Functionalization of polyisoprene: toward the mimic of natural rubber

Jeremie Grange

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Functionalization of Polyisoprene: Toward the mimic of Natural Rubber

Sous la direction de : Dr. Frédéric PERUCH et Pr. Stéphane GRELIER
Co-encadrant : Dr. Rachid MATMOUR

Soutenue le 23 janvier 2018

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The Mixed Tape  

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**Jidenna**  
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Par où commencer ?

Beaucoup, beaucoup de monde à remercier.

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Biensûr, je remercie également à la fois les acteurs du projet Rubbex ainsi que l’entreprise Michelin pour tout ce qu’ils ont pu m’apporter tout au long de cette thèse comme connaissances, à la fois sur le caoutchouc naturel mais aussi sur la chimie des polymères en général et sur le milieu industriel.

Ensuite vient le tour de mes chefs. Que dire ? Soit beaucoup, soit rien ! Je vais donc tâcher de faire preuve d’esprit de synthèse pour simplement vous dire merci pour tout ce que vous avez su m’apporter tant sur le plan scientifique que sur le plan humain. Je pense que vous en êtes conscients, mais vous êtes tous les deux de sacrés mentors pour moi et j’espère un jour être amené à retravailler avec vous quel que soit le projet, pour apprendre toujours plus à votre contact. Merci encore pour tout.

Et puis il y a les collègues. Ceux qui m’ont vu (supporté) pendant ces 3 ans et plus. J’ai hésité à faire une liste, mais je pense que les concernés se reconnaîtront d’eux-mêmes et savent très bien à quel point ils comptent pour moi (#N1-0autenthique). Merci à vous tous pour les rencontres, les discussions, les fous rires, les débats, etc… Pour paraphraser deux artistes que j’aime beaucoup je dirais que « je suis un peu de moi, mais beaucoup de vous tous quand j’y pense ». Bonne route à tous et j’espère vous recroiser rapidement.

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Résumé en français
Résumé en français

Ce projet de recherche se propose de générer de nouvelles connaissances sur le caoutchouc naturel (NR) afin d’essayer de gérer au mieux cette ressource renouvelable. En particulier, il a été largement montré que le caoutchouc naturel est composé d’environ 93% de polyisoprène (PI) 1,4-cis de très forte masse molaire, mais également de 7% d’autres composés (lipides, protéines, minéraux, …). Ces derniers sont vraisemblablement responsables de la très grande différence de propriétés observée entre NR et caoutchouc synthétique. De plus, de nos jours, le seul modèle rendant compte de ces observations est le modèle décrit par Tanaka, à savoir que les chaînes de PI sont fonctionnalisées en bout de chaîne par des protéines et des lipides (Figure 1). Ce modèle qui propose donc que les chaînes de PI soient plutôt sous la forme de copolymères est largement admis mais nécessite néanmoins d’être évalué afin de démontrer que la structure d’un tel copolymère peut rendre compte des propriétés supérieures du NR.

L’objectif principal de ce projet de thèse est donc la synthèse de copolymères modèles et l’évaluation de leurs propriétés afin de mieux comprendre la structuration du NR lors du stockage et le durcissement généralement observé. La synthèse des macromolécules mimant le modèle de Tanaka peut être décomposée en plusieurs étapes :

- L’obtention d’un noyau polyisoprène totalement « 1,4-cis »
- Le greffage d’un phospholipide en ω de la chaîne PI
- Le greffage d’une protéine en α de la chaîne PI

![Figure 1: Structure d’un polyisoprène bifonctionnel proposé par Tanaka, pouvant modéliser la structure du NR.](image)

L’obtention d’un noyau polyisoprène (PI) totalement « 1,4-cis » peut s’envisager de différentes manières. La synthèse chimique a été largement étudiée. Plusieurs voies sont possibles (radicalaire, anioniques, métathèse, coordination, cationique) mais aucune ne permet à la fois un bon contrôle de la microstructure de la chaîne, des extrémités de chaîne et de la masse molaire. En revanche, des travaux sur la dégradation chimique (métathétique ou oxydative) montrent des résultats d’intérêt pour l’application recherchée dans notre travail. En effet, cette voie permet d’obtenir une microstructure 100% 1,4-cis.
**Résumé en français**

De plus, ces méthodes permettent un parfait contrôle des extrémités de chaîne avec la possibilité de synthétiser des PI « homo » ou « hétéro » téléchéliques. Pour toutes ces raisons, la dégradation contrôlée du NR a été préférée.

Dans un premier temps, nous sommes partis de feuilles de NR issues de 2 clones (PB235 et RRIM600). Nous avons caractérisé en détail ces différents NR et mis en place des méthodes d’extraction afin de pouvoir ensuite dégrader les chaînes de polyisoprène. Les meilleurs résultats ont été obtenus avec le THF et le toluène.

Nous avons ensuite décidé de réaliser la dégradation oxydative du PI naturel ainsi obtenu par époxydation à l’acide méta-chloroperbenzoïque (m-CPBA) suivi d’une rupture à l’acide périodique (Figure 2).

![Figure 2: Schéma réactionnel de la dégradation oxidative du PI](image)

Différentes masses molaires ont ainsi pu être obtenues avec des dispersités relativement faibles. L’utilisation de deux matières premières naturelles et d’une matière première synthétique a également permis de déterminer des différences de réactivité au cours de la dégradation. En effet, à partir d’une certaine valeur de masse molaire, la dégradation devient difficile voir impossible avec le PI naturel. Dans le cas du PI synthétique en revanche, la dégradation est possible même à de très faible taux. Une masse de travail de 10 000 g/mol a été fixée pour le reste de l’étude, afin d’évaluer aisément l’efficacité des réactions mises en place et de permettre une bonne caractérisation des (co)polymères.

Dans un premier temps, un PI fonctionalisé avec deux chaines grasses, de longueur et structure variable, a été obtenu (Figure 3).

![Figure 3: Schéma réactionnel de la synthèse de PI doublement substitué par l’acide palmitique.](image)
L’analyse DSC de ces hybrides PI/Lipide a montré dans certains cas une cristallisation et une fusion du bout de chaîne lipidique à des températures variables selon la nature des chaînes grasses liées (Tableau 1). La température de cristallisation la plus haute a été obtenue après fonctionnalisation de la chaîne PI par deux acides lignocériques (C24:0).

Tableau 1: Températures de cristallisation et fusion de plusieurs hybrides PI/Lipides (10 000 g/mol) observées par DSC

<table>
<thead>
<tr>
<th>Chaîne grasse liée</th>
<th>$T_m$ a) (^°C)</th>
<th>$T_c$ b) (^°C)</th>
<th>$T_g$ d) (^°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acide undécènoïque (C11:1)</td>
<td>- c)</td>
<td>- c)</td>
<td>-65</td>
</tr>
<tr>
<td>Acide Linoléique (C18:2)</td>
<td>- c)</td>
<td>- c)</td>
<td>-65</td>
</tr>
<tr>
<td>Acide Myristique (C14:0)</td>
<td>- c)</td>
<td>- c)</td>
<td>-65</td>
</tr>
<tr>
<td>Acide Palmitique (C16:0)</td>
<td>-25</td>
<td>-37</td>
<td>-65</td>
</tr>
<tr>
<td>Acide Stéarique(C18:0)</td>
<td>-11</td>
<td>-18</td>
<td>-64</td>
</tr>
<tr>
<td>Acide nonadécanoïque (C19:0)</td>
<td>-5</td>
<td>-13</td>
<td>-64</td>
</tr>
<tr>
<td>Acide Lignocérique (C24:0)</td>
<td>22</td>
<td>17</td>
<td>-63</td>
</tr>
</tbody>
</table>

a) Température de fusion observée par DSC, b) Température de cristallisation observée par DSC; c) Pas de cristallisation (ou de fusion) observée, d) Transition vitreuse observée par DSC

L’influence de la taille de la chaîne polymère sur cette propriété a également été étudiée, montrant une disparition de la cristallisation pour des PI de 25 000 g/mol. En revanche, les températures de fusion et de cristallisation obtenues pour des hybrides de masse molaire 5 000 g/mol sont en tout point similaires à celles présentées dans le tableau ci-dessus. Il a donc été proposé que les chaînes lipidiques greffées présentent la capacité de créer des nodules de cristallisation regroupant ainsi plusieurs chaînes PI entre elles (Figure 4).
Ces hybrides PI/Lipides représentant des modèles de caoutchouc naturel, il a ensuite été proposé d’étudier leur propriété de cristallisation à -25°C, température définie dans la littérature comme étant la température la plus favorable à la cristallisation du PI. Des analyses DSC ont donc été menées sur des synthons PI de 10 000 g/mol fonctionnalisés (ou non) par des chaînes grasses ainsi qu’en présence (ou non) d’esters gras libres. Comme le montre la Figure 5, il a été montré que la fonctionnalisation par les lipides empêche la cristallisation du PI. En revanche, l’addition de lipides libres (Figure 6) a permis de retrouver une cristallisation du PI. Le meilleur résultat a été obtenu après ajout d’un mélange 4% (massique) d’acide stéarique / 4 % (massique) de linoléate de méthyle à l’hybride PI/Lipide initial (ici PI/acidé stéarique). Il a donc été conclu que si la fonctionnalisation du PI par des chaînes grasses empêche sa cristallisation à -25°C, l’ajout de lipides libres a, en revanche, un effet bénéfique sur la cristallisation du polymère. Ces résultats sont en tout point similaires à ceux existants dans la littérature pour le NR prouvant ainsi que l’hybride PI/Lipide synthétisé est un bon modèle de caoutchouc naturel.
Dans un second temps, le greffage de protéine en α de la chaîne PI principale a été étudié. Des synthons PI modifiés et accepteurs (Figure 7) de protéines ont été synthétisés via l’insertion en bout de chaîne polymère d’une fonction maléimide fortement réactive vis-à-vis des fonctions thiols des protéines (cystéines).
Deux protéines ont été utilisées pour le couplage avec la chaîne PI : la Lipase B issue de *Candida antarctica* (CALB) et l’albumine de sérum bovin (BSA). Dans les deux cas, des essais de couplage ont été menés, formant des émulsions très stables (Figure 8). Plusieurs tentatives de caractérisation de ces émulsions ont été réalisées (SEC, SDS-PAGE, RMN) mais n’ont pas permis de déterminer de façon claire la formation (ou non) de l’hybride PI/Protéine. En revanche, l’analyse par microscopie optique de ces émulsions a montré une différence dans les tailles des gouttes formées et ce, plus particulièrement dans le cas du couplage PIMal/BSA. Bien que ces analyses ne constituent pas une preuve suffisante, elles semblent être en faveur de la formation (certainement à faible rendement) du copolymère.

![Figure 8: Emulsions formées lors d’un essai de couplage entre un PIMal et CALB - A: Expérience témoin sans PI; B: Expérience témoin utilisant un PI non accepteur de protéine; C: Essai de couplage PIMal/CALB](image)

Enfin, aux vues des difficultés rencontrées pour le couplage PI/Protéine, il a été proposé d’étudier la possibilité de synthèse de copolymères à bloc PI/Polypeptide via la polymérisation par ouverture de cycle (ROP) de monomères de type N-carboxyanhydride (NCA). Cette polymérisation est une réaction utilisant généralement des amines primaires pour l’amorçage, fonction qu’il a été impossible d’obtenir en bout des chaîne PI malgré de nombreux essais. Cependant, nous avons été capables d’amorer la ROP des NCAs via l’usage de macro-amorceurs de PI porteurs de fonctions amino-alcools en bout de chaîne (Figure 9). Différentes architectures de copolymères PI/Polypeptide ont donc été synthétisées, ouvrant ainsi la voie à la synthèse d’un copolymère tri-bloc polypeptide-PI-lipide, proche du modèle de Tanaka.
En conclusion, ces travaux de thèse ont conduit à une meilleure compréhension du NR sous différents aspects. Tout d’abord, il a été caractérisé en détails deux clones d’*Hévéa* (RRIM 600 et PB 235), montrant de grandes différences en terme de composition. Ensuite, l’étude de la dégradation chimique des deux clones permettant d’obtenir des PI de faible masse molaire (10 000 g/mol) fonctionnels a permis de mettre en évidence des différences de comportement par rapport au polyisoprène synthétique. Enfin, de nombreuses synthèses nous ont permis d’avoir accès à un premier modèle de NR possédant deux lipides liés en bout de chaîne et montrant des propriétés de cristallisation à froid proche de celle du NR. Cette étude a permis de clarifier le rôle joué par les lipides libres et liés, présents dans le caoutchouc naturel, sur ses propriétés de cristallisation. D’autre part, malgré plusieurs tentatives, la synthèse d’un copolymère PI/Protéine n’a pas abouti mais, des copolymères PI/Polypeptide ont pu être obtenus par ouverture de NCA. Cette voie est aujourd’hui la plus prometteuse pour atteindre une structure proche du modèle proposé par Tanaka (Figure 1).
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Abbreviations
AcOH: Acetic acid
AE-DPNR: Acetone extracted deproteinized natural rubber
AE-NR: Acetone extracted natural rubber
ATRP: Atom transfer radical polymerization
BenzylGluNCA: Benzylglutamate N-carboxyanhydride
BSA: Bovine serum albumin
BTF: $\alpha,\alpha,\alpha$-Trifluorotoluene
CALB: Lipase B from *Candida Antartica*
CCr: Cold crystallization
CRP: Controlled radical polymerization
CTA: Control transfer agent
DA: Diels-Alder
DCE: Dichloroethane
DCM: Dichloromethane
DEAM: Diethanolamine
DMAPP: Dimethylallyl pyrophosphate
DMCOD: 1,5-dimethyl-1,5-cyclo-octadiene
DPNR: Deproteinized natural rubber
DSC: Differential scanning calorimetry
FPP: Farnesyl pyrophosphate
GGPP: Geranyl geranyl pyrophosphate
GPP: Geranyl pyrophosphate
HRP: Horse radish peroxidase
HTLNR: Hydroxy-telechelic liquid natural rubber
IPP: Isopentenyl pyrophosphate
IR: Isoprene rubber
LA: Lewis acid
LASCs: Lewis acid surfactant combined catalysts
LiA: Linoleic acid
LiNTf$_2$: Bis(trifluoromethane)sulfonimide lithium salt
LNR: Liquid natural rubber
MalChlo: Maleimidohexanoyl chloride
MalDAAm: 6-(4-(aminomethyl)-1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindol-2(3H)-yl)hexanoic acid
MalHex: Maleimidohexanoic acid
MalProt: Furan protected maleimido hexanoic acid
MEAM: Methylethanolamine
ML: Methyl linoleate
MSA: Methanesulfonic acid
NaBH$_4$: Sodium borohydride
NCA: N-carboxyanhydride
NIPAM: N-isopropylacrylamide
NMP: Nitroxide mediated polymerization
NMR: Nuclear magnetic resonance
NR: Natural rubber
PB: Phosphate buffer
PI: Polyisoprene
PIDeg: Hetero-telechelic (ketone/aldehyde) rubber.
PIDiLip: Hetero-telechelic (ketone/di-lipid) polyisoprene
PIDiLipLip: Hetero-telechelic (lipid/di-lipid) polyisoprene

PIDiLipMal: Hetero-telechelic (maleimide/di-lipid) polyisoprene

PIDiLipMalProt: Hetero-telechelic (furan protected maleimide/di-lipid) polyisoprene

PIDiLipOH: Hetero-telechelic (hydroxyl/lipid) polyisoprene

PIDiOH: Hetero-telechelic (diethanolamino/ketone) polyisoprene

PIDiP Pep: Hetero-telechelic (ketone/di-polypeptide) polyisoprene

PIMal: Hetero-telechelic (ketone/maleimide) polyisoprene

PImOH: Hetero-telechelic (ketone/methylethanolamine) polyisoprene

PIMonoLip: Hetero-telechelic (ketone/lipid) polyisoprene

PIMonoLipLip: Homo-telechelic (lipid/lipid) polyisoprene

PIMonoLipOH: Hetero-telechelic (hydroxyl/lipid) polyisoprene

PINH₂: Hetero-telechelic (ketone/primary amine) polyisoprene

PINHOH: Hetero-telechelic (ethanolamino/hydroxyl) polyisoprene

PIOHOH: Hetero-telechelic (ethylethanolamine/hydroxyl) polyisoprene

PIOH: Hetero-telechelic (ketone/hydroxyl) polyisoprene

PIOH: Hetero-telechelic (ketone/hydroxyle) polyisoprene

PI PPe pFur: Di-block copolymer PI/polypeptide obtained by diels-alder chemistry

p-MOS: Para-methoxystyrene

PPe p: Polypeptide

PPe pDMEA: Dimethylethanolamine terminated polypeptide

PPe pFur: Furan terminated polypeptide

RAFT: Reversible addition fragmentation polymerization

REF: Rubber elongation factor

ROMP: Ring opening metathesis polymerization

ROP: Ring opening polymerization

RP: Rubber particles

SEC: Size exclusion chromatography

SIC: Strain-induced crystallization

SRPP: Small rubber particle protein

STABH: Sodium triacetoxyborohydride

TEA: Triethylamine

TE-AE-DPNR: Transesterified acetone extracted deproteinized natural rubber

TE-DPNR: Transesterified deproteinized natural rubber

TE-NR: Transesterified natural rubber

TFA: Trifluoroacetic acid

T₆: Glass transition temperature

TGA: Thermo-gravimetric analysis

THF: Tetrahydrofuran

TLNR: Telechelic liquid natural rubber
Introduction
Introduction

For approximately 20 years and with the growth of environmental issues, like global warming or fossil fuel feedstock decrease, the chemical industry is slowly adapting and transforming toward more sustainable processes and starting materials. A wide variety of new materials (monomer, polymer, composites, etc…) emerged either inspired or directly taken from natural resources (lignin, vegetal oil, cellulosics, polysaccharides, etc…). More generally, this new approach usually starts from the observation of Nature, the understanding and/or characterization of the desired new process/molecule/material and either its direct use or modification to replace an existing material presenting any environmental or economical issue (like petro-sourcing for example). Figure 1 presents a schematic diagram of various biorefinery processes summarizing the raw-materials of interest and the final valuable products available by a “greener” approach witnessing of the change of mind that chemistry is currently living.

Figure 1: Schematic diagram of biorefinery for precursor-containing biomass - Reproduced from Kamm et al.¹

Natural rubber (NR) is an old biopolymer reported to be used by Mesoamerican people as early as 1600 BC². Contrarily to other compounds from biomass, NR has been industrially used for about 200 years now, after the discovery of vulcanization in 1838 by Charles Goodyear, and the global consumption is still increasing (Figure 2). It is produced by more than 2 500 plant species but the main industrial source of NR comes from Hevea brasiliensis, a tree, principally cultivated in South-East Asia (Thailand and Indonesia)³.
Depending on the plant species, the microstructure of natural polyisoprene varies from 100% 1,4-trans for Gutta-Percha to 100% 1,4-cis for Hevea tree. NR from Hevea exhibits thermo-mechanical properties (fatigue resistance, high hysteresis, etc...) that makes it essential for some applications like plane or truck tires, medical material or seismic anti-vibration systems. These astonishing properties can not be fully mimicked by synthetic rubbers as their origins are not still completely understood. Furthermore, as NR comes from biomass, its structure is highly dependent on parameters such as season, age of the tree, nature of the soil where it is farmed and can also face microbiologic attacks thus blocking the production, and causing variations in industrial processes.4

Figure 2: Global production of natural and synthetic rubber during the twentieth century

– Obtained from http://www.rubberstudy.com –

Isoprene rubber (IR) is the synthetic homologue of NR. It is obtained from the polymerization of isoprene, a monomer extracted from petroleum cracking fractions. This material presents several advantages compared to NR like the low production cost and fewer variation of structure after optimization of the synthetic process. This material was extensively studied to get independent from NR but unfortunately it was rapidly established that IR was not able to compete with the mechanical properties of the natural material. Among all the studies trying to determine the origins of the property differences between IR and NR, Tanaka and co-workers summarized about 30 years of their own researches5–8 on that field and gave birth to the only versatile explanation to date by postulating that the polyisoprene (PI) chain present in NR is not only a single linear chain of polymer but is substituted in α and ω positions by a proteinic and a lipidic moiety respectively (Figure 3).
Tanaka explained that such a molecule could self-assemble (Figure 4) creating micro-domains of either lipids and/or proteins, thus forming a physical network. Considering the presence in the material of free lipids and proteins, those anchors can be formed either only by chain-ends of various linear PI or with the inclusion of free compounds. This model also suits for the self-assembly of rubber particles in water media (i.e. in latex) with the formation of a lipidic membrane stabilized by the amphiphilic behavior of proteins (Figure 5) thus forming particles in water.
Introduction

Surprisingly, the veracity of this model has never been checked directly in the literature. 

The goal of this PhD work is then to synthesize the “tri-block” molecule reported in Figure 3 and to study the properties of such copolymer to validate (or not) the model proposed by Tanaka. To this end, a general chemical pathway was established starting from a pure 1,4-\textit{cis} hetero-telechelic PI, which will be functionalized at both chain-ends either by a lipidic moiety or a protein. Attention will also be paid on both di-blocks “PI-Lipid” and “PI-Protein” as none of them have already been reported in the literature. Figure 6 summarizes the different pathways considered during this PhD work. The choice of the pathways will be justified all along the different chapters of the document.

In a first chapter, a bibliographic study will present generalities about both IR and NR : NR biosynthesis and properties, the main property differences between NR and IR and also an overview of the synthesis of IR. A brief summary of the existing literature describing Polymer/Protein and Polymer/Lipid coupling will follow, showing that PI was never particularly studied in this field, thus enhancing the challenge of this PhD thesis.

The following chapters will focus on the synthetic pathway. First will be presented the synthesis of a 1,4-\textit{cis} pure heterotelechelic PI varying the length of the polymeric chain. Several reaction parameters will be studied. Then, the coupling of PI with lipids will be focused on. The different properties of this hybrid materials will be investigated in details. Finally, it will be presented the coupling of PI with proteins, which is the most challenging part of the thesis. This manuscript will end up with a general conclusion.
Figure 6: General chemical pathways developed in the manuscript

R, R', R'' = Alkyl groups or alkyl maleimide
REFERENCES:


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Bibliography
I. Overview of NR: Generalities, Biosynthesis and Establishment of Tanaka’s model

a. General considerations on NR

As briefly explained in the general introduction, NR is a material of strategic importance for industry. It is present in more than 40,000 commercial goods like tires but also in more than 400 medical products\(^1\). Many plants are able to produce NR but the resulting polymer is usually of low molar mass and is not suitable for mechanical applications\(^2\). Among this diversity, only a couple of plants namely *Hevea brasiliensis*, *Parthenium argentatum* (Guayule) and *Taraxacum kok-saghyz* (Russian Dandelion) were particularly studied for the production of rubber suitable for industrial applications. It is important to note that generally, “NR” designs Natural Rubber from *Hevea brasiliensis* as it is to date the main one to be used by companies. The previous sentence raises a problem widely demonstrated by Cornish *et al.*\(^3\)–\(^6\) that nowadays, the genomic diversity of the *Hevea brasiliensis* produced is very low which could lead to crop failure or fungal issues and thus to a decrease in the global supply of the material. For this reason the work of the team of Cornish focuses mainly on finding alternative sources of NR with properties comparable to the one extracted from *Hevea brasiliensis*. One good candidate for this substitution is the rubber extracted from Guayule which is comparable to traditional NR but is considered as non-allergenic compare to NR from *Hevea brasiliensis* which possesses many allergens mostly due to its protein content. However, Guayule presents one major drawback: the rubber is produced in all compartments of the plant (leaves, roots, limb, etc.). Consequently, the recovery of the rubber is difficult. The shrub has to be cut, milled and the rubber is then extracted with organic solvents. On the contrary, with *Hevea brasiliensis*, the rubber can be obtained directly by tapping the tree. Figure 1 shows the tapping of an *Hevea* tree and the recovery of the latex. By centrifugation, this latex can be fractionated into three main parts: namely the cream which is the hydrophobic part of the latex (mainly composed of rubber particles), the C-serum composed of the hydrophilic component (proteins, sugars, minerals etc…) and the lutoids also called “bottom fraction” in the literature. By coagulation of the latex in acidic conditions, NR “ball” can be directly recovered affording a processable material.
Rubber particles contained in the latex are indeed stored in the laticifers which are special plant channels (vessels) devoted to the transport and storage of latex solely\(^7\). To date, no particular evidence helped understanding the production of rubber by plants as no specific interest of this material was reported for the physiology of the vegetal\(^8\). It was, nevertheless proposed that latex could be used by the plant as a protection. Two size populations exist in rubber particles (RPs), one small (about 0.2 µm) and one big (about 1 µm) with only few differences reported between them. In both cases, they are composed of a core of PI surrounded by a lipidic membrane where proteins and other rubber components can be adsorbed\(^1\) (Figure 2). Berthelot et al.\(^9,10\) proved that among all the proteins present in the *Hevea brasiliensis* the two predominant ones are REF (Rubber Elongation Factor) and SRPP (Small Rubber Particle Protein), two relatively small proteins (15 kDa and 24 kDa respectively), rather hydrophobic and which are located on different RPs (REF is adsorbed in bigger RPs while SRPP is part on the small RPs membrane). The exact role of those proteins is still not perfectly understood but first insights seem to prove that SRPP plays a role in latex coagulation and that both of them have a positive effect on the rubber biosynthesis.
More generally, NR is a complex material as it is not only constituted of an hydrocarbon polymeric chain but also of a “non-isoprene” part. This part usually represents around 6 wt% of the dry material and is highly dependent, in composition, of the clonal origin of the rubber, the meteorological conditions, the nature of the soil where the tree was grown, etc… This “non-rubber” part is composed of proteins, carbohydrates, lipids and inorganic constituents and represents the main composition difference with IR. It is thus believed to be involved in the specific and superior properties of NR. Table 1 gives average values of the NR composition as reported by Vaysse et al.\textsuperscript{11} The lipid content of various clones of NR\textsuperscript{12–14} was highly studied by Vaysse and Liengpayoon, highlighting the variation of composition in function of the clonal origin previously mentionned.

<table>
<thead>
<tr>
<th></th>
<th>Latex</th>
<th>Dry Rubber</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% w/w</td>
<td>% w/w dry matter</td>
</tr>
<tr>
<td></td>
<td>fresh latex</td>
<td>dry matter of latex</td>
</tr>
<tr>
<td>Rubber hydrocarbon</td>
<td>35.0</td>
<td>87.0</td>
</tr>
<tr>
<td>Proteins</td>
<td>1.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>1.5</td>
<td>3.7</td>
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<tr>
<td>Lipids</td>
<td>1.3</td>
<td>3.2</td>
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<tr>
<td>Organic solutes</td>
<td>0.5</td>
<td>1.1</td>
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<tr>
<td>Inorganic substances</td>
<td>0.5</td>
<td>1.2</td>
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Approximate values only (highly dependant on clone, season and physiological status of the tree)
Regarding the polymer characteristics, NR is usually of high molar mass (~1 000 000 g/mol) with a quite broad dispersity (> 2). Moreover, it usually exhibits a bimodal molar mass distribution\(^2\). Nevertheless, three different SEC profiles can be obtained depending on the clonal origin of *Hevea*: a broad signal with only one population, two distinct signals with a predominance of one population toward the other or two distinct signals nearly of same intensity (Figure 3).

**Figure 3: Typical SEC chromatograms of commercial Hevea rubbers - Reproduced from Tanaka et al.\(^2\)**

It must be underlined that when NR is solubilized in organic solvents (toluene, THF, cyclohexane, DCM etc...) a gel fraction is obtained, which can also vary with the clonal origin of the rubber and is assumed to be formed along the biosynthesis of the material due to chain branching or even physical cross-linking\(^2\) induced by the presence of “non-rubber” constituent conferring self-assembly properties to the polymeric chain.

### b. Biosynthesis of NR

Many studies have been conducted to understand the biosynthesis of NR\(^15\) and are still ongoing nowadays\(^16\). The overall process belongs to the larger family of isoprenoid (low molar mass compounds) biosynthesis\(^1\). The monomer used for the synthesis of NR is isopentenyl pyrophosphate (IPP, Figure 4). It is produced from sucrose via the cytosolic mevalonate pathway (in cytoplasm) or the methylerythritol pathway (in chloroplast or bacteria).\(^11\) Once IPP is obtained, it is then isomerized to dimethylallyl pyrophosphate (DMAPP, Figure 4) by an enzyme named IPP isomerase. DMAPP was originally thought to be the initiator of the polymerization reaction\(^17\) and thus PI from NR was supposed to be composed of a long chain of 1,4-cis units starting from one dimethylallyl moiety. But, ozonolysis of NR and NMR studies contradicted this assumption. Indeed, no trace of acetone formation was observed for *Hevea brasiliensis* NR (coming from ozonolysis of the...
dimethylallyl moiety) and a small amount of 1,4-trans units was detected by $^{13}$C NMR analysis$^{2,7,18}$.

![Figure 4: Structure of IPP and DMAPP](image)

The initiator was then found out to be an oligomer of IPP and DMAPP but in the trans isomeric form as observed in isoprenoids. It is now established that the second step of the rubber biosynthesis is the formation of the initiating species resulting of the condensation of IPP onto DMAPP by a trans-prenyl transferase to form various molecules like geranylpyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranyl geranyl pyrophosphate (GGPP) which are the true initiators for the IPP polymerization, with nevertheless different activities as described by Archer$^7$.

![Figure 5: Structure of GPP, FPP and GGPP](image)

The elongation step (the propagation) consists in the successive condensation of IPP by a removal of the OPP moiety, thus generating a carbocation attacked by a new IPP molecule and then a removal of proton to recover a double bound in the cis configuration. This is known to take place in the active site of an enzyme called cis-prenyl transferase or rubber transferase, which is adsorbed/encapsulated in the lipidic membrane of the rubber particles. A metallic cofactor (Mg$^{2+}$) is needed for the enzyme to be active (Figure 6). Cornish et al.$^{5,19-21}$ reported about the structure of the cis-prenyl transferase representing it like a “tunnel” where initiator enters, binds to the active site and starts the polymerization. The presence of an
Finally, the last shade existing in the biosynthesis machinery concerns the termination step and the nature of the ω chain-end of the polymer. To date, no particular evidence exists to explain the termination step. It seems that at some point, the growing chain disconnects from the active site of the enzyme and is packed in the core of the RPs. It was originally thought that the polymer would grow until it reaches a limit molar mass thus triggering the termination step. Nevertheless, this is in contradiction with the general dispersity observed in NR which is usually broad while such a limit value of molar mass would induce a really narrow dispersity. About the terminal chain end of the polymer, infra-red and NMR analyses carried out by Tanaka et al.\textsuperscript{22–24} showed the existence of fatty acid chains linked to the PI backbone. It is generally accepted that the terminal group comes from the degradation of the pyrophosphate moiety but no evidence of an hydroxyl function coming from its hydrolysis was observed. The main possibility described is the esterification of the chain-end with lipids either in the form of phospholipids or simple ester groups formed after hydrolysis of the pyrophosphate group and reaction with a free fatty acid.

To conclude, this sub-chapter briefly presented NR and its biosynthesis. An overall vision of the knowledges on this field was given by Cornish and is reported here in Figure 7.
This figure exposes the complexity of the biomachinery involved in this interfacial polymerization. But also, this vision is a starting point for various works trying to mimic this biosynthesis using cationic polymerization for example as will be developed later in the manuscript.

Figure 7: General view of the NR biosynthesis in the RPs - Reproduced from Cornish et al.5

c. Tanaka’s model of NR

As previously mentioned, Tanaka’s team contributed a lot to the better understanding of NR structure, properties and biosynthesis. The genesis of the model presented in the introduction comes from the desire of finding an overall explanation to all the different parameters of NR like biosynthesis pathways, structure of the polymer, physico-chemical properties (gel fraction, molar mass distribution and bimodality) and thermo-mechanical properties (green strain, crystallization on strain, cold crystallization and global resistance of the material). In two successive reviews published in 200123 and 200922 Tanaka summarizes about 30 years of research presenting all the links he established between the structure of the material and the known properties of NR. In this subchapter, it will be presented how Tanaka developed the model previously discussed. It is important to mention that, in his work, Tanaka describes the protein chain-end as the ω part of the polymer and the lipidic chain-end as the α one.
This choice is questionable as the term $\alpha$ in polymer chemistry usually refers to the chain-end coming from the initiator which is, in the case of NR, the protein part. For the sake of homogeneity, in all the manuscript, the $\alpha$ chain-end will refer to the protein one and the $\omega$ chain end to the lipidic one.

**i. The trans units at the $\alpha$-chain-end**

As explained before, the presence of *trans* units at the beginning of the polymeric chain was due to the condensation of IPP with a *trans*-prenyl tranferase. As the molar mass of NR from *Hevea* is too high for precise NMR analysis, Tanaka studied NR from other sources (mushroom$^{25}$, *Goldenrod* leaves$^{26}$ and also short polyprenols$^{27}$) to characterize their structure and compare them with *Hevea* NR after fractionation to analyze only the shortest chains (Figure 8). Starting from the study of *Goldenrod*, several dyads of *trans* and *cis* units were assigned. Moreover, for *Goldenrod* and polyprenols, it is known that the biosynthesis proceeds starting from DMAPP and thus the signal of the terminal dimethylallyl group in these model molecules (named $\omega$ in the NMR spectrum) can be observed. Finally, it was demonstrated that this dimethylallyl group was linked to the *trans* units and that no *cis-trans* dyad could be observed in the model molecules but only *trans-cis* and *cis-cis* which was expectable regarding the “pure” microstructure of NR. *Goldenrod* rubber and polyprenols exhibit the same structure: DMAPP moiety as chain end, a couple of *trans* units and then a varying amount of pure *cis* structure. Comparing with NR from *Hevea* the same *trans* signals were visible which explains the *trans* units in Tanaka’s model.

![Figure 8: $^{13}$C NMR spectrum of rubber from Goldenrod and low molar mass fraction of NR - Reproduced from Tanaka et al.](image)
ii. Origin of the α proteic part

It is the most controverted part of the model. Originally, the structure of the α chain-end was supposed to be a dimethylallyl group. However, ozonolysis of NR did not show the formation of acetone and the $^{13}$C NMR analysis (see § 1.3.1.) showed the absence of the dimethylallyl group in NR. To clarify this point, deproteinized NR (DPNR) were produced by the use of proteolytic enzymes and acidic or basic treatments$^{28}$. Figure 9 presents a FTIR spectrum of different types of NR bearing a decreasing amount of proteins. It can be seen that a small band around 3300 cm$^{-1}$ is decreasing with the ratio of nitrogen present in the polymer. By comparison with model oligopeptides it was concluded that if even after harsh removal of proteins, the same absorption band as oligopeptide is still present, it is because the remaining proteins are linked to the polymer backbone. Nevertheless, this conclusion was denied by Tanaka himself$^{29}$ and it was demonstrated that in fact this band was also present in IR thus showing that it can not be a proof of linkage with proteins.

![Figure 9: FTIR spectra of purified NR and model oligopeptides](image)

Besides, the gel content decreased with the removal of proteins thus attesting that some of the branching points of the material were involving somehow proteins. The current hypothesis is that the real α chain-end is still unknown but it may interact (at least physically) with proteins or oligopeptides and play a role in the mechanical properties of the material.
The last part of the model is the linkage with phospholipids. As explained before, many lipids are present in the latex, either linked or not to the polyisoprene chains. Free lipids can be removed by a soxhlet acetone extraction. The obtained polymer is referred to as acetone extracted natural rubber (AE-NR) and, when applied to DPNR, the polymer is called (AE-DPNR). Moreover, linked fatty ester can be removed from the polymer backbone by transesterification using sodium methanolate and toluene as a solvent. The obtained polymer is referred to using the prefix “TE” for “transesterified” (for example, transesterified acetone extracted deproteinized natural rubber will be referred to as TE-AE-DPNR).

Figure 10 presents the two main evidences proposed by Tanaka for the presence of phospholipids at the chain end. The superimposition of FTIR spectra shows that in fresh NR two distinct bands are visible for fatty acids and fatty esters at 1710 and 1738 cm\(^{-1}\) respectively. After acetone extraction, only the band corresponding to ester moieties at 1738 cm\(^{-1}\) remains visible but disappears after transesterification.

This proves that fatty ester moieties are linked to the polymer backbone. The \(^{13}\)C NMR analysis of a low molar mass fraction of NR also showed the characteristic signals of fatty ester moieties (Figure 10b).

Figure 10: (a) FTIR spectra of natural rubber from pale creep: (A) control, (B) extracted with acetone and (C) transesterified / (b) \(^{13}\)C NMR spectrum of low molar-mass fraction of DPNR with \(M_n = 6.8 \times 10^4 \text{ g/mol} - Reproduced from Tanaka et al.\(^{23,24}\)
Moreover, AE-DPNR was shown to contain about one phosphorous atom and two fatty chain per chain of PI, leading to the conclusion that the linked fatty esters are phospholipid derivatives. Besides, $^{31}$P NMR analysis performed on AE-DPNR revealed the presence of characteristic signals of both mono and diphosphate moieties. Tanaka then postulated that these chain-ends arise from the pyrophosphate group present during the propagation which, after chemical modification either by a direct condensation of a phospholipid moiety or after hydrolysis (to yield a terminal hydroxyl group) and esterification lead to a lipid terminated PI. Furthermore, it was shown that the enzymatic suppression of phospholipid moieties induced a higher decrease of the gel fraction than the removal of proteins. Finally, after dissolution in toluene it appears that the addition of a small amount of polar and protic solvent (methanol) could partially break the gel fraction, thus indicating that the branching point could be attributed to hydrogen bonding between chains and especially between phospholipid moieties.

**iv. Conclusion**

In conclusion, Tanakas’s model of NR is, to date, the only model to give a link between biosynthesis of NR, physico-chemical properties of the final polymer and its internal structure. Nevertheless, it subsists lack of information regarding the $\alpha$ chain-end which was not clearly identified.

**d. Cold crystallization of NR and IR**

Cold crystallization (CCr) of polyisoprene refers to the capacity of the material to crystallize after being maintained at low temperature for a given time. Many works focused on this property due to the fact that NR exhibits a quicker CCr than its synthetic analogous. This ability of the natural material is one of the main particularity that was not clearly elucidated yet.

From the pioneering work of Wood in 1946 to the more recent work of Kawahara in 2004, many researches focused on a better understanding of CCr for both IR and NR. Wood studied many parameters such as the rate of crystallization of NR or the temperatures at which crystallization can be observed. NR exhibits its highest rate of crystallization at $-25^\circ$C where half of the final crystallinity is obtained within 2.5 hours. It is also shown that the rate of crystallization follows a Gaussian plot (Figure 11). For instance, sample of NR kept at $-50^\circ$C or $-78^\circ$C for 3 weeks did not exhibit any crystallization. The same samples underwent crystallization when temperature was raised to $-35^\circ$C.
Thus, below -50°C the crystallization of NR becomes negligible as well as for temperatures higher than 14°C (the time for a total crystallization was evaluated to be about a year).

