MHC complex (Figure I.8) and to the convergent studies showing the MHC anchors of CII(256-270), residue 264 is located in the center of the complex (residues at P1 and P4 being Ile^{260} and Phe^{263}). The carbohydrate moieties of the derived glycopeptides are thus located optimally for specific interactions with the T-cell receptor (Figure I.9).

[44] (a) Kihlberg, J. and Elofsson, M. *Curr. Med. Chem.* **1997**, *4*, 79. (b) Speir, J. A.; Abdel-Motal, U. M.; Jondal, M.; Wilson, I. A. *Immunity* **1999**, *10*, 51. (c) Glithero, A.; Tormo, J.; Haurum, J. S.; Arsequell, G.; Valencia, G.; Edwards, J.; Springer, S.; Twnsend, A.; Pao, Y. L.; Wormaid, M.; Dwek, R. A.; Jones, E. Y.; Elliot, T. *Immunity* **1999**, *10*, 63.

Figure I.9 A schematic description of the interactions formed in the « class II MHC molecule / GP2 / TCR complex »



I.4. Carbohydrate specificity of T-cell hybridomas

As mentioned above, the immunodominant epitope CII(256-270) recognized by the hybridomas was found to carry a β-D-galactopyranosyl residue attached to Hyl[264]. To investigate the carbohydrate specificity of these T-cell hybridomas, *Holm* and colleagues recently prepared modified galactosyl residues attached to Hyl at position 264.[45] As the peptides were also used to study whether or not glycosylation is important when arthritis is induced in mice that are transgenic for the human DR4 class II molecule, the sequence of the glycopeptides corresponded to residues 259-273 or 256-270 of CII.

I.4.1. Preparation of modified galactosyl moiety analogues of CII

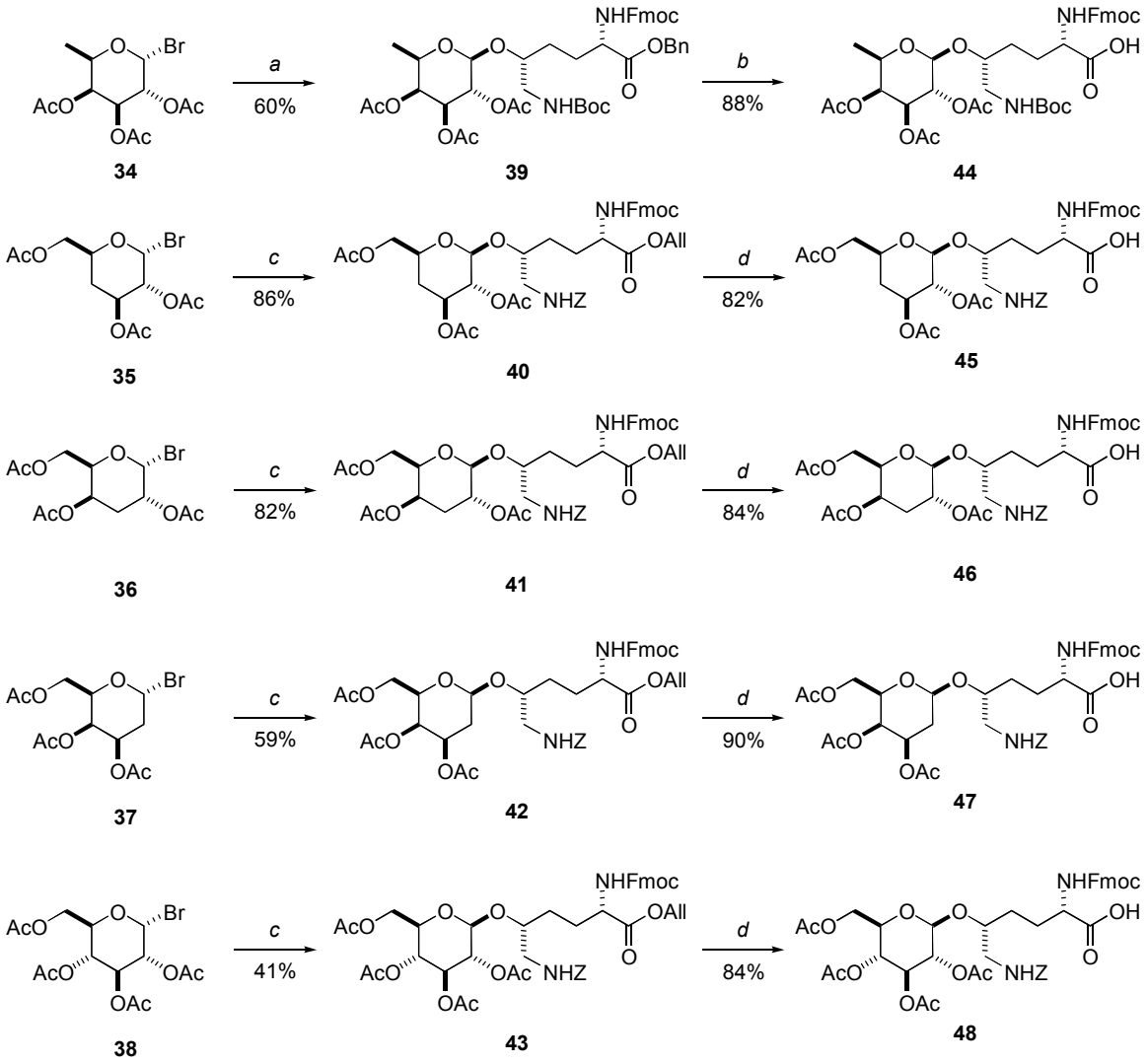
The modifications consisted of the sequential mono-deoxygenation of the four hydroxy groups on the galactosyl moiety and the inversion of the stereochemistry at C-4 (ie, by replacing D-galactose with D-glucose).

The procedure and protection strategy used were the ones published for the improved preparation of galactosylated hydroxylysine (Scheme I.10).[40] The coupling yields ranged from 41% for the less reactive acetobromoglucose **38** to 86%. Appropriate cleavage of the ester

[45] Holm, B.; Bäcklund, J.; Recio, M. A. F.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2002**, *3*, 1209.

groups gave the glycosylated building blocks **44-48** ready for use in solid-phase glycopeptide synthesis.

Scheme I.10 Preparation of hydroxylysine building blocks carrying different glycosyl moieties[45]

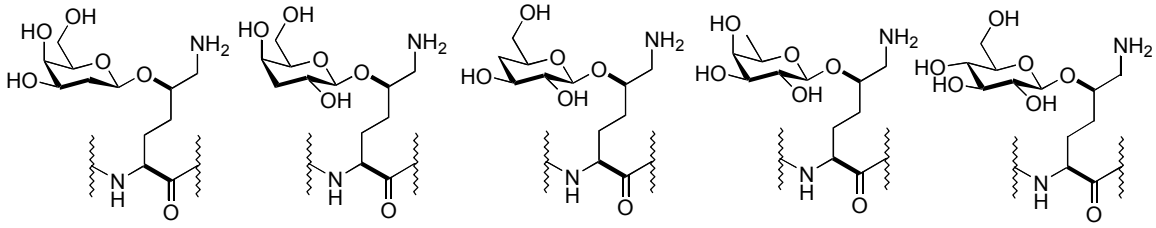


(a) silver silicate, **18**, CH_2Cl_2, MS, 0°C ; (b) H_2, Pd/C, AcOEt; (c) silver silicate, **19**, CH_2Cl_2, MS, 0°C ; (d) $(PPh_3)_4Pd(0)$, *N*-methylaniline, THF.

Glycopeptide synthesis was performed under standard conditions according to the Fmoc strategy (as previously described in section I.3).[45] Acid-catalyzed cleavage from the solid support, followed by deacylation and purification by RP-HPLC, gave the five target glycopeptides **GP9-GP13** in 13 to 43% yields according to the resin capacity (Figure I.10).

Figure I.10 Glycopeptides with Hyl^{264} carrying galactosyl analogues[45]

H-Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-**Xaa^{264}**-Gly-Glu-Gln-Gly-Pro-Lys-Gly-Glu-Thr^{273}-OH



2-deoxyGalHyl 3-deoxyGalHyl 4-deoxyGalHyl 6-deoxyGalHyl GlcHyl

Peptides	Xaa^{264}	Sequence
GP9	2-deoxyGalHyl	CII(259-273)
GP10	3-deoxyGalHyl	CII(259-273)
GP11	4-deoxyGalHyl	CII(259-273)
GP12	6-deoxyGalHyl	CII(256-270)
GP13	GlcHyl	CII(256-270)

I.4.2. Specificity of T-cell hybridomas obtained in CIA

The study revealed that the twenty hybridomas specific for **GP2** can be divided into four groups with different patterns of fine specificity for the galactosyl moiety (Table I.2).

Table I.2 Response of the GalHyl^{264} specific hybridomas toward glycopeptides carrying galactosyl analogues

	Group I	Group II	Group III	Group IV
nb of hybridomas	11	3	3	3
GP2	+++	+++	+++	+++
GP9	+++	+++	-	-
GP10	+++	-	++	-
GP11	-	++	-	-
GP12	+++	+++	++	+++
GP13	-	++	-	-

The sensitivity of the T cell hybridomas is given with an arbitrary scale: -, no reactivity; +, low reactivity; ++, medium reactivity; +++, high reactivity.

The reactivity of the eleven hybridomas belonging to group I has a strong dependency on the hydroxy group at C-4. The three hybridomas of group II are highly sensitive to the presence of HO-3 and HO-4 to a lesser extent. The three hybridomas in group III depend

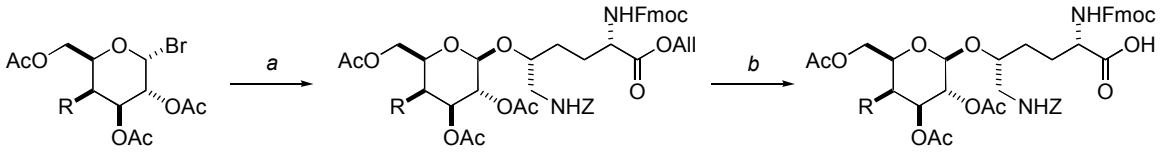
strongly on both HO-2 and HO-4, and weakly on HO-3 and HO-6. The last three hybridomas (group IV) are very sensitive to the loss of HO-2, HO-3, and HO-4, but not HO-6. In addition, the hybridomas in groups I-III have been shown previously to require the ε-amino group in the side chain of the glycosylated hydroxylysine (see Table I.1).[43]

Interestingly, only three out of the twenty hybridomas investigated had a « weak » dependence on the less sterically hindered - also more flexible - primary hydroxy group at C-6, but all hybridomas require HO-4 of galactose to generate a full response.

I.4.3. Further evaluation of the role of HO-4

Following these results, *Holm* and colleagues published the synthesis and evaluation of two CII(259-273)-derived glycopeptides carrying *β*-galactosyl residues modified at C-4 by *O*-methylation and exchange of the hydroxy group for a fluorine atom.[46]

Scheme I.11 Preparation of building blocks carrying *β*-galactosyl residues modified at C-4[46]



49 R = OMe
50 R = F

51 R = OMe (yield = 60%)
52 R = F (yield = 66%)

53 R = OMe (yield = 76%)
54 R = F (yield = 82%)

(a) silver silicate, **19**, CH_2Cl_2, MS, 0°C ; (b) $(PPh_3)_4Pd(0)$, *N*-methylaniline, THF.

The synthesis of the glycosylated hydroxylysine building blocks **53** and **54**, shown in Scheme I.11, was performed according to the procedure described for the preparation of mono-deoxygenated building blocks **45-48**. As previously described, glycopeptide synthesis followed by deacylation and purification gave the two desired glycopeptides **GP14** and **GP15**.

Evaluation of glycopeptides **GP14** and **GP15** with four T-cell hybridomas requiring HO-4 to generate a full response (ie, belonging to group I, Table I.2) confirmed the important role of HO-4 of the *β*-galactosylated Hyl[264] in interactions with TCR.

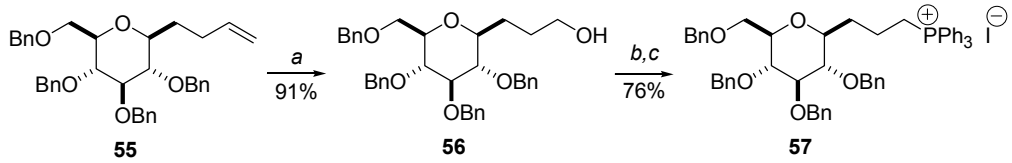
[46] Holm, B.; Baquer, S. M.; Holm, L.; Holmdahl, R.; Kihlberg, J. *Bioorg. Med. Chem.* **2003**, *11*, 3981.

I.5. Preparation and evaluation of a CII-analogue carrying a *C*-glycoside

I.5.1 Synthesis of *C*-galactosylated Hnv and incorporation into CII(256-270)

C-glycosylated amino acids are stable against both chemical and enzymatic degradation. An important property of *C*-glycosides is that they adopt conformations similar to the corresponding *O*-linked carbohydrates at the glycosidic bond.[47] *Wellner* and colleagues chose Hnv as the first target since three (among twenty) T-cell hybridomas have been found to respond equally well to the native glycopeptide **GP2** and to **GP6**. Moreover, this simplified analogue, which do not possess a stereogenic center at C-5, can easily be obtained by using the coupling method recently applied to the synthesis of *C*-glycosylated amino acid building blocks.[48]

Scheme I.12 Preparation of the phosphonium salt[48]



(a) O_3, $NaBH_4$, CH_2Cl_2/MeOH (2:5), -78°C → rt ; (b) PPh_3, imidazole, I_2, toluene ; (c) PPh_3, 120°C.

Preparation of the required ylide is presented in Scheme I.12. Ozonolysis of alkene **55** followed by a reductive work-up with sodium borohydride provided alcohol **56**, which was transformed into phosphonium salt **57** by a two-step procedure.

The corresponding ylide was coupled with *Garner* aldehyde[49] in a *Wittig* reaction to give *Z*-alkene **58** together with the corresponding *E*-isomer (Z/E > 14:1, 71%). Hydrogenation of the mixture of alkenes (using *Pearlman*'s catalyst) provided **59**, which was treated under

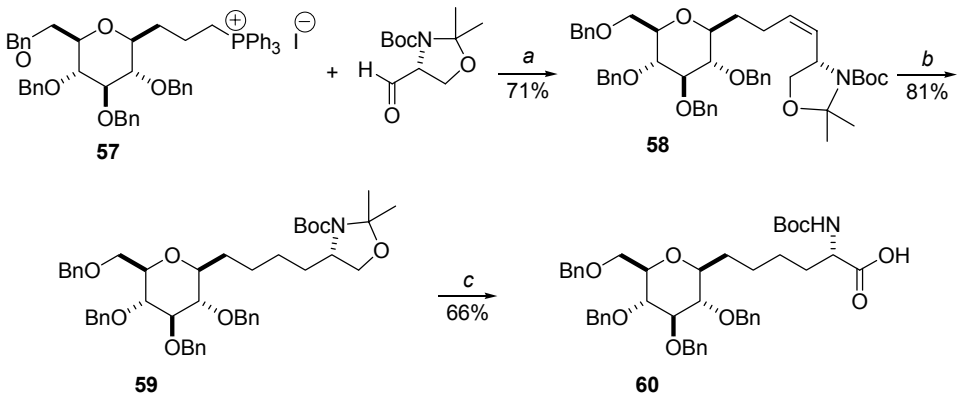
[47] (a) Goekjian, P. G.; Wu, T. C.; Kishi, Y. *J. Org. Chem.* **1991**, *56*, 6412. (b) Goekjian, P. G.; Wu, T. C.; Kang, H. Y.; Kishi, Y. *J. Org. Chem.* **1991**, *56*, 6422. (c) Wang, Y.; Goekjian, P. G.; Ryckman, D. M.; Miller, W. H.; Babirad, S. A.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 482. (d) Haneda, T.; Goekjian, P. G.; Kim, S. H.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 490.

[48] Wellner, E.; Gustafsson, T.; Bäcklund, J.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2000**, *1*, 272.

[49] (a) Garner, P. and Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361. (b) McKillop, A.; Taylor, R. J. K.; Watson, R. J.; Lewis, N. *Synthesis* **1994**, 31. (c) Dondoni, A. and Perrone, D. *Synthesis* **1997**, 527.

Jones oxidation conditions to give the desired protected *β*-D-galactosyl-CH_2-Hnv building block **60** (Scheme I.13).

Scheme I.13 Synthesis of the *C*-galactosylated building block[48]



(a) KHMDS, THF, -45°C → rt ; (b) H_2, $Pd(OH)_2$/C, AcOEt/MeOH (4:1) ; (c) *Jones* reagent, acetone.

A combination of the Boc and Fmoc protocols was used in the synthesis of the corresponding *C*-linked glycopeptide **GP16**. All amino acids, with the exception of Glu^{266} and *β*-D-Gal-CH_2-Hnv^{264}, were attached to the peptide resin carrying a N^{α}-Fmoc protective group. After cleavage from the resin and full deprotection by using TESOTf in TFA, purification by RP-HPLC gave **GP16** in 19% yield based on the resin capacity.

I.5.2. Immunological study

The response and specificity of the three hybridomas, which responded to both **GP2** and **GP6** (Table I.1, group IV), was investigated with *C*-linked glycopeptide **GP16**.[48]

Table I.3 Response of the $GalHyl^{264}$ specific hybridomas toward glycopeptide GP14

	Hybridoma I	Hybridoma II	Hybridoma III
GP2 or GP6	+++	+++	+++
GP16	+	+	-

The three hybridomas correspond to group IV of Table I.1; The sensitivity of the T cell hybridomas is given with an arbitrary scale: -, no reactivity; +, low reactivity; ++, medium reactivity; +++, high reactivity.

Two hybridomas recognized *C*-linked glycopeptide **GP16**, but at ten- to twenty-fold higher concentrations than required with **GP2** or **GP6**. Thus, even a minor structural change such as

replacement of an oxygen atom by a methylene group in a T-cell epitope has a significant influence on the T-cell response.

II. Objectives and Synthetic Issues

II.1. A brief statement

T-cells are known to be highly specific in their recognition of complexes between MHC molecules and peptide antigens. Slight structural modifications of amino acid side chains that contact the TCR can have a dramatic influence on the response of the T-cell, ranging from induction of selective stimulatory functions to completely turning off the functional capacity of the cell. Peptides in which TCR contact sites have been manipulated, but which retain the capacity to activate some TCR-mediated effecter functions, have been termed altered peptide ligands (APLs).[1] Importantly, such selective activation may result in induction of anergy ; a reduced ability of the T-cell to respond to a subsequent exposure to the stimulatory antigen. It can also lead to T-cell antagonism, defined as a down-modulation of agonist-induced T-cell proliferation when both agonist and APL are simultaneously presented to the T-cell. These data suggest that APLs could be used in immunotherapy of autoimmune diseases by inducing tolerance (ie, breaking autoimmunity). Promising results have already been obtained in animal models of experimental encephalomyelitis.[2]

Kihlberg and colleagues have shown that more than two-thirds of the twenty nine helper T-cell hybridomas obtained from mice immunized with CII responded to glycopeptide **GP2** in which Hyl^{264} carries a β-D-galactopyranosyl residue.[3] Additionally, removing the ε-amine function is detrimental for the recognition by TCR. In a parallel study, they also determined the relative position of the MHC anchor residues in the antigenic peptide (Ile^{260} and Phe^{263}), thus suggesting that the glycosylated hydroxylysine at position 264 may serve as the primary TCR contact.[4] In another paper, they investigated the dependence of the hybridomas on the

[1] (a) Evavold, B. D.; Sloan-Lancaster, J.; Allen, P. M. *Immunol. Today* **1993**, *14*, 602. for a review on altered peptide ligands, see: (b) Sloan-Lancaster, J. and Allen, P. M. *Annu. Rev. Immunol.* **1996**, *14*, 1.

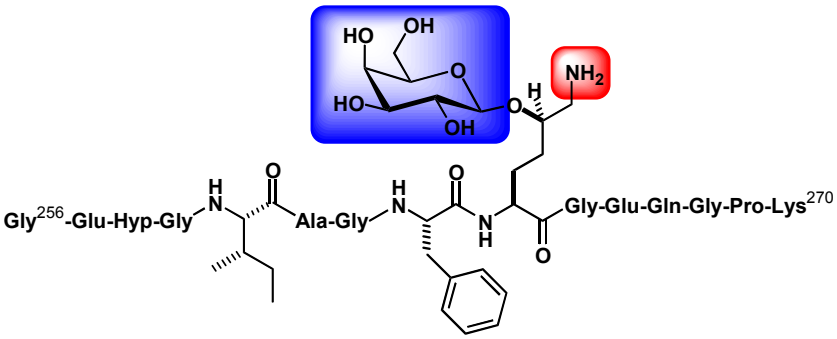
[2] (a) Nicholson, L. B.; Greer, J. M.; Sobel, R. A.; Lees, L. B.; Kuchroo, V. K. *Immunity* **1995**, *3*, 397. (b) Brocke, S.; Gijbels, K.; Allegratta, M.; Ferber, I.; Piercy, C.; Blankenstein, T.; Martin, R.; Utz, U.; Karin, N.; Mitchell, D.; Veromaa, T.; Waisman, A.; Gaur, A.; Conion, P.; Ling, N.; Fairchild, P. J.; Wraith, D. C.; O'Garra, A.; Fathman, C. G.; Steinman, L. *Nature* **1996**, *379*, 343.

[3] Corthay, A.; Bäcklund, J.; Broddefalk, J.; Michaëlsson, E.; Goldschmidt, T. J.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 2580.

[4] Kjellén, P.; Brunsberg, U.; Broddefalk, J.; Hansen, B.; Vestberg, M.; Ivarsson, I.; Engström, A.; Svejgaard , A.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 755.

different hydroxy groups of the galactose moiety.[5] The main result standing out of this study was the requirement of the secondary hydroxy group at C-4 to generate a full response.

Figure II.1 Key elements of the immunodominant T-cell epitope GP2



The galactose and the ε-primary amine being the two functional groups closely involved in the TCR-epitope interaction, fine modifications of the side chain of the galactosylated Hyl^{264} might allow indirect structural modulations of the TCR contacts. In vitro evaluation of glycopeptides incorporating galactosylated hydroxylysine mimetics with T-cell hybridomas from CIA that recognize galactosylated CII(256-270) (**GP2**) should afford a better understanding of the epitope recognition by TCR. In addition, slight structural modifications of the Hyl^{264} side chain could generate promising APLs.

II.2. Objectives : a new set of CII-glycopeptides for the determination of the fine specificity of T-cells

A structure / activity relationship study aimed at probing the fine specificity of glycopeptide-specific T-cells was envisioned by modulating key elements of the GalHyl residue and precisely analyzing their impact on the recognition by a panel of T-cell hybridomas. Several issues can be addressed in evaluating the role of Hyl side chain on the recognition pattern : (i) the permissiveness of the ε-functional group, (ii) the importance of the stereochemistry at C-5, (iii) the relative permissiveness of the carbohydrate anchorage

[5] (a) Holm, B.; Bäcklund, J.; Recio, M. A. F.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2002**, *3*, 1209. (b) Holm, B.; Baquer, S. M.; Holm, L.; Holmdahl, R.; Kihlberg, J. *Bioorg. Med. Chem.* **2003**, *11*, 3981.

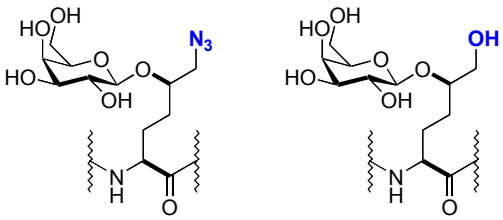
position relative to the ε-amino group and peptide backbone and (iv) the effect of steric hindrance at C-5.

The series of modifications on the natural epitope CII(256-270) carrying a galactosylated hydroxylysine residue at position 264 (shown in Figure II.2) were thus considered. The single shared element of the resulting glycopeptides making up this set is the *O*-linked galactosyl moiety. The modifications **A**-**F** can be classified into two subsets.

Figure II.2 The proposed modifications

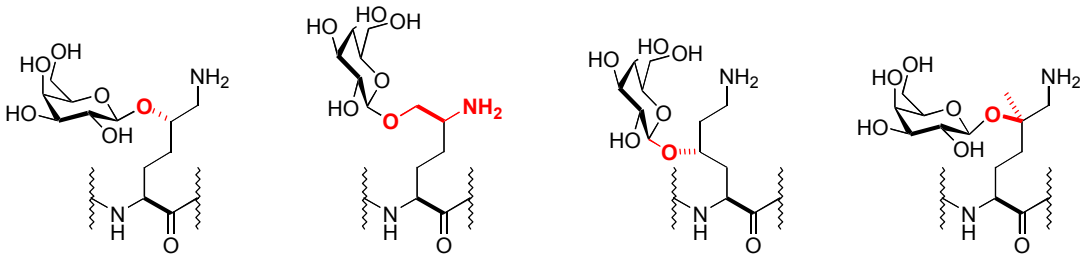
H-Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-**Xaa**^{264}-Gly-Glu-Gln-Gly-Pro-Lys^{270}-OH

Modulation of the primary amine :



A **B**

Modulation of the galactosyl anchorage :



C **D** **E** **F**

The first category, concerns the substitution of the primary ε-amino group. The detrimental effect of the absence of the primary amine on the epitope recognition has already been demonstrated,[3] but glycopeptides containing different ε-functional groups in place of this primary amine have not been evaluated so far. In residues **A** and **B**, the amino group is replaced by a hydrophobic azide function and by a hydrophilic hydrogen bond donor hydroxy group, respectively.

The second group of modifications (**C**-**F**) is intended to provide information on the requirements for positioning the galactosyl moiety, the ε-amine and the peptidic chain relative to each others in the TCR pocket. Modifications include inversion of stereochemistry at C-5 (**C**), permutation of the amino and galactosyl groups (**D**), anchorage of the *O*-linked *β*-galactosyl at C-4 (**E**), as well as introduction of a methylene group at C-5, while maintaining the (5*R*) stereochemistry of natural 5-hydroxylysine (**F**).

This study required research in organic and peptide chemistry, and addressed different synthetic issues to elaborate the new set of unnatural glycopeptides. Additionally, glycopeptides incorporating galactosylated hydroxylysine mimics will be evaluated in vitro with three T-cell hybridomas and one T-cell clone, all specific for CII.

II.3. Synthetic strategy

II.3.1. General considerations

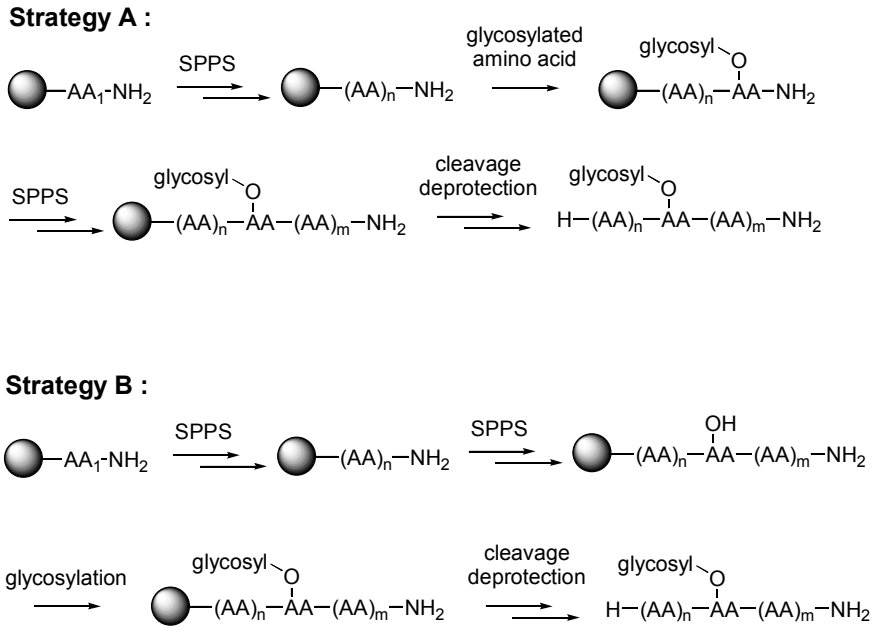
In the particular case of glycopeptide synthesis,[6] the choice of the method for solid phase assembly of glycopeptides is essential to elaborate the whole synthetic strategy.

There are two different approaches to the assembly of *O*-linked glycopeptides : (i) the building block approach and (ii) the direct condensation strategy. In the building block strategy (Figure II.3, strategy A), a glycosylated amino acid is prepared prior to glycopeptide synthesis and then incorporated into the growing peptide chain like any non-modified amino acid. Using the Fmoc strategy, this approach provides a particularly viable route to *O*-linked glycopeptides since it limits the exposure of the glycosidic linkage to acidic conditions to the final detachment step. The second approach, direct condensation (strategy B), reverses the two synthetic steps. It requires the preparation of a suitably protected peptide, which then serves as an acceptor in glycoside synthesis. This strategy has gained only little attention due

[6] for recent reviews, see: (a) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579. (b) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495. (b) Seitz, O. *ChemBioChem* **2000**, *1*, 214. (c) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317. (d) Kihlberg, J. and Elofsson, M. *Curr. Med. Chem.* **1997**, *4*, 85. (e) Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 294.

to poor yields and high consumption of glycosyl donors. However, few groups have recently presented successful glycosylations of resin-bound small peptides.[7]

Figure II.3 Approaches to solid phase synthesis of *O*-linked glycopeptides

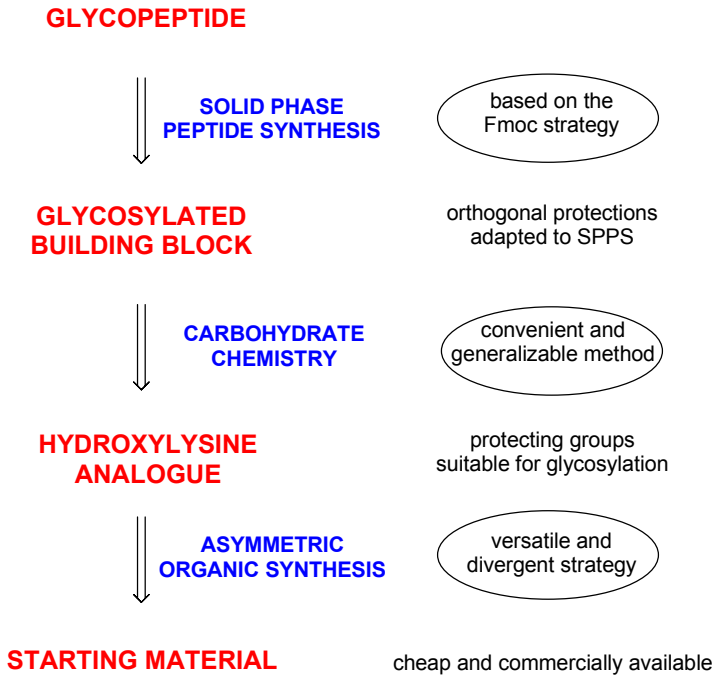


Today, assembly of Fmoc protected glycosylated amino acid building blocks still represents the most efficient and reliable method for preparing *O*-linked glycopeptides and the few research groups involved in the preparation of mono-galactosylated CII-derived glycopeptides (presented in section V) applied this strategy.

Our overall retrosynthetic strategy, from the final glycopeptide to the starting material through the three synthetic sequences, is depicted in Figure II.4. The major recurring issue of the synthesis concerned the simultaneous occurrence of diverse functionalities in a single molecule. Thus, the choice of an adapted protective group strategy was particularly important through the whole synthesis.

[7] for recent reviews, see: (a) Osborn, H. M. I. and Khan, T. H. *Tetrahedron* **1999**, *55*, 1807. for selected examples, see: (b) Halkes, K. M.; Gotfredsen, C. H.; Grotli, M.; Miranda, L. P.; Duus, J. O.; Meldal, M. *Chem. Eur. J.* **2001**, *7*, 3584. (c) Jobron, L. and Hummel, G. *Angew. Chem. Int. Ed.* **2000**, *39*, 1621.

Figure II.4 Global synthetic strategy for CII-derived glycopeptides synthesis



II.3.2. Synthesis of conveniently protected hydroxylysine analogues

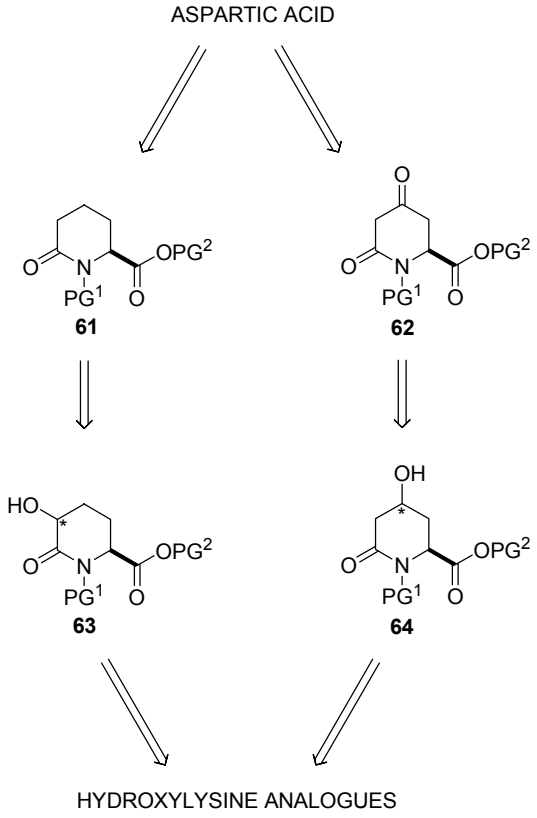
As I started my thesis, there was several routes to prepare the naturally occurring (2*S*,5*R*)-5-hydroxylysine (presented in section III).[8] However, these strategies were not versatile enough to afford an access to the desired hydroxylysine analogues. Our idea was to start from a single precursor and to develop a divergent strategy for the preparation of all hydroxylysine analogues.

II.3.2.1. The divergent approach

We reasoned that **A-F** could be accessible from a unique starting material : aspartic acid (Asp) ; a cheap and commercially available chiral building block. Our synthetic strategy relies on only two key intermediates : the enantiopure 5-hydroxy-6-oxo-1,2-piperidinedicarboxylate **63** and the enantiopure 4-hydroxy-6-oxo-1,2-piperidinedicarboxylate **64** (Figure II.5) ; both molecules sharing the piperidin-2-one ring structure.

[8] (a) Löhr, B.; Orlich, S.; Kunz, H. *Synlett.* **1999**, *7*, 1139. (b) van den Nieuwendijk, A. M. C. H.; Kriek, N. M. A. J.; Brussee, J.; van Boom, J. H.; van der Gen, A. *Eur. J. Org. Chem.* **2000**, 3683. (c) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron* **1999**, *55*, 63. (d) Allevi, P. and Anastasia, M. *Tetrahedron: Asymmetry* **2000**, *11*, 3151.

Figure II.5 Retrosynthesis of the divergent approach



The piperidin-2-one ring structure is a common structural feature in many natural products,[9] in synthetic molecules of biological interest (HIV protease inhibitors,[10a] glycosidase inhibitors,[10b] thrombin inhibitors,[10c] antagonists of the neurokinin-2 receptor[10d] and fibrinogen receptor antagonists[10e]) as well as in dipeptide surrogates and various

[9] selected examples: (a) Adalinine: Lognay, G.; Hemptinne, J. L.; Chan, F. Y.; Gaspar, C. H.; Marlier, M.; Braekman, J. C.; Daloze, D.; Pasteels, J. M. *J. Nat. Prod.* **1996**, *59*, 510. (b) Tasipeptins: Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 620. (c) Micropeptins: Reshef, V.; Carmeli, S. *Tetrahedron* **2001**, *57*, 2885. (d) Nostopeptins: Okino, T.; Qi, S.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 158. (e) Nostocyclin: Kaya, K.; Sano, T.; Beattie, K. A.; Geoffrey, A. *Tetrahedron Lett.* **1996**, *37*, 6725.

[10] (a) De Lucca, G. V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 501. (b) Nishimura, Y.; Adachi, H.; Satoh, T.; Shitara, E.; Nakamura, H.; Kojima, F.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 4871. (c) Minami, N. K.; Reiner, J. E.; Semple, J. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2625. (d) MacKenzie, A. R.; Marchington, A. P.; Middleton, D. S.; Newman, S. D.; Jones, B. C. *J. Med. Chem.* **2002**, *45*, 5365. (e) Chung, J. Y. L.; Hughes, D. L.; Zhao, D.; Song, Z.; Mathre, D. J.; Ho, G. J.; McNamara, J. M.; Douglas, A. W.; Reamer, R. A.; Tsay, F. R.; Varsolona, R.; McCauley, J.; Grabowski, E. J. J.; Reider, P. J. *J. Org. Chem.* **1996**, *61*, 215.

constrained peptidomimetics.[11,12] In addition, functionalized piperidin-2-ones are useful and versatile building blocks in organic synthesis. They serve as precursors in the synthesis of key constituents of bioactive molecules such as higher membered lactams,[13] enantiopure substituted piperidines,[14] pipecolic acids,[15] indolizidines,[16] quinolizidine[17] and isoquinolizidine[18] skeletons, as well as δ-amino acids.[19] Therefore, a number of approaches

[11] for reviews on bicyclic lactams as conformationally constrained dipeptide units, see: (a) Halab, L.; Gosselin, F.; Lubell, W. D. *Biopolymers* **2000**, *55*, 101. (b) Hanessian, S.; McNaughton-Smith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789.

[12] for representative examples, see: (a) Weber, K.; Ohnmacht, U.; Gmeiner, P. *J. Org. Chem.* **2000**, *65*, 7406. (b) Koulocheri, S. D.; Magiatis, P.; Haroutounian, S. A. *J. Org. Chem.* **2001**, *66*, 7915. (c) Al-Obeidi, F. A.; Micheli, B. J. M.; Barfield, M.; Padias, A. B.; Wei, Y.; Hall, H. K. Jr. *Macromolecules* **1999**, *32*, 6507. (d) Piro, J.; Rubiralta, M.; Giralt, E.; Diez, A. *Tetrahedron Lett.* **1999**, *40*, 4865. (e) De Laszlo, D. E.; Bush, B. L.; Doyle, J. J.; Greenlee, W. J.; Hangauer, D. G.; Halgren, T. A.; Lynch, R. J.; Schorn, T. W.; Siegl, P. K. S. *J. Med. Chem.* **1992**, *35*, 833. (f) Kemp, S.; McNamara, P. E. *J. Org. Chem.* **1985**, *50*, 5834.

[13] Suh, Y. G.; Kim, S. A.; Jung, J. K.; Shin, D. Y.; Min, K. H.; Koo, B. A.; Kim, H. S. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 3545.

[14] for a recent review, see: (a) Bailey, P. D.; Millwood, P. A.; Smith, P. D. *J. Chem. Soc., Chem. Commun.* **1998**, 633. for representative examples, see: (b) Micouin, L.; Varea, T.; Riche, C.; Chiaroni, A.; Quirion, J. C.; Husson, H. P. *Tetrahedron Lett.* **1994**, *35*, 2529. (c) Luker, T.; Hiemstra, H.; Speckamp, W. N. *J. Org. Chem.* **1997**, *62*, 3592. (d) Toyooka, N.; Yoshida, Y.; Yotsui, Y.; Momose, T. *J. Org. Chem.* **1999**, *64*, 4914. (e) Davis, F. A.; Chao, B.; Fang, T.; Szewczyk, J. M. *Org. Lett.* **2000**, *2*, 1041. (f) Agami, C.; Dechoux, L.; Ménard, C.; Hebbe, S. *J. Org. Chem.* **2002**, *67*, 7573. (g) Hanessian, S.; Seid, M.; Nilsson, I. *Tetrahedron Lett.* **2002**, *43*, 1991. (h) Hanessian, S.; van Otterlo, W. A. L.; Nilsson, I.; Bauer, U. *Tetrahedron Lett.* **2002**, *43*, 1995.

[15] for representative examples, see: (a) Davis, F. A.; Fang, T.; Chao, B.; Burns, D. M. *Synthesis* **2000**, *14*, 2106. (b) Battistini, L.; Zanardi, F.; Rassu, G.; Spanu, P.; Pelosi, G.; Gasparri Fava, G.; Belicchi Ferrari, M.; Casiraghi, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2975. (c) Koulocheri, S. D.; Magiatis, P.; Skaltsounis, A.-L.; Haroutounian, S. A. *Tetrahedron* **2002**, *58*, 6665. (d) Hanessian, S.; Reinhold, U.; Gentile, G. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1881.

[16] for a recent review, see: Nemr, A. E. *Tetrahedron* **2000**, *56*, 8579.

[17] Rubiralta, M.; Diez, A.; Vila, C.; Troin, Y.; Feliz, M. *J. Org. Chem.* **1991**, *56*, 6292.

[18] for selected examples, see: (a) Casamitjana, N.; Amat, M.; Llor, N.; Carreras, M.; Pujol, X.; Fernandez, M. M.; Lopez, V.; Molins, E.; Miravitlles, C.; Bosch, J. *Tetrahedron: Asymmetry* **2003**, *14*, 2033. (b) Dias, L. C.; Fernandes, A. M. A. P.; Zukerman-Schpector, J. *Synlett.* **2002**, *1*, 100. (c) Allin, S. M.; Vaidya, D. G.; James, S. L.; Allard, J. E.; Smith, T. A. D.; McKee, V.; Martin, W. P. *Tetrahedron Lett.* **2002**, *43*, 3661. (d) Roussi, F.; Quirion, J.-C.; Tomas, A.; Husson, H.-P. *Tetrahedron* **1998**, *54*, 10363. (e) Torisawa, Y.; Nakagawa, M.; Hosaka, T.; Tanabe, K.; Lai, Z.; Ogata, K.; Nakata, T.; Oishi, T.; Hino, T. *J. Org. Chem.* **1992**, *57*, 5741.

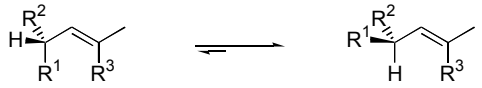
[19] (a) Kende, A. S.; Dong, H. Q.; Mazur, A. W.; Ebetino, F. H. *Tetrahedron Lett.* **2001**, *42*, 6015. (b) Casimir, J. R.; Didierjean, C.; Aubry, A.; Rodriguez, M.; Briand, J.-P.; Guichard, G. *Org. Lett.* **2000**, *2*, 895. (c) Muller, M.; Schoenfelder, A.; Didier, B. Mann, A.; Wermuth, C. G. *Chem. Commun.* **1999**, 683. (d) Karla, R.; Ebert, B.;

have been investigated for the preparation of substituted δ-lactams in enantiomerically pure form.[20] Although chiral auxiliaries have been utilized with great success,[21] amino acids proved to be particularly useful precursors for the asymmetric synthesis of substituted piperidin-2-ones.

We thus became interested in the stereocontrolled synthesis of *N*-acylated δ-lactams **63** and **64**, mono-hydroxylated at the α- and β- positions, respectively. This group of lactams has not received much attention so far, but *N*-acylated δ-lactams such as (2*S*)-6-oxo-1,2-piperidinedicarboxylate **61** and (2*S*)-4,6-dioxo-1,2-piperidinedicarboxylate **62** were thought to be useful precursors because of minimization of pseudo-allylic A(1,3) strain[22] which forces the ring substituent at the δ-position to adopt a pseudo-axial orientation. This pre-organization provides a high diastereofacial bias for further asymmetric transformations.

II.3.2.2. Pseudo-allylic A(1,3) strain

The notion of allylic A(1,3) strain was firstly defined by *Johnson*,[23] who stated that in the 3,3-disubstituted allylic system, those conformations in which the groups R^1 or R^2 and R^3 are coplanar, as in conformation A, are energetically unfavorable. The preferred conformation is B, in which the H-C-C=C-R^3 unit lies in one plan. Calculations[24] show that conformation B is almost exclusively populated, and that conformation A is approximately 3.5 kcal.mol^{-1} higher in energy.



CONFORMATION A CONFORMATION B

Thorkildsen, C.; Herdeis, C.; Johansen, T. N.; Nielsen, B.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1999**, *42*, 2053. (e) Rodriguez, M.; Aumelas, A.; Martinez, J. *Tetrahedron Lett.* **1990**, *31*, 5153.

[20] for a recent review, see: Weintraub, P. M.; Sabol, J. S.; Kane, J. M.; Borcherding, D. R. *Tetrahedron* **2003**, *59*, 2953.

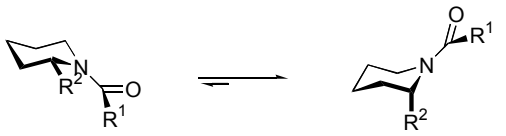
[21] (a) Meyers, A. I.; Brengel, G. P. *J. Chem. Soc., Chem. Commun.* **1997**, 1. (b) Micouin, L.; Jullian, V.; Quirion, J.-C.; Husson, H.-P. *Tetrahedron: Asymmetry* **1996**, *7*, 2839. (c) Enders, D.; Bartzen, D. *Liebigs Ann./Receuil* **1997**, 1115. (d) Enders, D.; Gröbner, R.; Raabe, G.; Runsink, J. *Synthesis* **1996**, 941.

[22] for reviews on allylic A(1,3) strain, see: (a) Hoffmann, R. H. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1124. (b) Hoffmann, R. H. *Chem. Rev.* **1989**, *89*, 1841.

[23] Johnson, F. *Chem Rev.* **1968**, *68*, 375.

[24] Broeker, J. L.; Hoffmann, R. W.; Houk, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 5006.

The phenomenon of allylic A(1,3) strain also applies to *N*-acylated lactams, due to the partial double-bond character of the N-CO bond. It has been known and understood for a long time that a ring substituents at position 2 of *N*-acyl piperidines preferentially adopts an axial arrangement (conformation D).



CONFORMATION C CONFORMATION D

Nature utilizes minimization of allylic A(1,3) strain to constrain flexible substances into an energetically-preferred spatial arrangement which can be related to biological activity.[25] In addition, this effect has proven to be a powerful tool to achieve conformational pre-organization and has allowed chemists to obtain high level of asymmetric induction in a predictable manner.[26] Herein, we would like to highlight a few particular examples related to the use of pseudo-allylic A(1,3) strain in *N*-acylated piperidine ring structures (Figure II.6).

Beak and *Zajdel* used the principle of minimization of pseudo-allylic A(1,3) strain to assign the orientation of a C-2 substituent of a *N*-acylated piperidine.[27] In 1988, *Brown* and colleagues described a highly stereoselective organocuprate attack on a *α,β*-unsaturated piperidin-4-one substrate in the total synthesis of (±)-Lasubine II.[28] More recently, *Mann*, *Wermuth* and colleagues[19c] as well as *Hanessian* and colleagues[14h,g] reported stereoselective 1,4-addition on *α,β*-unsaturated *δ*-substituted piperidin-2-ones. Interestingly, in the case of organocuprate attacks, the *cis*-isomer is predominantly formed when R^6 is a protected hydroxymethyl[14g,19c] or a phenyl[28] group. In the contrary, the *trans*-isomer is predominant when R^6 is an ester function.[14f,g,19c]

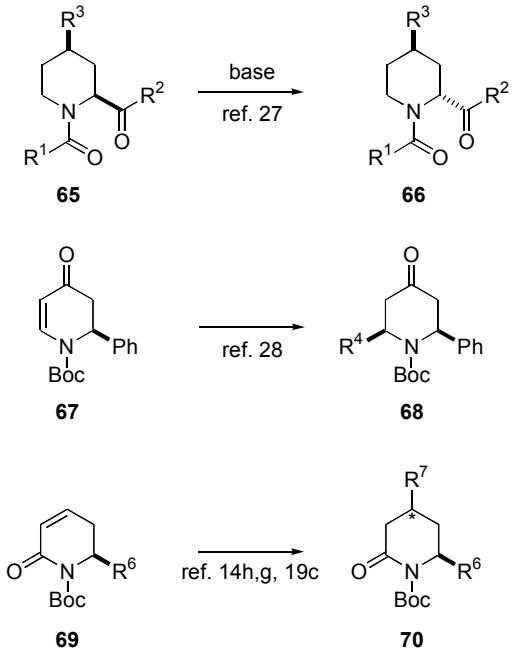
[25] for examples, see reference 22a and references cited therein.

[26] for utilization of allylic A(1,3) strain in the stereoselective alkylation of enolates derived from (a) 1-acyl-1,3-imidazolidin-4-ones and *N*-acyl-oxazolidin-5-ones, see: Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2708. (b) *N*-acyl-oxazin-2-ones, see: Dellaria, J. F.; Santarsiero, B. D. *J. Org. Chem.* **1989**, *54*, 3916. (c) *N*-acylmorpholin-3-ones, see: Fritch, P. C.; Kazmierski, W. M. *Synthesis* **1999**, *112*. Norman, B. H.; Kroin, J. S. *J. Org. Chem.* **1996**, *61*, 4990. Anthony, N. J.; Gomez, R. P.; Holtz, W. J.; Murphy, J. S.; Ball , R. G.; Lee, T. J. *Tetrahedron Lett.* **1995**, *36*, 3821. (d) pyroglutamic acid derivatives, see: Najera, C.; Yus, M. *Tetrahedron: Asymmetry* **1999**, *10*, 2245 and references therein.

[27] Beak, P. and Zajdel, W. J. *J. Am. Chem. Soc.* **1984**, *106*, 1010.

[28] Brown, J. D.; Foley, M. A.; Comins, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 7445.

Figure II.6 Applications of pseudo-allylic A(1,3) strain in *N*-acylated piperidine rings



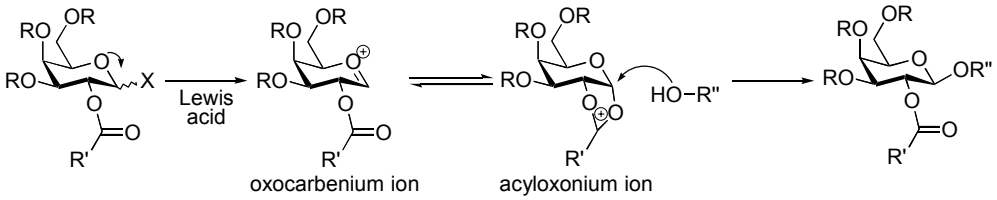
II.3.3. Synthesis of *β*-galactosylated building blocks

The general chemical approach to the formation of *O*-glycosidic bonds has remained almost unchanged since the beginning of the 20th century and the Koënigs-Knorr strategy (Figure II.7).[29,30] A glycosyl donor having an anomeric leaving group is converted to an oxocarbenium ion by action of a soluble or insoluble promoter (ie, *Lewis* acid). For the preparation of *β*-glycosides, the glycosyl donor usually carries a participating protective group at O-2 (ie, acyl). The subsequent attack of this reactive intermediate by the glycosyl acceptor leads to the formation of the *β*-glycoside.

[29] Koenigs, W. and Knorr, E. *Chem. Ber.* **1901**, *34*, 957.

[30] for reviews on methods for the synthesis of oligosaccharides and glycoconjugates, see: (a) Nicolaou, K. C. and Mitchell, H. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 1576. (b) Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212.

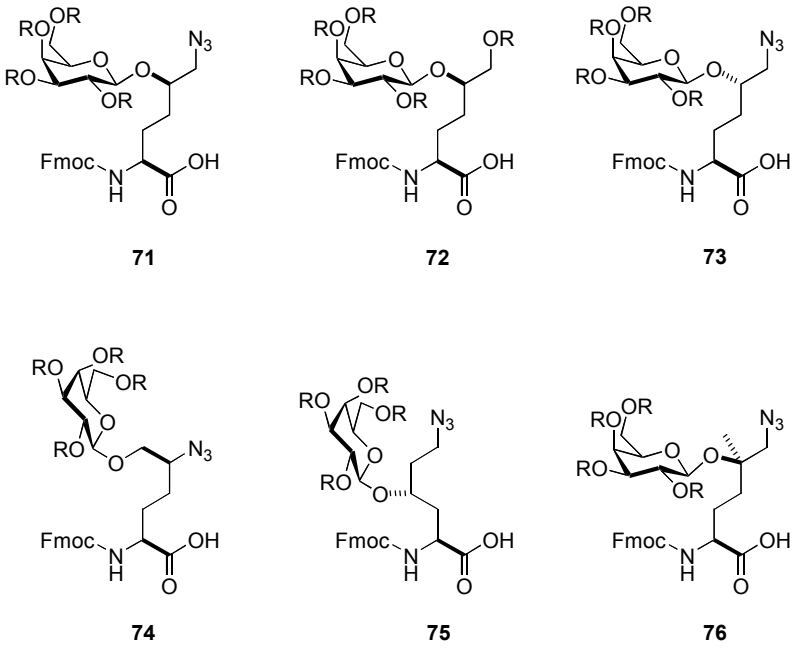
Figure II.7 General approach to the formation of *β*-glycosidic bonds



During hydroxylation, the initially formed oxocarbenium ion interacts with the acyl protective group to form a more stable cyclic acyloxonium ion. Nucleophilic attack at the anomeric center opens the acyloxonium ion to give the desired *β*-glycosides.

Few groups have already developed different conditions for the preparation of galactosylated (2*S*,5*R*)-5-hydroxylysine building blocks ready for use in SPPS (presented in section V).[31] Starting from the published glycosylations, our objective was to develop a unique procedure adapted to the preparation of all the building blocks presented in Figure II.8. Thus a common protective group strategy was required.

Figure II.8 Glycosylated building blocks



[31] (a) Holm, B.; Broddefalk, J.; Flodell, S.; Wellner, E.; Kihlberg, J. *Tetrahedron* **2000**, *56*, 1579. (b) Löhr, B. Orlich, S.; Kunz, H. *Proceeding of the 25th European Peptide Symposium, Edited by Bajusz, S. and Hudecz, F. Peptides* **1998**, 228. (c) Malkar, N. B.; Lauer-Fields, J. L.; Fields, G. B. *Tetrahedron Lett.* **2000**, *41*, 1137.

Building block **71** will be used in the preparation of the T-cell epitope **GP2** as well as the glycopeptide carrying an azide at the place of the *ε*-amino functionality. Building blocks **72-76** will be utilized in the preparation of the corresponding unnatural glycopeptides.

III. Preparation of 5-Hydroxylysine Analogues

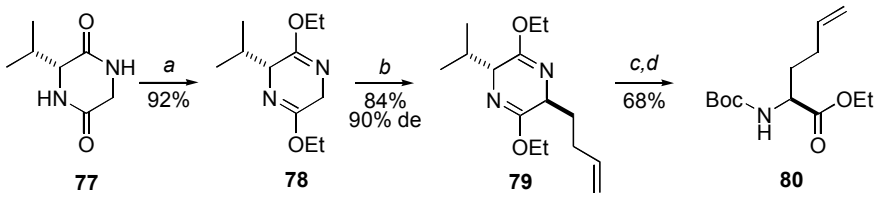
III.1. A new strategy for the preparation of (2*S*,5*R*)-5-hydroxylysine

III.1.1. Reported synthetic methods

Although (2*S*,5*R*)-5-hydroxylysine is commercially available, it is expensive and requires lengthy procedures for protection prior to its glycosylation and further use in glycopeptide synthesis (even if the procedure was recently improved by *Kihlberg* and colleagues,[1] see section I.3). Commercial (2*S*,5*R*)-5-hydroxylysine is commonly produced by a tedious procedure starting from gelatine acid hydrosylates.[2] Only recently, a few stereoselective approaches to the synthesis of protected derivatives of (2*S*,5*R*)-5-hydroxylysine have been investigated. Herein, we describe these methods together with other interesting strategies combining asymmetric steps and resolution procedures.

In the strategy developed by *Löhr* and colleagues,[3] Two successive asymmetric steps were used to create the two stereogenic centers. The 1,2-amino alcohol structure was incorporated by *Sharpless* asymmetric aminohydroxylation[4] (Scheme III.2) of a homoallyl glycine derivative **80** built using the *Schöllkopf* methodology (Scheme III.1).[5]

Scheme III.1 Schöllkopf synthesis for the stereoselective formation of the *α*-stereocenter[3]



77 **78** **79** **80**

(a) $BF_4(OEt)_3$; (b) nBuLi, 1-bromo-3-butene ; (c) HCl ; (d) Boc_2O.

[1] Holm, B.; Broddefalk, J.; Flodell, S.; Wellner, E.; Kihlberg, J. *Tetrahedron* **2000**, *56*, 1579.

[2] Sheehan, J. C. and Bolhofer, W. A. *J. Am. Chem. Soc.* **1950**, *72*, 2466.

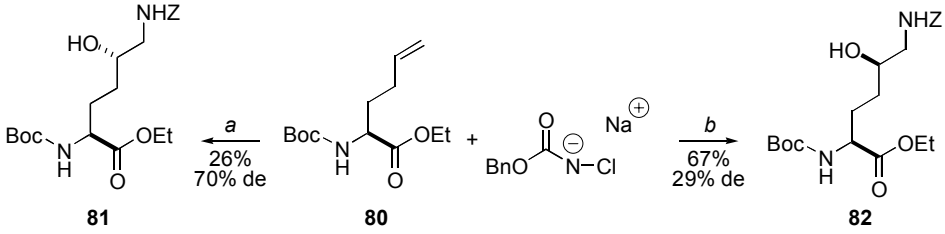
[3] Löhr, B.; Orlich, S.; Kunz, H. *Synlett.* **1999**, *7*, 1139.

[4] Li, G.; Han-Ting, C.; Sharpless, K. B. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 451.

[5] Schöllkopf, U.; Groth, U.; Chuanzeng, D. *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 798.

The diketopiperazine **77** required for the synthesis of the L-homoallyl glycine was obtained from D-valine and glycine. After transformation into the bislactim ether **78** and deprotonation, electrophilic attack by 1-bromo-3-butene gave the alkylated bislactim ether **79** in 84% yield and 90% diastereomeric excess (Scheme III.1).

Scheme III.2 Sharpless aminohydroxylation for the generation of the amino-alcohol moiety[3]



81 **80** **82**

(a) $K_2OsO_2(OH)_4$, $(DHQ)_2PHAL$; (b) $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$.

The use of the ligand $(DHQD)_2PHAL$ in the asymmetric aminohydroxylation of **80** led to the (2*S*,5*R*)-5-hydroxylysine derivative as the major product, while the ligand $(DHQ)_2PHAL$ produced the other isomer (Scheme III.2). Comparison of the yields and diastereoselectivities of the reactions shows that reactions with $(DHQ)_2PHAL$ give lower yields but higher diastereoslectivities than the reaction with $(DHQD)_2PHAL$, which gives a higher yield of the desired (2*S*,5*R*)-5-hydroxylysine derivative **82**, but a low diastereoselectivity.

More recently, *Brussee* and colleagues[6] developed a ten-step stereoselective synthesis involving the *Williams*' glycine template methodology[7] and (*R*)-hydroxynitrile lyase[8] for the introduction of the chirality at the 2- and 5-position, respectively. In the first step, (3*E*)-3-hexenal **83** was converted into its (*R*)-cyanohydrin with the aid of purified (*R*)-hydroxynitrile lyase. Upon treatment with TBDPSCl, *O*-protected cyanohydrin **84** was obtained. Conversion into the *O*,*N*-protected amino alcohol **85** was accomplished by a four-step, one-pot procedure. Subsequent removal of the TBDPS moiety, followed by acid-catalysed reaction with 2-methoxypropene, afforded oxazolidine **86** in 80% yield. Ozonolysis and reductive work-up

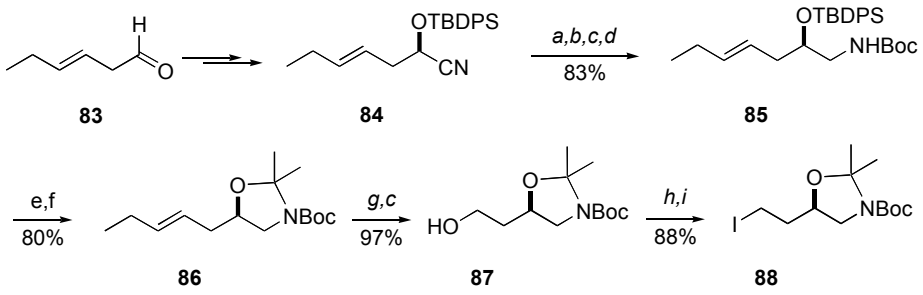
[6] van den Nieuwendijk, A. M. C. H.; Kriek, N. M. A. J.; Brussee, J.; van Boom, J. H.; van der Gen, A. *Eur. J. Org. Chem.* **2000**, 3683.

[7] (a) Williams, R. M.; Im, M. N. *J. Am. Chem. Soc.* **1991**, *113*, 9276. (b) Dellaria, J. F. and Santarsiero, B. D. *J. Org. Chem.* **1989**, *54*, 3916.

[8] Smitskamp-Wilms, E.; Brussee, J.; van der Gen, A.; van Scharrenburg, G. J. M.; Sloothak, J. B. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 209.

gave the primary alcohol **87** almost quantitatively. Iodide **88** was obtained by a two-step procedure (Scheme III.3).

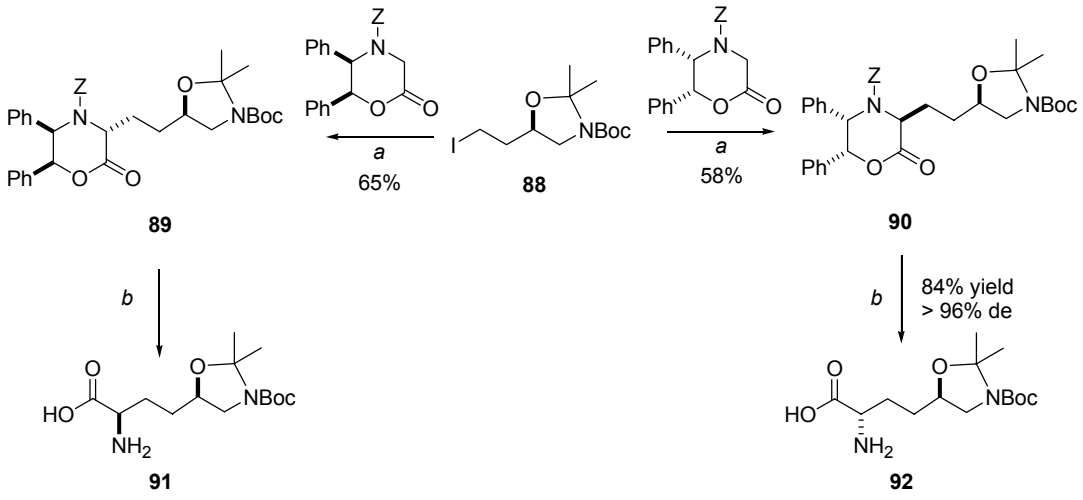
Scheme III.3 Preparation of the iodide 88[6]



(a) DIBAL ; (b) MeOH ; (c) $NaBH_4$; (d) Boc_2O ; (e) TBAF ; (f) 2-methoxypropene ; (g) O_3 ; (h) Tosyl chloride ; (i) NaI.

Alkylation of *Williams*' glycine template with iodide **88** gave **90** in 58% yield and >96% diastereomeric excess (de). Reductive deprotection of **90** gave protected (2*S*,5*R*)-5-hydroxylysine **92** in 84% yield (Scheme III.4). Additionally, alkylation of the enantiomer template and further deprotection according to the same procedure allowed the preparation of (2*R*,5*R*)-5-hydroxylysine **91**.

Scheme III.4 Synthesis of protected (2*S*,5*R*)- and (2*R*,5*R*)-5-hydroxylysine[6]

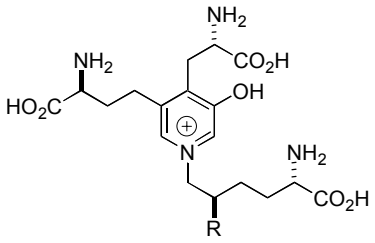


(a) NaHMDS ; (b) H_2, Pd/C.

This lengthy synthetic procedure afforded (2*S*,5*R*)-5-hydroxylysine in 28% overall yield starting from **84**.

Other reported strategies utilized (*S*)-glutamic acid as a starting material.[9] However, these routes are not stereoselective and the development of resolution methods was thus necessary.

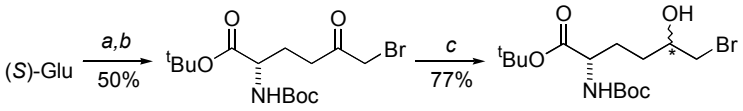
In the course of the synthesis of collagen cross-links pyridinoline **93** and deoxypyridinoline **94**, *Adamczyk* and colleagues[9a] prepared (2*S*,5*R*)-5-hydroxylysine.



93 R = OH
94 R = H

The bromo alcohol **96** was obtained in a three-step procedure as a 1:1 diastereomeric mixture by reduction of the α-bromo ketone **95** (Scheme III.5).

Scheme III.5 Synthesis of the hydroxy-bromo intermediate from (*S*)-glutamic acid[9a]



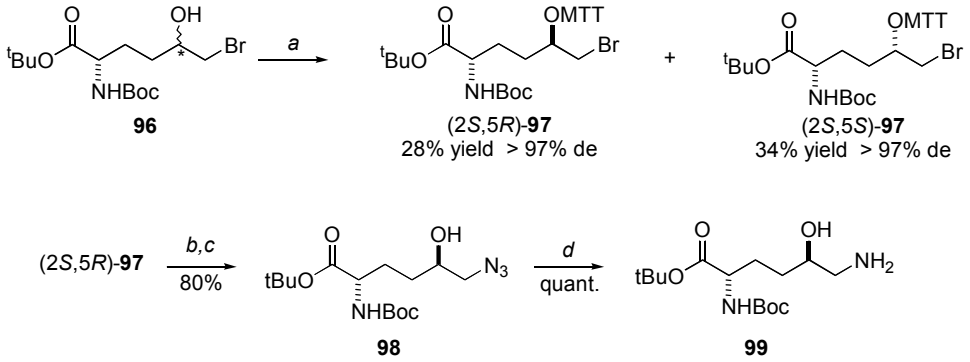
95 **96**

(a) Isobutyl chloroformate, NMM, THF, 0°C, then CH_2N_2, ether, 0°C→rt ; (b) 48% HBr, ether, –20°C ; (c) $NaBH_4$, MeOH, 0°C→rt.

As all the common separation procedures failed, **96** was esterified with *O*-methoxy-*N*-(*tert*-butoxycarbonyl)-*L*-thyroxine (MTT) prior to separation by preparative HPLC (Scheme III.6). (2*S*,5*R*)-**97** was treated with sodium azide and the resulting crude compound was hydrolyzed using lithium hydroxide to give **98** in 80% yield. Quantitative reduction of the azide function gave protected (2*S*,5*R*)-5-hydroxylysine **99**.

[9] (a) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron* **1999**, *55*, 63. (b) Allevi, P. and Anastasia, M. *Tetrahedron: Asymmetry* **2000**, *11*, 3151.

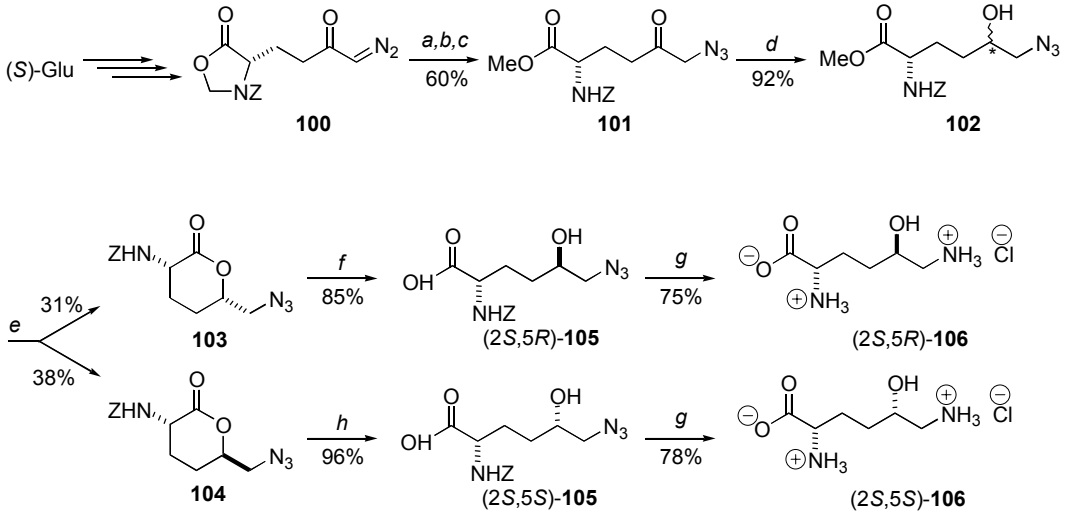
Scheme III.6 Preparation of protected (2*S*,5*R*)-5-hydroxylysine[9a]



(a) (i) MTT, DCC, CH_2Cl_2, rt, (ii) separation ; (b) NaN_3, DMF, 80°C ; (c) LiOH, THF/H_2O, rt ; (d) Pd/C, H_2, EtOH.

One year later, *Allevi* and *Anastasia*[9b] reported the synthesis of all 4 possible stereomers of 5-hydroxylysine, by a similar strategy. As *Adamczyk* and colleagues, they did not develop the stereoselective reduction of the *α*-azido ketone **101**, but a novel separation procedure based on lactonization of the corresponding hydroxy ester **102** (Scheme III.7).

Scheme III.7 Synthesis of (2*S*,5*R*)- and (2*S*,5*S*)-5-hydroxylysine[9b]



(a) $NaHCO_3$, MeOH, reflux ; (b) HBr 33% in AcOH, THF, 0°C ; (c) NaN_3, DMF, rt ; (d) $NaBH_4$, MeOH, 0°C ; (e) TFA, rt ; (f) Cs_2CO_3, MeOH/H_2O (1:1), rt ; (g) H_2, Pd/C, MeOH/H_2O (1:1), then pH 6.5-7.0 ; (h) *α*-chymotripsin, phosphate buffer/acetone, 25°C.

The diazo ketone **100**, prepared in three steps from (*S*)-glutamic acid, was transformed into the azido ketone **101**, which, by reduction with sodium borohydride, afforded the hydroxy ester **102**, as a mixture of two diastereomers. The unique efficient method for the diastereomers resolution was found to be their transformation into the corresponding lactones

103 and **104**, which were separated by chromatography on silica. Thus, (2*S*,5*R*)-**106** and (2*S*,5*S*)-**106** were obtained in a two-step procedure from **103** and **104**, respectively.

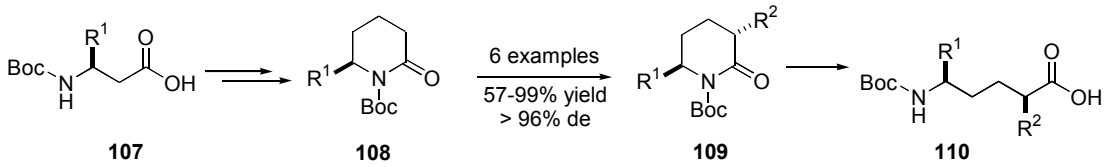
Using the same methodology, (*R*)-glutamic acid should allow the preparation of (2*R*,5*S*)- and (2*R*,5*R*)-5-hydroxylysine.

Overall, these methods suffer from limitations including the need for resolutions, lengthy synthetic procedures and / or separation of diastereomers.

III.1.2. The proposed strategy

None of the aforementioned syntheses has taken advantage of the α-stereogenic center of a starting α-amino acid to control the stereochemistry while the second stereogenic center at C-5 is introduced. We recently reported a short and stereoselective route to optically active 1,4-disubstituted δ-amino acids that allows the incorporation of various natural and non-natural side chain functionalities at the α-position.[10] The method involved the alkylation of *N*-Boc-protected 6-alkylated δ-lactams readily prepared from the *N*-Boc protected β^3-amino acids *via* reduction of the keto functionality of the corresponding β-aminoacyl *Meldrum*'s acid.

Scheme III.8 Synthesis of α,δ-disubstituted δ-amino acids[10]



107 **108** **109** **110**

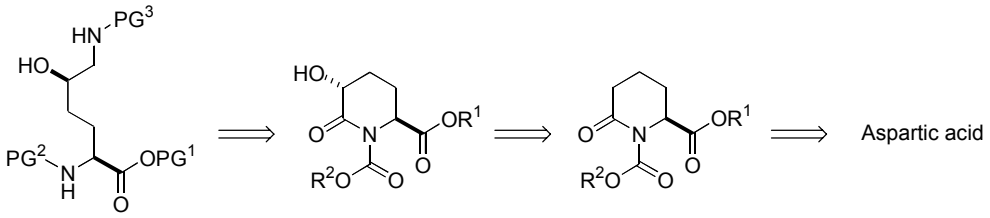
The high level of 1,4-asymmetric induction achieved during alkylation of the corresponding enolate anion is probably due to the minimization of the pseudo-allylic A(1,3) strain (see explanations in section II).[11] Indeed, the ring substituent at the 6-position is believed to adopt a quasi-axial conformation, which can provide a high diastereofacial bias in the alkylation step.

[10] Casimir, J. R.; Didierjean, C.; Aubry, A.; Rodriguez, M.; Briand, J. P.; Guichard, G. *Org. Lett.* **2000**, *2*, 895.

[11] for reviews on allylic A(1,3) strain, see: (a) Hoffmann, R. H. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1124. (b) Hoffmann, R. H. *Chem. Rev.* **1989**, *89*, 1841.

Herein, we propose a new expedient stereoselective synthesis of orthogonally protected (2*S*,5*R*)-5-hydroxylysine starting from aspartic acid as an inexpensive chiral educt, the existing *α*-stereogenic center serving for asymmetric induction at C-5. Our strategy (retrosynthetic analysis shown in Scheme III.9) is based on asymmetric oxidation of enolates generated from *δ*-lactams **61** to give the corresponding *α*-hydroxy carbonyl compounds **63**. In order to prepare CII-derived glycopeptides, the 5-hydroxylysine analogue **111** carrying suitable protecting groups (PG^1, PG^2 and PG^3) for glycosylation and SPPS was also prepared.

