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Geoffrey Hibert

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THÈSE PRÉSENTÉE
POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE DES SCIENCES CHIMIQUES
SPÉCIALITÉ: POLYMÈRES

Par **Geoffrey HIBERT**

**GLYCOLIPIDS: FROM SYNTHESIS AND SELF-ASSEMBLY
STUDIES TO THE DESIGN OF ORIGINAL BIOBASED
POLYMERS**

**Glycolipides : de la synthèse et de l'étude de l'auto-assemblage à la conception de
polymères bio-sourcés originaux**

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Soutenue le 28/11/2016

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"Les folies sont les seules choses que l'on ne regrettera jamais."

Oscar Wilde

"La vie est une fête."

KBB

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Résumé

Au vu de l'augmentation de la population mondiale, associée au développement économique des pays émergents, il est observé une demande toujours plus grandissante des ressources fossiles pour la production d'énergie et de tous les dérivés chimiques et matériaux couramment utilisés dans notre vie quotidienne. En raison de l'appauvrissement des ressources fossiles facilement exploitables,¹ les gaz et pétrole de schiste font l'objet depuis quelques années d'un intérêt croissant des grands groupes industriels. Néanmoins, l'exploitation de ces gaz de schiste est l'objet de nombreuses controverses car elle est considérée très polluante, entraînant la contamination des eaux et des sols ainsi que l'émission de polluants dans l'air (dioxyde de carbone, oxydes nitreux et sulfure d'hydrogène).² De plus, les problèmes environnementaux liés au réchauffement climatique provoqué par l'émission des gaz à effet de serre sont de plus en plus prégnants. Ainsi, la recherche académique et industrielle a le devoir de se tourner aujourd'hui vers des solutions plus durables et plus respectueuses de l'Homme et de l'environnement.

La biomasse apparaît être une alternative viable pour remplacer les ressources fossiles. En effet, elle constitue une source de carbone ayant un temps de régénération court. Ce dernier est de l'ordre de la dizaine d'années contrairement à celui des ressources fossiles qui se comptent en millions d'années pour se régénérer.³ Par conséquent, le concept de bioraffinerie a été développé pour produire des carburants, de l'énergie et des produits chimiques nécessaires à notre vie moderne.⁴⁻⁶ Une bioraffinerie est un complexe qui combine des équipements et des procédés de conversion de la biomasse en biocarburants, en énergie principalement sous forme de chaleur et en biomolécules chimiques (Figure 1). En sélectionnant la bio-ressource et la transformation appropriée (gazéification, extraction, pyrolyse ou fermentation) une importante variété de molécules peut être obtenue. Cependant, il est important de combiner ce concept de bioraffinerie avec les principes de la chimie verte développés durant les 25 dernières années. Les 12 principes de la chimie verte visent à développer des procédés pour synthétiser des produits chimiques renouvelables et durables en réduisant la production de déchets et l'utilisation de substances dangereuses et en limitant la consommation énergétique afin de produire de manière plus durable et plus respectueuse de l'environnement.^{7,8}

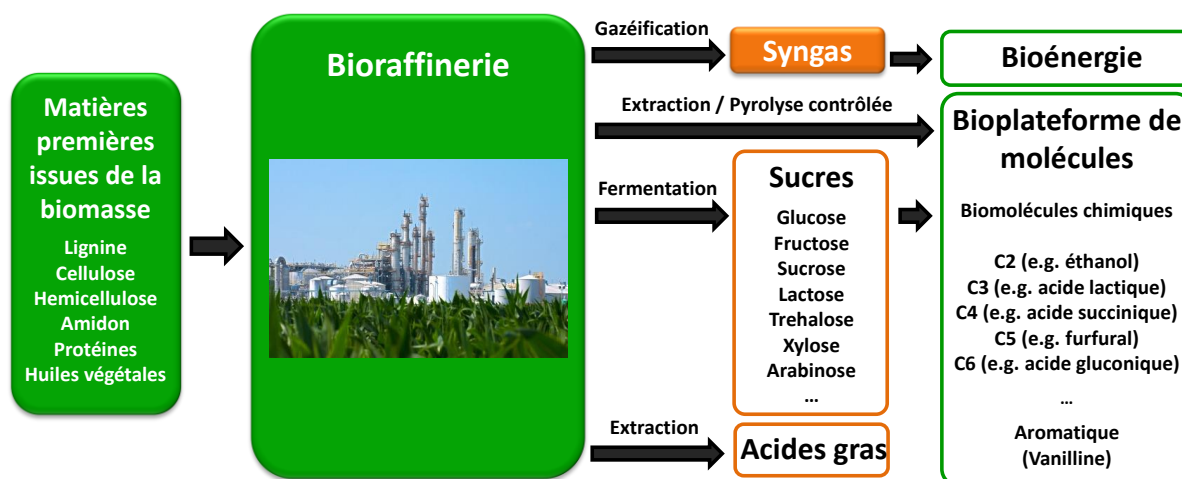


Figure 1: Schéma du concept de bioraffinerie

Parmi toutes les ressources renouvelables disponibles, l'amidon, la lignocellulose⁹ et les huiles végétales¹⁰ sont étudiés intensivement pour la production de produits chimiques. En effet, ces matières premières permettent l'accès à une large plateforme de produits chimiques. L'amidon est un polymère naturel qui sert de composé de réserve d'énergie pour les plantes. On le retrouve notamment dans les fruits, les graines et les racines des plantes. La lignocellulose est composée de trois principaux polymères naturels que sont la cellulose, les hémicelluloses et la lignine qui constituent la paroi des cellules des végétaux. Ainsi la dépolymérisation de l'amidon, de la cellulose et l'hémicellulose permettent de produire des glucides qui donnent l'accès à une large gamme de molécules hydrophiles et biocompatibles pouvant être utilisées pour la synthèse de produits chimiques et par conséquent de polymères bio-sourcés.¹¹

Les huiles végétales sont constituées de triglycérides qui sont composés de glycérol et d'acide gras saturés et insaturés possédant des chaînes alkyle contenant de 8 à 22 carbones. Les acides gras sont principalement employés de nos jours pour la production de biodiesel par réaction avec le méthanol. De plus, les huiles végétales sont également employées dans de nombreuses applications telles que les lubrifiants, les cosmétiques, les peintures et les revêtements grâce à leur large disponibilité et faible coût de production. En outre, elles peuvent être le substrat de nombreuses réactions chimiques sur les deux sites réactifs qu'elles contiennent: d'une part la fonction acide carboxylique des acides gras et, d'autre part, la double liaison présente sur les chaînes insaturées. Ainsi, elles permettent l'accès à une large gamme de molécules aliphatiques étant déjà employées pour la synthèse de polymères bio-sourcés.¹²

La combinaison des sucres et des acides gras permet l'obtention de glycolipides: des molécules amphiphiles avec des propriétés intéressantes.¹³ Les glycolipides sont des

molécules bio-sourcées qui peuvent être naturelles et biosynthétisées par des micro-organismes ou bien synthétiques et produites chimiquement. En plus d'être bio-sourcées, ces molécules sont biodégradables, non-toxiques et elles possèdent des propriétés tensio-actives. Ainsi grâce à tous ces avantages, ils sont déjà utilisés comme tensioactifs dans de nombreuses applications telles que l'agro-alimentaire, les cosmétiques, les lessives et les produits pharmaceutiques.^{14,15} De plus certains d'entre eux ont démontré une capacité intéressante de gélification de l'eau et de solvants organiques^{16,17} qui permet d'élargir encore les domaines d'applications de ces molécules renouvelables. Ainsi **ces travaux de thèse visent à synthétiser des glycolipides, à étudier leurs propriétés tensioactives et gélifiantes et à concevoir des polymères originaux** à partir de ces molécules amphiphiles.

Ainsi, comme il a été évoqué précédemment, les glycolipides sont constitués d'une part de sucres qui sont hydrophiles et, d'autre part, de dérivés d'acides gras qui sont hydrophobes. Or il existe une grande diversité de sucres (monosaccharides, disaccharides et polysaccharides) et une grande diversité de dérivés d'acide gras. Ainsi un état de l'art a été réalisé sur une famille restreinte de glycolipides: les esters de sucres.

Dans la littérature, trois principales voies de synthèse sont rapportées pour l'élaboration d'esters de sucres. La première est une voie biosynthétique où les esters de sucres sont produits naturellement par des bactéries, des levures ou des moisissures. Il existe trois principaux types d'esters de sucres naturels: les lipides de mannosylerythritol, les sophorolipides et les lipides de trehalose.¹⁸ Ils sont produits par une succession de réactions enzymatiques opérées au niveau des membranes cellulaires des micro-organismes. L'avantage de cette production est qu'elle s'effectue avec de bons rendements et que la chimie utilisée est respectueuse de l'environnement. Cependant il est impossible de faire varier les glucides ou les chaînes lipidiques car les microorganismes ne produisent qu'un seul type d'esters de sucre. Ainsi des voies chimiques ont été développées afin de synthétiser une plus grande variété d'esters de sucre. La principale voie de synthèse est une estérification en milieu basique des sucres avec des acides gras ou des esters gras.¹⁹ Cependant cette voie n'est pas sélective et tous les groupements hydroxyle des sucres peuvent être estérifiés conduisant à un mélange de polyesters de sucres avec différents degrés de substitution. Il est, de plus, difficile d'isoler les polyesters ayant des degrés de substitution différents. Enfin, des températures élevées ainsi que des solvants toxiques (DMF, pyridine, etc) sont nécessaires pour effectuer ces estérifications. Des techniques de synthèse ont donc été développées afin d'estérifier plus sélectivement les hydroxyles primaires des sucres et ainsi mimer les esters de sucres naturels. Des estérifications *via* des étapes de protection / deprotection des alcools secondaires sont

décrites dans la littérature.²⁰ Elles permettent une bonne sélectivité mais augmente le nombre de réactions. Des estérifications sans groupes protecteurs des alcools secondaires ont donc été développées afin de diminuer le nombre d'étapes. L'estérification de Mitsunobu²¹ permet l'estérification sélective des alcools primaires des sucres en utilisant la triphenyl phosphine et le diisopropyl azodicarboxylate. Une autre voie décrite dans la littérature utilise des agents de couplage peptidiques pour l'estérification sélective des alcools primaires.²² Ces estérifications étant sélectives, elles produisent cependant des sous-produits réactionnels et des solvants toxiques sont employés pour solubiliser les sucres et les acides gras. La dernière voie grandement décrite dans la littérature est l'estérification catalysée par des enzymes et principalement par des lipases.^{19,23} De nombreuses conditions ont été testées afin d'améliorer le rendement (enzyme, dérivé d'acide gras, température, solvant) mais généralement des esters gras vinyliques sont utilisés dans un solvant organique anhydre catalysé par la lipase B de la *Candida antarctica* immobilisée sur résine acrylique. Cette estérification est très prometteuse avec sa grande sélectivité, elle est de plus "verte" car des solvants peu toxiques sont utilisés et les enzymes peuvent être réutilisées après réaction. Cependant le rendement reste pour le moment faible.

Au vu des nombreux avantages de ces molécules amphiphiles, les esters de sucres apparaissent être de bons candidats pour la synthèse de polymères. Cependant très peu d'étude sont rapportées dans la littérature. Des polymères réticulés ont été élaborés à partir de polyesters de saccharose.²⁴ Les insaturations présentes sur les esters de saccharose ont été époxydées puis des réseaux époxyde ont été synthétisés par réticulation des groupements époxyde avec des anhydrides. Des polymères tridimensionnels ont également été obtenus par réactions entre des esters de methylglucoside ou de saccharose avec des diisocyanates.²⁵ Deux types de polymères linéaires sont également rapportés dans la littérature: les polymères possédant les sucres comme groupe pendent et ceux contenant le motif sucre à l'intérieur des chaînes polymères. Les polymères possédant les sucres comme motif pendent ont été synthétisés soit par polymérisation radicalaire²⁶ soit par polycondensation de groupements hydroxyle présents sur la chaîne grasse avec des diisocyanates.²⁵ Les polymères avec le motif sucre intracaténaire ont été synthétisés par polycondensation,²⁷ polymérisation par ouverture de cycle²⁸ (esters de sucre sous forme de lactone) ou par polymérisation par métathèse par ouverture de cycle.²⁹ Ces derniers sont des polymères bio-sourcés amphiphiles et biodégradables, grâce aux zones clivables présentes au sein des chaînes polymères (sucres et fonctions esters). Ce sont donc des candidats intéressants pour des applications biomédicales et pharmaceutiques. La faible quantité d'études rapportées sur le sujet, laisse de nombreuses possibilités à explorer pour la synthèse de nouveaux polymères à partir d'esters de sucres.

Dans le cadre de cette thèse, la stratégie développée a consisté à concevoir de nouveaux polymères linéaires contenant le motif sucre intracaténaire à partir d'esters de trehalose.

Le tréhalose a été sélectionné comme sucre pour la conception de glycolipides. En effet, ce disaccharide non réducteur contenant deux alcools primaires possède de nombreux avantages. Il est très résistant au pH et à la température. De plus, il possède des propriétés de cryo-protection des protéines et des cellules.³⁰ En outre, il peut être clivé en deux molécules de glucose par une enzyme présente dans l'intestin: la trehalase. Toutes ces propriétés font de lui un sucre très attractif pour la synthèse d'esters de sucres.

Ainsi, des esters de tréhalose ont été synthétisés par estérification des alcools primaires du tréhalose avec des acides gras de différentes natures (Figure 2). Deux voies de synthèse ont été retenues, une estérification sans groupement protecteur et une estérification enzymatique. L'estérification sans groupement protecteur a été développée par le groupe de Grindley.²² C'est une synthèse monotope en deux étapes, menée à température ambiante dans la pyridine en présence d'une base, la DIPEA, et utilisant un agent de couplage peptidique le TBTU. La première étape consiste en la formation d'un ester activé par réaction de l'acide gras avec le TBTU, la deuxième étape est une transesterification entre l'ester activé et les alcools primaires du tréhalose. Cette estérification permet la production d'un mélange de diester et de monoester, l'un ou l'autre pouvant être obtenu majoritairement en ajustant la stœchiométrie en acide gras. Ainsi, le nombre d'équivalence en acide gras a été ajusté pour favoriser la production en diesters mais des monoesters sont toujours formés. Contrairement aux résultats rapportés par le groupe de Grindley, le rendement en diesters est faible (15%) dû à la formation de triesters and polyesters de tréhalose, ceci pouvant être expliqué par la faible solubilité du trehalose dans le milieu réactionnel. Ainsi des tentatives d'optimisation ont été testées en changeant les conditions réactionnelles pour éviter la formation de ces polyesters de tréhalose et ainsi augmenter le rendement en diesters. La formation de polyesters de tréhalose n'a pu être évitée en dépit des différentes conditions testées. Des travaux sont donc encore nécessaires pour améliorer le rendement de synthèse des diesters de tréhalose. Malgré cela, des monoesters et diesters de tréhalose ont pu être isolés par chromatographie flash sur colonne de silice.

Dans un second temps, l'estérification enzymatique du tréhalose a également été réalisée par réaction avec des esters vinyliques gras catalysée par la lipase B de *Candida antarctica* immobilisée sur résine acrylique. Le choix des esters vinyliques permettent de tirer l'équilibre de la réaction vers la formation d'esters de tréhalose. En effet, lors de la transesterification, du

formaldéhyde est rejeté et facilement retiré du milieu réactionnel car cil est sous forme gazeuse à température ambiante. Les esters vinyliques gras ont été synthétisés par transvinylation des acides gras avec l'acétate de vinyle. Deux catalyseurs de transvinylation ont été testés, un catalyseur à l'iridium et un catalyseur au palladium. Le catalyseur au palladium a été sélectionné car il a permis une transvinylation de l'acide undécénoïque sans isomérisation de sa double liaison terminale contrairement au catalyseur à l'iridium. Ensuite l'estérification enzymatique dans le 2-méthyltétrahydrofurane a permis la synthèse de diesters sans formation de monoesters ou d'autres polyesters de tréhalose. Ainsi cette estérification est plus sélective; elle est effectuée dans un solvant moins toxique que la pyridine mais une étape de transvinylation est cependant nécessaire.

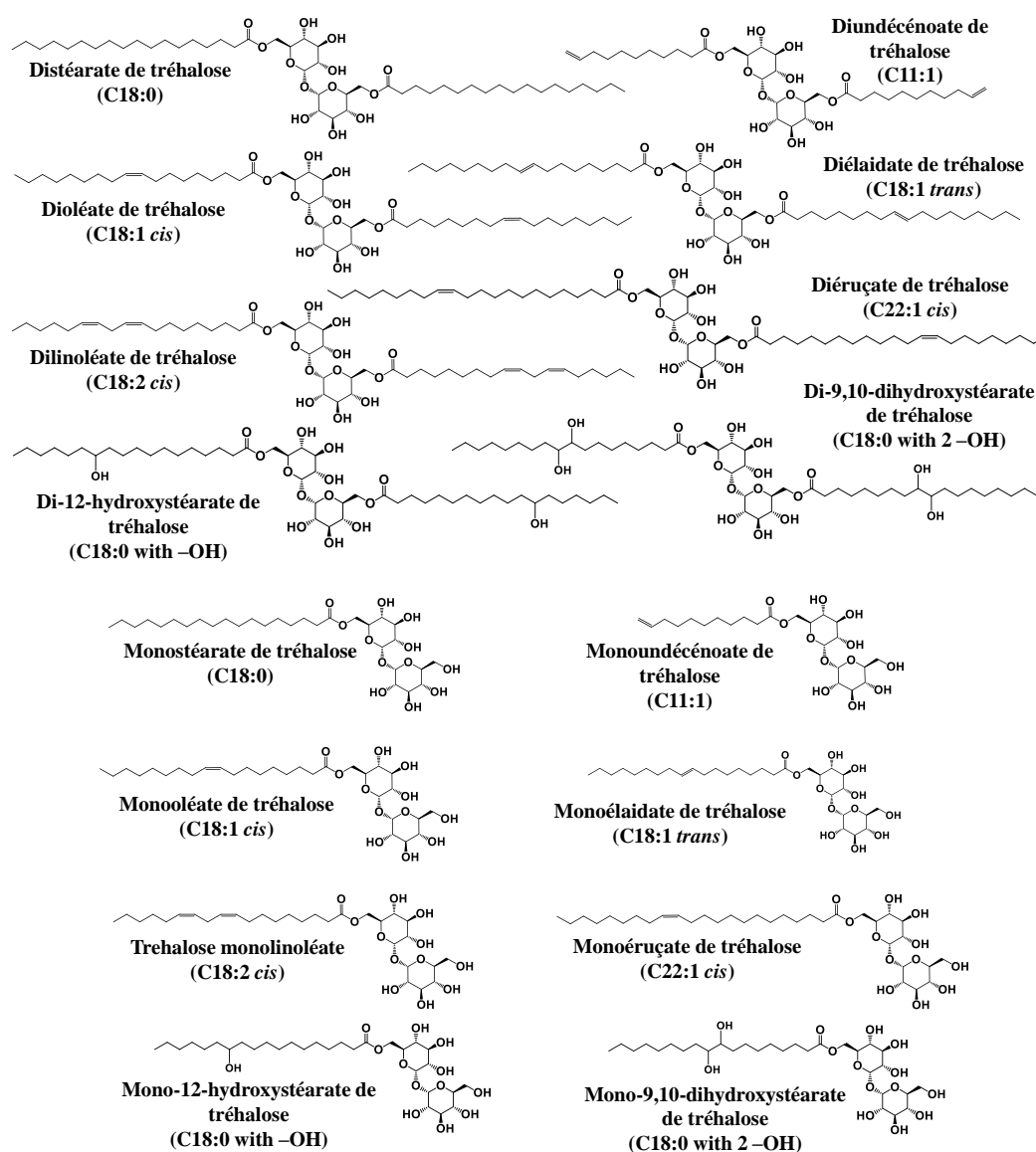


Figure 2: Esters de tréhalose synthétisés au cours de la thèse

Les propriétés d'auto-assemblage de ces esters de tréhalose ont ensuite été examinées (Figure 3). Les propriétés tensio-actives des monoesters de tréhalose ont été étudiées dans l'eau. En

effet, ces molécules sont constituées comme tous les tensioactifs d'une "tête" polaire et hydrophile et d'une "queue" apolaire et hydrophobe. La concentration micellaire critique (CMC) de ces composés a été déterminée en mesurant la tension de surface de solutions de monoesters de tréhalose dans l'eau par la méthode de la goutte pendante. Des valeurs de CMC comprises entre 0,16 g/L et 1,0 g/L ont ainsi été déterminées avec une tension de surface après CMC proche de 42 mN/m ce qui est cohérent avec les valeurs décrites dans la littérature. Ainsi, la nature de la chaîne alkyle n'a pas de grande influence sur la tension de surface mais a un impact sur les valeurs de CMC. Les monoesters de tréhalose s'auto-assemblent dans l'eau sous forme de micelles sphériques (10 - 20 nm) observables en microscopie électronique à transmission (MET). En augmentant la concentration au-delà de 30 g/L, le diester de tréhalose a la capacité de former des hydrogels. De par l'absence de cristallinité, il a été déduit que le réseau tridimensionnel de ces hydrogels est dû à l'enchevêtrement de micelles. Les propriétés rhéologiques de ces gels ont été étudiées montrant un comportement thixotrope. Contrairement aux monoesters, les diesters de tréhalose ne sont pas solubles et ne s'auto-assemblent pas dans l'eau. Cependant il a été montré dans la littérature qu'ils ont la capacité de gélifier des solvants organiques. D'autres diesters de sucres sont décrits comme étant de bons gélifiants d'huiles végétales. Ainsi la gélification des diesters de tréhalose dans trois huiles végétales (huile d'olive, huile de lin, huile de ricin) a été étudiée. Les différents diesters ont été capables de gélifier les trois huiles testées, à l'exception de ceux comportant des groupements hydroxyle sur leurs chaînes grasses. Ceux-ci sont seulement solubles même en augmentant la concentration jusqu'à 20 % massique. Des xérogels ont été réalisés à partir de gels dans l'acétate d'éthyle. Leur observation en microscopie optique et électronique à balayage (MEB) a montré que les diesters forment un réseau tridimensionnel composé de fibres cristallines (SAFIN). Ceci a pu être confirmé par analyse enthalpique différentielle (AED) et par diffraction/diffusion des rayons X. Ainsi, dans les huiles végétales ou les solvants organiques, les diesters de tréhalose s'empilent en couche formant des fibres qui, par enchevêtrement, forment le réseau tridimensionnel constituant le gel. Pour finir, les propriétés rhéologiques des gels ont été étudiées montrant une différence de propriétés suivant la concentration en gélifiant et la nature des chaînes lipidiques. En effet, l'augmentation de la concentration augmente le nombre de fibres et donc le nombre de points d'enchevêtrement, ce qui a pour effet de rendre le gel plus ferme mais plus fragile (hautes valeurs des modules G' et G'' et faible valeur de la déformation seuil σ_y). Les chaînes courtes permettent également d'obtenir des gels plus fermes mais donc plus fragiles et plus cassants. Une chaîne lipidique saturée semble aussi rendre les gels plus fermes en comparaison aux chaînes insaturées. Cependant, les valeurs des modules

G' et G'' ainsi que celles de σ_y sont très dépendantes de l'huile utilisée ; ainsi il a été difficile de dégager une réelle tendance entre la structure des gélifiants et les propriétés rhéologiques. Cependant, la large gamme de diesters de tréhalose permet de varier les propriétés rhéologiques et ainsi d'adapter les structures en fonction des applications visées.

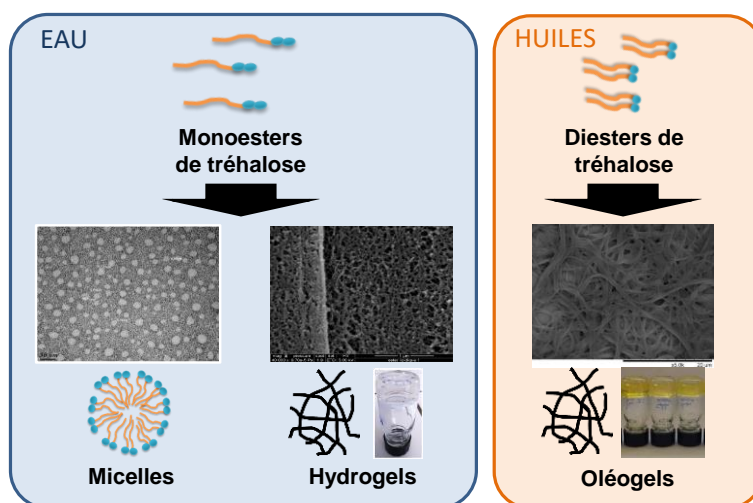


Figure 3: Auto-assemblage des monoesters et diesters de tréhalose

Finalement, de nouveaux polymères ont été synthétisés à partir de diesters de tréhalose (Figure 4). Ainsi des polyuréthanes ont été préparés à partir de trois diesters contenant des groupements hydroxyle sur leur chaîne lipidique et de deux diisocyanates. La polymérisation a été effectuée à 60°C en présence de dilaurate de dibutylétain comme catalyseur. Selon leur structure, les polyuréthanes synthétisés possèdent des masses molaires assez faibles comprises entre 4000 et 8000 g/mol. Des polyuréthanes (PUs) de structures plutôt ramifiées ont été obtenus, résultant de la réactivité des hydroxyles primaires et secondaires du tréhalose avec les diisocyanate.

Les isocyanates étant des composés toxiques issus du phosgène lui-même mortel, la recherche se tourne de plus en plus vers l'élaboration de polyuréthanes sans isocyanates. Ainsi des poly(hydroxyuréthane)s peuvent être obtenus par réaction entre des diamines et des carbonates cycliques. Dans ce but, deux diesters de tréhalose contenant des carbonates cycliques à 5 chaînons sur leur chaîne lipidique ont été synthétisés. Des poly(hydroxyuréthane)s (PHUs) amorphes ont pu ensuite être préparés par réaction entre des diamines et les carbonates cycliques présents sur les diesters de tréhalose. Ces polymères possèdent des masses molaires comprises entre 3000 et 10000 g/mol qui sont donc proches de celles obtenues avec les PUs et des températures de transition vitreuse comprises entre 10 et 50 °C selon leur structure.

Le diundécénoate de tréhalose a été (co)polymérisé par métathèse selon le procédé ADMET (Acyclic Diene METathesis) avec l'undécénoate d'undécényle (également bio-sourcé). Contrairement aux polyuréthanes et poly(hydroxyuréthane)s synthétisés précédemment la copolymérisation statistique des deux monomères a permis d'obtenir des polymères semi-cristallins. La fonction uréthane doit donc empêcher la cristallisation des PUs et PHUs. Il a été observé deux types de cristallisation dans ces copolymères, synthétisés par métathèse, pouvant être expliqué par la présence de partie hydrophile et lipophile au sein de mêmes chaînes. L'empilement des motifs entre les chaînes crée une organisation lamellaire à longue distance (2,56 nm) entre les motifs sucres alors que les chaînes lipidiques s'organisent à courte distance (0,40 nm) créant ainsi l'apparition de deux cristallisations distinctes. Des masses molaires plus élevées ont pu être obtenues pour ces polymères, jusqu'à 13000 g/mol.

Enfin le diundécénoate de tréhalose a été polymérisé par réaction thiol-ène avec des dithiols de différentes longueurs et natures. Ainsi des polymères semi-cristallins ont également été obtenus avec des masses molaires plus élevées comprises entre 8000 et 16000 g/mol, des températures de transition vitreuse autour de 70°C et des températures de fusion autour de 100°C. Les atomes de soufre présents sur les chaînes grasses ont ensuite été oxydés pour obtenir soit des groupements sulfoxyde par réaction avec du peroxyde d'hydrogène soit des sulfones par réaction avec l'acide métachloroperbenzoïque. Les polymères ainsi obtenus après oxydation sont devenus amorphes. Les fonctions sulfone et sulfoxyde empêchent donc la cristallisation de ces polymères.

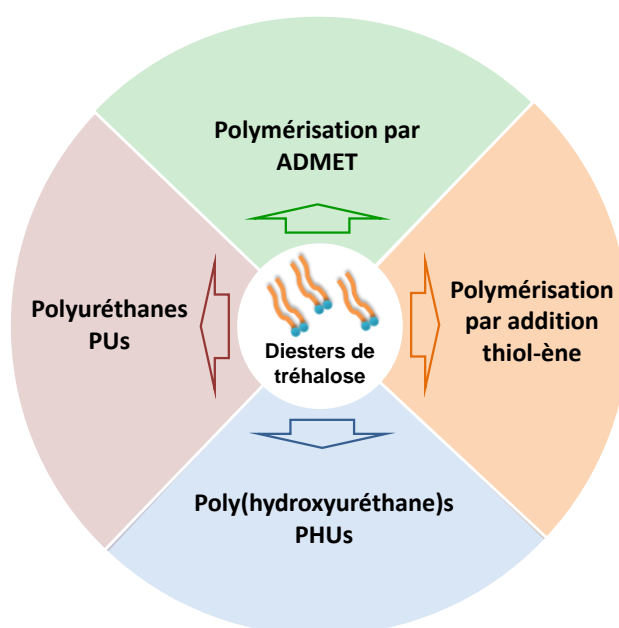


Figure 4: Polymères synthétisés à partir des diesters de tréhalose

Finalelement des tests préliminaires d'auto-assemblage des polymères synthétisés par ADMET et thiol-ène ont été faits. La technique de déplacement de solvant a été utilisée consistant à remplacer un solvant par un autre par dialyse. Des solutions de polymère dans le diméthylformamide (DMF) ont été dialysées dans de l'eau et des latex ont ainsi été obtenus. Ces latex ont été analysés par diffusion dynamique de la lumière (DLS) et par microscopie électronique à balayage (MET). Il a été observé que les polymères s'auto-assemblent en particules sphériques de tailles comprises entre 160 et 200 nm. Ces résultats préliminaires sont très prometteurs et ouvre la porte à de nombreuses applications.

L'objectif de cette thèse portait sur la conception de nouveaux polymères bio-sourcés à partir de glycolipides. La première partie de ce travail, traitant de la synthèse d'esters de tréhalose a permis l'obtention d'une plateforme de monoesters et de diesters de tréhalose. Les deux voies de synthèse étudiées sont prometteuses mais méritent encore des études plus avancées pour améliorer le rendement et limiter la formation de produits secondaires. Dans un second temps, les propriétés tensio-actives des monoesters et les propriétés gélifiantes des diesters ont été étudiées. Finalelement, les diesters de tréhalose ont permis la synthèse de polymères possédant diverses propriétés thermomécaniques et pouvant s'auto-assembler dans l'eau. Ces polymères semblent intéressants pour des applications biomédicales, dans l'encapsulation de principes actifs par exemple.

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List of abbreviations

Techniques:

DLS: dynamic light scattering
DMA: dynamic mechanical analysis
DSC: differential scanning calorimetry
FTIR: Fourier transform infrared
HPLC: high performance liquid chromatography
NMR: nuclear magnetic resonance
MALDI-TOF MS: matrix-assisted laser desorption ionization-time of flight mass spectrometry
NMR: nuclear magnetic resonance spectroscopy
SEC: size exclusion chromatography
SEM: scanning electron microscopy
TEM: transmission electron microscopy
TGA: thermogravimetric analysis chromatography

Chemicals:

CaH₂: calcium hydride
CALB: Candida Antarctica Lipase B
CDCl₃: deuterated chloroform
ChCl: choline chloride
DBTDL: dibutyltin dilaurate
DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene
DCM: dichloromethane
DIC12: 1,12-diisocyanatododecane
DIPEA: N,N-Diisopropylethylamine
DMC: dimethyl carbonate
DMPA: 2,2-dimethoxy-2-phenylacetophenone
DMF: dimethylformamide
DMSO: dimethylsulfoxide
EtOAc: ethyl acetate
EtOH: ethanol
G1: Grubbs 1st generation catalyst
G2: Grubbs 2nd generation catalyst
HG1: Hoveyda-Grubbs 1st generation catalyst

HG2: Hoveyda-Grubbs 2nd generation catalyst
HCl: hydrochloric acid
IPDA: isophorone diamine
IPDI: isophorone diisocyanate
K₂CO₃: potassium carbonate
KOH: potassium hydroxide
mCPBA: meta-chloroperbenzoic acid
mTHF: 2-methyltetrahydrofuran
MeOH: methanol
NaOAc: sodium acetate
NaOH: sodium hydroxide
TBD: 1,5,7-triazabicyclododecene
TBTU:
THF: tetrahydrofuran
U: urea
V70: 2,2'-Azobis(4-methoxy-2,4-dimethylvaleronitrile)

Characteristic parameters:

CMC: critical micelle concentration
D: dispersity
G': storage modulus
G'': loss modulus
HLB: hydrophilic lipophilic balance
MGC: minimal gelation concentration
 \bar{M}_n : number average molar mass
 \bar{M}_w : mass average molar mass
T_{d5%}: temperature corresponding to 5 wt% loss
T_{gel-to-sol}: gel to sol transition temperature
T_g: glass transition temperature
T_m: melting temperature

Polymerization techniques

ADMET: acyclic diene metathesis
ROMP: ring opening metathesis polymerization
ROP: ring opening polymerization

General introduction

General introduction

Due to the increase of the world population and to an economical growth, the need of oil for energy production and for all the chemical materials widely used in our daily life is drastically increased. Due to the oil price fluctuation with its history of booms and busts and in the meantime, the dramatical decrease of readily available fossil resource¹, shale gas has been subjected to an important concern from major industrial groups in the last several years. Nevertheless, the shale gas extraction is considered very polluting, leading to water and soil contamination.² In addition, a particular attention is drawn on environmental issues such as the global warming due to greenhouse gas emission. Thus, numerous researches are leading today on the development of sustainable solutions.

Biomass offers a viable alternative to fossil resources. Indeed, biomass constitutes a carbon source with a short regeneration time that can be measured in decades contrary to the regeneration time of fossil resources which require several million years.³ Therefore, to replace fossil resources by biomass as raw material for the production of fuels and chemicals, the concept of biorefinery has been developed.⁴⁻⁶ A biorefinery is a facility which combines biomass conversion processes and equipment to produce bio-fuels, energy and bio-chemical products. However, this concept has to be associated with the green chemistry principles largely developed in the last 25 years. The 12 principles of green chemistry aims at the development of renewable chemical products and sustainable processes in order to reduce wastes, to conserve energy and to limit the use of hazardous substances.^{7,8}

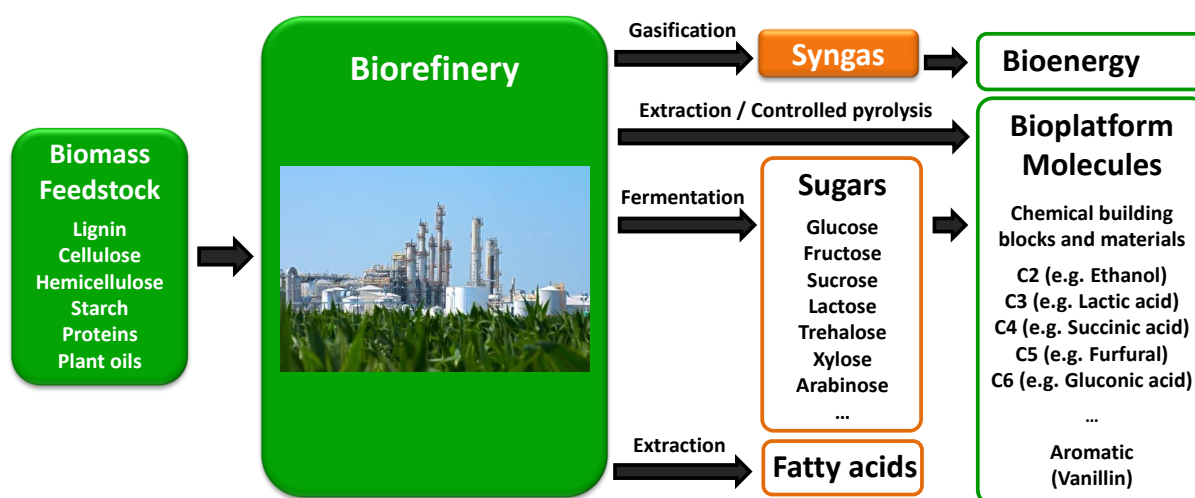


Figure 5: Schematic concept of an integrated biorefinery

Among the available biomass feedstocks, starch, lignocellulose⁹ and vegetable oils¹⁰ are intensively studied for the production of energy and chemicals.

Carbohydrates can be produced by the depolymerization of starch or lignocellulosic biomass. Starch is a natural polysaccharide constituted of glucose unit. Lignocellulosic biomass is constituted of three biopolymers: two polysaccharides, cellulose, hemicellulose and an aromatic polymer lignin. Glucose, a six-carbon sugar, can be produced by the depolymerization of starch and cellulose. This depolymerization is generally performed in concentrated acidic conditions. The depolymerization of the hemicelluloses allows the release of glucose and other six-membered sugars (mannose, galactose and rhamnose) but also five-membered sugars (xylose, arabinose).¹¹

Vegetable oils are constituted of triglycerides composed of glycerol and saturated or unsaturated fatty acids with alkyl chain containing from C8 to C22. The latter can be reacted with methanol to produce fatty acid methyl esters (FAME). Vegetable oils are also employed in many applications such as cosmetics, lubricants, paints and coatings thanks to their competitive cost and good availability. Indeed, the annual worldwide production of the main vegetable oils reached 177 million tons in 2015.¹² Moreover, these substrates can be subjected to chemical reactions through their reactive functions, the carboxylic acid group and the double bonds¹³ giving access to a large palette of original aliphatic molecules.

The combination of carbohydrates and fatty acids leads to new amphiphilic molecules: the glycolipids.¹⁴ These bio-sourced molecules can be natural and so biosynthesized by microorganisms¹⁵ but also synthetic and thus chemically produced.^{16,17} Therefore, these molecules are bio-based but they are also non-toxic and biodegradable. Besides, due to their amphiphilic feature, they possess interesting properties such as good surface-active properties. Glycolipids are already used in industry as biosurfactants in numerous applications such as, food, detergents, cosmetics and pharmaceuticals.^{18,19} Moreover, some of them have demonstrated good ability to gelify water²⁰ or organic solvent²¹ which could expand the application fields of these sustainable molecules. Finally, with their advantages exposed previously, and their huge diversity thanks to the vast choice of carbohydrates and fatty acids moieties, glycolipids are good candidates for polymer synthesis. However, to our knowledge, very few examples of glycolipids involved in polymerization reactions are described in the literature.²²⁻²⁴

In this context, this thesis led in the Laboratoire de Chimie des Polymères Organiques (LCPO) and financially supported by the Aquitaine Council and the Centre Technique

Industriel des Corps Gras (ITERG) focuses on the design of original glycolipids (trehalose esters) and the polymerization thereof, together with the investigation of their physicochemical properties. The self-assembly properties of these amphiphilic glycolipids and their use as surfactants or gelling agents were studied. The synthesis of original polymers from these glycolipids was also investigated.

This manuscript is divided into four chapters.

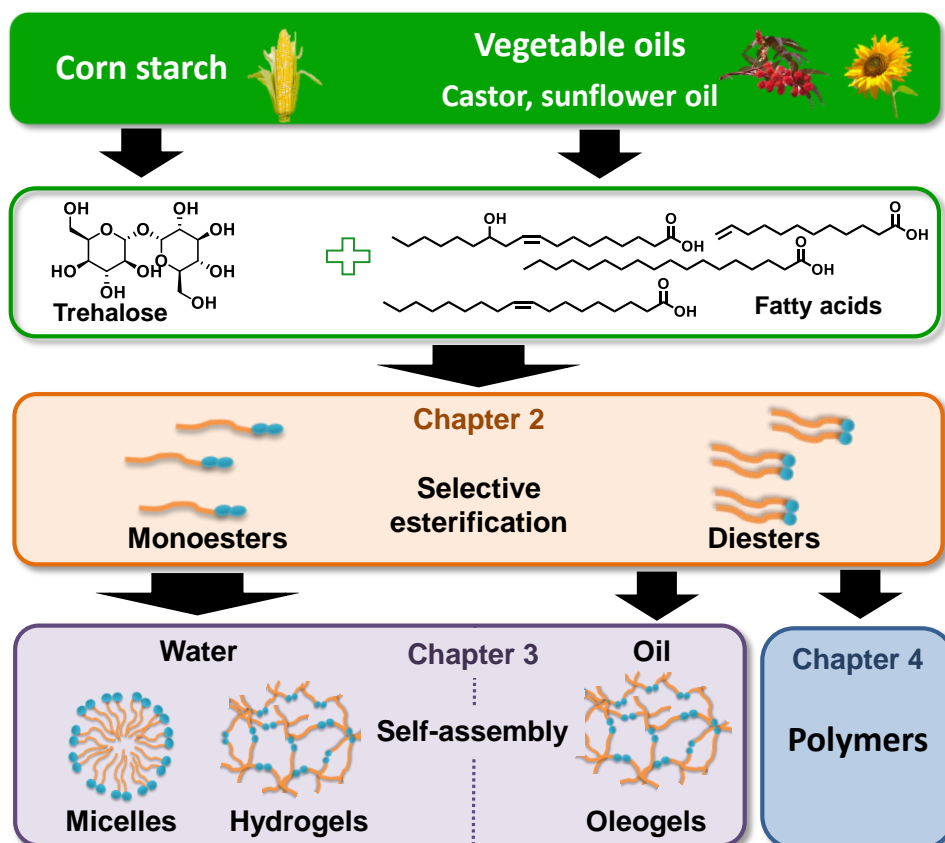


Figure 6: Outline of this PhD work

The first chapter is dedicated to the state of the art on glycolipids and more particularly on sugar esters. After an introduction dealing with their properties and applications, this bibliographic describes the different synthetic pathways to prepare sugar esters. This section shows the main limitations, drawbacks but also the advantages of each synthetic route to produce such amphiphilic molecules. In the last part of this chapter, the synthesis and properties of polymers prepared from sugar esters are discussed.

The second chapter is devoted to the synthesis of a range of trehalose esters with different alkyl chain lengths and structures. Two synthetic routes were used to selectively esterify the primary alcohols of the trehalose to produce preferentially monoesters and diesters of

trehalose. Thus, a selective chemical esterification pathway employing a peptide coupling agent was firstly used to produce a platform of trehalose monoesters and diesters. An enzymatic catalysed esterification was then investigated.

The third chapter is dedicated to the self-assembly properties of the so-formed trehalose esters. Indeed, trehalose monoesters show surfactant properties in water, and they self-assemble into micelles. One of these trehalose monoesters, trehalose monoerucate, exhibits gelation properties of water at high concentration. Therefore, morphological structure and rheological properties of hydrogels prepared from trehalose monoerucate were then studied. Contrary to trehalose monoesters, the trehalose diesters are not soluble in water but the latter showed a good ability to gelify vegetable oils. Thus, oleogels in three different vegetable oils (olive, linseed and castor) were prepared with trehalose diesters and the rheological properties of these oleogels were analyzed.

Finally, in the fourth chapter, the trehalose esters were derivatized to be used as monomers for polymerization. Novel thermoplastics polyurethanes, poly(hydroxyurethane)s, polyesters and thiopolyesters were successfully obtained by polycondensation, ADMET and thiol-ene polymerization, respectively. The structure-properties relationships of these new glycolipid-based polymers were evaluated. As the so-formed polymers contained trehalose moiety in the polymer backbone, complex crystallization behavior and self-assembly properties were observed.

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Chapter 1: State of the art on glycolipids: application, synthesis and use in polymerization

Key words: glycolipid, sugar esters, surfactant, biosurfactant, gel, selective esterification, enzymatic esterification, thermosets, thermoplastics, glycopolymers.

Mots clés: glycolipide, ester de sucre, tensioactif, biotensioactif, gel, estérification sélective, estérification enzymatique, polymères thermodurcissables, polymères thermoplastiques, glycopolymères.

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I. Glycolipids: general concepts

1. Introduction to glycolipids

Glycolipids are amphiphilic molecules present in all living organisms. They are composed of a sugar moiety, which can be monosaccharides or polysaccharides (the hydrophilic part) linked to a lipidic moiety (the hydrophobic part). In nature, glycolipids are essential constituents of cell membranes¹ (Figure 7). The carbohydrate part is at the outer part of the bilayer membrane and the lipidic part inside. Glycolipids play roles in immune response, in cell aggregation and dissociation and in the exchange of substances or signals.

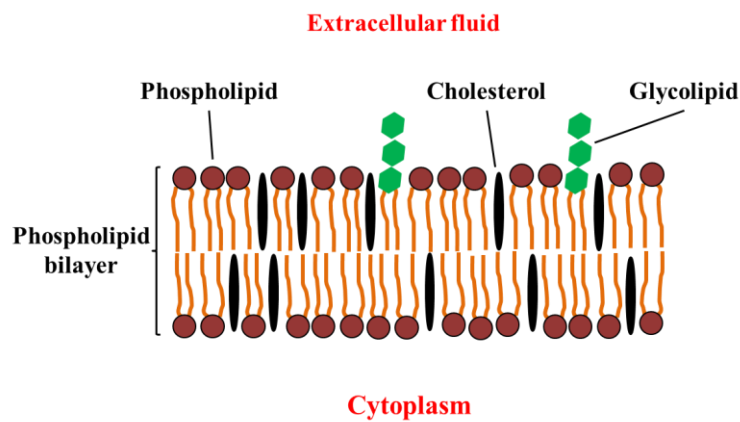


Figure 7: Scheme of a cell membrane

It exists a huge diversity of glycolipids due to the possible combination of different sugar moieties (mono-, di- and polysaccharides) (Figure 8) with a wide variety of fatty acids derivatives (Figure 9).²

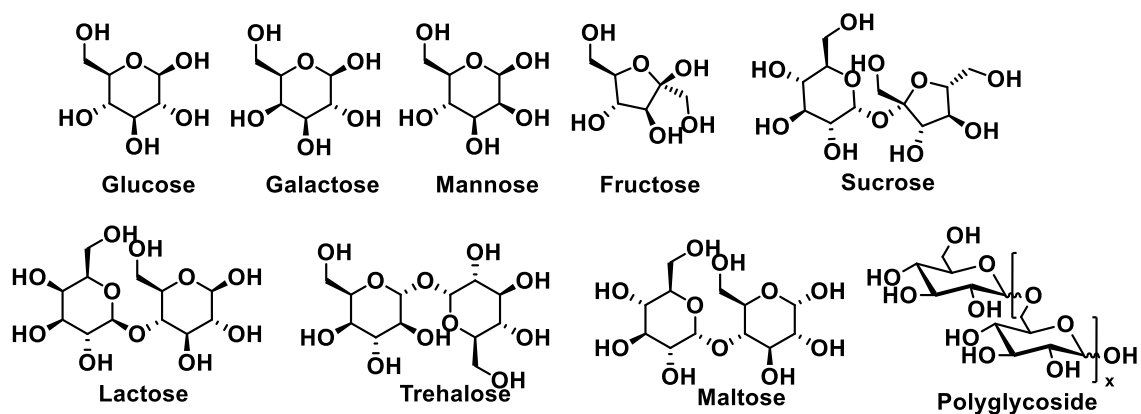


Figure 8: Example of different sugar moieties

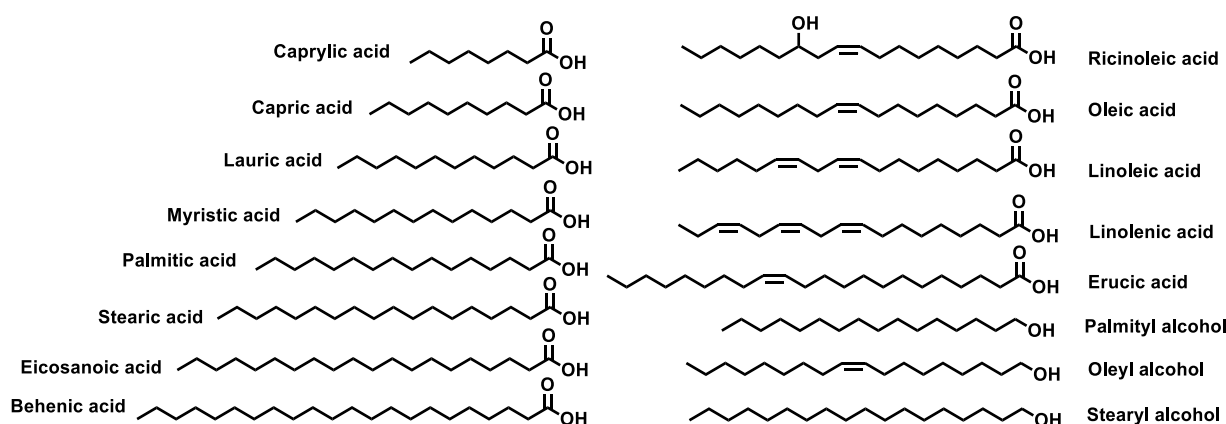
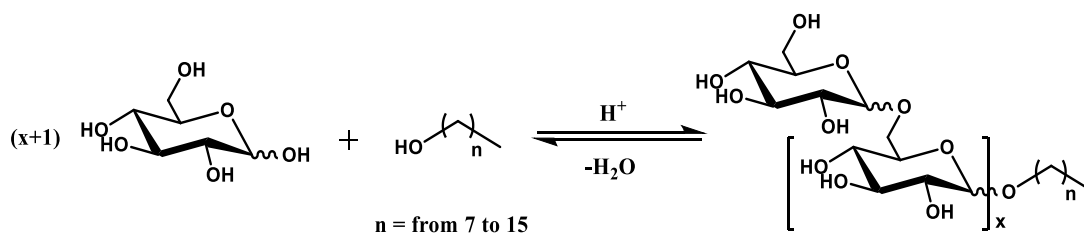


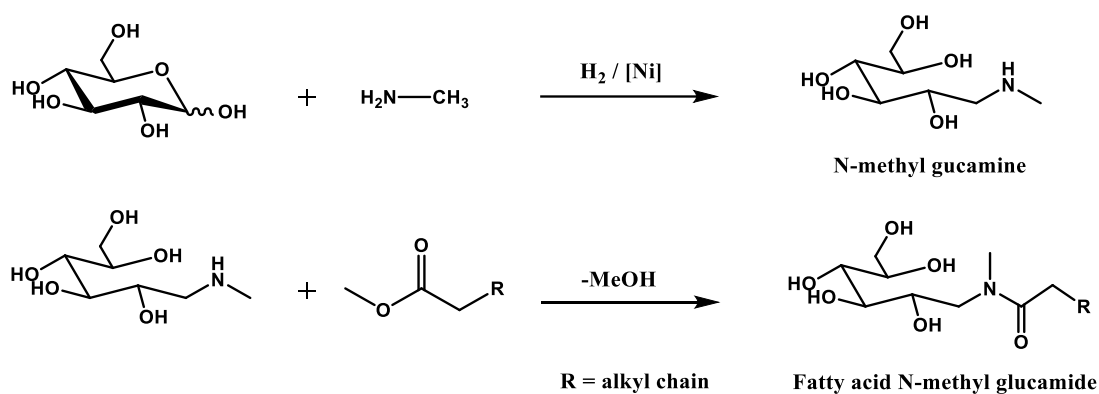
Figure 9: Examples of fatty acid derivatives

Three sorts of glycolipids are mainly synthesized and used in industry, mostly for their surfactant properties.^{1,3-7} Alkyl polyglycosides are synthesized by the Fischer reaction of glucose with long-chain alcohols with alkyl chains from C8 to C16. The reaction is an acid-catalysed acetylation of glucose in molar excess of fatty alcohols. The result of the reaction is a mixture of alkyl mono-, di- or oligoglycosides.^{8,9} The so-formed products are classified by the alkyl chain length and the average number of glucose units. The main manufacturers are AkzoNobel, BASF, Seppic, LG, Kao or Henkel. Alkyl polyglycosides are essentially used as surfactants in detergency and cosmetics due to their foam stabilizing abilities and their skin tolerance.



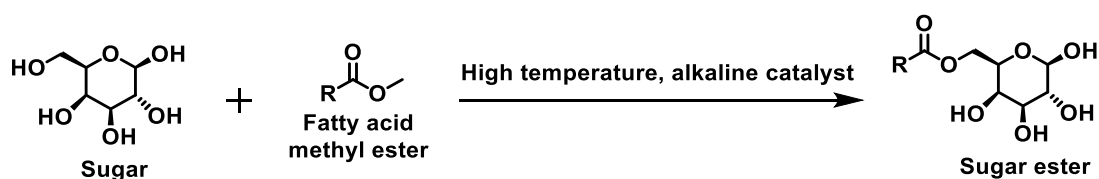
Scheme 1: Synthetic pathway of alkyl polyglycosides by acid-catalysed acetylation of glucose with molar excess of fatty alcohol

Fatty acid glucamides are a class of glycolipids essentially used in detergency and cosmetics. The latter are synthesized by the reaction of glucose with methylamine under reductive conditions to form the N-methyl glucamine. In a second reaction step, the N-methyl glucamine is converted with fatty acid methyl ester to fatty acid amide. Thus, one single carbohydrate is linked to a fatty amide chain.^{8,10}



Scheme 2: Synthetic pathway of fatty acid glucamide from glucose

Sugar esters obtained by direct esterification of carbohydrates (Scheme 3) constitute the largest class of glycolipids industrially synthesized and employed in many applications.^{6-8,11,12} This chapter will focus on the properties, applications, synthesis and polymerization of sugar esters.



Scheme 3: Synthetic pathway of glucose ester by alkaline-catalysed transesterification of glucose with fatty acid methyl ester

2. Sugar esters

Due to the huge diversity of carbohydrates and fatty acids, a wide variety of sugar esters are reported in literature.^{2,13-16} However, sucrose esters are essentially commercially used in different applications.^{6,17-20} Sugar esters can be divided in two classes: natural and synthetic sugar esters. The natural sugar esters are biosynthesized by microorganisms, fungi or plants. Three types of natural sugar esters are identified: mannosylerythritol lipids, lactonic sophorolipids and the trehalose lipids.^{21,22} Synthetic sugar esters are chemically synthesized with any carbohydrates or fatty acids.^{11,23,24} The synthesis of these glycolipids will be described in the following part of this chapter. Such glycolipids exhibit different properties according to their structures, i.e. depending on the carbohydrate part and the lipidic part but also on the degree of substitution. Indeed, several hydroxyl groups are present on a sugar, so several fatty acid chains can be esterified on a carbohydrate.

2.1. General properties

2.1.1. Surfactant properties

Surfactants are amphiphilic molecules composed of a hydrophilic “head” linked to a hydrophobic “tail”. The HLB introduced by Griffin²⁵ is the balance between the hydrophilic and the hydrophobic groups of a molecule. It allows predicting the surfactant properties of a molecule. Sugar esters are good surfactants since they possess hydrophilic and hydrophobic parts. According to the sugar moiety, the fatty acid chain length and the degree of esterification, a wide range of HLB (1-16) can be reached.²⁶ The hydrophilic or hydrophobic character of glycolipids can thus be tuned as a function of targeted specific applications. Sugar esters exhibit high emulsifying, stabilizing, foaming and detergency properties but, as most of the surfactants, their most important feature is their capacity to reduce the interfacial tension at the interface between liquids, solids and gases. The surface tension can be reduced until a minimum reached at the critical micelle concentration (CMC). The CMC is the concentration from which surfactant molecules self-assemble into micelles. As an example, sophorolipid²⁷ esters can reduce the surface tension of water from 72.8 to values below 38.7 mN·m⁻¹ with CMC of 5.10⁻⁵ mol/L. Moreover, Chen et al.²⁸ found that disaccharide monoesters synthesized from trehalose, maltose and cellobiose are able to reduce the surface tension of water below 38 mN·m⁻¹ with CMC of 10⁻⁴ mol/L, 10⁻³ mol/L and 5.10⁻³ mol/L respectively. Another example was reported by Neta *et al.* on the ability of fructose esters to reduce the surface tension to 35.8 mN·m⁻¹ to stabilize an emulsions.²⁹ Many other studies of surface-active sugar esters are reported in the literature.

2.1.2. Gelation properties

Amphiphilic molecules are able to gelify water and/or organic solvents at low concentration (~ 1 wt.%). The gel formation results in the self-assembly of the ‘solid’ glycolipids into a tridimensional network which traps the solvent molecules which is induced by the generation of weak interactions between the sugar esters. It is a combination of hydrogen-bonding interactions between sugar moieties and Van der Waals interactions between fatty acid chains. Dordick and coll. have demonstrated the gelation capacity of trehalose diesters with various alkyl chain lengths in organic solvents.^{30,31} Such organogels (gel formed in organic solvents) were obtained at very low concentration of gelators (less than 1 wt.%). John’s group has demonstrated the ability of sorbitol and mannitol diesters to gel various organic solvents.³²

These diesters self-assemble in a crystalline 3D-network to form organogels. The same authors have also demonstrated that such amphiphilic diesters could gelify vegetable oils^{33,34} (the so-formed gels are called oleogels). Moreover the latter could selectively gelify oil in oil/water mixture opening depollution applications.³² John's group has also prepared some hydrogels (gels in water) from self-assembled amygdalin esters.³⁵ The amygdalin is a cyanogenic glycoside produced by numerous plants. The gel was constituted of fibers, self-assembled by hydrogen bonding, π - π stacking and Van der Waals interactions.

2.1.3. Antimicrobial and antitumoral properties

The antimicrobial activity of different sugar monoesters has been evaluated in the literature. Fructose monoesters and particularly fructose monolaurate was described to be good antibacterial agents which can inhibit the cell growth of *Streptococcus mutans*.³⁶ Habulin *et al.* showed that fructose monolaurate and sucrose monolaurate inhibited the growth of *Bacillus cereus*, a bacterium which can cause food poisoning.³⁷ Recently, Zhang *et al.*³⁸ showed that sucrose monolaurate and maltose monolaurate were better antimicrobial than *S. aureus* bacterium. However, the latter could not inhibit *E. coli* and *C. albicans*, demonstrating that the sugar monoesters were more effective against Gram-positive than Gram-negative bacteria. The authors show that antimicrobial activity was dependent on the carbon chain length, the degree of esterification and the hydrophilic group. Moreover, Kato *et al.*³⁹ revealed that sucrose oleate and sucrose laurate allowed tumour cell inhibition of Ehrlich ascites tumour. Ferrer *et al.*⁴⁰ have demonstrated that monoester of a trisaccharide, such as the maltotriose, was an antitumor agent against two human hepatocarcinoma cancer cells. In addition, it was revealed in another study of Nishikawa *et al.*⁴¹ that antitumor activity of sugar ester, formed with long fatty acid chains, were higher than medium-chain fatty acids. Nevertheless, the exact mechanism of the antimicrobial and antitumor activity is still unclear but it should be due to interactions of the sugar esters with cell membrane of bacteria and tumour cells.

2.2. Applications

2.2.1. Cosmetics

Thanks to all these properties, biodegradability and biocompatibility, sugar esters are employed in numerous applications. Glycolipids such as sucrose esters are used in

cosmetics.^{3,42} Indeed, surfactants as emulsifiers and foaming agents are very important ingredients in cosmetic formulations. Some studies have shown that mannosylerythritol lipids have skin care properties. Thus, these glycolipids could have a great potential for novel cosmetics with moisturizing activity.⁴³

2.2.2. Food industry

In food industry, sucrose esters with low degree of substitution are employed as emulsifiers. The latter are referred as E473 in the European system of food additives.¹⁹ Sucrose esters with higher degree of substitutions are referred as sucrose polyesters. These glycolipids are used as substitutes to triglycerides in frying applications because they are not metabolized by the digestive lipases. They have been developed by Procter & Gamble¹⁹ to be used as non-caloric fat substitutes. Sugar esters are also used to retard staling, to solubilize flavour oils and to enhance organoleptic properties in bakery and ice cream.²² In bakery, surfactants such as polysorbates, diacetyl tartaric acid esters of monoglycerides are also used. Thus, sugar esters could replace these surfactants and may be used as additives in dough to develop soft crust and to improve the gluten network present in the dough system. They could also be used to control the staling of the bakery.⁴⁴ Thanks to their gelation properties, they could be also used to obtain oleogels or hydrogels. As already mentioned, sugar esters were reported to exhibit antimicrobial properties. Fructose laurate showed the highest inhibitory effect of bacteria growth.³⁶ Several studies have also reported that sugar esters were able to inhibit bacteria biofilm formation. For example, Furukawa *et al.*⁴⁵ showed that sucrose monomyristate could inhibit the biofilm formation of *Escherichia coli* and *Staphylococcus aureus*.

2.2.3. Detergency

Due to their ability to reduce surface tension and their non-toxicity, sugar esters are employed in detergent applications.²² As example, Procter & Gamble commercialize sucrose polyesters employed in detergents and the Japanese company Saraya Co, LTD has commercialized a dish washer containing sophorolipids.⁴⁶ Sophorolipids can also be applied in laundry detergents.⁴⁷ Sucrose esters with various degrees of esterification can also be used in detergents¹⁹ thanks to the emulsifiers properties.

2.2.4. Pharmaceutical and biomedical

Finally, thanks to their surfactants or gelation properties they can be used to encapsulate some drugs or molecules and may be used in pharmaceutical applications.⁴⁸ A lot of group have studied the sucrose esters ability to form various formulation systems for the loading of different active molecules. Thus, Mollee *et al.* prepared reversed vesicles in oil stabilised by sucrose palmitate. These vesicles was able to encapsulate p-aminobenzoic acid and cholesterol.⁴⁹ Honeywell-Nguyen *et al.* prepared elastic vesicles by using sucrose laurate and PEG-8-L as surfactants. Rotigotine was loaded is the vesicles for transdermal delivery.⁵⁰ Microspheres were prepared by Miyazaki *et al.* by an emulsion solvent evaporation process. These results revealed that micro-particles were obtained with sucrose esters and they exhibited efficient encapsulation properties for pharmaceuticals applications. Several others studies describe the preparation of hydrogels or solution stabilized by sucrose esters for drugs dermal absorptions.⁷ Thus, the glycolipid potential is huge in pharmaceutical and biomedical field.

II. Sugar esters synthesis

1. Biosynthesis

Three types of sugar esters are naturally produced by microbial synthesis. Most of them are synthesized from bacteria and a minority from yeasts and fungi. This section is dedicated to the different natural sugar esters also called biosurfactants and the synthetic approaches to produce them.

1.1. Mannosylerythritol lipids

Mannosylerythritol lipids (MELs) are glycolipids composed of fatty acid ester of either 4-O-D-mannopyranosyl-erythritol or 1-O-D-mannopyranosyl-erythritol.

1.1.1. Mannosylerythritol lipids diversity and microbial source

MELs are classified as MEL-A, -B, -C and -D according to the acetylation on the carbon at the C4 and C6 positions. MEL-A is diacetylated compound while MEL-B and MEL-C are

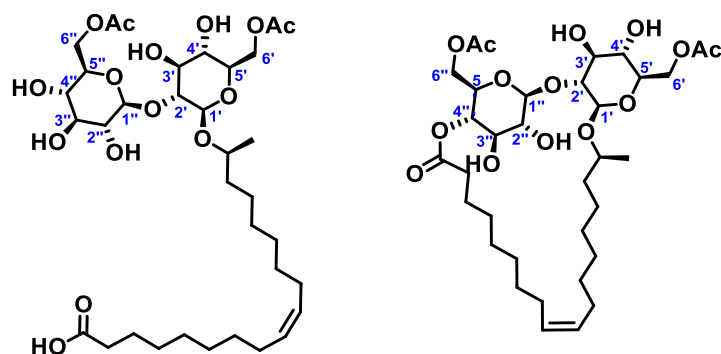


Figure 11: Majority structure of sophorolipid, acidic form (left) and lactonic form (right)

1.2.1. Microorganism producer

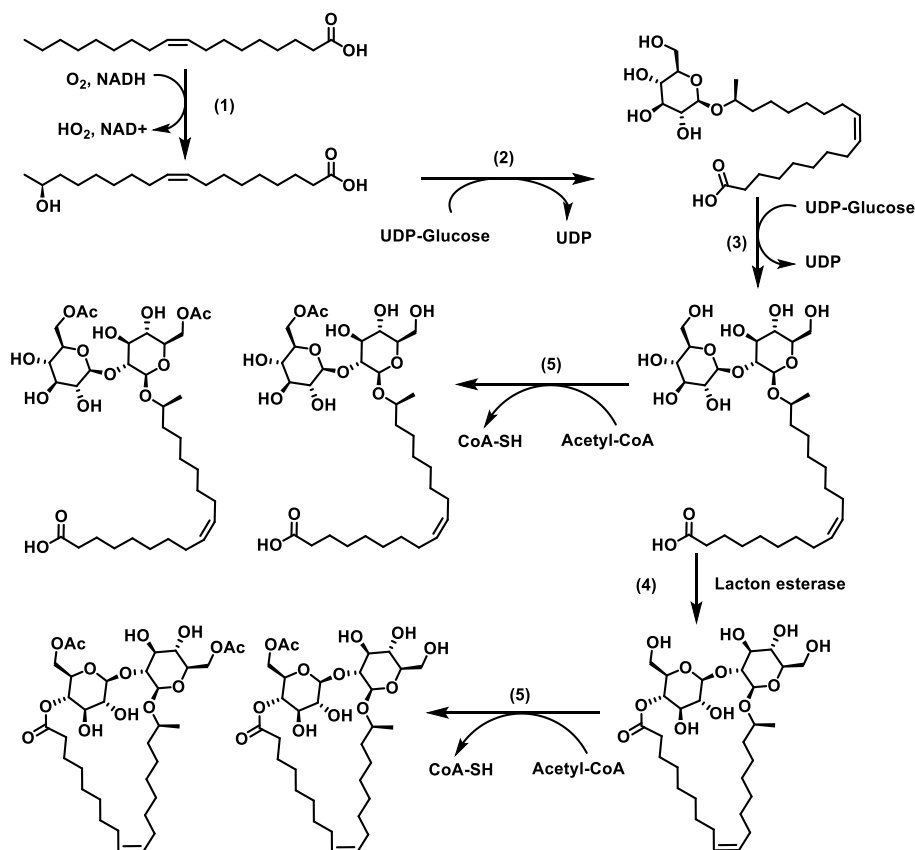
Sophorolipids are synthesized by non-pathogenic yeast. In the 1960s, Gorin *et al.* first described an extracellular glycolipid synthesis by *Torulopsis magnolia*.⁶² Nevertheless, the authors reported in 1968 that the producing strain was not well identified and the glycolipids were actually produced by *Torulopsis apicola* which is better known under the name *Candida apicola*.⁶³ The structure of this new glycolipid was determined as partially acetylated 2-*O*-β-D-glucopyranosyl-D-glucopyranose unit linked to 17-L-hydroxyoctadecanoic or 17-L-hydroxy-Δ⁹-octadecenoic acid. Turoch *et al.* have also reported in 1968 the discovery of a new sophorolipid from *Candida bogoriensis* also known as *Rhodotorula bogoriensis*.⁶⁴ Another producing yeast strain, *Candida bombicola*, was discovered by the same group. The structure of the sophorolipids produced by this yeast strain is quite identical to those of *Candida apicola*.⁶⁵

Recently, Chen *et al.* have reported the production of sophorolipids from a new strain *Wickerhamiella domericqiae*.⁶⁶ They observed more than six glycolipids and identified the major component as 17-L-(oxy)-octadecanoic acid 1,4''-lactone 6',6''-diacetate, which is identical to the main product produced by *Candida apicola* and *Candida bombicola*.

Candida bombicola is the most known and preferred by the majority of the research groups due to its good productivity: more than 400 g/L sophorolipids.⁶⁷

1.2.2. Synthetic pathway

The proposed mechanism of sophorolipid synthesis is described in Scheme 4.



Scheme 4: Scheme of the synthesis of sophorolipids according to Van Bogaert⁶⁸

Sophorolipids are synthesized from glucose and fatty acids. The fatty acids can be provided into the production medium as such. But fatty acid methyl or ethyl esters and triglycerides can be used as well. In this case, esterases present in yeasts will produce fatty acids from these substrates. Moreover, n-alkanes can also be used as feedstock. Indeed, sophorolipid-producing yeast strains such as *Candida bombicola* and *Candida apicola* can grow on alkanes. These yeast strains have enzymes to lead terminal oxidation of alkanes. Consequently, fatty acids are formed *in situ* when alkanes are used as feedstock.

In the first step of the sophorolipid synthetic pathway, fatty acids are converted to terminal (ω) or *subterminal* ($\omega-1$) hydroxyl fatty acids by a cytochrome P450 monooxygenase enzyme⁶⁹ (Scheme 4, (1)). In a second step, the hydroxylated fatty acid is β -glycosidically coupled with a molecule of glucose at the 1'-position through the action of a glycotransferase⁷⁰ (Scheme 4, (2)). Then, a second glucose molecule is glycosidically linked to the 2'-position of the first one by a glycotransferase II (Scheme 4, (3)). The so-formed sophorolipid obtained after the action of the glycotransferase II is under an acidic form but sophorolipids can also be modified to a lactonic form. The lactonization of sophorolipids generally occurs on the 4''-position catalysed by an extracellular lactone esterase.⁷¹ Finally, acetylation of sophorolipids (in acidic or lactonic form) is performed in the 6' and/or 6''-

position by an acetyl-coenzyme A (CoA) dependent acetyl transferase. The major product is the diacetylated with ω -1 hydroxylated monounsaturated sophorolipid lactone.

1.2.3. Culture condition for the production of sophorolipids

As it was described before, two main yeast strains are used for the production of sophorolipids: *Candida bombicola* and *Candida apicola*. The culture medium is composed of glucose (hydrophilic carbon source), yeast extract, urea and hydrophobic carbon source (fatty acid, fatty acid esters, triglycerides or n-alkanes). The production of sophorolipids is possible with only one type of carbon source^{70,72} (hydrophilic or hydrophobic) but the presence of both allows higher productivity.

The fermentation can be divided in two phases: the cell growth phase and the stationary phase.⁷³ During the cell growth phase, no sophorolipids are produced, pH drastically drops and it must be maintained at 3.5 by addition of NaOH to optimize the sophorolipid production.⁷⁴ When the yeast cells enter in the stationary phase, sophorolipids production starts under nitrogen-limiting conditions. The yeast cells are very sensitive to oxygen limitation, thus good aeration conditions are required to sophorolipid production. The optimal temperature fermentation for sophorolipids production is 21 °C, but generally the fermentations are run between 25 and 30 °C to facilitate the handling (for example: sample or oil addition). The temperature does not play an important role during the fermentation. Indeed, the production of sophorolipids is quite similar at 25°C or at 30°C.⁷⁴

After the fermentation, the sophorolipids are extracted with ethyl acetate and washed with hexane to remove residual fatty acids. Centrifugation can also be used to isolate the sophorolipids. Finally, lactic sophorolipids can be purified by crystallization.⁷⁵

1.3. Trehalose lipids

Trehalose lipids are glycolipids composed of fatty acids linked to a disaccharide, the trehalose. Trehalose is a non-reducing sugar in which two molecules of glucoses are linked in a α,α -1,1-glycosidic bond.

1.3.1. Trehalose mycolates and trehalose dicorynomylates

Trehalose lipids are the basic component of *Mycobacteria* and *Gorybacteria*. The latter were first discovered by Bloch in 1950 who observed that strains of *Mycobacteria tuberculosis* expressed a material named “cord factor” which was an important constituent of the cell wall for the pathogenesis of the bacteria.⁷⁶ This “cord factor” was identified as trehalose dimycolates. This is the most reported trehalose lipid. It is a α -branched-chain mycolic acid (Figure 12) esterified on the primary alcohols of the trehalose.

