Lipase-Mediated Enzymatic Catalysis For The Synthesis of New Chiral Polymers

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ABSTRACT

Immobilized lipase B from *Candida antarctica* (N435) was investigated as a potential biocatalyst to generate silicone-based chiral polymers from monomers derived from the enzymatic dihydroxylation of bromobenzene. Several conditions and parameters have been investigated for this purpose and lipase transesterification preference to each of the free secondary alcohols in the chiral monomers was documented. The N435 was challenged with a series of substrates where the free alcohol moieties were systematically protected in order to study the substrate preference(s) for the transesterification reactions.

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LIST OF ABBREVIATIONS

Ac	Acetyl
AIBN	2,2′-Azoisobutyronitrile
Bn	Benzyl
Boc	*tert*-butoxycarbonyl
Bz	Benzoyl
CALB	*Candida antarctica* lipase B
CALB-PCMC	*Candida antarctica* lipase B protein-coated microcrystals
CLEC	Cross-linked enzyme aggregates
COSY	(proton) Correlated spectroscopy
d	Days
DCE	1,2-Dichloroethene
DCM	Dichloromethane
DMAP	Dimethylamino pyridine
DME	Dimethoxyethane
DMF	Dimethylformamide
DMP	2,2-Dimethoxypropane

DMS	Dimethylsulfide
DMSO	Dimethylsulfoxide
EtOAc	Ethyl acetate
Et_3N	Triethylamine
g	gram
GPC	Gel permeation chromatography
h	Hours
HDL	16-hexadecanolide
Hex	Hexanes
HMBC	Heteronuclear multiple-bond correlation
HNLs	Hydroxynitrile lyases
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear Single Quantum Coherence
Imid	Imidazole
IPTG	β-isopropylthiogalactopyranoside,
IR	Infrared
MALDI	Matrix-assisted laser desorption/ionization

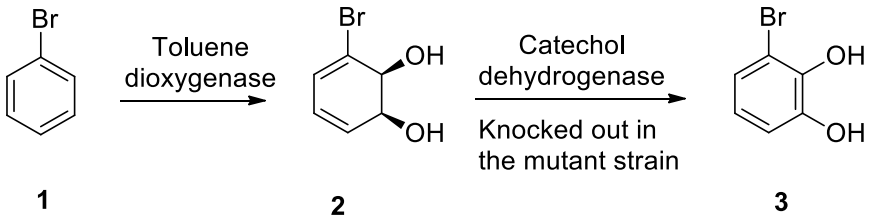
*m*CPBA	*meta*-Chloroperoxybenzoic acid
mg	Milligrams
min	Minutes
mL	Milliliter
mmol	Millimole
mol	Mole
MOMCl	Methyl chloromethyl ether
MOM	Methoxymethyl ether
N435®	Novozymes-435, CALB immobilized on Amberzyme, Lewatit
NDO	Naphthalene dioxygenase
NMO	N-methyl-morpholine-N-oxide
NMR	Nuclear magnetic resonance
NOSY	Nuclear Overhauser cross relaxation
OC	2-oxo-12-crown-4-ether
PBS	Polybutylene succinate
PCL	Polycaprolactone
PCMC	Protein coated microcrystals

Pd	Palladium
PET	Polyethylene terephthalate
PDMS	Polydimethylsiloxane
PDPS	Polydiphenylsiloxane
PDL	15-pentadecanolide
PLA	Polylactic acid
Pt	Platinum
p-TsOH	*p*-Toluenesulfonic acid
Py	Pyridine
R_f	Retardation factor
ROP	Ring-opening polymerization
rpm	Revolutions per minute
rt	room temperature
TAG	Triacylglycerol
TBAF	Tetrabutylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TBSCl	*t*-Butyldimethylsilyl chloride

TBS	*t*-Butyldimethylsilyl
TBSOTf	tert-Butyldimethylsilyl trifluoromethanesulfonate
t-BuOK	Potassium *tert*-butoxide
t-BuOH	*tert*-Butyl alcohol
TDO	Toluene dioxygenase
THF	Tetrahydrofuran
TFA	Trifluoroacetic acid
THSCl	Dimethylthexylsilyl chloride
TIPSOTf	Triisopropylsilyl trifluoromethanesulfonateso
TLC	Thin layer chromatography
TMAD	Tetramethylazodicarboxamide
TMS	Trimethylsilyl
TsCl	4-Toluenesulfonyl chloride

1. Introduction

Green chemistry is a term often used to describe processes or procedures that are environmentally friendly: these processes have a minimal impact on the environment by limiting the use and production of hazardous materials and decreasing energy consumption. Beside its environmental impact, green chemistry necessitates the establishment of sustainable procedures that assign an economic value to eliminating waste and polluting the environment. In terms of sustainability, green chemistry focuses on the usage of renewable resources for various basic and advanced chemical transformation.[1] In 1968 Gibson and coworkers reported the oxidative degradation of aromatics by soil bacteria.[2, 3] In 1970 his group reported the first example of a mutant, *Pp*39D, capable of blocking the further degradation of arene-*cis*-dihydro diols.[4, 5] These dioxgenase enzymes not only fulfill the green chemistry requirement for clean, sustainable chemical conversion, but provide the enantiopure arene-*cis*-dihydro diols **2** which are not attainable by conventional chemical synthesis. To date hundreds of substrates have been tested ranging from toluene to benzo[*a*]pyrene, and the enantiopure arene *cis*-diols produced have been utilized in the synthesis of complex natural or synthetic products that have high medical and industrial importance such as morphine, pancratistatin, and many more.[6]



Scheme 1. Catechol dehydrogenase gene knocked out in the mutant strain *Pp*39D prevents further degradation of the arene-*cis*-dihydro diol **2**.[4]

This project aims to exploit the benefits of two different green processes: the first one is the toluene dioxgenase mediated dihydroxlation of arenes by the recombinant strain *E.coli* JM 109 (pDTG 602), a genetically engineered species that over expresses toluene dioxygenase and is capable of converting bromobenzene to 3-bromo-*cis*-1,2-dihydroxycyclohexa-3,5-diene **2**. This arene-*cis*-dihydro diols will be used to synthesize various chiral building blocks aimed for polymer synthesis such as **4**, **5** and **6**.

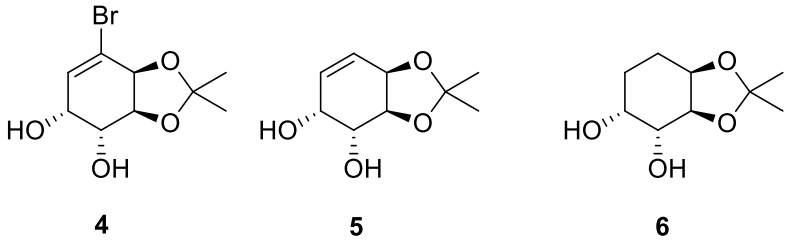


Figure 1. Chiral monomers derived from 3-bromo-*cis*-1,2-dihydroxycyclohexa-3,5-diene **2** that will be utilized in chiral polymer synthesis.

The polymer synthesis will utilize immobilized lipase B from *Candida antarctica* (N435) as an environmentally friendly biocatalyst. *Candida antarctica* lipase B (CALB) is a serine hydrolase that displays an extraordinary enantioselectivity toward secondary alcohols.

This project aims to produce optically active silicone-based chiral polymers, which have a wide range of potential applications, whether as chiral stationary phases for chiral chromatography of racemic mixtures or many other industrial application such as ferroelectric liquid crystals, nonlinear optical assemblies, optical data, storage media, etc.[7]

This project aims to investigate several conditions and parameters for the enzymatic polymerization of **4** - **6**. It also aims to investigate lipase transesterification preference to each of the secondary alcohols in monomeric chiral diols. A set of lipase B test substrates with one free alcohol moiety were synthesized, in order to investigate lipase B transesterification to one secondary alcohol when the other alcohol is already blocked.

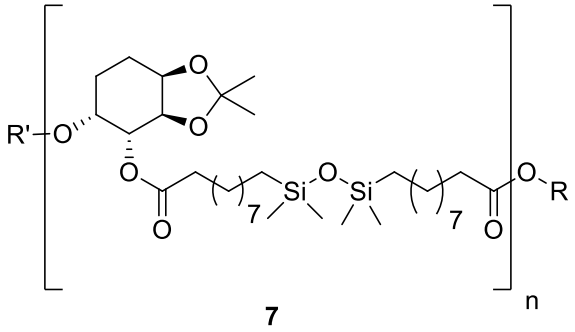


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Figure 2. One of the proposed disiloxane-containing chiral polymers.

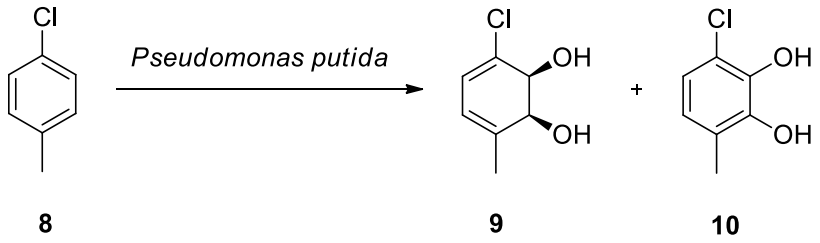
2. Historical

2.1 Toluene dioxygenase

2.1.1 Introduction

In 1968 Gibson proposed that *Pseudomonas putida* metabolizes benzene to pyrocatechol through a *cis*-l,2-dihydrocyc1ohexa-3,5-diene intermediate.[2, 3] Later that year he isolated and identified (1*S*,2*S*)-3-chloro-6-methylcyclohexa-3,5-diene-1,2-diol **9** as one of the metabolic products when *Pseudomonas putida* metabolized *p*-chlorotoluene **8**.[2] The identification of this metabolite provided evidence that metabolic oxidation of arenes proceeds through an arene-*cis*-dihydro diol intermediate.

In 1970 he successfully developed the mutant, *Pp*39D, incapable of further metabolism of the arene-*cis*-dihydro diol.[4] To date over 400 different arene-*cis*-dihydro diols have been identified as metabolic products of various aromatics dioxygenases and in the majority of these cases the product is enantiomerically pure.[5, 6, 40] These enantiopure arene-*cis*-dihydro diols are not attainable by conventional chemical synthesis and they are used in the enantioselective synthesis of various new compounds. Aromatic hydrocarbon dioxygenases also represent a potential solution for the bioremediation of various aromatic soil contaminants. This chapter will briefly review the history of biocatalysis and enzymatic dihydroxylation, the biology of aromatic hydrocarbon dioxygenases, substrate specificity of toluene dioxygenase, and the application of these arene-*cis*-dihydro diols in organic synthesis.



Scheme 2. Dihydroxylation of *p*-chlorotoluene by wild *Pseudomonas putida.*[2]

2.1.2 History of biocatalysis

It is very likely mankind discovered alcohol by accident, most likely through fermented fruits and honey. Analysis of artifacts from the neolithic village of Jiahu in northern China indicated the presence of fermented drinks made from rice, honey and fruits dating back more than 9000 years, to around the same time when beer was made from barley in Egypt and the middle east.[8]

Recent evidence suggests that ancient Egyptians had also fermented various other fruits in addition to fermenting barley. Chemical analysis of ancient Egyptian pottery from *ca.* 3150 BCE has revealed a wide range of fermented natural products, Grapes were used to produce wine for recreational usage, medical papyri dating back to *ca*. 1850 BCE shows that various fermented products were used for medicinal purposes.[8]

By the early 18th century yeast was identified as the causative agent for fermentation for either bread or alcoholic beverage manufacturing,[9] Samuel Johnson the author of the world's most famous English language dictionaries" A Dictionary of the English Language " from *ca.* 1755 describes yeast (known at that time as the "ferment")

"the ferment put into drink to make it work; and into bread, to lighten and swell it"[11]

At that time yeast was not considered a living organism, but rather a crude chemical, and not surprisingly most of the research done on fermentation was done by chemists and not biologists.[9]

Not many chemists at that time focused on fermentation or yeast studies. The first scientist to be interested in yeast studies was Antoine Lavoisier (1743-1794), who is considered to be the father of modern chemistry. [9]

Lavoisier is credited with changing chemistry from a qualitative science to a quantitative one; he recognized oxygen and hydrogen as elements, explained the phenomena of combustion, even predicted the existence of silicon and proposed the law of mass balance. [9] Lavoisier described fermentation as one of the most extraordinary chemical transformations. He approached fermentation in the same way he approached combustion, by focusing on the mass balance, whether it was the mass of sugar, water, yeast paste, carbon dioxide, or acetic acid. He derived the word alcohol from the Arabic word Al-Kuhl, a redistilled spirit. [9]

Lavoisier stated that for every 100 mass portions of sugar 60 mass portions of alcohol and 40 mass portions of carbon dioxide were produced, assuming that yeast only breaks sugar into two components. [9]

The new French republic subsequently invested a lot of efforts in understanding the fermentation processes, as alcoholic beverages, especially wine and brandy, held high economic value for France at that time.[9]

In 1815 Joseph Louis Gay-Lussac, using a method to prevent food from decomposing developed by his friend Appert, a French cook, sterilized a bottled of grape juice by

heating and sealed it from air. He observed that the grape juice would not ferment unless new yeast was added to the juice and the juice was briefly exposed to air. [9, 13]

One may think that Gay-Lussac should have concluded by now that yeast is a living organism that perishes due to heat exposure, but he only concluded that yeast is essential for alcohol production. He did, however, produce a more accurate mass analysis than Lavoisier. [9, 13]

It was not until 1827 that Desmaziéres observed the fermentation process from a biological point of view. He observed under a microscope that two different microorganisms were present during wine and beer fermentation, namely *Mycoderma cerevisiae* (*cerevisiae* meaning beer in Latin) and *Mycoderma vini* (*vini* meaning wine in Latin). Desmaziéres didn't conclude, however, that these organisms are the causative agent for alcohol production.[9, 14]

In 1835 Charles Cagniard de la Tour utilized a more powerful microscope for observing yeast and in doing so confirmed that yeasts are living organisms. Moreover, he confirmed budding as the asexual way yeast multiplies.[9, 15] La Tour's work, in combination with that of other contemporary scientists including Theodor Schwann and Christian Erxleben, established that yeasts are living, multiplying organism and not a non-living organic residue.[9] Our modern understanding of fermentation comes mainly from the work of Louis Pasteur. Pasteur repeated Lavoisier experiments and found that alcohol and carbon dioxide are not the only products of fermentation, and that significant amounts of glycerin, succinic acid, and amylic alcohol are also produced, suggesting that fermentation is a biological process.[10]

Pasteur conducted numerous experiments under several conditions and he managed to

finally link alcohol production to yeast multiplication, thus proving for the first time that alcohol is the product of a biological transformation and that living organisms are required for alcohol production.[10,16]

Pasteur also managed to differentiate between different microbiological fermentation processes and he proved that there were at least two types of fermentation: alcoholic and lactic fermentation.[10, 16]

Pasteur linked fermentation to living cell multiplication, but it was not until 1897 that German biochemist Eduard Büchner explained the possibility of fermentation using yeast extract and the absence of living yeast cells.[12, 17] Büchner was subsequently awarded the Nobel prize in chemistry for his work in cell-free fermentations. In his paper he refuted Pasteur's ideas of living cell fermentation and suggested that cell extracts and not living cells, are responsible for fermentation. [12, 17]

2.1.3 Dihydroxylation of arene substrates

The first evidence of bacterial metabolism of aromatic compounds was reported in 1908 by Störmer.[19] He isolated *Bacillus hexcarbavorum*, a bacterial species that utilize toluene and xylene for growth. Five years later Söhngen reported the ability of certain bacterial strains to utilize benzene, petroleum and paraffin as sources of energy, arguably the first reported research in the field of bioremediation.[20] In the same year, Wagner isolated and identified two of these bacterial species: *Bacterium benzoli a* which utilizes benzene, phenol, and pyrocatechol as a source of energy, and *Bacterium benzoli b* which consumes crude oil for the same purpose.[21]

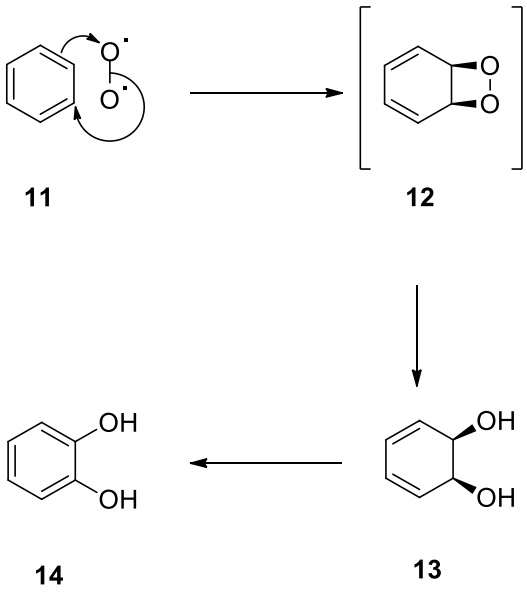
In 1947 Stainer attempted to elucidate the metabolic pathway of microbial oxidation though simultaneous adaptation.[22] Karström in 1937 was the first to propose and prove the concept of enzymatic simultaneous adaptation, which describes the process by which an enzyme will always consume the metabolic intermediates of its primary substrate.[23] Stainer performed a series of enzymatic simultaneous adaptation experiments using phenols, acids, aldehydes and DL-mandelic acid; through monitoring oxygen consumption and substrates level he concluded that phenols are metabolic products, not metabolic intermediates.[22]

Similar results were reported by Young in the same year.[24] Young conducted the same experiment only on different substrates and bacterial species. He proposed that bacterial oxidation did not proceed through a phenolic intermediate as *Pseudomonas aeruginosa* and *Mycobacterium rhodochrous* were unable to utilize phenol as a source of energy.[24]

In 1957 Haccius and Helfrich reported that catechol is the metabolic product of *Nocardia coralline* metabolization of benzene.[25] Marr and Stone reported similar results during their work on *Pseudomonas aeruginosa,* wherein they identified catechol as the primary metabolic product of benzene. They also reported, however, that bacterial flora can utilize catechol as an energy source and they proposed that the metabolic oxidation of benzene proceeds through *trans* cyclohexa-3,5-diene-l,2-diol in a similar fashion to the metabolic pathway of benzene in eukaryotes performed by cytochrome enzymes.[26]

In 1968 Gibson reported the metabolic oxidation of benzene, toluene, and ethyl benzene by *Pseudomonas putida*.[2, 3] He also conducted oxygen uptake experiments in which *Pseudomonas putida* was incubated with both *cis*- and *trans*-cyclohexa-3,5-diene-1,2-diols. The results demonstrated that the *cis* isomer is consumed at a rate six times faster

than the *trans* isomer. Based on this observation Gibson proposed the first mechanism of benzene oxidation. [3]



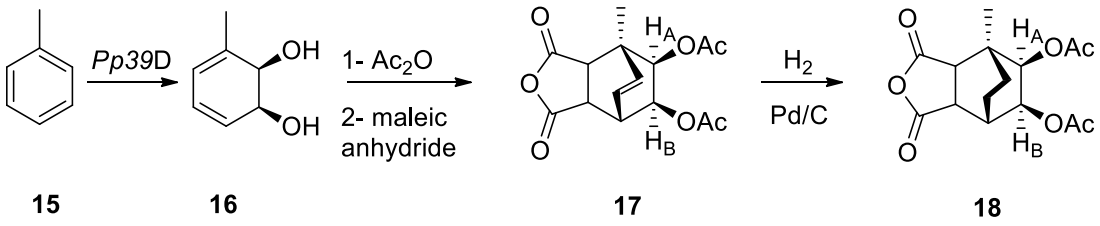
Scheme 3. Gibson's original proposed mechanism for oxidation of benzene.[3]

In order to support his proposed mechanistic pathway, Gibson extended his work to include the metabolic oxidation of halogenated aromatic derivatives. He reported that the bacterial oxidation of toluene, fluoro-, chloro-, bromo-, and iodobenzene produces the *meta*-substituted catechols. However, he isolated and identified (1*S*,2*S*)-3-chloro-6-methylcyclohexa-3,5-diene-1,2-diol **9** as one of the metabolic products when *Pseudomonas putida* metabolized *p*-chlorotoluene **8**,[2] which suggested that microbial oxidation of arenes goes through a *cis*-dihydrodiol intermediate.

Gibson also proposed that the *cis*-dihydrodiols were produced by an iron-mediated reaction with molecular oxygen in a mechanism that is similar to eukaryotic cytochromes. In order to support this hypothesis he incubated *P. putida* with benzene under an $^{18}O_2$

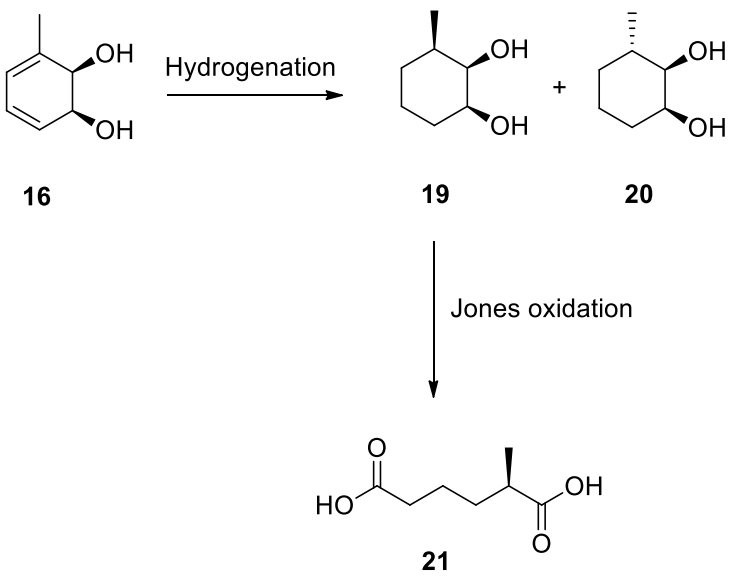
atmosphere.[27] The mass analysis of the catechol produced indicated the incorporation of two ^{18}O atoms.

In 1970 Gibson successfully developed the *Pseudomonas putida* mutant, *Pp39*D, which was blocked in the further oxidation of arene-*cis*-dihydro diols. Gibson aimed to utilize *Pp39*D in production of (+)-*cis*-2,3-dihydroxy-l-methylcyclohexa-4,6-diene **16** from toluene, and in doing so he confirmed that the *cis*-dihydrodiols are the sole product of metabolic dihydroxylation of toluene **15** by the enzyme toluene dioxygenase. NMR redundant studies of the diol produced were inconclusive with respect to proving the stereochemistry. Gibson managed to transform the acetylated derivative of arene-*cis*-dihydro diol **16** into a more rigid structure through a condensation with maleic anhydride to provide **17**, hydrogenation of which provided a stable and rigid derivative **18**. Nuclear magnetic resonance studies revealed that there is a *syn* relation between H_A and H_B.[28]



Scheme 4. Gibson's proof of relative stereochemistry of arene-*cis*-dihydro diol **16**.[28]

In 1973 Gibson managed to unambiguously prove the stereochemistry of **16**.[29] Hydrogenation of **16** yielded diols **20** and **19**, which were separable as monobenzoates. Diol **19** was later oxidized using Jones' reagent to yield (*R*)-(-)-methyladipic acid **21**. The absolute stereochemistry of (*R*)-(-)-methyladipic acid **21** had previously been elucidated by LaLonde in 1970.[30]



$[\alpha]_D$= -14.2, EtOH; Lit. $[\alpha]_D$= -13.4

Scheme 5. Gibson's proof of absolute stereochemistry for arene-*cis*-dihydro diol **16**.[29]

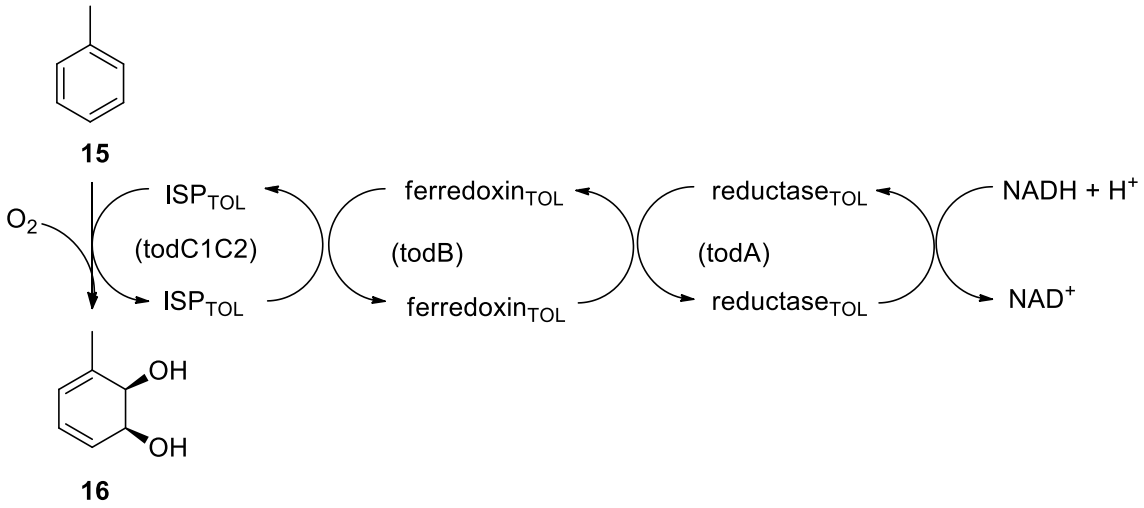
2.1.4 Mechanism of the enzymatic dihydroxylation

Gibson wanted to utilize bacterial aromatic hydrocarbon dioxgenases in bioremediation technology. Aromatic hydrocarbons are common contaminants of soil and water and they represent a significant portion of industrial chemical waste;[35] bioremediation is a potential solution to this problem. A key step in bioremediation is the breakdown of cyclic hydrocarbon into straight chains by ring fission dioxygenases. However, ring-fission enzymes cannot typically degrade xenobiotics that contain only carbon and hydrogen. Thus dihydroxylation of these compounds provides a chemical handle for ring fission enzymes. The majority of bacterial species do not possess a degradation pathway for all aromatic xenobiotics, therefore such a pathway can only be constructed through directed evolution or plasmid insertion.

Gibson's first attempt was to produce a mutant strain of *Pseudomonas putida* that accumulates arene-*cis*-dihydro diols and not pyrocatechol. He achieved this goal by

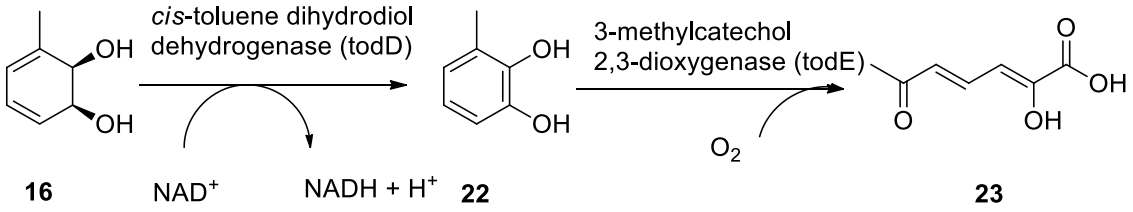
utilizing a *N*-methyl-*N*-nitrosoguanidine medium, which transformed the wild strain to the mutant *Pseudomonas putida* F39D that lacked the enzymes necessary to oxidize the arene-*cis*-dihydro diol intermediate to the corresponding pyrocatechol.[4, 31]

Gibson noticed that the mutant *Pseudomonas putida* F39D lacked two enzymes that are present in the original wild type strain (*todE, todD*) and he concluded that these two enzymes are responsible for the downstream processing of the arene-*cis*-dihydro diols. Based on that observation Gibson identified the gene sequence responsible for the multicomponent toluene dioxygenase protein complex which consists mainly of five enzymes (*todA, todB, todC1C2, todE, todD*). A genetically modified *E. coli* strain that carries the recombinant pDTG601 plasmid and expresses the (*todA, todB, todC1C2)* enzymes and lacks the gene encoding for (*todE* and *todD*) was later developed by Gibson. The genetically engineered strain accumulates arene-*cis*-dihydro diols similar to the mutant *Pseudomonas* putida F39D.[32]



Scheme 6. Metabolic pathway for the dihydroxylation of **15** to **16** by the toluene dioxygenase complex.[32]

As in many other similar enzymatic reaction, the electron source is the oxidation of nicotinamide adenine dinucleotide (NADH → $NAD^+ + H^+ + 2e-$). The electrons are first captured by a flavoprotien ($reductase_{TOL}$) which shuttles the electrons to the $ferrodoxin_{TOL}$ enzyme, a small iron-sulfur protein.[32] The reduction cycle continues through another iron-sulfur protein ISP_{TOL}, which ultimately reduces molecular oxygen. There are two main reasons as to why the recombinant clone *E. coli* JM 109 is more effective than the mutant strains *Pseudomonas putida* F39D. The mutant strains require induction of protein synthesis using chlorobenzene or toluene which can problematically interfere with substrate screening as arene-*cis*-dihydro diols generated from the inducer must be separated from any arene-*cis*-dihydro diols produced from the substrates being screened. The *E. coli* strain is induced by a sugar analogue, β-isopropylthiogalactopyranoside, removing this separation issue. In addition, protein translation in the *E. coli* strains occurs from the plasmid and not from the full genome as in the mutant strains, thus placing several copies of the plasmid in *Escherichia coli* JM109 (pDTG601) will increase protein production.[32]



Scheme 7. Metabolic pathway for the degradation of **16**, a pathway that is omitted in both the mutant stain *Pseudomonas putida* F39D and the genetically engineered strain *E. coli* JM 109.[31, 32]

The precise mechanism of the dihydroxylation by TDO is still unknown. The earliest proposal by Gibson suggested a 1,2-dioxetene intermediate, which is probably not the case as it involves high energy intermediates.

In 1998 the structure of the Naphthalene dioxygenase enzyme (NDO) an enzyme that performs similar *cis*-dihydroxylations on fused aromatics was solved. [33]

The structure showed that the active site contains a Rieske [2Fe-2S] center and a non heme-iron that are responsible for oxygen addition.[33, 34]

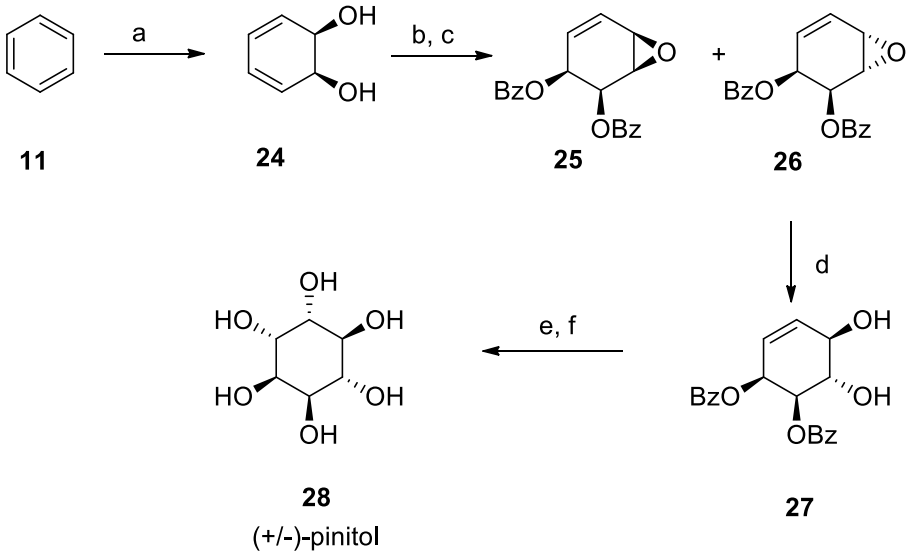
2.1.5 Toluene dioxgyenase substrate scope

To date over 400 different arene-*cis*-dihydro diols have been identified as metabolic products of various aromatic hydrocarbon dioxygenases and in the majority of these cases the products are enantiomerically pure.[5, 40] The chiral cyclohexadiene-*cis*-diols are ideal substrates for the synthesis of a variety of complex natural products and analogues. The most obvious utilization of such cyclohexadiene-*cis*-diols is to integrate the chirality of such diols into synthetic or natural products, such as carbohydrates, conduramines, and others.[6]

In 1987 Ley utilized the metabolic dihydroxylation of benzene in his synthesis of racemic pinitol.[36] Ley utilized benzene as the simplest substrate for microbial oxidation which was converted to *meso*-diol **24** by the mutant strain of *Pseudomonas putida* Fl. *Pseudomonas putida* Fl is very similar to the mutant *Pp*39D. It expresses the (*todA, todB, todC1C2)* enzymes that are responsible for the *cis*-dihydroxylation and lacks the genes encoding for (*todE, todD*) the two enzymes responsible for the further metabolism for the arene-*cis*-dihydro diol.

The *meso* diol **24** was converted to the dibenzoate, which was later subjected to

epoxidation with *m*CPBA. The epoxides produced **25** and **26**, were separated by chromatography and the desired epoxide **26** was opened using methanol and camphor sulfonic acid to produce **27**. Upjohn dihydroxylation was performed on **27** using OsO_4 as a catalyst and the product was deprotected to produce racemic pinitol **28**.[36]



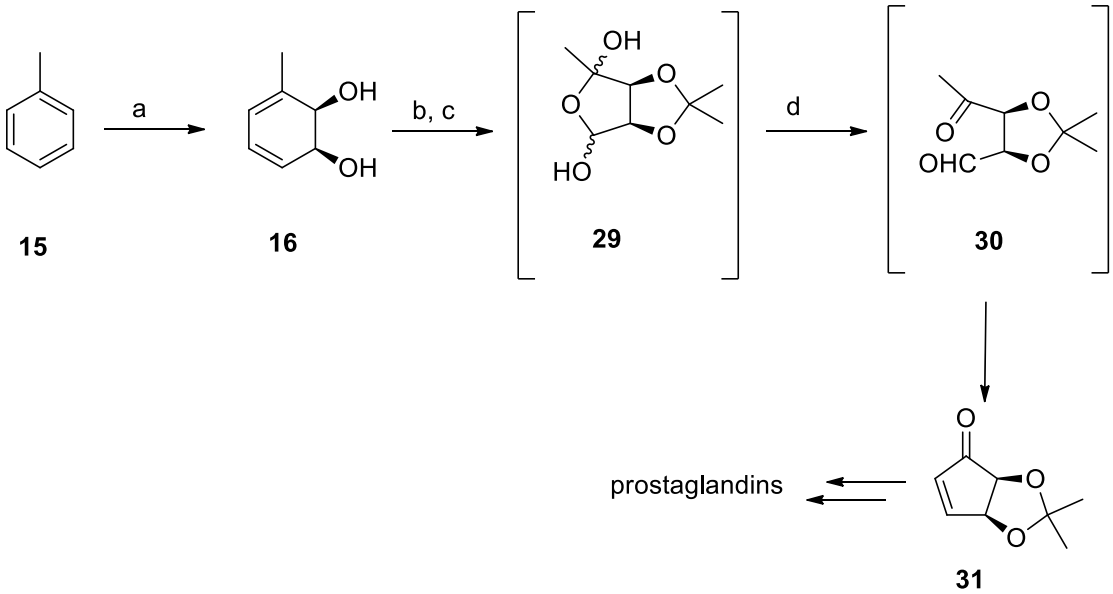
Reagents and conditions: (a) *Pseudomonas putita* F1 (b) PhCOCl, DMAP, pyridine (c) *m*CPBA, DCE, pH 8 phosphate buffer (d) MeOH, camphor sulfonic acid (e) OsO_4, NMO, H_2O (f) Et_3N, MeOH, H_2O.

Scheme 8. Ley's synthesis of racemic pinitol.[36]

In 1988, only one year after Ley's first use of the cyclohexadiene-*cis*-diols, Hudlický utilized diol **16,** produced from the microbial oxidation of toluene, in the synthesis of prostaglandin intermediates and perhydroazulene terpenes .[37] Hudlický's chemoenzymatic synthesis of a prostanoid intermediate was the first enantioselective chemoenzymatic synthesis that made use of the cyclohexadiene-*cis*-diols .[37]

A microbial fermentation utilizing *Pseudomonas putida* 39D was performed to transform toluene **15** to **16** which was protected as its corresponding acetonide, and subjected to

ozonolysis to give an isomeric mixture of the hemiacetal **29**. Further treatment with alumina, DME at reflux produced the α,β-unsaturated ketone **31** which had previously been converted to prostaglandins.[38]



Reagents and conditions: (a) *Pseudomonas putita* 39D (b) 2,2-dimethoxypropane, *p*TsOH, rt (85%) (c) O_2/O_3, dimethyl sulfide (d) neutral alumina, DME, reflux

Scheme 9. Key steps in Hudlický's chemoenzymatic synthesis of a prostanoid intermediate.[37]

In 1993 Boyd screened a series of 1,4-disubstituted benzene derivatives as substrates for toluene dioxygenase.[39] Based on the enantiomeric ratios of the chiral cyclohexadiene-*cis*-diols produced **32**, **33** and **34** Boyd proposed a model to predict the regio- and the stereochemistry of the dihydoxylation. He proposed that the enantiotopic differentiation by the enzyme depends on the size difference of the substituents.

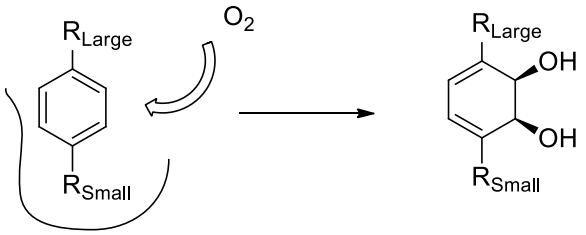


Figure 3. Boyd's model for predicting the stereo- and regioselectivity of the dihydroxylations.[39]

Since the late 1970s Gibson's group has screened and identified hundreds of substrates. Ribbons and coworkers investigated the dihydroxylation of various benzoic acids,[42] and Tomas Hudlický and coworkers have screened and identified hundreds of new substrates.[6] The reported substrates possess a variety of functionalities including halogens, alkanes, alkenes, alkynes, nitrile, phenyl, carboxylic acids, and other substituents. Single ring and fused rings aromatics have been investigated; yields vary depending on the structure and functionality.

The most comprehensive list of chiral cyclohexadiene-*cis*-diols can be found in Johnson's *Organic Reactions* chapter.[40] Only a fraction of the cyclohexadiene-*cis*-diols which have been identified have been utilized in synthesis. The most widely utilized substrates are mono-halo-benzenes, β -haloethyl benzene, styrene, and *m*-dibromobenzene.[6] Some of the key products of metabolic dihydroxylation are shown in Figure 4.

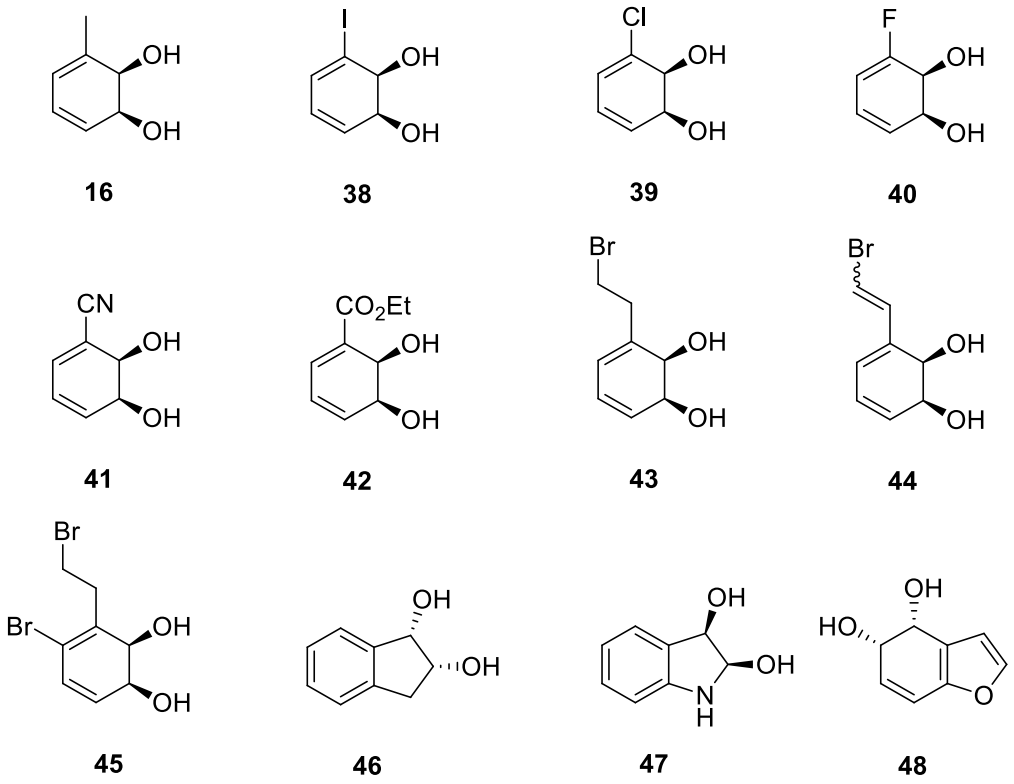


Figure 4. Selected key cyclohexadiene-cis-diols that have been utilized in chemical synthesis.[6, 40]

In 2013 Hudlický's group screened and identified several new para-substituted arenes suitable as substrates for toluene dioxygenase. Through the screening of a library of compounds many compounds were shown to be suitable substrates for toluene dioxgyenase, including 4-bromo-phenylacetylene, 4-bromobenzaldehyde. These compounds have great potential in the synthesis of various complex natural products. Some of the reported arene-cis-dihydro diols identified by this study are exemplified by compounds **35**, **36**, and **37**.[41]

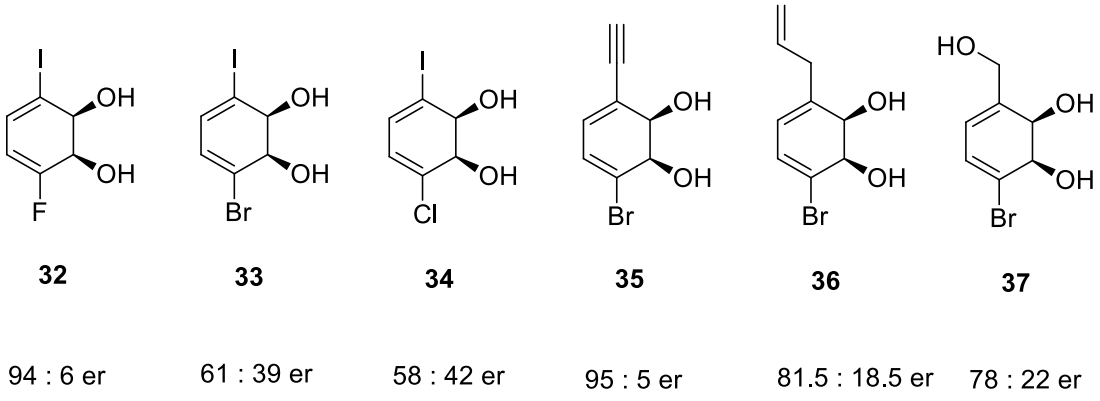


Figure 5. Screened 1,4-difunctionalized diene diols and their enantiomeric ratios.[41]

2.1.6 Utilization of toluene dioxygenase in chemical synthesis.

There is no doubt that the arene-*cis*-dihydro diols derived from the microbial oxidation of aromatics are ideal substrates for the synthesis of certain complex natural and synthetic products. They are very useful when the chirality induced by the aromatic oxidation is utilized to control the stereochemistry of the final products. Not only the two hydroxyl functionalities can be chemically differentiated, but all six carbon atoms can be subjected to selective functionalization. The olefins are sterically and electronically differentiated which open the door for various selective intra- or intermolecular Diels-Alder reactions as well as selective dihydroxylation, epoxidation, aziridation, and cyclopropanations.[6] The two hydroxyl functionalities are ideal for attaching various functionalities. The halogen present in some arene-*cis*-dihydro diols can be utilized for coupling.[6] Some of the reactions which are possible with bromo arene-*cis*-dihydro diol are shown in Figure 6.

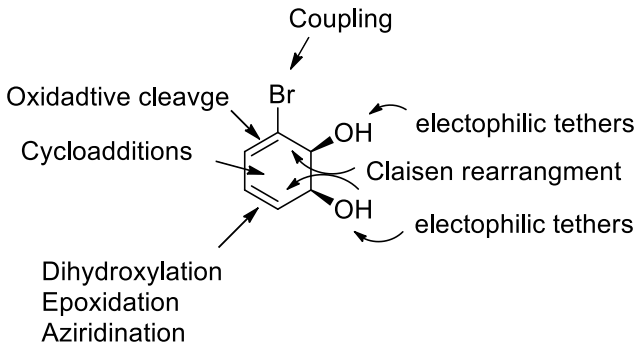
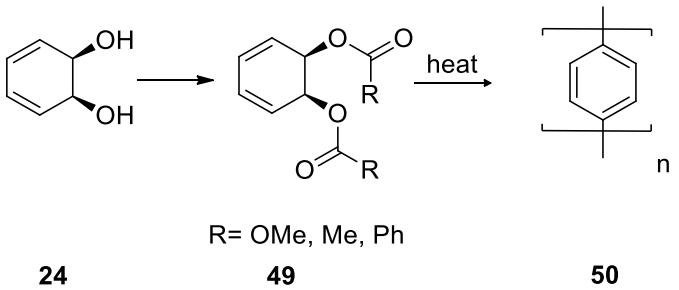


Figure 6. Possible reactions of the bromo arene-cis-dihydro diol.[6]

Since Gibson's first discovery and isolation of first arene-*cis*-dihydro diol in 1968 almost nineteen years passed before any of the arene-*cis*-dihydro diols were utilized in synthesis.[2, 3] The first utilization of arene-*cis*-dihydro diols in an organic synthesis was reported by Taylor in 1983.[43] Taylor and his coworkers at the Imperial Chemical Industries PLC utilized the arene-*cis*-dihydro diol **24** derived from benzene. The arene-*cis*-dihydro diol was converted to various diesters **49** and then heated to form polyphenylene **50**, Scheme 10. [43] Following Taylor's polyphenylene synthesis, Ley reported the synthesis of racemic pinitol, Scheme 8.[36] In 1988 Hudlický completed his chemoenzymatic preparation of a prostanoid intermediate, Scheme 9.[37]



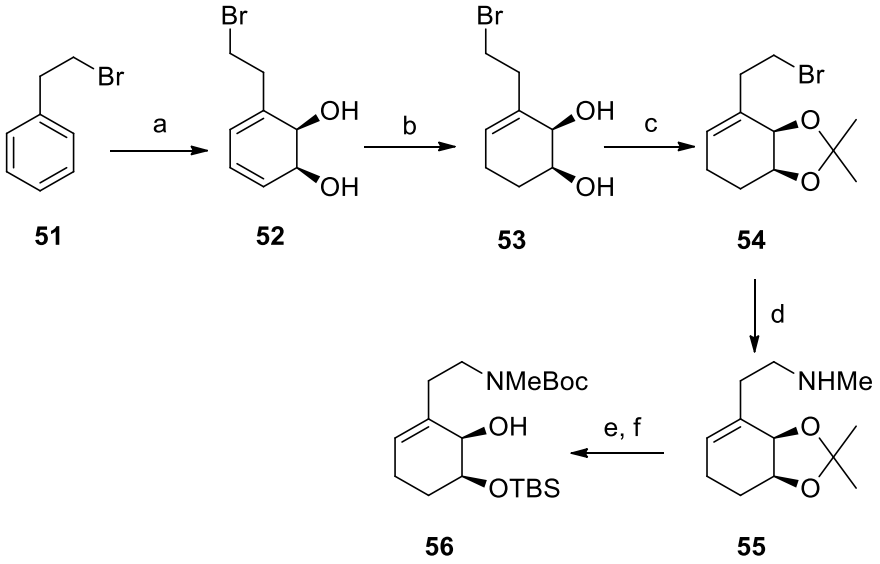
Scheme 10. First application of TDO in chemical synthesis. [43]

Since the late 1980s the utilization of toluene dioxgenase metabolites has vastly expanded, although out of the 400 metabolites of toluene dioxgenase discovered only seven compounds have been utilized in various synthetic projects. These seven arene-*cis*-dihydro diols are the metabolic product of bromobenzene, chlorobenzene, iodobenzene, *m*-dibromobenzene, toluene, styrene and β-bromoethylbenzene.[6] Chlorobenzene has been utilized as a starting material in the synthesis of various synthetic targets such as conduritol C,[44] D-chiro-inositol,[45]conduramine A-1,[46] (-)-pinitol,[44] hexoses,[47]aminohexoses,[48] carbasugars,[49] L-ascorbic acid, both D- and L-erythrose,[50] natural and unnatural sphingosines,[51] (+)-aspicillin,[52] (-)-cladospolide A,[53]cladospolide B.[54] Bromobenzene metabolite has been used numerous times in different syntheses such as the synthesis of pancratistatin,[55] 7-deoxypancratistatin,[56] and many other pancratistatin analogues. This compound has also been utilized in the synthesis of lycoricidine and amino-inositols.[57, 58]

2.1.7 Selected chemoenzymatic synthesis using toluene dioxygenase

In 2014 Vimal Varghese and Tomas Hudlický completed an extremely efficient chemoenzymatic total synthesis of *ent*-hydromorphone. Despite all the effort which has previously been invested in the synthesis and design of morphinans by many other scientists; a practical and efficient synthesis of morphine analogues has yet to be established. Hudlický's short, twelve step synthesis consist of five major transformation, the first one of course is the aromatic dihydroxylation of bromoethylbenzene to the corresponding *cis*-dihydrodiol, followed by a Mitsunobu coupling , oxidative dearomatization , subsequent [4+2] cycloaddition and intramolecular amination. The

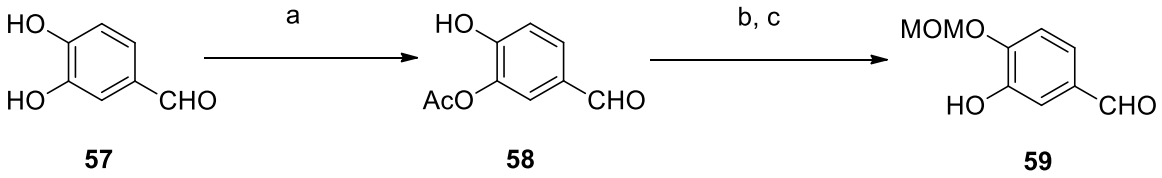
synthesis is by far the most effective route to the total synthesis of *ent*-hydromorphone.[59] The synthesis starts by designing two subunits that will later be joined *via* Mitsunoba coupling. The first subunit was designed through aromatic dihydroxylation of bromoethylbenzne **51** by *E. coli* JM 109 (pDTG601A) to produce the corresponding *cis* -dihydrodiol **52** which was subjected to selective reduction by potassium azodicarboxylate and protection of the diol moiety to give **54**. Bromine was later substituted with methylamine to yield **55**, which is subjected to protection with a Boc group. The acetonide was then removed under acidic conditions and regio-selective TBS protection was performed to yield **56** as shown in Scheme 11. [59]



Reagents and conditions: (a) *E. coli* JM 109 (pDTG601A)
(b) potassium azodicarboxylate, AcOH, MeOH (c) 2,2-dimethoxypropane, acetone, *p*TsOH
(d) $MeNH_2$, K_2CO_3, THF, sealed tube (e) 1. HCl, EtOH; 2. Boc_2O, $NaHCO_3$, EtOH
(f) TBSCl, imidazole, CH_2Cl_2, -78°C to rt.

Scheme 11. Design of the first subunit in *ent*-hydromorphone synthesis. [59]

The second subunit was synthesized through regioselective acetylation of 3,4-dihydroxybenzaldehyde **57** to yield **58** followed by selective MOM protection under mild basic conditions and hydrolysis of the acetyl to yield the second subunit **59** as shown in Scheme 12.[59]



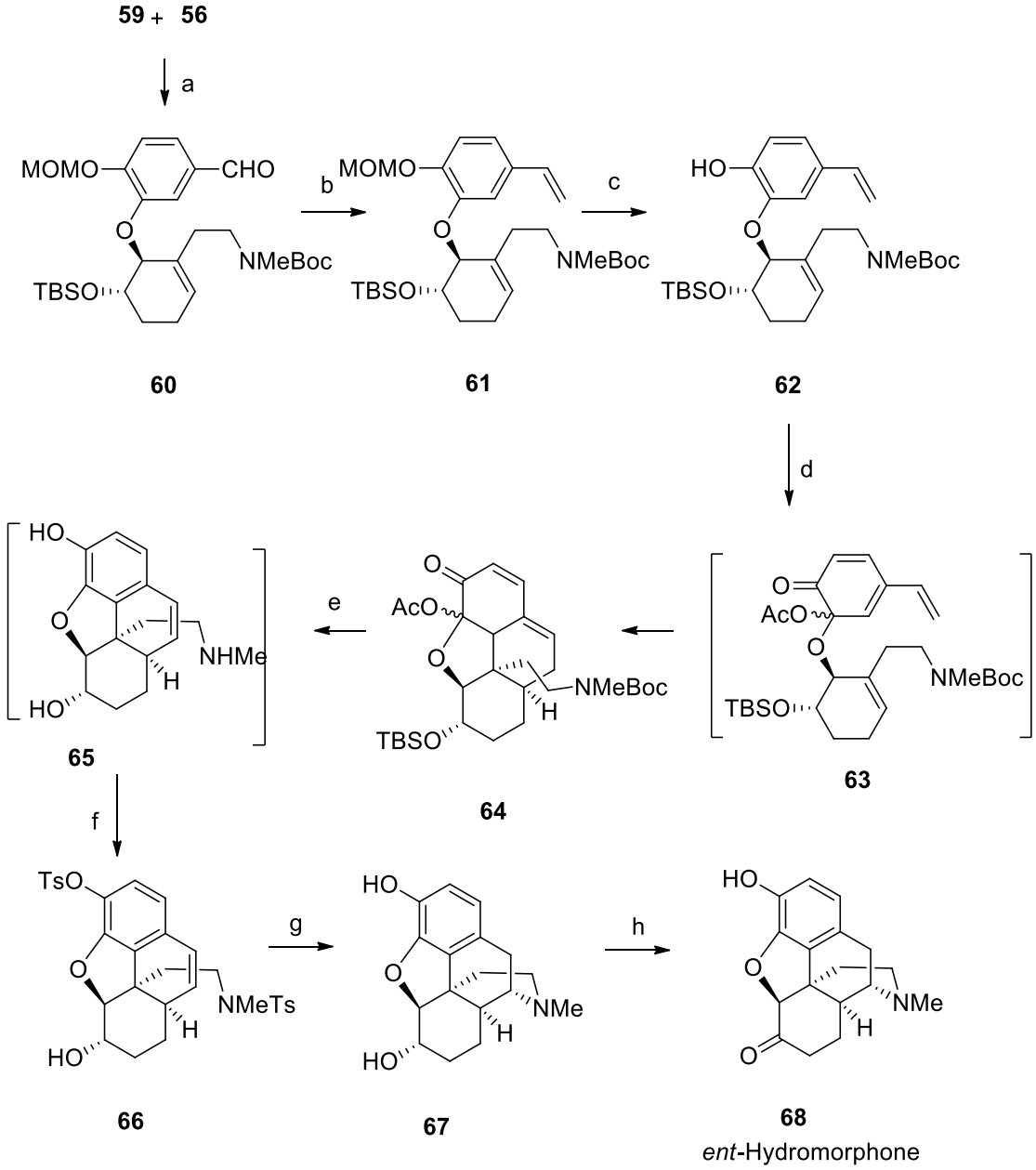
57 **58** **59**

Reagents and conditions: (a) Ac_2O, NaOH, THF, 0°C (b) MOMCl, K_2CO_3, DMF, 0°C to rt (c) K_2CO_3, MeOH

Scheme 12. Design of the second subunit in *ent*-hydromorphone synthesis. [59]

The two units were coupled using Mitsunobu conditions to produce aldehyde **60.** The aldehyde was converted to a vinyl group using a Wittig reaction to produce **61** and MOM group was cleaved under mild condition to produce **62**. Exposure of **62** to lead tetraacetate in refluxing dichloroethane lead to oxidative de-aromatization, and subsequent [4+2] cycloaddition to yield **64** . Subjecting **64** to trifluoroacetic acid lead to production of phenol **65** through re-aromatization and hydrolysis of the Boc group. Treatment with excess tosyl chloride phenol **65** provided the tosyl amide **66**. The ethylamino bridge was achieved by nitrogen centered radical cyclization. Oxidation of **67** to *ent*-hydromorphone **68** was accomplished with benzophenone and t-BuOK in 44%

yield.[59]

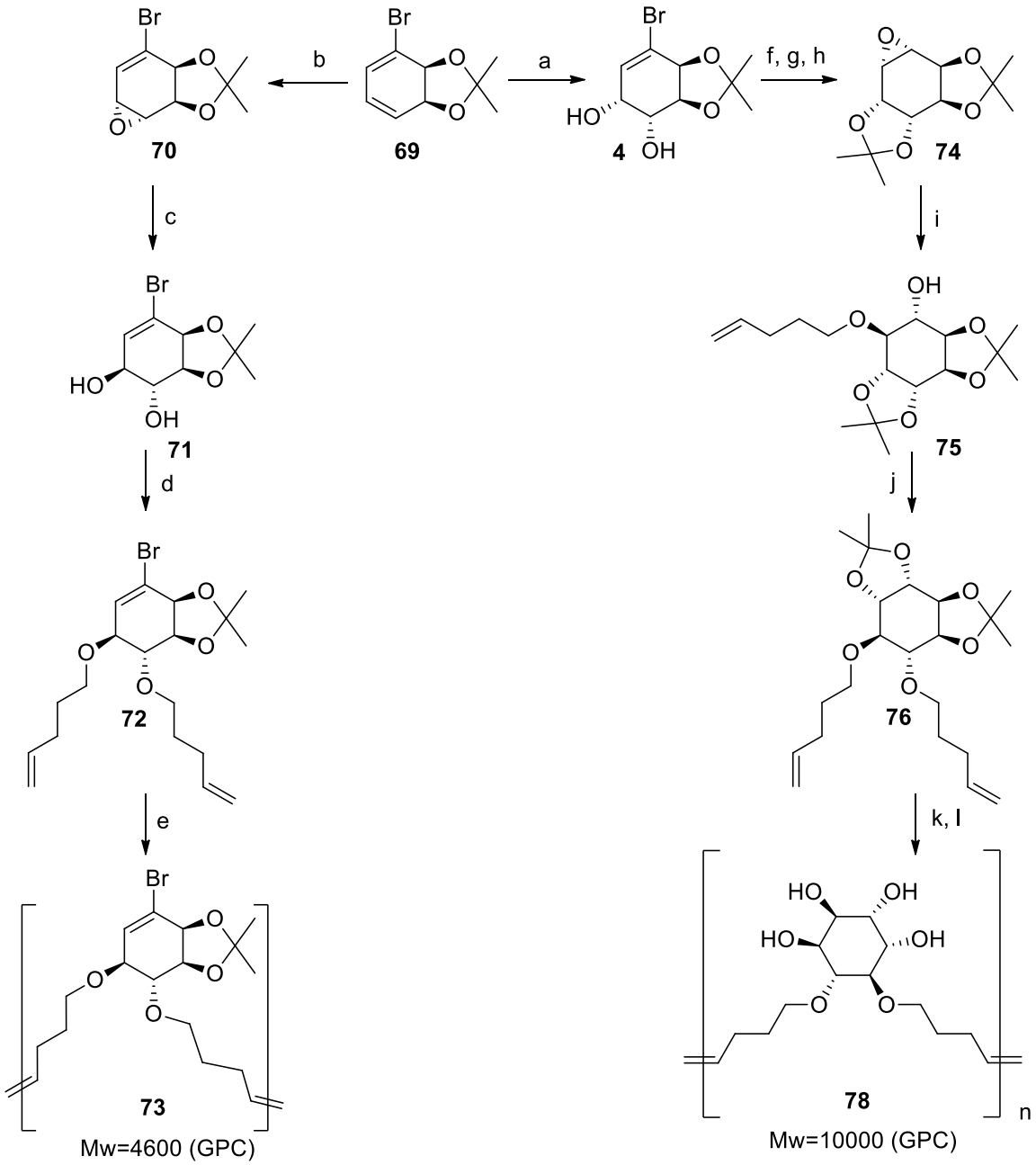


Reagents and conditions: (a) TMAD, PBu_3 (b) CH_3PPh_3Br, n-BuLi, THF, 78°C to 0°C then reflux for 4 h (c) $ZnBr_2$, $CH_3(CH_2)_{10}CH_2SH$, CH_2Cl_2 (d) $Pb(OAc)_4$, DCE, reflux, 4 h
(e) TFA, CH_2Cl_2, 0°C, 15 min
(f) TsCl, Et_3N, CH_2Cl_2 (g) Li, tBuOH, NH_3, THF, 78°C, 10 min
(h) t-BuOK, PhCOPh, $PhCH_3$/DME, 85°C, 8 h, 44%.

Scheme 13. Hudlický's *ent*-hydromorphone synthesis.[59]

2.1.8 Toluene dioxgyenase in polymer synthesis

In 2004 Hudlicky and coworkers reported the synthesis of chiral polyhydroxylated polymers derived from the arene *cis*-dihydro diols.[113] Diol **2** produced from microbial oxidation of bromobenzene was converted into various monomers that were later polymerized *via* Grubbs' metathesis. To produce the first monomeric unit diol **2** was converted to **69**, which was later converted to diol **4** *via* Upjohn dihydroxylation using osmium tetroxide. Further reduction and subsequent epoxidation with *m*CPBA provided epoxide **74**.[113] Epoxide **74** was opened with $BF_3 \cdot Et_2O$ and penten-1-ol to give alcohol **75**, which was alkylated with 5-bromopentene to result in the first chiral monomer **76**.[113] The second chiral monomer was produced by subjecting the acetonide protected arene *cis*-dihydro diol **69** to epoxidation conditions to yield epoxide **70**. The epoxide was opened to afford trans diol **71** which was alkylated to furnish the second monomer **72**.[113] Subjecting monomer **76** to Grubbs' first generation catalyst gave polymer **77**. Later deprotection of that polymer under acidic condition yielded the fully hydroxylated polymer **78** which was estimated to weigh 10,000 g/mol via GPC analysis. Monomer **72** was treated with Grubbs' second generation catalyst to yield polymer **73** with molecular weight of 4,600 g/mol .[113]



Reagents and conditions: (a) acetone, H_2O, NMO, OsO_4 (b) *m*CPBA, CH_2Cl_2 (c) Amberlyst resin (d) DMF, NaH, allyl bromide (e) Grubbs' catalyst (f) Bu_3SnH, AIBN, PhH (g) DMP, *p*TsOH (h) *m*CPBA, CH_2Cl_2 (i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4-penten-1-ol (j) DMF, NaH, 5-bromopentene (k) Grubbs' first-generation catalyst (l) THF: TFA:H_2O, 4:1:1.

Scheme 14. Chiral polyhydroxylated polymers from the arene-*cis*-dihydro diols.[113]

2.2 Lipases

2.2.1 Introduction

The story of lipases starts in 1848 when an infamous researcher named Claude Bernard discovered that pancreatic secretions could emulsify fatty substances.[60] A French physiologist born in 1813, he spent his early life writing comedy plays until he was convinced at the age of 21 that he lacked the intelligence to adopt literature as a profession and that he should seek a less creative profession like medicine.[60, 61]

Lacking normal social skills, Bernard focused on medical research more than the practice of medicine. He applied for a position at the Sorbonne but was not accepted, and so he stayed at the College de France until he was appointed as a full professor in 1855. At the time his work was commonly regarded as inferior to other research done in the field.[60, 61]

Today Bernard is regarded as one of the greatest men of science. Bernard was the first to introduce the term "Milieu intérieur", now known as homeostasis, he single handedly discovered the vaso-motor system and glycogenic function of the liver.[60, 61]

Bernard changed the way science is conducted. In his book " *An Introduction to the Study of Experimental Medicine* " Bernard established what we now know as the scientific protocol and experimental procedure. Bernard redefined scientific observation, proof, disproof, assumptions, and data statistics.[62]

Bernard's first major scientific discovery was in 1848 when he discovered that pancreatic juice can emulsify and saponify fatty substances, specifically a candle that was on his lab bench that was made of tallow. Bernard's discovery of the action of this pancreatic enzyme was later given the name lipase.[62, 61]

Enzyme technology is a multibillion industry; proteases dominate the market and lipases come in second place.[63] Lipases are excellent industrial biocatalysts due to their specificity, thermal stability, and tolerance to organic solvents.[63] The detergents industry accounts for 32% of the worldwide industrial lipase use. It is estimated that an average of a hundred tons of lipases are utilized in detergent manufacturing each year.[64]

Lipases are enzymes that catalyze the hydrolysis of triacylglycerols (TAGs) to glycerol and free fatty acids. they can also perform ester transesterification as well as ester synthesis. Lipases are different than esterases. Esterases only hydrolyze water-soluble short acyl chain esters, while lipases hydrolyze water-insoluble long chain triacylglycerols.[64]

Esterases are active in aqueous solution while lipases show very limited activity in aqueous solutions. Free lipases are only active when adsorbed to an oil/water emulsion interface. Immobilization plays an integral part in the utilization of lipases in an industrial setup. Immobilized lipases show different properties such as increased catalytic activity, temperature tolerance and solvent tolerance. Since the 1980s several new lipases have been discovered, cloned, and expressed. Also, different types of immobilization techniques have been utilized. This chapter aims to discuss the different sources of microbial lipases, lipases properties and mechanism, lipase catalyzed reactions, different immobilization techniques, industrial uses of lipases and finally a special focus on lipases in polymers synthesis.

2.2.2 Lipase-catalyzed reactions and mechanisms.

Normally, lipases hydrolyze TAGs. Lipases, however, are one of the most versatile and diverse enzymatic families. The lipase enzyme family contains distinctively different lipases, which differ in both function and structure. Some lipases are treated separately, such as phospholipases and sphingomyelinases, which are never regarded as regular lipases.

Most lipases possess a common mechanism of action. The lipase protein structure usually comprises an alpha/beta hydrolase fold, which is a very common shape between the hydrolytic enzymes, indicating their common evolutionary origin.[65]

A catalytic triad consisting of an acid, base, and nucleophile is present in the active site. Usually this triad consists of histidine (base), serine (nucleophile), and aspartate (acid), Figure 7. The same triad is also common to all serine hydrolases and is found in many other different enzymes such as trypsin and esterases. Lipases also possess an oxyanion hole in the active site, that consists of three hydrogen bond donors that stabilize the transition state and tetrahedral intermediate.[65, 66]

A nucleophilic attack by the serine on the carbonyl carbon results in an acyl-enzyme intermediate and an alcohol. The acyl enzyme intermediate is later hydrolyzed by water.[65, 66] Lipases have a very broad range of substrates; although triacylglycerols are the natural acyl donors for these enzymes, various esters, thioesters, amides and free carboxylic acids can act as acyl donors. Similarly, many nucleophiles other than water can deacylate the acyl-enzyme intermediate which leads to transesterification. Transesterification reactions are predominant in organic media due to the absence of water.