Later, Gent et al.\textsuperscript{34} went deeper into the understanding of the CCr of NR taking into account the “non-rubber” constituents of the material. As discussed previously, NR is composed of high molar mass PI with 4 to 6% of “non-rubber” molecules such as proteins, lipids, sugars, minerals, etc. As the major part of these latter are proteins and lipids, Gent focused on their impact on the CCr of NR by removing selectively either the proteins of NR (DPNR) or its lipidic part (AE-NR). After acetone extraction, the rate of crystallization of NR decreases drastically. Indeed, when for Pale Creep NR or Smoked Sheet, the initial half-life of crystallization is 126 minutes and 144 minutes respectively, after acetone extraction the time is shifted to 410 minutes and 450 minutes respectively. When small amounts (0.1, 1 and 4 wt %) of stearic acid (one of the main fatty acid present in NR) were added to AE-NR, an increase of the rate of crystallization was observed. Stearic acid acted then as a nucleating agent.

CCr of IR was then compared with NR. Burfield and Tanaka\textsuperscript{35,36} showed that IR even with a high rate of 1,4-\textit{cis} units exhibits really poor crystallization when compared to DPNR (i.e. 10% of its crystallinity). Moreover, the rate of crystallization decreased with the reduction of the 1,4-\textit{cis} content. It might be noted that AE-DPNR still crystallizes quicker than the best IR studied. Like for NR, stearic acid can also work as a nucleating agent for IR, enhancing its speed of crystallization. In conclusion, both the non-isoprene components of NR and its pure microstructure are responsible for it superior properties. Many other studies were performed to better understand the CCr of NR. For example, Tanaka showed a great decrease in the speed of crystallization of the AE-NR compared to NR.
Nevertheless, after transesterification of the NR, the initial speed of crystallization was recovered (Figure 12a). When TE-DPNR and AE-DPNR were doped with 1% (w/w) of lipids (methyl linoleate (ML) and stearic acid (SA) were selected as they represent the main lipids present in NR) (Figure 12b and c), whatever the fatty acid added, the resulting materials exhibited a higher rate of crystallization, the highest one being obtained with AE-DPNR doped with free fatty chains. It was thus suggested that the higher rate of crystallization of NR comes from a synergetic effect between linked and free fatty chains. Nevertheless, this synergetic effect is visible only in the case of AE-DPNR doped with methyl linoleate but not really in the case of doping with stearic acid. Figure 12b shows an equal rate of crystallization of TE-DPNR and AE-DPNR doped with SA whereas Figure 12c shows that the maximum rate of crystallization is obtained for AE-DPNR doped with ML. It indicates that SA nucleating effect is powerful enough to counter-balance the loss of linked fatty chains as AE-NR and TE-NR exhibits the same rate of crystallization. (Figure 12b plot D).


Tanaka investigated also the doping of IR with several fatty acids or esters. A plasticizing effect of some unsaturated fatty acids and of their corresponding methyl ester was shown (Table 2). For instance, addition of 30 wt % of linoleic acid (LiA), methyl oleate or methyl linoleate decreases significantly the $T_g$ of IR (~ 30°C).
According to the authors, IR samples mixed with even a huge amount of unsaturated fatty acids formed transparent materials (films) contrarily to saturated lipids. This transparency of the final admixture is considered as a proof of solubility of the fatty acid in the polymer matrix while translucency is a proof of immiscibility for pure aliphatic fatty chains. This main difference of behavior could also be related to the nucleating effect of stearic acid and the apparent plasticizing effect of linoleic acid and methyl linoleate.

Table 2: Carbon Number and $T_m$ of Fatty Acids and Their Esters, and $T_g$ of cis-1,4 Polyisoprene (IR) Containing 30 wt % of Acid or Ester – Reproduced from Tanaka et al.\textsuperscript{38}

<table>
<thead>
<tr>
<th>Specimen</th>
<th>$C_n$</th>
<th>$T_m$/°C</th>
<th>$T_g$/°C of cis-1,4 Polyisoprene Containing 30 Wt % Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>14</td>
<td>52.0</td>
<td>-67.8</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>16</td>
<td>59.5</td>
<td>-65.0</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18</td>
<td>65.9</td>
<td>-62.6</td>
</tr>
<tr>
<td>Elaidic acid</td>
<td>18</td>
<td>38.4</td>
<td>-63.3</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18</td>
<td>-21.5</td>
<td>-65.5</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18</td>
<td>-9.4</td>
<td>-80.8</td>
</tr>
<tr>
<td>Methyl palmitate</td>
<td>16</td>
<td>26.0</td>
<td>-65.8</td>
</tr>
<tr>
<td>Methyl stearate</td>
<td>18</td>
<td>34.8</td>
<td>-63.8</td>
</tr>
<tr>
<td>Methyl Oleate</td>
<td>18</td>
<td>-26.5</td>
<td>-96.0</td>
</tr>
<tr>
<td>Methyl linolate</td>
<td>18</td>
<td>-43.9</td>
<td>-90.2</td>
</tr>
</tbody>
</table>

It was also attempted to mimic the CCr of NR by both chemical modification and doping of a high “1,4-cis” rate IR (~ 98\%\textsuperscript{39,40}). Using the existing pendant 3,4-units, Kawahara \textit{et al.} introduced hydroxyl functions onto the polymeric backbone to further graft various fatty acids to mimic the linked fatty acid of NR. The amount of fatty chains linked to IR was 0.53 wt % as determined by IR analysis, corresponding to an average of 4 fatty chains linked per chain. After mixing this hybrid polymer with 1 wt % of ML, the rate of CCr increased to a level still lower than the DPNR but much bigger than the initial IR (Figure 13). It was also shown that this behavior is dependent on the nature of the linked fatty acid (Figure 14).
To finish with, it was demonstrated that the crystallization of PI showed a non-usual behavior\textsuperscript{41,42}. Indeed, crystallized PI shows two different endothermal peaks during melting (Figure 15). Kim\textsuperscript{41} explained that both transitions correspond to different types of crystallites which are similar in structure (same X-Ray diffraction pattern) but may differ from their stability and rate of formation. In the literature, both endotherms are always referred to as $\alpha$ and $\beta$ transitions for high and low crystallization temperature respectively.

As shown in Figure 15, the $\beta$ transition is the slowest as it appears only after quite a long time of crystallization. The proportion between the two transitions also varies with the degree of crystallinity: the higher the overall crystallinity of the PI, the higher the proportion of $\beta$ transition. It was also demonstrated that the propagation axes of the crystals are different as well as their thickness\textsuperscript{42}. For IR, the proportion of the $\beta$ transition is bigger than in NR after full crystallization of the sample.
In conclusion, the origin of the fast and high CCr of NR has been widely studied for the past fiftieth years as it is one of the main properties that cannot be reproduced with high 1,4-cis rate IR. The higher speed of crystallization was reported to be obtained at -25°C reaching 75% of the final crystallization rate after 3 hours of isotherm. It was demonstrated that this phenomenon was most probably due to the presence of fatty acid in NR, both free and linked fatty chains playing a role. The nature of the fatty acids present in the material is also important as SA exhibits a nucleating capacity toward PI whereas ML and LiA are described as plasticizers of the polymer. Both effects were described as acting synergistically in the case of NR. Tanaka and Kawahara synthesized a model of NR by grafting different fatty acids on an IR. Nevertheless, the amount of 1,4-cis units is not 100% and the grafted lipids are connected onto the polymeric backbone but not at the chain-ends. This would change the weight proportion between the lipidic and the polymeric chains and give different properties. Indeed, this model does not exactly follow the behavior of NR as the CCr of IR linked to fatty chains is higher than the pure IR (the reverse for NR if comparing TE-NR with AE-NR). It may thus be possible to get closer to Tanaka’s model of NR in order to improve the understanding of the phenomenon.

**e. Strain-induced crystallization of NR and IR**

Strain-induced crystallization (SIC) is the second major difference of behavior between IR and NR. Industrially, it is a property of high interest as it corresponds to the response of the material under solicitation which has an impact on the applications for both IR and NR.
Even if SIC was first established in 1925\textsuperscript{43} the reason for the superiority of the NR toward its synthetic analogue is still under debate. Here, it is proposed a brief overview of both positions developed in the literature without claiming to be exhaustive.

Both IR and NR (vulcanized or not) exhibit SIC phenomenon (even if for IR it requires a high 1,4-\textit{cis} rate) and usually NR present higher mechanical properties. The higher gap in performances is visible in non-vulcanized state. Stress-strain plots for non-vulcanized IR and NR (Figure 16.a black and red respectively) and for vulcanized ones (Figure 16.b IR in black and NR in red) showed that even in the non-vulcanized state NR exhibits a cross-linked behavior with a decrease of stress during the decrease of strain. This behavior could explain the fast SIC of unvulcanized NR as the PI chains will be easier to align (under stress) thanks to the cross-linking. In comparison, the stress observed in the case of non-vulcanized IR rapidly collapses with the increase of the strain behaving like a linear polymer.

This particular behavior was correlated to the existence in the natural material of anchor points at both chain-ends (Tanaka’s model)\textsuperscript{44–47} thus creating a pseudo-network that could explain the crosslinked-like behavior of NR. It could also be a clue toward the understanding of the high tensile-strength of vulcanized NR compared to vulcanized IR\textsuperscript{48}. Nevertheless, this structural organization exhibited by NR does not give any information about the quicker SIC of NR compared to the IR in vulcanized state (Figure 17). Indeed, the SIC of vulcanized NR is still quicker that the one of the synthetic homologue and the overall rate of crystallization of NR is also higher than that of IR. It is also noteworthy that the gap between crystallization and melting of both materials is the same\textsuperscript{49} and values for NR are just shifted to lower values. On Figure 17b it can be seen that after total crystallization of vulcanized NR it remains crystalline at higher temperature than the synthetic sample.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure16}
\caption{Stress-strain plots for (a) non-vulcanized NR (red) and IR (black); (b) vulcanized NR (red) and IR (black)- Reproduced from Toki et al\textsuperscript{44}.}
\end{figure}
These properties are an example of the limitation of the “pseudo-network” theory as it can not be used to clarify the high rate of crystallinity neither the fast crystallization of vulcanized NR under low strain rate.

Figure 17: Evolution of the crystallization rate (CI) in function of strain (a) and in function of temperature (b) for both vulcanized IR (red) and NR (blue) - Reproduced from Candeau et al. 49

By analogy with CCr, it was suggested that slow SIC of IR could come from the absence of non-rubber constituents like fatty acids 50,51 that were demonstrated to be nucleating agent of PI in the case of CCr or from defects induced by lowest rate of 1,4-cis units in the synthetic PI 44,52. Nevertheless, use of stearic acid as nucleating agent for the SIC was demonstrated to be a weak hypothesis by Kohija et al. 50 as the addition of various amounts of SA did not accelerate the SIC in IR. The starting point of the SIC could be the alignment of the polymer-chains which crystallize and thus play themselves the role of nucleating agent. This would be more favorable to the stereoregularity theory as the better the microstructure control, the higher the crystallization. This was also confirmed by Toki et al. 52 by studying the SIC of NR and two different IRs bearing different microstructures. They demonstrated that indeed the higher the 1,4-cis content, the higher the SIC, but they could not relate their results to the high tensile-strength of NR, as sample with various 1,4-cis exhibited similar values.

To the best of our knowledge, to date, the origin of the superior mechanical properties of NR (vulcanized or not) compared to IR is still not perfectly clear. Both hypotheses (“non-rubber constituents” or “high rate of 1,4-cis”) allow to explain part of those properties but an overall explanation is still expected.
II. Synthesis of Polyisoprene

a. Introduction

Isoprene is a quite versatile monomer which can be polymerized by all the traditional technics (i.e. anionic, cationic, radical, metathesis, coordination-insertion). A good control of the microstructure is a crucial parameter as the proportion of each isomer (Figure 18) could highly influence the properties of the final polymer. In the frame of the work presented here, the highest rate of 1-4 cis units was necessary to get as close as possible to Tanaka’s model. Moreover, in order to selectively functionalize the polymer backbone in α or ω position, a good control of both chain-ends as well as different reactive functionalities at both chain-end was required. In this sub-chapter, all the different synthetic methods are compared in order to determine if any polymerization method could be used in our case.

![Figure 18: Different microstructures of polyisoprene.](image)

b. Cationic polymerization

Cationic polymerization of isoprene might be both the most promising way of synthesizing PI but also the most challenging one\(^5\). Indeed, as described previously the mechanism involved in the biosynthesis of NR is close to a cationic process\(^5\). In general, the cationic polymerization of isoprene follows the mechanism presented in Figure 19. The initiating step is the ionization of the initiator to form either a carbocation or a simple proton (in the case of water as initiating agent) which then reacts with a first isoprene molecule to form a tertiary carbocation. This carbocation can re-arrange to various mesomeric forms before propagation. This rearrangement is responsible for the formation of various configurations in the final polymer. The terminating group might be variable as many termination reactions can take place in cationic polymerization: the β-elimination of a proton affording a terminal diene (principal termination observed), transfer to water giving a hydroxyl-group at the end of the chain and termination by the counter-ion putting back the “X” function at the end of the chain.
Table 3 gives an overview of various conditions used to perform this polymerization highlighting the great number of studies since the 60s. As reported, even if results are highly dependent on the conditions, some general trends can be established:

- the microstructure of the cationic PIs is mainly 1,4-trans, contrarily to NR which bears only 1,4-cis units
- a high amount of double bond is lost, associated to a high $T_g$. This was described to be due to the formation of mono-, di- or tri- cycles resulting from side reactions, creating either pendant or internal aliphatic rings. This would increase the glass transition of the final polymers as well as the chain rigidity. Their exact structure remaining unclear.
- Cationic PIs are also characterized by a broad molar mass distribution mainly due to extensive chain-transfer reactions. Moreover, most of the time, PIs are partially cross-linked
More recent studies of Kostjuk and Peruch bring new interesting results. They worked on the cationic polymerization in emulsion and tried also to mimic the biosynthesis of NR using analogs of the natural monomer and initiator. The first publication about water-phase cationic polymerization of isoprene was reported in 2011 by Kostjuk et al. Their pioneering work was compared with a traditional approach using organic solvents. Table 4 reports the results obtained varying the solvent from dichloromethane to α,α,α-Trifluorotoluene (BTF). Whatever the solvent used, an important loss of double bond is still visible even if this can be limited by decreasing the concentration of the initiating system.

It is also noteworthy that the higher the conversion, the higher the loss of double bond and the distribution of molar masses. The microstructure is highly 1,4-trans as usually reported for cationic PIs.

Table 3: Different conditions used for cationic polymerization of isoprene - Reproduced from Ouardad et al.

<table>
<thead>
<tr>
<th>Catalytic systems</th>
<th>Solvents</th>
<th>( e )</th>
<th>( T(\degree C) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF(_3)</td>
<td>Pentane</td>
<td>1.84</td>
<td>30</td>
</tr>
<tr>
<td>BF(_3)Cl</td>
<td>Dichloromethane</td>
<td>8.93</td>
<td>0</td>
</tr>
<tr>
<td>BF(_3)H(_2)O</td>
<td>Hexane</td>
<td>1.88</td>
<td>5</td>
</tr>
<tr>
<td>SnCl(_4)</td>
<td>Chloroform</td>
<td>4.81</td>
<td>-45 to 30</td>
</tr>
<tr>
<td>AlCl(_3)</td>
<td>Ethyl bromide, heptane, benzene</td>
<td>9.5; 9.2; 2.27; -78 to 80</td>
<td></td>
</tr>
<tr>
<td>AlCl(_3)</td>
<td>Heptane, benzene, nitrobenzene</td>
<td>1.92; 2.77; 1.56; -20 to 70</td>
<td></td>
</tr>
<tr>
<td>AlCl(_3)/TiCl(_4)</td>
<td>Heptane, benzene</td>
<td>1.92; 2.27; -78 to 80</td>
<td></td>
</tr>
<tr>
<td>MgBr/TiCl(_4)</td>
<td>Benzene</td>
<td>2.27</td>
<td>20</td>
</tr>
<tr>
<td>TiCl(_4)</td>
<td>Heptane</td>
<td>1.92</td>
<td>20</td>
</tr>
<tr>
<td>C(_6)H(_5)SbCl(_5)</td>
<td>Nitrobenzene</td>
<td>1.56</td>
<td>20</td>
</tr>
<tr>
<td>Ph(_3)C(_6)H(_5)Cl</td>
<td>Nitrobenzene</td>
<td>1.56</td>
<td>20</td>
</tr>
<tr>
<td>H(_2)SO(_4)</td>
<td>Dichloromethane</td>
<td>8.93</td>
<td>25</td>
</tr>
<tr>
<td>BuCl/TiCl(_4)</td>
<td>Dichloromethane</td>
<td>8.93</td>
<td>25</td>
</tr>
<tr>
<td>CCl(_3)/COOH/TiCl(_4)</td>
<td>Dichloromethane, benzene</td>
<td>8.93; 2.27</td>
<td>25</td>
</tr>
<tr>
<td>CumyUMe/TiCl(_4)</td>
<td>Methyl chloride/n-hexane</td>
<td>9.11; 1.88</td>
<td>40</td>
</tr>
<tr>
<td>DMAX/Lewis acid</td>
<td>Dichloromethane, cyclohexane</td>
<td>8.93; 2.02</td>
<td>-60 to 20</td>
</tr>
<tr>
<td>MeOPlEtOH/B(C(_6)F(_5))(_3)</td>
<td>Dichloromethane, water</td>
<td>8.93; 8.01</td>
<td>-30 to 20</td>
</tr>
</tbody>
</table>

More recent studies of Kostjuk and Peruch bring new interesting results. They worked on the cationic polymerization in emulsion and tried also to mimic the biosynthesis of NR using analogs of the natural monomer and initiator. The first publication about water-phase cationic polymerization of isoprene was reported in 2011 by Kostjuk et al. Their pioneering work was compared with a traditional approach using organic solvents. Table 4 reports the results obtained varying the solvent from dichloromethane to α,α,α-Trifluorotoluene (BTF). Whatever the solvent used, an important loss of double bond is still visible even if this can be limited by decreasing the concentration of the initiating system.

It is also noteworthy that the higher the conversion, the higher the loss of double bond and the distribution of molar masses. The microstructure is highly 1,4-trans as usually reported for cationic PIs.

Table 4: Results on the cationic polymerization of isoprene using the 1-(4-methoxyphenyl)ethanol/B(C\(_6\)F\(_5\))\(_3\) initiating system in two different solvents - Reproduced from Kostjuk et al.

<table>
<thead>
<tr>
<th>Run</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Conv (%)</th>
<th>( M_n ) (g.mol(^{-1}))</th>
<th>D</th>
<th>Unsaturation (^b) (%)</th>
<th>( \text{trans-} ) (^c) ( 1,4 ) (%)</th>
<th>( T_g ) (^d) (\degree C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH(_2)Cl(_2)</td>
<td>360</td>
<td>30</td>
<td>4910</td>
<td>2.9</td>
<td>72</td>
<td>94.0</td>
<td>-33.6</td>
</tr>
<tr>
<td>2</td>
<td>BTF</td>
<td>2</td>
<td>82</td>
<td>5580</td>
<td>3.9</td>
<td>63</td>
<td>93.4</td>
<td>-</td>
</tr>
<tr>
<td>3(^e)</td>
<td>BTF</td>
<td>120</td>
<td>21</td>
<td>3460</td>
<td>1.9</td>
<td>83</td>
<td>92.9</td>
<td>-25.8</td>
</tr>
<tr>
<td>4(^f)</td>
<td>BTF</td>
<td>360</td>
<td>26</td>
<td>2660</td>
<td>1.4</td>
<td>88</td>
<td>92.9</td>
<td>-32.4</td>
</tr>
</tbody>
</table>

\(^a\) Polymerization conditions : [B(C\(_6\)F\(_5\))\(_3\)] = 0.023 M ; [IP] = 1.67 M ; initiator : [1-(4-methoxyphenyl)ethanol] = 0.011 M; solvent (BTF or CH\(_2\)Cl\(_2\)) 5mL; temperature = -30\degree C.; \(^b\) Determined by \(^1\)H NMR: 100% corresponds to linear polyisoprene with one unsaturation per isoprene unit. \(^c\) Determined by \(^1\)H NMR and \(^13\)C NMR spectroscopy. \(^d\) Measured by DSC. \(^e\) [B(C\(_6\)F\(_5\))\(_3\)] = 0.01 M. \(^f\) [initiator] = 0.023 M.
Table 5 gives the results obtained for isoprene polymerization in aqueous media. Three conditions were tested: suspension, dispersion and emulsion. The water polymerization allows pretty high rates of double bonds after the reaction (~98 %) and quite narrow molar mass distribution. This improvement means that no side reaction occurs. As most of the potential side reactions come from β-elimination of proton it seems that even if a proton is released its affinity with water will “wash it” away from the polymer backbone and thus protect the polymer.

Table 5: Results from the cationic polymerization of isoprene using the 1-(4-methoxyphenyl)ethanol/B(C₆F₅)₃ initiating system in aqueous media - Reproduced from Kostjuk et al.⁶⁵.

<table>
<thead>
<tr>
<th>Run</th>
<th>Process</th>
<th>Time (h)</th>
<th>Conv (%)</th>
<th>Mₙ (g.mol⁻¹)</th>
<th>D</th>
<th>Unsaturation b (%)</th>
<th>trans-1,4 c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Suspension</td>
<td>138</td>
<td>51</td>
<td>1040</td>
<td>1.7</td>
<td>97</td>
<td>96.4</td>
</tr>
<tr>
<td>2</td>
<td>Dispersion</td>
<td>142</td>
<td>39</td>
<td>900</td>
<td>1.4</td>
<td>99</td>
<td>96.2</td>
</tr>
<tr>
<td>3</td>
<td>Emulsion</td>
<td>141</td>
<td>30</td>
<td>680</td>
<td>1.5</td>
<td>97</td>
<td>96.7</td>
</tr>
</tbody>
</table>

Polymerization conditions: [IP] = 1.72 M; [B(C₆F₅)₃] = 4.7x10⁻² M; [1-(4-methoxyphenyl)ethanol] = 1.86x10⁻¹ M; temperature = 20°C; a Determined by ¹H NMR: 100% corresponds to linear polyisoprene with one unsaturation per isoprene unit. b Determined by ¹H NMR and ¹³C NMR spectroscopy.

Molar masses are nevertheless quite low with this process (< 1 200 g/mol) and did not change much even with addition of monomer. Figure 20 describes briefly the polymerizing process in the case of polymerization in suspension but can be generalized to dispersion and emulsion just by addition of organic solvent or surfactant (respectively) into the “PI/isoprene droplet” presented in the scheme. The polymerization proceed at the interface with a lewis acid (LA) stable in water and remaining active in aqueous media. As the monomer and initiator are mostly hydrophobic, it is assumed that initiation starts at the interface where the chains continue to grow. The authors assumed that the flattening of the molar masses is due to a “DP effect” at which the polymer chain becomes too hydrophobic, loses its charge and goes deeper into the organic droplet.
Couple of years later, Kostjuk et al.\textsuperscript{58} went further in the water polymerization of isoprene using a special family of LA : Lewis acid surfactant combined catalysts (LASCs). They used a complex of ytterbium with sodium dodecyl benzene sulfonate that exhibited both surfactant properties and the capacity to carry the catalyst into the organic phase (i.e. PI / isoprene droplet from Figure 20) allowing to pursue the polymerization without any “DP effect”. In this case, protons coming from the interaction of pentachlorophenol with the LA initiate the polymerization. Table 6 summarizes the results obtained for various conditions. The molar masses obtained are high (more than 100 kg/mol for polystyrene/isoprene copolymer) without increasing too much the molar mass distribution.

For polyisoprene samples, high molar masses could also be reached with reasonable dispersities. Interestingly, the glass transition temperature are quite close from the one of NR attesting of a low level of cyclization and double bond loss ( > 94%). Finally, even 1,4-\textit{cis} units were obtained by this method ( around 20% ) opening the way to “artificial NR”.

![Figure 20: General scheme of cationic polymerization of isoprene in aqueous medium](image_url)

- Suspension process
Table 6: Emulsion cationic polymerization of styrene and isoprene catalyzed by water-dispersable LASCs

<table>
<thead>
<tr>
<th>Run</th>
<th>Monomer</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Conv. (%)</th>
<th>Mₙ (kg/mol)</th>
<th>Mₙ/Mₘ</th>
<th>Styrene/isoprene (%)</th>
<th>Tg (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Isoprene</td>
<td>40</td>
<td>13</td>
<td>89</td>
<td>97.0</td>
<td>3.8</td>
<td>n.a.</td>
<td>-58</td>
</tr>
<tr>
<td>11ᵇ</td>
<td>Isoprene</td>
<td>40</td>
<td>24</td>
<td>92</td>
<td>60.8</td>
<td>2.7</td>
<td>n.a.</td>
<td>-57</td>
</tr>
<tr>
<td>12</td>
<td>Styrene / Isoprene</td>
<td>40</td>
<td>15</td>
<td>100</td>
<td>124.9</td>
<td>4.9</td>
<td>47:53</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>Styrene / Isoprene</td>
<td>40</td>
<td>15</td>
<td>64</td>
<td>81.7</td>
<td>3.9</td>
<td>26:74</td>
<td>-16</td>
</tr>
<tr>
<td>14</td>
<td>Styrene / Isoprene</td>
<td>40</td>
<td>15</td>
<td>89</td>
<td>152.2</td>
<td>3.2</td>
<td>73:27</td>
<td>0, 64</td>
</tr>
</tbody>
</table>

ᵃ: Polymerization conditions: H₂O (3.5g); monomer (1.5mL); YbCl₃ x 6 H₂O (0.21g); DBSNa (0.78g); b: C₅Cl₅OH (0.14g) as initiator, c: Determined by ¹H NMR spectroscopy, n.a.: not applicable.

The second pathway mainly developed in our group for the cationic polymerization of isoprene is based on the assumption that the biosynthesis of NR is “pseudo-cationic”⁵⁴. The key steps in this process are both the abstraction of the pyrophosphate group to form a carbocation and the β-elimination of the proton which is highly selective in the case of the enzyme and gives either 1,4-cis or 1,4-trans units. As the abstraction of the pyrophosphate group is assumed to be carried out by a cationic metal present in the enzyme (Mg²⁺, Mn²⁺) an analogy could be easily made with the LA acid system developed in traditional cationic polymerization. Peruch et al. focused their approach on the use of DMAPP and IPP derivatives for the initiating system and the monomer units respectively. As pyrophosphate monomers are difficult to obtain and are rather unstable toward water, halogenated derivatives as well as hydroxylated ones were selected. In a first attempt⁶², DMAOH and IPOH were used with a boron LA as the catalyst. It was demonstrated that the LA could abstract the hydroxyl group to form a carbocation that could perform the polymerization of IPOH, but the final polymer is not a polyisoprene but a polyol as presented in Figure 22. The present mechanism differs from the biosynthesis as, before the β-elimination, a molecule of IPOH is added to the carbocation.

Figure 21: Chemical structure of DMAOH and IPOH
The DMAOH/LA system was used directly for isoprene polymerization\textsuperscript{63}. In this case, polyisoprene oligomers were obtained with similar properties as traditional ones obtained by cationic polymerization (i.e. 1,4-\textit{trans} microstructure, low molar masses, high $T_g$ and double bond loss). Other analogs of DMAPP (Figure 23) were used as initiators of the polymerization of isoprene. The nature of the function has a strong influence on the reaction: increase of the kinetics with halogenated compounds but also increase of the insoluble part and the molar mass distribution. Whatever the initiator used, the main characteristics of cationic PIs remained.

As most of the transfer reactions come from the $\beta$-elimination of a proton, a base (ditertiobutylpyridine) was added to the medium in order to trap all the released protons. As a consequence, molar mass distribution was drastically decreased ($< 2$ with the presence of the base and $> 4$ without), double bond loss was divided by a factor of two and $T_g$ that was around 30°C without the base shifted around -50°C. Nevertheless, the microstructure was still predominantly 1,4-\textit{trans} and the obtained molar masses were quite low ($< 3\ 000\ g/mol$).

In conclusion, cationic polymerization of isoprene is a tricky technique to obtain 1,4-\textit{cis} polyisoprene. In term of microstructure 1,4-\textit{trans} polyisoprene is the main architecture that can be obtained.
Talking about synthetic methods, the polymerization in emulsion seems to be the most promising way to achieve well defined architectures (i.e. no loss of double bond, quite high molar masses and average molar mass distribution) even if the maximum rate of 1,4-cis units is for the moment quite low. Thus, working in “water conditions” could be seen as a real performance as it remains close to the biosynthesis process of NR and uses “greener” conditions for the polymerization than the traditional methods but it does not suit for our project.

c. Anionic polymerization

The anionic polymerization of isoprene is certainly one of the oldest methods to obtain well defined and controlled materials. Nowadays, it is the principal pathway to industrially produce polydienes (i.e. polyisoprene or polybutadiene) and more generally all kind of rubber materials. Figure 24 gives the general procedure of the anionic polymerization of isoprene. An alkali organo-metallic compound (Li, K, Na) is used as the initiator, the carbanion will add onto isoprene and thus perform the propagation to form the final polymer. One of the main advantage of anionic polymerization is its livingness allowing to synthesize block-copolymers by sequential addition of a second monomer as in the case of SBS (styrene-butadiene-styrene) tri-block co-polymer for exemple.

Concerning the microstructure obtained by anionic polymerization, it was early described\textsuperscript{66} that among all alkali metals only lithium was able to produce a high 1,4-cis rate of isoprene units. For example, in bulk conditions, using lithium dispersed in isoprene, IR of about 94\% 1,4-cis was obtained. Table 7 summarizes the microstructures reported in the literature for a wide range of alkali metals and illustrates the unique capacity of lithium. But, the microstructure is not only highly dependent on the counter-ion but also on the monomer concentration and the solvent\textsuperscript{67}.
The best 1,4-cis rate (>95%\textsuperscript{68–70}) can be achieved in non polar and non-protic solvent (i.e. alkanes or cyclo-alkanes) using lithium metal or an organo-metallic derivatives (the best one being sec-butyl lithium), at high monomer concentration and low concentration of active species. The range of temperature is relatively adaptable as only few variation of the 1,4-cis content is observed while performing the reaction between -25 and 40°C\textsuperscript{71}.

Table 7: Microstructure of alkali metal-catalyzed polyisoprenes - Reproduced from Foster et al.\textsuperscript{72}

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>% 1,4 cis</th>
<th>% 1,4 trans</th>
<th>% 3,4</th>
<th>% 1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium</td>
<td>94</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sodium</td>
<td>0</td>
<td>43</td>
<td>51</td>
<td>6</td>
</tr>
<tr>
<td>Potassium</td>
<td>0</td>
<td>52</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>Rubidium</td>
<td>5</td>
<td>47</td>
<td>39</td>
<td>8</td>
</tr>
<tr>
<td>Cesium</td>
<td>4</td>
<td>51</td>
<td>37</td>
<td>8</td>
</tr>
</tbody>
</table>

Recently, Carlotti \textit{et al.}\textsuperscript{73–75} developed what is called the “retarded anionic polymerization” using trialkylaluminium derivatives and alkali metal hydrides to slow down the reaction process allowing to perform anionic polymerization at higher temperature without losing any control of the polymerization and, moreover, decreasing the cost of the process. It is important to note that in this approach the microstructure of the polydiene could be varied with a maximal 1,4 units (cis and trans) of about 80%. The stereoregularity can be tuned by the addition of alkoxyalkyl salts.

Anionic polymerization of isoprene is thus a powerful route to obtain high 1,4-cis polyisoprene with good control of the molar mass distribution. Nevertheless, this method can be tedious as it is highly dependent on the nature of the solvent, alkali metal and concentration. This approach constituted one of the most suitable pathway for our project as a high 1,4-cis rate could be obtained as well as a good control of the chain-ends.

d. Radical polymerization

A lot of efforts were also put on the development of radical polymerization of isoprene, more particularly toward controlled radical polymerization (CRP) techniques (Figure 25), like nitroxide mediated polymerization (NMP)\textsuperscript{76–81}, reversible addition-fragmentation polymerization (RAFT)\textsuperscript{82–86} and atom transfer radical polymerization (ATRP)\textsuperscript{87,88}. Generally, isoprene seems to be reluctant to polymerize by radical pathway as in many cases high temperature, high monomer concentration and long time of reaction were required but full conversion was never reached. Selected examples for each technic will be presented below.
i. **NMP polymerization**

NMP polymerization is the first CRP technic to have been investigated for isoprene. Literature clearly indicates the high fluctuation of the results obtained with the change of reaction conditions (nature of nitroxide, temperature, concentration and solvent). A conversion of 75% can be obtained after 36 h at 120°C using (2,2,6,6-Tetramethylpiperidin-1-yl)oxy (TEMPO). Well defined PI (10 000 g/mol) with narrow dispersity (1.07) were obtained by Benoit *et al.*

Regarding the microstructure, no precise details were given by the authors but they report a microstructure predominantly 1,4 (*cis* and *trans*) and comparable to the microstructure obtained in free radical polymerization which is expectable as far as, after the “un-capping” in CRP (Figure 25), the reaction processes as a free radical polymerization without any control of the stereo regularity of addition. Cross-linking and high molar mass fraction appeared when conversion was higher than 80% due to transfer to the polymer. Finally, the use of pyridine enhanced the polymerization rate (50% conversion in 16h) without any loss of the control. This effect was attributed to the stabilizing effect of the pyridine toward the radical of the nitroxide. To date, NMP polymerization might be a simple method to obtain PI by CRP. The only issue is the difficulty of synthesis of the nitroxide.
ii. RAFT polymerization

Jitchum et al.\textsuperscript{84} as well as Germack et al.\textsuperscript{83} reported the RAFT polymerization of isoprene in bulk. RAFT polymerization of isoprene needs high temperature (more than 110°C) and a long time of reaction (> 20h) to obtain reasonable conversions (> 30 %) with low dispersity (< 1.4). If temperature was raised higher than 130°C a total loss of control was observed leading to an increase of the molar mass distribution, side reactions and even degradation of the CTA in some cases. Again, like for NMP, when conversion is higher than 80%, a high molar mass fraction was observed certainly due to chain coupling or cross-linking. This was even more visible with the appearance of an insoluble fraction when conversion reached 95%. The microstructure observed for the PI formed was mainly composed of 1,4 units (75 %) without any distinction between \textit{cis} or \textit{trans} additionally to 5% of 1,2 units and 20% 3,4 units. Bar-Nes \textit{et al.}\textsuperscript{85} reported the synthesis of block-copolymers in emulsion involving isoprene. They copolymerized isoprene with both styrene and acrylic acid to obtain self-assembly properties. They reported that the isoprene polymerization was once again very slow and tedious to control. Nevertheless, the emulsion polymerization of isoprene was faster than the one in solution (50% of conversion after 10h). This was attributed to the presence of residual styrene moieties in the media that helps the transfer of radicals during the reaction and also because polymerizations are usually faster in emulsion (compared to solution) due to compartmentalization effect\textsuperscript{89}.

iii. ATRP polymerization

ATRP for isoprene was hardly described. Wootthikanokkhan \textit{et al.}\textsuperscript{87} reported the impossibility to obtain a PI in bulk by this method. They attributed this to the very poor solubility of the copper derivative (CuBr) in isoprene. Even with increased amount of copper, they only obtained 5% of conversion after 24h. Addition of solvent increased the homogeneity of the reaction medium but a decrease of the polymerization rate was observed. Recently Zhu \textit{et al.}\textsuperscript{88} reported a first example of polymerization of isoprene carried out with the ATRP method using a usually poorly reactive system (Copper (I) bromide/2-2’-bipyridine as a catalyst and 2-bromopropionate as initiator). Maintaining the system at high temperature for a long time (150°C for 72h) and using THF as a solvent for a better homogeneity, a high conversion of 71% was observed. The PI formed had a microstructure of 89% 1,4 units (64% \textit{trans}) with a molar mass up to 12 kg/mol and a dispersity of 1.6.
In conclusion, radical polymerization of isoprene is suitable for the synthesis of block copolymers due to its living nature but does not provide high amount of 1,4-cis units. Whatever the method of polymerization, it looks like a good range of molar masses can be achieved with relatively narrow molar mass distribution but no control on the microstructure.

e. Ring opening metathesis polymerization (ROMP)

To the best of our knowledge, there is only one article dealing with the synthesis of telechelic polyisoprene by ROMP of 1,5-dimethyl-1,5-cyclo-octadiene (DMCOD)\(^{90}\). Controlled homotelechelic polyisoprene (Figure 26) could be obtained in a wide range of molar masses (1500 – 25 000 g/mol ) with low molar mass distribution (1.22 – 1.63). A huge screening was made among the existing metathesis catalysts to find an efficient one, as DMCOD is known to be poorly reactive as it is a di-substituted cycle with a low cycle tension. The catalyst represented in Figure 26 is the one giving the best conversion ( ~ 99% ). No description of the final microstructure of the polymer was done. It is also important to highlight that the final PI (acetylated of hydroxylated) is homotelechelic which can be a limitation for specific substitution in α or ω position as both chain-end have the same reactivity.

![Figure 26: ROMP pathway to afford hydroxyltelechelic polyisoprene - Reproduced from Grubbs et al.\(^{90}\)](image)

In conclusion, ROMP of DMCOD is a suitable way to obtain homotelechelic PI bearing reactive functions (i.e. hydroxyl in this case) which can be highly valuable for the synthesis of ABA co-polymers. This technique presents several drawbacks : highly dry conditions have to be used as most of the time Grubb’s catalysts are highly sensitive to moisture and all the CTA can not be used as the catalytic system can interact with various functions (in the case presented here, it was not possible to work with a CTA bearing hydroxyl functions directly).
Coordination polymerization of isoprene is the most studied technic regarding the number of publications and patents. The discovery of Ziegler-Natta catalysis in the early 1950’s for the polymerization of olefins was quickly applied to diene monomers such as isoprene or butadiene. In 1979 Schoenberg et al. published a review on various methods for obtaining IR and, at that time, they already reported 1,4-cis content up to 97% as showed in Table 8. But these results were overtaken with the emergence of the neodymium based catalysts which replaced titanium in the Ziegler-Natta process. It became thus possible to obtain 99.7% 1,4-cis PI using specific catalyst that will be presented later in this sub-chapter. Most of the results presented here comes from a book published in 2006 by Friebe et al. retracing the development of this technology.

