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Iterative synthesis of sequence-defined polymers using solid and soluble supports

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Iterative synthesis of sequence-defined polymers using solid and soluble supports

Résumé

Dans ce travail, des méthodes itératives ont été étudiées afin de préparer des oligomères à séquences bien définies en utilisant des supports solides ou solubles. Trois stratégies de couplage de monomères ont été exploitées *(i)* AB + AB, *(ii)* AB + CD et *(iii)* AA + BB. La première méthode a permis la synthèse d'oligopeptides en utilisant les protocoles classiques de la synthèse peptidique à partir notamment d'acides aminés protégés par des groupements Fmoc. Les deux autres stratégies ont permis de préparer des oligo(alcoxyamine amide)s et des oligoamides, en l'absence de groupements protecteurs. Dans ces cas, le contrôle de la structure primaire de l'oligomère a été rendu possible soit par l'utilisation de réactions chimio-sélectives (AB + CD) soit en introduisant un large excès de monomères bifonctionnels (AA + BB). Ainsi, les oligo(alkoxyamine amide)s ont été préparés en utilisant des couplages successifs de bromo-anhydride et d'amino-nitroxide ; et les oligoamides ont été obtenus par couplages de diacides et de diamines.

L'approche pratique permettant la formation de ces oligomères à séquence contrôlée ainsi que leur caractérisation seront décrites dans cette thèse.

Mots clés : Synthèse itérative, structure primaire, polymère à séquence définie, support-solide, phase liquide, support-soluble, séquence contrôlée

Résumé en anglais

In this work, iterative methods have been studied to prepare sequence-defined oligomers on solid and soluble supports. Three model monomer coupling strategies have been exploited, *(i)* AB + AB, *(ii)* AB + CD and *(iii)* AA + BB, for the synthesis of oligopeptides, oligo(alkoxyamine amide)s and oligoamides, respectively. In the first strategy (AB + AB), oligopeptides have been synthesized using classical peptide synthesis protocols, in which Fmoc-protected amino acids were used. The other two strategies (AB + CD and AA + BB) are protecting-group free methods. In this case, the control over the oligomer primary structure has been achieved using chemoselective reactions (AB + CD) or a large excess of bifunctional monomers (AA + BB). The oligo(alkoxyamine amide)s have been prepared using successive coupling of bromo-anhydride and amino-nitroxide building blocks. The oligoamides have been obtained by sequential coupling of diacid and diamine building blocks. The practical approach to these primary structures using solid- and liquid-phase methodologies followed by the characterization of formed oligomers is the scope of this thesis.

Keywords: iterative synthesis; primary structure; sequence-defined polymer; solid-phase; liquid-phase; soluble support; sequence control

To my parents

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Anna

Dream big and dare to fail.

-Norman D. Vaughan-

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EXPERIMENTAL PART

ACRONYMS AND ABBREVIATIONS

ADMET	acyclic diene methathesis
AIBN	azobisisobutyronitrile
ARGET-ATRP	activator regenerated by electron transfer ATRP
ATRP	atom transfer radical polymerization
BOC	*tertio*-butyloxycarbonyl
BTA	benzene-1,3,5-tricarboxamide
Bipy	2,2'-bipyridine
CDI	1,1′-carbonyldiimidazole
2-ClTrt	2-chlorotrityl
CuAAC	copper-catalysed azide-alkyne cycloaddition
CuBr	copper (I) bromide
dNBipy	4,4'di-*n*-nonyl-2,2'-bipyridine
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxyde
EtOH	ethanol
ESI-MS	electrospray ionization mass spectrometry
Fmoc	fluorenylmethyloxycarbonyl chloride
FTIR	Fourier transform infrared spectroscopy
Gly (or G)	glycine
HMBA	4-hydroxymethyl benzoic acid
H	hexamethylenediamine

HMPA	4-hydroxymethylphenoxyacetic acid
HOBT	1-hydroxybenzotriazole hydrate
Lys (or K)	lysine
MALDI-TOF	matrix-assisted laser desorption/ionization – time-of-flight mass spectrometry
MBHA	(4-methyl)benzhydrylamine
MBP	methyl 2-bromopropionate
MeOH	methanol
Me_6TREN	tris(2-dimethylaminoethyl)amine
MeIm	1-methylimidazole
MSNT	l-(mesitylene-2-sulphonyl)-3-nitro-*1H*-l,2,4-triazole
Mtt	4-methyltrityl
NHS	*N*-hydroxysuccinimide
NMP	*N*-methyl-2-pyrrolidone
PCR	polymer chain reaction
PEB	1-phenylethyl bromide
PEG or PEGMA	(metoxy) poly(ethylene glycol) (methacrylate)
PMDETA	*N,N,N′,N″,N″*-pentamethyldiethylenetriamine
PROXYL	3-Amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy
PS	polystyrene
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
RAFT	reversible addition-fragmentation chain-transfer polymerization
S	succinic anhydride
SDP	bis(diphenylphosphino)-1,1′-spirobiindane
SASRIN	2-methoxy-4-alkoxybenzyl alcohol resin
SEC	size exclusion chromatography
SET-LRP	single-electron transfer living radical polymerization

$Sn(EH)_2$	tin(II) 2-ethylhexanoate
SPPS	solid-phase peptide synthesis
Sty	styrene
TBAF	tetra-*n*-butylammonium fluoride
TEA	triethylamine
TEMPO	4-amino-2,2,6,6-tetramethylpiperidine 1-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Tyr (or Y)	tyrosine

INTRODUCTION GENERALE EN FRANÇAIS

Dans la Nature, il existe de nombreux exemples de polymères biologiques, dont l'ADN, l'ARN ou les protéines.[1] Ces systèmes chimiques très avancés possèdent une habilité unique à encoder l'instruction génétique utilisée pour le développement et le fonctionnement des organismes vivants. Cette propriété est basée sur le contrôle précis de l'architecture macromoléculaire (**Figure 1**). Le paramètre clé de ces structures hautement organisées est la présence de séquences bien définies d'acides aminés qui se replient généralement pour adopter des conformations de chaînes plus complexes (*i.e.* structures primaire, secondaire et tertiaire).

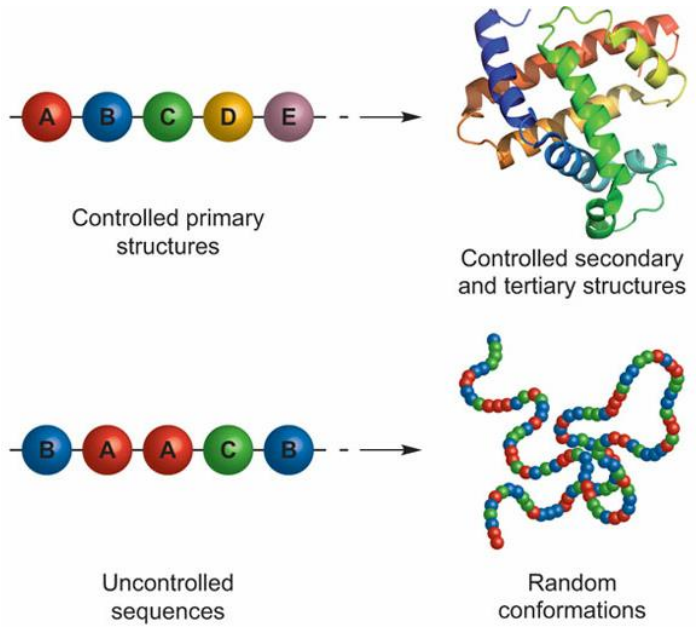


Figure 1. Importance de la régularité des séquences en synthèse de polymères. L'architecture principale bien définie des biopolymères conduit à la formation de structures secondaires et tertiaires *(haut)*, à l'inverse des structures peu contrôlées des polymères synthétiques usuels *(bas)*. Réimprimé avec la permission de Macmillan Publishers Ltd: Nature Chemistry,[2] copyright 2010.

En suivant l'exemple de la Nature, les polymères synthétiques à séquences contrôlées ont un avenir prometteur en science des polymères moderne. Cependant, les méthodes de polymérisation permettant la formation de composés à séquences spécifiques sont encore très peu explorées. De manière générale, le développement de nouveaux matériaux à structures primaires contrôlées peut être envisagé soit par des procédés biologiques ou par des techniques de polymérisations chimiques à séquence contrôlée.[3] La régulation des comonomères dans l'approche biologique a été optimisée par l'utilisation de matrices

d'ADN,[4] d'enzymes[5] ou d'organismes vivants.[6] L'utilisation de matrices d'ADN afin de réaliser le couplage des acides nucléiques et de leurs analogues peut s'apparenter à des réactions de polymérisations biologiques. De plus, des outils tels que la réaction en chaînes par polymérase (PCR) [7] et des techniques d'ingénierie de protéines [8] ont été développés. La PCR est certainement l'une des techniques biologiques les plus étudiées pour effectuer de la régulation de séquences ; cependant, l'utilisation de l'ingénierie protéique en science des polymères semble plus appropriée du fait de la possible utilisation de monomères moins conventionnels.

Bien que les stratégies basées sur des polymérisations biologiques offrent des avantages indiscutables pour la synthèse de polymères à longues séquences, elles sont souvent limitées à des monomères naturels. Afin de contourner ces restrictions, le contrôle de séquences de polymères a été obtenu par des synthèses chimiques. Jusqu'à présent, l'approche la plus communément utilisée pour réguler l'ordre des comonomères est l'attachement étape par étape de monomères. La croissance itérative d'une macromolécule à séquence ordonnée est généralement obtenue à partir d'un support auquel les molécules sont attachées de manière covalente. En particulier, la synthèse sur support-solide introduite par Bruce Merrifield est une technique adaptée à la préparation de longues séquences d'oligomères (*i.e.* oligopeptides contenant jusqu'à 50 acides aminés). Dans cette approche, la croissance itérative des macromolécules visées se déroule sur une résine composée de polymères réticulés insolubles qui permet d'éviter des étapes de purification fastidieuses après chaque ajout de monomères.

L'utilisation d'un polymère soluble comme support est une alternative à la méthode en phase solide pour la formation de polymères à séquences ordonnées.[9] En effet, dans cette approche en solution, le suivi de la réaction peut se faire par résonance magnétique nucléaire (RMN) ou par chromatographie d'exclusion stérique (SEC). Par ailleurs, le support soluble ne sert pas seulement d'outil de synthèse mais permet également d'améliorer les propriétés du copolymère final. Par exemple, l'ajout de poly(éthylène glycol) (PEG) à des principes actifs pharmaceutiques ou à des protéines thérapeutiques permet d'augmenter leur tolérance par le système immunitaire.[10] De plus, le support soluble peut être fonctionnalisé dans le but d'encoder le message chimique qu'il transporte pour des applications en bio-ingénierie [11] ou dans le domaine de l'électronique.[12]

Le but de cette thèse est d'explorer le domaine de la polymérisation à séquence-contrôlée *via* la croissance itérative. En particulier, un accent sera porté à la préparation de polymères à

séquence bien définie non-naturels *via* le couplage itératif de monomères sur des supports polymériques. Les synthèses itératives ont fait l'objet de nombreuses études depuis plusieurs décennies bien que ce soient des techniques qui prennent du temps. Deux aspects importants ont été étudiés dans cette thèse afin de simplifier les procédures : *(i)* l'utilisation d'un support soluble qui va faciliter le couplage en solution et améliorer les rendements de réactions, et *(ii)* le développement de réactions de synthèse ne nécessitant pas l'utilisation de groupements protecteurs.

Afin de mesurer le potentiel de ces synthèses itératives, trois catégories de polymères modèles ont été étudiées: des oligopeptides, des oligoamides et des oligo(alkoxyamine amide)s. Ceux-ci ont été préparés à partir de trois différentes stratégies de couplage : AB + AB, AA + BB et AB + CD (les lettres A, B, C et D indiquent les groupements réactifs terminaux de chaque composé qui pourront réagir ensemble) (**Figure 2**).

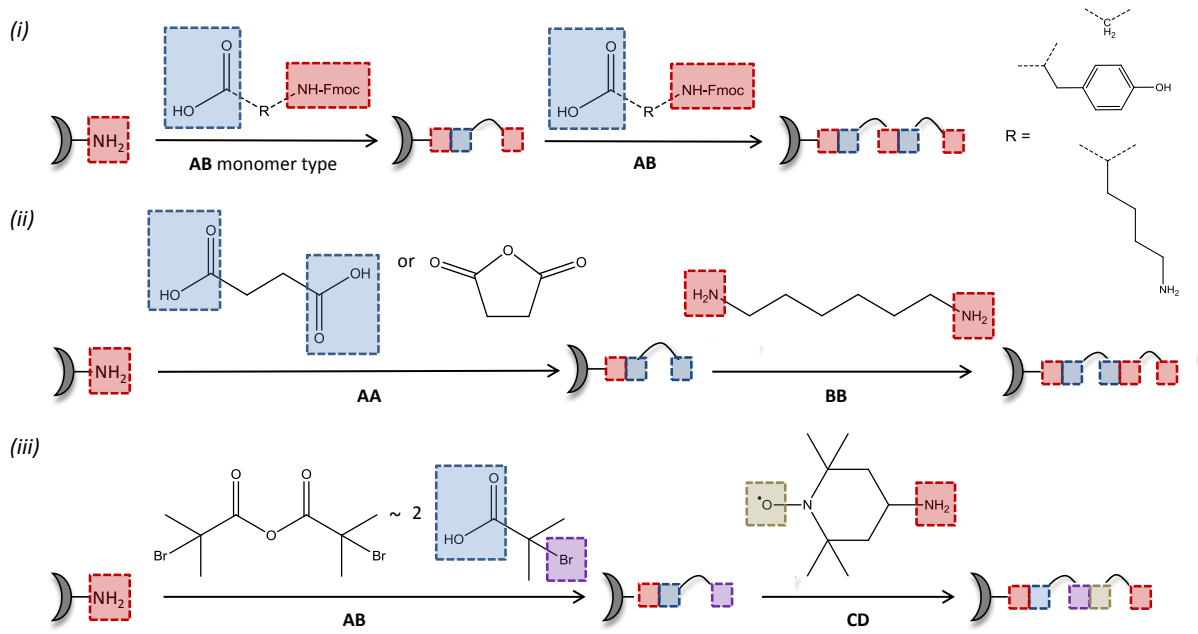


Figure 2. Polymères à séquences contrôlées préparés par une approche itérative à partir d'un support soluble ou solide enutilisant trois approches de couplage de monomères différentes: *(i)* AB + AB, *(ii)* AA + BB and, *(iii)* AB + CD.

La première approche (AB + AB) correspond à une synthèse peptidique classique dans laquelle des acides aminés protégés par des groupements Fmoc sont utilisés.

Les deux autres approches (AA + BB and AB + CD) sont basées sur des stratégies ne nécessitant pas l'utilisation de groupements protecteurs. Dans l'approche AA + BB, un large excès de monomères bifonctionnels est utilisé afin de contrôler la croissance itérative en

l'absence de groupements protecteurs. La stratégie AB + CD repose sur deux réactions chimio-sélectives successives, *i.e.* la formation d'une liaison amide impliquant les terminaisons A et D et la formation d'une liaison alcoxyamine impliquant les groupements B et C terminaux.

Comme mentionné précédemment, les polymères à séquences définies sont obtenus par deux approches: soit à partir d'un support-solide ou soit à partir d'un support soluble (**Figure 3**). La synthèse en phase solide repose sur l'utilisation d'un support insoluble qui permet la séparation des oligomères intermédiaires des réactifs et solvants solubles à la fin de la réaction par filtration. L'optimisation de cette technique a permis l'utilisation de synthétiseurs automatiques qui ont grandement simplifié et accéléré tout le processus. A l'inverse, l'approche en phase liquide se produit en milieu homogène qui permet aux réactifs d'avoir un meilleur accès au support. Par conséquent, cette méthode permet une meilleure efficacité de réaction. Le manuscrit va permettre de mettre en évidence les avantages et inconvénients de ces deux méthodes pour la préparation d'oligomères fonctionnels.

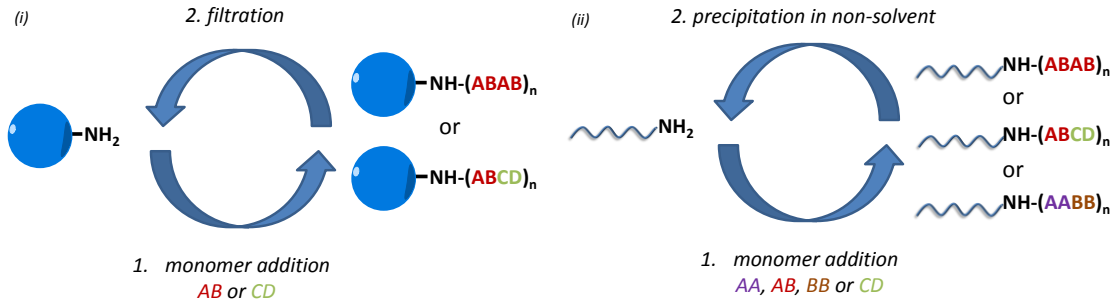


Figure 3. Approche pratique aux méthodologies utilisées dans cette étude: Synthèse en phase *(i)* solide et *(ii)* liquide.

Le premier chapitre de cette thèse est une introduction aux polymères à séquences contrôlées. Les progrès réalisés ces dernières décennies dans ce domaine et les méthodes pour contrôler les séquences de monomères seront décrites. Ce chapitre présentera également une comparaison des deux méthodes les plus utilisées pour préparer des macromolécules à séquences contrôlées : l'approche conventionnelle qui est la synthèse sur support-solide et une stratégie en phase liquide basée sur l'utilisation de supports polymères solubles. Enfin, un paragraphe concernant les techniques de polymérisations utilisées dans ce travail pour préparer les supports solubles viendra clore ce chapitre bibliographique.

Le chapitre II est dédié à l'oligomérisation par étape de monomères de type AB contenant une amine protégées par un groupement Fmoc d'un côté et un acide carboxylique de l'autre. Les monomères de type AB peuvent être polymérisés de manière séquentielle si l'une des deux fonctions réactives est désactivée temporairement. Cette approche est souvent utilisée en synthèse peptidique sur support-solide. Cependant, le polystyrène réticulé utilisé dans cette dernière stratégie n'est qu'un outil de synthèse et n'a aucun intérêt au niveau du composé final. L'intérêt de ce second chapitre est donc de démontrer l'utilité d'un support soluble qui ne va pas uniquement servir d'outil mais qui va contribuer aux propriétés finales du copolymère à blocs formés. En particulier, cette partie décrit une approche « inverse » permettant la synthèse de polymères bioconjugués à partir d'un support soluble à base de polystyèrene.[13] La préparation des supports solubles et la formation des polystyrène-oligopeptides conjugués est présentée dans ce chapitre. Enfin, nous démontrons la capacité de ces composés biohybrides à former des copolymères triblocs avec du poly(éthylène glycol) afin d'envisager des applications dans le domaine biomédical.

Le Chapitre III explore une nouvelle méthode pour préparer des polymères à séquences contrôlées basés sur des liaisons alcoxyamine dynamiques.[14] Le terme "liaison dynamique" réfère à une classe de liaisons chimiques qui peut se couper et se reformer de manière sélective et réversible (conditions d'équilibre). Les oligomères à séquences définies décris dans ce chapitre ont été obtenus en utilisant une stratégie de couplage de type AB + CD à partir de monomères hautement réactifs *via* la formation de liaisons dynamiques. De manière intéressante, la réaction n'a pas nécessité l'utilisation de groupements protecteurs. Le couplage des monomères s'effectue de manière rapide et efficace du fait de la réaction entre le radical formé sur l'halogène terminal d'un premier monomère et le radical persistent provenant de l'alcoxyamine. Cette nouvelle liaison a pour particularité de pouvoir encoder un message chimique qui pourra être lu sous certaines conditions (i.e changement de température). Ce processus de codage-décodage est examiné en détail dans cette troisième partie.

Dans le chapitre IV, l'objectif est la synthèse de copolymères à blocs de type polystyrène-*co*-oligoamide. Les oligoamides à séquences définies sont traditionnellement préparés par des réactions successives de diacides sur des diamines (monomères de types AA et BB). Il existe de nombreux exemples dans la littérature de synthèses sur support solides permettant l'obtention de ces structures.[15] [16] Cependant, l'utilisation de monomères non protégés afin d'effectuer des couplages sur des supports solubles n'a pas encore été mis en évidence.

Par conséquent, dans ce chapitre, les protocoles utilisés en phase solide pour former des oligoamides sont comparés aux méthodologies en phase liquide utilisant des supports solubles en l'absence de groupements protecteurs.

GENERAL INTRODUCTION

Nature contains many examples of biological polymers, such as DNA, RNA and proteins.[1] These advanced chemical systems possess a unique ability for encoding the genetic instructions used in the development and functioning of all known living organisms. This property relies on precise control over the macromolecular architecture (**Figure 1**). The keystone of these highly organized structures is a well-defined sequence of building blocks (*e.g.* amino acids) folding generally into complex chain conformations (*i.e.* primary, secondary and tertiary structures).

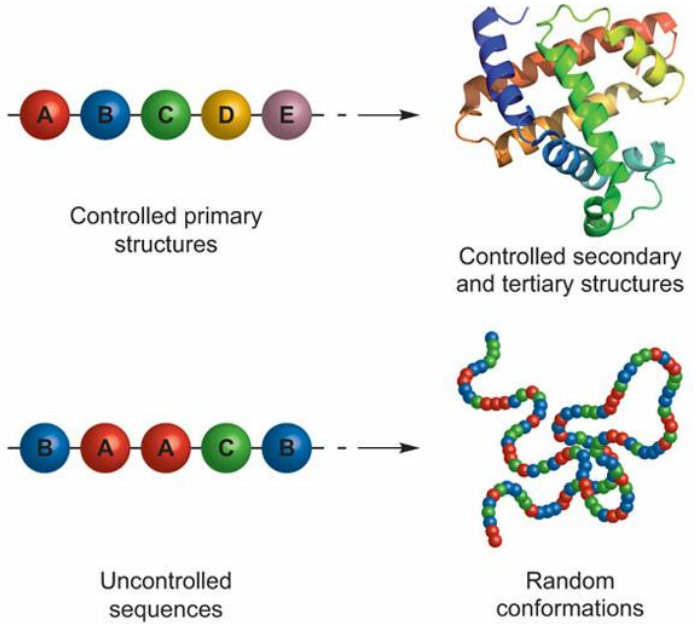


Figure 1. Importance of sequence regulation in polymer synthesis. Defined principal architecture of biopolymers leads to formation of secondary and tertiary structures *(top)*, in contrast to generally poorly controlled structures of synthetic common polymers *(bottom)*. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[2] copyright 2010.

Following Nature's example, synthetic sequence-controlled polymers appear to have a great potential in modern polymer science. However, sequence-specific polymerization methods are still underexplored. Development of new materials with controlled primary structures can be generally approached by the sequence-controlled biological and chemical polymerization processes.[3] Regulation of the comonomer order in the biological approach has been optimized by utilization of DNA templates,[4] enzymes[5] and living organisms.[6] Historically, utilization of DNA templates to direct the coupling of nucleic acids and their analogs was the fundamental adaptation of biologically-based polymerizations. Furthermore,

polymerase chain reaction (PCR)[7] and protein engineering[8] techniques have been developed. PCR technique is certainly the most widespread biological mechanism in the regulation of sequences; however utilization of protein engineering in polymer science appears more adequate due to its tolerance to noncanonical monomers.

Although biologically based polymerization strategies offer indisputable advantages in the synthesis of long-sequence polymers, they are often limited to a natural backbone. To overcome these restrictions, the control of polymer sequences has been attained by *sensu stricto* chemical synthesis. To date, the most common chemical approach to regulate comonomer order in polymer chain remains step-by-step attachment of the monomers. Iterative growth of a sequence-ordered macromolecule is typically obtained on a support, to which the molecule of interest is covalently attached. In particular, solid support synthesis introduced by Bruce Merrifield is a useful technique for preparation of long oligomer sequences (*i.e.* oligopeptides up to 50 amino acids). In this methodology, iterative synthesis of the targeted macromolecule occurs on an insoluble cross-linked resin that prevents tedious purification steps after attachment of each monomer. Alternatively, the use of soluble polymers as supports allows an insight into formation of the sequence-ordered polymer.[9] Indeed, monitoring of the reaction progress in this solution based approach can be attained by nuclear magnetic resonance (NMR) and size exclusion chromatography (SEC) techniques. Furthermore, the soluble supports not only play a role of a macromolecules' carrier, but often improve properties of the final copolymer. For instance, covalent attachment of polyethylene glycol (PEG) to a drug or therapeutic protein increases their tolerance in the immunological system.[10] Moreover, the soluble supports can be functionalized according to the purpose of encoding the chemical message they transport for applications in bioengineering[11] and electronics.[12]

From the scientific perspective, the goal of this thesis is to investigate the field of sequence-controlled polymers prepared *via* iterative growth. In particular, an emphasis is put here on the preparation of non-natural sequence-defined polymers *via* iterative coupling of monomers on polymer supports. Iterative processes have been investigated for several decades but they are, in general, time-consuming experimental approaches. Two important aspects have been studied in this thesis in order to simplify these step-by-step reactions: *(i)* the use of soluble supports that facilitate solution coupling and results in high yields, and *(ii)* the development of a protecting-group-free coupling chemistry.

To seize the potential of iterative synthesis, we have studied three model categories of sequence-defined polymers: oligopeptides, oligoamides and oligo(alkoxyamine amide)s. Consequently, there were three monomer coupling strategies exploited in this work: AB + AB, AA + BB and AB + CD building blocks coupling (A, B, C and D denote reactive termini, which can react with each other) (**Figure 2**).

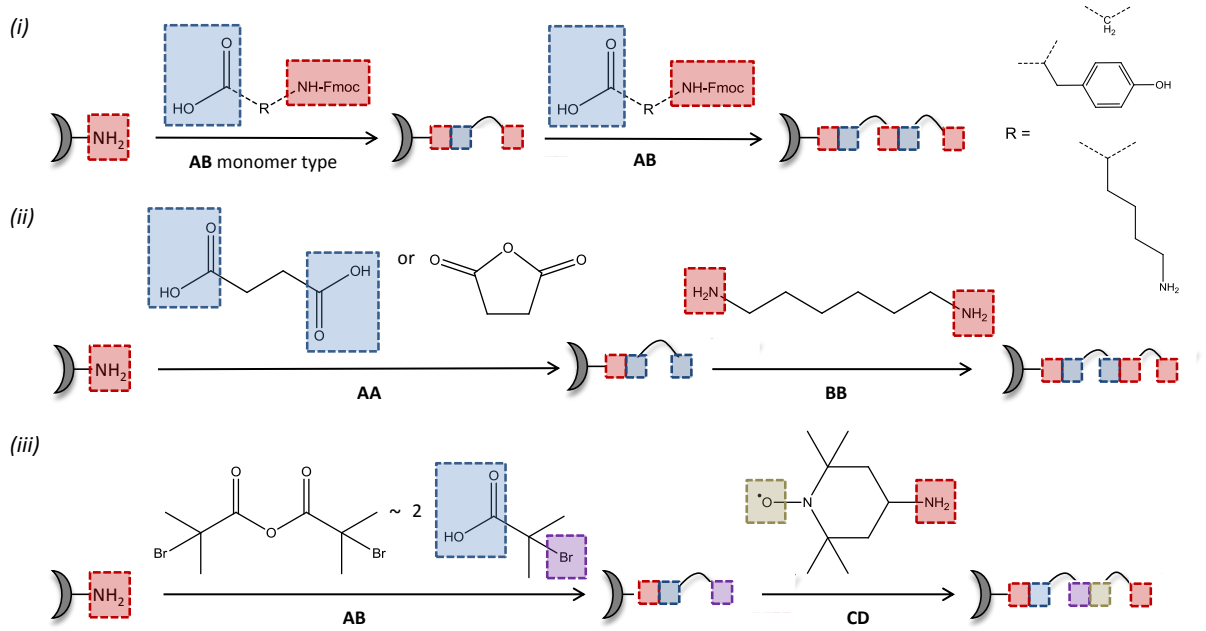


Figure 2. Sequence-controlled polymers prepared by iterative growth on a soluble/solid support *via* 3 different approaches of monomer coupling: *(i)* AB + AB, *(ii)* AA + BB and, *(iii)* AB + CD.

The first approach (AB + AB) is a classical peptide synthesis in which Fmoc-protected amino acids were used. The two other approaches (AA + BB and AB + CD) are protecting-group-free strategies. In the AA + BB, large excess of bifunctional monomers were used to control iterative growth in the absence of protecting groups. The AB + CD approach relies on two successive chemoselective reactions, *i.e.* the formation of an amide bond involving termini A and D and the formation of an alkoxyamine bond involving termini B and C.

As mentioned before, the sequence-defined polymers are obtained in these studies by 2 approaches: the solid- and the liquid-phase synthesis (**Figure 3**). The solid-phase principle relies on usage of an insoluble support that permits separation of the intermediate oligomer from soluble reagents and solvents at the end of the reaction (by filtration). Optimization of this technique allowed the use of automated synthesizers that greatly simplified and accelerated the whole process. On the contrary, the liquid-phase approach occurs in homogeneous conditions that gives reagents an easier access to the support. In consequence,

this method results in higher efficiency of the reaction. The manuscript investigates in details advantages and drawbacks of both of the two methods used for the preparation of functional oligomers.

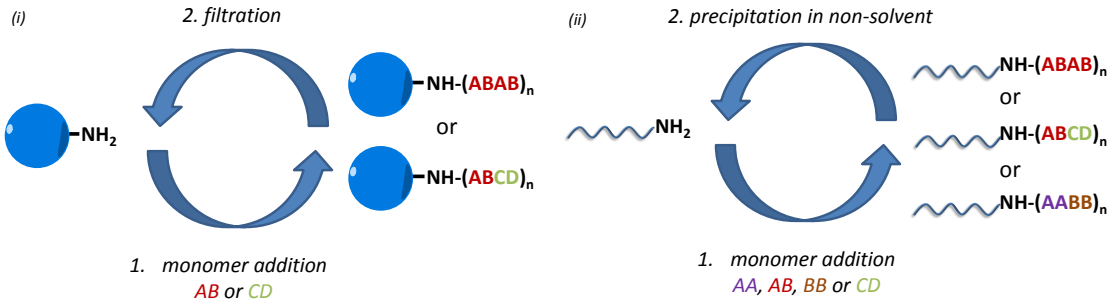


Figure 3. Practical approach to: *(i)* solid- and *(ii)* liquid-phase methodologies used in the present study.

The **first chapter** of this thesis is an introduction to sequence-controlled polymers. The progress made over the last few decades in this field and the methods for controlling monomer sequences are hereby described. Consequently, the chapter presents comparison between the two most used techniques for preparing sequence-ordered macromolecules: conventional solid-phase approach and a liquid-phase strategy based on the use of soluble polymer support. Finally, a short paragraph describes the polymerization methods used in this work for preparation of the soluble supports.

The central focus of **Chapter II** is a stepwise oligomerization of AB monomers containing a carboxyl terminus and a Fmoc-protected secondary amine. Reactive monomers of the AB type can be sequentially polymerized if one of two functions is temporarily deactivated. This approach is commonly used in solid-phase peptide synthesis. However, the cross-linked polystyrene support is at this point nothing but a disposable segment that possesses no further importance after the synthesis is completed. In connection with it, the purpose of this chapter is to demonstrate the utility of soluble supports that not only perform a carrier "duty", but also contribute to the properties of the final block copolymer. In particular, the second chapter describes an "inverse" pathway for synthesis of bioconjugates by utilization of a soluble polystyrene support.[13] Simultaneously, the preparation of soluble polymer supports and polystyrene-oligopeptide conjugates is shown. Finally, we demonstrate an ability of the outcoming biohybrid conjugate to form a triblock copolymer with PEG for the application in drug delivery field.

Chapter III explores a new method for preparing sequence-controlled polymers based on dynamic alkoxyamine bonds.[14] The term "dynamic bond" refers to a class of bonds that can selectively undergo reversible breaking and reformation, usually under equilibrium conditions. The sequence-defined oligomer studied in this chapter was obtained by AB + CD coupling of highly reactive monomers *via* formation of dynamic bonds. Interestingly, the reactive end-functions did not require protecting groups. Pairing of the polymer's building blocks occurs rapidly and efficiently due to reaction between the radical formed on halogen terminus of the first monomer and a persistent alkoxyamine radical of the second monomer. Specificity of the newly created chemical bonds allows for coding of a chemical message that can be scrambled under particular environmental conditions (*i.e.* temperature). The coding-decoding process is hereby investigated in details.

In **Chapter IV** we have focused on the synthesis of polystyrene-oligoamide block copolymers. Sequence-defined oligoamides are traditionally prepared by successive reaction of diacids and diamines (*i.e.* building blocks of the AA and BB type). There are several examples of solid-phase syntheses of such structures in the literature.[15],[16] However, development of non-protected monomer coupling on soluble supports has not yet been established. Therefore, in this chapter we examine the available solid-phase protocols for oligoamide synthesis against liquid-phase methodology employing soluble supports in absence of protecting groups.

Chapter I

OVERVIEW ON SEQUENCE-CONTROLLED POLYMERS

1. ORIGINS

The term “sequence-controlled polymers” refers to architecturally advanced macromolecules with sequential arrangement of the monomer units along their chains. The monomer organization, together with polymer topology (branched or linear polymer chains), chain length and tacticity (isotactic, atactic or syndiotactic) are important components of polymer architectures.

Manipulating the architecture of synthetic polymers was so far restricted to simple chain modifications (**Figure 4**). For instance, end-functionalized macromolecules or block copolymers represent the most elemental sequence-controlled polymers. Furthermore, formation of dendrimers or star polymers provides an insight into recently achieved polymer structural diversity.[17]

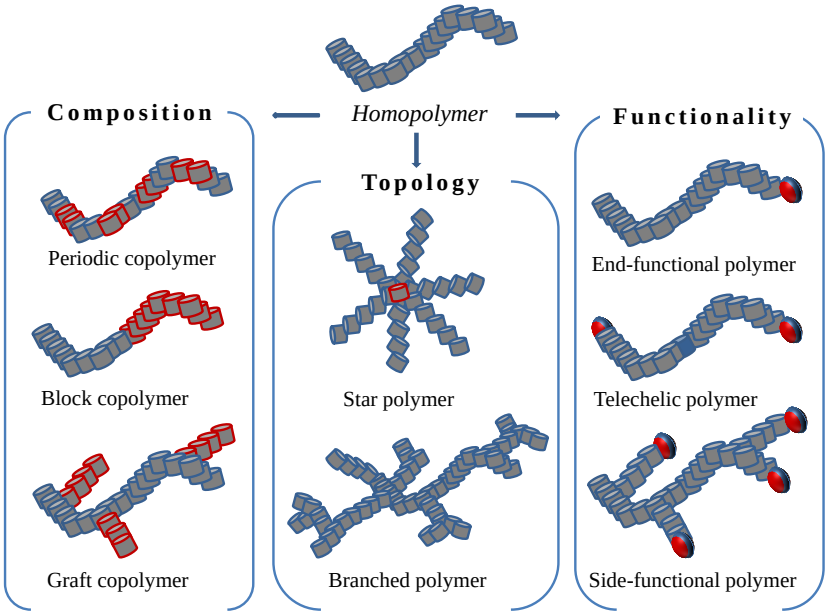


Figure 4. Schematic representation of polymer architectures: polymers with controlled composition, topology and functionality.

Nevertheless, a peerless example of the polymer complexity is given by Nature. The monomer sequence patterns found in biology, *e.g.* DNA, represent the highest level of macromolecular precision. Indeed, the double-stranded DNA helix is composed of four nucleotide monomer units that include a nucleobase (guanine, adenine, thymine or cytosine), sugars (deoxyribose), and phosphate groups (**Figure 5**).

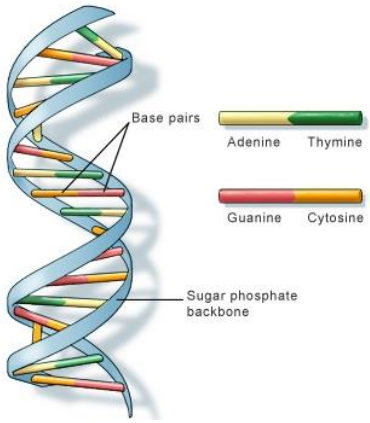


Figure 5. Ordered structure of a DNA. Source: U.S. National Library of Medicine.

Precise positioning of monomer units in biopolymers (DNA, RNA or proteins) allows these macromolecules to perform advanced tasks in living organisms. In particular, sequence-defined natural polymers serve as molecular information storage and participate in molecular recognition and biocatalysis processes. Moreover, it has been shown that potential of these sequence-ordered structures can be used in nanotechnology and material science.[18] For instance, sequence-defined oligopeptides and oligonucleotides can be used to form precise folded origami[18] to guide the self-assembly of synthetic polymers and nanoparticles.[19, 20]

In comparison with their biological counterparts, man-made polymers appear very primitive. However, taking into account that a defined primary structure seems to be crucial for high-performance materials, sequence organization of monomers become recently one of the fastest developing fields in polymer science.[3] These recent developments are described in the present chapter. A broad literature overview is presented in the following paragraphs. The first section (Chapter I, paragraph 2) focuses on the recent advances in attaining controlled primary, secondary and tertiary polymer structures. In addition, an emphasis is put in this part on the final properties of such macromolecules, such as folding and self-assembly. In the next section (Chapter I, paragraph 3), a broad overview of the biologically-inspired and purely synthetic approaches for the synthesis of sequence-controlled structures is given. The paragraph 4 contains information about iterative methods for the preparation of defined macromolecules, including solid- and liquid-phase methodologies. In the final paragraph of chapter I (paragraph 5), the controlled radical polymerization techniques used in this thesis for the synthesis of well-defined polymer supports are described.

2. RECENT APPLICATIONS AND PROMISES

Today, synthetic sequence-controlled polymers are far from exhibiting the degree of microstructural perfection found in biological macromolecules. Nevertheless an impressive progress has been made in this field over the last few years. In particular, control over primary structures, folding of the sequence-defined polymers and the recently identified single-chain technology[21] have shown interesting potential for variety of applications that will be discussed in the following sections.

2.1. CONTROLLED PRIMARY STRUCTURES

One of the most fascinating features of sequence-defined biological polymers is their ability to store and retrieve information. DNA stores, for example, the genetic information of plants and animals. Church and co-workers have recently reported that artificial DNA synthesized in laboratory conditions can be used to store digital information.[22] In the preliminary work, they have demonstrated creation of a book containing about 50,000 words. This was prepared from a library of oligonucleotides, in which each of the DNA's base encoded a single bit of information (0 for adenine and cytosine, 1 for guanine and thymine). Furthermore, it has been evidenced that DNA structure offer a much higher storage density of information in comparison with the existing technologies.

The storage of information can, in theory, also be attained using a synthetic sequence-defined copolymer. In principle, a binary code can be encoded on any copolymer composed of two monomers. Quite recently, Lutz and co-workers have reported the preparation of encoded oligomers using AB and CD building blocks (**Figure 6**).[23] Selection of the appropriate monomers linked with each other through chemoselective repeating cycles of amidification and CuAAC reactions led to creation of a (0,1) binary code. This code composed of one coding and one non-coding AB unit (A = acid, B = alkyne) has been complemented by a non-coding spacer monomer CD (C = amine, D = azide). The model encoding pentamer was obtained by stepwise coupling of three coding/non-coding monomers and two spacers on a Wang solid support. The possibility of different monomer arrangement permitted encoding of eight sequence-defined oligomers (**Figure 6**).

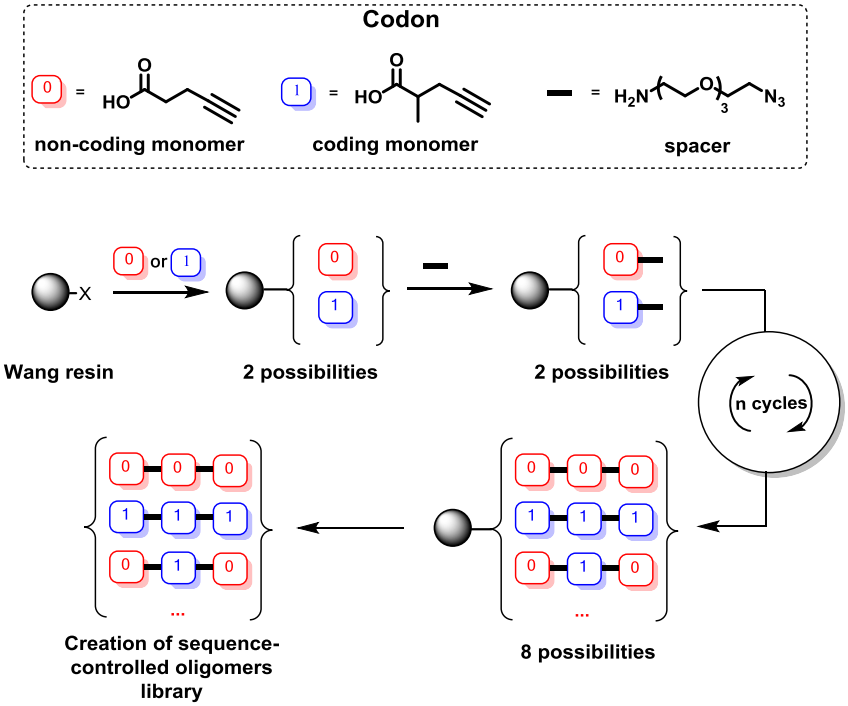


Figure 6. General strategy for synthesizing sequence-defined oligomers *via* an "AB + CD" iterative approach. Reprinted by permission from [23], © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Some techniques for "reading" molecular information encoded on polymer chains have also been reported. For instance, Colquhoun and co-worker have proposed a solution to read polymer codes.[24] They constructed molecular tweezers capable to recognize a polyimide sequence. The polyimide has been prepared from three different units (S, I and F arranged in a general pattern [-S-(I or F)-]$_n$).* The *S-I-S* and *I-S-I* triads were identified by sterically and electronically complementary molecular tweezers (**Figure 7**). The tweezers were formed from two electron-rich pyrene rings connected together *via* a joint structure particular for each of the tweezers type. Recognition of the triplet sequences relied on the difference in the affinity of pyrene pincers to the electron-poor aromatic surfaces of S, I and F.

Although still limited technologically, the sequence-controlled polymer storage systems offer great perspectives. Indeed, the data capacity of these organizations goes far beyond commercially available digital technology.

* S - biphenylene-disulfonyl-diamine; I – pyromellitimide and F – hexafluoroisopropylidene diphthalimide. It should not be confused with the abbreviations used in Chapter III and IV for succinic anhydride (S) and 2-bromoisobutyryl bromide (I).

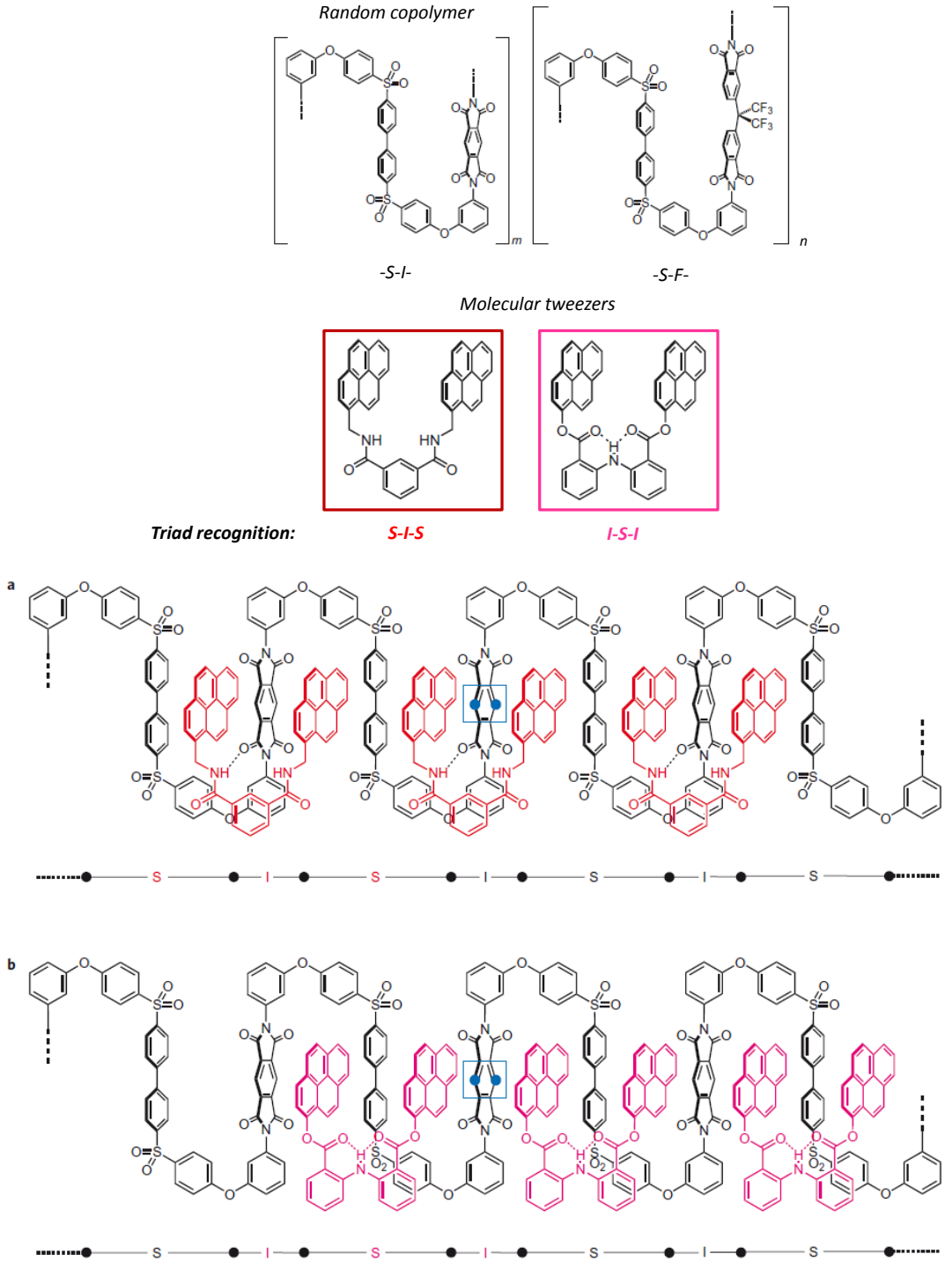


Figure 7. Visualisation of multiple binding to different polyimide triplet sequences by tweezer molecules (**a** and **b**). Constitution of a random copolymer prepared from S, I and F units is presented in the top panel and the structures of complementary molecular tweezers responsible for recognition of the triads *S-I-S* and *I-S-I* is displayed below. Preference for the *S-I-S* pattern has been revealed by the tweezers containing the 3-aminophenoxy residue (red), while the *I-S-I* sequence was binded to the intramolecularly hydrogen-bonded diester tweezers (magenta). Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[25] copyright 2010.

2.2. CONTROLLED SECONDARY AND TERTIARY STRUCTURES

Contemporary polymer chemistry allows building highly ordered structures by covalent or non-covalent association of polymer chains.[26] The structural control of a polymer influences the final properties of these macromolecules, such as folding[27] and self-assembly.[18] Since secondary and tertiary structures are fundamental in biology, folding of non-natural polymers opens new possibilities in synthetic chemistry.

2.2.1. FOLDAMERS

The term foldamers refers to a class of non-natural oligomeric sequences that possess unique ability of folding into well-defined conformations in solution. The field of foldamers has grown rapidly over the last twenty years bringing a vast amount of examples relevant to this topic, as highlighted in the reviews of Gellman,[28] Moore[26] and Seebach.[29] Some chosen examples of foldamers are described in this section. However, this part is not meant to be comprehensive.

Foldamers organize themselves by supramolecular interactions such as metal-ligand coordination, π-stacking or hydrogen bonding. For example, the group of Zuckermann introduced a concept of bioinspired foldamers based on a *N*-substituted glycine (*i.e.* peptoid) backbone.[30] Driven by a hydrophobic collapse, these peptidomimetic polymers were shown to form compact globular structures. To achieve the controlled folding, several peptoids have been further modified. For instance, peptoids containing chiral centers at the α position of their aromatic side chains were demonstrated to fold into a repeating helical structure resembling polyproline type I helix.[31] However, these polymers exhibited low water solubility. Optimization of this approach through the selection of additional monomers allowed Zuckermann and co-workers to prepare a 15-mer amphiphilic peptoid with a 3-fold periodicity (**Figure 8**).[32] A set of 15 different monomers has been used to design this oligomeric structure. The ionic (blue) and polar nonionic (green) side chains were incorporated to induce the helix. The hydrophobic (red) and hydrophilic side chains (blue and green) were placed along the main chain to balance the amphiphilic character of the peptoid. Moreover, the three variable positions indicated with Y[*] were used to explore potential interhelical electrostatic interactions.

[*] Y – carboxyl or amine group. Not to confuse with the abbreviation used in Chapter II for tyrosine.

Ultimately, Zuckerman and co-workers have designed a tertiary peptoid structure *via* covalent linkage of the previously studied amphiphilic 15-mer oligomers (**Figure 9**).[33] Chemical conjugation of the helical units has been accomplished through the formation of disulfide and oxime bridges between the peptoids' *N*- and *C*-modified termini.

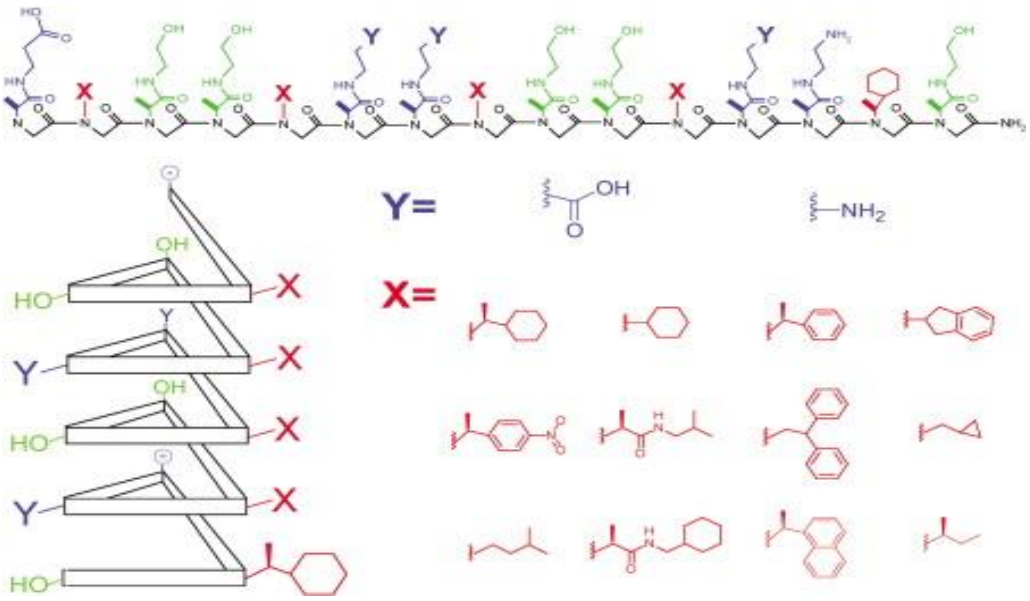


Figure 8. The 15-mer amphiphilic peptoid sequence with a 3-fold periodicity. Reprinted by permission from [32], copyright © 2002 Elsevier Ltd.

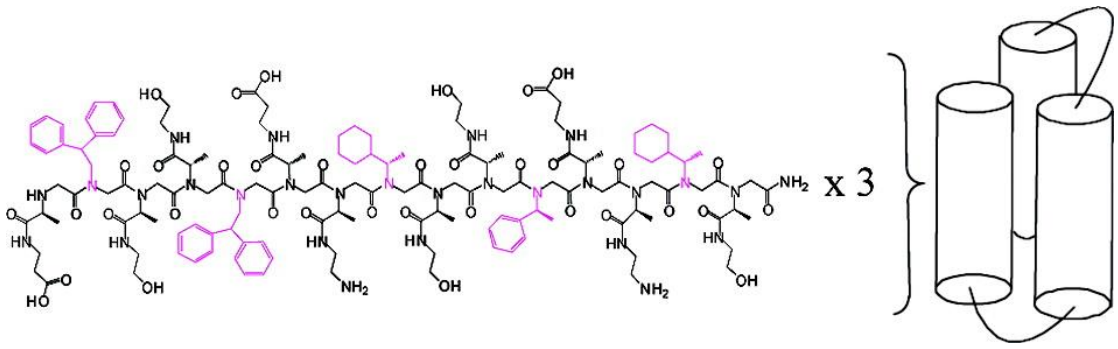


Figure 9. Creation of a three-dimensional fold by the chemical conjugation of helical peptoids. Nonpolar side-chains are presented in violet. Reprinted with permission from [33]. Copyright 2005 ACS.

2.2.2. SEMI-CRYSTALLINE PHASE OBTAINED WITH SEQUENCE-DEFINED POLYMERS

In recent years, it was also shown that monomer sequences greatly influences of the solid-phase behaviour of polymers. The group of Wagener has studied crystallization behavior of a model linear polyethylene prepared *via* acyclic diene metathesis polymerization (ADMET).[34] Taking advantage of ADMET's functional group tolerance, they have

designed block copolymers with a variety of reactive groups or pending side chains to yield unique architectures and properties. For instance, the acid- and ion-containing polyethylenes were found to form liquid-like, layered, or cubic morphologies (**Figure 10**).