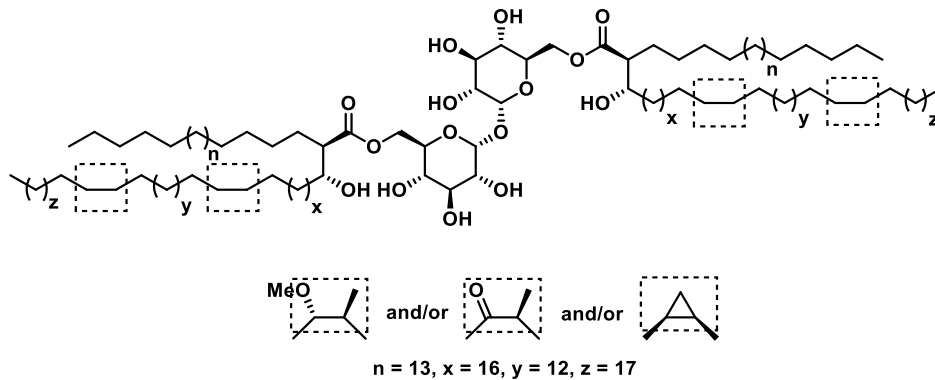
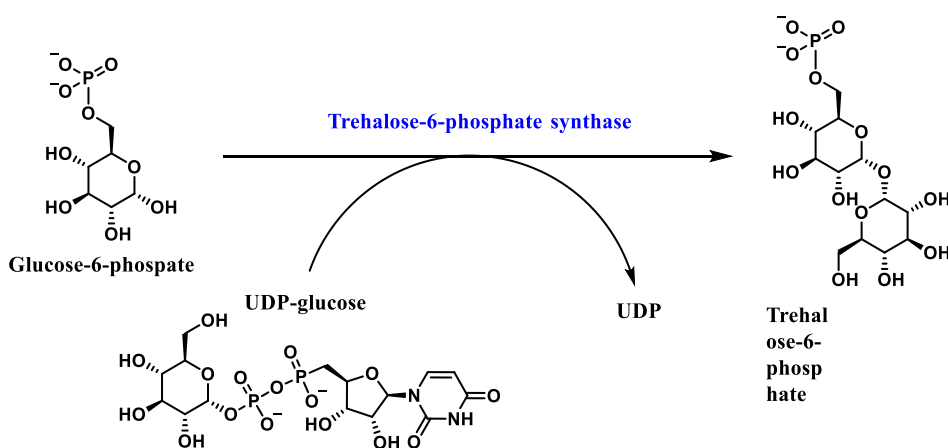


Figure 12: Chemical structure of trehalose dimycolates

There are different types of trehalose dimycolates, which are synthesized by different microorganisms such as *Arthrobacter*, *Nocardia*, *Rhodococcus* and *Gordonia*. The type and the amount of trehalose dimycolates synthesized are different according to the microorganism used.

The trehalose production dimycolates was elucidated to occur in the final stage of the synthesis of the cell wall. An intermediate of the reaction, the trehalose-6-phosphate is synthesized from UDP-glucose (uridine diphosphate glucose) and glucose-6-phosphate catalysed by a trehalose-6-phosphate synthase.



Scheme 5: Synthetic pathway for the biosynthesis of trehalose-6-phosphate

Mycolic acids synthesized from Claisen-condensation are transported and linked to peptidoglycan-arabinogalactan complex of the cell wall. The mycolyl group linked to polyketide synthase 13 (Pks13) is then transferred to d-mannopyranosyl-1-phosphoheptaprenol (Man-P-heptaprenol) by a cytoplasmic mycolyltransferase I to form 6-O-mycolyl-b-D-mannopyranosyl-1-phosphoheptaprenol (Myc-PL). The mycolyl group is then transferred to trehalose-6-phosphate (Treh-6-P) by a mycolyltransferase II to form trehalose monomycolate (TMM). Finally, trehalose monomycolates (TMM) are transferred outside of the cell by an ABC transporter where they are involved in the synthesis of trehalose dimycolates and cell wall arabinogalactan-mycolate. Free trehalose is also rejected during this last reaction.⁷⁷

1.3.2. Maradolipids

Another type of trehalose lipids are synthesized in the dauer larvae of *Caenorhabditis elegans*: maradolipids.^{78,79} When the *Caenorhabditis elegans* (*C. elegans*) is exposed to extremely dry conditions, it stops its reproductive life cycle and it generates a dauer larvae. These trehalose lipids present in the outer membrane of the dauer larvae, the maradolipids, are composed of a mixture of 60 derivatives of trehalose diesters in 6 and 6'-positions. The fatty acid chains linked to the trehalose can be symmetrical or not and about 2/3 of maradolipids have branched fatty acid side chain. The 6,6'-trehalose diester, 6-O-(13-methylmyristoyl)-6'-O-oleoyltrehalose is the most abundant component found in *C. elegans*.

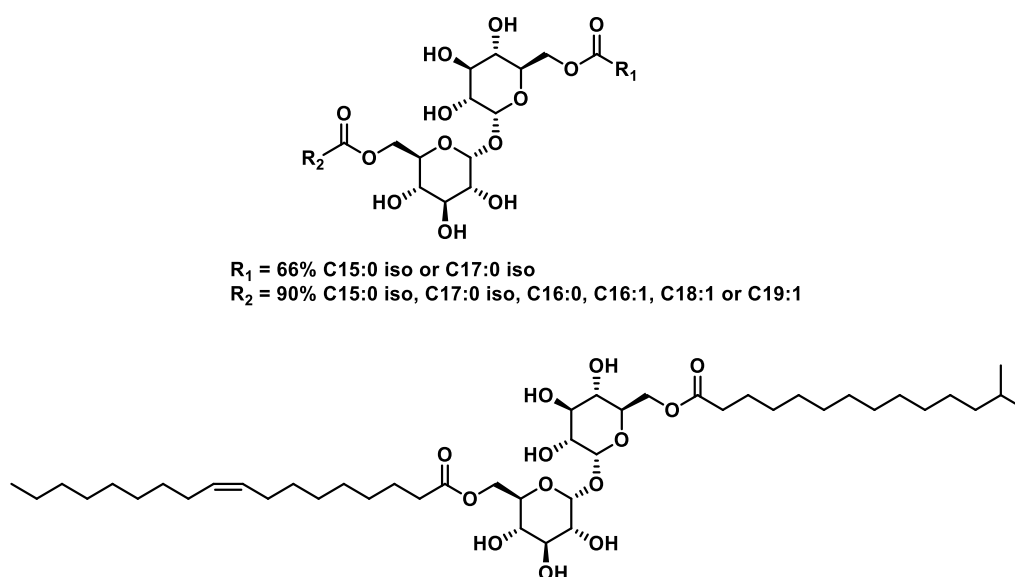


Figure 13: Chemical structure of maradolipids (top) and the most abundant component (bottom)

Conclusion

To conclude this section, a lot of glycolipids and particularly sugar esters are biosynthesized by different bio-organisms. The advantage is that it is a green process with high production yields. However, the sugar moieties and the fatty acid chains are totally dependent of the bio-organism. The glycolipids biosynthesized can be a mixture of different structures and it is difficult to isolate one type of sugar esters. Even by changing the feedstock, the sugar and the fatty acid of the glycolipid cannot be controlled.

2. Chemical synthesis of sugar esters

The following section will discuss the main chemical pathways developed for the synthesis of sugar esters.

2.1. Un-controlled esterification

Historically, the first synthesis of sugar esters and particularly sucrose esters was reported by Herzfeld in 1880, describing the preparation of octoacetate.⁸⁰ Further to the discovery of the interesting surfactants properties of sucrose esters, synthetic processes have been commercially developed for their production. Sucrose esters are nowadays the main sugar esters commercially produced and employed in many application fields (cosmetics, food industry and detergents).¹⁹

Since their discovery, numerous manufacturing processes have been patented to synthesize sucrose esters on commercial scale.⁸¹⁻⁸⁶ The first involves the esterification of fatty acid chlorides or anhydrides. However this process needs the use of toxic solvents to facilitate the mixing of the reactants and the production yields remain low. The second process was largely developed and is the major route employed today. It is the transesterification reaction of fatty acids esters with sugar at high temperature in the presence of an alkaline catalyst. Here again, the reaction is managed with toxic solvents. Generally, DMF is used as a solvent to solubilize both the carbohydrates and the fatty acids. This procedure was first developed by Snell and co-workers and patented later by Hass *et al.*⁸³ The process includes the reaction of triglycerides and fatty acid methyl esters with sucrose in the presence of sodium methoxide as catalyst in DMF. The reaction is performed with harsh conditions at 180 °C for 15 hours. Due to the toxicity of DMF, it has been subsequently replaced by dimethyl sulfoxide (DMSO) which is less toxic.⁸⁷ The product contains more than 50% of monoesters, more than 10% or

mixture of di- and higher esters and unreacted sucrose and fatty acid methyl esters.⁶ With the use of fatty acid methyl esters, methanol is produced during the transesterification and can be removed by distillation to drive the equilibrium to the sucrose esters production.

To improve the production yield of sucrose esters, Osipaw and Rosenblatt have developed the Nebraska-Snell process. This method consists in the addition of fatty acid methyl esters on micro-emulsion of sugar in propylene glycol in the presence of potassium carbonate. The propylene glycol is then distilled from the mixture and sucrose esters were produced in 85% yield of monoesters and 15% in diesters.⁸⁸

A solvent-free method was also developed by Feuge and his colleagues implying the reaction between molten sucrose with fatty acid methyl esters in the presence of alkali soap playing the role of solubilizers and catalysts.⁸⁴ The main issue of these two last routes is the use of high temperature (175-185°C), which leads to fast sucrose degradation before its reaction.

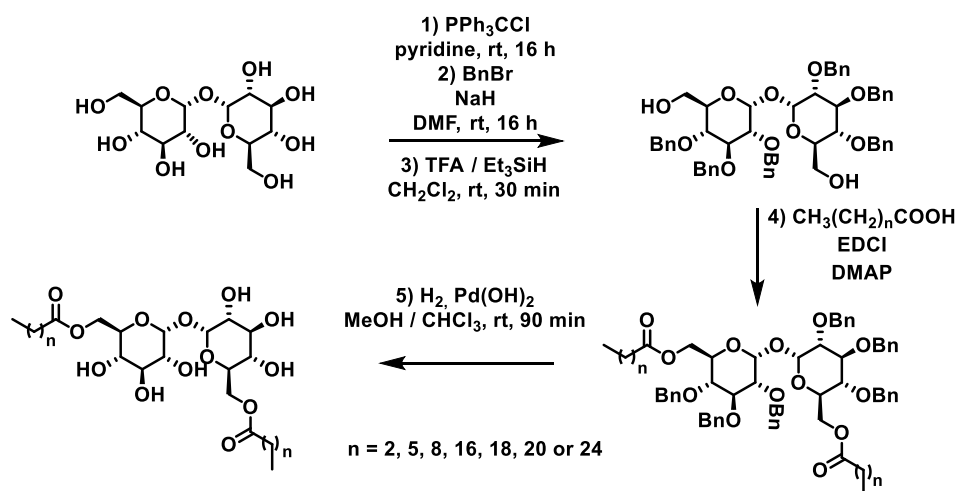
Many drawbacks are observed for these processes. The transesterification is not selective, thus a mixture of poly-esters of sugar is produced and it is difficult to isolate the species, the yield is low, toxic solvents (DMF, pyridine) are employed for the solubilisation of sucrose and, finally, harsh conditions are needed. However, these processes are still largely employed in industry to produce sucrose esters at 5000 tper year due to the low cost of production and feedstock.^{23,89}

2.2. Esterification *via* protection/deprotection strategy

The main employed chemical synthesis of sugar ester is not selective. However, specific biosynthesized glycolipids and particularly trehalose lipids exhibit interesting biological activities and could be used in several biomedical applications.⁹⁰ These glycolipids are trehalose diesters synthesized with high selectivity. To better understand the biological activity of these glycolipids, several groups have reported selective esterification of well-defined trehalose diesters using esterification pathways *via* protection/deprotection strategy to esterify only the primary hydroxyl groups and not the secondary ones.

Harland *et al.*⁹¹ have produced synthetic trehalose diesters with different chain structures in a five-step procedure (Scheme 6). The hydroxyl groups in 6,6'-position were selectively protected with trityl chloride, then the other hydroxyl groups were converted to benzyl ethers and the trityl groups were removed with trifluoroacetic acid and triethylsilane to get diols in 6,6'-position in good yield (68 %). Then, esterification mediated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), catalysed by 4-(dimethylamino)pyridine

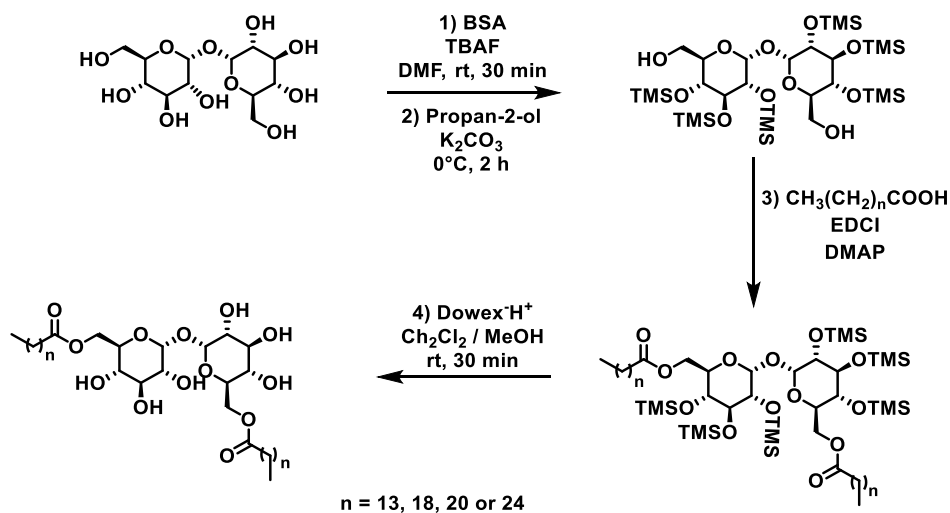
(DMAP), was used to couple various fatty acids in 6,6'-position. The last step of the synthesis was to remove the benzyl group with Pd(OH)₂ and H₂ in a mixture of methanol/chloroform (MeOH/CHCl₃). Trehalose diesters were obtained in global yield between 33 and 51 % for C₄-C₁₈ diesters. However, Khan *et al.*⁹² have demonstrated that it was impossible to get the trehalose diesters with longer fatty acids (C₂₀-C₂₆). Indeed, during the deprotection step of the benzyl groups, harsh conditions and protic solvents are needed to solubilize the diesters with long alkyl chains, which lead to the degradation of the C₂₀-C₂₆ derivatives. Thus, this protection/deprotection strategy appears to be an efficient procedure to selectively esterify trehalose with short fatty acids.



Scheme 6: Synthesis of trehalose diesters in a five-step procedure using benzyl ether protection of the secondary alcohols

Another protection/deprotection methodology developed by Toubiana *et al.*⁹³ in 1975 uses the trimethylsilyl group as protective group for the secondary alcohols of trehalose. The authors synthesized trehalose dimycolates (6,6'-di-O-mycoloyl- α,α -trehalose) from this procedure. With the same approach, Dulayymi *et al.*⁹⁴ have synthesized trehalose monomycolates and trehalose dimycolates. This strategy was also used by Khan *et al.*⁹² to synthesize other trehalose diesters with different alkyl chain lengths and structures. The trehalose diester synthesis is performed in five steps from trehalose. First, the hydroxyl groups of trehalose were protected with N,O-bis(trimethylsilyl)acetamide (BSA) catalysed by tetrabutylammonium fluoride (TBAF) (Scheme 7, (1)). Then, K₂CO₃ was used to remove the trimethylsilyl (TMS) group from the primary alcohols in 6 and 6'-position of the sugar (Scheme 7, (2)). Esterification mediated by EDCI catalyzed by DMAP was then used to couple various fatty acids in 6,6'-position (primary alcohols). Finally, the TMS group was removed with Dowex-H⁺ resin to form the target trehalose diesters with global yields between 33 and 58 %. Contrary to the previous route, the efficiency of this protection/deprotection

strategy is not dependent on the fatty acid chain length due to milder conditions employed during the deprotection step.



Scheme 7: Synthesis of trehalose diesters in a five-step procedure using trimethylsilyl protection of the secondary alcohols

Conclusion

The protection/deprotection approach appears to be very interesting regarding the selectivity of primary alcohols esterification. Indeed, the esterification reaction of the only primary alcohol of trehalose could be achieved in relatively good yield, around 50%. However, this approach is not in accordance with the green chemistry principles. Indeed, five reaction steps are needed, hazardous solvents are used (pyridine, DMF) and the atom economy is not achieved with all the protective groups used during such synthetic pathway.

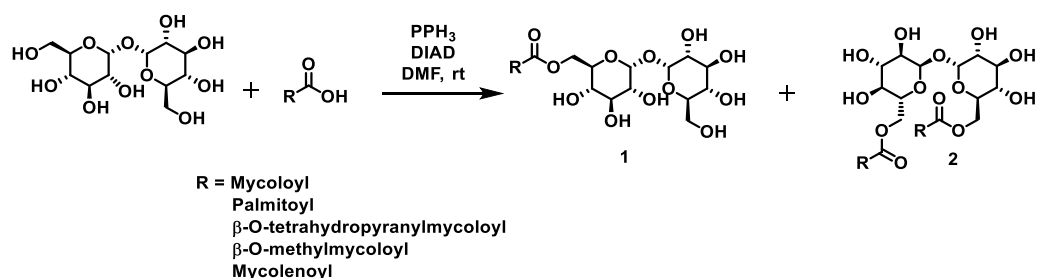
2.3. Protecting group-free selective esterification of primary alcohols

To reduce the number of reaction steps while keeping the selectivity of the esterification reaction, protecting group-free strategies were recently developed. Two main routes were reported and are described thereafter.

2.3.1. Mitsunobu reaction

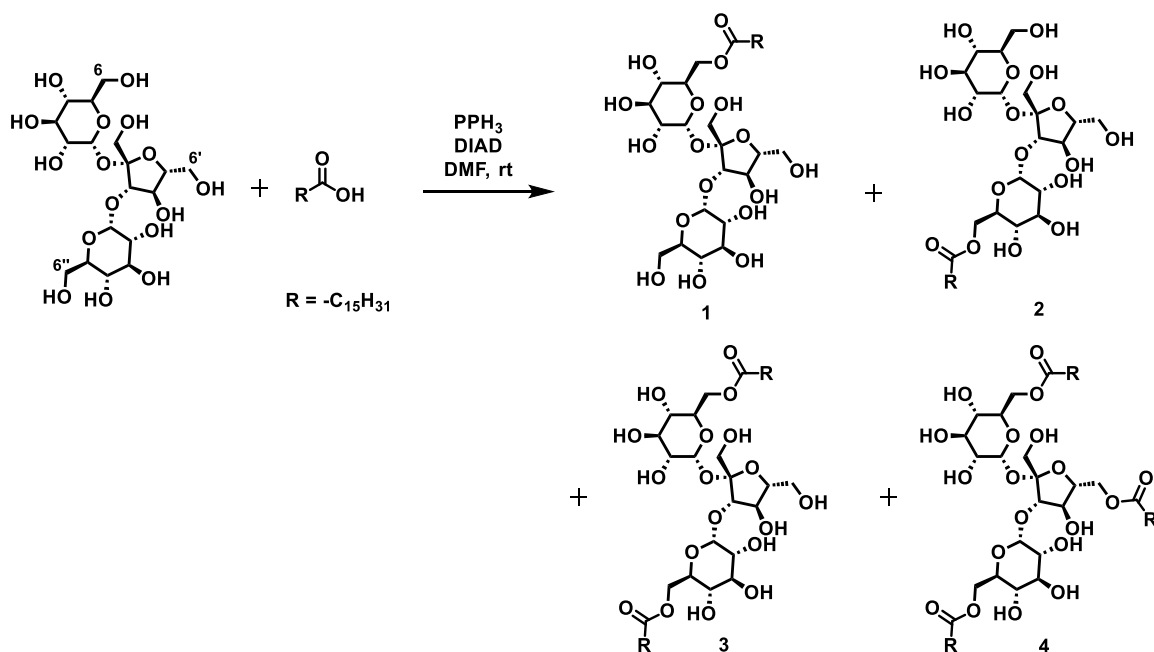
Mitsunobu esterification of trehalose has been reported by Jenkins *et al.*⁹⁵ in 1986. Well-controlled monoesters and diesters of trehalose were synthesized as a mimic of biosynthesized trehalose lipids. The esterification of the primary alcohols consists in a one-step selective

reaction. Trehalose, triphenyl phosphine (PPH₃), fatty acids and diisopropyl azodicarboxylate (DIAD) were reacted at room temperature to form, as main products according to the stoichiometry, trehalose diesters and trehalose monoesters in good yield (around 60%).



Scheme 8 : Synthesis of trehalose monoesters and trehalose diesters by Mitsunobu esterification⁹⁵

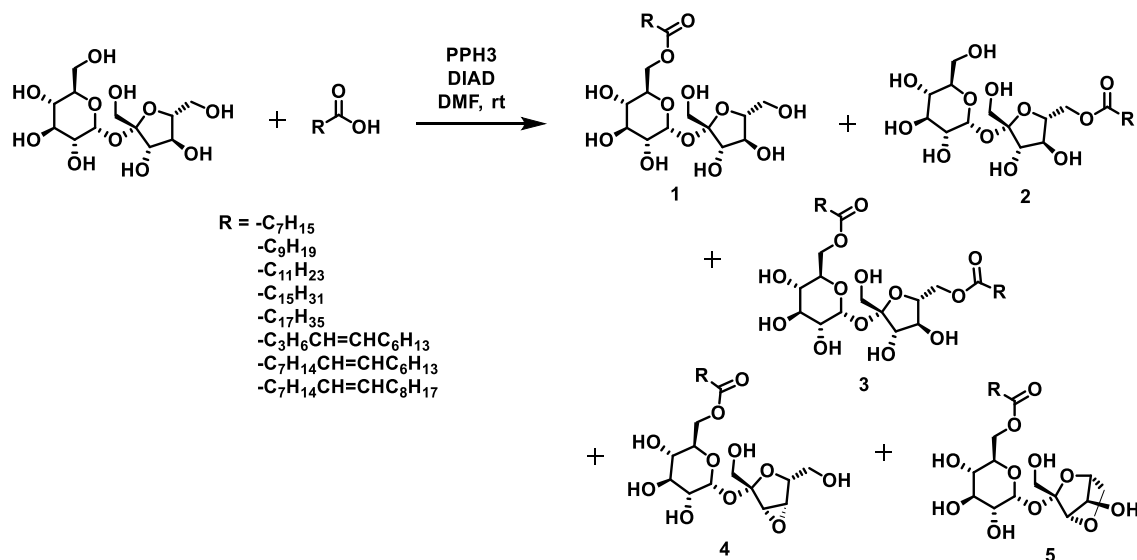
Queneau's group has reported the esterification of sucrose and melezitose^{17,96} with palmitic acid by Mitsunobu esterification. With melezitose, esterification selectively occurred on 6,6' and 6''-position due to the sensitivity of this reaction to the steric hindrance. Thus, mono-, di- and tri-esters of melezitose were obtained with a good selectivity (Scheme 9).



Scheme 9: Esterification of melezitose with palmitic acid under Mitsunobu esterification⁸⁹

However, Queneau and coll. revealed that intermolecular etherification occurred during sucrose esterification under Mitsunobu conditions¹⁷ and that anhydro- derivatives were formed as by-products (Scheme 10, species 4 and 5). Thus, esterification of sucrose was not

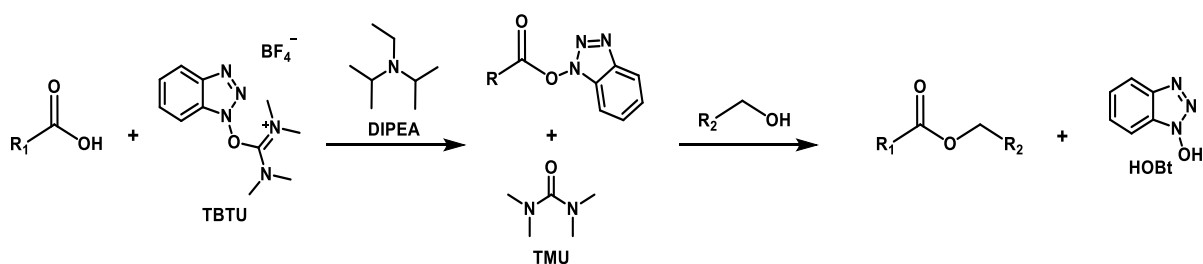
so selective under these conditions and diesters and monoesters were difficult to isolate and purify.



Scheme 10 : Synthesis of sucrose monoesters (1 and 2) and sucrose diesters (3) by Mitsunobu esterification according to Queneau and coll. ¹⁷

2.3.2. Peptide coupling agent

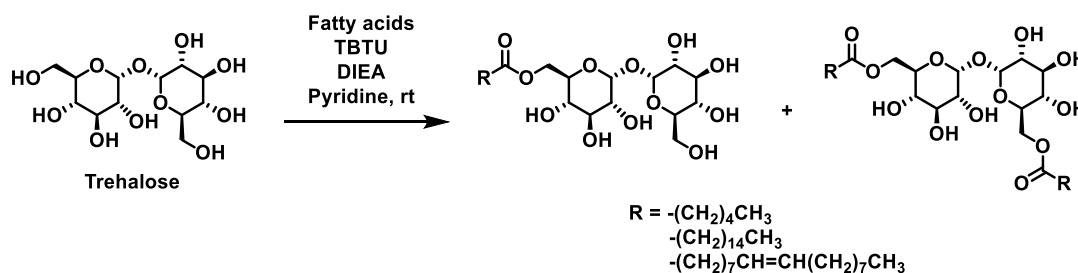
Recently, Grindley's group has described a selective esterification of primary alcohols using a peptide-coupling agent. Due to the steric hindrance and an appropriate choice of the organic base, the esterification is selective on the primary alcohols⁹⁷ (Scheme 11).



Scheme 11: Synthetic pathway of selective esterification of primary alcohol using a peptide coupling agent according to Grindley and coll. ⁹⁷

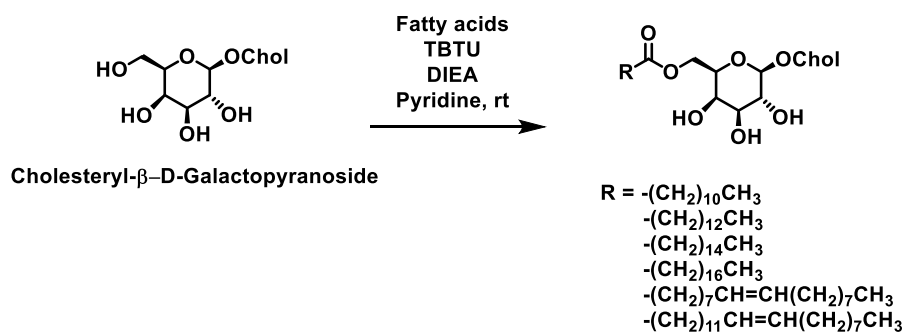
The authors showed that trehalose could be selectively esterified with this pathway.⁹⁸ Thus, trehalose monoesters were predominantly obtained in good yields (around 60 %) by esterification using 1 equivalent of fatty acid in pyridine at room temperature. With 2 equivalents of fatty acids, the esterification led to diesters in good yields as well (around 70

%). The monoesters can also react a second time with different fatty acids under the same conditions to get dissymmetrical diesters thus mimicking some biosynthesized maradolipids.



Scheme 12: Synthesis of trehalose monoesters and trehalose diesters⁹⁸

The authors also used this protecting group-free esterification to produce a library of Lyme disease glycolipid antigens.⁹⁹ Different fatty acids were regioselectively esterified on the primary alcohol of cholesteryl- β -D-galactopyranoside in good yields (80 %).



Scheme 13: Esterification of cholesteryl- β -D-galactopyranoside with different fatty acids⁹⁹

Conclusion

The pathways described in this section exhibit a good selectivity of primary alcohol esterification without using any protecting groups. Indeed, only the primary alcohols of the carbohydrate can be esterified without esterification of the secondary ones. Moreover, by adjusting the equivalent number of fatty acids, either monoesters or diesters of disaccharides can be obtained in good yields. However, this route shows some drawbacks such as the solvent used (DMF, pyridine) which is toxic and even classified as CMR substance as well as the use of TBTU or triphenylphosphine that goes against the atom economy principle.

3. Enzymatic catalyzed esterification

The high selectivity of enzymes and their high activity in mild conditions have interested a lot of research groups. A huge literature exists on the synthesis of sugar esters catalysed by

enzymes.^{2,13-16} Enzymatic synthesis of glycolipid is complicated because a lot of parameters can influence the reaction and have to be considered. In most cases, optimal reaction conditions are different for each specific synthesis. This section will discuss the major reaction parameters that influence the enzymatic synthesis of glycolipids.

3.1. The enzymes

Enzymes are proteins, which catalyse biological reactions. Enzymes are classified in 6 categories according to the reaction they catalyse. Among this classification, hydrolases and particularly lipases and proteases are enzymes used as biocatalysts for glycolipid synthesis.

Lipases are more commonly used and reported in literature for the catalysis of esterification¹². Most of the lipases used in ester synthesis come from microbial origin¹⁰⁰⁻¹⁰². Lipases from *Candida antarctica*, *Candida rugosa*, *Candida cylindracea*, *Rhizomucor miehei*, *Bacillus subtilis*, and *Bacillus licheniformis* are frequently used. The most employed is the *Candida antarctica* lipase B immobilized on acrylic resins due to its stability in organic solvents and its efficiency.^{12,16}

3.2. The substrates

Carbohydrates and fatty acids are the substrates involved in the synthesis of glycolipids. Numerous studies have been published on the enzymatic synthesis of glycolipids from various sugars such as glucose, mannose, galactose, sucrose, fructose, lactose, trehalose or maltose.^{2,3,5,103-109} The main problem for all these sugars is their solubility in organic solvents.

Regarding the fatty acids, the chain length has an impact on the properties of the final glycolipids but it has also some influences on the esterification rate and yield. For example, some lipases exhibit higher reactivity for long fatty acids while others are more reactive for short and branched fatty acids.^{14,103,106}

The esterification reaction is an equilibrated reaction and water produced during the reaction has to be removed to drive the synthesis towards the production of sugar esters. Otherwise, in the presence of water, sugar esters can be easily hydrolyzed by the enzyme. Therefore, the use of fatty (methyl, ethyl or vinyl) esters is preferred allowing the transesterification instead of the esterification route. In this case, the side products (alcohols) are easier to remove. Additionally, by using fatty vinyl esters, vinyl alcohol is produced during the reaction and its tautomer is acetaldehyde which evaporates above 20°C.¹¹⁰

Finally, the molar ratio of the two substrates plays an important role on the esterification reaction. Depending on the enzyme, a too high concentration of fatty acids could either decrease the activity of the enzymes but an excess of fatty acids (or fatty acid ester) compare to sugar could also favour the production of glycolipids.^{111,112}

3.3. The reaction medium of enzymatic esterification

3.3.1. Organic solvents

The selection of the solvent is very crucial because it must be able to dissolve all the substrates. The main issue is the sugar hydrophilicity and the fatty acids hydrophobicity. Thus, it is complex to have a homogeneous phase for the reaction with the two substrates completely soluble in the reaction medium. Moreover, the solvent must not deactivate the enzymes. Indeed, organic solvents can cause the enzyme dehydration which reduces its activity and stability.^{113,114} Immobilization of the enzyme appears to increase its resistance against organic solvents. Moreover, esterification reaction is usually performed in organic solvents with very low water content to prevent the hydrolysis of the ester function. Many glycolipid syntheses were performed in organic solvents like pyridine, DMF or dimethylpyrrolidone due to the solubility of both the sugars and fatty acids in these solvents.^{115,116} However, as mentioned earlier, these solvents are very toxic. Thus, many other organic solvents have been tested for enzymatic esterification of sugars. Very hydrophobic solvents like hexane show low esterification yield while water-miscible solvent as acetone, acetonitrile and tertiary alcohols are better candidates.¹¹⁷ The latter have the ability to partially solubilize the sugars and to well solubilize the fatty acids. Thus, numerous experiments have been reported employing 2-methyl-2-butanol and tert-butanol¹⁰⁸ for the synthesis of glycolipids. These tertiary alcohols are non-toxic; they provide high enzyme activity and are not reactive due to their steric hindrance.

3.3.2. Ionic liquids and deep eutectic solvents

Ionic liquids (ILs) can be an alternative to organic solvents. The latter are organic salts which are liquid at room temperature. These non-volatile, thermo- and chemically stable solvents have demonstrated a good ability to solubilize carbohydrates (mono-, di- or polysaccharides). Recently, some reports showed that lipases are still active when they are employed in ionic

liquids such as 1-butyl-3-methylimidazolium trifluoromethanesulfonate [Bmim][OTf] or 1-butyl-3-methylimidazolium hexafluorophosphate [Bmim][PF₆].^{118,119} ILs are the focus of increasing attention as solvent for the enzymatic synthesis of glycolipids. However, ILs show some limitations, like their high cost, their purification, their poor degradability and their toxicity is currently unclear.

Deep eutectic solvents (DES) are a new class of solvents which does not show the limitations of ILs¹²⁰⁻¹²³ and could represent a good alternative to ILs. DES consists in a mixture of an ammonium salt or a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD). In these solvents, the HBD interacts with the ammonium anion to form a complex, which induces a depression in the melting point of the mixture. Thus, the melting point of the mixture is much lower than the melting point of the individual compound. For examples, the main used DES is the mixture between choline chloride and urea in 1:2 molar ratio (with melting points of 247 °C and 133 °C, respectively) resulting in a deep eutectic solvent melting temperature around (12 °C). DES do not deactivate enzymes like lipases.¹²⁴ Pöhnlein *et al.*¹²⁵ have recently demonstrated that glucose can be enzymatically esterified with vinyl hexanoate in DES.¹²⁵ Thus, these particular solvents appear to be good alternatives to classical organic solvents.

3.4. Effect of temperature and water activity

The temperature can affect the activity of the enzymes. Commercially lipases used under immobilized form are quite thermo-stable. For example, lipase B from *Candida antarctica* immobilized on acrylic resin can be used between 60°C and 80 °C without loss of activity.^{126,127} The temperature can also enhance the reaction rate, the solubility of the substrates and the products. Thus, according to the substrates and solvent used, a range of temperature has to be tested to find the optimal conditions to improve the glycolipid synthesis.

The enzymes need some water to keep their three-dimensional conformation and flexibility. So, the amount of water in the organic solvent is a crucial parameter to maintain the enzyme activity.^{128,129} However, as mentioned previously, low water content in the organic solvents is necessary to avoid sugar ester hydrolysis. Thus, the water content should be a compromise to maintain the enzyme activity while avoiding the hydrolysis of sugar esters.

An example of lipase-catalysed synthesis using the main common condition is shown in Figure 14.

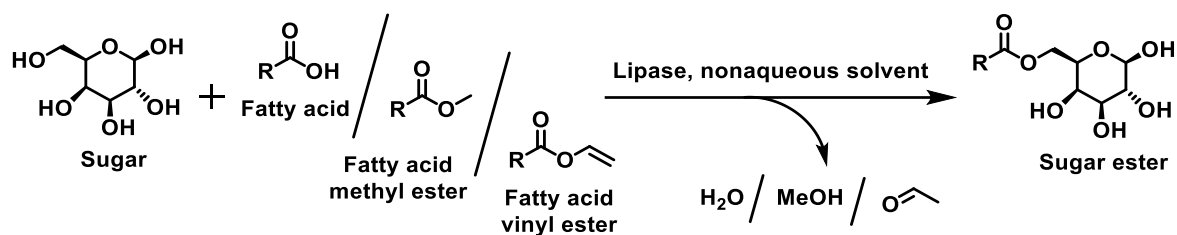


Figure 14: Example of lipase-catalyzed synthesis of sugar esters from glucose and different fatty acid derivatives performed in a non-aqueous solvent

Conclusion

Enzymatic esterification appears to be a good way to produce sugar esters with high selectivity. The glycolipids synthesis is possible in mild conditions. The use of non-aqueous and non-hazardous solvents compared to chemical routes allows greener glycolipid synthesis. Ionic liquids and deep eutectic solvents provide new opportunities to improve the solubility of the substrates and thus enhance the sugar esters production. For now, the use of enzymes is not very employed in industrial process for material synthesis, but thanks to their many advantages described previously, the use of enzyme tends to grow progressively.

III. Polymers from glycolipids

A lot of reviews have been reported on the synthesis of polymers containing sugar moieties (glycopolymers).^{130–132} Due to their self-assembly properties, their interaction with lectin and their biocompatibility, these polymers are potentially dedicated for nano-biotechnological, biomedical and pharmaceutical applications. Linear glycopolymers are mainly synthesized with pendent sugar moiety. The carbohydrate moieties are attached to functions which can then be polymerized by radical polymerization, ionic polymerization, ring-opening polymerization or ring-opening metathesis polymerization.¹³³

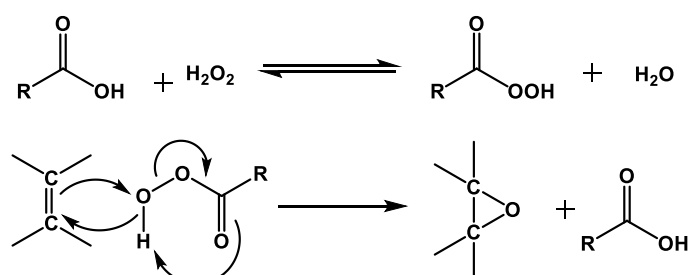
However, to the best of our knowledge, there are only few reports on polymers obtained from glycolipids. This section is dedicated to the 3D networks and linear polymers which have been synthesized from sugar esters.

1. Cross-linked polymers from sugar esters

Thermosets are polymeric materials formed by a cross-linked three-dimensional network. Thermosetting resins are in a liquid or viscous state at the initial step, which becomes solid

and totally insoluble after curing and that cannot be reshaped anymore. Thermosetting resins represent 20 % of the total polymer production.¹³⁴ Major examples are vinyl ester ‘resins’, phenolic and urea formaldehyde resins, polyesters resins, polyurethane resins, epoxy resins. Recently a lot of works have been reported on the synthesis of thermosetting resins from renewable resources.¹³⁵ However, to the best of our knowledge, very few research groups have reported the use of glycolipids and particularly sugar esters for the synthesis of thermosets.

Thermoset resins based on sucrose esters have been developed by the group of Webster.^{136,137} Sucrose esters were epoxidized (Figure 15) by peracetic acid generated *in situ* from the reaction between hydrogen peroxide (H_2O_2) and acetic acid catalysed by an acidic ion-exchange resin (Amberlite IR 120).



Scheme 14: Reactions mechanism of *in situ* epoxidation

The sucrose esters were industrially produced by Procter & Gamble, following a base-catalysed chemical esterification of sucrose with a mixture of fatty acid methyl esters. Indeed, the fatty acid methyl esters used for the transesterification process were not separated after vegetable oil refining. Thus, depending on the composition of the vegetable oil used, the sucrose esters were constituted of different ratios of saturated, mono-unsaturated, bis-unsaturated or tri-unsaturated lipidic chains and different degree of substitution (Figure 15). The sucrose esters are actually a mixture of tetraesters, pentaesters, hexaesters, heptaesters and octaesters.¹³⁶

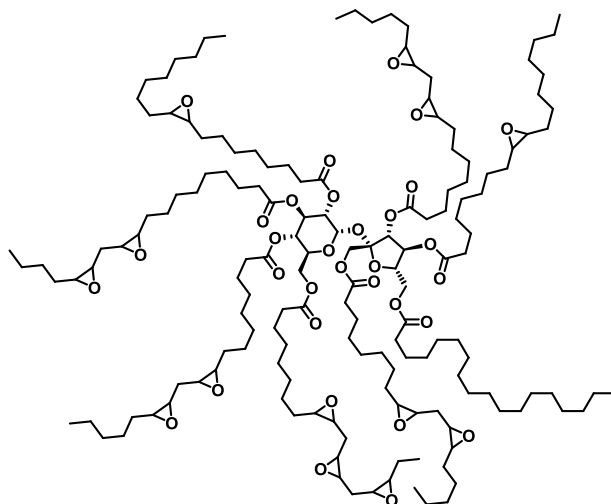
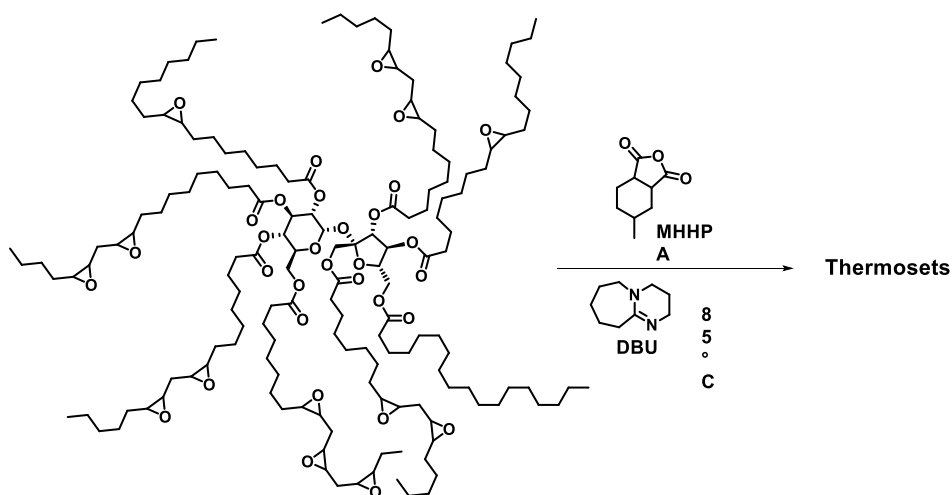


Figure 15: Epoxidized sucrose ester of fatty acids from Webster and coll.¹³⁷

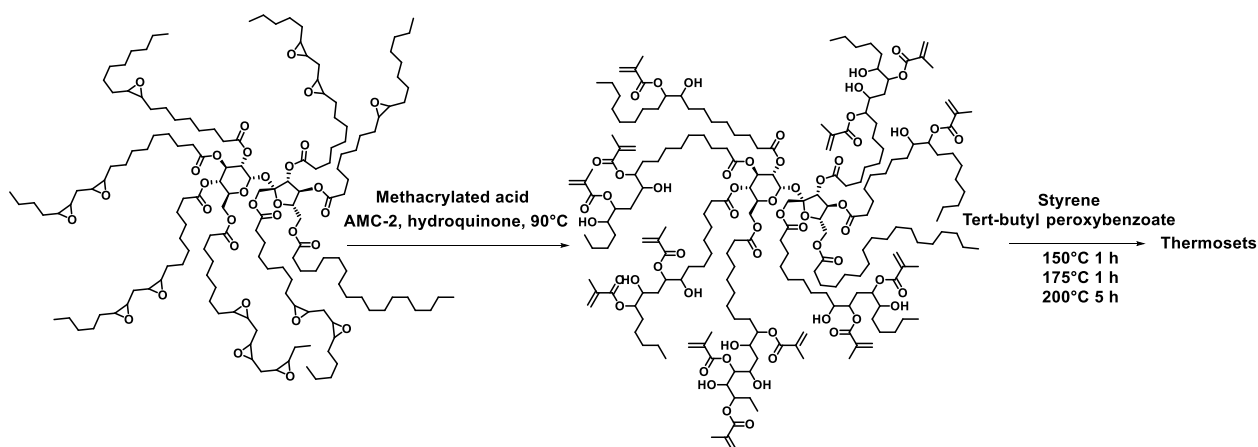
Epoxidized sucrose esters were directly cured with an anhydride, the 4-Methyl-1,2-cyclohexanedicarboxylic anhydride (MHHPA) catalysed by the 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) to get polyester thermosets¹³⁷ (Scheme 15). The cross-linked epoxidized sucrose esters show better performances than cross-linked epoxidized soybean oil. The latter exhibited high modulus (up to 1400 MPa), and were hard and ductile. Such high bio-based content thermosets (71-77 %) with good mechanical performance could be used in a lot of domains such as coatings and composites.



Scheme 15: Synthetic route to polyester thermosets from sucrose esters¹³⁷

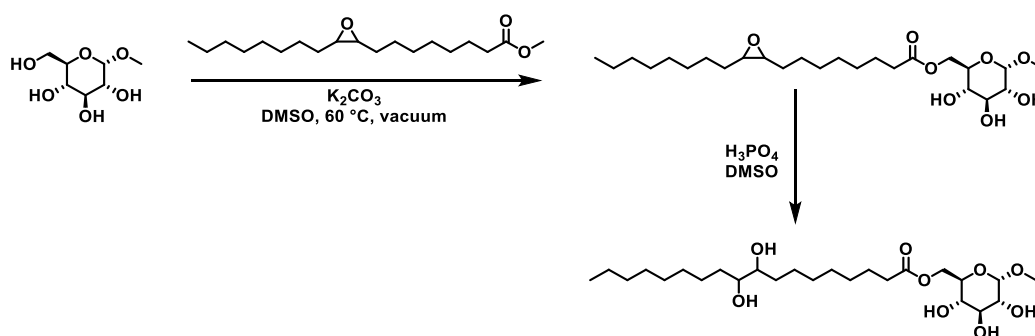
Epoxidized sucrose esters were reacted with methacrylic acid to produce vinyl ester cross-linkers. The vinyl ester resins were then cross-linked by free radical polymerization initiated by tert-butyl peroxybenzoate with or without the use of styrene as commoners¹³⁸ (Scheme 16). Here again, thermosets with high bio-based content (50 – 85 %) showed better thermo

mechanical properties than thermosets made with methacrylated epoxidized soybean oil. The thermosets possessed glass transition temperatures of 63–114°C, tensile strength between 9.1 and 28.3 MPa, modulus of 406 – 1060 MPa and elongation at break between 2.7 and 7.5%.



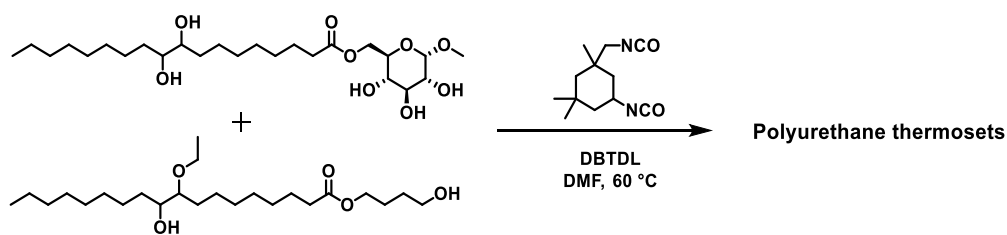
Scheme 16: Synthetic route to vinyl ester thermosets from epoxidized sucrose ester

Polyols were synthesized by our group from methyl α -D-glucoside and epoxidized methyl oleate.¹³⁹ The transesterification of methyl α -D-glucoside was performed in DMSO with potassium carbonate as a base to get the sugar monoester. The epoxide function was hydrolysed under acidic conditions to obtain the glycolipidic polyols (Scheme 17).



Scheme 17: Synthesis of polyols from methyl α -D-glucoside¹³⁹

Polyurethane thermosets were then synthesized from methyl α -D-glucoside monoester and a fatty acid based diol with isophorone diisocyanate as comonomer in DMF in the presence of DBTDL as a catalyst (Scheme 18). Thermosets exhibited glass transition temperature between -8 and 91 °C according to the amount of fatty acid based co-monomer which brings more flexibility to the thermosets.

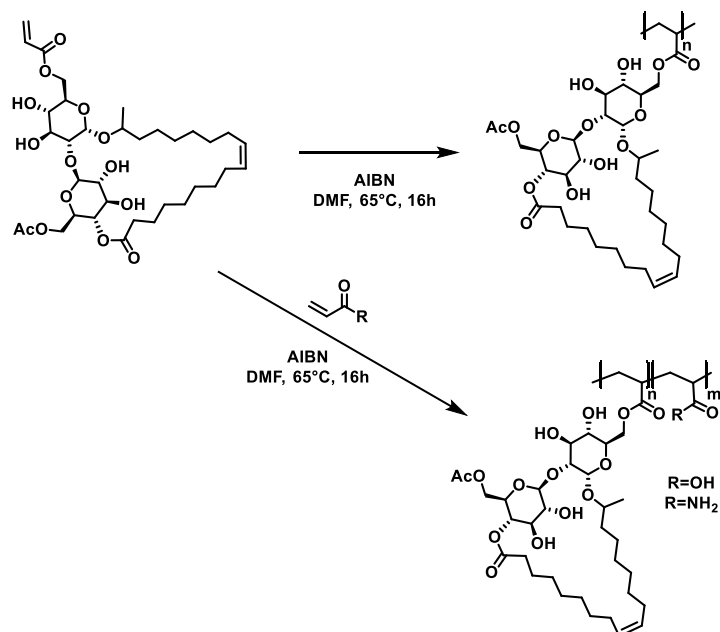
Scheme 18: Synthesis of polyurethane thermostets¹³⁹

2. Linear polymers from sugar ester

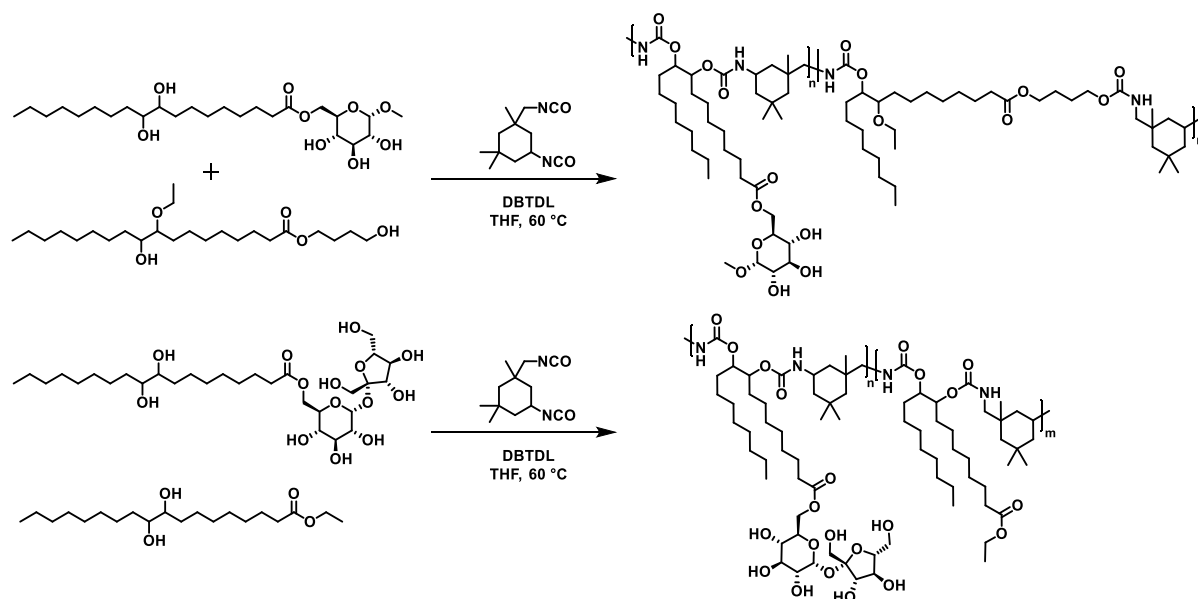
In thermoplastic polymers, the sugar moiety could be as pendant group or within the polymer backbone. Numerous works have been reported on the synthesis of thermoplastic polymers containing sugar moiety.¹⁴⁰ However, to our knowledge, very few research groups have reported the use of sugar esters for the synthesis of thermoplastic polymers.

2.1. Sugar moiety as pendant group

A lactonic sophorolipid was enzymatically modified by Bischt *et al.* to be polymerized.¹⁴¹ The lactonic sophorolipid was selectively acrylated in the 6'-position of the sugar moiety using enzymatic reaction. Free radical polymerization of acrylated lactonic sophorolipid was then performed with AIBN as initiator. To increase the hydrophilic character of the polymers, the sophorolipidic monomer was copolymerized with acrylic acid and acrylamide. A water-soluble polymer was synthesized with acrylamide as commoner.

Scheme 19: Synthesis of homo- and copolymers from acrylated lactonic sophorolipid¹⁴¹

Our group has also synthesized thermoplastic polymers from polyols described previously and another glycolipid synthesized from sucrose.¹³⁹ Thus, methyl α -D-glucoside monoester and sucrose monoester were synthesized by transesterification of sugar moiety with fatty acid methyl ester in DMSO in the presence of potassium carbonate. The epoxide function was hydrolysed under acidic conditions either before or after the transesterification to obtain the glycolipidic polyol. Then, the two glycolipids were polymerized with two different fatty acid based diols and isophorone diisocyanate. Then, the two glycolipids were polymerized with two different fatty acid based diols and isophorone diisocyanate.

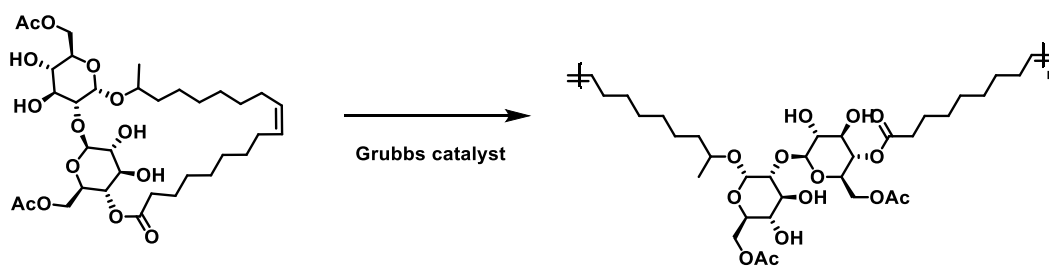


Scheme 20: Synthetic route to linear thermoplastic polyurethane¹³⁹

In this case, the hydroxyl groups of the sugar moiety did not react with the diisocyanate due to self-assembly of the glycolipid in THF.

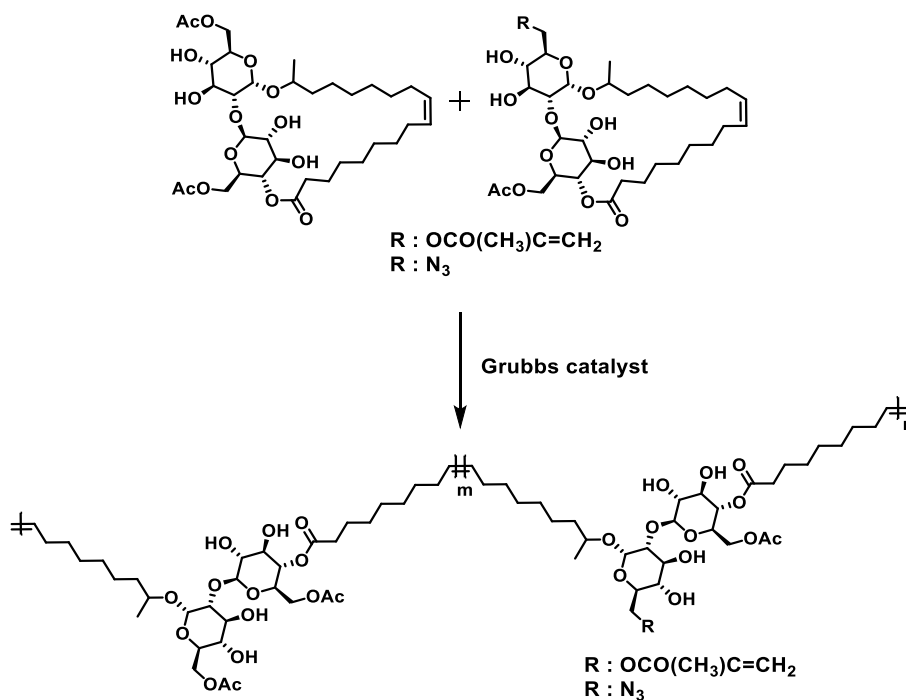
2.2. Polymers containing intra-chain sugar moiety

Park *et al.* published a study on polyester synthesis from trehalose and sucrose diesters.¹⁴² The esterification of carbohydrates was performed with divinyl adipate in acetone in the presence of Novozyme 435 as catalyst. As described previously, the lipase enabled the selective esterification of the primary hydroxyls of the trehalose. With an excess of divinyl adipate, the diester was only obtained using these conditions. The polycondensation was then performed in acetone with different diols. The polymerization was initiated by the addition of Novozyme 435 in the reaction mixture. Polyesters with molar masses between 17000 and 22000 $\text{g}\cdot\text{mol}^{-1}$ were obtained. Degradation of the polymers was performed in aqueous media with Novozyme 435. Thus, after degradation, sucrose, diols and diacids could be recovered. The enzyme can catalyse both the polymerization and the degradation.



Scheme 23: Synthetic route to polyester from lactonic sophorolipid¹⁴⁴⁻¹⁴⁶

In another publication, Gross and colleagues have reported the ROMP of chemo-enzymatically modified lactonic sophorolipids.¹⁴⁷ Sophorolipids were methacrylated and azidated with control of the clickable functional group. Copolymers of acetylated and modified sophorolipids were synthesized by ROMP with Grubbs catalysts. Polymers were then post-modified by click chemistry reactions. Functionalization of methacrylate was performed *via* a thiol-ene reaction with mercaptoethanol and functionalization of the azide was performed *via* azide-alkyne cyclo-addition reaction (Scheme 24). They showed that polymers synthesized from sophorolipids were compatible with human mesenchymal stem cells. Moreover, these polymers were biodegradable and the degradation could be controlled according to the functions linked to the sophorose moiety. Thus, this study reveals polymers from sophorolipids can be employed for biomedical applications.



Scheme 24: Synthesis of copolymers from modified lactonic sophorolipids¹⁴⁷

IV. Conclusion and perspectives

Due to their nontoxicity, biodegradability, biocompatibility and their surfactant and gelation properties, glycolipids have been developed in order to replace petroleum-based surfactants employed in industrial scale. They are already employed in many application fields such as cosmetics, food industry, biomedical application and detergency. However, only few types of glycolipids are industrially employed (alkyl glucoside, fatty acid glucamides and sugar esters) while still having good potential to be more developed further.

Among glycolipids, sugar esters represent the most important family studied in literature. The latter can be naturally produced in high yield by microorganisms, from raw renewable materials. However, synthetic sugar esters were equally chemically synthesized through different esterification pathways. To reach particular properties of natural sugar esters, fatty acids have to be linked on specific hydroxyl groups of the carbohydrates. Thus, protection/deprotection routes were developed to synthesize well-controlled sugar esters. However, to better fit with the green chemistry principles, protective-group free and selective synthetic pathways of sugar esters were elaborated. Another reported green way to synthesize sugar esters with high selectivity is the enzymatic esterification. This reaction can be performed in mild conditions and in non-toxic solvents. For all these reasons, this pathway is not so adapted in industrial processes. Thanks to the huge library of sugars and fatty acids, a large diversity of sugar esters is reported in the literature. According to the HLB and the degree of esterification, versatile surfactant properties can be obtained to target specific properties.

Despite the large number of researches driven on sugar esters, only few works are dedicated to their use as building blocks in polymerization courses. Numerous sugar-containing polymers (glycopolymers), have been described and studied in literature, but the monomers are rarely glycolipids.

In this context, this thesis aimed at developing a range of trehalose-based substrates, to study their self-assembly properties in terms of surfactant and gelation properties and to polymerize them in order to obtain novel linear glycopolymers with self-assembly properties.

In this regard, protective group-free selective chemical esterification and enzymatic-catalyzed esterification were performed and optimized to synthesize trehalose monoesters and trehalose diesters. A range of trehalose esters with different alkyl chain lengths and structures were thus

prepared (Chapter II). The self-assembly properties in water of these trehalose lipids together with their ability to gel water and vegetable oils were then investigated (Chapter III). Finally, some chemical modifications were successfully performed on the trehalose esters in order to be used as monomers for polymerization. Thus, novel thermoplastic polyurethanes, non-isocyanate polyurethanes, polyesters and thiopolyesters were successfully obtained by polycondensation, ADMET and thiol-ene polymerization, respectively (Chapter IV). The structure-properties relationship of these new glycolipid-based polymers was evaluated. As the so-formed polymers contained trehalose moiety in the polymer backbone, interesting crystallization behaviour and self-assembly properties were observed.

V. References

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Chapter 2: Synthesis of glycolipids: diesters and monoesters of trehalose

Key words: Trehalose diester, trehalose monoester, protecting group free esterification, peptide coupling agent, fatty vinyl ester, lipase-catalysed esterification.

Mots clés: Diester de trehalose, monoester de trehalose, estérification sans groupe protecteur, agent de couplage peptidique, ester vinylique gras, estérification catalysé par lipase.

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Introduction

As described in the previous chapter, a lot of sugars and fatty acids can be used as precursors to glycolipids. In this study, the trehalose has been selected as carbohydrate for the synthesis of new glycolipids. Indeed, this sugar, discovered by Wiggers¹ in 1832 and named trehalose by Berthelot² in 1858, is produced by many bacteria, fungi, plants and invertebrates but not by mammals. It is a non-reducing carbohydrate that means it can't be oxidized in water to form species containing aldehyde or ketone groups. Thus, it remains in its cyclic form. Due to the low energy of the glycosidic bond, this sugar is maybe the most stable thermodynamically and kinetically amongst the natural disaccharides.³ Moreover, it is not sensitive to pH like sucrose, it is resistant to temperature and not subject to the Maillard reaction³ and unreactive to peptides and proteins.³ However, it can be cleaved into two molecules of glucose by an enzyme, the trehalase^{4,5} (α,α -trehalose-1-C-glucohydrolase). In terms of applications, it plays a role of cryo-protection of proteins and mammals cells⁶ and is already used in cosmetics as a stabilizer and moisturizer.^{3,7,8} All these properties make trehalose a very attractive sugar for industrial and academic purposes.⁹

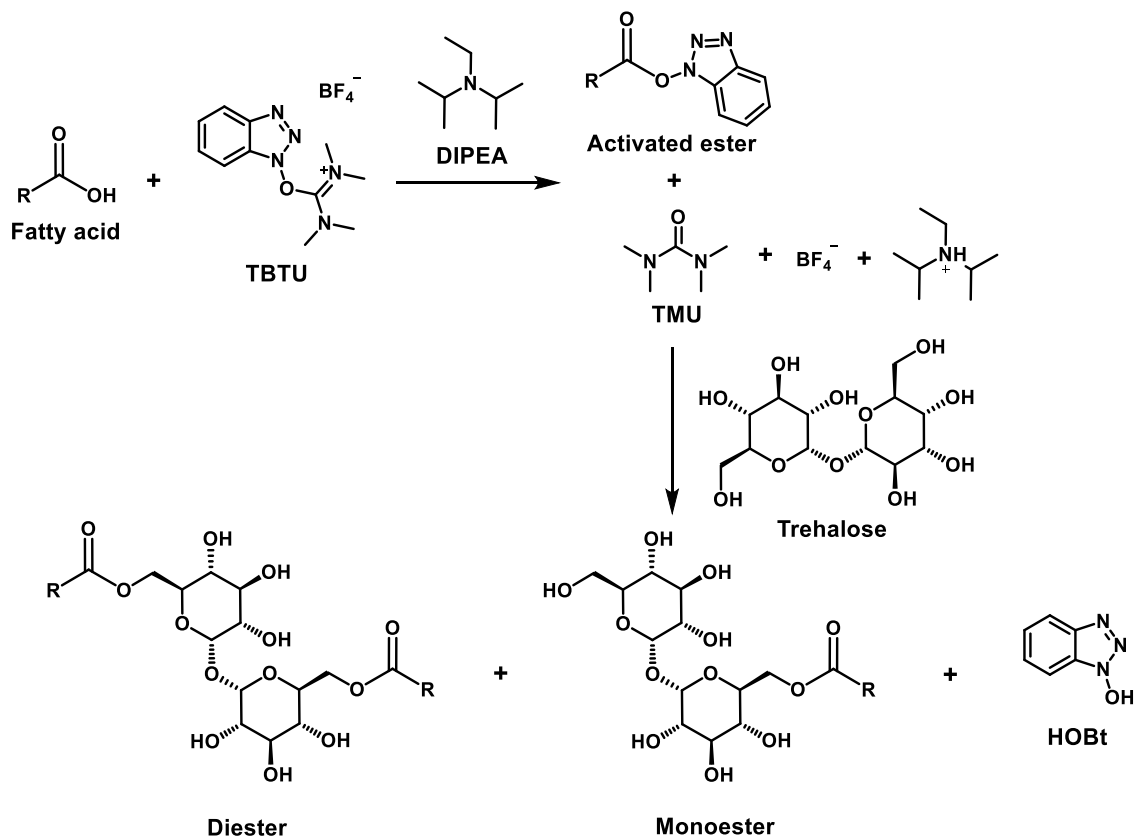
As described in the previous chapter, three main pathways are reported in the literature to synthesize sugar esters. We have chosen to mainly work on the chemical route and particularly the one developed by Grindley¹⁰ to synthesize a platform of trehalose diesters and monoesters. The synthesis and the purification of these compounds are described in the first part. The enzymatic pathway was studied in a second part and appeared to be very promising.

I. Chemical synthesis of glycolipids - monoesters and diesters of trehalose

1. Esterification and purification

The trehalose is a disaccharide having two primary alcohols. To selectively esterify these hydroxyl groups, either the secondary alcohols of the trehalose have to be protected or the esterification process has to be selective to esterify only the primary alcohol. In the first case, largely described in the literature,¹¹⁻¹⁶ steps of protection and deprotection increase the number of reaction steps. In the second case, a selective esterification has been reported by the Grindley's group; it is a two-step one pot selective esterification of the primary alcohols.^{10,17,18} The first step is the formation of an activated ester by the reaction between a

fatty acid and a peptide-coupling agent. Then, this so-formed activated ester reacts preferentially with the primary alcohol of the trehalose to form the trehalose esters.



Scheme 25: Selective esterification of trehalose using peptide coupling agent

The same conditions as the ones reported by Grindley's group¹⁰ were applied with different fatty acids. The synthesis of diesters was the first objective to have difunctional molecules, which could be used as monomers for the synthesis of linear polymers. Grindley's group demonstrated that by adjusting the equivalent number of fatty acids, diesters or monoesters can be selectively obtained with pretty good yields.¹⁰ Thus, with 1.1 equivalents of fatty acid, monoesters are produced in majority and if 2.1 equivalents of fatty acids are employed, diesters are mainly produced.

Undecenoic acid (2.1 eq), TBTU (2.1 eq) and DIPEA (4 eq) were dissolved in dry pyridine. 2.1 equivalents of fatty acids were employed in order to favor the synthesis of diesters. The resulting mixture was stirred at room temperature for 30 min under a nitrogen atmosphere to form the activated ester. A solution of trehalose in dry pyridine was then slowly injected into the first reaction mixture and stirred at room temperature for 72 h. Pyridine was evaporated and the resulting residue was washed with water to remove the by-product of the reaction

(DIPEA, 1-hydroxybenzotriazole (HOBt) and tetramethylurea). After centrifugation, the non-water soluble residue was purified by flash chromatography to isolate trehalose diesters and trehalose monoesters. The synthesis of trehalose diesters and trehalose monoester was confirmed by ^1H NMR spectroscopy (Figure 16). The esterification of the primary alcohol of the trehalose was confirmed by the shift of the peaks of the protons H6 from 3.48 and 3.55 to 4.28 and 4.02 ppm and the protons H5 from 3.65 to 3.89 ppm, respectively. All the peaks are shifted for the diesters and only the half for the monoesters. No shift of the protons H2, H3 and H4 were observed confirming that no esterification occurred on the secondary alcohols.

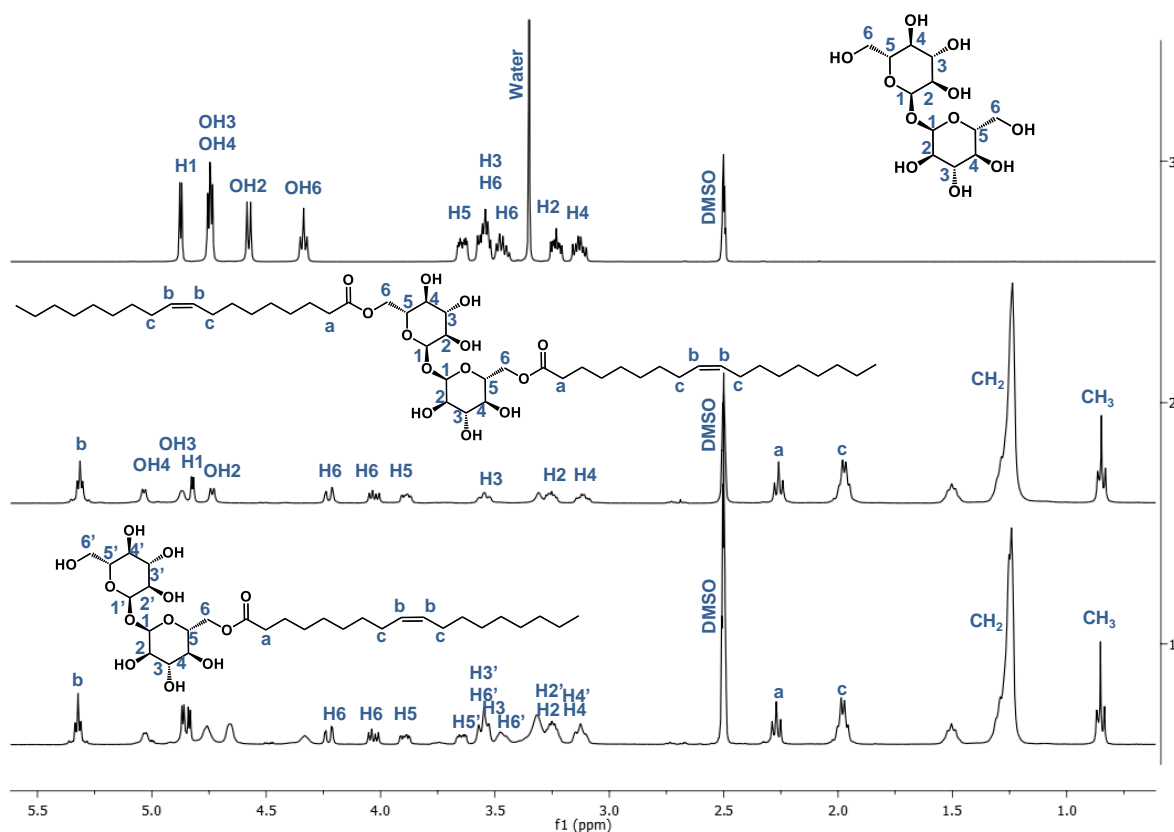


Figure 16: Stacked ^1H NMR spectra in DMSO- d_6 of trehalose (top), trehalose dioleate (middle) and trehalose monooleate (bottom)

Grindley has reported that even with 2.1 equivalent of fatty acid, the corresponding monoester of trehalose is also formed. Based on this procedure, a platform of diesters and monoesters were synthesized using fatty acids with various alkyl chain length (from C11 to C22), different unsaturation state (saturated, one unsaturation and two unsaturation) and several functionality (one hydroxyl group, two hydroxyl groups or none) (Figure 17, Figure 18 and Figures S1-S18).