Amines can also act as nucleophile, which leads to the production of amide products. Lipases are highly promiscuous and have even shown to catalyze epoxidation, aldol addition, and racemisation reactions.[81]

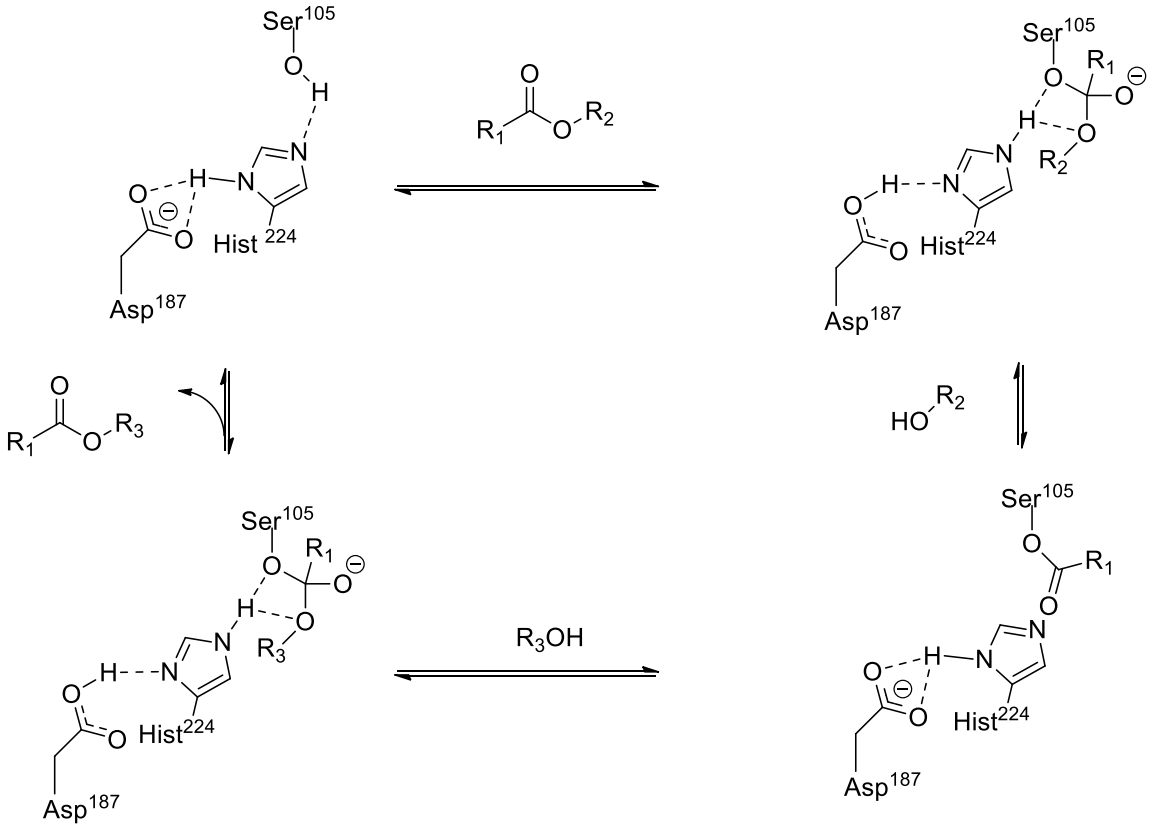


Figure 7. Mechanism of *Candida antarctica* lipase B-catalyzed transesterification.[65, 66]

2.2.3 General properties of lipases

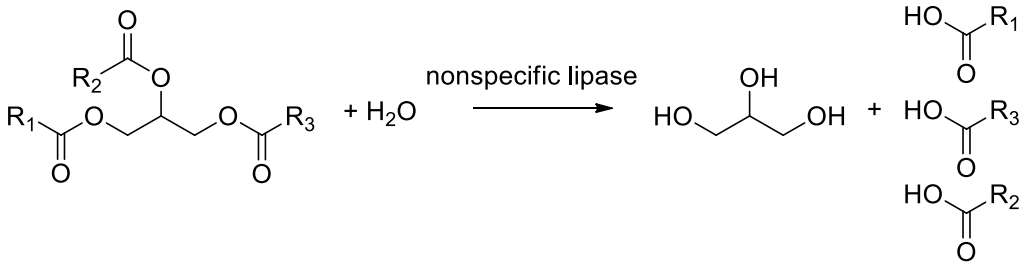
In nature lipases can function in neutral or alkaline pH, however, optimum hydrolytic activity is observed at pH 4.8.[67] Lipases exhibits optimum activity in a temperature range between 30-60 °C. Addition of ethylene glycol has enhanced the thermal stability of lipases from certain species.[67] Immobilized lipases have much higher thermal stability than free enzymes. In 2013 Zelisko and coworkers reported the synthesis of siloxane-containing polyesters and polyamides by using a commercial immobilized enzyme lipase B from *Candida antarctica* at elevated temperatures well above the normal denaturation temperature for free enzyme.[68]

Lipases differ greatly depending on their origin. Specific lipase properties may also depend on the cellular location of the lipase protein within the organism. Certain strains produce intra- and extra-cellular lipases, which make them an industrial favourite as they have a wide scope of acceptable substrates.[69]

Lipases can be found in different microbial, plant, and animal species. Although animal lipases were commercially exploited for decades their use now is limited to medical research. Production of microbial lipases is significantly easier than plant or animal lipases, as microbes can be mass cultured and genetically modified, and offer much easier purification and isolation protocols. Some distinctively industrial useful lipases originate from plant or animals, however, their production is usually carried through a cloned microbial host.[69]

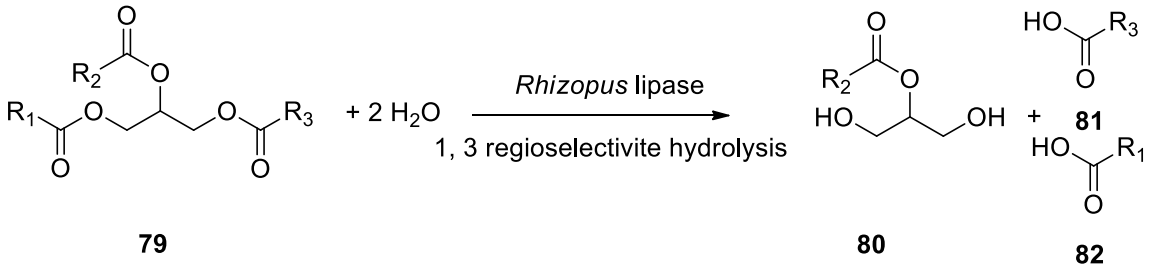
Using cloning technology microbial hosts can express foreign enzymes. Cloned organisms tend to produce foreign enzymes in great excess, due to placing more than one copy of the foreign gene in the cloned organism. Therefore they result in much better

production when compared to the wild strains. Wild strains also tend to express various enzymes in equal amounts which makes isolating one specific enzyme a difficult task.



Scheme 15. General lipase catalyzed reaction.

Although some lipases are nonspecific (Scheme 15), certain lipases are highly selective. *Candida antarctica* lipase B (CALB) is highly enantioselective.[68] *Rhizopus* lipases exhibit 1, 3-regioselectivity and hence are often utilized in the conversion of triglycerides **79** into the corresponding monoglycerides **80**.[71]



Scheme 16. *Rhizopus* lipase-catalyzed reaction. [71]

Fungal lipases are responsible for many of the flavours in cheese; commercially significant fungal lipases include lipases from *Penicillium roqueforti* which is responsible for the flavour of blue cheese. These lipases release acids from fats which are responsible for the strong flavour of certain cheeses.[70] Other virulent bacterial species such as *Staphylococcus haemolyticus* and *Staphylococcus aureus* also been reported to produce

lipases.[72] Fungal lipases are industrially very significant due to the fact that they exhibit better thermal stability than most bacterial enzymes.[69,70]

2.2.4 Production of commercial lipases

There is a high demand for thermally stable lipases that possess high catalytic activities regardless of harsh thermal and pH conditions and therefore the hunt for such lipases occurs in rather non-traditional places such as oil processing factories, oil products dumping grounds, dairy wastes and other various industrial waste sites.[73]

In 1993 Matthiasson and coworkers aimed to identify thermally stable lipases by investigating thermophilic bacteria dwelling in the Icelandic hot springs. [74] A similar idea was deployed when researchers investigated *Candida antarctica* for lipase activity; *Candida antarctica* is a thermally tolerable organism that dwells in extreme cold conditions. Immobilised lipases from *Candida antarctica* showed significant thermal stability specially under elevated temperatures.[68]

Production of commercial lipases of constitutes four distinctive steps. First, a conclusive screening of several microbial candidates for certain lipase activity, followed by optimizing production and growth parameters then purification of the lipase protein and finally immobilizing the lipase protein onto a mechanical carrier.

Lipases are distinctively different; therefore screening methods must be specific in terms of the enzymatic function assayed. Screening might be directly performed on crude cellular extract of a microorganism culture, depending on the nature and the virulence of the microorganism screened.[75, 76]

Plate screening methods are extremely common. Agar plates can offer cheap and basic

information about enzymatic activity. Since lipases hydrolyze lipids, agar plates prepared with lipid substrates are utilized for lipase activity screening. Lipases hydrolyze lipids into corresponding fatty acids resulting in clear or opaque spots on the agar plates, indicating lipase activity. [75, 76]

Colorimetric assays utilizing plate cultures prepared with a colorimetric pH indicator such as phenol red or victoria blue are commonly used. [75, 76] Active lipases result in a pH drop due to fatty acid production that subsequently that will lead to a pH indicator color change. Another commonly colorimetric method is the use of rhodamine B plates, where 4-nitrophenol esters of fatty acids are used for detection of lipase activity. Lipase hydrolyze these esters and release 4-nitrophenol, the amount of which is measured by spectrophotometric means and correlated to lipase activity. [75, 76] Other fairly uncommon screening methods include titration, radioactive assays and immunoassays. [75, 76]

Commercial lipases are produced mainly through submerged fermentation a technique that utilizes free flowing liquid substrates. Lipase production is similar to every other protein production. Protein production often depends on several factors such as temperature, pH, nitrogen, carbon and lipid sources, agitation, and dissolved oxygen levels.[73] Certain inducers and inhibitors can drastically affect the production rates.

Any fermentation process will require a carbon and nitrogen source to produce proteins. Lipids are an excellent carbon source, and lipase production in most cases is positively correlated to the lipid content. Lipase production can be also stimulated by other esters, and bile salts.[75,76] Other possible carbon sources are mainly sugars. A study by Srinivasan showed that in case of *Rhizopus nigricans,*glucouse fed bacteria exhibited maximum lipase production, and other sugars such as mannitol, galactose, sucrose, fructose, lactose,

maltose produced a reduced amount of lipase compared to glucouse.[77]

Triglyceride and fatty acids concentrations play an important role in lipase production. Although triglycerides and fatty acids can induce lipase production, very high concentrations of free fatty acids or triglycerides can decrease lipase production. Alternative carbon sources have been studied such as Tween 80 (Figure 8), a nonionic surfactant and emulsifier often used in foods and cosmetics. Slow release of oleic acid from Tween 80 provides the culture with essential carbon and does not cause suppression of the enzyme production.[76, 78]

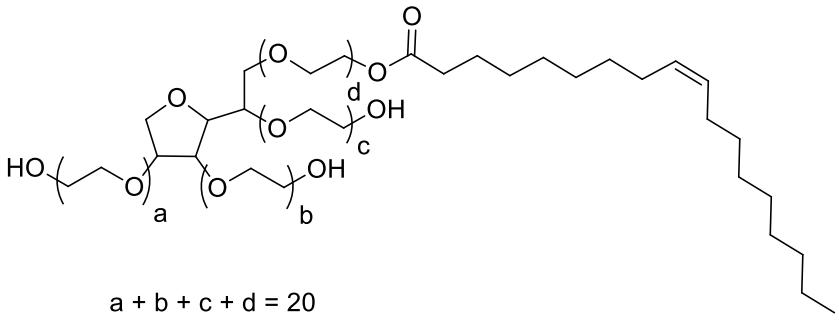


Figure 8. Tween 80 surfactant

The choice of a suitable nitrogen source in not as challenging as finding the suitable carbon source. There is no reported evidence that a specific nitrogen source that hinders lipase production.[76] Peptone is commonly used as a nitrogen source. Peptone is a mixture of polypeptides and amino acids formed by the partial hydrolysis of different proteins. Peptone has showed to increase lipase production in certain species.[77]

Temperature control plays a crucial role in any fermentation application. Similar to most microbial fermentations lipase production is maximum in the temperature range of 30-40 °C,[73] however, Oso reported a maximum lipase production from *Talaromyces emersonii* at 45 °C. *Talaromyces emersonii* is commercially used to produce a

thermostable enzyme that are extensively utilized in the bread manufacturing industry.[79] Microbial proteins in general can be purified by ammonium sulphate precipitation, gel filtration, ion exchange liquid-liquid extraction that relays on solubilizing specific biomolecules from aqueous fermentation culture.[80] Other less popular purification techniques include immunopurification which utilize monoclonal antibodies and membrane purification processes. Membrane purification relies on three different parameters to separate various biological macromolecules: pressure, carrier gas and the size of membrane pores. Through controlling all of these parameters selective isolation of certain biomacromolecules can be achieved.[80]

2.2.5 Immobilization of lipases

The use of biocatalysts in industries solely depends on the cost effectiveness of substituting regular chemical process with the biocatalytic ones. Enzymes will be only be utilized in industry if the enzymatic process is economical and financially more advantageous than the chemical one. Therefore, while the cost of production of such enzymes plays an important role, so does the recovery and the reusability.[81]

Free enzymes are labile and vulnerable to degradation through the manufacturing and recovery processes. They also exhibit fairly low activity and stability in organic solvents. All of these problems could be solved through immobilization; the use of immobilized enzymes not only improves enzyme stability under the reaction conditions, but also enhances enzyme activity and reusability of the enzyme. Immobilization also eases product and starting material isolation and recovery.[81]

Although immobilization is essential for the industrial use of biocatalysts, it is one of the main causes of enzyme deactivation. Lipases as many other protein share the same immobilization techniques, Figure 9.[81] Deactivation due to immobilization can occur when the enzyme is bound to the inert bead through its active sites (Figure 11) or the enzyme is placed in the middle of the inert bead thus become isolated from the reaction mixture.[81]

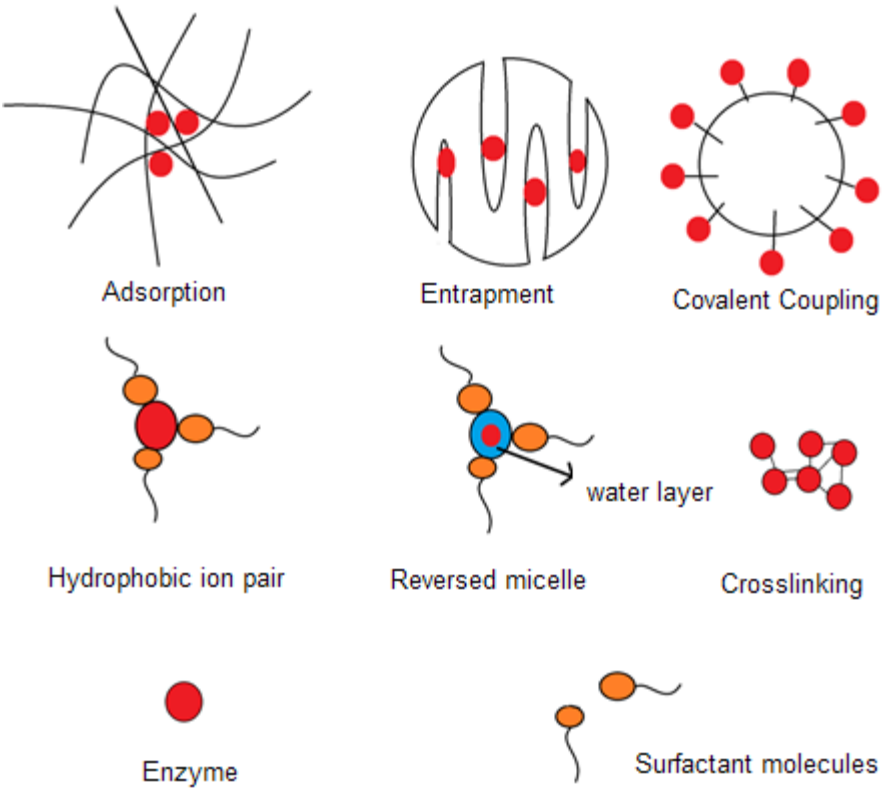


Figure 9. Various enzymes immobilization techniques.[81]

The choice of a certain immobilization techniques depends on how successful it is. The evaluation of the success of a technique depends on three different parameters: catalytic activity, stability, and yield. Stability is economically important; a stable enzyme has can be used more times.[81] It is also very important to maintain high catalytic activity; the immobilized enzyme should still be able to perform the same specific conversion as the free one.[81]Yield is an effective indicator of the success of a certain immobilization technique. Poor immobilization technique is usually translated to low yields and vice versa. A general practice when immobilizing active biological proteins is to compare the

activity of the immobilized enzyme to the "free" enzyme. This approach is not applicable in the case of lipases because free lipases in organic media are highly insoluble and their activity is fairly low.[81] Immobilization drastically improves the activity in organic solvents, thus comparing the activity of the immobilized enzyme to the free enzyme is not useful. A comparison between different immobilization techniques is more effective.[81]

Adsorption is the simplest method of enzyme immobilization. Lipase proteins are water-soluble and usually adhere to supporting material through hydrophobic interactions.[81] Not only is adsorption used for enzyme immobilization but it can also be used in enzyme purification. Adsorption also increases enzyme activity, which can be explained through the fact that when enzymes interact hydrophobically to the supporting material it induces structural alterations where the active site is more open.[81]

Adsorption of the enzyme to inorganic material such as diatomaceous earth has been reported.[81, 131] Although diatomaceous earth is cheap, inert and easy to filter, interactions between the enzyme and the diatomaceous earth are fairly weak and thus not very effective as an immobilization technique. A tremendous effort in research has devoted to immobilize lipases to various types of mesoporous silica.[81, 132] Mesoporous silica is an excellent carrier for biological enzymes because different parameters like porosity pore size and particle size can be controlled. During manufacturing of mesoporous silica, different concentration of surfactants in the manufacturing process results in different pore size. Interestingly pore sizes of the supporting material not only affect the yield and rates of lipases catalyzed reactions but can also affect enzyme specific activity. [81, 132]

Enzyme activity was also shown to be related to the hydrophobicity of the supporting material. A very hydrophobic supporting material, usually a material that contains long alkyl chain, results in much higher activity than a less hydrophobic material.[81]

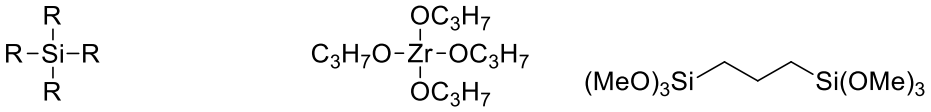
Several commercially available lipases are adsorbed on porous organic polymers. Organic polymers provide excellent support and excellent enzyme loading. For example commercially available Novozyme-435® is CALB immobilized on an acrylate-based polymer. The N435 preparation exhibits excellent activity and reusability in both industrial and research applications.[68] Since CALB is commercially and scientifically significant several attempts were carried to develop an ideal supporting material. Lipase B immobilized on polypropene resin exhibited better stability and reusability than N435.[81, 133] Since adsorption involves various aqueous solutions, immobilized enzymes have to be dried. Typical methods of drying such as freeze drying and vacuum drying can render the enzyme inactive, and so rinsing with organic solvent is the preferred method to eliminate water as it does not result in enzyme deformities.[81]

Protein coated microcrystals PCMC is an immobilization method that involves precipitation of an enzyme on the surface of certain crystals. Free enzymes are usually dissolved in water and a water soluble excipient is added. Slow addition of this solution to a compatible organic solvent will induce crystallization of the excipient salt and precipitation of the protein on the surface of those crystals. Protein coated microcrystals of lipases show 200 times more activity than the enzyme in "free" form,[134] and exhibit very good storage; 90% of CALB-PCMC survived after one year storage in a 1-propanol suspension.[135]

Enzyme cross-linking applied together with PCMC results in better preparation compared

to PCMCs alone, due to minimization of enzyme leakage.[81, 134]

Organic silanes are used to manufacture sol-gel material that are used to entrap various proteins. Lipases entrapped in sol-gels are fairly common. Silanes are hydrolyzed to form silicon dioxide which forms a gel that entraps protein particles. It is very important to choose silanes with hydrophobic substituents, as they arrange themselves away from the lipase hydrophilic active site. Silanes utilized for sol-gel are shown in Figure 10.[81, 136, 137] Zirconium sol-gel **88** was developed because it required much milder conditions for gel formation. However, enzymatic activity of the immobilized lipase was lower than of that of the free enzyme. [81, 138]



83 $R_1=R_2=R_3=R_4=OMe$
84 $R_1=C_2H_5$ $R_2=R_3=R_4=OMe$
85 $R_1=n\text{-}C_3H_7$ $R_2=R_3=R_4=OMe$
86 $R_1=n\text{-}C_4H_9$ $R_2=R_3=R_4=OMe$

88

87

Figure 10. Building units often utilized for sol-gel preparation. [81, 136, 137, 138]

Covalent coupling is a very common way to immobilize enzymes. There is always a certain degree of inactivation when covalent coupling is used. If inactivation is severe the covalent coupling is not a viable immobilization technique. Inactivation may result from to attachment of the enzyme through its active site (Figure 11) or in case of multipoint attachment enzyme cannot perform conformational changes which is essential for catalysis.[81]

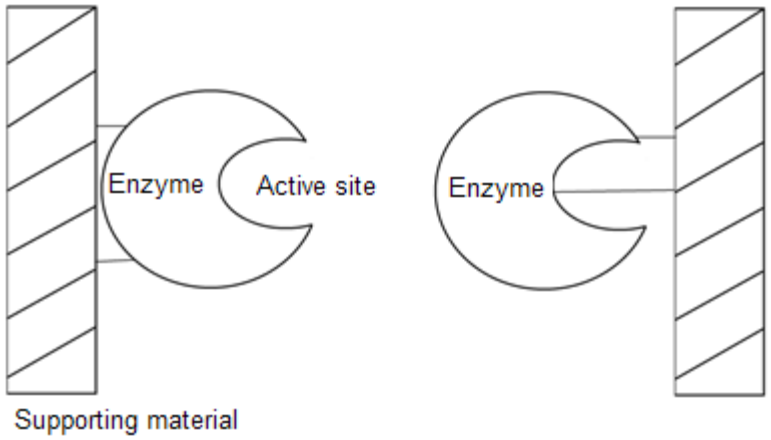


Figure 11. Inactivation of enzyme through covalent bonding

Commercially available covalently binding supporting material usually contain an epoxy group on its surface which interacts with the enzyme amine group. A simple conversion of the epoxy group to an amine group results in mechanical matrices containing amines on their surfaces; amines can then interact with the enzyme through a glutaraldehyde linker or directly interact with a previously oxidized enzyme, Figure 12.[81,82]

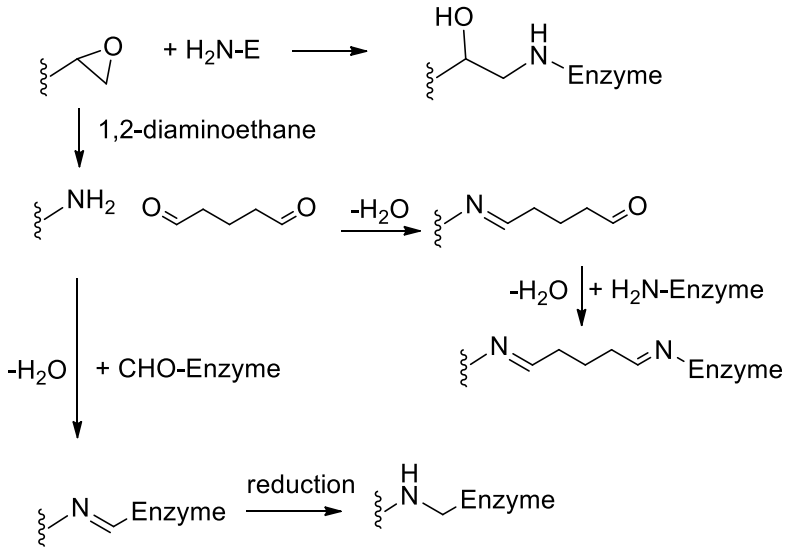


Figure 12. Different methods developed to covalently immobilize proteins.[81, 82]

Cross linking operates by the same mechanism as covalent binding, however this time enzyme proteins are linked to each other with no solid support. However, it is rarely utilized on its own but rather in combination with other techniques. Cross-linked enzyme crystals (CLECs) are protein crystals that are reinforced by crosslinking. The most common reagent utilized for crosslinking is glutaraldehyde.[81] Lipase B has very few lysine residues on its surface thus it is not a good substrate for covalent binding.[81]

Surfactants are often used with lipase preparation. It is known that adding a surfactant increase lipases activity, probably by increasing the ratio of lipases present in the active, open conformation. Besides increasing lipases activity, surfactants can be added to lipase proteins solely to increase enzyme stability in organic solvents.

Although not as effective as the other techniques lipases can be utilized in the form of hydrophobic ion pairs in organic solvents. The most common surfactant used to

manufacture these ion pairs is Aerosol OT.[81, 83] Span 60 is a surfactant can be used to form lipase microemulsions or surfactant-coated liapases.[139] Surfactants may inactivate the enzyme, thus the choice of the appropriate surfactant for lipase coating is extremely important. Certain surfactants may contain ester bonds which might be hydrolyzed by the lipase itself.[81]

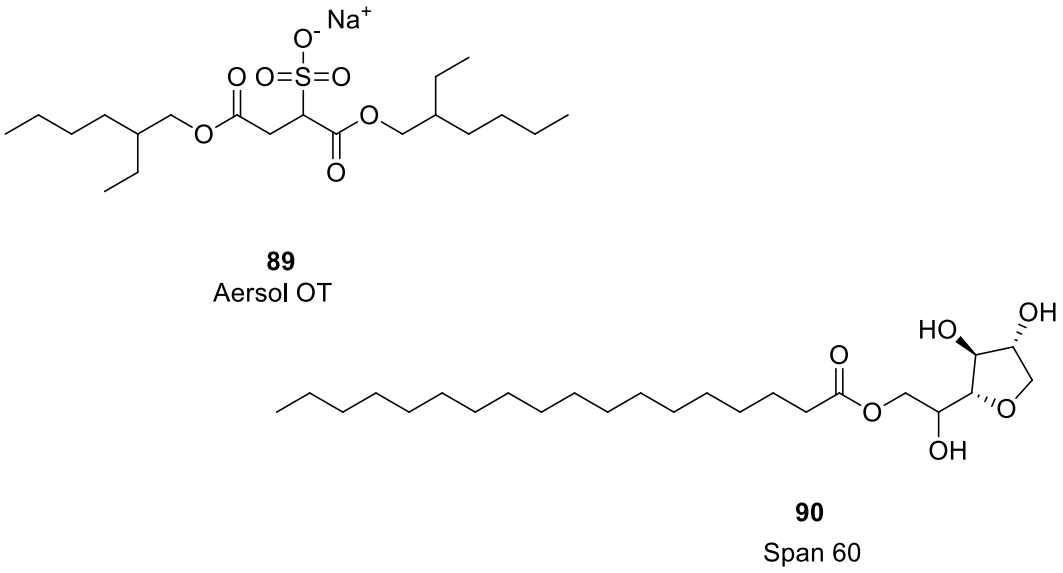


Figure 13. Aersol OT used to form ion pair, span 60 used for coating lipases.[81, 83, 139]

Usually the efficiency of certain immobilization technique is evaluated by comparing the free enzyme against the immobilized one. Such a comparison is not useful because the immobilized enzyme will most likely exhibit better properties than the free enzyme specially in organic media. Very few studies compare different immobilization techniques against each other using the same enzyme. Adlercreutz and coworkers compared different immobilization techniques for thermostable lipase and their effect on catalyzed esterification of 1-phenylethanol with caproic acid.[84] As expected the specific activity of the free enzyme was very low. Freeze drying techniques yielded enzymes with

very low reactivity as well. Coating the enzyme with Aersol OT and Span 65 exhibited much lower activity compared to coating with Span 60. Sol-gel entrapment exhibited the second best specific activity while adsorption to polypropene matrix exhibited the highest activity, Figure 14.[84]

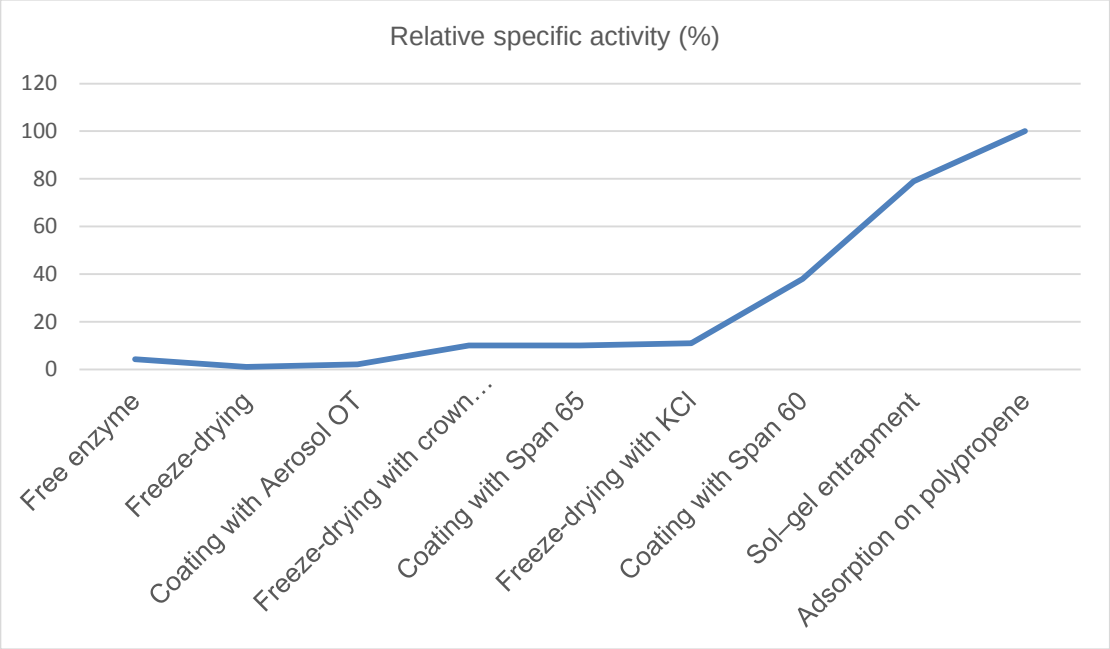
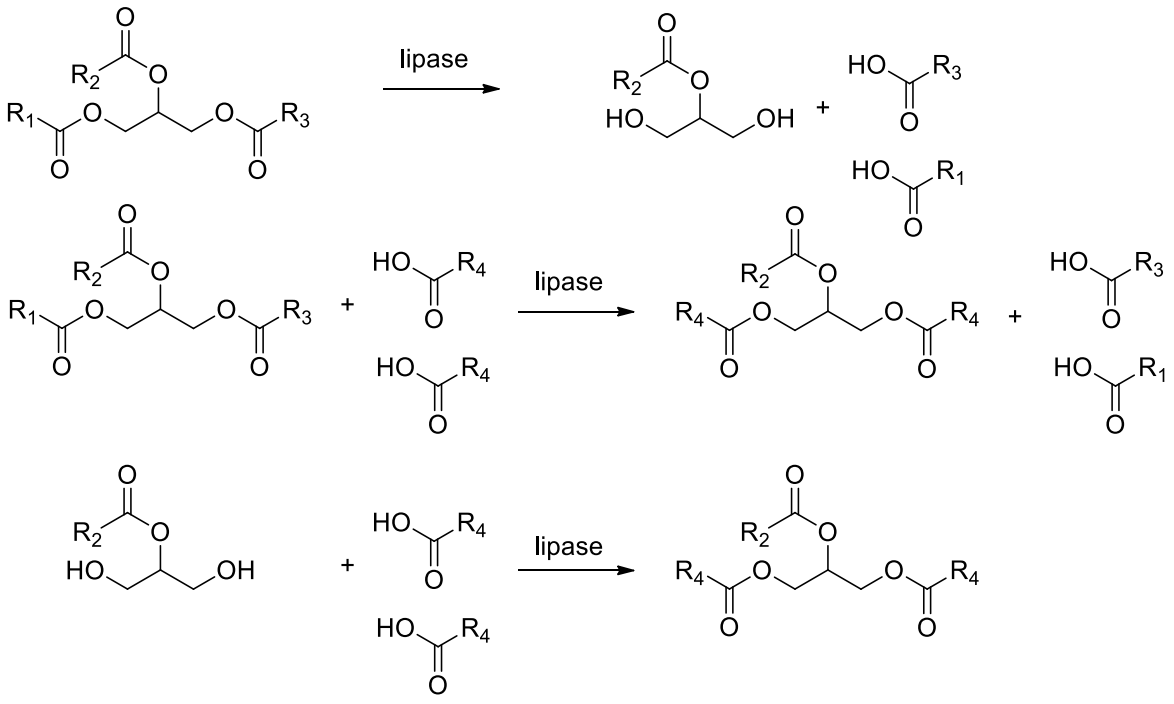


Figure 14. Comparison of immobilization techniques. [84]

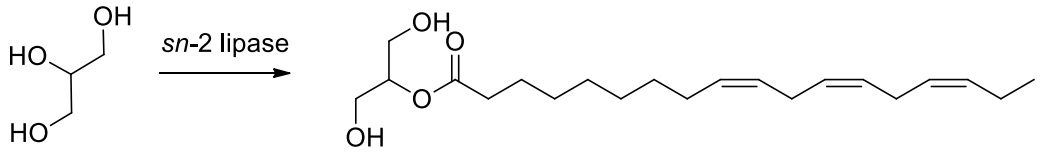
2.2.6 Industrial application of lipases

Lipases evolved over billions of years to maintain a single function, which is to process lipids. Therefore, it is not a surprise that lipases, biggest industrial application is fat processing. The fat industry is a multibillion dollar field; lipases are utilized to convert low value fats into higher value products. In the oil and fat industries lipase regiospecificity plays a major role. Some lipases are specific for acyl chains in the *sn*-1 and *sn*-3 positions which enables the controlled modification of triacylglycerols. Lipases have also shown specificity towards chain lengths and degree of saturation and can exhibit significant stereoselectivity which has been exploited in the manufacture of chiral triacylglycerols.[81]



Scheme 17. Lipases regioselective modification of triacylglycerols.[81]

Triacylglycerols are a major food component. One of the earliest example to produce modified triacylglycerols was the enzymatic manufacturing of coco butter. These modified triacylglycerols exhibited much higher quality and melting point. The lipases for this purpose were adsorbed on kieselguhr matrix; and the transesterfication reaction were done in petroleum ether as a solvent.[85] Modified lipids at the *sn*-1, 3 positions exhibit no health risks. In almost all cases fatty acids in the *sn*-1, 3 positions are hydrolysed before being uptaken by the body as 2-monoacylglycerols. Monoacylglycerols containing medically useful long chains omega fatty acids at the *sn*-2 position have been synthesized using regioselective *sn*-2 lipases, Scheme 18.[85]

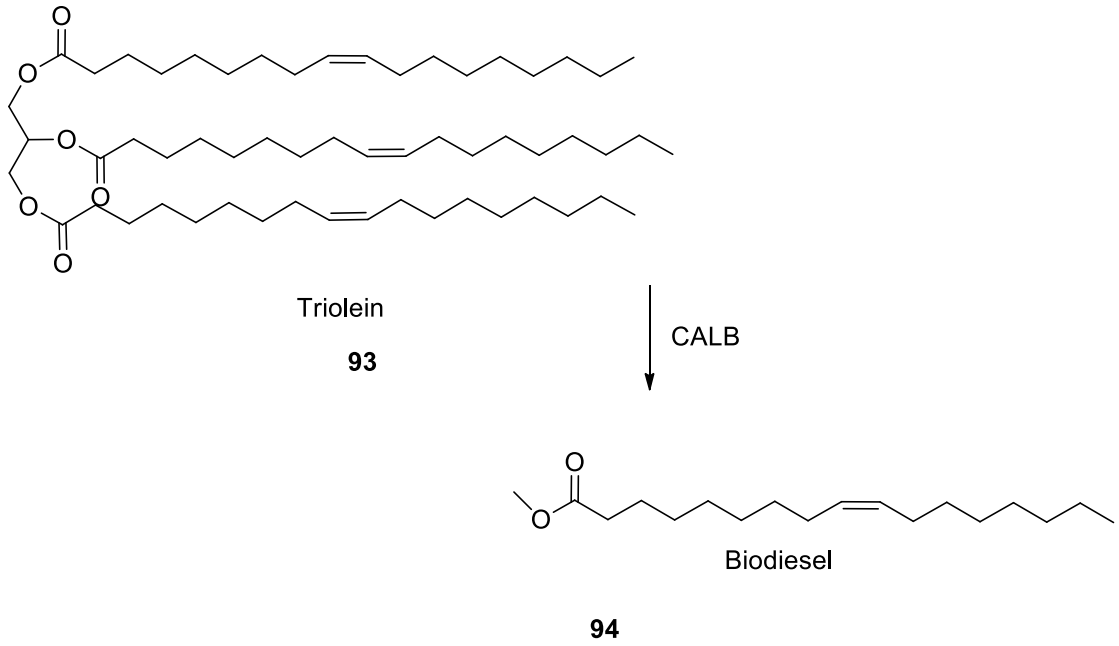


91 **92**

Scheme 18. Incorporation of long chain omega fatty acid using *sn*-2 lipases.

Lipases are widely used for interesterification of fats, especially in the margarine industry. Margarine manufacturing usually starts by mixing two ingredients, one of which has a high melting point and the other is low. Lipase mediated interesterification between fatty acids occurs till a homogenous product with desired melting point is achieved.[81] One of fastest growing fields nowadays is the production of sustainable biodiesels.. Combustible fuels usually constitute of methyl ester of fatty acids. Genetically modified plants are designed to produce triacylglycerols which are converted into the methyl esters of fatty acids, such conversion is usually performed through industrial acidic or alkaline catalysts that are fast, effective, and cheap. However, such conversion is not

environmentally benign and requires low water and a free fatty acid content. Lipases exhibit high transesterification rates for biodiesel **94** production. Immobilized lipases such as N435 and lipases from various *Pseudomonas* strains have been investigated for this purpose. CALB (Novozym-435®) can be applied directly to the Triolein **93** crude mixture even if it contains water, Scheme 19. [86] Lipases from *pseudomonas* strains can be deployed even when water content is up to 10%.[86]



Scheme 19. Production of biodiesel through lipases transesterification.[86]

Detergent industries are amongst the largest consumer of industrial enzymes. Besides being environmentally benign, enzymes provide milder cleaning conditions when compared to chemical detergents. Enzymatic detergents increase clothing and washing machines lifetimes, enhance fabric softness and do not produce static.[64] Lipases are used in detergents to lipolyse fats, it is rarely used alone and often in combinations of proteases, amylases and cellulases. Other significant industrial applications of lipases

include the paper industry, tea processing, biosensors, and medical diagnostics.[70, 75, 76]

2.2.7 Lipases in polymer synthesis

2.2.7.1 Introduction

Polyesters are the fourth most abundant natural biomacromolecules after nucleic acids, proteins, and polysaccharides. In modern civilisation polyesters are extremely important. Some of the commonly used polyesters are polybutylene succinate **95**, a biodegradable plastic; poly(ε-caprolactone) **96**, Poly(lactic acid) **97**, a biodegradable material, and polyethylene terephthalate **98**, a strong synthetic fiber, Figure 15.[87]

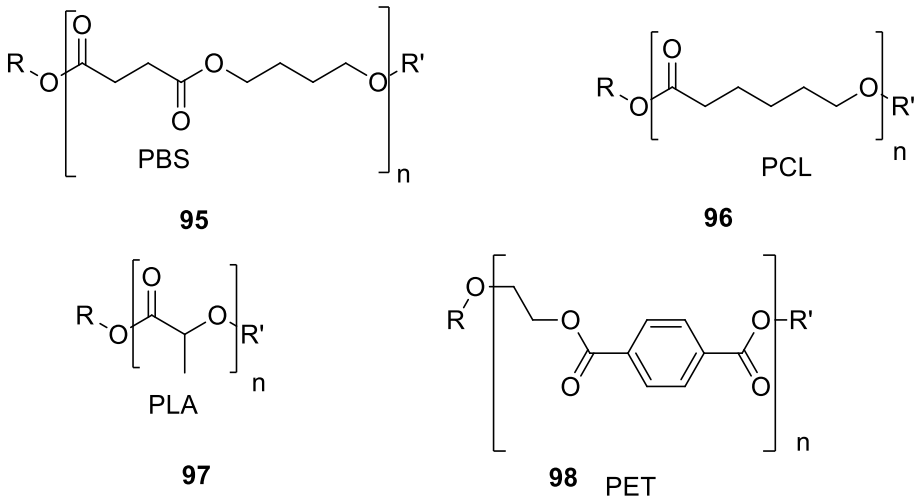
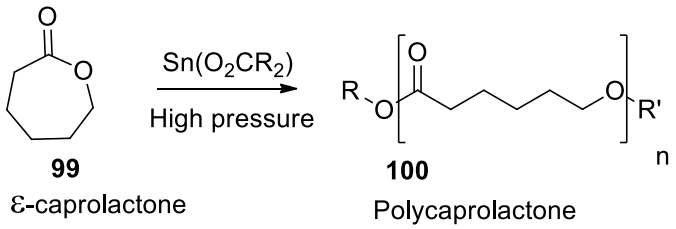


Figure 15. Example of widely used polyesters.[87, 140]

Industrially, polyesters are synthesized *via* condensation polymerization or ring-opening polymerization through the aid of toxic catalysts, Scheme 20. There is a huge demand for the establishment of sustainable and environmentally benign polyester production techniques, through the utilization of materials from renewable recourses and elimination

of the use of toxic catalysts.[87]



Scheme 20. Industrial synthesis of polyesters through ring-opening polymerization.[140]

In the early 1980s there were several reports concerning *in vivo* polymerization. Poly(hydroxyalkanoate)s were produced *via* living cell fermentation. Enzymatic polymerization did not receive much attention until Okumura in 1984 reported the *in vitro* synthesis of oligomers by lipase isolated from *Aspergillius niger* through a condensation reaction.[87] In 1993 two groups reported independently *in vitro* lipase catalyzed ring-opening polymerization, Scheme 21.[87] Since the early studies lipases have received great attention as green biocatalysts for various polymerization reactions. As discussed before, polymerization reactions are categorized into two main categories: condensation reactions and ring-opening reactions. Condensation may occur between different molecules of carboxylic acids and alcohols, or one molecule may contain both the alcohol and the acid functionalities. Ring-opening is usually performed on lactones or other cyclic monomers, Figure 16.[87] Naturally lipases perform ester bond hydrolysis and formation between glycerol and fatty acid; there are various reports that lipases are involved in sugar oxazoline polymerization *in vivo* to form natural polymers.[89] The mechanism by which the lipase operates involves ester hydrolysis if the nucleophile is water and transesterification if the nucleophile is alcohol.

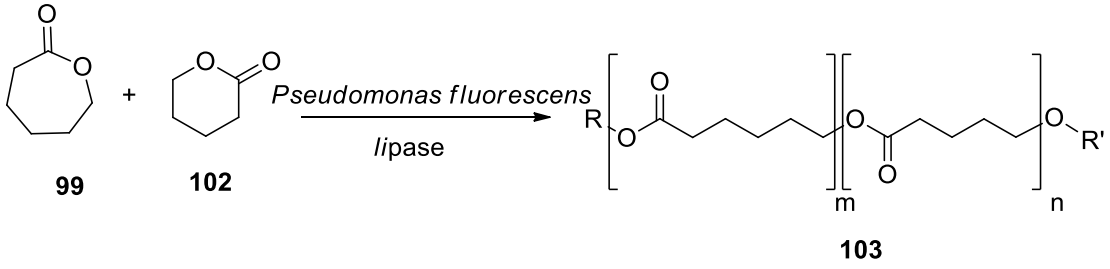
CALB is a commercially available as Novozym-435® is widely used in polymerization

reactions, due to its stability and high activity. Other lipases utilized for polymerization include lipases from *Candida cylindracea*, *Pseudomonas fluorescens* and *Aspergillus niger*.[87]

2.2.7.2 Lipase mediated ring-opening polymerization

Kobayashi and Kohn both reported ring-opening polymerization of ε-caprolactone **99**, a seven membered lactone, and δ-valerolactone **102**, a six membered lactone, through ring-opening polymerization using *Pseudomonas fluorescens* lipase.[90, 91] Polymerization of ε-caprolactone yielded a polymer of M_w of 7,700 g/mol and polydispersity index of 2.4.[90, 91] Polymerization of δ-valerolactone yielded a polymer of M_w of 1,900 g/mol and a polydispersity index of 3.0.[90, 91]

Kobayashi and coworkers successfully conducted a ring-opening copolymerization of ε-caprolactone and δ-valerolactone using *Pseudomonas fluorescens* lipase. The resulting copolymer had a M_w of 3700 g/mol and polydispersity index of 2.9 and a random arrangement, Scheme 21.[87, 96]



Scheme 21. Lipase-catalyzed ROP copolymerization of ε-caprolactone and δ-valerolactone.[87, 96]

Figure 16 shows various cyclic monomers and lactones that were successfully polymerized using microbial lipases.[87]

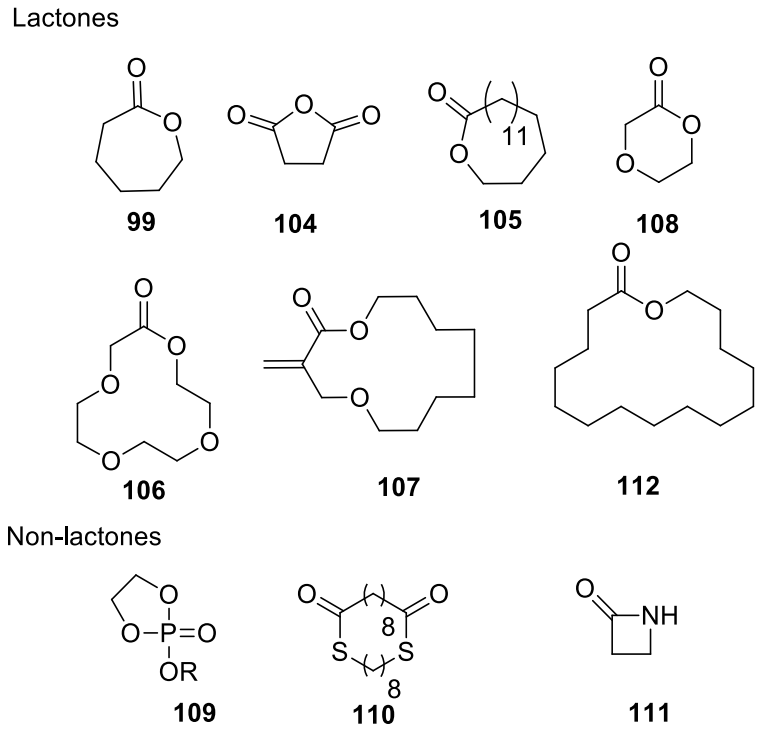
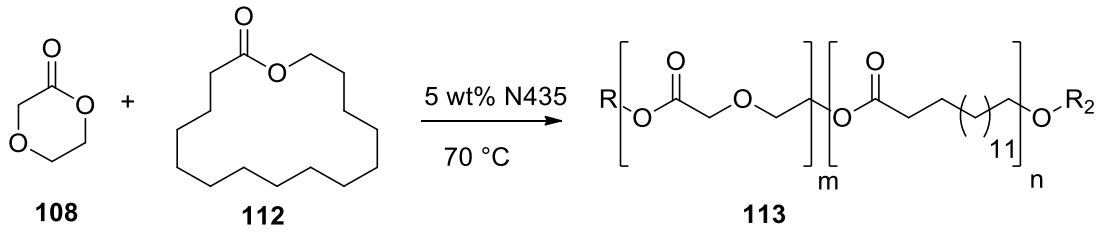


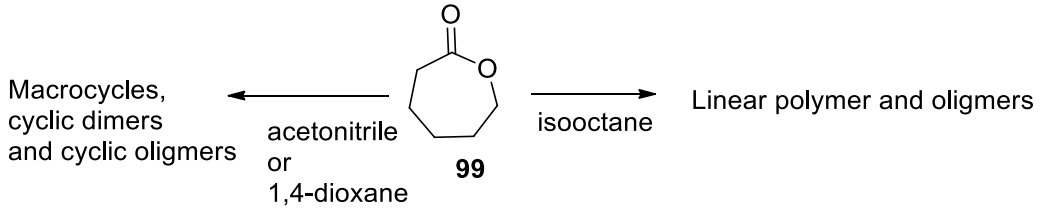
Figure 16. Various cyclic lactones and monomers that were successfully polymerized thorough enzymatic ring-opening polymerization.[87]

1,4-Dioxan-2-one **108** was successfully polymerized using lipase B from *Candida antarctica*. Polymers from 1,4-dioxan-2-one are highly desirable and they have various medical application.[87, 92] Metal-free polymerization, therefore, is an advantage as these polymers will later be utilized in biomedical applications. 1,4-Dioxan-2-one **108** was also copolymerized with ω-pentadecalactone **112** through enzymatic ring-opening polymerization at 70 °C catalyzed by 5 wt% N435 in dried toluene and diphenyl ether for 26 hours yielding copolymer **113** with M_w >30,000 g/mol.[87, 92]



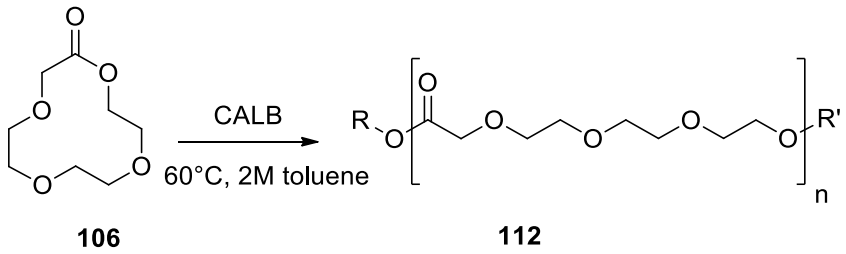
Scheme 22. 1,4-Dioxan-2-one enzymatic ring-opening polymerization.[87, 92]

Another interesting application of CALB is the work done on ring-opening polymerization of ε-caprolactone. Ring-opening polymerization ε-caprolactone **99** was performed using Novozym-435® a commercial version of CALB. When the reaction was performed in organic solvents such as *iso*octane, cyclic dimers of ε-caprolactone were formed in 2% yield, and linear polymer was formed in 98% yield. However, when the same reaction was performed in acetonitrile cyclic oligomers were produced up to 70% yield and only 30% of the starting material was converted to linear polymer. [87, 93]



Scheme 23. Ring-opening polymerization ε-caprolactone under various solvent conditions.[87, 90]

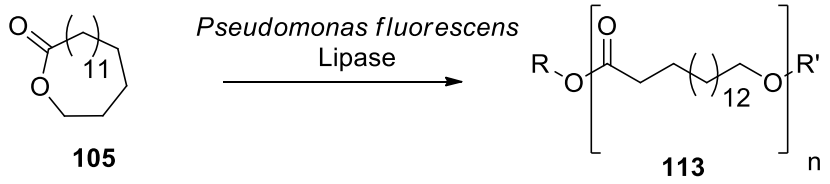
Twelve membered ring lactone 2-oxo-12-crown-4-ether **106** showed high polymerization rates with CALB at 60 °C in toluene, the poly(ester-ether) produced **112** had M_n value of 3500 g/mol and T_g value=40 °C and excellent water solubility (Scheme 24). [87, 94]



106 112

Scheme 24. Lipase catalyzed ring-opening polymerization of **106**. [87, 94]

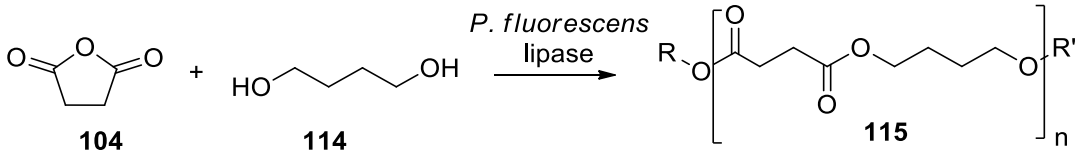
The seventeen-member lactone 16-hexadecanolide (HDL) **105** is the largest studied lactone subjected to lipase-mediated ring-opening polymerization. Lipases from *Candida antarctica, Candida cylindracea* and *Pseudomonas fluorescens* were successful in both bulk and organic solvent polymerization to yield polyHDL polymer **113** with M_n=5,800 g/mol and polydispersity index of nearly 2, Scheme 25. [87, 95]



105 113

Scheme 25. Enzymatic polyHDL polymer synthesis. [87, 95]

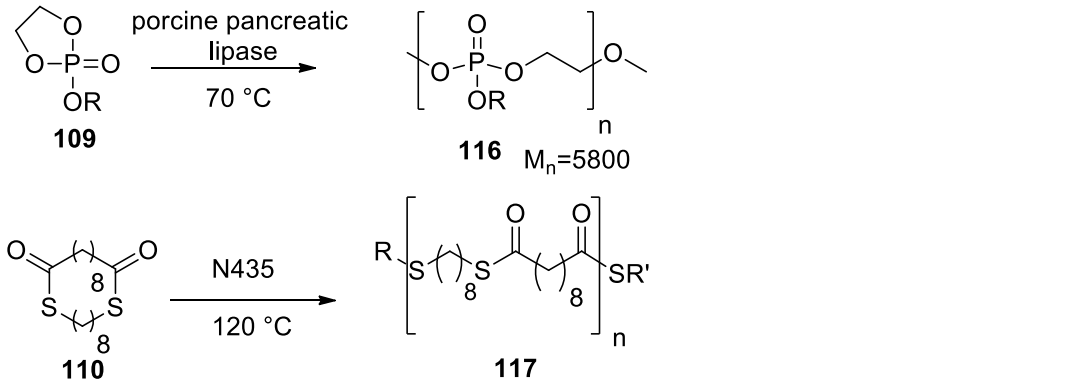
Beside lactones several other cyclic monomers were successfully polymerized. In 1993 Uyama and coworkers reported the ring-opening condensation copolymerization of acid anhydride **104** with glycol **114**, a reaction that was catalyzed by lipase from *Pseudomonas fluorescens* to yield polyester **115** with M_n=2,000 g/mol, Scheme 26.[97] Ten years later other anhydrides such as succinic, glutaric, and diglycolic anhydrides,were polymerized by CALB. [87, 98]



Scheme 26. Synthesis of polyesters from anhydrides. [87, 98]

It is worth mentioning that lipases also catalyze ring-opening polymerizations of various phosphorus and sulfur compounds. Polyphosphates have a wide range of application in biomedical applications and as retardants. Lipase mediated ring-opening polymerization of cyclic phosphates **109** was achieved in bulk utilizing silica-immobilized porcine pancreas lipase at 70°C, to produce polymer **116** that averaged M_n=5,800 g/mol.[99]

Polythioesters have been produced through a similar fashion. *Candida antarcatica* mediated ring-opening polymerization of the 18-membered cyclic monomer 1,6-hexanedithiol-sebacate **110** was achieved at 120 °C for 48 h to yield a polymer **117** of M_w= 12,000 g/mol, Scheme 27.[100] Interestingly monomer **110** was prepared using lipase from *Candida antarcatica* and the same lipase preparation was later used for polymerization.[87,100]



Scheme 27. Lipase mediated polymerization of thioesters and cyclic phosphates.[87, 99, 100]

2.2.7.3 Lipase catalyzed condensation reactions

The lipase-catalyzed polyester synthesis *via* condensation polymerization are divided into four categories: esterification through dehydration, transesterification through alcoholysis, acidolysis, and intermolecular esterification, Figure 17.[87]

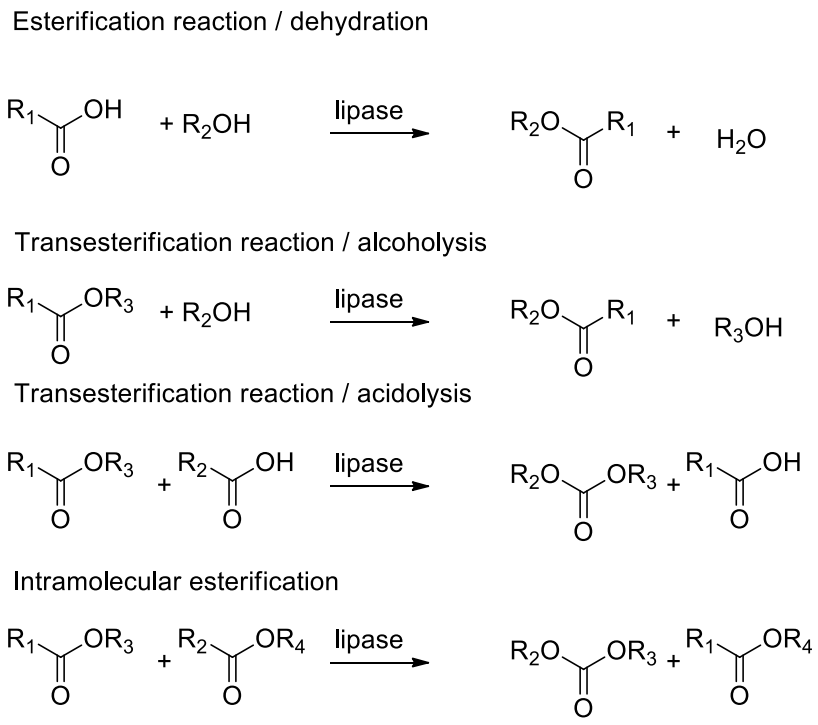
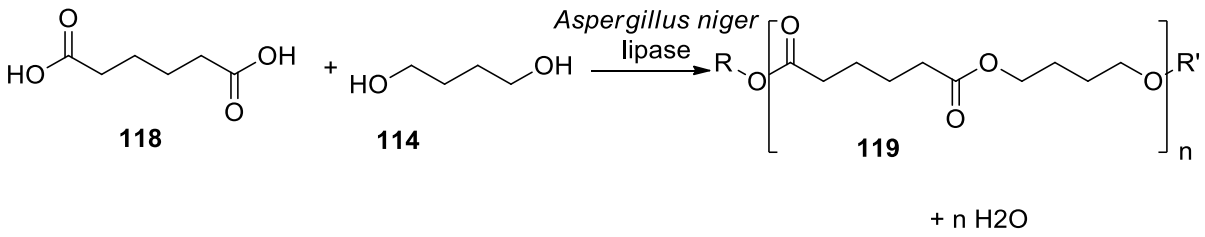


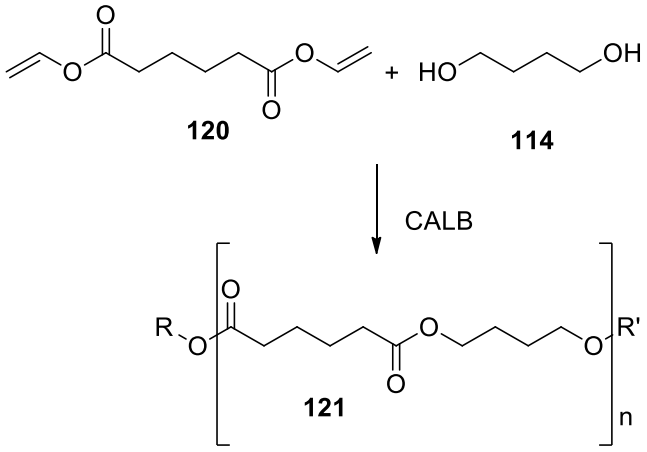
Figure 17. Four different types of lipase condensation reactions.[87]

The first example of a lipase-catalyzed condensation reaction was reported in 1984 by Tominaga, a simple dehydration polymerization of adipic acid **118** and 1,4-butanediol **114** in diisopropyl ether to produce oligomers **119** with degree of polymerization of 20. Tominaga first experimented with four different lipases and the best results were achieved with free lipase from *Aspergillus niger* at 30 °C.[88]



Scheme 28. Dehydration polymerization of adipic acid and 1,4-butanediol.[88]

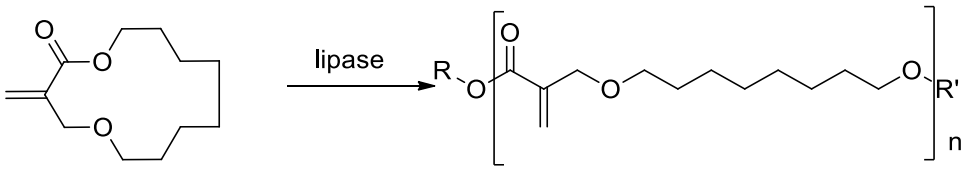
Supercritical carbon dioxide has been utilized as a solvent for lipase mediated polymerization.[101] Supercritical carbon dioxide is an inexpensive, inert, nontoxic, and nonflammable solvent. Supercritical carbon dioxide was used as a solvent for the lipase-catalyzed condensation of divinyl adipate **120** and 1,4-butanediol **114**. [101] *Candida antarctica* lipase was chosen as a biocatalyst and polymer **121** with M_n=39,000 g/mol was produced, Scheme 29.[87, 101]



Scheme 29. Condensation of divinyl adipate and 1,4-butanediol catalyzed by lipase with supercritical carbon dioxide as a solvent. [87, 101]

2.2.7.4 Selectively of lipase

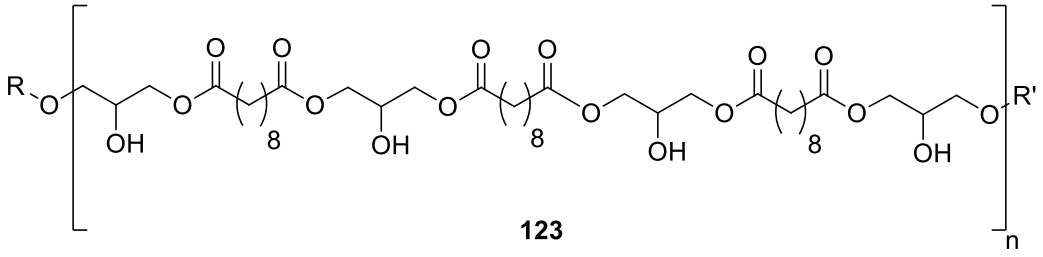
Lipases are highly chemoselective. For example, a lipase-catalyzed polymerization of 2-methylene-4-oxa-12-dodecanolide **107** yielded polymer **122** with the highly reactive exo-methylene group in the main chain, which is nearly impossible to achieve through traditional chemistry.[87, 102]



107 **122**

Scheme 30. Chemoselective activity of lipase during polymerization of 2-methylene-4-oxa-12-dodecanolide. [87, 102]

As discussed before lipases are highly regioselective. For example,
CALB-mediated polymerization of divinyl sebacate and glycerol yields a soluble polymer **123** with 1,3- diglyceride as the main unit constituting the polymer chain as shown by ^{1}H NMR analysis, Figure 18. [87, 103]

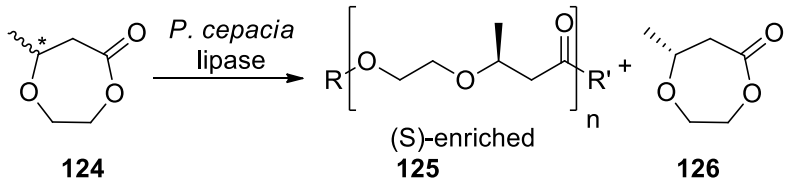


123

Figure 18. A branched polymer showing mainly 1,3-diglyceride connection. [87,103]

Candida antarctica lipase B is highly enantioselective, and therefore it is utilized in the synthesis of chiral polymers. CALB is often used for chiral resolution of monomeric units, or polymerization of already existing chiral monomers such as chiral sugars or amino acids.[87] CALB is widely used to manufacture chiral polymers through ring-opening polymerization. The earliest studies available about the enantioselective ring-opening polymerization was the production of optically active polymer through ring-opening of α methyl-β-propiolactone in toluene to produce an optically active (polymer with $[\alpha]_D^{25}$=+12 to +19. [87, 104]

Other related work includes ring-opening polymerization of racemic 3-methyl-4-oxa-6-hexanolide **124** catalyzed by lipase from *Pseudomonas cepacia*. The enantioselective lipase catalyzed the ring-opening polymerization of the (*S*)-isomer seven times faster than the (*R*)-isomer, leading to (*S*) enriched polymer **125** and residual (*R*)-isomer **126**.[87, 105]



Scheme 31. Enantioselective lipase catalyzed ring-opening polymerization of 3-methyl-4-oxa-6-hexanolide. [87, 105]

2.2.8 N435

Novozym-435® is an immobilized form of lipase B from *Candida antarctica*. Lipases from *Candida antarctica* were filed for patent at 1987 by Michiyo Ishii.[106] Ishii was researcher at Novo Nordisk, later acquired by Merck. His research aimed to identify thermally stable lipases, similar to lipases found in Icelandic hot springs. Ishii investigated the *Candida antarctica* species because of its ability to withstand harsh temperatures. In the original patent Ishii describes lipase A and lipase B as two nonspecific lipases that can function at temperatures as high as 60 °C and pH values between 7 and 8. Ishii reported that lipase A is the more thermostable, and lipase B is more alkali-resistant.[106] N435 can withstand temperatures almost double that of the free enzyme and is highly resistant to denaturation. By immobilizing CALB on acrylic resin it has enhanced enzyme activity, selectivity, stability, and reusability in organic solvents. CALA is less specific than CALB, and it has a unique activity towards the utilization of highly branched substrates where other lipases fail to utilize such branched compounds. CALB consists of 317 amino acid residues and has an α/β hydrolase fold. CALB possesses a tight and small active site, it contains a stereospecific pocket in which secondary alcohols have to direct one substituent to fit in that pocket.[126] Therefore CALB is highly enantioselective towards chiral secondary alcohols. Kazlauskas' rule generally predict which secondary alcohol react faster in reaction catalyzed by lipase B. In case of bulky substituents the *R*-enantiomer reacts faster than the *S*-enantiomer.[129] Recent molecular dynamic studies that combine enzyme kinetics with molecular modeling can accurately predict the fast reacting enantiomer.[130]

2.3 Silicone containing copolymers

2.3.1 Introduction

Silicone is a general term often given to polymeric materials with a Si-O backbone, with two monovalent organic groups attached to each silicon atom. The O-Si-O unit is always referred to as a "siloxane". Since these polymers constitute organic and inorganic materials, they offer an amazing set of properties that cannot be achieved through organic or inorganic polymers on their own. In general such polymers usually possess high flexibility, low glass transition temperatures, and excellent thermal and oxidative stability.[110] Silicone-containing polymers usually have very low surface tension with values ranges around 21–22 mN/m.[107] Added together these properties makes silicones highly biocompatible, therefore they have massive applications in biomedical research. It was also found that commonly known polymers such as PVC, when enriched with polydimethylsiloxane results in significant increase in the biocompatibility compared to the original polymers.[110]

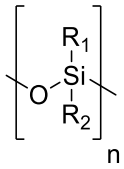


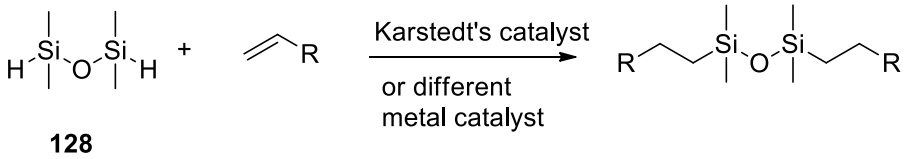
Figure 19. General siloxane polymer backbone.[110]

The difference between the Si-O bond and the organic C-O bond contributes to silicones unique characteristics. Theoretically an ideal C-O bond length is 1.43 Å and a Si-O bond length is 1.83 Å. [109, 110] However, X-ray crystallography shows that average C-O bond is 1.426 Å which is very close to theoretical value of 1.43 Å ,but that the average Si-O bond is 1.622 Å significantly deviating from the theoretical value.[109, 110] The difference between the theoretical and actual value can be attributed to the high ionic character of the Si-O bond due to the significant electronegativity difference between silicon and oxygen atoms. Moreover the Si-O bond has partial double bond character.[109, 110] This phenomena can be explained by examining the size and the electronegativity of Si, O and C atoms.[109, 110]

The atomic radius of the carbon atom is 0.77 Å and atomic radius of silicon atom is 1.17 Å and oxygen being the smallest in size has a radius of 0.66 Å. Oxygen has a high Pauling electronegativity value of 3.5 while carbon and silicon have values of 2.5 and 1.7 respectively. [109,110]

The double bond character enhances the thermal stability of siloxanes while the partial ionic character provides significant flexibility to the system.[110]

Siloxanes are commonly used in an addition reaction of a hydrosilyl group to a vinyl or allyl-terminated compound. This reaction known as hydrosilylation, is usually catalyzed by metal complexes of platinum, palladium, or rhodium, Scheme 32. [109,110]



Scheme 32. General scheme for hydrosilylation reactions. [109, 110]

2.3.2 Silicone copolymers

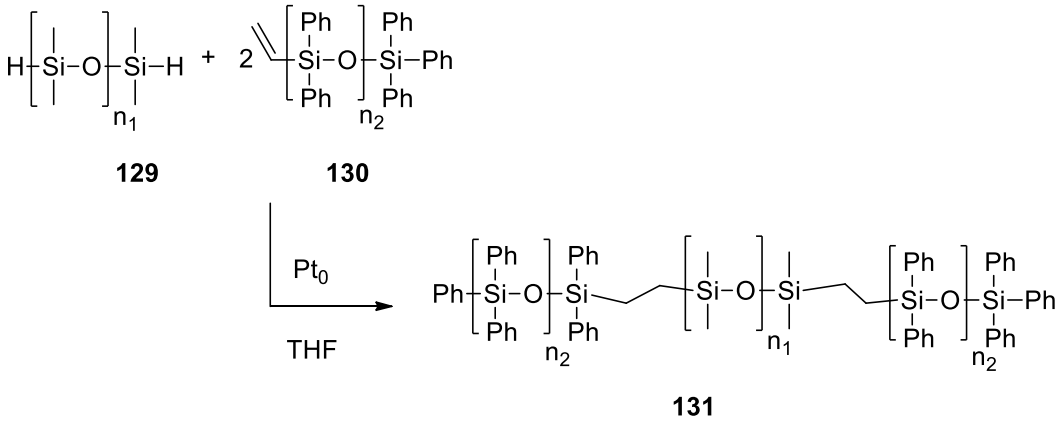
Copolymers are polymers derived from one or more monomer units. Homopolymers, on the other hand, are polymers that are derived from only one monomer unit. Copolymers are fairly common; nitrile rubber is a commonly used copolymer of 2-propenenitrile, 1,2-butadiene, and 1,3-butadiene. Each of those monomers will independently result in homopolymers that do not possess the tensile or thermal stability of the copolymer. Copolymers are divided into several categories, Table 1.[111]

	Description	Structure
Alternating copolymers	Monomeric units alternate in regular fashion.	A-B- A-B- A-B- A-B
Periodic copolymers	Monomeric units are arranged in a fixed repetitive sequence.	A-A-A-B-B-A-A-A-BB
Statistical copolymers	monomeric units are arranged according to certain statistical influences.	Various structures, may considered random
Block copolymers	Block copolymer usually refer to two or more homopolymers fused together. Graft copolymers are block copolymers where the side chains of certain polymers are different than the main chain.	Graft Copolymer: A-$[A]_n$-A-B-$[B]_n$-B-A-$[A]_n$-A

Table 1. General differences between various copolymers.[111]

The interest behind creating silicone copolymers aims to integrate silicone surface properties with the mechanical integrity of an organic polymers.[110]

One of the most favoured routes to fuse silicone based polymeric blocks is the hydrosilylation reaction. For example homopolymers were fabricated first through polymerization of hexamethylcyclotrisiloxane and hexaphenylcyclotrisiloxane independently to yield two distinctive homopolymers **129** and **130**.[112] The two polymers Polydimethylsiloxane (PDMS) **129** and Polydiphenylsiloxane (PDPS) **130** are later fused through a Pt-catalyzed hydrosilylation to yield a distinctive triblock graft copolymer **131**, Scheme 33.[112]

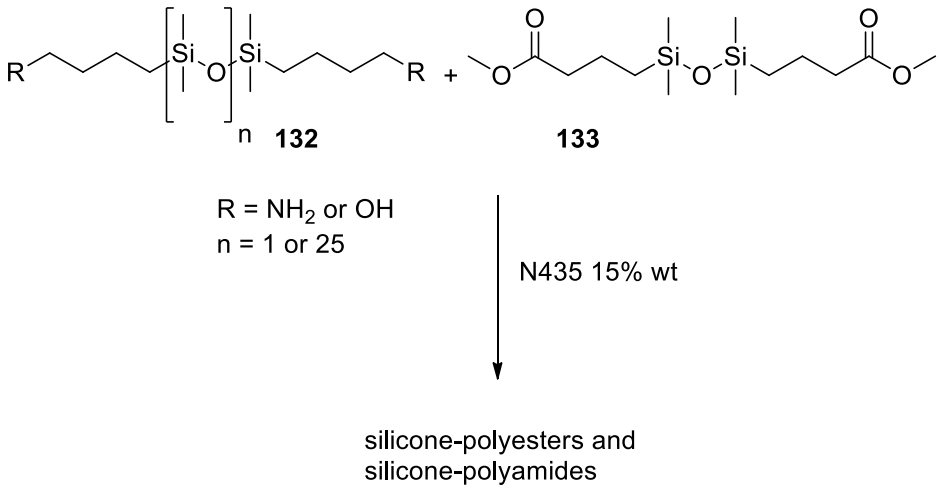


Scheme 33. Preparation of triblock copolymer via Pt-catalyzed hydrosilylation.