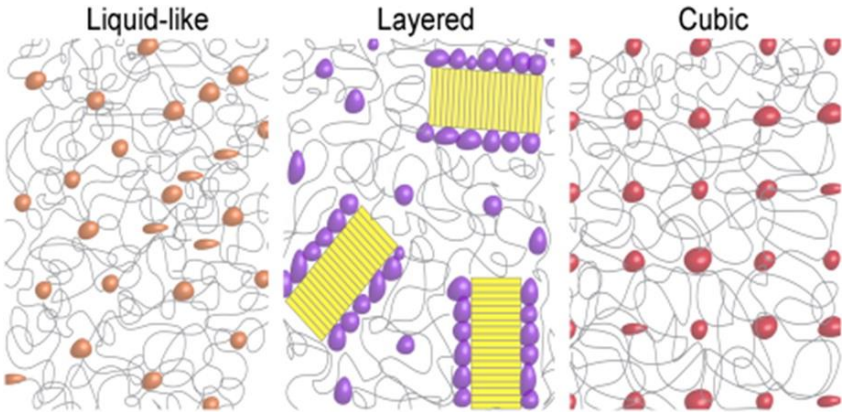


Figure 10. Morphology types of precise acid- and ion-containing polyethylenes. Reprinted with permission from [34]. Copyright 2013 ACS.

Orientation of the aggregates (colored globules) and the PE crystallites (yellow) depended on the copolymer composition. For the PE-based copolymers with acrylic or phosphonic acids, the morphology has been dominated by PE crystallization. Alternatively, strong ionic aggregates were formed for the copolymers with 1-methylimidazolium bromide.

The precise positioning of functional groups in oligomer chains is certainly a promising approach to complex synthetic structures. However, the reported foldamers are to date short stereoregular sequences. Indeed, examples of higher molecular weight foldamers are rare in the literature. However, other approaches to folding exist. For instance, it was recently shown by numerous authors that interesting folded objects can be obtained using atactic polymer precursors. In this case, the macromolecules are not really “folded” but are intramolecularly “compacted” into a defined structure.

Compaction of a polymer random coil using cooperative supramolecular interactions was demonstrated by Meijer and co-workers.[35] In this stepwise folding approach, the incorporation of benzene-1,3,5-tricarboxamide moieties into an atactic polymethacrylate chain induced self-assembling of the polymer *via* intramolecular hydrogen bonding (**Figure 11**).

Atactic polymers can also be cyclized *via* formation of covalent bridges. The arrangement of polymers into well-defined shapes by using covalent bonding has been commonly referred to

as macromolecular origami.[36] Good accuracy in intramolecular folding of linear polymer chains has been attained *via* synthetic strategies based on reactive units incorporated at precise locations. For instance, formation of P-shaped, Q-shaped, 8-shaped and α-shaped macromolecular origamis has been reported according to this approach (**Figure 12**).[37]

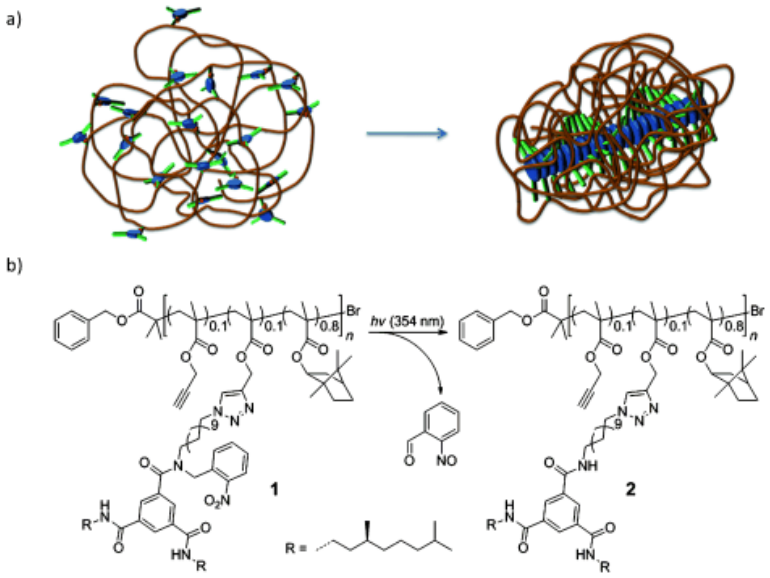


Figure 11. Folding of a polymethacrylate random coil into a helical secondary structure. Reprinted by permission from [35], copyright © 2011 Wiley Periodicals, Inc.

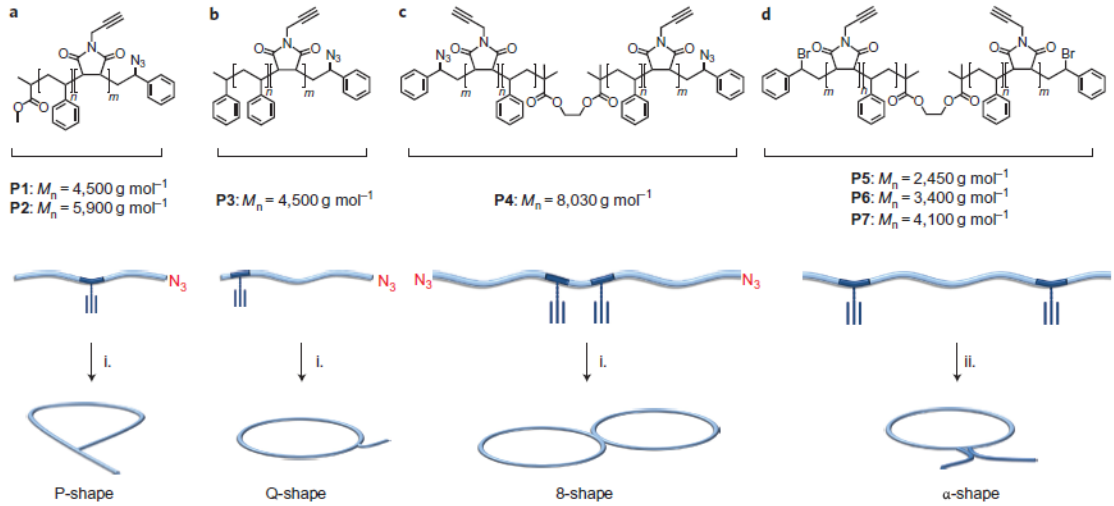


Figure 12. Covalent folding of linear synthetic polymer chains. An average comonomer composition of particular origamis prepared from *m* *N*-propargyl maleimide and *n* styrene units is displayed on the top of the figure. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[37] copyright 2011.

Incorporation of the functional maleimide units have been kinetically controlled during ATRP growth of polystyrene. Desired shapes of the folded structures were then obtained *via*

introduction of azide groups at one or both ends of the chain and their subsequent coupling with the alkyne moiety by CuAAC reaction (P-,Q- and 8-shapes). Alternatively, the α-shape has been obtained *via* direct Glaser coupling of terminal alkynes.

Creation of non-natural complex structures in atactic polymers can be also accomplished without incorporation of functional groups. An interesting approach to crystalline arrangement of anisotropic polymers *via* host-guest cross-polymerization has been presented by Kitagawa.[38] In this concept, styrene and methyl methacrylate monomers were polymerized inside the host porous coordination polymer (PCP) containing divinyl cross-linkers (**Figure 13**).

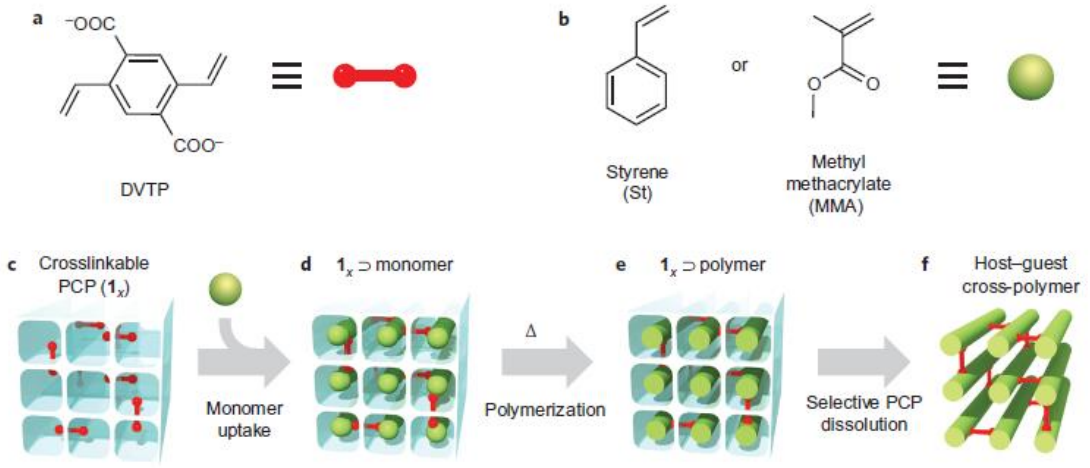


Figure 13. Schematic representation of a host–guest cross-polymerization (**c**-**f**). **a**, molecular structure of divinyl terephthalate cross-linking ligand (DVTP). **b**, monomers used in the host–guest cross-polymerizations. x – molar amount of DVTP used for the preparation of scaffold 1 from PCP. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[38] copyright 2013.

Host 1_x included vinyl monomers in its nanocavities and the heat triggered radical cross-polymerization in the presence of AIBN initiator, yielding $1_x \supset$ polymer nanocomposites. Selective decomposition of the PCP matrix generated highly ordered cross-linked polymers.

2.2.3. SINGLE-CHAIN TECHNOLOGY

At the molecular level, synthetic macromolecules can play a role of nanomachines that are responsible for execution of advanced tasks, such as catalysis or signal transport.[39] Engineering of these polymer nanodevices composed typically of one or few polymer chains has been called single-chain technology.[40]

Single-chain nanomachines can be directed to target applications in catalysis. For instance, Meijer and co-workers[41] have demonstrated the synthesis of an enzyme-mimicking water-soluble polymer carrying BTA substituents and catalytic sites (**Figure 14**). The polymer was obtained *via* copolymerization of PEG and BTA methacrylates with subsequent introduction of Ru and SDP at 50% conversion of the copolymer. Precise positioning of the catalytic sites in the middle of the polymer chain formed an apolar catalytic core stabilized by a hydrophilic shell. Folding of this system in aqueous medium resulted in a novel enzyme-like catalytic system.

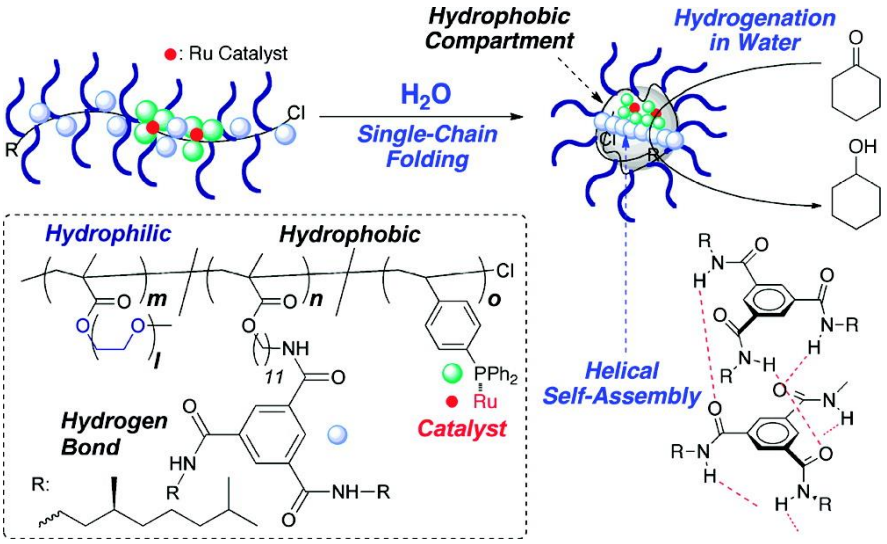


Figure 14. Folding of a sequence-controlled polymer containing three segments: water-soluble PEG methacrylate, stacking-imposing BTA methacrylate and Ru-coordinating SDP. Reprinted with permission from [41]. Copyright 2011 ACS.

Incorporation of sugar moieties in a polymer chain is another approach toward the formation of functional biodevices. Although it is relatively difficult to control the sequence of sugars along the polymer chains, several methods have been developed. For instance, Lutz and co-workers[42] demonstrated the synthesis of single-chain sugar arrays *via* sequence-controlled copolymerization of styrene and *N*-propargyl maleimides (PMI). The presence of PMI moieties allowed CuAAC of sugar model molecules (**Figure 15**).

The functional polymer backbone was prepared by NMP with precise incorporation of three PMIs containing protecting groups of different lability: trimethylsilyl (TMS), triethylsilyl (TES), and triisopropylsilyl (TIPS). The PMIs units were inserted at the beginning, in the middle and close to the end of the polymer chain. Selective deprotection and functionalization

of the three distinct alkyne sites led to a biologically active glycopolymer that constitutes mimics of a glycoprotein.

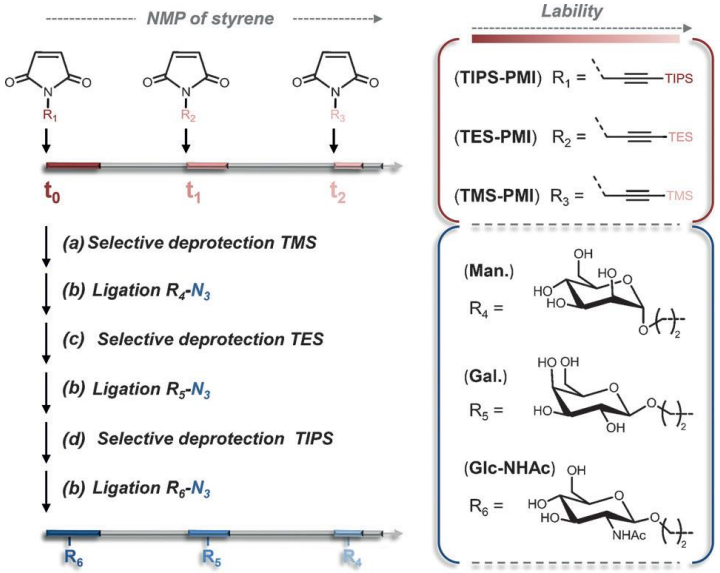


Figure 15. General strategy for the synthesis of single-chain sugar arrays. Reprinted by permission from[42], copyright © 2013 Wiley Periodicals, Inc.

The concept of sequence-controlled multiblock glycopolymer has been also investigated by Haddleton and co-workers.[43] A series of glycomonomers was first designed to perform the polymerization *via* stepwise addition of the monomers. The polymerization process was initiated from mannose glycomonomer that has been consumed after 12 h of reaction. Subsequently, equimolar amount of mannose glycomonomer has been injected into the reaction mixture. The complete conversion of the second monomer has been reached after 16 h. Alternative incorporation of the mannose and glucose glycomonomers led to preparation of a well-defined six-block glycopolymer (**Figure 16**).

Tailor-made macromolecules were also shown to act as single-chain functional devices by transferring chemical, electrical or optical signals. An interesting example of the energy transfer along polymer chain was presented in the group of Meyer.[44] Their polymer scaffold composed of ruthenium-based chromophore units was shown to transfer light from one chromophore to another until reaching ultimately a chemical reaction center (**Figure 17**). In this cascade of electron-transfer steps, light energy has been at the end converted in chemical energy.

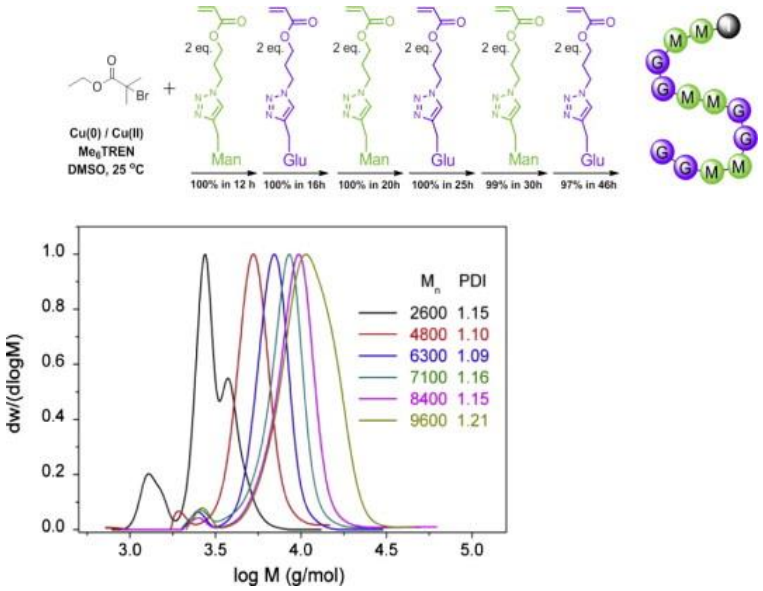


Figure 16. The synthesis of sequence-controlled mannose–glucose hexablock copolymer. Reprinted by permission from[43], copyright © 2010 Wiley Periodicals, Inc.

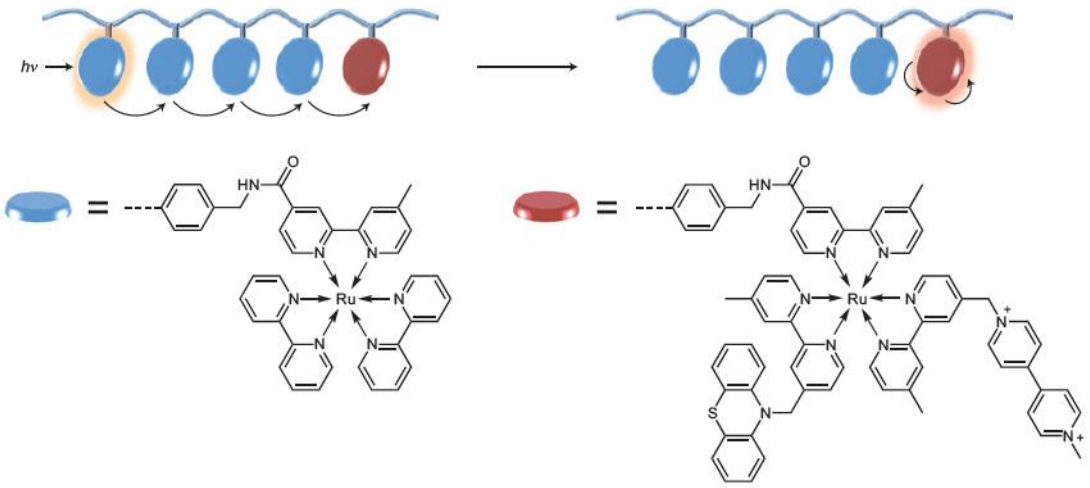


Figure 17. Schematic representation of a single-chain photosynthetic antenna system. The most-left blue disk acts as antenna fragment that collects light energy. The energy is then transferred through neighboring chromophores into the red disk reaction center. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[40] copyright 2011.

3. STRATEGIES FOR CONTROLLING POLYMER SEQUENCES

3.1. BIOLOGICAL APPROACHES

Biological processes, such as DNA replication or ribosomal protein synthesis are fascinating examples of sequence-controlled polymerizations. DNA's mechanism of replication and

transcription relies on interaction between DNA and RNA polymerases. Translation process is even more complex and requires assistance of ribosomes (**Figure 18**).

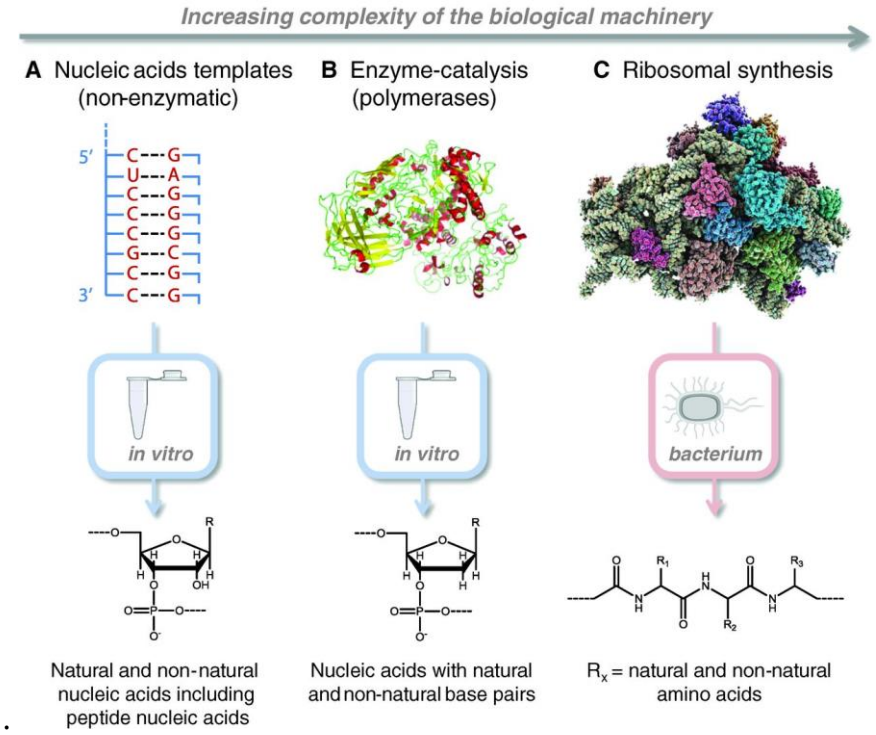


Figure 18. Biological approaches to sequence control presented on the evolution scale. Image from [3]. Reprinted with permission from AAAS

In the first step of copying genetic information, each strand of the original DNA molecule serves as template for the production of the complementary strand, a process referred to as semiconservative replication (**Figure 19**).

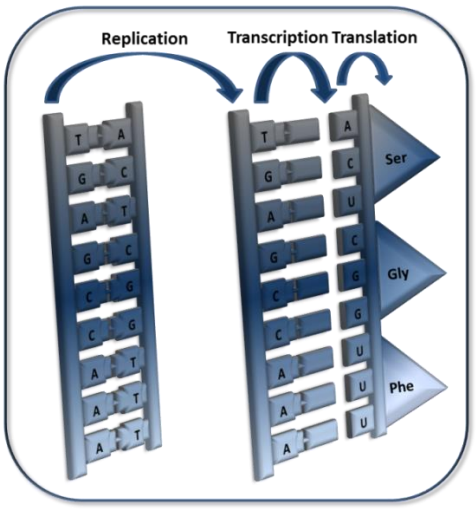


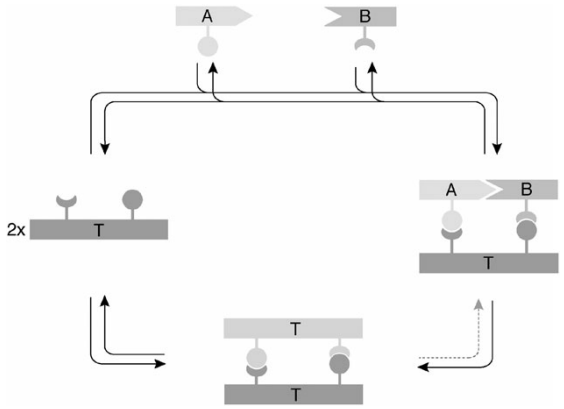
Figure 19. Process of coping genetic information stored in DNA.

Next, a particular segment of DNA is copied into RNA by the enzyme, RNA polymerase, in a process called transcription. As opposed to DNA replication, transcription results in an RNA complement that includes uracil (U) in all instances where thymine (T) would have occurred in a DNA complement. Eventually, in translation process, messenger RNA (mRNA) produced by transcription is decoded by a ribosome complex to produce a specific amino acid chain that will later fold into an active protein.

The progress made in the field of sequence-controlled polymers based on the biological approaches will be a scope of the following section.

3.1.1. NUCLEIC-ACID TEMPLATE POLYMERIZATION

In laboratory methods, the coupling of nucleic acids and their analogs can be driven by DNA templates. Self-replication of complementary nucleotide-based oligomers is the most fundamental biological polymerization approach. In its simplest form, the replicate is formed *via* catalyzed adjustment of the A and B building blocks to the corresponding T pattern (**Scheme 1**).



Scheme 1. Self-replication system based on a biological pattern (T) that imposes arrangement of the A and B precursors into original T template. Reproduced from [45] with permission of RSC.

The non-enzymatic template-directed reaction was reported for the first time in 1966 by Gilham and co-workers[46] The synthesis involved ligation of short thymidine oligonucleotide 5'-phosphates on oligoadenylate templates in the presence of a water-soluble carbodiimide. Further development of this method allowed ligation of the newly-formed strand, thus leading to autocatalytic replication.[47, 48] In 2002, a synthesis of DNA-nanoscaffolds has been achieved by van Kiedrowski[49] and Lynn.[50] One year later, Liu and co-workers[51] developed Lynn's concept of reductive amination reaction of peptide

nucleic acids (PNAs) by replacing it with amine acylation. Ultimately, the most recent discovery relies on building sequence-defined polymer structures unlike to nucleic acids.[52]

Using macrocyclic PNA adapters, a variety of synthetic polymers, such as β- and γ-peptides containing up to 90 amino acid residues has been obtained (**Figure 20**). To date, this approach of DNA-templated polymerization remains the most efficient non-enzymatic technique imitating replication, transcription and translation processes.

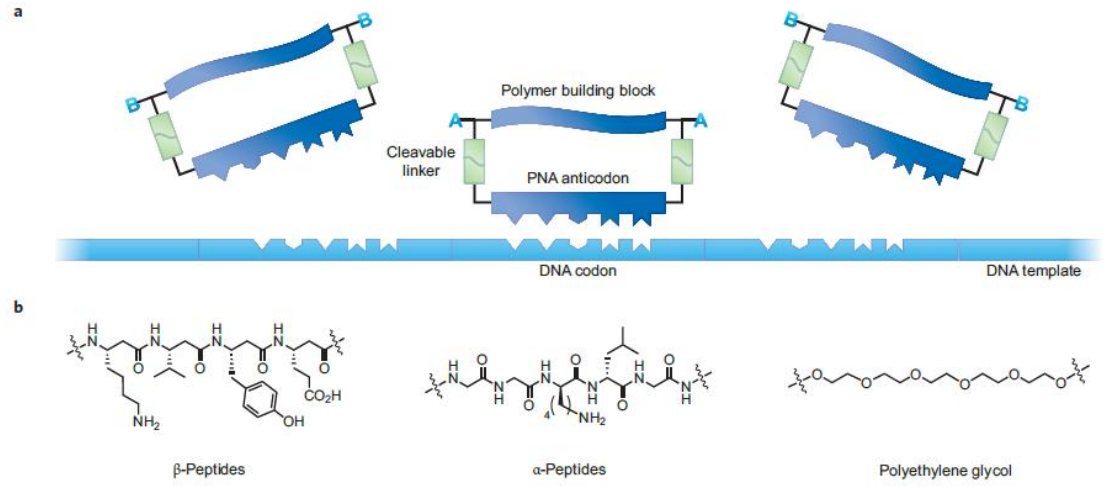


Figure 20. Sequence-controlled polymers prepared *via* macrocyclic building blocks with five-base-long PNA anticodons assembled on a DNA template and coupled through the reactive groups A and B (**a**). The bottom panel presents examples of polymer building blocks that can be stitched together to form sequence-defined materials using this approach. Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[53] copyright 2013.

3.1.2. Enzyme-catalyzed polymerization

The main advantage of the enzyme-catalyzed biologically based polymerization comprises synthesis of much longer nucleic acid sequences than those attained with the non-enzymatic replication. Polymerase chain reaction (PCR) is the most important example of such process. The PCR technique depends on the natural polymer backbone. In the first step of the PCR process, the reaction mixture is heated above DNA's denaturation temperature, allowing separation of the two complementary DNA strands (**Figure 21**). Next, the temperature is lowered to permit binding of a DNA-complementary primer (annealing). In the last step (extension), the temperature is raised again and at that time the DNA polymerase is able to extend the primers by adding nucleotides to the developing DNA strand. With each repetition

of these three steps, the number of copied DNA molecules doubles. This explains the great amplification potential of this method.

Coping of sequence-encoded template strands occurred initially in a presence of Klenow fragment. [*] [54, 55] However, PCR experimental protocols require thermally-stable polymerases, what constituted a restriction in the use of this enzyme. Identification of a heat-resistant *Taq* polymerase[†] [7] helped to optimize the PCR process.

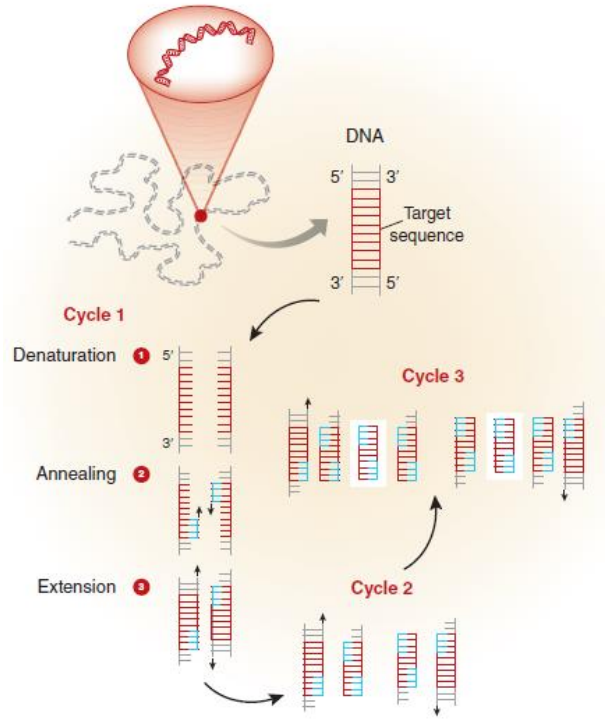


Figure 21. Principle of the polymerase chain reaction. Reprinted by permission from Macmillan Publishers Ltd: Journal of Investigative Dermatology,[56] copyright 2013.

PCR-based strategies are widely used to diagnose diseases, clone and sequence genes, or identify criminals. That makes the PCR the most widespread technique among the biological sequence regulation methods. However, PCR amplification involving noncanonical monomers remains challenging. The examples of polymerase-mediated unnatural base pairing are few,[56-59] but the field is likely to develop.

[*] Klenow fragment is a large protein fragment of *E. coli* DNA Polymerase I (*i.e.* an enzyme participating in the process of DNA replication).

[†] *Taq* polymerase was named after the thermophilic bacterium *Thermus aquaticus* from which it was originally isolated.

3.1.3. GENETIC ENGINEERING

Ultimately, high molecular weight monodisperse peptides can be prepared by the assistance of bacteria (usually *Escherichia Coli*). In this genetic engineering process, the bacteria play the role of a host for plasmid DNA that incorporates an artificial gene, encoding the protein of interest.[6] Bacterial expression of the gene results in the targeted protein that is eventually released from the host and purified (**Figure 22)**.

As demonstrated by Tirrell and co-workers,[60] the expression of artificial genes permitted preparation of extracellular matrix proteins that are studied for applications in tissue engineering. A biopolymer based on the elastin-mimetic repeating sequence was synthesized using this strategy.[61, 62] Other examples of targets for protein engineering include spider dragline silk,[63] mussel byssus thread[64] or helical polypeptides.[65]

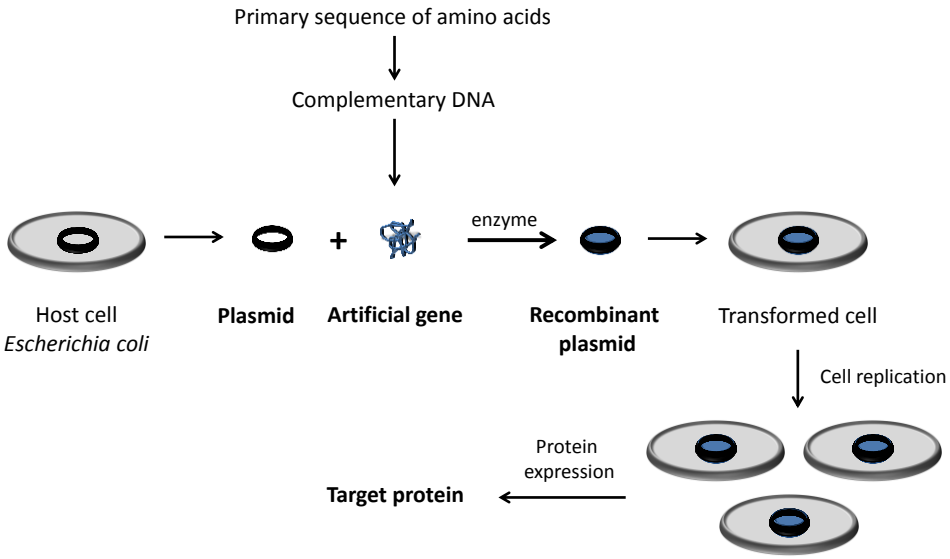


Figure 22. Protein expression on example of *Escherichia coli* expression system and DNA introduced in a plasmid expression vector.

Interestingly, noncanonical amino-acids can also be used in this protein engineering approach.[66, 67] In 1989, Schultz and Chamberlin developed independently a method to enable introduction of non-natural amino acids into proteins *via* "stop" codons.[68] These stop codons signal the termination of the growing polypeptide chain. However, there are specific tRNAs that insert an additional amino acid residue instead of ending the protein decoding process, when the stop codon appears. The use of these "suppressors" has been adapted to deliver the noncanonical amino acids. Furthermore, the method has been developed to recognize four- and five-base codons rather than the normal triplets.[69]

3.2. CHEMICAL APPROACHES

Sequence-controlled polymerizations based on biological approaches are exceptionally efficient techniques for preparation of biopolymers. However, in terms of structural diversity, synthetic chemistry offers many more possibilities. A summary of the modern methodologies for the synthesis of sequence-controlled polymers based on the chemical approaches is given below.

3.2.1. ITERATIVE GROWTH

Iterative growth is one of the most precise pathways for the chemical regulation of polymer sequences. The principle of this method relies on stepwise couplings of monomer units that possess reactive functional groups (**Figure 23**). In general, all the functions except one are inactive at the time of the reaction. The inactive groups are temporarily deactivated to avoid potential side reactions. The deactivation is achieved using selected protecting groups.

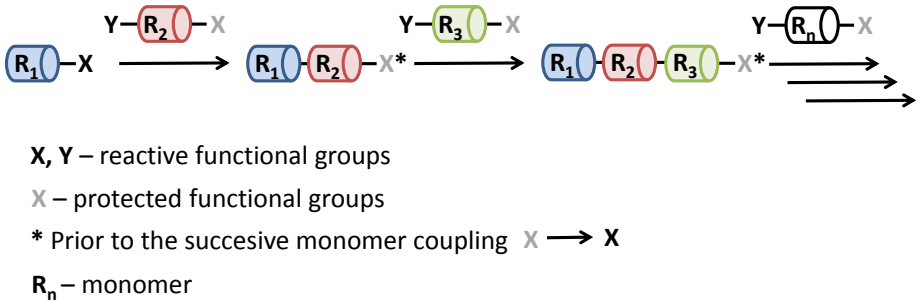


Figure 23. Schematic representation of the iterative growth approach.

One of the first reports on the iterative polymer synthesis was presented in 1954 by Katsoyannis and co-workers[70] who have obtained an octapeptide with the hormonal activity of oxitocin *via* condensation of a sequence of amino acids in solution. This example encouraged the scientists to look for a way to synthesize longer peptides[71] and other analogs of the successful hormone.[72, 73] However, although relatively efficient, the solution synthesis has been a demanding and time-consuming approach due to the tedious purification steps.

Preparing ordered structures *via* iterative growth approach has been greatly simplified with the discovery of a solid support.[74] The use of crossed-linked polystyrene beads as an insoluble support revolutionized the approach to sequence-defined macromolecules. Rapid product purification made this methodology to date the most widespread chemically-based

technique for preparation of sequence-ordered structures. Moreover, the usage of an automated peptide synthesizer allowed synthesis of oligopeptides containing up to 50 amino acids. A fully automated procedure could be also adapted in the synthesis of oligonucleotides allowing preparation of up to 200 sequence-ordered nucleotide units.

However, insoluble polymer supports have yet several shortcomings, such as limitations in the access to the functional groups of the solid support. The use of a soluble macromolecular carrier appeared thus as an alternative method for the preparation of sequence-defined polymers *via* the iterative growth approach.[75] The ordered structures are commonly synthesized on linear polystyrene or polyethylene glycol supports.[76]

Further details on the iterative growth strategy for controlling polymer sequences and examples of the structures prepared *via* this approach will be given in **paragraph 4** of this chapter.

3.2.2. SEQUENCE-REGULATED POLYMERIZATION STRATEGIES

Sequential control in the iterative approach appears moderately simpler in comparison with the continuous polymerization processes, such as step-growth or chain-growth polymerizations. The presence of highly reactive transient species in these processes makes controlling monomer sequences a challenging task. Propagation steps involving radicals or ions are difficult to regulate, thus in general leading the process to random microstructures. However, in some cases, sequences can be controlled as described in the following sub-paragraphs.

3.2.2.1. STEP-GROWTH

In 2007, Kamigaito and co-workers introduced a novel synthetic method to well-defined polymers based on vinyl and organic halides.[77] This radical polyaddition concept relied on the metal-assisted formation of carbon-centered radical species from the active C–X bond, followed by the addition to the C=C double bond and the formation of a new C–X bond. In the result of this step-growth approach, a polyester bearing an active C−Cl terminus was obtained. Although it is not the scope of this section, it should be mentioned that the presence of halogen atom in the final polyester allowed initiating a chain-growth process with vinyl monomers to yield block copolymers consisting of polyesters and vinyl polymers (**Figure 24**).

The use of the specially designed monomers containing styrene (a), vinyl chloride (b) and acrylate (c) moieties allowed synthesis of sequence-regulated vinyl copolymers forming an ABC pattern (**Figure 25**).

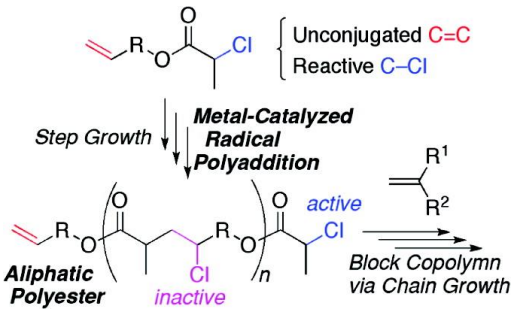


Figure 24. The concept of metal-catalyzed step- and chain-growth radical polymerization of designed monomers for sequence-regulated formation of vinyl copolymers. Reprinted with permission from[78]. Copyright 2009 ACS.

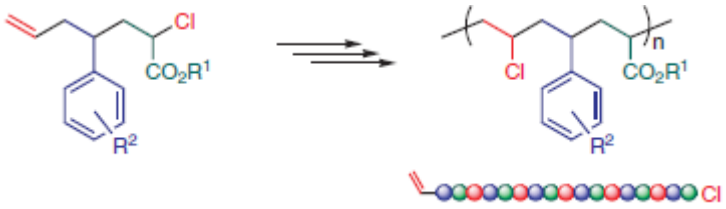


Figure 25. Step-growth radical polymerization of designed monomers with unconjugated C=C and reactive C-Cl bonds to yield ABC-sequence-regulated copolymers. Reprinted by permission from Macmillan Publishers Ltd: Nature Communications,[79] copyright 2010.

However, the radical polyaddition of the ABC-type monomers suffered from cyclization *via* a 5- or 6-membered ring formation. To reduce this intramolecular monomer cyclization, an ABCC-type monomer consisting of one styrene and two methyl acrylate units has been employed. Indeed, the analysis of the polyaddition process occurring using this building block revealed a remarkably improved efficiency in the formation of linear oligomers.

In another approach, Lutz and co-workers investigated the step-growth strategy for the preparation of periodic copolymers composed of styrene and maleimide units.[80] The final macromolecule has been obtained *via* "click" reaction of short polystyrene segments containing various maleimide moieties (**Figure 26**).

In the first step of the synthesis, styrene monomer has been polymerized *via* ATRP with kinetically-controlled addition of different maleimide units. The well-defined oligomers have been next chain-end modified to obtain azide and alkyne functionalities. Finally, the

transformed oligomers were linked together *via* CuAAC approach to form the functional periodic copolymer.

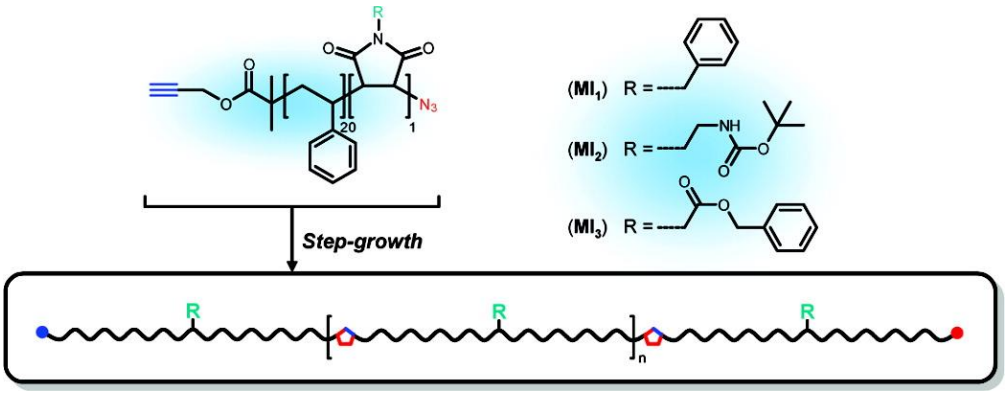


Figure 26. Step-growth approach for the synthesis of periodic functional copolymers of styrene and maleimides. Reprinted with permission from [80]. Copyright 2010 ACS.

Acyclic diene polymerization (ADMET) will be the last step-growth model polymerization described in this section. The ADMET process relies on the condensation of terminal dienes possessing a functional group in the middle of the chain. Release of the volatile ethylene drives the reaction to completion.

The use of ADMET allows preparation of various precise polymer structures. For instance, Li and co-workers[81] presented periodic copolymers containing γ-butyrolactones prepared by the ADMET approach (**Figure 27**).

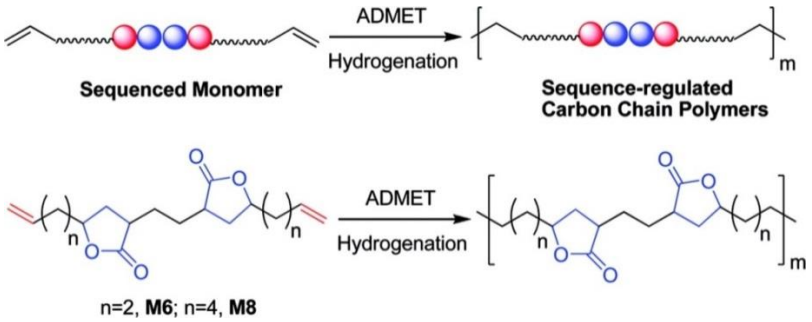


Figure 27. ADMET polymerization of structurally symmetric diene monomers containing two γ-butyrolactone units. Reprinted with permission from [81]. Copyright 2012 ACS.

Two symmetric diene monomers, M6 and M8, were polymerized using Grubbs catalysts, and subsequently hydrogenated to yield well-defined polyethylenes of high molecular weight. Moreover, thermal stability was preserved by the regularity of the positioned rigid γ-butyrolactone units.

3.2.2.2. CHAIN-GROWTH

3.2.2.2.1. Single monomer insertion

The single monomer addition strategy reported initially by Higashimura and co-workers[82] was used to control sequential oligomerization of vinyl ethers and styrene derivatives in living cationic polymerization process in the presence of HI and ZnI_2 (HI reacted with a first monomer to form a dormant adduct that was activated by ZnI_2 in the presence of a second monomer). Such addition/activation approach required gradual decrease in reactivity of the added monomers in order to favor the growth of the targeted oligomer as compared to homopolymerization. The imposed decreasing reactivity of the monomers was one of the drawbacks of this method.

Several years later, Sawamoto and co-workers[83] demonstrated a concept based on the insertion of a Boc-protected 2-aminoethyl vinyl ether (BocVE) into a growing poly(n-butyl vinyl ether) chain initiated by $SnCl_4$ catalyst. The propagating polymer was capped with BocVE *via* the formation of an adduct that sterically hampered further polymerization. Deprotection of the Boc group led to exposition of the amine group that could re-initiate the polymerization with a next feed of monomer and catalyst (**Figure 28**).

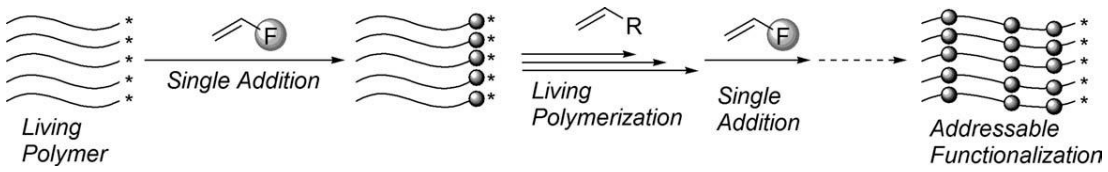


Figure 28. Selective single addition combined with a capping agent insertion in living polymerization. Reprinted by permission from [83], copyright © 2010 Wiley Periodicals, Inc.

Sequence controlled polymerization utilizing allyl alcohol as the monomer precursor has been also investigated in the ATRP process. In the work of Huang and co-workers,[84] a strategy based on a cycle of mono-addition–transformation–functionalization of a bromide-terminated poly(methyl acrylate) (PMA) has been proposed (**Figure 29**). The complete single-monomer-addition cycle consisted of four steps. At first, the PMA (1) was reacted with allyl alcohol and then the hydroxymethyl residue (2) was oxidized to a carboxylic acid (3). Next, isopropanol was used to react with the newly-formed carboxylic acid group (4). Finally, the second mono-addition of allyl alcohol was carried out at the new chain end to complete the polymer sequence regulation cycle (5).

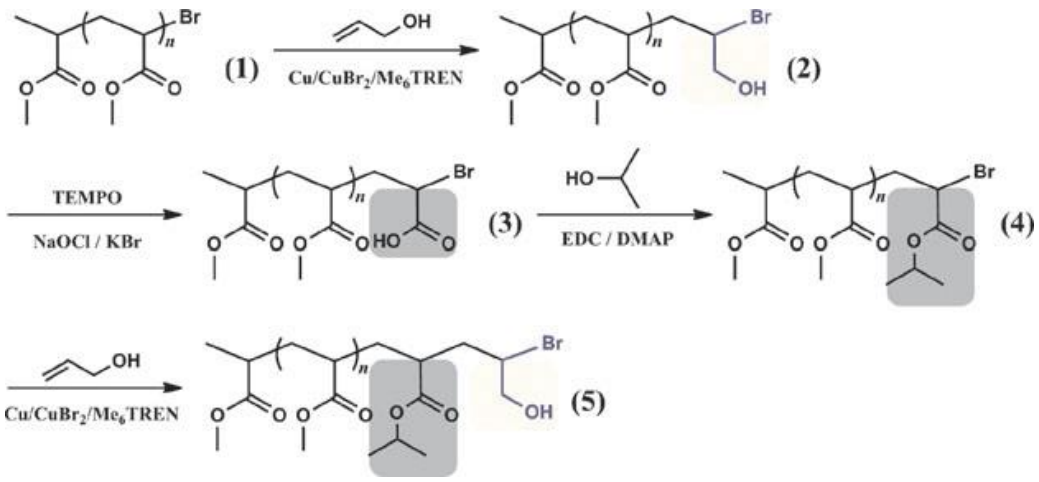


Figure 29. Sequence controlled polymerization of vinyl copolymers using PMA support. Reproduced from [84] with permission from RSC.

Insertion of consecutive monomer units in reducing quantities was demonstrated to overcome this issue in the RAFT controlled radical polymerization of acrylate oligomers.[85] Literature survey clearly shows that the optimization of this process should include a preparation of higher chain lengths molecules and an improved efficiency. To achieve it, Moad and co-workers presented an approach for the synthesis of an macro-RAFT agent by consecutive insertion of styrene and *N*-isopropylacrylamide (NIPAM) monomers into cyanoisopropyl trithiocarbonate.[86] The authors showed that a high transfer constant for the RAFT agent and a high rate of addition of the radical (R·) are the essential requirements of this strategy. The propagation rate was then determined by the rate of R adding to the monomer. The single monomer insertion strategy *via* the RAFT approach has been also demonstrated by Junkers and co-workers who synthesized two independent sets of sequence-controlled monodisperse oligoacrylates consisting of up to four monomer units.[87]

Ultimately, Meyer and co-workers defined a strategy employing single-monomer insertion in living ring-opening metathesis polymerization.[88] In this approach, a series of heterotelechelic oligo(phenylene-vinylene)s (OPV)s conjugates has been obtained *via* an iterative cycle of cross-metathesis and olefination (**Figure 30**). Further functionalization of these oligomers through the establishment of donor-acceptor complexes has shown an influence on the electronic and optical properties of these macromolecules.[43]

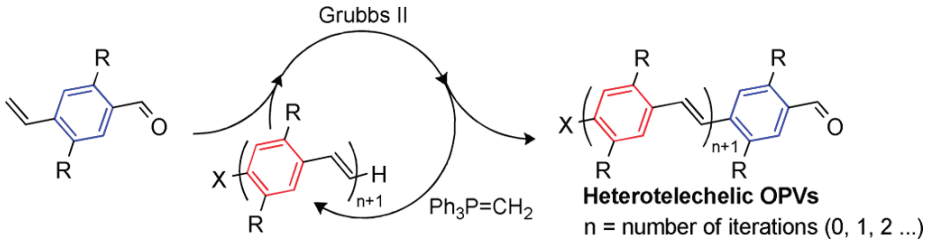


Figure 30. Synthetic route for the preparation of a series of heterotelechelic OPVs. Reprinted with permission from [88]. Copyright 2010 ACS.

3.2.2.2.2. Alternating block copolymers

Selecting monomers with particular reactivity is one of the successful concepts employed to regulate comonomer sequences in chain-growth polymerizations. Indeed, the system based on donor-acceptor building blocks can be adapted in an alternating polymerization providing an appropriate combination of monomers.[89] Numerous examples of AB alternating copolymers have been reported during the last three decades.[90-93] In most cases, the alternation is predetermined by the reactivity ratios of the comonomers. However, in some cases, the reactivity of the monomers can be tuned *in situ* by adding specific reagents (*e.g.* solvent or ligand).[94] For instance, the Lewis-acid assisted preparation of well-defined alternating copolymers of methyl methacrylate and styrene has been reported by Matyjaszewski and co-workers[95] The use of donor-acceptor monomer pair of styrene-methyl methacrylate complexed with Lewis acid has been examined in several CRP techniques. In result, RAFT process appeared to be the most successful polymerization for this comonomer system.

Interestingly, Kamigaito and co-workers[96] demonstrated a copolymerization resulting in an unconventional sequence order. The repetitive segments of AAB-type were obtained *via* copolymerization of a maleimide derivative (A, acceptor) with limonene (B, donor) in fluoroalcohol solvent. The studies provided by Kamigaito indicated that the ~~BA• radical favors A while ~~AA• exclusively reacts with B. The high selectivity of the propagating chain relied most probably on the interaction between the monomers and the solvent. The end-to-end sequence-regulation of the copolymer has been proved by mass spectrometry analysis (**Figure 31**).

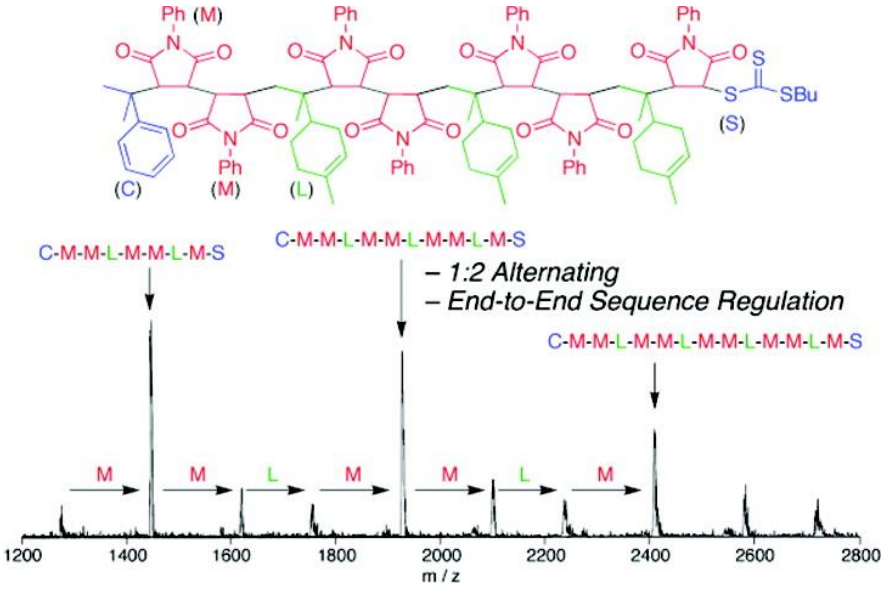


Figure 31. Structure of the copolymer of *d*-limonene and phenylmaleimide confirmed by MALDI-TOF spectrum. Reprinted with permission from [96]. Copyright 2010 ACS.

Beyond AB and AAB alternating copolymers, donor and acceptor comonomers can also be used to synthesize complex sequence-controlled microstructures. Such situation can be obtained when non-stoechiometric amounts of donor and acceptor comonomers are used in a living chain-growth polymerization. The first example of this type was reported by Lutz and co-workers[97] A series of *N*-substituted maleimides has been introduced into a growing polymer chain at desired time intervals (**Figure 32**).