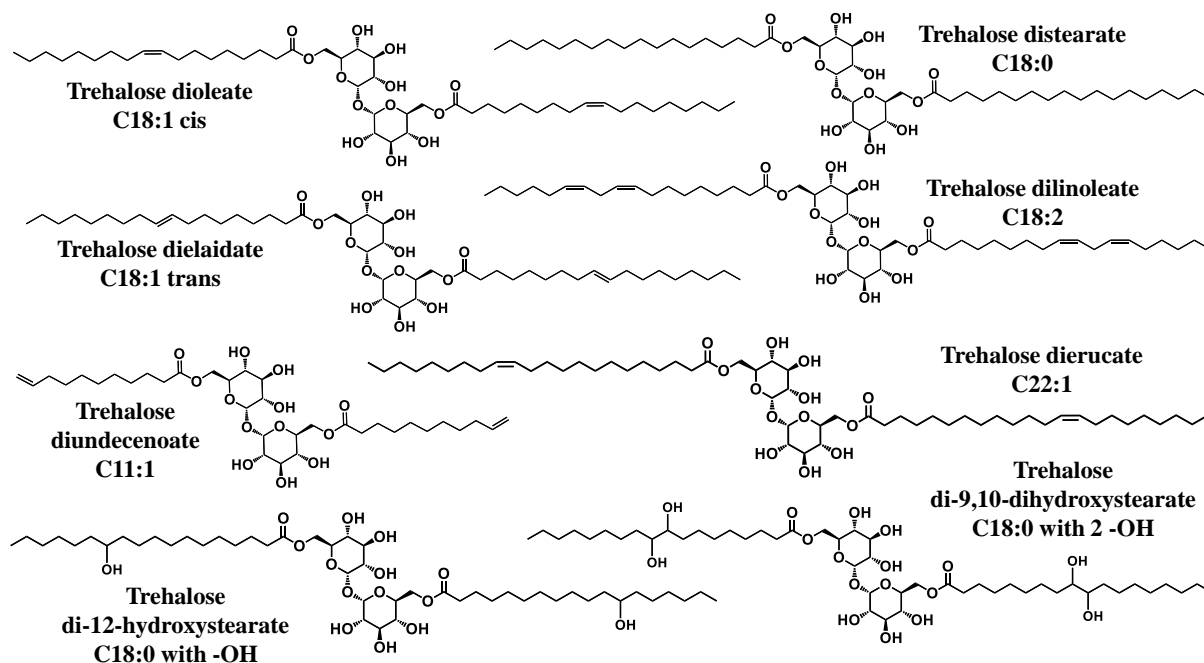


Figure 17: Platform of trehalose diesters synthesized according to Grindley's conditions¹⁰

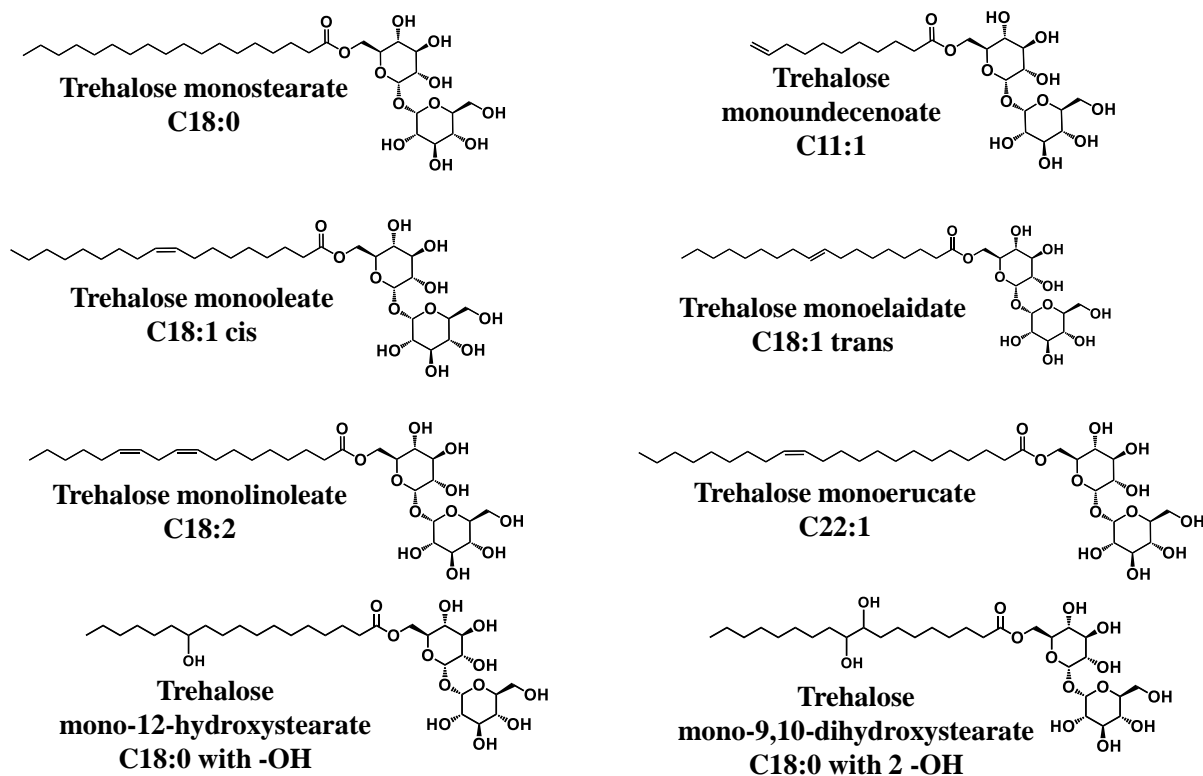


Figure 18 : Platform of trehalose monoesters synthesized according to Grindley's conditions¹⁰

After purification, regardless of the fatty acid used, very low yields were obtained (around 20 %) for all the diesters. Several by-products were also isolated by flash chromatography. A possible explanation could be the solubility of the reactants. This solubility issue is a classical challenge met in the chemical synthesis of glycolipids. Indeed, carbohydrates are hydrophilic molecules soluble in aqueous solutions and fatty acids are hydrophobic molecules soluble in organic solvents. Few solvents are able to solubilize both molecules to obtain a homogeneous reaction medium to manage the esterification reaction.

2. Investigation on the esterification

In order to determine why the diester yields were low in comparison to the ones obtained by Grindley's group (66% yield of trehalose dioleate and 18% yield of trehalose monooleate were obtained with 2.1 equivalent of oleic acid), aliquots were taken during the esterification and analyzed by HPLC in THF. HPLC of the fatty acid, diester and monoester was also performed.

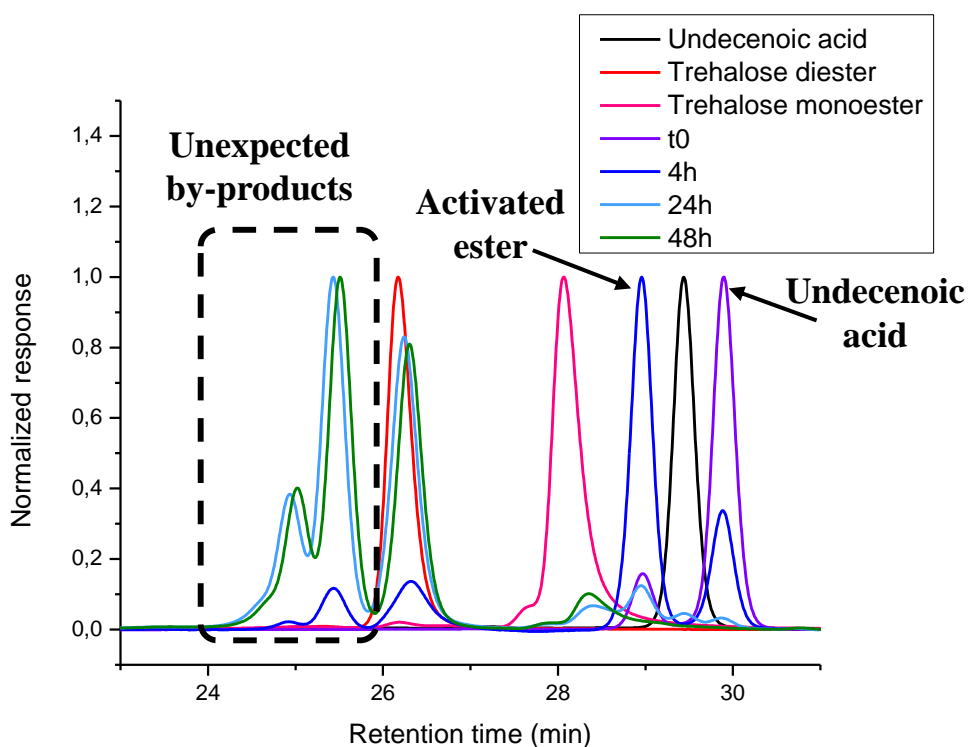


Figure 19 : HPLC in THF of crude mixture of esterification reaction at different times and HPLC of undecenoic acid, trehalose diundecenoate and trehalose monoundecenoate

As can be seen on the HPLC traces, two by-products are present in the reaction medium after 48h (57 wt.%). These by-products are already formed after 4 h reaction. Their formation could explain the low yield obtained in the cases of the diester (35 wt.%) and monoester (8 wt.%) syntheses. The formation of triester of trehalose is reported by Grindley¹⁰ (Scheme 26). In the presence of 3.5 equivalents of fatty acid, Grindley demonstrated that the triester was formed and this could also occur in our case. Thus, these compounds could correspond to triesters and polyesters of trehalose. The separation by flash chromatography of the two species shown in HPLC was not possible. But the mixture could be isolated and analyzed by ¹H NMR spectroscopy (Figure 20). Thus, the ¹H NMR analysis of the two unexpected species could confirm the formation of the “polyesters”.

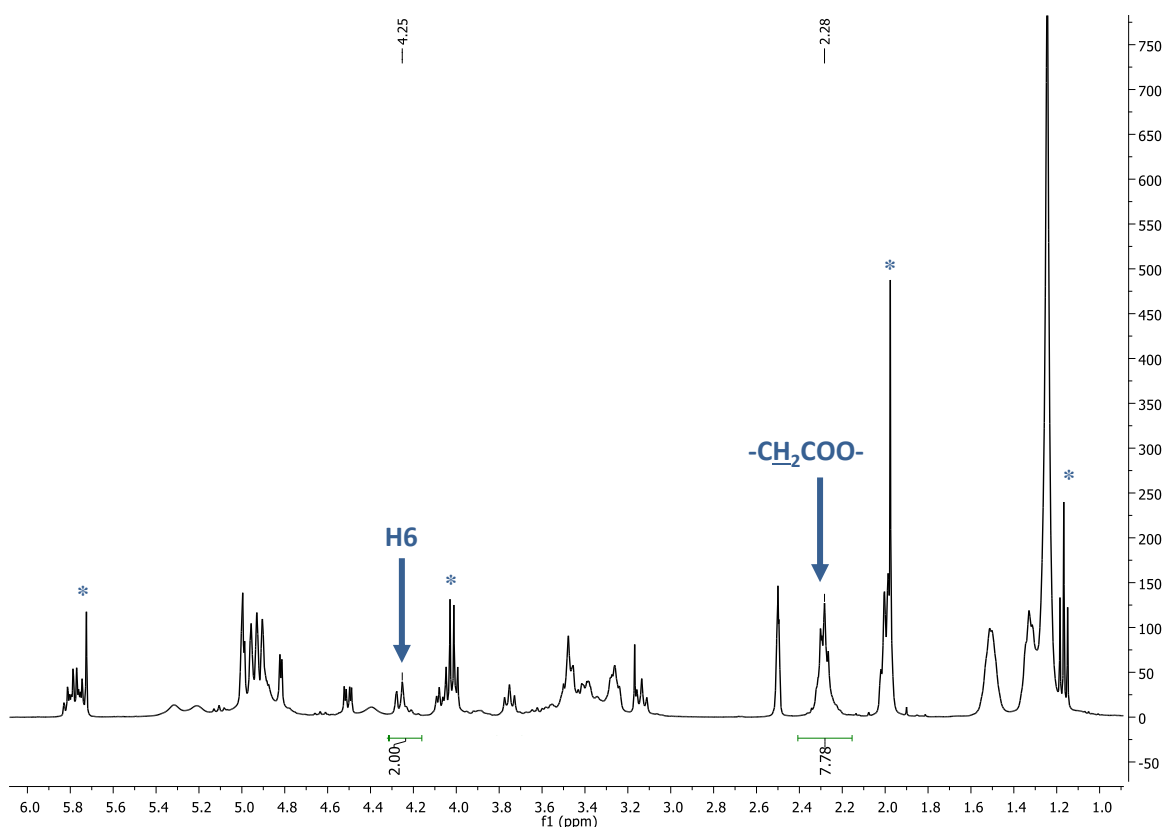
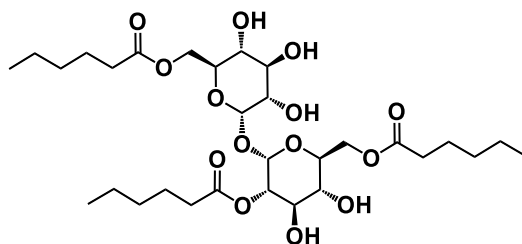


Figure 20: ¹H NMR spectrum in DMSO-d₆ of trehalose of flash chromatography fraction corresponding to trehalose “polyesters”. (*) Impurities.

The proton in 6-position of trehalose (H6) integrates for 2 protons. In order to determine the number of fatty acids esterified on the trehalose, the proton nearby the ester function at 2.28 ppm was integrated. In the case of the monoester with only one fatty acid chain esterified on the trehalose, the integration of this peak at 2.28 ppm is 2. In the case of the “polyester” fraction (Figure 20), the integration is 7.79, which means that this fraction was composed of a mixture of triesters and quadriesters with a degree of substitution of 3.9.

The presence of “polyesters” is certainly due to the low solubility of trehalose in pyridine. Indeed, when the mixture of fatty acid, TBTU and DIPEA is added to the trehalose, partially solubilized in pyridine (it was visible to the naked eye), the equivalent number of activated ester formed during the first reaction step is superior to 2.1 and it could explain why “polyesters” were obtained after few hours of reaction.



Scheme 26: Triester formed using 3.5 eq of hexanoic acid, 3.5 eq of TBTU and 3.5 eq of DIPEA for 1eq of trehalose at room temperature for 48h described by Grindley

3. Optimization of the esterification

We tried to optimize the esterification experimental conditions in order to improve the yield of the diesters and also enhance the selectivity. To avoid the formation of these by-products and to favor the formation of the diesters, the reaction medium was diluted (use of 80 mL of solvent instead of 20 mL) and the addition of the reaction mixture containing fatty acid, TBTU and DIPEA was done drop-wise. These conditions did not allow reducing the amount of by-products formed during the esterification (still 56 wt.% of “polyesters”).

Other solvents were tested to improve the homogeneity of the reaction medium. Thus, Acetonitrile, THF, methyl-THF, DMF, DMSO and a deep eutectic solvent: choline chloride / urea (1 : 2) were used as solvent for the esterification. The conversion was determined by ^1H NMR spectroscopy after 3 days.

Trehalose is poorly soluble in acetonitrile, THF and methyl-THF so the reaction mixtures in these solvents were not homogeneous and the esterification did not occur. Trehalose is soluble in the deep eutectic solvent choline chloride / urea but the fatty acid is not, here again the mixtures were not homogeneous and the esterification did not occur. The reaction medium was homogeneous in DMF and DMSO, it was translucent but, surprisingly, the esterification did not occur.

To understand why the esterification reaction was not occurring in DMSO and DMF, aliquots were taken after the first step of the esterification performed in DMF (formation of the activated ester) and at the end of the esterification before purification. They were analyzed by ^1H NMR spectroscopy (Figure 21).

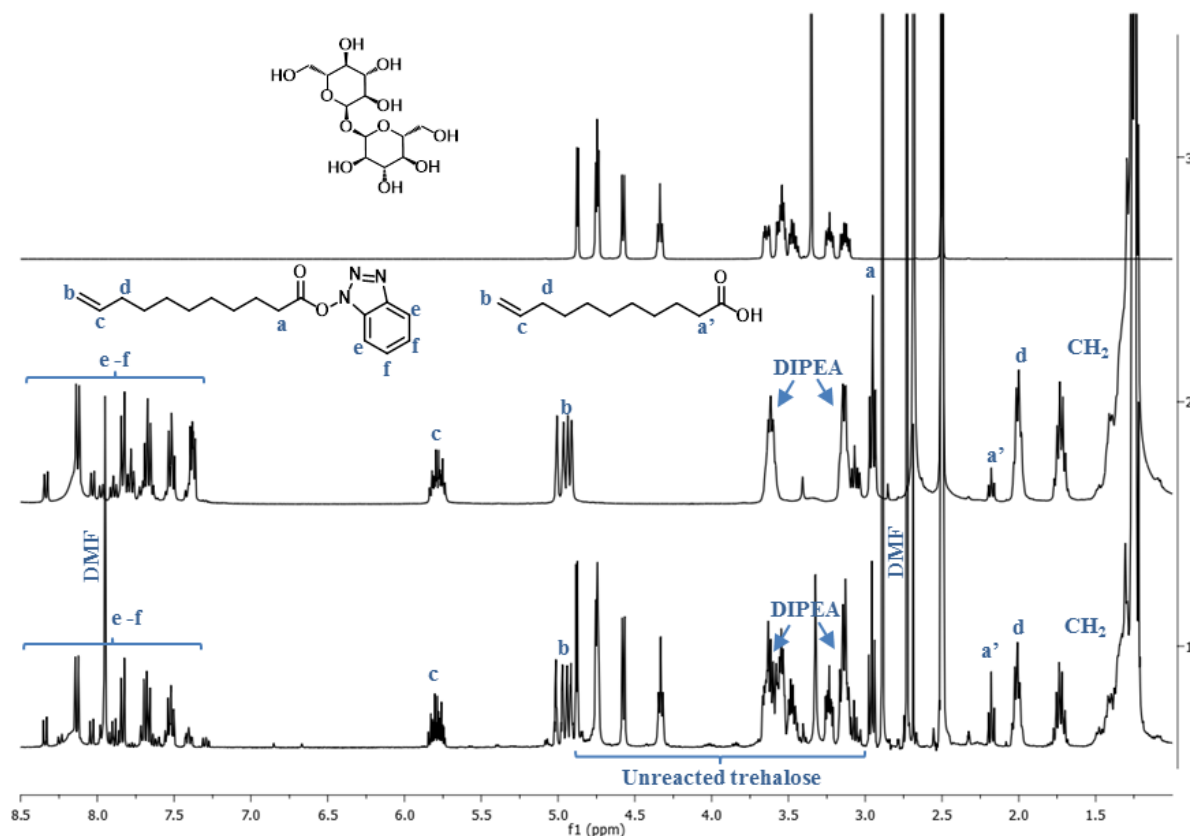


Figure 21 : Stacked ^1H NMR spectra in DMSO- d_6 of trehalose (top), activated ester (middle) and crude reaction mixture performed in DMF (bottom)

On the ^1H NMR spectrum of the crude reaction mixture, the peaks of unreacted trehalose are present but the peak corresponding to the proton nearby the ester function is also present, indicating that the first step occurred but not the second one (transesterification of the activated ester with the trehalose). We thus tried to use a stronger organic base to see if this second step could happen in DMF. The reaction was performed using the same conditions as previously but by replacing DIPEA by DBU. A sample of the crude mixture was analyzed by ^1H NMR (Figure 22).

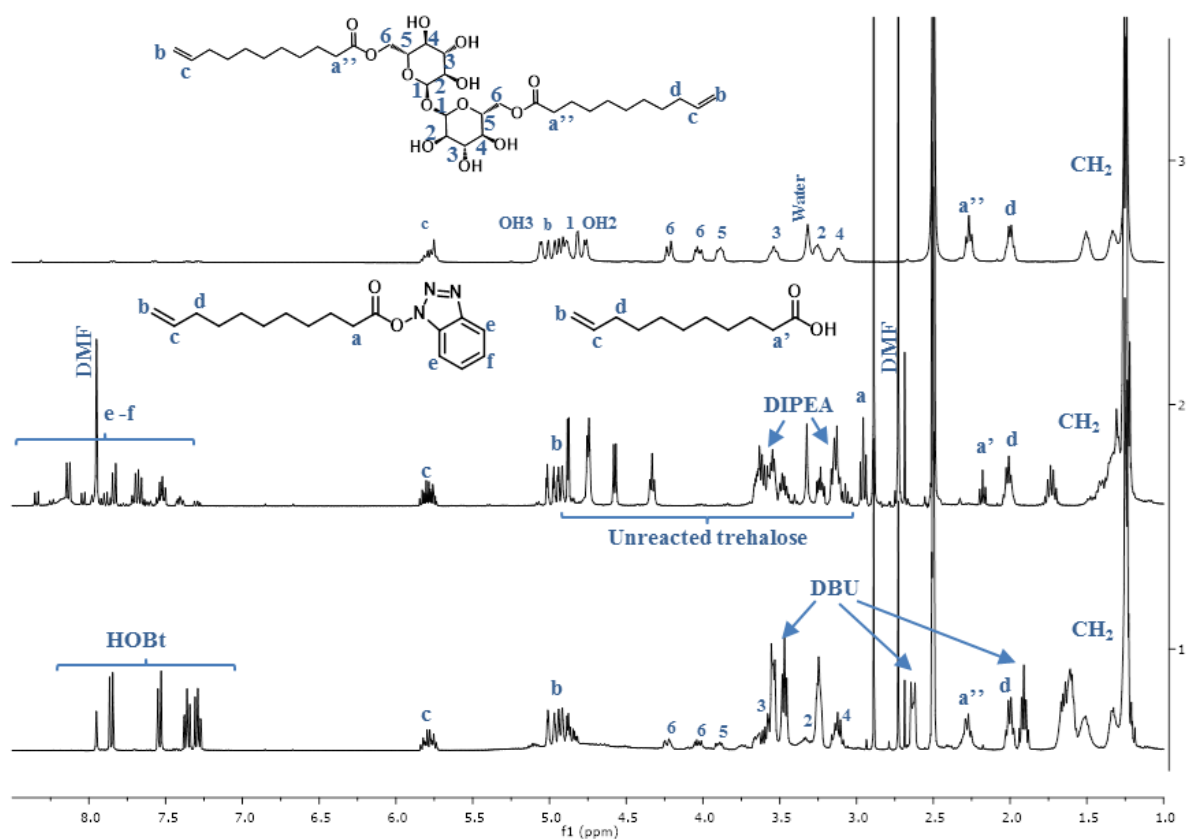


Figure 22 : Stacked ¹H NMR spectra in DMSO-d₆ of trehalose (top), activated ester (middle) and crude reaction mixture performed in DMF (bottom)

By using DBU as a base for the esterification instead of the DIPEA, diesters of trehalose were obtained. It was confirmed by ¹H NMR spectroscopy where peaks corresponding to protons H6 (at 4.24 ppm and 4.02 ppm) and H5 (at 3.89 ppm) of the trehalose diesters appeared. However, the triesters and quadriesters by-products are still present (57 %, determined by HPLC) using these conditions.

Contrary to what is reported in the literature,¹⁰ the trehalose esterification developed by Grindley is not so selective and deserve improvements to increase the diesters and monoesters yield formation in comparison to the triesters and polyesters. Besides, pyridine or DMF are classified CMR (carcinogenic, mutagen or toxic for reproduction) and have to be manipulated with caution and the use of peptide coupling agent produce two by-products, tetramethylurea and 1-hydroxybenzotriazole. For all these reasons, another greener pathway could be considered for the synthesis of such sugar esters. As it was described previously, the enzymatic esterification could be a better pathway to synthesize glycolipids. The enzymatic esterification of trehalose has been thus studied during this thesis and the results are described in the next part.

4. Experimental

General procedure for esterification of trehalose

In an oven-dried round bottomed flask equipped with a magnetic stir bar, fatty acid (2.1 equiv), TBTU (2.1 equiv) and DIPEA (4 equiv) were dissolved in dry pyridine. The resulting mixture was stirred at room temperature for 30 min under a nitrogen atmosphere. A solution of trehalose (1 eq) in dry pyridine was then slowly injected into the reaction mixture and stirred at room temperature for 72 h. Pyridine was evaporated under vacuum. The resulting residue was washed with water to remove the by-product of the reaction (DIPEA and peptide coupling agent) and it was purified by flash chromatography using an elution gradient of 5-25% methanol in EtOAc-DCM (1:1) to give pure trehalose diesters and trehalose monoesters.

Trehalose

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 4.87 (d, 2H, $-\underline{\text{C}}\text{H}-$, H1), 4.75 (m, 4H, $-\underline{\text{O}}\text{H}$, H3 – H4), 4.58 (d, 2H, $-\underline{\text{O}}\text{H}$, H2), 4.34 (d, 2H, $-\underline{\text{O}}\text{H}$, H6), 3.64 (m, 2H, $-\underline{\text{C}}\text{H}-$, H5), 3.53 (d, 4H, $-\underline{\text{C}}\text{H}-$, H6 – H3), 3.48 (m, 2H, $-\underline{\text{C}}\text{H}-$, H6), 3.23 (m, 2H, $-\underline{\text{C}}\text{H}-$, H2), 3.14 (m, 2H, $-\underline{\text{C}}\text{H}-$, H4).

Trehalose dioleate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.31 (m, 4H, $-\underline{\text{C}}\text{H}=\text{CH}-$), 5.04 (m, 2H, $-\underline{\text{O}}\text{H}$, H4), 4.87 (d, 2H, $-\underline{\text{O}}\text{H}$, H3), 4.83 (d, 2H, $-\underline{\text{C}}\text{H}-$, H1), 4.74 (d, 2H, $-\underline{\text{O}}\text{H}$, H2), , 4.24 (d, 2H, $-\underline{\text{C}}\text{H}-$, H6), 4.03 (m, 2H, $-\underline{\text{C}}\text{H}-$, H6), 3.88 (m, 2H, $-\underline{\text{C}}\text{H}-$, H5), 3.54 (m, 2H, $-\underline{\text{C}}\text{H}-$, H3), 3.25 (m, 2H, $-\underline{\text{C}}\text{H}-$, H2), 3.12 (m, 2H, $-\underline{\text{C}}\text{H}-$, H4), 2.26 (t, 4H, $-\underline{\text{C}}\text{H}_2\text{-COO-CH}_3$), 1.98 (m, 4H, $-\text{CH}=\underline{\text{C}}\text{H}_2-$), 1.50-1.24 (m, 44H, aliphatic $-\underline{\text{C}}\text{H}_2-$), 0.85 (t, 6H, aliphatic $-\underline{\text{C}}\text{H}_3$). $T_m = 135^\circ\text{C}$.

Trehalose monooleate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.32 (m, 2H, $-\underline{\text{C}}\text{H}=\text{CH}-$), 5.04 (d, 2H, $-\underline{\text{O}}\text{H}$, H4), 4.88 (d, 1H, $-\underline{\text{C}}\text{H}-$, H1), 4.86 (d, 1H, $-\underline{\text{O}}\text{H}$, H3), 4.82 (d, 1H, $-\underline{\text{C}}\text{H}-$, H1), 4.76 (d, 1H, $-\underline{\text{O}}\text{H}$, H2), 4.76 (m, 2H, $-\text{OH}$, H3 – H4), 4.66 (d, 1H, $-\text{OH}$, H2), 4.33 (d, 1H, $-\text{OH}$, H6'), 4.24 (d, 1H, $-\underline{\text{C}}\text{H}-$, H6), 4.04 (m, 1H, $-\underline{\text{C}}\text{H}-$, H6), 3.90 (m, 1H, $-\underline{\text{C}}\text{H}-$, H5), 3.65 (m, 1H, $-\underline{\text{C}}\text{H}-$, H5'), 3.55 (m, 3H, $-\underline{\text{C}}\text{H}-$, H6 – H3), 3.48 (m, 1H, $-\underline{\text{C}}\text{H}-$, H6'), 3.26 (m, 2H, $-\underline{\text{C}}\text{H}-$, H2), 3.13 (m, 2H, $-\underline{\text{C}}\text{H}-$, H4), 2.27 (t, 2H, $-\underline{\text{C}}\text{H}_2\text{-COO-}$), 1.99 (m, 2H, $-\underline{\text{C}}\text{H}_2\text{-CH}=\text{CH}-$), 1.50-1.24 (m, 22H, aliphatic $-\underline{\text{C}}\text{H}_2-$), 0.85 (t, 3H, aliphatic $-\underline{\text{C}}\text{H}_3$).

Trehalose distearate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.05 (m, 2H, -OH, H4), 4.90 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.75 (d, 2H, -OH, H2), , 4.21 (d, 2H, -CH-, H6), 4.03 (m, 2H, -CH-, H6), 3.89 (m, 2H, -CH-, H5), 3.55 (m, 2H, -CH-, H3), 3.25 (m, 2H, -CH-, H2), 3.12 (m, 2H, -CH-, H4), 2.26 (t, 4H, -CH₂-COO-CH₃), 1.50-1.23 (m, 60H, aliphatic -CH₂-), 0.85 (t, 6H, aliphatic -CH₃).

Trehalose monostearate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.04 (d, 2H, -OH, H4), 4.88 (d, 1H, -CH-, H1), 4.87 (d, 1H, -OH, H3), 4.84 (d, 1H, -CH-, H1), 4.76 (d, 1H, -OH, H2), 4.76 (m, 2H, -OH, H3 - H4), 4.66 (d, 1H, -OH, H2), 4.34 (d, 1H, -OH, H6'), 4.24 (d, 1H, -CH-, H6), 4.04 (m, 1H, -CH-, H6), 3.89 (m, 1H, -CH-, H5), 3.65 (m, 1H, -CH-, H5'), 3.55 (m, 2H, -CH-, H6' - H3), 3.47 (m, 1H, -CH-, H6'), 3.25 (m, 2H, -CH-, H2), 3.12 (m, 2H, -CH-, H4), 2.27 (t, 2H, -CH₂-COO-), 1.50-1.24 (m, 30H, aliphatic -CH₂-), 0.85 (t, 3H, aliphatic -CH₃). T_m = 148 °C.

Trehalose diundecenoate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.78 (m, 2H, -CH=CH₂), 5.05 (d, 2H, -OH, H4), 4.97 (m, 4H, CH₂=CH-), 4.87 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.74 (d, 2H, -OH, H2), 4.24 (d, 2H, -CH-, H6), 4.02 (m, 2H, -CH-, H6), 3.89 (m, 2H, -CH-, H5), 3.54 (m, 2H, -CH-, H3), 3.28 (m, 2H, -CH-, H2), 3.13 (m, 2H, -CH-, H4), 2.27 (t, 4H, -CH₂-COO-), 2.01 (m, 4H, -CH₂-CH=CH-), 1.51-1.25 (m, 28H, aliphatic -CH₂-).

Trehalose monoundecenoate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.78 (m, 1H, -CH=CH₂), 5.04 (d, 2H, -OH, H4), 4.97 (m, 2H, CH₂=CH-), 4.88 (d, 1H, -CH-, H1), 4.87 (d, 1H, -OH, H3), 4.83 (d, 1H, -CH-, H1), 4.76 (d, 1H, -OH, H2), 4.76 (m, 2H, -OH, H3 - H4), 4.68 (d, 1H, -OH, H2), 4.34 (d, 1H, -OH, H6'), 4.24 (d, 1H, -CH-, H6), 4.04 (m, 1H, -CH-, H6), 3.88 (m, 1H, -CH-, H5), 3.65 (m, 1H, -CH-, H5'), 3.55 (m, 2H, -CH-, H3 - H6'), 3.46 (m, 1H, -CH-, H6'), 3.25 (m, 2H, -CH-, H2), 3.13 (m, 2H, -CH-, H4), 2.27 (t, 2H, -CH₂-COO-), 2.01 (m, 2H, -CH₂-CH=CH-), 1.51-1.25 (m, 14H, aliphatic -CH₂-). T_m = 152 °C.

Trehalose dierucate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.32 (m, 4H, -CH=CH-), 5.04 (m, 2H, -OH, H4), 4.88 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.75 (d, 2H, -OH, H2), , 4.25 (d, 2H, -CH-,

H6), 4.02 (m, 2H, -CH-, H6), 3.87 (m, 2H, -CH-, H5), 3.55 (m, 2H, -CH-, H3), 3.25 (m, 2H, -CH-, H2), 3.11 (m, 2H, -CH-, H4), 2.26 (t, 4H, -CH₂-COO-CH₃), 1.98 (m, 8H, -CH=CH₂-), 1.50-1.24 (m, 60H, aliphatic -CH₂-), 0.85 (t, 6H, aliphatic -CH₃). T_m = 151 °C.

Trehalose monoerucate

¹H NMR (DMSO-d₆, 400MHz, δ (ppm)): 5.32 (m, 2H, -CH=CH-), 5.04 (d, 2H, -OH, H4), 4.86 (d, 1H, -CH-, H1), 4.86 (d, 1H, -OH, H3), 4.82 (d, 1H, -CH-, H1), 4.77 (d, 1H, -OH, H2), 4.77 (m, 2H, -OH, H3 – H4), 4.67 (d, 1H, -OH, H2), 4.34 (d, 1H, -OH, H6'), 4.24 (d, 1H, -CH-, H6), 4.04 (m, 1H, -CH-, H6), 3.91 (m, 1H, -CH-, H5), 3.65 (m, 1H, -CH-, H5'), 3.55 (m, 2H, -CH-, H3 - H6'), 3.47 (m, 1H, -CH-, H6'), 3.26 (m, 2H, -CH-, H2), 3.12 (m, 2H, -CH-, H4), 2.27 (t, 2H, -CH₂-COO-), 1.99 (m, 4H, -CH₂-CH=CH-), 1.50-1.24 (m, 30H, aliphatic -CH₂-), 0.85 (t, 3H, aliphatic -CH₃).

Trehalose dilinoleate

¹H NMR (DMSO-d₆, 400MHz, δ (ppm)): 5.32 (m, 8H, -CH=CH-), 5.03 (m, 2H, -OH, H4), 4.87 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.73 (d, 2H, -OH, H2), , 4.24 (d, 2H, -CH-, H6), 4.04 (m, 2H, -CH-, H6), 3.90 (m, 2H, -CH-, H5), 3.55 (m, 2H, -CH-, H3), 3.26 (m, 2H, -CH-, H2), 3.11 (m, 2H, -CH-, H4), 2.73 (t, 4H, -CH=CH-CH₂-CH=CH-), 2.26 (t, 4H, -CH₂-COO-CH₃), 2.02 (m, 8H, -CH=CH₂-), 1.50-1.25 (m, 32H, aliphatic -CH₂-), 0.86 (t, 6H, aliphatic -CH₃). T_m = 138 °C.

Trehalose monolinoleate

¹H NMR (DMSO-d₆, 400MHz, δ (ppm)): 5.32 (m, 4H, -CH=CH-), 5.03 (d, 2H, -OH, H4), 4.87 (d, 1H, -CH-, H1), 4.86 (d, 1H, -OH, H3), 4.82 (d, 1H, -CH-, H1), 4.76 (d, 1H, -OH, H2), 4.76 (m, 2H, -OH, H3 – H4), 4.65 (d, 1H, -OH, H2), 4.33 (d, 1H, -OH, H6'), 4.24 (d, 1H, -CH-, H6), 4.04 (m, 1H, -CH-, H6), 3.89 (m, 1H, -CH-, H5), 3.66 (m, 1H, -CH-, H5'), 3.55 (m, 2H, -CH-, H3 - H6'), 3.48 (m, 1H, -CH-, H6'), 3.25 (m, 2H, -CH-, H2), 3.13 (m, 2H, -CH-, H4), 2.73 (t, 2H, -CH=CH-CH₂-CH=CH-), 2.27 (t, 2H, -CH₂-COO-), 1.99 (m, 2H, -CH₂-CH=CH-), 1.50-1.24 (m, 16H, aliphatic -CH₂-), 0.86 (t, 3H, aliphatic -CH₃).

Trehalose dielaidate

¹H NMR (DMSO-d₆, 400MHz, δ (ppm)): 5.35 (m, 4H, -CH=CH-), 5.04 (m, 2H, -OH, H4), 4.88 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.75 (d, 2H, -OH, H2), , 4.24 (d, 2H, -CH-, H6), 4.03 (m, 2H, -CH-, H6), 3.89 (m, 2H, -CH-, H5), 3.54 (m, 2H, -CH-, H3), 3.27 (m, 2H, -

$\underline{\text{CH}}_2$ -, H2), 3.11 (m, 2H, $-\underline{\text{CH}}_2$ -, H4), 2.26 (t, 4H, $-\underline{\text{CH}}_2\text{-COO-CH}_3$), 1.93 (m, 4H, $-\text{CH}=\underline{\text{CH}}_2$ -), 1.50-1.24 (m, 44H, aliphatic $-\underline{\text{CH}}_2$ -), 0.85 (t, 6H, aliphatic $-\underline{\text{CH}}_3$). Tm = 148 °C.

Trehalose monoelaidate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.36 (m, 2H, $-\underline{\text{CH}}=\text{CH-}$), 5.03 (d, 2H, $-\underline{\text{OH}}$, H4), 4.9 (d, 1H, $-\underline{\text{CH}}_2$ -, H1), 4.86 (d, 1H, $-\underline{\text{OH}}$, H3), 4.82 (d, 1H, $-\underline{\text{CH}}_2$ -, H1), 4.76 (d, 1H, $-\underline{\text{OH}}$, H2), 4.76 (m, 2H, $-\underline{\text{OH}}$, H3 – H4), 4.66 (d, 1H, $-\underline{\text{OH}}$, H2), 4.33 (d, 1H, $-\underline{\text{OH}}$, H6'), 4.24 (d, 1H, $-\underline{\text{CH}}_2$ -, H6), 4.04 (m, 1H, $-\underline{\text{CH}}_2$ -, H6), 3.90 (m, 1H, $-\underline{\text{CH}}_2$ -, H5), 3.65 (m, 1H, $-\underline{\text{CH}}_2$ -, H5'), 3.55 (m, 2H, $-\underline{\text{CH}}_2$ -, H3 - H6'), 3.48 (m, 1H, $-\underline{\text{CH}}_2$ -, H6'), 3.26 (m, 2H, $-\underline{\text{CH}}_2$ -, H2), 3.13 (m, 2H, $-\underline{\text{CH}}_2$ -, H4), 2.27 (t, 2H, $-\underline{\text{CH}}_2\text{-COO-}$), 1.99 (m, 2H, $-\underline{\text{CH}}_2\text{-CH}=\text{CH-}$), 1.50-1.24 (m, 22H, aliphatic $-\underline{\text{CH}}_2$ -), 0.85 (t, 3H, aliphatic $-\underline{\text{CH}}_3$).

Trehalose Di-12-hydroxystearate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.05 (d, 2H, $-\underline{\text{OH}}$, H4), 4.87 (d, 2H, $-\underline{\text{OH}}$, H3), 4.82 (d, 2H, $-\underline{\text{CH}}_2$ -, H1), 4.76 (d, 2H, $-\underline{\text{OH}}$, H2), , 4.21 (d, 2H, $-\underline{\text{CH}}_2$ -, H6), 4.17 (m, 2H, $-\text{CH-}\underline{\text{OH}}$), 4.02 (m, 2H, $-\underline{\text{CH}}_2$ -, H6), 3.88 (m, 2H, $-\underline{\text{CH}}_2$ -, H5), 3.55 (m, 2H, $-\underline{\text{CH}}_2$ -, H3), 3.30 (m, 2H, $-\underline{\text{CH}}_2\text{-OH}$), 3.25 (m, 2H, $-\underline{\text{CH}}_2$ -, H2), 3.12 (m, 2H, $-\underline{\text{CH}}_2$ -, H4), 2.27 (t, 4H, $-\underline{\text{CH}}_2\text{-COO-CH}_3$), 1.50-1.23 (m, 56H, aliphatic $-\underline{\text{CH}}_2$ -), 0.85 (t, 6H, aliphatic $-\underline{\text{CH}}_3$). Tm = 118 °C.

Trehalose Mono-12-hydroxystearate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.03 (d, 2H, $-\underline{\text{OH}}$, H4), 4.87 (d, 1H, $-\underline{\text{CH}}_2$ -, H1), 4.87 (d, 1H, $-\underline{\text{OH}}$, H3), 4.84 (d, 1H, $-\underline{\text{CH}}_2$ -, H1), 4.76 (d, 1H, $-\underline{\text{OH}}$, H2), 4.76 (m, 2H, $-\underline{\text{OH}}$, H3 – H4), 4.67 (d, 1H, $-\underline{\text{OH}}$, H2), 4.34 (d, 1H, $-\underline{\text{OH}}$, H6'), 4.25 (d, 1H, $-\underline{\text{CH}}_2$ -, H6), 4.17 (m, 1H, $-\text{CH-}\underline{\text{OH}}$), 4.04 (m, 1H, $-\underline{\text{CH}}_2$ -, H6), 3.89 (m, 1H, $-\underline{\text{CH}}_2$ -, H5), 3.65 (m, 1H, $-\underline{\text{CH}}_2$ -, H5'), 3.55 (m, 2H, $-\underline{\text{CH}}_2$ -, H3 - H6'), 3.47 (m, 1H, $-\underline{\text{CH}}_2$ -, H6'), 3.30 (m, 1H, $-\underline{\text{CH}}_2\text{-OH}$), 3.27 (m, 2H, $-\underline{\text{CH}}_2$ -, H2), 3.12 (m, 2H, $-\underline{\text{CH}}_2$ -, H4), 2.27 (t, 2H, $-\underline{\text{CH}}_2\text{-COO-}$), 1.51-1.24 (m, 28H, aliphatic $-\underline{\text{CH}}_2$ -), 0.85 (t, 3H, aliphatic $-\underline{\text{CH}}_3$).

Trehalose Di-9,10-dihydroxystearate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.04 (d, 2H, $-\underline{\text{OH}}$, H4), 4.87 (d, 2H, $-\underline{\text{OH}}$, H3), 4.83 (d, 2H, $-\underline{\text{CH}}_2$ -, H1), 4.76 (d, 2H, $-\underline{\text{OH}}$, H2), , 4.24 (d, 2H, $-\underline{\text{CH}}_2$ -, H6), 4.17 (m, 4H, $-\text{CH-}\underline{\text{OH}}$), 4.11 (m, 4H, $-\text{CH-}\underline{\text{OH}}$), 4.04 (m, 2H, $-\underline{\text{CH}}_2$ -, H6), 3.89 (m, 2H, $-\underline{\text{CH}}_2$ -, H5), 3.55 (m, 2H, $-\underline{\text{CH}}_2$ -, H3), 3.30 (m, 2H, $-\underline{\text{CH}}_2\text{-OH}$), 3.25 (m, 2H, $-\underline{\text{CH}}_2$ -, H2), 3.20 (m, 4H, $-\underline{\text{CH}}_2\text{-OH}$), 3.12 (m, 2H, $-\underline{\text{CH}}_2$ -, H4), 2.27 (t, 4H, $-\underline{\text{CH}}_2\text{-COO-CH}_3$), 1.51-1.23 (m, 52H, aliphatic $-\underline{\text{CH}}_2$ -), 0.85 (t, 6H, aliphatic $-\underline{\text{CH}}_3$).

Trehalose Mono-9,10-dihydroxystearate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.04 (d, 2H, $-\text{OH}$, H4), 4.9 (d, 1H, $-\text{CH}$, H1), 4.87 (d, 1H, $-\text{OH}$, H3), 4.84 (d, 1H, $-\text{CH}$, H1), 4.76 (d, 1H, $-\text{OH}$, H2), 4.76 (m, 2H, $-\text{OH}$, H3 – H4), 4.66 (d, 1H, $-\text{OH}$, H2), 4.34 (d, 1H, $-\text{OH}$, H6'), 4.24 (d, 1H, $-\text{CH}$, H6), 4.17 (m, 2H, $-\text{CH-OH}$), 4.04 (m, 1H, $-\text{CH}$, H6), 3.89 (m, 1H, $-\text{CH}$, H5), 3.65 (m, 1H, $-\text{CH}$, H5'), 3.55 (m, 2H, $-\text{CH}$, H3 - H6'), 3.47 (m, 1H, $-\text{CH}$, H6'), 3.25 (m, 2H, $-\text{CH}$, H2), 3.20 (m, 2H, $-\text{CH-OH}$), 3.12 (m, 2H, $-\text{CH}$, H4), 2.27 (t, 2H, $-\text{CH}_2\text{-COO-}$), 1.50-1.24 (m, 26H, aliphatic $-\text{CH}_2-$), 0.85 (t, 3H, aliphatic $-\text{CH}_3$).

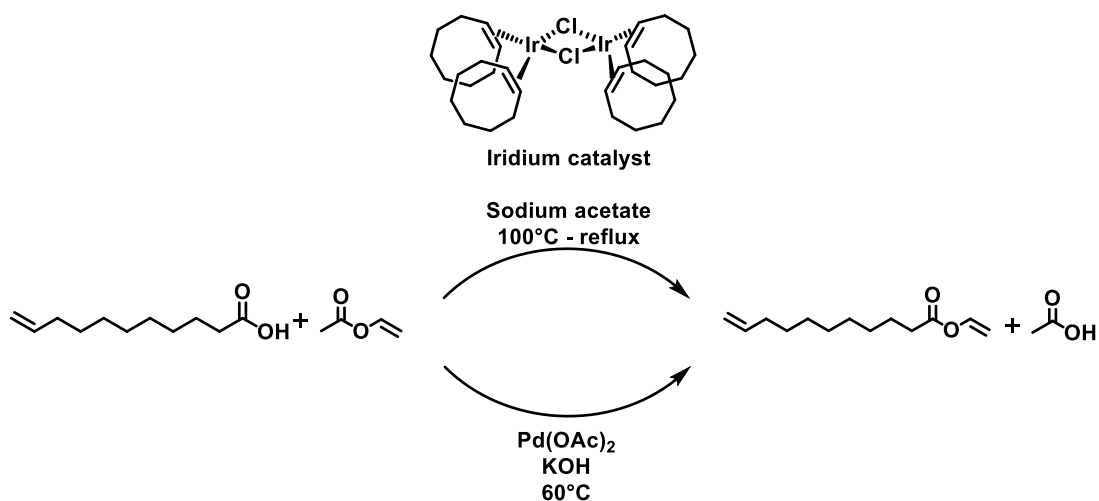
II. Enzymatic synthesis

Enzymes are proteins which could catalyse some specific chemical reactions. The lipase B *Candida antarctica* immobilized on acrylic resin (Novozyme 435) is an enzyme known to catalyse esterification or transesterification reactions. The use of such enzyme for sugar esterification is widely reported in the literature.^{19–22} Several groups have used this enzyme to synthesize trehalose esters and diesters.^{23–27} Another possibility is to use vinyl esters for the transesterification of carbohydrates.²³ Indeed, during the reaction with vinyl esters, acetaldehyde is produced and is readily removed from the reaction medium as it is a gas at room temperature. Vinyl esters have been synthesized from different fatty acids, and then enzymatic esterification of trehalose was performed to synthesize trehalose esters.

1. Vinyl esters synthesis

Different catalysts are used in the literature to synthesize vinyl esters from vinyl acetate and fatty acids.^{28–32} Two routes using two different catalysts were tried. First iridium-based catalyst was used for this transvinylation reaction between fatty acids and vinyl acetate.

In this part, an iridium- or palladium-based were compared for the transvinylation of undecenoic acid with vinyl acetate. Undecenoic acid was dissolved in vinyl acetate, then, either sodium acetate and chlorobis(cyclooctene)iridium(I)dimer (iridium catalyst) or potassium hydroxide and palladium acetate were added and the reaction mixture was stirred under nitrogen atmosphere for 24 h (Scheme 27). Vinyl esters were obtained after purification by flash chromatography.



Scheme 27 : Transvinylation of undecenoic acid employing an iridium-based catalyst (top) or palladium acetate as catalyst (bottom)

Samples were analysed by ^1H NMR spectroscopy to confirm the formation of the vinyl esters.

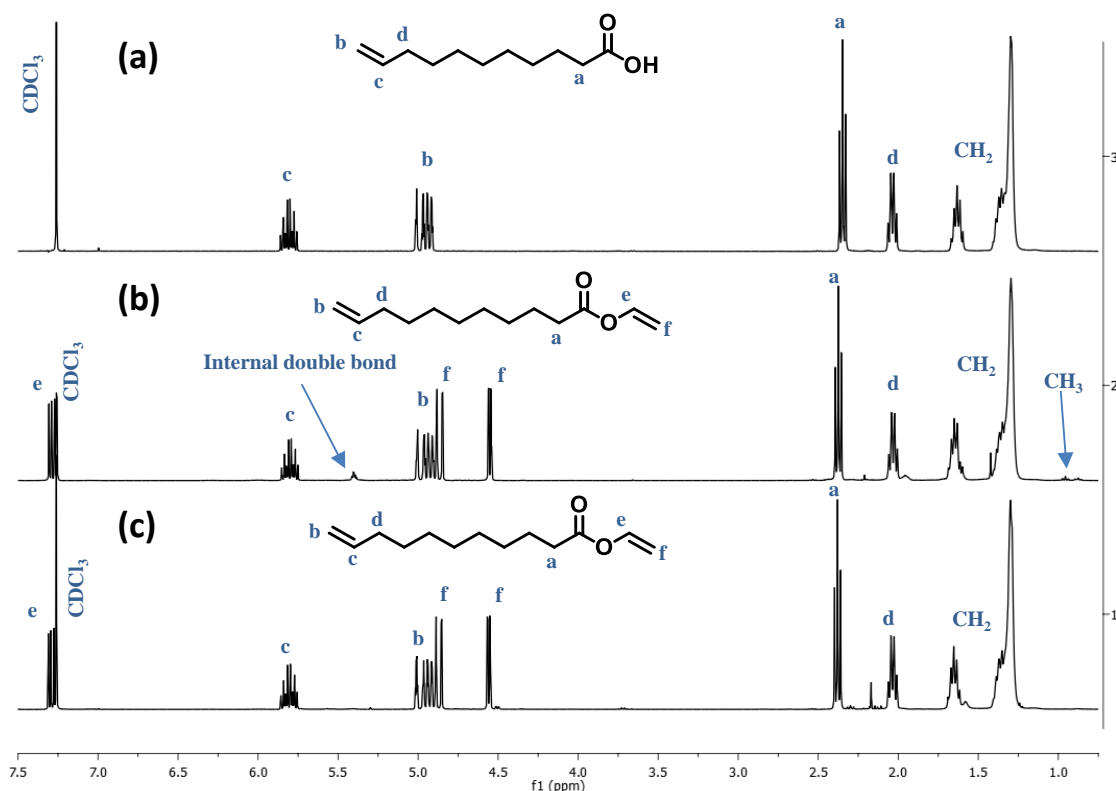


Figure 23 : Stacked ^1H NMR spectra performed in CDCl_3 of (a) undecenoic acid, (b) purified vinyl undecenoate synthesized with iridium-based catalyst and (c) purified vinyl undecenoate synthesized with palladium acetate as catalyst

The ^1H NMR spectrum confirms the formation of the vinyl undecenoate with the appearance of the peaks corresponding to the protons of the vinyl ester function at 4.56, 4.88 and 7.29 ppm (Figure 23). However, even after purification of vinyl undecenoate by flash chromatography, signals at 5.40 ppm and 0.9 ppm still remain that can be attributed to internal double bonds and CH_3 groups. Such signals could be due to the presence of isomerization of the terminal double bond. Indeed, the catalyst used as other iridium-based catalysts are reported to catalyse double bonds isomerization. In this case, isomerization took place on the terminal double bond to form some internal ones.³³ Thus, 9 % of isomers (determined by ^1H NMR) are present with vinyl undecenoate under these conditions.

By using palladium acetate as catalyst, no isomerization on the terminal double bond of the undecenoic acid was observed after the completion of the reaction (no peaks at 5.40 ppm and 0.9 ppm on ^1H NMR spectroscopy were observed) (Figure 23 and Figure S19).

This condition was selected to be applied on three other fatty acids, oleic acid, linoleic acid and hydrogenated ricinoleic acid. Good yield were also obtained for all the fatty acids without observation of any side reactions (Figure 26 and Figure S20-S22).

Entry	Fatty acids	Vinyl esters	Yield
1			95 %
2			95 %
3			80 %
4			80 %

Table 1 : Vinyl esters synthesized with palladium acetate from undecenoic acid, oleic acid, linoleic acid and hydrogenated ricinoleic acid

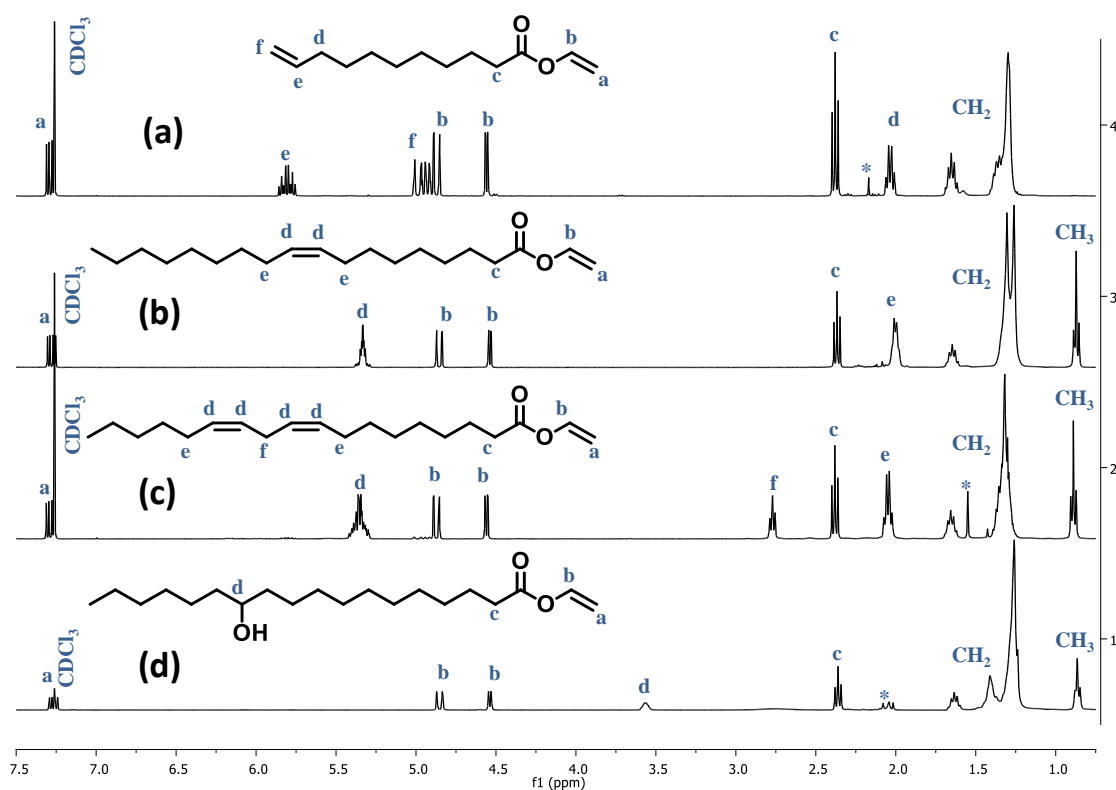


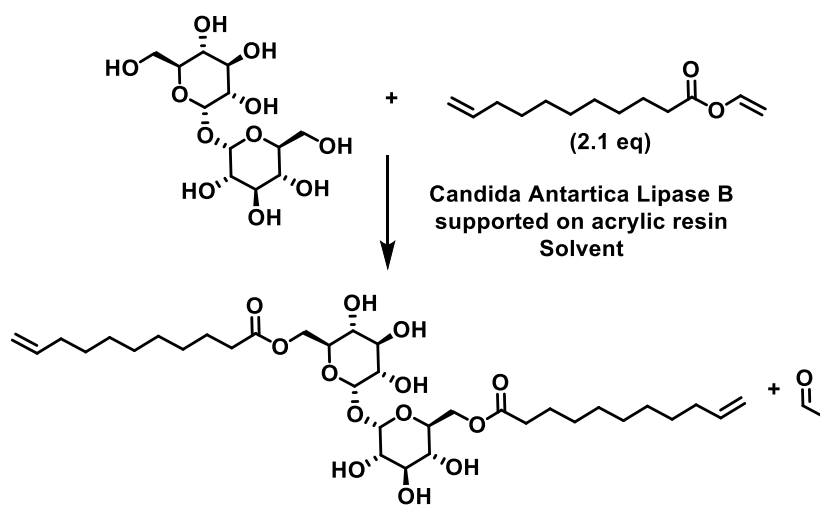
Figure 24 : Stacked ^1H NMR spectra performed in CDCl_3 of (a) vinyl undecenoate, (b) vinyl oleate, (c) vinyl linoleate and (d) vinyl hydrogenated ricinoleate

It could be noted that Deleuze's group³⁴ also described the same transvinylation reaction in a microwave reactor. We also performed the transvinylation reaction on undecenoic acid under

microwave at 60°C for 2h using the same amount of reagents and 5 mol% of palladium acetate as catalysts as previously discussed. The formation of vinyl undecenoate was confirmed by ^1H NMR spectroscopy and no isomerization or side products were observed in these conditions. The use of microwaves allowed us decreasing the reaction time from 24h to 2h with nearly the same conversion in comparison to classical heating.

2. Enzymatic trehalose esters synthesis and purification

The enzymatic transesterification of trehalose to obtain trehalose esters was then investigated first with vinyl undecenoate (Scheme 28). Trehalose was esterified employing a lipase B from *Candida antarctica* (Novozyme 435) in dry solvents.²³ The reaction mixture was stirred for 72 h. Different conditions have been tested and are summarized in Table 2. After the reaction time, the reaction mixture was filtered to remove the enzyme and unreacted trehalose and then the solvent was evaporated. The obtained crude product was analyzed by ^1H NMR spectroscopy to determine the conversion in trehalose esters.



Scheme 28 : Enzymatic transesterification of trehalose with vinyl undecenoate

As described in the literature and contrary to the chemical route previously studied, neither monoester of trehalose nor by-products (triesters or quadriesters) were formed as proved by ^1H NMR analysis. Only diesters were selectively formed during the reaction whatever the conditions employed. It could be confirmed by the disappearance of the peaks at 3.65, 3.55 and 3.47 ppm corresponding respectively to proton H5 and H6 of the unesterified trehalose (Figure 25). A peak at 2.17 ppm was observed on the ^1H NMR spectrum, which could be attributed to the proton nearby the acidic function of undecenoic acid. Indeed during the

reaction, vinyl undecenoate hydrolysis could occur due to the trace of water present in the reaction media, even if the reaction was performed in dry atmosphere.

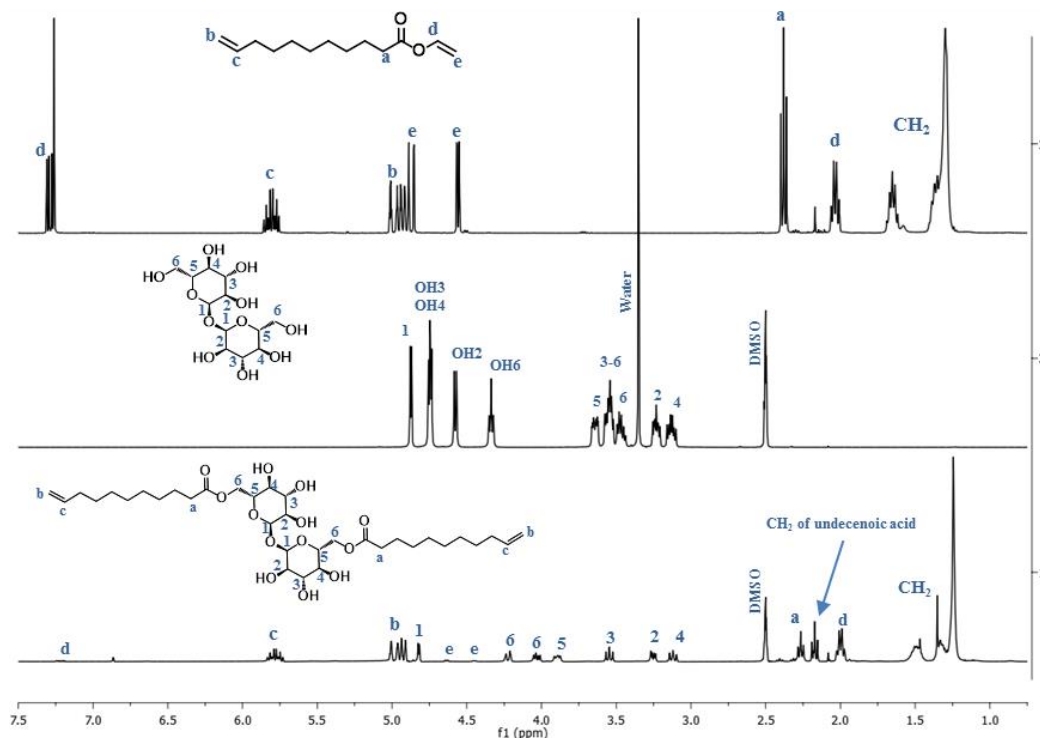


Figure 25 : Stacked ^1H NMR spectra of vinyl undecenoate (top) performed in CDCl_3 , trehalose (middle) and crude reaction mixture of esterification in mTHF(bottom) performed in DMSO-d_6

Entry	Vinyl undecenoate (equivalent number)	Novozyme 435		Solvent	Temperature	Conversion in trehalose diundecenoate
		(weight percentage / weight of substrate)				
1	2,5 eq	25 wt%		Acetone	45°C	30 %
2	2,5 eq	25 wt%		THF	45°C	12 %
3	2,5 eq	40 wt%		Acetone	45°C	40 %
4	2,1 eq	25 wt%		Tert-buthyl alcohol	60°C	40 %
5	2,1 eq	25 wt%		mTHF	60°C	50 %

Table 2: Results on the enzymatic transesterification performed during 72h: equivalent number of vinyl undecenoate, solvent used, temperature and conversion in trehalose diundecenoate determined by ^1H NMR spectroscopy

Trehalose was first esterified in acetone as described by John and coll.³⁵ (Table 2, entry 1). A conversion of 30 % in trehalose diundecenoate was obtained. By increasing the enzyme weight percentage, the conversion in trehalose diundecenoate can be slightly increased. Different solvents and temperature were tested to find out the best conditions and then increase the conversion. The highest conversion was obtained with 2-methyltetrahydrofurane at 60°C in the presence of 2.1 equivalents of vinyl esters and 25 wt.% of lipase per weight of substrates (trehalose and vinyl ester). These conditions were applied to the vinyl esters synthesized in the previous part. Indeed, trehalose dioleate, trehalose dilinoleate and trehalose di-12-hydroxystearate were obtained with conversions of 50 %. The conversion in trehalose diesters was not dependent of the nature of the vinyl ester used for the esterification.

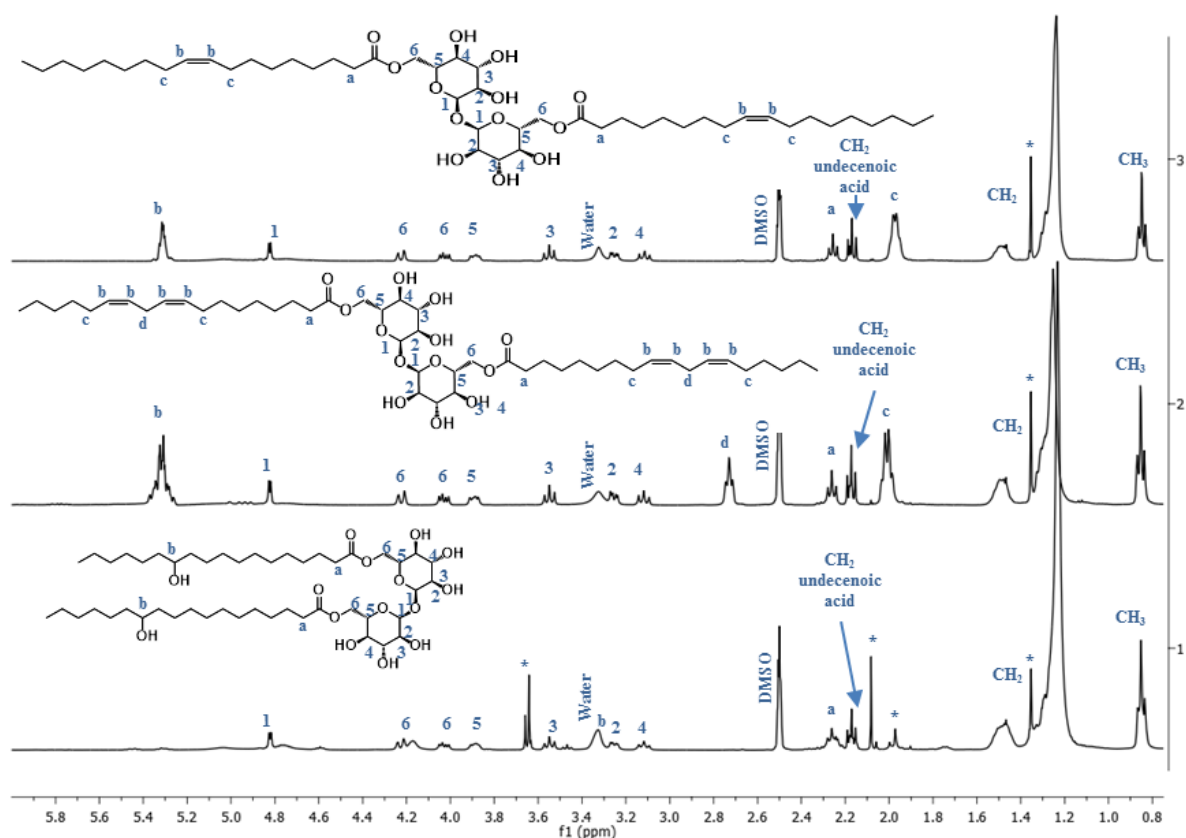


Figure 26 : Stacked ¹H NMR spectra of crude reaction mixture of trehalose dioleate (top), crude reaction mixture of trehalose dilinoleate (middle) and crude reaction mixture of trehalose di-12-monohydroxydistearate (bottom) performed in DMSO-d₆

Finally, trehalose diesters could be isolated (with around 49 % yield) by silica column flash chromatography using as eluent MeOH in DCM/EtOAc (1:1).

3. Experimental

General procedure for fatty acid transvinylation with iridium catalyst

Fatty acid (1 eq) and a 10 eq. excess of vinyl acetate (VAc) was poured in an oven-dried round bottom flask equipped with a condenser. Then, the sodium acetate (3 mol%), and the iridium catalyst (Chlorobis(cyclooctene)iridium(I)dimer) (1 mol%) were added and the resulting reaction mixture was stirred at reflux at 100 °C for 24 h under nitrogen atmosphere. The reaction mixture was then poured into water and extracted with dichloromethane (DCM). The organic phase was dried over magnesium sulfate and DCM and vinyl acetate were removed with a rotary evaporator. The resulting residue was purified by silica gel flash chromatography using an elution gradient of 2-5% MeOH in DCM to give the vinyl ester.

General procedure for fatty acid transvinylation with palladium acetate in oil bath

Fatty acid (1 eq) and a 15 eq. excess of vinyl acetate (VAc) was poured in an oven-dried round bottom flask. Then, the palladium acetate (0.05 eq.), and the potassium hydroxide (0.10 eq.) were added and the resulting reaction mixture was stirred at 60 °C for 24 h. The reaction mixture was diluted in DCM and then filtrated over celite to remove the palladium acetate, before removing the solvent with a rotary evaporator. The resulting residue was purified by silica gel flash chromatography using an elution gradient of 2-5% MeOH in DCM to give the vinyl ester.

General procedure for fatty acid transvinylation under microwave

Fatty acid (1 eq) and a 15 eq. excess of vinyl acetate (VAc) was poured in a vial for the microwave reactor. Then, the palladium acetate (0.05 eq.), and the potassium hydroxide (0.10 eq.) were added and the resulting reaction mixture was stirred under microwave at 60 °C for 2 h. The reaction mixture was diluted in DCM and then filtrated over celite to remove the palladium acetate, before removing the solvent with a rotary evaporator. The resulting residue was purified by silica gel flash chromatography using an elution gradient of 2-5% MeOH in DCM to give the vinyl ester.

Vinyl undecenoate

^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 7.29 (m, 1H, = $\underline{\text{C}}\text{H}$ -OCO-), 5.81 (m, 1H, - $\underline{\text{C}}\text{H}=\text{CH}_2$), 4.97 (m, 2H, $\underline{\text{C}}\text{H}_2=\text{CH}$ -), 4.88 (d, 1H, $\text{CH}_2=\underline{\text{C}}\text{H}$ -OCO-), 4.56 (d, 1H, $\text{CH}_2=\underline{\text{C}}\text{H}$ -OCO-), 2.37 (t, 4H, - $\underline{\text{C}}\text{H}_2$ -COO-), 2.04 (m, 4H, - $\underline{\text{C}}\text{H}_2$ -CH=CH-), 1.67 (m, 4H, - $\underline{\text{C}}\text{H}_2$ -CH₂-COO-), 1.30 (m, 20H, aliphatic - $\underline{\text{C}}\text{H}_2$ -).

Vinyl oleate

^1H NMR (DMSO- d^6 , 400MHz, δ (ppm)): 7.29 (m, 1H, =CH-OCO-), 5.81 (m, 1H, -CH=CH₂), 4.97 (m, 2H, CH₂=CH-), 4.88 (d, 1H, CH₂=CH-OCO-), 4.56 (d, 1H, CH₂=CH-OCO-), 2.37 (t, 4H, -CH₂-COO-), 2.04 (m, 4H, -CH₂-CH=CH-), 1.67 (m, 4H, -CH₂-CH₂-COO-), 1.30 (m, 20H, aliphatic -CH₂-), 0.87 (t, 3H, aliphatic -CH₃).

Vinyl linoleate

^1H NMR (DMSO- d^6 , 400MHz, δ (ppm)): 7.29 (m, 1H, =CH-OCO-), 5.81 (m, 1H, -CH=CH₂), 4.97 (m, 2H, CH₂=CH-), 4.88 (d, 1H, CH₂=CH-OCO-), 4.56 (d, 1H, CH₂=CH-OCO-), 2.37 (t, 4H, -CH₂-COO-), 2.06 (m, 4H, -CH₂-CH=CH-), 1.66 (m, 4H, -CH₂-CH₂-COO-), 1.30 (m, 20H, aliphatic -CH₂-), 0.89 (t, 3H, aliphatic -CH₃).

Vinyl hydroxystearate

^1H NMR (DMSO- d^6 , 400MHz, δ (ppm)): 7.29 (m, 1H, =CH-OCO-), 5.81 (m, 1H, -CH=CH₂), 4.97 (m, 2H, CH₂=CH-), 4.88 (d, 1H, CH₂=CH-OCO-), 4.56 (d, 1H, CH₂=CH-OCO-), 2.37 (t, 4H, -CH₂-COO-), 2.04 (m, 4H, -CH₂-CH=CH-), 1.67 (m, 4H, -CH₂-CH₂-COO-), 1.30 (m, 20H, aliphatic -CH₂-), 0.87 (t, 3H, aliphatic -CH₃).