2.3.3 Lipase B and silicone polyesters

Gross and coworkers were first to investigate lipase B mediated catalysis in the synthesis of siloxane-based polymers.[128] As discussed before, organic-silicone copolymers are highly beneficial as they possess silicone outstanding surface properties and the stability of a traditional organic polymer. However, these polymers are synthesized under harsh

reaction conditions which may lead to uncontrolled redistribution of the siloxane backbone and side reactions.[128] Gross reported the synthesis of polyesteramides with M_n= 7,000 g/mol using N435 as a catalyst at 70 °C.[128] Zelisko and coworkers reported the efficiency and reusability of N435 in the synthesis of disiloxane-containing polyesters.[116] N435 exhibited high catalytic activity and can be reused up to ten times in separate reactions without significant drop in efficiency. The excellent reusability of N435 opens the door for further industrial applications.[116] Moreover, Zelisko reported the synthesis of various disiloxane and polysiloxane polyesters and polyamides utilizing N435, Scheme 34.[68] Moreover through the course of their investigation it was proven that high enzymatic esterification rates can be achieved at temperatures as high as 130 °C, a temperature way above the denaturation temperature of the free enzyme.[68] High conversion rates were associated with both low and high molecular weight acyl acceptor proving the versatility of N435. A Polysiloxane polyester was produced up to 25,650 g/mol at a reaction temperature of 110 °C. Disiloxane polyesters were produced at 4,000 g/mol at a temperature of 100 °C.[68]



Scheme 34. Lipase-mediated production of silicone polyester and silicone polyamides.[68]

3. Results and discussion

3.1 Introduction

In the world of polymer science momentous efforts have been devoted to synthesizing chiral polymers. Polymers containing heteroatoms in their main chain have been widely reported, however, only few of those are optically active. [7] Optically active polymers are immensely different from optically inactive polymers, possessing a wide range of characteristics that are not present in optically inactive polymers. Optically active polymers containing silicon are very rare and potentially extremely useful. The pharmaceutical industry is moving towards non-racemic medicines; optically active silicon polymers, therefore, can be utilized as either stationary phases for the separation and purification of chiral drugs or as drug delivery vehicles. In addition to the drug industry, optically active silicon polymers have other industrial applications in material science such as ferroelectric liquid crystals, nonlinear optical assemblies and data storage.[7] A practical approach to produce chiral polymers is by simply integrating chiral units into the polymer chain such technique is commonly used, for example by Chujo in and coworkers in 2007 introduced sterogenic phosphorus centers to optically inactive materials to produce an optically active polymer **134**.[115]

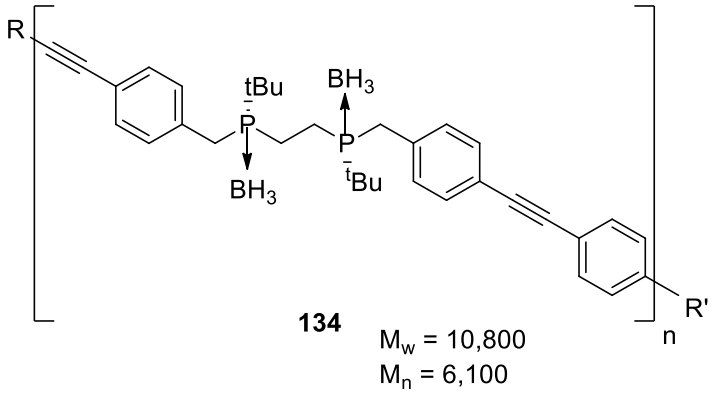
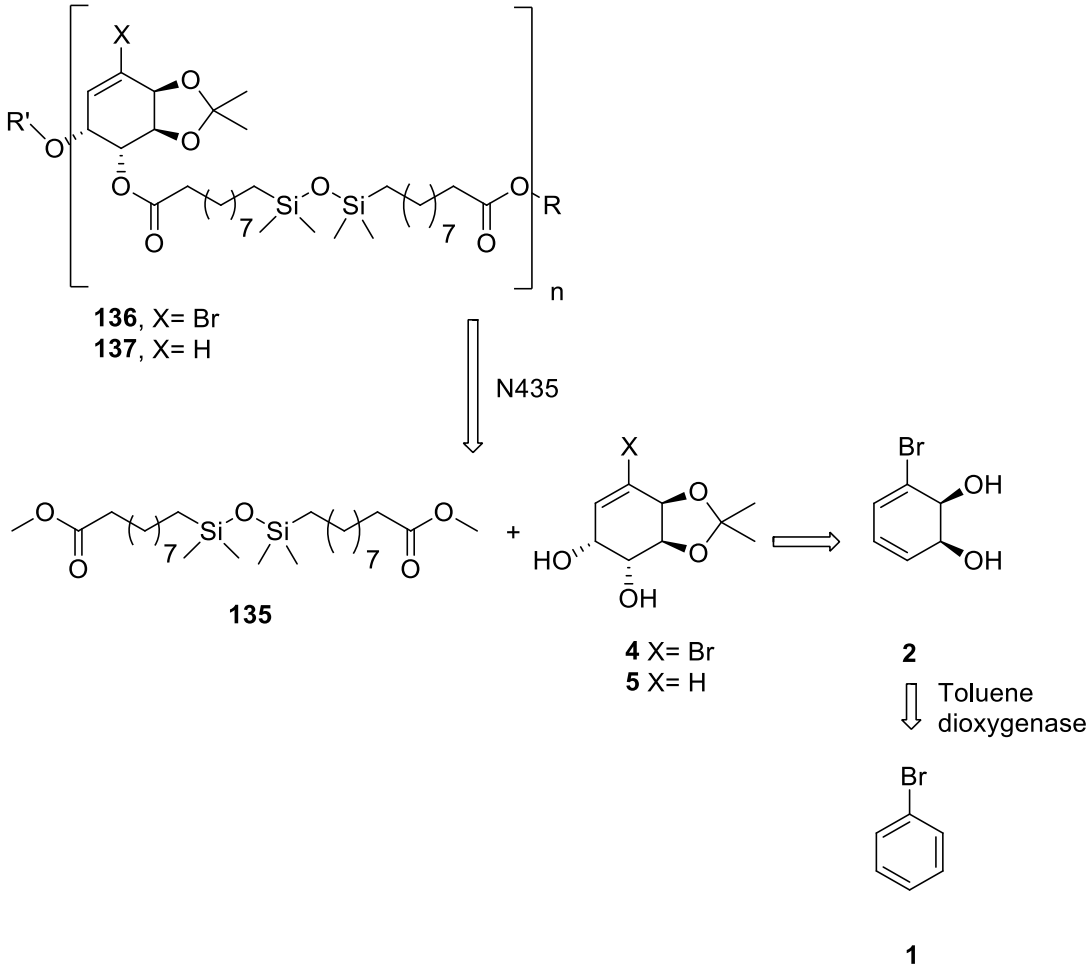


Figure 20. An example of an optically active polymer possessing a sterogenic phosphorus center.[115]

The research presented here aims to achieve two main goals. First, to synthesize several chiral monomers and incorporate them with silicon-based linkers in an attempt to produce optically active silicone polymers. The chiral monomeric units are derived from enantiopure arene-*cis*-dihydrodiols. In our lab the enantiopure arene-*cis*-dihydrodiols are obtained through microbial oxidation of simple aromatics by the genetically engineered strain *Escherichia coli* JM109 (pDTG601) that over-expresses the toluene dioxygenase enzyme cable of such chemical conversion. Transsterification is catalyzed by the environmentally benign lipase B enzyme from *Candida antarctica*, a commercially available immobilized enzyme (N435). Various conditions have been tested for the polymerization reactions to determine the most productive method and the most suitable monomeric unit. The second part of this research aims to closely investigate the preference of the immobilized lipase B enzyme for each of the secondary alcohols in the chiral monomeric diols, through closely investigating the polymerization reaction as well as synthesizing N435 test substrates with only one free secondary alcohol and the other

alcohol is blocked by a methyl group, to investigate lipase transesterification once one alcohol moiety is blocked.



Scheme 35. Retrosynthetic analysis for the production of optically active silicon-containing polymers.

3.2 Synthesis of the monomers

3.2.1 Production of the arene-*cis*-dihydrodiols

The enantiopure arene-*cis*-dihydrodiols are produced in our lab through a highly efficient medium-scale fermentation with *Escherichia coli* JM109 (pDTG601). As discussed in the historical section, Gibson first reported the production of arene-*cis*-dihydrodiols by fermentation with the blocked mutant *P. putida* 39D in 1970 a genetically engineered strain *Escherichia coli* JM109 (pDTG601) is advantageous for two reasons: *Escherichia coli* JM109 (pDTG601) contains a plasmid that encodes for the aromatic dioxygenase which means that enzyme production can be up regulated by placing several copies of the plasmid; the second reason is that the *P. putida* 39D requires induction of protein synthesis using chlorobenzene or toluene which problematically interfere with other aromatic substrate screening as arene-*cis*-dihydro diols generated from the inducer must be separated from the arene-*cis*-dihydro diols produced from the screened substrates. The exact protocol for efficiently producing arene-*cis*-dihydrodiols through whole cell fermentation was optimized in the Hudlický group 12 years ago;[117] a similar protocol is still used to this day. Bromobenzene is the best substrate discovered so far for aromatic dihydroxylation through toluene dioxygenase in terms of yields. One medium scale whole cell fermentation of bromobenzene **1**(10 -15 L) can produce up to 90-120 grams of the corresponding arene-*cis*-dihydrodiols **2**. The arene-*cis*-dihydrodiols produced exhibit significant chemical stability, for those two reasons bromobenzene metabolic dihydroxylation was chosen as the starting material for chiral monomers production. The process starts by culturing a previously thawed *E. coli* JM 109 (pDTG601) on agar

plates consisting of regular bactoagar, yeast extract and the antibiotic ampicillin. *E. coli* JM 109 (pDTG601) is engineered to be an ampicillan resistant species, thus ampicillan helps eliminate other undesired microorganisms. The streaked agar plates are stored at an incubator for two days. Two separate cell colonies are later chosen for the preparation of the preculture.

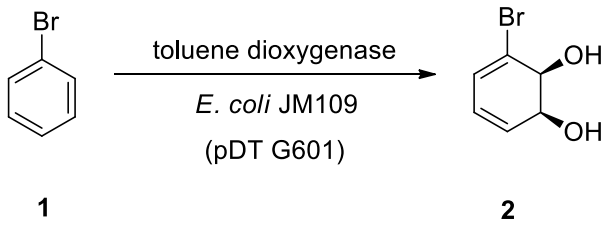
Each of the chosen cell colonies is placed in a two liter fernbach flask, which has been previously sterilized using an autoclave. Glucose, yeast extract, buffer salts, and various mineral salts are added to the growing preculture. The preculture is allowed to grow in an orbital shaker at 35°C for twelve hours.

As the preculture is allowed to grow, the fermentor is sterilized and charged with the production medium which consists of monopotassium phosphate, citric acid, ferric ammonium sulphate, magnesium sulfate and a solution of trace metals. The fermenter is allowed to cool, thiamine hydrochloride and ampicillan is added to the solution.

Based on visual inspection, only one of the precultures is transferred to the fermenter. pH is adjusted using ammonium hydroxide. A sample is taken immediately after the preculture is added to the fermenter to act as a blank reference to monitor any change in optical density. Glucose is added to the growing culture and the addition rate is increased as the cells multiply. As more glucose is added the more oxygen is consumed. The oxygen level is maintained by controlling the stirring in the fermenter; faster stirring is translates to higher oxygen levels. Bacteria continue to grow leading to the decrease of oxygen levels. When the optical density of the culture is 15 times the initial value, β-isopropylthiogalactopyranoside (IPTG) is added to induce the toluene dioxgyenase production.

The pH is adjusted to 7.0 and another sample is taken to act as an optical density reference before adding the aromatic substrate. The production of the arene-*cis*-dihydrodiol is monitored through measurement of the corresponding chromophore absorbance at 275 nm. Different aromatic substrates are added at different rates; in this case the rate of addition of bromobenzene is 35 g h^{-1}.[117] If substrate is added too fast bacterial cells will die and thus an optimum rate of addition is required. An antifoam is added to the culture to prevent foaming which can cause blockage of the tubing and interfere with the fermentation monitoring. Bromobenzene is usually added over 3 hours of fermentation. The substrate addition is stopped when the optical density measurement indicates no more diols are produced.

After the stopping the feeding the pH is adjusted to the slightly basic pH 7.6, the broth is later centrifuged and the cell mass is later autoclaved for safe disposal. The supernatant is extracted with equivalent amount of EtOAC through three physical extractions. The organic solution is washed with basic Na_2CO_3 solution to eliminate trace amounts of acids and phenols. The organic layer is later dried over pellets of sodium sulfate, filtered and then evaporated under reduced pressure. On average each fermentation produces 90-120 grams of the corresponding diol **2**. The produced diol **2** is re-crystallized from EtOAc/hexanes and physical data are compared to literature values.[39]



Scheme 36. Production of diol **2** by live cell fermentation.

3.2.2 Synthesis of the chiral monomers

The enantiopure arene-*cis*-dihydrodiol **2** produced through the toluene dioxygenase mediated biotransformation is utilized in the synthesis of several chiral monomeric units **4**, **5** and **6**. Chiral silicone-based polymers can be achieved by insertion of asymmetric chiral units into the polymeric framework.

Through the course of this research optically active chiral monomers **4**, **5**, and **6** derived from the enantiopure arene-*cis*-dihydrodiol **2** are utilized in polymerization reaction with optically inactive disiloxanes in order to produce optically active copolymers.

This subchapter describes the synthesis of the chiral monomeric units.

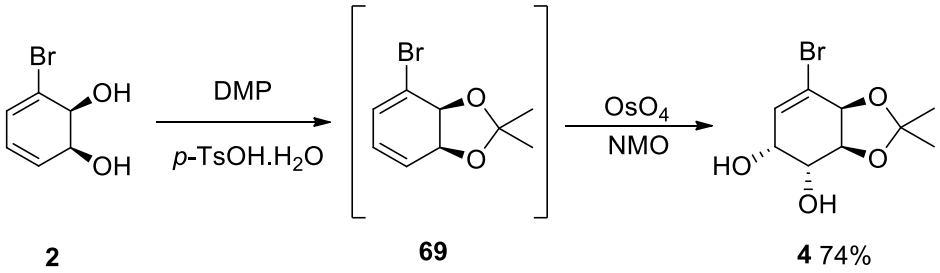
The first chiral monomer was synthesized before by Hudlický, Price, Rulin and Tsunoda in 1990.[118] This molecule was a key intermediate in an enantiodivergent synthesis of (+)-pinitol. The pinitol synthesis utilizes enantiopure arene-*cis*-dihydrodiols as the source of chirality; with special care to symmetry the arene-*cis*-dihydrodiol **2** can be converted to either pinitol enantiomers.

Diol **2** is dissolved in 2,2-dimethoxypropane which was treated with catalytic *p*-toluenesulfonic acid. The reaction usually runs for 10-15 minutes, after which time the reaction mixture is quenched with sodium bicarbonate and then concentrated under reduced pressure. The residue is then extracted three times using EtOAc.

The separated organic layer is base washed, dried using sodium sulphate, filtered then concentrated under reduced pressure.

Acetonide **69** is cooled to 0 °C dissolved in water and acetone mixture and subjected to Upjohn dihydroxylation conditions using catalytic OsO_4 and stoichiometric *N*-methylmorpholine *N*-oxide as an oxidant. The only modification applied to the original

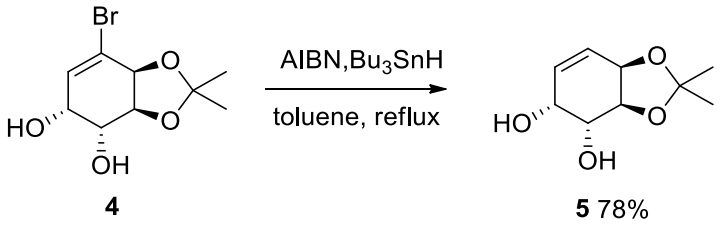
protocol is the addition of 1-2 mL of isopropanol which increases the stability of the diene **69** decreasing the amount of the resulting Diels-Alder side product or phenol. Yields for this reaction are very good, reaction time averages 72 hours.



2 **69** **4** 74%

Scheme 37. Synthesis of diol **4** through upjohn dihydroxylation.

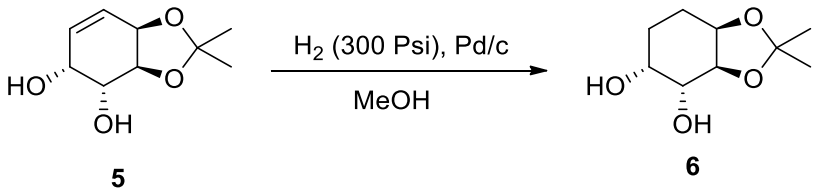
The second chiral monomeric unit is simply the debrominated analogue of diol **4**. Tributyltin hydride is used for this reduction. Tributyltin hydride is an excellent reducing agent for carbon-bromine bonds, due to the relativity weak bond between tin and hydrogen compared to the strong bond between carbon and hydrogen. The resulting diol **5** is produced in relatively high yields, up to 92%. However handling such reactions can be very challenging due to the high toxicity and fat solubility of tin adducts. The purification of such compounds by column chromatography results in contaminated glassware and silica. A much easier way to work up such reaction is to perform a hexane/acetonitrile extraction where the desired diol **5** is extracted by acetonitrile while the lipophilic tin adducts are dissolved in hexanes. Both **4** and **5** are re-crystallized from EtOAc/hexanes and their physical data are matched to their literature values.



4 **5** 78%

Scheme 38. Synthesis of diol **5** through tin mediated dehalogenation.

Diol **6** is the fully saturated analogue of diol **5**, and is prepared through Pd-catalyzed hydrogenation. The hydrogenation of the double bond in diol **5** was only successful under 300 psi of H_2 using MeOH as a solvent. High pressure hydrogenation was required as both faces of the molecules are blocked by oxygen atoms. Diol **6** was also re-crystallized from EtOAc/hexanes. All the diols are purified and sent for combustion analysis for purity confirmation before being utilized in enzyme-meditated polymerization.



5 **6**

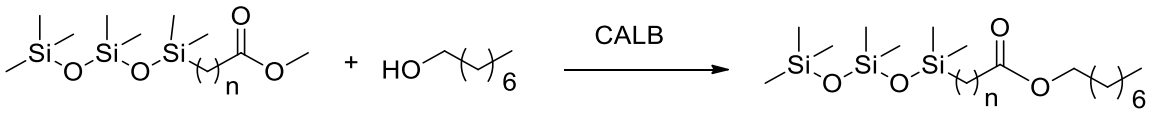
Scheme 39. Synthesis of diol **6** through Pd catalyst hydrogenation.

Solvent	Pressure (Psi)	Pd 10 %/C (catalyst loading)	Yield
MeOH	300	10%	69%
EtOH	300	5%	-
MeOH	30	10%	10-15%
EtOH	30	10%	-
EtOAc	30	10%	-
MeOH/EtOAc (1:1)	30	10%	5%

Table 2. Various reaction conditions and results for diol **6** synthesis.

3.2.3 Synthesis of the disiloxane monomer

In 2014 Frampton and Zelisko published a comprehensive report about chain length selectivity during the polycondenstation of siloxanes containing esters and alcohols.[119] This research combined with previous investigations from the same group indicates a very clear trend about the effect of chain length and the distance between the ester and the siloxane moiety on the rate of the enzymatic condensation reaction by lipase B. Trisiloxane esters containing two to five methylene units were least favoured by the enzyme and it was clearly noted that increasing the number of carbons between the silicon atoms and the esters group lead to higher conversion rates.[119] Which indicates that the degree of transesterification increases with the increase of the chain length of the trisiloxanes acyl donor, this probably occurs because longer chains place the bulky trisiloxane group outside of the enzyme active site, thus improving the degree of transesterification.[119]



138 n=5 Conversion=13±1
139 n=6 Conversion=35±4
140 n=7 Conversion=43±1
141 n=8 Conversion=73±6

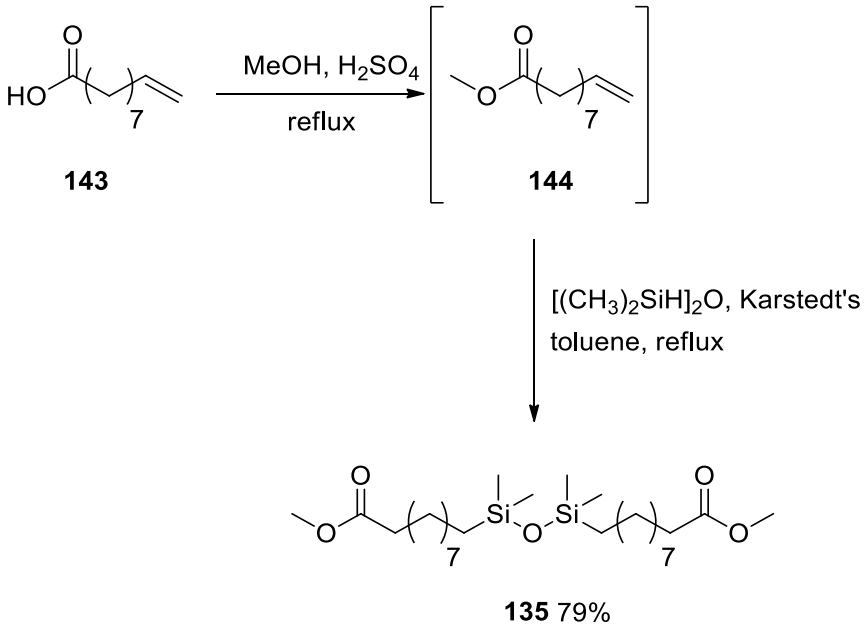
142

Scheme 40. Chain length selectivity during the polycondenstation of siloxane containing esters and alcohols.[119]

Since the aim of this project is to synthesize a siloxane-based polymer through lipase B mediated catalysis, the disiloxane monomer must be designed to achieve good active site compatibility, which can be achieved by increasing the chain arm length by placing nine

methylene carbons between the disiloxane and the two terminal ester groups.

The starting material is the commercially available 9-decenoic acid, was subjected to a Fischer-Speier esterification through refluxing in methanol in presence of H_2SO_4 to produce the methyl dec-9-enoate.[141] The methyl ester was subjected to a hydrosilyation reaction with 1,1,3,3-tetramethyldisiloxane in the presence of platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane as the catalyst.[120]



143 **144**

135 79%

Scheme 41. Synthesis of the disiloxane diester **135**.

3.3 Enzymatic polymerization reactions

3.3.1 Introduction

In an effort to replace traditional metal-based catalysts often utilized in the synthesis of silicon-based polymers, N435, an immobilized *Candida antarctica* lipase B was utilized to catalyze the transesterification reactions between the chiral monomers **4**, **5**, and **6** with the disiloxane diester **135**. N435 was chosen as the biocatalyst because it possesses a broad substrate scope, high efficiency, and stability. Moreover, there is substantial literature precedence concerning utilizing N435 in the synthesis of silicone based polyesters.[68, 116, 128]. Seguin in 2013 reported the consumption of **135** during N435-mediated transesterification with **4**, **5** and **6**. Similar results were not obtained when lipases from *Rhizomucor miehei* and *Thermomyces lanuginosus* were utilized.[120]

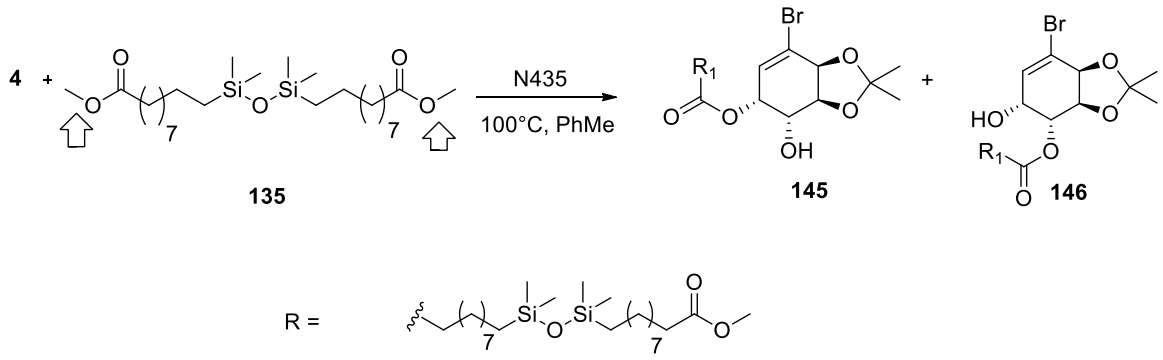
In 2013 Zelisko and coworkers explored the efficiency and reusability of N435 in the synthesis of disiloxane-containing polyesters.[116] N435 exhibited high catalytic activity up to ten times in separate reactions without a significant drop in conversion. The excellent reusability of N435 opens the door for further industrial applications.

3.3.2 Parameters utilized in monitoring enzymatic polymerization reaction

3.3.2.1 Consumption of starting material

The consumption of the disiloxane diester is monitored during each reaction. Such monitoring is possible by evaluating the ^{1}H NMR integration of the methoxy peak. For example a 50% percent decrease in the intensity of the methoxy peak may indicate that the substrates **4** and **135** are converted into the dimers **145** or **146** (AB unit), Scheme 42.

Ester hydrolysis to the corresponding acid will also lead also a decrease in the intensity of the methoxy peak in the ^{1}H NMR spectra, therefore background reactions are always performed.



Scheme 42. Dimer formation leads to 50% reduction in the methoxy peak intensity.

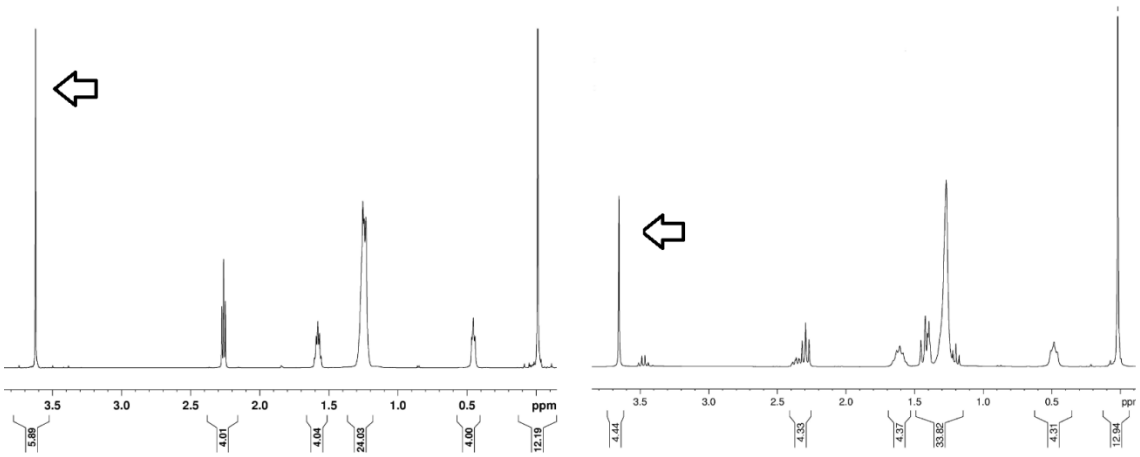


Figure 21. Decrease in the methoxy signal peak relates quantitatively to the transesterification process

3.3.2.2 Mass analysis

In order to assess various polymer functions, whether mechanical or physical traits, Polymer molecular weight must be determined. Synthetic polymers are polydisperse, polymer molecular weight is not a single value but rather a mathematical average of the

weight of the different chains present in the polymer mixture. Common methods used to determine polymeric molecular weights includes gel permeation chromatography (GPC), size exclusion chromatography (SEC) or Matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF)

MALDI-ToF is a soft ionization techniques used in mass analysis, often for large biological molecules and organic compounds that tend to fragment using other harsher analytical methods. MALDI-ToF operates through two distinct steps. First the desired sample is mixed with a matrix material that tends to heavily absorb a UV laser beam. The UV laser beam vaporizes the upper layer of the matrix surface (1 μm). The fumes produced from such an ablation contain charged and neutral matrix and sample species. The detector picks charged species consisting of the sample molecule [M] with an added ion. ($[M+H]^+$ in the case of a proton, $[M+Na]^+$ in the case of sodium ion).[121]

MALDI-ToF analysis was performed using the dithranol matrix which was selected based on it is well-known applicability in polymer analysis together with commonly used sodium chloride salts.[121]

GPC analysis is an advanced technique of size exclusion chromatography. GPC is extremely advantageous when is applied to polymer analysis. It exhibits high efficiency as it does not depend on the ionization of the sample molecules nor fragmentation, thus eliminating the problem of MALDI, that causes fragmentation and does not report to non-charged molecular species. GPC is therefore more accurate in detecting a variety of molecular species, A major GPC disadvantage is that is does not differentiate between species that have less than a 10% mass difference and usually recognizes them as one species. However, modern GPC columns exhibit improvement in that field.[142]

Regardless of the mass analysis technique used they all tend to report two parameters with regard to the sample analyzed mass and intensity. The different masses and intensities of each sample analyzed are later used to report three different polymer characteristics: Number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (P_i).[122] M_n is a simple statistical average that focuses on the number of different species in a given polymer, therefore M_n represents a molecular weight distribution where equal number of molecules lies on either side of it.[122] M_n is usually derived from mass analysis techniques that report masses and intensities but it can also be reported from other techniques such as NMR end-group analysis.[122]

$$M_n = \frac{\sum N_i M_i}{\sum N_i}$$

Where M_i is the mass of a given chain and N_i is the number or intensity that corresponds to such mass.

M_w focuses more on the weight of the chain rather than its intensity, which means that the bigger that chain the more it contributes to the M_w final value. M_w is also a statistical average but in regards of weight not number, which means that M_w represents a molecular weight distribution where equal weight of molecules lies on either side of it.[122]

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

Where M_i is the mass of a given chain and N_i is the number or intensity that corresponds to such mass

PDI measures the broadness of the weight distribution in the given polymer.

The larger the weight distribution of the polymer chains the bigger the P_i value.

Biological macromolecules have a PDI of 1. Controlled synthetic polymers have a polydispersity of 1.02 to 1.1, A value of 2.0 usually indicates a step growth polymerization reactions. Step growth polymerization means that monomers react to form first dimers, then trimers, then oligomers and eventually long chain polymers. The polydispersity index is measured through dividing the M_w value by the M_n value.[122]

$$\text{PDI} = \frac{M_w}{M_n}$$

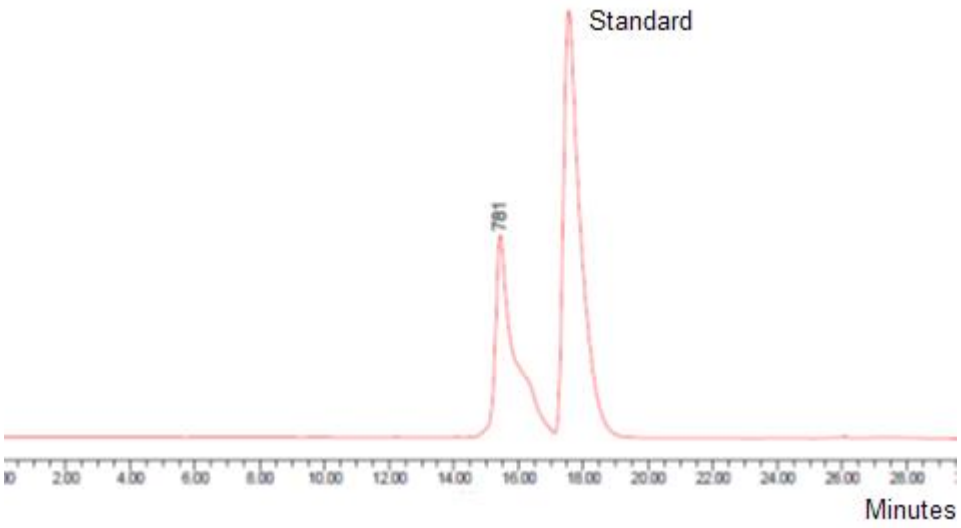


Figure 22. Normalized GPC Chromatogram.

3.3.3 Consumption of the diester starting material

The degree of consumption of the diester starting material is an excellent indicator about the extent of transesterification performed by N435. The degree of consumption of the diester **135** was determined by ^{1}H NMR analysis by integrating the protons alpha to the carbonyl of the diester and the methyl protons of the ester. Comparing both of these integrals is used to determine the degree of consumption of the diester starting material. In order to utilize such data, control reactions had to be performed. The first control reaction was reported by Seguin and Zelisko.[120] It aimed to determine the extent of hydrolysis of the diester starting material in presence of residual water by N435. Water is known to act as a nucleophile in lipase-mediated esterifications and result in ester hydrolysis. The diester starting material **135** was incubated with 10 wt% N435 without any chiral diols. Reactions were performed in toluene and solvent-free conditions at 100 °C for 24 h and 7 d; maximum hydrolysis observed was 7%, while Seguin reported a maximum hydrolysis of 5%.[120] Other control reaction performed involved incubating the diester **135** with chiral diols **4**, **5** and **6** without any added N435. Reactions were performed in toluene and solvent-free conditions at 100 °C for 24 h and 7d, transesterification was not observed. All of the following reactions were performed in toluene or solvent-free sealed tube at 100 °C for one or seven days. Each of the diols **4**, **5**, and **6** are mixed with the diester **135** in 1:1 ratio. Solvated reactions were performed in toluene; 1 mL of toluene was used for every 0.2 mmol of diol starting material. Reactions are catalyzed by 10 wt% N435 (with regards to the total weight of the starting materials). Reactions were performed under an air atmosphere and stirred continuously at 150 rpm. For every set of conditions three identical reactions were run for 24 h and another three

for 7 d. Reactions were always terminated by the addition of 3 mL of diethyl ether, filtered through a medium porosity Büchner funnel, and concentrated under reduced pressure.

Starting material	Conditions	Percent consumption of diester **135**	
		24 h reactions	7 d reactions
4 + **135**	Solvent-free	17 ± 2.8	55 ± 5.7
	Toluene	14.6 ± 1.9	27.9 ± 6.5
5 + **135**	Solvent-free	11.3 ± 1.5	25.2 ± 3.9
	Toluene	12 ± 2	22.5 ± 0.8
6 + **135**	Solvent-free	29.2 ± 3.4	49.2 ± 2.1
	Toluene	33.1 ± 2.6	58.3 ± 5.6

Table 3. Degree of consumption of the diester **135** when subjected to N435-mediated transesterification with diols **4**, **5**, and **6**.

With diols **4** and **5** solvent-free conditions result in higher consumption of the siloxane diester **135** when compared to solvated reactions. However, the highest consumption of **135** was achieved with diol **6** in toluene for 7 d, with consumptions of **135** up to 58%. Diol **6** is fully saturated and lacks the double bond found in **4** and **5** which means it is the least constrained among the 3 diols, which results in better fitting in the enzyme active site and higher consumption of the starting material.

3.3.4 Enzymatic catalysis in toluene

As discussed before chiral diols and the diester **135** were subjected to N435-mediated transesterification in toluene. Crude unfractionated reaction products were analyzed by MALDI-ToF.

Starting material	24 h reactions		7 d reactions	
	M_w (g/mol)	M_n (g/mol)	M_w (g/mol)	M_n (g/mol)
4 + **135**	675.5 ± 89	625.7 ± 51	624.9 ± 44.6	597.6 ± 38.0
5 + **135**	645 ± 37	615 ± 22	897 ± 35.0	757.3 ± 50.3
6 + **135**	756 ± 1.2	743.3 ± 0.5	940.5 ± 72.3	850.4 ± 50.8

Table 4. MALDI-ToF analysis of enzymatic transesterification of the chiral diols with diester **135** in toluene.

MALDI-ToF analysis revealed that higher molecular weight molecules were synthesized when diol **6** was employed. However, the values suggested that most of the products formed were dimers.

It has been well documented that not all polymeric species are detected by MALDI-ToF. Therefore GPC analysis was performed for the same unfractionated reaction products. The following table represents a comparison between GPC analysis and MALDI-ToF analysis of selected unfractionated reaction products.

Starting material	GPC		MALDI-ToF	
	M_w (g/mol)	M_n (g/mol)	M_w (g/mol)	M_n (g/mol)
4 + **135**	826	803	658	626
5 + **135**	823	801	686	638
6 + **135**	1432	1124	977	856

Table 5. A comparison between GPC analysis and MALDI-ToF analysis of selected unfractionated reaction products.

Table **5** suggests that MALDI-ToF analysis failed to detect larger species. Although GPC analysis did not indicate any polymer formation, diol **6** exhibited the best results at values of M_w = 1,432 g/mol and M_n= 1,124 g/mol which suggest the formation of tetrameric species.

Polydispersity index for transesterification of diol **4** and **5** with **135** ranged between 1.01 and 1.12 which indicated controlled esterification reactions, while the polydispersity index for transesterification of diol **6** with **135** ranged between 1.1 and 1.3.

3.3.5 Enzymatic catalysis in solvent-free conditions

Solvent-free reactions are highly desirable in industry due to the toxicity and flammability of organic solvents, and the easiness of product recovery. Solvent-free N435 catalyzed enzymatic catalysis has been reported, occasionally with better results than solvated catalysis.[93, 116] Regular solvent-free reactions proved unsuccessful, as the three diol substrates tend to phase separate from the diester **135**, probably due to the difference in polarity. A sealed tube, solvent-free reaction was attempted to overcome this problem. A small sized sealed tube (< 0.5 cm^3) was used to perform the solvent-free reactions. GPC analysis was later utilized to compare solvent-free technique with enzymatic catalysis in toluene. Reactions performed in toluene and in solvent-free conditions were both run for 7 d at 100 °C and 10 wt% N435, reactions were terminated in the regular fashion.

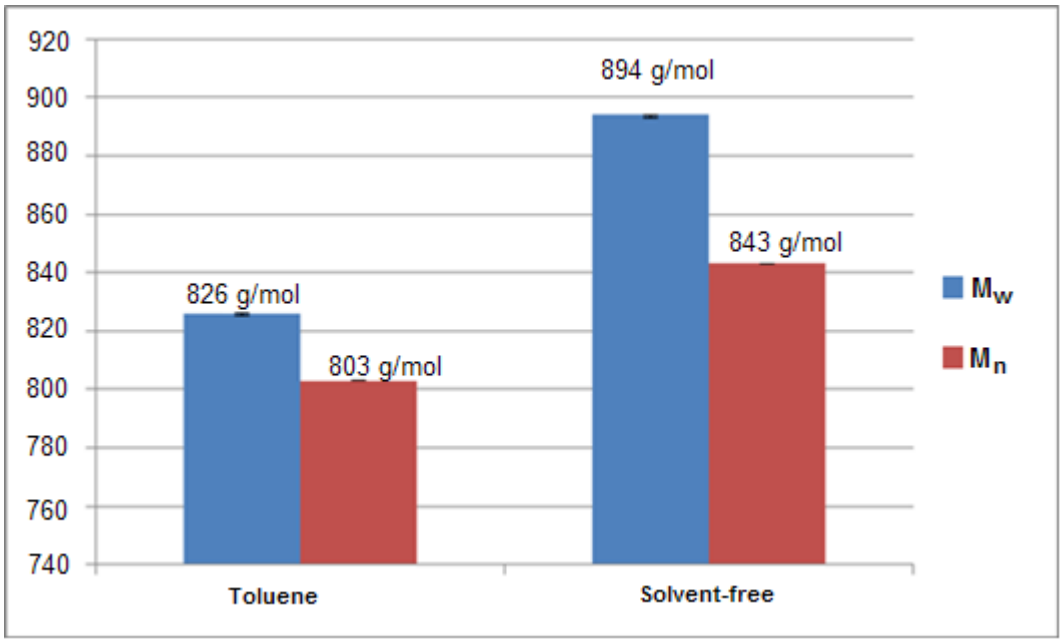


Figure 23. GPC analysis of N435-catalyzed unfractionated transesterification reaction of diol **4** with diester **135** in solvent-free conditions and in toluene.

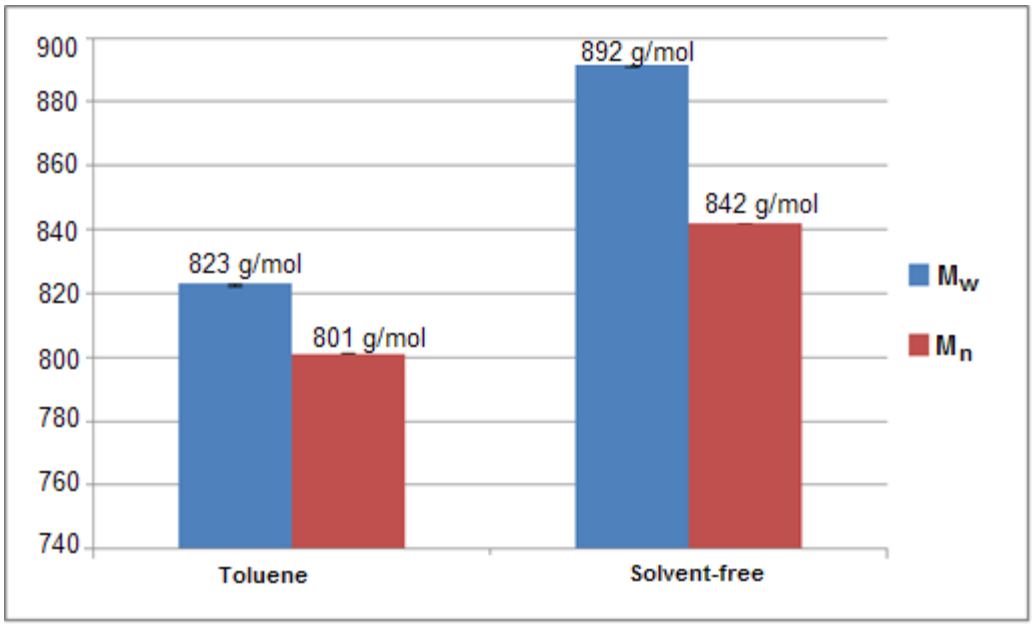


Figure 24. GPC analysis of N435-catalyzed unfractionated transesterification reaction of diol **5** with diester **135** in solvent-free conditions and in toluene.

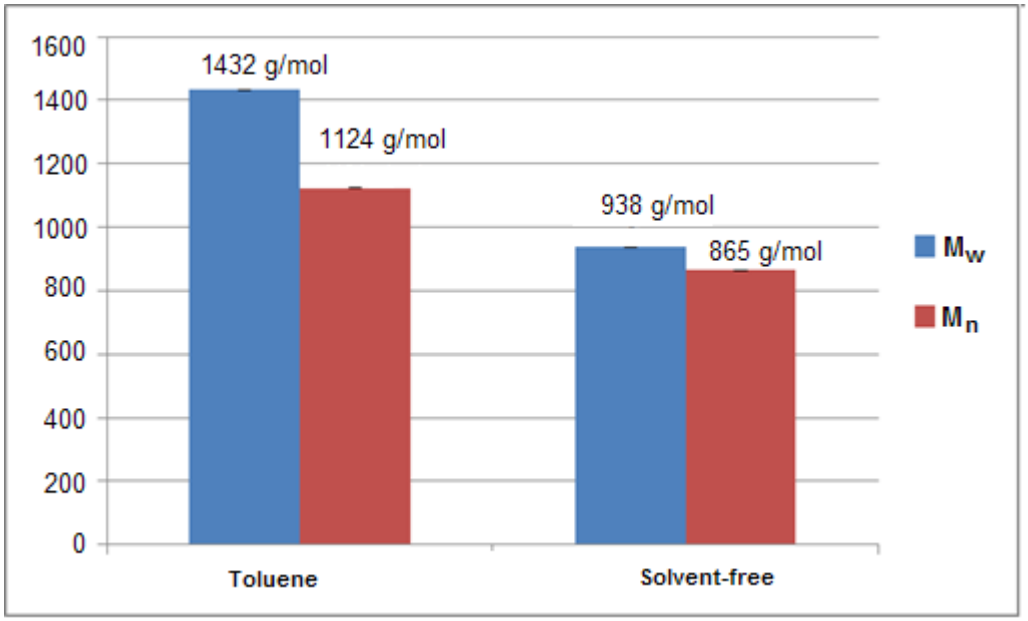


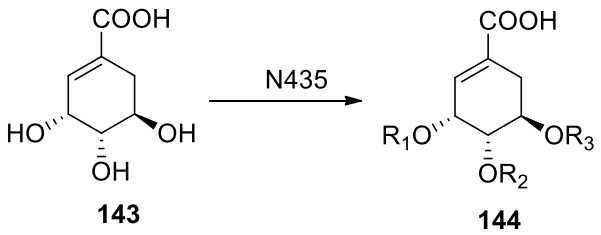
Figure 25. GPC analysis of N435-catalyzed unfractionated transesterification reaction of diol **6** with diester **135** in solvent-free conditions and in toluene.

GPC analysis of various unfractionated reactions indicates the formation of larger oligomeric species with diols **4** and **5** in solvent-free conditions compared to reactions performed in toluene at the same temperature, which indicated better transesterification conditions. However, diol **6** exhibits better transesterification in toluene when compared to solvent-free reaction, resulting in M_w of 1432 g/mol that corresponds to the formation of tetrameric species.

3.3.6. Isomeric ratios of the dimers produced

Mass analysis alone did not provide any information about the enzymatic preference for each of the secondary alcohols in diols **4**, **5** and **6** towards transesterification with diester **135**. Successful isolation and characterization of dimmers **145** – **150** indicated that both secondary alcohols in **4**, **5** and **6** are accessible to N435-mediated transesterification with **135**. Further investigation indicated a preference towards the transesterification of secondary alcohol distal to the acetonide.

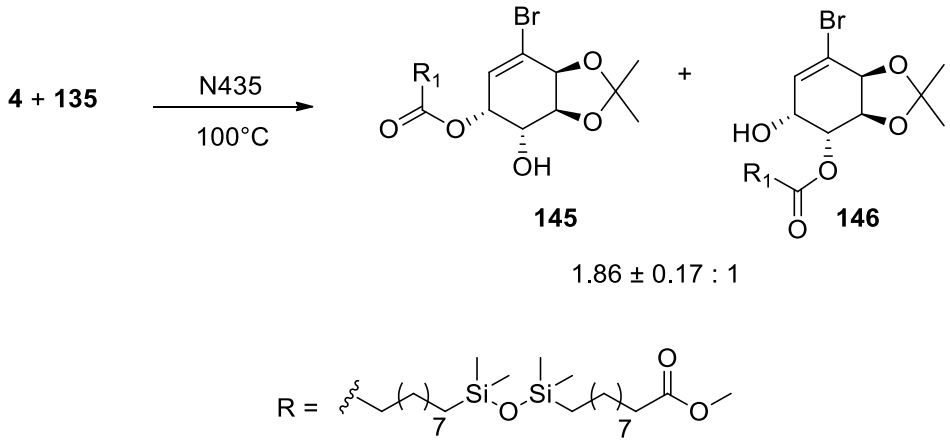
Regioselective protection and deprotection of polyoxygenated carbacycles is an extremely beneficial asset in modern synthetic chemistry. A chemically challenging aspect is regioselective protection and deprotection of chemically similar secondary alcohol. Gotor in 2002 reported a selective protection of the secondary alcohols of shikimic acid utilizing various types of lipase. The enzyme mainly esterified C-3 and C-5 hydroxy groups; monoacetates were obtained in 45 and 37% yield, respectively. Through his experimenting with lipases Gotor reported the selectivity of CAL-A with short chain acyl donors. However, when acyl donors have longer chains, better results are obtained by N435.[123]



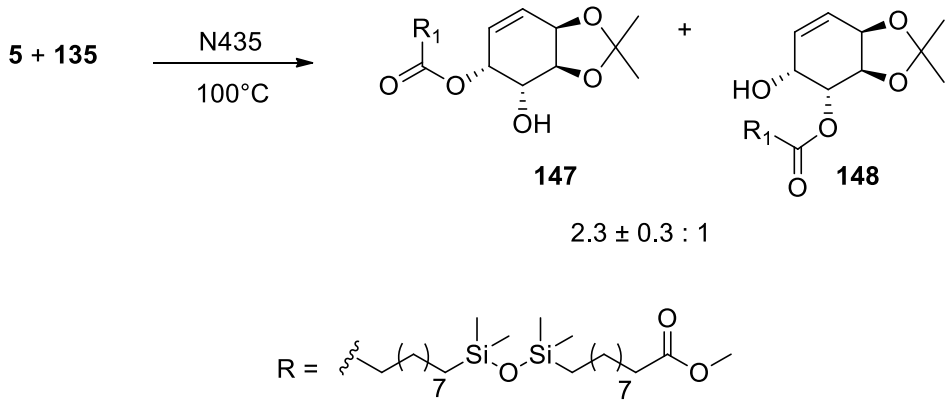
144a R_1= Ac, R_2= H, R_3= H 45%
144b R_1= H, R_2= Ac, R_3= H 18%
144c R_1= H, R_2= H, R_3= Ac 37%

Scheme 43. Lipase esterification preference to shikimic acid secondary alcohols.[123]

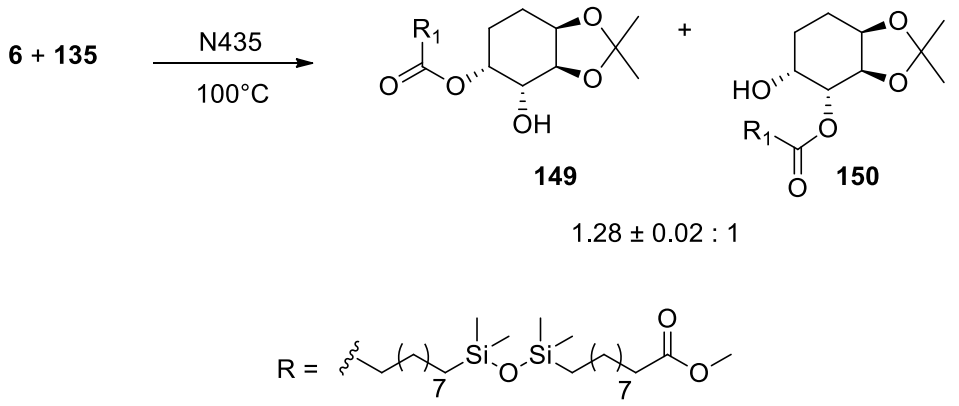
The selectivity towards the allylic alcohol can be attributed to being chemically more reactive, while low reactivity towards the C-4 secondary alcohol can be justified by hindrance by two adjacent secondary alcohols at C-3 and C-5 positions.[123] Through this research special attention has been directed to the selectivity of immobilized *Candida antarctica* lipase B (Novozym-435®) towards the esterification of the two secondary alcohols present in the chiral monomeric units. The chiral monomer investigated does not possess similar hindrance found in the shikimic acid, also diol **6** synthesized doesn't possess a double bond, therefore provides a better idea about enzyme selectivity when the two secondary alcohols present are chemically equal. The results showed a clear and distinctive preference for the transesterification of the secondary alcohol distal to the acetonide group by (Novozym-435®) in the unsaturated monomers **4** and **5**, and slight preference towards the same alcohol moiety in the saturated diol **6**.



Scheme 44. N435 transesterification preference for each of the secondary alcohols in diol **4** with diester **135**.



Scheme 45. N435 transesterification preference for each of the secondary alcohols in diol **5** with diester **135**.



Scheme 46. N435 transesterification preference for each of the secondary alcohols in diol **6** with diester **135**.

Ratios of isomers **145** and **146** were determined by ^{1}H NMR analysis of the mixture of isomers separated from the starting material; the ratio could not be obtained from the crude mixture due to signal overlaying with the diol **4** resonance.

Ratios between isomers **147** and **148**, **149** and **150** were determined by ^{1}H NMR ratio of crude reaction mixtures. Reported ratios are obtained from both solvent-free and reactions in toluene. All the previously reported esters **145 - 150** were isolated by column chromatography and fully characterized.

It is worth mentioning that **145**, **146**, **147**, and **148** are not stable to room temperature storage, while **149** and **150** showed significant stability under the same conditions. This may explain why higher oligomers were only formed during the transesterification of diol **6**. The better results associated with diol **6** may also be attributed to the flexibility of the ring due to absence of the double bond. This flexibility could enable fitting into the enzyme active site.

3.3.7 Effect of catalyst loading and substrates concentration

In an attempt to push the enzymatic transesterification towards larger macromolecules with higher molecular weight several reactions were conducted including increasing the catalyst loading per reaction. Solvent-free reactions were conducted for 7 d and catalyzed by 10, 20, and 30 wt% N435 (with regards to the total weight of the starting materials). GPC analysis performed shows the effect on increasing the catalyst loading in each of the following sealed tube reactions.

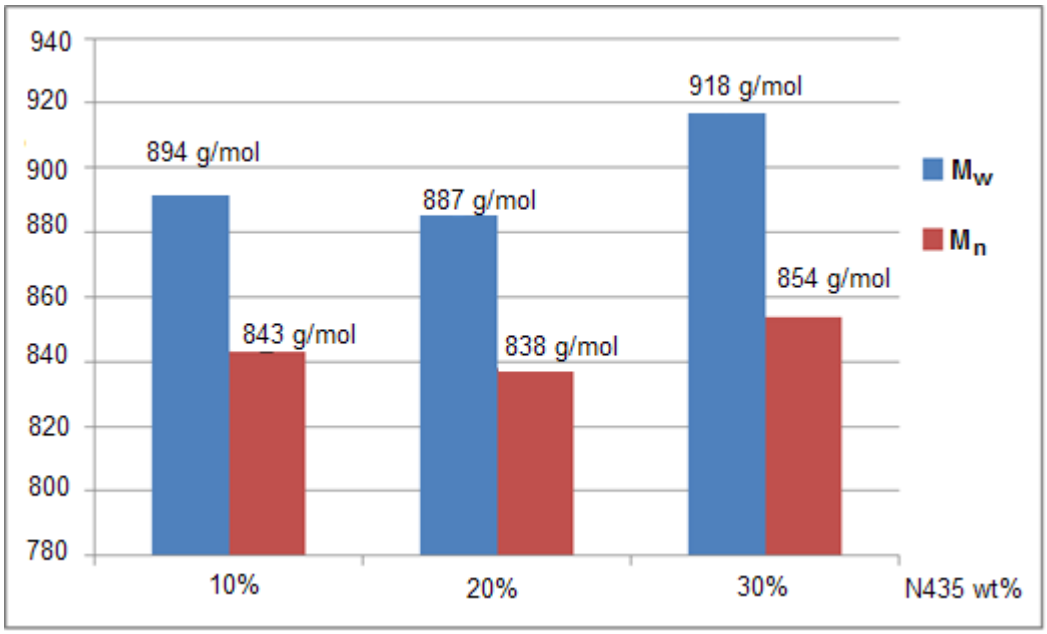


Figure 26. GPC analysis of 10, 20, and 30 wt% N435 catalyzed transesterification of diol **4** with diester **135** in solvent-free conditions for 7 d.

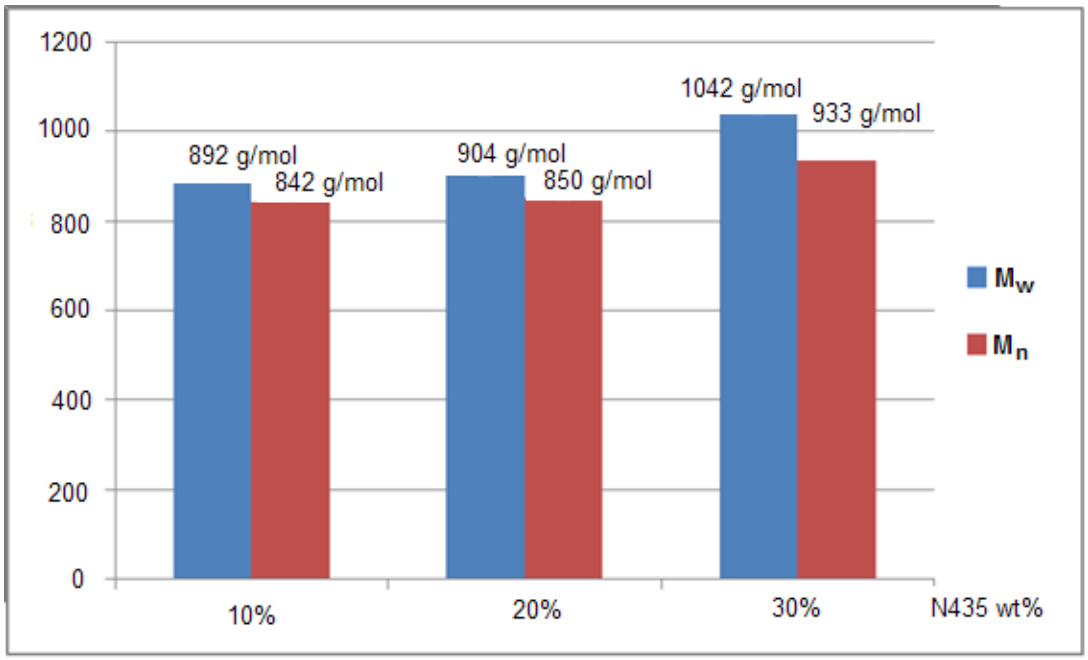


Figure 27. GPC analysis of 10, 20, and 30 wt% N435 catalyzed transesterification of diol **5** with diester **135** in solvent-free conditions for 7 d.

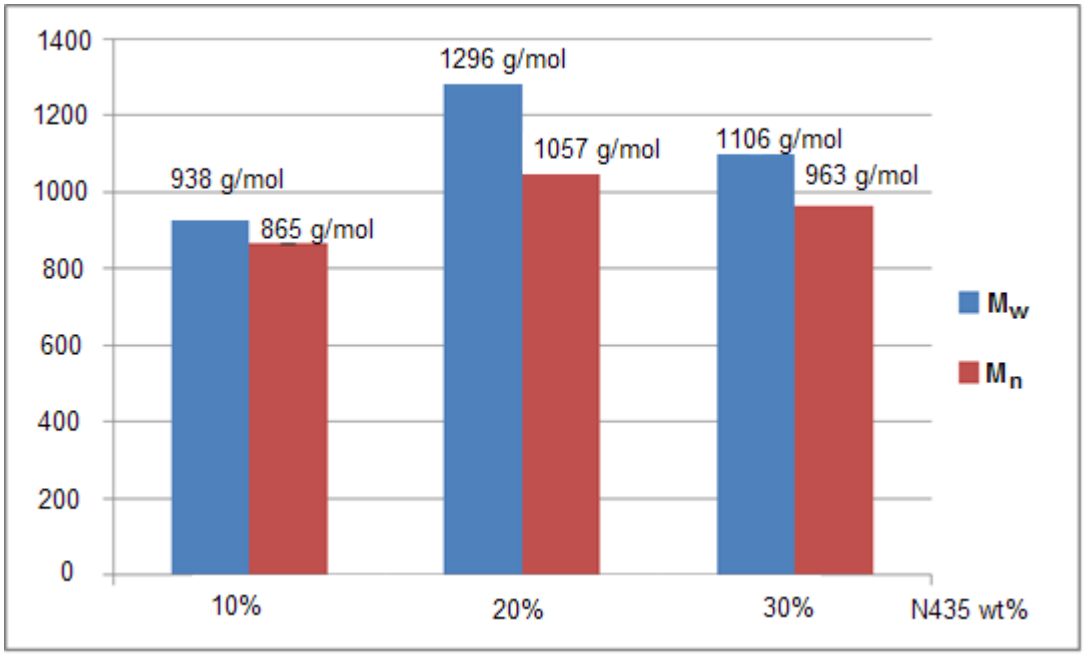


Figure 28. GPC analysis of 10, 20, and 30 wt% N435 catalyzed transesterification of diol **6** with diester **135** in solvent-free conditions for 7 d.

Results indicated a slight improvement associated with diols **4** and **5** in the case of doubling or tripling the biocatalyst amount. No significant improvement was observed with increasing the N435 amount with diol **6**. Solvated enzymatic catalysis for diol **6** still represent the best conditions for N435-mediated transesterification reactions.

Solvent-free reactions and reactions performed in toluene are usually conducted on a diol and diester that are mixed in 1:1 molar ratio. This brief investigation aimed to determine the effect of altering one of the substrates concentrations and its subsequent effect on the molecular weight of the unfractioned reaction product.

Diol **5** was chosen for such investigation. N435- mediated transesterification reaction was performed on diol **5**: diester **135** ratio of 1:2 and 2:1. Reactions were performed in solvent-free sealed tube for 7 d and catalyzed by 10 wt% N435 (with regards to the total weight of the starting materials), results were analysed by GPC.