### Table 8: Polymerization of Isoprene with (AlHNR)ₙ and TiCl₄ - Reproduced from Schoenberg et al.⁹¹

<table>
<thead>
<tr>
<th>Cocatalyst</th>
<th>Al / Ti mole ratio</th>
<th>Cis-1,4 by IR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AlHN-C₂H₅)ₙ</td>
<td>1.4</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td>1.55</td>
<td>95.0</td>
</tr>
<tr>
<td>(AlHN-iC₃H₇)ₙ</td>
<td>1.1</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>97.0</td>
</tr>
<tr>
<td>(AlHN-nC₄H₉)ₙ</td>
<td>1.5</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>97.0</td>
</tr>
</tbody>
</table>

Basically neodymium (Nd) systems are considered as Ziegler-Natta catalysts and proceed the same way as the traditional “Aluminium – Titanium” mechanism (Figure 27). In the literature, various Nd complexes have been designed playing on the “X” group and on the addition of ligands to improve several parameters (solubility, selectivity, etc.).
Four groups of Nd catalysts emerged:

- NdX₃ : with X an halogenous atom. This family was the first one reported in the literature.
- Nd(OR) : OR being an alcoholate (Figure 28a)
- NdO or Nd⁴O : were O suits for carboxylate groups (usually aliphatic chains). (Figure 28b) and “O” suits for isoctanoate.
- NdP : were P corresponds to phosphate or phosphonate groups (Figure 28c).

It appears that many parameters can influence the microstructure of the polymer like the temperature, the molar ratio of Nd toward co-catalyst, the solubility of the catalyst, the nature of the co-catalyst (aluminium or magnesium for example), the ratio monomer/co-catalyst, the solvent, etc..
The following trends can be drawn from all the published studies\textsuperscript{92}:

- The solubility of the catalyst can be improved by the nature of “X” groups attached to the Nd as previously mentioned. As the first generation of catalysts (NdX\textsubscript{3}) were acting heterogeneously in aliphatic solvents, the use of fatty chains as in the case of Nd(OR), Nd(O) and NdP helped the homogeneity of the system and thus the efficiency of the catalytic system. The use of ligands such as alcohols, phosphates, sulfoxide, boron derivatives or pyridine on NdX\textsubscript{3} was also demonstrated to improve the solubility.

- Co-catalysts used are generally alkyl aluminium derivatives like TIBA (Tri-isobutyl aluminium), DIBAH (Di-isobutyl aluminium hydride) or DEAH (Diethyl aluminium hydride) giving high rates of 1,4-cis units (> 98\%). On the contrary, alkyl magnesium derivatives give high 1,4-trans rate.

- The molar mass and the dispersity of the polymer as well as the microstructure depend on the molar ratio of co-catalyst and Nd. In general, the rate of 1,4-cis and the molar mass drop when this ratio is increased contrarily to the dispersity. For all “generations” of Nd, it has to be mentioned that an excess of aluminium derivative is used (from 1-5 eq with NdP system to 8-100 eq in the case of NdO).

- The solvents used for the polymerization are alcanes or cyclo-alcanes in order to avoid poisoning of the catalyst or side reaction.

- Increase of the temperature can potentially damage the catalytic system (~ above 60°C) or decrease the molar masses.

- This technique proceeds in a “quasi-living” manner. The formation of di-block copolymer is limited to a diene/diene polymer and even the polymerization of styrene can be tricky. Control of chain-ends was not well described.

In 2002, Laubry \textit{et al.}\textsuperscript{93–95} from Michelin company patented a method to obtain a “quasi-pure” 1,4-cis PI (99.7% reported) in large scale using relatively smooth conditions (room temperature, small amount of aluminum, bulk conditions). Moreover, they managed to perform the polymerization directly from the C5 fraction of petroleum cracking without any further purification of isoprene. To date, this is one of the highest 1,4-cis reported in the literature even if some other examples attended the same rate like the work of Zhang\textsuperscript{96} who used the same type of catalysis but varying the metal (Lutetium and Yterbium) and the ligands (diphenylphosphine derivatives).
To conclude, coordination polymerization is the most interesting system to obtain high 1,4-cis rate in IRs, even industrially. There are only few drawbacks as it is a well understood system with clearly identified key parameters. This polymerization process can even be performed on C5 crack fraction of petroleum and provides a wide range of molar masses in a controlled manner. The main disadvantage is the control of chain-ends which is generally not particularly discussed in the literature. This is an important issue for the selective functionalization aimed in the project.

**g. General conclusion on polyisoprene synthesis**

Isoprene polymerization is a vast domain of investigation regarding the different technics applicable and the variability of the IR formed in term of microstructure, molar mass, dispersity, etc. As it was explained in the introduction of this sub-chapter our goal was not to be exhaustive and to present all the different systems precisely, but to give an overview of what can be achieved to obtain a high 1,4-cis rate in IR and controlled chain-ends to be employed for the synthesis of Tanaka’s co-polymer. Table 9 summarizes the different advantages and drawbacks of each synthetic method taking into account our specifications. As we are targeting a high rate of 1,4-cis units, cationic polymerization as well as CRP and metathesis can not be taken into account. For the remaining systems (i.e. anionic and coordination), both present determinant disadvantages such as poor control of the chain-ends in the case of coordination polymerization and really sensitive conditions for anionic polymerization (microstructure dependent on many parameters). For all those reasons, in the frame of this work, chemical degradation of NR was chosen in order to obtain hetero-telechelic 100% 1,4-cis PI. This method will be presented in the next chapter.

<table>
<thead>
<tr>
<th>Synthetic method</th>
<th>1,4-cis rate</th>
<th>Control of the chain-ends</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic</td>
<td>(-) (-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Anionic</td>
<td>(+) (+) (+)</td>
<td>(+) (+)</td>
</tr>
<tr>
<td>NMP</td>
<td>(-) (-)</td>
<td>(+) (+)</td>
</tr>
<tr>
<td>RAFT</td>
<td>(-) (-)</td>
<td>(+) (+) (+)</td>
</tr>
<tr>
<td>ATRP</td>
<td>(-) (-)</td>
<td>(+) (+)</td>
</tr>
<tr>
<td>Metathesis</td>
<td>(-) (-)</td>
<td>(+) (+) (+)</td>
</tr>
<tr>
<td>Coordination</td>
<td>(+) (+) (+)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(+) : the system exhibits this property / (-) : The system does not exhibit this property
III. Polymer coupling: Grafting lipids and proteins

For the synthesis of Tanaka’s model molecule, it was important to know what had already been described for the coupling of polymers with both lipids and proteins in order to define a pathway applicable for our project. The Polymer-Lipid coupling was not highly studied in the literature. On the contrary, polymer-protein coupling has been extensively investigated. In this sub-chapter, we aim to present an overview of the methods described to perform the coupling reactions.

a. Polymer – Lipid coupling

In 1984, Reusch\textsuperscript{97} introduces the term of “lipopolymer” into the scientific community as referring to “molecules that contain a polymer chain that is bound covalently to a lipid moiety”. Some works focused then on the study of such architectures, but working with hydrophilic polymers such as PEG\textsuperscript{98–101} or polyethyleneimine\textsuperscript{102,103}. These amphiphilic systems were mostly studied as drug carriers for targeted drug delivery. Recently, other studies reported the functionalization of a phospholipid moiety in order to obtain a RAFT\textsuperscript{104,105} agent suitable for the growing of a polymer chain. In this case, the studied monomers were morpholin derivatives used as the hydrophilic blocks.

No particular interest was paid, to the best of our knowledge, to the coupling of lipids with a hydrophobic polymer. The only example is the one already described in the “CCr subchapter”, where Kawahara et al.\textsuperscript{39,106} linked fatty chains of various chain-length to a backbone of PI through hydroboration reaction. Such a low amount of documents on the hydrophobic-hydrophobic coupling is surprising but also challenging as the impact of the fatty chains on hydrophobic polymer backbones seems to be out of knowledge.

b. Polymer – Protein coupling

Polymer-Protein materials are commonly referred to as Bioconjugates or Macromolecular Chimeras. This last term was introduced in 2003 by Antonietti\textsuperscript{107} to define this kind of complex architecture even if the oldest papers dealing with protein-polymer coupling were from 1977\textsuperscript{108,109}. Since this date, the main application of Bioconjugates was the biomedical field focusing on the self-assembly properties of those chimeras and their capacity of being used for drug delivery.
Even if it is a field of high interest, this is out of purpose for our project and that is why we decided to focus more on the grafting methods existing than to the properties of these systems especially regarding the fact that most of the bioconjugates were synthesized starting from hydrophilic polymers contrarily to polyisoprene. The following sections will thus present the two pathways that can be used for coupling.

i. Grafting to

The first synthetic approach reported in the literature is the “grafting to” method which consists in connecting directly both blocks (polymer and protein) using specific chemistry (Figure 29). This method is quite versatile regarding the amount of accessible reactive functions in the peptide backbone\textsuperscript{110} like primary amines, carboxylic acids, thiols, etc.. Moreover, the progress in polymer chemistry usually allow to have a great control of the chain-end, which can be chosen to be reactive toward one of the function present on the protein backbone\textsuperscript{111–113}. One of the main drawback of this strategy is the selectivity of the reaction as one peptide can be present more than once on the backbone of the protein, thus leading to multiple grafting of polymer onto the polypeptide backbone. To prevent this kind of side reaction, modification of the protein was proposed by introducing a controlled amount of a specific function that was absent in the native protein. For example the high selectivity demonstrated by Huisgen cyclo-addition can be used in bioconjugate synthesis by either introducing the azide function\textsuperscript{114} or the alkyne function\textsuperscript{115} on the protein.

Another strategy widely used is the thiol chemistry using cysteine as the amount of free thiol in proteins can be controlled by the reduction of disulfide bridges for example\textsuperscript{115}. In this case, another type of “click-chemistry” called “Thiol-Maleimide” chemistry\textsuperscript{116–118}, a metal-free reaction, extracted from the family of Michael additions\textsuperscript{115,119,120} can be used.
Nevertheless, the main drawback of this strategy, in addition to the selectivity, is the difficulty to graft two polymers together (and especially in a one-to-one ratio) as the chance of encountering of the reactive functions is limited by other parameters such as the molar mass of both blocks, the solubility of both polymers, etc.. For these reasons, many authors preferred to grow one of the two blocks starting from the other.

**ii. Grafting from**

Figure 30 represents a general scheme of the grafting from pathway. Here, two examples are given: the growth of a polypeptide from a polymer through ring-opening polymerization (ROP) of N-carboxyanhydride (NCA) monomers and the growth of a polymer from a protein. In this last case, CRPs were selected among all the other existing methods as it can generally bear aqueous conditions (the best/only solvent of most of the proteins) and as less side reactions due to the peptide pendant functions were observed. In 2014, Cobo et al.\textsuperscript{121} published a review retracing various polymerization pathways. The polyacrylamide family was highly studied thanks to its LCST behavior, the polymeric chain can grow in aqueous media and the self-assembly can be induced by temperature\textsuperscript{112,122–124}. Moreover, N-isopropylacrylamide (NIPAM) is a versatile monomer as it can be polymerized by most of the known CRP pathways.

Even if the « grafting from » approach is a powerful tool already widely studied and reported, it is not applicable in the frame of the project presented here as it is impossible to grow a PI chain fully 1,4-\textit{cis} via CRP as seen before.

On the contrary, the work of Deming on the synthesis of « protein like » polymers from the ring opening polymerization (ROP) of NCA was more attractive for our project. Generally, this polymerization can proceed through two different mechanisms depending on the conditions used\textsuperscript{125} (Figure 31).
Through the activated monomer mechanism, high molar masses can be obtained, but without controlling the polymerization. The balance between basicity and nucleophilicity of the initiator is a key factor. For this reason, the amine mechanism using primary amines is preferred as they are more nucleophilic than basic. But, using primary amine is usually not enough to completely prevent side reactions. The decrease of temperature or the nature of the solvent were also important parameters for the control of the reaction.

The polymer thus obtained is a polypeptide (PPep) possessing a helicoidal morphology as can be encountered in proteins. Furthermore, the « R » group of the monomer can be varied and copolymerization reactions could lead to model proteins. This implies that the polymerization must be controlled. One possibility is to initiate the polymerization with primary amines and to perform the ROP at 0°C in DMF. 99% of the chain-ends were still “living” at the end of the polymerization process in this case. Deming et al. developed the use of Cobalt and Nickel catalysts to initiate the polymerization through a different pathway, affording well defined polymers that could be composed of a broad range of monomers bearing different functions. Other methods like the use of aminosilanes, protonated amines or even the alkylation of the NCA monomer thus blocking the activated monomer mechanism were also reported, all leading to a good control of the polymerization. It is noteworthy that this polymerization is never referred to as a “grafting from” method in the field of Bioconjugate synthesis but NCA polymers can be seen as “synthetic protein”. Interestingly, a couple of works already studied the self-assembly properties of block copolymers formed by NCA polymer (mostly polybenzylglutamate) and PI.

Figure 31: "Activated monomer" and "amine" mechanism for the ROP of NCAs

The polymer thus obtained is a polypeptide (PPep) possessing a helicoidal morphology as can be encountered in proteins. Furthermore, the « R » group of the monomer can be varied and copolymerization reactions could lead to model proteins. This implies that the polymerization must be controlled. One possibility is to initiate the polymerization with primary amines and to perform the ROP at 0°C in DMF. 99% of the chain-ends were still “living” at the end of the polymerization process in this case. Deming et al. developed the use of Cobalt and Nickel catalysts to initiate the polymerization through a different pathway, affording well defined polymers that could be composed of a broad range of monomers bearing different functions. Other methods like the use of aminosilanes, protonated amines or even the alkylation of the NCA monomer thus blocking the activated monomer mechanism were also reported, all leading to a good control of the polymerization. It is noteworthy that this polymerization is never referred to as a “grafting from” method in the field of Bioconjugate synthesis but NCA polymers can be seen as “synthetic protein”. Interestingly, a couple of works already studied the self-assembly properties of block copolymers formed by NCA polymer (mostly polybenzylglutamate) and PI.
In these works, primary amine terminated PI was obtained by anionic polymerization and used as macroinitiator for the growth of the peptide block(s). Different architectures (di-block or tri-block co-polymers) were studied exhibiting self-assembly properties affording micelles or membranes.

In conclusion, the “Grafting from” pathway usually gives better control toward the linkage between Polymer and Protein (or “protein-like”) as it starts by the functionalization of one block to grow the other. For that purpose, CRP techniques are the most employed. Only few examples deal with the direct coupling of hydrophobic polymers with proteins as will be discussed in the following paragraph.

### iii. Giant amphiphiles

“Giant amphiphiles” can be seen as a sub-collection of the family of Bioconjugates as it characterizes the architecture obtained by the linkage of an hydrophobic polymer chain with a protein. This term was first introduced in 2001 by Hannink et al. as they were the first to report the linkage of a polystyrene chain with a protein, the horse radish peroxidase (HRP). These particular bioconjugates are usually formed by the “grafting to” pathway and have been developed essentially by two teams (Velonia et al. and Nolte et al.) through Thiol-Maleimide coupling, Huisgen click-chemistry or cofactor reconstitution. This last method comes from the specific recognition of various proteins toward a special substrate called “co-factor”. Usually, this type of linkage is non-covalent but the strength of the bonding is such that it can be considered as irreversible. The most famous example is the Biotin / Streptavidin interaction which is characterized by a linkage energy of 21 kcal.mol$^{-1}$ and that is widely used in biochemistry. In most of the works of Velonia et al., the proteins that have been used are Lipase B from *Candida antarctica* (CALB) and BSA and the hydrophobic polymer is a PS chain, generally of 5 kg/mol with controlled chain-ends. The coupling was usually performed in heterogeneous conditions in a mixture of THF and water in various proportions to best solubilize either the protein or the polymer. CALB presents two advantages: it is a relatively tough protein that can bear various conditions and organic solvents without facing any denaturation or degradation and it is a relatively small protein (about 35 kDa), thus rendering more accessible the desired amino-acid for the coupling with the PS chain. On another end, BSA presents the advantage of bearing natively a free cysteine available for the coupling without necessity for disulfur bridge breaking prior to coupling reaction which is the case for the lipase.
Nevertheless, the coupling reactions usually take long time to be performed (from 1 night to 1 week), are highly dependent of the conditions and poor yields are generally observed, needing the use of extensive dialysis for purification. Figure 32 presents various morphologies of giant amphiphiles observed by Nolte and Velonia. As expected, these systems exhibit particular self-assembly behaviours once placed in water or THF.

![Various morphologies of Giant Amphiphiles](image)

**Figure 32:** Various morphologies of Giant Amphiphiles reported in the literature\textsuperscript{115,119,147}. a: PS-CALB fibrils (observed by TEM), b: aggregates of complexes formed between PS and HRP in aqueous solution (observed by TEM), c: TEM picture of a BSA-PS giant amphiphile in aqueous media (observed by TEM)

In conclusion, bioconjugates is a wide field in the polymer science being more and more investigated due to the promising results already obtained for drug-delivery and biomedical applications.

Various methods exist to attach a polymeric chain to a protein backbone either by direct coupling or by growing one block from the other. In both cases, the obtained chimera usually presents self-assembly properties forming vesicles, polymersomes, fibrils etc.. The most studied macromolecular chimeras involve “smart polymers” like PNIPAM or PEG that can be grafted in water and become hydrophobic by increasing the temperature thus achieving amphiphilic macromolecules. To date, only few attention was paid on the grafting of hydrophobic polymers to proteins certainly due to the restrictive conditions to be used and the difficulty to graft the polymeric chain on the protein. BSA–PS and Lipase–PS are, to date, the only members of the Giant Amphiphile family with interesting self-assembly properties. Beside these approaches, the ROP of NCA seems a functional pathway to achieve “protein-like” block co-polymers.
This bibliographical study highlighted the origins and the challenge represented by this PhD project. Indeed, despite the fact that NR is widely used in industry, this material is not perfectly understood regarding its biosynthesis or the origin of some of its superior properties toward IR. Tanaka offered a piece of explanation making bridges between the known pathway of the biomachinery, the structure of the material and some of the properties of NR. But, this model was never demonstrated to be true as no attempt of synthesizing the “tri-block” structure was proposed. For that, a good control of the microstructure of the polymer (pure 1,4-cis) and of the chain-ends (need for different reactivities in $\alpha$ and $\omega$) was mandatory. But it appears that, among all the synthetic pathways to afford IR, a method corresponding to this specifications could not be obtained. Another pathway was thus selected that will be presented in the next chapter. Finally, no clue could be obtained from the literature for the synthesis of the PI-Lipid hybrid as this field was never investigated. Contrarily to that, the PI-Protein coupling seems to be possible using the coupling pathway developed by Velonia et al. which uses the thiol-maleimide chemistry and CALB or BSA as proteins. This indicates that a maleimide terminated polyisoprene might be compulsory for the rest of the study.
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Natural Rubber

and Chemical Degradation
I. Introduction

This chapter will focus on the chemical degradation of NR. As explained in the bibliographic part, the synthesis of a pure 1,4-cis PI chain with controlled chain-ends can be tedious and restrictive as no polymerization technique would be suitable. Nevertheless, taking advantage of the pure 1,4-cis microstructure of NR, other methods were developed affording PIs with rather low molar masses and exhibiting pure 1,4-cis microstructure by degradation of the natural material. Furthermore, according to the method employed, the nature of the chain-ends can also be tuned.

First, a brief bibliographical part will give an overview of the existing methods for chemical degradation of NR. In a second step, it will be presented the starting materials (two different clones of NR) used in the frame of this work. They will be fully characterized. Finally, the chemical degradation of both NR clones will be investigated and compared to the one of a synthetic PI.

II. Bibliography

Liquid natural rubber (LNR) are natural rubber derivatives of low molar masses (<20 000g/mol) obtained from the chemical degradation of NR. The term “liquid” refers to the fact that these compounds are generally liquids due to their low molar mass\(^1\). This family of polymer was developed essentially to perform chemistry, as NR is generally difficult to process regarding its high molar mass (~1 000 000 g/mol) and the difficulty to solubilize it due to the gel fraction. To date, no production on a large scale of these derivatives was reported but they have been widely used for chemical reaction such as chain-extension or block co-polymer formation\(^2\)–\(^10\). More specifically, the synthesis of telechelic liquid natural rubber (TLNR) was deeply investigated as these latter can be post-modified, opening a wide range of potential applications. The main chemical pathways described in the literature for the degradation of NR as well as the structure of the resulting TLNR are presented on Figure 1. Each method will be developed below.
Natural Rubber and Chemical Degradation

Figure 1: Main chemical degradation pathways described in the literature.
a. Ozonolysis

The ozonolysis of NR was studied since more than 70 years, either to produce TLNR \(^{11}\) or for a better understanding of the structure of NR by analysis of the degradation products.\(^{12,13}\) Many articles dealt also with the ozonolysis of synthetic rubbers.\(^{14,15}\) Nor reported that oligomers of NR (about 900 g/mol) could be obtained within 20 minutes at 0°C in chloroform but with a lack of control toward the chain-end formation. Infra-Red analysis allowed to show the appearance of new functional groups like carboxylic acid, ketone, aldehyde and hydroxyl groups. It was also reported the presence of residual molozonide groups most probably located onto the polymer backbone. Figure 2 presents the possible reaction pathways that occur during the ozonolysis of a polydiene. The author concluded that the exact structure of the chain-ends could not be given due to the great number of possibilities.

![Figure 2: Possible reactions following ozonolysis of a diene-containing polymer - Reproduced from Phyniocheep\(^{16}\)](image)

b. Photodegradation

The photodegradation of NR was first reported by Cunneen \textit{et al}. in 1973\(^{17}\). Ill-defined structures of PI were obtained after exposure of a solution of NR to UV. The molar masses obtained were around 3 000 g/mol attesting the photodegradation of the material.
Later, Gupta et al. as well as Ravidran et al. reported the photodegradation of NR in a toluene solution and in the presence of hydrogen peroxide yielding hydroxyl-telechelic liquid natural rubber (HTLNR), with a molar mass around 3 000 g/mol, a functionality of 1.4 due to side reactions and the formation of carbonyl groups. Ravidran reported that molar masses from 5 000 g/mol to 200 000 g/mol could be obtained by varying the time of exposure. The source of UV was also varied from a UV lamp to sunlight thus showing that both sources could be used. Even if the time of exposure to reach 5 000 g/mol was long (about 50 h), the system remained economically interesting regarding the use of sunlight. One main drawback of the system is the presence of about 10% of side products consisting mostly of a cross-linked phase. The degradation mechanism proposed by the author is depicted on Figure 3.

\[
\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O}_2^* \\
\text{H}_2\text{O}_2^* \rightarrow 2\text{OH} \\
\] 

\[
\begin{array}{c}
\text{H}_2\text{C} = \text{C} \quad \text{H}_2\text{C} = \text{C} \\
\text{H} \quad \text{H} \quad \text{H} \end{array} + \text{OH} \rightarrow 
\begin{array}{c}
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \\
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \end{array} \\
\begin{array}{c}
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \\
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \end{array} \\
\begin{array}{c}
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \\
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \end{array} \\
\begin{array}{c}
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H} \\
\text{H}_2\text{C} \quad \text{CH}_2 \quad \text{H}
\end{array}
\]

Figure 3: NR Degradation pathway proposed by Ravidran et al. - Reproduced from the original article

**c. Oxydo-reduction degradation**

The oxydo-reduction degradation of NR uses also free radicals but supplied from another source than light. The most studied system was phenylhydrazine/O\(_2\) that generates phenyl free radicals which can perform the chain degradation (Figure 4). This degradation reaction leads to a hetero-telechelic PI terminated by a methyl ketone and a phenyl ketone.
Natural Rubber and Chemical Degradation

Figure 4: Mechanical pathway for the oxydo-reductive degradation of NR using phenylhydrazine
- Reproduced from Brosse et al.24

Molar masses from 3 to 35 kg/mol were reported for this process with quite narrow molar mass distribution compared to the starting NR (< 2)1. Few drawbacks were reported about this method except that the degradation slows down with increasing quantity of phenylhydrazine24. Moreover, the formation of epoxides and hydroxyl functions were also reported as side products of the reaction20. The main advantage of this method is that it can be applied in the latex phase directly.21

d. Metathesis

Metathetic degradation of NR is a less studied route as only few papers deal with it. Originally, the use of different types of catalysts (Schrock’s catalyst based on molybdenum25 as well as tungsten chloride26) was described, but side reactions like internal cyclization were present. The development of this field is mostly due to the appearance of the first and second generation of Grubbs catalysts that presents more stability among chemical functions (hydroxyl, carboxylic acid or ester) thus allowing to vary the chain-end termination of TLNR27. Moreover, the triple substitution of the double-bond from the polymer backbone usually reduces the activity of the catalyst which was improved in the case of second generation Grubbs catalyst28.
Natural Rubber and Chemical Degradation

It has to be noted that the degradation usually proceeds via the use of a substituted vinylic chain-transfer agent which gives access to functional chain-ends by playing on its substituents. Most of the recent works with the Grubbs catalysts were described by Pilard and coll. In 2005, Solanky et al. reported the degradation of a synthetic polyisoprene using second generation of Grubbs catalyst and a diacetate as the chain transfer agent (CTA, Figure 5). The use of a CTA bearing hydroxyl functions to obtain directly hydroxy-telechelic polymer lowered the activity of the catalyst. A range of molar masses going from 900 to 22000 g/mol could be obtained with reasonable molar mass distribution (~ 2). The same reaction was also performed directly into the latex phase in the presence of small amount of DCM. A degradation was still observed (about 40 kg/mol TLNR obtained) but the catalyst was not really adapted to such media.

Later, it was reported the same procedure on waste tires in toluene and on NR in ionic liquids. The metathetic degradation of waste tires afforded small oligomers (~ 400 g/mol) in a poor yield due to the non-rubber constituents present in tires and the difficulty to purify these oligomers. Nevertheless, the obtained telechelic polymers presented the good functionality at the chain-end thus opening the way to recyclability of waste tires. In the case of ionic liquid, TLNRs were obtained in a range of molar masses from 25 to 80 kg/mol (depending on the time of reaction) and a rather low molar mass distribution around 2.

In 2011, Gutierrez et al. reported a similar process using β-pinene as CTA. TLNR could be obtained in a range of 700 to 3000 g/mol, with a molar mass distribution comparable to the one reported previously (1.6- 2.5). The structures and yields of the obtained oligomers are reported in Figure 6.

Figure 5: Chemical pathway reported by Solanky et al. for the chemical degradation of NR using metathesis

Figure 6: Structure and yields (detected by GC/MS) of the products of metathesis degradation of NR using β-pinene as CTA – Reproduced from literature
In conclusion, metathetic degradation of NR is an interesting pathway giving access to TLNR in a wide range of molar masses and reasonable molar mass distributions.

**e. Oxidative degradation**

The oxidative degradation of NR is the most described method to obtain TLNR in a controlled manner. It was first reported by Reyx and Campistron in 1997\(^{31}\) and was then highly developed and used by the team from Université du Maine\(^{7,32–40,41,42}\) to design various functional oligomers and to synthesize block copolymers. This method is based on the use of periodic acid for the cleavage of the carbon-carbon double bound of the polymer backbone previously epoxidized or not. The exact mechanism of cleavage is not perfectly understood yet\(^{41,43}\) but it is believed to proceed through the formation of vicinal diol that rearranges, leading to the chain cleavage and the formation of carbonyl groups (Figure 7). Gilliet-Ritoit\(^{41}\) reported that the degradation using periodic acid alone is usually slower than when applied to epoxidized rubbers (4h vs 1h), suggesting that the acid alone encounters a limitative step which is the formation of epoxide and/or vicinal diols.

As previously mentioned, functional TLNRs were widely produced for the synthesis of different polyisoprene/polyurethane block co-polymers\(^{7,36–40,44}\). The range of molar masses obtained is from 1 500 g/mol to 5 000 g/mol with a molar mass distribution from 1.5 to 3. Reaction conditions are quite gentle (epoxidation at 0°C and acidic degradation at room temperature). Moreover, the difference of reactivity between the aldehyde and the ketone chain-ends was used to functionalize the obtained polymer backbone selectively\(^ {36} \).
f. Conclusion

To conclude, various methods were already described for the chemical degradation of NR. Most of them allowed to produce TLNR with various chain-ends. For our project, as both chain-ends have to be selectively modified, the oxidative degradation using periodic acid and epoxidation was selected as it gives access to two different chain-ends (ketone and aldehyde) that possess different chemical reactivities.

III. Characterization of the starting material

First, we had to obtain information about the NR used as starting material. Indeed, two different unsmoked NR sheets were supplied by our partner in Thailand (UMR IATE/LBTNR / Katsetsart University) (Figure 8). These sheets were obtained from recovery of fresh latex, acidic coagulation of the rubber, passing through a rolling mill and drying. These two materials are coming from two different clonal origin of *Hevea brasiliensis* namely RRIM 600 and PB 235. There are differences between these two clones, for example the lipidic composition as reported by Vaysse *et al.*

Figure 8: Picture of the unsmoked NR sheets supplied in the frame of Rubbex project
Natural Rubber and Chemical Degradation

a. Extraction of polyisoprene from Natural Rubber

As explained in the bibliographic part, the solubilization of NR can be tricky due to the high molar mass of the PI chains but also to the gel phase formed. It was thus first studied the possible extraction of natural PI by solubilization of NR in different solvents, to remove the gel fraction and most of the non-rubber components. To perform this first study, four good solvents of PI namely cyclohexane, DCM, Toluene and THF were employed.

Results are summarized in Table 1. It can be seen that whatever the solvent and the clone used, the overall recovery is pretty good (always > 90%). On the contrary, the solubilized and gel fractions are highly dependent on the solvent and the clone:

* in cyclohexane, RRIM600 became more and more soluble with time, whereas PB235 was hardly soluble even after 5 days of stirring and remained in the state of a swollen gel in this solvent.

* in THF, half of the RRIM600 was soluble whatever the solubilization time, whereas PB235 was almost fully soluble, even after 1 day of stirring.

* in DCM, both clones presented almost similar solubility (between 60 and 80% of soluble fraction). Nevertheless, the extraction after centrifugation was quite difficult as the gel fraction was above the solution phase. This could have an impact of the figures reported here.

* in toluene, PB235 was visually fully soluble (no gel fraction recovered after centrifugation) and RRIM600 was almost fully soluble (less than 10% of gel fraction, Figure 10). In that case, the evaporation of the solvent was sometime difficult, explaining some overall recoveries higher than 100% in some cases.

In conclusion, regardless the nature of the clone, toluene is the best solvent for the solubilization of NR. THF gives both a rather good yield of extraction and an easy separation and drying using centrifugation and vacuum. As a consequence, when it was possible (\(^1\)H and \(^{13}\)C NMR spectra), NR was solubilized in toluene but, for other analyses, THF was used thus analysing only the soluble part of the sample. Degradation reactions were, however, performed in THF as it is known to be a good solvent of periodic acid.
**Natural Rubber and Chemical Degradation**

![Image of solubilization process](image)

**Figure 9:** Solubilization of RRIM 600 in THF and centrifugation

![Image of solubilization process](image)

**Figure 10:** Solubilization of both NR clones in toluene after 24h.

Table 1: Summary of the data obtained for the extraction of natural PI from RRIM 600 and PB235 using different solvents. [NR] = 20 g/L.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Solvent</th>
<th>Solubilization time</th>
<th>Overall recovery (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Solubilized Natural PI (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gel fraction (%)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRIM 600</td>
<td>Cyclohexane</td>
<td>1 day</td>
<td>90</td>
<td>38</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 days</td>
<td>90</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days</td>
<td>93</td>
<td>87</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>THF</td>
<td>1 day</td>
<td>90</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 days</td>
<td>94</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days</td>
<td>95</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>1 day</td>
<td>94</td>
<td>77</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 days</td>
<td>88</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days</td>
<td>98</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>1 day</td>
<td>91</td>
<td>85</td>
<td>14</td>
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<tr>
<td></td>
<td></td>
<td>3 days</td>
<td>115</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days</td>
<td>101</td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>PB235</td>
<td>Cyclohexane</td>
<td>1 day</td>
<td>88</td>
<td>18</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 days</td>
<td>89</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days</td>
<td>86</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>THF</td>
<td>1 day</td>
<td>98</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 days</td>
<td>93</td>
<td>91</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days</td>
<td>97</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>1 day</td>
<td>91</td>
<td>61</td>
<td>39</td>
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<td></td>
<td></td>
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<td>94</td>
<td>84</td>
<td>16</td>
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<td>5 days</td>
<td>88</td>
<td>62</td>
<td>38</td>
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<tr>
<td></td>
<td>Toluene</td>
<td>1 day</td>
<td>97</td>
<td>100</td>
<td>0</td>
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<td></td>
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<td></td>
<td></td>
<td>5 days</td>
<td>94</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Overall recovery (solubilized + gel fractions) starting from 1g of NR;  
<sup>b</sup> Percentage of the solubilized PI fraction;  
<sup>c</sup> Percentage of the gel fraction
Natural Rubber and Chemical Degradation

b. Characterization of the raw material

i. SEC analysis

To obtain well defined chromatograms of both starting NRs, it was necessary to find the right concentration of sample to inject due to the molar mass of the polymer and the gel fraction that could not be filtered. Finally, by adjusting the concentration of NR to 1 mg/mL, both clones could be analyzed by SEC in THF. Figure 11 presents the chromatograms given by the RI detector. As already described, RRIM 600 possesses a bi-modal profile whereas the PB 235 is nearly mono-modal.

![SEC Chromatograms](image)

Figure 11: SEC chromatograms of both NR clones in THF injected at 1mg/mL.

The molar mass of PB 235 is evaluated to be 1 200 000 g/mol with a quite narrow molar mass distribution of 1.6. In the case of RRIM 600, the molar mass was calculated to be 500 000 g/mol with a molar mass distribution of 2.6. Integrating separately the 2 peaks, the molar mass ($M_n$) of the high molar mass and low molar mass fractions were estimated to be 1 200 000 g/mol and 200 000 g/mol respectively. This is in agreement with the results generally reported in the literature$^{48}$.

ii. NMR analysis

Figure 12 and Figure 13 present the $^1$H and $^{13}$C NMR analysis of PB 235 respectively. The NMR analysis was performed in deuterated toluene as it is the only solvent to solubilize entirely the PB235 sample when the concentration was adjusted to 20 mg/mL. Only this clonal form is presented here as the same results were achieved with RRIM 600.
Natural Rubber and Chemical Degradation

$^1$H NMR spectrum shows the characteristic signals of a 1,4-PI (signal at 5.26 ppm corresponding to the vinylic proton, signal at 2.17 ppm corresponding to the “-CH$_2$” groups in α position of the CH=CCH$_3$ double bond and signal at 1.75 ppm corresponding to the CH$_3$ group of the double bond) with the absence of any 1,2 or 3,4 units. In the zone 0-3 ppm, one can see other signals badly defined and of very small intensities. They could correspond to aliphatic moieties either coming from lipids or proteins. $^{13}$C NMR spectrum confirms the high purity of 1,4-$cis$ units with the signals 5 at 32.2 ppm and 3 at 23.4 ppm corresponding to the “-CH$_2$” group in α position of the quaternary carbon and the “-CH$_3$” group respectively. For $trans$ units, the signals would be at 40.4 and 16.3 ppm respectively which were not observed in the spectrum.

Figure 12: $^1$H NMR spectrum of PB235 in toluene d$_8$

Figure 13: $^{13}$C NMR spectrum of PB235 in toluene d$_8$
Natural Rubber and Chemical Degradation

iii. Elementary analysis

To go further, elementary analysis was also performed on both clones. The results are given in Table 2. As it can be observed, their composition is highly similar. There are only small variations of the nitrogen and oxygen contents. The nitrogen content could be related to the proteins present in both samples. These figures seem to indicate that RRIM 600 contains more proteins that PB 235. Table 2 presents also the results obtained for the natural PIs obtained by extraction with THF for 24h. Unfortunately, the oxygen content could not be determined for the THF extract of PB 235 but, the analysis of the nitrogen and oxygen content of the gel phase highly increased compared to the one of the extracts. This suggests that the extraction of natural polyisoprene using THF has also a purifying effect by the removal of some “non-rubber” constituents. The enrichment of the gel phase in nitrogen content must correspond to a concentration of proteins. The origin of the enrichment of the gel phase in oxygen is not so easy to attribute as the source of oxygen could come from some specific peptides in proteins as well as from lipidic moieties (free fatty acids, triglycerides, diglycerides, etc…).

Table 2: Elementary analysis results obtained for both NR clones and their extracts with THF

<table>
<thead>
<tr>
<th>NR Clone</th>
<th>Carbon (wt %)</th>
<th>Hydrogen (wt %)</th>
<th>Oxygen (wt %)</th>
<th>Nitrogen (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRIM 600</td>
<td>85.7</td>
<td>11.3</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>RRIM extract – THF – 24h</td>
<td>86.2</td>
<td>11.3</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>RRIM gel phase – THF – 24h</td>
<td>86.3</td>
<td>10.9</td>
<td>3.6</td>
<td>1.3</td>
</tr>
<tr>
<td>PB 235</td>
<td>86.1</td>
<td>11.3</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>PB 235 extract – THF – 24h</td>
<td>86.9</td>
<td>12.5</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>PB 235 gel phase – THF – 24h</td>
<td>80.2</td>
<td>11.7</td>
<td>2.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

iv. Conclusion

Both clones present very similar structure (only 1,4-cis units) as confirmed by NMR analyses. The main differences come from SEC analysis as the RRIM 600 exhibits a bi-modal profile whereas the PB 235 SEC trace is mono-modal and from elementary analysis with a slightly higher content of oxygen and nitrogen for RRIM 600. We also showed the possibility to extract natural PI from both NR clones using various solvents. It was observed that NR behaves differently regarding the solvent used.
Natural Rubber and Chemical Degradation

Cyclohexane seemed to be relatively “bad” for the solubilization whereas toluene is able to dissolve entirely the sample. The natural PI thus extracted is “freed” from a part of the non-rubber constituent (most probably proteins) as the nitrogen content of the extracted sample decreases compared to the original material.