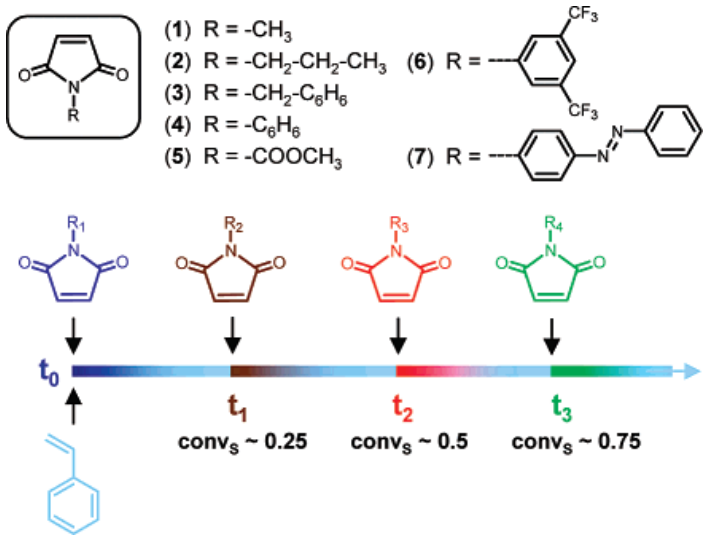


Figure 32. Time-controlled monomer insertion in the ATRP of styrene. Reprinted with permission from [97]. Copyright 2007 ACS.

It has been shown that maleimides possess a low tendency for homopolymerization, and simultaneously a high affinity for cross-propagation with styrene.[98] Local functionalization of the polystyrene chain has been achieved by adding discrete amounts of maleimide comonomers at the beginning of the polymerization and at 25, 50 and 75% conversion of the monomer. Kinetically-controlled incorporation of the maleimides allowed synthesis of a sequential copolymer with a relatively high precision. Further development of these studies permitted an extraordinary accuracy in the maleimides insertion.[99]

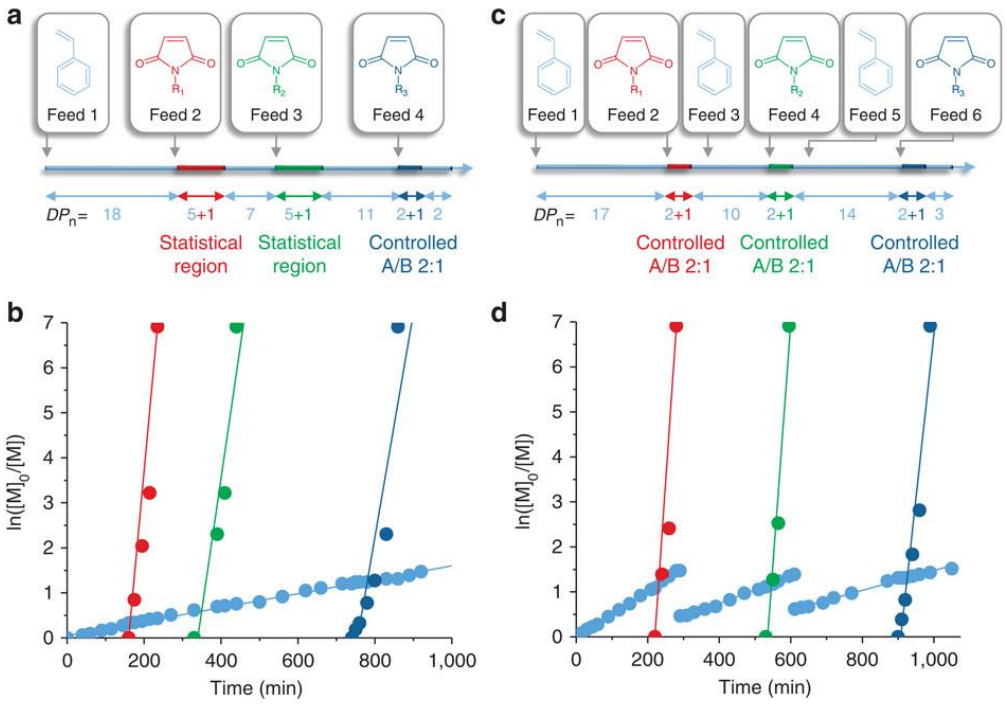


Figure 33. Strategy studied for the synthesis of sequence-regulated polymers by controlled/'living' radical copolymerization of a large excess of a donor monomer (styrene) and an acceptor comonomer (*N*-substituted maleimide). Depending on the time of addition of the acceptor comonomer during the copolymerization, microstructures containing broad (a) or narrow statistical region (c) can be obtained. Plots (b) and (d) are representations of monomer conversion versus time recorded for these reactions. Reprinted by permission from Macmillan Publishers Ltd: Nature Communications,[99] copyright 2010

The approach of the kinetic control of comonomer sequences has been also applied in anionic polymerization.[100] Nevertheless, the optimized polymerization protocols did not completely eliminate the chain-to-chain deviations in length or in the polymer composition.

3.2.2.2.3. Template polymerization

As mentioned earlier in this chapter, polymer templates play a key role in biological polymerizations. Thus, over the last decades, many attempts of templated polymerization

have been reported. For instance, **paragraph 3.1.1** describes *in vitro* polymerization methods employing nucleic acids templates. However, fully synthetic templates were also tested for controlling polymer sequences. Generation of highly selective recognition sites in man-made macromolecules was applied for the first time in 1972 by Gullf.[101] His work on the assembly of a cross-linked polymer matrix around templating moieties has been termed "polymer imprinting". Removal of such template upon specific conditions created the molecular imprint (**Figure 34**).

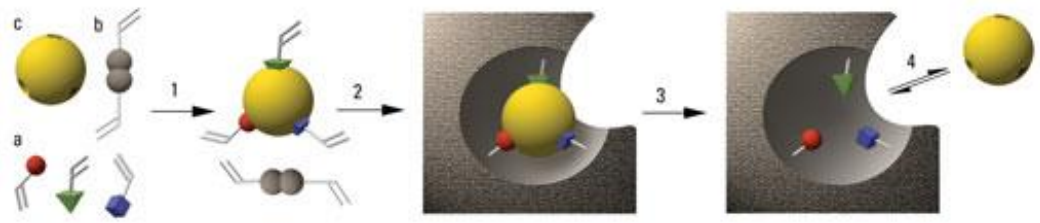


Figure 34. Schematic representation of the molecular imprinting of a synthetic polymer: (a) functional monomers, (b) a cross-linker, and (c) a template molecule are mixed together. Reprinted with permission from [102]. Copyright 2003 ACS.

In the first step of this process, the functional monomers form a complex with the template molecule (1). Next (2), the monomers copolymerize with the cross-linker. As polymerization proceeds, an insoluble, highly cross-linked polymeric network is formed around the template (3). In the final step (4), removal of the template liberates complementary binding sites that can re-accommodate the template in a highly selective manner. Although the sequence pattern of the polymer imprint has not been the main focus of this approach, the presented example is a good introduction to the polymerization methods using a molecular-scale template.

The studies toward sequence-controlled polymerization upon template effect have been investigated by Ouchi and Sawamoto.[103] The authors have examined the use of template-bearing initiators to provide precise monomer arrangement in a growing polymer chain. The template-initiator has been prepared from a heterobifuctionalized halide and subsequently coupled with metal catalysts in the living cationic polymerization process (**Figure 35**).

The designed template-initiator **1** has been first reacted with a Boc-protected 2-aminoethyl vinyl ether (BocVE) in the presence of $SnCl_4$ catalyst (**Figure 36**). Deprotection of the Boc group led to exposition of the amine group, affording **2**. Next, a radical addition of methacrylic acid to the template-bearing halide **2** was initiated with the ruthenium complex

catalyst. Based on the ionic interactions, the specific recognition of the template amine with the acid group in MAA has been observed.

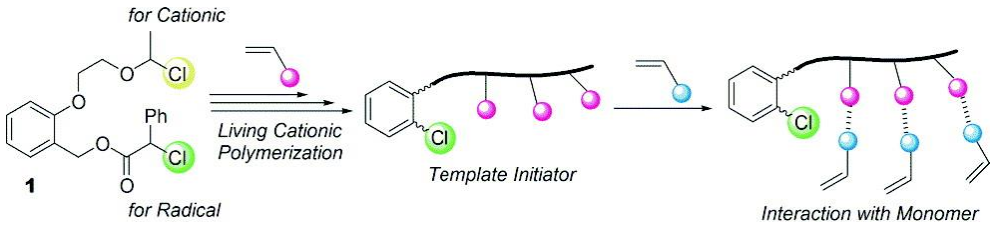


Figure 35. Template-assisted living radical polymerization preceded by the synthesis of the template-initiator. Reprinted with permission from [103]. Copyright 2009 ACS.

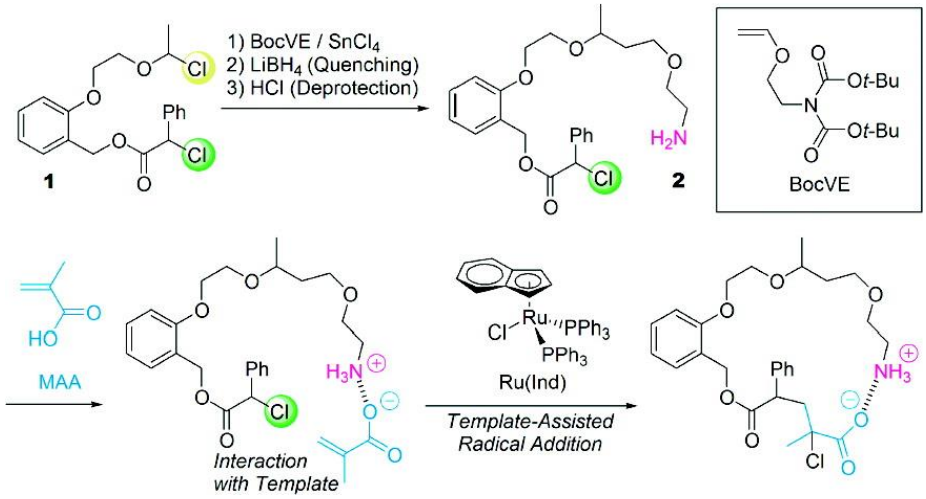


Figure 36. Radical MMA addition to the template-initiator bearing an amino site for the recognition of the monomer's acid function. Reprinted with permission from [103]. Copyright 2009 ACS

Selective radical addition of the MAA over its ester derivative, methyl methacrylate, with an amino-functionalized template initiator **2** was the first example of the substrate-template recognition approach to sequence-controlled polymers. Beyond the acid-amine pair, Sawamoto and co-workers[104] presented an alternative crown ether recognition site to select ionic monomers according to their cation size. Size-specific recognition by lariat crown ether embedded close to reactive halide in the modified template initiator **1** has been crucial in the selection of two kinds of methacrylates carrying sodium and ammonium cations (**Figure 37**).

At last, O'Reilly and co-workers have shown polymerization of a nucleobase-containing vinyl monomer in the presence of a complementary block copolymer template.[105] The control of the polymerization has been accomplished by segregation of the propagating radicals in

discrete micelle cores. The segregation process occurred due to the assembly of dynamic template polymers (**Figure 38**).

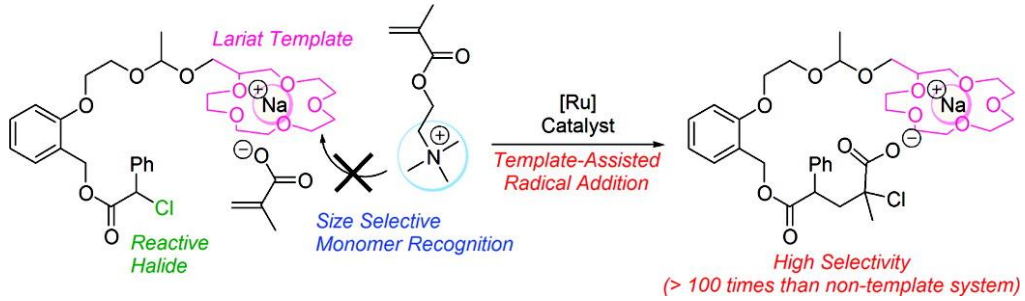


Figure 37. Size-selective monomer recognition by lariat capture of the designed template initiator. Reprinted with permission from [104]. Copyright 2010 ACS.

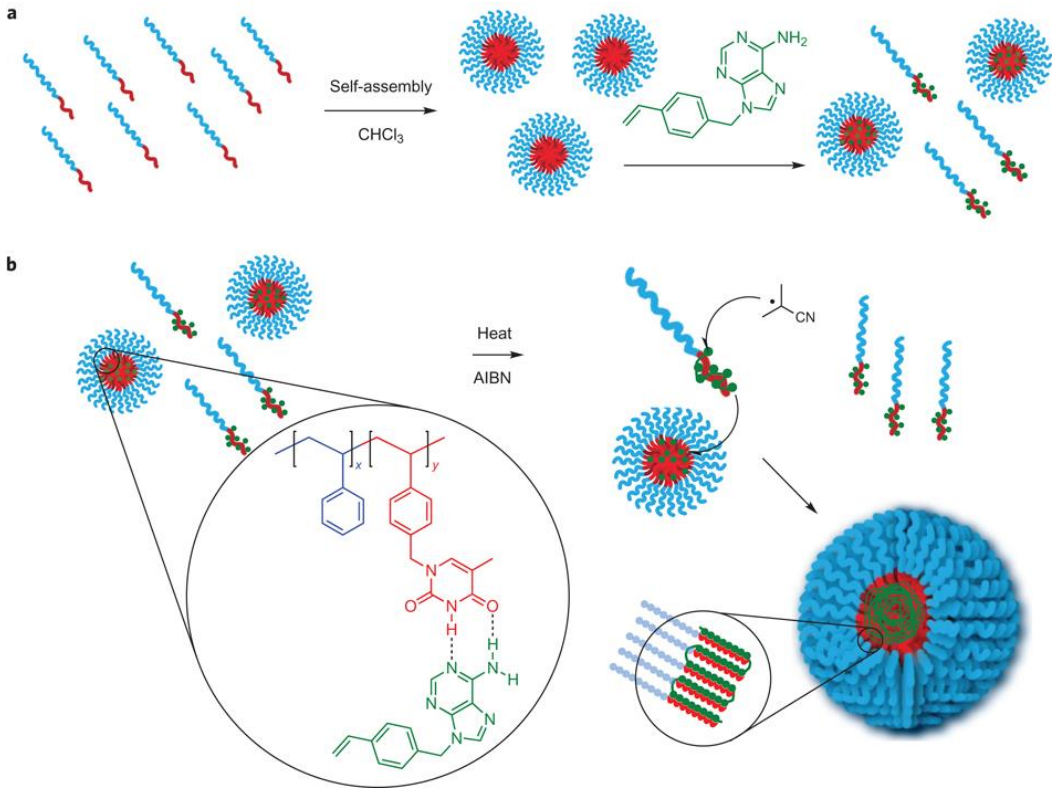


Figure 38. Assembly of template followed by addition of a complementary monomer (a). Schematic representation of template polymerization *via* cooperative assembly (b). Reprinted by permission from Macmillan Publishers Ltd: Nature Chemistry,[105] copyright 2012.

Synthetic nucleic acid analogue, poly(styrene-*b*-vinylbenzyl thymine), PS-*b*-PVBT, was used in this strategy as a template block copolymer. In chloroform, this block copolymer form micelles with PVBT core. Addition of a complementary adenine monomer (VBA) induced dynamic exchange of VBA-loaded templates. Controlled radical polymerization initiated with

AIBN and heat led to formation of polymers of very low polydispersity (≤1.08) and high molecular weight (up to 400,000 $g \cdot mol^{-1}$).

Although the design of templates containing specific build-in recognition sites is challenging, this technique offers interesting perspectives for the future development of sequence-specific radical polymerizations.

3.2.2.2.4. Catalyst-driven polymerization

A concept based on the use of specific catalysts proposed by Thomas and co-workers[106] is another remarkable approach to sequence regulation. In their work, the authors demonstrated the synthesis of alternating polyester *via* ring-opening polymerization of a mixture of enantiomerically pure but different monomers using an yttrium complex as initiator. This novel approach is an alternative to the common catalyst chain-end control in the polymerization of racemic monomer mixtures. (**Figure 39**)

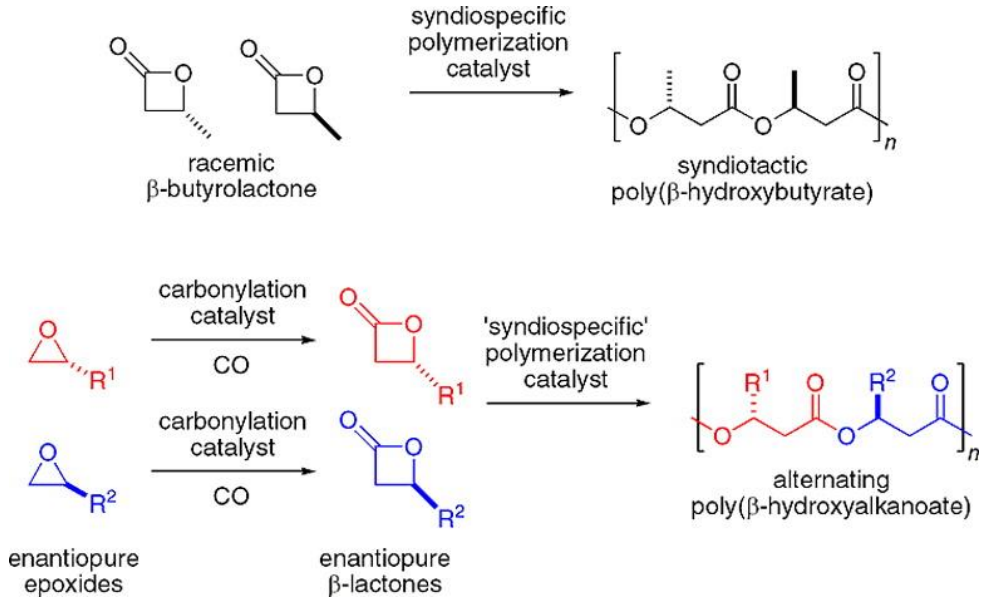


Figure 39. Synthesis of syndiotactic poly(β-hydroxybutyrate) and an alternating poly(β-hydroxyalkanoate) in the presence of a syndiospecific polymerization catalyst. Reprinted with permission from [106]. Copyright 2009 ACS.

The control over stereoselective ring-opening polymerization can be also provided by the aluminum-based catalysts.[107] This catalyst-driven sequence control approach is fascinating, but still underexplored process. For instance, inspired by Leigh's and co-workers latest discovery,[108] one could imagine development of an artificial catalytic nanomachine resembling ribosomes (**Figure 40**). Such an advanced chemical design is to date beyond our

capabilities; however the sequence-specific peptide synthesis by an artificial small-molecule machine that moves along a molecular strand, picking up amino acids that block its path,[109] opens up promising opportunities (**Figure 40**).

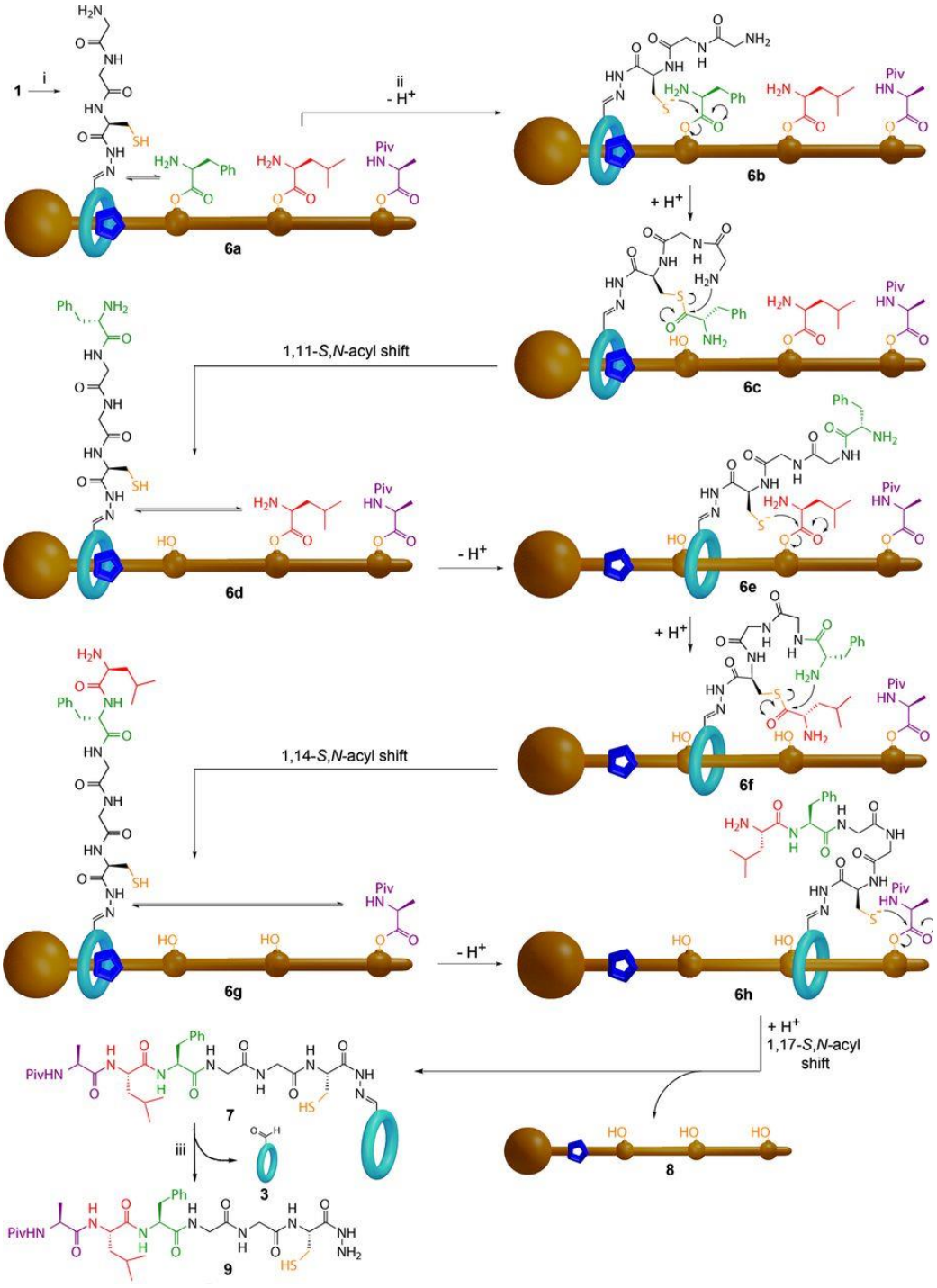


Figure 40. Proposed mechanism for sequence-specific peptide synthesis by a rotaxane-based molecular machine (1). Image from [109]. Reprinted with permission from AAAS

4. ITERATIVE SYNTHESIS

Sequence-defined segments were prepared for the first time in the late 1940s by connecting monomer units in an iterative growth manner. Stepwise preparation of ordered structures included oligosaccharides,[110] oligonucleotides,[111] or alkenols containing isoprene units.[112] The introduction of solid-phase chemistry in 1963 by Bruce Merrifield greatly simplified the whole process.[74] Merrifield's strategy for synthesis of a tetrapeptide relied on repeating cycles of deprotection/amidification of amino acids linked to a cross-linked poly(styrene-*co*-divinyl benzene) bead. The method will be described in details in **paragraph 4.1**. Moreover, it should be mentioned that this technique is not restricted to peptides, but can be theoretically extended to numerous types of monomer sequences.

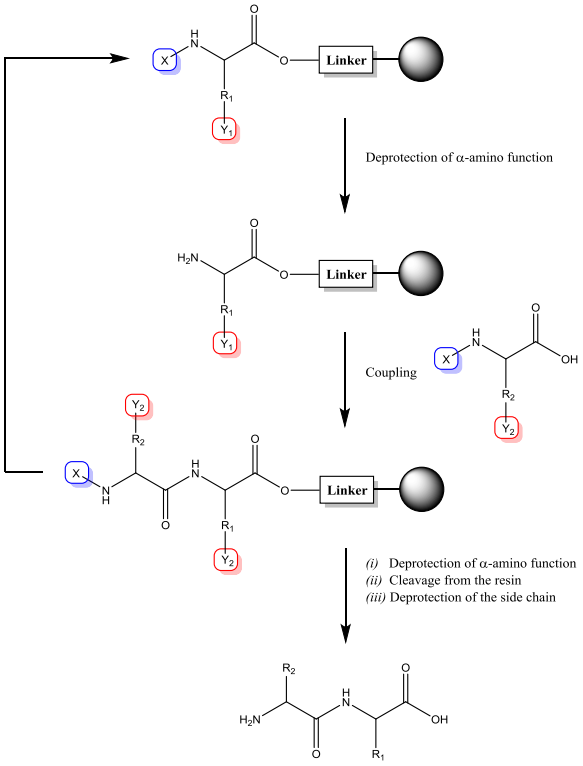
Several years later, in 1971, Mütter, Bayer and co-workers revealed a concept of employing soluble poly(ethylene glycol) supports[113, 114] for peptide synthesis. In contrast to the solid-phase approach, the synthesis in solution is a homogenous reaction, giving in consequence better yields and decreasing probability of failure sequences. On the other hand, undisputable advantage of the solid phase method of eliminating purification steps brings us to discussion about the superiority of one method over the other. However, the choice of the technique is dictated by the purpose of the synthesis (length of the desired sequence, scale of the process, etc.) Recent advancements using both methods will be described in the following subchapters.

4.1. ITERATIVE SOLID-PHASE SYNTHESIS

4.1.1. BACKGROUND

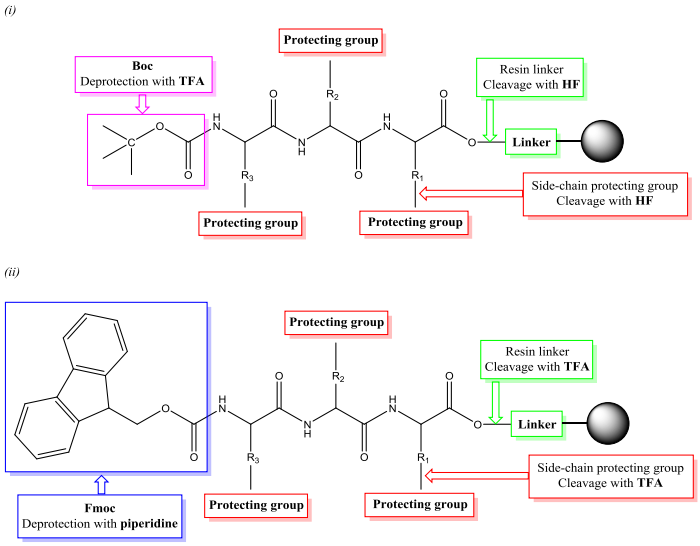
Typically, solid-phase methodology relies on the usage of insoluble supports (*e.g.* cross-linked polymer beads or solid nanoparticles) that allow binding end-functionalized monomers (*e.g.* amino acids) in a step-by-step manner. In order to avoid side-reactions, the monomer reactive groups are generally protected, with exception of the group participating directly in the formation of the chemical bond with the support. After reaction, the excess of reagents is washed away and the second monomer's active side is deprotected to allow its attachment. The cycle of coupling-washing-deprotection-coupling is repeated until the targeted oligomer sequence is achieved. The final macromolecule is then cleaved from the resin *via* a cleavable linker with simultaneous removal of all the protection groups. The solid-phase chemistry has

found a broad application in the synthesis of peptides. The concept of solid phase peptide synthesis (SPPS) is outlined in (**Scheme 2**)



Scheme 2. General synthetic route in SPPS.

While a large library of protecting groups in organic synthesis has been developed,[115] the Fmoc and Boc strategies remained the most used in the solid-phase approach (**Scheme 3**).[116]



Scheme 3. Representation of the *(i)* Boc and *(ii)* Fmoc protecting strategies.

Introduced by Merrifield butyloxycarbonyl (Boc) approach has been optimized for the synthesis of base-sensitive peptide analogues.[117] Removal of the Boc protecting group requires strong acidic conditions (*i.e.* hydrofluoric acid), thus the method has been preferentially avoided. Instead, 9-fluorenylmethoxycarbonyl (Fmoc) strategy presented by Sheppard has been adapted.[118] The use of this approach allowed orthogonal syntheses by selective removal of the Fmoc base-sensitive protecting group in contrary to acid-sensitive side-chain protecting groups. Being relatively safe in comparison with other methods, the Fmoc strategy evolved into a routinely used technique for SPPS.

Attachment of the first residue to the solid support is crucial. Thus to ensure reasonable yields of this coupling, the choice of an appropriate anchoring functionality has to be accomplished. Selection of the reactive functional group implies the choice of the resin. Indeed, based on the reactive functional groups, resins have been divided into different categories according to the type of a linker they constitute. The most commonly used linkers are hydroxymethyl, trityl chloride and aminomethyl. Among hydroxymethyl-based resins, one can distinguish the Wang[118] and Rink acid[119] type of linkers. In addition, commercialized products involve HMPA, HMBA or SASRIN linkers (**Figure 41** left). Loading of the first residue on the resins containing the hydroxyl-linker occurs *via* esterification. Formation of the ester bond can proceed *via* three strategies:[120] *(i)* symmetrical anhydride method (preparation of symmetrical anhydride from its acid derivative), *(ii)* dichlorobenzoyl chloride approach (formation of a mixed anhydride by reaction of an acid and 2,6-dichlorobenzoyl chloride in DMF/pyridine),[121] and *(iii)* MSNT/Melm strategy (treatment of the amino acids with MSNT and Melm).[122]

2-chlorotrityl chloride linker is a good alternative to the previously mentioned hydroxymethyl resin[123] (**Figure 41** middle). These extremely moisture sensitive resins seem ideal for a peptide synthesis with the participation of *C*-terminal histidine and cysteine, since coupling to trityl chloride-type resins does not require pre-activation of amino acids.[124] Moreover, this technique permits to avoid the side reaction effects observed when using the symmetrical anhydride strategy. Thus, the method has been advised for the synthesis of peptides containing *C*-terminal proline, methionine, or tryptophan residues.[125]

No pre-activation necessity is also a main characteristic of resins containing an aminomethyl linker. Although convenient for the formation of amide bond, the resin often suffers from inconsistent quality and presence of residual ketone functionalities due to the lack of efficient

aminomethyl resin preparation protocols. However, an effort to improve the existing procedures has been done during the last decade. For instance, Matsueda has established an experimental protocol for the synthesis of MBHA-resin in order to form peptides of high purity. Enhanced grade of the obtained polymer beads allowed commercialization of aminomethyl-based resin in the form of products containing Rink-amide, Sieber or PAL linkers (**Figure 41** right).

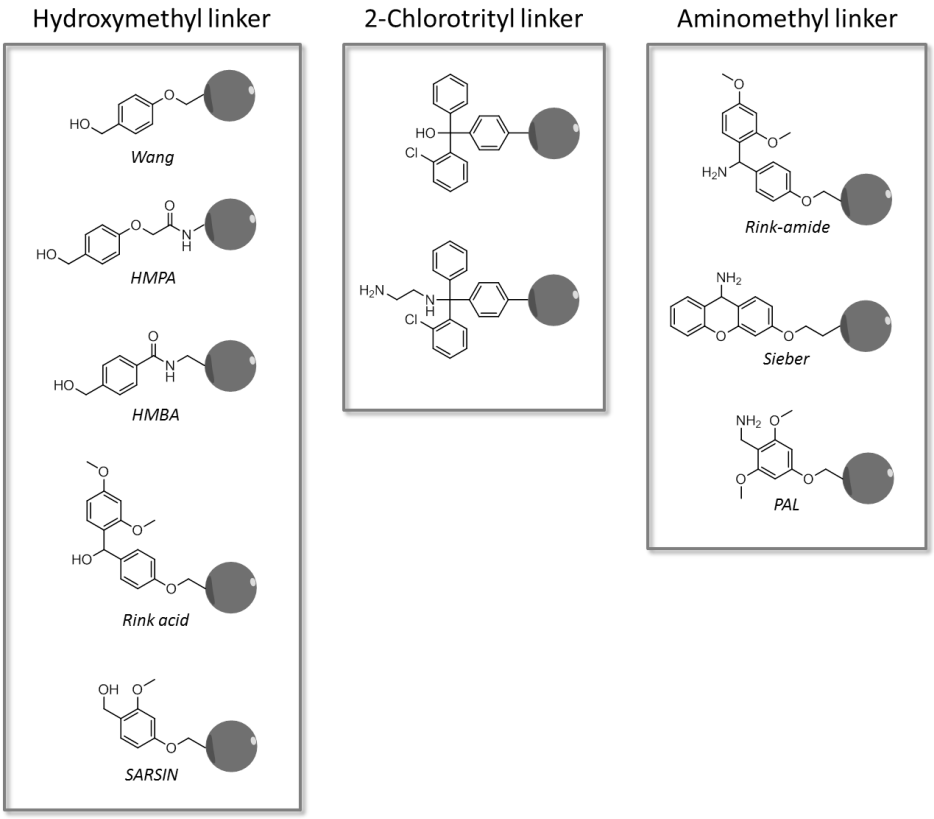


Figure 41. Variety of resin-bounded linkers commonly used in solid phase synthesis.

Development of solid phase organic chemistry has brought new challenges in the choice of the linker. Immobilization of a target molecule on the resin became no longer the unique criterium for linker selection.[126] Indeed, traceless,[127] partial release[128] or biocompatible[129, 130] linkers are among the wide collection of available structures.

4.1.2. SEQUENCE-CONTROLLED POLYMERS PREPARED ON SOLID SUPPORTS

The solid-phase method has rapidly evolved into one of the most powerful and versatile technique for the construction of biopolymers, with particular regard to oligopeptides, oligonucleotides or oligosaccharides. However, the method is not restricted to nature-mimicking structures. Adopting this approach in combinatorial synthesis gave the polymer

chemists access to various highly defined and functionalized macromolecules. In addition, the great advantage of potentially automated processes encouraged numerous research groups to exploit the solid-phase approach for the preparation of oligocarbamates, oligoureas, oligoamides or oligoesters. Syntheses of these structures have been generally accomplished *via* one of the model monomer coupling strategies: AB + AB, AA + BB or AB + CD. These strategies will be discussed in details in the following sections. It is important to specify that the letters A, B, C and D denote here chemical functions. This should not be confused with the AB terminology used in the case of alternating copolymers (**paragraph 3** in Chapter I), where A and B denotes monomer units.

4.1.2.1. AB + AB APPROACH

The strategy of AB + AB sequential polymerization refers conventionally to a reaction between monomers possessing two different functionalities with mutual chemical affinity. In solid-phase methodology, the most common examples of such structures are amino acids (*i.e.* A = primary amine, B = acid), traditionally employed in the synthesis of peptides.

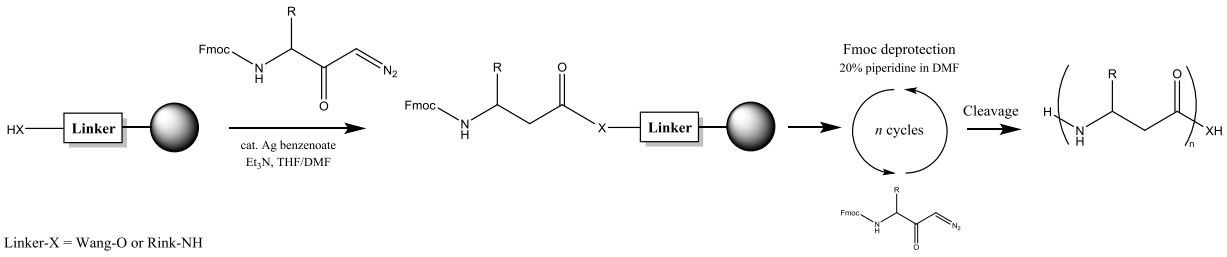
The AB + AB approach on solid support has been demonstrated for the first time in the preparation of a tetrapeptide. The solid-phase peptide synthesis has been gradually developing through the synthesis of a bradykinin nonamer,[131] neurotensin 12-mer and rhodopsin multimer.*[132] Chemical route to these naturally occurring α-peptides has been described in the previous section.

Beside peptide synthesis, the solid-phase approach has been utile for preparation of various pseudo-peptide structures, such as β-peptides,[133] γ-peptides,[134] peptide nucleic acids[134] and unnatural glycopeptides.[135] These peptide analogues were shown to have mediating potential in different biological processes, thus attracting attention of biochemists in the search of new drugs. Moreover, these macromolecules revealed an ability to form stable secondary structures that have been briefly described in (**paragraph 2.2.1** in Chapter I**)**.

An example of the solid-phase synthesis approach to β-peptides using the AB + AB approach has been reported by Bair and co-workers[136] Experimental protocol of this reaction was

* Bradykinin – peptide involved in the regulation of blood pressure.
Neurotensin – peptide involved in the regulation of central nervous system.
Rhodopsin – peptide involved in the regulation of light perception.

established based on Arndt-Eistert homologation* of Fmoc-protected amino acid diazoketones (**Scheme 4**).



Scheme 4. Synthesis of β-peptides *via* Arndt-Eistert homologation on solid support.

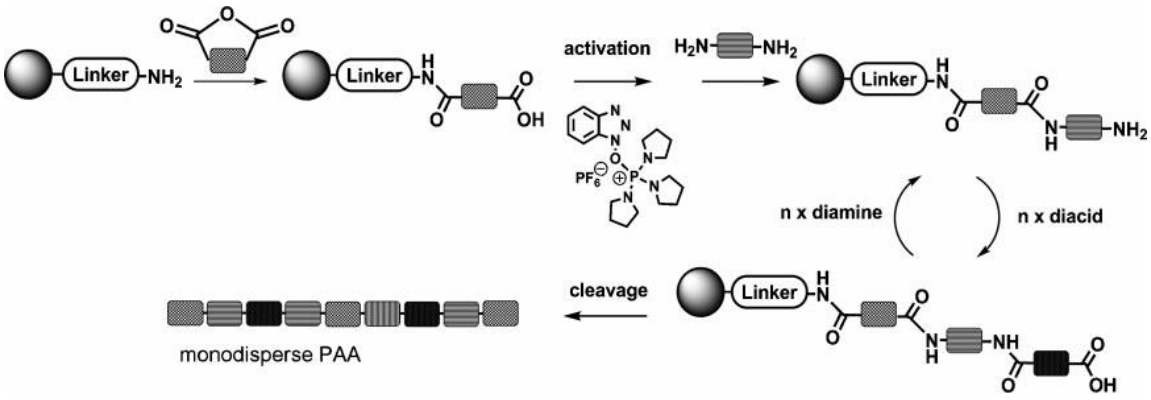
Besides the Arndt-Eistert homologation, other classical reactions, such as enolate alkylation or the syntheses based on Kolbe, Michael or Wittig reaction mechanisms were used to access the β-peptides.[29] The AB + AB solid-phase approach proved thus to be compatible with a wide variety of monomers providing the use of temporarily protecting groups.

4.1.2.2. AA + BB APPROACH

Selection of orthogonal protecting groups and their selective manipulation in the AB + AB solid-phase approach is a relatively challenging task. In alternative iterative solid-phase approaches based on AA + BB monomer coupling strategy, the presence of protecting groups is not compulsory. Indeed, as demonstrated by Rose and Vizzavona, solid-phase synthesis of well-defined polyamides can be accomplished without the use of protecting groups.[137] In this method, stepwise addition of commercially available diacid (succinic anhydride) and diamine (PEG-based diamine or 1,6-diaminohexane) led to formation of biocompatible polyamides with a defined number of repeat units. It should be highlighted that modulation of chain hydrophobicity by selecting an appropriate diacid and diamine for each coupling step allowed avoiding cross-linking (bridging) of the activated carboxyl groups with the diamine.

Furthermore, Börner and co-workers employed the AA + BB strategy in a fully automated manner for the synthesis of monodisperse poly(amidoamine)s (PAA).[138] These biocompatible macromolecules were obtained *via* alternating coupling of selected functional diacids and diamines on solid support (**Scheme 5**).

* In the first step of Arndt-Eistert homologation, the starting acid is converted into acid chloride (*e.g.* with thionyl chloride). Next, a reaction between acid chloride and diazomethane gives a diazoketone that forms the desired acid homologue in the presence of a nucleofile (*e.g.* Et_3N) and a metal catalyst (e.g. Ag benzenoate).



Scheme 5. Solid-phase-supported synthesis of monodisperse poly(amidoamine)s performed on automated synthesizer. Reprinted by permission from [138], © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Precise positioning of the reactive groups along PAA chain allowed next the synthesis of peptide-*b*-PAA and poly(ethylene oxide)-*b*-PAA conjugates.[139] The latter was found to interact with DNA. Moreover, the authors showed that they could tune the complexation of DNA varying the cationic nature of PAA segment.

4.1.2.3. AB + CD APPROACH

AB + CD monomer coupling strategy represents the final approach in the stepwise formation of well-defined polymers. The principle of controlling such reaction relies on the selection of monomers that react with each other in a chemoselective manner.

The AB + CD iterative approach opens many opportunities in designing sequence-specific macromolecules. For instance, the aforementioned peptoids (**paragraph 2.2.1**) are examples of the structures prepared *via* this approach. Peptoids are commonly obtained *via* solid-phase repeating cycles of acylation with bromoacetic acid followed by the replacement of the halide with a primary amine (**Figure 42**).

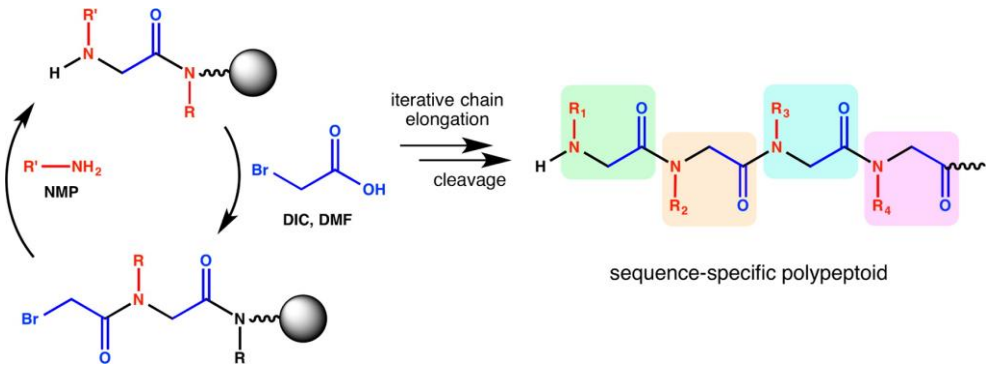


Figure 42. Solid-phase strategy to peptoids. Reprinted with permission from [140]. Copyright 2013 ACS.

The concept of AB + CD iterative growth has been also employed by Lutz and co-workers in the synthesis of sequence-ordered oligomers *via* solid- and liquid-phase methodologies.[76] The targeted macromolecules have been prepared *via* 1,3-dipolar cycloaddition of terminal alkynes (A) and azides (D) and amidification of carboxylic acids (B) with primary amines (C). Importantly, protecting groups have not been required in this approach since function A reacted exclusively with function C, and function B with function D.

On the other hand, Meier and co-workers have recently developed approach based on iterative application of the versatile Passerini three-component reaction (P-3CR) in combination with efficient thiol–ene addition reactions.[141] By adapting this approach, different side chain were introduced to the polymer/oligomer backbone in a simple way and at a defined position within the macromolecule. Thus, successful synthesis of a tetramer as well as a block copolymer containing a sequence-defined block of five units and bearing five different side chains was accomplished

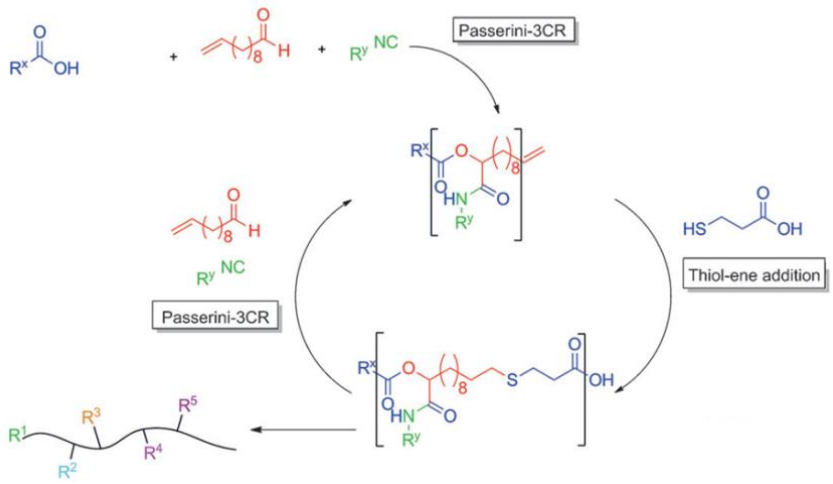


Figure 43. Synthesis of a sequence-defined tetramer (or block copolymer) by iterative application of the P-3CR and the thiol–ene addition reaction. Reprinted by permission from [141], copyright © 2014 Wiley Periodicals, Inc.

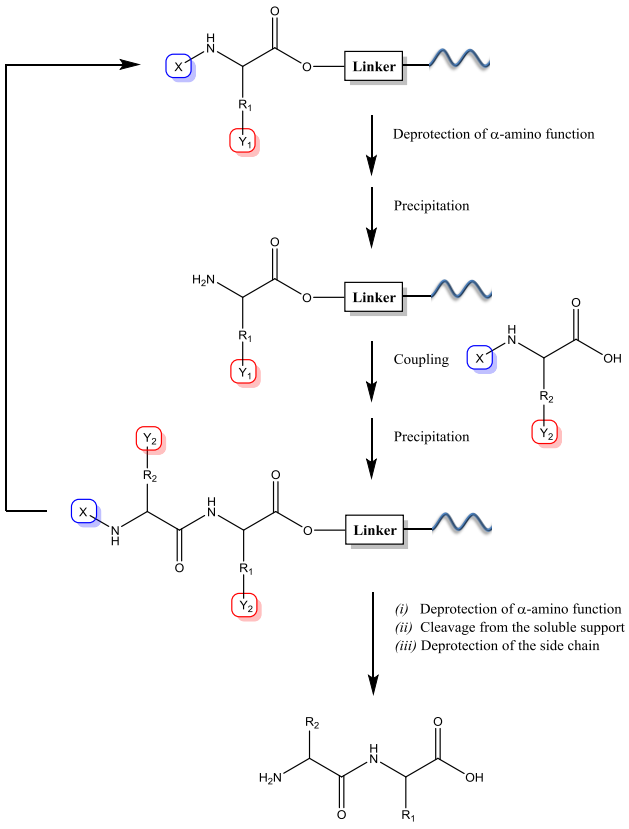
4.2. ITERATIVE LIQUID-PHASE SYNTHESIS

4.2.1. BACKGROUND

Soluble polymer supports represent an interesting alternative to conventional solid-phase supports for organic synthesis, catalysis, and biochemistry. Presented in 1965 by Shemyakin and co-workers liquid-phase methodology for the synthesis of oligonucleotides has been designed to overcome the shortcomings of the heterogeneous reaction conditions associated with the solid-phase approach.[142] By replacing insoluble cross-linked resin with a

homogeneous support, steric factors causing incompletion of coupling reactions could be avoided. In addition to the quantitative coupling, the soluble supports can serve as functional polymer platforms by being a part of the build-up block copolymer. [76]

The first successful attempt to liquid-phase approach was performed on poly(ethylene glycol).[143] Due to its biological compatibility, this water-soluble polymer has been widely used for synthesis of peptides. Conventionally, the construction of a peptide chain on soluble supports processes *via* linkage of the carboxylic group of an amino acid and an amine/alcohol functionality of the polymer (**Scheme 6**). Subsequently, any other functional group in the amino acid needs to be protected to ensure regioselective coupling. As in the solid-phase synthesis, attention should be drawn to the attachment of the first amino acid residue. A quantitative coupling in this step is crucial for the efficiency of the entire peptide synthesis. Most of the coupling methods resulting in amide bond formation require pre-activation of the amino acid's carboxylic group. Among all, several milder activation techniques have been identified as suitable for peptide synthesis. For instance, formation of acyl halides, acyl azides, acylimidazoles, anhydrides or active esters has been reported as appropriate strategies.[144]



Scheme 6. General synthetic route in liquid-phase synthesis of peptides.

Purification of the support-oligomer conjugate after each step of coupling is certainly more demanding in comparison with the simple "wash-off" in the solid-phase approach. The method used for separating polymers from the reaction mixture depends on the chemical nature of the synthesized block copolymer (**Figure 44**).

Most frequently, the macromolecule is isolated from the reagents by selective precipitation in a non-solvent (**Figure 44** A).[145] The resulting precipitate is then filtered, dried and consequently used for the next coupling step. For instance, the two commonly used poly(ethylene glycol) and polystyrene linear supports can be easily purified in diethyl ether or methanol solutions, respectively. Another commonly used method for the isolation of the polymer support is thermal precipitation (**Figure 44** B). This technique relies on the differences in solubility of the mixture entities in the precipitation solvent according to the used temperature. Recovery of the soluble polymer-bound oligomers can be also attain by selective solubility of the polymer in one phase of a biphasic mixture.[146] This separation technique is routinely used in organic synthesis; nevertheless limited to water-soluble polymers in regards to the discussed here systems. In particular, considerably much attention has been denoted to fluorinated polymers having selective solubility in a fluorous phase of a biphasic organic/fluorocarbon system (**Figure 44** C).[147]

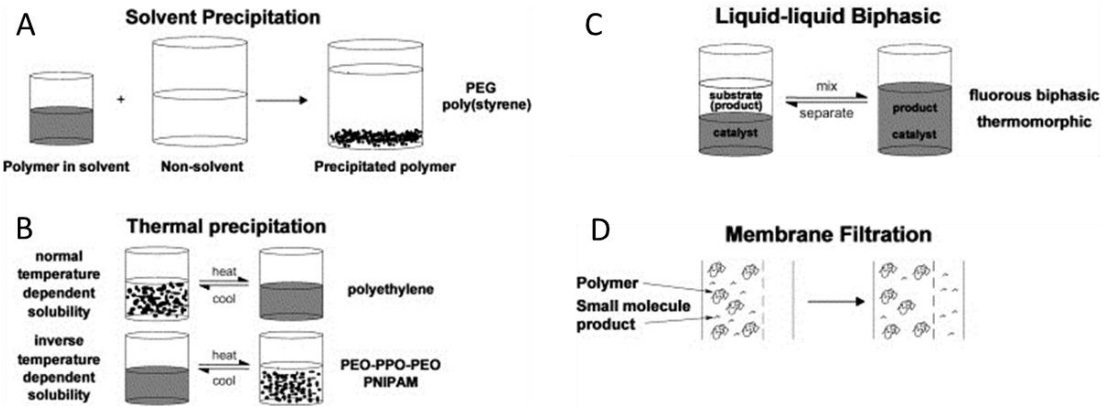


Figure 44. Polymer support purification methods used in liquid-phase synthesis. Reprinted by permission from 132, copyright © 2001 Elsevier Ltd.

Alternatively, product separation can be accomplished by dialysis using semipermeable membrane, *i.e.* in membrane filtration process (**Figure 44** D).[148] The ultrafiltration has been demonstrated for the isolation of polymer-peptides conjugates containing hydrophilic macromolecules, such as poly(ethyleneimine) or poly(acrylic acid).[149] Moreover, this technique is particularly appropriate for separation of dendritic supports because of the

globular structure of these hyperbranched polymers.[150] Eventually, polymer separation can be achieved with assistance of gel permeation and adsorption chromatography.[151]

Introduction of second amino acid unit in peptide chain on soluble supports is preceded by removal of temporary protecting group of the amine function of the polymer-linked first residue. The commonly used peptide protection strategies are similar to the methods used in the solid-phase approach. An excess of the second amino acid and presence of the carboxylic acid group activating agents lead to the formation of second amide bond. After coupling, the polymer-supported conjugate is purified by one of the aforementioned techniques. The cycle "coupling-deprotection-coupling" is repeated until formation of the desired peptide sequence. Ultimately, the targeted peptide can be released from the support providing presence of a cleavable linker (the different type of linkers were described in **paragraph 4.1.1** in Chapter I).

4.2.2. ORGANIC SYNTHESIS ON SOLUBLE POLYMER SUPPORTS

Iterative synthesis on soluble polymer supports can be accomplished by employment of variety of polymers. Ideally, a macromolecular carrier should possess some important features, such as *(i)* high solubilizing power in compromise with high polymer loading capacity, *(ii)* well-defined molecular weight and molecular weight distribution, *(iii)* ability to withstand the reaction conditions, *(iv)* good recovery after each coupling, and *(v)* suitability to chain-end functionalization.

The linear polystyrene and poly(ethylene glycol) polymers mentioned before belong to the most commonly used soluble supports. In addition, the possible use of commercially available poly(vinyl alcohol),[152, 153] polyethylene[154] or poly(*N*-isopropylacrylamide)[155] for liquid-phase synthesis has been demonstrated. A list of potential soluble polymer supports has been established by Gravert and Janda (**Figure 45**).[156]

Multiple reports on oligopeptides, oligonucleotides, oligosaccharides and further smaller compounds prepared using soluble supports have been published over the last two decades.[157] Furthermore, special consideration on these macromolecular grafting platforms was dedicated to their application in catalyst and ligand recovery.[149, 158] In the following sections, the utility of liquid-phase methodology for the synthesis of such structures will be screened. Simultaneously, the synthesis of oligopeptides will be here omitted as it has been

already described in **paragraph 4.1.2.1** (Chapter I) as the example of AB + AB iterative approach to sequence-controlled polymers.

