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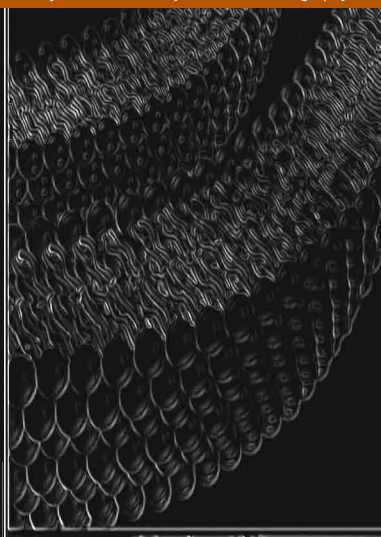

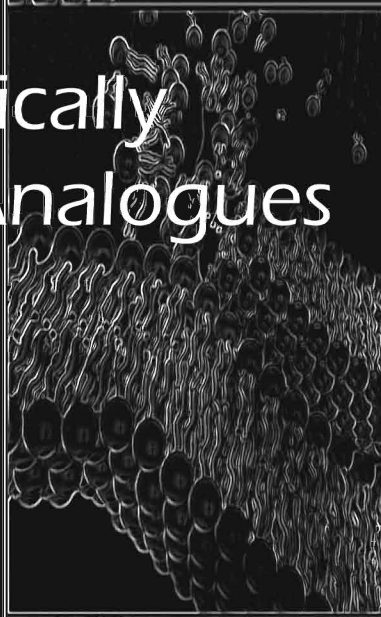

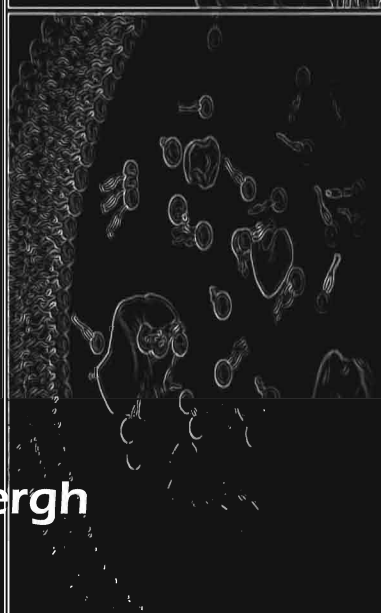

Synthesis of Biologically Relevant Sphingolipid Analogues

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SYNTHESIS OF BIOLOGICALLY RELEVANT SPHINGOLIPID ANALOGUES

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Prof. dr. apr. S. Van Calenbergh

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TABLE OF CONTENTS

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Gent, september 2005

SUMMARY

This Ph.D. study constitutes part of an extensive project dealing with the synthesis of biologically relevant sphingoid and ceramide analogues. In this context, we effected the synthesis of two different classes of sphingolipid analogues.

In a first part, we aimed at the synthesis of hybrid PDMP analogues, based both on PDMP and styryl analogues of natural ceramide. Since preliminary synthetic approaches based on a mono-protective strategy resulted in undesired cyclisation products, a double-protective strategy was devised in which the secondary alcohol and the *tert*-Boc protected amine were locked in an oxazolidine ring. Hence, the desired *E*-styryl PDMP analogues have been synthesised in 16 steps starting from D-serine. The synthetic route was developed such that future introduction of different aryl groups is straightforward. Throughout the synthetic coarse, we gained access to a number of structural analogues, which provided more insight into the structure-activity relationship of this class of compounds. Biological evaluation, both *in vitro* on rat liver Golgi extracts as *in vivo* on HEK-293 and COS-7 cells revealed two lead compounds with comparable inhibitory potency as PDMP, which could be elaborated to more potent inhibitors.

In a second part, we aimed at the synthesis of a new class of homoceramides, named N-homoceramides, which contain an extra methylene-spacer between the N-acyl chain and C2 of the sphingoid backbone. To this aim, we evaluated the introduction of branching by use of a cyano group, both in a sugar approach as in regioselective epoxide opening. Since these experiments failed to provide a reliable means for introduction of branching, we opted to introduce an extra methylene spacer by use of 1,3-dithiane. Hence, the desired N-homo(dihydro)ceramides have been synthesized in 20 steps starting from D-ascorbic acid, a common food preservative. Key reaction in this synthetic approach was a fully regioselective epoxide opening with lithium 1,3-dithiane. In addition, a fully stereoselective Grignard reaction gave access to D-*ribo*-N-homophytoceramide.

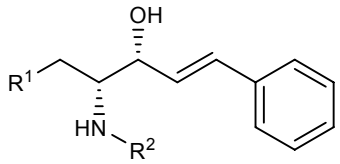
SAMENVATTING

Dit doctoraatswerk maakt deel uit van een omstandig project over de synthese van biologisch relevante sfingoïd- en ceramide-analogen. In dit opzicht werden twee verschillende klassen van verbindingen gesynthetiseerd.

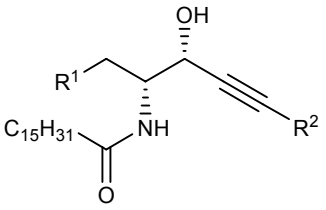
In een eerste deel werd er geopteerd om hybride PDMP analogen the synthetiseren, die zowel op PDMP als op *E*-styreen analogen van natuurlijk ceramide gebaseerd zijn. Aangezien preliminaire synthetische routes die gebaseerd waren op enkelvoudige bescherming van de sfingoïd stikstof resulteerden in ongewenste cyclisatie reakties, werd er geopteerd om een dubbel bescherming in te voeren waarbij de *tert*-Boc beschermde amine functie en het secundare alcohol van de sfingoïd keten in een oxazolidine ring werden vastgelegd. Bijgevolg werden de gewenste *E*-styreen analogen uitgaande van D-serine gesynthetiseerd in 16 stappen. De synthetische route werd zodanig opgevat dat verschillende aromaten op eenvoudige wijze zullen ingevoerd kunnen worden bij verdere synthetische modificaties. Er werd eveneens toegang verkregen tot een aantal structuuranalogen die meer inzicht konden verschaffen in de sructuur-aktiviteitsrelatie van deze klasse van verbindingen. *In vitro* biologische evaluatie op Golgi extracten en *in vivo* evaluatie op HEK-293 en COS-7 cellen bracht twee gelijkwaardige verbindingen verbindingen in vergelijking met PDMP aan het licht, die verder kunnen uitgewerkt worden tot meer potente inhibitoren.

In een tweede luik werd de synthese van een nieuwe klasse van homoceramiden, N-homoceramiden genaamd, uitgewerkt. Deze verbindingen bevatten een extra koolstof atoom tussen de N-acyl keten en C2 van de sfingoïd keten. Om dit te bewerkstelligen werd zowel vanuit een suiker als door middel van regioselectieve epoxide opening getracht om een cyanide in te voeren. Aangezien deze benaderingswijze niet voldeed voor de synthese van de gewenste verbindingen, werd geopteerd om de vertakking in te voeren door middel van 1,3-dithiaan. Bijgevolg werden de gewenste N-homo(dihydro)ceramiden in 20 stappen gesynthetiseerd, uitgaande van D-isoascorbinezuur, een veel gebruikt bewaarmiddel in de voedingsindustrie. De sleutelreaktie in deze benaderingswijze was de volledig regioselectieve epoxide opening met lithium 1,3-dithiaan. Daarenboven stelde een volledig regioselective Grignard reaktie ons in staat om D-*ribo*-homophytoceramide te synthetiseren.

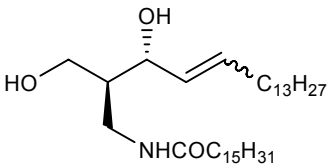
OVERVIEW OF SYNTHESIZED COMPOUNDS



Compound	R^1	R^2
2.39a	morpholinyl	$-C_{16}H_{33}$
2.39b	pyrrolidinyl	$-C_{16}H_{33}$
2.39c	$-NH_2$	$-C_{16}H_{33}$
2.41a	morpholinyl	$-C(O)C_{15}H_{31}$
2.41b	piperidinyl	$-C(O)C_{15}H_{31}$
2.41c	pyrrolidinyl	$-C(O)C_{15}H_{31}$

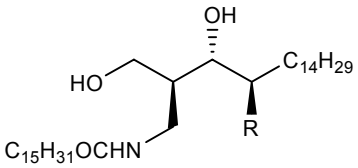


Compound	R^1	R^2
2.38a	morpholinyl	phenyl
2.38b	piperidinyl	phenyl
2.38c	pyrrolidinyl	phenyl
2.38d	$-N_3$	phenyl
2.38e	$-NH_2$	phenyl
2.43	pyrrolidinyl	H



3.22: 4*Z*

3.23: 4*E*



3.24: R = -H

3.25: R = -OH

LIST OF ABBREVIATIONS

4-DMAP	4-Dimethylaminopyridine
AC	Acid ceramidase
AMPDNJ	N-(5-Adamantane-1-yl-methoxypentyl)-DNJ
aSMase	Acid sphingomyelinase
BBB	Blood-brain barrier
BMT	Bone marrow transplantation
Bn	Benzyl
Cbz	Carbobenzoxy
Cer	Ceramide
CM	Cross-metathesis
COSY	Correlated spectroscopy
DAD	Diode-array detector
DAG	Diacylglycerol
DET	Diethyltartrate
DiBAlH	Diisobutylaluminum hydride
DIPEA	Diisopropylethylamine
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	Dimethylsufloxide
ee	Enantiomeric excess
ER	Endoplasmatic reticulum
ERT	Enzyme replacement therapy
ES	Electrospray
EtOH	Ethanol
GlcCer	Glucosyl ceramide
GPCR	G-Protein-coupled receptors
GSLs	Glycosphingolipids
HDL	High density lipoprotein
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
HMQC	Heteronuclear multiple quantum coherence
HOBT	1-Hydroxybenzotriazole

HPLC	High pressure liquid chromatography
Hünig's base	Diisopropylethylamine; DIPEA
LacCer	Lactosyl ceramide
MDR	Multi Drug Resistance
MeOH	Methanol
MS	Mass spectrum
MsCl	Methanesulfonyl chloride
NBD	7-Nitrobenzo-2-oxa-1,3-diazole
NBDNJ	*N*-Butyldeoxynojirimycin
nBuLi	n-Butyllithium
NGDGJ	*N*-Butyl-deoxygalactonojirimycin
NGF	Nerve growth factor
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect
nSMase	Neutral sphingomyelinase
P4	D-threo-(1*R*,2*R*)-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol
PCC	Pyridinium chlorochromate
PDGF	Platelet-derived growth factor
PDMP	D-threo-(1*R*,2*R*)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol
Phth	Phthalimide
PPTS	Pyridinium *para*-toluenesulfonate
p-TsCl	*para*-Toluenesulfonyl chloride
p-TsOH	*para*-Toluenesulfonic acid
RT	Room temperature
S1P	Sphingosine-1-phosphate
SAP	Sphingolipid activator protein
SLs	Sphingolipids
SM	Sphingomyelin
SMase	Sphingomyelinase
SPT	Serine palmitoyl transferase
sSMase	Secretory sphingomyelinase
TBAF	Tetrabutylammonium fluoride

TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
TBHP	*tert*-Butylhydroperoxide
TEA	Triethylamine
tert-Boc	*tert*-Butoxycarbonyl
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSI	Trimethylsilyl iodide
TNF-α	Tumor necrosis factor α
Triflate	Trifluoromethane sulfonate
Trt	Trityl

Chapter

STATE-OF-THE-ART AND RESEARCH OBJECTIVES

1 SPHINGOLIPIDS

1.1. HISTORICAL BACKGROUND

Sphingolipids (SLs), a class of natural products, have been described for the first time in 1884 by a German physician, J. L. W. Tudichum.[1] He named the three related lipids, isolated from human brain by fractional crystallisation, sphingomyelin, cerebroside and cerebrosulfatide. Hydrolysis led to a long carbon-chain aliphatic amine, called sphingosine, a name derived from Greek mythology (sphinx) and referring to the enigmatic behaviour of this compound. The structure of sphingosine (Figure 1.1) was elucidated by Carter in 1947.[2]

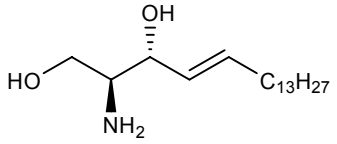


Figure 1.1: Structure of D-*erythro*-sphingosine.

The structural identification of SLs and the investigation of their metabolism were facilitated by autopsy material from patients who had suffered from rare metabolic defects. These sphingolipidoses are characterized by storage of specific SLs as a consequence of mutations that lead to deficiency of proteins involved in their lysosomal degradation.[3]

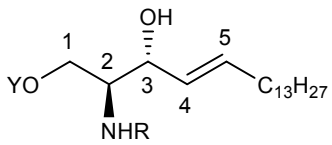
Sphingolipids (SL) have gained much attention over the last decade since it became clear that they not only act as structural elements of cell membranes, but also participate in a myriad of biological processes. An exponentially growing research interest in the role of SLs in signal transduction pathways and as mediators of cell growth, senescence, differentiation, adhesion and apoptosis,[4] was triggered by the discovery of sphingosine as an inhibitor of protein kinase C.[5]

1.2. STRUCTURE AND CLASSIFICATION OF SPHINGOLIPIDS

At least 300 different SLs are biosynthesized in various mammalian cell types. Although structurally diverse, SLs share a hydrophobic component, generally referred to as ceramide. Ceramide is comprised of a sphingosine ((2*S*,3*R*,4*E*)-2-amino-1,3-

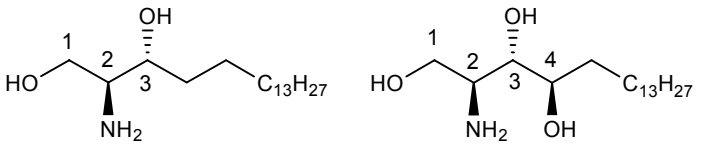
dihydroxy-4-octadecene) backbone and a fatty acid, joined in an amide bond. The important features for biological activity are located at carbons 1-5 of the sphingosine backbone and include a primary and secondary hydroxyl group at positions C1 and C3 (Table 1.1).

Table 1.1: Structural composition of SLs.



	Head group (Y)	**R**
Sphingosine	H	H
Neutral Sphingolipids		
Ceramides	H	$C(O)(CH_2)_nCH_3$ (n = 14-22)
1-*O*-Acyl ceramides	$C(O)(CH_2)_nCH_3$ (n = 14-22)	$C(O)(CH_2)_nCH_3$ (n = 14-22)
Phosphosphingolipids		
Spingomyelins	Phosphocholinyl	$C(O)(CH_2)_nCH_3$ (n = 14-22)
Sphingosine-1-phosphate	PO_3^{2-}	H
Ceramide-1-phosphate	PO_3^{2-}	$C(O)(CH_2)_nCH_3$ (n = 14-22)
Sphingosylphosphorylcholine	Phosphocholinyl	H
Glycosphingolipids		
Neutral glycosphingolipids	Glucosyl, galactosyl, lactosyl	$C(O)(CH_2)_nCH_3$ (n = 14-22)
Sulfatides	Complex sugar with sulphate moieties	$C(O)(CH_2)_nCH_3$ (n = 14-22)
Gangliosides	Complex sugar with sialic acid residues	$C(O)(CH_2)_nCH_3$ (n = 14-22)
Lysosphingolipids	Any group mentioned above	H

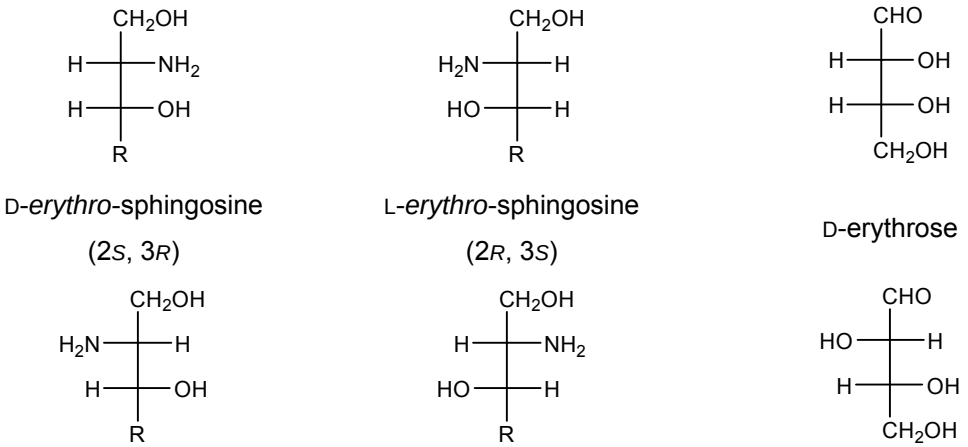
Sphingoids or ***sphingoid*** base refers to ***sphinganine*** (dihydrosphingosine; Figure 1.2), its homologues and stereomers, as well as hydroxylated and unsaturated analogues.[6] The chain length of natural sphingoids varies from 14 to 22 carbon atoms. These carbon chains may contain methyl or hydroxyl groups as branches, as well as double bonds, in particular between C4 and C5.



Sphinganine D-*ribo*-4-hydroxysphinganine

Figure 1.2: Structures of sphinganine and phytosphingosine.

Sphingosine bearing two chiral centers prevails in four stereomeric forms (Figure 1.3). The diastereomeric pairs are named D- and L-*erythro*- and *threo*-sphingosine. Naturally occurring SLs possess a D-*erythro* configuration. When both the secondary hydroxyl and amino groups are on the same side as depicted in the Fisher projection, the diastereomers are *erythro*, in analogy to erythrose. In contrast, when these groups are on opposite sides, the isomers are referred to as *threo* in analogy to threose. The D- and L-descriptors are determined by the position of the C3-hydroxyl group. A hydroxyl group to the right refers to D whereas the hydroxyl group is oriented to the left in the L-configuration.



R = *trans*-CH=CH-$C_{13}H_{27}$

Figure 1.3: Fischer projections of sphingosine stereomers.

D-*ribo*-4-Hydroxysphinganine (***phytosphingosine***; Figure 1.2) occurs mainly in yeasts and other lower eukaryotes,[7] but is also the characteristic sphingoid base of SLs in keratinocytes.[8] The function of the additional free hydroxyl group is presumed to be to increase rigidity of intercellular lipid aggregates through formation of a large number of hydrogen bonds and hence reduce transepidermal water loss.[9] The

structure of the derived ceramides differs in a characteristic manner from other mammalian ceramides by the presence of unusually long fatty acid residues up to 34 carbon atoms and sphingoid bases that are hydroxylated at various positions.[10]

Sphingomyelin (SM) is made up of a sphingoid-base, an amide-linked acyl chain and a phosphorylcholine headgroup. The acyl chain composition may vary among tissues, for example, brain gray matter sphingomyelin contains predominantly stearic acid.[11] In contrast, non-neuronal tissue seems to be comprised of a mixed population of sphingomyelins.[12] In mammalian cells, SM accounts for 5 -10% of membrane phospholipids.

Glycosphingolipids (GSLs) have a sugar residue β-glycosidically attached to the primary hydroxyl group of a ceramide. Classification is based on the carbohydrate composition. Neutral GSLs contain sugars such as glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine and fucose.

Gangliosides are a subclass of GSLs, predominantly localized in the central nervous system. The carbohydrate contains one or more acid sugar residues, derived from sialic acid (*N*-acetylneuraminic acid; Figure 1.4), which is α-glycosidically linked to other sugars. GSLs with one or more sulphate groups in the carbohydrate moiety are called ***sulfatides*** and are highly abundant in myelin sheaths. Both gangliosides and sulfatides may contain up to 20 monosaccharide units.

Lysosphingolipids are glyco- or phosphosphingolipids without *N*-acyl chain.

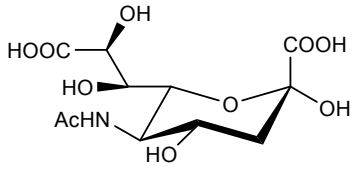
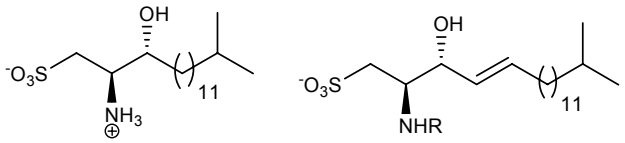


Figure 1.4: Structure of *N*-acetyl-neuraminic acid.

Another, less known, class comprises the ***sulfonosphingolipids***, which contain a C1 sulfonate group. The first known example of this class was isolated from *Nitzschia alba*[13] and has a 1-deoxyceramide-1-sulfonic acid structure. Other representatives of this class have emerged and the best-known examples (Figure 1.5) are the sulfobacins (*Chryseobacterium sp.*)[14] and capnine,[15] which has been isolated from cell envelopes of bacteria (*Capnocytophaga*).



Capnine Suflobacins

R = acyl or hydroxylated acyl

Figure 1.5: General structure of capnines and sulfobacins.

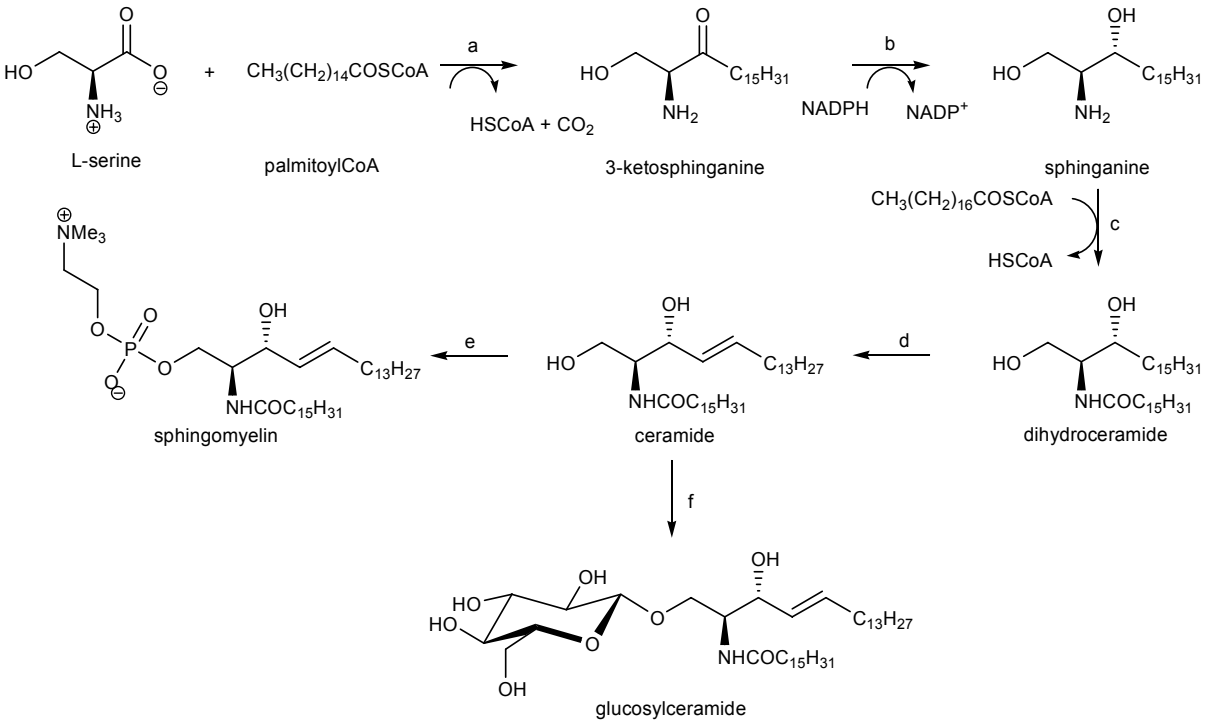
1.3. METABOLISM OF SPHINGOLIPIDS

1.3.1 BIOSYNTHESIS OF SPHINGOLIPIDS

Biosynthesis of SLs takes place in the endoplasmatic reticulum and the Golgi apparatus.[16] *De novo* biosynthesis (Figure 1.6) starts with the condensation of L-serine and a coenzyme A activated fatty acid residue, usually palmitoyl coenzyme A, affording 3-ketosphinganine. This rate-limiting step for SL biosynthesis is catalysed by ***serine palmitoyl transferase*** (SPT), a pyridoxal phosphate-dependent heterodimeric enzyme.[17] Since SPT determines the rate of SL biosynthesis, its function might be a major point of regulation. However, regulatory mechanisms remain largely unknown to date.

In a subsequent reaction 3-ketosphinganine is reduced to D-*erythro*-sphinganine by ***3-ketosphinganine reductase*** in a NADPH-dependent reaction.[18] Sphinganine is subsequently acylated by ***ceramide synthase*** (sphinganine *N*-acyltransferase) yielding dihydroceramide. The enzyme shows a preference for the coenzyme A esters of stearic and palmitic acid.[19] The ensuing desaturation of dihydroceramide to ceramide follows, which is mediated by ***dihydroceramide desaturase***. It is noteworthy that sphingosine is not involved in the biosynthesis of SLs but is formed during degradation of SLs.

All enzymes for the initial steps in the biosynthesis of SLs are located on the cytosolic leaflet of the ER membrane.[20] Further modifications take place at the Golgi membranes.



a) serine palmitoyl transferase; b) 3-ketosphinganine reductase; c) sphinganine *N*-acyltransferase; d) dihydroceramide desaturase; e) sphingomyelin synthase; f) glucosylceramide synthase.

Figure 1.6: Biosynthesis of SLs.

Sphingomyelin is formed on the luminal part of the Golgi membranes,[21] but other sites have also been suggested.[22] This reaction, which is catalyzed by ***sphingomyelin synthase*** (SMase), involves the transfer of a phosphorylcholine headgroup from phosphatidylcholine to ceramide yielding sphingomyelin and 1,2-diacylglycerol (DAG) as a by-product.

Biosynthesis of GSLs requires the stepwise addition of carbohydrate moieties to the SL which serves as a membrane anchor.[23] The first step in this sequence is the formation of glucosylceramide (GlcCer), which is catalyzed by ***glucosyl ceramide synthase*** (GlcCer synthase). Hence, glucose is β-glycosidically linked to ceramide in an UDP-glucose dependent reaction. The human enzyme has been cloned[24] and is located on the cytosolic leaflet of the Golgi apparatus.[25] From this point, GlcCer can go directly to the plasma membrane[26] or it can be modified by further glycosylation on the luminal side of the Golgi apparatus. Transport of GlcCer to the Golgi apparatus occurs both by vesicular and protein mediated transport mechanisms.[27]

Recently, a new ceramide trafficking protein, CERT, has been identified[28] which demonstrated the ability to extract ceramides from membranes of the ER and

traffic them specifically to the Golgi apparatus. However, the role of CERT in regulation of cellular ceramide levels remains unknown.

Introduction of the next sugar residue, galactose, is mediated by galactosyl transferase I[29] and gives rise to lactosylceramide (LacCer), the common precursor of the major GSLs in vertebrates. The sequential addition of nucleotide-activated sugar residues to LacCer requires the action of membrane-bound glycosyltransferases in the lumen of the Golgi apparatus.

1.3.2 INHIBITORS OF SPHINGOLIPID BIOSYNTHESIS

Most inhibitors of SL biosynthesis act at an early stage and have a lipid-like structure. Specific and potent inhibitors play a crucial role in the investigation of SL metabolic pathways. Moreover, membrane-permeating inhibitors are interesting therapeutic agents.[30]

1.3.2.1. INHIBITORS OF SERINE PALMITOYLTRANSFERASE

L-Cycloserine, β-chloro- and β-fluoro alanine (Figure 1.7) are non-specific suicide inhibitors of SPT. Indeed, inhibition of other pyridoxal phosphate-dependent enzymes has been observed which makes them unsuitable biochemical tools to study effects of reduced SL metabolism.

Sphingofungins, which have structural similarity to SL, have been isolated from yeasts (*Aspergillus fumigatus*[31] and *Paecilimyces variotii*[32]) and inhibit SPT competitively with respect to serine. Their action can be reversed by phytosphingosine, but not sphingosine, which is not unexpected since phytosphingosine is the main sphingoid base in yeast.

Myriocin, a further structural analog of the sphingoid backbone, is an extremely potent immunosuppressant (K_i = 0.28 nM).[33] Inhibition of SPT is accompanied by suppression of IL-2 mediated T cell growth, which explains the immunosuppressive effect.

Lipoxamycin, a secondary metabolite from actinomycetes, has also been reported as a potent inhibitor of SPT (IC_{50} = 21 nM)[34] and exhibits strong antifungal activity.