IV. Controlled degradation of NR

This sub-chapter will then focus on the synthesis of TLNRs obtained from the successive epoxidation and acidic degradation of high molar mass rubber, varying the origin of the starting material. Indeed, natural PIs coming from 24 h THF extraction of both natural clones (ExtraNR) will be used as well as a 600 000 g/mol IR of high 1,4-cis content (97%).

a. Purification process and side reaction

It was rapidly observed the appearance of side reactions during the degradation. Indeed, some samples presented impurities (Figure 14) and values of molar masses calculated by NMR were quite far from the ones obtained by SEC as reported in Table 3. By replacing the parameter $i_{al}$ by $(i_{al} + i_{?})$ in the formula reported below, values really close to the $M_n$ (SEC) could be obtained (4 700 g/mol for the degraded ExtraNR and 7 000 for the degraded IR). Moreover, the ratio $i_?/i_{al}$ was around 6-7 either in the case of the ExtraNR or the degraded IR. To finish with, no modification of the ketone side of the degraded rubber was observed, which means that this side reaction consumes only aldehydes.

| Sample           | $M_n^{th}$ (g/mol) | $M_n^{NMR}$ (g/mol) | $M_n^{SEC}$ (g/mol) | $^{1}H$ NMR integrals 
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Degraded ExtraNR</td>
<td>4 000</td>
<td>7 100</td>
<td>4 600</td>
<td>$i_{al}$  $i_{iso}$ $i_{?}$ $i_{?}'$</td>
</tr>
<tr>
<td>Degraded IR</td>
<td>6 000</td>
<td>12 700</td>
<td>7 700</td>
<td>1 104 0,6 4,0</td>
</tr>
</tbody>
</table>

$^{a}$: Targeted molar mass

$^{b}$: Calculated from the $^1H$ NMR integrals by using the formula: $M_n^{NMR} = \left(\frac{i_{iso}}{i_{al}} \times 68\right) + 100$

$^{c}$: Obtained by SEC in THF with LS detector, using a $dn/dc$ value of 0.130

$^{d}$: Obtained by integration of $^1H$ NMR signals. $i_{al}$ corresponds to the integration of the signal from the aldehydic proton, $i_{iso}$ corresponds to the integration of the signal from the diene proton of the polymer backbone (shift: 5.15 ppm in Figure 14), $i_{?}$ corresponds to the integration of the signal at 4.33 ppm in Figure 14, $i_{?}'$ corresponds to the integration of the signal at 3.51 ppm in Figure 14.
This side reaction was explained thanks to the literature, where Li et al. characterized an acetal having a structure close to what could be obtained during the degradation process (Figure 15). 2D NMR analysis of a rubber exhibiting the impurity signals (Figure 16) confirmed this structure and explained the ratio around 6 between both signals. Moreover, it explained well the selectivity toward aldehydes as this function is more reactive than ketone toward the formation of acetal functions.

This impurity formation can be explained by the presence of acid and the wide excess of methanol used for the precipitation of the polymer. For this reason, it was proposed that the precipitation of the polymer would take place in methanol but bearing a small amount of alkaline water in order to degrade the last traces of acid and to be in the presence of water thus rendering less favourable the formation of the acetal.
b. Epoxidation

It was then investigated the influence of the nature of the starting material (RRIM 600, PB 235 or IR) on the epoxidation efficiency, as well as the influence of the reaction time. The natural samples used came from the 24 h THF extraction of both clones affording ExtraRRIM 600 and ExtraPB 235 (when obtained from RRIM 600 and PB 235 respectively). The experimental rate of epoxidation can be easily calculated by $^1$H NMR (Figure 17), from the signals of the protons present on the oxirane rings and on the double bonds. Results are summarized in Table 4. As it can be seen, the experimental epoxidation rates were very close, even if a bit lower than the theoretical ones. No particular effect of the nature of the rubber can be observed. The reaction is also pretty fast as the epoxidation rates are very similar after 2 and 4h of reaction. The quantity of m-CPBA ($m_w$) to add to the reaction medium for a targeted epoxidation rate was calculated as follows:

$$m_w = \frac{t_x \times m_R \times M_{m-CPBA}}{P \times M_{iso}}$$

where:
- $t_x$ is the targeted rate of epoxidation (%)
- $m_R$ is the mass of rubber (g)
- $M_{m-CPBA}$ is the molar mass of m-CPBA (g/mol)
- $P$ is the purity of m-CPBA (wt %)
- $M_{iso}$ is the molar mass of isoprene (g/mol)
The only difference between Extra NR and IR epoxidation is that, after 4h of reaction, the epoxidation rate in ExtraNR is higher than the one in IR. This can be explained by the equation reported above for the calculation of the quantity of m-CPBA used. Indeed, in the equation, the epoxidation rate is calculated from the molar ratio of epoxidizing agent toward the molar amount of isoprene units. To obtain this molar amount, the rubber sample is considered as entirely constituted of isoprene units which is true only in the case of IR. For ExtraNR we showed that the samples were not only composed of PI but also of other constituents. Thus, by dividing mR by the Miso the corresponding molar quantity of isoprene is overestimated in the case of ExtraNR thus explaining the difference of the obtained epoxidation rate between ExtraNR and IR.

Table 4. Epoxidation of different starting materials

<table>
<thead>
<tr>
<th>Targeted epoxidation rate (%)</th>
<th>Reaction time (h)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ExtraRRIM 600</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
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<td>2</td>
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</table>

* experimental epoxidation rate determined by $^1$H NMR according to the following formula: $\frac{i_{2}i_{2}'}{i_{2}+i_{2}'} \times 100$; b) not performed

Figure 17: $^1$H NMR analysis of an ExtraRRIM 600 epoxidized at 20 % - CDCl$_3$
Natural Rubber and Chemical Degradation

c. Acidic cleavage

The epoxidized rubbers were then reacted with periodic acid to cleave the oxirane rings. It was decided to use only 1.1 equivalent of periodic acid (compared to the epoxide units) in order to avoid unwanted cleavage of the polymeric chains as periodic acid alone is able to degrade NR (see bibliography sub-chapter). It was first investigated the influence of the reaction time by following the variation of the molar mass with time by SEC (Figure 18). It appears clearly that acidic degradation proceeds rapidly as after 1h the bimodality of the sample had totally disappeared and only compounds of lower molar masses can be observed. The molar mass distribution also decreased to values around 1.5. After 2 h, a slight decrease of the molar mass could still be noticed, but no more evolution can be noticed for longer reaction times. The reaction seemed thus to reach completion after 2 h, but the molar mass of the final polymer was about 40 kg/mol whereas a molar mass of 13 kg/mol was expected (experimental epoxidation rate of 0.53 % calculated by $^1$H NMR).

![SEC chromatograms of epoxidized ExtraRRIM 600 after different time of degradation, compared to the starting ExtraRRIM 600.](image)

The partial degradation was further confirmed by $^1$H NMR analysis (Figure 19) with the appearance of a signal at 9.77 ppm corresponding to the protons of the aldehyde moiety, but also with the presence of a residual signal corresponding to the protons of the epoxide units at 2.68 ppm. In the case of IR, the signal of the residual epoxide units had almost totally disappeared (Figure 19) and the measured molar mass ($M_n \sim 25$ kg/mol by SEC) is close to the targeted one. It was further confirmed by $^1$H NMR that in this case, a molecular mass of 23 kg/mol was obtained using the aldehyde proton as a reference.
This could indicate that in Extra NR, the acidic cleavage of the epoxides is “disturbed” most probably by some “impurities” (the non-rubber compounds). The addition of 2.2 equivalents of periodic acid (instead of 1.1 equivalent initially) allowed to cleave all the epoxide units (no more signal for oxirane protons on Figure 20). In this case, the $M_n$SEC was 15 kg/mol, still a bit higher than the theoretical value which can be related to the slight difference existing between the experimental epoxidation rate and the targeted one (Table 4). It can be concluded from these preliminary studies that the optimal conditions to obtain TLNR (from the two clones used in this study) are to perform first the epoxidation with m-CPBA for 2h, followed by acidic cleavage for 2h with 2.2 eq of periodic acid.

Figure 19: $^1$H NMR spectra of IR and ExtraRRIM 600 degraded with 1,1 eq of periodic acid.

Figure 20: $^1$H NMR spectra of ExtraRRIM 600 degraded with 1,1 or 2,2 eq of periodic acid.
d. Comparison between extracted PI and NR sheet:

It was then decided to determine the benefit of the “purification step” by comparing the TLNRs obtained from ExtraRRIM 600 and from the RRIM 600 raw sheet directly. The same experimental conditions (0.6 % of epoxidation rate and 2.2 equivalents of periodic acid, targeting a final molar mass of 10 000 g/mol) were thus applied to both samples. Figure 21 and Figure 22 presents, respectively, the \(^1\)H NMR spectra and the SEC chromatograms obtained in both cases. No significant difference is observed between the TLNR obtained from the extracted PI and the one obtained from the NR sheet. This would mean that the gel fraction does not interfere in the degradation process and/or is also chemically cleaved during the reaction.

![Figure 21: \(^1\)H NMR spectra of degraded PIs coming from THF extraction (ExtraRRIM 600) and the unsmoked sheet (RRIM 600)](image1)

![Figure 22: SEC chromatograms of two degraded PIs coming from THF extraction (ExtraRRIM 600) and the unsmoked sheet (RRIM 600)](image2)
Regarding this result, for the rest of the work presented in the manuscript, the degradation will be performed directly onto the raw NR.

To go further, we decided to perform experiments to construct abacus curves for the two NR clones and the IR. Results are depicted on Figure 23. In the case of IR degradation (blue curve), even if the values are higher than the expected ones, final molar masses evolve linearly with the invert of the epoxidation rate over a wide range. On the contrary, for both NR clones, a different behaviour is observed (orange and grey curves). For high epoxidation rates both NRs follow a linear variation with the invert of the epoxidation rate as expected. But both clones quickly reach a rate at which the degradation becomes less efficient thus forming telechelic polymers with molar mass values far much higher than the expected ones. The limit values for both clones correspond to an epoxidation rate of 0.5% as can be observed from the zoom of the top left corner of Figure 23.

Figure 23: Study of the evolution of the experimental molar masses (determined by SEC-THF) of TLNRs versus \(1/(\text{rate of epoxidation})\).

Figure 24 presents the \(^1\text{H} \text{NMR}\) spectra of degraded RRIM 600 obtained for 3 different epoxidation rates. It can be observed that above the limit defined previously, no residual epoxides could be observed and a clear signal of aldehyde is visible. But for an epoxidation rate of 0.25 % (i.e. below the limit) the signal of residual epoxides becomes stronger and the one of the aldehydic proton is almost absent. In the case of IR (Figure 25), it can be observed that for the same epoxidation rates, there is no residual epoxide and that the decrease of the intensity of the signal of aldehyde could in this case be attributed only to the increase of the molar mass (less chain-end). These results confirm that during the chemical degradation of NR, a part of the periodic acid used for the cleavage of the chains is disabled.
The origin of this phenomenon was not further determined but it proves that in order to afford high molar mass LTNR a higher amount of acid is compulsory to cleave all the epoxides.

Figure 24: $^1$H NMR spectra of degraded ExtraRRIM 600 with 1 %, 0.5 % and 0.25 % of epoxidation (from the bottom to the top, respectively).

Figure 25: $^1$H NMR spectra of degraded IR starting from 1 %, 0.5 % and 0.25 % of epoxidation (from the bottom to the top, respectively)
e. Conclusion

In conclusion, we showed the possibility of degradation of both natural and synthetic rubbers with various optimisations of the degradation process as well as the purification one. It was shown that the natural material presented internal constituents that reduce the efficiency of the acidic degradation by preventing the cleaving power of the acid. This could be avoided by increasing the amount of acid used, but an optimization of the quantity has to be done for each molar mass targeted. Moreover, it was shown that depending on the clonal origin of the natural material, the behaviour toward degradation is different. This result is brand new as to the best of our knowledge, no comparable study has been done in the literature. The determination of the presence of side reaction (acetalization) of the aldehydic part of the degraded PI permitted to change the approach of the purification process thus rendering it easier than the pathway described in the literature\(^7,36-40\) consisting in washing the reaction solution with different saline aqueous solutions. This evidence is again, to the best of our knowledge, a first example in the literature as this side reaction was never reported. Finally, it was shown unnecessary the extraction of natural PI as the degradation pathway proceeds the same way for crude NR or natural PI coming from THF extraction. All this work allowed to define a clear chemical pathway for the degradation of rubbers affording TLNR of about 10 000 g/mol (while targeting an epoxidation rate of 0.8%) with a molar mass distribution between 1,4 and 1,6. The Figures 26 and 27 can be used as reference for the rest of the PhD work.

Figure 26: \(^1\)H NMR spectrum of a degraded natural PI obtained from RRIM 600
V. Conclusion

In conclusion of this chapter, an investigation of the composition of two clones of NR (RRIM 600 and PB 235) allowed to obtain more information about the non-rubber constituents of both samples and, more generally, gave information about the differences existing between both clones of *Hevea*.

In a second step, it was possible to obtain hetero-telechelic liquid rubbers from different origin (natural or synthetic PIs) bearing two different chain-ends. Among all the existing methods, the acidic degradation was chosen and studied. After the optimization of some parameters and a better comprehension of its chemical pathway, well defined PI could be obtained and characterized. The molar mass of 10 000 g/mol was selected as it permits a good characterization of the polymer by NMR. The increase of the molar mass was shown to be possible but more optimisation toward the quantity of acid to be used were necessary. The obtained hetero-telechelic PI could then be used for the functionalization and the modification of chain-end to obtain reactive synthons for the synthesis of the desired tri-block polymer as will be developed in the next chapter.
Natural Rubber and Chemical Degradation

VI. Experimental part

a. Natural PI extraction

NR (1 g), taken from the sheets received from Thailand, was immersed in 50 mL of the selected solvent for various times (1 day, 3 days and 5 days) under vigorous stirring. The obtained admixture was then centrifuged (speed: 7000 rpm – 20 minutes) and the gel phase was separated from the solution. Both phases were then dried under vacuum overnight and weighed. A “recovery yield” was then established for each solvent.

b. Degradation of NR

IR, NR from the unsmoked sheet or natural PI obtained from extraction (1 g) was solubilized in 40 mL of THF overnight under vigorous stirring. The flask is then cooled to 0°C with an ice bath while 28 mg (0.12 mmol, targeted epoxidation rate of 0.8%) of m-CPBA are dissolved in 5mL of THF. The solution of m-CPBA is then added dropwise to the rubber solution. After the addition, the cooling bath is removed and the solution is let stirring at room temperature for 2 h. Periodic acid (61 mg, 0.26 mmol, 2.2 eq) are then solubilized in 5 mL of THF and added dropwise to the epoxidized rubber solution. A decrease of viscosity is rapidly observed as well as a yellowish coloration and the disappearance of the gel fraction in the case of NR. The solution is still inhomogeneous as small brown particles could be observed. After 2 h of reaction the solution is filtered to remove the suspended particles affording a homogeneous yellow solution which is then concentrated under rotative evaporation. The concentrated solution is then precipitated in a huge excess of methanol (150 mL) in presence of alkaline water (water/KOH solution, pH ~ 8). The precipitated polymer is then solubilized in Et₂O, filtered through Celite® and dried first with the rotative evaporation and then at 40°C overnight affording a viscous yellowish liquid. Yield ~ 85%, $M_n$ (NMR) = 11 400 g/mol, $M_n$ (SEC) = 11 200 g/mol), $\bar{D}$ ~ 1.5.

$^1$H NMR (CD₂Cl₂) δ (ppm): 9.77 (t, 1H, COH$_{aldehyde}$), 5.14 (s, 1H, CHCCH₃), 2.49 (t, 2H, CH₂COH), 2.43 (t, 2H, CH₂COCH₃), 2.34 (t, 2H, CH₂CH₂COH), 2.05 (s, 4H, CH₂CH / CH₂CCH₃), 1.67 (s, 3H, CH₃CCH)
REFERENCES:


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Gondard, C. *Polymer* 2005, 46 (18), 6869.


(44) Kébir, N. Elaboration de nouveaux polyuréthanes à partir de cis-1,4-oligoisoprènes hétérocarbonyltélécéliques issus de la dégradation contrôlée du cis-1,4-polyisoprène de haute masse. Etude de leurs propriétés mécaniques, thermiques et biocides, 2005. Le Mans Université


Selective Chain-end Functionalization
Selective Chain-end Functionalization

I. Introduction:

The first step of the project was the synthesis of TLNR bearing two different functions at the α and ω chain-ends as shown in the previous chapter (PIDeg). The present chapter will focus on the functionalization of this telechelic polymer in order to be able to perform later the synthesis of di-blocks PI-lipid, PI-protein and the desired tri-block protein-PI-lipid to mimic Tanaka’s model.

First, a brief state of the art will be presented explaining the chemical pathway given below (Figure 1) and how this multistep synthesis was built. This will be followed by the presentation of the results obtained for the synthesis of functional PIs but decomposing each step of the synthesis. The experimental procedure will be given at the end of this chapter which will be concluded by a table summarizing all the molecules synthesized. Voluntarily, only few information will be given in this chapter about the use of the synthesized functional PIs as this part of the manuscript was thought to be seen as a toolbox which will be referred to in the other chapters.
Selective Chain-end Functionalization

![Chemical pathways diagram]

Figure 1: General chemical pathways developed for chain-end functionalization

R, R' = alkyl groups or alkyl maleimide
Many functional PI derivatives were already synthesized from degraded NR, mainly for the synthesis of polyurethanes. The reductive amination of degraded PI\textsuperscript{1,2,5} was particularly studied using primary and secondary amines as well as ammonium salt to selectively functionalize either the ketone or the aldehyde chain-end (Figure 2). It was demonstrated that primary amines were not selective as reductive amination occurred at both chain-ends. Indeed, with 2 equivalents of the amine, the double functionalization was obtained while with 1.2 equivalent of the amine, an admixture of both ketone and aldehyde functionalized PI was observed. When the same reaction was performed with secondary amines or ammonium salt, only aldehyde modification was observed, regardless the quantity of amine used for the reaction.

This difference was explained by the decrease of the nucleophilicity of the electron pair going from alkyl primary amine to functional secondary amines and ammonium salt and by the difference of steric hindrance between aldehyde and ketone on the one hand and between primary and secondary amines on the other hand. This was further supported by Abdel-Magid et al\textsuperscript{8,9} who investigated these reactions for many ketone or aldehyde functionalized-molecules.
Selective Chain-end Functionalization

It was also shown that with the use of sodium triacetoxyborohydride (STABH) as reducing agent it was possible to be selective for the reduction of aldehyde towards ketone even without any use of amine. Moreover, this reducing agent presents the advantage of being cheap and widely commercially available as well as less toxic than its homologous sodium tricyanoborohydride usually used for reductive aminations.

Finally, the selective reduction of ketone was also reported toward esters using sodium borohydride (NaBH₄) as the reducing agent\(^2\) (Figure 3) which will also be used in our strategy.

![Figure 3: Selective reduction of chain-ends using NaBH₄ reported from Kebir et al.]()

To conclude, reductive amination (as well as selective reduction) of degraded PI is possible and was already studied. By using secondary amines or ammonium salt, we should be able to selectively functionalize the aldehyde chain-end of our TLNR which could lead to the synthesis of the first di-block PI/Lipid or PI/protein. Moreover, the use of STABH as reducing agent could also permit to selectively reduce the aldehyde chain-end without degradation of the ketone part also allowing selective functionalization of our polymer. Finally, the use of NaBH₄ for the selective reduction of the ketone chain-end toward ester is also of interest and leads the way to the tri-block formation. It appears that the most important aspect of functionalizing chain-ends will be to go back and forth between activation of the chain-end and functionalization in order to obtain the desired final tri-block.

III. Chain-end functionalization

a. Results and discussion

i. Synthesis of a heterotelechelic ketone/maleimide PI (PIMal)

The first functional PI to be synthesized was a PI terminated by a maleimide group. This synthon was originally designed for the synthesis of a di-block PI-Protein via a thiol-maleimide “click chemistry” between a cysteine of the protein and the PIMal.
Selective Chain-end Functionalization

The synthesis starts with the selective reduction of the aldehyde chain-end of the degraded PI using NaBH(OAc)$_3$ affording a heterotelechelic ketone/hydroxyl PI (PIOH) (Figure 4).

On the $^1$H NMR spectrum (Figure 5), the total disappearance of signals 7’ (2.48 ppm) and 8’ (9.77 ppm), corresponding to the “-CH$_2$” group in α position of the aldehyde’s carbonyl and the aldehyde proton respectively, confirmed the total reduction of the aldehyde. The remaining signal 2’ (2.43 ppm) attests the selectivity of the reaction as this signal corresponds to the “-CH$_2$” group in α position of the ketone chain-end. The signal 8 (3.63 ppm) that appeared during the reaction corresponds to the “-CH$_2$” group in α position of the hydroxyl function generated and is in good agreement with the literature$^7$. 

![Chemical pathway for the synthesis of PlMal](image)

![1H NMR spectra of PIDeg and PIOH](image)
Selective Chain-end Functionalization

The second step to synthesize PIMal is an esterification reaction. To this end, carboxylic acid terminated maleimide (MalHex) was first reacted with oxalyl chloride to obtain an acyl chloride (MalChlo). Contrarily to fatty acyl chlorides (see later in the chapter) which were not particularly sensitive molecules, maleimide derivatives faced more side reactions. The chlorination process had to be optimized to obtain the desired compound. The main side reaction observed was the decrease of the integral of signal 5’ (3.5 ppm) corresponding to the “CH₂” group in α position of the nitrogen atom. A decrease of the integration of the double bond of maleimide was concomitantly observed. This could suggest the opening of the maleimide ring and/or the degradation of the double bond. After optimization, full conversion was confirmed by the shift of signal 1 (2.32 ppm) becoming 1’ (2.87 ppm) in Figure 6 as well as by the splitting of signal 2-4 (1.61 ppm) becoming two distinct signals 2’ (1.72 ppm) and 4’ (1.60 ppm). In $^{13}$C NMR analysis, the same shift of the carbonyl from signal 1 (179.7 ppm) to 1’ (173.7 ppm) was observed as in the case of lipidic acyl chlorides (see later in the chapter). Nevertheless, the MalChlo remained highly sensitive to water and difficult to retain contrarily to the fatty acyl chlorides that were rather hydrophobic and self-protected by the long aliphatic tail. For that reason, fresh MalChlo had to be synthesized prior to each esterification.

Figure 6: $^1$H NMR spectra of MalChlo and MalHex
The last step of the synthesis was then the esterification reaction. A similar reaction had already been reported by Goodyear in 2013\textsuperscript{10}. As can be seen from Figure 8, the appearance of the characteristic signals of the maleimide function (13’, 3.51 ppm / 14’ and 15’, 6.66 ppm) as well as the one from the \textquotedblleft CH\textsubscript{2}\textquotedblright group in \(\alpha\) position of the carbonyl ester (9’, 2.27 ppm) confirmed the formation of the targeted compound. Again, the maleimide moiety induced side reactions as can be seen from the SEC chromatograms (Figure 9). It can be observed a tiny broadening of the peak after esterification considering the refractive index detector (full line). But, on the light scattering detector (dashed line) a shoulder appeared at higher molar mass. Nevertheless, this population can be neglected as it appeared to be highly minoritary.
ii. **Synthesis of fatty acyl chlorides**

In order to obtain fatty acid terminated PIs, esterification pathway was chosen using fatty acyl chlorides. A relatively large panel of fatty chains was used for the coupling:

- Saturated ones: Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Nonadecanoic acid (C19:0) and Lignoceric acid (C24:0)
- Unsaturated ones: Undecenoic acid (C11:1), Linoleic acid (C18:2)
As fatty acids are cheap and easily available, it was preferred to synthesize most of the acyl chlorides prior to the esterification, starting from the acid and using oxalyl chloride as the chlorinating agent. Few drops of DMF were also used as catalyst (Figure 10).

As can be seen from $^1$H and $^{13}$C NMR analysis (Figure 11 and Figure 12 respectively), the chlorination of carboxylic acid went to total conversion regarding the shift of the signal corresponding to the "-CH$_2$" group in α position of the carbonyl group from 2.34 ppm (carboxylic acid) to 2.87 ppm (acyl chloride) on the $^1$H NMR spectrum and the shift of the carbonyl signal from 180.1 ppm (carboxylic acid) to 174.4 ppm (acyl chloride) in $^{13}$C NMR analysis. Comparison with commercial acyl chlorides confirmed that the new carbonyl formed was the expected one. Besides, long carboxylic acid (C$_{24:0}$) was rather insoluble in DCM and the reaction had to be conducted in heterogeneous conditions. This did not seem to have any negative impact as the yield of this reaction was also 100%.

Figure 10: Proposed mechanism for the chlorination of carboxylic acid using oxalyl chloride and DMF as the catalyst

Figure 11: $^1$H NMR spectra of C$_{14:0}$Chlo, C$_{16:0}$Chlo and C$_{24:0}$Chlo synthesized with oxalyl chloride
Figure 12: $^{13}$C NMR spectra of $C_{14:0}$Chlo, $C_{16:0}$Chlo and $C_{24:0}$Chlo synthesized with oxalyl chloride

iii. Synthesis of a homotelechelic lipid/lipid PI (PIMonoLipLip)

The first PI/Lipid hybrid synthesized was a PI functionalized at each chain-end by one fatty ester. Its multi-step synthesis is described in the following paragraph.

As various fatty acids will be used later in the manuscript, a precision is given about the denomination:

- $\text{PIMonoLip}$, $\text{PIMonoLipLip}$, $\text{PIDiLip}$ or $\text{PIDiLipLip}$ represents the molecule in general without any precision about the fatty chains linked.
- $\text{PIMonoC}_{n:p}$, $\text{PIMonoC}_{n:p}C_{n:p}$, $\text{PIDiC}_{n:p}$ or $\text{PIDiC}_{n:p}C_{n:p}$ represents a specific molecule following the nomenclature of fatty acids where « n » is the number of carbon of the lipidic backbone and p the number of unsaturation of the lipid. As an example, $\text{PIDiC}_{16:0}$ is the nomenclature applied for a PI terminated by two palmitic moieties at the same chain end.

The PIMonoLip, like the PIMal, were obtained by the esterification of a PIOH with fatty acyl chlorides (Figure 13). The $^1$H NMR spectrum obtained after the esterification is given in Figure 14 (here in the case of a PIMonoC$_{24:0}$). The shift of signal 8 (3.63 ppm) to 8’ (4.03 ppm) corresponding to the “CH$_2$” group in α position of the chain-end as well as the appearance of the signal 11’ (0.88 ppm) corresponding to the terminal “CH$_3$” group of the fatty chain and of signal 9’ (2.26 ppm) corresponding to the “CH$_2$” group in α position of the ester carbonyl attest that the reaction is quantitative.
In order to graft a fatty chain at the other chain-end of the PIMonoLip formed, the terminal ketone was first selectively reduced into a hydroxyl function with NaBH₄. This reaction afforded a heterotelechelic hydroxyl/lipid PI (PIMonoLipOH). Again, the conditions had to be optimized. Contrarily to the literature where the reaction was performed in pure THF⁴, MeOH was needed as co-solvent in order to have a better solubilization of the reducing agent and thus reach full conversion in 1h at room temperature. Indeed, it can be seen on the ¹H NMR spectrum (Figure 15 showing the spectrum obtained in the case of a PIMonoC₂₄:₀OH) the total disappearance of signal 2 (2.43 ppm) corresponding to the “CH₂” group in α position of the ketone function and the appearance of signal 12’ (3.80 ppm) corresponding to the “CH” group in α position of the newly formed hydroxyl function.
Selective Chain-end Functionalization

The integration of the signals from the fatty ester confirmed the selectivity of the reduction as the value of integrals did not change before and after reduction (integral of signal 8’ at 4.03 ppm = 2).

Finally, the last step of this synthesis was the grafting of a second fatty chain at the α chain-end of the PIMonoLipOH previously obtained, via an esterification reaction using fatty acyl chlorides. First, the same reaction conditions as for the synthesis of PIMonoLip were applied. Even after 20h of reaction at room temperature, only about 50% of conversion was reached (Figure 16 presenting the \(^1\)H NMR spectrum obtained in the case of a PIMonoC\(_{24:0}\)C\(_{24:0}\)). The signal 5 (4.82 ppm) corresponding to the “CH” group in α position of the new ester can be observed but the signal noted “*” (3.80 ppm) corresponding to the “CH” group in α position of the hydroxyl from PIMonoLipOH is still visible. TEA was then replaced by DMAP as it is more nucleophilic than TEA. Indeed, a higher nucleophilicity would increase the speed of the substitution of chloride by DMAP and thus render more reactive the lipidic moiety (Figure 17). The DMAP could also activate the alcohol by hydrogen bonding. Furthermore, DMAP will still act as proton trap and thus protect the double bonds from degradation. The \(^1\)H NMR spectrum of PIMonoLipLip obtained by this process is given in Figure 18, presenting the \(^1\)H NMR spectrum of a PIMonoC\(_{24:0}\)C\(_{24:0}\) as an exemple.
Selective Chain-end Functionalization

In this case, the esterification is quantitative regarding the total disappearance of the signal corresponding to the “CH” group in α position of the hydroxyl of PIMonoLipOH (3.80 ppm).

Figure 16: $^1$H NMR spectrum of a PIMonoC$_{24:0}$C$_{24:0}$ obtained after the first attempt of esterification using fatty acyl chloride and TEA.

Figure 17: Proposed mechanical pathway for the effect of DMAP

Figure 18: $^1$H NMR spectrum of a PIMonoC$_{24:0}$C$_{24:0}$ synthesized using DMAP
iv. Synthesis of a heterotelechelic di-lipid/lipid PI (PIDiLipLip)

This synthon is an analogue of PIMonoLipLip but with 2 fatty chains at the ω chain-end of the polymer. Again, the synthesis proceeds in a multistep pathway. Each step will be presented successively. The first step is the reductive amination of the terminal aldehyde of PIDeg by diethanol amine (DEA) (Figure 19). The obtained polymer is a heterotelechelic ketone/di-hydroxyl PI (PIDiOH). This reaction gives access to a PI bearing two reactive hydroxyl functions at one chain-end and is probably one of the most important brick of the overall functional PIs presented in this chapter as it will allow getting closer to Tanaka’s model.

Again, the reaction conditions described in the literature had to be optimized. The solvent was thus changed from a dichloroethane/DMF admixture to dry THF, the temperature was raised from room temperature to 40°C and 4.5 and 4.2 equivalents of diethanolamine and reducing agents were used instead of 2.1 and 2.8 equivalents respectively. In these conditions, the reaction was quantitative as confirmed on $^1$H NMR spectrum (Figure 20) with the total disappearance of signals 8 (9.77 ppm) and 7 (2.48 ppm) corresponding to the aldehydic proton and to the “CH$_2$” group in α position of the aldehyde respectively. The appearance of signals 8’ (2.52 ppm), 9’ (2.64 ppm) and 10’ (3.57 ppm) corresponding to the “CH$_2$” groups in α position of the nitrogen, β and α position of the new hydroxyl group respectively further confirmed the achievement of the reaction. The remaining signal 2 (2.43 ppm) in PIDiOH corresponding to the “CH$_2$” group in α position of the ketone function indicates the selectivity of the reaction. The integration of the new chain-end signals allowed to establish that the hydroxyl functionality of the polymer is 2 as expected. During this study, it was observed that these chain-ends were sensitive towards acidic protons as when the NMR was performed in CDCl$_3$ instead of CD$_2$Cl$_2$, broad signals were observed for the polymer (Figure 21 bottom spectrum) that could be shifted back by the addition of an excess of triethylamine (Figure 21 upper spectrum).
Selective Chain-end Functionalization

This protonation can be observed from the signals $8''$ (3.12 ppm), $9''$ (3.27/3.33 ppm) and $10''$ (4.05 ppm) that are shifted to signals $8'$ (2.52 ppm), $9'$ (2.64 ppm) and $10'$ (3.57 ppm).

Figure 20: $^1$H NMR spectra in CD$_2$Cl$_2$ of PIDeg and PIDiOH

Figure 21: $^1$H NMR spectra in CDCl$_3$ of a PIDiOH protonated (bottom spectrum) and a PIDiOH deprotonated by TEA (upper spectrum).
The second step of the synthesis of PIDiLipLip is the esterification of PIDiOH with fatty acids affording a heterotelechelic ketone/di-lipid PI (PIDiLip). The obtained polymer will present a structure of \( \omega \) chain-end close to Tanaka’s model. The procedure is similar to the one described for PIMonoLip, using fatty acyl chlorides as reactants and TEA as both, a catalyst and a proton trap.

Silica beads functionalized with primary amines had to be used for the purification of the sample as, during the first experiments, it was observed the formation of di-ketene as can be seen from Figure 22. The formation of ketene could happen when acyl chlorides are mixed with TEA due to the basicity of the amine and the acidity of the proton in \( \alpha \) position of the carbonyl group\(^{11}\). The ketene formed can then dimerize to give a 4 membered ring lactone referred to as “di-ketene” (Figure 23). During the purification of the polymer, the di-ketene precipitated also and it was thus not possible to purify the PIDiLip. As primary amines react quickly with di-ketenes\(^{12}\), at the end of the reaction, it was added amine functional beads to trap the diketene and remove them by simple filtration. Figure 24 presents the \(^1\)H NMR spectra comparison between a contaminated PIDiC\(_{24:0}\) before (bottom spectrum) and after (upper spectrum) purification with silica beads. Signals 3 and 4 are no longer visible after the treatment attesting the success of this purification method. With this new purification method, pure compounds could be obtained.

![Figure 22: \(^1\)H NMR spectrum of a PIDiC\(_{24:0}\) polluted by di-ketene formation](image-url)
Selective Chain-end Functionalization

![Chemical pathway for the formation of ketene and its dimerization](image1)

Figure 23: Chemical pathway for the formation of ketene and its dimerization

![Purification of a PIDiC using amino functional beads](image2)

Figure 24: Purification of a PIDiC$_{24:0}$ using amino functional beads (before purification on the bottom).

However, the esterification reaction was quantitative as it can be seen on $^1$H NMR spectrum (Figure 25). Here, in the case of PIDiC$_{24:0}$, signals 8 (2.52 ppm), 9 (2.64 ppm) and 10 (3.57 ppm) were completely shifted to signals 8’ (2.50 ppm), 9’ (2.74 ppm) and 10’ (4.09 ppm) corresponding to the “CH$_2$” groups in α position of the nitrogen atom and in β and α position of the newly ester formed, respectively.
It may be highlighted that signals 9 and 10 (becoming 9’ and 10’) are shifted to higher ppm values, whereas the signal 8 (becoming 8’) is shifted to lower ppm values. This can be attributed to the hydrogen bonding that can be formed between the proton of the hydroxyl group and the free doublet of nitrogen in the case of a tertiary amine (Figure 26)\textsuperscript{13}. It is thus reasonable to expect this hydrogen bonding to have an influence on the shift of the “-CH\textsubscript{2}” group in α position of the nitrogen (PI side), effect that disappears after esterification as no proton is available anymore.

Then, as in the case of PIMonoLipOH, heterotelechelic hydroxyl/di-lipid PIs (PIDiLipOH) were further synthesized with the same procedure (NaBH\textsubscript{4} in THF/MeOH for 1h at RT). The reaction was again quantitative as can be seen on \textsuperscript{1}H NMR spectrum (Figure 27 presenting the \textsuperscript{1}H NMR spectrum obtained in the case of a PIDiC\textsubscript{24:0}OH) with the total disappearance of the signal 2 (2.43 ppm) corresponding to the “CH\textsubscript{2}” group in α position of the ketone chain-end and the appearance of the signal 14’ (3.74 ppm) corresponding to the “CH” group in α position of the newly hydroxyl function formed.
Finally, heterotelechelic lipid/di-lipid PIs (PIDiLipLip) were synthesized with the same chemical procedure as in the case of PIMonoLipLip, affording the desired compound with a quantitative conversion. Indeed, it can be seen on $^1$H NMR spectrum (Figure 28 presenting the $^1$H NMR spectrum obtained in the case of a PIDiC$_{24:0}$C$_{24:0}$) the total disappearance of the signal 5’ (3.74 ppm) corresponding to the “CH” group in α position of the terminal hydroxyl function and the appearance of the signal 5 (4.89 ppm) corresponding to the “CH” group in α position of the new ester group formed. This is further confirmed by the shift of signal 4’ (1.16 ppm) to signal 4 (1.20 ppm) corresponding to the “CH$_3$” group in α position of the new ester formed.
Selective Chain-end Functionalization

v. Synthesis of a hetero-telechelic methylethanolamino/ketone PI (PImOH):

This synthon was synthesized to be used as a macro-initiator in the synthesis of a di-block copolymer PI/Polypeptide in order to mimic the PI/Protein coupling. More information will be given later, in the chapter dedicated to bio-conjugation (chapter V). The reaction conditions are similar to the one used for the synthesis of PIDiOH but changing the amine from DEA to N-methylethanolamine (MEAM) (Figure 29).

The reaction was quantitative as confirmed by $^1$H NMR analysis with the total disappearance of the signal of the aldehydic proton at 9.74 ppm. To further confirm the synthesis of the expected product, HSQC analysis was performed, as the signals corresponding to the “CH$_2$” groups in α position of the ketone (2’ 2.41 ppm) and of the nitrogen atom (PI side) (8’ 2.39 ppm) are overlaid. For sake of clarity, only a zoom of the zone corresponding to the chain-ends is presented in Figure 31 A. It can be seen that signal 8’ (2.39 ppm) obtained from the proton analysis of PImOH couples with two different carbons in HSQC (57.9 ppm and 44.0 ppm). By comparing with the HSQC analysis of the PIdeg (Figure 31 B.), it can be confirmed that the signal at 44.0 ppm corresponds to the carbon of the “CH$_2$” group in α position of the ketone chain-end. The right compound was thus well synthesized and characterized.

Figure 29: Chemical pathway for the synthesis of PImOH

Figure 30: $^1$H NMR spectra of PImOH and PIdeg
Selective Chain-end Functionalization

Figure 31: HSQC analysis of a PImOH (A.) and a PIDeg (B.)

vi. Synthesis of a primary amine terminated PI

1. Reductive amination using ammonium salt: (PINH$_2$)

Comparably to PImOH, PINH$_2$ could be of high interest as a macroinitiator for the synthesis of di-block copolymer PI/Polypeptide as the ROP of NCA was mainly described using primary amine as initiator. Moreover, it was found in the literature that by reacting a primary amine with maleic anhydride it is possible to obtain a maleimide function$^{14}$. Applying this chemistry to PINH$_2$ could therefore generate a maleimide terminated PI thus rendering the polymer backbone functionalizable with proteins via “thiol-maleimide” click chemistry as reported in the general bibliographic part.
To the best of our knowledge, the synthesis of PINH$_2$ has only been reported in literature once via the selective reductive amination of the terminal aldehyde of PIDeg using ammonium acetate. It was reported that PINH$_2$ is not really stable as it can undergo chain-extension when heated up to 40°C. We then attempted to carry out the synthesis of PINH$_2$, either following the described procedure or adapting it (change of solvent or quantity of ammonium acetate). Whatever the solvent or the amount of ammonium acetate, the salt could not be solubilized and the reaction had to be carried out in heterogeneous conditions. The $^1$H NMR spectra obtained for several conditions are given in Figure 32. It is reported in the literature a triplet characteristic of the “-CH$_2$” group in α position of the primary amine around 2.6 ppm. Such a signal was never obtained in our case. Nevertheless, in all conditions, a total disappearance of the aldehydic proton (9.7 ppm) was observed whereas the signal due to “-CH$_2$” protons in α position of the ketone chain-end (2.4 ppm) remained or slightly decreased in intensity. On spectrum B, two new signals were observed (3.6 and 3.4 ppm respectively) which did not fit with the shift presented in the literature. The $^1$H NMR analysis thus showed no evidence that a PINH$_2$ was obtained but showed that some reaction occurred at the aldehyde chain-end, rather selectively regarding the integration of the “-CH$_2$” group in α position of the ketone chain-end.

