

# Peptide-polymer conjugates: divergent synthesis from the initiating peptides

Valentin Puchelle

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Valentin Puchelle. Peptide-polymer conjugates : divergent synthesis from the initiating peptides. Polymers. Sorbonne Université, 2020. English. NNT : 2020SORUS472 . tel-04028228

### HAL Id: tel-04028228 https://theses.hal.science/tel-04028228

Submitted on 14 Mar 2023  $\,$ 

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# Sorbonne Université

Ecole doctorale 397 – Physique et Chimie des Matériaux Institut Parisien de Chimie Moléculaire (IPCM – UMR 8232) / Équipe Chimie des Polymères

# Peptide-polymer conjugates: divergent synthesis from the initiating peptides

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Thèse de doctorat de Physique et Chimie des Matériaux

Dirigée par Philippe Guégan et co-encadrée par Nicolas Illy

Présentée et soutenue publiquement le 29 septembre 2020

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« L'un m'anime le cœur, l'autre retient mon bras. »

Le Cid P. Corneille

# Remerciements

Ce projet de doctorat a été réalisé à l'Institut Parisien de Chimie Moléculaire (IPCM), au sein de l'équipe Chimie des Polymères dirigée par le Dr. Laurent Bouteiller.

Je tiens à remercier Laurent Bouteiller qui m'a, en premier lieu, permis d'effectuer mon stage de Master 2 au sein de son laboratoire avant d'intégrer officiellement l'équipe Chimie des Polymères.

J'adresse également mes remerciements aux membres du Jury qui ont accepté d'évaluer ce travail : la Professeure Fabienne Barroso du DIPC à San Sebastian, la Docteure Sylvie Boileau Directrice de recherche émérite à l'ICMPE, le Professeur Stéphane Carlotti de l'Université de Bordeaux, le Docteur Jacques Penelle Directeur de recherche à l'ICMPE ainsi que la Professeure Laurence Rozes de Sorbonne Université. (I would like to thank the Professor Fabienne Barroso for taking part of the committee to assess this work).

Ce projet je l'ai fait évoluer pendant 3 ans. Il a réussi à grandir et s'épanouir grâce à mon étroite collaboration avec les deux personnes suivantes :

En premier lieu je remercie mon directeur de thèse, le Professeur Philippe Guégan, de m'avoir fait confiance pour défendre ce projet tout d'abord devant l'ED puis tout au long des 3 ans. J'ai ainsi pu mener à bien ces expériences qui ont pu faire avancer la science.

Je tiens à vous remercier pour toutes les discussions scientifiques ou non que nous avons pu avoir. Je me suis toujours senti écouté, même lorsque nous n'étions pas d'accord. J'emporte avec moi tous vos conseils distillés lors des nombreuses discussions que nous avons eues jusqu'à parfois tard le soir.

Ce projet n'aurait pas pu être aussi bien mené sans l'intervention du Docteur Nicolas Illy. Un grand merci, à toi Nicolas, pour l'écoute dont tu as su faire preuve, la franchise qui te caractérise (un peu trop peut-être), ton esprit scientifique affûté et ton immense disponibilité. Ton sérieux et ta malice ont pu faire de notre collaboration à trois une interaction qui fonctionne.

A l'ensemble de l'équipe Guégan, Véronique Bennevault, Cécile Huin, Haiqin Du, Pauline Lebellec et les quelques stagiaires, comme Anissa et Simon que j'ai pu côtoyer, je garderais en mémoire avec profonde nostalgie l'ensemble de nos pérégrinations dans le 5<sup>e</sup> arrondissement pour trouver LE restaurant, la perle rare, de nos déjeuners et dîners d'équipe. Ces moments de partage hors laboratoire ont surement été la clef d'une belle cohésion entre nous. Merci à vous !

Le volet synthèse de ce projet a été appuyé par une partie analytique et caractérisation. Claire, merci d'avoir été là pour chaque question qui ont souvent été répétitives « Alors le Maldi ? Il est réparé ? ». Merci pour ta rigueur et ta bonne humeur communicative.

Merci également à Gaëlle Pembouong pour ton infinie patience avec le parc de GPC(s). Je n'ai pas toujours été très « docile » mais analyser nos échantillons en GPC ne serait pas aussi facile sans tout le travail de maintenance que tu fais au quotidien.

Sylvie Noinville, merci d'avoir toujours pu te libérer pour nos sessions d'infrarouge. Ton œil avisé a toujours su m'apporter les réponses qui me manquaient.

J'ai également une pensée pour Lydia Sosa Vargas, merci pour ton dévouement sans limite. L'engouement que tu places dans chaque chose que tu entreprends me rend admiratif. J'espère que le stand « Sliiiiiiime » lors de la Fête de la science sera toujours autant couronné de succès.

De façon plus générale je tiens à remercier l'ensemble des membres de l'équipe Chimie des Polymères. C'est avec grand plaisir que j'ai fait avancer ce projet pendant ces 3 ans. Merci à vous !

J'ai une pensée toute particulière pour la Docteure Özgül Tezgel qui m'a mis sur les rails du projet à mon arrivée. Quel enfer j'ai dû te faire vivre ! Merci pour ta patience et ton aide plus que précieuse qui m'a permis de gagner une grande autonomie. Tu as dignement gagné ton titre de « Maman du Labo », alors que j'étais un enfant plutôt dissipé.

Ces remerciements ne seraient pas complets sans un mot sur le lien qui s'est tissé avec mes « collègues » du 4<sup>e</sup>. Mes premiers mots vont à Gwendoline et Laetitia, Laetitia, aussitôt débarqué dans ce bureau aussitôt intégré. Merci pour ta bienveillance qui fait ta force. Gwendoline, merci d'avoir su toujours être là et pour tes conseils avisés de présentations. Finalement merci à toutes les deux d'avoir supporté l'enfant parfois terrible que je suis, mon caractère réfractaire au changement, et finalement d'avoir su créer une belle cohésion dans ce bureau 401. Mais la fine équipe ne serait pas au complet sans nos comparses Gaëlle et Pauline de l'équipe Radicalaire. Gaëlle, je me revois encore en train de faire de la visco pendant que tu étais dans ton labo et qu'on discutait déjà autour de votre rota grinçant ! Merci pour ta gentillesse. Et Pauline…il y a toujours un moment où notre connexion est allée beaucoup trop loin mais malheureusement ce n'était pas quand on était les plus lucides. Merci pour ta bonne humeur. Ces 3 ans se sont si bien déroulés aussi grâce à vous. Et surtout n'oubliez pas : attention au calcul de concentrations !

Je souhaite exprimer mes sincères remerciements à mes amis qui m'ont toujours poussé et ont su m'apporter l'oxygène et le recul dont j'ai parfois eu besoin.

Enfin je remercie mes parents, ma sœur, Marie et Camille pour leur soutien sans faille qu'ils m'ont apporté.

# Abbreviations

<sup>13</sup> C NMR	Carbon nuclear magnetic resonnance
<sup>1</sup> H NMR	Proton nuclear magnetic resonnance
ACN	Acetonitrile
AGE	Allyl glycidyl ether
AGET-ATRP	Activator generated by electron transfer-ATRP
ARGET ATRP	Activator regenerated by electron transfer ATRP
AROP	Anion ring-opening polymerization
ATR-FTIR	Attenuated total reflection-Fourrier-transform infrared specroscopy
ATRP	Atom transfer radical polymerization
BDS	Broadband dielectric spectroscopy
BEMP	2- <i>tert</i> -butylimino-2-diethylamino-1,3-dimethylperhydro -1,3,2-diazaphosphorine
BnGE	Benzyl glycidyl ether
BO	1,2-Butylene oxide
BSA	Bovine serum albumin
COSY	Correlation spectroscopy
CRP	Controlled radical polymerization
СТА	Chain transfer agent
Ð	Dispersity
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIBO	Dibenzocyclooctine
DKP	2,5-diketopiperazine
DMAC	<i>N</i> , <i>N</i> -dimethyl acetamide
DMAEMA	2-dimethylaminoethyl methacrylate
DMF	N,N-Dimethylformamide
DPMP-Li	1,1-diphenyl-3-methylpentyllithium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPH-Li	Diphenylhexyl lithium
DTT	Dithiothreitol
ECH	Epichlorohydrin
EEGE	Ethoxyethyl glycidyl ether
EGE	Ethyl glycidyl ether
EO	Ethylene oxide
EPICH	Epicyanohydrin
Et <sub>3</sub> Al (TEA)	Triethylaluminum
Et <sub>3</sub> B (TEB)	Triethylborane
GA	Glycidyl azide
GME	Glycidyl methyl ether
GPE	Glycidyl phenyl ether
HA	Hyaluronic acid
HEMA	Hydroxyethyl methacrylate

HMBC	Heteronuclear muliple-bond correlation spectroscopy
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single-quantum correlation spectroscopy
<i>i-</i> Bu <sub>3</sub> Al (TiBA)	Triisobutylaluminum
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
MeTHF	2-methyltetrahydrofuran
MMA	Methyl methacrylate
M <sub>n</sub>	Number-average molar masse
MPEGA	Monomethoxy poly(ethylene glycol)-methacrylate
MTBD	7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene
$M_{ m w}$	Weight-average molar mass
NCA	N-carboxyanhydride
NHC	N-heterocyclic carbene
NHO	N-heterocyclic olefins
NMP	Nitroxide-mediated polymerization
Oligo(EG)	Oligo(ethylene glycol)
PB	Poly(butadiene)
PBS	Phopshate buffer solution
PDMAEMA	Poly(N,N-dimethylaminoethyl methacrylate
PEG	Poly(ethylene glycol)
PEGA	Poly(ethylene glycol)-acrylate
PEGMA	Poly(ethylene glycol)-methacrylate
PG	Polyglycidol
PGE	Phenyl glycidyl ether
Ph <sub>3</sub> Al	Triphenylaluminum
PHEA	Poly((2-hydroxyethyl) acrylate)
PHEA	Poly(hydroxyethyl acrylate
PHEMA	Poly(2-hydroxyethyl methacrylate
PHMPA	Poly(N-(2hydroxypropyl) methacrylamide
PI	Poly(1,4-isoprene)
PMDETA	<i>N</i> , <i>N</i> , <i>N</i> ', <i>N</i> '', <i>N</i> ''-pentamethyldiethylenetriamine
PMMA	Poly(methyl methacrylate)
PMPC	Poly(2-methacryloyloxyethyl phosphorylcholine)
PNIPAAm	Poly(N-isopropylacrylamide)
PO	1,2-propylene oxide
POZ	Polyoxazoline
PPO	Poly(propylene oxide)
PSA	Polysialic acid
PTAD	4-phenyl-3 <i>H</i> -1,2,4-triazole-3,5(4 <i>H</i> )-dione
PVP	poly(N-vinylpyrrolidone)
RAFT	Reversible/addition fragmentation chain transfer polymerization
RDRP	Reversible deactivation radical polymerization
RI	Refractive index
ROMP	Ring-opening metathesis polymerization

ROP	Ring-opening polymerization
RT	Room temperature
SEC	Size exclusion chromatography
SPPS	Solid phase peptide synthesis
PEG-SS-4TP	4-(PEG-disulfanyl)pyridine
<i>t</i> BuGE	Tert-butyl glycidyl ether
tBuP <sub>1</sub>	1-tert-butylimino-tris(dimethylamino)phosphorane
tBuP <sub>2</sub>	1- <i>tert</i> -butyl-2,2,4,4,4-pentakis(dimethylamino) -2Λ5,4Λ5-catenadi(phosphazene)
<i>t</i> BuP <sub>4</sub>	1- <i>tert</i> -butyl-4,4,4-tris(dimethylamino)-2,2-bis [tris(dimethylamino)phosphoranylidenamino] -2Λ5,4Λ5-catenadi(phosphazene)
<i>t</i> BuP <sub>n</sub>	Phosphazene base
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TiBP	2,8,9-triisobutyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane
TOCSY	Total correlation spectroscopy
UV	Ultraviolet



Name	Abbrevi	ation
Alanine	Ala	Α
Arginine	Arg	R
Asparagine	Asn	Ν
Aspartic acid	Asp	D
Cysteine	Cys	С
Glutamic acid	Glu	Е
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	Н
Isoleucine	lle	I
Leucine	Leu	L
Lysine	Lys	Κ
Methionine	Met	М
Phenylalanine	Phe	F
Proline	Pro	Ρ
Serine	Ser	S
Threonine	Thr	Т
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

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— General introduction —

Peptide-polymer or protein-polymer conjugates are a class of macromolecular architectures combining the advantages of each component to overcome some of their limitations. Conjugation of a polymer to a peptide/protein induces consequential changes in physicochemical properties of the initial biological material. The most common forms of conjugates are based on poly(ethylene glycol) (PEG) polymer. PEGylation is a well known strategy to improve properties of peptides/proteins such as solubility, stability or bioavailability, which is opening the way to a vast range of applications in the biomedical field.

Conjugates can be synthesized according to two approaches, convergent synthesis or divergent synthesis. Most of the conjugates are synthesized by the convergent synthesis due the straightforward character of this approach. The convergent technique can bind a wide variety of polymer chains to peptides/proteins. However, the approach faces low yields and requires chemical activation of the concerned functions before functionalization. Advances in radical-based polymerizations allowed the divergent synthesis of polymers from biomolecules. Controlled Radical Polymerizations (CRP) are commonly used to synthesize conjugates. Indeed, CRPs proceed under mild reaction conditions compatible with modification of peptides or proteins. However, the CRP techniques limit the scope of the targeted macromolecules to vinyl-based polymer chains and require modification of the polymerization.

Those traditional ways to synthesize conjugates rely on specific amino-acids residues, and no work has been reported on the functionalization of the most common function in peptide and protein i.e. secondary amide functions. Generally speaking, developments to improve the site-specificity of the modification require several synthetic steps or specific biochemical procedures. In this project, we propose a synthetic approach independent of the amino acids composition of the peptides/proteins.

Our group shows that it is possible to control the Anionic Ring-Opening Polymerization (AROP) of oxirane using amide-based molecules as initiators. We propose to apply this strategy to the functionalization of peptides and develop a divergent synthetic method for the functionalization of peptides on amide functions by polyethers. Amide functions or peptide bonds are ubiquitous among proteins. Polyethers present the advantages of the hetero atoms-based backbone, with a tunable hydrophilicity regarding the polymerized oxirane units.

General introduction

However, main characteristics of the AROP of oxiranes make very challenging its use with biological materials. The high basicity of the medium and nucleophilicity of propagating species can be harmful for the peptide/protein structure. This project aims to apply our method to NH amide(s) of peptides and develop reactions conditions for a successful functionalization. Phosphazene bases will be used as promoters, to generate reactive amidates by deprotonation, then able to initiate a polymer chain. Control of the polymerization will also be modulated through the use of Lewis acids to decrease the reactivity of the propagating centers.

The first chapter of this manuscript is a literature review. In a first part, the different strategies to synthesize peptide-polymer conjugates, convergent and divergent syntheses, will be introduced, with a discussion on their main advantages, drawbacks and their recent advances. In a second part, oxirane polymerizations by AROP to yield chain-ends functionalized polymers will be disclosed as well as the use of organocatalysts to promote the AROP from various protic initiators.

In the three following chapters, strategies to synthesize conjugates in a controlled manner from different peptides initiators will be developed. The complexity of the initiating peptides will be increased step by step along the chapters. In chapter 2, small cyclic dipeptides, 2,5-diketopiperazines (DKP), will be used to initiate the AROP of oxiranes, with a targeted functionalization on the amide functions. Due to the presence of a limited number of initiating sites and their simple structure, DKPs are going to be used as model compounds to prove the modification of peptides by AROP. Then, in chapter 3 the attention will be switched to a protected linear peptide Z-Val-Phe-OMe. The purpose is to investigate reaction conditions compatible with the protective functions ester and carbamate. Finally, the chapter 4 will focus on the attempts to *N*-modify a protected version of tri-alanine peptide and the unprotected form of the tri-alanine.

Along those chapters main concerns will be made about the polymerization from the peptides without side reactions.

The Chapter 5 is dedicated to the experimental procedures used to perform the synthesis, the analytical techniques and the parameters used to characterize the macromolecules.

# — **CHAPTER 1** —

# Literature review

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#### Introduction

The aim of the project is the synthesis of peptide-polymer conjugates in a controlled manner, using anionic ring-opening polymerization of oxirane monomers from different peptides. This literature review will be divided into two major parts. In a first part, we will focus on peptide/protein-polymer conjugates, and the different approaches that can be used to synthesize them. To begin with, convergent synthesis, and specifically the binding of PEG chains to biological materials will be described. Focus will be turned to the parameters to reach a high degree of selectivity during conjugation. Then divergent synthesis, by the mean of controlled radical polymerization, will be reported here. Attempts of conjugation by grafting through will also be described. The main advantages and drawbacks of each conjugation techniques and recent advances will be highlighted during this first part.

In a second part attention will then be turned to the chemistry involved in the polymers synthesis, namely poly(ether)s synthesis using oxirane-based monomers initiated by unusual organic functions. Conventional anionic ring-opening polymerization will be covered by the mean of alkali metal-based initiators. Ability of the initiating systems to yield well-defined homo or heterotelechelic polymers will be highlighted. Then growing interest for metal free organocatalyzed AROP through the use of highly basic molecules will be considered. Insight of specific uses of phosphazene bases as deprotonating agent is going to be provided, for the contributions made into the field of macromolecular engineering. Finally, the addition of a Lewis acid in the AROP reaction medium will be addressed to introduce monomer-activated AROP, and benefit in term of polymerizable monomers or control of the polymerization will be mentioned.

As a conclusion, an innovative strategy for the synthesis of peptide-polymer conjugates will be suggested. Based on techniques discussed in this chapter, synthesis of polyether chains bound to small peptides by the mean of anionic polymerization will be addressed.

## I. Peptide/protein-polymer conjugates

Peptides are ubiquitous in nature and defined as small chains of amino acids (less than 50 units). In biological systems they fulfill a broad range of tasks such as DNA replication, immune defenses, hormones regulation... In the biomedical field they can also be used as therapeutic agent and offers many advantages. They can bind with exquisite specificity to an *in vivo* target, thus reducing side effects. They usually have a short *in vivo* half-life and their products of degradation are amino acids, which limit the risk of toxicity. <sup>1</sup>

Apart for the range of highly exigent biomedical applications for peptide/protein-polymer conjugates, they are also used in non-biological environments. Material-based applications such as filtration tools, <sup>2</sup> optoelectronic devices <sup>3</sup> or catalysis <sup>4</sup> purposes, are taking advantage of the self-assembly capacity of the bio-conjugates. However, this literature review will focus exclusively on biomedical application due to the potential utilization of the project in this particular field.

#### 1. Interest of conjugates

Even though peptide-based drugs show great potency to be used as a medicine, some drawbacks are a limitation to a larger scale use. Such as, a short circulating plasma half-life, indeed their small size is favoring a rapid clearance by liver or kidneys. <sup>5</sup> They also tend to display a low hydrolytic stability towards proteolytic enzymes of the digestive system or blood plasma. And depending on the amino acids sequence some can have a poor solubility into aqueous/organic medium. <sup>6</sup>

A way to improve the bioavailability and biodistribution of the therapeutic peptides is to proceed via chemical modifications. The structural modification of the peptide can be achieved by covalent binding of a polymer giving a hybrid material, peptide/protein-polymer conjugate.

Peptide/protein-polymer conjugates were defined by Klok as "Hybrid constructs combining a defined number of protein segments with uniform chain lengths and defined monomer sequences (amino acids units) with a defined number of synthetic polymer chains. From this design arised benefits from the synergetic behavior of both components".<sup>7</sup>

Those mentioned advantages, arise from the increase of the overall mass of the peptide. The mass increase is altering the general biodistribution and improves the circulation time. <sup>8</sup> One of the most important features acquired by polymer conjugation is the shield constituted by the random coil conformation of the PEG chains. <sup>9</sup> This behavior is lowering interaction with the immune system components leading to reduce the elimination of the protein by this mean. Additionally, it was reported that conjugation of PEG chains to protein leads to an enhanced solubility in aqueous and organic media and an increased temperature stability. <sup>10</sup>

These gains in term of properties are controlled by key parameters which are: <sup>7,11–14</sup>

(1) The nature of the polymer backbone. As reviewed by Klok and Gauthier, the side-chain functionality of the repetitive units can interact with the biologic and modify his properties. <sup>15</sup> However, they reported that advantages and drawbacks are specific to a given system polymer/protein and should be considered case by case.

(2) The molar mass of the conjugate and the number of conjugated chains. Length/number of conjugated polymers modify the pharmacokinetic properties of the protein, but predicting the effects due to polymer conjugation cannot be only directed by the  $M_n$  of the macromolecules.

(3) The structure of the conjugate can have a design varying from the common head-to-tail architecture to comb-shaped or dendritic structures. The architecture employed is highly dependent upon the application.

(4) The location of the conjugation on the peptide/protein. For instance, a site-specific modification far from the active site has better chance to preserve the protein activity.

(5) Coupling chemistries employed for the synthesis are introducing new organic function in the peptide/protein. Those groups can alter the biological function of the peptide or the protein. Reactive conditions to perform the ligation can also harm the integrity of the peptide/protein.

Three strategies are available to synthesize peptide/protein-polymer conjugates (Figure 1):

- Grafting-to, a pre-synthesized polymer is grafted to a protein.
- Grafting-from, the polymer is grown from the peptide/protein initiator, previously modified, or not, for having such role in the polymerization.
- Grafting-through, monomers are functionalized with a peptide/protein pendant group and are subsequently polymerized in a polymeric backbone, bearing biologically active pendant groups.



Figure 1: General methods for synthesis of peptide/protein-polymer conjugates.

For all the three mentioned approaches, the synthetic procedures require a straightforward purification. And the final conjugates are required to observe a minimal loss of biological activity regarding the native protein.

The three strategies forenamed are going to be described in more details. Contributions to the field of conjugates as well as limitations will be discussed in the next pages.

# 2. Grafting-to synthesis for peptide/protein-polymer conjugates production

PEG is the gold standard for conjugation: it is highly soluble in water and common organic solvents, neutral and inert, nontoxic, and mostly non-immunogenic. PEGylation that is, the binding of poly(ethylene glycol) (PEG) chains to a peptide or a protein, has become the preferred technique for improving pharmacokinetic properties of protein-based drugs.<sup>16</sup>

PEGylation usually occur by grafting-to approach due to the straightforwardness and flexibility offered by this strategy. In such approach the biomolecule and the polymer are independently synthesized and then coupled together to form the conjugate. This method requires the use of polymers chains functionalized in  $\alpha$  or  $\omega$  positions. Those reactive handles on the polymer can be generated by post-polymerization modifications, by using an appropriate initiator with interesting organic functions or using a desired quencher. <sup>17,18</sup>

In the following parts main effort of conjugation to protein/peptide using PEG will be covered. PEGylation of  $1^{st}$  and  $2^{nd}$  generation will be tackled.

#### a. 1st generation PEGylation strategy

The first generation of PEGylation is based on the coupling of activated alcohol function from PEG and a primary amine from the peptide/protein.

Heterotelechelics PEG-hydroxy were chemically modified to generate more reactive derivatives (see Figure 2). (1) PEG-dichlorotriazine, (2) PEG-tresylate, (3) PEG-succinimidyl esters, (4) PEG-benzotriazole, (5) PEG-p-nitrophenyl carbonate, (6) PEG-trichlorophenylcarbonate, (7) PEG-carbonylimidazole were synthetized.



Figure 2: First-generation amine reactive PEG derivatives.

The first reported protein-polymer conjugate by Abuchowski *et al.* used (1) PEGdichlorotriazine for modification of bovine serum albumin (BSA) <sup>19</sup> and bovine liver catalase. <sup>20</sup> However the technique was facing a lack of selectivity and crosslinking between proteins was observed during the synthesis, due to the reactivity of all the Cl atoms around the triazine heterocycle. The linkage of (2) PEG-tresylate to primary amine-containing molecules was also used for alkylation purposes. <sup>21</sup>

Following PEG-organic functions were used as acylating agents for conjugation to peptides and proteins: PEG-succinimidyl carbonate (3), <sup>22</sup> (4) PEG-benzotriazole carbonate, <sup>23</sup> (5) PEG-*p*-nitrophenylcarbonate, (6) PEG-trichlorophenylcarbonate, (7) PEG-carbonylimidazole. <sup>24</sup> By using the specific reactivity of the hydroxy groups with various activating agents, PEG chain ends could actually be bound to amino groups. But the 1<sup>st</sup> generation chemistry lacked the ability to form high molar mass monofunctional pure PEG derivatives. <sup>25</sup> The absence of selectivity regarding the  $NH_2$  groups, the occurring of side reactions and the various degrees of bifunctional diol-PEGs in a batch were great challenges to be solved by the second generation of PEGylation reagents. <sup>26</sup>

# b. 2<sup>nd</sup> generation PEGylation strategy

The second generation of PEGylating agents is using more specific chain end functions, which are able to react with limited range of organic groups. <sup>27</sup> For selectivity purpose, the coupling reactions peptide/protein to polymer need to be bioorthogonals. <sup>28</sup> Meaning that the functionalization will be selective under biocompatible conditions and that only one or a limited number of PEG copies will be grafted to the biological active compound.

PEGylation agents from the 2<sup>nd</sup> generation are usually targeting specifying organic functions, which can be borne by natural amino-acid residues or non-canonical residues.

#### i. Natural amino acids-based reactions

Proteins are made up from a standard 20 amino acids list (see abbreviations list). They usually contain some of the residues if not all, which can then be used as conjugation sites, depending on their side chains functionality, abundance and location. **Table 1** to **Table 4** lists some of the most commonly used reactions for polymers ligation to proteins. The displayed reactions are not exhaustive but are shown for their high specificity towards the targeted organic functions. (Complete overview of the reactions towards the different residues can be found in literature  $^{7,14,29,30}$ )

Aliphatic side-chains-based amino acids (Ala, Gly, Leu, Pro, Val, Ile) lack a reactive handle for conjugation and therefore are not displayed in this list.

Polar non ionizable amino acids (Gln, Asn) are difficult to target for selective modifications due to poor reactivity. Met can be target through oxaziridines without harnessing other residues but few articles reported the use of this site for conjugation.

#### Chapter 1: Literature review

Concerning the aromatic side chains (Phe, His), limited methods of conjugations exist for their targeting, and they usually observe transfer reactions between other aromatic residues. Trp can be selectively modified but they are of low abundance and generally located near the active site.

The polar ionizable-side chains (Ser, Thr) are poor nucleophilic groups compared to other residues like Lys or Cys which are generally favored.

Asp, Glu and C-ter functions are easily targeted but the lack of selectivity regarding one COOH function render the approach less common.

The chemistry to modify Arg residues can involve other highly reactive amino acids such as Lys or Cys. Amongst the 20 canonical amino acids, Cysteine, Lysine, Tyrosine and N ter are the most popular functions concerned for modifications, because of the easy targeting of the side-chains and the high reactivity of the organic function.

#### • Primary amino groups-specific conjugation

The 2<sup>nd</sup> generation PEGylation offers synthetic method more specific to amino functions allowing precise modification with limited side reactions.

Primary amines in lysine and N-ter functions are nitrogen-based organic functions which can be selectively modified due to the high nucleophilicity of the side function.

By using squaric acid and squarate derivatives, BSA protein was selectively modified by carbohydrates and PEG chains on lysines and N-terminal NH<sub>2</sub>. <sup>31,32</sup> (see **Table 4**)

Targeting selectively N-ter amino groups can be achieved by exploiting  $pK_a$  difference between the NH<sub>2</sub>(Lysine) ( $pK_a \approx 10$ ) and NH<sub>2</sub>(N-ter) ( $pK_a \approx 8$ ). The use of reductive amination through reducing agents such as NaBH<sub>4</sub> or NaCNBH<sub>3</sub> was exploited by Kinstler and coworkers. They specifically linked PEG to final amino group without altering lysine residues. <sup>33</sup> (see **Table 2**)

However,  $NH_2$  containing residues are the least specific linkage sites. Indeed, Lys amino acids are abundant residues on protein and most part of the lysines are exposed at the surface of the 3D structure. Furthermore, targeting amino groups lead to modification of the protein charge, which can result in a lower biological activity.

#### **Table 1:** Typical reactions towards amino acid residues bearing a polar non ionizable side-chain.

Target group	Amino acid	Natural Abundance (%)	Location	Reaction	Ref.
Met-S-	Met	2.39	Core	Polymer $N \rightarrow N $	34

**Table 2:** Typical reactions towards terminal functions of the proteins/peptides.

Target group	Amino acid	Natural Abundance (%)	Location	Reaction	Ref.
NH <sub>2</sub>	N-ter			Polymer $H$ $H$ $H$ $N$ Protein $NH_2$ $H$ $N$ Protein $NaBH_4/NaCNBH_3$	33
СООН	C-ter			Protein OH Polymer—NH <sub>2</sub> $\rightarrow$ Polymer OH Polymer	35

*Chapter 1: Literature review* **Table 3:** Typical reactions towards amino acids residues bearing an aromatic side-chain.

Target group	Amino acid	Natural Abundance (%)	Location	Reaction	Ref.
ОН	Tyr	3.00	Core	$(1)$ $\xrightarrow{Polymer} (1)$	36-38
				(3) Protein $\longrightarrow$ OH Polymer $\longrightarrow$ OAc $\longrightarrow$ Protein $\longrightarrow$ Protein $\longrightarrow$ OH Protein $\longrightarrow$ OH	
Indol	Trp	1.13	Core	$(9)  \text{Polymer} \qquad \qquad$	39-41
				(10) Potymer $\overset{\text{Protein}}{\overset{\text{NH}}{\overset{\text{H}}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}}{\overset{\text{H}}{\overset{\text{H}}}{\overset{\text{H}}{\overset{\text{H}}}{\overset{\text{H}}}}}}}}}}$	
Imidazole	His	2.30	Intermediate	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	42

**Table 4:** Typical reactions towards amino acids residues bearing a polar ionizable side-chain.

Target group	Amino acid	Natural Abundance (%)	Location	Reaction	Ref.
NH <sub>2</sub>	Lys	5.91	Surface	Polymer NH OEt Protein (NH <sub>2</sub> Polymer NH OEt Polymer NH HN-Protein	31,32
SH	Cys	1.50	Core	$(4) \text{ Polymer}  \qquad  \qquad \qquad  $	43-46
СООН	Asp	5.33	Surface	Protein	47
СООН	Glu	6.66	Surface	Polymer— $NH_2$ $\longrightarrow$ Polymer $N$ $Polymer$	
Guanidium	Arg	5.44	Intermediate	$\begin{array}{c} & HN \\ & HN \\ & HN \\ & H^{2} $	48

#### • Tyrosine specific conjugation

Tyrosines are an interesting alternative for selective modifications. Barbas and coworker established a selective modification of the tyrosine through click chemistry. They used PEG-4-phenyl-3H-1,2,4-triazole-3,5(4H)-dione (PEG-PTAD) for the selective modification of tyrosine residues over several proteins like BSA, myoglobin, and chymotrypsinogen (see (1) in **Table 3**). <sup>36</sup> Similar to PTAD, diazo functional-PEG was used by Haddleton for selective modification of Tyr residues of salmon calcitonin (see (2) in **Table 3**). <sup>38</sup> Through a three components Mannich type reaction, Francis and coworkers reported the

modification of chymotrypsinogen selectively on Tyr residues under mild conditions assisted by Palladium complexes (see (3) in **Table 3**). <sup>37</sup>

Tyrosine offers the advantage of being uncharged, and thus conjugation will not change the ionization potential of the protein. But Tyr residues are highly abundant near the active sites of peptides/proteins. A chemical modification on Tyr side-chains thus may have influence on the biological activity of the compound.

• Cysteine specific conjugation

Cysteines are the residues which are often targeted for conjugation. They are of interest because of their low abundance and the reactivity of the thiol group, which can undergo modifications specifics to thiols chemistry.

Michael addition is the most common reaction for targeting cysteine residues. PEGmaleimide (see (4) in **Table 4**) is by far one of the most used reagent <sup>43</sup> but PEG-vinyl sulfone (see (5) in **Table 4**) and PEG-iodoacetamide (see (6) in **Table 4**), which reacts more slowly with thiols, were also used for selective conjugation to enzymes. <sup>44</sup> As example of applications, Stayton and Hoffman used poly(N-isopropylacrylamide) (PNIPAAm) functionalized with maleimide and vinyl sulfone for cysteine targeting, and synthesized conjugates with stimuli-responsive properties. <sup>49,50</sup>

Another approach for selective targeting of cysteines is the disulfide formation. The thioldisulfide exchange can be carried out under a large range of pH and the process is entirely reversible by using a reducing agent, like dithiothreitol (DTT). Another highly reported end-group activated polymer, for disulfide formation, is orthopyridyl disulfide (see (7) in **Table 4**). The first conjugation using this synthetic approach was reported by Stein and coworker, who conjugated 4-(PEG-disulfanyl)pyridine (PEG-SS-4TP) to Papain resulting in a successful selective PEGylation on the cys residues. <sup>45</sup> Maynard *et al.* developed a strategy involving direct use of pyridyl disulfide initiator, to overstep the postpolymerization modification. The synthesis of poly(2-hydroxyethyl methacrylate) (poly(HEMA)) by ATRP via a specifically designed initiator was followed by conjugation to BSA protein. <sup>51</sup>

Thiols are capable of binding to alkenes via thiol-ene coupling. This reaction involves a bioorthogonal radical addition mechanism, and has a high tolerance regarding water, oxygen, and other functional groups. The capacity of conjugation was demonstrated by reaction of cysteine amino acids on 1,2-poly(butadiene) having pendant alkenyl group. <sup>46</sup>

Chemical modifications on those residues may be specifics and reversible, but can alter the 3D structure and so the activity of the protein. Cys amino acids are generally located deep into the structure and can be involved in conformational constraints of the edifice, by taking part in disulfide bonds.

Peptide, polypeptides and proteins are made of several repetitive units of each amino acid, making the targeting of a specific residue at a precise location rather difficult. Recent advances in the introduction of non-natural amino acids in the protein sequence are paving the way for site-specific modifications using bioorthogonal chemical reactions.

#### ii. Non-canonical amino acids-based reactions

Integration of non-canonical amino acids, non-natural residues, along the protein backbone can be achieved via a chemical pathway based on SPPS (Solid Phase Peptide Synthesis) or using biosynthetic tools. <sup>26</sup> Introduction of those organic groups allows a precise site-specific conjugation. Due to the high specificity of the reactions in **Table 5**, syntheses of peptide/protein-polymer conjugates using non-canonical functions are very efficient and can be done in mild conditions. **Table 5** displays a list of common click chemistry reactions applied to ligation of polymer to non-canonical amino acids residues.
Reaction type	Reagents	Product	Ref.
Alkyne-azido coupling	$R_1$ + $R_2$ + $N_3$	$R_1$ $N = N$ $N = R_2$	52,53
	+ R <sub>2</sub> N <sub>3</sub>	N <sup>2</sup> N <sup>-R</sup> 2 R <sub>1</sub>	54
Staudinger	$\begin{array}{ c c c } & & & & \\ & & & & \\ & & & & \\ & & & & $	R <sub>2</sub> H R <sub>1</sub>	55,56
inguitonis	$\begin{array}{ c c c c c } Ph & Ph & Ph \\ Ph & S & R_1 + R_2 \\ \hline Ph & O \\ \hline \end{array}$	$R_2 \xrightarrow{H} R_1$	
Hydrazone ligation	$R_2 + R_1 + R_3 + NH_2$	$R_2$ $R_1$ $H$ $R_3$	57
Oxime ligation	$R_2$ $R_1$ $R_3$ $NH_2$	$R_2 R_1$	58

 Table 5: Bioorthogonal polymer conjugation reactions to peptide/protein.

Azides are one of the most common non-canonical organic functions introduced in biological material. Azide moiety born by amino acid residues allow the conjugation by Huisgen cycloaddition, with alkyne-terminated-polymers <sup>52,53</sup> or via copper free ligation, with dibenzocyclooctine(DIBO)-PEG. <sup>54</sup> Staudinger ligation was also exploited for conjugation purpose of phosphane-terminated-PEG to azide-proteins. <sup>55,56</sup>

A carbonyl function can be introduced in the protein by using a non-natural amino acid bearing ketone or aldehyde side group. Those organic groups were used for the conjugation of hydrazine or hydroxylamine modified polymers via oxime or hydrazine ligation. <sup>57,58</sup>

Conjugation chemistries with non-canonical amino acid residues present the advantages to be bioorthogonal and to generate unstable linkages in some cases. However, integration of non-natural amino acids in a peptide is a challenging process. Via biosynthesis using cellular machinery, the efficiency is low and requires intensive work. <sup>59</sup>

Via SPPS the length of the peptide is limited to about 50 residues and side-groups of amino acids must be compatible with coupling and deprotection steps.

In this part, particular attention was given to the most common ways to conjugate, via grafting-to, a polymer to a peptide or a protein in a more selective manner. But the difficulty of grafting-to technique relies in the reduced accessibility of functional end groups on polymer chain. Purification of the conjugate from a mixture of un-reacted polymers, peptides and byproducts can be also a difficult process. Even though shortcomings are inherent to this approach, it is still the most straightforward and used. Various PEGylated proteins-based drugs, are synthesized by grafting-to approach and are already on the market, Oncaspar®, Peg-intron®, Neulasta®... <sup>60</sup> In these three examples, the PEGylated versions posses the same biological activity as the native biologic and exhibit an increased serum half-life compared to the protein alone. However, the attachment of PEG chains is nonspecific and is believed to happen on a range of reactive residues on the proteins.

## 3. Grafting-from peptide/protein initiators

Grafting-from consists in the growing of a polymer chain from a peptide/protein. This approach lowers the steric hindrance during the ligation and simplifies the purification procedures. Radical based polymerizations are of interest because of the mild reaction conditions. Ionic polymerization methods preclude the grafting-from a peptide/protein due to harsh environment in the reaction medium. Some attempts were made with ring-opening metathesis polymerization (ROMP), but this approach reveals to be complex due to fine tuning of the catalyst required to control the polymerization in aqueous medium. Additionally very slow ROMP kinetics were observed when the polymerization was done in water buffered solutions. <sup>61</sup>

## a. Controlled Radical Polymerization

The synthesis of conjugates via divergent synthesis has been made possible due to developments in RDRP (Reversible Deactivation Radical Polymerization). These techniques permitted a diversification of polymers conjugated beyond the PEG standard.

Commonly used CRP (Controlled Radical Polymerization) reactions for grafting-from including RAFT and ATRP will be reviewed in the following parts. NMP polymerization was also reported for the divergent synthesis of peptide/protein-polymer conjugates but will not be introduced here. <sup>62–64</sup> Compared to ATRP and RAFT, NMP exhibited limitations in term of range of monomers with controllable polymerization, high temperature of the process, and chain-end functionalization. <sup>65</sup>

CRPs require modification of the peptide/protein prior polymerization. A reactive handle capable of initiation or mediation have to be inserted onto the biomolecule. To do so, a wide range of selective modifications were extensively reviewed in the literature, to be able to grow a polymer chain from a specific site of the peptide/protein. <sup>7,14</sup>

#### i. ATRP

ATRP is a living-controlled radical polymerization developed independently by Matyjaszewski group and Sawamoto group in 1995. <sup>66,67</sup> A typical ATRP process (**Scheme 1**) involves a rapid exchange of a halide atom between a propagating center and a dormant specie. The equilibrium is shifted towards the dormant specie to ensure a good control of the polymerization. The reversible deactivation of the radicals is catalyzed by transition metal complex (usually from Cu). ATRP can be used to produce polymers with predictable molar masses, from a wide range of monomers, and low dispersities.





Börner and coworkers were actually the first to synthesize a polymer from an oligopeptide macro-initiator. The N terminal group of the peptide was acylated using 2-bromopropionic acid. *N*-butyl acrylate was polymerized in DMSO, with copper bromide and PMDETA as ligand. Conjugates of a poly(*N*-butylacrylate) were exhibiting a  $M_n = 11\ 000\ \text{g.mol}^{-1}$  and  $M_w/M_n = 1.19$ .<sup>68</sup>

To compare the efficiency of the grafting-from and the grafting-to strategies, Matyjaszewski and Russell modified lysine residues on the chymotrypsin with 2-bromoisobutyramide. The chymotrypsin initiator was used with CuBr and 2,2'-bipyridine to successfully polymerized, monomethoxy poly(ethylene glycol)-methacrylate (MPEGA) and 2-(dimethylamino) ethyl methacrylate (DMAEMA).<sup>69</sup> The results shown that the grafting-from approach leads to protein-polymer conjugates with overall better monodispersed sample than the grafting-to approach.