Scheme III.9 Retrosynthesis of orthogonally protected (2*S*,5*R*)-5-hydroxylysine



111 **63** **61** **112**

Additionally, this strategy, which is versatile, was used to prepare other valuable organic compounds (ie, *δ*-hydroxylated *δ*-amino acids and (+)-pyridinoline precursors) as well as new unnatural 5-hydroxylysine derivatives for use in preparation of new APLs[12] of the immunodominant epitope CII(256-270).

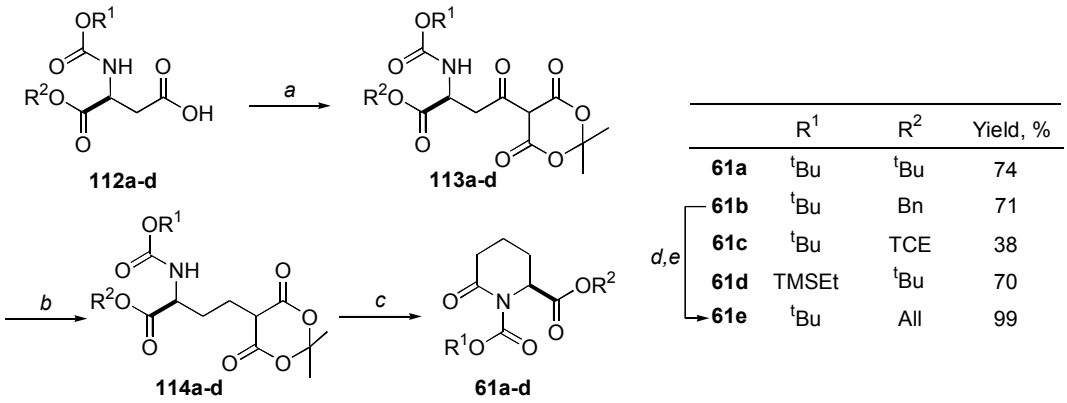
III.1.3. *δ*-Lactams synthesis

Several (2*S*)-6-oxo-1,2-piperidinedicarboxylates **61a-e** varying at R^1 or R^2 were synthesized for the purpose of studying the stereodirecting effect of the ester substituent at C-2 and the influence of the *N*-protecting groups. Piperidinones **61a-d** were prepared from the conveniently protected aspartic acid derivatives **112a-d** using a procedure similar to that previously described by us for the synthesis of *N*-protected 6-alkylated *δ*-lactams[10] and by *Smrcina* and colleagues for the synthesis of pyrrolidin-2-ones[13] (Scheme III.10).

[12] for a review on altered peptide ligands, see: Sloan-Lancaster, J. and Allen, P. M. *Annu. Rev. Immunol.* **1996**, *14*, 1.

[13] Smrcina, M.; Majer, P.; Majerova, E.; Guerassina, T. A.; Eissenstat, M. A. *Tetrahedron* **1997**, *53*, 12867.

Scheme III.10 Preparation of δ-lactam derivatives

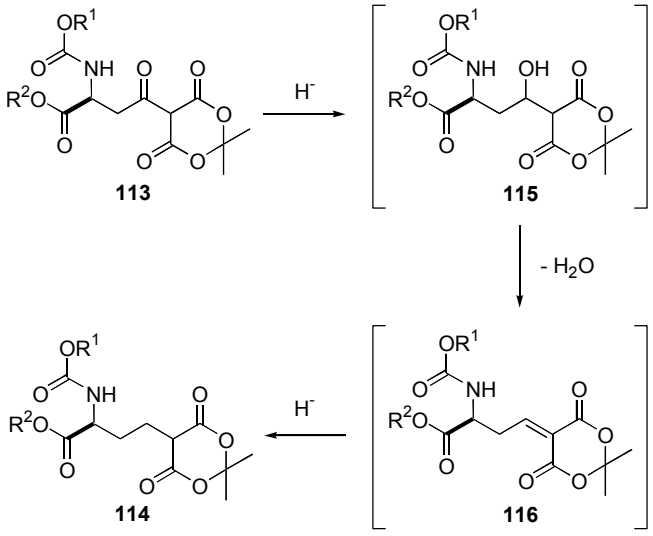


	R^1	R^2	Yield, %
61a	^{t}Bu	^{t}Bu	74
61b	^{t}Bu	Bn	71
61c	^{t}Bu	TCE	38
61d	TMSEt	^{t}Bu	70
61e	^{t}Bu	All	99

(a) *Meldrum*'s acid, EDC, DMAP, CH_2Cl_2 ; (b) $NaBH_4$, CH_2Cl_2/AcOH (10:1) ; (c) toluene, reflux ; (d) H_2, Pd/C, EtOH ; (e) allyl bromide, DBU, CH_3CN.

The condensation of **112** with *Meldrum*'s acid afforded **113**, which was used in the next step without further purification. Treatment of **113** with sodium borohydride in CH_2Cl_2 / AcOH at room temperature resulted in complete reduction of its ketone functionality to give **114**. It is likely that, according to the previously reported mecanism,[13] **113** first underwent reduction of its ketone functionality to the β-hydroxy diester, followed by dehydration to the unsaturated ester, which was further reduced to **114** *via Michael* addition of hydride ion (Scheme III.11).

Scheme III.11 Mecanism of reduction of 113 into 114



These compounds were obtained in excellent yield and easily purified by crystallization with the exception of **114b**, for which a flash chromatography was necessary. Decarboxylative ring closure of **114** in refluxing toluene afforded lactams **61** in moderate to good yields after flash chromatography. Additionally, we prepared the piperidinone **61e** in a two-step procedure starting from **61b**. The quantitative reduction of the benzyl ester gave the free carboxylic acid, which was directly protected by reaction with allyl bromide in the presence of DBU to yield **61e**.

III.1.4. α-Hydroxylation studies

Initial oxidation studies were conducted on the enolate of di-*tert*-butyl (2*S*)-6-oxo-1,2-piperidinedicarboxylate **61a**. Various oxidizing agents were evaluated, including *Vedejs*' MoOPH and *Davis*' oxaziridines.[14,15] In all experiments described herein, **61a** was converted to the corresponding enolate by treatment with 1.1 equiv of LiHMDS or NaHMDS at −78 °C in THF for 2.5 h, before reaching the required temperature for oxidation and addition of the oxidizing agent. Even if most of the oxidizers tested are commercially available, we chose to prepare them following the procedures described in literature.

III.1.4.1. Preparation of the oxidizing agents

Preparation of MoOPH (MoO_5•Py•HMPA): *Mimoun* and colleagues[16] have firstly described the isolation of crystalline molybdenum peroxides having a variety of ligands. But *Vedejs* and colleagues[14a,b] were the first to describe the use of these complexes in oxidation reactions. We followed their two-step procedure to obtain MoOPH in a good yield on a 20g

[14] for oxidations using MoOPH, see: (a) Vedejs, E. *J. Am. Chem. Soc.* **1974**, *94*, 5944. (b) Vedejs, E.; Engler, D. A.; Telschow, J. E. *J. Org. Chem.* **1978**, *43*, 188. for a comparison between MoOPH and (±)-PPO, see: (c) Davis, F. A.; Vishwakarma, L. C.; Billmers, J. M. *J. Org. Chem.* **1984**, *49*, 3243. (d) Natale, N. R.; McKenna, J. I.; Nio, C. S.; Borth, M. *J. Org. Chem.* **1985**, *50*, 5660. for oxidations using (±)-PPO and / or (+)- and (-)-CSO, see: (e) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346. (f) Davis, F. A.; Wei, J.; Sheppard, A. C.; Gupernick, S. *Tetrahedron Lett.* **1987**, *28*, 5115. (g) Smith, A. B., III; Dorsey, B. D.; Ohba, M.; Lupo, A. T., Jr; Malamas, M. S. *J. Org. Chem.* **1988**, *53,* 4314. (h) Davis, F. A.; Sheppard, A. C.; Chen, B. C.; Serajul Haque, M. *J. Am. Chem. Soc.* **1990**, *112*, 6679.

[15] for reviews on the chemistry of *N*-sulfonyl oxaziridines, see: (a) Davis, F. A.; Sheppard, A. C. *Tetrahedron* **1989**, *45*, 5703. (b) Davis, F. A.; Chen, B. C. *Chem. Rev.* **1992**, *92*, 919.

[16] for the preparation of MoOPH, see: Mimoun, H.; Seree de Roch, I.; Sajus, L. *Bull. Soc. Chim.* **1969**, *5*, 1481.

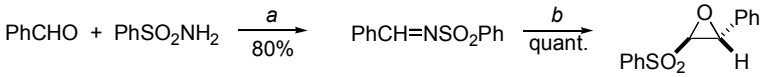
scale (Scheme III.12). A solution of $H_2Mo_2O_{11}$ was obtained by dissolving MoO_3 in 30% H_2O_2 at 40°C. Addition of HMPA to this solution afforded crystalline $MoO_5 \bullet H_2O \bullet$HMPA easily recovered by filtration. This complex was quantitatively transformed into the corresponding anhydrous peroxide under high vacuum in the presence of P_2O_5. After dissolution of the anhydrous peroxide in THF and addition of one equivalent of pyridine, MoOPH precipitated as a yellow crystalline solid. The analytical data of MoOPH were in accordance with the literature,[16] but ^{1}H NMR characterization showed a low contamination with the hydrate complex.

Scheme III.12 Preparation of MoOPH[16]

(a) H_2O_2, 40°C, then HMPA ; (b) P_2O_5, high vacuum ; (c) pyridine, rt.

Preparation of (±)-trans-2-(phenylsulfonyl)-3-phenyloxaziridine ((±)-PPO): (±)-PPO was prepared in a two-step procedure starting from benzaldehyde and benzenesulfonamide (Scheme III.13). In our hands, the reductive amination, described by *Vishwakarma* and colleagues,[17] gave *N*-benzylidenebenzenesulfonamide in 80% yield. For the oxidation step, we chose to replace *m*CPBA by oxone, as described by *Davis* and colleagues,[18] which gave us (±)-PPO in quantitative yield. For both compounds ((±)-PPO and the intermediate) the analytical data were in accordance with the literature.[17]

Scheme III.13 Preparation of (±)-PPO[17,18]



(a) amberlyst 15, MS, toluene, reflux ; (b) oxone, $NaHCO_3$, toluene/H_2O.

Preparation of (+)-(2*R*,8a*S*)-10-(camphorsulfonyl)oxaziridine ((+)-CSO): (-)-10-camphorsulfonyl chloride was obtained in 83% yield (20g scale) from commercial (+)-10-camphorsulfonic acid by using the procedure described by *Bartlett* and *Knox*.[19] For the next

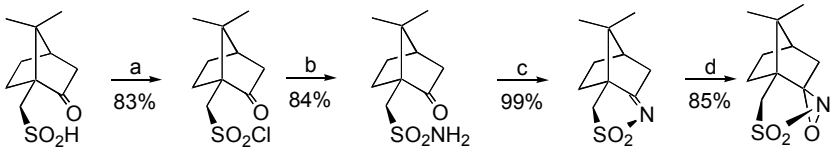
[17] Vishwakarma, L. C.; Stringer, O. D.; Davis, F. A. *Org. Synth.* **1987**, *66*, 203.

[18] Davis, F. A.; Chattopadhyay, S.; Towson, J. C.; Lal, S.; Reddy, T. *J. Org. Chem.* **1988**, *53*, 2087.

[19] Bartlett, P. D.; Knox, L. H. *Organic Syntheses*; Wiley: New York, **1973**; *Collect. Vol. V*, pp 196.

three steps, we initially followed the methodology developed by *Towson* and colleagues.[20] However, for the oxidation of the imine into the oxaziridine, oxone did not seem to be the reagent of choice. Indeed, the variable reactivity of the batches gave inconsistent oxidation rates, which rendered the reaction difficult to monitor. We thus preferred the procedure recently described by *Bulman Page* and colleagues,[21] which in our hands gave (+)-CSO in 85% yield.

Scheme III.14 Preparation of (+)-CSO[19,20,21]



(a) PCl_5, O°C→rt ; (b) NH_4OH, CH_2Cl_2, 0°C ; (c) Amberlyst 15, MS, toluene, reflux ; (d) H_2O_2, K_2CO_3, MeOH, rt.

(−)-CSO was prepared using the same procedure but starting from (−)-10-camphorsulfonic acid. In both syntheses, the analytical data of intermediates and final compounds were in accordance with the literature.[20]

III.1.4.2. Optimization of the hydroxylation step

The results presented in Table III.1 show the influence of the counterion (Li^+ or Na^+) and the conjugated effects of temperature and reaction time for the four electrophiles mentioned above.

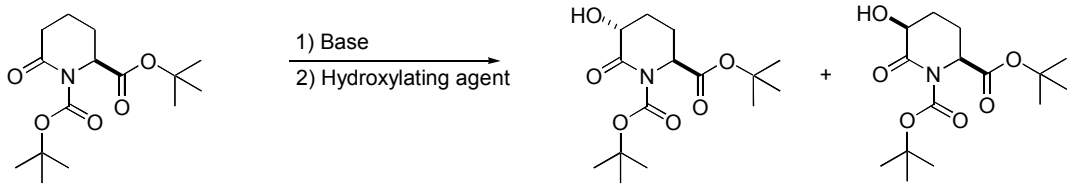
Initial oxidation studies with *Vedejs*' reagent confirmed the stereodirecting effect of the *tert*-butyl ester substituent. When the lithium enolate of **61a** was treated with MoOPH for 1 h, the corresponding hydroxylayed adduct **63a** was obtained in low yield with 76% de. The ratio between the two diastereomers was determined by RP-HPLC of the crude product using a C_{18} column. The major diastereomer, subsequently identified as (5*R*)-**63a**, (*vide infra*) was isolated in 20% yield (Table III.1, entry 1). Recovery of 73% of starting material **61a** suggested that the low yield was attributable to a slow oxidation rate at −78°C. Attempts to improve the yield by a slow increase of the temperature over a long period of time (entry 2)

[20] Towson, J. C.; Weismiller, M. C.; Sankar Lal, G. Org. Synth. 1990, 69, 158.

[21] Bulman Page, P. C.; Heer, J. P.; Bethell, D.; Lund, A.; Collington, E. W.; Andrews, D. M. *J. Org. Chem.* **1997**, *62*, 6093.

did not give any hydroxylated product and resulted in nearly complete degradation of **61a**. Similarly, no hydroxylation took place at −78°C with the more nucleophilic sodium enolate (entry 3). However, performing the experiment with Li-enolate at −50°C during 0.5 h gave both a dramatic increase in yield and good stereoselectivity (entry 4). Replacing LiHMDS by NaHMDS improved the yield but resulted in lower diastereoselectivity (entry 5 vs 4). In all cases, the remaining starting material made the flash chromatography of **63a** difficult, and a non-negligible amount of product was lost during purification.

Table III.1 Counterion and hydroxylating agent study for preparation of 63a



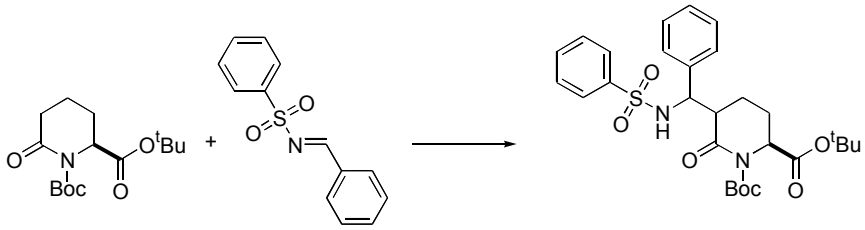
61a (5*R*)-**63a** (5*S*)-**63a**

Entry	Base	Electrophile	T, °C	Time, h	Dr[a], (5*R*)-**63a**:(5*S*)-**63a**	Purified yield of (5*R*)-**63a**, %
1	LiHMDS	MoOPH[b]	−78	1.0	88:12	20 (73)[c]
2	LiHMDS	MoOPH[b]	−78 → rt	16.0	nd	< 5
3	NaHMDS	MoOPH[b]	−78	3.0	-	0
4	LiHMDS	MoOPH[b]	−50	0.5	91:9	77
5	NaHMDS	MoOPH[b]	−50	0.5	83:17	83
6	LiHMDS	(±)-PPO[d]	−78	1.0	97:3	< 10
7	NaHMDS	(±)-PPO[d]	−78	0.5	96:4	38
8	NaHMDS	(±)-PPO[d]	−78	0.25	94:6	48
9	NaHMDS	(±)-PPO[d]	−78	3.0	nd	< 5
10	LiHMDS	(+)-CSO[e]	−78	0.25	99:1	42 (53)[c]
11	LiHMDS	(+)-CSO[e]	−50	0.25	97:3	77
12	**LiHMDS**	**(+)-CSO[e]**	**−78**	**16.0**	**98:2**	**92**
13	LiHMDS	(−)-CSO[e]	−78	0.25	99:1	21 (78)[c]
14	LiHMDS	(−)-CSO[e]	−50	0.25	97:3	62
15	LiHMDS	(−)-CSO[e]	−78	16.0	98:2	76

[a] Ratio determined by analytical C_{18} RP-HPLC of the crude product ; [b] MoOPH = MoO_5•pyridine•HMPA ; [c] % of recovered starting material ; [d] PPO = trans-2-(phenylsulfonyl)-3-phenyloxaziridine ; [e] CSO = (10-camphorsulfonyl)-oxaziridine.

Oxidation of the lithium enolate of **61a** by (±)-PPO for 1 h (entry 6) resulted in the formation of the imino-aldol **117** (Scheme III.15) as the major product together with **63a** in low yield but 94% de. **117** was unambiguously assigned by ^{1}H NMR and resulted from the addition of the enolate to the sulfonimine generated from (±)-PPO.[14e-h,15]

Scheme III.15 Side reaction, formation of the imino-aldol 117



61a **117**

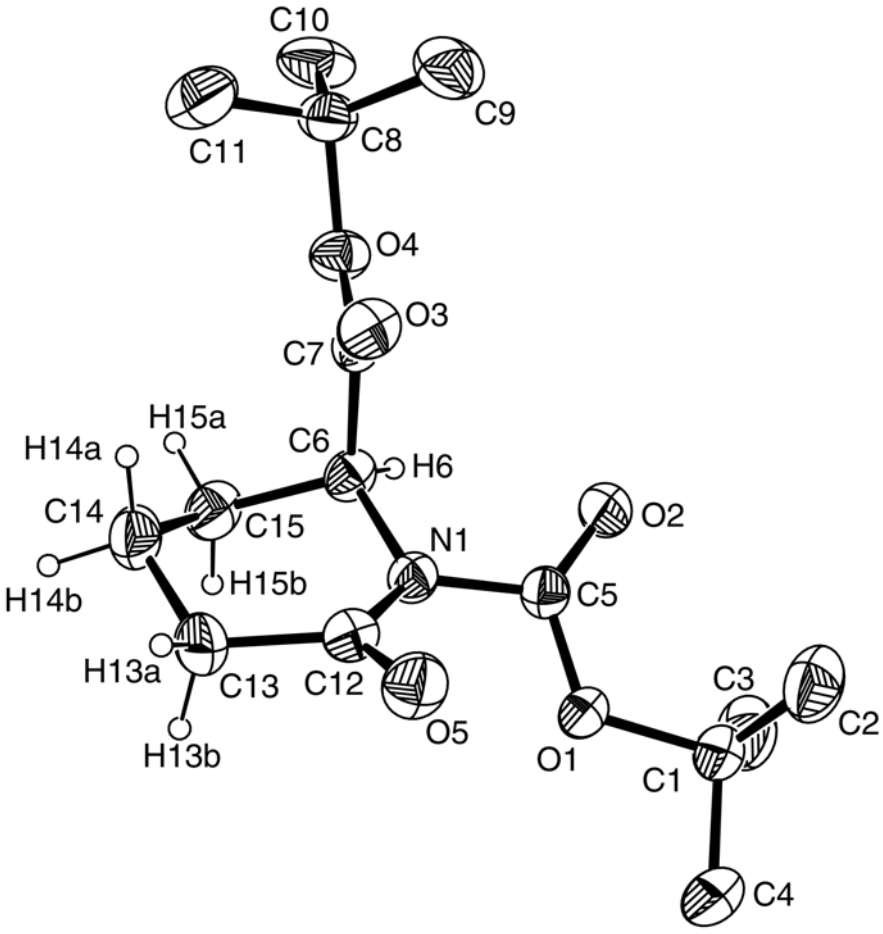
(a) Base (LiHMDS or NaHMDS), THF, –78°C.

This side reaction is generally avoided by using sodium enolates. However, in the case of **61a**, replacing the lithium enolate by the sodium enolate (entry 7 vs 6) did not eliminate the imino-aldol but only reduced its amount to the advantage of **63a**. Reduction of the reaction time from 0.5 to 0.25 h (entry 8 vs 7) further improved the yield with only a slight decrease of diastereoslectivity. Long reaction times (entry 9) were detrimental for the reaction, and only a mixture of degradation products and starting material was recovered. In all these reactions the presence of the imino-aldol **117** made the purification extremely difficult, and the yields of isolated (5*R*)-**63a** (entries 6-8) do not reflect the actual extent of the reaction. With the aim of circumventing the problem of the imino-aldol formation, we next investigated (+)- and (–)-CSO as hydroxylating agents.[14g-h,15] The experiments were performed in parallel (entries 10-12 vs 13-15) and resulted in very clean reactions (no degradation). In the first series of experiment, the lithium enolate of **61a** was treated at −78 °C for 0.25 h with (+)-CSO or (–)-CSO (entries 10 and 13). Excellent diastereoselectivities but modest yields were obtained as a result of the steric hindrance of CSO. Performing the reaction at −50 °C resulted in better yield and only slightly lower de. The reaction was brought to completion by performing the oxidation at −78 °C overnight (entries 12 and 15). When using (+)-CSO under these conditions, the hydroxylation reaction was found to proceed almost quantitatively with a high diastereoselectivity (96% de) : (5*R*)-**63a** was isolated in 92% yield. From these experiments with CSO, two general trends can be noted : (i) on one hand, yields are consistently higher

with (+)-CSO than with (–)-CSO ; (ii) on the other hand, (+)- and (–)-CSO provide the same product stereochemistry.

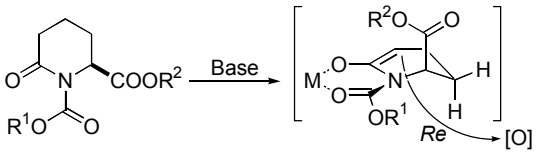
Structural evidence for the high diastereofacial bias in the hydroxylation reaction was obtained by determination of the X-ray crystal structure of **61a**. The structure shown in Figure III.1 confirms the expected axial orientation of the C-2 ring substituent (numbered C-6 on the ortep plot) resulting from the minimization of the allylic A(1,3) strain.

Figure III.1 Ortep plot of 61a



Its enolate form is believed to adopt a half chair conformation with the metal coordinating both oxygen atoms O-2 and O-5 (Figure III.2). In these conditions, the C-2 ring substituent (numbered C-6 on the ortep plot) should block the *Si* face of the enolate, leaving only the *Re* face free for the approach of the oxidizing agent. This mechanism leads to the formation of the *trans*-hydroxylated piperidinone.

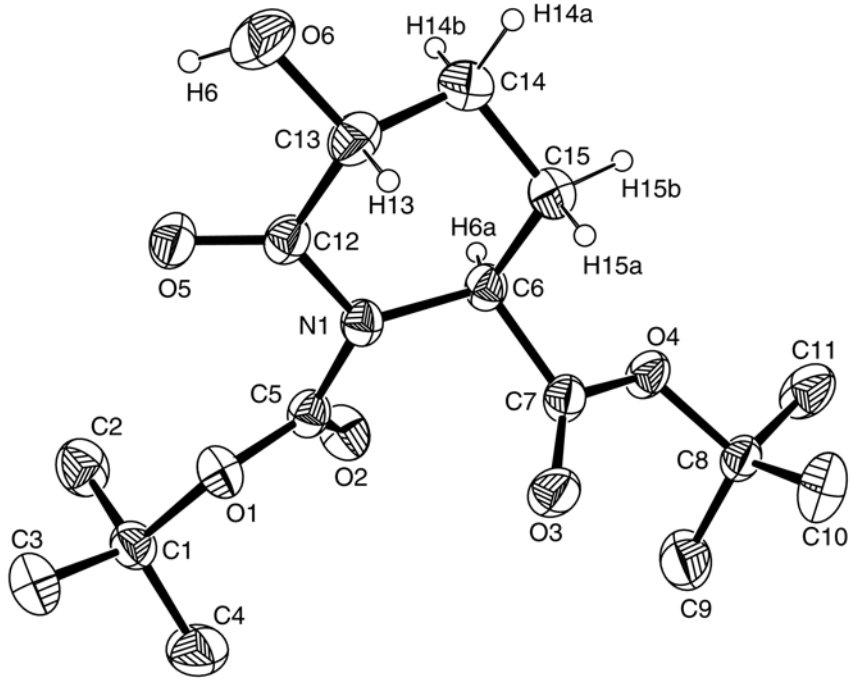
Figure III.2 Proposed enolate conformation



61 **118**

The stereochemical outcome of the reaction was confirmed by X-ray crystal structure analysis of the major diastereomer, which was unambiguously assigned to (5*R*)-**63a** (Figure III.3).

Figure III.3 ORTEP plot of compound (5*R*)-63a



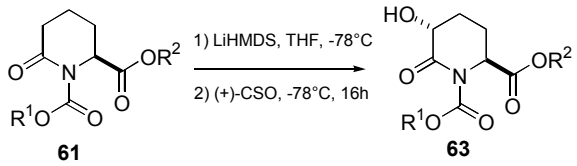
The conformational modification of the piperidinone ring with a semi-equatorial orientation of the 2 bulky substituents (COO^{t}Bu and OH) exerts a minimal effect of allylic A(1,3) strain on the conformation of (5*R*)-**63a**.

III.1.4.3. Extension to other piperidinones

Influence of the protecting groups: the influence of R^1 and R^2 in **61** on the outcome of the hydroxylation reaction was investigated under our optimal conditions : Li-enolate / (+)-

CSO / −78 °C / 16 h. Results shown in Table III.2 indicate that the nature of the ester group R^2 has only little influence on yield and stereoselectivity. Only in the case of the trichloroethyl ester protection, the yield was found to be consistently lower. The replacement of Boc by Teoc (compound **61d**) had no significant effect.

Table III.2 Influence of the protecting groups



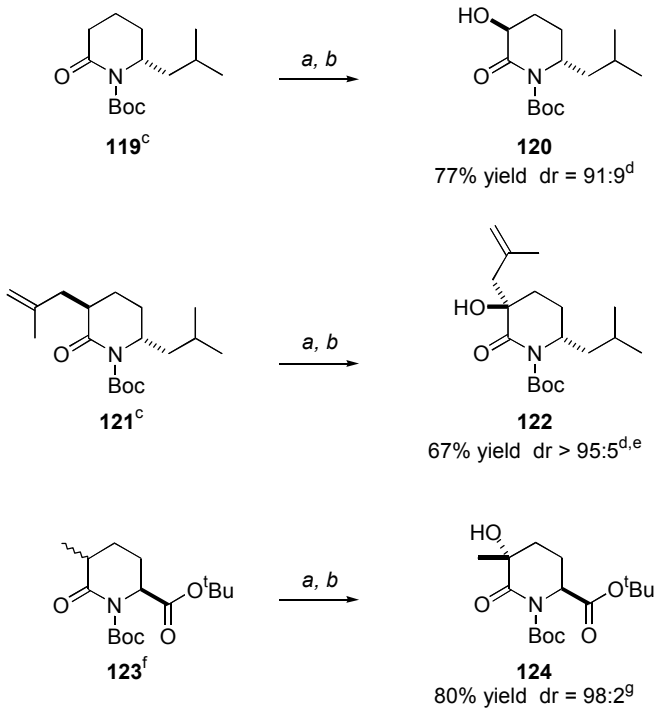
Compound	R^1	R^2	Dr[a], (5*R*)-**63**: (5*S*)-**63**	Purified yield of (5*R*)-**63**, %
61b	^{t}Bu	Bn	99:1	88
61c	^{t}Bu	TCE	100:0	73
61d	TMSEt	^{t}Bu	99:1	87
61e	^{t}Bu	All	99:1	89

[a] Ratio determined by analytical C_{18} RP-HPLC of the crude product.

Extension to functionalized piperidinones: in order to extent this hydroxylation reaction to others piperidinones, the hydroxylation reaction of the related 6-alkylated or 3,6-disubstituted δ-lactams **119**, **121**, and **123** (Scheme III.16) was investigated. Compounds **119** and **121** were prepared using the strategy previously described.[10] Compound **123** was prepared by alkylation of the Li-enolate of lactam **61a** with methyl iodide.

When the ester group in **61** was replaced by a *i*Bu side chain (**119**), a significant decrease in diastereoselectivity was observed under our standard conditions with de = 82%. This reaction was not optimized for higher diastereoselectivities. Conversely, high diastereofacial bias was obtained in the hydroxylation of the enolate generated from the δ-lactam **121**. The corresponding 3-alkylated-3-hydroxylated adduct **122** was obtained in 67% yield together with 18% of unreacted **121**. In good agreement with the result obtained for **61a**, hydroxylation of the mixture of diastereomers **123** proceeded in good yield and gave **124** as a sole diastereomer (dr = 98:2) in 80% yield.

Scheme III.16 Stereoselective hydroxylation of diverse δ-lactams



(a) LiHMDS, THF, −78°C ; (b) CSO, −78°C, 16 h ; (c) preparation previously described by us (ref. 10) ; (d) ratio determined by ^{1}H NMR ; (e) **122** is the only diastereomer detected ; (f) resulting from alkylation of **61a** with MeI, equimolar mixture of diastereomers ; (g) ratio determined by C_{18} RP-HPLC of the crude product.

III.1.4.4. A possible improved strategy

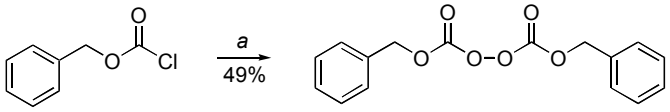
Even if this procedure is particularly efficient on a gram scale, the use of CSO is indirectly a drawback. After the work-up, the crude product is contaminated by a large amount of unreacted CSO and the corresponding imine. This last side-product can be partly eliminated by precipitation, but pure hydroxylated compounds cannot be isolated except by a flash chromatography. It rendered the scale-up of this procedure difficult and the strategy unattractive for industrial applications.

Later on the project, we tried another oxidizing agent : dibenzyl peroxydicarbonate (DPD). It can be easily prepared in a single step from aqueous hydrogen peroxide and benzyl chloroformate (Scheme III.17).[22] The analytical data were in accordance with the literature[22b]

[22] for the preparation of DPD, see: (a) Strain, F.; Bissinger, W. E.; Dial, W. R.; Rudoff, H.; DeWitt, B. J.; Stevens, H. C.; Langston, J. H. *J. Am. Chem. Soc.* **1950**, *72*, 1254. (b) Gore, M. P. and Vederas, J. C. *J. Org. Chem.* **1986**, *51*, 3700.

and this peroxydicarbonate is an unexpectedly stable non hygroscopic material that can be stored indefinitely without decomposition.

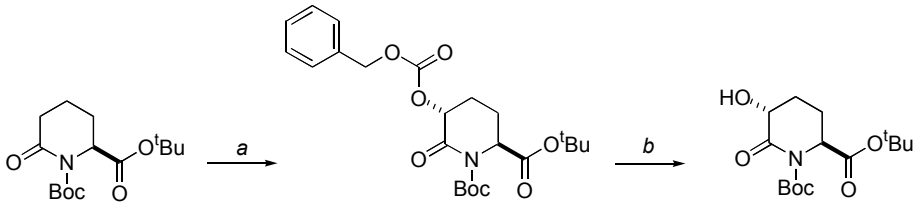
Scheme III.17 Preparation of dibenzyl peroxydicarbonate[22]



(a) Benzyl chloroformate, H_2O_2, NaOH, hexane.

The effectiveness of this oxidizer was investigated under the optimal conditions found for (+)-CSO : Li-enolate / –78°C / 16 h. These conditions, which are clearly not optimized for DPD, gave quantitatively **125** together with the corresponding diastereomer (determined by C_{18} RP-HPLC of the crude product).

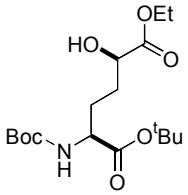
Scheme III.18 *α*-Hydroxylation using dibenzyl peroxydicarbonate



61a **125** **63a**
56% yield dr = 88:12[c]

(a) (i) LiHMDS, THF, –78°C, (ii) DPD, THF, –78°C ; (b) H_2, Pd/C, EtOH ; (c) ratio determined by C_{18} RP-HPLC of the crude product.

Hydrogenation of crude **125**, gave the desired *α*-hydroxylated *δ*-lactam (5*R*)-**63a** in 58% overall yield (dr = 88:12) starting from **61a**. The unexpected low yield can be explained by the formation of ester **126** (> 15%), which might be avoided by replacing ethanol with ethyl acetate.



126

Alternatively, since the use of DPD resulted in an extremely clean two-step sequence to give (5*R*)-**63a**, one could envision an alternative route to the optimized oxidation step we described previously. However, (+)-CSO proved to be efficient for oxidations on gram scale and instead of indulging ourselves in optimization of the DPD procedure, we chose to concentrate our efforts on the preparation of (2*S*,5*R*)-5-hydroxylysine analogues.

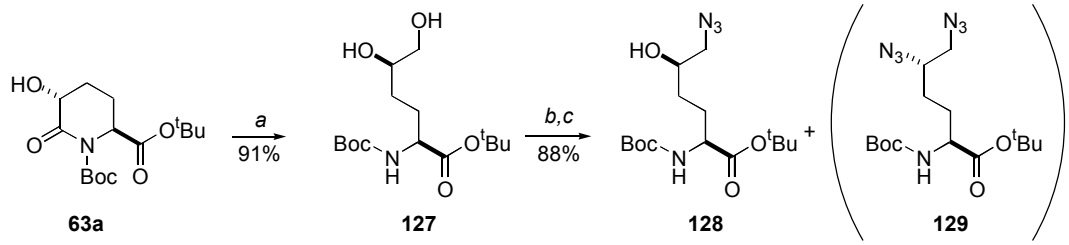
III.1.5. Synthesis of protected (2*S*,5*R*)-5-hydroxylysine derivative

With pure hydroxylated piperidinone (5*R*)-**63a** in hand, the transformation to the diol **127** then became the key step in our approach to the synthesis of natural (2*S*,5*R*)-5-hydroxylysine (Scheme III.19) and its derivatives. We studied methods involving direct ring-opening of (5*R*)-**63a** under reductive conditions ; the two-step procedure consisting of ring opening of lactam (5*R*)-**63a** by treatment with LiOH followed by reduction of the newly formed acidic function to the expected diol **127** was not investigated.[23] Initially, we tried to reduce the lactam function using lithium borohydride in the presence of one equivalent of water. This procedure, proposed by *Penning* and colleagues[24] for the reduction of sterically hindered and alkene-containing acyloxazolidines, failed to give us the desired product when starting from (5*R*)-**63a**. Protection of the secondary alcohol prior to the reduction did not bring any improvement. However, we found that the *N*-Boc-protected lactam (5*R*)-**63a** could be opened using sodium borohydride in ethanol, the 1,2-diol **127** being formed in high yield (Scheme III.19). The protection of the acidic function as a *tert*-butyl ester was mandatory in this step. All attempts to convert lactams **63b** and **63e** to the corresponding diols under these conditions failed and gave only degradation byproducts. The *N*-Teoc-protected lactam **63d** could be converted to the corresponding 1,2-diol under these conditions, but the yield was lower.

[23] Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424.

[24] Penning, T. D.; Djuric', S. W.; Haack, R. A.; Kalish, V. J.; Miyashiro, J. M.; Rowell, B. W.; Yu, S. S. *Synth. Commun.* **1990**, *20*, 307.

Scheme III.19 Preparation of intermediate 128

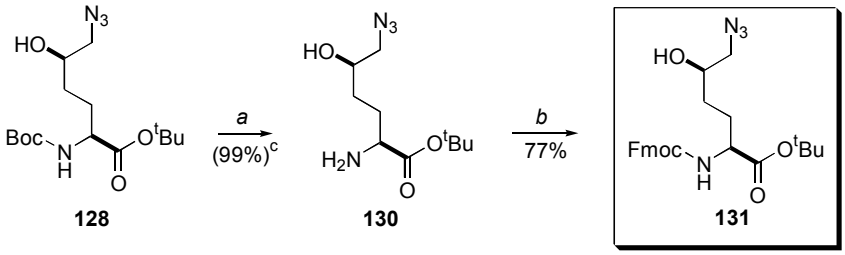


(a) $NaBH_4$, EtOH ; (b) MsCl, collidine, CH_2Cl_2 ; (c) NaN_3, DMF.

The primary alcohol in **127** was selectively mesylated in a very clean reaction,[25] thus averting the need for a purification before the following step. The monomesylate was obtained in 88% yield together with the dimesylate derivative as a byproduct. The crude mesylate was quantitatively converted into the 1,2-azido alcohol **128** by nucleophilic substitution with NaN_3. In this step, the 5,6-diazidonorleucine **129**, formed by conversion of the dimesylate, was isolated as the only side product (12%).

Compound **129** was not interesting in the present project, but it was conserved as an highly valuable precursor of 5,6-diaminonorleucine (5-aminolysine). Alternatively, transformation of **127** into the dimesylate followed by nucleophilic substitution with NaN_3 should offer **129** in a quantitative yield.

Scheme III.20 Preparation of orthogonally protected (2*S*,5*R*)-5-hydroxylysine



(a) PTSA, CH_3CN, 0°C→rt ; (b) FmocOSu, $NaHCO_3$, THF/H_2O ; (c) the reaction yield was determined without purifying crude **130**.

The obtention of orthogonally protected synthons ready for glycosylation and further use in solid phase synthesis of glycopeptides was essential. To fulfill these conditions, the *N*-Boc protecting group, which is too sensitive for the acid conditions of glycosylation (the Boc strategy is also inappropriate for the preparation of glycopeptides) had to be selectively removed and replaced by a *N*-Fmoc protection.

We studied different methods reported in the literature,[26] but only one was satisfactory in our case. The *N*-Boc functional protecting group was selectively cleaved by *p*-toluenesulfonic acid (PTSA) in acetonitrile.[26c] This selective deprotection required a monitoring by TLC because the reaction kinetic and equilibrium of deprotection of Boc preferentially to the *tert*-butyl ester were very sensitive to time and temperature. An ammonia work-up gave the free amine **130** which was immediately reprotected into the Fmoc derivative **131**. This strategy afforded **131** in an overall yield of 42% starting from the aspartic acid derivative **112a**. The protected 5-hydroxylysine **131** was the key intermediate in our strategy since it is orthogonally protected and ready for glycosylation and further use in solid phase peptide synthesis (SPPS). In this monomer, the azide is used as a temporary protecting group for the ε-amino function of 5-hydroxylysine which will be reduced at the end of peptide synthesis on the solid support (section VI).[27]

III.1.6. Determination of the stereomeric purity of synthetic 5-hydroxylysine

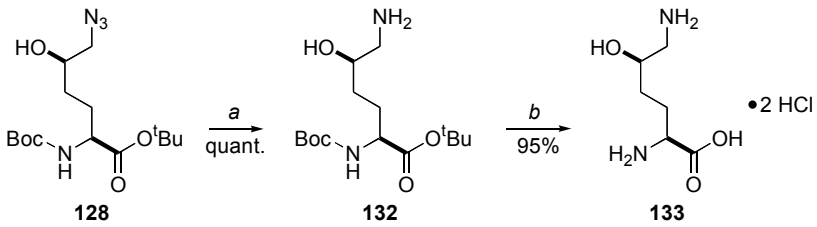
Our diastereoselective strategy was validated by the preparation of unprotected 5-hydroxylysine (**133**) and its comparison with a commercial sample of (2*S*,5*R*)-5-hydroxylysine. The reduction of the azide function from **128** gave the 1,2-amino alcohol Boc-Hyl-O^{t}Bu (**132**), a known precursor of natural hydroxylysine,[9a] in quantitative yield. The final deprotection of the Boc and *tert*-butyl ester functional groups afforded **133**.

[25] O'Donnell, C. J. and Burke, S. D. *J. Org. Chem.* **1998**, *63*, 8614.

[26] (a) for examples of selective deprotection by using HCl, see: August, R. A.; Khan, J. A.; Moody, C. M.; Young, D. W. *J. Chem. Soc., Perkin Trans. I* **1995**, 507. Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216. Stanley, M. S. *J. Org. Chem.* **1992**, *57*, 6421. (b) for an example of selective deprotection by using sulfuric acid, see: Lin, L. S.; Lanza, T. Jr.; de Laszlo, S. E.; Truong, Q.; Kamenecka, T.; Hagmann, W. K. *Tetrahedron Lett.* **2000**, *41*, 7013. (c) for examples of selective deprotection by using PTSA, see: Baldwin, J. E.; Adlington, R. M.; Godfrey, C. R. A.; Gollins, D. W.; Schofield, C. J. *Tetrahedron* **1991**, *47*, 5835. Goodacre, J.; Ponsdorf, R. J.; Stirling, I. *Tetrahedron Lett.* **1975**, *42*, 3609.

[27] (a) Meldal, M.; Juliano, M. A.; Jansson, A. M. *Tetrahedron Lett.* **1997**, *38*, 2531. (b) Lundquist, J. T. and Pelletier, J. C. *Org. Lett* . **2001**, *3*, 781.

Scheme III.21 Preparation of (2S,5*R*)-5-hydroxylysine

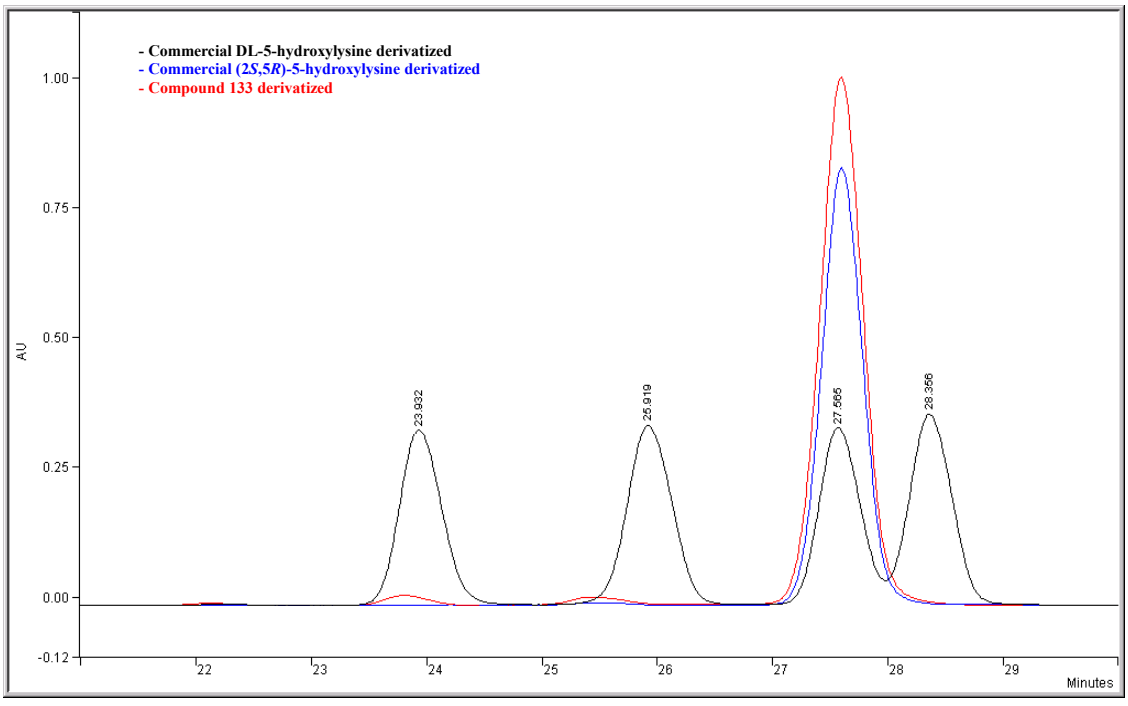


128 **132** **133**

(a) H_2, Pd/C, EtOH ; (b) HCl, dioxane.

The stereomeric purity of 5-hydroxylysine (**133**) prepared from (5*R*)-**63a** was determined after derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (*Marfey*'s reagent),[28] by RP-HPLC analysis using a C_{18} column and a linear gradient of 0.1% aqueous TFA and MeOH (30-65% MeOH in 35 min). Under these conditions, the four diastereomers of 5-hydroxylysine were baseline separated. Comparison with a commercial sample of (2*S*,5*R*)-5-hydroxylysine revealed that 5-hydroxylysine **133** was 97% pure (Figure III.4).

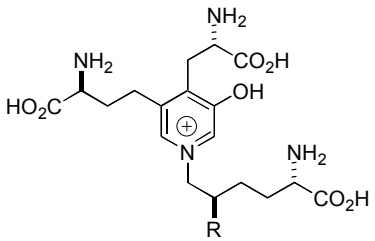
Figure III.4 RP-HPLC analysis of derivatized 5-hydroxylysine



[28] Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.

III.1.7. Synthesis of (+)-pyridinoline precursors

The structure of collagen is stabilized by intra- and intermolecular (+)-pyridinoline (**93**) and (+)-deoxypyridinoline (**94**) cross-links formed between adjacent lysyl and hydroxylysyl residues. Because of the usefulness of **93** and **94** for the diagnosis of osteoporosis and other metabolic bone diseases, as well as difficulties in its isolation from natural sources, numerous approaches to the synthesis of **93** and **94** have been proposed in recent years.[29] The preparation of **93** involved either the use of **132** or the related (2*S*,5*R*)-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate **134** and *tert*-butyl (2*S*)-2-[(tert-butoxycarbonyl)amino]-4-[(2*R*)-oxiranyl]butanoate **136** as intermediates.

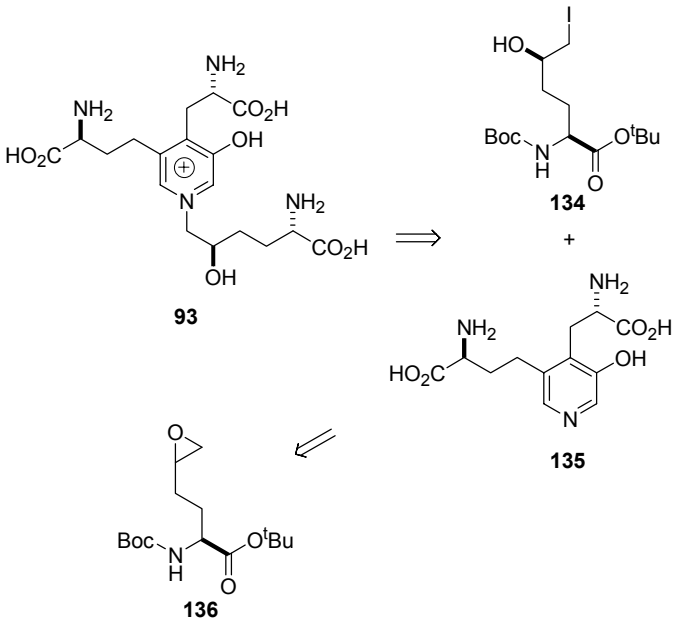


93 R = OH
94 R = H

The total synthesis of (+)-pyridinoline **93** (Scheme III.22), described independently by *Waechli* and colleagues and *Adamczyk* and colleagues,[29a,b] involves the construction of an appropriately functionalized 3-hydroxy-4,5-disubstituted pyridine derivative **135**, followed by the quaternization of nitrogen with the iodo compound **134**. **135** was prepared starting from the epoxy amino acid **136** in a biomimetic pathway (Scheme III.22).

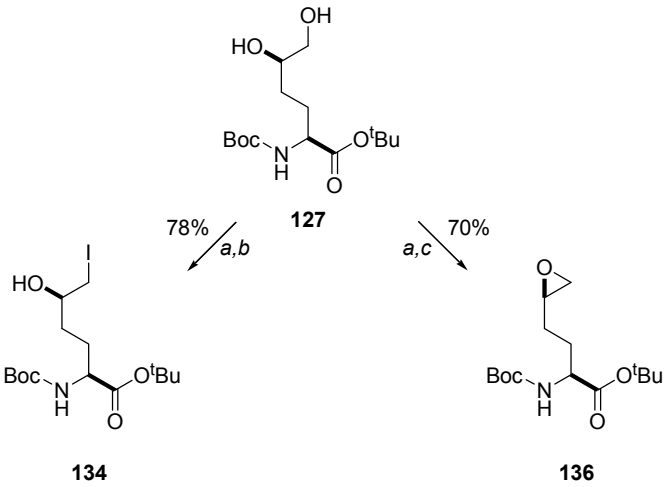
[29] for the synthesis of **93**, see: (a) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron* **1999**, *55*, 63. (b) Waelchli, R.; Beerli, C.; Meigel, H.; Révész, L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2831. (c) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron: Asymmetry* **2000**, *11*, 2289. for the synthesis of **94**, see: (d) Allevi, P.; Longo, A.; Anastasia, M. *Chem Commun.* **1999**, 559. (e) Allevi, P.; Longo, A.; Anastasia, M. *J. Chem. Soc. Perkin Trans. 1* **1999**, 2867. (f) Adamczyk, M.; Akireddy, S. R.; Reddy, R. E.; *Tetrahedron: Asymmetry* **1999**, *10*, 3107.

Scheme III.22 Retrosynthesis of (+)-pyridinoline



We found that the diol **127** was a versatile precursor in the synthesis of related amino acids **134** and **136**, since only two steps were required for these transformations (Scheme III.23). The preparation of these precursors of (+)-pyridinoline (**93**) is a first example of the high versatility of our strategy which allows the access to new or already described compounds functionally derived from 5-hydroxylysine such as **129**, **134** and **136**.

Scheme III.23 Preparation of the two intermediates from the synthesis of 93



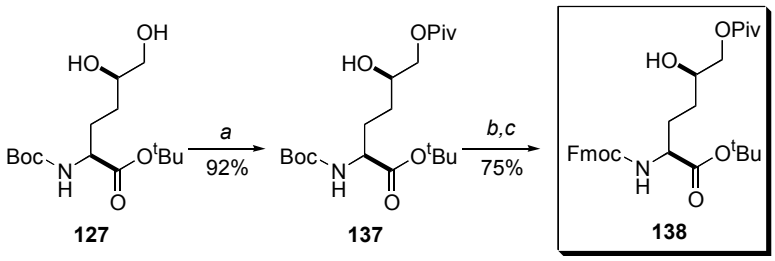
(a) MsCl, collidine, CH_2Cl_2 ; (b) NaI, acetone ; (c) K_2CO_3, acetone.

III.2. Synthesis of unnatural 5-hydroxylysine analogues

III.2.1. Synthesis of (2*S*,5*R*)-5,6-dihydroxynorleucine derivative

The primary alcohol in the 1,2-diol **127** was selectively protected with a pivaloyl functional group.[30] Selective removal of the N^{α}-Boc protection with PTSA in acetonitrile immediately followed by a washing with a 1N NH_4OH solution gave the free amine. Due to the sensitivity of the pivaloate protecting group to basic aqueous media, the N^{α}-Fmoc protection was performed in the presence of FmocOSu in CH_2Cl_2 without any base. The orthogonally protected (2*S*,5*R*)-5,6-dihydroxynorleucine derivative **138** was obtained in 69% overall yield from **127**.

Scheme III.24 Synthesis of the (2*S*,5*R*)-5,6-dihydroxynorleucine derivative



(a) PivCl, Et_3N, CH_2Cl_2 ; (b) PTSA, CH_3CN ; (c) FmocOSu, CH_2Cl_2.

III.2.2. Synthesis of (2*S*,5*S*)-5-hydroxylysine derivative

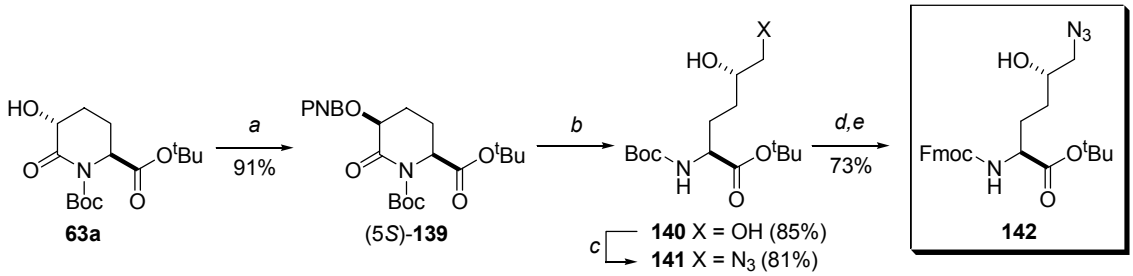
Starting from the hydroxylated compound **63a**, the inversion of the stereochemistry at C-5 was cleanly performed by the *Mitsunobu* reaction[31] and provided **139a** in 91% yield. The reductive ring-opening of (5*S*)-**139a** was accompanied by the removal of the *p*-nitrobenzoate functional group (PNB) to yield the 1,2-diol **140**. Selective mesylation of the primary alcohol from **140** and quantitative conversion of the crude mesylate by nucleophilic substitution with NaN_3 gave the 1,2-azido alcohol **141**. Selective deprotection of the N^{α}-Boc functional group and reaction of the crude primary amine with FmocOSu under basic aqueous conditions gave

[30] Yamada, S.; Sugaki, T.; Matsuzaki, K. *J. Org. Chem.* **1996**, *61*, 5932.

[31] (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) for a review on the Mitsunobu reaction, see: Hughes, D. L. *Organic Reactions* **1992**, *42*, 335.

(2*S*,5*S*)-5-hydroxylysine derivative **142** (46% overall yield from **63a**) ready for glycosylation and further use in glycopeptide synthesis.

Scheme III.25 Synthesis of the (2*S*,5*S*)-5-hydroxylysine derivative



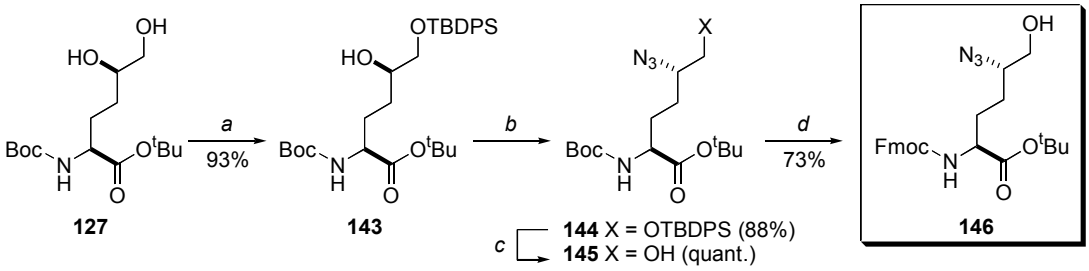
(a) *p*-nitrobenzoic acid, DIAD, PPh_3, THF ; (b) $NaBH_4$, ethanol ; (c) (i) MsCl, collidine, CH_2Cl_2, (ii) NaN_3, DMF ; (d) PTSA, CH_3CN ; (e) FmocOSu, $NaHCO_3$, THF/H_2O.

The ratio between the two diastereomers (5*S*)-**139** and (5*R*)-**139** was determined by comparison between the signals obtained for the H—C-5 proton in both compounds. This signal was observed at 4.55 ppm and 4.74 ppm in (5*S*)-**139** and (5*R*)-**139**, respectively. For comparison, pure (5*R*)-**139** was prepared by reaction of **63a** with *p*-nitrobenzoic acid chloride (PNBCl).

III.2.3. Synthesis of (2*S*,5*S*)-5-azido-6-hydroxynorleucine

We chose to start from the 1,2-diol **127** and to develop a strategy based on the selective protection of the primary alcohol with a *tert*-butyl diphenylsilyl (TBDPS) functional group (Scheme III.26). Silyl ether **143** was obtained in 93% yield and subjected to methanesulfonylation, followed by nucleophilic substitution with sodium azide (as previously described) to give **144** in 88% yield. Removal of the TBDPS protection in the presence of fluoride ions (TBAF) quantitatively yielded **145**. Finally, the previously described protection / deprotection sequence gave the (2*S*,5*S*)-5-azido-6-hydroxynorleucine derivative **146** in 73% yield.

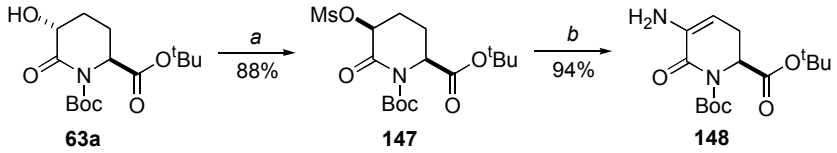
Scheme III.26 Synthesis of the (2*S*,5*S*)-5-azido-6-hydroxynorleucine derivative



(a) TBDPSCl, imidazole, CH_2Cl_2 ; (b) (i) MsCl, DIEA, CH_2Cl_2, (ii) NaN_3, DMF, 80°C ; (c) TBAF, CH_2Cl_2 ; (d) (i) PTSA, CH_3CN, (ii) FmocOSu, K_2CO_3, acetone/H_2O.

Alternatively, the most direct way for the preparation of **146** is the conversion of the secondary alcohol into an azide from the *α*-hydroxylated piperidin-2-one **63a**. Actually, mesylation of this alcohol allowed us to isolate and characterize the desired intermediate **147**, but the nucleophilic substitution resulted in the formation of the *α*-amino enone **148** in 94% yield (Scheme III.27). As already reported, the *α*-azido ketone rearranges into the *α*-amino enone **148** *via* the corresponding *α*-imino ketone.[32]

Scheme III.27 Preparation of the *α*-amino enone 148



(a) MsCl, DIEA, CH_2Cl_2 ; (b) NaN_3, DMF, 80°C.

Reduction of **148**,[32c,f,33] followed by direct protection of the primary amine with a Z group and reductive opening of the lactam (as previously described) should allow the preparation of a *N*-protected analogue of **146**.

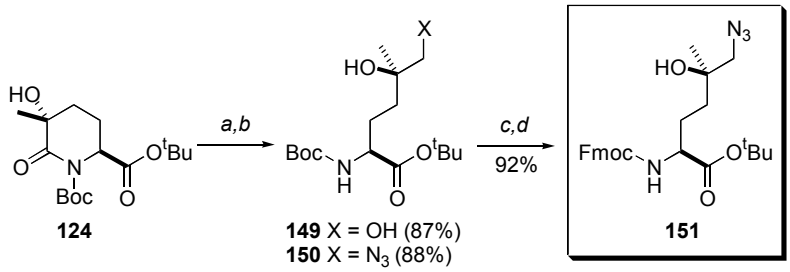
[32] for selected examples, see: (a) Patonay, T.; Juhasz-Toth, E.; Bényei, A. *Eur. J. Org. Chem.* **2002**, 285. (b) Patonay, T.; Hoffman, R. V. *J. Org. Chem.* **1995**, *60*, 2368. (c) DeWald, H. A.; Heffner, T. G.; Jaen, J. C.; Lustgarten, D. M.; McPhail, A. T.; Meltzer, L. T.; Pugsley, T. A.; Wise, L. D. *J. Med. Chem.* **1990**, *33*, 445. (d) Penz, G. and Zbiral, E. *Chem. Ber.* **1985**, *118*, 4131. (e) Litkei, G.; Mester, T.; Patonay, T.; Bognar, R. *Liebigs Ann. Chem.* **1979**, 174. (f) Patonay, T.; Rakosi, M.; Litkei, G.; Bognar, R. *Liebigs Ann. Chem.* **1979**, 162.

[33] for selected examples, see: (a) Couladouros, E. A. and Apostolopoulos, C. D. *Synlett* **1996**, 341. (b) Varela, O.; Nin, A. P.; de Lederkremer, R. M. *Tetrahedron Lett.* **1994**, *35*, 9359.

III.2.4. Synthesis of (2*S*,5*R*)-5-hydroxy-5-methyllysine derivative

The 5-hydroxylysine derivative methylated at the 5-position was prepared in a manner similar to (2*S*,5*R*)-5-hydroxylysine, but starting from the *α*-methylated, *α*-hydroxylated lactam **124** (Scheme III.27). As previously mentioned, this lactam was prepared from *α*-methylated derivative **123** obtained as a 1:1 mixture of diastereomers. Hydroxylation proceeded in 80% yield and gave **124** as sole diastereomer (dr = 98:2). Following oxidation, the 1,2-diol **149** was formed in high yield by opening the lactam **124** using sodium borohydride.

Scheme III.27 Synthesis of the (2*S*,5*R*)-5-hydroxy-5-methyllysine derivative



124 **149** X = OH (87%) **150** X = N_3 (88%) **151**

(a) $NaBH_4$, ethanol ; (b) (i) MsCl, collidine, CH_2Cl_2, (ii) NaN_3, DMF ; (c) PTSA, CH_3CN ; (d) FmocOSu, $NaHCO_3$, THF/H_2O.

Selective mesylation of the primary alcohol directly followed by nucleophilic substitution with sodium azide gave the 1,2-azido alcohol **150** in 88% yield. The *N*-Fmoc protected (2*S*,5*R*)-5-hydroxy-5-methyllysine derivative **151** was obtained in 92% yield using the two-step procedure described for the preparation of the others 5-hydroxylysine analogues.

IV. Preparation of 4-Hydroxylysine Analogues

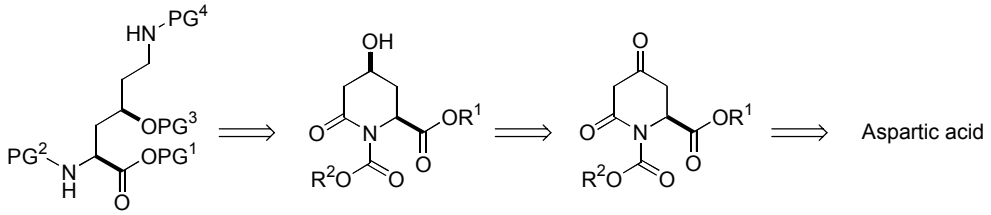
IV.1. A versatile strategy

There was no reported strategies for the preparation of enantiopure 4-hydroxylysine derivatives at the time we decided to prepare glycopeptides incorporating this unnatural amino acid. As we did for the preparation of 5-hydroxylysine, we focused on the development of a versatile route which would allow modulation of the amino acid side-chain, glycosylation, as well as application in the preparation of others highly valuable organic compounds.

IV.1.1. Retrosynthesis

In this section, we present a stereocontrolled synthesis of orthogonally protected (2*S*,4*S*)-4-hydroxylysine starting from aspartic acid as an inexpensive chiral adduct. As previously reported for the preparation of 5-hydroxylysine (section III), the existing α-stereogenic center served for asymmetric induction in the creation of the second stereogenic center at C-4. Our strategy (retrosynthetic analysis shown in Scheme IV.1) is based on asymmetric reduction of ketones **62** to the corresponding β-hydroxy carbonyl compounds **64**. In order to prepare glycopeptides, the 4-hydroxylysine analogue **152** should carry suitable protecting groups (PG^1, PG^2, PG^3 and PG^4) for glycosylation and SPPS.

Scheme IV.1 Retrosynthesis of orthogonally protected (2*S*,4*S*)-4-hydroxylysine

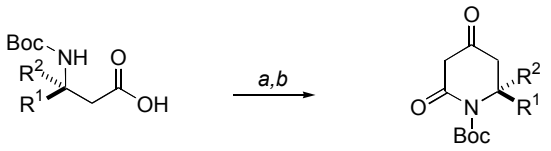


152 **64** **62** **112**

IV.1.2. 4,6-Dioxopiperidines synthesis

By analogy with the synthesis of chiral tetramic acids from *N*-protected α-amino acids,[1] condensation of *N*-protected 3-amino propanoic acids with *Meldrum*'s acid followed by cyclization at reflux in ethyl acetate provided a short entry to enantiopure *N*-acylated 2-substituted 4,6-dioxopiperidines. This procedure was already reported by *Murray* and *Starkey*[2] for the preparation of **62b** and is similar to that we described for the preparation of piperidin-2-ones (section III). Dioxopiperidines **62a-f** were obtained in good to excellent yields starting from the protected aspartic acid derivatives **62a-b** and β^3-amino acids **62c-f** (Table IV.1). Compounds **62a** and **62c-f** were easily purified by recrystallization, but a flash chromatography was necessary for the purification of **62b**.

Table IV.1 Preparation of 4,6-dioxopiperidines



112a-f **62a-f**

compound	R^1	R^2	purified yield, %
62a	COO^tBu	H	77
62b	COOBn	H	85
62c	H	iBu	68
62d	H	Bn	87
62e	H	iPr	63
62f	H	Me	93

(a) Meldrum's acid, EDC, DMAP, CH_2Cl_2 ; (b) AcOEt, reflux.

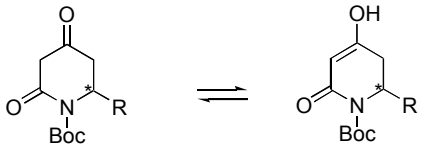
[1] for representative examples, see: (a) Jouin, P. and Castro B. *J. Chem. Soc. Perkin Trans. I* **1987**, 1177. (b) Fehrentz, J. A.; Bourdel, E.; Califano, J. C.; Chaloin, O.; Devin, C.; Garrouste, P.; Lima-Leite, A. C.; Llinares, M.; Rieunier, F.; Vizavonna, J.; Winternitz, F.; Loffet, A.; Martinez, J. *Tetrahedron Lett.* **1994**, *35*, 1557. (c) Galeotti, N.; Poncet, J.; Chiche, L.; Jouin, P. *J. Org. Chem.* **1993**, *58*, 5370. (d) Decicco, C. P. and Grover, P. *J. Org. Chem.* **1996**, *61*, 3534. (e) Ma, D.; Ma, J.; Ding, W.; Dai, L. *Tetrahedron: Asymmetry* **1996**, *7*, 2365.

[2] Murray, P. J. and Starkey, I. D. *Tetrahedron Lett.* **1996**, *37*, 1875.

IV.1.3. The keto-enolic equilibrium in the dioxopiperidines

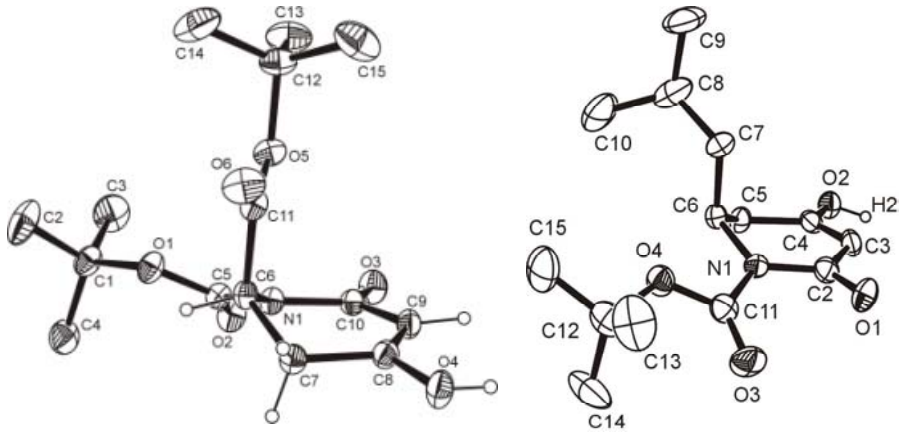
Dioxopiperidines **62** exist in equilibrium with the thermodynamically stable enol form **153** (Figure IV.1). Very similar to what is observed with other 1,3-dicarbonyl compounds and *N*-protected chiral tetramic acids in particular, the keto form **62** exclusively is populated in $CDCl_3$, while ^{1}H and ^{13}C NMR in DMSO-d_6, show only the enol form **153**.

Figure IV.1 Keto-enolic equilibrium in 4,6-dioxopiperidines



X-ray diffraction studies on crystals of **62a** and **62c** reveal the enol tautomers **153a** and **153c**, respectively (Figure IV.2). Both compounds share very similar structural features.

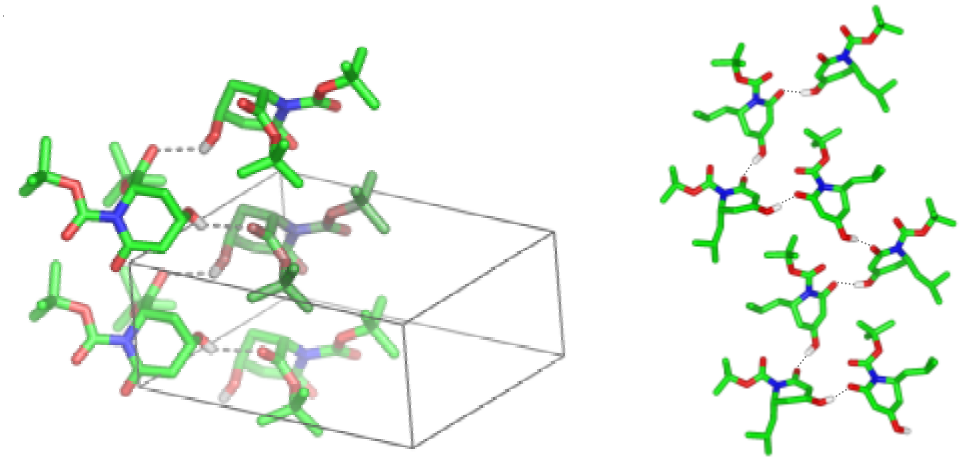
Figure IV.2 Ortep of 153a and 153c



The carboxylate and the isobutyl side chains form a dihedral angle of ca 77° and ca 78° with the piperidine ring in **153a** and **153c**, respectively, confirming the expected axial orientation of the C-6 ring substituent due to the minimization of the pseudo-allylic A(1,3)

strain.[3] This side-chain orientation should provide a high diastereofacial bias in the following reduction step. In both molecules, the piperidine ring assumes a sofa like conformation, with the C-2 atoms deviating by 0.599(3) Å and 0.556(5) Å from the least-square plane defined by the 5 other atoms. The hydroxyl group at the 4-position of **153a** and **153c** is involved as a strong proton donor in the crystal packing of both molecules. On one hand, strong hydrogen bonds involving this hydroxyl group at C-4 and the carbonyl oxygen at position C-6 (d(O···O) : 2.625(2) Å) as proton acceptor link the molecules in infinite C(6) chains running along the b axis. In the crystal packing of **153c**, *Van der Waals* interactions between the chains produce layers with aliphatic groups at the surfaces, and finally the layers pack together to produce a loosely held three dimensional structure.

Figure IV.3 Crystal packing of 153a and 153c along the b axis



153a **153c**

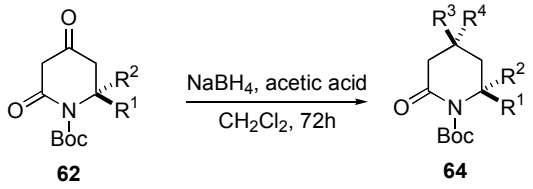
IV.1.4. Diastereoselective reduction studies

N-acylated chiral tetramic acids can be reduced stereoselectively to the corresponding *cis*-4-hydroxy derivatives either by treatment with $NaBH_4$ in CH_2Cl_2 / AcOH[1a-c] or by hydrogenation with PtO_2 (*Adam*'s catalyst) in AcOEt.[1d] Both procedures have been evaluated for the reduction of *N*-acylated 4,6-dioxopiperidines **62**.

[3] for reviews on allylic A(1,3) strain, see: (a) Hoffmann, R. H. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1124. (b) Hoffmann, R. H. *Chem. Rev.* **1989**, *89*, 1841.

Reduction with $NaBH_4$: Treatment of **62** with $NaBH_4$ in CH_2Cl_2 / AcOH (9:1) for 72 h resulted in quantitative reduction of the keto functionality[4] and gave the expected 4-hydroxylated adduct **64** with a diastereomeric excess in the range 68-98% as determined by C_{18} RP-HPLC of the crude products (Table IV.2).

Table IV.2 Influence of the C-2 side chain



dioxopiperidine	R^1	R^2	R^3	R^4	dr[a] of **64**	yield,[b] %
62a	COO^tBu	H	OH	H	7:93 (0:100)[c]	89 (82)[c]
62b	COOBn	H	OH	H	15:85	85
62c	H	iBu	H	OH	90:10 (0:100)[c]	93 (71)[c]
62d	H	Bn	H	OH	91:9	91
62e	H	iPr	H	OH	100:0	95
62f	H	Me	H	OH	84:16	87

[a] (2*S*,4*R*)-**64**:(2*S*,4*S*)-**64**, ratio determined by analytical C_{18} RP-HPLC of the crude product ; [b] mixture of (2*S*,4*R*)-**64** and (2*S*,4*S*)-**64** obtained after purification of the crude product by a flash column chromatography ; [c] values in parentheses are for pure compounds isolated by recrystallization.