General procedure for optimized enzymatic esterification of trehalose

The lipase (0.7 g, 20 wt%) was added to a mixture of trehalose (1 eq) and vinyl ester (2.1 eq) in dry 2-methyltetrahydrofuran (20 mL). The reaction mixture was stirred at 60 °C for 72 h. After the reaction time, the reaction mixture was filtered to remove the enzyme and the solvent was removed with a rotary evaporator. The obtained crude product was purified by silica gel flash chromatography using an elution gradient of 5-25% methanol in EtOAc-DCM (1:1) to give the pure trehalose diester.

III. Conclusions and perspectives

The chemical esterification of trehalose in the presence of a peptide-coupling agent was performed to synthesize trehalose diesters and monoesters. It was observed that triesters and polyesters were also produced during the reaction. Even by diluting the reaction medium or by changing the solvent, by-products were formed, proving that such chemical reaction was not so selective. However, following this procedure, pure trehalose diesters and trehalose monoesters were successfully produced with various fatty substrates and isolated by flash chromatography. Enhancement of this esterification pathway should still be done to avoid the production of by-products.

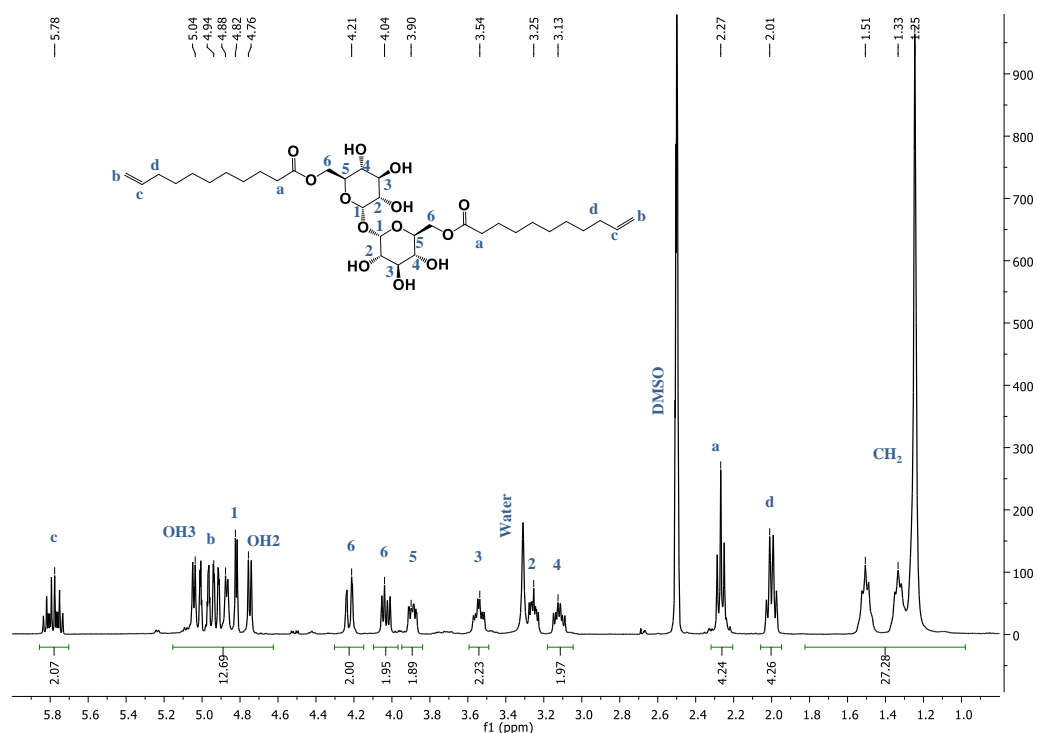
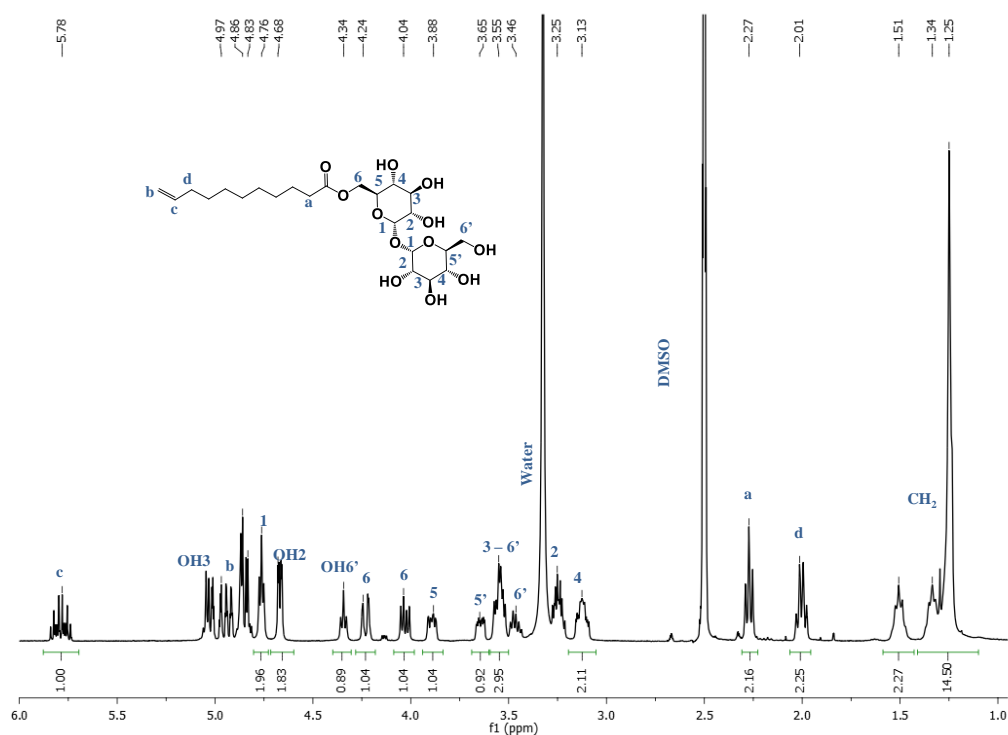
Two transvinylation routes were investigated to obtain some vinyl esters from fatty acid and vinyl acetate, in the presence of iridium-based and palladium acetate catalysts, respectively. In the first route, isomerization of the double bond was observed. In the second route, vinyl undecenoate without isomerization of the terminal double bond was successfully formed. This strategy was then selected and applied to different fatty acids. A series of diesters of trehalose were also obtained by enzymatic catalysis. Lipase B from *Candida antarctica* was selected, allowing a good selectivity of primary alcohol esterification. No monoesters or by-products were obtained in these conditions. Pure trehalose diesters were obtained with yield around 50 %. The conditions of this enzymatic transesterification should be improved as this transesterification route is very promising for the synthesis of trehalose diesters. Several advantages can be noticed concerning the enzymatic esterification compare to the chemical pathway. Indeed, low toxic methyl-tetrahydrofuran (mTHF) was used as solvent instead of pyridine which is classified CMR. Besides, mTHF can be bio-based. Thus, due to the absence of side-products, the enzymatic route appeared to be a better route for the synthesis of trehalose diesters. However, trehalose monoesters cannot be synthesized by this way.

IV. References

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V. Supporting information

Figure S1: ^1H NMR spectrum of trehalose diundecenoate performed in DMSO-d_6 Figure S2: ^1H NMR spectrum of trehalose monoundecenoate performed in DMSO-d_6

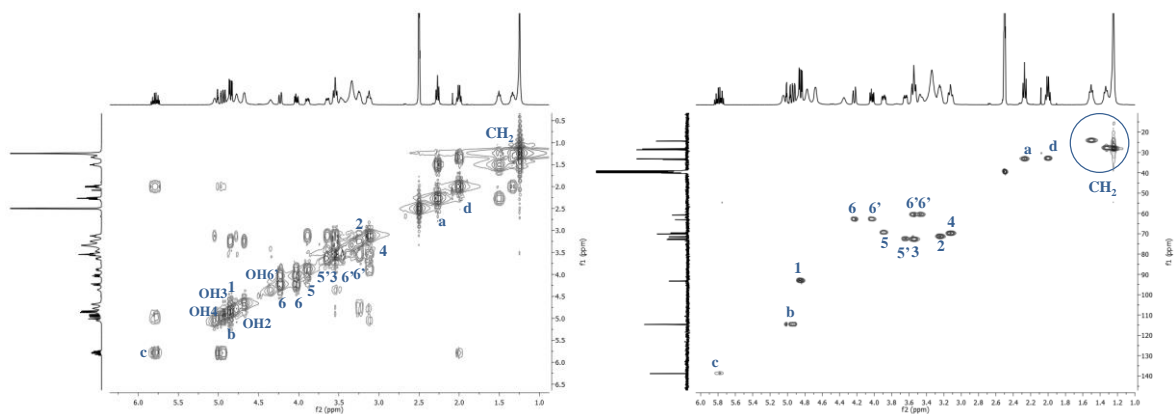


Figure S3: ^1H - ^1H COSY NMR spectrum (left) and ^1H - ^{13}C HSQC NMR spectrum (right) of trehalose monoundecenoate performed in DMSO-d_6

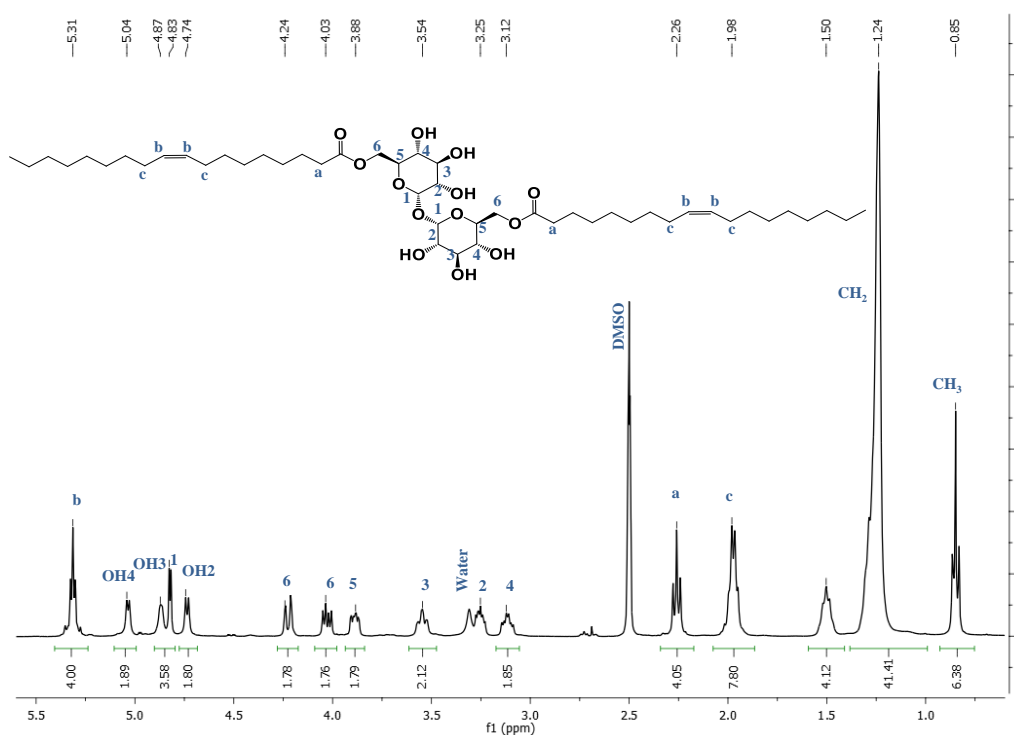


Figure S4: ^1H NMR spectrum of trehalose dioleate performed in DMSO-d_6

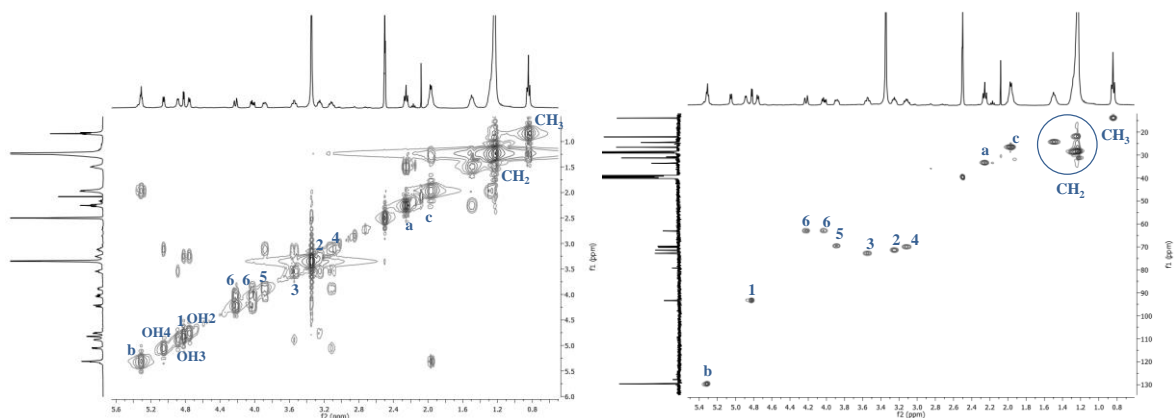


Figure S5: ^1H - ^1H COSY NMR spectrum (left) and ^1H - ^{13}C HSQC NMR spectrum (right) of trehalose dioleate performed in DMSO-d_6

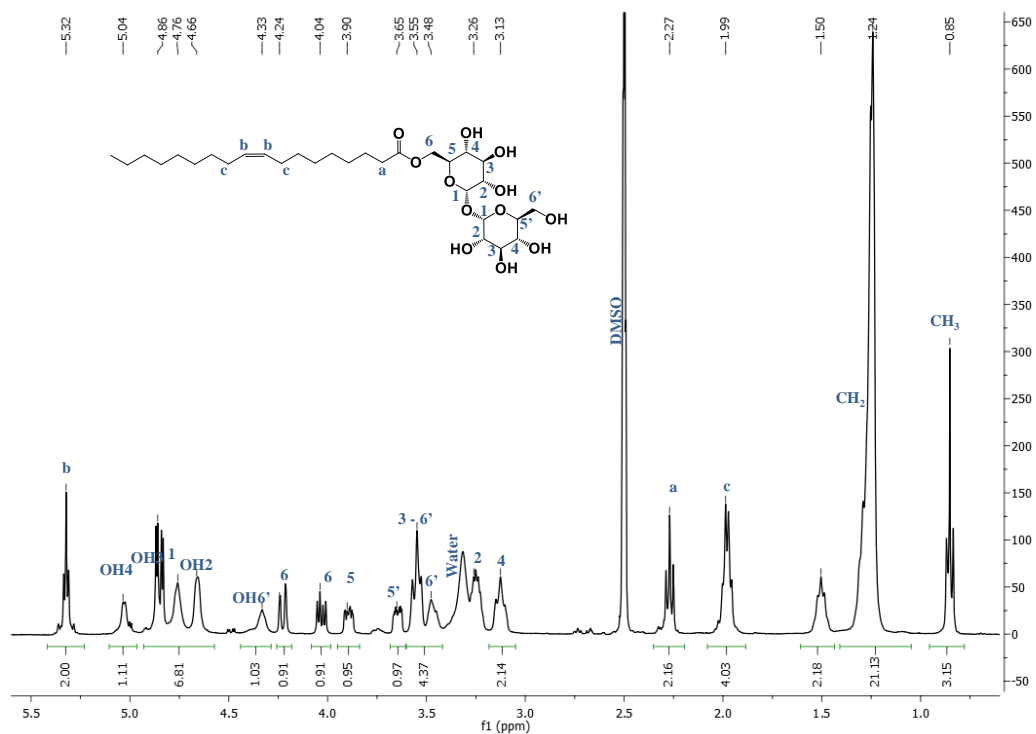


Figure S6: ¹H NMR spectrum of trehalose monooleate performed in DMSO-d₆

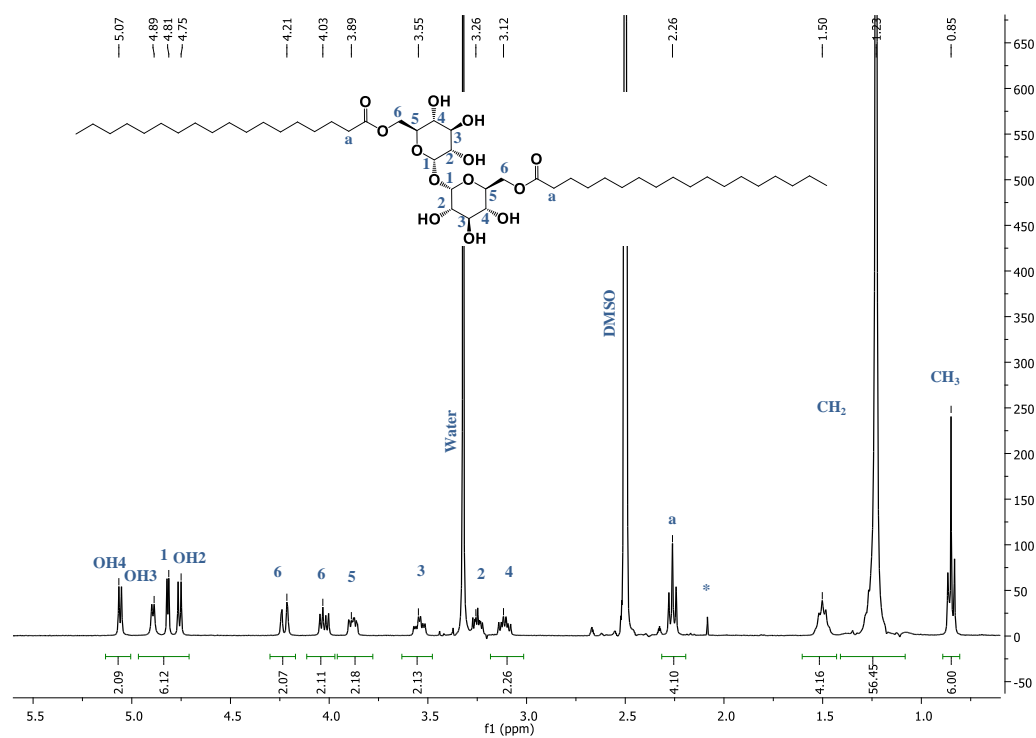


Figure S7: ¹H NMR spectrum of trehalose distearate performed in DMSO-d₆

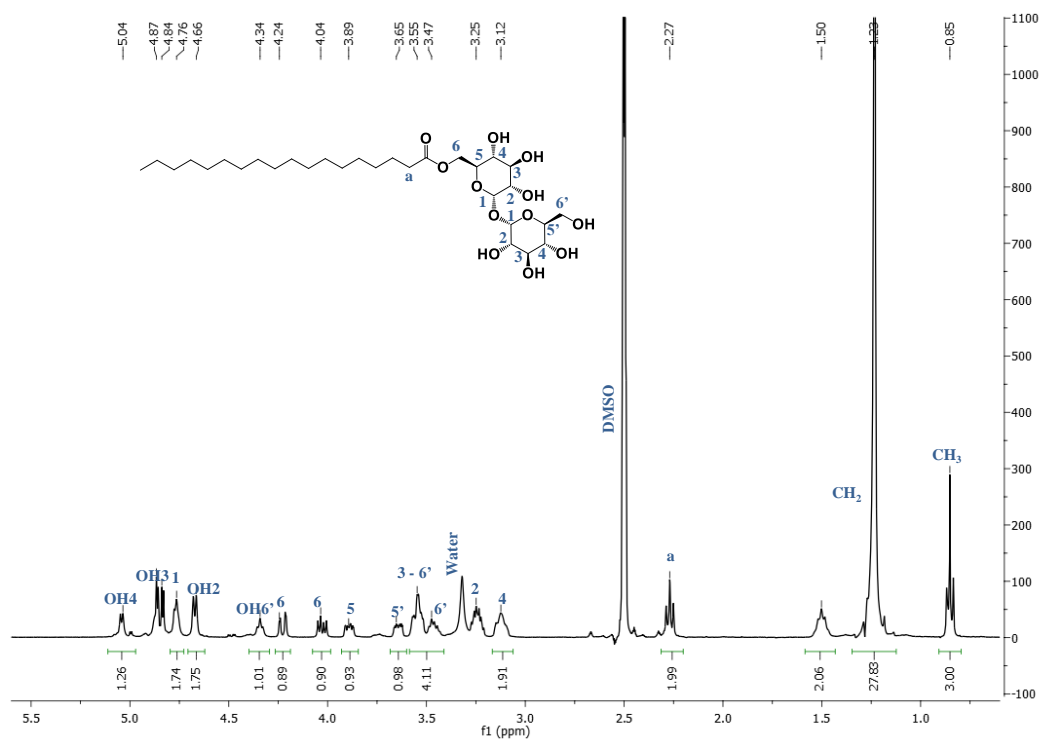


Figure S8: ^1H NMR spectrum of trehalose monostearate performed in DMSO-d6

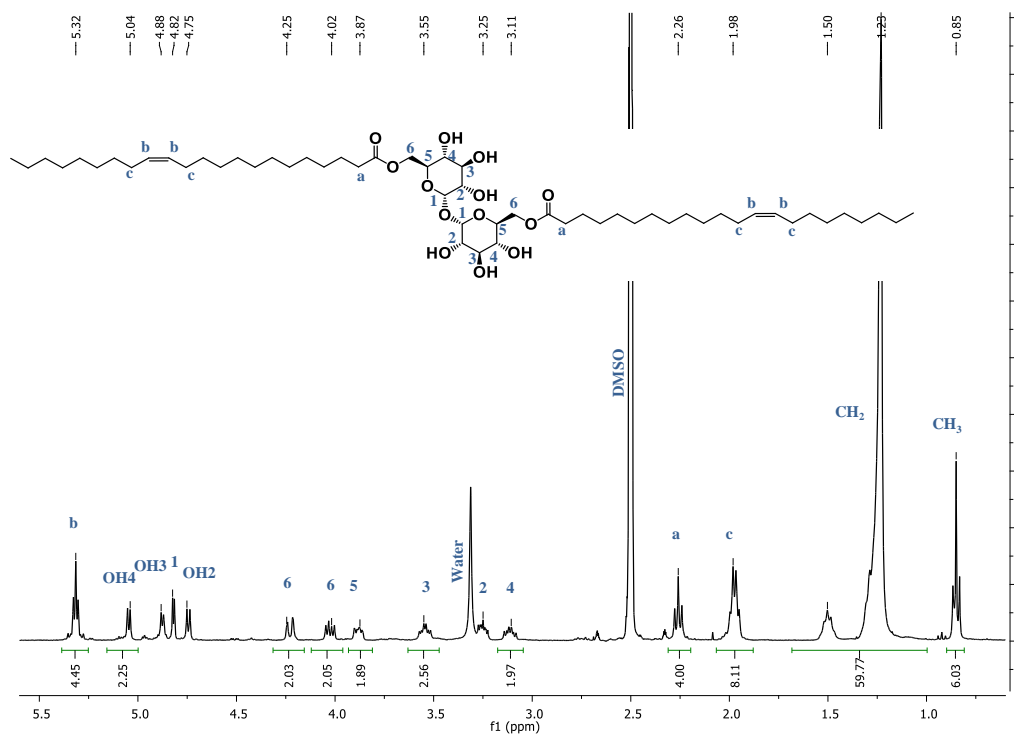


Figure S9: ^1H NMR spectrum of trehalose dierucate performed in DMSO-d6

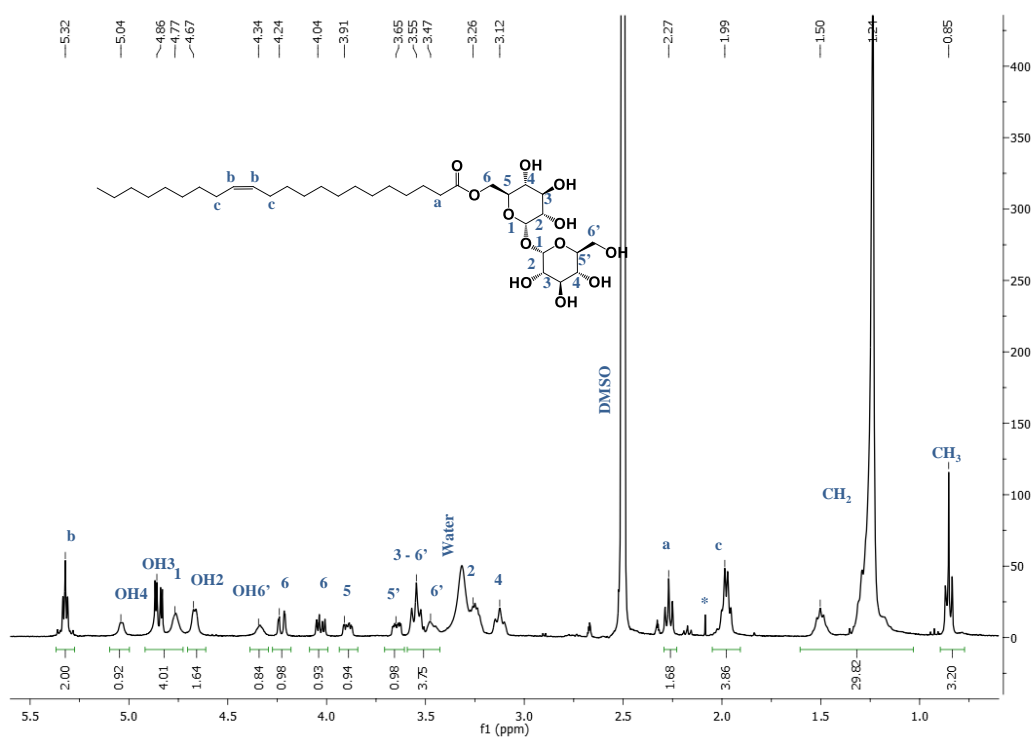


Figure S10: ¹H NMR spectrum of trehalose monoerucate performed in DMSO-d₆

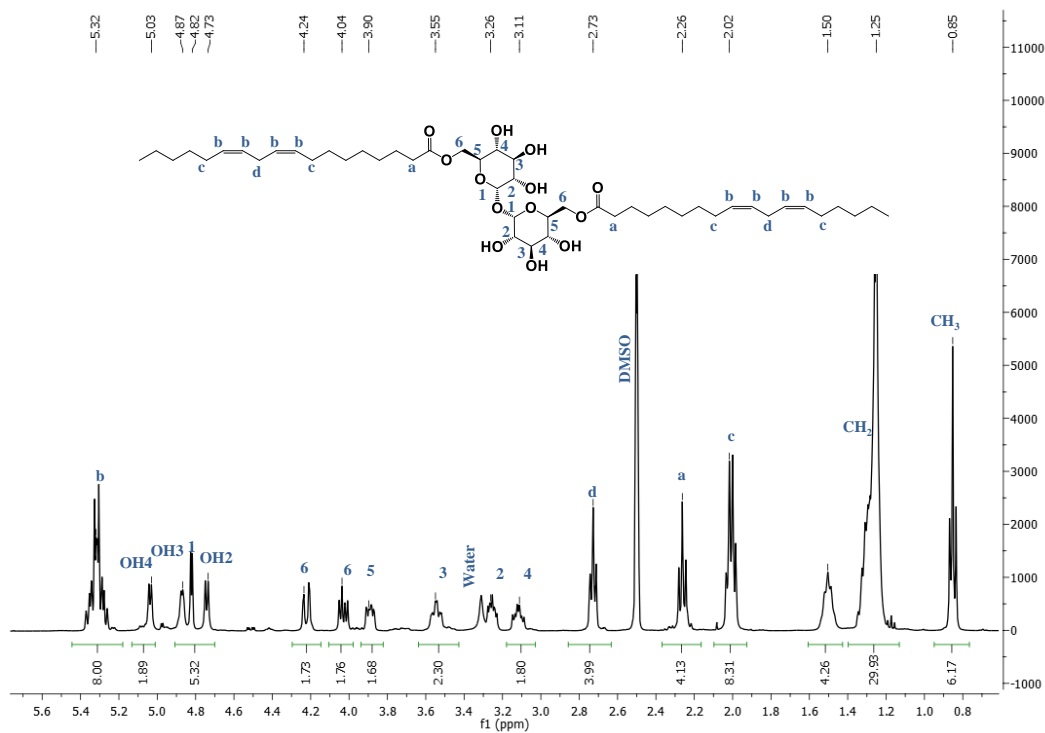


Figure S11: ¹H NMR spectrum of trehalose dilinoleate performed in DMSO-d₆

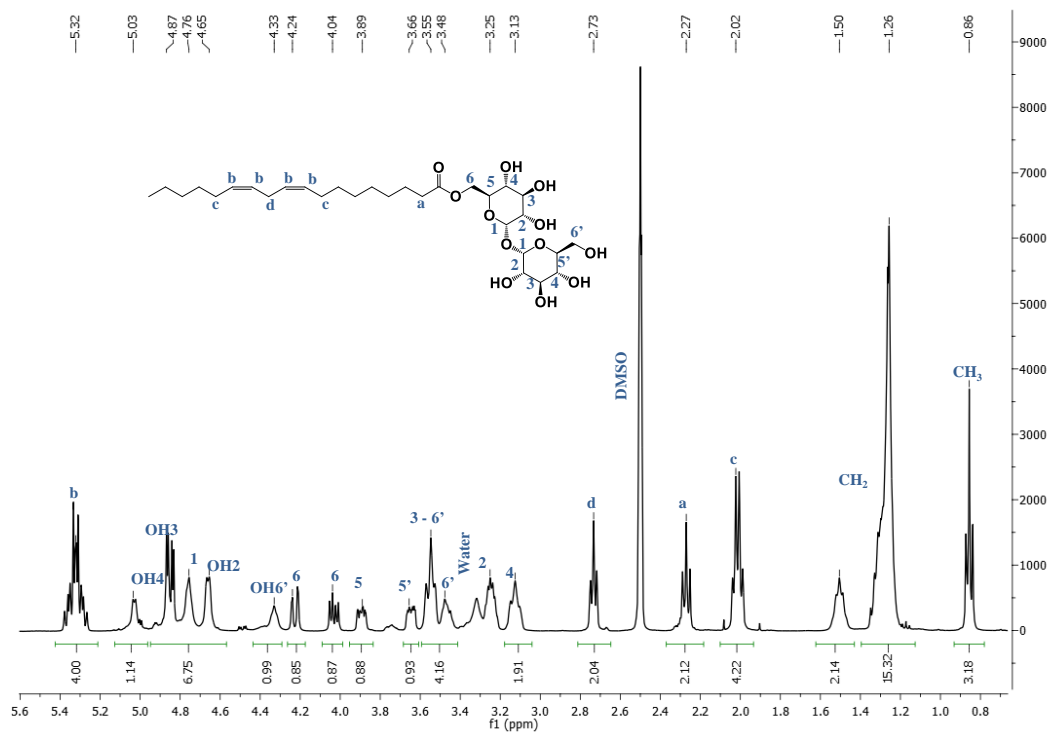


Figure S12: ¹H NMR spectrum of trehalose monolinoleate performed in DMSO-d₆

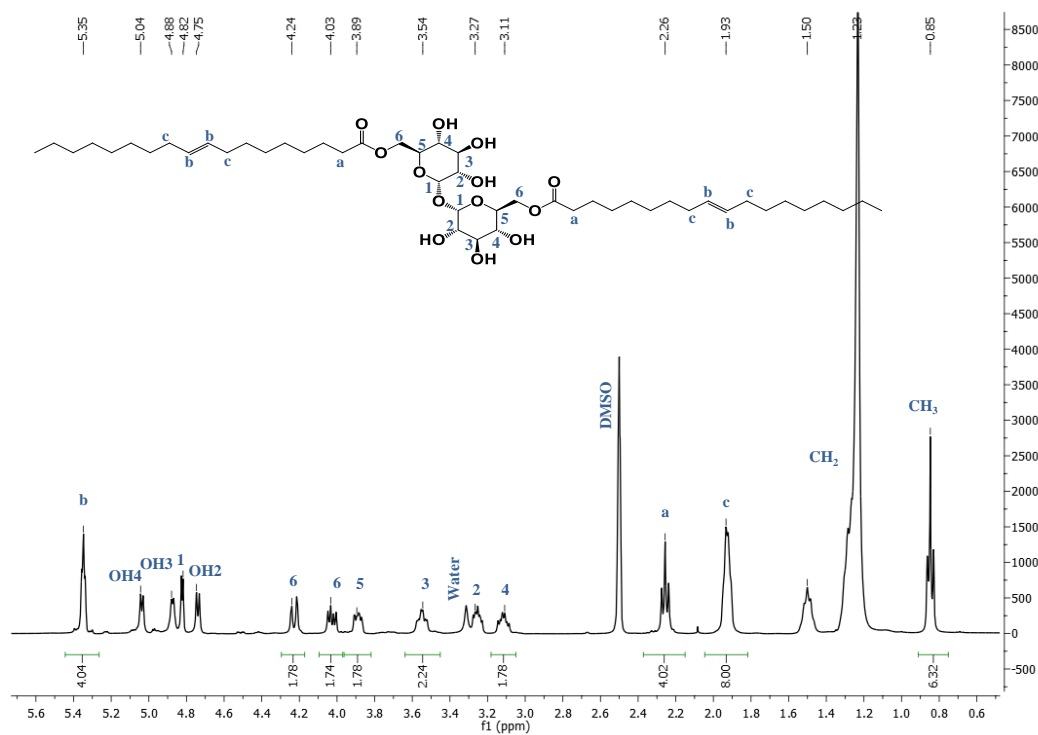


Figure S13: ¹H NMR spectrum of trehalose dielaidate performed in DMSO-d₆

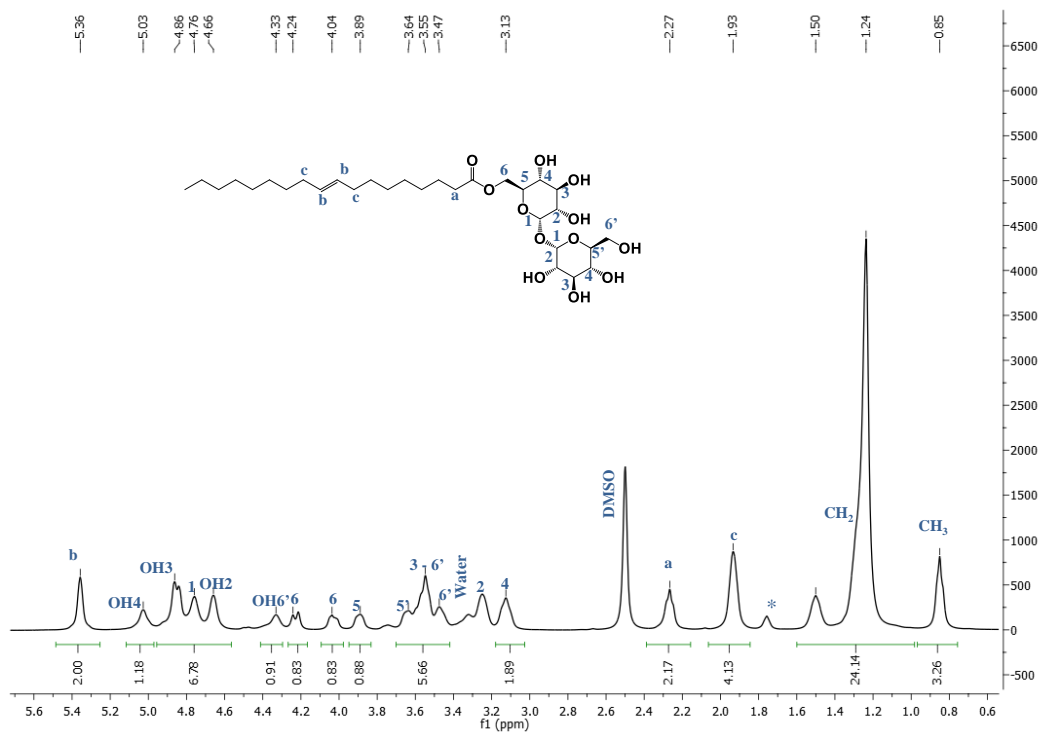


Figure S14: ^1H NMR spectrum of trehalose monoelaidate performed in DMSO-d_6

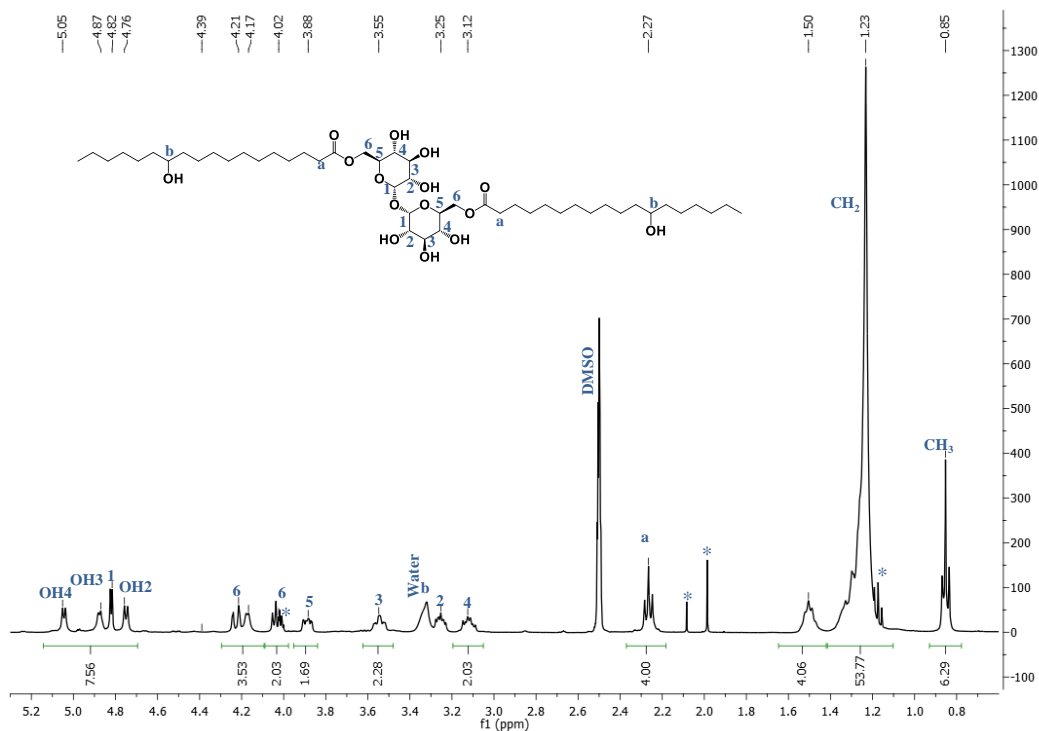


Figure S15: ^1H NMR spectrum of trehalose monohydroxydistearate performed in DMSO-d_6

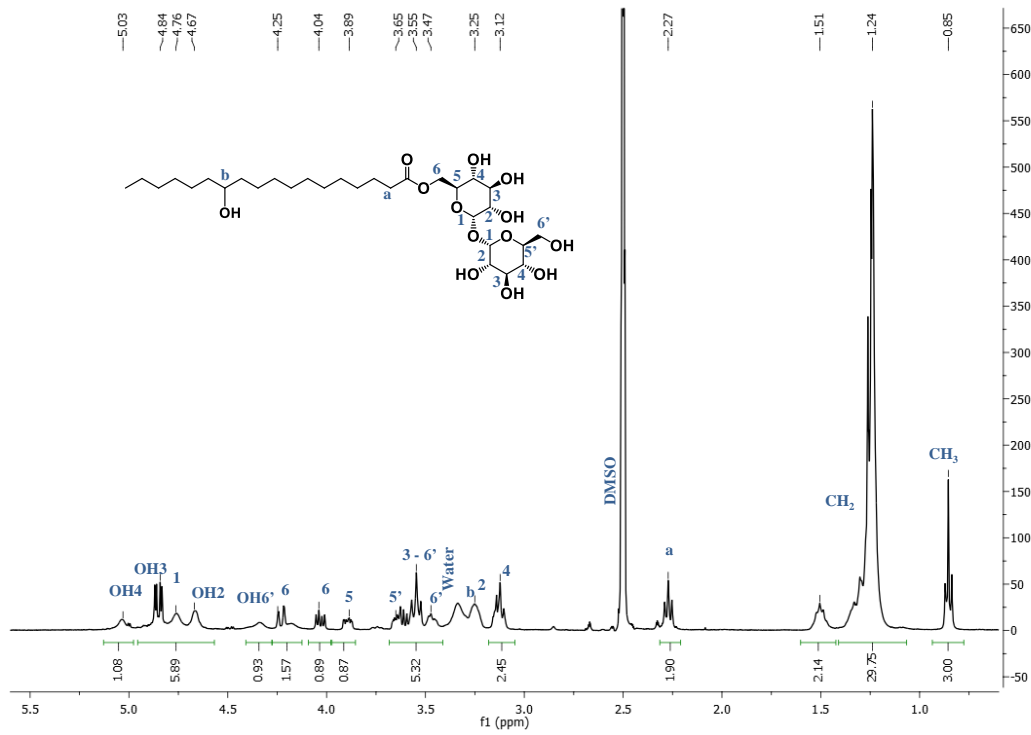


Figure S16: ^1H NMR spectrum of trehalose monohydroxymonostearate performed in DMSO-d_6

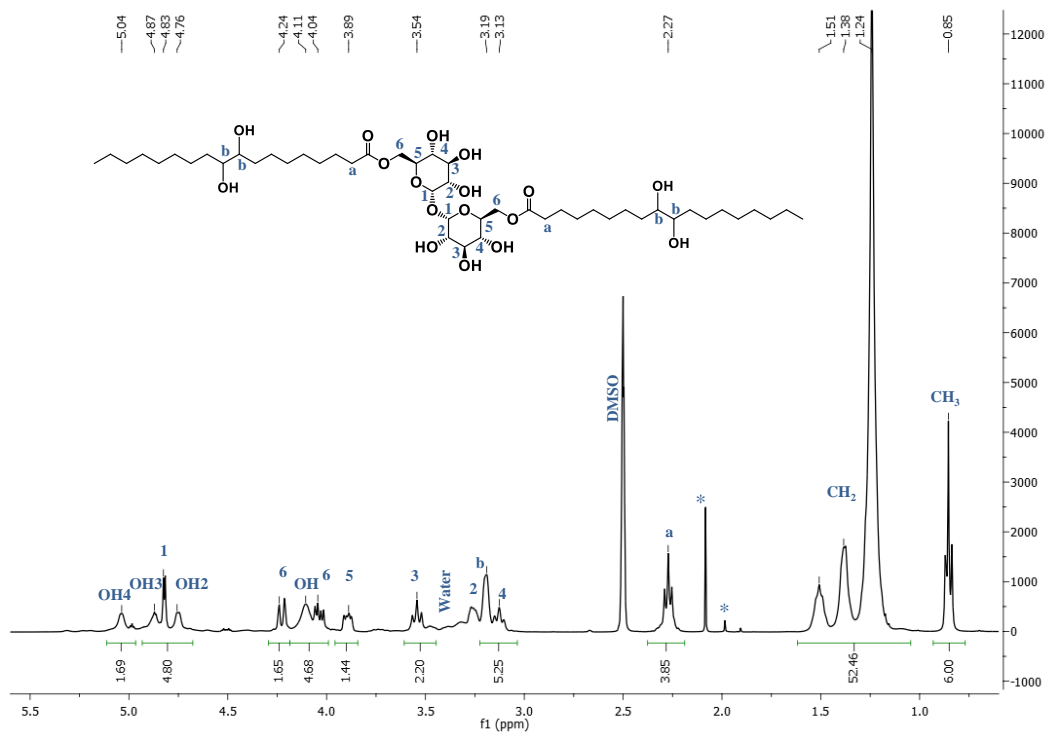


Figure S17: ^1H NMR spectrum of trehalose dihydroxydistearate performed in DMSO-d_6

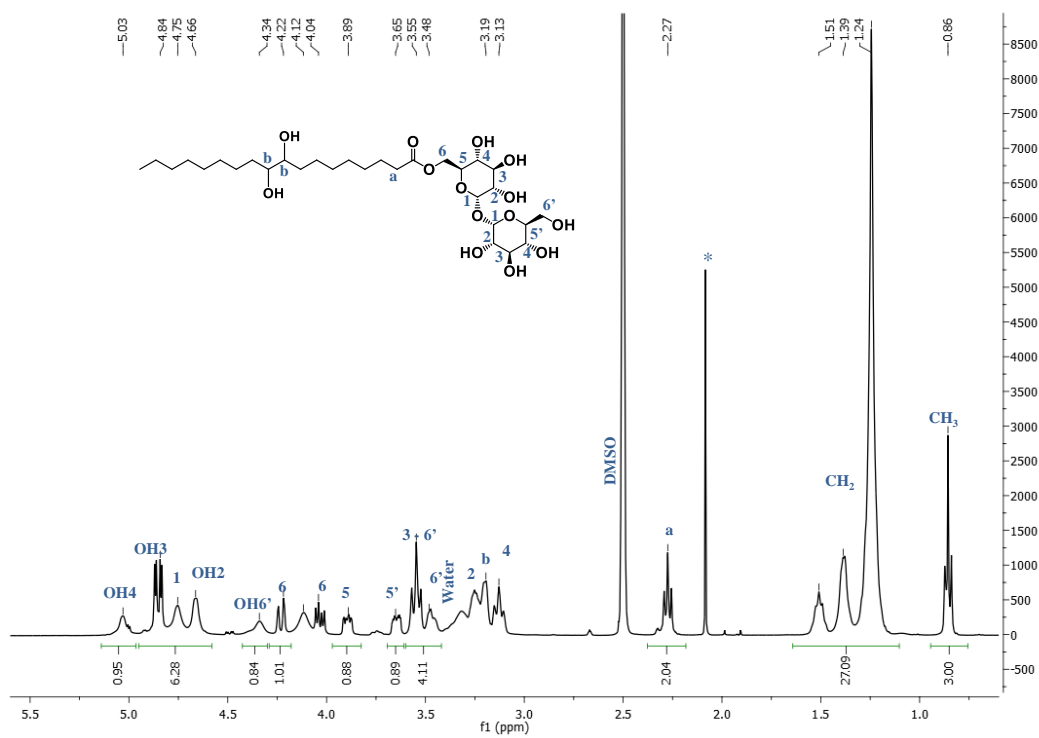


Figure S18: ¹H NMR spectrum of trehalose dihydroxymonostearate performed in DMSO-d₆

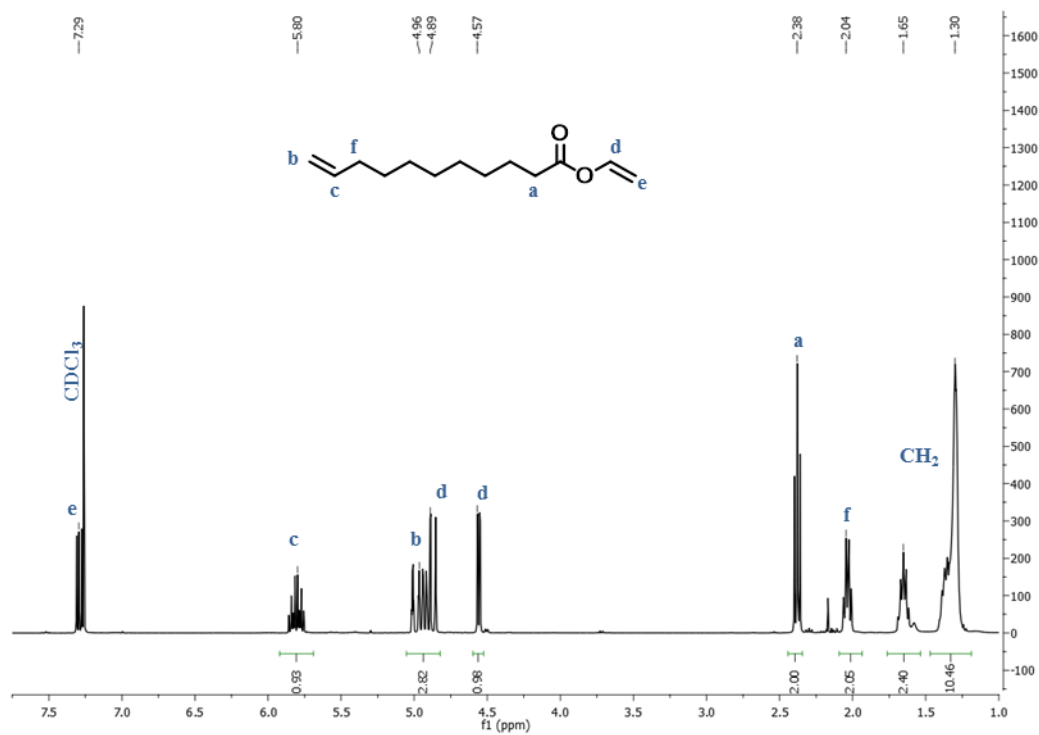
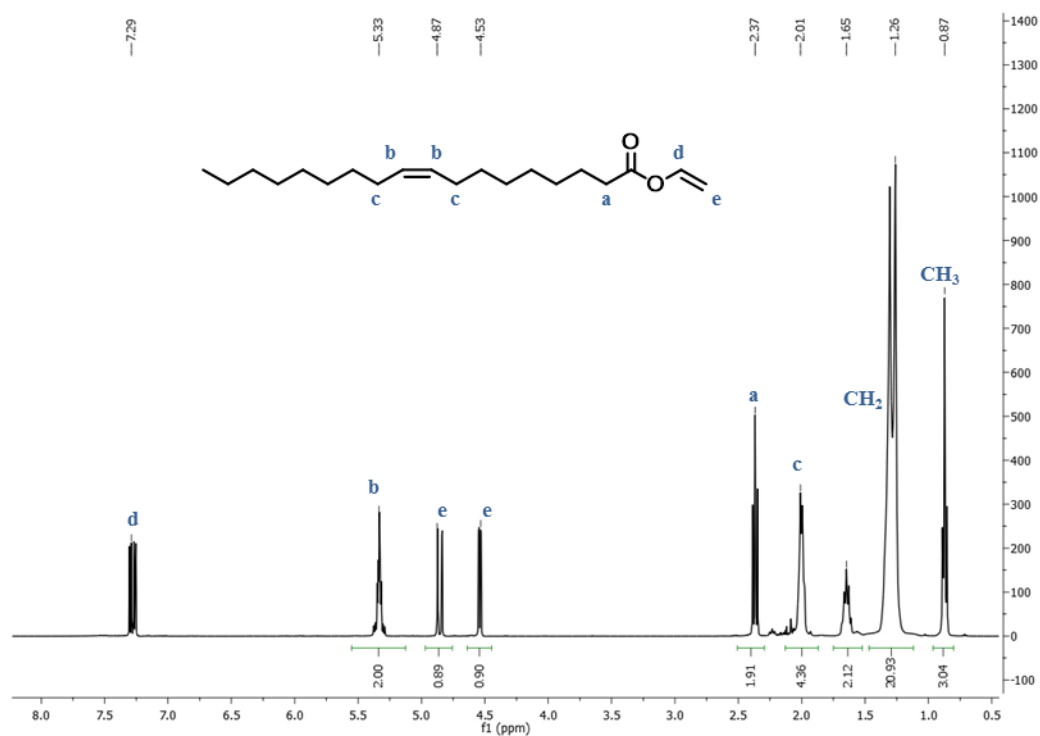
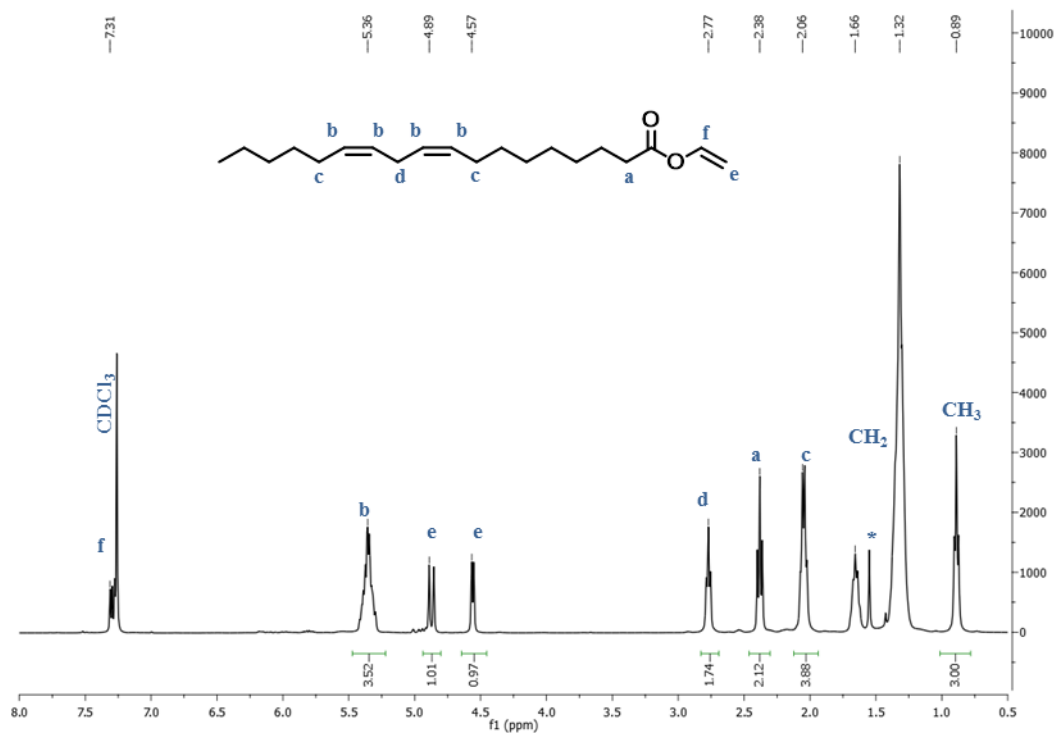


Figure S19: ¹H NMR spectrum of vinyl undecenoate performed in CDCl₃

Figure S20: ¹H NMR spectrum of vinyl oleate performed in CDCl₃Figure S21: ¹H NMR spectrum of vinyl linoleate performed in CDCl₃

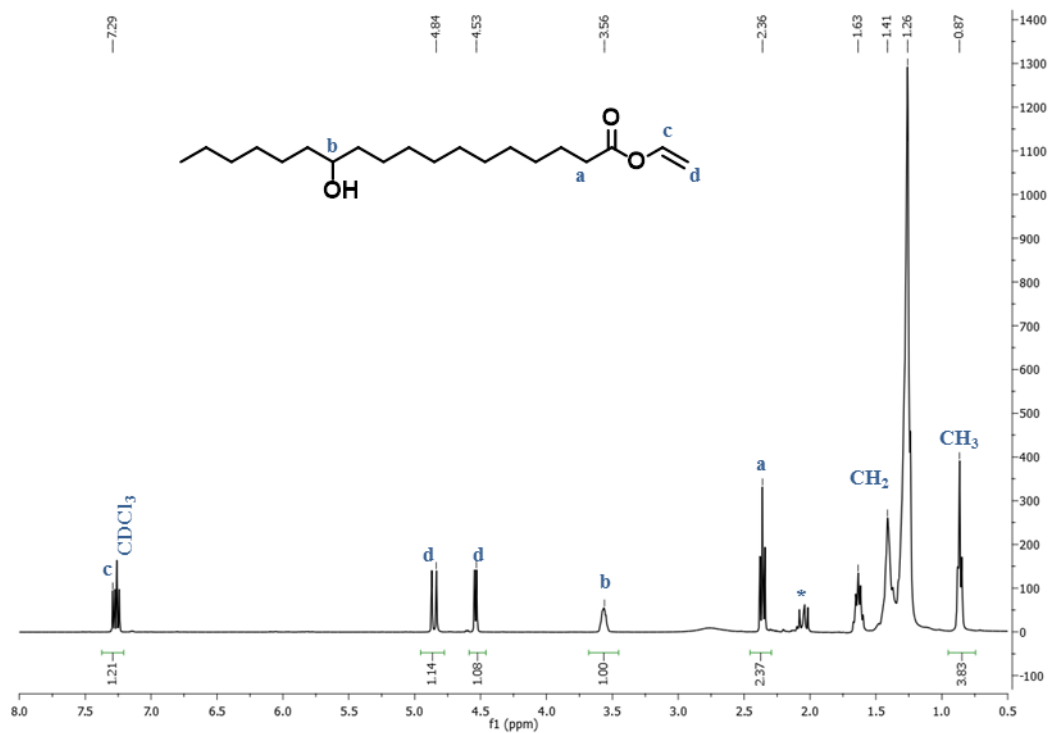


Figure S22: ^1H NMR spectrum of vinyl hydroxystearate performed in CDCl_3

Chapter 3: Self-assembly properties of trehalose monoesters in water and trehalose diesters in vegetable oils

Key words: Surfactant, hydrophilic lipophilic balance, critical micelle concentration, surface tension, hydrogel, oléogel.

Mots clés: Tensioactif, balance hydrophile lipophile, concentration micellaire critique, tension de surface, hydrogel, oleogel.

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Introduction

The self-assembly of trehalose monoesters and trehalose diesters previously synthesized has been investigated. The so-formed monoesters have the ‘classical’ shape of a surfactant with a hydrophilic polar “head” and a lipophilic carbon “tail”. Thus, the surfactant properties of these monoesters of trehalose are described in the first part of this chapter. At higher concentration than the critical micelle concentration (CMC), trehalose monoerucate can form hydrogels. This feature will also be described in the beginning of the second part of this chapter.

Contrary to the monoesters, the trehalose diesters precipitate in water and do not exhibit any self-assembly properties in water. Indeed, the latter are too hydrophobic due to the two fatty acid chains linked to the trehalose. Diesters of trehalose are already reported as good gelators for organic solvents.¹ Other sugar-based diesters (from mannitol or sorbitol) were also described to exhibit good gelation properties of vegetable oils.^{2,3} Therefore, in the second part of this chapter, the gelation capacity of the trehalose monoerucate in water and trehalose diesters in vegetable oils is presented.

I. Surfactant properties of trehalose monoesters in water

Surfactants are amphiphilic molecules with a hydrophilic “head” and a lipophilic “tail”. Sugar esters are more and more valorized as surfactants. The latter have a low toxicity; they can be biocompatible and have the advantage to emanate from renewable resources.^{4,5} Sucrose esters are examples of sugar esters which are already commercially produced and used for many applications as cosmetics, detergents and pharmaceutical products.^{4,6,7} The trehalose monoesters which the synthesis was previously described have the classical shape of surfactants with a hydrophilic “head” and a lipophilic “tail”. The surfactant properties of these monoesters of trehalose have been studied. They have the capacity to self-assemble in water and form micelles.⁸⁻¹¹ The surfactant and so the self-assembly properties of the trehalose monoesters are described thereafter.

Eight trehalose monoesters have been synthesized from different fatty acids in order to explore the structure/self-assembly properties relationship (Figure 27).

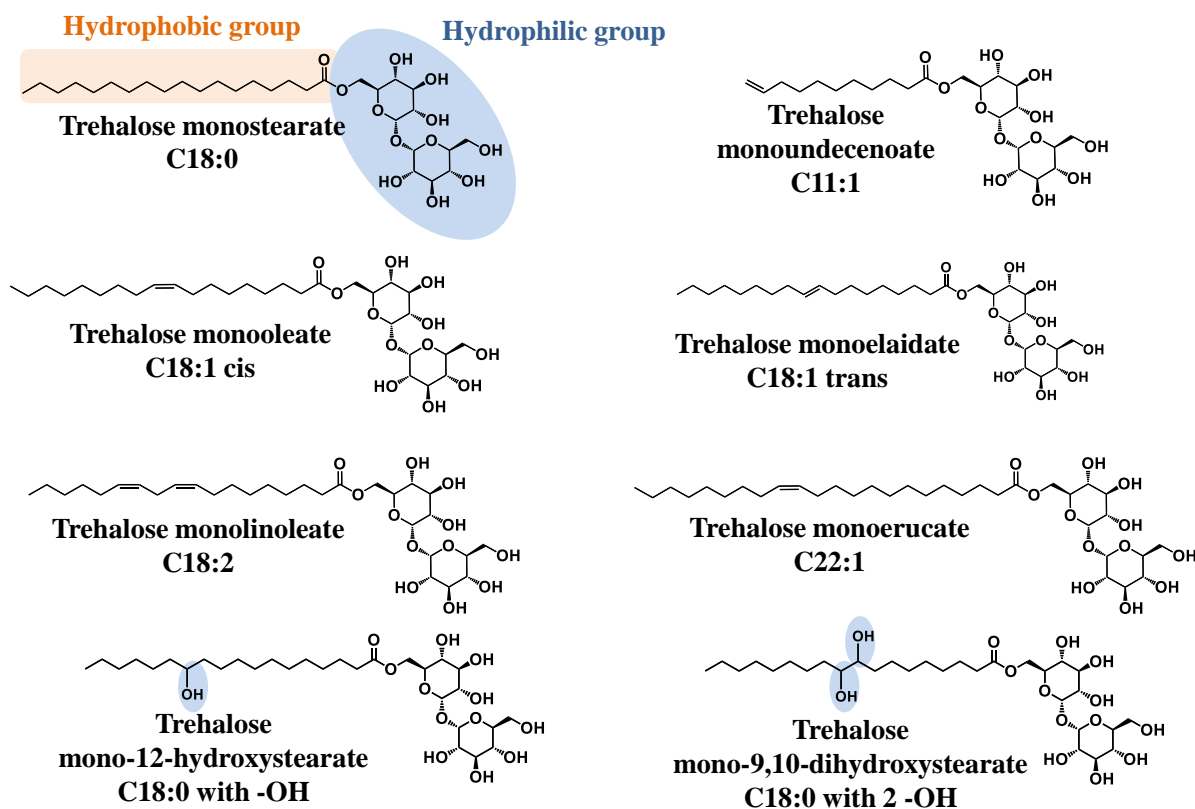


Figure 27 : Platform of trehalose monoesters: trehalose monostearate, trehalose monoundecenoate, trehalose monooleate, trehalose monoelaidate, trehalose monolinoleate, trehalose monoerucate, trehalose 12-mono-hydroxymonostearate and trehalose 9,10-dihydroxymonostearate. Hydrophobic group is depicted in orange and hydrophilic group in blue.

1. HLB Hydrophilic Lipophilic balance

The HLB concept, introduced by Griffin¹² is the balance between the hydrophilic and the lipophilic groups of a molecule. It can be calculated following the formula adapted to non-ionic surfactants as follows:

$$HLB = 20 \frac{\text{Hydrophilic group molar mass}}{\text{Total surfactant molar mass}}$$

According to this method, the HLB must be comprised between 0 and 20 where 0 corresponds to a completely lipophilic molecule and 20 to a completely hydrophilic molecule. Thus, it permits to empirically predict the surfactant properties of a molecule:

- If the HLB < 10, the molecule is not soluble in water
- If the HLB > 10, the molecule is soluble in water
- Between 1.5 and 3: the molecule is an antifoaming agent

- Between 3 and 6: the molecule is a water-in-oil emulsifier
- Between 7 and 9: the molecule is a wetting and spreading agent
- Between 10 and 16: the molecule is an-oil-in water emulsifier
- Between 13 and 15: the molecule is a detergent
- Between 15 and 18: the molecule is a solubilizing agent

Hydrophilic and hydrophobic parts were determined by ^1H NMR spectroscopy. Indeed, the hydrophilic protons are gathered between 3.0 and 5.2 ppm and separated from the lipophilic proton between 0.5 and 3.0 ppm and between 5.2 and 7.0 ppm.

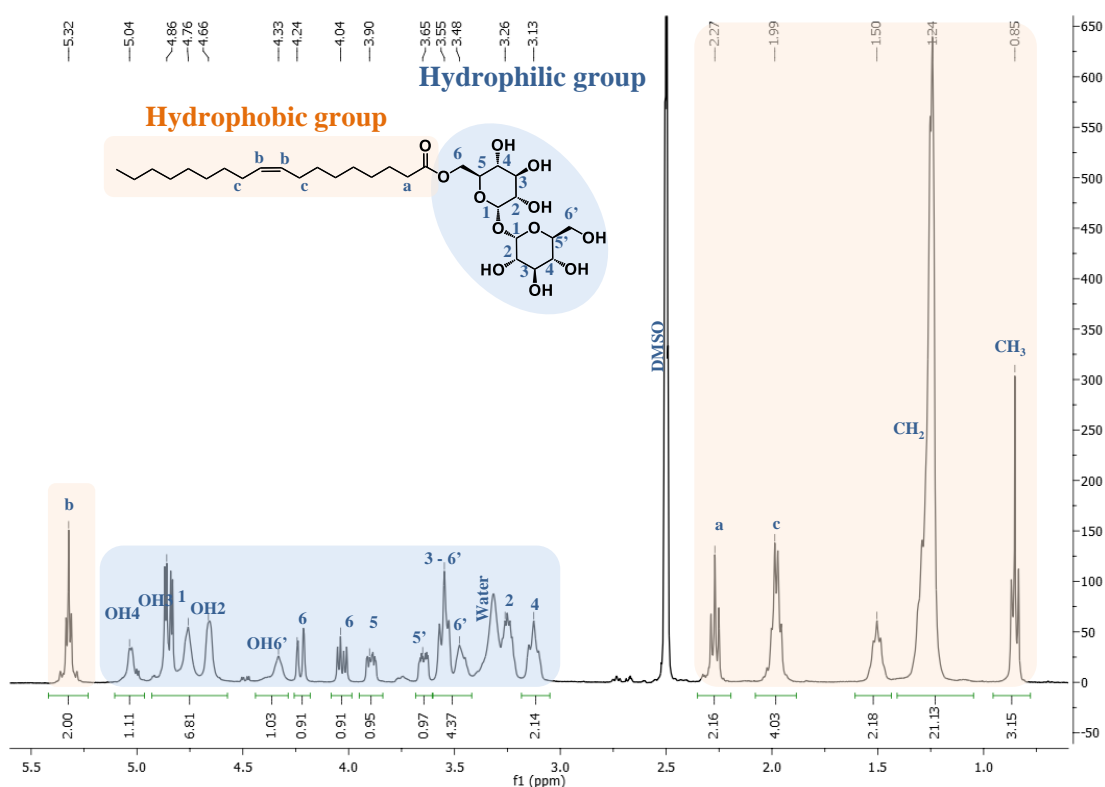


Figure 28: ^1H NMR spectrum of trehalose monooleate performed in DMSO-d_6 . Hydrophobic group is depicted in orange and hydrophilic group in blue.

The HLB determination for trehalose mono-12- hydroxystearate and trehalose mono-9,10-di-hydroxystearate were particular due the presence of hydrophilic moieties (hydroxyl group) in the hydrophobic part. Thus, the protons of the hydroxyl moieties and the ones nearby the hydroxyl were considered hydrophilic in the HLB calculation.

The HLB of the trehalose monoesters were calculated following the Griffin's formula; data are summarized in Table 3.

Trehalose monoesters	HLB
Trehalose monoundecenoate (C11:1)	13.42
Trehalose monostearate (C18:0)	11.21
Trehalose monooleate (C18:1 <i>cis</i>)	11.25
Trehalose monoelaidate (C18:1 <i>trans</i>)	11.25
Trehalose monolinoleate (C18:2)	11.29
Trehalose monoerucate (C22:1 <i>cis</i>)	10.30
Trehalose mono-12- hydroxystearate (C18:0; 1 –OH)	11.50
Trehalose mono-9,10-dihydroxystearate (C18:0; 2 –OH)	11.78

Table 3 : HLB of the trehalose monoesters calculated from Griffin's formula

All the monoesters exhibit HLB superior to 10 and proved to be fully soluble in water. In agreement with their HLB values comprised between 10.30 and 13.42, the trehalose monoesters could be oil-in-water emulsifier and form self-assemblies (micelles) in water.

2. Surface tension and critical micelle concentration (CMC)

Each trehalose monoesters was dissolved in deionized water at different concentrations. The air-water surface tensions were measured by the pendant drop method at 25°C. The surface tension was plotted as a function of the concentration. The CMC was determined graphically from the slope change in the surface tension versus the concentration plot (Figure 29). The surface tension data are summarized in

	CMC (g.L⁻¹)	CMC (mol.L⁻¹)	γ_{CMC} (mN.m⁻¹)
Trehalose Monoundecenoate	1.0	1.9×10^{-3}	40.2
Trehalose Monooleate	1.8×10^{-1}	2.9×10^{-4}	39.2
Trehalose Monoelaidate	6.0×10^{-1}	9.8×10^{-4}	40.2
Trehalose Monolinoleate	5.7×10^{-1}	9.4×10^{-4}	39.4
Trehalose Monoerucate	1.6×10^{-1}	2.4×10^{-4}	42.4
Trehalose mono-12- hydroxystearate	4.2×10^{-1}	6.7×10^{-4}	39.7
Trehalose mono-9,10-dihydroxystearate	4.6×10^{-1}	7.2×10^{-4}	40.2

Table 4.