The scope of the ATRP technique was covered with various protein initiators. <sup>18</sup> Monomers used for classical radical polymerization were also used, such as MMA <sup>70</sup> or HEMA <sup>71</sup>. In parallel, the Haddleton group also achieves the copolymerization of methacrylate based monomers and PEGMA <sup>72</sup> initiated by a protein macroinitiator.

Even though the direct ATRP technique comes in very handy for the preparation of conjugates, the high sensitivity towards oxygen and low efficiency in water limit the implementation of this technique.<sup>73</sup>

The Matyjaszewski group developed advanced ATRP conditions compatible with aqueous media. AGET (Activator Generated by Electron Transfer) ATRP relies on the introduction of a reducing agent to produce the Cu(I) species *in situ*. By this mean they induced a better tolerance to oxygen and a better control in aqueous media at lower temperatures. <sup>74,75</sup>

Maynard and coworkers selectively modified insulin through a single lysine residue. The insulin modification on multiple amino groups was followed by semipreparative HPLC to isolate the mono-modified proteins. Then the latter insulin was used to subsequently initiate AGET ATRP of methacrylate trehalose monomer in PBS.<sup>76</sup>

Continuous screening of the polymerization conditions to improve the control, leads to ARGET (Activator ReGenerated by Electron Transfer) ATRP. The technique relies on a slow feeding of the reducing agent, enabling to continuously regenerate the Cu(I) catalyst. <sup>77,78</sup>

The main advantages of ARGET over AGET is the reduce amount of Cu catalyst used while retaining high degree of conversion. ARGET ATRP was used to design BSA conjugates and further investigated for polymerization from peptides and proteins initiators. <sup>79,80</sup>

However, it was observed that Cu catalyst employed during the ATRP was interacting with the proteins in a competitive manner. <sup>68,81</sup> This was leading to a faster polymerization process and a loss of control. This downside is a limitation for a general implementation of ATRP in the synthesis of peptide/protein-polymer conjugates.

#### ii. RAFT

RAFT polymerization is a living-controlled radical polymerization first reported in 1998 by the CSIRO laboratory. <sup>82</sup> RAFT method is able to control the polymerization for most classical monomers used in radical polymerization (methacrylate, acrylate, acrylamide, methacrylamide, vinyl monomers, dienes...).



Scheme 2: General mechanism of the RAFT process.

The RAFT process shown in **Scheme 2** is governed by a reversible transfer reaction between a growing macromolecule and a Chain Transfer Agent (CTA) (or RAFT agent).

The RAFT CTA is a small organic molecule typically based on thiocarbonylthio compounds including dithiocarbamates, trithiocarbonates, and xanthates.

The CTA is the key element of the RAFT process, and judicious choice of the R and Z substituents can ease the control of the polymerization.  $^{83,84}$ 

Approaches of immobilizing the peptide/protein via the R-group and via the Z group (Scheme 3) were adopted and examples of each strategy will be developed. RAFT polymerization offers the advantage of being free of metal catalyst and tolerant to low concentrations of air and moisture. The method is also more versatile than ATRP for controlling the polymerization of a wide range of monomers under mild conditions.



Scheme 3: R-Group (top) and Z-Group (bottom) strategy for the synthesis of protein-polymer conjugates via RAFT polymerization.

The first grafting-from a protein using RAFT was reported by Davis, Bulmus and coworkers. BSA protein was modified with a trithiocarbonate to act as CTA and was located on the Z-group side. The polymerization of PEG-acrylates (PEGA) proceeded successfully in water/DMF but gave polymers with a high dispersity ( $M_w/M_n > 2$ ). This value was assigned to the steric hindrance around the CTA implied by the BSA, which tends to make the access difficult of the propagating center to the thiocarbonythio group. Furthermore, an inhibition time was observed due to slow fragmentation rate of the RAFT agent at the early stage of the polymerization. <sup>85</sup>

The same Z-group strategy was used to prepare well-defined BSA-PNIPAAm or BSA-PHEA conjugates with retained enzymatic activity. <sup>86</sup>

The R-group strategy was introduced by Summerlin and coworkers using again the BSA model protein, depicted in **Scheme 3**. By using the BSA-macroCTA combined with NIPAAm in PBS, the group afforded relatively monodisperse ( $M_w/M_n = 1.38$ ) thermoresponsive conjugates, which activity could be tuned by the temperature. No loss of biological activity was observed for the BSA-poly(NIPAAm) conjugate compare to the native BSA. <sup>87</sup> The R-group strategy offers better control than the Z-group, due to an improved accessibility of the CTA during the polymerization. Also, it produces polymer chains bearing biological material on a side and thiocarbonylthio group on the other side, allowing broad range of post-polymerization chemical modifications.

Both examples displayed here, used the only free surface cysteine residue of BSA, Cys-34, for modification to act as a CTA. Other proteins with significant amount of free Cys are prone to side reactions between SH functions and thiocarbonylthio CTA, thus require selective protection of Cys residues. <sup>88</sup> Additionally, Levesque *et al.* reported the limited stability of thiocarbonylthio compounds in conditions compatible with protein modifications. The thioester was subject to hydrolysis enhanced by raising the temperature (25-35°C) or by increasing the pH (7-9). In these conditions, the reaction time must be restricted to few hours to preserve the CTA and the control of the polymerization. <sup>89</sup> Even though RAFT has shown capabilities into grafting-from conjugation, this polymerization approach suffers from clear limitations coming from transfer reactions with other amino acids and stability of the CTA.

The grafting-from technique provides many advantages over the grafting-to: the profound steric hindrance encounter between two macromolecules during the ligation reaction is avoided, and the purification process is easier.

The developments and usages of new monomers have broadened the possibilities for conjugation of polymers other than PEG, although macromolecules are limited to C-C backbone. Other drawbacks arising from the use of RDRP techniques are the lack of full functionalization on the polymer chains, and the need to modify the peptide before polymerization. Additionally thiocarbonylthio extremities were found to be cytotoxic during *in vitro* studies. <sup>90</sup>

## 4. Grafting-through

This strategy defines the polymerization of (macro)monomers functionalized with a peptide pendant group. Typical procedure involves the introduction of polymerizable group on a peptide, resulting in the production of comb-shaped structures where peptide segments are pendant groups along the polymer backbone. Grafting-through is of high interest to reach high density of grafting along the backbone, where grafting-to is usually limited to low grafting concentration in such architecture. The comb-shape is appealing for biomedical applications requiring close proximities between the multiple copies of the peptide/protein such as drug released materials. <sup>91</sup> Amino acids and peptides pendant groups can confer interesting properties in terms of self-organization by non-covalent bonds of the grafted macromolecules. <sup>92,93</sup>

The first reported grafting-through polymerization was achieved by Murata *et al.* Leu-Ala dipeptide was modified with methacrylate <sup>94</sup> and methacrylamide <sup>95</sup> functions and further used for free radical polymerization.

Then RDRP techniques were used to offer better control of the polymerization. At first, methacrylate modified VPGVG was polymerized under ATRP conditions leading to well-defined high molar mass polymers with a fairly narrow dispersity. <sup>96</sup> The same monomer was used under RAFT conditions. The RAFT polymerization reached higher degree of conversions and a lower dispersity. The higher  $M_w/M_n$  in ATRP was explained by complexation and deactivation of the copper catalyst by the peptide bonds. <sup>97</sup>

Polymerization of modified peptides/proteins can require complex chemistry for the macromonomers synthesis, and control of the polymerization can be difficult to reach for high degree of polymerization.



Scheme 4: Grafting through via polymerization of macromonomer functionalized with a protein pendant group.

Early examples of grafting-through approach also included norbornenes modified with a peptide pendant group. Grubbs and coworkers polymerized macromonomers of norbornenes with a peptide pendant group by ROMP using a catalyst based on Ruthenium. <sup>98,99</sup> But the use of transition-metal based catalyst remain challenging with peptide/protein based system due to the possible reactivity of the amino-acid residues under such conditions.

The grafting-through definition can also be applied to amino acid-based monomers functionalized by a polymer chain. Condensation of those amino acids generates a comb-shaped structure with a polypeptide backbone. This synthetic biomaterial exhibits biodegradable properties, <sup>100</sup> can form stable secondary structures such as  $\alpha$ -helix or  $\beta$ -sheet, <sup>101</sup> and the peptide backbone can have stimuli responsive properties. <sup>102</sup>

Deming and coworkers reported the polymerization of oligo(ethylene glycol)-modified *N*-carboxyanhydride (NCA) (**Scheme 5**). They synthesized poly(amino acids) backbone, with oligo(ethylene glycol) (oligo(EG)) pendant chains. This strategy introduces self-assembly properties into conjugates, while taking advantages of the oligo(EG) water solubility and passivating capacities. <sup>103</sup> A variety of polypeptides backbones was then synthesized with various ethylene glycol lengths and architectures. <sup>104,105</sup> However, the technique is limited to NCA functionalized with small oligo(EG). The polymerization of longer oligo(EG) chains-NCA is reported to proceed in an uncontrolled manner, due to difficult purification of the monomer NCA having long oligo(EG) pendant chains. <sup>106</sup>



Scheme 5: Polypeptide functionalized with polymer side-chain via 2 synthetic approaches.

PEGylated amino acids were also synthetized (**Scheme 5**). Various size of oligo(EG) up to 5000 g.mol<sup>-1</sup> PEGylated amino acids were used for synthesis of peptide/polymer conjugates via SPPS. Even though the technique allows a site-specific PEGylation, limitations are brought by the SPPS. In term of size, SPPS is limited up to 50 amino acids, the numerous synthetic steps and required purifications are drawbacks that restrict this approach to specialized applications. <sup>107,108</sup>

Grafting-through is a useful tool for creating comb-shape structured conjugates. In each strategy mentioned the biological material can either take the place as pendant group or main backbone. Site-specific modifications could be achieved via incorporation of PEG-amino acids during SPPS. However, the final conjugate can shows limited solubility in aqueous or organic medium due to high density of peptide materials. This explains the intricacy of this technique and makes this approach less common.

# 5. Conclusion and challenges

The three grafting procedures were highlighted in the latter part. Grafting-to is a straightforward approach but facing low yields due to difficult purifications. Yields can be enhanced using grafting-from strategy but main limitation is the absence of full polymers functionality. While grafting-through can benefits the close proximity of the peptide/protein units but shortcomings as for the size of the proteins grafted or polypeptide backbone, as well as the length of the polymers pendant groups limit the use of the technique. In all the mentioned approaches, only canonical amino acids or non-canonical functions were targeted for grafting purposes (see **Table 1** and **Table 5**). And to the best of our knowledge no work has been done on the functionalization of peptide bonds (amide function), whereas the modification of peptides on N atoms of amide groups shown great improvement in term of therapeutic properties and bioavailability.<sup>109</sup>

Additionally the amide functions were revealed by Dentzer *et al.* to be a suitable initiation site for anionic ring opening polymerization of oxiranes, but the N-H groups of peptides were never used for such an application.

A wide range of polymers were used for conjugation to a peptide/protein, a non-exhaustive list is displayed in **Table 6**. Beyond the PEG standard, vinyl based polymers generally observed a low biocompatibility. Concerning polysaccharides, the high dispersities and the heterogeneity, inherent to the product himself, make it less attractive than a synthetic polymer. Concerning PG, Du *et al.* reported better repellant properties of polyglycidol chains versus PEG for different proteins. This polymer represents a good alternative to the PEG in the *N*-modified peptide-polymers conjugates synthesis for biological applications, and opens new area in the biohybrid peptide-polymers conjugates field. <sup>110</sup>

Non-degradable synthetic polymer	Structure	Ref.
PEG (poly(ethylene glycol))	$( \sim )_n$	21,27
PVP (poly( <i>N</i> -vinylpyrrolidone)		111,112
PG (polyglycidol)		113,114
PHMPA (poly( <i>N</i> -(2hydroxypropyl) methacrylamide))	HN O HO	115,116
PNIPAAm (poly( <i>N</i> -isopropylacrylamide))	O NH	50,87
PMMA (poly(methyl methacrylate))		70
PEGMA (poly(ethylene glycol) methyl methacrylate))	t - n of of mto	69,72,80
PDMAEMA (poly( <i>N</i> , <i>N</i> -dimethylaminoethyl methacrylate))		69
PHEA (poly(hydroxyethyl acrylate))	страния осто он	86
PHEMA (poly(2-hydroxyethyl methacrylate))	t , он	51,71

**Table 6:** Polymers used for conjugation to a peptide or a protein

PMPC (poly(2-methacryloyloxyethyl phosphorylcholine)		117
POZ (polyoxazoline)	R = O $f \sim D_n$	118
Naturally degradable polymers		
Dextran		119,120
Dextrin		121,122
PSA (polysialic acid)		123,124
HA (hyaluronic acid)	HO COH OHO COH OHO NH OH OY NH	125,126

# II. Unusual chemical functions as initiators for oxiranes polymerization

## a. Introduction

Schlaad and coworker <sup>127</sup> and Dentzer *et al.* <sup>128</sup> reported the use of secondary amide functions as initiator for the AROP of epoxide monomers (**Scheme 6**). In the latter study the controlled polymerization of BO was successfully initiated by amide-based molecules. Many other initiating organic functions were reported for the AROP of oxirane monomers. Those works on organic initiating groups are paving the way for peptide/protein grafting-from conjugation at specific site and by polymers having various backbones.



Scheme 6: Polymerization of oxirane initiated by amide based initiator.

In this part, main focus will be turned to initiation of the AROP of 1,2 epoxides monomers by specific organic functions. Conditions required for a successful initiation without transfer reaction and for a controlled AROP will be reviewed. Comprehensive reviews have been published in the literature covering all aspects of oxirane polymerization, <sup>129–132</sup> herein only specific features of the AROP will be covered.

## b. General considerations

Polyoxiranes or polyethers are interesting materials with a low value of glass-transition temperature. Their specific backbone is made of a repetitive unit of 2 methylene carbons and 1 oxygen atom. They are used in a broad range of applications in fields such as biomedical, food additives, cosmetics or lubricants... Polyethers are mostly obtained by ring opening polymerization (ROP) of epoxides monomers (IUPAC: oxiranes) following three different mechanisms: cationic, anionic and metal mediated (coordination polymerization).

#### Chapter 1: Literature review

Coordination polymerization relies on the use of organometallic compounds. It is a powerful technique to synthesize high molar mass polymers, but control of the chain ends functionalization is not possible by this approach. Ionic polymerizations are of interest for precise polymerization process allowing better control over targeted  $M_n$  and dispersities. Since the cationic polymerization of oxirane monomers is producing an important amount of cyclic byproducts, due to intramolecular chain transfers during the ROP, the anionic ring-opening polymerization (AROP) is of interest for 1,2-epoxides polymerization.<sup>129</sup> In light of those considerations, this literature overview will only cover the polymerization of oxirane monomers by AROP.

For 3-members cycle monomers, the ring strain is the driving force of the ROP. The strain is mainly due to distorted angle between bonds, bond stretching or compression, electronic repulsion and interaction between the cycle and the substituent. For EO, the ring strain was evaluated to be  $\approx 112$  kJ.mol<sup>-1</sup>, thus rendering the polymerization thermodynamically possible. <sup>133</sup> The ability of cyclic monomers to undergo a polymerization is also driven by reaction conditions like temperature, solvent and the use of additives, such as catalysts or complexing agents.

During anionic polymerization various active species can take part in the process: aggregates, ions pairs, solvent separated ions pairs, and free ions (**Scheme 7**). Szwarc reported that each structure contributes to the propagation with his own propagating rate constant. <sup>134</sup> Generally speaking, the reactivity of the propagating species in anionic polymerization increases from aggregate being the least reactive to free ions having higher rates of propagation. Modulations of the reaction conditions aforementioned (T°, solvent, or additives) allow to tune the reactivity of the system, by modification of the deaggregation constant, separation and dissociation constant of associated structures.

For an ideal living and controlled polymerization, the reaction should occurs without transfer nor termination, which are parameters allowing to reach the targeted molar masses with a low dispersities. In 1940, Flory described the mechanism for EO polymerization via anionic ringopening polymerization (AROP). It was actually the first system satisfying the criteria for living process, having the expected molar masses and a predicted Poisson-type distribution. <sup>135</sup>



Scheme 7: Ionic species taking part in the polymerization process during AROP.

## 1. Conventional Anionic Ring-Opening Polymerization

Conventional AROP is a chain polymerization reaction divided in 3 steps (Scheme 8). The initiation consists in the nucleophilic substitution  $(SN_2)$  of the anion, leading to the formation of an alkoxide propagating center. The latter alkoxide is able to further attack an epoxide monomer giving a polyether chain. The termination step occurs after addition of a protic compound in the medium, such as diluted acidic solution, alcohol, or simply water. This mode of termination generates hydroxy end function.



Scheme 8: Anionic Ring-Opening Polymerization (AROP) of oxiranes initiated by alkali metal based initiators.

In this part, initiating systems for the conventional AROP of epoxide-based monomers will be covered. Their efficiency to lead to a controlled polymerization will be highlighted as well as their contributions to the synthesis of end functionalized polymer chains.

## a. Alkali metal derivatives as initiators

Alkali metal-based compounds are the most used initiators for the AROP of epoxides. The main requirement for such initiators is to have a high nucleophilicity capable of ring-opening the oxiranes. They are composed of an anionic specie interacting with a counter cation from the alkali metal group, where Li, Na, K and Cs are commonly reported for initiating purposes. Lithium alkoxides are not capable of polymerization of EO, due to strong aggregation propensity in ether solvents. <sup>136</sup> Kazanskii and coll. shown that initiation of a living and controlled polymerization of EO was possible using naphthalene alkali metal initiators, from Na, K and Cs. <sup>137</sup> Moreover they reported that the rate of propagation was increasing with the size of the cation Na<sup>+</sup> > K<sup>+</sup> > Cs<sup>+</sup> in THF. <sup>138</sup>

Derivatives such as alkali metal hydrides, alkyls, aryls and amines <sup>139</sup> compounds of sodium, potassium and cesium were used to produce high molar masses polymers of EO. <sup>140,141</sup> Those organic functions, mostly derivated from carbanionic and carbazyl, represent somehow a limited range of organic functions to be used as initiators.

Alkali metal alkoxides were also studied as initiating systems, because of their structural similarities with the propagating alkoxide species. <sup>142,143</sup> They are produce from the activation of an alcohol by a metal-ion, forming the initiating alkoxide. They are of particular interest for their ability to bring terminal functionality to polyethers (**Table 7**). By using a wide variety of bifunctional initiators, a range of organic functions could be inserted in  $\alpha$  chain-end position, leading to heterotelechelic polymers. Heterotelechelic polyethers can be synthesized via direct polymerization or by post-polymerization modification. The second strategy was investigated to generate amine terminated polyethers using a protected amine bearing initiator in order to avoid the transfer reaction to the amine group. <sup>144,145</sup>

By using mercapto-carboxylic acid compounds as initiators Zeng and Allen, were able to yield carboxy-functionnalized PEG. Surprisingly for such initiating agent, the polymerization proceeded via the thiolate only leaving the carboxylate function inactive. <sup>146</sup>

Functionnalized initiator	Structure	Ref.
Protected amino group	R OH N R Si N K	139,147,148
Alcene-OH	R	149
Alkyne-OH		150
Formyl-OH	ОСОН	151
Fatty acid-OH	О ОН	152
Acid cleavable funct-OH	R O OH	153
Carboxylic acid-thiol	HS () OH	146

Table 7: Metal alkoxide initiators used for the synthesis of heterotelechelic polymer by AROP

The use of metal alkoxide initiators was also extended to polyalcohol molecules for the synthesis of star-shape PEG. <sup>154,155</sup> The technique was successful for the synthesis of high MW structures having a low dispersity. The low dispersity values are explained by the high rate of proton exchange between -OH and -O<sup>-</sup> compared to the rate of propagation. <sup>156</sup> However the harsh reaction conditions of the AROP limit the scope of the multifunctional cores. <sup>157</sup>

For the polymerization of epoxides, alkali metal based and alkali alkoxide initiating systems provoke transfer reaction to the monomer as depicted in **Scheme 9**. The transfer reaction was reported for glycidyl ether and glycidyl amine derivatives using various metal alkoxide initiators. <sup>158–161</sup>

Price and coworkers also reported that the polymerization of PO was limited to PPO up to 6000 g/mol due to proton abstraction on the substituent of the monomer, leading to the formation of unsaturated species. <sup>162</sup>



Scheme 9: Transfer reaction occurring to the monomer in the AROP of PO.

## b. Tertiary amines and quaternary amoniums as initiators

In order to introduce metal-free AROP, higher nucleophilic reagents were used. Nitrogenbased initiators do not require metallic activation prior polymerization to fulfill their purpose. Tertiary amines such as benzyldimethylamine, pyridine, triethylamine or 2,4,6tris(dimethylaminomethyl)phenol, as well as imidazole are commonly used for the polymerization of phenyl glycidyl ether (PGE) as model monomer for the polymerization of polyfunctional epoxides monomers. The initiation mechanism is still unclear and two hypotheses are reported in the literature. <sup>163,164</sup> However, both suggested mechanisms result in the formation of an alkoxide propagating center, and a living polymerization process. The main disadvantages of this initiating system are the slow reaction rate, induction period and a limited molar masses due to high degree of chain transfer reactions (**Scheme 10**). <sup>165</sup>

Concerning ammonium-based initiators, tetrabutylammonium fluoride was used with GPE. The molar masses were controlled up to 4000 g/mol but achieving high dispersity values. Also the chain-ends functionality was not controlled for all the chains in the sample. <sup>166,167</sup>

#### Initiation (2 supposed mechanisms)



Scheme 10: Suggested mechanism for the epoxide AROP initiated by tertiary amines.

Those initiating systems (Alkali metal derivatives and amines-based) for the conventional AROP exhibit generally slow polymerization rates and low  $M_n$  are generally obtained. Functionality can be introduced in chain-end positions of the polymers with functional initiators. However, via direct synthesis, the organic functions introduced needs to be compatible with reaction conditions and are limited to a small range of alcohol-based molecules.

# 2. Organocatalyzed Anionic Ring-Opening Polymerization

Metal-free/organocatalyzed polymerizations are of interest for the high level of chain-end functionality that can be brought to polymers. Concerning epoxides AROP, organocatalysts acting as base are one of the most used classes of organocatalysts (**Table 8**). <sup>168,169</sup> By activation of the monomer, the initiator or both, organobases have broadened the range of organic functions used as initiator, and thus the possibility to synthesize hetero-homotelechelic polymers in a controlled manner.

Organobase	Structure	pKa [ACN]	Ref.
NHC	R∼ <mark>N∕</mark> N∕R \/	27.4-34.9	170
NHO	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	26.6-28.2	171
TiBP		33.53	172
BEMP		27.6	172
MTBD		25.4	173
DBU		24.3	174
<i>t</i> BuP <sub>1</sub>		26.9	172
tBuP <sub>2</sub>		33.5	172
tBuP₄	$ \begin{array}{c c} & & & & \\ & & & & \\ & & & & \\ & & & & $	42.7	172

Table 8: Organobases used in epoxides polymerization, pKa value are given in acetonitrile.

## a. N-heterocyclic carbenes (NHCs)

NHC are versatile compounds used in synthetic organic chemistry. They are known for their excellent capacity to be ligand for organometallic catalysts but also to be organocatalysts on their own. Their nucleophilic properties as well as Bronsted bases ability justify the increasing interest in the NHC organocatalytic reactions (**Table 8**). <sup>175</sup>

Taton *et al* were the first to introduce the use of *N*-heterocyclic carbenes for the polymerization of oxirane –based monomers. In their works the NHC compound is sufficiently nucleophilic to play the role of initiator for the AROP of EO. The reaction of the NHC with the epoxide gives an imidazolium alkoxide capable of further reaction with EO. Chain end functionality was introduced by attack of a termination agent to yield  $\alpha,\omega$ -difunctionalized PEG chains. <sup>176</sup> Via this approach they were able to bring azide, hydroxyl, benzyl organic function in  $\alpha$  position.

This initiating system was successful for the synthesis of poly(EO) in a living and controlled manner, but for PO polymerization the system was limited to small MW polymers due to transfer reactions to the monomer. <sup>177</sup>



Scheme 11: Mechanism for EO polymerization initiated by NHC and additives.

NHCs could also be used as organocatalyst with a chain regulator (Nu-E) which also served as functionalization agent. Compared to NHC initiated AROP, the amount of NHCorganocatalyst is significantly reduced and the polymerization does not require long quenching time. The mechanism is still unclear and two pathways are hypothesized: **Scheme 10** (A) the initiation is performed by the NHC and Nu-E is involved in a reversible exchange with the propagating center releasing dormant species, **Scheme 10** (B) the initiation is realized by the activated Nu-E via interaction between the NHC and the E. This technique using NHCs as catalyst lead to the formation of heterodifunctionalized poly(EO) chains, with Nu in  $\alpha$  position (azide, propargyl, benzyl) and E in  $\omega$  position (hydroxy, trimethylsilyl).<sup>178</sup>

The use of NHCs is an interesting strategy for the polymerization of EO in a living/controlled manner and for the functionalization of chains-ends during the polymers synthesis. However, the polymerization of other oxiranes was not reported and the initiating system shown limitations to reach high MW poly(PO) due to transfer reactions.

## b. N-heterocyclic olefins (NHOs)

Because of their higher nucleophilicity than NHCs, NHOs (**Table 8**) <sup>179</sup> were used to organocatalyzed the PO polymerization. Dove and coworkers used NHO catalysts with benzyl alcohol to initiate the polymerization of propylene oxide following the same mechanism described for NHCs. They reported the synthesis of high MW polymers with highly controlled chain-end functionality. <sup>180</sup>

However, the sensitivity of the NHO catalyst to moisture and temperature, and the fact that they are not commercially available make their use rather difficult for the moment.

NHCs and NHOs employed as organocatalysts are interesting for the controlled polymerization of oxirane monomers into polymers, besides it is also a great strategy to introduced functional groups at chain-ends. Although, such *N*-heterocyclic bases are limited to EO and PO AROP and to linear architectures.

## c. Phosphazene bases (*t*BuP<sub>n</sub>)

Phosphazene bases are a family of extremely strong uncharged Brönsted bases, containing at least a central phosphorous atom bonded to four nitrogen atoms of three amines and one imine substituent. Most of these bases were synthesized by Schwesinger, and he defined their nomenclature based on the number of unit P=N- encounter in the bases (see Scheme 12 and Table 8).<sup>172</sup>



Scheme 12: General formula of phosphazene bases.

The main feature of phosphazene bases is their high basicity, which increase with the number of P=N- units as demonstrated in **Table 8**. This behavior is explained by the increasing delocalization of the charge within the phosphazenium cation. They are capable of generating highly reactive and stable anions with soft phosphazenium counter cation. <sup>181,182</sup> As a consequence the *t*BuP<sub>n</sub> are of interest to transform weak protic functions into a sufficiently nucleophilic reagent.

Additionally, those uncharged superbase are highly tolerant to oxidation and hydrolysis, <sup>183</sup> exhibit an elevated thermal resistance, <sup>182</sup> and are soluble in apolar solvents (Hexane, THF, toluene...) as well as more polar solvents (ACN, DMSO, DMAC...).

However, phosphazene bases are hygroscopic reagents and have to be stored consequently, besides, even though they are commercially available, they still exhibit a high price for such compound.

Phosphazene bases introduced a way to have access to a broad range of polymers from vinyl and heterocyclic monomers. But also extend the scope of macromolecular engineering into complex macromolecular structures. <sup>184,185</sup> Herein only epoxides monomers will be covered due to the interest we had into synthesizing functional polyethers.

In ionic polymerization phosphazene bases can be used according to two strategies, (i) the complexation of the metal counter ion or (ii) the deprotonation of an acidic co-initiator.

## i. Phosphazene bases as complexing agents of Li

The complexing strategy is usually applied to lithium alkoxide initiators, which are unable to initiate the AROP of epoxides monomers by themselves. The addition of  $tBuP_n$  in the medium complexes Li cations and efficiently overcomes the strong aggregation of the propagating ion pairs, and thus favor the propagation. The role of  $tBuP_n$  are the same as cryptates and crown ether reported by Boileau <sup>186</sup> and Penczek <sup>187</sup>.

Möller and Esswein were the first to report the EO polymerization from BuLi:*t*BuP<sub>4</sub> as initiating system, in a living and controlled manner.<sup>188</sup>

From this work many other organolithium compounds were used as initiators combined with  $tBuP_4$  as complexing agent, ranging from small molecules to lithium terminated polymers (**Table 9**). The initiating systems based on RLi: $tBuP_n$  are efficient for the polymerization of various oxirane-based monomers without transfer reactions.

Monomer	Co-initiator	Ref.
EO	Bu-Li	188
EO	sec-Bu-Li tBuO-Li DPH-Li	189
EO	PB-Li PI-Li	190
EO	PB- <i>block</i> -PI-Li	191
EEGE	DPMP-Li	192
EGE/GME	PVP- <i>block</i> -PEO-Li	193

Table 9: AROP of epoxides monomers from alkyl lithium : *t*BuP<sub>4</sub> as initiating system.

This strategy to use  $tBuP_n$ , as complexing agent, still requires the activation of the initiator by an organolithium compound. Since metal-free syntheses are favored for electronic applications, the use of phosphazene bases as deprotonating agent seems to be a straightforward approach.

#### ii. Phosphazene bases as deprotonating agents

As already mentioned, phosphazene bases are highly basic reagents which are able to turn a weak acid into a highly reactive anion.  $tBuP_n$  can generate *in situ* an anionic initiator by deprotonation of acidic organic function which can further react with an epoxide monomer. Amongst the acidic functions used as initiators, alcohols are the most reported due to their prevalence in organic compounds. Other weak acid functions were used for initiating purpose, such as primary and secondary amides, alkynes and more readily accessible acids such as carboxylic acids. Using phosphazene bases as co-initiator allow the direct synthesis of highly functional polyethers and extend the scope of chemical functions used as initiator.

• Alcohol-initiated AROP

The range of alcohol-containing organic molecules is vast, and a wide range of compounds was used as initiators combined with phosphazene base (see **Table 10**). As reported by Misaka and coworkers, only the  $tBuP_4$  is capable of polymerizing epoxides in a reasonable amount of time, <sup>194</sup> while  $tBuP_2$  exhibits a low rate of polymerization for EO, <sup>195</sup> and other milder bases such as BEMP, TiBP and MTBD didn't show any capacity in promoting the polymerization from an alcohol. The use of 1 equivalent of  $tBuP_n$  regarding the ROH initiator is an efficient ratio for alcohol initiation. However, thanks to a fast rate of exchange reaction between alkoxide and alcohol, the quantity of  $tBuP_4$  can be lowered to 0.01 equivalents. <sup>196</sup> Using minimal amounts of phosphazene base are only lowering the reactivity of the initiating system, and thus increase polymerization rate, while preserving the integrity of the initiator. <sup>194,197</sup>

Recently conducted kinetic experiments in our group, allowed us to determined that benzyl alcohol: $tBuP_4$  is generating propagating ion pairs. From this initiating system, we demonstrated that a wide variety of epoxide-based monomers could be polymerized with controllable  $M_n$  and low  $M_w/M_n$ .<sup>198</sup>

Mono-alcohol functional initiators promoted by  $tBuP_4$ , were able to initiate the synthesis of heterotelechelic polyethers. Even biological molecules such as terpenes alcohol were used for a grafting from synthesis of PEG chains. Zhao *et al* reported the polymerization of PEO initiated by terpene alcohol molecules such as cholesterol. The initiating system terpene: $tBuP_4$  leads to an efficient initiation of EO polymerization in a living and controlled manner.<sup>197</sup>

Initiator	Monomer(s)	Ref.	
Linear polymers			
— — ОН	EO	199	
Н₃С—ОН ∕∕∕∕ОН	EO	200	
Л ОН	Glycidyl amine derivatives	201	
	BO, PO, EEGE, BnGE, tBuGE	194	
Terpene-OH	EO	197	
ОН	PO, BO, <i>t</i> BuGE, AGE, EEGE, BnGE, azidoalkyl glycidyl ethers	198,202	
OH	Glycidyl azido based monomers	203	
но	PG-block-PBO	110	
Star-shape macromolecules			
НО ОН НО ОН	РВО	204	
НОСОН	PBO- <i>b</i> -PEO- <i>b</i> -polyester	205	
но он	РЕО	200	
Cyclodextrin $HO \rightarrow O \rightarrow$	PBO and PBO-block-PEEGE	206	
Brush like architecture			
стр ОН	PEO, PEO- <i>block</i> -PPO,	207–209	

# Table 10: Alcohol:tBuP<sub>n</sub> initiated AROP of epoxide monomers

The use of phosphazene bases was extended to multifunctional alcohols molecules, allowing the synthesis of well-defined macromolecular architectures such star-shape polymers.  $^{200,204,205}$  For example Faye and coworkers described the synthesis of polyethers chains initiated by modified  $\beta$ -cyclodextrin. 14 OH functions were efficiently promoted by *t*BuP<sub>4</sub> to act as initiators for the AROP of BO or EEGE, giving 14-arms star macromolecules.  $^{206}$ The grafting-from approach was also applied to polymer backbone leading to brush like copolymers.  $^{208,209}$  OH functions of poly(*p*-hydroxystyrene) were used as initiators with *t*BuP<sub>4</sub> for the polymerization of EO and/or PO, yielding a high grafting density with high molar masses and low dispersities.  $^{208}$ 

• Carboxylic acid initiated AROP

COOH organic functions are far more strong acid than the previous mentioned OH groups. Reaction of epoxides with carboxylic acids is well known for the production of ester functions. Though, the presence of protic compounds in the solution precludes the polymerization due to re-protonation of the generated alkoxides.<sup>210</sup>

Hadjichristidis and coworkers used carboxylic acid with  $tBuP_4$  to initiate the AROP of EO. Characterization of the polymers revealed that a fast transfer reaction to the initiator COOH and to the ester functions newly formed was occurring. Those reactions lead to the formation of diester by-products and a mix of hetero-homotelechelic polymers.<sup>196</sup>

• Amide initiated AROP

Nitrogen containing functions such as primary and secondary amides were used as initiators combined with phosphazene base. Dentzer and coworkers reported the synthesis of heterotelechelic poly(BO) chains. The synthesis was achieved using various secondary amide-based molecules as initiator and  $tBuP_4$  as co-initiator. Proper reactions conditions were able to reach control of the molar masses and low dispersities.

Additionally to linear structures, brush-like macromolecular structures were designed using poly(N,N-dimethylacrylamide-co-acrylamide) and poly(NIPAM) as initiating scaffold. The primary amides and secondary amides of the polymer backbone were successfully deprotonated, thanks to  $tBuP_n$ , and used as initiators for the AROP of EO as pendant chains. <sup>127,211</sup>

Concerning poly(NIPAM)-g-PEO, dispersity remain fairly low for the grafted structures and thermoresponsive properties were tuned regarding the density of PEO chains grafted along the polymer backbone. However, precise information about the grafting density were not provided in related works.

Alcohols, amides, and carboxylic acids were reviewed in this chapter due to their commonly presence in peptide chemistry but other organic function were used as initiator such as thiols  $^{212,213}$ , CH acidic compounds  $^{214}$  or  $\alpha$ -methylbenzyl cyanide  $^{199}$ ...

Phosphazene bases-promoted AROP revealed itself to be successful for the polymerization of various epoxide-based monomers, from various types of low acidic organic groups. This technique was also highly efficient for the synthesis of well-defined macromolecular structures which could not be reached with other organobases or via conventional AROP. However, transfer reactions to monomers have been reported, which limit the molar masses for EEGE, <sup>158</sup> and some highly protic groups such as carboxylic acid lead to an uncontrolled end-chains functionality.

## 3. Monomer-activated AROP

The polymerization of epoxide monomers to synthesize high molar masses macromolecules, without transfer reactions, is a difficult task to achieve by conventional AROP and organocatalyzed AROP. For instance, PO polymerization suffers generally from transfer reaction from the monomer and molar masses are usually limited around 10 000 g/mol. Using a Lewis acid combined with an AROP initiator allows the synthesis of high  $M_n$  PPO in a controlled manner with fairly narrow dispersities.<sup>215</sup>

## a. Principle and advantages

This new synthetic approach using a Lewis acid is named monomer-activated AROP and relies on a 3 molecular initiating system: anion-counter cation-Lewis acid (**Scheme 13**). In such conditions, the Lewis acid is involved in formation of complexes with the anion/alkoxide propagating center, which leads to a significant decrease of the reactivity of anionic species.

Excess of Lewis acid compound in the medium activates the oxirane by complexation of the oxygen of the heterocycle, resulting in an electron withdrawing effect on the  $\alpha$ -carbons, making them more prone to nucleophilic attack by the complexed anionic species.<sup>216</sup>



Scheme 13: Monomer-activated AROP, combining phosphazene base, protic initiator and triisobutylaluminum for the synthesis of poly(oxirane).

Billouard *et al* were the first to report the combination of alkali metal alkoxide initiator with Lewis acid for the polymerization of epoxides. By using *i*-Bu<sub>3</sub>Al (TiBA) as Lewis acid they were able to favor propagation of PO over transfer to the monomer, yielding high  $M_n$  polymer in a short matter of time. PO polymerizations exhibited kinetic of 0 order regarding the monomer concentration. Besides, they achieved control of the molar masses up to 24000 g.mol<sup>-1</sup> with low  $M_w/M_n$ .<sup>216</sup>

Other initiating systems like onium salts, were used for a faster polymerization than the ones initiated with metal alkoxide initiators. Onium salts + triisobutylaluminum were able to bring  $\alpha$ -functionality on the polymer chains like halogen atoms or azido functions, and to control the polymerization of various epoxides as shown in **Table 11**.<sup>215,217–219</sup>

Many epoxides, bearing organic function having a strong electron withdrawing effect on the side chain, were not able to polymerize under classical AROP conditions. Such monomers like epichlorohydrin (ECH)<sup>220</sup> or epicyanohydrin (EPICH),<sup>221</sup> are subject to preponderant transfer reactions to the monomer rather than polymerization. But association of highly nucleophilic initiator and TiBA allowed the (co)polymerization of those monomers.

Concerning copolymers, Frey and coworkers reported the influence of the AROP mechanism on reactivity ratios of EO and glycidyl ether monomers. Conventional AROP yield random copolymers, while the use of a Lewis acid decreases the reactivity of glycidyl ether and produce tapered structures. <sup>222–225</sup>

Monomer	initiator	Ref.
$\bigtriangledown$	NR <sub>4</sub> X	217,218
$\bigtriangledown$	$NR_4X$	218,220
√° <sup>−</sup> ° <sup>−</sup>	$NR_4X$	217,219
v o k	$NR_4X$	219
	$NR_4X$	223,226
	$NR_4X$	227
CI	$NR_4X$	217,220
C ≈N	$NR_4X$	221
	$NR_4X$	224
$ \begin{array}{c c} & & & \\ & & \\ \hline \\ & & \\ & \\ & \\ & \\ & \\$	MePPh <sub>3</sub> Br	228
	MePPh <sub>3</sub> Br	225

Table 11: Monomer-activated AROP of various oxiranes based on onium salt initiating system

Monomer-activated AROP is a great strategy to synthesize high molar mass polymers from a broad range of epoxide monomers. Besides the high compatibility with various organic functions born by oxirane, the macromolecules synthesized exhibit a highly controlled structure. However, results obtained with initiating systems based on organic salts + TiBA can be extended using the advances made in the fields of organocatalyzed AROP.

Organocatalyzed AROP by organobases can be combined with the monomer-activated strategy to achieve a rapid and controlled polymerization in mild conditions.

Very recently NHC catalyzed AROP, which was limited to EO polymerization, has shown improvements to polymerize PO in a controlled fashion, when combined with *i*-Bu<sub>3</sub>Al. <sup>229</sup>

Dialkyl magnesium and Grignard reagents were also used as bases for deprotonation purposes, and associated with *i*-Bu<sub>3</sub>Al to control the polymerization of epoxides. But a limited control over the molar masses could be achieved and the polymerization was facing transfer reactions, leading to the formation of by-products. <sup>230,231</sup>

Highly basic phosphazene bases were combined as well with TiBA to control the polymerization of BO, from alcohol initiators as reported by Carlotti and coll. <sup>197</sup> Secondary carbamates were also used as initiators. Actually, under highly basic conditions carbamate molecules undergo a deprotonation and a self-dissociation. The addition of *i*-Bu<sub>3</sub>Al in the medium leads to the stabilization of the carabamate by interaction with the carbonyl group, which impedes his self-disruption (**Scheme 14**). <sup>232</sup>



Scheme 14: AROP mechanism of BO with carbamate initiator and tBuP<sub>4</sub>.