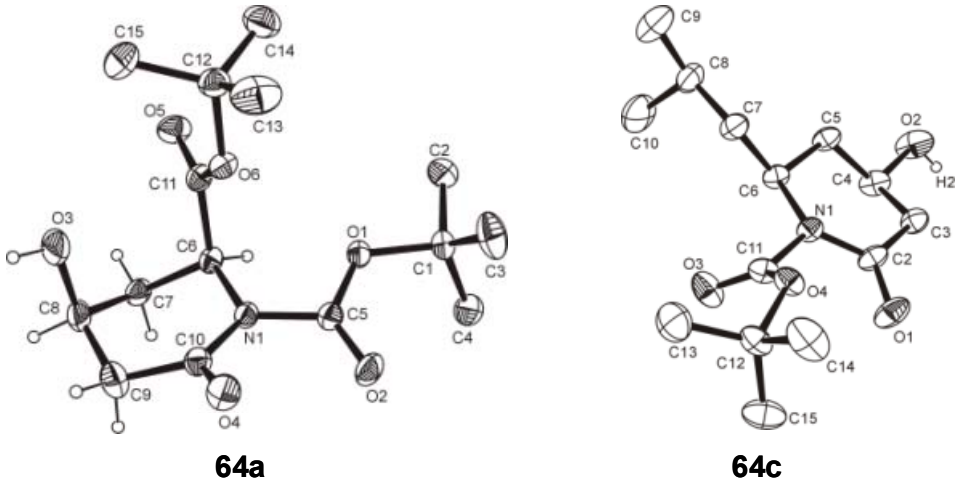
The selectivity of the reaction is significantly influenced by the bulk of the side chain at C-2, the lowest and the highest selectivities being observed for the methyl and the isopropyl groups, respectively. In the case of carboxylate side chains, the *tert*-butyl ester group in **62a** exert a stronger stereodirecting effect than the corresponding benzyl ester. Compounds **64a** and **64c** were obtained in diastereomerically pure form (> 99% de) following a single recrystallization step (Table IV.2, entry 1 and 3) and their absolute configuration at C-4 was confirmed by X-ray crystal structure determination (Figure IV.4).

Compounds **64a** and **64c** adopt a chair conformation with atoms N-1 and C-4 displaced on opposite sides of the C-2,C-3,C-5 and C-6 mean plane by –0.355(4)Å and 0.657(1)Å,

[4] no reduction took place when **62a** was treated with $NaBH_4$ in the absence of AcOH.

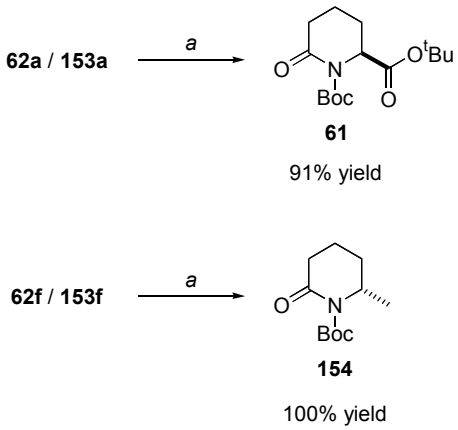
respectively for **64a** and –0.221(5)Å and 0.659(1)Å, respectively for **64c**. The hydroxy and the *tert*-butyl carboxylate groups in the 4- and 6-positions of the piperidine ring in **64a** assume an axial orientation, as can be seen from the angles of 5.7(2)° and of 16.8(1)° between the normal to the mean plane of the ring atoms and the bonds C-4—O-1 and C-2—C-12, respectively. In contrast, the hydroxy and the isobutyl groups in the crystal structure of **64c** assume an equatorial orientation, as can be seen from the torsional angles C-2—C-3—C-4—O-2 of ca 170° and C-4—C-5—C-6—C-7 of ca 167°.

Figure IV.4 Ortep plot of 64a and 64c



Hydrogenation: In contrast to *N*-acylated chiral tetramic acids,[1d] hydrogenation of *N*-acylated 4,6-dioxopiperidines **62** with *Adam*'s catalyst (PtO_2) in AcOEt failed to yield the expected 4-hydroxylated derivatives. Hydrogenation of **62a** and **62f** resulted in quantitative formation of the fully reduced *N*-acylated piperidin-2-ones **61** and **154** (Scheme IV.2). Changing the solvent (ie, THF, chloroform) or the amount of catalyst (5-15%) did not improve the selectivity of the reaction and the yields of **64a** and **64f** were consistently below 10%. Monitoring the progression of the reaction by C_{18} RP-HPLC revealed that **61** starts to form immediately. Its amount increased as the starting material was consumed ; the amount of **64a** remaining low. No reaction took place when *Adam*'s catalyst was replaced by Pd on carbon or by *Pearlman*'s catalyst.

Scheme IV.2 Reduction by hydrogenation



(a) PtO_2, H_2, AcOEt, 1 atm.

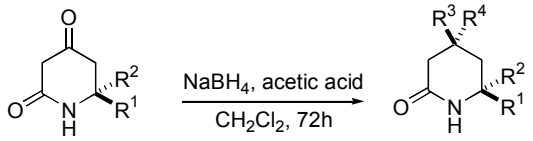
IV.1.5. Influence of the *N*-acylation

The reduction of the 6-substituted-2,4-dioxopiperidines **155** in a fashion similar to substituted cyclohexanones[5] is believed to be driven by torsional effects that favor attack of the hydride reagent across the axial face of the C=O. Dioxopiperidines **155** were prepared in quantitative yield by treatment of **62** with trifluoroacetic acid (TFA) and their reduction was studied under the same conditions for comparison (Table IV.3). Overall, yields of 4-hydroxylated product **156** were lower. In the absence of pseudo-allylic A(1,3) strain, the *cis*-4-hydroxy isomer is still obtained as the major product, thus confirming the attack across the axial face of the C=O, but diastereomeric excesses were lower (58-68%) and the stereoselectivity is essentially independent of the bulk of the side chain at C-6 (Table IV.3). A similar selectivity (85:15 cis / trans) was reported by *Davis* and colleagues in the reduction of the related enantiopure (*R*)-6-phenylpiperidin-2,4-dione.[6]

[5] Hutchins, R. O.; Su, W. Y.; Sivakumar, R.; Cistone, F.; Stercho, Y. P. *J. Org. Chem.* **1983**, *48*, 3412.

[6] Davis, F. A.; Fang, T.; Chao, B.; Burns, D. M. *Synthesis* **2000**, 14, 2106.

Table IV.3 Reduction of 6-substituted-2,4-dioxopiperidines 155



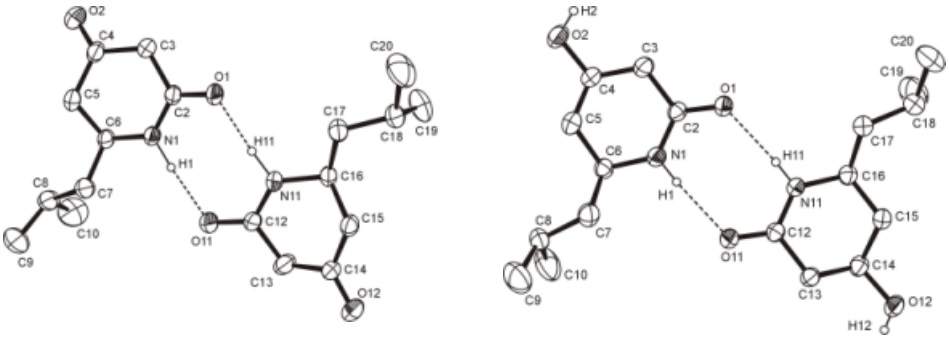
155 **156**

dioxopiperidine	R^1	R^2	R^3	R^4	dr[a] of **156**	yield,[b] %
155b	COOBn	H	OH	H	21:79	89
155c	H	iBu	H	OH	82:18	62
155d	H	Bn	H	OH	84:16	86
155e	H	iPr	H	OH	84:16	49
155f	H	Me	H	OH	80:20	37

[a] (2*S*,4*R*)-**156**:(2*S*,4*S*)-**156**, ratio determined by analytical C_{18} RP-HPLC of the crude product ; [b] mixture of (2*S*,4*R*)-**156** and (2*S*,4*S*)-**156** obtained after purification of the crude product by a flash column chromatography.

Monocrystals were obtained from **155c** and X-ray crystal structure determination confirmed the absolute configuration of the hydroxylated dioxopiperidine **156c** (Figure IV.5). The piperidine ring of **156c** adopts a half-chair like conformation with the carbon atoms at the 4-position displaced from the mean plane, defined by the five other atoms of the ring, by 0.641(5) Å. The hydroxy group in the 4-position of the piperidine ring assumes an equatorial orientation, as can be seen from the torsional angle C-12—C-13—C-14—O-12 of ca 169°.

Figure IV.5 Ortep plot of 155c and 156c



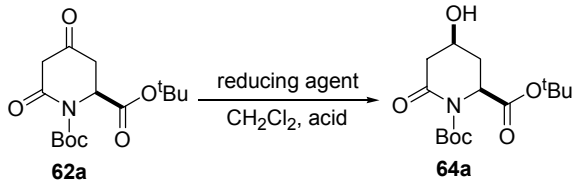
155c **156c**

IV.1.6. Evaluation of new reducing conditions

In an attempt to further improve the selectivity of the reduction of **62a** to **64a** with a view to synthesizing 4-hydroxylysine derivatives and 4-hydroxypipecolates, other reducing agents and carboxylic acids were examined (Table IV.4). Of all other borohydride reagents considered, $NaBH_4$ gave the best results. Substituting $NaBH(OAc)_3$ for $NaBH_4$ (entry 2) improved the stereoselectivity, but the reaction never reached completion. It is worth mentioning that replacing Na^+ by the bulkier Me_4N^+ countercation[7] resulted in complete degradation of **62a** and no hydroxylated product was obtained (entry 3).[8] When $NaBH_3CN$ was used, the reduction was completed within 16 h, but the de was only 50% (entry 4).

The nature of the carboxylic acid exerts a moderate effect on the stereoselectivity of the reaction. However, pivalic acid gave slightly better results than AcOH (entry 5 vs 1).

Table IV.4 Effect of the borohydride reagent and the carboxylic acid on the reduction of 62a



62a **64a**

entry	reducing agent	carboxylic acid	time, h	dr[a] of **64a**	yield,[b] %
1	**$NaBH_4$**	**acetic[c]**	**72**	**7:93**	**89**
2	$NaBH(OAc)_3$	acetic[c]	72	4:96	61
3	$Me_4NBH(OAc)_3$	acetic[c]	16	-	0[d]
4	$NaBH_3CN$	acetic[c]	16	25:75	93
5	$NaBH_4$	pivalic[e]	72	5:95	93
6	$NaBH_4$	Ac-L-Pro[e]	16	16:84	90
7	$NaBH_4$	Ac-D-Pro[e]	16	11:89	90

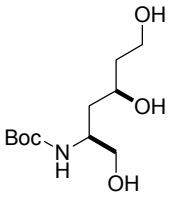
[a] (2*S*,4*R*)-**64a**:(2*S*,4*S*)-**64a**, ratio determined by analytical C_{18} RP-HPLC of the crude product ; [b] mixture of (2*S*,4*R*)-**64a** and (2*S*,4*S*)-**64a** obtained after purification of the crude product by a flash column chromatography ; [c] 10% $^{v}/_{v}$ of acetic acid ; [d] this reaction resulted in complete degradation of the starting material ; [e] 10 equivalents of acid.

[7] $Me_4NBH(OAc)_3$ was described to be an excellent reagent in the reduction of *β*-hydroxy ketones, see: Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560.

[8] in contrast the reduction of **62c** with $Me_4NBH(OAc)_3$ resulted in a clean deprotection of the Boc group to give 4,6-dioxopiperidine **155c** in 84% yield.

IV.1.7. Synthesis of (2*S*,4*S*)-4-hydroxylysine

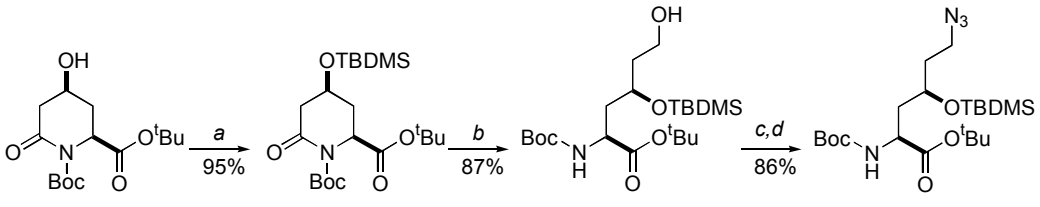
In contrast to the result obtained with the 5-hydroxylated piperidinone which could be opened directly using sodium borohydride in ethanol to give the corresponding 1,2-diol in high yield (section III), ring-opening of **64a** under the same conditions failed to give us the desired 1,3-diol derivative. Instead, triol **157**, unambiguously characterized by ^{1}H and ^{13}C NMR, was isolated as the major side product in 32% yield.



157

However, upon protection of the secondary alcohol with a TBDMS group prior to the reduction, the *O*-protected 1,3-diol **159** could be obtained in 85% yield from **64a**. After purification by filtration through a short plug of silica, the primary alcohol was converted into the corresponding azide **160** by mesylation followed by nucleophilic substitution with NaN_3. The azide can serve as a temporary protection for the amino group of 4-hydroxylysine, the reduction of the azide function being quantitatively and cleanly performed on the solid support at the end of the elongation of the peptidic chain (section VI).[9] This reaction sequence afforded the 4-hydroxylysine derivative **160** in 71% overall yield starting from **64a**.

Scheme IV.3 Preparation of *N*-Boc protected 4-hydroxylysine



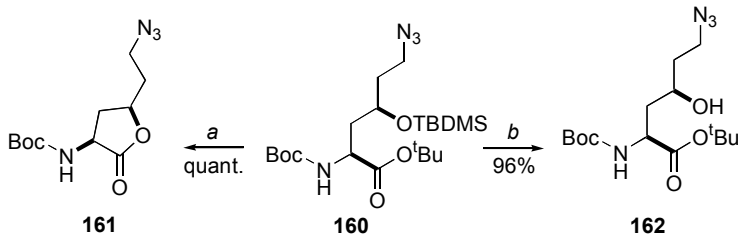
64a **158** **159** **160**

(a) TBDMSCl, imidazole, CH_2Cl_2 ; (b) $NaBH_4$, EtOH ; (c) MsCl, Et_3N, CH_2Cl_2 ; (d) NaN_3, DMF.

[9] (a) Meldal, M.; Juliano, M. A.; Jansson, A. M. *Tetrahedron Lett.* **1997**, *38*, 2531. (b) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781.

Our first attempt to remove the TBDMS protection in presence of TBAF[10] resulted in quantitative formation of the undesired compound **161** (Scheme IV.4). The formation of this lactone can be easily explained by intramolecular condensation. After removal of the TBDMS group, the anion displaced the *tert*-butyl ester to form the highly favorable lactone ring. This side reaction was completely reversed to the benefit of the desired 4-hydroxylysine derivative **162** by simply performing the reaction in a mixture of acetic acid and THF as solvent (Scheme IV.4).[10a,11]

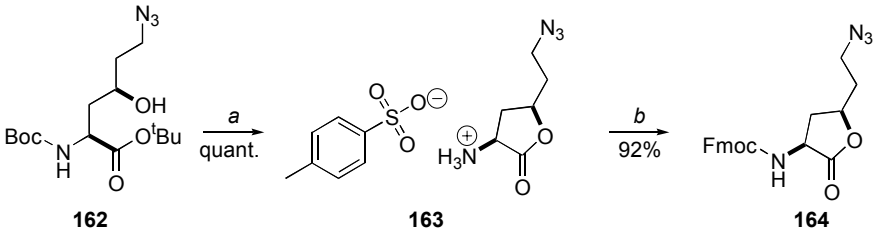
Scheme IV.4 Deprotection of the secondary alcohol



(a) TBAF, THF ; (b) TBAF, AcOH, THF.

However, in the case of **162**, the conditions previously developed for selective deprotection of the *N*-Boc group in the presence of the *tert*-butyl ester were unsuitable.

Scheme IV.5 Formation of the *N*-Fmoc protected lactone 164



(a) PTSA, CH_3CN ; (b) FmocOSu, $NaHCO_3$, acetone/H_2O.

Instead as previously observed with **160** in the presence of TBAF, treatment of **162** with PTSA gave the corresponding lactone as a PTSA salt (Scheme IV.5). Treatment of the *O*-protected derivative **160** to the same conditions (ie, PTSA in acetonitrile) resulted also in the formation of lactone **163**. Thus, the intramolecular condensation resulting in the formation of

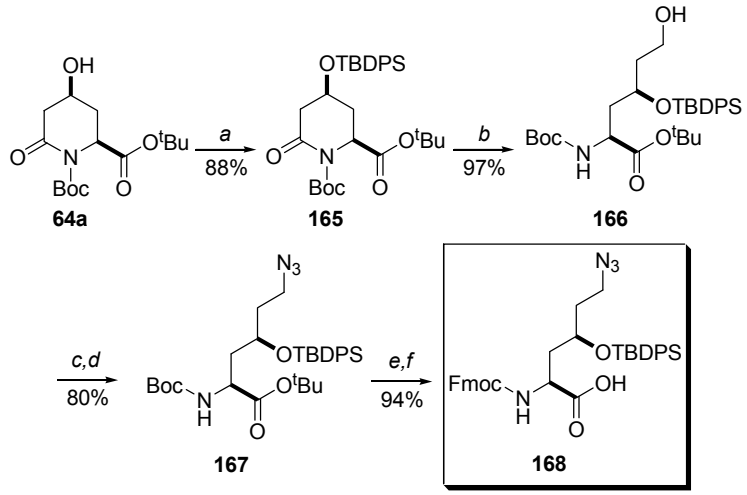
[10] (a) Greene, T. W. and Wuts, P. G. M. Protective Groups in Organic Syntheses (third edition), *Wiley Interscience* **1999**, 133. (b) Corey, E. J. and Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

[11] Smith, III, A. B. and Ott, G. R. *J. Am. Chem. Soc.* **1996**, *118*, 13095.

the lactone ring could also be performed in acidic conditions. The *N*-Fmoc protected lactone **164** could be easily obtained by reaction with FmocOSu in presence of $NaHCO_3$ as a base.

With regards to these disappointing results, we first chose to replace the TBDMS protection by the similar protecting group TBDPS. This slight modification allowed us to envision exactly the same strategy but with a protecting group hundred times more stable to acidic conditions.[12] The 1,3-azido alcohol **167** was prepared following the procedure described for the preparation of **160**. Using a TBDPS protecting group gave higher yields than TBDMS. This new strategy turned out to be advantageous because the TBDPS protection of the secondary alcohol was completely stable to the conditions developed for selective deprotection of the Boc and *tert*-butyl ester groups.

Scheme IV.6 Preparation of orthogonally protected 4-hydroxylysine



(a) TBDPSCl, imidazole, CH_2Cl_2 ; (b) $NaBH_4$, EtOH ; (c) MsCl, Et_3N, CH_2Cl_2 ; (d) NaN_3, DMF ; (e) TFA/CH_2Cl_2 (1:1) ; (f) FmocOSu, K_2CO_3, acetone/H_2O.

We thus developed an efficient stereocontrolled synthesis of orthogonally protected (2*S*,4*S*)-4-hydroxylysine derivative (40% overall yield, starting from **112a**) useful for the incorporation into peptides using the Fmoc strategy.[13] However, the issue concerning the preparation of a 4-hydroxylysine derivative ready for glycosylation could not be solved by this strategy because of difficulties to find an orthogonal deprotection scheme.

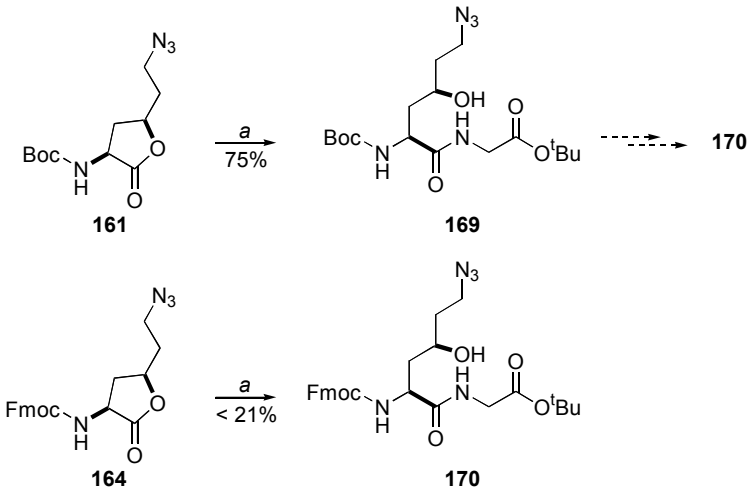
[12] Greene, T. W. and Wuts, P. G. M. Protective Groups in Organic Syntheses (3rd edition), *Wiley Interscience* **1999**, 141.

[13] Felix, A.; Moroder, L.; Toniolo, C.; Murray G. Methods of Organic Chemistry, Vol. E22a (4th Ed.), *Thieme* **2002**, 359.

IV.1.8. Preparation of a 4-hydroxylysine aglycon

As the two lactones **161** and **164** were both isolated as side products from our synthesis of 4-hydroxylysine, we envisioned the preparation of the *N*-protected 4-hydroxylysyl-glycine *tert*-butyl ester dipeptides **169** and **170** *via* ring opening of the lactones in the presence of H-Gly-O^{t}Bu (Scheme IV.7).[14] Preliminary results gave **169** in 75% yield and **170** in less than 21% yield from **161** and **164**, respectively.

Scheme IV.7 Ring opening of lactones 161 and 164



(a) H-Gly-O^{t}Bu, THF.

As **170** can serve as a possible 4-hydroxylysine aglycon, this approach would solve the protecting group issue en route to the synthesis of *N*-Fmoc protected glycosylated 4-hydroxylysine building blocks by taking advantage of the side-reactions giving **161** and **164** in almost quantitative yield.

[14] for selected examples, see: (a) Mues, H.; Kazmaier, U. *Synlett* **2000**, *7*, 1004. (b) Martin, S. F.; Dwyer, M. P.; Hartmann, B.; Knight, K. S. *J. Org. Chem.* **2000**, *65*, 1305. (c) Adamczyk, M.; Reddy, R. E.; Rege, S. D. *Synth. Commun.* **2000**, *30*, 3281.

IV.2. Synthesis of *N*-Fmoc protected 4-hydroxypipecolic acids

IV.2.1. Interests in 4-hydroxypipecolic acids

4-Hydroxypipecolic acids **171** are naturally occurring non-proteinogenic amino acids that have been isolated from the leaves of *Calliandra pittieri*, *Strophantus scandeus* and *Acacia oswaldii*.[15] With their rigid structure and multiple functionality, they make ideal candidates for use as scaffolds around which compound libraries may be designed for drug discovery. Indeed, molecules derived from **171** have been demonstrated to possess biological activity in diverse examples.[16]

N-Boc protected derivatives of **171** are commercially available from Neosystem and Acros Organics. Recently, a number of asymmetric strategies for the preparation of 4-hydroxypipecolic acid derivatives have been disclosed.[17] However, a short and direct strategy

[15] (a) Romeo, J. T.; Swain, L. A.; Bleeker, A. B. *Phytochemistry* **1983**, *22*, 1615. (b) Schenk, V. W.; Schutte, H. R. *Flora* **1963**, *153*, 426. (c) Clark-Lewis, J. W.; Mortimer, P. I. *J. Chem. Soc.* **1961**, 189.

[16] for a NMDA receptor agonist, see: (a) Pellicciari, R.; Natalini, B.; Luneia, R.; Marinozzi, M.; Marinella, R.; Rosato, G. C.; Sadeghpour, B. M.; Snyder, J. P.; Monahan, J. B.; Moroni, F. *Med. Chem. Res.* **1992**, *2*, 491. for a HIV protease inhibitor (Palinavir), see: (b) Anderson, P. C.; Soucy, F.; Yoakim, C.; Lavallée, P.; Beaulieu, P. L. *U.S. Patent 5,545,640*, **1996**. (c) Lamarre, D.; Croteau, G.; Bourgon, L.; Thibeault, D.; Wardrop, E.; Clouette, C.; Vaillancourt, M.; Cohen, E.; Pargellis, C.; Yoakim, C.; Anderson, P. C. *Antimicrob. Agents Chemother.* **1997**, *41*, 965.

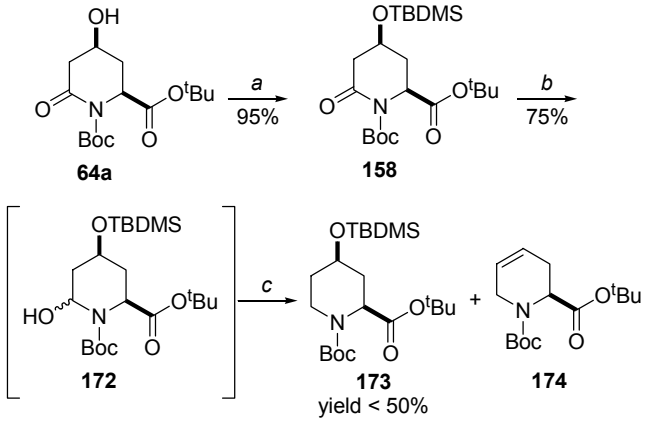
[17] for previous syntheses of *cis*-4-hydroxypipecolates, see: (a) ref. 6. (b) Lloyd, R. C.; Smith, M. E. B.; Brick, D. Taylor, S. J. C.; Chaplin, D. A.; McCague, R. *Org. Process Res. Dev.* **2002**, *6*, 762. (c) Celestini, P.; Danieli, B.; Lesma, G.; Sacchetti, A.; Silvani, A.; Passarella, D.; Virdis, A. *Org. Lett.* **2002**, *4*, 1367. (d) Kulesza, A.; Mieczkowski, A.; Jurczak, J. *Tetrahedron: Asymmetry* **2002**, *13*, 2061. (e) Rutjes, F. P. J. T.; Veerman, J. J. N.; Meester, W. J. N.; Hiemstra, H.; Schoemaker, H. E. *Eur. J. Org. Chem.* **1999**, 1127. (f) Brooks, C. A.; Comins, D. L. *Tetrahedron Lett.* **2000**, *41*, 3551. (g) Di Nardo, C.; Varela, O. *J. Org. Chem.* **1999**, *64*, 6119. (h) Haddad, H.; Larchevêque, M. *Tetrahedron: Asymmetry* **1999**, *10*, 4231. (i) Bousquet, Y.; Anderson, P. C.; Bogri, T.; Duceppe, J. S.; Grenier, L.; Guse, I. *Tetrahedron* **1997**, *53*, 15671. (j) Gillard, J.; Abraham, A.; Anderson, P. C.; Beaulieu, P. L.; Bogri, T.; Bousquet, Y.; Grenier, L.; Guse, I.; Lavallée, P. *J. Org. Chem.* **1996**, *61*, 2226. (k) Nin, A. P.; Varela, O.; de Ledermaker, R. M. *Tetrahedron* **1993**, *49*, 9459.

was still required for large quantity synthesis. Herein, we have investigated the synthesis of *cis*-4-hydroxypipecolic acid starting from the 4-hydroxylated lactam intermediate **64a**.

IV.2.2. Preparation of (2*S*,4*R*)-4-hydroxypipecolic acid

With (2*S*,4*S*)-4-hydroxy-6-oxo-1,2-piperidinedicarboxylate **64a** in hand, we first envisioned its two-step conversion into the 4-hydroxypipecolate **173** via the corresponding hemiaminal. This method had previously been reported for the reduction of pyrroglutamates to proline derivatives[18] and *N*-Boc protected piperidin-2-ones to the corresponding pipecolates[2,18f] in high yields. However, partial reduction of **64a** with $LiEt_3BH$ (Super Hydride®) did not provide the desired hemiaminal but gave degradation byproducts. Protection of the secondary alcohol prior to the reduction was mandatory. The resulting hemiaminal **172** was isolated in 75% and characterized by ^1H and ^{13}C NMR. The reduction of crude **172** with Et_3SiH / $Et_2O{\bullet}BF_3$, proved to be difficult because of partial removal of the TBDMS group. The desired hydroxypipecolate **173** was finally recovered in low yield together with the pipecolate **174** (Scheme IV.8).

Scheme IV.8 First attempts to reduce the lactam ring

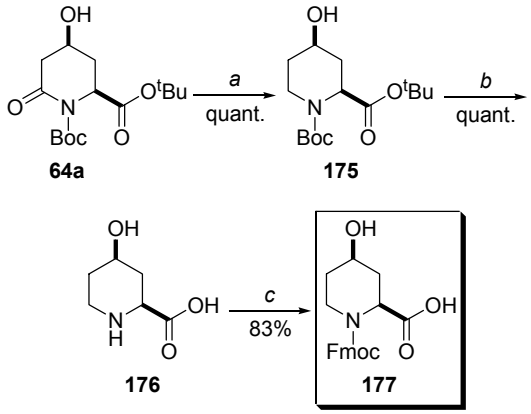


(a) TBDMSCl, imidazole, CH_2Cl_2 ; (b) $LiEt_3BH$, THF, −78°C ; (c) Et_3SiH, $Et_2O{\bullet}BF_3$, CH_2Cl_2, −78°C.

Compound **174** was unambiguously characterized by COSY NMR experiments. Because compounds **173** and **174** are extremely close on TLC and almost inseparable by chromatography, this route was not investigated further.

Alternatively, direct reduction of **64a** with BH_3•SMe_2 was considered.[19] Although this reagent achieves complete reduction of lactam functionality in one single step, it has not often been used. One possible reason could be the need of heating and long reaction times. Nevertheless, in our hands, lactam **64a** was cleanly reduced in 16 h at room temperature and gave pure **175** in quantitative yield after work-up.

Scheme IV.9 Preparation of N^{α}-Fmoc protected (2*S*,4*R*)-4-hydroxypipecolate



(a) BH_3•SMe_2, THF ; (b) HCl, dioxane ; (c) FmocOSu, K_2CO_3, acetone/H_2O.

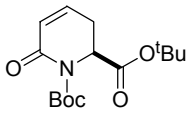
Simultaneous deprotection of the Boc and *tert*-butyl ester functional groups yielded the (2*S*,4*R*)-4-hydroxypipecolic acid **176**. Protection of the secondary amine by a Fmoc group afforded the *cis*-hydroxypipecolate **177** in an overall yield of 52% starting from the aspartic acid derivative **112a**.

[18] for recent examples, see: (a) Oba, M.; Miyakawa, A.; Nishiyama, K. *J. Org. Chem.* **1999**, *64*, 9275. (b) Oba, M.; Terauchi, T.; Miyakawa, A.; Nishiyama, K. *Tetrahedron: Asymmetry* **1999**, *10*, 937. (c) Escribano, A.; Carreno, C.; Garcia Ruano, J. L. *Tetrahedron Lett.* **1994**, *35*, 2053.

[19] for reductions of substituted pyrrolidinones with BH_3•SMe_2, see: (a) Courcambeck, J.; Bihel, F.; De Michelis, C.; Quéléver, G.; Kraus, J. L. *J. Chem. Soc. Perkin Trans. I* **2001**, 1421. (b) Moody, C. M. and Young, D. W. *J. Chem. Soc. Perkin Trans. I* **1997**, 3519. (c) Moody, C. M. and Young, D. W. *Tetrahedron Lett.* **1994**, *35*, 7277. (d) Moody, C. M.; Starkmann, B. A.; Young, D. W. *Tetrahedron Lett.* **1994**, *35*, 5485. (e) Herdeis, C. and Hubmann, H. P. *Tetrahedron: Asymmetry* **1994**, *5*, 351. (f) Heffner, R. J. and Joullié, M. M. *Tetrahedron Lett.* **1989**, *30*, 7021.

IV.2.3. Preparation of (2*S*,4*S*)-4-hydroxy pipecolic acid

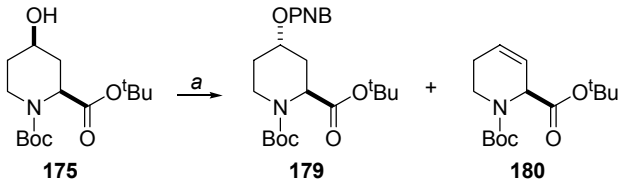
For the preparation of the corresponding *trans*-(2*S*,4*S*)-4-hydroxypipecolate, we initially tried to invert the C-4 stereocenter directly on **64a** under *Mitsunobu* conditions.[20] Unfortunately, all our attempts resulted in *β*-elimination and yielded *α*,*β*-unsaturated lactam **178**[21] as the sole product.



178

Starting from *cis*-hydroxypipecolate, the inversion reaction has already been mentioned, but not fully described.[22] Herein, treatment of **175** with *p*-nitrobenzoic acid under *Mitsunobu* conditions gave the desired pipecolate **179** with inverted configuration at C-4 in 53% yield together with the 3,4-unsaturated pipecolate **180** (Scheme IV.10).

Scheme IV.10 *Mitsunobu* inversion using *p*-nitrobenzoic acid



175 **179** **180**

53% yield 32% yield

(a) PNBOH, DIAD, PPh_3, THF.

Both pipecolates **179** and **180** were unambiguously characterized by X-ray diffraction studies (Figure IV.5 and IV.6, respectively).

[20] Mitsunobu, O. *Synthesis* **1981**, 1.

[21] enantiopure *N*-acylated 2-substituted-4,5-unsaturated *δ*-lactams are useful building blocks which can be further transformed in a stereocontrolled manner to give the corresponding 4-substituted derivatives, see: (a) Hanessian, S.; van Otterlo, W. A. L.; Nilsson, I.; Bauer, U. *Tetrahedron Lett.* **2002**, *43*, 1995. (b) Hanessian, S.; Seid, M.; Nilsson, I. *Tetrahedron Lett.* **2002**, *43*, 1991. (c) Muller, M.; Schoenfelder, A.; Didier, B.; Mann, A.; Wermuth, C. G. *Chem. Commun.* **1999**, 683.

[22] Bellier, B.; Da Nascimento, S.; Meudal, H.; Gincel, E.; Roques, B. P.; Garbay, C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1419.

Figure IV.5 Ortep plot of 179

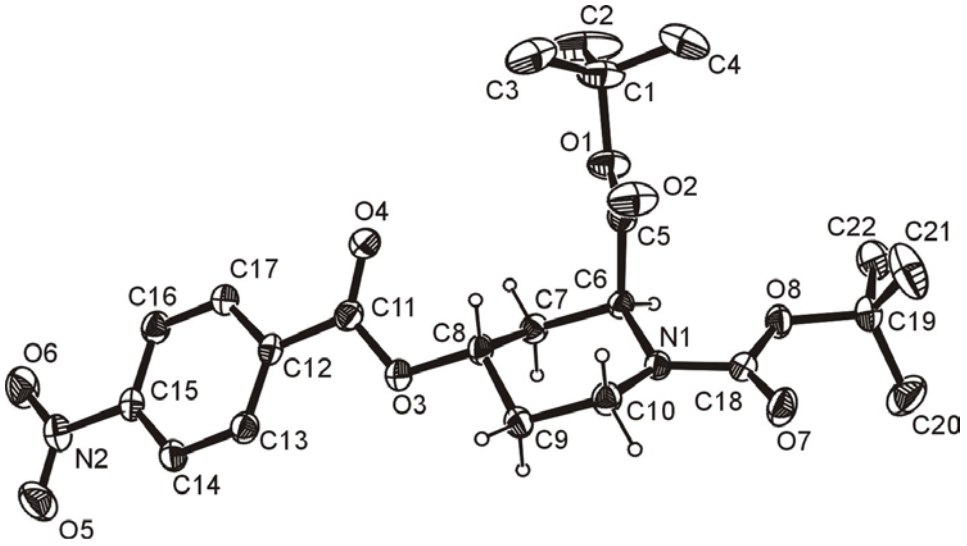
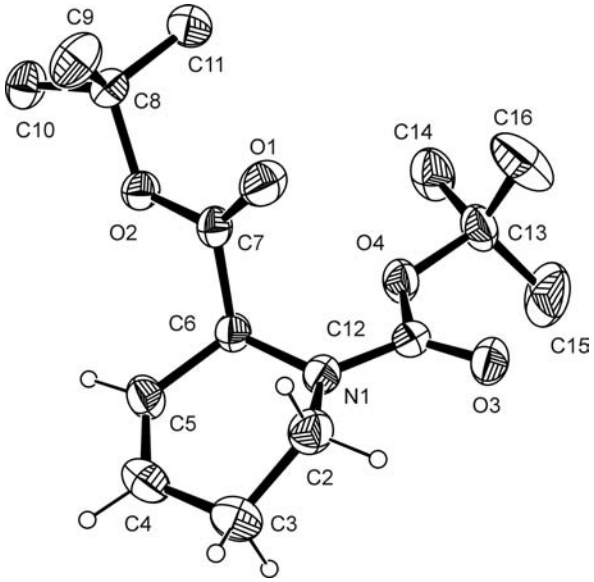
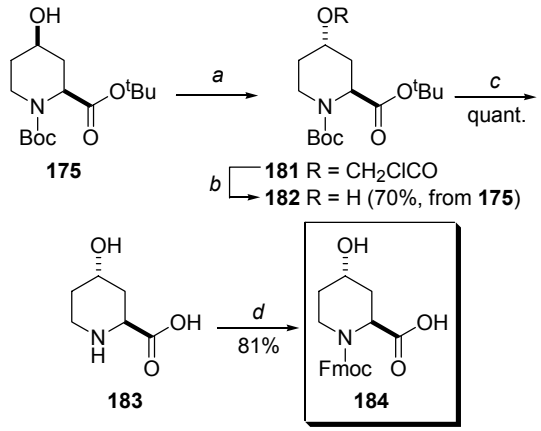


Figure IV.6 Ortep plot of 180



Chloroacetic acid was subsequently found to be the best acid to promote inversion at C-4. *Mitsunobu* reaction of **175** with chloroacetic acid afforded chloroacetate **181** which was immediately cleaved under basic conditions to give the corresponding *trans*-4-hydroxypipecolate **182** in 70% yield (2 steps). Deprotection of the *N*-Boc and *tert*-butyl ester functional groups yielded the (2*S*,4*S*)-4-hydroxypipecolic acid **183** which was reprotected in **184** with an overall yield of 57% from **175**.

Scheme IV.11 Preparation of N^{α}-Fmoc protected (2*S*,4*S*)-4-hydroxypipecolate



(a) $CH_2ClCOOH$, DIAD, PPh_3, THF ; (b) 1N NaOH, dioxane/H_2O ; (c) HCl, dioxane ; (d) FmocOSu, K_2CO_3, acetone/H_2O.

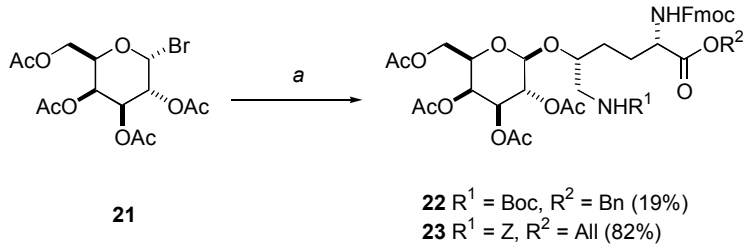
In conclusion, by analogy to chiral tetramic acids, reduction of *tert*-butyl 2-substituted-4,6-dioxo-1-piperidinecarboxylate **62** by $NaBH_4$ in CH_2Cl_2 / AcOH afforded the corresponding *cis*-4-hydroxy *δ*-lactams in good yield and stereoselectivity. 4-Hydroxy-6-oxo-1,2-piperidinedicarboxylate **64a**, readily accessible from Boc-Asp-O^{t}Bu (3 steps, 63% overall yield) has proven to be an excellent building block for the synthesis of a protected 4-hydroxylysine derivative **168** (41% overall yield) and for the synthesis of *cis*- and *trans*-4-hydroxypipecolates **177** and **184** (52 and 36% overall yield, respectively). Alternatively, **64a** is a key intermediate in the promising preparation of the 4-hydroxylysine aglycon **170**.

V. Synthesis of Glycosylated Building Blocks

V.1. Reported syntheses of *β*-galactosylated 5-hydroxylysine

Kihlberg's research group was the first one involved in the preparation of glycosylated hydroxylysine building blocks for use in the solid phase synthesis of glycopeptides (section I). We already reviewed the procedure they developed for the preparation of the mono-galactosylated building blocks **22** and **23** (Scheme V.1).[1] The galactosylation was performed with the peracetylated galactosyl bromide **21** under promotion of silver silicate. In the case of the galactosyl acceptor **5**, the low yield was explained by the inadequate stability of the N^{ε}-Boc protective group to silver silicate. In contrast, protection of the aglycon with the set of non acid-sensitive functional groups (ie, Fmoc, Z and allyl ester) gave *β*-glycoside **23** in high yield.

Scheme V.1 Galactosylation with acetylated galactosyl bromide under promotion by silver silicate[1]



21

22 R^1 = Boc, R^2 = Bn (19%)
23 R^1 = Z, R^2 = All (82%)

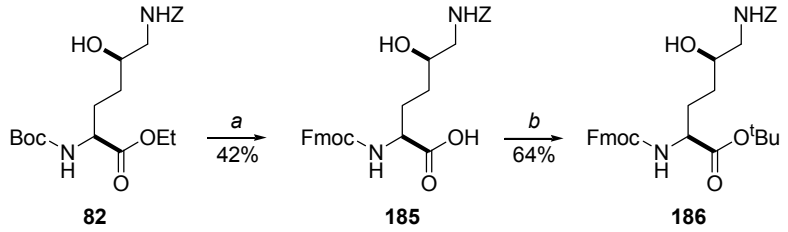
(a) silver silicate, CH_2Cl_2, MS, 0°C, then **5** or **6**.

Two other groups, already cited in section III for their preparation of protected (2*S*,5*R*)-5-hydroxylysine, have developed different strategies for galactosylation of hydroxylysine. Firstly, *Löhr* and colleagues[2] employed the tricholoroacetimidate methodology. Starting from the hydroxylysine derivative **82**, the galactosyl acceptor **186** was obtained in 27% yield by a two-step sequence (Scheme V.2).

[1] Holm, B.; Broddefalk, J.; Flodell, S.; Wellner, E.; Kihlberg, J. *Tetrahedron* **2000**, *56*, 1579.

[2] Löhr, B. Orlich, S.; Kunz, H. *Proceeding of the 25th European Peptide Symposium, Edited by Bajusz, S. and Hudecz, F. Peptides* **1998**, 228.

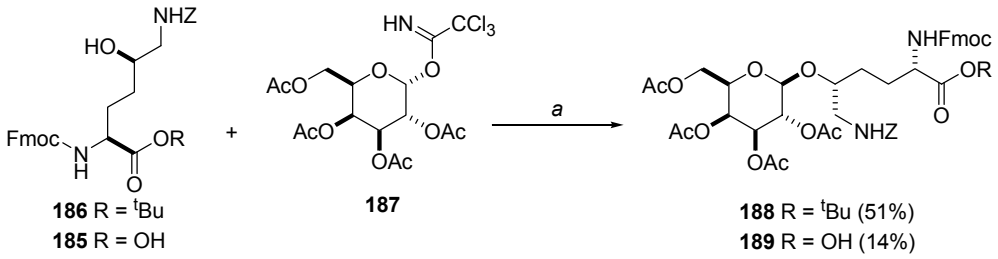
Scheme V.2 Preparation of the aglycons 185 and 186[2]



(a) (i) LiOH, (ii) HCl, (iii) FmocOSu ; (b) DCC, ^{t}BuOH, CuCl.

Galactosylation of the fully protected hydroxylysine **186** with the peracetylated galactosyl tricholoroacetimidate **187** under promotion by trimethylsilyl triflate (TMSOTf) gave the galactosylated derivative **188** in 51% yield. Under the same conditions, galactosylation of **185** gave the corresponding carboxylic acid **189** in only 14% yield (Scheme V.3). Despite the poor yield, this second strategy allowed the economy of one protecting step.

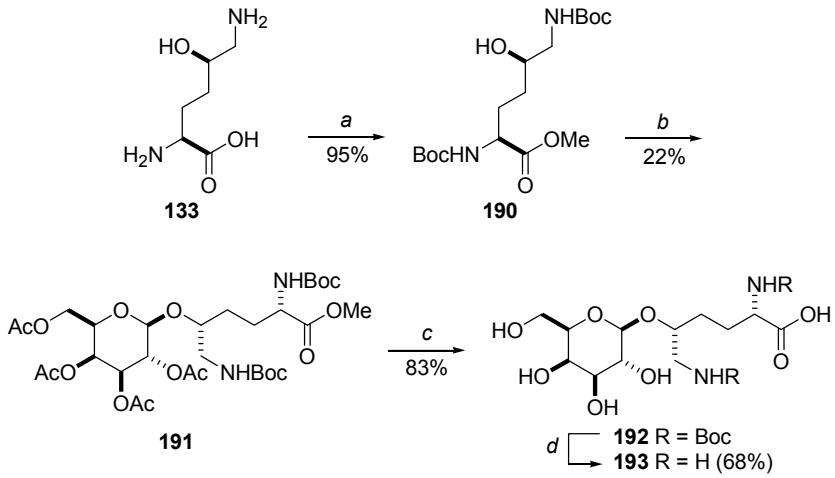
Scheme V.3 Galactosylation with acetylated galactosyl trichloroacetimidate[2]



(a) TMSOTf, CH_2Cl_2 or CH_2Cl_2/CH_3CN.

Adamczyk and colleagues[3] used the peracetylated galactosyl bromide **21**, but chose mercury (II) cyanide for promoting the galactosylation. In contrary to *Löhr* and colleagues, they did not start from one protected intermediate of their synthesis of (2*S*,5*R*)-5-hydroxylysine (section III), but they developed a full procedure starting from commercially available (2*S*,5*R*)-5-hydroxylysine. This strategy was not devoted to the preparation of a building block incorporable in solid phase synthesis of glycopeptides. Compound **193** was prepared for use as a calibrator, a control and a clinical reference standard in the development of immunoassays.

Scheme V.4 Preparation of 193 starting from commercial hydroxylysine[3]

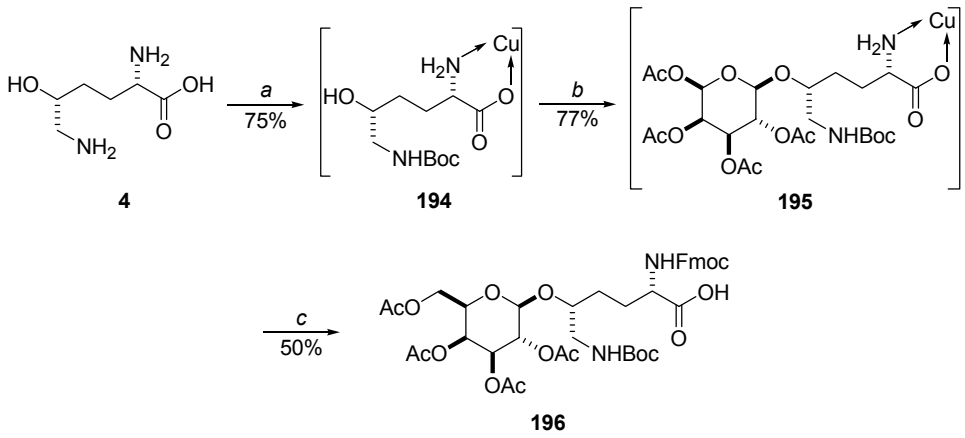


(a) (i) Boc_2O, $NaHCO_3$, THF/H_2O, (ii) CH_2N_2, ether ; (b) **21**, $Hg(CN)_2$, toluene, 75°C ; (c) LiOH, THF/H_2O ; (d) TFA, CH_2Cl_2.

By this procedure the galactosylated hydroxylysine derivative **191** was obtained in only 22% yield. The authors did not discuss this issue, but it appeared clearly that the protective group strategy (ie, *N*-Boc protection of the two amine functions) was not adapted to the glycosylation conditions).

The same year, *Malkar* and colleagues[4] published a methodology also starting from the commercially available (2*S*,5*R*)-5-hydroxylysine.

Scheme V.5 Preparation of a galactosylated building block from commercial hydroxylysine[4]



(a) (i) $CuCO_3 \bullet Cu(OH)_2$, H_2O, reflux, (ii) Boc_2O, NaOH, H_2O/dioxane ; (b) NaH, CH_3CN, then **21** ; (c) (i) Chelex 100, H_2O/CH_3OH, (ii) FmocOSu, $NaHCO_3$, H_2O/acetone.

[3] Adamczyk, M.; Reddy, R. E.; Rege, S. D. *Synth. Commun.* **2000**, *30*, 3281.
[4] Malkar, N. B.; Lauer-Fields, J. L.; Fields, G. B. *Tetrahedron Lett.* **2000**, *41*, 1137.

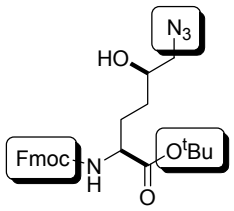
This synthesis was convenient, since it allowed the formation of the building block **196** (ready for use in SPPS) in only five steps and 29% overall yield (Scheme V.5). Hydroxylysine was transformed into the copper complex which allowed regioselective protection of the *ε*-amino group to yield **194**. Formation of the corresponding alcoholate prior to addition of galactosyl donor **21** gave the galactosylated complex **195** in 77% yield. After dissociation of the chelate, the *α*-amino function was protected with a Fmoc group to yield the galactosylated hydroxylysine derivative **196**.

Unfortunately, in our hands, the procedure described by *Malkar* and colleagues never gave the desired compound **196** and we abandoned this strategy after several trials due to the prohibitive price of commercial (2*S*,5*R*)-5-hydroxylysine.[5]

V.2. Optimized conditions for our aglycons

Comparison of the four galactosyl acceptors reported for the synthesis of *β*-galactosylated (2*S*,5*R*)-5-hydroxylysine presented previously reveals that the main difference resides in the protecting groups on the galactosyl acceptor. The choice of the protecting group strategy thus has a determinant role in the outcome of the glycosylation procedure. General protecting group scheme developed for our aglycons is shown in Figure V.1.

Figure V.1 Our general protecting group scheme



131

The N^{α}-amino functionality was protected with a Fmoc group for convenient SPPS. As *Löhr* and colleagues did,[2] we chose to protect the acid function with a *tert*-butyl ester. This protection is sensitive to acidic conditions, and thus to Lewis acids, but it allows quantitative orthogonal deprotection after the glycosylation step. Besides these common protecting groups, the azide function was considered as a temporary protection for the N^{ε}-amine ; the

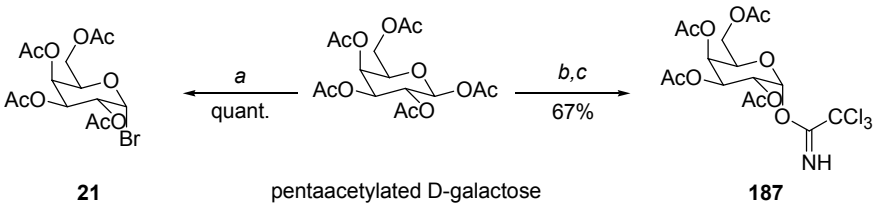
[5] (5*R*)-5-Hydroxy-L-lysine dihydrochloride monohydrate: Fluka 55501, € 169.50 for 1 gram.

reduction of the azide function being quantitatively and cleanly performed on the solid support at the end of the elongation of the peptidic chain (section VI).[6] Glycosylations have already been performed on acceptors carrying azide and this functionality should not interfere with the glycosylation reaction.[7]

V.2.1. Strategies using acetylated galactosyl donors

Initial galactosylation studies were conducted on the protected (2*S*,5*R*)-5-hydroxylysine **131** as a model template. The two galactosyl donors **21** and **187**, already used in the previous synthesis of galactosylated hydroxylysine, were evaluated together with various promoters, including trifluoroboron diethyletherate (BF_3•Et_2O), TMSOTf and silver silicate ($AgSiO_4$).

Scheme V.7 Preparation of the galactosyl donors 21 and 187



21 pentaacetylated D-galactose **187**

(a) HBr 33% in AcOH, CH_2Cl_2 ; (b) hydrazine acetate, DMF, 50°C ; (c) CCl_3CN, DBU, CH_2Cl_2.

Galactosyl bromide **21** and tricholoroacetimidate **187** were easily prepared from the commercial pentaacetylated D-galactose according to the published procedures (Scheme V.7). The analytical data of **21** and **187** were in accordance with the literature.

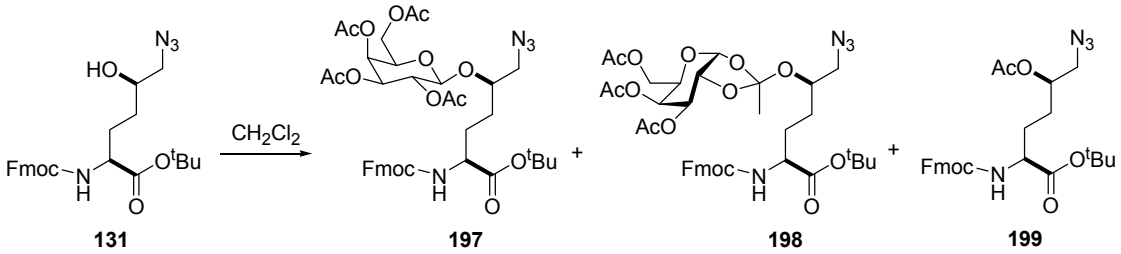
The experimental conditions are summarized in Table V.1. The major isolated products, together with the corresponding yields, are indicated in the last column of the table. These compounds have been purified by flash column chromatography and further characterized by ^1H and ^{13}C NMR for each experiment. All conditions tested with both galactosyl donors **21** and **187** failed to give us the desired galactosylated 5-hydroxylysine derivative **197**. Instead, the two major compounds isolated were the corresponding orthoester **198** and the C-5

[6] (a) Meldal, M.; Juliano, M. A.; Jansson, A. M. *Tetrahedron Lett.* **1997**, *38*, 2531. (b) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781.

[7] for examples of a glycosyl acceptor carrying azide functionalities, see: (a) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Seeberger, P. H. *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 2128. (b) Chen, Y.; Heeg, M. J.; Braunschweiger, P. G.; Xie, W.; Wang, P. G. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 1768. (c) Yuasa, H. and Hashimoto, H. *J. Am. Chem. Soc.* **1999**, *121*, 5089.

acetylated 5-hydroxylysine analogue **199**. Formation of these byproducts will be discussed later in this section.

Table V.1 Donor and promoter study for preparation of galactosylated hydroxylysine



131 **197** **198** **199**

entry	donor	*Lewis* acid [equiv]	temp, °C	time, h	isolated compound (yield, %)
1	**21**	$AgSiO_4$	rt	72	**198** (60)
2	**21**	$AgSiO_4$	0	4	**198** (31)
3	**187**	$BF_3•Et_2O$ [0.25]	−78 → rt	16	**199** (57)
4	**187**	$BF_3•Et_2O$ [0.50]	0	1	**199** (56)
5	**187**	$BF_3•Et_2O$ [0.25][a]	−78 → rt	16	**198** (70)
6	**187**	TMSOTf [0.15]	–20	0.5	**198** (71)
7	**187**	TMSOTf [0.30]	0	1	**199** (82)
8	**187**	TMSOTf [0.10]	−78 → rt	16	**199** (55) + **197** (35)

[a] CH_3CN instead of CH_2Cl_2 as solvent

Treatment of **131** with the galactosyl donor **21** in presence of $AgSiO_4$ gave exclusively the orthoester **198** (entry 1 and 2). This derivative was unambiguously characterized by ^1H and ^{13}C NMR. Treatment of **131** with the tricholoroacetimidate **187** in presence of catalytic amounts of $BF_3•Et_2O$ gave the acetylated 5-hydroxylysine **199** in 55% yield (entry 3-4). Changing the solvent ($CH_2Cl_2 \rightarrow CH_3CN$) only reversed the outcome of the reaction in favor of the orthoester **198** (entry 5). Replacing $BF_3•Et_2O$ by TMSOTf did not give more convincing results. This stronger Lewis acid gave also the orthoester **198** or the rearranged acetylated 5-hydroxylysine **199**, both isolated in high yields (entry 6-7). Only one trial allowed us to detect the desired galactosylated 5-hydroxylysine derivative **197** (entry 8). However, **197** was obtained in only 35% yield together with **199** (55% yield).

V.2.2. Orthoester formation and rearrangement

In the present case, the major drawback to the preparation of the 1,2-*β*-galactosidic linkage is the formation of the corresponding orthoester.[8] Indeed, the attack of oxygen doublet at the acyl carbon, instead of the anomeric carbon, leaded to the formation of the corresponding orthoester (Figure V.2). Under the acidic conditions used for glycoside formation the orthoester can rearrange into the corresponding glycoside. In the literature, conversion of orthoesters into glycosidic products under the action of an acidic promoter is the most cited method.[9] In more atypical examples, the glycoside was successfully obtained from the corresponding orthoester by simply increasing the catalytic amount of promoter,[10] by replacing silver carbonate with TMSOTf,[11] or by substituting the milder Lewis acid BF_3•Et_2O for TMSOTf.[12] Moreover, sugar 1,2-orthoesters have already been used as donors in the construction of 1,2-*β*-glycosidic linkage.[13] Unfortunately, all our attempts failed and the orthoester rearrangement gave rise to transesterification of the acceptor in an irreversible way, as illustrated in Figure V.2. This mechanism explains the formation of **199** in galactosylations with donors carrying C-2 acetyl protections.

[8] Ferse, F. T.; Floeder, K.; Hennig, L.; Findeisen, M.; Welzel, P. *Tetrahedron* **1999**, *55*, 3749.

[9] (a) Yang, Z.; Lin, W.; Yu, B. *Carbohydr. Res.* **2000**, 329, 879. (b) Wang, W. and Kong, F. *J. Org. Chem.* **1998**, *63*, 5744. (c) Saunders, W. J.; Manning, D. D.; Koeller, K. M.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 16391. (d) Gass, J.; Strobl, M.; Loibner, A.; Kosma, P.; Zaehringer, U. *Carbohydr. Res.* **1993**, *244*, 69. (e) Urban, F. J.; Moore, B. S.; Breitenbach, R. *Tetrahedron Lett.* **1990**, *31*, 4421.

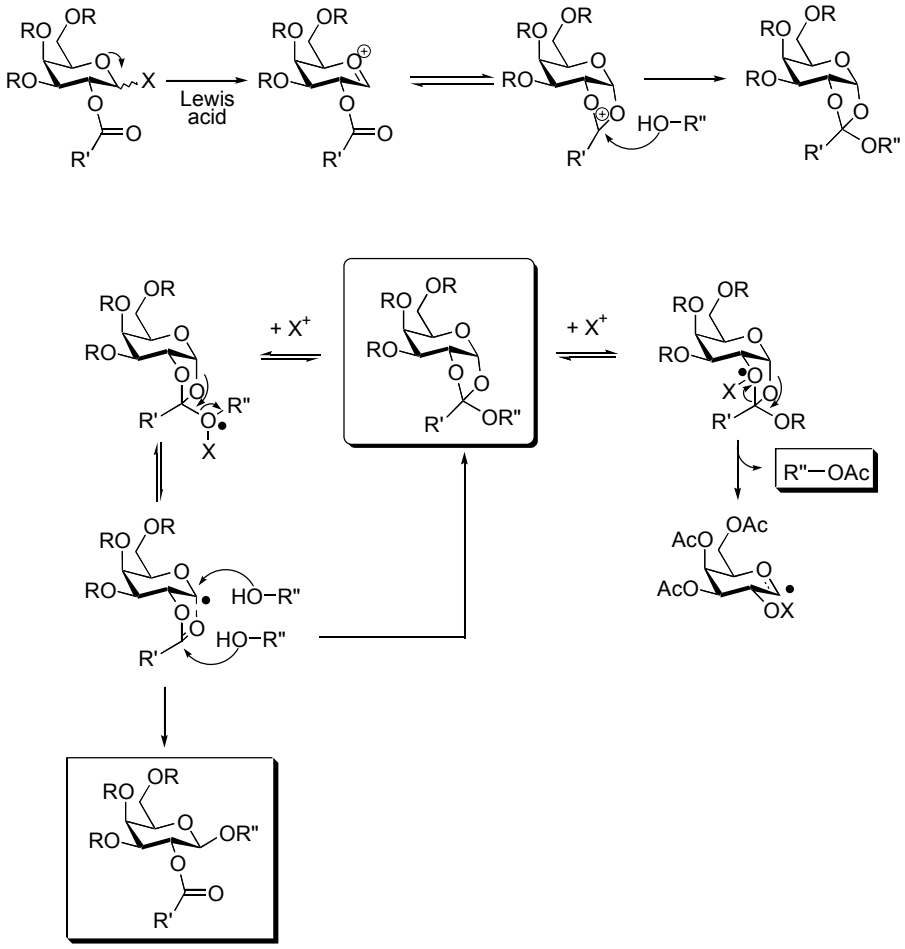
[10] Eisele, T.; Windmüller, R.; Schmidt, R. R. *Carbohydr. Res.* **1998**, *306*, 81.

[11] Lindhorst, T.; Kötter, S.; Kubisch, J.; Krallmann-Wenzel, U.; Ehlers, S.; Kren, V. *Eur. J. Org. Chem.* **1998**, 1669.

[12] Halkes, K. M.; Gotfredsen, C. H.; Grotli, M.; Miranda, L. P.; Duus, J. O.; Meldal, M. *Chem. Eur. J.* **2001**, *7*, 3584.

[13] the construction of 1,2-*trans*-glycosidic linkages starting from 1,2-orthoesters as glycosyl donors have particularly been studied by Kochetkov and colleagues, see: (a) Kochetkov, N. K.; Nepogod'ev, S. A.; Backinowsky, L. V. *Tetrahedron* **1990**, *46*, 139 and references cited therein.

Figure V.2 Orthoester formation and rearrangement



However, the formation of these two byproducts can be limited when a benzoate or a pivaloate is used as participating group instead of an acetate. Use of the charge delocalizing benzoate[14] stabilizes the acyloxonium ion, whereas the use of the bulky pivaloyl ester[15] hinders the formation of the orthoester.

V.2.3. A modified protecting group strategy

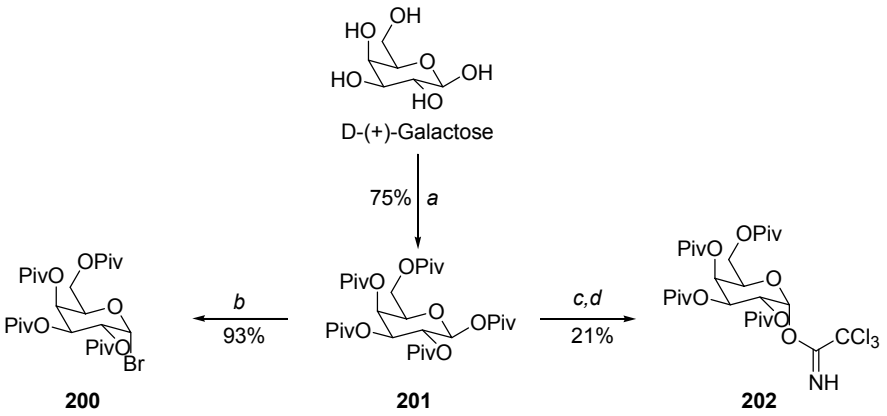
We then changed our strategy and tried to eliminate the formation of the orthoester **198** and the derived byproduct **199** by replacing the acetyl protections of the galactosyl donor with the bulkier pivaloyl functional groups.[15a] As previously, (2*S*,5*R*)-5-hydroxylysine **131** served

[14] for a selected example, see: Mitchell,S. A.; Pratt, M. R.; Hruby, V. J.; Polt, R. *J. Org. Chem.* **2001**, *66*, 2327.

[15] for selected examples, see: (a) Kunz, H. and Harreus, A. *Liebigs Ann. Chem.* **1982**, 41. (b) Sato, S.; Nunomura, S.; Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 4097.

as a model template in a new study to evaluate pivaloylated galactosyl donors **200** and **202** in the presence of the same promoters (ie, BF_3•Et_2O, TMSOTf and $AgSiO_4$).

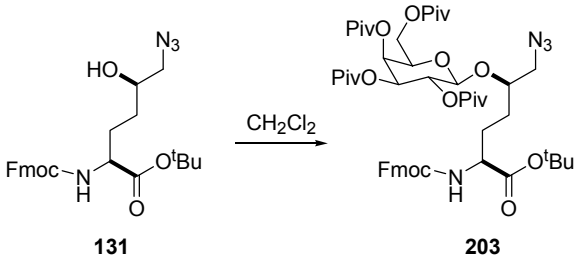
Scheme V.7 Preparation of the galactosyl donors 200 and 202



(a) PivCl, pyridine, $CHCl_3$; (b) HBr 33% in AcOH, CH_2Cl_2 ; (c) hydrazine acetate, DMF, 50°C ; (d) CCl_3CN, DBU, CH_2Cl_2.

Although pentapivaloylated D-galactose is commercially available, we preferred to prepare it in large quantities from the cheap D-galactose. Galactosyl bromide **200** and tricholoroacetimidate **202** were prepared from pentapivaloylated galactose **201** according to the procedures described previously for the preparation of the acetylated galactosyl donors **21** and **187**. The analytical data of **200** and **202** were in accordance with the literature.

Table V.2 Conditions study with pivaloylated galactosyl donors



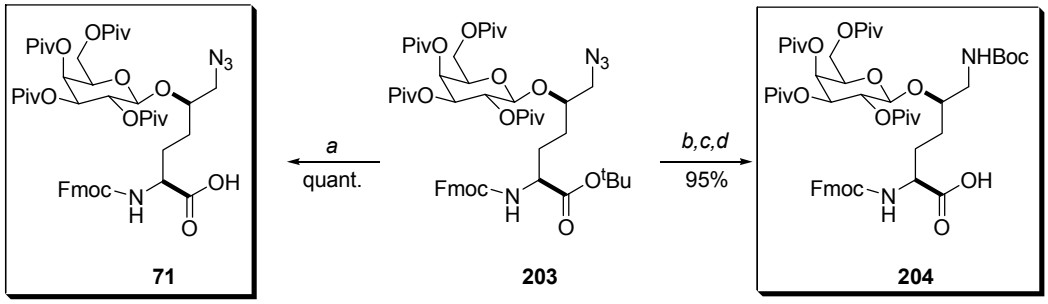
donor	*Lewis* acid	temp, °C	time, h	yield, %
200	silver silicate	rt	72	62
200	**silver silicate**	**rt**	**16**	**83**
202	$BF_3.Et_2O$	rt	24	46
202	TMSOTf	–10	0.5	64

To our satisfaction, the formation of orthoester was suppressed in the galactosylation reaction. Following optimization, the galactosylated derivative **203** was finally obtained in 83% yield, the clean reaction being easily monitored by C_{18} RP-HPLC.

V.2.4. *β*-Galactosylated (2*S*,5*R*)-5-hydroxylysine building blocks

Acid-catalyzed cleavage of the *tert*-butyl ester functional group gave quantitatively the derived building block **71**. If required, the azide function can be transformed into a N^{ε}-Boc protection by a three-step sequence. Reduction of the azide using zinc in presence of AcOH afforded quantitatively the amino derivative. Quantitative cleavage of the *tert*-butyl ester prior to Boc protection of the primary amine gave the building block **204** in 95% overall yield.

Scheme V.8 Synthesis of galactosylated (2*S*,5*R*)-5-hydroxylysine building blocks



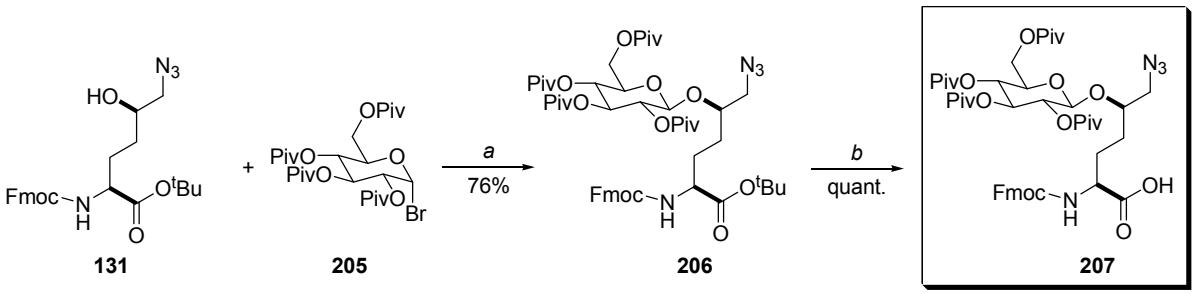
(a) TFA, CH_2Cl_2 ; (b) Zn, AcOH, THF ; (c) HCl/dioxane ; (d) Boc_2O, DIEA, THF.

Both building blocks **71** and **204** were found to be suitable for the synthesis of the corresponding CII-derived glycopeptides as described in the following section.

V.2.5. Glucosylated (2*S*,5*R*)-5-hydroxylysine building block

Applying this two-step optimized protocol (ie, glycosylation and *tert*-butyl ester cleavage) in presence of the glucosyl donor **205** yielded the glucosylated (2*S*,5*R*)-5-hydroxylysine building block **207** (Scheme V.8).

Scheme V.8 Synthesis of glucosylated (2*S*,5*R*)-5-hydroxylysine

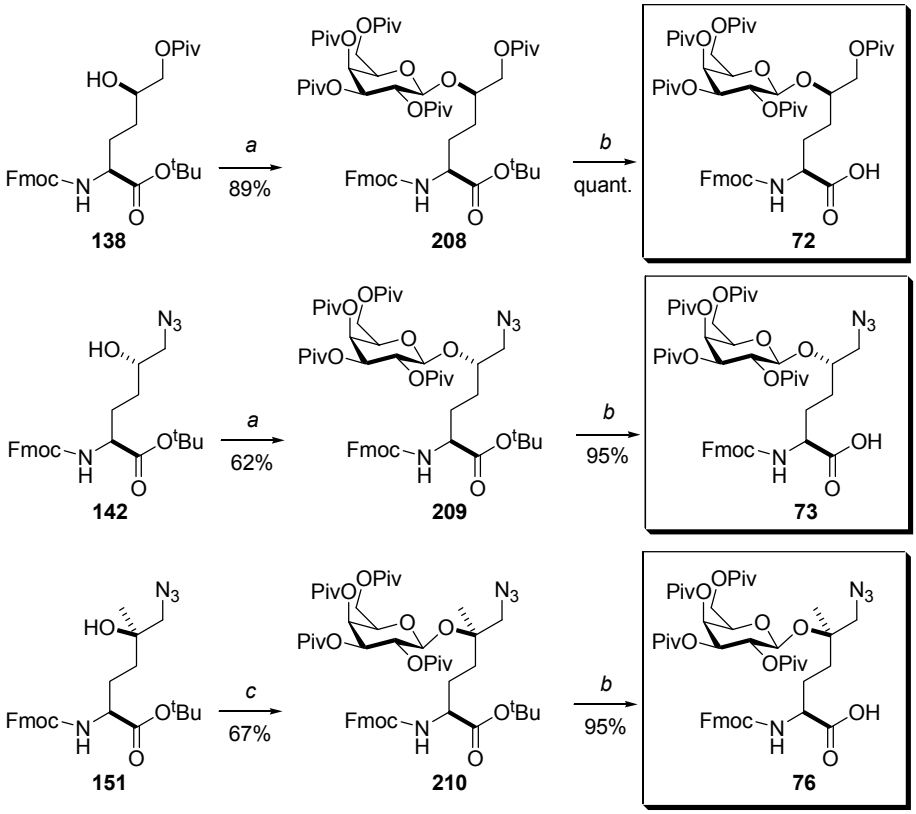


(a) silver silicate, 4Å MS, CH_2Cl_2 ; (b) TFA, CH_2Cl_2.

V.3. Galactosylation of the 5-hydroxylysine mimetics

All 5-hydroxylysine analogues **138**, **142**, **146** and **151** were galactosylated using the optimized procedure developed previously in this section. Galactosylation was performed using the pivaloylated galactosyl bromide **200**.

Scheme V.9 Synthesis of the galactosylated building blocks ready for use in SPPS



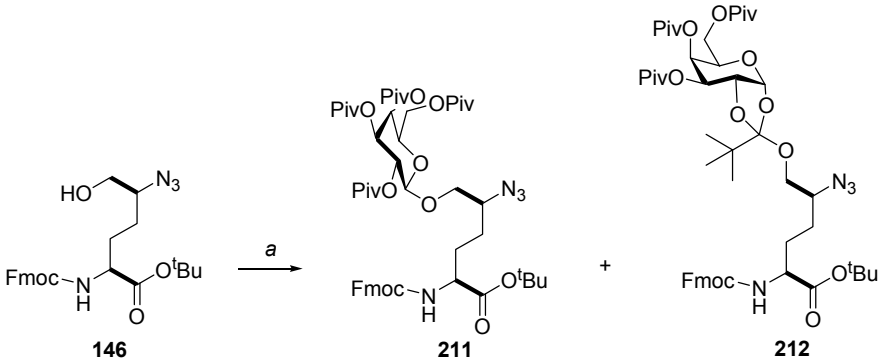
(a) **200**, silver silicate, 4Å MS, CH_2Cl_2; (b) TFA, CH_2Cl_2 ; (c) procedure (a) + CH_2Cl_2/cyclohexane (1:3).

The galactosylated hydroxylysine analogues **208**, **209** and **210** were obtained in good to excellent yields (Scheme V.9). The low reactivity of the tertiary alcohol in acceptor **151** necessitated a slight modification in the common procedure used for all other derivatives. In this particular case, the same amount of galactosyl donor was used, but it was divided into six equal portions added every 12 h. Additionally, a mixture of CH_2Cl_2 and cyclohexane was used as a solvent to reduce the degradation rate of galactosyl bromide **200**. C_{18} RP-HPLC monitoring of the reaction indicated completion after three days.

In all cases, final deprotection of the *tert*-butyl ester group gave synthons **72**, **73** and **76** in high, if not quantitative yield. These three building blocks, together with the glycosylated (2*S*,5*R*)-5-hydroxylysine **71** and **207**, were all used in the preparation of glycopeptides derived from CII(256-270).

Surprisingly, galactosylation of **146** under our optimized procedure resulted in the formation of the corresponding orthoester **212** which was recovered in 81% yield together with the desired galactosylated building block **211** (Scheme V.10). Attempts to rearrange the orthoester **212** to the desired glycosylated derivative **211** failed so far.

Scheme V.10 Attempt to prepare the galactosylated building block 211



146 **211** **212**

19% yield 81% yield

(a) **200**, silver silicate, 4Å MS, CH_2Cl_2.