Figure 32: $^1$H NMR spectra obtained after 4 different attempts of synthesis of PINH$_2$: A [PI] = 20 mmol/L, [NH$_4$OAc] = 600 mmol/L, [NaBH(OAc)$_3$] = 56 mmol/L, [AcOH] = 20 mmol/L; B : Same conditions as A but decreasing the quantity of ammonium acetate (200 mmol/L); C : Same conditions as A but changing the solvent from DCM to THF; D : Same conditions as A but changing the solvent from DCM to dichloroethane (DCE).
Selective Chain-end Functionalization

By analysing these polymers in SEC, it can be seen for the attempts B, C and D, an increase of the molar mass (Table 1). This suggests that during the synthesis, the polymer undergoes side reactions increasing its molar mass. The main hypothesis to explain this increase and the disappearance of the aldehydic proton in NMR would be that, due to the low solubility of the ammonium salt, whenever a first primary amine is formed, it preferentially reacts either with another aldehyde or ketone chain-end of another polymer chain thus explaining the increase of the molar mass (Figure 33).

Table 1: Molar mass and dispersity values obtained for the four attempts of PINH$_2$ synthesis

<table>
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<th>Attempt</th>
<th>$M_n$ (g/mol)$^a$</th>
<th>$D^a$</th>
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<td>PIDeg</td>
<td>8 000</td>
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</tr>
<tr>
<td>A</td>
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<td>B</td>
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<tr>
<td>C</td>
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<tr>
<td>D</td>
<td>32 000</td>
<td>1,3</td>
</tr>
</tbody>
</table>

$^a$: Obtained by SEC in THF

Figure 33: Proposed side-reaction pathway faced during the synthesis of PINH$_2$
Selective Chain-end Functionalization

Finally, as the synthon could never be synthesized properly, this pathway was abandoned. Nevertheless, as aminated PI could be a key molecule, we investigated another possibility to obtain the polymer.

2. **Diels-Alder reaction using furfurylamine (PIDA)**

It was proposed to use Diels-Alder chemistry between furfurylamine and PiMal to afford the desired amine terminated polymer. As a first step, a model reaction was studied between MalHex and furfurylamine (Figure 34).

![Figure 34: Diels-Alder model reaction studied for the synthesis of PIDA](image)

Multiple NMR analyses (\(^1\)H, \(^{13}\)C, \(^{13}\)C-DEPT 135, HSQC, HMBC) were compulsory for the characterization of the product of the reaction and revealed that the obtained molecule was different from the expected one (Figure 36 to Figure 39). The main difference comes from the signals 6a and 6b (on Figure 36, 2.83 and 2.44 ppm respectively) which were determined by \(^{13}\)C–DEPT 135 to be protons from a “CH\(_2\)” group not existing in the expected molecule. Furthermore, by looking in the literature, the same side reaction had already been described\(^{15}\). This behavior is specific from the use of furfurylamine in DA conditions. However, it was shown that when the primary amine was turned into an amide, the targeted DA adduct could be formed. As the primary amine is the function targeted in our case, this reaction was also abandoned and not applied to PI. The structure of the undesired compound formed is given in Figure 35.

![Figure 35: Structure of the unexpected compound formed](image)
Figure 36: $^1$H NMR spectrum of the product obtained after DA reaction

Figure 37: $^{13}$C NMR spectrum of the product obtained after DA reaction
vii. **Synthesis of a heterotelechelic di-lipid/maleimide PI (PIDiLipMal)**

All the chemistries developed in this sub-chapter were finally combined to obtain a PI functionalized in α and ω positions by a maleimide and two fatty esters respectively. This molecule is the key-stone to obtain the desired “tri-block” of Tanaka’s model as one chain-end is already functionalized with two lipids and the maleimide can be used for the grafting of a protein via thiol-maleimide “click chemistry”. This molecule was then crucial for the rest of the project.
Selective Chain-end Functionalization

The easiest way to obtain PiDiLipMal would be the esterification of a PIDiLipOH using a maleimide acyl chloride and DMAP as catalyst regarding the results obtained for the synthesis of PIDiLipLip (or PIMonoLipLip). Unfortunately, in this case, an unexpected pink coloration of the polymer as well as an increase of the molar mass (even reaching 300 000 g/mol while starting with a 10 000 g/mol PIDiLipOH) were observed. Moreover, $^1$H NMR analysis revealed that most of the maleimide double bond was consumed with the disappearance of the corresponding signal at 6.66 ppm. Two different approaches were thus considered: either the protection of the maleimide double bond prior to esterification (Figure 40 A.), or a more powerful esterification pathway as the one using MalChlo and DMAP might be too slow compared to the degradation of the maleimide group (Figure 40 B.). The use of furan for the protection of the maleimide function was already described in the literature.\textsuperscript{16} The reaction between both functions is reported to be generally fast and quantitative and the deprotection facilitated by the fact that the boiling point of furan is low (~ 30°C) compared to the temperature of deprotection (usually ~ 130°C). The furan can thus be easily removed from the reaction media, by evaporation, during the deprotection process. However, it increases the number of steps to afford the desired compound and presents a risk, during deprotection, of cyclization from the PI double bounds as the polymer cannot handle high temperature for too long. Two deprotection pathways were then investigated, one in bulk using vacuum to remove the furan generated and one in toluene solution distilling the furan.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure40.png}
\caption{Protection (A.) and direct esterification (B.) pathways proposed for the synthesis of PiDi(or mono)LipMal}
\end{figure}
The first step was thus the synthesis of a protected maleimide (MalProt). As $^1$H and $^{13}$C NMR spectra were difficult to assign directly, various NMR characterizations (COSY, HSQC, HMBC not presented here) were used to determine the exact structure of the compound. It appeared that, as could be expected, both “endo” (noted “+” in Figure 41 and Figure 42) and “exo” isomers were formed. The reaction was found to be quantitative with the total disappearance of the signal corresponding to the double bond of the maleimide (6.66 ppm) and the appearance of two new signals (6.37 and 6.49 ppm) both corresponding to the new double bond formed by the DA reaction for the endo and exo compound respectively. About 60% of “endo” compound was obtained.

**Figure 41:** $^1$H NMR spectrum of the compound obtained after furan protection of MalHex

**Figure 42:** $^{13}$C NMR spectrum of the compound obtained after furan protection of MalHex
Concerning the esterification, the reaction proceeded via Steglich esterification using dicyclohexylcarbodiimide (DCC) and DMAP as catalysts to afford a heterotelechelic MalProt/di-lipid PI (PIDiLipMalProt). This reaction was very effective as, on the $^1$H NMR spectrum (Figure 43), the total shift of signal 13 (3.74 ppm) corresponding to the “CH” group in α position of the terminal hydroxyl group of PIDiLipOH to signal 13’ (4.89 ppm) after esterification, corresponding to the same “CH” group but, now, in α position of an ester function was observed.

![Figure 43: $^1$H NMR spectrum obtained after after the esterification of a PIDiLipOH with MalProt](image)

Finally, the deprotection of the maleimide function was investigated to obtain a heterotelechelic maleimide/di-lipid PI (PIDiLipMal). The deprotection in “bulk” was the first one to be tested. Various reaction times and temperatures were tried but it appeared that going over 140°C, the polymer presented cross-linking signs (total or partial insolubility in solvent, coloration, increase of viscosity). A partial deprotection (up to 70% calculated by $^1$H NMR) was observed for a heating at 120°C for 20 minutes without any visible sign of cross-linking.
Increasing the heating time up to 40 minutes (still at 120°C) allowed a nearly total deprotection (about 95% calculated by $^1$H NMR analysis, Figure 44) even if a small signal corresponding to the protected form was still visible (signal noted “$^*$” on the NMR analysis). But, these conditions also led to a broadening of the SEC trace (Figure 45). This might be due to cross-linking occurring via side reaction(s) with the double bonds of PI. It was thus thought that addition of solvent was mandatory to avoid this problem.

Figure 44: $^1$H NMR spectrum of a PIDiLipMal obtained by bulk deprotection

Figure 45: SEC chromatogram of a PIDiLipMal obtained by deprotection in bulk
Selective Chain-end Functionalization

The deprotection in solution appeared to be more suitable to reach the targeted functional polymer (Figure 46 and Figure 47). Indeed, the rate of deprotection is about 90% (calculated by $^1$H NMR analysis) and no sign of cross-linking can be observed on the SEC trace. Even if this deprotection pathway can still be optimized, it represents a powerful manner to obtain a PIDiLipMal without any side reaction.

Figure 46: $^1$H NMR spectrum of a PIDiLipMal obtained by solution deprotection

Figure 47: SEC chromatogram of a PIDiLipMal obtained by deprotection in solution and by direct esterification compared with the starting PIDiLipOH

In order to avoid the problems encountered during the deprotection step, the direct esterification pathway was also tested. Furthermore, Steglich esterification was demonstrated to be a powerful reaction (PIDiLipMalProt) using low amount of catalyst (DMAP) without generation of acid (like with acyl chlorides) thus preventing any damage of the PI backbone.
Selective Chain-end Functionalization

However, it still implies to keep in contact DMAP and the double bound of maleimide which are suspected to be the reason of the pink polymer and cross-linking faced at the beginning of this sub-chapter. This reaction was not optimized and huge quantities of reactants were used in order to complete the reaction quickly and avoid thus side reactions between maleimide and DMAP.

In 40 seconds, a rate of esterification of about 85% (calculated by $^1$H NMR analysis through the appearance of the signal at 4.89 ppm corresponding to the “CH” group in α position of the newly ester bond) can be reached with the presence of residual PIDiLipOH (Figure 48 indicated by a “*”) but without any visible cross-linking according to the SEC traces showed in Figure 47. Contrarily to the previous method, the 15% residual PIDiLipOH still contain a hydroxyl function that could possibly interfere in the coupling with proteins.

Figure 48: $^1$H NMR spectrum of a PIDiLipMal obtained by direct esterification pathway
Selective Chain-end Functionalization

IV. Conclusion

In conclusion, this chapter described the synthesis of diverse functional and functionalized PIs without particularly focusing on their application that will be the scope of the coming chapters. All the structures obtained are given in Table 2 as well as experimental values of integrals for various PI synthons in Table 3. Taking advantage of the selectivity of some reactions or reactants, it was possible to obtain hetero-telechelic PIs both functionalized with two lipids at one chain-end and with a maleimide moiety at the other one. This particular synthon is postulated to be the keystone leading to the final tri-block copolymer of Tanaka by using the “thiol-maleimide” click-chemistry between cysteine and the maleimide chain-end. This coupling chemistry will be developed in the last chapter of the manuscript. Else, PIDiOH and PImOH were synthesized to be used as macro initiators of a polypeptide block which will be also discussed in the last chapter of the manuscript. Finally, diverse architectures of PI/Lipid hybrids were synthesized varying the number of fatty esters linked. The thermo-mechanical properties obtained with these structures will be developed in the next chapter.
### Table 2: Summary of all the PI synthesized

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<th>Chemical structure</th>
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### Selective Chain-end Functionalization

![Chemical Structures]

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<td><img src="image5" alt="Structure" /></td>
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<tr>
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<td><img src="image6" alt="Structure" /></td>
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#### α-lipid / ω-lipid

<table>
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<th>Chain-ends</th>
<th>Chain-end Functionalization</th>
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</thead>
<tbody>
<tr>
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#### α-maleimide / ω-lipid

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#### Table 3: Experimental values of integrals for various PI synthons

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<th>Name</th>
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<th>Chain-ends</th>
<th>Integration</th>
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<td>PiDeg</td>
<td>-CH=CCH&lt;sub&gt;3&lt;/sub&gt;-</td>
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<td>-CHO</td>
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<tr>
<td>PIOH</td>
<td>-CH=CCH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>140</td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;OH</td>
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<td></td>
<td></td>
<td></td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;COCH&lt;sub&gt;3&lt;/sub&gt;</td>
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<tr>
<td>PIDiOH</td>
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<td>143</td>
<td>-CH&lt;sub&gt;2&lt;/sub&gt;COCH&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>-CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;</td>
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</table>
Selective Chain-end Functionalization

V. Experimental part

a. Synthesis of fatty acyl chlorides

Figure 49: General structure of fatty acyl chlorides

A solution of the desired fatty acid was prepared in dry DCM ([fatty acid] = 0.5M). Oxalyl chloride (3 eq) was then added to the mixture under vigorous stirring followed by 3 drops of DMF. A vigorous bubbling was observed. The reaction conversion was followed by connecting the reaction flask to a bubbler containing a KOH solution. When no bubbling was still visible, the reaction mixture was evaporated under vacuum to remove the solvent and the excess of oxalyl chloride. The final product was then dried overnight at 40°C under dynamic vacuum affording a yellowish liquid for $\text{C}_{18:0}\text{Chlo}$ and $\text{C}_{14:0}\text{Chlo}$ and a white powder for $\text{C}_{24:0}\text{Chlo}$. Yield: >90%.

$\text{C}_{14:0}\text{Chlo}$: $^1\text{H}$ NMR (CDCl$_3$) $\delta$ (ppm): 2.87 (t, 2H, CH$_2$CCl), 1.70 (m, 2H, CH$_2$CH$_2$Cl), 1.25 (s, 20H, CH$_2$-fatty chain), 0.88 (t, 3H, CH$_3$CH$_2$); $^{13}\text{C}$ NMR (CDCl$_3$) $\delta$ (ppm): 174.21 (1C, COCl), 47.28 (1C, COCH$_3$), 31.93 (1C, COCH$_2$CH$_2$), [29.65, 29.64, 29.61, 29.52, 29.36, 29.33, 29.07, 28.43, 25.07, 22.71] (10C, CH$_2$-fatty chain), 14.11 (1C, CH$_2$CH$_3$).

$\text{C}_{18:0}\text{Chlo}$: $^1\text{H}$ NMR (CDCl$_3$) $\delta$ (ppm): 2.87 (t, 2H, CH$_2$CCl), 1.70 (m, 2H, CH$_2$CH$_2$Cl), 1.25 (s, 28H, CH$_2$-fatty chain), 0.88 (t, 3H, CH$_3$CH$_2$); $^{13}\text{C}$ NMR (CDCl$_3$) $\delta$ (ppm): 174.21 (1C, COCl), 47.28 (1C, COCH$_3$), 31.93 (1C, COCH$_2$CH$_2$), [29.81, 29.75, 29.66, 29.52, 29.46, 29.21, 28.57, 25.20, 22.83] (14C, CH$_2$-fatty chain), 14.25 (1C, CH$_2$CH$_3$).

$\text{C}_{24:0}\text{Chlo}$: $^1\text{H}$ NMR, (CDCl$_3$) $\delta$ (ppm): 2.87 (t, 2H, CH$_2$CCl), 1.70 (m, 2H, CH$_2$CH$_2$Cl), 1.25 (s, 40H, CH$_2$-fatty chain), 0.88 (t, 3H, CH$_3$CH$_2$); $^{13}\text{C}$ NMR (CDCl$_3$) $\delta$ (ppm): 174.21 (1C, COCl), 47.28 (1C, COCH$_3$), 31.93 (1C, COCH$_2$CH$_2$), [29.86, 29.76, 29.68, 29.52, 29.49, 29.22, 28.59, 25.21, 22.86] (20C, CH$_2$-fatty chain), 14.28 (1C, CH$_2$CH$_3$)
Selective Chain-end Functionalization

b. Synthesis of PIMal

i. Synthesis of PIOH

![Structure of PIOH](image)

PIDeg (2 g, 0.2 mmol of aldehyde function) was dissolved in 5 mL of dry THF. Triacetoxyborohydride (0.19 g, 4 eq, 0.8 mmol) were then added to the obtained solution as well as 12 µL (1 eq, 0.2 mmol) of acetic acid. The reaction mixture was stirred at 40°C overnight and the final product was obtained after two successive precipitations into a large excess of cold methanol, solubilized in Et₂O, filtrated through Celite® and dried overnight at 40°C under dynamic vacuum. Yield: ~ 90%

^1^H NMR (CDCl₃) δ (ppm): 5.12 (t, 1H, CHCH₃), 3.62 (t, 2H, CH₂OH), 2.42 (t, 2H, CH₂COCH₃), 2.05 (s, 4H, CH₂CH / CH₂CCH₃), 1.67 (s, 3H, CH₃CCH)

ii. Synthesis of MalChlo

![Structure of MalChlo](image)

MalHex (0.5 g, 0.05 mmol) was solubilized into 3 mL of dry DCM. Oxalyl chloride (0.4 mL, 2 eq, 0.1 mmol) was then added under flux of argon as well as few drops of DMF. A vigorous bubbling was observed. The reaction conversion was followed by connecting the reaction flask to a bubbler containing a KOH solution. When no bubbling was still visible, the reaction mixture was evaporated under vacuum to remove the solvent and the excess of oxalyl chloride. The final product was then dried overnight under dynamic vacuum affording an orange powder. Yield: > 90%
Selective Chain-end Functionalization

$^1$H NMR (CDCl$_3$) δ (ppm): 6.68 (s, 2H, $CH_{\text{double bond}}$), 3.51 (t, 2H, $CH_2N$), 2.87 (t, 2H, $CH_2COCl$), 1.72 (q, 2H, $CH_2CH_2COCl$), 1.60 (q, 2H, $CH_2CH_2N$), 1.33 (m, 2H, $CH_2CH_2CH_2$) ; $^{13}$C NMR (CDCl$_3$) δ (ppm): 173.12 (1C, $COC$), 170.9 (2C, $CO_{\text{cycle}}$), 134.22 (2C, $CH_{\text{double bond}}$), 46.95 (1C, $CH_2COCl$), 37.48 (1C, $CH_2N$), 28.14 (1C, $CH_2CH_2COCl$), 25.63 (1C, $CH_2CH_2N$), 24.60 (1C, $CH_2CH_2CH_2$)

iii. Synthesis of PIMal

![Structure of PIMal](image)

PIOH (1 g, 0.1 mmol) was solubilized in 4 mL of dry THF. Dry TEA (50 µL, 4 eq, 0.4 mmol) was then added to the polymer solution followed by 51 mg (2 eq, 0.2 mmol) of MalChlo. A precipitate was quickly observed and the heterogeneous media stirred at room temperature for 1h. The final polymer was recovered after two successive precipitations into a large excess of cold methanol, solubilization in Et$_2$O, filtration through Celite® and overnight drying under dynamic vacuum at room temperature. Yield: ~ 80%

$^1$H NMR (CDCl$_3$) δ (ppm): 6.68 (s, 2H, $CH_{\text{double bond}}$), 5.12 (t, 1H, $CHCCH_3$), 4.02 (t, 2H, $CH_2OCO$), 3.51 (t, 2H, $CH_2N$), 2.42 (t, 2H, $CH_2COCH_3$), 2.27 (t, 2H, $CH_2COO$), 2.05 (s, 4H, $CH_2CH / CH_2CCH_3$), 1.67 (s, 3H, $CH_3CCH$)

c. Synthesis of PIMonoLipLip

i. Synthesis of PIMonoLip

![General structure of a PIMonoLip](image)

PIOH (1 g, 0.1 mmol) was dissolved in 4 mL of dry THF. Dry TEA (60 µL, 4 eq, 0.4 mmol) was added followed by 2 eq (0.2 mmol) of the desired acyl chloride. After 1 h of reaction, 3-aminopropyl-functionalized silica particles were added (~1 NH$_2$ eq toward acyl chloride) and the obtained mixture was stirred for 2h.
Selective Chain-end Functionalization

The final polymer was obtained after two successive precipitations into a large excess of cold methanol, solubilizing in Et₂O, filtration through Celite® and overnight drying at 40°C under dynamic vacuum. Yield: ~ 80%,

\[ ^1H \text{NMR (CDCl}_3) \delta (ppm): 5.12 \,(s, \, 1H, \, CHCCH}_3), \, 4.03 \,(t, \, 2H, \, CH_2OCO), \, 2.43 \,(t, \, 2H, \, CH_2CCH}_3), \, 2.26 \,(t, \, 2H, \, OCOCH}_2) \, 2.05 \,(s, \, 4H, \, CH_2CH / CH_2CCH}_3), \, 1.67 \,(s, \, 3H, \, CH_3CCH), \, 1.26 \,(s, \, CH_2fatty\_chain), \, 0.88 \,(t, \, 3H, \, CH_2CH_3) \]

\[ ii. \, \text{Synthesis of PIMonoLipOH} \]

PIMonoLip (1 g, 0.1 mmol) was solubilized in 3.7 mL of a MeOH/THF admixture (10/90 v/v%). NaBH₄ (21 mg, ~5 eq, 0.5 mmol) was then added to the solution. A strong degassing was observed and the reaction allowed to proceed at room temperature for 1h. The polymer was then recovered by two successive precipitations in cold methanol followed by a solubilization in Et₂O, filtration through Celite® and overnight drying at 40°C under dynamic vacuum. Yield ~ 85%.

\[ ^1H \text{NMR (CDCl}_3) \delta (ppm): 5.12 \,(t, \, 1H, \, CHCCH}_3), \, 4.03 \,(t, \, 2H, \, CH_2OCO), \, 3.80 \,(m, \, 1H, \, CHCH}_3OH), \, 2.28 \,(t, \, 2H, \, CH_2COO), \, 2.05 \,(s, \, 4H, \, CH_2CH / CH_2CCH}_3), \, 1.67 \,(s, \, 3H, \, CH_3CCH), \, 1.25 \,(s, \, CH_2fatty\_chain), \, 1.18 \,(d, \, 3H, \, CH_3CHOH), \, 0.88 \,(t, \, 2H, \, CH_3CH_2) \]

\[ iii. \, \text{Synthesis of PIMonoLipLip} \]

PIMonoLipOH (0.1 g, 0.01 mmol) was dissolved in 0.7 mL of dry THF as well as 11 mg of DMAP (10 eq, 0.1 mmol). The desired fatty acyl chloride (4 eq, 0.04 mmol) was then added to the solution and the reaction allowed to proceed at 40°C for 3 h.
Selective Chain-end Functionalization

3-aminopropyl-functionalized silica particles were added (~1 NH$_2$ eq toward acyl chloride) and the obtained mixture was stirred for 2 h at room temperature. The final polymer was then recovered by two successive precipitations into a large excess of methanol, solubilization in Et$_2$O, filtration through Celite® and drying overnight at 40°C under dynamic vacuum. Yield ~ 80%.

$^1$H NMR (CDCl$_3$) δ (ppm): 5.12 (t, 1H, CHCCH$_3$), 4.82 (m, 1H, CHOCH$_3$), 4.02 (t, 2H, CH$_2$OCO), 2.21 (t, 2H, CH$_2$COOCH$_2$), 2.18 (t, 2H, CH$_2$COOCHCH$_3$), 1.96 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.60 (s, 3H, CH$_3$C), 1.18 (s, CH$_2$ fatty chain), 0.80 (t, 6H, (CH$_2$CH$_3$)$_2$)

d. Synthesis of PIDiLipLip

i. Synthesis of PIDiOH

![Figure 56: Structure of PIDiOH](image)

PIDeg (3.7 g, 0.37 mmol) was dissolved in 10 mL of dry THF. Diethanolamine (0.19 g, 4.5 eq, 1.6 mmol) was then added and the reaction was stirred at 40°C for 2 h. Finally, 0.36 g (4.2 eq, 1.55 mmol) of sodium triacetoxyborohydride were added to the reaction mixture followed by 30µL (1.3 eq, 0.48 mmol) of acetic acid and the non-homogeneous solution was stirred at 40°C overnight. The reaction mixture was then directly precipitated into a large excess of cold methanol under vigorous stirring. The methanol was then removed and the polymer dissolved in DCM and precipitated again in cold methanol. The polymer was then dissolved in Et$_2$O, filtrated on Celite® and dried under dynamic vacuum at 40°C overnight affording a colorless viscous liquid. Yield: ~ 85%

$^1$H NMR (CD$_2$Cl$_2$) δ (ppm): 5.14 (s, 1H, CHCCH$_3$), 3.57 (t, 4H, (CH$_2$OH)$_2$), 2.64 (t, 4H, N(CH$_2$)$_2$), 2.52 (t, 2H, CH$_2$N), 2.42 (t, 2H, CH$_2$COCH$_3$), 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_3$CH)
Selective Chain-end Functionalization

ii. Synthesis of PIDiLip:

![Figure 57: general structure of PIDiLip](image)

PIDiOH (2 g, 0.2 mmol) was dissolved in 7 mL of dry THF. Dry TEA (180 µL, 6 eq, 1.2 mmol) was then added followed by 3 eq (0.6 mmol) of the desired acyl chloride. After 1 h of reaction, 3-aminopropyl-functionalized silica particles were added (~1 NH$_2$ eq toward acyl chloride) and the obtained mixture was stirred for 2 h. The reaction media was then precipitated in a large excess of cold methanol, dissolved in Et$_2$O, filtered through Celite® and dried overnight at 40°C under vacuum. The final compound was a colorless viscous liquid except for PIDiC$_{24:0}$ which was a colorless paste. Yield: ~ 80%

$^1$H NMR (CD$_2$Cl$_2$) $\delta$ (ppm): 5.12 (s, 1H, CHCCH$_3$), 4.09 (t, 4H, (CH$_2$OCO)$_2$), 2.74 (t, 4H, N(CH$_2$)$_2$), 2.51 (t, 2H, CH$_2$N), 2.43 (t, 2H, CH$_2$CCH$_3$), 2.26 (t, 4H, OCOCH$_2$) 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_2$CCH), 1.26 (s, CH$_2$, fatty chain), 0.88 (t, 6H, CH$_2$CH$_3$).

iii. Synthesis of PIDiLipOH

![Figure 58: General structure of PIDiLipOH](image)

PIDiLip (1 g, 0.1 mmol) was solubilized in 3.7 mL of a MeOH/THF admixture (10/90 v/v%). NaBH$_4$ (21 mg, ~5 eq, 0.5 mmol) was then added to the polymer solution. A strong degassing was observed and the reaction allowed to proceed at room temperature for 1h. The polymer was then recovered by two successive precipitations into a large excess of cold methanol followed by a solubilization in Et$_2$O, filtration through Celite® and overnight drying at 40°C under dynamic vacuum. Yield ~ 85%.
Selective Chain-end Functionalization

$^1H$ NMR (CD$_2$Cl$_2$) $\delta$ (ppm): 5.12 (s, 1H, CHCCH$_3$), 4.09 (t, 4H, (CH$_2$OCO)$_2$), 3.74 (m, 1H, CHOCH$_3$), 2.74 (t, 4H, N(CH$_2$)$_2$), 2.51 (t, 2H, CH$_2$N), 2.26 (t, 4H, (OCOCH$_2$)$_2$), 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_3$CCH), 1.26 (s, CH$_2$, fatty chain), 1.16 (d, 3H, CH$_3$CHOH), 0.88 (t, 6H, CH$_2$CH$_3$).

iv. **Synthesis of PIDiLipLip**

![Figure 59: General structure of PIDiLipLip](image)

PIDiLipOH (0.1 g, 0.01 mmol) was dissolved in 0.7 mL of dry THF as well as 11 mg of DMAP (10 eq, 0.1 mmol). The desired acyl chloride (4 eq, 0.04 mmol) was then added to the polymer solution and the reaction allowed to proceed at 40°C for 3 h. 3-aminopropyl-functionalized silica particles were added (~1 NH$_2$ eq toward acyl chloride) and the obtained mixture was stirred for 2 h at room temperature. The final polymer was then recovered by two successive precipitations into a large excess of methanol, solubilization in Et$_2$O, filtration through Celite® and drying overnight at 40°C under dynamic vacuum. **Yield:** ~80%.

$^1H$ NMR (CD$_2$Cl$_2$) $\delta$ (ppm): 5.12 (s, 1H, CHCCH$_3$), 4.89 (m, 1H, CHOCH$_3$), 4.09 (t, 4H, (CH$_2$OCO)$_2$), 2.74 (t, 4H, N(CH$_2$)$_2$), 2.51 (t, 2H, CH$_2$N), 2.43 (t, 2H, CH$_2$CCH$_3$), 2.28 (t, 4H, (OCOCH$_2$)$_2$), 2.25 (t, 2H, CH$_2$COOCHCH$_3$), 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_3$CCH), 1.26 (s, CH$_2$, fatty chain), 1.20 (d, CH$_3$CHOCH), 0.88 (t, 9H, CH$_2$CH$_3$).

e. **Synthesis of PImOH**

![Figure 60: Structure of PImOH](image)

PIDeg (1 g, 0.1 mmol) was dissolved in 3 mL of dry THF. N-methylethanolamine (33 mg, 4.5 eq, 0.45 mmol) was then added and the reaction was stirred at 40°C for 2 h.
Selective Chain-end Functionalization

Finally, 0.12 g (4.2 eq, 0.42 mmol) of sodium triacetoxyborohydride were added to the reaction mixture followed by 10 µL (1.3 eq, 0.13 mmol) of acetic acid and the non-homogeneous solution was stirred at 40°C overnight. The final polymer was obtained after two successive precipitations into a large excess of cold methanol, solubilization in Et₂O, filtration through Celite® and drying overnight at 40°C under dynamic vacuum. Yield: ~ 85%

\[ ^1H \text{ NMR (CD}_2\text{Cl}_2) \delta (ppm): 5.12 (t, 1H, CHCCH}_3), 3.25 (t, 2H, CH}_2OH), 2.50 (t, 2H, CH}_2CH}_2OH), 2.41 (t, 2H, CH}_2COCH}_3), 2.39 (t, 2H, CH}_2N), 2.04 (s, 4H CH2CH / CH}_2CCH}_3), 1.67 (s, 3H, CH}_3CCH) \]

f. Synthesis of a heterotelechelic ketone/amine PI (PINH\(_2\))

![Structure of PINH\(_2\)](image)

PlDeg (1 g, 0.1 mmol) was solubilized in 5 mL of DCM. Ammonium acetate (0.25 g, 30 eq, 3 mmol) was then added to the polymer solution as well as 65 mg (2.8 eq, 0.28 mmol) of NaBH(OAc)\(_3\) and 6 µL (1 eq, 0.1 mmol) of glacial acetic acid. The reaction was then stirred overnight at room temperature and the final polymer recovered after two successive precipitations into a large excess of cold methanol, solubilization in Et₂O, filtration through Celite® and dried overnight at room temperature under dynamic vacuum.

g. DA reaction between MalHex and Furfurylamine (MalDAAm)

![Structure of MalDAAm](image)

MalHex (0.1 g, 0.47 mmol) was solubilized into 1 mL of DCM and Furfurylamine (0.46 g, 10 eq, 4.7 mmol) was then added to the solution.
Selective Chain-end Functionalization

After stirring at room temperature overnight, the final compound was obtained by evaporation of the solvent and the excess of furfurylamine under dynamic vacuum.

Characterization of the undesired compound formed:

$^1$H NMR (CDCl$_3$): $\delta$ (ppm) 7.36 (d, 1H, CHONH), 6.30 (m, 1H, CH$_{\text{double bond}}$), 6.22 (m, 1H, CH$_{\text{double bond}}$), 3.84 (m, 2H, CH$_2$NHC), 3.70 (m, 1H, CHCOCH$_2$), 3.45 (t, 2H, CH$_2$N), 2.83/2.44 (dd, 2H, CH$_2$COCH), 2.18 (t, 2H, CH$_2$COOH), 1.53 (m, 4H, CH$_2$CH$_2$N / CH$_2$CH$_2$COOH), 1.27 (m, 2H, CH$_2$CH$_2$CH$_2$)

$^{13}$C NMR (CDCl$_3$): $\delta$ (ppm) 178.49 (1C, COOH), 177.83 (1C, COCH), 175.43 (1C, COCH$_2$), 152.26 (1C, CHCH$_2$), 142.59 (1C, CHNHCH), 110.56 (1C, CH$_{\text{double bond}}$), 108.24 (1C, CH$_{\text{double bond}}$), 55.06 (1C CHCO), 44.15 (1C, CH$_2$NH), 37.81 (1C, CH$_2$N), 36.13 (1C, CH$_2$CO), 35.11 (1C, CH$_2$COOH), 27.38 (1C, CH$_2$CH$_2$N), 26.43 (1C, CH$_2$CH$_2$COOH), 24.82 (1C, CH$_2$CH$_2$CH$_2$)

h. Synthesis of PDiLipMal

i. Synthesis of MalProt

MalHex (0.5 g, 2.36 mmol) was solubilized into 2.3 mL of DCM. Furan (1.6 mL, 10 eq, 23.6 mmol) was then added to the solution and the admixture stirred at room temperature overnight. The final compound was obtained by evaporation of the solvent and the excess of furan under dynamic vacuum at 40°C. Yield: 100%.

$^1$H NMR (CDCl$_3$) $\delta$ (ppm): endo compound: 6.37 (s, 2H, CH$_{\text{double bond}}$), 5.30 (m, 2H, (CHOCHCH$_2$)$_2$), 3.50 (m, 2H, (CHCOCH)$_2$), 3.30 (t, 2H, CH$_2$N), 2.31 (t, 2H, CH$_2$COOH), 1.55 (m, 2H, CH$_2$CH$_2$COOH), 1.43 (m, 2H, CH$_2$CH$_2$N), 1.27 (m, 2H, CH$_2$CH$_2$CH$_2$) exo compound: 6.49 (s, 2H, CH$_{\text{double bond}}$), 5.24 (t, 2H, (CHOCHCH)$_2$), 3.46 (t, 2H, CH$_2$N), 2.82 (s, 2H, (CHCOCH)$_2$), 2.31 (t, 2H, CH$_2$COOH), 1.60 (m, 4H, CH$_2$CH$_2$N / CH$_2$CH$_2$COOH), 1.27 (m, 2H, CH$_2$CH$_2$CH$_2$)
Selective Chain-end Functionalization

$^{13}$C NMR (CDCl$_3$) $\delta$ (ppm): endo compound: 179.98 (1C, COOH), 175.03 (2C, (COCHN)$_2$), 134.3 (2C, CH$_{\text{double bond}}$), 79.37 (2C, (COCHCH)$_2$), 45.8 (2C, (CHCOCH)$_2$), 38.15 (1C, CH$_2$N), 33.57 (1C, CH$_2$COOH), 27.02 (1C, CH$_2$CH$_2$N), 25.96 (1C, CH$_2$CH$_2$CH$_2$), 23.98 (1C, CH$_2$CH$_2$COOH) exo compound: 179.98 (1C, COOH), 176.15 (2C, (COCHN)$_2$), 136.5 (2C, CH$_{\text{double bond}}$), 80.85 (2C, (COCHCH)$_2$), 47.28 (2C, (CHCOCH)$_2$), 38.55 (1C, CH$_2$N), 33.57 (1C, CH$_2$COOH), 27.02 (1C, CH$_2$CH$_2$N), 25.96 (1C, CH$_2$CH$_2$CH$_2$), 23.98 (1C, CH$_2$CH$_2$COOH)

ii. Synthesis of PIDiLipMalProt

Figure 64: General structure of PIDiLipMalProt

PIDiLipOH (0.4 g, 0.04 mmol) was dissolved into 1.5mL of dry THF. MalProt (50 mg, 4 eq, 0.16 mmol) was then added to the polymer solution as well as 40 mg of DCC (4.4 eq, 0.17 mmol) and 4 mg of DMAP (0.8 eq, 0.03 mmol). The reaction solution was then stirred overnight at room temperature. The final polymer was obtained after two successive precipitations into a large excess of cold methanol, solubilization in Et$_2$O, filtration through Celite® and overnight drying at 40°C under dynamic vacuum. Yield: ~ 85%

$^1$H NMR (CD$_2$Cl$_2$) $\delta$ (ppm): endo compound: 6.37 (s, 2H, CH$_{\text{double bond}}$), 5.30 (m, 2H, (CHOCHCH)$_2$), 5.12 (s, 1H, CHCCH$_3$), 4.88 (m, 1H, CHOOCOCH$_3$), 4.09 (t, 4H, (CH$_2$OCO)$_2$), 3.50 (m, 2H, (CHCOCH)$_2$), 3.30 (t, 2H, CH$_2$N), 2.74 (t, 4H, N(CH$_2$)$_2$), 2.51 (t, 2H, CH$_2$N), 2.43 (t, 2H, CH$_2$CCH$_3$), 2.31 (t, 2H, CH$_2$COOH), 2.26 (t, 4H, OCOCH$_2$) 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_2$CCH), 1.26 (s, CH$_2$, fatty chain), 0.88 (t, 6H, CH$_2$CH$_3$), exo compound: 6.49 (s, 2H, CH$_{\text{double bond}}$), 5.24 (m, 2H, (CHOCHCH)$_2$), 5.12 (s, 1H, CHCCH$_3$), 4.88 (m, 1H, CHOOCO), 4.09 (t, 4H, CH$_2$OCO), 3.46 (t, 2H, CH$_2$N), 2.82 (m, 2H, (CHCOCH)$_2$), 2.74 (t, 4H, NCH$_2$), 2.51 (t, 2H, CH$_2$N), 2.43 (t, 2H, CH$_2$CCH$_3$), 2.31 (t, 2H, CH$_2$COOH), 2.26 (t, 4H, OCOCH$_2$) 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_2$CCH), 1.26 (s, CH$_2$, fatty chain), 0.88 (t, 6H, CH$_2$CH$_3$)
Selective Chain-end Functionalization

iii. Synthesis of PIDiLipMal

![General structure of PIDiLipMal](image)

1. Deprotection of PIDiLipMalProt

**Bulk**: 0.1 g of PIDiLipMalProt (0.01 mmol) were stirred and heated up to 120°C for 45 minutes under dynamic vacuum.

**Solution**: 0.1 g of PIDiLipMalProt (0.01 mmol) were dissolved into 10 mL of toluene. The solution was then heated up to 120°C for 2h under stirring, distilling the vapors. The final polymer was recovered by evaporation of the solvent and drying overnight at 40°C under dynamic vacuum.

2. Direct esterification

PIDiLipOH (0.1 g, 0.01 mmol) was solubilized into 1 mL of dry THF. MalHex (30 mg, 10 eq, 0.1 mmol) was then added to the solution as well as 30 mg of DCC (11 eq, 0.11 mmol) and 2 mg of DMAP (1 eq, 0.01 mmol). The reaction mixture was then stirred for 40 seconds at room temperature and then precipitated twice in a large excess of cold methanol. The polymer was then solubilized in Et₂O, filtrated through Celite® and dried overnight at room temperature under dynamic vacuum.