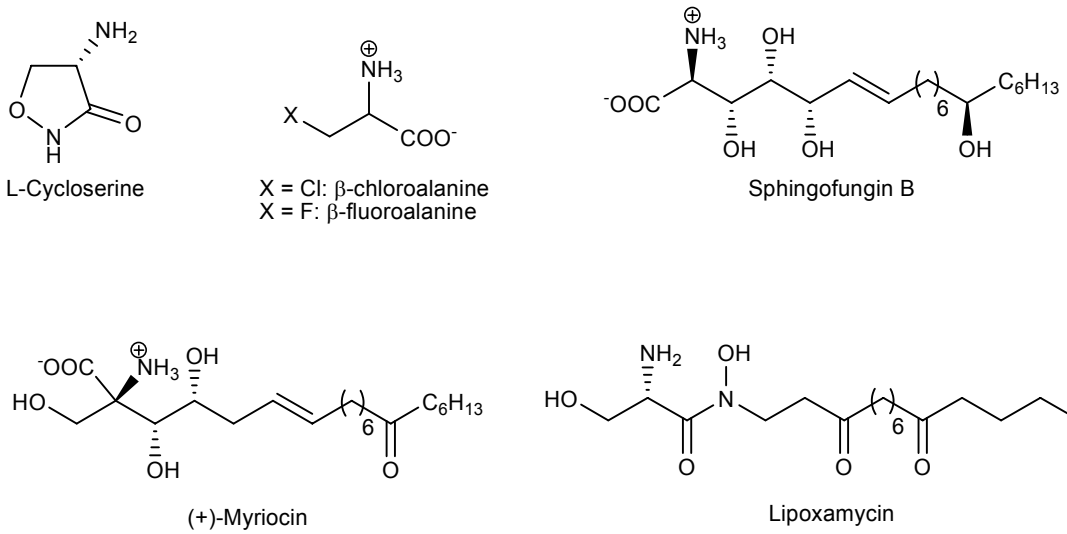


Figure 1.7: Known inhibitors of serine palmitoyltransferase.

1.3.2.2. INHIBITORS OF CERAMIDE SYNTHASE

Corn and cereals are frequently infected by *Fusarium verticillioides*, a fungus that produces fumonisins (Figure 1.8).[35] Consumption of contaminated food results in various, often neurological, diseases in animals and has been associated with esophageal cancer in humans.[36] Fumonisins have been identified as inhibitors of ceramide synthase (IC_{50} = 0.1 μM) and have shown to be valuable compounds in the elucidation of SL metabolism. They show structural similarity with sphinganine, but have increased metabolic stability due to the absence of the primary alcohol. Analogues have been synthesised as substrates and inhibitors of ceramide synthase, though all exhibiting decreased activity.[37] Inhibition of ceramide synthase leads to accumulation of sphinganine, which explains the toxic and mitogen effects of fumonisins.

Alternaria toxin (IC_{50} = 1 μM), a phytotoxin, has a sphingoid backbone similar to fumonisin B1. Since it exhibits lower biological activity relative to fumonisin B1, it is of limited use in metabolism studies.[38]

Australifungin, a potent antifungal, has been identified as an in-vitro inhibitor of ceramide synthase with an IC_{50} comparable to fumonisin B1.[39]

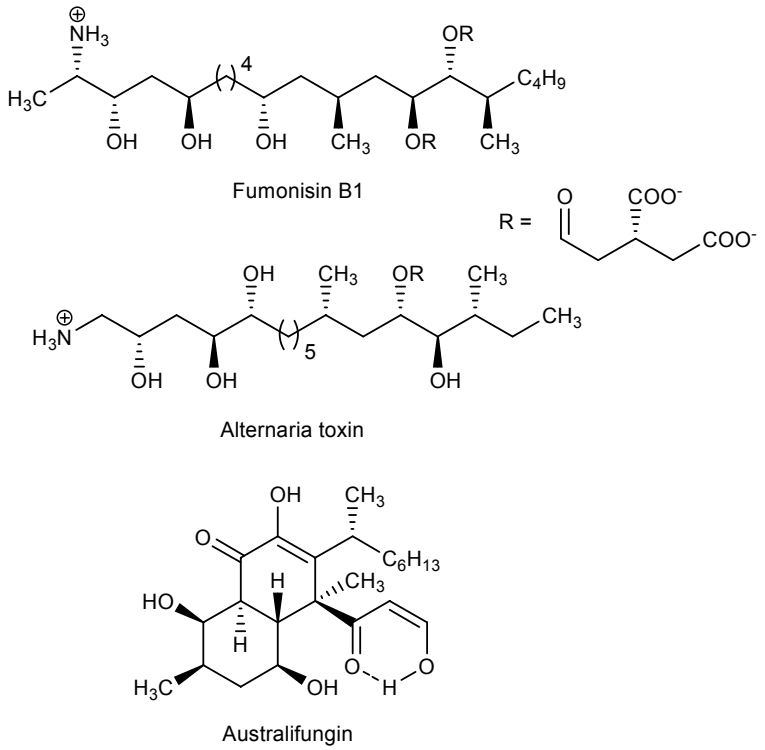


Figure 1.8: Structure of known inhibitors of ceramide synthase

1.3.2.3. INHIBITORS OF GLUCOSYLCERAMIDE SYNTHASE

Potent inhibitors of GlcCer synthase are suitable for functional analysis and have therapeutic potential. Two different classes of GlcCer synthase inhibitors have been described to date (Figure 1.9 and Figure 1.10). The more studied class comprises D-*threo*-PDMP (D-*threo*-(1*R,2R*)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; IC_{50} = 2.5 – 10 μM)[40] and structural analogues. *N*-butyldeoxynojirimycin (NBDNJ; IC_{50} = 20 μM)[41] is the representative of the second class of GlcCer synthase inhibitors.

a. PDMP and analogues

Since the discovery of ***PDMP***,[42] a relatively small number of analogues has been described. The core structure of these compounds is based on that of GlcCer, although only D-*threo* isomers exhibit inhibitory activity.[43] PDMP consists of three parts including a phenyl group, an acylamino group and a morpholino group. Structural analogy to GlcCer is illustrated in Figure 1.9. Both compounds have an acylaminogroup, whereas the phenyl group mimics the 4,5-*E* double bond in GlcCer. The cyclic amine is proposed to mimic the sugar transition state during glycosylation.

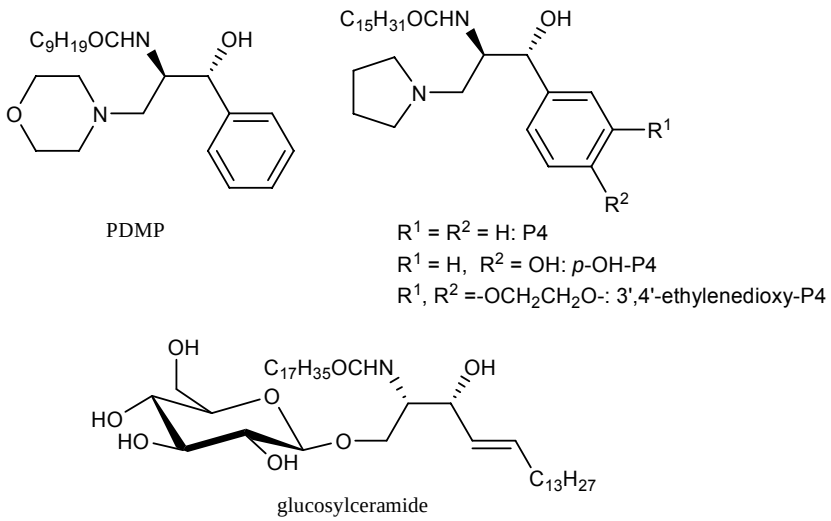


Figure 1.9: Structures of GlcCer, PDMP and PDMP analogues.

Early attempts to diversify the structure of PDMP addressed the acylamino group. It was shown that chain elongation from decanoyl to myristoyl or palmitoyl significantly increased inhibitory activity.[44] Replacement of the morpholino group by other cyclic amines resulted in the discovery of ***P4*** (D-*threo*-1-phenyl-2-aminopalmitoyl-3-pyrrolidino-1-propanol; IC_{50} = 0.5 μM) which shows a tenfold increase in activity compared to PDMP. Modification of the phenyl group of P4 resulted in a number of very potent inhibitors. From preliminary experiments,[45] it was shown that there exists a linear relationship between the log (IC_{50}) and the sum of the hydrophobic (σ) and electronic (π) properties of a substituent on the phenyl ring. These results indicated that a more negative value for $\sigma + \pi$ would result in more potent inhibitors.[46] A series of new compounds was designed to test this hypothesis. The compounds ***p-OH-P4*** (IC_{50} = 90 nM) and ***3',4'-ethylenedioxy-P4*** (IC_{50} = 100 nM) exhibit excellent inhibitory activities thereby supporting the proposed linear relationship. Replacement of the *N*-acyl chain of P4 by a benzyloxycarbonyl moiety also resulted in an increase in inhibitory potency (IC_{50} = 0.3 μM).[47]

Cytotoxic effects of PDMP have been associated with an increase in intracellular ceramide levels, although the underlying reasons have not yet been elucidated. A major advantage of these newer inhibitors is the dissociation of inhibition of GlcCer synthesis from intracellular ceramide accumulation. Aliphatic analogues in which the phenyl substituent is replaced by a usual ceramide *E*-alkenyl chain also lack ceramide accumulation, but proved to be less potent that the aromatic counterparts.[45]

It is noteworthy that ceramide accumulation also might exhibit beneficial effects. Indeed, it has been demonstrated that *in vivo* administration of PDMP and P4 did not result in remarkable toxicity in rats, mice and fish.[48] However, these compounds killed over 80 kinds of human cancer cell lines at 5 μM. Therefore, PDMP and its analogues might represent usefull agents in the treatment of cancer. Moreover, evidence has arisen that PDMP and related compounds are able to reverse multi-drug resistance (MDR) in cancer cells, although the underlying mechanism remains controversial.[49]

b. Deoxynojirimycin and analogues

NBDNJ (IC_{50} = 20 μM) and analogues (Figure 1.10) are iminosugars in which a nitrogen atom replaces the ring oxygen of natural monosaccharides. Surprisingly, modelling experiments (Figure 1.11) suggest that these compounds are structural mimics of ceramide and not of the glucose transition state.[50] IC_{50} values seem to depend on the length of the *N*-alkyl chain since lower inhibitory activity is found for *N*-nonyl derivatives compared to *N*-butyl derivatives. This phenomenon could be explained by the presence of two distinct binding sites on GlcCer synthase as suggested by Butters *et al*.[51]

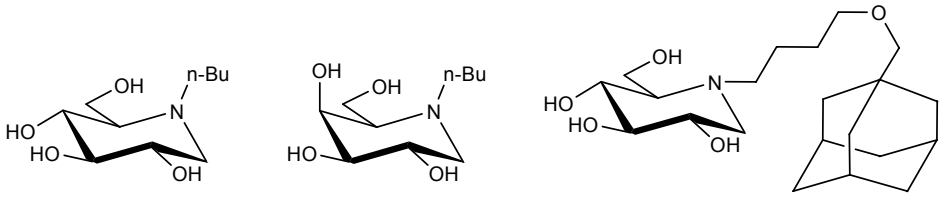


Figure 1.10: Structures of known inhibitors of glucosylceramide synthase

Structural analogues of NBDNJ have been synthesised, the most potent being ***NGDGJ*** (*N*-butyl-deoxygalactonojirimycin; IC_{50} = 41 μM)[52] and ***AMPDNJ*** (*N*-(5-adamantane-1-yl-methoxypentyl)-DNJ; IC_{50} = 25 nM).[53] A significant advantage of these new compounds is their specificity towards GlcCer synthase inhibition. Indeed, NBDNJ shows low specificity and contradictorily even inhibits β-glucocerebrosidase, the enzyme that catabolizes GlcCer, at higher concentrations (IC_{50} = 0.52 mM).

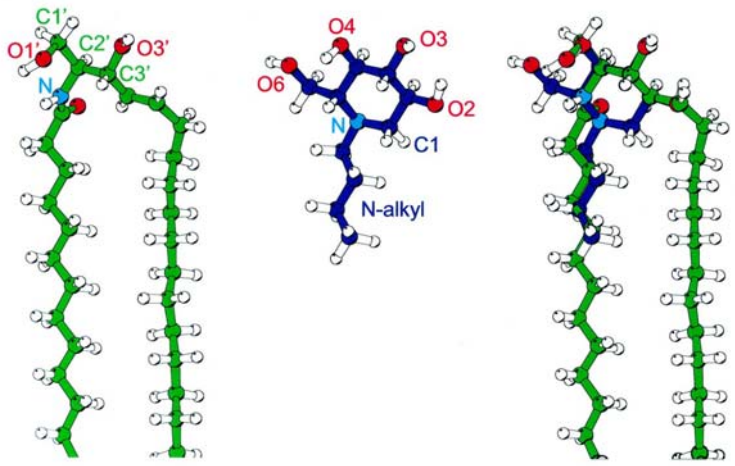


Figure 1.11: Modelled structures of ceramide (left), NBDNJ (center) and overlay of ceramide and NBDNJ. Reprint from reference 50.

1.3.3 CATABOLISM OF SPHINGOLIPIDS

Degradation of SLs occurs in the late endosomes or lysosomes.[4,16] Apart from lysosomal degradation, SM can also be degraded in extralysosomal membranes in response to extra- and intracellular stimuli. Consequently, lipid messengers are formed on demand allowing the cell to transmit stress signals.[3,4]

1.3.3.1. LYSOSOMAL DEGRADATION

Degradation of GSLs (Figure 1.12) takes place in the lysosomal compartments of the cell. Hence, membrane structures are digested by lysosolic ***hydrolases***, which cleave individual sugar residues from the non-reducing end of GSLs.

Digestion starts with the formation of endosomes which traffic through the endosomal compartments to reach the lysosome. The final degradation product in this pathway is ceramide, which is in turn deacylated by an ***acid ceramidase*** to form sphingosine. The cleaved fragments (sugar residues, fatty acids and sphingoid bases) are then able to leave the lysosome and can be degraded further or re-enter the biosynthetic pathway. If degradation fails due to a defect in the proteins involved, nondegradable GSLs accumulate in the lysosome resulting in lysosomal sphingolipid storage diseases (Section 2, p 27). Interestingly, all known defects in GSLs metabolism affect the degradation pathway.

It is necessary to note that the lysosomal degradation products are not available for signalling processes within and outside of the cell.

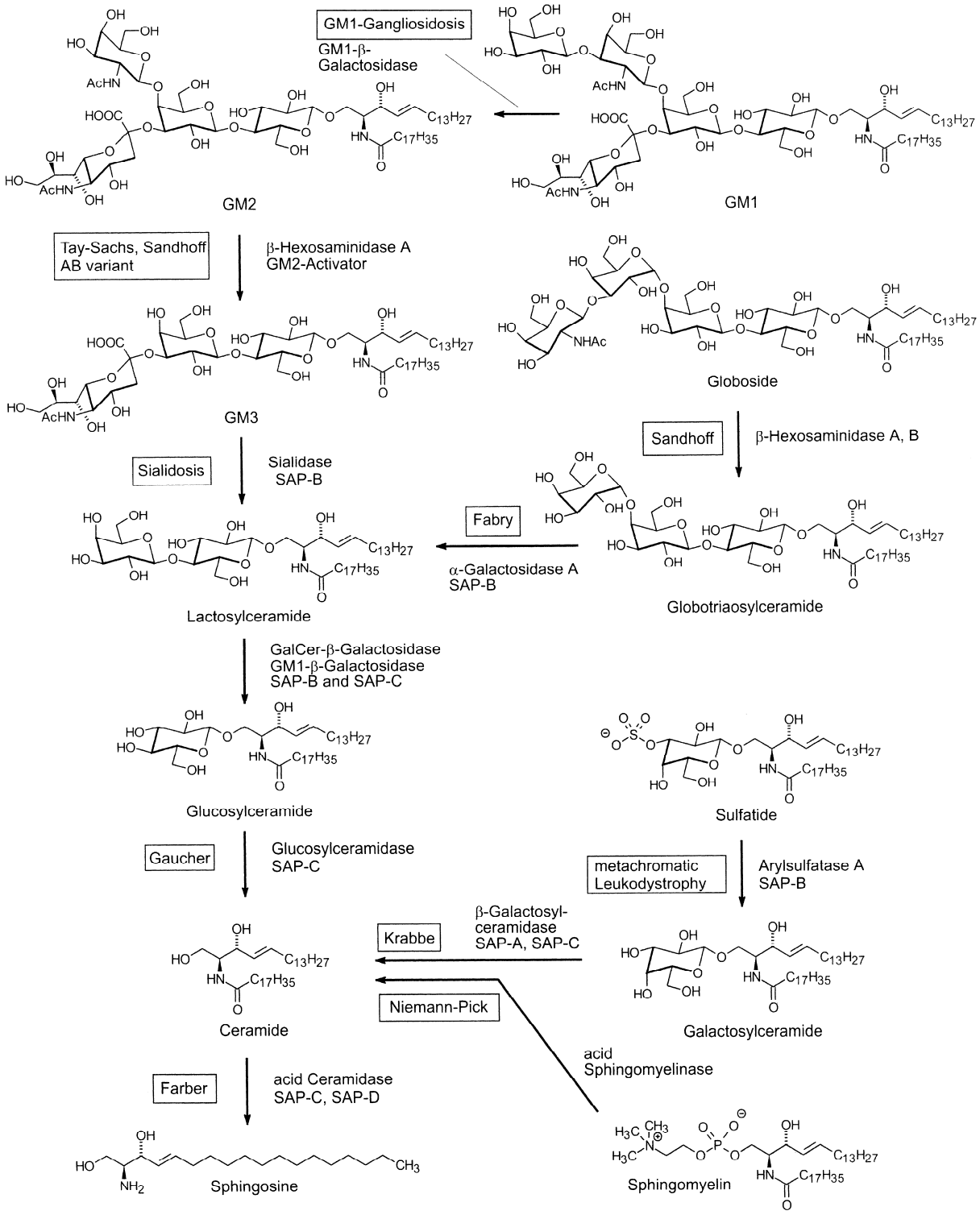


Figure 1.12: Lysosomal sphingolipid degradation. Eponyms of known metabolic diseases are indicated. Heterogeneity in the lipid part is not indicated. Reprint from reference 16.

1.3.3.2. NONLYSOSOMAL DEGRADATION.

In contrast to constitutive SL degradation, which takes place in the lysosomes, sphingomyelin degradation (Figure 1.13) is highly regulated.[54]

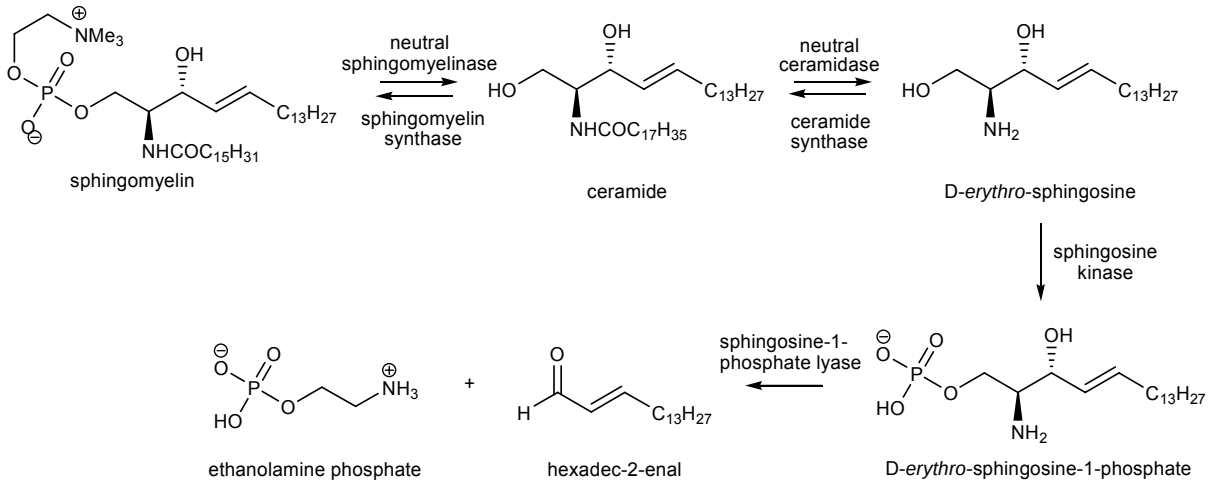


Figure 1.13: Nonlysosomal SL degradation.

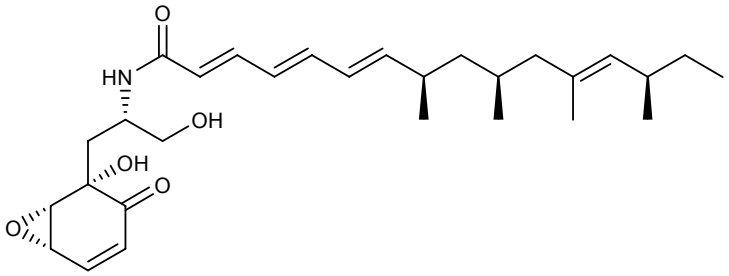
Sphingomyelin can be cleaved by ***sphingomyelinases*** (SMases)[55] of various subcellular localisations thereby releasing ceramide, which is a metabolic intermediate, as well as a signalling substance. Phosphorylation of ceramide has been observed,[56] though the biological relevance remains unclear. The primary alcohol of sphingosine can also be phosphorylated by a cytosolic ***sphingosine kinase***[57] thereby yielding sphingosine-1-phosphate (S1P). Additionally, the reverse mechanism has also been observed in which sphingosine-1-phosphate is dephosphorylated by ***phosphatidic acid phosphorylase***.[58] In a final step, sphingosine-1-phosphate is cleaved by ***sphingosine-1-phosphate lyase*** in a pyridoxalphosphate-dependent reaction[59] producing ethanolamine phosphate and hexadec-2-enal.

1.3.4 INHIBITORS OF SPHINGOLIPID DEGRADATION

Inhibition of SL degradation could provide a useful means of interfering with signalling processes. Moreover, covalent inhibitors can serve as independent tools for analysis of the active site of SL degrading enzymes.

1.3.4.1. INHIBITORS OF SPHINGOMYELINASES

Inhibition of sphingomyelinase could be useful in the treatment of inflammatory or infectious diseases. Indeed, it has been demonstrated that acid sphingomyelinase (aSMase) activity is necessary for infection of non-phagocytizing cells by *Neisseria gonnorhoea*.[60] The most studied SMase inhibitor is scyphostatin, a constituent of *Dasyscyphus mollissimus*. Scyphostatin shows moderate selectvitiy towards neutral sphingomyelinase (nSMase; IC_{50} = 1 μM) compared to aSMase (IC_{50} = 49 μM). It served as a lead for the elaboration of more simplified analogues with comparable potency.[61]

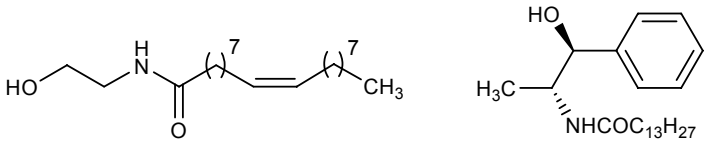


Scyphostatin

Figure 1.14: Structure of scyphostatin.

1.3.4.2. INHIBITORS OF CERAMIDASE

Ceramidase inhibitors are useful tools in the investigation of SL mediated signal transduction processes. *N*-oleoylethanolamine[62] and (1*S*,2*R*)-D-*erythro*-2-(*N*-myristoylamino)1-phenyl-1-propanol (MAPP)[63] are the only representatives of this class (Figure 1.15).



N-oleoylethanolamine D-*erythro*-MAPP

Figure 1.15: Inhibitors of ceramidase.

1.3.4.3. INHIBITORS OF SPHINGOSINE KINASE

The best known inhibitor of sphingosine kinase is *N,N*-dimethylsphingosine (IC_{50} = 5 μM).[64] Although of limited use for functional analysis since they are easily metabolised,[65] short-chain sphingoid bases have also shown to be moderate inhibitors of sphingosine kinase.[66]

1.3.5 THE SPHINGOMYELIN CYCLE: ENZYMES INVOLVED IN GENERATION AND INACTIVATION OF CYTOSOLIC CERAMIDE

The observation that extracellular agents can induce SM hydrolysis paved the way to the discovery of the SM cycle (Figure 1.16). To date a variety of agents are known to interfere with SM hydrolysis (Table 1.3, p.23). The cellular and molecular effects of these extracellular agents can be mimicked by addition of exogenous ceramide, while exogenous dihydroceramide lacks this effect.[67] This result suggests the specific interaction between ceramide and a binding protein. Few downstream effectors of ceramide have been discovered to date.

SM hydrolysis is considered to produce the main source of ceramide for signal transduction.[68] The reaction is mediated by a group of ***sphingomyelinases*** (SMase), thereby producing ceramide and phosphorylcholine. Currently, seven distinct mammalian enzymes have been identified based upon their pH optima, cellular localisation and cation dependence.[69] Despite many studies, the role of individual SMases in cell signalling remains ambiguous.

The first identified member of this class was acid sphingomyelinase (***aSMase***) which is located in the lysosomes.[70] Deficiency of this enzyme results in type A or type B Nieman-Pick disease.[71] Cells from Nieman-Pick patients have proven to be invaluable in ascertaining a signalling role for aSMase. Indeed, it has been shown that they are resistant to both γ-irradiation and chemotherapeutic agents[72] thereby providing proof for the necessary role of aSMase in stress response to various stimuli. Additionally, a Zn^{2+}-dependent aSMase that is secreted by diverse cell types has been found in serum[73] (hence being designated secreted SMase or ***sSMase***).

Neutral membrane-bound, Mg^{2+}-independent SMase (***nSMase***) is the most intensively studied member of this class, and biochemical and cellular control mechanisms are on the verge of being elucidated.[74]

Additionally, an ***alkaline SMase*** with a pH optimum of 9 has been identified in the gastrointestinal tract of rats.[75]

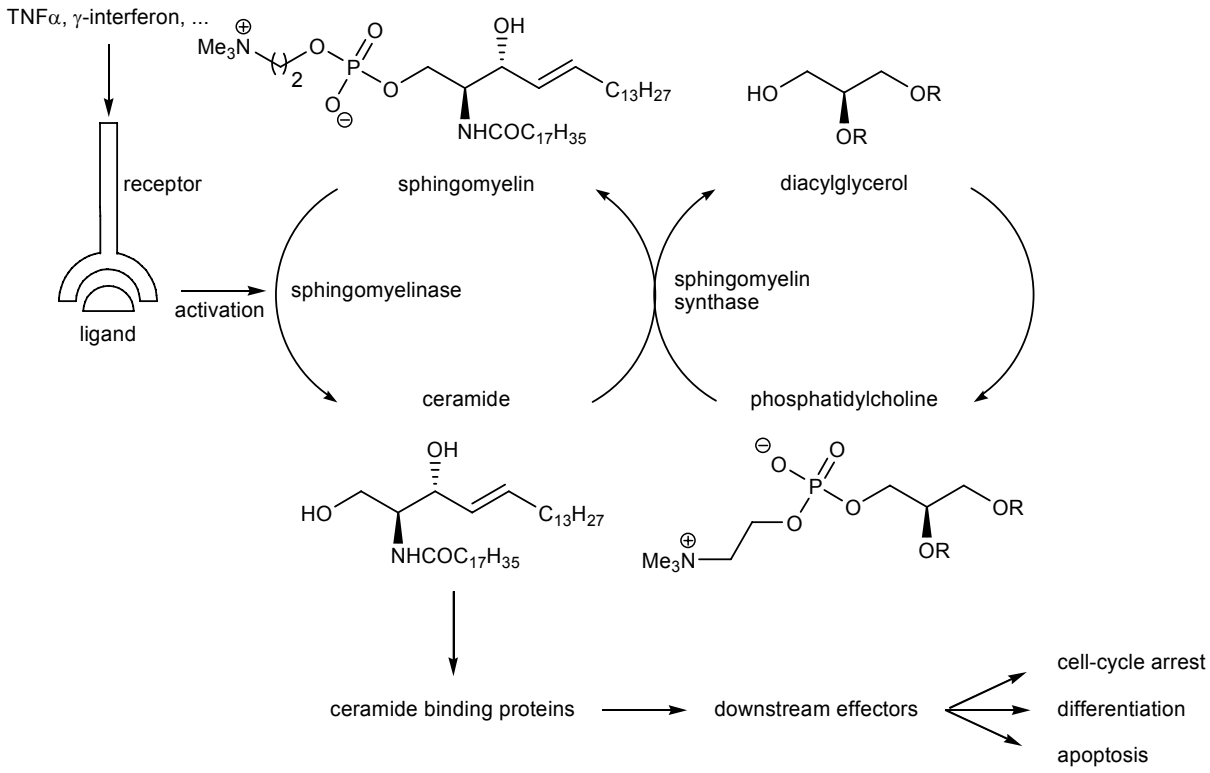


Figure 1.16: Sphingomyelin cycle (R = acyl group).