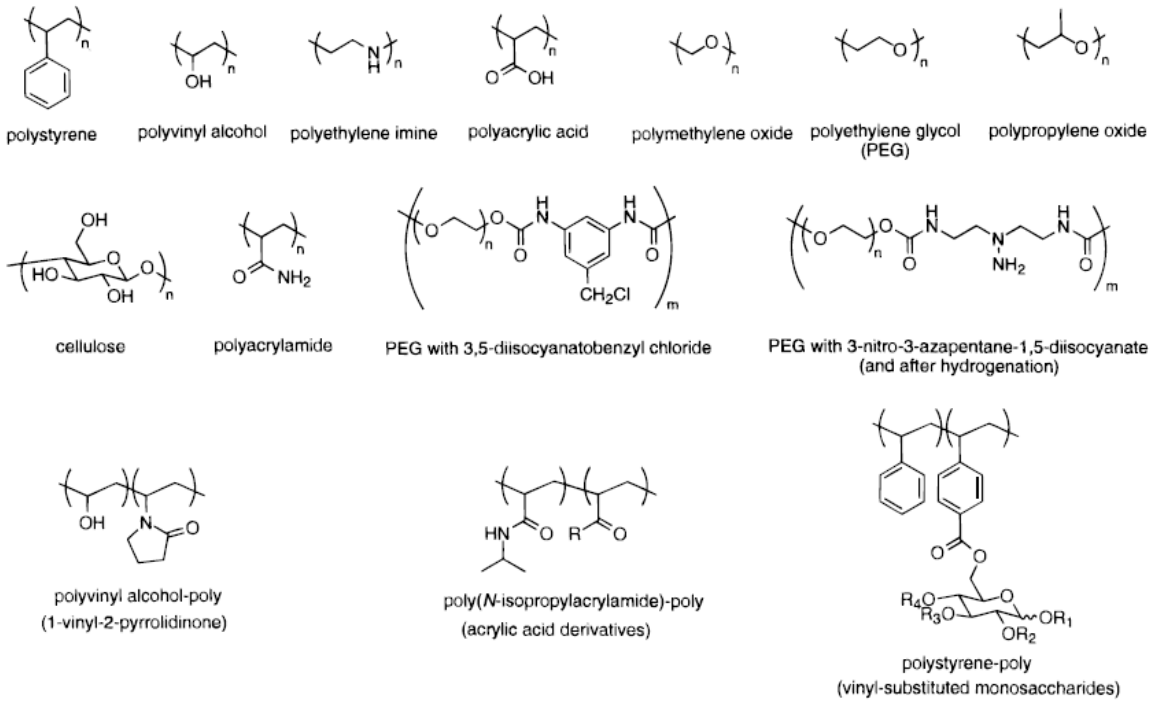


Figure 45. Soluble polymers utilized in liquid-phase synthesis. Reprinted with permission from [157]. Copyright 1997 ACS.

4.2.2.1. SYNTHESIS OF OLIGONUCLEOTIDES

Oligonucleotide chain growth on a soluble polymer carrier requires addition of water or pyridine solvents, thus generally favoring hydrophilic polymers.[159] Indeed, satisfactory reaction rates obtained on polystyrene supports were yet inconvincible in comparison with problematic separation of this hydrophobic polymer.[160] However, the synthesis of oligonucleotides on water-soluble poly(vinyl alcohol) has revealed another important aspect of liquid-phase synthesis. High loading capacity of this polymer possessing numerous hydroxyl anchoring groups resulted in the decrease of solubility of the support. This effect occurred in consequence to the oligonucleotide attachment.[161] Moreover, a successful application of the liquid-phase oligonucleotides synthesis has resulted from the use of poly(ethylene glycol). Indeed, the employment of this soluble support has led to development of a high-efficiency liquid phase method (HELP)[159] for PEG-oligonucleotide synthesis in a large scale *via* phosphotriester[162] or H-phosphonate[163] procedures.

4.2.2.2. SYNTHESIS OF OLIGOSACCHARIDES

The potential for soluble polymer supports to facilitate oligosaccharides synthesis was discovered few years later. Unlike peptides and oligonucleotides, synthesis of the oligosaccharides requires particular precautions due to the presence of chiral bonds in the macromolecule. Initial attempts to control formation of the stereochemically sensitive glycosidic bond were characterized by low yields and the presence of side-product impurities as indicated by Paulsen.[164] In 1991, Krepinsky and co-workers proposed an improved protocol for a more efficient synthesis of oligosaccharides on PEG soluble support.[165] In this approach, poly(ethylene glycol) monomethyl ether of M_w = 5000 $g \cdot mol^{-1}$ has been linked to the different hydroxyl groups of the oligosaccharide *via* ester bond of succinic acid. Such PEG bonding allowed the glycosylation reaction to be completed by repeated additions of the glycosylating agent. The removal of non-polar decomposed reactants occurred through washing off the mixture, while more polar impurities were separated from the product by PEG-*b*-oligosaccharide crystallization from ethanol. Isolated products were obtained in 85-95% yield. This result demonstrated the advantage of poly(ethylene glycol) support for the synthesis of biopolymers. In addition, polyacrylamide and its copolymers have equally shown the ability to support oligosaccharide synthesis, although the coupling was in this case less efficient and required mediation of enzymes.[166]

4.2.2.3. SYNTHESIS OF SMALLER COMPOUNDS

Finally, the advantages of soluble polymer supports were demonstrated for the synthesis of a large number of molecules with different functionalities, including ligands and catalysts. The liquid-phase methodology allows an excess of reagents, thus proving optimum yields. Numerous examples in the literature refer to azatides,[167] optoelectronic liquid crystallines,[168] sulfonoamid antitumor agents,[169-171] mimetics of neomycin B[172] and many more.[173] Such diversity of compounds obtained *via* soluble-support approach shows benefits of this methodology and encourages for further research.

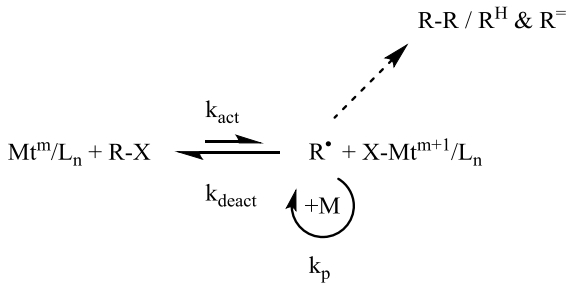
5. CONTROLLED POLYMERIZATION TECHNIQUES

Limited control over molecular weight, molecular weight distribution and polymer chain-end functionalities in the free-radical polymerization encouraged polymer chemists to look for an alternative technique for a synthesis of well-defined macromolecules. Foundations to development of controlled polymerization have been given in 1956 by Szwarc.[174] His

discovery of living anionic polymerization demonstrated possibility of continuous polymer chain growth until complete consumption of the monomer. However, the drawbacks of using drastic experimental conditions and the limited choice of monomers compatible with the reactive centers prevented expansion of this method. Subsequently, control radical polymerization (CRP) techniques have been introduced to merge the advantages offered by the free-radical and the anionic polymerizations. In consequence, several efficient methods have been found, including atom transfer radical polymerization (ATRP),[175] activator regenerated by electron transfer (ARGET) ATRP,[176] nitroxide mediated polymerization (NMP),[177] reversible addition-fragmentation chain-transfer (RAFT)[178] and single-electron transfer living radical polymerization (SET-LRP).[179] The main principle of the CRP methods is equilibrium between dormant and active polymer chains undergoing propagation. Importantly, to supress terminations occurring traditionally in radical reactions, CRP process must favour the formation of dormant chains. Further insight into selected CRP methods is given below.

5.1. ATOM TRANSFER RADICAL POLYMERIZATION

ATRP is one of the most widely spread techniques of controlled radical polymerizations. It has been independently discovered in 1995 by Krzysztof Matyjaszewski[180] and Mitsuo Sawamoto.[181] Broad range of polymers prepared *via* this method (styrenes,[182-184] (meth)acrylates,[185-187] acrylonitriles,[188] or (meth)acrylamides[189, 190], *etc.*) led to commercialization of the ATRP technique in industry, *e.g.* in the field of coatings and adhesives. The mechanism of ATRP is based on a reversible redox process that occurs between alkyl halides and transition metal complexes (**Scheme 7**).



Scheme 7. Schematic representation of the ATRP mechanism.

The first step in the polymerization is a homolytical cleavage of an alkyl halide bond forming a carbon-centered radical on the alkyl. Generation of the radical occurs through one-electron oxidation of the transition metal complex and subsequent abstraction of the halogen (X) from a dormant species (R-X). As in all CRP techniques, a low concentration rate of the radicals is essential to maintain the equilibrium of the reaction. Indeed, control over the polymerization is highly dependent on the amount of dormant and active species present in the reaction mixture. In consequence, the overall rate of ATRP process is controlled by a redox potential of the metal complexes. Propagating species grow by the addition of the intermediate radicals to the monomers as in the conventional free-radical polymerization. Termination reactions also occur, however, in a well-controlled ATRP, termination concerns no more than a few percent of the polymer chains.

An appropriate selection of a monomer, initiator, catalyst and ligand is crucial to provide uniform growth of the polymer. Monomers typically used in this polymerization were already mentioned at the beginning of the paragraph. Importantly, all of these structures contain substituents stabilizing the propagating radicals.[191]

The initiator should ensure fast initiation that in consequence allows reducing terminations and transfers. Most of the ATRP initiators are alkyl bromides and chlorides; however alkyl iodides have been also reported.[192] An overview of the structural variety of the initiators is presented in **Figure 46**.

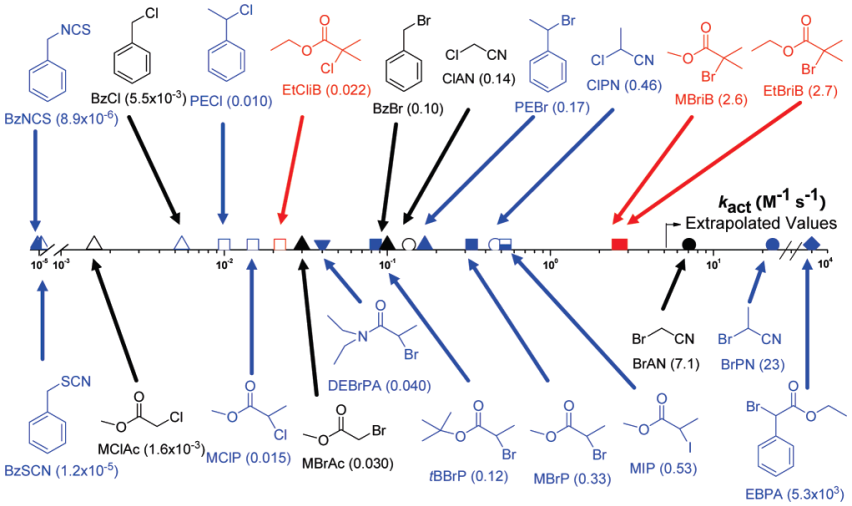


Figure 46. ATRP activation rate constants for various initiators with $Cu^{I}X$/PMDETA (X = Br or Cl) in MeCN at 35 °C: 3°, red; 2°, blue; 1°, black; isothiocyanate/thiocyanate, left half-filled; chloride, open; bromide, filled; iodide, bottom half-filled; amide, ▼; benzyl, ▲; ester, □; nitrile, ○; phenyl ester, ◊. Reprinted with permission from [193]. Copyright 2007 ACS.

The activity of alkyl group for the ATRP initiators follows the order of 3° > 2° > 1° and phenyl ester > cyanide > ester > benzyl > amide. Furthermore, the activity of the leaving group decreases in the order of I > Br > Cl >> SCN ≈ NCS. It seems thus obvious that the use of alkyl iodides is preferential; however a low stability of metal iodide complexes favoured the employment of bromides and chlorides.

Since the appropriate selection of an initiator is a simple method for introduction of an α-functionality into the polymer chain, the field of end-functionalized polymers has developed rapidly. Indeed, introduction of a hydroxyl group in the initiator's structure permits post-modification of the polymer with a carboxylic acid/isocyanate groups,[194] presence of an azide or alkyl group allows click chemistry-type reactions,[195, 196] silyl moieties let modifications on the surface,[197] *etc*. **Figure 47** displays examples of such functional initiator structures.

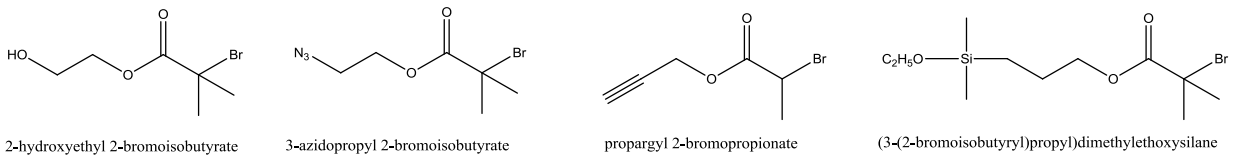


Figure 47. Examples of functional ATRP initiators.

Moreover, the initiator structure can determine architecture of the polymerized macromolecule. For instance, polymerization conducted with an initiator containing multiple alkyl halide sites concentrated on the same core will lead to a star-shape polymer.[198]

The most important component of ATRP is however a transition metal complex formed by a catalyst and a ligand that regulate equilibrium of the polymerization. Efficient transition metal catalyst should possess at least two oxidation states varied by an electron. Furthermore, it should have affinity toward a halogen. Ultimately, it needs to form a strong complex with the ligand. From the limited number of the transition metals fulfilling these requirements, copper based catalyst was found to be the most efficient.

In general, there are three roles of the ligand in the ATRP system: *(i)* solubilization of the metal ion in the organic medium, *(ii)* selectivity of the metal center by a steric fitting and, *(iii)* adjustment of the redox potential of the final metal complex.[199] For the copper-mediated ATRP, chelating ligands containing a nitrogen atom(s) were found to be the most efficient.

Figure 48 displays a summary of the activity of copper based catalysts with different *N*-based ligands.

To date, Bipy, d*n*Bipy, PMDETA and Me_6TREN remain the most frequently employed ligands. Selection of the suitable ligand is determined by the rate constants k_{act} and k_{deact} (k_{act} describes generation of radicals, k_{deact} – formation of alkyl halides), thus dependent at the same time on the initiator's nature.

In addition to the selection of relevant polymerization components, optimization of the ATRP process requires adjustment of the reaction solvent, temperature and time. ATRP can be carried out in bulk, solution or a heterogeneous system, such as emulsion or suspension. Depending on the monomer, different solvents can be applied, including water.[200] Nevertheless, the use of polar solvents should be carefully examined due to potential side reactions, such as *e.g.* chain transfer or elimination of the halogen from polystyryl halides.

ATRP is generally conducted in the range of temperatures from 60 to 120 °C. Higher temperatures increase the rate of polymerization, thus better control can be observed. However, chain transfer and other side reactions become more pronounced at elevated

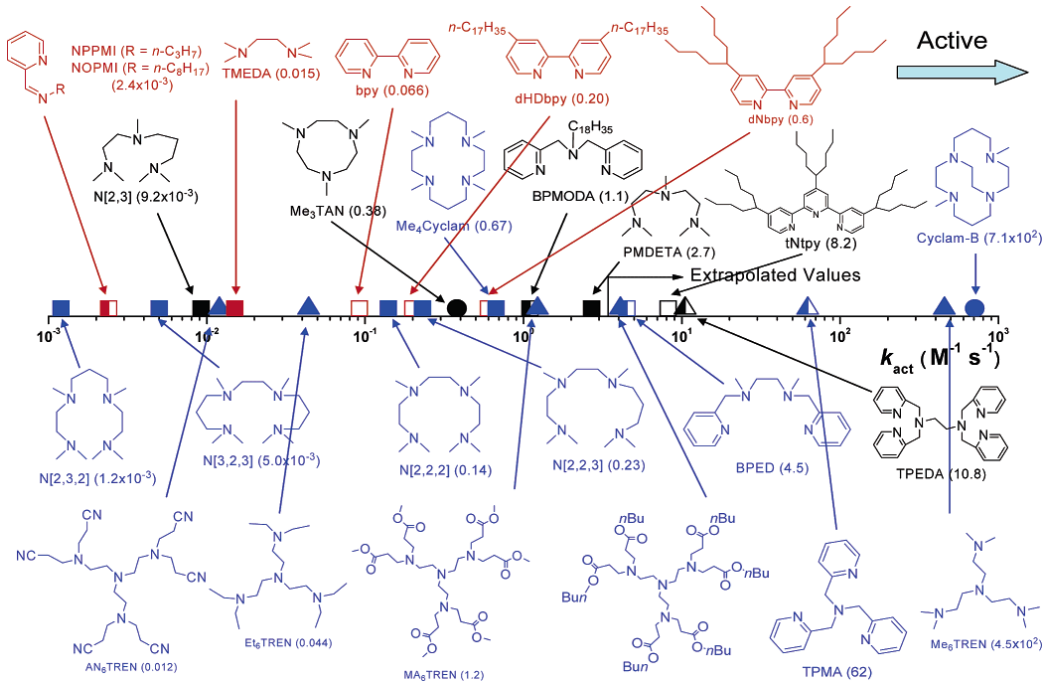


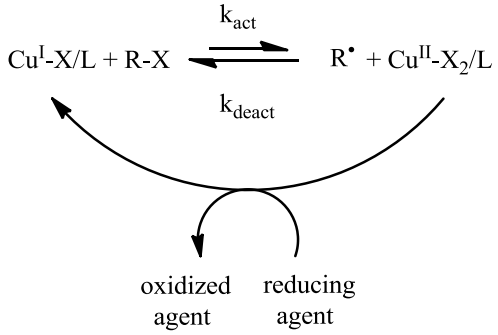
Figure 48. ATRP activation rate constants for various ligands with ethyl 2-bromoisobutyrate in the presence of $Cu^{I}X$ (X = Br or Cl) in MeCN at 35 °C: N2, red; N3, black; N4, blue; amine/imine, solid; pyridine, open; mixed, left-half solid; linear, □; branched, ▲; cyclic, ○. Reprinted with permission from [201]. Copyright 2006 ACS.

temperatures.[202] Eventually, the optimal temperature is dictated by targeted molecular weight and the monomer and catalyst used in the reaction.

To well maintain the polymerization time, the reaction should not be led to complete, since high monomer conversions induce loss of end groups. Indeed, at prolonged reaction times, the rate of propagation slows down, while the rate of side reactions remains constant due to its independency on monomer concentration. The recommended polymer conversion should thus not exceed 95%.

5.2. ACTIVATOR REGENERATED BY ELECTRON TRANSFER ATRP

Adapting ATRP to industrial production was one of the motivation for development of a polymerization technique that requires small amount of the copper catalyst. Moreover, lowering quantity of the residual copper traces increases suitability of the final product for biological applications. The novel approach called activator regenerated by electron transfer ATRP allowed not only to reduce concentration of the catalyst, but in addition extended the level of oxygen toleration. The method has been discovered by Matyjaszewski and co-workers in 2006.[203] ARGET ATRP system differs from the original ATRP by presence of a reducing agent that permits regeneration of the catalyst. A mechanism of the process is shown in **Scheme 8**.



Scheme 8. Schematic representation of the ARGET ATRP mechanism.

Owning to the reducing agent, the inactive Cu^{II} species are continuously converted in their active Cu^{I} form throughout polymerization. Apart from that, the ARGET ATRP process follows earlier described ATRP mechanism.

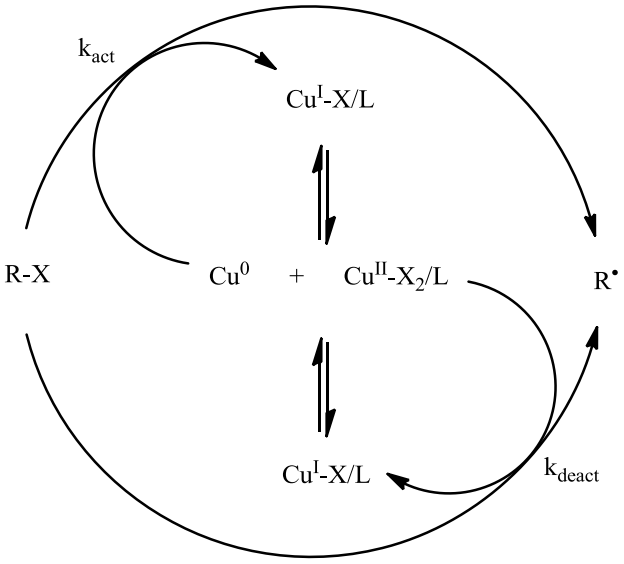
ARGET ATRP is compatible with numerous reducing agents, including environmentally friendly compounds, such as tin(II) 2-ethylhexanoate ($Sn(EH)_2$), glucose,[176] hydrazine and

ascorbic acid.[204] As the choice of reducing agent impacts equilibrium of the reaction, strong reducing agents should be used with consideration. For instance, ascorbic acid can only be employed in a heterogeneous polymerization mixture that limits the activity of this strong reducing agent.

Besides the advantages of a "green polymerization" and partial tolerance to the air, ARGET ATRP has proved a capability to synthesise polymers not feasible with ATRP. For instance, Yamamoto and co-workers have demonstrated polymerization of di(ethylene glycol) methyl ether with 80% conversion, instead of 50% reached with ATRP.[205]

5.3. SINGLE ELECTRON TRANSFER LIVING RADICAL POLYMERIZATION

SET-LRP is an alternative polymerization method using low concentration of copper catalyst. It has been discovered in 2006 by Percec and co-workers.[206] The method relies on the usage of initiators and ligands employed in ATRP and ARGET ATRP with subsequent replacement of the copper salts with an elemental copper as an activating species. The mechanism of SET-LRP is displayed in **Scheme 9**.



Scheme 9. Schematic representation of the SET-LRP mechanism.

In SET-LRP, generation of the radical occurs through a Cu^0-mediated outer-sphere electron transfer. The radicals undergo continuous reactions of activation-deactivation as in the aforementioned ATRP and ARGET ATRP processes. Again, to maintain a good control over the polymerization, equilibrium between the dormant and active species must be preserved. Activation and deactivation steps create Cu^I species that are steadily disproportionated into

Cu^0 and Cu^{II} by assistance of an appropriate ligand and solvent. Disproportionation-supporting ligands include Me_6TREN, TREN or PMDETA. Simultaneously, the process is stimulated by polar or coordinating solvents, such as DMSO,[206] alcohols[207] or mixture of water and other organic solvents.[208]

SET-LRP has been successfully employed for the synthesis of acrylates,[209] methacrylates[210] and acrylamides.[211] There are two advantages of this technique over the corresponding low-catalyst ARGET ATRP: *(i)* high polymer conversions are possible to attain at room temperature, *(ii)* suitability of the process to various polar or coordinating solvents, and *(iii)* simplicity of the removal of elemental Cu^0 (traditionally introduced to the system in a form of a wire).

Nevertheless, SET-LRP and ARGET ATRP methods possess the same drawback. To achieve a good control of the final polymer properties, high molar excess of the ligand is required. To date, the use of most common SET-LRP and ARGET ATRP ligands, such as Me_6TREN and TPMA, is too expensive for industrial polymer production.

Chapter II

SEQUENCE-CONTROLLED SYNTHESIS OF POLYMER-PEPTIDE CONJUGATES USING AN AB + AB APPROACH

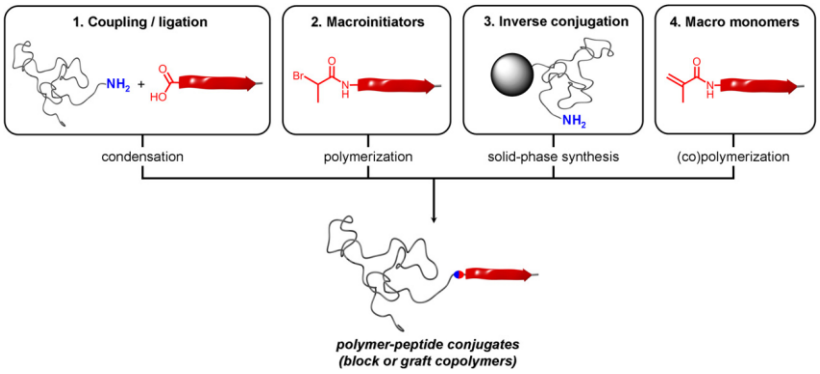
1. INTRODUCTION

Polymer bioconjugates are macromolecular structures that consist of a biologically active molecule covalently attached to a synthetic polymer. Biohybrids have proved utility in number of fields including drug delivery (enhancing solubility and therapeutic activity of bonded biological molecules),[212] biomimetic sensors,[213] gene therapy[214] or biomineralization.[215-217] While most of the applications of bioconjugates are still far from being commercialized, few examples already existing on the market are worth to be mentioned.[218, 219] For instance, employment of immobilized enzymes in pharmaceutical industry (preparation of 6-aminopenicillanic acid from immobilized penicillin amidase),[220] food industry (isomerization of fumaric acid to malic acid catalyzed by immobilized fumarase)[221] or chemical industry (production of acrylamide from acrylonitrile by usage of immobilized nitrile hydratase)[222] has been established. Furthermore, polymer bioconjugates have been used for transportation of bioactive peptides and proteins. For instance, this concept has been commercialized to treat hepatitis B, hepatitis C and multiple sclerosis in a form of PEGylated interferon.[223] Indeed, covalent attachment of poly(ethylene glycol) to therapeutically active compounds has been found to improve solubility and reduce toxicity of carried drugs.[224, 225] It is thus clear that although the scientific potential of polymer bioconjugates has been discovered only in the last decade; evolution of this field brings many promises in modern polymer chemistry.

Peptide-polymer conjugates can be synthesized using a variety of approaches. Traditionally, design of the biohybrid polymers consists of three steps: *(i)* introduction of chemoselective groups onto natural or synthetic peptides/proteins, *(ii)* introduction of chemoselective groups onto the side-chain or chain-end of synthetic polymers, and *(iii)* convergent or divergent assembling of polymers and peptides/proteins.[226] **Scheme 10** presents a summary of featured strategies used for preparing polymer bioconjugates.

Due to its versatility, direct polymerization from a pre-defined peptide (**Scheme 10** *(2))* is the most promising approach to access well-defined polymer–peptide bioconjugates. The peptide fragment is first synthesized *via* standard solid phase peptide synthesis methodology. Next, the molecule is chemically modified with a low molecular weight initiator moiety to be subsequently used as macroinitiator for the polymerization of synthetic monomers. Although the polymer block is not restricted in molecular weight, the polymerization process needs to

be carefully examined with respect to the used proteins. Controlled radical polymerization techniques were proved to have broad tolerance to the presence of peptide segments and proteins. For instance, atom transfer radical polymerization,[175] reversible addition-fragmentation chain-transfer polymerization[178] or nitroxide mediated polymerization[177] have all been tested for preparation of polymer "biocarriers". Similarly, the CRP techniques were applied in copolymerizations incorporating peptide macromonomers (**Scheme 10** *(4)*).[227]



Scheme 10. Graphic illustration of the principal approaches towards polymer bioconjugates: *(1)* coupling of peptide segments to preformed polymer blocks using one or multiple reactive sites, *(2)* "grafting from" approach: growth of polymer block from the peptide segment, *(3)* sequential assembling of the peptide on a preformed synthetic polymer, *(4)* polymerization of short peptide segments that possess a polymerizable functionality. Reprinted with permission from [228]. Copyright 2013 ACS.

Although competitively efficient, the macroinitiator and macromonomer strategies can be limited in the choice of peptides compatible with CRP techniques. Alternatively, linkage of polymer and peptide segments together may be performed *via* reactive anchor sites of peptide's building blocks (**Scheme 10** *(1)*). This technique was initially used to couple PEO to peptides or proteins by formation of an amide bond.[229] Peptide ligation reactions require utilization of selective chemistries that allow binding of end-functionalized polymer chains to a specific position in a peptide sequence in case of presence of two or more functional groups.

Regioselectivity is a central feature of another commonly used technique for preparation of bioconjugates – solid supported synthesis (**Scheme 10** *(3)*). Conjugation of the monomers occurs in this approach in a stepwise manner *via* bonding of deprotected amino acids end groups. Eventually, the target peptide is cleaved from the support which is no longer useful.

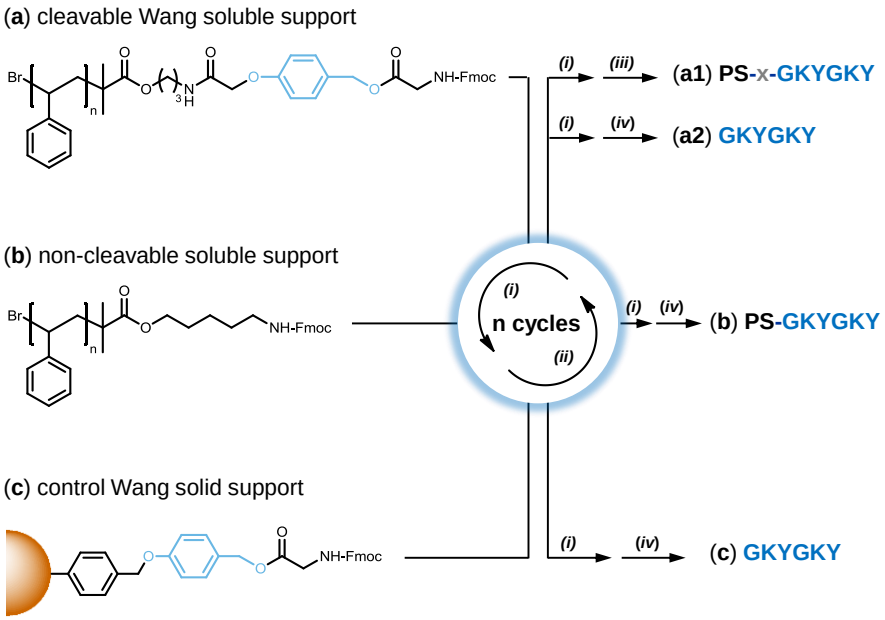
The methodology for preparation of bioconjugates applied in this work combines the efficiency of solid-phase approach with "economization" of the traditionally wasted polymer

support. Indeed, by employing liquid-phase methodology our approach allowed preserving of the support. Thus, in a strategy of "inverse bioconjugation", we aim to synthesize a bioconjugate from a soluble polymer platform being support for iterative growth of peptides.[230]

In order to demonstrate this concept, two types of polystyrene soluble supports have been prepared: with and without a cleavable linker (**Scheme 11** (a) and (b)). Presence of the linker (an acid-labile *p*-alkoxybenzyl ester moiety) allowed cleavage and characterization of the final peptide sequence, while the synthesis on polymer support without cleavable site led to formation of permanent biohybrid block copolymer. A Gly-Lys-Tyr-Gly-Lys-Tyr (GKYGKY) sequence has been chosen as the model oligopeptide in this study. This specific oligopeptide sequence is a short chain analog of the so-called "mussels glue" – a substance that allows mussels binding to underwater surfaces *via* formation of cooperative ionic and catechol anchors.[231] In addition, we performed a control solid-phase synthesis on a commercially available Wang resin (**Scheme 11** (c)) in order to examine validity of our solution-based approach.

Well-defined polystyrene supports were prepared by atom transfer radical polymerization. As mentioned before, ATRP has been proved to serve as an efficient method for preparation of tailor-made polymers. Similarly, because of its ease of manipulation and high recovery yields, polystyrene is a well-recognized polymer support for solution-based syntheses.[9]

Peptide synthesis by stepwise amino acids coupling required utilization of protection groups for the amino acid side-chain. Depending on the selected approach, two types of protecting groups have been employed. Boc and *t*Bu structures for the synthesis on non-cleavable PS support (**Scheme 11** (b)), and Mtt and 2ClTrt structures for the reaction on cleavable PS support (**Scheme 11** (a)). The Boc and Mtt moieties were used to protect the amino side-chain group of lysine, while the use of *t*Bu and 2ClTrt groups deactivated tyrosine's hydroxyl function. Mtt and 2ClTrt structures can be removed in mild acidic conditions, thus allowing for potential deprotection of the peptide segment without cleaving it from the support (*reminder:* the cleavable support contains an acid-labile linker). This result can be equivalently obtained by the removal of Boc and *t*Bu groups in our second approach (where mild cleavage conditions are not required).



Scheme 11. Strategies examined herein for the synthesis of peptide-polymer conjugates: (a) liquid phase synthesis using a cleavable Wang support, (b) liquid phase synthesis using a non-cleavable support, (c) control peptide synthesis on a conventional cross-linked Wang resin. Experimental conditions: *(i)* piperidine/CH_2Cl_2 1:1, *(ii)* PyBOP, DIPEA, CH_2Cl_2. The Fmoc protected amino acids Lys(Mtt), Tyr(2ClTrt), Gly, Lys(Mtt), and Tyr(2ClTrt) were used in this order in approach **a**. The Fmoc protected amino acids Gly, Lys(*t*Boc), Tyr(*t*Bu), Gly, Lys(*t*Boc), and Tyr(*t*Bu) were used in this order in approach **b**. The Fmoc amino acids Lys(*t*Boc), Tyr(*t*Bu), Gly, Lys(*t*Boc), and Tyr(*t*Bu) were used in this order in approach **c**. *(iii)* TFA/CH_2Cl_2 3:97, *(iv)* TFA/CH_2Cl_2 1:1. Reproduced from [232] with permission of RSC.

Polystyrene-peptide block copolymer was next utilized as a platform for PEGylation. Solid-phase methodology has been conveniently applied to peptide–PEG conjugate synthesis by either sequential amino acids coupling on a PEG-preloaded resin (inverse conjugation)[233] or introducing end functional PEG to a supported peptide.[234] Solid support coupling strategies have strong advantages due to ease of purification and sequence specificity, however the peptide-PEG block copolymer was formed with a low efficiency, in particular in case of high molecular weight PEG chains.[235] For instance, coupling with CH_3O-PEG-COOH of M_n = 2000 g·mol^{-1} leads to a conversion of only 40% (despite of large excess of PEGylation agent).[236] In this context, we present in this chapter a concept to improve peptide-PEG coupling rate *via* liquid-phase methodology. On the contrary to solid-phase strategy, our system is free from diffusion limitations of the resin-based heterogeneous reactions. Well-defined polystyrene supports prepared by ATRP were utilized as a platform for iterative synthesis of Tyr-Lys-Tyr-Lys-Gly peptide. PEGylation process occurred *via* binding to *N*-terminal amino group of the supported pentapeptide. To evaluate the potential of

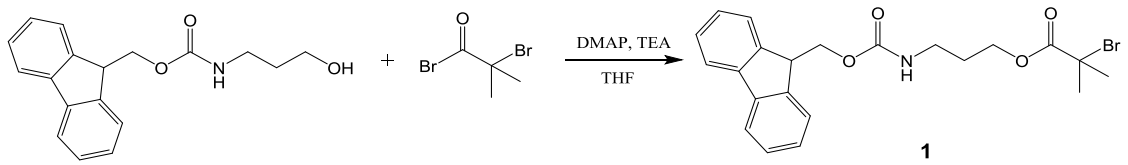
the polystyrene soluble support, we investigated the linkage of PEGs of two different molecular weights: M_n = 2000 g·mol^{-1} and 5000 g·mol^{-1} (PEG2k and PEG5k).

2. PREPARATION OF SOLUBLE POLYMER SUPPORTS

2.1. SELECTION OF APPROPRIATE POLYMERIZATION METHOD

Soluble polymer supports were prepared by controlled radical polymerization of styrene. To achieve the best quality of the support (controlled molecular weight and narrow molecular weight distribution), the following CRP methods have been compared: ATRP, ARGET ATRP and SET-LRP.

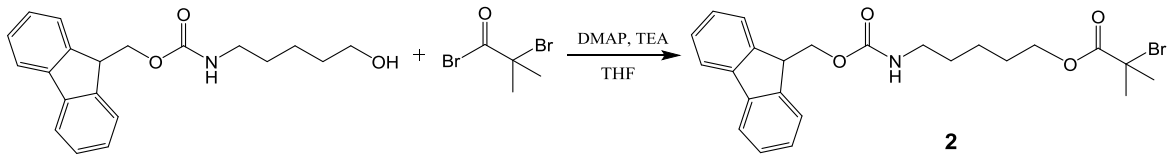
Preparation of tailor-made polystyrene supports suitable for our studies required utilization of functional initiators. An ATRP initiator 1 containing Fmoc-protected primary amine function was synthesized using the strategy displayed in **Scheme 12**.[237]



Scheme 12. Synthesis of the ATRP initiator 1.

2-bromo-2-methylpropionic acid 3-(Fmoc-amino) propyl ester (initiator 1) was obtained from the reaction of 3-(Fmoc-amino)-1-propanol with an excess of 2-bromoisobutyrylbromide and triethylamine. The pure product was isolated with 84% yield. The initiator was then characterized by ^{1}H NMR (**Figure 49**). Observation of the specific peaks in the area of 4.41-7.76 ppm indicated presence of Fmoc protecting group (signals **a**, **b** and **c**). Appearance of the signal at 1.96 ppm (**g**) confirmed formation of an ester bond between the bromoisobutyryl moiety and hydroxyl end group of propanol (signals of propanol's main structure: **d**, **e** and **f**).

Alternatively, an ATRP initiator 2 with extended aliphatic backbone was prepared using the same experimental protocol. 2-bromo-2-methylpropionic acid 5-(Fmoc-amino) pentyl ester was obtained from the reaction of 5-(Fmoc-amino)-1-propanol with an excess of 2-bromoisobutyrylbromide and triethylamine (**Scheme 13**).



Scheme 13. Synthesis of the ATRP initiator 2.

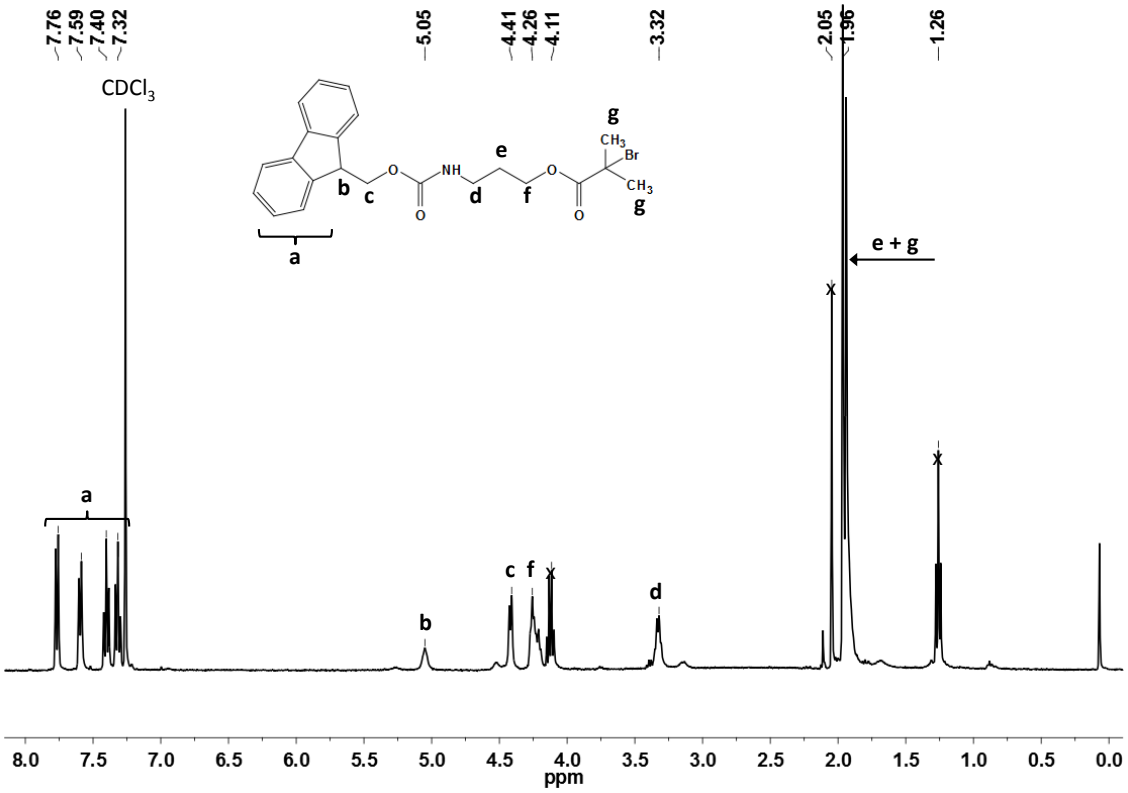


Figure 49. ^{1}H NMR spectrum recorded in $CDCl_3$ for the ATRP initiator **1**.

The initiator 2 was then characterized by ^{1}H NMR (**Figure 50**). Having almost the same molecular structure, the NMR spectrum of initiator 2 corresponds to NMR spectrum of initiator 1. A single difference between them can be visible in the region of 1.45-1.71 ppm (**e**). The three evident pentet signals have been assigned to 3 methylene groups located in the middle of initiator 2 aliphatic structure. Presence of the Fmoc protecting group (4.40-7.75 ppm; **a**, **b** and **c**) and the methyl groups of bromoisobutyryl moiety (1.93 ppm; **g**) has ultimately proved the structure of initiator 2.

The initiators were then tested in polymerization of styrene using the three CRP techniques mentioned earlier in the paragraph. General experimental conditions for these reactions are summarized in **Table 1**.

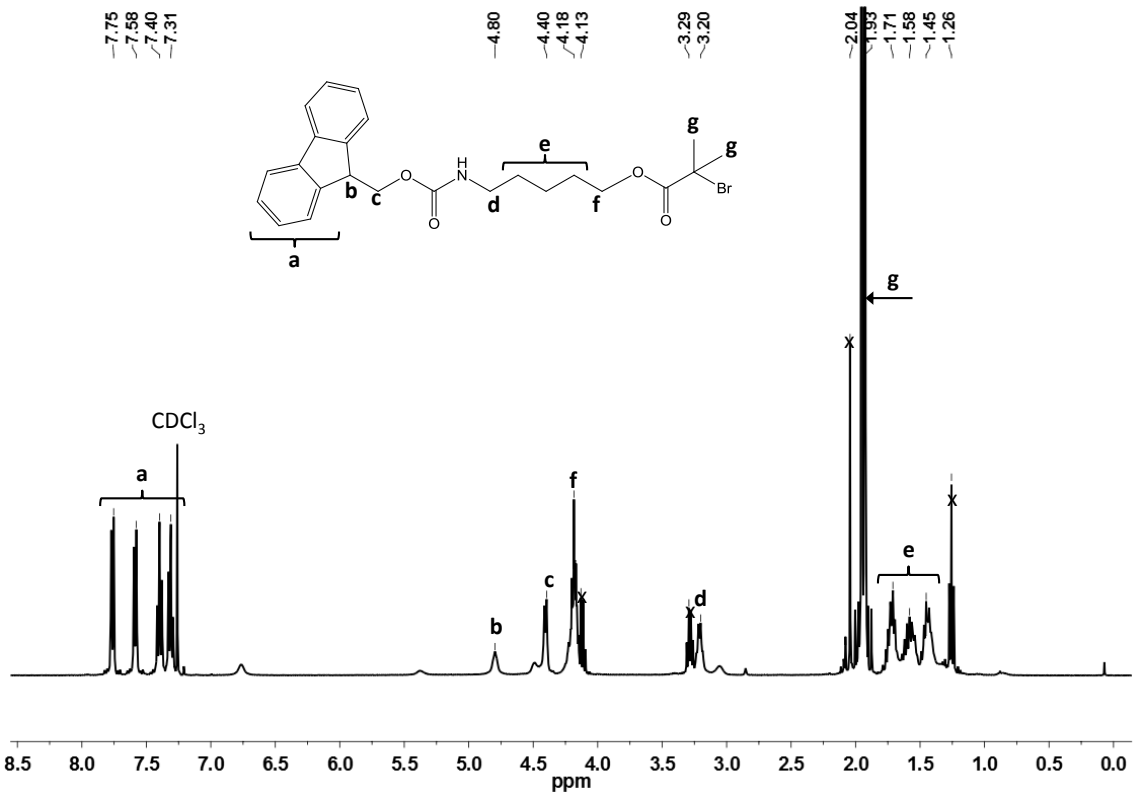
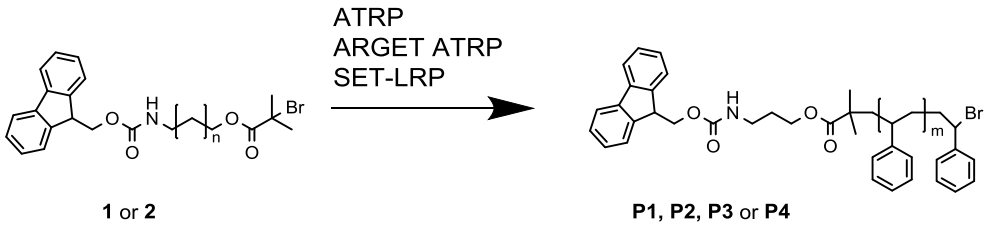


Figure 50. ^{1}H NMR spectrum recorded in $CDCl_3$ for the ATRP initiator **2**.

Table 1. Summary of the compounds used in the polymerization methods applied in this thesis.

Polymerization type	Polymer	Initiator	Catalyst	Ligand	Reducing agent
ATRP	P1	1	CuBr	dnBipy	–
	P2	2	CuBr		
ARGET ATRP	P3	1	CuBr	Me_6TREN	$Sn(EH)_2$
SET-LRP	P4	1	$Cu^0/CuBr_2$	PMDETA	–

Relatively low molecular weight polystyrene (DP = 40-50) has been chosen as a model support due to its well-recognized potential in organic synthesis (calculated loading capacity of approximately 0.22 mmol/g allow to keep equilibrium between good solubilizing power and high recovery yields of the polymer).[9] **Scheme 14** presents a common synthetic route for the aforementioned polymerizations.



Scheme 14. Schematic representation of the polymer synthesis using the initiator **1** or **2** *via* ATRP, ARGET ATRP and SET-LRP techniques.

First, ATRP was examined. The polymerization was conducted in argon atmosphere at 110°C following the conditions: [Sty]/[Initiator]/[Catalyst]/[Ligand] = 40/1/1/2. Resulting polymers **P1** and **P2** exhibited controlled molecular weight and narrow molecular weight distribution (M_n ($P1_{(1)}$) = 4095 g·mol^{-1}; M_w/M_n ($P1_{(1)}$) = 1.16; M_n (P2) = 4390 g·mol^{-1}; M_w/M_n (P2) = 1.18, **Appendix 1**). Formation of well-defined polystyrene samples was also indicated by ^{1}H NMR (**Figure 51**).

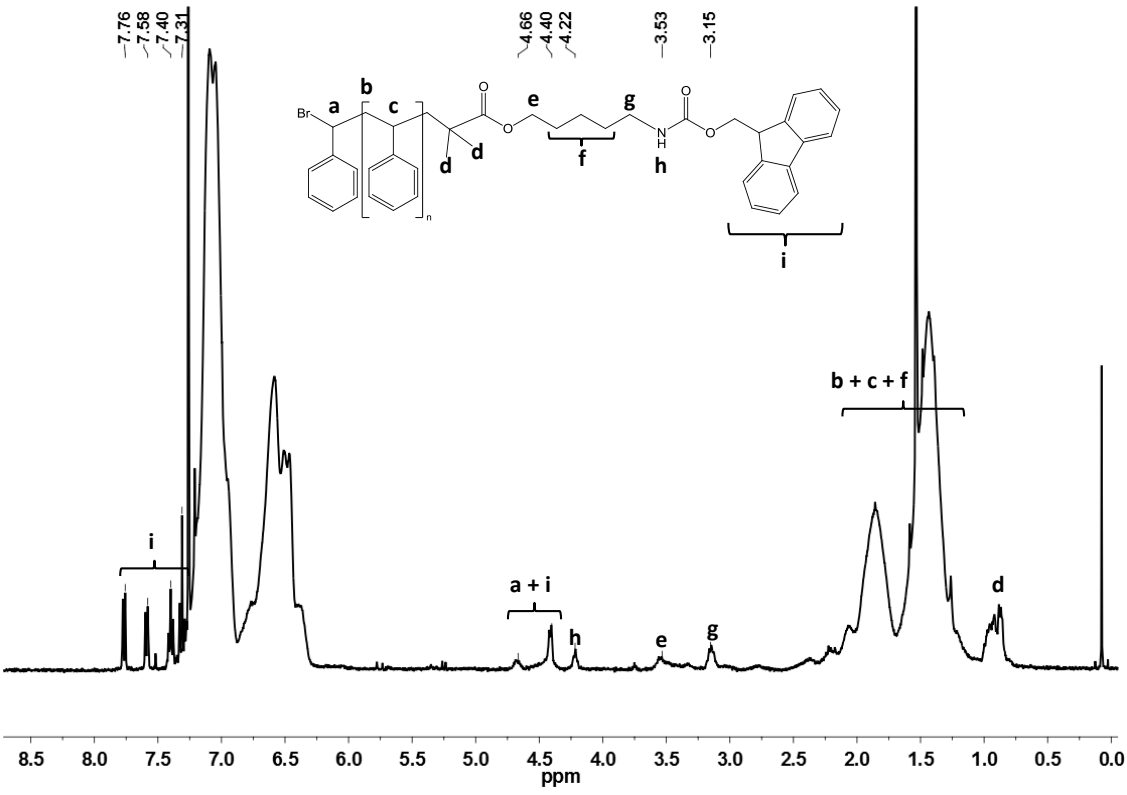


Figure 51. ^{1}H NMR spectrum recorded in $CDCl_3$ for the polystyrene support **P2** prepared by ATRP.

Figure 51 shows a representative spectrum of ATRP polystyrene support prepared using the initiator **2**. Methylene groups of the initiator (**f**) are at this point hindered by the broad signal of polystyrene aliphatic protons (**b** and **c**). Due to proximity of oxygen and nitrogen atoms,

the **e** and **g** CH_2-type protons of the initiator were deshielded to 3.53 and 3.15 ppm, respectively. Moreover, the Fmoc protons appeared in the region of 7.31-7.76 ppm and 4.40-4.66 ppm (**f**), the latter together with a single aliphatic proton of the chain-end styrene unit (**a**). As calculated from the raw NMR spectra, polymer $P1_{(1)}$ was isolated at 82% conversion, while the conversion of $P2_{(1)}$ reached 94% causing bimolecular weight terminations (**Figure 52).**

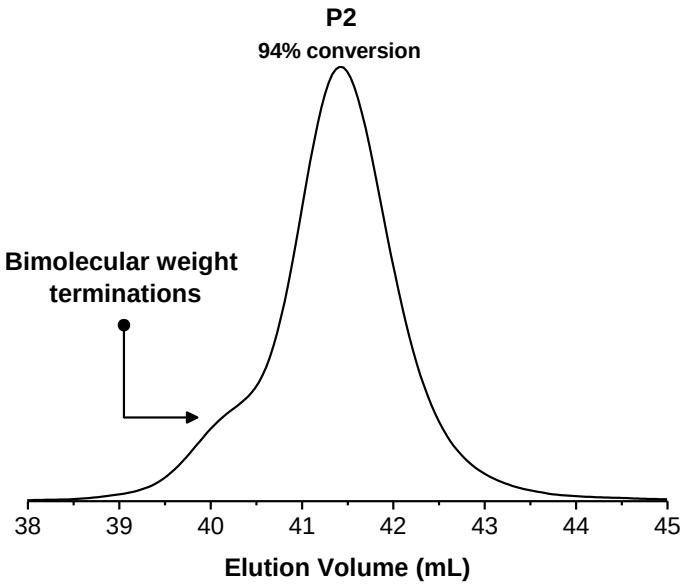


Figure 52. SEC chromatogram recorded in THF for the polystyrene support P2.

Well-defined polystyrene supports obtained *via* ATRP technique are suitable for variety of synthetic purposes. For instance, P1 and P2 have been utilized for preparation of oligopeptides that will be described in the course of this chapter. However, considering potential medical application of this approach, high amounts of toxic copper bromide should be avoided. Therefore, two novel polymerization techniques characterized by the use of reduced amounts of catalyst have been tested using initiator **1.** Primarily, a series of ARGET ATRP supports was prepared according to **Scheme 14**.

Polymer **P3** was obtained in a wide range of molecular weights and molecular weight distributions following the conditions displayed in **Table 2**.

The ARGET ATRP experiments were carried out at 110 °C in argon atmosphere with a constant ratio of catalyst/reducing agent $[CuBr]/[Sn(EH)_2]$ = 0.005/0.1. All polymerizations but entry 5 were initiated using initiator 1 (the initiator for entry 5 was MBP). The reactions were conducted in bulk (entry 1 and 2) or in toluene and the quantities of used monomer and

ligand are listed in the table. The best control over molecular weight distribution (M_w/M_n = 1.18) has been achieved with $P3_{(1);}$ however, the molecular weight of this polymer (M_n = 5100 g·mol^{-1}) has exceeded the expected value (theoretical M_n of 86% polymerized styrene of DP_{40} is 3440 g·mol^{-1}). This has been caused by the partial removal of Fmoc initiator protecting group (>50% as indicated by ^{1}H NMR). The Fmoc group has been probably affected by the presence of a basic ligand (Me_6TREN). Indeed, excess of the ligand (0.2 Eq.) was used in the 2nd polymerization leading to increased molecular weight and polydispersity (M_n = 5300 g·mol^{-1}, M_w/M_n = 1.86) at the same conversion. Polymerization $P3_{(3)}$ and $P3_{(4)}$ were thus conducted with addition of solvent to evaluate the influence of dilution effect on preservation of the polymer chain-end functionality. The polymerization mixtures $P3_{(3)}$ and $P3_{(4)}$ were prepared with the addition of 20% and 50% of toluene, respectively (in regards to the volume of styrene). Simultaneously, the molar equivalent of the ligand has been increased to 0.1 in order to preserve good polymerization rate. Nevertheless, both of the reactions proved to be too slow to maintain a good control over molecular weight and molecular weight distribution (entry 3 and 4 in **Table 2**). On the contrary, a well-defined polystyrene prepared from MBP initiator in the same conditions as for polymer $P3_{(4)}$ has been obtained (M_n = 3300 g·mol^{-1}, M_w/M_n = 1.21). It is thus evident that ARGET ATRP has a limited use with our initiator 1-based polymerization system.

Table 2. Series of ARGET ATRP experiments prepared using styrene monomer and initiator 1. * Permanent experimental conditions: [CuBr]/[$Sn(EH)_2$] = 0.005/0.1. ** Polymerization using MBP initiator.