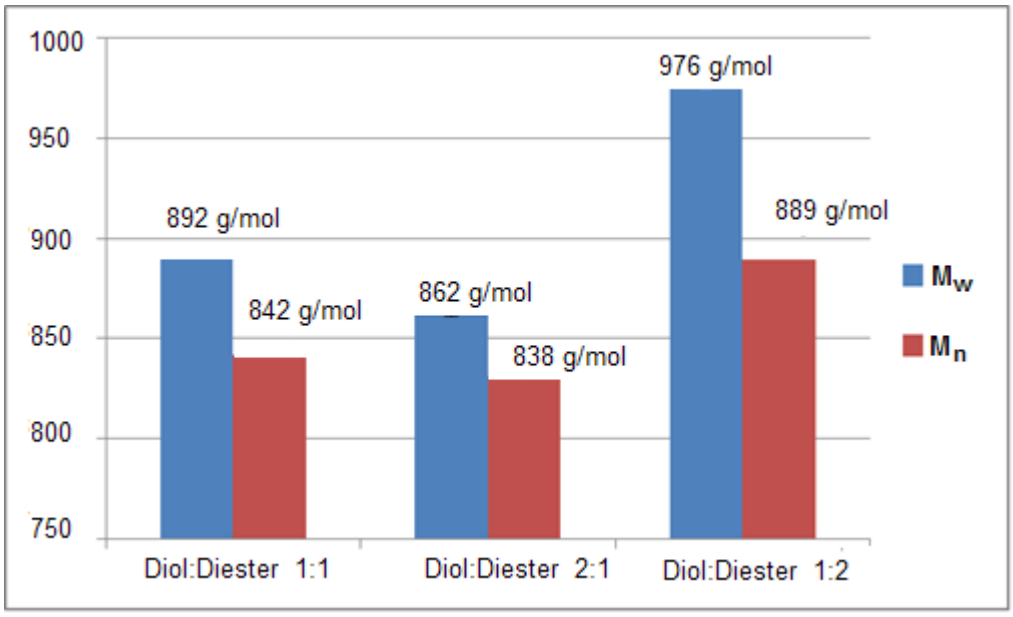


Figure 29. GPC analysis of N435 catalyzed transesterification of various ratios of diol **5** with diester **135**.

GPC analysis results showed an increase in M_w and M_n of the unfractioned reaction product resulting from doubling the ratio of the diester **135** substrate compared to diol **5** substrate.

3.4 Synthesis of N435 test substrates

A set of N435 test substrates were synthesized in order to further investigate the lipase-mediated transesterification reactions. A direct investigation of the transesterification of the free diols gives information about preference of the N435 to each of the free secondary alcohols in monomers **4**, **5**, and **6**. However, synthesizing versions of these diols with only one free secondary alcohol provides information about N435 transesterification of this alcohol when the other secondary alcohol is already blocked. Various attempts have been tried before to synthesize such compounds but they either did not succeed or were not efficient enough. A later successful protocol was developed based on TIPS group selective protection reported by Banwell.[124]

This research aims to assess N435 preference to each of the free secondary alcohols when the other secondary alcohol is already blocked, such information can be extremely valuable in understanding the propagation or halting of the polymerization reaction. This required the synthesis of six analogues of diols **4, 5** and **6**, where in each case only one of the secondary alcohol groups is free, later these analogues will be subjected to the same transesterification conditions and this will provide information about N435-mediated transesterification of the free alcohols. An important characteristic for the protecting

group is that it must be small in size so as not to interfere with the substrate fitting in the enzyme pocket, and also cannot be an ester group as the lipase enzyme could cleave an ester functionality. A methyl ether was thus chosen as the blocking group due to its small size and stability.

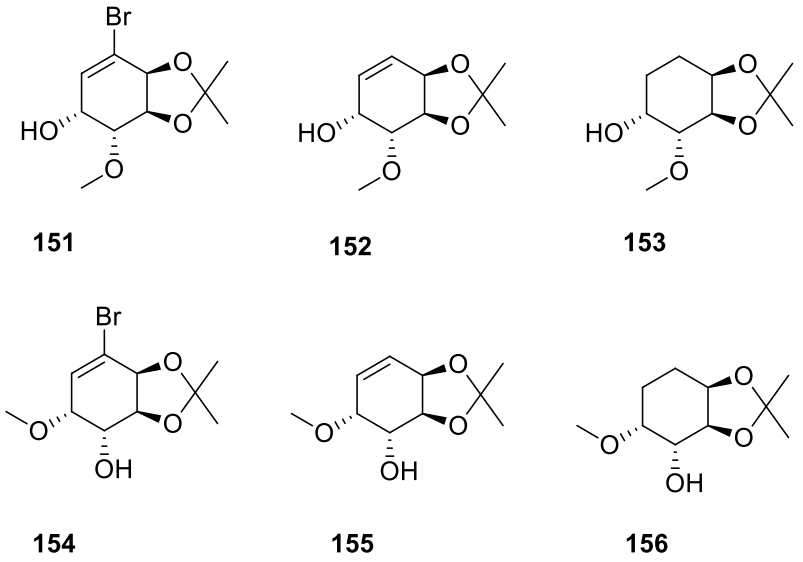
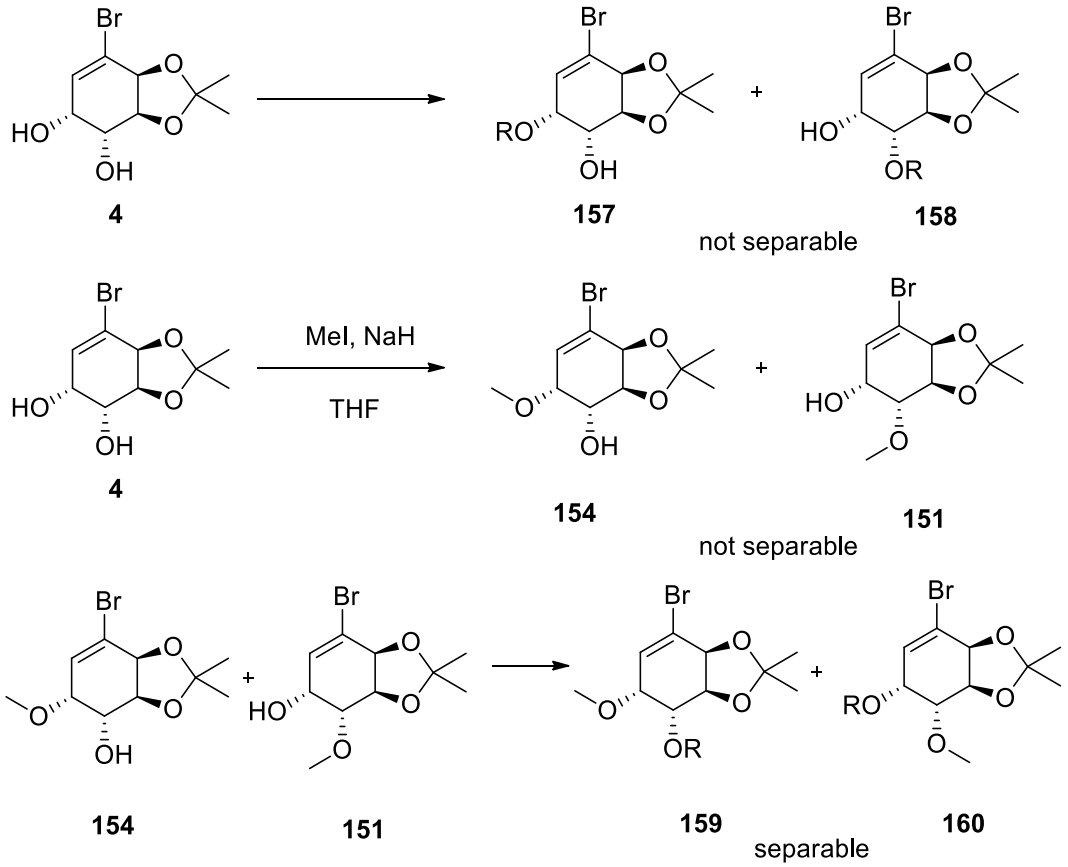


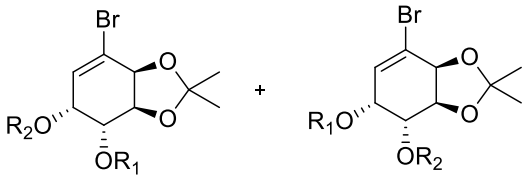
Figure 30. N435 test substrates.

The major obstacle in synthesizing these test substrates is that introducing and protecting group will result in two isomers that do not show any difference in elution values. Subsequent work aimed to introduce another protecting group to these two isomers in order to get separation between the two produced isomers.



Scheme 47. Proposed solution for isomeric separation

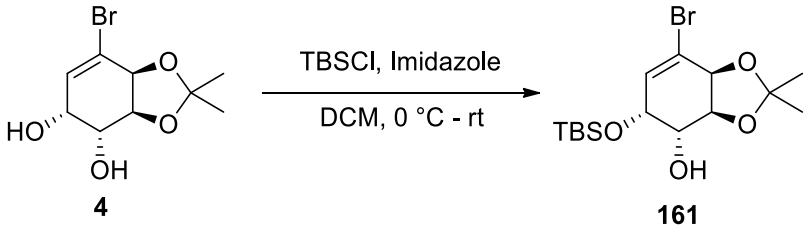
Several protecting group were tried, best results were achieved through TBS protecting of isomers **151** and **154**, which resulted in isomers that exhibited slight separation. Although the process was successful to produce the desired analogues it resulted in very low yields.



R_1	R_2	Result
H	Me	No separation
H	MOM	No separation
H	TBS	No separation
H	Ac	No separation
Me	TMS	No separation
Me	Ac	No separation
Me	Bn	Poor separation
Me	TBS	Poor separation
Me	TBDPS	Poor separation

Table 6. Various protecting group and elution variance according to TLC.

Selective protection is very desirable in this case as it will eliminate the need for separation of the two isomers. The first procedure attempted to achieve selective protection utilized TBSCl and imidazole at 0 °C.[125]

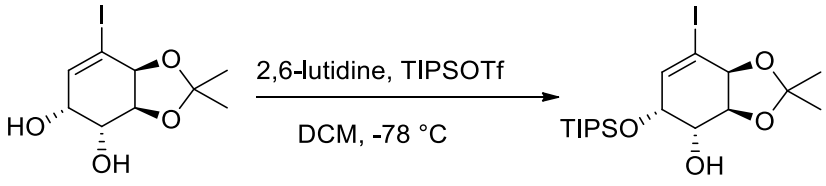


4 **161**

Scheme 48. Selective TBS protection.

This procedure was not very useful as TBS group tend to migrate very easily thus eliminating the purpose of selective protection.

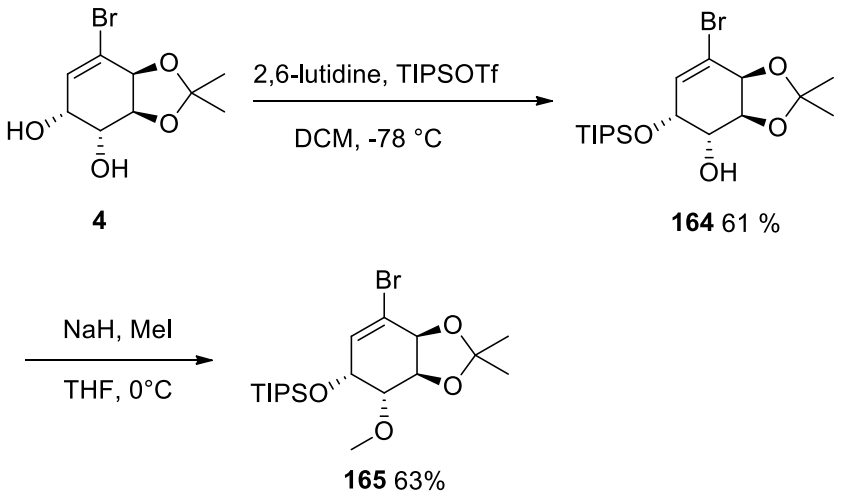
In July 2014, during their chemoenzymatic total synthesis of the phytotoxic geranylcyclohexentriol (−)-phomentrioloxin, Banwell and coworkers introduced a TIPS protecting group selectively to C-5 secondary alcohol of **162**. TIPS is more useful to this project as it exhibits less tendency to migrate. The selective protection was achieved by using TIPSOTf and 2,6-lutidine as the base in DCM at -78°C.[124]



162 **163** 63%

Scheme 49. Selective TIPS protection.[124]

Selective protection of the allylic secondary alcohol in diol **4** was performed, followed by subsequent methyl group protection of the homoallylic alcohol with NaH and MeI to yield **165** in good yield..



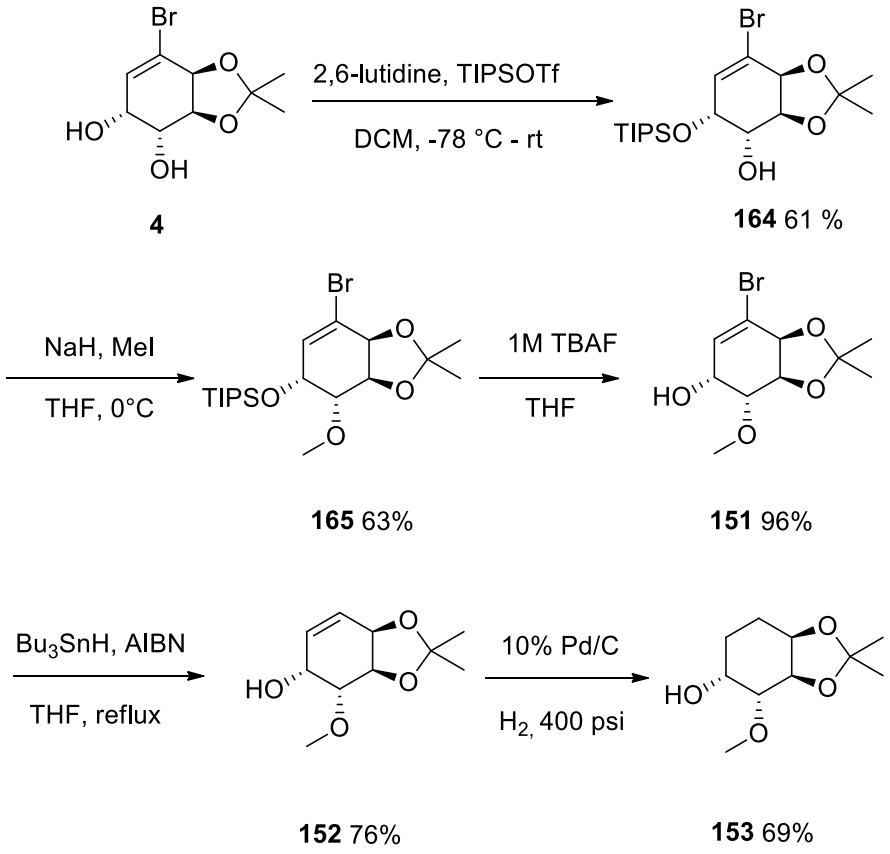
4

164 61 %

165 63%

Scheme 50. Utilizing selective TIPS protection in synthesizing **165**.

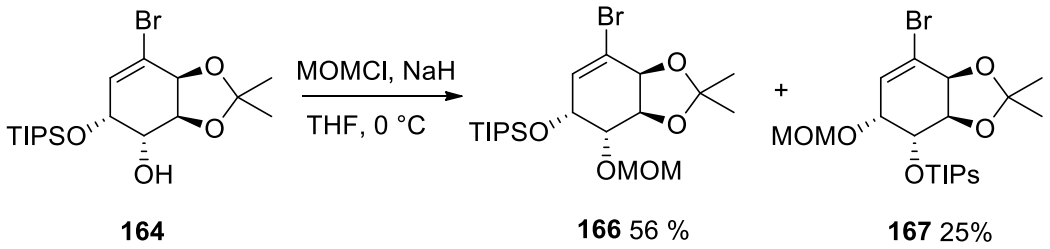
The desired product **165** was isolated through chromatography, a very efficient and fast deprotection of TIPS using 1M TBAF solution yielded **151**, the first desired test substrate. Subsequent reduction of **151** using tributyltin hydride and AIBN yields **152** which was later reduced through a palladium catalyzed hydrogenation to yield **153** in good overall yield.



Scheme 51. Synthesis of N435 test substrates **151**, **152** and **153**.

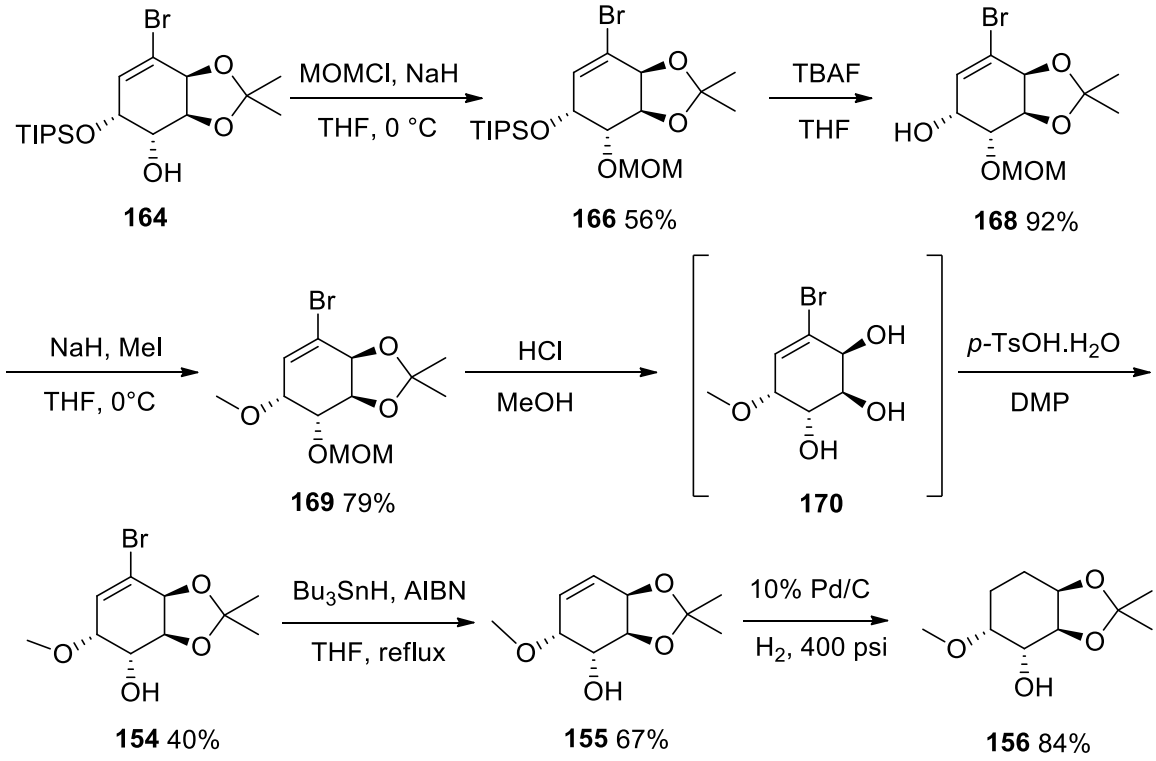
A route utilizing the protection of the homoallylic alcohol with a MOM group was utilized in order to access the rest of the lipase B test substrates. Longer reaction time are needed to install the MOM group compared to the methoxy group, TIPS migration is observed and **167** is formed, Scheme 53. Chromatographic separation of **166** wass performed. The TIPS protecting group was removed using 1M solution of TBAF, the allylic alcohol was protected with a methyl group to yield **169**. The MOM group was later cleaved under acidic conditions which also tended to remove the acetonide group. Subsequent addition of the the acetonide protecting group was performed to yield **154** and a similar set of reduction reactions were performed to access the reduced analogues

155 and **156**.



164 **166** 56 % **167** 25%

Scheme 52. TIPS group migration during MOM protection.



164 **166** 56% **168** 92%

169 79% **170**

154 40% **155** 67% **156** 84%

Scheme 53. Synthesis of test substrates **154**, **155**, and **156.**

3.5 Experiments utilizing the N435 test substrates

Successful isolation and characterization of dimmers **145** – **150** indicated that both secondary alcohols in **4**, **5**, and **6** were accessible to N435-mediated transesterification with **135**. However, mass analysis of the unfractionated reaction mixture did not indicate the formation of polymers. This raised the question of whether N435 was able to efficiently transesterify the secondary alcohol moiety in the chiral monomers if the other secondary alcohol was already blocked. Synthesizing versions of these diols with only one free secondary alcohol will provide information about N435 transesterification of this alcohol when the other secondary alcohol is blocked. Utilizing an ester group for such task was not feasible, as ester group has been noticed to migrate between the two secondary alcohols of diols **4**, **5**. Moreover N435 will catalyze ester hydrolysis and transesterification which will alter the data of this investigation. So methyl ether was thus chosen as the blocking group due to its small size and stability.

In order to assess N435 preference towards transesterifying each of the secondary alcohols in each isomer, corresponding isomers are mixed together in 1:1 ratio. Later each pair of the corresponding isomers will be subjected to N435-mediated transesterification with **135**, in an exact same protocol conducted for N435-mediated transesterfication in toluene. Each pair of isomers will be subjected to three 24 h reactions and three 7 d reaction.

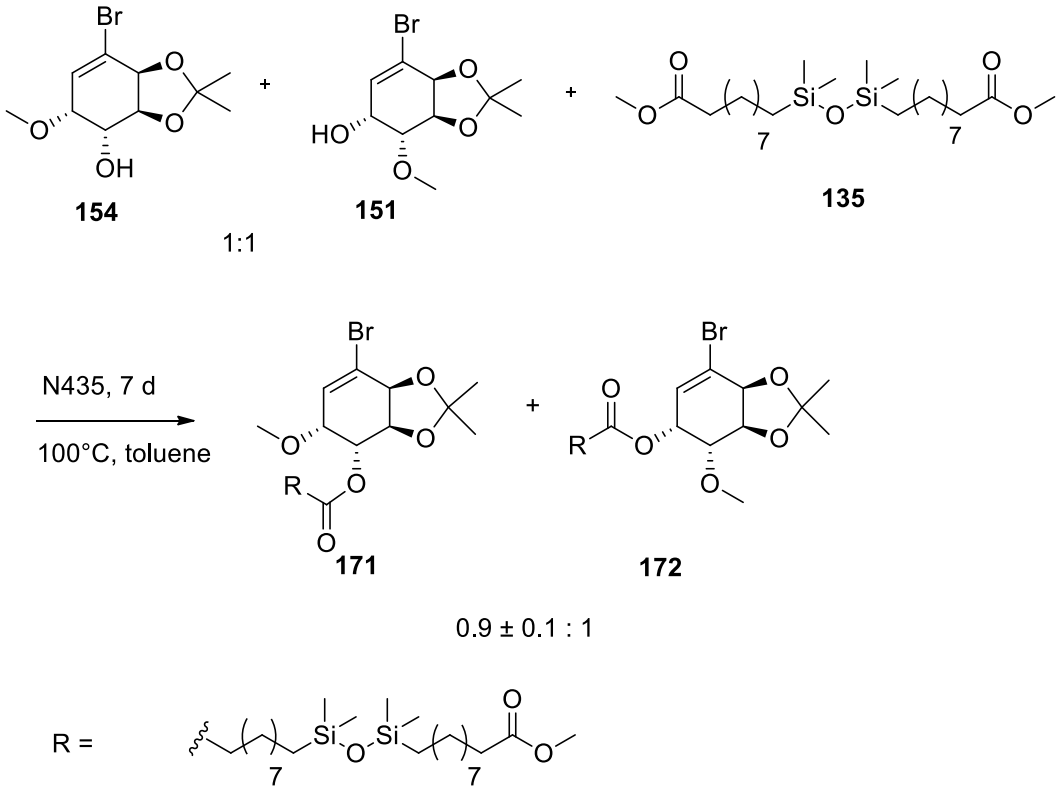
^{1}H NMR analysis of the crude reactions mixture will provide information about the lipase preference to each of the secondary alcohol moiety found in the two isomers.

In order to ease product separation and characterization each one of the testing substrates will be subjected separately to N435-mediated transesterification with **135**.

The first set of reactions was performed on isomers **154** and **151** for 24 h and 7 d reactions. **151**, **154** and **135** were mixed together in 1:1:1 ratio.

Reactions were performed in toluene at 100 °C in 1 mL of toluene. Reactions were catalyzed by 10 wt% N435 (with regards to the total weight of the starting materials). Reactions were performed under an air atmosphere and stirred continuously at 150 rpm. 24 h reactions showed no formation of esters **171** and **172**; in fact products only started to form at the seventh day in a very low yield. This result indicated that once one secondary alcohol was blocked the N435 was significantly retarded in terms of transesterifying the other secondary alcohol.

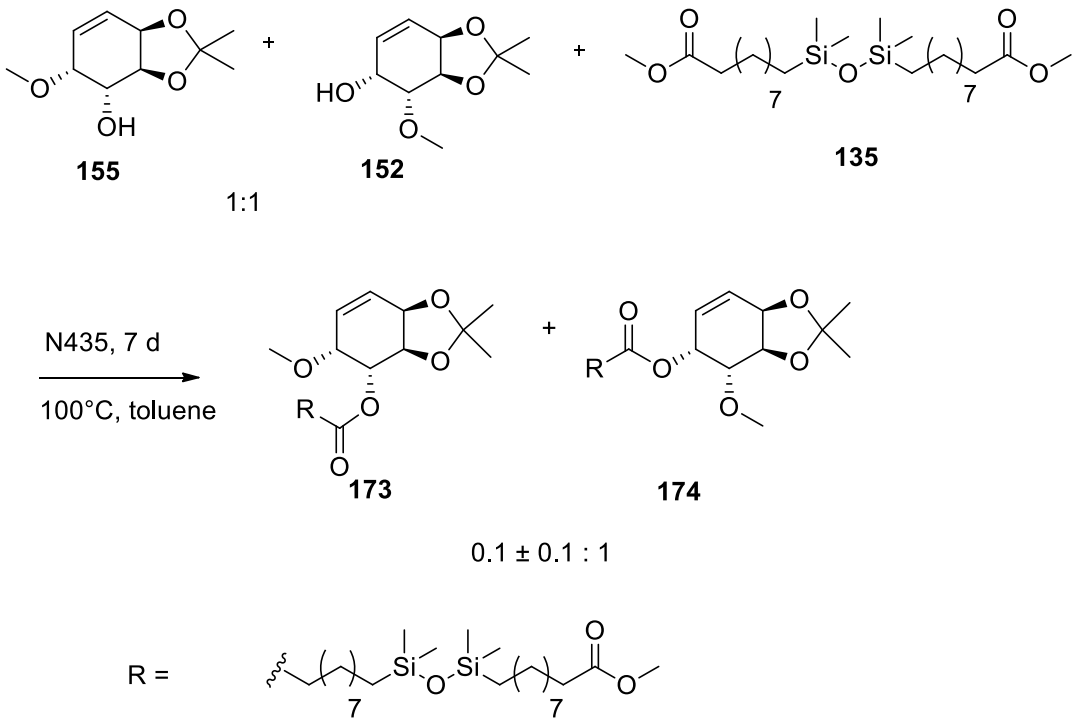
Ratios of the ester produced from **151** were slightly higher than ester produced from **154** which agreed with the previous finding on the lipase preference to transesterify the secondary alcohol distal to the acetonide group.



Scheme 54. N435-mediated transesterification of **151** and **154** with diester **135** shows slight preference to transesterify **151** to form ester **172**.

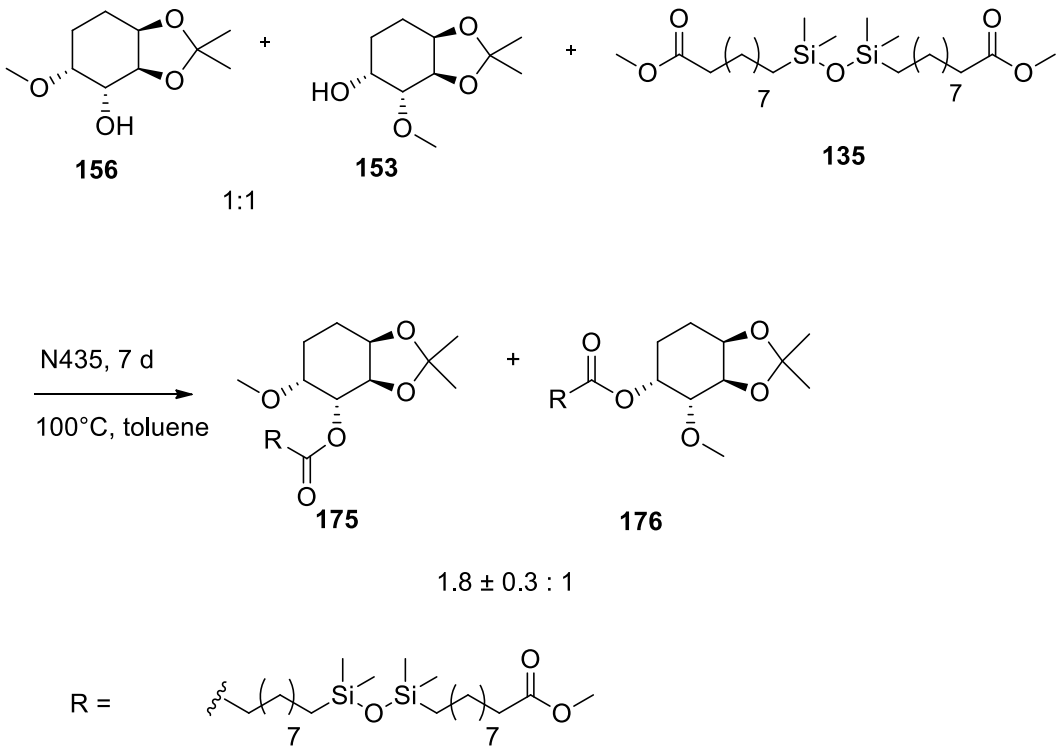
152 and **155** were tested in similar fashion; the first set of reactions was performed on isomers **155** and **152** for 24 h and 7 d reactions. **155**, **152** and **135** were mixed together in 1:1:1 ratio. The 24 h reactions showed no formation of esters **173** and **174**. Product formation was noticed during the sixth and the seventh day of the reaction, which agreed with the hypothesis that once one secondary alcohol is blocked, N435 is significantly retarded in terms of transesterifying the other secondary alcohol.

Ratios of the ester produced from **152** were significantly higher than the ester produced from **154** which agreed with the previous finding on the lipase preference to transesterify the secondary alcohol distal to the acetonide group.



Scheme 55. N435-mediated transesterification of **152** and **155** with diester **135** shows significant preference to transesterify **152** to form ester **174**.

153 and **156** were tested in a similar fashion; N435 catalyzed reactions were performed on isomers **153** and **156** for 24 h and 7 d reactions. **153**, **156**, and **135** were mixed together in a 1:1:1 ratio. The 24 h reactions showed no formation of esters **175** and **176**.



Scheme 56. N435-mediated transesterification of **153** and **156** with diester **135** shows slight preference to transesterify **156** to form ester **175**.

Analysis of ^{1}H NMR spectra of crude reaction mixtures showed the production of **175** in double the amount of **176,** thus indicating N435 transesterfication preference towards the secondary alcohol proximal to the acetonide, presenting the only case in which N435 transesterification favours the secondary alcohol proximal to the acetonide group. Although such reaction can propagate into larger molecules, Any larger species than the dimes were not observed at all or existed in extremely low concentration as shown in the MALDI spectra, < 1%. Esters **171** – **176** were isolated and fully characterized.

4. Conclusion and future work

N435 was used to catalyze the transesterification reactions between various chiral monomers and a disiloxane diester in an effort to produce optically active silicon-based polymers. Various conditions have been tested for the transesterification reactions and GPC analysis indicated the formation of oligomer species. Best transesterification conditions were observed with diol **6** and diester **135** in toluene, where oligomeric species reached M_w = 1,432 g/mol indicating the formation of tetrameric species. Optically active dimers were isolated and characterized. The enzymatic preference for each of the secondary alcohols in the chiral monomers towards transesterification with diester **135** was identified. Moreover, a set of lipase B test substrates with only one free secondary alcohol were synthesised and tested, which provided information about lipase B transesterification of the free secondary alcohol when the other secondary alcohol is not free. N435 transesterification preference to the hydroxyl group distal to the acetonide group was observed in most cases and significant retardation of the transesterification process was observed during the N435-mediated transesterification when the test substrates were examined. Results indicates that once one secondary alcohol is not free N435 is significantly attenuated in terms of transesterifying the other secondary alcohol, which may explain why polymerization reactions do not propagate as promptly once one alcohol is esterified.

Future work may include incorporating the data presented in this thesis into a computer-based docking study. 'High-throughput' molecular docking methods can be used to

predict in where and in which relative orientation a substrate binds to a protein, this information will assist in designing new substrates as well as screening potential leads. Such study may be very valuable to the industries and researchers utilizing the immobilized CALB in their work.

CALB possesses a tight and small active site, it contains a stereospecific pocket in which secondary alcohols have to direct one substituent to fit in that pocket.[126] In order to overcome steric interactions within the CALB active site; smaller, less hindered chiral monomers may result in much better transesterification, because of easier fitting in the enzyme active site. Monomers **177**, **178**, and **179** are potential substrates for N435-mediated polymerization. *Candida antarctica* lipase A possesses a high thermal stability and excellent activity towards sterically hindered alcohols, thus it could be used for the polymerization of bulky substrates.[127]

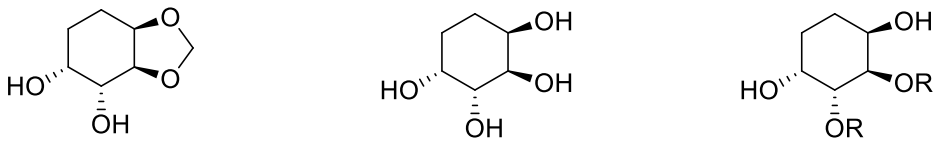


Figure 31. Potential substrates for N435-mediated polymerization.

5. Experimental Section

5.1 General experimental

Reactions were carried out under inert atmosphere in flame dried glassware unless stated otherwise. Solvents were distilled: CH_2Cl_2, DMF, iPr_2Net, DCE and pyridine from CaH_2; MeOH from magnesium methoxide; THF from Na/benzophenone; toluene from Na. Qualitative TLC was done with pre-coated silica gel aluminium sheets (EMD silica gel 60 F254); detection by UV or by spraying with "CAM" solution (5 g of $(NH_4)_6Mo_7O_{24}.4H_2O$, 1 g of $Ce(SO_4)_2$, 100 ml of 10% H_2SO_4) or 0.5% aqueous $KMnO_4$ solution followed by heating. Column chromatography was performed using silica gel SiliaFlash P60 from Silicycle (40-66 µm). IR spectra was recorded as solutions in $CHCl_3$ or as a neat sample. ^{29}Si NMR spectra were acquired at 60, 80, and 120 MHz ^{1}H NMR and ^{13}C NMR spectra were recorded at 300, 400 or 600 MHz and 75, 100 or 150 MHz, respectively, and were calibrated to the solvent signal or tetramethylsilane; the chemical shifts are reported in ppm. Mass spectra were recorded on Kratos/MsI Concept lS mass spectrometer at Brock University. Combustion analyses were performed by Atlantic Microlabs, Norcross, GA. Melting points were recorded on a Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured on a Mandel Rudolph Research Analytical Automatic Polarimeter at 589 nm with a cell length of 50 mm and was measured at 18-22 °C; concentration *c* in g/100 ml. IR spectra were obtained on a Bruker ATR FT-IR Spectrometer and PerkinElmer SpectrumOne. Mass Spectrometry (Electron Impact [EI] and Matrix Assisted Laser Desorption Time of Flight [MALDI-ToF]) Electron impact mass spectrometry (EI-MS) was carried out using a Kratos/MSI Concept 1S high resolution mass spectrometer in positive ion mode. MALDI-ToF MS spectra

were acquired on a Bruker Autoflex MALDI-ToF mass spectrometer in the positive ion mode. Samples were dissolved into HPLC grade THF or acetone, sonicated, and combined with a NaCl/THF (acetone) mixture and sonicated a second time. A small sample was transferred to a stainless steel plate that was preloaded with a dried dithranol spot deposited from a THF solution. Gel Permeation Chromatography (GPC) Polymer molecular weights and polydispersity indices (relative to polystyrene standards) were analyzed via GPC using a Waters 2695 Separations Module equipped with a Waters 2414 refractive index detector, a Waters 2996 photodiode array detector, and three Jordi Fluorinated DVB mixed bed columns. THF was used as the eluent at a flow rate of 1.0 mL/min.

5.2 General procedure for enzymatic reactions

A borosilicate, 20 mL Radley's reaction vessel attached to a water cooled condenser is utilized for enzymatic reactions in toluene, while a borosilicate vial (0.5 cm^3) with teflon cap is utilized for solvent-free sealed tube reactions.

Chiral diols and diester are mixed together in a 1:1 molar ratio for 5 minutes at 100 ° C. After the mixture turns homogenous, N435 is added, generally unless mentioned otherwise, reactions are catalysed by 10 wt% N435 (with respect to the total weight of the starting material). Toluene is added to the solvated reaction, 1 mL of toluene is added for every 0.2 mmol of diol present in the reaction. Water jacketed condensers are used for solvated reactions. Reactions are incubated at 100 °C for 24 h or 7 d. Once reaction is completed, it is cooled down to room temperature. 3 mL of Et_2O are added to quench the reaction; reaction is stirred for 5 minutes before being filtered. Enzyme beads are filtered through a medium porosity Büchner funnel, N435 beads are washed with 5 ml Et_2O and 5

mL of $CHCl_3$. The organic phases were concentrated under reduced pressure. Control reactions are conducted in similar fashion; however the addition of the diols and the enzymes is omitted in each case.

Diol **4**	Diester **135**	Conditions	MALDI-ToF		GPC	
			M_w g/mo)	M_n g/mol	M_w g/mol	M_n g/mol
54.8 mg (0.2 mmol)	104.1 mg (0.2 mmol)	7 d, toluene, 100 °C, 10 wt% N435, 150 rpm.	617.6	586.34	826	803
49.0 mg (0.18 mmol)	93.1 mg (0.18 mmol)		574.4	554.7		
46.9 mg (0.17 mmol)	89.1 mg (0.17 mmol)		658.7	626.0		
45.0 mg (0.17 mmol)	84.4 mg (0.17 mmol)	24 h, toluene, 100 °C, 10 wt% N435, 150 rpm.	651.2	621.2		
45.6 mg (0.17 mmol)	89.1 mg (0.17 mmol)		775.0	679.0		
44.9 mg (0.17 mmol)	86 mg (0.17 mmol)		769.1	649.3		
Diol **5**	Diester **135**					
29 mg (0.15 mmol)	79.6 mg (0.15 mmol)	7 d, toluene, 100 °C, 10 wt% N435, 150 rpm.	857.2	704.4		
28 mg (0.15 mmol)	76.1 mg (0.18 mmol)		912.3	804.6		
30.9 mg (0.17 mmol)	84.1mg (0.17 mmol)		922.8	764.5		
31 mg (0.17 mmol)	85.4 mg (0.17 mmol)	24 h, toluene, 100 °C, 10 wt%	613.0	594.2		

31 mg (0.17 mmol)	84 mg (0.17 mmol)	N435, 150 rpm.	686.9	638.8	823	801
32.9 mg (0.17 mmol)	87 mg (0.17 mmol)		636.8	612.7		
Diol **6**	Diester **135**					
35 mg (0.17 mmol)	86 mg (0.15 mmol)	7 d, toluene, 100 °C, 10 wt% N435, 150 rpm.	857.2	796.8		
36 mg (0.17 mmol)	86.1 mg (0.18 mmol)		987.1	897.9		
35 mg (0.17 mmol)	90.1mg (0.18 mmol)		977.32	856.6	1432	1124
35 mg (0.17 mmol)	85.4 mg (0.17 mmol)	24 h, toluene, 100 °C, 10 wt% N435, 150 rpm.	754.7	743.1		
35 mg (0.17 mmol)	86 mg (0.17 mmol)		756.1	744.5		
22.9 mg (0.11 mmol)	55.7 mg (0.11 mmol)		757.3	743.1		

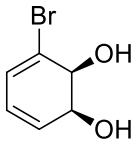
Table 7. Selected experimental amounts for N435-mediated transesterification reactions in toluene.

Diol **4**	Diester **135**	Conditions	GPC	
			M_w	M_n
36 mg (0.14 mmol)	67 mg (0.13mmol)	Solvent-free, 100 °C, 10 wt% N435	894	843
41mg (0.15 mmol)	77 mg (0.15mmol)	Solvent-free, 100 °C, 20 wt% N435	887	838
37 mg (0.14 mmol)	78 mg (0.15mmol)	Solvent-free, 100 °C, 30 wt% N435	918	854
Diol **5**	Diester **135**			
32 mg (0.14 mmol)	88 mg (0.18mmol)	Solvent-free, 100 °C, 10 wt% N435	892	842
27 mg (0.15 mmol)	74 mg (0.15mmol)	Solvent-free, 100 °C, 20 wt% N435	904	850
24 mg (0.13 mmol)	72 mg (0.14mmol)	Solvent-free, 100 °C, 30 wt% N435	1042	933
61mg (0.32 mmol)	77 mg (0.15mmol)	Solvent-free, 100 °C, 10 wt% N435	862	838
15 mg (0.08mmol)	72 mg (0.14mmol)		976	889
Diol **6**	Diester **135**			
25mg (0.13 mmol)	67 mg (0.13mmol)	Solvent-free, 100 °C, 10 wt% N435	938	865
27mg (0.14 mmol)	72 mg (0.14mmol)	Solvent-free, 100 °C, 20 wt% N435	1296	1057
28mg (0.15 mmol)	78 mg (0.15mmol)	Solvent-free, 100 °C, 30 wt% N435	1106	963
Reactions are performed over 7 d, 150 rpm, in a sealed tube < 0.5cm^3.				

Table 8. Selected experimental amounts for N435-mediated transesterification reactions in solvent-free conditions.

5.3 Experimental procedures

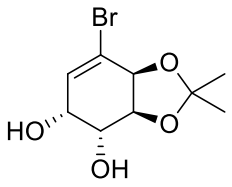
(1*S*,2*S*)-3-Bromocyclohexa-3,5-diene-1,2-diol (2)[117]



The enantiopure arene-*cis*-dihydrodiols are produced through medium-scale fermentation with *Escherichia coli* JM109 (pDTG601). Medium scale whole cell fermentation of bromobenzene **1** (10 -15 L) can produce up to 90-120 grams of the corresponding arene-*cis*-dihydrodiols **2**. Fermentations were conducted according to literature protocols.[117] The produced diol **2** is re-crystallized through EtOAc/hexanes and physical data are compared to literature values.[39]

2: R_f = 0.3 [hexanes/EtOAc (40:60)]; mp 91 ^{o}C (EtOAc/), [lit.[39] 91-94°C (EtOAc)]; $[\alpha]_D^{20}$ = 24 (*c* = 1.2, MeOH) [lit.[39] $[\alpha]_D^{20}$ = 20 (*c* = 2.8, MeOH)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, *J* = 5.8 Hz, 1H), 5.94 (dd, *J* = 9.5, 2.6 Hz, 1H), 5.84 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.49 (s, 1H), 4.27 (d, *J* = 6.2 Hz, 1H), 2.74 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 129.3, 127.00, 126.03, 123.9, 72.5, 69.2.

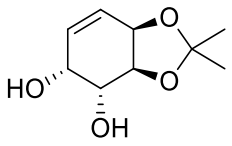
(3a*S*,4R,5*R*,7a*S*)-7-Bromo-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxole-4,5-diol (4)[44]



A magnetically stirred solution of **2** (2.0 g, 10.4 mmol) in 2,2-dimethoxypropane (25 mL) was treated with *p*-toluenesulfonic acid (40 mg, 0.21 mmol). After stirring for 10 min, the reaction mixture was quenched with concentrated solution of sodium bicarbonate (1 mL) and the resulting mixture was concentrated under reduced pressure. The residue thus obtained was dissolved in CH_2Cl_2 (25 mL). The separated aqueous phase was extracted with CH_2Cl_2 (1 × 10 mL), and the combined organic phases were washed with $NaHCO_3$ (1 × 10 mL of a saturated aqueous solution) before being dried ($MgSO_4$), filtered, and then concentrated under reduced pressure. The residue presumed to contain the acetonide, was dissolved in acetone/water (50 ml of 80:20 v/v mixture), the resulting solution was cooled to 0 °C and then treated with *N*-methylmorpholine *N*-oxide (2.50 g, 21.34 mmol) and osmium tetroxide (21 mg, 0.08 mmol). Isopropanol (2 mL) was added to the reaction mixture. Stirring was continued for 72 h, during which time the reaction mixture was maintained at 0 °C. Reaction was monitored by TLC, after the consumption of the starting material the reaction mixture was treated with sodium metabisulfite (5 mL of a saturated aqueous solution). The aqueous phase was extracted with EtOAc (4 × 40 mL), and the combined organic phases were then dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resulting yellow residue was

subjected to flash chromatography hexanes/EtOAc (50:50) and concentration of the appropriate fractions afforded **4** 2.0 g (74% over two steps) as a white, crystalline solid. **4:** R_f = 0.34 [hexanes/EtOAc (50:50)]; mp 114 ^{o}C (EtOAc/hexanes), [lit.[44] 113-114 °C (EtOAc/hexanes)]; $[\alpha]_D^{20}$ = –9.8 (*c* = 1.2, $CHCl_3$) [lit.[44] $[\alpha]_D^{20}$ = -11.6 (*c* = 2.8, CHCl3)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13 (d, *J* = 3.1 Hz, 1H), 4.63 (d, *J* = 5.2 Hz, 1H), 4.50 – 4.33 (m, 1H), 4.29 (s, 1H), 4.16 (s, 1H), 4.11 (s, 2H), 1.40 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.1, 123.6, 110.3, 76.4, 76.2, 69.4, 67.4, 27.7, 26.8; Anal. Calcd for $C_9H_{13}BrO_4$: C, 40.78; H, 4.94. Found C, 40.99; H, 5.01.

(3a*S*,4*R*,5*R*,7a*R*)-2,2-Dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxole-4,5-diol (5)[44]

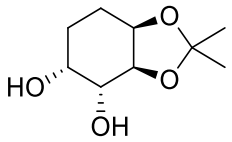


To a flame-dried round-bottom with attached reflux condenser was charged a suspension of **4** (2.0 g, 7.5 mmol) and tributyltin hydride (2.5 g, 8.5 mmol) in THF (50 mL). Argon was bubbled through the mixture for 10 min. AIBN (0.16 g, 1 mmol) was added the reaction mixture was immersed in pre-heated oil bath at 90 ˚C. After 8 h, the mixture was concentrated under reduced pressure, and the residue was dissolved in a mixture of hexanes and acetonitrile (50:50), the acetonitrile fraction was concentrated under reduced pressure, the resulting solid was purified by column chromatography on silica gel

hexanes/EtOAc (50:50) to give 1.1 g (78%) of **5** as a white crystalline solid.

5: R_f = 0.31 [hexanes/EtOAc (25:75)]; mp 67°C (EtOAc/hexanes), [lit.[44] 66-67 °C (EtOAc/hexanes)]; $[\alpha]_D^{20}$ = –149 (*c* = 2.5, $CHCl_3$) [lit.[44] $[\alpha]_D^{20}$ = -151.1 (*c* = 2.8, CHCl3)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 – 5.92 (m, 2H), 4.66 (dd, *J* = 5.9, 2.5 Hz, 1H), 4.39 – 4.31 (m, 2H), 4.01 – 3.96 (m, 1H), 2.39 (d, *J* = 4.1 Hz, 1H), 2.29 (d, *J* = 4.9 Hz, 1H), 1.44 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.8, 127.6, 109.5, 75.7, 71.8, 71.2, 65.9, 27.9, 25.9; Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found C, 57.75; H, 7.54.

(3a*S*,4*R*,5*R*,7a*R*)-2,2-Dimethylhexahydrobenzo[d][1,3]dioxole-4,5-diol (6)

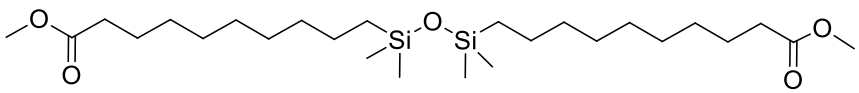


To a solution of **5** (1.0 g, 5.3 mmol) in MeOH (10 mL) was added 10 % Pd/C (100 mg). Hydrogen was bubbled through the mixture for 5 min then the reaction mixture was stirred under hydrogen pressure (300 psi). After 8 h, the catalyst was filtered off and the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel hexanes/EtOAc (10:90) to yield 0.7 g (69%) of **6** as colourless crystalline solid.

6: R_f = 0.58 [MeOH/EtOAc (10:90)]; mp 101-102°C (hexanes/EtOAc), $[\alpha]_D^{20}$ = – 90.6 (*c* = 0.5, $CHCl_3$); IR (ATR) *v* 3450, 3398, 3274, 2982, 2944, 2877, 1415, 1378, 1333, 1241, 1222, 1051, 874 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.31 – 4.29 (m, 1H), 4.07 (dd, *J* =

7.0, 5.3 Hz, 1H), 4.04 – 4.02 (m, 1H), 3.65 (dd, *J* = 7.0, 2.6 Hz, 1H), 3.05 (s, 1H), 2.61 (s, 1H), 2.12 – 2.05 (m, 1H), 1.90 – 1.86 (m, 1H), 1.79 – 1.70 (m, 2H), 1.49 (s, 3H), 1.36 (s, 3H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.5, 78.9, 73.9, 73.8, 69.3, 28.4, 26.3, 25.0, 21.1; MS (EI) *m/z* (%) 57 (42), 67 (80), 95 (41), 173 (100); HRMS (EI) calcd for $C_9H_{16}O_4$ (M – CH_3 species) 173.0814. Found 173.0807; Anal. Calcd for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found C, 57.46; H, 8.61.

Dimethyl 10,10'-(1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(decanoate) (135) [120, 141]

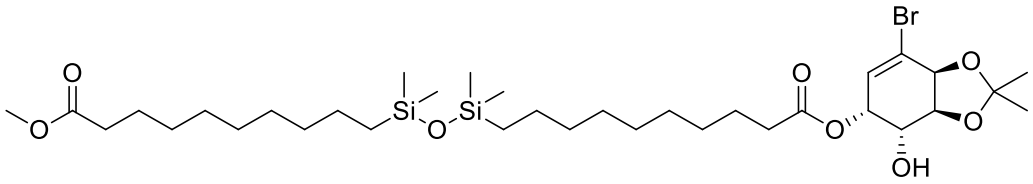


To a solution of methyl dec-9-enoate (5.0 g, 27.1 mmol) in toluene (30 mL) was added 1,1,3,3-Tetramethyldisiloxane (1.8 g, 13.4 mmol). Argon was bubbled through the mixture for 5 min then Karstedt's catalyst was added (20μL). The mixture was heated to reflux for 9 h then the catalyst was filtered off and the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel hexanes/EtOAc (90:10) to yield 5.3 g (79%) of **135** as colourless oil.

135: R_f = 0.63 [Hexanes/EtOAc (90:10)]; IR (ATR) *v* 2920, 2852, 1740, 1435, 1360, 1251, 1170, 1504, 840, 794 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.66 (s, 6H), 2.29 (t, *J* = 7.5 Hz, 4H), 1.63 – 1.56 (m, 4H), 1.27 (bs, 24H), 0.48 (t, *J* = 7.4 Hz, 4H), 0.02 (s, 12H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 51.4, 34.1, 33.4, 29.4, 29.39, 29.31, 29.2, 25.0, 23.3, 18.4, 0.4; ^{29}Si NMR (60 MHz, $CDCl_3$) δ 7.26; MS (EI) *m/z* (%) 89 (100), 163 (91);

HRMS (EI) calcd for $C_{26}H_{54}O_5Si_2$ (M – CH_3 species) 487.3275 Found 487.3274; Anal. Calcd for $C_{26}H_{54}O_5Si_2$: C, 62.10; H, 10.82. Found C, 62.24; H, 10.66.

(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-4-hydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (145)

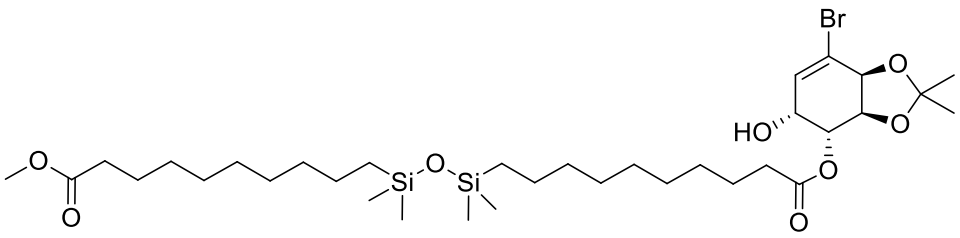


135 (85 mg, 0.17 mmol) was added to a stirred solution of diol **4** (45 mg, 0.17 mmol) and stirred for 5 min in toluene (0.85 mL) maintained at 100 °C. The resulting mixture was charged with N435 (14 mg). The reaction mixture was heated 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (97:3) and deactivated silica (10 wt% H_2O) to give 5 mg (4%) of **(145)** as colorless oil.

145: R_f = 0.32 [Hexanes/EtOAc (80:20)]; $[\alpha]_D^{20}$ = 15.1 (*c* = 0.35, $CHCl_3$);); IR (ATR) *v* 3466, 2922, 2853, 1741,1437, 1372, 1250, 1163, 1051, 838, 787 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.11 (d, *J* = 2.4 Hz, 1H), 5.42 – 5.40 (m, 1H), 4.69 (d, *J* = 5.3 Hz, 1H), 4.45 – 4.42 (m, 1H), 4.29 – 4.26 (m, 1H), 3.67 (s, 3H), 2.40 - 2.28 (m, 4H), 1.75 – 1.57

(m, 4H), 1.46 (s, 3H), 1.41 (s, 3H), 1.28 (bs, 24H), 0.49 (bs, 4H), 0.02 (bs, 12H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 172.7, 127.5, 125.2, 110.5, 76.3, 76.1, 69.6, 68.3, 51.4, 34.2, 34.1, 33.4, 29.4, 29.34, 29.31, 29.2, 29.18, 29.12, 27.7, 26.1, 25.0, 24.9, 23.3, 18.4, 0.4; ^{29}Si NMR (80 MHz, $CDCl_3$) 7.3; MS (EI) *m/z* (%); 133 (34), 287 (60), 317 (100) HRMS (EI) calcd for $C_{34}H_{63}BrO_8Si_2$: (^{81}Br, M – CH_3 species)721.2991. Found 721.2973.

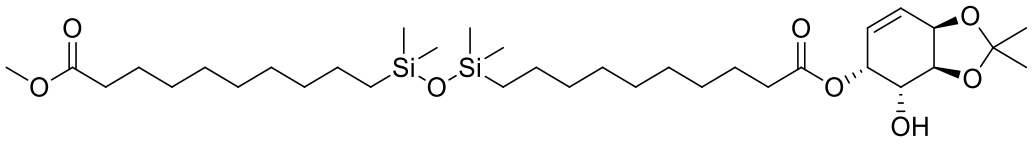
(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-5-hydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (146)



135 (85 mg, 0.17 mmol) was added to a stirred solution of diol **4** (45 mg, 0.17 mmol) and stirred for 5 min in toluene (0.85 mL) maintained at 100 °C. The resulting mixture was charged with N435 (14 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (97:3) and deactivated silica (10 wt% H_2O) to give 2 mg (1.6%) of **146** as colorless oil.

146: R_f = 0.37 [Hexanes/EtOAc (80:20)]; $[\alpha]_D^{20}$ = -9.8 (*c* = 0.30, $CHCl_3$);); IR (ATR) *v* 3431, 2920, 2852, 2323, 2041, 1994, 1904, 1740, 1655, 1459, 1438, 1371, 1250, 1163, 1047, 840, 792 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.20 (d, *J* = 2.7 Hz, 1H), 5.42 – 5.39 (m, 1H), 4.60 (d, *J* = 5.1 Hz, 1H), 4.49 – 4.44 (m, 2H), 3.66 (s, 3H), 2.40 – 2.28 (m, 4H), 1.63 – 1.57 (m, 4H), 1.44 (s, 3H), 1.39 (s, 3H), 1.27 (bs, 24H), 0.49 (bs, 4H), 0.02 (bs, 12H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 173.4, 131.4, 123.4, 110.7, 76.3, 74.0, 70.9, 66.1, 51.5, 34.2, 34.1, 33.4, 29.4, 29.34, 29.32, 29.2, 29.1, 27.5, 26.2, 24.99, 25.95, 23.3, 18.4, 0.4; ^{29}Si NMR (80 MHz, $CDCl_3$) 7.3; MS (EI) *m/z* (%); 133 (33), 287 (64), 317 (100) HRMS (EI) calcd for $C_{34}H_{63}BrO_8Si_2$: (^{81}Br, M – CH_3 species)721.2991. Found 721.2991.

(3a*S*,4*R*,5*R*,7a*R*)-4-Hydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (147)

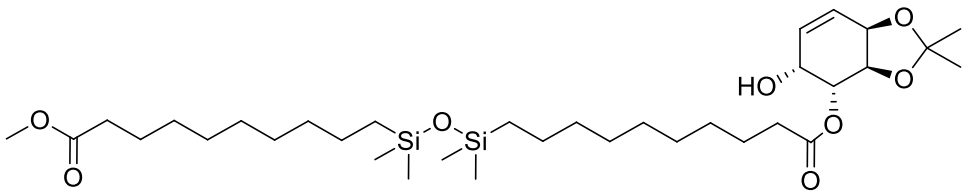


135 (85 mg, 0.17 mmol) was added to a stirred solution of diol **5** (33 mg, 0.18 mmol) and stirred for 5 min in toluene (0.9 mL) maintained at 100 °C. The resulting mixture was charged with N435 (12 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic

phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) and deactivated silica (10 wt% H_2O) to give 9 mg (8%) of **(147)** as colorless oil.

147: R_f = 0.46 [Hexanes/EtOAc (70:30)]; $[\alpha]_D^{20}$ = -69.1 (*c* = 0.75, MeOH); IR (ATR) *v* 3460, 2922, 2853, 1739, 1459, 1437, 1371, 1250, 1214, 1165, 1050, 920, 838, 789, 518 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.00 – 5.97 (m, 1H), 5.89 (dd, *J* = 10.2, 4.2 Hz, 1H), 5.40 – 5.38 (m, 1H), 4.70 – 4.67 (m, 1H), 4.35 (dd, *J* = 7.0, 6.1 Hz, 1H), 4.09 (dd, *J* = 7.0, 3.6 Hz, 1H), 3.66 (s, 3H), 2.37 – 2.28 (m, 4H), 1.64 – 1.59 (m, 4H), 1.46 (s, 3H), 1.39 (s, 3H), 1.27 (bs, 24H), 0.49 (t, *J* = 7.5 Hz, 4H), 0.02 (bs, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 173.2, 129.1, 126.6, 109.7, 75.6, 71.8, 69.9, 68.5, 51.5, 34.3, 34.1, 33.4, 29.43, 29.41, 29.35, 29.34, 29.31, 29.2, 29.1, 27.9, 25.8, 25.0, 23.3, 18.4, 0.4. ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.28; MS (EI) *m/z* (%) 57 (100), 69 (48), 71 (65), 85 (48), 97 (35), 149 (31), 317 (14); HRMS (EI) calcd for $C_{34}H_{64}O_8Si_2$ (M- CH_3 species): 641.3906. Found 641.3894.

(3a*R*,4*R*,5*R*,7a*R*)-5-Hydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (148)

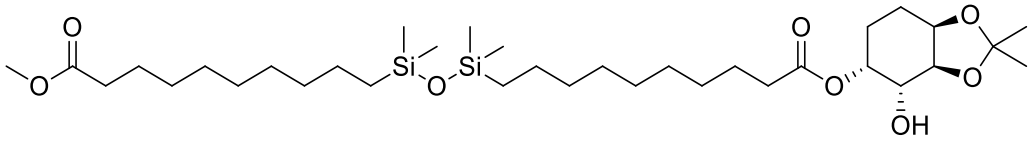


135 (85 mg, 0.17 mmol) was added to a stirred solution of diol **5** (33 mg, 0.18 mmol) and stirred for 5 min in toluene (0.9 mL) maintained at 100 °C. The resulting mixture was charged with N435 (12 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) and deactivated silica (10 wt% H_2O) to give 3 mg (3%) of **(148)** as colorless oil.

148: R_f = 0.41 [Hexanes/EtOAc (70:30)]; $[\alpha]_D^{20}$ = - 41.8 (*c* = 0.15, MeOH); IR (ATR) *v* 3429, 2920, 2852, 1739, 1461, 1438, 1374, 1250, 1216, 1163, 1057, 920, 839, 790, 515, 425 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.95 (d, *J* = 2.3 Hz, 2H), 5.23 (dd, *J* = 7.2, 3.6 Hz, 1H), 4.65 (dd, *J* = 5.8, 1.8 Hz, 1H), 4.45 (dd, *J* = 7.1, 5.9 Hz, 1H), 4.42 (bs, 1H)., 3.67 (s, 3H), 2.41 – 2.28 (m, 4H), 1.99 (bs, 1H) 1.68 – 1.60 (m, 4H), 1.44 (s, 3H), 1.37 (s, 3H), 1.27 (bs, 24H), 0.49 (t, *J* = 6.4 Hz, 4H), 0.03 (s, 12H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 173.5, 129.9, 127.6, 109.8, 73.1, 72.9, 72.0, 64.9, 51.5, 34.3, 34.1, 33.4, 29.4, 29.41, 29.36, 29.32, 29.2, 29.1, 27.7, 26.0, 25.0, 23.3, 18.4, 0.4; ^{29}Si NMR (80 MHz,

$CDCl_3$) δ 7.29; MS (EI) *m/z* (%) 57 (100), 69 (52), 71 (67), 83 (46), 111 (27), 149 (62), 287 (36), 317 (56); HRMS (EI) calcd for $C_{34}H_{64}O_8Si_2$ (M- CH_3 species): 641.3906. Found 641.3942.

(3a*S*,4*R*,5*R*,7a*R*)-4-Hydroxy-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-5-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (149)

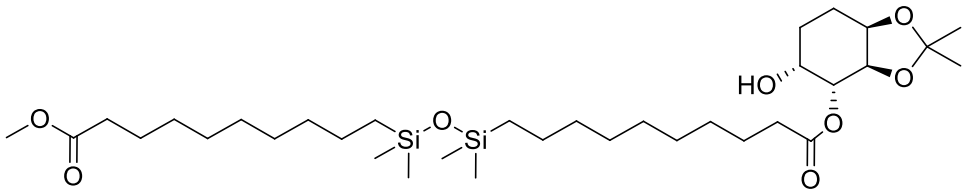


135 (87 mg, 0.17 mmol) was added to a stirred solution of diol **6** (33 mg, 0.175 mmol) and stirred for 5 min in toluene (0.87 mL) maintained at 100 °C. The resulting mixture was charged with N435 (12 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) and deactivated silica (10 wt% H_2O) to give 14 mg (12%) of **(149)** as colorless oil.

149: R_f = 0.44 [Hexanes/EtOAc (70:30)]; $[\alpha]_D^{20}$ = - 35.3 (*c* = 0.7, MeOH); IR (ATR) *v* 3468, 2922, 2853, 1737, 1437, 1369, 1249, 1369, 1249, 1215, 1163, 1055, 1036, 838, 789, 703, 512 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.18 – 5.16 (m, 1H), 4.35 – 4.31 (m,

1H)., 4.06 (dd, *J* = 6.9, 5.4 Hz, 1H), 3.81 – 3.77 (m, 1H), 3.66 (s, 3H), 2.35 – 2.28 (m, 4H), 2.20 (d, *J* = 4.3 Hz, 1H), 1.96 – 1.91 (m, 2H), 1.83 – 1.79 (m, 2H), 1.63 – 1.50 (m, 4H), 1.50 (s, 3H), 1.37 (s, 3H), 1.27 (bs, 24H), 0.48 (t, *J* = 7.4 Hz, 4H), 0.02 (bs, 12H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 173.5, 108.7, 78.7, 73.5, 72.3, 72.1, 51.4, 34.5, 34.1, 33.4, 29.44, 29.40, 29.33, 29.31, 29.2, 29.1, 28.4, 26.2, 25.1, 25.0, 23.3, 23.0, 22.0, 18.4, 0.7, 0.4, 0.1; ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.27; MS (EI) *m/z* (%) 55 (55), 67 (66), 95 (60), 155 (65), 325 (100); HRMS (EI) calcd for $C_{34}H_{66}O_8Si_2$ (M- CH_3 species): 643.4062 Found 643.4039; Anal. Calcd for $C_{34}H_{66}O_8Si_2$: C, 61.96; H, 10.09 Found C, 61.77; H, 10.09.

(3a*R*,4*R*,5*R*,7a*R*)-5-Hydroxy-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-4-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (150)

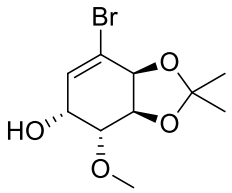


135 (87 mg, 0.17 mmol) was added to a stirred solution of diol **6** (33 mg, 0.175 mmol) and stirred for 5 min in toluene (0.87 mL) maintained at 100 °C. The resulting mixture was charged with N435 (12 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the

organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) and deactivated silica (10 wt% H_2O) to give 19 mg (16%) of **(150)** as colorless oil.

150: R_f = 0.48 [Hexanes/EtOAc (70:30)]; $[\alpha]_D^{20}$ = -30.7 (*c* = 0.9, MeOH); IR (ATR) *v* 3467, 2922, 2853, 1738, 1437, 1380, 1249, 1216, 1162, 1058, 838, 788, 704, 512 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.94 (dd, *J* = 7.8, 2.6 Hz, 1H), 4.36 – 4.33 (m, 1H), 4.21 (dd, *J* = 7.8, 5.1 Hz, 1H), 4.11 – 4.08 (m, 1H), 3.66 (s, 3H), 2.42 – 2.28 (m, 4H), 2.17 – 2.08 (m, 1H), 1.99 – 1.73 (m, 3H), 1.69 – 1.58 (m, 4H), 1.51 (s, 3H), 1.36 (s, 3H), 1.27 (bs, 24H), 0.49 (t, *J* = 7.5 Hz, 4H), 0.02 (bs, 12H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.4, 173.3, 108.7, 75.7, 75.7, 74.1, 68.3, 51.5, 34.4, 34.1, 33.44, 33.41, 29.45, 29.41, 29.33, 29.31, 29.2, 29.1, 28.1, 26.4, 25.0, 25.0, 24.8, 23.3, 20.6, 18.4, 0.7, 0.4, 0.1; ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.29; MS (EI) *m/z* (%) 55 (100), 57 (66), 67 (50), 95 (53), 317 (82), 325 (48); HRMS (EI) calcd for $C_{34}H_{66}O_8Si_2$ (M- CH_3 species): 643.4062 Found 643.4046; Anal. Calcd for $C_{34}H_{66}O_8Si_2$: C, 61.96; H, 10.09 Found C, 61.76; H, 9.98.