Ceramide can be recycled to SM by transfer of a phosphocholine head group from phosphatidyl choline. This reaction is catalyzed by SM synthase, which is predominantly localized in the Golgi apparatus,[76] although a plasma-membrane bound fraction has been identified.[77] It is noteworthy from this reaction that a by-product, diacylglycerol (DAG), can activate protein kinase C and thus initiates a separate signal transduction cascade.[78]

In addition to SM hydrolysis, ceramide can directly be formed by acylation of sphingosine by ***ceramide synthase***, which is predominantly located on the cytosolic leaflet of the ER membrane.[79] Evidence has emerged that the enzyme plays a crucial role in cell cycle progression[80] and apoptosis.[81]

Introduction of a 4,5-double bond in dihydroceramide by ***dihydroceramide dehydrogenase***[20,82] is an alternative means for generation of cytosolic ceramide. The enzyme shows a preference for C_{18}-sphingoid bases and is located on the cytosolic leaflet of the ER.

Ceramidases or ***N-acylsphingosine deacylases*** are the most important catabolic enzymes. Their interplay with SMases regulates the availability of ceramide for signalling processes. Different forms of ceramidases have been identified. ***Acid ceramidase*** (AC) is activated by saposins A, C and D (SAP-A, SAP-C and SAP-D) and requires the presence of anionic lysosomal lipids for activity.[83] Saposins are essential cofactors for lysosomal degradation of membrane associated SL and are ubiquitously expressed in different tissues of the body.[84] The *in vivo* regulatory role of saposins on ceramide levels remains unclear. Because free sphingosine, a known inhibitor of protein kinase C, is available anly through the action of ceramidases, these enzymes might play a crucial role in the regulation of cell growth and differentiation.[5,85] A non-lysosomal ***neutral ceramidase*** has been described in a variety of cells and tissues[86] and an ***alkaline ceramidase*** has been isolated from guinea pig skin.[87] Currently, the relationship of acid, neutral and alkaline ceramidase is not entirely clear.

Ceramide kinase phosphorylates ceramide[88] thereby producing ceramide-1-phosphate and is predominantly located in the plasma membrane.[89] It has been suggested that ceramide kinase might play a critical role in phagocytosis, but the mechanistic background remains enigmatic.[89]

Phosphorylation of sphingosine is mediated by ***sphingosine kinase***, a ubiquitously expressed cytosolic enzyme. An increase in activity was observed in response to several growth promoting agents.[90]

1.4. BIOLOGICAL ACTIVITY OF SPHINGOLIPIDS

1.4.1 BIOLOGICAL FUNCTIONS OF CERAMIDE

1.4.1.1. APOPTOSIS

Ceramide-mediated cell death was observed in early attempts to examine the cellular effects of ceramide. Careful observation of this phenomenon revealed that this cell death was accompanied by DNA fragmentation, a hallmark of apoptosis.[91] Eukaryotic cells are able to commit suicide by activation of this self-destruction program in case of infection or damage. Failure of apoptosis may thus result in cancer or autoimmune diseases. Overactivation of apoptosis may in contrast attribute to aggravation of certain neurodegenerative processes or HIV infection.

The exact physiological function of ceramide in apoptosis remains controversial. A major regulation role in this event has been attributed to caspases,[92] a class of cysteine proteases, which serve to enhance both ceramide generation and ceramide downstream targets (death receptor pathway for apoptosis). Another class of important apoptosis regulators is the Bcl-2 family which is mediated by PI-3 kinase, a direct effector enzyme of ceramide. Bcl-2 family is a large group of proteins which regulates mitochondrial membrane permeability (mitochondrial pathway for apoptosis) thereby directly influencing the release of important pro-apoptotic substances from mitochondria.[93]

1.4.1.2. GROWTH ARREST

An increase in ceramide levels has been observed during the G_1-phase of cell division, presumably mediated by SMase activity.[94] Addition of short chain ceramides to cells resulted in dephosphorylation of the retinoblastoma protein Rb, which normally induces expression of genes required for cell proliferation.[95] Additional evidence was provided by the observation that ceramide only poorly affects growth arrest in cells that lack the Rb gene. Since protein kinase C activators, which are able to inhibit ceramide induced apoptosis, had no affect on ceramide-mediated growth arrest, ceramide seems to affect apoptosis and growth arrest independently.[94]

1.4.1.3. DIFFERENTIATION

Early studies on HL-60 cell lines demonstrated that vitamin D3, which had been reported to induce differentiation of HL-60 cells to monocytic/macrophage-like cells,[96] increased ceramide formation. Additionaly, it was shown that exogenous ceramide could mimic the effect of vitamin D3, which suggests that ceramides have a crucial role in differentiation of HL-60 cells.

1.4.2 BIOLOGICAL FUNCTIONS OF SPHINGOSINE

Inhibition of protein kinase C was the first biological role attributed to sphingosine (IC_{50} = 100 μM).[5,85] However, considering the high IC_{50}, the *in vivo*

relevance might be questioned.[97] This breakthrough was, nevertheless, the onset of the discovery of other sphingosine-mediated kinases (Table 1.2). The most important effector proteins are the sphingosine-dependent protein kinases (SDKs). These protein kinases have been shown to phosphorylate a variety of cellular proteins,[98,106] thus tempting one to speculate that sphingosine might regulate signal transduction events.

Despite major research efforts, much remains to be uncovered of the biological functions of sphingosine since controversial reports continue to emerge. Numerous studies concerning the mitogen effect[99] of sphingosine were shown to be PKC-independent. Contradictorily, other reports put sphingosine in the frontline of apoptosis[100] and growth arrest.[101] These controversial effects might arise from sphingosine metabolic instability (phosphorylcholination, phosphorylation or acylation) which can result in the observation of mixed effects.

Table 1.2: Effector enzymes of sphingosine.

Target	Effect
Insulin receptor tyrosine kinase[102]	Inhibition
Calmodulin dependent kinase[103]	Inhibition
Diacylglycerol kinase[104]	Enhance
Casein kinase II[105]	Enhance
Sphingosine-dependent protein kinases (SDKs)[106]	Activator

1.4.3 BIOLOGICAL FUNCTIONS OF SPHINGOMYELIN

Sphingomyelin primarily acts as a reservoir of important signalling molecules and is regulated through the action of SMases. SMase activity is triggered by a myriad of stimuli (Table 1.3), although underlying control mechanisms have not yet fully been elucidated.[107]

Sphingomyelin preferentially concentrates in the outer leaflet of the plasma membrane of mammalian cells in association with cholesterol, by a heterogenous lateral distribution called ***lipid rafts*** or sphingolipid-based microdomains. These rafts showed to be less fluid than the bulk liquid-disordered phospholipids based on diacylglycerol. However, rafts should not be considered as static entities, but rather as dynamic microdomains that can cluster or disintegrate upon specific stimuli.

Clustering is associated with enhanced recruitment of propagator proteins[108] resulting in the creation of a signalling centre[109] that informs the cell about the contact to which it has been engaged. Rafts have also been associated with pathogen entries in cells. For example, HIV-1 enters the cell by binding to CD4, a protein that is associated with rafts.[110]

Table 1.3: Stimuli triggering sphingomyelin hydrolysis.

Inflammatory cytokines	Damaging agents	Inducers of differentiation	Inducers of apoptosis
IL-1α and IL-1β	Heat shock	TNFα	TNFα
IFγ	Ionizing radiation	NGF	Fas ligand
LPS	Daunorubicin	Vitamin D3	Dexamethasone
TNFα	Vincristine	Retinoic acid	Nitric oxide
	Oxidative stress	Progesterone	Staurosporine
	UV light	Serum deprivation	

1.4.4 BIOLOGICAL FUNCTIONS OF SPHINGOSINE-1-PHOSPHATE

1.4.4.1. INTRACELLULAR SIGNALLING

Intracellular S1P mobilizes Ca^{2+} from internal sources and affects many signalling pathways leading to proliferation and suppression of apoptosis.[111] Sphingosine kinase, the enzyme that forms S1P from sphingosine, is activated by many stimuli including platelet-derived growth factor (PDGF), nerve growth factor (NGF), muscarinic acetylcholine agonists and TNF-α.[112] Hence, S1P and ceramide/sphingosine elicit opposing cellular signalling cascades thereby determining cell fate. This sphingolipid rheostat concept[111] has important clinical consequences. For example, the balance between S1P and sphingosine has been suggested to determine allergic responsiveness of mast cells.[113] Additionally, HDL (high density lipoprotein) mediated protection against arteriosclerosis has been associated with resetting of the SL rheostat.[114]

Inhibitors of sphingosine kinase that could selectively block intracellular Ca^{2+} mobilization, cellular proliferation and survival all induced by various stimuli, provided evidence for the involvement of S1P in these processes. Inversely, ceramide-

induced apoptosis could be reversed by S1P. These findings support the second messenger function of S1P rather than the involvement of S1P G-protein-coupled receptors (S1P GPCRs or EDG receptors; see section 1.4.4.2). Moreover, this assumption is supported by the fact that sphinganine-1-phosphate can also bind to EDG receptors without influencing cell survival.[115]

Recent findings question the involvement of S1P in mitosis.[116] Disruption of the S1P lyase gene in mice provoked accumulation of S1P in the cells. However, no distinct effect on DNA synthesis could be observed thereby implying that not S1P, but rather products from S1P lyase are responsible for cell proliferation.

1.4.4.2. EXTRACELLULAR SIGNALLING

Interest in S1P has accelerated remarkably since it proved to mediate cellular signalling processes through the action of the G-protein-coupled receptor EDG1 ($S1P_1$).[115,117] To date, five distinct members, EDG1 ($S1P_1$), EDG5 ($S1P_2$), EDG3 ($S1P_3$), EDG6 ($S1P_4$) and EDG8 ($S1P_5$), have been identified[118] and are commonly referred to as S1PRs (sphingosine-1-phosphate receptors). These S1PRs are ubiquitously expressed and coupled to a variety of G-proteins. Moreover, they are involved in multiple cellular processes as summarized in Table 1.4.

Table 1.4: Overview of S1PRs: functions and major expression sites.

S1PR	Function	Cell types
$S1P_1$	Cell migration (chemotaxis): vascular maturation/angiogenesis	Cardiovascular, nervous, reproductive and immune systems
$S1P_2$	Inhibition of cell migration during embryogenesis	Cardiovascular system, brain
$S1P_3$	Cell migration	Fibroblasts, brain, cardiovascular system
$S1P_4$	Cytokine receptor?	Lymphocytes and hematopoietic tissue
$S1P_5$	Antiproliferative	Oligodendrocytes – astrocytes

Extracellular S1P is generated from SM through the action of plasma membrane-bound sphingomyelinase (nSMase), ceramidase and sphingosine kinase, and subsequently bound to albumin and other plasma proteins where they provide stable reservoirs and ensure efficient delivery to cell-surface receptors.[119]

Pathological implications of these findings are numerous, and research effort directed towards the development of S1PR agonists and antagonists has resulted in the discovery of FTY720 (Figure 1.17; EC_{50} ($S1P_1$) = 1.3 nM; EC_{50} ($S1P_3$) = 2 nM),[120] a non-specific agonist of four of the five S1PRs. Currently, FTY720 is in phase II clinical trials for the prevention of allograft rejection,[121] which probably manifests itself through stimulation of the $S1P_1$ receptor. However, unselective agonism resulted in undesired cardiovascular side-effects, including mild bradycardia, presumably through interaction with the $S1P_3$ receptor. Therefore, current research efforts mainly focus on the uncoupling of $S1P_1$ and $S1P_3$ agonism.[122]

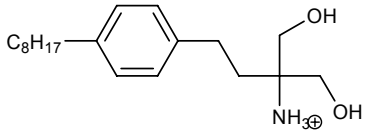


Figure 1.17: Structure of FTY720.

1.4.5 BIOLOGICAL FUNCTIONS OF GLYCOSPHINGOLIPIDS.

Evidence has accumulated that GSLs might exhibit signalling functions. For example, lactosylceramide has shown to be a potent mitogen for aortic smooth muscle cells[123] and epithelial cells.[124] Moreover, it might be involved in the pathogenesis of arteriosclerosis.

Gangliosides were shown to be involved in embryogenesis,[125] as well as in pathological conditions including tumour onset and progression.[126] Moreover, evidence is accumulating that they are key players in the induction of invasion and metastasis. For example, melanoma cells contain and secrete high amounts of GD3, whereas it is almost absent in normal melanocytes.[127] Metastatic melanoma cells exhibited have higher GD3 content than cells that are poorly metastatic.[128]

Studies on the physiological role of GlcCer have revealed its involvement in mitosis.[129] Therefore, inhibitors of GlcCer synthase might present useful agents in the treatment of proliferative disorders like tumour development and lysosomal sphingolipid storage diseases. GSLs are highly abundant in rafts in association with various functional membrane proteins involved in cell adhesion and cell signalling. The extruding carbohydrate moieties of plasma membrane GSLs also act as receptors for attachment of viruses, bacteria, yeast and parasites.[130]

2 LYSOSOMAL SPHINGOLIPID STORAGE DISEASES

2.1. INTRODUCTION

Degradation of SLs is associated with the occurrence of inherited diseases.[131] Catabolism involves about 40 hydrolases, including glycosidases, lipases, nucleases, phosphatases, phospholipases, proteases and sulfatases. Lysosomal sphingolipid storage diseases originate from catabolic enzymatic deficiency thereby causing the blockage and storage of undegradable substrate. Lysosomal sphingolipid storage diseases have a collective frequency of 1 in 18,000 births and are the most common cause of paediatric neurodegenerative diseases.

With the exception of Fabry's disease, which is an X-linked recessive disorder, lysosomal sphingolipid storage diseases are autosomal recessive disorders. The majority of underlying deficiencies has been elucidated to date, and detection of heterozygotes and prenatal diagnosis[132] have become common practice in medicine in countries with high prevalence. However, deficiencies are not restricted to enzymatic degradation since defects in transport and activator proteins have been described.[133]

Clinical consequences of lysosomal sphingolipid storage diseases primarily depend upon the cell type that is predominantly affected. Accumulation of ceramide and glucosylceramide affects mainly visceral organs and skin, whereas gangliosides accumulation results in neurological complications. Severity is typically determined by residual enzyme activity. Infantile manifestation of a disease usually indicates complete absence of enzymatic activity. A schematic overview of biochemical pathways involved in lysosomal sphingolipid storage diseases is depicted in Figure 2.1 (in analogy to Figure 1.12, p.15).

2.2. OVERVIEW

2.2.1 GANGLIOSIDOSES

Gangliosidoses are caused by defective ganglioside degradation, mainly in the central nervous system and to some extent in the viscera. Two subtypes have been identified: GM1 and GM2 gangliosidoses.

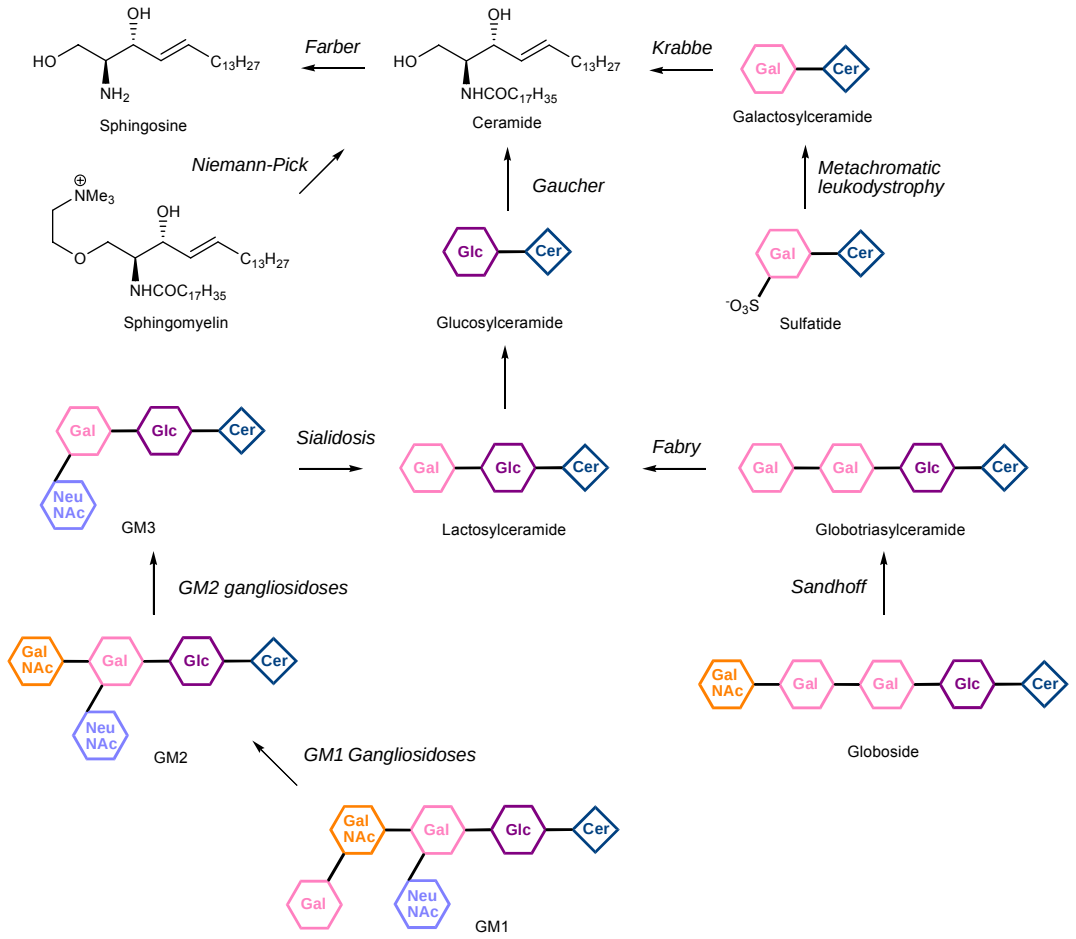


Figure 2.1: Schematic overview of lysosomal sphingolipid storage diseases.

2.2.1.1. GM1 GANGLIOSIDOSES

GM1 gangliosidosis was reported for the first time in 1959 by Norman and co-workers[134] whereas the underlying enzymatic deficiency was identified in 1967.[135] Deficiency of lysosomal GM1 β-galactosidase expresses in two separate diseases, GM1 gangliosidosis and Morquio Type B syndrome.

GM1 gangliosidosis affects mainly the central nervous system. Three distinct clinical forms have been identified depending on the age of onset. In type 1 or the infantile form, neurological symptoms appear within the first 6 months of life leading to death within two years. This form is characterized by brain GM1 accumulation up to 3-5 times normal values. Cherry-red spots on the patient's ocular fundi, hepatosplenomegaly, facial dysmorphism and skeletal deformations are typical symptoms.

Type 2 or the juvenile form usually presents symptoms at 6 - 12 months after birth and shows an identical, though less pronounced, pathological profile with a life expectancy around 10 years.

Type 3 or the adult (chronic) form is characterised by mild, slow progressive neurological disorders without involvement of skeleton deformations.[136]

Morquio Type B syndrome presents a mild form of GM1 β-galactosidase deficiency with mainly skeletal deformation without neurological involvement.

2.2.1.2. GM2 GANGLIOSIDOSES

GM2 gangliosidoses result from deficiency in β-hexosaminidases, the enzymes that degrade GM2 ganglioside. Three subtypes (β-hexosaminidases A, B and S) have been identified and cause distinct types of GM2 gangliosidoses (Table 2.1). Three polypeptides (α and β chains and GM2-activator protein) encoded by three different genes, are involved in enzymatic cleavage.[137] Classification of GM2 gangliosidosis subtypes is based on detection of enzymes that are still present in tissues of patients.[138]

Table 2.1: Overview of GM2 gangliosidoses.

Deficient protein	Disease
β-hexosaminidase A and S	B-variant; infantile onset: Tay-Sachs disease
β-hexosaminidase A and B	O-variant or Sandhoff's disease
GM2 activator protein	AB-variant

a. B-variant of GM2 gangliosidoses

Tay-Sachs disease is characterised by early onset of neurological symptoms, including psychomotoric retardation and tonic-clonic seizures, and results in early death a few years after birth. The disease is widespread among Ashkenazi Jews (1/27 births).

The juvenile form exhibits a clinical profile comparable to Tay-Sachs disease with slowly progressing symptoms appearing between the age of 2 - 5 years after birth. Life expectancy of the patients reaches up to 40 years.

Adult forms show multiple symptoms, including psychosis and muscular atrophy. In contrast to infantile and juvenile forms, mental retardation is not observed. Worldwide, 50 patients of this type have been identified, mostly among Ashkenazi Jews.

b. O-variant of GM2 gangliosidoses or Sandhoff's disease

Sandhoff's disease is comparable to Tay-Sachs disease, but has additional peripheric manifestations including hypertrophy of internal organs and bone deformations.[139]

c. AB-variant of GM2 gangliosidoses

This variant of GM2 gangliosidoses is characterized by massive storage of GM2 in the brain[140] due to the absence of GM2 activator protein, which is believed to act as a surfactant. Clinical progression is identical to Tay-Sachs, but symptoms are rather delayed.

2.2.2 NIEMANN-PICK'S DISEASE

Niemann-Pick's disease is classified into several subtypes (A, B and C).[141] Types A and B of Niemann-Pick are characterized by aSMase deficiency, whereas type C has normal aSMase activity and results from impaired cholesterol trafficking.[142]

Type A Niemann-Pick mainly displays neuropathological symptoms, starting in early childhood and characterized by advancing psychomotoric retardation and hepatosplenomegaly. Death follows usually around the age of three years.

Type B Niemann-Pick shows no neuropathological involvement but mainly affects organs. Patients generally can reach adulthood

Niemann-Pick disease shows a panethnic distribution, but prevails mainly in Ashkenazi Jews.

2.2.3 GALACTOSIALIDOSIS

Galactosialidosis is a combination of two lysosomal sphingolipid storage diseases, GM1 gangliosidoses and sialidosis. Deficiency of both β-galactosidase and sialidase results in the storage of sialic acid- and galactose-containing substrates

such as GM3 and GM1. Both enzymes join to form a so-called "protective protein",[143] which combines different enzymatic activities (serine esterase, carboxypeptidase and deamidase activities). The exact physiological role of this complex is not entirely clear but it has shown to be identical to a protein that is released after thrombin stimulation of blood platelets.

Galactosialidosis has been diagnosed in about 70 patients worldwide and is characterized by cherry-red spots on the ocular fundi, foam cells in the spinal chord, vacuolized lymphocytes and facial dysmorphism.[144] Three different phenotypes have been identified, based upon the onset of symptoms.

2.2.4 SIALIDOSIS

Sialidase deficiency results in accumulation of sialylated oligosaccharides. The infantile form of this disease (Type II sialidosis) is also called mucolipidosis I. The typical clinical profile is characterized by skeletal deformations, megaly of internal organs and mental retardation. The juvenile form is also known as mucolipidosis IV.

2.2.5 FABRY'S DISEASE

Fabry's disease results form deficient α-galactosidase A activity leading to the accumulation of α-glycosidically bound galactose substrates.
Renal failure, painful skin lesions but little neurological involvement characterize the disease. Pathogenesis results form blockage of small blood vessels by lipid depositions. Symptoms usually start in infancy or adolescence.

2.2.6 METACHROMATIC LEUKODYSTROPHY

Deficiency of arylsulfatase A causes metachromatic leukodystrophy, results in the accumulation of sulfatides[145] in different organs and is identified by the presence of metachromatic granula in affected cells. This deficiency is mainly observed in myelin-forming cells in the central nervous system and results in progressive demyelination.

Three clinical subtypes have been recognized. The infantile variant results in death a few months after birth while the late infantile variant results in death at the age of 2 - 3 years. The adult form is rarely observed and is characterized by personality changes, dementia, psychosis and peripheral neuropathy.

2.2.7 MULTIPLE SULFATASE DEFICIENCY OR AUSTIN'S DISEASE

Twelve different sulphatases have been identified in humans and deficiency of these enzymes results in Austin's disease. Clinical manifestations mainly comprise skeletal abnormalities, hepatosplenomegaly and neuropathological symptoms identical to metachromatic leukodystrophy.[146]

2.2.8 GAUCHER'S DISEASE

Gaucher's disease results from deficiency of β-glucocerebrosidase, the enzyme that cleaves the glucose residue from GlcCer, and is the most common of the sphingolipidoses. Storage primarily occurs throughout the reticulo-endothelial system. Hence, lysosomes within macrophages expand due to excessive accumulation of GlcCer by phagocytosis, thereby forming so-called Gaucher cells.

Early classification systems distinguished three phenotypes based on the absence or presence and rate of progression of neuropathic manifestations.

Type I is the most common phenotype. Worldwide prevalence is 1 in 57.000[147] and 1 in 1000 in Ashkenazi Jews. The disease presents at any age and is characterized predominantly by hepatosplenomegaly, bone deformations and pulmonary impairment due to invasion by Gaucher cells.

In the acute neuropathic type II, symptoms present at the age of 3 months and progressive neurological complications result in death within the first year of life.

Subacute neuropathic type III Gaucher (juvenile form) presents in later childhood with slow progressive neurological manifestations. This type prevails predominantly in the Swedish province of Norrbotten.

However, recent clinical evaluation of Gaucher patients has led to the conclusion that Gaucher's disease is more correctly characterized as a continuum of phenotypes (Figure 2.2, p.33)[148] subdivided in patients with and without neuropathological complications. More than 200 mutations have been detected in the

gene encoding for GlcCer synthase, ensuing in multiple genotypes. However, no close relationship has been observed between genotype and fenotype, nor is there a connection between residual enzyme activity or the amount of stored lipid and the fenotype.

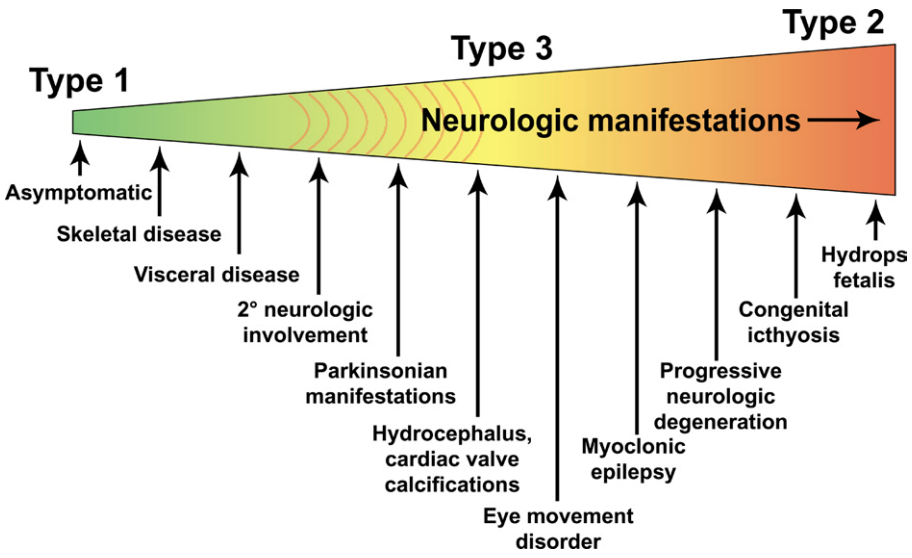


Figure 2.2: Gaucher disease as a wide spectrum of phenotyppes. Reprint from ref. 148.

2.2.9 KRABBE'S DISEASE

Krabbe's disease results from impaired activity of lysosomal galactocerbrosidase, the enzyme that catalyzes cleavage of the galactose residue from GalCer.[149] The clinical spectrum is comparable to that of metachromatic leukodystrophy. Neurological symptoms usually start within the first 6 months after birth and progressive psychomotoric delay and retrogression result in death, usually before the age of 2 years. Later onset patients have rarely been reported.