The combination organobase and Lewis acid allowed the polymerization of new monomers like glycidyl phtalimide. The initiating system ROH:*t*BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al was efficiently applied to the first reported AROP of glycidyl phthalimide. <sup>233</sup>

As demonstrated in the few aforementioned examples, *i*-Bu<sub>3</sub>Al is the most used Lewis acid in the monomer-activated AROP. Other Lewis acids based on aluminum such as Et<sub>3</sub>Al were used, but did not exhibit as good efficiency as TiBA to produce high molar masses polymers in rather short matter of time. In all the TiBA-related work an increase of the dispersity values can be observed compared to experiment without Lewis acid, indicating a loss of control. And combination of organobases with *i*-Bu<sub>3</sub>Al can lead to transfer reaction to the Lewis acid like hydride initiation. Those issues are related to, what we assume to be linked to, the low stability of TiBA-based complexes. Improvements could be achieved by investigation of other Lewis acids to control the monomer-activated AROP. <sup>198,232</sup>

## a. Influence of the Lewis acid's chemical structure

Compared to alkylaluminum, organoborane compounds are fairly stable Lewis acid used in ring-opening polymerization reaction. <sup>234</sup> Borane-based Lewis acids offer the possibility to initiate and control polymerization reactions via metal-free chemical tools. Et<sub>3</sub>B (TEB), a mild Lewis acid, was used instead of TiBA and had shown impressive results in the organocatalyzed monomer-activated AROP of oxiranes. <sup>235</sup> Gnanou *et al.* reported that TiBA was reacting with the monomer glycidyl azide (GA) impeaching its polymerization. Instead they introduce TEB as Lewis acid catalyst in the reaction medium. By changing the Lewis acid they were able to successfully initiate the polymerization of GA and synthesize high molar masses poly(glycidyl azide) up to 11 000 g.mol<sup>-1</sup> with low dispersities  $M_w/M_n \le 1.2$ . <sup>236</sup>

On a mechanistic point of view, the organoborane Lewis acid is suggested to have the same role as *i*-Bu<sub>3</sub>Al. Based on this consideration, the kinetic order towards [M] would be the same between TiBA and TEB assisted polymerizations. However, a 1<sup>st</sup> order kinetic regarding the monomer concentration was obtained with Et<sub>3</sub>B. <sup>235,237,238</sup> To date these unexpected results remain unexplained and further mechanistic investigations needs to be pursued to clarify the precise role of the organoborane Lewis acids.

TEB was used with organobases, such as NHC <sup>238</sup> and  $tBuP_n$ . Surprisingly, less basic organocatalysts such as DBU and MTBD were also reported for a successful polymerization of epoxide monomers in a controlled manner. <sup>235</sup> Poly(PO) up to 210 kg/mol were obtain with a  $M_w/M_n = 1.07$  with no sign of transfer reactions.

Very recently the AROP of epoxides monomers by COOH initiation was revealed to be efficient, when assisted with phosphazene bases and TEB. Chen *et al.* combined RCOOH:*t*BuP<sub>2</sub>:Et<sub>3</sub>B to initiate the polymerization of PO, BO and AGE. Polymerization reactions were occurring without transesterification side reactions to the polymer backbone, to yield heterotelechelic polyethers with cleavable end groups.<sup>237</sup>

Unlike boron based Lewis acid, to the best of our knowledge no Lewis acid "strength values" were determined for aluminum based systems. <sup>239</sup> Thus the comparison of Lewis acidity between different compounds from Al or B is only based on reactivity data, from different reported polymerization initiating systems using Lewis acid as catalyst.

# **Conclusion and objectives**

In this part we discussed the polymerization of oxirane monomers through anionic ringopening polymerizations. We highlighted that the organocatalysis of the AROP was as successful method for the polymerization of epoxide monomers in a living/controlled manner. The use of phosphazene bases has shown great improvements in generating highly reactive anions from weak acid organic functions, such as alcohols and more interestingly amides. Those reactive anions were used as initiators and have proven to be powerful to polymerize a wide range of monomers in a control manner. While the use of superbases have broaden the scope of organic functions used as initiator, the introduction of a Lewis acid such as TiBA and TEB reduced the reaction time and increase the targeted  $M_n$  via the monomer-activated AROP. This technique allowed a specific suppression of the transfer reactions to the monomer and the initiator, giving access to the synthesis of high molar masses and welldefined difunctional polymers. By permitting anionic ring-opening polymerizations from amides or carboxylic acids functions, those reaction conditions render possible the controlled synthesis of polyethers from organic functions ubiquitous in peptides and proteins, opening a new way to perform divergent synthesis of conjugates.

Those AROP techniques to improve control of the process will be applied for the first time to peptides initiators for grafting-from of polyether chains. Using phosphazene bases on amide functions, we wish to generate highly reactive amidates to initiate the polymerizations of epoxide monomers without transfer reactions and in a controlled fashion.

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# — CHAPTER 2 —

# Synthesis of conjugates from small cyclic dipeptides: asymmetric DKPs as initiators

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## Introduction

The use of amide functions as initiators for oxiranes AROP in the presence of  $tBuP_4$ , was demonstrated five years ago by our group. Extension of this seminal work to biomacromolecules is a continuity of this work, and the peptide family represents a natural opportunity. In this part, we will address the functionalization of amides on small cyclic peptides, 2,5-diketopiperazines (DKP). DKP molecules were chosen based on their simplicity, to serve as model compound compared to large proteins. The advantage of the ring structure is the absence of amine and carboxylic acid terminal groups. Parasitic initiations from those organic functions are prevented, and NH amides are the only potential initiating sites.

For DKPs, the methylation on amides is usually achieved by sodium hydride base. <sup>1</sup> When alkali hydrides are not efficient for the alkylation of the NHs amide, the use of mild phosphazene bases, like BEMP, was reported to be useful on the DKP ring in either DMF or DCM with an alkyl bromine reagent. <sup>2</sup>

Based on the apparent stability of the bioactive material in strong basic conditions, we proposed in this first part to synthesize peptide-polymer conjugates from DKPs using phosphazene bases as co-initiator.

In the present part reaction conditions for the polymerization of oxiranes initiated by DKP will be developed in order to better control the AROP process.

First, focus will be made on proline-based DKP peptides as initiators having only one accessible amide function for functionalization. The aim is to test the ability of the simplest initiating system DKP-proline: $tBuP_n$ , to control the polymerization of epoxide monomers.

Then complexity of the system will be increased by having asymmetric DKPs displaying two amide functions. The introduction of bi-functional initiators raises the question of mono or biinitiation of oxirane monomers in AROP conditions.

For each part careful characterization by NMR, SEC, ATR-FTIR, and MALDI-TOF will be covered for the detection of side reactions.

# I. Proline-based DKPs as initiators for the AROP of epoxide monomers

### 1. Conventional AROP promoted by t-BuP<sub>4</sub>



Scheme 1: Polymerization of BO initiated by proline-based DKP.

In this first part, 4 different 2,5-diketopiperazines were used to evaluate the initiating capacities of the amides in DKP (**Scheme 1**). Proline based-DKPs are having one amide function susceptible of AROP initiation. To focus on the specific initiation by the secondary amide functions, the side chains of the second amino acid residue is an aliphatic hydrocarbon chain. It exists a large choice of hydrocarbon side chains, different by their numbers of carbon atoms and their architectures. Therefore it is an opportunity to investigate the influence of the side chain nature on the rate of polymerization. Cyclo(Glycine-Proline), cyclo(Alanine-Proline), cyclo(Leucine-Proline), and cyclo(Phenylalanine-Proline) disclosed in **Scheme 2** were used with increasing steric hindrance and hydrophobicity around the NH function.



Scheme 2: Cyclic proline-based dipeptides

Compound	pKa value (DMSO)	Ref.		
tBuP <sub>4</sub>	30.2	3		
tBuP <sub>2</sub>	21.5	3		
	11.3-11.5	Estimated values from MarvinSketch 20.8 software		

**Table 1:** Comparative table of pKa values

pKa values shown in **Table 1** suggest that both phosphazene bases  $tBuP_2$  and  $tBuP_4$  are likely to generate a reactive anion from deprotonation of the NH amide function, then able to initiate the AROP of epoxides.

As aforementioned, amide functions combined with  $tBuP_4$  in toluene were successfully used as initiators for the anionic ring-opening polymerization of BO in a living and controlled manner. <sup>4</sup> 2,5-diketopiperazines are hydrophilic peptide materials and exhibit a low solubility in organic solvent, thus THF was used as solvent due to is higher polarity than toluene. BO was used as model monomer to optimize the reaction conditions, because BO is regarded as rather safe monomer and was proven to be efficiently polymerized from amide-based initiators. <sup>4</sup> Given the fact that the BO AROP was successfully initiated at [Amide]/[ $tBuP_4$ ] ratio = 1 at 25°C, the same conditions were applied to proline-based DKPs.

The four cyclic peptides were used as potential initiators in AROP conditions, as already reported. <sup>5</sup> The polymerization conditions and results are gathered in **Table 2**.

A polymerization reaction is occurring regardless the used DKP. Furthermore, a high conversion was reached within 24 hours for C(A-P), C(G-P) and C(L-P). But the reaction was slower for C(F-P), as two days were necessary to reach almost full conversion. This effect can be assigned to the cumbersome environment generated by the aromatic ring substituent of the Phe side chain.

Run	Initiator	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub>	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	<i>M</i> <sup>n</sup> <sup>b</sup> <sup>13</sup> C NMR (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
P1-1	C(A-P)	30/1/1	24	95	2200	2500	2500	1.13
P1-2	C(G-P)	30/1/1	24	95	2200	n.d.	2300	1.16
P1-3	C(L-P)	30/1/1	24	93	2200	3400	2800	1.20
P1-4	C(F-P)	30/1/1	46	95	2250	3500	2900	1.25

**Table 2:** Polymerizations of BO initiated by Proline-based DKPs: $tBuP_4$  (1:1), at 25°C in THF,  $[M]_0 = 2M$ .

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.
<sup>b</sup>: Determined by quantitative <sup>13</sup>C NMR in CDCl<sub>3</sub>.
<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For DKPs with less hindered cycles, P1-1 and P1-2, SEC elugrams display a symmetric and monomodal peak (see Figure 4). For the two experiments P1-1 and P1-2, a good agreement between the experimental molar masses, given by SEC and quantitative <sup>13</sup>C NMR, and the theoretical  $M_n$  is obtained. This observation on  $M_n$  is suggesting that no side reactions are occurring during the polymerization. For the determination of  $M_n$ , regular <sup>1</sup>H NMR could not be used due to overlapping of the signals on spectra. See appendix for a typical determination of  $M_n$  via <sup>13</sup>C NMR in Figure A4. Those preliminary results for P1-1 and P1-2 suggest that the polymerizations were quantitatively initiated by our system (DKPs:*t*BuP<sub>4</sub>).

<sup>1</sup>H NMR spectrum in Figure 1 and <sup>13</sup>C NMR spectrum in Figure 2 for P1-1, display signals from the cyclic peptide with signals from the polymer chain even after passing through alumina. The high polarity of alumina suggests that un-functionalized DKPs are likely to be retained during the filtration. Additional NMR spectra of P1-2, P1-3 and P1-4 can be seen in appendix Figure A5, Figure A6, Figure A7, Figure A8, Figure A9 and Figure A10.

For the more hydrophobic DKPs, P1-3 and P1-4, experimental  $M_n$  values are higher than the theoretical  $M_n$  and dispersities tends to be quite low ( $M_w/M_n = 1.20 - 1.25$ ). Also, it can be noted that  $M_n$  NMR are higher than  $M_n$  SEC, suggesting the occurrence of side reactions. This indicates that all the polymer chains are not functionalized with a DKP.







A comparative kinetic study was made with C(G-P) and C(F-P) (see **Figure 3**). For both DKP, the kinetic plots exhibit an induction period at the beginning of the polymerization. This effect is related to a slow initiation stage, assigned to aggregation of the anionic amidate<sup>+</sup> $HtBuP_4$  complexes due to their low solubility in THF. After insertion of few monomer units, the system become soluble and allows deaggregation. Then a linear dependence can be noted of the logarithmic concentration with time during the rest of polymerization process, proving a first order dependence regarding the monomer concentration (**Figure 3**). This result is in agreement with other reported works of AROP initiated by combination of a protic organic function and phosphazene base  $tBuP_4$ . <sup>4,6</sup> Additionally, from the slope of each plot we can deduce that the rate of polymerization is higher for C(G-P) than the rate for C(F-P). This difference can be explained by the more hindered environment around the NH amide in C(F-P) than in C(G-P) explaining a lower accessibility and lower initiation efficiency. A difference of the propagating rate of both polymerizations using different DKPs initiators is however surprising. More experimental data and computational simulation should be undertaken to fully explain this observation.



Figure 3: Comparison of  $\ln([M]_0/[M]) = f(t)$  curves for the BO polymerization initiated by C(G-P) and C(F-P) in THF at 25°C.

On the SEC traces of the two latter experiments, a slight tailing towards the small molar masses can be observed for C(F-P)-poly(BO) (**Figure 4**). Both results from kinetic and SEC agree to indicate that the initiation in the case of C(F-P) is slow.



Figure 4: SEC traces comparison for the poly(BO) polymers initiated by cyclo(Gly-Pro) and by cyclo(Phe-Pro) recorded in SEC THF.

MALDI-TOF analyses of the samples from **Table 2** were carried out. For all the samples, the expected population was detected. Example spectrum of P1-1 in **Figure 5** exhibit a major distribution assigned to a DKP initiated poly(BO) chains with hydroxy function in  $\omega$  position. Spectra of P1-2, P1-3 and P1-4 can be seen in appendix in **Figure A11**, **Figure A12**, and **Figure A13**, respectively.



Figure 5: (a) MALDI-TOF spectrum recorded in reflectron mode and (b) zoom in area for P1-1.

An additional population was detected by MALDI-TOF of P1-1, suggesting the occurrence of side reactions. One side reaction, that we hypothesize, is the transesterification reaction of a propagating center on a DKP ring. We assume that the alkoxide reactive center is nucleophilic enough to attack the carbonyls of the cyclic peptide initiator, leading to the opening of the ring and formation of an ester bond in the polymer backbone (**Scheme 3**).



Scheme 3: Suggested reaction mechanism for the opening of the DKP ring.

The transesterification reaction considered gave theoretical mass values which correspond to a population observed on P1-3 and P1-4 spectra, but not on the mass spectrometry analysis of P1-1 in **Figure 5**. This observation suggests that side reactions may depend on the chemical nature of the used DKP. Transfer reactions to the monomer were also considered but no agreement was found between experimental masses and calculated values. See in appendix for suggested additional populations and masses list in **Figure A1**. The population issued of side reaction in P1-1 could not be identified, however a DKP ring-opening reaction is occurring when the initiating DKP are C(L-P) and C(F-P) in these polymerization conditions.

In order to suppress those side reactions, optimizations from the initial procedure will be made in the following part. Main efforts will be directed to the C(A-P) due to the neutral substituent of Ala residue. Alanine is used in biology for determination of the role of the amino acids side chains on the function and stability of peptides. The method is called Alanine mapping and consists in the replacement of amino acids by Ala residues.<sup>7</sup>

### 2. Monomer-activated AROP from proline-based DKPs

#### a. Role of *i*-Bu<sub>3</sub>Al and mechanism of the polymerization

The use of trialkylaluminum as Lewis acid during the anionic ring-opening polymerization of epoxides monomers has already been reported through the monomer-activated AROP. For the PO polymerization via alkali metal alkoxide-initiated AROP, the addition of *i*-Bu<sub>3</sub>Al leads to the inhibition of the transfer reaction to the monomer, and the promotion of the propagation. <sup>8</sup> Alkylaluminum Lewis acid was reported to operate by complexation of the propagating center and the monomer, decreasing the reactivity of the alkoxide and activating the epoxide. In the use of carbamates as initiators with *t*BuP<sub>4</sub>, triisobutylaluminum shown a good efficiency to stabilize the carbamate and suppress the side reactions. <sup>9</sup> In this part the solvent has to be switched to MeTHF to avoid any competitive complexation of THF by the aluminum compound. <sup>10</sup>

**Table 3** gathers the polymerizations of BO initiated by C(A-P) in monomer-activated AROP conditions.

Run	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [ <i>i</i> -Bu <sub>3</sub> Al] <sub>0</sub>	T (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>13</sup> C NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>c</sup>
P2-1	30/1/1/1	25	158	0	-	-	-	-
P2-2	30/1/1/2	25	3	100	2300	4100	3900	1.39
P2-3	30/1/1/3	25	3	100	2300	4600	5200	1.52
P2-4	30/1/1/2	10	17	100	2300	3800	4000	1.36
P2-5	30/1/1/2	0	3	100	2300	4100	4000	1.45
P2-6	30/1/1/2	-10	69	100	2300	3400	2300	1.53
P2-7	30/1/1/2	-20	15	100	2300	4500	4300	1.45
P2-8 <sup>d</sup>	30/1/1/2	0	23	100	2300	5200	5300	1.31
P2-9 <sup>d</sup>	30/1/1/2	-20	20	96	2200	4100	4200	1.47
P2-10	30/1/1.25/2	25	1	100	2300	4000	3900	1.33
P2-11	30/1/1.25/2	-20	15	94	2200	4500	3600	1.49
P2-12e	30/1/1.25/2	25	1	100	2300	3000	3100	1.33
P2-13 <sup>f</sup>	30/1/1.25/2	25	1.5	100	2300	3200	3200	1.32
P2-14 <sup>g</sup>	30/1/1 25/2	25	1.5	100	2300	2500	2800	1 35

**Table 3:** Polymerizations of BO initiated by C(A-P) using *t*BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al as catalytic system, in MeTHF;  $[M]_0 = 2M.$ 

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by quantitative <sup>13</sup>C NMR in CDCl<sub>3</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

<sup>d</sup>: Stepwise monomer addition 25µl/h.

As already reported, 1 eq of the aluminum compound is demanded by the anionic center to form an initiating aluminum-anion-counter cation complex (Scheme 4). To trigger the polymerization, an excess of *i*-Bu<sub>3</sub>Al is needed to activate the monomer by O coordination, increasing the reactivity of the methylene C atom on the oxirane ring.

The polymerization of BO initiated by the initiating system  $[C(A-P)]_0/[tBuP_4]_0$ : 1/1 in the presence of 1 equivalent of *i*-Bu<sub>3</sub>Al did not occurred.

Then the amount of Lewis acid was raised to 2 and 3 equivalents. Poly(BO) polymers could be synthesized within 3 hours. Much faster reaction rates were observed compared to the reaction performed without trialkylaluminum, highlighting the activation effect of the Lewis acid. P2-2 SEC trace in Figure 6 shows a monomodal signal with a tailing towards high retention volumes.

<sup>e</sup>: Dropwise monomer addition at 25°C.

<sup>f</sup>: Dropwise monomer addition at -20°C.

<sup>g</sup>: Dropwise *i*-Bu<sub>3</sub>Al addition at -20°C.

The same result was observed for P2-3. For P2-2 and P2-3 the experimental molar masses are higher than the theoretical values and dispersities are broader ( $M_w/M_n = 1.39 - 1.52$ ) than experiments performed without *i*-Bu<sub>3</sub>Al ( $M_w/M_n = 1.13 - 1.25$ ). This broadening effect of monomer-activated AROP on the *D* was reported in a comparative kinetic work conducted recently.<sup>11</sup>

MALDI-TOF spectrum of P2-2 displayed in **Figure 6** exhibits a single population being poly(BO) chains end capped by C(A-P) and hydroxy function. The addition of *i*-Bu<sub>3</sub>Al sufficiently reduces the nucleophilicity of the propagating center to impeach occurrence of side reactions. Observations made from SEC, MALDI-TOF and <sup>13</sup>C NMR analyses suggest a slow initiation process. It was reported by Louis Gold that high value of  $k_p/k_i$ , for low  $X_n$ , induces a low initiation efficiency and an increase of the dispersity. <sup>12</sup> In comparison, the reactivity of the amidate seems to be more affected than the nucleophilicity of the alkoxide leading to a higher  $k_p/k_i$  ratio e.g.  $[(k_p/k_i)_{TiBA} > (k_p/k_i)_{no TiBA}]$  causing a broadening of the dispersity.



Figure 6: Characterization of P2-2, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

#### Chapter 2: Asymmetric DKPs

For  $[i-Bu_3A1]/[C(A-P)]$  ratios  $\geq 2$ , the exclusion of side reactions, allow to synthesize macromolecules fully functionalized by DKP at one end and a hydroxy function at the other. However, the control of the polymerization needs to be improved in order to decrease the discrepancy between measured and theoretical molar masses and the broad dispersities. As the P2-2 experiment has demonstrated the best results so far, these conditions will be used as starting point for further optimizations.



Scheme 4: Proposed mechanism of the polymerization initiated by the system DKP:tBuP4:i-Bu3A1

#### **b.** Effect of temperature

The introduction of 2 eq of *i*-Bu<sub>3</sub>Al leads to broad dispersities, induced by the increased  $k_p/k_i$  ratio, compared to ratios without Lewis acid  $[(k_p/k_i)_{TiBA} > (k_p/k_i)_{no TiBA}]$ . We hypothesize that the activation energies of the initiation and propagation are differents. By lowering the temperature of the polymerization, we expect to decrease the  $k_p/k_i$  ratio and thus improve the control on  $M_n$  and lower  $M_w/M_n$ .

Reactions were conducted at 10°C, 0°C, -10°C and -20°C (P2-4, P2-5, P2-6, and P2-7, respectively) and reached full conversion. The broad dispersities values ( $M_w/M_n = 1.36 - 1.53$ ) and higher experimental  $M_n$  than theoretical  $M_n$  revealed that no improvements can be achieved by decreasing the temperature.

#### c. Step-wise monomer addition

Groll and coworkers reported the polymerization of AGE and EEGE by slow feeding the monomer into the reaction medium. By doing so they were able to suppress the transfer reactions to the monomer and to reduce significantly the dispersities of the samples  $(M_w/M_n = 1.30 \text{ to } 1.16)$ . <sup>13</sup> Based on this procedure we added the BO in a step-wise manner (25 µl of monomer per hour) in a reactor already filled with C(A-P):*t*BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al in MeTHF at either 0°C (P2-8) or -20°C (P2-9). SEC elugrams exhibited high dispersities for both samples and no improvements in term of the control of the molar masses.

#### d. Effect of excess *t*BuP<sub>4</sub>

By using 25% excess of phosphazene base we assume to favor the equilibrium towards the amidate form of DKP (see Scheme 4). For condition using 1.25 eq of  $tBuP_4$  at 25°C (P2-10) the dispersity was slightly improved compared to using an equimolar ratio of initiator and base (P2-2  $M_w/M_n$ = 1.39; P2-10  $M_w/M_n$ = 1.33). But no improvement was made on the control of the  $M_n$ . Though  $M_n$  determined by NMR is in the same range of the mass value determined by SEC suggesting that no side reactions were taking place during the polymerization.

The same reaction was performed at -20°C (P2-11), even though the molar mass was lower than before, the low temperature induced a broadening of the dispersity.

#### e. Effect of dropwise monomer addition

For epoxide monomers the ring strain is so important that the ring-opening polymerization of those monomers is highly exothermic. <sup>14</sup> The use of i-Bu<sub>3</sub>Al in monomer-activated AROP results in high polymerization rate, which could cause an increase of the temperature of the reaction mixture. The released heat could occasion a loss of control of the AROP.

In order to control the amount of released heat, we decided to proceed through a drop-wise addition of the monomer in the reaction medium ( $\approx 75 \ \mu l.min^{-1}$ ) at 25°C and -20°C, P2-12 and P2-13, respectively. For P2-12 experiment, the peak on the SEC elugram is monomodal and exhibits a slight tailing towards high retention volumes (see **Figure 8**). The same behavior was observed for P2-13. The experimental molar masses of P2-12 and P2-13 were closer to the theoretical values than before; additionally the dispersities were the lowest obtained up to this point. However, under monomer-activated AROP conditions with *i*-Bu<sub>3</sub>Al, the kinetic is following a 0 order regarding the monomer concentration, thus modulation of the monomer concentration during the polymerization is expected to have no effects on the control of polymerization. Those improved results are interesting and highlight a phenomenon we do not fully understand at the moment.



**Figure 7:** MALDI-TOF spectrum in reflectron mode for P2-13 (a), zoom in region of the spectrum (b). MALDI-TOF spectrum of the sample P2-13 (**Figure 7**), where the addition of the monomer was performed at -20°C, displays a major population corresponding to the expected polymer, DKP initiated poly(BO) terminated by an hydroxy function, and a very minor population which could not be assigned. See appendix for suggested side reactions **Figure A1**, **Figure A2** and **Figure A3**.

While the only population detected on the P2-12 mass spectra in **Figure 8**, is assigned to poly(BO) chains, end caped by C(A-P) and hydroxy function.



Figure 8: Characterization of P2-12, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.

<sup>1</sup>H and <sup>13</sup>C NMR experiments of P2-12 coupled with 2D NMR (**Figure 9**), allowed to identify signals attributed to the DKP and to the polymer and so to confirm the expected structure. See appendix for 2D NMR spectra in **Figure A14**, **Figure A15** and **Figure A16**.



Figure 9: <sup>1</sup>H NMR spectra P2-12 recorded in CDCl<sub>3</sub> at RT.

The addition order of the reagents was then changed, and *i*-Bu<sub>3</sub>Al was added drop-wise at -20°C then the reaction was heated up to 25°C for polymerization (P2-14). The <sup>13</sup>C NMR and SEC molar masses are in good agreement with the theoretical value, besides the dispersity is kept relatively low (1.35). Nevertheless, the MALDI-TOF analysis detected a major population corresponding to the one expected and a minor population indicating the occurrence of side reactions during the AROP, which could not be identified (see Figure 10). See appendix for the suggested side reactions **Figure A1**, **Figure A2** and **Figure A3**.


Figure 10: MALDI-TOF spectrum recorded in reflectron mode P2-13 (a); Zoom in area of the spectrum (b).

To conclude on this optimization part, 2 equivalents of *i*-Bu<sub>3</sub>Al are efficient to suppress side reactions. The triisobutylaluminum decreases the reactivity of the anionic active center and activates the monomer. A limitation of this reduced reactivity is the low efficiency of the amidates complexes as initiators, producing higher molar masses polymers with broader dispersities than without *i*-Bu<sub>3</sub>Al. However, we demonstrated that a slight excess of  $tBuP_4$  combined with a drop-wise addition of the monomer could enhance the control of the polymerization and yield polymers with a relatively low dispersities.

The best conditions we identified being the ones for P2-12, these were applied to the other DKPs to synthesized peptide-polymer conjugates in **Table 4**.

Run	Initiator	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [ <i>i</i> -Bu <sub>3</sub> Al] <sub>0</sub>	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> SEC <sup>b</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>b</sup>
P3-1	C(G-P)	30/1/1.25/2	1	100	2300	3900	1.37
P3-2	C(L-P)	30/1/1.25/2	1	100	2400	2700	1.32
P3-3	C(F-P)	30/1/1.25/2	1	100	2400	3400	1.38

**Table 4:** Polymerizations of 1,2-butylene oxide initiate by proline-based DKPs: $tBuP_4$ : $i-Bu_3Al$  (1:1.25:2) in MeTHF; [BO]<sub>0</sub> = 2M at 25°C.

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.

<sup>b</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Poly(BO) initiated by Cyclo(L-P) has a molar mass in good agreement with the theoretical  $M_n$  and  $M_w/M_n = 1.32$ . MALDI-TOF spectrum of P3-2 exhibits a single population (Figure 11) being polymer chains initiated by C(L-P) and terminated by an alcohol group.



Figure 11: Characterization of P3-2, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.

For polymerizations initiated by C(G-P) and C(F-P) (**Table 4**, P3-1 and P3-3, respectively), the experimental values of  $M_n$  are higher than theoretical  $M_n$ . Using the optimized conditions for these two DKPs leads to a significant decrease but not a full suppression of the side reactions, as shown on MALDI-TOF spectra in **Figure 12**. Besides, the m/z of the additional distribution, on P3-1 and P3-3 mass spectra, are the same, which is suggesting that side reactions detected are not specific to a given DKP.

These results confirm that there is a considerable importance of the DKP chemical structure on the characteristics of resulting polymers. We have shown that having a benzyl group on the side chain next to the amide function slows down the kinetic of AROP. The slow rate of initiation observed along the paragraph, could be explained by the H rate of exchange of the amide functions, directly linked to the acidity of the N-H function.

In the case of amides from amino acids bearing aromatic side groups it has been reported a lower rate of proton exchange for those N-H function also related to a cumbersome environment having steric blocking effect. <sup>15–17</sup> In C(F-P)-initiated poly(BO), the high  $M_n$  and broad dispersities are explained by the low accessibility of the N-H and related to low initiation efficiency of the peptide.

The limited control of AROP initiated by C(G-P) regarding the ones initiated by C(L-P) and C(F-P) is explained by the absence of electron donating side chains in Glycine and a more accessible amide function making the carbonyl group more prone to nucleophilic attacks.



Figure 12: MALDI-TOF spectra recorded in reflectron mode. (a) MALDI-TOF spectrum of P3-1; (b) Zoom in area of the spectrum of P3-1; (a') MALDI-TOF spectrum of P3-3; (b') Zoom in area of the spectrum of P3-3.

#### f. Monomer-activated AROP of EEGE from cyclo(Leucine-Proline) and cyclo(Phenylalanine-Proline)

In this part the monomer was switched from BO to EEGE, to extend the nature of polymer chains that can be synthesized from our initiating system DKP:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al. The purpose is to demonstrate the possibility to functionalize the DKP with a water-soluble polymer.

By replacing BO to EEGE, we expect to have better results in term of control of the polymerization due to the lower reactivity of EEGE compared to BO. <sup>11</sup> C(L-P) and C(F-P) were chosen due to their easily assigned NMR signature compared to the two other DKPs. We used the optimized conditions determined in the latter part to polymerize EEGE from C(L-P) and C(F-P) (Table 5).

**Table 5:** Polymerizations of EEGE initiated by proline based DKP using t-BuP<sub>4</sub>/i-Bu<sub>3</sub>Al as a catalytic system (Initiator/ $tBuP_4/i$ -Bu<sub>3</sub>Al = 1/1.25/2) at [M]=2M in MeTHF, 25°C.

Run	Initiator	[M] <sub>0</sub> /[I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [ <i>i</i> -Bu <sub>3</sub> Al] <sub>0</sub>	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sup>b</sup> <sup>1</sup> H NMR (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
P4-1	C(L-P)	5/1/1.25/2	1	100	940	1500	1600	1.30
P4-2	C(L-P)	20/1/1.25/2	1	100	3100	2800	2200	1.39
P4-3	C(L-P)	30/1/1.25/2	2	95	4300	4500	4900	1.25
P4-4	C(F-P)	5/1/1.25/2	2	100	970	2000	1600	1.38
P4-5	C(F-P)	10/1/1.25/2	2	100	1700	4800	3300	1.20
P4-6	C(F-P)	30/1/1.25/2	2	94	4300	6200	5100	1.24

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (DMF, 60°C, PMMA standards).

EEGE polymerizations reported in Table 5, were conducted in MeTHF at 25°C initiated by DKP/t-BuP<sub>4</sub>/*i*-Bu<sub>3</sub>Al = 1/1.25/2. All experiments proceeded to full conversion in a short matter of time. For C(L-P), the same trend is observed between AROP of BO and EEGE. SEC trace of P4-1 in Figure 17 exhibit a monomodal peak and same results were obtained for the other polymers of Table 5. Additionally dispersities of the 6 experiments are fairly narrow  $M_{\rm w}/M_{\rm n} = 1.20 - 1.39.$ 

For C(L-P)-initiated poly(EEGE), the molar masses determined by <sup>1</sup>H NMR and SEC are in the same range and agree with the theoretical  $M_n$  values. This is suggesting a quantitative initiation process from C(L-P), for these two oxirane monomers.

For C(F-P)-initiated polymers, <sup>1</sup>H NMR  $M_n$  are significantly higher than SEC  $M_n$ , and experimental  $M_n$  display higher polymerization degrees than expected.

MALDI-TOF analyses of all the samples detected three populations, the major one being poly(EEGE) chains, end capped by DKP at one end and a hydroxy function at the other. The second population was assigned to DKP-poly(EEGE) polymer having one deprotected unit, due to acidic quench at the end of the polymerization.

The peaks corresponding to an additional deprotected EEGE unit have the same theoretical mass values as the expected population thus could not be detected. Concerning the third detected distribution, mass values were not fitting to the supposed transesterification reaction (Scheme 3) or the reactions considered in appendix. (Figure A1, Figure A2 and Figure A3). Typical MALDI-TOF spectra are displayed in Figure 13 and Figure 14 for both initiators.



Figure 13: MALDI-TOF spectrum for P4-1 recorded in reflectron mode (a), and Zoom in area (b).



Figure 14: MALDI-TOF spectrum for P4-4 recorded in reflectron mode (a), and Zoom in area (b).

Additional IR analysis in **Figure 15** demonstrates the presence of ester functions which could explain the populations unassigned on MALDI-TOF spectrum.



Figure 15: ATR-FTIR spectra of poly(EEGE) conjugated (black curve) to C(L-P); (red curve) to C(F-P).

The poly(EEGE) chains were deprotected via acidic hydrolysis in HCl (0.5M in methanol) and then dialyzed against water to remove excess acid. High conversion of acetal groups into alcohol function was reached within 3 hours, as seen on a typical NMR spectrum displayed in **Figure 16**. Also, the change in water solubility for the product was an evidence of successful deprotection. Conjugates of poly(glycidol) were characterized by SEC in DMF and by MALDI-TOF analysis. Results are gathered in **Table 6**.



**Figure 16:** <sup>1</sup>H NMR spectra of P4-1 (top) and after acidic cleavage of the protecting group P4-1d (bottom) recorded in MeOD<sub>4</sub> at RT.

Run	Initiator	Deprotection <sup>a</sup> (%)	<i>M</i> <sub>n</sub> theo <sup>b</sup> (g/mol)	M <sup>a</sup> <sup>1</sup> H NMR (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	M <sub>w</sub> /M <sub>n</sub> (SEC) <sup>c</sup>
P4-1d	C(L-P)	99.1	900	1200	1000	1.54
P4-2d	C(L-P)	99.5	1200	2200	1800	1.34
P4-3d	C(L-P)	99.8	2600	3200	3600	1.32
P4-4d	C(F-P)	99.3	900	1300	1000	1.57
P4-5d	C(F-P)	99.3	1800	3200	2600	1.19
P4-6d	C(F-P)	99.7	2700	3600	3800	1.31

 Table 6: C(X-P)-poly(glycidol) conjugates characterization.

<sup>a</sup>: Determined by <sup>1</sup>H NMR in MeOD<sub>4</sub>. <sup>b</sup>: Calculated from *M*<sub>n</sub> SEC of C(X-P)-poly(EEGE) conjugates in **Table 5**. <sup>c</sup>: Determined by SEC (DMF, 60°C, PMMA standards).

All the polymers were successfully deprotected within 3 hours, with a high percentage of deprotection (Table 6). The molar masses decrease induced by the deprotection of EEGE units, was evidence by SEC stacking of the elugrams in Figure 17. The downwards shift to higher retention volumes for the traces in blue, corresponding to poly(glycidol) conjugates, clearly indicates a mass loss compared to the traces in black, for poly(EEGE) conjugates.

A good agreement between NMR and SEC  $M_n$  can be noted for all the polymers.

Excepted for P4-1d and P4-4d exhibiting measured masses similar to the theoretical values, all the experimental masses show a higher value than the theoretical  $M_n$ . This observation has been already made for C(F-P) - EEGE conjugates while C(L-P) - EEGE conjugates were having good agreement between measured and theoretical  $M_{\rm n}$ .



**Figure 17:** SEC traces stacking of poly(EEGE) in black and poly(glycidol) in (blue) in SEC with DMF as eluent. (a) conjugate from C(L-P); (b) conjugate from C(F-P).

MALDI-TOF spectra of P4-1d in **Figure 18** and of P4-4d in **Figure 19** show a major population being the one of poly(glycidol) chains, end functionalized by a DKP and a hydroxy function cationized with a lithium ion. It should be mentioned that for poly(glycidol) conjugates the combination matrix/salt was changed to  $\alpha$ -cyano-4-hydroxycinnamic/LiCl. And a minor population assigned to poly(glycidol) chains also end capped by a DKP and alcohol function, but cationized with a sodium ion. Indeed, the most common salts for cationization are implying the use of Na based products for MALDI-TOF. Residual sodium in the high vacuum atmosphere of the MALDI-TOF can lead to a "contamination" of the analyzed populations. Side reactions on EEGE conjugates, are not visible anymore on the spectra of the glycidol conjugates. This disappearance occurs at the same time than the peak at high retention volume on SEC elugrams in **Figure 17**.

MALDI-TOF analysis of all the polymers in **Table 6** confirmed the nature of the chain ends after deprotection.



Figure 18: MALDI-TOF spectrum for P4-1d recorded in reflectron mode (a), and Zoom in area (b).



Figure 19: MALDI-TOF spectrum of P4-4d recorded in reflectron mode (a), and Zoom in area (b).

<sup>1</sup>H NMR spectra of P4-1d in **Figure 20** and P4-4d in **Figure 21** allowed us to identify signals from the initiating DKPs, evidencing the grafting of polymers on cyclic peptides even after acidic treatment. We also identify a proton at 5.23 ppm, which could be assigned to H placed near an ester function. This specific attribution can be explained by the transfer reaction mechanism suggested in (Scheme 3), leading to a ring-opening of a DKP and formation of an ester bond. <sup>18</sup> Identification of the NMR signals was achieved with 1D and 2D experiments and spectra can be seen in appendix **Figure A17**, **Figure A18**, **Figure A19**, and **Figure A20**.



Figure 20: <sup>1</sup>H NMR spectrum of VP4-1d recorded in MeOD<sub>4</sub> at RT.



Figure 21: <sup>1</sup>H NMR spectrum of VP4-4d recorded in MeOD<sub>4</sub> at RT.

The ATR-FTIR spectra in **Figure 22** exhibit a main peak at  $\approx 1660 \text{ cm}^{-1}$  signature of a C=O amide bond stretching, while the peak observed at 1735 - 1738 cm<sup>-1</sup> is assigned to C=O ester bond stretching. Both NMR and IR spectra are evidencing the presence of minor side reactions products in the polymer.

By using optimized conditions, we demonstrated the conjugation of poly(glycidol) polymers to two DKPs. We proved that the post-polymerization deprotection preserved the structure of the initiating cyclic peptide.

Literature reported proline-based DKPs as being biologically active for antimicrobial applications. Conjugation of poly(glycidol) to C(L-P) and C(F-P) could have effect on the pharmacokinetic properties and stability of the peptides. Conjugates DKP-polymers in **Table 6** are under biological testing to assess their bioactive capacity compared to the native peptide. Molar mass influence will be probed for the role played in the stability of the product in biological conditions, or his effects on the pharmacokinetic properties.



Figure 22: ATR-FTIR spectra of PG conjugated (black curve) to cyclo(Leu-Pro); (red curve) to cyclo(Phe-Pro).

In this first part (I. Proline-based DKPs as initiators for the AROP of epoxide monomers) focused on proline-based DKPs, we demonstrated for the first time the amides modification of cyclic dipeptides, through AROP, using an initiating system based on  $tBuP_4$  and i-Bu<sub>3</sub>Al. The use of the trialkylaluminum Lewis acid allowed us to eliminate most part of the side reactions, and to yield polymer chains fully functionalized by a single DKP. By using different DKPs, we illustrated that AROP can be conducted regardless the hydrophobic side chain of the amino acid even for bulky groups. Hydrophobic and hydrophilic polymers were conjugated to DKPs, poly(BO) and poly(glycidol) respectively, demonstrating the versatility of the investigated technique for various epoxides monomers. However, improvements towards control of the polymerization remain challenging, but modulations by the strength of the Lewis acid could be an optimization option for further studies.