\(^1H\) NMR (CD₂Cl₂): δ (ppm) 6.66 (s, 2H, CH\text{double bond}), 5.12 (s, 1H, CHCCH₃), 4.88 (m, 1H, CHOCOCH₃), 4.09 (t, 4H, (CH₂OCO)₂), 3.48 (m, 2H, CH₂N), 2.74 (t, 4H, N(CH₂)₂), 2.51 (t, 2H, CH₂N), 2.27 (t, 6H, CH₂COO), 2.05 (s, 4H, CH₂CH / CH₂CCH₃), 1.67 (s, 3H, CH₃CCH), 1.26 (s, CH₂, fatty chain), 1.18 (d, CH₃CHO), 0.88 (t, 6H, CH₂CH₃)
Selective Chain-end Functionalization

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(6) Kébir, N. Elaboration de nouveaux polyuréthanes à partir de cis-1,4-oligoisoprènes hétérocarbonyltélécéliques issus de la dégradation contrôlée du cis-1,4-polyisoprène de haute masse. Etude de leurs propriétés mécaniques, thermiques et biocides, 2005.
Polyisoprene / Lipid Coupling
Polyisoprene / Lipid Coupling

I. Introduction

The previous chapter described the synthesis of various PI-Lipid hybrid polymers constituted of a polymeric chain functionalized by one, two or three fatty chains. This chapter will focus first on the thermo-mechanical properties of this new hybrid polymers and the influence of the nature of the fatty chain linked to the polymer. Then, by studying the CCr of those hybrid materials, the influence of linked and free fatty chains on the CCr of PI will be investigated. As those hybrid polymers are close models of NR, this study will probably allow a better understanding of the superior CCr property of the natural polymer toward IR.

II. Chain-end crystallization

a. Study of PIDiLip

DSC analysis was first performed on the PIDiLip to investigate the influence of the grafted fatty chain on the PI crystallization properties. Figure 1 presents the DSC profiles of NR, PIDeg and PIDiOH. Those profiles were recorded as references for the rest of the study. No significant difference between NR and its shorter derivatives was observed. In all cases, a $T_g$ around -63°C was measured.

![Figure 1: DSC thermograms of NR (RRIM 600), a PIDeg and a PIDiOH](image)

When analyzing the various PIDiLip synthesized, several DSC profiles were observed. Depending on the nature of the fatty chain linked, both a crystallization and a melting peak can appear (Figure 2 and Annexes 1 to 6). For the unsaturated fatty ester used (C_{11:1} / C_{18:2}) as well as for the shorter saturated one (C_{14:0}), only a $T_g$ around -65°C was measured, which is close to the value observed for initial NR, PIDeg and PIDiOH.
On the contrary, for longer saturated fatty chains, both a crystallization and a melting peak are observed. The results are also summarized in Table 1.

<table>
<thead>
<tr>
<th>Grafted fatty acid</th>
<th>T&lt;sub&gt;m&lt;/sub&gt;&lt;sup&gt;a)&lt;/sup&gt; (°C)</th>
<th>T&lt;sub&gt;c&lt;/sub&gt;&lt;sup&gt;b)&lt;/sup&gt; (°C)</th>
<th>T&lt;sub&gt;g&lt;/sub&gt;&lt;sup&gt;d)&lt;/sup&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undecenoic acid (C11:1)</td>
<td>- c)</td>
<td>- c)</td>
<td>-65</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>- c)</td>
<td>- c)</td>
<td>-65</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>- c)</td>
<td>- c)</td>
<td>-65</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>-25</td>
<td>-37</td>
<td>-65</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>-11</td>
<td>-18</td>
<td>-64</td>
</tr>
<tr>
<td>Nonadecanoic acid (C19:0)</td>
<td>-5</td>
<td>-13</td>
<td>-64</td>
</tr>
<tr>
<td>Lignoceric acid (C24:0)</td>
<td>22</td>
<td>17</td>
<td>-63</td>
</tr>
</tbody>
</table>

<sup>a)</sup> Melting temperature observed by DSC; <sup>b)</sup> Crystallization temperature observed by DSC; <sup>c)</sup> No crystallization or melting observed by DSC; <sup>d)</sup> Glass transition observed by DSC

Comparison of the crystallization temperatures of the free saturated fatty methyl esters alone with the ones obtained in the case of PiDiLip (Figure 3) showed that the crystallization temperature of the PiDiLips and of the free methyl esters alone varies similarly versus the lipidic chain length, but with a shift to lower values (i.e. free methylC<sub>18:0</sub> alone crystallizes at 40°C while it crystallizes at -18°C when attached to the PI chain-end). Here, the values used for the crystallization temperature of fatty methyl esters alone are data from the supplier.
The trendline associated to the crystallization temperature variation of the PIDiLip was the following:

\[
y = -0.3148x^2 + 19.285x - 264.62
\]

where:
- \( y \) represents the crystallization temperature of a PIDiLip and
- \( x \) represents the size of a saturated fatty ester (in carbon number)

It was postulated that below the glass transition temperature of PI, no crystallization of chain-ends could be observed as the system would be in a glassy state. The previous equation was thus used to determine the “low limit” of size of fatty ester than can be grafted to the polymer backbone and would afford crystallization. The value of \( x \) for \( y = -63^\circ C \) was thus calculated. As the equation is a second order one, it gives two solutions, \( x_1 = 48 \) and \( x_2 = 13 \). Considering the solution \( x_2 \), it would mean that for all the saturated fatty esters bearing less than 13 carbons and linked to a 10 000 g/mol PI, the crystallization temperature of the chain-ends would be below the \( T_g \) of the polymer, thus not visible by DSC. It can be extrapolated that if the melting temperature of a fatty ester alone is below 5°C (melting temperature of the methyl-n-tridecanoate), then it will no more crystallize when attached to a 10 000 g/mol PI chain. Those two limits (13 carbons, and 5°C for the crystallization temperature) well explain the behavior of the non-crystallinity of PIDiC_{11:1}, PIDiC_{18:2} and PIDiC_{14:0}.
Indeed, in the case of unsaturated fatty chains, the crystallization temperature of methylundecenoate and methyllinoleate alone are -24°C and -35°C respectively, far below the limit of 5°C proposed previously. This mean that, while being attached to the PI chain, those esters would crystallize below the $T_g$ of the polymer. The case of PIDiC$\text{14:0}$ can be related to the limit of 13 carbons obtained from the trendline equation. Two hypotheses can be formulated. Either the low limit of 13 is not very accurate due to the fact that it is calculated from a trendline, either as the PIDiC$\text{14:0}$ might possess a crystallization temperature close to the $T_g$, the heat flow used for the analysis ($10^\circ$C/min) was too fast compared to the speed of crystallization of the chain-end and the glass transition was reached prior to the beginning of crystallization of the fatty chains. This could also be related to the case of PIDiC$\text{16:0}$ which crystallizes only partially during the cooling cycle (Annexe 3).

Interestingly, in the case of PIDiC$\text{24:0}$ (Annexe 6), it was possible to observe the crystallization of the material at room temperature ($T_{\text{cryst}} = 17^\circ$C). A total change of viscosity was thus observed with a material behaving more like a paste than a viscous liquid (general behavior of the other 10 000 g/mol PIs). This hybrid was studied by optical microscopy using a heating and cooling plate under polarized light. Figure 4 presents 3 pictures of the same sample submitted to a cooling cycle (from 60°C to 5°C) followed by a heating cycle (from 5°C to 60°C) performed at a heat rate of 10°C/ min in order to be comparable with the DSC program.

The pictures “a” and “c” correspond to the hybrid polymer observed at 60°C and show an amorphous matrix of PI. On the contrary, picture “b” represents the same sample but cooled down to 5°C. Crystallites appeared under the shape of “shinny dots” thanks to the polarized light. The appearance of cristallites explains the changing of behavior of the PIDiC$\text{24:0}$ as compared to the other hybrids. Figure 5 presents our proposition of the self-assembly of a PIDiLip below the crystallization temperature of the lipidic chain-ends.
The nodules of crystallization created by the fatty chains are forming micro-domains anchoring the PI but keeping the ketone chain-end free. This vision of the phenomenon raised the hypothesis of a potential thermal cross-linking functionalizing both α and ω chain-ends by fatty esters. This will be discussed later in this chapter.

Figure 5: Schematic representation of the self assembly of a PIDiLip below its crystallization temperature

b. Variation of the PI chain-length

It was then proposed to investigate the influence of the chain-length of PI on this phenomenon. For that purpose, various PIDiLips were obtained starting either from PIDiOH of 5 000 g/mol or 27 000 g/mol.

i. 5 000 g/mol

As can be seen on Figure 6 (as well as in Table 2 which summarizes the results and on Annexes 7 to 9), the decrease of the PI chain length did not interfere at all in the crystallization and melting phenomena. Indeed, the temperatures obtained for both the crystallization and melting of the lipidic chain-end are identical from the one obtained in the case of 10 000 g/mol PIDiLips. The main difference existing between the two experiments is the values of the enthalpy obtained during crystallization or melting that increased from ~ 8 J/g (10 kg/mol PIDilip) to ~ 14 J/g (5 kg/mol PIDiLip). This result was expectable as the mass proportion of fatty esters for PIDiLip 5 kg/mol is twice the one of PIDiLip 10 kg/mol.
### Polyisoprene / Lipid Coupling

#### Table 2: Crystallization and melting temperatures of various PiDiLip (5 kg/mol) observed by DSC

<table>
<thead>
<tr>
<th>Grafted fatty acid</th>
<th>$T_m$ a)</th>
<th>$T_c$ b)</th>
<th>$T_g$ d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid (C14:0)</td>
<td>-c)</td>
<td>-c)</td>
<td>-64</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>-24</td>
<td>-35</td>
<td>-64</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>-9</td>
<td>-16</td>
<td>-64</td>
</tr>
<tr>
<td>Lignoceric acid (C24:0)</td>
<td>24</td>
<td>19</td>
<td>-64</td>
</tr>
</tbody>
</table>

a) Melting temperature observed by DSC; b) Crystallization temperature observed by DSC; c) No crystallization or melting observed by DSC; d) Glass transition observed by DSC

---

**Figure 6: DSC thermogram of PIDiC\textsubscript{18:0} 5 kg/mol**

**ii. 27 000 g/mol**

The second approach was to increase the molar mass of the PI chain up to 27 000 g/mol and to functionalize it with C\textsubscript{16:0} fatty esters affording a PIDiC\textsubscript{16:0}. The DSC thermogram obtained after its analysis is reported in Figure 7. No crystallization was obtained for this polymer. This could come from the increasing of the viscosity of the polymer higher than the 10 000 g/mol synthons. At low temperature, the viscosity becoming even higher, rendered the mobility of chains difficult and, as a matter of fact, prevents the crystallization of the chain-ends. Additionally, the mass percentage of fatty esters is nearly 3 times lower than with the 10 000 g/mol PIDiLip which could also renders not favourable the crystallization. Another hypothesis could be that 27 000 g/mol is higher than the molar mass of entanglement of PI (~ 14 000 g/mol) thus rendering difficult the mobility of the polymer chains and, thus, the encountering of the chain-ends to crystallize.
c. Variation of the number of linked fatty chains

The effect of the number of linked fatty chains on the crystallization behavior was then studied. To this end, the crystallization properties of both PIMonoLip and PIDiLipLip (Figure 8) were then determined.

![Figure 8: General structures of PIMonoLip and PIDiLipLip](image)

**i. PIMonoLip**

As described in the previous chapter, various hybrid polymers (PIMonoC\textsubscript{16:0}, PIMonoC\textsubscript{18:0} and PIMonoC\textsubscript{24:0}) were synthesized starting from PI of 5 and 10 kg/mol. DSC analyses were performed and surprisingly, with the 10 000 g/mol series, only the PIMonoC\textsubscript{24:0} exhibited a weak crystallization. Moreover, the temperature of crystallization and melting (-32°C and -18°C respectively, Figure 9) were shifted to lower values when compared to PIDiC\textsubscript{24:0}. The explanation was originally thought to be the mass fraction represented by the fatty esters in PIMonoLip which is twice lower than in the case of PIDiC\textsubscript{24:0}. 

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<table>
<thead>
<tr>
<th>Figure 7: DSC thermogram of PIDiC\textsubscript{16:0} of 27 kg/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="DSC thermogram" /></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Figure 8: General structures of PIMonoLip and PIDiLipLip</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="General structures" /></td>
</tr>
</tbody>
</table>
But, when PIMonoLip of 5 000 g/mol were analyzed, again, only the PIMonoC\textsubscript{24:0} exhibited a crystallization and at the same temperature as PIMonoC\textsubscript{24:0} of 10 000 g/mol. It was nevertheless more intense than for the latter.

Those results deny the preliminary hypothesis of the influence of the mass fraction as for PIMonoC\textsubscript{24:0} 5 000 g/mol the proportion of fatty chains is the same as for PIDiC\textsubscript{24:0} 10 000 g/mol. They, however, suggest that one important parameter that governs the crystallization behavior is the shape of the hybrid. Indeed, for the same weight fraction of fatty chains attached to the PI (PIDiC\textsubscript{24:0} 10 kg/mol and PIMonoC\textsubscript{24:0} 5 kg/mol), when two lipids are attached at the same chain-end (PIDiLip) instead of one (PIMonoLip), a crystallization at higher temperature is observed.
Polyisoprene / Lipid Coupling

It could be proposed that the lipidic domain, in the case of a “Y” shaped hybrid is bigger than in the case of a linear one thus shifting the crystallization to higher temperatures. The peaks noted “*” on the figures corresponds to ketene impurities as, at this point of the project, the removal method had not yet been found.

\[ \text{ii. PIDiLipLip} \]

As described before, fatty chains could form nodules of crystallization. This raised the hypothesis that if the ketone chain-end of the PI backbone could also be functionalized with a fatty chain (PIDiLipLip or PIMonoLipLip) it would be possible to induce the formation of a physical network formed by crystals. In the same vein, a recent work described the synthesis triblock copolymers composed of an amorphous core of branched poly(n-butyl acrylate) functionalized at both chain-ends by a “crystallizable” statistic copolymer of polyoctadecyl acrylate and polydocosylacrylate\(^1\). It was demonstrated that different self-assembled structures could be obtained, including physical cross-linking (Figure 11).

![Figure 11: Schematic representation of the different architectures affordable playing on the length of the branches from the amorphous core - Reproduced from Daniel et al.\(^1\)](image)

Two synthons were thus synthesized starting from the same PIDiC\(_{24:0}\) (10 000 g/mol): a PIDiC\(_{24:0}\)\(_{24:0}\) and a PIDiC\(_{24:0}\)\(_{16:0}\). The DSC thermograms are given in Figure 12 and Figure 13 respectively. For PIDiC\(_{24:0}\)\(_{24:0}\), it can be observed that the addition of one extra fatty chain does not have any influence on the crystallization (or melting) temperature if compared to the PIDiC\(_{24:0}\).
The only difference was again the enthalpy of crystallization which increased slightly from 8 to 12 J/g. Moreover, only one peak of crystallization (or melting) is visible on the thermogram which would confirm the co-crystallization of both chain-ends and the potential physical cross-linking. In the case of PIDiC$_{24:0}$C$_{16:0}$, a slight decrease of the crystallization temperature is observed as compared to the value of PIDiC$_{24:0}$ alone which could be explained by the co-crystallization of the C$_{24:0}$ and the C$_{16:0}$. Again, only one exotherm is visible for the crystallization thus also attesting the co-crystallization of both chain-ends and thus the possible physical cross-linking. No further investigation on the reversible cross-linking of the PIDiLipLip was performed, mostly due to lack of time.

Figure 12: DSC thermogram of PIDiC$_{24:0}$C$_{24:0}$

Figure 13: DSC thermogram of a PIDiC$_{24:0}$C$_{16:0}$
d. Addition of free lipids

To complete this study, it was proposed to find a method to increase the crystallization temperature of the hybrids in order to try to obtain a material physically cross-linked at room temperature. It was thus proposed to add free fatty acids or esters in order to reinforce the crystals.

Experiments were first carried out on PIDiLip. Various amounts of methyl palmitate (MetPalmitate) were first added to PIDiC_{16:0}. To this end, the fatty ester and the polymer were dissolved in diethyl ether to get a homogeneous solution. After drying, the final material was analysed by DSC. The amount of methyl ester introduced was varied (0.1, 0.3, 1, 2 and 10 wt%). Figure 14 presents the DSC thermograms obtained for each composition. As it can be observed, the crystallization temperature increased with the amount of MetPalmitate shifting from -32.2°C to 9°C. As a reference, the crystallization temperatures of PIDiC_{16:0} alone and MetPalmitate alone are -35°C and 34°C respectively. Interestingly, except in the case of 10% MetPalmitate, only one exotherm was observed thus confirming the co-crystallization of the chain-ends with the free lipids. This experiment demonstrates the real potential of the “doping” as a gap of approximately 20°C could be obtained between the initial hybrid and the 2 wt% doped one.

Figure 14: DSC thermograms of admixtures of PIDiC_{16:0} with 0.1, 0.3, 1, 2 and 10 wt% of MetPalmitate
In a second step, free fatty acids were added instead of methyl fatty esters as the crystallization temperature of fatty acids is higher than the corresponding fatty ester. Again, various amounts (0.1, 0.3, 1, 2 and 10 wt%) of free fatty acids were used. Contrarily to the previous experiment, here, stearic acid (noted here “SteAcid”) was added to PIDiC$_{18:0}$. Figure 15 presents an overlay of the DSC thermograms obtained for each composition. Different behaviour can be observed as two distinct exotherms were quickly observed. For small quantities of SteAcid (0.1 and 0.3 wt%) the exotherm corresponding to the crystallization of chain-ends (peak A / -18°C) was less intense and a second broad peak appeared (A’ / -3.2°C). This new exotherm certainly corresponds to the co-crystallization of both free and linked fatty chains. But for higher amounts of SteAcid (1, 2 and 10 wt%), the peak A totally disappeared as the peak A’ increased and a new exotherm appeared, which shifted to higher temperatures with the increase of the doping amount (peak C, C’ and C’’). Taking into account that the stearic derivatives (acid or esters) are not soluble in PI$^2$, when a certain amount of SteAcid is reached in the PI matrix, the chain-end still co-crystallizes with a part of the free lipids (peak A’) but the rest of the free lipids crystallizes at higher temperature without any mixing with the chain-ends (peaks C, C’ and C’’). The increase of the temperature between C, C’ and C’’ comes from the fact that the amount of free fatty acids, not co-crystallized with the chain-ends, is increasing and tends to the crystallization temperature of pure stearic acid (68°C). This renders totally incompatible the acidic doping with a cross-linked material at room temperature.

![Figure 15: DSC thermograms of admixtures of PIDiC$_{18:0}$ with 0.1, 0.3, 1, 2 and 10 wt% of SteAcid](image)
Polyisoprene / Lipid Coupling

From the previous experiments, it can be observed that the best co-crystallization effects were observed with fatty ester derivatives. In order to generate physically cross-linked networks, PIDiC<sub>24:0</sub>C<sub>24:0</sub> was mixed with 0.1, 1, 2 and 10 wt% of methyl lignocerate (noted here “MeLignocerate”) and analyzed by DSC (Figure 16). After addition of 2 wt% of MeLignocerate, the temperature of crystallization doubled (from 17°C to 36°C) with only one visible exotherm, thus attesting of the good co-crystallization of the free and linked fatty chains. For even higher amount of doping agent (10 wt%) a strange profile was obtained showing 3 distinct exotherms and the temperature reached 46°C. It seems, however, possible to increase the crystallization temperature of the chain-ends of a PIDiLipLip with the use of fatty esters carrying the temperature around 40°C. As a reference, the crystallization temperatures of PIDiC<sub>24:0</sub> and MeLignocerate alone are 17°C and 59°C respectively thus attesting of the efficiency of the doping.

![Figure 16: DSC thermograms of admixtures of PIDiC<sub>24:0</sub>C<sub>24:0</sub> with 0.1, 0.3, 1, 2 and 10 wt% of MeLignocerate](image)

e. Conclusion

To conclude on this first part of the chapter, it was observed that when grafted to PI, fatty esters were able to crystallize even in the presence of a huge amorphous matrix that represents the polymer. The fatty esters were able to form microdomains thus creating nodules of crystallization comparable to thermal anchors for the polymeric chains. Moreover, the temperature of crystallization can be monitored by either the nature of the fatty ester linked and/or the addition of free fatty esters.
Polyisoprene / Lipid Coupling

It was, however, proved that the increase of the polymeric chain length prevents this phenomenon and that the fatty acids can not be used as doping agents as they are not mixable with the polymer. Finally, the functionalization of the polymer at both chain-ends could, according to the literature, lead to the formation of a reversible network formed by the crystallites. More experiments have to be done in this field in order to obtain any elastomeric material.

III. Cold Crystallization

a. Introduction

The origin of the synthesis of PIDiLip was to generate a good model of NR which could be helped to a better understanding of its properties. As explained in the bibliographic part, among the different properties of NR, CCr was proved to be related to the presence of fatty chains in NR. We thus had the opportunity of studying the influence of both linked and free lipids on the CCr capacity of PI by using a model possessing a structure close to the natural polymer according to Tanaka. The results will be presented under the form of a scientific article.

b. New insight into the Cold Crystallization of Natural Rubber: the role of linked and free fatty chains

INTRODUCTION

Natural Rubber (NR) is one of the most important natural polymer as it is widely used in the industry for various applications (tires, gloves, etc…).\(^3\) It exhibits specific thermo-mechanical properties that cannot be obtained with synthetic polyisoprene (IR) such as strain induced crystallization (SIC), high green-strength, excellent crack resistance and fast cold crystallization (CCr).\(^4\)\(^–\)\(^7\) This later property corresponds to the crystallization of PI at low temperature (usually -25°C) without any external perturbations, contrarily to SIC which implies the deformation of the material.\(^8\) The origins of the superior capacities of NR are not yet fully understood despite the wide amount of studies carried out on this topic. Nevertheless, Tanaka \textit{et al.}\(^9\)\(^–\)\(^12\) showed that it could arise from the structure of the polymer which was described to be composed of a polyisoprene (PI) chain functionalized at one chain-end by a protein and at the other by a phospholipidic moiety (Figure 17). These chain-ends could self-assemble to form a dynamic network in the material thus explaining the superior mechanical resistance of NR toward IR.\(^9\)
In 1946, Wood\textsuperscript{13} studied the crystallization of NR and showed that the fastest crystallization rate was obtained by keeping the material at \(-25^\circ\text{C}\) for several hours. Burfield established later that 75\% of the final crystallinity could be reached after 3 h of isotherm and that total crystallization was observed after 16 h.\textsuperscript{14} CCr was shown to be correlated to the suggested structure of NR (Figure 17) and more specifically to the lipidic chain-end. The influence of free fatty acid/esters present in NR was also investigated as well as their interaction with the PI chain.\textsuperscript{2,15–21} Kawahara \textit{et al.} demonstrated that saturated fatty acids/esters (stearic acid being the main studied) enhanced the crystallization of NR and that unsaturated ones (methyl linoleate for example) had a plasticizing effect on the PI chain.\textsuperscript{2} Regardless the nature of the fatty acid, it was shown a synergetic effect between linked and free fatty chains as well as between stearic acid and methyl linoleate to promote the cold crystallization of the material.\textsuperscript{20,22} Similar studies were also performed on synthetic polyisoprenes. To this end, lipids were grafted onto IR by hydroboration using the pendant 3,4-units, leading to a model of NR.\textsuperscript{21} A fast crystallization was observed when the backbone was functionalized with 0.5 wt \% of stearic acid and mixed with 1 wt \% of methyl linoleate, but even if it was faster than the starting IR, it was still slower than NR. This difference could be related to the microstructure of NR, which is commonly accepted to be pure 1,4-\textit{cis} contrarily to the IR used by Kawahara which contained about 2 \% of 1,2 and 3,4 units, as it was demonstrated to play a role on the crystallization of PI.\textsuperscript{23}

In this study, we investigated the synthesis of new hybrid polymers formed from the functionalization of a pure 1,4-\textit{cis} PI (molar mass of \(~10 000 \text{ g/mol}\)) with one or two fatty chains at the \(\omega\) chain-end as a closer model of NR and we studied their cold crystallization properties. Even if isoprene polymerization was already studied by many techniques (cationic,\textsuperscript{24,25} anionic,\textsuperscript{26} radical,\textsuperscript{27–29} metathesis,\textsuperscript{30} and coordination polymerization\textsuperscript{31}), none of them allowed to reach a pure 1,4-\textit{cis} microstructure with controlled chain-ends.
Oxidative degradation of NR by successive epoxidation and acidic degradation was preferred as it could lead to pure 1,4-cis PI with ketone and aldehyde reactive chain-ends.\textsuperscript{32} Moreover, the molar mass could be controlled by the quantity of epoxidized units. Reactive chain-ends could then be easily modified to graft lipidic moieties in $\omega$ position. The general synthetic strategy developed in this study is depicted on Figure 18. In this paper, the influence of the nature of fatty acids on the crystallization properties will be discussed as well as the influence of the addition of free lipids. Finally, modification of a synthetic polyisoprene will also be studied to try to mimic the crystallization properties of NR.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure18.png}
\caption{General synthetic route to obtain PIDi(or Mono)Lip}
\end{figure}

**EXPERIMENTAL**

**Materials.**

Natural rubber (NR) RRIM600 was kindly provided by Katsetsart University in Thailand. Cis-1,4-polyisoprene (IR) (97% cis-1,4, $M_n = 600$ kg/mol, $D = 2.8$) was purchased from Scientific Polymer Products, Inc. 3-Chloroperoxybenzoic acid (mCPBA) (70-75%, Acros), periodic acid (H$_3$IO$_6$) (≥ 99%, Aldrich), acetic acid (99%, Aldrich), potassium hydroxide (KOH) (85%, Aldrich), sodium triacetoxyborohydride (NaBH(OAc)$_3$) (97%, Aldrich), diethanolamine (DEA) (99%, Alfa Aesar), stearic acid (SA) (95%, Aldrich), myristic acid (99%, Aldrich), lignoceric acid (> 99%, Aldrich), methyl linoleate (ML) (99%, Aldrich), linoleoyl chloride (>99%, Aldrich), 10-undecenoyl chloride (97%, Aldrich), palmitoyl chloride (98%, Aldrich), 3-aminopropyl-functionalized silica gel
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(~1 mmol/g NH\textsubscript{2} loading, 40-63µm, Aldrich), oxalyl chloride (>99%, Aldrich) were used without further purification. Tetrahydrofuran (THF) and dichloromethane (DCM) were dried on alumina column. Triethylamine (TEA) was dried on KOH pellets and distilled prior to use. Methanol, diethyl ether and dimethylformamide (DMF) (reagent grade, Aldrich) were used as received as well as Celite\textsuperscript{®} (R566, Aldrich).

**Characterization.**

Liquid-state \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra were recorded at 298 K on a Bruker Avance 400 spectrometer operating at 400 MHz and 100 MHz respectively in appropriate deuterated solvents. Polymer molar masses were determined by size exclusion chromatography (SEC) using tetrahydrofuran as the eluent (THF with 250 ppm of Butylated hydroxytoluene as inhibitor, Aldrich). Measurements were performed on a Waters pump equipped with Waters RI detector and Wyatt Light Scattering detector. The separation is achieved on three Tosoh TSK gel columns (300 × 7.8 mm) G5000 HXL, G6000 HXL and a Multipore HXL with an exclusion limits from 500 to 40 000 000 g/mol, at a flow rate of 1 mL/min. The injected volume was 100 µL. Columns’ temperature was 40 °C. \(M_n\) and \(Đ\) values were calculated using \(dn/dc(\text{polyisoprene})=0.130\). Data were processed with Astra software from Wyatt. Differential scanning calorimetry (DSC) measurements were performed using a DSC Q100 LN\textsubscript{2} or a DSC Q100 RSC apparatus from TA Instruments depending on the experiment. With DSC Q100 LN\textsubscript{2}, the samples were first heated to 80°C during 20 minutes to suppress any traces of solvent then cooled to -100°C and heated back to 120°C at the rate of 10°C min\textsuperscript{-1}. Consecutive cooling and heating run were also performed at 10°C min\textsuperscript{-1}. The analyses were carried out in a helium atmosphere with aluminum pans. DSC Q100 RSC device was used for isothermal analysis. The samples were heated at 80°C during 20 minutes prior to use to suppress any traces of solvent, then cooled to -25°C during predetermined time and then heated to 120°C at a heating rate of 10°C min\textsuperscript{-1}. Fourier Transformed Infra-Red-Attenuated Total Reflection (FTIR-ATR) spectra were recorded between 4000 and 400 cm\textsuperscript{-1} on a Bruker VERTEX 70 instrument (4 cm\textsuperscript{-1} resolution, 32 scans, DLaTGS MIR) equipped with a Pike GladiATR plate (diamond crystal) for attenuated total reflectance (ATR) at room temperature.
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Synthesis.

Synthesis of heterotelechelic keto-aldehyde PI (PIDeg) (1). 5 g of NR were dissolved overnight in 250 mL of THF under vigorous stirring. The viscous solution obtained was then cooled to 0°C and 50 mL of mCPBA (0.14 g, 0.6 mmol) solution in THF were added dropwise to the NR solution. The reaction was then allowed to warm up to room temperature for 2 h. 0.3 g of periodic acid (2.2 eq to mCPBA, 1.33 mmol) were dissolved in 50 mL of THF and added dropwise to the epoxidized NR solution. After 2 h of stirring at room temperature, the reaction mixture was filtered affording a yellow solution which was then concentrated in vacuum and precipitated into a large excess of cold methanol containing 2 mL of alkaline water. The polymer was then dissolved in diethyl ether (~ 100 mL) and the obtained cloudy solution was filtered on Celite®. The final product was obtained by evaporation of the Et₂O and drying overnight at 40°C under dynamic vacuum affording a yellowish and transparent viscous liquid. Yield: ~ 80 %, Mₙ = 9 620 g/mol, Đ = 1.6 (Figure S1).

(Figure S2); ¹H NMR (CDCl₃) δ (ppm): 9.77 (t, 1H chain-end, CH₂CHO), 5.13 (t, 1H CH=CH₂CH₃), 2.49 (t, 2H, CH₂CHO), 2.44 (t, 2H, CH₂COCH₃), 2.35 (t, 2H, CH₂CH₂CHO), 2.13 (s, 3H, CH₃COCH₂), 2.05 (s, 4H, CH₂CH/CH₂CH₃), 1.68 (s, CH₃CCH).

(Figure S3); FTIR: νH–C=C : 3035 cm⁻¹; νCH₂, CH₃ : 2900–2730 cm⁻¹; νC=O : 1722 cm⁻¹; δC=C : 1664 cm⁻¹; nCH₂CH₃ cis-1,4- isoprene : 1446, 1375 cm⁻¹; δC=C–H : 833 cm⁻¹.

Synthesis of heterotelechelic keto-diol PI (PIDiOH) (2). 3.7 g of PIDeg (0.37 mmol of aldehyde groups) were dissolved in 10 mL of dry THF. 0.19 g (4.5 eq, 1.66 mmol) of diethanolamine were added and the reaction was stirred at 40°C for 2 h. Finally, 0.36 g (4.2 eq, 1.55 mmol) of sodium triacetoxyborohydride were added to the reaction mixture followed by 30µL (1.3 eq, 0.48 mmol) of acetic acid and the heterogeneous medium was stirred at 40°C overnight. The reaction mixture was then directly precipitated into a large excess of cold methanol under vigorous stirring. The polymer was further dissolved in DCM and precipitated again in cold methanol, dissolved in Et₂O, filtered on Celite® and dried under vacuum at 40 °C overnight affording a colorless viscous liquid. Yield: ~ 85 %

(Figure S4); ¹H NMR (CDCl₂) δ (ppm): 5.14 (s, 1H, CH=CH₂CH₃), 3.57 (t, 4H, (CH₂)₂OH), 2.64 (t, 4H, N(CH₂)₂), 2.52 (t, 2H, CH₂N), 2.42 (t, 2H, CH₂COCH₃), 2.05 (s, 4H, CH₂CH / CH₂CCH₃), 1.67 (s, 3H, CH₃CCH)
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Synthesis of heterotelechelic keto-hydroxyl PI (PIOH) (3). 2 g of PIDeg (0.2 mmol) were dissolved in 5 mL of dry THF. 0.19 g (4 eq, 0.8 mmol) of triacetoxyborohydride were then added to the obtained solution as well as 12 µL (1 eq, 0.2 mmol) of acetic acid. The reaction mixture was stirred at 40 °C overnight and the final product was obtained after two successive precipitations into a large excess of cold methanol, dissolution in Et₂O, filtration through Celite® and drying overnight at 40°C under dynamic vacuum. The PIOH is a viscous and colorless liquid. Yield: ~ 90 % (Figure S5); ¹H NMR (CDCl₃) δ (ppm): 5.12 (t, 1H, CH=CH₂CH₃), 3.62 (t, 2H, CH₂OH), 2.42 (t, 2H, CH₂COCH₃), 2.05 (s, 4H, CH₂CH / CH₂CCH₃), 1.67 (s, 3H, CH₃CCH)

Synthesis of fatty acyl chlorides (C₁₄:0 Chlo / C₁₈:0 Chlo / C₂₄:0 Chlo). A solution of the desired fatty acid was prepared in dry DCM ([fatty acid] = 0.5M). 3 eq of oxalyl chloride were then added to the mixture under vigorous stirring followed by 3 drops of DMF. A vigorous bubbling was observed. The reaction conversion was followed by connecting the reaction flask to a bubbler containing a KOH solution. When no bubbling was visible, the reaction mixture was evaporated under vacuum to remove the solvent and the excess of oxalyl chloride. The final product was then dried overnight at 40°C under dynamic vacuum affording a yellowish liquid for C₁₈:0 Chlo and C₁₄:0 Chlo and a white powder for C₂₄:0 Chlo. Yield: > 90 %.

C₁₄:0 Chlo: (Figure S6) ¹H NMR (CDCl₃) δ (ppm): 2.87 (t, 2H, CH₂CCl), 1.70 (m, 2H, CH₂CH₂Cl), 1.25 (s, 20H, CH₂-fatty chain), 0.88 (t, 3H, CH₃CH₂) / (Figure S7) ¹³C NMR (CDCl₃) δ (ppm): 174.21 (1C, COCl), 47.28 (1C, COCH₂), 31.93 (1C, COCH₂CH₂), [29.65, 29.64, 29.61, 29.52, 29.36, 29.33, 29.07, 28.43, 25.07, 22.71] (10C, CH₂-fatty chain), 14.11 (1C, CH₂CH₃).

C₁₈:0 Chlo: (Figure S6) ¹H NMR, (CDCl₃) δ (ppm): 2.87 (t, 2H, CH₂CCl), 1.70 (m, 2H, CH₂CH₂Cl), 1.25 (s, 28H, CH₂-fatty chain), 0.88 (t, 3H, CH₃CH₂) / (Figure S7) ¹³C NMR (CDCl₃) δ (ppm): 174.21 (1C, COCl), 47.28 (1C, COCH₂), 31.93 (1C, COCH₂CH₂), [29.81, 29.75, 29.66, 29.52, 29.46, 29.21, 28.57, 25.20, 22.83] (14C, CH₂-fatty chain), 14.25 (1C, CH₂CH₃).

C₂₄:0 Chlo: (Figure S6) ¹H NMR, (CDCl₃) δ (ppm): 2.87 (t, 2H, CH₂CCl), 1.70 (m, 2H, CH₂CH₂Cl), 1.25 (s, 40H, CH₂-fatty chain), 0.88 (t, 3H, CH₃CH₂) / (Figure S7) ¹³C NMR (CDCl₃) δ (ppm): 174.21 (1C, COCl), 47.28 (1C, COCH₂), 31.93 (1C, COCH₂CH₂), [29.86, 29.76, 29.68, 29.52, 29.49, 29.22, 28.59, 25.21, 22.86] (20C, CH₂-fatty chain), 14.28 (1C, CH₂CH₃).
Polyisoprene / Lipid Coupling

Synthesis of heterotelechelic keto-dilipid PI (PIDiLip) (4). 2 g of PIDiOH (0.2 mmol) were dissolved in 7 mL of dry THF. 180 µL (6 eq, 1.2 mmol) of TEA were then added followed by 3 eq (0.6 mmol) of the desired acyl chloride. After 1 h of reaction, 3-aminopropyl-functionalized silica particles were added (~1 NH$_2$ eq toward acyl chloride) and the mixture was stirred for 2 h. The reaction medium was then precipitated in a large excess of cold methanol, dissolved in Et$_2$O, filtered through Celite® and dried first on rotary evaporator and then overnight at 40°C under vacuum. The final compound was a colorless viscous liquid except for PIDiC$_{24:0}$ which was a colorless paste. Yield: ~ 80 %

(Figure S8): $^1$H NMR (CD$_2$Cl$_2$) δ (ppm): 5.12 (s, 1H, CH=CCH$_3$), 4.09 (t, 4H, (CH$_2$)$_2$OCO), 2.74 (t, 4H, N(CH$_2$)$_2$), 2.51 (t, 2H, CH$_2$N), 2.43 (t, 2H, CH$_2$CCH$_3$), 2.26 (t, 4H, OCOCH$_2$) 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_3$CCH), 1.26 (s, CH$_2$$_2$, fatty chain), 0.88 (t, 6H, CH$_2$CH$_3$)

Synthesis of heterotelechelic keto-monolipid PI (PImonoLip) (5). 1 g of PIOH (0.1 mmol) was dissolved in 4 mL of dry THF. 60 µL (4 eq, 0.4 mmol) of TEA were added followed by 2 eq (0.2 mmol) of the desired acyl chloride. After 1 h of reaction, 3-aminopropyl-functionalized silica particles were added (~1 NH$_2$ eq toward acyl chloride) and the obtained mixture was stirred for 2 h. The final polymer was obtained after two successive precipitations into a large excess of cold methanol, dissolving in Et$_2$O, filtration through Celite® and overnight drying at 40°C under dynamic vacuum. Yield: ~ 80 %.

(Figure S9): $^1$H NMR (CDCl$_3$) δ (ppm): 5.12 (s, 1H, CH=CCH$_3$), 4.03 (t, 2H, CH$_2$OCO), 2.43 (t, 2H, CH$_2$CCH$_3$), 2.26 (t, 2H, OCOCH$_2$) 2.05 (s, 4H, CH$_2$CH / CH$_2$CCH$_3$), 1.67 (s, 3H, CH$_3$CCH), 1.26 (s, CH$_2$$_2$, fatty chain), 0.88 (t, 3H, CH$_2$CH$_3$)

RESULTS AND DISCUSSION

Polymer modification.

Synthesis of PIDeg (1)

In this study, 10 000 g/mol PIDeg were targeted. The epoxidation and the acidic degradation (Figure 18) were performed successively without intermediate purification. During the precipitation step in methanol, the use of alkaline water was compulsory to prevent acetal formation at the aldehyde chain-end.
The targeted molar mass can, nevertheless, be adjusted by using the following formula:

\[ \bar{M}_n = \frac{100 \times 68}{T_x} + 100 \]

\[ T_x = \left[ \text{epoxidized units} \right] / \left[ \text{monomer units} \right] \times 100\% \]

\( \bar{M}_n \) : Targeted molar mass (g/mol)

The occurring of the degradation reaction was monitored by SEC, \( ^1 \)H NMR and FTIR-ATR analyses (Figures S1, S2 and S3 respectively). By SEC, it was observed a decrease of the molar mass from 500 000 g/mol (NR) to 10 000 g/mol (PIDeg) as well as a decrease of the molar mass distribution from 2.6 (NR) to 1.6 (PIDeg). The formation of carbonyl chain-ends as confirmed by \( ^1 \)H NMR with the appearance of a triplet at 9.77 ppm characteristic of the aldehyde proton as well as the appearance of 3 triplets at 2.49, 2.44 and 2.35 ppm corresponding to “CH₂” groups in α and β position of the aldehyde and in α position of ketone chain-end respectively. Moreover, the appearance of a band at 1722 cm\(^{-1}\) in FTIR-ATR also confirmed the generation of carbonyl functions.