The white substance of the brain and peripheral nerves exclusively are affected resulting in complete absence of SM in the terminal phase.

2.2.10 FARBER'S DISEASE

Deficient acid ceramidase activity, entailing ceramide accumulation, results in Farber's disease, a very rare sphingolipidosis that usually results in death within one year after birth.[150] To date, only 43 patients have been reported worldwide. Symptoms are comparable to Niemann-Pick disease, but histological differences

have been described. Seven different subclasses have been defined, 6 being ascribed to a defect in lysosomal acid ceramidase and one attributed to deficiencies in SL activator proteins, SAP-C and SAP-D, which fail to execute their cofactor function. Since both neutral and alkaline ceramidase exhibit different tissue distribution, they cannot compensate for the defective acid ceramidase.

2.3. TREATMENT

Two basic approaches can be considered for the treatment of lysosomal sphingolipid storage diseases. One strategy relies on increasing the enzyme levels to compensate for the underlying defect and a second aiming at reduction of the quantity of SLs being synthesised. Current clinical approaches have predominantly focused on increasing enzyme levels through protein replacement strategies. Enzyme substitution can be either direct by infusion or indirect by bone marrow transplantation or gene therapy to replenish the source of fully functional enzyme.[151]

The second approach pursues balancing of impaired SL degradation and biosynthesis.[152] This therapeutic strategy has been named substrate deprivation therapy.[153]

2.3.1 BONE-MARROW TRANSPLANTATION

Bone-marrow transplantation (BMT) is particularly useful in addition to enzyme replacement therapy (ERT) for the treatment of sphingolipidoses with neuropathic manifestations. Hence, BMT could overcome restrictions in ERT by surpassing the blood-brain barrier (BBB) since bone-marrow macrophages can cross the BBB to a small extent and, therefore, are able to supply the deficient enzyme in the central nervous system.[154] Results of BMT are variable,[155] and the success of this approach is limited by the requirement of well-matched donors and the severity of the procedure. BMT in animal models has led to improvement in neurological symptoms, but recovery has not been achieved.[156] Therefore, it does not yet present a reliable approach for the treatment of sphingolipidoses.

2.3.2 GENE THERAPY

It has been shown that as much as 40% of newly synthesised lysosomal enzymes are secreted.[157] This fact makes gene therapy particularly interesting since it implies that a normal cell could cross-correct the metabolic defect by secretion of the deficient enzyme. Moreover, no examples of toxicity have been observed in massive overexpression of lysosomal enzymes, thereby avoiding the need to control expression levels. Thus, genetic modification of a limited number of cells could provide a useful means of supplying large amounts of the deficient enzyme to virtually all cells. This idea can be effected by viral transduction and/or transplantation in a gene therapy approach. The primary goal in gene therapy is to provide sufficient enzyme activity in the central nervous system, which is predominantly affected in a majority of the sphingolipidoses.

The therapeutic potential of gene therapy has been thoroughly evaluated in engineered or spontaneous animal models of several sphingolipidoses. Most studies demonstrated peripheric reconstitution to a certain extent, although tissue and storage disease specificity was observed thereby clearly indicating that feasibility of gene therapy needs to be assessed separately for every enzyme deficiency. In contrast, all experiments failed to enhance enzyme activity in the central nervous system. This fundamental issue might be overcome by direct injection of viral vectors or transplantation of enzyme-overexpressing cells into the brain.[158]

2.3.3 ENZYME REPLACEMENT THERAPY

The first sphingolipidosis that was successfully treated with enzyme replacement therapy was Gaucher type I. In early trials, it had been observed that administration of purified placental β-glucocerebrosidase resulted in reduction of hepatic glucocerebroside in only half of the patients.[159] Later on, it was shown that most of the enzyme had accumulated in hepatocytes of non-responsive patients and was therefore unable to reach the macrophages, which are predominantly affected in nonneuropathic Gaucher's disease. Accumulation of β-glucocerebrosidase, which has four oligosaccharide side chains with terminal *N*-acetylneuraminic acid or galactose residues, was attributed to the presence of a particularly strong lectin for galactose in hepatocytes.[160] Modification of placentally derived β-

glucocerebrosidase so that *N*-glycans terminate in mannose, resulted in a 50-fold increase in the uptake in macrophages.[161] Clinical trials with this modified enzyme unequivocally demonstrated that twice-weekly intravenous administration of the mannose-terminated placental β-glucocerebrosidase (Ceredase™, Genzyme, USA) resulted in remarkable improvement in organomegaly and correction of heamotological parameters.[162] To date, a recombinant form of the enzyme is available (Cerezyme™, Genzyme, USA), which reduces potential infection risks associated with intravenous infusion of the enzyme derived from pooled human tissue.[163] At present, over 3000 patients with type I Gaucher's disease are receiving ERT.

However, ERT suffers from considerable drawbacks. The yearly cost for treatment of adult patients is estimated between 50,000 and 500,000 US$. Moreover, little is known about optimal dosing regimens during maintenance therapy. Since systemic administered β-glucocerebrosidase is unable to pass the blood-brain barrier, the effect of ERT in neuropathic forms of Gaucher's disease is restricted to alleviation of peripheric symptoms.[164] However, mild forms of type III neuropathic Gaucher's disease responded well to high dose ERT.[165]

Recent clinical trials with cultured α-galactosidase A from cancer cell lines proved to be very effective in alleviating peripheric symptoms in Fabry's disease.[166] This result has led to the approval of cultured α-galactosidase A by several countries (Fabrazyme™ from Chinese hamster ovary cells and Replagal™ from human cells)[167] for the treatment of Fabry's disease.

Currently, aSMase for the treatment of Niemann-Pick's disease is in preclinical phase.

2.3.4 SUBSTRATE DEPRIVATION THERAPY

The ultimate aim in substrate deprivation therapy is reduction of substrate influx to a level that matches residual enzyme activity. Even if this goal cannot be achieved, the patient would avoid disease progression if the rate of accumulation could be kept below a toxic threshold. However, this method necessitates minimal residual lysosomal enzyme activity, a condition which is the case in most juvenile and adult forms. In contrast, infantile onset of lysosomal sphingolipid storage diseases is characterized by no or very little residual enzyme activity.[168] Nevertheless, if

nonexistent residual enzyme activity would be partially restored exogeneously by gene therapy or BMT, combination with substrate deprivation therapy could provide a useful means of treating infantile onset forms. Substrate deprivation therapy is not a new or unique concept since it has proven useful, for example, in treatment of lactose intolerance by reduction of dietary lactose.

GlcCer synthase presents an attractive target for substrate deprivation therapy since it acts as a hub for biosynthesis of more complex, GlcCer-based SLs. Therefore, all sphingolipidoses upstream of GlcCer are potential disease targets for GlcCer synthase inhibitors. An overview of GlcCer synthase inhibitors has been presented in section 1.3.2.3 of this chapter (p.11).

In vivo evaluation of the feasibility of the substrate deprivation concept has been focused predominantly on NBDNJ. NBDNJ had originally been developed as an anti-HIV agent. Its mode of action relied on inhibition of α-glucosidase I and II, thereby inhibiting viral replication *in vivo.*[169] Phase II clinical trials of NBDNJ revealed that serum levels needed for α-glucosidase inhibition could not be reached without the incidence of severe gastro-intestinal side effects and, hence, no significant effect on viraemia was observed. Despite the lack of antiviral efficacy, these trials provided important information. It was shown that NBDNJ was generally well tolerated, the major side effect being osmotic diarrhoea. Moreover, serum levels through oral administration ranged between 10 - 50 μM, levels which were known to inhibit GlcCer synthase both *in vitro* and *in vivo*.

Three clinical studies[170] using NBDNJ (Zavesca^{TM}, OGT 918, miglustat) for the treatment of type I Gaucher's disease have been conducted. Typical dosage of NBDNJ ranged between 50 and 100 mg/day. Results from these trials indicate that treatment with NBDNJ leads to an overall improvement in clinical parameters measuring disease progression.[171] Moreover, clinical outcome improved if NBDNJ was combined with ERT. Diarrhoea and weight loss are the most prevalent side effects of treatment with NBDNJ. Some rare cases of neuropathy have also been observed. Currently, the galactose analogue (NBDGJ), which exhibits higher selectivity than NBDNJ towards GlcCer synthase, is in phase I clinical trial (CDP 923, Celltech, UK).

Potent analogues of PDMP, the representative of the other known class of GlcCer synthase inhibitors, have only been discovered since 1999,[52] and one drug

candidate (3',4'-ethylenedioxy-P4; Genz-112638, Genzyme General, USA) is currently in phase I clinical trial for the treatment of type I Gaucher's disease. Genzyme claims that 3',4'-ethylenedioxy-P4 was found to be 5000 times more potent in vitro than NBDNJ and 100 times more potent than AMPDNJ, the most potent iminosugar analogue known to date. In vivo, it showed to be 20 times more potent than AMPDNJ. Moreover, even at high doses, no significant toxicity has been observed.

3 OBJECTIVES

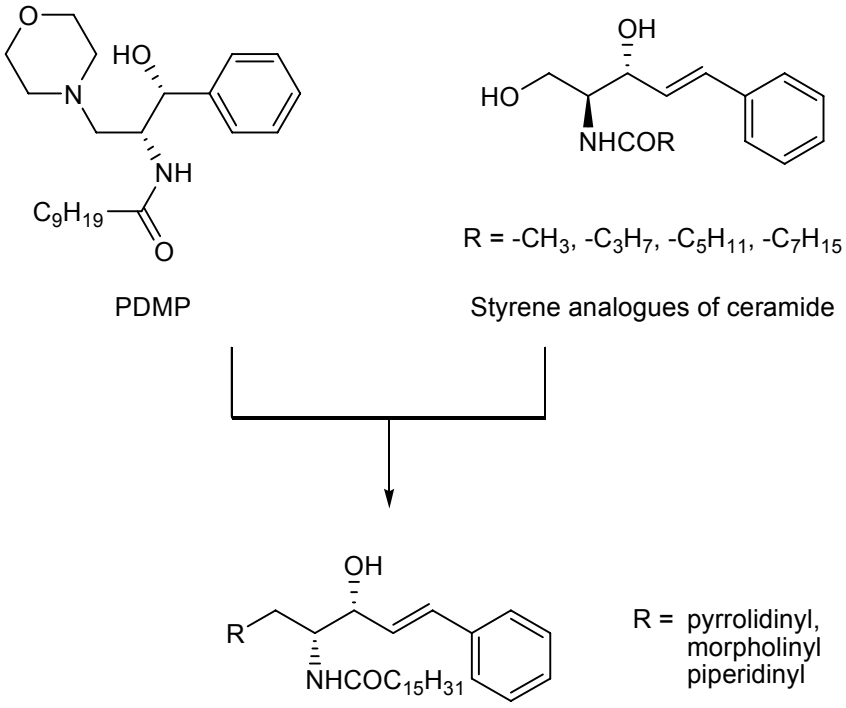
This Ph.D. study constitutes part of an extensive project dealing with the synthesis of biologically relevant sphingoid and ceramide analogues. Chemical approaches provide useful extentions of molecular and cell biology in elucidating the biochemistry and functions of SLs. Moreover, bioorganic chemistry is able to furnish new conceptual therapeutics as demonstrated by the development of GlcCer synthase inhibitors.

The first part of this study presents the development of a convenient synthetic route to access new hybrid GlcCer synthase inhibitors. In a second part, we want to disclose a new class of ceramide homologues.

3.1. SYNTHESIS OF HYBRID PDMP ANALOGUES

An earlier study from this group[172] demonstrated that substitution of the alkenyl chain of ceramide by a styryl group did not affect the capacity to reverse the inhibitory effect of fumonisin B1 on the axonal growth of hippocampal neurons. Since metabolisation of ceramide to GlcCer is a prerequisite to sustain growth of hippocampal neurons,[173] these results prove that styrene analogues are substrates for GlcCer synthase and, therefore, are metabolised to their glucosylated form. Thus, combination of these styrene analogues with basic structural features required for GlcCer synthase inhibition might provide new potential therapeutic compounds (Scheme 3.1).

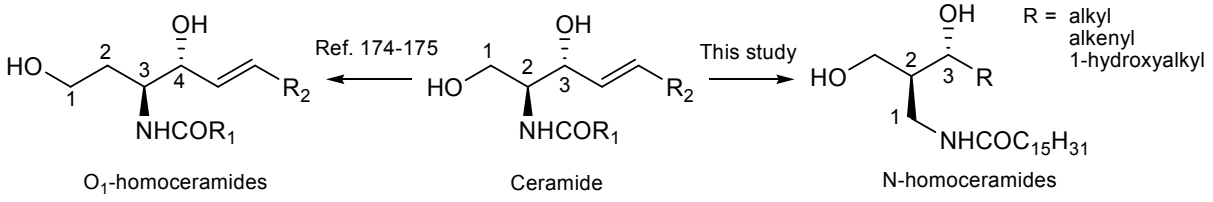
Since further structural modifications could involve the introduction of substituted aromatic rings in analogy to more potent PDMP analogues (1.3.2.3a, p.11), we aimed at the elaboration of a synthetic route towards a single advanced intermediate to allow straightforward introduction of the aryl group. Since our primary concern was whether these merged analogues would still exhibit biological activity, initial optimization of the synthetic scheme will be accomplished with the unsubstituted aromatic analogue.



Scheme 3.1: Overview of structural modifications.

3.2. SYNTHESIS OF N-HOMOCERAMIDES.

Homologation is a classical tool in medicinal chemistry to enhance biological properties of endogenous compounds. Recently, our group reported an expedient route for the synthesis of D-*erythro*-O_1-homoceramides[174] (Scheme 3.2), which were later shown to exhibit considerable apoptotic activity.[175] In analogy to these findings, we aim at the synthesis of a new class of ceramide homologues which contain an additional methylene group between the *N*-acyl chain and C2.

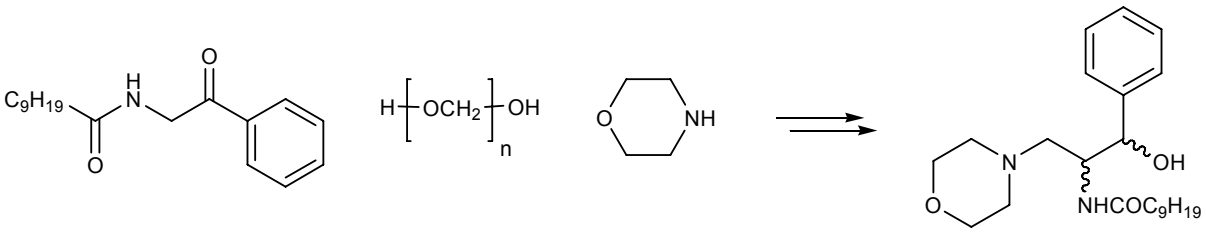


Scheme 3.2: Overview of structural modifications.

4 REPORTED SYNTHETIC STRATEGIES

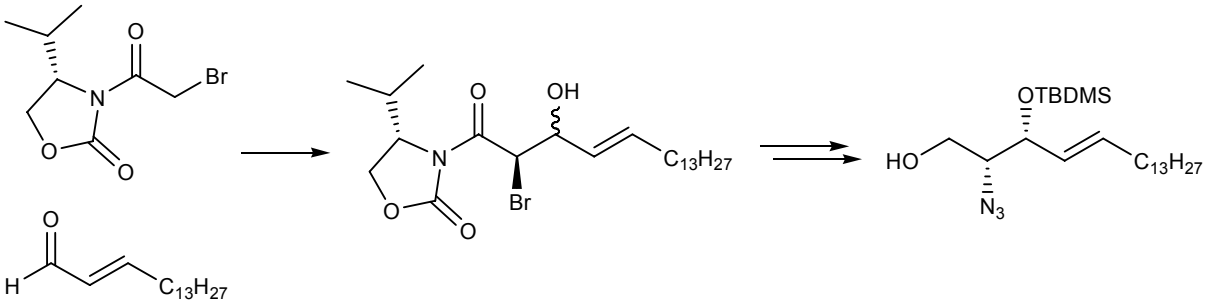
4.1. SYNTHESIS OF PDMP AND ANALOGUES.

In 1980,[42] Radin and Vunnam reported the first synthesis of PDMP by a Mannich reaction of 2-*N*-decanoylaminoacetophenone, paraformaldehyde and morpholine and subsequent reduction with $NaBH_4$, thereby yielding a mixture of (*D*,*L*)-*threo*- and -*erythro*-PDMP (Scheme 4.1). In a subsequent paper,[43] Radin and Inokuchi described the procedure for isolation of all isomers of PDMP by combination of HPLC and crystallisation of the dibenzoyl-tartaric acid salts. Hence, the authors were able to assign inhibitory activity of PDMP to the D-*threo* isomer.



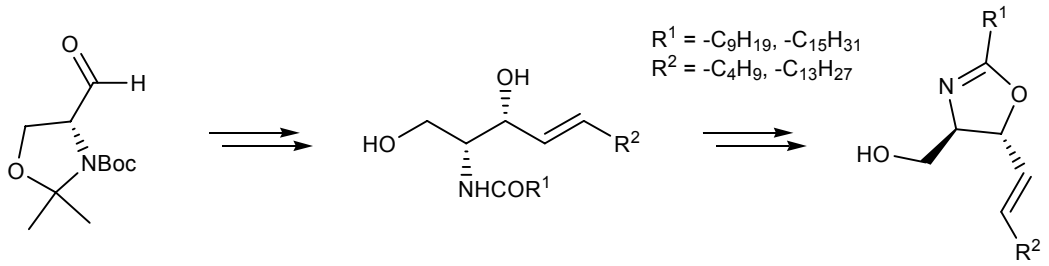
Scheme 4.1: Synthesis of PDMP according to Radin and Vunnam.

Stereoselective aldol condensation between the boron enolate of bromoacetyl oxazolidinone with *E*-2-hexadecenal, as originally devised by Nicolaou *et al*.,[176] was the key reaction in the synthetic approach presented by Carson and Ganem[177] (Scheme 4.2). Replacement of the bromine with sodium azide, protection of the secondary alcohol with TBDMSCl and subsequent deprotection of the oxazolidinone with $LiBH_4$ furnished the 1,2-azidoalcohol, which could be easily transformed in five steps (61% yield) to the *E*-alkenyl PDMP analogue (7% total yield over 9 steps). Preparation of the homochiral starting material required 4 additional steps.



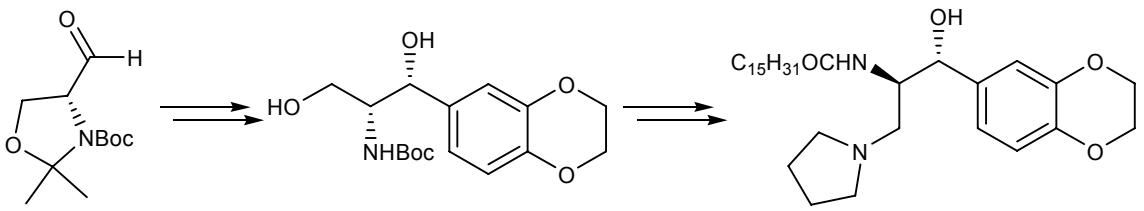
Scheme 4.2: Synthesis of PDMP analogues according to Carson and Ganem.

An alternative procedure, starting from D-Garner's aldehyde, was reported by Miura *et al.*[178] (Scheme 4.3). The authors accessed several D-*threo*-ceramide analogues in a five-step sequence. Selective mesylation of the primary alcohol of ceramide and subsequent treatment with morpholine surprisingly yielded the 2,3-oxazoline (20 – 50%). Triflation followed by substitution with morpholine or pyrrolidine and final cleavage of the oxazoline gave access to *E*-alkenyl PDMP analogues in low yield (2 - 4 % overall yield).



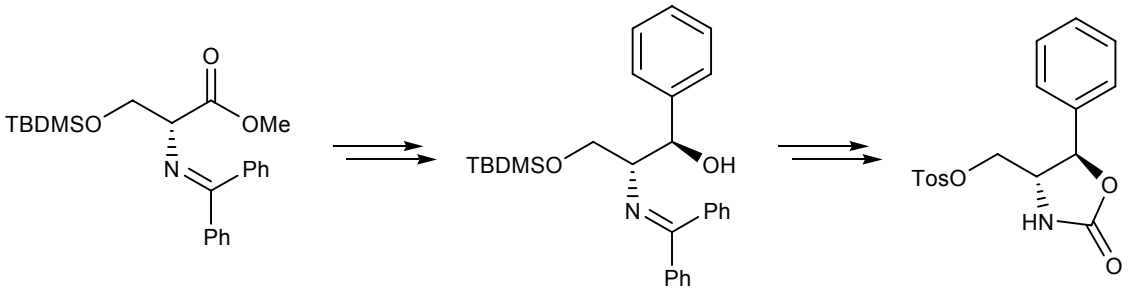
Scheme 4.3: Synthesis of PDMP analogues according to Miura *et al.*

A comparable strategy was presented by Hussain and Ganem[179] (Scheme 4.4). Hence, CuI-Me_2S mediated Grignard addition to D-Garner's aldehyde followed by selective acetal cleavage provided the intermediate 1,3-diol, which was converted in a 4 step sequence to the desired PDMP analogue.



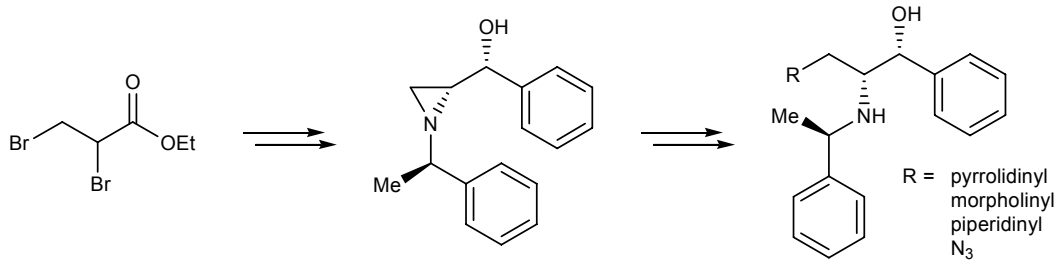
Scheme 4.4: Synthesis of 3',4'-ethylenedioxy-P4 according to Hussain and Ganem.

Starting from a D-serine derived Schiff base, Mitchell *et al*.[180] have accessed PDMP analogues with different cyclic amines (Scheme 4.5). Thus, treatment of the imine with *i*-Bu_5Al_2H followed by Grignard reaction with PhMgBr provided the PDMP core structure. Substitution of the tosylate, which was prepared from the Grignard adduct in 4 steps, gave access to various cyclic amine derivatives that were smoothly transformed to the desired D-*threo*-PDMP analogues. An identical procedure was employed by Slavish *et al.*[181] for the synthesis of PDMP analogues with altering aromatic rings.



Scheme 4.5: Synthesis of PDMP analogues according to Mitchell *et al.*

Shin *et al.* devised a new method for regioselective ring opening of non-activated aziridines and applied it towards the elaboration of PDMP analogues (Scheme 4.6).[182] Thus, TMSI-mediated ring opening of the aziridine, obtained in 4 steps from ethyl 2,3-dibromopropanoate, produced 2,3-aminoalcohol ring opening products in high yield (87 - 99%). Subsequent hydrogenolysis of the α-methyl benzyl moiety followed by selective acylation of the unmasked primary amine afforded PDMP analogues in high overall yield (70 – 80%).

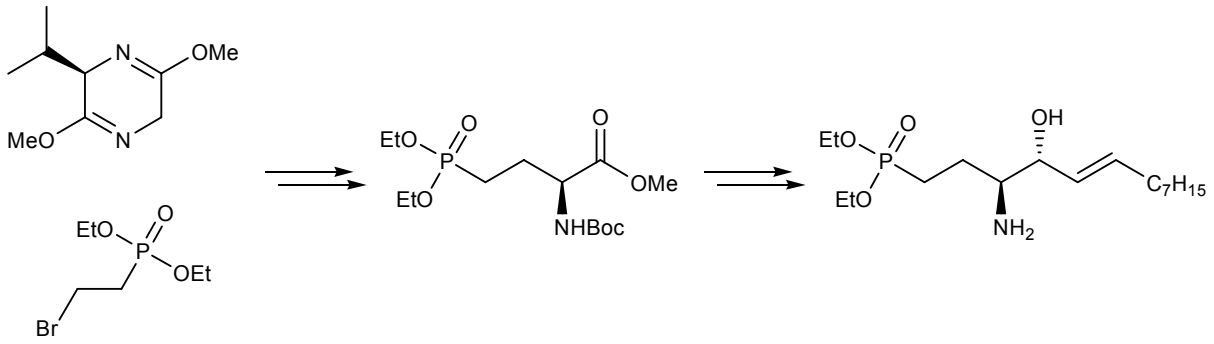


Scheme 4.6: Access to PDMP analogues according to Shin *et al.*

4.2. *SYNTHESIS OF HOMOCERAMIDES*

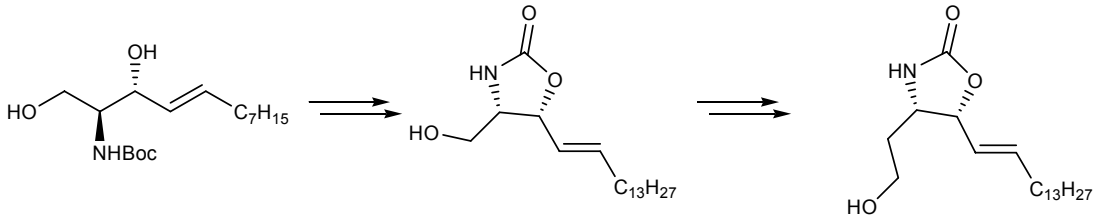
Homologation in ceramides has been focussing primarily on the synthesis of sphingosine-1-phosphate derivatives with improved metabolic stability, which can be effected by replacement of the 1-phosphate by a phoshonate group.

Sandhoff and coworkers[183] reported the first synthesis of the phosphonate analogue of sphingosine-1-phosphate (Scheme 4.7). Starting with stereoselective alkylation of the bis-lactimether of cyclo(-L-Val-Gly) with 2-bromoethylphosphonate followed by mild acidic hydrolysis of the bislactim ether and subsequent *tert*-Boc protection of the liberated primary amine afforded the phosphonate intermediate, which was transformed to the desired phosphonate analogue of sphingosine-1-phosphate in a four-step sequence in moderate yields (28-31%).



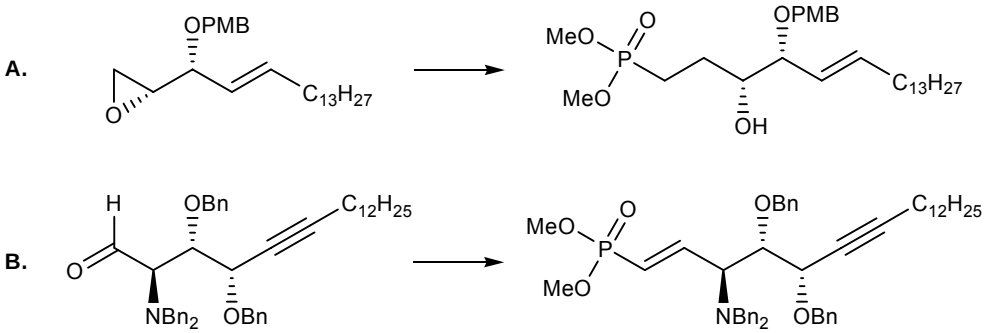
Scheme 4.7: Synthesis of the phophonate analogue of sphingosine-1-phospate by Sandhoff and coworkers.