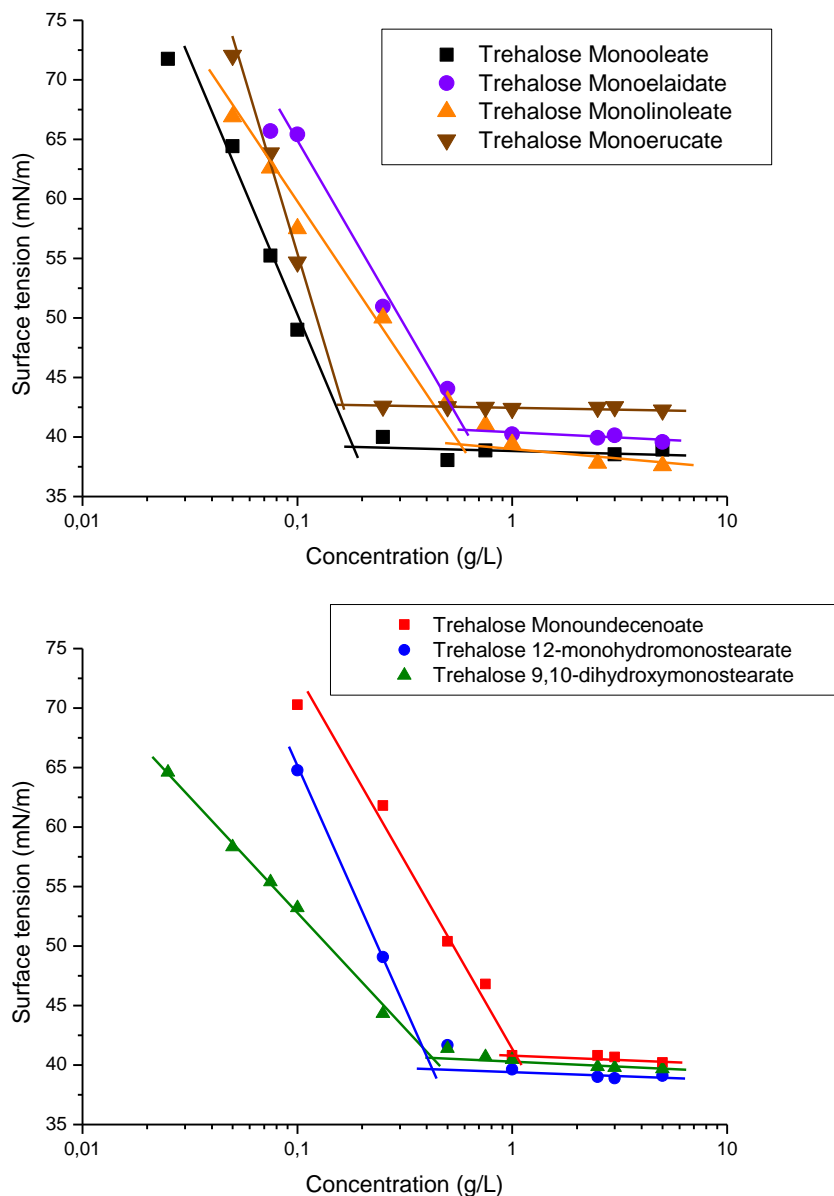


Figure 29: Surface tension of aqueous solution of trehalose monooleate, trehalose monoelaidate, trehalose monoerucate and trehalose monolinoleate (top) and trehalose monoundecenoate, trehalose 12-monohydroxymonostearate and trehalose 9,10-dihydroxymonostearate (bottom) at various concentrations

Trehalose monostearate was low soluble in water at 25°C, so the CMC of this monoester was not determined. In agreement with literature data,¹⁰ the CMC of the trehalose monoesters decreases with the increase of the fatty acid chain length. As expected, trehalose monoundecenoate with the shortest fatty acid chain (11 carbons) exhibits the highest CMC value (1 g.L⁻¹) and trehalose monoerucate (22 carbons) the longest one (0.16 g.L⁻¹). In the case of the trehalose monoesters bringing hydroxyl groups on the C18 fatty acid chain (trehalose mono-12-hydroxystearate and trehalose mono-9,10-dihydroxystearate), the latter should exhibit higher CMC values than the ones of monoesters without hydroxyl groups. Indeed, these

glycolipids are less hydrophobic due to the presence of the hydroxyl groups. However, trehalose monolinoleate and trehalose monoelaidate had higher CMC than trehalose mono-12-hydroxystearate and trehalose mono-9,10-dihydroxystearate. Due to the presence of unsaturation on these latter trehalose monoesters, it is difficult to evaluate the effect of the hydroxyl group on the CMC values. Trehalose monooleate with one unsaturation had lower CMC than trehalose monolinoleate which has 2 unsaturations. Thus, the CMC increases when the degree of unsaturation increases. An exception could be noticed concerning trehalose monoelaidate which exhibits a *trans* unsaturation. Indeed, the latter shows a higher CMC value than the one of trehalose monooleate (*cis* unsaturation) for a same number of unsaturation and carbon atoms on the fatty chain. This feature proves the effect of the stereochemistry of the fatty acid chain on the self-assembly properties. This lower CMC value of trehalose monoelaidate (containing the *trans* unsaturation) is difficult to explain but can be attributed to the spread out spatial conformation brought by the *trans* unsaturation.

All the monoesters have a surface tension at the CMC (γ_{CMC}) closed to 40 mN.m⁻¹. These values are very closed to the ones reported in the literature for trehalose monoesters¹³ (between 40 and 45 mN.m⁻¹)

	CMC (g.L ⁻¹)	CMC (mol.L ⁻¹)	γ_{CMC} (mN.m ⁻¹)
Trehalose Monoundecenoate	1.0	1.9×10^{-3}	40.2
Trehalose Monooleate	1.8×10^{-1}	2.9×10^{-4}	39.2
Trehalose Monoelaidate	6.0×10^{-1}	9.8×10^{-4}	40.2
Trehalose Monolinoleate	5.7×10^{-1}	9.4×10^{-4}	39.4
Trehalose Monoerucate	1.6×10^{-1}	2.4×10^{-4}	42.4
Trehalose mono-12- hydroxystearate	4.2×10^{-1}	6.7×10^{-4}	39.7
Trehalose mono-9,10-dihydroxystearate	4.6×10^{-1}	7.2×10^{-4}	40.2

Table 4: CMC values and surface tension properties of trehalose monoesters

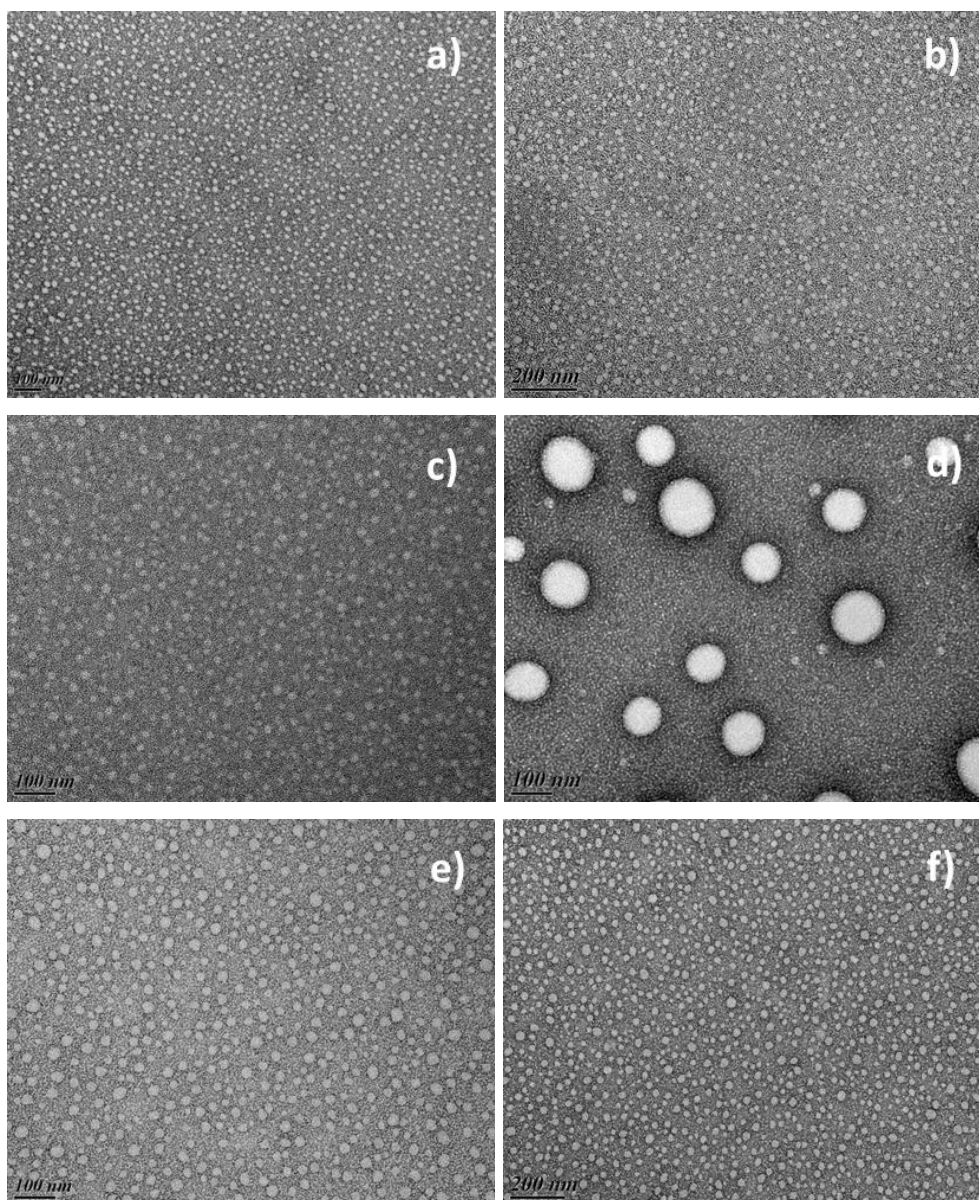
3. Micelle observation

The trehalose monoesters were dissolved in water at a concentration of 5 g.L⁻¹. These solutions were observed by TEM.

Apart from trehalose monoerucate, spherical micelles with diameters between 10 and 20 nm were observed by TEM (Figure 30). In the case of trehalose monoerucate, spherical micelles of around 30 nm could be observed, but larger spherical objects of around 150 nm could also

be detected (Figure 30d). These bigger spherical objects could be attributed to aggregates of small micelles.

The trehalose monoesters solutions were analyzed by dynamic light scattering (DLS). However the results were not relevant and could not be exploited due to the small size of the micelles observed in TEM. Indeed, they were in the limit of measurement of the DLS.



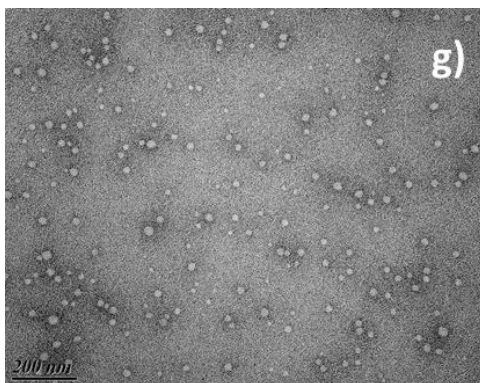


Figure 30: TEM images from dispersion of (a) trehalose monooleate, (b) trehalose monoelaidate, (c) trehalose linoleate, (d) trehalose monoerucate, (e) trehalose monoundecenoate, (f) trehalose 12-monohydroxymonostearate and (g) trehalose 9,10-dihydroxymonostearate in water

4. Experimental part

The surface tension was determined using the pendant drop method with a goniometer Krüss DSA100 equipped with a camera (J1H High Speed Camera) with 8420 CCD image sensor.

Image of micelles were obtained by TEM. For the Tem grids preparation, 3 μL of trehalose monoesters solution were dropped off on copper grids coated with formvar/carbon films. The, the grids were stained with a aqueous solution of uranyl acetate at 10 wt.%.

II. Study of gelation properties of trehalose monoerucate in water and trehalose diesters in vegetable oil

Numerous examples of gels are reported in the literature.¹⁴⁻¹⁸ A gel is 3D network of gelators in a solvent. Therefore, gelators have the ability to turn a liquid into a solid-like phase by trapping the solvent molecules in a 3D network. A gel can be physical or chemical. Physical gels are reversible and according to the chemical structure of the gelator molecules, their self-assembly is driven by weak interactions such as hydrogen bonding, electrostatic forces, π - π stacking or Van Der Waals bonds interactions. In chemical gels, the gelator molecules are linked covalently to form the 3D network, and the gels are irreversible.

There are a huge number of gelators described in the literature. The latter can be organic or inorganic and among the organic ones, there are two groups. Either polymeric gelators with

molar masses superior to $3000 \text{ g}\cdot\text{mol}^{-1}$ or low molecular weight gelators (LMWGs)^{19–21} with molar masses lower than $3000 \text{ g}\cdot\text{mol}^{-1}$. This thesis will focus exclusively on LMWG.

Gelation by LMWG is thermally reversible; gels formed are of physical type. Commonly the gelator molecules form a self-assembled fibrillar network (SAFiN). Thus, in this case, the network is composed of entangled crystalline fibrils of gelators. Gels are thus formed by the dissolution of small amount of gelator in a hot solvent (water for hydrogel and organic solvents for organogels). Then the mixture is cooled below the temperature of gelation which is the temperature from which the complete volume of solvent is immobilized and no flow is observed. Nevertheless, in some cases, the self-assembly is not fibrillar, the network is composed of non-crystalline entangled worm-like micelles.

Many gelators are generally amphiphilic molecules.¹⁷ Due to environmental issues, a growing interest is shown on the use of bio-based amphiphilic gelators to replace the petroleum-based ones.²² Thanks to biorefineries, a huge platform of bio-based tunable molecules can be produced and notably amphiphilic molecules. Numerous studies were conducted on the use of bio-sourced amphiphilic molecules as gelators.²³ As it is mentioned earlier, different sugar diesters are described in literature to be good gelators of water^{24,25} and organic solvents.^{3,26,27} Therefore, the ability of trehalose monoerucate to gelify water and the capacity of trehalose diesters to gelify vegetable oil are described in the next section of this chapter.

1. Gelation properties of trehalose monoerucate in water

As shown by TEM, trehalose monoerucate self-assembled into large spherical assemblies (around 150 nm) above the CMC. Interestingly, by increasing the concentration above $30 \text{ g}\cdot\text{L}^{-1}$ the viscosity of the solution increases until the formation of a gel. This ability of the trehalose monoerucate to gelify water was thus studied, as detailed in the following part.

1.1. Hydrogel preparation and minimal gelation concentration (MGC)

A series of hydrogels were prepared by adding a precise amount of trehalose monoerucate into water. After diluting the monoester in water under shaking at 80°C , homogeneous mixtures were obtained and cooled down to room temperature to obtain translucent gels. The bottles were inverted to confirm the gelation of water (Figure 31).



Figure 31: Hydrogel of trehalose monoerucate in water (5 wt%)

The minimal gelation concentration (MGC) was determined by diluting the gel until getting a solution after the gelation procedure (homogenization by heating and shaking, and then cooling). The MGC in water of trehalose monoerucate was found equal to 3 wt% (or 30 g. L⁻¹). In the cases of the other synthesized trehalose monoesters, the viscosity of the solution was increasing with the concentration of the glycolipid but no hydrogel could be obtained even by increasing the concentration up to 20 wt%.

1.2. Morphological study

Different types of gelators are reported in the literature.^{19,23,28–30} As trehalose monoerucate has a low molar mass, it belongs to the low molecular weight gelators (LMWG).^{14,15,19,23} Contrary to the pure trehalose monoerucate which is crystalline, no crystallization or melting could be observed with the hydrogel according to polarized optical microscopy and DSC analysis in hermetic pan (Figure 32). Thus, this system did not have a gel-to-sol transition temperature surprisingly.

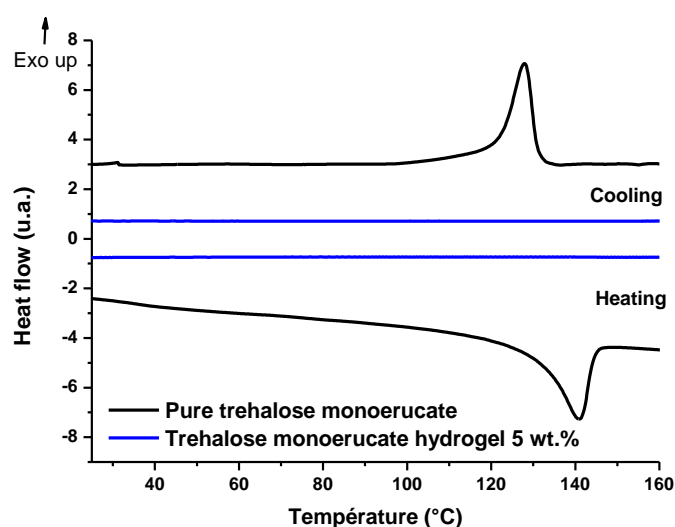


Figure 32: DSC curves of pure trehalose monoerucate (black) and hydrogels formed by trehalose monoerucate in water at 5wt.% (blue).

This thermal analysis proves that trehalose monoerucate does not self-assemble into a crystalline fibrillar network. At low concentration above the CMC (0.16 mg.mL^{-1}), this glycolipid could self-assemble into micelles leading to a gel at higher concentration (above 30 mg.mL^{-1}). Similar feature has been reported in the case of erucyl dimethyl amidopropyl betaine (EDAB)³¹ which self-assemble in a tridimensional network composed of entangled worm-like micelles. It could be an explanation how trehalose monoerucate self-assembles in water at high concentration.

The hydrogel at 5 wt.% were frozen by cryofixation at 2050 bars and -196°C and they were subsequently observed by cryo-SEM. On the cryo-SEM images (Figure 33). A 3D network of fibers is observed on the cryo-SEM images. This observation gives an information on the hydrogel morphology but this is not sufficient to demonstrate the formation of entangled wormlike micelles.

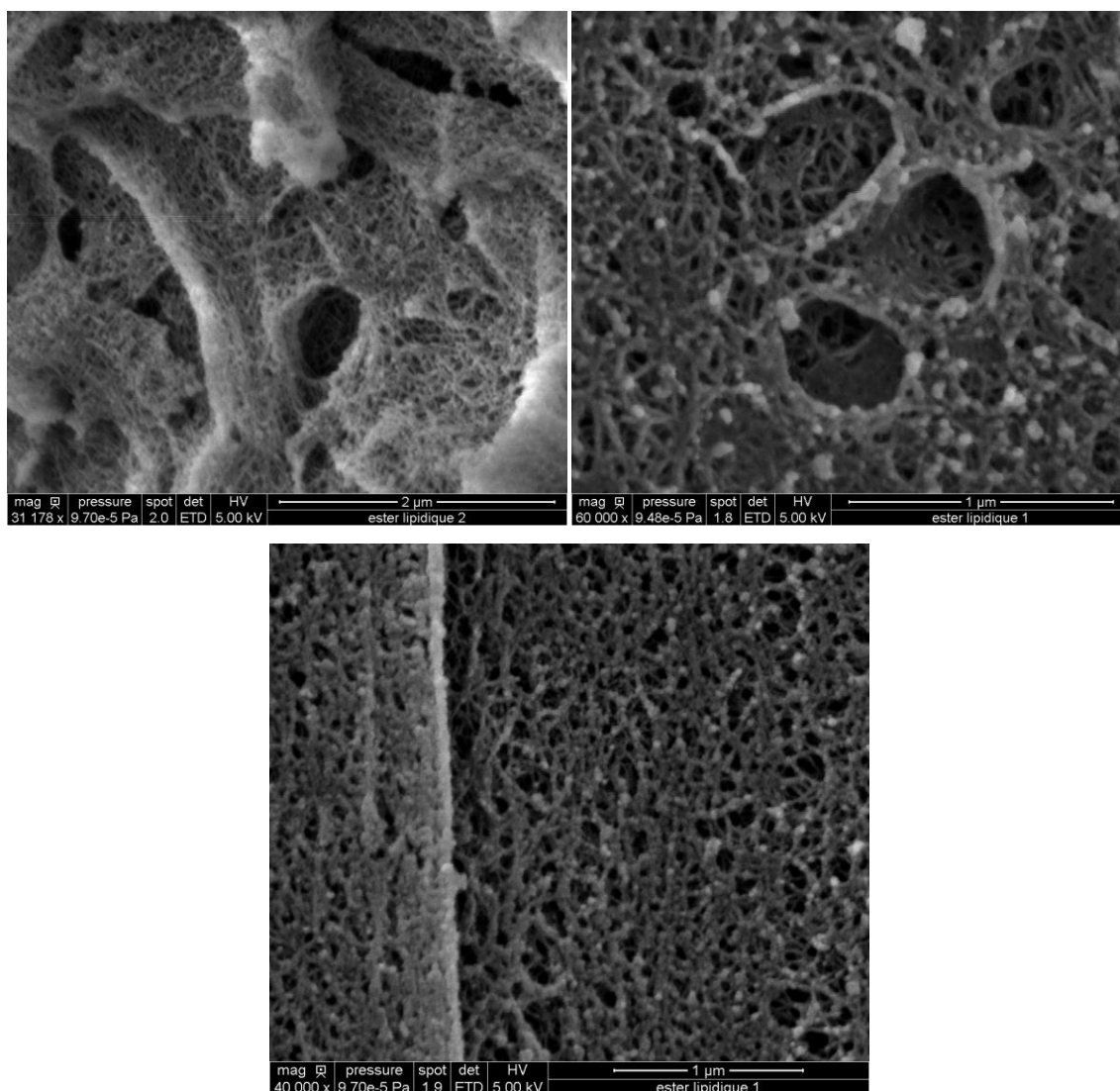


Figure 33: Cryo-SEM images of the xerogel of trehalose monoerucate at 5 wt%/v in water

1.3. Rheological behavior

Dynamic oscillatory rheology was performed on the hydrogel to determine several mechanical parameters: the storage modulus G' and the loss modulus G'' , corresponding to the solid and to the liquid viscous behaviors, respectively. First, an oscillatory strain sweep measurement was performed between 1 % and 1200 % at 1 Hz. The curves of G' and G'' were typical of a gel. The first region corresponds to an elastic solid behavior with G' predominant to G'' (linear viscoelastic region) (Figure 34, (region a)). Over this region, the G' value exceeded that of G'' . After this region a slightly increase of G'' value could be observed corresponding to a break of interaction points (Figure 34, (region b)) of the gel. Finally, the value of G'' became superior to the ones of G' but the two curves sharply decreased. In this last region, the gel was totally destroyed and flowed like a viscous liquid (Figure 34, (c)). These results can be compared with reported studies on wormlike hydrogels made from EDAB. Kumar *et al.*³¹ showed that hydrogel of EDAB exhibit G' values of 10 Pa, for a concentration of 50 mmol.L⁻¹. The trehalose monoerucate exhibit G' values of 10 Pa for a concentration of 75 mmol.L⁻¹ (= 5 wt.% = 50 g.L⁻¹).

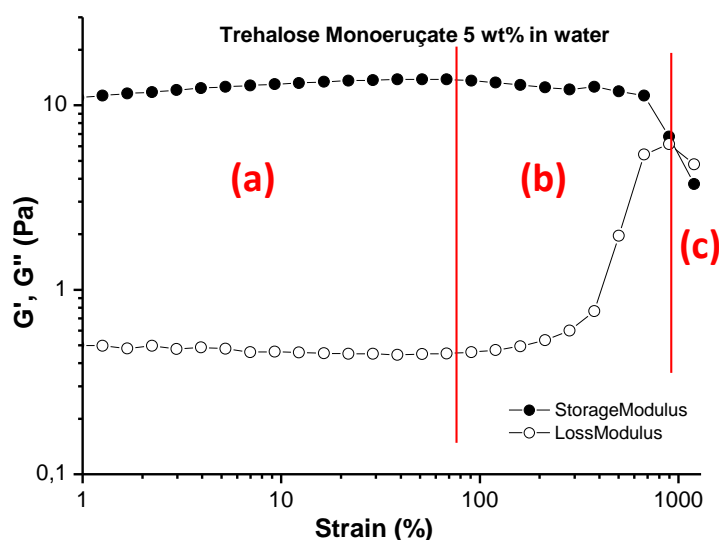


Figure 34: Oscillatory strain sweep of trehalose monoerucate in water (5 wt.%) performed at 1Hz. (a) Linear viscoelastic region, (b) break of entanglement points, (c) destruction of the gel.

Solutions with 3 different concentrations of trehalose monoerucate (1, 3 and 5 wt.%) were made in water and their rheological properties were analyzed. These measurements showed

that all the solutions have a characteristic response of gels. However at 1 wt.%, the concentration is below the MGC and no gel should be formed. The solution flows and does not be “solidified”. Thus, the gel formed at 1 wt% is a very soft gel.

The rheological measurements showed that G' and G'' values increased with the concentration (Figure 35). The increase of the gel strength with the concentration of gelators could be attributed to the density increase of the network. Higher amount of gelators in the oil leads to higher degrees of entanglement.

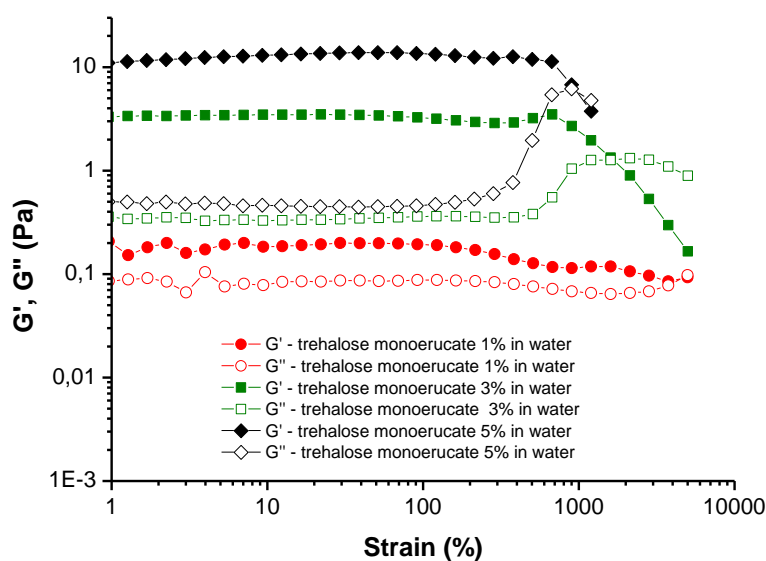


Figure 35: Oscillatory strain sweep of trehalose monoerucate at different concentrations in water performed at 1 Hz

We finally studied the thixotropic properties of the hydrogel. Thixotropy corresponds to the ability of a gel to recover its initial mechanical properties after a strong shear leading to its destabilization. A strong shear was applied on the hydrogel and then the G' and G'' values were measured during a low shear to evaluate the thixotropic properties. Thus, the hydrogel was sheared at a constant strain of 2000 % for 7 minutes in order to disrupt the gel. After this step, G' and G'' were then measured for a shear at a constant strain of 10 % (located in the linear viscoelastic region) as function of time (Figure 36). The curves of G' and G'' recovered their initial value after 13 minutes (16 Pa for G' and 0.5 Pa for G'') which means that the hydrogel was thixotropic.

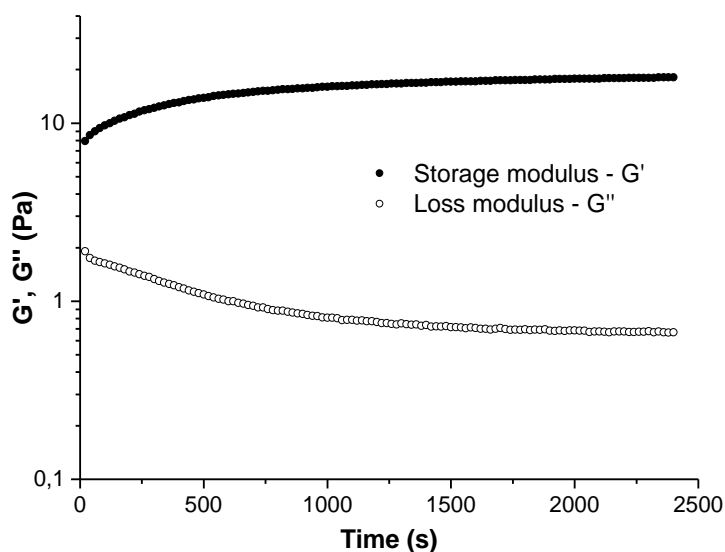


Figure 36: Mechanical properties recovery of gel with trehalose monoerucate in water at 5wt.%. G' and G'' values during sinusoidal strain sweep at 1 Hz of hydrogel after a shearing step at 2000 % at 1 Hz during 7 minutes

Conclusion

To conclude on this part, the self-assembly properties of different trehalose monoesters chemically synthesized have been studied. These glycolipids exhibit surfactant properties and self-assemble into micelles. It has been shown that the configuration (*cis* or *trans*) of the unsaturated fatty acid chain had an influence on the CMC value. One glycolipid, the trehalose monoerucate exhibits gelation properties in water. The structure of the hydrogels obtained still need to be studied further. The hydrogels obtained present two very interesting characteristics for future developments: they are transparent and show a thixotropic feature.

2. Gelation properties of trehalose diesters in vegetable oils

As the trehalose diesters had two fatty acid chains linked to the trehalose, they are too hydrophobic to self-assemble in water. This feature could be confirmed by the HLB which were lower than 10 proving the insolubility in water. The HLB were calculated following the same method as for trehalose monoesters; data are summarized in Table 5.

Trehalose diesters	HLB
Trehalose diundecenoate (C11:1)	10.0
Trehalose distearate (C18:0)	7.78
Trehalose dioleate (C18:1 <i>cis</i>)	7.81
Trehalose dielaidate (C18:1 <i>trans</i>)	7.81
Trehalose dilinoleate (C18:2)	7.85
Trehalose dierucate (C22:1 <i>cis</i>)	6.92
Trehalose di-12- hydroxystearate (C18:0; 1 –OH)	8.30
Trehalose di-9,10-dihydroxystearate (C18:0; 2 –OH)	8.78

Table 5: HLB of the trehalose diesters calculated from Griffin's formula

Nevertheless, the latter were able to gelify some organic solvent and vegetable oils. The study of the gels prepared with trehalose diesters in some vegetable oils is thus described in the next section.

2.1. Oleogel preparation and MGC determination

The physical gels were prepared by adding a precise quantity of trehalose diesters to a desired solvent (vegetable oils and pharmaceutical grade oils). To that purpose, the diesters were dissolved in the solvent by stirring the mixture at 130°C, temperature allowing the dissolution of the diesters in the vegetable oils. After getting a homogeneous solution, the mixture was cooled down to room temperature. The sample vials were inverted to confirm the gel formation (Figure 37).

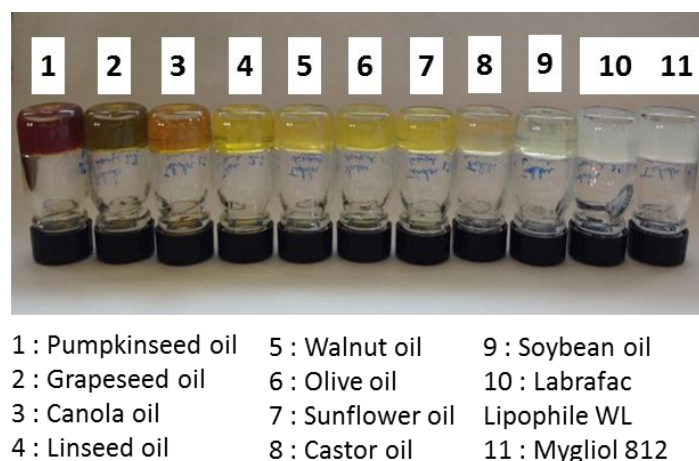
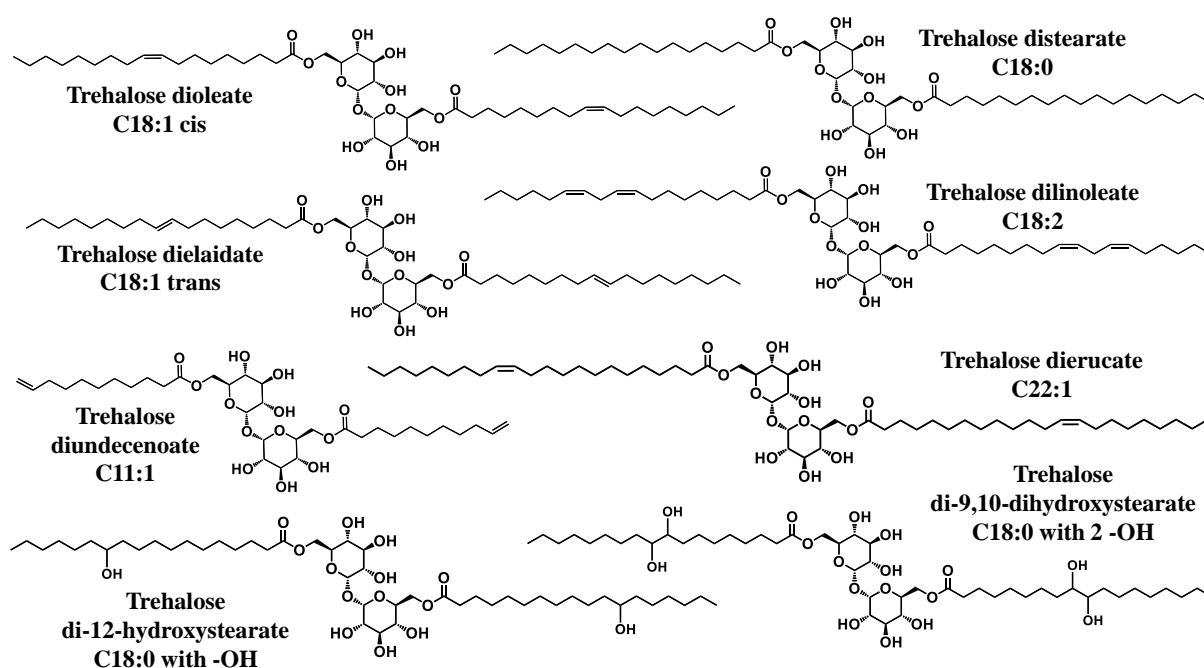


Figure 37 : Gels obtained from different oils and trehalose dioleate as gelator (2wt%/v)

As an example, trehalose dioleate was used to gelify various vegetable oils and two pharmaceutical oils (Figure 37). In the following part, only the gels obtained with three vegetable oils (olive, linseed, and castor) were studied in more details and in order to get better understanding on the gelation mechanism and properties. Indeed, these three oils have been selected due to their different composition, the olive oil is rich in oleic acid (C18:1 *cis*), the linseed oil in linoleic acid (C18:2) and the castor oil in ricinoleic acid (C18:1-OH).

As already described in the previous chapter, a platform of trehalose diesters has been synthesized. Different fatty acids have been chosen to evaluate the influence of the lipophilic structure on the gel properties.



Scheme 29: Platform of the synthesized trehalose diesters

To evaluate the efficiency of these trehalose diesters as gelators, the MGC of each diester was determined in the three vegetable oils previously cited. As already explained, the minimal gelation concentration (MGC) was determined by diluting the gel until getting a solution after the gelation procedure (homogenization by heating and shaking, and then cooling). The data are summarized in Table 6.

All the glycolipids displayed excellent gelation properties in vegetable oil with low MGC values around 0.5 wt.%/v. Gels were obtained in the three selected vegetable oils except for 12-monohydroxy- and 9,10-dihydroxy-distearate in olive and linseed oils. Indeed, these two glycolipids revealed fully soluble in olive and linseed oils up to 20 wt.% while they could

gelify castor oil, this latter oil being essentially composed of ricinoleic acid (C18:1(OH)). The structure similarity of the vegetable oil and of the glycolipid appears to be an important parameter with respect to the gelation phenomenon; i.e. the gelation of castor oil being only possible with trehalose 9,10-dihydroxy-distearate and trehalose 12-monohydroxy-distearate.

Minimal gelation concentration [wt%/v]			
	Olive oil	Linseed oil	Castor oil
Trehalose diesters	(majority C18:1)	(majority C18:2)	(majority C18:1(OH))
Trehalose dioleate	G (0.75)	G (0.5)	G (0.5)
Trehalose distearate	G (0.75)	G (1.0)	G (0.5)
Trehalose di-12-hydroxystearate	S	S	G (0.5)
Trehalose di-9,10-dihydroxystearate	S	S	G (1.0)
Trehalose diundecenoate	G (0.5)	G (0.5)	G (0.25)
Trehalose dieruçate	G (0.5)	G (0.5)	G (0.5)
Trehalose dilinoleate	G (0.5)	G (0.5)	G (0.25)
Trehalose dielaidate	G (0.5)	G (0.5)	G (0.25)

Table 7: MGC values of gels in olive, linseed and castor oils with different trehalose diesters as gelators. (G: gel, S: soluble).

2.2. Morphological study by microscopy, DSC and XRS

Due to the presence of the vegetable oil, the observation of the physical gel by optical microscopy or scanning electron microscopy (SEM) was difficult. Indeed, it was not possible to remove the vegetable oil to well observe the network without disrupting the gel structure. However, the synthesized trehalose diesters could also gelify some organic solvents such as ethyl acetate. Thus organogels in ethyl acetate (EtOAc) at 2 wt%/v were prepared and EtOAc were evaporated to obtain xerogels. Therefore, as referred in the literature for such kind of gel,¹ the observation of the xerogels was possible by optical and scanning electron microscopy after evaporation of the organic solvent. The xerogels were observed in optical microscopy by phase contrast microscopy and dark field microscopy and they were also observed by SEM. Observation in optical microscopy revealed that trehalose diesters self-

assembled in a 3D-network of fibers (Figure 38). Such morphology was irrespective of the trehalose diester used for the gelation.

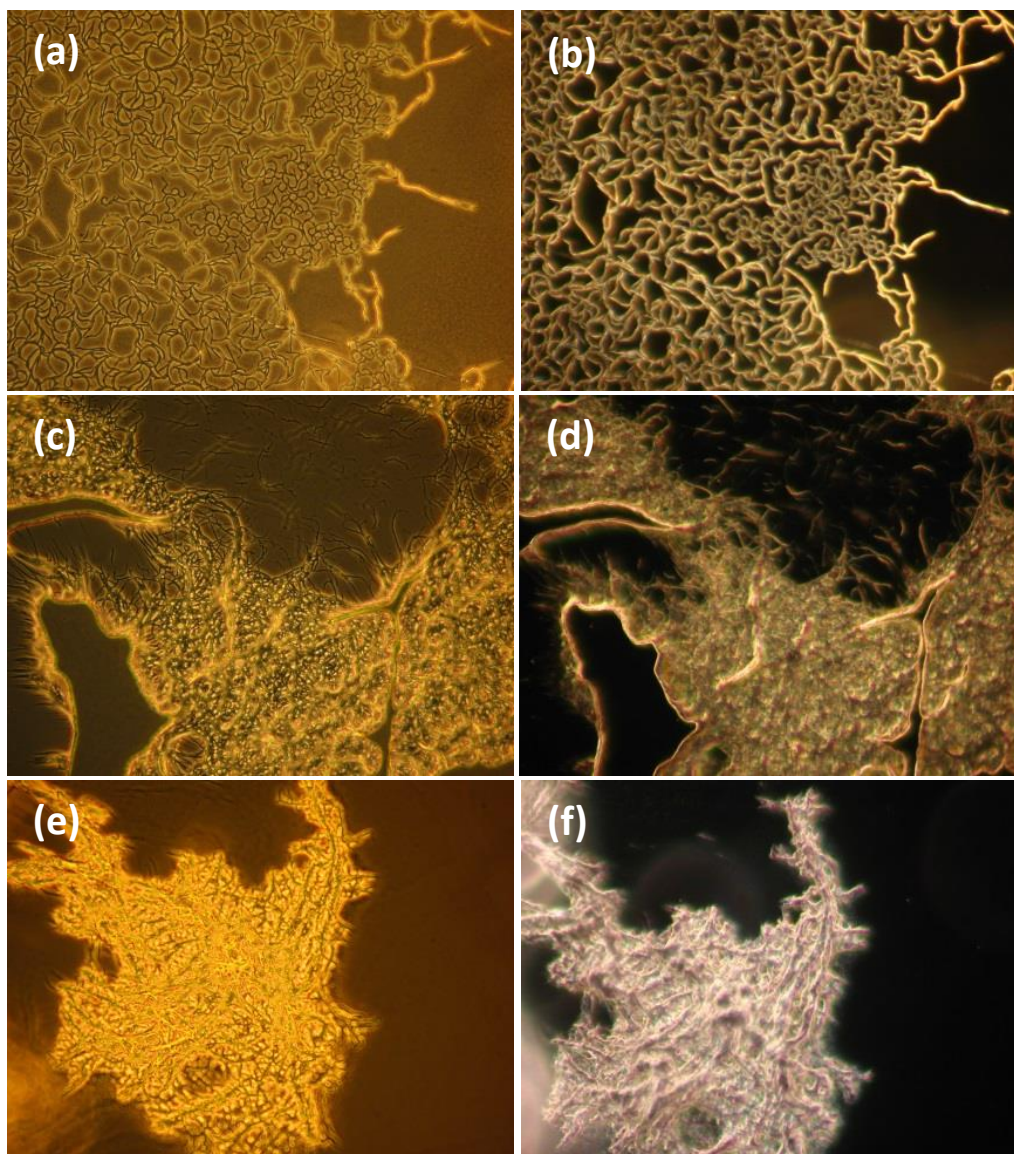


Figure 38 : Optical micrograph of xerogel of trehalose dioleate 2 wt%/v in EtOAc observed in phase contrast (a) and observed in dark field (b); Optical micrograph of xerogel of trehalose dielaidate 2 wt%/v in EtOAc observed in phase contrast (c) and observed in dark field (d), Optical micrograph of xerogel of trehalose dioleate 2 wt%/v in EtOAc observed in phase contrast (e) and observed in dark field (f)

A crystalline fibrous network was also observed by SEM (Figure 11). Thus, the trehalose diesters form a self-assembled fibrillar network (SAFiN). However, it was difficult to evaluate the size of the fibers. Indeed, the fibers are always resulting from the assembly of smaller fibrils.

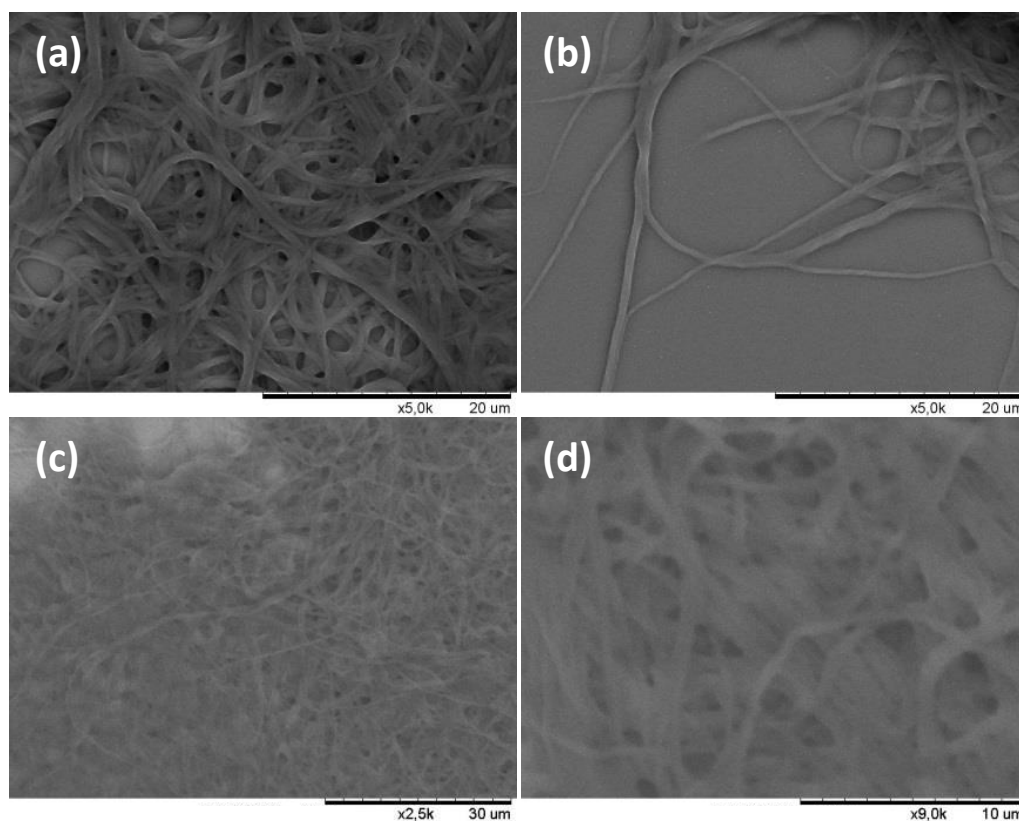


Figure 39 : SEM images of xerogel of trehalose dielaidate 2 wt%/v in EtOAc (a) and (b), SEM images of xerogel of trehalose dioleate 2 wt%/v in EtOAc (c) and (d)

DSC was also performed on the oleogels to determine the temperature $T_{\text{gel-sol}}$ corresponding to the gel-to-sol transition temperature. This temperature is attributed to the melting of the crystalline tridimensional network (solid content of a gel).

It was reasonably observed that such $T_{\text{gel-sol}}$ increases with the concentration of gelator, phenomenon explained by an increase of the 3D network. The $T_{\text{gel-sol}}$ were comprised between 100 and 120 °C in castor oil and between 120 and 140 °C in olive and linseed oils and appeared irrespective of the gelator used (Table S1, page 139).

X-Ray scattering (XRS) was performed on gels in castor oil and on the pure corresponding trehalose diester (Figure 40 and Table 8. The XRS patterns of the gels show well-ordered long d-spacing values (between 2.2 and 4.8 nm) which differ with respect to the trehalose diester employed. Moreover the d-spacing values of the gels were relatively close to the ones observed for the pure trehalose diesters. Thus, the self-assembly of trehalose diesters in vegetable oils was relatively close to the one of the pure trehalose diesters. These results are

similar to those reported concerning the self-assembly of trehalose diesters in organic solvents or other sugar diesters in vegetable oils.^{1,2}

	molecular length^a of pure trehalose diesters	d-spacing pure trehalose diesters	d-spacing Gel in castor oil (5 wt%/v)
Trehalose Dioleate	4.5	3.2 nm	3.3 nm
Trehalose Distearate	4.3	3.8 nm / 3.2nm	4.8 nm / 3.3 nm
Trehalose Diundecenoate	1.9	2.2 nm	2.3 nm
Trehalose Dierucate	5.2	4.25 nm / 3.7 nm	4.5 nm / 3.7 nm
Trehalose Dilinoleate	3.6	3.2 nm	3.2 nm
Trehalose Dielaidate	4.8	4.21 nm / 3.18 nm	4.11 nm / 3.28 nm

Table 9: Characteristic distance obtained from XRS analysis in pure trehalose diesters and in corresponding gel made in castor oil at 5 wt%. ^aEnd-to-end length of the trehalose diesters determined by 3D modelization and after energy-minimized calculations

John and co-workers^{1,2} showed that the d-spacing distances in the gel observed by X-Ray was almost the same as the molecular length of sugar diesters determined by molecular modelling studies using energy-minimized calculations. Based on this, they proposed an arrangement of the sugar diesters in organic solvents or vegetable oils. Thus, the sugar diesters are stacked into multilayers to form some fibers. The combination of these fibers leads to fibrous (bigger fibers). By entanglement of these fibrous, a 3D network is obtained. The driving force of this self-assembly is the formation of hydrogen bonds between each trehalose moieties, but also with Van der Waals interaction between the fatty acid chains.

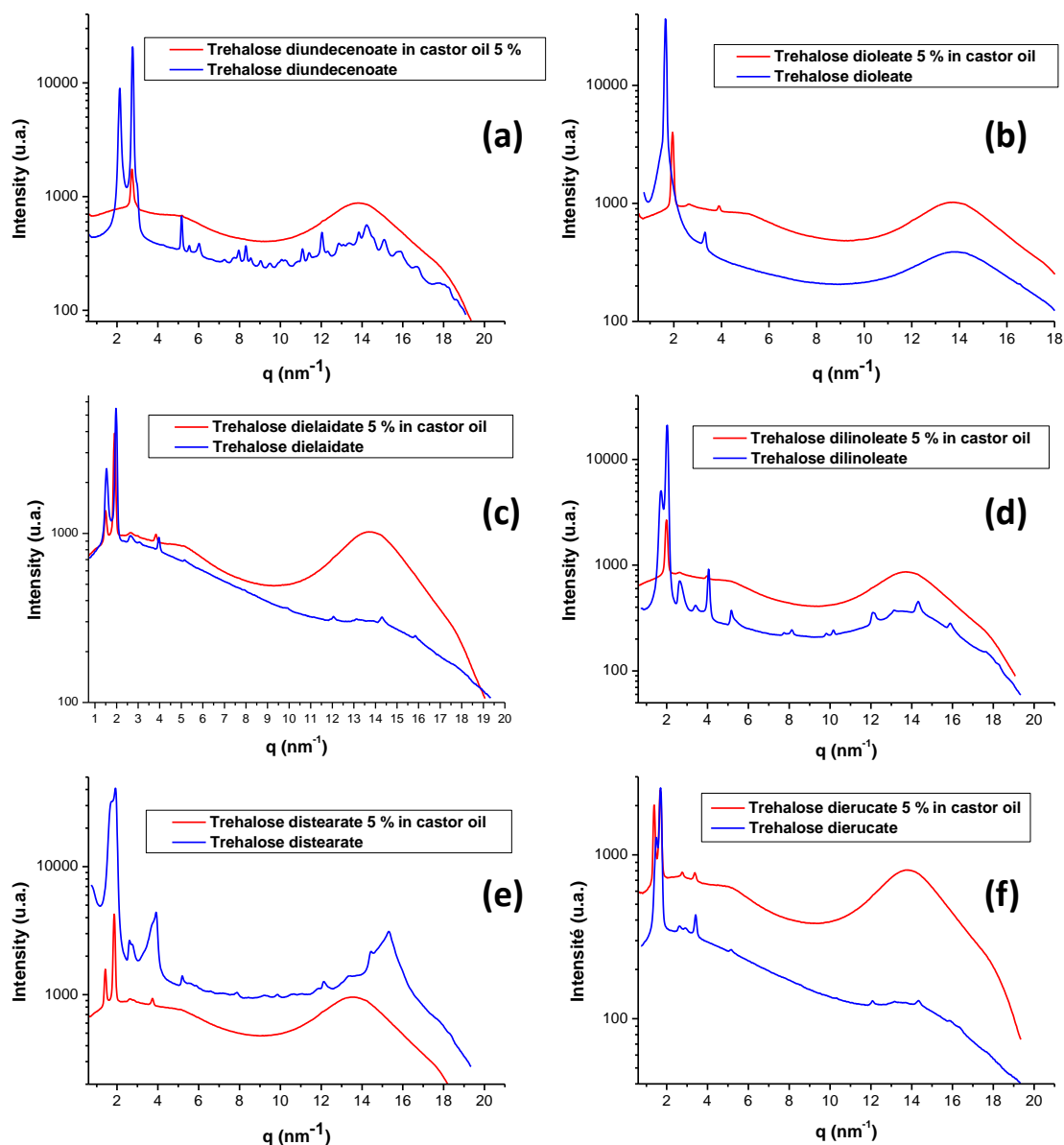


Figure 40: X-Ray spectra of pure trehalose diesters (blue curve) and of castor oil gel (5wt.%) of corresponding trehalose diesters (red curve) using (a) Trehalose diundecenoate, (b) Trehalose dioleate, (c) Trehalose dielaidate, (d) Trehalose dilinoleate, (e) Trehalose dierucate

The trehalose diesters have been modeled in 3 dimensions on Chem3D (a 3D molecule editor). The molecular length of the trehalose diesters have been measured after energy-minimized calculations (Figure 41). It was observed that the modelled molecular length of the trehalose diesters was almost the same as the d-spacing distance of the pure glycolipids and the d-spacing of the gels, respectively. Thus, the arrangement of sugar diesters in vegetable oils proposed by John's group could be applied to the trehalose diesters. Indeed, the latter self-assemble in stacked lamellar multilayers. This stacking generates some fibers leading to the tridimensional network (i.e. the gel) (Figure 42).

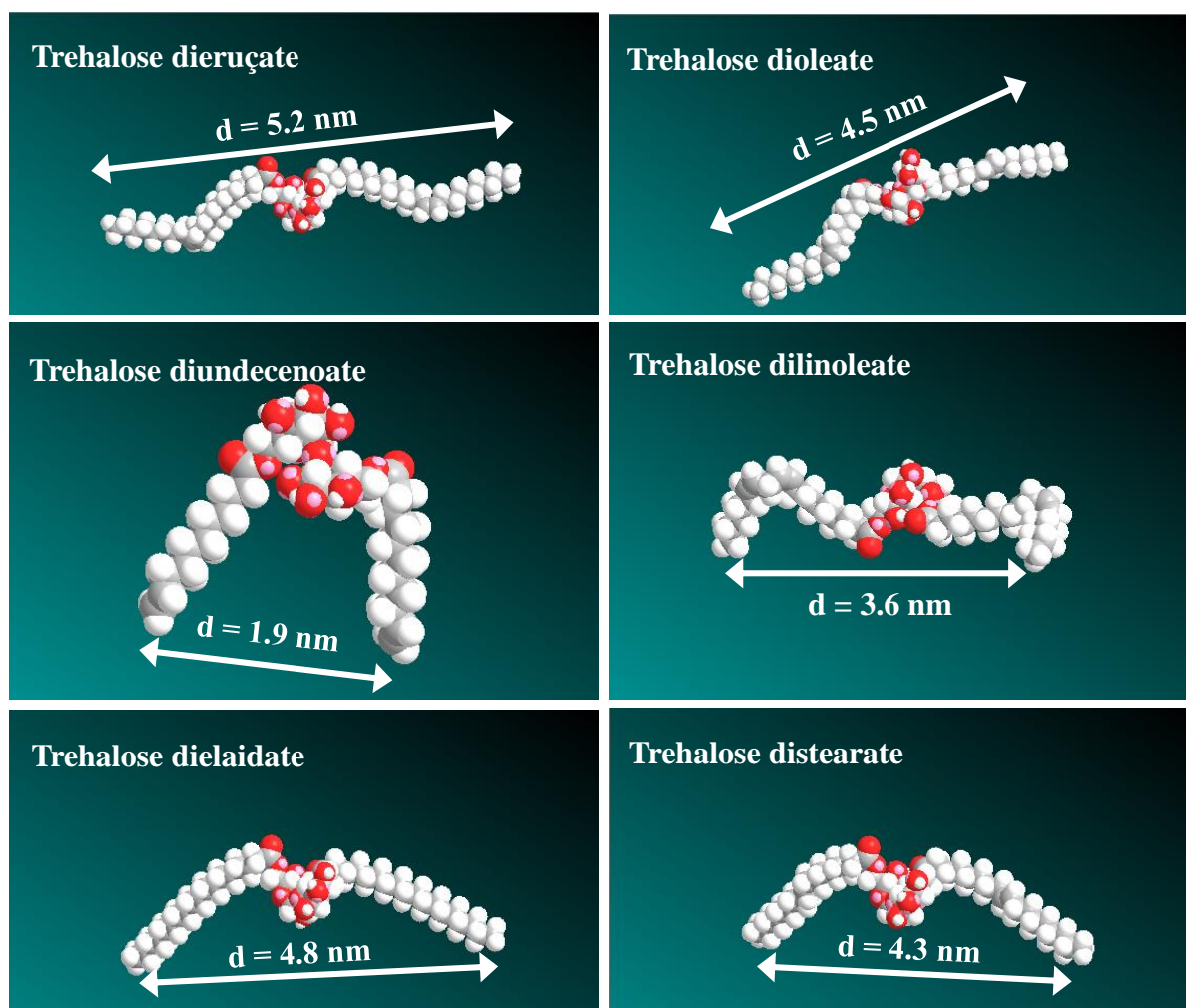


Figure 41: Tridimensional representation of trehalose diesters on Chem3D after energy-minimized calculation

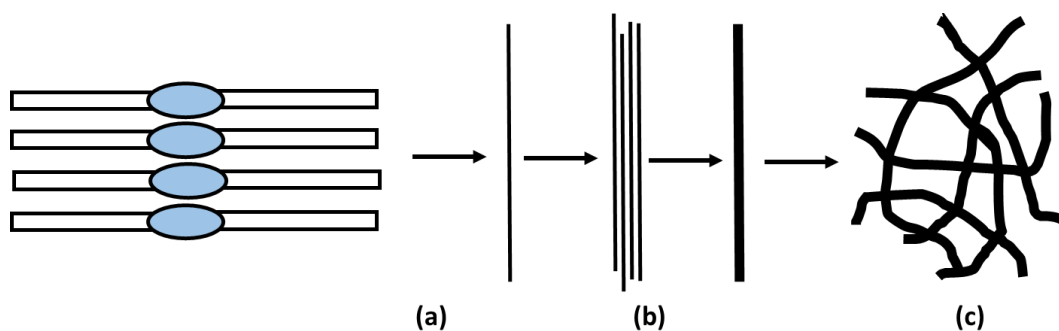


Figure 42: Model of glycolipid self-assembly in the gel. (a) multilayer lamellar stacking to form fibers, (b) aggregates of fibers to form larger ones, (c) entanglement of the fibers to form the 3D network

2.3. Rheological study of the oleogels

As previously described for the hydrogel, dynamic rheology was performed on the oleogels. First, an oscillatory strain sweep measurement was performed between 0.01 % and 100 % at a

constant frequency of 1 Hz. Here again, the curves of G' and G'' were typical of a gel. The first region corresponds to an elastic solid behavior (Figure 43, (a)). After this region a slightly increase of G'' value could be observed (Figure 43, (b)) and finally, the value of G'' became superior to the ones of G' but the two curves sharply decreased (Figure 43, (c)) corresponding to the deconstruction of the gel.

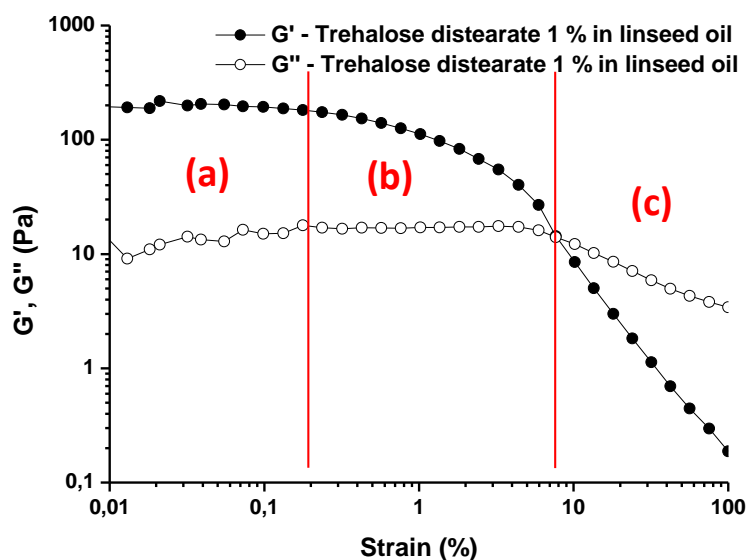


Figure 43: Strain sweep measurement of organogel of trehalose distearate in linseed oil at 1 wt%. (a) Linear viscoelastic region, (b) break of entanglement points, (c) destruction of the gel.

It could be noticed that the organogels obtained with the trehalose diesters were stronger than the hydrogel formed with the trehalose monoerucate. Indeed, G' and G'' values of the organogels were more than 100 times superior compared to the values of the hydrogels.

An oscillatory frequency sweep measurement was then performed between 0.02 and 10 Hz at a constant strain of 0.1 %. The frequency curves showed that G' stayed higher to G'' which is a typical response for gels. This behavior is in agreement with the structure of a gel and shows that rheological properties were independent with the frequency.

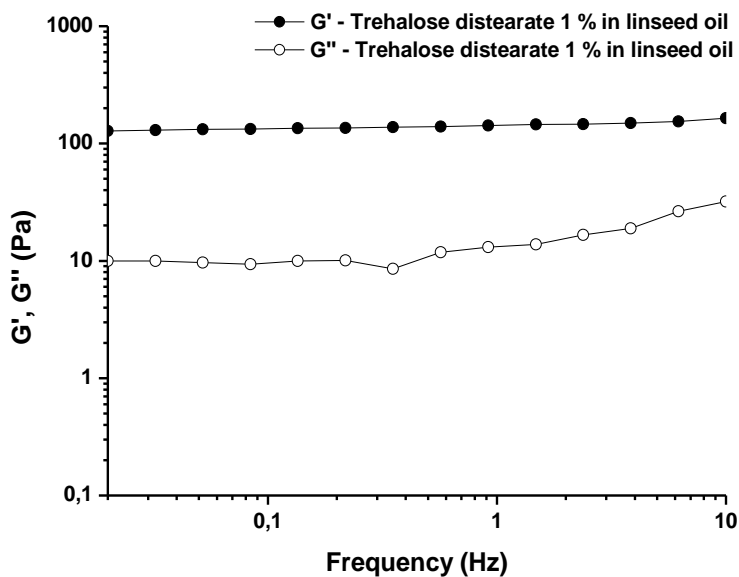


Figure 44: Frequency sweep measurement of organogel of trehalose distearate in linseed oil at 1wt.%

It was easier to see the end of the linear viscoelastic domain by measuring the shear stress variation as a function of the strain. The shear stress stayed linear throughout the linear viscoelastic domain (between 0.01 % and 0.18 % in Figure 45, (regiona)). From this curve, the yield strain noted σ_y can be evaluated as the strain corresponding to the end of the elastic domain.

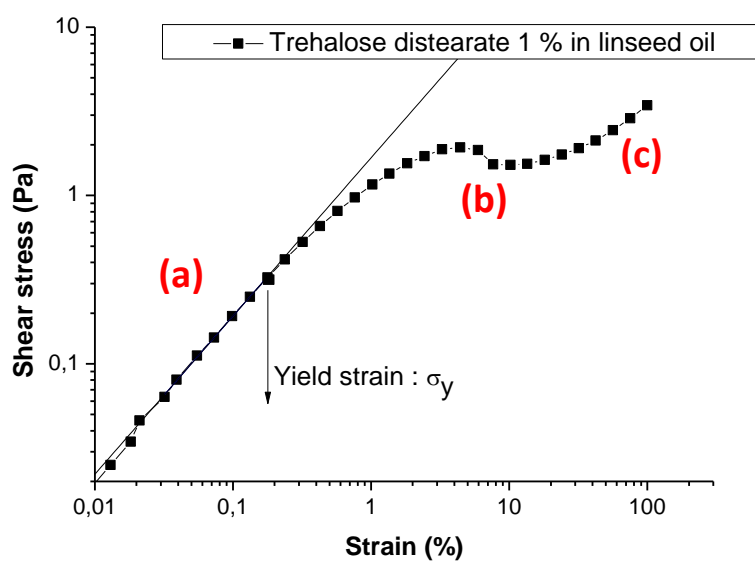


Figure 45: Shear stress curve of trehalose distearate at 1 wt.% in linseed oil obtained from strain sweep

Above the linear domain (Figure 45, (region a)), a decrease of the shear stress was observed (Figure 45, (region b)) and then the shear stress increased again (Figure 45, (region c)). Normally, during a shearing, the stress should not decrease. The same phenomenon has been already reported and was called “shear banding”.³² At the molecular level, two phases are in equilibrium into the gel: the solvent which is liquid and the network which is solid. However, at the macroscopic level, the gel seems to be homogeneous and one phase is observed. When a shearing is applied, the equilibrium is disrupted and the tridimensional network is broken. Thus, the macroscopic phase was subdivided locally into two phases with different viscoelasticity properties. This phenomenon could be observed macroscopically with the apparition of aggregates and with an oil layer at the surface of the gel. Therefore, the measurements obtained above the linear viscoelastic domain were not reproducible and not really relevant. Indeed, this subdivision of the macroscopic phase could not be controlled. Effects of parameters such as the concentration of the gelators, the length and the structure of the lipidic chain on the rheological properties were examined by comparing the G' and G'' values in the viscoelastic domain and the yield strain σ_y .

2.3.1. Effect of the concentration of gelators

Gels with 3 different concentrations of gelator (1, 3 and 5 wt.%) were made in the three selected vegetable oils (olive oil, linseed oil and castor oil). The following 6 gelators: trehalose dioleate, trehalose distearate, trehalose diundecenoate, trehalose dierucate, trehalose dilinoleate and trehalose dielaidate were investigated. Strain sweep and frequency sweep measurements were performed on these gels. These measurements showed that G' and G'' values increased and that σ_y decreased with the concentration (Figure 46). This behavior was observed for all gelators in the three oils tested. The increase of the gel strength with the concentration of gelators could be attributed to the density increase of the network. Higher amount of gelators in the oil leads to higher degrees of entanglement. In that case, gels are stronger, so the rheological parameters (G' and G'') are amplified. However, the gels are less elastic and thus less resistant to strain. This feature could explain the decrease of σ_y with the concentration of gelator.

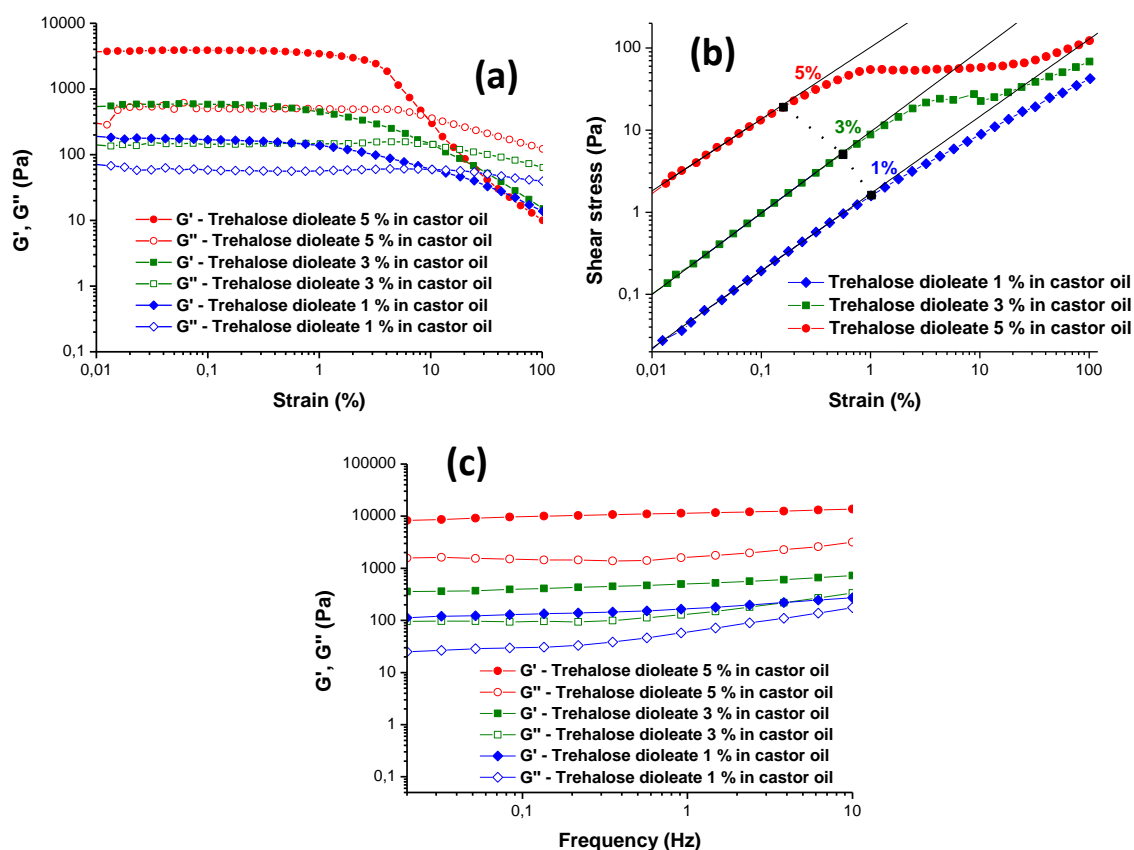


Figure 46: Effect of the concentration of trehalose dioleate on the oscillatory strain sweep (a and b), and on the oscillatory frequency sweep (c). Gel at 5 wt.% in castor oil (red curve), at 3 wt.% (green curve) and at 1 wt.% (blue curve).

2.3.2. Effect of the lipidic chain structure

The effect of the structure of the fatty acid chain on the rheological properties of the gel was studied. The effect of the *cis-trans* stereochemistry of the double bond present on trehalose diesters was first studied (Figure 47). Trehalose dielaidate and trehalose dioleate having one unsaturation with respectively a *trans* or a *cis* configuration showed similar yield strain. However, gels formed with the trehalose dielaidate (*trans* configuration) showed higher G' than the ones formed with the trehalose dioleate. Thus, the *trans* configuration brings more hardness to the gels. It could be explained by the difference of the spatial chain arrangement. Indeed, in the case of the *trans* configuration, the lipidic chain can be extended contrarily to the situation of the *cis* configuration, where the lipidic chain is bent. This behavior was validated for the three selected vegetable oils investigated.

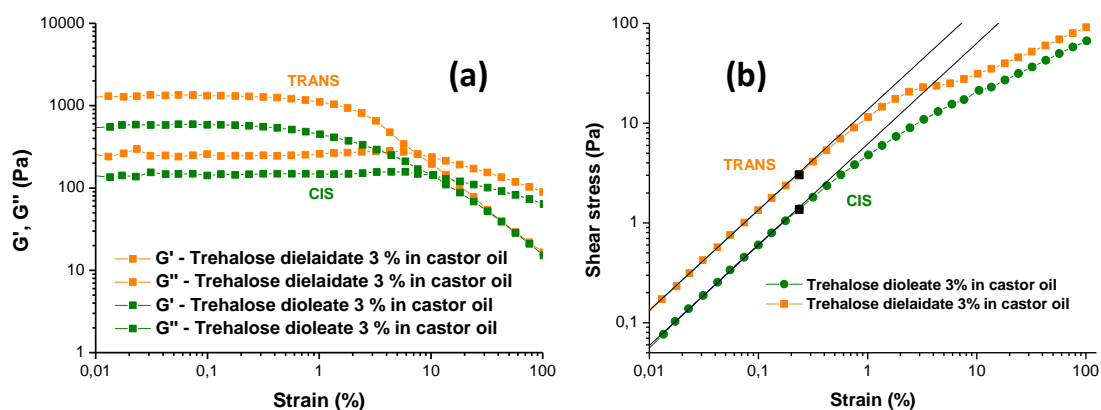


Figure 47: Effect of the *cis-trans* configuration on the rheological properties of oleogel in castor oil. Oscillatory strain sweep performed on gel made from trehalose dioleate (a) and trehalose dielaidate (b) in castor oil at 3 wt.%

The effect of the number of unsaturations of the lipidic chain on the rheological properties of the gel was then determined. Indeed, the trehalose diesters could have no unsaturation, one or two double bonds with a *cis* configuration, i.e. the trehalose distearate, trehalose dioleate and trehalose dilinoleate, respectively. The saturated trehalose diester exhibited higher G' and G'' with a lower σ_y than the unsaturated diesters for each oil. Stronger gels were thus obtained with the saturated glycolipids. This confirmed the statement exposed before with the *trans* unsaturated trehalose dielaidate. Indeed, here again, the saturated glycolipid could be totally extended in the oil contrarily to the unsaturated ones, which are bent due to the *cis* configuration of their double bond. The trehalose diesters with two unsaturations on their lipidic moieties have higher G' values than the mono-unsaturated one. Such feature is difficult to explain. Indeed, the fatty acid chains with two *cis* unsaturations (trehalose dilinoleate) should be more bent, than the mono unsaturated one (trehalose dioleate).