No	**Varying experimental conditions***			**$\Delta t_{polym.}$** [min]	**Conversion** [%, NMR]	**M_n** [g·mol^{-1}]	**M_w/M_n**
	Sty [Eq]	**$V_{tol.}$:V_{Sty}**	**Me_6TREN [Eq]**				
$P3_{(1)}$	40	bulk	0.05	300	86	5100	1.18
$P3_{(2)}$	40	bulk	0.2	315	87	5300	1.86
$P3_{(3)}$	50	1:5	0.1	660	75	5400	1.41
$P3_{(4)}$	50	1:2	0.1	510	45	3000	1.41
$P3_{(5)}$**	40	1:2	0.1	330	87	3300	1.21

Restricted use of ARGET ATRP in the synthesis of functional soluble supports from initiator 1 encouraged us to look for an alternative polymerization technique. In consequence, single electron transfer living polymerization has been applied (**Scheme 14**).

SET-LRP was examined with two polymerization systems: (*i*) using standard MBP initiator and (*ii*) using initiator 1. In both cases, the polymers were obtained in the following conditions: [Sty]/[Initiator]/[Cu^0]/[$CuBr_2$]/[Ligand] = 40/1/0.25/0.1/0.35. SEC outcome of these polymerizations is exhibited in **Figure 53.**

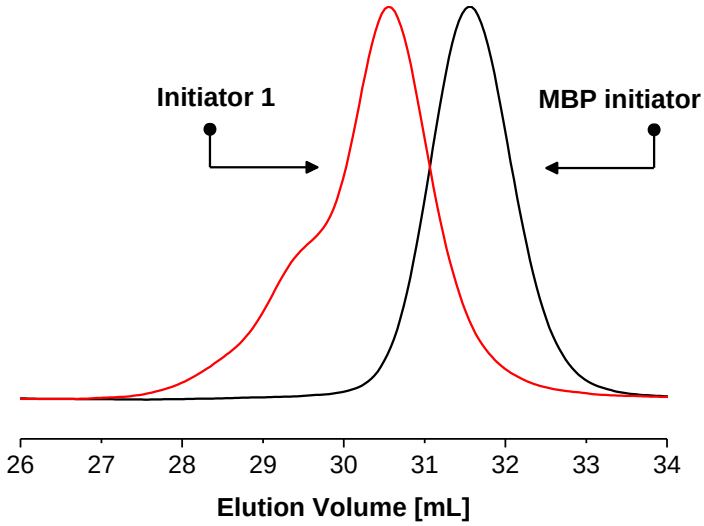


Figure 53. SEC chromatograms recorded in THF for the polystyrene support **P4** and a model polystyrene prepared *via* SET-LRP technique.

SEC measurements evidenced that SET-LRP process can be successfully initiated by methyl 2-bromopropionate, resulting in a well-defined polymer (M_n = 3200 g·mol^{-1}, M_w/M_n = 1.12). On the contrary, despite low conversion of styrene (60%), the polymer prepared from initiator 1 appeared inhomogeneous (**Figure 53**). Indeed, the obtained molecular weight (M_n = 6200 g·mol^{-1}) was too high. In addition, the formation of bimolecular structures represented by the high molecular weight shoulder visible on the chromatogram of P4 contributed to increased molecular weight distribution (1.28).

ARGET ATRP and SET-LRP were shown to be suitable for preparation of well-defined polymers initiated by MBP. However, defects in the course of polymerization have been observed upon initiator modification.

2.2. Elimination of terminal bromide

Removal of the polymer terminal bromide has been realized to suppress potential side reactions related to the nucleophilic nature of the halogen. The chain-end post-modification was first conducted on standard polystyrene prepared from MBP. Bromide moiety was

removed in an overnight reaction with a 10-fold excess of tetra-*n*-butylammonium fluoride (TBAF), which has a strong halogen affinity. The resulting polymer was then characterized by NMR to state disappearance of the proton in α position of the bromide (signal around 4.5 ppm; **a**) and simultaneous appearance of a double bond at the polymer chain-end (signal in the area of 6-6.5 ppm) (**Figure 54**).

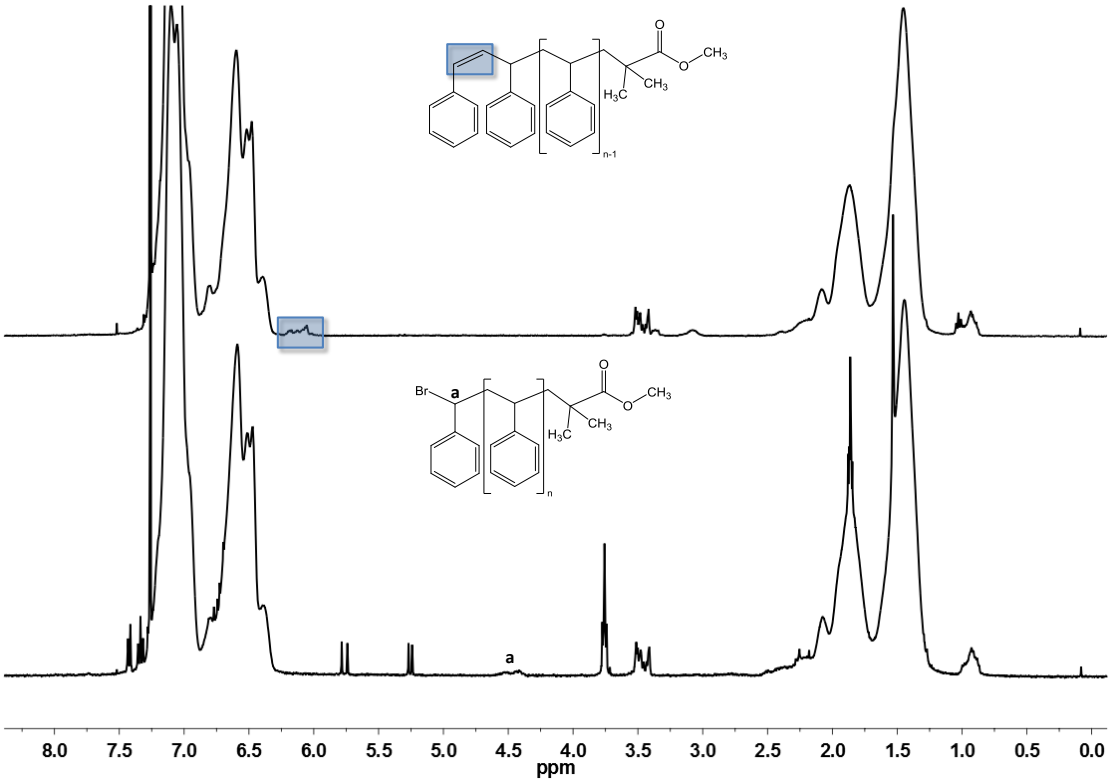


Figure 54. ^{1}H NMR spectra recorded in $CDCl_3$ for the polystyrene support prepared *via* ATRP technique from MBP before (*bottom*) and after (*top*) TBAF treatment. The blue box indicates formation of a double bond at the polymer α-chain end.

Subsequently, ARGET ATRP and SET-LRP have been performed using the build-in initiator **1.** The formed PS-chain has been post-modified using the same procedure. As shown in **Figure 55**, control over molecular weight and molecular weight distribution upon bromide elimination has been assured.

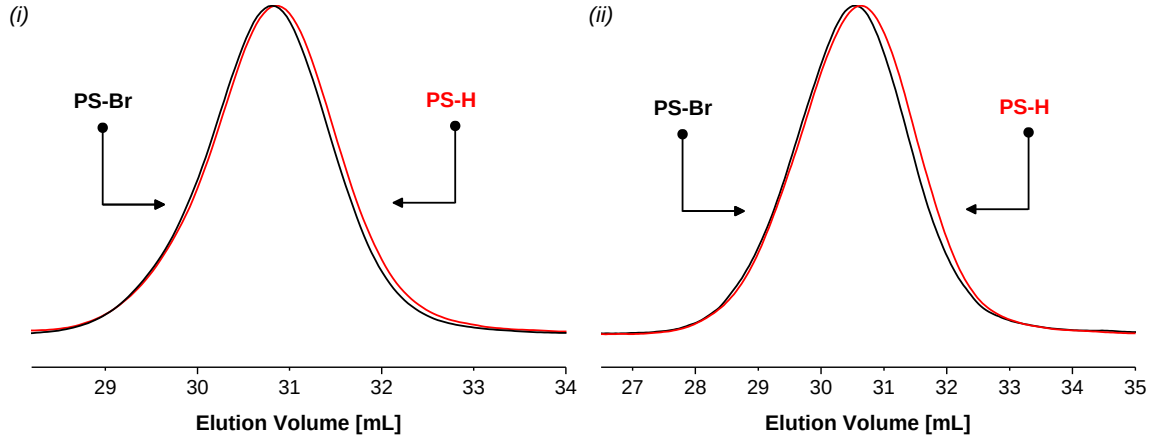


Figure 55. SEC chromatograms recorded in THF for polystyrene support prepared by *(i)* ARGET ATRP and *(ii)* SET-LRP techniques before (black curves) and after TBAF treatment (red curves).

However, NMR studies revealed simultaneous removal of the Fmoc initiator protecting group (**Figure 56**).

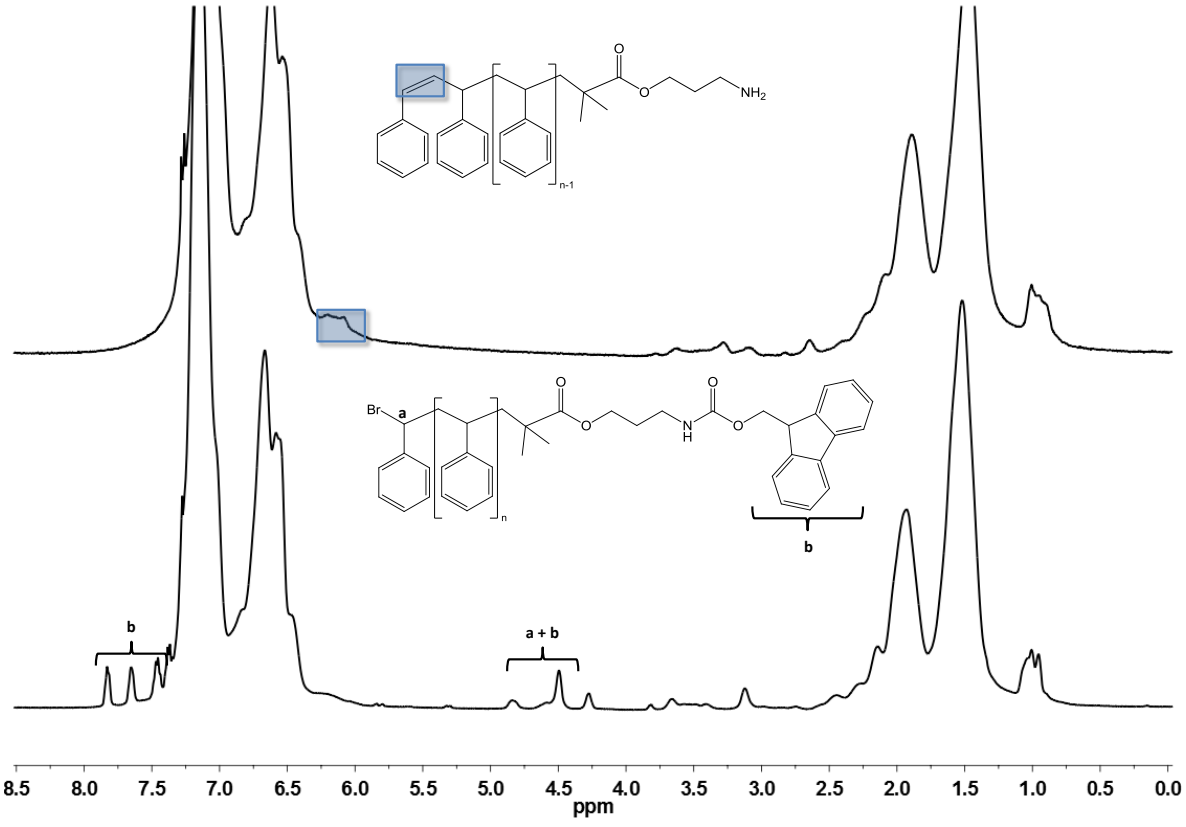


Figure 56. ^{1}H NMR spectra recorded in $CDCl_3$ for the polystyrene support prepared by ARGET ATRP technique from initiator **1** (*bottom*) and treated with TBAF (*top*).

The removal of the Fmoc group is in agreement with SEC data. Indeed, the molecular weight shift shown in **Figure 55** is higher than expected ($\Delta Mp_{(i)}$ = 217 g·mol^{-1} and $\Delta Mp_{(ii)}$ = 260 g·mol^{-1}, while M_{Br} = 80 g·mol^{-1}).

Moreover, TBAF treatment of the polystyrene prepared by ATRP using initiator 1 results in the formation of bimolecular structures (**Figure 57**). This effect is probably related to the reaction between the α-bromide chain end and the Fmoc-deprotected ω-chain end of the polymer.

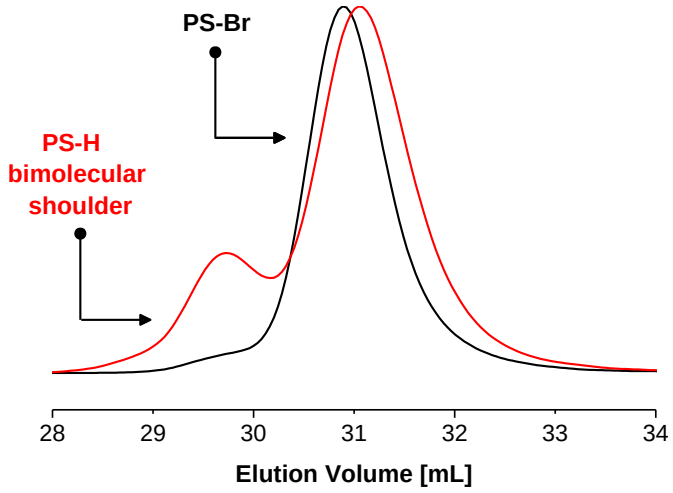
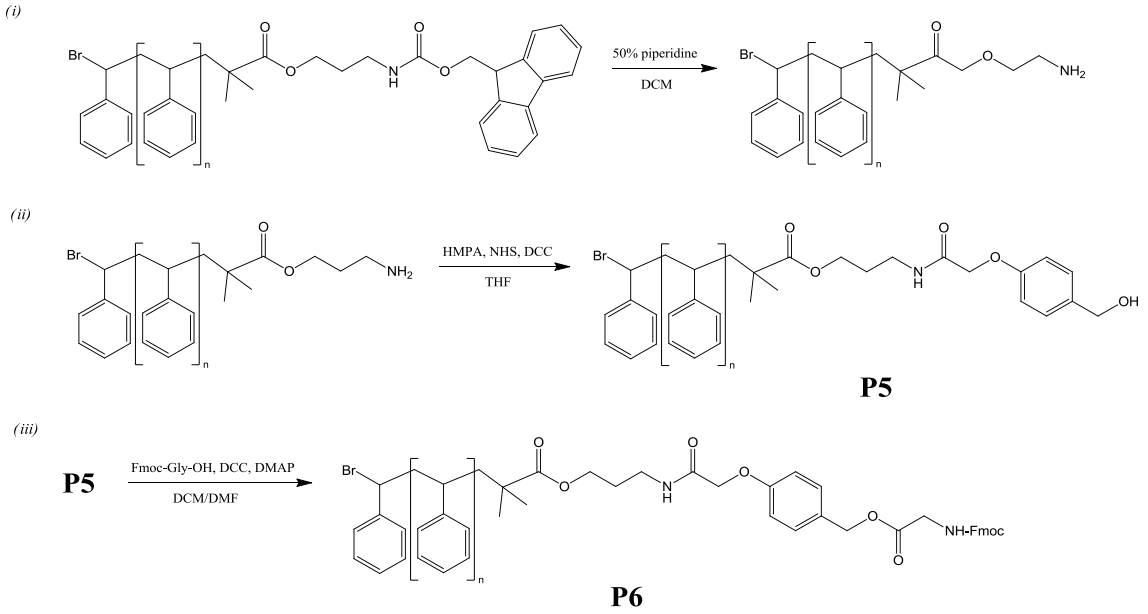


Figure 57. SEC chromatograms recorded in THF for polystyrene support prepared by ATRP from initiator **1** before (black curve) and after TBAF treatment (red curve).

As a result of demonstrated studies, soluble polymer supports were principally prepared *via* ATRP technique, providing no significance of remained bromide in the formation of a support-oligomer block. Alternatively, ARGET ATRP methodology was employed, as manifested in the synthesis of reactive polyamide structures (**Chapter IV**).

2.3. Synthesis of cleavable and non-cleavable supports

Polymers of P1, P2 and P3 type prepared from initiator 1 or 2 were utilized directly as permanent supports in the iterative synthesis of various oligomers. Their non-cleavable structure allowed monitoring monomer coupling process, however characterization of the obtained precise oligomer remained restricted. To permit analysis of these oligomers, a polymer support containing a cleavable linker has been prepared in four steps from P1 (**Scheme 15**).



Scheme 15. 3-step synthesis of a polystyrene cleavable support: *(i)* deprotection of Fmoc group from P1, *(ii)* attachment of a Wang linker and, *(iii)* loading of P5 with Fmoc-protected glycine.

As displayed above, the soluble cleavable polystyrene support (P5) has been obtained *via* attachment of a hydroxymethyl group. This commonly used moiety, known as Wang linker, permits esterification of a large library of monomers. Prior to the synthesis of the Wang-functionalized soluble support, Fmoc protecting group of $P1_{(1)}$ was removed. The resulting amine function was then reacted with 4-(hydroxymethyl)phenoxyacetic acid in the presence of NHS and DCC, affording $P5_{(1)}$. Ultimately, the Wang-functionalized soluble support was linked with Fmoc-glycine through a DCC-mediated "symmetrical anhydride" approach, resulting in $P6_{(1)}$. SEC measurements evidenced that the molecular structure of the polymer remained well-defined (M_n = 4500 g·mol^{-1}; M_w/M_n = 1.1) (**Figure 58**). Additionally, ^{1}H NMR confirmed quantitative chain-end modifications (**Figure 59)**.

An overlay of NMR spectra characterizing the 3-step preparation process of the cleavable support (**Scheme 15**) is displayed in **Figure 59**. Spectrum of the starting polymer prepared by ATRP from initiator 1 has been described earlier in the paragraph. The Fmoc polymer protecting group (**f**) has been removed prior to the attachment of an acid-labile ester linker. $P1_{(1)}$ was then modified by reaction with HMPA as highlighted by the appearance of the benzene ring-linked methylene signals at 4.62 (**g**) and 4.47 ppm (**h**) presented in the middle spectrum. At the end, the Wang soluble polystyrene support was loaded with Fmoc-protected glycine resulting in a re-appearance of the Fmoc protecting group signals (**j**). In addition,

introduction of the glycine unit decreased electron density around **h**, subsequently deshielding the signal at 5.14 ppm.

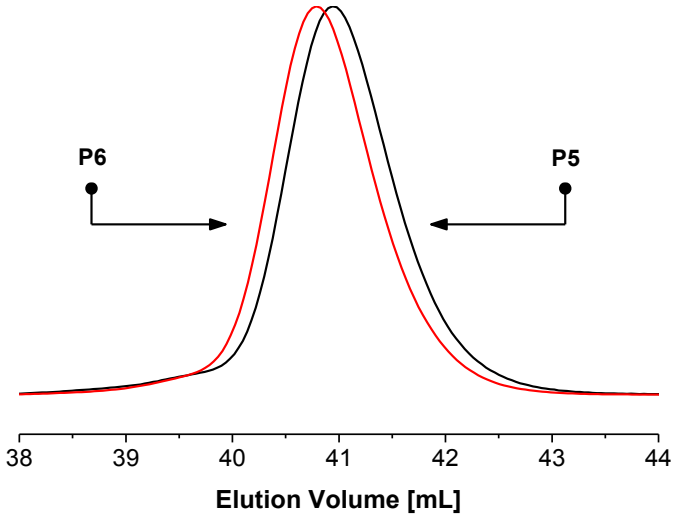


Figure 58. SEC chromatograms recorded in THF for the polystyrene supports $P5_{(1)}$ (PS with a Wang functionalization) and $P6_{(1)}$ (PS with the Wang functionalization preloaded with glycine).

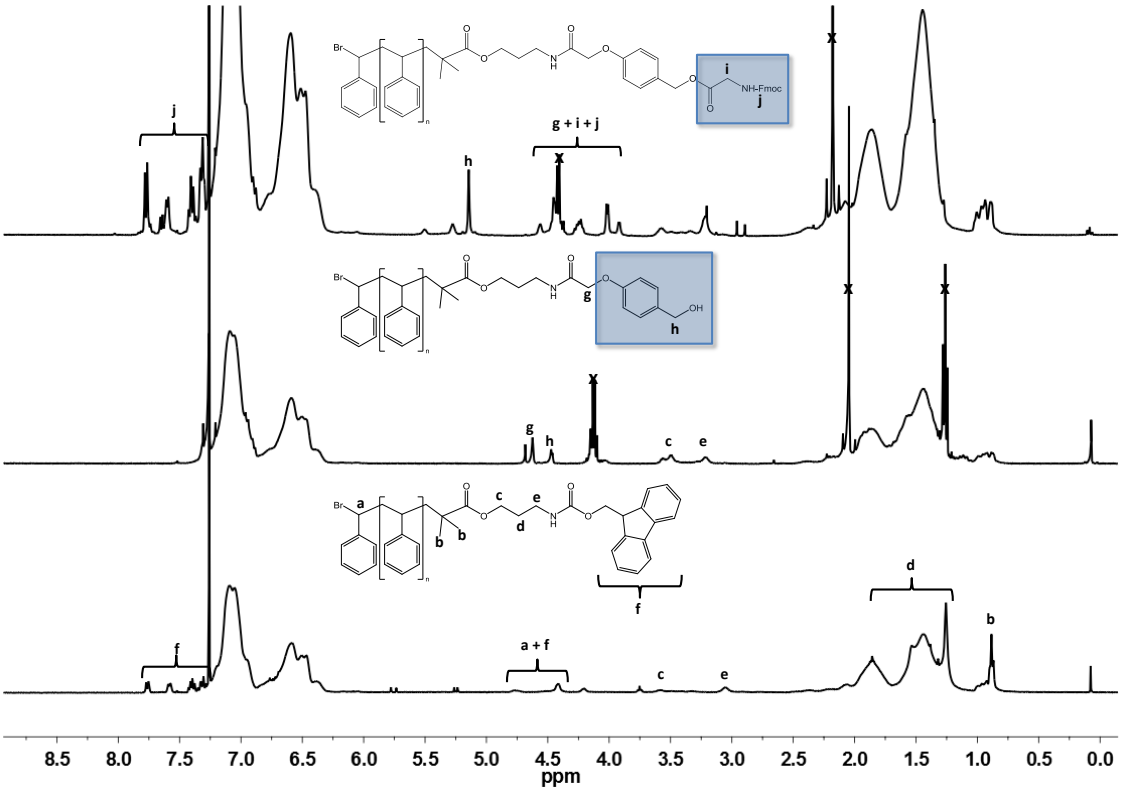
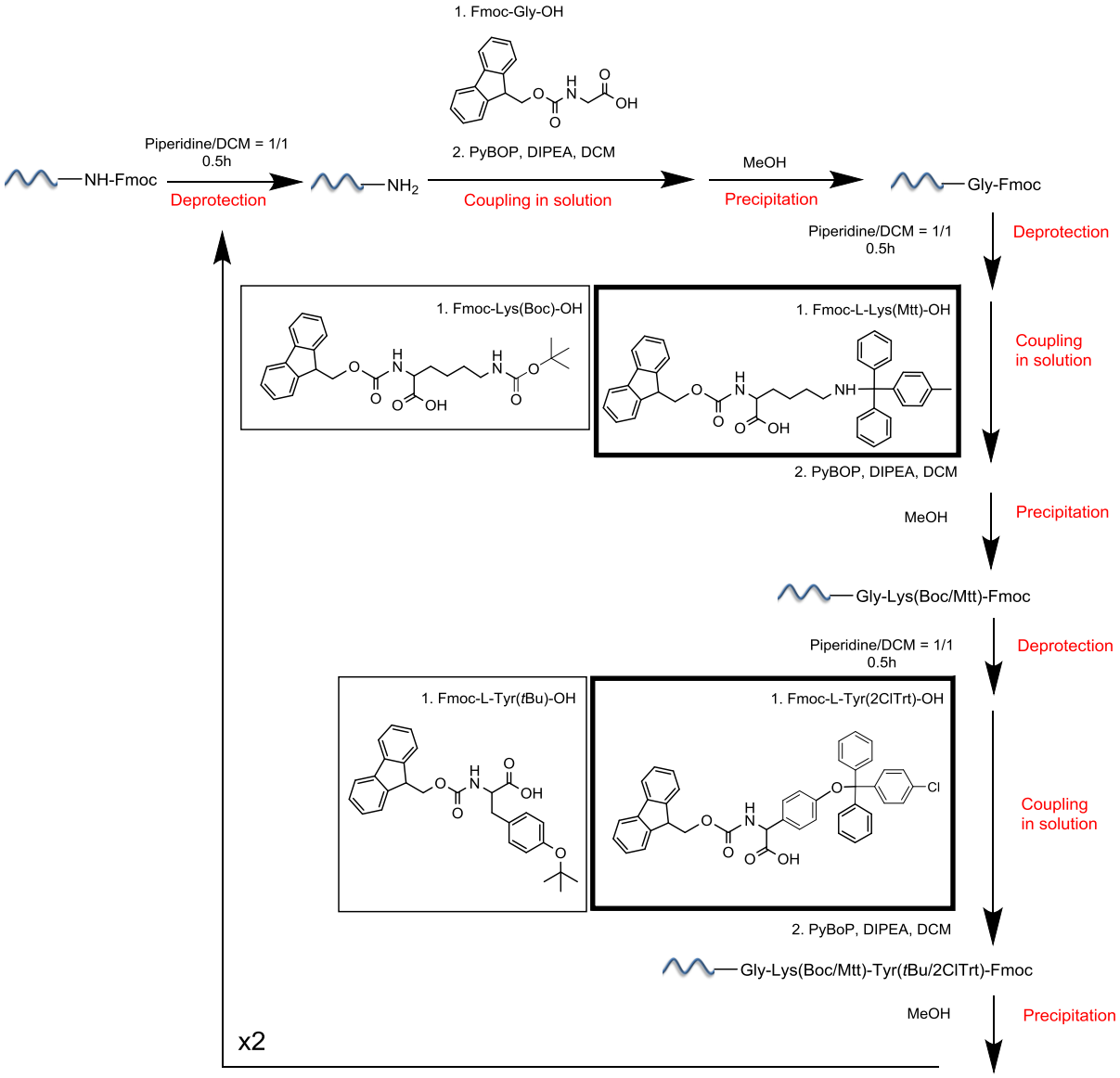


Figure 59. ^{1}H NMR spectra recorded in $CDCl_3$ for the subsequent preparation of a cleavable Wang support preloaded with glycine-Fmoc ($P6_{(1)}$): polystyrene obtained *via* ATRP from initiator 1 (bottom), polystyrene possessing an acid-labile *p*-alkoxybenzyl ester linker (*i.e.* Wang functionality) (middle), and Fmoc-glycine-loaded Wang soluble polystyrene support $P6_{(1)}$ (top).

3. GROWTH OF POLYSTYRENE-OLIGOPEPTIDE CONJUGATES

The permanent supports P1, P2, P3, and P6 (bearing the Wang linker) were tested for peptide synthesis. Hexapeptides Gly-Lys(Mtt)-Tyr(2ClTrt)-Gly-Lys(Mtt)-Tyr(2ClTrt)-Fmoc and Gly-Lys(Boc)-Tyr(*t*Bu)-Gly-Lys(Boc)-Tyr(*t*Bu)-Fmoc were prepared in an iterative fashion using standard Fmoc protocols (**Scheme 16**).



Scheme 16. Synthesis of –(Gly-Lys-Tyr)$_2$ oligomer sequence on polystyrene soluble supports. Amino acid structures placed in bolded frames were bonded with the support containing a cleavable linker, as opposite to the oligopeptide containing Lys(Boc) and Tyr(*t*Bu) units that was synthesized on a non-cleavable support.

Oligopeptide synthesis exhibited in **Scheme 16** is a representation of the AB + AB iterative synthesis model. Amino acids were linked together with assistance of protecting groups that

prevented side reactions. According to the support used, the oligopeptide was obtained either as an individual oligomer or as a part of a well-defined block copolymer. Acid-sensitive trityl-based structures, *i.e.* 4-methyltrityl and 2-chlorotrityl, were used to protect side-chain amine and hydroxyl functions of lysine and tyrosine monomers, respectively. In this way, tuning acidity of the cleavage mixture led either to the deprotection of the peptide (mild acidic conditions) or to the cleavage and deprotection occurring simultaneously (strong acidic conditions). In contrast, the removal of *tert*-butyloxycarbonyl and *tert*-butyl, demands strong acidic conditions. Thus, these protecting groups were used only in the case of non-cleavable support. Indeed, deprotection of the synthesized polystyrene-hexapeptide block copolymer in presence of the acid-labile linker would lead in any case directly to cleavage. Boc and *t*Bu strategy has been therefore limited to permanent supports P1, P2 and P3.

After each deprotection/coupling cycle, the modified polymer support was precipitated and analyzed by ^{1}H NMR and SEC. **Figure 60** shows the iterative growth of the polymer bioconjugate on a cleavable Wang support ($P5_{(1)}$) recorded by SEC.

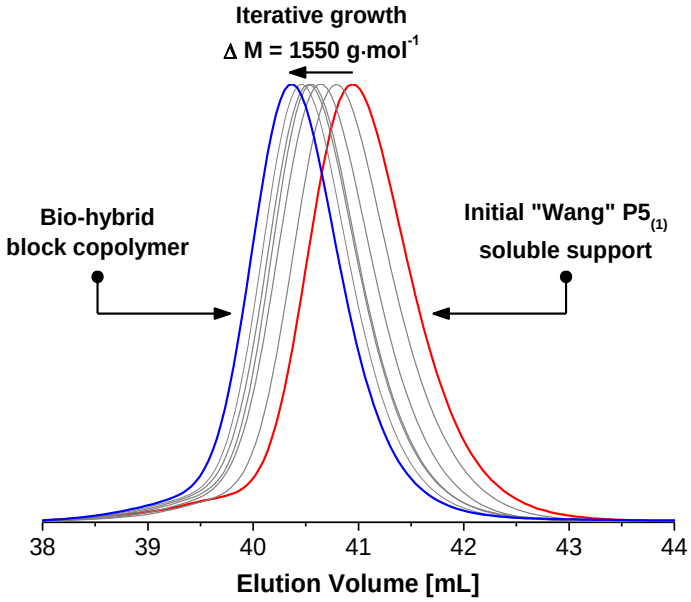


Figure 60. SEC chromatograms recorded in THF for step-by-step growth of the polystyrene-peptide bioconjugate performed on a Wang cleavable support ($P5_{(1)}$). The polystyrene support $P5_{(1)}$ is represented by a red line and the resulting peptide-polymer conjugate by a blue line. The grey traces show intermediate situations recorded after each amino acid coupling.

Clear shifts observed after each amino acid coupling and the molecular weight difference between initial Wang soluble support and the biohybrid block copolymer represented by the red and blue curves in **Figure 60** indicate high efficiency of this approach. Indeed, the

molecular weight deviation of 1550 g·mol^{-1} has been found in good correlation with theoretical molecular weight of the protected hexapeptide (1972 g·mol^{-1}), taking into account a PS calibration of the SEC. Furthermore, distribution of the molecular weight remained uniform at all the stages of the synthesis ($M_w/M_n \approx 1.1$).

Similarly, **Figure 61** exhibits SEC overlay of the iterative growth of the polymer bioconjugate on a non-cleavable support ($P2_{(1)}$). Also in this case, a progressive shift of the chromatogram towards high molecular weight can be observed. The difference of maximum peak values of the initial permanent support and the resulting biohybrid polymer has been calculated as 1475 g·mol^{-1}. This is in agreement with the expected molecular weight of the hexapeptide (1218 g·mol^{-1}) taking into account the imprecision of polystyrene calibration.

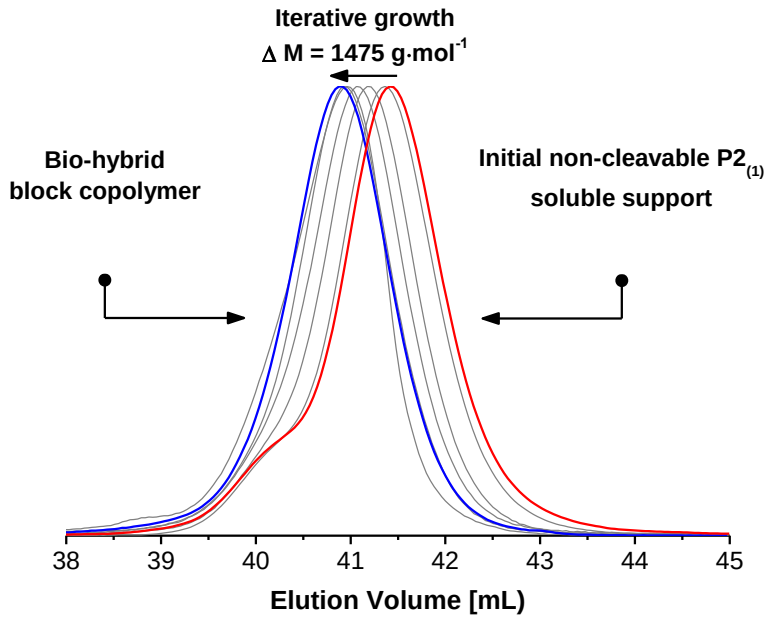


Figure 61. SEC chromatograms recorded in THF for the iterative growth of the polystyrene-peptide bioconjugate performed on a non-cleavable support ($P2_{(1)}$). The polystyrene support $P2_{(1)}$ is represented by a red line, the resulting peptide-polymer conjugate by the blue line and the grey traces show intermediate situations recorded after each amino acid coupling.

Furthermore, precipitation of polystyrene supports in methanol was found to be an efficient method for support recovery (**Table 3** and **Table 4**).

Table 3. Yields obtained after each step of the synthesis of a polymer bioconjugate on a cleavable Wang soluble support (Approach **a** in **Scheme 11**). In each case, these yields were calculated after reaction and precipitation of the conjugate in methanol.

	Isolated polymer	**Yield [%]**
1	PS-x-Gly-Fmoc ($P6_{(1)}$)	78
2	PS-x-Gly-NH_2	90
3	PS-x-Gly-Lys(Mtt)-Fmoc	99
4	PS-x-Gly-Lys(Mtt)-NH_2	72
5	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-Fmoc	94
6	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-NH_2	91
7	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-Gly-Fmoc	95
8	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-Gly-NH_2	90
9	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-Gly-Lys(Mtt)-Fmoc	99
10	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-Gly-Lys(Mtt)-NH_2	76
11	PS-x-Gly-Lys(Mtt)-Tyr(2ClTrt)-Gly-Lys(Mtt)-Tyr(2ClTrt)-Fmoc	92

In average, the yields of support recovery approached 90% in both cases presented in this study of polymer bioconjugates. However, potential effectiveness of this liquid-phase methodology can be certainly increased providing appropriate consideration in handling of the precipitation process (*i.e.* high concentration of the polymer solution, precipitation in a cold non-solvent, *etc.*). As displayed above, recuperation of the soluble supports can be achieved in up to 99%, which makes this approach equally attractive as the commonly used solid-phase synthesis.

Table 4. Yields obtained after each step of the synthesis of a polymer bioconjugate on a non-cleavable soluble support (Approach **b** in **Scheme 11**). As before, the yields were calculated after reaction and precipitation of the conjugate in methanol.

	Isolated polymer	**Yield [%]**
1	PS-Gly-Fmoc ($P2_{(1)}$)	98
2	PS-Gly-NH_2	96
3	PS-Gly-Lys(Boc)-Fmoc	78
4	PS-Gly-Lys(Boc)-NH_2	94
5	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-Fmoc	97
6	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-NH_2	72
7	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-Gly-Fmoc	99
8	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-Gly-NH_2	97
9	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-Gly-Lys(Boc)-Fmoc	79
10	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-Gly-Lys(Boc)-NH_2	87
11	PS-Gly-Lys(Boc)-Tyr(*t*Bu)-Gly-Lys(Boc)-Tyr(*t*Bu)-Fmoc	89

4. CLEAVAGE AND ANALYSIS OF THE PEPTIDE.

In order to fully assess the quality of the performed peptide synthesis, the hexapeptide was cleaved from the polystyrene support (approach **a2** in **Scheme 11**) and analyzed by MALDI-TOF and ESI-MS (**Figure 62**). Electrospray ionization mass spectrometry has been recorded in a negative mode in order to observe the ions created by the removal of protons from the hydroxyl groups of the hexapeptide. Observation of the negatively charged ions allowed extraction of 3 signals of the target oligopeptide from the spectrum: 713.55, 714.56 and 715.24 $g \cdot mol^{-1}$ that correspond to theoretical ionization signals of the molecule (713.36, 714.37 and 715.37 $g \cdot mol^{-1}$, respectively). Next, MALDI-TOF spectra were recorded to compare the oligopeptide prepared on both supports. As displayed in the output of this mass spectrometry, the hexapeptide prepared *via* solution-based approach has been identical with its molecular equivalent obtained conventionally *via* solid phase technique (the signal at 715.36 corresponds to the theoretical molecular weight of a positively charged molecule). The bottom spectrum was recorded for the bare CHCA matrix, therefore the peak at m/z = 568.06 that appeared on the middle spectrum is due to the matrix and occurred as a consequence of a

low concentration of the analysed sample. Comparison of the two methods gave thus equal results, suggesting high efficiency of the adopted strategies.

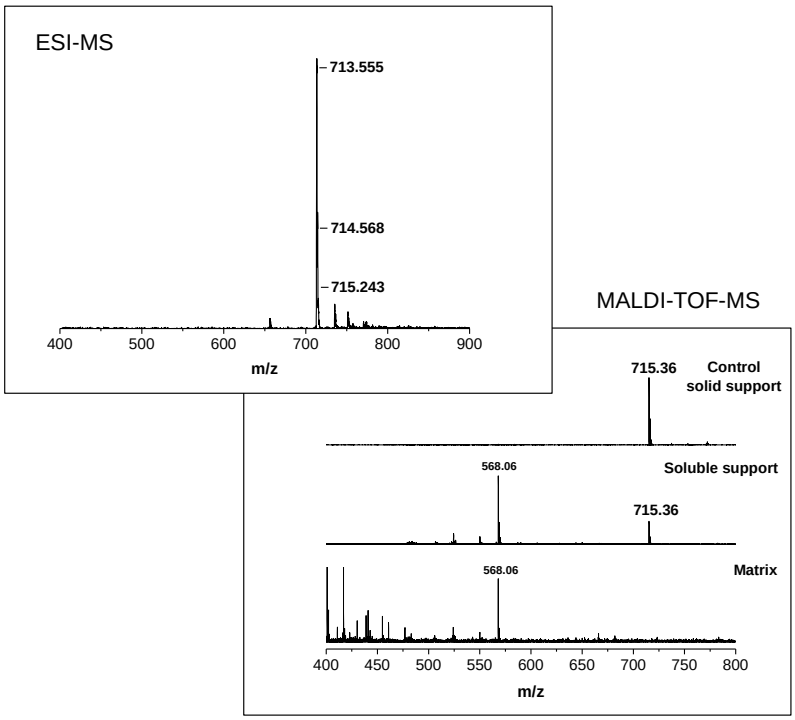


Figure 62. ESI-MS and MALDI-TOF-MS spectra recorded for the hexapeptide GKYGKY after deprotection and cleavage from the soluble and solid supports.

In fact, quality of the obtained hexapeptide relied on a well-designed methodology to polymer-supported peptide bioconjugates. Evaluation of the approaches **a1** and **b** in **Scheme 11** has been first accomplished by NMR. As depicted in **Figure 63**, deprotection of the polystyrene-*b*-GKYGKY block copolymer resulted in disappearance of the signals of aromatic protecting groups (corresponding to the removal of Mtt, 2ClTrt and Fmoc structures in the present example).

Next, SEC measurements were conducted to assure that no side effects occurred during the deprotection process (**Figure 64**). The analyses have been performed in NMP to provide good solubility of the sample and subsequently decrease affinity of unprotected amine and hydroxyl groups to the columns.

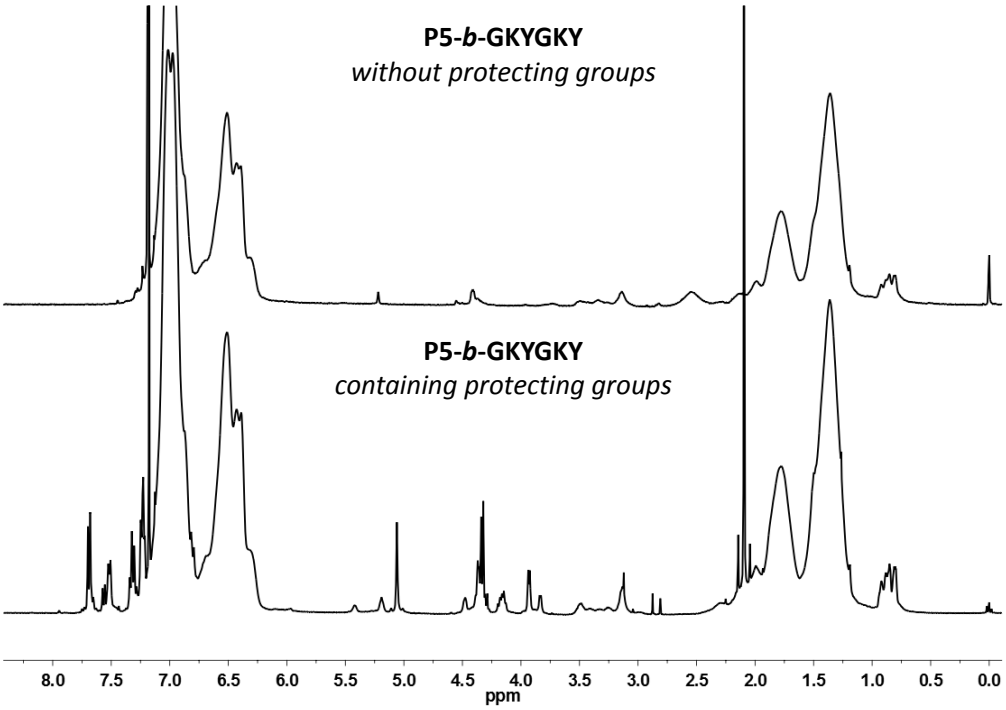


Figure 63. ^{1}H NMR spectra recorded in $CDCl_3$ for the conjugate $P5_{(1)}$-*b*-GKYGKY with (*bottom*) and without protecting groups (*top*).

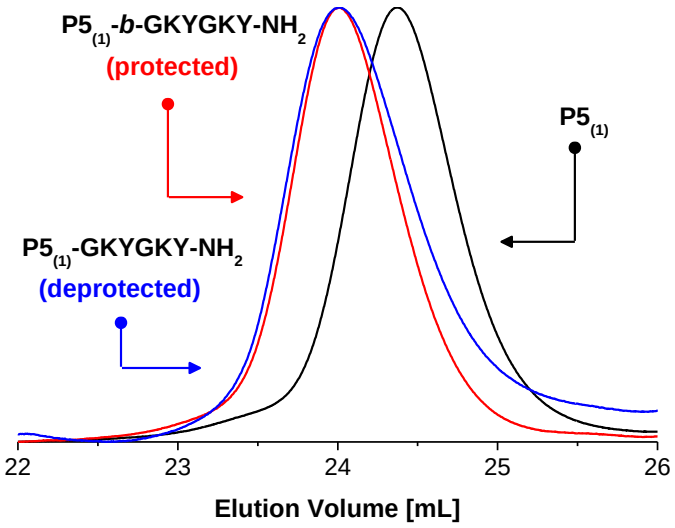


Figure 64. SEC chromatograms recorded in NMP for: the cleavable soluble support $P5_{(1)}$ (black curve), the corresponding peptide-polymer bioconjugate after removal of the Fmoc protecting group (red curve), and the final peptide-polymer bioconjugate PS-*b*-GKYGKY after complete deprotection (blue curve).

Complete deprotection of the polymer bioconjugate has not remarkably influenced the molecular weight of the block copolymer. A visible difference between the peptide-polymer

hybrids represented by the red and blue curves in **Figure 64** concerns the molecular weight distribution. However, the presence of deprotected oligopeptide side-chain groups contributed to the increase in polydispersity of the final biohybrid.

Similarly, removal of the Fmoc, Boc and *t*Bu protecting groups in the approach **b** allowed obtaining the conjugate polystyrene-*b*-GKYGKY from the support $P2_{(1)}$ **(Figure 65)**.

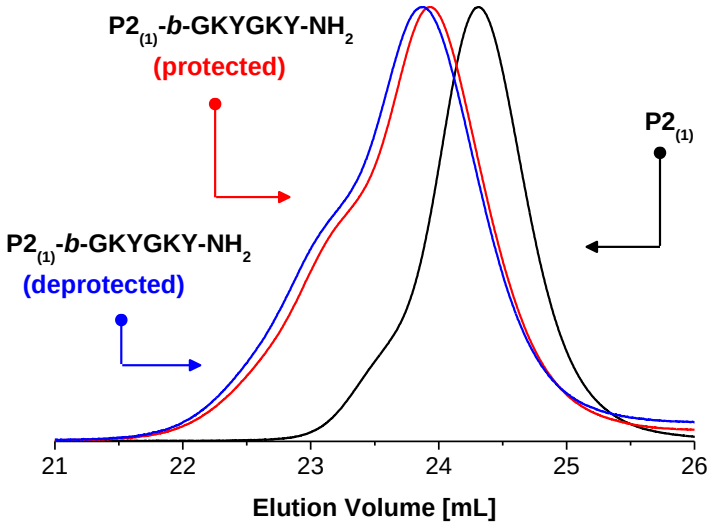


Figure 65. SEC chromatograms recorded in NMP for: the non-cleavable soluble support $P2_{(1)}$ (black curve), the corresponding peptide-polymer bioconjugate after removal of the Fmoc protecting group (red curve), and the final peptide-polymer bioconjugate PS-*b*-GKYGKY after complete deprotection (blue curve).

Similarly to the previous observation, a slight increase of polydispersity is noticed (red and blue curves in **Figure 65**). This effect occurred in response to the appearance of unprotected amine and hydroxyl groups of the hexapeptide. In addition, formation of a triblock bioconjugate visualized on the graph by the presence of a high molecular weight shoulder occurred in consequence of a coupling with polymer dead chains already existing in the initial $P2_{(1)}$ support.

5. PEGylation of PS-pentapeptide

The end-functional homogenous polystyrene has been employed as an alternative support in the peptide PEGylation process. The glycine-preloaded Wang cleavable support **P6** was first utilized as a support for the sequential synthesis of a tetrapeptide Tyr-Lys-Tyr-Lys (**Figure 66**) following the previously described protocols.

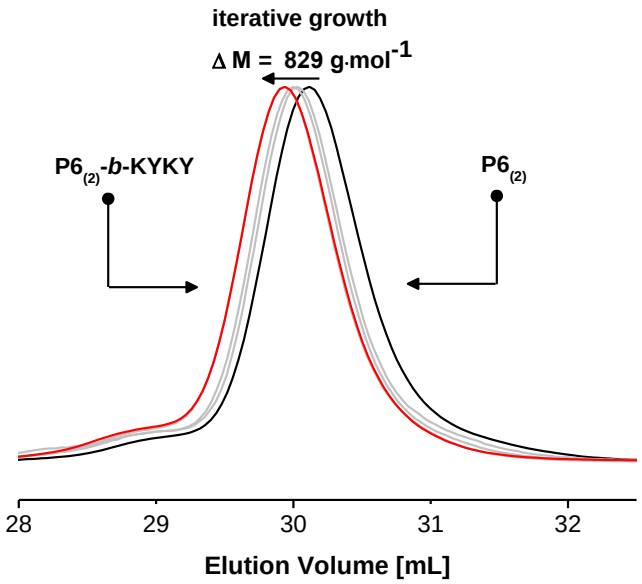


Figure 66. SEC chromatograms recorded in THF for iterative growth of Tyr(*t*Bu)-Lys(Boc)-Tyr(*t*Bu)-Lys(Boc) peptide on a glycine-loaded Wang-functionalized polystyrene soluble support.

The obtained well-defined polymer-pentapeptide conjugate (M_n = 8761 g·mol^{-1}, M_w/M_n = 1.13) was then characterized by ESI-MS (**Figure 67**).

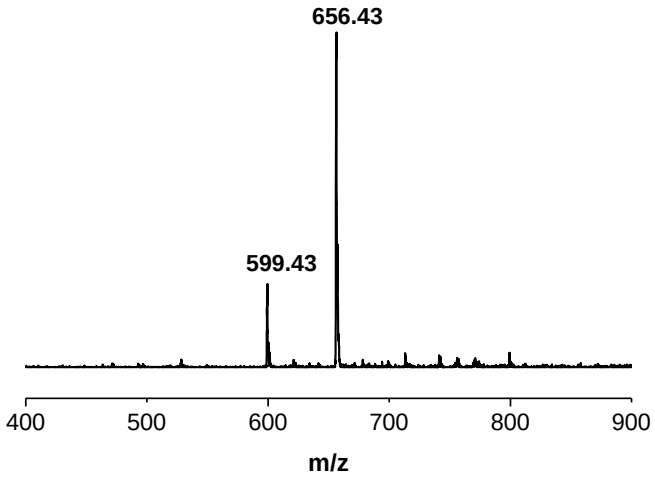


Figure 67. Negative mode ESI-MS of Gly-Lys-Tyr-Lys-Tyr molecule cleaved from P5$_{(2)}$-*b*-Gly-Lys(Boc)-Tyr(*t*Bu)-Lys(Boc)-Tyr(*t*Bu) polymer conjugate (protection groups removed simultaneously).

The spectrum visible in **Figure 67** is in good agreement with theoretical mass of the target pentapeptide ([Gly-Lys-Tyr-Lys-Tyr - H]$^-$ = 656.35 g·mol^{-1}). Value of 599.43 g·mol^{-1} shown as the second peak on the graph corresponds to the oligopeptide sequence with a deletion of glycine monomer ([Lys-Tyr-Lys-Tyr - H]$^-$ = 599.33 g·mol^{-1}).

The polymer-peptide conjugate was then examined in PEGylation reaction by coupling PEGMA to the *N*-terminal amino group of the supported peptide. The aim of this reaction was

evaluate the potential of homogeneous support in comparison with available solid-phase methodology. The coupling of PEGs (M_n = 2000 g·mol^{-1} and 5000 g·mol^{-1}) to the support was performed under inert gas atmosphere for 5 days using 1.15 equiv. of PEG. Unlike solid-phase methodology, the solution-based synthesis did not require large excess of the reagents to drive the reaction to full conversion. In addition, activation of the peptide was facilitated by addition of PyBOP/DIPEA (2/4 equiv.) in DCM. SEC results of the peptide's PEGylation on the soluble support $P5_{(2)}$ as evidenced by SEC are exposed in **Figure 68**.

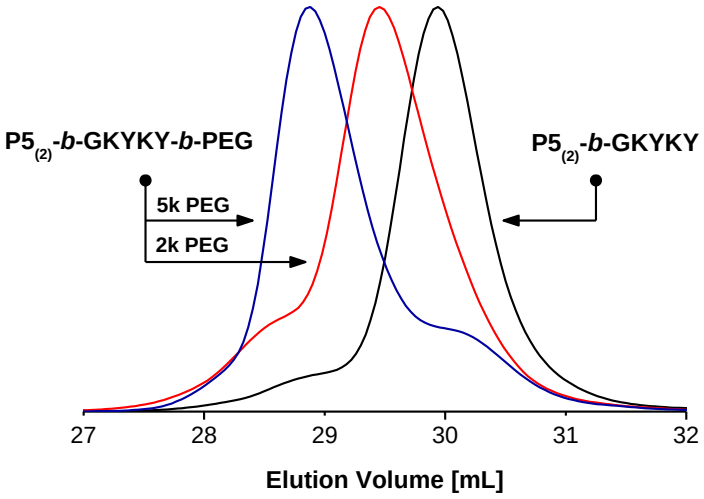


Figure 68. SEC chromatograms recorded in THF for PEGylation of the pentapeptide GKYKY on the homogeneous support $P5_{(2)}$. Initial chromatogram recorded for YKYKG-*b*-$P5_{(2)}$ is represented here by a black curve, while the red and blue chromatograms address PEG2k-*b*-YKYKG-*b*-$P5_{(2)}$ and PEG2k-*b*-YKYKG-*b*-$P5_{(2)}$ triblock copolymers, respectively.

As expected, utilization of a homogeneous support facilitated the access of PEG to peptide's anchor side, resulting in high yields of PEG coupling confirmed by the shifts of SEC chromatograms in **Figure 68**. Indeed, apparent molecular weight shifts of about 3000 and 7000 g·mol^{-1} have been subsequently observed after linkage of PEG2k and PEG5k. It should be noted that the samples were precipitated in MeOH prior to analysis, thus the blue and red chromatograms do not correspond to the unreacted PEGs. Secondly, a small molar excess of PEG has been used in these reactions (1.15 equiv. of PEG with respect to the amino functionalities). In these regards, PEGylation of the peptide-PS conjugate to a soluble polymer seems to be easy in handling and at the same time an efficient method.

Coupling with PEG2k represented by the red curve in **Figure 68** led to formation of two chain populations – the main peak corresponding to PEG2k-*b*-YKYKG-*b*-$P5_{(2)}$ triblock copolymer and the side peak revealing presence of hexablocks (PEG-*b*-YKYKG-*b*-$PS)_2$ formed in consequence of the presence of the ATRP byproducts possessing 2 reactive chain ends mentioned previously. Creation of such structures has been hampered in the case of the coupling with PEG5k. This phenomenon is possibly due to the presence of steric hindrance appearing in the course of attachment of the first PEG chain. Nevertheless, existence of the hexablocks does not impose further complications since cleavage and analysis of the PEG-peptide conjugates is not affected. Besides, calibration of the SEC has been realized using PS standards, thus giving an approximate molecular weight difference values. Precise yields of the PEGylation reaction have been then calculated by ^{1}H NMR (**Figure 69** & **Figure 70**).