# II. Bifunctional asymmetric DKPs as initiators for the AROP of epoxide monomers

The main work of this part will be done on three bifunctional DKPs having different amino acids compositions (**Scheme 5**). By using those new cyclic dipeptides, we want to investigate if polymerization is possible from bifunctional initiators. We also aim to determine the required conditions for a living and controlled polymerization. (**Scheme 6**)



Cyclo(Glycine-Valine) Cyclo(Glycine-Phenylalanine) Cyclo(Leucine-Phenylalanine) Scheme 5: Bifunctional asymmetric dipeptides

In a first part, the influence of the monomer structure will be discussed, phosphazene base  $tBuP_4$  will be used combined with the DKPs for initiation of the AROP. Balance of  $tBuP_2$  and  $tBuP_4$  will be covered as a mean to control the polymerization. And finally, the structure of the Lewis acid will be assessed as a regulator of the monomer-activated AROP from the dipeptide.



Scheme 6: Polymerization of oxirane based monomers initiated by asymmetric DKP and the suggested structures synthesized.

#### 1. Determination of the best monomer for the synthesis of protected linear glycidols from DKPs

Herein we report the usage of two glycidyl ether monomers, EEGE and tBuGE, combined with cyclo(Gly-Val) for the production of conjugates (see Table 7). The advantage of these two monomers is that they can generate alcohol functions along the polymer backbone after acidic deprotection. Based on the results obtained for proline-based DKP, in the first part only the phosphazene base tBuP<sub>4</sub> will be considered as a co-initiator for the AROP of glycidyl ether monomers. The influence of the tBuP<sub>4</sub> number of equivalents on the control of the polymerization will be investigated in Table 7.

Table 7: Polymerizations of glycidyl ether monomers using cyclo(Gly-Val):tBuP<sub>4</sub> as initiating system in THF at  $[M]_0 = 1.5M$ .

Run	Monomer	. [M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>c</sup>
VP6-1	EEGE	30/1/1	25	7	100	4500	4100	4200	1.21
VP6-2	EEGE	30/1/2	25	9	100	4500	4100	4300	1.30
VP6-3	EEGE	30/1/0.5	25	141	100	4500	3600	3400	1.13
VP6-4	tBuGE	30/1/1	25	19	100	4100	4200	4100	1.11
VP6-5	<i>t</i> BuGE	30/1/2	25	19	100	4100	4600	4500	1.12
VP6-6	tBuGE	30/1/0.5	25	19	86	3500	3400	4400	1.09
VP6-7	<i>t</i> BuGE	30/1/0.5	40	24	100	4100	4400	3300	1.23

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For all the polymerizations reported in Table 7, high conversion was reached. Having two possible initiating sites (NH functions) on the initiator does not prevent the polymerization.

For poly(EEGE) conjugates, SEC traces in Figure 23 exhibit asymmetric peaks with a shoulder at higher retention volumes.

While the SEC traces of poly(*t*BuGE) conjugates in Figure 25 are monomodal and the peaks exhibit a relatively good symmetry with a reduced shoulder at low retention volumes but no signal at higher retention volumes.

Apart for the experiments conducted with 0.5 equivalents of  $tBuP_4$  regarding Cyclo(Gly-Val), polymers in **Table 7**, display a good agreement between SEC  $M_n$ , NMR  $M_n$  and theoretical molar masses. Additionally the dispersities are quite low  $M_w/M_n = 1.09 - 1.30$ , and tend to increase with the increasing amount of phosphazene base in the medium. However, polymerizations using EEGE produce polymers with higher dispersities than *t*BuGE polymers. This observation is suggesting a less controlled polymerization in the case of EEGE, which is correlated to a reported higher reactivity of EEGE under conventional AROP conditions than *t*BuGE.<sup>11</sup>

Polymerization conditions VP6-3, VP6-6 and VP6-7, for which 0.5 equivalents of  $tBuP_4$  is sufficient for the initiation of the AROP by one equivalent of DKP ( $M_n$  theo  $\approx M_n \exp$ ), indicate an efficient transfer reaction between the propagating center and NH amides. Indeed, if the transfer reaction were not occurring, experimental number-average molar masses should be twice higher as expected.

MALDI-TOF spectra of VP6-1, VP6-2 and VP6-3 in **Figure 23**, exhibit distributions of small  $M_n$  separated from distributions at higher  $M_n$  with different chain end functions. In the mass range (m/z) = 1000 to 3000, at least three populations can be seen. Those populations are poly(EEGE) chains but extremities could not be identify since mass values does not fit with any hypothetical structures we tried. See in appendix for suggested side reactions **Figure A1**, **Figure A2**, **Figure A3** and **Figure A22**. At higher molar masses, two populations are identified as the actual poly(EEGE) initiated by cyclo(Gly-Val) and terminated by a hydroxy function and the second population being the polymer with one deprotected glycidol unit. Detailed typical spectrum can be found in appendix **Figure A21**.



Figure 23: Influence of the quantity of tBuP<sub>4</sub> on the MALDI-TOF spectra recorded in reflectron mode and SEC elugrams in THF for poly(EEGE). (a) VP6-3 SEC trace; (b) VP6-3 MALDI-TOF spectrum; (a') VP6-1 SEC trace; (b') VP6-1 MALDI-TOF spectrum; (a'') VP6-2 SEC trace; (b'') VP6-2 MALDI-TOF spectrum.

By increasing the number of equivalents of phosphazene base, the shoulder on the SEC elugrams at high retention volumes is increasing (see **Figure 23**). The increase of the low molar masses chains is also observed on MALDI-TOF spectra which could prove less side reactions in the case of a reduced amount of  $tBuP_4$  used.

ATR-FTIR spectrum of VP6-2 in **Figure 24**, displays a major peak at 1669 cm<sup>-1</sup> for the C=O amide bond stretching, and a very weak peak at 1740 cm<sup>-1</sup> for C=O ester bond stretching suggesting a limited occurrence of the transesterification reaction.



Figure 24: ATR-FTIR spectrum of VP6-2.

MALDI-TOF spectra of VP6-6, VP6-4 and VP6-5 in **Figure 25**, display a major population attributed to poly(*t*BuGE) functionalized by cyclo(Gly-Val) at one end and hydroxy function at the other end (typical detailed spectrum is disclosed in appendix **Figure A23**). Several very minor populations can be seen in the small molar masses range.

One can be assigned to water-initiated poly(*t*BuGE), and at least two additional poly(*t*BuGE) populations can be seen but which extremities could not be identified. See in appendix for suggested side reactions **Figure A1**, **Figure A2**, **Figure A3**.



**Figure 25:** Influence of the quantity of *t*BuP<sub>4</sub> on the MALDI-TOF spectra recorded in reflectron mode and SEC elugrams for poly(*t*BuGE). (a) VP6-6 SEC trace; (b) VP6-6 MALDI-TOF spectrum; (a') VP6-4 SEC trace; (b') VP6-4 MALDI-TOF spectrum; (a'') VP6-5 SEC trace; (b'') VP6-5 MALDI-TOF spectrum.



Figure 26: ATR-FTIR spectrum of VP6-5.

VP6-5 (2 eq  $tBuP_4$ ; tBuGE) was further analyzed in infrared spectroscopy for ester bond detection. The spectrum in **Figure 26**, show a single peak for C=O amide bond stretching.

In order to enhance the reactivity of VP6-6 (0.5 eq *t*BuP<sub>4</sub>; *t*BuGE; 25°C), the temperature of the AROP was raised to 40°C in VP6-7. But for the sample, it only leads to a broadening of the dispersity  $M_w/M_n = 1.23$ .

By switching to *t*BuGE monomer, we efficiently managed to suppress the side reaction between alkoxide and the carbonyl groups of the DKP. In a more general way, when comparing the characterizations of cyclo(Gly-Val) - poly(EEGE) (**Figure 23**) and cyclo(Gly-Val) - poly(*t*BuGE) (**Figure 25**), we can clearly see a significant reduction of the side reactions for poly(*t*BuGE) polymers and a good control of the molar masses. These observations can be explained by the minor reactivity of *t*BuGE compared to EEGE in conventional AROP conditions with phosphazene base.<sup>11</sup>

However, analytical results shown above did not provide information on a mono or bifunctionalization on the DKP.

From those results we can deduced that *t*BuGE is a better monomer than EEGE to generate poly(glycidyl ether) conjugates. Thus *t*BuGE will be then used for further optimization and polymerization from other DKPs.

### 2. Synthesis of cyclo(Gly-Phe)-poly(tBuGE) conjugates using *t*BuP<sub>4</sub>

The cyclo(Gly-Val)/ $tBuP_4$  initiating system allowed the controlled polymerization of tBuGE. Thus, we extended this findings to a new DKP, having a phenyl side group instead of the isopropyl side-chain, i.e. the cyclo(Gly-Phe). The polymerization conditions were kept the same as for cyclo(Gly-Val) and the results are gathered in Table 8.

Run	Initiator	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	<i>M</i> <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP7-1	C(G-F)	30/1/1	25	24	77	3200	3600	3300	1.23
VP7-2	C(G-F)	30/1/2	25	24	100	4100	6700	4300	1.28
VP7-3	C(G-F)	30/1/0.5	25	69	87	3600	3400	2700	1.14
VP7-4	C(G-F)	30/1/0.5	40	48	100	4100	4100	3400	1.17
VP7-16	C(G-F)	30/1/0.5	55	21	100	4100	5600	3200	1.19

**Table 8:** Polymerizations of *t*BuGE using cyclo(Gly-Phe):  $tBuP_4$  as initiating system in THF,  $[M]_0 = 1.5M$ .

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Under similar conditions used in the previous part, polymerization of tBuGE proceed to high conversion when initiated by cyclo(Gly-Phe) (Table 8).

VP7-1 gave polymers exhibiting the expected molar masses but a slightly broad  $M_w/M_n$ . SEC trace of VP7-1 shown in Figure 27 exhibit a monomodal peak with a slight tailing.

The MALDI-TOF spectrum of VP7-1 displays several populations (Figure 27). The major one is assigned to poly(tBuGE) chains, end capped by C(G-F) and hydroxy function. A second population (blue) could correspond to a water-initiated polymer chain or product or transesterification regarding calculated mass values. And at least three others poly(tBuGE) distributions were detected, but which end functions could not be identified. Detailed MALDI-TOF spectrum of VP7-1 in the range m/z = 900-2000 is provided in appendix Figure A24. The number of equivalents of the base was raised to 2 equivalents. SEC elugram of VP7-2 exhibits a bimodal peak and NMR  $M_n$  is way higher than SEC and theoretical values.

This result suggests high content of side reactions. SEC elugram and MALDI-TOF spectrum of VP7-2 are show in appendix **Figure A25**.



**Figure 27:** Characterization of VP7-1, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum. ?-poly(*t*BuGE) either corresponds to water initiated polymer or product of transesterification reaction.

The cyclo(Gly-Phe):*t*BuP<sub>4</sub> ratio was lowered to 1:0.5. For VP7-3 at 25°C, completion of the reaction was reached in almost 3 days. In order to reduce the polymerization time, the temperature was raised to 40°C, VP7-4, and to 55°C for VP7-16.

As previously, the experiments at 0.5 equivalents of  $tBuP_4$  demonstrate a very efficient transfer reaction between alkoxide propagating center and NH amides. The temperature increases, leads to decrease the polymerization time and increasing the dispersities  $M_w/M_n = 1.14 (25^{\circ}C) - 1.17 (40^{\circ}C) - 1.19 (55^{\circ}C)$ .

The increase of temperature has also effects on the shape of the peaks on SEC elugrams. In **Figure 28** we can notice a dissymmetry of the signal of VP7-4 and VP7-16 suggesting the presence of two distributions.

For the all three polymers synthetized with 0.5 eq of  $tBuP_4$ , the MALDI-TOF spectra in **Figure 28** are multimodal and exhibit at least 4 populations. The major one is assigned to poly(tBuGE) initiated by C(G-F), and cationized by Na<sup>+</sup> (in green), a second on to water-initiated poly(tBuGE) or product of transesterification, and at least two populations were detected but not assigned. Supposed side reactions can be seen in appendix **Figure A1**, **Figure A2**, **Figure A3** and **Figure A22**.

On SEC traces and MALDI-TOF spectra in Figure 28, by increasing the temperature an upward shift, toward the high molar masses, is clearly visible. (See in appendix for typical detailed spectrum Figure A26). Those observations, from Figure 28, can be a witness of the double initiation on the bifunctional DKP. Using cyclo(Gly-X), we assume that the polymerization would be endorsed by the NH on Gly residue side. In the first part (prolinebased DKPs), we showed that NH close to Phe amino acid was less reactive than any other NH function born by a less hydrophobic amino acid residue. In the conditions used here, initiation could occurs on both amides at the same time, yielding DKP bi-functionalized by two polymer chains on the N atoms of the cycle. When both initiating possibilities, mono and double, are in the same reaction medium, the consumption of the monomer is twice important for a double-sided initiator as for a mono sided initiator. It results in higher molar masses for double initiated polymers than mono-initiated polymers. The mass difference, between the two peaks in MALDI-TOF spectrum of Figure 28, is believed to evidence the mono or double initiation phenomenon. The first peak of the expected population  $(m/z) \approx 2500$  is guessed to belong to mono-initiated polymer chains while the second peak at  $(m/z) \approx 4000$  is assigned to a bi-initiated poly(*t*BuGE).

This hypothesis is based on an equal reactivity of the alkoxide species. But those interpretations are only hypothetical and need further investigations to be confirmed.

<sup>15</sup>N NMR and <sup>1</sup>H-<sup>15</sup>N HSQC NMR could be considered for identification of the amides functions NH or *N*-PBO, to provide information about mono or double sided modifications.



Figure 28: Influence of the temperature on the MALDI-TOF spectra recorded in reflectron mode and SEC elugrams in THF for poly(*t*BuGE). (a) VP7-1 SEC trace; (b) VP7-1 MALDI-TOF spectrum; (a') VP7-4 SEC trace; (b') VP7-4 MALDI-TOF spectrum; (a'') VP7-16 SEC trace; (b'') VP7-16 MALDI-TOF spectrum. ?-poly(*t*BuGE) either corresponds to water initiated polymer or product of transesterification reaction.



Figure 29: ATR-FTIR spectrum of VP7-16.

ATR-FTIR spectrum of VP7-16 in **Figure 29** displays a peak assigned to C=O ester bond stretching suggesting a ring-opening of the DKP by a transesterification reaction. (Mechanism in **Scheme 3**)

<sup>13</sup>C NMR analysis was conducted on the three samples but the low sensitivity of the technique did not allow to gain information on the structure of the product.

In this part we achieved the synthesis of poly(tBuGE) from cyclo(Gly-Phe). By using phosphazene  $tBuP_4$  in stoichiometric amount, we managed to synthesize rather controlled polymers with the expected  $M_n$  while maintaining the dispersities low. The bimodal distributions for Cyclo(Gly-Phe) and the monomodal distributions for Cyclo(Gly-Val) seems to indicate that temperature can favor a mono or bi-sided functionalization.

### **3.** Combination of *t*BuP<sub>2</sub> and *t*BuP<sub>4</sub> bases for DKP-polymer conjugates synthesis

It has been reported, that the use of combined  $tBuP_4$  and  $tBuP_2$  bases can lead to controlled polymerizations from symmetric DKPs. <sup>18</sup> Mixing phosphazene bases can have an effect on the reactivity of the anionic species in the mechanism,  $tBuP_4$  is known to lead to highly reactive species, while  $tBuP_2$  decreases drastically the reactivity of the anions.

Tezgel et al. demonstrated that the 1:1:0.25 ratio for DKP:tBuP<sub>2</sub>:tBuP<sub>4</sub> was having the best results in term of control of molar masses and side reactions limitation.

In this third part, we applied the same approach, hoping that it could help to suppress the secondary reactions. As tBuGE shown great potency in the latter parts, it will be used here for AROP from DKPs. Reaction conditions are gathered in Table 9.

Table 9: Polymerizations of tBuGE initiated by phophazene bases tBuP<sub>n</sub> and Cyclo(G-X) in THF;  $[M]_0 = 1.5M.$ 

Run	Initiator	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>2</sub> ] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP6-9	C(G-V)	30/1/1/0.25	25	96	90	3700	4200	2700	1.09
VP6-8	C(G-V)	30/1/1/0.25	40	24	97	4000	3100	3500	1.08
VP7-6	C(G-F)	30/1/1/0.25	25	96	24	980	1700	1100	1.15
VP7-11	C(G-F)	30/1/1/0.15	40	96	81	3300	3300	1600	1.22
VP7-5	C(G-F)	30/1/1/0.25	40	48	74	3000	3300	2300	1,14
VP7-12	C(G-F)	30/1/1/0.50	40	24	86	3500	3800	2900	1.16
VP7-13	C(G-F)	30/1/1/0.75	40	24	98	4000	4400	3300	1.22

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Polymerizations reported in Table 9 proceed to high conversion, but compared to *t*BuP<sub>4</sub>-only promoted AROP, polymerizations time are longer. This result confirms the expected decrease of the propagating centers reactivity.

For VP6-9 completion of the polymerization is reached in nearly 4 days. The temperature was increased to 40°C and high conversion was achieved within 24 hours (VP6-8).

SEC trace of VP6-8 exhibits a symmetric peak in Figure 30 and the same result was obtained for VP6-9. For the experiments VP6-9 and VP6-8, SEC and NMR molar masses are in close agreement, but experimental  $M_n$  are lower than the theoretical  $M_n$ . Additionally the dispersities are low even at elevated temperature  $M_w/M_n = 1.08-1.09$ .

MALDI-TOF spectrum of VP6-8 in Figure 30, compared to previously shown spectra in Figure 25, exhibits no clear differences from the previous polymerization conditions.

The major population is cyclo(Gly-Val)-initiated poly(tBuGE), minor second population is assigned to water-initiated polymer chains, and at least 3 populations of poly(tBuGE) can be seen in the small  $M_n$  area but could not be attributed. Same results were obtained for VP6-9. See appendix for detailed spectrum of VP6-8 in small  $M_n$  range, **Figure A27** and suggested side reactions in **Figure A1**, **Figure A2**, **Figure A3**, **Figure A22**.



Figure 30: Characterization of VP6-8, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.

ATR-FTIR spectrum of VP6-8 (Figure 31) exhibits a single peak at 1670 cm<sup>-1</sup> assigned to C=O amide bond stretching suggesting that no transesterification reaction occured.



Figure 31: ATR-FTIR spectrum of VP6-8

By using a combination of  $tBuP_4$  and  $tBuP_2$  bases at 40°C, we were actually able to yield polymer chains initiated by cyclo(Gly-Val) with controlled molar masses and low dispersities.

For cyclo(Gly-Phe) the same ratio as VP6-9 was applied to the polymerization of poly(*t*BuGE) i.e. 1:1:0.25 (DKP:*t*BuP<sub>2</sub>:*t*BuP<sub>4</sub>). For VP7-6 at 25°C, the polymerization did not go to completion over 4 days. Raising the temperature to 40°C allows a significant reduction of the reaction time. For this specific DKP, the influence of the quantity of *t*BuP<sub>4</sub> used combined with *t*BuP<sub>2</sub> base was investigated to identify controlled polymerization conditions. For the experiments from VP7-11 to VP7-13 the amount of *t*BuP<sub>4</sub> was increased from 0.15 to 0.75 equivalents, while keeping constant the number equivalent to 1 for *t*BuP<sub>2</sub>. In the 3 cases, VP7-5 to VP7-13, NMR  $M_n$  are higher than SEC values and those experimental molar masses are lower than theoretical  $M_n$  suggesting the occurrence of side reactions. Additionally disperisties tend to increase with the amount of *t*BuP<sub>4</sub> used (VP7-11 value apart due to the low  $M_n$  of the polymer).



Figure 32: Influence of the tBuP<sub>2</sub>:tBuP<sub>4</sub> ratios on the SEC elugrams and MALDI-TOF spectra recorded in reflectron mode. (a) SEC trace in THF of VP7-11; (a') MALDI-TOF spectrum of VP7-11; (b) SEC trace in THF of VP7-5; (b') MALDI-TOF spectrum of VP7-5; (c) SEC trace in THF of VP7-12; (c') MALDI-TOF spectrum of VP7-12; (d) SEC trace in THF of VP7-13; (d') MALDI-TOF spectrum of VP7-13. ?-poly(tBuGE) either corresponds to water initiated polymer or product of transesterification reaction.

We can see by SEC and mass spectrometry analyses shown in **Figure 32**, that the synthesized polymers exhibit bimodal SEC traces. With increasing the amount of  $tBuP_4$ , the SEC elugrams shift towards high  $M_n$ . This behavior can also be seen on the mass spectra, by the upward shift of the expected population (green) towards higher molar masses.

MALDI-TOF of the samples displayed in **Figure 32** exhibit the expected population, polymer chains end-capped by cyclo(Gly-Phe) and hydroxy function (green). Detailed spectra are disclosed in appendix **Figure A28**. For all the experiments a water contamination or product of transesterification reactions can be seen (blue). Increasing the quantity of  $tBuP_4$  leads to an intensification of side reactions, as demonstrated by the poly(tBuGE) distribution(s) with undetermined chain-end functions (red).

It was previously demonstrated, that the transfer reaction between alkoxide and NH amides is highly efficient for cyclo(Gly-Val):*t*BuP<sub>4</sub> initiated polymerizations. Here, the apparition of this bimodal profile seems to indicate a less efficient transfer reaction. We assume that this is due to the steric hindrance of the aromatic group. By increasing the amount of *t*BuP<sub>4</sub>, the reactivity of the propagating center is increased, and transfer reaction between alkoxide and NH are more probable, favoring the double-initiation (**Scheme 7**). Like aforementioned, double-sided initiated polymers have a higher molar mass than the mono-sided initiated ones, when synthesized from the same reaction medium. We can then hypothesize that the first peak on the MALDI-TOF spectrum ((m/z)  $\approx 2000$ ) belongs to a mono-initiated polymers and the second peak ((m/z)  $\approx 3000 - 4000$ ) can be assigned to double-initiated polymers. However, further investigations are required to confirm those conclusions.



Scheme 7: Suggested mechanism of the transfer reaction between an alkoxide and an amide

ATR-FTIR spectra for VP7-11 and VP7-13 are displayed in **Figure 33**. For  $tBuP_2:tBuP_4$  ratio 1:0.15 a single peak is detected at 1669 cm<sup>-1</sup> signature of C=O amide bond stretching while for the 1:0.75 ratio an extra peak is seen at 1705 cm<sup>-1</sup> assigned to C=O ester bond stretching. This suggests the occurrence of transesterification reaction.



Figure 33: ATR-FTIR spectra of VP7-11 (black curve) and VP7-13 (green curve).

Water initiation was also subject to investigations. In order to suppress what we assigned to be initiation by residual water, two drying procedures were attempts. The cyclo(Gly-Phe) was dried for 3 hours by freeze-drying then used as initiator and another try was made by placing cyclo(Gly-Phe) under high vacuum for 3 hours at room temperature. But no improvements were noticeable on the MALDI-TOF analyses.

ATR-FTIR analyses (Figure 33) and results from the drying procedures attempted, suggest that the distribution outlined in blue on MALDI-TOF spectra of Figure 32 is composed of mixed population, water-initated polymers and products of transesterification having the same mass values.

For polymerization of epoxides-based monomers promoted by  $tBuP_2$  using a catalytic amount of  $tBuP_4$  we proposed the following equilibrium between reactive species, see **Scheme 8**.



**Scheme 8:** Equilibrium between the propagating species for *t*BuP<sub>2+</sub>*t*BuP<sub>4</sub>-based organocatalytic system

Based on the pKa values of **Table 1**, amide functions can be deprotonated by phosphazene bases  $tBuP_4$  and  $tBuP_2$ . Concerning  $tBuP_2$ , it was reported that epoxides polymerization in this case is particularly slow and takes several days to reach completion. <sup>19</sup> Therefore, we assume that propagation is mainly operated by the complexes from <sup>+</sup>H $tBuP_4$ , considered as active form, and the one with <sup>+</sup>H $tBuP_2$  can be considered as dormant anionic species. A permanent exchange equilibrium between counter ions <sup>+</sup>H $tBuP_4$  and <sup>+</sup>H $tBuP_2$  in the anionic complexes allows the quantitative polymerization from deprotonated initiators (**Scheme 8**). Increasing the amount of  $tBuP_4$  in the AROP conditions, makes the anionic center spend more time in the active form than in the inactive configuration, leading to an increased reactivity and faster rate of polymerization. (See VP7-11 to VP7-13 in **Table 9**).

For cyclo(Gly-Val) and cyclo(Gly-Phe), using a combination of organobases modulates the reactivity of the anionic center, allowing successful limitation of the secondary reactions. Telechelic polymers were synthetized with targeted  $M_n$  and low dispersities. However the small amount of side reactions detected can be detrimental for further applications in the biomedical field.

## 4. Effect of the chemical structure of Lewis acids on the AROP of oxiranes from asymmetric DKPs

The synthesis of cyclo(Gly-X) – poly(tBuGE) with controlled  $M_n$  and without side reactions can be achieved by using  $tBuP_2$  with a catalytic amount of  $tBuP_4$ . In order to provide an alternative approach, we suggest using the monomer-activated AROP conditions to reach the controlled divergent synthesis of polymers from amide functions of cyclic peptides.

#### a. Synthesis of N-poly(tBuGE)-C(G-F) conjugates

As described in the latter parts, the synthesis of asymmetric DKP-polymer conjugates is a challenging task. The occurrence of side reactions during the AROP is the main concern of this study. In this part, various Lewis acids will be used so as to control the reactivity of the propagating center and activate the monomer. <sup>8</sup> The solvent will be switched to MeTHF to avoid any competitive complexations of the Lewis acid with the solvent. <sup>10</sup>

Optimizations will be first performed on cyclo(Gly-Phe) because of the difficulty to control the AROP from this dipeptide, then the best conditions will be applied to cyclo(Gly-Val) and cyclo(Leu-Phe).

4 Lewis acids will be used for the monomer-activated AROP of *t*BuGE initiated by cyclo(Gly-Phe) (**Scheme 9**). Triisobutylaluminum (*i*-Bu<sub>3</sub>Al) is the most used alkylaluminum catalyst for AROP of epoxides. <sup>8</sup> Triethylborane (Et<sub>3</sub>B) was recently reported in the AROP of epoxides using *t*BuP<sub>n</sub> bases by Chen *et al.* <sup>20</sup> Triethylaluminum (Et<sub>3</sub>Al) was used in the first reported monomer activated AROP, but compared to triisobutylaluminum Et<sub>3</sub>Al was not as efficient. <sup>8</sup> Triphenylaluminum (Ph<sub>3</sub>Al) had never been reported for monomer-activated AROP of epoxides, but the cumbersome environment around the metallic center could modulate the reactivity of the system.

The 4 aforementioned Lewis acids were used here (**Table 10**) to assess the influence of the catalyst's structure on the polymerization, and to further guide the choice of Lewis acid compounds.



Scheme 9: Lewis acids used as catalysts for the monomer-activated AROP.

The [Lewis acid]/[Cyclo(Gly-Phe)] ratio was automatically set above 1 due to the requirements of the monomer-activated anionic ring-opening polymerization mechanism. First assumptions to run these experiments are based on suggested mechanism Scheme 4, as previously discussed.

The [Cyclo(Gly-Phe)]/[tBuP<sub>4</sub>] ratio was kept to 1 for all the experiment displayed in Table 10, while the amount of Lewis acid was alternated between 2 and 3 equivalents.

Run	Lewis Acid	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> /[L.A] <sub>0</sub>	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	Mn <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP-AN10	Ph <sub>3</sub> Al	30/1/1/2	168	0	-	-	-	-
VP7-38	Ph <sub>3</sub> Al	30/1/1/3	1	100	4100	6400	7300	1.72
VP7-25	<i>i</i> -Bu <sub>3</sub> Al	30/1/1/2	31	79	3200	3600	2800	1.24
VP7-26	<i>i</i> -Bu <sub>3</sub> Al	30/1/1/3	4	88	3600	4500	4200	1.76
VP7-29	Et <sub>3</sub> Al	30/1/1/2	70	75	3100	3000	2300	1.15
VP7-30	Et <sub>3</sub> Al	30/1/1/3	1	100	4100	4400	4400	1.33
VP7-27	$Et_3B$	30/1/1/2	4	100	3300	3800	3800	1.13
VP7-28	Et <sub>3</sub> B	30/1/1/3	1	100	4100	3800	3700	1.13

Table 10: Polymerizations of tBuGE by the initiating system cyclo(Gly-Phe):tBuP4:Lewis acid; in MeTHF at  $25^{\circ}$ C; [M]<sub>0</sub> = 1.5M.

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards)

The polymerization of BO by the initiating system cyclo(Gly-Phe):*t*BuP<sub>4</sub>:Ph<sub>3</sub>Al at a 1:1:2 ratio did not occur. But when the quantity of Ph<sub>3</sub>Al was raised to 3 eq, the AROP goes to completion in 1 hour. We suggest that the bulkiness of the trialkyaluminum compound was impeaching the efficient monomer activation due to steric hindered environment around the Al center.

For the Lewis acids *i*-Bu<sub>3</sub>Al, Et<sub>3</sub>Al and Et<sub>3</sub>B the polymerization proceed to high conversion values. The experiments with 3 eq have a faster rate of polymerization than the experiments with 2 eq, explained by the increasing concentration of activated monomers. The fact that even 2 equivalents-based experiments are able to proceed to polymerization suggests a high rate of R<sub>3</sub>Met exchange between the complexes [oxirane-R<sub>3</sub>Met] and [C=O-R<sub>3</sub>Met] in the medium.

The SEC elugram of VP7-38 displayed in **Figure 34** shows a tailing towards high retention volumes. The tailing combined with higher molar mass values than expected, and broad dispersity indicate a slow initiation step. MALDI-TOF spectrum of VP7-38 in **Figure 34** detected the expected population being poly(*t*BuGE) initiated by cyclo(Gly-Phe) and terminated by an hydroxyl function, and very minor distributions.


Figure 34: Characterization of VP7-38, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.

Then *i*-Bu<sub>3</sub>Al was used in VP7-25 and VP7-26 with 2 and 3 number of equivalents, respectively. SEC elugrams of both polymers, in **Figure 35**, display asymmetric peaks. The experimental molar masses are in the range of the theoretical  $M_n$ , but using an increased amount of *i*-Bu<sub>3</sub>Al leads to a significant increase of the dispersities. Like already reported, by kinetic experiments, triisobutylaminum decreases the reactivity of the propagating centers while strongly activating the monomer, enhancing the overall reactivity of the system leading to a slight loss of polymerization control. <sup>11</sup> MALDI-TOF spectrum of VP7-25 in **Figure 35**, exhibits a major population assigned to the expected one end, and at least 3 minor populations amongst them one is attributed to water initiated polymer chains or products of transesterification reaction. Whereas for VP7-26, the mass spectrometry analysis only detected the expected distribution i.e. cyclo(Gly-Phe)-poly(*t*BuGE) cationized with Na<sup>+</sup>. Detailed MALDI-TOF spectra are provided in appendix **Figure A29**.



**Figure 35:** Characterization of VP7-25 and VP7-26, (a) SEC trace with THF as eluant of VP7-25; (a') MALDI-TOF spectrum recorded in reflectron mode of VP7-25; (b) SEC trace with THF as eluant of VP7-26; (b') MALDI-TOF spectrum recorded in reflectron mode of VP7-26. ?-poly(*t*BuGE) either correspond to water initiated polymer or product of transesterification reaction.

Infrared spectra for both experiments (VP7-25 and VP7-26) in **Figure 36** display a single peak attributed to C=O amide bond stretching.



Figure 36: ATR-FTIR stacked spectra of VP7-25 and VP7-26

A less bulky Lewis acid, Et<sub>3</sub>Al, was afterwards used. SEC elugrams of VP7-29 and VP7-30 in **Figure 37** show symmetric peaks with broadening of the signal for VP7-30. Additionally for the two experiments, SEC and NMR molar masses are in close agreement, suggesting that no side reactions occurred during the AROP. VP7-29 is showing a slight deviation of the experimental  $M_n$  compared to the theoretical molar mass while the dispersity is quite low. Increasing the amount of Lewis acid in the medium (VP7-30) leads to broadening of the  $M_w/M_n = 1.33$  but having a better control over  $M_n$ . In term of side reactions detection, the mass spectrometry analysis of the sample VP7-29 and VP7-30 in **Figure 37**, detected only the expected population. Detailed reflectron spectra are disclosed in appendix **Figure A30**.



**Figure 37:** MALDI-TOF spectra and SEC elugrams of VP7-29 and VP7-30, (a) SEC trace in THF of VP7-29; (a') MALDI-TOF spectrum recorded in reflectron mode of VP7-29; (b) SEC trace in THF of VP7-30; (b') MALDI-TOF spectrum recorded in reflectron mode of VP7-30.

ATR-FTIR spectra of VP7-29 and VP7-30 in **Figure 38** exhibit a peak at 1670 cm<sup>-1</sup> signature of the C=O amide.



Figure 38: ATR-FTIR stacked spectra for VP7-29 and VP7-30.

From the experiments in **Table 10** performed with Ph<sub>3</sub>Al, *i*-Bu<sub>3</sub>Al and Et<sub>3</sub>Al, little information were conclude from the bulkiness of the trialkylaluminum compound-role in the AROP. However, using increasing amounts of Lewis acids, leads to reduced polymerization times and polymers with high dispersities. This observation can be correlated with the influence of the  $k_p/k_i$  ratio on the dispersity already discussed in the first part of this chapter (p86). For a given initiation rate, high propagation rates induce an increase of the  $k_p/k_i$  ratio producing polymers with high D.

Like mentioned in the literature review part, Et<sub>3</sub>B was used because of his milder Lewis acid activity compared to other Lewis acid like *i*-Bu<sub>3</sub>Al. <sup>20,21</sup> Here, two experiments were set, using 2 and 3 equivalents of Et<sub>3</sub>B, VP7-27 and VP7-28, respectively. The purpose was to examine the influence of the atom center between Et<sub>3</sub>Al and Et<sub>3</sub>B. Full conversion of the reactions VP7-27 and VP7-28 was reached in a shorter period of time compared to Et<sub>3</sub>Al experiments. The  $M_n$  given by SEC and NMR are in good agreement with the theoretical values and unlike previous experiment, by increasing the amount of Lewis acid, the dispersities remain low. Despite those encouraging results, the SEC traces and distributions detected via MALDI-TOF are not monomodal and a shoulder toward low molar masses is clearly visible on the SEC elugrams. Mass spectra of VP7-27 and VP7-28 display three populations in **Figure 39** (detailed mass spectra in appendix **Figure A31**). Even though the major population in MALDI-TOF corresponds to poly(*t*BuGE) chains end capped by cyclo(Gly-Phe) and hydroxy function, minor populations were detected.



Figure 39: MALDI-TOF spectra and SEC elugrams of VP7-27 and VP7-28, (a) SEC trace in THF of VP7-27; (a') MALDI-TOF spectrum recorded in reflectron mode of VP7-27; (b) SEC trace in THF of VP7-28; (b') MALDI-TOF spectrum recorded in reflectron mode of VP7-28. ?-poly(*t*BuGE) either corresponds to water initiated polymer or product of transesterification reaction.

The two peaks profile of SEC and MALDI-TOF analyses could indicates a mixed initiation phenomenon between mono-initiated and double-initiated polymers. Looking at the molar masse values on the mass spectrum, the maximum of the first peak is located at  $(m/z) \approx 2000$  while the mass of the second peak is  $(m/z) \approx 4000$ . This two-time fold difference, could be an evidence of a mono-initiated poly(*t*BuGE) for the first peak and a double initiated poly(*t*BuGE) for the second one. However, without further analysis, those conclusions remain purely hypothetical.

Additionally, infrared spectra in **Figure 40** for VP7-27 and VP7-28, exhibit a peak at 1699  $cm^{-1}$  signature of C=O ester bond stretching. This indicates the ring-opening of the DKP by an attack of alkoxide propagating center via transesterification reaction.



Figure 40: ATR-FTIR stacked spectra for VP7-27 and VP7-28.

Among the 4 Lewis acids tried in **Table 10**,  $Ph_3Al$  and *i*-Bu<sub>3</sub>Al induce a loss of control by producing polymers with broad dispersities, and  $Et_3B$  does not prevent alteration of the peptide structure during the AROP, since ester functions were detected by infrared spectroscopy. The results obtained from  $Et_3Al$  experiments, especially using 2 eq (VP7-29), were successful regarding the control of the molar mass and the purity of the DKP structure. Under those conditions, the side reactions were completely suppressed.

Since the sample from VP7-29 display the best results so far, the resulting polymer will be further analyzed to try to determine how many amides were actually concerns by the initiation step.



Proton NMR spectrum of the native cyclo(Gly-Phe) is displayed in **Figure 41**. Both NH amides are visible and can be attributed, 7.74 ppm NH on the glycine side and 7.96 ppm NH on the phenylalanine side. These attributions were made via <sup>1</sup>H and <sup>13</sup>C NMR coupled with 2D NMR experiments (COSY, HSQC <sup>1</sup>H-<sup>13</sup>C, HMBC, TOCSY). TOCSY and <sup>13</sup>C NMR spectra are displayed in appendix **Figure A32** and **Figure A33**.



**Figure 42:** <sup>1</sup>H NMR spectrum of VP7-29 in DMFd<sub>7</sub> at RT.

<sup>1</sup>H NMR spectrum of VP7-29 in **Figure 42** display signals assigned to the DKP and to the polymer. Integration values on the spectrum are normalized with signals from the proton of the aromatic ring. An integration value of  $\approx$  2 for the hydroxy chain-end function signal (7) suggests the presence of two OH groups. Besides, no signals for NH functions could be seen at room temperature on the spectrum of VP7-29. The temperature during <sup>1</sup>H NMR experiment was increased gradually, to break the H-bonds which could "trap" the signal of the proton from the secondary amides. Resulting spectra are stacked in **Figure 43**. Apart for the signals arising with the temperature between 8.2 ppm and 8.3 ppm which was regarded as impurity, no improvements are obtained from this approach. The values of integrations and the complete disappearance of NH signals suggest that functionalization occurs on both amide groups. HSQC <sup>1</sup>H-<sup>15</sup>N NMR experiments was realized on VP7-29 but no correlation spots were seen to indicate an H born by an N atom.



Figure 43: Zoom in of <sup>1</sup>H NMR experiment at several temperatures recorded in DMFd<sub>7</sub> for VP7-29.

Comparison of the <sup>13</sup>C NMR spectra of VP7-29 to the native cyclopeptide in **Figure 44**, indicates a downward shift of the two C=O signals. It can as well be seen that the C=O signals from VP7-29 are not as well defined as the ones observed from the pure DKP.



**Figure 44:** <sup>13</sup>C NMR spectra in DMFd<sub>7</sub>, (top) spectrum of the native C(G-F); (middle) spectrum of the VP7-29; (bottom) same sample analyzed 24 days after

The absence of correlations between the spectrum of the native peptide and the spectrum from the conjugate, could also indicate that the functionalization concerned both NH amide functions on the DKP ring. However, the modification of a single NH can have influence on both carbonyls since the structure of the cyclic peptide is very constraint.

By using various Lewis acids with different metallic centers, we demonstrated significant differences in term of control of the AROP, and side reactions suppression. According to the two previous parameters, Et<sub>3</sub>Al-catalyzed AROP represent the best conditions. Sample synthesized from the latter procedure was analyzed via NMR to investigate the initiation phenomenon, and results are in favor of a double-sided growth of poly(*t*BuGE) from cyclo(Gly-Phe).

#### b. Optimized conditions for Cyclo(Gly-Val) and C(Leu-Phe)

Et<sub>3</sub>Al, the more efficient Lewis acid was applied to the synthesis of conjugate from cyclo(Gly-Val) and cyclo(Leu-Phe) in Table 11.

**Table 11:** Polymerizations of *t*BuGE by the initiating system DKP: $tBuP_4$ :Et<sub>3</sub>Al in MeTHF at 25°C, [M]<sub>0</sub> = 1.5M

Run	Initiator	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> /[Et <sub>3</sub> Al] <sub>0</sub>	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	Mn <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}/M_{\rm n}}{({\rm SEC})^{\rm c}}$
VP6-23	C(G-V)	30/1/1/2	71	95	3900	3600	4600	1.15
VP6-24	C(G-V)	30/1/1/3	8	86	3500	3800	4100	1.32
VP21-1	C(L-F)	30/1/1/2	56	68	2900	3400	3300	1.22
VP21-2	C(L-F)	30/1/1/3	23	100	4200	4200	4200	1.29

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Polymers were synthesized using a DKP:tBuP<sub>4</sub> ratio of 1:1 with variation of the quantity of Et<sub>3</sub>Al between 2 and 3 number of equivalents.