**Synthesis of PIDiLip (4)**

For the synthesis of PIDiLip (4) the reductive amination of the aldehyde chain-end of (1) with diethanolamine and NaBH(OAc)\(_3\) was first performed to yield PIDiOH (2). Compared to the procedure described in the literature\(^{33}\), the solvent was changed (THF instead of DMSO/dichloroethane mixture) to reach a higher reaction rate. \(^1\)H NMR analysis (Figure S4) showed a quantitative conversion with the total disappearance of the signals at 9.7, 2.49 and 2.35 ppm corresponding to the aldehydic proton and both “CH₂” groups in α and β position of the aldehyde chain-end respectively. Furthermore, the appearance of signals at 3.57, 2.64 and 2.52 ppm corresponding to both “CH₃” groups in α and β position of the hydroxyl function and to the “CH₂” group in α position of the nitrogen atom respectively also confirmed the good control of the reaction. Finally, the selectivity was confirmed by the presence of the signal at 2.43 ppm corresponding to the “CH₂” group in α position of the ketone chain-end. The following step was to graft two fatty chains through acylation reactions affording (4). The corresponding fatty acyl chlorides were first synthesized through the reaction of fatty acids with oxalyl chloride in dichloromethane with excellent yields (Figure S6 and S7). As confirmed by the total shift of the signal at 2.35 ppm in \(^1\)H NMR corresponding to the “CH₂” group in α position of the carboxylic acid carbonyl to a signal at 2.87 ppm after the chlorination.
Moreover, in $^{13}$C NMR analysis the shift of the signal at 182 ppm corresponding to the carbon of the carboxylic acid carbonyl to a signal at 174.21 ppm after chlorination also confirms the full conversion. These acyl chlorides were then reacted with (2) in dry THF in the presence of triethylamine. Again, $^1$H NMR analysis (Figure S8) confirmed full conversion with the total shift of the signal at 3.57 ppm corresponding to the “CH$_2$” group in α position of the hydroxyl chain-end to a new signal at 4.09 ppm corresponding to the “CH$_2$” group in α position of the ester formed. The use of the silica beads functionalized by amino-propyl groups is important as it prevents contamination of the product by di-ketene (side product) formed during the reaction and removes the excess of acyl chloride used. No change on the SEC chromatogram was observed thus attesting that no cross-linking occurred during the overall pathway.

**Synthesis of PIMonoLip (5)**

The synthesis of PIMonoLip (5) starts with the selective reduction of the aldehyde chain-end of PIDeg (1) to yield PIOH (3). It was then used NaBH(OAc)$_3$ as a reducing agent known for its selectivity toward aldehydes against ketone. Again, $^1$H NMR analysis (Figure S9) confirmed the quantitative conversion with the disappearance of the signals at 9.77, 2.49 and 2.35 from (1) corresponding to the aldehydic proton and both “CH$_2$” groups in α and β position of the aldehyde chain-end respectively. A new signal appeared at 3.62 ppm corresponding to the signal of the “CH$_2$” group in α position of the newly formed hydroxyl function.

The last step was the grafting of one fatty chain via an esterification reaction similarly to the synthesis of (4). Again, the reaction was carried out in dry THF using fatty acyl chlorides and triethylamine as a proton trap. The $^1$H NMR analysis (Figure S9) showed a quantitative conversion with the total shift of the signal at 3.62 ppm corresponding to the “CH$_2$” group in α position of the hydroxyl chain-end to a signal at 4.03 ppm corresponding to the same group in α position of the new ester function. Again, no change in the SEC chromatogram was observed attesting that no side reaction occurred.

All the synthesized molecules (varying the number of fatty chains and their nature) are summarized in Table 3.
**Table 3: Summary of all PIDiLip and PIMonoLip synthesized**

<table>
<thead>
<tr>
<th>General structure</th>
<th>R</th>
<th>Corresponding name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PIDiLip</strong></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;11:1&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;18:2&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;14:0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;16:0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;18:0&lt;/sub&gt;</td>
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<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;19:0&lt;/sub&gt;</td>
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<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIDiC&lt;sub&gt;24:0&lt;/sub&gt;</td>
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<tr>
<td><strong>PIMonoLip</strong></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIMonoC&lt;sub&gt;16:0&lt;/sub&gt;</td>
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<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIMonoC&lt;sub&gt;18:0&lt;/sub&gt;</td>
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<tr>
<td></td>
<td><img src="image.png" alt="Image" /></td>
<td>PIMonoC&lt;sub&gt;24:0&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

**Thermal analysis of the hybrid polymers synthesized**

First, DSC analyses of the PIDiLip were performed. Figure 19 shows the DSC thermogram obtained for PIDiC<sub>18:0</sub>. It can be observed a T<sub>g</sub> at -63°C as well as a crystallization (and a melting) at low temperature for the hybrid polymer, whereas for NR, PIDeg and PIDiOH (Figures S10 to S12), only a T<sub>g</sub> at -63°C was observed. This could not be due to PI crystallization which was reported to be slow, occurring only while maintaining the rubber at low temperature (usually -25°C) for a long time. It can then be assumed that the lipids linked at the chain-ends could crystallize. Moreover, it was possible to tune the crystallization and melting temperature of the chain-ends by varying the nature of the fatty acid as indicated on Table 4. Indeed, T<sub>m</sub> and T<sub>c</sub> increased with the fatty acid chain length (Figures S13 to S18). For instance, T<sub>m</sub> increased from -25 to 22°C for C<sub>16:0</sub> and C<sub>24:0</sub> respectively.
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Nevertheless, for unsaturated fatty acids (C\textsubscript{11:1} and C\textsubscript{18:2}) and smaller saturated fatty acid (C\textsubscript{14:0}), no crystallization occurred. For PIDiC\textsubscript{24:0}, as the melting temperature is close to room temperature, the material became more viscous due to partial crystallization. In the case of PIMonoLip, only PIMonoC\textsubscript{24:0} presented a crystallization but at a temperature much lower than PIDiC\textsubscript{24:0} (-35°C instead of 18°C). This suggests that the number of chains grafted to the polymer backbone is also an important parameter for the chain-end crystallization.

![DSC thermogram of PIDiC\textsubscript{18:0}](image)

Table 4. Crystallization and melting temperatures of various PiDiLip observed by DSC

<table>
<thead>
<tr>
<th>Grafted fatty acid</th>
<th>T\textsubscript{m}\textsuperscript{a)} (°C)</th>
<th>T\textsubscript{c}\textsuperscript{b)} (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undecenoic acid (C\textsubscript{11:1})</td>
<td>-\textsuperscript{c)}</td>
<td>-\textsuperscript{c)}</td>
</tr>
<tr>
<td>Linoleic acid (C\textsubscript{18:2})</td>
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</tr>
<tr>
<td>Palmitic acid (C\textsubscript{16:0})</td>
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<td>-37</td>
</tr>
<tr>
<td>Stearic acid (C\textsubscript{18:0})</td>
<td>-11</td>
<td>-18</td>
</tr>
<tr>
<td>Nonadecanoic acid (C\textsubscript{19:0})</td>
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<td>-13</td>
</tr>
<tr>
<td>Lignoceric acid (C\textsubscript{24:0})</td>
<td>22</td>
<td>17</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Melting temperature observed by DSC; \textsuperscript{b)} Crystallization temperature observed by DSC; \textsuperscript{c)} No crystallization or melting observed by DSC

In a second step, as PIDiLip were designed to be simple models of NR, it was decided to study their cold crystallization behavior. DSC analysis was used to study the crystallization of the starting NR after 2 and 8 h of isotherm at -25 °C (Figure 20).
It can be seen, as reported in the literature, that the crystallization process is quite long as only a small melting endotherm can be observed after 2 h at -25 °C. Interestingly, exothermal crystallization peak can be observed during the isotherm as a function of time (Figure 21). It was defined in the literature different characteristic times: tₖ (induction time, corresponding to the starting time of isothermal analysis), tₘ (time of maximum crystallization, i.e. the time at which the maximum of the plot is observed) and tₑ (extrapolated time, i.e. the time at which the crystallization is effectively starting). We added a fourth value, t₇, corresponding to the time at which the crystallization is finished (see Figure 21 line b). Similar analyses were then achieved for PIDeg and PIDiOH (Figure 21). All the characteristic times are summarized in Table 5 as well as the melting temperatures obtained for each sample by performing DSC heating cycle following isothermal crystallization. For PIDiOH and PIDeg, the crystallization started with a delay (tₑ: 93 and 82 min for PIDeg and PIDiOH respectively) but maximum of heat flow was achieved after similar crystallization time for all three polymers (tₘ-tₑ~150), as well as crystallization finished in ~320 min (t₇-tₑ) in all three cases. In the literature, such a delay was only observed for acetone extracted NR (AE-NR) (removal of the free fatty chains by acetone extraction) or trans-esterified NR (TE-NR) (removal of both linked and free fatty chains). In our case, it could thus mean that controlled degradation removed free and/or linked fatty chains from the initial NR but that the reductive amination had no effect. Finally, an increase of the overall crystallinity of the PIDeg and PIDiOH was observed compared to the initial NR (higher enthalpy), which is in agreement with the literature, as Kawahara reported a delayed crystallization of TE-NR compared to NR but a higher final crystallinity.

Figure 20: DSC thermograms of NR obtained after 2h and 8h at -25°C
Table 5. Characteristic values of CCr of NR, PIDeg and PIDiOH obtained for 8 h isothermal crystallization at -25°C

<table>
<thead>
<tr>
<th>Sample</th>
<th>( t_i ) ( ^a )</th>
<th>( t_e ) ( ^a )</th>
<th>( t_m ) ( ^a )</th>
<th>( t_f ) ( ^a )</th>
<th>( \Delta H_m ) ( ^b )</th>
<th>( T_m ) ( ^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR (RRIM 600)</td>
<td>0</td>
<td>0</td>
<td>146</td>
<td>338</td>
<td>14</td>
<td>-1</td>
</tr>
<tr>
<td>PIDeg</td>
<td>0</td>
<td>93</td>
<td>249</td>
<td>408</td>
<td>23</td>
<td>-1</td>
</tr>
<tr>
<td>PIDiOH</td>
<td>0</td>
<td>82</td>
<td>236</td>
<td>396</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>

\( ^a \) Characteristic times obtained from the DSC thermograms; \( ^b \) Melting enthalpy calculated from the area of the melting endotherm on the DSC thermograms. \( ^c \) Melting temperatures obtained from the DSC thermograms.

The cold crystallization of PIDiC\textsubscript{18:0} was then investigated and compared to the one of PIDiOH (Figure 22). Endotherm peaks were visible in both cases. Nevertheless, in the case of PIDiOH, the observed endotherm corresponded to the melting of the crystallized PI chains, whereas for PIDIC\textsubscript{18:0}, the endotherm was due to the melting of the chain-ends as the melting temperature is lower than the one of PI chains and corresponds to the \( T_m \) of the chain-ends during regular DSC cycle (Figure 3, Table 2). The grafted fatty chains at one chain-end seemed then to prevent (or at least significantly decrease) the CCr of the polymeric chains. This observation is in good agreement with the literature as AE-NR (only containing linked lipids) presented a huge decrease in the crystallization rate. The effect of the number of linked fatty chains was also investigated.
Polyisoprene / Lipid Coupling

In the case of PIMonoLip, no PI crystallization was observed (Figure S19). Tanaka suggests that grafting fatty chains onto PI decreases the “purity” of the sample thus rendering the crystallization less favorable.\textsuperscript{16} In our case, the absence of crystallization of PI even after 8 h of isotherm can be related to the mass fraction of lipids which acted as a huge quantity of “impurities” in the case of PIDiC\textsubscript{18:0} (5.7 wt%).

Figure 22: DSC thermograms of a PIDiC\textsubscript{18:0} and PIDiOH after 8 h of isothermal crystallization at -25 °C

In a last step, free lipids were added to try to recover a cold crystallization for the hybrid material. Indeed, methyl linoleate (ML) and stearic acid (SA), which represent the major part of the lipids in the \textit{Hevea} NR\textsuperscript{36}, were reported to have a nucleating effect for SA and a plasticizing effect for ML on the CCr of NR. PIDiC\textsubscript{18:0} was investigated first and was mixed with 4 % SA, 4 % ML and 4 % ML + 4 % SA (wt%). DSC thermograms are given on Figure 23. The addition of 4 % of ML did not induce any crystallization of the polymer and only the melting of the lipidic chain-ends was present. On the contrary, the addition of 4 % SA or 4 % ML + 4 % SA favored CCr of the PI chains with a higher melting enthalpy when both free lipids were present, showing the synergetic effect of SA and ML. Nevertheless, even in the presence of 4 % of both SA and ML the overall crystallinity was much lower than that of NR. This is a different behavior than AE-NR for which addition of SA with ML allowed to recover the original crystallinity\textsuperscript{20,21}. This can be due to a much higher weight content of linked fatty chains in our case as well as the low molar mass used for this study (~10 000 g/mol).
By mixing PIDiOH with 4 % of SA + 4 % of ML, it can be seen on Figure 24 that the addition of free fatty chains increased the crystallization rate which became even higher than the one of NR (t_m and t_f of 91 and 182 min vs 146 and 338 min respectively) with an overall crystallinity comparable to the one of PIDiOH reported in Table 5. It can be assumed that the nucleating effect of SA created the first nodules of crystallization and that the plasticizing capacity of ML conferred mobility to the chains even at low temperature.

A similar study was then realized with a high molar mass IR (M_n~600 000 g/mol) having high content of 1,4-cis units (~ 97%). The same chemical procedures were followed to synthesize again hybrid polymer (IRDic18:0) with a molar mass of polyisoprene of 10 000 g/mol.
The chain-end crystallization occurred exactly the same way as for PIDiC\textsubscript{18:0} synthesized from NR (Figure S20). Nevertheless, contrarily to NR, no crystallization was observed for the IRDiOH (Figure S21), neither for the starting IR maintained at -25°C for 8 h. The time of isotherm was thus extended to 60 h and in this case crystallization appeared only for the initial IR (Figure S22) but not for IRDeg (Figure S23). These results suggest that the CCr of IR is much slower than the one of NR certainly due to the presence of 1,2- and 3,4-units in the microstructure but also that the higher the molar mass, the quicker the CCr. As expected, IRDiC\textsubscript{18:0} did not exhibit any crystallization in agreement with the absence of CCr for IRDeg. Both samples were then mixed with free lipids (4 % ML + 4 % SA) and maintained at -25°C for 60 h. The DSC thermograms obtained are reported in Figure 25. Both the IRDiC\textsubscript{18:0} and the IRDeg recovered a CCr. In the case of the hybrid, only a weak endotherm can be observed which is partially covered by the melting of the fatty chain-end. But, like in the case of PI Deg mixed with free lipids, the IRDeg exhibits a huge endotherm corresponding to the crystallization of the PI. Moreover, the size of the endotherm obtained in the case of IRDeg mixed with free lipids is even bigger than the one of the starting IR. Again, this confirms the boosting role of the free lipids for the crystallization of PI.

![Figure 25: DSC thermograms of a high molar mass IR, IRDeg mixed with free fatty chains and IRDiC\textsubscript{18:0} also mixed with free fatty chains. Obtained after 60 h of crystallization at -25 °C.](image-url)
CONCLUSION.

In this study, several hybrid polyisoprenes ($M_n\approx 10\,000\,g/mol$) bearing one or two fatty esters at one chain-end were synthesized either from NR (100% 1,4-cis units) or from IR (97% 1,4-cis units) as models of NR. Before the grafting of the fatty chain, polymers coming from NR exhibited a high crystallinity after isothermal treatment at -25°C, whereas polymers coming from IR showed no crystallinity probably due to the presence of other units than 1,4-cis. Detailed DSC analyses of the hybrid polymers showed that the grafting of lipidic chains prevented cold crystallization of polyisoprene, a general feature of NR. On the contrary, chain-ends could crystallize relatively quickly despite the huge amorphous matrix. The addition of free fatty acids to the hybrid polymer allowed to recover a partial cold crystallization in a lower amount than the initial NR. Nevertheless, addition of both SA and ML presented a synergetic effect to enhance CCr of all the polymers either natural or synthetic. All these observations allow to clarify the role of both free and linked fatty chains on the CCr of NR and allowed to obtain a crystallization rate of a 10 000 g/mol IR comparable to the one of a 600 000 g/mol IR.

IV. General conclusion:

This chapter focused on the study of properties of various PI-Lipid hybrids synthesized as models of NR. It was shown that the chain-ends were able to crystallize despite the amorphous PI matrix. Moreover, the crystallization temperature can be monitored by varying the length of the fatty chain linked and/or the addition of free fatty esters. It opened the possibility to get access to cross-linked materials, where the anchoring points would be fatty chain crystallites. It was further studied the CCr of NR and of PI with linked and free fatty chains. Linked fatty chains prevented the crystallization of PI. On another hand, free fatty chains were demonstrated to highly increase the crystallization rate of PI under isothermal conditions at -25°C thus allowing to induce a faster crystallization of a 10 000 g/mol IR than a 600 000 g/mol IR.
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V. Supporting information:

![SEC chromatogram of PiDeg](image1)

**Figure S1:** SEC chromatogram of PiDeg

![1H NMR spectrum of PIDeg in CDCl₃](image2)

**Figure S2:** $^1$H NMR spectrum of PIDeg in CDCl₃
Polyisoprene / Lipid Coupling

Figure S3: ATR-FTIR spectrum of PIDeg

Figure S4: $^1$H NMR spectrum of PIDiOH in CD$_2$Cl$_2$
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Figure S5: $^1$H NMR spectrum of PIOH in CDCl$_3$.

Figure S6: $^1$H NMR spectra of myristic acid and C$_{14}$OChlo, C$_{16}$OChlo and C$_{24}$OChlo in CDCl$_3$. 
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Figure S7: $^{13}$C NMR spectra of myristic acid and C$_{14:0}$Chlo, C$_{18:0}$Chlo and C$_{24:0}$Chlo in CDCl$_3$

Figure S8: $^1$H NMR spectra of PIDiOH and PIDiC$_{24:0}$ in CD$_2$Cl$_2$
**Polyisoprene / Lipid Coupling**

Figure S9: $^1$H NMR spectra of PiMonoOH and PiMonoC24:0 in CDCl$_3$

Figure S10: DSC thermogram of NR
Polyisoprene / Lipid Coupling

Figure S11: DSC thermogram of PIDeg

Figure S12: DSC thermogram of PIDiOH

Figure S13: DSC thermogram of PIDiC24:0
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Figure S14: DSC thermogram of PIDiC\textsubscript{14:0}

Figure S15: DSC thermogram of PIDiC\textsubscript{16:0}

Figure S16: DSC thermogram of PIDiC\textsubscript{18:2}
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Figure S17: DSC thermogram of PIDiC11:1

Figure S18: DSC thermogram of PIDiC19:0

Figure S19: DSC thermogram of a PIMonoC24:0 OH after 8h of isothermal crystallization at -25°C
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Figure S20: DSC thermogram of IRDiC_{18:0}

Figure S21: DSC thermograms of initial IR and IRDiOH after 8 h of isothermal crystallization at -25 °C

Figure S22: DSC thermogram of high molar mass IR after isothermal crystallization at -25 °C for 60 h.
Figure S23: DSC thermogram of IRDeg after isothermal crystallization at -25 °C for 60 h.
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Polyisoprene / Protein Coupling
Polyisoprene / Protein Coupling

I. Introduction

This final chapter will focus on the PI / Protein coupling. As a reminder, the targeted coupling chemistry is a thiol-maleimide “click reaction” involving the thiol group of a cysteine for the protein part and a maleimide chain-end for the polymer side. This coupling chemistry was demonstrated in the literature to be effective\(^1\) affording one of the first example of Giant Amphiphile by coupling a lipase to a polystyrene chain. At this stage of the project, two synthons were available for the coupling: PIMal and a PIDiLipMal. In this chapter, various attempts of coupling will be presented emphasizing the difficulties to characterize the final product. Finally, an alternative solution was proposed to afford a protein-like block via the use of N-carboxyanhydride (NCA) polymerization (Figure 1).

II. Polymer-Protein coupling

\textbf{a. Reduction of disulfure bridges and PI/Lipase coupling}

Lipase B from \textit{Candida antarctica} (CALB) was first selected to be coupled to PI as it had already been used in the literature for a coupling with polystyrene. CALB presents two main advantages. First, this protein is relatively resistant to organic solvents thus avoiding any irreversible denaturation during the coupling.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{General scheme representing the PI-Protein coupling strategy (A) and the PI-Polypeptide synthesis (B.)}
\end{figure}
The other major advantage of this protein is that its molar mass is quite low, ~ 35 kDa, which is only 3 times more than the functional PI used, and not too far from the REF and SRPP, which are the final targeted proteins. However, under its native form, the 6 thiol groups of CALB cysteines are engaged in disulfure bridges which necessitate their reduction prior to any attempt of coupling with PI. Figure 2 presents the structure of the three most common reducing agents used for the disulfure bridges reduction of proteins. Among them, β-mercapto ethanol is the most powerful, usually able to reduce all disulfure bridges regardless the structure of the protein. Nevertheless, it stays connected to the newly formed thiol as represented in Figure 3, rendering it impossible to use for the project as the objective is to obtain free thiol functions.

![Figure 2: Structure of the 3 commonly used reducing agents for disulfure bridges cleavage](image)

DTT appeared to be a good candidate for the disulfure reduction as it is another powerful reducing agent and leads to free thiol groups after the reduction as illustrated in Figure 4. Moreover, this reducing agent was used by Velonia et al.\(^1\) for the reduction of lipase and gave good results. Nevertheless, it has to be used in excess (~ 2 eq to cleave 1 disulfure bridge), and as DTT contains thiol groups, it could interfere in the PI-Protein coupling by reacting with the maleimide chain-end if the excess is not removed. To prevent this side-reaction, extensive dialysis has to be performed which slows down the overall process.
TCEP was thus selected as a “sulfur-free” reducing agent. It was reported in the literature to be a powerful candidate for the disulfide bridge reduction\(^2\) presenting the advantage of not interfering in the thiol maleimide reaction as compared to other reducing agent able to couple with the maleimide function\(^3\). This renders even more attractive the use of TCEP as even if traces remain after the purification of the reduced protein it would not interfere with the thiol-maleimide coupling reaction.

Surprisingly, after the reducing process and the purification step, only 30 wt% of protein was recovered. This is probably due to the purity of the starting protein (see below) and/or the adsorption of proteins to the dialysis membrane. The recovered protein was then analysed by SDS-PAGE method and compared to both, the native protein and a sample reduced with a great excess of β-mercaptoethanol (Figure 5). It can be observed that the commercial CALB (column 2) exhibits several bands, whereas only one should be present for a pure sample. This could partially explain the mass loss during the reduction process as those unknown impurities could potentially pass through the dialysis membrane. Moreover, it could be noticed that the difference of migration between the fully reduced CALB (column 3) and the native one (column 2) is very small (see the zoom). Nevertheless, the band in column 4 corresponding to the CALB reduced with TCEP lies in between the native CALB and the one reduced with β-mercaptoethanol. The reduction with TCEP was thus less efficient and gave only a partially reduced CALB. A higher amount of TCEP (up to 100 equivalents) did not increase the reduction rate as the same SDS-PAGE profile was obtained. The Ellman’s titration\(^4\) performed to determine the quantity of free thiol present on the protein failed for an unknown reason.
A first attempt of coupling of the partially reduced CALB with PIMal was nevertheless carried out. The solvent conditions reported in the literature (THF/water admixture 16/84 v/v%)\(^1\) were abandoned as the PIMal, first solubilized in THF, precipitated instantaneously when added to the water solution containing the protein. THF was then substituted by Toluene which is an excellent solvent of PI and can also bear a slight fraction of water. Two blank experiments were also performed.

- A “blank protein” experiment without PI.
- A “blank PI” using PIOH instead of PIMal, as it should not couple with the protein due to the absence of maleimide group.

Figure 6 presents some pictures of the coupling attempts after addition of the PI phase on the protein phase (Figure 6.1) and after 24 h of stirring followed by overnight phase separation (Figure 6.2). Before stirring, no PI precipitation was observed contrarily to the attempt of coupling carried out using THF. The cloudiness observed for the sample 1.C came from the presence of TEA chloride salt that remained from the grafting of maleimide at the PI chain-end (see chapter IV). After stirring, 3 emulsions were obtained (Figure 6.2) and demonstrated to be stable as no phase separation occurred overnight. A sample of each emulsion was observed by optical microscopy (Figure 7).
The 3 emulsions present three distinct particle profiles. On the “blank protein” experiment (Figure 7.A) a nearly perfect spherical emulsion stabilized by the protein itself thanks to its amphiphilic behaviour can be observed. The “blank PI” experiment presents relatively ill-defined bigger particle size. In both cases, the emulsion might be stabilized by the proteins but it is nevertheless difficult to explain the difference of behaviour (size and shape of the particles). Finally, for the coupling experiment; a different behaviour compared to the two other samples is noticeable. The size of the particles formed is in between the two other experiments and the aspect of the droplets changed. Nevertheless, it is difficult to conclude that coupling occurred.

The use of other methods to characterize the sample and demonstrate the coupling (SEC, NMR, SDS-PAGE) failed as it was impossible to solubilize the entire sample in one common solvent. As an example, in the case of $^1$H NMR analysis, when the sample was solubilized in CDCl$_3$, only the signals of PIMal could be observed.
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This result could have been expected as only the chloroform-soluble fraction is analysed and as PIMal is used in excess compared to the protein. It was then decided to study the coupling of PI with Bovine Serum Albumin (BSA).

b. PI/BSA coupling

BSA is a bigger protein than CALB (66kDa instead of 35kDa) but presents the advantage of bearing a free thiol function in the native state\(^5\) on the Cys-34, which avoids the reduction step and allow to gain time in the overall pathway. The same coupling conditions as for CALB were applied to BSA. Reaction time was 24 h (Figure 8.1) or 3 days (Figure 8.2). Again, in all cases, emulsions were obtained. In the case of the coupling attempt carried out for 3 days (Figure 8.2.C), a phase inversion can be observed (emulsion in the aqueous phase). A sample of each emulsion was taken, dispersed into pure water and analyzed by optical microscopy (Figure 9). Whatever the reaction time, well defined emulsions were obtained in the case of the “blank protein” experiment with the formation of spherical droplets. In the case of the “blank PI” experiment, the aspect of the spheres obtained are really similar to the one obtained with the “blank protein” experiment and seemed to indicate that, as expected, no coupling occurred. The coupling attempt after 24h (Figure 9.1.C) presents the same aspect of particles than the ones obtained with CALB. But, after 3 days of coupling (Figure 9.2.C), a phase inversion is observed, and the microscopy analysis presents a double population of particles, one colourless similar to a foam and a second, darker, forming spherical particles of different sizes. We could then think that the coupling occurred with the formation of big spherical droplets totally distinct from the “blank protein” experiment regarding the diversity of sizes and their global aspect but, still, this does not constitute a direct proof.

Figure 8: PI-Protein coupling attempt / 1. 24h of coupling + overnight phase separation / 2. 3 days coupling + overnight phase separation. (A: “blank protein” / B: “blank PI” / C: coupling).
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Figure 9: Optical microscopy analysis of the 3 emulsions obtained after the coupling attempts: 1. 24h of coupling + overnight phase separation / 2. 3 days of coupling + overnight phase separation
(A: “blank protein” / B: “blank PI” / C: coupling)

Only few further analyses (SEC in water and DMF) were performed, at the moment of the writing of the manuscript, mostly due to lack of time and troubles of solubilization. The only SEC analyses that were carried out did not give any result most probably due to solubility issues. To date, optical microscopy analysis is the only method allowing to analyze the entire sample at the same time. The resulting pictures are difficult to interpret but seem to go in the sense of the occurring of the coupling even if they do not constitute an undoubtful proof of it. Another strategy was then proposed, using N-carboxyanhydride polymerization with a PI macro-initiator to afford a PI-Polypeptide di-block with the polypeptide block mimicking proteins.
III. PI-polypeptide co-polymer synthesis

In this subchapter will be presented two distinct methods to afford a di-block PI-b-Polypeptide (Figure 10). The first one is based on Diels-Alder click-chemistry and proposes to synthesize a furan terminated polypeptide block that will be grafted to the already discussed PIMal (Figure 10.a). The polypeptide furan terminated could be obtained by ROP of NCA using furfurylamine as the initiator. Indeed, it was already demonstrated that DA reaction between maleimide and furfurylamine did not lead to the expected product (see chapter IV), but this behavior no longer exist if the furfurylamine is turned into a furfurylamide. The second strategy (Figure 10.b) is based on the capacity of PIDiOH and PImOH to be used as macroinitiators of the ROP of N-carboxyanhydrides (NCA). A bibliographic part will briefly present the chemistry involved and the possibilities existing for the alcohol initiation of NCA also demonstrating the novelty of the system proposed and will be followed by the results obtained using this method. Finally, it will be shown that this strategy might be the most promising one to afford a structure close to Tanaka’s model, regarding the difficulties encountered with the protein coupling.
Figure 10: Two different pathways investigated for the synthesis of PI-Polypeptide diblock
a. **Synthesis of benzylglutamate N-carboxyanhydride (BenzylGluNCA)**

Regardless the strategy targeted, the starting point of the study was the synthesis of the NCA monomer. BenzylGluNCA was chosen, as it appeared to be the easiest monomer to obtain and one of the most stable among all existing NCAs. To this end, benzylglutamate was reacted with triphosgene in dry THF. The occurring of the reaction can be easily followed as benzylglutamate was insoluble in THF, but became soluble as soon as the NCA ring was formed. The product was fully characterized by NMR analysis (\(^1\)H, \(^{13}\)C and HSQC/ Figure 11 and Figure 12). All spectra are comparable to the ones described in the literature.\(^1^2\) Full conversion was obtained with a good purity of the monomer.

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**Figure 11:** \(^1\)H and \(^{13}\)C NMR spectra of BenzylGluNCA in CDCl\(_3\)

**Figure 12:** HSQC NMR analysis of BenzylGluNCA - CDCl\(_3\)
Polyisoprene / Protein Coupling

b. Synthesis of a di-block PI-Polypeptide via DA reaction:

The first step was then the synthesis of a polypeptide furan terminated. It proceeded via a classic polymerization of NCA using a primary amine, furfurylamine, as initiator. A good control of the reaction was obtained in dry DMF at 0°C. When performed in CDCl$_3$, the $^1$H NMR analysis gived poorly defined spectra. This was reported to be due to the structure of the polymer, forming an $\alpha$-helix architecture, preventing a good solubilization in some solvents. To improve the solubility, trifluoroacetic acid (TFA), deuterated or not, must be used as a co-solvent. The $^1$H NMR spectrum, the SEC chromatogram as well as the MALDI-TOF spectrum of the polymer are given in Figure 13 to Figure 15 respectively. The assignment could be made thanks to literature$^{10}$. It can be noted that the furan chain-ends (signals 7 and 8 at 6.27 and 6.22 ppm respectively on Figure 14) allowed to estimate the molar mass by the use of the following formula:

$$M_n (NMR) = \frac{i(5) \times 2}{i(7-8)} \times M_n (\text{unit}) + M_n (\text{initiator})$$

With:
- $M_n (NMR)$ : The molar mass of the polymer calculated by $^1$H NMR
- $i(5)$ : the value of the integral of signal “5” on Figure 13 corresponding to the “-CH” group of the repeating unit
- $i(7-8)$: the value of the integral of both signals “7” and “8” on Figure 13 corresponding to “CH” protons of the furan chain-end
- $M_n (\text{unit})$: The value of the molar mass of a repeating unit (219 g/mol)
- $M_n (\text{initiator})$: The value of the molar mass of the initiator (97 g/mol)

The calculated molar mass was then estimated to be $M_n (NMR) = 4450$ g/mol which is in good agreement with the targeted molar mass (5000 g/mol). The dispersity was evaluated by SEC in DMF (Figure 14) revealing a quite narrow distribution (1.05) attesting the good control of the polymerization. The molar mass obtained (3700 g/mol) is in the same range of the targeted one but is not reliable as it is based on polystyrene standards. DMF was also used to solubilize the matrix and the polymer to perform MALDI-TOF analysis (Figure 15). Two distinct populations could be observed, one of small molar masses (1435-2530 g/mol) and of weak intensity and a second, more intense, representing higher molar masses (3180-6250 g/mol). The simulation revealed an exact correspondence between the obtained spectrum and the expected compound.
Indeed, it is possible to calculate the exact degree of polymerization of each peak by using the formula:

$$DP(\text{peak}) = \frac{M(\text{peak}) - Mn(\text{Na}^0) - Mn(\text{furfurylamine})}{Mn(\text{unit})}$$

With:
- $DP(\text{peak})$: The degree of polymerization of a precise population of polymer
- $M_n(\text{Na}^0)$: the molar mass of native sodium used for ionisation (23.00 g/mol)
- $M_n(\text{furfurylamine})$: the molar mass of furfurylamine used as the initiator (97.12 g/mol)
- $M_n(\text{unit})$: the molar mass of a repetition unit (219.24 g/mol)

As an example, applying this formula to the main peak of the major population of Figure 15 gives a $DP$ of 20 for a $M_n(\text{peak})$ of 4504.3 g/mol. Surprisingly, applying the same formula to the main peak of the minor population ($M_n(\text{peak}) = 1873.8$ g/mol) gives a $DP$ of 8. This would mean that the minor population presents the same structure as the major one but is made of shorter chains. To date, no explanation was found for this double population, as no shoulder or bimodal distribution was observed by SEC. However, all those characterizations attest for the formation of the desired polymer bearing the expected furanic chain-end.

\[\text{Figure 13: } ^{1}H \text{NMR spectrum of a PPepFur - CDCl}_3/\text{TFA 2/1 (v/v)}\]
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Figure 14: SEC chromatogram of a PPepFur in DMF.

Figure 15: MALDI-TOF spectrum of PPepFur
The next step of the synthesis was the coupling via Diels-Alder reaction between both blocks. As a model of PIDiLipMal, a PIMal was used and reacted with the previously synthesized PPeFur. The choice of the conditions appeared to be tricky as PIMal and PPeFur can be hardly solubilized in the same solvent. Chloroform happened to be the only candidate, even if, as already said, NMR spectra are not perfectly defined in this solvent probably due to solubility issues. A homogeneous solution (at least visually) was obtained. After 48h of heating at 60°C, no coupling occurred. It was then added 5% (v%) of DMF in order to help the solubilization of the peptidic chain to allow the reaction to proceed, again at 60°C for 48h. The $^1$H NMR analysis of the final polymer is given in Figure 16 and compared to the starting PIMal. No TFA could be used in this case to characterize the compound as it could degrade the PI backbone. On the NMR spectrum, no visible sign of coupling appeared and, furthermore, the signals of the chain-ends of both polymers remained perfectly visible (singlet at 6.68 ppm corresponding to the double bond of maleimide and two singlets at 6.27 and 6.22 corresponding to “–CH” groups of the furanic function). The bad solvatation of the PPeFur in chloroform, despite the small amount of DMF used, might be one explanation for this impossibility of coupling. Indeed the polypeptide chain might adopt a conformation blocking the accessibility to the furan chain-end. Another reason might be the temperature which could be too low for the occurring of the DA reaction. For these reasons, this di-block coupling was abandoned and never applied to a PIDiLipMal.

![Figure 16: $^1$H NMR spectra of a PIMal and an attempt of DA coupling to afford PI-b-PPep – CDCl$_3$](image)
c. ROP of NCA initiated by PIDiOH:

i. Bibliography:

As presented in the “bibliography part” (see chapter 1), the NCA polymerization generally proceeds via a primary amine initiation and at low temperature to keep a good control of the reaction (Figure 17). As, it was not possible to obtain primary amine terminated PI, we turned towards alcohol initiation of NCA as recently described in the literature.

Indeed in 2015, Zhao et al.\(^6,7\) introduced a new polymerization system based on the use of amino-alcohols as initiators and the use of a thiourea derivative (TU) as the catalyst (Figure 18). This alcohol initiated polymerization is rendered possible thanks to a multiple hydrogen bonds system. The first addition of monomer proceeds with the activation of the monomer by the thiourea concomitantly to the internal activation of the initiator by hydrogen bonding between the tertiary amine and the hydroxyl group.

Figure 17: General chemical pathway for the ROP of NCA initiated by a primary amine

Figure 18: General chemical pathway proposed by Zhao et al.\(^7\) for the ROP of NCA using hydroxylamines as initiating system
This first addition leads to a decarboxylation of the chain-end to recover a terminal primary amine. It allows the propagation step to occur using the “classic” polymerization process. The role of thiourea was also important during chain-growth. Indeed, the terminal tertiary amine could act as a base to deprotonate a monomeric unit and initiate a new chain. This phenomenon is prevented here by the interaction between the chain-end and the thiourea, thus reducing the basicity of the terminal tertiary amine. Moreover, polymerization could be performed at room temperature without loss of the reaction control, again thanks to the thiourea interaction with the propagating primary amine thus slowing down the reactivity. With the use of 3 equivalents of thiourea (compared to the initiator), the reaction reaches full conversion within 90 minutes with a dispersity of 1.02 whereas only 57% of conversion were obtained after 240 minutes when 10 molar equivalents of thiourea were used but still with a narrow molar mass distribution (1.05). Multifunctional amino-alcohols can be used as plurifunctional initiators (Figure 19) to obtain different architectures of polypeptide (linear, 3-arms and 4-arms stars).

More recently, Gradišar \textit{et al}.\textsuperscript{8} presented another method for the ROP of NCA using alcohols as initiating species and acid as catalyst. In this case, the reaction pathway proceeds in two steps (Figure 20):

- First, the initiation step uses an organic acid to activate the monomer (3 equivalents of methanesulfonic acid (MSA) compared to the initiator) and to block the propagation by protonation of the formed primary amine.
- Then, the propagation step starts by the addition of a base (N-ethyl-diisopropylamine) in a slight default compared to the acid (2.5 equivalents of base compared to the initiator).

![Figure 19: Multifunctional hydroxylamines used by Zhao et al\textsuperscript{7}.](image1.png)

![Figure 20: Mechanism proposed for the ROP of NCA using alcohol as initiating system and acid as a catalyst](image2.png)
This NCA polymerization allows to form well defined polypeptides with molar masses around 6 000 g/mol and molar mass distributions varying from 1.1 to 1.4. To date, those two methods are the only ones reported for the controlled ROP of NCA using alcohols as initiators.