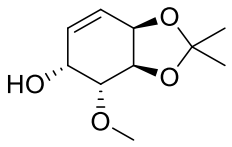
(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-4-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol (151).



To a solution of **165** (3.4 g, 7.8 mmol) in THF (30 mL) stirred under argon atmosphere, was added 5 ml of tetrabutylammonium fluoride solution (1.0 M in THF). After 1 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography hexanes/EtOAc (50:50) to yield 2.1 g (96%) of **151** as a white crystalline solid.

151: R_f = 0.56 [Hexanes/EtOAc (50:50)]; mp 65-67 ˚C (EtOAc); $[\alpha]_D^{20}$ = -7.5 (*c* = 1.1, $CHCl_3$); IR ($CHCl_3$) *v* 3613, 3025, 2991, 2936, 1646, 1454, 1383, 1375, 1229, 1212, 1077, 1049 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.15 (d, *J* = 2.9 Hz, 1H), 4.61 – 4.59 (m, 1H), 4.53 (t, *J* = 5.1 Hz, 1H), 4.35 – 4.30 (m, 1H), 3.76 (t, *J* = 4.2 Hz, 1H), 3.54 (s, 3H), 2.66 (d, *J* = 9.6 Hz, 1H), 1.43 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.0, 123.3, 110.3, 78.7, 76.2, 73.9, 66.3, 59.2, 27.6, 26.2; MS (EI) *m/z* (%) 124 (15), 115 (100), 59 (10), 55, (11), 43 (26); HRMS (EI) calcd for $C_{10}H_{15}BrO_4$: 278.0149. Found 278.0153; Anal. Calcd for $C_{10}H_{15}BrO_4$: C, 43.03; H, 5.42. Found C, 44.17; H, 5.44.

(3a*R*,4*R*,5*R*,7a*R*)-4-Methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol (152).

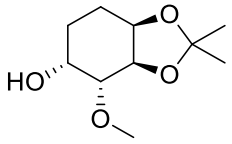


To a flame-dried argon purged round-bottom with attached reflux condenser was charged a suspension of **151** (2.0 g, 7.1 mmol) and tributyltin hydride (2.5 g, 8.5 mmol) in THF (50 mL). Argon was bubbled through the mixture for 30 min. AIBN (0.16 g, 1 mmol) was added to the mixture before it was immersed in a pre-heated oil bath at 90 ˚C. After 8 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel hexanes/EtOAc (50:50) to give 1.1 g (76%) of **152** as yellow oil.

152: R_f = 0.29 [Hexanes/EtOAc (50:50)]; $[\alpha]_D^{20}$ = -96.28 (*c* = 1.0, $CHCl_3$); IR (ATR) *v* 3448, 2983, 1736.7, 1457, 1215, 1160, 1055, 910 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.93 – 5.85 (m, 2H), 4.62 – 4.59 (m, 1H), 4.41 (t, *J* = 6.1 Hz, 1H), 4.30 (bs, 1H), 3.51 (bs, 4H), 2.65 (d, *J* = 6.1 Hz, 1H), 1.41 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.1, 127.6, 109.2, 80.5, 73.8, 71.9, 64.1, 58.7, 27.8, 25.8; MS (EI) *m/z* (%) 185 (10), 127 (12), 115 (100), 97 (20), 81 (14), 55 (13), 43(32); HRMS (EI) calcd for $C_{10}H_{16}O_4$: 200.1049. Found 200.1048. ; Anal. Calcd for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05. Found C, 59.22; H, 8.25.*

*Inseparable impurities in the same chromatographic region.

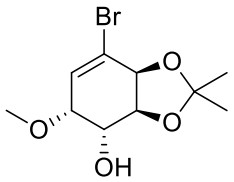
(3a*R*,4*R*,5*R*,7a*R*)-4-Methoxy-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-5-ol (153).



To a solution of **152** (1.0 g, 4.99 mmol) in MeOH (10 mL) was added 10 % Pd/C (100 mg, 1.06 mmol). Hydrogen was bubbled through the mixture for 5 min then the mixture was stirred under hydrogen pressure (400 psi). After 8 h, the catalyst was filtered off and the solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel Hexanes/EtOAc (50:50) to yield 0.7 g (69%) of **153** as yellow oil.

153: R_f = 0.26 [Hexanes/EtOAc (50:50)]; $[\alpha]_D^{20}$ = -62.16 (*c* = 1.2, $CHCl_3$); IR (ATR) *v* 3465, 2983, 2933, 1442, 1377, 1241, 1213, 1157, 1051, 872 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.25 (bs, 1H), 4.06 – 4.02 (m, 2H), 3.45 (s, 3H), 3.14 – 3.11 (m, 1H), 2.49 (s, 1H), 2.04 - 1.99 (m, 1H), 1.84-1.65 (m, 3H), 1.45 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.1, 83.0, 77.5, 74.0, 66.3, 57.7, 28.3, 26.2, 24.3, 20.7; MS (EI) *m/z* (%) 187 (100), 127 (16.3), 100 (17.7), 95 (19.0), 87 (33.2), 84(54.1), 71(42.2), 67(33.5), 59(33.8), 43 (59.5); HRMS (EI) calcd for $C_{10}H_{18}O_4$: 202.1204. Found 202.1205; Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found C, 59.09; H, 8.97.

(3aS,4R,5R,7aS)-7-Bromo-5-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (154)

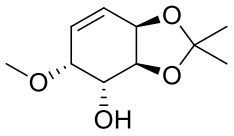


Concentrated Hydrochloric acid (2 mL) was added to a stirred solution of **166** (1.4 g, 4.3 mmol) in dry MeOH (50 mL) maintained at 0 °C under a argon atmosphere. Stirring was continued for 4 h at 0 °C then the reaction mixture was treated with ice−water (10 mL). The aqueous phase was extracted with EtOAc (3 × 25 mL) and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was dissolved in 2,2-dimethoxypropane (30 ml) and catalytic amount of *p*-toluenesulfonic acid (20 mg) was added, stirring was continued for 6 h, then reaction mixture was treated with concentrated solution of $NaHCO_3$ (2 × 1 mL). The reaction mixture was concentrated under reduced pressure The resulting aqueous phase was extracted with EtOAc (2 × 25 mL) and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography hexanes/EtOAc (50:50) to yield 0.5 g (40%) of **154** as a white crystalline solid.

154: R_f = 0.56 [Hexanes/EtOAc (50:50)]; mp 68-69 ˚C (EtOAc); $[\alpha]_D^{20}$ = -52.9 (*c* = 0.85, $CHCl_3$); IR (ATR) *v* 3512, 2995, 2935, 2899, 2829, 1640, 1342, 1198, 999 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.15 (dd, *J* = 2.5, 0.9 Hz, 1H), 4.64 (dd, *J* = 5.4, 1.3 Hz, 1H), 4.47

(t, *J* = 5.1 Hz, 1H), 4.33 (t, *J* = 3.9 Hz, 1H), 3.98 (t, *J* = 3.9 Hz, 1H), 3.47 (s, 3H), 2.44 (s, 1H), 1.42 (s, 3H), 1.40 (s, 3H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.2, 124.2, 110.0, 76.1, 67.1, 57.1, 27.6, 26.1; MS (EI) *m/z* (%) 115 (100), 124 (11), 15); HRMS (EI) calcd for $C_{10}H_{15}BrO_4$ (M-CH_3 species) 26.9997. Found 263.9951; Anal. Calcd for $C_{10}H_{15}BrO_4$: C, 43.03; H, 5.42. Found C, 43.33; H, 5.39.

(3a*S*,4*R*,5*R*,7a*R*)-5-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (155).



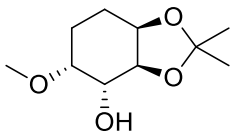
To a flame-dried argon purged round-bottom with attached reflux condenser was charged a suspension of **154** (0.5 g, 2.5 mmol) and tributyltin hydride (0.87 g, 3 mmol) in THF (50 mL). Argon was bubbled through the mixture for 30 min. AIBN (0.16 g, 1 mmol) was added to the mixture before it was immersed in pre-heated oil bath at 90 ˚C. After 8 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel hexanes/EtOAc (25:75) to give 0.34 g (67%) of **155** as colorless oil.

155: R_f = 0.26 [hexanes/EtOAc (50:50)]; $[\alpha]_D^{20}$ = -162.5 (*c* = 3.0, MeOH); IR (ATR) *v* 3439, 2985, 2931, 2825, 1643, 1457, 1375, 1218, 1159, 1098, 1045, 928, 865, 791 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (d, *J* = 1.2 Hz, 2H), 4.49 (d, *J* = 6.0 Hz, 2H), 4.24 (t, *J* = 5.8 Hz, 1H), 4.02 – 4.00 (m, 1H), 3.73 – 3.72 (m, 1H), 3.30 (s, 3H), 2.98 (s, 1H), 1.25 (s, 3H), 1.21 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 127.8, 127.3, 109.0, 75.4, 74.7, 71.6, 68.6, 56.8, 27.6, 25.8; MS (EI) *m/z* (%) 53 (10), 55 (17), 81 (19), 97 (25), 115 (100); HRMS (EI) calcd for $C_{10}H_{16}O_4$ (M- CH3 species): 185.0814. Found 185.0810; Anal. Calcd for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05. Found C, 59.36; H, 8.14.*

*Inseparable impurities in the same chromatographic region.

(3a*S*,4*R*,5*R*,7a*R*)-5-Methoxy-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-4-ol (156).

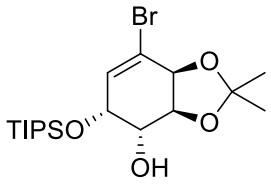


To a solution of **155** (1.4 g, 7 mmol) in MeOH (10 mL) was added 10 % Pd/C (100 mg, 1.06 mmol). Hydrogen was bubbled through the mixture for 5 min then the reaction mixture was stirred under hydrogen pressure (400 psi). After 8 h, the catalyst was filtered off and the solution was concentrated under reduced pressure. The residue was purified by column chromatography hexanes/EtOAc (50:50) to yield 1.2 g (84%) of **156** as colorless oil.

156: R_f = 0.23 [Hexanes/EtOAc (50:50)]; $[\alpha]_D^{20}$ = -116.5 (*c* = 0.87, MeOH); IR (ATR) *v* 3450, 2984, 2934, 2879, 2827, 1634, 1456, 1373, 1336, 1243, 1214, 1160, 1054, 1011, 909, 875, 857, 825, 788, 720; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 – 4.14 (m, 1H), 3.95 –

3.92 (m, 1H), 3.58 (bs, 1H), 3.47 – 3.45 (m, 1H), 3.23 (s, 3H), 2.93 (d, *J* = 5.7 Hz, 1H), 1.84 – 1.67 (m, 3H), 1.55 – 1.45 (m, 1H), 1.36 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 108.2, 79.0, 78.4, 73.6, 72.45, 56.4, 28.3, 26.1, 21.7, 21.2. MS (EI) *m/z* (%) 67 (36), 84 (34), 127 (18), 187 (100); HRMS (EI) calcd for $C_{10}H_{18}O_4$: (M - CH_3 species) 187.0970. Found 187.0974; Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found C, 59.10; H, 9.00.

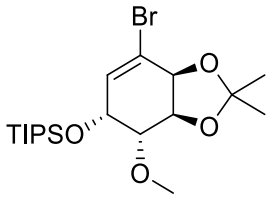
(3a*S*,4*S*,5*R*,7a*S*)-7-Bromo-2,2-dimethyl-5-((triisopropylsilyl)oxy)-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (164).



Triisopropylsilyl trifluoromethanesulfonate (7.2 mL, 27 mmol) was added dropwise to a stirred solution of diol **4** (6.0 g, 22.6 mmol) and 2,6-lutidine (5.3 mL, 45 mmol) in CH_2Cl_2 (115 mL) maintained at −78 °C under a argon atmosphere.[124] The resulting mixture was allowed to warm to rt over 3 h, then treated with NH_4Cl (60 mL of a saturated aqueous solution).[124] The separated aqueous phase was extracted with CH_2Cl_2 (3× 40 mL) and the combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography hexanes/EtOAc (97:3) to give 5.8 g (61%) of **164** as yellow oil.

164: R_f = 0.35 [Hexanes/EtOAc (90:10)]; $[\alpha]_D^{20}$ = -25.5 (*c* = 0.33, $CHCl_3$);); IR ($CHCl_3$) *v* 3560, 2941, 2866, 1644, 1461, 1370, 1339, 1230, 1146, 1077, 1053, 879, 679 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.01 – 5.98 (m, 1H), 4.63 – 4.61 (m, 1H), 4.50 – 4.47 (m, 2H), 4.24 (t, *J* = 3.9 Hz, 1H), 2.67 (d, *J* = 1.4 Hz, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.12 – 1.01 (m, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.8, 123.5, 110.0, 75.9, 75.7, 69.3, 68.3, 27.5, 26.2, 18.0, 12.1; MS (EI) *m/z* (%) 376 (16), 322 (21), 321 (100), 319 (98), 303 (32), 301 (31), 240 (52), 159 (62); HRMS (EI) calcd for $C_{17}H_{30}BrO_4Si$: 405.1098. Found 405.1096.

(((3a*S*,4*S*,5*R*,7a*S*)-7-Bromo-4-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl)oxy)triisopropylsilane (165)

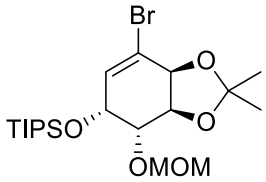


Sodium hydride (0.58 g, 24 mmol) was added to a stirred solution of alcohol **164** (8.5 g, 20.1 mmol) and iodomethane (1.6 mL, 26 mmol) in dry THF (70 mL) maintained at 0 °C under a argon atmosphere. Stirring was continued for 4 h at 0 °C then the reaction mixture was treated with ice−water (10 mL). The separated aqueous phase was extracted with EtOAc (3 × 25 mL) and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting light yellow oil was

subjected to flash chromatography hexanes/EtOAc (90:10) to give 5.5 g (63%) of **165** as a white crystalline solid.

165: R_f = 0.34 [Hexanes/EtOAc (90:10)]; mp 62-63 ˚C (EtOAc); $[\alpha]_D^{20}$ = -55.8 (*c* = 1.5, $CHCl_3$); IR (ATR) *v* 2940, 2889, 2865, 1650, 1462, 1040, 880 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.16 (s, 1H), 4.63 (d, *J* = 5.2 Hz, 1H), 4.57 (s, 1H), 4.50 – 4.38 (m, 1H), 3.71 (s, 1H), 3.55 (s, 3H), 1.42 (s, 3H), 1.39 (s, 3H), 1.09 (bs, 21H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.1, 122.2, 109.9, 80.3, 75.1, 68.1, 59.7, 27.5, 26.0, 18.0, 12.3; MS (EI) *m/z* (%) 75 (50), 89 (43), 145 (100), 254 (49), 393 (36); HRMS (EI) calcd for $C_{19}H_{35}BrO_4Si$(M-CH_3): 421.1234. Found 421.1229; Anal. Calcd for $C_{19}H_{35}BrO_4Si$: C, 52.40; H, 8.10. Found C, 52.68; H, 8.09.

(((3a*S*,4*S*,5*R*,7a*S*)-7-Bromo-4-(methoxymethoxy)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl)oxy)triisopropylsilane (166)

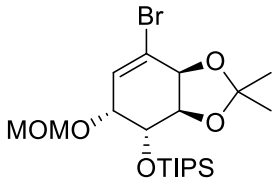


Sodium hydride (0.72 g, 30 mmol) was added to a stirred solution of alcohol **164** (11.0 g, 26 mmol) and chloromethyl methyl ether (2.4 mL, 30 mmol) in dry THF (100 mL) maintained at 0 °C under a argon atmosphere. Stirring was continued for 12 h at 0 °C, then the reaction mixture was treated with ice−water (10 mL) and NH_4Cl (10 mL). The separated aqueous phase was extracted with EtOAc (2 × 40 mL) and the combined

organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography hexanes/EtOAc (90:10) to give 6.9 g (56%) of **166** as clear colorless oil.

166: R_f = 0.58 [Hexanes/EtOAc (90:10)]; $[\alpha]_D^{20}$ = -85.65 (*c* = 0.4, MeOH); IR (ATR) *v* 2940, 2889, 2865, 1650, 1462, 1040, 880 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.14 (d, *J* = 2.3 Hz, 1H), 4.87 (d, *J* = 6.7 Hz, 1H), 4.72 (d, *J* = 6.7 Hz, 1H), 4.65 (d, *J* = 5.5 Hz, 1H), 4.58 (s, 1H), 4.47 (t, *J* = 5.3 Hz, 1H), 4.16 – 4.14 (m, 1H), 3.39 (s, 3H), 1.42 (s, 3H), 1.40 (s, 3H), 1.10 – 0.95 (m, 21H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 133.2, 122.3, 110.1, 97.2, 76.0, 75.9, 68.2, 55.7, 27.5, 26.2, 18.03, 18.0, 12.3; MS (EI) *m/z* 75 (56), 117 (62), 133 (100), 145 (100); HRMS (EI) calcd for $C_{20}H_{37}BrO_5Si$ (M – CH_3 species) 449.1359 Found 449.1357.

(((3a*R*,4*R*,5*R*,7a*S*)-7-Bromo-5-(methoxymethoxy)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)oxy)triisopropylsilane. (167)

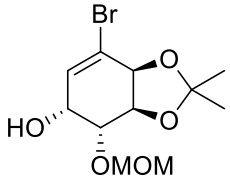


Sodium hydride (0.72 g, 30 mmol) was added to a stirred solution of alcohol **164** (11.0 g, 26 mmol) and chloromethyl methyl ether (2.4 mL, 30 mmol) in dry THF (100 mL) maintained at 0 °C under a argon atmosphere. Stirring was continued for 12 h at 0 °C, then the reaction mixture was treated with ice−water (10 mL) and NH_4Cl (10 mL). The separated aqueous phase was extracted with EtOAc (2 × 40 mL) and the combined

organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography hexanes/EtOAc (90:10) to give 3.1 g (25%) of **167** as clear colorless oil.

167: R_f = 0.63 [Hexanes/EtOAc (90:10)]; $[\alpha]_D^{20}$ = -29.9 (*c* = 0.52, MeOH); IR (ATR) *v* 2942, 2866, 1463, 1381, 1236, 1211, 1154, 1103, 1076, 1043 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 – 6.13 (m, 1H), 4.74 – 4.67 (m, 2H), 4.63 (dd, *J* = 5.4, 1.3 Hz, 1H), 4.42 – 4.40 (m, 1H), 4.37 – 4.34 (m, 1H), 4.26 – 4.23 (m, 1H), 3.38 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H), 1.11 – 1.00 (m, 21H).^{13}C NMR (75 MHz, $CDCl_3$) δ 130.3, 122.4, 110.1, 96.0, 77.2, 77.1, 73.5, 70.8, 55.6, 27.5, 26.2, 18.04, 18.00, 12.5. MS (EI) *m/z* 75 (51), 117 (56), 133 (96), 145 (100); HRMS (EI) calcd for $C_{20}H_{37}BrO_5Si$ (M – CH_3 species) 449.1359 Found 449.1354.

(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-4-(methoxymethoxy)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-ol (168)

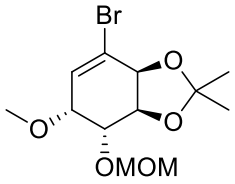


To a solution of **166** (2.9 g, 6.3 mmol) in THF (30 mL) stirred under argon atmosphere, was added 5 mL of tetrabutylammonium fluoride solution (1.0 M in THF). After 1 h, the reaction mixture was treated with ice−water (10 mL). The separated aqueous phase was extracted with EtOAc (2 × 25 mL) and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting light yellow oil

was subjected to flash chromatography hexanes/ EtOAc (50:50) to yield 1.77 g (92%) of **168** as colourless oil.

168: R_f = 0.52 [Hexanes/ EtOAc (50:50)]; $[\alpha]_D^{20}$ = 4.1 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$) *v* 3431, 2935, 1644, 1373, 1227, 1072, 1028, 917 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.11 (d, *J* = 2.6 Hz, 1H), 4.73 (s, 2H), 4.56 (dd, *J* = 5.2, 0.9 Hz, 1H), 4.45 (t, *J* = 5.1 Hz, 1H), 4.31 (bs, 1H), 4.07 (t, *J* = 4.2 Hz, 1H), 3.37 (s, 3H), 3.31 (d, *J* = 9.0 Hz, 1H), 1.38 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CHCl_3$,75MHz) δ 132.3, 122.8, 110.3, 97.6, 77.6, 76.5, 75.3, 66.5, 56.0, 27.5, 26.3; MS (EI) *m/z* (%) 205 (14), 191 (14), 161 (13), 146 (32), 145 (100), 110 (21), 97 (16), 59 (50), 45 (9), 43 (53) ; HRMS (EI) calcd for $C_{11}H_{17}BrO_5$: 308.0259 Found 308.0259; Anal. Calcd for $C_{11}H_{17}BrO_5$: C, 42.74; H, 5.54. Found C, 42.91; H, 5.44.

(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-5-methoxy-4-(methoxymethoxy)-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxole (169).

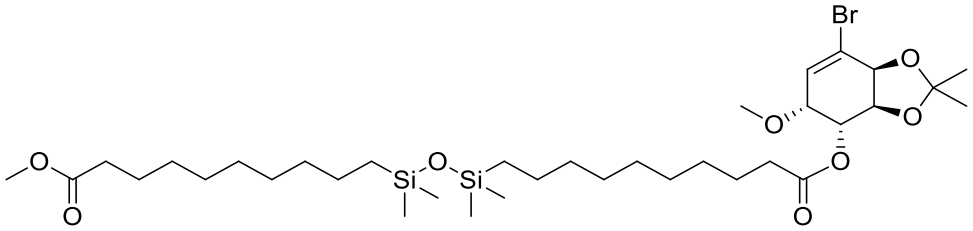


Sodium hydride (0.18 g, 7.5 mmol) was added to a stirred solution of alcohol **168** (1.7 g, 5.5 mmol) and iodomethane (0.44 mL, 7.01 mmol) in dry THF (55 mL) maintained at 0 °C under a argon atmosphere. Stirring was continued for 6 h at 0 °C, then the reaction

mixture was treated with ice−water (10 mL). The separated aqueous phase was extracted with EtOAc (2 × 25 mL) and the combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography on silica gel hexanes/EtOAc (50:50) to give 1.45 g (79%) of **169** as colourless oil.

169: R_f = 0.68 [hexanes/EtOAc (50:50)]; $[\alpha]_D^{20}$ = -67.34 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$) *v* 2985, 2932, 1643, 1454, 1372, 1340, 1216, 1149, 1035, 917 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.26 (d, *J* = 3.1 Hz, 1H), 4.79 – 4.72 (m, 2H), 4.65 (d, *J* = 5.3 Hz, 1H), 4.47 (t, *J* = 5.7 Hz, 1H), 4.19 – 4.16 (m, 1H), 3.96 – 3.94 (m, 1H), 3.43 (s, 3H), 3.39 (s, 3H), 1.43 (s, 3H), 1.39 (s, 3H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.8, 123.5, 110.1, 96.9, 77.0, 75.4, 75.9, 73.6, 57.4, 55.7, 27.6, 26.0; MS (EI) *m/z* (%) 145 (74), 87 (5), 73 (6), 45 (100), 43 (15); HRMS (EI) calcd for $C_{12}H_{19}BrO_5$: 322.0416. Found 322.04159; Anal. Calcd for $C_{12}H_{19}BrO_5$: C, 44.60; H, 5.93. Found C, 44.61; H, 5.84.

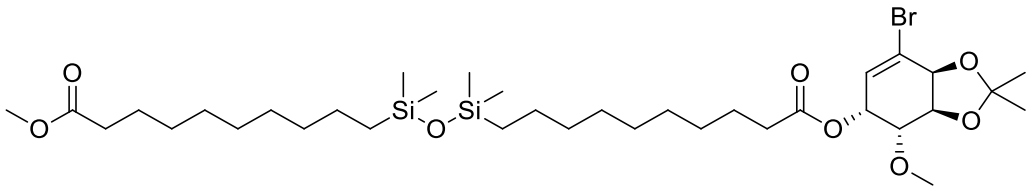
(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-5-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (171)



135 (88 mg, 0.17 mmol) was added to a stirred solution of **154** (47 mg, 0.17 mmol) and stirred for 5 min in toluene (0.7 mL) maintained at 100 °C. The resulting mixture was charged with N435 (13 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) to give 11 mg (9%) of **171** as colorless oil.

171: R_f = 0.50 [hexanes/EtOAc (80:20)]; $[\alpha]_D^{20}$ = -43.5 (*c* = 0.65, MeOH); IR (ATR) *v* 2940, 2889, 2865, 1650, 1462, 1040, 880 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.24 (d, *J* = 3.2 Hz, 1H), 5.51 (dd, *J* = 5.5, 3.6 Hz, 1H), 4.64 (d, *J* = 5.3 Hz, 1H), 4.43 (t, *J* = 5.7 Hz, 1H), 4.04 – 3.91 (m, 1H), 3.66 (s, 3H), 3.39 (s, 3H), 2.37 – 2.34 (m, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.62 – 1.59 (m, 4H), 1.44 (s, 3H), 1.39 (s, 3H), 1.28 – 1.26 (m, 24H), 0.49 (t, *J* = 7.4 Hz, 4H), 0.02 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.2, 130.2, 123.3, 110.5, 76.7, 74.6, 74.1, 68.4, 57.7, 51.5, 34.2, 34.1, 33.46, 33.43, 29.47, 29.42, 29.37, 29.34, 29.32, 29.2, 29.0, 27.5, 26.1, 24.99, 24.97, 23.3, 18.4, 0.4. ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.24; MS (EI) *m/z* (%) 57 (45), 91 (32), 115 (61), 124 (25), 133 (30), 203 (30), 317 (100); HRMS (EI) calcd for $C_{34}H_{62}BrO_8Si_2$: 733.3167. Found 733.3169.

(3a*S*,4*R*,5*R*,7a*S*)-7-Bromo-4-methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (172)

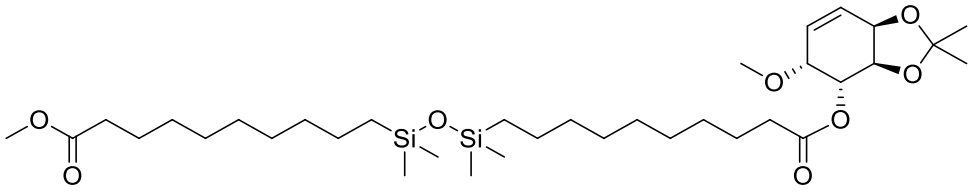


135 (87 mg, 0.17 mmol) was added to a stirred solution of **151** (47 mg, 0.17 mmol) and stirred for 5 min in toluene (0.8 mL) maintained at 100 °C. The resulting mixture was charged with N435 (13 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction mixture was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) to give 13 mg (10%) of **172** as colorless oil.

172: R_f = 0.48 [hexanes/EtOAc (80:20)]; $[\alpha]_D^{19}$ = -44.06 (*c* = 0.69, $CHCl_3$); IR (ATR) *v* 2921, 2853, 1739, 1648, 1437, 1371, 1250, 1165, 1115, 1043, 841, 794 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.19 (d, *J* = 4.2 Hz, 1H), 5.48 (t, *J* = 3.7 Hz, 1H), 4.69 (d, *J* = 5.8 Hz, 1H), 4.44 (t, *J* = 6.2 Hz, 1H), 3.72 (dd, *J* = 6.4, 3.5 Hz, 1H), 3.66 (s, 3H), 3.48 (s, 3H), 2.37 – 2.28 (m, 4H), 1.65 – 1.59 (m, 4H), 1.41 (s, 3H), 1.39 (s, 3H), 1.27 (bs, 24H),

0.49 (t, J = 7.3 Hz, 4H), 0.02 (s, 12H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.3, 173.0, 128.3, 125.3, 110.3, 77.8, 76.7, 74.9, 67.6, 59.2, 51.4, 34.2, 34.1, 33.42, 33.40, 29.43, 29.40, 29.35, 29.32, 29.30, 29.2, 29.1, 27.7, 25.9, 25.0, 23.3, 18.4, 0.40; ^{29}Si NMR (120 MHz, $CDCl_3$) δ 7.29; MS (EI) *m/z* (%) 57 (35), 85 (15), 115 (14), 149 (100), 317 (45); HRMS (EI) calcd for $C_{34}H_{62}BrO_8Si_2$ (M - CH_3 species): 733.3167. Found 733.3159

(3a*R*,4*R*,5*R*,7a*R*)-5-Methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (173)

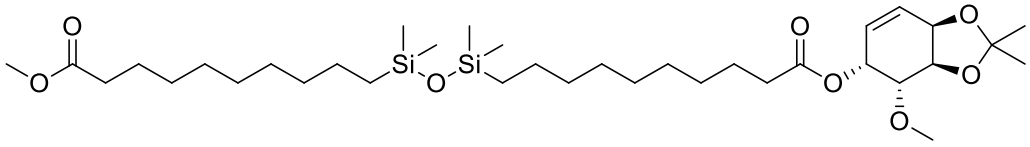


135 (79 mg, 0.16 mmol) was added to a stirred solution of **155** (34 mg, 0.17 mmol) and stirred for 5 min in toluene (0.85 mL) maintained at 100 °C. The resulting mixture was charged with N435 (12 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction mixture was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) to give 4 mg (3.5%) of **173** as colorless oil.

173: R_f = 0.40 [(Hex/EtOAc (80:20)]; $[\alpha]_D^{18}$ = -58.22 (c = 0.945, $CHCl_3$); IR (ATR) ν

2922, 2854, 1740, 1460, 1372, 1251, 1162, 1101, 1041, 840, 788, 621 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 – 5.92 (m, 2H), 5.27 (dd, *J* = 7.2, 3.4 Hz, 1H), 4.68 – 4.66 (m, 1H), 4.44 (t, *J* = 6.7 Hz, 1H), 3.97 (t, *J* = 3.5 Hz, 1H), 3.66 (s, 3H), 3.38 (s, 3H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.70 – 1.59 (m, 4H), 1.42 (s, 3H), 1.37 (s, 3H), 1.27 (s, 24H), 0.48 (t, *J* = 7.0 Hz, 4H), 0.02 (s, 12H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.3, 173.5, 128.7, 127.9, 109.7, 73.3, 73.2, 72.2, 71.3, 57.7, 51.4, 34.4, 34.1, 33.4, 33.4, 29.5, 29.4, 29.3, 29.3, 29.3, 29.2, 29.1, 27.6, 25.8, 25.0, 23.3, 18.4, 0.4.; ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.28; MS (EI) *m/z* (%) 45 (91), 57 (39), 69 (46), 71 (42), 115 (57), 125 (54), 149 (55), 163 (36), 317(100), 318 (48); HRMS (EI) calcd for $C_{10}H_{16}O_4$ (M- CH3 species): 655.4062 Found 655.4054.

(3a*R*,4*R*,5*R*,7a*R*)-4-Methoxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-5-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (174)

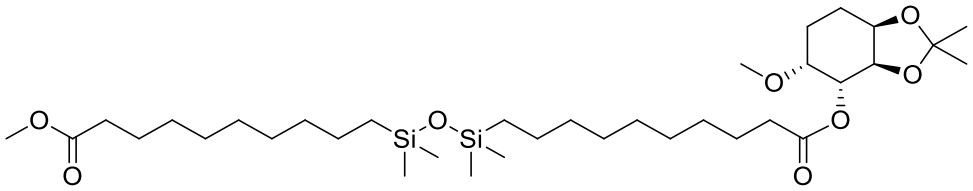


135 (160 mg, 0.32 mmol) was added to a stirred solution of **152** (65 mg, 0.32 mmol) and stirred for 5 min in toluene (1.5 mL) maintained at 100 °C. The resulting mixture was charged with N435 (22 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic

phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) to give 21 mg (9.6%) of **174** as colorless oil.

174: R_f = 0.38 [(Hex/EtOAc (80:20)]; $[\alpha]_D^{20}$ = -38.4(*c* = 1.15, MeOH); IR (ATR) *v* 2922, 2853, 1738, 1460, 1372, 1250, 1197, 1164, 1120, 1054, 839, 789, 621 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.04 – 5.93 (m, 2H), 5.51 (t, *J* = 4.0 Hz, 1H), 4.70 (dd, *J* = 6.3, 3.2 Hz, 1H), 4.37 (t, *J* = 6.8 Hz, 1H), 3.66 (s, 3H), 3.51 (dd, *J* = 7.6, 3.4 Hz, 1H), 3.47 (s, 3H), 2.34 – 2.27 (m, 4H), 1.63 – 1.59 (m, 4H), 1.47 (s, 3H), 1.38 (s, 3H), 1.27 (bs, 24H), 0.48 (t, *J* = 7.3 Hz, 4H), 0.02 (s, 12H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 173.3, 129.6, 126.7, 109.4, 79.3, 74.4, 72.3, 65.3, 58.5, 51.4, 34.3, 34.1, 33.43, 33.41, 29.45, 29.40, 29.36, 29.31, 29.2, 29.1, 27.8, 25.4, 25.0, 24.98, 23.29, 23.28, 18.4, 0.4; ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.28; MS (EI) *m/z* (%) 59 (22), 81 (24), 97 (27), 115 (24), 125 (41), 149 (39), 317(100); HRMS (EI) calcd for $C_{10}H_{16}O_4$ (M- CH3 species): 655.4062 Found 655.4034.

(3a*R*,4*R*,5*R*,7a*R*)-5-Methoxy-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-4-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (175)

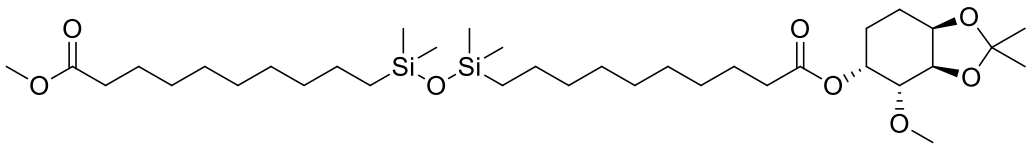


135 (135 mg, 0.27 mmol) was added to a stirred solution of **156** (56 mg, 0.28 mmol) and stirred for 5 min in toluene (1.35 mL) maintained at 100 °C . The resulting mixture was charged with N435 (22 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction mixture was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) to give 11 mg (6%) of **175** as colorless oil.

175: R_f = 0.25 [hexane/EtOAc (80:20)]; $[\alpha]_D^{20}$ = - 41.4 (*c* = 0.55, EtOAc);); IR (ATR) *v* 2923, 2853, 1739, 1437, 1369, 1249, 1215, 1164, 1109, 1056, 923, 836, 786, 705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.91 (dd, *J* = 8.1, 2.6 Hz, 1H), 4.33 – 4.31 (m, 1H), 4.21 (dd, *J* = 8.1, 5.2 Hz, 1H), 3.66 (bs, 4H), 3.31 (s, 3H), 2.41 – 2.37 (m, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 2.01 – 1.81 (m, 3H), 1.78 – 1.69 (m, 1H), 1.68 – 1.61 (m, 4H), 1.49 (s, 3H), 1.35 (s, 3H), 1.27 (bs, 24H), 0.48 (t, *J* = 7.3 Hz, 4H), 0.02 (s, 12H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ 174.4, 173.7, 108.6, 76.9, 75.8, 75.1, 74.1, 56.9, 51.4, 34.5, 34.1, 33.44, 33.41, 29.5, 29.4, 29.35, 29.31, 29.2, 29.08, 28.15, 26.4, 25.0, 24.98, 23.3, 21.9, 20.9, 18.4, 0.4; ^{29}Si NMR (80 MHz, $CDCl_3$) δ 7.29; MS (EI) *m/z* (%) 55 (93), 57 (100), 67 (58), 71 (96), 83 (54),127 (43), 163 (48), 187 (29), 243 (32), 317 (83); HRMS (EI) calcd for $C_{35}H_{68}O_8Si_2$ (M - CH_3): 657.4212. Found 657.4205; Anal. Calcd for $C_{35}H_{68}O_8Si_2$: C, 22.46; H, 10.18. Found C, 62.64; H, 10.28.

(3a*R*,4*R*,5*R*,7a*R*)-4-methoxy-2,2-dimethylhexahydrobenzo[d][1,3]dioxol-5-yl 10-(3-(10-methoxy-10-oxodecyl)-1,1,3,3-tetramethyldisiloxanyl)decanoate (176)



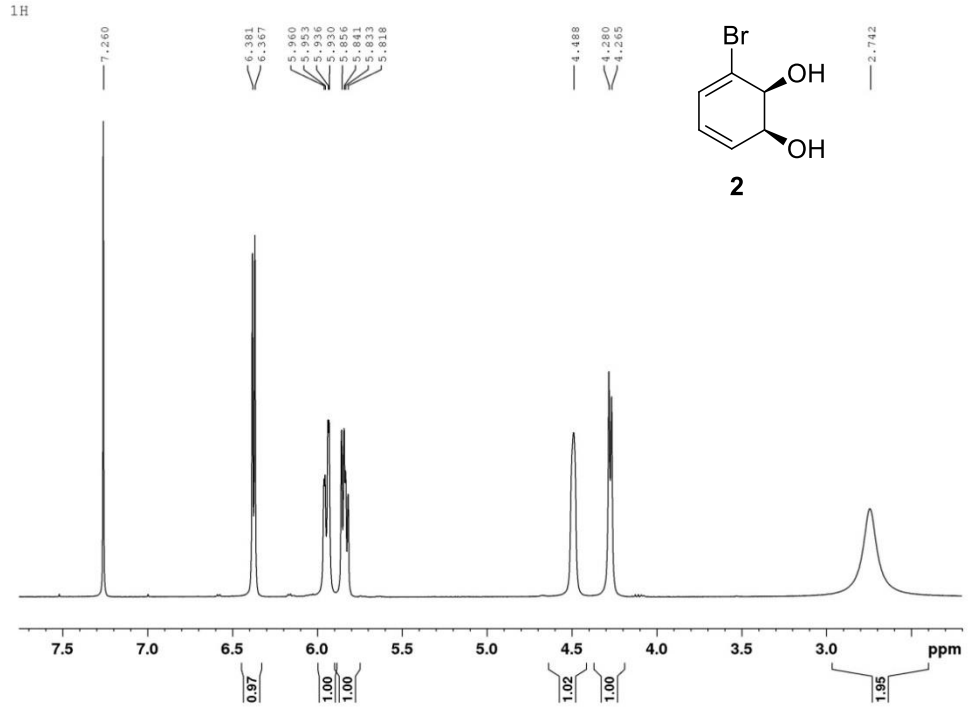
135 (370 mg, 0.74 mmol) was added to a stirred solution of **153** (150 mg, 0.74 mmol) and stirred for 5 min in toluene (3.5 mL) maintained at 100 °C. The resulting mixture was charged with N435 (52 mg). The reaction mixture was heated at 100 °C for 7 d. After 7 d the reaction mixture was cooled down to room temperature, then treated with Et_2O (3 mL). The reaction mixture was filtered through medium porosity Büchner funnel and the organic phases were concentrated under reduced pressure. The resulting light yellow oil was subjected to flash chromatography with hexanes/EtOAc (90:10) to give 31 mg (6%) of **176** as colorless oil.

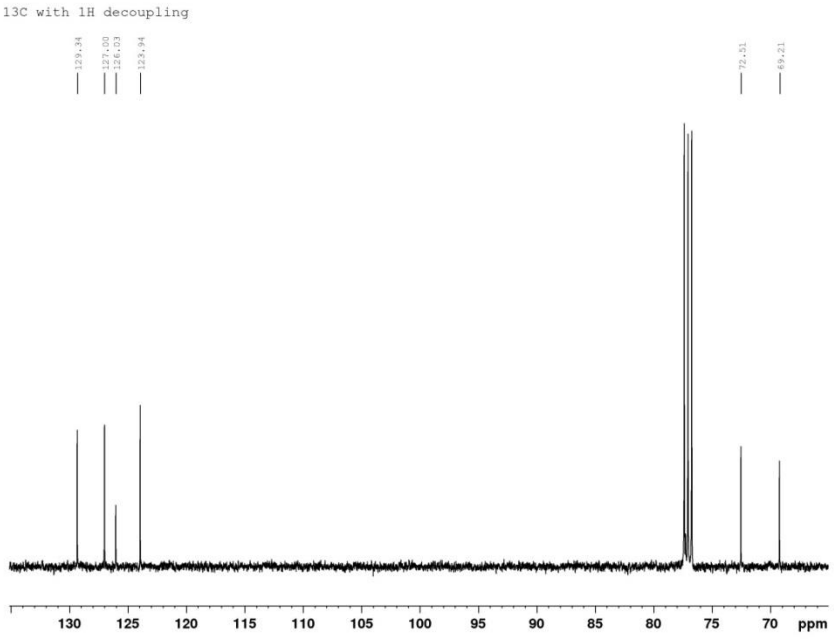
176: R_f = 0.23 [hexane/EtOAc (80:20)]; $[\alpha]_D^{19}$ = -36.1 (*c* = 1.52, EtOAc);); IR (ATR) *v* 2924, 2854, 2430, 1789, 1739, 1250, 1216, 1168, 1117, 1057, 923, 833, 792 cm^{-1}; ^{1}H

NMR (400 MHz, $CDCl_3$) δ 5.38 – 5.36 (m, 1H), 4.32 (dd, *J* = 8.3, 3.5 Hz, 1H), 4.07 (dd, *J* = 7.3, 5.4 Hz, 1H), 3.65 (s, 3H), 3.42 (s, 3H), 3.23 (dd, *J* = 7.4, 2.7 Hz, 1H), 2.33 – 2.27 (m, 4H), 1.97 – 1.71 (m, 4H), 1.65 – 1.54 (m, 4H), 1.51 (s, 3H), 1.36 (s, 3H), 1.27 (bs, 24H), 0.48 (t, *J* = 7.3 Hz, 4H), 0.02 (s, 12H).; ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3, 173.2, 108.4, 81.3, 77.4, 73.7, 67.8, 57.7, 51.4, 34.5, 34.1, 33.43, 33.40, 29.4, 29.39, 29.33, 29.32, 29.30, 29.2, 29.1, 28.4, 26.2, 25.1, 25.0, 23.3, 23.0, 21.6, 18.4, 0.4; ^{29}Si NMR (120 MHz, $CDCl_3$) δ 7.23; MS (EI) *m/z* (%) 55 (90), 57 (100), 67 (48), 71 (80), 83 (50), 127 (26), 149 (52), 243 (25), 317 (52); HRMS (EI) calcd for $C_{35}H_{68}O_8Si_2$ (M - CH_3): 657.4212. Found 657.4205; Anal. Calcd for $C_{35}H_{68}O_8Si_2$: C, 22.46; H, 10.18. Found C, 62.61; H, 10.40.

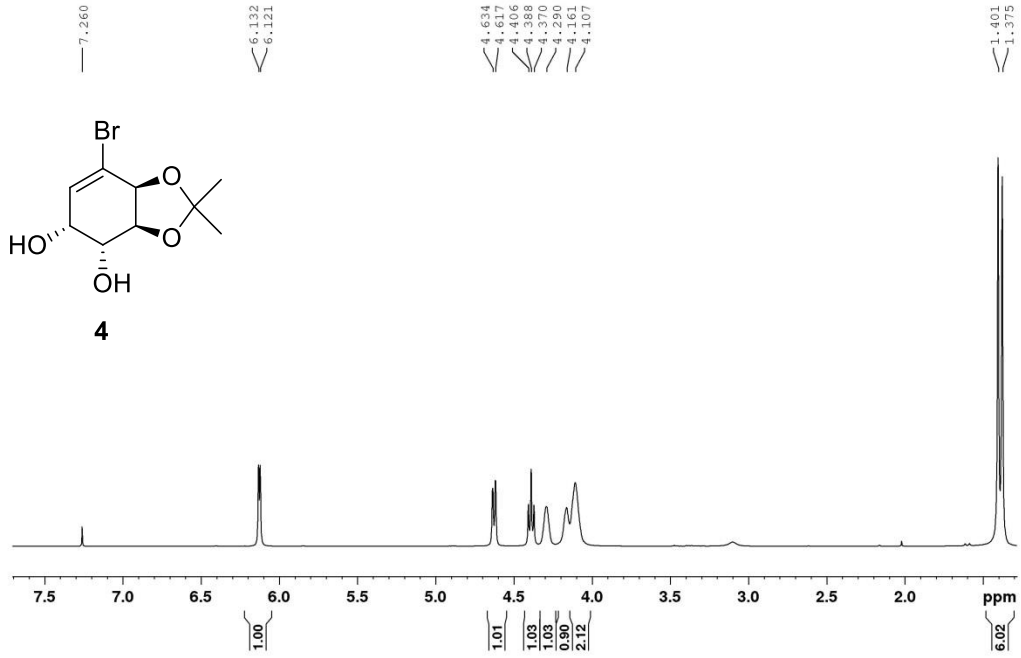
6. Selected spectra

6.1 NMR spectra

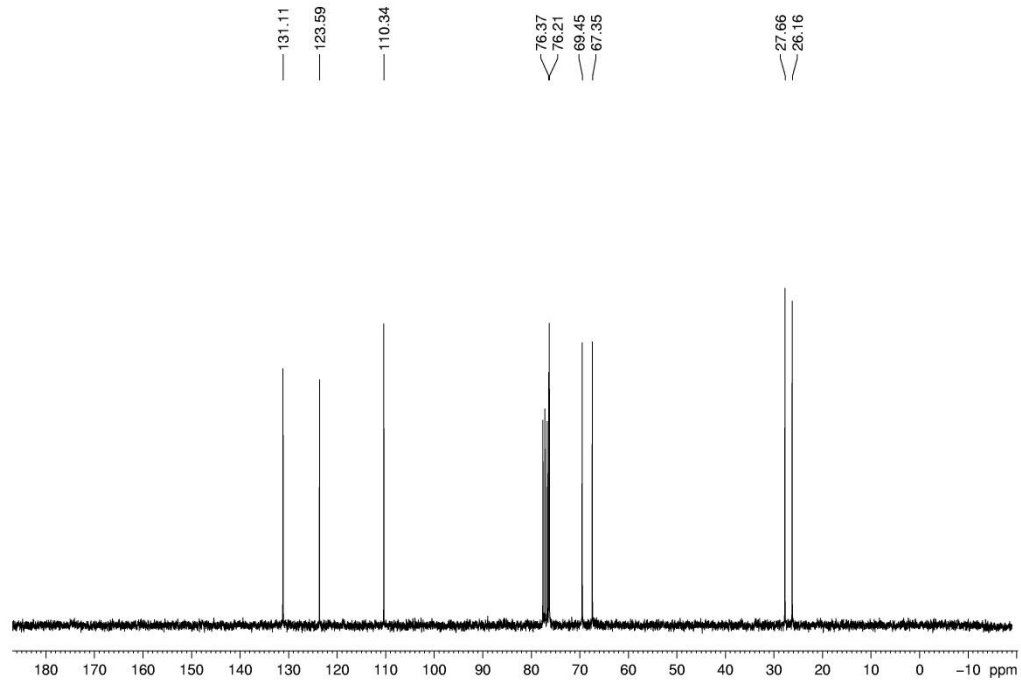


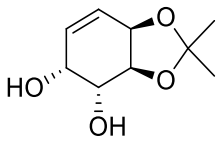


1D proton

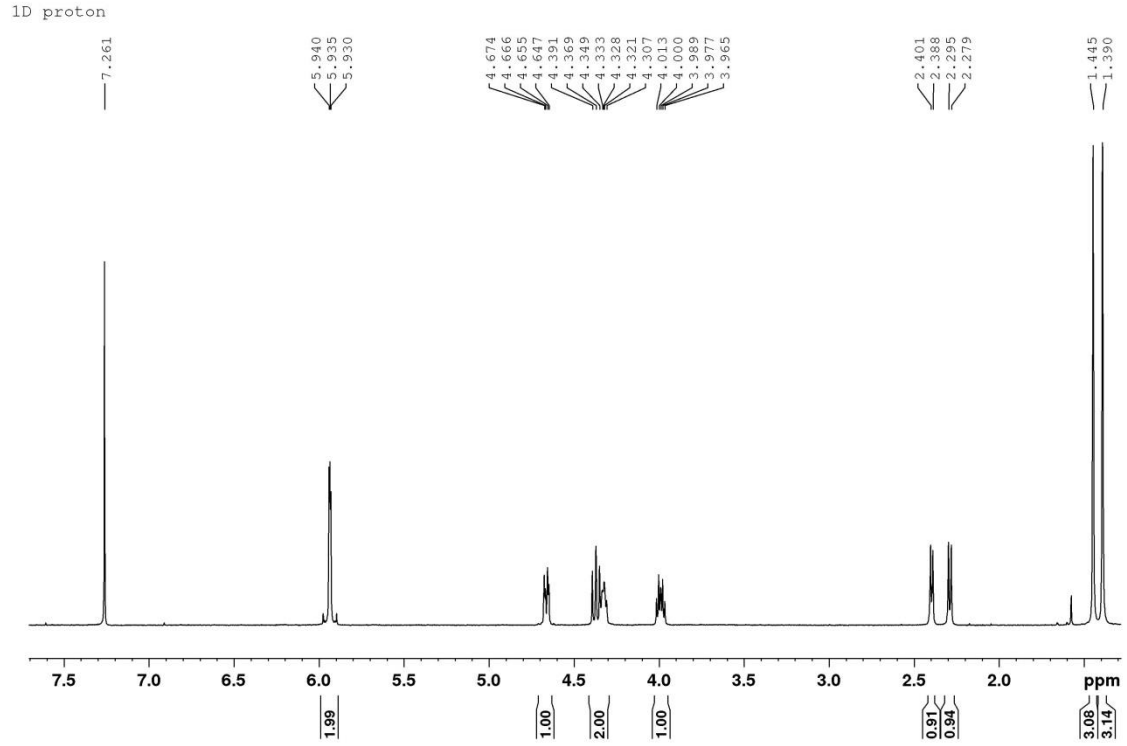


1D carbon with proton decoupling

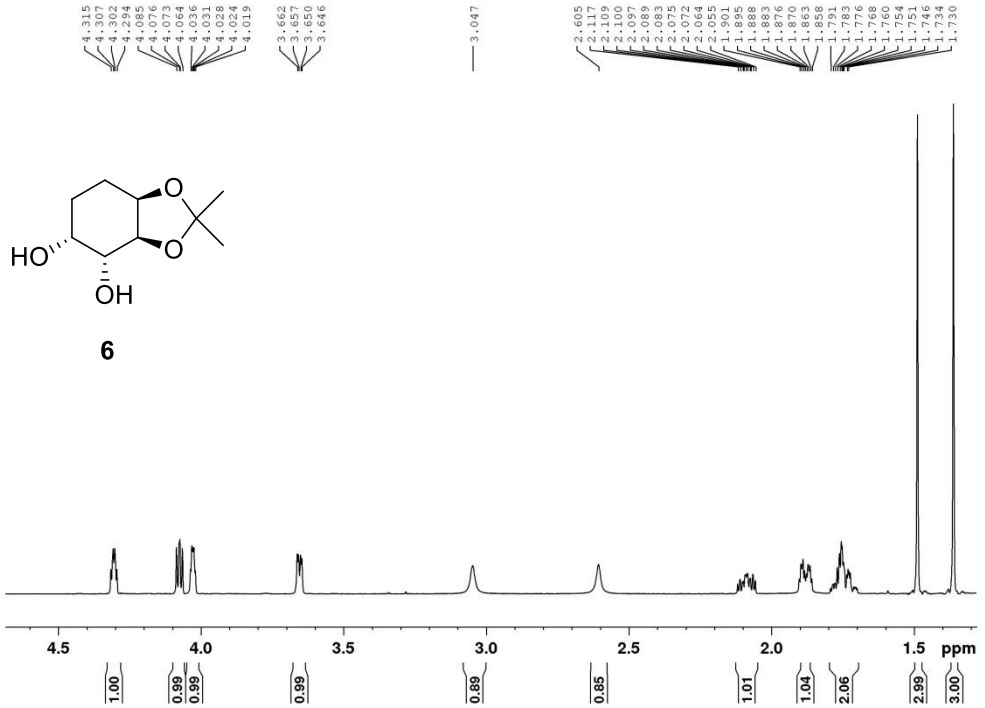



HO
O
O
OH

5

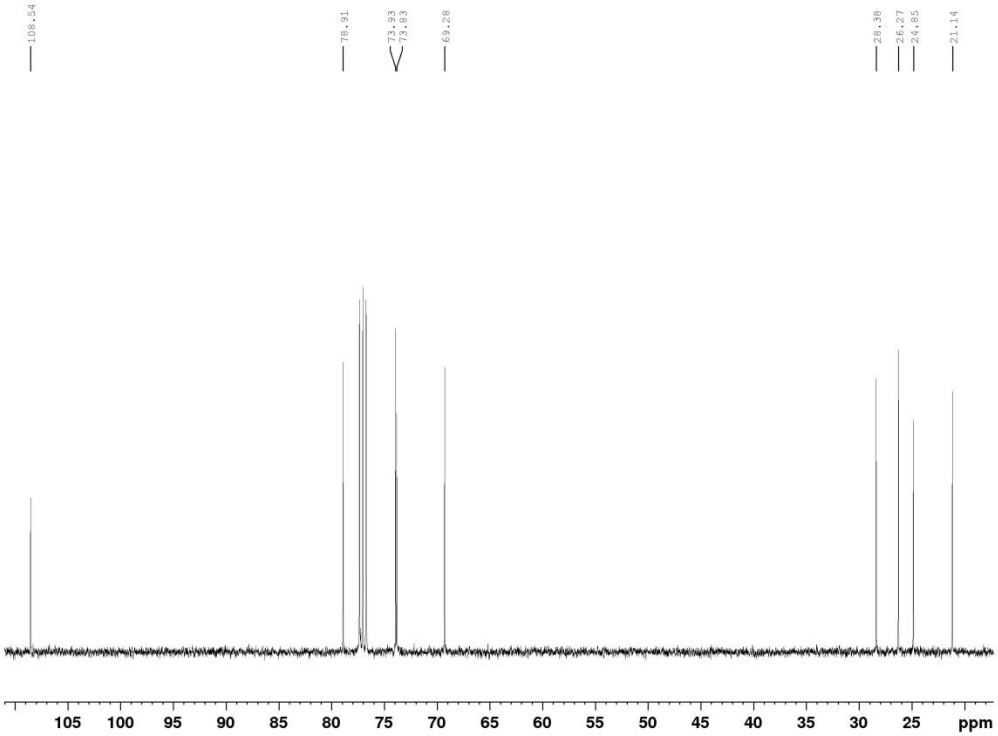

1D proton
7.261
5.940
5.935
5.930
4.674
4.666
4.655
4.647
4.391
4.369
4.349
4.333
4.328
4.321
4.307
4.013
4.000
3.989
3.977
3.965
2.401
2.388
2.295
2.279
1.445
1.390
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.99
1.00
2.00
1.00
0.91
0.94
3.08
3.14

1d proton

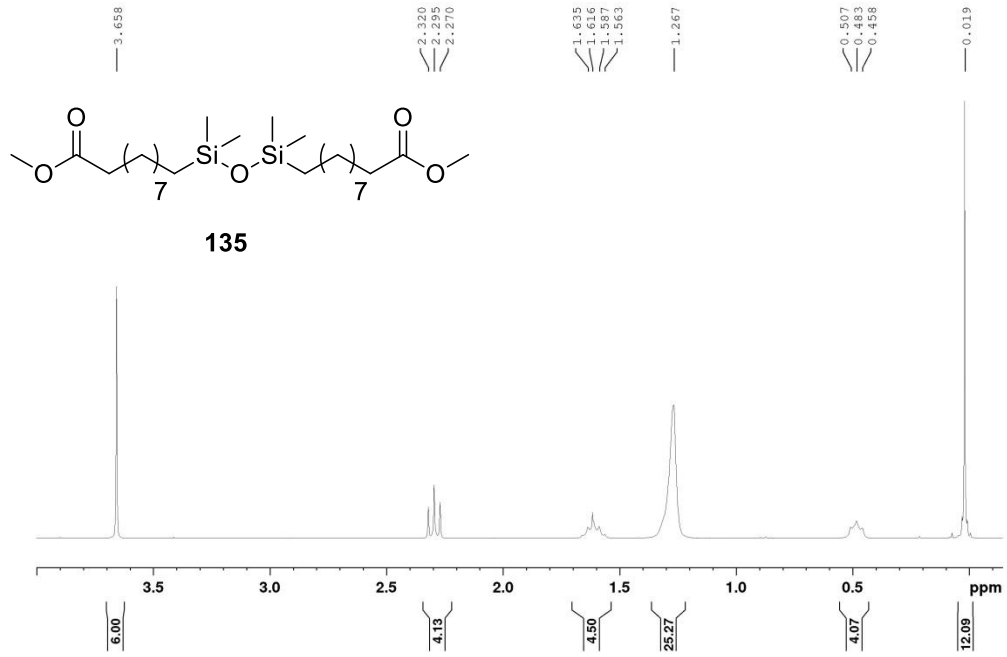


6

13C with 1H decoupling

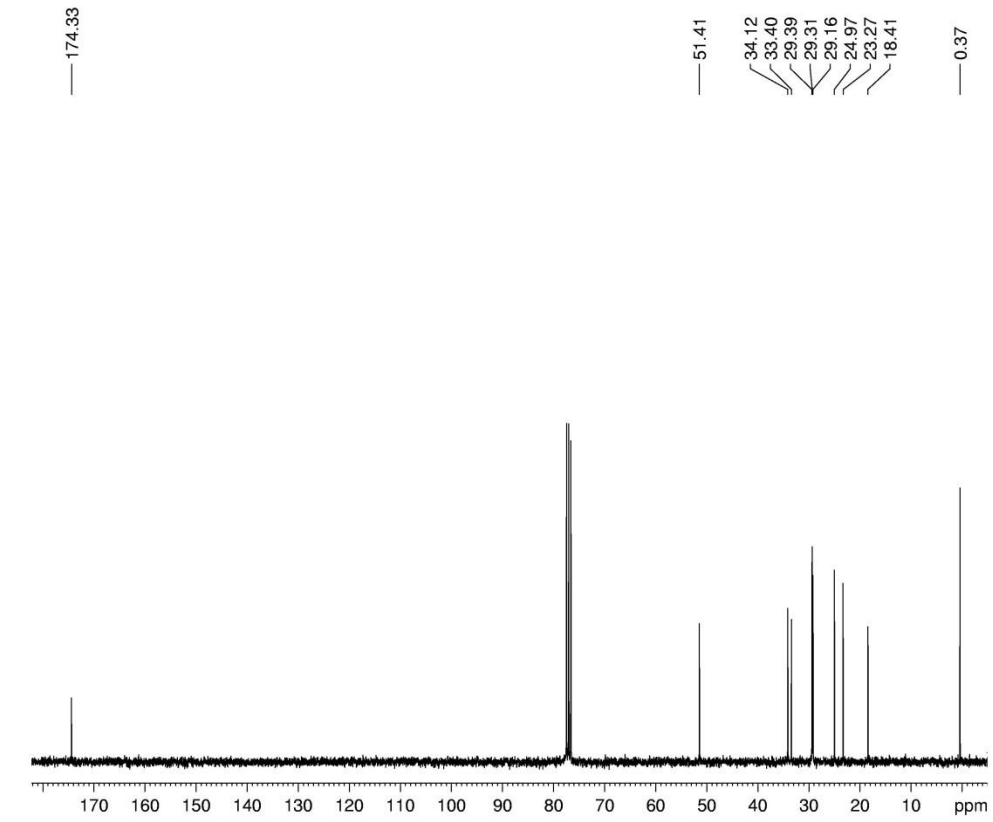


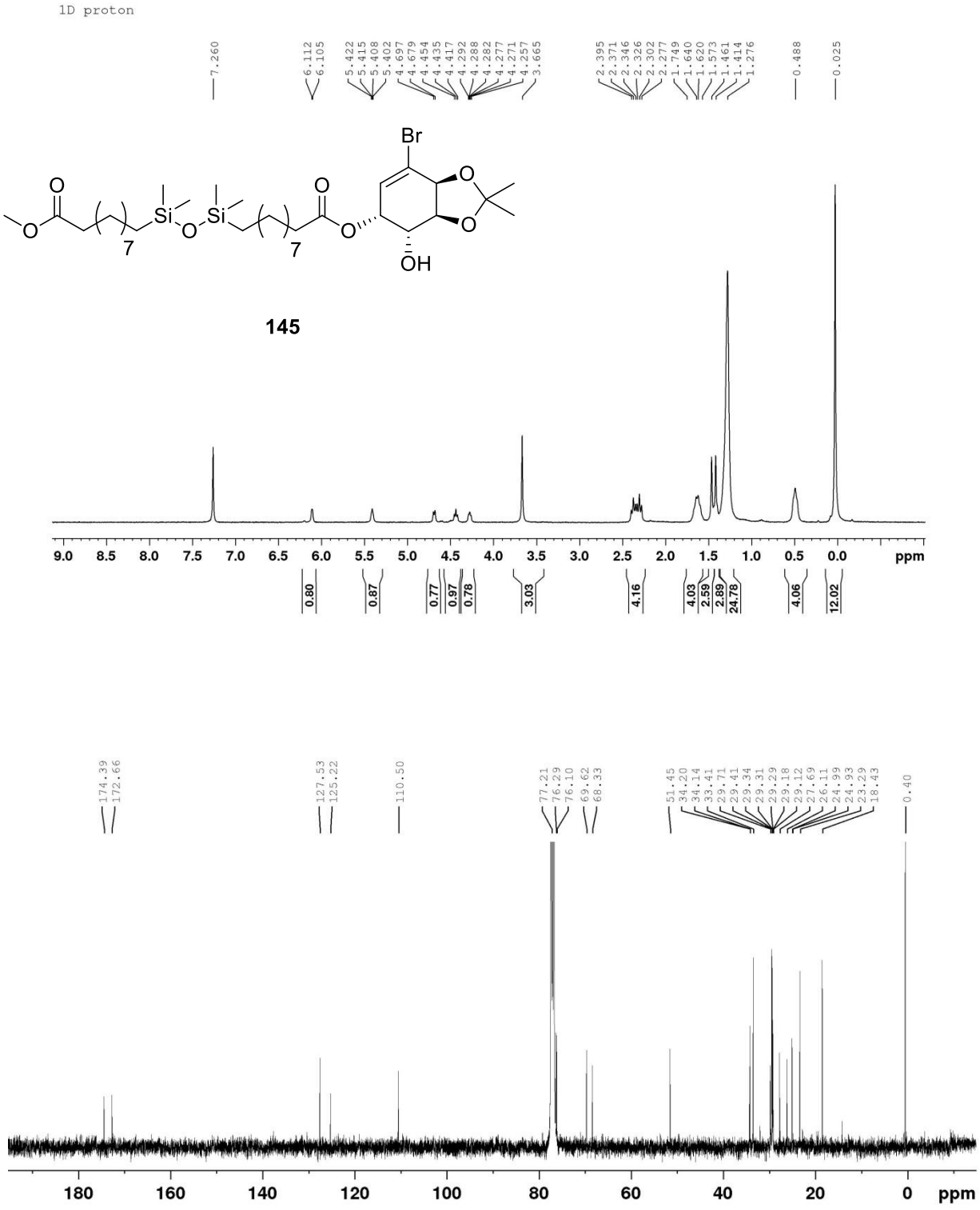
1D proton

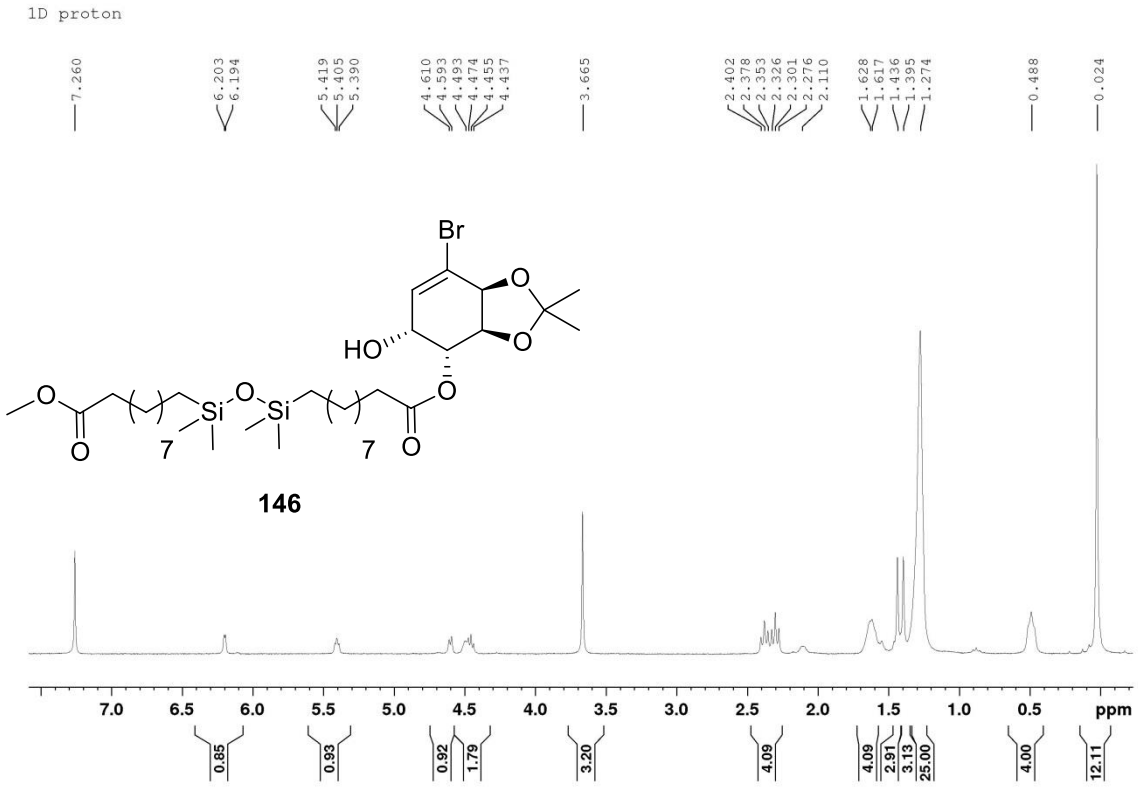


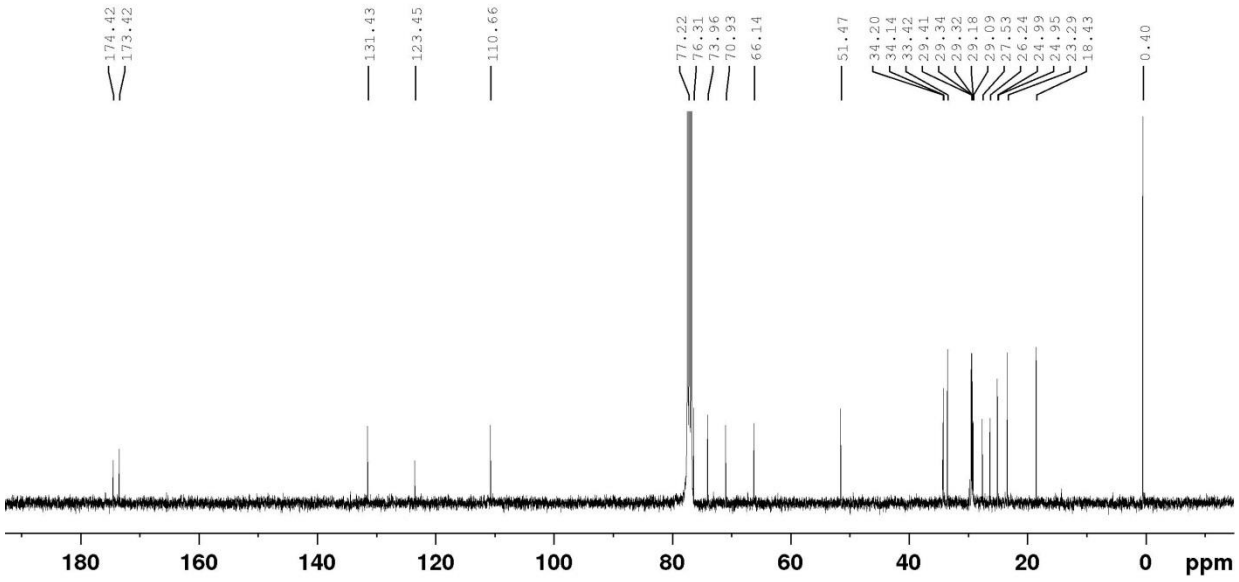
135

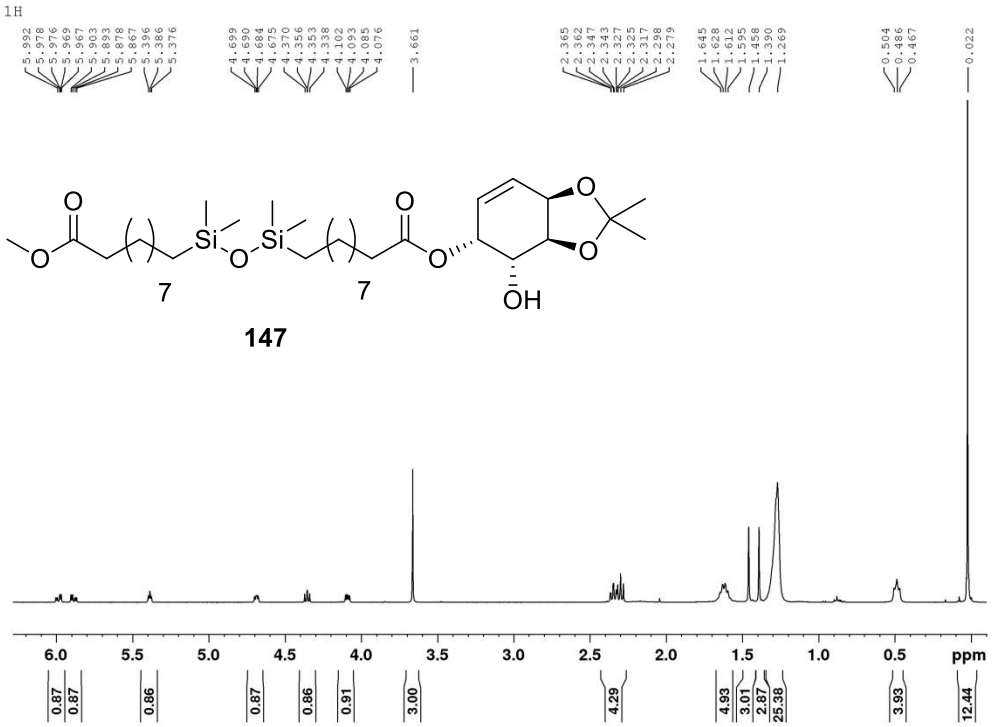
1D carbon with proton decoupling



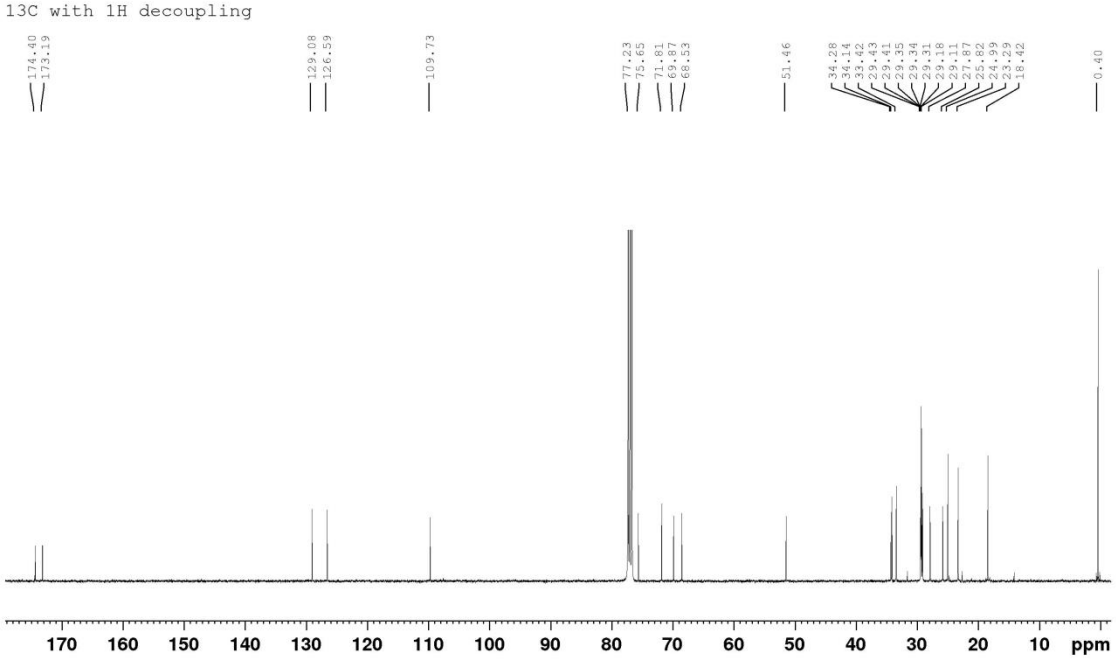

1D proton
7.260
6.112
6.105
5.422
5.415
5.408
5.402
4.697
4.679
4.454
4.435
4.417
4.292
4.288
4.282
4.277
4.271
4.257
3.665
2.395
2.371
2.346
2.326
2.302
2.277
1.749
1.640
1.620
1.573
1.461
1.414
1.276
0.488
0.025
Br
O
Si
OH
7
145
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
0.80
0.87
0.77
0.97
0.78
3.03
4.16
4.03
2.59
2.89
24.78
4.06
12.02
174.39
172.66
127.53
125.22
110.50
77.21
76.29
76.10
69.62
68.33
51.45
34.20
34.14
33.41
29.71
29.41
29.34
29.31
29.29
29.18
29.12
27.69
26.11
24.99
24.93
23.29
18.43
0.40
180 160 140 120 100 80 60 40 20 0 ppm