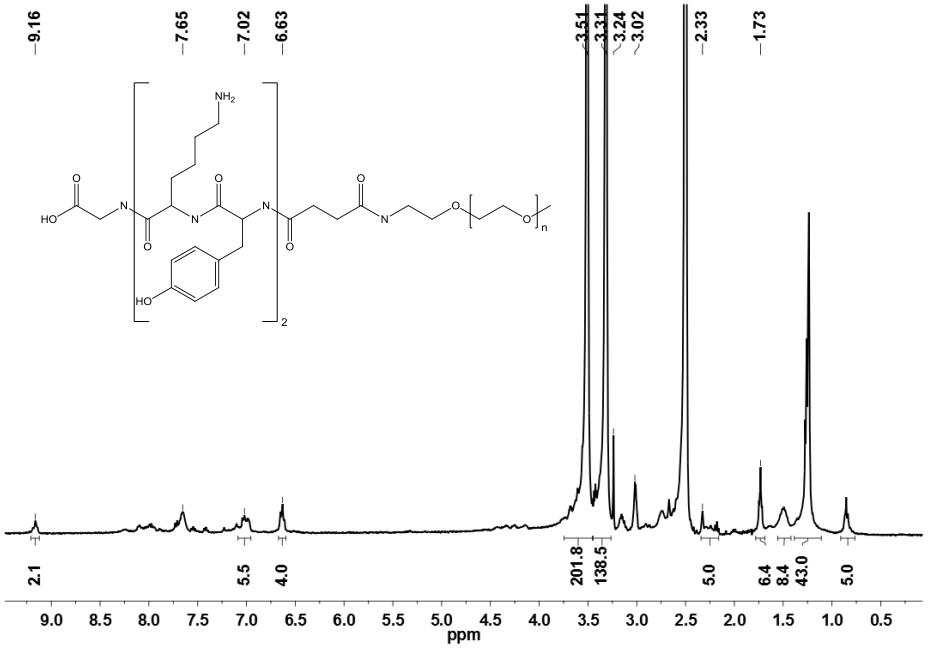


Figure 69. ^{1}H NMR spectrum recorded in $CDCl_3$ for MeO-PEG2k-Tyr-Lys-Tyr-Lys-Gly-COOH synthesized on PS soluble support $P5_{(2)}$.

Coupling efficiencies of PEG2k and PEG5k to peptide-polymer were determined after cleavage of the PEG-peptide conjugates from the soluble support. Cleavage of the conjugate was performed by treatment with TFA/DCM mixture (50 vol.%) during two hours. The ratio of peptide vs. PEG2k was determined by comparison of integral intensities of 4H from $CH^{Ar}Tyr_1$ at 6.63 ppm and 202H of PEG at 3.94-3.42 ppm, to obtain a quantitative coupling efficiency (95%). In case of PEG5k, the coupling efficiency reached 35% (determined by

comparison of integral intensities of 8H from $CH^{Ar}Tyr_{1+2}$ at 7.36-6.45 ppm and 152H of PEG at 3.95-3.44 ppm).

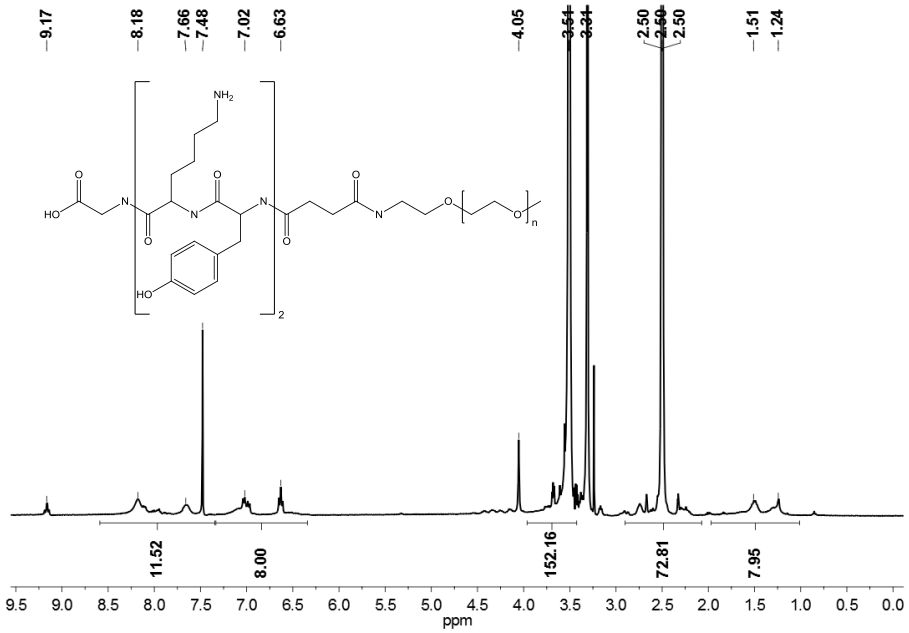


Figure 70. ^{1}H NMR spectrum recorded in $CDCl_3$ for MeO-PEG5k-Tyr-Lys-Tyr-Lys-Gly-COOH synthesized on PS soluble support $P5_{(2)}$.

Ultimately, the cleaved peptide-PEG bioconjugates were analysed by SEC (**Figure 71**).

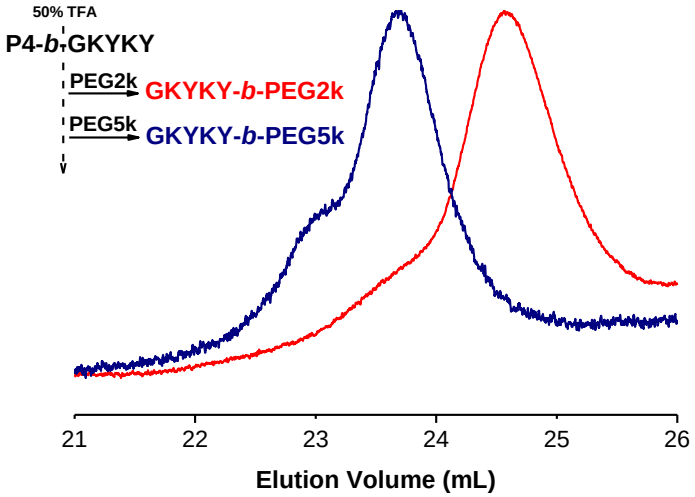


Figure 71. SEC chromatograms recorded in NMP for the peptide-PEG bioconjugates after cleavage from the polystyrene support. The peptide-PEG2k is represented by a red curve and the peptide-PEG5k by a blue curve.

The increased molecular weight distribution ($M_w/M_n \approx 1.3$) is most probably the effect of deprotection of the peptide's functional groups. Furthermore, recombination of the unprotected peptide-PEG chain ends was perhaps a reason for the appearance of the molecular shoulder of the GKYKY-*b*-PEG block copolymers. The molecular weight values of the obtained peptide-PEG conjugates are displayed in **Table 5**.

Table 5. SEC and theoretical molecular weight values of the peptide-PEG conjugates cleaved from the soluble polymer support. * Measurement in NMP. ** Based on the theoretical molecular weight of the peptide and the added PEG.

Conjugate	**$M_{n\ SEC}$* [g·mol^{-1}]**	**$M_{theor.}$** [g·mol^{-1}]**
Peptide-PEG2k	5497	2656
Peptide-PEG5k	11009	5656

6. CONCLUSIONS

The synthesis of polymer bioconjugates using soluble supports has been successfully accomplished. Moreover, it has been shown that the strategy established in this chapter can be applied in the PEGylation process. The key-feature of this method relied in preparation of well-defined cleavable and non-cleavable polystyrene supports of narrow polydispersity and controlled molecular weight. Importantly, the use of the controlled radical polymerization techniques, in particular ATRP, combined with post-modifications of the polymer allowed preparation of functionalized supports. Liquid-phase iterative synthesis of oligopeptides on such tailor-made platforms resulted in well-defined polymer-peptide bioconjugates. In addition, the employed methodology permitted analyses of the intermediates by ^{1}H NMR and SEC after each amino acid coupling.

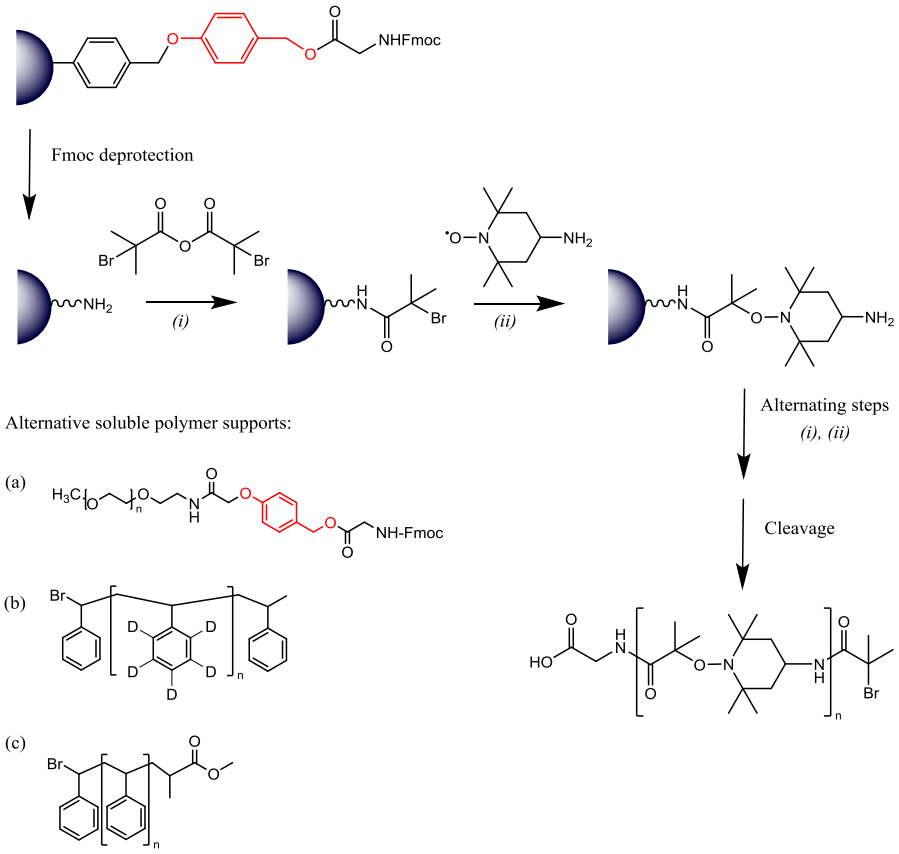
It has been then shown that the polymer-linked peptides are more suitable for PEG chemical grafting than conventionally used microgel resins. Indeed, as demonstrated in this chapter, only 1.15 equivalent excess of PEG compared to the peptide has been required for a quantitative coupling with PEG2k. Although coupling of larger residues, *e.g.* PEG of M_n = 5000 g·mol^{-1} led to lower conversion (35%), this result is yet remarkable considering its pharmaceutical applications.

Chapter III

SEQUENCE-CONTROLLED SYNTHESIS OF OLIGO(ALKOXYAMINE AMIDE)S USING AN AB + CD APPROACH

1. INTRODUCTION

In this chapter, the synthesis of sequence-controlled oligo(alkoxyamine amide)s using liquid- and solid-phase synthesis approaches is described. **Scheme 17** shows the "AB + CD" synthetic strategy that was studied for preparing these polymers.



Scheme 17. Iterative approach to (alkoxyamine amide)s using a cleavable Wang solid support *via* alternating coupling of the monomers of AB and CD type (steps *(i)* and *(ii)*, respectively). Alternative soluble polymer supports are displayed on the left. The iterative syntheses using supports (b) and (c) were performed *via* invers order of the alternating steps *(i)* and *(ii)*.

The oligo(alkoxyamine amide)s have been obtained *via* iterative coupling of amino-nitroxide and bromoanhydride building blocks on polymer supports. Two kind of supports were employed in this strategy: a commercially available glycine-preloaded Wang resin solid support and polystyrene and poly(ethylene glycol) soluble supports (**Scheme 17**). Similarly to the Wang resin, the PEG soluble support contains a cleavable *p*-hydroxybenzyl linker and a Fmoc-protected primary amine terminus. The polystyrene support is non-cleavable. It was prepared by ATRP and contains therefore a bromine-terminus.

The primary amine-terminated Wang resin and PEG soluble supports were first reacted with a bromoanhydride building-block (**Scheme 17** *(i)*) in the presence of an excess of DIPEA base. Next, the bromo-terminated polymer was transformed into a carbon-centered radical using $Cu^{I}Br$ activation and reacted *in situ* with an amino-nitroxide building-block. The nitroxide coupling occurred in a rapid and efficient way in the presence of $Cu^{I}Br$ and Me_6TREN in DMSO (**Scheme 17** *(ii)*). Typically, the radical coupling reaction is completed within several minutes. The iterative coupling of alternating bromoanhydride and nitroxide building blocks was repeated until the formation of the targeted polymer sequence.

The oligo(alkoxyamine amide)s synthesized on the bromide-terminated polymer supports have been prepared using the same iterative strategy. However, due to the bromine chain-end of the soluble support, the nitroxide building-block was used first. Afterwards, the alternating iterative steps were performed as discussed above.

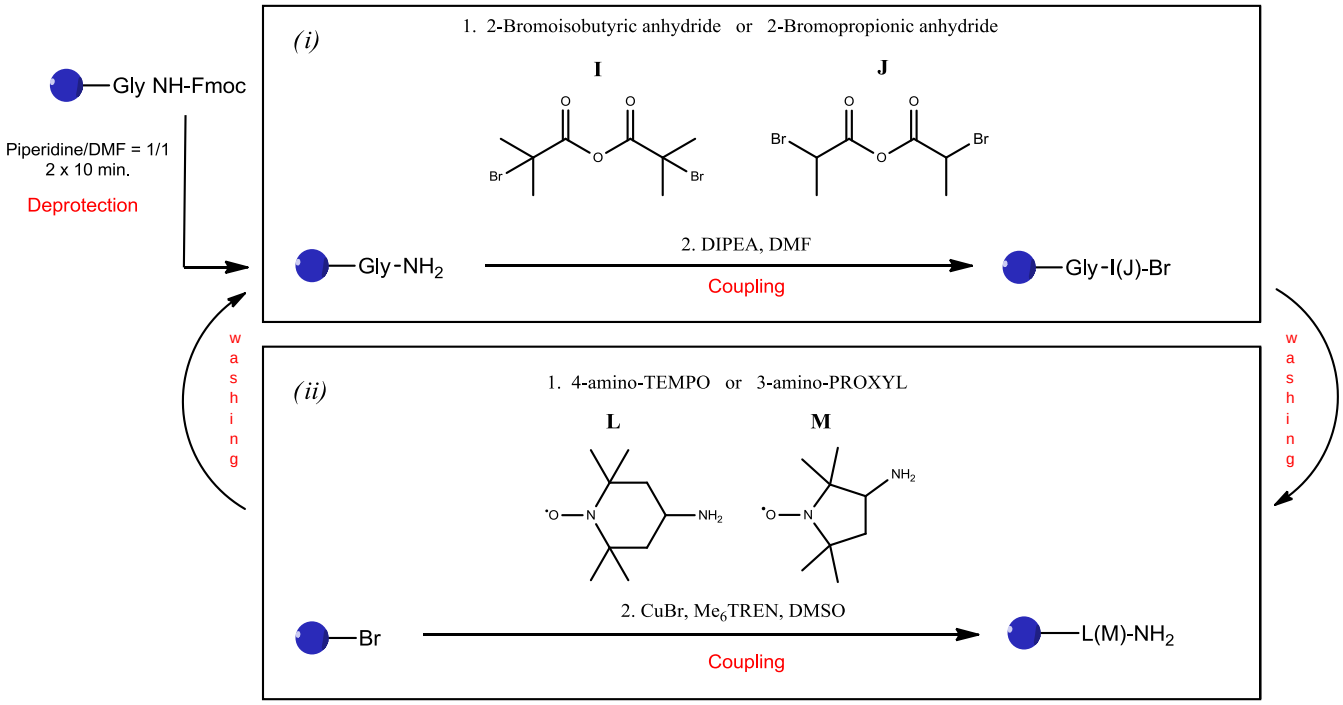
The commercially available solid support was used at first in this study in order to verify the validity of this new "AB + CD" strategy. Afterwards, the PS and PEO soluble supports were tested. Well-defined PS soluble supports (P7 and P8) were prepared by ATRP using MBP and PEBr initiators, while the cleavable support P9 was obtained from a commercially available PEG following the protocols described in **paragraph 2.2 in** Chapter II. The presence of a labile linker on the PEO support allowed cleavage and characterization of the formed sequence-defined oligomers. The iterative synthesis of oligo(alkoxyamine amide)s exploited in this study represents an "AB + CD" protecting-group free model coupling strategy. High efficiency and short reaction times are the main characteristics of this innovative coupling approach. These interesting findings are described in details within the next pages.

The formed sequence-defined oligo(alkoxyamine amide)s contain multiple thermo-labile alkoxyamine bonds in their chains. Thus, these polymers can be easily degraded by heating. A preliminary investigation of the thermal degradation of these polymers is described at the end of this chapter.

2. INVESTIGATION OF THE CONCEPT ON A RESIN

The iterative synthesis of oligo(alkoxyamine amide)s was first studied on a commercially available glycine-preloaded Wang resin. In order to obtain an optimal accessibility of the active sites and a good diffusion of the reactants in the resin, the beads were swollen in DCM

prior to the alkoxyamine amide growth. The importance of swelling is described in **paragraph 4.1.1** in Chapter I. The solid-phase protocol for the synthesis of monodisperse oligo(alkoxyamine amide)s is shown in **Scheme 18**.



Scheme 18. Strategy for the synthesis of (alkoxyamine amide)s on Wang-Gly-Fmoc solid support.

First, the terminal Fmoc-protecting group of the support was removed using a solution of piperidine in DMF (1/1 v:v). The attachment of the first bromoanhydride building-block was performed in the presence of an excess of DIPEA. This reaction is fast and proceeds in general in a few minutes in homogeneous solution. However, solid-phase chemistry requires usually longer times to obtain quantitative yields, and therefore a coupling step of 50 minutes was studied in the present work. In the second step and as shown in **Scheme 17**, the bromine atom of the modified resin was activated in the presence of CuBr/Me_6TREN/DMSO and reacted with an amino-nitroxide building-block. The nitroxide radical coupling reaction was typically completed within 15 min. The iterative growth of the oligo(alkoxyamine amide) was then realized by the sequential attachment of bromoanhydride and amino-nitroxide building-blocks. As shown in Scheme 18, different types of anhydrides (I and J) and nitroxides (L and M) were tested in this work. These different monomers allow potentially the synthesis of oligomers containing encrypted primary structures. The iterative chemistry on the solid-support was characterized using different analytical methods. Completion of each step has

been verified by a Kaiser test.[238] In addition, the cleaved oligomer sequences were characterized by SEC and ^{1}H NMR. The presence of a multiple alkoxyamine bond in the oligomer structure prevented analysis of these samples by mass spectrometry techniques (*i.e.* ESI-MS and MALDI-TOF) under standard conditions. Soft ionization protocols could be potentially used to analyze these polymers,[239] but were not tested within the time-frame of this thesis.

2.1. SYNTHESIS OF A MODEL ALTERNATING SEQUENCE

In order to test the validity of our chemical strategy, an alternating oligo(alkoxyamine amide)s was first prepared on the solid support. This polymer was obtained using 2-bromoisobutyryl anhydride (I) and amino-TEMPO (L) as building blocks. Monodisperse model oligomers containing 3-, 5-, 7- and 9-units were prepared and characterized. In order to monitor the progress of the iterative synthesis on the resin, a primary amine color test (*i.e.* Kaiser test) was performed after each building-block attachment (**Figure 72**). Kaiser mixture was prepared by mixing ninhydrine, phenol and potassium cyanide solutions.[238] This mixture is yellow. Usually, in the presence of primary amine groups (*i.e.* positive Kaiser test), extensively heated resin beads immersed in the Kaiser mixture turn dark blue. In the present case, the positive Kaiser test turns the mixture into dark (-IL and –ILIL resin-conjugates in **Figure 72**). This unusual color change is due to the presence of primary amine groups and probably some free amino-TEMPO in the vials due to degradation of the oligomer by heating. In contrast, the absence of primary amine groups results in no color change as compared to the original Kaiser mixture (*i.e.* the mixture remains yellow, as in the case of resin-I, resin-ILI and resin-ILILI conjugates in **Figure 72**).

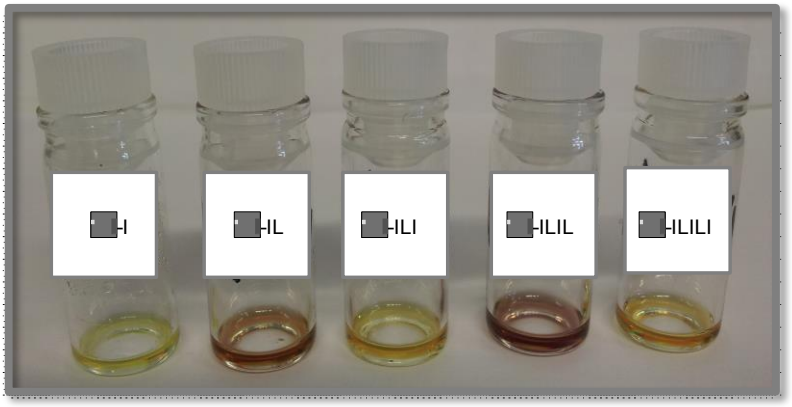


Figure 72. Kaiser test conducted after each coupling of a sequential synthesis of an alkoxyamine amide pentamer.

After reaction, the resin-bound molecules were cleaved from the support using a TFA/DCM mixture (1/1). **Figure 73** shows the SEC chromatograms recorded for the obtained oligomer chains. Since primary amines interact with SEC analytical columns, only bromine–terminated oligomers were designed in this study. (*i.e.* SEC measurements were conducted after attachment of an I building block).

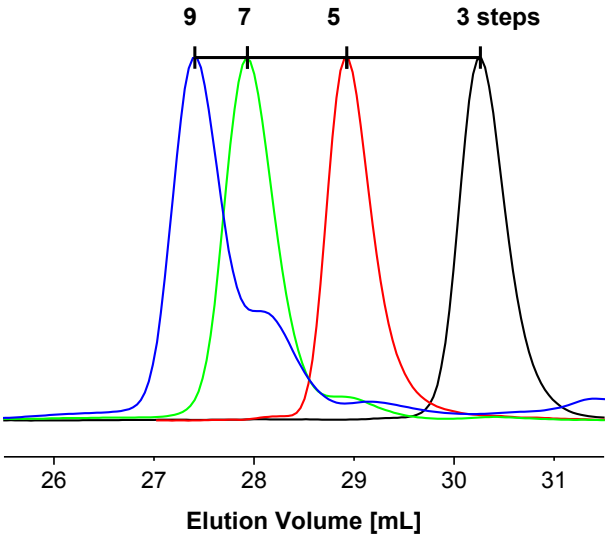


Figure 73. SEC chromatograms recorded in THF for the cleaved oligo(alkoxyamine amide)s prepared from 2-bromoisobutyryl anhydride and amino-TEMPO building blocks on a Wang solid support. The oligomers of different length are represented by the following colors: black – 3 units, red – 5 units, green – 7 units and blue – 9 units.

The results presented in **Figure 73** indicate that the protecting-group-free iterative "AB + CD" approach works efficiently on a solid-support. Indeed, fully monodisperse chromatograms were obtained for the oligomers containing 3, 5 and 7 units.

However, the appearance of a low molecular weight shoulder in the chromatogram of the nonamer suggests a decrease of the coupling efficiency when longer sequences are targeted. These defects seem to occur mostly when the bromo-anhydride building blocks are attached, as evidenced by the chromatograms overlay. Indeed, the low molecular weight shoulder of the blue chromatogram eluted at the elution volume as the heptamer. Extension of the reaction time from 50 min to 1.5 h, as well as repetition of the bromoanhydride attachment step helped to increase reaction coupling efficiency. However, complete suppression of the low molecular weight shoulder in the chromatogram of the nonamer has not yet been achieved. Molecular

weight values of the formed oligomers, calculated according to PS standards, were found to be in very good agreement with the theoretical molecular weight values (**Table 6**).

Monodispersity and controlled molecular weight of the synthesized oligo(alkoxyamine amide)s is also in agreement with the NMR analysis of the molecule. **Figure 74** displays the NMR spectrum of the oligomer prepared *via* alternated coupling of 3I and 2L building blocks (*i.e.* 5 steps).

Table 6. SEC and theoretical molecular weight values of the alkoxyamine amide oligomers prepared from 2-bromoisobutyryl anhydride and amino-TEMPO building blocks.

Oligomer sequence	**$M_{p\ (SEC)}$ [g/mol]**	**$M_{theor.}$ [g/mol]**
GILI	510	464
GILILI	811	705
GILILILI	1124	945
GILILILILI	1369	1185

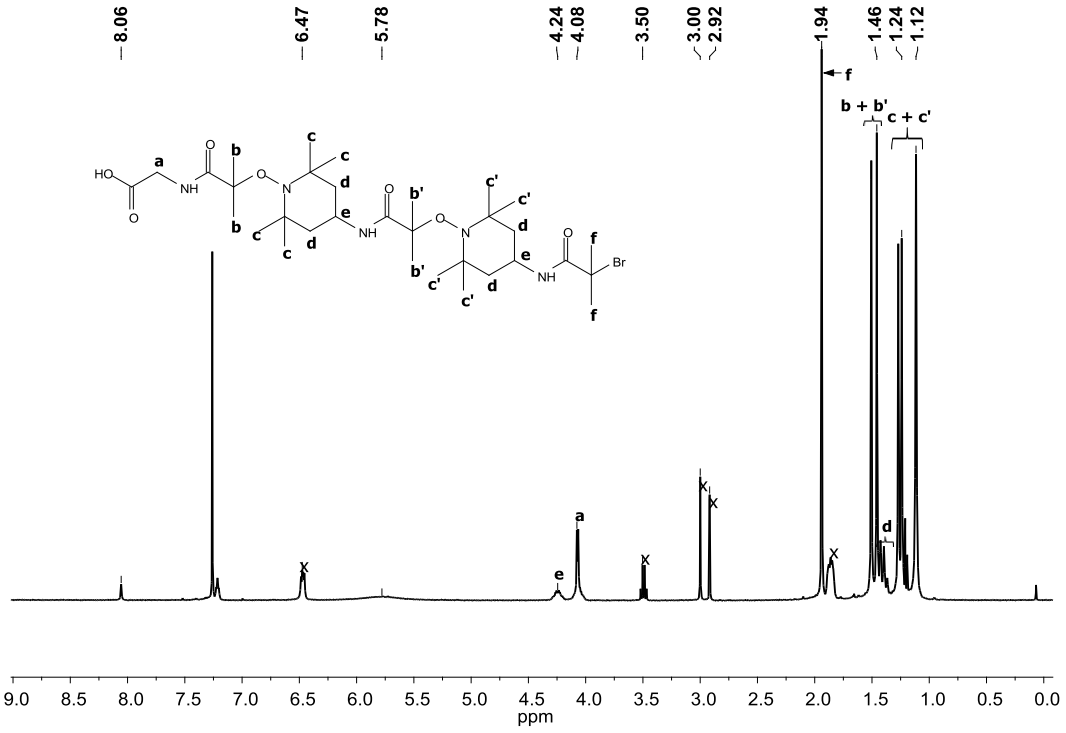


Figure 74. ^{1}H NMR-spectrum recorded in $CDCl_3$ for a pentamer prepared from 2-bromoisobutyryl anhydride and amino-TEMPO building blocks on a Wang solid support.

^{1}H NMR signals of the GILILI pentamer presented in **Figure 74** confirmed the synthesis of an oligo(alkoxyamine amide) oligomer. Signal of the solid support residual glycine unit can be seen at 4.08 (**a**). Next, the peaks in the region of 1.46-1.55 ppm have been attributed to bromoanhydride methyl groups (**b** and **b'**). Subsequently, the methyl groups of the terminal I unit appeared as a singlet at 1.94 ppm (**f**). Finally, the methyl groups of the TEMPO units have been identified in the region of 1.12-1.24 ppm (**c and c'**). Furthermore, the protons included in the nitroxide cyclohexane ring appeared at 1.33-1.43 ppm (**d**) and at 4.24 ppm (**e**).

2.2. SYNTHESIS USING BROMOPROPIONIC ANHYDRIDE AS A BUILDING BLOCK

In the previous paragraph, it was shown that the "AB + CD" iterative concept works. However, this was demonstrated using two monomers only (*i.e.* alternating sequences of I and L). In order to create more complex primary structures, it was important to demonstrate that other building-blocks can be used in this approach. For instance, another bromo-anhydride, 2-bromopropionic anhydride (J),was tested. The synthesis of alternating (alkoxyamine amide)s constructed from 2-bromopropionic anhydride and amino-TEMPO units was studied on the Wang solid support. In particular, model oligomers with 3-, 5- and 7-units were prepared. After synthesis, the resin-bound oligomers were cleaved from the support using a TFA/DCM mixture (1/1 v:v).

The synthesis of the $G(JL)_nJ$ oligomers seemed to be less efficient due to the presence of small molecular weight shoulders at high elution volume in SEC. Positions of the shoulders on the chromatogram suggested incomplete yields of the bromoanhydride coupling steps. Indeed, the formation of a tertiary carbon radical ($R_3C\cdot$) is generally favored over secondary ($R_2HC\cdot$). This could explain the observed differences in reactivity between anhydrides I and J. Nevertheless, the formed oligomers were near monodisperse and the molecular weight values calculated by SEC using PS standards were in agreement with the theoretical values.

The formed oligomers were also characterized by NMR. As an example, **Figure 75** shows the NMR spectrum measured for the heptamer. It has been recorded in deuterated acetone due to a poor solubility of the $G(JM)_3J$ oligomer in $CDCl_3$. Due to the oligomeric structure, the observed signals of the molecule were complex. Still, the most characteristic peaks were assigned. Signals of the solid support residual glycine methylene protons (**a**) can be visible at 3.98-4.45 ppm, together with the methine protons of amino-TEMPO (**e**) and 2-

bromopropionic anhydride (**g**). Methyl groups of the J units (**b**) were found in the region 1.29-1.36 ppm, with exception of the methyl group of terminal J unit at 2.06 ppm (**b'**). The group of intense peaks in the region of 1.17-1.29 ppm was recognized as methyl units attached to the heterocyclic nitroxide structures (**c**). The methylene groups included in the TEMPO heterocycle (**d**) appeared in the region of 1.36-1.73 ppm.

Table 7. SEC and theoretical molecular weight values of the alkoxyamine amide oligomers prepared from 2-bromopropionic anhydride and amino-TEMPO building blocks.

Oligomer sequence	**$M_{p\ (SEC)}$ [g/mol]**	**$M_{theor.}$ [g/mol]**
GJLJ	534	436
GJLJLJ	791	663
GJLJLJLJ	1066	889

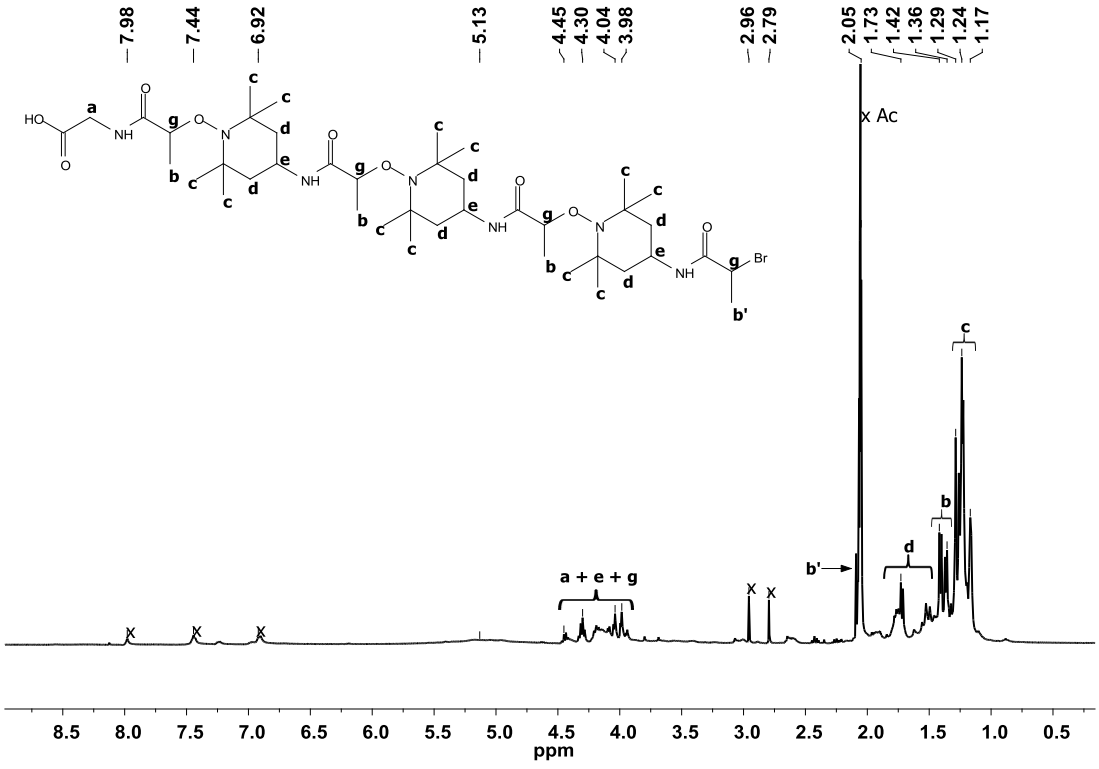


Figure 75. ^{1}H NMR-spectrum recorded in $(CD_3)_2CO$ for the heptamer prepared from 2-bromopropionic anhydride and amino-TEMPO building blocks on a Wang solid support.

2.3. SYNTHESIS USING TWO DIFFERENT ANHYDRIDES

Next, sequence-defined oligo(alkoxyamine amide)s were prepared on the solid support using amino-TEMPO as a CD spacer and either 2-bromoisobutyryl anhydride or 2-bromopropionic anhydride as an AB building block. This synthesis was performed in order to show that the "AB+CD" strategy can be used to encode binary molecular information in the oligomer chains. The sequence GJLJLJLILI was chosen arbitrarily and was characterized by ^{1}H NMR (**Figure 76**).

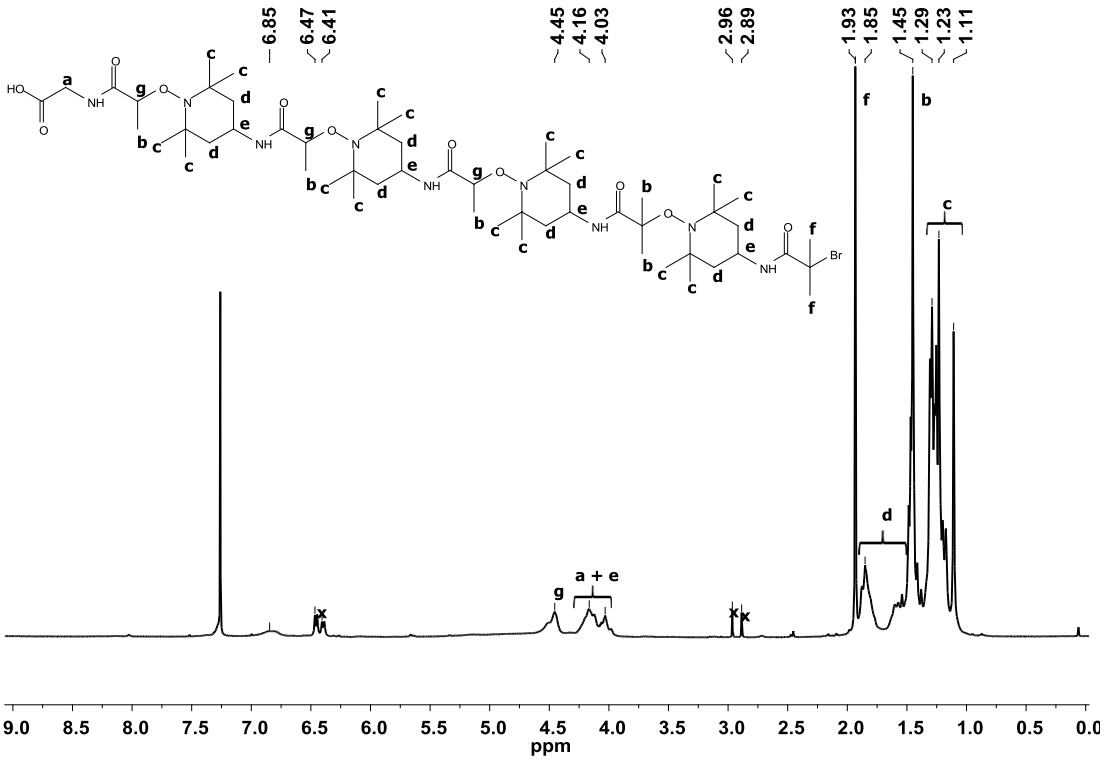


Figure 76. ^{1}H NMR-spectrum recorded in $CDCl_3$ for the nonamer prepared from 2-bromoisobutyryl anhydride, 2-bromopropionic anhydride and amino-TEMPO building blocks on a Wang solid support.

In line with the previous analyses, the spectrum of GJLJLJLILI comprised signals characteristic for the 2-bromoisobutyryl anhydride, 2-bromopropionic anhydride and amino-TEMPO building blocks. In particular, the nitroxide's methyl protons appeared as a group of signals in the area of 1.11-1.29 ppm (**c**) and the neighboring peaks at 1.45 and 1.93 ppm have been assigned to $-CH_3$ moieties of the bromoanhydride units (**b** and **f**). Methylene protons of the solid support residual glycine unit (**a**) were found in the region of 4.03-4.16 ppm, together with the nitroxide's methine protons (**e**). Next, the peak at 4.45 ppm has been related to

methine protons of the 2-bromopropionic anhydride units (**g**). The methylene protons of the TEMPO heterocycle were identified in the region of 1.45-1.9 ppm (**d**).

The SEC data shown in **Figure 77** compares the chromatogram of the oligomer GJLJLJLILI to the one of the alternating IL nonamer (synthesized in **paragraph 2.1** in this chapter). The difference in molecular weight peak values between these two chromatograms (ΔM = 99 $g \cdot mol^{-1}$) roughly corresponds to the variation of the nonamer structure (theoretically, ΔM = 45 $g \cdot mol^{-1}$). Moreover, the molecular weight of the GJLJLJLILI oligomer ($M_{n(GJLJLJLILI)}$ = 1202 $g \cdot mol^{-1}$) is in a good agreement with the theoretical molecular weight value ($M_{theor.(GJLJLJLILI)}$ = 1143 $g \cdot mol^{-1}$). However, as already observed in previous experiments, a shoulder at low molecular weight is observable in the SEC chromatogram.

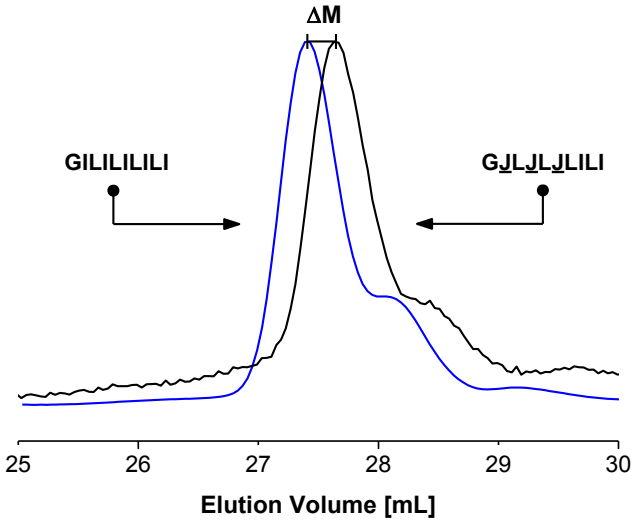


Figure 77. SEC chromatograms recorded in THF for the nonamers prepared on a Wang solid support from 2-bromopropionic anhydride, 2-bromoisobutyryl anhydride and amino-TEMPO building blocks (GJLJLJLILI – black curve and GILILILILI – blue curve).

2.4. SYNTHESIS USING AMINO-PROXYL AS A SPACER

Ultimately, the solid-phase methodology has been used to construct an alkoxyamine amide consisting of alternated 2-bromoisobutyryl and amino-PROXYL (M) units. Following the previously described procedure, the resin-linked molecules were cleaved from the support by the use of a TFA/DCM mixture (1/1). SEC values assigned to the cleaved oligomer chains were found in a good agreement with the theoretical values of the molecules (**Table 8**). However, the chromatograms of the oligomer obtained using M as a CD spacer where overall less well-defined than those obtained with amino-TEMPO.

Table 8. SEC and theoretical molecular weight values of the alkoxyamine amide oligomers prepared from 2-bromoisobutyryl anhydride and amino-PROXYL building blocks.

Oligomer sequence	$M_{p\ (SEC)}$ [g/mol]	$M_{theor.}$ [g/mol]
GIMI	528	450
GIMIMI	741	675
GIMIMIMI	955	901

The ^{1}H NMR characterization of the formed oligo(alkoxyamine amide) is shown in **Figure 78**.

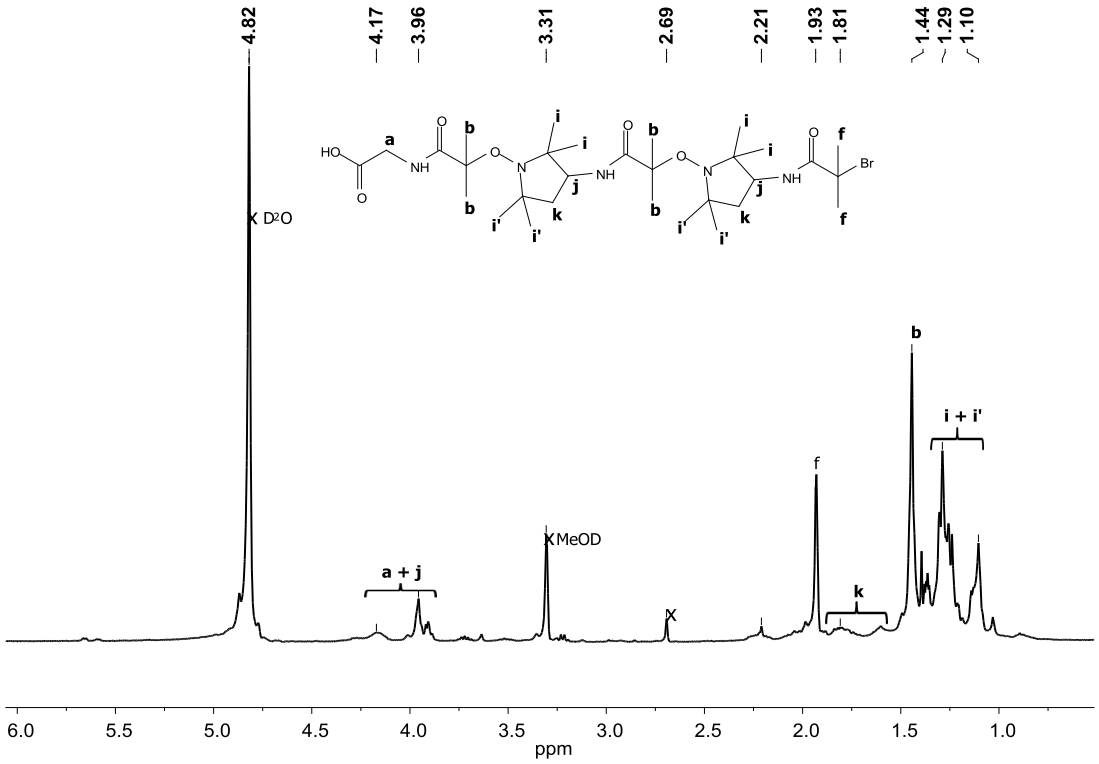


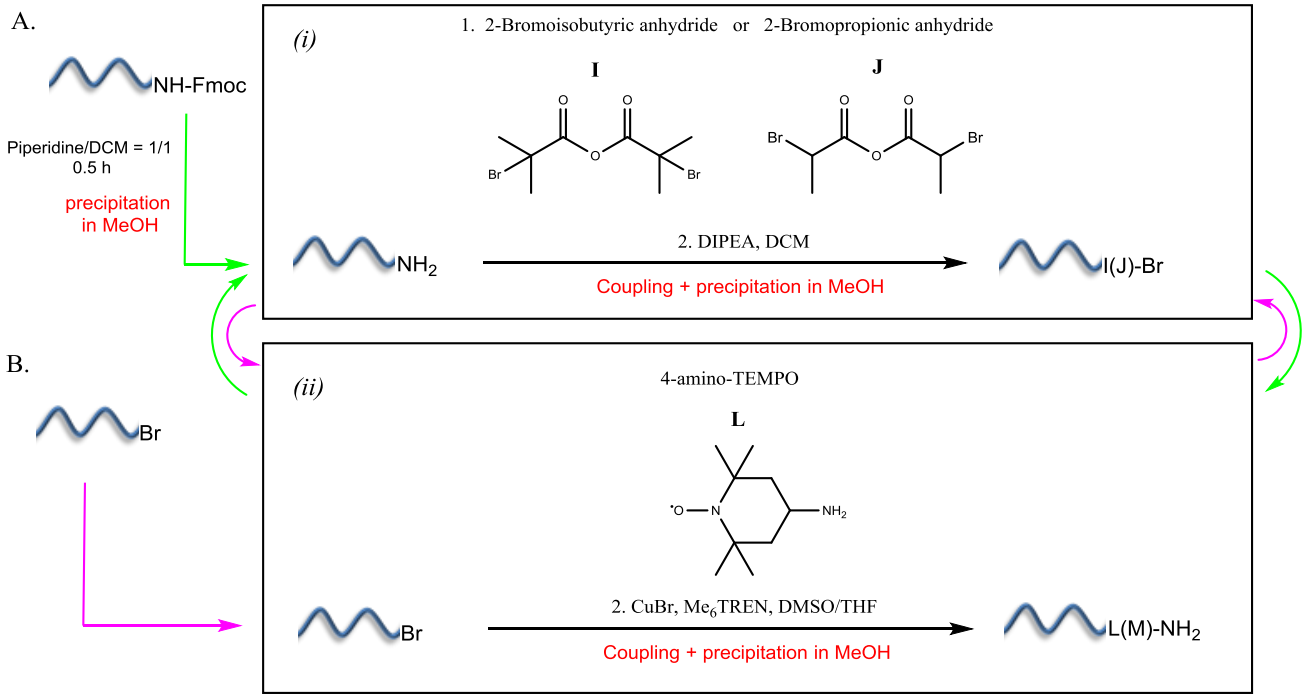
Figure 78. ^{1}H NMR-spectrum recorded in CD_3OD for the pentamer prepared from 2-bromoisobutyryl anhydride and amino-PROXYL building blocks on a Wang solid support.

The spectrum indicates that the pentamer structure was obtained. Traditionally, methylene protons of the residual glycine unit and methine protons of the amino-PROXYL unit were identified in the region of 3.96-4.17 ppm (**a** and **j**). Next, methyl protons of the bromoanhydride units built-in the pentamer structure (**b**) were found at 1.44 ppm; whereas the peak of the terminal bromoanhydride methyl groups appeared at 1.93 ppm (**f**). The methylene protons of the PROXYL units (**k**) appeared in the area of 1.6-1.84 ppm. Presence of the

PROXYL units has been additionally confirmed by the intense response of methyl protons at 1.1-1.29 ppm (**i** + **i'**).

3. SYNTHESIS OF OLIGO(ALKOXYAMINE AMIDE)S ON SOLUBLE SUPPORT

The iterative syntheses performed on the Wang solid-support indicated that the "AB + CD" strategy is a very efficient method for preparing monodisperse sequence-defined oligo(alkoxyamine amides). However, it was observed that some coupling steps were incomplete when long oligomeric structures were targeted (*i.e.* DP9 or higher). This aspect could be due to a low accessibility of the formed oligomers in the resin network. Thus, soluble polymer supports were investigated to prepare oligo(alkoxyamine amide)s. As discussed in **Chapter 1**, the use of soluble polymer supports allows performing iterative chemistry in homogeneous solutions and leads therefore often to better coupling yields than solid-supports.



Scheme 19. Strategy employed for the synthesis of oligo(alkoxyamine amide)s on soluble poly(ethylene glycol) (A) and polystyrene (B) supports. The sequence of monomer attachment to A and B supports is indicated by the green and magenta arrows, respectively.

Scheme 19 presents a liquid-phase methodology for the protection-group-free synthesis of oligo(alkoxyamine amide)s. The liquid-phase approach to oligo(alkoxyamine amide)s has been accomplished *via* utilization of two kinds of soluble supports: polystyrene without cleavable site and poly(ethylene glycol) containing a labile *p*-hydroxybenzyl moiety (**Scheme 17**). Both polymeric supports were prepared according to the procedures described in **Chapter II**.

In the case of the well-defined polymer supports **P9**, the Fmoc-protected primary amine terminus was first deprotected in a mixture of piperidine and DCM (1/1 v:v). Next, the first bromoanhydride unit was attached to the support in the presence of an excess of DIPEA (**Scheme 19** *i*). In the second step, amino-TEMPO was used in the presence of CuBr and Me_6TREN in DMSO. In addition, an appropriate amount of THF was used to provide homogeneous reaction conditions. While the attachment of bromoanhydride units required usually about 50 min, the nitroxide radical coupling was completed within 15 min. The modified polymer support was purified by precipitation in diethyl ether after each coupling step.

In the case of the polystyrene soluble supports **P7** and **P8,** the stepwise formation of the oligo(alkoxyamine amide)s was initiated differently. First, amino-TEMPO was reacted with the bromine-end group of the support (**Scheme 19** *ii*). Afterwards, steps *i* and *ii* were repeated several times.

3.1. SYNTHESIS OF A MODEL ALTERNATING SEQUENCE ON PS SUPPORTS

The liquid-phase approach to oligo(alkoxyamine amide)s has been found a highly-efficient strategy to obtain monodisperse sequence-controlled polymer chains containing multiple repetitive units. **Figure 79** shows the SEC characterization of the synthesis of a 24-building block oligo(alkoxyamine amide) on the P7 support. This polymer was prepared using I as a AB building-block and L as a CD spacer (*i.e.* model alternating IL microstructure).

Figure 79 clearly shows that a controlled iterative growth occurred on the soluble support. Indeed, an incremental increase of the average molecular weight of the modified support is clearly noticeable. It should be again noted that only bromine-terminated polymers are shown in this figure (*i.e.* polymers obtained after reaction with a bromoanhydride). Indeed, due to the

interactions of primary amines with the SEC columns, the chromatograms of the amine-terminated polymers (*i.e.* polymers obtained after reaction with amino-TEMPO) are omitted.

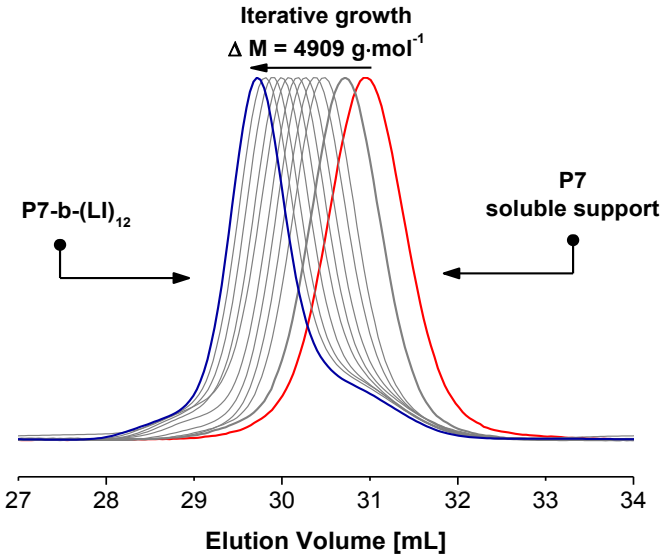


Figure 79. SEC chromatograms recorded in THF for the iterative growth of a polystyrene-alkoxyamine amide conjugate performed on a permanent soluble support (P7). The polystyrene Fmoc-protected support P7 is represented by a red line and the resulting alkoxyamine amide-polymer conjugate by a blue line. The grey traces show intermediate situations recorded after each bromoanhydride coupling with an exception of the most-right grey curves corresponding to P7-*b*-$(LI)_2$ and P7-*b*-$(LI)_4$ intermediates.

Table 9 shows the details of the iterative growth as monitored by SEC. Typically, an average molecular weight shift of about 425 g·mol^{-1} has been measured between two consecutive bromoanhydride attachments. This difference do not fully correspond to the theoretically calculated molecular weight shift (*i.e.* 241 g·mol^{-1}) due to PS calibration of the SEC measurement. After all iterative steps, the formed PS-*b*-$(LI)_{24}$ conjugate exhibited a molecular weight M_n of 7460 g·mol^{-1} and a narrow molecular weight distribution (M_w/M_n = 1.17). The small molecular weight shoulder observable on the blue chromatogram in **Figure 79** is probably due to the dead polymer chains of the PS support (*i.e.* dead by coupling during the ATRP process) that do not participate to the iterative growth. Furthermore, the molecular weight difference between the final PS-*b*-$(LI)_{24}$ conjugate and the P7 soluble support (*i.e.* difference between the blue and red chromatograms in **Figure 79**) is ΔM = 4909 g·mol^{-1}. As before, this difference do not fully correspond to the theoretically calculated molecular weight shift (*i.e.* 2940 g·mol^{-1}) due to PS calibration of the SEC measurement. All these results show that ATRP-made PS are very efficient supports for oligo(alkoxyamine amide) synthesis.