### ii. Results and Discussion

The synthesis of a di-block “PI-Protein like” was studied applying the chemistry developed by Zhao et al.\(^6,7\) to PI bearing an amino-alcohol chain-end. Among the functional PI described in Chapter 4, PIDiOH and PImOH were good candidates. With PIDiOH a “Y” shaped polymer could be obtained if both hydroxyl groups could initiate the polymerization. This synthon was then preferred.

In a first step, the ROP of NCA using dimethylethanolamine (DMEA) as the initiator was performed following the procedure reported in the literature. The \(^1\)H NMR analysis of the obtained polymer is given in Figure 21 as well as the SEC analysis performed in DMF reported in Figure 22. The \(^1\)H NMR analysis obtained is similar to the one described in the literature\(^7\) thus confirming the occurring of the polymerization. The molar mass was estimated to be 10 000 g/mol by NMR for a targeted molar mass of 13 000 g/mol by using the formula:

\[
M_n(RMN) = \frac{3 \times i(5)}{i(8)} \times M(unit) + M_n(initiator)
\]

Where:
- \(M_n(RMN)\) is the molar mass of the polymer calculated by \(^1\)H NMR
- \(i(5)\) and \(i(8)\) are the values of the integral of signals 5 and 8 in Figure 21 respectively
- \(M\) (unit) is the molar mass of a repetition unit (219 g/mol)
- \(M_n\) (initiator) is the molar mass of the initiator (89 g/mol)

The SEC analysis showed two different populations, one with a \(M_n\) of 11 000 g/mol which is in agreement with the NMR analysis and the targeted molar mass, and a second population around 30 000 g/mol. This could indicate the presence of a small amount of side reactions.
As described in the literature, the amount of TU used during the polymerization is the key point to prevent side reactions. Nevertheless, a too high amount of TU prevents the polymerization due to hydrogen bonding between TU and the propagating primary amine. We decided to establish a balance between both phenomena increasing the amount of TU to 3 equivalents (compared to initiator) and increasing the reaction time to 2 h.
Those conditions were used for the polymerization of NCA using PIDiOH as the initiator. $^1$H NMR analysis comparing the obtained polymer with a PPeP initiated by DMEA is given in Figure 23 as well as a SEC analysis performed in THF comparing the obtained polymer with the starting PIDiOH in Figure 24. On $^1$H NMR analysis, it is difficult to identify the linkage between both blocks due to the bad definition of the polypeptide in CDCl$_3$. When TFA was added, the sample was degraded rapidly preventing the analysis. Nevertheless, the comparison of the spectrum of the obtained polymer with the PPeP initiated by DMEA showed that all the signals corresponding to the polypeptide block are present in the copolymer. Moreover, the signal corresponding to the “-CH$_2$” group in $\alpha$ position of the ketone chain-end of the PI block remains visible at 2.43 ppm. Finally, the DOSY NMR analysis confirmed the coupling as two different diffusion coefficient were found for the macroinitiator and the final copolymer. The formation of the diblock was also confirmed by the SEC analysis in THF (Figure 24). After the polymerization, an increase of the molar mass is observed by using MALLS detection whereas the elution time of the co-polymer shifted to higher retention time. This is proposed to be due to the interactions between the polypeptide block and the stationary phase of the column and thus confirms the obtention of the good compound. Indeed, by looking at the variation of the molar mass in function of the elution time (two lines in the zoom of Figure 24) it can be observed that for the co-polymer the molar mass is practically constant along the time contrarily to the macro-initiator which presents an important decrease of the molar mass with the increase of the elution time. Finally, the increase of molar mass is about 10 000 g/mol which is in agreement with the targeted molar mass.

![Figure 23: $^1$H NMR spectra of a PIDiPPep and a PPeP initiated by DMEA in CDCl$_3$](image)

Figure 23: $^1$H NMR spectra of a PIDiPPep and a PPeP initiated by DMEA in CDCl$_3$
In conclusion, the ROP of NCA using PI as the initiator was possible thanks to the amino-alcohol chain-end. It is a convenient method to afford a di-block copolymer PI-Polypeptide with relatively simple chemistry. However, as the frame of the work presented here is to obtain a tri-block co-polymer Protein-PI-Lipid, it was compulsory to develop the same chemistry but using the ketone chain-end of PIDeg as the aldehyde side must be used for the synthesis of PIDiLip. The main problem came from the impossibility of functionalizing the ketone side with a secondary amine to afford an amino-alcohol chain end. A new approach was thus developed using two successive reductive aminations.

iii. **Synthesis of a heterotelechelic ethylethanolamine/hydroxyl PI (PINOHOH)**

![Figure 25: General chemical pathway for the synthesis of PINOHOH](image)

To synthesize a PI amino-alcohol terminated at the α chain-end (ketone chain-end in PIDeg), a reductive amination of the ketone chain-end with ethanolamine must be performed to afford a hetero-telechelic (ethanolamine/hydroxyl) PI (PINOHOH). Before using a PIMono(or Di)Lip, it was proposed to use a model compound, PIOH (described in Chapter 4), as it is easier and quicker to synthesize than the PIMono(orDi)Lip.
Polyisoprene / Protein Coupling

The $^1$H NMR spectrum of the compound synthesized is reported in Figure 26 and compared to the initial PIOH. The signal of the “$-\text{CH}_2$” group in α position of the ketone chain-end at 2.43 ppm totally disappeared confirming a quantitative reaction. New signals appeared at 3.6 ppm ($8^\prime$-11’) and at 2.79, 2.74 and 2.68 ppm ($9^\prime$-10’) and were assigned to newly formed chain-end thanks to HSQC and HMBC experiments. No significant change could be observed in SEC thus attesting of the absence of side reactions. The next step was another reductive amination using acetaldehyde and PINHOH in order to obtain a tertiary amine that could initiate NCA polymerization. It was proposed that, ideally, both reductive aminations (from PIOH to PINOHOH) could be done successively as they involve the same reaction conditions. The $^1$H NMR spectrum of PINOHOH is given in Figure 27 and compared to PINHOH. The assignment was tricky regarding the number of signals and HSQC and HMBC experiments were again needed to assign each signal. The total shift of the signal from the “$-\text{CH}_2$” group in α position of the hydroxyl group (from 3.63 to 3.43 ppm) confirmed full conversion. The increase of the number of signals between 2.3 and 2.8 ppm corresponding to the “$-\text{CH}$” and “$-\text{CH}_2$” groups in α position of the nitrogen atom (namely “$9^\prime$”, “$10^\prime$” and “$12^\prime$” in Figure 27) is also a proof that the reaction occurred. Moreover, no particular change in the molar mass of the polymer was observed in SEC thus attesting of the absence of side reactions. It was thus possible to synthesize a PINOHOH functionalized at the α chain-end (the ketone side in PiDeg) by an amino-alcohol function that could be used for NCA polymerization.

Figure 26: $^1$H NMR spectra of PINHOH and PIOH in CD$_2$Cl$_2$
iv. **Synthesis of Polypeptide initiated by PINOHOH (PIPPeNOHOH)**

Finally, the ROP of NCA was carried out using PINOHOH as the initiator and TU as the catalyst. The $^1$H NMR spectrum of the polymer obtained is given in Figure 28 and compared to the one of the macro-initiator. Again, TFA could not be used to improve the quality of the spectrum as it would cause the degradation of the PI block. The characteristic signals of the polypeptide block were present on the spectrum (18’, 17’, 14’, 15’ and 16’ at 7.25, 5.02, 3.96, 2.61 and 2.27 ppm respectively) as well as the signal 11 corresponding to the “-CH$_2$” group in $\alpha$ position of the hydroxyl group (α chain-end) of the macro-initiator at 3.43 ppm totally disappeared, indicating efficient initiation of the polymerization. Moreover, the presence of the signal at 3.63 ppm corresponding to the “-CH$_2$” group in $\alpha$ position of the hydroxyl group (ω chain-end) confirms the selectivity of the initiation by the amino-alcohol group.
SEC analysis was also performed in THF containing Bis(trifluoromethane)sulfonimide lithium salt in order to avoid the “stacking” of the polypeptide block on the column (Figure 24). Figure 29 presents the SEC chromatograms obtained for the di-block copolymer and the starting macro-initiator. The molar mass after polymerization goes from 11 000 g/mol to 16 000 g/mol, in good agreement with the targeted molar mass of the polypeptide block (targeted molar mass of the NCA block around 5 000 g/mol). A narrowing of the signal was observed but only from the small molar mass side of the Gaussian plot. This can be explained by the high control of the NCA polymerization. Indeed, the molar mass distributions being small (<1.1)<sup>9,10</sup>, blocks of the same length were added to all the PI macroinitiator, this latter having a broader molar mass distribution. Assuming that the hydrodynamic volume of polypeptide is different from the one of polyisoprene, the impact of the co-polymerization on low molar mass PI will be greater than on the high molar mass ones and thus explains the behavior observed here. Finally, no trace of homopolypeptide was observed in the SEC chromatogram also confirming that the signals observed in proton NMR of polypeptide does not come from an admixture of two homopolymers.
Polyisoprene / Protein Coupling

To conclude, the ROP of NCA was studied as a simple model of a protein block. The coupling attempts via the use of DA reaction did not lead to the expected structure, certainly due to the difference of solubility of both blocks and probably due to difficult encountering of both terminal functions. As a new approach, the NCA polymerization using PI macro-initiator was developed and lead to the formation of two distinct polymer architectures: "Y" shape in the case of the use of PIDiOH as initiator and a linear di-block in the case of the use of PINOHOH as a starting material. The latter also presents the advantage of bearing the polypeptide block at its α chain-end, allowing the possibility to apply this chemistry to PIDiLip and for the synthesis of the tri-block co-polymer close to Tanaka’s model.

IV. Conclusion

The protein/PI coupling was the most challenging part of this PhD work. The strategy proposed for the coupling, using thiol-maleimide click chemistry was demonstrated in the literature to work but appeared to be highly difficult to perform regarding the difference of solubility of both blocks. Some attempts were nevertheless performed showing formation of particles of variable shapes. Unfortunately, no direct proof of coupling could be obtained despite the different analysis methods used.

As a substitute, ROP of NCA was applied to obtain various PI/Polypeptide co-polymers. Moreover, the chemistry developed for the polymerization is an alternative of the classic primary amine synthesis as it uses amino-alcohols as initiators and TU as catalyst.
Finally, it was shown the possibility to apply this chemistry from the α chain-end of the PI chain (the ketone chain-end in PIDeg) in order to be able to synthesize a polypeptide block starting from PIDiLip and, thus, afford a tri-block co-polymer close in structure to Tanaka’s model.

V. Experimental part

a. Reduction of disulfure bridge (TCEP / CALB)

CALB (0.5 g) was dissolved in 5 mL of a phosphate buffer (PB) 1M (pH = 7.4). TCEP.HCl (41 mg, 10 eq) was then added to the solution as well as NaHCO₃ to obtain a final solution at pH ~ 8. Two cycles of vacuum/argon were applied to afford a final solution under inert atmosphere. The solution was then stirred at room temperature overnight. The excess of salt was removed by dialysis in pure water, and the final proteins were recovered by freeze-drying. Yield: 30%

b. Coupling CALB / PIMal

The reduced CALB (50 mg) was dissolved in 0.6 mL of PB 1M and mixed with 4 drops of TEA. PIMal (71 mg, 5 eq) was dissolved in 0.6 mL of toluene. The PI solution was then added to the protein solution and the heterogeneous solution vigorously stirred for 24h. The admixture was kept at room temperature overnight to allow phase separation.

c. Coupling BSA / PIMal

Native BSA (50 mg) was dissolved in 0.3 mL of PB 1M and mixed with 4 drops of TEA. PIMal (38 mg, 5 eq) was dissolved in 0.3 mL of toluene. The polymer solution was then added to the protein solution and the heterogeneous solution vigorously stirred for 24 hours. The admixture was kept at room temperature overnight to allow phase separation.

d. Synthesis of BenzylGluNCA

![Structure of BenzylGluNCA](image-url)
Benzylglutamate (2 g, 7.58 mmol) was put in suspension in 20 mL of dry THF. Triphosgene (1.35 g, 0.6 eq, 4.55 mmol) was then added and the reaction was allowed to proceed at 50°C with the reaction flask connected to a bubbler fulfilled with a KOH solution. The reaction media became slightly yellow and homogeneous with the formation of the NCA. After 2 h of reaction, the media was concentrated using vacuum and then directly precipitated in cold Et₂O. The white powder formed was then recovered by filtration, wash 2 times with Et₂O and dried under dynamic vacuum overnight. Yield ~ 93%.

¹H NMR (CDCl₃) δ (ppm): 7.34 (m, 5H, CH₆Ar), 6.84 (s, 1H, NH), 5.12 (s, 2H, CH₂O), 4.37 (t, 1H, CHNH), 2.56 (t, 2H, CHCOO), 2.24/2.12 (m, 1H/1H, CH₂CHNH)

¹³C NMR (CDCl₃) δ (ppm): 172.4 (1C, COOCO), 169.5 (1C, OCONH), 152.2 (1C, CH₂OCO), 135.3 (1C, CH₂C₆Ar), 128.8/128.6/128.4 (5C, CH₆Ar), 67.3 (1C, CH₂OCO), 56.9 (1C, COCHNH), 29.7 (1C, OCOCH₂), 26.7 (1C, CH₂CH₂CH)

e. Synthesis of PPepFur

BenzylGluNCA (1 g, 3.8 mmol) was dissolved in 5 mL of dry DMF and cooled to 0°C using an ice bath. Furfurylamine (15 µL, 0.04 eq, 0.16 mmol) was added to the solution and the reaction maintained at 0°C during 6 h under stirring, with the reaction flask connected to a bubbler. The final polymer was recovered by precipitation into cold Et₂O and overnight drying under vacuum, affording a white solid. Yield ~ 70%.

¹H NMR (CDCl₃/TFA: 2/1 v/v) δ (ppm): 7.30 (m, 5H, CH₆Ar), 6.27 (s, 1H_chain-end, OCH=CHCH), 6.22 (s, 1H_chain-end, CHCH=CH), 5.11 (q, 2H, CH₂C₆Ar), 4.69 (m, 1H, COCHNH), 2.47 (m, 2H, COCH₂CH₂), 2.14/1.96 (m, 2H, CH₂CH₂CH)
Polyisoprene / Protein Coupling

f. Synthesis of PIPPepFur

First, 20 mg of P PepFur (4 µmol) were dissolved in chloroform with 40 mg (1 eq, 4 µmol) of
PIMal. The Diels-Alder reaction was performed at 60°C during 48h under stirring. The final
polymer was precipitated in cold methanol and dried overnight at 40°C under vacuum.

g. Synthesis of P PepDMEA

BenzylGluNCA (0.35 g, 1.33 mmol) and TU (11 mg, 0.016 eq, 22 µmol) were dissolved in 7
mL of dry DCM. Then, 2.2 µL of dry DMEA were added to the reaction medium and the
reaction flask was connected to a bubbler. The reaction was allowed to proceed at room
temperature during 40 minutes and the final polymer was obtained by precipitation into a
large excess of cold methanol and drying overnight under dynamic vacuum. Yield ~ 90%

$^1$H NMR (CDCl$_3$/TFA: 2/1 v/v) δ (ppm): 7.30 (m, 5H, $CH_A$), 5.11 (q, 2H, $CH_2C_A$), 4.69 (m,
1H, COCHNH), 2.47 (m, 2H, COCH$_2$CH$_2$), 2.14/1.96 (m, 2H, CH$_2$CH$_2$CH)
Polyisoprene / Protein Coupling

h. Synthesis of PIDiPPep

![Figure 34: Structure of PIDiPPep](image)

BenzylGluNCA (0.25 g, 0.95 mmol) and TU (30 mg, 60 µmol, 0.06 eq) were dissolved in 5 mL of dry dichloromethane. Separately, 0.17 g of PIDiOH (19 µmol, 0.02 eq) were dissolved in 5 mL of dry DCM. The NCA/TU solution was then added to the PI solution and the reaction was allowed to proceed for 2h at room temperature. The reaction flask was connected to a bubbler to evacuate the CO\(_2\) generated during the reaction. The final polymer was recovered by two successive precipitations into a large excess of cold methanol and drying overnight at 40°C under dynamic vacuum. The obtained polymer was a yellowish sticky solid material.

Yield: ~ 90%

i. Synthesis of PINHOH

![Figure 35: Structure of PINHOH](image)

PIOH (0.26 g, 26 µmol) were solubilized in 1 mL of dry THF. Then, 15.6 µL of ethanolamine (5eq, 130 µmol) were added to the solution and stirred at 40°C during 2h with the polymer. Finally, 57 mg (9 eq, 234 µmol) of NaBH(OAc)\(_3\) were then added to the reaction flask as well as 2 µL (1.5 eq, 34 µmol) of acetic acid and the reaction was maintained at 40°C overnight. The final polymer was then recovered by two successive precipitations into cold methanol, solubilisation in Et\(_2\)O, filtration through Celite\textsuperscript{®} and overnight drying at 40°C under dynamic vacuum. Yield: ~ 85%
Polyisoprene / Protein Coupling

$^1$H NMR (CD$_2$Cl$_2$): $\delta$ (ppm): 5.14 (m, 1H, CH=CCH$_3$), 3.60 (m, 4H, CH$_2$OH$_a$/CH$_2$OH$_b$), 2.79/2.74/2.68 (m, 3H, NHCH$_2$CH$_2$/CHCH$_3$), 2.06 (m, 4H, CH$_2$CH=CCH$_3$CH$_2$), 1.69 (m, 3H, CH=CCCH$_3$)

j. Synthesis of PINOOH

PINOOH (0.2 g, 20 µmol) was dissolved in 1 mL of dry THF. Then, 10 µL (10 eq, 200 µmol) of acetaldehyde were added to the solution and stirred 2h with the polymer. Finally, 93 mg (20 eq, 400 µmol) of NaBH(OAc)$_3$ and 3 µL (2 eq, 52 µmol) of acetic acid were then added to the solution and the reaction was allowed to proceed at 40°C overnight. The final polymer was recovered by two successive precipitations into cold methanol, solubilization into Et$_2$O, filtration through Celite$^\text{®}$ and overnight drying at 40°C under dynamic vacuum.

Yield: ~ 85%

$^1$H NMR (CD$_2$Cl$_2$): $\delta$ (ppm): 5.14 (m, 1H, CH=CCH$_3$), 3.60 (m, 2H, CH$_2$OH$_a$), 3.43 (m, 2H, NCH$_2$CH$_2$OH), 2.73/2.57/2.48/2.36 (m, 5H, CHN(CH$_2$)$_2$), 2.06 (m, 4H, CH$_2$CH=CCH$_3$CH$_2$), 1.69 (m, 3H, CH=CCCH$_3$)

k. Synthesis of PIPPePOOH

$^1$H NMR (CD$_2$Cl$_2$): $\delta$ (ppm): 5.14 (m, 1H, CH=CCH$_3$), 3.60 (m, 2H, CH$_2$OH$_a$), 3.43 (m, 2H, NCH$_2$CH$_2$OH), 2.73/2.57/2.48/2.36 (m, 5H, CHN(CH$_2$)$_2$), 2.06 (m, 4H, CH$_2$CH=CCH$_3$CH$_2$), 1.69 (m, 3H, CH=CCCH$_3$)
Polyisoprene / Protein Coupling

PINOHOH (0.11 g, 11 µmol) was dissolved into 1.5 mL of dry DCM. Then, 71 mg of BenzylGluNCA (273 µmol, 25 eq) was dissolved into 1 mL of dry DCM as well as 12.6 mg of TU (25 µmol, 2.2 eq). The solution of PINOHOH is then added to the solution of monomer, the reaction flask is linked to a bubbler and the reaction allowed to stir at room temperature for 40 minutes. The final polymer is obtained by precipitation in cold methanol containing a small amount of water (2 mL in 150 mL of methanol), filtration through glass filter and overnight evaporation at 40°C under dynamic vacuum.

Yield: ~ 90%
REFERENCES


(8) Gradišar, Š.; Žagar, E.; Pahovnik, D. *ACS Macro Lett.* **2017**, *6* (6), 637.


Conclusion and Outlooks
Conclusion and Outlooks

The main objective of this PhD work was the synthesis of a tri-block copolymer composed of a core of pure 1-4 \textit{cis} PI functionalized at both chain-ends (namely $\alpha$ and $\omega$, respectively) by a protein and one or two fatty chains respectively. This molecule had been reported to be a good model of NR and the study of the tri-block properties could lead to a better understanding of the natural polymer. The strategy developed to afford such an architecture was thus to graft selectively each block, one after the other, to the PI backbone. It was also proposed, in parallel, to study the properties of each di-block (PI-Lipids / PI-Protein) separately prior to study the properties of the tri-block. It is believed that applying this chemical pathway to synthetic PI could allow to develop a new kind of hybrid material possessing properties close to NR, or at least better than synthetic PI alone.

In order to graft selectively each block at each chain-end, the starting PI should be hetero-telechelic. In the literature, many IRs were developed bearing various terminal chain-ends but the control of the microstructure could be an issue. In order to mimic NR, the microstructure of the hetero-telechelic PI should be 100\% 1,4-\textit{cis}. This lead us to the use of the chemical degradation of NR, already described in the literature, leading to pure 1,4-\textit{cis} microstructure (as the starting material is NR), bearing a ketone function at the $\alpha$ chain-end and an aldehyde function at the $\omega$ one. It was demonstrated that those functions could undergo selective functionalization using, for example, reductive amination chemistry, playing with the nature of the amine or of the reducing agent.

The first chapter focused on the characterization of two NRs coming from two different \textit{Hevea brasiliensis} clones (RRIM 600 and PB 235). Both clones present different molar masses and dispersity ($M_n \sim 500\ 000$ g/mol and $D \sim 2.6$ for RRIM 600 against $M_n \sim 1\ 000\ 000$ g/mol and $D \sim 1.5$ for PB 235) with a bimodality observed in RRIM 600 attesting the existence of 2 distinct populations of PI in the natural material. Elementary analysis showed that the nitrogen content of RRIM 600 (0.6 wt\%) was slightly higher than the one of PB 235 (0.4 wt\%) which could be related to a higher amount of proteins in RRIM 600. Finally, solubilization trials showed that both clones were rather well soluble in toluene, poorly soluble in cyclohexane and DCM and quite soluble also in THF. This later was then used for the degradation pathway as it allows to solubilize all the reactants.

The chemical degradation of both clones was then studied through the partial epoxidation of the double bounds with m-CPBA followed by the acidic cleavage of the oxirane groups with periodic acid to yield ketone/aldehyde hetero-telechelic PI (PiDeg).
First attempts showed that some periodic acid was consumed by the non-rubber compounds present in NR, preventing to cleave all the oxirane functions. As a consequence, experimental molar masses could be quite far from the targeted ones. This could be nevertheless improved by increasing the amount of acid to 2 equivalents compared to oxiranes. However, for small rates of epoxidation (targeting molar masses higher than 20,000 g/mol) the acidic cleavage appeared again less efficient leading to PIDeg of higher molar masses than expected and bearing remaining oxirane units. The same reactions were applied to IR confirming that this deviation from targeted molar mass was due to non-rubber compounds present in NR as no deviation was observed in the case of IR degradation. It was possible to obtain well-defined 10,000 g/mol hetero-telechelic PI with a 100% 1,4-cis microstructure.

The selective functionalization of the PIDeg was then studied after having defined the synthetic strategies to follow for grafting proteins and/or lipids. The “thiol-maleimide” chemistry was chosen for the protein coupling as it seemed to be selective and was already reported for the coupling of a protein with a PS chain. The lipidic coupling was not particularly studied in the literature. It was then decided to synthesize a PI chain bearing two hydroxyl groups at the ω chain-end (the α chain-end remaining intact) to graft fatty chains through esterification reactions to yield PIDiLip. Next step was to synthesize a PI chain bearing at α and ω chain-ends a maleimide function and two fatty esters respectively (PIDiLipMal). The ketone α chain-end of PIDiLip was then selectively reduced to yield PIDiLipOH. Maleimide grafting was a bit tricky but PIDiLipMal could be obtained in pretty good yield.

The properties of PIDiLip were first investigated, as they should be a good model of NR (half of the Tanaka’s model). It was first shown by DSC that the fatty esters grafted at the polymer chain-end could crystallize despite the amorphous PI matrix. Moreover, the crystallization temperature varied with the size of the linked fatty esters. The highest crystallization temperature was obtained with lignocerates (C\textsubscript{24:0}) reaching 20°C. Linked fatty chains were thus able to create nodules of crystallization in the material, suggesting that, if both chain-ends (α and ω) could be functionalized with lipids, a physically cross-linked material could be prepared. The addition of free fatty chains to “dope” the material and increase its crystallization temperature was investigated. Fatty acids were not suitable, contrarily to fatty esters which could increase the crystallization temperature up to 40°C in the case of methyl lignocerate. Then, the role of linked and free fatty chains onto the cold crystallization of NR was studied.
Conclusion and Outlooks

It was observed that linked fatty chains prevent totally the cold crystallization of PI, regardless the number of fatty chains linked or the position (α and/or ω chain-end). Addition of free lipids allowed a partial recovery of the crystallinity as already described in the literature during the study of the cold crystallization of NR. The PIDiLip can thus be considered as a good model of the natural material. Similar results were obtained for IR models (quick cold crystallization when an admixture of methyl linoleate and stearic acid were added).

In the last chapter, coupling of protein and PI was investigated. Lipase B from *Candida antarctica* (CALB) was first selected to be coupled with PI because of its tolerance to organic solvent. As the native CALB does not present any free cysteine, the reduction of one or more disulfure bridges was investigated. TCEP lead to the partial reduction of the protein as characterized by SDS-PAGE analysis. The reduced protein was then tried to be coupled with an α-malemido-terminated PI (PIMal). A stable emulsion was obtained with the formation of particles of various sizes. Unfortunately, despite the use of various analysis technics, we did not succeed to have any direct proof that the coupling reaction occurred. Similar study was performed with *Bovine Serum Albumin* (BSA) which natively bears a free thiol. Again, it was not possible to be 100% sure that the coupling reaction occurred. As the PI/Protein coupling appeared to be difficult, the N-carboxyanhydride (NCA) ring opening polymerization (ROP) was investigated to afford a polypeptide block, which could mimic the behaviour of proteins. The coupling of PI maleimide and PPre furane remained unsuccessful. On the contrary, it was possible to initiate the ROP of NCA from a PI bearing an amino-alcohol function at the chain end (PIDiOH, PINOHOH). Diverse architectures of PI-Polypeptide di-block could thus be obtained, opening the route to the synthesis of a tri-block Polypeptide-PI-Lipid.

The principal outlook of this PhD work would be to demonstrate the feasibility of the PI-Protein coupling and to manage to characterize the amphiphilic properties of the hybrid polymer. Only few insights were developed here and a deeper investigation on this topic would be of high interest as PI-Protein hybrids have never been reported in the literature. Of course, applying the coupling method to the PIDiLip would allow to form the tri-block of Tanaka and would certainly lead to interesting self-assembly properties.
Another interesting axis to develop concerns the ROP initiated by PI. Indeed, here, we focused on the ROP of NCA to obtain a polypeptide block, but the chemistry developed using TU as catalyst is a chemistry that could be applied to other monomers like carbonate, lactones, lactide etc… It could be interesting to develop a panel of di-block or tri-block copolymers using the same ROP chemistry but varying the cycles to open.

Finally, the most promising outlook of this PhD work would be to cross-link the PI-Lipid hybrids and to study the influence of linked and unlinked fatty chains on the strain induced crystallization of the material to, potentially, confer new properties to any IR and a greater resistance.
MATERIAL AND METHODS

Material.

Natural rubbers (NRs) RRIM600 and PB 235 were kindly provided by UMR iATE/LBTNR/Katsetsart University in Thailand. Cis-1,4-polyisoprene (IR) (97% cis-1,4, \(M_n = 600 \text{ kg/mol, } D = 2.8\)) was purchased from Scientific Polymer Products, Inc. 3-Chloroperoxybenzoic acid (mCPBA) (70-75%, Acros), periodic acid (\(H_2IO_6\)) (≥ 99%, Aldrich), acetic acid (99%, Aldrich), potassium hydroxide (KOH) (85%, Aldrich), sodium borohydride (>96%, Aldrich), furfurylamine (>99%, Aldrich), dimethylaminopyridine (>98%, Aldrich), N,N’-Dicyclohexylcarbodiimidesodium (>98% Aldrich), triacetoxyborohydride (NaBH(OAc)\(_3\)) (97%, Aldrich), diethanolamine (DEA) (99%, Alfa Aesar), ammonium acetate (98%, Aldrich), Bis(trichloromethyl)carbonate (>99%, Aldrich), (ethanolamine (99%, Alfa Aesar), methylethanolamine (99%, Aldrich), stearic acid (SA) (95%, Aldrich), myristic acid (99%, Aldrich), lignoceric acid (> 99%, Aldrich), methyl linoleate (ML) (99%, Aldrich), linoleoyl chloride (>99%, Aldrich), 10-undecenoyl chloride (97%, Aldrich), palmitoyl chloride (98%, Aldrich), 3-aminopropyl-functionalized silica gel (~1 mmol/g NH\(_2\) loading, 40-63µm, Aldrich), oxalyl chloride (>99%, Aldrich), Lipase B from *Candida antarctica* (Chiral Vision), *Bovine serum albumin* (Fraction V, 99%, Aldrich) and 6-maleimidohexanoic acid (>90%, Aldrich) were used without further purification. Tetrahydrofuran (THF), dimethylformamide (DMF) and dichloromethane (DCM) were dried on alumina column. Triethylamine (TEA) and dimethylethanolamine (99%, Aldrich) were dried on KOH pellets and distilled prior to use. Methanol, toluene and diethyl ether (reagent grade, Aldrich) were used as received as well as Celite\(^\text{®}\) (R566, Aldrich).

NMR analysis

Liquid-state \(^1\)H NMR and \(^{13}\)C NMR, HSQC and HMBC spectra were recorded at 298 K on a Bruker Avance 400 spectrometer operating at 400 MHz and 100 MHz respectively in appropriate deuterated solvents.
**Conclusion and Outlooks**

**SEC analysis**

Most of the polymer molar masses were determined by size exclusion chromatography (SEC) using tetrahydrofuran as the eluent (THF with 250 ppm of Butylated hydroxytoluene as inhibitor, Aldrich) and trichlorobenzene as a flow marker. Measurements were performed on a Waters pump equipped with Waters RI detector and Wyatt Light Scattering detector. The separation is achieved on three Tosoh TSK gel columns (300 × 7.8 mm) G5000 HXL, G6000 HXL and a Multipore HXL with an exclusion limits from 500 to 40 000 000 g/mol, at a flow rate of 1 mL/min. The injected volume was 100µL. Columns’ temperature was 40 °C. $M_n$ and Đ values were calculated using $dn/dc(polyisoprene)=0.130$. Data were processed with Astra software from Wyatt.

For polypeptide homopolymers, polymer molar masses were determined using dimethylformamide (DMF + lithium bromide LiBr 1g/L) as the eluent. Measurements in DMF were performed on a PL GPC50 integrated system from Agilent equipped with RI and UV (260 nm) detectors and two KD-804 Shodex gel columns (300 x 8 mm) (exclusion limits from 4000 g/mol to 200 000 g/mol) at a flowrate of 0.8 mL/min. Columns temperature was held at 50°C. Polystyrene was used as the standard.

For PI-Polypeptide copolymers, polymer molar masses were determined using tetrahydrofuran (THF + Lithium(I) bis(trifluoromethanesulfonyl)imide (LiNTf$_2$) 10mM) as the eluent. Measurements were performed on an Ultimate 3000 system from Thermoscientific equipped with diode array detector DAD. The system also includes a multi-angle light scattering detector MALS and differential refractive index detector dRI from Wyatt technology. Polymers were separated one PSS SDV linear S column (300 x 8 mm) (exclusion limits from 100 g/mol to 150 000 g/mol) at a flowrate of 1 mL/min. Columns temperature was held at 36°C.

**DSC analysis**

Differential scanning calorimetry (DSC) measurements were performed using a DSC Q100 LN$_2$ or a DSC Q100 RSC apparatus from TA Instruments depending on the experiment. With DSC Q100 LN$_2$, the samples were first heated to 80°C during 20 minutes to suppress any traces of solvent then cooled to -100°C and heated back to 120°C at the rate of 10°C min$^{-1}$. Consecutive cooling and heating run were also performed at 10°C min$^{-1}$. The analyses were carried out in a helium atmosphere with aluminum pans. DSC Q100 RSC device was used for isothermal analysis.
Conclusion and Outlooks

The samples were heated at 80°C during 20 minutes prior to use to suppress any traces of solvent, then cooled to -25°C during predetermined time and then heated to 120°C at a heating rate of 10°C min⁻¹.

**MALDI-TOF analysis**
MALDI-TOF spectra were performed by the CESAMO (Bordeaux, France) on a Voyager mass spectrometer (Applied Biosystems). Spectra were recorded in the positive-ion mode using the reflectron and with accelerating voltage of 20kV. Samples were dissolved in DMF at 10 mg/mL. The matrix solution (2,5-Dihydroxybenzoic acid, DHB) was prepared by dissolving 10 mg in 1 mL of DMF. A MeOH solution of cationisation agent (NaI, 10 mg/mL) was also prepared. Solutions were combined in a 10:1:1 volume ratio of matrix to sample to cationizing agent.

**FTIR-ATR analysis**
Fourier Transformed Infra-Red-Attenuated Total Reflection (FTIR-ATR) spectra were recorded between 4000 and 400 cm⁻¹ on a Bruker VERTEX 70 instrument (4 cm⁻¹ resolution, 32 scans, DLaTGS MIR) equipped with a Pike GladiATR plate (diamond crystal) for attenuated total reflectance (ATR) at room temperature.

**SDS-PAGE analysis**
Protein samples were analyzed by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), using 4-20% precast mini gels (Mini-PROTEAN® TGX™ Gels, BIO-RAD). One volume of protein sample was mixed with one volume of sample buffer (65.8 mM Tris-HCl, pH 6.8, 2.1% SDS, 26.3% (w/v) glycerol, 0.01% bromophenol blue) containing 2-mercaptoethanol (1.36 M). For non-reducing conditions, 2-mercaptoethanol was not added into the sample loading buffer. Samples were then denatured for 3 min at 95°C, and loaded onto the gel. Electrophoresis was performed in TGS buffer (25 mM Tris pH 8.3, 192 mM glycine, 0.1% SDS) at constant amperage (25 mA/gel). Gels were stained for 30 min. with Coomassie colloidal blue (InstantBlue, Expeideon), and destained with water baths. To estimate the size of the protein of interest, a protein ladder was run simultaneously in every gel (Precision Plus Protein™ Unstained Standards, BIO-RAD).
Annexes
Annexe 1: DSC thermogram of PIDiC$_{11:1}$

Annexe 2: DSC thermogram of PIDiC$_{14:0}$

Annexe 3: DSC thermogram of PIDiC$_{16:0}$
Annexe 4: DSC thermogram of PIDiC$_{19:0}$

Annexe 5: DSC thermogram of PIDiC$_{18:2}$

Annexe 6: DSC thermogram of PIDiC$_{24:8}$
Annexe 7: DSC thermogram of PIDiC\textsubscript{14:0} 5 kg/mol

Annexe 8: DSC thermogram of PIDiC\textsubscript{16:0} 5 kg/mol

Annexe 9: DSC thermogram of PIDiC\textsubscript{24:0} 5 kg/mol
Fonctionnalisation de Polyisoprène : Vers un modèle du caoutchouc naturel

Ce travail de thèse porte, de manière globale, sur une meilleure compréhension du caoutchouc naturel (NR). En effet, bien que ce matériau soit fortement utilisé dans l’industrie et ce depuis des dizaines d’années, plusieurs de ses propriétés restent à ce jour mal comprises. Antérieurement à nos travaux, il a été fait un lien entre la biosynthèse du polymère et ces propriétés et il a été proposé que le caoutchouc naturel était constitué d’une chaîne polyisoprène (PI) 100% 1,4-cis de forte masse molaire (> 500 000 g/mol) fonctionnalisée en α et ω par une protéine et un motif phospholipidique respectivement. Ces bouts de chaîne seraient capables de s’auto-assembler pour créer un réseau physique qui confère au NR ses propriétés si intéressantes. L’objet de cette thèse a donc été de synthétiser un copolymère tri-bloc Protéine/PI/Lipides afin de confirmer cette hypothèse en produisant en laboratoire un homologue de NR. Pour ce faire, un PI hétéro-téléchélique cétone/aldéhyde a été obtenu par dégradation chimique de NR. Cette méthode a permis d’obtenir un PI 100 % 1,4-cis possédant deux fonctions chimiques différentes en bout de chaîne permettant ainsi le greffage sélectif d’une protéine où d’un motif lipidique. Ces deux couplages ont ensuite été étudiés séparément (PI/Protéine puis PI/Lipides) révélant des propriétés intéressantes dans le cas du copolymère di-bloc PI/Lipide. Le couplage PI/Protéine s’est avéré plus compliqué et seul des copolymères di-blocs PI/Polypeptide ont pu être obtenus avec certitude, en utilisant des synthons PI comme macro-amorceurs. Une voie de synthèse a également été dégagée pour un tri-block Polypeptide/PI/Lipide présentant une structure très proche du modèle de Tanaka.

Mots clés: Caoutchouc naturel ; Polyisoprène ; Fonctionnalisation ; Modèle de Tanaka

Functionalization of Polyisoprene: Toward the mimic of Natural Rubber

This PhD work focuses on a better comprehension of natural rubber (NR). Indeed, despite the fact that this material has been used for a long time in industry, some properties remain unclear. Previous works of Tanaka allowed to make a link between the biosynthesis of the material and its properties. It was thus suggested that NR was composed of a high molar mass chain of polyisoprene (PI, > 500 000 g/mol) functionalized at the α and ω chain-end by a protein and a phospholipidic moiety respectively. These chain-ends would be able to self-assemble into a pseudo-physical network which would explain some of the superior properties of NR. The goal of this PhD work is to synthesize a Protein/PI/Lipid tri-block copolymer in order to check this hypothesis and to synthesize hybrid material close to NR. First, a 1,4-cis hetero-téléchelic (ketone/aldéhyde) PI of 10 000 g/mol was obtained by chemical degradation of NR, yielding a polymeric chain bearing two different functions at the chain-ends, allowing to perform a selective functionalization with both a lipidic moiety and a protein. Both di-block copolymers (PI/Lipid and PI/Protein) were synthesized and studied separately. The PI/Lipid di-block copolymer revealed interesting properties. The synthesis of PI/Protein di-block copolymer revealed more difficult and only PI/Polypeptide di-block copolymer could have been obtained. To this end, PI macro-initiator allowed the Ring-Opening Polymerization od N-carboxyanhydride. Finally, a chemical pathway was established, allowing to synthesize a Polypeptide/PI/Lipid tri-block close to Tanaka’s model.

Key words: Natural rubber ; Polyisoprene ; Functionalization ; Tanaka’s model