Some years later, Tarnowski *et al.*[184] presented an alternative synthetic route towards homo-sphingosine-1-phoshonate starting from an *N-tert*-Boc protected ceramide, prepared from D-galactose and D-xylose (Scheme 4.8). Regioselective mesylation of the protected ceramide followed by acid catalyzed removal of the *tert*-Boc group and treatment with *N,N'*-carbonyldiimidazole led to the intermediate cyclic urethane. Substitution of the mesylate group with KCN, followed by reduction of the nitrile with DiBAlH and $NaBH_4$ afforded the intermediate primary alcohol, which was transformed in 4 steps to the desired homo-sphingosine-1-phosphonate. Recently, an alternative approach starting from D-galactose was presented by the same authors.[185]



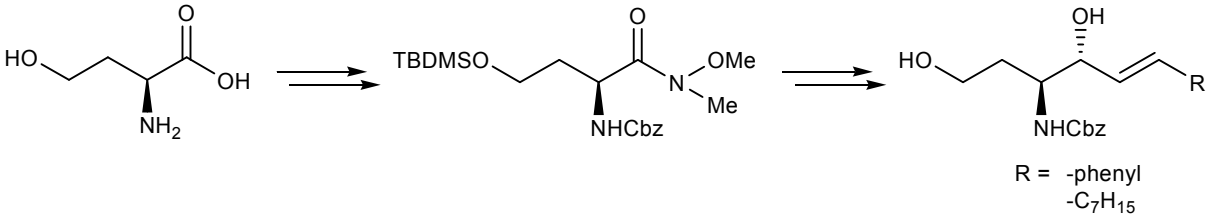
Scheme 4.8: Synthesis of sphingosine-1-phosphate homologues according to Tarnowski *et al.*

Bittman and co-workers developed two different routes towards the synthesis of phosphonate analogues of sphingolipids. In a first synthetic strategy, the authors introduced chain elongation through regioselective epoxide opening with lithium dimethyl methylphosphonate (Scheme 4.9 A).[186] In a second paper, the synthesis of L-*lyxo*-phytosphingosine-1-phosphonate was described starting from D-(-)-tartaric acid. Introduction of the methylene spacer was achieved by Horner-Wadsworth-Emmons olefination between an intermediate aldehyde and tetramethyl methylenediphosphonate (Scheme 4.9 B).[187]



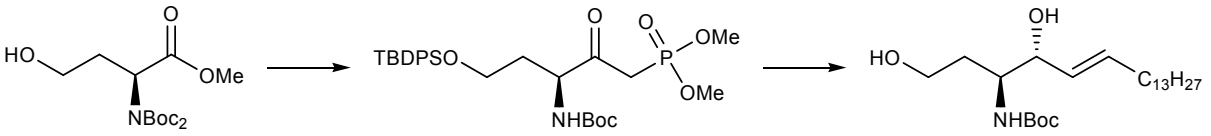
Scheme 4.9: Synthetic routes towards sphingosine-1-phosphonate and L-*lyxo*-phytosphingosine-1-phosphonate according to Bittman and co-workers

Recently, our group reported an expedient route for the synthesis of D-*erythro*-O_1-homoceramides (Scheme 4.10).[174] L-Homoserine served as a chiral building block and was transformed to a protected Weinreb amide in 4 steps. Reaction of the Weinreb amide with the appropriate lithium acetylide followed by stereoselective reduction of the thus formed alkynone and subsequent silyl deprotection with TBAF gave access to the 1,4-diol intermediate, which was converted to the desired O_1-homoceramide in two steps involving a one-pot alkyne reduction and Cbz deprotection and final acylation of the primary amine.



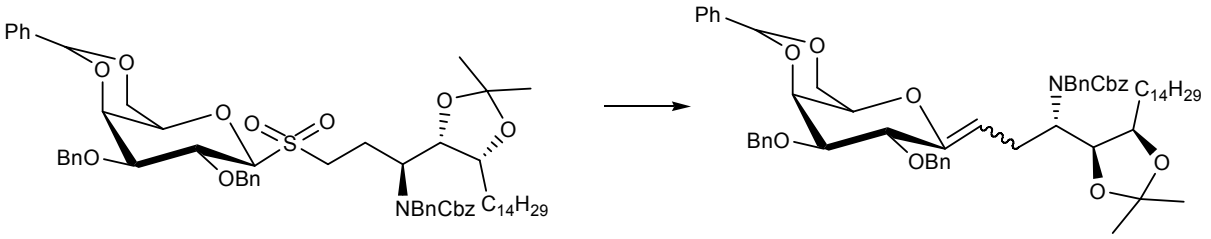
Scheme 4.10: Synthesis of O_1-homoceramides according to De Jonghe *et al.*

Later, an alternative route towards D-*erythro*-O_1-homoceramides was presented by Ogino and co-workers[175] (Scheme 4.11). Di-*tert*-Boc protected *L*-homoserine methyl ester, obtained in 4 steps from L-aspartic acid, served as starting material and gave access to the intermediate key phosphonate in 3 steps. Horner-Wadsworth-Emmons olefination followed by stereoselective reduction of the ketone and TBAF mediated desilylation produced the intermediate 1,4-diol, which was further transformed in two steps to the coveted O_1-homoceramide.



Scheme 4.11: Synthesis of O_1-homoceramides according to Ogino and co-workers..

Recently, the synthesis of the C-glycoside analogue of α-galactosylceramide has been reported. In a first report,[188] protected D-*ribo*-homophytosphingosine, derived in six steps from L-homoserine, was attached to a thioglycoside. Key transformation was the Ramberg-Bäcklund reaction thereby stitching the homophytosphingosine side chain to the galactose moiety (Scheme 4.12). The same group later presented an alternative procedure, employing a cross-metathesis (CM) approach.[189]



Scheme 4.12: Synthesis of the C-glycoside of α-galactosylceramide according to Franck and co-workers.

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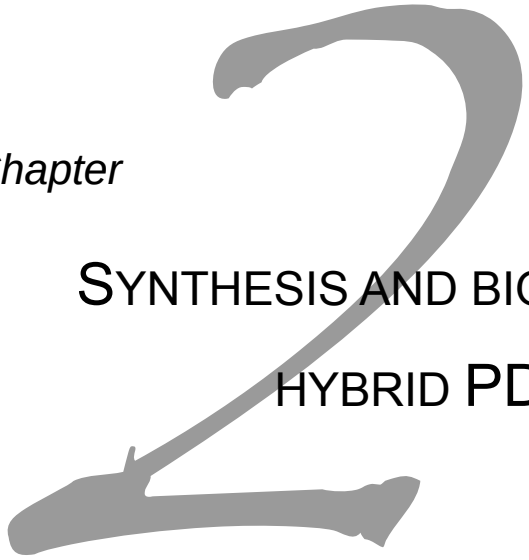
Chapter



SYNTHESIS AND BIOLOGICAL EVALUATION OF HYBRID PDMP ANALOGUES

6 MONO-PROTECTIVE STRATEGY FOR THE SYNTHESIS OF HYBRID PDMP ANALOGUES.

6.1. INTRODUCTION - RETROSYNTHESIS

Stereochemical and structural features of inexpensive and readily available chiral pool compounds such as amino acids and sugars have been extensively exploited for the synthesis of SLs.[1] Amongst these chiral pool compounds, serine has shown to be particularly popular since all four stereoisomers of the sphingoid backbone (Figure 1.3, p. 5) are readily accessible depending on the choice of either D- or L-serine as starting material.

Initially, we pursued a similar synthetic strategy as earlier advanced by this group[2] starting from D-Garner's aldehyde (Figure 6.1), which can by obtained from commercially available D-serine by an established literature procedure.[3]

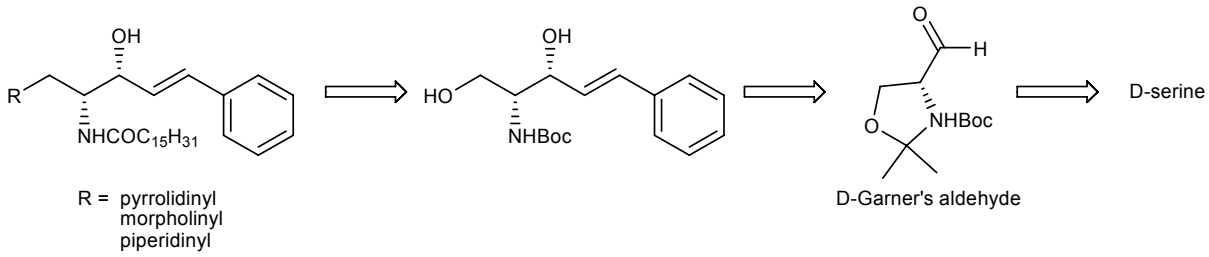


Figure 6.1: Retrosynthesis of hybrid PDMP analogues.

Stereoselective addition of an appropriate lithium acetylide to Garner's aldehyde results in either *threo*- or *erythro*-alkynols, depending on the specific reaction conditions.[4] While application of a cation-complexing cosolvent accounts for high *erythro*-selectivity, addition in the presence of a Lewis acid, such as anhydrous $ZnBr_2$, produces predominantly *threo*-alkynols (Figure 6.2). Thus, in the presence of a Lewis acid, the chelation-controlled conformation comprises a synperiplanar orientation of the aldehyde and the *tert*-Boc carbonyl, resulting in preferential attack at the *Si*-face of the carbonyl group, which gives rise to the *threo*-epimer.

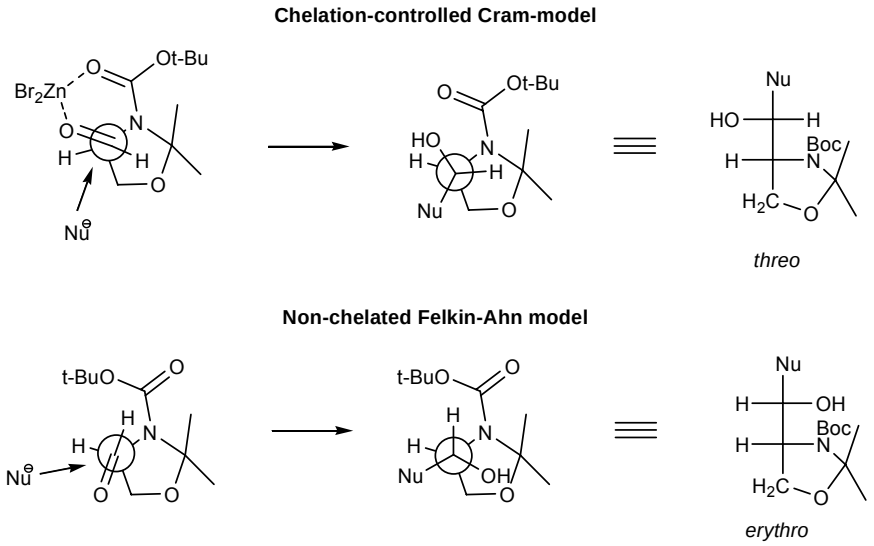


Figure 6.2: Diastereoselectivity of nucleophilic additions to Garner's aldehyde (Nu = nucleophile).

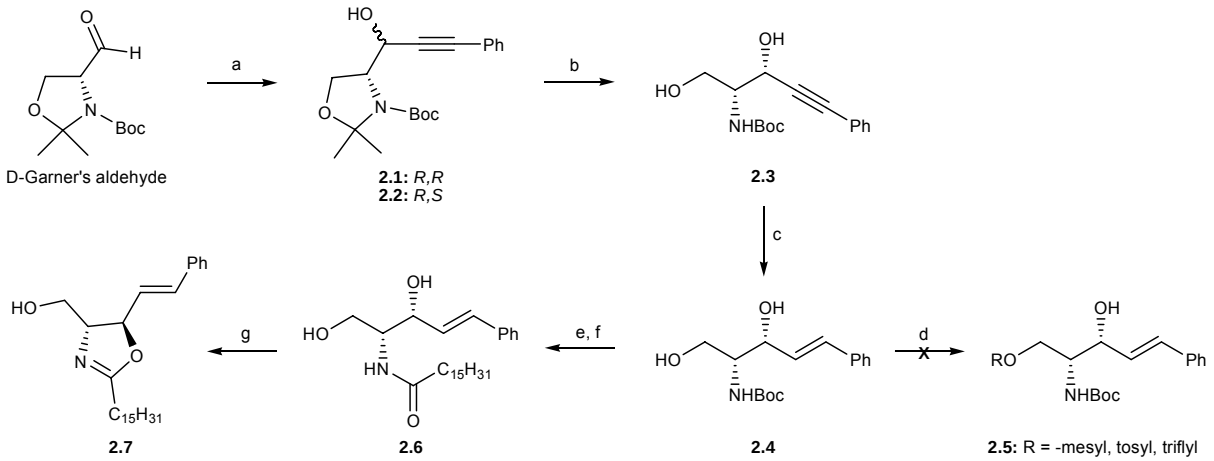
In contrast, the preferred transition state in the absence of complexation is based on a conformation in which the bulkiest α-ligand positions itself perpendicular to the plane of the carbonyl group, thereby generating an *anti*-relationship towards the incoming nucleophile. The medium-sized substituent is placed synclinal with respect to the carbonyl function and hence, attack occurs preferentially at the least hindered *Re*-face of the aldehyde, leading to the *erythro*-product.

Subsequent reduction of the alkyne to the *E*-alkene followed by introduction of the cyclic amine on the *N-tert*-Boc protected ceramide, as previously described by Ganem and Husain,[5] was expected to provide the *N-tert*-Boc protected intermediate which could straightforwardly be transformed to the desired D-*threo*-PDMP analogues in a two-step sequence involving *tert*-Boc deprotection and selective acylation of the thus unmasked primary amine.

6.2. SYNTHESIS

A solution of D-Garner's aldehyde in Et_2O was treated with lithiumphenyl acetylide at -78°C in the presence of excess $ZnBr_2$, thereby producing an epimeric mixture of *threo*- and *erythro*-alkynols **2.1** and **2.2** (*threo*:*erythro* 9:1), which was repeatedly purified by flash chromatography, ultimately affording pure *threo*-alkynol **2.1** and a mixture of both epimers (Scheme 6.1). *Threo*-stereochemistry was assigned by comparison of NMR data with a previous report.[2]

Selective cleavage of the oxazolidine ring of **2.1** with *p*-TsOH produced *N*-*tert*-Boc protected 1,3-diol **2.3**, which was treated with Red-Al® to afford *E*-alkene **2.4** in very good yield (88%). By analogy to a literature report by Husain and Ganem,[5] **2.4** was subjected to selective mesylation. Unfortunately, treatment of **2.4** with MsCl in the presence of TEA (1 eq. each) in CH_2Cl_2 failed to produce the desired mesylate, but instead gave rise to a complex reaction mixture as observed by TLC.



Scheme 6.1: a) lithium phenylacetylide, $ZnBr_2$, Et_2O, -78°C (**2.1**: 44%; **2.1** + **2.2**: 28%); b) *p*-TsOH, MeOH, RT, 36 h (70%); c) Red-Al, Et_2O, -78°C to RT, overnight (88%); d) see Table 6.1 ; e) 1N HCl:dioxane (1:1), reflux, 30' (72% crude yield); f) 50% NaOAc:THF (1:1), palmitoyl chloride, RT, 2 h, (58%); g) MsCl, TEA, CH_2Cl_2, 0°C to rt, 16 h (38%).

A literature survey revealed that 1,2-*N*-*tert*-Boc-amino alcohols are prone to undergo oxazolidinone ring closure[6] upon attempts to activate the alcohol. The nucleophilic nature of the *tert*-Boc group might account for the observed complex reaction mixtures. Such reactivity of the *tert*-Boc group arises on one hand from the polarized carbonyl bond and on the other hand, from the presence of the *tert*-butyl group, which can be expelled as a cation, and hence, the presence of any general base gives rise to isobutylene (Figure 6.3, B), although a concerted reaction course is possible too (A). Reaction outcome greatly depends upon specific reaction conditions including reaction temperature, base and solvent. Hence, based on different literature reports, a set of altered reaction conditions was devised in order to circumvent previous issues. Unfortunately, none of these adjustments (Table 6.1) changed the reaction outcome, nor did changing from MsCl to *p*-TsCl or triflic anhydride.

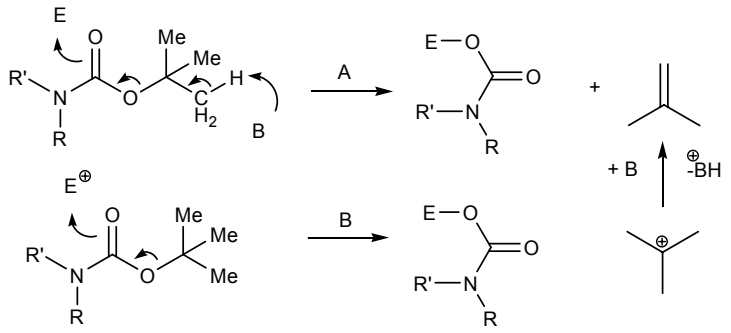


Figure 6.3: Nucleophilic reactivity of the *tert*-Boc group.

Therefore, we decided to switch to an alternative approach in which oxazoline formation is exploited as protective strategy to allow selective introduction of a leaving group on C1 of the sphingoid backbone, followed by substitution with an appropriate secondary amine, as was advanced previously by *Miura et al.*[7] Thus, deprotection of the *tert*-Boc group with 1N HCl, followed by acylation with palmitoyl chloride under Schotten-Baumann conditions[8] afforded styrene ceramide analogue **2.6**. Treatment of alcohol **2.6** with MsCl at 0°C in the presence of TEA afforded oxazoline **2.7** in disappointing yield (38%). Substitution of TEA by DIPEA or MsCl by *p*-TsCl resulted in even lower yields.

Table 6.1: Overview of mesylation conditions (1 eq. MsCl)

Base (eq.)	Solvent	4-DMAP	Time	Temperature
Pyridine	Pyridine	Cat.	24 h	-20 °C→ rt
DIPEA (1)	CH_2Cl_2	Cat.	4 h[a]	0 °C
DIPEA (1)	CH_2Cl_2	-	8 h[a]	0 °C
4-DMAP (1)	Pyridine	Used as base	2 h[a]	0 °C

[a:] Reaction time required for complete consumption of starting material as judged by TLC.

In view of the potentially low yields involved in conversion of the primary alcohol of **2.7** to a tertiary amine (20 – 24% for the aliphatic counterparts),[7] we did not consider further elaboration to the desired PDMP analogues since it had become clear that our envisioned synthetic approach would not provide a sufficiently reliable means for further structural modifications. Nonetheless, these unsatisfactory results clearly indicated that a double-protective strategy, in consideration of the reactivity of 1,2-aminoalcohols towards intramolecular cyclisation, might provide a more versatile approach.

7 DOUBLE-PROTECTIVE STRATEGY FOR THE SYNTHESIS OF HYBRID PDMP ANALOGUES

7.1. REGIOSELECTIVE AZIRIDINE OPENING AS KEY REACTION IN THE CONSTRUCTION OF THE PDMP BACKBONE

7.1.1 INTRODUCTION - RETROSYNTHESIS

The search for alternative methods for efficient construction of the hybrid PDMP core structure drew our attention to recent report by *Shin et al.*[9] The authors devised an efficient and short synthesis of D-*threo*-PDMP based on regioselective aziridine ring opening with various amine nucleophiles.

By analogy, we wanted to explore the application of this new methodology in the quest for our envisioned hybrid D-*threo*-PDMP analogues. On one hand, the aziridine could serve as a protecting group during introduction of the acetylene side chain, while on the other hand, it provides the functional requirements for introduction of cylic amines.

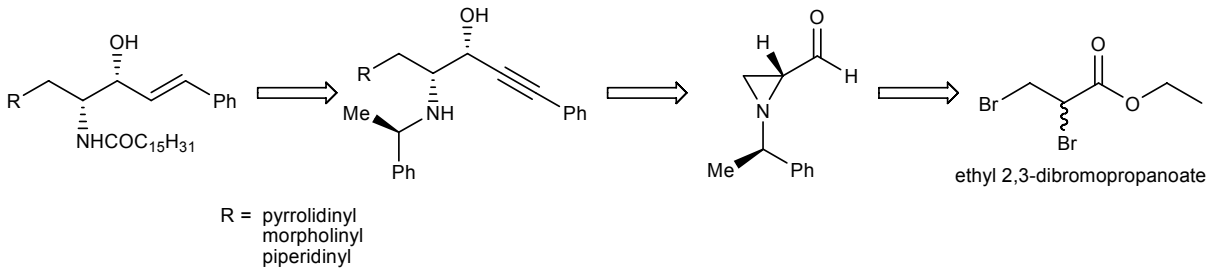


Figure 7.1: Retrosynthesis.

Thus, starting from ethyl 2,3-dibromopropanoate (Figure 7.1), the intermediate configurationally stable aldehyde could be prepared in 3 steps providing the required stereochemistry at C2 of the sphingoid backbone by use of a chiral anchor which concomitantly serves as *N*-protecting group. Introduction of the acetylene side chain, followed by regioselective aziridine ring opening in the presence of TMSI would provide the intermediate ring-opened product. The proposed mechanism for aziridine ring-opening is depicted in Figure 7.2.[9] Thus, upon treatment with TMSI, the aziridine ring opens up regioselectively providing an intermediate alkyl iodide, which

subsequently reacts with amine nucleophiles. Aqueous work-up cleaves the TMS-group, thereby providing the α-methylbenzyl protected secondary amine.

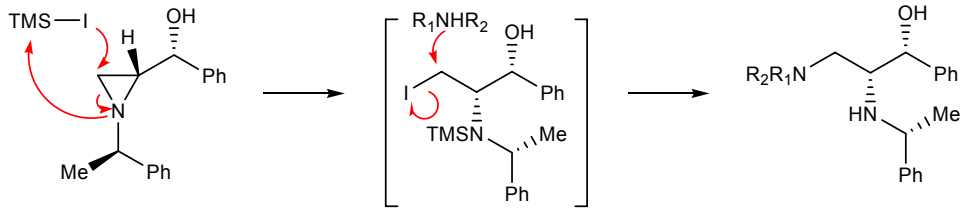


Figure 7.2: Proposed mechanism for regioselective aziridine ring opening with TMSI.

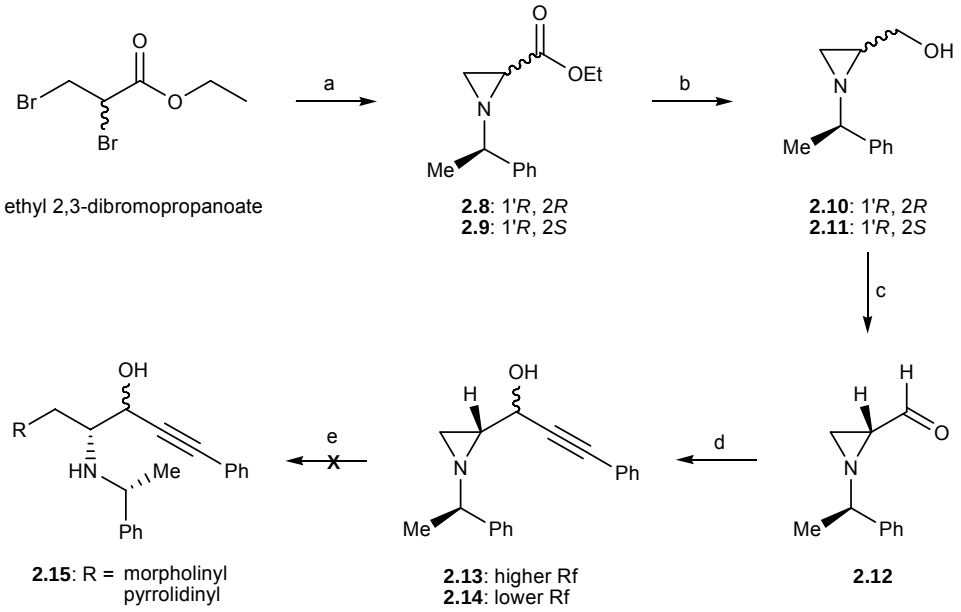
Reduction of the alkyne followed by deprotection of the α-methylbenzyl group and final acylation would furnish the desired PDMP analogues. Since neither the double bond in styrene nor the alkyne intermediate would withstand catalytic hydrogenation conditions involved in removal of the α-methylbenzyl protecting group, an alternative deprotection strategy should be anticipated. A literature survey revealed that heating in formic acid (50 - 60 °C) had been successfully applied for the deprotection of a *N*-α-methylbenzyl moiety thereby leaving *E*-double bonds unaffected.[10]

7.1.2 SYNTHESIS.

Treatment of ethyl 2,3-dibromopropanoate with *R*-(+)-(1-phenylethyl)amine in the presence of TEA[11] produced a mixture (1:1) of epimers **2.8** and **2.9**, which could easily be separated by flash chromatography (Table 7.1). Stereochemistry could not be assigned at this stage since optical rotations for these compounds have not been published. However, reduction of the esters with $LiAlH_4$ furnished primary alcohols **2.10** and **2.11** with known optical rotations[12] (Table 7.1).

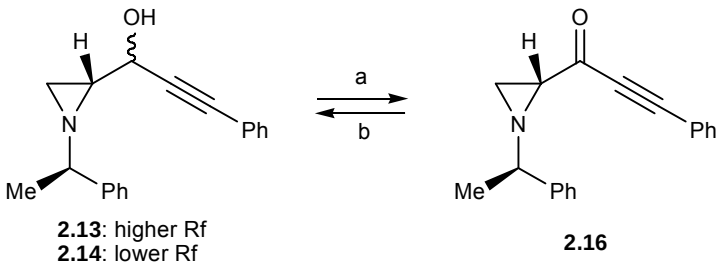
Table 7.1: Overview of optical rotations of **2.8**-**2.11** (measured in $CHCl_3$).

Compound	Measured $[\alpha]^D_{20}$	Literature[12] $[\alpha]^D_{20}$	Stereochemistry
2.8	+63.3 (c 0.85)	---	1'*R*,2*R*
2.9	+42.5 (c 0.79)	---	1'*R*,2*S*
2.10	+74.5 (c 1.0)	+71.8 (c 1.0)	1'*R*,2*R*
2.11	+53.7 (c 1.0)	+53.3 (c 10.1)	1'*R*,2*S*



Scheme 7.1: a) *R*-(+)-α-methylbenzylamine, TEA, 0°C to reflux, 3 h (**2.8**: 19%; **2.9**: 17%; **2.8** + **2.9**: 60 %); b) $LiAlH_4$, THF, 0°C to RT, overnight (**2.10**: 97%; **2.11**: 95%); c) oxalylchloride, DMSO, TEA, CH_2Cl_2, -78°C to 0°C, 2.5 h (72%); d) lithium phenylacetylide, LiCl, THF, -78°C to RT, overnight (**2.13**: 57%, **2.14**: 10%, **2.13** + **2.14**: 17%); e) see text.

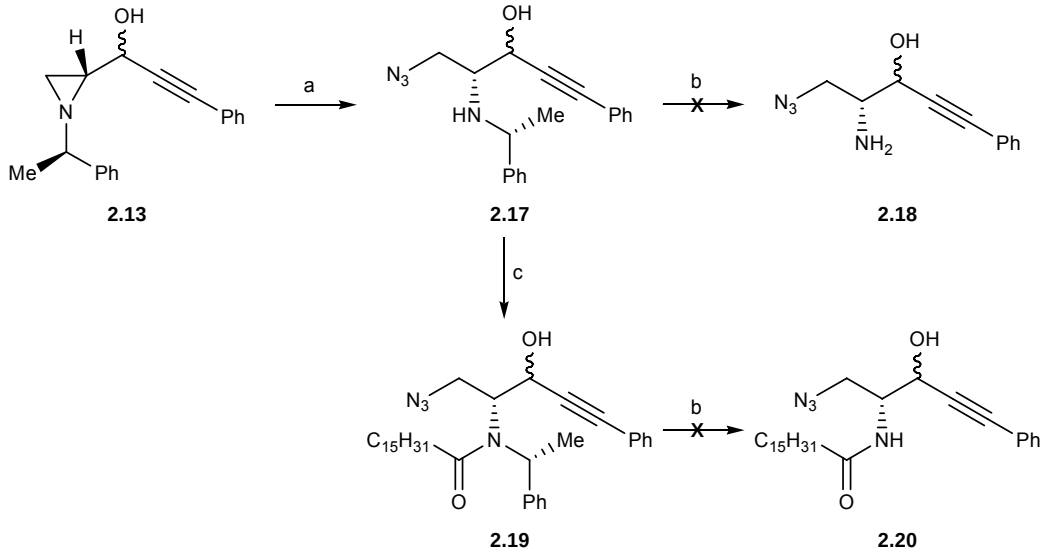
Swern oxidation of **2.10** provided aldehyde **2.12**, which could be purified by column chromatography without loss of optical purity.[13] Treatment of the aldehyde with lithium phenylacetylide in the presence of excess LiCl produced a mixture of diastereomers (6:1; **2.13**: faster eluting; **2.14**: slower eluting). Addition of LiCl to the reaction medium previously had been shown to enhance stereoselectivity in favour of the *threo*-epimer,[13] therefore, we expected that the faster eluting diastereomer possessed the *threo*-configuration as judged by TLC analysis. This presumption was confirmed by oxidation of a mixture of **2.13** and **2.14** to ketone **2.16** and subsequent reduction with L-Selectride, a reducing agent that is known to favour *threo*-selectivity (Scheme 7.2).[14]



Scheme 7.2: a) Dess-Martin periodinane, CH_2Cl_2, 0°C to RT, 4 h (82%); b) L-Selectride, LiBr, THF, -78°C, 1 h (**2.13**: 52%, **2.14**: 9%, **2.13** + **2.14**: 15%).