1D proton
7.260
6.203
6.194
5.419
5.405
5.390
4.610
4.593
4.493
4.474
4.455
4.437
3.665
2.402
2.378
2.353
2.326
2.301
2.276
2.110
1.628
1.617
1.436
1.395
1.274
0.488
0.024
Br
HO
O
Si
7
146
ppm
0.85
0.93
0.92
1.79
3.20
4.09
4.09
2.91
3.13
25.00
4.00
12.11

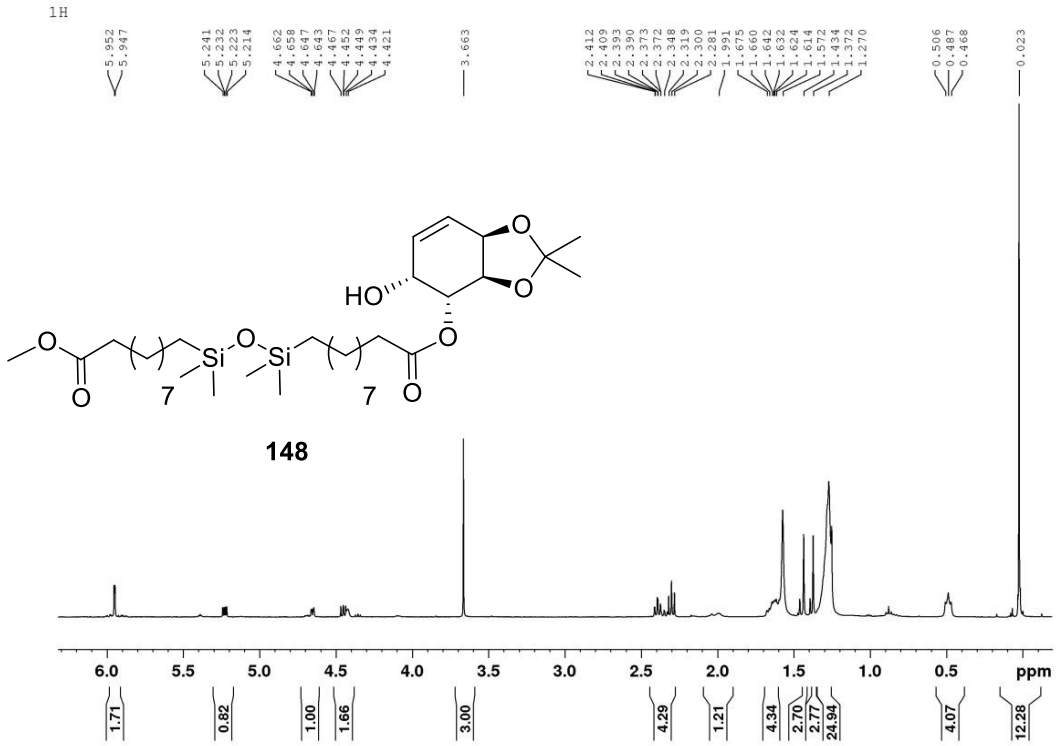

174.42
173.42
131.43
123.45
110.66
77.22
76.31
73.96
70.93
66.14
51.47
34.20
34.14
33.42
29.41
29.34
29.32
29.18
29.09
27.53
26.24
24.99
24.95
23.29
18.43
0.40
ppm


1H
5.992
5.978
5.976
5.969
5.967
5.903
5.893
5.878
5.867
5.396
5.386
5.376
4.699
4.690
4.684
4.675
4.370
4.356
4.353
4.338
4.102
4.093
4.085
4.076
3.661
2.365
2.362
2.347
2.343
2.327
2.325
2.317
2.298
2.279
1.645
1.628
1.612
1.595
1.458
1.390
1.269
0.504
0.486
0.467
0.022
O
O
Si
O
Si
O
O
O
O
7
7
OH
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.87
0.87
0.86
0.87
0.86
0.91
3.00
4.29
4.93
3.01
2.87
25.38
3.93
12.44

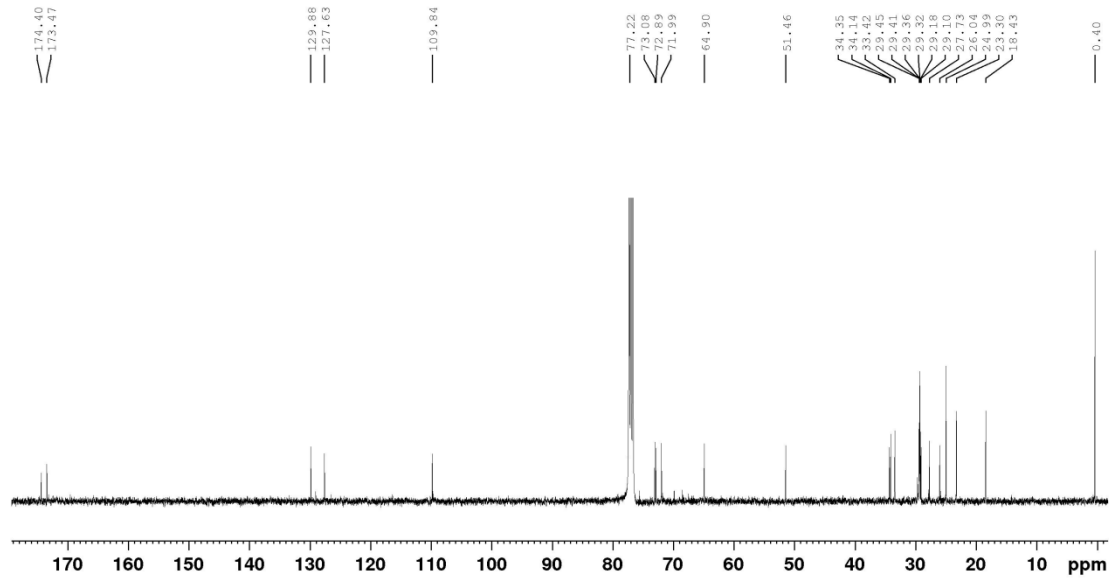
147

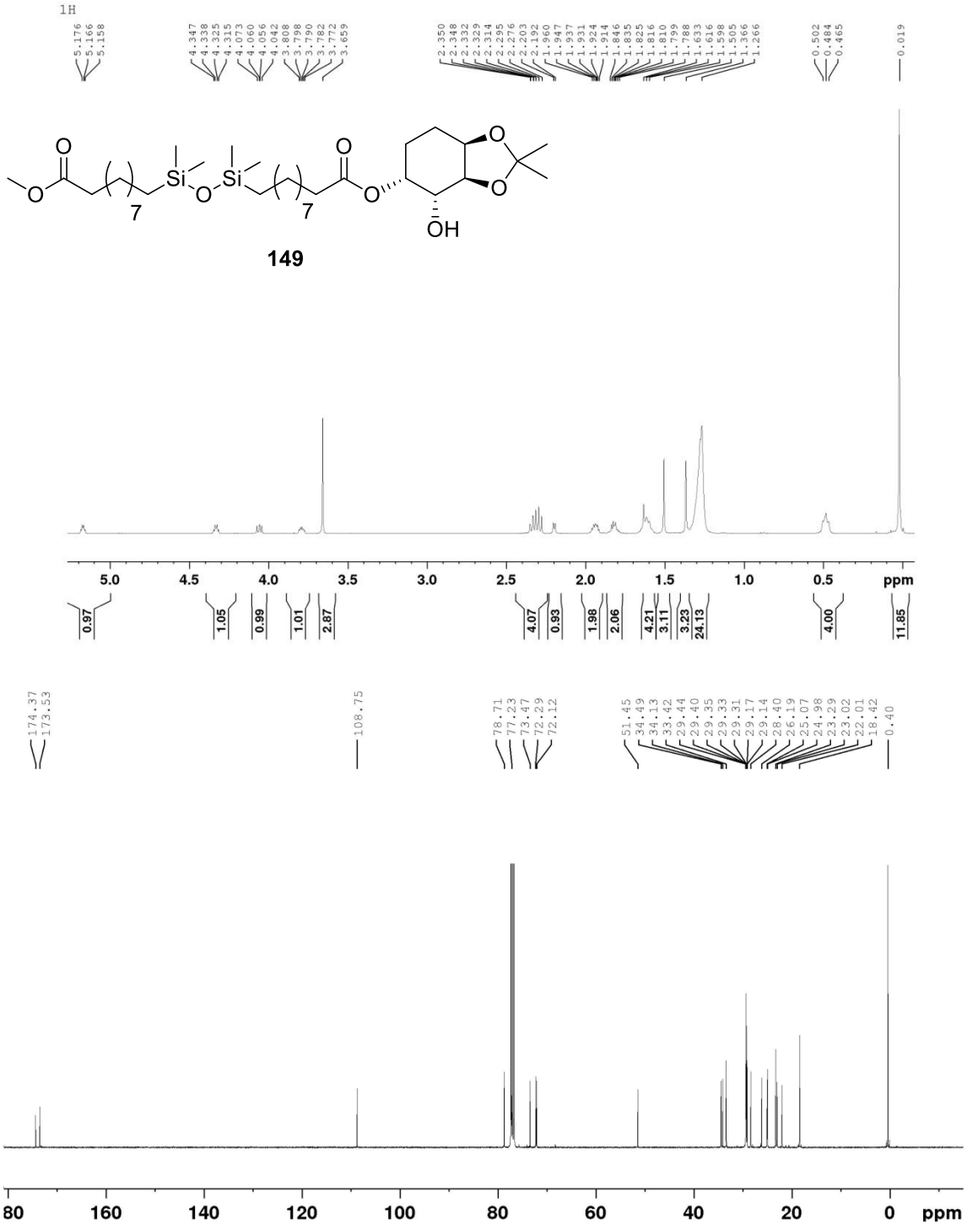

13C with 1H decoupling
174.40
173.19
129.08
126.59
109.73
77.23
75.65
71.81
69.87
68.53
51.46
34.28
34.14
33.42
29.43
29.41
29.35
29.34
29.31
29.18
29.11
27.87
25.82
24.99
23.29
18.42
0.40
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

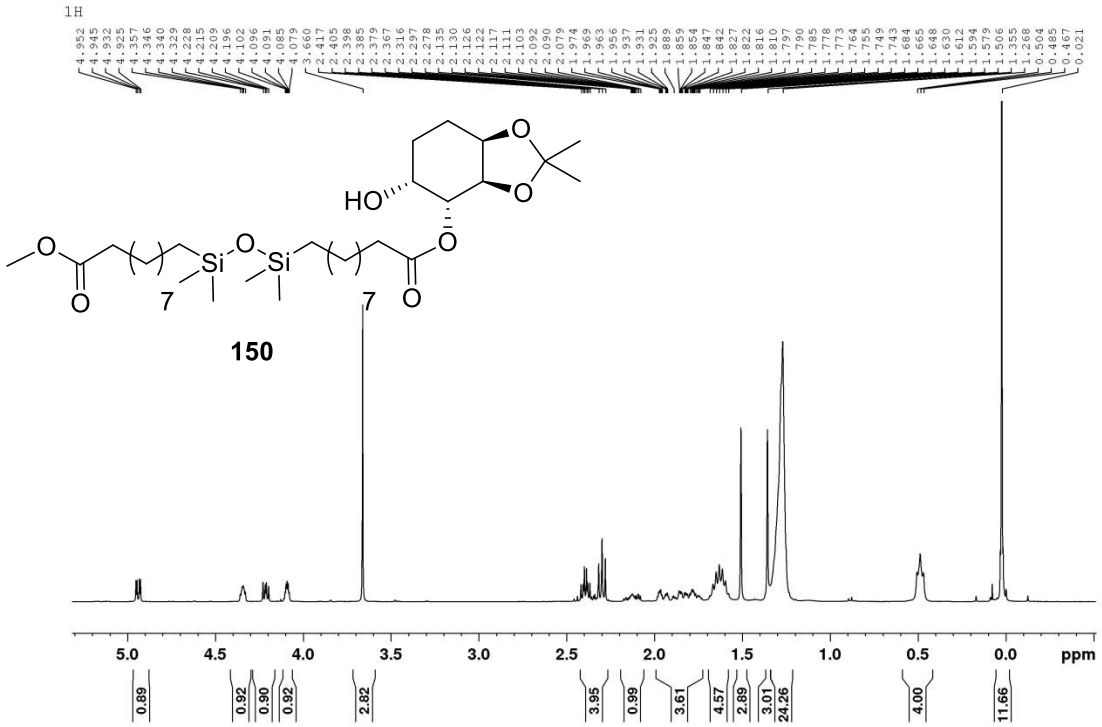
1H

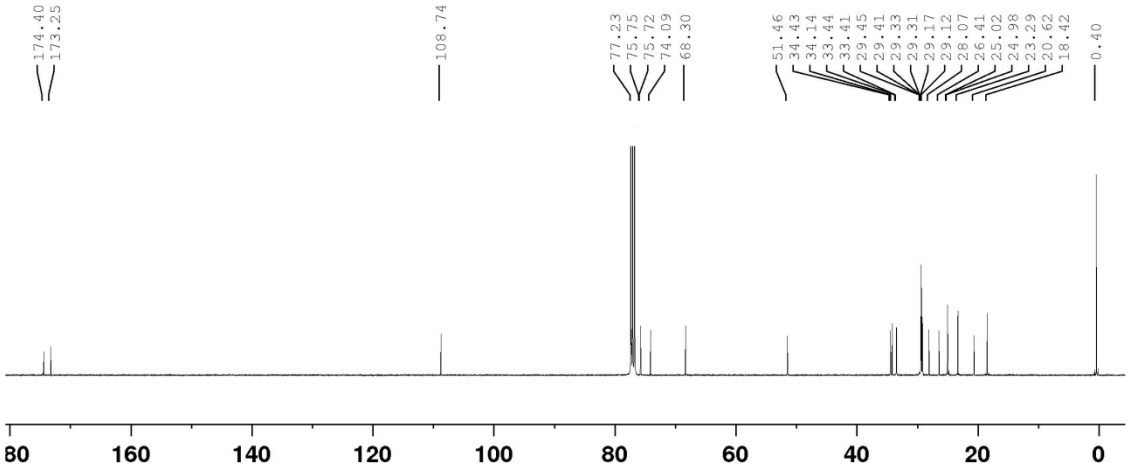


13C with 1H decoupling




1H
5.176
5.166
5.158
4.347
4.338
4.325
4.315
4.073
4.060
4.056
4.042
3.808
3.798
3.790
3.782
3.772
3.659
2.350
2.348
2.332
2.329
2.314
2.295
2.276
2.203
2.192
1.960
1.947
1.937
1.931
1.924
1.914
1.846
1.835
1.825
1.816
1.810
1.799
1.788
1.633
1.616
1.598
1.505
1.366
1.266
0.502
0.484
0.465
0.019
O
O
Si
O
Si
O
O
O
O
O
7
7
OH
149
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.97
1.05
0.99
1.01
2.87
4.07
0.93
1.98
2.06
4.21
3.11
3.23
24.13
4.00
11.85
174.37
173.53
108.75
78.71
77.23
73.47
72.29
72.12
51.45
34.49
34.13
33.42
29.44
29.40
29.35
29.33
29.31
29.17
29.14
28.40
26.19
25.07
24.98
23.29
23.02
22.01
18.42
0.40
80
160
140
120
100
80
60
40
20
0
ppm


1H
HO
O
Si
7
150
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
0.89
0.92
0.90
0.92
2.82
3.95
0.99
3.61
4.57
2.89
3.01
24.26
4.00
11.66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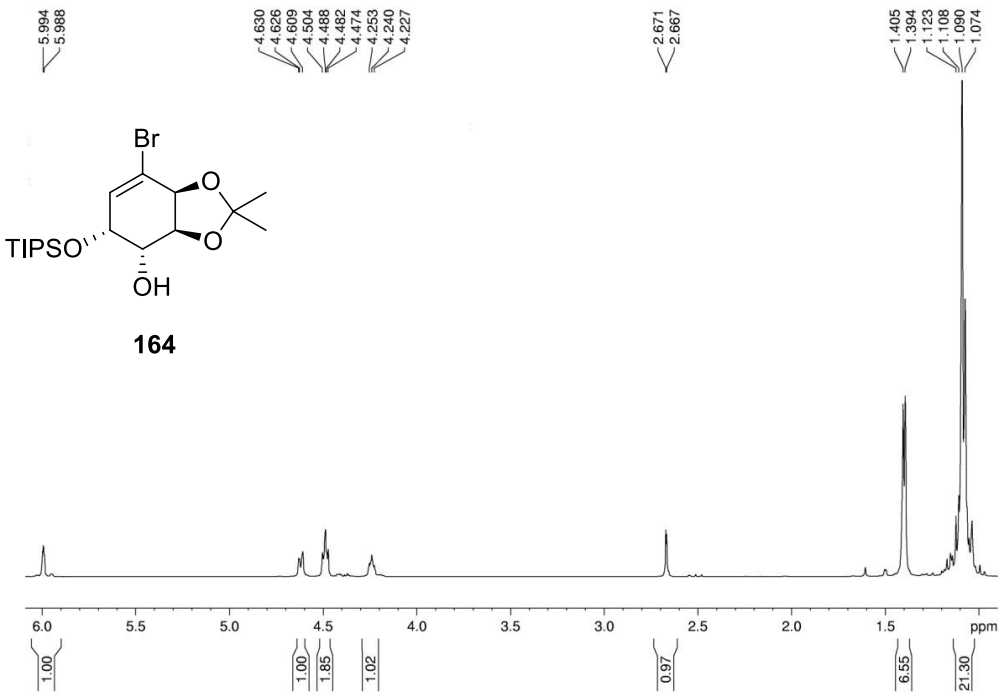
173.25
108.74
77.23
75.75
75.72
74.09
68.30
51.46
34.43
34.14
33.44
33.41
29.45
29.41
29.33
29.31
29.17
29.12
28.07
26.41
25.02
24.98
23.29
20.62
18.42
0.40
80
160
140
120
100
80
60
40
20
0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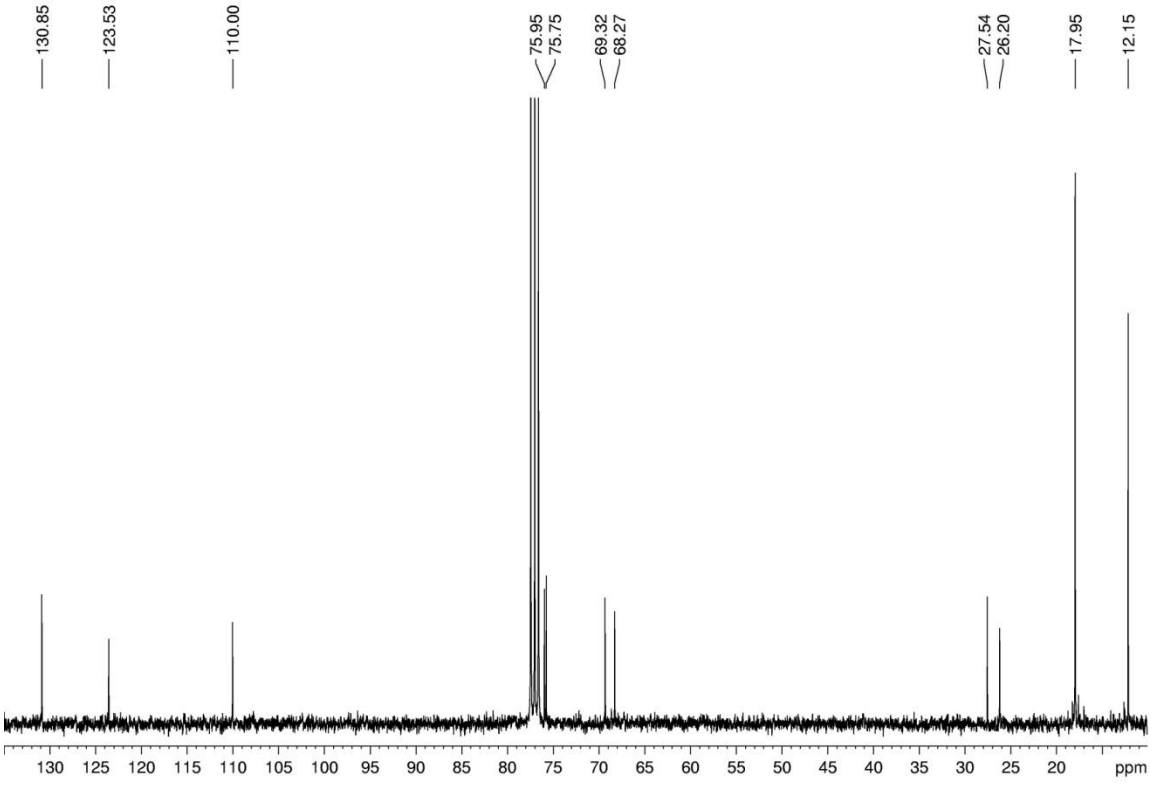
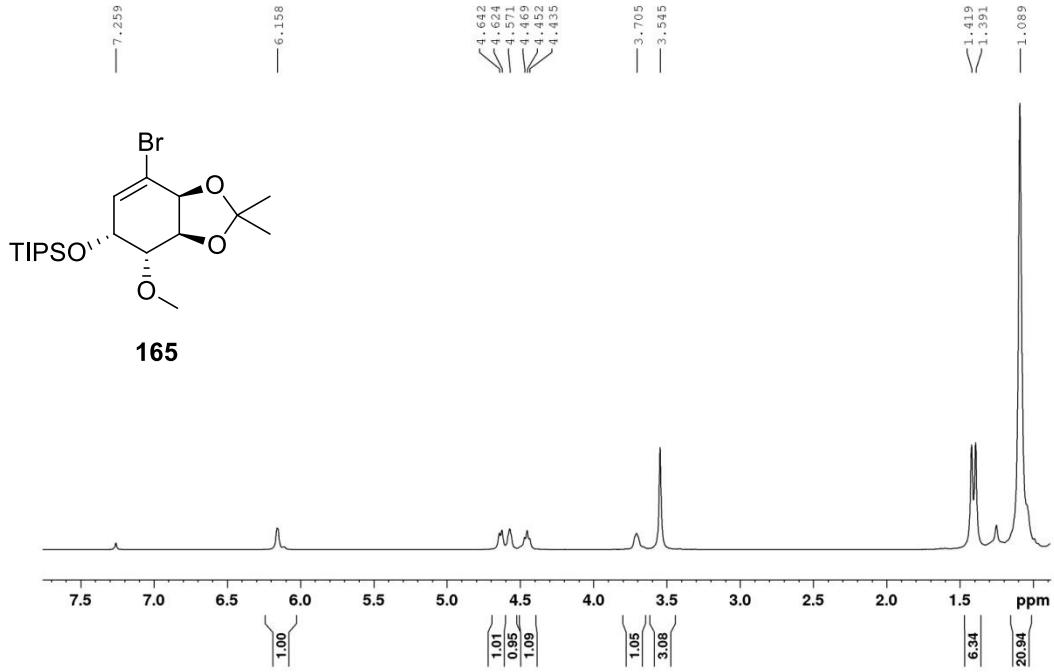
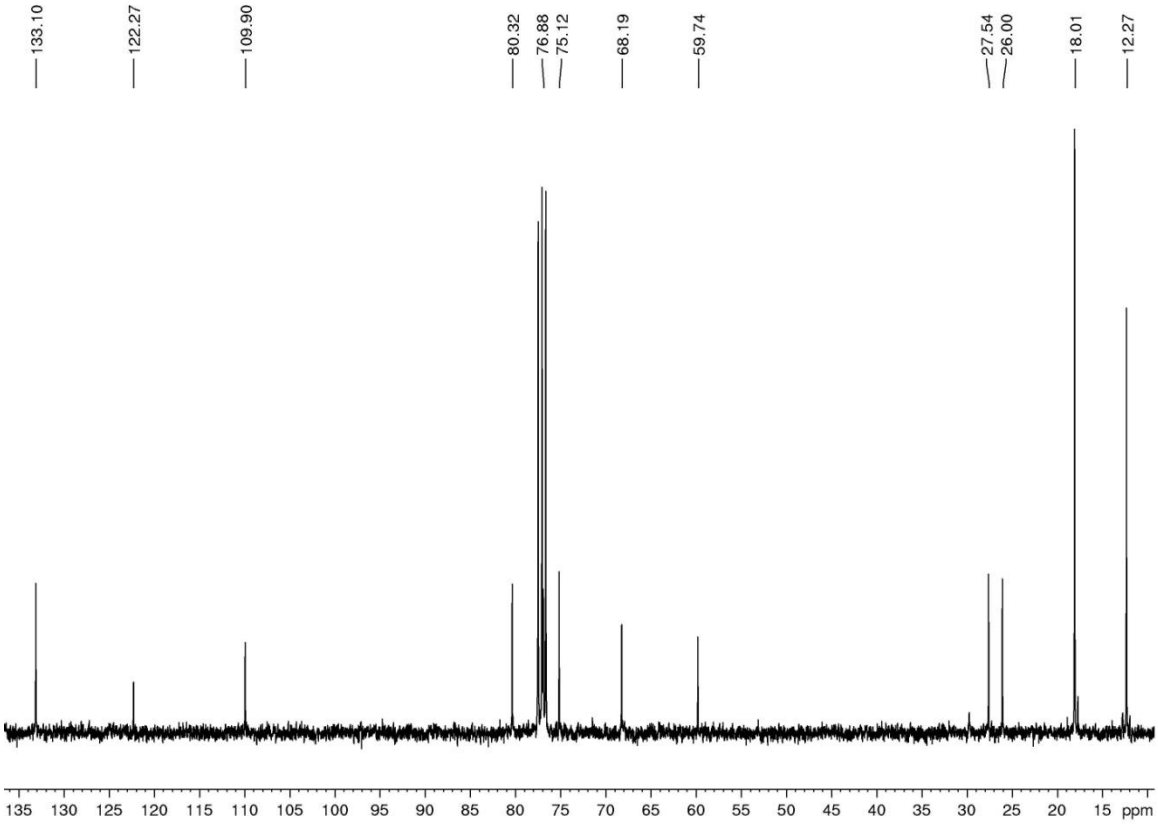
5.988
4.630
4.626
4.609
4.504
4.488
4.482
4.474
4.253
4.240
4.227
2.671
2.667
1.405
1.394
1.123
1.108
1.090
1.074
Br
O
O
TIPSO
OH
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
1.00
1.85
1.02
0.97
6.55
21.30

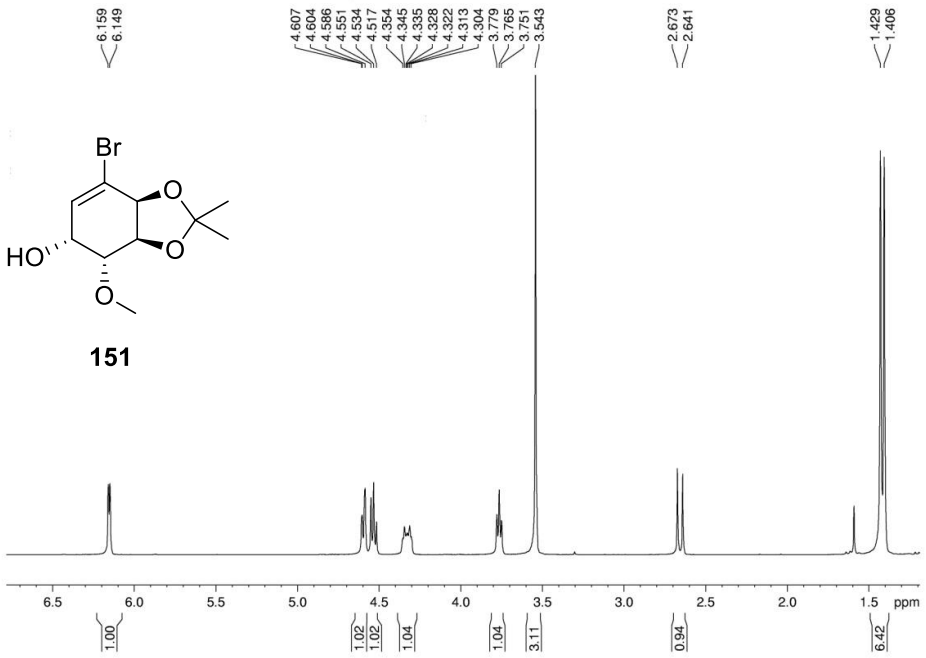
164


130.85
123.53
110.00
75.95
75.75
69.32
68.27
27.54
26.20
17.95
12.15
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
ppm

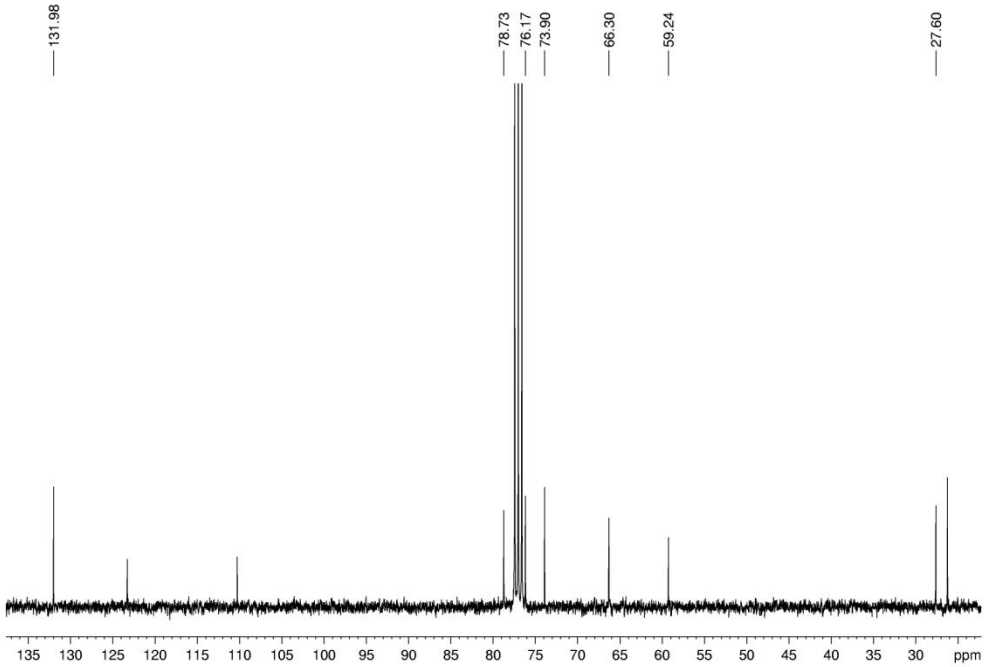

7.259
6.158
4.642
4.624
4.571
4.469
4.452
4.435
3.705
3.545
1.419
1.391
1.089
Br
O
O
TIPSO
O
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
1.01
0.95
1.09
1.05
3.08
6.34
20.94

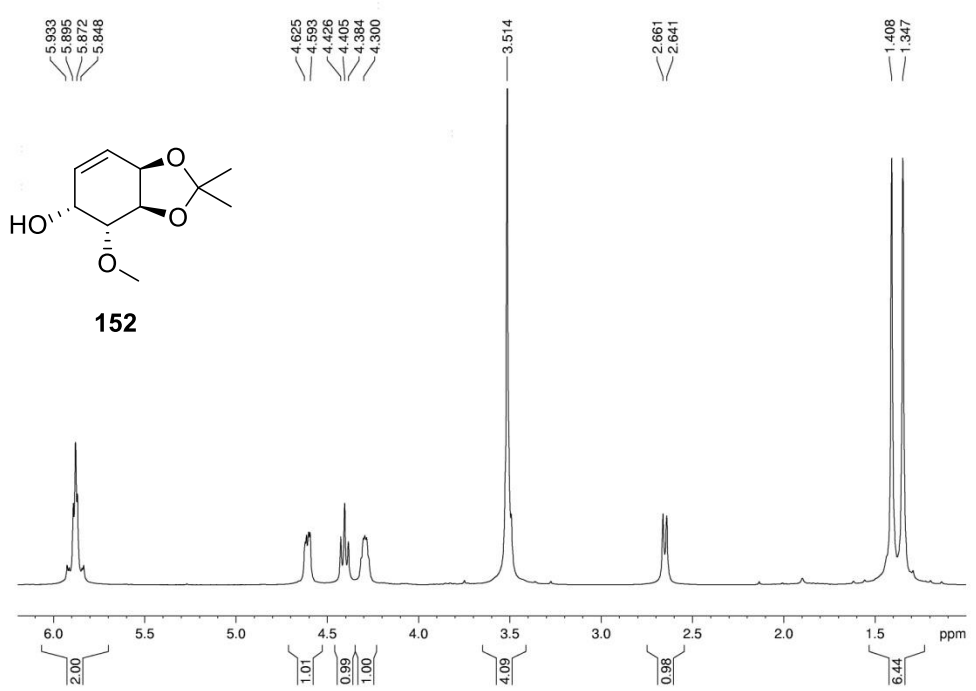
165


133.10
122.27
109.90
80.32
76.88
75.12
68.19
59.74
27.54
26.00
18.01
12.27
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
ppm

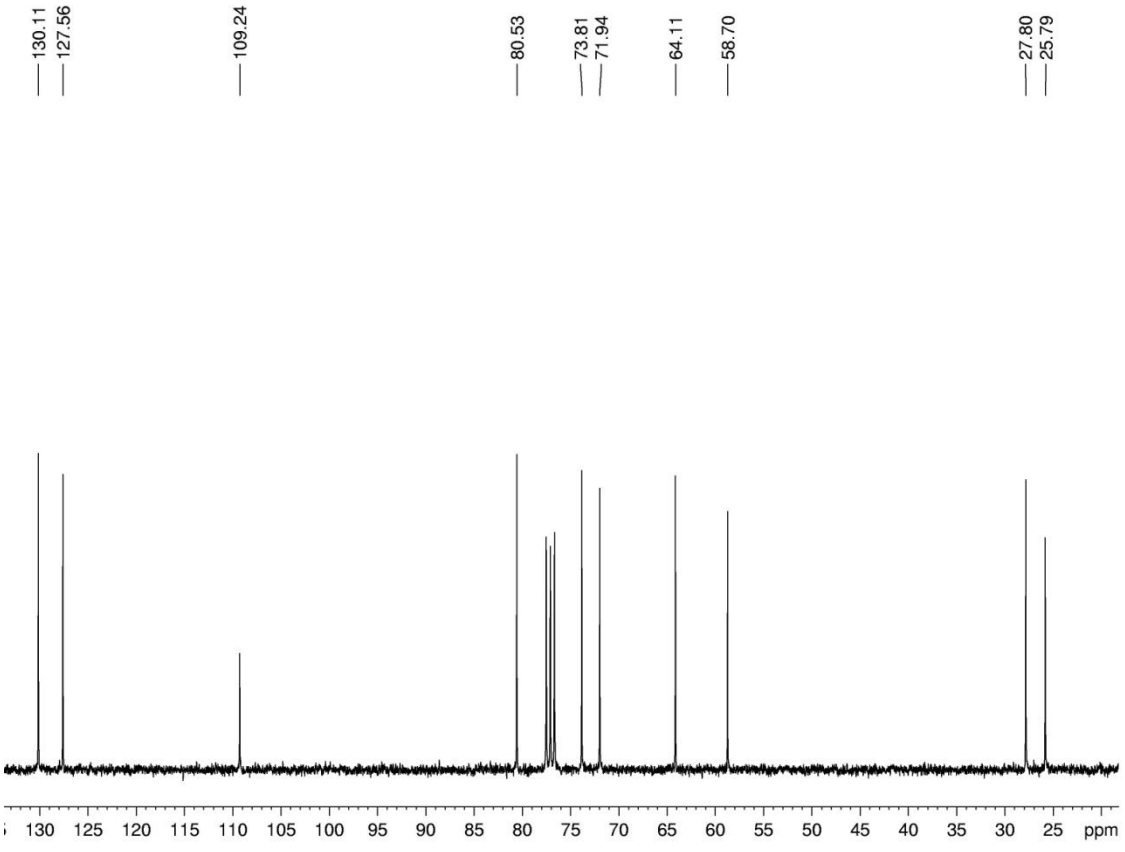

6.159
6.149
4.607
4.604
4.586
4.551
4.534
4.517
4.354
4.345
4.335
4.328
4.322
4.313
4.304
3.779
3.765
3.751
3.543
2.673
2.641
1.429
1.406
Br
O
O
HO
O
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
1.02
1.02
1.04
1.04
3.11
0.94
6.42

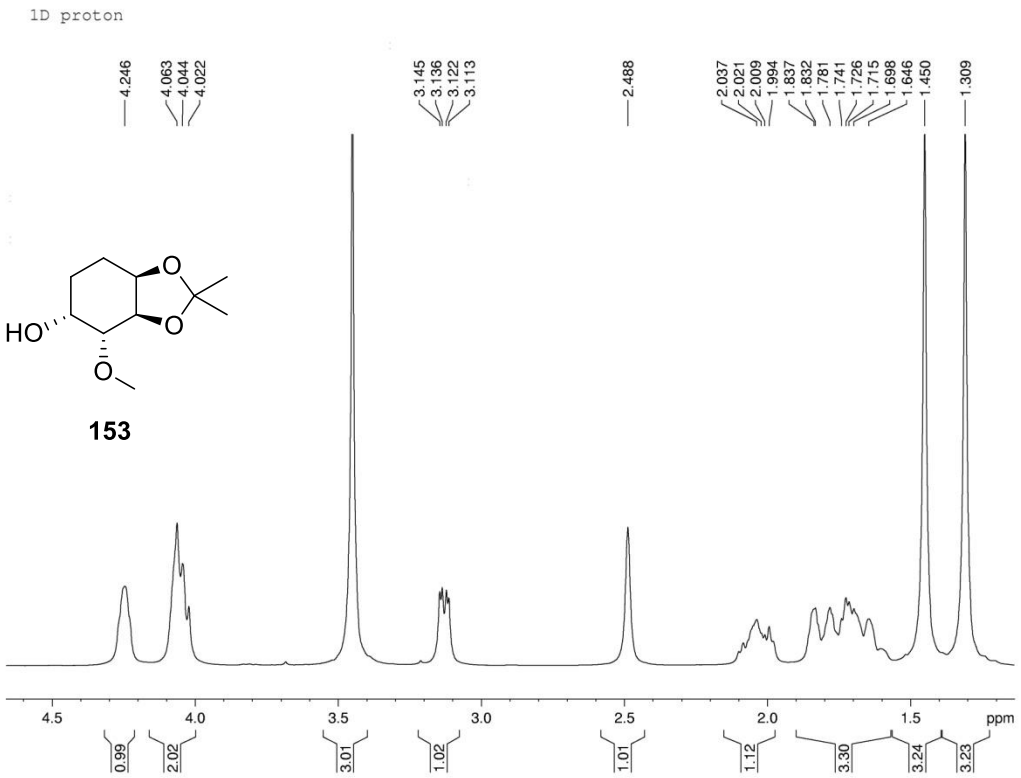
151

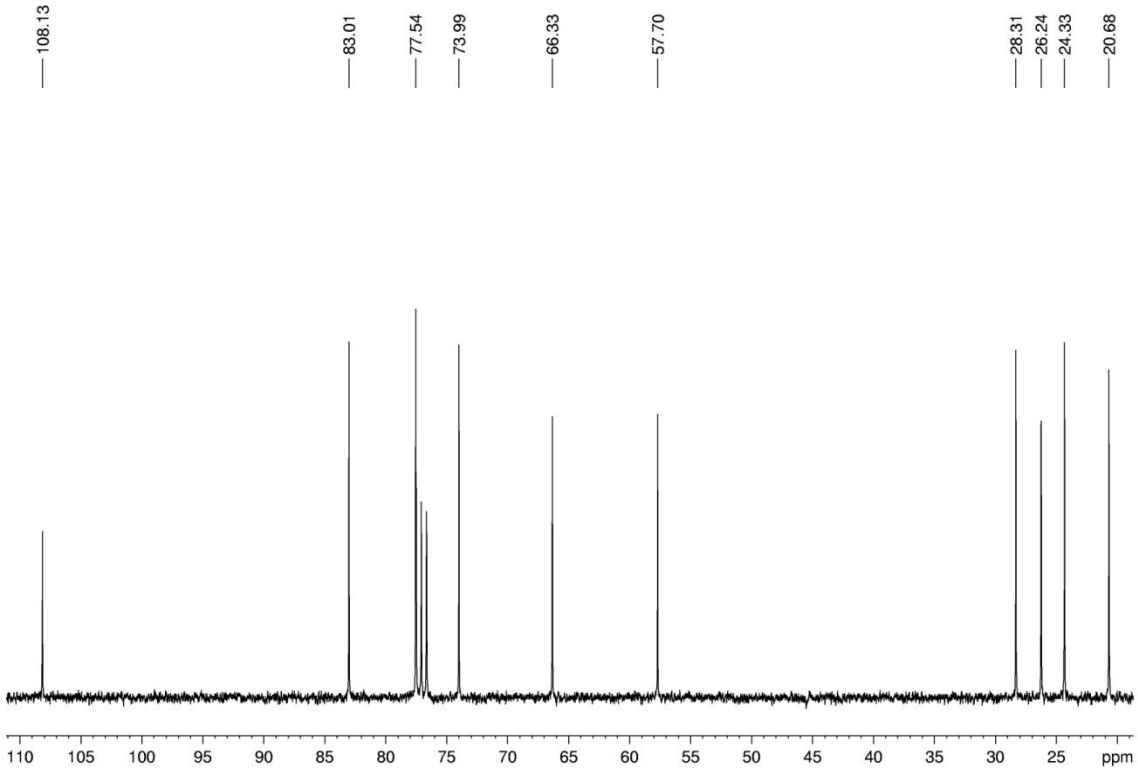

131.98
78.73
76.17
73.90
66.30
59.24
27.60
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
ppm

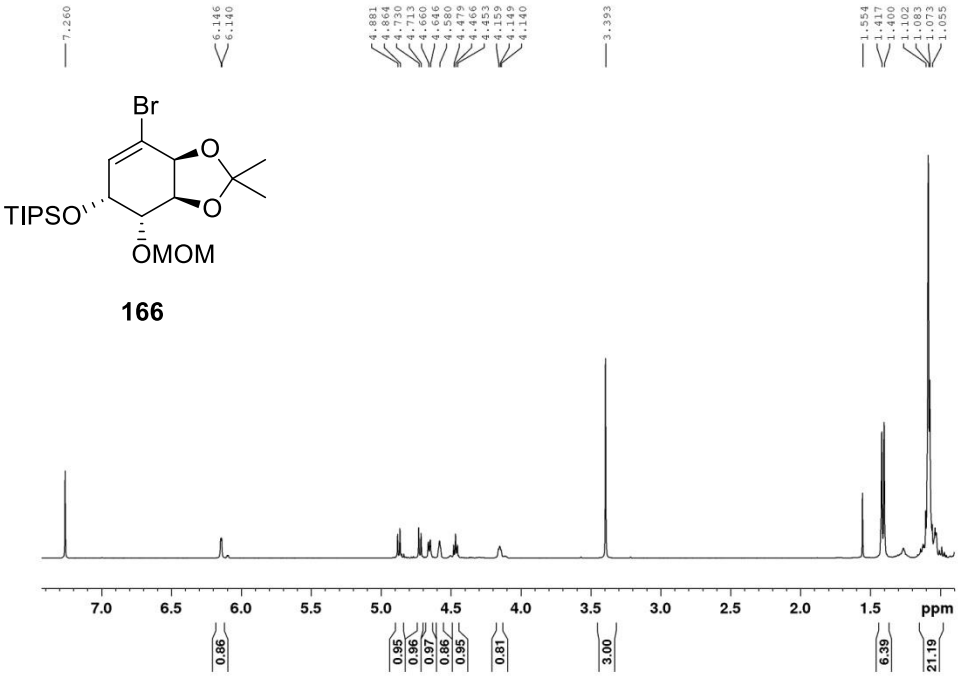

5.933
5.895
5.872
5.848
4.625
4.593
4.426
4.405
4.384
4.300
3.514
2.661
2.641
1.408
1.347
HO
O
O
O
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
2.00
1.01
0.99
1.00
4.09
0.98
6.44

152

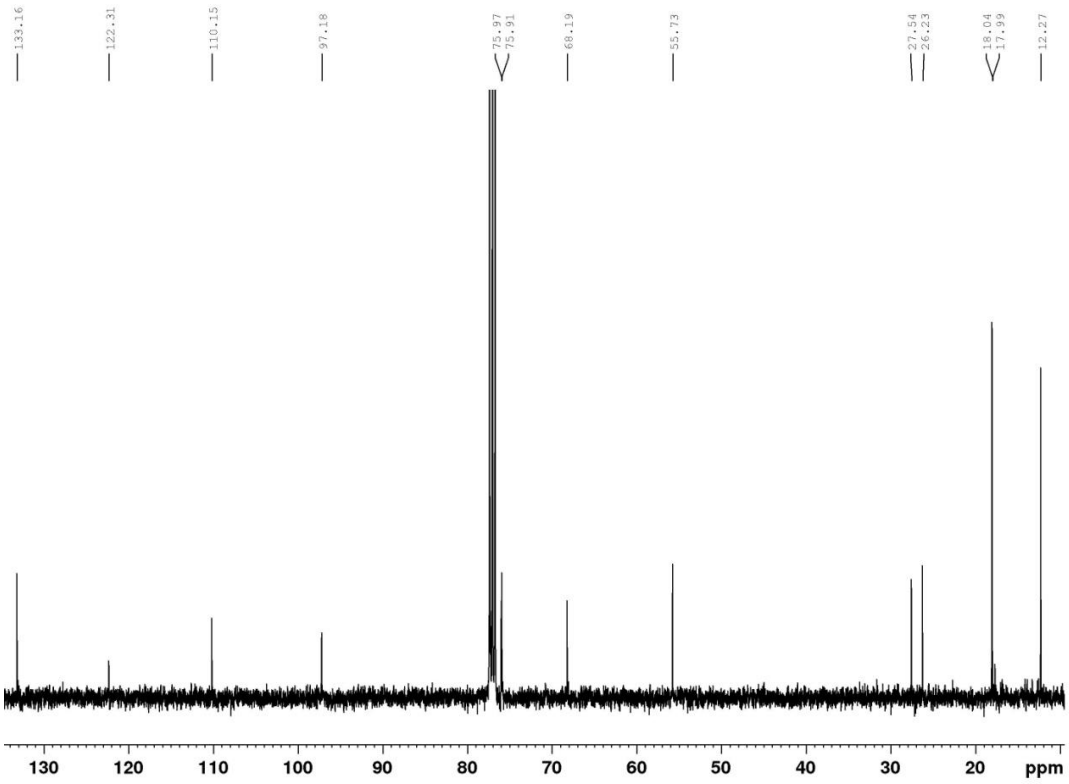

130.11
127.56
109.24
80.53
73.81
71.94
64.11
58.70
27.80
25.79
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

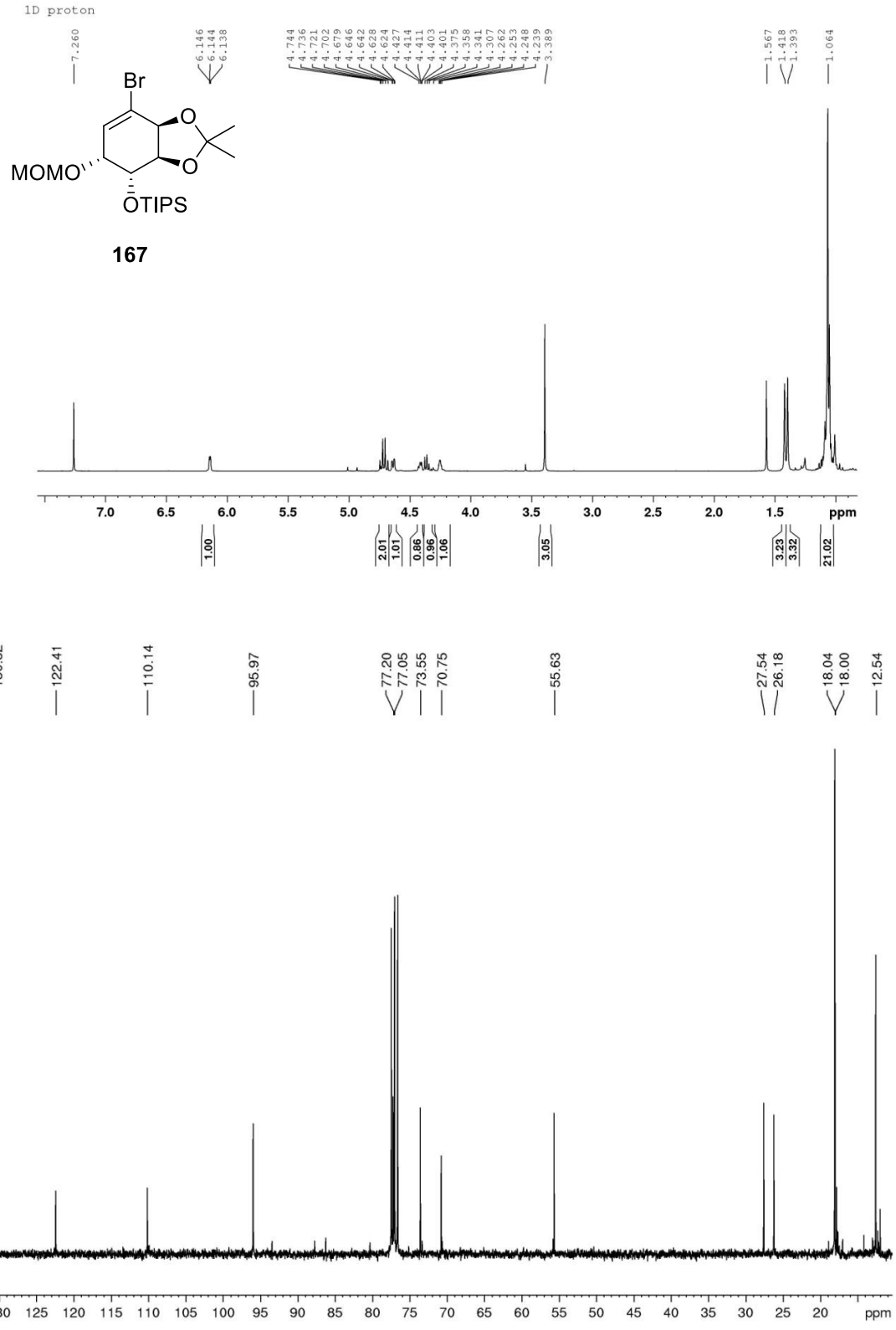

1D proton
4.246
4.063
4.044
4.022
3.145
3.136
3.122
3.113
2.488
2.037
2.021
2.009
1.994
1.837
1.832
1.781
1.741
1.726
1.715
1.698
1.646
1.450
1.309
HO
O
O
O
153
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
0.99
2.02
3.01
1.02
1.01
1.12
3.30
3.24
3.23

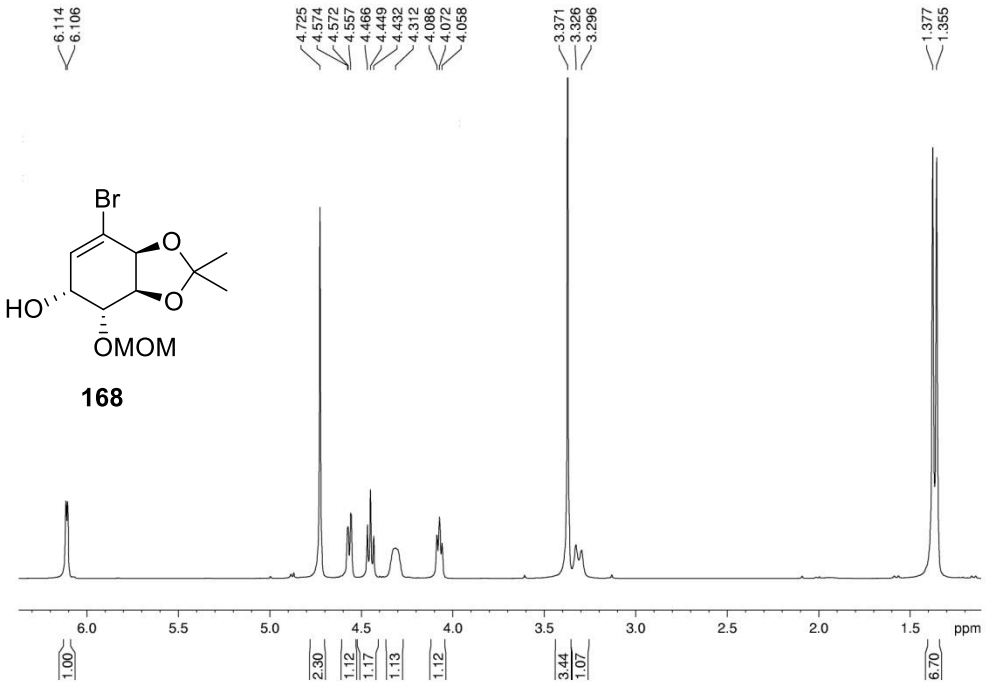

108.13
83.01
77.54
73.99
66.33
57.70
28.31
26.24
24.33
20.68
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

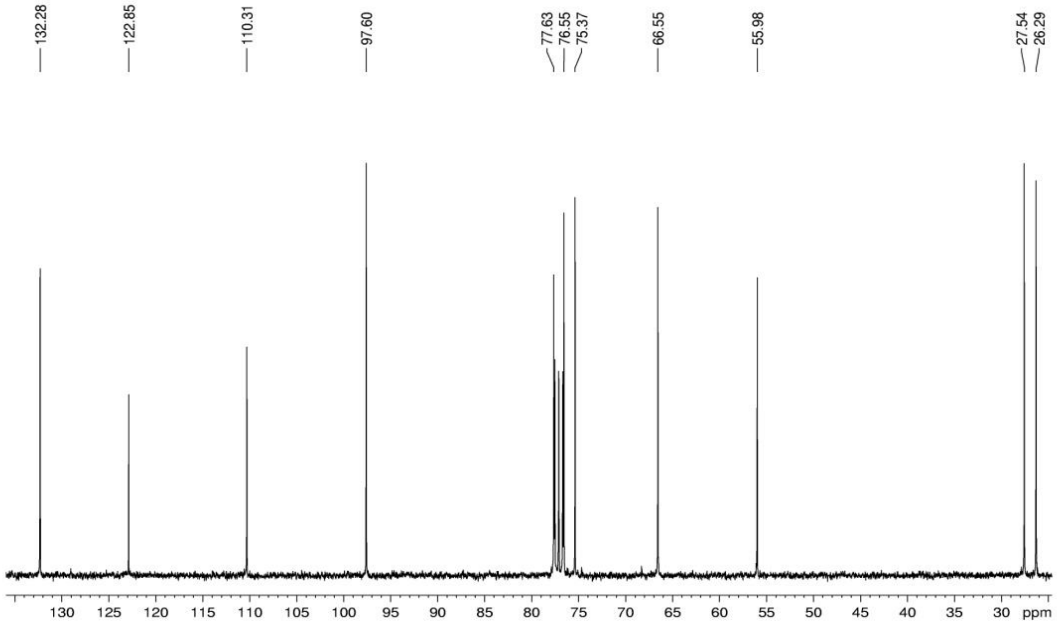

7.260
6.146
6.140
4.881
4.864
4.730
4.713
4.660
4.646
4.580
4.479
4.466
4.453
4.159
4.149
4.140
3.393
1.554
1.417
1.400
1.102
1.083
1.073
1.055
Br
O
O
TIPSO
OMOM
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
0.86
0.95
0.96
0.97
0.86
0.95
0.81
3.00
6.39
21.19

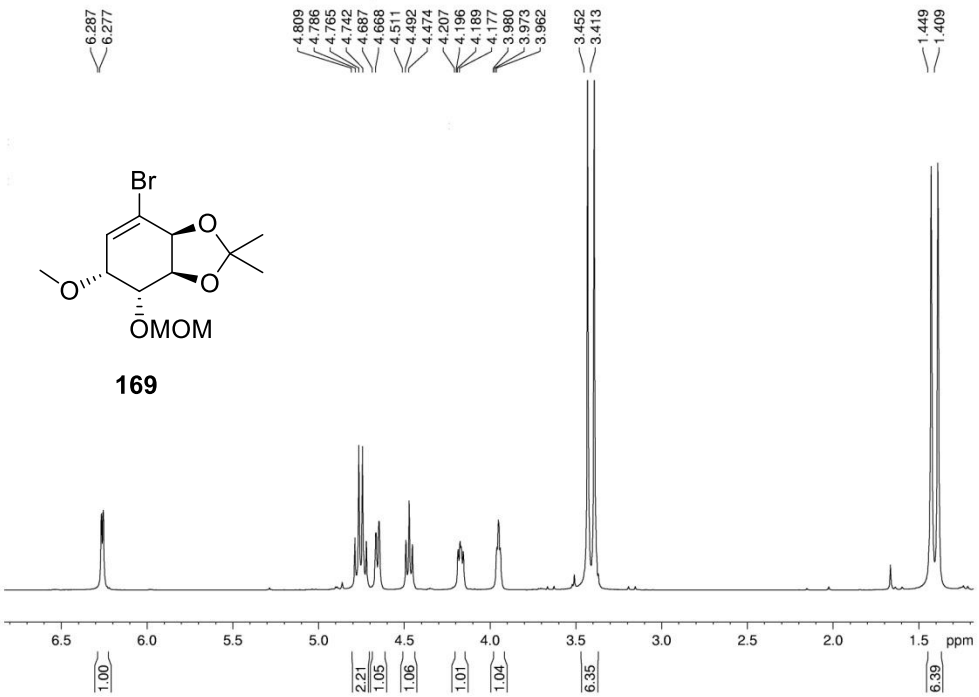
166


133.16
122.31
110.15
97.18
75.97
75.91
68.19
55.73
27.54
26.23
18.04
17.99
12.27
130
120
110
100
90
80
70
60
50
40
30
20
ppm

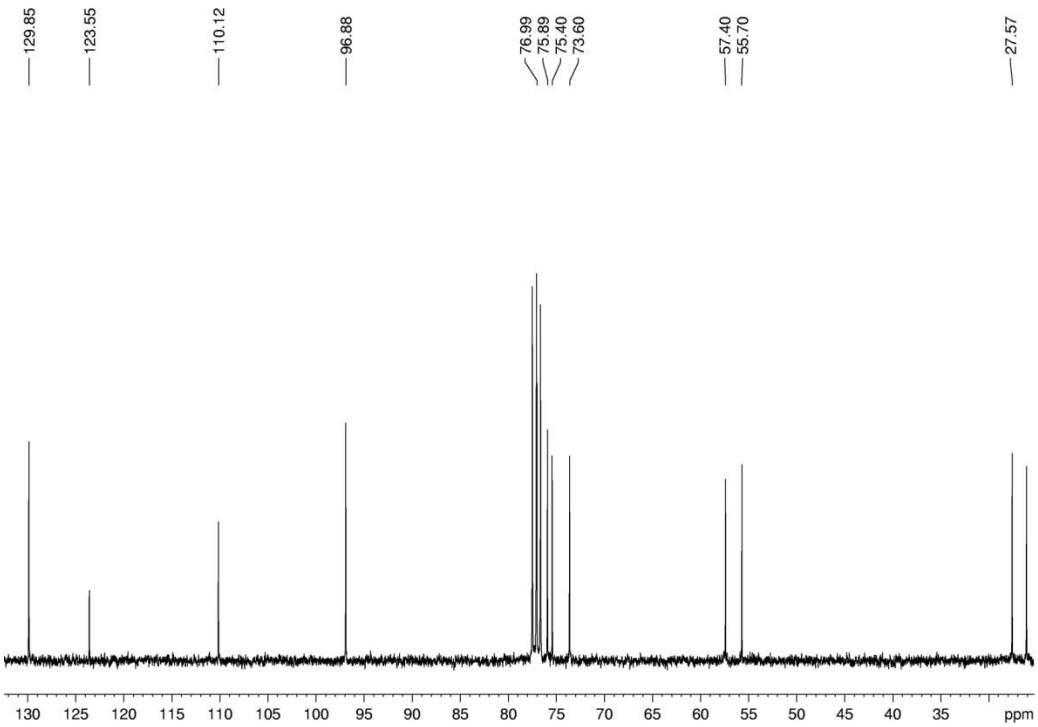

1D proton
Br
O
O
MOMO
OTIPS
167
7.260
6.146
6.144
6.138
4.744
4.736
4.721
4.702
4.679
4.646
4.642
4.628
4.624
4.427
4.414
4.411
4.403
4.401
4.375
4.358
4.341
4.307
4.262
4.253
4.248
4.239
3.389
1.567
1.418
1.393
1.064
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
2.01
1.01
0.86
0.96
1.06
3.05
3.23
3.32
21.02
130.32
122.41
110.14
95.97
77.20
77.05
73.55
70.75
55.63
27.54
26.18
18.04
18.00
12.54
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
ppm