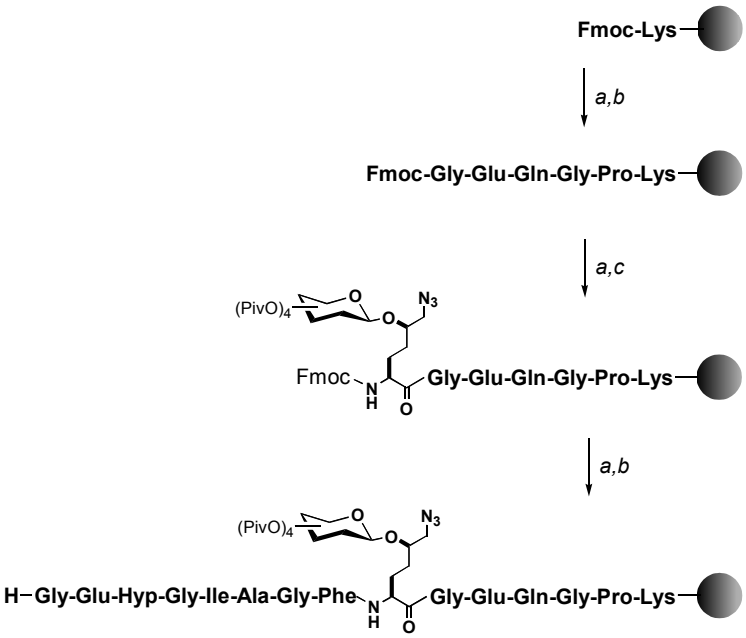
VI. Preparation of CII-derived Peptides and Glycopeptides

With *N*-Fmoc protected glycosylated building blocks **71-73**, **76** and **207** in hand, we were ready to prepare natural and non-natural glycopeptides related to sequence CII(256-270). Synthesis was performed on solid support using standard Fmoc / ^{t}Bu peptide chemistry.[1] Optimization of azide reduction and pivaloyl deprotection steps was performed on the naturally occurring and already described glycopeptide **GP2**.

VI.1. Elongation of the peptidic chain on solid support

All glycopeptides were synthesized using a home-made semi-automatic peptide synthesizer[2] on a polystyrene resin (purchased from Neosystem). The *C*-terminal amino acid Lys^{270} was attached to the resin via a *p*-hydroxymethylphenoxy linker (*Wang* resin).[3]

Figure VI.1. General procedure for the solid phase assembly (example of GP2)



(a) 20% piperidine in DMF ; (b) Fmoc-Xaa-OH, BOP, HOBt, DIEA, DMF ; (c) **71**, BOP, HOBt, DIEA, DMF.

[1] *Fmoc Solid Phase Peptide Synthesis – A Practical Approach*, edited by Chan, W. S. and White, P. D. © Oxford University Press **2000**.

[2] Neimark, J. and Briand, J. P. *Pept. Res.* **1993**, *6*, 219.

[3] Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328.

All amino acids were coupled as their benzotriazolyl esters, which were generated by addition of BOP and HOBt to a solution of the amino acid in DMF. A large excess of DIEA was added at the time of coupling. Commercial *N*-Fmoc amino acids (5 equivalents) having standard side chains were introduced *via* a double coupling (2 times 20 min). The highly valuable building blocks **71**-**73**, **76** and **207** were coupled with the same chemical strategy (ie, BOP / HOBt / DIEA). However, the amount of reactants was reduced (only 2 equivalents) and the duration of the first coupling increased (60 min). After each step, the resin was washed automatically and the reaction was monitored by the *Kaiser* test.[4] Fmoc deprotection was performed automatically with 20% piperidine in DMF and monitored by the same test. At the end of the elongation of the peptidic chain (ie, after the final deprotection), the resin was washed with CH_2Cl_2 and dried with Et_2O.

At this stage of the synthesis and for the **GP2** glycopeptide, the purity of the crude product was controlled by partial cleavage of the peptidic chain from the resin and found to be of 92% (determined by C_{18} RP-HPLC, see Figure VI.3 picture A).

VI.2. Deprotection strategy

VI.2.1. Reduction of the azido function and cleavage from solid support

The reduction of the azido function into the corresponding primary amine which is required for the preparation of glycopeptides **GP2**, **GP13**, **GP17**, **GP18** and **GP23**-**GP25** was performed on solid support following elongation of the peptidic chain. The conditions were optimized for **GP2**.

Table VI.1. Reduction of the azide using phosphines (studies conducted on GP2 intermediate)

phosphine	% of conversion azide → amine			
	12 h	24 h	48 h	72 h
PMe_3	64	85	-	94
PPh_3	54	85	96	97

[4] Kaiser, E.; Colescott, R. L.; Bosinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595.

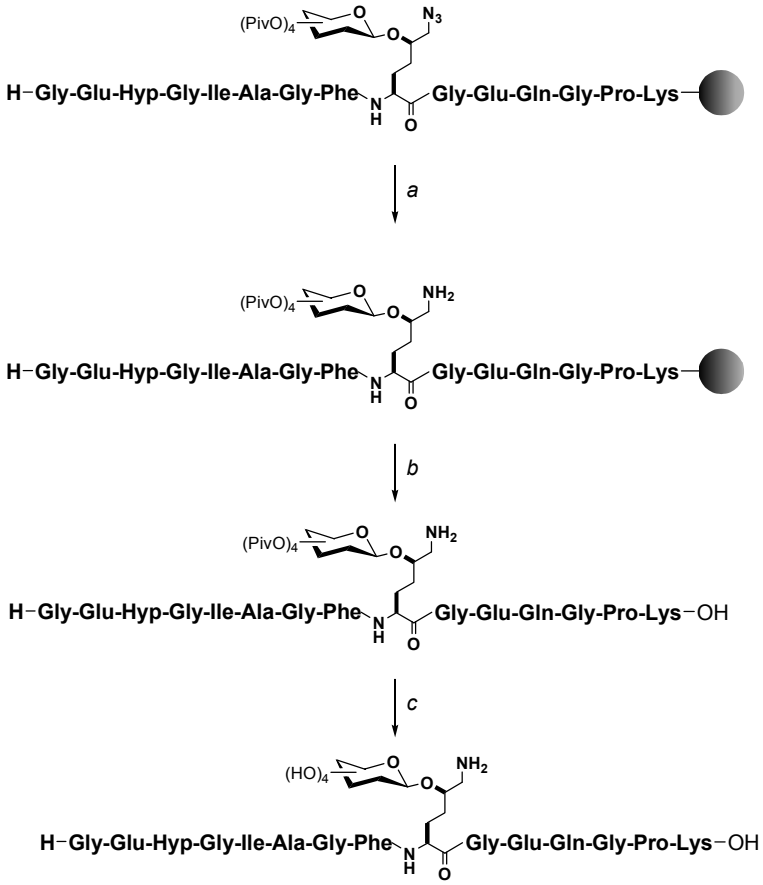
We found that treatment of the resin with both trimethylphosphine (PMe_3) and triphenylphosphine (PPh_3) in THF resulted in clean and quantitative reduction of all azido function after 72 h (Table VI.1).

Cleavage from the resin was performed with TFA containing water, triisopropylsilane (TIPS) and dithiothreitol (DTT) as scavengers. These conditions also removed the standard side-chain protecting groups (ie, Boc for Lys, *tert*-butyl for Glu and Hyp, and triphenylmethyl for Gln). At this stage of the synthesis, the purity of the glycopeptide corresponding to **GP2** was 87% (determined by C_{18} RP-HPLC, see Figure VI.3 picture B).

VI.2.2. Deprotection of the glycosyl moiety

An additional deprotection step was necessary for the removal of the pivaloyl esters protecting groups used for the saccharide moieties. To avoid epimerization of the amino acids α-stereogenic centers along the peptidic chain, a diluted solution of NaOMe in MeOH (40 mM) was used. This concentration is only two times higher than the concentration commonly used for cleavage of acetyl groups from glycopeptides. However, the time required for full deprotection was largely increased. Monitoring the reaction by C_{18} RP-HPLC indicated the complete cleavage of all four pivaloyl groups after 8-12 h and without formation of degradation byproducts.

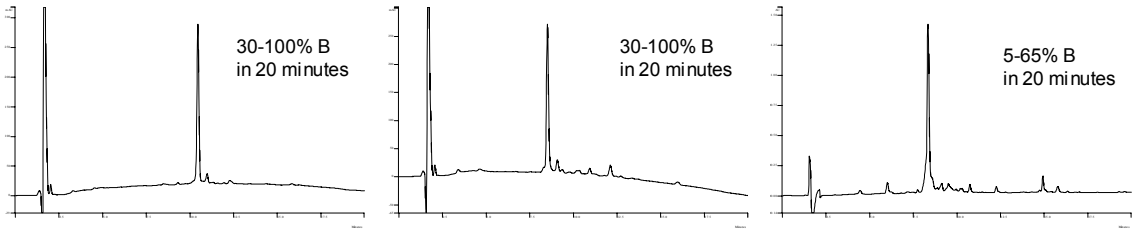
Figure VI.2. General procedure for deprotection and cleavage from the resin



(a) PPh_3, THF/H_2O ; (b) TFA/H_2O/TIPS/DTT (8.8:0.5:0.2:0.5) ; (c) NaOMe, MeOH.

At this stage of the synthesis, the purity of the naturally occurring glycopeptide **GP2** was 71% (determined by C_{18} RP-HPLC, see Figure VI.3 picture C).

Figure VI.3. HPLC profiles of the three GP2 intermediates at different stages of the synthesis



picture A picture B picture C

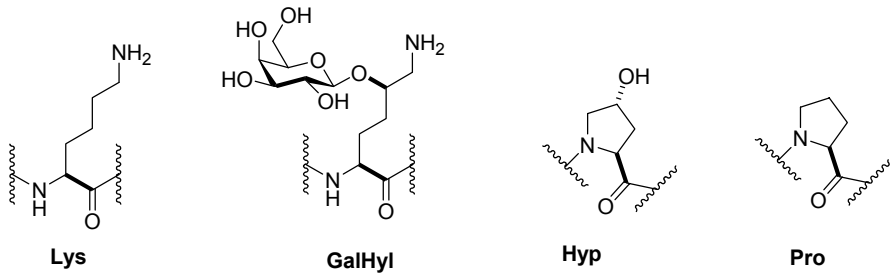
Finally, purification by C_{18} RP-HPLC furnished all CII-derived peptides and glycopeptides in high purity (> 99%). All peptides and glycopeptides were characterized by mass analysis (MALDI-TOF) and the overall yields, based on the resin capacity, were calculated.

VI.3. List of peptides and glycopeptides

Several naturally occurring CII(256-270) peptides carrying various degrees of post-translational modifications were prepared (Figure VI.4). **GP19** and **GP20** are non glycosylated peptides with a Lys residue at position 264 that differ only at residue 258 (Hyp vs Pro). The two glycopeptides **GP2** and **GP17** exhibit the same modulation at position 258, but residue 264 is a galactosylated (2*S*,5*R*)-5-hydroxylysine (GalHyl). Additionally, glycopeptide **GP18**, the mCII analogue of **GP2**, was prepared by substituting a Asp for the Glu^{266}.

Figure VI.4 Naturally occurring synthetic CII-derived peptides and glycopeptides

H-Gly^{256}-Glu-**Xaa^{258}**-Gly-Ile-Ala-Gly-Phe-**Xaa^{264}**-Gly-**Xaa^{266}**-Gln-Gly-Pro-Lys^{270}-OH

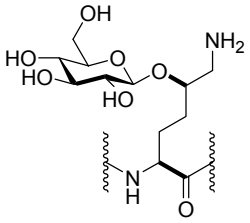


Peptides	Xaa^{258}	Xaa^{264}	Xaa^{266}	Calc. Mass	Found	Yield, %
GP2	Hyp	GalHyl	Glu	1666	1667.11	59
GP17	Pro	GalHyl	Glu	1650	1651.18	56
GP18	Hyp	GalHyl	Asp	1652	1653.66	58
GP19	Hyp	Lys	Glu	1488	1489.03	95
GP20	Pro	Lys	Glu	1472	1473.08	95

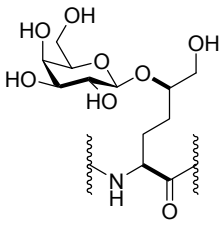
Starting from the synthetic building blocks described in the previous section, we also prepared six unnatural glycopeptides derived from CII(256-270) shown in Figure VI.5. The modification concerned exclusively the residue 264, which was replaced by the glucosylated building block **207** or by one of the galactosylated building blocks **72**, **73** and **76**. While peptide **GP13** is an analogue of **GP2** modified at the carbohydrate moiety, all the other peptides carry modifications at the Hyl side-chain. The ε-amino group is replaced by an azido group or a hydroxy function in **GP21** and **GP22**. The stereochemistry at the 5-position is reversed in **GP23** and a bulky methyl group is present at the C-5 position in **GP24**. Finally, peptide **GP25** corresponds to **GP2** carrying a fully protected galactosyl moiety.

Figure VI.5 Unnatural synthetic CII-derived peptides and glycopeptides

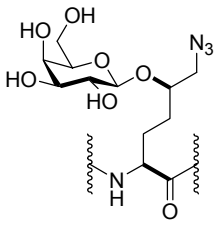
H-Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-**Xaa^{264}**-Gly-Glu-Gln-Gly-Pro-Lys^{270}-OH



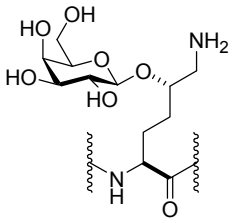
GlcHyl



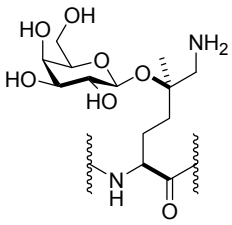
GalDhn



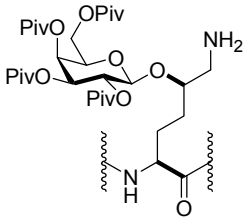
GalHyl(N_3)



Gal(5*S*)Hyl



GalHyl(Me)



Gal(Piv_4)Hyl

Peptides	Xaa^{264}	Calc. Mass	Found	Yield, %
GP13	GlcHyl	1666	1667.28	55
GP21	GalHyl(N_3)	1692	1693.02	59
GP22	GalDhn	1667	1667.01	58
GP23	Gal(5*S*)Hyl	1666	1667.84	58
GP24	GalHyl(Me)	1680	1680.40	57
GP25	Gal(Piv_4)Hyl	2002	2002.65	73

All glycopeptides were obtained in good yields (55-73%) based on the resin capacity and have been used in in-vitro assays to evaluate their recognition by CII-specific T-cells (following section).

VII. Immunological Assays

The synthetic glycopeptides **GP2**, **GP13** and **GP17-GP25** served to elucidate the fine specificity of three T-cell hybridomas (namely, A2G10, A8E2 and A9E5)[1] and one T-cell clone (namely, A9.2)[2] all specific for CII. The in-vitro evaluations were performed by the group of *Fournier* and colleagues (INSERM U567, Hôpital Cochin, Paris) who collaborate with us on this project.

VII.1. Materials and methods

VII.1.1. Generation of CII-specific T-cell hybridomas

The three anti-CII T-cell hybridomas required for this study were isolated by *Chiocchia* and colleagues few years ago.[1] The T-cell hybridomas were derived by fusion of lymph node cells from CII-primed DBA/1 (H-2^q) mice (immunized with bCII emulsified in CFA) and the mutant BW5147 thymoma cells (H-2^k). These $CD4^+$ T-cell hybridomas recognized CII from many species (except the mouse) and produced IL-2 in response to CII peptides presented by I-A^q molecules. More precisely, the reactivity was directed against the immunodominant CB11 fragment.

VII.1.2. Generation of the CII-specific T-cell clone

The anti-CII T-cell clone A9.2 was recently isolated by *Doncarli* and colleagues.[2] Six T-cell clones were generated in-vitro from the lymph nodes of CII-immunized DBA/1 mice in three independent experiments. All isolated T-cell clones were found to be reactive to the glycosylated dominant epitope CII(256-270). Following sequencing, TCR of these clones were found to be identical. When transferred to CII-immunized DBA/1 mice, the representative clone A9.2 increased the incidence, aggravated significantly the clinical signs

[1] Chiocchia, G.; Manoury-Schwartz, B.; Boissier, M. C.; Gahery, H.; Marche, P. N.; Fournier, C. *Eur. J. Immunol.* **1994**, *24*, 2775.

[2] Doncarli, A.; Chiocchia, G.; Stasiuk, L. M.; Herbage, D.; Boutillon, M. M.; Fournier, C.; Abehsira-Amar, O. *Eur. J. Immunol.* **1999**, *29*, 3636.

of CIA and greatly enhanced the anti-CII antibody response. Interestingly, the TCR of A9.2 is very similar to that of the three T-cell hybridomas previously generated (previous paragraph).[1,2]

VII.1.3. Measurement of T-cells reactivity

The T-cell hybridomas were co-cultured with DBA/1 irradiated spleen cells or M12.C10 cells in the presence of varying concentrations of glycopeptides. After 24 h of incubation at 37°C, supernatants were collected and frozen at –20°C. Thawed supernatants were tested for their ability to support CTLL-2 proliferation. CTLL-2 growth was assayed by the mean of [^{3}H] thymidine incoporation determined by liquid scintillation counter. The results were expressed as the mean of triplicate experiments after division by the mean background obtained by co-cultures of T-cell hybridomas and spleen cells without peptide.

In a same manner, the T-cell clone A9.2 was co-cultured with M12.C10 in the presence of varying concentrations of glycopeptides. After 48 h of incubation, [^{3}H] thymidine was added and T-cell proliferation was determined by mean of [^{3}H] thymidine incoporation. The results were expressed as the mean of triplicate experiments after division by the mean background obtained by co-cultures of A9.2 and M12.C10 without peptide.

VII.2. T-cell recognition of glycopeptides

VII.2.1. Evaluation of the natural peptides

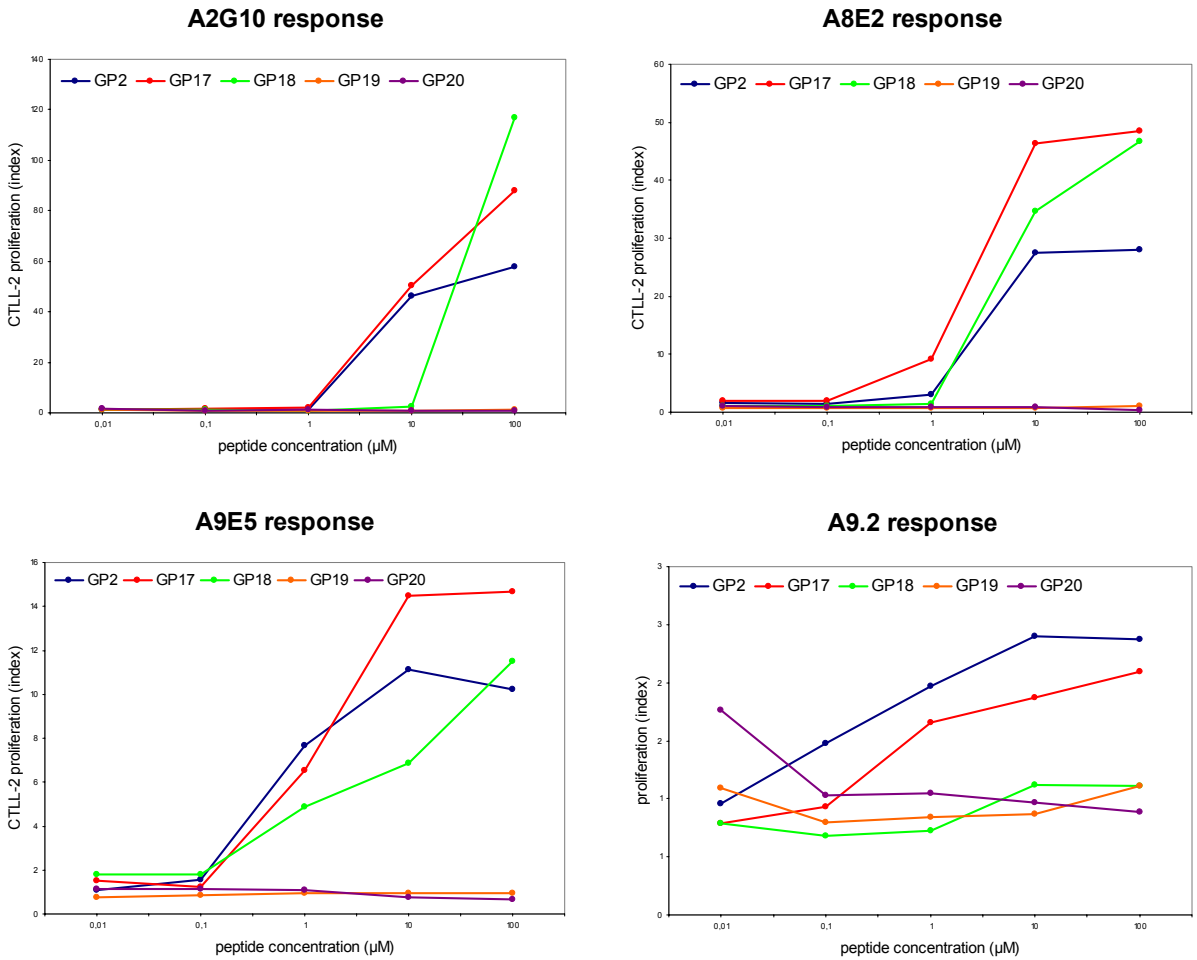
Among the eleven CII(256-270) peptides prepared, five are natural CII-derived peptides (ie, **GP2**, **GP17**, **GP18**, **GP19** and **GP20**) carrying various degrees of post-translational modifications (ie, hydroxylation and glycosylation). These peptides were used as control to verify the role of glycosylation on T-cell recognition in our assay as well as to study the effect of Pro / Hyp substitution at position 258 and Glu / Asp substitution at position 266 (bCII vs mCII).

The non glycosylated analogues **GP19** and **GP20** were not recognized by any of the three T-cell hybridomas nor by the T-cell clone (Figure VII.1). Glycopeptides **GP2** and **GP17**,

varying only at residue 258 (Hyp vs Pro), were recognized by the three T-cell hybridomas with similar proliferation indexes as well as by the T-cell clone (Figure VII.1). According to these results, the post-translational hydroxylation of Pro^{258} is seemingly not required for the recognition by T-cells and has no influence on the recognition pattern.

The fifth naturally occurring CII(256-270) peptide **GP18**, which corresponds to the autologous mCII ($Glu^{266} \rightarrow$ Asp) analogue of **GP2**, is generally recognized by T-cells but to a much lower extent compared to the heterologous CII-derived glycopeptide **GP2**. Nevertheless, the recognition of **GP18** by the three T-cell hybridomas and the T-cell clone could support a possible role for homologous CII in the development of CIA after immunization by heterologous CII.

Figure VII.1 Evaluation of the five naturally occurring peptides GP2 and GP17-GP20

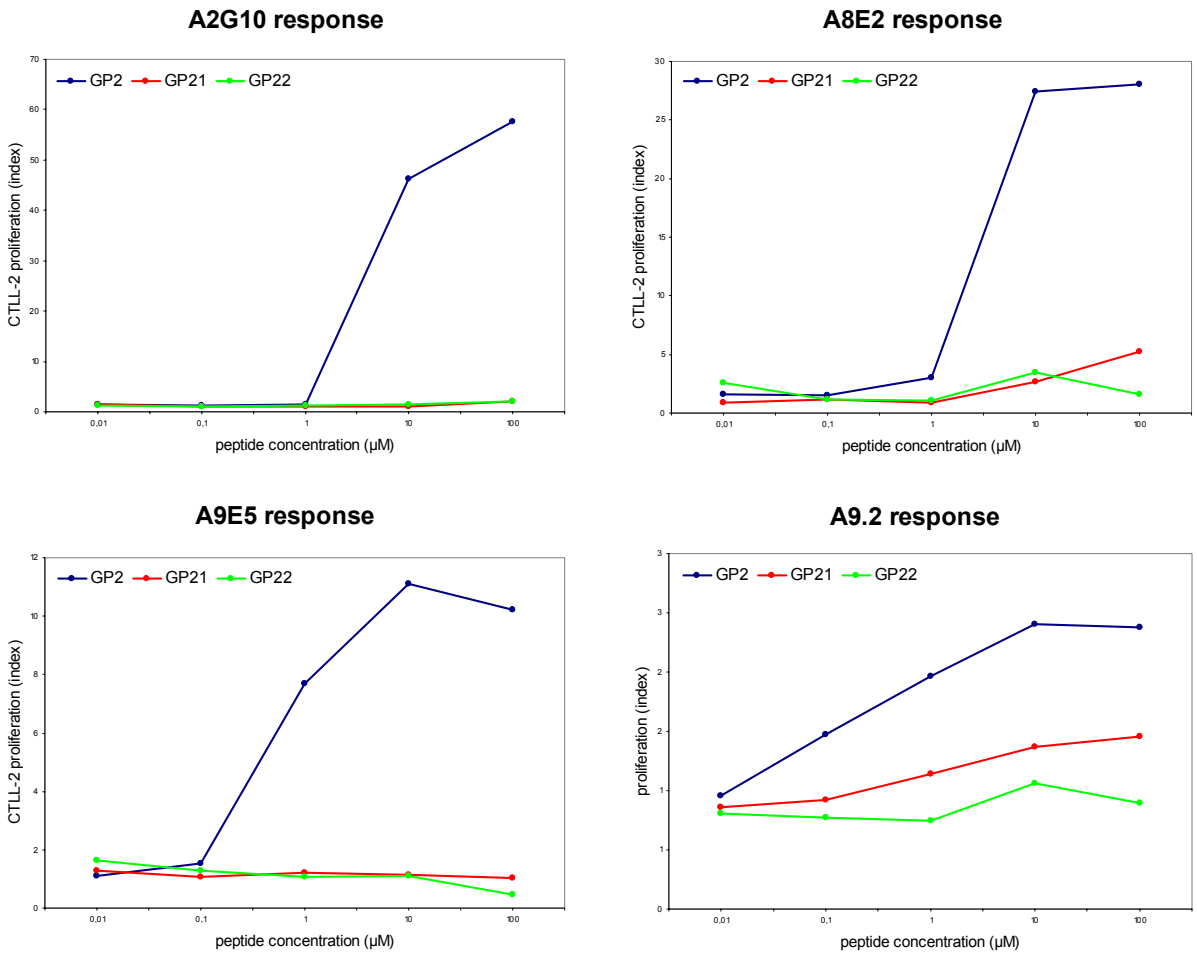


In the following assays, non natural glycopeptides **GP13** and **GP21-GP25** were tested to study the fine specificity of CII-derived T-cells. The response to the natural glycopeptide **GP2** was plotted on the graphs for comparison.

VII.2.2. Glycopeptides modified at the ε-primary amine

CII-derived T-cells are extremely sensitive to modification of the ε-primary amine notwithstanding the presence of the galactosyl group. The azido derivative **GP21** was not recognized by the three CII-specific T-cell hybridomas tested nor by the T-cell clone (Figure VII.2). This result is not surprising because the highly hydrophobic azido function is extremely different from the naturally occurring amine present in the **GP2** glycopeptide.

Figure VII.2 Evaluation of the 2 glycopeptides modified at the ε-primary amine GP21 and GP22



The isosteric modification (ie, substitution of OH for NH_2) in glycopeptide **GP22** was expected to be at least partially tolerated. However, similar to **GP21**, **GP22** did not induce proliferation of the CII-specific T-cells (Figure VII.2).

These results confirmed the importance of the N^{ε}-amino group in epitope recognition demonstrated by *Corthay* and colleagues,[3] but the evaluation of **GP22** also suggested the lack of permissiveness at this position. The primary amine at the ε position (i) is a hydrogen bond donor and can be involved in the formation of H-bonds with residues of the TCR and, (ii) when protonated, can be involved in electrostatic interactions. The finding that **GP22**, with a hydroxy function in place of the amino group at the ε position, was not recognized by T-cells supports the hypothesis that protonated N^{ε}-amine of Hyl^{264} is involved in electrostatic interactions with negatively charged residues present at the surface of the TCR. Another hypothesis would be that protonated N^{ε}-amine of Hyl^{264} could form an intramolecular salt bridge with the Glu^{266} side-chain, thus positioning and stabilizing the galactosyl moiety for T-cell recognition.

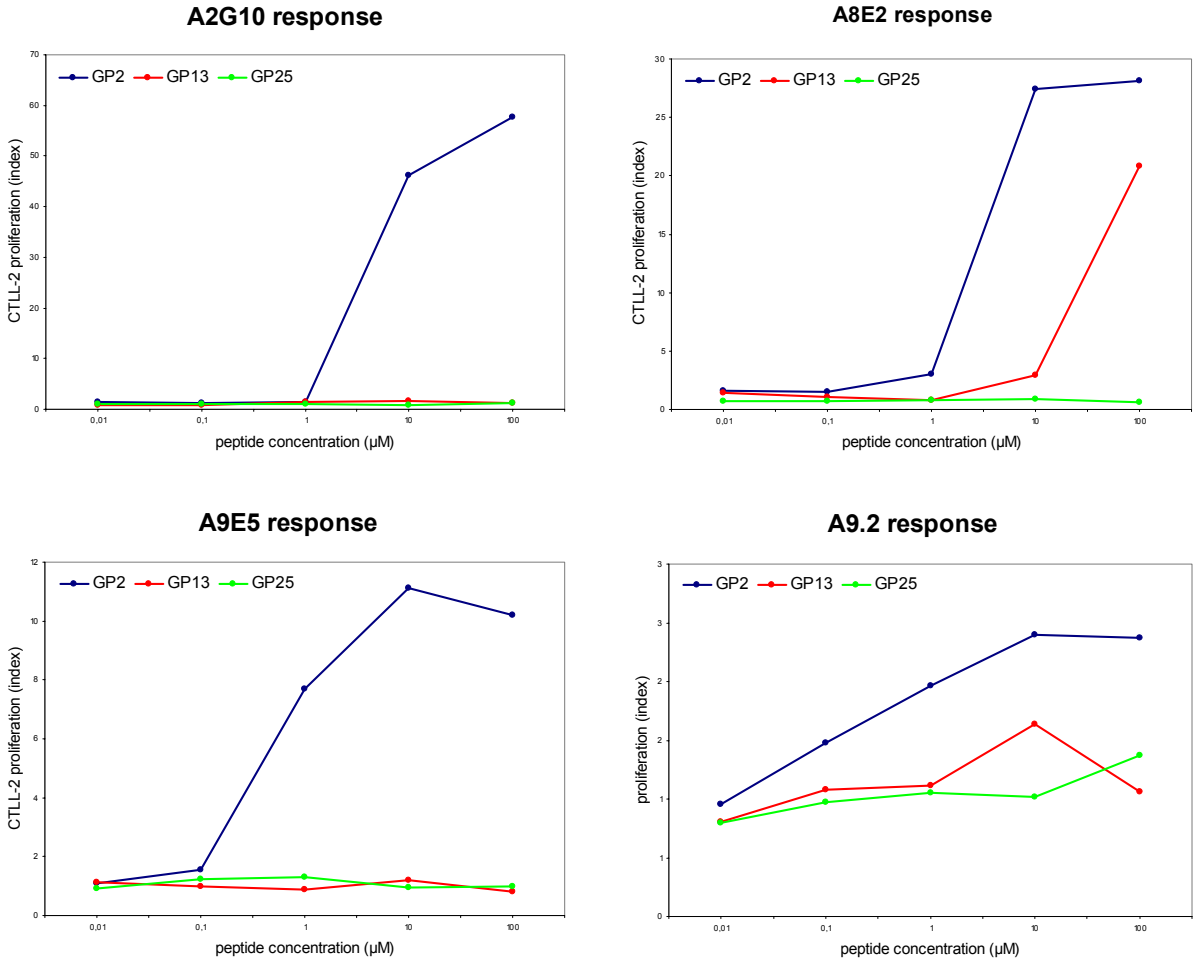
VII.2.3. Modulation of the galactosyl moiety

As recently reported by *Holm* and colleagues,[4] **GP13** (ie, carrying a glucosyl moiety) completely turned off T-cell proliferation (Figure VII.3). In **GP13**, the axial HO-4 required to generate a full response was substituted by an equatorial hydroxy group (ie, galactosyl vs glucosyl). In the same manner, **GP25**, which carries a fully protected galactosyl moiety, did not induce T-cell proliferation (Figure VII.3). This result was obviously predicted, but glycopeptide **GP25** could prove useful as a pro-drug for in-vivo assays. Indeed, the pivaloyl ester protections of the galactosyl moiety can be expected both to improve the admission rate of the glycopeptide by dramatically increasing its hydrophobicity and to release the natural epitope **GP2** by enzymatic hydrolysis of the acyl protecting groups.

[3] Corthay, A.; Bäcklund, J.; Broddefalk, J.; Michaëlsson, E.; Goldschmidt, T. J.; Kihlberg, J.; Holmdahl, R. *Eur. J. Immunol.* **1998**, *28*, 2580.

[4] Holm, B.; Bäcklund, J.; Recio, M. A. F.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2002**, *3*, 1209.

Figure VII.3 Evaluation of the two glycopeptides GP13 and GP25

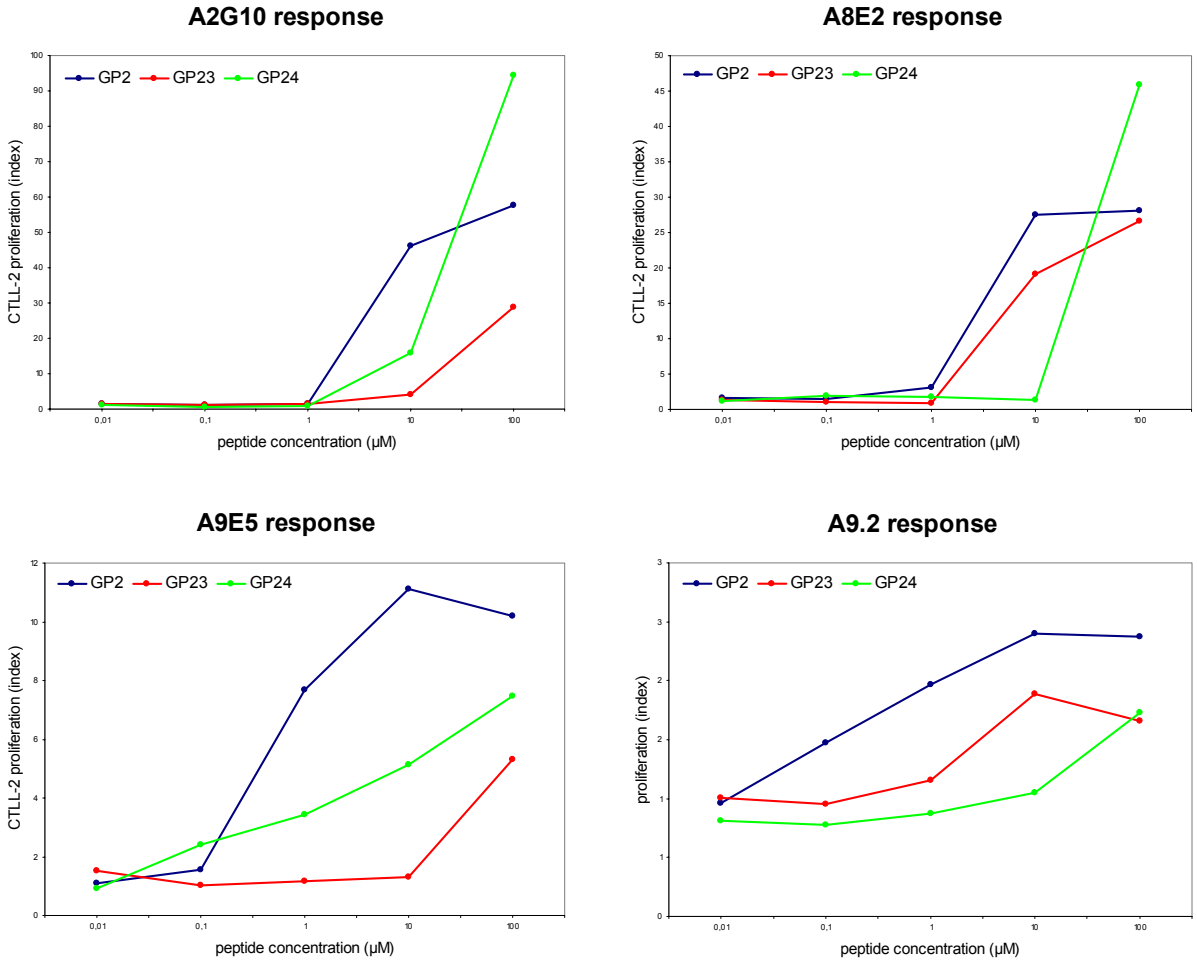


VII.2.4. Glycopeptides with GalHyl derivatives modified at C-5

Comparison of the proliferation indexes obtained in the presence of peptides **GP2** and **GP23** revealed the importance of the configuration at C-5 of GalHyl[264] residue (Figure VII.4). Although the inversion of the configuration at C-5 was found to be detrimental for binding to and for stimulation of A9E5 hybridoma, peptide **GP23** was nevertheless recognized by A8E2 and A2G10 hybridomas, albeit at high concentration. Thus, provided that the required pharmacophores, namely the HO-4 group of the galactosyl residue and the primary amino group at the ε position are present, slight changes in their relative orientation and / or position are partially tolerated. This is confirmed by the results obtained with **GP24** (Figure VII.4) bearing a disubstituted C-5 carbon (ie, hydroxylated and methylated). Like **GP2**, albeit to a lower extent, **GP24** is recognized by the three hybridomas. Although this modification creates

some steric congestion in the vicinity of the galactosyl moiety and the ε-primary amino group, it is well tolerated by the TCR which in this case demonstrates some plasticity.

Figure VII.4 Evaluation of the two glycopeptides modulated at the galactosyl anchorage GP23 and GP24



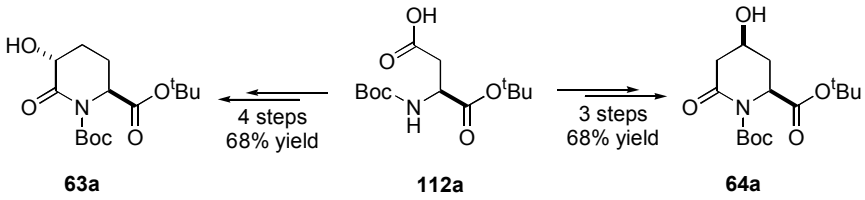
Taking together, these results give information about the relative position of the elements composing the recognition pattern. Interestingly, even if key elements have been identified for the interaction with the TCR (ie, the ε-primary amine and the HO-4 of the galactosyl moiety), their position relative to each others in the epitope is not necessary frozen to generate a T-cell response. Seemingly, the ternary interaction show some plasticity, since both the inversion of stereochemistry and the introduction of a methyl group at C-5 are authorized to a certain extent.

VIII. Conclusion and Perspectives

VIII.1. The divergent stereocontrolled strategy

In the course of this project aimed at determining the fine specificity of CII-specific T-cells, we developed a divergent asymmetric strategy for the preparation of 4- and 5-hydroxylysine analogues. All syntheses started from Asp as a cheap and commercially available chiral building block. These divergent strategies rely two key intermediates, namely the enantiopure 5-hydroxy-6-oxo-1,2-piperidinedicarboxylate **63a** and the enantiopure 4-hydroxy-6-oxo-1,2-piperidinedicarboxylate **64a** ; both compounds being prepared by asymmetric transformation in 68% overall yield (four steps from Boc-Asp-O^{t}Bu) for **63a** and 68% overall yield (three steps from Asp) for **64a** (Figure VIII.1).

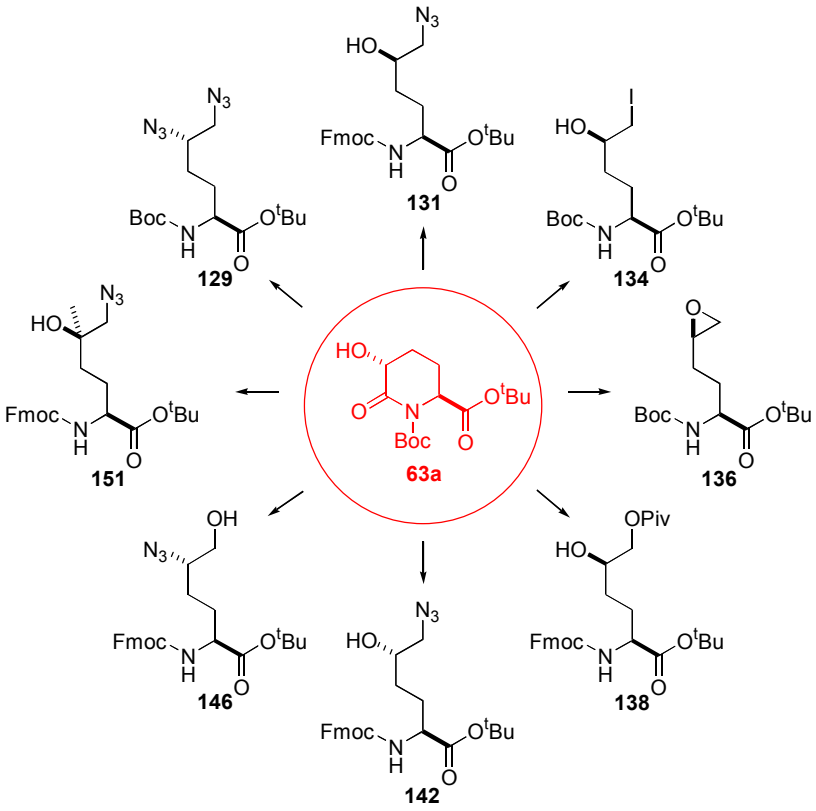
Figure VIII.1 Synthesis of the two key intermediates 63a and 64a



63a **112a** **64a**

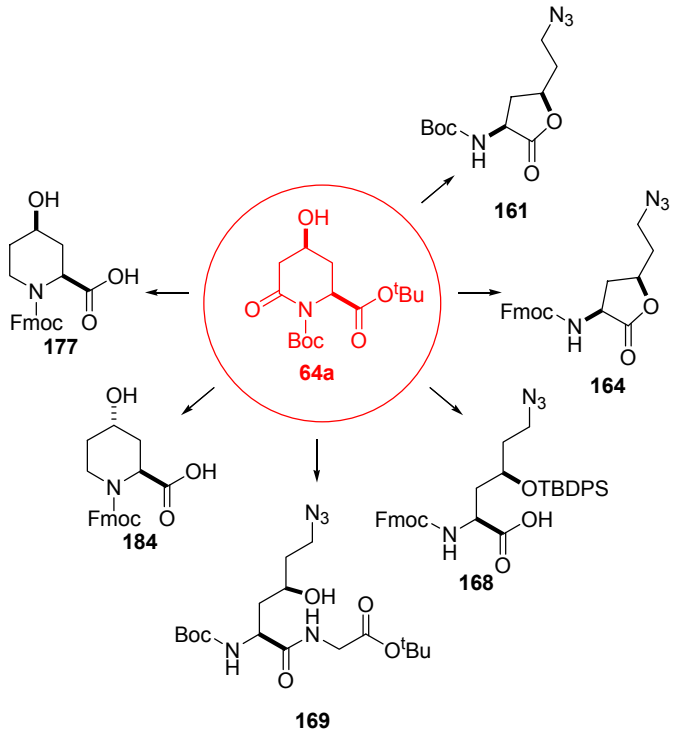
The 5-hydroxy-6-oxo-1,2-piperidinedicarboxylate **63a** served in the preparation of seven valuable 5-hydroxylysine analogues **129**, **131**, **134**, **136**, **138**, **142**, **146** and **151**. The amino acids **131**, **138**, **142**, **151** have been glycosylated and incorporated into CII-derived glycopeptides for further use in the present study aimed at determining the fine specificity of T-cells in CIA. Compounds **134** and **136** are described in the literature as key intermediates for the preparation of (+)-pyridinoline **93**, a useful synthetic molecule for the diagnosis of osteoporosis and other metabolic bone diseases.

Figure VIII.2 Analogues of 5-hydroxylysine from 63a



The 4-hydroxy-6-oxo-1,2-piperidinedicarboxylate **64a** served in the preparation of the 4-hydroxylysine analogue **168** and the 4-hydroxypipecolate derivatives **177** and **184**. The *N*-Fmoc protected (2*S*,4*S*)-4-hydroxylysine **168** is ready for use in SPPS using standard Fmoc / ^{t}Bu peptide chemistry. In route to the preparation of aglycons hydroxylated at C-4, the *N*-Boc and *N*-Fmoc protected lactones **161** and **164** have been isolated. After preliminary experiments, the lactone **161** was found to be a precursor of choice for the preparation of the dipeptide aglycon **169**, which could serve in the synthesis of glycopeptides incorporating glycosylated 4-hydroxylysine. The *N*-Fmoc protected (2*S*,4*S*)-4-hydroxypipecolic acid **177** and (2*S*,4*R*)-4-hydroxypipecolic acid **184** can also be incorporated into biologically relevant peptides using standard Fmoc / ^{t}Bu peptide chemistry. Natural or synthetic molecules incorporating a 4-hydroxypipecolate residue possess interesting biological activities. Our short and direct route has been found to be suitable for scale-up. A larger batch synthesis (starting from >20g of **63a**) of 4-hydroxypipecolic acid **177** has been performed in collaboration with Neosystem (Strasbourg) where it is now commercially available.

Figure VIII.3 Derivatives prepared from 64a



VIII.2. Determination of the fine specificity of T-cells

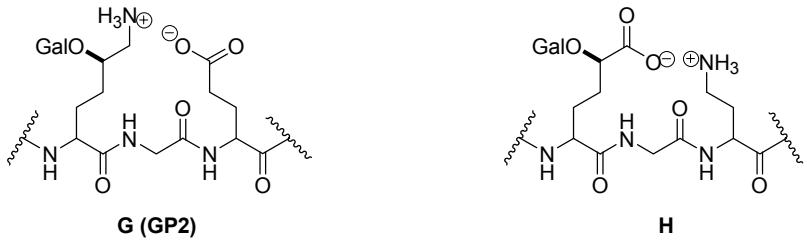
The in-vitro evaluation of the eleven peptides with three CII-specific T-cell hybridomas and one T-cell clone allowed us to characterize more precisely the pharmacophores of the immunodominant T-cell epitope.

The evaluation of the naturally occurring peptides (**GP2**, **GP17-GP20**) suggest (i) that the post-translational hydroxylation of Pro^{258} is seemingly not required for the recognition by T-cells and has no influence on the recognition pattern and (ii) a possible role for homologous CII in the development of CIA after immunization by heterologous CII.

On the other hand, the structure / activity relationship study gave information about the pharmacophores of the epitope CII(256-270). The evaluation of **GP21** and **GP22** confirmed the important role of the ε-primary amino group of Hyl^{264} in epitope recognition and support the view that upon protonation, this N^{ε}-amine could be involved in electrostatic interactions with negatively charged residues present at the surface of the TCR. Another scenario, however, would be that protonated N^{ε}-amine of Hyl^{264} could form an intramolecular salt bridge with the Glu^{266} side-chain thus positioning and stabilizing the galactosyl moiety for T-

cell recognition. To test this hypothesis, one could envision the synthesis of peptide **H** (Figure VIII.4).

Figure VIII.4 A possible intramolecular salt bridge in peptides G (GP2) and analogue H



G (GP2) **H**

The evaluation of **GP13** confirmed the importance of HO-4 to generate a full response. Finally, the evaluation of **GP23** and **GP24** suggest a certain plasticity of the TCR in accomodation of the two pharmacophores (ie, the *ε*-primary amine and the HO-4 of the galactosyl moiety) ; their position relative to each others is not frozen for recognition by T-cells.

VIII.3. Perspectives : synthesis of *S*- and *C*-glycoside analogues of GalHyl

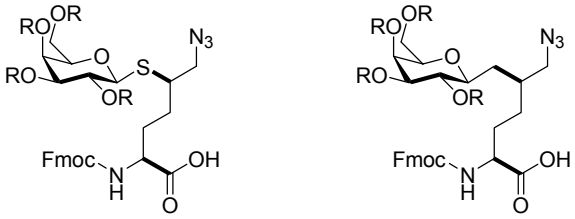
Structure / activity relationship studies aimed at probing the fine specificity of CII-specific T-cells have been undertaken independently by *Kihlberg*'s research group and ourselves. These studies conducted on natural and unnatural glycosylated analogues of the immunodominant epitope CII(256-270) have delineated the role and the importance of the pattern of recognition : (i) the four different hydroxy groups composing the galactosyl moiety, (ii) the *ε*-primary amine functionality and (iii) the position of the galactosyl moiety, the *ε*-amine and the peptidic chain relative to each others. However, one parameter has remained unexplored so far : the glycosidic linkage itself.

S- and *C*-glycosylated amino acids are stable against both chemical and enzymatic degradation and have gained considerable attention in recent years.[1] As previously mentioned (section II), by introducing fine structural modifications of amino acid side-chains in contact with the TCR, one might generate APLs with useful effects on T-cells (ie, induction of

[1] Marcaurelle,L. A. and Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384

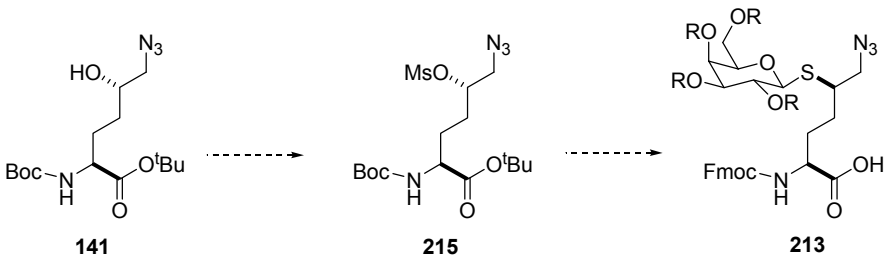
tolerance).[2] Thus, the study of peptides incorporating *S*- and *C*-glycosylated Hyl at position 264 might be of particular interest. Additionally, it would be an advantage if the synthetic transformation of an autoimmune epitope to an APL, useful to break autoimmunity, was accompanied by an increased stability of the peptide toward degradation. *Wellner* and colleagues have recently reported a synthesis of the *C*-galactosylated analogue of GalHnv and its incorporation into the CII dominant epitope (section I).[3] In this case, C-glycosylation does not affect the pattern of recognition of the T-cell subset that recognize the CII peptide with GalHnv at position 264 ; however, the affinity for the TCR is considerably diminished. The synthesis of *S*- and *C*-glycosylated Hyl derivative **213** and **214** (Figure VIII.5) and of the corresponding CII-derived peptide have not been described so far.

Figure VIII.5 *S*- and *C*-galactosylated building blocks



While the preparation of the *S*-analogue **213** can be easily envisioned by simple organic transformations starting from the *N*-Boc / *tert*-butyl ester protected derivative **141** as presented in Figure VIII.6 (preliminary work by *A. Violette*, IBMC, Strasbourg). The design of an efficient and stereocontrolled route to the *C*-glycosylated derivative is more problematic.

Figure VIII.6 Preparation of the *S*-galactosylated building block 213



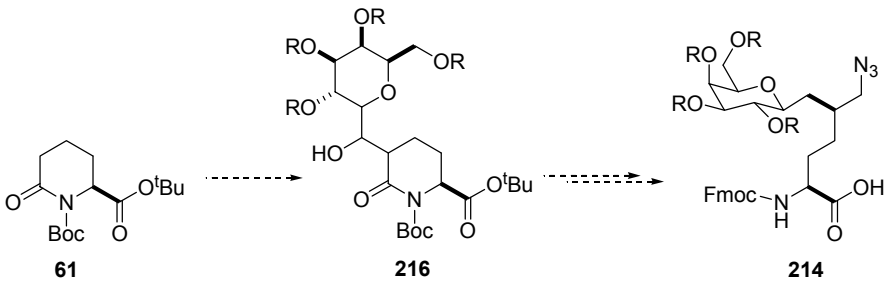
[2] Sloan-Lancaster, J. and Allen, P. M. *Annu. Rev. Immunol.* **1996**, *14*, 1.

[3] Wellner, E.; Gustafsson, T.; Bäcklund, J.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2000**, *1*, 272.

Among the numerous routes reported for the preparation of *C*-glycosylated amino acids,[4] none appeared really suitable to access the desired *C*-galactosylated hydroxylysine analogue **214** in enantiomerically pure form and in a limited number of steps. Indeed, all the strategies have been tailored for the introduction of only two stereogenic centers (ie, the anomeric center of the *C*-glycoside and the α-amino acid moiety). Most of the reported asymmetric approaches are based on an alkylation step using chiral auxiliaries for fusing the glycoside and the α-amino acid moieties. Additional homologation steps served to modulate the length of the linkage (ie, the amino acid side-chain). Recently, *Gustafsson* and colleagues published the synthesis of a *C*-galactosylated analogue of galactosylthreonine[5] (14 steps); the three stereogenic centers (ie, anomeric center, C(α) and C(β) of the threonine) being created by the reaction sequence. A similar strategy should allow the preparation of a *C*-galactosylated analogue of GalHyl, but this route would be extremely long.

Our idea is to start from the (2*S*)-6-oxo-1,2-piperidinedicarboxylate **61** and to develop an asymmetric aldol reaction using the galactosyl aldehyde developed and largely used by *Dondoni* and colleagues (Figure VIII.7).[6] If successful, this strategy will be the first synthesis of a *C*-glycosylated analogue of GalHyl (preliminary work by *N. Trouche* and *J. Marin*, IBMC, Strasbourg).

Figure VIII.7 Preparation of the *C*-galactosylated building block 214



This new example further enhances usefulness of piperidinone ring structures in asymmetric synthesis and highlights the unique place of intermediate **61** in our global strategy for preparing CII-derived glycopeptides.

[4] Dondoni, A. and Marra, A. *Chem. Rev.* **2000**, *100*, 4395.

[5] Gustafsson, T.; Saxin, M.; Kihlberg, J. *J. Org. Chem.* **2003**, *68*, 2506.

[6] Dondoni, A. and Scherrmann, M. C. *J. Org. Chem.* **1994**, *59*, 6404.

IX. Experimental Section

IX.1. General

Unless stated otherwise, the reactions were performed under an atmosphere of argon. THF was distilled from Na / benzophenone ; CH_2Cl_2 was distilled from CaH_2 ; cyclohexane was distilled from CaH_2 ; toluene was distilled over Na. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) with detection by UV light and charring with 1% $^{w}/_{w}$ ninhydrin in ethanol followed by heating. Flash column chromatography was carried out on silica gel (0.063-0.200 mm). HPLC analysis was performed on a Nucleosil C_{18} column (5 μm, 3.9 × 150 mm) by using a linear gradient of A (0.1% TFA in H_2O) and B (0.08% TFA in CH_3CN) at a flow rate of 1.2 ml/min with UV detection at 214 nm. Optical rotations were recorded with a Perkin-Elmer polarimeter. ^{1}H and ^{13}C NMR spectra were recorded using a BRUKER *AVANCE* apparatus. Mass spectrum have been recorded using a MALDI-TOF apparatus (BRUKER ProteinTOF).

IX.2. Materials

Amino acid derivatives were purchased from Neosystem. Boc-Asp-OBn (**90b**) is commercially available. Boc-Asp-O^{t}Bu (**90a**),[1] Boc-Asp-OTCE (**90c**) and Teoc-Asp-O^{t}Bu (**90d**)[2] were prepared starting from the commercially available Boc-Asp(Bn)-OH.

IX.3. Compounds cited in section III

Preparation of Meldrum's derivatives 114a-d: 1.5 equiv of EDC, 1.5 equiv of DMAP and 1.0 equiv of Meldrum's acid were added to a 0.25 M solution of PG^{1}-Asp-OPG^{2} in CH_2Cl_2 at 0°C. The mixture was allowed to reach room temperature, stirred for 3 h, and then washed with 1N $KHSO_4$. The organic layer was dried over Na_2SO_4 and filtered prior to the

[1] (a) Mathias, L. J. *Synthesis* **1979**, 561. (b) Bergmeier, S. C.; Cobas, A. A.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 2369.

[2] Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346.

addition of CH_2Cl_2 (to afford a 0.05M solution) and 10% $^{v}/_{v}$ of acetic acid. 3.0 equiv of $NaBH_4$ were added portion-wise to the previous solution stirred at rt. After 48 h, the mixture was diluted with brine and the organic layer was washed using water and dried over Na_2SO_4, filtered and evaporated to afford a residue which was purified by crystallization or by flash chromatography.

***tert*-butyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)butanoate (114a)**

Recrystallization of the crude product from CH_2Cl_2/pentane gave **114a** (5.44 g, yield = 98%): HPLC t_R 10.98 (linear gradient, 30-100% B, 20 min); white crystals; $[\alpha]_D$ = +11.2 (*c* = 1.0, $CHCl_3$); mp: 100-102 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (bd, *J* = 7.1 Hz, 1H), 4.18 (bs, 1H), 3.77 (bs, 1H), 2.24-2.18 (m, 1H), 2.09-2.01 (m, 2H), 1.83-1.77 (m, 1H), 1.80 (s, 3H), 1.74 (s, 3H), 1.45 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.4 (C), 165.2 (2 C), 155.6 (C), 105.0 (C), 82.3 (C), 79.9 (C), 53.2 (CH), 45.4 (CH), 29.6 (CH_2), 28.6 (CH_3), 28.3 (3 CH_3), 28.0 (3 CH_3), 26.4 (CH_3), 21.8 (CH_2); Anal. Calcd for $C_{19}H_{31}NO_8$: C, 56.84; H, 7.78; N, 3.49; Found: C, 56.84; H, 7.92; N, 3.47.

benzyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)butanoate (114b)

Recrystallization of the crude product from CH_2Cl_2/pentane gave **114b** (6.34 g, yield = 95%): HPLC t_R 12.34 (linear gradient, 30-100% B, 20 min); white crystals; $[\alpha]_D$ = +6.5 (*c* = 1.0, $CHCl_3$); mp: 94-96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.30 (m, 5H), 5.17 (m, 2H), 5.16 (m, 1H), 4.37 (m, 1H), 3.73 (m, 1H), 2.25-2.18 (m, 1H), 2.16-2.07 (m, 2H), 1.86-1.81 (m, 1H), 1.80 (s, 3H), 1.74 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.2 (C), 165.1 (C), 165.0 (C), 156.0 (C), 135.3 (C), 128.7 (5 CH), 105.1 (C), 80.4 (C), 67.3 (CH_2), 52.8 (CH), 45.4 (CH), 29.5 (CH_2), 28.6 (CH_3), 28.3 (3 CH_3), 26.5 (CH_3), 21.9 (CH_2).

trichloroethyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)butanoate (114c)

Recrystallization of the crude product from CH_2Cl_2/pentane gave **114c** (3.66 g, yield = 90%): HPLC t_R 11.92 (linear gradient, 30-100% B, 20 min); white crystals; $[\alpha]_D$ = –8.8 (*c* = 0.9, MeOH); mp: 124-125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (bd, *J* = 7.7 Hz, 1H), 4.87 (d, *J* = 11.8 Hz, 1H), 4.69 (d, *J* = 11.8 Hz, 1H), 4.44 (bs, 1H), 3.73 (bs, 1H), 2.29-2.24 (m, 1H), 2.24-2.15 (m, 2H), 1.90-1.85 (m, 1H), 1.81 (s, 3H), 1.74 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.9 (C), 165.1 (2 C), 155.5 (C), 105.2 (C), 94.5 (C), 80.5 (C), 74.4 (CH_2),

53.0 (CH), 45.5 (CH), 29.1 (CH_2), 28.5 (CH_3), 28.3 (3 CH_3), 26.5 (CH_3), 22.0 (CH_2); Anal. Calcd for $C_{17}H_{24}Cl_3NO_8$: C, 42.83; H, 5.07; N, 2.94; Found: C, 43.04; H, 4.87; N, 2.90.

***tert*-butyl (2*S*)-4-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-yl)-2-({[(trimethylsilyl)ethoxy]carbonyl}amino)butanoate (114d)**

Purification of the crude product by flash column chromatography gave **114d** (5.35 g, yield = 98%): HPLC t_R 13.10 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = +12.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (bd, *J* = 5.3 Hz, 1H), 4.22 (bs, 1H), 4.14-4.09 (m, 2H), 3.69 (bs, 1H), 2.22-2.13 (m, 1H), 2.09-1.98 (m, 2H), 1.85-1.76 (m, 1H), 1.79 (s, 3H), 1.73 (s, 3H), 1.45 (s, 9H), 0.98-0.94 (m, 2H), 0.00 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.4 (C), 165.1 (2 C), 156.4 (C), 105.0 (C), 82.4 (C), 63.4 (CH_2), 53.6 (CH), 45.5 (CH), 29.5 (CH_2), 28.5 (CH_3), 28.0 (3 CH_3), 26.5 (CH_3), 21.8 (CH_2), 17.7 (CH_2), -1.4 (3 CH_3); Anal. Calcd for $C_{20}H_{35}NO_8Si$: C, 53.91; H, 7.92; N, 3.14; Found: C, 54.15; H, 8.02; N, 3.09.

Preparation of piperidin-2-ones derivatives 61a-d: A 0.1M solution of **114** in dry toluene was heated at reflux overnight. The solvent was evaporated and the residue dissolved in AcOEt and washed with saturated $NaHCO_3$, brine and 1N $KHSO_4$. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 2:8) to yield pure **61**.

di(*tert*-butyl) (2*S*)-6-oxo-1,2-piperidinedicarboxylate (61a)

Purification of the crude product by flash column chromatography gave **61a** (2.03 g, yield = 76%): HPLC t_R 10.44 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –12.3 (*c* = 1.1, MeOH); mp: 46-48 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (dd, *J* = 5.8, 3.6 Hz, 1H), 2.57 (ddd, *J* = 17.5, 5.6, 4.5 Hz, 1H), 2.47 (ddd, *J* = 17.4, 9.8, 7.0 Hz, 1H), 2.19-2.13 (m, 1H), 2.05-1.96 (m, 1H), 1.82-1.74 (m, 2H), 1.48 (s, 9H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.6 (C), 170.4 (C), 152.3 (C), 83.2 (C), 82.1 (C), 59.0 (CH), 34.5 (CH_2), 27.9 (6 CH_3), 25.9 (CH_2), 18.3 (CH_2); Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68; Found: C, 60.30; H, 8.64; N, 4.65.

2-benzyl 1-(*tert*-butyl) (2*S*)-6-oxo-1,2-piperidinedicarboxylate (61b)

Purification of the crude product by flash column chromatography gave **61b** (3.19 g, yield = 75%): HPLC t_R 11.09 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –10.2 (*c* = 1.0, $CHCl_3$); mp: 43-44 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.29 (m, 5H), 5.22 (d, *J* = 12.2 Hz, 1H), 5.14 (d, *J* = 12.2 Hz, 1H), 4.73 (m, 1H), 2.55-2.49 (m, 1H), 2.44 (ddd, *J* = 17.6, 9.9, 6.8 Hz, 1H), 2.18-2.11 (m, 1H), 2.06-1.97 (m, 1H), 1.77-1.69 (m, 1H), 1.67-1.60 (m, 1H),

1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.4 (C), 170.2 (C), 152.2 (C), 135.3 (C), 128.7 (2 CH), 128.5 (CH), 128.4 (2 CH), 83.6 (C), 67.3 (CH_2), 58.6 (CH), 34.5 (CH_2), 27.9 (3 CH_3), 25.9 (CH_2), 18.3 (CH_2); Anal. Calcd for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20; Found: C, 64.52; H, 7.01; N, 4.28.

2-trichloroethyl 1-(*tert*-butyl) (2*S*)-6-oxo-1,2-piperidinedicarboxylate (61c)

Purification of the crude product by flash column chromatography gave **61c** (1.00 g, yield = 42%): HPLC t_R 11.87 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D = -1.0$ (c = 1.1, MeOH); mp: 63-65 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.00 (d, J = 11.9 Hz, 1H), 4.87 (dd, J = 6.0, 3.5 Hz, 1H), 4.68 (d, J = 11.9 Hz, 1H), 2.63 (ddd, J = 17.5 , 5.8, 4.4 Hz, 1H), 2.53 (ddd, J = 17.5, 9.8, 7.2 Hz, 1H), 2.35-2.28 (m, 1H), 2.21-2.11 (m, 1H), 1.91-1.77 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.1 (C), 169.8 (C), 152.5 (C), 94.5 (C), 84.0 (C), 74.5 (CH_2), 58.3 (CH), 34.5 (CH_2), 28.0 (3 CH_3), 25.8 (CH_2), 18.3 (CH_2); Anal. Calcd for $C_{13}H_{18}Cl_3NO_5$: C, 41.68; H, 4.84; N, 3.74; Found: C, 41.78; H, 4.84; N, 3.81.

2-(*tert*-butyl) 1-[(trimethylsilyl)ethyl] (2*S*)-6-oxo-1,2-piperidinedicarboxylate (61d)

Purification of the crude product by flash column chromatography gave **61d** (2.87 g, yield = 71%): HPLC t_R 13.74 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D = -10.1$ (c = 1.1, MeOH); mp: 52-54 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (dd, J = 5.9, 3.5 Hz, 1H), 4.35-4.30 (m, 2H), 2.59 (ddd, J = 17.3, 5.8, 4.1 Hz, 1H), 2.48 (ddd, J = 17.3, 10.0, 7.1 Hz, 1H), 2.23-2.16 (m, 1H), 2.06-1.97 (m, 1H), 1.85-1.73 (m, 2H), 1.48 (s, 9H), 1.12-1.08 (m, 2H), 0.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.4 (C), 170.3 (C), 154.4 (C), 82.3 (C), 65.6 (CH_2), 59.1 (CH), 34.4 (CH_2), 28.0 (3 CH_3), 25.8 (CH_2), 18.3 (CH_2), 17.5 (CH_2), -1.6 (3 CH_3); Anal. Calcd for $C_{16}H_{29}NO_5Si$: C, 55.95; H, 8.51; N, 4.08; Found: C, 55.56; H, 8.44; N, 4.09.

2-allyl 1-(*tert*-butyl) (2*S*)-6-oxo-1,2-piperidinedicarboxylate (61e)

Palladium on activated carbon (150 mg, 10% $^w/_w$ Pd) was added to a solution of 1.50 g (4.50 mmol) of **61b** in 20.0 mL of AcOEt at room temperature. The mixture was stirred for 2 h and filtered through Celite®. Evaporation of the solvent gave 1.10 g of the free-acid intermediate. 1.00 g (4.11 mmol) of the free-acid derivative and 497 mg (4.11 mmol) of allyl bromide were dissolved in 15.0 mL of acetonitrile. The mixture was cooled to 0°C and 751 mg (4.93 mmol) of DBU were added. After stirring for 6 h, acetonitrile was evaporated and the residue was dissolved in AcOEt. The solution was washed with saturated $NaHCO_3$, brine and 1N $KHSO_4$. Drying over Na_2SO_4 and evaporation of the filtrate afforded pure **61e** (1.15 g, yield = 99%

starting from **61b**): HPLC t_R 8.48 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = –8.9 (*c* = 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.95-5.85 (m, 1H), 5.34 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.25 (dd, *J* = 10.4, 1.4 Hz, 1H), 4.72 (dd, *J* = 5.9 , 3.8 Hz, 1H), 4.66-4.63 (m, 2H), 2.57 (ddd, *J* = 17.5, 6.0, 4.3 Hz, 1H), 2.47 (ddd, *J* = 17.5, 9.9, 6.8 Hz, 1H), 2.21-2.15 (m, 1H), 2.10-2.01 (m, 1H), 1.83-1.67 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.2 (C), 170.2 (C), 152.3 (C), 131.4 (CH), 119.0 (CH_2), 83.6 (C), 66.1 (CH_2), 58.6 (CH), 34.5 (CH_2), 27.9 (3 CH_3), 25.9 (CH_2), 18.3 (CH_2); Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94; Found: C, 59.62; H, 7.70; N, 5.01.

General hydroxylation procedures.

Enolate generation: the desired piperidinone derivative was placed in an argon-filled round-bottom flask, dissolved in anhydrous THF to give a ca. 0.3M solution, and cooled to –78°C. The indicated base (NaHMDS or LiHMDS; 1.1 equiv) was introduced as a 1.0M solution in THF *via* a hypodermic syringe. After being stirred for 2.5 h, the desired enolate was ready for hydroxylation.

Enolate hydroxylation. Procedure A: to the cold enolate solution (–78°C) prepared as described above, was added the indicated amount of crystalline MoOPH (2.0 equiv). The temperature and duration of the oxidation were specific to each experiment. The reaction was quenched by the addition of a saturated solution of Na_2SO_3 (1 mL/mmol of enolate). The resulting 2-phase solution was vigorously stirred for 15 min. The THF was evaporated under reduced pressure and replaced with AcOEt which was washed with water. The organic layer was dried over Na_2SO_4, concentrated and chromatographed on silica gel (AcOEt/Hex, 3:7).

Procedure B: to the cold enolate solution (–78°C) prepared as described above, was added the indicated amount of oxaziridine (1.5 equiv) dissolved in the minimum volume of THF. The temperature and duration were specific to each experiment. The reaction was quenched by the introduction of a saturated aqueous solution of NH_4Cl (1 mL/mmol of enolate), the THF was evaporated under reduced pressure, replaced with AcOEt which was washed with water. The organic layer was dried over Na_2SO_4, concentrated and chromatographed on silica gel (MeOH/CH_2Cl_2, 2:98).

di(*tert*-butyl) (2*S*, 5*R*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate ((5*R*)-63a)

Using the best conditions (table III.1, entry 12), (5*R*)-**63a** was obtained as a yellowish oil which was purified by flash column chromatography as described in procedure B (640 mg,

yield = 92%): HPLC t_R 7.72 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –12.1 (*c* = 1.0, MeOH); mp: 88-90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (t, *J* = 6.4 Hz, 1H), 4.11 (dd, *J* = 9.7, 7.2 Hz, 1H), 3.61 (bs, 1H), 2.34-2.26 (m, 1H), 2.25-2.16 (m, 1H), 2.13-2.04 (m, 1H), 1.78-1.68 (m, 1H), 1.52 (s, 9H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.8 (C), 169.8 (C), 151.6 (C), 84.2 (C), 82.7 (C), 68.3 (CH), 58.4 (CH), 27.9 (6 CH_3), 26.8 (CH_2), 22.7 (CH_2); Anal. Calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44; Found: C, 56.91; H, 8.20; N, 4.46.

Procedure using dibenzyl peroxodicarbonate as the oxidizer: 100 mg (0.334 mmol) of **63a** were placed in an argon-filled round-bottom flask, dissolved in 2.0 mL of anhydrous THF and cooled to –78°C. 368 µL (0.368 mmol) of a 1.0M solution of LiHMDS in THF were added *via* a hypodermic syringe. After being stirred for 2.5 h, 101 mg (0.334 mmol) of DPD in solution in the minimum volume of anhydrous THF were added. The reaction mixture was stirred for 16 h at –78°C and quenched by the addition of AcOH (1.0 equiv) *via* a hypodermic syringe. The mixture was allowed to reach 0°C and a large volume of water was added. THF was evaporated under reduced pressure, replaced with AcOEt (20.0 mL) which was washed with water (2 × 15.0 mL). The organic layer was dried over Na_2SO_4 and concentrated to give **125**. Crude **125** was dissolved in 10.0 mL of ethanol and 15 mg of Pd/C were added to the solution. The resulting suspension was vigorously stirred at room temperature under an H_2 atmosphere for 3 h. Pd/C was filtrated on Celite® and washed with ethanol. The filtrate was concentrated and chromatographed on silica gel (AcOEt/Hex, 3:7) to afford pure (5*R*)-**63a** (61 mg, yield = 58%).

di(*tert*-butyl) (2*S*, 5*S*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate ((5*S*)-63a)

HPLC t_R 7.48 (linear gradient, 30-100% B, 20 min); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.49 (m, 1H), 4.06 (dd, *J* = 11.3, 6.2 Hz, 1H), 2.34-2.02 (m, 3H), 1.80-1.66 (m, 1H), 1.50 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.6 (C), 170.2 (C), 153.1 (C), 83.9 (C), 82.7 (C), 69.9 (CH), 59.9 (CH), 27.9 (6 CH_3), 26.7 (CH_2), 23.9 (CH_2).

2-benzyl 1-(*tert*-butyl) (2*S*,5*R*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate (63b)

Using the procedure developed for (5*R*)-**63a** (table III.1, entry 12), **63b** was obtained as a yellowish oil which was purified by flash column chromatography (1.81 g, yield = 88%): HPLC t_R 8.39 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = –7.8 (*c* = 1.2, MeOH); mp: 88-90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.29 (m, 5H), 5.22 (d, *J* = 12.1 Hz, 1H), 5.15 (d, *J* = 12.2 Hz, 1H), 4.83 (t, *J* = 6.3 Hz, 1H), 4.08 (dd, *J* = 9.6, 6.9 Hz, 1H), 2.27-2.19 (m, 2H), 2.12-2.06 (m, 1H), 1.77-1.68 (m, 1H), 1.46 (s, 9H); ^{13}C NMR (100 MHz,

$CDCl_3$) 173.7 (C), 170.7 (C), 151.4 (C), 135.0 (C), 128.8 (CH), 128.7 (2 CH), 128.4 (2 CH), 84.5 (C), 68.3 (CH), 67.6 (CH_2), 58.0 (CH), 27.8 (3 CH_3), 26.7 (CH_2), 22.7 (CH_2); Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01; Found: C, 61.87; H, 6.88; N, 3.95.