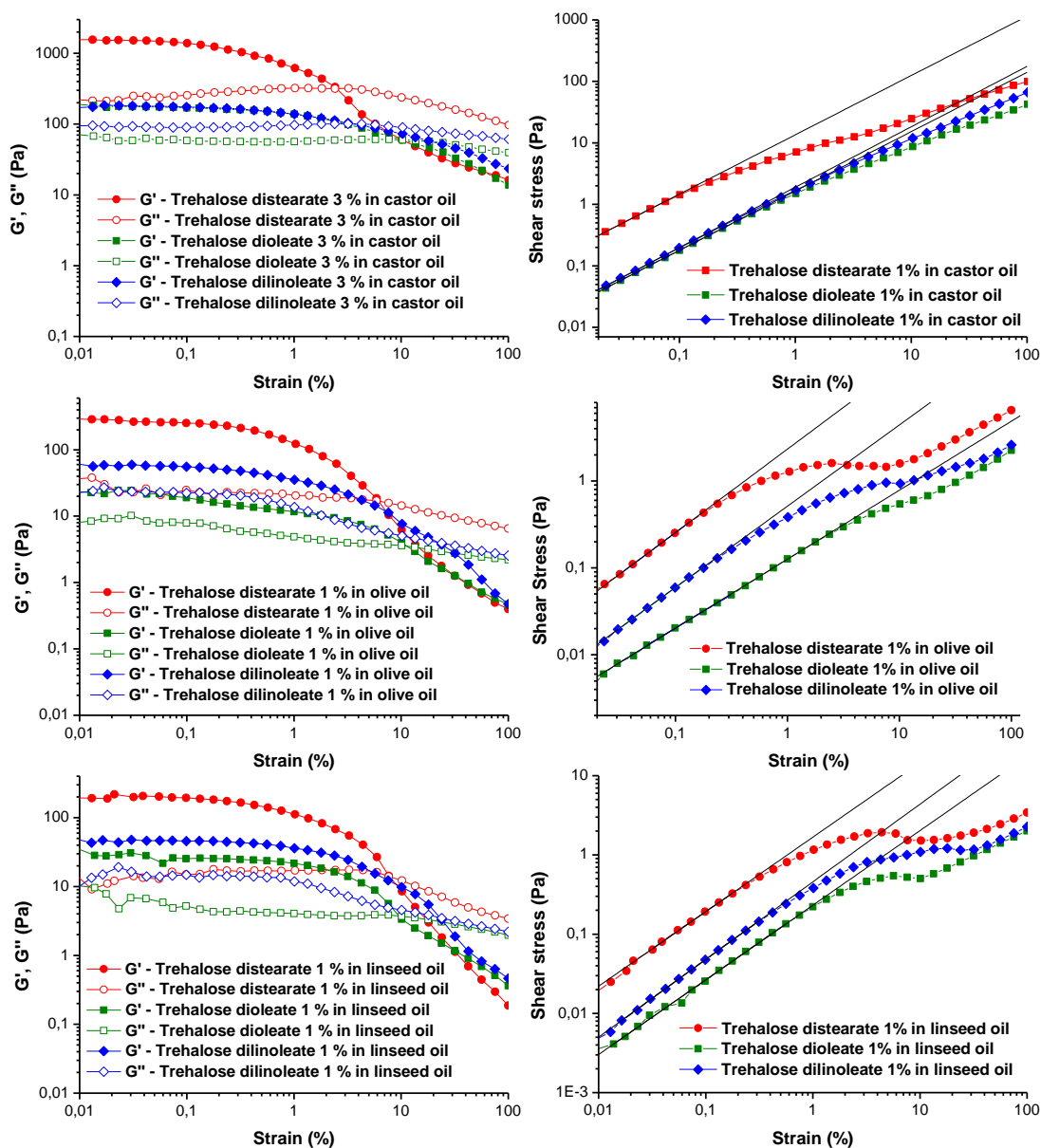


Figure 48 : Effect of the unsaturation number of the alkyl chain on rheological properties of gel. Oscillatory strain sweep performed on gel

Finally, the effect of the lipidic chain length was investigated. Three trehalose diesters with different lipidic chain length were compared: trehalose diundecenoate (C12), trehalose dioleate (C18) and trehalose dierucate (C22). The shortest diester (Figure 49, black curve) showed higher G' and lower σ_y than the two others. Short lipidic chains seem to favor the formation of gels with higher mechanical properties. Indeed, the gels prepared with trehalose dioleate (C18) (Figure 49, green curve) and trehalose dierucate (C22) (Figure 49, purple curve) exhibit lower mechanical properties. G' and G'' values of trehalose dioleate and trehalose dierucate were 100 times inferior than the ones of trehalose diundecenoate. This

could be explained by the presence of the internal double in *cis* configuration which bent the lipidic chain.

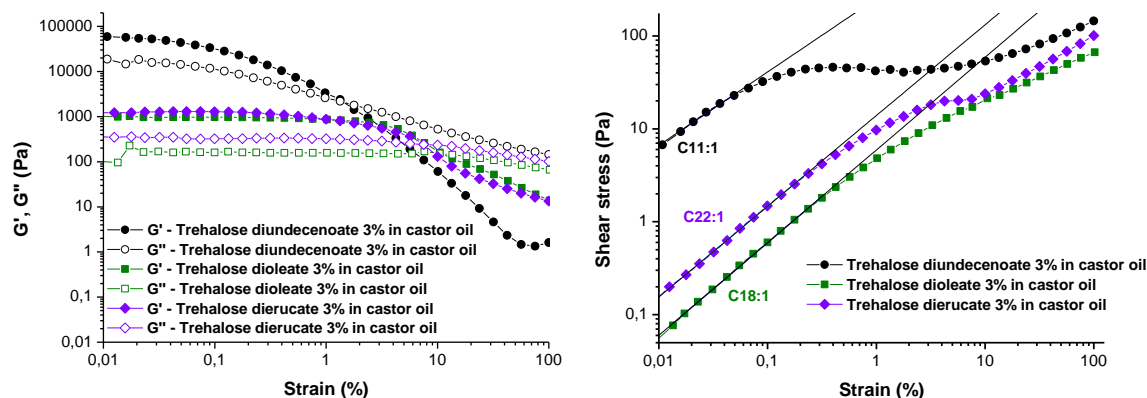


Figure 49: Effect of the lipid chain length on the rheological properties of gels. Oscillatory strain sweep performed on gel made from trehalose diundecenoate (black curve), trehalose dioleate (green curve) and trehalose dierucate (purple curve) in castor oil at 3 wt.%

3. Experimental part

Preparation of the gels

Gels were prepared by adding the selected weight percentage of trehalose diesters in oil (1, 3 and 5 wt % / v based on oil). The mixture of gelator and oil was homogenized at 130 °C under agitation. When the mixture was homogeneous and translucent, the sol was cooled to room temperature to obtain the gel. To confirm the formation of the gel, the vial containing the mixture was inverted.

Characterization of the gels

Gelation efficiency was determined with the MGC (minimal gelation concentration). The concentration of gelator was decreased in the gel by adding a small volume of oil. If after the oil addition, the gel is still formed (the mixture did not fall by inverting the vial), more oil was added. The MGC is reached when, after adding oil, the mixture flowed by inverting the vial.

The gel-to-sol transition temperature was determined by DSC. Gelation mechanism was studied by using X-Ray Scattering performed on pure glycolipids and on corresponding gels obtained in castor oil at 5 wt.%, optical microscopy and SEM. Microscopy sample was prepared by deposit of the gel prepared in ethyl acetate as solvent. The latter then removed by drying other night to obtain a xerogel. For the SEM analysis, the sample was coated with gold during 40 s at 50 mA.

Rheological measurement

Dynamic rheological measurements were performed on a rheometer equipped with a cone and plate geometry. The bottom plate was used to vary the temperature by Peltier effect. To assure the reproducibility and to not destroy the gel during its positioning on the bottom plate, the latter was dropped off and heated above the $T_{\text{gel-sol}}$ then cooled down to room temperature. It was let at rest during 10 minutes before performing the rheological measurements.

Strain sweep was performed from 0.01 % to 100 % with a constant shear frequency of 1 Hz.

Frequency sweep was performed from 10 Hz to 0.02 Hz with a constant shear strain of 0.1 %.

III. Conclusions and perspectives

In this chapter, trehalose monoesters have shown some surfactant properties. Surface tension and CMC in water were determined. The CMC ranged between 0.16 and 1.0 g.L⁻¹ according to the lipidic chain nature of the trehalose monoester but the surface tension values at the CMC were all around 42 mN.m⁻¹. The latter self-assembled as spherical micelles in water which were observed by TEM. At concentration higher than 30 g.L⁻¹, the trehalose monoerucate with the largest lipidic chain was able to gelify water. It was speculated that the hydrogel was a tridimensional network of entangled large micelles formed by the glycolipid. The rheological properties of this hydrogel were studied showing a thixotropic behavior.

Trehalose diesters are not soluble in water, but they exhibited self-assembly properties in vegetable oils and organic solvents. The ability of the trehalose diesters to gelify 3 vegetable oils (olive oil, linseed oil and castor oil) was then studied. Microscopy and X-ray scattering allowed determining the gel morphology. The so-formed organogels were a tridimensional network composed of crystalline fibers. The fibers were constituted of stacked multi-layer lamellas of trehalose diesters. The rheological properties were also investigated, showing an influence of the lipidic chain nature. It was observed that short chain length favored the formation of harder gels (higher G' and G'' values) but also more brittle under a strain (lower σ_y). Moreover, saturated diesters compared to unsaturated ones favored also the formation of harder gels with high G' and G'' values. In addition, the firmness of the gel increased with the concentration of the gelator due to an increase of the gelator concentration. However the values of G', G'' and σ_y are also very dependent on the vegetable oil and they cannot be predicted. Therefore, it should be necessary to evaluate the rheological properties for each diester depending on the oil to reach the best properties according to specific application.

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V. Supporting information

Glycolipids	Vegetable oil	wt. %		
		1	3	5
		T _{gel-to-sol} (°C)		
Trehalose Distearate (C18:0)	Olive oil	131	133	136
	Linseed oil	131	132	134
	Castor oil	nd	nd	124
Trehalose Dioleate (C18:1)	Olive oil	119	132	124
	Linseed oil	122	124	125
	Castor oil	nd	111	114
Trehalose Dilinoleate (C18:2)	Olive oil	nd	112	120
	Linseed oil	Nd	115	123
	Castor oil	97	104	104
Trehalose Dielaidate (C18:1 trans)	Olive oil	nd	124	126
	Linseed oil	nd	124	127
	Castor oil	94	105	120
Trehalose Diundecenoate (C11:1)	Olive oil	134	136	140
	Linseed oil	nd	131	140
	Castor oil	102	118	119
Trehalose Dierucate (C22:1)	Olive oil	nd	103	103
	Linseed oil	nd	114	118
	Castor oil	nd	106	nd

Table S1: Gel-to-sol transition temperature of gel prepared with trehalose diesters at various concentration

Chapter 4: Towards new polymers from trehalose diesters

Key words: Trehalose diesters, polyurethane, poly(hydroxyurethane), ADMET, Thiol-ene polymerization, sulphur oxidation, self-assembly polymers

Mots clés: Diesters de trehalose, polymérisation par métathèse de diènes acycliques, polymérisation par thiol-ène, oxydation des soufres, polymères auto-assemblés

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Introduction

In this chapter, the derivatization and the polymerization of the trehalose diesters synthesized in the previous chapters is described. Trehalose diesters were either modified in order to synthesize polyurethanes, poly(hydroxyurethane)s or directly polymerized by acyclic diene metathesis or thiol-ene reaction.

I. Polyurethanes (PUs)

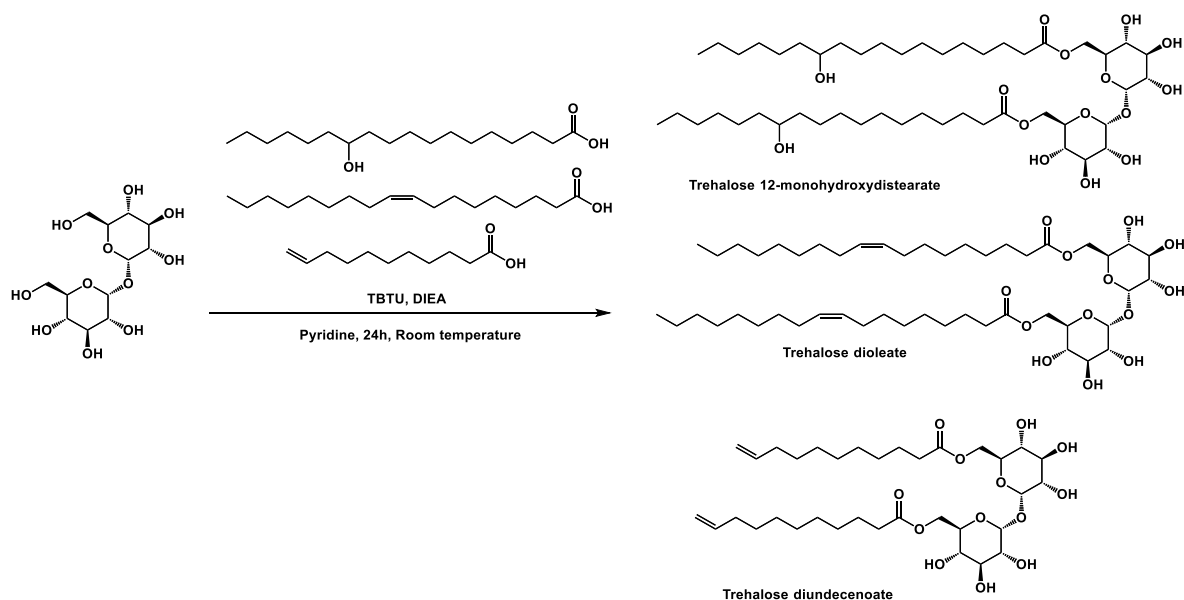
Polyurethanes (PUs) have the ability to be shaped into different forms as thermosets (foams, adhesives, coatings) thermoplastic elastomers, fibers, etc. Thanks to their large potential, PUs are employed in many applications and notably in medicine because of their biocompatibility. However it is very difficult to degrade them thanks to their stability to hydrolysis. The insertion of sugar moiety or ester function in the polymer backbone could represent an interesting alternative to enhance their biodegradability.

Numerous examples of sugar-derived polyurethanes are described in the literature. Different carbohydrates were polymerized with diisocyanates to obtain polyurethanes and poly(urea-urethane)s^{1,2} Hashimoto *et al.* have also reported the synthesis of polyurethanes from saccharidic lactones and the authors studied their degradation.³ They showed that these polyurethanes decomposed easily in phosphate buffer, so these polymers could be used as degradable polymer materials. Recently, xylitol was used to synthesize polyurethanes with HMDI and MDI. In contrast to polyurethanes without hydroxyl groups, polyurethanes synthesized from xylitol exhibited degradation in aqueous buffer.^{4,5} More recently, isosorbide was used to synthesize polyurethanes with a fatty acid-based diisocyanates.⁶ These novel PUs were almost fully synthesized from renewable resources (92 wt.%). Many other studies have been reported on the use of fatty acids for the synthesis of polyurethanes for their sustainability and biodegradability.⁷ Nevertheless, few polymers synthesized from glycolipids are reported in the literature. Our group has described the use of sugar esters as polyols for the synthesis of thermoplastic and thermoset polyurethanes.⁸

Based on this work, we have decided to employ a series of original diesters of trehalose in order to get access to novel sugar-based polyurethanes.

1. PU synthesis, molecular structure and dimension

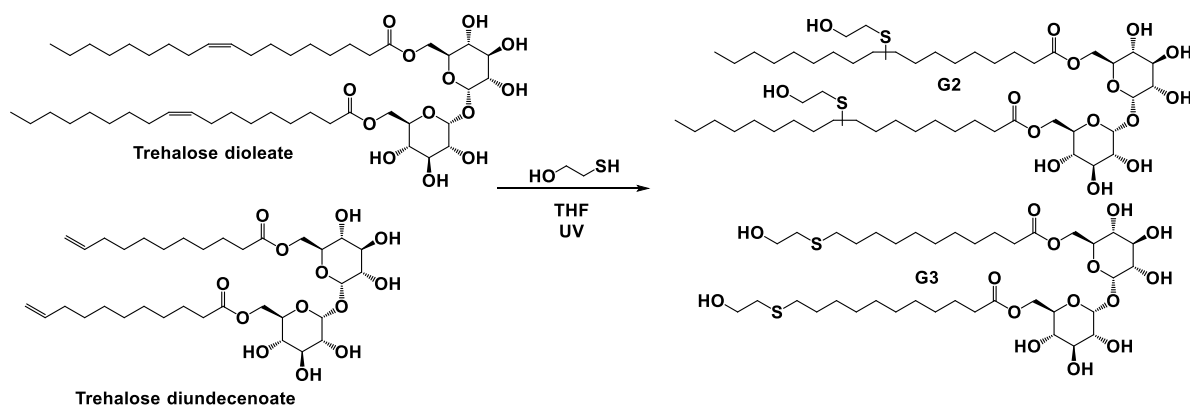
Three trehalose diesters were first prepared by the protective group-free esterification described in the chapter 2. After purification by flash chromatography, three pure trehalose diesters were obtained, trehalose di-12-hydroxystearate, trehalose dioleate and trehalose diundecenoate (Scheme 30).



Scheme 30: Synthetic pathway of trehalose 12-monohydroxydistearate, trehalose dioleate and trehalose diundecenoate

In the next step, trehalose dioleate and trehalose diundecenoate were hydroxylated by thiol-ene addition of 2-mercapto-ethanol onto the double bond of the fatty chain moiety.

The thiol-ene addition is a radical addition between a thiol and an alkene. The thiol-ene reaction was performed in THF under UV irradiation at room temperature. The completion of the reaction was followed by ^1H NMR spectroscopy in DMSO-d_6 with the disappearance of the double bond proton peak (at 5.6 ppm for internal double bond, and 5.7 and 4.9 ppm for the terminal double bond) (Figure 50). After purification, two polyols were obtained (G2 and G3) (Scheme 31).



Scheme 31: Thiol-ene modification of trehalose dioleate and trehalose diundecenoate with mercaptoethanol

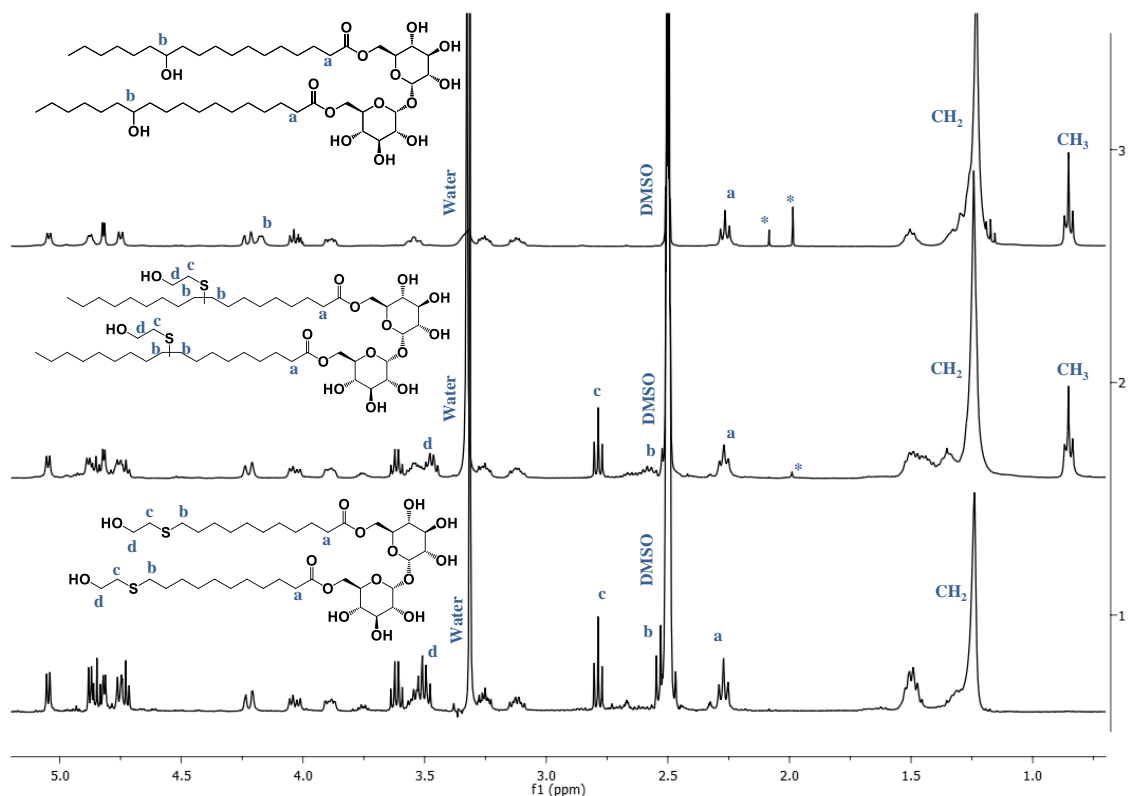
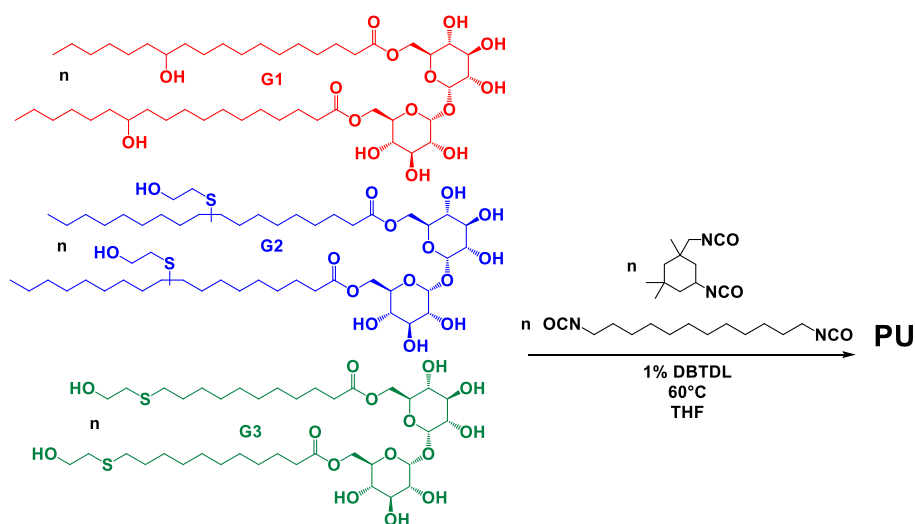


Figure 50 : Stacked ^1H NMR spectra of monomer G1 (top), G2 (middle) and G3 (bottom) performed in DMSO-d_6 . (*) Impurities.

The latter glycolipid monomers were then polymerized in THF at 60°C with two different diisocyanates, an aliphatic one, the 1,12-diisocyanatododecane (DIC12) and a cyclo-aliphatic one, the isophorone diisocyanate, (IPDI) (Scheme 32). The DIC12 is an aliphatic diisocyanate selected to introduce flexibility in the PUs whereas the cyclic IPDI was selected to introduce rigidity in the polyurethanes.



Scheme 32 : Synthetic pathway to polyurethanes synthesis from monomers G1, G2 and G3

PU	Glycolipid	Diisocyanate	\bar{M}_n (g.mol ⁻¹)	\bar{D}^a	T _g (°C)	T _{d5%} (°C)
PU1	G1	DIC12	5500	8.7	nd	224
PU 2		IPDI	8600	6.2	96	274
PU 3	G2	DIC12	4300	4.0	nd	210
PU 4		IPDI	3900	2.2	64	225
PU 5	G3	DIC12	5400	6.0	nd	262
PU 6		IPDI	4300	4.1	70	235

Table 10 : Properties of the PUs synthesized from glycolipids in THF. (nd) not determined

The so-formed glycolipids have eight hydroxyl functions, one on each fatty chain and 6 on the sugar moiety. Thus, to get linear polyurethanes, a NCO/OH_{total} ratio equal to 0.25 was chosen with the goal that only the hydroxyl groups of the fatty chain react with the diisocyanates. The formation of the PUs was monitored by FTIR spectroscopy with the disappearance of the isocyanate band at 2250 cm⁻¹ and the appearance of the urethane band at 1530 cm⁻¹ (Figure 51 and Figure 52). After 6 h reaction, no more isocyanate band appeared in the FTIR spectrum (see Figure 51), which indicates the completion of the polymerization.

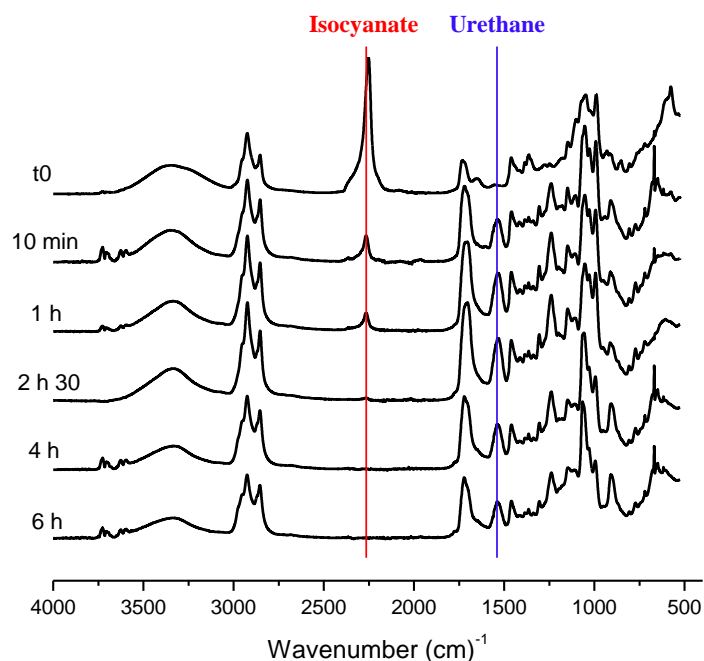


Figure 51 : PU synthesis monitored by FTIR spectroscopy of glycolipid G2 with IPDI

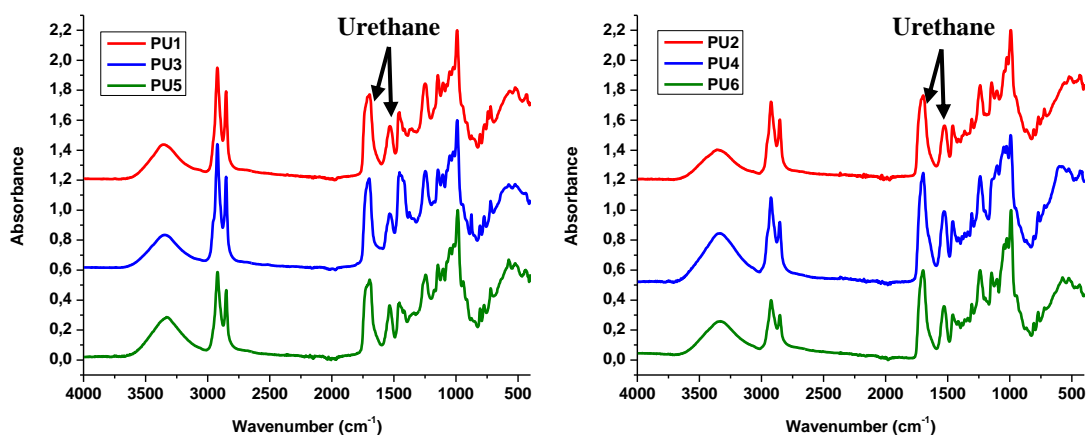


Figure 52 : Stacked FTIR spectra of PU synthesized from DIC12 (left), and PU synthesized from IPDI (right) with monomers G1 (red), G2 (blue) and G3 (green)

The so-formed PUs are soluble in DMSO and DMF so they are not cross-linked. They were characterized by ¹H NMR spectroscopy in order to see whether the hydroxyl groups of the sugar moiety also reacted with the isocyanate functions. The presence of signals attributed to these OH groups in the PU spectrum seems to indicate that the latter did not participate to the polymerization. However, it is difficult to integrate these peaks (between 3.00 and 4.30 ppm on ¹H NMR spectra) (Figure 54) and to really evaluate if the secondary hydroxyls of the sugar didn't react in a certain proportion. It is worth mentioning that hydroxyl groups of the fatty chain of G2 and G3 are primary alcohols while the ones from the sugar moiety are secondary. This can logically explain that isocyanate preferably reacted with the more reactive primary hydroxyl groups linked to the fatty chains. In the cases of PU1 and PU2 synthesized from G1, the reaction of diisocyanate with the hydroxyl group from the sugar can be envisaged as G1 possesses only secondary alcohol groups. Thus, diisocyanates could react with any hydroxyls of this glycolipid. On the ¹H NMR spectra of PU1 and PU2, peaks corresponding to the protons of the hydroxyls of the sugar moiety are not so distinct. Thus, it is complicated to confirm a possible reaction of diisocyanates with alcohol functions from the sugar moiety. The formation of PU was confirmed by the appearance of the signal of the urethane proton (–NH–COO–) at 7.07 ppm. The presence of urea was also observed on ¹H NMR spectra (urea proton peak –NH–COO–NH– at 5.75 ppm). The formation of urea could be due to the presence of water traces in the trehalose diesters, as trehalose moiety is hygroscopic and difficult to dry.

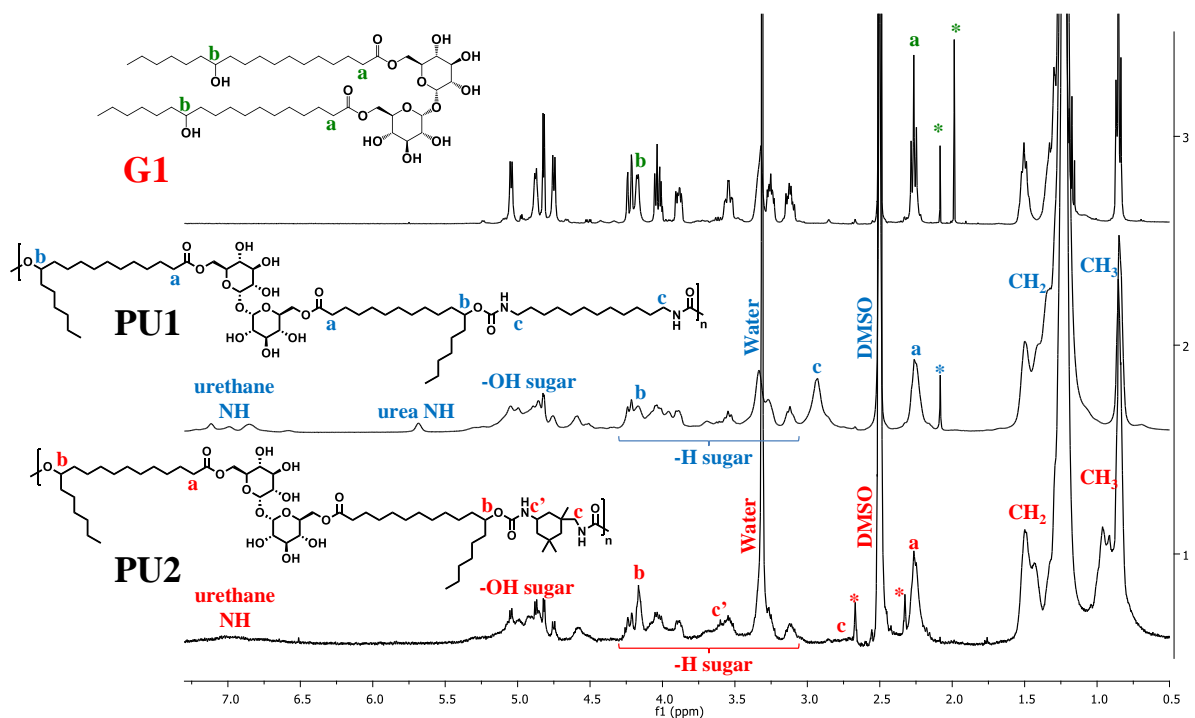


Figure 53: Stacked ^1H NMR spectra of glycolipid G1 (top), and PU1 synthesized from DIC12 (middle) and PU2 synthesized from IPDI (bottom)

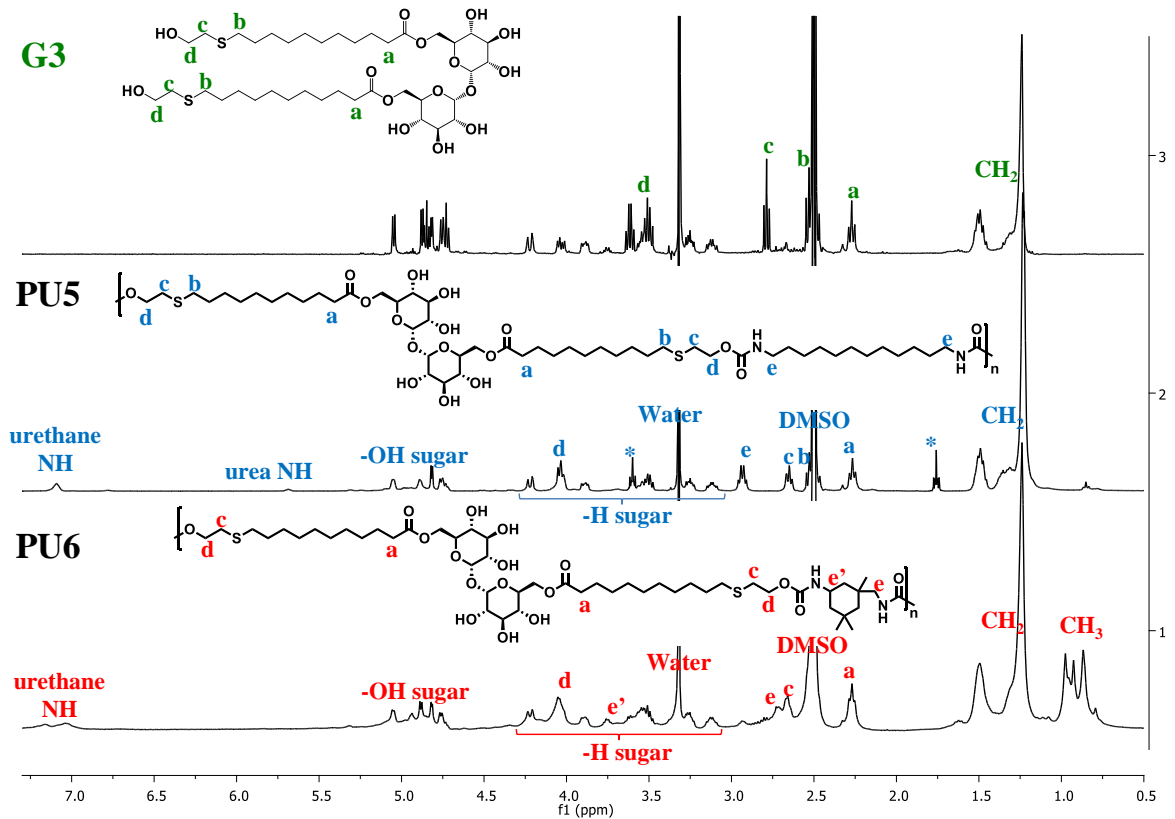


Figure 54 : Stacked ^1H NMR spectra of glycolipid G3 (top), and PU5 synthesized from DIC12 (middle) and PU6 synthesized from IPDI (bottom)

PUs were analyzed by SEC in DMF (LiBr) with the help of PS standard (Figure 55). All PUs appeared to be soluble in DMF with molar masses ranging from 3900 to 8600 g.mol⁻¹. As the polymers are fully soluble (no cross-linked fraction was noticed), one can suspect that reaction of isocyanates with the hydroxyl groups of the sugar was very limited as already indicated through NMR analysis. However, molar masses of PU1 and PU2 (synthesised from G1) were higher than the molar masses of PUs synthesized from G2 and G3. Thus, contrary to what we could have considered, the monomer G1 was not less reactive than G2 and G3. Moreover, the dispersity of the PUs is wide. It could be explained by the presence of water in the reaction and so the formation of urea. This can also confirm that the secondary alcohols of the sugar have reacted. Thus, as the so-formed polymers were soluble, they are certainly branched.

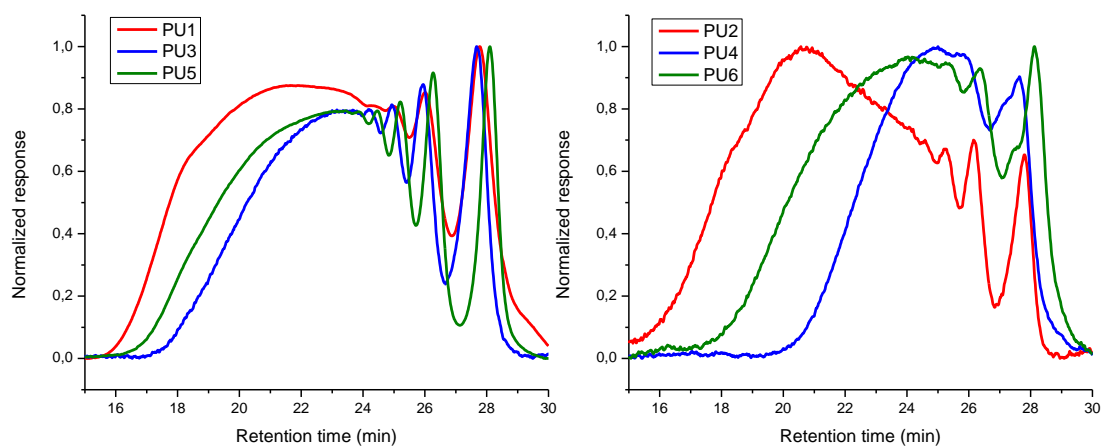


Figure 55 : SEC traces of PUs synthesized from DIC12 (lef) and IPDI (right) with monomers G1 (red), G2 (blue) and G3 (green)

2. Thermal properties of PUs

The thermal stability of the PUs was evaluated by thermogravimetric analysis (TGA) under nitrogen at a heating rate of 10 °C.min⁻¹ (Figure 56). The 5 wt.% of degradation was picked up. It can be noticed that PUs synthesized from IPDI exhibit a higher stability with respect to PUs synthesized from 1,12-diisocyanatododecane; the cyclo-aliphatic diisocyanate bringing more thermal stability to the polymers. Moreover, TGA traces show that PU deteriorated according to a multi-step degradation process and that PUs synthesized from DIC12 have more degradation step than the others.

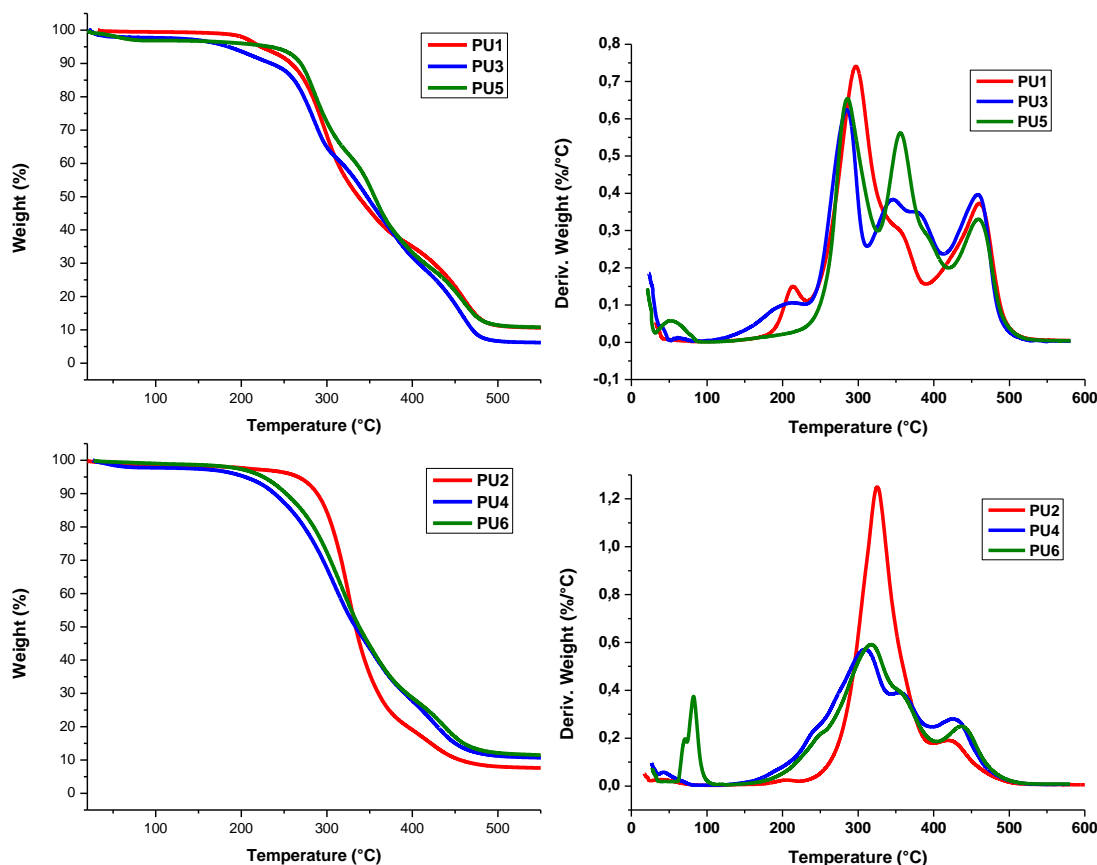


Figure 56 : TGA traces of PUs synthesized from DIC12 (top left) and IPDI (bottom left). Derivative TGA traces of PUs synthesized from DIC12 (top right) and IPDI (bottom right)

The phase transitions of the PUs were determined by differential scanning calorimetry (DSC). All analyses were carried out under a nitrogen atmosphere with a $10\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ heating rate (Figure 57). All the PUs were found amorphous. The T_g of PUs synthesized from DIC12 (PU1, PU3 and PU5) could not be determined precisely. Indeed they were too large ranging between 0 and $60\text{ }^{\circ}\text{C}$ (Figure 57). DMA should be performed to accurately determine the T_g . Unfortunately, the low amount of polymer did not allow further thermal experiments. PU2, PU4 and PU6 obtained from IPDI had T_g higher than 60°C , ($96\text{ }^{\circ}\text{C}$, $64\text{ }^{\circ}\text{C}$ and $70\text{ }^{\circ}\text{C}$ respectively). Therefore, as expected, PUs prepared from cyclo-aliphatic diisocyanate had higher T_g than the ones from aliphatic diisocyanate. Indeed, the cyclo-aliphatic diisocyanate which brings more rigidity to the polymer backbone increases the T_g value. While comparing the different glycolipids used, PU2 from G1 exhibits higher T_g than PU6 from G3, which has higher T_g than PU4 from G2. This feature can be explained by the presence of sulfur atom in PU4 and PU6. Finally, PU4 obtained from G2 exhibited the lowest T_g due to the presence of pendant alkyl chains on the polymer backbone, which increases the free volume of the polymer chain.

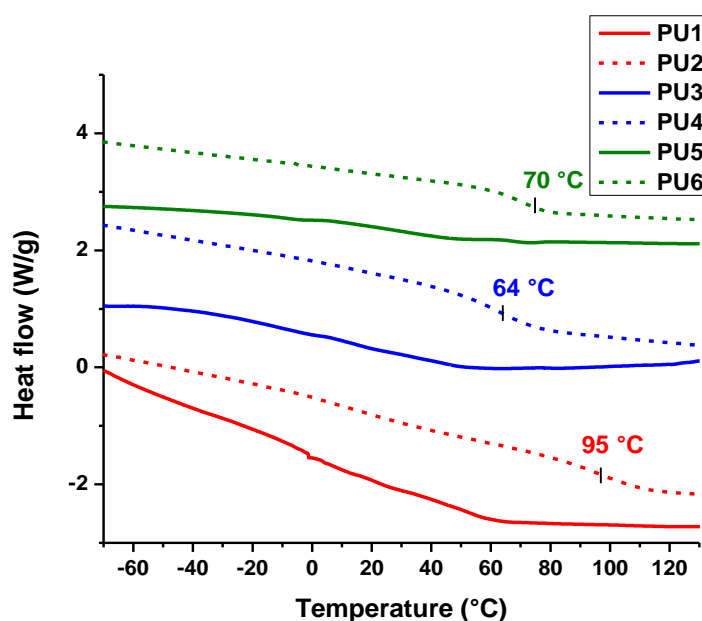


Figure 57 : DSC curves of PUs. Second heating run performed at 10°C/min

Conclusion

New glycolipids and particularly, new trehalose diesters have been synthesized by a selective esterification of the primary alcohols from the trehalose and three different fatty acids. Two of these sugar diesters have been modified by thiol-ene “click” chemistry in order to bring hydroxyl functions on the fatty chain. The latest have been polymerized with two diisocyanates to get novel glyco-polyurethanes with carbohydrate moiety inside the polymer backbones. Due to the wide dispersity of these glycolipids, the reaction of hydroxyl groups of the sugar with diisocyanates could be envisaged but PUs are soluble in DMF. Thus, the so-formed polyurethanes appear to be branched. More characterization should be done to verify this feature, MALDI for exemple. However, the low amount of polymer did not allow further experiments.

3. Experimental

Trehalose Di-12-monohydroxystearate (monomer G1)

(See chapter 2 for the synthesis)

Thiol-ene of trehalose dioleate (monomer G2) with mercaptoethanol

Trehalose dioleate (0.2 g, 0.23 mmol) and 2-mercaptoethanol (20 eq, 0.36 g, 4.6 mmol) were dissolved in THF. Then, 2,2-dimethoxy-2-phenylacetophenone (DMPA) (10 mol.%, 6 mg)

was added and the reaction mixture was stirred under irradiation using UV light ($\lambda=365$ nm), until disappearance of the double bond signal followed by ^1H NMR spectroscopy. Then the reaction was quenched by turning off the UV light and the THF was evaporated. The residue was dissolved in chloroform and washed with water (4 times) to remove the unreacted 2-mercaptoethanol. The organic phase was dried over anhydrous magnesium sulfate and the chloroform evaporated with a rotary evaporator. ^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.05 (d, 2H, -OH, H4), 4.89 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.76 (d, 2H, -OH, H2), , 4.24 (d, 2H, -CH-, H6), 4.04 (m, 2H, -CH-, H6), 3.88 (m, 2H, -CH-, H5), 3.55 (m, 2H, -CH-, H3), 3.45 (m, 4H, -CH₂-OH), 3.25 (m, 2H, -CH-, H2), 3.12 (m, 2H, -CH-, H4), 2.79 (t, 4H, -CH-S-CH₂-CH₂-OH), 2.60 (m, 2H, -CH-S-CH₂-), 2.27 (t, 2H, -CH₂-COO-CH₃), 1.51-1.25 (m, 28H, aliphatic -CH₂-), 0.85 (t, 3H, aliphatic -CH₃).

Thiol-ene of trehalose diundecenoate (monomer G3) with mercaptoethanol

Trehalose diundecenoate (0.2 g, 0.3 mmol.) and 2-mercaptoethanol (10 eq., 0.23 g, 3.0 mmol) were dissolved in THF and the reaction mixture was stirred under irradiation using UV light ($\lambda=365$ nm), until disappearance of the double bond signal followed by ^1H NMR spectroscopy. Then the reaction was quenched by turning off the UV light and the THF was evaporated. The residue was dissolved in chloroform and washed with water (4 times) to remove the unreacted 2-mercaptoethanol. The organic phase was dried over anhydrous magnesium sulfate and the chloroform evaporated with a rotary evaporator. ^1H NMR (DMSO- d_6 , 400MHz, δ (ppm)): 5.05 (d, 2H, -OH, H4), 4.89 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.76 (d, 2H, -OH, H2), , 4.24 (d, 2H, -CH-, H6), 4.04 (m, 2H, -CH-, H6), 3.88 (m, 2H, -CH-, H5), 3.55 (m, 2H, -CH-, H3), 3.45 (m, 4H, -CH₂-OH), 3.25 (m, 2H, -CH-, H2), 3.12 (m, 2H, -CH-, H4), 2.79 (t, 4H, -S-CH₂-CH₂-OH), 2.55 (m, 2H, -CH₂-S-CH₂-), 2.27 (t, 2H, -CH₂-COO-CH₃), 1.51-1.25 (m, 16H, aliphatic -CH₂-).

Polymerization

Monomer G1 (0.2 g, 0.22 mmol) or G2 (0.1 g, 0.10 mmol) or G3 (0.1 g, 0.12 mmol) were mixed in THF with IPDI (1 eq.) or 1,12-diisocyanatododecane (1 eq.) and DBTDL (2 wt.%). The reaction mixture was then stirred at 60 °C under nitrogen atmosphere. During the polymerization, aliquots were sampled out and analyzed by FTIR spectroscopy. After the complete disappearance of the isocyanate bands, the solvent was removed under vacuum.

II. Poly(hydroxyurethane)s (PHUs)

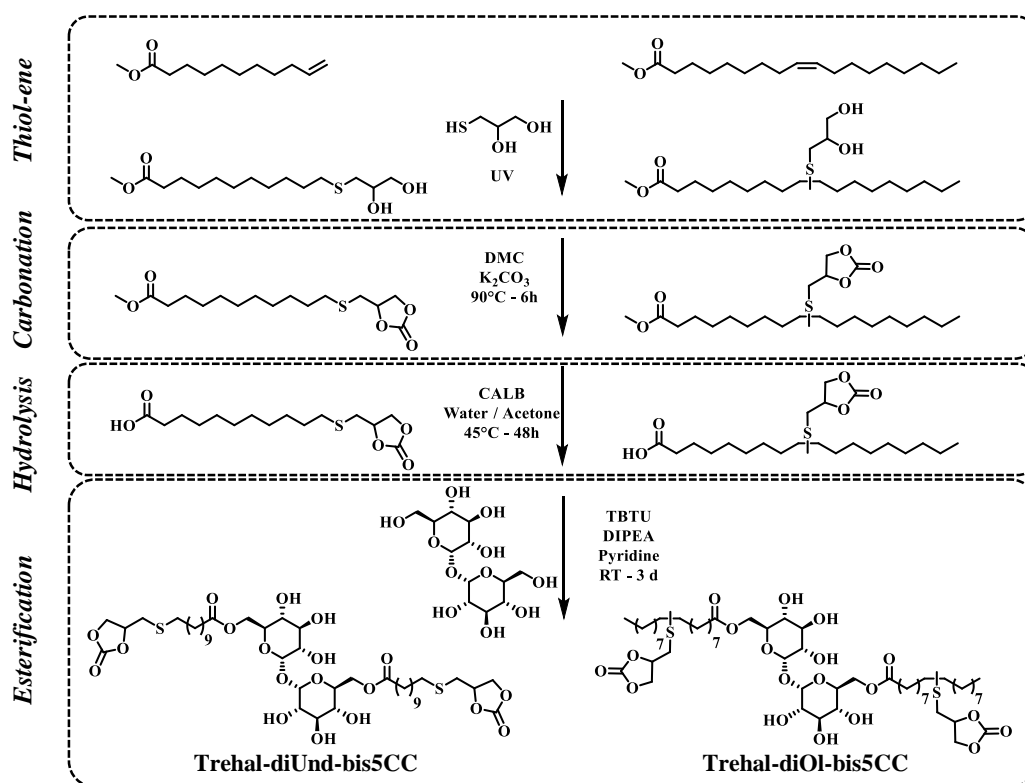
As discussed before, polyurethanes constitute widely used polymers. The common pathway to synthesize them is the reaction between alcohols and isocyanates. The latter are rather toxic and synthesized from phosgene, which is a mortal gas. Consequently, industrial and academic researchers try to develop alternatives in the manufacture of polyurethanes. A possible route is the synthesis of poly(hydroxyurethane)s (PHU) which are obtained by the ring-opening of bis-cyclic carbonates with diamines. Moreover, with the actual context of fossil fuel depletion and global warming, it is important to replace petroleum-based monomers by bio-based monomers for the synthesis of polyurethanes. In this purpose, the synthesis of bio-based bis-cyclic carbonates and bio-based diamines has been reported in literature.¹⁰⁻¹³

Besides, it has been demonstrated that 5-membered cyclic carbonates exhibited slow polymerization rate with amines. To tackle this challenge, some research groups developed larger cyclic carbonates, for example 6-membered cyclic carbonates known to be more reactive than the 5-membered ones.¹²⁻¹⁶ Another possibility is to insert a heteroatom nearby the 5-membered cyclic carbonate to improve its reactivity with amines.^{17,18} In the literature, few examples of polyurethanes synthesized from sugar esters have been described but no example of poly(hydroxyurethane)s is reported.

1. PHU synthesis, molecular structure and dimension

In this study, trehalose diesters bearing 5-membered cyclic carbonate onto the fatty chain were developed in order to design new poly(hydroxyurethane)s containing trehalose moiety in the polymer backbone.

A pathway in four steps was developed to synthesize two novel diesters of trehalose bearing 5-membered bis-cyclic carbonates (Scheme 33).



Scheme 33 : Synthetic pathway to 5-membered bis-cyclic carbonate trehalose diesters

Firstly, thiol-ene click reaction was performed, under UV irradiation without any catalyst, on the fatty methyl esters with 1-thioglycerol to bring a 1,2-diol moiety. A full conversion was obtained after 2 h for methyl undecenoate and after 24 h for methyl oleate. Indeed, it has been reported that a terminal double bond is much more reactive than an internal one.¹⁹ The so-formed diol esters were obtained as white solids after purification by flash chromatography (yield = 90 %). The thiol-ene reaction was monitored by ¹H NMR spectroscopy. The disappearance of the peak corresponding to the double bond at 5.8 and 4.9 ppm for the methyl undecenoate and 5.3 ppm for the methyl oleate, respectively, was observed (Figure 58 and Figure 59).

The fatty acid methyl esters were chosen as starting reactants instead of the fatty acids because no carbonate was obtained during the carbonation step in the presence of acidic moiety. This feature was explained by the reaction between the acidic function and K₂CO₃, which prevents the deprotonation of the hydroxyl groups and thus their carbonation with the DMC.

The carbonation of the diol was then performed with dimethyl carbonate using potassium carbonate as catalyst (Figure 58 and Figure 59). The carbonation was followed by ¹H NMR

spectroscopy. After 6 h of reaction under reflux, the full conversion of the two diols was observed with the disappearance of the peak assigned to the protons nearby the diol (3.7 and 3.5 ppm) and the appearance of the peaks corresponding to the carbonate (4.9, 4.6 and 4.2 ppm). Moreover, no side products were observed using these conditions. Finally, the resulting methyl ester carbonates were purified by flash chromatography (yield = 85 %) (Figure 58 and Figure 59).

The methyl ester function was then hydrolyzed into acidic moiety. Indeed, the last step of the pathway is the chemical esterification of the primary alcohols of the trehalose, and this reaction only proceeds on an acidic function. The hydrolysis of the methyl esters was performed in a water/acetone solution, using lipase B from *Candida antarctica* immobilized on acrylic resin (also known as Novozyme 435) as a catalyst. The enzymatic hydrolysis was chosen compared to a classical way, using a strong base such as KOH, to prevent the opening of the cyclic carbonate linked to the fatty chain. Actually, the lipase only reacts with the ester moiety and does not react with the carbonate. After 72 h stirring at 60 °C, the reaction mixture was filtrated to remove the enzyme and the acid was purified by flash chromatography to obtain the fatty acid cyclic carbonate as a white solid (yield = 70 %). The hydrolysis was confirmed by means of ¹H NMR spectroscopy by the disappearance of the methyl ester peak at 3.6 ppm. There was no shift or disappearance of the peaks corresponding to the cyclic carbonate confirming its inertness in this step (Figure 58 and Figure 59).

The last reaction step is a two-step one-pot selective esterification of the primary alcohol developed by Grindley⁹. After purification by flash chromatography, trehalose diesters with sulfur activated 5-membered bis-cyclic carbonates were obtained as gummy solid. The chemical structure of the two glycolipids was checked by ¹H NMR spectroscopy ((Figure 58, Figure 59 and Figure 60). The esterification of the primary alcohol was confirmed by the shift of the peaks of the protons H6 from 3.48 and 3.55 to 4.28 and 4.02 ppm and the protons H5 from 3.65 to 3.89 ppm, respectively.

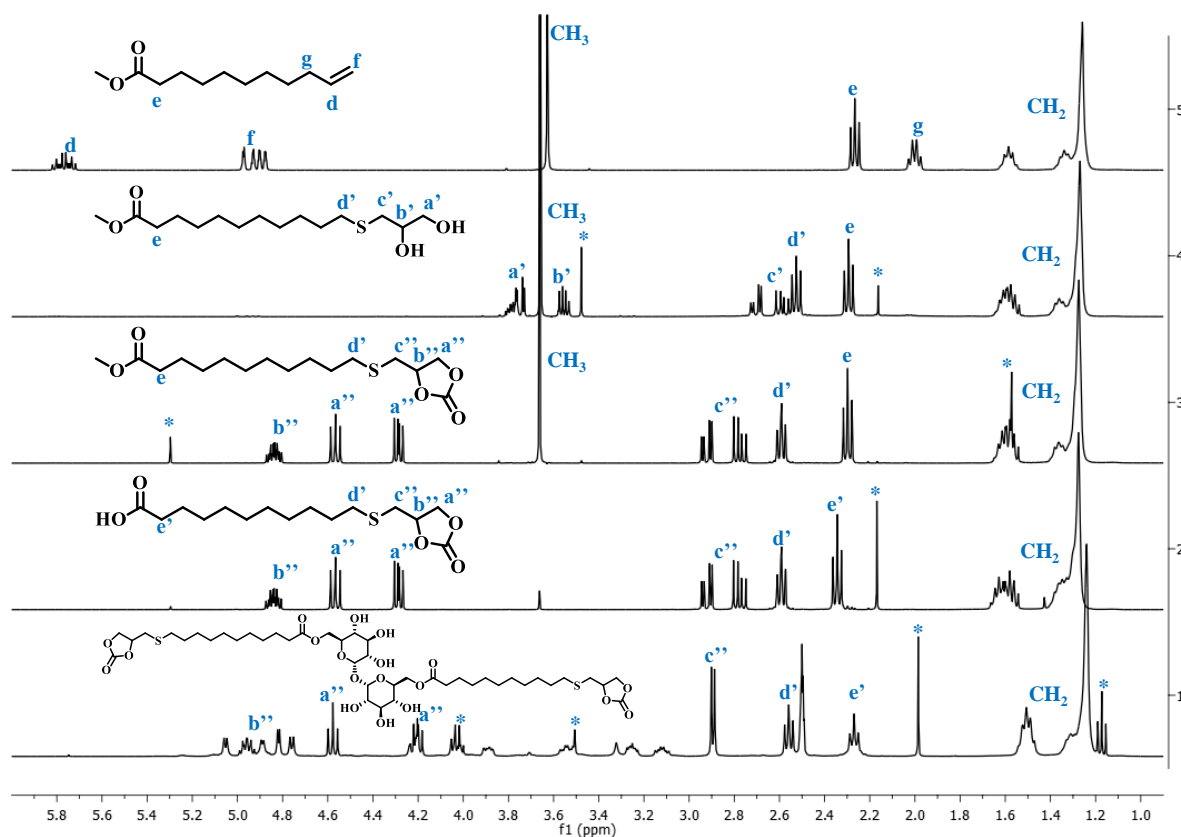


Figure 58: Stacked ^1H NMR spectra of intermediates of Trehal-diUnd-bis5CC(*). Impurities.

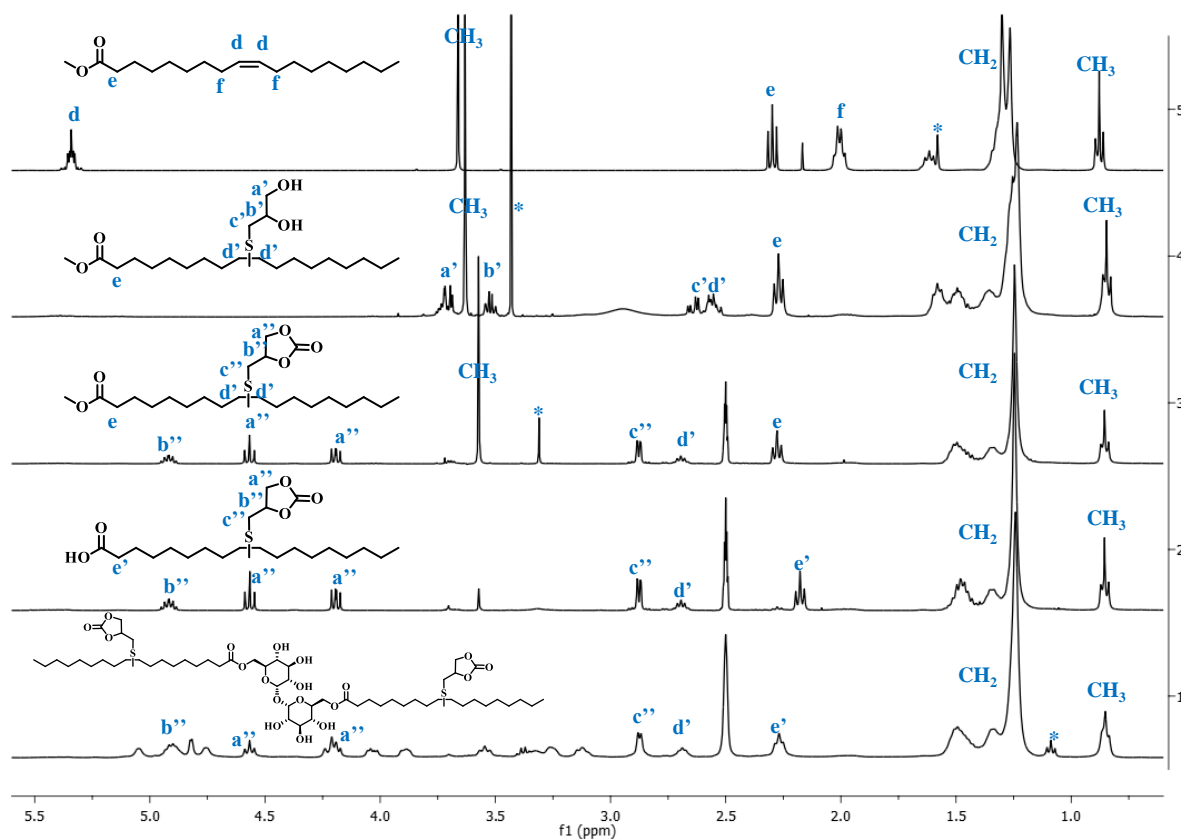


Figure 59: Stacked ^1H NMR spectra of intermediates of Trehal-diOl-bis5CC.

(*) Impurities.

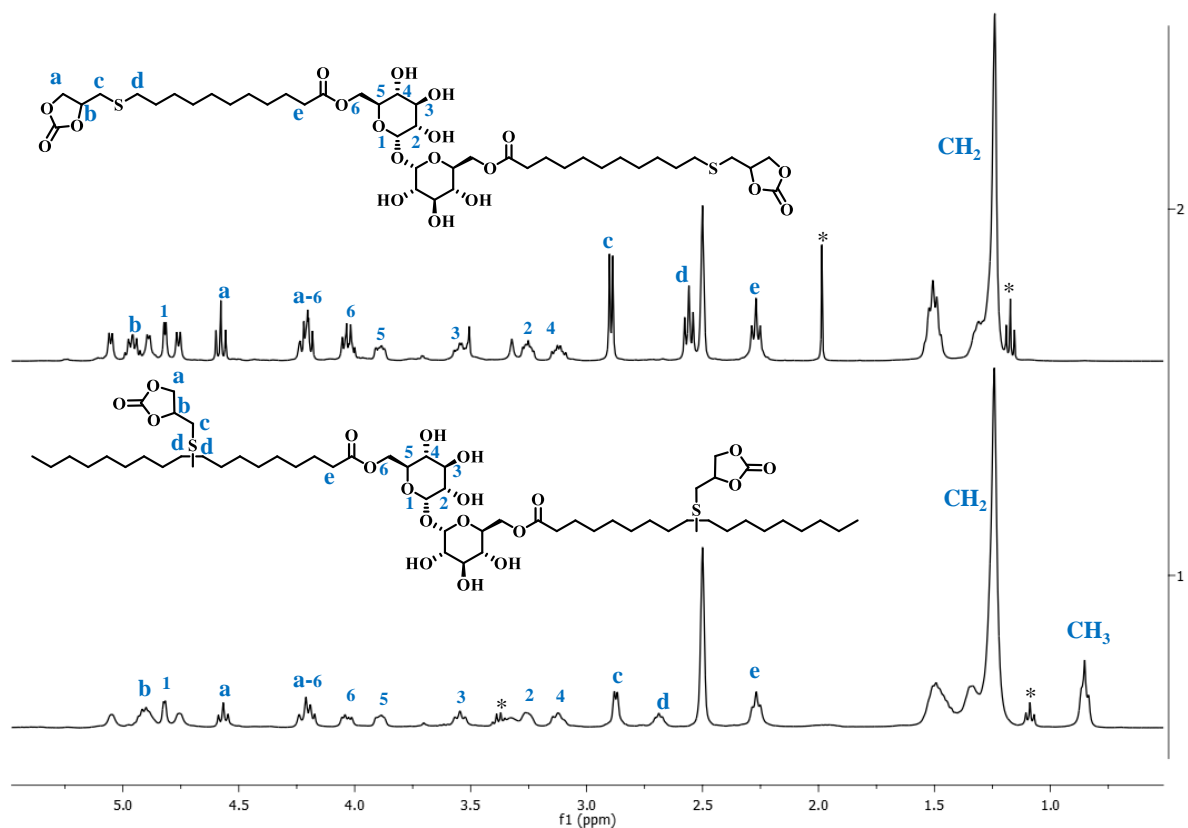
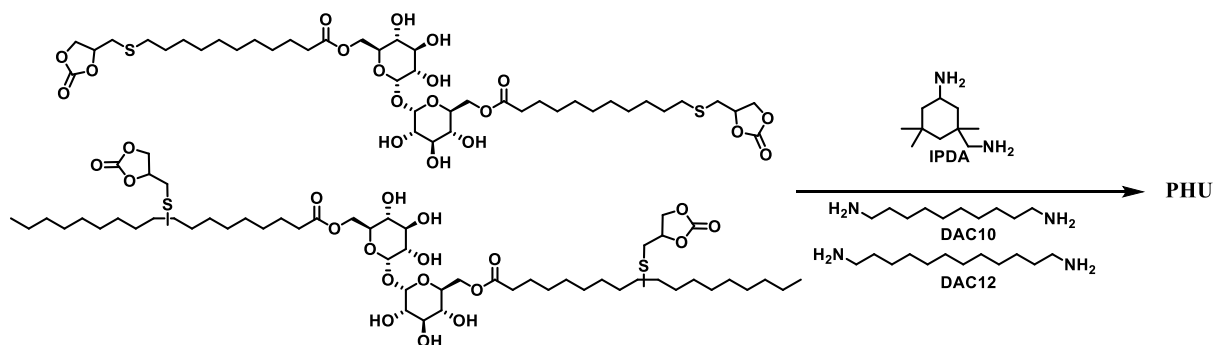


Figure 60 : Stacked ^1H NMR spectra of Trehal-diUnd-bis5CC (up) and Trehal-diOl-bis5CC (bottom) in DMSO-d_6 , (*) Impurities.

A series of poly(hydroxyurethane)s were synthesized using **Trehal-diUnd-bis5CC** and **Trehal-diOl-bis5CC** as activated 5-membered bis-cyclic carbonates and three different diamines, two aliphatic ones, 1,10-diaminododecane, 1,12-diaminododecane and a cyclic one the isophorone diamine (Scheme 34 and Table 2). The polymerization was performed in DMF at 60 °C under nitrogen atmosphere without any catalyst.

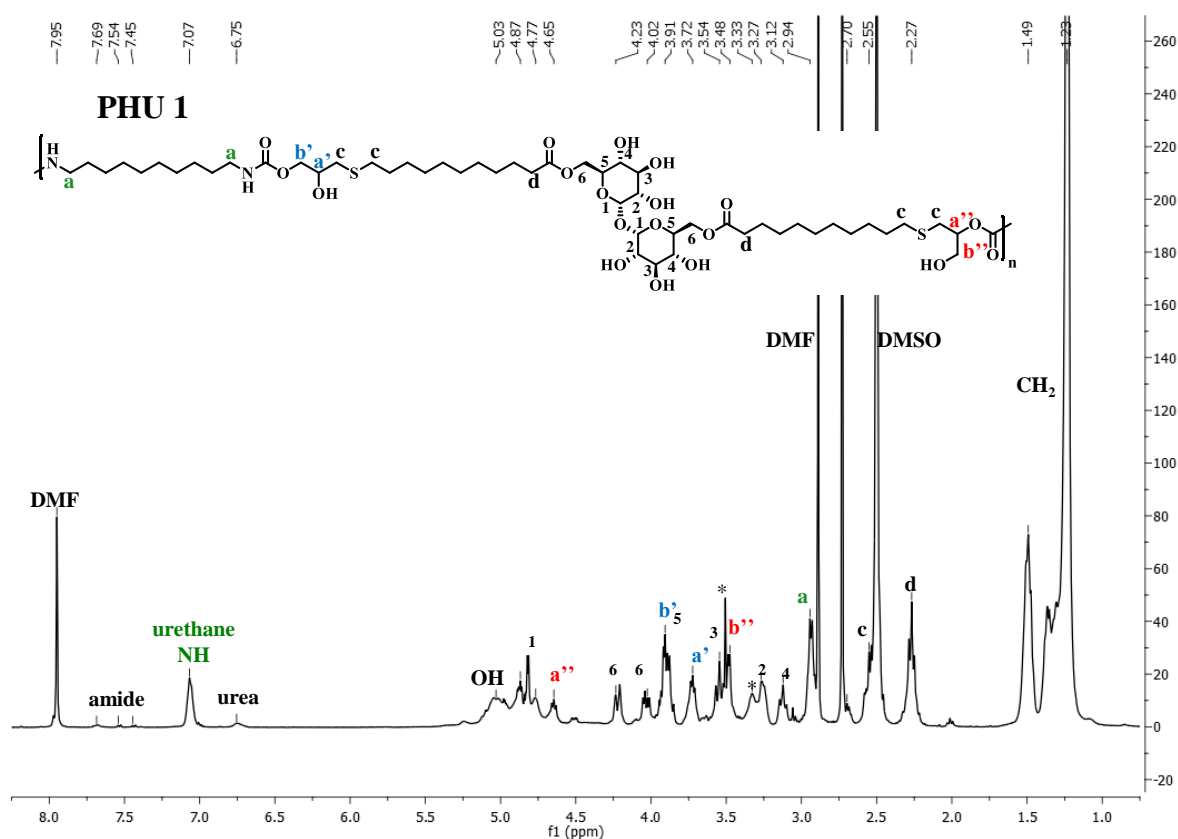


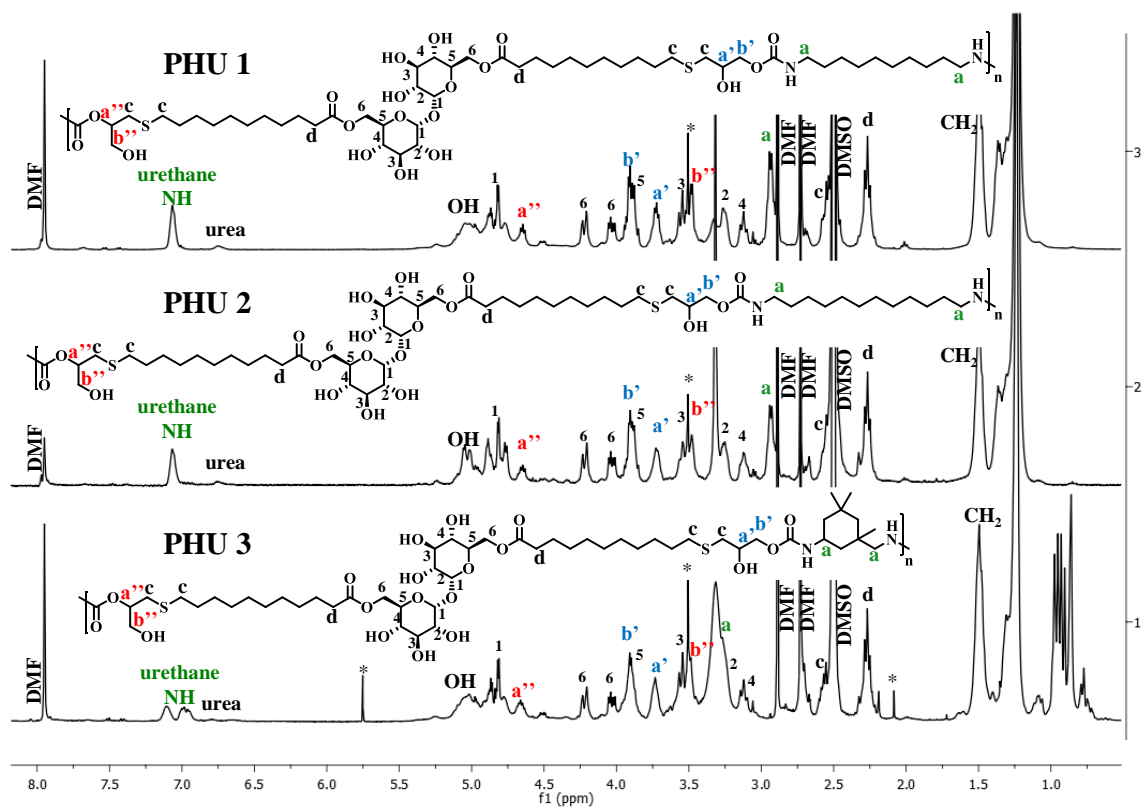
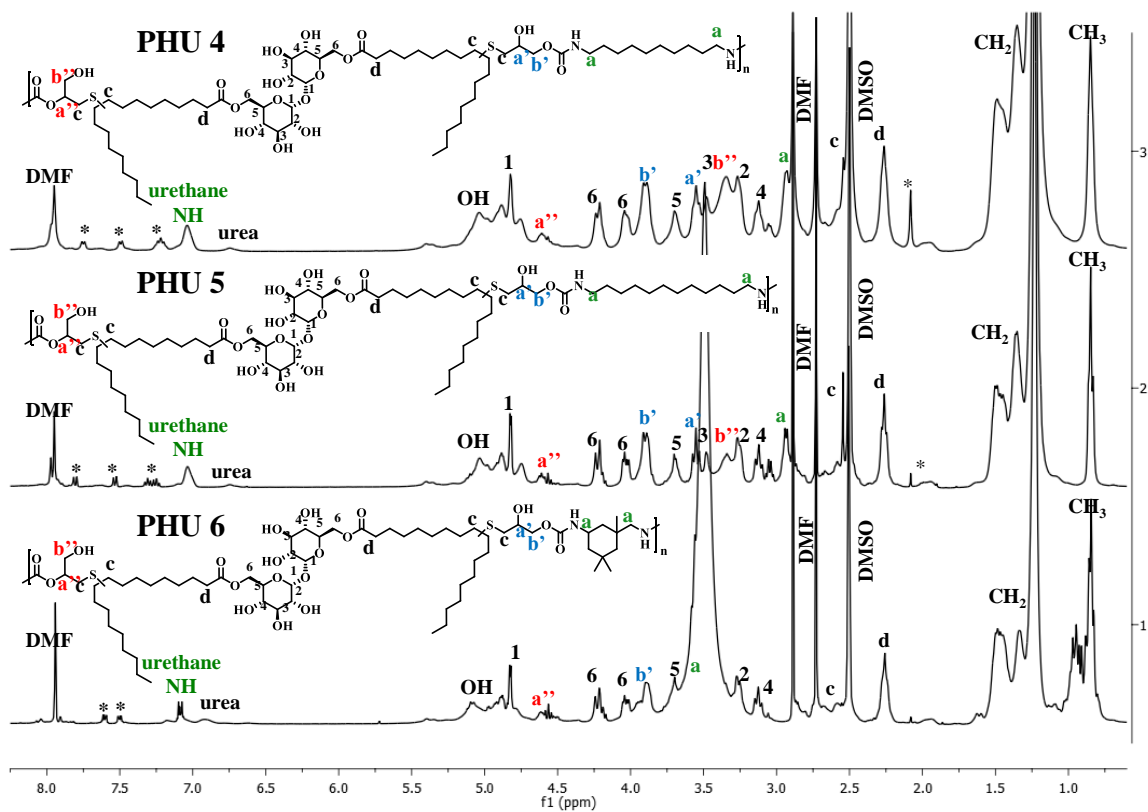
Scheme 34: Synthetic pathway to poly(hydroxyurethane)s obtained from bis-cyclic carbonates and diamines

PHU	Biscarbonate	Diamine	\bar{M}_n (g.mol ⁻¹)	\bar{D}^a	T _g (°C)	T _{d5%} (°C)
PHU1	Trehal-diUnd-bis5CC	DAC10	10000	3.7	34	262
PHU2	Trehal-diUnd-bis5CC	DAC12	7800	3.7	30	258
PHU3	Trehal-diUnd-bis5CC	IPDA	3000	3.4	36	253
PHU4	Trehal-diOl-bis5CC	DAC10	3200	1.9	16	246
PHU5	Trehal-diOl-bis5CC	DAC12	3000	2.4	10	246
PHU6	Trehal-diOl-bis5CC	IPDA	2800	1.9	56	240

Table 11 : Characterization of the PHUs

The so-formed PHUs were characterized by ¹H NMR, COSY, HSQC (Figure S1) and FTIR spectroscopy without quenching or purification, after 5 days of polymerization. Indeed, it has been reported that the kinetic of polymerization was slow. As an example, Maisonneuve *et al.* as reported that the polymerization was slow and was led up to 13 days to reach high conversion.²⁰ Thus, the polymerization was carried out during 5 days to reach high conversions and high molar masses.