However, the absolute stereochemistry could not be assigned at this point since comparison of NMR data with similar reported compounds was non-conclusive.[15] Nonetheless, oxazolidinone ring formation between the proparagylic alcohol and the secondary amine of the aziridine ring-opening products in a later stage would allow unambiguous assignment of the absolute stereochemistry by comparison of NMR data for both epimers.

Unfortunately, regioselective ring-opening of the aziridine failed in our hands with various secondary amines under different conditions. Reaction of **2.13** with 3 equivalents of TMSI followed by addition of a secondary amine[9] (pyrrolidine, morpholine) didn't furnish the desired tertiary amines (**2.15**), even after prolonged reaction times (72 h). Instead, a complex reaction mixture was obtained as observed by TLC. *In situ* generation of TMSI by reaction of TMSCl with NaI did not affect the outcome of the reaction. When **2.10** was subjected to these reaction conditions, a similar reaction pattern was observed.



2.13 **2.17** **2.18** **2.19** **2.20**

Scheme 7.3: a) $TMSN_3$, CH_2Cl_2, RT, 16 h (100%); b) see Table 7.2; c) palmitoyl chloride, DIPEA, CH_2Cl_2, 0°C to RT, overnight (78%).

On the other hand, reaction of **2.13** with $TMSN_3$ under analoguous reaction conditions yielded azido derivative **2.17** in quantitative yield (Scheme 7.3). Since amines are readily accessible from azides by reduction under Staudinger conditions, leaving the styrylmoiety unaffected, azide **2.17** could serve as a valuable building block for further elaboration to selected cyclic tertiary amines. However, treatment of

2.17 with formic acid at elevated temperatures failed to deprotect the α-methylbenzyl group but produced a complex reaction mixture instead. Several other debenzylation methods also failed to produce primary amine **2.18** (Table 7.2). Since we expected that debenzylation of acylated amines might proceed more smoothly, **2.17** was acylated with palmitoyl chloride affording protected amide **2.19**. Analogous to our previous results, debenzylation failed under a variety of reaction conditions (Table 7.2). These disappointing results forced us to explore alternative synthetic strategies to access the desired hybrid PDMP analogues.

Table 7.2: Debenzylation of **2.17** and **2.19**.

Reagent	Temperature	Time	Result
HCOOH[10]	60°C – 80 °C	16 h – 96 h	No reaction - decomposition
Li naphtalenide[16]	-78°C to 0°C	3 h – 9 h	Decomposition
Li/NH_3[17]	-78°C to rt	6 h – 18 h	Decomposition
AcBr[18]	0°C to rt	2 h – 6 h	Decomposition

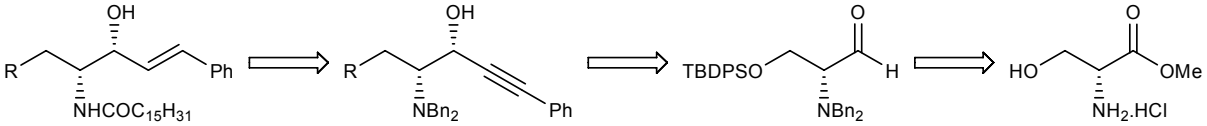
7.2. N,N-DIBENZYL PROTECTING GROUP

7.2.1 INTRODUCTION – RETROSYNTHESIS

N,N-dibenzyl protected α-amino aldehydes have shown to be valuable organic building blocks for the synthesis of pharmaceutically and biologically interesting substances,[19] not only since they exhibit remarkable configurational stability, but also because they allow excellent control of diastereoselectivity in addition reactions.[20] However, special care should be paid to deprotection since classical hydrogenation conditions affect both double and triple bonds and are therefore not appropriate for our specific needs. Furthermore, in contrast to the removal the α-methylbenzyl group, a myriad of alternative benzyl deprotection methods with enhanced functional group tolerance have been described.[21]

Thus, addition of lithium phenylacetylide to the modified Reetz's aldehyde,[20] accessible in three steps from commercially available D-serine methyl ester hydrochloride according to a literature procedure, should predominantly provide the *threo*-diastereomer under chelating conditions. Deprotection of the TBDPS group,

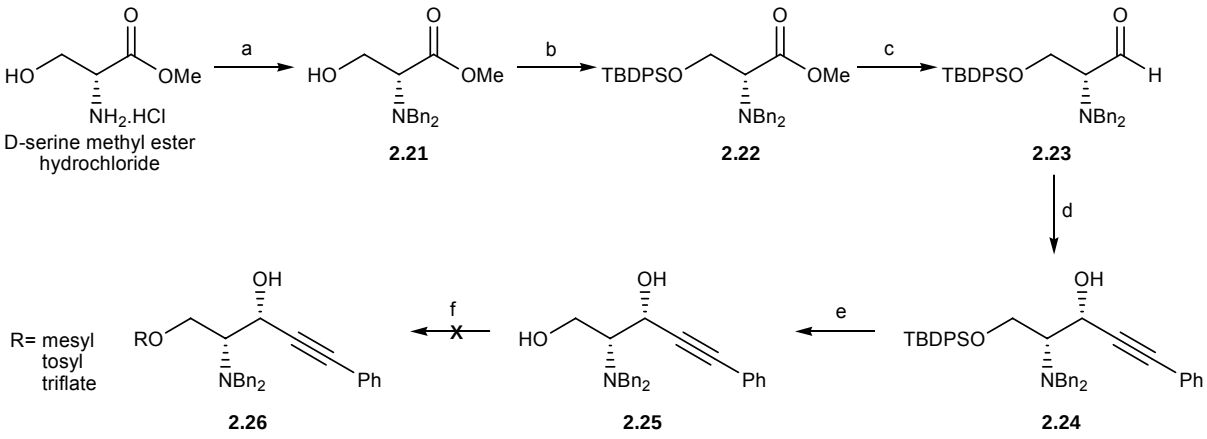
followed by conversion of the primary alcohol to a leaving group and subsequent substitution with a cyclic secondary amine should provide the intermediate 1,2-aminoalcohol. Reduction of the alkyne followed by *N*,*N*-dibenzyl deprotection and final acylation of the primary amine should give access to the desired PDMP analogues.



Scheme 7.4: Retrosynthetic scheme for the synthesis of hybrid PDMP analogues starting from D-serine methyl ester hydrochloride.

7.2.2 SYNTHESIS

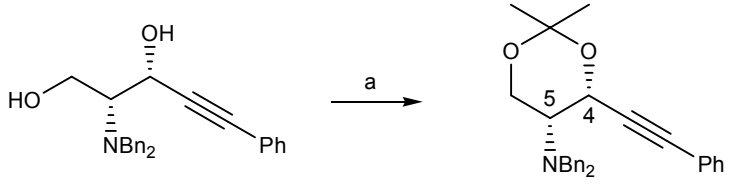
Chemoselective dibenzylation of *D*-serine methyl ester with benzylbromide gave the desired amino ester **2.21**,[22] which was quantitatively converted to TBDPS ether **2.22** (Scheme 7.5). Treatment of ester **2.22** with DiBAlH produced the corresponding aldehyde **2.23** which was used without further purification since it had been reported that silica gel can catalyze the decomposition of *N,N*-dibenzylamino aldehydes.[23]



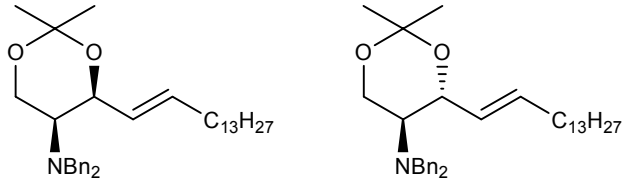
Scheme 7.5: a) benzyl bromide, K_2CO_3, MeCN, RT, 24 h (87%); b) TBDPSCl, imidazole, DMF, 0°C to RT, 5h, (100%); c) DiBAlH, toluene, -78°C, 3 h; d) lithium phenylacetylide, -78°C to RT, overnight (64%); e) TBAF, THF, RT, 30' (84%); f) see Table 7.3.

Addition of lithium phenylacetylide to aldehyde **2.23** in the presence of $ZnBr_2$ gave access to *threo*-1,2-aminoalcohol **2.24** (*threo:erythro* 95:5) in excellent

diastereoselectivity. Deprotection of the TBDPS group with TBAF afforded 1,3-diol **2.25**. *Threo*-stereochemistry was confirmed by conversion of **2.25** to its dioxolane derivative **2.27** and comparison of ^{1}H NMR data with similar reported compounds[20b] (Scheme 7.6).



2.25 **2.27**: $^{3}J_{4,5}$ = 4.11 Hz



threo: $^{3}J_{4,5}$ = 4.1 Hz *erytrho*: $^{3}J_{4,5}$ = 9.8 Hz

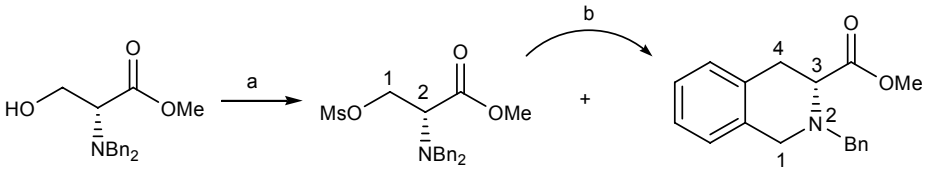
Scheme 7.6: Synthesis of dioxolane **2.27** and comparison of ^{1}H NMR data with previously reported compounds.[20b] a) 2,2-dimethoxypropane/DMF (1:1), PPTS, RT, 2 h (85%).

Surprisingly, all attempts to transform the primary alcohol of **2.25** into a leaving group failed and resulted in complex reaction mixtures (Table 7.3).

Table 7.3: Overview of reaction conditions that failed to transform **2.25** to **2.26**.

Solvent/base	Reagents (eq.)	Temp.	Time
pyridine/pyridine	*p*-TsCl (1.05 + 0.4), 4-DMAP (cat.)	0 °C to rt	33 h
pyridine/pyridine	MsCl (1.1)	0°C to 50 °C	39 h
CH_2Cl_2 /TEA	MsCl (1.1)	0°C to rt	34 h
DMF/DIPEA	MsCl (1.1)	0 °C to rt	34 h
CH_2Cl_2/pyridine (1:1)	Tf_2O (1.05)	-20 °C	2 h

In order to investigate the underlying reason for the unsuccessful transformation of **2.25**, we explored the reaction outcome when a more simplified model compound was subjected to analogous reaction conditions. Thus, when **2.21** was treated with MsCl in CH_2Cl_2, TLC analysis indicated formation of two distinct new products. Apart from mesylate **2.26**, a compound with higher Rf could be isolated (**2.26**:**2.28** 4:1). Continued stirring at room temperature for 15 h resulted in a 1:1 ratio of both compounds.



2.21 **2.26** **2.28**

Scheme 7.7: a) MsCl, DIPEA, 4-DMAP, CH_2Cl_2, 0°C to RT, 15 h (**2.26**:**2.28** 1:1); b) morpholine, DMF, 50°C, 1 h (100%).

The structure of **2.28** was elucidated from the ^{1}H NMR and NOESY data. The ^{1}H NMR spectrum indicated the presence of only 9 aromatic protons and disappearance of the primary alcohol, although the typical mesyl $-CH_3$ was lacking, and the C(4) protons showed a remarkable upfield shift compared to **2.26** (Table 7.4). Moreover, NOESY revealed an unexpected contact between C(4)H_a and the aromatic region. This spatial interaction did not manifest itself in the NOESY spectrum of **2.26**. Treatment of mesylate **2.26** with morpholine in DMF at elevated temperature, resulted in a quick and clean conversion to **2.28** (100%). A literature survey indicated that an identical reaction outcome has been reported when **2.21** was subjected to tosylation conditions[24]. This unexpected electrophilic cyclisation might account for the complex reaction mixtures observed in the attempted transformation of the primary alcohol of **2.25** to a leaving group.

Table 7.4: Comparison of NMR data between **2.26** and **2.28**.

	2.26			**2.28**	
	δ	*J* (Hz)		δ	*J* (Hz)
-C(1)H_a	4.42	7.03, 10.55	-C(4)H_a	2.90	5.57, 13.19
-C(1)H_b	4.51	6.45, 10.56	-C(4)H_b	3.20	9.38, 13.19
-benzyl CH_2 (A)	3.59	13.78	-C(1)H_2	3.57	13.48
-benzyl CH_2 (B)	3.81	13.78	-benzyl CH_2	3.68	13.48
-C(2)*H*	3.60-3.66	m	-C(3)*H*	4.18	5.86, 9.38

Since all alternative procedures failed to access the envisioned hybrid PDMP analogues, we decided to revisit our original synthetic strategy (Section 5, p.61) and introduce double protection by fixing the *tert*-Boc protected amine and the secondary alcohol in an oxazolidine ring.

7.3. *N-TERT-BOC OXAZOLIDINE FORMATION AS DOUBLE-PROTECTIVE STRATEGY*

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PDMP ANALOGUES

Ulrik Hillaert, Swetlana Boldin-Adamsky, Jef Rozenski, Roger Busson, Anthony H. Futerman, Serge Van Calenbergh

Submitted to Chembiochem

Abstract

Sphingolipids (SL) have gained much attention over the last decade since it became clear that they not only act as structural elements of cell membranes, but also are involved in a myriad of biological processes. Since the discovery of D-threo-1-phenyl-2-aminodecanoyl-3-morpholinopropanol (D-threo-PDMP or PDMP), an inhibitor of glucosylceramide synthase (GlcCer synthase), a number of more potent analogues have been developed. A new series of hybrid PDMP analogues, based both on PDMP and styryl analogues of natural ceramide, has been synthesised from D-serine. The synthetic route was developed such that future introduction of different aryl groups is straightforward. Biological evaluation, both in vitro on rat liver Golgi fractions as well as in vivo in HEK-293 and COS-7 cells, revealed two lead compounds with comparable inhibitory potency as PDMP, which could be elaborated to more potent inhibitors.

Introduction

A plethora of biological effects has been assigned to sphingolipids (SLs) over the last two decades. Whereas SLs initially were regarded as inert structural components of cell membranes, it has now become clear that they play an important role in the regulation of a myriad of cellular processes including cellular differentiation, growth, adhesion, senescence, apoptosis and signal transduction.[1] It is obvious that disruption of this fragile cellular equilibrium, for example by impaired lysosomal degradation of SLs, could have severe pathophysiological consequences. Lysosomal storage diseases, such as Gaucher and Tay-Sachs diseases, are caused by the defective catabolism of glycosphingolipids (GSLs), resulting in substrate accumulation.[2]

Since glucosylceramide (GlcCer; Figure 1) acts as a hub for the synthesis of more complex GSLs, it has been suggested[3] that partial reduction of the

biosynthesis of GlcCer might offer a valuable strategy for treatment of storage diseases such as Gaucher disease and other sphingolipid storage diseases.

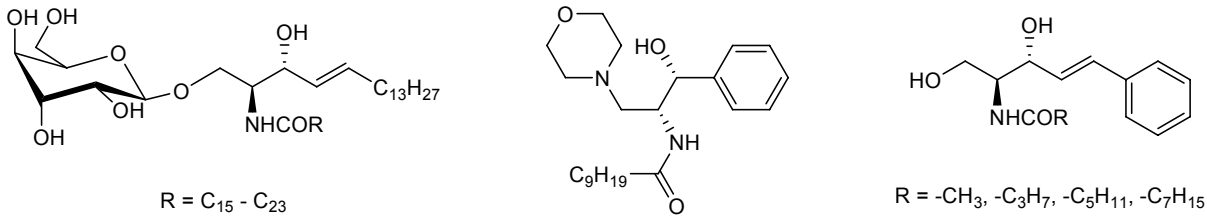


Figure 1. Structures of GlcCer, D-*threo*-PDMP and *E*-styryl-ceramides.

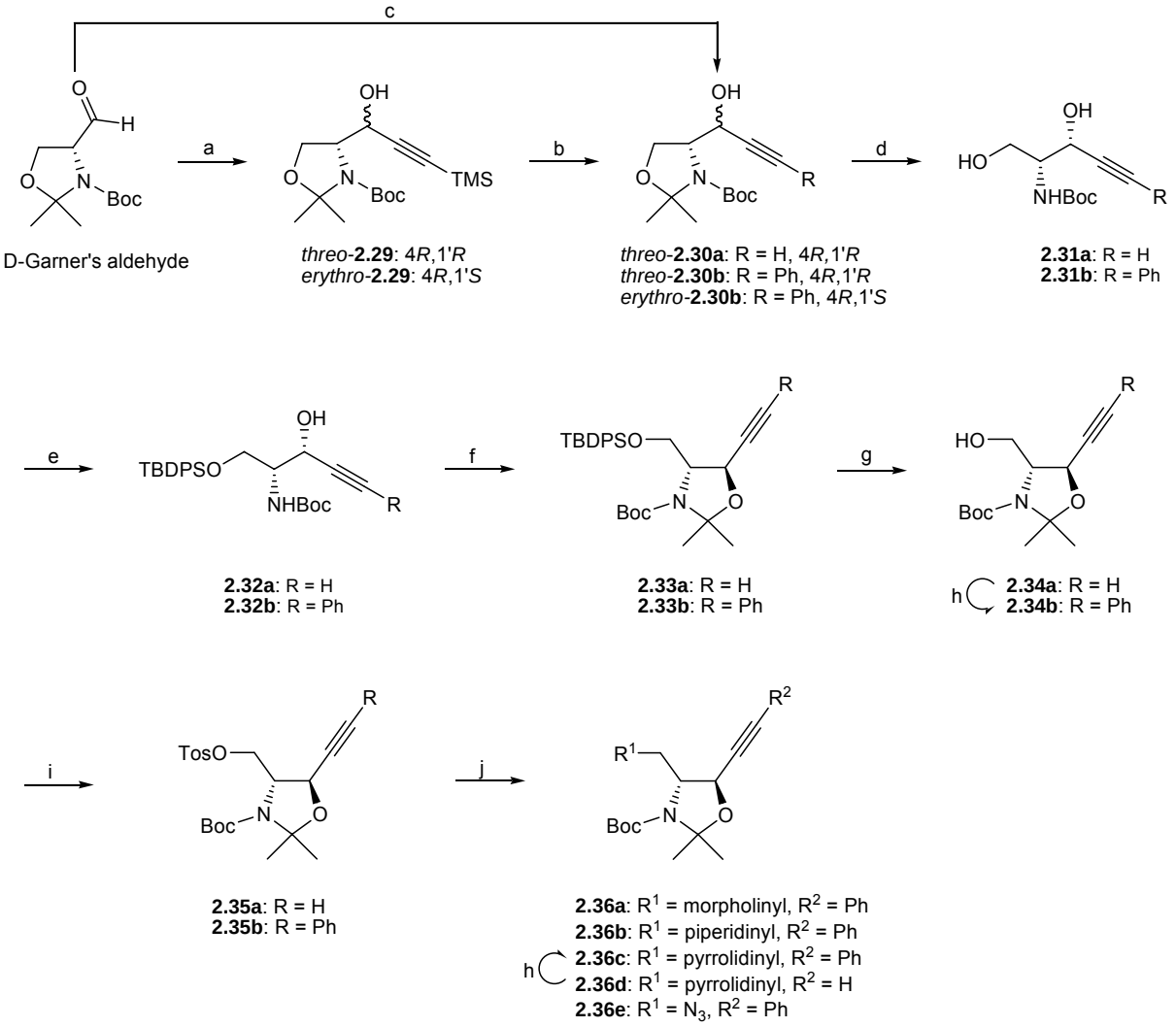
PDMP[4] and related compounds[5] have been developed as potent inhibitors of GlcCer synthase. Surprisingly, only the D-threo isomer specifically inhibits GlcCer synthase.[6] Furthermore, it was shown that elongation of the acyl chain from decanoyl to palmitoyl[5a] and introduction of electron rich aromatic substituents[5b] drastically enhanced the inhibitory capacity. Moreover, a pyrrolidino head group was proposed to be the best mimic of the sugar transition state.

In our approach we aimed at the synthesis of hybrid structures based on *E*-styryl analogues of natural ceramide. It was indeed previously shown that such analogues are recognised by GlcCer synthase[7] and subsequently metabolised to the glucosylated form. Our primary concern was whether these hybrid analogues would still exhibit biological activity.

Our strategy focused mainly on the elaboration of a synthetic route towards a single advanced intermediate for introduction of the aryl moiety by Sonogashira coupling between a terminal alkyne and an appropriate aryl iodide, thereby avoiding reworking of the entire synthetic scheme for each compound. Styryl analogue **2.41a** (Scheme 2) was first synthesised to test our hypothesis. In addition, the intermediates of this synthetic route served as a back-up to monitor Sonogashira coupling in the terminal alkyne route. Throughout the synthetic scheme, we gained access to a number of structural analogues which could provide more insight into the structure-activity relationship of this class of compounds.

Synthesis

The known Garner aldehyde (derived in 5 steps from D-serine[8]) served as a chiral building block since it allows good control of stereochemistry in nucleophilic additions and it has shown to be configurationally stable.[9] Indeed, addition of the appropriate lithium acetylide to the aldehyde under chelating conditions ($ZnBr_2$) yielded predominantly *threo*-adducts **2.29** and **2.30b** (41% and 44% isolated yields respectively; *threo:ertythro* 9:1; Scheme 1).



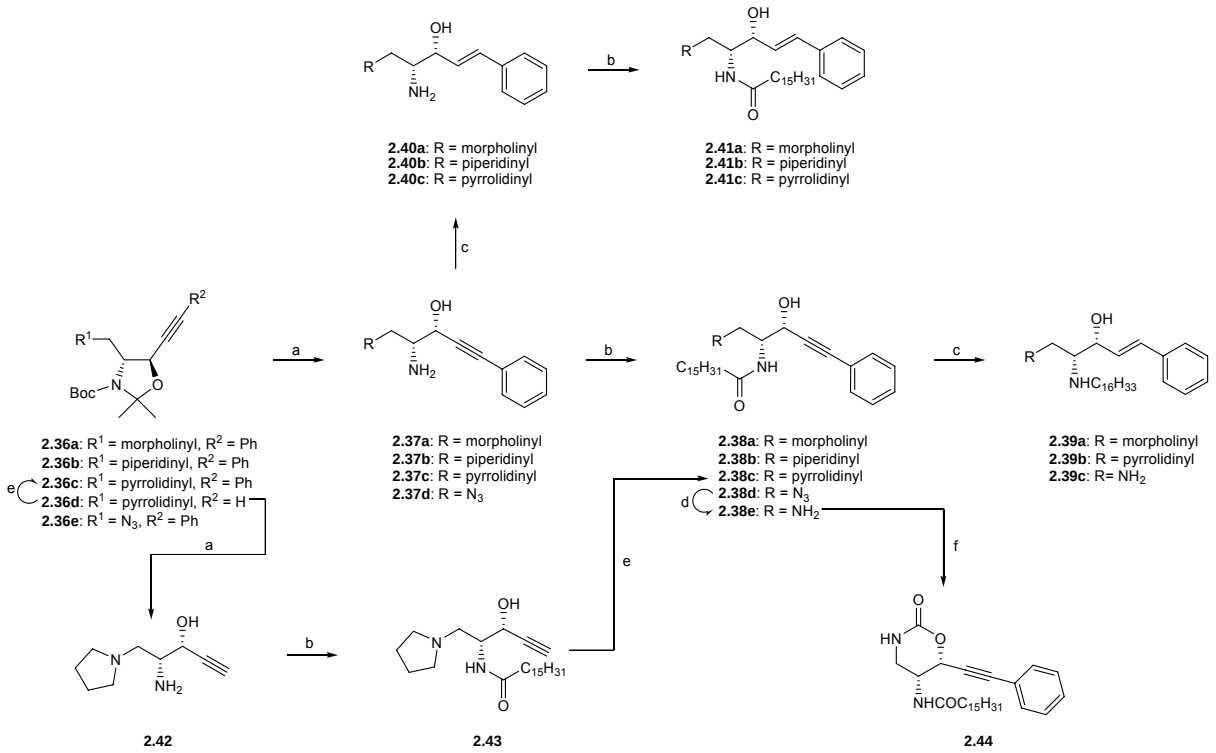
Scheme 1. Conditions: a) lithium trimethylsilylacetylide, $ZnBr_2$, Et_2O, -78°C to RT, overnight; b) TBAF, THF, RT, 1 h; c) lithiumphenylacetylide, ZnBr2, -78°C to RT, overnight; d) 90% acetic acid, 60°C, 5 h or *p*-TsOH, MeOH, RT, 36 h; e) TBDPSCl, imidazole, 4-DMAP, DMF, RT, 16 h; f) *p*-TsOH, Me_2CO, 2,2-dimethoxypropane, reflux, 6 h; g) TBAF, THF, RT, 1 h; h) $PdCl_2(PPh_3)_2$, iodobenzene, piperidine, 70°C, overnight; i) *p*-TsCl, 4-DMAP, pyridine, 0°C to RT, 35 h; j) See experimental part.

Removal of the TMS group with TBAF from *threo*-**2.29** produced terminal alkyne *threo*-**2.30a** (93%). Subsequent opening of the oxazolidine ring with 90% acetic acid or *p*-TsOH in MeOH followed by selective silylation of the primary alcohol gave the protected sphingosine analogues **2.32a** (83% from *threo*-**2.30a**) and **2.32b** (59% from *threo*-**2.30b**). Since it had become clear from preliminary experiments that a double amino-protective strategy would be crucial for successful elaboration to the desired compounds, acid mediated oxazolidine ring formation followed by desilylation allowed access to alcohols **2.34a** (94% from **2.32a**) and **2.34b** (76% from **2.32b**). Sonogashira coupling of terminal alkyne **2.34a** with iodobenzene rendered alcohol **2.34b** in excellent yield (98%).

Although we succeeded in converging both synthetic pathways at this point, it would still be more convenient to introduce the aromatic ring at a later stage to avoid the separate introduction of amine substituents for each individual aryl analogue. Therefore, primary alcohols **2.34a** and **2.34b** were protected as the respective tosylates **2.35a** (94%) and **2.35b** (64%). The lower yield of **2.35b** might be ascribed to decomposition during work-up. Indeed, the reaction mixture became more complex when solvent removal was carried out at 40-50°C. In contrast, when the temperature was strictly kept below 35°C, few side products were observed. The nucleophilic nature of the *tert*-Boc group,[10] susceptible to formation of a bicyclic oxazolidine-oxazolidinone,[11] might be responsible for this phenomenon. Treatment of **2.35a** and **2.35b** with the appropriate nucleophile in DMF at elevated temperatures gave access to key intermediates **2.36a-e** (62-99%). Sonogashira coupling of **2.36d** with iodobenzene proceeded smoothly to produce **2.36c** (93%).

Acid mediated deprotection of **2.36a-e** followed by acylation gave alkyne analogues **2.38a-d** (48-89%) and **2.43** (60%; Scheme 2). Unfortunately, Sonogashira coupling of **2.43** with iodobenzene showed to be a bridge too far since it failed to give alkyne **2.38c** in satisfactory yields (32%). Therefore, key intermediate **2.36d** should be regarded as a solid base for future introduction of aryl substituents. Reduction of **2.38d** under Staudinger conditions gave amine **2.38e** in excellent yield (97%). Since Birch reduction of **2.38a** and **2.38b** produced complex mixtures, we opted to reduce the alkyne with Red-Al®, although we were aware that controversial results[12] had been obtained in the presence of amides. Unfortunately, upon treatment of amides **2.38a**, **2.38b** and **2.38e** with Red-Al® at -78°C, the corresponding *E*-styryl ceramines **2.39a** (60%) **2.39b** (quant.) and **2.39c** (45%) were isolated as the sole reaction

products. Nonetheless, comparison of the biological activities of these amines with the amide counterparts could provide more insight into the role of the amide function in binding to GlcCer synthase since no D-*threo* ceramines have been evaluated to date as potential inhibitors.