2-trichloroethyl 1-(*tert*-butyl) (2*S*,5*R*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate (63c)

Using the procedure developed for (5*R*)-**63a** (table III.1, entry 12), **63c** was obtained as a yellowish oil which was purified by flash column chromatography (181 mg, yield = 73%): HPLC t_R 9.45 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –17.3 (*c* = 1.0, MeOH); mp: 91-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (d, *J* = 11.8 Hz, 1H), 4.93 (t, *J* = 6.5 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.15-4.10 (m, 1H), 3.59 (bs, 1H), 2.37-2.26 (m, 2H), 2.23-2.15 (m, 1H), 1.81-1.71 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.3 (C), 169.6 (C), 151.5 (C), 94.4 (C), 84.8 (C), 74.5 (CH_2), 68.3 (CH), 57.6 (CH), 27.9 (3 CH_3), 26.6 (CH_2), 22.5 (CH_2); Anal. Calcd for $C_{13}H_{18}Cl_3NO_6$: C, 39.97; H, 4.64; N, 3.59; Found: C, 40.27; H, 4.65; N, 3.54.

2-(*tert*-butyl) 1-[(trimethylsilyl)ethyl] (2*S*,5*R*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate (63d)

Using the procedure developed for (5*R*)-**63a** (table III.1, entry 12), **63d** was obtained as a yellowish oil which was purified by flash column chromatography (90 mg, yield = 87%): HPLC t_R 11.99 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = –10.4 (*c* = 0.8, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.76 (dd, *J* = 6.3, 6.0 Hz, 1H), 4.37-4.32 (m, 2H), 4.12 (dd, *J* = 9.1, 7.6 Hz, 1H), 3.60 (bs, 1H), 2.25-2.07 (m, 3H), 1.78-1.69 (m, 1H), 1.45 (s, 9H), 1.12-1.06 (m, 2H), 0.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.7 (C), 169.7 (C), 153.4 (C), 82.9 (C), 68.2 (CH), 66.6 (CH_2), 58.3 (CH), 27.9 (3 CH_3), 26.6 (CH_2), 22.6 (CH_2), 17.6 (CH_2), –1.6 (3 CH_3).

2-allyl 1-(*tert*-butyl) (2*S*,5*R*)-5-hydroxy-6-oxo-1,2-piperidinedicarboxylate (63e)

Using the procedure developed for (5*R*)-**63a** (table III.1, entry 12), **63e** was obtained as a yellowish oil which was purified by flash column chromatography (890 mg, yield = 89%): HPLC t_R 6.64 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = –9.0 (*c* = 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91-5.86 (m, 1H), 5.31 (dd, *J* = 17.3, 1.4 Hz, 1H), 5.28 (dd, *J* = 10.4, 1.4 Hz, 1H), 4.83 (t, *J* = 6.5 Hz, 1H), 4.68-4.64 (m, 2H), 4.13 (dd, *J* = 9.3, 6.4 Hz, 1H), 3.61 (bs, 1H), 2.32-2.24 (m, 2H), 2.18-2.05 (m, 1H), 1.82-1.74 (m, 1H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.6 (C), 170.6 (C), 151.4 (C), 131.2 (CH), 119.4 (CH_2),

84.5 (C), 68.3 (CH), 66.4 (CH_2), 58.0 (CH), 27.9 (3 CH_3), 26.7 (CH_2), 22.7 (CH_2); Anal. Calcd for $C_{14}H_{21}NO_6$: C, 56.18; H, 7.07; N, 4.68; Found: C, 56.31; H, 7.19; N, 4.76.

***tert*-butyl (3*S*, 6*R*)-3-hydroxy-6-isobutyl-2-oxo-1-piperidinecarboxylate (120)**

Lactam **120** was prepared according to the procedure B using (−)-CSO as oxidizing agent. The solution was stirred at −78°C during 16 h. After the workup, a flash column chromatography (MeOH/CH_2Cl_2, 2:98) of the crude product afforded pure **120** (96 mg, yield = 77%): HPLC t_R 9.27 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = +17.2 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.33-4.25 (m, 1H), 4.17 (t, *J* = 7.9 Hz, 1H), 3.73 (bd, *J* = 1.4 Hz, 1H), 2.41-2.30 (m, 1H), 2.06-1.99 (m, 1H), 1.77-1.65 (m, 2H), 1.65-1.58 (m, 1H), 1.52 (s, 9H), 1.43 (m, 2H), 0.92 (d, *J* = 5.3 Hz, 3H), 0.90 (d, *J* = 5.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.3 (C), 151.9 (C), 83.8 (C), 67.7 (CH), 54.4 (CH), 43.8 (CH_2), 27.8 (3 CH_3), 26.8 (CH_2), 25.0 (CH), 24.2 (CH_2), 23.5 (CH_3), 21.4 (CH_3); Anal. Calcd for $C_{14}H_{25}NO_4$: C, 61.97; H, 9.29; N, 5.16; Found: C, 61.82; H, 9.05; N, 5.37.

***tert*-butyl (3*S*, 6*R*)-3-hydroxy-6-isobutyl-3-(2-methylallyl)-2-oxo-1-piperidinecarboxylate (122)**

Compound **122** was prepared according to the procedure B using (−)-CSO as oxidizing agent. The solution was stirred at −78°C during 16 h. After the workup, a flash column chromatography (MeOH/CH_2Cl_2, 2:98) of the crude product afforded pure **122** (73 mg, yield = 67%): HPLC t_R 14.68 (linear gradient, 30-100% B, 20 min); colorless crystals; $[\alpha]_D$ = −31.6 (*c* = 1.0, $CHCl_3$); mp: 42-43 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.89 (dd, *J* = 2.2, 1.5 Hz, 1H), 4.73 (dd, *J* = 2.0, 0.8 Hz, 1H), 3.99-3.92 (m, 1H), 3.57 (bs, 1H), 2.39 (d, *J* = 10.1 Hz, 1H), 2.32 (d, *J* = 10.2 Hz, 1H), 2.06-2.00 (m, 1H), 1.75 (s, 3H), 1.72-1.68 (m, 3H), 1.45 (s, 9H), 1.32-1.21 (m, 1H), 1.18-1.05 (m, 1H), 0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 176.3 (C), 153.8 (C), 140.5 (C), 115.7 (CH_2), 83.8 (C), 72.4 (C), 60.9 (CH), 46.8 (CH_2), 36.7 (CH_3), 30.5 (CH_2), 27.6 (3 CH_3), 25.8 (CH_2), 24.0 (CH), 18.6 (CH_2), 12.8 (CH_3), 12.0 (CH_3); Anal. Calcd for $C_{18}H_{31}NO_4$: C, 66.43; H, 9.60; N, 4.30; Found: C, 66.37; H, 9.82; N, 4.38.

di(*tert*-butyl) (2*S*)-5-methyl-6-oxo-1,2-piperidinedicarboxylate (123)

Compound **61a** (100 mg, 0.334 mmol) was placed in an argon-filled round-bottomed flask, dissolved in 2.0 mL of anhydrous THF and cooled to –78°C. 0.334 mL of a 1N solution of LiHMDS in THF were introduced by the mean of a hypodermic syringe. After 2.5 h, 142 mg (1.0 mmol) of MeI were added *via* a hypodermic syringe. After being stirred at –78°C during 16 h, the reaction was quenched by adding 0.35 mL of a saturated aqueous solution of NH_4Cl. THF was evaporated under reduced pressure, replaced with AcOEt which was washed with

water. The organic layer was dried over Na_2SO_4, concentrated and chromatographed on silica gel (AcOEt/Hex, 2:8) to give **123** (97 mg, yield = 92%) as an equimolar mixture of diastereomers: HPLC t_R 11.32 (linear gradient, 30-100% B, 20 min); yellowish oil; $[\alpha]_D$ = −14.8 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.54 (dd, *J* = 6.2, 5.0 Hz, 1H), 4.46-4.45 (m, 1H), 2.51-2.44 (m, 1H), 2.43-2.35 (m, 1H), 2.14-1.80 (m, 8H), 1.45 (s, 9H), 1.44 (s, 9H), 1.40 (s, 9H), 1.40 (s, 9H), 1.18 (d, *J* = 2.2 Hz, 3H), 1.17 (d, *J* = 2.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.1 (C), 173.9 (C), 170.8 (C), 170.7 (C), 153.1 (C), 152.7 (C), 83.1 (C), 82.9 (C), 82.0 (C), 82.0 (C), 59.8 (CH), 58.9 (CH), 39.3 (2 CH), 38.1 (CH), 38.0 (CH), 28.0 (3 CH_3), 28.0 (3 CH_3), 27.9 (6 CH_3), 27.2 (CH_2), 26.3 (CH_2), 25.7 (CH_2), 23.6 (CH_2), 17.5 (CH_3), 16.9 (CH_3); Anal. Calcd for $C_{16}H_{27}NO_5$: C, 61.32; H, 8.68; N, 4.47; Found: C, 61.44; H, 8.93; N, 4.52.

di(*tert*-butyl) (2*S*,5*R*)-5-hydroxy-5-methyl-6-oxo-1,2-piperidinedicarboxylate (124)

Compound **124** was prepared according to the procedure B using (+)-CSO as oxidizing agent. The solution was stirred at −78°C during 16 h. After the workup, a flash column chromatography (MeOH/CH_2Cl_2, 2:98) of the crude product afforded pure **124** (84 mg, yield = 80%): HPLC t_R 8.57 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = +44.5 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.56 (t, *J* = 5.7 Hz, 1H), 3.12 (bs, 1H), 2.25-2.13 (m, 1H), 2.04-1.91 (m, 2H), 1.84-1.79 (m, 1H), 1.49 (s, 9H), 1.46 (s, 9H), 1.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 176.0 (C), 170.1 (C), 151.9 (C), 131.2 (CH), 83.8 (C), 82.3 (C), 72.6 (C), 59.8 (CH), 32.3 (CH_3), 28.1 (CH_2), 27.9 (3 CH_3), 22.9 (CH_2); Anal. Calcd for $C_{16}H_{27}NO_6$: C, 58.34; H, 8.26; N, 4.25; Found: C, 58.33; H, 8.44; N, 4.06.

***tert*-butyl (2*S*, 5*R*)-2-[(*tert*-butoxycarbonyl)amino]-5,6-dihydroxyhexanoate (127)**

Sodium borohydride (935 mg, 24.75 mmol) was added to a solution of 1.56 g (4.95 mmol) of α-hydroxy lactam (5*R*)-**63a** in 20.0 mL of absolute ethanol at 0°C. The mixture was allowed to reach room temperature and stirred for 4 h. After being quenched by water, the mixture was stirred for a further 10 min. Evaporation of the solvent gave a solid, which was dissolved in AcOEt. The solution was washed with water and dried over Na_2SO_4. Evaporation of the filtrate afforded an oil which was subjected to filtration through a silica pad (AcOEt/AcOH, 99:1). Pure **127** was recovered (1.44 g, yield = 91%): HPLC t_R 5.85 (linear gradient, 30-100% B, 20 min); $[\alpha]_D$ = +12.2 (*c* = 1.1, $CHCl_3$); waxy white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (bd, 1H), 4.15-4.10 (m, 1H), 3.92 (bs, 2H), 3.69-3.63 (m, 1H), 3.56 (dd, *J* = 11.0, 2.9 Hz, 1H), 3.38 (dd, *J* = 11.0, 7.3 Hz, 1H), 1.93-1.86 (m, 1H), 1.68-1.59 (m, 1H), 1.47-1.42 (m,

2H), 1.41 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.0 (C), 155.8 (C), 82.0 (C), 79.9 (C), 71.7 (CH), 66.6 (CH_2), 53.8 (CH), 29.3 (CH_2), 28.7 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3); Anal. Calcd for $C_{15}H_{29}NO_6$: C, 56.41; H, 9.15; N, 4.39; Found: C, 56.20; H, 9.42; N, 4.35.

***tert*-butyl (2*S*, 5*R*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxyhexanoate (128)**

Collidine (6.0 mL, 45.10 mmol) was added to a solution of 1.44 g (4.51 mmol) of the 1,2-diol **128** in 90.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 568 mg (4.96 mmol) of MsCl dissolved in 5.5 mL of CH_2Cl_2 was added. After being stirred for 20 h at 0°C, the reaction was quenched by the addition of water (35.0 mL), the phases were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with 1N $KHSO_4$, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in 20.0 mL of DMF. 586 mg (9.02 mmol) of NaN_3 were added to the solution which was heated to 80°C for 8 h. After being allowed to cool to room temperature, water was added to the solution which was extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 3:7) to yield pure **128** (1.37 g, yield = 88%): HPLC t_R 10.07 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = +13.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (bd, 1H), 4.21-4.14 (m, 1H), 3.76 (bs, 1H), 3.39-3.36 (m, 1H), 3.26 (dd, *J* = 12.4, 4.0 Hz, 1H), 3.21 (dd, *J* = 12.4, 6.6 Hz, 1H), 1.96-1.87 (m, 1H), 1.69-1.58 (m, 1H), 1.53-1.48 (m, 2H), 1.42 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.7 (C), 155.8 (C), 82.2 (C), 80.0 (C), 70.4 (CH), 57.0 (CH_2), 53.5 (CH), 29.8 (CH_2), 29.6 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3); Anal. Calcd for $C_{15}H_{28}N_4O_5$: C, 52.31; H, 8.19; N, 16.27; Found: C, 52.32; H, 8.42; N, 16.09.

***tert*-butyl (2*S*, 5*S*)-5,6-diazido-2-[(*tert*-butoxycarbonyl)amino]hexanoate (129)**

HPLC t_R 13.39 (linear gradient, 30-100% B, 20 min); yellowish oil; $[\alpha]_D$ = –4.6 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.12 (bd, *J* = 7.5 Hz, 1H), 4.20-4.14 (m, 1H), 3.53-3.44 (m, 1H), 3.38 (dd, *J* = 12.8, 4.2 Hz, 1H), 3.29 (dd, *J* = 12.6, 7.1 Hz, 1H), 1.93-1.82 (m, 1H), 1.78-1.68 (m, 1H), 1.65-1.54 (m, 1H), 1.51-1.42 (m, 1H), 1.44 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.3 (C), 155.4 (C), 82.4 (C), 79.9 (C), 61.3 (CH), 54.9 (CH2), 53.1 (CH), 29.4 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 27.4 (CH_2).

***tert*-butyl (2*S*, 5*R*)-6-azido-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-5-hydroxyhexanoate (131)**

p-Toluenesulfonic acid (2.26 g, 11.88 mmol) was added to a solution of 2.04 g (5.92 mmol) of **128** in 30.0 mL of CH_3CN at 0°C. The mixture was stirred at 0°C for 2 h and consistently checked by TLC (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1). The mixture was then allowed to reach room temperature and stirred for an additional 2 h. The reaction was quenched by the addition of 250 mL of aqueous 1N NH_4OH. The solution was extracted with 240 mL (3 × 80.0 mL) of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 15.0 mL of THF and the same volume of water was added to the solution. Solid $NaHCO_3$ (995 mg, 11.84 mmol) and FmocOSu (2.40 g, 7.10 mmol), dissolved in the minimum volume of THF, were added to the mixture stirred at ambient temperature. After 3 h, THF was evaporated and replaced by AcOEt. The solution was washed with a saturated $NaHCO_3$ solution, water, 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2) to yield pure **131** (2.10 g, yield = 76% for 2 steps): HPLC t_R 13.23 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +7.3 (*c* = 1.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (m, 2H), 5.59 (bd, *J* = 7.8 Hz, 1H), 4.40 (d, *J* = 6.9 Hz, 2H), 4.34-4.30 (m, 1H), 4.22 (t, *J* = 6.9 Hz, 1H), 3.83-3.77 (m, 1H), 3.37-3.20 (m, 2H), 2.87 (bs, 1H), 2.07-1.97 (m, 1H), 1.78-1.68 (m, 1H), 1.62-1.52 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.3 (C), 156.1 (C), 143.8 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.0 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 82.5 (C), 70.4 (CH), 67.0 (CH_2), 57.0 (CH_2), 53.8 (CH), 47.2 (CH), 29.6 (2 CH_2), 28.0 (3 CH_3).

Determination of the stereomeric purity of synthetic 5-hydroxylysine.

(2*S*,5*R*)-5-hydroxylysine dihydrochloride (133)

Palladium on activated carbon (5 mg, 10% $^w/_w$ Pd) was added to a solution of 100 mg (0.29 mmol) of **128** in 2.0 mL of ethanol. The mixture was vigorously stirred at room temperature for 4 h under an atmosphere of hydrogen and filtered through Celite®. Evaporation of the solvent gave **132** in quantitative yield as a colorless oil. The residue was dissolved in 2.0 mL of a 4N solution of HCl in dioxane and stirred at room temperature for 2 h. Evaporation of the solvent gave **133** (65 mg, yield = 95%); white solid.

Determination of the stereomeric purity of 133: 5-hydroxylysine **133** (1 mg) was dissolved in 1.0 mL of 50% ($^v/_v$) aqueous acetonitrile containing 0.4% ($^v/_v$) of triethylamine. A 200 µL aliquot of this solution was mixed with 200 µL of Marfey's reagent in acetone (2.5

mg in 1.0 mL). The mixture was thermostated at 40°C for 2 h and diluted to 1.0 mL with water prior to injection. The same procedure was repeated using the commercially available (2*S*,5*R*)-5-hydroxylysine and a commercial mixture of the 4 diastereomers. HPLC analysis was performed on a Nucleosil C_{18} column (5 μm, 3.9 × 150 mm) by using a linear gradient (30-65% B, 35 min) of A (0.1% TFA in H_2O) and B (MeOH) at a flow rate of 1.0 ml/min with UV detection at 214 nm: D,L-5-hydroxy-D,L-lysine: t_R 23.93 min, 25.92 min, 27.57 min, 28.36 min; commercial **133**: t_R 27.59 min; synthesized **133**: t_R 27.59 min. Synthesized 5-hydroxylysine **133** was 97% pure (limit of detection of analytical RP-HPLC : 0.1%).

***tert*-butyl (2*S*,5*R*)-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate (134)**

Collidine (0.83 mL, 6.26 mmol) was added to a solution of 200 mg (0.626 mmol) of **127** in 11.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 79.0 mg (6.88 mmol) of MsCl dissolved in 0.75 mL of CH_2Cl_2 was added. After being stirred for 20 h at 0°C, the reaction was quenched by water, the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (5.0 mL). The combined organic extracts were washed with 1N $KHSO_4$, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in 10.0 mL of acetone. 188 mg (1.252 mmol) of NaI were added to the solution which was heated at reflux for 16 h. Acetone was evaporated and replaced by AcOEt. The solution was washed with water, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 2:8) to yield pure **134** (210 mg, yield = 78%): HPLC t_R 10.82 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = +14.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.23 (bd, 1H), 4.18-4.14 (m, 1H), 3.55-3.49 (m, 1H), 3.26 (m, 2H), 3.18 (dd, *J* = 10.1, 5.9 Hz, 1H), 1.94-1.86 (m, 1H), 1.68-1.51 (m, 3H), 1.41 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.6 (C), 155.7 (C), 82.2 (C), 79.9 (C), 70.6 (CH), 53.5 (CH), 31.9 (CH_2), 29.7 (CH_2), 28.4 (3 CH_3), 28.1 (3 CH_3), 15.4 (CH_2); Anal. Calcd for $C_{15}H_{28}INO_5$: C, 41.97; H, 6.57; N, 3.26; Found: C, 42.04; H, 6.69; N, 3.28.

***tert*-butyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-[(2*R*)oxiranyl]butanoate (136)**

Collidine (0.83 mL, 6.26 mmol) was added to a solution of 200 mg (0.626 mmol) of **127** in 11.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 79.0 mg (6.88 mmol) of MsCl dissolved in 750 μL of CH_2Cl_2 were added. After being stirred for 20 h at 0°C, the reaction was quenched by water, the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (5.0 mL). The combined organic extracts were washed with 1N $KHSO_4$, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in

3.0 mL of methanol. 173 mg (1.252 mmol) of K_2CO_3 were added to the solution which was stirred at room temperature during 4 h. The mixture was filtered, the solvent was evaporated and replaced by AcOEt. The solution was washed with water, brine, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by filtration through a plug of silica (AcOEt/Hex, 3:7) to yield **136** (132 mg, yield = 70%): HPLC t_R 9.93 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = +18.3 (c = 1.0, $CHCl_3$); mp: 43-45 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 (bd, 1H), 4.15-4.12 (m, 1H), 2.88-2.84 (m, 1H), 2.70 (dd, J = 4.9, 4.0 Hz, 1H), 2.43 (dd, J = 4.9, 2.7 Hz, 1H), 1.95-1.87 (m, 1H), 1.72-1.63 (m, 1H), 1.60-1.52 (m, 2H), 1.40 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.6 (C), 155.4 (C), 82.0 (C), 79.6 (C), 53.7 (CH), 51.7 (CH), 47.0 (CH_2), 29.2 (CH_2), 28.4 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3); Anal. Calcd for $C_{15}H_{27}NO_5$: C, 59.78; H, 9.03; N, 4.65; Found: C, 59.64; H, 9.30; N, 4.91.

***tert*-butyl (2*S*,5*R*)-2-[(*tert*-butoxycarbonyl)amino]-6-[(2,2-dimethylpropanoyl)oxy]-5-hydroxyhexanoate (137)**

Pivaloyl chloride (188 mg, 1.56 mmol) dissolved in 1.0 mL of CH_2Cl_2 was added to a solution of 250 mg (0.78 mmol) of 1,2-diol **127** in a mixture of 2.5 mL of CH_2Cl_2 and 2.5 mL of pyridine at 0°C. After being stirred for 2 h at 0°C, the solution was quenched by water. The solvent was evaporated and replaced by AcOEt. The solution was washed with 1N $KHSO_4$, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography to yield pure **137** (290 mg, yield = 92%): HPLC t_R 11.19 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = –8.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.21 (bd, J = 8.0 Hz, 1H), 4.23-4.14 (m, 1H), 4.04 (dd, J = 11.3, 4.0 Hz, 1H), 3.96 (dd, J = 11.3, 6.2 Hz, 1H), 3.86-3.78 (m, 1H), 2.01-1.90 (m, 1H), 1.72-1.62 (m, 1H), 1.56-1.48 (m, 2H), 1.42 (s, 9H), 1.40 (s, 9H), 1.17 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.6 (C), 171.7 (C), 155.6 (C), 82.0 (C), 79.8 (C), 69.6 (CH), 68.3 (CH_2), 53.6 (CH), 38.8 (C), 29.5 (CH_2), 29.0 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 27.0 (3 CH_3). Anal. Calcd for $C_{20}H_{37}NO_7$: C, 59.53; H, 9.24; N, 3.47. Found: C, 59.29; H, 9.57; N, 3.50.

***tert*-butyl (2*S*,5*R*)-6-[(2,2-dimethylpropanoyl)oxy]-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-hydroxyhexanoate (138)**

The protected 1,2-diol **137** (100 mg, 0.248 mmol) was dissolved in 2.0 mL of CH_3CN cooled at 0°C. *p*-Toluenesulfonic acid (94 mg, 0.496 mmol) was added and the resulting mixture was stirred at 0°C for 2 h. The mixture was then allowed to reach room temperature and stirred for

an additional 2 h and consistently checked by TLC (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1). The reaction was quenched by the addition of 30.0 mL of aqueous 1N NH_4OH. The solution was extracted with 30.0 mL (2 × 15.0 mL) of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 2.0 mL of CH_2Cl_2 and FmocOSu (100 mg, 0.297 mmol) was added. The solution was stirred at room temperature and checked by TLC (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1). After complete consumption of the starting amine, CH_2Cl_2 was evaporated and replaced by AcOEt. The solution was washed with 1N $KHSO_4$ and water, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2) to yield pure **138** (97 mg, yield = 75% for 2 steps): HPLC t_R 14.30 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +4.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4 Hz, 2H), 7.60 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.31 (t, J = 7.3 Hz, 2H), 5.57 (bd, J = 8.0 Hz, 1H), 4.39 (d, J = 7.0 Hz, 2H), 4.37-4.29 (m, 1H), 4.22 (t, J = 7.0 Hz, 1H), 4.10 (dd, J = 11.3, 3.8 Hz, 1H), 4.00 (dd, J = 11.2, 6.4 Hz, 1H), 3.90-3.82 (m, 1H), 2.70 (bs, 1H), 2.11-1.98 (m, 1H), 1.81-1.67 (m, 1H), 1.61-1.52 (m, 2H), 1.47 (s, 9H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.7 (C), 171.5 (C), 156.1 (C), 143.9 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 82.3 (C), 69.7 (CH), 68.4 (CH_2), 67.0 (CH_2), 54.0 (CH), 47.2 (CH), 38.8 (C), 29.4 (CH_2), 28.9 (CH_2), 28.0 (3 CH_3), 27.2 (3 CH_3). Anal. Calcd for $C_{30}H_{39}NO_7$: C, 68.55; H, 7.48; N, 2.66. Found: C, 68.83; H, 7.88; N, 2.31.

di(*tert*-butyl) (2*S*,5*S*)-5-[(4-nitrobenzoyl)oxy]-6-oxo-1,2-piperidinedicarboxylate ((5*S*)-139)

p-Nitrobenzoic acid (436 mg, 2.61 mmol) and PPh_3 (685 mg, 2.61 mmol) were added to a solution of 550 mg (1.74 mmol) of **63a** in 30.0 mL of THF at ambient temperature. The solution was cooled to 0°C and DIAD (528 mg, 2.61 mmol) dissolved in 8.0 mL of THF was introduced *via* a hypodermic syringe. The mixture was allowed to reach room temperature and stirred overnight. Evaporation of the solvent gave an oil, which was dissolved in AcOEt. The solution was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 3:7) to yield pure (5*S*)-**139** (738 mg, yield = 91%): HPLC t_R 13.87 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –12.1 (c = 1.0, $CHCl_3$); mp = 48-49 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32-8.24 (m, 4H), 5.59 (dd, J = 12.1, 6.4 Hz, 1H), 4.59-4.56 (m, 1H), 2.38-2.22 (m, 3H), 2.15-2.03 (m, 1H), 1.51 (s, 9H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.1 (C),

167.2 (C), 163.8 (C), 152.5 (C), 150.6 (C), 135.0 (C), 131.1 (2 CH), 123.4 (2 CH), 83.9 (C), 82.7 (C), 71.0 (CH), 59.1 (CH), 27.9 (3 CH_3), 27.8 (3 CH_3), 25.1 (CH_2), 23.9 (CH_2). Anal. Calcd for $C_{22}H_{28}N_2O_9$: C, 56.89; H, 6.08; N, 6.03. Found: C, 56.59; H, 6.21; N, 5.89.

di(*tert*-butyl) (2*S*,5*R*)-5-[(4-nitrobenzoyl)oxy]-6-oxo-1,2-piperidinedicarboxylate ((5*R*)-139)

Pyridine (128 μL, 1.58 mmol) was added to a solution of 100 mg of (0.32 mmol) of **63a** in 2.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and *p*-nitrobenzoic chloride (71 mg, 0.38 mmol) was added. The solution was allowed to reach room temperature and stirred for 2 h. After being quenched by water, the solvent was evaporated and the residue dissolved in AcOEt. The solution was washed with saturated $NaHCO_3$, brine, 1N $KHSO_4$, and dried over Na_2SO_4. Evaporation of the filtrate afforded a residue which was subjected to filtration through a short plug of silica (AcOEt/Hex, 3:7). Pure (5*R*)-**139** was recovered (146 mg, yield = 99%): HPLC t_R 13.92 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –7.9 (*c* = 1.0, $CHCl_3$); mp = 136-138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28-8.20 (m, 4H), 5.55 (dd, *J* = 8.4, 6.7 Hz, 1H), 4.75-4.72 (m, 1H), 2.36-2.16 (m, 3H), 2.11-2.01 (m, 1H), 1.50 (s, 9H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.7 (C), 166.6 (C), 163.5 (C), 151.8 (C), 150.7 (C), 134.9 (C), 131.1 (2 CH), 123.5 (2 CH), 84.2 (C), 82.9 (C), 70.4 (CH), 58.1 (CH), 27.8 (6 CH_3), 24.8 (CH_2), 22.5 (CH_2).

***tert*-butyl (2*S*,5*S*)-2-[(*tert*-butoxycarbonyl)amino]-5,6-dihydroxyhexanoate (140)**

Sodium borohydride (155 mg, 4.09 mmol) was added to a solution of 380 mg (0.82 mmol) **139a** in 4.0 mL of absolute ethanol at 0°C. The mixture was allowed to reach room temperature and stirred for 4 h. After being quenched by water, the mixture was stirred for further 10 min. The solvent was evaporated and the residue dissolved in AcOEt. The resulting solution was washed with saturated $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (AcOEt) to give pure **140** (222 mg, yield = 85%): HPLC t_R 5.85 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +3.5 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.34 (bd, *J* = 7.9 Hz, 1H), 4.12-4.01 (m, 1H), 3.68-3.59 (m, 1H), 3.54 (dd, *J* = 11.2, 3.1 Hz, 1H), 3.37 (dd, *J* = 11.2, 7.5 Hz, 1H), 1.80-1.69 (m, 2H), 1.41-1.33 (m, 2H), 1.40 (s, 9H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.1 (C), 155.7 (C), 81.9 (C), 79.7 (C), 71.5 (CH), 66.5 (CH_2), 53.9 (CH), 28.8 (CH_2), 28.7 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3). Anal. Calcd for $C_{15}H_{29}NO_6$: C, 56.41; H, 9.15; N, 4.39. Found: C, 56.35; H, 9.28; N, 4.60.

***tert*-butyl (2*S*,5*S*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxyhexanoate (141)**

Collidine (534 μL, 4.01 mmol) was added to a solution of 128 mg (0.40 mmol) of the 1,2-diol **140** in 8.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 50 mg (0.44 mmol) of MsCl dissolved in 0.5 mL of CH_2Cl_2 was added. After being stirred for 20 h at 0°C, the reaction was quenched by water (3.0 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with 1N $KHSO_4$, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 5.0 mL of DMF. NaN_3 (52 mg, 0.80 mmol) was added to the solution, which was heated to 80°C for 16 h. After being cooled to room temperature, water was added to the solution, which was extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 3:7) to yield pure **141** (110 mg, yield = 80%): HPLC t_R 9.55 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +5.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.17 (bd, *J* = 7.3 Hz, 1H), 4.21-4.12 (m, 1H), 3.80-3.72 (m, 1H), 3.32 (dd, *J* = 12.4, 3.8 Hz, 1H), 3.23 (dd, *J* = 12.4, 7.1 Hz, 1H), 1.89-1.69 (m, 2H), 1.56-1.43 (m, 2H), 1.44 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.7 (C), 155.5 (C), 82.1 (C), 79.8 (C), 70.1 (CH), 56.9 (CH_2), 53.6 (CH), 29.8 (CH_2), 29.0 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3). Anal. Calcd for $C_{15}H_{28}N_4O_5$: C, 52.31; H, 8.19; N, 16.27. Found: C, 52.42; H, 8.45; N, 16.11.

***tert*-butyl (2*S*,5*S*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-hydroxyhexanoate (142)**

p-Toluenesulfonic acid (88 mg, 0.463 mmol) was added to a solution of 80 mg (0.232 mmol) of **141** in 2.0 mL of CH_3CN at 0°C. The mixture was stirred at 0°C for 4 h and consistently checked by TLC (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1). The mixture was then allowed to reach room temperature and stirred for an additional 4 h (until **141** disappeared). The reaction was quenched by the addition of 30.0 mL of aqueous 1N NH_4OH. The solution was extracted with 30.0 mL (2 × 15.0 mL) of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 2.0 mL of THF and the same volume of water was added to the solution. Solid $NaHCO_3$ (39 mg, 0.464 mmol) and a solution of FmocOSu (94 mg, 0.279 mmol) in 0.5 mL of THF were added to the solution, which was stirred at room temperature for 3 h. THF was evaporated and replaced by AcOEt. The solution was washed with a saturated $NaHCO_3$ solution, brine, 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column

chromatography (CH_2Cl_2/MeOH, 98:2) to yield pure **142** (79 mg, yield = 73%): HPLC t_R 13.22 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +3.5 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.59 (bd, *J* = 7.9 Hz, 1H), 4.40 (d, *J* = 7.0 Hz, 2H), 4.32-4.25 (m, 1H), 4.21 (t, *J* = 6.9 Hz, 1H), 4.82-4.73 (m, 1H), 3.34-3.30 (m, 1H), 3.27-3.19 (m, 1H), 2.80 (bs, 1H), 1.89-1.77 (m, 2H), 1.60-1.43 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.5 (C), 156.1 (C), 143.9 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 82.4 (C), 70.1 (CH), 67.0 (CH_2), 57.0 (CH_2), 54.0 (CH), 47.2 (CH), 29.6 (CH_2), 28.9 (CH_2), 28.0 (3 CH_3). Anal. Calcd for $C_{25}H_{30}N_4O_5$: C, 64.36; H, 6.48; N, 12.01. Found: C, 64.59; H, 6.57; N, 11.94.

***tert*-butyl (2*S*,5*R*)-2-[(*tert*-butoxycarbonyl)amino]-6-{[tert-butyl(diphenyl)silyl]oxy}-5-hydroxyhexanoate (143)**

Imidazole (85 mg, 1.25 mmol) and TBDPSCl (327 mg, 1.19 mmol) dissolved in 1.0 mL of CH_2Cl_2 were added to a solution of 190 mg (0.595 mmol) of **127** in 5.0 mL of CH_2Cl_2 at 0°C. The mixture was stirred for 30 min and immediately quenched by the addition of water. After evaporation of CH_2Cl_2, the residue was dissolved in AcOEt, washed with water, dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by a flash column chromatography (AcOEt/Hex, 2:8) yielded pure **143** (308 mg, yield = 93%): HPLC t_R 16.03 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D$ = +8.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67-7.64 (m, 4H), 7.41-7.34 (m, 6H), 5.22 (bd, *J* = 8.0 Hz, 1H), 4.21-4.15 (m, 1H), 3.75-3.68 (m, 1H), 3.62 (dd, *J* = 10.1, 4.0 Hz, 1H), 3.51 (dd, *J* = 10.1, 7.0 Hz, 1H), 2.78 (bs, 1H), 2.00-1.90 (m, 1H), 1.70-1.58 (m, 1H), 1.53-1.48 (m, 2H), 1.43 (s, 9H), 1.42 (s, 9H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.9 (C), 155.5 (C), 135.5 (4 CH), 133.1 (2 C), 129.8 (2 CH), 127.8 (4 CH), 81.6 (C), 79.5 (C), 71.6 (CH), 67.9 (CH_2), 53.9 (CH), 29.3 (CH_2), 28.7 (CH_2), 28.3 (3 CH_3), 27.8 (3 CH_3), 26.8 (3 CH_3), 19.2 (C). Anal. Calcd for $C_{31}H_{47}NO_6Si$: C, 66.75; H, 8.49; N, 2.51. Found: C, 66.52; H, 8.47; N, 2.76.

***tert*-butyl (2*S*,5*S*)-5-azido-2-[(*tert*-butoxycarbonyl)amino]-6-{[tert-butyl(diphenyl)silyl]oxy}hexanoate (144)**

N,*N*-Diisopropylethylamine (56 mg, 0.433 mmol) was added to a solution of 120 mg (0.215 mmol) of **143** in 3.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 62 mg (0.429 mmol) of MsCl dissolved in 0.5 mL of CH_2Cl_2 were added. After removing the ice bath, the reaction was stirred at room temperature until total consumption of **143** (checked by TLC). CH_2Cl_2 was evaporated and replaced by AcOEt. The organic phase was

washed with 1N $KHSO_4$, brine, a saturated solution of $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 3.0 mL of DMF. Sodium azide (42 mg, 0.646 mmol) was added to the solution, which was heated to 80°C for 16 h. After being cooled to room temperature, water was added to the solution, which was extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:9) to yield pure **144** (110 mg, yield = 88%): HPLC t_R 18.77 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D$ = –1.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75-7.67 (m, 4H), 7.46-7.26 (m, 6H), 5.07 (bd, *J* = 8.0 Hz, 1H), 4.24-4.18 (m, 1H), 3.73 (dd, *J* = 10.6, 3.8 Hz, 1H), 3.63 (dd, *J* = 10.6, 6.6 Hz, 1H), 3.45-3.35 (m, 1H), 1.83-1.71 (m, 3H), 1.54-1.35 (m, 1H), 1.47 (s, 9H), 1.43 (s, 9H), 1.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.5 (C), 155.4 (C), 135.6 (4 CH), 132.9 (2 C), 129.8 (2 CH), 127.8 (4 CH), 82.1 (C), 79.7 (C), 67.1 (CH_2), 63.3 (CH), 53.4 (CH), 29.6 (CH_2), 28.3 (3 CH_3), 27.8 (3 CH_3), 26.7 (3 CH_3), 25.9 (CH_2), 19.1 (C). Anal. Calcd for $C_{31}H_{46}N_4O_5Si$: C, 63.89; H, 7.96; N, 9.61. Found: C, 63.51; H, 7.86; N, 9.28.

***tert*-butyl (2*S*,5*S*)-5-azido-2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (145)**

Tetrabutylammonium fluoride supported on silica (750 mg, 1.0-1.5 mmol/g) were added to a solution of **144** (250 mg, 0.429 mmol) in 5.0 mL of anhydrous THF. The suspension was gently stirred for 2 h and checked by TLC (AcOEt/Hex, 3:7). Supported-TBAF was filtered and washed with AcOEt. The filtrate was evaporated and crude **145** was filtered through a short pad of silica (AcOEt/Hex, 3:7) to afford pure **145** (148 mg, yield = 100%): HPLC t_R 10.22 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +15.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.12 (bd, *J* = 7.0 Hz, 1H), 4.23-4.16 (m, 1H), 3.70 (dd, *J* = 11.2, 4.2 Hz, 1H), 3.59 (dd, *J* = 11.3, 6.8 Hz, 1H), 3.53-3.46 (m, 1H), 2.06 (bs, 1H), 1.97-1.85 (m, 1H), 1.81-1.72 (m, 1H), 1.69-1.57 (m, 2H), 1.47 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.4 (C), 155.5 (C), 82.3 (C), 79.9 (C), 65.1 (CH_2), 63.4 (CH), 53.2 (CH), 29.6 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 26.2 (CH_2). Anal. Calcd for $C_{15}H_{28}N_4O_5$: C, 52.31; H, 8.19; N, 16.27. Found: C, 52.36; H, 8.46; N, 16.07.

***tert*-butyl (2*S*,5*S*)-5-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-6-hydroxyhexanoate (146)**

p-Toluenesulfonic acid (132 mg, 0.694 mmol) was added to a solution of 120 mg (0.348 mmol) of **145** in 2.0 mL of CH_3CN at 0°C. The mixture was stirred until **145** totally disappeared (checked by TLC, AcOEt/pyridine/acetic acid/water, 8:2:0.5:1). The reaction was

quenched by the addition of 30.0 mL of aqueous 1N NH_4OH. The solution was extracted with 30.0 mL (2 × 15.0 mL) of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 2.0 mL of acetone and the same volume of water was added to the solution. Solid K_2CO_3 (144 mg, 1.04 mmol) and a solution of FmocOSu (176 mg, 0.522 mmol) in 1.0 mL of acetone were added to the solution, which was stirred at room temperature for 3 h. Acetone was evaporated and replaced by AcOEt. The solution was washed with a saturated $NaHCO_3$ solution, brine and 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2) to yield pure **146** (118 mg, yield = 73%): HPLC t_R 13.69 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +9.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.31 (m, 2H), 5.49 (bd, *J* = 7.9 Hz, 1H), 4.40 (d, *J* = 7.1 Hz, 2H), 4.33-4.26 (m, 1H), 4.21 (t, *J* = 7.0 Hz, 1H), 3.72-3.69 (m, 1H), 3.61-3.55 (m, 1H), 3.51-3.44 (m, 1H), 2.23 (bs, 1H), 2.00-1.76 (m, 2H), 1.70-1.58 (m, 1H), 1.52-1.40 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.2 (C), 156.0 (C), 143.8 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 82.6 (C), 67.0 (CH_2), 65.2 (CH_2), 63.5 (CH), 53.8 (CH), 47.1 (CH), 29.4 (CH_2), 28.0 (3 CH_3), 26.1 (CH_2). Anal. Calcd for $C_{25}H_{30}N_4O_5$: C, 64.36; H, 6.48; N, 12.01. Found: C, 64.05; H, 6.54; N, 11.88.

di(*tert*-butyl) (2*S*,5*R*)-5-[(methylsulfonyl)oxy]-6-oxo-1,2-piperidinedicarboxylate (147) *N*,*N*-Diisopropylethylamine (164 mg, 1.27 mmol) was added to a solution of 200 mg (0.215 mmol) of **63a** in 2.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 145 mg (1.26 mmol) of MsCl dissolved in 0.5 mL of CH_2Cl_2 was added. After removing the ice bath, the reaction was stirred at room temperature for 16 h. CH_2Cl_2 was evaporated and replaced by AcOEt. The organic phase was washed with 1N $KHSO_4$, brine, a saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography (AcOEt/Hex, 3:7) gave pure **147** (220 mg, yield = 88%): HPLC t_R 11.30 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = +19.7 (*c* = 1.0, $CHCl_3$); mp: 121-123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.03 (m, 1H), 4.60 (m, 1H), 3.20 (s, 3H), 2.31-2.17 (m, 2H), 2.15-2.03 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.6 (C), 166.0 (C), 151.1 (C), 84.5 (C), 83.0 (C), 76.6 (CH_3), 58.4 (CH), 39.1 (CH), 27.8 (3 CH_3), 27.7 (3 CH_3), 26.1 (CH_2), 21.7 (CH_2).

di(*tert*-butyl) (2*S*)-5-amino-6-oxo-3,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (148)

Compound **147** (70.0 mg, 0.178 mmol) was dissolved in 2.0 mL of DMF. NaN_3 (58.0 mg, 0.892 mmol) was added to the solution, which was heated to 45°C for 16 h. After being cooled to room temperature, water was added to the solution, which was extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:1) to yield pure **148** (52 mg, yield = 94%): HPLC t_R 6.00 (linear gradient, 30-100% B, 20 min); colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.35-5.31 (m, 1H), 4.76-4.72 (m, 1H), 3.67 (bs, 1H), 2.68-2.62 (m, 2H), 1.49 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.0 (C), 161.9 (C), 152.2 (C), 135.9 (C), 103.6 (CH), 83.3 (C), 82.3 (C), 57.2 (CH), 27.9 (3 CH_3), 27.8 (3 CH_3), 25.1 (CH_2).

***tert*-butyl (2*S*,5*R*)-2-[(*tert*-butoxycarbonyl)amino]-5,6-dihydroxy-5-methylhexanoate (149)**

Sodium borohydride (575 mg, 15.20 mmol) was added to a solution of 1.00 g (3.03 mmol) of **124** in 12.0 mL of absolute ethanol at 0°C. The mixture was allowed to reach room temperature and stirred overnight. After being quenched by water, the mixture was stirred for further 10 min. The solvent was evaporated and the residue dissolved in AcOEt and washed with saturated $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography (AcOEt) to give pure **149** (882 mg, yield = 87%): HPLC t_R 6.63 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +9.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.41 (bd, J = 7.9 Hz, 1H), 4.01-3.93 (m, 1H), 3.75 (bs, 1H), 3.44 (bs, 1H), 3.26 (s, 2H), 1.79-1.67 (m, 1H), 1.59-1.46 (m, 1H), 1.43-1.26 (m, 2H), 1.31 (s, 9H), 1.28 (s, 9H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.0 (C), 155.7 (C), 81.6 (C), 79.5 (C), 72.3 (C), 69.3 (CH_2), 54.2 (CH), 33.5 (CH_2), 28.2 (3 CH_3), 27.8 (3 CH_3), 22.9 (CH_2), 20.8 (CH_3). Anal. Calcd for $C_{16}H_{31}NO_6$: C, 57.64; H, 9.37; N, 4.20. Found: C, 57.44; H, 9.58; N, 4.19.

***tert*-butyl (2*S*,5*R*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-5-methylhexanoate (150)**

Collidine (2.28 mL, 17.12 mmol) was added to a solution of 570 mg (1.71 mmol) of **149** in 36.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 206 mg (1.80 mmol) of MsCl dissolved in 2.0 mL of CH_2Cl_2 was added. After being stirred for 20 h at 4°C, the reaction was quenched by water (13.0 mL), the phases were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with 1N $KHSO_4$, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in

7.0 mL of DMF. Sodium azide (333 mg, 5.12 mmol) was added to the solution, which was heated to 80°C for 16 h. After being cooled to room temperature, water was added to the solution, which was extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 3:7) to yield **150** (540 mg, yield = 88%): HPLC t_R 11.09 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +9.0 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.17 (bd, *J* = 7.7 Hz, 1H), 4.30-4.23 (m, 1H), 3.23 (s, 2H), 2.58 (bs, 1H), 1.97-1.89 (m, 1H), 1.69-1.57 (m, 3H), 1.46 (s, 9H), 1.44 (s, 9H), 0.93 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.6 (C), 151.5 (C), 82.1 (C), 79.9 (C), 72.4 (C), 61.3 (CH_2), 53.6 (CH), 33.9 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3), 27.5 (CH_2), 21.0 (CH_3). Anal. Calcd for $C_{16}H_{30}N_4O_5$: C, 53.61; H, 8.44; N, 15.63. Found: C, 54.04; H, 8.30; N, 15.39.

***tert*-butyl (2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-hydroxy-5-methylhexanoate (151)**

p-Toluenesulfonic acid (531 mg, 2.79 mmol) was added to a solution of 500 mg (1.39 mmol) of **150** in 10.0 mL of CH_3CN at 0°C. The mixture was stirred for 9 h and consistently checked by TLC (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1). The reaction was quenched by the addition of 100 mL of aqueous 1N NH_4OH. The solution was extracted with 100 mL (2 × 50.0 mL) of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in 8.0 mL of THF and the same volume of water was added to the solution. Solid $NaHCO_3$ (233 mg, 2.78 mmol) and FmocOSu (518 g, 1.54 mmol), dissolved in 2.0 mL of THF, were added to the mixture stirred at ambient temperature. After 3 h, THF was evaporated and replaced by AcOEt. The solution was washed with a saturated $NaHCO_3$ solution, brine, 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo. Purification of the crude product by flash column chromatography (CH_2Cl_2/MeOH, 98:2) gave pure **151** (618 mg, yield = 92%): HPLC t_R 14.50 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +5.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.71 (bd, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 7.1 Hz, 2H), 4.40-4.31 (m, 1H), 4.22 (t, *J* = 7.1 Hz, 1H), 3.22 (s, 2H), 2.72 (bs, 1H), 2.04-1.94 (m, 1H), 1.80-1.59 (m, 3H), 1.49 (s, 9H), 1.20 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.5 (C), 156.2 (C), 143.9 (C), 143.8 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 82.4 (C), 72.3 (CH_2), 67.0 (C), 60.9 (CH_2), 54.2 (CH), 47.1 (CH), 34.2 (CH_2), 28.0 (3 CH_3), 27.1 (CH_2), 24.3

(CH_3). Anal. Calcd for $C_{26}H_{32}N_4O_5$: C, 64.98; H, 6.71; N, 11.66. Found: C, 65.16; H, 6.96; N, 11.34.

IX.4. Compounds cited in section IV

Preparation of 4,6-dioxopiperidines 62a-f: EDC (1.5 equiv), DMAP (1.5 equiv) and Meldrum's acid (1.0 equiv) were added to a 0.25M solution of acid **112** in CH_2Cl_2 at 0°C. The mixture was allowed to reach room temperature, stirred for 3 h and then washed with 1N $KHSO_4$. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in AcOEt to afford a 0.1M solution which was heated at reflux for 5 h. After being allowed to cool to room temperature, the mixture was washed with 1N $KHSO_4$ and brine. Drying over Na_2SO_4 and evaporation of the filtrate afforded crude **62** which was purified by recrystallization or by flash chromatography.

di(*tert*-butyl) (2*S*)-4,6-dioxo-1,2-piperidinedicarboxylate (62a)

Recrystallization of the crude product from CH_2Cl_2/pentane gave **62a** (5.82 g, yield = 77%): HPLC t_R 8.14 (linear gradient, 30-100% B, 20 min); yellowish crystals; $[\alpha]_D$ = +98.0 (c = 1.0, $CHCl_3$); mp 124-126°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.04 (dd, J = 6.8, 2.2 Hz, 1H), 3.50 (d, J = 19.5 Hz, 1H), 3.34 (d, J = 19.5 Hz, 1H), 2.99 (dd, J = 17.5, 2.2 Hz, 1H), 2.81 (dd, J = 17.6, 6.8 Hz, 1H), 1.52 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 200.1 (C), 168.9 (C), 165.4 (C), 151.2 (C), 84.6 (C), 83.9 (C), 54.6 (CH), 50.3 (CH_2), 40.9 (CH_2), 27.9 (3 CH_3), 27.8 (3 CH_3). Anal. Calcd for $C_{15}H_{23}NO_6$: C, 57.50; H, 7.40; N, 4.47. Found: C, 57.36; H, 7.48; N, 4.38.

di(*tert*-butyl) (2*S*)-4-hydroxy-6-oxo-3,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (153a)

^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 4.92 (d, J = 1.8 Hz, 1H), 4.81 (dd, J = 6.9, 1.7 Hz, 1H), 3.34 (bs, 1H), 3.00 (ddd, J = 17.7, 6.9, 1.8 Hz, 1H), 2.59 (dd, J = 17.7, 1.7 Hz, 1H), 1.43 (s, 9H), 1.37 (s, 9H).

2-benzyl 1-(*tert*-butyl) (2*S*)-4,6-dioxo-1,2-piperidinedicarboxylate (62b)

Purification of the crude product by flash column chromatography (AcOEt/Hex/AcOH, 5:5:0.1) gave **4b** (2.19 g, yield = 85%): HPLC t_R 9.05 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +76.1 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.27 (m, 5H), 5.24 (dd, J = 6.9, 2.0 Hz, 1H), 5.19 (s, 2H), 3.45 (d, J = 19.4, Hz, 1H), 3.31 (d, J = 19.4 Hz, 1H), 3.04 (dd, J = 17.9, 2.0 Hz, 1H), 2.84 (dd, J = 17.9, 6.9 Hz, 1H), 1.48 (s, 9H); ^{13}C

NMR (100 MHz, $CDCl_3$) 199.8 (C), 169.9 (C), 165.1 (C), 151.1 (C), 134.5 (C), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.4 (CH), 128.0 (CH), 84.9 (C), 68.3 (CH_2), 53.9 (CH), 50.5 (CH_2), 40.5 (CH_2), 27.8 (3 CH_3); Anal. Calcd for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.09; N, 4.03. Found: C, 62.32; H, 6.07; N, 4.19.

***tert*-butyl (2*S*)-2-isobutyl-4,6-dioxo-1-piperidinecarboxylate (62c)**

Recrystallization of the crude product from CH_2Cl_2/pentane gave **62c** (2.69 g, yield = 68%): HPLC t_R 8.61 (linear gradient, 30-100% B, 20 min); colourless crystals; $[\alpha]_D$ = –78.9 (*c* = 1.0, $CHCl_3$); mp 116-119°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.69-4.63 (m, 1H), 3.45 (d, *J* = 20.7 Hz, 1H), 3.35 (d, *J* = 20.7 Hz, 1H), 2.75 (dd, *J* = 16.8, 5.3 Hz, 1H), 2.65 (dd, *J* = 16.8, 2.6 Hz, 1H), 1.63-1.51 (m, 2H), 1.53 (s, 9H), 1.39-1.30 (m, 1H), 0.92 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.4 (C), 166.2 (C), 151.5 (C), 84.1 (C), 50.2 (CH), 49.1 (CH_2), 43.7 (CH_2), 43.0 (CH_2), 27.9 (3 CH_3), 25.1 (CH), 23.1 (CH_3), 21.5 (CH_3). Anal. Calcd for $C_{14}H_{25}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.80; H, 8.67; N, 5.08.

di(*tert*-butyl) (2*S*)-4-hydroxy-6-oxo-3,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (153c)

^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 4.94 (m, 1H), 4.40-4.34 (m, 1H), 3.32 (bs, 1H), 2.85 (m, 1H), 2.18-2.07 (m, 1H), 1.56-1.34 (m, 3H), 1.43 (s, 9H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.8 Hz, 3H).

***tert*-butyl (2*S*)-2-benzyl-4,6-dioxo-1-piperidinecarboxylate (62d)**

Recrystallization of the crude product from CH_2Cl_2/pentane gave **62d** (567 mg, yield = 87%): HPLC t_R 9.12 (linear gradient, 30-100% B, 20 min); yellowish crystals; $[\alpha]_D$ = –92.6 (*c* = 1.0, $CHCl_3$); mp 130-132°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30-7.24 (m, 3H), 7.16-7.13 (m, 2H), 4.79-4.73 (m, 1H), 3.37 (d, *J* = 20.7 Hz, 1H), 3.26 (d, *J* = 20.8 Hz, 1H), 3.07 (dd, *J* = 13.3, 5.1 Hz, 1H), 2.69 (dd, *J* = 13.3, 9.5 Hz, 1H), 2.64 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.2 (C), 166.1 (C), 151.5 (C), 135.9 (C), 129.5 (2 CH), 128.9 (2 CH), 127.3 (CH), 84.2 (C), 53.5 (CH), 49.1 (CH_2), 42.1 (CH_2), 41.0 (CH_2), 27.9 (3 CH_3). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.57; H, 7.09; N, 4.51.

***tert*-butyl (2*S*)-2-isopropyl-4,6-dioxo-1-piperidinecarboxylate (62e)**

Recrystallization of the crude product from CH_2Cl_2/pentane gave **62e** (645 mg, yield = 63%): HPLC t_R 7.32 (linear gradient, 30-100% B, 20 min); white crystals; $[\alpha]_D$ = –67.7 (*c* = 1.0, $CHCl_3$); mp 101-103°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.41-4.35 (m, 1H), 3.44 (d, *J* = 20.7 Hz, 1H), 3.33 (d, *J* = 20.7 Hz, 1H), 2.82 (dd, *J* = 17.0, 2.4 Hz, 1H), 2.67 (dd, *J* = 17.0, 5.7 Hz, 1H), 1.80-1.68 (m, 1H), 1.53 (s, 9H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.7 (C), 166.2 (C), 152.3 (C), 84.0 (C), 57.1 (CH), 49.2 (CH_2),

41.9 (CH_2), 33.0 (CH), 27.9 (3 CH_3), 19.6 (CH_3), 19.5 (CH_3). Anal. Calcd for $C_{13}H_{21}NO_4$: C, 61.16; H, 8.29; N, 5.49. Found: C, 61.00; H,8.52; N, 5.37.

***tert*-butyl (2*S*)-2-methyl-4,6-dioxo-1-piperidinecarboxylate (62f)**

Recrystallization of the crude product from CH_2Cl_2/pentane gave **62f** (625 mg, yield = 93%): HPLC t_R 13.17 (linear gradient, 30-100% B, 20 min); whyte crystals; $[\alpha]_D$ = –108.5 (*c* = 1.0, $CHCl_3$); mp 127-129°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71-4.65 (m, 1H), 3.45 (d, *J* = 20.7 Hz, 1H), 3.33 (d, *J* = 20.7 Hz, 1H), 2.81 (dd, *J* = 16.7, 5.9 Hz, 1H), 2.55 (dd, *J* = 16.6, 2.2 Hz, 1H), 1.53 (s, 9H), 1.31 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.2 (C), 165.9 (C), 151.5 (C), 84.1 (C), 49.0 (CH_2), 48.0 (CH), 45.3 (CH_2), 27.9 (3 CH_3), 20.5 (CH_3). Anal. Calcd for $C_{11}H_{17}NO_4$: C, 58.14; H, 7.54; N, 6.16. Found: C, 57.96; H,7.51; N, 6.38.

General procedure for 4,6-Dioxopiperidine Reduction. The desired dioxopiperidine **62** was placed in an argon-filled round-bottom flask, dissolved in distilled CH_2Cl_2 to give a ca 0.15M solution and cooled to 0°C. The indicated carboxylic acid was introduced *via* a hypodermic syringe and the mixture was stirred for 5 min. The indicated amount of reducing agent was added portion-wise and the mixture was allowed to reach room temperature. The reaction time was specific to each experiment. The reaction was quenched by the addition of water and stirred for 10 min. CH_2Cl_2 was evaporated, replaced by AcOEt and the organic layer was washed with saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4, concentrated and purified by recrystallization or by flash chromatography to yield **64**.

di(*tert*-butyl) (2*S*,4*S*)-4-hydroxy-6-oxo-1,2-piperidinedicarboxylate (64a)

Recrystallization of the crude product from CH_2Cl_2/diisopropyl ether gave **64a** (820 mg, yield = 82%, de > 99%): HPLC t_R 6.19 (linear gradient, 30-100% B, 20 min); colourless crystals; $[\alpha]_D$ = –18.0 (*c* = 1.0, $CHCl_3$); mp 132-134°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.61 (dd, *J* = 6.5, 4.1 Hz, 1H), 4.23 (m, 1H), 2.74 (dd, *J* = 17.4, 4.8 Hz, 1H), 2.69 (bs, 1H), 2.60 (ddd, *J* = 17.4, 4.6, 1.6 Hz, 1H), 2.41-2.33 (m, 1H), 2.20 (ddd, *J* = 14.1, 6.6, 2.9 Hz, 1H), 1.48 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.0 (C), 168.9 (C), 151.8 (C), 83.4 (C), 82.4 (C), 63.9 (CH), 56.2 (CH), 43.2 (CH_2), 32.9 (CH_2), 27.9 (3 CH_3), 27.8 (3 CH_3). Anal. Calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 56.85; H, 7.95; N, 4.44.

2-benzyl 1-(*tert*-butyl) (2*S*,4*S*)-4-hydroxy-6-oxo-1,2-piperidinedicarboxylate (64b)

Purification of the crude product by flash column chromatography (AcOEt/AcOH, 10:0.1) gave **64b** (86 mg, yield = 85%): HPLC t_R 7.87 (linear gradient, 30-100% B, 20 min);

colourless oil; $[\alpha]_D$ = –19.8 (*c* = 1.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.30 (m, 5H), 5.19 (d, *J* = 12.3 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 4.78 (dd, *J* = 6.6, 3.7 Hz, 1H), 4.28-4.23 (m, 1H), 2.75 (dd, *J* = 17.4, 4.8 Hz, 1H), 2.63 (ddd, *J* = 17.5, 3.8, 1.8 Hz, 1H), 2.53-2.44 (m, 1H), 2.22 (ddd, *J* = 14.2, 6.4, 2.5 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.8 (C), 169.0 (C), 151.7 (C), 135.3 (C), 128.5 (2 CH), 128.3 (CH), 128.3 (2 CH), 83.7 (C), 67.4 (CH_2), 63.5 (CH), 55.5 (CH), 43.0 (CH_2), 32.9 (CH_2), 27.8 (3 CH_3). Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 61.76; H, 6.67; N, 3.99.

***tert*-butyl (2*S*,4*R*)-4-hydroxy-2-isobutyl-6-oxo-1-piperidinecarboxylate (64c)**

Recrystallization of the crude product from CH_2Cl_2/pentane gave **64c** (572 mg, yield = 71%, de > 99%): HPLC t_R 7.51 (linear gradient, 30-100% B, 20 min); colourless crystals; $[\alpha]_D$ = +66.3 (*c* = 1.0, $CHCl_3$); mp 94-95°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.18-4.09 (m, 2H), 2.82 (ddd, *J* = 16.6, 5.7, 1.8 Hz, 1H), 2.59 (bs, 1H), 2.48 (dd, *J* = 16.6, 8.7 Hz, 1H), 2.30-2.21 (m, 1H), 1.71-1.57 (m, 3H), 1.51 (s, 9H), 0.92 (d, *J* = 4.2 Hz, 3H), 0.90 (d, *J* = 4.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.0 (C), 152.4 (C), 83.4 (C), 64.1 (CH), 52.6 (CH), 44.8 (CH_2), 43.3 (CH_2), 35.5 (CH_2), 27.9 (3 CH_3), 24.8 (CH), 23.8 (CH_3), 21.2 (CH_3); Anal. Calcd for $C_{14}H_{25}NO_4$: C, 61.97; H, 9.29; N, 5.16. Found: C, 62.10; H, 9.48; N, 5.22.

***tert*-butyl (2*S*,4*R*)-2-benzyl-4-hydroxy-6-oxo-1-piperidinecarboxylate (64d)**

Purification of the crude product by flash column chromatography (AcOEt/Hex, 6:4) gave **64d** (92 mg, yield = 91%): HPLC t_R 8.51 (linear gradient, 30-100% B, 20 min); colorless oil; $[\alpha]_D$ = +32.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30-7.22 (m, 5H), 4.37-4.28 (m, 1H), 4.19-4.11 (m, 1H), 3.13 (dd, *J* = 13.0, 4.2 Hz, 1H), 2.95 (dd, *J* = 13.0, 10.0 Hz, 1H), 2.82 (ddd, *J* = 16.6, 5.5, 1.5 Hz, 1H), 2.51 (dd, *J* = 16.6, 7.7 Hz, 1H), 2.33 (bs, 1H), 2.02-1.94 (m, 1H), 1.76-1.67 (m, 1H), 1.54 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.9 (C), 152.5 (C), 137.5 (C), 129.6 (2 CH), 128.6 (2 CH), 126.7 (CH), 83.6 (C), 64.3 (CH), 55.8 (CH), 43.5 (CH_2), 41.3 (CH_2), 33.8 (CH_2), 28.0 (3 CH_3). Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 67.12; H, 7.71; N, 4.50.

***tert*-butyl (2*S*,4*R*)-2-isopropyl-4-hydroxy-6-oxo-1-piperidinecarboxylate (64e)**

Purification of the crude product by flash column chromatography (AcOEt/Hex, 7:3) gave **64e** (94 mg, yield = 95%): HPLC t_R 6.00 (linear gradient, 30-100% B, 20 min); white crystals; $[\alpha]_D$ = +68.5 (*c* = 0.8, $CHCl_3$); mp 56-58°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.11-4.01 (m, 1H), 3.95 (dt, *J* = 9.0, 5.8 Hz, 1H), 2.80 (ddd, *J* = 16.3, 5.5, 2.4 Hz, 1H), 2.60 (bs, 1H), 2.39 (dd, *J* = 16.2, 10.4 Hz, 1H), 2.17-2.04 (m, 2H), 1.68-1.57 (m, 1H), 1.50 (s, 9H), 0.91 (d, *J* = 3.3 Hz, 3H), 0.89 (d, *J* = 3.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.1 (C), 153.2 (C), 83.6

(C), 64.0 (CH), 58.6 (CH), 43.2 (CH_2), 31.2 (CH_2), 30.7 (CH), 27.8 (3 CH_3), 18.9 (CH_3), 16.0 (CH_3). Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.96; H, 9.21; N, 5.35.

***tert*-butyl (2*S*,4*R*)-2-methyl-4-hydroxy-6-oxo-1-piperidinecarboxylate (64f)**

Purification of the crude product by flash column chromatography (AcOEt/Hex, 8:2) gave **64f** (83 mg, yield = 87%): HPLC t_R 11.56 (linear gradient, 5-65% B, 20 min); colorless oil; $[\alpha]_D$ = +35.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.12-4.05 (m, 2H), 2.79 (ddd, *J* = 16.4, 7.3, 2.0 Hz, 1H), 2.46 (dd, *J* = 16.6, 9.0 Hz, 1H), 2.28-2.19 (m, 1H), 2.25 (bs, 1H), 1.72-1.60 (m, 1H), 1.51 (s, 9H), 1.34 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.6 (C), 152.9 (C), 83.6 (C), 64.0 (CH), 50.1 (CH), 43.0 (CH_2), 38.7 (CH_2), 27.8 (3 CH_3), 21.8 (CH_3). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.51; H, 8.51; N, 6.05.

Preparation of 4,6-dioxopiperidines 155b-f. The desired dioxopiperidine was placed in an argon-filled round-bottom flask, dissolved in distilled CH_2Cl_2 to give a ca. 0.15M solution. The same volume of trifluoroacetic acid was introduced *via* a hypodermic syringe and the mixture was stirred for 2 h at room temperature. The solvents were evaporated and the residue was purified by filtration through a plug of silica.

benzyl (2*S*)-4,6-dioxo-2-piperidinecarboxylate (155b)

Purification of the crude product by filtration on silica (AcOEt) gave **155b** (106 mg, yield = 99%): TLC Rf 0.22 (AcOEt); white solid; $[\alpha]_D$ = +50.2 (*c* = 1.1, MeOH); mp 102-103°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (bs, 1H), 7.41-7.29 (m, 5H), 5.19 (m, 2H), 4.41-4.36 (m, 1H), 3.33 (d, *J* = 20.0 Hz, 1H), 3.24 (d, *J* = 20.0 Hz, 1H), 2.88 (dd, *J* = 16.9, 5.7 Hz, 1H) 2.72 (dd, *J* = 16.9, 10.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 200.9 (C), 169.8 (C), 168.8 (C), 134.4 (C), 128.9 (CH), 128.8 (2 CH), 128.5 (2 CH), 68.2 (CH_2), 50.7 (CH), 47.5 (CH_2), 40.7 (CH_2). Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.16; H, 5.47; N, 5.64.

(2*S*)-2-isobutyl-4,6-dioxopiperidine (155c)

Purification of the crude product by filtration on silica (CH_2Cl_2/MeOH, 9:1) gave **155c** (645 mg, yield = 100%): TLC Rf 0.52 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +30.6 (*c* = 1.0, MeOH); mp 122-124°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (bs, 1H), 3.75 (m, 1H), 3.29 (m, 2H), 2.70 (dd, *J* = 16.3, 4.2 Hz, 1H), 2.34 (dd, *J* = 16.3, 8.8 Hz, 1H), 1.77-1.68 (m, 1H), 1.56-1.47 (m, 1H), 1.42-1.34 (m, 1H), 0.95 (d, *J* = 2.7 Hz, 3H), 0.93 (d, *J* = 2.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.5 (C), 170.6 (C), 47.1 (CH), 46.7 (CH_2), 44.5 (CH_2), 44.4 (CH_2), 24.3

(CH), 22.4 (CH_3), 22.1 (CH_3). Anal. Calcd for $C_9H_{15}NO_2$: C, 63.88; H, 8.93; N, 8.28. Found: C, 64.16; H, 9.00; N, 8.24.

(2*S*)-2-benzyl-4,6-dioxopiperidine (155d)

Purification of the crude product by filtration on silica (AcOEt) gave **155d** (67 mg, yield = 100%): TLC Rf 0.24 (AcOEt); white solid; $[\alpha]_D$ = +9.2 (*c* = 1.0, MeOH); mp 156-158°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.26 (m, 3H), 7.18-7.15 (m, 2H), 7.06 (bs, 1H), 3.95-3.88 (m, 1H), 3.24 (d, *J* = 20.1 Hz, 1H), 3.12 (dd, *J* = 20.1, 0.6 Hz, 1H), 2.87-2.84 (m, 2H), 2.66 (dd, *J* = 16.2, 4.5 Hz, 1H), 2.43 (ddd, *J* = 16.1, 8.5, 0.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.8 (C), 169.1 (C), 135.4 (C), 129.3 (2 CH) 129.0 (2 CH), 127.5 (CH), 49.8 (CH), 47.1 (CH_2), 44.0 (CH_2), 42.0 (CH_2). Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.96; H, 6.36; N, 6.62.

(2*S*)-2-isopropyl-4,6-dioxopiperidine (155e)

Purification of the crude product by filtration on silica (CH_2Cl_2/MeOH, 9:1) gave **155e** (548 mg, yield = 100%): TLC Rf 0.40 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +35.3 (*c* = 1.0, MeOH); mp 88-90°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (bs, 1H), 3.53-3.48 (m, 1H), 3.27 (m, 2H), 2.62 (dd, *J* = 16.1, 4.6 Hz, 1H), 2.45 (dd, *J* = 16.1, 8.7 Hz, 1H), 1.87-1.78 (m, 1H), 0.99 (d, *J* = 6.1 Hz, 3H), 0.97 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 202.9 (C), 170.6 (C), 54.4 (CH), 46.6 (CH_2), 41.1 (CH_2), 32.2 (CH), 18.0 (CH_3), 17.7 (CH_3). Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.61; H, 8.46; N, 8.75.

(2*S*)-2-methyl-4,6-dioxopiperidine (155f)

Purification of the crude product by filtration on silica (CH_2Cl_2/MeOH, 9:1) gave **155f** (56 mg, yield = 100%): TLC Rf 0.20 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +83.8 (*c* = 1.0, MeOH); mp 124-127°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (bs, 1H), 3.81-3.75 (m, 1H), 3.22 (m, 2H), 2.64 (dd, *J* = 16.3, 3.7 Hz, 1H), 2.29 (dd, *J* = 16.3, 9.7 Hz, 1H), 1.30 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 203.4 (C), 169.5 (C), 47.1 (CH_2), 46.2 (CH_2), 44.4 (CH), 21.3 (CH_3). Anal. Calcd for $C_6H_9NO_2$: C, 56.68; H, 7.13; N, 11.02. Found: C, 56.95; H, 7.11; N, 10.98.

Reduction of 155 into 156. The desired dioxopiperidine **155** was placed in an argon-filled round-bottom flask, dissolved in distilled CH_2Cl_2 to give a ca. 0.15 M solution. 10% $^v/_v$ of acetic acid were introduced *via* a hypodermic syringe and the mixture was cooled to 0°C. $NaBH_4$ (3.0 equiv) was added portion-wise and the mixture was allowed to reach room temperature. The reaction was stirred for 72 h and monitored by C_{18} RP-HPLC. The reaction

was quenched by the addition of water and stirred for 10 min. CH_2Cl_2 was evaporated, replaced by AcOEt and the organic layer was washed with saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4, concentrated and purified by flash chromatography.

benzyl (2*S*,4*S*)-4-hydroxy-6-oxo-2-piperidinecarboxylate (156b)

Purification of the crude product by flash column chromatography (AcOEt/AcOH, 10:0.1) gave **156b** (90 mg, yield = 89%): TLC Rf 0.19 (AcOEt/AcOH, 10:0.1); colorless oil; $[\alpha]_D$ = –1.1 (*c* = 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.26 (m, 5H), 6.84 (bs, 1H), 5.17 (m, 2H), 4.17-4.13 (m, 1H), 4.09-4.05 (m, 1H), 3.86 (bs, 1H), 2.58 (dd, *J* = 17.6, 4.6 Hz, 1H), 2.44-2.38 (m, 1H), 2.31-2.23 (m, 1H), 2.18-2.09 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.4 (C), 170.8 (C), 135.0 (C), 128.6 (2 CH), 128.5 (CH), 128.3 (2 CH), 67.5 (CH_2) 63.6 (CH), 51.8 (CH), 39.8 (CH_2), 32.4 (CH_2). Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.84; H, 6.05; N, 5.47.

(2*S*,4*R*)-4-hydroxy-2-isobutyl-6-oxopiperidine (156c)

Purification of the crude product by flash column chromatography (CH_2Cl_2/MeOH, 9:1) gave **156c** (63 mg, yield = 62%): TLC Rf 0.36 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +31.4 (*c* = 0.8, MeOH); mp 179-180°C; ^{1}H NMR (300 MHz, CD_3OD) δ 4.01-3.92 (m, 1H), 3.49-3.40 (m, 1H), 2.64-2.56 (m, 1H), 2.20-2.15 (m, 2H), 1.80-1.70 (m, 1H), 1.46-1.26 (m, 3H), 0.94 (d, *J* = 3.9 Hz, 3H), 0.92 (d, *J* = 3.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD 172.3 (C), 64.0 (CH), 48.1 (CH), 45.4 (CH_2), 40.0 (CH_2), 37.4 (CH_2), 23.8 (CH), 22.0 (CH_3), 21.2 (CH_3). Anal. Calcd for $C_9H_{17}NO_2$: C, 63.13; H, 10.01; N, 8.18. Found: C, 62.83; H, 10.05; N, 8.14.

(2*S*,4*R*)-4-hydroxy-2-benzyl-6-oxopiperidine (156d)

Purification of the crude product by flash column chromatography (CH_2Cl_2/MeOH, 9:1) gave **156d** (78 mg, yield = 86%): TLC Rf 0.32 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +57.3 (*c* = 1.0, MeOH); mp 133-134°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.25 (m, 3H), 7.19-7.15 (m, 2H), 5.84 (bs, 1H), 4.06-3.97 (m, 1H), 3.63-3.54 (m, 1H), 2.88 (dd, *J* = 13.5, 5.4 Hz, 1H), 2.74-2.63 (m, 2H), 2.27 (dd, *J* = 17.0, 10.2 Hz, 1H), 2.20-2.15 (m, 1H), 2.55-1.43 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.0 (C), 136.3 (C), 129.2 (2 CH), 129.0 (2 CH), 127.2 (CH), 64.8 (CH), 51.3 (CH), 43.0 (CH_2), 40.8 (CH_2), 38.0 (CH_2). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.20; H, 7.57; N, 6.76.

(2*S*,4*R*)-4-hydroxy-2-isopropyl-6-oxopiperidine (156e)

Purification of the crude product by flash column chromatography (CH_2Cl_2/MeOH, 9:1) gave **9e** (99 mg, yield = 49%): TLC Rf 0.18 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +11.4 (*c* =

1.2, MeOH); mp 149-152°C; ^{1}H NMR (300 MHz, CD_3OD) δ 4.01-3.90 (m, 1H), 3.32-3.28 (m, 1H), 2.60 (ddd, *J* = 17.0, 5.7, 2.2 Hz, 1H), 2.13 (ddd, *J* = 17.0, 10.6, 0.7 Hz, 1H), 2.06-1.98 (m, 1H), 1.88-1.77 (m, 1H), 1.40-1.28 (m, 1H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) 172.8 (C), 64.2 (CH), 55.1 (CH), 40.1 (CH_2), 32.4 (CH_2), 31.5 (CH), 16.9 (CH_3), 15.5 (CH_3). Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.34; H, 9.77; N, 8.85.