Figure 61: ¹H NMR of the PHU1 synthesized from Trehal-diUnd-bis5CC and the 1,10-diaminodecane in DMSO-d₆

Figure 62 : Stacked ^1H NMR spectra of PHU 1 to 3 in DMSO-d_6 Figure 63 : Stacked ^1H NMR spectra of PHU 4 to 6 performed in DMSO-d_6

PHUs formation was proved by the disappearance of the carbonate protons signal at 4.92, 4.57 and 4.19 ppm and the appearance of the signal of the urethane proton ($-\text{NH}-\text{COO}-$) at 7.07 ppm. The formation was also confirmed by FTIR spectroscopy with the disappearance of the carbonate band at 1800 cm^{-1} and the appearance of bands at 1680 and 1530 cm^{-1} , which correspond respectively to $\text{C}=\text{O}$ and NH vibrations of the urethane function (Figure 64). Moreover, the chemical structures of PHU were determined thanks to ^1H NMR (Figure 61), ^{13}C NMR and 2D NMR (COSY, HSQC).

As it was already reported in the literature,^{18,20} side reactions could occur during the polymerization. It is known that primary amines could react with ester functions from the glycolipid to form amide linkages. Another possibility is the reaction between primary amine with urethane functions leading to urea. Indeed, the formation of urea was confirmed by ^1H NMR, with the appearance of peaks corresponding to the labile protons urea at 6.75 ppm. The formation of amide could not be determined due to signal overlapping on ^1H NMR spectra with the ones of impurities. However, the proportion between urea and urethane formed during the polymerization could be evaluated by ^1H NMR and is summarized in Table 12. It can be noticed that the percentage of urea was almost the same between the PHUs. Thus, the formation of urea was independent of the trehalose diester structure.

PHU	Bis-carbonate	Diamines	Proportion (%)	
			Urethane	Urea
1	Trehal-diUnd-bis5CC	C10	92	8
2	Trehal-diUnd-bis5CC	C12	94	6
3	Trehal-diUnd-bis5CC	IPDA	91	9
4	Trehal-diOl-bis5CC	C10	98	2
5	Trehal-diOl-bis5CC	C12	96	4
6	Trehal-diOl-bis5CC	IPDA	93	7

Table 12: Proportion of urethane and urea in the PHU

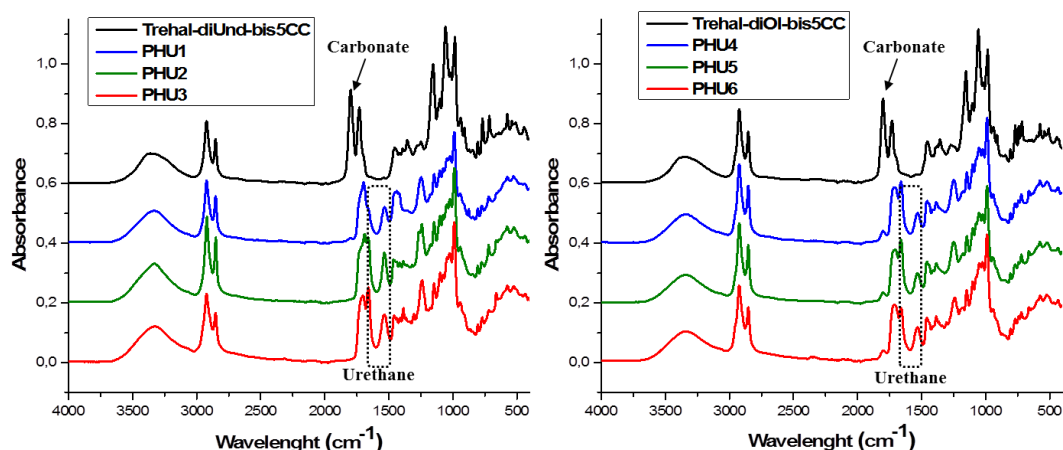


Figure 64 : Stacked FTIR spectra of the PHU synthesized from the Trehal-diUnd-bis5 (left) and from the Trehal-diOI-bis5CC (right)

The poly(hydroxyurethane)s molar masses were determined by SEC in DMF using a polystyrene standards. The SEC traces are shown Figure 65 and data are summarized in Table 11. PHUs exhibit molar masses in the range 2800 to 10000 $\text{g}\cdot\text{mol}^{-1}$. The PHU1, 2 and 3 synthesized from the Trehal-diUnd-bis5CC exhibit higher molar masses than the PHU4, 5 and 6 obtained with Trehal-diOI-bis5CC. The lowest PHUs molar masses were obtained in the case of isophorone diamine. It could be due to the steric hindrance of the cyclic diamine, which doesn't permit to reach higher molar masses. The highest molar masses were obtained with the 1,10-diaminodecane and the Trehal-diUnd-bis5CC.

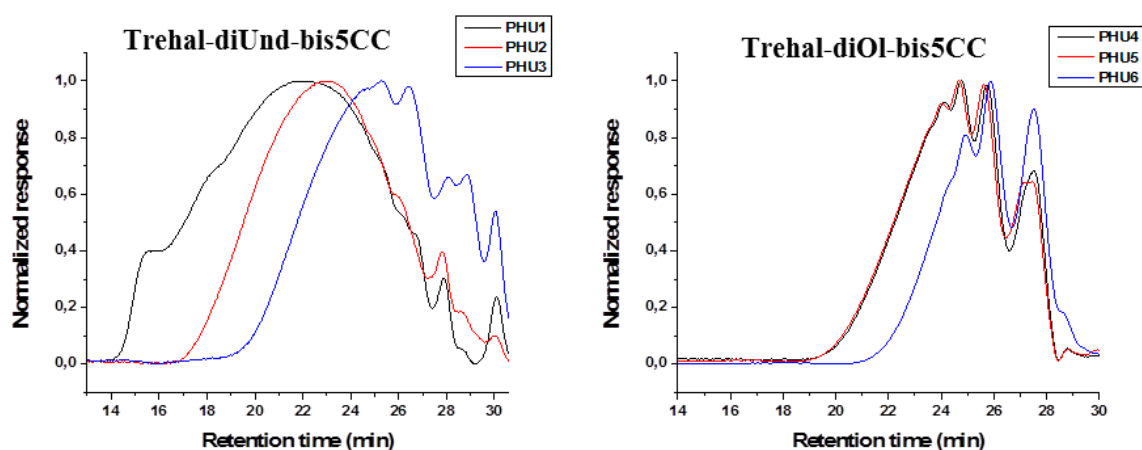


Figure 65 : SEC traces of the PHUs after 5 days of polymerization (SEC in DMF, PS standards)

Compared to PUs obtained in the previous section, no side reaction could occur on hydroxyl group of the sugar moiety; consequently the so-formed poly(hydroxyurethane)s were mostly linear and not branched.

2. Thermal properties of PHUs

The thermal properties of the PHUs were determined by thermogravimetric analyses (Figure 66) and DSC analyses (Figure 67). Data are summarized in Table 11.

An isotherm at 160 °C for 15 min was performed on the thermogravimetric analyses to remove the traces of DMF; TGA was then performed at 10°C.min⁻¹ under nitrogen until 600 °C. Figure 66 shows the TGA curves of the PHU after DMF removal. The thermal stabilities of the PHU are very similar even if the temperature after 5 wt.% loss is slightly lower for the polymers synthesized from the Trehal-diOl-bis5CC.

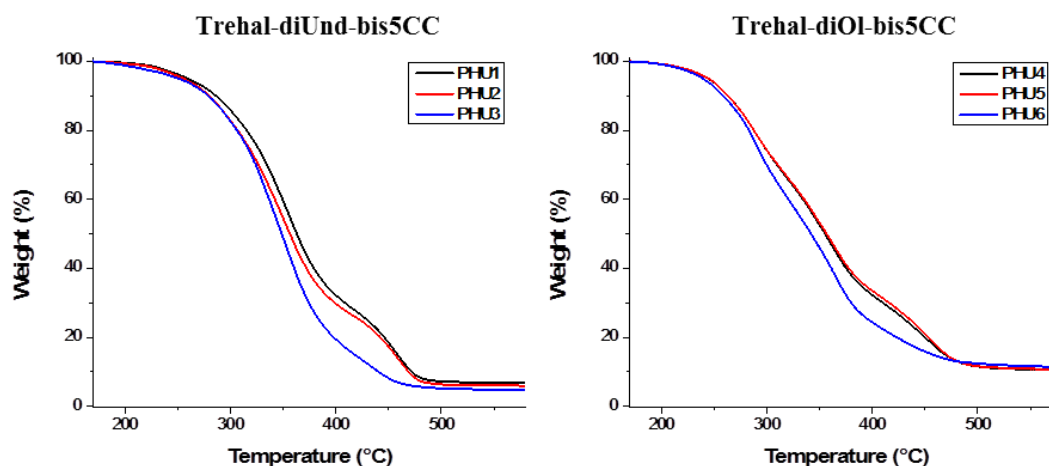


Figure 66 : TGA curves of the PHUs 1 to 6 synthesized from Trehal-diUnd-bis5CC (left) and Trehal-diOl-bis5CC (right) after the isothermal performed at 160°C to remove the DMF residue

As expected, all the PHUs were found amorphous like the PUs obtained in the previous section. In the cases of PHUs synthesized from the aliphatic diamines (PHU1, PHU2, PHU4 and PHU5), T_g around 30 °C were found with Trehal-diUnd-bis5CC while lower values, around 10 °C, were obtained for the PHUs obtained with Trehal-diOl-bis5CC. It was due to the presence of aliphatic pendant chains on the polymer backbone, which act as internal plasticizer, and consequently decreased the T_g . In the case of PHUs synthesized from isophorone diamine, higher T_g were obtained, 36°C for PHU3 and 56°C for PHU6,

respectively. Such values can be explained by the cyclic diamine, which brings rigidity to the polymer backbone.

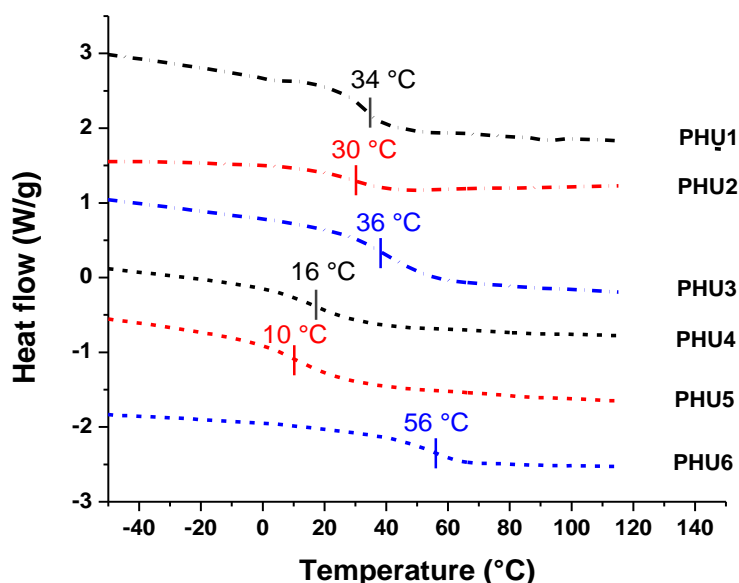


Figure 67 : DSC curves of the PHUs 1 to 6. Second heating run performed at 10°C/min

To conclude, two novel diesters of trehalose with sulfur 5-membered bis-cyclic carbonate on the fatty chain were synthesized in a four-stage process. Poly(hydroxyurethane)s were successfully synthesized from these two new monomers and different diamines. The monomer with no pendant alkyl chains (Trehal-diUnd-bis5CC) allowed us obtaining PHUs with higher molar masses and few side reactions.

3. Experimental

Thiol-ene modification of fatty acids with thioglycerol

Methyl-Und-thioglycerol

Methyl 10-undecenoate (10 g, 50 mmol.) and 1-thioglycerol (2 eq., 10 g, 100 mmol) were stirred during 2 h under irradiation using UV light ($\lambda=365$ nm). Then the reaction was quenched by turning off the UV light. Purification over silica gel flash chromatography was performed using DCM/MeOH 98/2 eluent. Yield: 80%. ^1H NMR (400 MHz, CDCl_3 , δ (ppm)): 3.74 (m, 2H, $-\text{CH}_2\text{-OH}$), 3.64 (s, 3H, $-\text{COO-CH}_3$), 3.53 (m, 1H, $-\text{CH-OH}$), 2.65 (m, 2H, $-\text{S-CH}_2\text{-CH-}$), 2.51 (m, 2H, $-\text{CH}_2\text{-CH}_2\text{-S-CH}_2\text{-}$), 2.28 (t, 2H, $-\text{CH}_2\text{-COO-CH}_3$), 1.59-1.25 (m, 16H, aliphatic $-\text{CH}_2\text{-}$).

Methyl-Ol-thioglycerol

Methyl oleate (10 g, 33 mmol.) and 1-thioglycerol (10 eq., 36 g, 337 mmol) were stirred under irradiation using UV light ($\lambda=365$ nm) until total conversion of the internal double bond. Then the reaction was quenched by turning off the UV light. Purification over silica gel flash chromatography was performed using DCM/MeOH 98/2 eluent. Yield: 76 %. $^1\text{H NMR}$ (400 MHz, CDCl_3 , δ (ppm)): 3.72 (m, 2H, $-\text{CH}_2\text{-OH}$), 3.63 (s, 3H, $-\text{COO-CH}_3$), 3.53 (m, 1H, $-\text{CH-OH}$), 2.63 (m, 3H, $-\text{CH}_2\text{-CH}_2\text{-S-CH}_2\text{-CH-}$), 2.27 (t, 2H, $-\text{CH}_2\text{-COO-CH}_3$), 1.58-1.24 (m, 26H, aliphatic $-\text{CH}_2\text{-}$), 0.85 (t, 3H, aliphatic $-\text{CH}_3$).

Carbonation***Und(ester)-5CC***

In a flame-dried round bottom flask equipped with a reflux condenser, (3 mol.%, 68 mg, 0.5 mmol) potassium carbonate (K_2CO_3) was added to a solution of Methyl-Und-thioglycerol (5 g, 16.3 mmol) dissolved in dimethyl carbonate (DMC) (25 eq., 34 mL, 0.4 mol). The reaction mixture was stirred during 6 h under reflux. At the end of the reaction, the DMC was evaporated with a rotary evaporator. Purification over silica gel flash chromatography was performed using DCM/MeOH 98/2 eluent. $^1\text{H NMR}$ (400 MHz, CDCl_3 , δ (ppm)): 4.83 (m, 1H, $-\text{CH-O-}$), 4.57 (m, 1H, $-\text{CH}_2\text{-O-}$), 4.28 (m, 1H, $-\text{CH}_2\text{-O-}$), 3.66 (s, 3H, $-\text{COO-CH}_3$), 2.91 (m, 2H, $-\text{S-CH}_2\text{-CH-}$), 2.59 (t, 2H, $-\text{CH}_2\text{-CH}_2\text{-S-CH}_2\text{-}$), 2.30 (t, 2H, $-\text{CH}_2\text{-COO-CH}_3$), 1.56-1.27 (m, 16H, aliphatic $-\text{CH}_2\text{-}$).

Ol(ester)-5CC

In a flame-dried round bottom flask equipped with a reflux condenser, potassium carbonate (K_2CO_3 3 mol.%, 48 mg, 0.3 mmol.) was added to a solution of methyl-Ol-thioglycerol (5 g, 11.6 mmol.) dissolved in dimethyl carbonate (DMC 25 eq., 24 mL, 0.3 mol.). The reaction mixture was stirred during 6 h under reflux. At the end of the reaction, the DMC was evaporated with a rotary evaporator. Purification over silica gel flash chromatography was performed using DCM/MeOH 98/2 eluent. $^1\text{H NMR}$ (400 MHz, DMSO-d_6 , δ (ppm)): 4.92 (m, 1H, $-\text{CH-O-}$), 4.57 (m, 1H, $-\text{CH}_2\text{-O-}$), 4.19 (m, 1H, $-\text{CH}_2\text{-O-}$), 3.57 (s, 3H, $-\text{COO-CH}_3$), 2.88 (m, 2H, $-\text{S-CH}_2\text{-CH-}$), 2.69 (t, 2H, $-\text{CH}_2\text{-CH}_2\text{-S-CH}_2\text{-}$), 2.28 (t, 2H, $-\text{CH}_2\text{-COO-CH}_3$), 1.51-1.25 (m, 16H, aliphatic $-\text{CH}_2\text{-}$), 0.86 (t, 3H, aliphatic $-\text{CH}_3$).

Saponification***Und(acid)-5CC***

In a round bottom flask, Methyl-Und-5CC (5 g, 15 mmol.) was dissolved in a solution water/acetone (2:5) (2 mL/10 mL). Then, lipase B from candida antartica (1g, 20 wt.%) was added to the reaction medium. The reaction mixture was stirred during 48 h at 60°C. At the end of the reaction, the solvent was evaporated with a rotary evaporator. Purification over silica gel flash chromatography was performed using DCM/MeOH 98/2. ¹H NMR (400 MHz, CDCl₃, δ (ppm)): 4.83 (m, 1H, -CH-O-), 4.57 (m, 1H, -CH₂-O-), 4.28 (m, 1H, -CH₂-O-), 2.91 (m, 2H, -S-CH₂-CH-), 2.59 (t, 2H, -CH₂-CH₂-S-CH₂-), 2.30 (t, 2H, -CH₂-COO-CH₃), 1.56-1.27 (m, 16H, aliphatic -CH₂-).

Ol(acid)-5CC

In a round bottom flask, Methyl-Ol-5CC (5 g, 11 mmol.) was dissolved in a solution water/acetone (2:5) (4 mL/10 mL). Then, lipase B from candida antartica (1g, 20 wt.%) was added to the reaction medium. The reaction mixture was stirred during 48 h at 60°C. At the end of the reaction, the solvent was evaporated with a rotary evaporator. Purification over silica gel flash chromatography was performed using DCM/MeOH 98/2. ¹H NMR (400 MHz, DMSO-d₆, δ (ppm)): 4.92 (m, 1H, -CH-O-), 4.57 (m, 1H, -CH₂-O-), 4.19 (m, 1H, -CH₂-O-), 2.88 (m, 2H, -S-CH₂-CH-), 2.69 (t, 2H, -CH₂-CH₂-S-CH₂-), 2.28 (t, 2H, -CH₂-COO-CH₃), 1.51-1.25 (m, 16H, aliphatic -CH₂-), 0.86 (t, 3H, aliphatic -CH₃).

Chemical esterification of trehalose

Trehal-diUnd-bis5CC

In a flame-dried round bottom flask equipped, Und-5CC (2.1 eq., 3.9 g, 12 mmol.), TBTU (2.1 eq., 3.9 g, 12 mmol.) and DIPEA (2 eq., 1.9 mL, 11 mmol) were dissolved in dry pyridine (20 mL). The resulting mixture was stirred at room temperature for 30 min under a nitrogen atmosphere. Then the reaction mixture was slowly injected into a solution of trehalose (1 eq, 5.8 mmol) in dry pyridine (20 mL) and stirring was continued at room temperature for 72 h. Pyridine was removed under vacuum and the resulting residue centrifuged in water to remove the by-products of the reaction. The precipitate was dried and purified by flash chromatography using an elution gradient of 5-25% methanol in EtOAc-DCM (1:1) to give diester of trehalose as a gummy solid. ¹H NMR (DMSO-d₆, 400MHz, δ (ppm)): 5.06 (d, 2H, -OH, H4), 4.96 (m, 1H, -CH-O-), 4.90 (d, 2H, -OH, H3), 4.81 (d, 2H, -CH-, H1), 4.77 (d, 2H, -OH, H2), 4.58 (m, 2H, -CH₂-O-), 4.21 (d, 2H, -CH-, H6), 4.20 (m, 2H, -CH₂-O-), 4.04 (m, 2H, -CH-, H6), 3.88 (m, 2H, -CH-, H5), 3.54 (m, 2H, -CH-, H3), 3.25 (m, 2H, -CH-, H2), 3.11 (m, 2H, -CH-, H4), 2.90 (m, 2H, -S-CH₂-CH-), 2.54 (t, 2H, -CH₂-CH₂-S-CH₂-), 2.27 (t, 2H, -CH₂-COO-CH₃), 1.51-1.24 (m, 16H, aliphatic -CH₂-).

Trehal-diOl-bis5CC

In a flame-dried round bottom flask equipped, Ol-5CC (2.1 eq., 2.5 g, 6.1 mmol.), TBTU (2.1 eq., 1.9 g, 6.1 mmol) and DIPEA (2 eq., 0.9 mL, 5.8 mmol) were dissolved in dry pyridine (15 mL). The resulting mixture was stirred at room temperature for 30 min under a nitrogen atmosphere. Then the reaction mixture was slowly injected into a solution of trehalose (1 eq., 5.8 mmol) in dry pyridine (15 mL) and stirring was continued at room temperature for 72 h. Pyridine was removed under vacuum and the resulting residue centrifuged in water to remove the by-products of the reaction. The precipitate was dried and purified by flash chromatography using an elution gradient of 5-25% methanol in EtOAc-DCM (1:1) to give diester of trehalose as a gummy solid. ¹H NMR (DMSO-d₆, 400MHz, δ (ppm)): 5.04 (d, 2H, -OH, H4), 4.92 (m, 1H, -CH-O-), 4.89 (d, 2H, -OH, H3), 4.82 (d, 2H, -CH-, H1), 4.76 (d, 2H, -OH, H2), 4.57 (m, 2H, -CH₂-O-), 4.24 (d, 2H, -CH-, H6), 4.19 (m, 2H, -CH₂-O-), 4.03 (m, 2H, -CH-, H6), 3.88 (m, 2H, -CH-, H5), 3.54 (m, 2H, -CH-, H3), 3.25 (m, 2H, -CH-, H2), 3.12 (m, 2H, -CH-, H4), 2.88 (m, 2H, -S-CH₂-CH-), 2.69 (t, 2H, -CH₂-CH₂-S-CH₂-), 2.28 (t, 2H, -CH₂-COO-CH₃), 1.51-1.25 (m, 16H, aliphatic -CH₂-), 0.86 (t, 3H, aliphatic -CH₃).

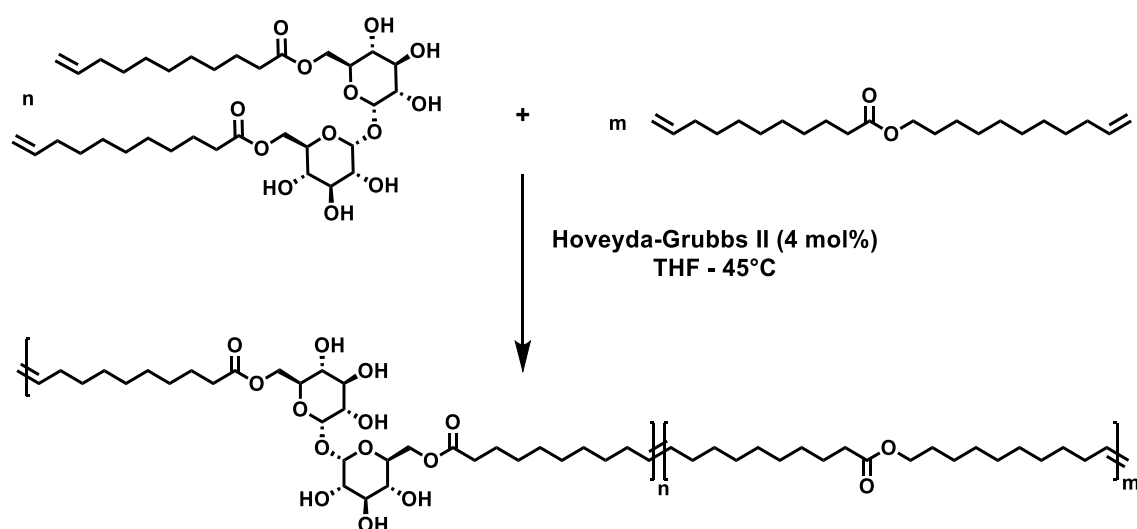
Polymerization

In a flame-dried Schlenk, 5-membered bis-cyclic carbonate (1 eq.) and corresponding diamine (1eq.) were dissolved in DMF (0.1 mL). The reaction medium was stirred at 60 °C for 5 days under nitrogen atmosphere.

III. Glyco-polyesters *via* ADMET polymerization

1. Glyco-polyester synthesis and characterization of glyco-polyesters

ADMET polymerization is performed on α,ω -diene monomers to produce unsaturated polymers. In this case, it is a step-growth polymerization driven by the release of ethylene. When the melting points of the monomers are not too high ($<100^{\circ}\text{C}$), the polymerization is conducted in bulk and under vacuum to remove the ethylene from the reaction medium. Thus, the equilibrium of the reaction is driven towards the polymer formation. But in our case, the melting point of the trehalose diundecenoate synthesized in the previous chapter is around 145°C which is incompatible with the catalyst stability to perform the polymerization in bulk. So, ADMET homopolymerizations and copolymerizations with a fatty acid-based diene, the undecenyl undecenoate, were conducted in THF for 24 hours at 45°C (Scheme 35).



Scheme 35: Synthesis of glyco-polyesters by ADMET polymerization

The first step consists in the study of the reactivity of the glycolipid diene toward the ruthenium-based metathesis catalyst. The efficiency of the catalyst depends on several factors, like the solubility of the monomer or the tolerance of the catalyst toward chemical functions present on the monomers. In order to select the best catalyst, trehalose diundecenoate was first reacted in THF with 4 mol.% Grubbs 1st (G1), Grubbs 2nd (G2), Hoveyda-Grubbs 1st (HG1) and Hoveyda-Grubbs 2nd (HG2) generation metathesis catalyst (Figure 68).

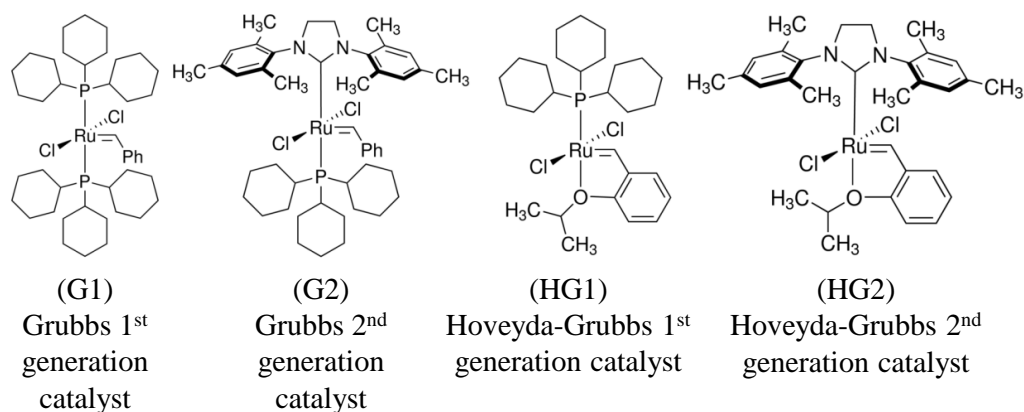
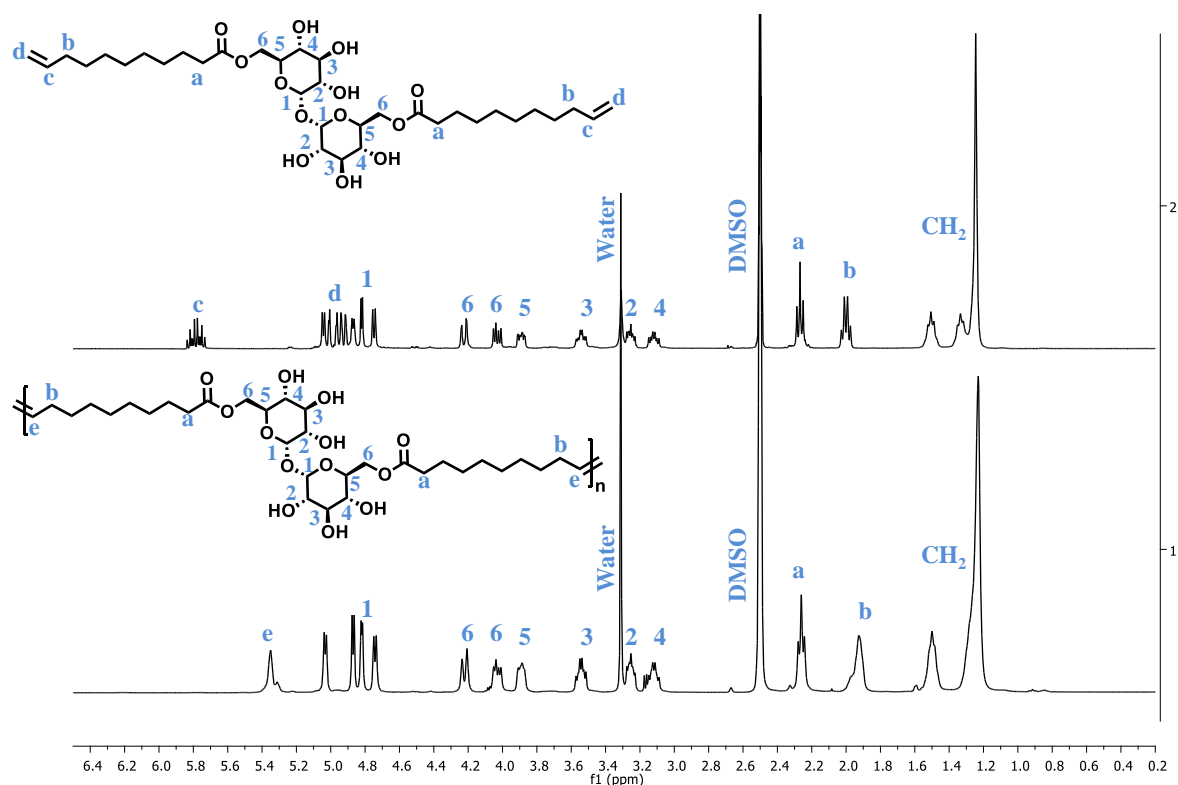


Figure 68: Chemical structures of ruthenium-based metathesis catalyst

The completion of the reaction was determined by ^1H NMR. For each polymer the disappearance of the terminal double bond peaks at 5.00 and 5.8 ppm and the appearance of the internal double bond peak at 5.35 ppm confirmed the polymer formation.

Figure 69 : ^1H NMR spectra of trehalose diundecenoate (top) and the glycol-polyester (bottom) performed in DMSO-d_6

The polymers were then purified by precipitation in cold methanol. The molar masses of the glyco-polyesters were determined by SEC in DMF and are summarized in Table 13.

Entry	Catalyst	\bar{M}_n^a (g.mol ⁻¹)	\bar{D}^a
1	G1	10000	1.9
2	G2	17000	3.1
3	HG1	4700	1.3
4	HG2	13200	2.1

Table 13: Macromolecular characteristics of glyco-polyesters synthesized by ADMET

^aDetermined by SEC in DMF, PS calibration

The SEC profiles of the glyco-polyesters synthesized by employing respectively HG2, G1, HG1 and G2, respectively are shown in Figure 70.

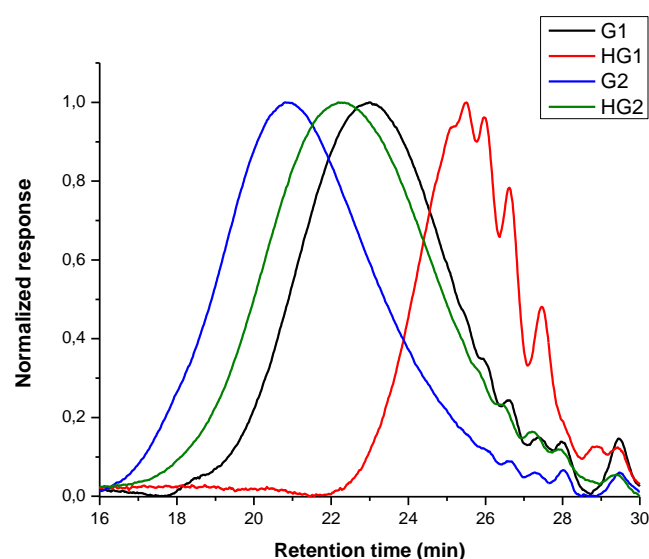


Figure 70 : SEC traces of glyco-polyesters synthesized by ADMET polymerization with different metathesis catalysts performed in DMF (LiBr, PS standards)

Hoveyda-Grubbs 2 (HG2) catalyst appears to be more relevant than the other catalysts. Indeed, molar masses around 13000 g.mol⁻¹ can be obtained after 24 hours of reaction and the dispersity is closed to 2. Consequently, HG2 was retained as a catalyst in the following. Indeed, compared to 1st generation catalyst, the 2nd generation metathesis catalysts are reported to be more reactive²¹ and Hoveyda-Grubbs 2nd generation catalyst exhibit good a moisture tolerance.²¹ In trehalose diesters, trace of water could remain due to the hygroscopy of trehalose. Thus it could explain why HG2 was the best catalyst for trehalose diundecenoate polymerization.

The undecenyl undecenoate was synthesized from esterification reaction between methyl undecenoate and undecenol catalyzed by TBD (Figure 71).

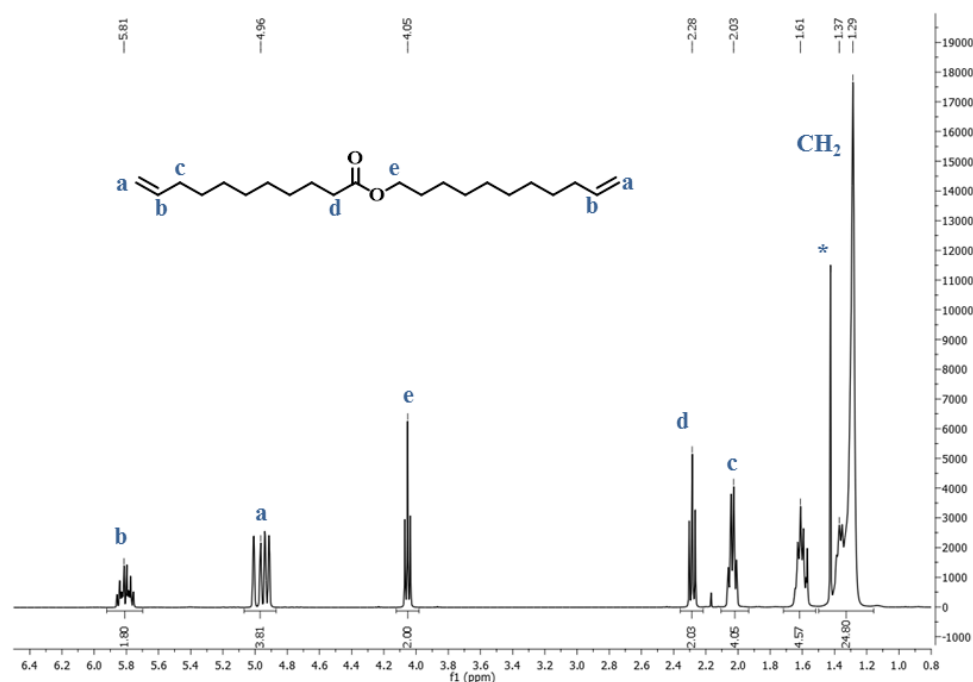


Figure 71 : ^1H NMR spectra of undecenyl undecenoate performed in CDCl_3

The latter was added as comonomer to trehalose diundecenoate in different proportions, from 33 mol.% to 67 mol.%, to get random copolymers. MALDI-TOF confirmed the formation of the copolymers (Figures S2 - S5). The completion of the polymerization reactions and the composition of each copolymer were evaluated by ^1H NMR. As previously discussed, the completion of the reaction was confirmed with the disappearance of the terminal double bond signal (5.00 and 5.8 ppm) and the appearance of the internal double bond signal (5.35 ppm). The copolymer composition was estimated using the 4.2 ppm chemical shift of protons 6' on the trehalose. The experimental polymer composition was found slightly different (Figure 72 and Table 14).

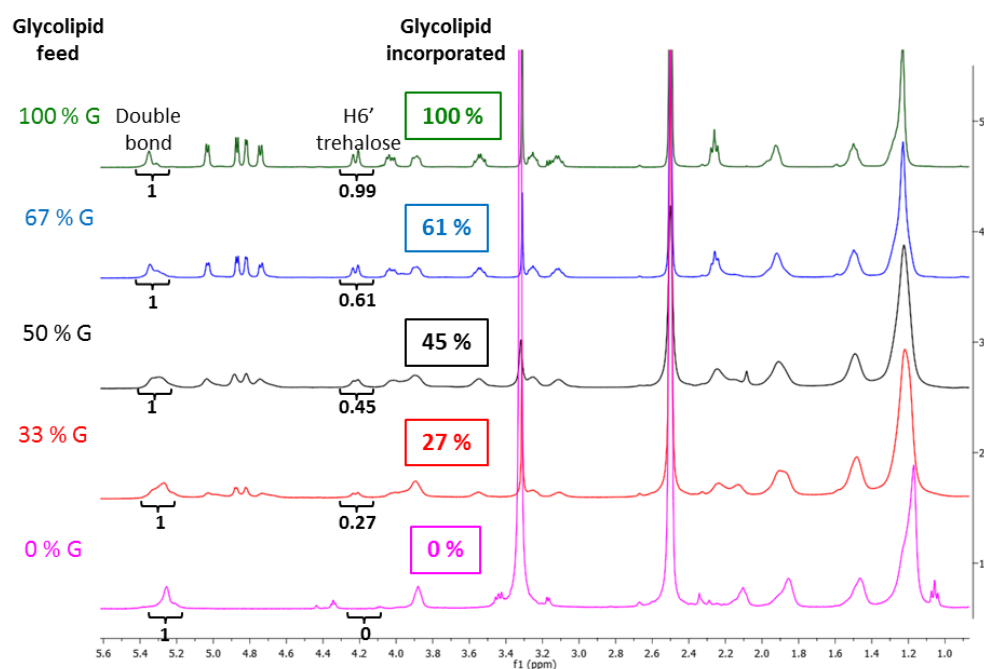


Figure 72 : ^1H NMR spectra in DMSO of the copolymers obtained by ADMET, with ratio of trehalose undecenoate incorporated (100 % G = 100 % trehalose undecenoate)

The SEC traces of the (co)polymers obtained by ADMET polymerization were determined by SEC in DMF and in THF due to the difference of solubility. The data are summarized in Table 14.

Polymers	Glycolipid feed (mol.%)	Glycolipid incorporated (mol.%)	\bar{M}_n^a (g.mol $^{-1}$)	D^a
GPes100	100	100	13200	2.1
GPes67	67	61	6000	1.7
GPes50	50	45	9600	1.6
GPes33	33	27	5500 - 3500 ^b	1.5 - 1.3 ^b
GPes0	0	0	2500 ^b	1.2 ^b

Table 14: SEC results of copolymers synthesized by ADMET polymerization

^aDetermined by SEC in DMF, PS calibration

^bDetermined by SEC in THF, PS calibration

The SEC traces of the copolymers obtained by ADMET polymerization are shown in Figure 73. The molar masses of the copolymers increase by increasing the glycolipid content and are ranging from 2500 to 13200 g.mol⁻¹ with a dispersity around 2.

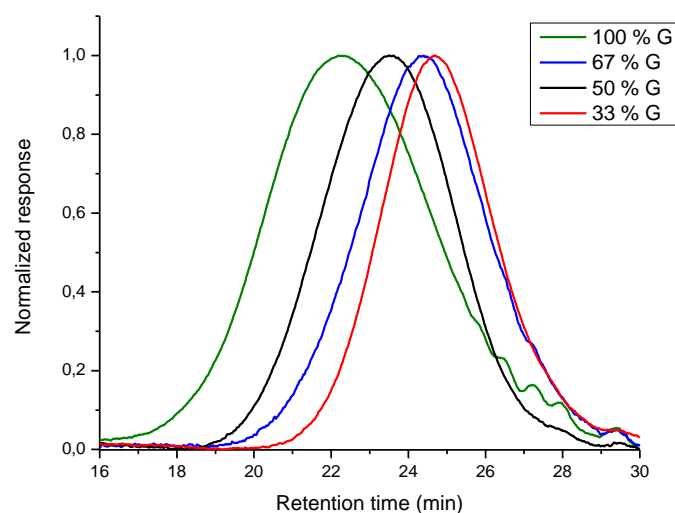


Figure 73: SEC traces of trehalose diundecenoate/undecenyl undecenoate copolymers after ADMET polymerization

2. Thermal properties and crystallization of glyco-polyesters

2.1. Thermal stability of copolymers synthesized by ADMET

The thermal stability of the homopolymers and copolymers was evaluated by thermogravimetric analysis (TGA) under nitrogen at a heating rate of 10 °C.min⁻¹. The (co)polymers containing glycolipids are thermally stable, up to 200 °C with a 5 wt.% loss occurring around 280°C. The “fatty” homopolymer is much more stable, up to 350°C with a 5 wt.% loss occurring at 380°C. In the TGA curves, two thermal decompositions can be observed, the first one could be attributed to the sugar part around 330°C and the second one to the lipidic part around 420°C (Figure 74).

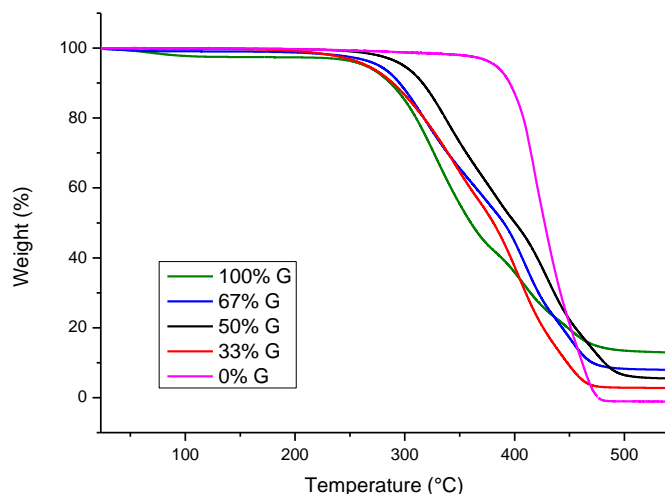


Figure 74: TGA curves of (co)polymers obtained by ADMET polymerization (percentage of glycolipid is indicated)

2.2. Thermal behavior of copolymers synthesized by ADMET

The thermal behavior of the (co)polymers was determined by DSC. All analyses were carried out under a nitrogen atmosphere with a $10\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ heating rate. The DSC traces are shown in Figure 75.

Polymers	Glycolipid feed (mol.%)	T_m ($^{\circ}\text{C}$)	ΔH (J/g)	$T_{d5\%}$ ($^{\circ}\text{C}$)
GPes100	100	156	19.57	275
GPes67	67	130	13.60	280
GPes50	50	12 - 145	9.2 – 9.9	278
GPes33	33	24 - 110	32.67 – 5.43	275
GPes0	0	35	83	380

Table 15 : Thermal properties of the (co)polymers obtained by ADMET polymerization from trehalose diundecenoate and undecenyl undecenoate

All the polymers so-formed were semi-crystalline but no glass transition temperature (T_g) could be detected by DSC even after quenching the samples in liquid nitrogen. GPes100 and GPes0 present a melting temperature (T_m) at $145\text{ }^{\circ}\text{C}$ and $35\text{ }^{\circ}\text{C}$, respectively. When 33 mol.% of glycolipid is incorporated in the copolymer, two melting temperatures are observed, one corresponding to the “lipidic part” at $25\text{ }^{\circ}\text{C}$ and one at $110\text{ }^{\circ}\text{C}$ corresponding to the “sugar part”. At a ratio of 50 mol.% of incorporated glycolipid, the melting temperature of the “sugar

part” increases to 145 °C and the melting temperature of the fatty counterpart stays around 20 °C. Above 50 mol.%, only one melting temperature can be observed at 130 °C.

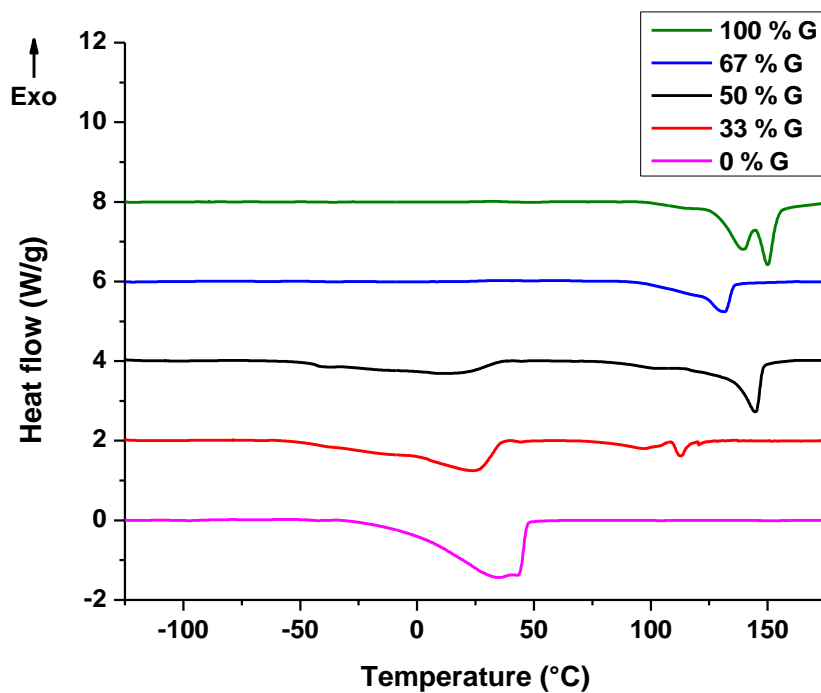


Figure 75 : DSC curves of the (co)polymers obtained by ADMET polymerization (percentage of glycolipid are indicated)

2.3. Structural characterization

To better understand the double crystallization of the copolymers, small-angle X-ray scattering experiments were performed. Figure 76 shows X-Ray spectrum at 30 °C of the powdery (co)polymers.

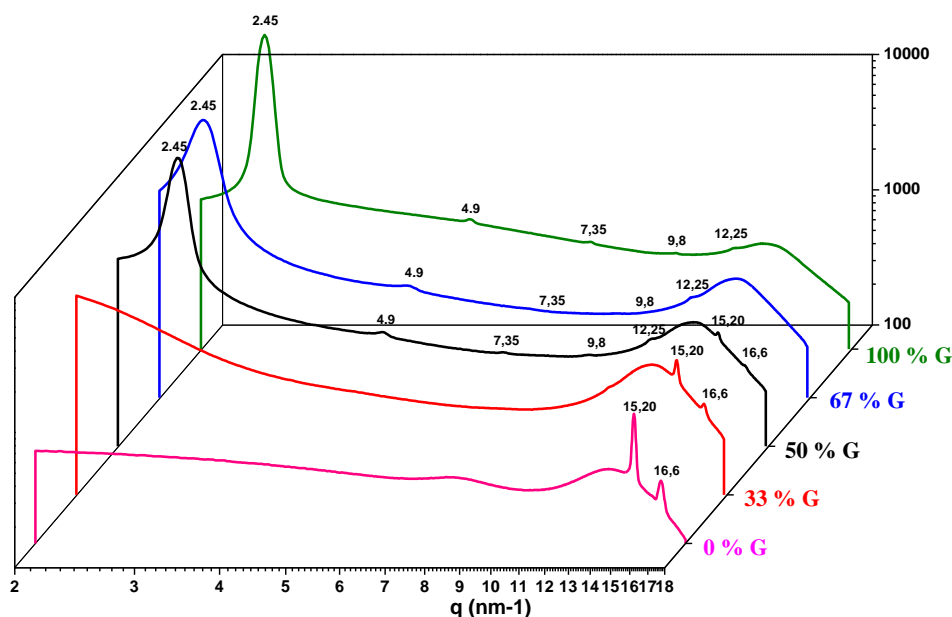


Figure 76: X-Ray spectra of the (co)polymers at 30°C after cooling from 160 °C

The X-Ray spectrum of the “fatty” homopolymer (0 % G) shows no small-angle reflections but wide-angle reflections ($q = 15.20$ and 16.6 nm^{-1}) corresponding to characteristic distance around 0.40 nm , which could be associated with the distance between aliphatic chains. These short distances of crystallization of long polyesters was already reported and described in the literature.²²

The diffractogram of the homopolymer synthesized from trehalose undecenoate (100 % G) shows characteristic peaks in the small-angle region, with a periodicity of q which corresponds to a lamellar packing ($q = 2.45, 4.9, 7.35, 9.8$ and 12.5 nm^{-1}). The first intense small-angle reflection observed at $q = 2.45 \text{ nm}^{-1}$ is assigned to a long period of 2.56 nm ($d=2\pi/q$). This long period could be attributed to the distance between two trehalose units in the polymer backbone. These observations are in agreement with earlier work²³ on polysphorolipids. However, no short distance peak that may reflect the lipid phase crystallization is observed on the spectrum. This feature could be explained by the presence of the trehalose units in the polymer backbone. Indeed, the sugar creates inter-chain spacing, which may prevent the crystallization of the lipid fraction.

Where 33 % of glycolipid is incorporated in the copolymer, the crystallization of the lipidic phase at short distance is observed. However, a double melting point is observed on the DSC

curves. The too small amount of trehalose units incorporated in the polymer backbone cannot induce a lamellar organization to be observed by X-ray scattering.

With 50 % of glycolipid in the copolymer, the two types of organizations are observed. There is enough sugar and lipidic units to provide the two crystallizations.

When 67 % of glycolipid is incorporated in the polymer chains, the lamellar organization of the trehalose is only observed. The large amount of sugar present in the backbone prevents the crystallization of the lipidic phase, as it is the case for the homo-glyco-polyester (100 % G).

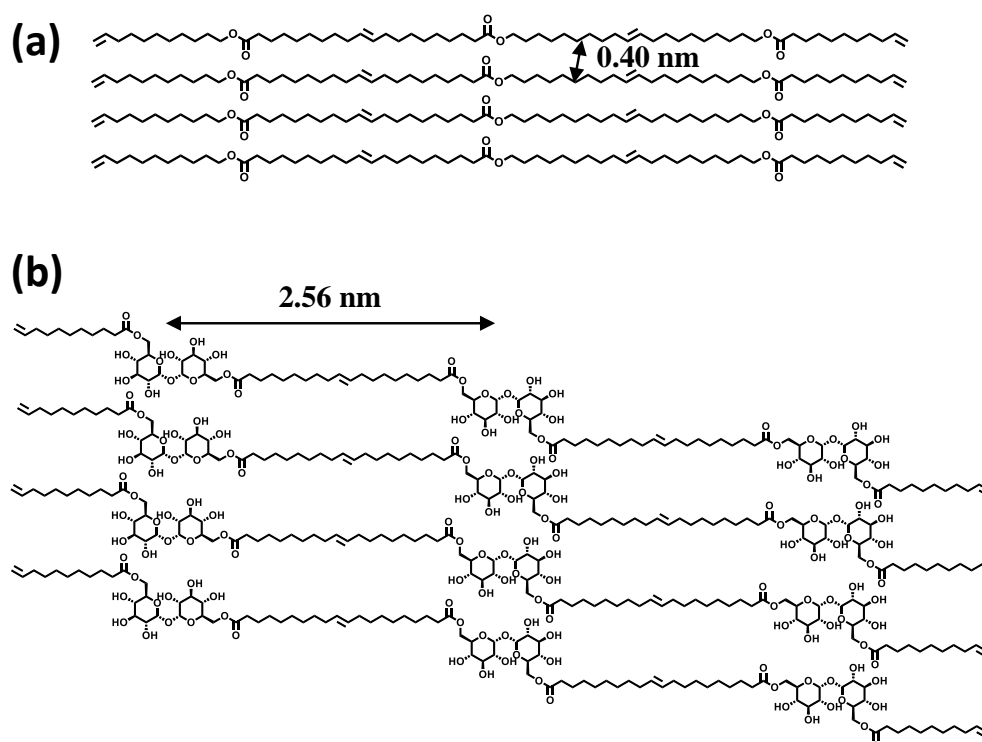


Figure 77 : (Co)polymers packing model, lamellar packing of the homopolymer synthesized with 100 % of glycolipid (a), lipid crystallization of the homopolymer synthesized with 0 % of glycolipid

Figure 78 and Figure 79 show X-Ray spectra at 100 °C and 160 °C of the powdery (co)polymers. It could be noted that the long period ($d = 2.56$ nm) observed in X-ray spectra of the polymers are close to the distance observed in X-ray spectra of the gels ($d = 2.3$ nm). This could be confirmed that in the gels the trehalose diesters are self-assembled into lamellar multi-layer stackings.

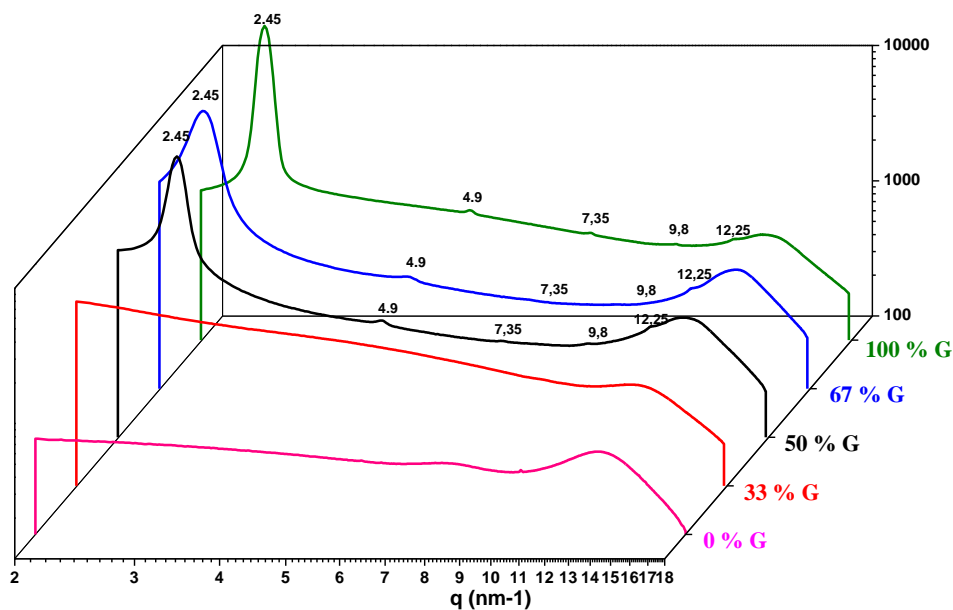


Figure 78: X-Ray spectra of the (co)polymers at 100°C after cooling from 160 °C

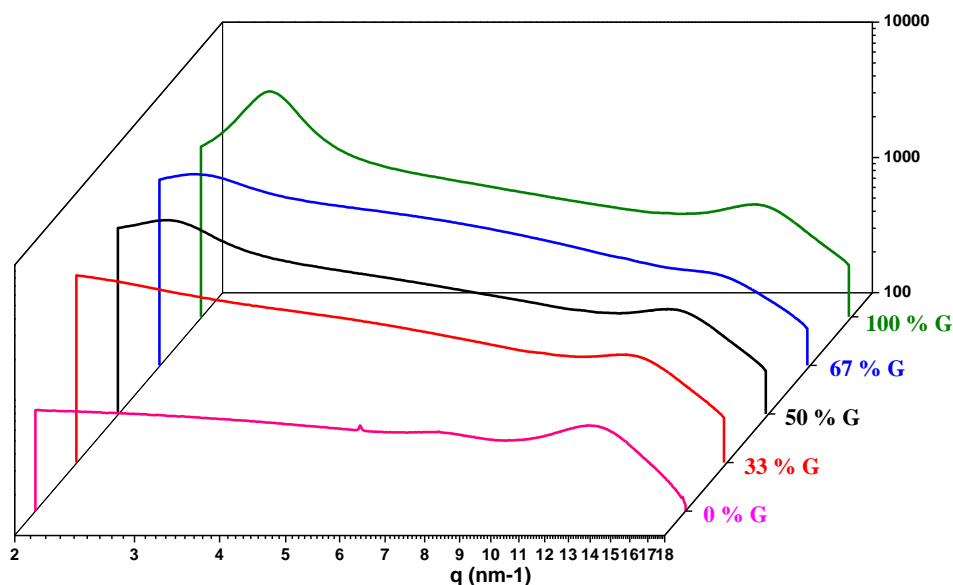


Figure 79: X-Ray spectra of the (co)polymers at 160°C

On the X-Ray spectra at 100 °C (Figure 78), the short distance reflections ($d = 0.40$ nm) disappeared in the cases of GlycoP0 and of the copolymers containing 33 % and 50 % of glycolipid (GlycoP33 and GlycoP50), showing the loss of the lipidic phase crystallization. This statement is in agreement with DSC data. At 160 °C (Figure 79), above the melting temperature, the long period reflections ($d=2.56$ nm), corresponding to the lamellar packing

between sugars along the chains, disappears. That corresponds to the second fusion observed by DSC.

3. Experimental

Synthesis of undecenyl undecenoate

Undecenol (12.8 g, 0.08 mol.) was blended with 10-methylundecenoate (15 g, 0.08 mol.). TBD (5% mol.) was added as a catalyst. The reaction was performed under a nitrogen flow at 120°C for 2h, then the temperature was increased to 160 °C for 2h more under dynamic vacuum. Purification over silica gel flash chromatography was performed using cyclohexane/ethyl acetate 94/6 eluent. Yield: 76%. ¹H NMR (400MHz, CDCl₃, δ (ppm)): 5.8 (m, 1H, -CH=CH₂), 4.9 (m, 2H, CH₂=CH-), 4.0 (t, 2H, -CH₂COO-), 2.2 (t, 2H, -COOCH₂-), 2.0 (m, 4H, -CH₂-CH=CH-), 1.5-1.2 (m, 26H, aliphatic -CH₂-).

ADMET polymerization:

Homopolymerization:

Into a flame-dried Schlenk flask equipped with bubbler, trehalose diundecenoate (0.2 g, 0.3 mmol.) dried over-night under vacuum was dissolved in 2 mL of dry THF. Metathesis catalyst (4 mol.%) was added and the reaction mixture was stirred under nitrogen atmosphere for 24 h at 45 °C. Then, 3 ml of ethyl vinyl ether were introduced into the flask to quench the reaction. The final glyco-polyester was purified by precipitation in cold methanol.

Copolymerization:

Into a flame-dried round Schlenk flask, trehalose diundecenoate (0.2 g, 0.3 mmol.) and the corresponding amount of undecenyl undecenoate (see Table 14) were mixed and dried over-night under vacuum. Then, the diene monomers were dissolved in 2 mL of dry THF and Hoveyda-Grubbs 2nd generation (4 mol.%) was added. The reaction mixture was stirred at 45 °C under nitrogen atmosphere for 24 h. Then, 3 mL of ethyl vinyl ether and 4 mL of THF were introduced to the flask. The final copolymers were purified by precipitation in cold methanol.

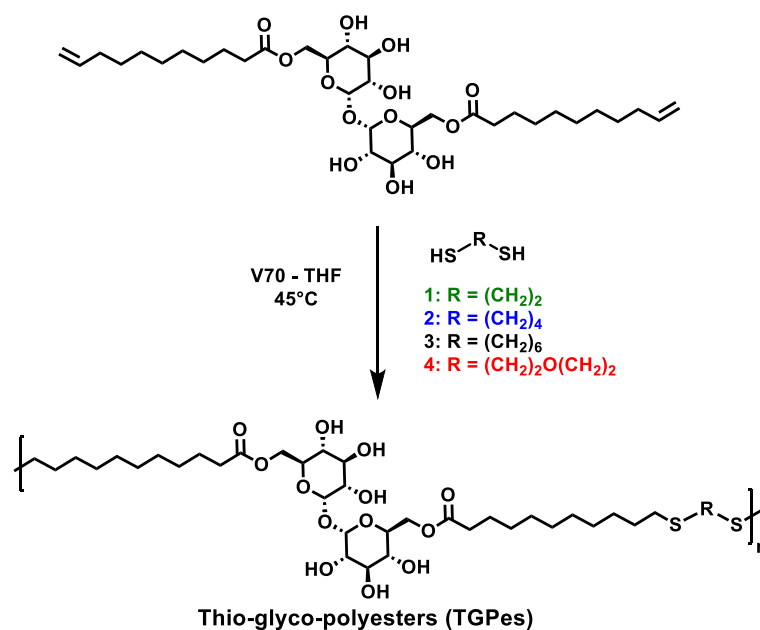
IV. Thio-glyco-polyesters *via* thiol-ene polymerization

Thiol-ene polymerization is considered as click reaction due to the high reaction yield, the mild reaction condition and the non-production of by-products. This polymerization is growing in the field of biomedical applications. Thanks to its versatility and simplicity, thiol-ene polymerization was used to polymerize the trehalose diundecenoate synthesized in the previous chapter. This study is described in this section.

1. Thio-glyco-polyesters synthesis, molecular structure and dimension

The trehalose diundecenoate was polymerized by thiol-ene addition with different dithiols. Four of them were employed to evaluate the influence of the carbon chain length and the presence of the ether moiety on the polymer behaviors (Scheme 36). It is important to notice that the reaction kinetic depends on the structure of the dithiol. Indeed, a terminal double bond is much more reactive than an internal one. In our case, the diene possesses terminal double bond, that makes it more suitable for a thiol-ene polymerization in comparison to internal ones. Generally, the thiol-ene polymerization is either not catalyzed or catalyzed by a thermal initiator (usually the AIBN) or a photo initiator (usually the DMPA). In this case, we selected the V70 (2,2'-Azobis(4-methoxy-2,4-dimethylvaleronitrile)) as an initiator, because it possesses a lower decomposition temperature than AIBN. It allowed us conducting the polymerization at 45 °C in THF to keep the same polymerization conditions as for the ADMET polymerization.

Based on ^1H NMR analyses, the polymerizations were considered complete after 24h. It was confirmed for each thio-glyco-polyesters (TGPeS) with the disappearance of the terminal double bond located at 5.00 and 5.8 ppm (Figure 80). At the end of the reaction, the polymers were purified by precipitation in cold methanol.



Scheme 36: Synthesis of thio-glyco-polyesters TGPEs 1 to 4 by thiol-ene polymerization from trehalose diundecenoate and different dithiols (up) and its oxidized products SOGPES, SGPEs (bottom)

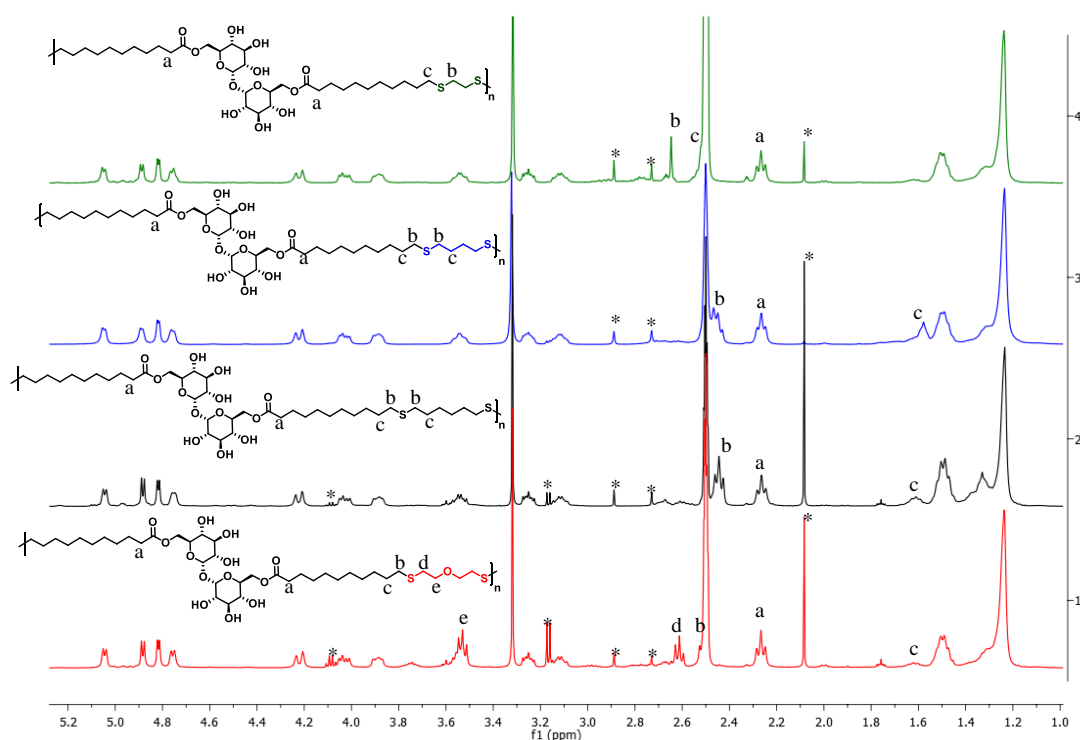


Figure 80 : ^1H NMR spectra of the thio-glyco-polyester obtained by thiol-ene polymerization

The thio-glyco-polyester molar masses were determined by SEC in DMF and are summarized in Table 16 and SEC traces are shown in Figure 81.

Polymer	Dithiol	\bar{M}_n (g.mol ⁻¹)	\bar{D}^a
TGPes1	1,2-Ethanedithiol	7800	2.5
TGPes2	1,4-Butanedithiol	16800	2.3
TGPes3	1,6-Hexanedithiol	13700	2.3
TGPes4	2-Mercaptoethyl ether	11500	2

Table 16 : SEC data of the polymers synthesized by thiol-ene polymerization (Determined by SEC in DMF, LiBr, PS calibration) from trehalose diundecenoate and different dithiols.