For cyclo(Gly-Val)-initiated polymers,  $M_n$  SEC are in the range of the theoretical values but  $M_n$  SEC and  $M_n$  NMR are not in close agreement. Whereas for cyclo(Leu-Phe)-initiated polymers, experimental  $M_n$  are in good agreement with the theoretical molar masses.

For all the polymers in Table 11, dispersities values tend to increase with the quantity of Lewis acid, suggesting a slow initiating system in case of 3 eq of Et<sub>3</sub>Al.

For the 2 eq experiment (VP6-23 and VP21-1), mass spectrometry analyses of the products exhibit a major population being the expected one and two minor additional populations. For the experiments based on 3 eq of Et<sub>3</sub>Al (VP6-24 and VP21-2), only the expected population was detected via MALDI-TOF (Figure 45 and Figure 46).



Figure 45: Characterization of VP6-24, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.

The need of an additional equivalent of Et<sub>3</sub>Al to control the polymerization for those two DKPs cyclo(Gly-Val) and cyclo(Leu-Phe) compared to cyclo(Gly-Phe) is not quite understood.



Figure 46: Characterization of VP21-2, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.

In this second part we addressed the synthesis of DKP-polymer conjugates. Polymerization of tBuGE was initiated by bifunctional initiators. By using the right Lewis acid in a sufficient number of equivalents, DKP could actually be fully functionalized by polymers chain(s).

#### c. Synthesis of cyclo(Gly-X) – poly(BO) for structural investigation

In 2019, Barroso-Bujans and coll. used broadband dielectric spectroscopy (BDS) to investigate the growth of polymer from a bifunctional initiator. Ethylene glycol was used as initiator, and the AROP of glycidyl phenyl ether (GPE) was promoted via two ways, via  $tBuP_4$  and  $tBuP_4$ :*i*-Bu<sub>3</sub>Al. By using two synthetic pathways they investigated the influence of the reaction conditions on the arm symmetry. Using BDS analysis they probed the relaxation of the polymers as a mean to analyze and quantify the polymeric structures in the samples.

In this manner, they reveal that the *t*BuP<sub>4</sub> promoted AROP was producing 2 symmetric arms polymers from ethylene glycol, while monomer-activated AROP with tBuP<sub>4</sub>:i-Bu<sub>3</sub>Al leads to unidirectional growth of polymers.<sup>22</sup>

Here, polymers from cvclo(Gly-Val) and cvclo(Gly-Phe) will be synthesized to be further analyzed by BDS, to investigate the microstructure of the macromolecules. By using the best conditions for side reactions suppression, which are tBuP<sub>4</sub>:Lewis acid promoted AROP, we want to clarify if polymerization conditions are favoring mono or double-sided initiated polymers, and thus if the amino acid composition has an influence on the selectivity of initiation.

The monomer was switched from tBuGE to BO. Requirements of this BDS technique is that the polymer chain must have an important dipole moment, do not crystallize and molar masses should be comprised between 3000 g.mol<sup>-1</sup> and 10 000 g.mol<sup>-1</sup>. In the light of this considerations, poly(BO) is reported and regarded as an good alternative to poly(GPE).<sup>23</sup> For the following AROP experiments reported in Table 12 and Table 13 poly(BO) molar masses were set accordingly to the mass limits of the analysis.

Run	Lewis Acid	[M] <sub>0</sub> /[I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [L.A] <sub>0</sub>	Time (h)	Conv (%) <sup>a</sup>	<i>M</i> <sub>n</sub> theo (g/mol)	Mn <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP6-17	<i>i</i> -Bu <sub>3</sub> Al	50/1/1/2	21	100	3800	6900	4800	1.26
VP6-18	<i>i</i> -Bu <sub>3</sub> Al	50/1/1/3	3	100	3800	6400	6400	1.63
VP6-19	Et <sub>3</sub> Al	50/1/1/2	8	91	3500	4700	4500	1.14
VP6-20	Et <sub>3</sub> Al	50/1/1/3	3	88	3300	2200	4700	1.33

**Table 12:** BO polymerizations promoted by the initiated system cyclo(Gly-Val):*t*BuP<sub>4</sub>:Lewis acid at 25°C, in MeTHF,  $[M]_0 = 1.5M$ .

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Run	Lewis Acid	[M] <sub>0</sub> /[I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [L.A] <sub>0</sub>	Time (h)	Conv (%) <sup>a</sup>	M <sub>n</sub> theo (g/mol)	Mn <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP7-33	<i>i</i> -Bu <sub>3</sub> Al	50/1/1/2	21	100	3800	3600	3700	1.26
VP7-34	<i>i</i> -Bu <sub>3</sub> Al	50/1/1/3	3	100	3800	6000	6000	1.62
VP7-35	Et <sub>3</sub> Al	40/1/1/2	6	91	2800	3000	3500	1.15
VP7-36	Et <sub>3</sub> Al	40/1/1/3	3	98	3000	3200	3800	1.41

Table 13: BO polymerizations promoted by the initiated system cyclo(Gly-Phe):*t*BuP<sub>4</sub>:Lewis acid at 25°C, in MeTHF,  $[M]_0 = 1.5M$ 

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in dichloromethane-d<sub>2</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

From the AROP experiments reported in Table 12 and Table 13 all the polymerizations proceeded to full conversion in less than a day. For both Lewis acids used, we can notice that increasing the amount of trialkyl aluminum catalyst leads to a significant increase of the dispersities. For VP7-35 and VP7-36 the [M]<sub>0</sub>/ [I]<sub>0</sub> ratio had to be lower to 40, due to a gellification of the reaction medium occurring at high polymerization degree targeted with Et<sub>3</sub>Al, thus limiting the conversion of the AROP.

For VP6-18 and VP6-19, SEC  $M_n$  and NMR  $M_n$  are in close agreement, but the experimental values are higher than expected. For VP6-17 and VP6-20, experimental  $M_{\rm n}$  are not in greement with the theoretical  $M_{\rm n}$ .

For cyclo(Gly-Phe) conjugates, experimental molar masses are in the range of the theoretical  $M_{\rm n}$ , excepted for VP7-34.



**Figure 47:** Influence of the number equivalents of *i*-Bu<sub>3</sub>Al used on SEC traces and MALDI-TOF spectra for cyclo(Gly-Val)-based conjugates. (a) SEC trace in THF of VP6-17; (b) MALDI-TOF spectrum recorded in reflectron mode for VP6-17; (a') SEC trace in THF for VP6-18; (b') MALDI-TOF spectrum recorded in reflectron mode for VP6-18.



**Figure 48:** Influence of the number equivalents of Et<sub>3</sub>Al on SEC elugrams and MALDI-TOF spectra for Cyclo(Gly-Val)-based conjugates. (a) SEC trace in THF for VP6-19; (b) MALDI-TOF spectrum recorded in reflectron mode VP6-19; (a') SEC trace in THF for VP6-20; (b') MALDI-TOF spectrum recorded in reflectron mode VP6-20.



**Figure 49:** Influence of the number equivalents of *i*-Bu<sub>3</sub>Al on SEC elugrams and MALDI-TOF spectra for Cyclo(Gly-Phe)-based conjugates. (a) SEC trace in THF for VP7-33; (b) MALDI-TOF spectrum recorded in reflectron mode VP7-33; (a') SEC trace in THF for VP7-34; (b') MALDI-TOF spectrum recorded in reflectron mode VP7-34.



**Figure 50:** Influence of the number equivalents of Et<sub>3</sub>Al on SEC elugrams and MALDI-TOF spectra for Cyclo(Gly-Phe)-based conjugates. (a) SEC trace in THF for VP7-35; (b) MALDI-TOF spectrum recorded in reflectron mode VP7-35; (a') SEC trace in THF for VP7-36; (b') MALDI-TOF spectrum recorded in reflectron mode VP7-35; (a) SEC trace in THF for VP7-36.

For the SEC traces shown in **Figure 47** and **Figure 49**, a slight tailing can be observed which is increasing with the amount of *i*-Bu<sub>3</sub>Al. This phenomenon noted for the polymers using *i*-Bu<sub>3</sub>Al as catalyst is due to a slow initiation process due to the lower reactivity of the complexes amidate-<sup>+</sup>HtBuP<sub>4</sub>-*i*-Bu<sub>3</sub>Al.

While peaks from SEC traces in **Figure 48** and **Figure 50** are symmetrical but having the dispersity increasing with the equivalents of Et<sub>3</sub>Al.

MALDI-TOF spectra displayed in **Figure 47** and in **Figure 48** for cyclo(Gly-Val) initiated polymerization and in **Figure 49** and **Figure 50** for cyclo(Gly-Phe) exhibit a major population assigned to poly(BO) initiated by cyclo(Gly-X) and terminated by an hydroxy function. Detailed mass spectra are shown in appendix **Figure A35** and **Figure A36**.

MALDI-TOF spectra of 2 eq of Lewis acid experiments from cyclo(Gly-Val) (VP6-17 and VP6-19) (see **Figure 47** and **Figure 48**), seem to indicate a multimodal distribution from the shape of the expected population (green). Otherwise we can see that increasing the amount of Lewis acid in the medium leads to a total suppression of the side reactions and water initiated poly(BO).

For reactions performed with cyclo(Gly-Phe) and triethylaluminum, (VP7-33 and VP7-34) (see **Figure 49**) mass spectra contain parasitic initiation from side reactions. While *i*-Bu<sub>3</sub>Albased reactions are capable of complete inhibition of those secondary reactions (see MALDI-TOF spectrum in **Figure 50**).

The 8 samples were analyzed though IR spectroscopy. Spectra in (**Figure 51**) exhibit a single peak assigned to C=O amide bond stretching.



Figure 51: ATR-FTIR of (a) *N*-poly(BO)-Cyclo(Gly-Val); (b) *N*-poly(BO)-Cyclo(Gly-Phe).

From this study using a different monomer, the results in terms of MALDI-TOF analysis were the same as the ones obtained with *t*BuGE. Lewis acid catalysis is a great alternative to  $tBuP_4:tBuP_2$  promoted AROP in term of side reactions suppression. The poly(BO) – cyclo(Gly-X) (X = Val or Phe) will be further analyzed through BDS, to define if the initiation is occurring on both amide functions or if the process is selective. And if the initiation is partially selective, we will be able to quantify the amount of DKP concerned by a double-sided and mono-sided polymer growth.

# Conclusion

In this chapter we reported the first synthesis of 2,5-diketopiperazine-polyethers conjugates by AROP.

In a first part, mono-functional DKPs based on proline were used combined with phosphazene base to initiate the polymerization of epoxide monomers. Addition of *i*-Bu<sub>3</sub>Al as catalyst allows the partial suppression of transfer reactions to the initiator while maintaining the control of the AROP. The technique was used to synthesize DKP-polyglycidol conjugates for further biological activity testing.

Then the conventional AROP conditions using *t*BuP<sub>4</sub> base was implemented with bifunctional DKPs, bearing two different side groups. Optimization allowed the polymerization of *t*BuGE promoted by *t*BuP<sub>2</sub> with a catalytic amount of *t*BuP<sub>4</sub> bringing very limited side reactions. An alternative method for complete clearance of side reactions was developed from Lewis acid catalysis via the monomer-activated AROP. Influence of the Lewis acids chemical structure was highlighted through the control of the polymerization and the occurrence of side reactions. Even though NMR experiments are in favor of bi-functionalized DKP, further analysis on DKP-poly(BO) conjugates will be pursued in order to investigate the structure of the conjugate and clarify the mono- or double-sided initiation phenomena.

The functionalization method was applied to various types of DKPs. Polymers of different nature were grafted to the cyclic dipeptides to tune the solubility of the biological material and allowing a further use in biomedical applications.

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# Appendix





Figure A1: Possible mechanism for the chain transfer to the oxirane and the calculated mass of the product.



Figure A2: Possible mechanism for the chain transfer to the triisobutylaluminum and the calculated mass of the product.



Figure A3: Possible mechanism for the chain transfer to the triisobutylaluminum and the calculated mass of the product.



**Figure A4:** <sup>13</sup>C quantitative NMR spectrum recorded in CDCl<sub>3</sub> of poly(BO) polymer initiated by C(A-P):*t*BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (Table 2, run P2-12) and equation used to determined its number -average molar mass.













Figure A11: MALDI-TOF spectrum of P1-2 recorded in reflectron mode (a), and Zoom in area (b).



Figure A12: MALDI-TOF spectrum of P1-3 recorded in reflectron mode (a), and Zoom in area (b).



Figure A13: MALDI-TOF spectrum of P1-4 recorded in reflectron mode (a), and Zoom in area (b) and (c).

Chapter 2: Asymmetric DKPs



Figure A14: <sup>1</sup>H-<sup>1</sup>H COSY NMR spectrum of P2-12 recorded in CDCl<sub>3</sub> at RT.



Figure A15: <sup>1</sup>H-<sup>13</sup>C HSQC NMR spectrum of P2-12 recorded in CDCl<sub>3</sub> at RT.



Figure A16: <sup>1</sup>H-<sup>13</sup>C HMBC NMR spectrum of P2-12 recorded in CDCl<sub>3</sub> at RT.



**Figure A17:** <sup>1</sup>H-<sup>1</sup>H COSY NMR spectrum of P4-1d in MeOD<sub>4</sub> at RT.



Figure A18: <sup>1</sup>H-<sup>13</sup>C HSQC NMR spectrum of P4-1d in MeOD<sub>4</sub> at RT.


**Figure A19:** <sup>1</sup>H-<sup>1</sup>H COSY NMR spectrum of P4-4d in MeOD<sub>4</sub> at RT.



Figure A20: <sup>1</sup>H-<sup>13</sup>C HSQC NMR spectrum of P4-4d in MeOD<sub>4</sub> at RT.



Figure A21: MALDI-TOF spectrum recorded in reflectron mode of VP6-2, (a) zoom in area m/z = 1660-1930; (b) zoom in area m/z = 4000-4550.





Figure A22: Postulated mechanism for the reaction of DKP ring-opening.



Figure A23: MALDI-TOF spectrum recorded in reflectron mode of VP6-6, (a) zoom in area m/z = 1400-1660; (b) zoom in area m/z = 3000-3350.



Figure A24: MALDI-TOF spectrum recorded in reflectron mode of VP7-1, zoom in area m/z = 980-1225.



**Figure A25:** Characterization of VP7-2, (a) SEC trace with THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) and (d) Zoom in region of the MALDI-TOF spectrum. ?-poly(*t*BuGE) either corresponds to water initiated polymer or product of transesterification reaction.



Figure A26: MALDI-TOF spectrum recorded in reflectron mode of VP7-16, (a) zoom in area m/z = 980-1260; (b) zoom in area m/z = 2800-3050.



Figure A27: MALDI-TOF spectrum recorded in reflectron mode of VP6-8, insert m/z = 1120 - 1350.



Figure A28: MALDI-TOF spectrum recorded in reflectron mode insert m/z = 1800 – 2200 and 980 - 1220, (a) Mass spectrum of VP7-11; (b) and (c) Mass spectrum of VP7-13.



Figure A29: MALDI-TOF spectrum recorded in reflectron mode insert m/z = 1990 – 2325 or 1000 - 1260, (a) and (b) Mass spectrum of VP7-25; (a') Mass spectrum of VP7-26.



Figure A30: MALDI-TOF spectrum recorded in reflectron mode insert m/z = 1990 – 2325, (a) Mass spectrum of VP7-29; (a') Mass spectrum of VP7-30.



Figure A31: MALDI-TOF spectrum recorded in reflectron mode insert m/z = 1990 - 2325, (a) Mass spectrum of VP7-27; (a') Mass spectrum of VP7-28.



Figure A32: TOCSY NMR spectrum of cyclo(Glycine-Phenylalanine) recorded in DMFd7 at RT.



Figure A33: <sup>13</sup>C NMR spectrum of cyclo(Glycine-Phenylalanine) recorded in DMFd<sub>7</sub> at RT.



Figure A34: (a)  ${}^{1}$ H- ${}^{1}$ H COSY NMR spectrum of VP7-29 recorded in DMFd<sub>7</sub> at RT; (b)  ${}^{1}$ H- ${}^{13}$ C HSQC NMR spectrum recorded in DMFd<sub>7</sub> at RT.



Figure A35: MALDI-TOF spectrum recorded in reflectron mode insert m/z = 1700 - 1950, (a) Mass spectrum of VP6-17; (b) Mass spectrum of VP6-18; (a') Mass spectrum of VP6-19; (b') Mass spectrum of VP6-20.



Figure A36: MALDI-TOF spectrum recorded in reflectron mode insert m/z = 1700 - 1950, (a) Mass spectrum of VP7-33; (b) Mass spectrum of VP7-34; (a') Mass spectrum of VP7-35; (b') Mass spectrum of VP7-36.

## — CHAPTER 3 —

# Protected dipeptide as initiator: polymer synthesis from Z-Val-Phe-OMe

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#### Introduction

In this chapter we extended previous results to the *N*-modification of protected linear peptide. Herein, we report the polymerization of epoxide monomers from the dipeptide Z-Val-Phe-OMe, using NH functions as initiators. Divergent synthesis of polyethers from the dipeptide without side reactions was of prime concern for this work.

The dipeptide size was chosen to limit the number of initiating sites. The peptide is composed of Valine and Phenylalanine residues. Both amino acids bear non-ionizable side groups, precluding secondary reactions due to the side chains. Furthermore, Val and Phe possess specific chemical signatures, allowing a precise identification of the NH functions by spectroscopy analysis.

The protected version of the dipeptide was used to prevent parasitic initiations, from the amino or carboxylic acid terminal functions. Among the protecting groups, we choose a peptide protected by a benzyl carbamate (Z) on N-ter side and by an ester function (OMe) on C-ter side. Both organic functions offer the advantages to be easily cleavable by post-modification.

Moreover, having an ester and carbamate functions on the same peptide backbone with the amide, will allow to investigate reaction conditions compatible with those protecting groups, and to explore the site selectivity of the modification regarding the NH amide or NH carbamate.

### I. Z-Val-Phe-OMe functionalization by poly(BO) via conventional AROP

In this first part, the polymerization of 1,2-butylene oxide (BO) from the dipeptide Z-Valine-Phenylalanine-OMe (Z-Val-Phe-OMe) is reported (see **Scheme 1**). Initially the monomer BO was used as model monomer for optimization of the reaction conditions. BO is rather safe to handle, and is not subject to transfer reactions, for moderate targeted  $M_n$ , during organocatalyzed AROP. Phosphazene bases  $tBuP_4$  and  $tBuP_2$  will be used in this part.



Scheme 1: Polymerization of 1,2-butylene oxide initiated by Z-Val-Phe-OMe

Compound	pKa value (DMSO)	Ref.
tBuP <sub>4</sub>	30.2	1
tBuP <sub>2</sub>	21.5	1
<b>NH</b> amide	12.2	Estimated values from
NH carbamate	13.6	MarvinSketch 20.8 software

Carbamate and amide functions of the peptide have estimated pKa values of 13.6 and 12.2, respectively (Table 1), much lower than  $tBuP_n$ . Given the pKa values of  $tBuP_4$  and  $tBuP_2$ , both bases seem to be likely to promote AROP from both NH functions. Since the estimated pKa value of the amide is lower than the one of the carbamate, we hypothesize that the functionalization will preferably occurs on the amide group rather than on the carbamate group.

Polymerization reactions from Table 2 were conducted in THF at various temperatures, and targeting a  $X_n = 30$ . The polymerization degree was set reasonably low to obtain polymers in molar masses range easily characterizable by MALDI-TOF. Only 1 equivalent of organobase was used regarding the peptide initiator, due to the proven efficiency of such ratio to initiate the AROP of BO.<sup>2,3</sup>

Run	<i>t</i> BuP <sub>n</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP11	tBuP <sub>4</sub>	25	47	93	2400	4500	1000	1.35
VP11-4	tBuP <sub>4</sub>	-20	22	100	1500	2200	860	2.17
VP11-50	<i>t</i> BuP <sub>2</sub>	25	24	0	-	-	-	-
VP11-51	<i>t</i> BuP <sub>2</sub>	-20	24	0	-	-	-	-

**Table 2:** Polymerizations of BO by the initiating system Z-VF-OMe: $tBuP_{n=} 1:1$ , in THF,  $[M]_0 = 1.3M$ .

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For VP11 using tBuP<sub>4</sub>, the polymerization reached a high conversion within 2 days. SEC  $M_n$ is lower than the theoretical value, while the NMR  $M_n$  is higher though dispersity is fairly narrow. Discrepancy between measured and theoretical molar masses suggests the occurrence of side reactions during the AROP. The reactions using  $tBuP_2$  lead to no polymerization in the reaction vessel after 1 day. This result is consistent with the state of the art, reporting that only *t*BuP<sub>4</sub> base is capable of promoting the AROP of epoxides in a reasonable matter of time.

MALDI-TOF spectrum of VP11 in Figure 1 exhibits two populations, one assigned to methanolate initiated poly(BO), and the other one to benzyl alcoholate initiated poly(BO), moreover no population initiated by the peptide could be detected.



Figure 1: MALDI-TOF spectrum recorded in reflectron mode for VP11 (left) and zoom in area (right).

Those two alcohol-initiated polymer chains result from two side reactions occurring during the polymerization. Concerning the benzyl alcohol (green), Illy and coworkers already identified that carbamate functions, under highly basic conditions, undergo a deprotonation and a self-dissociation, releasing an alkoxide and a cyclic byproduct following the mechanism given in **Scheme 2 (a)**. <sup>4</sup> The reaction concerning the ester is a transesterification between alkoxide propagating center and carbonyl function of the ester, releasing methanolate in the medium (**Scheme 2 (b)**). <sup>5</sup> Both deprotonated alcohols generated by those side reactions, further reacted with a monomer unit to initiate a polymer chain.

To improve the stability of the protecting groups, the temperature was decreased to -20°C in VP11-4. We assume that the activation energy of the side reactions aforementioned would be different from the propagation reaction. The molar mass given by NMR is in agreement with the theoretical value while the  $M_n$  given by SEC is lower than expected  $M_n$ .

Decreasing the temperature to -20°C, leads to a significant broadening of the dispersity. Moreover MALDI-TOF analysis does not display improvements.



Scheme 2: Mechanisms of the two side reactions occurring to the peptide initiator. (a) carbamate dissociation (b) transesterification reaction.

Using phosphazene bases combined with Z-VF-OMe demonstrated that the ester and carbamate functions are not stable under such basic conditions. Thus, no functionalization can be performed using this system. In their work about secondary carbamate-initiated AROP, Illy and coworkers used *i*-Bu<sub>3</sub>Al as a mean to stabilize the carbamate initiators. The use of *i*-Bu<sub>3</sub>Al as Lewis acid will be investigated in the following parts, and his ability to keep the integrity of the peptide will be monitored.

### II. Z-Val-Phe-OMe functionalization by poly(BO) via Monomer-activated AROP

In this part we will discuss the Z-Val-Phe-OMe-initiated AROP, organocatalyzed by combination of  $tBuP_n$  and Lewis acid in the medium, to synthesize peptide-polymers conjugates. Investigations will be conducted (1) to explore the stability of the protecting groups under polymerization conditions, and (2) to identify the site of modification on the peptide, on samples displaying the best results by MALDI-TOF spectrometry.

#### 1. *t*BuP<sub>4</sub> combined with *i*-Bu<sub>3</sub>Al

Phosphazene base  $tBuP_4$  was used in this part. The solvent was switched to MeTHF in order to avoid competitive complexation of the Lewis acid by the solvent. Influence of the temperature, and of the number of equivalents used *i*-Bu<sub>3</sub>Al will be investigated to limit the side reactions during the polymerizations reported in **Table 3**.

Due to the requirements of the monomer-activated mechanism the [*i*-Bu<sub>3</sub>Al]/[Z-Val-Phe-OMe] ratio was automatically set above 1.

2 eq of *i*-Bu<sub>3</sub>Al were not sufficient to initiate the polymerization. Lewis acids based on aluminum center exhibit a high oxophilicity, and are known to build coordination bonds with heteroatoms. Using Z-Val-Phe-OMe peptide, containing 3 carbonyl groups, we suggest that 3 equivalents of *i*-Bu<sub>3</sub>Al would be the minimum amount of Lewis acid to stabilize the peptide, but not to polymerize BO with the monomer-activated AROP. Indeed, an excess of aluminum compound is required for monomer activation.

Based on those considerations, a  $[i-Bu_3A1]/[Z-Val-Phe-OMe]$  ratio > 4 would be necessary for the Z-Val-Phe-OMe-initiated AROP of BO (see Scheme 3).

We suggest that the Lewis acid near the deprotonation site is used to complex the amidate, or the propagating alkoxide and  $^{+}H$ -*t*BuP<sub>4</sub> counter ion.

Run	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [ <i>i</i> -Bu <sub>3</sub> Al] <sub>0</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>c</sup>
VP11-7	30/1/1/2	-20	144	-	-	-	-	-
VP11-19	30/1/1/3	25	24	100	2600	5100	2900	1.56
VP11-17	30/1/1/3	-20	24	92	2400	4700	3900	2.02
VP11-10	30/1/1/4	25	16	100	2600	5600	5000	1.69
VP11-5	30/1/1/4	-20	38	100	2600	3900	3000	2.20
VP11-8	30/1/2/4	-20	158	70	1800	8600	2600	1.62
VP11-55	30/1/1/5	25	0.67	100	2600	4800	4900	1.90
VP11-59	30/1/1/5	-20	1	100	2600	4400	4800	1.87
VP11-56	30/1/1/6	25	0.67	100	2600	4100	4600	1.90
VP11-60	30/1/1/6	-20	1	100	2600	4600	4800	1.90

Table 3: Polymerizations of BO via monomer-activated AROP initiated by Z-Val-Phe-OMe in MeTHF,  $[M]_0 = 1.3M.$ 

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For  $[i-Bu_3A1]/[Z-Val-Phe-OMe]$  ratio  $\geq 3$ , polymerization proceeds. High conversion values, over 70%, were reached, calculated with <sup>1</sup>H NMR comparing the signals integrations of the monomer and the polymer, from sampling in the reaction medium. The fact that already with 3 equivalents the polymerization proceeds, indicates a fast rate of *i*-Bu<sub>3</sub>Al exchange between the different coordination sites [*i*-Bu<sub>3</sub>Al-carbonyl] and [*i*-Bu<sub>3</sub>Al-epoxide] (see Scheme 3).



Scheme 3: Complexation sites in the reaction medium for *i*-Bu<sub>3</sub>Al.

At 25°C (VP11-19) the  $M_n$  given by SEC is in good agreement with the theoretical value, while the RMN  $M_n$  is higher than the SEC  $M_n$ , suggesting the occurrence of side reactions. MALDI-TOF analysis of VP11-19 detected 4 populations in **Figure 2**. (detailed spectrum in appendix **Figure A9**). The major one (purple) being poly(BO) chains initiated by hydride and terminated by an hydroxyl, suggests a transfer reaction involving the *i*-Bu<sub>3</sub>Al (see appendix for proposed mechanism **Figure A2**). A poly(BO) initiated by Z-Val-Phe-OMe (green) and hydroxy terminated is detected. Two minor populations were attributed to poly(BO) initiated by a methanolate and benzyl alcoholate (red), and terminated by hydroxy functions.

The detection of the expected population demonstrates the possible functionalization of the peptide by poly(BO) chains. By using triisobutylaluminum, the stability of the protecting groups was enhanced, allowing initiation by Z-Val-Phe-OMe.

To further increase the sturdiness of the ester and the carbamate, the temperature was dropped to -20°C (VP11-17). The relative good agreement between the SEC molar masses and NMR molar masses suggest the presence of a peptide end-group at the end of each polymer chain. However, the decrease of the temperature induces higher experimental values of  $M_n$  than theoretical, and a significant broadening of the dispersity from  $M_w/M_n = 1.56$  (VP11-19, 25°C) to 2.02 (VP11-17, -20°C). SEC traces in **Figure 2** show a tailing towards small  $M_n$ , which indicates a slow initiation process.

MALDI-TOF spectra of VP11-17 in **Figure 2**, partially confirmed our previous assumption: the major population is now poly(BO) initiated by Z-Val-Phe-OMe terminated by OH, and cationized by Na<sup>+</sup>, while only a minor population corresponding to hydride-initiated poly(BO) was detected. Detailed spectrum is provided in appendix **Figure A9**.

Based on those results the synthesis of the expected conjugate was demonstrated. *i*-Bu<sub>3</sub>Al confirms its ability (1) to stabilize the carbamate function under highly basic conditions, and (2) to lower the reactivity of the alkoxide preventing nucleophilic attack on the carbonyls (**Scheme 4**). However, the addition of triisobutylaluminum in the medium can leads to side reactions, such as hydride initiation.



**Figure 2:** SEC elugrams with Refractive index trace and UV trace, and MALDI-TOF spectra recorded in linear mode.(a) SEC traces of VP11-19; (b) MALDI-TOF spectrum VP11-19; (a') SEC traces of VP11-17; (b') MALDI-TOF spectrum VP11-17.



Scheme 4: Proposed mechanism of ester and carbamate stabilization using *i*-Bu<sub>3</sub>Al.

VP11-10 was carried out with 4 eq of *i*-Bu<sub>3</sub>Al at 25°C. Compared to VP11-19 (3 eq *i*-Bu<sub>3</sub>Al; 25°C), polymer from VP11-10 exhibits a close agreement between SEC and NMR molar masses. For VP11-10, measured  $M_n$  are higher than theoretical, and a high dispersity is obtained. On SEC trace of VP11-10 in **Figure 3**, an additional signal towards high retention volumes ( $\approx$ 28 ml) was identified to be non-modified peptide, suggesting a rather low initiation efficiency of the peptide, consequence of a slow initiation process. SEC elugram of Z-Val-Phe-OMe is displayed in appendix **Figure A8**.

However, the comparison of VP11-19 and VP11-10 MALDI-TOF spectra clearly shows a considerable reduction of the side reactions. On MALDI-TOF spectrum of VP11-10 in **Figure 3**, the major population is the expected one, Z-Val-Phe-OMe - poly(BO) – OH, cationized by Na<sup>+</sup>, and only a minor distribution was detected, but population could not be assigned to suggested side reaction exhibited in appendix **Figure A1**, **Figure A2**, **Figure A3**, **Figure A4**, **Table A1**, **Table A2**. Detailed mass spectrum of VP11-10 is disclosed in appendix **Figure A10**.

Under those conditions, the integrity of the peptide structure is mostly retained, due to improved stability of the ester and carbamate functions.

To reduce the amount of secondary reactions the temperature was decreased to  $-20^{\circ}$ C in VP11-5. For this sample the experimental  $M_n$  are slightly higher than theoretical molar mass, and decreasing the temperature leads to a significant broadening of the dispersity  $M_w/M_n = 2.20$ . The results suggest that the amounts of products from side reactions are limited at - 20°C. Additionally, when comparing the reflectron spectra of VP11-17 (**3eq** *i*-Bu<sub>3</sub>Al; -20°C) in **Figure 2** and of VP11-5 (**4eq** *i*-Bu<sub>3</sub>Al; -20°C) in **Figure 3**, slight improvement can be seen on 4 eq Lewis acid-based spectrum.

We recently published a study demonstrating the broadening of the  $M_w/M_n$  by introduction of triisobutylaluminum in the medium.<sup>2</sup> Moreover, for experiments performed at -20°C, a tailing can be observed on the SEC traces. Combination of factors, low temperature and addition of *i*-Bu<sub>3</sub>Al leads to dispersities above 2. This result can be explained by a slow initiation step, increasing the  $k_p / k_i$  ratio (p86), inducing a low initiation efficiency.



**Figure 3:** SEC elugrams with Refractive index trace and UV trace, and MALDI-TOF spectra recorded in linear mode.(a) SEC traces of VP11-10; (b) MALDI-TOF spectrum VP11-10; (a') SEC traces of VP11-5; (b') MALDI-TOF spectrum VP11-5.

The number of equivalents of *i*-Bu<sub>3</sub>Al was raised to 5 and 6 eq, in order to test if an increasing amount of triisobutylaluminum, could enhance the protection of the peptide, and induce a significant reduction of side reactions. For VP11-55 (5eq *i*-Bu<sub>3</sub>Al; 25°C), VP11-49 (5eq *i*-Bu<sub>3</sub>Al; -20°C), VP11-56 (6eq *i*-Bu<sub>3</sub>Al; 25°C) and VP11-60 (6eq *i*-Bu<sub>3</sub>Al; -20°C), the experimental molar masses are higher than the theoretical values, and dispersities are high,  $M_w/M_n \ge 1.87$ .

RI SEC trace of VP11-55 in **Figure 4** exhibits a tailing towards high retention volumes. On RI and UV SEC traces a peak at high retention volumes ( $\approx 28$  ml) was assigned to crude Z-Val-Phe-OMe .(SEC elugram of Z-Val-Phe-OMe in appendix **Figure A8**).

MALDI-TOF analysis of VP11-55 displayed in **Figure 4** exhibits two populations. The major one being poly(BO) with Z-Val-Phe-OMe at one end and an hydroxy function at the other, and the second population is poly(BO) initiated by methanolate. The same results were observed for VP11-49, VP11-56 and VP11-60.



Figure 4: Characterization of VP11-55, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

By increasing the number equivalents of *i*-Bu<sub>3</sub>Al, we favored the side reaction to the ester function. In synthetic chemistry, alkylaluminum Lewis acid are a common way to increase the C atom electrophilicity and to activate the carbonyl functions (**Scheme 5**). By increasing the amount of triisobutylaluminum, we might have actually activated the ester function, and made it more prone to nucleophilic attack by the propagating center.



Scheme 5: Ester activation upon the addition of Lewis acid (*i*-Bu<sub>3</sub>Al).

In VP11-8, 2eq of  $tBuP_4$  and 4eq of *i*-Bu<sub>3</sub>Al were used at -20°C to increase the reactivity of the initiating peptide, and thus increase the rate of initiation. Those conditions did manage to reduce the dispersity, but polymer display NMR  $M_n$  value higher than SEC and theoretical values, suggesting the occurrence of side reactions. MALDI-TOF analysis of VP11-8 in **Figure 5** display only two undesired populations of poly(BO). The use of 2 equivalents of phosphazene base leads to a significant disruption of the Z-Val-Phe-OMe structure, thus the amount of  $tBuP_4$  will be limited to 1 for further optimization.



Figure 5: (a) MALDI-TOF spectrum recorded in reflectron mode for VP11-8; (b) zoom in area.
Up to this point, experiments based on 1 eq of  $tBuP_4$  and 4 equivalents of *i*-Bu<sub>3</sub>Al are successful in side reactions limitation. Composition of the initiating system was set accordingly [Z-Val-Phe-OMe]<sub>0</sub>/[ $tBuP_4$ ]<sub>0</sub>/[*i*-Bu<sub>3</sub>Al]<sub>0</sub>/[M]<sub>0</sub>: 1/1/4/30 and kept constant for optimization. The challenge was to try to reduce the dispersity by introducing a deprotonation time. The hypothesis is: addition of *i*-Bu<sub>3</sub>Al before the phosphazene base could hinder the structure of the peptide, and render the access to NH functions difficult for deprotonation. By implementing a deprotonation time of the peptide, and changing the addition order of the reagents it is supposed that it might increase the amount of NH functions concerned by deprotonation, and thus minimize the  $M_w/M_n$ . To test the hypothesis 3 experiments were set with 2 hours deprotonation time at different temperatures. Polymerization conditions are gathered in **Table 4**.

**Table 4:** BO polymerizations from the initiating system  $[Z-Val-Phe-OMe]_0/[tBuP_4]_0/[i-Bu_3Al]_0/[M]_0$ : 1/1/4/30, with 2h deprotonation time, in MeTHF,  $[M]_0=1.3M$ .

Run	Temp. (°C) Deprotonation/ polymerization	Time (h)	Conv <sup>a</sup> (%)	<i>M</i> n theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>c</sup>
VP11-9	25/25	86	100	2600	4000	4900	2.54
VP11-6	25/-20	17	100	2600	5700	6500	2.21
VP11-30	-20/-20	3	100	2600	4700	5600	2.23

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.

<sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

The three experiments exhibit experimental  $M_n$  higher than theoretical  $M_n$  and broad dispersities. Those results suggest even lower initiation efficiency than for regular polymerization method, thus, further optimization will be made without deprotonation time.

To conclude, the results obtained so far demonstrated the first functionalization of Z-Val-Phe-OMe by poly(BO) chains. By using a sufficient amount of Lewis acid, we managed to significantly improve the stability of the protecting groups. From all the conditions tested, experiments based on 4 equivalents of i-Bu<sub>3</sub>Al are the only ones, for a successful stabilization of the protective ester and carbamate functions on the peptide. For less than 4 equivalents, there is not enough Lewis acid to keep the protections and with more than 4 equivalents, the activation of the carbonyls overpasses the protective role of *i*-Bu<sub>3</sub>Al. Even though with 4 eq we actually managed to limit the side reactions leading to alcoholates initiation, there is still secondary reaction(s) occuring during the polymerization, and for some we don't know the mechanism or the chemical entities implied. We suggest that the high nucleophilicity of the anions generated by  $tBuP_4$  might be responsible for those secondary reactions.

#### 2. *t*BuP<sub>2</sub> combined with *i*-Bu<sub>3</sub>Al

In order to decrease the reactivity of the anionic center,  $tBuP_2$  will be used instead of  $tBuP_4$ . We recently reported that  $tBuP_4$ -promoted AROP was propagating through ion pairs. <sup>2</sup> Using  $tBuP_2$  the same phenomenon is expected to occur. The ionic radius of the <sup>+</sup>H $tBuP_2$  (3.3 Å) is smaller than <sup>+</sup>H $tBuP_4$  (5.0 Å), <sup>6</sup> which explains a shorter inter-ionic distance in the case of  $tBuP_2$ , and a stronger interaction with the amidate/alkoxide, decreasing the reactivity of the anion, and slowing down the kinetic of  $tBuP_2$ -promoted AROP. <sup>7</sup>

The influence of the number of equivalents of i-Bu<sub>3</sub>Al and the temperature will be investigated in **Table 5** to tune the stability of the ester and carbamate protecting groups. 3 eq of i-Bu<sub>3</sub>Al were used as a start to take advantage of the exchange dynamic of the Lewis acid between the complexation sites, and allowing a limitation of the quantity of this highly reactive compound

For all conditions reported in **Table 5**, a polymerization was initiated, and reached a high conversion in less than a day. On the SEC traces a signal at higher retention volumes suggest the presence of remaining peptide. Polymers in **Table 5**, exhibit higher experimental  $M_n$  than theoretical, and broad dispersities,  $M_w/M_n = 1.81 - 2.56$ . Those observations suggest a slow initiation step inducing low initiation efficiency.

According to MALDI-TOF, polymerization conditions of VP11-15 are the only one to allow a complete suppression of the secondary reactions (**Figure 6**).

Run	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [ <i>i</i> -Bu <sub>3</sub> Al] <sub>0</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	Mn <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP11-22	30/1/1/3	25	20	100	2600	4600	5900	1.86
VP11-18	30/1/1/3	-20	24	90	2300	5700	5300	1.87
VP11-48	30/1/1/4	40	0.75	100	2600	4600	5200	1.87
VP11-11	30/1/1/4	25	13	100	2600	4400	5000	1.83
VP11-35	30/1/1/4	0	3	100	2600	4600	5100	2.16
VP11-15	30/1/1/4	-20	21	100	2600	4700	7100	2.09
VP11-53	30/1/1/5	25	1	100	2600	5300	5000	1.85
VP11-57	30/1/1/5	-20	1	100	2600	3800	4700	1.81
VP11-54	30/1/1/6	25	1	100	2600	4100	4200	1.95
VP11-58	30/1/1/6	-20	1	100	2600	3400	4100	1.95

**Table 5:** BO polymerizations initiated by Z-Val-Phe-OMe: $tBuP_2:i-Bu_3Al$  in MeTHF,  $[M]_0 = 1.3$  M.

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Considering the successful suppression of side reactions for VP11-15, the reactions conditions were used as reference for kinetic experiment. The kinetic plot reported in Figure 7 shows a linear dependence regarding the logarithmic concentration of BO vs time which establish a 1<sup>st</sup> order kinetic regarding the monomer, in contrary to Puchelle et al. who demonstrated a 0 order to the monomer.<sup>2</sup> The mechanism responsible for this behavior is still unclear, and further experiments will be pursued to precise the role of the Lewis acid.