(2*S*,4*R*)-4-hydroxy-2-methyl-6-oxopiperidine (156f)

Purification of the crude product by flash column chromatography (CH_2Cl_2/MeOH, 9:1) gave **156f** (67 mg, yield = 37%): TLC Rf 0.14 (CH_2Cl_2/MeOH, 9:1); white solid; $[\alpha]_D$ = +36.8 (*c* = 1.0, MeOH); mp 181-183°C; ^{1}H NMR (300 MHz, CD_3OD δ 4.02-3.92 (m, 1H), 3.53-3.41 (m, 1H), 2.59 (ddd, *J* = 17.2, 5.7, 2.0 Hz, 1H), 2.18-2.09 (m, 2H), 1.37-1.25 (m, 1H), 1.21 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) 172.3 (C), 63.9 (CH), 45.7 (CH), 39.6 (CH_2), 39.4 (CH_2), 20.9 (CH_3). Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 56.05; H, 8.64; N, 10.77.

***tert*-butyl *N*-[(1*S*,3*S*)-3,5-dihydroxy-1-(hydroxymethyl)pentyl]carbamate (157)**

Sodium borohydride (48 mg, 1.27 mmol) was added to a solution of **64a** (80 mg, 0.254 mmol) in 2.0 mL of EtOH at 0°C. The mixture was allowed to reach room temperature and stirred for 6 h. After being quenched by water, the mixture was stirred for a further 10 min. Evaporation of the solvent gave an oil which was dissolved in AcOEt. The solution was washed with water and dried over Na_2SO_4. Evaporation of the filtrate afforded an oil which was subjected to filtration through a silica pad (CH_2Cl_2/MeOH, 9:1). Pure **157** was recovered (20 mg, yield = 32%): TLC Rf 0.42 (CH_2Cl_2/MeOH, 9:1); colourless oil; $[\alpha]_D$ = −11.4 (*c* = 0.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15 (bd, *J* = 8.8 Hz, 1H), 4.64 (m, 1H), 3.91-3.80 (m, 3H), 3.72 (dd, *J* = 10.9, 4.0 Hz, 1H), 3.62 (dd, *J* = 10.9, 4.6 Hz, 1H), 3.32 (bs, 1H), 2.76 (bs, 1H), 1.96 (bs, 1H), 1.81-1.64 (m, 2H), 1.62-1.55 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 157.4 (C), 80.2 (C), 68.5 (CH), 65.3 (CH_2), 61.8 (CH_2), 49.1 (CH), 40.4 (CH_2), 38.0 (CH_2), 28.3 (3 CH_3).

di(*tert*-butyl) (2*S*,4*S*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-6-oxo-1,2-piperidinedicarboxylate (158)

Imidazole (430 mg, 6.32 mmol) and TBDMSCl (476 mg, 3.16 mmol) were added to a solution of 500 mg (1.58 mmol) of **64a** in 5.0 mL of CH_2Cl_2 at 0°C. The mixture was allowed to reach room temperature and stirred overnight. After evaporation of the solvent, the residue

was dissolved in AcOEt. The solution was washed with 1N $KHSO_4$, brine and water, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by filtration through a plug of silica (AcOEt/Hex, 2:8) to yield **158** (650 mg, yield = 95%): HPLC t_R 18.16 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = -29.4 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (dd, *J* = 7.6, 6.7 Hz, 1H), 4.12-4.03 (m, 1H), 2.74 (ddd, *J* = 16.6, 5.3, 1.8 Hz, 1H), 2.49 (dd, *J* = 16.6, 8.5 Hz, 1H), 2.37-2.28 (m, 1H), 2.08-1.96 (m, 1H), 1.50 (s, 9H), 1.46 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.0 (C), 168.9 (C), 152.0 (C), 83.5 (C), 82.2 (C), 64.4 (CH), 56.6 (CH), 44.4 (CH_2), 34.9 (CH_2), 27.9 (3 CH_3), 27.9 (3 CH_3), 25.8 (3 CH_3), 18.1 (C), –4.7 (CH_3), –4.8 (CH_3). Anal. Calcd for $C_{21}H_{39}NO_6Si$: C, 58.71; H, 9.15; N, 3.26. Found: C, 59.01; H, 9.14; N, 3.46.

***tert*-butyl (2*S*,4*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-{[*tert*-butyl(dimethyl)silyl]oxy}-6-hydroxyhexanoate (159)**

Compound **158** (500 mg, 1.16 mmol) was dissolved in 10.0 mL of EtOH and cooled to 0°C. After portion-wise addition of $NaBH_4$ (220 mg, 5.82 mmol), the mixture was allowed to reach room temperature and stirred overnight. After being quenched by water, the mixture was stirred for a further 10 min. Evaporation of the solvent gave an oil which was dissolved in AcOEt. The solution was washed with water and dried over Na_2SO_4. Evaporation of the filtrate afforded an oil which was subjected to filtration through a silica pad (AcOEt/Hex, 1:1). Pure **159** was recovered (440 mg, yield = 87%): HPLC t_R 16.12 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = -2.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.26 (bd, *J* = 7.3 Hz, 1H), 4.16-4.08 (m, 1H), 4.08-4.00 (m, 1H), 3.82-3.68 (m, 2H), 2.22 (bs, 1H), 1.98-1.63 (m, 4H) 1.45 (s, 9H), 1.42 (s, 9H), 0.90 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.0 (C), 155.3 (C), 81.6 (C), 79.5 (C), 68.5 (CH), 59.5 (CH_2), 52.0 (CH), 38.8 (CH_2), 38.4 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 25.8 (3 CH_3), 17.9 (C), –4.5 (CH_3), –4.8 (CH_3). Anal. Calcd for $C_{21}H_{43}NO_6Si$: C, 58.16; H, 9.99; N, 3.23. Found: C, 57.89; H, 10.35; N, 3.23.

***tert*-butyl (2*S*,4*R*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-4-{[*tert*-butyl(dimethyl)silyl]oxy}hexanoate (160)**

N,*N*-Diisopropylethylamine (194 µL, 1.14 mmol) was added to a solution of 330 mg (0.76 mmol) of **159** in 5.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 88 µL (1.14 mmol) of MsCl were added *via* a hypodermic syringe. The mixture was allowed to reach room temperature and stirred for 3 h. The reaction was quenched by water, CH_2Cl_2 was evaporated and replaced by AcOEt. The organic phase was washed with 1N

$KHSO_4$, brine, saturated $NaHCO_3$ and water, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in 10.0 mL of DMF. 148 mg (2.28 mmol) of NaN_3 were added to the solution which was heated to 80°C for 8 h. After the solution was cooled to room temperature, water was added and the solution was extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered, and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:9) to yield pure **160** (298 mg, yield = 86%): HPLC t_R 16.80 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D$ = +1.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.22 (bd, *J* = 7.1 Hz, 1H), 4.18-4.12 (m, 1H), 3.97-3.89 (m, 1H), 3.36 (m, 2H), 1.94-1.83 (m, 1H), 1.81-1.68 (m, 3H); 1.46 (s, 9H), 1.43 (s, 9H), 0.90 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.8 (C), 155.3 (C), 81.7 (C), 79.5 (C), 67.0 (CH), 51.9 (CH), 47.5 (CH_2), 39.1 (CH_2), 35.8 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 25.8 (3 CH_3), 17.9 (C), –4.5 (CH_3), –4.8 (CH_3). Anal. Calcd for $C_{21}H_{42}N_4O_5Si$: C, 54.99; H, 9.23; N, 12.22. Found: C, 55.23; H, 9.30; N, 12.44.

***tert*-butyl *N*-[(3*S*,5*R*)-5-(azidoethyl)-2-oxotetrahydro-3-furanyl]carbamate (161)**

Tetrabutylammonium fluoride (543 mg, 2.08 mmol) was added to a solution of **160** (250 mg, 0.429 mmol) in 20.0 mL of THF at 0°C. The mixture was allowed to reach rt and stirred for 2 h. The reaction was quenched by adding a saturated solution of NH_4Cl, THF was evaporated and replaced by AcOEt. The organic layer was washed with 1N $KHSO_4$, brine and saturated $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated in vacuo. Crude **161** was filtered through a short plug of silica (AcOEt/Hex, 1:1) to afford pure **161** (281 mg, yield = 75%): HPLC t_R 7.56 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +57.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.12 (bs, 1H), 4.56-4.46 (m, 1H), 4.42-4.35 (m, 1H), 3.53-3.48 (m, 2H), 2.89-2.79 (m, 1H), 2.02-1.81 (m, 3H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.3 (C), 155.3 (C), 80.6 (C), 74.8 (CH), 51.3 (CH), 47.4 (CH_2), 36.5 (CH_2), 34.6 (CH_2), 28.2 (3 CH_3). Anal. Calcd for $C_{11}H_{18}N_4O_4$: C, 48.88; H, 6.71; N, 20.73. Found: C, 49.15; H, 6.72; N, 20.66.

***tert*-butyl (2*S*,4*R*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoate (162)**

Acetic acid (58 μL, 1.00 mmol) was added to a solution of **160** (92 mg, 0.200 mmol) in 2.0 mL of THF at 0°C. 262 mg (1.00 mmol) of TBAF were added and the solution was stirred for 96 h at rt. The reaction was quenched by adding a saturated solution of NH_4Cl, THF was evaporated and replaced by AcOEt. The organic layer was washed with 1N $KHSO_4$, brine and saturated $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated in vacuo. Purification of the

crude product by flash column chromatography (AcOEt/Hex, 2:8) gave pure **162** (66 mg, yield = 96%): HPLC t_R 10.93 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +15.1 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.39 (bd, J = 7.7 Hz, 1H), 4.41-4.33 (m, 2H), 3.70 (bt, J = 9.7 Hz, 1H), 3.50-3.37 (m, 2H), 1.90-1.80 (m, 1H), 1.76-1.57 (m, 3H), 1.44 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.7 (C), 157.0 (C), 82.5 (C), 80.6 (C), 64.1 (CH), 51.0 (CH), 48.3 (CH_2), 42.2 (CH_2), 35.6 (CH_2), 28.2 (3 CH_3), 27.9 (3 CH_3).Anal. Calcd for $C_{15}H_{28}N_4O_5$: C, 52.31; H, 8.19; N, 16.27. Found: C, 52.59; H, 8.14; N, 16.28.

(3*S*,5*R*)-5-(azidoethyl)-2-oxotetrahydro-3-furanaminium 4-methyl-1-benzenesulfonate (163)

p-Toluenesulfonic acid (55 mg, 0.289 mmol) was added to a solution of 50 mg (0.145 mmol) of **162** in 2.0 mL of CH_3CN at 0°C. The mixture was stirred for 6 h (until complete consumption of **162**). A large volume of cold Et_2O was added and the suspension was filtered. The precipitate was washed with CH_2Cl_2 / Et_2O and dried under high vacuum to afford pure **163** (50 mg, yield = 100%): TLC Rf 0.45 (AcOEt/pyridine/acetic acid/water, 8:2:0.5:1); white solid; $[\alpha]_D$ = +25.4 (*c* = 1.0, MeOH); mp = 234-240°C (decomposition); ^{1}H NMR (300 MHz, CD_3OD) δ 7.70 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 4.72-4.63 (m, 1H), 4.40 (dd, *J* = 8.7, 12.0 Hz, 1H), 3.55-3.45 (m, 1H), 2.87-2.78 (m, 1H), 2.37 (s, 3H), 2.03-1.91 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.0 (C), 138.6 (C), 126.8 (2 CH), 124.0 (2 CH), 74.4 (CH), 47.6 (CH), 32.2 (CH_2), 31.4 (CH_2), 18.3 (CH_3). Anal. Calcd for $C_{13}H_{18}N_4O_5S$: C, 45.61; H, 5.30; N, 16.36. Found: C, 45.76; H, 5.31; N, 16.19.

9*H*-fluoren-9-ylmethyl *N*-[(3*S*,5*R*)-5-(azidoethyl)-2-oxotetrahydro-3-furanyl]carbamate (164)

Compound **163** (75 mg, 0.219 mmol) was dissolved in 1.0 mL of H_2O. Solid K_2CO_3 (90 mg, 0.651 mmol) and FmocOSu (110 mg, 0.326 mmol), dissolved in 1.0 mL of acetone, were added to the solution stirred at ambient temperature. After 3 h, acetone was evaporated and replaced by AcOEt. The solution was washed with 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo. Purification of the crude product by flash column chromatography (AcOEt/Hex, 1:1) gave pure **164** (79 mg, yield = 92%): HPLC t_R 12.47 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = +48.0 (*c* = 1.0, $CHCl_3$); mp = 132-134°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.48 (bd, *J* = 4.8 Hz, 1H), 4.51-4.43 (m, 1H), 4.42 (d, *J* = 6.6 Hz, 2H), 4.21 (t, *J* = 6.6 Hz, 1H), 3.51-3.47 (m, 2H), 2.84 (m, 1H), 2.04-1.83 (m, 1H), 1.80-

1.59 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 174.0 (C), 155.9 (C), 143.6 (2 C), 141.3 (2 C), 127.8 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 74.9 (CH), 67.3 (CH_2), 51.6 (CH), 47.4 (CH_2), 47.0 (CH), 36.1 (CH_2), 34.5 (CH_2).

di(*tert*-butyl) (2*S*,4*S*)-4-{[*tert*-butyl(diphenyl)silyl]oxy}-6-oxo-1,2-piperidinedicarboxylate (165)

Imidazole (432 mg, 6.34 mmol) and TBDPSCl (872 mg, 3.17 mmol) were added to a solution of 500 mg (1.58 mmol) of **64a** in 20.0 mL of CH_2Cl_2 at 0°C. The mixture was allowed to reach room temperature and stirred overnight. After evaporation of the solvent, the residue was dissolved in AcOEt. The solution was washed with 1N $KHSO_4$, brine and water, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by filtration through a plug of silica (AcOEt/Hex, 2:8) to yield **165** (770 mg, yield = 88%): HPLC t_R 17.30 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D = -19.5$ (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66-7.60 (m, 4H), 7.44-7.38 (m, 6H), 4.30 (dd, J = 8.3, 6.6 Hz, 1H), 4.06-3.97 (m, 1H), 2.65 (ddd, J = 16.6, 5.5, 2.0 Hz, 1H), 2.53 (dd, J = 16.6, 9.0 Hz, 1H), 2.30-2.21 (m, 1H), 2.07-1.97 (m, 1H), 1.47 (s, 9H), 1.45 (s, 9H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.0 (C), 168.7 (C), 151.9 (C), 135.6 (4 CH), 133.4 (C), 133.1 (C), 130.0 (2 CH), 127.8 (4 CH), 83.6 (C), 82.2 (C), 65.0 (CH), 56.6 (CH), 44.0 (CH_2), 34.7 (CH_2), 27.9 (3 CH_3), 27.8 (3 CH_3), 26.9 (3 CH_3), 19.0 (C). Anal. Calcd for $C_{31}H_{43}NO_6Si$: C, 67.24; H, 7.83; N, 2.53. Found: C, 67.02; H, 7.72; N, 2.65.

***tert*-butyl (2*S*,4*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-{[*tert*-butyl(diphenyl)silyl]oxy}-6-hydroxyhexanoate (166)**

Compound **165** (710 mg, 1.28 mmol) was dissolved in 10.0 mL of EtOH and cooled to 0°C. After portion-wise addition of $NaBH_4$ (242 mg, 6.40 mmol), the mixture was allowed to reach room temperature and stirred overnight. After being quenched by water, the mixture was stirred for a further 10 min. Evaporation of the solvent gave an oil which was dissolved in AcOEt. The solution was washed with water and dried over Na_2SO_4. Evaporation of the filtrate afforded an oil which was subjected to filtration through a silica pad (AcOEt/Hex, 4:6). Pure **166** was recovered (690 mg, yield = 97%): HPLC t_R 15.66 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D = +4.8$ (c = 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71-7.68 (m, 4H), 7.44-7.37 (m, 6H), 5.30 (bd, J = 7.9 Hz, 1H), 4.14-4.04 (m, 2H), 3.63-3.49 (m, 2H), 1.96-1.92 (m, 1H), 1.75-1.71 (m, 1H), 1.57 (m, 2H), 1.42 (s, 9H), 1.36 (s, 9H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.7 (C), 155.3 (C), 135.9 (2 CH), 135.8 (2 CH),

133.6 (C), 133.2 (C), 129.9 (CH), 129.8 (CH), 127.8 (2 CH), 127.7 (2 CH), 81.6 (C), 79.5 (C), 69.6 (CH), 50.0 (CH_2), 52.0 (CH), 38.4 (CH_2), 38.3 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 26.9 (3 CH_3), 19.3 (C). Anal. Calcd for $C_{31}H_{47}NO_6Si$: C, 66.75; H, 8.49; N, 2.51. Found: C, 66.88; H, 8.65; N, 2.56.

***tert*-butyl (2*S*,4*R*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-4-{[*tert*-butyl(diphenyl)silyl]oxy}hexanoate (167)**

N,*N*-Diisopropylethylamine (307 µL, 1.80 mmol) was added to a solution of **166** (670 mg, 1.20 mmol) in 20.0 mL of CH_2Cl_2 at ambient temperature. The solution was cooled to 0°C and 139 µL (1.80 mmol) of MsCl were added *via* a hypodermic syringe. The mixture was allowed to reach room temperature and stirred for 3 h. The reaction was quenched by water, CH_2Cl_2 was evaporated and replaced by AcOEt. The organic phase was washed with 1N $KHSO_4$, brine, saturated $NaHCO_3$ and water, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in 10.0 mL of DMF. Sodium azide (234 mg, 3.60 mmol) was added to the solution which was heated to 80°C for 8 h. After being cooled to room temperature, the solution was diluted with water and further extracted twice with AcOEt. The combined organic layers were washed with water, dried over Na_2SO_4, filtered, and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:9) to yield pure **167** (560 mg, 80%): HPLC t_R 18.55 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D = -10.7$ (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71-7.67 (m, 4H), 7.44-7.35 (m, 6H), 5.21 (bd, J = 7.6 Hz, 1H), 4.15-4.08 (m, 1H), 3.96-3.92 (m, 1H), 3.16 (t, J = 7.1 Hz, 2H), 1.97-1.83 (m, 2H), 1.75-1.68 (m, 2H); 1.43 (s, 9H), 1.37 (s, 9H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.6 (C), 155.3 (C), 135.8 (4 CH), 133.6 (C), 133.0 (C), 129.9 (2 CH), 127.8 (4 CH), 81.7 (C), 79.5 (C), 69.0 (CH), 51.8 (CH), 47.5 (CH_2), 38.6 (CH_2), 35.2 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3), 26.9 (3 CH_3), 19.3 (C). Anal. Calcd for $C_{31}H_{46}N_4O_5Si$: C, 63.89; H, 7.96; N, 9.61. Found: C, 63.72; H, 8.11; N, 9.79.

(2*S*,4*R*)-6-azido-4-{[*tert*-butyl(diphenyl)silyl]oxy}-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic acid (168)

Compound **167** (200 mg, 0.343 mmol) was dissolved in 2.0 mL of CH_2Cl_2 and cooled to 0°C. 2.0 mL of TFA were slowly added *via* a hypodermic syringe and the solution was stirred for 3 h. The mixture was allowed to reach room temperature and stirred for an additional 3 h. The reaction was checked by TLC (AcOET/pyridine/acetic acid/water, 8:2:0.5:1) and stirred at room temperature until **167** was not detected. The solution was cooled to 0°C and quenched by the addition of 50.0 mL of a 1N NH_4OH solution. The solution was extracted twice with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated in

a vacuum. The residue was dissolved in 5.0 mL of acetone and the same volume of water was added to the solution. 142 mg (1.03 mmol) of K_2CO_3 and 139 mg (0.412 mmol) of FmocOSu dissolved in 1.0 mL of acetone were added to the solution. The mixture was stirred overnight. Acetone was evaporated and replaced by AcOEt prior to washing the solution with 1N $KHSO_4$, brine and water. The crude product was purified by flash column chromatography (AcOEt/Hex/AcOH, 3:7:0.1) to yield **168** (210 mg, 94%): HPLC t_R 15.58 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D$ = +2.5 (*c* = 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80-7.70 (m, 6H), 7.63-7.58 (m, 2H), 7.46-7.29 (m, 10H), 5.57 (bd, *J* = 6.9 Hz, 1H), 4.51-4.45 (m, 1H), 4.41-4.35 (m, 2H), 4.24-4.20 (m, 1H), 4.07-3.98 (m, 1H), 3.22-3.17 (m, 2H), 2.12-1.94 (m, 2H), 1.81-1.75 (m, 2H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.4 (C), 156.1 (C), 143.8 (C) 143.7 (C), 141.3 (2 C), 135.9 (2 CH), 135.8 (2 CH), 133.4 (C), 132.7 (C), 130.1 (CH), 130.0 (CH), 127.9 (2 CH), 127.8 (2 CH), 127.7 (2 CH), 127.1 (2 CH), 125.2 (CH), 125.1 (CH), 120.0 (2 CH), 68.6 (CH), 67.1 (CH_2), 51.5 (CH), 47.4 (CH_2), 47.1 (CH), 37.4 (CH_2), 35.3 (CH_2), 27.0 (3 CH_3), 14.2 (C). Anal. Calcd for $C_{37}H_{40}N_4O_5Si$: C, 68.49; H, 6.21; N, 8.64. Found: C, 68.57; H, 6.06; N, 8.40.

***tert*-butyl 2-({(2*S*,4*R*)-6-azido-2-[(*tert*-butoxycarbonyl)amino]-4-hydroxyhexanoyl}amino)acetate (169)**

Lactone **161** (45 mg, 0.166 mmol) was placed in a 10 mL round-bottom flask and dissolved in 2.0 mL of distilled THF. 79 mg (0.830 mmol) of H-Gly-O^{t}Bu, previously dissolved in 1.0 mL of THF, were introduced *via* a hypodermic syringe. The white suspension was stirred at room temperature and the reaction was checked by TLC (AcOEt/Hex, 1:1). After 1 weak, **161** completely disappeared. THF was evaporated and replaced by AcOEt. The solution was washed with 1N $KHSO_4$, brine and water and evaporated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:1) to yield **169** (50 mg, yield = 75%): HPLC t_R 8.77 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +14.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (m, 1H), 5.70 (d, *J* = 7.9 Hz, 1H), 4.46-4.41 (m, 1H), 3.92 (d, *J* = 5.1 Hz, 2H), 3.81 (m, 1H), 3.51-3.37 (m, 2H), 1.87-1.64 (m, 4H), 1.45 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.9 (C), 168.9 (C), 156.7 (C), 82.5 (C), 80.5 (C), 65.1 (CH), 51.5 (CH), 48.4 (CH_2), 42.0 (CH_2), 41.9 (CH_2), 35.9 (CH_2), 28.2 (3 CH_3), 28.0 (3 CH_3).

***tert*-butyl 2-({(2*S*,4*R*)-6-azido-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-4-hydroxyhexanoyl}amino)acetate (170)**

Lactone **164** (75 mg, 0.191 mmol) was placed in a 10 mL round-bottom flask and dissolved in 2.0 mL of distilled THF. 91 mg (0.956 mmol) of H-Gly-O^{t}Bu, previously dissolved in 1.0 mL of THF, were introduced *via* a hypodermic syringe. The white suspension was stirred at room temperature and the reaction was checked by TLC (AcOEt/Hex, 1:1). After 96 h the reaction was stopped. THF was evaporated and replaced by AcOEt. The solution was washed with 1N $KHSO_4$, brine and water and evaporated in vacuo. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:1) to yield **170** (21 mg, yield = 21%): HPLC t_R 12.71 (linear gradient, 30-100% B, 20 min); colourless oil; $[\alpha]_D$ = +6.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.45 (t, *J* = 7.5 hz, 2H), 7.36 (dt, *J* = 7.5 Hz, 0.8 Hz, 2H), 6.71-6.69 (m, 1H), 6.07 (d, J = 7.7 Hz, 1H), 4.55-4.43 (m, 3H), 4.26 (t, *J* = 7.0 Hz, 1H), 4.06-4.00 (m, 1H), 3.98-3.92 (m, 1H), 3.90-3.83 (m, 1H), 3.51-3.48 (m, 2H), 1.88-1.64 (m, 4H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.6 (C), 168.8 (C), 157.2 (C), 143.7 (C), 143.6 (C), 141.4 (2 C), 82.8 (C), 67.4 (CH_2), 65.7 (CH), 52.3 (CH), 48.4 (CH_2), 47.2 (CH), 42.1 (CH_2), 41.7 (CH_2), 35.9 (CH_2), 28.1 (3 CH_3).

di(*tert*-butyl) (2*S*,4*R*)-4-{[*tert*-butyl(dimethyl)silyl]oxy}-1,2-piperidinedicarboxylate (173)

A 1M solution of $LiEt_3BH$ in THF (307 μL, 0.307 mmol) was added to a solution of 110 mg (0.256 mmol) of **158** in 2.0 mL of THF at –78°C and the mixture was stirred for 30 min. The reaction was quenched by 0.6 mL of saturated $NaHCO_3$ and allowed to reach 0°C. H_2O_2 was added *via* a hypodermic syringe (5 drops) and the mixture was stirred for 30 min at 0°C. The solvents were evaporated and replaced by CH_2Cl_2. The organic phase was washed with water, dried over Na_2SO_4, filtered and concentrated in vacuo to give the intermediate **172**. Crude **172** and Et_3SiH (30 mg, 0.256 mmol) were dissolved in 5.0 mL of CH_2Cl_2 and cooled to –78°C. BF_3•Et_2O (40 mg, 0.282 mmol) was introduced by drop-wise addition and the mixture was stirred for 30 min. Et_3SiH (30 mg, 0.256 mmol) and BF_3•Et_2O (40 mg, 0.282 mmol) were consecutively added *via* a hypodermic syringe and the mixture was stirred for an additional 2 h. The reaction was quenched by 0.6 mL of a saturated $NaHCO_3$ solution and allowed to reach room temperature. After addition of a large volume of a saturated $NaHCO_3$ solution, the mixture was extracted with CH_2Cl_2. The organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo. Purification of the crude product by flash column chromatography (AcOEt/Hex, 1:9) gave **173** (yield < 50%): TLC Rf 0.36 (AcOEt/Hex, 1:9); colourless oil; $[\alpha]_D$ = –31.4 (*c* = 1.0, $CHCl_3$); appeared as a mixture of two conformers ^{1}H NMR (300 MHz,

$CDCl_3$) δ 4.61-4.52 (m, 0.5H) and 4.47-4.38 (m,0.5H), 4.07-4.03 (m, 1H), 3.88-3.68 (m, 1H), 3.40-3.19 (m, 1H), 2.35-2.32 (m, 0.5H) and 2.30-2.26 (m, 0.5H), 1.85-1.77 (m, 1H), 1.61-1.53 (m, 2H), 1.44 (s, 18H), 0.87 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.0 (C), 155.7 (C), 80.7 (C), 79.5 (C), 64.1 (CH), 52.4 (CH), 34.9 (CH_2), 33.1 (CH_2), 32.2 (CH_2), 28.3 (3 CH_3), 28.2 (3 CH_3), 26.0 (3 CH_3), 18.5 (C), –4.8 (CH_3), –4.9 (CH_3). Anal. Calcd for $C_{21}H_{41}NO_5Si$: C, 60.68; H, 9.94; N, 3.37. Found: C, 60.83; H, 10.03; N, 3.37.

di(*tert*-butyl) (2*S*)-3,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (174)

Pure **174** was isolated in low yield after flash column chromatography (AcOEt/Hex, 1:9) from the crude product obtained for the preparation of **173**: TLC Rf 0.33 (AcOEt/Hex, 9:1); colorless oil; $[\alpha]_D$ = –9.3 (*c* = 1.0, $CHCl_3$); appeared as a mixture of two conformers ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.77-5.71 (m, 1.5H) and 5.64-5.59 (m, 0.5H), 4.94-4.91 (m, 0.5H) and 4.74-4.71 (m, 0.5H), 4.09-3.99 (m, 1H), 3.91-3.66 (m, 1H), 2.66-2.56 (m, 1H), 2.47-2.37 (m, 1H), 1.48 (s, 4.5 H) and 1.46 (s, 4.5H), 1.42 (s, 4.5H) and 1.41 (s, 4.5H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.7 (C), 155.8 and 155.3 (C), 124.2 and 123.9 (CH), 122.5 and 121.9 (CH), 81.4 and 81.3 (C), 80.0 (C), 53.0 and 51.6 (CH), 42.2 and 41.7 (CH_2), 28.4 (3 CH_3), 28.0 (3 CH_3), 26.6 (CH_2). Anal. Calcd for $C_{15}H_{25}NO_4$: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.28; H, 8.79; N, 4.74.

di(*tert*-butyl) (2*S*,4*R*)-4-hydroxy-1,2-piperidinedicarboxylate (175)

Compound **64a** (200 mg, 0.634 mmol) was dissolved in 4.0 mL of anhydrous THF, and cooled to 0°C. BH_3•SMe_2 (301 µL, 3.17 mmol) was introduced *via* a hypodermic syringe. The mixture was allowed to reach room temperature and stirred overnight. After being cooled to 0°C, the reaction was quenched by the addition of 1.0 mL of MeOH and stirred for a further 10 min. Evaporation of the solvent gave a residue, which was dissolved in AcOEt. The solution was washed with saturated $NaHCO_3$, water, 1N $KHSO_4$ and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was placed under high vacuum to eliminate the dimethylsulfide derivatives and gave pure **175** (yield = 100%): TLC Rf 0.30 (AcOEt/Hex, 1:1); white crystals; $[\alpha]_D$ = –54.6 (*c* = 0.9, $CHCl_3$); mp 91-93°C; appeared as a mixture of conformers ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.68-4.62 (m, 0.5H) and 4.50-4.43 (m, 0.5H), 4.10-4.06 (m, 1H), 3.85-3.68 (m, 1H), 3.36-3.17 (m, 1H), 2.36-2.31 (m, 1H), 2.10 (bs, 1H), 1.85-1.77 (m, 1H), 1.73-1.54 (m, 2H), 1.42 (bs, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.0 (C), 155.6 (C), 81.3 (C), 79.7 (C), 63.1 (CH), 52.2 and 51.1 (CH), 35.9 and 34.9 (CH_2), 33.1 (CH_2), 31.0 (CH_2), 28.3 (3 CH_3), 27.9 (3 CH_3). Anal. Calcd for $C_{15}H_{27}NO_5$: C, 59.78; H, 9.03; N, 4.65. Found: C, 59.50; H, 9.14; N, 4.67.

(2*S*,4*R*)-1-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-4-hydroxy-2-piperidinecarboxylic acid (177)

Pipecolate **175** (250 mg, 0.829 mmol) was placed in a round-bottom flask and dissolved in 2.0 mL of trifluoroacetic acid. The solution was stirred for 2 h at room temperature and checked by TLC. Trifluoroacetic acid was co-evaporated with hexane and the residue was dried under vacuum. Crude **176** was dissolved in 3.0 mL of water. K_2CO_3 (344 mg, 2.49 mmol) and FmocOSu (280 mg, 0.829 mmol) dissolved in 3.0 mL of acetone were consecutively added. After the pH (> 9) of the resulting solution had been checked, the mixture was stirred overnight. Acetone was evaporated and the residual solution was diluted with water. The aqueous solution was acidified with solid $KHSO_4$ prior to extraction with AcOEt. The organic phase was washed with 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in a vacuum to yield a yellowish oil. Purification of the crude product by flash column chromatography (AcOEt/AcOH, 10:0.1) gave **177** (253 mg, yield = 83%): HPLC t_R 8.77 (linear gradient, 30-100% B, 20 min); white solid; $[\alpha]_D$ = –9.5 (*c* = 1.0, $CHCl_3$); mp 200-201°C; appeared as a mixture of conformers ^{1}H NMR (300 MHz, CD_3OD) δ 7.82 (d, *J* = 7.3 Hz, 2H), 7.68-7.58 (m, 2H), 7.41 (t, *J* = 7.3 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 2H), 4.68 (d, *J* = 5.8 Hz, 0.5H) and 4.53 (d, *J* = 6.7 Hz, 0.5H), 4.47-4.39 (m, 2H), 4.31-4.22 (m, 1H), 4.08-4.03 (m, 1H), 3.87-3.74 (m, 1H), 3.60-3.43 (m, 1H), 2.47-2.38 (m, 1H), 1.92-1.81 (m, 1H), 1.77-1.58 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) 175.2 (C), 158.1 and 157.8 (C), 145.3 (C), 145.2 (C), 145.1 (C), 142.6 (C), 128.8 (2 CH), 128.1 (2 CH), 126.1 (2 CH), 120.9 (2 CH), 68.8 (CH_2), 63.7 (CH), 52.5 and 52.2 (CH), 48.4 (CH), 37.1 and 36.8 (CH_2), 34.2 (CH_2), 32.0 and 31.9 (CH_2); Anal. Calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.47; H, 6.09; N, 3.63.

di(*tert*-butyl) (2*S*)-6-oxo-3,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (178)

p-Nitrobenzoic acid (239 mg, 1.43 mmol) and PPh_3 (374 mg, 1.43 mmol) were added to a solution of **64a** (300 mg, 0.951 mmol) in 15.0 mL of distilled THF. The mixture was cooled to 0°C prior to the drop-wise addition of a solution of DIAD (288 mg, 1.42 mmol) in 4.0 mL of dry THF. The solution was protected from light and stirred overnight with a slow increase of temperature from 0°C to rt. THF was evaporated and replaced by AcOEt. The solution was washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 3:7) to yield pure **178** (243 mg, yield = 86%): HPLC t_R 9.30 (linear gradient, 30-100% B, 20 min); colourless crystals; $[\alpha]_D$ = +11.6 (*c* = 1.0, $CHCl_3$); mp 99-102°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55-6.50 (m, 1H), 5.88-5.84 (m, 1H), 4.82-4.79 (m, 1H), 2.82-2.73 (m, 1H), 2.71-2.61 (m, 1H),

1.45 (s, 9H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.5 (C), 162.5 (C), 152.1 (C), 139.7 (CH), 126.5 (CH), 83.1 (C), 82.5 (C), 56.2 (CH), 27.9 (3 CH_3), 27.7 (3 CH_3), 27.2 (CH_2); Anal. Calcd for $C_{15}H_{23}NO_5$: C, 60.59; H, 7.80; N, 4.71. Found: C, 60.13; H, 7.82; N, 4.77.

di(*tert*-butyl) (2*S*,4*S*)-4-[(4-nitrobenzoyl)oxy]-1,2-piperidinedicarboxylate (179)

p-Nitrobenzoic acid (83 mg, 0.497 mmol) and PPh_3 (130 mg, 0.496 mmol) were added to a solution of **175** (100 mg, 0.332 mmol) in 5.0 mL of distilled THF. The mixture was cooled to 0°C prior to the drop-wise addition of a solution of DIAD (101 mg, 0.499 mmol) in 1.0 mL of dry THF. The solution was protected from light and stirred overnight with a slow increase of temperature from 0°C to rt. THF was evaporated and replaced by AcOEt. The solution was washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:9) to yield pure **179** (80 mg, yield = 53%): HPLC t_R 17.08 (linear gradient, 30-100% B, 20 min); white crystals; $[\alpha]_D$ = –20.5 (c = 1.0, $CHCl_3$); mp 165-167°C; appeared as a mixture of two conformers ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 8.9 Hz, 2H), 8.16 (d, J = 8.8 Hz, 2H), 5.04-4.93 (m, 1H) and 4.77-4.71 (m, 1H), 4.19-4.00 (m, 1H), 3.21-3.03 (m, 1H), 2.64-2.53 (m, 1H), 2.16-2.04 (m, 1H), 1.89-1.76 (m, 1H), 1.71-1.57 (m, 1H), 1.48 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.0 (C), 163.8 (C), 155.1 (C), 150.5 (C), 135.6 (C), 130.7 (2 CH), 123.5 (2 CH), 82.2 (C), 80.4 (C), 70.3 (CH), 55.0 and 54.0 (CH), 40.1 and 39.5 (CH_2), 31.9 (CH_2), 30.5 and 30.4 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3). Anal. Calcd for $C_{22}H_{30}N_2O_8$: C, 58.66; H, 6.71; N, 6.22. Found: C, 58.75; H, 6.96; N, 6.22.

di(*tert*-butyl) (2*S*)-5,6-dihydro-1,2(2*H*)-pyridinedicarboxylate (180)

Pure **180** was isolated in 32% yield after flash column chromatography (AcOEt/Hex, 1:9) of the crude product obtained for the preparation of **179**: TLC Rf 0.52 (AcOEt/Hex, 1:9); colorless oil; $[\alpha]_D$ = –207.9 (c = 1.1, $CHCl_3$); appeared as a mixture of two conformers ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.96-5.87 (m, 1H), 5.82-5.69 (m, 1H), 4.82-4.77 (m, 0.5H) and 4.67-4.62 (m, 0.5H), 4.18-4.12 (m, 0.5H) and 4.03-3.97 (m, 0.5H), 3.11-3.02 (m, 0.5H) and 2.99-2.89 (m, 0.5H), 2.25-2.14 (m, 1H), 2.07-1.93 (m, 1H), 1.44 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 169.7 (C), 155.0 (C), 127.6 and 127.2 (CH), 122.9 and 122.1 (CH), 81.4 (C), 79.9 (C), 56.4 and 55.5 (CH), 38.9 and 37.6 (CH_2), 28.3 (3 CH_3), 28.0 (3 CH_3), 24.8 and 24.6 (CH_2). Anal. Calcd for $C_{15}H_{25}NO_4$: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.79; H, 9.16; N, 4.88.

di(*tert*-butyl) (2*S*,4*S*)-4-hydroxy-1,2-piperidinedicarboxylate (182)

Chloroacetic acid (141 mg, 1.49 mmol) and PPh_3 (392 mg, 1.49 mmol) were added to a solution of **175** (300 mg, 0.995 mmol) in 10.0 mL of distilled THF. The mixture was cooled to 0°C prior to the drop-wise addition of a solution of DIAD (302 mg, 1.49 mmol) in 5.0 mL of dry THF. The solution was protected from light and stirred overnight with a slow increase of temperature from 0°C to room temperature. THF was evaporated and replaced by AcOEt. The solution was washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated in a vacuum to give **181**. The crude product was dissolved in 1.0 mL of dioxane and the same volume of water was added. A 1N NaOH solution was added drop by drop until the pH reached the value of 9. The mixture was stirred for 30 min and checked by TLC. The reaction was quenched by the addition of a large volume of 1N $KHSO_4$ before extraction with AcOEt. The organic phases were dried over Na_2SO_4, filtered and evaporated in a vacuum. The crude product was purified by flash column chromatography (AcOEt/Hex, 1:1) to yield **182** (211 mg, yield = 70%): TLC 0.44 (AcOEt/Hex, 1:1); colourless oil; $[\alpha]_D = -38.7$ (c = 1.1, $CHCl_3$); appeared as a mixture of conformers ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.78 (bd, J = 4.9 Hz, 0.5H) and 4.60 (bd, J = 5.5 Hz, 0.5H), 4.03-3.88 (m, 1H), 3.61-3.50 (m, 1H), 2.99-2.84 (m, 1H), 2.69 (bs, 1H), 2.39-2.32 (m, 1H), 1.89-1.80 (m, 1H), 1.60-1.48 (m, 1H), 1.44-1.26 (m, 1H), 1.39 (s, 9H), 1.37 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.7 and 170.5 (C), 155.4 (C), 81.7 (C), 80.1 (C), 65.9 and 65.7 (CH), 55.2 and 54.2 (CH), 40.5 and 39.9 (CH_2), 35.3 and 35.2 (CH_2), 34.0 and 33.8 (CH_2), 28.2 (3 CH_3), 27.9 (3 CH_3). Anal. Calcd for $C_{15}H_{27}NO_5$: C, 59.78; H, 9.03; N, 4.65. Found: C, 59.98; H, 9.22; N, 4.63.

(2*S*,4*S*)-1-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-4-hydroxy-2-piperidinecarboxylic acid (184)

Pipecolate **182** (110 mg, 0.365 mmol) was placed in a round-bottom flask and dissolved in 2.0 mL of trifluoroacetic acid. The solution was stirred for 2 h at room temperature and checked by TLC. Trifluoroacetic acid was co-evaporated with hexane and the residue was dried under vacuum. Crude **183** was dissolved in 1.5 mL of water. K_2CO_3 (151 mg, 1.09 mmol) and FmocOSu (123 mg, 0.365 mmol) dissolved in 1.5 mL of acetone were consecutively added. After checking the pH of the resulting solution, the mixture was stirred overnight. Acetone was evaporated and the residual solution was diluted with water. The aqueous solution was acidified with solid $KHSO_4$ prior to extraction with AcOEt. The organic phase was washed with 1N $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo to yield a yellowish oil. Purification of the crude product by flash column chromatography (AcOEt/AcOH, 10:0.1) gave **184** (109 mg, yield = 81%): HPLC t_R 8.28 (linear gradient, 30-100% B, 20 min); white

solid; $[\alpha]_D$ = –8.2 (*c* = 1.0, $CHCl_3$); mp 65-67°C; appeared as a mixture of conformers ^{1}H NMR (300 MHz, CD_3OD) δ 7.82-7.80 (m, 2H), 7.65-7.57 (m, 2H), 7.43-7.38 (m, 2H), 7.34-7.29 (m, 2H), 4.91 (d, *J* = 5.7 Hz, 0.5 H) and 4.74 (d, *J* = 5.7 Hz, 0.5H), 4.48-4.37 (m, 2H), 4.28-4.20 (m, 1H), 4.13-4.05 (m, 0.5H), 4.00-3.91 (m, 0.5H), 3.65-3.56 (m, 1H), 3.16-3.00 (m, 1H), 2.50-2.40 (m, 1H), 1.95-1.83 (m, 1H), 1.60-1.47 (m, 1H), 1.38-1.23 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) 174.0 (C), 157.8 and 157.4 (C), 145.3 and 145.2 (C), 145.1 and 145.0 (C), 142.6 (2 C), 128.8 (2 CH), 128.1 (2 CH), 126.1 and 126.0 (2 CH), 120.9 (2 CH), 69.0 and 68.9 (CH_2), 66.4 (CH), 55.5 and 55.4 (CH), 48.3 (CH), 41.5 (CH_2), 36.2 and 36.1 (CH_2), 34.8 and 34.7 (CH_2); Anal. Calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.83; H, 6.08; N, 3.61.

IX.5. Compounds cited in section V

General galactosylation procedure (using tetra-acetylated donors): 131 was dissolved in the required solvent to give a 0.1M solution. The desired galactosyl donor (1.5 equiv) and 4Å MS were added and the solution was stirred at room temperature for 30 min. The mixture was cooled down to the desired temperature (for reactions at room temperature, the addition was performed at 0°C) and the required amount of *Lewis* acid was added (in solution in 1.0 mL of CH_2Cl_2 for BF_3•Et_2O and TMSOTf). The reaction was quenched by the addition of Et_3N when the Lewis acid was BF_3•Et_2O or TMSOTf and the mixture was filtered through a pad of Celite®. The crude product was purified by flash column chromatography (AcOEt/Hex, 4:6).

***tert*-butyl (2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)hexanoate (197)**

Compound **197** was detected in low yield from some experiments making up the promoters and tetra-acetylated donors study (Table V.1). Purification by flash column chromatography allowed us to separate and to characterize the different compounds obtained (ie, **197**, **198** and **199**). After pooling and evaporation of the fractions containing **197**, a second purification by flash column chromatography (AcOEt/Hex, 4:6) afforded pure **197**: HPLC t_R 15.25 (linear gradient, 30-100% B, 20 min); TLC Rf 0.34 (AcOEt/Hex, 4:6); colourless oil; $[\alpha]_D$ = +4.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.2 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.2 Hz, 2H), 7.29 (t, *J* = 7.2 Hz, 2H), 5.42-5.34 (m, 2H), 5.20 (dd, *J* = 10.4,

7.8 Hz, 1H), 5.00 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.57 (d, *J* = 7.8 Hz, 1H), 4.45-4.34 (m, 2H), 4.25-4.10 (m, 4H), 3.89 (t, *J* = 6.4 Hz, 1H), 3.74 (m, 1H), 3.42-3.37 (m, 2H), 2.14 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.70-1.52 (m, 4H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.1 (C), 170.4 (C), 170.3 (C), 170.1 (C), 169.2 (C), 155.8 (C), 143.7 (2 C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 101.3 (CH), 82.6 (C), 79.0 (CH), 70.9 (CH), 70.8 (CH), 69.0 (CH), 67.0 (CH_2), 67.0 (CH), 61.4 (CH_2), 54.4 (CH_2), 54.0 (CH), 47.1 (CH), 29.7 (CH_2), 28.6 (CH_2), 28.0 (3 CH_3), 20.6 (2 CH_3), 20.5 (2 CH_3). Anal. Calcd for $C_{39}H_{48}N_4O_{14}$: C, 58.79; H, 6.07; N, 7.03. Found: C, 58.92; H, 6.24; N, 6.31.

Corresponding orthoester (198)

Orthoester **198** was detected in good to moderate yields from some experiments making up the promoters and tetra-acetylated donors study (Table V.1) Purification by flash column chromatography (AcOEt/Hex, 4:6) yielded pure **198**: TLC Rf 0.50 (AcOEt/Hex, 4:6); colourless oil; $[\alpha]_D$ = +40.3 (*c* = 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.3 Hz, 2H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.43-7.38 (m, 2H), 7.31 (tt, *J* = 7.2, 1.3 Hz, 2H), 5.81 (d, *J* = 4.8 Hz, 1H), 5.44 (t, *J* = 2.9 Hz, 1H), 5.20-5.16 (m, 1H), 5.02 (dd, *J* = 6.7, 3.3 Hz, 1H), 4.36-4.05 (m, 6H), 3.80 (m, 1H), 3.40-3.23 (m, 2H), 2.08 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.70 (s, 3H), 1.70-1.53 (m, 4H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.1 (C), 170.4 (C), 170.0 (C), 169.7 (C), 156.2 (C), 143.7 (2 C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 122.1 (C), 120.0 (2 CH), 97.3 (CH), 82.6 (C), 75.9 (CH), 71.6 (CH), 71.5 (CH), 69.5 (CH), 67.0 (CH_2), 66.0 (CH), 61.1 (CH_2), 54.5 (CH_2), 54.2 (CH), 47.1 (CH), 29.7 (CH_2), 28.4 (CH_2), 28.0 (3 CH_3), 25.1 (CH_3), 20.7 (2 CH_3), 20.5 (1 CH_3).

***tert*-butyl (2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-(acetyloxy)hexanoate (199)**

Compound **199** was detected in good to moderate yields from some experiments making up the promoters and tetra-acetylated donors study (Table V.1). Purification by flash column chromatography (AcOEt/Hex, 4:6) yielded pure **199**: HPLC t_R 15.11 (linear gradient, 30-100% B, 20 min); TLC Rf 0.67 (AcOEt/Hex, 4:6); colourless oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 5.40 (bd, J = 7.8 Hz, 1H), 5.03-4.95 (m, 1H), 4.39 (d, *J* = 7.0 Hz, 2H), 4.29-4.19 (m, 2H), 3.39-3.33 (m, 2H), 2.09 (s, 3H), 1.89-1.61 (m, 4H), 1.48 (s, 9H).

General galactosylation procedures (using bromide 200). Procedure C: 1.0 equiv of the desired hydroxylysine analogue (ie, **131**, **138**, **142** or **146**) and the required glycosyl donor (1.5 equiv) were placed in an argon-filled round-bottom flask and dissolved in dry

CH_2Cl_2 to give a ca. 0.1M solution of acceptor. Powdered 4 Å molecular sieve was added and the suspension was stirred for 30 min. The mixture was protected from light and $AgSiO_4$ (5 equiv) was added and the reaction was stirred for 8 h at room temperature. The conversion rate was checked by RP-HPLC and bromide (1.5 equiv) was added for a second time. After stirring for further 8 h, RP-HPLC showed completion, and the dark-brown suspension was filtered through a plug of Celite®. The solvent was evaporated to give a brownish residue which was purified by flash column chromatography (Et_2O/Pent, 3:7).

Procedure D: 1.0 equiv of the desired hydroxylysine analogue (ie, **151**) and the required galactosyl donor (0.5 equiv) were placed in an argon-filled round-bottom flask and dissolved in dry CH_2Cl_2 to give a ca. 0.1M solution of acceptor. The solution was diluted by addition of distilled cyclohexane *via* a hypodermic syringe to give a ca. 0.1M solution of galactosyl acceptor. Powdered 4 Å molecular sieve was added and the suspension was stirred for 30 min. The mixture was protected from light and $AgSiO_4$ (5 equiv) was added and the reaction was stirred for 24 h at room temperature. The conversion rate was checked by RP-HPLC, bromide (0.5 equiv) was added for a second time and the reaction was stirred again for 24 h. This operation was repeated every 24 h 4 times more (overall addition of galactosyl donor : 3.0 equiv). After 6 days, RP-HPLC showed completion, and the dark-brown suspension was filtered through a plug of Celite®. The solvent was evaporated to give a brownish residue which was purified by flash column chromatography (Et_2O/Pent, 3:7).

Procedure E: the glycosylated building block (**203**, **206**, **208**, **209** or **210**) was dissolved in CH_2Cl_2 to give a ca. 0.1M solution. The equal volume of TFA was added and the resulting solution was stirred for 2 h at ambient temperature. Following addition of a large volume of hexane, the solvents were evaporated to afford the glycosylated building blocks ready for use in SPPS.

***tert*-butyl (2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)Hyl-O^{t}Bu (203)**

Galactosylated building block **203** was prepared according to procedure C. Purification of the crude product gave **203** (850 mg, yield = 79%): HPLC t_R 18.56 (linear gradient, 50-100% B, 20 min); white foam; $[\alpha]_D$ = +3.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.31 (dt, *J* = 7.5, 1.3 Hz, 2H), 5.39 (d, *J* = 2.8 Hz, 1H), 5.36 (bd, *J* = 7.9 Hz, 1H), 5.23 (dd, *J* = 10.4, 7.7 Hz, 1H), 5.09 (dd, *J* = 10.4, 3.1 Hz, 1H), 4.69 (d, *J* = 7.9 Hz, 1H), 4.42 (dd, *J* = 10.6, 7.3 Hz, 1H), 4.36-4.29 (m, 1H), 4.24-4.13 (m, 3H), 4.06-3.94 (m, 2H), 3.80 (m, 1H), 3.82-3.73 (m, 1H), 3.45 (dd, *J* =

13.0, 4.2 Hz, 1H), 3.25 (dd, *J* = 13.0, 4.2 Hz, 1H), 1.91-1.80 (m, 1H), 1.70-1.55 (m, 3H), 1.48 (s, 9H), 1.26 (s, 9H), 1.17 (s, 9H), 1.13 (s, 9H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.8 (C), 177.3 (C), 176.9 (C), 176.3 (C), 171.0 (C), 155.8 (C), 143.8 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 100.5 (CH), 82.6 (C), 78.1 (CH), 71.0 (2 CH), 69.0 (CH), 67.1 (CH_2), 66.7 (CH), 61.3 (CH_2), 53.8 (CH), 53.7 (CH_2), 47.1 (CH), 39.0 (2 C), 38.7 (2 C), 28.6 (CH_2), 28.0 (3 CH_3), 27.4 (CH_2), 27.1 (12 CH_3). Anal. Calcd for $C_{51}H_{72}N_4O_{14}$: C, 63.47; H, 7.52; N, 5.81. Found: C, 63.10; H, 7.69; N, 5.36.

(2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)Hyl-OH (71)

The *N*-Fmoc protected derivative **71** was obtained according to procedure E (565 mg, yield = 100%): HPLC t_R 16.51 (linear gradient, 50-100% B, 20 min); white foam; $[\alpha]_D$ = +5.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (m, 2H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.31 (dt, *J* = 7.3, 1.1 Hz, 2H), 5.39 (d, *J* = 3.3 Hz, 1H), 5.37 (m, 1H), 5.23 (dd, *J* = 10.4, 7.7 Hz, 1H), 5.10 (dd, *J* = 10.4, 3.1 Hz, 1H), 4.68 (d, *J* = 7.9 Hz, 1H), 4.48-4.34 (m, 3H), 4.24-4.15 (m, 2H), 4.06-3.94 (m, 2H), 3.78 (m, 1H), 3.49-3.44 (m, 1H), 3.29-3.24 (m, 1H), 2.10-1.91 (m, 1H), 1.78-1.60 (m, 3H), 1.26 (s, 9H), 1.18 (s, 9H), 1.13 (s, 9H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.9 (C), 177.4 (C), 177.0 (C), 176.5 (C), 175.7 (C), 156.0 (C), 143.7 (C), 143.6 (C), 141.3 (2 C), 127.8 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 100.7 (CH), 78.2 (CH), 71.0 (2 CH), 69.0 (CH), 67.2 (CH_2), 66.7 (CH), 61.4 (CH_2), 53.8 (CH_2), 53.7 (CH), 47.1 (CH), 39.0 (2 C), 38.7 (2 C), 28.1 (CH_2), 27.9 (CH_2), 27.1 (12 CH_3). Anal. Calcd for $C_{47}H_{64}N_4O_{14}$: C, 62.10; H, 7.10; N, 6.16. Found: C, 61.84; H, 7.22; N, 5.72.

***tert*-butyl (2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)hexanoate: Fmoc-(GlcPiv$_4$)Hyl-O^{t}Bu (206)**

Galactosylated building block **206** was prepared according to procedure C. Purification of the crude product gave **206** (340 mg, yield = 76%): HPLC t_R 18.87 (linear gradient, 50-100% B, 20 min); white foam; $[\alpha]_D$ = +6.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.59 (m, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.30 (dt, *J* = 7.4, 1.0 Hz, 2H), 5.39 (bd, *J* = 7.7 Hz, 1H), 5.30 (t, *J* = 9.5 Hz, 1H), 5.11 (t, *J* = 9.9 Hz, 1H), 5.01 (dd, *J* = 9.5, 7.9 Hz, 1H), 4.66 (d, *J* = 7.9 Hz, 1H), 4.42 (dd, *J* = 10.4, 7.1 Hz, 1H), 4.36-4.30 (m, 1H), 4.27-4.19 (m, 3H), 4.00 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.78 (m, 1H), 3.72-3.68 (m, 1H), 3.45 (dd, *J* = 12.8, 4.2 Hz, 1H), 3.25 (dd, *J* = 12.8, 4.4 Hz, 1H), 1.88-1.77 (m, 1H), 1.70-1.53 (m, 3H), 1.47 (s, 9H), 1.21 (s, 9H), 1.14 (s, 9H), 1.12 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.9

(C), 177.1 (C), 176.3 (C), 176.1 (C), 170.9 (C), 155.8 (C), 143.8 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 100.1 (CH), 82.6 (C), 77.9 (CH), 72.2 (2 CH), 71.4 (CH), 67.9 (CH), 67.1 (CH_2), 61.7 (CH_2), 53.7 (CH), 53.7 (CH_2), 47.1 (CH), 38.8 (C), 38.7 (C), 38.6 (2 C), 28.5 (2 CH_2), 28.0 (3 CH_3), 27.1 (12 CH_3). Anal. Calcd for $C_{51}H_{72}N_4O_{14}$: C, 63.47; H, 7.52; N, 5.81. Found: C, 62.64; H, 7.54; N, 5.91.

(2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)hexanoate: Fmoc-(GlcPiv$_4$)Hyl-OH (207)

The *N*-Fmoc protected derivative **207** was obtained according to procedure E (565 mg, yield = 100%): HPLC t_R 17.02 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D$ = +6.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.59-7.55 (m, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.28 (t, *J* = 7.4 Hz, 2H), 5.55 (m, 1H), 5.29 (t, *J* = 9.3 Hz, 1H), 5.08 (t, *J* = 9.9 Hz, 1H), 4.98 (t, *J* = 8.6 Hz, 1H), 4.58 (m, 1H), 4.36 (m, 2H), 4.26-4.18 (m, 3H), 3.97-3.94 (m, 1H), 3.76-3.61 (m, 1H), 3.47-3.38 (m, 1H), 3.26-3.15 (m, 1H), 1.96-1.83 (m, 1H), 1.71-1.55 (m, 3H), 1.20 (s, 9H), 1.14 (s, 9H), 1.10 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.1 (C), 177.2 (C), 176.5 (C), 176.3 (C), 175.1 (C), 156.6 (C), 143.8 (C), 143.6 (C), 141.2 (2 C), 127.8 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 100.4 (CH), 82.6 (C), 78.2 (CH), 72.2 (2 CH), 71.5 (CH), 67.8 (CH), 67.3 (CH_2), 61.7 (CH_2), 53.6 (CH), 53.6 (CH_2), 47.0 (CH), 38.8 (C), 38.7 (C), 38.6 (2 C), 29.7 (CH_2), 27.8 (CH_2), 27.1 (6 CH_3), 27.0 (6 CH_3). Anal. Calcd for $C_{47}H_{64}N_4O_{14}$: C, 62.10; H, 7.10; N, 6.16. Found: C, 62.31; H, 7.28; N, 5.98.

***tert*-butyl (2*S*,5*R*)-6-[(2,2-dimethylpropanoyl)oxy]-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)Dhn(OPiv)-O^{t}Bu (208)**

Galactosylated building block **208** was prepared according to procedure C. Purification of the crude product gave **208** (382 mg, yield = 89%): HPLC t_R 19.30 (linear gradient, 50-100% B, 20 min); white foam; $[\alpha]_D$ = –1.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (bd, *J* = 7.3 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (dt, *J* = 7.5, 1.3 Hz, 2H), 5.39 (bd, *J* = 2.5 Hz, 1H), 5.32 (bd, *J* = 8.0 Hz, 1H), 5.19 (dd, *J* = 10.4, 7.5 Hz, 1H), 5.09 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.66 (d, *J* = 7.7 Hz, 1H), 4.43 (dd, *J* = 10.6, 7.3 Hz, 1H), 4.35-4.29 (m, 1H), 4.24-4.19 (m, 1H), 4.14-4.08 (m, 4H), 4.04-3.95 (m, 2H), 3.87 (m, 1H), 1.97-1.83 (m, 1H), 1.70-1.54 (m, 3H), 1.47 (s, 9H), 1.25 (s, 9H), 1.20 (s, 9H), 1.16 (s, 9H), 1.13 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.1 (C), 177.8 (C), 177.3 (C), 176.8 (C), 176.4 (C), 171.1 (C), 155.9 (C), 143.8 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH),

125.0 (2 CH), 120.0 (2 CH), 100.0 (CH), 82.5 (C), 76.2 (CH), 71.1 (CH), 70.9 (CH), 69.0 (CH), 67.1 (CH_2), 66.7 (CH), 65.2 (CH_2), 61.2 (CH_2), 54.0 (CH), 47.1 (CH), 39.0 (2 C), 38.7 (3 C), 28.5 (CH_2), 28.0 (3 CH_3), 27.4 (CH_2), 27.1 (9 CH_3), 27.0 (3 CH_3), 26.9 (3 CH_3). Anal. Calcd for $C_{56}H_{81}NO_{16}$: C, 65.67; H, 7.97; N, 1.37. Found: C, 65.48; H, 8.06; N, 1.26.

(2*S*,5*R*)-6-[(2,2-dimethylpropanoyl)oxy]-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)Dhn(OPiv)-OH (72)

The *N*-Fmoc protected derivative **72** was obtained according to procedure E (322 mg, yield = 100%): HPLC t_R 17.84 (linear gradient, 50-100% B, 20 min); colourless oil; $[\alpha]_D$ = +5.0 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.3 Hz, 2H), 7.61-7.57 (m, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.30 (dt, *J* = 7.5, 1.1 Hz, 2H), 5.45 (bd, *J* = 8.0 Hz, 1H), 5.38 (d, *J* = 3.1 Hz, 1H), 5.19 (dd, *J* = 10.4, 7.5 Hz, 1H), 5.10 (dd, *J* = 10.4, 3.1 Hz, 1H), 4.65 (d, *J* = 7.5 Hz, 1H), 4.46-4.34 (m, 3H), 4.22-3.94 (m, 6H), 3.91-3.84 (m, 1H), 2.10-1.96 (m, 1H), 1.81-1.54 (m, 3H), 1.25 (s, 9H), 1.21 (s, 9H), 1.19 (s, 9H), 1.12 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.3 (C), 177.9 (C), 177.4 (C), 176.9 (C), 176.7 (C), 175.8 (C), 156.1 (C), 143.7 (C), 143.6 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.0 (2 CH), 120.0 (2 CH), 100.2 (CH), 76.2 (CH), 71.1 (CH), 70.9 (CH), 69.0 (CH), 67.2 (CH_2), 66.7 (CH), 65.0 (CH_2), 61.2 (CH_2), 53.5 (CH), 47.1 (CH), 39.0 (C), 38.7 (2 C), 38.7 (2 C), 27.9 (CH_2), 27.4 (CH_2), 27.1 (15 CH_3). Anal. Calcd for $C_{52}H_{73}NO_{16}$: C, 64.51; H, 7.60; N, 1.45. Found: C, 64.04; H, 7.80; N, 1.42.

***tert*-butyl (2*S*,5*S*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)(5*S*)Hyl-O^{t}Bu (209)**

Galactosylated building block **209** was prepared according to procedure C. Purification of the crude product gave **209** (295 mg, yield = 62%): HPLC t_R 19.19 (linear gradient, 50-100% B, 20 min); white foam; $[\alpha]_D$ = +1.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 5.45 (bd, *J* = 8.0 Hz, 1H), 5.39 (dd, *J* = 3.3, 0.9 Hz, 1H), 5.20 (dd, *J* = 10.4, 7.7 Hz, 1H), 5.10 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.71 (d, *J* = 7.7 Hz, 1H), 4.38-4.35 (m, 2H), 4.33-4.26 (m, 1H), 4.21 (t, *J* = 7.0 Hz, 1H), 4.14 (dd, *J* = 10.8, 6.6 Hz, 1H), 4.06-4.00 (m, 1H), 3.95 (t, *J* = 6.8 Hz, 1H), 3.82-3.73 (m, 1H), 3.31 (bd, *J* = 5.7 Hz, 2H), 1.95-1.77 (m, 3H), 1.68-1.54 (m, 1H), 1.47 (s, 9H), 1.25 (s, 9H), 1.17 (s, 9H), 1.16 (s, 9H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.7 (C), 177.3 (C), 176.8 (C), 176.6 (C), 171.4 (C), 155.9 (C), 143.9 (C), 143.8 (C), 141.3 (2 C), 127.7 (2 CH), 127.0 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 99.8 (CH), 82.3 (C), 75.6 (CH),

71.1 (CH), 71.0 (CH), 68.8 (CH), 67.0 (CH_2), 66.7 (CH), 61.1 (CH_2), 54.7 (CH_2), 53.8 (CH), 47.1 (CH), 39.0 (C), 38.8 (C), 38.7 (C), 38.7 (C), 28.3 (CH_2), 28.2 (CH_2), 27.9 (3 CH_3), 27.1 (12 CH_3). Anal. Calcd for $C_{51}H_{72}N_4O_{14}$: C, 63.47; H, 7.52; N, 5.81. Found: C, 63.03; H, 7.60; N, 5.53.

(2*S*,5*S*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)(5*S*)Hyl-OH (73)

The *N*-Fmoc protected derivative **73** was obtained according to procedure E (198 mg, 95% yield): HPLC t_R 17.04 (linear gradient, 50-100% B, 20 min); white foam; $[\alpha]_D$ = +2.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (bs, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.61-7.58 (m, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.30 (dt, *J* = 7.5, 1.1 Hz, 2H), 5.50 (d, *J* = 8.1 Hz, 1H), 5.39 (d, *J* = 3.1 Hz, 1H), 5.22 (dd, *J* = 10.4, 7.7 Hz, 1H), 5.11 (dd, *J* = 10.4, 3.1 Hz, 1H), 4.74 (d, *J* = 7.9 Hz, 1H), 4.44-4.33 (m, 3H), 4.24-4.13 (m, 2H), 4.05-3.94 (m, 2H), 3.87 (m, 1H), 3.36-3.26 (m, 2H), 1.84-1.72 (m, 1H), 1.67-1.52 (m, 3H), 1.25 (s, 9H), 1.18 (s, 9H), 1.16 (s, 9H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.9 (C), 177.4 (C), 177.0 (C), 176.9 (C), 175.8 (C), 156.2 (C), 143.8 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 99.9 (CH), 75.1 (CH), 71.0 (2 CH), 69.0 (CH), 67.1 (CH_2), 66.7 (CH), 61.2 (CH_2), 54.8 (CH_2), 52.9 (CH), 47.1 (CH), 39.0 (2 C), 38.8 (C), 38.7 (C), 28.1 (CH_2), 27.9 (CH_2), 27.0 (12 CH_3).

***tert*-butyl (2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)-5-methylhexanoate: Fmoc-(GalPiv$_4$)Hyl(Me)-O^{t}Bu (210)**

Galactosylated building block **210** was prepared according to procedure D. Purification of the crude product gave **210** (683 mg, yield = 67%): HPLC t_R 18.64 (linear gradient, 60-100% B, 20 min); white foam; $[\alpha]_D$ = +0.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 5.42-5.35 (m, 2H), 5.22 (dd, *J* = 10.4, 7.7 Hz, 1H), 5.09 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.79 (d, *J* = 7.7 Hz, 1H), 4.45-4.33 (m, 2H), 4.27-4.16 (m, 2H), 4.12-4.02 (m, 3H), 3.96-3.92 (m, 1H), 1.89-1.78 (m, 1H), 1.72-1.60 (m, 3H), 1.48 (s, 9H), 1.25 (s, 9H), 1.23 (s, 3H), 1.18 (s, 9H), 1.14 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.8 (C), 177.3 (C), 177.0 (C), 176.2 (C), 171.1 (C), 155.8 (C), 143.9 (C), 143.8 (C), 141.3 (2 C), 127.7 (2 CH), 127.0 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 95.8 (CH), 82.4 (C), 79.3 (C), 71.1 (CH), 71.0 (CH), 68.8 (CH), 67.1 (CH_2), 66.9 (CH), 61.9 (CH_2), 57.9 (CH_2), 54.2 (CH), 47.1 (CH), 39.0 (C), 38.8

(C), 38.7 (2 C), 33.6 (CH_2), 28.0 (3 CH_3), 27.2 (3 CH_3), 27.1 (9 CH_3), 20.6 (CH_3). Anal. Calcd for $C_{52}H_{74}N_4O_{14}$: C, 63.78; H, 7.62; N, 5.72. Found: C, 63.62; H, 7.64; N, 5.41.

(2*S*,5*R*)-6-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbony]amino}-5-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)-5-methylhexanoate: Fmoc-(GalPiv$_4$)Hyl(Me)-OH (76)

The *N*-Fmoc protected derivative **76** was obtained according to procedure E (125 mg, 95% yield): HPLC t_R 13.79 (linear gradient, 60-100% B, 20 min); white foam; $[\alpha]_D$ = +4.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (bs, 1H), 7.74 (d, *J* = 7.3 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 2H), 7.28 (dt, *J* = 7.4, 0.9 Hz, 2H), 5.55 (bd, *J* = 7.5 Hz, 1H), 5.39 (d, *J* = 3.3 Hz, 1H), 5.22 (dd, *J* = 10.2, 7.5 Hz, 1H), 5.10 (dd, *J* = 10.2, 3.3 Hz, 1H), 4.77 (d, *J* = 7.5 Hz, 1H), 4.42-4.30 (m, 3H), 4.19 (t, *J* = 7.0 Hz, 1H), 4.13-4.02 (m, 2H), 3.97-3.92 (m, 1H), 3.54 (d, *J* = 12.8 Hz, 1H), 3.07 (d, *J* = 12.8 Hz, 1H), 1.96-1.87 (m, 1H), 1.73-1.58 (m, 3H), 1.25 (s, 9H), 1.22 (s, 3H), 1.18 (s, 9H), 1.13 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 178.0 (C), 177.4 (C), 177.1 (C), 176.6 (C), 176.5 (C), 156.3 (C), 143.8 (C), 143.7 (C), 141.2 (2 C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 95.9 (CH), 79.3 (C), 71.1 (2 CH), 68.9 (CH), 67.2 (CH_2), 66.8 (CH), 61.8 (CH_2), 57.8 (CH_2), 53.9 (CH), 47.0 (CH), 39.0 (C), 38.7 (C), 38.6 (2 C), 34.0 (CH_2), 27.2 (6 CH_3), 27.1 (6 CH_3), 26.2 (CH_2), 20.7 (CH_3). Anal. Calcd for $C_{48}H_{66}N_4O_{14}$: C, 62.46; H, 7.21; N, 6.07. Found: C, 62.58; H, 7.12; N, 5.93.

***tert*-butyl (2*S*,5*S*)-5-azido-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-6-*O*-(2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosyl)hexanoate: Fmoc-(GalPiv$_4$)Hnl(5N$_3$)-O^{t}Bu (211)**

Galactosylated building block **211** was prepared according to procedure C. Purification of the crude product gave **211** (65 mg, yield = 19%): HPLC t_R 19.35 (linear gradient, 50-100% B, 20 min); TLC Rf = 0.33 (Et_2O/Pent, 3:7); white foam; $[\alpha]_D$ = +4.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 5.40 (d, *J* = 2.6 Hz, 1H), 5.37 (bd, J = 8.0 Hz, 1H), 5.24 (dd, *J* = 10.4, 7.9 Hz, 1H), 5.09 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.56 (d, *J* = 7.9 Hz, 1H), 4.39-4.37 (m, 2H), 4.27-4.14 (m, 3H), 4.06-3.94 (m, 2H), 3.86-3.83 (m, 1H), 3.56-3.43 (m, 2H), 1.93-1.78 (m, 3H), 1.58-1.40 (m, 1H), 1.48 (s, 9H), 1.26 (s, 9H), 1.18 (s, 18H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.8 (C), 177.3 (C), 176.9 (C), 176.5 (C), 171.1 (C), 155.9 (C), 143.9 (C), 143.7 (C), 141.3 (2 C), 127.7 (2 CH), 127.0 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 101.3 (CH), 82.6 (C), 71.9 (CH_2), 71.1 (CH), 70.9 (CH), 68.5 (CH), 67.0 (CH_2), 66.6 (CH), 61.4 (CH), 61.1 (CH_2), 53.8 (CH), 47.1 (CH), 39.0 (C), 38.8 (C), 38.7 (2 C), 29.4 (CH_2), 28.0 (3

CH_3), 27.1 (3 CH_3), 27.0 (9 CH_3), 26.6 (CH_2). Anal. Calcd for $C_{51}H_{72}N_4O_{14}$: C, 63.47; H, 7.52; N, 5.81. Found: C, 63.35; H, 7.63; N, 5.54.

Corresponding orthoester (212)

Orthoester **212** was prepared according to procedure C. Purification of the crude product gave **212** (280 mg, yield = 81%): TLC Rf = 0.60 (Et_2O/Pent, 3:7); colourless oil; $[\alpha]_D$ = +33.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 5.80 (d, *J* = 4.7 Hz, 1H), 5.57-5.55 (m, 1H), 5.41-5.38 (m, 1H), 5.39 (bd, *J* = 8.0 Hz, 1H), 5.04 (dd, *J* = 6.2, 2.4 Hz, 1H), 4.41-4.33 (m, 3H), 4.28-4.18 (m, 2H), 3.96 (dd, *J* = 11.0, 7.7 Hz, 1H), 3.59-3.52 (m, 1H), 3.50-3.41 (m, 2H), 1.97-1.81 (m, 3H), 1.64-1.53 (m, 1H), 1.48 (s, 9H), 1.25 (s, 9H), 1.19 (s, 9H), 1.17 (s, 9H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.9 (C), 177.3 (C), 176.3 (C), 171.1 (C), 155.9 (C), 143.9 (C), 143.7 (C), 141.3 (2 C), 127.9 (C), 127.7 (2 CH), 127.1 (2 CH), 125.1 (2 CH), 120.0 (2 CH), 96.9 (CH), 82.6 (C), 78.7 (CH), 72.5 (CH), 70.8 (CH), 67.0 (CH_2), 66.0 (CH), 64.9 (CH_2), 61.1 (CH), 60.2 (CH_2), 53.8 (CH), 47.1 (CH), 39.0 (2 C), 38.7 (2 C), 29.6 (CH_2), 28.0 (3 CH_3), 27.2 (3 CH_3), 27.1 (6 CH_3), 26.6 (CH_2), 25.2 (3 CH_3).

IX.6. Peptides cited in section VI

General procedure for SPPS (Procedure F): peptides **GP19** and **GP20** as well as glycopeptides **GP2**, **GP13**, **GP17**, **GP18** and **GP21-GP25** were synthesized on *Wang* resin[3] using a home-made semi-automatic peptide synthesizer.[4] For each coupling step, the reactants were introduced manually as a solution in 2.0 mL of dry DMF. N^{α}-Fmoc amino acids (5.0 equiv) with standard side-chain protecting groups were coupled 2 times by using BOP (5.0 equiv), HOBt (5.0 equiv) and DIEA (10.0 equiv) in dry DMF for 20 min. Glycosylated building blocks **71-73**, **76** and **207** (2 equiv) were coupled 2 times by using BOP (2 equiv), HOBt (2 equiv) and DIEA (4.0 equiv) in dry DMF for 60 min (1st coupling) and 20 min (2nd coupling). The washing of the resin as well as Fmoc deprotection (by using a freshly prepared solution of 20% piperidine in DMF) were performed automatically. The coupling and

[3] Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328.

[4] Neimark, J. and Briand, J. P. *Pept. Res.* **1993**, *6*, 219.

deprotection steps were monitored by the *Kaiser* test.[5] At the end of the elongation of the peptidic chain, the resin was washed with CH_2Cl_2 and dried with Et_2O.

General procedure for azide reduction (Procedure G): the resin was placed in a syringe equipped with a frit and swallowed by the addition of 1.0 mL of THF. A solution of PPh_3 (10 equiv) in dry THF were added and the mixture was gently shaken for 72 h. The resin was washed with THF and CH_2Cl_2 and dried with Et_2O before the next step.

General procedure for cleavage from the resin (Procedure H): 10.0 mL of TFA / H_2O / TIPS / DTT (8.8:0.5:0.2:0.5) were added to the resin. The mixture was gently shaken for 2.5 h and the resulting solution was flushed through a frit in cold Et_2O. The precipitate was recovered by centrifugation, dissolved in a mixture of AcOH and H_2O and freeze-dried.