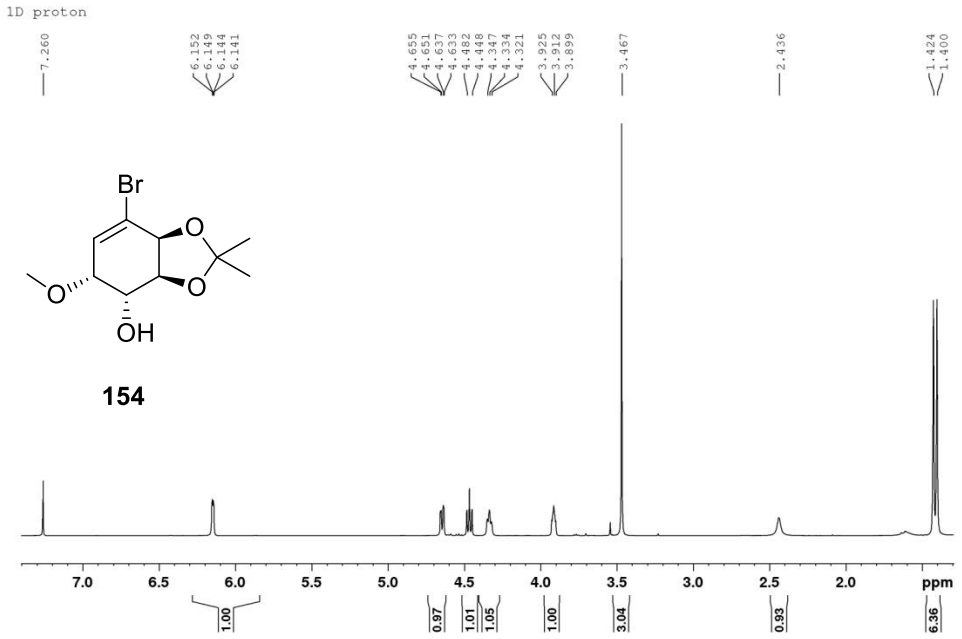

6.114
6.106
4.725
4.574
4.572
4.557
4.466
4.449
4.432
4.312
4.086
4.072
4.058
3.371
3.326
3.296
1.377
1.355
Br
O
O
HO
OMOM
168
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
2.30
1.12
1.17
1.13
1.12
3.44
1.07
6.70


132.28
122.85
110.31
97.60
77.63
76.55
75.37
66.55
55.98
27.54
26.29
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
ppm

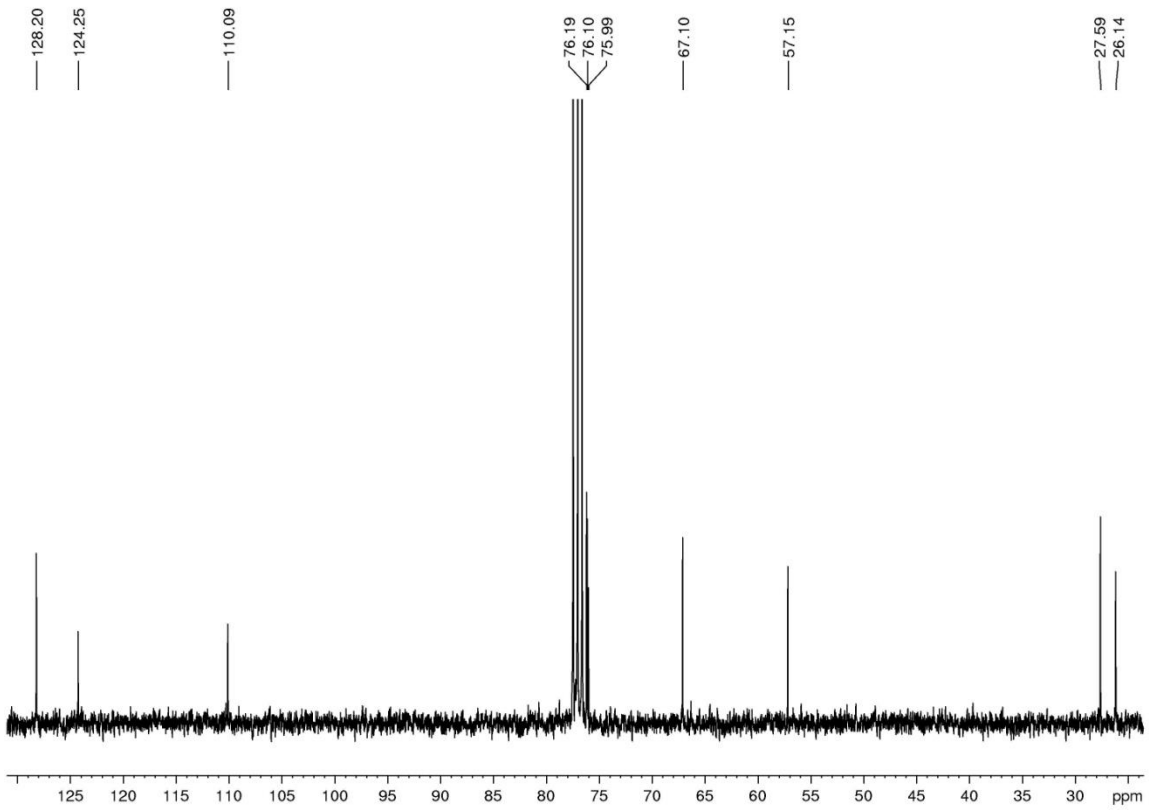

6.287
6.277
4.809
4.786
4.765
4.742
4.687
4.668
4.511
4.492
4.474
4.207
4.196
4.189
4.177
3.980
3.973
3.962
3.452
3.413
1.449
1.409
Br
O
O
O
OMOM
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
2.21
1.05
1.06
1.01
1.04
6.35
6.39

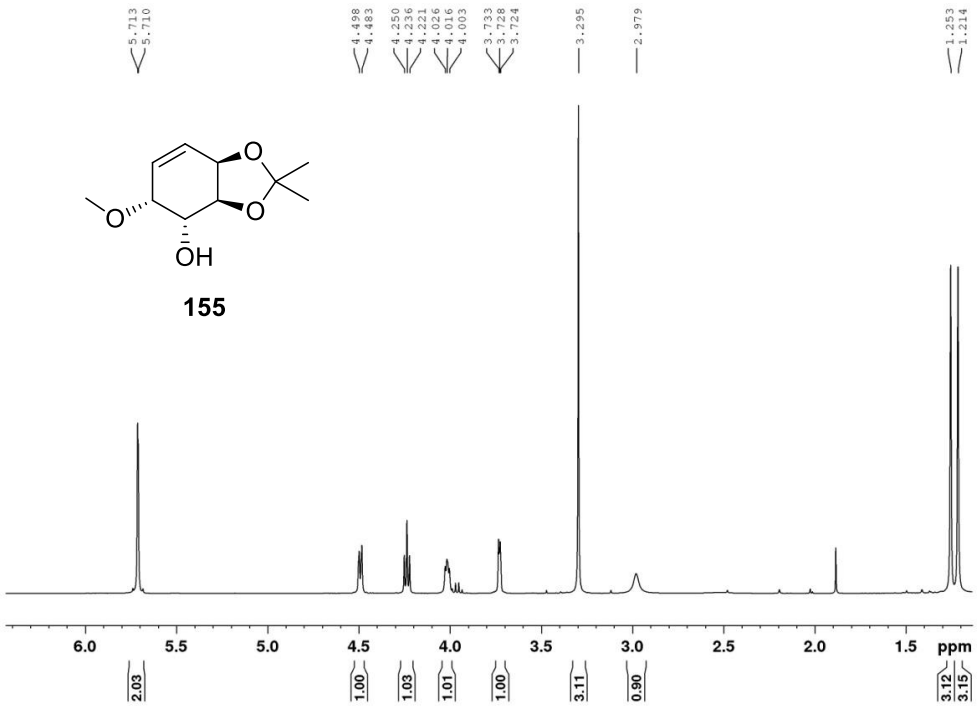
169


129.85
123.55
110.12
96.88
76.99
75.89
75.40
73.60
57.40
55.70
27.57
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
ppm


1D proton
7.260
6.152
6.149
6.144
6.141
4.655
4.651
4.637
4.633
4.482
4.448
4.347
4.334
4.321
3.925
3.912
3.899
3.467
2.436
1.424
1.400
Br
O
O
O
OH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.00
0.97
1.01
1.05
1.00
3.04
0.93
6.36

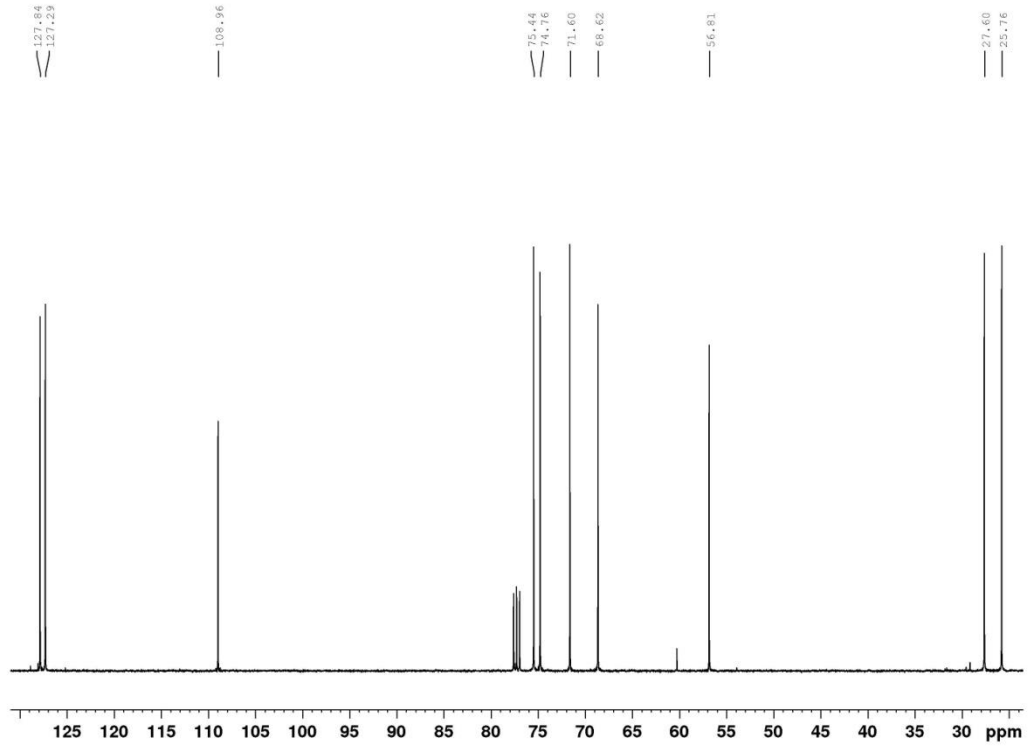
154

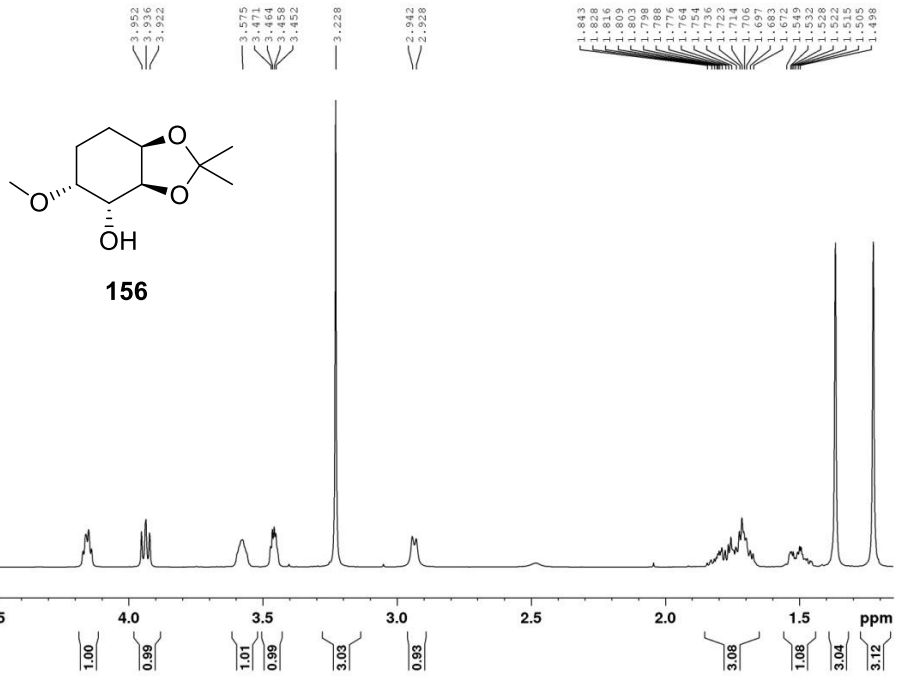

128.20
124.25
110.09
76.19
76.10
75.99
67.10
57.15
27.59
26.14
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
ppm



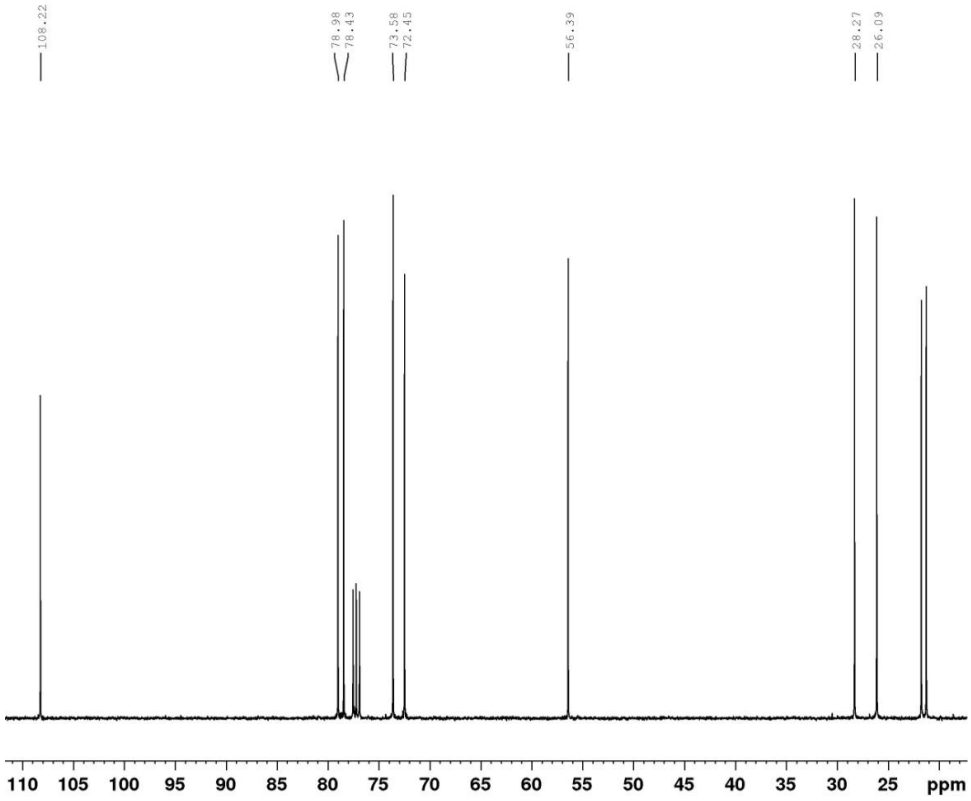
155

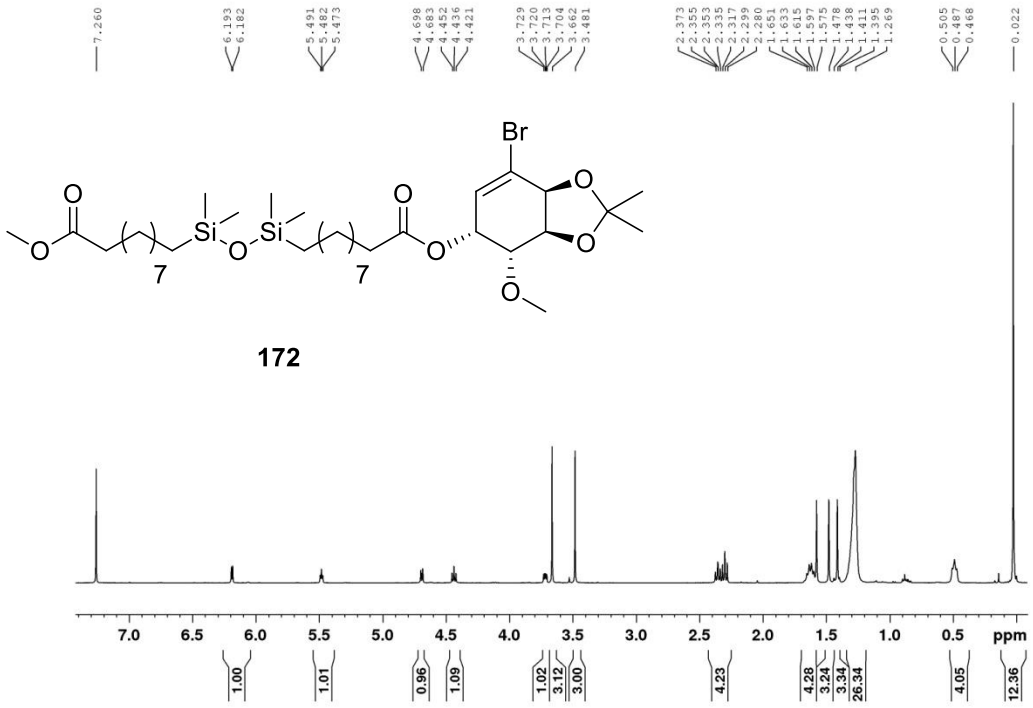
13C with 1H decoupling



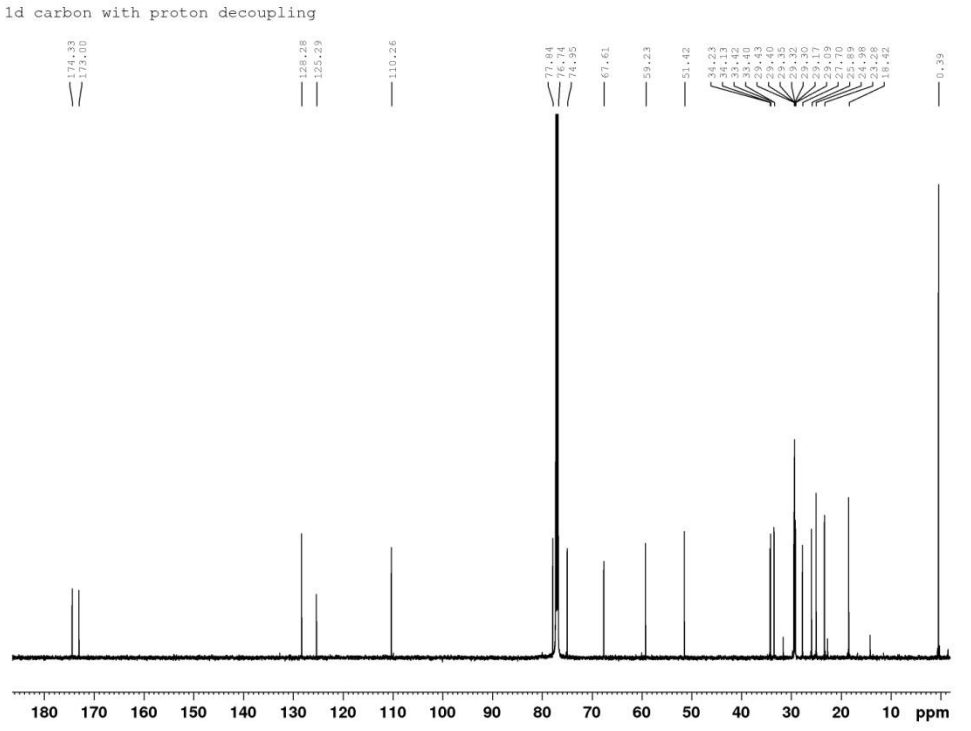

3.952
3.936
3.922
3.575
3.471
3.464
3.458
3.452
3.228
2.942
2.928
1.843
1.828
1.816
1.809
1.803
1.798
1.788
1.776
1.764
1.754
1.736
1.723
1.714
1.706
1.697
1.683
1.672
1.549
1.532
1.528
1.522
1.515
1.505
1.498
O
O
O
OH
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
0.99
1.01
0.99
3.03
0.93
3.08
1.08
3.04
3.12

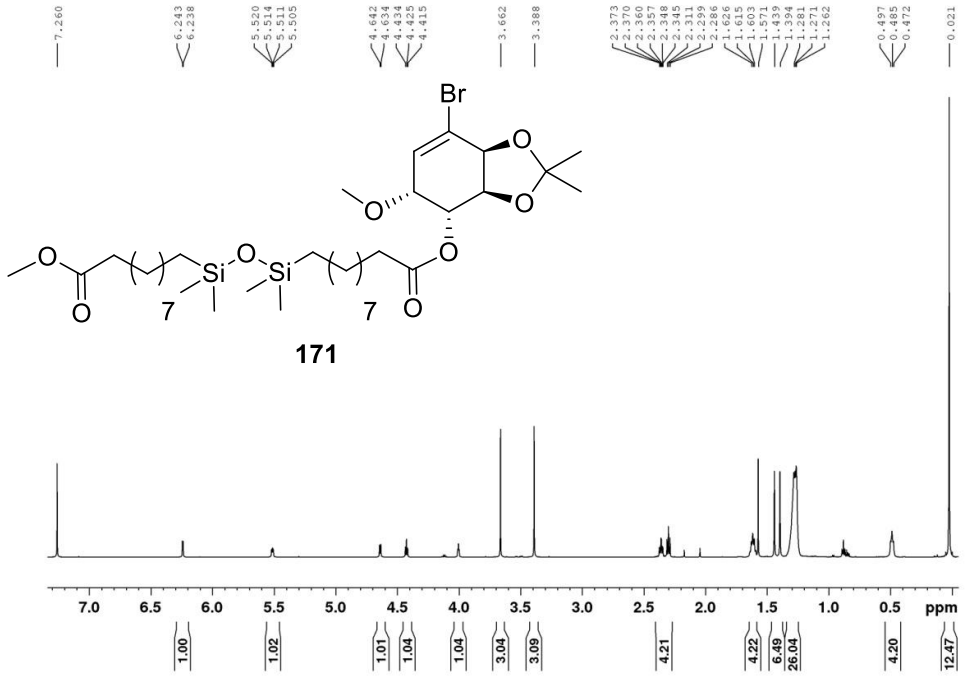
156


108.22
78.98
78.43
73.58
72.45
56.39
28.27
26.09
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
ppm

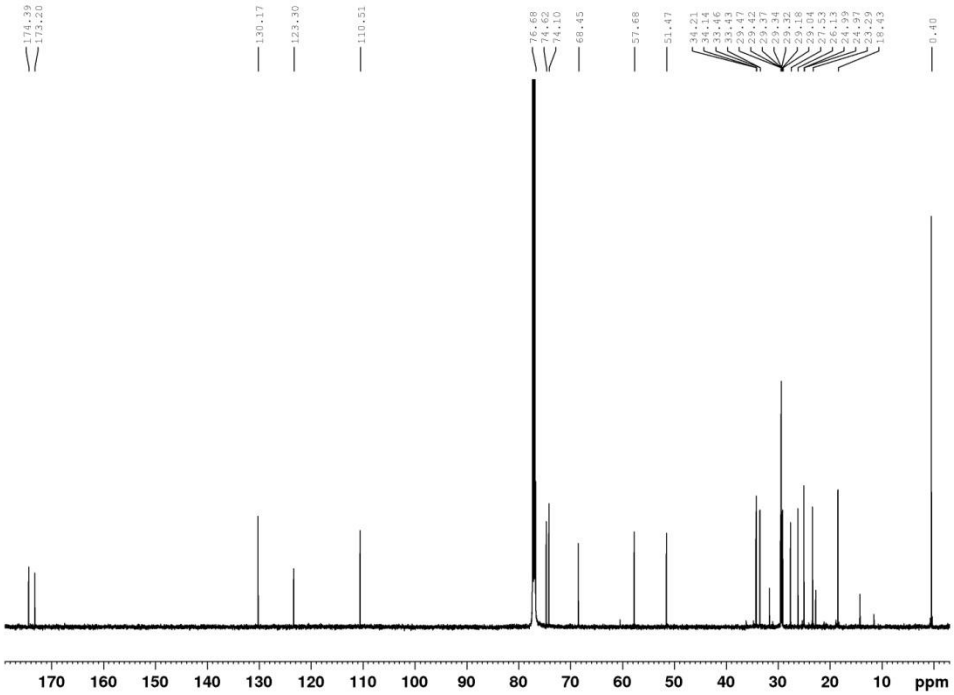

7.260
6.193
6.182
5.491
5.482
5.473
4.698
4.683
4.452
4.436
4.421
3.729
3.720
3.713
3.704
3.662
3.481
2.373
2.355
2.343
2.335
2.317
2.299
2.280
1.651
1.633
1.615
1.597
1.575
1.478
1.438
1.411
1.395
1.269
0.505
0.487
0.468
0.022
Br
O
O
O
O
O
O
Si
Si
7
7
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.01
0.96
1.09
1.02
3.12
3.00
4.23
4.28
3.24
3.34
26.34
4.05
12.36

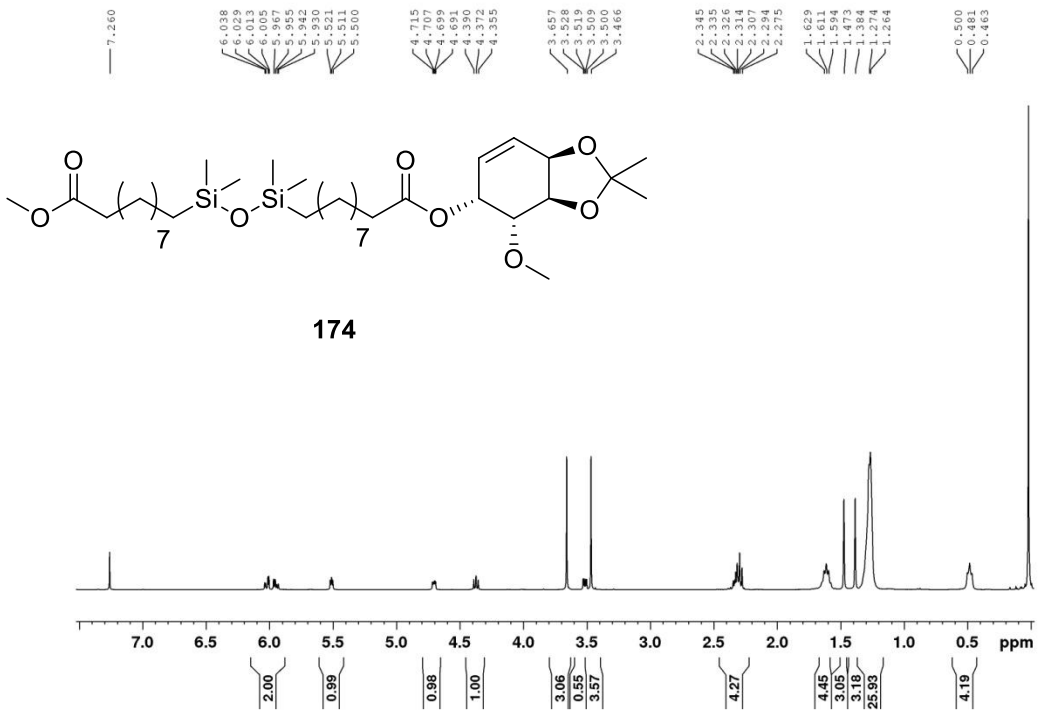
172


1d carbon with proton decoupling
174.33
173.00
128.28
125.29
110.26
77.84
77.14
76.35
67.61
59.23
51.42
34.23
34.13
33.42
33.40
29.43
29.40
29.35
29.32
29.30
29.17
29.09
27.70
25.89
24.98
23.28
18.42
0.39
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

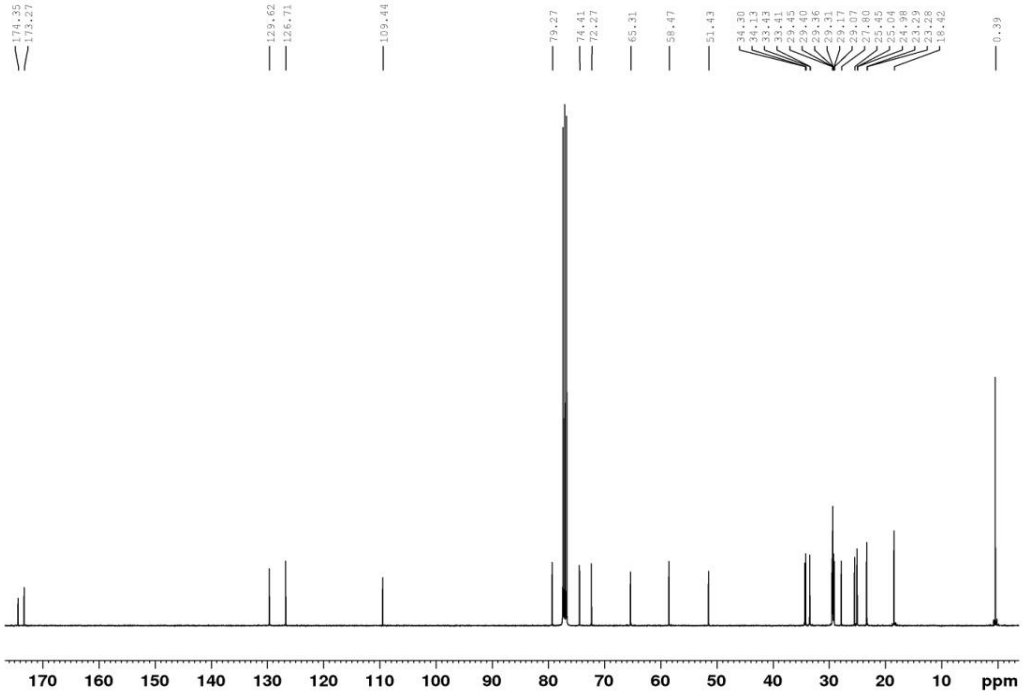

7.260
6.243
6.238
5.520
5.514
5.511
5.505
4.642
4.634
4.434
4.425
4.415
3.662
3.388
2.373
2.370
2.360
2.357
2.348
2.345
2.311
2.299
2.286
1.615
1.603
1.571
1.439
1.394
1.281
1.271
1.262
0.497
0.485
0.472
0.021
Br
O
O
O
O
O
Si
O
Si
O
7
7
O
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.02
1.01
1.04
1.04
3.04
3.09
4.21
4.22
6.49
26.04
4.20
12.47

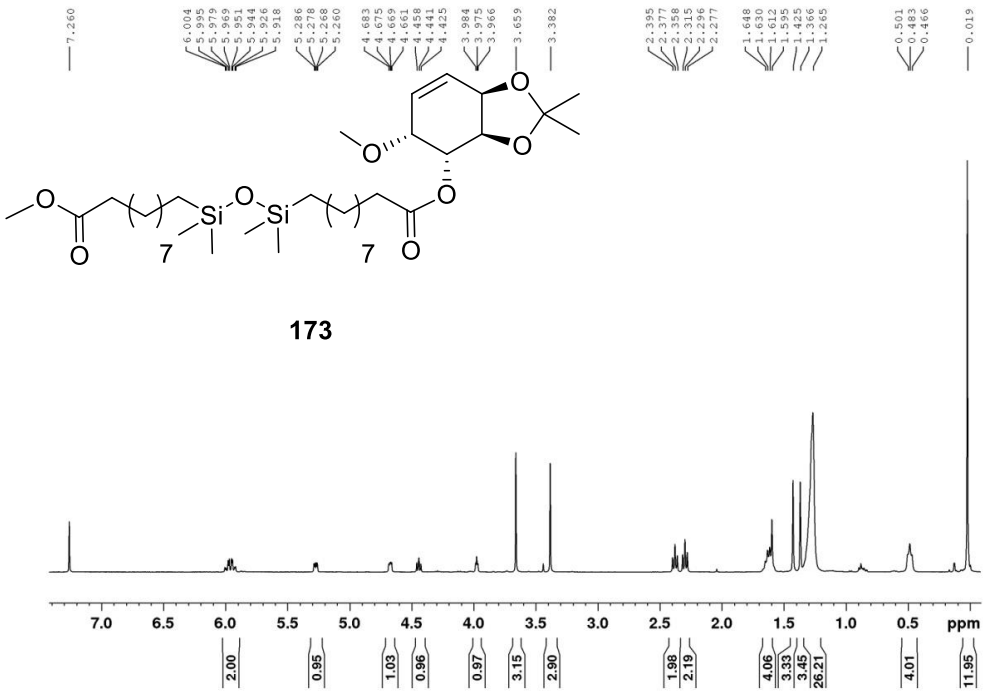
171


174.39
173.20
130.17
123.30
110.51
76.68
74.62
74.10
68.45
57.68
51.47
34.21
34.14
33.46
33.43
29.47
29.42
29.37
29.34
29.32
29.18
29.04
27.53
26.13
26.09
24.97
23.29
18.43
0.40
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

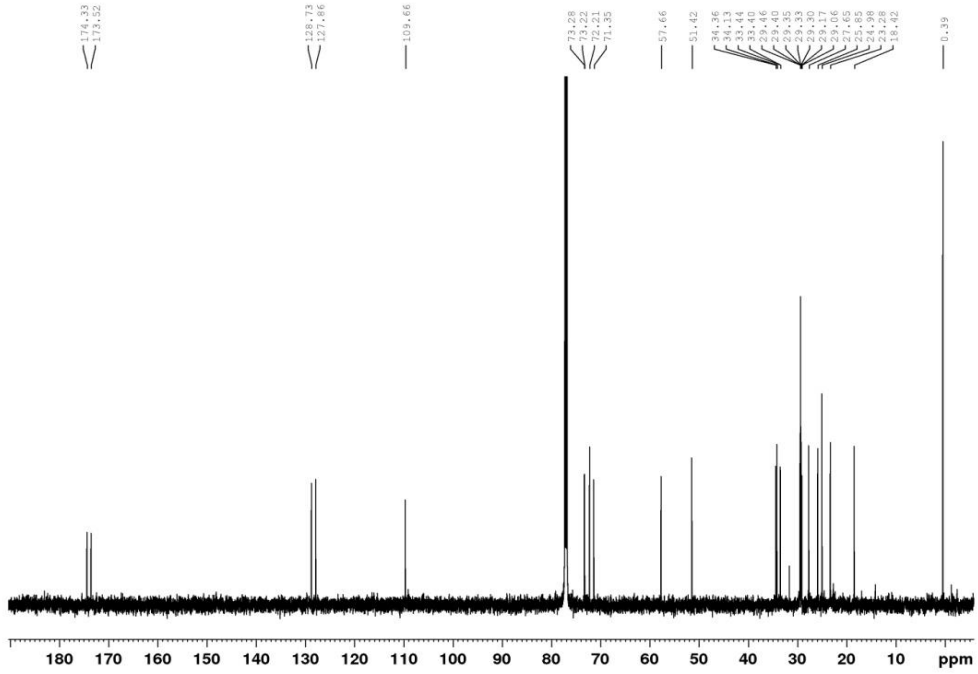

7.260
6.038
6.029
6.013
6.005
5.967
5.955
5.942
5.930
5.511
5.511
5.500
4.715
4.707
4.699
4.691
4.390
4.372
4.355
3.657
3.528
3.519
3.509
3.500
3.466
2.345
2.335
2.326
2.314
2.307
2.294
2.275
1.629
1.611
1.594
1.373
1.364
1.274
1.264
0.500
0.481
0.463
O
O
Si
O
Si
O
O
O
O
O
7
7
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.00
0.99
0.98
1.00
3.06
0.55
3.57
4.27
4.45
3.05
3.18
25.93
4.19

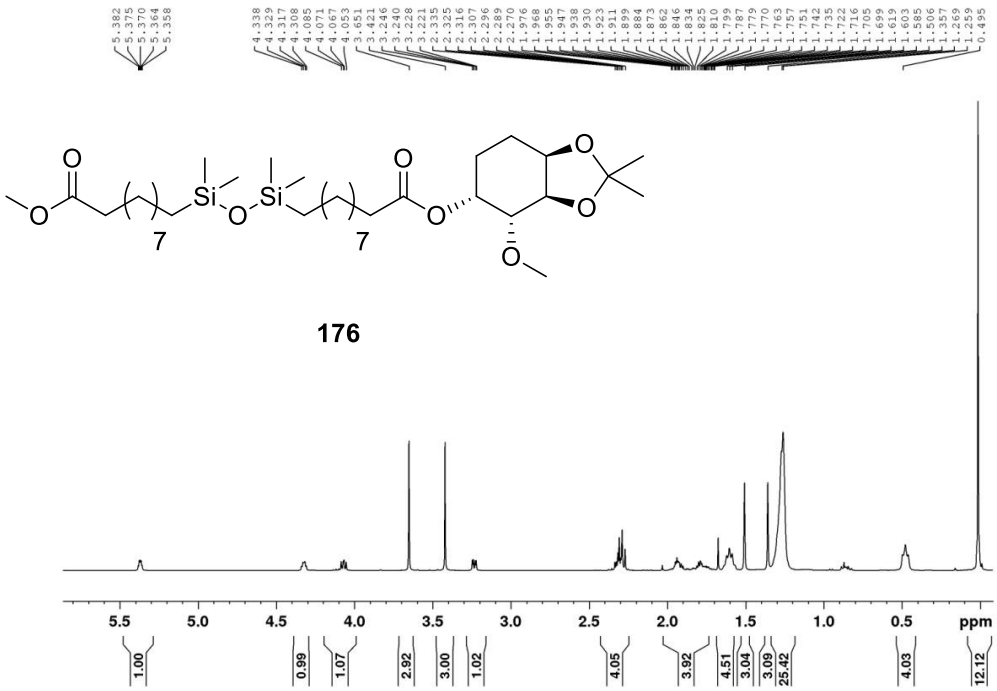
174


174.35
173.27
129.62
126.71
109.44
79.27
74.41
72.27
65.31
58.47
51.43
34.30
34.13
33.43
33.41
29.45
29.40
29.36
29.31
29.17
29.07
27.80
25.45
25.04
24.98
23.29
23.28
18.42
0.39
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.260
6.004
5.995
5.979
5.969
5.931
5.944
5.926
5.918
5.286
5.278
5.268
5.260
4.683
4.675
4.669
4.661
4.458
4.441
4.425
3.984
3.975
3.966
3.659
3.382
2.395
2.377
2.358
2.315
2.296
2.277
1.648
1.630
1.612
1.595
1.455
1.366
1.265
0.501
0.483
0.466
0.019
O
O
O
O
O
O
O
Si
Si
7
7
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
2.00
0.95
1.03
0.96
0.97
3.15
2.90
1.98
2.19
4.06
3.33
3.45
26.21
4.01
11.95

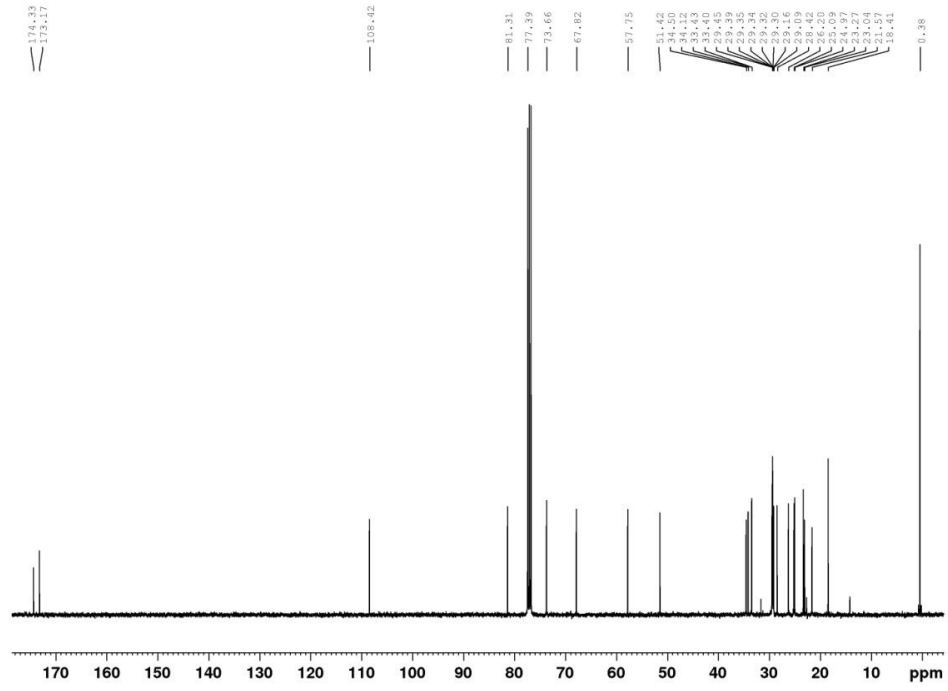
173

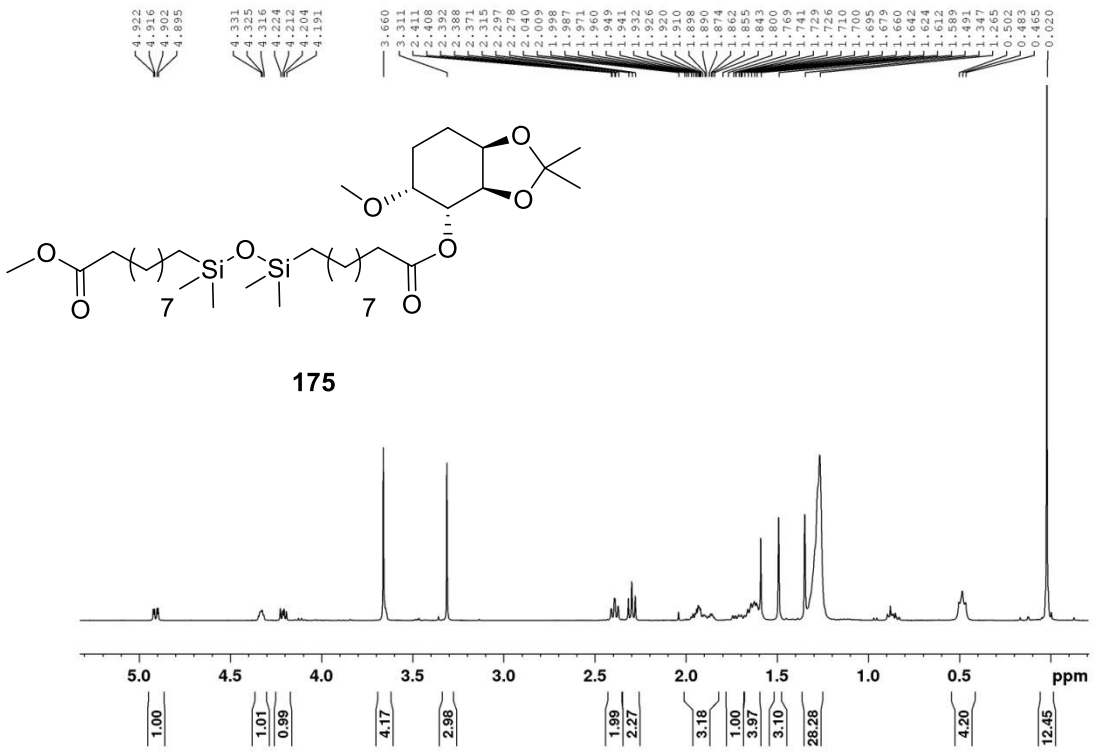

174.33
173.52
128.73
127.86
109.66
73.28
73.22
72.31
71.35
57.66
51.42
34.36
34.13
33.44
33.16
29.46
29.40
29.35
29.33
29.30
29.17
29.06
27.65
25.85
24.98
23.28
18.42
0.39
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm



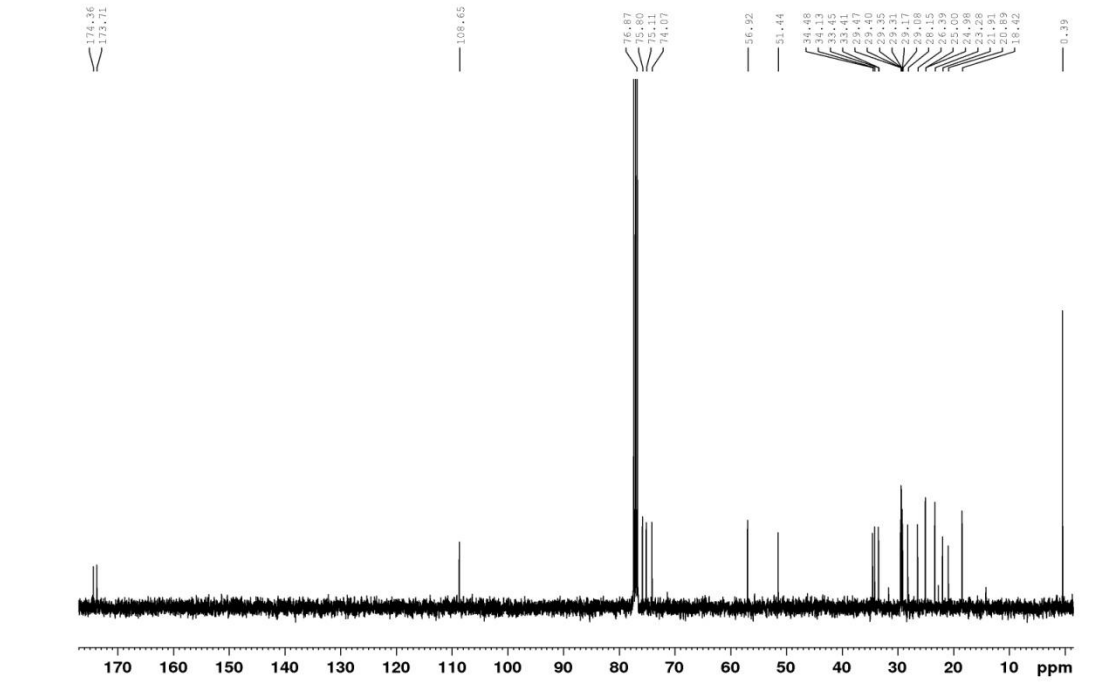
176

13C with 1H decoupling




4.922
4.916
4.902
4.895
4.331
4.325
4.316
4.224
4.212
4.204
4.191
3.660
3.311
2.411
2.408
2.392
2.388
2.371
2.315
2.297
2.278
2.040
2.009
1.998
1.987
1.971
1.960
1.949
1.941
1.932
1.926
1.920
1.910
1.898
1.890
1.874
1.862
1.855
1.843
1.800
1.769
1.741
1.729
1.726
1.710
1.700
1.695
1.679
1.660
1.642
1.624
1.612
1.589
1.491
1.347
1.265
0.502
0.483
0.465
0.020
O
O
O
O
O
Si
Si
O
O
7
7
O
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
1.01
0.99
4.17
2.98
1.99
2.27
3.18
1.00
3.97
3.10
28.28
4.20
12.45

175

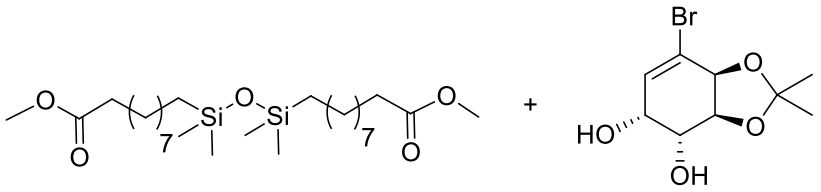

174.36
173.71
108.65
76.87
75.80
75.11
74.07
56.92
51.44
34.48
34.13
33.45
33.41
29.47
29.40
29.35
29.31
29.17
29.08
29.05
26.39
25.00
24.98
23.28
21.91
20.89
18.42
0.39
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

6.2 Selected MALDI-ToF spectra

Selected MALDI analysis of crude unfractioned N435-mediatied transesterification reactions between diol **4** (A) with diester **135** (B), showed that reactions performed in toluene for 24 h resulted in formation of the AB and the A_2B units in 97:3 ratio respectively. 7 d reactions performed in toluene resulted in formation of the following species AB:A_2B:A_2B_2:AB_{cyclic} in 90:7:2:1 ratios respectively. 7 d solvent-free reactions resulted in formation of AB:A_2B:AB_2:A_2B_2 species in roughly 62:18:11:9 ratios respectively.

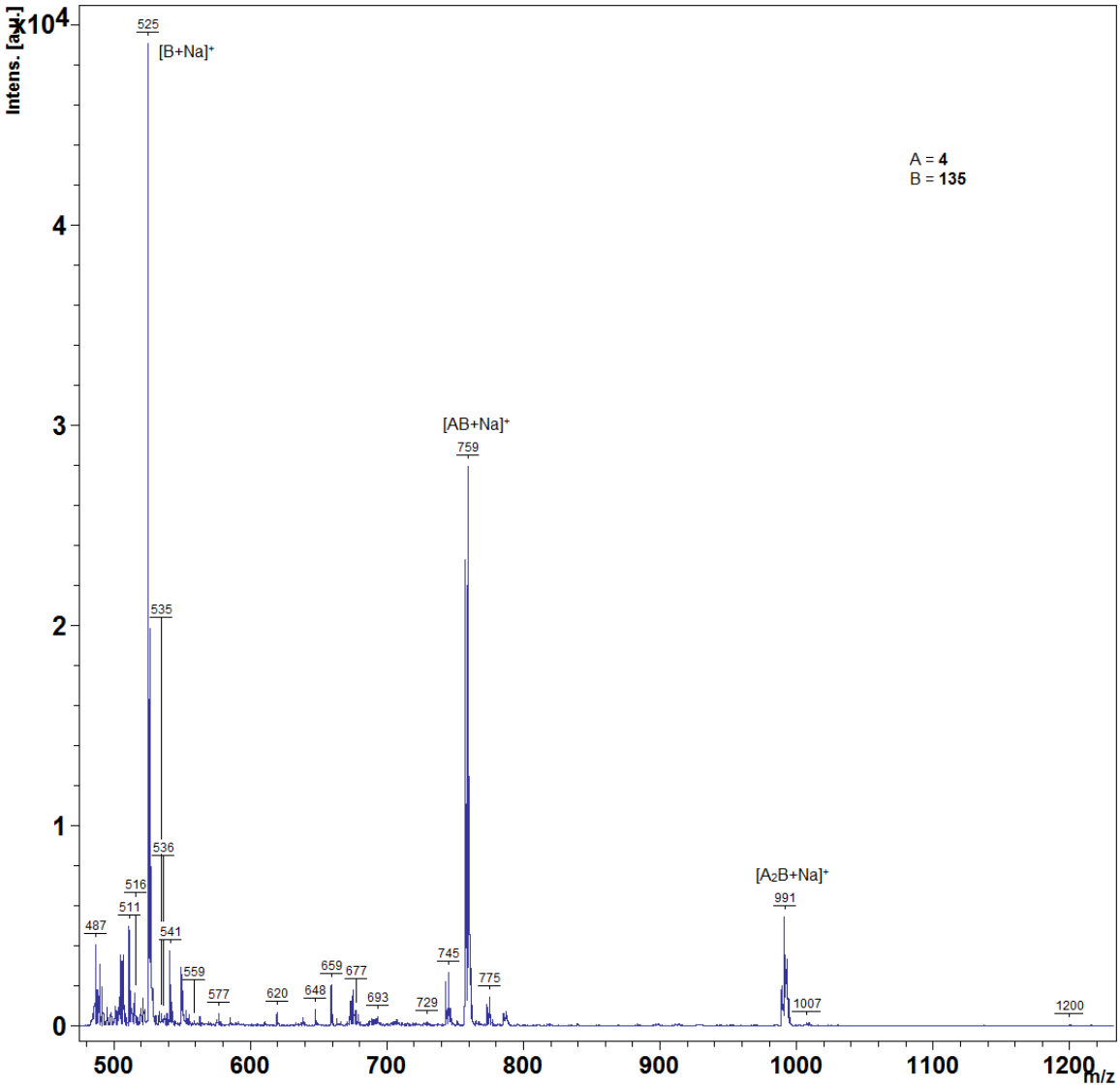
Selected MALDI analysis of crude unfractioned N435-mediatied transesterification reactions between diol **5** (A) with diester **135** (B), showed that reactions performed in toluene for 24 h resulted in formation of the AB:A_2B:AB_2 species in 81:15:4 ratios respectively. 7 d reactions performed in toluene resulted in formation of species AB:A_2B:AB_2:A_2B_2 in the following ratios 78:16:5:1 respectively. 7 d solvent-free reactions resulted in formation of AB:A_2B:AB_2:A_2B_2: A_2B_3 species in roughly 73:3:21:2:1 ratios respectively.

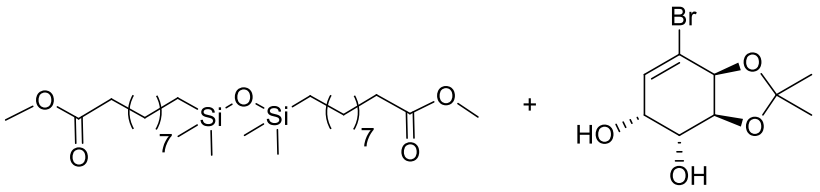
Selected MALDI analysis of crude unfractioned N435-mediatied transesterification reaction between diol **6** (A) with diester **135** (B), showed that reactions performed in toluene for 24 h resulted in formation of the species AB:A_2B in roughly 34:66 ratio respectively. 7 d reactions performed in toluene resulted in formation of species AB:A_2B:AB_2:A_2B_2 in roughly 20:60:10:10 ratios respectively. 7 d solvent-free reactions resulted in formation of AB:A_2B:AB_2:A_2B_2 species in roughly 50:14:23:2 ratios respectively.



135 **4**

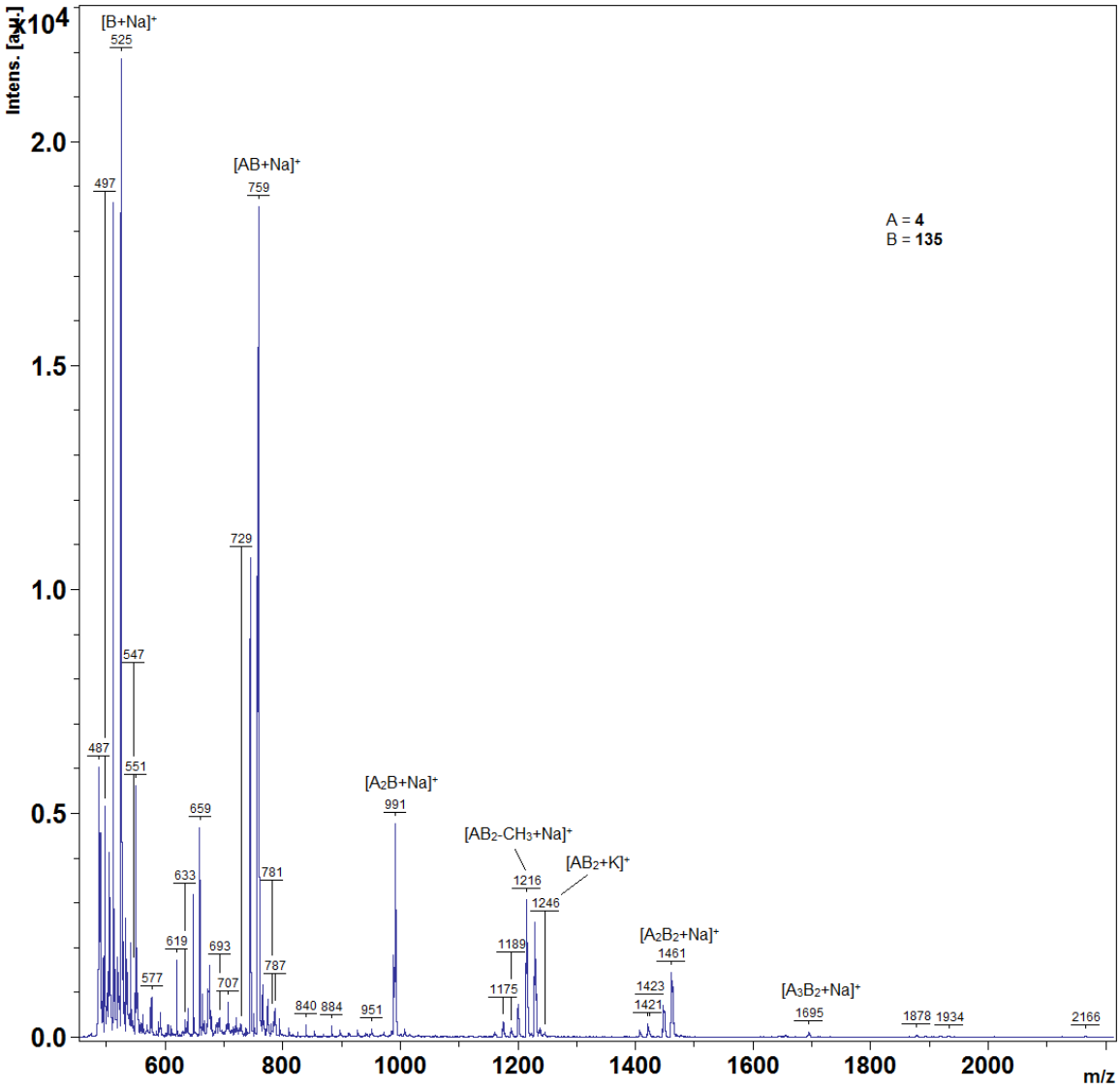
7 d, toluene, 100 °C, 10 wt% N435, 150 rpm

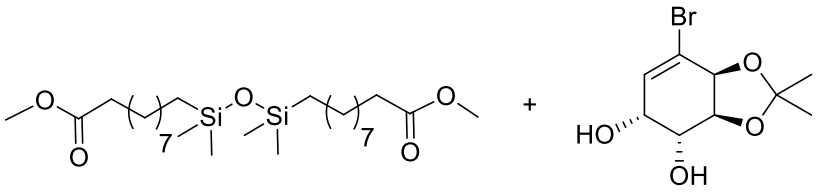




135 **4**

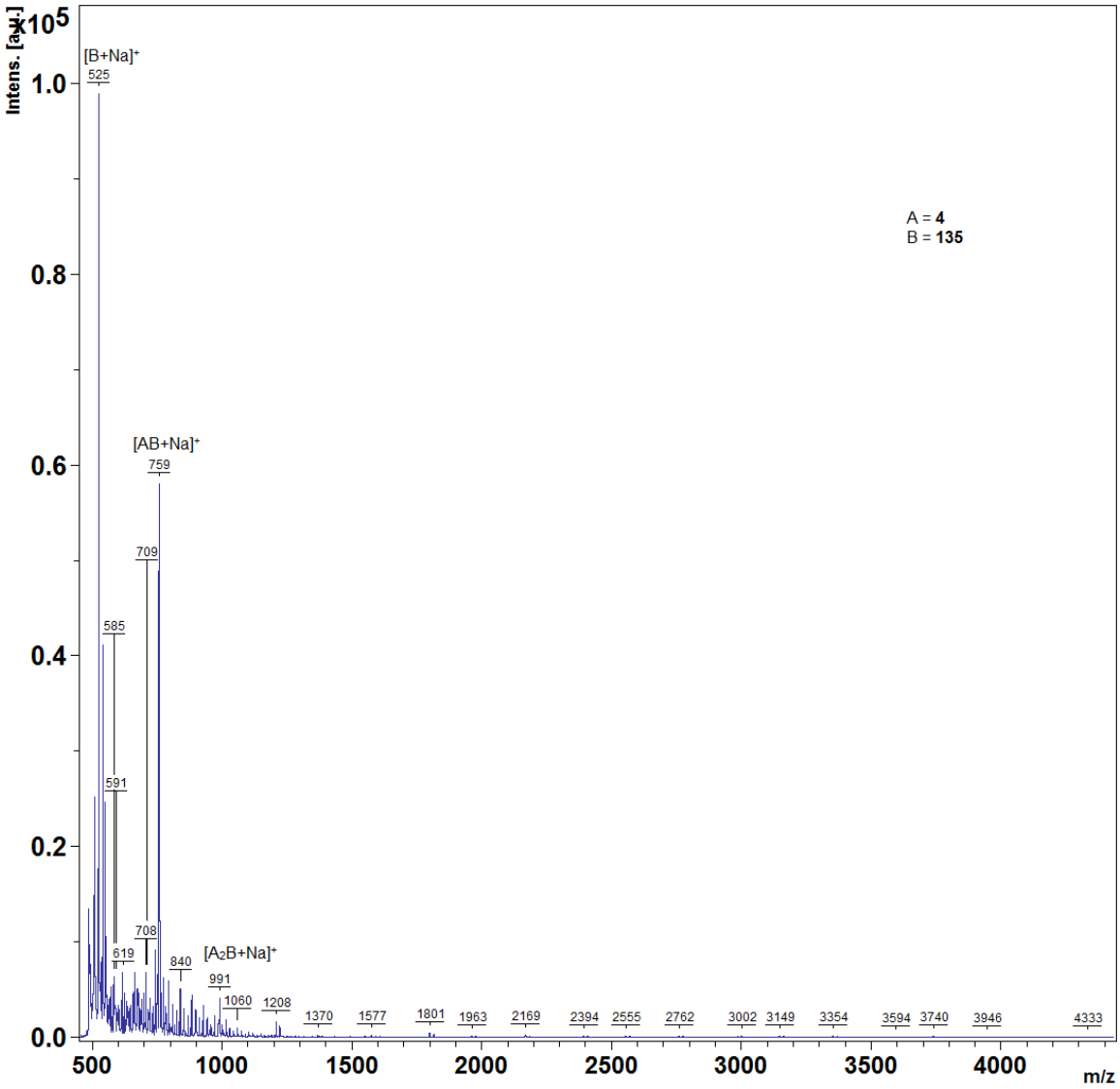
7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm

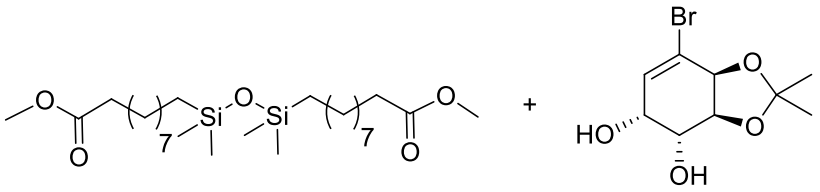




135 **4**

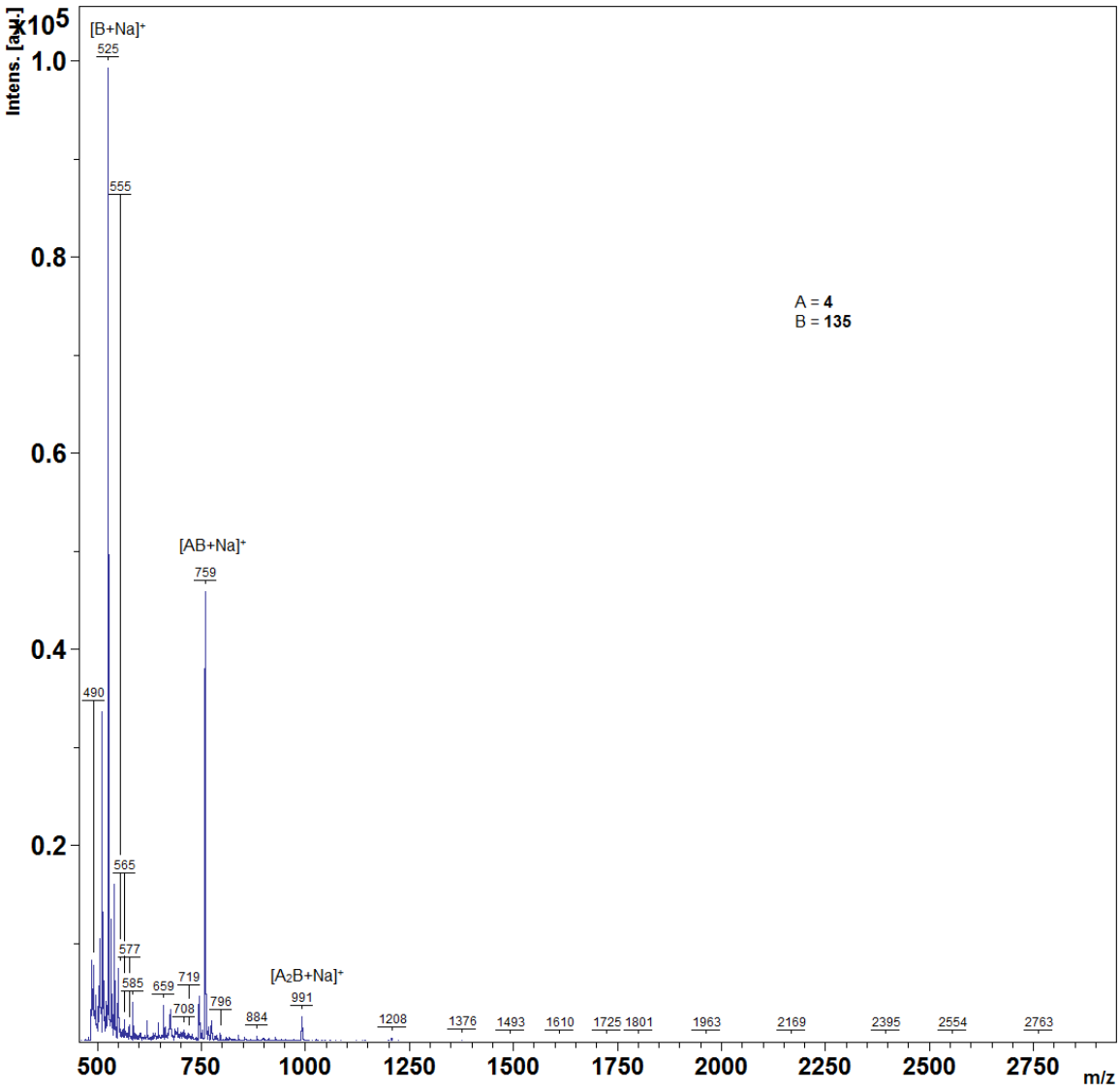
24 h, solvent-free, 100 °C, 10 wt% N435, 150 rpm.