Furthermore, it should be specified that the yields of recovery by precipitation of the PS supports were good after each iterative step (average 92% of recovery).

Table 9. Molecular weight differences between intermediate couplings represented by the grey traces in **Figure 79**. The right column indicates the polymer recovery yields that were calculated after gravimetric precipitation of the conjugate in methanol.

	Isolated polymer	**ΔM [g·mol^{-1}]**	**Yield [% of recovery in the respect to preceeding amino-TEMPO unit attachment]**
1	PS-*b*-$(LI)_2$	856	98
2	PS-*b*-$(LI)_4$	663	97
3	PS-*b*-$(LI)_5$	498	97
4	PS-*b*-$(LI)_6$	324	99
5	PS-*b*-$(LI)_7$	332	94
6	PS-*b*-$(LI)_8$	634	93
7	PS-*b*-$(LI)_9$	367	86
8	PS-*b*-$(LI)_{10}$	393	77
9	PS-*b*-$(LI)_{11}$	410	95
10	PS-*b*-$(LI)_{12}$	532	81

The iterative process was also monitored by ^{1}H NMR. In general, the NMR spectra of formed oligo(alkoxyamine amide)s are complex and they overlap with the NMR signals of the polystyrene support. Thus, in the present case, a deuterated polystyrene support (P8) was synthesized. This support allows visualizing solely the NMR spectra of the oligomers. **Figure 80** presents the ^{1}H NMR spectra recorded for the synthesis of a model hexamer.

Appearance of the signals specific for the amino-TEMPO attachment can be observed in the region of 1.1-1.2 ppm (marked in grey in **Figure 80)**. In particular, the growing peak (*bottom –> up*) corresponds to the methyl groups of TEMPO. Moreover, increase in the signal integration (*bottom –> up*) is proportional to the amount of coupled TEMPO units. Linkage of the first anhydride building block to the PS_d-*b*-L conjugate reveals a signal at 1.97 ppm that corresponds to a $–CH_3$ group of the bounded unit (marked in blue in **Figure 80**). Subsequently, attachment of the second amino-TEMPO molecule shifts the protons at 1.47

ppm (marked in red in **Figure 80)**. In the next step, both of the signals can be detected due to the presence of a chain-build-in and a chain-terminal bromoanhydride building blocks. Eventually, the following nitroxide and anhydride couplings to the support trigger the aforementioned mechanism of the "on-off" switches related to the modifications of the alkoxyamine amide ω-chain end.

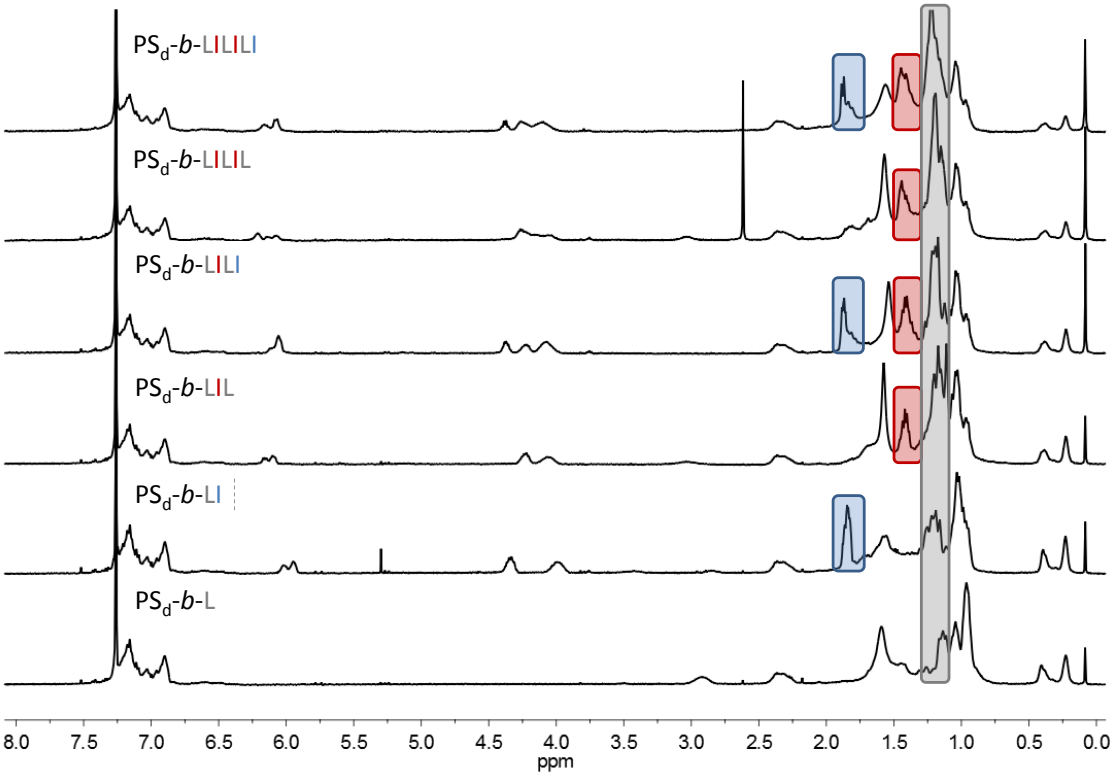


Figure 80. ^{1}H NMR spectra recorded in $CDCl_3$ for the stepwise preparation of a hexamer on a deuterated non-cleavable polystyrene support. The blue and red boxes indicate the bromoanhydrides' methyl groups (attached to the chain-end and build-in the chain, respectively), while the grey stripe designates $-CH_3$ groups of the amino-TEMPO units.

3.2. SYNTHESIS OF A MODEL ALTERNATING SEQUENCE ON PEG CLEAVABLE SUPPORT

The results of the previous paragraph shows that soluble polymer supports are much more promising for synthesizing sequence-defined oligo(alkoxyamine amide)s than solid-supports. However, the PS soluble supports studied in the previous paragraph were non-cleavable and it was therefore not possible to isolate, purify and analyze the formed oligomers. Thus, in the present paragraph, a cleavable support was studied. An example of cleavable PS support was described in **Chapter II** of this thesis (paragraph 2.2.2). However, this support, synthesized

by ATRP, could not be used for the synthesis of oligo(alkoxyamine amide)s because it contains a terminal bromine atom, that can react during the amino-TEMPO coupling step. Thus, a cleavable support was prepared by modification of a commercial amino-terminated poly(ethylene glycol). A cleavable *p*-hydroxybenzyl linker and a Fmoc-protected glycine unit were coupled to this PEG polymer following the procedure described in **Chapter II** for PS. The construction of a pentamer on the PEG cleavable support (P9) was monitored by SEC (**Figure 81**).

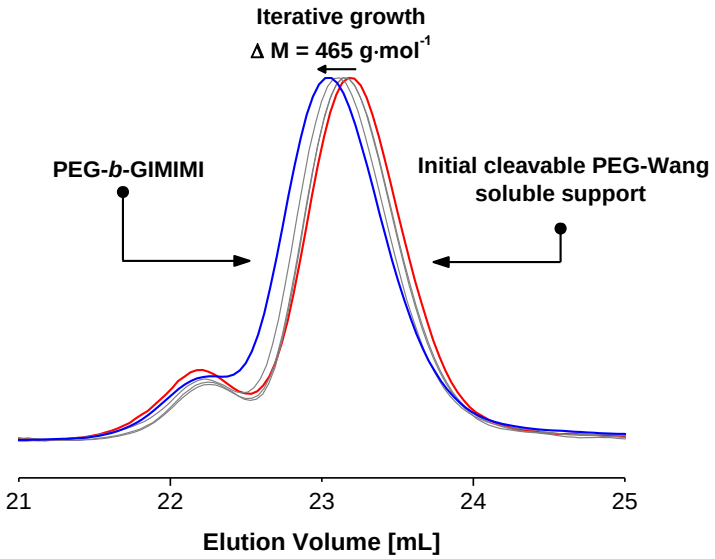


Figure 81. SEC chromatograms recorded in THF for a step-by-step growth of the poly(ethylene glycol)-*b*-pentamer conjugate performed on a cleavable soluble support (P9). The PEG Fmoc-protected support P9 is represented by a red line and the resulting (alkoxyamine amide)-polymer conjugate by a blue line. The grey traces show intermediate situations recorded after each bromoanhydride coupling.

Similarly to the approach of the non-cleavable soluble support, addition of each monomer unit has been confirmed by NMR signal shifts described earlier in **Figure 80**.

Oligomer sequence	**M_n [g/mol]**	**$M_{p\ (SEC)}$ [g/mol]**
PEG-Wang	5127	5070
PEG-b-GILILI	5141	5535

Synthesis of the alkoxyamine amide on PEG cleavable support has been finalized by cleavage and characterization of the obtained oligomer. The cleavage of PEG occurred in a mixture of TFA/DCM (v/v = 1/1, 2h). **Figure 82** compares the SEC chromatogram of the pentamer

obtained on the PEG soluble support to those of the oligo(alkoxyamine amide)s obtained on the Wang solid support (**paragraph 2.1** of this chapter).

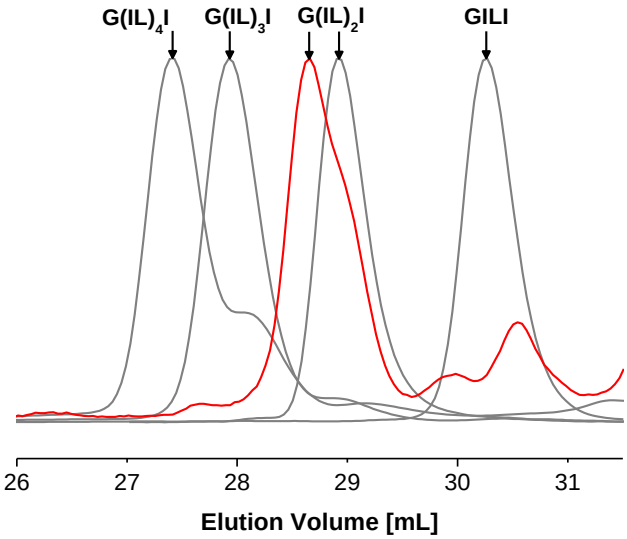


Figure 82. SEC chromatograms recorded in THF for the cleaved (alkoxyamine amide)s prepared from 2-bromoisobutyryl anhydride and amino-TEMPO building blocks on a cleavable soluble support (red curve) and a Wang solid support (grey curves).

SEC characterization of the pentamer represented by a red curve in **Figure 82** is in line with the series of oligomers prepared on a solid support represented by grey curves. A slight molecular weight shift between the two graphs of $G(IL)_2I$ oligomers can be explained by a change in calibration of the SEC instrument that occurred in the time difference between the two experiments ($M_{GILILI\ \_soluble\ support}$ = 903 g·mol^{-1}, $\Delta M(_{GILILI\_soluble\ support\ -\ GILILI\_solid\ support})$ = 92 g·mol^{-1}).

Cleavage of the alkoxyamine amide turned out to be incomplete as revealed by ^{1}H NMR analysis of the sample (**Figure 83**). Indeed, some residual PEG chains can be observed at chemical shifts of 3.03 and 3.62 ppm. The methyl groups of 2-bromoisobutyryl anhydride units appeared on the spectrum at 1.45 ppm (built-in units) and 2.04 ppm (terminal unit) and are marked in blue and green, respectively. The red box represents methyl groups of the amino-TEMPO building blocks visible in the region of 1.11-1.26 ppm. Still, this spectrum shows major differences with the resin-made pentamer displayed in **Figure 74**. Apart from the presence of solvent residual peaks, these discrepancies can be imputed to the oligomer degradation. It should be also noted that two different deuterated solvents were used for the NMR analysis of this oligomer. This reaction needs to be optimized in the future.

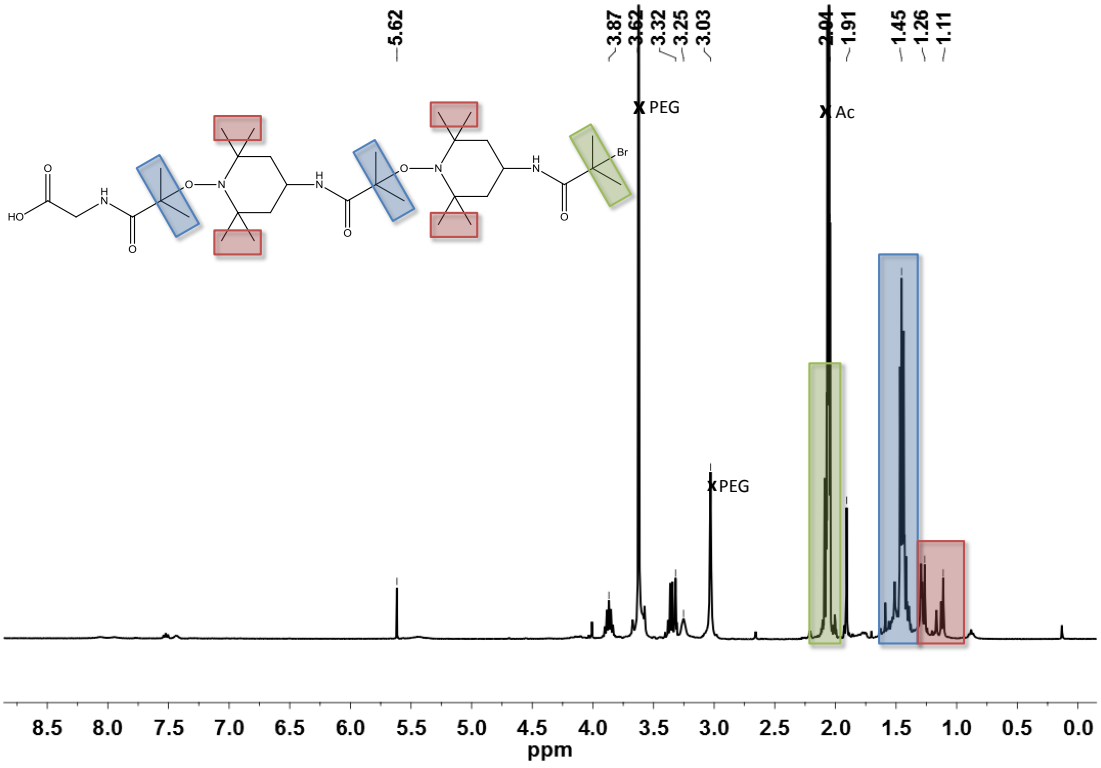


Figure 83. ^{1}H NMR spectra recorded in $(CD_3)_2CO$ for the pentamer cleaved from a PEG Wang soluble support (P9).

4. THERMAL DEGRADATION OF (ALKOXYAMINE AMIDE)S

As mentioned in the introduction of this chapter, oligo(alkoxyamine amide)s contain multiple alkoxyamine bonds and can therefore be thermo-degraded. Thus, the thermal degradation of a model oligo(alkoxyamine amide) described in **paragraph 2.3** was studied (*i.e.* oligo(alkoxyamine amide) prepared using two different anhydrides; $M_{n(GJLJLJLILI)}$ = 1202 g·mol^{-1}). Four series of experiments have been conducted at the following temperatures: 50, 75, 100 and 125 °C. The polymer was dissolved in anisole (assuming 0.02 M concentration of the alkoxyamine groups) and heated for 24h in an oxygen-free environment. The polymer degradation was monitored by SEC (**Figure 84**).

As evidenced in **Figure 84**, heating of the oligo(alkoxyamine amide) at 50 °C did not lead to any noticeable change of the chromatogram *(i)*. These results are consistent with the minimum cleavage temperature of TEMPO-based alkoxyamines, which is in general around 60°C. A significant deformation of the nonamer chromatogram occurs only at 75 °C *(ii)*. A slight deviation of the curve can be noticed already after 9h of the reaction; however, a clear progress of the thermal degradation process can be noticed after 24h. At temperatures above

100 °C *(iii* and *iv)*, a fast thermal degradation of the oligo(alkoxyamine amide) was observed. It is important to note that the shape of the violet chromatogram in **Figure 84** *iii* is fully different from the original chromatogram of the nonamer (*i.e.* black curve), thus indicating formation of low molecular weight degradation products. Typically, full degradation was accomplished after 24h of heating of the GJLJLJLILI oligomer at 100 °C. Similar results were obtained at higher temperature (*i.e.* 125 °C) already after 3h (orange curve in **Figure 84** *iv*). Interestingly, when the experiment was continued for 24h at 125°C, a high molecular weight shoulder was observed (*i.e.* violet chromatogram in *iv*). This may correspond to the step-growth polymerization of some low molecular weight degradation products.

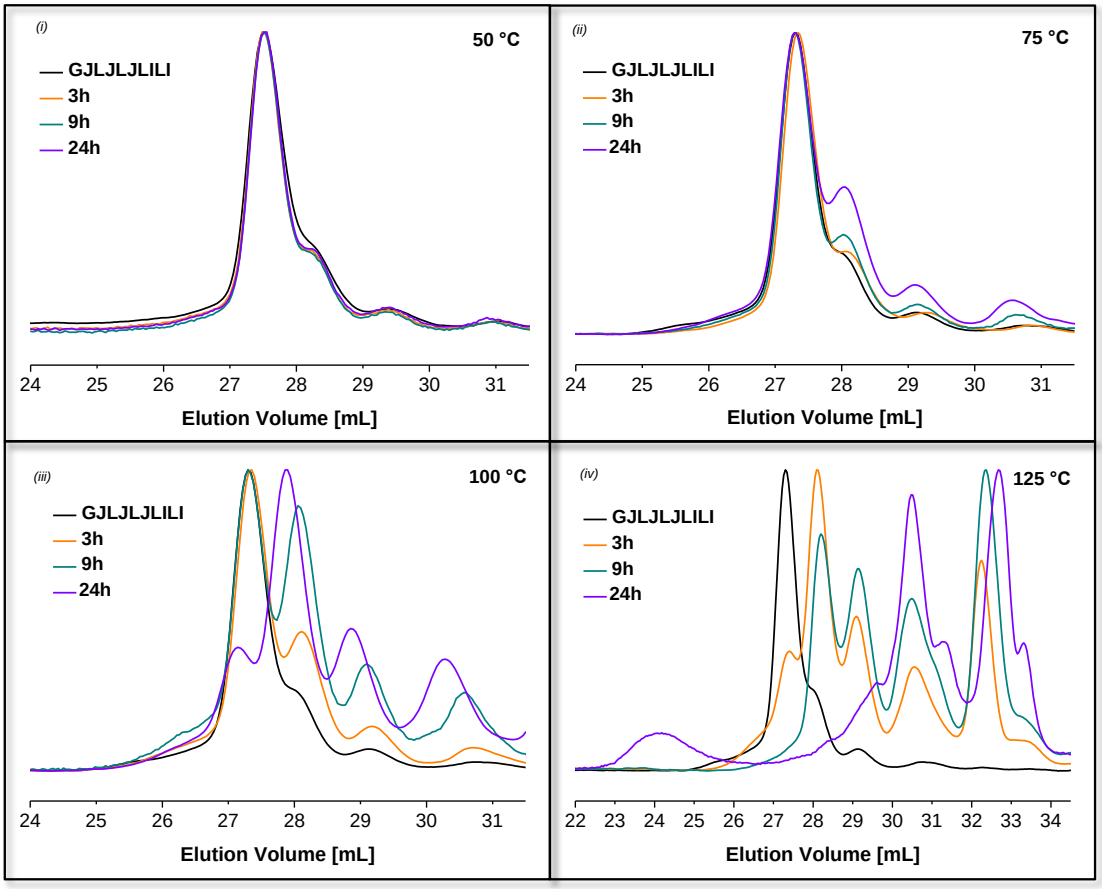


Figure 84. SEC chromatograms recorded in THF for the thermal degradation of the oligo(alkoxyamine amide) (GJLJLJLILI) in anisole (0.02 M) conducted at the following temperatures: *(i)* 50 °C, *(ii) 75* °C, *(iii)* 100 °C and *(iv)* 125 °C. The black curve represents the initial dynamic polymer and the remaining curves correspond to the reorganized polymer after 3h, 9h and 24h (the orange, green and violet lines, respectively).

5. CONCLUSIONS

A new chemoselective and protecting-group-free "AB + CD" method was tested for the synthesis of monodisperse sequence-defined oligo(alkoxyamine amides). Successive amidification and nitroxide radical coupling reactions were used to construct these oligomers. As described in this chapter, the synthesis has been accomplished *via* two approaches: *(i)* solid-phase methodology using a commercially available Wang resin and *(ii)* liquid-phase methodology using soluble PS and PEG supports. The latter strategy allowed preparation of block copolymers consisted of a well-defined polymer support and a precisely designed alkoxyamine amide oligomer. Presence of a labile *p*-hydroxybenzyl linker in the PEG chain permitted cleavage and analysis of the functional alkoxyamine amide microstructure.

The sequence regulation of the alkoxyamine amide has been accomplished using AB and CD building blocks possessing four different active groups: acid (A), halogen (B), nitroxide (C) and amine (D). The experiments conducted using various monomers (I, J, K and L) indicated that the efficiency of the synthesis is higher when I and K are used than their counterparts J and L. The appropriate selection of the monomers has been foundation for efficient and rapid synthesis of these (alkoxyamine amide)s. Indeed, the required time of radical couplings for the two consecutive monomer pairing is about 1h. In particular, the high input in diminution of the reaction time was attributed to the nitroxide radical coupling that exhibits properties of a "click" reaction, including high efficiency and selectivity of the formed product. In addition, the unique reversibility aspect of the nitroxide bonding has been revealed in response to an elevated temperature (>100 °C). A series of experiments demonstrated fractionation of the monodisperse SEC signal of the oligo(alkoxyamine amide) upon temperature increase. The process initiates in 75 °C, however complete transformation of the original graph is achieved in 100 °C after 24h or alternatively in 125 °C after 3h of the reaction. Although analysis of the obtained structure by ^{1}H NMR has been demanding, causing difficulties in interpretation of the spectra, it is certain that the polymer structure has changed resulting in generation of a new macromolecular pattern.

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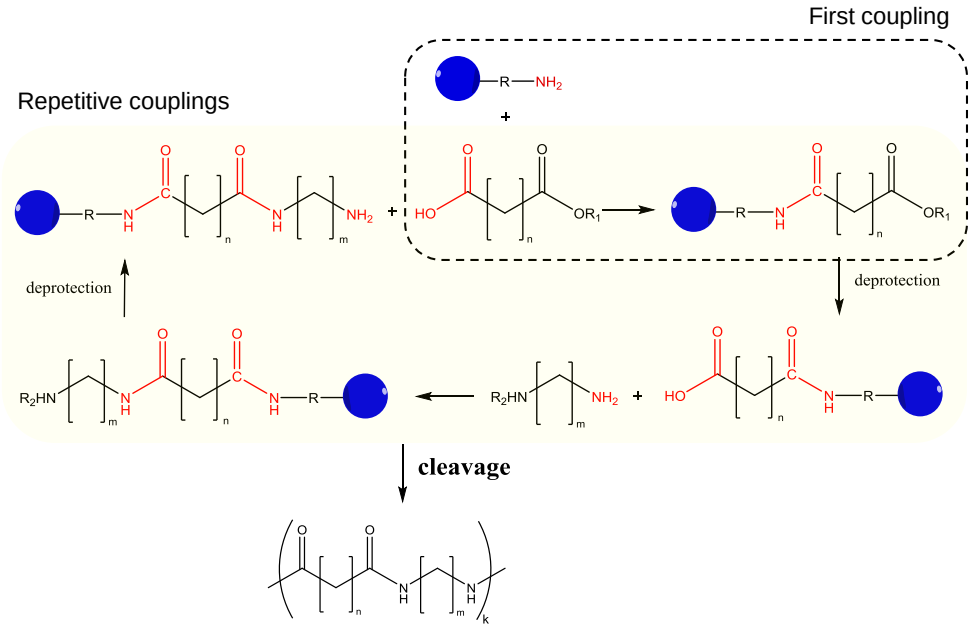
Chapter IV

SYNTHESIS OF OLIGOAMIDES ON SOLUBLE POLYMER SUPPORTS USING AN AA + BB APPROACH

1. INTRODUCTION

Since few decades, design and synthesis of functional oligoamides have been of great interest for synthetic chemists. In particular, peptide and protein mimicry using non-natural sequence-specific oligomeric backbone has been intensively investigated due to the potential therapeutic applications.[240] Such pseudo-peptides are commonly constructed on a solid-support *via* stepwise oligomerization of AB monomers containing a carboxyl terminus and a protected secondary amine function (e.g. polymers of nylon n type). This approach was used *i.a.* for the preparation of polyamides containing pyrrole and imidazole units,[241] quinoline-based monomers[242] or asymmetrically branched amino acids developed from diethylenetriamine.[243]

Sequential addition of diamine and diacid building blocks (*i.e.* AA and BB type) on a solid-support is another approach to amide bond formation (**Scheme 20**).[244] In this case, the resulting oligoamides are of the nylon n+2, m type.



Scheme 20. Schematic representation of conventional solid-phase approach to polyamides formation using protecting groups. R_1 and R_2 are respectively protecting groups of the carboxyl and amine functions, while R represents a resin-bond chemical linker. Final polyamide structure is obtained after cleavage from the support with simultaneous removal of the protecting groups.

Experimental protocols for polyamide syntheses are conventionally based on successive condensation of amines and carboxylic acids or their derivatives. In such reactions, the acids

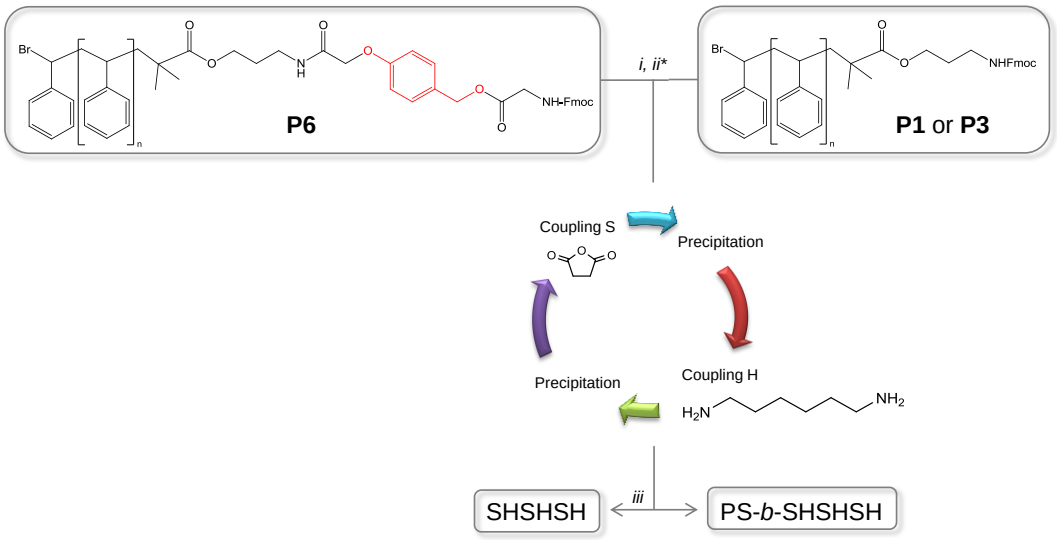
are usually either preactivated to form active esters (or acyl chlorides)[245] or *in situ* activated upon addition of stoichiometric amounts of coupling reagents.[64] The last procedure is extensively used in peptide synthesis. Due to necessity of protecting groups' elimination, the polyamidation process has been classified as environmentally unfriendly. Indeed, the amidation reaction generates a high amount of chemical wastes.

The synthesis of monodisperse sequence-defined oligoamides does not always require protecting group as shown in **Scheme 20**. Monodisperse polyamides were obtained by Rose and Vizzavona in an automated solid-phase supported protecting-group free synthesis. In this approach the absence of protecting groups greatly simplified the process.[137] To target future demands of oligoamides in medicine, control over macromolecular architecture, molecular weight and molecular weight distribution need to be attained. Biocompatible polyamides with a defined number of repeating units were prepared *via* a three-step procedure, starting from an amine-functionalized resin: *(i)* acylation with diacid derivative, *(ii)* activation with carbonyldiimidazole and, *(iii)* aminolysis with diamine. Similarly, Börner *et al.* have demonstrated the formation of sequence-defined poly(amidoamine)s with up to 10 segments with good control over the molecular weight and monomer sequence. These oligomers have been then conjugated with PEG to improve their biofunctionality.[246]

Yet, in these studies, the polymer supports were no longer useful after cleavage of the target oligoamide. Given broad importance of conjugated polymers in medicine and technology, we intend to present here a polymer-supported concept for the synthesis of monodisperse polyamides in solution. These structures were prepared on polystyrene soluble supports synthesized according to previously described protocols (**paragraph 2.2** in Chapter II). In particular, three sorts of polymeric platforms have been utilized in this work: *(i)* ATRP permanent support P1, *(ii)* ATRP support possessing a cleavable site and pre-loaded with glycine P6 and, *(iii)* ARGET ATRP non-cleavable support P3. Providing homogeneous conditions, efficiency of the reaction can be increased, therefore leading to an "atom economic" synthesis. Furthermore, the direct protection-group-free amidation procedure used in the present study prevents tedious protection/deprotection steps, therefore decreasing toxic waste generation.

Synthesis of polyamides was accomplished by stepwise coupling of diacid (or anhydride) and diamine building blocks. Initial model polyamide composition relied on nylon 6,6. To obtain the desired structure, adipic acid and hexamethylenediamine were employed. In order to favor

higher reaction efficiency, adipic acid has been next substituted by succinic anhydride (**Scheme 21**).



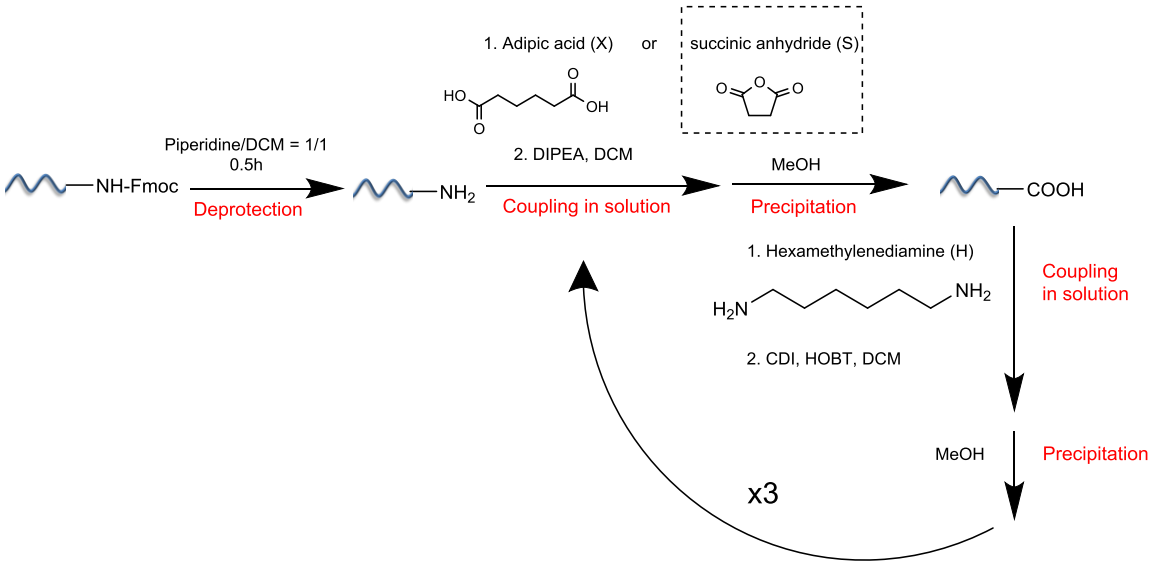
Scheme 21. Strategies examined for the synthesis of PS-*b*-PA conjugates. Terminal amine groups of polymers P1, P3 and P6 were deprotected prior to the assembly of the polyamide (*i*). Support P3 was additionally modified by treatment with TBAF to eliminate the bromide moiety at the end of the polymer chain (*ii*). After sequential coupling with succinic anhydride (S) and hexamethylenediamine (H) building blocks, the oligoamide was cleaved from the support under strong acidic conditions (*iii*).

Amidation reactions have been supported by utilization of soluble polystyrene supports P1, P3 and P6 prepared by controlled radical polymerization techniques. Post-modification of the final polymer (by TBAF treatment) was employed in the case of P3 in order to ensure complete removal of residual bromides in the polymer chain. This strategy has been adapted to eliminate the limitations encountered in the preparation of polyamides on a standard ATRP support. Moreover, the use of the cleavable support P6 allowed isolation of the obtained oligoamide and its subsequent analysis in order to evaluate the liquid-phase polymer-supported synthesis of polyamides in absence of protecting groups.

2. GROWTH OF POLYSTYRENE-OLIGOAMIDE CONJUGATES

Scheme 22 shows a liquid-phase protecting-group-free route for the synthesis of PS-*b*-PA conjugates. Iterative growth of the polyamide in this liquid-phase methodology approach addresses a model coupling strategy of AA + BB type. In the first step, one of the two carboxylic groups of the diacid (or anhydride) was attached to a deprotected amine function of the well-defined polystyrene support. The coupling process was facilitated by the use of an

excess of DIPEA. Next, the second carboxylic extremity was activated by CDI and HOBT to react with one amine moiety of the hexamethylenediamine. The polymer conjugate was purified by selective precipitation in MeOH after each coupling. The iterative growth was conducted until the formation of the hexaamide.



Scheme 22. Strategy for the synthesis of a *hexa*amide on a polystyrene soluble support in the absence of protecting groups. Succinic anhydride molecule has been placed in a dashed box to emphasize its foremost use in the assembly of polyamide building blocks as compared to adipic acid.

2.1. ITERATIVE SYNTHESIS INVOLVING ADIPIC ACID (X)

Adipic acid was initially considered as a diacid-type building block for the assembly of polyamide. The monomer was used in a 10 equivalent excess compared to the amine function of the support. *In situ* carboxy activation was in this case achieved by PyBOP addition in the presence of DIPEA. Combination of the two reactive amplified efficiency of the amide bond formation. SEC chromatogram of the resulting conjugate is displayed in **Figure 85**. Attachment of adipic acid to the polymer support resulted in an apparent molecular weight shift of 83 $g \cdot mol^{-1}$ (difference between the red and black curves on the graph). Subsequently, the theoretical molecular weight shift has been calculated as a value of -111 $g \cdot mol^{-1}$ (the negative value means in reality a shift into smaller molecular weight). Calibration of this SEC measurement has been set according to PS standards, thus the values should be considered as estimation. Furthermore, formation of the amide bond contributed to the appearance of a high molecular weight shoulder that corresponds to bimolecular coupling. This observation together with the high molecular weight shift value, suggest an overactivation of the adipic

acid. Assuming low reactivity of the monomer in the absence of PyBOP, growth of the bimolecular weight shoulder could be probably suppressed; however completion of the reaction would not be achieved. Considering this fact, utilization of a more reactive diacid derivative with a subsequent elimination of the phosphonium activator has been next employed to optimize the experimental protocol of diacid coupling.

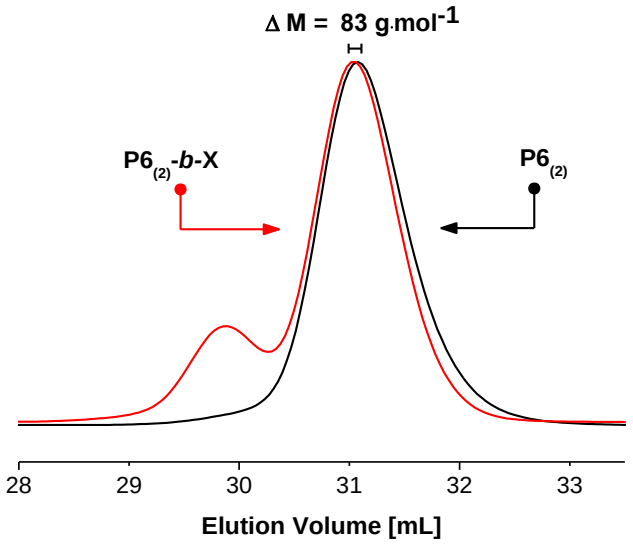


Figure 85. SEC chromatograms recorded in THF for the polystyrene cleavable support $P6_{(2)}$ (black curve) and the $P6_{(2)}$-*b*-X conjugate (red curve).

2.2. ITERATIVE SYNTHESIS INVOLVING SUCCINIC ANHYDRIDE (S)

Another protocol, using succinic anhydride, was adopted for the formation of polyamides to increase the reactivity of the building blocks. **Figure 86** presents the SEC chromatograms of the subsequent coupling of succinic anhydride and hexamethylediamine to the polymeric support P1. Similarly to the adipic acid attachment, a clear shift of 139 $g \cdot mol^{-1}$ can be observed after coupling of the anhydride (molecular weight difference between the black and red curves). Importantly, polydispersity of the resulted conjugate was maintained at a comparable level. Replacement of the diacid molecule with its cyclic analogue was then found to avoid undesired coupling. Succinic anhydride was used in a 10 equivalent excess compared to the amine functionality of the support. Control of the reaction was assured by the presence of DIPEA.

Deviation of the original $P1_{(3)}$ chromatogram in the form of a high molecular weight shoulder appeared after attachment of a diamine unit (blue curve in **Figure 86**). The SEC chromatogram of the PS-*b*-SH conjugate shifted to smaller molecular weight values. This

effect is probably due to interaction of the terminal amine group of the oligoamide with the SEC columns. Moreover, *N*-methyl-2-pyrrolidone solvent employed in this study following the experimental protocols on the solid support[137] did not solubilize all the compounds. The heterogeneity issue has been overcome by the use of a methanol/dichloromethane mixture (in a ratio 1:1). Indeed, methanol has been found a good solvent for the dissolution of hexamethylenediamine and HOBT, while dichloromethane was efficient for the solubilization of polystyrene and CDI.

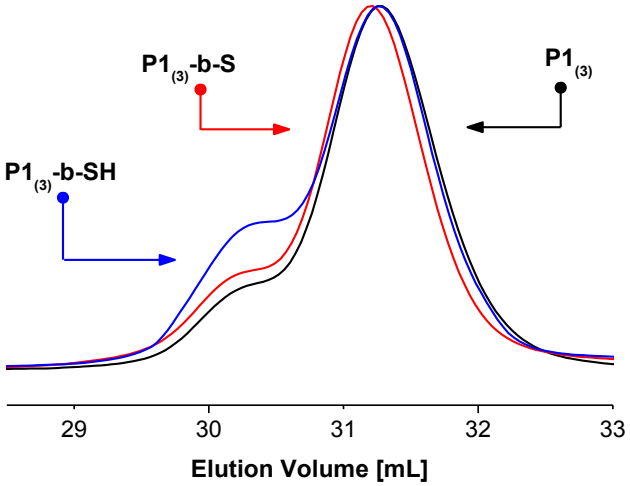


Figure 86. SEC chromatograms recorded in THF for the polystyrene permanent support $P1_{(3)}$ (black curve) and two consecutive steps of the oligoamide iterative growth: *(i)* addition of succinic anhydride represented by a red curve, and *(ii)* addition of hexamethylenediamine represented by a blue curve.

A three-step oligoamide synthesis using the modified procedure for the diamine coupling is exhibited in **Figure 87**. No bimolecular interactions between the diamine and the polymer chain have been observed in these SEC measurements. The molecular weight distribution of $P6_{(4)}$-*b*-S and $P6_{(4)}$-*b*-SH has not been affected by the coupling reactions. As compared with the previous observation, a molecular weight shift of 48 $g \cdot mol^{-1}$ was noticed after attachment of S. However, a regress of the blue chromatogram has been once more observed in consequence of the diamine attachment. This is probably due to interactions of the SEC columns with the terminal amine group of the $P6_{(4)}$-*b*-SH conjugate. Nevertheless, the amidation reaction conducted in homogeneous conditions provided by the mixture of MeOH and DCM allowed reducing undesired side reactions.

The coupling of the second succinic anhydride unit to the support resulted in an unexpected increase of the high molecular weight shoulder that is probably due to chain coupling.

Formation of such molecular "bridges" can probably be diminished by decreasing concentration of the polymer in the reaction mixture (ideally, concentration of the polymer should not exceed 100 mg/mL). An emphasis to meet these criteria has been put in the design of the next experiments.

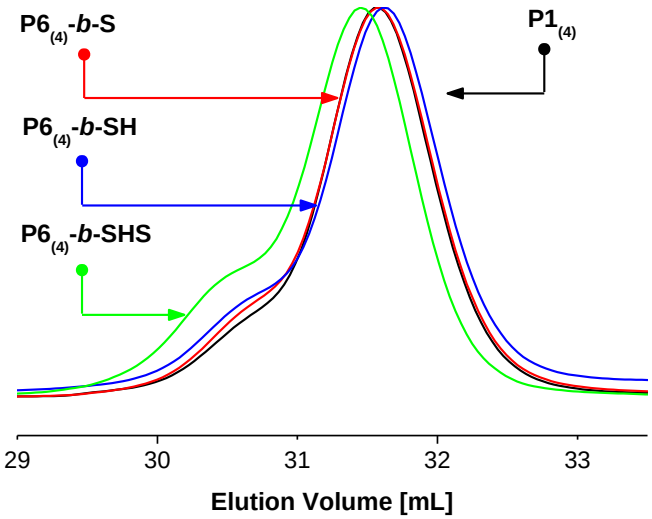


Figure 87. SEC chromatograms recorded in THF for the polystyrene permanent support $P6_{(4)}$ (black curve) and the three consecutive steps of the oligoamide's iterative growth (red, blue and green curves).

Nevertheless, an apparent molecular weight shift of $\Delta M_p = 25$ g·mol^{-1} can be observed in this three-step oligoamide growth. Yet, considering the PS calibration, this difference is in a relative good agreement with the theoretical molecular weight shift of 93 g·mol^{-1} calculated from the molecular weight difference between Fmoc-protected polystyrene support and the non-protected short-chain sequence of SHS oligoamide.

2.3. Repetition of the iterative couplings

The experimental procedure established for the polymer-supported synthesis of protecting-group-free polyamides in solution was next examined for the synthesis of longer sequences containing SH repetitive units. **Figure 88** shows the iterative growth of a tetraamide on a Br-free non-cleavable ARGET ATRP support P3 observed by SEC. Coupling of the first succinic anhydride unit to the support resulted in a significant regress of the molecular weight and a subsequent increase in the polydispersity of the obtained adduct (red line). This effect is probably due to interaction of the terminal amine group of the oligoamide with the SEC columns. Apart of a slight deviation of the P3-*b*-SHS chromatogram (blue line), a clear progression of the apparent molecular weight can be observed in the next steps. Indeed, theoretical molecular weight of the tetraamide (M = 415 g·mol^{-1}) has been correlated to the

molecular weight calculated as a difference between the **pink** and **black** curves (ΔM = 563 g·mol^{-1}). In this calculation, the molecular weight of the Fmoc protecting group was not taken into account assuming its pre-removal during ARGET ATRP process (**paragraph 2.2** in Chapter II). SEC characterization of the intermediate steps in the tetraamide synthesis process is presented in **Table 10**.

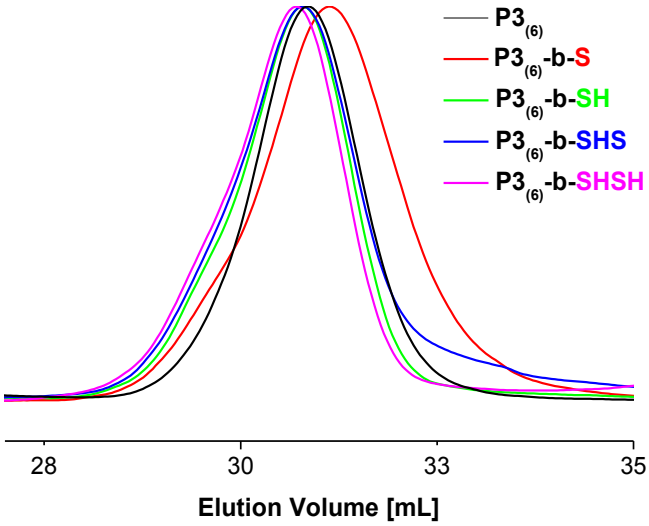


Figure 88. SEC chromatograms recorded in THF for the step-by-step growth of a polystyrene-tetraamide conjugate performed on a Br-free non-cleavable ARGET ATRP support $P3_{(6)}$. The four building blocks of oligoamide attached to the support are represented by the following colors: S – red, SH – green, SHS – blue, and SHSH – pink.

Table 10. Molecular weight and molecular weight distribution values recorded by SEC for the sequential assembly of a tetraamide on the polymer support $P3_{(6)}$.

Entry	Conjugate structure	M_p [g·mol^{-1}]	ΔM_p [g·mol^{-1}]	M_w/M_n
1	$P3_{(6)}$	5282		1.21
2	$P3_{(6)}$-*b*-S	4600	-682	1.54
3	$P3_{(6)}$-*b*-SH	5625	+1025	1.37
4	$P3_{(6)}$-*b*-SHS	5559	-66	1.73
5	$P3_{(6)}$-*b*-SHSH	5845	+286	1.29

In line with this context, iterative synthesis of the oligoamide has been extended to a six building blocks structure. As for the synthesis of tetraamide, the hexaamide preparation has

been performed on a Br-free non-cleavable ARGET ATRP support $P3_{(7)}$. SEC representation of these stepwise couplings is presented in **Figure 89** and **Table 11**.

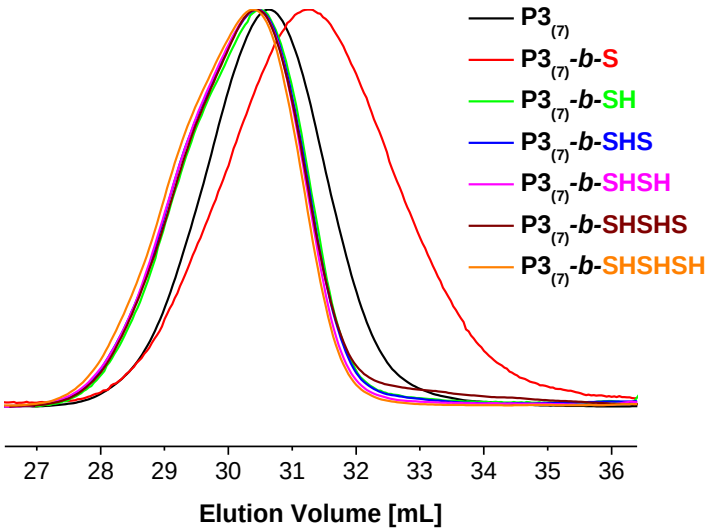


Figure 89. SEC chromatograms recorded in THF for the iterative growth of a hexaamide on a Br-free non-cleavable ARGET ATRP support $P3_{(7)}$. The polystyrene support $P3_{(7)}$ is represented by a black curve and the resulting polymer-hexaamide conjugate by an orange line. The rest of the colored traces show intermediate situations recorded after each diacid/diamine coupling.

Table 11. Molecular weight and molecular weight distribution values recorded by SEC for the sequential assembly of a hexaamide on the polymer support $P3_{(7)}$.

Entry	Conjugate structure	M_p [g·mol^{-1}]	ΔM_p [g·mol^{-1}]	M_w/M_n
1	$P3_{(7)}$	6163		1.37
2	$P3_{(7)}$-*b*-S	4273	-1890	1.83
3	$P3_{(7)}$-*b*-SH	6593	+2320	1.4
4	$P3_{(7)}$-*b*-SHS	6784	+191	1.32
5	$P3_{(7)}$-*b*-SHSH	6740	-44	1.31
6	$P3_{(7)}$-*b*-SHSHS	6786	+46	1.77
7	$P3_{(7)}$-*b*-SHSHSH	6931	145	1.31

In analogy to the previously described synthesis on the P3 Br-free support, attachment of the first succinic anhydride unit resulted in a visible shift of the chromatogram into smaller

molecular weight values. Broad molecular weight distribution attributed to this reaction (M_w/M_n = 1.83) has been diminished in the next steps to reach the value close to the one of the initial support. The molecular weight value of the final product (ΔM = 768 g·mol^{-1}) has been found close to the expected value (ΔM = 613 g·mol^{-1}). However, analysis of the single coupling steps revealed inconsistency in the hexaamide growth. Indeed, the detailed summary of this stepwise growth displayed in **Table 11** prevented determination of a trend that could clearly state a progress of the reaction. The overall yield of polyamide assembly reached in this case 67%.

3. CHARACTERIZATION OF THE POLYAMIDE STRUCTURES

In order to fully assess quality of the oligoamide synthesis, the hexaamide was cleaved from polystyrene support $P6_{(8)}$ and analyzed by MALDI-TOF and ^{1}H NMR techniques. While the multiplicity of signals obtained by mass spectrometry caused difficulties in interpretation, the attribution of the oligoamide signals given by NMR has been relatively less complex. **Figure 90** displays the aforementioned spectrum.

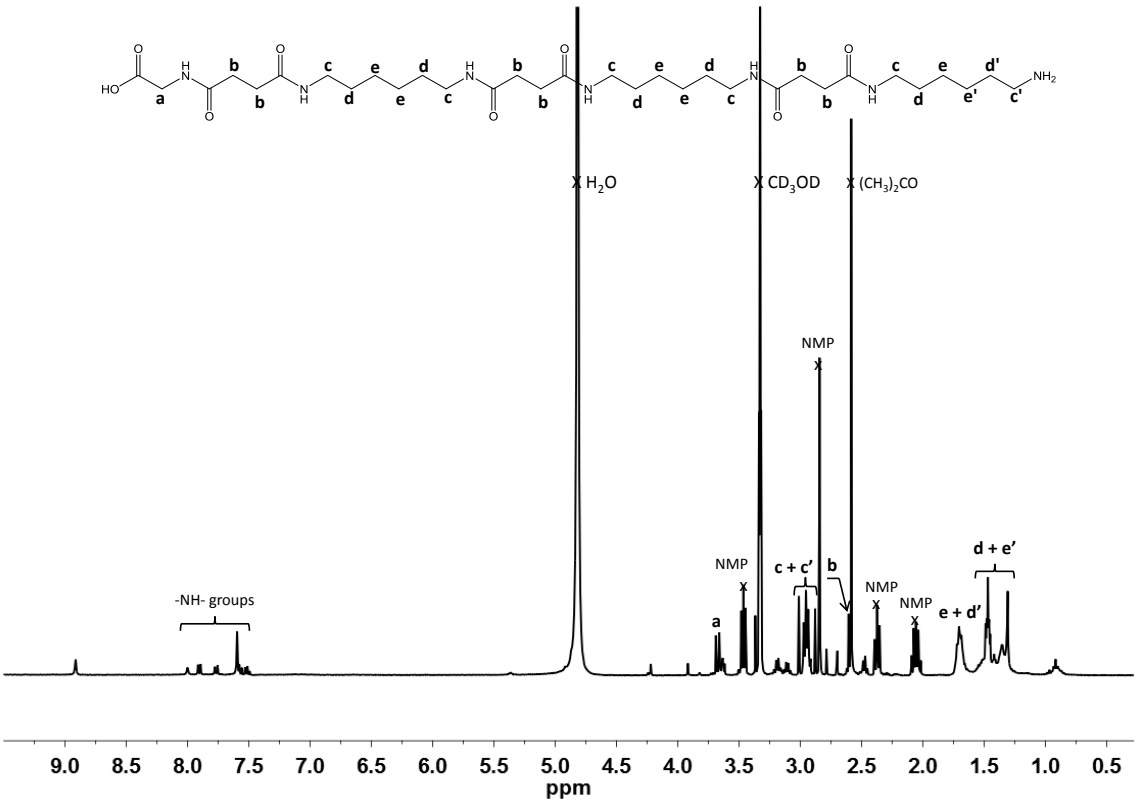


Figure 90. ^{1}H NMR spectra recorded in deuterated methanol (CD_3OD) for the hexaamide synthesized on polymer support P6.

Chemical structure of the target oligoamide has been evidenced in **Figure 90**. Individual signals, such as the methylene groups of succinic anhydride and hexamethylenediamine units were assigned at 2.5 ppm (**b**), 2.87-3.0 ppm (**c + c'**), 1.2-1.57 (**d + e'**) and 1.69 ppm (**e + d'**). Moreover, the group of peaks in the region of 7.5-8.0 ppm was identified as the protons of the primary amines. Still, the interpretation of the signals remains difficult due to the presence of solvent residual peaks.

In order to deeply understand the processes occurring during iterative growth of the oligoamide on polymer soluble support, the $P6_{(8)}$-*b*-SHSHSH conjugate was subjected to several physico-chemical analyses. For instance, to validate the presence of aggregates, dynamic light scattering measurement was carried out. Prior to the DLS measurements, a thermogravimetric analysis has been performed in order to indicate the maximum temperature for conducting the DLS. As presented in **Figure 91**, decomposition of the block copolymer initiated around 185° C.

Relatively high thermal stability of the sample is a consequence of the block copolymer composition containing an amorphous polystyrene part (T_{TGA} = 163° C),[247] and a crystalline analog of nylon 6.6 segment (T_{TGA} = 210° C).[248]

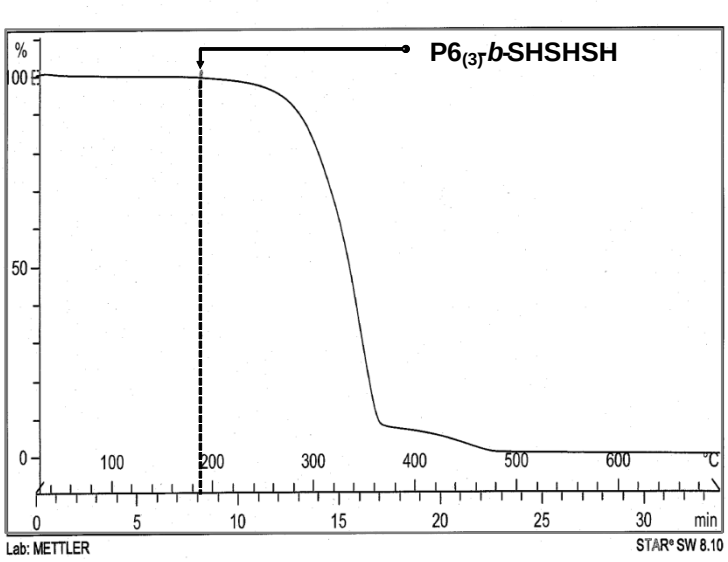


Figure 91. `Thermogravimetric analysis of $P6_{(8)}$-*b*-SHSHS conjugate decomposition.