General procedure for deprotection of the glycosyl moiety (Procedure I): The glycopeptide (white powder) was placed in a round bottom flask and dissolved in a freshly prepared 40 mM solution of NaOMe in MeOH. The deprotection was monitored by C_{18} RP-HPLC. After full deprotection of the glycosyl moiety, the solution was neutralized by drop-wise addition of AcOH and MeOH was evaporated under vacuum.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-GalHyl-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP2)

The synthesis of **GP2** was performed with building block **71** on resin (60 μmol) according to the procedures F-I. The purity of the crude peptide was 72% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP2** (59 mg, 59% yield and > 99% purity): HPLC t_R 8.44 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1666. Found: $[M+H^+]$ = 1667.11.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-GlcHyl-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP13)

The synthesis of **GP13** was performed with building block 207 on resin (80 μmol) according to the procedures F-I. The purity of the crude peptide was 75% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP13** (73 mg, 55% yield and > 99% purity): HPLC t_R 8.77 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1666. Found: $[M+H^+]$ = 1667.28.

Gly^{256}-Glu-Pro-Gly-Ile-Ala-Gly-Phe-GalHyl-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP17)

The synthesis of **GP17** was performed with building block **71** on resin (80 μmol) according to the procedures F-I. The purity of the crude peptide was 80% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP17** (74 mg, 56% yield and > 99% purity): HPLC t_R 8.74 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM

[5] Kaiser, E.; Colescott, R. L.; Bosinger, C. D.; Cook, P. *Anal. Biochem.* **1970**, *34*, 595.

= 1650. Found: $[M+H^+]$ = 1651.18.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-GalHyl-Gly-Asp-Gln-Gly-Pro-Lys^{270} (GP18)

The synthesis of **GP18** was performed with building block **71** on resin (60 μmol) according to the procedures F-I. The purity of the crude peptide was 63% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP18** (57 mg, 58% yield and > 99% purity): HPLC t_R 8.41 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1652. Found: $[M+H^+]$ = 1653.66.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-Lys-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP19)

The synthesis of **GP19** was performed with commercial amino acids on resin (25 μmol) according to the procedures F and H. The purity of the crude peptide was 85% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP19** (35 mg, 95% yield and > 99% purity): HPLC t_R 8.73 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1488. Found: $[M+H^+]$ = 1488.85.

Gly^{256}-Glu-Pro-Gly-Ile-Ala-Gly-Phe-Lys-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP20)

The synthesis of **GP20** was performed with commercial amino acids on resin (25 μmol) according to the procedures F and H. The purity of the crude peptide was 86% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP20** (35 mg, 95% yield and > 99% purity): HPLC t_R 9.15 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1472. Found: $[M+H^+]$ = 1473.08.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-GalHyl(N_3)-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP21)

The synthesis of **GP21** was performed with building block **71** on resin (60 μmol) according to the procedures F, H and I. The purity of the crude peptide was 70% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP21** (60 mg, 59% yield and > 99% purity): HPLC t_R 9.45 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1692. Found: $[M+H^+]$ = 1692.92.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-GalHyl(OH)-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP22)

The synthesis of **GP22** was performed with building block **72** on resin (60 μmol) according to the procedures F, H and I. The purity of the crude peptide was 67% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP22** (58 mg, 58% yield and > 99% purity): HPLC t_R 8.64 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1667. Found: $[M+H^+]$ = 1667.01.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-Gal(5*S*)Hyl-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP23)

The synthesis of **GP23** was performed with building block **73** on resin (60 μmol) according to the procedures F-I. The purity of the crude peptide was 83% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP23** (58 mg, 58% yield and > 99% purity): HPLC t_R 8.52 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1666. Found: $[M+H^+]$ = 1667.84.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-GalHyl(Me)-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP24)

The synthesis of **GP24** was performed with building block **76** on resin (60 μmol) according to the procedures F-I. The purity of the crude peptide was 70% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP24** (57 mg, 57% yield and > 99% purity): HPLC t_R 8.62 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 1680. Found: $[M+H^+]$ = 1680.17.

Gly^{256}-Glu-Hyp-Gly-Ile-Ala-Gly-Phe-Gal(Piv$_4$)Hyl-Gly-Glu-Gln-Gly-Pro-Lys^{270} (GP25)

The synthesis of **GP25** was performed with building block **71** on resin (60 μmol) according to the procedures F-H. The purity of the crude peptide was 86% (determined by C_{18} RP-HPLC). Purification by semi-preparative C_{18} RP-HPLC gave the glycopeptide **GP25** (88 mg, 73% yield and > 99% purity): HPLC t_R 16.84 (linear gradient, 5-65% B, 20 min). Calcd Mass: MM = 2002. Found: $[M+H^+]$ = 2002.65.

IX.7. Supplementary Material

IX.7.1. Crystal data and structure refinement for 61a

Table IX.1. Crystal data and structure refinement for **61a**.

Identification code	compound **61a**	
Empirical formula	C15 H25 N O5	
Formula weight	299.36	
Temperature	293(2) K	
Wavelength	0.71070 Å	
Crystal system	Orthorhombic	
Space group	P	
Unit cell dimensions	a = 5.9780(4) Å	α= 90°.
	b = 10.5680(8) Å	β= 90°.
	c = 27.154(2) Å	γ = 90°.
Volume	1715.5(2) Å^3	
Z	4	
Density (calculated)	1.159 Mg/m^3	
Absorption coefficient	0.086 mm^{-1}	
F(000)	648	

Crystal size	.3 x .2 x .2 mm^3
Theta range for data collection	2.44 to 25.02°.
Index ranges	0<=h<=7, 0<=k<=12, -31<=l<=31
Reflections collected	3693
Independent reflections	2485 [R(int) = 0.0330]
Completeness to theta = 25.02°	90.0 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2485 / 0 / 191
Goodness-of-fit on F^2	1.051
Final R indices [I>2sigma(I)]	R1 = 0.0629, wR2 = 0.1677
R indices (all data)	R1 = 0.0867, wR2 = 0.1905
Extinction coefficient	0.036(9)
Largest diff. peak and hole	0.247 and -0.187 e.$Å^{-3}$

Table IX.2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **61a**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	3587(8)	9833(4)	2212(2)	77(1)
C(2)	5949(9)	9440(6)	2111(2)	103(2)
C(3)	2701(11)	9346(7)	2691(2)	111(2)
C(4)	3340(15)	11241(6)	2187(3)	152(4)
O(1)	2121(5)	9443(2)	1801(1)	75(1)
C(5)	1778(6)	8233(4)	1699(2)	59(1)
O(2)	2392(5)	7344(3)	1932(1)	78(1)
N(1)	376(5)	8118(3)	1284(1)	56(1)
C(6)	-873(6)	6920(4)	1259(2)	58(1)
C(7)	446(6)	5918(3)	985(1)	57(1)
O(3)	1903(5)	6154(3)	688(1)	74(1)
O(4)	-317(4)	4777(2)	1102(1)	69(1)
C(8)	480(7)	3608(4)	857(2)	76(1)
C(9)	2974(8)	3472(5)	930(3)	106(2)
C(10)	-779(9)	2589(4)	1116(3)	106(2)
C(11)	-179(11)	3719(6)	308(2)	109(2)
C(12)	533(7)	8905(4)	875(2)	65(1)
O(5)	2124(6)	9596(3)	810(1)	84(1)
C(13)	-1454(9)	8918(5)	536(2)	81(1)
C(14)	-2787(8)	7714(5)	500(2)	83(1)
C(15)	-3107(7)	7133(4)	1006(2)	77(1)

Table IX.3. Bond lengths [Å] and angles [°] for **61a**.

C(1)-O(1)	1.478(5)
C(1)-C(3)	1.495(8)
C(1)-C(4)	1.497(8)
C(1)-C(2)	1.497(7)
C(2)-H(2A)	0.9600
C(2)-H(2B)	0.9600
C(2)-H(2C)	0.9600
C(3)-H(3A)	0.9600
C(3)-H(3B)	0.9600
C(3)-H(3C)	0.9600
C(4)-H(4A)	0.9600
C(4)-H(4B)	0.9600
C(4)-H(4C)	0.9600
O(1)-C(5)	1.324(4)
C(5)-O(2)	1.191(4)
C(5)-N(1)	1.411(5)

N(1)-C(12)	1.391(5)
N(1)-C(6)	1.471(5)
C(6)-C(7)	1.516(6)
C(6)-C(15)	1.519(6)
C(6)-H(6)	0.9800
C(7)-O(3)	1.212(4)
C(7)-O(4)	1.328(5)
O(4)-C(8)	1.482(5)
C(8)-C(10)	1.491(7)
C(8)-C(9)	1.511(7)
C(8)-C(11)	1.547(8)
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(12)-O(5)	1.212(5)
C(12)-C(13)	1.502(6)
C(13)-C(14)	1.504(7)
C(13)-H(13A)	0.9700
C(13)-H(13B)	0.9700
C(14)-C(15)	1.516(7)
C(14)-H(14A)	0.9700
C(14)-H(14B)	0.9700
C(15)-H(15A)	0.9700
C(15)-H(15B)	0.9700
O(1)-C(1)-C(3)	110.6(4)
O(1)-C(1)-C(4)	100.6(4)
C(3)-C(1)-C(4)	110.3(5)
O(1)-C(1)-C(2)	110.0(4)
C(3)-C(1)-C(2)	113.5(5)
C(4)-C(1)-C(2)	111.1(6)
C(1)-C(2)-H(2A)	109.5
C(1)-C(2)-H(2B)	109.5
H(2A)-C(2)-H(2B)	109.5
C(1)-C(2)-H(2C)	109.5
H(2A)-C(2)-H(2C)	109.5
H(2B)-C(2)-H(2C)	109.5
C(1)-C(3)-H(3A)	109.5
C(1)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	109.5
C(1)-C(3)-H(3C)	109.5
H(3A)-C(3)-H(3C)	109.5
H(3B)-C(3)-H(3C)	109.5
C(1)-C(4)-H(4A)	109.5
C(1)-C(4)-H(4B)	109.5
H(4A)-C(4)-H(4B)	109.5
C(1)-C(4)-H(4C)	109.5
H(4A)-C(4)-H(4C)	109.5
H(4B)-C(4)-H(4C)	109.5
C(5)-O(1)-C(1)	121.2(3)
O(2)-C(5)-O(1)	127.2(4)
O(2)-C(5)-N(1)	122.6(4)
O(1)-C(5)-N(1)	110.0(3)
C(12)-N(1)-C(5)	123.1(3)
C(12)-N(1)-C(6)	120.8(3)

C(5)-N(1)-C(6)	114.4(3)
N(1)-C(6)-C(7)	111.1(3)
N(1)-C(6)-C(15)	109.9(3)
C(7)-C(6)-C(15)	109.8(3)
N(1)-C(6)-H(6)	108.7
C(7)-C(6)-H(6)	108.7
C(15)-C(6)-H(6)	108.7
O(3)-C(7)-O(4)	126.4(3)
O(3)-C(7)-C(6)	123.8(3)
O(4)-C(7)-C(6)	109.7(3)
C(7)-O(4)-C(8)	122.6(3)
O(4)-C(8)-C(10)	103.1(4)
O(4)-C(8)-C(9)	109.7(4)
C(10)-C(8)-C(9)	111.6(5)
O(4)-C(8)-C(11)	106.8(4)
C(10)-C(8)-C(11)	112.5(5)
C(9)-C(8)-C(11)	112.7(5)
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(8)-C(11)-H(11A)	109.5
C(8)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(8)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
O(5)-C(12)-N(1)	122.0(4)
O(5)-C(12)-C(13)	121.8(4)
N(1)-C(12)-C(13)	116.1(4)
C(12)-C(13)-C(14)	116.8(4)
C(12)-C(13)-H(13A)	108.1
C(14)-C(13)-H(13A)	108.1
C(12)-C(13)-H(13B)	108.1
C(14)-C(13)-H(13B)	108.1
H(13A)-C(13)-H(13B)	107.3
C(13)-C(14)-C(15)	110.5(4)
C(13)-C(14)-H(14A)	109.5
C(15)-C(14)-H(14A)	109.5
C(13)-C(14)-H(14B)	109.5
C(15)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	108.1
C(14)-C(15)-C(6)	111.0(3)
C(14)-C(15)-H(15A)	109.4
C(6)-C(15)-H(15A)	109.4
C(14)-C(15)-H(15B)	109.4
C(6)-C(15)-H(15B)	109.4
H(15A)-C(15)-H(15B)	108.0

Table IX.4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **61a**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	86(3)	68(3)	75(3)	-10(2)	-26(2)	1(2)
C(2)	72(3)	128(5)	108(4)	-23(4)	-4(3)	-17(3)
C(3)	101(4)	147(5)	84(4)	-30(4)	-10(3)	10(4)
C(4)	194(8)	78(4)	185(7)	-44(4)	-111(6)	13(4)
O(1)	91(2)	54(2)	82(2)	-7(1)	-34(2)	-3(1)
C(5)	53(2)	61(2)	61(2)	-4(2)	-4(2)	0(2)
O(2)	89(2)	61(2)	85(2)	6(2)	-26(2)	0(2)
N(1)	55(2)	54(2)	59(2)	-1(2)	-2(2)	-5(1)
C(6)	52(2)	60(2)	63(2)	-4(2)	5(2)	-11(2)
C(7)	47(2)	61(2)	61(2)	-3(2)	-2(2)	-6(2)
O(3)	59(2)	79(2)	84(2)	-11(2)	14(2)	-7(2)
O(4)	63(2)	57(2)	86(2)	-4(2)	11(1)	-4(1)
C(8)	62(2)	62(3)	105(3)	-17(2)	-1(2)	5(2)
C(9)	68(3)	95(4)	156(6)	-16(4)	2(3)	14(3)
C(10)	88(3)	55(2)	176(6)	-6(3)	20(4)	-2(2)
C(11)	121(4)	98(4)	109(4)	-37(3)	-5(4)	1(4)
C(12)	70(2)	57(2)	68(2)	-3(2)	-4(2)	-1(2)
O(5)	91(2)	76(2)	84(2)	11(2)	-3(2)	-17(2)
C(13)	93(3)	84(3)	64(2)	-2(2)	-22(2)	14(3)
C(14)	63(3)	99(3)	87(3)	-17(3)	-18(2)	8(2)
C(15)	48(2)	89(3)	92(3)	-24(3)	5(2)	-5(2)

Table IX.5. Hydrogen coordinates ($x\ 10^4$) and isotropic displacement parameters ($Å^2 x\ 10^3$) for **61a**.

	x	y	z	U(eq)
H(2A)	5953	8732	1889	133
H(2B)	6662	9203	2414	133
H(2C)	6747	10133	1964	133
H(3A)	1773	8621	2631	144
H(3B)	1836	9995	2848	144
H(3C)	3927	9110	2900	144
H(4A)	2310	11458	1929	198
H(4B)	4769	11618	2120	198
H(4C)	2782	11550	2496	198
H(6)	-1151	6621	1595	76
H(9A)	3554	4227	1080	138
H(9B)	3684	3344	617	138
H(9C)	3269	2760	1140	138
H(10A)	-1248	2889	1434	138
H(10B)	169	1863	1157	138
H(10C)	-2068	2359	926	138
H(11A)	-220	4595	215	142
H(11B)	-1628	3349	258	142
H(11C)	902	3282	109	142
H(13A)	-935	9135	208	105
H(13B)	-2452	9587	643	105
H(14A)	-2013	7118	288	108
H(14B)	-4235	7892	355	108
H(15A)	-3884	6331	974	100
H(15B)	-4023	7689	1206	100

Table IX.6. Torsion angles [°] for **61a**.

C(3)-C(1)-O(1)-C(5)	-61.0(6)
C(4)-C(1)-O(1)-C(5)	-177.5(5)
C(2)-C(1)-O(1)-C(5)	65.2(6)
C(1)-O(1)-C(5)-O(2)	6.6(7)
C(1)-O(1)-C(5)-N(1)	-178.3(4)
O(2)-C(5)-N(1)-C(12)	-145.4(4)
O(1)-C(5)-N(1)-C(12)	39.3(5)
O(2)-C(5)-N(1)-C(6)	20.0(5)
O(1)-C(5)-N(1)-C(6)	-155.3(3)
C(12)-N(1)-C(6)-C(7)	76.0(4)
C(5)-N(1)-C(6)-C(7)	-89.7(4)
C(12)-N(1)-C(6)-C(15)	-45.6(5)
C(5)-N(1)-C(6)-C(15)	148.6(3)
N(1)-C(6)-C(7)-O(3)	-23.4(5)
C(15)-C(6)-C(7)-O(3)	98.3(4)
N(1)-C(6)-C(7)-O(4)	159.6(3)
C(15)-C(6)-C(7)-O(4)	-78.7(4)
O(3)-C(7)-O(4)-C(8)	-3.2(6)
C(6)-C(7)-O(4)-C(8)	173.7(3)
C(7)-O(4)-C(8)-C(10)	178.5(4)
C(7)-O(4)-C(8)-C(9)	59.5(6)
C(7)-O(4)-C(8)-C(11)	-62.8(5)
C(5)-N(1)-C(12)-O(5)	13.2(6)
C(6)-N(1)-C(12)-O(5)	-151.3(4)
C(5)-N(1)-C(12)-C(13)	-162.7(4)
C(6)-N(1)-C(12)-C(13)	32.9(5)
O(5)-C(12)-C(13)-C(14)	153.4(4)
N(1)-C(12)-C(13)-C(14)	-30.8(6)
C(12)-C(13)-C(14)-C(15)	42.5(6)
C(13)-C(14)-C(15)-C(6)	-55.4(5)
N(1)-C(6)-C(15)-C(14)	55.8(5)
C(7)-C(6)-C(15)-C(14)	-66.6(5)

IX.7.2. Crystal data and structure refinement for (5*R*)-63a

Table IX.7. Crystal data and structure refinement for (5*R*)-**63a**.

Identification code	compound (5*R*)-**63a**	
Empirical formula	C15 H25 N O6	
Formula weight	315.36	
Temperature	293(2) K	
Wavelength	0.71070 Å	
Crystal system	Orthorhombic	
Space group	P	
Unit cell dimensions	a = 8.6160(2) Å	α= 90°.
	b = 11.6880(3) Å	β= 90°.
	c = 17.1570(4) Å	γ = 90°.
Volume	1727.78(7) Å^3	
Z	4	
Density (calculated)	1.212 Mg/m^3	
Absorption coefficient	0.093 mm^{-1}	
F(000)	680	
Crystal size	.2 x .1 x .1 mm^3	
Theta range for data collection	3.17 to 25.33°.	
Index ranges	-10<=h<=10, -14<=k<=14, -20<=l<=20	
Reflections collected	1812	
Independent reflections	1812 [R(int) = 0.0000]	

Completeness to theta = 25.33°	99.4 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1812 / 0 / 200
Goodness-of-fit on F^2	0.955
Final R indices [I>2sigma(I)]	R1 = 0.0424, wR2 = 0.1065
R indices (all data)	R1 = 0.0657, wR2 = 0.1202
Absolute structure parameter	4.1(19)
Largest diff. peak and hole	0.142 and -0.155 e.Å^{-3}

Table IX.8. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for (5*R*)-**63a**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	10384(4)	3264(3)	2317(2)	59(1)
C(2)	8770(4)	3701(4)	2477(2)	82(1)
C(3)	11613(4)	4099(3)	2603(2)	75(1)
C(4)	10655(5)	2073(3)	2626(2)	91(1)
O(1)	10666(2)	3283(2)	1459(1)	56(1)
C(5)	9710(3)	2746(3)	979(2)	52(1)
O(2)	8799(3)	2004(2)	1135(1)	69(1)
N(1)	9951(3)	3128(2)	205(1)	48(1)
C(6)	9384(3)	2302(2)	-396(2)	49(1)
C(7)	10111(3)	1141(2)	-253(2)	53(1)
O(3)	11315(3)	981(2)	76(1)	72(1)
O(4)	9229(2)	347(2)	-587(1)	60(1)
C(8)	9688(4)	-874(2)	-608(2)	54(1)
C(9)	9735(7)	-1321(3)	202(2)	105(2)
C(10)	11192(4)	-1002(4)	-1038(3)	88(1)
C(11)	8388(4)	-1417(3)	-1076(3)	89(1)
C(12)	10069(3)	4285(2)	43(2)	52(1)
O(5)	9923(3)	5021(2)	532(1)	68(1)
C(13)	10417(4)	4616(3)	-794(2)	62(1)
O(6)	10182(4)	5801(2)	-884(2)	95(1)
C(14)	9458(4)	3934(3)	-1354(2)	69(1)
C(15)	9816(4)	2687(3)	-1219(2)	61(1)

Table IX.9. Bond lengths [Å] and angles [°] for (5*R*)-**63a**.

C(1)-O(1)	1.491(3)
C(1)-C(2)	1.507(5)
C(1)-C(4)	1.508(5)
C(1)-C(3)	1.522(5)
C(2)-H(2A)	0.9600
C(2)-H(2B)	0.9600
C(2)-H(2C)	0.9600
C(3)-H(3A)	0.9600
C(3)-H(3B)	0.9600
C(3)-H(3C)	0.9600
C(4)-H(4A)	0.9600
C(4)-H(4B)	0.9600
C(4)-H(4C)	0.9600
O(1)-C(5)	1.324(3)
C(5)-O(2)	1.200(3)
C(5)-N(1)	1.416(4)
N(1)-C(12)	1.384(4)
N(1)-C(6)	1.495(3)
C(6)-C(7)	1.514(4)
C(6)-C(15)	1.528(4)

C(6)-H(6A)	0.9800
C(7)-O(3)	1.196(4)
C(7)-O(4)	1.329(3)
O(4)-C(8)	1.481(4)
C(8)-C(9)	1.486(5)
C(8)-C(10)	1.499(5)
C(8)-C(11)	1.516(5)
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(12)-O(5)	1.208(3)
C(12)-C(13)	1.518(5)
C(13)-O(6)	1.408(4)
C(13)-C(14)	1.496(5)
C(13)-H(13)	0.9800
O(6)-H(6)	0.8200
C(14)-C(15)	1.507(4)
C(14)-H(14A)	0.9700
C(14)-H(14B)	0.9700
C(15)-H(15A)	0.9700
C(15)-H(15B)	0.9700
O(1)-C(1)-C(2)	109.0(3)
O(1)-C(1)-C(4)	109.7(3)
C(2)-C(1)-C(4)	113.0(3)
O(1)-C(1)-C(3)	101.3(2)
C(2)-C(1)-C(3)	111.4(3)
C(4)-C(1)-C(3)	111.8(3)
C(1)-C(2)-H(2A)	109.5
C(1)-C(2)-H(2B)	109.5
H(2A)-C(2)-H(2B)	109.5
C(1)-C(2)-H(2C)	109.5
H(2A)-C(2)-H(2C)	109.5
H(2B)-C(2)-H(2C)	109.5
C(1)-C(3)-H(3A)	109.5
C(1)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	109.5
C(1)-C(3)-H(3C)	109.5
H(3A)-C(3)-H(3C)	109.5
H(3B)-C(3)-H(3C)	109.5
C(1)-C(4)-H(4A)	109.5
C(1)-C(4)-H(4B)	109.5
H(4A)-C(4)-H(4B)	109.5
C(1)-C(4)-H(4C)	109.5
H(4A)-C(4)-H(4C)	109.5
H(4B)-C(4)-H(4C)	109.5
C(5)-O(1)-C(1)	120.4(2)
O(2)-C(5)-O(1)	127.6(3)
O(2)-C(5)-N(1)	122.3(3)
O(1)-C(5)-N(1)	110.0(2)
C(12)-N(1)-C(5)	120.5(2)
C(12)-N(1)-C(6)	121.1(2)
C(5)-N(1)-C(6)	113.2(2)
N(1)-C(6)-C(7)	109.4(2)
N(1)-C(6)-C(15)	111.5(2)

C(7)-C(6)-C(15)	108.2(2)
N(1)-C(6)-H(6A)	109.2
C(7)-C(6)-H(6A)	109.2
C(15)-C(6)-H(6A)	109.2
O(3)-C(7)-O(4)	126.2(3)
O(3)-C(7)-C(6)	125.1(3)
O(4)-C(7)-C(6)	108.6(2)
C(7)-O(4)-C(8)	122.0(2)
O(4)-C(8)-C(9)	108.8(3)
O(4)-C(8)-C(10)	109.9(3)
C(9)-C(8)-C(10)	113.7(3)
O(4)-C(8)-C(11)	102.6(2)
C(9)-C(8)-C(11)	111.6(3)
C(10)-C(8)-C(11)	109.7(3)
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(8)-C(11)-H(11A)	109.5
C(8)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(8)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
O(5)-C(12)-N(1)	123.3(3)
O(5)-C(12)-C(13)	119.7(3)
N(1)-C(12)-C(13)	117.0(3)
O(6)-C(13)-C(14)	112.0(3)
O(6)-C(13)-C(12)	109.1(3)
C(14)-C(13)-C(12)	111.2(3)
O(6)-C(13)-H(13)	108.1
C(14)-C(13)-H(13)	108.1
C(12)-C(13)-H(13)	108.1
C(13)-O(6)-H(6)	109.5
C(13)-C(14)-C(15)	107.7(3)
C(13)-C(14)-H(14A)	110.2
C(15)-C(14)-H(14A)	110.2
C(13)-C(14)-H(14B)	110.2
C(15)-C(14)-H(14B)	110.2
H(14A)-C(14)-H(14B)	108.5
C(14)-C(15)-C(6)	112.2(3)
C(14)-C(15)-H(15A)	109.2
C(6)-C(15)-H(15A)	109.2
C(14)-C(15)-H(15B)	109.2
C(6)-C(15)-H(15B)	109.2
H(15A)-C(15)-H(15B)	107.9

–

Table IX.10. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for (5*R*)-**63a**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	66(2)	63(2)	47(2)	-3(2)	0(2)	-1(2)
C(2)	77(2)	101(3)	68(2)	-12(2)	10(2)	5(2)
C(3)	83(2)	82(3)	61(2)	-15(2)	0(2)	-12(2)
C(4)	113(3)	79(2)	82(2)	20(2)	-14(3)	-1(3)
O(1)	57(1)	64(1)	48(1)	-6(1)	1(1)	-12(1)
C(5)	46(2)	48(2)	61(2)	-7(1)	1(1)	-2(2)
O(2)	70(1)	70(2)	66(1)	0(1)	4(1)	-24(1)
N(1)	49(1)	45(1)	51(1)	-5(1)	-1(1)	-3(1)
C(6)	44(1)	47(2)	57(2)	-10(1)	-5(1)	-3(1)
C(7)	52(2)	48(2)	58(2)	-9(1)	-1(2)	2(1)
O(3)	60(1)	61(1)	97(2)	-16(1)	-30(1)	9(1)
O(4)	55(1)	44(1)	81(1)	-15(1)	-16(1)	2(1)
C(8)	56(2)	44(2)	63(2)	-9(1)	-2(2)	5(1)
C(9)	178(5)	66(2)	73(2)	2(2)	2(3)	6(3)
C(10)	79(2)	72(2)	112(3)	-29(2)	19(2)	1(2)
C(11)	86(2)	51(2)	131(3)	-18(2)	-30(2)	0(2)
C(12)	45(2)	45(2)	66(2)	-6(2)	-4(2)	1(1)
O(5)	76(2)	50(1)	78(2)	-14(1)	-7(1)	4(1)
C(13)	68(2)	47(2)	71(2)	4(2)	6(2)	1(2)
O(6)	135(2)	50(1)	99(2)	15(1)	5(2)	7(2)
C(14)	83(2)	67(2)	58(2)	7(2)	-4(2)	3(2)
C(15)	66(2)	60(2)	56(2)	-7(2)	-6(2)	1(2)

Table IX.11. Hydrogen coordinates ($x\ 10^4$) and isotropic displacement parameters ($Å^2 x\ 10^3$) for (5*R*)-**63a**.

	x	y	z	U(eq)
H(2A)	8672	4466	2280	106
H(2B)	8585	3700	3029	106
H(2C)	8026	3215	2225	106
H(3A)	11375	4854	2417	98
H(3B)	12612	3870	2410	98
H(3C)	11629	4100	3162	98
H(4A)	11681	1826	2489	119
H(4B)	9905	1560	2405	119
H(4C)	10549	2076	3184	119
H(6A)	8253	2235	-357	64
H(9A)	8759	-1175	453	137
H(9B)	10550	-947	485	137
H(9C)	9925	-2130	192	137
H(10A)	11068	-736	-1564	114
H(10B)	11491	-1794	-1044	114
H(10C)	11981	-560	-783	114
H(11A)	8418	-1135	-1601	116
H(11B)	7408	-1225	-844	116
H(11C)	8515	-2233	-1078	116
H(13)	11513	4452	-898	80
H(6)	9969	6086	-461	123
H(14A)	9709	4145	-1885	90
H(14B)	8364	4080	-1267	90
H(15A)	10916	2558	-1302	79
H(15B)	9249	2230	-1594	79

Table IX.12. Torsion angles [°] for (5*R*)-**63a**.

C(2)-C(1)-O(1)-C(5)	-55.3(4)
C(4)-C(1)-O(1)-C(5)	68.9(4)
C(3)-C(1)-O(1)-C(5)	-172.9(3)
C(1)-O(1)-C(5)-O(2)	-18.8(4)
C(1)-O(1)-C(5)-N(1)	164.3(2)
O(2)-C(5)-N(1)-C(12)	136.4(3)
O(1)-C(5)-N(1)-C(12)	-46.4(4)
O(2)-C(5)-N(1)-C(6)	-18.4(4)
O(1)-C(5)-N(1)-C(6)	158.7(2)
C(12)-N(1)-C(6)-C(7)	150.9(3)
C(5)-N(1)-C(6)-C(7)	-54.4(3)
C(12)-N(1)-C(6)-C(15)	31.2(4)
C(5)-N(1)-C(6)-C(15)	-174.1(3)
N(1)-C(6)-C(7)-O(3)	-25.0(4)
C(15)-C(6)-C(7)-O(3)	96.7(4)
N(1)-C(6)-C(7)-O(4)	158.1(2)
C(15)-C(6)-C(7)-O(4)	-80.2(3)
O(3)-C(7)-O(4)-C(8)	-1.9(5)
C(6)-C(7)-O(4)-C(8)	174.9(3)
C(7)-O(4)-C(8)-C(9)	64.6(4)
C(7)-O(4)-C(8)-C(10)	-60.5(4)
C(7)-O(4)-C(8)-C(11)	-177.1(3)
C(5)-N(1)-C(12)-O(5)	-2.7(5)
C(6)-N(1)-C(12)-O(5)	150.2(3)
C(5)-N(1)-C(12)-C(13)	176.7(2)
C(6)-N(1)-C(12)-C(13)	-30.4(4)
O(5)-C(12)-C(13)-O(6)	-12.5(4)
N(1)-C(12)-C(13)-O(6)	168.1(3)
O(5)-C(12)-C(13)-C(14)	-136.6(3)
N(1)-C(12)-C(13)-C(14)	44.1(4)
O(6)-C(13)-C(14)-C(15)	179.0(3)
C(12)-C(13)-C(14)-C(15)	-58.7(4)
C(13)-C(14)-C(15)-C(6)	61.9(4)
N(1)-C(6)-C(15)-C(14)	-46.8(4)
C(7)-C(6)-C(15)-C(14)	-167.1(3)

IX.7.3. Crystal data and structure refinement for 64a

Table IX.13. Crystal data and structure refinement for **64a**.

Identification code	compound **64a**	
Empirical formula	C15 H25 N O6	
Formula weight	315.36	
Temperature	293(2) K	
Wavelength	0.71070 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 10.0100(3) Å	α= 90°.
	b = 6.2490(2) Å	β= 101.1450(10)°.
	c = 13.8790(5) Å	γ = 90°.
Volume	851.79(5) Å^3	
Z	2	
Density (calculated)	1.230 Mg/m^3	
Absorption coefficient	0.095 mm^{-1}	
F(000)	340	
Crystal size	0.2 x 0.2 x 0.1 mm^3	
Theta range for data collection	3.29 to 27.53°.	

Index ranges	0<=h<=13, -7<=k<=8, -18<=l<=17
Reflections collected	150057
Independent reflections	2125 [R(int) = 0.0420]
Completeness to theta = 27.53°	99.1 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2125 / 1 / 199
Goodness-of-fit on F^2	1.037
Final R indices [I>2sigma(I)]	R1 = 0.0417, wR2 = 0.0998
R indices (all data)	R1 = 0.0778, wR2 = 0.1252
Largest diff. peak and hole	0.127 and -0.160 e.Å^{-3}

Table IX.14. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for **64a**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(6)	4039(2)	3193(3)	2306(1)	50(1)
O(4)	1421(2)	1118(3)	2613(1)	53(1)
O(3)	-81(2)	3803(4)	2203(2)	71(1)
O(2)	1033(2)	6611(3)	1126(2)	69(1)
N(1)	1537(2)	3055(4)	1275(2)	44(1)
O(5)	4774(2)	275(4)	1610(2)	71(1)
C(2)	2476(3)	1376(5)	1062(2)	45(1)
C(12)	3904(3)	1568(5)	1683(2)	49(1)
C(6)	1426(3)	5018(5)	785(2)	51(1)
C(7)	865(3)	2767(4)	2069(2)	48(1)
O(1)	4128(3)	4058(6)	92(2)	116(1)
C(3)	2509(3)	1288(6)	-35(2)	59(1)
C(13)	5352(3)	3675(6)	2960(2)	61(1)
C(8)	830(3)	285(6)	3432(2)	58(1)
C(5)	1739(4)	5008(7)	-236(3)	74(1)
C(10)	1774(4)	-1553(7)	3791(3)	77(1)
C(4)	2825(3)	3448(7)	-402(2)	69(1)
C(11)	-585(4)	-573(7)	3031(3)	81(1)
C(16)	5800(4)	1793(7)	3626(3)	84(1)
C(15)	4975(5)	5546(8)	3544(4)	103(2)
C(14)	6384(4)	4278(9)	2340(3)	96(1)
C(9)	872(6)	1976(8)	4202(3)	100(1)

Table IX.15. Bond lengths [Å] and angles [°] for **64a**.

O(6)-C(12)	1.324(3)
O(6)-C(13)	1.477(4)
O(4)-C(7)	1.334(3)
O(4)-C(8)	1.474(3)
O(3)-C(7)	1.191(3)
O(2)-C(6)	1.201(4)
N(1)-C(6)	1.397(4)
N(1)-C(7)	1.409(3)
N(1)-C(2)	1.476(3)
O(5)-C(12)	1.207(3)
C(2)-C(12)	1.524(4)
C(2)-C(3)	1.530(4)
C(2)-H(2)	0.9800
C(6)-C(5)	1.508(4)
O(1)-C(4)	1.404(4)
O(1)-H(1)	0.8200
C(3)-C(4)	1.498(5)
C(3)-H(3A)	0.9700

C(3)-H(3B)	0.9700
C(13)-C(16)	1.510(5)
C(13)-C(15)	1.511(5)
C(13)-C(14)	1.515(5)
C(8)-C(9)	1.498(5)
C(8)-C(10)	1.510(5)
C(8)-C(11)	1.515(5)
C(5)-C(4)	1.510(5)
C(5)-H(5A)	0.9700
C(5)-H(5B)	0.9700
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(4)-H(4)	0.9800
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(16)-H(16A)	0.9600
C(16)-H(16B)	0.9600
C(16)-H(16C)	0.9600
C(15)-H(15A)	0.9600
C(15)-H(15B)	0.9600
C(15)-H(15C)	0.9600
C(14)-H(14A)	0.9600
C(14)-H(14B)	0.9600
C(14)-H(14C)	0.9600
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(12)-O(6)-C(13)	121.6(2)
C(7)-O(4)-C(8)	121.5(2)
C(6)-N(1)-C(7)	119.2(2)
C(6)-N(1)-C(2)	121.4(2)
C(7)-N(1)-C(2)	119.0(2)
N(1)-C(2)-C(12)	113.2(2)
N(1)-C(2)-C(3)	111.2(2)
C(12)-C(2)-C(3)	111.5(2)
N(1)-C(2)-H(2)	106.8
C(12)-C(2)-H(2)	106.8
C(3)-C(2)-H(2)	106.8
O(5)-C(12)-O(6)	125.8(3)
O(5)-C(12)-C(2)	120.8(3)
O(6)-C(12)-C(2)	113.3(2)
O(2)-C(6)-N(1)	122.5(2)
O(2)-C(6)-C(5)	121.2(3)
N(1)-C(6)-C(5)	116.2(3)
O(3)-C(7)-O(4)	126.0(3)
O(3)-C(7)-N(1)	124.6(3)
O(4)-C(7)-N(1)	109.4(2)
C(4)-O(1)-H(1)	109.5
C(4)-C(3)-C(2)	110.7(3)
C(4)-C(3)-H(3A)	109.5
C(2)-C(3)-H(3A)	109.5
C(4)-C(3)-H(3B)	109.5
C(2)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	108.1
O(6)-C(13)-C(16)	109.9(3)
O(6)-C(13)-C(15)	101.7(3)
C(16)-C(13)-C(15)	110.5(3)
O(6)-C(13)-C(14)	109.0(3)

C(16)-C(13)-C(14)	112.8(3)
C(15)-C(13)-C(14)	112.4(4)
O(4)-C(8)-C(9)	109.9(3)
O(4)-C(8)-C(10)	101.8(2)
C(9)-C(8)-C(10)	111.8(3)
O(4)-C(8)-C(11)	109.2(3)
C(9)-C(8)-C(11)	114.0(3)
C(10)-C(8)-C(11)	109.4(3)
C(6)-C(5)-C(4)	115.6(3)
C(6)-C(5)-H(5A)	108.4
C(4)-C(5)-H(5A)	108.4
C(6)-C(5)-H(5B)	108.4
C(4)-C(5)-H(5B)	108.4
H(5A)-C(5)-H(5B)	107.4
C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
O(1)-C(4)-C(3)	108.4(3)
O(1)-C(4)-C(5)	112.2(4)
C(3)-C(4)-C(5)	108.9(2)
O(1)-C(4)-H(4)	109.1
C(3)-C(4)-H(4)	109.1
C(5)-C(4)-H(4)	109.1
C(8)-C(11)-H(11A)	109.5
C(8)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(8)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(13)-C(16)-H(16A)	109.5
C(13)-C(16)-H(16B)	109.5
H(16A)-C(16)-H(16B)	109.5
C(13)-C(16)-H(16C)	109.5
H(16A)-C(16)-H(16C)	109.5
H(16B)-C(16)-H(16C)	109.5
C(13)-C(15)-H(15A)	109.5
C(13)-C(15)-H(15B)	109.5
H(15A)-C(15)-H(15B)	109.5
C(13)-C(15)-H(15C)	109.5
H(15A)-C(15)-H(15C)	109.5
H(15B)-C(15)-H(15C)	109.5
C(13)-C(14)-H(14A)	109.5
C(13)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
C(13)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5

Table IX.16. Anisotropic displacement parameters ($Å^2 x\ 10^3$)for **64a**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(6)	40(1)	55(1)	54(1)	-7(1)	8(1)	2(1)
O(4)	51(1)	62(1)	48(1)	10(1)	20(1)	7(1)
O(3)	67(1)	71(1)	87(2)	14(1)	42(1)	25(1)
O(2)	80(2)	46(1)	87(2)	4(1)	28(1)	6(1)
N(1)	41(1)	47(1)	46(1)	0(1)	15(1)	2(1)
O(5)	64(1)	83(2)	65(1)	-15(1)	12(1)	27(1)
C(2)	44(2)	49(2)	45(1)	-6(1)	15(1)	-1(1)
C(12)	51(2)	53(2)	47(2)	-2(1)	18(1)	4(2)
C(6)	49(2)	48(2)	56(2)	3(2)	11(1)	-6(1)
C(7)	50(2)	46(2)	49(2)	-1(1)	13(1)	4(1)
O(1)	71(2)	189(4)	86(2)	53(2)	7(1)	-52(2)
C(3)	48(2)	83(2)	48(2)	-12(2)	14(1)	8(2)
C(13)	52(2)	67(2)	61(2)	-4(2)	4(2)	-4(2)
C(8)	60(2)	71(2)	47(2)	5(2)	23(1)	3(2)
C(5)	94(3)	66(2)	66(2)	15(2)	29(2)	-2(2)
C(10)	72(2)	86(3)	74(2)	24(2)	12(2)	9(2)
C(4)	58(2)	110(3)	42(2)	6(2)	15(1)	-12(2)
C(11)	61(2)	101(3)	84(2)	28(2)	21(2)	-7(2)
C(16)	84(3)	94(3)	66(2)	5(2)	-4(2)	-4(2)
C(15)	89(3)	98(3)	111(3)	-44(3)	-14(3)	-4(2)
C(14)	57(2)	129(4)	102(3)	13(3)	16(2)	-27(2)
C(9)	148(4)	98(3)	63(2)	-13(2)	44(3)	-5(3)

Table IX.17. Torsion angles [°] for **64a**.

C(6)-N(1)-C(2)-C(12)	88.4(3)
C(7)-N(1)-C(2)-C(12)	-83.8(3)
C(6)-N(1)-C(2)-C(3)	-38.1(4)
C(7)-N(1)-C(2)-C(3)	149.7(2)
C(13)-O(6)-C(12)-O(5)	3.8(4)
C(13)-O(6)-C(12)-C(2)	-178.8(2)
N(1)-C(2)-C(12)-O(5)	178.1(3)
C(3)-C(2)-C(12)-O(5)	-55.6(4)
N(1)-C(2)-C(12)-O(6)	0.6(3)
C(3)-C(2)-C(12)-O(6)	126.9(3)
C(7)-N(1)-C(6)-O(2)	16.1(4)
C(2)-N(1)-C(6)-O(2)	-156.1(3)
C(7)-N(1)-C(6)-C(5)	-159.9(3)
C(2)-N(1)-C(6)-C(5)	27.9(4)
C(8)-O(4)-C(7)-O(3)	4.5(4)
C(8)-O(4)-C(7)-N(1)	-173.1(2)
C(6)-N(1)-C(7)-O(3)	21.6(4)
C(2)-N(1)-C(7)-O(3)	-166.0(3)
C(6)-N(1)-C(7)-O(4)	-160.8(2)
C(2)-N(1)-C(7)-O(4)	11.6(3)
N(1)-C(2)-C(3)-C(4)	53.8(3)
C(12)-C(2)-C(3)-C(4)	-73.7(3)
C(12)-O(6)-C(13)-C(16)	-60.3(3)
C(12)-O(6)-C(13)-C(15)	-177.4(3)
C(12)-O(6)-C(13)-C(14)	63.7(4)
C(7)-O(4)-C(8)-C(9)	-62.6(4)
C(7)-O(4)-C(8)-C(10)	178.7(2)
C(7)-O(4)-C(8)-C(11)	63.1(4)
O(2)-C(6)-C(5)-C(4)	150.5(3)

N(1)-C(6)-C(5)-C(4)	-33.4(4)
C(2)-C(3)-C(4)-O(1)	62.5(3)
C(2)-C(3)-C(4)-C(5)	-59.9(3)
C(6)-C(5)-C(4)-O(1)	-70.3(4)
C(6)-C(5)-C(4)-C(3)	49.7(4)

Table IX.18. Hydrogen bonds for **64a** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(5)#1	0.82	2.29	2.892(3)	130.7

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,y+1/2,-z

IX.7.4. Crystal data and structure refinement for 64c

Table IX.19. Crystal data and structure refinement for **64c**.

Identification code	compound **64c**	
Empirical formula	C14 H25 N O4	
Formula weight	271.35	
Temperature	293(2) K	
Wavelength	1.54060 Å	
Crystal system	monoclinic	
Space group	P21	
Unit cell dimensions	a = 5.563(2) Å	α= 90°.
	b = 9.891(3) Å	β= 95.02(2)°.
	c = 14.800(3) Å	γ = 90°.
Volume	811.2(4) $Å^3$	
Z	2	
Density (calculated)	1.111 Mg/m^3	
Absorption coefficient	0.657 mm^{-1}	
F(000)	296	
Crystal size	? x ? x ? mm^3	
Theta range for data collection	2.99 to 69.85°.	
Index ranges	0<=h<=6, -12<=k<=10, -18<=l<=17	
Reflections collected	2974	
Independent reflections	2974 [R(int) = 0.0000]	
Completeness to theta = 69.85°	99.9 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2974 / 0 / 176	
Goodness-of-fit on F^2	1.136	
Final R indices [I>2sigma(I)]	R1 = 0.0665, wR2 = 0.1749	
R indices (all data)	R1 = 0.1017, wR2 = 0.2273	
Absolute structure parameter	0.1(5)	
Extinction coefficient	0.008(2)	
Largest diff. peak and hole	0.226 and -0.216 $e.Å^{-3}$	

Table IX.20. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **64c**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	9478(7)	3131(5)	7965(3)	58(1)
C(2)	11221(11)	3297(7)	8788(5)	87(2)
C(3)	7828(10)	1951(6)	8070(6)	94(2)
C(4)	10683(12)	3021(8)	7109(5)	91(2)
O(1)	7746(5)	4280	7917(2)	55(1)
C(5)	8533(8)	5540(5)	7866(3)	52(1)

O(2)	10469(6)	5875(4)	7638(3)	80(1)
N(1)	6723(6)	6474(4)	8046(2)	48(1)
C(6)	6481(7)	7648(5)	7412(3)	47(1)
C(7)	5989(9)	7126(6)	6436(3)	62(1)
C(8)	6052(10)	8179(7)	5702(3)	80(2)
C(9)	5299(17)	7561(11)	4781(4)	127(3)
C(10)	8471(14)	8849(10)	5693(5)	125(3)
C(11)	4469(8)	8579(5)	7646(3)	54(1)
C(12)	4528(9)	8862(5)	8638(3)	61(1)
O(3)	2631(9)	9768(5)	8783(3)	95(2)
C(13)	4276(8)	7561(5)	9137(3)	57(1)
C(14)	5994(8)	6481(5)	8905(3)	54(1)
O(4)	6714(8)	5627(4)	9451(2)	79(1)

Table IX.21. Bond lengths [Å] and angles [°] for **64c**.

C(1)-O(1)	1.488(5)
C(1)-C(4)	1.488(8)
C(1)-C(2)	1.498(8)
C(1)-C(3)	1.501(7)
C(2)-H(2A)	0.9600
C(2)-H(2B)	0.9600
C(2)-H(2C)	0.9600
C(3)-H(3A)	0.9600
C(3)-H(3B)	0.9600
C(3)-H(3C)	0.9600
C(4)-H(4A)	0.9600
C(4)-H(4B)	0.9600
C(4)-H(4C)	0.9600
O(1)-C(5)	1.326(5)
C(5)-O(2)	1.203(5)
C(5)-N(1)	1.409(5)
N(1)-C(14)	1.367(5)
N(1)-C(6)	1.491(5)
C(6)-C(11)	1.513(6)
C(6)-C(7)	1.535(6)
C(6)-H(6A)	0.9800
C(7)-C(8)	1.509(7)
C(7)-H(7A)	0.9700
C(7)-H(7B)	0.9700
C(8)-C(10)	1.501(9)
C(8)-C(9)	1.519(10)
C(8)-H(8A)	0.9800
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(11)-C(12)	1.493(6)
C(11)-H(11A)	0.9700
C(11)-H(11B)	0.9700
C(12)-O(3)	1.415(6)
C(12)-C(13)	1.496(7)
C(12)-H(12A)	0.9800
O(3)-H(3)	1.03(10)
C(13)-C(14)	1.493(6)
C(13)-H(13A)	0.9700
C(13)-H(13B)	0.9700
C(14)-O(4)	1.212(5)

O(1)-C(1)-C(4)	110.7(4)
O(1)-C(1)-C(2)	109.0(4)
C(4)-C(1)-C(2)	113.1(5)
O(1)-C(1)-C(3)	101.5(3)
C(4)-C(1)-C(3)	110.9(5)
C(2)-C(1)-C(3)	111.1(5)
C(1)-C(2)-H(2A)	109.5
C(1)-C(2)-H(2B)	109.5
H(2A)-C(2)-H(2B)	109.5
C(1)-C(2)-H(2C)	109.5
H(2A)-C(2)-H(2C)	109.5
H(2B)-C(2)-H(2C)	109.5
C(1)-C(3)-H(3A)	109.5
C(1)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	109.5
C(1)-C(3)-H(3C)	109.5
H(3A)-C(3)-H(3C)	109.5
H(3B)-C(3)-H(3C)	109.5
C(1)-C(4)-H(4A)	109.5
C(1)-C(4)-H(4B)	109.5
H(4A)-C(4)-H(4B)	109.5
C(1)-C(4)-H(4C)	109.5
H(4A)-C(4)-H(4C)	109.5
H(4B)-C(4)-H(4C)	109.5
C(5)-O(1)-C(1)	120.4(3)
O(2)-C(5)-O(1)	125.7(4)
O(2)-C(5)-N(1)	123.0(4)
O(1)-C(5)-N(1)	111.1(3)
C(14)-N(1)-C(5)	117.0(3)
C(14)-N(1)-C(6)	124.3(3)
C(5)-N(1)-C(6)	114.7(3)
N(1)-C(6)-C(11)	111.0(3)
N(1)-C(6)-C(7)	109.2(3)
C(11)-C(6)-C(7)	110.2(3)
N(1)-C(6)-H(6A)	108.8
C(11)-C(6)-H(6A)	108.8
C(7)-C(6)-H(6A)	108.8
C(8)-C(7)-C(6)	115.6(4)
C(8)-C(7)-H(7A)	108.4
C(6)-C(7)-H(7A)	108.4
C(8)-C(7)-H(7B)	108.4
C(6)-C(7)-H(7B)	108.4
H(7A)-C(7)-H(7B)	107.4
C(10)-C(8)-C(7)	112.8(5)
C(10)-C(8)-C(9)	110.3(6)
C(7)-C(8)-C(9)	110.3(6)
C(10)-C(8)-H(8A)	107.7
C(7)-C(8)-H(8A)	107.7
C(9)-C(8)-H(8A)	107.7
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5

H(10B)-C(10)-H(10C)	109.5
C(12)-C(11)-C(6)	112.7(3)
C(12)-C(11)-H(11A)	109.0
C(6)-C(11)-H(11A)	109.0
C(12)-C(11)-H(11B)	109.0
C(6)-C(11)-H(11B)	109.0
H(11A)-C(11)-H(11B)	107.8
O(3)-C(12)-C(11)	108.4(4)
O(3)-C(12)-C(13)	111.5(4)
C(11)-C(12)-C(13)	109.2(3)
O(3)-C(12)-H(12A)	109.2
C(11)-C(12)-H(12A)	109.2
C(13)-C(12)-H(12A)	109.2
C(12)-O(3)-H(3)	110(5)
C(14)-C(13)-C(12)	114.3(4)
C(14)-C(13)-H(13A)	108.7
C(12)-C(13)-H(13A)	108.7
C(14)-C(13)-H(13B)	108.7
C(12)-C(13)-H(13B)	108.7
H(13A)-C(13)-H(13B)	107.6
O(4)-C(14)-N(1)	120.5(4)
O(4)-C(14)-C(13)	121.7(4)
N(1)-C(14)-C(13)	117.7(3)

Table IX.22. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **64c**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	52(2)	34(2)	86(3)	-3(2)	5(2)	10(2)
C(2)	86(3)	59(3)	110(4)	5(3)	-23(3)	16(3)
C(3)	77(3)	41(3)	165(7)	2(3)	17(4)	1(2)
C(4)	95(4)	84(4)	95(4)	-9(3)	15(3)	25(3)
O(1)	51(2)	31(1)	81(2)	1(1)	6(1)	5(1)
C(5)	55(2)	33(2)	67(3)	3(2)	-4(2)	2(2)
O(2)	59(2)	50(2)	133(3)	11(2)	23(2)	-4(2)
N(1)	58(2)	33(2)	52(2)	7(1)	4(1)	2(1)
C(6)	61(2)	31(2)	50(2)	9(2)	2(2)	0(2)
C(7)	76(3)	59(3)	49(2)	0(2)	4(2)	6(2)
C(8)	87(3)	97(4)	57(3)	20(3)	17(2)	19(3)
C(9)	146(6)	174(9)	62(4)	14(4)	10(4)	-7(6)
C(10)	122(6)	156(8)	100(5)	47(5)	23(4)	-34(6)
C(11)	74(3)	39(2)	47(2)	3(2)	2(2)	6(2)
C(12)	82(3)	41(2)	58(2)	-11(2)	-7(2)	11(2)
O(3)	136(4)	83(3)	64(2)	-21(2)	-4(2)	55(3)
C(13)	71(3)	50(2)	49(2)	-5(2)	2(2)	6(2)
C(14)	75(3)	37(2)	48(2)	5(2)	-3(2)	-2(2)
O(4)	130(3)	47(2)	59(2)	14(2)	9(2)	15(2)

Table IX.23. Hydrogen coordinates ($x\ 10^4$) and isotropic displacement parameters ($Å^2 x\ 10^{\ 3}$) for **64c**.

	x	y	z	U(eq)
H(2A)	10350	3349	9318	113
H(2B)	12135	4112	8733	113
H(2C)	12297	2536	8839	113
H(3A)	7113	2027	8635	122
H(3B)	8736	1126	8064	122

H(3C)	6582	1945	7579	122
H(4A)	11760	3772	7064	118
H(4B)	9487	3030	6601	118
H(4C)	11580	2192	7110	118
H(6A)	8000	8155	7461	62
H(7A)	7174	6436	6335	80
H(7B)	4415	6698	6378	80
H(8A)	4871	8880	5817	104
H(9A)	3732	7157	4791	166
H(9B)	6446	6882	4645	166
H(9C)	5242	8253	4325	166
H(10A)	8939	9219	6282	163
H(10B)	8375	9561	5251	163
H(10C)	9645	8195	5541	163
H(11A)	4595	9426	7323	70
H(11B)	2930	8171	7442	70
H(12A)	6074	9283	8844	79
H(3)	2880(160)	10170(100)	9430(60)	124
H(13A)	4517	7739	9783	74
H(13B)	2642	7229	9007	74

Table IX.24. Torsion angles [°] for **64c**.

C(4)-C(1)-O(1)-C(5)	-65.5(5)
C(2)-C(1)-O(1)-C(5)	59.4(6)
C(3)-C(1)-O(1)-C(5)	176.7(4)
C(1)-O(1)-C(5)-O(2)	19.0(7)
C(1)-O(1)-C(5)-N(1)	-165.8(4)
O(2)-C(5)-N(1)-C(14)	-118.6(5)
O(1)-C(5)-N(1)-C(14)	66.0(5)
O(2)-C(5)-N(1)-C(6)	39.9(6)
O(1)-C(5)-N(1)-C(6)	-135.6(4)
C(14)-N(1)-C(6)-C(11)	-24.4(5)
C(5)-N(1)-C(6)-C(11)	178.9(3)
C(14)-N(1)-C(6)-C(7)	-146.1(4)
C(5)-N(1)-C(6)-C(7)	57.2(4)
N(1)-C(6)-C(7)-C(8)	-171.6(4)
C(11)-C(6)-C(7)-C(8)	66.3(5)
C(6)-C(7)-C(8)-C(10)	61.7(7)
C(6)-C(7)-C(8)-C(9)	-174.4(5)
N(1)-C(6)-C(11)-C(12)	45.4(5)
C(7)-C(6)-C(11)-C(12)	166.5(4)
C(6)-C(11)-C(12)-O(3)	178.3(4)
C(6)-C(11)-C(12)-C(13)	-60.0(5)
O(3)-C(12)-C(13)-C(14)	171.2(4)
C(11)-C(12)-C(13)-C(14)	51.4(5)
C(5)-N(1)-C(14)-O(4)	-7.2(6)
C(6)-N(1)-C(14)-O(4)	-163.3(4)
C(5)-N(1)-C(14)-C(13)	173.5(4)
C(6)-N(1)-C(14)-C(13)	17.3(6)
C(12)-C(13)-C(14)-O(4)	150.1(5)
C(12)-C(13)-C(14)-N(1)	-30.6(6)

Table IX.25. Hydrogen bonds for **64c** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(3)-H(3)...O(4)#1	1.03(10)	1.72(10)	2.743(5)	172(8)

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,y+1/2,-z+2

IX.7.5. Crystal data and structure refinement for 153a

Table IX.26. Crystal data and structure refinement for **153a**.

Identification code	compound **153a**	
Empirical formula	C15 H24 N O6	
Formula weight	314.35	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 6.1880(5) Å	α= 90°.
	b = 9.6290(8) Å	β= 98.870(4)°.
	c = 14.7320(12) Å	γ = 90°.
Volume	867.30(12) $Å^3$	
Z	2	
Density (calculated)	1.204 Mg/m^3	
Absorption coefficient	0.093 mm^{-1}	
F(000)	338	
Crystal size	0.3 x 0.2 x 0.2 mm^3	
Theta range for data collection	3.81 to 26.74°.	
Index ranges	-7<=h<=7, -12<=k<=12, -18<=l<=18	
Reflections collected	5512	
Independent reflections	1943 [R(int) = 0.0390]	
Completeness to theta = 26.74°	99.1 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1943 / 1 / 199	
Goodness-of-fit on F^2	1.030	
Final R indices [I>2sigma(I)]	R1 = 0.0423, wR2 = 0.1009	
R indices (all data)	R1 = 0.0608, wR2 = 0.1116	
Largest diff. peak and hole	0.140 and -0.142 $e.Å^{-3}$	

Table IX.27. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **l53a**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(3)	4382(3)	4569(2)	4154(2)	66(1)
O(4)	8356(3)	422(2)	4769(1)	63(1)
O(5)	6327(3)	3336(2)	2017(1)	53(1)
O(1)	9059(3)	5997(2)	2826(1)	56(1)
O(2)	7065(3)	6687(2)	3906(1)	67(1)
O(6)	9018(4)	1751(2)	2075(2)	73(1)
N(1)	7684(3)	4362(2)	3660(1)	43(1)
C(8)	7979(4)	1681(3)	4395(2)	47(1)
C(7)	9881(4)	2255(3)	4018(2)	47(1)
C(6)	9149(4)	3369(2)	3308(2)	43(1)
C(9)	6145(4)	2422(3)	4379(2)	50(1)
C(5)	7847(4)	5796(3)	3494(2)	45(1)
C(10)	5960(4)	3831(3)	4069(2)	46(1)
C(11)	8156(4)	2712(3)	2398(2)	47(1)
C(1)	9591(5)	7418(3)	2546(2)	60(1)
C(12)	5131(5)	2886(3)	1115(2)	66(1)
C(4)	11017(6)	8133(4)	3320(3)	83(1)
C(13)	3307(7)	3950(5)	960(3)	97(1)
C(14)	6613(7)	3011(6)	391(2)	107(2)
C(3)	7529(7)	8210(5)	2210(4)	107(1)

C(2)	10865(8)	7118(4)	1761(3)	106(2)
C(15)	4204(7)	1462(5)	1210(4)	105(1)

Table IX.28. Bond lengths [Å] and angles [°] for **l53a**.

O(3)-C(10)	1.230(3)
O(4)-C(8)	1.338(3)
O(4)-H(4)	0.8200
O(5)-C(11)	1.327(3)
O(5)-C(12)	1.482(3)
O(1)-C(5)	1.340(3)
O(1)-C(1)	1.481(3)
O(2)-C(5)	1.195(3)
O(6)-C(11)	1.202(3)
N(1)-C(10)	1.401(3)
N(1)-C(5)	1.408(3)
N(1)-C(6)	1.466(3)
C(8)-C(9)	1.337(4)
C(8)-C(7)	1.483(3)
C(7)-C(6)	1.518(3)
C(7)-H(7A)	0.9700
C(7)-H(7B)	0.9700
C(6)-C(11)	1.524(4)
C(6)-H(6)	0.9800
C(9)-C(10)	1.430(4)
C(9)-H(9A)	0.9300
C(1)-C(4)	1.497(5)
C(1)-C(3)	1.504(5)
C(1)-C(2)	1.525(5)
C(12)-C(15)	1.501(6)
C(12)-C(14)	1.515(5)
C(12)-C(13)	1.515(5)
C(4)-H(4A)	0.9600
C(4)-H(4B)	0.9600
C(4)-H(4C)	0.9600
C(13)-H(13A)	0.9600
C(13)-H(13B)	0.9600
C(13)-H(13C)	0.9600
C(14)-H(14A)	0.9600
C(14)-H(14B)	0.9600
C(14)-H(14C)	0.9600
C(3)-H(3A)	0.9600
C(3)-H(3B)	0.9600
C(3)-H(3C)	0.9600
C(2)-H(2A)	0.9600
C(2)-H(2B)	0.9600
C(2)-H(2C)	0.9600
C(15)-H(15A)	0.9600
C(15)-H(15B)	0.9600
C(15)-H(15C)	0.9600
C(8)-O(4)-H(4)	109.5
C(11)-O(5)-C(12)	121.5(2)
C(5)-O(1)-C(1)	120.8(2)
C(10)-N(1)-C(5)	120.8(2)
C(10)-N(1)-C(6)	117.9(2)
C(5)-N(1)-C(6)	120.89(19)
C(9)-C(8)-O(4)	125.8(2)
C(9)-C(8)-C(7)	121.0(2)
O(4)-C(8)-C(7)	113.2(2)
C(8)-C(7)-C(6)	110.58(19)

C(8)-C(7)-H(7A)	109.5
C(6)-C(7)-H(7A)	109.5
C(8)-C(7)-H(7B)	109.5
C(6)-C(7)-H(7B)	109.5
H(7A)-C(7)-H(7B)	108.1
N(1)-C(6)-C(7)	110.62(19)
N(1)-C(6)-C(11)	113.22(19)
C(7)-C(6)-C(11)	110.5(2)
N(1)-C(6)-H(6)	107.4
C(7)-C(6)-H(6)	107.4
C(11)-C(6)-H(6)	107.4
C(8)-C(9)-C(10)	122.5(2)
C(8)-C(9)-H(9A)	118.7
C(10)-C(9)-H(9A)	118.7
O(2)-C(5)-O(1)	125.8(2)
O(2)-C(5)-N(1)	124.8(2)
O(1)-C(5)-N(1)	109.4(2)
O(3)-C(10)-N(1)	120.2(2)
O(3)-C(10)-C(9)	122.8(2)
N(1)-C(10)-C(9)	117.0(2)
O(6)-C(11)-O(5)	125.6(3)
O(6)-C(11)-C(6)	121.6(2)
O(5)-C(11)-C(6)	112.7(2)
O(1)-C(1)-C(4)	110.1(3)
O(1)-C(1)-C(3)	110.3(3)
C(4)-C(1)-C(3)	112.8(3)
O(1)-C(1)-C(2)	101.5(2)
C(4)-C(1)-C(2)	110.6(3)
C(3)-C(1)-C(2)	110.9(3)
O(5)-C(12)-C(15)	109.2(3)
O(5)-C(12)-C(14)	109.6(3)
C(15)-C(12)-C(14)	114.8(4)
O(5)-C(12)-C(13)	101.2(2)
C(15)-C(12)-C(13)	110.2(3)
C(14)-C(12)-C(13)	111.0(3)
C(1)-C(4)-H(4A)	109.5
C(1)-C(4)-H(4B)	109.5
H(4A)-C(4)-H(4B)	109.5
C(1)-C(4)-H(4C)	109.5
H(4A)-C(4)-H(4C)	109.5
H(4B)-C(4)-H(4C)	109.5
C(12)-C(13)-H(13A)	109.5
C(12)-C(13)-H(13B)	109.5
H(13A)-C(13)-H(13B)	109.5
C(12)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5
C(12)-C(14)-H(14A)	109.5
C(12)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
C(12)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
C(1)-C(3)-H(3A)	109.5
C(1)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	109.5
C(1)-C(3)-H(3C)	109.5
H(3A)-C(3)-H(3C)	109.5
H(3B)-C(3)-H(3C)	109.5
C(1)-C(2)-H(2A)	109.5

C(1)-C(2)-H(2B)	109.5
H(2A)-C(2)-H(2B)	109.5
C(1)-C(2)-H(2C)	109.5
H(2A)-C(2)-H(2C)	109.5
H(2B)-C(2)-H(2C)	109.5
C(12)-C(15)-H(15A)	109.5
C(12)-C(15)-H(15B)	109.5
H(15A)-C(15)-H(15B)	109.5
C(12)-C(15)-H(15C)	109.5
H(15A)-C(15)-H(15C)	109.5
H(15B)-C(15)-H(15C)	109.5

Table IX.29. Anisotropic displacement parameters ($Å^2$x 10^3)for **153a**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(3)	54(1)	67(1)	83(1)	0(1)	27(1)	12(1)
O(4)	53(1)	58(1)	81(1)	26(1)	21(1)	3(1)
O(5)	64(1)	48(1)	47(1)	-5(1)	7(1)	12(1)
O(1)	77(1)	38(1)	58(1)	3(1)	24(1)	1(1)
O(2)	81(1)	48(1)	77(1)	-8(1)	31(1)	9(1)
O(6)	86(1)	65(1)	68(1)	-15(1)	12(1)	26(1)
N(1)	46(1)	39(1)	47(1)	-2(1)	13(1)	2(1)
C(8)	45(1)	48(1)	48(1)	7(1)	8(1)	0(1)
C(7)	43(1)	46(1)	53(1)	6(1)	10(1)	4(1)
C(6)	40(1)	39(1)	51(1)	2(1)	15(1)	3(1)
C(9)	43(1)	54(2)	55(1)	9(1)	12(1)	-5(1)
C(5)	49(1)	41(1)	47(1)	-1(1)	10(1)	5(1)
C(10)	40(1)	54(1)	44(1)	-2(1)	11(1)	4(1)
C(11)	55(2)	39(1)	50(1)	1(1)	18(1)	6(1)
C(1)	84(2)	40(1)	59(2)	8(1)	19(1)	-1(1)
C(12)	79(2)	64(2)	51(2)	-13(1)	-1(1)	10(2)
C(4)	103(2)	63(2)	86(2)	-2(2)	21(2)	-24(2)
C(13)	95(2)	104(3)	82(2)	-10(2)	-19(2)	31(2)
C(14)	122(3)	148(4)	52(2)	-1(2)	14(2)	26(3)
C(3)	108(3)	85(3)	121(3)	50(3)	1(2)	17(2)
C(2)	171(4)	67(2)	95(3)	5(2)	74(3)	-19(3)
C(15)	100(3)	78(3)	127(4)	-19(2)	-16(2)	-8(2)

Table IX.30. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **153a**.

	x	y	z	U(eq)
H(4)	7281	166	4988	94
H(7A)	10611	1515	3738	56
H(7B)	10915	2648	4513	56
H(6)	10457	3880	3202	51
H(9A)	4944	2005	4576	61
H(4A)	12304	7585	3509	125
H(4B)	10228	8245	3827	125
H(4C)	11434	9029	3118	125
H(13A)	3921	4859	919	145
H(13B)	2492	3923	1464	145
H(13C)	2353	3741	399	145
H(14A)	7158	3943	384	161
H(14B)	5802	2790	-201	161
H(14C)	7817	2378	530	161

H(3A)	6755	8386	2715	160
H(3B)	6622	7673	1751	160
H(3C)	7892	9076	1948	160
H(2A)	12172	6613	1991	158
H(2B)	11248	7978	1496	158
H(2C)	9976	6575	1300	158
H(15A)	5374	799	1317	158
H(15B)	3245	1224	656	158
H(15C)	3399	1451	1717	158

Table IX.31. Torsion angles [°] for **153a**.

C(9)-C(8)-C(7)-C(6)	-24.2(3)
O(4)-C(8)-C(7)-C(6)	158.5(2)
C(10)-N(1)-C(6)-C(7)	-48.0(3)
C(5)-N(1)-C(6)-C(7)	139.4(2)
C(10)-N(1)-C(6)-C(11)	76.6(3)
C(5)-N(1)-C(6)-C(11)	-95.9(3)
C(8)-C(7)-C(6)-N(1)	48.3(3)
C(8)-C(7)-C(6)-C(11)	-77.9(3)
O(4)-C(8)-C(9)-C(10)	171.9(2)
C(7)-C(8)-C(9)-C(10)	-5.0(4)
C(1)-O(1)-C(5)-O(2)	1.4(4)
C(1)-O(1)-C(5)-N(1)	-176.8(2)
C(10)-N(1)-C(5)-O(2)	24.3(4)
C(6)-N(1)-C(5)-O(2)	-163.4(2)
C(10)-N(1)-C(5)-O(1)	-157.5(2)
C(6)-N(1)-C(5)-O(1)	14.8(3)
C(5)-N(1)-C(10)-O(3)	11.7(3)
C(6)-N(1)-C(10)-O(3)	-160.8(2)
C(5)-N(1)-C(10)-C(9)	-167.9(2)
C(6)-N(1)-C(10)-C(9)	19.5(3)
C(8)-C(9)-C(10)-O(3)	-171.1(3)
C(8)-C(9)-C(10)-N(1)	8.6(4)
C(12)-O(5)-C(11)-O(6)	-0.2(4)
C(12)-O(5)-C(11)-C(6)	178.2(2)
N(1)-C(6)-C(11)-O(6)	-170.3(2)
C(7)-C(6)-C(11)-O(6)	-45.6(3)
N(1)-C(6)-C(11)-O(5)	11.2(3)
C(7)-C(6)-C(11)-O(5)	135.9(2)
C(5)-O(1)-C(1)-C(4)	65.3(3)
C(5)-O(1)-C(1)-C(3)	-59.9(4)
C(5)-O(1)-C(1)-C(2)	-177.5(3)
C(11)-O(5)-C(12)-C(15)	67.2(4)
C(11)-O(5)-C(12)-C(14)	-59.3(4)
C(11)-O(5)-C(12)-C(13)	-176.6(3)

Table IX.32. Hydrogen bonds for **153a** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(4)-H(4)...O(3)#1	0.82	1.84	2.625(2)	159.4

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,y-1/2,-z+1

IX.7.6. Crystal data and structure refinement for 153c

Table IX.33. Crystal data and structure refinement for **153c**.
Identification code compound **153c**

Empirical formula	C14 H23 N O4	
Formula weight	269.33	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 2	
Unit cell dimensions	a = 14.6670(3) Å	α= 90°.
	b = 34.0857(8) Å	β= 90°.
	c = 6.33340(10) Å	γ = 90°.
Volume	3166.29(11) $Å^3$	
Z	8	
Density (calculated)	1.130 Mg/m^3	
Absorption coefficient	0.082 mm^{-1}	
F(000)	1168	
Crystal size	0.1 x 0.1 x 0.1 mm^3	
Theta range for data collection	3.31 to 25.35°.	
Index ranges	0<=h<=17, 0<=k<=40, 0<=l<=7	
Reflections collected	14369	
Independent reflections	3294 [R(int) = 0.0470]	
Completeness to theta = 25.35°	98.3 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3294 / 0 / 344	
Goodness-of-fit on F^2	1.016	
Final R indices [I>2sigma(I)]	R1 = 0.0473, wR2 = 0.1074	
R indices (all data)	R1 = 0.0935, wR2 = 0.1347	
Largest diff. peak and hole	0.141 and -0.158 $e.Å^{-3}$	

Table IX.34. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **153c**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(2)	5368(2)	7324(1)	3879(5)	59(1)
O(1)	7125(2)	6391(1)	7551(5)	71(1)
O(24)	7074(2)	8050(1)	12173(5)	60(1)
O(21)	5791(2)	7828(1)	6841(4)	63(1)
O(4)	7177(2)	5730(1)	2400(5)	64(1)
O(23)	6866(2)	7542(1)	9922(5)	66(1)
O(22)	3113(2)	8367(1)	9349(5)	64(1)
N(21)	5789(2)	8036(1)	10282(4)	45(1)
N(1)	6753(2)	6270(1)	4110(5)	47(1)
C(3)	6174(2)	6850(1)	5880(7)	51(1)
O(3)	8146(2)	5985(1)	4805(6)	88(1)
C(23)	4486(2)	8155(1)	8038(6)	50(1)
C(6)	6032(2)	6316(1)	2475(6)	46(1)
C(24)	3983(2)	8269(1)	9659(6)	45(1)
C(2)	6717(3)	6496(1)	5930(7)	54(1)
C(26)	5401(2)	8319(1)	11820(6)	43(1)
C(25)	4364(2)	8270(1)	11844(6)	44(1)
C(22)	5388(2)	7992(1)	8310(6)	47(1)
C(4)	5793(2)	6979(1)	4106(6)	47(1)
C(5)	5850(2)	6751(1)	2126(6)	48(1)
C(11)	7439(3)	5989(1)	3857(7)	57(1)
C(27)	5719(2)	8737(1)	11321(6)	50(1)
C(28)	5525(3)	9047(1)	12962(7)	65(1)
C(31)	6623(2)	7843(1)	10710(7)	50(1)
C(32)	7965(2)	7912(1)	12991(7)	62(1)
C(7)	5192(2)	6081(1)	3125(7)	61(1)
C(33)	7857(3)	7533(1)	14161(7)	68(1)

C(34)	8646(3)	7879(2)	11188(9)	94(2)
C(30)	5929(4)	8959(2)	15082(8)	105(2)
C(12)	7791(3)	5418(1)	1668(9)	78(1)
C(8)	4470(3)	6033(1)	1446(11)	95(2)
C(35)	8215(3)	8238(2)	14499(11)	109(2)
C(29)	5867(3)	9444(1)	12209(9)	93(2)
C(15)	7212(4)	5222(2)	-28(11)	118(2)
C(14)	8640(4)	5596(2)	677(12)	130(3)
C(13)	7987(5)	5138(2)	3422(10)	126(2)
C(10)	4823(5)	5835(3)	-508(11)	166(4)
C(9)	3645(3)	5808(2)	2351(14)	160(4)

Table IX.35. Bond lengths [Å] and angles [°] for **153c**.