Further analyses to determine the modification site(s) on the peptide were achieved on VP11-15, since the mass spectra did not show secondary populations.

First, the polymer from VP11-15 experiment was dialyzed against methanol to eliminate the crude peptide (VP11-15 -> VP11-15dl). By doing so we ensure to be able to only see signals assigned to Z-Val-Phe-OMe bind to a poly(BO) chains.



Figure 6: Characterization of VP11-15, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum



Figure 7: First-order plot (slope = 0.0466 min<sup>-1</sup>,  $R^2 = 0.976$ ) for the polymerization of BO at -20°C in MeTHF, [Z-Val-Phe-OMe]/[*i*BuP\_2]/[*i*-Bu\_3Al] = 1/1/4; [M]\_0 = 1.3M.

On both <sup>1</sup>H NMR spectrum and <sup>13</sup>C NMR spectrum of VP11-15dl, **Figure 8** and **Figure 9**, respectively, signals from the dipeptide, and the polymer can be seen even after passing through alumina, and dialysis against methanol. This information is evidencing the grafting of polymers on Z-Val-Phe-OMe. Additional 2D spectra can be seen in appendix **Figure A11** and **Figure A12**.





Figure 9: <sup>13</sup>C NMR (Jmod) spectrum of VP11-15dl recorded in CDCl<sub>3</sub> at RT.

# <sup>15</sup>N NMR experiment was tried but the low sensibility of the experiment displays unsatisfactory results in terms of identification of grafting sites.

Identification of the NH functions was performed on the initial peptide by <sup>1</sup>H-<sup>15</sup>N HSQC (**Figure 10**) to detect H born by N atom, and by TOCSY experiment (**Figure 11**). TOCSY is an NMR experiment for establishing <sup>1</sup>H-<sup>1</sup>H connectivity like COSY, but provides information by identifying larger and interconnected groups of indirectly coupled hydrogens. TOCSY is commonly used for structure elucidation in peptide materials.



Figure 10: Zoom in area of <sup>1</sup>H-<sup>15</sup>N HSQC of Z-Val-Phe-OMe recorded in CDCl<sub>3</sub> at RT.



Figure 11: Zoom in area TOCSY NMR spectrum of Z-Val-Phe-OMe recorded in CDCl<sub>3</sub> at RT.

The structure of the peptide was first confirmed via <sup>1</sup>H and <sup>13</sup>C NMR experiments coupled with COSY, HSQC and HMBC (see in appendix for additional 2D spectra Figure A5, Figure A6, and Figure A7). From the TOCSY spectrum (Figure 11) we can clearly identified the two NH functions, with correlation spots in agreement with the attribution in Figure 10.



Figure 12: <sup>1</sup>H-<sup>15</sup>N HSQC zoom in area spectrum of VP11-15dl recorded in CDCl<sub>3</sub> at RT



Figure 13: TOCSY NMR spectra of VP11-15 recorded in CDCl<sub>3</sub>. (top) VP11-15 spectra before dialysis; (bottom) VP11-15dl after dialysis, recorded in CDCl<sub>3</sub> at RT.

On the <sup>1</sup>H-<sup>15</sup>N HSQC spectrum of VP11-15dl in **Figure 12** only one spot is visible on the spectrum, and comparing the chemical shift of the signal with the non-modified peptide, the NH can be assigned to NH carbamate.

TOCSY experiment of VP11-15dl in **Figure 13**, demonstrates the disappearance of signal between the crude and dialyzed product suggesting the elimination of non-modified peptide. Additionally, H born by N atoms have correlation spots in agreement with NH function from the carbamate.



Figure 14: Stacked ATR-FTIR spectrum of Z-Val-Phe-OMe (black) and VP11-15dl (red).

On ATR-FTIR spectrum of VP11-15dl in **Figure 14**, we can observe a disappearance of the peak at 1536 cm<sup>-1</sup> assigned to NH amide/carbamate. This confirms the functionalization on the NH functions. On the peptide spectrum (black), the peaks observed at 1656 cm<sup>-1</sup> and 1691 cm<sup>-1</sup> are assigned to C=O amide bond stretching, and C=O carbamate bond stretching, respectively. The peak at 1745 cm<sup>-1</sup> is a signature of C=O ester bond stretching. The shift observed on the product's spectrum to 1650 cm<sup>-1</sup> and 1722 cm<sup>-1</sup> suggest a modification on both NH functions.

ATR-FTIR seems to indicate a double functionalization, while NMR spectra of VP11-15dl indicate that at least a fraction of the carbamate functions remains non-modified on the peptide, and suggest that functionalization is preferential on amide functions.

In conclusion, combination of  $tBuP_n$  with 4 equivalents of *i*-Bu<sub>3</sub>Al helped to stabilize the protective functions of the peptide in the reaction medium. Furthermore, the AROP had to be conducted at low temperature to prevent alteration of the peptide. All the side reactions were completely eliminated for [Z-Val-Phe-OMe]/[ $tBuP_2$ ]/[*i*-Bu<sub>3</sub>Al] : 1/1/4 at -20°C. We demonstrated the full functionalization of macromolecules with a peptide at one end and hydroxy function at the other end. However,  $M_n$  and dispersities are not controlled for all the reaction conditions tried.

Kinetic experiment indicated a 1<sup>st</sup> order dependence to the monomer concentration contrary to previously reported results demonstrating a 0 order kinetic. This could suggest a different mechanism from the already reported mechanism of the monomer-activated AROP, which is not fully understood. Thus, further investigations need to be conducted to precise the molecular species engaged in this specific monomer-activated AROP mechanism. Finally, by employing this functionalization approach we induced a site-preferential modification on amide bonds, as demonstrated by IR, and 2D NMR experiments on the polymer obtained from the best conditions.

# III. Z-Val-Phe-OMe functionalization by poly(glycidyl ether) derivatives via monomer-activated AROP

In the precedent part, we demonstrated the divergent synthesis of poly(BO) chains from a protected peptide, Z-Val-Phe-OMe. By using phosphazene bases and *i*-Bu<sub>3</sub>Al at low temperature, the synthesis of the conjugate was successfully conducted without side reactions. In this part we will use the polymerization conditions based on  $tBuP_4$  and  $tBuP_2$ , previously optimized, to synthesize a protected version of poly(glycidol) polymers. Poly(EEGE) and poly(tBuGE) will be grafted, to demonstrate the feasibility of peptide functionalization by different nature of polyether chains. By switching from BO to glycidyl ether, we wish to be able to modify the peptide with hydrophilic polymer chains.

#### 1. *t*BuP<sub>4</sub> combined with *i*-Bu<sub>3</sub>Al

Optimized conditions will be applied to EEGE and *t*BuGE polymerizations. AROP were conducted in MeTHF, with initiating system based on  $[Z-Val-Phe-OMe]_0/[tBuP_4]_0/[i-Bu_3Al]_0$ : 1/1/4 at -20°C, and results are gathered in **Table 6**.

**Table 6:** monomer-activated AROP of oxirane monomers,  $[Z-Val-Phe-OMe]/[tBuP_4]/[i-Bu_3Al] : 1/1/4 at - 20°C; <math>[M]_0 = 1.3M; X_n \text{ (targeted)} = 30.$ 

Run	Monomer	Time (h)	Conv <sup>a</sup> (%)	M₁ theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP11-5	BO	21	100	2600	3900	3000	2.20
VP11-38	EEGE	15	100	4800	7600	9500	2.12
VP11-39	tBGE	15	100	4000	5900	9900	1.72

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.

<sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For EEGE and *t*BuGE monomers, reaction conditions lead to polymerization, and full conversion was reached in less than a day. Polymers exhibit SEC molar masses higher than NMR molar masses, the two experimental  $M_n$  are higher than the theoretical values, and dispersities are broad.

For VP11-39, RI and UV SEC traces in **Figure 15**, exhibit a monomodal profile with an additional peak at high retention volume assigned to non-modified peptide. MALDI-TOF analysis of VP11-39 in **Figure 15** detected a single population corresponding to poly(*t*BuGE) chain terminated by Z-Val-Phe-OMe and hydroxy function. Same results were obtains for VP11-38.



Figure 15: Characterization of VP11-39, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

VP11-38 (poly(EEGE)) and VP11-39 (poly(*t*BuGE)) were dialyzed for 48 hours against methanol to evacuate un-reacted peptide initiator (VP11-38dl and VP11-39dl). Then samples were analyzed by TOCSY, and HQSC <sup>1</sup>H-<sup>15</sup>N NMR in **Figure 16** and in **Figure 17**, respectively. Only spectra from VP11-39dl are shown due to the absence of correlation spots and low signals content from VP11-38dl spectra, which did not allow identification of NH.



Figure 16: <sup>1</sup>H-<sup>15</sup>N HSQC zoom in area spectrum of VP11-39dl recorded in CDCl<sub>3</sub> at RT.



Figure 17: TOCSY NMR spectra of VP11-39dl recorded in  $CDCl_3$  at RT.

In **Figure 16**, the <sup>1</sup>H-<sup>15</sup>N HSQC analysis of VP11-39dl detected two signals corresponding to H born by a N atom. The chemical shift of the H = 5.49 ppm and 5.35 ppm, suggest that the signals are signature of NH function of the carbamate. Signal of NH<sub>a</sub> is split into two peaks which could results from conformational change in the peptide. Attribution was achieved by 2D NMR but reasons of such behavior are not fully understood. From the TOCSY spectrum of VP11-39dl in **Figure 17**, absence of correlation signals for NH amides suggests a functionalization of the amide functions.



Figure 18: Stacked ATR-FTIR spectrum of Z-Val-Phe-OMe (black) and VP11-39dl (red).

On IR spectrum of VP11-39dl in **Figure 18**, no signals for NH can be seen at 1536 cm<sup>-1</sup>, while shift of the peaks at 1650 cm<sup>-1</sup> for C=O amide bond stretching and 1730 cm<sup>-1</sup> for C=O carbamate bond stretching suggest functionalization on both NH functions.

Those IR-based results are different from the one from NMR experiments evidencing remaining NH function and preferential functionalization on the amide.

In conclusion, by using EEGE and *t*BuGE, the initiating system [Z-Val-Phe-OMe]/[tBuP<sub>4</sub>]/[i-Bu<sub>3</sub>Al] : 1/1/4 at -20°C is able to entirely repress side reactions, compared to BO AROP. Concerning poly(tBuGE)-based conjugate, the polymerization conditions generate site-preferential modification.

#### 2. *t*BuP<sub>2</sub> combined with *i*-Bu<sub>3</sub>Al

AROP experiments were set with initiating system based on the best condition determined in part II of this chapter;  $[Z-Val-Phe-OMe]/[tBuP_2]/[i-Bu_3Al]$  : 1/1/4 at -20°C in MeTHF. Polymerizations conditions of EEGE and *t*BuGE are reported in **Table 7**.

**Table 7:** monomer-activated AROP of oxirane monomers,  $[Z-Val-Phe-OMe]/[tBuP_2]/[i-Bu_3A1] : 1/1/4 at - 20°C; <math>[M]_0 = 1.3M; X_n \text{ (targeted)} = 30.$ 

Run	Monomer	Time (h)	Conv <sup>a</sup> (%)	M₁ theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>c</sup>
VP11-15	BO	21	100	2600	4700	7100	2.09
VP11-26	EEGE	15	100	4600	6300	20 000	1.62
VP11-25	<i>t</i> BuGE	15	100	4300	7100	13 000	1.59

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.

<sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.

<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards)

Polymerization conditions in **Table 7** lead to full conversion. For VP11-26 and VP11-25,  $M_n$  determined by SEC are higher than NMR  $M_n$ . The measured molar masses are higher than the theoretical values, and synthesized polymers display high dispersities. Additional peak towards high retention volumes on RI and UV SEC traces in **Figure 19**, suggests the presence of un-functionalized peptide.

On MALDI-TOF analysis of VP11-25 in **Figure 19**, a single population is detected, and assigned to poly(*t*BuGE) chains initiated by Z-Val-Phe-OMe and cationized by Na<sup>+</sup>. Same conclusions were drawn out from VP11-26 results.

The two samples were dialyzed against methanol for 48 hours, VP11-25->VP11-25dl and VP11-26->VP11-26dl. Then samples were further analyzed via TOCSY and <sup>1</sup>H-<sup>15</sup>N HSQC NMR experiments.



Figure 19: Characterization of VP11-25, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

On <sup>1</sup>H-<sup>15</sup>N HSQC spectrum of VP11-25dl in **Figure 20** a single signal corresponding to H born by a N atom can be seen on the spectrum. Chemical shift of the H  $\approx$  5.2 ppm, allows the attribution of the signal to NH function from the carbamate. On TOCSY spectrum of VP11-25dl in **Figure 21** the absence of correlation spots for NH amide suggest a functionalization on amide functions.

Like noted in precedent part, poly(EEGE) conjugate did not allow the identification of NH function by <sup>1</sup>H-<sup>15</sup>N HSQC experiment. However, TOCSY spectrum of VP11-26dl in **Figure 22** exhibits correlation spots suggesting a preferential functionalization on amide functions.

The infrared spectrum of VP11-25dl in **Figure 23**, shows peaks at 1651 cm<sup>-1</sup> assigned C=O amide bond stretching, at 1729 cm<sup>-1</sup> signature of C=O carbamate bond stretching and at 1743 cm<sup>-1</sup> for C=O ester bond stretching.



Figure 20: <sup>1</sup>H-<sup>15</sup>N HSQC zoom in area spectrum of VP11-25dl recorded in CDCl<sub>3</sub> at RT.



Figure 21: TOCSY NMR spectra of VP11-25dl recorded in CDCl<sub>3</sub> at RT.



Figure 22: TOCSY NMR spectra of VP11-26dl recorded in CDCl<sub>3</sub> at RT.



Figure 23: Stacked ATR-FTIR spectrum of Z-Val-Phe-OMe (black) and VP11-25dl (red).

VP11-26dl was deprotected under acidic treatment in HCl (0.5M in methanol) and then dialyzed against water, to remove excess acid. High conversion of acetal groups into alcohol functions is reached within 3 hours, as seen on the NMR spectrum displayed in **Figure 24**. Also, the change in water solubility for the product was an evidence of successful deprotection. VP11-26 and VP11-26D were characterized by SEC in DMF to obtain  $M_n$  values gathered in **Table 8**.



**Figure 24:** <sup>1</sup>H NMR spectra of VP11-26 (top) and after acidic cleavage of the protecting group VP11-26D (bottom) recorded in MeOD<sub>4</sub> at RT.

Table 8	: Characte	erization of	of VP11-	-26 and	VP11-26	6D by S	EC in	DMF
						- 2		

Run	Repetitive unit	<i>M</i> <sub>n</sub> theo (g/mol)	<i>M</i> n <sup>1</sup> H NMR <sup>a</sup> (g/mol)	M <sub>n</sub> SEC <sup>b</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>b</sup>
VP11-26	EEGE	4600	6300	22 700	1.38
VP11-26D	Glycidol	12 100 <sup>c</sup>	7700	16 900	1.45
<sup>a</sup> : Determined b	y <sup>1</sup> H NMR of the	final product in	methanol-d <sub>4</sub> . <sup>c</sup> : calcul	ated from $M_{\rm n}({\rm SI})$	EC) of VP11-26.

<sup>b</sup>: Determined by SEC (DMF, 60°C, PMMA standards).

For VP11-26D, the mass reduction is identified by the shift of the peak towards higher retention volumes on SEC elugram in **Figure 25**.



Figure 25: SEC elugrams of VP11-26 and VP11-26D recorded in DMF with RI detector.

MALDI-TOF spectrum of VP11-26D in **Figure 26** exhibits several distributions compared to the one of VP11-26. Two populations were assigned to poly(glycidol) chains functionalized by Z-Val-Phe-OMe and a hydroxy function cationized by Na<sup>+</sup> or by Li<sup>+</sup>, while the other distributions are poly(glycidol) chains with unidentified extremities. Suggested additional populations can be seen in appendix **Figure A1**, **Figure A2**, **Figure A3** and **Figure A13**. The additional distributions suggest that deprotection should be carried out under milder conditions to retain the structure of the peptide.



**Figure 26:** MALDI-TOF spectrum before and after acidic treatment of Z-Val-Phe-OME-poly(EEGE). (a) MALDI-TOF spectrum recorded in reflectron mode of VP11-26; (b) Zoom in area of the spectrum from VP11-26; (a') MALDI-TOF spectrum recorded in reflectron mode of VP11-26D; (b') Zoom in area of the spectrum from VP11-26D.



Figure 27: Stacked ATR-FTIR spectrum of Z-Val-Phe-OMe (black) and VP11-26D (red).

Infrared spectrum of VP11-26D in **Figure 27** exhibits signals for remaining NH functions at 1535 cm<sup>-1</sup>, peaks at 1645 cm for C=O amide, and at 1710 cm<sup>-1</sup> the broad signal could be assigned to C=O carboxylic acid bond stretching from ester cleavage in acid or to C=O carbamate bond stretching. A <sup>13</sup>C NMR was performed but spectrum did not allow settlement of this question. (see spectrum in appendix **Figure A14**) Due to the high molar mass of the sample, and solubility of the conjugates in limited solvents, chain end functions were not visible.

To conclude, using glycidyl ether monomers, we managed to modify the peptide Z-Val-Phe-OMe with protected poly(glycidol) chains. We achieved 100% peptide-functionalized polymer chains. Moreover by NMR, we demonstrated that the modification preferentially occurs on the amide function, which is a similar result compared to functionalization with poly(BO). However, the attempt of deprotection of the alcohols functions along the polymer backbone affected the integrity of the peptide-protecting groups.

# IV. Influence of Lewis acid structure on the control of the monomer-activated AROP

In previous parts, we demonstrated that under specific reactions conditions, the AROP initiation from the peptide Z-Val-Phe-OMe could be achieved for various oxirane monomers, yielding polymer chains end-functionalized by a peptide and a hydroxy function. However, optimized AROP conditions are producing polymers with higher  $M_n$  than expected, and with high dispersities values,  $M_w/M_n \ge 1.59$ . This is suggesting a slow initiation step and low efficiency of functionalization.

In the Chapter 2, we demonstrated that the Lewis acid nature has an influence on the peptide initiation efficiency. Herein two Lewis acids will be used in order to improve control of molar masses, and decrease the dispersities, while stabilizing the protective functions of the peptide. Results will be compared to previous ones obtained with *i*-Bu<sub>3</sub>Al. Mixing of Lewis acids will also be investigated.

Optimized reaction conditions using  $tBuP_2$  were used for the experiments reported in **Table 9**. Experiment using Et<sub>3</sub>B (VP11-61) was conducted at 0°C, because at -20°C the triethylborane compound had the tendency to freeze in the medium, impeaching the polymerization.

Table 9: AROP of BO catalyzed by various Lewis acids combined with the initiating system Z-Val-Phe-OMe: $tBuP_2$ , at  $[M]_0 = 1.3M$ , in MeTHF;  $X_n$  (targeted) = 30.

Run	Lewis acid	[I] <sub>0</sub> / [ <i>t</i> BuP <sub>2</sub> ] <sub>0</sub> / [L.A] <sub>0</sub>	Temp (°C)	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> <sup>1</sup> H NMR <sup>b</sup> (g/mol)	M <sub>n</sub> SEC <sup>c</sup> (g/mol)	$M_{\rm w}/M_{\rm n}$ (SEC) <sup>c</sup>
VP11-15	<i>i</i> -Bu <sub>3</sub> Al	1/1/4	-20	21	100	2600	4700	7100	2.09
VP11-63	Et <sub>3</sub> Al	1/1/4	-20	29	77	2000	3400	3000	1.79
VP11-61	$Et_3B$	1/1/4	0	3	100	2600	2700	3900	1.07
VP11-42	<i>i</i> -Bu <sub>3</sub> Al /Et <sub>3</sub> B	1/1/(3/1)	25	3	100	2600	4100	4300	1.95
VP11-40	<i>i</i> -Bu <sub>3</sub> Al	1/1/(3/0.1)	25	2	100	2600	3600	7000	1.62

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.
<sup>b</sup>: Determined by <sup>1</sup>H NMR of the final product in acetone-d<sub>6</sub>.
<sup>c</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For all the conditions in Table 9, polymerization proceeded, and reached a high conversion in a reasonable amount of time.

MALDI-TOF analysis of VP11-63 in Figure 28 detected the expected population and minor distributions suggesting side reactions. The major population is Z-Val-Phe-OMe - poly(BO) -OH, one of the minor populations was assigned to macrocyclic compound from intramolecular transesterification reaction and the additional distribution is of poly(BO), but which extremities could not be assigned. The suggested mechanism of the intramolecular transesterification is displayed in Scheme 2 and mass list in appendix Table A1 and Table A2. Surprisingly the methanolate initiated poly(BO) population from transesterification reaction was not detected. Compared to the results obtained with *i*-Bu<sub>3</sub>Al, Et<sub>3</sub>Al is not as efficient for limitation of the side reactions.



**Figure 28:** Characterization of VP11-63, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

VP11-61 mass spectrometry analysis (**Figure 29**) detected a single population assigned to poly(BO) initiated by benzyl alcoholate. This result demonstrates that  $Et_3B$  is efficient for the protection of the ester function, but is not able to stabilize the carbamate protecting group. This selective protecting behavior of Lewis acid suggests that using a N-ter protective function more stable under basic condition, such as acetamides, <sup>8</sup> we could allow the AROP from protected peptide using  $Et_3B$  as catalyst.



Figure 29: Characterization of VP11-61, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

For the three Lewis acids used, only triisobutylaluminum is able to catalyze the AROP of BO from Z-Val-Phe-OMe without side reactions at low temperature.

For lower and higher amount of *i*-Bu<sub>3</sub>Al than 4 equivalents, MALDI-TOF demonstrated that initiation by methanolate was occurring. Since Et<sub>3</sub>B is efficient for stabilization of the ester protective function, and *i*-Bu<sub>3</sub>Al for the benzyl carbamate protective function, we suggest combining the two Lewis acids. Additionally, it was reported that the addition of *i*-Bu<sub>3</sub>Al leads to increase the dispersity probably due to an increase of the  $k_p / k_i$  ratio So as to limit this effect it could be interesting to lower the amount of *i*-Bu<sub>3</sub>Al used.

In the following part the mixing of two Lewis acids will be considered.

For VP11-42 and VP11-40 the  $[i-Bu_3Al]_0/[Et_3B]_0$  ratio was set to 3/1 and 3/0.1, respectively. Reaction conditions lead to polymerization, and full monomer conversion was reached within 3 hours.  $M_n$  determined by NMR and SEC are higher than the theoretical  $M_n$ , and high dispersities are obtained. A small fraction of unreacted peptide can be seen on RI and UV SEC traces of VP11-40 in **Figure 30**. For VP11-40 and VP11-42, the expected species, poly(BO) functionalized by Z-Val-Phe-OMe and hydroxy function, is the only detected population by MALDI-TOF. Mass spectrum example for VP11-40 in **Figure 30**.

 $Et_3B$  taken alone is not able to procure full stabilization of the peptide, but when associated to *i*-Bu<sub>3</sub>Al, a complete suppression of the side reactions occurs. Besides, the combination of Lewis acids did actually manage to reduce the dispersity.



Figure 30: Characterization of VP11-40, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron; (c) Zoom in region of the MALDI-TOF spectrum.

Since VP11-40 sample exhibits the narrower dispersity and the best results in MALDI-TOF for the 4 new experiments listed in **Table 9**, the polymer was dialysized prior 2D NMR analyses with TOCSY and <sup>1</sup>H-<sup>15</sup>N HSQC experiments (VP11-40->VP11-40dl).

<sup>1</sup>H-<sup>15</sup>N HSQC spectrum in **Figure 31** exhibits correlation spots suggesting a single type of N-H function remaining in the sample. The chemical shift of the signal at  $\approx 5.3$  ppm indicates NH from carbamate function. Absence of correlation signals for NH amide on the TOCSY spectrum in **Figure 32**, suggests a functionalization on amide functions.



Figure 31: <sup>1</sup>H-<sup>15</sup>N HSQC zoom in area spectrum of VP11-40dl recorded in CDCl<sub>3</sub> at RT.



Figure 32: TOCSY NMR spectra of VP11-40dl recorded in CDCl<sub>3</sub> at RT.

The ATR-FTIR spectrum of VP11-40dl in **Figure 33**, exhibits peaks at 1530 cm<sup>-1</sup> for remaining NH, at 1650 cm<sup>-1</sup> for C=O amide bond stretching, at 1625 cm<sup>-1</sup> for C=O carbamate bond stretching, and 1745 cm<sup>-1</sup> signature of C=O ester bond stretching.



Figure 33: Stacked ATR-FTIR spectrum of Z-Val-Phe-OMe (black) and VP11-40dl (red).

To conclude, unlike *i*-Bu<sub>3</sub>Al, Et<sub>3</sub>B and Et<sub>3</sub>Al were not as efficient for the stabilization of the ester and the carbamate functions. Interestingly combination of triisobutylaluminum and triethylborane in  $[i-Bu_3Al]_0/[Et_3B]_0$  ratios = 3/1 or 3/0.1, achieves elimination of side reactions. We also demonstrate that mixing Lewis acids can improves the dispersities. However, the mechanism of action of *i*-Bu<sub>3</sub>Al+Et<sub>3</sub>B is still unclear, and further work needs to be pursued to identify the role of each Lewis acid in the reaction medium.

By 2D NMR experiments, the grafting-from is shown to occur preferentially on the amide function of the peptide Z-Val-Phe-OMe.

### Conclusion

In this part we reported the first functionalization of a linear peptide by grafting-from synthesis, using AROP of oxiranes.

The association of  $tBuP_n$  with *i*-Bu<sub>3</sub>Al Lewis acid allowed a significant reduction of the side reactions, and improvement of the stability of the protecting groups. Polymerization conditions were identified for a successful stabilization of the ester and the carbamate, allowing the synthesis of 100% Z-Val-Phe-OMe-functionalized polymers chains. However higher experimental  $M_n$  value than theoretical and high dispersities are evidences of slow initiation step inducing a low efficiency since un-reacted peptide was detected via SEC.

Preferential functionalization on the NH amide site is observed. Further quantitative analyses are needed to determine if some carbamate groups are also functionalized.

It was also demonstrated that the nature of Lewis acid reagents had an influence on the control of the polymerization, and the occurrence of side reactions. Usage of a mix of Lewis acids is an interesting strategy that needs further investigations.

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1193.874

## Appendix



Figure A1: Possible mechanism for the side reaction to the oxirane and the calculated mass of the product.

Na⁺



Figure A2: Possible mechanism for the side reaction to the triisobutylaluminum and the calculated mass of the product.



Figure A3: Possible mechanism for the side reaction to the triisobutylaluminum and the calculated mass of the product.



X <sub>n</sub>	Calculated exact mass	Structural formula
13	1262.904	

Figure A4: Possible mechanism for internal cyclization of Z-VF-OMe after deprotonation and the calculated mass of the product.

X <sub>n</sub>	Calculated exact mass	Structural formula
17	1280.040	
16	1284.010	

**Table A1:** Mass list of the alkoxide initiated polymers from side reactions.

Table A2: Mass list of the structures from side reactions targeting carbamate and ester functions.

X <sub>n</sub>	Calculated exact mass	Structural formula
12	1263.902	$ \begin{array}{c} & & \\ & & $
6	1247.716	$Na^{+}$
12	1267.884	NH Na <sup>+</sup>



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Figure A8: SEC elugram of Z-Val-Phe-OMe, RI trace and UV trace.



Figure A9: Zoom in area of MALDI-TOF spectra recorded in reflectron mode. (a) VP11-19 spectrum; (a') VP11-17 spectrum.



Figure A10: Zoom in area of MALDI-TOF spectra recorded in reflectron mode. (a) VP11-10 spectrum; (a') VP11-5 spectrum.







Figure A13: Mass list of the suggested additional populations.



# — **CHAPTER 4** —

Tripeptides functionalization: from Ac-Ala-Ala-Ala-OMe to the unprotected version Ala-Ala-Ala

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#### Introduction

In this part, we will address the synthesis of *N*-substituted linear tripeptides by polyether chains. Indeed, after the successful functionalization of the protected linear peptide Z-Val-Phe-OMe, we decided to further increase the complexity of the initiating system. Tri-alanine-based peptides will be used to evaluate if AROP of BO can still be carried out with initiator having three possible initiating sites. The peptide was chosen due to the neutral behavior of Alanine residues not prone to side reactions. Indeed, the CH<sub>3</sub> side chain strictly limits the possible interaction with other compounds while maintaining the conformation of the peptide. BO is a rather safe monomer to use for this application, since it exhibits a lower reactivity than PO or glycidyl ether monomers. <sup>1</sup>

First, to avoid any side reactions or parasitic initiations from amine and carboxylic acid, a protected version of the peptide will be used: Ac-Ala-Ala-Ala-OMe. Carboxylic acid is protected via a methyl ester which can be easily deprotected afterward, besides this function was proven to be stable under specific polymerization conditions in Chapter 3. And the amine end function is protected with an acetyl group, which is stable under acidic and basic conditions.  $^2$ 

In a second part, Ala-Ala-Ala peptide will be used to examine if polymerization can still be carried out in the presence of carboxylic acid and amino end-functions. Attempts to modify the Ala-Ala-Ala will allow evaluating the need of protecting groups for future functionalization.

## I. Ac-Ala-Ala-OMe functionalization by poly(BO)

In the precedent chapter, the combination of  $tBuP_n$  base and Lewis acid confirmed to be a successful strategy to promote the AROP of oxirane, without side reactions, from linear protected dipeptide (See Chapter 3). Here, triisobutylaluminum will be used along with phosphazene base and protected tri-alanine, to promote the *N*-functionalization of Ac-Ala-Ala-OMe by poly(BO) in a controlled manner. (see Scheme 1)



Scheme 1: Functionalization of Ac-Ala-Ala-Ala-OMe by poly(BO) via monomer-activated AROP.

Compound	pK <sub>a</sub> value (DMSO)	Ref.
tBuP <sub>4</sub>	30.2	3
tBuP <sub>2</sub>	21.5	3
NH	12.2	
NH	12.4	Estimated values from MarvinSketch 20.8 software
NH	12.6	

Table 1: pKa values comparison between phosphazene bases and amide.

Considering the pKa values reported in **Table 1**,  $tBuP_4$  and  $tBuP_2$  are basic enough to deprotonate amide functions on the peptide, and generate reactive anions. According to the simulations, the 3 amide groups have similar pKa suggesting that no function will preferentially initiate the polymerization.

Within the Chapter 3 describing the synthesis of conjugates from Z-Val-Phe-OMe, we highlighted that given the oxophilic nature of *i*-Bu<sub>3</sub>Al, 1 equivalent per carbonyl function should be added to the reactive media, to complete the entire coordination sites on the peptide (**Scheme 2**), and an excess of Lewis acid for monomer activation. The Ac-Ala-Ala-Ala-OMe peptide bears 4 carbonyl functions (**Scheme 2**), thus a [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratio > 5 should be, in theory, necessary for the monomer-activated AROP of BO from the protected peptide. It was also suggested, that the Lewis acid unit near the deprotonation NH site is used to complex the newly generated amidate and further the propagating alkoxide-<sup>+</sup>H*t*BuP<sub>n</sub>. This hypothesis is based on the dynamic exchange of *i*-Bu<sub>3</sub>Al between the complexation sites.



Scheme 2: Coordination sites for *i*-Bu<sub>3</sub>Al in the reaction medium

#### 1. Monomer-activated AROP based on tBuP<sub>4</sub>

The strategies developed in previous chapters were applied in **Table 2**. The influence of the quantity of Lewis acid and the temperature were investigated to control the monomeractivated AROP. Polymerization conditions are gathered in **Table 2**.

Polymerization proceed for all the investigated [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios in the considered range. A high conversion was reached within 2 hours or less, for all the samples. All the polymers have experimental molar masses higher than the theoretical values. Moreover, dispersities are high and are increasing with the Lewis acid content in the medium or by lowering the temperature to -20°C, which is consistent with the results obtained in the previous chapters. <sup>1</sup>H-NMR molar masses were not determined due to the low resolution of the peptide signals and the overlapping with poly(BO) repetitive units.

Run	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [ <i>i</i> -Bu <sub>3</sub> Al] <sub>0</sub>	Temp. (°C)	Time (min)	Conv <sup>a</sup> (%)	<i>M</i> <sub>n</sub> theo (g/mol)	M <sub>n</sub> SEC <sup>b</sup> (g/mol)	$\frac{M_{\rm w}/M_{\rm n}}{({ m SEC})^{ m b}}$
VP4-13	30/1/1/4	25	50	100	2500	6600	1.60
VP4-14	30/1/1/5	25	50	100	2500	6600	1.62
VP4-15	30/1/1/6	25	50	100	2500	6000	1.69
VP4-16	30/1/1/7	25	40	100	2500	4700	1.75
VP4-17	30/1/1/8	25	40	100	2500	4000	1.76
VP4-36	30/1/1/8	-20	120	91	2300	5200	2.11
VP4-18	30/1/1/9	25	40	100	2500	4500	1.77
VP4-41	30/1/1/9	-20	60	96	2400	4800	2.07
VP4-19	30/1/1/10	25	40	100	2500	4800	1.76
VP4-42	30/1/1/10	-20	60	96	2400	5700	1.87
VP4-33 <sup>c</sup>	30/1/1/10	-20	120	84	2400	9400	1.96

**Table 2:** BO polymerizations by the initiating system Ac-Ala-Ala-Ala-OMe: $tBuP_4$ : $i-Bu_3Al$  in MeTHF at  $[BO]_0 = 1.3M$ .

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture.

<sup>b</sup>:Determined by SEC (THF, 40°C, PMMA standards).

Based on the results provided by MALDI-TOF, for experiments conducted at [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios lower than 8, we were not able to detect the expected distribution. (see typical SEC elugram and MALDI-TOF spectra in appendix **Figure A5**). While the polymerization conditions VP4-17, VP4-18 and VP4-19 exhibit the best results. Suspected side reactions mechanisms are displayed in appendix **Figure A7**, **Figure A8**, **Figure A9**, **Figure A10**, **Table A1**, **Figure A11** and **Table A2**.

SEC elugram and MALDI-TOF spectrum are displayed in **Figure 1** for VP4-18. Via mass spectrometry analysis two distributions were detected, the major one corresponds to methanolate initiated poly(BO) and terminated by an hydroxy group, and the minor one is assigned to the expected distribution bearing one Ac-Ala-ala-ala-OMe and one hydroxy end groups. VP4-17 and VP4-19 have similar MALDI-TOF results.



Figure 1: SEC trace and MALDI-TOF spectra of VP4-18. (a) SEC elugram in THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in area of MALDI-TOF spectrum recorded in reflectron mode.

The methanolate initiation is due to a transesterification reaction between an alkoxide propagating center and the ester protecting group, according to the mechanism in **Scheme 3**. Unfortunately the complementary species generated by the transesterification and bearing an ester function could not be detected by MALDI-TOF or NMR.



Scheme 3: Supposed mechanism for the transesterification reaction to the ester protecting group.

<sup>1</sup>H NMR spectrum and <sup>13</sup>C NMR spectrum of VP4-18 in **Figure 2** and in **Figure 3**, respectively, exhibit signals assigned to the peptide and to the polymers. Attributions were realized with 1D NMR (<sup>1</sup>H and <sup>13</sup>C) and 2D NMR experiments (COSY, HSQC, HMBC). 2D spectra of VP4-18 are provided in appendix in **Figure A3** and **Figure A4**. See appendix for the NMR spectra of Ac-Ala-Ala-Ala-OMe **Figure A1** and **Figure A2**.



Figure 2: <sup>1</sup>H NMR spectrum of VP4-18 recorded in MeOD<sub>4</sub> at RT.





Comparison of the ATR-FTIR spectra of VP4-18 and of Ac-Ala-Ala-Ala-OMe is displayed in **Figure 4**. On the spectrum of the native peptide, 3 signals are detected in the CO amide bond stretching area. At 1630 cm<sup>-1</sup> the peak is assigned to C=O acetamide, at 1645 cm<sup>-1</sup> the signal is attributed to C=O amide on the ester side, and 1682 cm<sup>-1</sup> is signature of the central C=O amide (see **Figure 5**). In VP4-18 spectrum, we can observe that there is peak for C=O amide bond stretching at 1642 cm<sup>-1</sup>, between the two C=O amide at 1630 cm<sup>-1</sup> (C=O acetamide) and 1655 cm<sup>-1</sup> (C=O amide) (see **Figure 5**). The modification of the signal suggests a modification on the two amide functions (C=O acetamide and C=O amide), since the peak at 1682 cm<sup>-1</sup> (C=O amide) remains unchanged. Additionally, the spectrum suggests the presence of remaining NH functions on the conjugate, detected at 1549 cm<sup>-1</sup> and assigned to NH amide bond bending.



Figure 4: ATR-FTIR spectra of VP4-18 (red) and Ac-Ala-Ala-OMe (black).



Figure 5: Infrared attributions of the C=O amides bond stretching.

MALDI-TOF combined with NMR demonstrated that a fraction of the polymer is actually initiated by Ac-Ala-Ala-Ala-OMe, for [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios  $\geq$  8. However, even for high amount of *i*-Bu<sub>3</sub>Al, a complete suppression of the transesterification reaction on the terminal ester was not achieved. The high nucleophilicity of the anions generated by *t*BuP<sub>4</sub> is considered to be responsible of the occurrence of the side reactions.

#### 2. Monomer-activated AROP based on tBuP<sub>2</sub>

In the previous part (**1.Monomer-activated AROP based on** *tBuP4*), we demonstrated the *N*-functionalization of the peptide Ac-Ala-Ala-Ala-OMe. In order to lower the reactivity of the propagating center towards carbonyl functions, the phosphazene base  $tBuP_2$  will be used instead of  $tBuP_4$ . Same strategy about the temperature and the number equivalents of *i*-Bu<sub>3</sub>Al applied in the latter part, will be repeated herein. Reaction conditions are gathered in **Table 3**. As demonstrated in precedent part, it is necessary to conduct reactions at [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios > 5 to allow the polymerization to proceed.

Polymerization proceed for all the investigated [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios. A high conversion value was reached within less than 2 hours for all the samples. SEC traces of the samples display rather broad and symmetrical peaks (see Figure 6 and in appendix Figure A6). Additionally, polymers synthesized have higher experimental molar masses than the theoretical values and high dispersities, especially at -20°C.

Experiments conducted at [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios lower than 7 did not allow the detection of the expected population by mass spectrometry analysis. A typical spectrum can be seen in appendix Figure A6 and suspected side reactions mechanism in appendix from Figure A7, Figure A8, Figure A9, Figure A10, Table A1, Figure A11 and Table A2.

Run	[M] <sub>0</sub> / [I] <sub>0</sub> / [ <i>t</i> BuP <sub>2</sub> ] <sub>0</sub> / [ <i>i</i> - Bu <sub>3</sub> Al] <sub>0</sub>	Temp. (°C)	Time (h)	Conv <sup>a</sup> (%)	M₁ theo (g/mol)	M <sub>n</sub> SEC <sup>b</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>b</sup>
VP4-9	30/1/1/4	25	1h25	95	2400	6400	1.65
VP4-10	30/1/1/5	25	1h25	100	2500	6700	1.63
VP4-11	30/1/1/6	25	1h25	100	2500	6600	1.65
VP4-25	30/1/1/7	25	1	100	2500	4700	1.80
VP4-37	30/1/1/7	-20	1	85	2100	7700	1.98
VP4-26	30/1/1/8	25	1	100	2500	5900	1.76
VP4-38	30/1/1/8	-20	1	85	2100	7800	1.93
VP4-27	30/1/1/9	25	1	100	2500	5500	1.80
VP4-39	30/1/1/9	-20	1	90	2300	6600	2.03
VP4-28	30/1/1/10	25	1	100	2500	4800	1.80
VP4-40	30/1/1/10	-20	1	85	2100	5100	2.19

Table 3: BO polymerizations by the initiating system Ac-Ala-Ala-Ala-Ala-OMe:tBuP<sub>2</sub>:i-Bu<sub>3</sub>Al in MeTHF at  $[BO]_0 = 1.3M.$ 

<sup>a</sup>: Determined by <sup>1</sup>H NMR of the reaction mixture. <sup>b</sup>: Determined by SEC (THF, 40°C, PMMA standards).