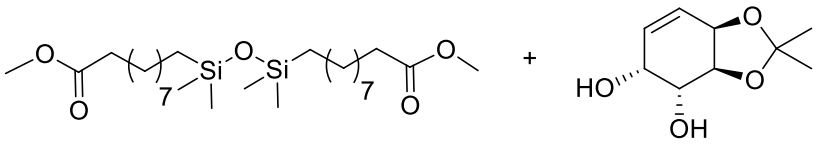




135 **4**

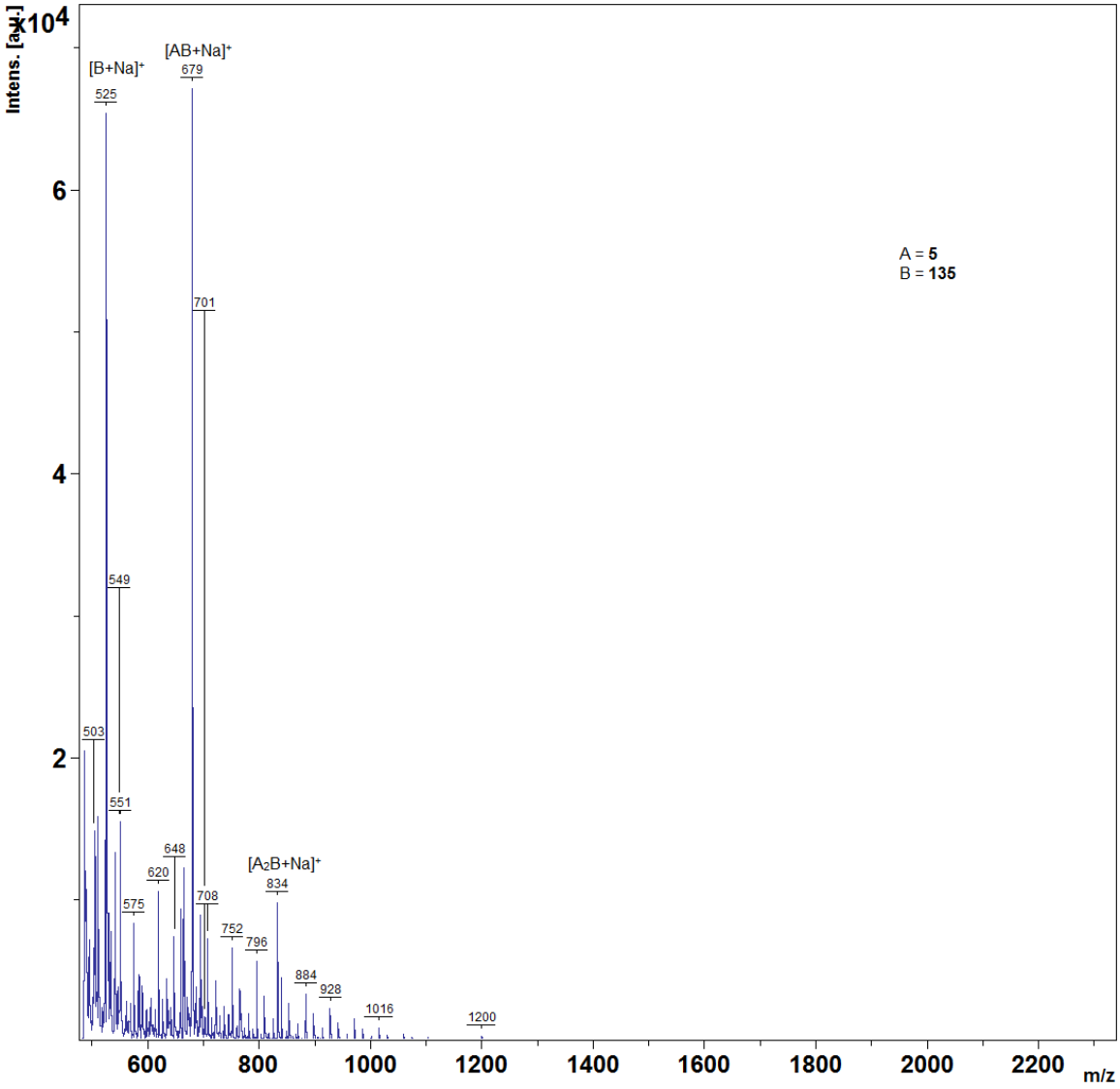
24 h, toluene, 100 °C, 10 wt% N435, 150 rpm

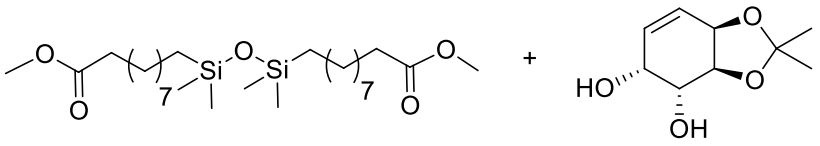




135 **5**

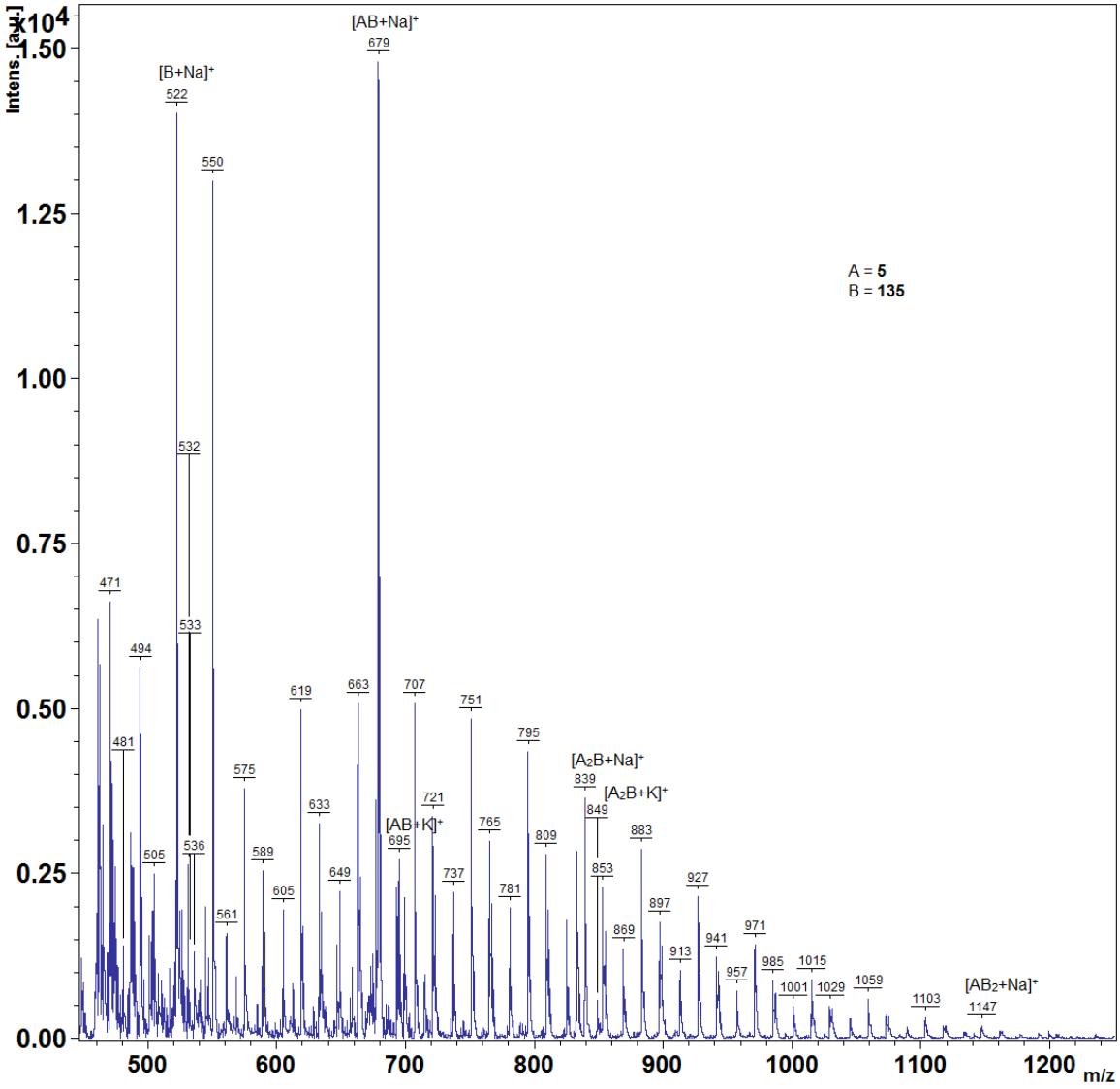
24 h, toluene, 100 °C, 10 wt% N435, 150 rpm

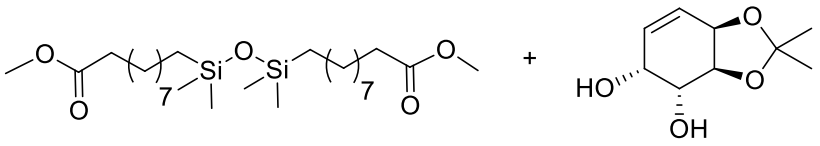




135 **5**

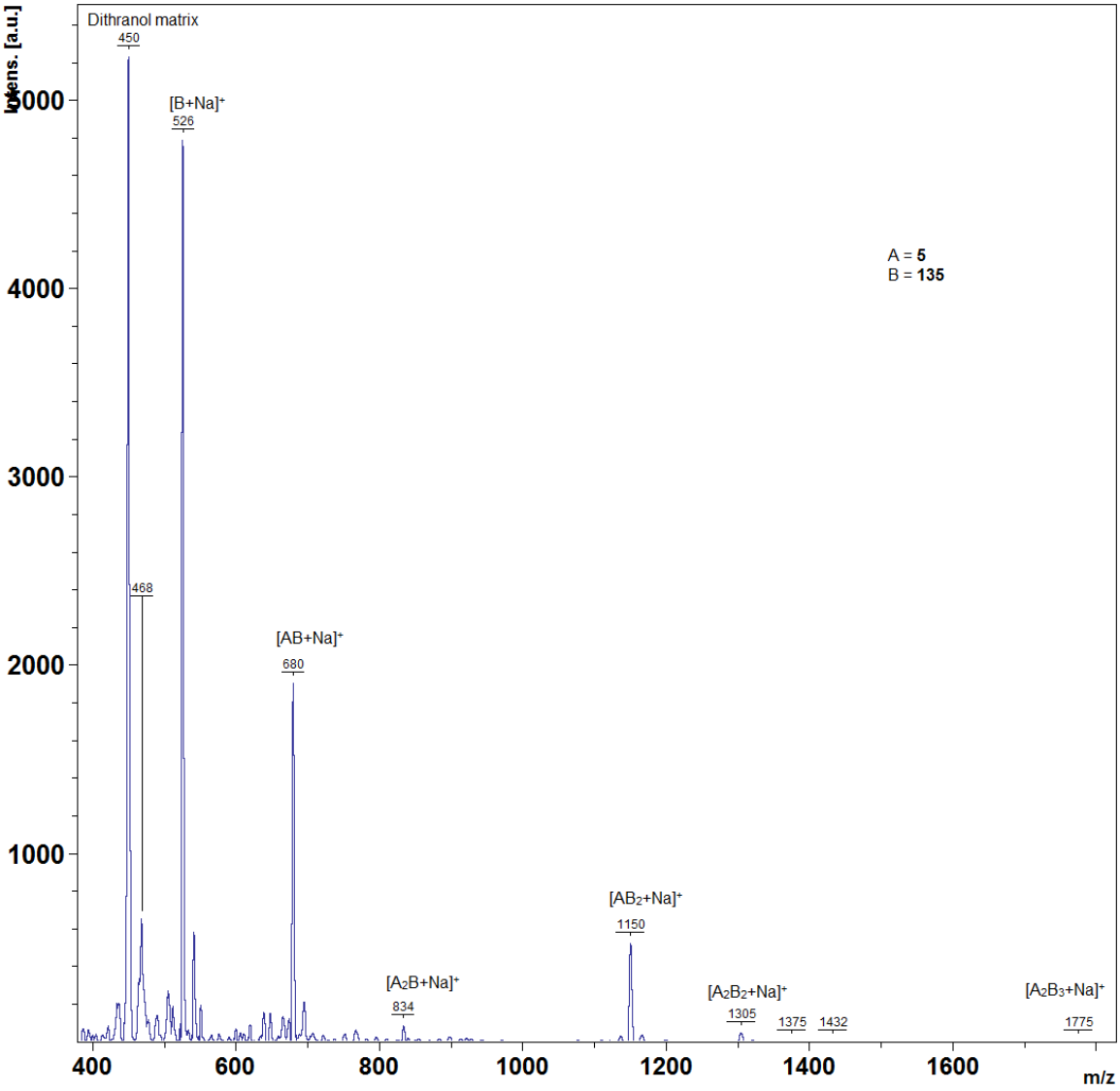
7 d, toluene, 100 °C, 10 wt% N435, 150 rpm

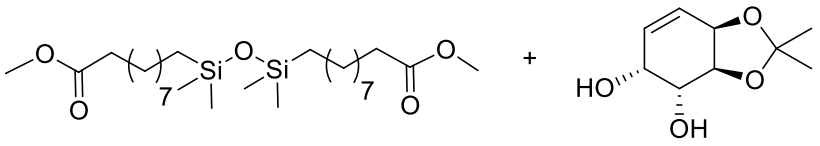




135 **5**

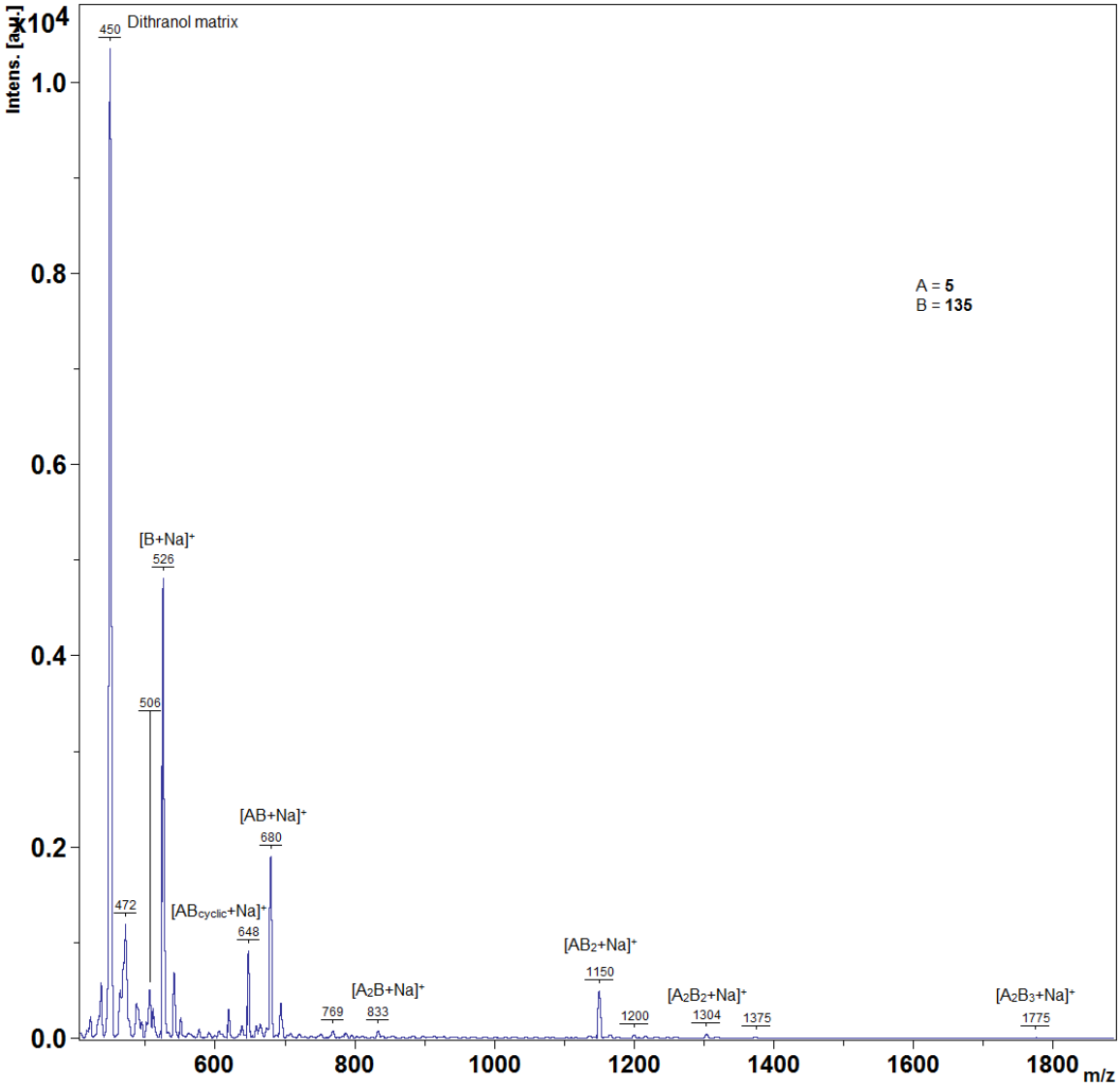
7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm

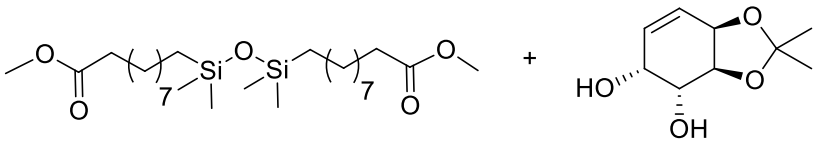




135 **5**

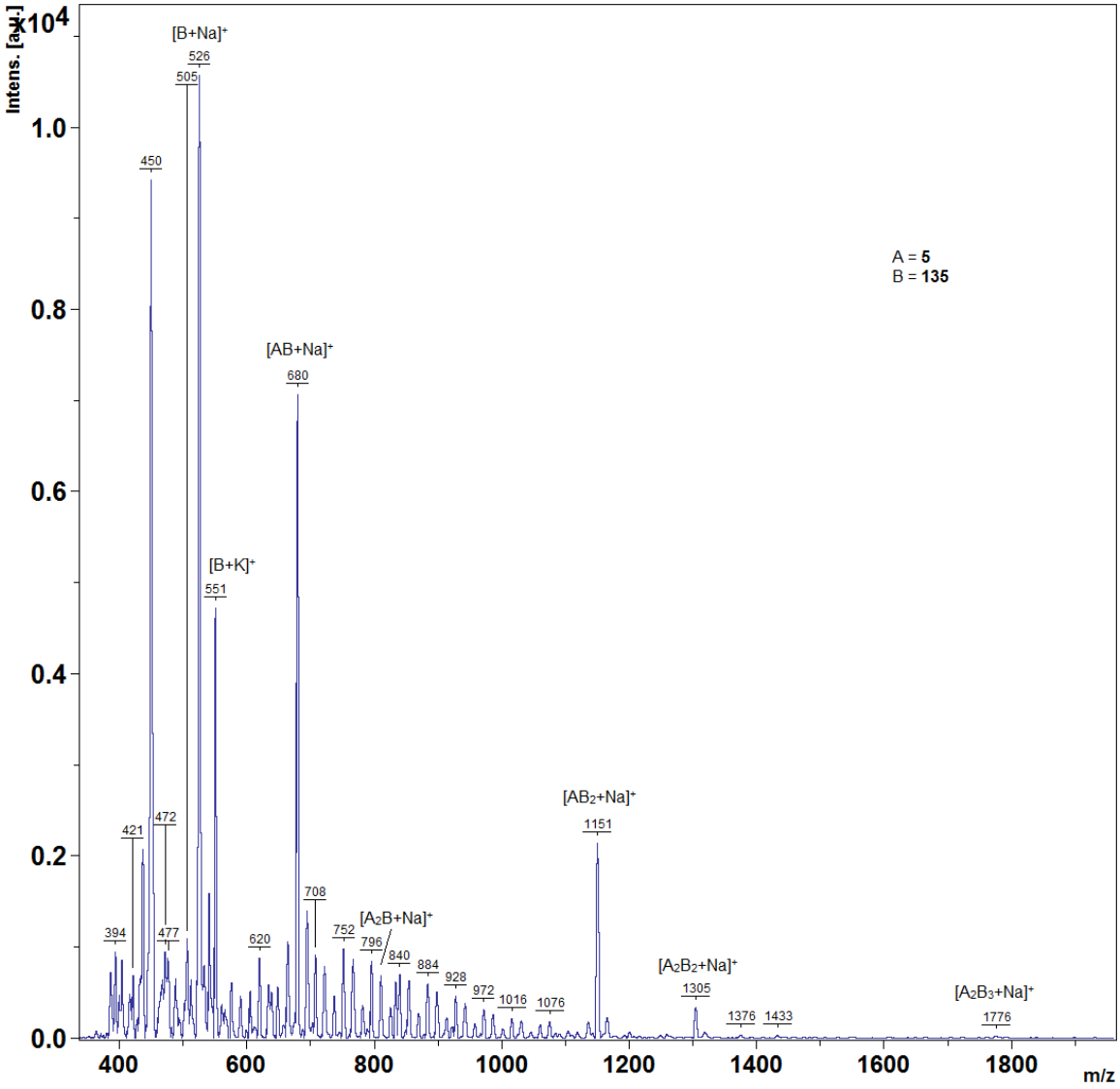
7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm

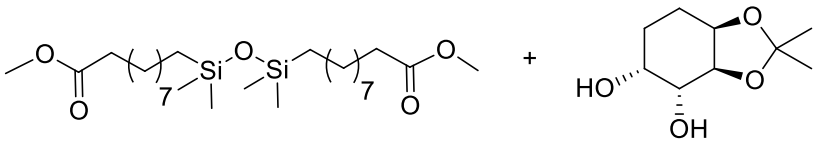




135 **5**

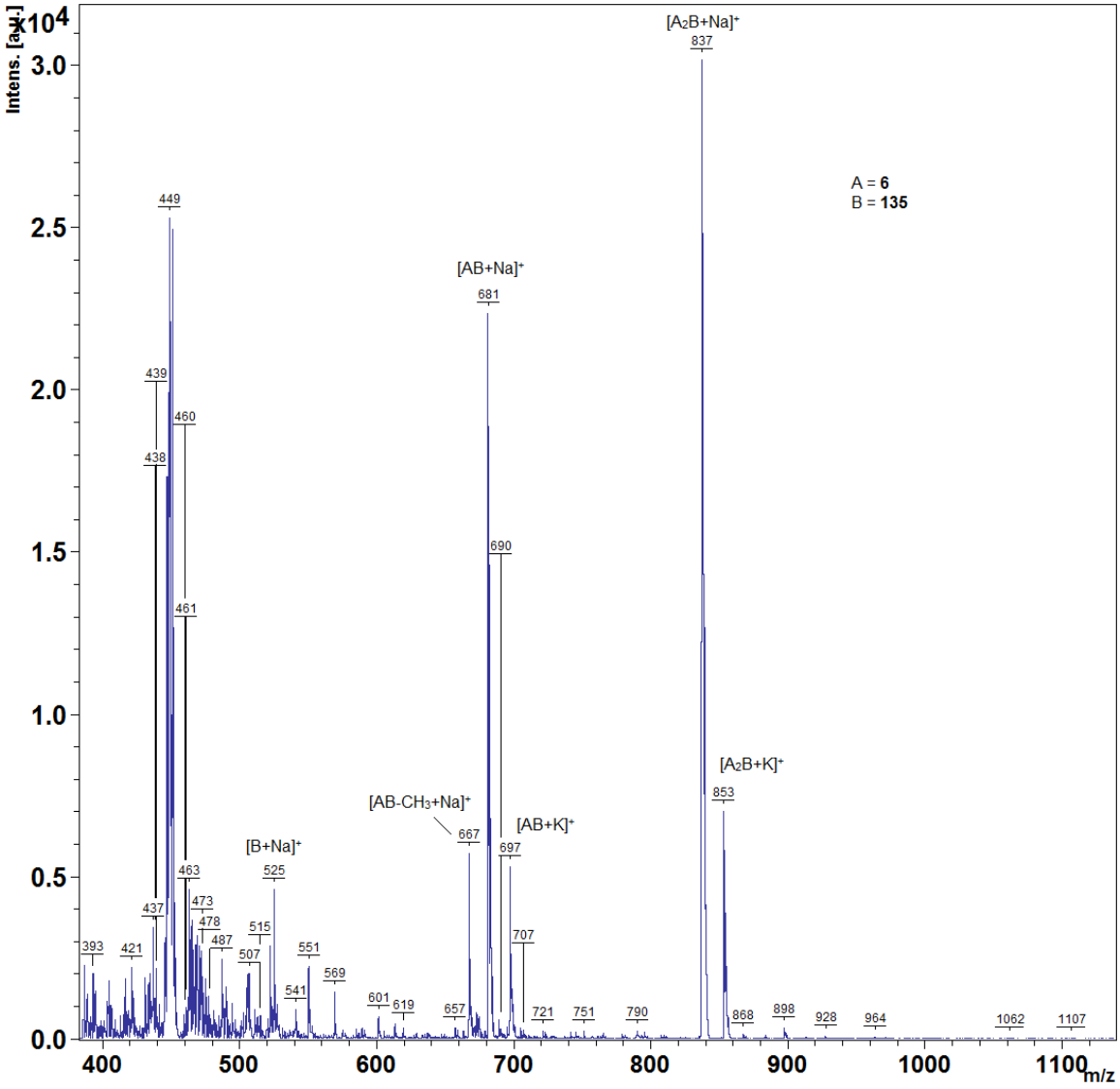
7 d, solvent-free, 100 °C, 20 wt% N435, 150 rpm

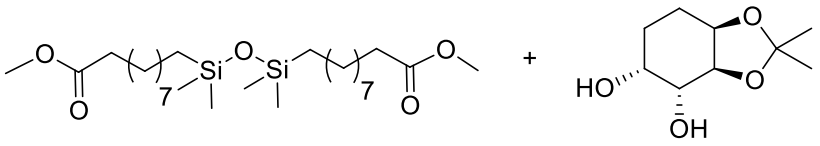




135 **6**

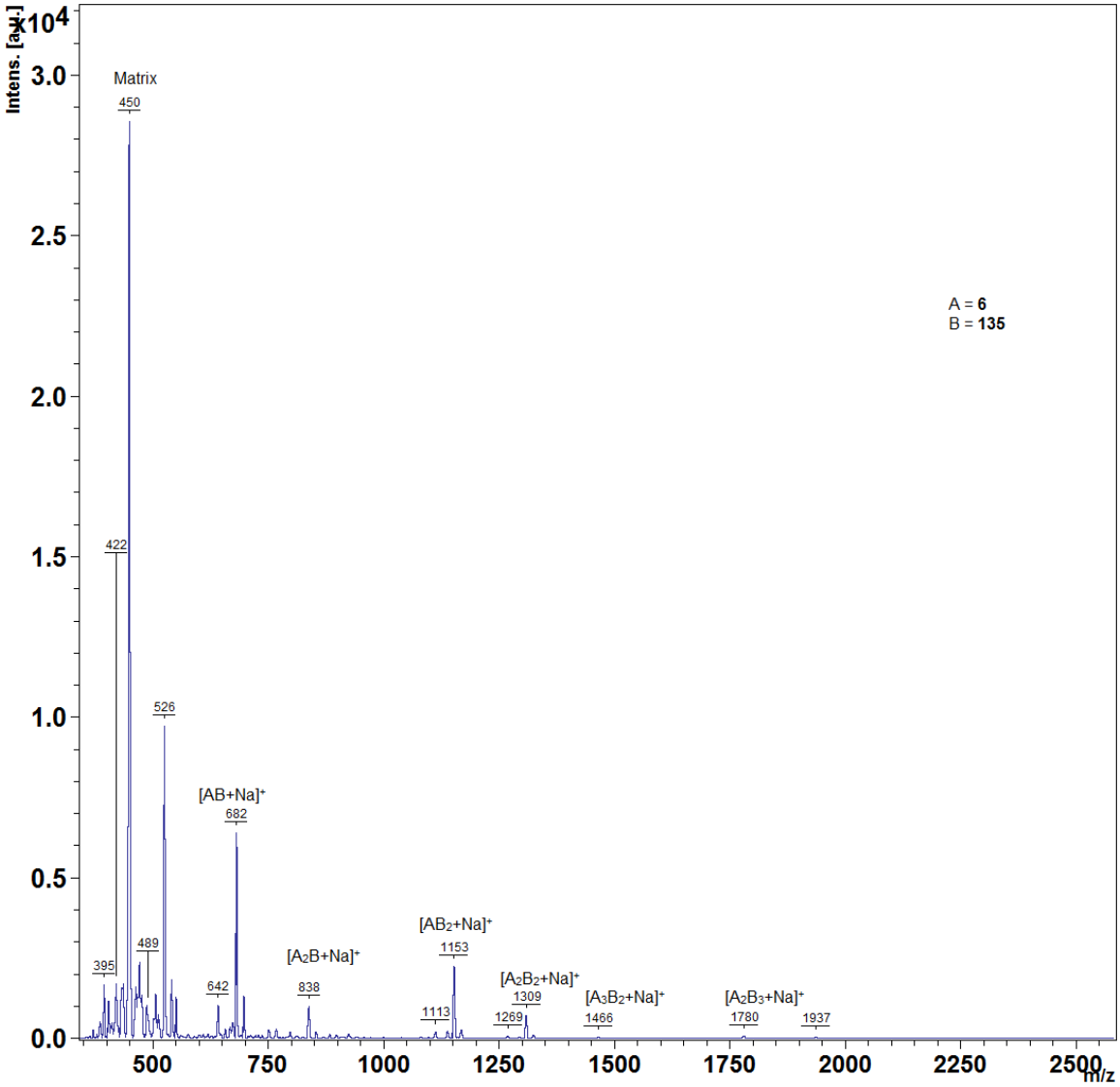
7 d, toluene, 100 °C, 10 wt% N435, 150 rpm

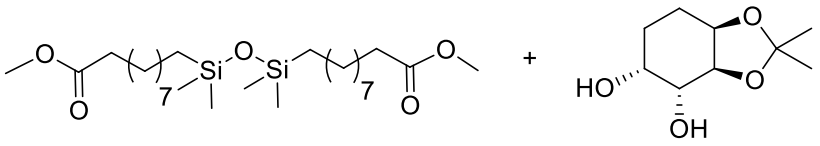




135 **6**

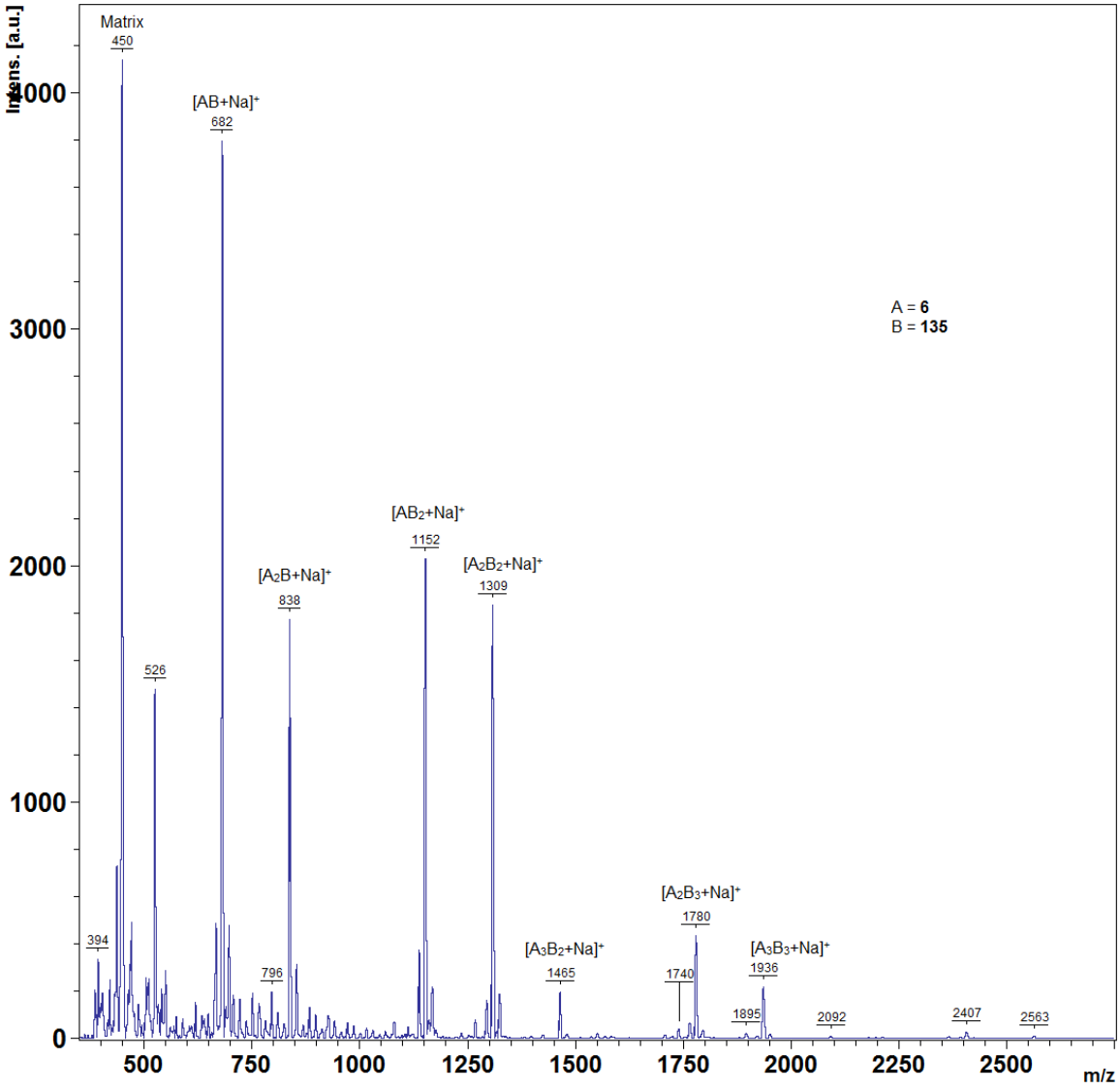
7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm



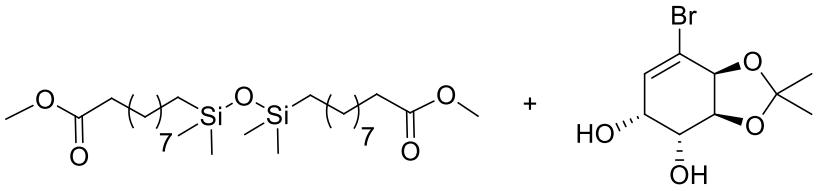


135 **6**

7 d, solvent-free, 100 °C, 20 wt% N435, 150 rpm

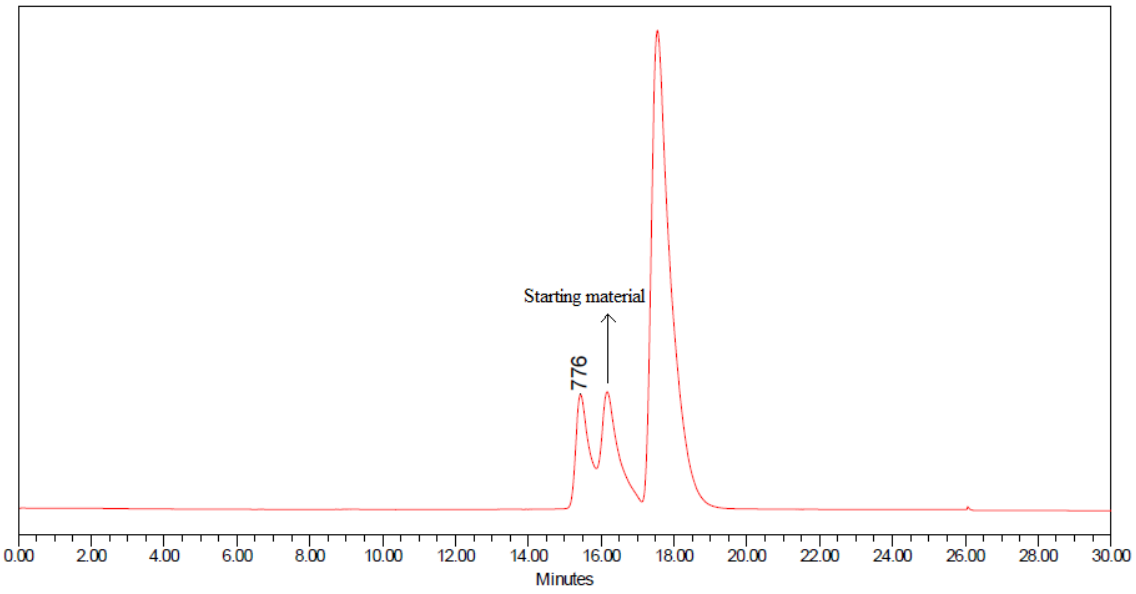


6.3 Selected GPC spectra



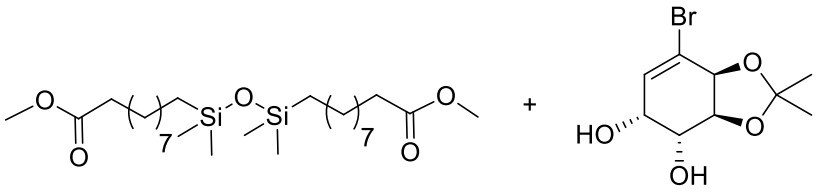
135 **4**

7 d, toluene, 100 °C, 10 wt% N435, 150 rpm



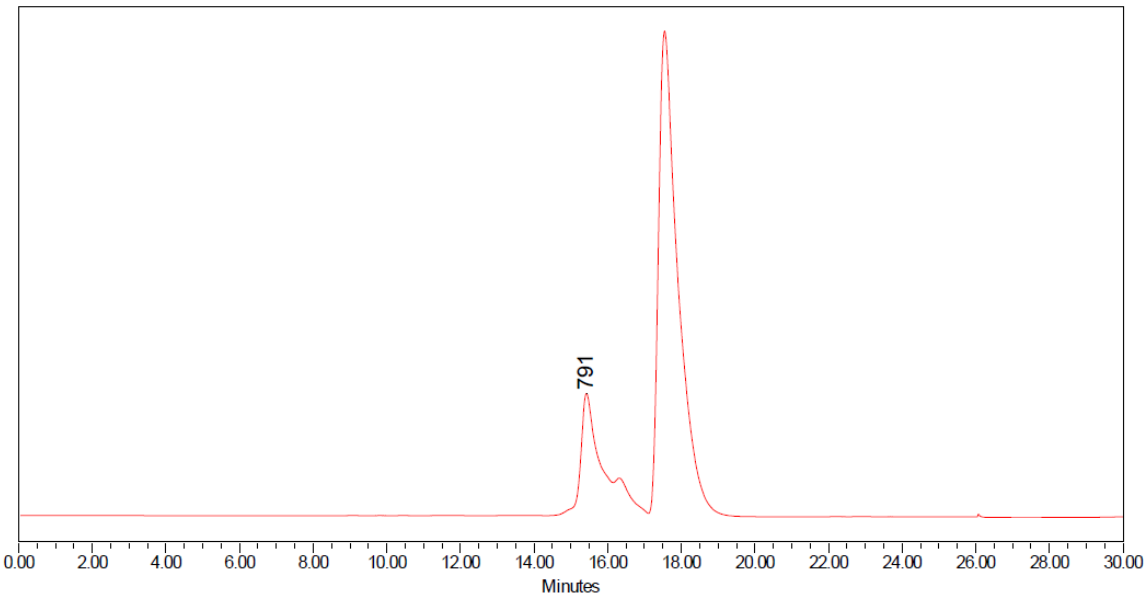
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		803	826	776	851	878		1.029		



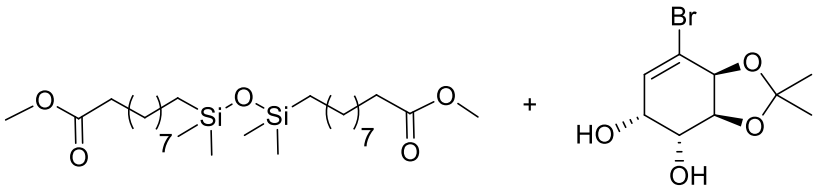
135 **4**

7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm



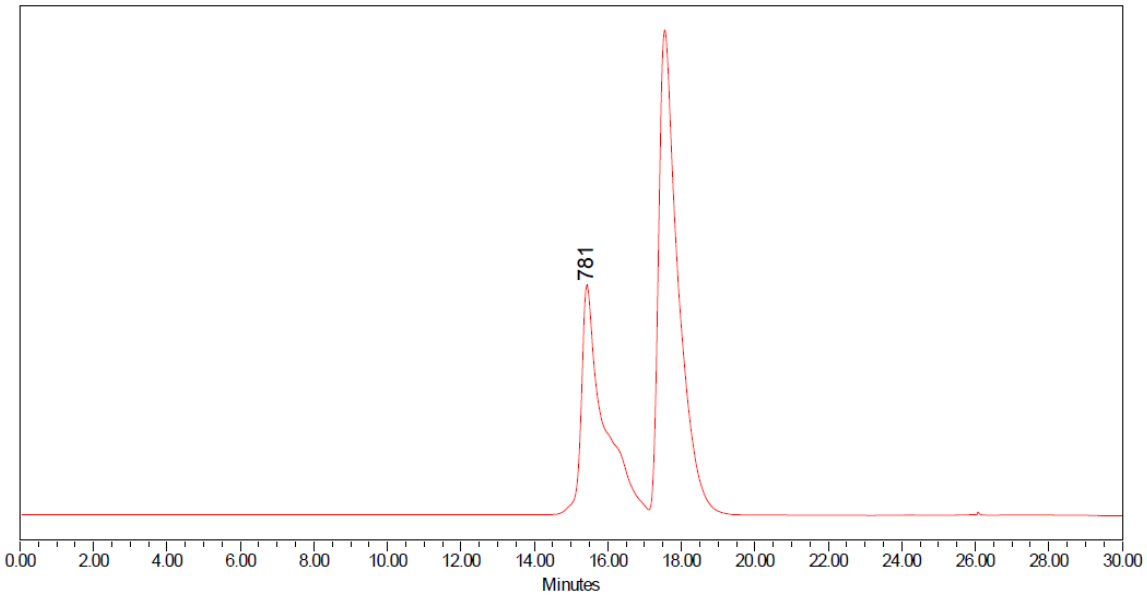
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		843	894	791	970	1086		1.061		



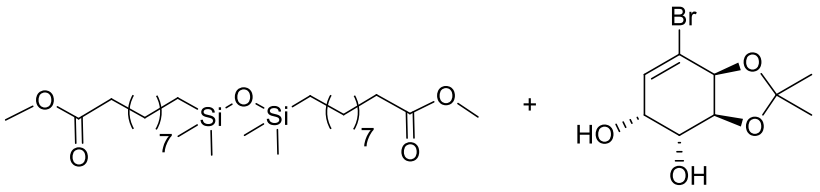
135 4

7 d, solvent-free, 100 °C, 20 wt% N435, 150 rpm



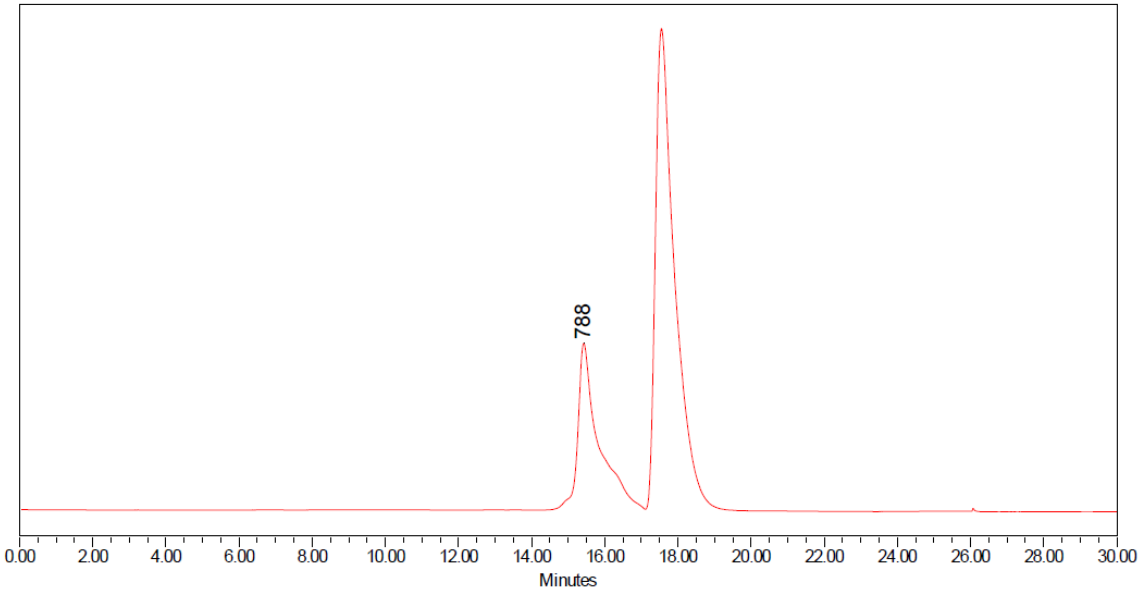
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		838	887	781	962	1078		1.059		



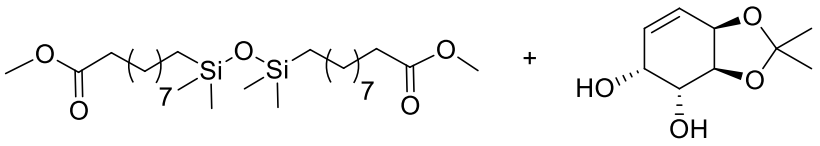
135 **4**

7 d, solvent-free, 100 °C, 30 wt% N435, 150 rpm



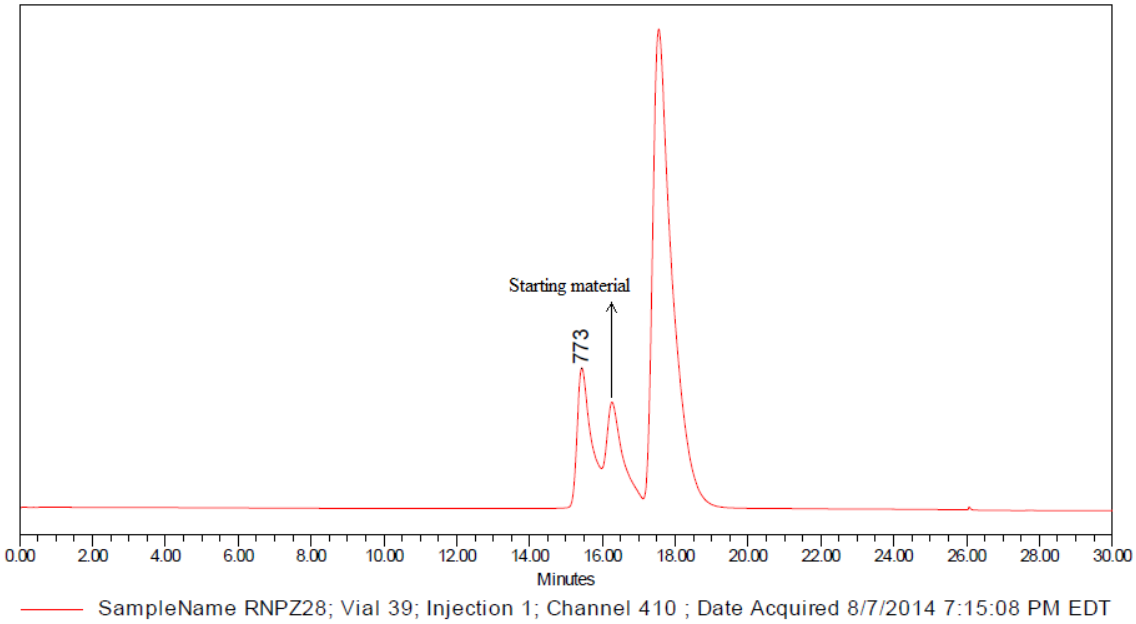
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		854	918	788	1019	1180		1.074		



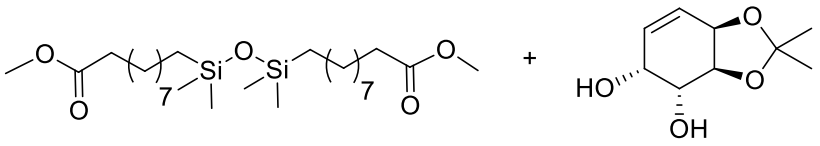
135 **5**

7 d, toluene, 100 °C, 10 wt% N435, 150 rpm



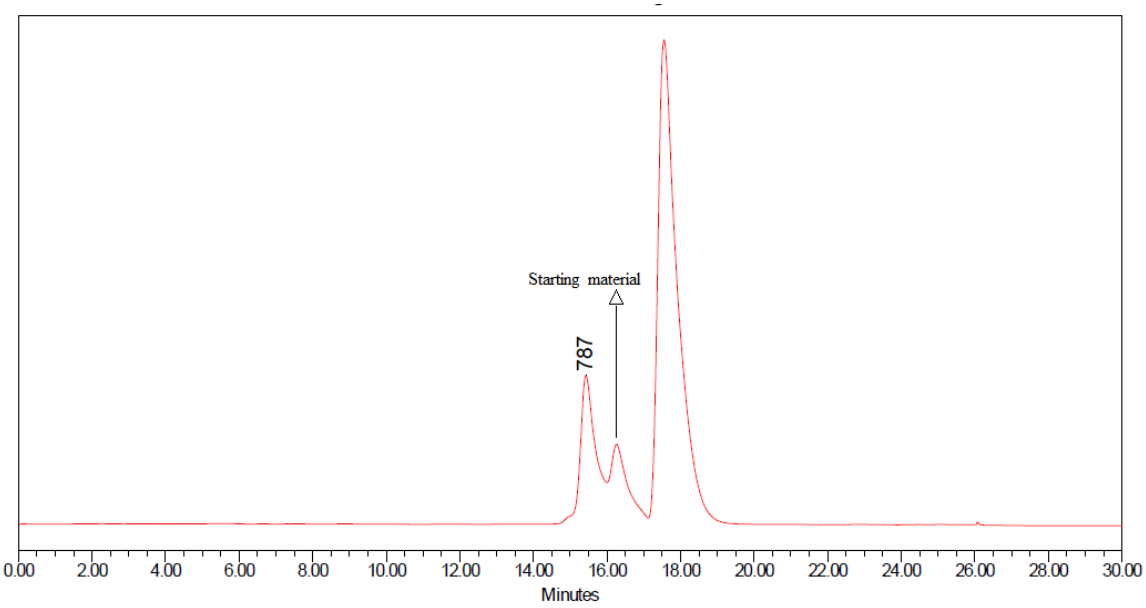
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		801	823	773	848	874		1.028		



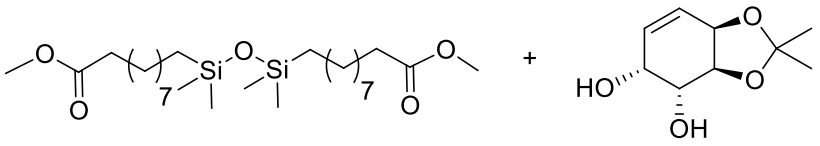
135 **5**

7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm



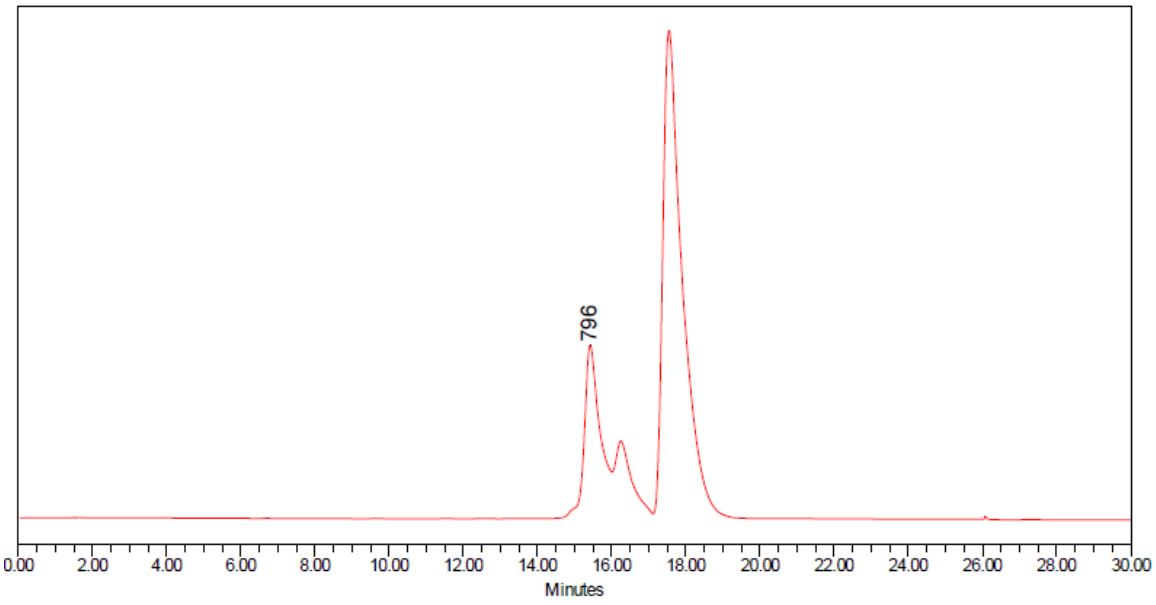
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		842	892	787	966	1077		1.060		



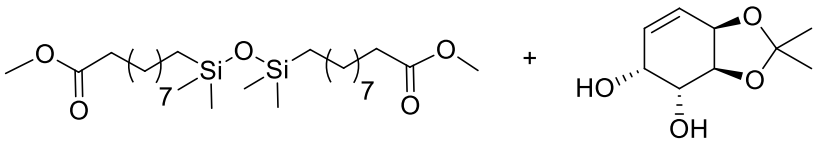
135 **5**

7 d, solvent-free, 100 °C, 20 wt% N435, 150 rpm



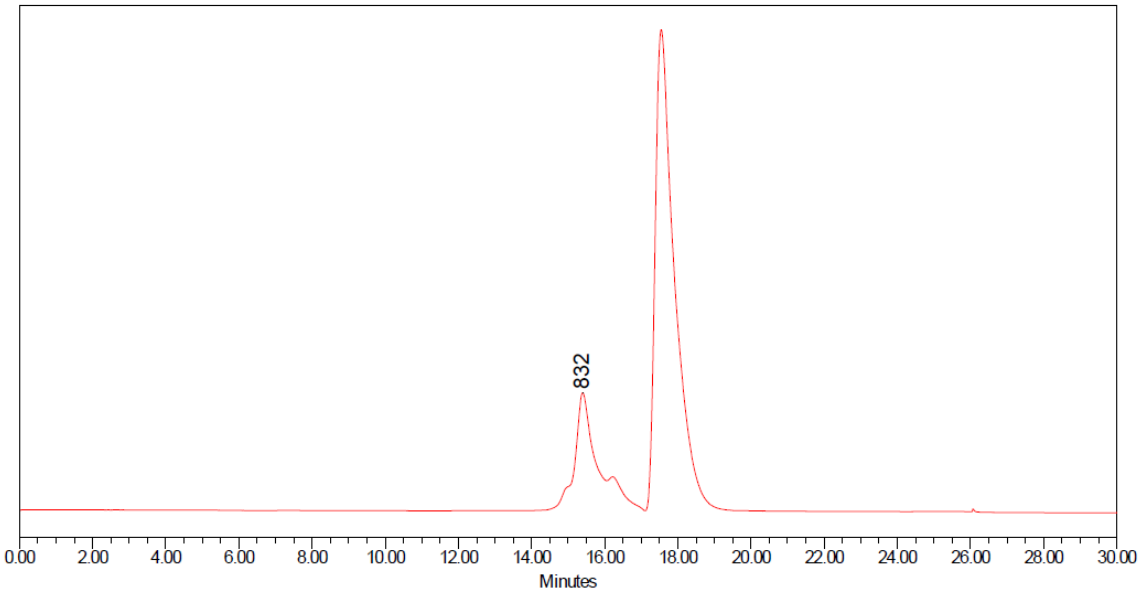
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		850	904	796	984	1103		1.063		



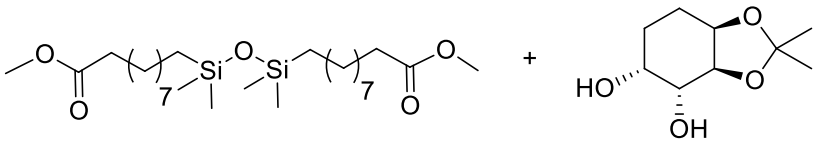
135 **5**

7 d, solvent-free, 100 °C, 30 wt% N435, 150 rpm



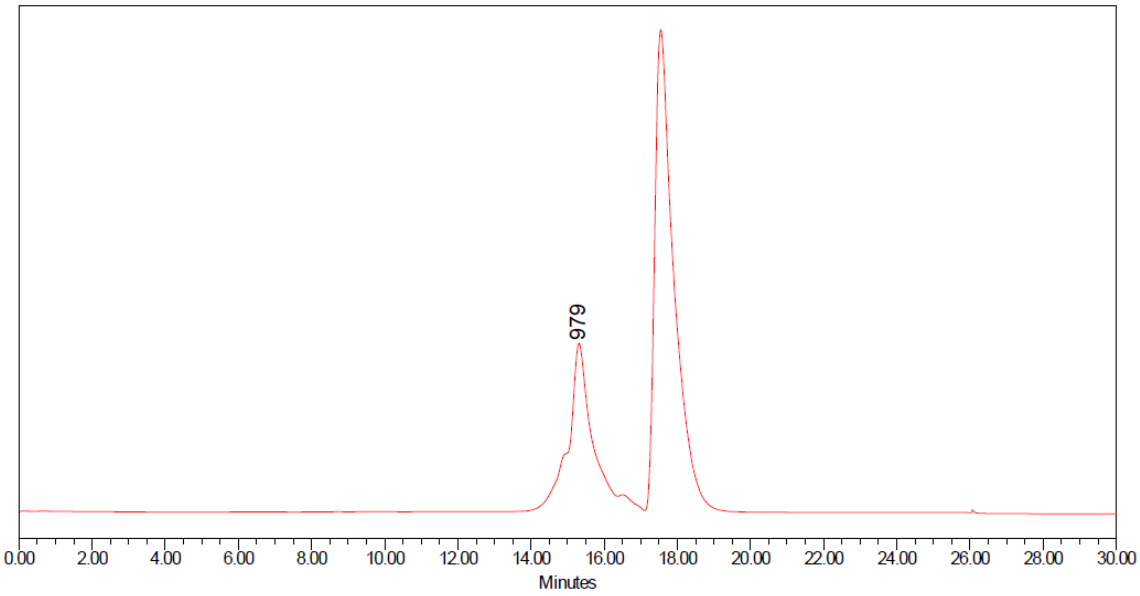
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		933	1042	832	1206	1428		1.117		



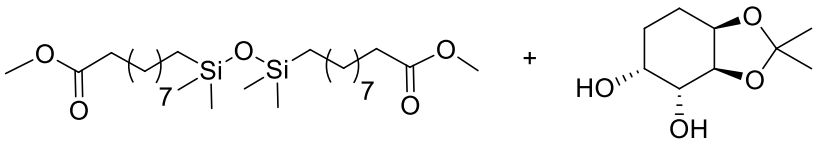
135 **6**

7 d, toluene, 100 °C, 10 wt% N435, 150 rpm



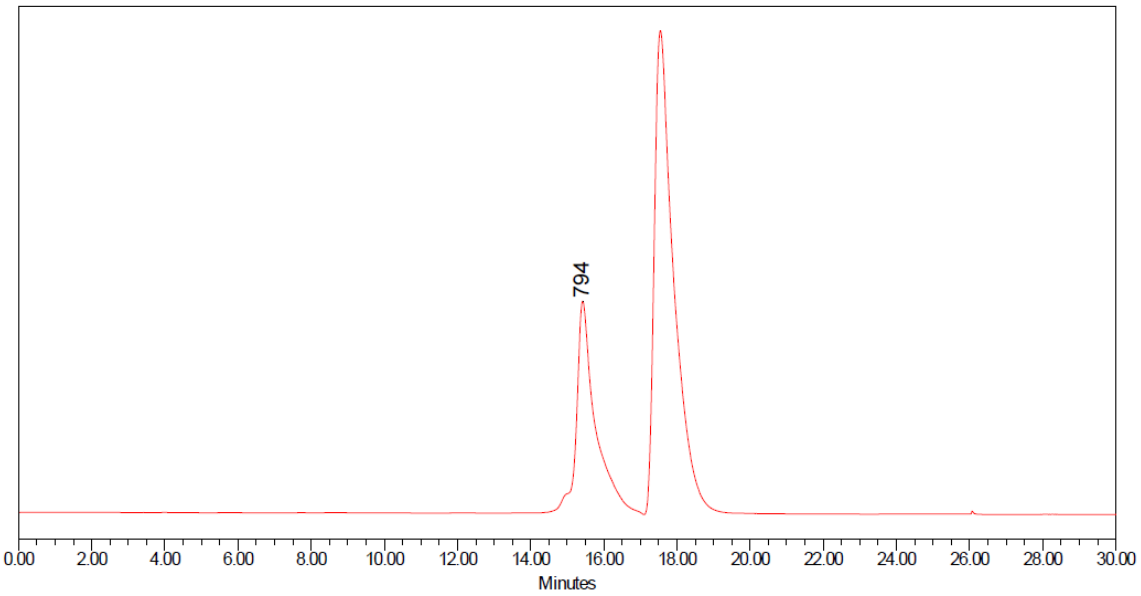
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		1124	1432	979	2006	2853		1.275		



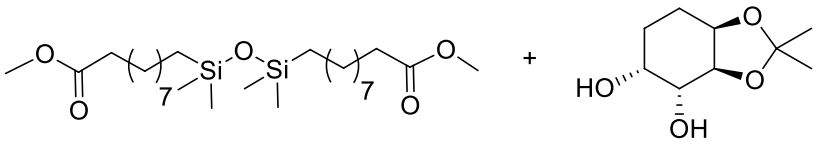
135 **6**

7 d, solvent-free, 100 °C, 10 wt% N435, 150 rpm



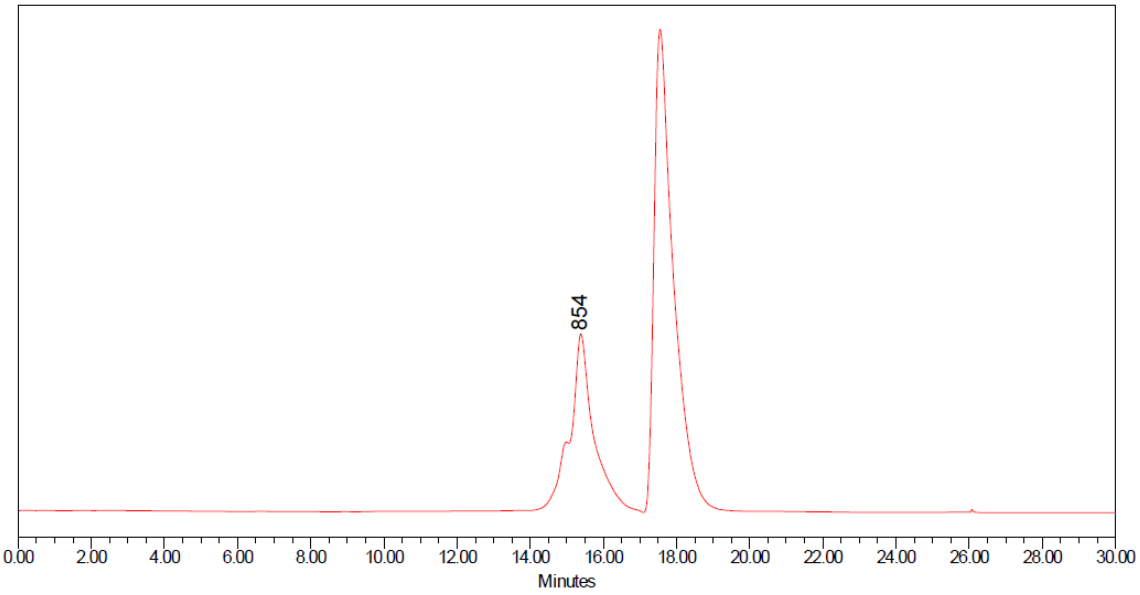
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		865	938	794	1054	1235		1.084		



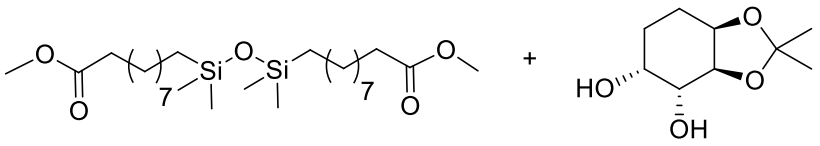
135 **6**

7 d, solvent-free, 100 °C, 20 wt% N435, 150 rpm



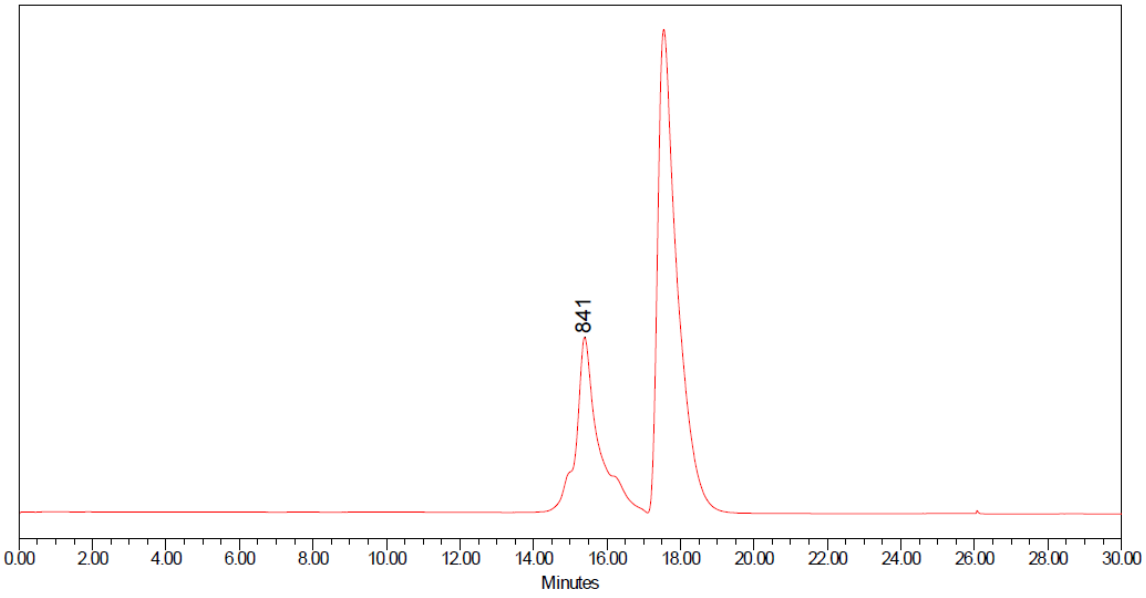
GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		1057	1296	854	1692	2238		1.226		



135 **6**

7 d, solvent-free, 100 °C, 30 wt% N435, 150 rpm



GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		963	1106	841	1336	1663		1.148		

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8. **VITA**

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