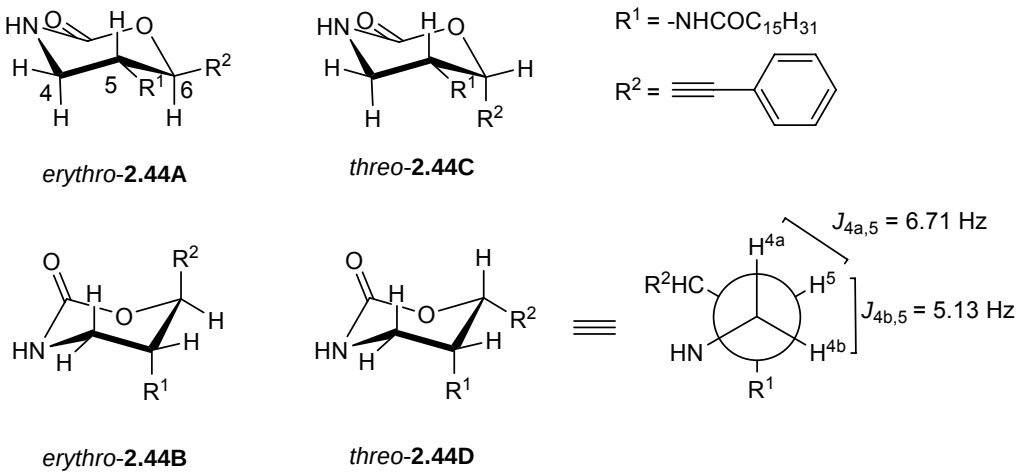


Scheme 2. Conditions: a) 3N HCl, MeOH, 50°C, 12 h; b) *p*-nitrophenylpalmitate, HOBT, pyridine, 50°C, 48 h; c) Red-Al, Et_2O, -78°C to RT, overnight; d) PPh_3, THF, RT for 30' then H_2O, RT, 48 h; e) $PdCl_2(PPh_3)_2$, iodobenzene, piperidine, 70°C, overnight; f) triphosgene, TEA, CH_2Cl_2, 0°C to RT, 1h**.**

In order to circumvent the reduction of the amide group, amines **2.37a-c** were first treated with Red-Al® at -78°C followed by acylation with *p*-nitrophenylpalmitoate to give access to PDMP analogues **2.41a-c** (84, 78 and 71% respectively).

Stereochemical assignment of the *threo* configuration was achieved by treatment of amine **2.38e** with triphosgene yielding oxazolidinone **2.44** (88%). Based on the small coupling constant $^3J_{5,6}$ = 3.42 Hz, an axial-axial orientation can be excluded (Scheme 3, conformer *erythro*-**2.44A**). The remaining question was whether C(5)*H* was in axial or equatorial position. The values of the coupling constants $^3J_{4a,5}$ = 6.71 Hz and $^3J_{4b,5}$ = 5.13 Hz indicate a pseudo-axial-axial orientation. Selective irradiation of C(6)*H* (δ = 5.40 ppm) and NOEDIF observation showed an increase of C(5)*H* (5.0%) and a weaker, but still significant increase of

C(4)H_b (2%). Only conformer *threo*-**2.44D** would give a NOE contact between C(6)*H* and C(4)H_b indicating a *cis*-relationship of the substituents on C(5) and C(6). Performing these experiments at higher temperature (60°C) did not affect the coupling constant, demonstrating the presence of a single conformer.



erythro-**2.44A** *threo*-**2.44C**

erythro-**2.44B** *threo*-**2.44D**

Scheme 3. Possible conformers of *erythro/threo*-**2.44**.

Biological evaluation

In a preliminary, exploratory in vitro assay, using a short acyl chain analogue of ceramide, *N*-[6-[(7-nitrobenzo-2-oxa-1,3-diazol-4-yl)amino]hexanoyl]D-*erythro*-sphingosine (C6-NBD-D-*erythro*-ceramide), as substrate for GlcCer synthase (Figure 2), compounds **2.38a,b,d,e**, **2.39a-c**, **2.41a-c, 2.43** and PDMP were evaluated as potential inhibitors of GlcCer synthase in rat liver Golgi membrane homogenates. Moreover, specificity of inhibition towards GlcCer synthase was assessed by monitoring sphingomyelin (SM) synthesis from C6-NBD-D-*erythro*-ceramide.[13] Indeed, toxicity of PDMP analogues has been associated with increased intracellular ceramide (Cer) levels.[5b] Inhibition of sphingomyelin synthase (SM synthase),[14] as well as different mechanisms, have been proposed[5a,5b] to cause this phenomena.

Interestingly, almost all compounds showed some inhibition of GlcCer synthesis (Figure 2A). Comparison of the inhibitory activities of the morpholino series **2.38a**, **2.39a** and **2.41a** clearly shows that the presence of an amide carbonyl increases inhibitory activity. Moreover, alkyne **2.38a** and *E*-alkene **2.41a** seem to be equally potent. In contrast, piperidine analogues **2.38b** and **2.41b** show a small difference in favour of the *E*-alkene analogue.

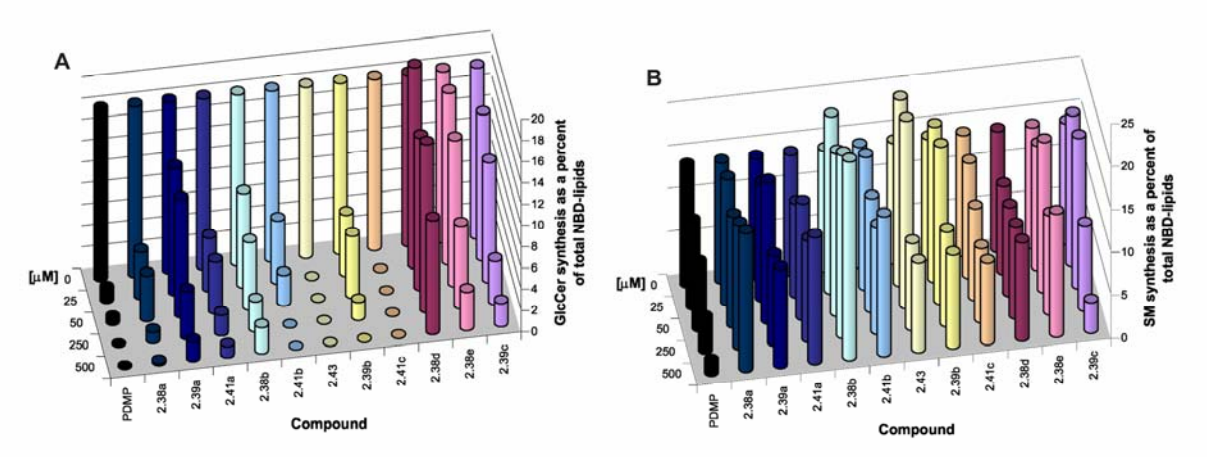


Figure 2. Effects of inhibitors on GlcCer (**A**) and SM (**B**) synthesis assayed in vitro in Golgi fractions. The reactions were carried out in the presence or absence of varying concentrations of inhibitors.

Surprisingly, analogue **2.43** revealed that the presence of the aromatic ring was not a prerequisite for inhibitory activity. Furthermore, terminal alkyne **2.43** demonstrated comparable potency with respect to its aromatic *E*-alkene counterpart **2.41c**. Data for ceramine **2.39b** confirmed the necessity of the amide for distinct inhibitory activity although this analogue still reduced GlcCer synthesis by 65% at 25 μM. By switching to non-cyclic nitrogen substituents as in compounds **2.38d**, **2.38e** and **2.39c**, the inhibitory activity drastically dropped, thereby clearly indicating the requirement of cyclic amines as sugar transition state mimics, as previously assumed.[5a] In agreement with published results for PDMP analogues,[5a] data for **2.41a**, **2.41b** and **2.41c** demonstrated that a pyrrolidine substituent on C1 is undoubtedly favourable over a morpholino or piperidino head group.

Most analogues showed a concentration-dependent decrease in SM synthesis similar to PDMP, as depicted in Figure 2B. However, treatment with 25 or 50 μM concentrations did not significantly affect SM synthesis, except for **2.38d**, which induced a substantial decrease in SM synthesis. When higher molar concentrations were applied, a significant decrease in SM synthesis was noticed, except for **2.38b,** which showed an increase in SM at all inhibitor concentrations.

An interesting feature was noticed in the assays of compounds **2.38e** and **2.39c**. Apart from the expected SLs, a new "upper band" was observed on TLC with an Rf which was slightly larger than the Rf of C6-NBD-ceramide. It is difficult to speculate on the nature of this metabolite, but based on its apolar behaviour, one could assume that acylation of C1-OH by 1-*O*-acyl transferase[15] might have

occurred, thereby yielding a compound with larger Rf than C6-NBD-ceramide. Further investigation will be necessary to reveal this metabolite's identity.

In a subsequent set of assays, the biological profile of the most potent inhibitors, **2.41c** and **2.43**, was examined in detail both in vitro on rat liver Golgi fractions and in vivo using HEK-293 cells. Both compounds were equally as potent as PDMP in inhibiting GlcCer synthase in Golgi fractions, as depicted in Figure 3A. The calculated IC_{50} values from these experiments for PDMP, **2.41c** and **2.43** are 5.33, 5.44 and 4.23 μM respectively. The value for PDMP is in good agreement with published data (5 μM).[6] Within the concentration range of this assay, neither analogues affected SM synthesis (Figure 3B).

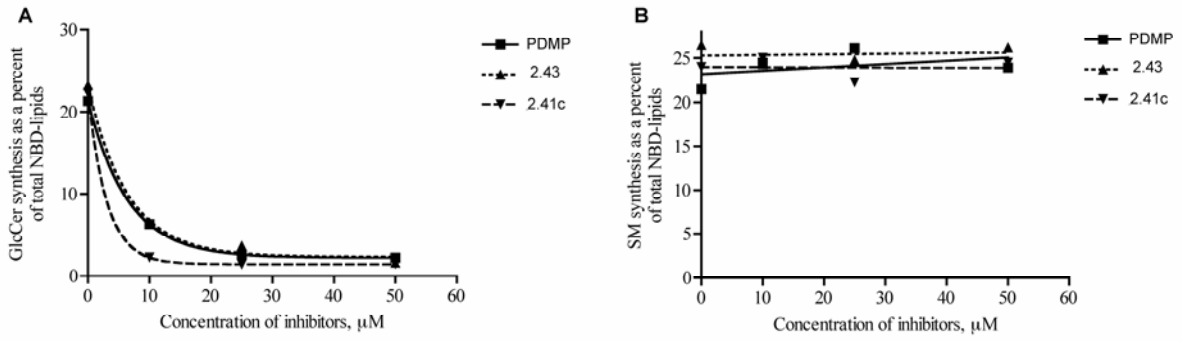


Figure 3. Effects of analogues **2.41c** and **2.43** on GlcCer (**A**) and SM (**B**) synthesis measured in vitro in Golgi fractions. The reactions were carried out in the presence and absence of various inhibitor concentrations.

In vivo inhibition was assessed by incubating HEK-293 cells for 3 h in the presence of 10, 25 and 50 μM of analogues **2.41c** and **2.43,** together with C6-NBD-ceramide (Figure 4A). Again, both compounds inhibited GlcCer synthesis to the same extent as PDMP. Data concerning SM synthesis in HEK-293 cells correlate well with findings in vitro (Figure 4B). Even at 50 μM no effect on SM synthesis was observed.

We next examined de novo synthesis of other SLs, using ^{3}H-serine as a precursor, in HEK-293 (Figure 5) and COS-7 cells (data not shown), following pre-treatment with the inhibitors. GlcCer synthesis was moderately inhibited in both cell lines. While values for **2.41c** (62% of control) were comparable to PDMP (52% of control), **2.43** (37% of control) proved to be somewhat more effective.

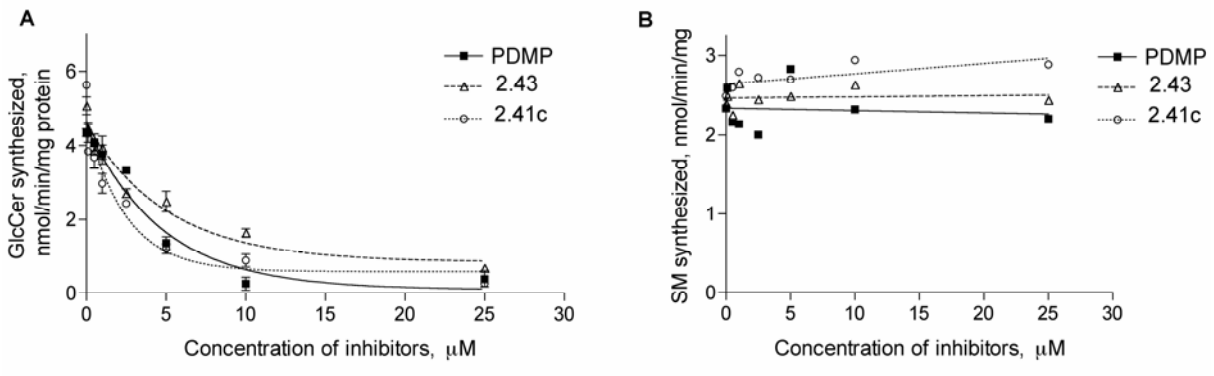


Figure 4. Effects of analogues **2.41c** and **2.43** on GlcCer (**A**) and SM (**B**) synthesis assayed in vivo in HEK-293 cells using C6-NBD-ceramide. The reactions were carried out in the presence and absence of various inhibitor concentrations.

Lactosylceramide (LacCer) levels slightly decreased upon treatment with both PDMP and **2.41c** (48% of control), whereas no effect could be observed upon incubation with **2.43** (118% of control). Cer levels substantially increased (up to two-fold) upon treatment with the inhibitors. In contrast, SM levels were only very slightly affected by both **2.41c** (81% of control) and **2.43** (118% of control) at this concentration. These findings indicate that inhibition of SM synthesis is not responsible for the observed ceramide accumulation in these cell lines. Therefore, other ceramide salvage pathways must be involved. The only way to disclose the true nature of the specific enzyme(s) involved in ceramide accumulation is by rigorously monitoring cellular levels of all known SLs.

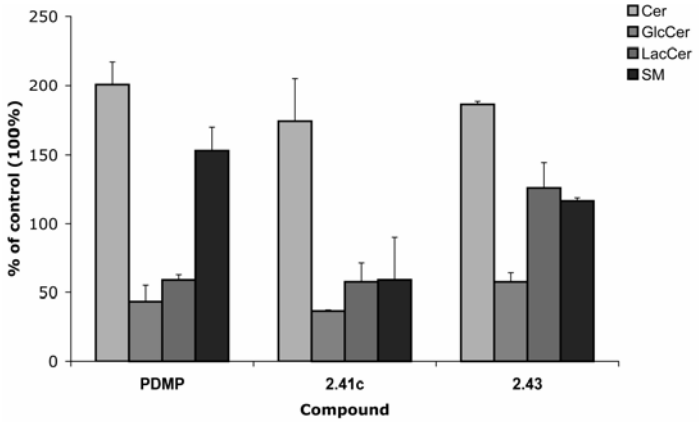


Figure 5. Effects of **2.43** and **2.41c** on de novo sphingolipid synthesis assayed using L-[3-^{3}H]-serine in vivo in HEK-293 cells. Analyses were performed after 3 h incubation following 1 h pre-treatment with 50 μM of the inhibitors.

In conclusion, we have developed a flexible synthetic route towards a new series of hybrid PDMP analogues. A key reaction in our approach was the

Sonogashira coupling between an aryl iodide and a terminal alkyne intermediate. The influence of the synthesized compounds on GlcCer and SM synthesis was evaluated both in vitro and in vivo. An increase in inhibitory activity was observed when a pyrrolidine head group was combined with an amide-linked fatty acid. Interestingly, substitution of the aromatic moiety by a terminal alkyne resulted in an equally potent analogue, compared to its *E*-alkenyl counterpart. This simplified terminal alkyne PDMP analogue provides a solid lead for future introduction of different aryl groups in our search for more potent PDMP analogues.

Experimental

General:

All reactions were carried out under inert (N_2) atmosphere. Precoated Macherey-Nagel (Düren, Germany) silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and/or revealed by sulphuric acid-anisaldehyde spray or phosphomolybdic acid spray. Column chromatography was performed on ICN silica gel (63-200 μM, ICN, Asse Relegem, Belgium). NMR spectra were obtained with a Varian Mercury 300 or 500 spectrometer (Varian, Palo Alto, California, USA). Chemical shifts are given in parts per million (δ) relative to residual solvent peak. All signals assigned to amino and hydroxyl groups were exchangeable with D_2O. Numbering for ^1H assignment is based on the IUPAC name of the compounds. Structural assignment was confirmed with COSY, HMQC and/or NOEDIF if necessary. Splitting of ^{13}C signals was often observed for oxazolidine intermediates due to the presence of rotamers. Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Intermediate alkynes 2.29 and 2.30:

Trimethylsilyl protected alkynes *erythro/threo*-**2.29**:

To a solution of trimethylsilylacetylene (12.53 mL, 88 mmol, 1.66 eq.) in anhydrous Et_2O (450 mL) at -78°C, *n*BuLi (50 mL of 1.6M in hexanes, 80 mmol, 1.51 eq.) was

added dropwise. The mixture was stirred for 1 h at 0°C and 1 h at room temperature and was subsequently cooled to 0°C. After addition of $ZnBr_2$ (23.89 g, 106.1 mmol, 2 eq.), the reaction mixture was stirred at room temperature for 1 h and subsequently cooled to -78°C. D-Garner's aldehyde (12.160 g, 53.07 mmol) was dissolved in anhydrous Et_2O (50 mL), cooled to -78°C and added dropwise to the above solution. The mixture was allowed to reach room temperature overnight and after cooling to 0°C, saturated NH_4Cl (100 mL) was added in one portion. The aqueous layer was extracted with Et_2O (2 x 100 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc 9:1) affording *threo*-**2.29** (7.167 g, 41%) and a mixture of *erythro/threo*-**2.29** (4.736 g, 27%), both as a slightly yellow oil.

Sample data for *threo*-**2.29:**

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.11 (s, 9H, (C*H*$_3$)$_3$Si), 1.35 – 1.52 (m, 15H, 2 x -C*H*$_3$ and *tert*-butyl), 3.75 - 3.85 (m, 1H, -C(4)*H*), 3.95 (td, 1H, *J* = 3.51 and 9.08 Hz, -C(5)*H*$_a$), 4.05 (1H, dd, *J* = 5.28 and 8.21 Hz, -C(5)*H*$_b$), 4.67 (m, 1H, -C(4)C*H*OH), 5.77 (m, 1H, -C(4)CHO*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: -0.30, -0.20, 23.51, 24.77, 25.54, 26.40, 27.87, 60.12, 60.65, 61.56, 63.65, 64.09, 79.24, 79.58, 93.58, 93.94, 106.10, 106.27, 151.88, 151.19.

Exact mass (ESI-MS) calculated for $C_{16}H_{30}NO_4Si$ $[M+H]^+$: 328.1944, found: 328.1943.

Alkyne *threo*-**2.30a**:

TBAF (38.5 mL of a 1M solution in THF, 38.5 mmol, 1.2 eq.) was added to a solution of *threo*-**2.29** (10.5 g, 32.05 mmol) in THF (10 mL). The solution was stirred for 1 h at room temperature and the solvent was subsequently removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc 4:1) yielding *threo*-**2.30a** (7.61 g, 93%) as a white solid.

Sample data for *threo*-**2.30a:**

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.35 – 1.42 (m, 12H, -C*H*$_3$ and *tert*-butyl), 1.50 (s, 3H, -C*H*$_3$), 3.20 (d, 1H, *J* = 4.4 Hz, alkyne *H*), 3.81 (m, 1H, -C(4)*H),* 3.94 (m, 1H, -C(5)*H*$_a$), 4.02 (dd, 1H, *J* = 2.64 and 9.09 Hz, -C(5)*H*$_b$), 4.61 (br s, 1H, -C(4)C*H*OH), 5.71 (m, 1H, -C(4)CHO*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 23.17, 24.49, 25.77, 26.58, 60.18, 60.66, 60.96, 63.39, 63.81, 75.59, 75.77, 79.23, 79.65, 83.50, 93.63, 93.94, 151.31, 152.00.

Exact mass (ESI-MS) calculated for $C_{13}H_{21}NO_4Na$ $[M+Na]^+$: 278.1368, found: 278.1364.

Alkynes *erythro/threo*-**2.30b**:

To a stirred and cooled (0°C) solution of lithium phenylacetylide (42.7 mL of a 1M solution in THF, 42.7 mmol, 2 eq.) in anhydrous Et_2O (200 mL), $ZnBr_2$ (10.11 g, 44.88 mmol, 2.1 eq.) was added and the mixture was stirred for 1 h at 0°C and 1 h at room temperature and was subsequently cooled – 78°C. D-Garner's aldehyde (4.90 g, 21.37 mmol) was dissolved in anhydrous Et_2O (25 mL), the resulting solution cooled to -78°C and added dropwise to the above solution. The mixture was allowed to reach room temperature overnight and after cooling to 0°C, treated with sat. NH_4Cl (50 mL). After separation of the phases, the aqueous layer was extracted with Et_2O (2 x 100 mL) and the combined organic phase was dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography (hexanes:EtOAc 9:1 → 85:15) affording *threo*-**2.30b** (3.125 g; 44%) and a mixture of *erythro*- and *threo*-**2.30b** (2.014 g, 28%), both as a yellow oil.

Sample data for *threo*-**2.30b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.46 – 1.49 (m, 12H, -C*H*$_3$ and *tert*-butyl), 1.50 (s, 3H, -C*H*$_3$), 3.85 – 4.06 (m, 2H, -C(4)*H* and –C(5)*H*$_a$), 4.14 (dd, 1H, *J* = 2.35 and 9.09 Hz, –C(5)*H*$_b$), 4.82- 4.92 (m, 1H, -C(4)C*H*OH), 5.88 (m, 1H, -C(4)CHO*H*), 7.35 – 7.40 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.78, 27.59, 60.68, 61.11, 63.65, 78.93, 83.84, 89.59, 93.34, 122.12, 127.87, 128.06, 131.02, 151.44.

Exact mass (ESI-MS) calculated for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1862, found: 332.1864.

Diols 2.31a and 2.31b:

Diol **2.31a**:

A solution of *threo*-**2.30a** (7.57 g, 29.65 mmol) in 90% acetic acid was stirred for 5 h at 60°C. The solvent was removed under reduced pressure, and the residue was coevaporated twice with isooctane (25 mL). The residue was purified by flash chromatography (hexanes:EtOAc 1:1) yielding **2.31a** (5.935 g, 93%) as a slightly yellow, viscous oil.

Sample data for **2.31a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.36 (s, 9H, *tert*-butyl), 3.21 (d, 1H, *J* = 2.06 Hz, alkyne *H*), 3.32 – 3.52 (m, 3H, -C(1)H_2 and -C(2)*H*), 4.28 – 4.35 (m, 1H, -C(3)*H*), 4.61 (t, 1H, *J* = 5.57 Hz, -C(1)O*H*)), 5.38 (d, 1H, *J* = 6.45 Hz, -C(3)O*H*), 6.24 (d, 1H, *J* = 7.63 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 28.20, 56.98, 59.62, 59.95, 75.03, 77.80, 84.25, 155.41.

Exact mass (ESI-MS) calculated for $C_{10}H_{17}NO_4Na$ $[M+Na]^+$: 238.1055, found: 238.1047.

Diol **2.31b:**

To a solution of *threo*-**2.30b** (5.91 g, 17.83 mmol) in MeOH (70 mL), *p*-TsOH.H_2O (339 mg, 1.78 mmol, 0.1 eq.) was added, and the resulting solution was stirred for 36 h at room temperature. TEA (3 mL) was added to the cooled (0°C) solution, and the solvent was removed in vacuo. The residue was dissolved in EtOAc (100 mL) and the resulting solution extracted with sat. $NaHCO_3$ (2 x 25 mL) and brine (25 mL). After drying over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (hexanes:EtOAc 3:2) producing **2.31b** (3.64 g, 70%) as a white foam.

Sample data for **2.31b:**

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.38 (s, 9H, *tert*-butyl), 3.25 – 3.68 (m, 3H, -C(1)H_2 and -C(2)*H*), 4.56 (dd, 1H, *J* = 3.82 and 6.75 Hz, -C(3)*H*), 4.64 (t, 1H, *J* = 5.57 Hz, -

C(1)O*H*), 5.47 (d, 1H, *J* = 6.45 Hz, -C(3)O*H*), 6.36 (d, 1H, *J* = 8.50 Hz, -N*H*), 7.32 – 7.42 (m, 5H, arom. *H*).

Exact mass (ESI-MS) calculated for $C_{16}H_{22}NO_4$ $[M+H]^+$: 292.1549, found: 292.1545.

Silyl ethers 2.32a and 2.32b:

Typical procedure: TBDPSCl (9.5 mmol) was added dropwise to a cooled solution (0°C) of **2.31a** or **2.31b** (10 mmol)**,** imidazole (30 mmol) and 4-DMAP (cat.) in anhydrous DMF (20 mL). The mixture was stirred overnight and the solvent was subsequently removed under reduced pressure. The residue was partitioned between Et_2O (25 mL) and sat. $NaHCO_3$ (12 mL). After separation of the phases, the organic layer was washed with sat. $NaHCO_3$ (12 mL) and brine (12 mL). The organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc 9:1) affording **2.32a** (89%) and **2.32b** (84%) as very viscous, slightly yellow oils.

Sample data for **2.32a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.97 (s, 9H, *tert*-butyl silyl), 1.37 (s, 9H, *tert*-butyl), 3.25 (d, 1H, *J* = 2.05 Hz, alkyn *H*), 3.60 – 3.82 (m, 3H, -C(1)H_2 and –C(2)*H*), 4.39 – 4.47 (m, 1H, -C(3)*H*), 5.49 (d, 1H, *J* = 6.74 Hz, -C(3)O*H*), 6.41 (d, 1H, *J* = 8.21 Hz, -N*H*), 7.35 – 7.44 (m, 4H, arom. *H*), 7.59 – 7.65 (m, 6H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 18.82, 26.53, 28.19, 56.90, 60.04, 62.56, 75.30, 77.77, 83.89, 127.78, 129.77, 133.01, 135.02, 155.39.

Exact mass (ESI-MS) calculated for $C_{26}H_{35}NO_4SiNa$ $[M+Na]^+$: 476.2233, found: 476.2234.

Sample data for **2.32b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.07 (s, 9H, *tert*-butyl silyl), 1.37 (s, 9H, *tert*-butyl), 3.66 – 3.73 (m, 1H, -C(1)H_a), 3.76 – 3.92 (m, 2H, -C(1)H_b and C(2)*H*), 4.56 – 4.67 (m, 1H, -C(3)*H*), 5.48 – 5.64 (m, 1H, -C(3)O*H*), 6.56 (d, 1H, *J* = 7.92 Hz, -N*H*), 7.28 – 7.48 (m, 10H, arom. *H*), 7.58 – 7.67 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 18.81, 26.54, 28.20, 57.10, 60.86, 62.83, 77.75, 84.00, 89.80, 122.24, 127.77, 128.50, 129.76, 131.27, 133.00, 133.03, 135.01, 155.46.

Exact mass (ESI-MS) calculated for $C_{32}H_{40}SiNO_4$ $[M+H]^+$: 530.2727, found: 530.2721.

Oxazolidines 2.33a and 2.33b:

Typical procedure: To a solution of **2.32a** or **2.32b** (10 mmol) in a mixture of acetone/2,2-dimethoxypropane (2.6:1, 40 mL), *p*-TsOH.H_2O (5 mol%) was added in one portion and the reaction was refluxed for 6 h. Removal of the solvent under reduced pressure, followed by flash chromatography (hexanes:EtOAc 95:5) afforded **2.33a** (98%) and **2.33b** (84%) as colourless oils.

Sample data for **2.33a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.96 (s, 9H, *tert*-butyl silyl), 1.15 – 1.44 (m, 12H, *tert*-butyl and –CH_3), 1.62 (s, 3H, -CH_3), 3.48 – 3.85 (m, 3H, alkyne *H* and –C(4)CH_2), 3.90 – 4.05 (m, 1H, -C(4)*H*), 4.82 – 4.97 (m, 1H, -C(5)*H*), 7.35 – 7.63 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 18.77, 26.50, 26.84, 27.24, 27.77, 61.12, 62.29, 64.27, 64.51, 65.33, 65.94, 77.03, 79.48, 79.79, 82.43, 82.92, 94.72, 95.27, 127.91, 129.98, 132.43, 134.99, 150.53, 151.01.