For [Lewis acid]<sub>0</sub>/[Peptide]<sub>0</sub> ratios  $\geq$  7, MALDI-TOF spectra show poly(BO) distribution bearing Ac-Ala-Ala-Ala-OMe and a hydroxy end groups. An additional distribution is visible on the spectrum and attributed to poly(BO) initiated by MeO<sup>-</sup>. However, the complementary structure generated by the transesterification, and bearing an ester function (Scheme 3), could not be observed on the spectrum.



Figure 6: SEC trace and MALDI-TOF spectra of VP4-40. (a) SEC elugram in THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in area MALDI-TOF spectrum recorded in reflectron mode.

For all the conditions tested in **Table 2** and **Table 3**, VP4-40 is identified to be the sample with the higher content of expected population (**Figure 6**). Compared to VP4-18 MALDI-TOF spectrum in **Figure 1**, the MALDI-TOF spectrum of VP4-40 in **Figure 6**, exhibits a higher ratio of expected population over methanolate initiate poly(BO) population.

To conclude, we slightly improved the functionalization of Ac-Ala-Ala-Ala-OMe by poly(BO), via lowering the basicity of  $tBuP_n$ . However, the control of the polymerization remains limited and transesterification reactions are not fully suppressed.

Previous results obtained in chapter 3 suggest that a milder Lewis acid could be an interesting way of optimization but was not further investigated in this part. Et<sub>3</sub>B is an interesting choice since this latter Lewis acid demonstrated his efficiency for specific stabilization of the ester function.

# II. NH<sub>2</sub>-Ala-Ala-COOH functionalization by poly(BO)

In this part, the functionalization of unprotected  $NH_2$ -Ala-Ala-Ala-COOH will be attempted. The peptide bears 3 protic functions on the backbone, which can serve as initiation site for AROP.



 $R_3 = H \text{ or poly(BO)}$ 

Scheme 4: Polymerization of 1,2-butylene oxide initiated by Ala-Ala-Ala.

Compound	pK <sub>a</sub> value (DMSO)	Ref.
tBuP <sub>4</sub>	<i>t</i> BuP <sub>4</sub> 30.2	
NH	12.3	
NH	12.5	Estimated values from
R-COOH	3.7	MarvinSketch 20.8 software
R-NH <sub>2</sub>	32.3	

 Table 4: Comparison of pKa values.

By comparison of the pKa values of the base and protic functions along the peptide backbone (**Table 4**),  $tBuP_4$  is able to generate reactive anions from amides, and carboxylic acids. C ter acid function is suggested to be the first concerned by deprotonation since the pKa of carboxylic acid is the lowest of the list in **Table 4**.

The various strategies that were developed in previous chapters were applied to the Ala-Ala-Ala:*t*BuP<sub>4</sub> promoted AROP, and the monomer-activated AROP. Polymerization conditions used are gathered in **Table 5**.

**Table 5:** BO polymerizations using Ala-Ala:*t*BuP<sub>4</sub>:R<sub>3</sub>Met as initiating system, in MeTHF at 25°C at [BO]<sub>0</sub> = 2M

Run	Lewis acid	[M] <sub>0</sub> /[I] <sub>0</sub> / [ <i>t</i> BuP <sub>4</sub> ] <sub>0</sub> / [L.A] <sub>0</sub>	Time (h)	Conv <sup>a</sup> (%)	M <sub>n</sub> theo (g/mol)	M <sub>n</sub> SEC <sup>b</sup> (g/mol)	$\frac{M_{\rm w}}{M_{\rm n}}$ (SEC) <sup>b</sup>	MALDI-TOF	ATR-FTIR	NMR
VP8-1	-	30/1/0.1/0	89	0	-	-	-			
VP8-2	-	30/1/0.2/0	89	0	-	-	-			
VP8-3	-	30/1/0.5/0	89	0	-	-	-			
VP8-4	-	30/1/1/0	89	0	-	-	-			
VP8-5	-	30/1/2/0	39	100	2400	2700	1.21	Figure A14	Figure A15	Figure A16
VP8-8	<i>i</i> -Bu <sub>3</sub> Al	30/1/2/4	56	64	1500	1000	1.80	Figure A17		
VP8-9	<i>i</i> -Bu <sub>3</sub> Al	30/1/2/5	2.5	99	2400	8200	1.69	Figure A17	Figure A19	Figure A18
VP8-10	<i>i</i> -Bu <sub>3</sub> Al	30/1/2/6	1	94	2300	11000	1.91	Figure A17		
VP8-16	Et <sub>3</sub> Al	30/1/2/4	96	84	2000	1500	1.67	Figure A21		
VP8-17	Et <sub>3</sub> Al	30/1/2/5	1	100	2400	1800	1.74	Figure A21	Figure A22	Figure A20
VP8-18	Et <sub>3</sub> Al	30/1/2/6	1	100	2400	1300	1.74	Figure A21		
VP8-13	Et <sub>3</sub> B	30/1/2/4	1	100	2400	6000	1.45	Figure A23		
VP8-14	Et <sub>3</sub> B	30/1/2/5	1	100	2400	3700	2.09	Figure A23		
VP8-15	Et <sub>3</sub> B	30/1/2/6	1	100	2400	6000	1.59	Figure A23		
<sup>a</sup> : Determined	by <sup>1</sup> H NMR o	f the reaction mixture.	<sup>b</sup> : Determ	nined by SEC (	THF, 40°C, PM	MMA standards	).			

<sup>o</sup>: Determined by SEC (THF, 40°C, PMMA standards).

Conventional AROP conditions did not allow the polymerization of BO, except for  $[tBuP_4]_0/[I]_0$  ratio = 2. The various attempts using the monomer-activated AROP conditions lead to polymers with SEC molar masses different from the theoretical values, either higher either lower.

MALDI-TOF spectra are displayed in appendix, see referred figures in **Table 5**. None of the MALDI-TOF spectra, i.e. reaction conditions, allowed synthesizing poly(BO) initiated by the peptide. Various side reactions are hypothesized, and reported in appendix **Figure A7**, **Figure A8**, **Figure A9**, **Figure A24**, **Table A3**, **Figure A25** and **Table A4**. Presence of dihydroxy-poly(BO) is evidenced on each spectrum, while other distributions could not be identified. This population could be issued of either water initiation or transfer reaction, as already discussed by Hadjichristidis *et al.* (see **Scheme 5**). <sup>4</sup> However, the difunctionalized polymer chains were not detected via MALDI-TOF, but evidence of ester functions was observed by Infrared spectroscopy. See ATR-FTIR spectra in appendix **Figure A15** and **Figure A19**.



Scheme 5: Proposed mechanism for the side reaction to the newly formed ester function.

Even if ATR-FTIR spectra exhibit weak signals signature of C=O amide bond stretching assigned to the initiating peptide, NMR analyses were not able to detect signals corresponding to the biological material.

To conclude this second part (**II.NH2-Ala-Ala-Ala-COOH**), polymerizations of BO from Ala-Ala and promoted by  $tBuP_4$  are attempted for  $[tBuP_4]_0/[I]_0$  ratios  $\geq 2$ . Combining Lewis acid (*i*-Bu<sub>3</sub>Al, Et<sub>3</sub>B, and Et<sub>3</sub>Al) with  $tBuP_4$ , to try eliminate the side reaction to the carboxylic acid, was not successful, and only lead to polymers with high discrepancy between experimental and theoretical  $M_n$  and high  $M_w/M_n$ .

Recent advances in the literature suggest interesting outlooks, however they were not investigated here. The efficient initiation of AROP of oxirane from carboxylic acid with  $tBuP_2:Et_3B$  catalytic system has been reported. <sup>5</sup> In addition Hadjichristidis and coworkers reported the use of MTBD to promote the polymerization of aziridine-based monomers from carboxylic acids initiators. <sup>6</sup>

Both articles have in common the use of mild reaction conditions with weak nucleophilic activators. These conditions represent an opportunity for further optimization of the synthesis of Ala-Ala-Poly(BO) conjugates.

# Conclusion

In this part the functionalization of tri-alanine based peptides was investigated.

Concerning Ac-Ala-Ala-Ala-OMe, the modification on amide functions was demonstrated. The combination of  $tBuP_n$  and *i*-Bu<sub>3</sub>Al allowed a reduction of the side reactions and increased the stability of the protective functions. However, full suppression of the side reactions was not reached by modulation of the reaction conditions.

For Ala-Ala, using  $tBuP_4$  with different Lewis acids did not promotes the modification of the peptide by poly(BO) chain(s).

Modulation of the initiating system with milder bases, and less reactive Lewis acids, could be an efficient way to conduct AROP in a living/controlled manner from Ac-Ala-Ala-Ala-OMe and Ala-Ala-Ala.

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# Appendix



Figure A1: <sup>1</sup>H NMR spectrum of Ac-Ala-Ala-Ala-OMe recorded in MeOD<sub>4</sub> at RT.



Figure A2: <sup>13</sup>C NMR spectrum of Ac-Ala-Ala-Ala-OMe recorded in MeOD<sub>4</sub> at RT.



Figure A3: COSY NMR spectrum of VP4-18 recorded in MeOD<sub>4</sub> at RT.





**Figure A5:** SEC trace and MALDI-TOF spectra of VP4-36. (a) SEC elugram in THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in area MALDI-TOF spectrum recorded in reflectron mode.



Figure A6: SEC trace and MALDI-TOF spectra of VP4-9. (a) SEC elugram in THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode; (c) Zoom in area MALDI-TOF spectrum recorded in reflectron mode.



X <sub>n</sub>	Calculated exact mass	Structural formula
21	1608.368	

Figure A7: Suggested mechanism of the side reactions to the monomer.


Figure A8: Suggested mechanism for the side reaction implying *i*-Bu<sub>3</sub>Al.



Figure A9: Suggested mechanism for the side reaction implying *i*-Bu<sub>3</sub>Al.



Figure A10: Suggested mechanisms for internal cyclization of Ac-Ala-Ala-Ala-OMe from attack via an amidate or an alkoxide. Each number refers to a mechanism and to a hypothetical structure in Table A1.

Chapter 4: Ac-AAA-OMe and AAA

 Table A1: Structures obtained from the suggested mechanisms in Figure A10.





Figure A11: Suggested mechanisms for inter molecular side reactions via an amidate or an alkoxide. Each number refers to a mechanism and to a hypothetical structure in Table A2.

#### Chapter 4: Ac-AAA-OMe and AAA

#### Table A2: Structures obtained from the suggested mechanisms in Figure A11.

X <sub>n</sub>	Calculated exact mass	Structural formula
		$(8) \xrightarrow{H^{0}}_{H^{0}} \xrightarrow{Na^{+}}_{H^{0}} (8^{*}) \xrightarrow{I_{H^{0}}}_{H^{0}} \xrightarrow{Na^{+}}_{H^{0}} (10) \xrightarrow{Na^{+}}_{H^{0}} (10) \xrightarrow{Na^{+}}_{H^{0}} \xrightarrow{OHN} \xrightarrow{O_{H^{0}}}_{H^{0}} \xrightarrow{O_{H^{0}}}_{H$
14	1606.126	$(10') \xrightarrow{H_{H}}_{O} \xrightarrow{(10')}_{H_{H}}_{O} \xrightarrow{(10')}_{O} \xrightarrow{H_{H}}_{O} \xrightarrow{(12')}_{H_{H}}_{O} \xrightarrow{(12')}_{H_{H}}_{O} \xrightarrow{(12')}_{H_{H}}_{H_{H}} \xrightarrow{(12')}_{H_{H}}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}} \xrightarrow{(12')}_{H_{H}}$
17	1577.169	$(9) \xrightarrow{\downarrow}_{N} \xrightarrow{\downarrow}_{N+} \xrightarrow{\downarrow}_{N+} \xrightarrow{\downarrow}_{N+} (9') \xrightarrow{\downarrow}_{N+} \xrightarrow{I} \xrightarrow{I}_{N+} \xrightarrow$
18	1565.208	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} $
16	1576.146	$(11) \xrightarrow{\downarrow}_{HN} \xrightarrow{\downarrow}_{Na^{+}} (11^{\prime}) \xrightarrow{\downarrow}_{H} \xrightarrow{\downarrow}_{H}$
19	1566.230	$ \begin{array}{c} Na^{+} \\ Na^{+} $
15	1575.123	$(13) \xrightarrow{\overset{H}{}_{n} \downarrow_{n}} \overset{Na^{+}}{\underset{O}{}_{n} \downarrow_{n} \downarrow_{n} \downarrow_{n}} \overset{I}{\underset{H}{}_{n} \downarrow_{n} \downarrow_{n} \downarrow_{n}} \overset{I}{\underset{H}{}_{n} \downarrow_{n} \downarrow_{n} \downarrow_{n}} (13') \xrightarrow{Na^{+}} \overset{I}{\underset{H}{}_{n} \downarrow_{n} \downarrow_{n} \downarrow_{n} \downarrow_{n}} \overset{I}{\underset{H}{}_{n} \downarrow_{n} $
14	1574.100	$(14) \xrightarrow{\circ}_{V} \stackrel{i}{\mapsto}_{V} $



9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 **Figure A12:** <sup>1</sup>H NMR spectrum of Ala-Ala-Ala recorded in DMSO-d<sub>6</sub> + few drops of trifluoroacetic acid (TFA) at RT.



**Figure A13:** <sup>13</sup>C NMR spectrum of Ala-Ala-Ala recorded in DMSO-d<sub>6</sub> + few drops of trifluoroacetic acid (TFA) at RT.



Figure A14: Characterization of VP8-5, (a) SEC trace with THF as eluant, (b) MALDI-TOF sepctrum recorded in reflectron mode; (c) Zoom in region of the MALDI-TOF spectrum.



Figure A15: ATR-FTIR spectrum of VP8-5.



VP8-5 recorded in CDCl<sub>3</sub> at RT.

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Figure A17: Comparison of the SEC elugrams and MALDI-TOF spectra regarding the number equivalents of *i*-Bu<sub>3</sub>Al used. (a) SEC trace of VP8-8 using THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode of VP8-8; (c) Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-9; (a') SEC trace of VP8-9 using THF as eluant;
 (b') MALDI-TOF spectrum recorded in reflectron mode of VP8-9; (c') Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-9; (a'') SEC trace of VP8-9 using THF as eluant;
 (b'') MALDI-TOF spectrum recorded in reflectron mode of VP8-9; (c'') Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-10 using THF as eluant;





Figure A20: ATR-FTIR spectrum of VP8-17.

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**Figure A21:** Comparison of the SEC elugrams and MALDI-TOF spectra regarding the number equivalents of Et<sub>3</sub>Al used. (a) SEC trace of VP8-16 using THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode of VP8-16; (c) Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-16; (a') SEC trace of VP8-17 using THF as eluant; (b') MALDI-TOF spectrum recorded in reflectron mode of VP8-17; (c') Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-17; (a'') SEC trace of VP8-18 using THF as eluant; (b'') MALDI-TOF spectrum recorded in reflectron mode of VP8-17; (c'') Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-18.



**Figure A22:** (top) <sup>1</sup>H NMR spectrum of VP8-17 recorded in CDCl<sub>3</sub> at 25°C; (bottom) <sup>13</sup>C NMR spectrum of VP8-17 recorded in CDCl<sub>3</sub> at 25°C.

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Figure A23: Comparison of the SEC elugrams and MALDI-TOF spectra regarding the number equivalents of Et<sub>3</sub>B used. (a) SEC trace of VP8-13 using THF as eluant; (b) MALDI-TOF spectrum recorded in reflectron mode of VP8-13; (c) Zoom in area MALDI-TOF spectrum recorded in reflectron mode of VP8-14; (a') SEC trace of VP8-14 using THF as eluant; (b') MALDI-TOF spectrum recorded in reflectron mode of VP8-14; (a') SEC trace of VP8-14; (a') SEC trace of VP8-14; (a') SEC trace of VP8-15 using THF as eluant; (b') MALDI-TOF spectrum recorded in reflectron mode of VP8-14; (a') SEC trace of VP8-15.



Figure A24: Suggested mechanisms for the internal cyclization from a nucleophilic attack by an amidate or an alkoxide. Each number is related to a proposed mechanism and to a structure referred in Table A3.



#### Table A3: Structures obtained from the suggested mechanisms in Figure A24



Figure A25: Suggested mechanism for inter molecular side reactions via an amidate or an alkoxide. Each number refers to a mechanism and to a hypothetical structure in Table A4.



Figure A26: Expected population sctructure and theoretical mass (m/z).

X <sub>n</sub>	Calculated exact mass	Structural formula				
11	1259.883	$(5) \qquad \qquad$				
12	1260.906	$(6) \underset{H_2N}{\overset{Na^+}{\underset{0}{\overset{0}{\overset{0}{\overset{0}{\overset{0}{\overset{0}{\overset{0}{0$				
16	1264.998					
10	1205.834	$(7) + H_{2} + H_{2} + H_{1} + H_{2} + H_{1} + H_{2} $				
13	1261.929	$(9) \xrightarrow{NH_2} (9') \xrightarrow{NH_2} (9') \xrightarrow{NH_2} Na^+ (9') \xrightarrow{NH_2} Na^+ Na^+ Na^+ Na^+ Na^+ NH_2$				
15	1263.975	$Na^{+}$ $Ho \downarrow \downarrow \stackrel{0}{\downarrow} \downarrow \stackrel{1}{\downarrow} \downarrow \stackrel{1}{\downarrow} (\downarrow \circ)_{n}^{H}$				

 Table A4: Structures obtained from the suggested mechanisms in Figure A25.

### — CHAPTER 5 —

# Analytical and experimental procedures

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#### I. Materials

THF and toluene as polymerization solvents were used dried with an MBRAUN MB SPS-800 solvent purification system under nitrogen. 2-methyltetrahydrofuran (MeTHF) (98%, Sigma-Aldrich) was used as received.

Oxirane monomers were purchase from chemical suppliers (**Table 1**). Ethoxyethyl glycidyl ether was synthesized from glycidol and ethyl vinyl ether following the procedure reported by Fitton *et al.*<sup>1</sup> All monomers were dried and cryo-condensate two times over CaH<sub>2</sub> (93%, 0-2 mm grain size, Acros Organic) prior to use. Aluminum Oxide (Al<sub>2</sub>O<sub>3</sub>, activated, neutral, Brockmann Activity I) was used for purification of the polymers.

Peptide initiators, phosphazene bases and Lewis acid solutions listed in **Table 1** were used as received.

Table 1: List of the reagents used
------------------------------------

Name	Purity (%) / Concentration (mol/l)	Supplier	Molar mass (g/mol)	Density at 25°C (g/ml)
tBuP <sub>4</sub>	0.8M in Hexane	Sigma-Aldrich	633.72	0.863
tBuP <sub>2</sub>	2M in THF	Sigma-Aldrich	367.45	
1,2-butylene oxide	99	Sigma-Aldrich	72.11	0.829
tert-Butyl glycidyl ether	99	Sigma-Aldrich	130.18	0.917
triisobutylaluminum	1M in Hexane	Sigma-Aldrich	198.32	0.685
triethylaluminum	1M in Hexane	Sigma-Aldrich	114.16	0.692
triphenylaluminum	1M in Diethyl ether	Sigma-Aldrich	258.29	0.868
triethylborane	1M in Hexane	Sigma-Aldrich	97.99	0.675
Cyclo(Glycine-Proline)	>99	Bachem	154.17	
Cyclo(Alanine-Proline)	>99	Bachem	168.20	
Cyclo(Leucine-Proline)	>99	Bachem	210.28	
Cyclo(Phenylalanine-Proline)	>99	Bachem	244.29	
Cyclo(Glycine-Valine)	>99	Sigma-Aldrich	156.18	
Cyclo(Glycine-Phenylalanine)	>99	Bachem	204.23	
Cyclo(Leucine-Phenylalanine)	>99	Bachem	260.34	
Z-Valine-Phenylalanine-OMe	>99	Bachem	412.49	
Ac-(Alanine) <sub>3</sub> -OMe	>99	Bachem	287.31	
NH <sub>2</sub> -(Alanine) <sub>3</sub> -COOH	>99	Bachem	231.25	

#### **II.** Instrumentation

#### 1. Nuclear Magnetic Resonance (NMR)

Routine analyses such as <sup>1</sup>H NMR experiments were recorded on either 300 MHz Bruker Avance III Nanobay or 400 MHz Bruker Avance III Nanobay. For NMR experiments requiring a long analysis time such as 2D experiments (<sup>1</sup>H-<sup>1</sup>H COSY, <sup>1</sup>H-<sup>13</sup>C HSQC, <sup>1</sup>H-<sup>13</sup>C HMBC, <sup>1</sup>H-<sup>1</sup>H TOCSY, <sup>1</sup>H-<sup>15</sup>N HSQC) a 400 MHz Bruker Avance I was used.

For proton NMR 10-20 mg of the product was used while for <sup>13</sup>C NMR spectrum 40-60mg of product was used with 0.5 ml of deuterated solvent. Depending on the solubility of the product, acetone-d<sub>6</sub>, DMSO-d<sub>6</sub>, DMF-d<sub>7</sub>, MeOD<sub>4</sub>, CDCl<sub>3</sub>, dichloromethane-d<sub>2</sub>, toluene-d<sub>8</sub> were used as analytical solvents for NMR.

#### 2. Size Exclusion Chromatography (SEC)

The number-average molar masses  $(M_n)$ , the weight-average molar masses  $(M_w)$  and the dispersities  $(D = M_w/M_n)$  were determined by SEC with a calibration curve based on narrow poly(methyl methacrylate) (PMMA) standards (from Polymer Standard Services), using the RI detector.

Poly(BO), poly(EEGE) and poly(*t*BuGE) were analyzed in THF SEC while poly(glycidol) soluble in polar solvent was analyzed in DMF SEC.

#### a. SEC in THF

Three PLGel Mixte C 5  $\mu$ m columns (7.5 × 300 mm; separation limits: 0.2–2000 kg.mol–1) maintained at 40 °C coupled with two modular detectors: a differential refractive index (RI) detector Viscotek 3580 and a diode array UV detector Shimadzu SPD20-AV. THF was used as the mobile phase at a flow rate of 1 ml/min, and toluene was used as a flow rate marker. All polymers were injected (100  $\mu$ l) at a concentration of 5 mg/ml after filtration through a 0.45  $\mu$ m pore-size membrane.

#### **b. SEC in DMF**

The analyses were performed at 60°C, and at a flow rate of 0.8 ml/min, at a polymer concentration of 5 mg/ml after filtration through 0.22  $\mu$ m pore-size membrane. The steric exclusion was carried out on two PSS GRAM 1000 Å columns (8 × 300 mm; separation limits: 1–1000 kg/mol) and one PSS GRAM 30 Å (8 × 300 mm; separation limits: 0.1–10 kg/mol) coupled with three detectors (Viscotek, TDA 305): a differential refractive index (RI) detector and an UV detector.

#### 3. Matrix-Assisted Laser Desorption and Ionization Time-Of-Flight

Mass spectra were recorded by matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) using a Bruker autoflex III smartbeam mass spectrometer, equipped with a laser that produces pulses at 337 nm using: (a) dithranol as a matrix and NaI as a cationizing agent for poly(BO), poly(EEGE) and poly(*t*BuGE) or (b)  $\alpha$ -cyanohydroxycinnamic acid as a matrix and LiBr as a cationizing agent for polyglycidols. Spectra were recorded in reflectron and linear mode at an accelerating potential of 20 kV.

Samples were prepared by dissolving the polymer in THF for matrix and salt (a) and in methanol for matrix and salt (b) at a concentration of 5 mg.ml<sup>-1</sup>. A 10 µl aliquot of this solution was mixed with 20 µl of matrix solution (at 20 mg.ml<sup>-1</sup> in THF or MeOH) and 10 µl of salt solution (at 10 mg.ml<sup>-1</sup> in THF or MeOH). Standards (poly(ethylene oxide) of known structures,  $M_n$ = 1470 g.mol<sup>-1</sup> purchased from Polymer Standards Service) were used to calibrate the mass scale.

#### 4. InfraRed spectroscopy

FTIR spectra in the attenuated total reflection (ATR) mode were recorded on a Bruker Equinox 55 spectrometer using a spectral resolution of 4 cm<sup>-1</sup>. The spectrometer was continuously purged with dry air to avoid water vapor absorption in the spectral range of amide and ester carbonyl absorptions. 60  $\mu$ l of the polymer solution in dichloromethane or methanol (20 mg.ml<sup>-1</sup>) was deposited on the bare ATR element made of germanium allowing 15 useful internal reflections and the solvent was allowed to evaporate before collecting 128 scans. Each recorded spectrum was transformed by ATR correction to the absorption spectrum.

#### III. Synthetic procedures

#### 1. AROP of oxiranes from peptide initiators

Polymerizations were carried out according the following procedure (Example VP6-4 (cyclo(Gly-Val) – poly(*t*BuGE))). The initiator cyclo(Gly-Val) (19 mg, 0.12 mmol) was weighted in the reaction vessel and transferred into a glove box to pursue the addition of the reagents. 1.8 ml of THF solvent was added, then *t*BuP<sub>4</sub> (153  $\mu$ l, 0.12 mmol) was added. The solution turned light yellowish and was stirred until complete dissolution of the cyclo(Gly-Val) ( $\approx 5 \text{ min}$ ). Finally the monomer *t*BuGE was added (520  $\mu$ l, 3.66 mmol). After closure of the vial the reaction was stirred at 25°C and left to react for the required period of time. Small aliquots of the reaction medium were sampled though the septum at various reaction times to determine the conversion by <sup>1</sup>H NMR. The polymerization was quenched by addition of 0.1 ml of acetic acid 1% and let to stir for 5 min at RT.

The crude reaction medium was evaporated and re-dissolved in 50 ml of chloroform with 6 g of alumina. The suspension was left to stir for 1 hour.

Then the content was filtered on sintered glass and wash off with 200 ml of ethyl acetate on clean alumina (wash with 50 ml DCM prior use). After filtration, solvents were evaporated and the product was dried overnight in a vacuum oven set at 50°C, to yield colorless viscous oil. Yield > 75%.

Variations from the procedure are specified in the different tables in this manuscript.

## 2. Monomer-activated AROP of oxiranes from peptide initiators

#### a. DKP-based initiating systems

Polymerizations were carried out according the following procedure (Example of VP7-25 (cyclo(Gly-Phe)-poly(*t*BuGE))). The initiator cyclo(Gly-Phe) (18.5 mg, 0.09 mmol) was weighted in the reaction vessel and transferred into a glove box to pursue the addition of the reagents. 1.1 ml of MeTHF solvent was added, then *t*BuP<sub>4</sub> (113 µl, 0.09 mmol) was added. The solution turned light yellowish and was stirred until complete dissolution of the cyclo(Gly-Phe) ( $\approx$  5 min). Then *i*-Bu<sub>3</sub>Al was added (181 µl, 0.18 mmol) followed by the monomer *t*BuGE (385 µl, 2.71 mmol). The addition of *i*-Bu<sub>3</sub>Al provoked a temporary rise of the temperature inside the reaction vessel above 25 °C.

After closure of the vial the reaction was stirred at 25°C and left to react for the required period of time. Small aliquots of the reaction medium were sampled though the septum at various reaction times to determine the conversion by <sup>1</sup>H NMR. The polymerization was quenched by addition of 0.1 ml of acetic acid 1% and let it stir for 5min at RT.

The crude reaction medium was evaporated and dissolved in 50 ml of chloroform with 6 g of alumina. The suspension was left to stir for 1 hour.

Then the content was filtered on sintered glass and wash off with 200 ml of ethyl acetate on clean alumina (wash with 50 ml DCM prior use). After filtration, solvents were evaporated and the product was dried overnight in a vacuum oven set at  $50^{\circ}$ C, to yield colorless viscous oil. Yield > 75%.

Variations from the procedure are specified in the different tables in this manuscript.

#### b. Linear peptide-based initiating systems

Linear peptide category comprises Z-Val-Phe-OMe, Ac-Ala-Ala-Ala-OMe and NH<sub>2</sub>-Ala-Ala-Ala-Ala-COOH.

#### i. polymerizations conducted at 25°C.

Polymerizations were carried out according the following procedure (Example for VP11-11 (Z-Val-Phe-OMe – poly(BO))). The initiator Z-Val-Phe-OMe (55 mg, 0.13 mmol) was weighted in the reaction vessel and transferred into a glove box to pursue the addition of the reagents. 2 ml of MeTHF solvent was added, then *i*-Bu<sub>3</sub>Al (535  $\mu$ l, 0.53 mmol) and the solution was stirred for 15 min. The monomer BO (350  $\mu$ l, 4.02 mmol) was added, and finally *t*BuP<sub>2</sub> (67  $\mu$ l, 0.13 mmol). The addition of *i*-Bu<sub>3</sub>Al provoked a temporary rise of the temperature inside the reaction vessel above 25 °C.

After closure of the vial the reaction was stirred at 25°C and left to react for the required period of time. Small aliquots of the reaction medium were sampled though the septum at various reaction times to determine the conversion by <sup>1</sup>H NMR. The polymerization was quenched by addition of 0.1 ml of acetic acid 1% and let it stir for 5 min at RT.

The crude reaction medium was evaporated and dissolved in 50ml of chloroform with 6 g of alumina. The suspension was left to stir for 1 hour.

Then the content was filtered on sintered glass and wash off with 200 ml of ethyl acetate on clean alumina (wash with 50 ml DCM prior use). After filtration, solvents were evaporated and the product was dried overnight in a vacuum oven set at 50°C, to yield colorless viscous oil. Yield > 75%.

#### ii. polymerizations conducted at low T°

Polymerizations were carried out according the following procedure (Example for VP11-15). The initiator Z-Val-Phe-OMe (49.3 mg, 0.12 mmol) was weighted in the reaction vessel and transferred into a glove box to pursue the addition of the reagents. 1.3 ml of MeTHF solvent was added, then *i*-Bu<sub>3</sub>Al (480  $\mu$ l, 0.48 mmol) and the solution was stirred for 15 min. The monomer BO (310  $\mu$ l, 3.56 mmol) was added, and the vial was closed with a septum. In a second vessel, *t*BuP<sub>2</sub> (60  $\mu$ l, 0.12 mmol) and 0.5 ml of MeTHF were mixed and vial was closed. Both vials were immerged at -20°C for 15 min. After that time, the content of the second vial (*t*BuP<sub>2</sub> in MeTHF) was withdraw with a syringe and quickly injected into the reaction vessel containing the peptide.

The reaction was stirred at -20°C and left to react for the required period of time. Small aliquots of the reaction medium were sampled though the septum at various reaction times to determine the conversion by <sup>1</sup>H NMR. The polymerization was quenched by addition of 0.1 ml of acetic acid 1% and let it stir for 5 min at RT.

The crude reaction medium was evaporated and dissolved in 50 ml of chloroform with 6 g of alumina. The suspension was left to stir for 1 hour.

Then the content was filtered on sintered glass and wash off with 200 ml of ethyl acetate on clean alumina (wash with 50 ml DCM prior use). After filtration, solvents were evaporated and the product was dried overnight in a vacuum oven set at  $50^{\circ}$ C, to yield colorless viscous oil. Yield > 75%.

#### 3. Poly(EEGE) deprotection into poly(glycidol)

#### Example with P4-6d (VP12-9D)

331.4 mg of the polymer VP12-9 were weighted in a vial and covered with 10 ml of HCl (0.5M in MetOH) [2.5 ml of HCl(2M) in MetOH + 7.5 ml of MetOH]. The mixture was left to stir at RT for 3h. Before the treatment, the solution was cloudy white and after the mixture turned clear with a white precipitate at the bottom of the vial. In order to keep the solution without risking any side damage to the product, the final acid solution was neutralized with about 1 ml of NaOH solution 5M and pH was checked with universal paper indicator. The solution was kept in fridge overnight

Then the next day, the content of the vial was poured into a dialysis membrane (1000 Da) so as to remove salts. The dialysis against water was left to run for 10h. (water was changed after 1h, then after 2h and every 3h)

Yield calculation:

 $n_{before\ deprot} = \frac{m}{M} = \frac{331.4 \times 10^{-3}}{5200} = 6.37 \times 10^{-5}$  mole

where M is the Mn before deprotection given by SEC in DMF

 $m_{theo} = n * M = 6.37.10^{-5} * 3800 = 0.242 g$ 

where M is the Mn post deprotection given by SEC in DMF

$$y = \frac{m_{exp}}{m_{theo}} = \frac{0.185}{0.242} \approx 0.76$$

#### IV. NMR $M_n$ determination examples

#### 1. Proline-based DKPs



**Figure 1:** <sup>1</sup>H NMR spectrum in dichloromethane-d<sub>2</sub> of P4-1 and the equation used to determine its numberaverage molar mass.



**Figure 2:** <sup>1</sup>H NMR spectrum in dichloromethane-d<sub>2</sub> of P4-4 and the equation used to determine its numberaverage molar mass.



**Figure 3:** <sup>1</sup>H NMR spectrum in methanol-d<sub>4</sub> of P4-1d and the equation used to determine its numberaverage molar mass.


**Figure 4:** <sup>1</sup>H NMR spectrum in methanol- $d_4$  of P4-4d and the equation used to determine its numberaverage molar mass.

# 2. Asymmetrics DKPs



**Figure 5:** <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of VP6-8 and the equation used to determine its number-average molar mass.

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**Figure 6:** <sup>1</sup>H NMR spectrum in dichloromethane-d<sub>2</sub> of VP6-17 and the equation used to determine its number-average molar mass.



**Figure 7:** <sup>1</sup>H NMR spectrum in dichloromethane-d<sub>2</sub> of VP7-11 and the equation used to determine its number-average molar mass.



**Figure 8:** <sup>1</sup>H NMR spectrum in dichloromethane-d<sub>2</sub> of VP7-35 and the equation used to determine its number-average molar mass.



**Figure 9:** <sup>1</sup>H NMR spectrum in dichloromethane-d<sub>2</sub> of VP21-1 and the equation used to determine its number-average molar mass.

# 3. Z-Valine-Phenylalanine-OMe



**Figure 10:** <sup>1</sup>H NMR spectrum in acetone-d<sub>6</sub> of VP11-15 and the equation used to determine its numberaverage molar mass.

# References

1 A. O. Fitton, J. Hill, D. E. Jane and R. Millar, Synthesis of Simple Oxetanes Carrying Reactive 2-Substituents, *Synthesis*, 1987, **1987**, 1140–1142.

— General conclusion —

In this project we demonstrated the first functionalization of amide functions of peptides by the divergent synthesis, using AROP of oxiranes.

Several peptide-based initiators for the polymerization of epoxides were used, such as proline-based 2,5-diketopiperazines (DKP), asymmetric bi-functional DKPs, and a protected linear peptide Z-Val-Phe-OMe. In each case, the optimization of the reaction conditions allowed the synthesis of 100% peptide-functionalized polyether chains without side reactions. We also demonstrated the versatility of the technique to modify the peptides, with either hydrophobic or hydrophilic polymer chains, to broaden the scope of application for our conjugates.

However, the *N*-modification of the tri-alanine based peptides in a controlled manner could not be realized during the time course of the PhD. Indeed, it was noted throughout the project that the high reactivity of the propagating center was responsible for the occurrence of side reactions.

MALDI-TOF, NMR and ATR-FTIR analyses evidenced transesterification reactions suggested to involve the propagating alkoxide and carbonyl functions on the peptide. Others secondary distributions were detected on the mass spectra but none of the suggested mechanisms for side reactions allowed identification of the chain-ends functions. To limit the side reactions, the nucleophilicity of the propagating center was decreased through different strategies: the use of less basic phosphazene base, combination of  $tBuP_2$  and a catalytic amount of  $tBuP_4$  and addition of the right amount of Lewis acid in the reaction medium. Each aforementioned approach leads to a successful reduction of the reactivity of the propagating centers and suppression of the side reactions.

However this decrease of the reactivity of the propagating centers leads to slower initiation step than conventional AROP conditions inducing a low initiation efficiency of the peptides and polymers with high dispersities.

Outlooks should be regarded to find a balance between a sufficient reduction of the reactivity to prevent the occurrence of side reactions and a good control of the polymerization to lower the dispersity. To do so, the use of a milder base like  $tBuP_2$  combined with less reactive Lewis acids is an interesting strategy. Indeed, recent publications combined milder organobases with borane-based Lewis acids for a living/controlled AROP of epoxides from low and high acidic initiators.

### General conclusion

Also the basicity of the propagating center could be lowered by using different monomers such as episulfides. Indeed, thiolates propagating centers exhibit lower basicity than alkoxides (example in DMSO: pKa (MeSH)  $\approx$  10.4 vs pKa (MeOH)  $\approx$  15.5) thus a limitation of the side reactions towards carbonyls is suggested. Additionally, given the pKa values, a reduction of the transfer reaction to amide functions is expected. This would be an interesting way to favor the mono-functionalization of bi-functional initiators like asymmetric DKPs.

Very recently, our group developed a method for the Anionic Ring-Opening co-Polymerization of thiobutyrolactone (TBL) and oxiranes monomers. <sup>1</sup> We demonstrated that the polymerization was living/controlled and occurred without side reactions even though the copolymers display ester functions along the backbone.

Concerning protected peptides, the protective end-functions should be chosen considering their stability towards the polymerization conditions, to enhance the sturdiness of the peptide and decrease the quantity of Lewis acid in the medium.

Initiating systems based on NH amide (from small DKP):*t*BuP<sub>4</sub>:Lewis acid reveal to be efficient to lead to a controlled AROP of oxiranes. The same strategy could be considered for the functionalization of protected amino-acids. Those amino acids-polymer conjugates could be further incorporated during peptide synthesis, to yield polypeptide backbone with pendant polymer chains.

V. Puchelle, Y. Latreyte, M. Girardot, L. Garnotel, L. Levesque, O. Coutelier, M. Destarac, P. Guégan, N. Illy, *Macromolecules* 2020, 53, 5188.

# Polymer Chemistry

# PAPER

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Cite this: Polym. Chem., 2020, **11**, 3585

Received 25th March 2020, Accepted 30th April 2020 DOI: 10.1039/d0py00437e

rsc.li/polymers

### Introduction

Polyethers exhibit specific characteristics, such as low glass transition temperatures and hydrophilicity. Thus, they have found application in a wide range of fields ranging from biomedical science to solar energy harvesting or energy storage in batteries.<sup>1-3</sup> Polyethers are commonly synthesized by AROP of epoxide monomers initiated by either alkali metal derivatives such as hydrides or alkoxides combined with metal counter ions.<sup>4-6</sup> However, since the beginning of 2010, organocatalyzed polymerization has gained interest and recent developments have been applied to AROP.7-10 Organocatalysts allow in particular to enhance the propagation rates and to improve the selectivity of the polymerization toward competing reactions. Among the large variety of commercially-available organocatalysts, the extremely strong, sterically hindered, nonionic phosphazene bases are of particular interest due to their ability to strongly increase the polymerization rate and to access new macromolecular architectures.<sup>11</sup> Tetrameric phos-

# Polymerization of epoxide monomers promoted by tBuP<sub>4</sub> phosphazene base: a comparative study of kinetic behavior<sup>†</sup>

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The kinetics of the anionic ring-opening polymerizations (AROP) of epoxide monomers, 1,2-epoxybutane (BO), 1,2-epoxypropane (PO), *tert*-butyl glycidyl ether (tBuGE), allyl glycidyl ether (AGE), benzyl glycidyl ether (BnGE), and ethoxyethyl glycidyl ether (EEGE), was investigated using benzyl alcohol/tBuP<sub>4</sub> as the initiating system. All the polymerizations proceed in a controlled manner following a first order kinetics with respect to the monomer. The influence of the side chains borne by the oxirane ring was evidenced. Propagating centers derived from epoxide bearing heretoatom-containing side chains display higher reactivities and propagation rates. A reactivity scale has been established and is as follows,  $k_{p,BnGE} > k_{p,AGE} > k_{p,EEGE} \gg k_{p,tBuGE} \approx k_{p,PO} > k_{p,BO}$ . Using BO as the model monomer and different initiator concentrations, the nature of the propagating species has been identified as ion pairs. The influence of a Lewis acid addition on the monomer reactivities and on the control of the polymerization was also investigated. In the presence of triisobutylaluminum (iBu<sub>3</sub>AI), polymerization kinetics was faster but led to a broadening of the molar mass distributions. The monomer reactivity scale was also strongly modified with  $k_{p,PO} > k_{p,BO} > k_{p,EEGE} \approx k_{p,rAGE} > k_{p,RBGE} \approx k_{p,tBuGE}$ . The polymerizations of PO, BO and tBuGE follow zero order kinetics which is not the case for the other oxirane monomers.

phazene base tBuP<sub>4</sub> is able to deprotonate very low acidic molecules (XH), generating extremely reactive anions associated with very large, highly stable, soft phosphazenium cations. The range of chemical moieties used as initiators in combination with tBuP4 has been extended to carboxylic acids,<sup>12</sup> thiols,<sup>13</sup> CH acidic compounds,<sup>14</sup> or nitrogen containing functions such as primary<sup>15</sup> and secondary amides<sup>16-18</sup> or carbamates.<sup>19</sup> In particular, Möller and coworkers were the first to demonstrate the ability of these superbases to initiate the AROP of ethylene oxide using an alcohol initiator.<sup>20</sup> The alcohol moiety is probably one of the most commonly found chemical functional groups. Thus, tBuP<sub>4</sub>-alcohol initiating systems have then been extended to several oxirane monomers, such as 1,2-epoxypropane (PO), 1,2-epoxybutane (BO), ethoxyethyl glycidyl ether (EEGE), benzyl glycidyl ether (BnGE), tert-butyl glycidyl ether (tBuGE) and glycidylamine derivatives.<sup>2,21–23</sup> To the best of our knowledge,  $tBuP_4$  is the only organic base that has been successfully used with alcohols as initiating systems for the polymerization of epoxides. Polymerization did not occur with milder organic bases such as tBuP<sub>1</sub>, tBuP<sub>2</sub>, TBD, DBU, TiBP or MTBD.<sup>2,14,20,21,24,25</sup> In order to increase the reactivity of the system and lower the transfer reaction occurrence, Carlotti and coworkers were the first to combine alcohol-tBuP4 initiating systems with a Lewis acid, tri-isobutylaluminum (iBu<sub>3</sub>Al), to initiate the AROP of

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propylene oxide, and perform an activated monomer mechanism with the oxirane monomer family.<sup>26</sup> Unfortunately, even if numerous papers described the polymerization of epoxides initiated by alcohol– $tBuP_4$  or by alcohol– $tBuP_4$ – $iBu_3Al$  initiating systems, less kinetics data, such as propagation rate constants, are available. A comparison of the different epoxide monomer reactivities in the presence of  $tBuP_4H^+$  phosphazenium counter-ions is very difficult due to the variety of experimental conditions used in the literature. A rational study involving most common monomers under standardized reaction conditions is lacking. In addition, to the best of our knowledge, the nature of the propagating species involved in the presence of phosphazenium as the counter ion has not been elucidated.