O(2)-C(4)	1.338(4)
O(2)-H(2)	0.8200
O(1)-C(2)	1.242(5)
O(24)-C(31)	1.341(5)
O(24)-C(32)	1.483(4)
O(21)-C(22)	1.236(4)
O(4)-C(11)	1.335(5)
O(4)-C(12)	1.467(5)
O(23)-C(31)	1.195(4)
O(22)-C(24)	1.333(4)
O(22)-H(22)	0.8200
N(21)-C(22)	1.389(4)
N(21)-C(31)	1.414(5)
N(21)-C(26)	1.485(4)
N(1)-C(2)	1.389(5)
N(1)-C(11)	1.398(5)
N(1)-C(6)	1.487(5)
C(3)-C(4)	1.330(5)
C(3)-C(2)	1.445(5)
C(3)-H(3)	0.9300
O(3)-C(11)	1.199(5)
C(23)-C(24)	1.322(5)
C(23)-C(22)	1.445(5)
C(23)-H(23)	0.9300
C(6)-C(7)	1.525(5)
C(6)-C(5)	1.526(5)
C(6)-H(6)	0.9800
C(24)-C(25)	1.493(5)
C(26)-C(25)	1.530(5)
C(26)-C(27)	1.531(5)
C(26)-H(26)	0.9800
C(25)-H(25A)	0.9700
C(25)-H(25B)	0.9700
C(4)-C(5)	1.477(5)
C(5)-H(5A)	0.9700
C(5)-H(5B)	0.9700
C(27)-C(28)	1.510(6)
C(27)-H(27A)	0.9700
C(27)-H(27B)	0.9700
C(28)-C(30)	1.498(6)
C(28)-C(29)	1.522(6)
C(28)-H(28)	0.9800
C(32)-C(33)	1.497(6)
C(32)-C(35)	1.511(6)
C(32)-C(34)	1.521(6)
C(7)-C(8)	1.510(7)

C(7)-H(7A)	0.9700
C(7)-H(7B)	0.9700
C(33)-H(33A)	0.9600
C(33)-H(33B)	0.9600
C(33)-H(33C)	0.9600
C(34)-H(34A)	0.9600
C(34)-H(34B)	0.9600
C(34)-H(34C)	0.9600
C(30)-H(30A)	0.9600
C(30)-H(30B)	0.9600
C(30)-H(30C)	0.9600
C(12)-C(13)	1.494(7)
C(12)-C(14)	1.520(7)
C(12)-C(15)	1.524(7)
C(8)-C(10)	1.501(9)
C(8)-C(9)	1.542(8)
C(8)-H(8)	0.9800
C(35)-H(35A)	0.9600
C(35)-H(35B)	0.9600
C(35)-H(35C)	0.9600
C(29)-H(29A)	0.9600
C(29)-H(29B)	0.9600
C(29)-H(29C)	0.9600
C(15)-H(15A)	0.9600
C(15)-H(15B)	0.9600
C(15)-H(15C)	0.9600
C(14)-H(14A)	0.9600
C(14)-H(14B)	0.9600
C(14)-H(14C)	0.9600
C(13)-H(13A)	0.9600
C(13)-H(13B)	0.9600
C(13)-H(13C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(4)-O(2)-H(2)	109.5
C(31)-O(24)-C(32)	120.6(3)
C(11)-O(4)-C(12)	121.4(3)
C(24)-O(22)-H(22)	109.5
C(22)-N(21)-C(31)	119.3(3)
C(22)-N(21)-C(26)	119.8(3)
C(31)-N(21)-C(26)	120.5(3)
C(2)-N(1)-C(11)	120.2(3)
C(2)-N(1)-C(6)	119.5(3)
C(11)-N(1)-C(6)	120.2(3)
C(4)-C(3)-C(2)	121.8(4)
C(4)-C(3)-H(3)	119.1
C(2)-C(3)-H(3)	119.1
C(24)-C(23)-C(22)	122.1(4)
C(24)-C(23)-H(23)	119.0
C(22)-C(23)-H(23)	119.0
N(1)-C(6)-C(7)	109.3(3)
N(1)-C(6)-C(5)	109.1(3)
C(7)-C(6)-C(5)	114.0(3)
N(1)-C(6)-H(6)	108.1
C(7)-C(6)-H(6)	108.1
C(5)-C(6)-H(6)	108.1

C(23)-C(24)-O(22)	119.6(3)
C(23)-C(24)-C(25)	120.8(3)
O(22)-C(24)-C(25)	119.6(3)
O(1)-C(2)-N(1)	120.5(4)
O(1)-C(2)-C(3)	121.7(4)
N(1)-C(2)-C(3)	117.8(4)
N(21)-C(26)-C(25)	108.4(3)
N(21)-C(26)-C(27)	110.6(3)
C(25)-C(26)-C(27)	114.0(3)
N(21)-C(26)-H(26)	107.8
C(25)-C(26)-H(26)	107.8
C(27)-C(26)-H(26)	107.8
C(24)-C(25)-C(26)	111.3(3)
C(24)-C(25)-H(25A)	109.4
C(26)-C(25)-H(25A)	109.4
C(24)-C(25)-H(25B)	109.4
C(26)-C(25)-H(25B)	109.4
H(25A)-C(25)-H(25B)	108.0
O(21)-C(22)-N(21)	121.5(3)
O(21)-C(22)-C(23)	121.4(3)
N(21)-C(22)-C(23)	117.0(3)
C(3)-C(4)-O(2)	125.3(3)
C(3)-C(4)-C(5)	121.3(3)
O(2)-C(4)-C(5)	113.3(3)
C(4)-C(5)-C(6)	113.5(3)
C(4)-C(5)-H(5A)	108.9
C(6)-C(5)-H(5A)	108.9
C(4)-C(5)-H(5B)	108.9
C(6)-C(5)-H(5B)	108.9
H(5A)-C(5)-H(5B)	107.7
O(3)-C(11)-O(4)	125.9(4)
O(3)-C(11)-N(1)	125.1(4)
O(4)-C(11)-N(1)	109.0(3)
C(28)-C(27)-C(26)	116.9(3)
C(28)-C(27)-H(27A)	108.1
C(26)-C(27)-H(27A)	108.1
C(28)-C(27)-H(27B)	108.1
C(26)-C(27)-H(27B)	108.1
H(27A)-C(27)-H(27B)	107.3
C(30)-C(28)-C(27)	113.7(4)
C(30)-C(28)-C(29)	109.2(4)
C(27)-C(28)-C(29)	110.2(4)
C(30)-C(28)-H(28)	107.8
C(27)-C(28)-H(28)	107.8
C(29)-C(28)-H(28)	107.8
O(23)-C(31)-O(24)	126.4(3)
O(23)-C(31)-N(21)	125.2(4)
O(24)-C(31)-N(21)	108.3(3)
O(24)-C(32)-C(33)	110.7(3)
O(24)-C(32)-C(35)	101.6(3)
C(33)-C(32)-C(35)	110.4(4)
O(24)-C(32)-C(34)	109.9(3)
C(33)-C(32)-C(34)	112.1(4)
C(35)-C(32)-C(34)	111.8(4)
C(8)-C(7)-C(6)	115.7(4)
C(8)-C(7)-H(7A)	108.3
C(6)-C(7)-H(7A)	108.4
C(8)-C(7)-H(7B)	108.4
C(6)-C(7)-H(7B)	108.4
H(7A)-C(7)-H(7B)	107.4

C(32)-C(33)-H(33A)	109.5
C(32)-C(33)-H(33B)	109.5
H(33A)-C(33)-H(33B)	109.5
C(32)-C(33)-H(33C)	109.5
H(33A)-C(33)-H(33C)	109.5
H(33B)-C(33)-H(33C)	109.5
C(32)-C(34)-H(34A)	109.5
C(32)-C(34)-H(34B)	109.5
H(34A)-C(34)-H(34B)	109.5
C(32)-C(34)-H(34C)	109.5
H(34A)-C(34)-H(34C)	109.5
H(34B)-C(34)-H(34C)	109.5
C(28)-C(30)-H(30A)	109.5
C(28)-C(30)-H(30B)	109.5
H(30A)-C(30)-H(30B)	109.5
C(28)-C(30)-H(30C)	109.5
H(30A)-C(30)-H(30C)	109.5
H(30B)-C(30)-H(30C)	109.5
O(4)-C(12)-C(13)	110.3(4)
O(4)-C(12)-C(14)	110.2(4)
C(13)-C(12)-C(14)	113.9(5)
O(4)-C(12)-C(15)	101.5(4)
C(13)-C(12)-C(15)	110.5(5)
C(14)-C(12)-C(15)	109.9(5)
C(10)-C(8)-C(7)	112.8(4)
C(10)-C(8)-C(9)	110.8(5)
C(7)-C(8)-C(9)	110.0(5)
C(10)-C(8)-H(8)	107.7
C(7)-C(8)-H(8)	107.7
C(9)-C(8)-H(8)	107.7
C(32)-C(35)-H(35A)	109.5
C(32)-C(35)-H(35B)	109.5
H(35A)-C(35)-H(35B)	109.5
C(32)-C(35)-H(35C)	109.5
H(35A)-C(35)-H(35C)	109.5
H(35B)-C(35)-H(35C)	109.5
C(28)-C(29)-H(29A)	109.5
C(28)-C(29)-H(29B)	109.5
H(29A)-C(29)-H(29B)	109.5
C(28)-C(29)-H(29C)	109.5
H(29A)-C(29)-H(29C)	109.5
H(29B)-C(29)-H(29C)	109.5
C(12)-C(15)-H(15A)	109.5
C(12)-C(15)-H(15B)	109.5
H(15A)-C(15)-H(15B)	109.5
C(12)-C(15)-H(15C)	109.5
H(15A)-C(15)-H(15C)	109.5
H(15B)-C(15)-H(15C)	109.5
C(12)-C(14)-H(14A)	109.5
C(12)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
C(12)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
C(12)-C(13)-H(13A)	109.5
C(12)-C(13)-H(13B)	109.5
H(13A)-C(13)-H(13B)	109.5
C(12)-C(13)-H(13C)	109.5
H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5

C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5

Table IX.36. Anisotropic displacement parameters ($Å^2$x 10^3)for **153c**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(2)	68(2)	54(2)	55(2)	-9(1)	-1(1)	7(1)
O(1)	85(2)	81(2)	48(2)	3(2)	-19(2)	2(2)
O(24)	47(1)	55(2)	79(2)	-12(2)	-15(1)	4(1)
O(21)	63(2)	79(2)	48(2)	-20(2)	8(2)	4(1)
O(4)	66(2)	59(2)	66(2)	-9(2)	0(2)	12(1)
O(23)	72(2)	53(2)	73(2)	-11(2)	2(2)	13(1)
O(22)	48(2)	95(2)	50(2)	-6(2)	-2(1)	4(1)
N(21)	44(2)	48(2)	42(2)	-7(2)	1(2)	3(1)
N(1)	47(2)	51(2)	44(2)	-4(2)	-2(2)	4(1)
C(3)	60(2)	54(2)	40(2)	-7(2)	3(2)	-4(2)
O(3)	65(2)	99(3)	98(3)	-7(2)	-21(2)	23(2)
C(23)	50(2)	65(2)	34(2)	-4(2)	0(2)	-2(2)
C(6)	47(2)	49(2)	41(2)	-6(2)	-2(2)	1(2)
C(24)	39(2)	51(2)	44(2)	3(2)	-2(2)	-2(2)
C(2)	54(2)	61(3)	46(3)	2(2)	0(2)	-10(2)
C(26)	44(2)	46(2)	38(2)	-4(2)	-5(2)	2(2)
C(25)	49(2)	47(2)	37(2)	-1(2)	5(2)	-4(2)
C(22)	48(2)	51(2)	41(2)	-5(2)	6(2)	-7(2)
C(4)	45(2)	47(2)	48(2)	-5(2)	7(2)	-4(2)
C(5)	48(2)	53(2)	43(2)	-1(2)	-5(2)	0(2)
C(11)	55(3)	63(3)	54(3)	5(2)	-5(2)	0(2)
C(27)	49(2)	50(2)	50(2)	3(2)	-3(2)	-4(2)
C(28)	68(3)	47(2)	79(3)	-13(2)	-3(2)	-6(2)
C(31)	49(2)	51(2)	50(2)	2(2)	5(2)	-3(2)
C(32)	41(2)	61(3)	84(3)	2(2)	-9(2)	2(2)
C(7)	53(2)	55(2)	74(3)	-7(2)	-1(2)	-11(2)
C(33)	52(2)	78(3)	72(3)	9(3)	-2(2)	3(2)
C(34)	52(2)	107(4)	123(5)	40(4)	14(3)	0(2)
C(30)	184(6)	75(4)	58(3)	-10(3)	-17(4)	-22(3)
C(12)	83(3)	54(3)	98(4)	-2(3)	17(3)	20(2)
C(8)	76(3)	72(3)	135(6)	-7(3)	-34(4)	-12(2)
C(35)	76(3)	84(4)	168(6)	-27(4)	-62(4)	3(2)
C(29)	120(4)	52(3)	108(4)	0(3)	-20(4)	-17(2)
C(15)	147(5)	84(4)	122(5)	-35(4)	8(5)	25(3)
C(14)	117(4)	99(4)	175(7)	-16(5)	68(5)	15(3)
C(13)	177(6)	74(4)	126(6)	16(4)	-2(5)	37(4)
C(10)	145(6)	268(10)	85(5)	-30(6)	-26(5)	-95(6)
C(9)	72(3)	150(6)	257(10)	-84(7)	10(5)	-46(3)

Table IX.37. Hydrogen bonds for **153c** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2)...O(21)	0.82	1.81	2.620(4)	171.9
O(22)-H(22)...O(1)#1	0.82	1.80	2.576(4)	157.8

Symmetry transformations used to generate equivalent atoms:
#1 x-1/2,-y+3/2,-z+2

IX.7.7. Crystal data and structure refinement for 155c

Table IX.38. Crystal data and structure refinement for **155c**.

Identification code	compound **155c**	
Empirical formula	C9 H15 N O2	
Formula weight	169.22	
Temperature	293(2) K	
Wavelength	0.71070 Å	
Crystal system	triclinic	
Space group	P1	
Unit cell dimensions	a = 5.0760(2) Å	α= 95.6910(12)°.
	b = 10.0760(4) Å	β= 102.5290(13)°.
	c = 10.1210(5) Å	γ = 103.730(2)°.
Volume	484.65(4) $Å^3$	
Z	2	
Density (calculated)	1.160 Mg/m^3	
Absorption coefficient	0.081 mm^{-1}	
F(000)	184	
Crystal size	? x ? x ? mm^3	
Theta range for data collection	2.72 to 26.26°.	
Index ranges	-5<=h<=6, -12<=k<=12, -12<=l<=12	
Reflections collected	3727	
Independent reflections	3727 [R(int) = 0.0000]	
Completeness to theta = 26.26°	99.2 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3727 / 2 / 220	
Goodness-of-fit on F^2	0.934	
Final R indices [I>2sigma(I)]	R1 = 0.0451, wR2 = 0.1120	
R indices (all data)	R1 = 0.0769, wR2 = 0.1232	
Absolute structure parameter	-0.9(12)	
Largest diff. peak and hole	0.139 and -0.144 $e.Å^{-3}$	

Table IX.39. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **155c**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	13829(5)	1972(3)	127(3)	57(1)
C(1)	12802(5)	644(3)	168(3)	55(1)
O(1)	14130	-10	920	67(1)
C(2)	9896(6)	-76(3)	-705(3)	70(1)
C(3)	8618(7)	670(3)	-1759(3)	64(1)
O(2)	6162(5)	260(3)	-2347(3)	92(1)
C(4)	10538(7)	1936(3)	-2032(3)	68(1)
C(5)	12386(5)	2820(3)	-712(3)	56(1)
C(6)	14533(6)	4041(3)	-980(4)	74(1)
C(7)	13375(7)	5235(4)	-1403(4)	92(1)
C(8)	15262(10)	6116(5)	-2158(5)	135(2)
C(9)	13050(10)	6095(4)	-168(5)	139(2)

N(11)	9451(5)	1276(3)	2809(2)	53(1)
C(11)	10409(5)	2629(3)	2816(3)	60(1)
O(11)	9306(4)	3248(2)	1950(2)	76(1)
C(12)	12864(6)	3402(3)	3961(4)	87(1)
C(13)	14771(6)	2592(3)	4598(3)	63(1)
O(12)	17102(5)	3185(3)	5277(3)	100(1)
C(14)	13720(6)	1058(3)	4315(3)	60(1)
C(15)	10540(5)	520(3)	3878(3)	51(1)
C(16)	9549(6)	-1009(3)	3296(3)	63(1)
C(17)	10338(6)	-1989(3)	4276(3)	69(1)
C(18)	9205(9)	-1895(5)	5519(4)	113(1)
C(19)	9511(11)	-3457(4)	3505(5)	134(1)

Table IX.40. Bond lengths [Å] and angles [°] for **155c**.

N(1)-C(1)	1.325(3)
N(1)-C(5)	1.469(3)
N(1)-H(1)	1.027(10)
C(1)-O(1)	1.247(3)
C(1)-C(2)	1.510(4)
C(2)-C(3)	1.488(4)
C(2)-H(2A)	0.9700
C(2)-H(2B)	0.9700
C(3)-O(2)	1.210(3)
C(3)-C(4)	1.500(4)
C(4)-C(5)	1.511(4)
C(4)-H(4A)	0.9700
C(4)-H(4B)	0.9700
C(5)-C(6)	1.527(4)
C(5)-H(5A)	0.9800
C(6)-C(7)	1.518(4)
C(6)-H(6A)	0.9700
C(6)-H(6B)	0.9700
C(7)-C(9)	1.511(5)
C(7)-C(8)	1.529(5)
C(7)-H(7A)	0.9800
C(8)-H(8A)	0.9600
C(8)-H(8B)	0.9600
C(8)-H(8C)	0.9600
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
N(11)-C(11)	1.332(3)
N(11)-C(15)	1.470(3)
N(11)-H(11)	1.005(10)
C(11)-O(11)	1.238(3)
C(11)-C(12)	1.495(4)
C(12)-C(13)	1.492(4)
C(12)-H(12A)	0.9700
C(12)-H(12B)	0.9700
C(13)-O(12)	1.209(3)
C(13)-C(14)	1.487(4)
C(14)-C(15)	1.524(3)
C(14)-H(14A)	0.9700
C(14)-H(14B)	0.9700
C(15)-C(16)	1.515(3)
C(15)-H(15A)	0.9800
C(16)-C(17)	1.526(3)
C(16)-H(16A)	0.9700
C(16)-H(16B)	0.9700

C(17)-C(18)	1.497(4)
C(17)-C(19)	1.519(4)
C(17)-H(17A)	0.9800
C(18)-H(18A)	0.9600
C(18)-H(18B)	0.9600
C(18)-H(18C)	0.9600
C(19)-H(19A)	0.9600
C(19)-H(19B)	0.9600
C(19)-H(19C)	0.9600
C(1)-N(1)-C(5)	125.5(2)
C(1)-N(1)-H(1)	117.9(15)
C(5)-N(1)-H(1)	116.5(15)
O(1)-C(1)-N(1)	122.1(2)
O(1)-C(1)-C(2)	119.7(2)
N(1)-C(1)-C(2)	118.2(2)
C(3)-C(2)-C(1)	117.3(2)
C(3)-C(2)-H(2A)	108.0
C(1)-C(2)-H(2A)	108.0
C(3)-C(2)-H(2B)	108.0
C(1)-C(2)-H(2B)	108.0
H(2A)-C(2)-H(2B)	107.2
O(2)-C(3)-C(2)	120.7(2)
O(2)-C(3)-C(4)	123.0(2)
C(2)-C(3)-C(4)	116.2(2)
C(3)-C(4)-C(5)	111.2(2)
C(3)-C(4)-H(4A)	109.4
C(5)-C(4)-H(4A)	109.4
C(3)-C(4)-H(4B)	109.4
C(5)-C(4)-H(4B)	109.4
H(4A)-C(4)-H(4B)	108.0
N(1)-C(5)-C(4)	109.7(2)
N(1)-C(5)-C(6)	109.79(18)
C(4)-C(5)-C(6)	111.7(2)
N(1)-C(5)-H(5A)	108.5
C(4)-C(5)-H(5A)	108.5
C(6)-C(5)-H(5A)	108.5
C(7)-C(6)-C(5)	114.6(2)
C(7)-C(6)-H(6A)	108.6
C(5)-C(6)-H(6A)	108.6
C(7)-C(6)-H(6B)	108.6
C(5)-C(6)-H(6B)	108.6
H(6A)-C(6)-H(6B)	107.6
C(9)-C(7)-C(6)	110.5(3)
C(9)-C(7)-C(8)	111.2(3)
C(6)-C(7)-C(8)	110.2(3)
C(9)-C(7)-H(7A)	108.2
C(6)-C(7)-H(7A)	108.2
C(8)-C(7)-H(7A)	108.2
C(7)-C(8)-H(8A)	109.5
C(7)-C(8)-H(8B)	109.5
H(8A)-C(8)-H(8B)	109.5
C(7)-C(8)-H(8C)	109.5
H(8A)-C(8)-H(8C)	109.5
H(8B)-C(8)-H(8C)	109.5
C(7)-C(9)-H(9A)	109.5
C(7)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(7)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5

C(11)-N(11)-C(15)	124.6(2)
C(11)-N(11)-H(11)	114.4(15)
C(15)-N(11)-H(11)	120.1(15)
O(11)-C(11)-N(11)	122.7(2)
O(11)-C(11)-C(12)	120.3(2)
N(11)-C(11)-C(12)	116.9(2)
C(13)-C(12)-C(11)	116.8(2)
C(13)-C(12)-H(12A)	108.1
C(11)-C(12)-H(12A)	108.1
C(13)-C(12)-H(12B)	108.1
C(11)-C(12)-H(12B)	108.1
H(12A)-C(12)-H(12B)	107.3
O(12)-C(13)-C(14)	122.5(2)
O(12)-C(13)-C(12)	119.9(2)
C(14)-C(13)-C(12)	117.5(2)
C(13)-C(14)-C(15)	114.23(19)
C(13)-C(14)-H(14A)	108.7
C(15)-C(14)-H(14A)	108.7
C(13)-C(14)-H(14B)	108.7
C(15)-C(14)-H(14B)	108.7
H(14A)-C(14)-H(14B)	107.6
N(11)-C(15)-C(16)	107.75(19)
N(11)-C(15)-C(14)	108.81(18)
C(16)-C(15)-C(14)	112.90(18)
N(11)-C(15)-H(15A)	109.1
C(16)-C(15)-H(15A)	109.1
C(14)-C(15)-H(15A)	109.1
C(15)-C(16)-C(17)	116.09(19)
C(15)-C(16)-H(16A)	108.3
C(17)-C(16)-H(16A)	108.3
C(15)-C(16)-H(16B)	108.3
C(17)-C(16)-H(16B)	108.3
H(16A)-C(16)-H(16B)	107.4
C(18)-C(17)-C(19)	112.3(3)
C(18)-C(17)-C(16)	113.5(2)
C(19)-C(17)-C(16)	109.9(2)
C(18)-C(17)-H(17A)	106.9
C(19)-C(17)-H(17A)	106.9
C(16)-C(17)-H(17A)	106.9
C(17)-C(18)-H(18A)	109.5
C(17)-C(18)-H(18B)	109.5
H(18A)-C(18)-H(18B)	109.5
C(17)-C(18)-H(18C)	109.5
H(18A)-C(18)-H(18C)	109.5
H(18B)-C(18)-H(18C)	109.5
C(17)-C(19)-H(19A)	109.5
C(17)-C(19)-H(19B)	109.5
H(19A)-C(19)-H(19B)	109.5
C(17)-C(19)-H(19C)	109.5
H(19A)-C(19)-H(19C)	109.5
H(19B)-C(19)-H(19C)	109.5

Table IX.41. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **l55c**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	60(1)	53(1)	52(1)	10(1)	-1(1)	15(1)
C(1)	61(1)	55(2)	44(1)	10(1)	5(1)	15(1)
O(1)	72(1)	60(1)	62(1)	18(1)	-4(1)	21(1)

C(2)	67(2)	66(2)	61(2)	14(1)	-4(1)	7(1)
C(3)	67(2)	70(2)	48(1)	7(1)	-1(1)	18(1)
O(2)	66(1)	102(2)	88(1)	20(1)	-13(1)	12(1)
C(4)	72(2)	72(2)	56(2)	21(1)	1(1)	21(1)
C(5)	56(1)	56(1)	56(2)	14(1)	4(1)	20(1)
C(6)	69(2)	62(2)	84(2)	21(1)	3(1)	15(1)
C(7)	83(2)	61(2)	112(3)	30(2)	-12(2)	10(2)
C(8)	153(3)	89(2)	149(4)	59(3)	12(3)	11(2)
C(9)	157(4)	87(3)	178(5)	10(3)	31(3)	56(2)
N(11)	52(1)	57(1)	50(1)	15(1)	4(1)	18(1)
C(11)	50(1)	60(2)	66(2)	18(1)	0(1)	15(1)
O(11)	72(1)	64(1)	82(1)	27(1)	-4(1)	14(1)
C(12)	70(2)	62(2)	103(2)	10(2)	-23(2)	10(1)
C(13)	50(2)	73(2)	57(2)	17(1)	2(1)	8(1)
O(12)	74(1)	91(2)	106(2)	25(1)	-25(1)	4(1)
C(14)	50(1)	74(2)	58(2)	20(1)	8(1)	22(1)
C(15)	54(1)	60(2)	44(1)	15(1)	10(1)	21(1)
C(16)	72(2)	62(2)	58(2)	16(1)	12(1)	22(1)
C(17)	73(2)	61(2)	78(2)	21(1)	12(1)	28(1)
C(18)	160(3)	118(3)	95(2)	60(2)	58(2)	59(2)
C(19)	201(4)	75(2)	133(3)	24(2)	33(3)	56(3)

Table IX.42. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **l55c**.

	x	y	z	U(eq)
H(1)	15740(30)	2480(20)	780(20)	74
H(2A)	9953	-955	-1168	90
H(2B)	8662	-277	-99	90
H(4A)	9435	2473	-2535	88
H(4B)	11698	1659	-2595	88
H(5A)	11202	3182	-203	73
H(6A)	16038	4383	-155	96
H(6B)	15330	3712	-1696	96
H(7A)	11520	4850	-2033	119
H(8A)	15379	5551	-2954	176
H(8B)	17099	6492	-1560	176
H(8C)	14492	6857	-2434	176
H(9A)	11825	5522	273	181
H(9B)	12270	6830	-456	181
H(9C)	14849	6481	463	181
H(11)	7660(30)	820(20)	2100(20)	69
H(12A)	13964	4154	3618	113
H(12B)	12165	3813	4674	113
H(14A)	14406	692	5135	78
H(14B)	14489	707	3599	78
H(15A)	9775	685	4670	67
H(16A)	10303	-1156	2507	82
H(16B)	7526	-1258	2970	82
H(17A)	12384	-1714	4598	90
H(18A)	9827	-958	5989	147
H(18B)	9864	-2491	6119	147
H(18C)	7196	-2175	5247	147
H(19A)	10340	-3474	2738	174
H(19B)	7510	-3770	3181	174
H(19C)	10159	-4054	4107	174

Table IX.43. Torsion angles [°] for **l55c**.

C(5)-N(1)-C(1)-O(1)	-179.0(2)
C(5)-N(1)-C(1)-C(2)	-0.5(4)
O(1)-C(1)-C(2)-C(3)	-169.4(2)
N(1)-C(1)-C(2)-C(3)	12.1(4)
C(1)-C(2)-C(3)-O(2)	-168.6(3)
C(1)-C(2)-C(3)-C(4)	11.3(4)
O(2)-C(3)-C(4)-C(5)	135.9(3)
C(2)-C(3)-C(4)-C(5)	-44.0(3)
C(1)-N(1)-C(5)-C(4)	-32.5(3)
C(1)-N(1)-C(5)-C(6)	-155.6(2)
C(3)-C(4)-C(5)-N(1)	52.8(3)
C(3)-C(4)-C(5)-C(6)	174.7(2)
N(1)-C(5)-C(6)-C(7)	-160.4(2)
C(4)-C(5)-C(6)-C(7)	77.7(3)
C(5)-C(6)-C(7)-C(9)	78.2(3)
C(5)-C(6)-C(7)-C(8)	-158.4(3)
C(15)-N(11)-C(11)-O(11)	-174.6(2)
C(15)-N(11)-C(11)-C(12)	3.4(4)
O(11)-C(11)-C(12)-C(13)	-155.5(3)
N(11)-C(11)-C(12)-C(13)	26.4(4)
C(11)-C(12)-C(13)-O(12)	162.1(3)
C(11)-C(12)-C(13)-C(14)	-15.6(4)
O(12)-C(13)-C(14)-C(15)	160.3(2)
C(12)-C(13)-C(14)-C(15)	-22.0(4)
C(11)-N(11)-C(15)-C(16)	-163.1(2)
C(11)-N(11)-C(15)-C(14)	-40.4(3)
C(13)-C(14)-C(15)-N(11)	47.5(3)
C(13)-C(14)-C(15)-C(16)	167.1(2)
N(11)-C(15)-C(16)-C(17)	-177.02(18)
C(14)-C(15)-C(16)-C(17)	62.8(3)
C(15)-C(16)-C(17)-C(18)	60.1(3)
C(15)-C(16)-C(17)-C(19)	-173.3(3)

Table IX.44. Hydrogen bonds for **155c** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1)...O(11)#1	1.027(10)	1.873(10)	2.897(2)	175(2)
N(11)-H(11)...O(1)#2	1.005(10)	1.869(10)	2.870(2)	174(2)

Symmetry transformations used to generate equivalent atoms:
#1 x+1,y,z #2 x-1,y,z

IX.7.8. Crystal data and structure refinement for 156c

Table IX.45. Crystal data and structure refinement for **156c**.

Identification code	compound **156c**	
Empirical formula	C9 H17 N O2	
Formula weight	171.24	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 12.9121(6) Å	α= 90°.
	b = 5.3871(3) Å	β= 109.692(2)°.
	c = 14.8945(7) Å	γ = 90°.
Volume	975.45(8) Å^3	

Z	4
Density (calculated)	1.166 Mg/m^3
Absorption coefficient	0.081 mm^{-1}
F(000)	376
Crystal size	0.2 x 0.1 x 0.1 mm^3
Theta range for data collection	2.56 to 26.32°.
Index ranges	0<=h<=16, 0<=k<=6, -18<=l<=17
Reflections collected	18084
Independent reflections	2196 [R(int) = 0.0790]
Completeness to theta = 26.32°	99.3 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2196 / 1 / 225
Goodness-of-fit on F^2	1.088
Final R indices [I>2sigma(I)]	R1 = 0.0589, wR2 = 0.1477
R indices (all data)	R1 = 0.0817, wR2 = 0.1680
Largest diff. peak and hole	0.487 and -0.451 $e.Å^{-3}$

Table IX.46. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **156c**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(11)	8169(2)	2590(5)	9493(2)	29(1)
O(1)	6797(2)	6833(5)	10497(2)	31(1)
N(11)	8348(2)	2785(7)	11050(2)	28(1)
O(12)	10680(2)	-2872(6)	11761(2)	34(1)
N(1)	6821(2)	7076(7)	8992(2)	29(1)
C(2)	6461(3)	7831(8)	9686(2)	27(1)
C(13)	9376(3)	-383(8)	10533(2)	29(1)
C(12)	8593(3)	1808(7)	10330(2)	26(1)
C(14)	10113(3)	-540(7)	11570(2)	27(1)
C(17)	7946(3)	2356(9)	12523(2)	31(1)
C(15)	9409(3)	-331(8)	12198(2)	32(1)
C(16)	8849(3)	2200(8)	12071(2)	28(1)
C(18)	8351(3)	2045(10)	13609(3)	40(1)
C(10)	7615(4)	7625(14)	6381(3)	61(2)
C(19)	9153(4)	4066(11)	14118(3)	60(1)
C(9)	5983(4)	4897(11)	5614(3)	60(2)
C(20)	7353(4)	1934(15)	13941(3)	69(2)
C(7)	6437(3)	6093(8)	7313(3)	35(1)
C(8)	6464(3)	6942(10)	6341(3)	39(1)
C(4)	5349(5)	11097(15)	8534(3)	77(2)
C(3)	5634(3)	9885(9)	9485(3)	36(1)
C(5)	5461(5)	9606(11)	7798(3)	61(1)
O(2)	4405(2)	12579(8)	8265(2)	55(1)
C(6)	6537(3)	8154(8)	8031(2)	31(1)

Table IX.47. Bond lengths [Å] and angles [°] for **156c**.

O(11)-C(12)	1.253(4)
O(1)-C(2)	1.258(4)
N(11)-C(12)	1.325(4)
N(11)-C(16)	1.472(4)
N(11)-H(11)	0.8600
O(12)-C(14)	1.433(5)
O(12)-H(12)	0.8200
N(1)-C(2)	1.332(4)
N(1)-C(6)	1.473(4)
N(1)-H(1)	0.8600

C(2)-C(3)	1.496(6)
C(13)-C(12)	1.518(6)
C(13)-C(14)	1.519(5)
C(13)-H(13A)	0.9700
C(13)-H(13B)	0.9700
C(14)-C(15)	1.512(5)
C(14)-H(14)	0.9800
C(17)-C(18)	1.532(5)
C(17)-C(16)	1.532(5)
C(17)-H(17A)	0.9700
C(17)-H(17B)	0.9700
C(15)-C(16)	1.525(6)
C(15)-H(15A)	0.9700
C(15)-H(15B)	0.9700
C(16)-H(16)	0.9800
C(18)-C(19)	1.518(7)
C(18)-C(20)	1.529(6)
C(18)-H(18)	0.9800
C(10)-C(8)	1.513(6)
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(19)-H(19A)	0.9600
C(19)-H(19B)	0.9600
C(19)-H(19C)	0.9600
C(9)-C(8)	1.523(7)
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(20)-H(20A)	0.9600
C(20)-H(20B)	0.9600
C(20)-H(20C)	0.9600
C(7)-C(6)	1.516(5)
C(7)-C(8)	1.530(5)
C(7)-H(7A)	0.9700
C(7)-H(7B)	0.9700
C(8)-H(8)	0.9800
C(4)-O(2)	1.398(6)
C(4)-C(5)	1.406(8)
C(4)-C(3)	1.489(6)
C(4)-H(4)	0.9800
C(3)-H(3A)	0.9700
C(3)-H(3B)	0.9700
C(5)-C(6)	1.528(6)
C(5)-H(5A)	0.9700
C(5)-H(5B)	0.9700
O(2)-H(2)	0.8200
C(6)-H(6)	0.9800
C(12)-N(11)-C(16)	127.6(3)
C(12)-N(11)-H(11)	116.2
C(16)-N(11)-H(11)	116.2
C(14)-O(12)-H(12)	109.5
C(2)-N(1)-C(6)	126.6(3)
C(2)-N(1)-H(1)	116.7
C(6)-N(1)-H(1)	116.7
O(1)-C(2)-N(1)	121.2(3)
O(1)-C(2)-C(3)	120.0(3)
N(1)-C(2)-C(3)	118.9(3)
C(12)-C(13)-C(14)	113.1(3)
C(12)-C(13)-H(13A)	109.0

C(14)-C(13)-H(13A)	109.0
C(12)-C(13)-H(13B)	109.0
C(14)-C(13)-H(13B)	109.0
H(13A)-C(13)-H(13B)	107.8
O(11)-C(12)-N(11)	121.9(3)
O(11)-C(12)-C(13)	119.8(3)
N(11)-C(12)-C(13)	118.2(3)
O(12)-C(14)-C(15)	108.4(3)
O(12)-C(14)-C(13)	110.7(3)
C(15)-C(14)-C(13)	108.9(3)
O(12)-C(14)-H(14)	109.6
C(15)-C(14)-H(14)	109.6
C(13)-C(14)-H(14)	109.6
C(18)-C(17)-C(16)	114.7(3)
C(18)-C(17)-H(17A)	108.6
C(16)-C(17)-H(17A)	108.6
C(18)-C(17)-H(17B)	108.6
C(16)-C(17)-H(17B)	108.6
H(17A)-C(17)-H(17B)	107.6
C(14)-C(15)-C(16)	110.4(3)
C(14)-C(15)-H(15A)	109.6
C(16)-C(15)-H(15A)	109.6
C(14)-C(15)-H(15B)	109.6
C(16)-C(15)-H(15B)	109.6
H(15A)-C(15)-H(15B)	108.1
N(11)-C(16)-C(15)	110.1(3)
N(11)-C(16)-C(17)	107.9(3)
C(15)-C(16)-C(17)	113.2(3)
N(11)-C(16)-H(16)	108.5
C(15)-C(16)-H(16)	108.5
C(17)-C(16)-H(16)	108.5
C(19)-C(18)-C(20)	111.9(5)
C(19)-C(18)-C(17)	112.4(4)
C(20)-C(18)-C(17)	108.8(3)
C(19)-C(18)-H(18)	107.8
C(20)-C(18)-H(18)	107.8
C(17)-C(18)-H(18)	107.8
C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
C(18)-C(19)-H(19A)	109.5
C(18)-C(19)-H(19B)	109.5
H(19A)-C(19)-H(19B)	109.5
C(18)-C(19)-H(19C)	109.5
H(19A)-C(19)-H(19C)	109.5
H(19B)-C(19)-H(19C)	109.5
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(18)-C(20)-H(20A)	109.5
C(18)-C(20)-H(20B)	109.5
H(20A)-C(20)-H(20B)	109.5
C(18)-C(20)-H(20C)	109.5
H(20A)-C(20)-H(20C)	109.5

H(20B)-C(20)-H(20C)	109.5
C(6)-C(7)-C(8)	115.1(4)
C(6)-C(7)-H(7A)	108.5
C(8)-C(7)-H(7A)	108.5
C(6)-C(7)-H(7B)	108.5
C(8)-C(7)-H(7B)	108.5
H(7A)-C(7)-H(7B)	107.5
C(10)-C(8)-C(9)	111.3(4)
C(10)-C(8)-C(7)	111.8(3)
C(9)-C(8)-C(7)	108.9(4)
C(10)-C(8)-H(8)	108.3
C(9)-C(8)-H(8)	108.3
C(7)-C(8)-H(8)	108.3
O(2)-C(4)-C(5)	114.3(4)
O(2)-C(4)-C(3)	115.9(4)
C(5)-C(4)-C(3)	115.7(5)
O(2)-C(4)-H(4)	102.7
C(5)-C(4)-H(4)	102.7
C(3)-C(4)-H(4)	102.7
C(4)-C(3)-C(2)	116.5(3)
C(4)-C(3)-H(3A)	108.2
C(2)-C(3)-H(3A)	108.2
C(4)-C(3)-H(3B)	108.2
C(2)-C(3)-H(3B)	108.2
H(3A)-C(3)-H(3B)	107.3
C(4)-C(5)-C(6)	116.1(4)
C(4)-C(5)-H(5A)	108.3
C(6)-C(5)-H(5A)	108.3
C(4)-C(5)-H(5B)	108.3
C(6)-C(5)-H(5B)	108.3
H(5A)-C(5)-H(5B)	107.4
C(4)-O(2)-H(2)	109.5
N(1)-C(6)-C(7)	109.3(3)
N(1)-C(6)-C(5)	109.4(3)
C(7)-C(6)-C(5)	111.1(3)
N(1)-C(6)-H(6)	109.0
C(7)-C(6)-H(6)	109.0
C(5)-C(6)-H(6)	109.0

Table IX.48. Anisotropic displacement parameters ($Å^2 x\ 10^3$)for **156c**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(11)	29(1)	30(2)	25(1)	3(1)	7(1)	4(1)
O(1)	29(1)	32(2)	32(1)	6(1)	11(1)	3(1)
N(11)	27(1)	30(2)	27(1)	5(1)	7(1)	6(2)
O(12)	33(1)	34(2)	39(1)	10(1)	16(1)	13(1)
N(1)	32(2)	27(2)	31(2)	2(2)	11(1)	3(2)
C(2)	24(2)	26(2)	31(2)	3(2)	10(1)	-1(2)
C(13)	29(2)	29(2)	30(2)	-2(2)	11(1)	0(2)
C(12)	23(2)	22(2)	32(2)	-1(2)	7(1)	-2(2)
C(14)	26(2)	21(2)	33(2)	1(2)	9(1)	-2(2)
C(17)	31(2)	33(2)	30(2)	1(2)	11(1)	5(2)
C(15)	33(2)	33(2)	27(2)	6(2)	9(1)	7(2)
C(16)	27(2)	28(2)	26(2)	0(2)	4(1)	-1(2)
C(18)	45(2)	46(3)	32(2)	6(2)	16(2)	13(2)
C(10)	56(3)	97(5)	33(2)	3(3)	18(2)	6(3)
H(10A)	90(40)	100(50)	50(30)	0(40)	40(30)	-10(40)
C(19)	84(3)	54(3)	35(2)	-5(2)	12(2)	2(3)

C(9)	74(3)	59(4)	36(2)	-10(3)	4(2)	17(3)
C(20)	66(3)	106(5)	47(2)	18(3)	34(2)	26(4)
C(7)	36(2)	29(2)	36(2)	-3(2)	5(2)	2(2)
C(8)	46(2)	38(3)	29(2)	1(2)	5(2)	15(2)
C(4)	72(3)	124(6)	45(3)	31(3)	32(2)	65(4)
C(3)	39(2)	35(2)	37(2)	7(2)	17(2)	12(2)
C(5)	87(4)	51(3)	45(2)	13(3)	24(2)	37(3)
O(2)	44(2)	81(3)	47(2)	29(2)	24(1)	31(2)
C(6)	40(2)	24(2)	29(2)	2(2)	10(2)	1(2)

IX.7.9. Crystal data and structure refinement for 179

Table IX.49. Crystal data and structure refinement for **179**.

Identification code	compound **179**	
Empirical formula	C22 H29 N2 O8	
Formula weight	449.47	
Temperature	293(2) K	
Wavelength	0.71070 A	
Crystal system, space group	Orthorhombic, P 21 21 21	
Unit cell dimensions	a = 6.2400(2) A	alpha = 90 deg.
	b = 13.5460(4) A	beta = 90 deg.
	c = 29.7090(9) A	gamma = 90 deg.
Volume	2511.21(13) A^3	
Z, Calculated density	4, 1.189 Mg/m^3	
Absorption coefficient	0.091 mm^-1	
F(000)	956	
Crystal size	0.3 x 0.2 x 0.2 mm	
Theta range for data collection	3.64 to 23.27 deg.	
Limiting indices	-6<=h<=6, -15<=k<=15, -32<=l<=32	
Reflections collected / unique	2091 / 2091 [R(int) = 0.0000]	
Completeness to theta =	23.27 99.1 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2091 / 0 / 290	
Goodness-of-fit on F^2	1.009	
Final R indices [I>2sigma(I)]	R1 = 0.0435, wR2 = 0.1084	
R indices (all data)	R1 = 0.0610, wR2 = 0.1182	
Extinction coefficient	0.009(2)	
Largest diff. peak and hole	0.307 and -0.173 e.A^-3	

Table IX.50. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **179**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(3)	2134(4)	8086(2)	6638(1)	61(1)
O(4)	2095(4)	7779(2)	5895(1)	74(1)
O(8)	-3378(4)	11901(2)	6574(1)	64(1)
O(7)	-4982(4)	11170(2)	7173(1)	74(1)
O(1)	-1562(4)	10301(2)	5596(1)	81(1)
N(1)	-2257(4)	10418(2)	6794(1)	48(1)
O(2)	-4182(5)	9700(3)	6019(1)	96(1)
O(6)	11432(5)	5126(2)	6219(1)	105(1)
C(7)	883(5)	9711(2)	6407(1)	53(1)
C(12)	4842(5)	7069(2)	6351(1)	52(1)
N(2)	10392(5)	5354(2)	6546(1)	75(1)
C(13)	5391(6)	6759(3)	6776(1)	60(1)

C(15)	8429(6)	5960(2)	6480(1)	58(1)
C(17)	6118(6)	6807(3)	5986(1)	65(1)
C(11)	2887(6)	7679(2)	6264(1)	58(1)
O(5)	10867(5)	5127(3)	6927(1)	116(1)
C(18)	-3674(6)	11167(3)	6874(1)	52(1)
C(6)	-1072(5)	10388(2)	6373(1)	46(1)
C(5)	-2494(6)	10088(3)	5980(1)	56(1)
C(10)	-2679(6)	9490(2)	7030(1)	58(1)
C(8)	234(5)	8715(2)	6589(1)	53(1)
C(9)	-692(6)	8852(3)	7056(1)	60(1)
C(16)	7925(6)	6251(3)	6049(1)	65(1)
C(14)	7200(6)	6192(3)	6845(1)	62(1)
C(19)	-4652(6)	12807(3)	6584(1)	70(1)
C(22)	-3757(9)	13377(3)	6191(1)	96(1)
C(20)	-4269(11)	13361(3)	7013(1)	109(2)
C(21)	-7007(8)	12561(4)	6511(2)	129(2)
C(1)	-2511(8)	10028(4)	5154(1)	98(2)
C(3)	-2813(12)	8932(5)	5131(2)	147(2)
C(4)	-4582(12)	10613(6)	5100(2)	167(3)
C(2)	-796(12)	10376(7)	4834(2)	205(5)

Table IX.51. Bond lengths [A] and angles [deg] for **179**.

O(3)-C(11)	1.325(4)
O(3)-C(8)	1.467(4)
O(4)-C(11)	1.210(4)
O(8)-C(18)	1.348(4)
O(8)-C(19)	1.462(4)
O(7)-C(18)	1.207(4)
O(1)-C(5)	1.313(4)
O(1)-C(1)	1.488(5)
N(1)-C(18)	1.366(4)
N(1)-C(6)	1.455(4)
N(1)-C(10)	1.462(4)
O(2)-C(5)	1.183(4)
O(6)-N(2)	1.209(4)
C(7)-C(8)	1.509(5)
C(7)-C(6)	1.530(4)
C(7)-H(2A)	0.9700
C(7)-H(2B)	0.9700
C(12)-C(13)	1.375(4)
C(12)-C(17)	1.391(5)
C(12)-C(11)	1.496(5)
N(2)-O(5)	1.210(4)
N(2)-C(15)	1.488(5)
C(13)-C(14)	1.380(5)
C(13)-H(12)	0.9300
C(15)-C(14)	1.364(5)
C(15)-C(16)	1.377(5)
C(17)-C(16)	1.369(5)
C(17)-H(14)	0.9300
C(6)-C(5)	1.520(5)
C(6)-H(18)	0.9800
C(10)-C(9)	1.514(5)
C(10)-H(20A)	0.9700
C(10)-H(20B)	0.9700
C(8)-C(9)	1.517(4)
C(9)-H(22A)	0.9700
C(9)-H(22B)	0.9700

C(16)-H(23)	0.9300
C(14)-H(24)	0.9300
C(19)-C(20)	1.498(5)
C(19)-C(22)	1.507(6)
C(19)-C(21)	1.522(6)
C(22)-H(26A)	0.9600
C(22)-H(26B)	0.9600
C(22)-H(26C)	0.9600
C(20)-H(27A)	0.9600
C(20)-H(27B)	0.9600
C(20)-H(27C)	0.9600
C(21)-H(28A)	0.9600
C(21)-H(28B)	0.9600
C(21)-H(28C)	0.9600
C(1)-C(3)	1.497(7)
C(1)-C(2)	1.506(8)
C(1)-C(4)	1.525(8)
C(3)-H(30A)	0.9600
C(3)-H(30B)	0.9600
C(3)-H(30C)	0.9600
C(4)-H(31A)	0.9600
C(4)-H(31B)	0.9600
C(4)-H(31C)	0.9600
C(2)-H(32A)	0.9600
C(2)-H(32B)	0.9600
C(2)-H(32C)	0.9600
C(11)-O(3)-C(8)	116.4(3)
C(18)-O(8)-C(19)	122.1(3)
C(5)-O(1)-C(1)	122.5(3)
C(18)-N(1)-C(6)	119.9(3)
C(18)-N(1)-C(10)	116.0(2)
C(6)-N(1)-C(10)	118.6(2)
C(8)-C(7)-C(6)	110.2(2)
C(8)-C(7)-H(2A)	109.6
C(6)-C(7)-H(2A)	109.6
C(8)-C(7)-H(2B)	109.6
C(6)-C(7)-H(2B)	109.6
H(2A)-C(7)-H(2B)	108.1
C(13)-C(12)-C(17)	119.7(3)
C(13)-C(12)-C(11)	122.0(3)
C(17)-C(12)-C(11)	118.3(3)
O(5)-N(2)-O(6)	123.8(3)
O(5)-N(2)-C(15)	117.7(3)
O(6)-N(2)-C(15)	118.4(3)
C(12)-C(13)-C(14)	120.6(3)
C(12)-C(13)-H(12)	119.7
C(14)-C(13)-H(12)	119.7
C(14)-C(15)-C(16)	123.0(3)
C(14)-C(15)-N(2)	119.1(3)
C(16)-C(15)-N(2)	117.9(3)
C(16)-C(17)-C(12)	120.4(3)
C(16)-C(17)-H(14)	119.8
C(12)-C(17)-H(14)	119.8
O(4)-C(11)-O(3)	124.5(3)
O(4)-C(11)-C(12)	123.4(3)
O(3)-C(11)-C(12)	112.1(3)
O(7)-C(18)-O(8)	125.2(3)
O(7)-C(18)-N(1)	124.5(3)
O(8)-C(18)-N(1)	110.2(3)
N(1)-C(6)-C(5)	111.8(2)

N(1)-C(6)-C(7)	111.4(2)
C(5)-C(6)-C(7)	110.9(2)
N(1)-C(6)-H(18)	107.5
C(5)-C(6)-H(18)	107.5
C(7)-C(6)-H(18)	107.5
O(2)-C(5)-O(1)	125.3(3)
O(2)-C(5)-C(6)	124.2(3)
O(1)-C(5)-C(6)	110.5(3)
N(1)-C(10)-C(9)	111.6(3)
N(1)-C(10)-H(20A)	109.3
C(9)-C(10)-H(20A)	109.3
N(1)-C(10)-H(20B)	109.3
C(9)-C(10)-H(20B)	109.3
H(20A)-C(10)-H(20B)	108.0
O(3)-C(8)-C(7)	109.7(2)
O(3)-C(8)-C(9)	106.7(3)
C(7)-C(8)-C(9)	108.7(3)
C(10)-C(9)-C(8)	109.5(3)
C(10)-C(9)-H(22A)	109.8
C(8)-C(9)-H(22A)	109.8
C(10)-C(9)-H(22B)	109.8
C(8)-C(9)-H(22B)	109.8
H(22A)-C(9)-H(22B)	108.2
C(17)-C(16)-C(15)	118.2(3)
C(17)-C(16)-H(23)	120.9
C(15)-C(16)-H(23)	120.9
C(15)-C(14)-C(13)	118.1(3)
C(15)-C(14)-H(24)	120.9
C(13)-C(14)-H(24)	120.9
O(8)-C(19)-C(20)	110.5(3)
O(8)-C(19)-C(22)	102.3(3)
C(20)-C(19)-C(22)	110.1(4)
O(8)-C(19)-C(21)	109.8(4)
C(20)-C(19)-C(21)	112.7(5)
C(22)-C(19)-C(21)	111.1(4)
C(19)-C(22)-H(26A)	109.5
C(19)-C(22)-H(26B)	109.5
H(26A)-C(22)-H(26B)	109.5
C(19)-C(22)-H(26C)	109.5
H(26A)-C(22)-H(26C)	109.5
H(26B)-C(22)-H(26C)	109.5
C(19)-C(20)-H(27A)	109.5
C(19)-C(20)-H(27B)	109.5
H(27A)-C(20)-H(27B)	109.5
C(19)-C(20)-H(27C)	109.5
H(27A)-C(20)-H(27C)	109.5
H(27B)-C(20)-H(27C)	109.5
C(19)-C(21)-H(28A)	109.5
C(19)-C(21)-H(28B)	109.5
H(28A)-C(21)-H(28B)	109.5
C(19)-C(21)-H(28C)	109.5
H(28A)-C(21)-H(28C)	109.5
H(28B)-C(21)-H(28C)	109.5
O(1)-C(1)-C(3)	109.7(4)
O(1)-C(1)-C(2)	101.3(4)
C(3)-C(1)-C(2)	111.7(6)
O(1)-C(1)-C(4)	107.5(4)
C(3)-C(1)-C(4)	113.8(6)
C(2)-C(1)-C(4)	111.9(5)
C(1)-C(3)-H(30A)	109.5

C(1)-C(3)-H(30B)	109.5
H(30A)-C(3)-H(30B)	109.5
C(1)-C(3)-H(30C)	109.5
H(30A)-C(3)-H(30C)	109.5
H(30B)-C(3)-H(30C)	109.5
C(1)-C(4)-H(31A)	109.5
C(1)-C(4)-H(31B)	109.5
H(31A)-C(4)-H(31B)	109.5
C(1)-C(4)-H(31C)	109.5
H(31A)-C(4)-H(31C)	109.5
H(31B)-C(4)-H(31C)	109.5
C(1)-C(2)-H(32A)	109.5
C(1)-C(2)-H(32B)	109.5
H(32A)-C(2)-H(32B)	109.5
C(1)-C(2)-H(32C)	109.5
H(32A)-C(2)-H(32C)	109.5
H(32B)-C(2)-H(32C)	109.5

Table IX.52. Anisotropic displacement parameters (A^2 x 10^3) for **l79**.
The anisotropic displacement factor exponent takes the form:
$-2\ pi^2\ [\ h^2\ a^{*2}\ U11 + ... + 2\ h\ k\ a^*\ b^*\ U12\ ]$

	U11	U22	U33	U23	U13	U12	
O(3)		60(1)	60(1)	63(1)	-7(1)	-1(1)	16(1)
O(4)	74(2)	81(2)	67(2)	-18(1)	-11(1)	19(2)	
O(8)	78(2)	51(1)	63(1)	5(1)	10(1)	21(1)	
O(7)	83(2)	69(2)	70(2)	0(1)	29(2)	19(2)	
O(1)		76(2)	118(2)	48(1)	6(1)	4(1)	-16(2)
N(1)	52(2)	47(2)	46(1)	2(1)	5(1)	9(1)	
O(2)	59(2)	162(3)	68(2)	-8(2)	4(1)	-25(2)	
O(6)	91(2)	113(2)	110(2)	6(2)	26(2)	47(2)	
C(7)	44(2)	59(2)	56(2)	-7(2)	4(2)	1(2)	
C(12)		55(2)	37(2)	63(2)	-7(2)	2(2)	3(2)
N(2)	69(2)	63(2)	94(3)	7(2)	6(2)	15(2)	
C(13)		63(2)	56(2)	60(2)	-3(2)	7(2)	8(2)
C(15)		54(2)	44(2)	76(2)	-3(2)	1(2)	9(2)
C(17)		71(3)	61(2)	63(2)	-6(2)	-4(2)	16(2)
C(11)		57(2)	55(2)	63(2)	-10(2)	-2(2)	0(2)
O(5)		102(2)	144(3)	103(2)	24(2)	-2(2)	55(2)
C(18)		57(2)	50(2)	50(2)	-5(2)	0(2)	7(2)
C(6)	46(2)	46(2)	46(2)	-3(2)	4(1)	3(2)	
C(5)	44(2)	70(2)	52(2)	3(2)	6(2)	9(2)	
C(10)		60(2)	57(2)	57(2)	4(2)	10(2)	6(2)
C(8)	47(2)	50(2)	62(2)	0(2)	6(2)	15(2)	
C(9)	67(2)	56(2)	58(2)	6(2)	3(2)	14(2)	
C(16)		65(2)	62(2)	67(2)	-2(2)	15(2)	9(2)
C(14)		66(2)	58(2)	61(2)	1(2)	2(2)	13(2)
C(19)		77(3)	52(2)	82(2)	2(2)	-2(2)	18(2)
C(22)		127(4)	61(3)	100(3)	15(2)	-4(3)	11(3)
C(20)		166(5)	69(3)	91(3)	-23(2)	10(3)	4(3)
C(21)		76(3)	90(4)	220(6)	21(4)	-30(4)	14(3)
C(1)	98(3)	152(5)	45(2)	8(3)	-7(2)	-16(4)	
C(3)		202(7)	154(6)	87(3)	-39(4)	-25(4)	5(6)
C(4)		188(7)	209(7)	103(4)	6(4)	-65(4)	62(6)
C(2)		203(7)	361(12)	52(3)	8(5)	11(4)	-118(9)

Table IX.53. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **179**.

	x	y	z	U(eq)
H(2A)	1526	9632	6112	64
H(2B)	1941	10007	6605	64
H(12)	4537	6934	7020	71
H(14)	5742	7011	5698	78
H(18)	-549	11057	6312	55
H(20A)	-3801	9132	6873	70
H(20B)	-3183	9633	7332	70
H(22A)	-1054	8215	7184	72
H(22B)	362	9163	7250	72
H(23)	8789	6074	5807	78
H(24)	7569	5974	7131	74
H(26A)	-4004	13016	5918	144
H(26B)	-2244	13471	6232	144
H(26C)	-4451	14008	6173	144
H(27A)	-4839	12991	7262	163
H(27B)	-4964	13992	6998	163
H(27C)	-2757	13454	7055	163
H(28A)	-7169	12208	6233	193
H(28B)	-7824	13161	6499	193
H(28C)	-7513	12160	6755	193
H(30A)	-1455	8610	5169	221
H(30B)	-3407	8758	4843	221
H(30C)	-3772	8726	5365	221
H(31A)	-4279	11305	5123	250
H(31B)	-5572	10426	5333	250
H(31C)	-5202	10474	4811	250
H(32A)	-640	11078	4859	308
H(32B)	-1194	10208	4531	308
H(32C)	538	10062	4908	308

Table IX.54. Torsion angles [deg] for **179**.

C(17)-C(12)-C(13)-C(14)	0.0(5)
C(11)-C(12)-C(13)-C(14)	179.6(3)
O(5)-N(2)-C(15)-C(14)	-3.2(5)
O(6)-N(2)-C(15)-C(14)	177.7(4)
O(5)-N(2)-C(15)-C(16)	177.6(4)
O(6)-N(2)-C(15)-C(16)	-1.5(5)
C(13)-C(12)-C(17)-C(16)	-0.5(5)
C(11)-C(12)-C(17)-C(16)	179.9(3)
C(8)-O(3)-C(11)-O(4)	-1.3(5)
C(8)-O(3)-C(11)-C(12)	179.0(3)
C(13)-C(12)-C(11)-O(4)	-163.0(3)
C(17)-C(12)-C(11)-O(4)	16.6(5)
C(13)-C(12)-C(11)-O(3)	16.7(4)
C(17)-C(12)-C(11)-O(3)	-163.7(3)
C(19)-O(8)-C(18)-O(7)	0.2(5)
C(19)-O(8)-C(18)-N(1)	-178.9(3)
C(6)-N(1)-C(18)-O(7)	166.9(3)
C(10)-N(1)-C(18)-O(7)	13.2(4)
C(6)-N(1)-C(18)-O(8)	-14.0(4)
C(10)-N(1)-C(18)-O(8)	-167.6(3)

C(18)-N(1)-C(6)-C(5)	-73.2(3)
C(10)-N(1)-C(6)-C(5)	79.7(3)
C(18)-N(1)-C(6)-C(7)	162.1(3)
C(10)-N(1)-C(6)-C(7)	-45.0(3)
C(8)-C(7)-C(6)-N(1)	51.4(3)
C(8)-C(7)-C(6)-C(5)	-73.8(3)
C(1)-O(1)-C(5)-O(2)	-2.9(6)
C(1)-O(1)-C(5)-C(6)	175.6(3)
N(1)-C(6)-C(5)-O(2)	-17.1(5)
C(7)-C(6)-C(5)-O(2)	107.8(4)
N(1)-C(6)-C(5)-O(1)	164.3(3)
C(7)-C(6)-C(5)-O(1)	-70.8(4)
C(18)-N(1)-C(10)-C(9)	-159.7(3)
C(6)-N(1)-C(10)-C(9)	46.3(4)
C(11)-O(3)-C(8)-C(7)	-78.2(3)
C(11)-O(3)-C(8)-C(9)	164.2(3)
C(6)-C(7)-C(8)-O(3)	-177.6(2)
C(6)-C(7)-C(8)-C(9)	-61.3(3)
N(1)-C(10)-C(9)-C(8)	-53.2(4)
O(3)-C(8)-C(9)-C(10)	-179.6(3)
C(7)-C(8)-C(9)-C(10)	62.2(4)
C(12)-C(17)-C(16)-C(15)	0.2(5)
C(14)-C(15)-C(16)-C(17)	0.7(6)
N(2)-C(15)-C(16)-C(17)	179.9(3)
C(16)-C(15)-C(14)-C(13)	-1.2(5)
N(2)-C(15)-C(14)-C(13)	179.7(3)
C(12)-C(13)-C(14)-C(15)	0.8(5)
C(18)-O(8)-C(19)-C(20)	63.3(5)
C(18)-O(8)-C(19)-C(22)	-179.6(3)
C(18)-O(8)-C(19)-C(21)	-61.6(5)
C(5)-O(1)-C(1)-C(3)	-58.5(6)
C(5)-O(1)-C(1)-C(2)	-176.8(5)
C(5)-O(1)-C(1)-C(4)	65.7(6)

IX.7.10. Crystal data and structure refinement for 180

Table IX.55. Crystal data and structure refinement for **180**.

Identification code	compound **180**	
Empirical formula	C15 H25 N O4	
Formula weight	283.36	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Trigonal	
Space group	P 31	
Unit cell dimensions	a = 9.8807(4) Å	α= 90°.
	b = 9.8807(4) Å	β= 90°.
	c = 14.9490(10) Å	γ = 120°.
Volume	1263.92(11) $Å^3$	
Z	3	
Density (calculated)	1.117 Mg/m^3	
Absorption coefficient	0.080 mm^{-1}	
F(000)	462	
Crystal size	0.2 x 0.1 x 0.1 mm^3	
Theta range for data collection	3.62 to 26.24°.	
Index ranges	0<=h<=12, -10<=k<=0, -18<=l<=18	
Reflections collected	1684	
Independent reflections	1684 [R(int) = 0.0000]	
Completeness to theta = 26.24°	98.2 %	
Absorption correction	None	

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1684 / 1 / 181
Goodness-of-fit on F^2	1.027
Final R indices [I>2sigma(I)]	R1 = 0.0421, wR2 = 0.0940
R indices (all data)	R1 = 0.0652, wR2 = 0.1052
Largest diff. peak and hole	0.104 and -0.143 e.Å^{-3}

Table IX.56. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for **180**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(2)	9430(2)	4934(2)	5902(1)	60(1)
O(4)	6913(2)	6950(2)	5703(1)	69(1)
O(1)	8374(3)	5581(3)	7040(2)	84(1)
O(3)	7236(2)	8774(3)	6743(2)	82(1)
N(1)	9256(3)	8461(3)	6300(2)	60(1)
C(6)	9737(3)	7445(3)	5868(2)	56(1)
C(12)	7744(3)	8106(3)	6299(2)	61(1)
C(8)	8877(3)	3308(3)	6201(2)	59(1)
C(13)	5213(3)	6223(4)	5638(2)	81(1)
C(11)	7122(4)	2393(4)	6135(3)	86(1)
C(10)	9609(4)	2726(4)	5526(2)	75(1)
C(2)	10296(4)	9494(4)	6996(2)	72(1)
C(3)	11896(4)	10547(4)	6607(3)	86(1)
C(9)	9459(5)	3293(5)	7132(2)	89(1)
C(4)	12430(4)	9638(4)	6079(2)	82(1)
C(5)	11474(3)	8245(4)	5761(2)	71(1)
C(7)	9090(3)	5885(3)	6359(2)	56(1)
C(14)	4804(4)	4921(5)	4982(3)	105(1)
C(15)	4805(6)	7394(7)	5261(5)	143(2)
C(16)	4505(5)	5579(7)	6539(3)	137(2)

Table IX.57. Bond lengths [Å] and angles [°] for **180**.

O(2)-C(7)	1.333(3)
O(2)-C(8)	1.484(3)
O(4)-C(12)	1.355(3)
O(4)-C(13)	1.463(3)
O(1)-C(7)	1.189(3)
O(3)-C(12)	1.208(3)
N(1)-C(12)	1.353(4)
N(1)-C(6)	1.458(3)
N(1)-C(2)	1.460(4)
C(6)-C(5)	1.496(4)
C(6)-C(7)	1.529(4)
C(6)-H(6)	0.9800
C(8)-C(11)	1.505(5)
C(8)-C(9)	1.509(4)
C(8)-C(10)	1.512(4)
C(13)-C(14)	1.504(5)
C(13)-C(16)	1.505(6)
C(13)-C(15)	1.512(6)
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(10)-H(10A)	0.9600
C(10)-H(10B)	0.9600
C(10)-H(10C)	0.9600
C(2)-C(3)	1.508(5)

C(2)-H(2A)	0.9700
C(2)-H(2B)	0.9700
C(3)-C(4)	1.477(5)
C(3)-H(3A)	0.9700
C(3)-H(3B)	0.9700
C(9)-H(9A)	0.9600
C(9)-H(9B)	0.9600
C(9)-H(9C)	0.9600
C(4)-C(5)	1.309(5)
C(4)-H(4)	0.9300
C(5)-H(5)	0.9300
C(14)-H(14A)	0.9600
C(14)-H(14B)	0.9600
C(14)-H(14C)	0.9600
C(15)-H(15A)	0.9600
C(15)-H(15B)	0.9600
C(15)-H(15C)	0.9600
C(16)-H(16A)	0.9600
C(16)-H(16B)	0.9600
C(16)-H(16C)	0.9600
C(7)-O(2)-C(8)	121.54(19)
C(12)-O(4)-C(13)	121.4(2)
C(12)-N(1)-C(6)	121.3(2)
C(12)-N(1)-C(2)	118.7(2)
C(6)-N(1)-C(2)	116.1(2)
N(1)-C(6)-C(5)	111.5(2)
N(1)-C(6)-C(7)	111.4(2)
C(5)-C(6)-C(7)	112.0(2)
N(1)-C(6)-H(6)	107.2
C(5)-C(6)-H(6)	107.2
C(7)-C(6)-H(6)	107.2
O(3)-C(12)-N(1)	124.4(3)
O(3)-C(12)-O(4)	125.4(3)
N(1)-C(12)-O(4)	110.1(2)
O(2)-C(8)-C(11)	108.7(2)
O(2)-C(8)-C(9)	110.6(2)
C(11)-C(8)-C(9)	112.6(3)
O(2)-C(8)-C(10)	103.0(2)
C(11)-C(8)-C(10)	110.7(3)
C(9)-C(8)-C(10)	110.8(2)
O(4)-C(13)-C(14)	102.4(2)
O(4)-C(13)-C(16)	108.9(3)
C(14)-C(13)-C(16)	110.7(4)
O(4)-C(13)-C(15)	109.2(3)
C(14)-C(13)-C(15)	110.6(4)
C(16)-C(13)-C(15)	114.3(4)
C(8)-C(11)-H(11A)	109.5
C(8)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(8)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(8)-C(10)-H(10A)	109.5
C(8)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(8)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5
N(1)-C(2)-C(3)	109.6(3)
N(1)-C(2)-H(2A)	109.7

C(3)-C(2)-H(2A)	109.7
N(1)-C(2)-H(2B)	109.7
C(3)-C(2)-H(2B)	109.7
H(2A)-C(2)-H(2B)	108.2
C(4)-C(3)-C(2)	110.9(3)
C(4)-C(3)-H(3A)	109.5
C(2)-C(3)-H(3A)	109.5
C(4)-C(3)-H(3B)	109.5
C(2)-C(3)-H(3B)	109.5
H(3A)-C(3)-H(3B)	108.1
C(8)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	109.5
C(8)-C(9)-H(9C)	109.5
H(9A)-C(9)-H(9C)	109.5
H(9B)-C(9)-H(9C)	109.5
C(5)-C(4)-C(3)	123.0(3)
C(5)-C(4)-H(4)	118.5
C(3)-C(4)-H(4)	118.5
C(4)-C(5)-C(6)	123.4(3)
C(4)-C(5)-H(5)	118.3
C(6)-C(5)-H(5)	118.3
O(1)-C(7)-O(2)	126.0(3)
O(1)-C(7)-C(6)	124.2(2)
O(2)-C(7)-C(6)	109.8(2)
C(13)-C(14)-H(14A)	109.5
C(13)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
C(13)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
C(13)-C(15)-H(15A)	109.5
C(13)-C(15)-H(15B)	109.5
H(15A)-C(15)-H(15B)	109.5
C(13)-C(15)-H(15C)	109.5
H(15A)-C(15)-H(15C)	109.5
H(15B)-C(15)-H(15C)	109.5
C(13)-C(16)-H(16A)	109.5
C(13)-C(16)-H(16B)	109.5
H(16A)-C(16)-H(16B)	109.5
C(13)-C(16)-H(16C)	109.5
H(16A)-C(16)-H(16C)	109.5
H(16B)-C(16)-H(16C)	109.5

Table IX.58. Anisotropic displacement parameters ($Å^2$x 10^3)for **180**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(2)	66(1)	57(1)	60(1)	-2(1)	9(1)	34(1)
O(4)	52(1)	72(1)	87(1)	-12(1)	-6(1)	34(1)
O(1)	114(2)	78(1)	74(1)	13(1)	35(1)	59(1)
O(3)	71(1)	75(1)	112(2)	-18(1)	3(1)	45(1)
N(1)	56(1)	54(1)	74(1)	-6(1)	-3(1)	30(1)
C(6)	56(2)	58(2)	58(1)	2(1)	5(1)	32(1)
C(12)	57(2)	50(2)	78(2)	4(1)	3(1)	28(1)
C(8)	65(2)	55(2)	62(2)	-2(1)	-2(1)	34(1)
C(13)	47(2)	88(2)	102(2)	-18(2)	-3(2)	30(2)
C(11)	68(2)	66(2)	116(3)	-2(2)	3(2)	26(2)
C(10)	84(2)	79(2)	75(2)	-22(2)	-10(2)	51(2)

C(2)	72(2)	67(2)	77(2)	-11(1)	-7(2)	35(2)
C(3)	67(2)	72(2)	101(3)	-8(2)	-8(2)	20(2)
C(9)	129(3)	96(2)	68(2)	-8(2)	-15(2)	76(2)
C(4)	56(2)	82(2)	87(2)	12(2)	8(2)	20(2)
C(5)	56(2)	79(2)	77(2)	8(2)	13(1)	35(2)
C(7)	56(1)	62(2)	55(1)	-5(1)	4(1)	35(1)
C(14)	69(2)	111(3)	123(3)	-38(2)	-20(2)	37(2)
C(15)	108(3)	157(5)	206(5)	-30(4)	-47(4)	97(4)
C(16)	76(3)	145(4)	118(3)	-25(3)	22(2)	1(3)

Table IX.59. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **180**.

	x	y	z	U(eq)
H(6)	9283	7216	5266	67
H(11A)	6811	2437	5532	129
H(11B)	6683	2833	6533	129
H(11C)	6755	1324	6297	129
H(10A)	10726	3322	5579	112
H(10B)	9306	2841	4934	112
H(10C)	9258	1643	5638	112
H(2A)	10369	8877	7478	86
H(2B)	9878	10120	7239	86
H(3A)	11862	11325	6227	104
H(3B)	12631	11088	7087	104
H(9A)	10580	3890	7140	133
H(9B)	9119	2236	7306	133
H(9C)	9048	3745	7542	133
H(4)	13494	10075	5968	98
H(5)	11896	7725	5452	85
H(14A)	5264	5353	4412	157
H(14B)	3689	4311	4919	157
H(14C)	5196	4266	5195	157
H(15A)	5074	8217	5688	215
H(15B)	3704	6883	5139	215
H(15C)	5377	7828	4717	215
H(16A)	4780	6426	6949	206
H(16B)	4897	4927	6760	206
H(16C)	3389	4973	6485	206

Table IX.60. Torsion angles [°] for **180**.

C(12)-N(1)-C(6)-C(5)	166.3(3)
C(2)-N(1)-C(6)-C(5)	-36.3(3)
C(12)-N(1)-C(6)-C(7)	-67.8(3)
C(2)-N(1)-C(6)-C(7)	89.7(3)
C(6)-N(1)-C(12)-O(3)	169.4(3)
C(2)-N(1)-C(12)-O(3)	12.6(4)
C(6)-N(1)-C(12)-O(4)	-13.2(3)
C(2)-N(1)-C(12)-O(4)	-170.1(2)
C(13)-O(4)-C(12)-O(3)	-9.5(4)
C(13)-O(4)-C(12)-N(1)	173.2(3)
C(7)-O(2)-C(8)-C(11)	65.8(3)
C(7)-O(2)-C(8)-C(9)	-58.3(3)
C(7)-O(2)-C(8)-C(10)	-176.8(2)
C(12)-O(4)-C(13)-C(14)	-173.9(3)
C(12)-O(4)-C(13)-C(16)	-56.7(4)
C(12)-O(4)-C(13)-C(15)	68.8(4)

C(12)-N(1)-C(2)-C(3)	-142.8(3)
C(6)-N(1)-C(2)-C(3)	59.2(3)
N(1)-C(2)-C(3)-C(4)	-48.3(4)
C(2)-C(3)-C(4)-C(5)	20.2(5)
C(3)-C(4)-C(5)-C(6)	2.2(5)
N(1)-C(6)-C(5)-C(4)	4.7(4)
C(7)-C(6)-C(5)-C(4)	-120.9(3)
C(8)-O(2)-C(7)-O(1)	2.0(4)
C(8)-O(2)-C(7)-C(6)	-177.0(2)
N(1)-C(6)-C(7)-O(1)	-4.7(4)
C(5)-C(6)-C(7)-O(1)	120.9(3)
N(1)-C(6)-C(7)-O(2)	174.4(2)
C(5)-C(6)-C(7)-O(2)	-60.0(3)