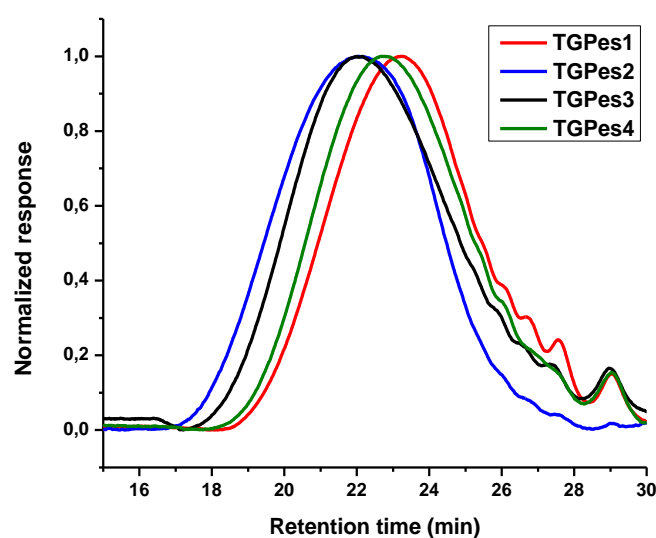
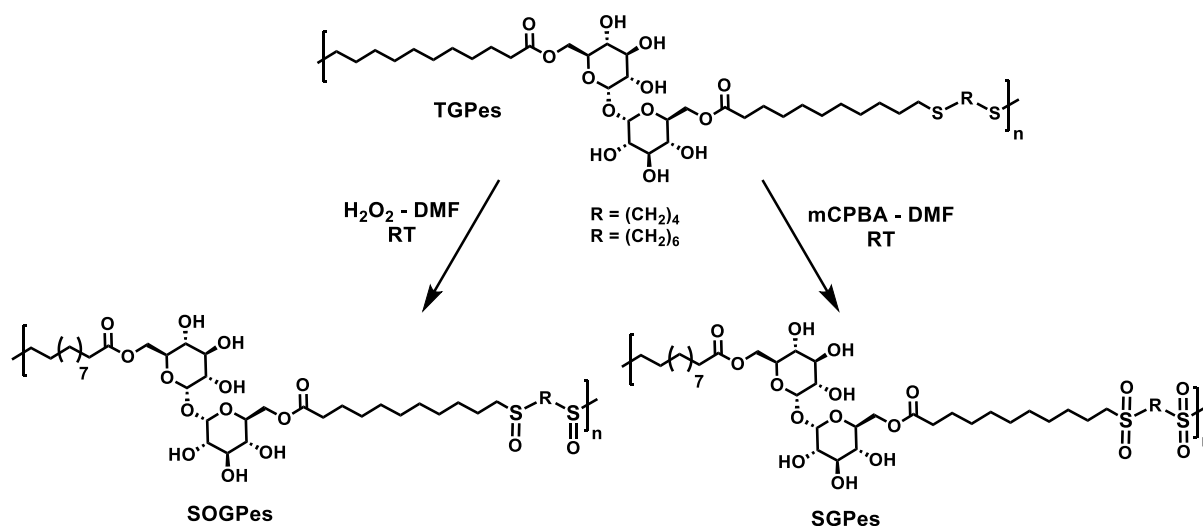


Figure 81 : SEC traces of thio-glyco-polyesters after thiol-ene polymerization performed in DMF (LiBr, PS standards)

SEC analysis indicated that the polymers were successfully formed. The lowest molar masses were obtained for the polymer synthesized with the shortest dithiol. For the others, the molar masses are above 11000 g.mol⁻¹, with dispersity value around 2.3. Dispersities of 2 were expected due to the step-growth mechanism of the thiol-ene condensation polymerization.

The sulfur atoms on the polymer backbone were then oxidized at room temperature with hydrogen peroxide and meta-chloroperoxybenzoic acid (mCPBA) to obtain respectively sulfoxides (SOGPes) and sulfones (SGPes) groups on the polymers in order to increase their hydrophilicity and to see the impact on the thermal properties.



Scheme 37: Synthetic pathways of oxidized products SOGPes (left) and SGPes (right) from thio-glyco-polyesters TGPes2 and TGPes4

The oxidation of TGPes2 and TGPes3 were assessed by FTIR and ^1H NMR spectroscopy (Figure 82 and Figure 83). After stirring overnight with H_2O_2 in DMF and purification, ^1H NMR analysis confirmed the quantitative sulfoxide formation. Indeed, the peak at 2.46 ppm corresponding to protons in alpha to the sulfur atoms disappeared, and a new peak at 2.70 ppm corresponding to protons adjacent to sulfoxide appeared. The oxidation of TGPes2 and TGPes3 in SGPes2 and SGPes3 was characterized similarly. After 5h stirring with mCPBA in DMF and purifying, ^1H NMR analysis validated the quantitative formation of sulfone as proved by the disappearance of the peak at 2.46 ppm and the appearance of peaks at 3.15 ppm corresponding to protons nearby the sulfone moiety. No formation of sulfoxide is observed in ^1H NMR, as no characteristic sulfoxide peak appeared. The sulfone formation was also confirmed by FTIR with the appearance of bands between 1250 cm^{-1} and 1380 cm^{-1} .

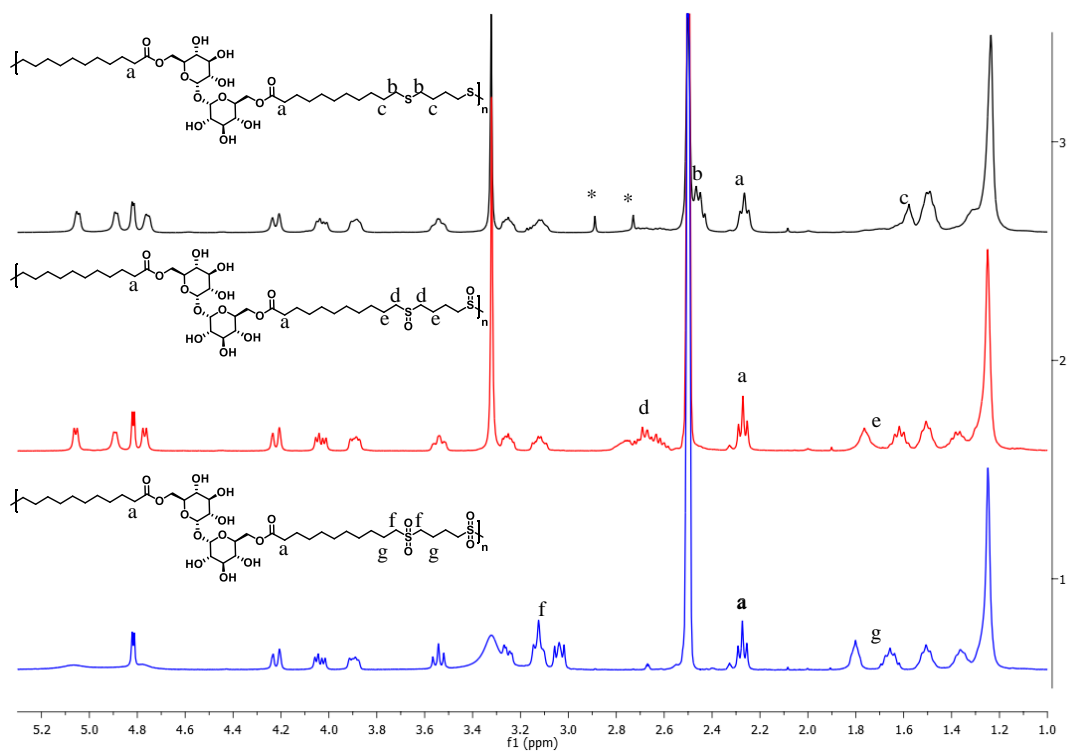


Figure 82: Stacked ^1H NMR spectra of TGPes2 (black), SOGPes2 (red) and SGPes2 (blue) in DMSO-d_6

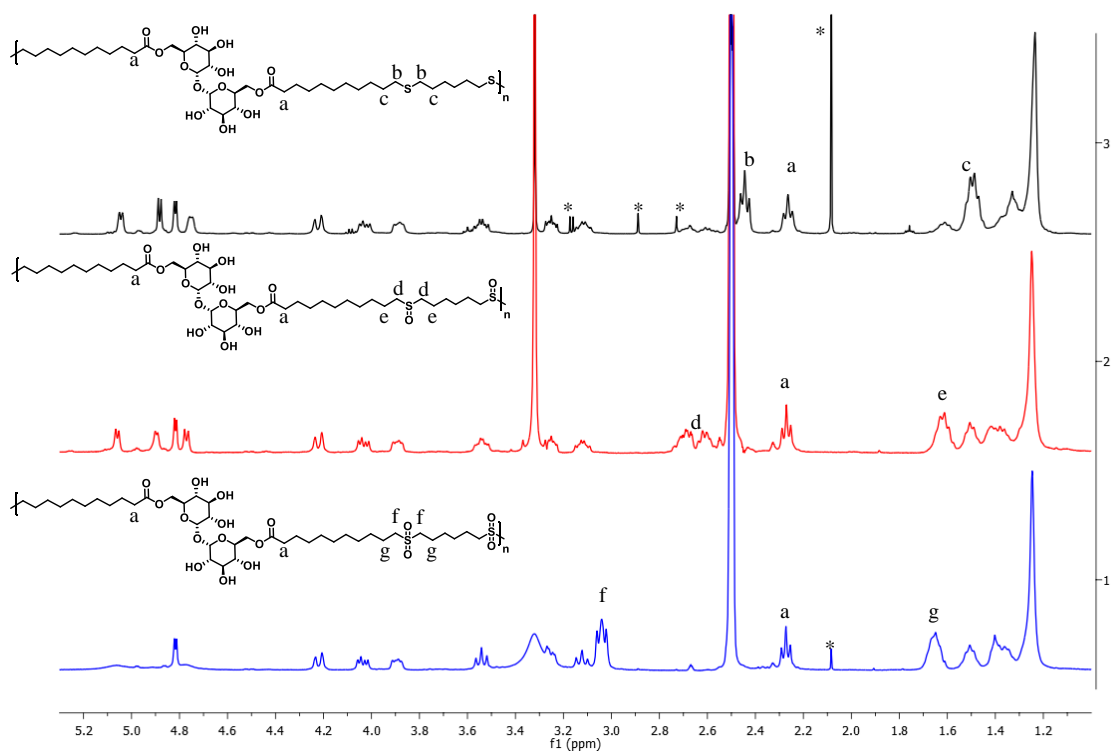


Figure 83 : Stacked ^1H NMR spectra of TGPes3 (black), SOGPes3 (red) and SGPes3 (blue) in DMSO-d_6

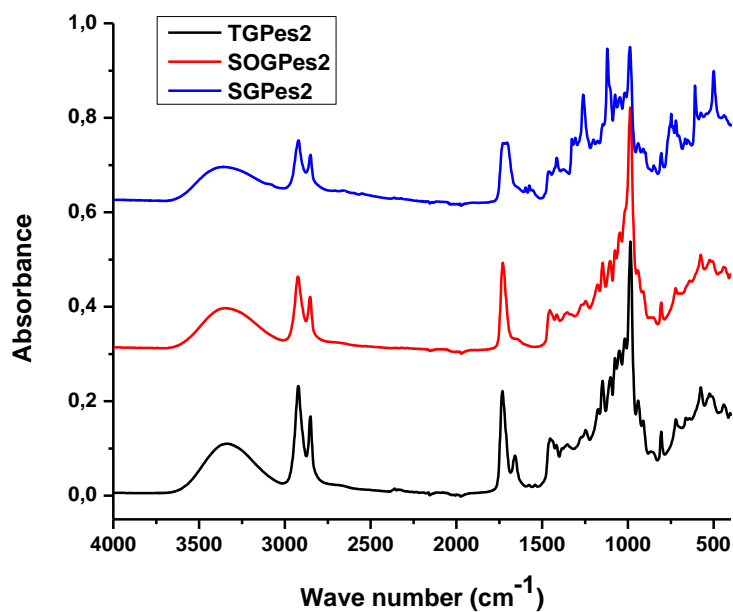


Figure 84: FTIR spectra of TGPes2, SOGPes2 (sulfoxide) and SGPes2 (sulfone)

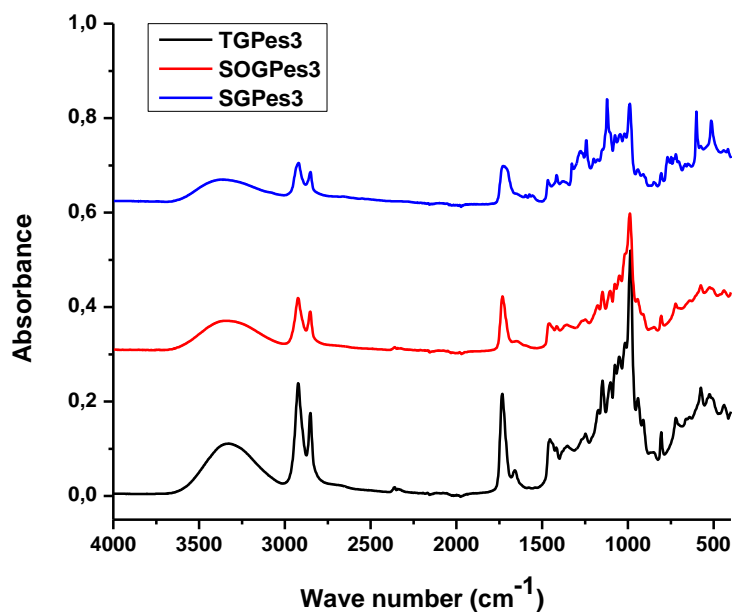


Figure 85: FTIR spectra of TGPes2, SOGPes2 (sulfoxide) and SGPes2 (sulfone)

Hydrolysis of glycosidic and ester bonds did not occur under these conditions, as no characteristic peaks (acid or glucose) appeared in ¹H NMR spectra. SEC in DMF was performed on SOGPes2, SOGPes3, SGPes2 and SGPes3 to attest that hydrolysis did not occurred during the oxidation (Figure 86).

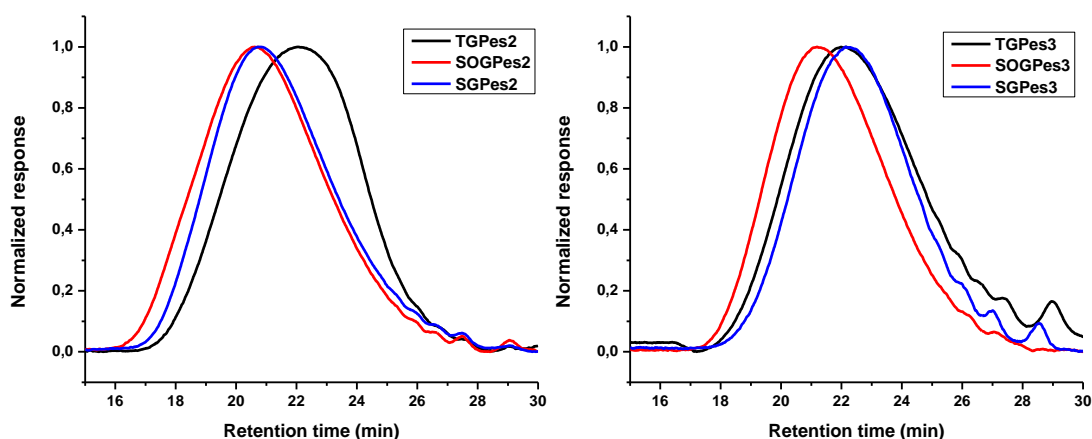


Figure 86 : SEC traces of TGPes2 and its oxidized products SOGPes2 and SGPes2 (on the left) and SEC traces of TGPes3 and its oxidized products SOGPes3 and SGPes3 (on the right) performed in DMF (LiBr, standards)

2. Thermal properties of thio-glyco-polyesters

The thermal stability and properties of all the thio-glyco-polyesters were evaluated by TGA and DSC (Figure 87). Data are summarized in Table 17. The thio-glyco-polyesters 1-4 are thermally stable, up to 200 °C with 5 wt.% loss occurring around 270 °C.

Polymer	Dithiol	T _g	T _m	T _{d5%}
TGPes1	1,2-Ethanedithiol	76 °C	107 °C	270 °C
TGPes2	1,4-Butanedithiol	75 °C	110 °C	270 °C
SOGPes2	-	76 °C	-	-
SGPes2	-	54 °C	110 °C	-
TGPes3	1,6-Hexanedithiol	73 °C	104 °C	276 °C
SOGPes3	-	74 °C	-	-
SGPes3	-	51 °C	-	-
TGPes4	2-Mercaptoethyl ether	64 °C	102 °C	266 °C

Table 17: Thermal properties of the polymers obtained by thiol-ene polymerization and of oxidized polymers (second heating run performed at 10°C.min⁻¹)

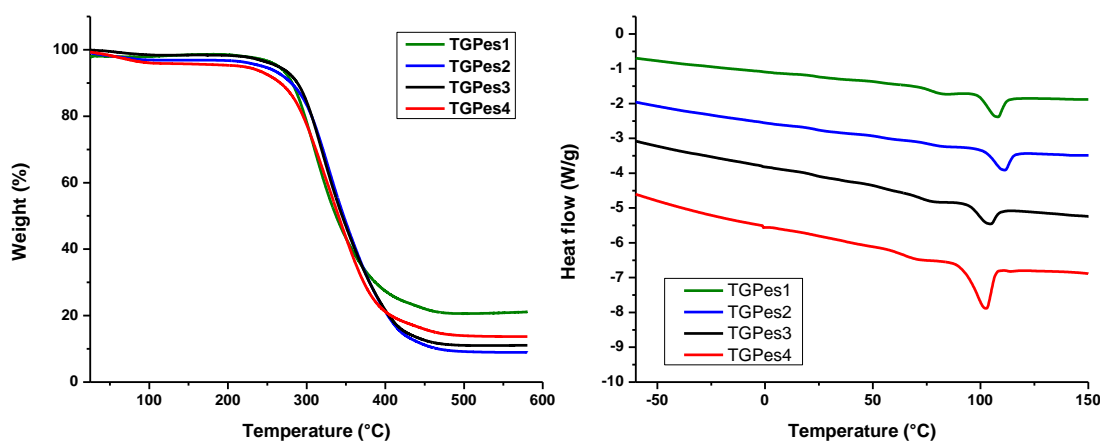


Figure 87 : TGA curves of polymers obtained by thiol-ene polymerization (left). DSC traces of the thio-glyco-polyesters (second heating run at $10^{\circ}\text{C}\cdot\text{min}^{-1}$) (right).

Differential scanning calorimetry analyses show that all the thio-glyco-polyesters are semi-crystalline with a melting temperature around 105°C . When the carbon chain length is increased between the sulfur atoms (from 2 to 6), the glass transition temperature decreases from 76 to 64°C .

The thermal stability of the oxidized polymers was compared to the one of the non-oxidized ones. Indeed, SOGPes2 and SOGPes3 present two thermal degradations, the first one at 225°C and the second one at 345°C while the TGA traces of SGPes2 and SGPes3 exhibit three main degradations at 237°C , 320°C and 405°C . The higher the oxygen content in the polymer, the lower its thermal stability.

The oxidized polymers were also analyzed by DSC. As it was shown before, all the thio-glyco-polyesters are semi-crystalline.

SOGPes2 and SOGPes3 are amorphous polymer with a T_g at 76°C and 74°C respectively. Thus, the sulfur oxidation into sulfoxide disturbs the polymer crystallinity. Concerning the polymers containing sulfone moieties, SGPes2 exhibits a T_g at 54°C and a melting temperature at 110°C while SGPes3 is amorphous with a T_g at 51°C . It is nevertheless difficult to explain the crystallinity of SGPes2.

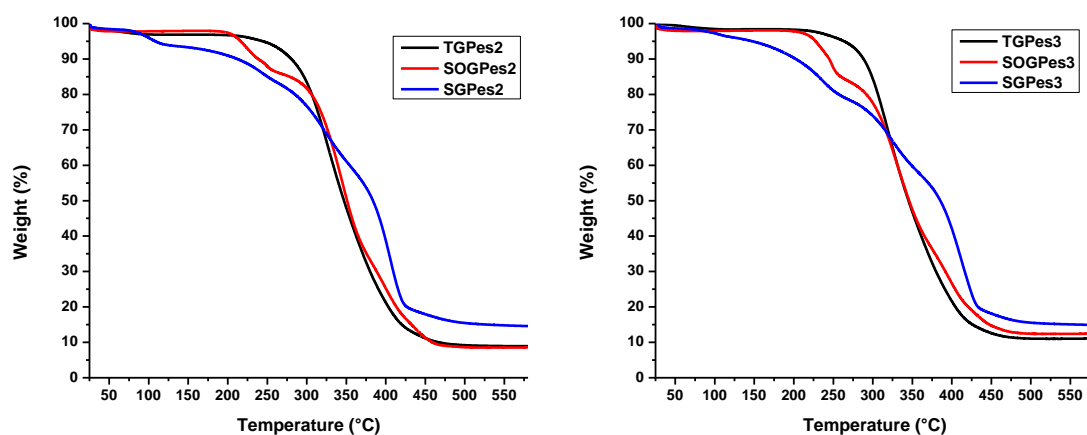


Figure 88 : TGA curves of TGPes2 and its oxidized products SOGPes2 and SGPes2 (on the left) and TGA curves of TGPes3 and its oxidized products SOGPes3 and SGPes3 (on the right)

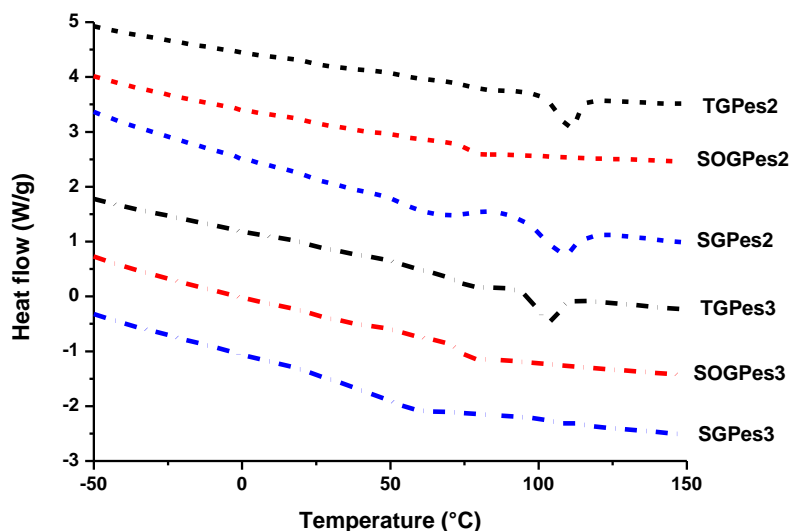


Figure 89 : DSC curves of TGPes2 and its oxidized products SOGPes2 and SGPes2 and TGPes3 and its oxidized products SOGPes3 and SGPes3 (second heating cycle)

3. Experimental Part

Thiol-ene polymerization:

Into a flame-dried round Schlenk flask, were introduced the diesters of trehalose (1 eq.) and the dithiols (1 eq.). The latter were dissolved in THF (2 mL) and V70 (10 mol.%) was added. The reaction mixtures were stirred at 45 °C for 24 h under nitrogen atmosphere, then precipitated into cold methanol to get the pure glyco-thio-polymers.

Oxidation of thio-glyco-polyesters into sulfoxides

Into a Schlenk flask, thio-glyco-polyesters (30 mg, 0.04 mmol.) were introduced and dissolved into DMF (0.5 mL). Then a solution of hydrogen peroxide 30 wt.% in water (4 eq, 15 μ L) was added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was then purified by dialysis into de-ionized water to remove H₂O₂ and DMF and was dried by freeze-drying to obtain the final pure polymers.

Oxidation of thio-glyco-polyesters into sulfones

Into a Schlenk flask, thio-glyco-polyesters (30 mg, 0.04 mmol.) were introduced and dissolved into DMF (0.5 mL). Then a solution of mCPBA (4 eq., 84 mg, 0.16 mmol.) in DMF (0.54 mL) was added and the reaction mixture was stirred 5 h at room temperature. The reaction mixture was then purified by dialysis into de-ionized water to remove the mCPBA and the DMF was dried by freeze-drying to obtain the final pure polymers.

V. Preliminary study of polymers self-assembly properties in water

1. Polymer assemblies study

The self-assembly properties in water of the glyco-polyesters and thio-glyco-polyesters were preliminary studied following the solvent displacement method (also referred to nanoprecipitation). This study was not done on the PUs and PHUs due to a too low amount of polymers.

As such, to induce self-assembly, solutions of polymers in DMF (a good solvent for both the lipid and sugar moieties) were dialyzed against water, which is a non-solvent for the lipidic part. Therefore, by replacing the organic solvent by water, the self-assembly of the polymers was induced. Milky solutions were obtained after the dialysis. The concentration of the polymer dispersion in water was adjusted to $0.5 \text{ mg}\cdot\text{mL}^{-1}$ by dilution. The resulting assemblies were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS). The hydrodynamic diameters and polydispersity indices (PDIs) of the polymer assemblies were determined and are summarized in Table 18. TEM images revealed that all assemblies were spherical in shape (Figure 90).

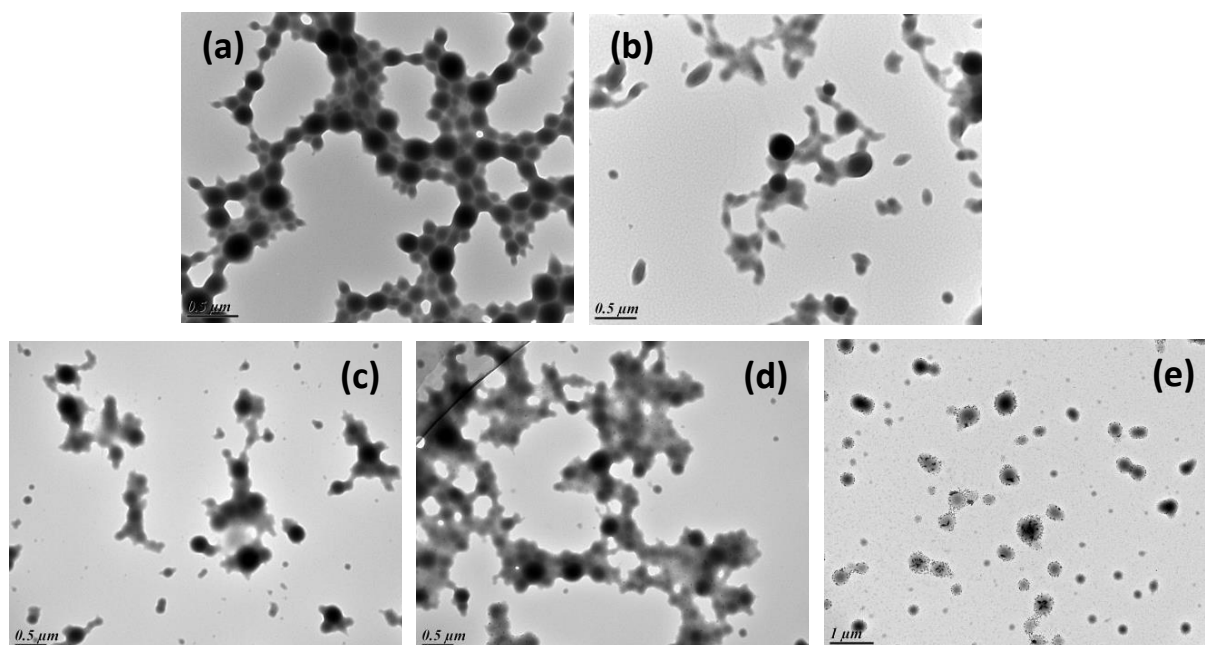


Figure 90: TEM images of assemblies: (a) GPes100, (b) GPes50, (c) TGPes2, (d) TGPes3 and TGPes4 obtained by dialyzing DMF solution against water. The samples are all unstained.

Polymers	Polymerization	d_H (nm)	PDI
GPes100		200	0.01
GPes67	ADMET	160	0.22
GPes50		160	0.07
GPes33		160	0.11
TGPes2		190	0.07
TGPes3	Thiol-ene polymerization	200	0.08
TGPes4		200	0.06
SOGPes2		190	0.04
SGPes2	Sulfur oxidation after thiol-ene polymerization	570	0.43
SOGPes3		170	0.08
SGPes3		220	0.22

Table 18: Hydrodynamic diameters and polydispersity indices (PDIs) measured by DLS of the assemblies formed from glyco-polyesters (GPes), thio-glyco-polyesters (TGPes), sulfoxide-glyco-polyesters (SOGPes) and sulfone-glyco-polyesters (SGPes)

DLS analysis (Figures 43-45) showed that all the polymers exhibit only one relaxation time and so a monomodal distribution, proving that the entire polymers are able to self-assemble in water by nanoprecipitation. DLS measurements showed that except for SGPes2, all the polymers have a hydrodynamic diameter (d_H) between 160 and 220 nm with relatively low PDI (below 0.25).

Therefore, the incorporation of the fatty monomer in the copolymer synthesized by ADMET does not have an influence on the self-assembly. Besides, the different alkyl chain length between the sulphur atoms in the thio-glycopolyesters and the sulfoxide or sulfone moieties do not have a real impact on the self-assembly as well. Indeed, all the hydrodynamic volumes were in the range. The sulfone-glyco-polyester SGPes2 had a largely higher hydrodynamic diameter (570 nm) than the others polymers which could not be explained.

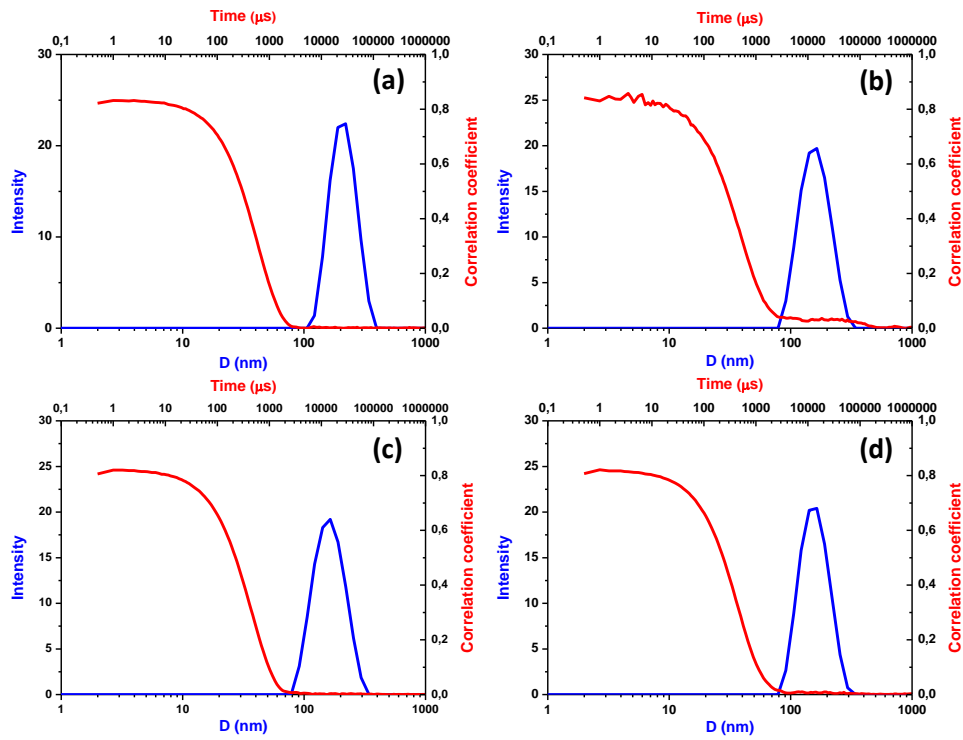


Figure 91: Auto-correlation functions (red) and relaxation time distribution (blue) (determined at 90°) scattering obtained from DLS for assemblies in water: (a) GPeS100, (b) GPeS67, (c) GPeS50, (d) GPeS33

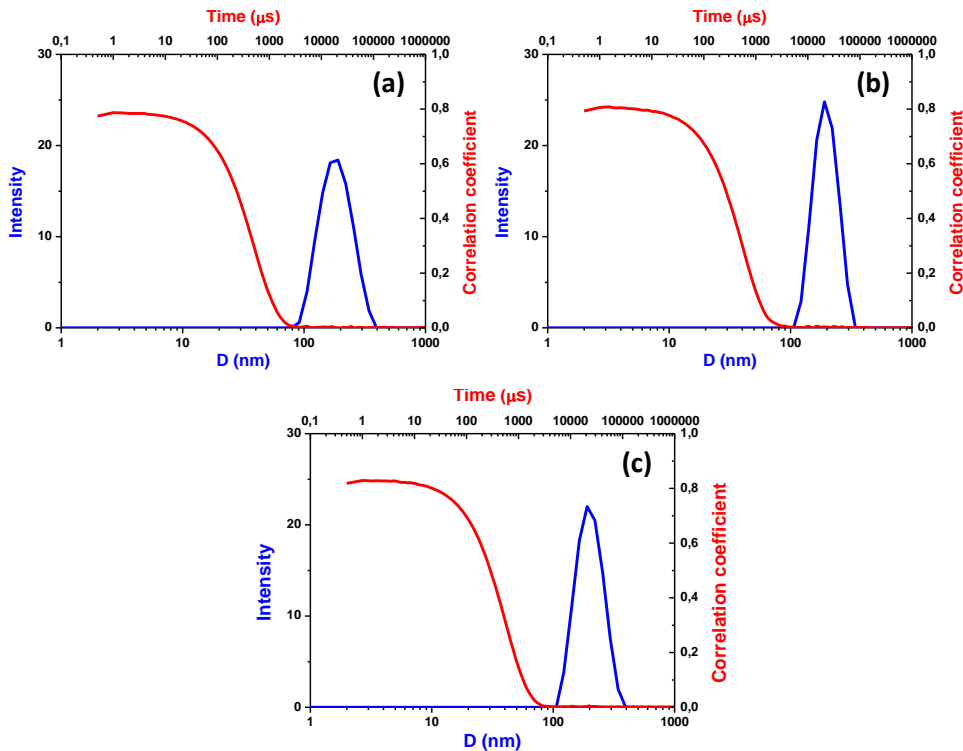


Figure 92: Auto-correlation functions (red) and relaxation time distribution (blue) (determined at 90°) scattering obtained from DLS for assemblies in water: (a) TGPes2, (b) TGPes3, (c) TGPes4

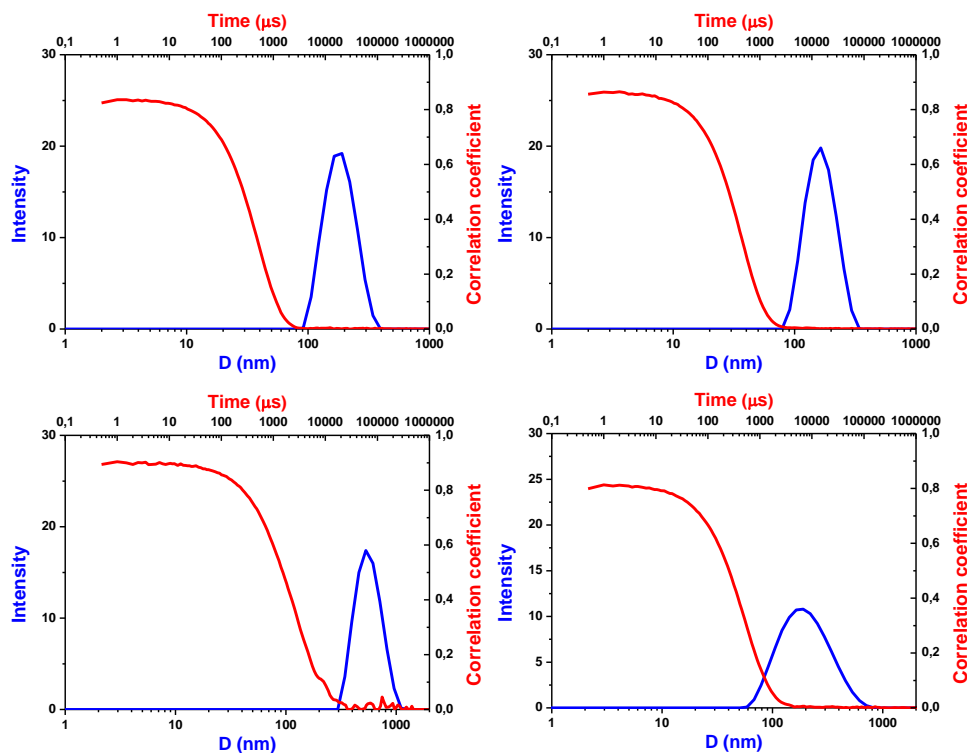


Figure 93: Auto-correlation functions (red) and relaxation time distribution (blue) (determined at 90°) scattering obtained from DLS for assemblies in water: (a) SOGPes2, (b) SOGPes3, (c) SGPes2, (d) SGPes3

2. Experimental

Solvent displacement (nanoprecipitation)

Polymers were solubilized in DMF and polymer solutions were then poured into dialysis membranes with molecular weight cutoff of 1kD. The membranes were beforehand soaked for 15 min and rinsed with deionized water. The membranes were then submerged in 2 L of deionized water and dialyzed under gentle magnetic stirring for 12 h. After 2 h and 4 h, the dialysis solution was replaced by fresh deionized water. At the end of the dialysis, milky solutions present inside the dialysis membranes were recover.

VI. Conclusions and perspectives

In this chapter, polymers were synthesized from different trehalose diesters. Thermoplastics polyurethanes, poly(hydroxyurethane)s and polyesters prepared by ADMET or thiol-ene were successfully obtained. Polyurethanes were prepared from three different trehalose diesters containing hydroxyl groups on the alkyl chain and two diisocyanates. It appeared that branched polyurethanes were synthesized due to the possible reaction between the secondary hydroxyl group of the trehalose moiety and the diisocyanates. Then, poly(hydroxyurethane)s were achieved by the reaction between diamines and two different trehalose diesters containing 5-membered bis-cyclic carbonates. Thus, linear PHUs with reasonable molar masses (from 3000 to 10000 g.mol⁻¹) were prepared by ring opening of the cyclic carbonates with different diamines. Copolymers were also obtained by ADMET polymerization between trehalose diundecenoate and undecenyl undecenoate and with molar masses up to 13000 g.mol⁻¹. These novel glyco-polyesters exhibit unique properties due to the lipidic and carbohydrate part that induced two different crystallization types within the materials. Furthermore, thio-glyco-polyesters with molar masses between 7000 and 16000 g.mol⁻¹ were synthesized from thiol-ene polymerization between trehalose diundecenoate and dithiols with various chain lengths and structures. The sulfur atoms present on these latest polymers were successfully oxidized to selectively obtained sulfoxide or sulfone group on the polymer backbone. Thus, the structure of these thio-glyco-polyesters can be easily tuned by oxidation.

Finally, in this chapter, a preliminary investigation was performed on these new polymers to evaluate their self-assembly in water. The polymers were nanoprecipitated by solvent displacement to form spherical assemblies with hydrodynamic diameters around 200 nm. This preliminary physicochemical investigation showed promising results with respect to the glycopolymers, opening the way to future development in particular in the biomedical field. However a series of important questions still need to be addressed such as the toxicity and the biodegradability of these materials.

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VII. Supporting information

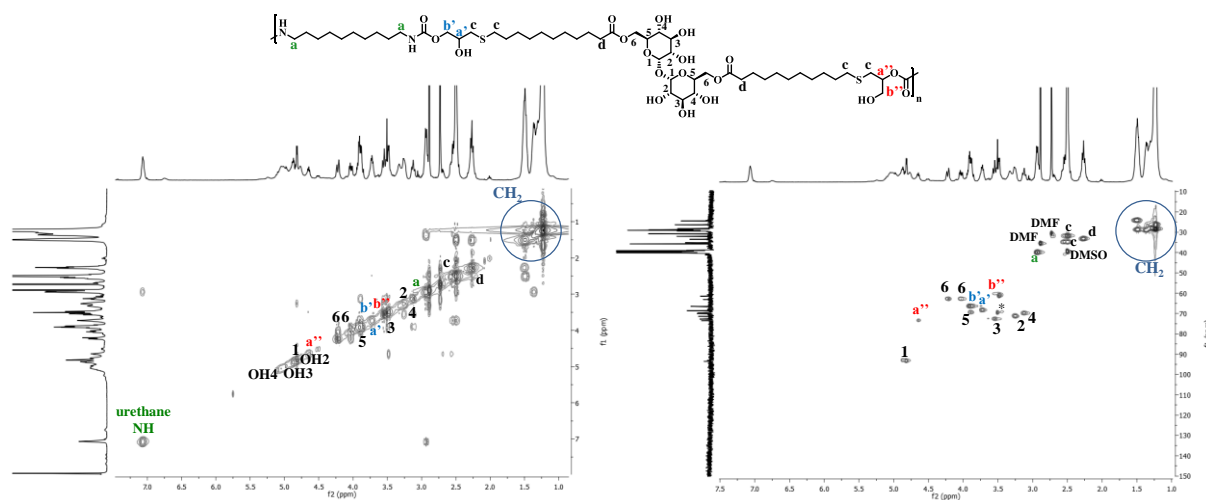
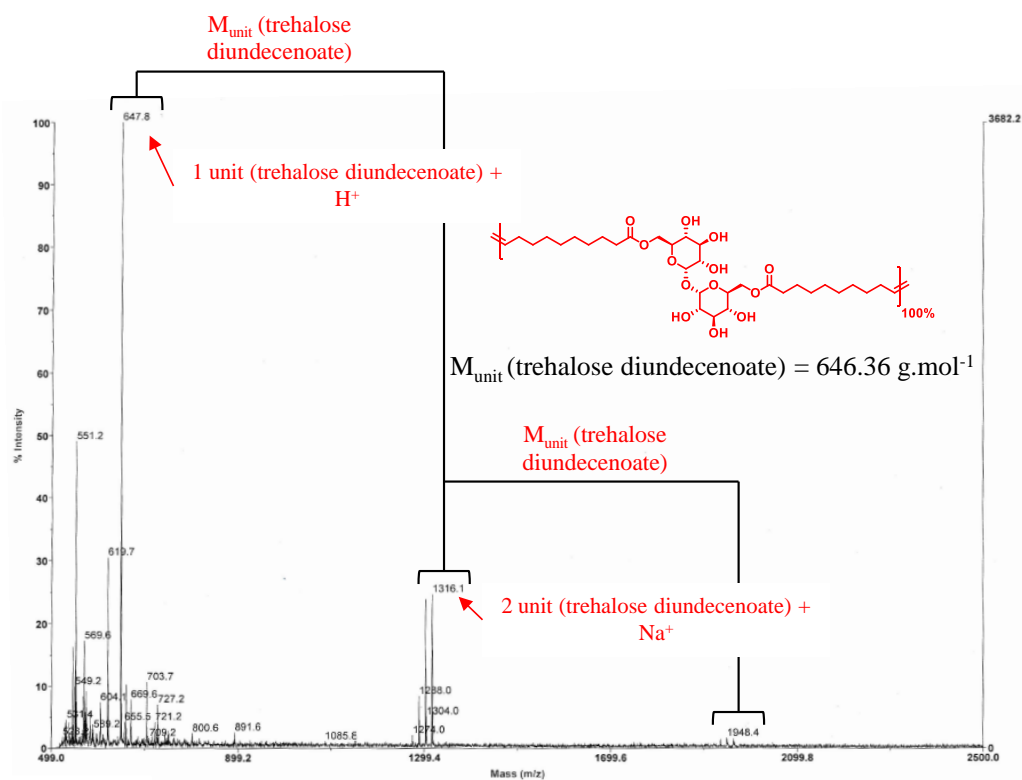
Figure S23: ¹H-¹H COSY NMR spectrum (left) and ¹H-¹³C HSQC NMR spectrum (right) of the PHU1 in DMSO-d₆

Figure S24: MALDI-TOF analysis of GPEs100 (Matrix 2,5-dihydroxybenzoic acid (DHB))

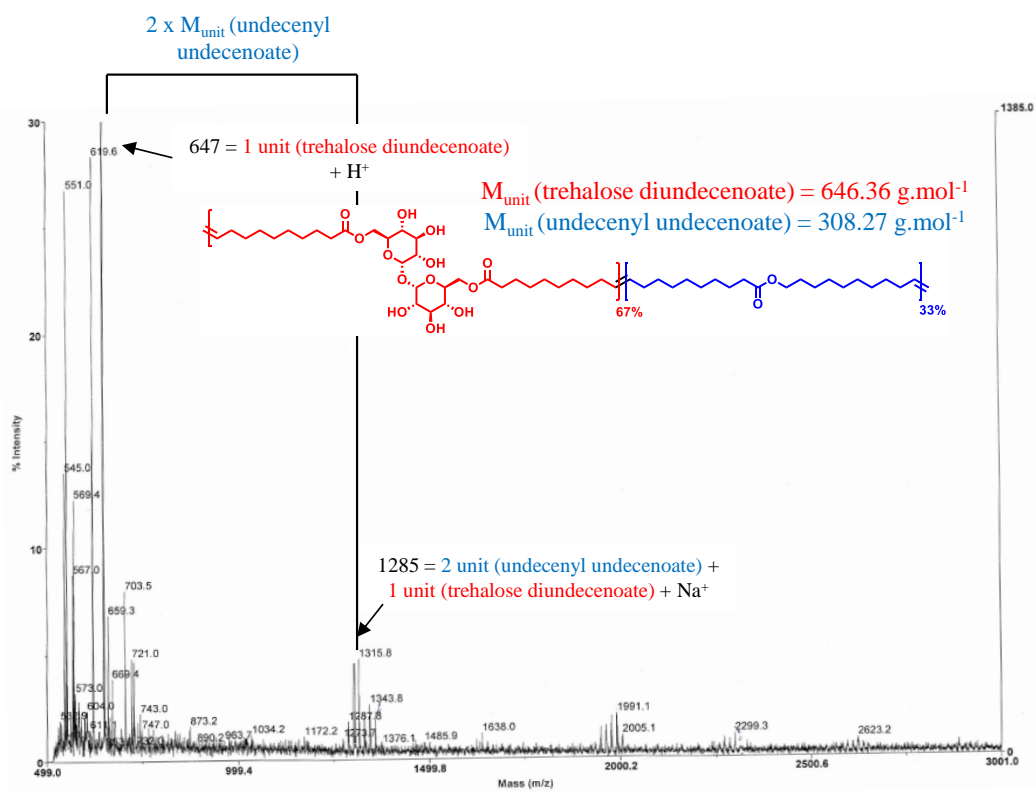


Figure S25: MALDI-TOF analysis of GPes67 (Matrix 2,5-dihydroxybenzoic acid (DHB))

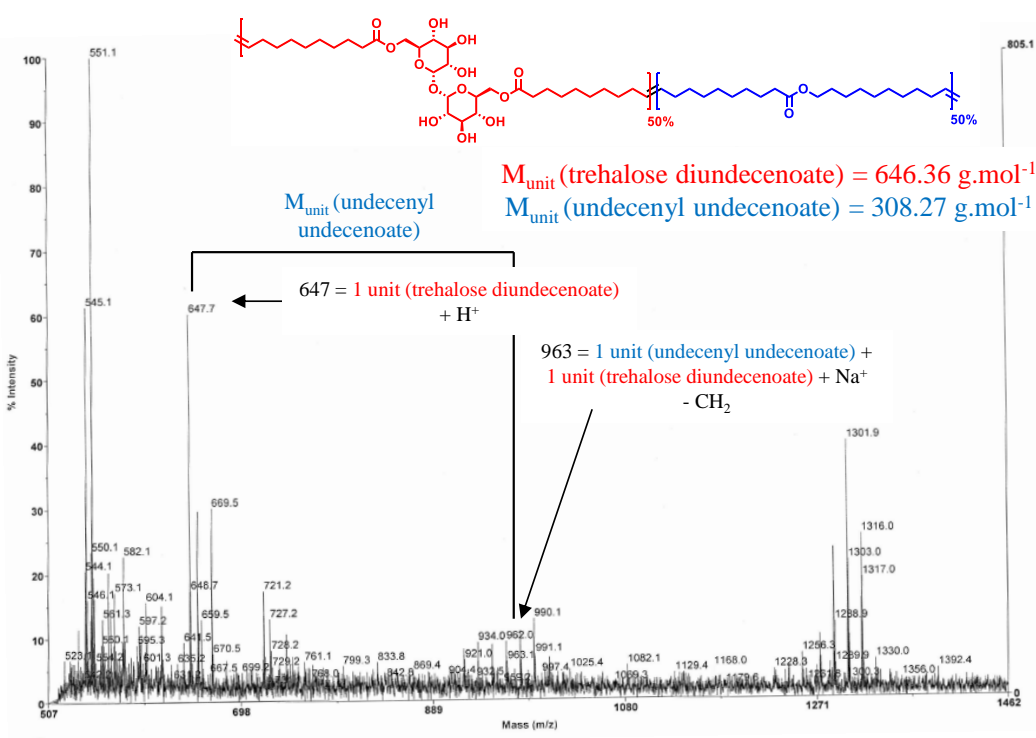


Figure S26: MALDI-TOF analysis of GPes50 (Matrix 2,5-dihydroxybenzoic acid (DHB))

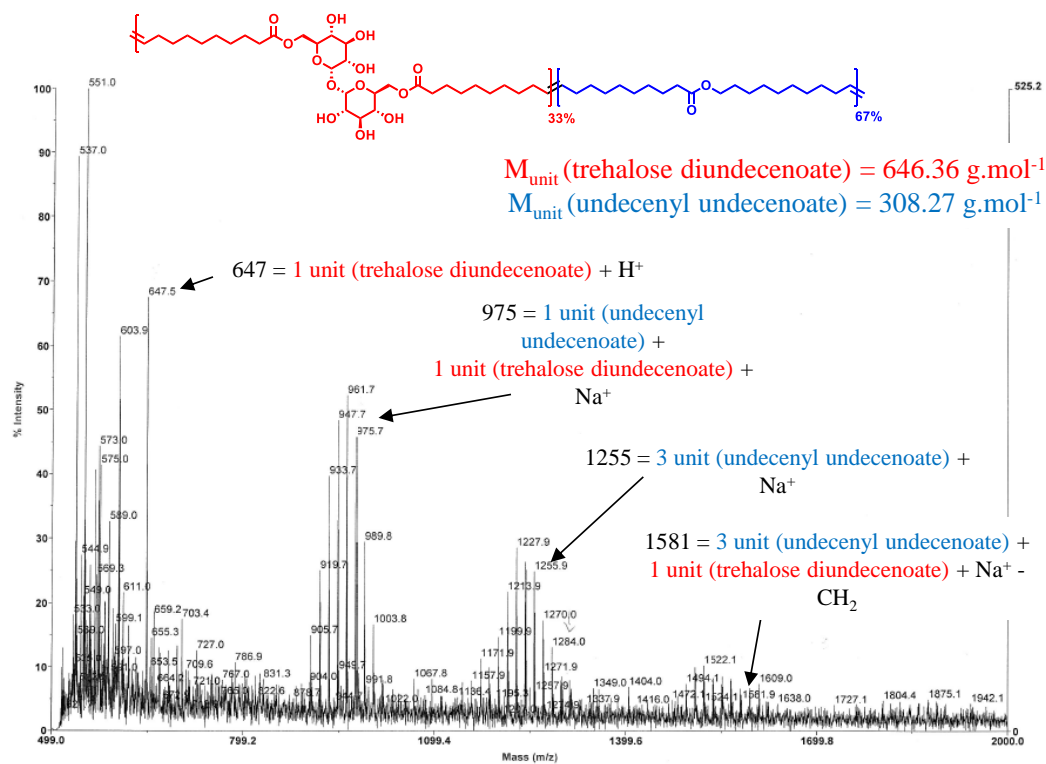


Figure S27: MALDI-TOF analysis of GPes33 (Matrix 2,5-dihydroxybenzoic acid (DHB))

General conclusions and perspectives

General conclusions and perspectives

Environmental issues, worries regarding oil depletion and price fluctuations have been led the scientific community to find out sustainable solutions. To this end, biomass represents the main resource for the production of energy and the synthesis of chemical products. Biorefineries have been developed to convert the biomass into bio-chemical products to progressively replace the fossil-based products. Important works have already been developed to convert vegetable oils and polysaccharides into monomers and corresponding bio-based polymers. Nevertheless, very few data are reported so far about glycolipids as monomers to design high added-value polymers.

One of the aims of this thesis was to use glycolipids as monomer for the synthesis of novel bio-based polymers. A disaccharide, the trehalose and several fatty acids with different alkyl chain structures were selected for the synthesis of trehalose esters. The self-assembly properties of these amphiphilic molecules were then investigated. Afterwards, the difunctional trehalose diesters were modified to be used as monomers for the synthesis of thermoplastic polymers by polycondensation, ADMET and thiol-ene polymerization.

Initially, trehalose esters were synthesized by selective esterification of the primary alcohol of the trehalose in order to target the synthesis of trehalose diesters. A protective-group free esterification was firstly selected. This method is a two-step one pot esterification using a peptide coupling agent (TBTU). The first step is the formation of an activated ester by the reaction between a fatty acid and TBTU under the presence of an amine as a base. Then the activated ester is transesterified with the trehalose to get the trehalose esters. However, this esterification was proved not to be so selective. Indeed, due to trehalose solubility issue in organic solvents, trehalose triesters and trehalose polyesters were also formed (57 wt.%) in addition to trehalose diesters (35 wt.%) and trehalose monoesters (8 wt.%). Different solvents were tested to improve the solubility of the trehalose but the formation of these polyesters could not be avoided. However, flash chromatography allowed us isolating the trehalose diesters and the trehalose monoesters. Therefore, a range of trehalose monoesters and trehalose diesters were obtained from this chemical esterification. The reaction conditions of this esterification still have to be enhanced to improve the selectivity of the reaction on the primary alcohols and thus avoid the formation of the trehalose polyesters. Actually, this side-

product issue results from the classical challenge of the sugar esterification which is to find a good solvent for both the trehalose and the fatty acid.

Then a lipase-catalyzed esterification was studied to synthesize such trehalose diesters. The enzymatic esterification of trehalose was performed with fatty vinyl esters. Two transvinylation routes were investigated to obtain some vinyl undecenoate from undecenoic and vinyl acetate, in the presence of iridium and palladium-based catalysts, respectively. The palladium catalyzed route was selected and applied to different fatty acids. Indeed, in this pathway, no isomerization of the terminal double bond of the vinyl undecenoate was observed. Then, a series of trehalose diesters (60 % yields) were synthesized by enzymatic catalyzed transesterification using lipase B from *Candida antarctica*. By comparison with the protective group-free esterification, the enzymatic-catalyzed esterification produces neither trehalose monoesters nor polyesters were produced. Thus, the selectivity through the enzymatic process was much better and moreover lesser toxic solvents could be employed. However, diester yields should still be improved by using different solvents and/or enzymes.

Afterwards, the self-assembly properties of the trehalose monoesters and diesters were evaluated. The trehalose monoesters exhibit surfactant properties. Thus, surface tension and CMC in water were determined. It appeared that the lipidic chain nature has an influence of the CMC values which range between 0.16 and 1.0 mg.L⁻¹ but it has little impact on the surface tension which remains around 42 mN.m⁻¹ for all the trehalose monoesters. The latter self-assembled in spherical micelles in water which were observed by TEM. Trehalose monoerucate (C22:1) was also able to gelify water at concentration higher than 30 g.L⁻¹. The so-formed hydrogel was speculated to be a 3D network of entangled large micelles. The rheological properties were evaluated showing that the hydrogels were flexible. Indeed, G' and G'' values were low (16 Pa) at the highest concentration tested (5 wt.%). Moreover, rheological measurement performed on these hydrogels revealed that they exhibit a thixotropic behavior.

On the contrary, trehalose diesters are too hydrophobic to be soluble or self-assembled in water. However, they are able to gelify organic solvents and vegetable oils at low concentration (less than 1 wt.%). Therefore, oleogels were prepared in three vegetable oils (olive, linseed and castor oil). Microscopy, X-Ray scattering and DSC allowed us demonstrating that all the gels are 3D network of crystalline fibers formed by the stacking of trehalose diesters (SAFiN). The rheological properties were also evaluated showing an influence of the concentration of trehalose diesters on the gel strength. The higher the concentration, the harder the gels. Indeed, with an increase of the concentration, the density of

fibers in the 3D network also increases creating more entanglements. The rheological studies also showed that lipidic chain nature has an impact on the gel properties. It was observed that short chain length favored the formation of hard but brittle gels (high G' and G'' values, low σ_y). Besides, saturated diesters compared to unsaturated ones also favored the formation of harder gels. In addition, the rheological properties were found very dependent on the vegetable oil and were difficult to predict.

Finally, different thermoplastic polyurethanes were synthesized from the trehalose diesters. Polyurethanes were synthesized from three trehalose diesters containing hydroxyl group on their lipidic chains and two diisocyanates. Thus, branched amorphous polyurethanes were obtained due to the reaction of the secondary hydroxyls of trehalose with the diisocyanates. Due to the toxicity of isocyanates, the synthesis of non-isocyanate polyurethanes was performed. Amorphous poly(hydroxyurethane)s were successfully prepared by the reaction of diamines with two trehalose diesters bearing 5-membered bis-cyclic carbonates. Linear poly(hydroxyurethane)s with reasonable molar masses (from 3000 to 10000 $\text{g}\cdot\text{mol}^{-1}$) were obtained the ring opening of the cyclic carbonates with the diamines. Moreover, trehalose diundecenoate was polymerized by ADMET and thiol-ene polymerization. Copolymers were obtained by ADMET polymerization between trehalose diundecenoate and undecenyl undecenoate with molar masses ranging from 2500 to 13000 $\text{g}\cdot\text{mol}^{-1}$. These novel glyco-polyesters are semi-crystalline and exhibit unique organization within the material induced by the lipidic and carbohydrate parts. Trehalose diundecenoate were also polymerized by thiol-ene reaction with different dithiols. Thus, semi-crystalline thio-glyco-polyesters with molar masses between 7000 and 16000 $\text{g}\cdot\text{mol}^{-1}$ were obtained. The sulphur atoms present in the polymer chains were selectively oxidized either as sulfoxide or sulfone moieties.

Lastly, a preliminary study on the glyco-polyesters and thio-glyco-polyesters self-assembly was carried out. Stable nanoparticles were then prepared by solvent displacement method where polymer solutions in DMF were dialyzed against water. Spherical assemblies between 160 and 200 nm were observed in TEM and DLS. It could be explained by the presence of hydrophilic and hydrophobic domains in the polymers backbone.

To conclude, the main objective of this thesis which was the design of novel polymers from trehalose esters was fulfilled. Four families of polymers were obtained through different polymerization reactions. Moreover, thanks to their amphiphilic characters trehalose esters exhibit surfactant properties in water and self-assemble in 3D network to gelify water of vegetable oils.

Research perspectives on this subject are numerous. The protective group-free esterification reaction to produce the diesters with good selectivity has to be improved. Ionic liquid or deep eutectic solvents could be used to solubilize both the trehalose and the fatty acids. Otherwise, as the trehalose is soluble in aqueous solution and the fatty acids in organic solvents, the esterification could be performed at the interface between an aqueous and organic phase and phase transfer catalysts could be used during this esterification. Regarding the enzymatic-catalyzed esterification, others enzymes could be tested to improve the diester yields.

Among the synthesized trehalose monoesters, trehalose monoerucate is the only one which has the ability to gelify water at low percentage (<5%). Monoesters of trehalose with alkyl chains longer than C22 could be synthesized to study the influence on the water gelation. Regarding the oleogels, rheological properties are different according to the trehalose diester employed. Oleogels could be prepared from mixtures of diesters to investigate the impact on the rheological properties. Hybrid-diester containing two different fatty acids could be also synthesis and there properties evaluated in regards to the corresponding diesters. It would be necessary to investigate the toxicity of these trehalose esters to target cosmetic and agro-food applications for these gels.

Four families of polymers were obtained during this thesis. Higher amount of these polymers should be produced in order to perform further analysis notably mechanical analysis. The (bio)degradability/biocompatibility of the polymers was not studied; the presence of ester functions and glycosidic bonds in the polymer backbone should favor this feature. The exploratory work exposed in the last part on the polymer self-assembly appears to be very promising. Indeed, these substrates have to be more investigated to be used as fully bio-based materials for drug delivery applications.

Materials and methods

I. Materials

Oleic acid (90%), methyl oleate (99%), erucic acid (90%), stearic acid (98%), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%), O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) (97%), N,N-Diisopropylethylamine (DIPEA) (99%), calcium hydride (CaH₂) (95%), potassium carbonate (98%), Chlorobis(cyclooctene)iridium(I)dimer (97%), potassium hydroxide (KOH, pellet), 2-methyltetrahydrofuran (99%), sodium acetate (99%), vinyl acetate (99%), tert-butanol, Grubbs 1st generation metathesis catalyst, Grubbs 2nd generation metathesis catalyst, Hoveyda-Grubbs 1st generation metathesis catalyst, Hoveyda-Grubbs 2nd generation metathesis catalyst, 2-mercaptoethanol (98%), 1-thioglycerol (99%), benzophenone (99%), 1,4-butanedithiol (97%), 1,6-hexanedithiol (97%), urea, 1,12-diisocyanatododecane (97%), dimethyl carbonate (DMC, 99%), 1,5,7-triazabicyclodec-5-ene (TBD, 98%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1-thioglycerol (99%), *meta*-chloroperbenzoic acid (mCPBA, 77%), Lipase B from *Candida antarctica* (CALB), hydrogen peroxide (30% v/v), were obtained from Sigma-Aldrich.

10-undecen-1-ol (99%), 10-undecenoic acid, sodium sticks (in mineral oil) (99%), isophorone diisocyanate (98%), choline chloride (98%) were supplied from Alfa Aesar.

Methyl 10-undecenoate (99%), 2-Mercaptoethyl ether (97%), palladium acetate (98%), 1,10-diaminododecane (98%), dibutyltin dilaurate (95%), isophorone diamine (99%), 1,12-diaminododecane (98%) were purchased from TCI.

Anhydrous trehalose (99%), linoleic acid (99%), 1,2-ethanedithiol (97%), anhydrous pyridines (99.8%), were bought from Fisher.

2,2'-Azobis(4-methoxy-2,4-dimethylvaleronitrile) (V70) was purchased from Wako.

Elaidic acid (99%) was obtained from Nu-Check Prep.

Labrafac Lipophile WL were kindly supplied by Gattefossé.

Mygliol 812 were kindly furnished by Cremer.

Olive oil, linseed oil, pumpkinseed oil, grapeseed oil, canola oil, walnut oil, sunflower oil, were purchased at Biocoop.

Uranyl acetate dehydrate was supplied by TAAB.

12-hydroxystearic acid, 9,10-dihydroxystearic acid and castor were kindly provided by ITERG.

All product and solvents (reagent grade) were used as received otherwise mentioned. The solvents were of reagent grade quality and were purified wherever necessary according to the methods reported in the literature.

II. Methods

Nuclear Magnetic Resonance (NMR)

All NMR experiments were performed at 298 K on a Bruker Avance 400 spectrometer operating at 400MHz. CDCl₃ and DMSO, were used as solvent depending on the sample and are mentioned in the legends of the NMR spectra.

Fourier Transformed Infra-Red-Attenuated Total Reflection (FTIR-ATR)

Fourier transform infrared (FTIR) 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. The spectra were acquired

Size exclusion chromatography (SEC)

Polymer molar masses were determined by Size Exclusion Chromatography (SEC) using THF as the eluent. Measurements in THF were performed on a PL GPC50 integrated system with RI and UV detectors and four TSK columns: HXL-L (guard column), G4000HXL (particles of 5 mm, pore size of 200A, and exclusion limit of 400 000 g/mol), G3000HXL (particles of 5 mm, pore size of 75A, and exclusion limit of 60 000 g/mol), G2000HXL (particles of 5 mm, pore size of 20 A, and exclusion limit of 10 000 g/mol) at an elution rate of 1mL/min. Polystyrene was used as the standard.

Size exclusion chromatography analyses of PUs were performed in DMF (25°C) on a PL-GPC 50 plus Integrated GPC from Polymer laboratories-Varian with a series of three columns from Polymer Laboratories (PLgel: PLgel 5 μ m Guard (guard column 7.5 mm ID x 5.0 cm L); PLgel 5 μ m MIXED-D (7.5 mm ID x 30.0 cm L) and PLgel 5 μ m MIXED-D (7.5 mm ID x 30.0 cm L)). In both cases, the elution times of the filtered samples were monitored using RI detectors.

In both cases, the elution times of the filtered samples were monitored using UV and RI detector and SEC were calibrated using PS standard.

High-performance liquid chromatography (HPLC)

HPLC was performed at the ITERG using a Shimadzu instrument equipped with an Agilent PLgel column (300 mm, 7.5 mm diameter) and compounds were detected by a RID detector at 40°C. The samples were diluted in THF at 10 mg.L⁻¹ and filtered before injection in the column. The analyses were performed with THF as eluent at 40°C.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) measurements were performed on DSC Q100 (TA Instruments). The sample was heated from -150°C to 170°C at a rate of 10°C.min⁻¹. Consecutive cooling and second heating run were also performed at 10°C.min⁻¹. The glass transition temperatures and melting points were calculated from the second heating run.

Thermogravimetric analysis (TGA)

Thermogravimetric analyses (TGA) were performed on TA Instruments Q50 from room temperature to 600°C at a heating rate of 10°C.min⁻¹. The analyses were investigated under nitrogen atmosphere with platinum pans.

Optical microscopy

A standard glass slide containing a small portion of gel was mounted on an Axioskop 40 Zeiss microscope equipped with a digital camera (Canon Powershot A640) connected to the computer.

Scanning electron microscopy (SEM)

Scanning electron microscopy images were performed on xerogels on a MEB HITACHI TM-1000 having acceleration voltage of 15kV. Samples were previously coated with gold film (15 - 20 nm).

Cryo-Scanning electron microscopy (Cryo-SEM)

Cryo-SEM sample preparation and observation were performed at the Bordeaux Imaging Center (BIC). Hydrogels were frozen by cryofixation on Cu/Au Ø 3,0 x 0,8 mm support at 2050 bars and -196°C on a HPM100 Leica apparatus. They were then conserved in liquid nitrogen until the use by the cryo-module Quorum. The observation were obtained on Quanta FEG 250 FEI microscope having acceleration voltage of 5kV.

Transmission electron microscopy (TEM)

Transmission Electron Microscopy images were recorded at the Bordeaux Imaging Center (BIC) on a Hitachi H7650 microscope working at 80kV in high resolution mode. Samples were prepared either onto a copper grid (200 mesh coated with carbon/formvar) by droplet deposition of aqueous solution of trehalose monoesters and subsequently stained by droplet deposition of aqueous solution of uranium acetate (10 mg/mL). Or they were prepared by spraying a 0.5 mg/mL aqueous solution of polymer nanoparticles onto a copper grid (200 mesh coated with carbon) using a homemade spray tool. No stained were applied in this case.

Dynamic light scattering

Dynamic light scattering measurements were performed at 25°C with a Malvern Instrument Nano-ZS equipped with a He-Ne laser (1 ¼ 632.8 nm). Samples were introduced into cells (pathway: 10 mm) after filtration through 0.45 µm cellulose micro-filters. The measurements were performed at a scattering angle of 90°.

Flash chromatography

Flash chromatography was performed on a Grace Reveleris apparatus, employing cartridges from Grace and gradient solvent (precised in the manuscript for each case) equipped with ELSD and UV detectors at 254 and 280 nm. Elution solvents are dependent on the sample and are mentioned in the experimental parts.

X-ray scattering

X-ray diffraction patterns at wide angles (WAXS) were obtained by Ahmed Bentaleb from the Centre de Recherche Paul Pascal (CRPP) with a microfocus rotating anode X-ray source (Rigaku MicroMax-007 HF) combined with performant multi-layers optics and a 3-pinholes collimation that provide an intense X-ray intensity on the sample. The sample, mounted on X-Y stage, was held in a Lindemann capillary and placed in an oven providing a temperature control of 0.1 K. A 2-dimensionnal detector (Image plate from Mar Research) was collecting

the scattered radiations. Calibration of the sample-detector distance was performed using silver behenate as a reference.

Rheological measurements

Rheological properties of the gels were assessed on a Anton Paar MCR 301 stress controlled rheometer. Temperature was controlled on the bottom plate by Peltier effect. The geometry used for the measurements was a cone-and-plate (50 mm / 2°). The gels were loaded between the cone-and-plate and melted at 130 °C for 3 min and then cooled down to room temperature. The measurements were started after 10 min of sample rest.

Time-Of-Flight Mass Spectrometer with Matrix-Assisted Laser Desorption/Ionization (MALDI-TOF MS)

MALDI-MS spectra were performed by the Centre d'Etude Structurale et d'Analyse des Molécules Organiques (CESAMO) on a Voyager mass spectrometer (Applied Biosystems). The instrument is equipped with a pulsed N₂ laser (337 nm) and a time-delayed extracted ion source. Spectra were recorded in the positive-ion mode using the reflectron and with an accelerating voltage of 20 kV. Samples were dissolved DMF at 10 mg/mL. The DHB matrix (2,5-dihydroxybenzoic acid) solution was prepared by dissolving 10 mg in 1 mL of DMF. A methanol solution of cationisation agent (NaI, 10 mg/mL) was also prepared. The solutions were combined in a 10:1:1 volume ratio of matrix to sample to cationisation agent. One to two microliters of the obtained solution was deposited onto the sample target and vacuum-dried.

Glycolipides : de la synthèse et l'étude de l'auto-assemblage à la conception de polymères bio-sourcés originaux

Résumé :

Ces travaux de thèse traitent de l'étude de glycolipides et plus précisément d'esters de trehalose pour la synthèse de nouveaux polymères bio-sourcés. Des monoesters et diesters de trehalose ont ainsi été synthétisés par estérification des alcools primaires du trehalose avec des acides gras selon deux voies de synthèse. La première utilisant un agent de couplage peptidique ne nécessite pas l'utilisation de groupement protecteur pour estérifier sélectivement les alcools primaires. La deuxième est une estérification sélective catalysée par une lipase. L'auto-assemblage des esters de trehalose a ensuite été étudié. Les monoesters possèdent des propriétés tensio-actives dans l'eau et le trehalose monoerucate a la capacité de gélifier l'eau. Les diesters, quant à eux sont de bons gélifiants pour les solvants organiques et les huiles végétales. Par conséquent, des gels ont été préparés dans trois huiles végétales, puis leur morphologie et leur propriété rhéologique ont été étudiées. Ensuite, les diesters ont été fonctionnalisés et polymérisés selon différentes stratégies. Ainsi, des polyuréthanes et des poly(hydroxyuréthane)s ont été synthétisés par polycondensation tandis que des glyco-polyesters ont été obtenus par polymérisation par métathèse et addition thiol-ène. Finalement, les propriétés d'auto-assemblage de ces polymères ont été étudiées. Ces derniers peuvent former des nanoparticules par la méthode de déplacement de solvants.

Mots clés : Glycolipides, esters de trehalose, auto-assemblage, tensioactif, gel, polyuréthane, poly(hydroxyuréthane), polymérisation par métathèse, polymérisation par addition thiol-ène.

Glycolipids: from synthesis and self-assembly studies to the design of original bio-based polymers

Abstract :

The aim of this thesis was to study glycolipids and particularly trehalose esters for the synthesis of new bio-sourced polymers. Trehalose monoesters and diesters were synthesized by two esterification pathways of the primary alcohol of trehalose with different fatty acids. The first synthetic route is a protective group-free esterification using a peptide coupling agent and the second one is a lipase-catalyzed esterification. The self-assembly properties of the trehalose esters were investigated. Trehalose monoesters showed surfactant properties in water and trehalose monoerucate was even able to form gels in water. The trehalose diesters appeared to be good gelators for organic solvent and vegetable oil. Thus, gels in three vegetable oils were prepared and their morphology and rheological properties were studied. Afterwards, trehalose diesters were functionalized and polymerized with different strategies. Thus, polyurethanes and poly(hydroxyurethane)s were obtained by polycondensation whereas glyco-polyesters were synthesized by acyclic diene metathesis (ADMET) and thiol-ene polymerization. Finally, the self-assembly properties of these polymers were investigated. The latter were able to form some nanoparticles by solvent displacement method.

Keywords : Glycolipids, trehalose esters, self-assembly, surfactant, gel, polyurethane, poly(hydroxyurethane), ADMET, thiol-ene polymerization,

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