In this work, we aim to address these gaps in fundamental knowledge, and to provide a rational study involving the most common epoxide monomers. The AROP of 6 different epoxides, namely PO, BO, AGE, EEGE, BnGE and tBuGE, was carried out under identical reaction conditions with benzyl alcohol-tBuP<sub>4</sub> as the initiating system. Kinetic studies have been performed, and the propagation rate constants have been calculated. The reactivities of epoxides have been compared and the influence of the chemical nature, the electron density and the steric hindrance of the lateral substituents has been highlighted. The nature of the phosphazenium alkoxide propagating species has been investigated using BO as the model monomer. In the last part, kinetic studies were performed under the same conditions but in the presence of the iBu<sub>3</sub>Al Lewis acid, under activated monomer mechanism conditions. A monomer reactivity scale has been established and compared with the previous one.

### Experimental

#### Materials and methods

Benzyl alcohol (anhydrous, 99.8%, Sigma-Aldrich), phosphazene base  $tBuP_4$  solution (0.8 mol L<sup>-1</sup> in hexane, Sigma-Aldrich), triisobutyl aluminum (iBu<sub>3</sub>Al) solution (1 mol L<sup>-1</sup> in hexane), and 2-methyltetrahydrofuran (MeTHF) (98%, Sigma-Aldrich) were used as received. 1,2-Epoxybutane (>99%, Sigma-Aldrich), 1,2-epoxypropane, (≥99.5, Sigma-Aldrich), *tert*-butyl glycidyl ether (99%, Sigma-Aldrich), and allyl glycidyl ether (≥99%, Sigma-Aldrich) were distilled over CaH<sub>2</sub> (93%, 0–2 mm grain size, Acros Organic) two times prior to use. Benzyl glycidyl ether (99%, Sigma-Aldrich) was dried over activated molecular sieves (3 Å). Tetrahydrofuran was dried with an MBRAUN MB SPS-800 solvent purification system under nitrogen. Ethoxyethyl glycidyl ether was synthesized from glycidol and ethyl vinyl ether following the procedure reported by Fitton *et al.*<sup>27</sup> and distilled twice over CaH<sub>2</sub> prior to use.

#### Instrumentation

<sup>1</sup>H analyses were performed on a Bruker Avance 300 MHz spectrometer. CDCl<sub>3</sub> was used as the deutered solvent. Polymer molar masses were determined by Size Exclusion

Chromatography (SEC) with three PLGel Mixte C 5  $\mu$ m columns (7.5 × 300 mm; separation limits: 0.2–2000 kg mol<sup>-1</sup>) maintained at 40 °C coupled with two modular detectors: a differential refractive index (RI) detector Viscotek 3580 and a diode array UV detector Shimadzu SPD20-AV. THF was used as the mobile phase at a flow rate of 1 ml min<sup>-1</sup>, and toluene was used as a flow rate marker. All polymers were injected (100  $\mu$ l) at a concentration of 5 mg ml<sup>-1</sup> after filtration through a 0.45  $\mu$ m pore-size membrane. OmniSEC 4.7 software was used for data acquisition and data analysis. The number-average molar masses ( $M_n$ ), the weight-average molar masses ( $M_w$ ) and the molar mass distribution ( $D = M_w/M_n$ ) were determined by SEC with a calibration curve based on narrow poly(methyl methacrylate) (PMMA) standards (from Polymer Standard Services), using the RI detector.

Matrix-assisted laser desorption and ionization time-offlight. Mass spectra were recorded by matrix-assisted laser desorption and ionization time-of-flight (MALDI-Tof) mass spectrometry (MS) using a Bruker autoflex III smartbeam mass spectrometer, equipped with a laser that produces pulses at 337 nm using dithranol as a matrix and NaI as a cationizing agent. Spectra were recorded in reflectron and linear mode at an accelerating potential of 20 kV. Samples were prepared by dissolving the polymer in THF at a concentration of 5 mg ml<sup>-1</sup>. A 10 µL aliquot of this solution was mixed with 20 µl of dithranol solution as a matrix (at 20 mg ml<sup>-1</sup> in THF) and 10 µl of NaI solution (at 10 mg ml<sup>-1</sup> in THF). Standards (poly (ethylene oxide) of known structures,  $M_n = 1470$  g mol<sup>-1</sup> purchased from Polymer Standards Service) were used to calibrate the mass scale.

#### Procedures

All polymerizations were carried out at 25 °C, under nitrogen in a glass reactor fitted with a Rotaflo and equipped with a stirring bar and closed with a septum. The following typical procedure was applied. In a glove box, the phosphazene base tBuP<sub>4</sub> solution (242  $\mu$ l, 0.194 mmol) was introduced into the polymerization reactor. Then hexane was evaporated using high vacuum from a vacuum line ( $\approx 10^{-6}$  hPa). The reaction vessel was transferred back into the glovebox and dried THF was added (4.15 ml) followed by benzyl alcohol (20 µl, 0.193 mmol). Finally, the monomer BO was added (840 µl, 9.66 mmol). After closure of the reactor, the reaction was stirred at 25 °C and left to react for the required period of time. For polymerization carried out in the presence of iBu<sub>3</sub>Al, the Lewis acid was added after tBuP4 and just before the monomer. It may be noted that the addition of iBu<sub>3</sub>Al provoked a temporary rise of the temperature inside the reaction vessel above 25 °C. Kinetic studies were followed by removing aliquots at various reaction times, and conversion and molar masses were determined by <sup>1</sup>H NMR and SEC, respectively (Fig. S1-S11<sup>†</sup>). The reaction was quenched by addition of 0.1 ml of 10% acid acetic. The polymer was dissolved in chloroform and purified by passing through aluminum oxide and by removing the solvent under vacuum at 50 °C to give a colorless viscous liquid. Yield ≥80%. Kinetic experiments were

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run at least twice to confirm the behavior of each monomer towards the initiating system considered.

## **Results and discussion**

Ring-opening polymerizations of alkyl-substituted epoxides (PO, BO) and glycidyl ether monomers (*t*BuGE, EEGE, AGE, BnGE) were first performed under a N<sub>2</sub> atmosphere with benzyl alcohol:*t*BuP<sub>4</sub> as the initiating system (Scheme 1). These epoxide monomers are among the most common epoxide monomers used to prepare well-defined polyethers.<sup>2</sup> The monomer concentration was fixed to  $[M]_0 = 2 \text{ mol } L^{-1}$  and the [*t*BuP<sub>4</sub>]<sub>0</sub>/[initiator]<sub>0</sub> ratio was fixed to 1/1. A reaction temperature of 25 °C was chosen and is expected to ensure reaction completion in a reasonable amount of time. Preliminary experiments dealing with the nature of the propagating species were conducted with BO, a quite safe monomer providing the usual characteristics of substituted epoxide.

#### Investigation of the nature of the propagating species

First, the chemical nature identification of the propagating centers was carried out. Various  $[BO]_0/[Initiator]_0$  ratios were used, and the  $[tBuP_4]_0/[initiator]_0$  ratio is set to 1 (Table 1). The initiator concentration  $[I]_0$  ranged from 7.5 mmol L<sup>-1</sup> to 60 mmol L<sup>-1</sup> for a fixed monomer concentration  $[M]_0 = 2.0 \text{ mol } \text{L}^{-1}$ . Kinetic data could be fitted according to a first-order equation. For all  $[M]_0/[I]_0$  ratios, the reactions were first order with respect to the monomer over the entire conversion range (Fig. 1), which is in good agreement with previously



Scheme 1  $tBuP_4$  catalyzed AROP of oxirane monomers using BnOH :  $tBuP_4$  as the initiating system.



**Fig. 1** First order plot for the polymerization of 1,2-epoxybutane initiated by benzyl alcohol in decreasing concentration of initiator; in THF at 25 °C (BnOH :  $tBuP_4$  (1 : 1) as the initiating system at  $[M]_0 = 2$  mol  $L^{-1}$ ).

reported results for AROP of BO initiated by low acidic molecules– $tBuP_4$  initiating systems.<sup>16,22</sup>

Furthermore, the poly(BO) molar mass increases linearly with the monomer conversion up to the final yield. Experimental molar masses  $M_{\rm n}$  are in good agreement with the theoretical values and the dispersity D remains narrow over the entire conversion range. These results are illustrated in Fig. S13,<sup>†</sup> which corresponds to the experimental data obtained for  $[M]_0/[I]_0$  ratio = 50/1 (Table 1 run 4), but the conclusion applies equally well to the five  $[M]_0/[I]_0$  ratios. The above kinetic results argue in favor of a living polymerization mechanism occurring without transfer or termination reactions under the investigated conditions. The kinetic results obtained for all the [M]<sub>0</sub>/[I]<sub>0</sub> ratios are superimposed on a pseudo first order plot (Fig. 1). A linear dependence regarding ln([M]<sub>0</sub>/[M]) versus time can be observed for each ratio. From each curve, a value of propagating constant  $k_{p,app}$  was determined using the slope  $(k_p[I]_0)$  of the linear regression.

Anionic polymerization in a polar organic solvent proceeds *via* a multiple state mechanism involving contact ions, solvent separated ion pairs and free anions.<sup>28</sup> Szwarc and Schulz have demonstrated that the overall polymerization rate constant,  $k_p$ , is a function of the concentration of active species ( $c^*$ ) according to eqn (1) where  $k_{\pm}$  is the rate constant of the ion pairs

Table 11,2-Epoxybutane polymerization, initiated by benzyl alcohol :  $tBuP_4$  (1 : 1) at 25 °C in THF;  $[M]_0 = 2.0 \text{ mol } L^{-1}$ 

Run	[BnOH] (10 <sup>-2</sup> mol L <sup>-1</sup> )	[BO]/[BnOH]/[tBuP <sub>4</sub> ]	Time	%Conv. <sup>a</sup>	$k_{p}[I]_{0}^{b}(\min^{-1})$	$k_{\rm p} \ (10^{-2} \ {\rm L} \ { m mol}^{-1} \ { m min}^{-1})$
1	0.75	267/1/1	73 h 50	74	$3.06  imes 10^{-4}$	4.08
2	1.5	133/1/1	53 h 15	86	$5.85  imes 10^{-4}$	3.90
3	3	67/1/1	33 h 40	92	$1.19 \times 10^{-3}$	3.96
4	4	50/1/1	22 h 30	94	$1.80 \times 10^{-3}$	4.51
5	6	33/1/1	23 h	98	$2.70  imes 10^{-3}$	4.50

<sup>a</sup> Determined by <sup>1</sup>H NMR in CDCl<sub>3</sub>. <sup>b</sup> Slope of the curves in Fig. 3.

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**Fig. 2** Dependence of the propagation rate constant  $k_{p,app}$  towards the reciprocal square root concentration of benzyl alcohol for BO polymerization in THF at 25 °C.

(contact and solvent separated ion pairs),  $k_{-}$  is the rate constant of free ions and  $K_{\rm D}$  is the overall dissociation constant between the total concentration of ion pairs and free ions.<sup>28–30</sup>

$$k_{\rm p} = k_{\pm} + (k_{-} - k_{\pm}) K_{\rm D}^{1/2} \ (c*)^{-1/2} \tag{1}$$

In order to determine the contribution of the different propagating species, we plotted  $k_{p,app} = f([I]^{-0.5})$ . Fig. 2 shows that  $k_{p,app}$  is constant with respect to the reciprocal square root concentration of the benzyl alcohol initiator and has an average value of 0.0419  $\pm$  0.0026 L mol<sup>-1</sup> min<sup>-1</sup>. This result suggests that the propagating active species are exclusively ion pairs. For the polymerization of BO in non-polar toluene, the  $k_{p,app}$  values reported by Dentzer *et al.*<sup>16</sup> and by Misaka *et al.*<sup>21</sup> using secondary amide–*t*BuP<sub>4</sub> and alcohol–*t*BuP<sub>4</sub> as initiating systems are equal to 0.0336 L mol<sup>-1</sup> min<sup>-1</sup> and 0.0480 L mol<sup>-1</sup> min<sup>-1</sup>, respectively. Propagation rate constants have very similar values in THF and in toluene, suggesting comparable interactions between H*t*BuP<sub>4</sub><sup>+</sup> counter-ions and alkoxides in both solvents and that ion pairs are mainly contact ion pairs.

#### Influence of the epoxide substituent on the propagation rates

Kinetic experiments were carried out with 6 epoxide monomers in THF at 25 °C using benzyl alcohol :  $tBuP_4$  as the initiating system (Table 2 and Fig. S12–S17†). Poly(propylene oxide) (polyPO, Table 2 run 1), poly(butylene oxide) (polyBO, Table 2 run 2), poly(*tert*-butyl glycidyl ether) (polytBuGE, Table 2 run 3), poly(allyl glycidyl ether) (polyAGE, Table 2 run 4), poly (ethoxyethyl glycidyl ether) (polyEEGE, Table 2 run 5) and poly (benzyl glycidyl ether) (polyBnGE, Table 2, run 6) were successfully synthesized with very narrow dispersities. For all runs, experimental number-average molar masses measured by endgroup analysis of the <sup>1</sup>H NMR spectra are in excellent agreement with the theoretical ones. We can thus conclude that the initiation is quantitative and that each benzyl alcohol molecule generates one macromolecule. In addition, the  $M_{\rm p}$  values of polyPO, polyBO, polyAGE, and polyEEGE determined by SEC in THF are also in very good agreement with the theoretical number-average molar masses, suggesting the absence or a negligible amount of transfer reactions. A discrepancy can be noticed in the case of polyether bearing bulky hydrophobic lateral substituents like polytBuGE and of polyBnGE. However, the dispersity remains low for each sample. A first explanation could be that SEC in THF using PMMA standards significantly underestimated the molar masses for these polyethers. In all cases, the reactions were first order with respect to the monomer over the entire conversion range, and the degrees of polymerization increased linearly with conversion over the same conversion range as shown in Fig. S12-S17.† The above kinetic results, coupled to the very narrow molar mass distributions (D < 1.1), provide experimental evidence that supports a living and controlled polymerization mechanism (no transfer or termination reactions) and a fast initiation step under the investigated experimental conditions, bearing in mind the relatively low targeted molar masses.

The first order kinetic graphs of the AROP of the six epoxide monomers are superimposed in Fig. 3; 2 distinctive groups having propagation rate constants with similar orders of magnitude are clearly visible: (i) BnGE, AGE and EEGE are the most reactive monomers having propagation rate constants ranging from 2.23 to  $4.12 \times 10^{-1} \text{ L mol}^{-1} \text{ min}^{-1}$ ; (ii) BO, PO and *t*BuGE are less reactive having propagation rate constants ranging from 4.51 to  $5.43 \times 10^{-2}$  L mol<sup>-1</sup> min<sup>-1</sup>. The monomer reactivity order determined here with the BnOH-tBuP4 initiating system is:  $k_{p,BnGE} > k_{p,AGE} > k_{p,EEGE} \gg k_{p,tBuGE} \approx k_{p,PO} >$  $k_{\rm p,BO}$ . When a potassium hydride suspension in THF with 18-crown-6 is used as the initiating system, a similar tendency was observed by Stolarzewicz et al.<sup>31</sup> with  $k_{p,AGE} \gg k_{p,PO} >$  $k_{p,BO}$ . But, it is worth noting that the monomer reactivity scale is also strongly dependent on the initiating system as illustrated by the results obtained by Möller et al.<sup>32</sup> in diglyme at 120 °C using potassium 3-phenyl-1-propanol as the initiator where  $k_{p,EEGE} > k_{p,AGE} > k_{p,tBuGE}$ .

Table 2 Ring-opening polymerization of epoxide monomers initiated by benzyl alcohol :  $tBuP_4$  (1 : 1) at 25 °C in THF;  $[M]_0 = 2 \text{ mol } L^{-1}$ 

Run	Monomer	Time	%Conv.	$k_{ m p,app}$ (L mol <sup>-1</sup> min <sup>-1</sup> )	$M_{ m n,theo}$ (g mol <sup>-1</sup> )	$M_{ m n,NMR}$ (g mol <sup>-1</sup> )	$M_{ m n,SEC}$ (g mol <sup>-1</sup> )	$\frac{M_{ m w}/M_{ m n}}{ m (SEC)}$
1	РО	25 h	96	$5.38  imes 10^{-2}$	2800	2800	2900	1.09
2	BO	22 h 30	92	$4.51  imes 10^{-2}$	3400	3200	3100	1.09
3	tBuGE	22 h 40	94	$5.43  imes 10^{-2}$	6200	6100	4900	1.10
4	AGE	4 h	96	$3.00 \times 10^{-1}$	5600	5700	5100	1.08
5	EEGE	5 h	94	$2.23 \times 10^{-1}$	7000	7400	6200	1.08
6	BnGE	4 h 30	99	$4.12\times 10^{-1}$	8200	N.A.	4700	1.08

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Fig. 3 First-order-plot in the polymerization of BnGE, AGE, EEGE, tBuGE, PO, and BO at 25 °C in THF. Monomer/initiator = 50/1, [Monomer] = 2.0 mol  $L^{-1}$ .

We previously demonstrated that only one propagating species exists in the case of the polymerization of BO, and we suggest a similar trend for tBuGE, PO. However, polymerizations of BnGE, AGE, and EEGE provide much faster kinetics that seem to follow a similar behavior. The reactivity differences between the two sets of monomers could be explained by the interactions between the alkoxide propagating center and the counter-ion phosphazenium HtBuP4<sup>+</sup>. It is known that the anionic ring-opening polymerization mechanism is based on a tri-molecular transition state valid for ionic pairs propagating species.<sup>33</sup> This state of transition is composed of the propagating center, the counter cation and a monomer unit. The phosphazene base is acting like a Lewis acid and interacts with the following nucleophilic species: the oxanionic propagating center and the monomer unit to be incorporated in the chain as shown in Fig. 4. The coordination between alkoxide and HtBuP4<sup>+</sup> stabilizes the transition state and initiates the epoxide cleavage.<sup>34</sup> The substituents present on the monomer are known to play an important role in the transition state<sup>35</sup>



Fig. 4 Transition-state structure scheme for the monomer addition regarding the substituent on the epoxide.

which is confirmed in our case. The methyl and ethyl groups of PO and BO do not interact with the phosphazenium counter-ion in the transition state (Fig. 4). In contrast, in the case of BnGE, EEGE and AGE, multiple interactions are possible between the counter-ion  $HtBuP_4^+$  and the oxygen atoms in the  $\beta$ -position of the lateral group of the last propagating chain monomer unit and of the monomer being incorporated (Fig. 4). These additional interactions tend to weaken the main alkoxide-HtBuP4<sup>+</sup> interaction and to increase the nucleophilicity of the alkoxide propagating center and thus, the propagation rate. A similar effect has already been observed for the sodium phenolate initiated AROP of methyl glycidyl ether and thiomethyl glycidyl ether compared to that of propylene oxide.35 More recently Lynd and coll. published a DFT calculation study reporting that for the AROP initiated by potassium alkoxide, the energetic transition state barrier was lower in the case of the addition of one AGE unit than one EO unit.<sup>36</sup> It can be noted that tBuGE has a propagation rate constant similar to those of PO and BO despite the presence of an oxygen atom in the  $\beta$  position of its lateral substituent. We assume that in this case, the steric hindrance due to the bulky tert-butyl group prevents the coordination effect with the bulky phosphazenium counter-ion.

#### Polymerization in the presence of a Lewis acid

In this part, the influence of the addition of a Lewis acid in the reaction mixture will be investigated. To the best of our knowledge, no kinetic studies have ever been reported for the AROP of epoxide using a phosphazene base/iBu<sub>3</sub>Al as a polymerization promoter. Polymerizations were carried out at the same monomer concentration in MeTHF at 25 °C with  $alcohol: tBuP_4: iBu_3Al$  (1:1:2) as the catalytic system (Table 3). The solvent was switched from THF to 2-methyltetrahydrofuran (MeTHF), to avoid competitive complexation of the aluminum compound by THF.37 As previously reported,<sup>38</sup> a ratio of  $[iBu_3Al]/[benzyl alcohol] > 1$  is required: one equivalent of aluminum derivative is consumed by the low-reactive propagating center, the excess activates the monomer and allows for the propagation. As expected compared to the polymerization without iBu<sub>3</sub>Al, the addition of a Lewis acid leads to faster kinetics. However for these moderate targeted polymerization degrees, a loss of polymerization control suggested by the increase of the dispersity (1.35 < D < D)1.60) is concomitantly observed (Fig. S18<sup>†</sup>). All the samples were analyzed by MALDI TOF: only the expected populations could be detected (ESI Fig. S25-S30†) suggesting living polymerization mechanisms without transfer reactions.

In order to determine the monomer order, monomer conversion and  $\ln([M]_0/[M])$  were plotted as functions of time (Fig. S19–S24†). All the polymerizations proceed at high speed: less than 30 minutes were actually needed to reach full conversion. The polymerization of PO, BO and *t*BuGE was zero-order with respect to the monomer. These results are similar to those obtained by Carlotti and coll. who have reported that the polymerizations of PO with alkoxide/iBu<sub>3</sub>Al,<sup>38</sup> onium salt/iBu<sub>3</sub>Al,<sup>39</sup> and tetraalkylammonium salt/iBu<sub>3</sub>Al as initiating

Table 3	Monomer-activated	ring-opening	polymerization	of epoxides	initiated by	/ benzyl	alcohol: tBuP4	ı:iBu₃Al (1:1:2	2) at 25 °(	C in THF;	$[M]_0 =$
2 mol L <sup>-1</sup>											

Run	Monomer	Time (min)	%Conv.	$M_{ m n,theo} \ ({ m g\ mol}^{-1})$	$M_{n,NMR}$ (g mol <sup>-1</sup> )	$M_{ m n,SEC}$ (g mol <sup>-1</sup> )	$\frac{M_{ m w}/M_{ m n}}{ m (SEC)}$
1	BO	5	92	3400	3700	4800	1.52
2	PO	2,5	96	2900	3300	5400	1.54
3	tBuGE	22	81	5400	8000	7900	1.38
4	AGE	6	85	4900	7700	9900	1.43
5	EEGE	9	94	7000	7200	6700	1.59
6	BnGE	24	85	7100	N.A.	5700	1.35

systems<sup>40</sup> are following a zero order kinetics according to the equation  $R_{\rm p} = k_{\rm p}[I]_0.([iBu_3Al]_0 - [I]_0)$ , where  $k_{\rm p}$  is the propagation rate constant and  $[I]_0$  is the initiator concentration. For AGE, EEGE and BnGE, kinetic data could not be fitted according to a zero order equation or a first order equation. Carlotti suggests that an induction period is observed at the early stage, and could be explained by the aggregation of complexes between low molar mass propagating macromolecules, the phosphazenium counter-ion and iBu<sub>3</sub>Al. After the insertion of a few additional monomer units the complex becomes fully soluble and the polymerization rate increased. At high monomer conversion, a reactivity decrease is observed due to the competitive complexation of iBu<sub>3</sub>Al by the polyether chain.41 Attempts to increase the number of aluminum equivalents with respect to the benzyl alcohol did not lead to significant improvement of the linearity of the kinetics.

In order to scale the monomer reactivity, the plots of monomer conversion *versus* time have been superimposed in Fig. 5. The presence of iBu<sub>3</sub>Al strongly modifies the monomer reactivity scale:  $k_{p,PO} > k_{p,BO} > k_{p,EEGE} \approx k_{p,AGE} > k_{p,BnGE} \approx k_{p,tBuGE}$ . Trialkylaluminum deactivates the alkoxide propagating center but in the meantime activates the monomer through its coordination with the oxygen atom of the oxirane ring, which is increasing the electrophilicity of the  $\alpha$ -methylene carbon. These opposing effects result in an

overall improved reactivity of all the monomers, demonstrating the key role of monomer complexation in the polymerization kinetics. The new reactivity scale can therefore be explained by the strength of the monomer coordination by iBu<sub>3</sub>Al (Fig. 6). For PO and BO, iBu<sub>3</sub>Al can only be complexed by the oxygen atom of the epoxide ring resulting in strong monomer activation, high reactivity and fast polymerization. The activation of glycidyl ether monomers, such as EEGE and AGE, is reduced by the presence of additional oxygen atoms in the lateral substituent, which are also interacting with the Lewis acid and thus, slightly decreasing the expected electrophilicity of the  $\alpha$ -methylene carbon, and then their propagation rates. Similar reactivity differences have already been reported for the copolymerization of ethylene oxide (EO) with glycidyl ether monomers (EEGE and glycidyl methyl ether (GME)) under conventional oxyanionic or monomer-activated mechanisms.<sup>42,43</sup> Lastly, the lower reactivity of tBuGE and BnGE can be explained by the steric hindrance induced by their bulky side chain which is added to the complexation of the Lewis acid by the oxygen atoms of their side-chains. This hypothesis is supported by the fact that the activated-monomer AROP mechanism is already proceeding through bulky aluminum complexes,<sup>44</sup> and by the bulkiness of *t*BuP<sub>4</sub>,<sup>11</sup> which contributes to the re-enforcement of the cumbersome environment around the propagating center.



**Fig. 5** Conversion *versus* time plots for the polymerization of alkylene oxide monomers BO and PO and for the protected glycidol monomers AGE, EEGE, tBuGE, and BnGE (MeTHF, 25 °C,  $[M]_0 = 2 \text{ mol } L^{-1}$ , [benzyl alcohol] =  $3.7 \times 10^{-2} \text{ mol } L^{-1}$ , benzyl alcohol : tBuP<sub>4</sub> : iBu<sub>3</sub>Al (1:1:2)).



Fig. 6 Complexation of triisobutylaluminum by various epoxide monomer structures.

## Conclusions

The kinetics of the AROP of epoxyde monomers initiated by alcohol/tBuP<sub>4</sub> has been investigated. The propagating species have been identified to be exclusively ion pairs and the polymerization of epoxide monomers occurred via a living controlled polymerization process. A reactivity scale has been established for a library of 6 of the most commonly used epoxide monomers: 2 alkyl-substituted epoxides and 4 glycidyl ether monomers:  $k_{p,BnGE} > k_{p,AGE} > k_{p,EEGE} \gg k_{p,tBuGE} \approx k_{p,PO} >$  $k_{\rm p,BO}$ . The epoxide lateral substituents have a significant influence on the monomer reactivity. Lateral substituents allowing an additional complexation of the phosphazenium couterions led to loose ion pairs and systems displaying higher reactivities. The addition of a Lewis acid in the reaction mixture, typical of monomer-activated ring-opening polymerization, led not only to an expected drastic increase of the kinetics, but also to a full modification of the monomer reactivity scale due to differences in the strength of the monomer activation:  $k_{p,PO}$  $> k_{p,BO} > k_{p,EEGE} \approx k_{p,AGE} > k_{p,BnGE} \approx k_{p,\ell BuGE}$ .

## Conflicts of interest

There are no conflicts to declare.

### Acknowledgements

H. D. acknowledges the Ph.D. scholarship offered by the China Scholarship Council (CSC).

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# **Supporting Information**

Polymerization of epoxide monomers promoted by  $tBuP_4$  phosphazene base: A comparative study of kinetic behavior.

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**Figure S1.** <sup>1</sup>H NMR spectrum in  $CDCl_3$  of the reaction mixture of PO polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> at 25°C in THF (Table 2, run 1) after 3 hours and the equation use to determine the monomer conversion.



**Figure S2.** <sup>1</sup>H NMR spectrum in  $CDCI_3$  of the reaction mixture of BO polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> at 25°C in THF (Table 2, run 2) after 6 hours and the equation use to determine the monomer conversion.



**Figure S3.** <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of the reaction mixture of *t*BuGE polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> at 25°C in THF (Table 2, run 3) after 9 hours and the equation use to determine the monomer conversion.



**Figure S4.** <sup>1</sup>H NMR spectrum in  $CDCl_3$  of the reaction mixture of AGE polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> at 25°C in THF (Table 2, run 4) after 2 hours and the equation use to determine the monomer conversion.



$$p = 1 - \frac{l_{C_{M2}}}{\left(l_f + l_{f_M}\right)} = 1 - \frac{1}{2.37} = 0.58$$

**Figure S5.** <sup>1</sup>H NMR spectrum in  $CDCl_3$  of the reaction mixture of EEGE polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> at 25°C in THF (Table 2, run 5) after 1.5 hours and the equation use to determine the monomer conversion.



**Figure S6.** <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of the reaction mixture of BnGE polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> at 25°C in THF (Table 2, run 6) after 6 hours and the equation use to determine the monomer conversion.



**Figure S7.** <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of polyPO polymerization initiated by benzyl alcohol- $tBuP_4$  (Table 2, run 1) and the equation use to determine its number-average molar mass.



**Figure S8.** <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of polyBO polymerization initiated by benzyl alcohol- $tBuP_4$  (Table 2, run 2) and the equation use to determine its number-average molar mass.



**Figure S9.** <sup>1</sup>H NMR spectrum in  $CDCl_3$  of polytBuGE polymerization initiated by benzyl alcohol-*t*BuP<sub>4</sub> (Table 2, run 3) and the equation use to determine its number-average molar mass.



**Figure S10.** <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of polyAGE polymerization initiated by benzyl alcohol- $tBuP_4$  (Table 2, run 4) and the equation use to determine its number-average molar mass.



**Figure S11.** <sup>1</sup>H NMR spectrum in  $CDCl_3$  of polyEEGE polymerization initiated by benzyl alcohol- $tBuP_4$  (Table 2, run 5) and the equation use to determine its number-average molar mass.



**Figure S12.** AROP of PO initiated by benzyl alcohol-*t*BuP<sub>4</sub> in THF at 25°C ( $[M]_0 = 2 \text{ M}$ ;  $[I]_0 = 4 \times 10^{-2} \text{ mol.L}^{-1}$ ): (a) First order plot ( $k_{p, app}$ (black) = 5.38 x 10<sup>-2</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>;  $k_{p, app}$ (blue) = 5.38 x 10<sup>-2</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>; average value  $k_{p, app} = 5.38 \times 10^{-2} \text{ L.mol}^{-1}$ .min<sup>-1</sup>); relationship observed between number-average molar mass (full squares; linear trend curve is indicated by the dashed line) or dispersity (empty squares) and monomer conversion.


**Figure S13.** AROP of BO initiated by benzyl alcohol-*t*BuP<sub>4</sub> in THF at 25°C ( $[M]_0 = 2 M$ ;  $[I]_0 = 4 \times 10^{-2} mol.L^{-1}$ ): (a) First order plot (slope of the linear regression = 0.0018 min<sup>-1</sup>, R<sup>2</sup> = 0.994; k<sub>p, app</sub> = 4.51 x 10<sup>-2</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>); relationship observed between number-average molar mass (full squares; linear trend curve is indicated by the dashed line) or dispersity (empty squares) and monomer conversion.



**Figure S14.** AROP of *t*BuGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in THF at 25°C ( $[M]_0 = 2 \text{ mol.L}^{-1}$ ;  $[I]_0 = 4 \text{ x}$ 10<sup>-2</sup> mol.L<sup>-1</sup>): (a) First order plot ( $k_{p, app}$ (black) = 5.35 x 10<sup>-2</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>;  $k_{p, app}$ (blue) = 5.52 x 10<sup>-2</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>; average value  $k_{p, app} = 5.43 \text{ x} 10^{-2} \text{ L.mol}^{-1}.min^{-1}$ ); relationship observed between numberaverage molar mass (full squares; linear trend curve is indicated by the dashed line) or dispersity (empty squares) and monomer conversion.



**Figure S15.** AROP of EEGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in THF at 25°C ([M]<sub>0</sub> = 2 mol.L<sup>-1</sup>; [I]<sub>0</sub> = 4 x  $10^{-2}$  mol.L<sup>-1</sup>): (a) First order plot ( $k_{p, app}$ (black) = 2.28 x  $10^{-1}$  L.mol<sup>-1</sup>.min<sup>-1</sup>;  $k_{p, app}$ (blue) = 2.19 x  $10^{-1}$  L.mol<sup>-1</sup>.min<sup>-1</sup>; average value  $k_{p, app}$  = 2.23 x  $10^{-1}$  L.mol<sup>-1</sup>.min<sup>-1</sup>); relationship observed between number-average molar mass (full squares; linear trend curve is indicated by the dashed line) or dispersity (empty squares) and monomer conversion.

(b)



**Figure S16.** AROP of AGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in THF at 25°C ( $[M]_0 = 2 \text{ mol.L}^{-1}$ ;  $[I]_0 = 4 \text{ x}$  10<sup>-2</sup> mol.L<sup>-1</sup>): (a) First order plot ( $k_{p, app}$ (black) = 3.00 x 10<sup>-1</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>;  $k_{p, app}$ (blue) = 3.00 x 10<sup>-1</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>; average value  $k_{p, app} = 3.00 \text{ x} 10^{-1} \text{ L.mol}^{-1}$ .min<sup>-1</sup>); relationship observed between number-average molar mass (full squares; linear trend curve is indicated by the dashed line) or dispersity (empty squares) and monomer conversion.



**Figure S17.** AROP of BnGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in THF at 25°C ( $[M]_0 = 2 \text{ mol.L}^{-1}$ ;  $[I]_0 = 4 \times 10^{-2} \text{ mol.L}^{-1}$ ): (a) First order plot ( $k_{p, app}$ (black) = 3.95 x 10<sup>-1</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>;  $k_{p, app}$ (blue) = 4.30 x 10<sup>-1</sup> L.mol<sup>-1</sup>.min<sup>-1</sup>; average value  $k_{p, app} = 4.12 \times 10^{-1} \text{ L.mol}^{-1}.min^{-1}$ ); relationship observed between number-average molar mass (full squares; linear trend curve is indicated by the dashed line) or dispersity (empty squares) and monomer conversion.

(b)



**Figure S18**. SEC traces of the poly(BO) classical AROP without *i*-Bu<sub>3</sub>Al (solid line), monomer-activated AROP with *i*-Bu<sub>3</sub>Al (dashed line), detected by RI detector (eluent: THF; 40°C; flow rate: 1 ml.min<sup>-1</sup>)



**Figure S19**. Monomer conversion and  $ln([M]_0/[M])$  vs time for the AROP of PO initiated by benzyl alcohol-*t*BuP<sub>4</sub> in the presence of *i*Bu<sub>3</sub>Al in MeTHF at 25°C ([M]<sub>0</sub> = 2 mol.L<sup>-1</sup>; [I]<sub>0</sub> = 3.7 x 10<sup>-2</sup> mol.L<sup>-1</sup>, Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S20.** Monomer conversion and  $ln([M]_0/[M])$  vs time for the AROP of BO initiated by benzyl alcohol-*t*BuP<sub>4</sub> in the presence of *i*Bu<sub>3</sub>Al in MeTHF at 25°C ([M]<sub>0</sub> = 2 mol.L<sup>-1</sup>; [I]<sub>0</sub> = 3.7 x 10<sup>-2</sup> mol.L<sup>-1</sup>, Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S21.** Monomer conversion and  $ln([M]_0/[M])$  vs time for the AROP of AGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in the presence of *i*Bu<sub>3</sub>Al in MeTHF at 25°C ( $[M]_0 = 2 \text{ mol.L}^{-1}$ ;  $[I]_0 = 3.7 \times 10^{-2} \text{ mol.L}^{-1}$ , Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S22.** Monomer conversion and  $ln([M]_0/[M])$  vs time for the AROP of EEGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in the presence of *i*Bu<sub>3</sub>Al in MeTHF at 25°C ([M]<sub>0</sub> = 2 mol.L<sup>-1</sup>; [I]<sub>0</sub> = 3.7 x 10<sup>-2</sup> mol.L<sup>-1</sup>, Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S23.** Monomer conversion and  $ln([M]_0/[M])$  vs time for the AROP of *t*BuGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in the presence of *i*Bu<sub>3</sub>Al in MeTHF at 25°C ( $[M]_0 = 2 \text{ mol.L}^{-1}$ ;  $[I]_0 = 3.7 \times 10^{-2} \text{ mol.L}^{-1}$ , Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S24.** Monomer conversion and  $ln([M]_0/[M])$  vs time for the AROP of BnGE initiated by benzyl alcohol-*t*BuP<sub>4</sub> in the presence of *i*Bu<sub>3</sub>Al in MeTHF at 25°C ( $[M]_0 = 2 \text{ mol.L}^{-1}$ ;  $[I]_0 = 3.7 \times 10^{-2} \text{ mol.L}^{-1}$ , Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S25.** MALDI-TOF spectrum of poly(PO) (MeTHF, 25°C,  $[M]_0 = 2 \text{ mol.L}^{-1}$ , [benzyl alcohol] = 3,7 x 10<sup>-2</sup> mol.L<sup>-1</sup>, Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S26.** MALDI-TOF spectrum of poly(BO) (MeTHF, 25°C,  $[M]_0 = 2 \text{ mol.L}^{-1}$ , [benzyl alcohol] = 3,7 x10<sup>-2</sup> mol.L<sup>-1</sup>, Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S27.** MALDI-TOF spectrum of poly(tBuGE) (MeTHF, 25°C,  $[M]_0 = 2 \text{ mol.L}^{-1}$ , [benzyl alcohol] = 3,7 x 10<sup>-2</sup> mol.L<sup>-1</sup>, Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).



**Figure S28.** MALDI-TOF spectrum of poly(AGE) (MeTHF, 25°C,  $[M]_0 = 2 \text{ mol.L}^{-1}$ ,  $[benzyl alcohol] = 3,7 x 10^{-2} \text{ mol.L}^{-1}$ ,  $Benzyl Alcohol:t-BuP_4:i-Bu_3Al (1:1:2)$ ).



**Figure S29.** MALDI-TOF spectrum of poly(EEGE) (MeTHF, 25°C,  $[M]_0 = 2 \text{ mol.L}^{-1}$ ,  $[benzyl alcohol] = 3,7 x 10^{-2} \text{ mol.L}^{-1}$ , Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>:*i*-BuP<sub>4</sub>



**Figure S30.** MALDI-TOF spectrum of poly(BnGE) (MeTHF, 25°C,  $[M]_0 = 2 \text{ mol.L}^{-1}$ ,  $[benzyl alcohol] = 3,7 x 10^{-2} \text{ mol.L}^{-1}$ , Benzyl Alcohol:*t*-BuP<sub>4</sub>:*i*-Bu<sub>3</sub>Al (1:1:2)).