Analysis of the dynamic light scattering data indicated that the polymer-oligoamide system forms aggregates of an average 25 nm radius (**Figure 92)**. The measurement was performed at two temperatures: 22 °C and 30 °C in 1,2,4-trichlorobenzene (1,2,4-TCR) that was found to be the best solvent for this system.[249] Based on the refractive index of 1,2,4-TCR and its

viscosity at 22 °C and 30 °C, an analysis conducted with CONTIN algorithm[250] has determined the presence of aggregates with an average radius values of 26.9 ± 0.4 nm and 27.5 ± 0.7 nm, at 22 °C and 30 °C, respectively. In addition, the CONTIN analysis has revealed uniform size distribution of the aggregates.

The presence of crystallizable hexaamide segment in the PS-*b*-PA conjugate has encouraged us to foresee formation of a higher morphological organization, such as the ribbon-like structures reported by Atkins co-workers.[251] An accurate characterization of the crystallization behavior and crystalline morphology of the block copolymer was carried out with transmission electron microscopy. The micrographs of PS-*b*-PA copolymers are visualized in **Figure 94**. The structures observed by TEM are strongly dependent on the applied crystallization procedure. Differences in the images are in a relation with the solvent evaporation process. The micrographs presented in **Figure 91** were prepared by observation of sample's edges affected by faster evaporation of the solvent. The synthesized PS-*b*-PA block copolymer appeared on the micrographs in a random arrangement.

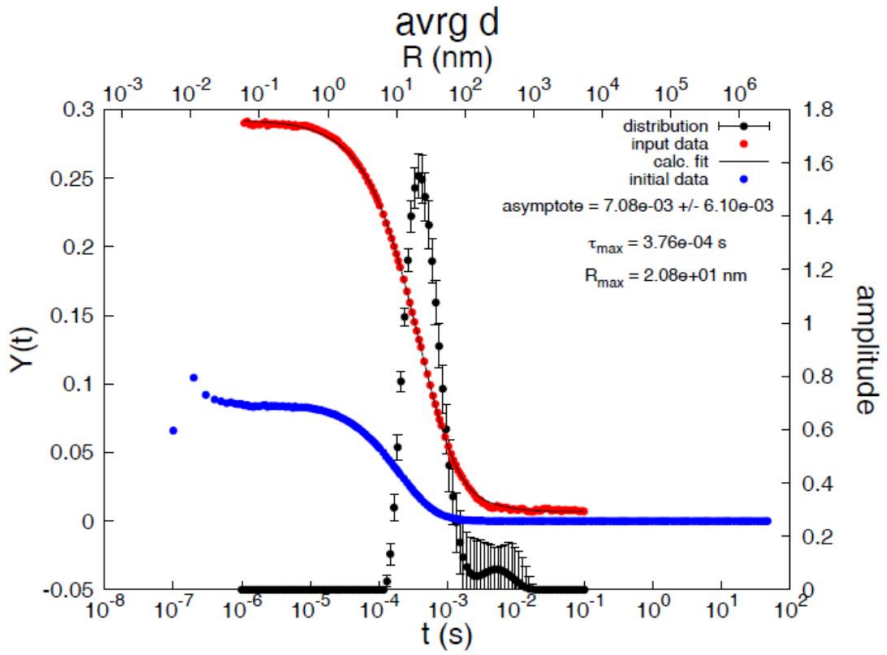


Figure 93. Composite plot of the polystyrene-hexaamide conjugate chains distribution in trichlorobenzene solution calculated by CONTIN algorithm. Correlation of the values used for analysis by CONTIN (red line) from the initial values (blue line) resulted in the black curve indicating an average size of the studied block copolymer system, *e.g.* 50 nm.

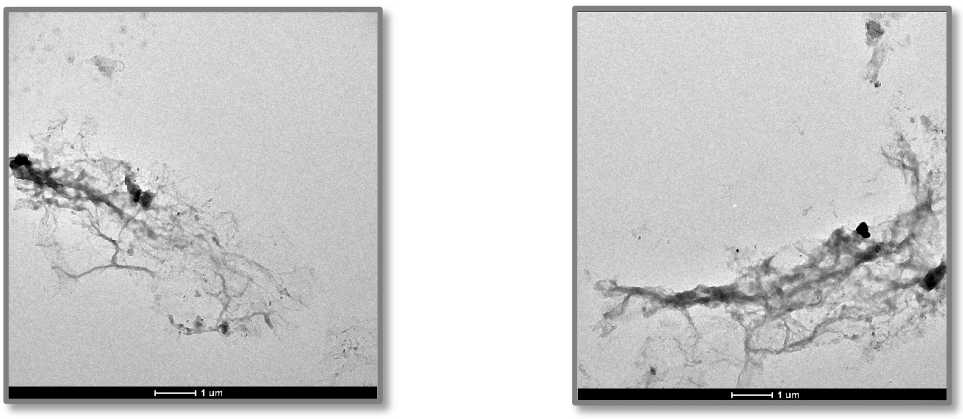


Figure 94. TEM micrographs of PS-*b*-hexaamide block copolymers containing about 10% wt. hexaamide.

4. CONCLUSIONS

Polyamide synthesis using soluble supports in the absence of protecting groups seems to be not entirely a valid pathway for the preparation of monodisperse functional PS-*b*-PA block copolymers. Moderately well-defined cleavable or non-cleavable polystyrene supports with polydispersity in the range of 1.1-1.5 were prepared by atom transfer radical polymerization or activator regenerated by electron transfer ATRP. Replacement of the ATRP technique with the recently developed ARGET ATRP polymerization method allowed minimization of bromide content in the final polymer in parts per million (ppm).[252] Despite the marginal quantity of active bromide, stepwise reactions of non-protected diacids and diamines were still found to be affected. In response to it, an experimental protocol of terminal bromide removal has been employed (paragraph **2.2**). However, SEC measurements highlighted defects in the synthesis of the PS-*b*-S conjugate. This phenomenon was related to the terminal bromide elimination occurring during ARGET ATRP. Impaired quality of the ARGET ATRP support in comparison to earlier examined ATRP support has been caused by partial removal of the Fmoc chain-end protecting group (imposed by utilization of basic Me_6TREN ligand) that led to *in situ* intermolecular reactions between the α and ω chain-ends of the polymers.

Furthermore, the liquid-phase methodology employed in the present study allowed characterization of intermediate conjugates by NMR and SEC. Defects observed for the iterative growth of polyamides have permit to conclude that good solubility and an appropriate concentration of the polymer in the reaction mixture are the principal requirements to avoid formation of interchain hydrogen bonds.

However, strong intermolecular interactions could not be fully eliminated. In consequence, we have focused on investigation of the PS-*b*-PA block copolymer microstructure. Characterizations were carried out by thermogravimetry, dynamic light scattering and transmission electron microscopy. These measurements revealed that no crystalline structure was formed. Nonetheless, the synthesized conjugates have tendency to form aggregates. Yet, the formation of relatively stable semi-crystalline networks can be envisaged assuming a well-defined primary structure of the block copolymers containing elongated polyamide crystalline segments.

SUMMARY

The iterative approach has proved to be a powerful and convenient tool for the preparation of sequence-controlled polymers. This procedure has been used in the synthesis of oligopeptides, oligo(amine amide)s and oligoamides. The iterative synthesis protocol has been realized using solid and soluble polymer supports. The solid-phase synthesis has been performed on a commercially available Wang resin preloaded with glycine. The liquid-phase synthesis has been attained using tailor-made polystyrene and poly(ethylene glycol) supports. The well-defined soluble supports were prepared *via* controlled radical polymerization techniques, in particular ATRP and ARGET ATRP. The near-monodisperse supports of desired molecular weight have been imperative to the development and understanding of the conjugated oligomers.

For the preparation of oligomers, three iterative approaches have been targeted in this work: *(i)* AB + AB, *(ii)* AB + CD and *(iii)* AA + BB (the letter code represents general arrangement of the monomers' reactive functions). The approach *(i)* has been described in **Chapter II**. The oligomer composed of repetitive amino acids was obtained *via* protective-group coupling strategy. The monomers containing temporarily deactivated carboxylic acid and amine functions were coupled on the soluble support to yield well-defined polystyrene-*b*-oligopeptide block copolymer. In parallel, a control strategy has been applied on the solid support. The regular SEC progress in the stepwise monomer couplings along with the expected molecular weight of the formed oligopeptide confirmed an ordered structure of the targeted oligomer. The liquid-phase strategy has been thus presented as an interesting alternative to the commonly used solid-phase approach in the synthesis of biopolymers. Moreover, it has been shown that the PEGylation process conducted in homogeneous reaction conditions using the polymer-supported peptide is remarkably more efficient in comparison with the available solid-phase protocols.

The synthesis of sequence-regulated oligomers containing ABCD repetitive segments has been demonstrated in **Chapter III**. In this approach, the ordered oligomer was obtained *via* stepwise couplings of bromoanhydride and nitroxide radical building blocks. These chemoselective reactions permitted to work in the absence of the

protecting groups. The oligo(alkoxyamine amide)s of various structures have been prepared following the solid- and liquid-phase methodologies. The latter proved to be especially efficient for the synthesis of longer oligomeric structures containing up to 24 units. Alternatively, the solid-phase approach has been utile in comparison of the short sequences prepared from the library of bromoanhydride and nitroxide radical monomers. In addition, due to the presence of reversible nitroxide bonds, the well-defined oligo(alkoxyamine amide)s were shown to possess a unique ability of structural reorganization. The proof of concept has been provided by SEC analysis of a heated nonamer.

At last, we have investigated a liquid-phase strategy to attain the sequence-ordered polymers of the AABB pattern. The targeted oligoamides were prepared in a protection-group free approach. This method described in **Chapter IV** has been developed complimentary to the available solid-phase protocols. However, it turned out that complete control over comonomer sequences is difficult to achieve *via* this liquid-phase protection-group free approach. The absence of protecting groups has been probably a reason for the formation of aggregates as confirmed by DLS.

In larger context, the studies on the sequence-controlled polymers accomplished in this thesis have shown that the iterative synthesis approach is a convenient way for the preparation of precisely ordered macromolecules of different chemical nature. Beside the commonly used solid-phase methodology, we have introduced a strategy based on the tailor-made polymer soluble supports that allowed conducting the reaction in homogeneous conditions. This unconventional method led to preparation of well-defined block copolymers consisting of a monodisperse soluble polymer part and a monomer-regulated short chain oligomer. However, the application of this iterative approach in technology would require several improvements. First of all, the polymers of higher molecular weight should be achieved without the loss of control over their polydispersity. Secondary, the method should be optimized for yield, including automation of the liquid-phase methodology. Ultimately, the secondary and tertiary interactions of the targeted polymers should be investigated to attain the highest level of molecular complexity.

The exploration of methods to control monomer sequences will continue to be important, especially as sequence-specific polymers gain increased attention due to

their potential applications. Our work in this field provides a valuable basis for the design of sequence-defined materials with well-controlled primary structures.

Experimental part

1. CHEMICALS

Compounds and solvents placed on the lists below were used as received.

Compound	**Supplier**	**Purity [%]**
5-(Fmoc-amino)-1-pentanol	Aldrich	98
3-(Fmoc-amino)-1-propanol	Iris Biotech	98
2-Bromoisobutyrylbromide	Alfa Aesar	97
4-Dimethylaminopyridine	Acros Organics	99
Methoxypolyethylene glycol amine 5000 (H_2N-PEG-OCH_3); loading ≥0.17 mmol/g NH_2	Fluka	
1-phenylethyl bromide (PEBr)	Alfa Aesar	97
Sodium sulphate anhydrous	SDS	99.6
4,4'-Di-n-nonyl-2,2'-bipyridine	Alfa Aesar	97
Piperidine	Sigma-Alrdich	99
4-Hydroxymethylphenoxyacetic acid	Novabiochem/Merck	97
N-Hydroxysuccinimide	Aldrich	98
N,*N*-Dicyclohexylcarbodiimide (DCC)	Aldrich	99
Fmoc-Gly-OH	Novabiochem/Merck	98
Fmoc-L-Lys(Boc)-OH	Polypeptide Lab	99
Fmoc-L-Lys(Mtt)-OH	Polypeptide Lab	98.5
Fmoc-Tyr(*t*Bu)-OH	Novabiochem/Merck	100
Fmoc-Tyr(2-ClTrt)-OH	Novabiochem/Merck	98
N,*N*-Diisopropylethylamine (DIPEA)	Sigma-Aldrich	>99
Tin (II) 2-ethylhexanoate ($Sn(EH)_2$)	Alfa Aesar	96
Trifluoroacetic acid (TFA)	Sigma-Aldrich	99
Tris(2-dimethylaminoethyl)amine (Me_6TREN)	Alfa Aesar	>99
(Benzotriazol-1-yloxy) tripyrrolidinophospho-nium hexafluorophosphate (PyBOP)	Novabiochem/Merck	99
Copper powder, spherical, 100 mesh	Alfa Aesar	99.5

Copper (II) bromide	Alfa Aesar	99
N,N,N′,N″,N‴-Pentamethyldiethylenetriamine (PMDETA)	Aldrich	97
Triethylamine	Aldrich	99.5
Succinic anhydride	Sigma-Aldrich	>99
Hexamethylenediamine	Aldrich	98
1,1′-Carbonyldiimidazole (CDI)	Aldrich	>99
4-Amino-2,2,6,6-tetramethylpiperidine 1-Oxyl (Amino-TEMPO)	TCI	97
3-Amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (amino-TEMPO)	Acros Organics	99
2-Bromoisobutyric acid	Alfa Aesar	98
Fmoc-Gly-Wang resin, 0.40 - 1.00 mmol/g loading	Novabiochem/Merck	

THF (99%, Aldrich, stabilized with BHT) was dried and distilled over sodium-benzophenone. Copper (I) bromide (Sigma-Aldrich, 98%) was washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried. Styrene (Sigma-Aldrich, 99%) was distilled over calcium hydride under vacuum and stored under argon at -15 °C before use.

Solvent	**Supplier**	**Purity [%]**
Methanol (MeOH)	Carlo Erba	99.9
Dichloromethane (DCM)	Carlo Erba	99.9
DCM anhydrous	Sigma-Aldrich	99.8, with amylene
Diethyl ether	Sigma-Aldrich	>99
Dimethylformamide (DMF)	Sigma-Aldrich	>99
DMF anhydrous	Alfa Aesar	>99
Tetrahydrofuran for SEC (THF)	Sigma Aldrich	99.8, HPLC, stabilizer-free
N-Methyl-2-pyrrolidone for SEC (NMP)	Sigma Aldrich	≥99, CHROMASOLV® Plus, HPLC

2. EXPERIMENTAL PROTOCOLS

2.1. SYNTHESIS OF POLYMERIZATION INITIATORS

2.1.1. SYNTHESIS OF INITIATOR 1

2.5 g of 3-(Fmoc-amino)-1-propanol (8.2 mmol, 1 Eq.) and 0.2 g of DMAP (1.65 mmol, 0.2 Eq.) were dissolved in 100 mL dry THF in a 250 mL-two neck round bottom flask and cooled down to 0 °C. 2.87 mL of triethylamine (20.6 mmol, 2.5 Eq.) was then added to the mixture. Subsequently, 2.55 mL of 2-bromoisobutyrylbromide (20.6 mmol, 2.5 Eq.) was added dropwise to the solution. The experimental mixture was slowly allowed to warm up to room temperature and stirred for 22h. After reaction, triethylamine hydrobromide was removed by filtration and the solvent was dried by rotary evaporation at 40 °C. The crude reaction mixture was mixed with water (100 mL, sodium chloride saturated) and extracted with dichloromethane (100 mL and 2 x 50 mL). The organic phases were combined, washed with water (20 mL), dried over anhydrous Na_2SO_4, and concentrated on rotavapor. The product was purified by column chromatography using n-hexane/ethyl acetate (volume ratio 4:1) as eluent. Yield: 84%. ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 1.96 (t, 2H, NH-CH_2-$\underline{CH_2}$-CH_2-O), 1.96 (s, 6H, CH_3), 3.32 (m, 2H, $\underline{CH_2}$-NH), 4.26 (m, 2H, CH_2-$\underline{CH_2}$-O), 4.41 (d, 2H, CH (cyclopentane)-$\underline{CH_2}$-O), 5.05 (broad s, 1H, Fmoc (cyclopentane)-CH_2-O), 7.32 (t, 2H, 2H aromatic Fmoc I) 7.40 (t, 2H, 2H aromatic Fmoc II) 7.59 (d, 2H, aromatic Fmoc III), 7.76 (d, 2H, aromatic Fmoc IV). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) = 30.7 (1C, NH-CH_2-$\underline{CH_2}$-CH_2-O), 32.5 (2C, CH_3), 38.0 (1C, $\underline{CH_2}$-NH), 47.3 (1C, Fmoc-CH_2-O), 55.8 (1C, $\underline{C}$-(CH_3)Br), 63.7 (1C, CH_2-$\underline{CH_2}$-O), 66.6 (1C, Fmoc-$\underline{CH_2}$-O), 120.0 (2C, aromatic Fmoc I), 124.8, 127.0, 127.7 (6C, aromatic Fmoc II-IV), 141.3, 144.0 (4C, Fmoc cyclopentane), 156.4 (1C, CO-NH), 171.7 (1C, CO-C-(CH_3)Br).

2.1.2. SYNTHESIS OF INITIATOR 2

2.5 g of 5-(Fmoc-amino)-1-pentanol (7.7 mmol, 1Eq.) was dissolved in 100 mL of dry THF in a 250 mL-two neck round bottom flask and cooled down to 0 °C. 3.21 mL of triethylamine (23 mmol, 3 Eq.) was then added to the mixture. Subsequently, 2.85 mL of 2-bromoisobutyrylbromide (23 mmol, 3 Eq.) was added dropwise to the solution. The experimental mixture was slowly allowed to warm up to room temperature and stirred for 20h. After reaction, the solution was filtered to remove the triethylamine hydrobromide and concentrated by rotary evaporation at 40 °C. The crude reaction mixture was mixed with

water (100 mL, sodium chloride saturated) and extracted with dichloromethane (1 x 100 mL and 2 x 50 mL). The organic phases were combined, washed with water (20 mL), dried over Na_2SO_4, and concentrated on rotavapor. The product was purified by column chromatography using *n*-hexane/ethyl acetate (volume ratio 4:1) as eluent. Yield: 44%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.45 (m, 2H, NH-CH_2-CH_2-$\underline{CH_2}$-CH_2-CH_2-O), 1.59 (m, 2H, NH-CH_2-$\underline{CH_2}$-CH_2-CH_2-CH_2-O), 1.71 (m, 2H, NH-CH_2-CH_2-CH_2-$\underline{CH_2}$-CH_2-O), 1.93 (s, 6H, CH_3), 3.20 (m, 2H, $\underline{CH_2}$-NH), 4.18 (t, 2H, CH_2-$\underline{CH_2}$-O), 4.40 (m, 2H, CH (cyclopentane)-$\underline{CH_2}$-O), 4.80 (broad s, 1H, Fmoc (cyclopentane)-CH_2-O), 7.31 (t, 2H, 2H aromatic Fmoc I) 7.40 (t, 2H, 2H aromatic Fmoc II) 7.58 (d, 2H, aromatic Fmoc III), 7.75 (d, 2H, aromatic Fmoc IV). ^{13}C NMR (400 MHz, $CDCl_3$): δ(ppm) = 28.0 (1C, NH-CH_2-CH_2-$\underline{CH_2}$-CH_2-CH_2-O), 29.5 (1C, NH-CH_2-$\underline{CH_2}$-CH_2-CH_2-CH_2-O), 30.7 (1C, NH-CH_2-CH_2-CH_2-$\underline{CH_2}$-CH_2-O). 32.3 (2C, CH_3), 40.2 (1C, $\underline{CH_2}$-NH), 47.3 (1C, Fmoc-CH_2-O), 56.0 (1C, $\underline{C}$-(CH_3)Br), 65.7 (1C, CH_2-$\underline{CH_2}$-O), 66.5 (1C, Fmoc-$\underline{CH_2}$-O), 120.0 (2C, aromatic Fmoc I), 125.0, 127.0, 127.7 (6C, aromatic Fmoc II-IV), 141.3, 144.0 (4C, Fmoc cyclopentane), 156.4 (1C, CO-NH), 171.7 (1C, CO-C-(CH_3)Br).

2.2. SYNTHESIS OF POLYMERIC SUPPORTS

2.2.1. **P1** (ATRP)

The present example corresponds to the synthesis of polymeric support $P1_{(1)}$ and can be understood as a general procedure for the synthesis of polymeric support of the P1-type.

Initiator 1 (1 g, 1 Eq.), copper (I) bromide (0.34 g, 1 Eq.) and 4,4'-Di-n-nonyl-2,2'-bipyridine (1.95 g, 2 Eq.) were added into a 25 mL round bottom flask equipped with a magnetic stirrer and sealed with a rubber septum. The mixture was degassed and purged with dry argon for a few minutes. Then, degassed styrene (10.9 mL, 40 Eq.) was added using a degassed syringe and the flask was introduced into an oil bath thermostated at 110 °C. After 5h and 15 min of reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The purified polymer $P1_{(1)}$ was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (conv. = 82%, M_n = 4095 g·mol^{-1}, M_w/M_n = 1.16).

Characterization of the successive P1 polymers can be found in **Appendix 1**.

2.2.2. **P2** (ATRP)

Initiator 2 (0.2 g, 1 Eq.), copper (I) bromide (0.063 g, 1 Eq.) and 4,4'-di-*n*-nonyl-2,2'-bipyridine (0.36 g, 2 Eq.) were added into a 25 mL round bottom flask equipped with a magnetic stirrer and sealed with a rubber septum. The mixture was degassed and purged with dry argon for few minutes. Then, degassed styrene (2 mL, 40 Eq.) was added using a degassed syringe and the flask was introduced into an oil bath thermostated at 110 °C. After 6h of reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The purified polymer was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (conv. = 94%, M_n = 4390 g·mol^{-1}, M_w/M_n = 1.18).

2.2.3. **P3** (ARGET ATRP)

The present example corresponds to the synthesis of polymeric support $P3_{(1)}$ and can be understood as a general procedure for the synthesis of polymeric support of the P3-type. Variation of the experimental conditions introduced for $P3_{(2)}$-$P3_{(5)}$ can be found in **Table 2**.

Initiator 1 (146 mg, 1 Eq.), copper (I) bromide (0.235 mg, 0.005 Eq.), tris(2-dimethylaminoethyl)amine (8.7 μL, 0.1 Eq.) and tin (II) 2-ethylhexanoate (13 mg, 0.1 Eq.) were added into a 25 mL round bottom flask equipped with a magnetic stirrer and sealed with a rubber septum. The mixture was degassed and purged with dry argon for a few minutes. Then, degassed styrene (1.5 mL, 40 Eq.) was added using a degassed syringe and the flask was introduced into an oil bath thermostated at 110 °C. After 5h of reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The purified polymer $P3_{(1)}$ was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (conv. = 86%, M_n = 5082 g·mol^{-1}, M_w/M_n = 1.18).

Characterization of the successive $P3_{(6)}$-$P3_{(7)}$ polymers synthesized in accordance with the conditions for $P3_{(1)}$ can be found in **Appendix 1**.

2.2.4. **P4** (SET-LRP)

Initiator 1 (97 mg, 1 Eq.) copper powder (3.5 mg, 0.25 Eq.), copper (II) bromide (4.9 mg, 0.1 Eq.) and PMDETA (13 mg, 0.35 Eq.) were added into a 25 mL round bottom flask equipped with a magnetic stirrer and sealed with a rubber septum. The mixture was degassed and purged with dry argon for few minutes. Then, degassed styrene (1 mL, 40 Eq.) was added

using a degassed syringe and the flask was introduced into an oil bath thermostated at 90 °C. After 2.5h of reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The purified polymer was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (conv. = 62%, M_n = 6227 g·mol^{-1}, M_w/M_n = 1.28).

2.2.5. **P5** (WANG LINKER ATTACHMENT)

The present example corresponds to the synthesis of polymeric support $P5_{(1)}$ and can be understood as a general procedure for the synthesis of polymeric support of the P5-type.

2.2.5.1. DEPROTECTION OF FMOC GROUP

Polymer $P1_{(1)}$ (8.8 g) was dissolved in a mixture of piperidine/DCM (1/1, 20 mL) and the solution was stirred for 1.5h at RT. After the reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The deprotected polymer was characterized by ^{1}H NMR in $CDCl_3$.

2.2.5.2. ATTACHMENT OF A WANG LINKER

Deprotected polymer $P1_{(1)}$ (7.5 g, 1 Eq.), 4-hydroxymethylphenoxyacetic acid (0.5 g, 1.5 Eq.), *N*-hydroxy succinimide (0.25 g, 1.2 Eq.), dicyclohexylcarbodiimide (0.42 g, 1.1 Eq.), and dry THF (100 mL) were added into a 250 mL round bottom flask. The mixture was stirred for 21h at RT. After reaction, the mixture was filtrated and the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in vacuum oven at room temperature overnight. The purified polymer $P5_{(1)}$ was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (M_n = 4127 g·mol^{-1}, M_w/M_n = 1.13).

Characterization of the successive P5 polymers can be found in **Appendix 1**.

2.2.6. **P6** (GLYCINE LOADING)

The present example corresponds to the synthesis of polymeric support $P6_{(1)}$ and can be understood as a general procedure for the synthesis of polymeric support of the P6-type.

Fmoc-Glycine (1 g, 10 Eq.) was dissolved in 10 mL dry DCM (several drops of DMF were added in order to complete dissolution). DCC (0.34 g, 5 Eq.) was then added to the solution and the mixture was stirred for 15 min at 0 °C. Next, $P5_{(1)}$ (1.36 g, 1 Eq.) and DMAP (0.004

g, 0.1 Eq.) were added and the reaction mixture was stirred for 1h at RT. After reaction, dicyclohexylurea was removed by filtration and the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The polymer support loaded with glycine was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (M_n = 4471 g·mol^{-1}, M_w/M_n = 1.10).

Characterization of the successive P6 polymers can be found in **Appendix 1**.

2.2.7. **P7** (ATRP)

MBP (0.06 mL, 1 Eq.), copper (I) bromide (0.038 g, 0.5 Eq.) and PMDETA (0.05 mL, 0.5 Eq.) were added into a 25 mL round bottom flask equipped with a magnetic stirrer and sealed with a rubber septum. The mixture was degassed and purged with dry argon for few minutes. Then, degassed styrene (3 mL, 50 Eq.) was added using a degassed syringe and the flask was introduced into an oil bath thermostated at 90 °C. After 4 hours of reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The purified polymer was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (conv. = 82%, M_n = 4006 g·mol^{-1}, M_w/M_n = 1.11).

2.2.8. **P8** (ATRP)

PEBr (0.35 mL, 1 Eq.), copper (I) bromide (0.37 g, 1 Eq.) and dNbpy (2.09 g, 2 Eq.) were added into a 25 mL round bottom flask equipped with a magnetic stirrer and sealed with a rubber septum. The mixture was degassed and purged with dry argon for few minutes. Then, degassed deuterated styrene (4.4 mL, 15 Eq.) was added using a degassed syringe and the flask was introduced into an oil bath thermostated at 110 °C. After 1h and 45 min. of reaction, the polymer was precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The purified polymer was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (M_n = 1816 g·mol^{-1}, M_w/M_n = 1.07).

2.2.9. **P9** (WANG LINKER ATTACHMENT)

Commercial H_2N-PEG-OCH_3 (1.0 g, 1 Eq.), 4-hydroxymethylphenoxyacetic acid (0.055 g, 1.5 Eq.), *N*-hydroxy succinimide (0.028 g, 1.2 Eq.), dicyclohexylcarbodiimide (0.045 g, 1.1 Eq.), and the mixture of anhydrous DCM (8.3 mL) and anhydrous DMF (0.7 mL) were added

into a 25 mL round bottom flask. The mixture was stirred for 21h at RT. After reaction, the mixture was filtrated and the polymer was precipitated in diethyl ether. The precipitate was collected by filtration, washed with diethyl ether and dried in vacuum oven at room temperature overnight. The purified polymer P9 was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (M_n = 5127 g·mol^{-1}, M_w/M_n = 1.11).

2.3. ITERATIVE COUPLING OF PEPTIDES

2.3.1. LIQUID-PHASE PEPTIDE COUPLING

The present example corresponds to coupling of Fmoc-Lys(Mtt)-OH to the polymer support loaded with glycine (P6) and can be understood as a general procedure for peptide coupling.

The Fmoc-protected P6 polymer (0.975 g, 1 Eq.) was dissolved in 4 mL of piperidine/DCM (1/1) and the mixture was stirred for 15 min at RT. The deprotected polymer was then precipitated in methanol. The precipitate was collected by filtration, washed with methanol and dried in vacuum oven at room temperature for 2 h. Next, the deprotected polymer, Fmoc-Lys(Mtt)-OH (0.681 g, 5 Eq.), PyBOP (0.556 g, 4.9 Eq.) and DIPEA (0.38 mL, 10 Eq.) were dissolved in DCM (several drops of DMF were needed in order to complete dissolution) and the mixture was stirred for 2h at room temperature. The polymer was then obtained following the same procedure as after Fmoc deprotection. The polymer was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF after each step of new amino acid coupling.

2.3.1 CLEAVAGE OF THE PEPTIDE FROM THE CLEAVABLE SUPPORT

0.1 g of PS-*b*-Gly-Lys(Mtt)-Tyr(2-ClTrt)-Gly-Lys(Mtt)-Tyr(2-ClTrt)-OH was treated with 1 mL of TFA/CH_2Cl_2 mixture (1/1) for 4h at RT. After reaction, the polymer support was isolated by selective precipitation in methanol. The precipitate was collected by filtration and the remained solution was then removed by rotavapor, giving the peptide as a result. The polymer support was characterized by ^{1}H NMR in $CDCl_3$ and SEC in THF (M_n = 4785 g·mol^{-1}, M_w/M_n = 1.28), and the formation of the desired peptide sequence was determined by ESI-MS (exact mass of the peptide: 714.3701 g·mol^{-1}; m/z found: $[M-H]^-$ = 713.383 g·mol^{-1}).

2.3.2. SELECTIVE DEPROTECTION OF THE SIDE GROUPS

0.1 g of PS-*b*-peptide containing a cleavable linker was treated with 1 mL TFA/CH_2Cl_2 mixture (3/97) for 0.5h at RT. In parallel, 0.1 g PS-*b*-peptide containing a non-cleavable linker

was treated with 1 mL TFA/CH_2Cl_2 mixture (1/1) for 4h at RT. After the reactions, the deprotected PS-*b*-peptide copolymers were isolated by selective precipitation in methanol. The precipitates were collected by filtration, washed with methanol and dried in a vacuum oven at room temperature overnight. The deprotected copolymers were characterized by ^{1}H NMR in $CDCl_3$ and SEC in NMP (PS-*b*-peptide containing a cleavable linker: M_n = 5043 g·mol^{-1}, M_w/M_n = 1.16) and in THF (PS-*b*-peptide containing a non-cleavable linker: M_n = 5443 g·mol^{-1}, M_w/M_n = 1.22).

2.3.3. SOLID-PHASE AUTOMATED SYNTHESIS OF THE CONTROL PEPTIDE GKYGKY

The peptide synthesis was performed on an Applied Biosystems ABI 433a peptide synthesizer in a 0.1 mmol scale, using a Gly preloaded Wang-PS resin as solid support. Fmoc-amino acid derivatives were coupled following standard ABI-Fastmoc protocols (single coupling, no capping) in NMP facilitated by HBTU/DIPEA. After final Fmoc removal the resin was washed with NMP and DCM and dried overnight under vacuum at 25 °C. Cleavage of the control peptide from the resin was performed with TFA/water/TES 90:9:1 v/v for 2 h, to obtain the fully deprotected peptide. The peptide was isolated by diethylether precipitation, centrifugation, and lyophilization from water. The obtained product: 153 mg; yield = 85%. Molecular characterization was performed by MALDI-TOF. Exact mass of the peptide: 714.3701 g·mol^{-1}, m/z found: 715.350 $[M+H]^+$ (100%); 737.338 $[M+Na]^+$ (2%); and 772.382 (4%) $[M+tBu+H]^+$.

2.4. PEGYLATION OF POLYMER-BOUNDED PENTAPEPTIDE

0.1 g (1 Eq.) of a Fmoc-deprotected PS-bounded pentapeptide (H_2N-Tyr(tBu)-Lys(Boc)-Tyr(tBu)-Lys(Boc)-Gly-*b*-$P5_{(2)}$) was solved in 1 mL of DCM. Subsequently, 1.15 Eq. of MeO-PEG2k or MeO-PEG5k, 2Eq. of PyBOP and 4 Eq. of DIPEA were added. The mixture was stirred for 5 days at room temperature and progress of the reaction was monitored by SEC analyses of the samples taken in the 3rd, 4th and 5th day of stirring.

2.4.1. CLEAVAGE OF PEGYLATED PEPTIDE

0.1 g of MeO-PEG-Tyr(tBu)-Lys(Boc)-Tyr(tBu)-Lys(Boc)-Gly-*b*-$P5_{(2)}$ conjugate was treated with 1 mL of TFA/CH_2Cl_2 mixture (1/1) for 4h at RT. After reaction, the MeO-PEG-Tyr-Lys-Tyr-Lys-Gly-COOH was isolated by precipitation in methanol and separation from the

precipitated PS. The filtrate was concentrated on rotavapor and analyzed by ^{1}H NMR spectroscopy.

^{1}H NMR of cleaved PEG2k-pentapeptide (400 MHz, DMSO-d_6, δ in ppm): 9.16 (m, 2H, OH Tyr_1, OH Tyr_2), 8.3-6.9 (m, 12H, CONH Tyr_2, CONH Gly, CONH Tyr_1, CONH Lys_1,CONH Lys_2, CONH PEG derivative, $CONH_2$ acid amide, NH_2Lys_1, NH_2Lys_2), 6.63 (m, 8H, $4xCH^{Ar}Tyr_1$, $4xCH^{Ar}Tyr_2$), 4.5-4.0 (m, 3H, CH_3), 3.94-3.42 (m, 202H, CH_2PEG), 3.1-2.7 (m, 10 H, CH_2Gly, $C^{\beta}H_2Tyr_1$, $C^{\beta}H_2Tyr_2$, $C^{\varepsilon}H_2Lys_1$, $C^{\varepsilon}H_2Lys_2$) 1.8-1.11 (m, 12H, $6xC^{\beta}H_2Lys_1$, $C^{\beta}H_2Lys_2$,$C^{\delta}H_2Lys_1$, $C^{\delta}H_2Lys_2$,$C^{\gamma}H_2Lys_1$, $C^{\gamma}H_2Lys_2$). The ratio of peptide vs. PEG was determined by comparison of integral intensities of 4H from $CH^{Ar}Tyr_1$ at 6.63 ppm and 202H of PEG at 3.94-3.42 ppm to obtain a quantitative coupling efficiency.

^{1}H NMR of cleaved PEG5k-pentapeptide (400 MHz, DMSO-d_6, δ in ppm): 9.16 (m, 2H, OH Tyr_1, OH Tyr_2), 8.3-7.5 (m,12H, CONH Tyr_2, CONH Gly, CONH Tyr_1, CONH Lys_1,CONH Lys_2, CONH PEG derivative, $CONH_2$ acid amide, NH_2Lys_1, NH_2Lys_2), 7.5-6.3 (m, 8H, $4xCH^{Ar}Tyr_1$, $4xCH^{Ar}Tyr_2$), 4.5-4.0 (m, 3H, CH_3), 3.9-3.4 (m, 152H, CH_2PEG), 3.1-2.7 (m, 10 H, CH_2Gly, $C^{\beta}H_2Tyr_1$, $C^{\beta}H_2Tyr_2$, $C^{\varepsilon}H_2Lys_1$, $C^{\varepsilon}H_2Lys_2$), 2.0-1.11 (m, 12H, $6xC^{\beta}H_2Lys_1$, $C^{\beta}H_2Lys_2$,$C^{\delta}H_2Lys_1$, $C^{\delta}H_2Lys_2$,$C^{\gamma}H_2Lys_1$, $C^{\gamma}H_2Lys_2$), The ratio of peptide vs. PEG was determined by comparison of integral intensities of 8H from $CH^{Ar}Tyr_{1+2}$ at 7.5-6.3 ppm and 152H of PEG at 3.9-3.4 ppm to obtain a coupling efficiency of 35%.

2.5. ITERATIVE COUPLING OF (ALKOXYAMINE AMIDE)S

2.5.1. SYNTHESIS OF 2-BROMOISOBUTYRYL ANHYDRIDE[253]

2-Bromoisobutyric acid (10.0 g, 59.9 mmol) was dissolved in CH_2Cl_2 (75 mL). *N*,*N*′-dicyclohexylcarbodiimide (6.80 g, 32.9 mmol) was added, and the milky mixture was stirred at ambient temperature overnight. The precipitate was filtered off, and the filtrate was concentrated using rotary evaporation and precipitated into ice cold *n*-pentane. The residue was filtered, washed with cold *n*-pentane, and dried under reduced pressure. The resulting white solid was afforded in 51% yield. ^{1}H NMR ($CDCl_3$): δ 2.00 ppm (s, 12H, CH_3).

2.5.2. SOLID-PHASE ALKOXYAMINE AMIDE COUPLING

The present example corresponds to an alkoxyamine amide nonamer prepared from the alternative coupling of 2-bromoisobutyric anhydride and amino-TEMPO to the Gly-preloaded

Wang resin and can be understood as a general procedure for alkoxyamine amide coupling on the solid support.

2.5.2.1. PREPARATION OF RESIN

0.3 g (0.237 mmol, 1 Eq.) of Gly-preloaded Wang-PS resin (loading 0.79 mmol/g) was used as a solid support and placed in a fritted plastic funnel. Prior to the synthesis, the resin was swollen by gentle shaking in DCM for 0.5h. Next, the Fmoc protecting group of Gly was removed by 2 x 10 min reaction with piperidine/DCM (2 mL/2 mL). Kaiser test made on few resin beads revealed presence of amine groups by coloring the resin in red.

2.5.2.2. LIGATION OF BROMOANHYDRIDE UNIT

Solution of 2-Bromoisobutyric anhydride (0.3745 g, 5 Eq.) and DIPEA (0.95 mL, 23 Eq.) in anhydrous DMF (4 mL) was added to the funnel with resin beads and the mixture was shaked for 50 min on a mechanical shaker. After draining the solution from the filter and washing the beads with DMF (several times), Kaiser test confirmed no presence of amine groups by coloring the resin in yellow.

2.5.2.3. LIGATION OF NITROXIDE RADICAL UNIT

Amino-TEMPO (0.1218 g, 3 Eq.) was dissolved in 5 mL of anhydrous dimethylsulfoxide (DMSO) and placed in the funnel with the bromoanhydride-loaded resin together with CuBr (0.0442 g, 1.3 Eq.) and Me_6TREN (0.07 mL, 1.3 Eq.). The funnel was then sealed with rubber septum and the reaction mixture was purged with Ar for few minutes. Afterwards, the mixture was shaked for 15 min in an inert atmosphere. After draining the solution from the filter and washing the beads with DMF, Kaiser test confirmed presence of amine groups (red color beads).

Subsequent couplings with 2-bromoisobutyric anhydride and amino-TEMPO were repeated in the same way until obtaining a 9-unit alkoxyamine amide.

2.5.2.4. CLEAVAGE OF ALKOXYAMINE AMIDE

Cleavage of the alkoxyamine amide from the resin was performed in TFA/DCM solution (1/1) for 2 h. The nonamer was isolated by cold diethylether precipitation. The obtained product: 51.6 mg from 100 mg of resin; yield = 17%. SEC characterization:

M_n = 1273 g·mol^{-1}, M_p = 1369 g·mol^{-1}, M_w/M_n = 1.03. ^{1}H NMR characterization has been presented in the main part of the manuscript.

2.5.3. LIQUID-PHASE ALKOXYAMINE AMIDE COUPLING

The present example corresponds to a 24-unit alkoxyamine amide prepared from the alternative coupling of 2-bromoisobutyric anhydride and amino-TEMPO to the permanent polymer support P7 and can be understood as a general procedure for alkoxyamine amide coupling on the soluble polystyrene support. The liquid-phase procedure using cleavable PEG soluble support required modification of the solvent system that is detailed in the previous procedures. Attachment of the first unit to P7 occurred in the reaction with nitroxide radical, while attachment of the first unit to P9 occurred in the reaction with bromoanhydride.

2.5.3.1. LIGATION OF NITROXIDE RADICAL UNIT

Polymer P7 (1.8 g, 1 Eq.), amino-TEMPO (0.23 g, 3 Eq.), CuBr (0.084 g, 1.3 Eq.) and Me_6TREN (0.14 mL, 1.3 Eq.) were dissolved in a mixture of dry THF and DMSO (2:1 v/v; for PEG support P9, only DMSO was used) and placed in a round-bottom flask sealed with rubber septum. The solution was then purged with Ar for few minutes and stirred for 15 min. The polymer was precipitated in cold MeOH (for PEG support P9, the polymer was precipitated in cold diethyl ether) and the precipitate was collected by filtration, washed with methanol (or diethyl ether) and dried in a vacuum oven for 2h.

2.5.3.2. LIGATION OF BROMOANHYDRIDE UNIT

The nitroxide-loaded polymer P7 (1.75 g, 1 Eq.) was mixed with 2-bromoisobutyric anhydride (0.69 g, 5 Eq.) in a solution of DIPEA (1.75 mL, 23 Eq.) and anhydrous DCM (4 mL). The mixture was stirred for 50 min and then the polymer was precipitated in MeOH (for PEG support P9, the polymer was precipitated in cold diethyl ether). The precipitate was collected by filtration, washed with methanol (or diethyl ether) and dried in a vacuum oven for 2h.

Subsequent couplings with amino-TEMPO and 2-bromoanhydride were repeated in the same way until the formation of a 24-unit alkoxyamine amide. The resulting polymer conjugate (0.4 g) was characterized by SEC in THF: M_n = 7460 g·mol^{-1}, M_p = 8940 g·mol^{-1} and M_w/M_n = 1.17.

2.5.3.3. CLEAVAGE OF ALKOXYAMINE AMIDE

Cleavage of the alkoxyamine amide was performed on a cleavable PEG support coupled with a pentamer. The cleavage reaction was conducted in TFA/DCM solution (1/1) for 2h. The pentamer was isolated by precipitation in cold diethylether. The final product: 15.4 mg from 100 mg of the polymer conjugate taken for cleavage. SEC chromatogram composed of 3 peaks: M_{p1} = 803 g·mol^{-1} (main peak), M_{p2} = 549 g·mol^{-1} and M_{p3} = 468 g·mol^{-1}.

2.6. ITERATIVE COUPLING OF POLYAMIDES

The present example corresponds to hexamide prepared from the alternative coupling of succinic anhydride and hexamethylenediamine on the polystyrene soluble support $P3_{(7)}$ and can be understood as a general procedure for liquid-phase iterative coupling of polyamides.

2.6.1. LIGATION OF SUCCINIC ANHYDRIDE UNIT

Polymer $P3_{(7)}$ (1.7 g, 1 Eq.) and succinic anhydride (0.31 g, 10 Eq.) were dissolved in the mixture of DCM and NMP (13 mL/3 mL) in a round-bottom flask. Next, DIPEA was added (1.24 mL, 23 Eq.) and the solution was stirred for 1h. After reaction, the polymer was precipitated in MeOH and the precipitate was collected by filtration, washed with methanol and dried in a vacuum oven for 2h.

2.6.2. LIGATION OF HEXAMETHYLENEDIAMINE UNIT

Hexamethylenediamine (1.32 g, 60 Eq.) was dissolved in 1.5 mL of MeOH in a round-bottom flask. Next, 0.135 g of HOBT was added to the flask (0.5 M in MeOH) and the solution was stirred for 1 h. After 1h, the solution was transferred to another flask with a mixture of PS-*b*-S (1 g, 1 Eq.) and CDI (0.82 g, 26.7 Eq.) in DCM (7.5 mL). Simultaneously, few droplets of DMF were added to prevent precipitation of the polymer support in MeOH. The mixture was stirred for 1h. After reaction, the polymer was precipitated in MeOH and the precipitate was collected by filtration, washed with methanol and dried in a vacuum oven for 2h.

Subsequent couplings with succinic anhydride and hexamethylenediamine were repeated in the same way until formation of a hexaamide. The resulting polymer conjugate (0.4 g) was characterized by SEC in THF: M_n = 7539 g·mol^{-1}, M_p = 6931 g·mol^{-1} and M_w/M_n = 1.31.

2.6.3. CLEAVAGE OF OLIGOAMIDE

Cleavage of the oligoamide was performed on a cleavable support $P6_{(8)}$ coupled with a hexamer. The cleavage reaction was conducted in TFA/DCM solution (1/1) for 2h. The hexaamide was isolated by precipitation in cold diethylether. The final product: 6.8 mg from 100 mg of the polymer conjugate. No SEC analysis has been performed due to insolubility of the product in the solvents compatible with the measuring instrument. MALDI-TOF analysis resulted in a wide mass spectrum of separated peaks. Exact mass of the hexaamide: 669.44 $g \cdot mol^{-1}$; m/z found: $[M+H]^+$ = 670.24 $g \cdot mol^{-1}$). ^1H NMR (400 MHz, CD_3OD): δ (ppm) = 1.2-1.55 (m, 12H, NH-CH_2-CH_2-$\underline{CH_2}$-$\underline{CH_2}$-CH_2-CH_2-NH), 1.59-1.7 (broad s, 12H, NH-CH_2-$\underline{CH_2}$-CH_2-CH_2-$\underline{CH_2}$-CH_2-NH), 2.82 (s, 12H, O-$\underline{CH_2}$-$\underline{CH_2}$-O), 2.94 (t, 12H, NH-$\underline{CH_2}$-CH_2-CH_2-CH_2-CH_2-$\underline{CH_2}$-NH), 9.0 (s, 1H, COOH).

3. INSTRUMENTATION

3.1. SIZE EXCLUSION CHROMATOGRAPHY (SEC)

3.1.1. SEC IN THF

Molecular weights and molecular weight distributions were determined using a SEC system equipped with a Shimadzu RiD_10A refractive index detector and five PLgel 10µ Mixed-B columns. The mobile phase was THF with a flow rate of 1 $mL \cdot min^{-1}$ using a Shimadzu LC20AD pump. Toluene was used as internal reference. The molecular weight calibration was based on sixteen narrow molecular weight linear polystyrene standards from Polymer Laboratories.

3.1.2. SEC IN NMP

Molecular weights and molecular weight distributions of deprotected PS-*b*-oligopeptide molecules were determined using a SEC system equipped with viscometer, Refractive index and right angle light scattering (90 °) detector (TDA Viscotek) and three PLgel 10µ Mixed-B columns. The mobile phase was NMP with 0.1M LiBr with a flow rate of 0.5 $mL \cdot min^{-1}$ at 60 °C using a Shimadzu LC20AD pump. Toluene was used as internal reference. The molecular weight calibration was based on fourteen narrow molecular weight linear polystyrene standards from Polymer Laboratories.

3.2. NUCLEAR MAGNETIC RESONANCE (NMR)

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or in CD_3OD on either a Bruker Avance 400 MHz or on a Bruker Avance 600 MHz spectrometers equipped with Ultrashield magnets.

3.3. ELECTROSPRAY MASS SPECTROMETRY (ESI-MS)

ESI-MS was carried out on a Waters Acquity UPLC-SQD apparatus equipped with a PDA detector (190-500 nm, 80Hz), using a reverse phase column (Waters, BEH C18 1.7 mm, 2.1mm x 50 mm) and the MassLynx 4.1 – XP software. The mobile phase was methanol/water with 0.005% ammonia, and a solution of the peptide in methanol was prepared for the analysis.

3.4. MALDI-TOF MASS SPECTROMETRY

Mass measurements were carried out on an Ultraflex^{TM} MALDI-TOF/TOF mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany). This instrument was used at a maximum accelerating potential of 25 kV in positive mode and was operated in mode reflector at 26 kV. The delay extraction was fixed at 110 ns and the frequency of the laser (nitrogen 337 nm) was set at 20 Hz. The acquisition mass range was set to 400-800 m/z with a matrix suppression deflection (cut off) set to 400 m/z. The equipment was first externally calibrated with a standard peptide calibration mixture that contained 7 peptides (Bruker Peptide Calibration Standard #206196, Bruker Daltonics GmbH, Bremen, Germany). Sample preparation was performed with the dried droplet method using a mixture of 0.5 µl of sample with 0.5 µl of matrix solution dry at room temperature. The matrix solution was prepared from a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) in water/acetonitrile 50/50 diluted three times in water/acetonitrile/trifluoroacetic acid 50/49.9/0.1.

3.5. TRANSMISSION ELECTRON MICROSCOPY (TEM)

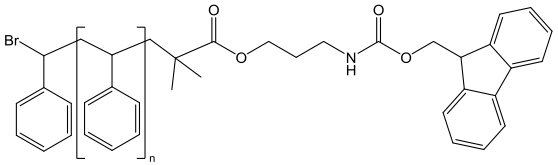
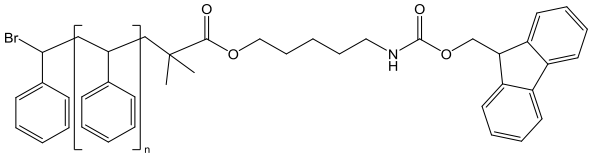
Transmission electron microscopy observations were carried out with Tecnai G2 (FEI) electron microscope and Eagle 2k (FEI) ssCCD camera. The analytical sample was prepared by the deposition of 5 µL of the 0.002 M solution of hexaamide in 1,2,4-trichlorobenzene on a 400 mesh copper grid diameter of 3 mm. The sample was then dried on a filter paper and immobilized on the surface by chemical metallization.

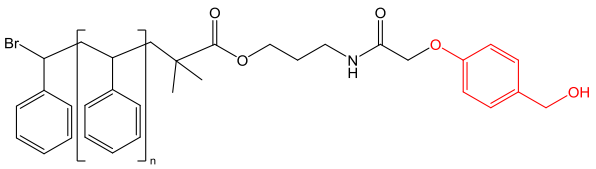
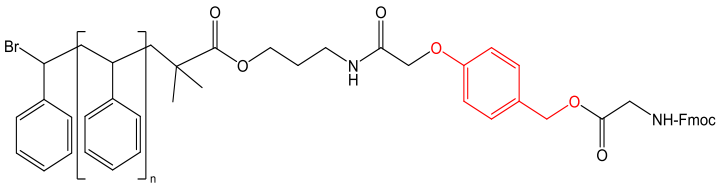
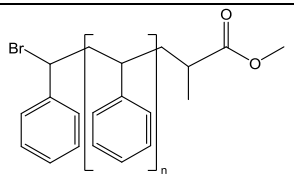
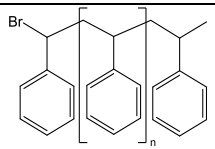
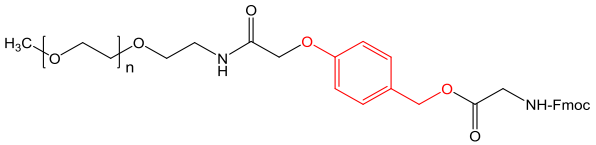
3.6. DYNAMIC LIGHT SCATTERING (DLS)

Quasi-static light scattering was performed using the ALV/DLS/SLS-5020F experimental setup (ALV Laser Vertriebsgesellschaft mbH, Langen, Germany), consisting in a He–Ne laser (22 mW, $\lambda 0$ = 632.8 nm), a compact ALV/CGS-8 goniometer system, and an ALV-5000/E multi-tau correlator. Intensity–intensity correlation functions (ICFs) were measured at a fixed scattering angle θ = 90° and were analyzed with Contin software. Temperature was regulated to 22 and 30 °C. Samples were filtered through 0.45 μm Millipore filters.

3.7. THERMOGRAVIMETRIC ANALYSIS (TGA)

The thermogravimetric analysis was carried out on Metler TC10A/TC15 TA controller equipped with Metler TG50 thermobalance. A sample of 10 mg was weighted and heated from 25 to 700 °C at a rate of 10 °C/min in order to determine the decomposition temperature.

Abbreviation	Polymer structure	Polymerization technique	Batch	M_n [g·mol^{-1}]	M_p [g·mol^{-1}]	M_w/M_n
P1		ATRP	1	4095	4419	1.16
			2	6141	7630	1.24
			3	4504	4512	1.12
			4	3157	3430	1.17
P3		ARGET ATRP	6	4890	5282	1.21
			7	5267	6163	1.37
			8	4262	7391	1.82
P4		SET-LRP	1	6227	6210	1.28
P2		ATRP	1	4390	4842	1.18

P5		ATRP	1 2 4	4100 7209 3933	4538 7635 3991	1.11 1.14 1.15
		ARGET ATRP	8	6446	8794	1.50
P6		ATRP	1 2 4	4471 7208 3773	4920 7932 3795	1.10 1.11 1.12
		ARGET ATRP	8	6760	8943	1.49
P7		ATRP	1	4006	4254	1.11
P8		ATRP	1	1816	1896	1.07
P9		Commercial sample prepared by living anionic polymerization	1	5127	5070	1.11

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