Exact mass (ESI-MS) calculated for $C_{29}H_{40}SiO_4N$ $[M+Na]^+$: 516.2546, found: 516.2554.

Sample data for **2.33b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.95 – 1.05 (s, 9H, *tert*-butyl silyl), 1.18 – 1.50 (m, 12H, *tert*-butyl and –CH_3), 1.67 (s, 3H, -CH_3), 3.60 – 3.92 (m, 2H, -C(4)*H* and -C(4)CH_a), 3.98 – 4.10 (m, 1H, -C(4)CH_b), 5.08 – 5.20 (m, 1H, C(5)*H*), 7.35 – 7.50 (m, 10H, arom. *H*), 7.58 – 7.65 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 18.79, 26.55, 27.82, 61.32, 62.17, 64.26, 64.51, 65.23, 65.82, 77.03, 79.33, 79.64, 87.37, 87.82, 95.78, 96.02, 121.36, 127.92, 128.75, 129.09, 130.00, 131.33, 132.52, 135.07, 150.55, 151.03.

Exact mass (ESI-MS) calculated for $C_{35}H_{44}SiO_4N$ $[M+H]^+$: 570.3040, found: 570.3043.

Alcohols 2.34a and 2.34b:

Typical procedure: To a solution of **2.33a** or **2.33b** (10 mmol) in THF (50 mL), TBAF (15 mmol) was added in one portion, and the solution was stirred for 75 min at room temperature. The solvent was subsequently removed under reduced pressure, and the residue was dissolved in EtOAc (100 mL). The organic layer was washed with saturated $NaHCO_3$ (3 x 25 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (hexanes:EtOAc 4:1) yielding **2.34a** (96%) and **2.34b** (91%) as white solids.

Alcohol **2.34b** from **2.34a**:

To a solution of **2.34a** (651 mg, 2.55 mmol) and $PdCl_2(PPh_3)_2$ (36 mg, 51 μmol, 2 mol%) in piperidine (5 mL), iodobenzene (429 μL, 3.82 mmol, 1.5 eq.) was added and the mixture was stirred overnight at 70°C. The solvent was subsequently removed under reduced pressure and the residue was purified by column chromatography rendering (hexanes:EtOAc 4:1) **2.34b** (841 mg, 99%) as a white solid.

Sample data for **2.34a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.40 (s, 9H, *tert*-butyl), 1.60 – 1.63 (m, 6H, 2 x –CH_3), 3.15 – 3.26 (m, 1H, -C(4)CH_a), 3.45 – 3.53 (m, 1H, -C(4)CH_b), 3.58 (d, 1H, *J* = 2.35, alkyne *H*), 3.76 - 3.90 (m, 1H, -C(4)*H*), 4.72 – 4.78 (m, 1H, -C(5)*H*), 5.05 – 5.15 (m, 1H, -C(4)CH_2O*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.51, 26.84, 27.19, 28.01, 59.50, 60.24, 65.05, 65.32, 65.48, 65.89, 76.79, 79.42, 79.88, 83.60, 94.60, 94.95, 150.74, 151.07.

Exact mass (ESI-MS) calculated for $C_{13}H_{21}NO_4Na$ $[M+Na]^+$: 278.1368, found: 278.1364.

Sample data for **2.34b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.41 (m, 9H, *tert*-butyl), 1.44 (s, 3H, -CH_3), 1.68 (s, 3H, -CH_3), 3.24 – 3.36 (m, 1H, -C(4)CH_a), 3.51 – 3.62 (m, 1H, -C(4)CH_b), 3.88 - 4.20 (m, 1H, -C(4)*H*), 5.02 (d, 1H, *J* = 1.53 Hz, -C(5)*H*), 5.06 – 5.17 (br s, 1H, -C(4)CH_2O*H*), 7.34 – 7.45 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.61, 27.06, 27.94, 59.51, 60.25, 65.16, 66.17, 66.49, 79.42, 79.81, 84.87, 89.01, 94.53, 94.78, 121.49, 128.70, 128.94, 131.21, 157.75.

Exact mass (ESI-MS) calculated for $C_{19}H_{26}O_4N$ $[M+H]^+$: 332.1862, found: 332.1865.

Tosylates 2.35a and 2.35b:

Typical procedure: Solid *p*-TsCl (3 mmol) was added to an ice-cold solution of **2.34a** or **2.34b** (1 mmol) in anhydrous pyridine (1 mL) and after stirring for 35 h at room temperature, the reaction mixture was evaporated to dryness at high vacuum (< 35°C for **2.35a,** 40 – 50°C for **2.35b**). The residue was purified by column chromatography (hexanes:EtOAc 85:15) to yield **2.35a** (94%) and **2.35b** (64%) as white solids.

Sample data for **2.35a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.34 (s, 9H, *tert*-butyl), 1.58 – 1.61 (m, 6H, 2 x -C*H*$_3$), 2.41 (s, 3H, tosyl -C*H*$_3$), 3.64 (d, 1H, *J* = 2.34 Hz, alkyne *H*), 4.00 – 4.10 (m, 3H, -C(4)C*H*$_2$ and -C(4)*H*), 4.70 – 4.75 (br s, 1H, -C(5)*H*), 7.47 (d, 2H, *J* = 8.21 Hz, arom. *H*), 7.78 (d, 2H, *J* = 8.21 Hz, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 20.77, 25.40, 26.67, 27.77, 61.52, 61.79, 65.37, 65.92, 67.26, 68.11, 77.52, 80.13, 80.44, 82.40, 95.22, 95.54, 127.65, 130.30, 131.85, 145.29, 150.26, 150.87.

Exact mass (ESI-MS) calculated for $C_{20}H_{27}NO_6SNa$ $[M+Na]^+$: 432.1457, found: 432.1453.

Sample data for **2.35b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.33 (s, 9H, *tert*-butyl), 1.41 (s, 3H, -C*H*$_3$), 1.65 (s, 3H, -C*H*$_3$), 2.38 (s, 3H, tosyl -C*H*$_3$), 4.04 – 4.22 (m, 3H, -C(4)C*H*$_2$ and -C(4)*H*), 4.93 – 5.20 (m, 1H, -C(5)*H*), 7.34 – 7.44 (m, 5H, arom. *H*), 7.47 (d, 2H, *J* = 8.21 Hz, arom. *H* tosyl), 7.78 (d, 2H, *J* = 8.21 Hz, arom. *H* tosyl).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 21.12, 25.56, 25.62, 26.88, 27.17, 27.83, 61.53, 61.85, 66.07, 66.63, 67.35, 68.20, 80.18, 80.48, 85.55, 87.84, 95.16, 95.58, 121.21, 127.74, 128.84, 130.36, 131.37, 131.88, 145.37, 150.37, 150.97.

Exact mass (ESI-MS) calculated for $C_{26}H_{32}O_6NS$ $[M+H]^+$: 486.1950 found: 486.1956.

Intermediates 2.36a-e:

Typical procedure for intermediates **2.36a-d**:

The secondary amine (4 mmol) was added to a solution of **2.35a-b** (0.25 mmol) in anhydrous DMF (3 mL) and the mixture was heated at 45°C for 72 h. The residue, resulting from removal of the solvent in vacuo, was purified by column chromatography (hexanes:EtOAc 4:1 for **2.36a-b**, CH_2Cl_2:MeOH 99:1 for **2.36c-d**) yielding **2.36a** (62%), **2.36b** (85%), **2.36c** (82%) and **2.36d** (89%) as slightly brown solids.

Intermediate **2.36c** from **2.36d**:

An identical procedure as for the preparation of **2.34b** from **2.34a** gave **2.36d** (93%) as a slightly brown solid.

Intermediate **2.36e**:

NaN_3 (268 mg, 4.12 mmol, 10 eq.) was added to a solution of **2.35b** (200 mg, 0.412 mmol) in anhydrous DMF (10 mL) and the mixture was heated at 45°C for 72 h. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography (hexanes:EtOAc 97:3) rendering **2.36e** (145 mg, 99%) as a white solid.

Sample data for **2.36a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.43 (s, 9H, *tert*-butyl), 1.48 (s, 3H, -C*H*$_3$), 1.71 (s, 3H, -C*H*$_3$), 2.32 – 2.46 (m, 4H, C*H*$_2$-N-C*H*$_2$ morpholine), 2.50 – 2.62 (m, 2H, -C(4)C*H*$_2$), 3.50 – 3.65 (m, 4H, C*H*$_2$-O-C*H*$_2$ morpholine), 3.98 – 4.18 (m, 1H, -C(4)*H*), 4.96 – 5.05 (m, 1H, -C(5)*H*), 7.36 – 7.47 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.77, 27.41, 28.04, 53.70, 60.20, 61.06, 66.26, 68.24, 79.65, 82.07, 85.08, 94.87, 121.52, 128.85, 129.13, 131.36, 150.60.

Exact mass (ESI-MS) calculated for $C_{23}H_{33}O_4N_2$ $[M+H]^+$: 401.2440, found: 401.2439.

Sample data for **2.36b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.30 – 1.54 (m, 18H, *tert*-butyl, -C*H*$_3$, C*H*$_2$-C*H*$_2$-C*H*$_2$ piperidine), 1.68 – 1.72 (s, 3H, –C*H*$_3$), 2.23 – 2.60 (m, 6H, -C(4)C*H*$_2$ and C*H*$_2$-N-C*H*$_2$

piperidine), 4.92 – 4.16 (br s, 1H, -C(4)*H*), 4.82 - 5.04 (br s, 1H, -C(5)*H*), 7.34 – 7.45 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 23.84, 25.57, 27.02, 27.38, 27.97, 28.97, 54.55, 60.37, 61.60, 68.24, 79.51, 84.97, 89.31, 94.87, 121.49, 128.77, 129.04, 131.28, 150.59.

Exact mass (ESI-MS) calculated for $C_{24}H_{35}O_3N_2$ $[M+H]^+$: 399.2648, found: 399.2640.

Sample data for **2.36c**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.40 (s, 9H, *tert*-butyl), 1.44 (s, 3H, -CH_3), 1.64 – 1.74 (m, 7H, CH_2-CH_2-CH_2-CH_2 pyrrolidine and -CH_3), 2.20 – 2.90 (br s, 5H, CH_2-N-CH_2 pyrrolidine and -C(4)CH_a), 3.23 – 3.34 (m, 1H, -C(4)CH_b), 3.95 – 4.21 (m, 1H, -C(4)*H*), 4.82 – 5.20 (br s, 1H, -C(5)*H*), 7.32 – 7.44 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 23.12, 25.70, 27.43, 27.97, 53.80, 56.25, 57.58, 61.82, 62.47, 67.33, 67.96, 79.57, 79.95, 85.06, 89.14, 94.63, 94.93, 121.52, 128.76, 129.04, 131.28, 150.61, 151.00.

Exact mass (ESI-MS) calculated for $C_{23}H_{33}O_3N_2$ $[M+H]^+$: 385.2491, found: 385.2487.

Sample data for **2.36d**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.48 – 1.52 (m, 12H, *tert*-butyl and -CH_3), 1.60 – 1.70 (m, 7H, -CH_3 and CH_2-CH_2-CH_2-CH_2 pyrrolidine), 2.30 – 2.60 (m, 6H, CH_2-N-CH_2 pyrrolidine and -C(4)CH_2), 3.57 (d, 1H, *J* = 2.34 Hz, alkyne *H*), 3.86 – 4.00 (m, 1H, -C(4)*H*), 4.68 – 4.73 (br s, 1H, -C(5)*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 23.16, 25.51, 27.49, 27.96, 53.71, 57.62, 62.59, 67.23, 76.77, 79.48, 83.72, 94.97, 150.62.

Exact mass (ESI-MS) calculated for $C_{17}H_{29}O_3N_2$ $[M+H]^+$: 309.2178, found: 309.2180.

Sample data for **2.36e**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.43 (s, 9H, *tert*-butyl), 1.50 (s, 3H, -CH_3), 1.69 (s, 3H, -CH_3), 3.45 – 3.78 (m, 2H, -C(4)CH_2), 4.00 – 4.14 (br s, 1H, -C(4)*H*), 4.96 (d, 1H, *J* = 2.93 Hz, -C(5)*H*), 7.34 – 7.46 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.37, 26.89, 27.83, 49.97, 51.15, 62.56, 66.69, 67.28, 85.56, 87.56, 95.37, 121.30, 128.68, 129.08, 131.31, 150.48.

Exact mass (ESI-MS) calculated for $C_{19}H_{25}N_4O_3$ $[M+H]^+$: 357,1927, found: 357,1929.

Amides 2.38a-e and **2.43**:

Typical procedure for amides **2.38a-d** and **2.43**:

A solution of oxazolidines **2.36a-e** (0.8 mmol) in a mixture of MeOH/3N HCl (1:2, 30 mL) was heated at 50°C for 12 h and the solvent was subsequently removed under reduced pressure. The residue was covered with chloroform (3 x 20 mL) and the volatiles evaporated thereby quantitatively affording crude amines **2.37a-d** and **2.42** as their hydrochloride salts which were used without further purification.

To a cooled solution (0°C) of crude amines **2.37a-d** and **2.42** (0.8 mmol) and HOBT (10 mol %) in anhydrous pyridine (25 mL), *p*-nitrophenylpalmitate (0.8 mmol) dissolved in anhydrous DMF (5 mL) was added dropwise and the mixture was heated for 48 h at 50°C. The solvent was subsequently removed under reduced pressure and the residue was purified by column chromatography (hexanes:EtOAc:TEA 65:34:1 for **2.38a-c** and **2.43;** hexanes:EtOAc 7:3 for **2.38d**) producing **2.38a** (67%), **2.38b** (48%), **2.38c** (89%), **2.38d** (62%) and **2.43** (60%) as colourless solids.

Amide **2.38c** from **2.43**:

An identical procedure as for the preparation of **2.34b** from **2.34a** afforded **2.38c** (32%) as a colourless solid.

Amide **2.38e** from **2.38d**:

To a solution of **2.38d** (51.4 mg, 0.113 mmol) in THF (250 μL), PPh_3 (59 mg, 0.226 mmol, 2 eq.) was added. After stirring for 10 min at room temperature, H_2O (250 μL) was added and stirring was continued for 48 h. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography (CH_2Cl_2:MeOH(10% 6N NH_3 in MeOH) 9:1) affording **2.38e** (47 mg, 97%) as a white solid.

Sample data for **2.38a**:

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 0.81 (t, 3H, *J* = 6.52 Hz, -C*H*$_3$ acyl), 1.10 – 1.30 (m, 24H, acyl *H*), 1.50 – 1.63 (m, 2H, -COCH$_2$-C*H*$_2$-$C_{13}H_{27}$), 2.14 (t, 2H, *J* = 7.50 Hz, -CO-C*H*$_2$-$C_{14}H_{29}$), 2.45 – 2.80 (m, 5H, -C(1)*H*$_a$ and *CH*$_2$-N-C*H*$_2$ morpholine), 2.58 (dd, 1H, *J* = 10.81 and 11.57 Hz, -C(1)*H*$_b$), 3.63 – 3.74 (m, 4H, C*H*$_2$-O-C*H*$_2$ morpholine),

4.41 – 4.54 (m, 1H, -C(2)*H*), 4.72 (d, 1H, *J* = 3.73 Hz, -C(3)*H*), 5.55 – 5.90 (m, 1H, -N*H*), 7.31 – 7.40 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 13.12, 13.17, 20.06, 21.68, 24.62, 28.19, 28.34, 28.45, 28.63, 28.67, 30.90, 35.72, 46.00, 52.88, 58.61, 65.25, 65.55, 85.58, 86.08, 121.06, 127.39, 127.79, 130.78, 172.18.

Exact mass (ESI-MS) calculated for $C_{31}H_{51}O_3N_2$ $[M+H]^+$: 499.3899, found: 499.3902.

Sample data for **2.38b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (t, 3H, *J* = 0.83 Hz, -C*H*$_3$ acyl), 1.12 – 1.29 (m, 24H, acyl *H*), 1.30 – 1.40 (m, 2H, -COCH$_2$-C*H*$_2$-C$_{13}$H$_{27}$), 1.41 – 1.53 (m, 6H, C*H*$_2$-C*H*$_2$-C*H*$_2$ piperidine), 2.09 (t, 2H, *J* = 6.98 Hz, -CO-C*H*$_2$-C$_{14}$H$_{29}$), 2.32 – 2.45 (m, 5H, -C(1)*H*$_a$, C*H*$_2$-N-C*H*$_2$ piperidine), 2.65 (dd, 1H, *J* = 6.74 and 12.61 Hz, -C(1)*H*$_b$), 4.04 – 4.14 (m, 1H, -C(2)*H*), 4.56 (d, 1H, *J* = 3.52 Hz, -C(3)*H*), 5.80 – 6.20 (br s, 1H, -C(3)O*H*), 7.31 -7.42 (m, 5H, arom. *H*), 7.60 – 7.65 (d, 1H, *J* = 8.21 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.93, 22.08, 23.76, 25.52, 28.48, 28.69, 28.86, 28.93, 28.99, 29.02, 31.28, 35.38, 50.08, 54.23, 58.11, 62.20, 83.74, 90.04, 122.51, 128.37, 128.49, 131.35, 172.25.

Exact mass (ESI-MS) calculated for $C_{32}H_{53}O_2N_2$ $[M+H]^+$: 497.4107, found: 497.4101.

Sample data for **2.38c**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (t, 3H, *J* = 7.03 Hz, -C*H*$_3$ acyl), 1.12 – 1.28 (m, 24H, acyl *H*), 1.42 – 1.51 (m, 2H, -COCH$_2$-C*H*$_2$-C$_{13}$H$_{27}$), 1.62 – 1.68 (m, 4H, C*H*$_2$-C*H*$_2$ pyrrolidine), 2.09 (dt, 2H, *J* = 2.93 and 7.04 Hz, -CO-C*H*$_2$-C$_{14}$H$_{29}$), 2.41 – 2.53 (m, 5H, -C(1)*H*$_a$ and C*H*$_2$-N-C*H*$_2$ pyrrolidine), 2.76 (dd, 1H, *J* = 5.57 and 12.02 Hz, -C(1)*H*$_b$), 4.05 (ddd, 1H, *J* = 3.81, 7.92 and 11.43 Hz, -C(2)*H*), 4.57 (d, 1H, *J* = 3.81 Hz, -C(3)*H*), 5.75 – 5.87 (br s, 1H, -OH), 7.31 – 7.40 (m, 5H, arom. *H*), 7.60 (d, 1H, *J* = 8.50 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.88, 22.02, 23.13, 25,47, 28.45, 28.63, 28.80, 28.88, 28.96, 31.22, 35.35, 51.94, 53.72, 55.11, 62.00, 86.57, 90.12, 122.53, 128.28, 128.43, 131.29, 172.19.

Exact mass (ESI-MS) calculated for $C_{31}H_{51}O_2N_2$ $[M+H]^+$: 483.3951, found: 483.3953.

Sample data for **2.38d**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (t, 3H, *J* = 6.65 Hz, -C*H*$_3$ acyl), 1.10 – 1.30 (m, 24H, acyl *H*), 1.42 – 1.54 (m, 2H, -COCH$_2$-C*H*$_2$-C$_{13}$H$_{27}$), 2.12 (dt, 2H, *J* = 2.64 and 7.04 Hz, -CO-C*H*$_2$-C$_{14}$H$_{29}$), 3.45 (dd, 1H, *J* = 8.79 and 12.60 Hz, -C(1)*H*$_a$), 3.53 (dd, 1H, *J* = 4.69 and 12.60 Hz, -C(1)*H*$_b$), 4.02 – 4.12 (m, 1H, -C(2)*H*), 4.53 (app. t, 1H, *J* = 4.83 Hz, -C(3)*H*), 5.89 (d, 1H, *J* = 5.57 Hz, -C(3)O*H*), 7.32 – 7.44 (m, 5H, arom. *H*), 7.96 (d, 1H, *J* = 8.50 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.93, 13.93, 22.08, 25.34, 28.53, 28.69, 28.83, 28.91, 28.98, 29.01, 31.28, 35.41, 50.11, 53.23, 54.90, 61.43, 84.25, 88.89, 122.21, 128.51, 128.56, 131.40, 172.65.

Exact mass (ESI-MS) calculated for $C_{27}H_{43}O_2N_4$ $[M+H]^+$: 455.3386, found: 455.3380.

Sample data for **2.38e**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (t, 3H, -C*H*$_3$ acyl), 1.11 – 1.28 (m, 24H, acyl *H*), 1.41 – 1.53 (m, 2H, -COCH$_2$-C*H*$_2$-C$_{13}$H$_{27}$), 2.02 – 2.19 (m, 2H, -CO-C*H*$_2$-C$_{14}$H$_{29}$), 2.68 (dd, 1H, *J* = 7.62 and 12.90 Hz, -C(1)*H*$_a$), 2.85 (dd, 1H, *J* = 6.16 and 12.90 Hz, -C(1)*H*$_b$), 3.10 - 3.50 (br s, 2H, -N*H*$_2$), 3.76 – 3.88 (m, 1H, -C(2)*H*), 4.64 (d, 1H, *J* = 4.11 Hz, -C(3)*H*), 7.31 – 7.42 (m, 5H, arom. *H*), 7.61 (d, 1H, *J* = 8.50 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.95, 22.08, 25.51, 28.57, 28.69, 28.86, 28.93, 29.02, 31.28, 38.96, 54.90, 55.25, 61.74, 83.74, 90.13, 122.53, 128.36, 128.50, 131.31, 172.60.

Exact mass (ESI-MS) calculated for $C_{27}H_{45}O_2N_2$ $[M+H]^+$: 429.3481, found: 429.3483.

Sample data for **2.43**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.82 (t, 3H, *J* = 6.74 Hz, -C*H*$_3$ acyl), 1.12 – 1.30 (m, 24H, acyl *H*), 1.38 – 1.50 (m, 2H, -COCH$_2$-C*H*$_2$-C$_{13}$H$_{27}$), 1.58 – 1.66 (m , 4H, -C*H*$_2$-C*H*$_2$- pyrrolidine), 2.06 (t, 2H, *J* = 7.62 Hz, -CO-C*H*$_2$-C$_{14}$H$_{29}$), 2.37 – 2.45 (m, 5H, -C(1)*H*$_a$ and C*H*$_2$-N-C*H*$_2$ pyrrolidine), 2.68 (dd, 1H, *J* = 5.86 and 12.02, -C(1)*H*$_b$), 3.18 (d, 1H, *J* = 2.34 Hz, alkyne *H*), 3.86 – 3.96 (m, 1H, -C(2)*H*), 4.32 (dd, 1H, *J* = 1.76 and 3.82 Hz, -C(3)*H*), 7.54 (d, 1H, *J* = 8.21 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*; hydrochloride salt of **2.43**) δ: 14.36, 23.33, 23.68, 23.89, 26.31, 26.47, 30.00, 30.18, 30.25, 30.42, 30.61, 32.64, 36.79, 51.38, 52.98, 53.63, 55.13, 63.15, 86.00, 88.42, 174.27.

Exact mass (ESI-MS) calculated for $C_{25}H_{47}O_2N_2$ $[M+H]^+$: 407.3638, found: 407.3632.

Ceramines 2.39a-c:

Typical procedure: Red-Al® (4 mmol) was added to a cooled (-78°C) solution of **2.38a**, **2.38b** or **2.38e** (0.4 mmol) in anhydrous Et_2O (10 mL), and the resulting mixture was stirred overnight at room temperature. After quenching the reaction (0°C) by addition of MeOH (10 mL), sat. disodiumtartrate (20 mL) was added, and the mixture was stirred for an additional 4 h. Et_2O (25 mL) and sat. $NaHCO_3$ (25 mL) were added, the aqueous layer was extracted with Et_2O (3 x 25 mL) and the combined organic phase was dried over $MgSO_4$. Flash chromatography (hexanes:EtOAc:TEA 60:39:1 for **2.39a** and **2.39b**, CH_2Cl_2:MeOH (5% 6N NH_3 in MeOH) 4:1 for **2.39c**) afforded **2.39a** (60%), **2.39b** (100%) and **2.39c** (45%) as colourless oils.

Sample data for **2.39a**:

^{1}H NMR (300 MHz; DMSO-*d6* + D_2O) δ: 0.83 (t, 3H, *J* = 6.70 Hz, -C*H*$_3$ alkyl), 1.12 – 1.42 (m, 28H, alkyl *H*), 2.19 (dd, 1H, *J* = 7.62 and 12.31 Hz, -C(5)*H*$_a$), 2.24 – 2.42 (m, 5H, C*H*$_2$-N-C*H*$_2$ morpholine and -C(5)*H*$_b$), 2.58 (dt, 2H, *J* = 2.35 and 7.04 Hz, -NH-C*H*$_2$-$C_{15}H_{31}$), 2.68 – 2.75 (m, 1H, -C(4)*H*), 3.52 (t, 4H, *J* = 4.69 Hz, C*H*$_2$-O-C*H*$_2$ morpholine), 4.17 (app. t, 1H, *J* = 3.81 Hz, -C(3)*H*), 6.38 (dd, 1H, *J* = 5.28 and 15.83 Hz, -C(2)*H*), 6.53 (d, 1H, *J* = 15.83 Hz, -C(1)*H*), 7.15 – 7.40 (m, 5H, arom).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.94, 22.08, 26.64, 28.68, 28.86, 28.99, 29.74, 31.28, 47.74, 53.70, 58.77, 59.19, 66.32, 71.36, 126.07, 127.04, 128.43, 128.55, 132.05, 137.06.

Exact mass (ESI-MS) calculated for $C_{31}H_{55}O_2N_2$ $[M+H]^+$: 487.4264, found: 487.4268.

Sample data for **2.39b**:

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 0.88 (t, 3H, *J* = 6.66 Hz, -C*H*$_3$ alkyl), 1.41 – 1.51 (m, 26H, alkyl *H*), 1.32 – 1.42 (m, 2H, -NHCH$_2$-C*H*$_2$-$C_{14}H_{29}$), 1.75 – 1.83 (m, 4H, C*H*$_2$-C*H*$_2$ pyrrolidine), 2.56 – 2.77 (m, 8H, -C(5)*H*$_2$, -NH-C*H*$_2$-$C_{15}H_{31}$ and C*H*$_2$-N-C*H*$_2$ pyrrolidine), 2.93 (dt, 1H, *J* = 4.17 and 6.99 Hz, -C(4)*H*), 4.16 (app. t, 1H, *J* = 4.90 Hz, -C(3)*H*), 6.30 (dd, 1H, *J* = 5.43 and 15.93 Hz, -C(2)*H*), 6.71 (dd, 1H, *J* = 1.03 and 15.92 Hz, -C(1)*H*), 7.20 – 7.43 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 14.12, 22.69, 23.59, 27.19, 29.36, 29.49, 29.61, 29.69, 30.23, 31.92, 48.63, 54.51, 57.69, 59.53, 73.20, 126.64, 127.35, 128.50, 130.49, 130.66, 137.11.

Exact mass (ESI-MS) calculated for $C_{31}H_{55}ON_2$ $[M+H]^+$: 471.4314, found: 471.4308.

Sample data for **2.39c**:

^{1}H NMR (300 MHz; DMSO-*d6* + D_2O) δ: 0.83 (t, 3H, *J* = 6.73 Hz, -C*H*$_3$ alkyl), 1.15 – 1.30 (m, 26H, alkyl *H*), 1.33 – 1.40 (m, 2H, -NHCH$_2$-C*H*$_2$-$C_{14}H_{29}$), 2.36 – 2.73 (m, 5H, -C(5)*H*$_2$, -NH-C*H*$_2$-$C_{15}H_{31}$ and –C(4)*H*), 4.35 (app. t, 1H, *J* = 4.06 Hz, -C(3)*H*), 6.33 (dd, 1H, *J* = 5.61 and 16.00 Hz, -C(2)*H*), 6.55 (d, 1H, *J* = 16.14 Hz, -C(1)*H*), 7.16 – 7.42 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.94, 22.08, 26.78, 28.69, 29.07, 30.07, 31.28, 37.02, 47.38, 57.84, 64.91, 71.55, 126.10, 127.09, 128.54, 128.74, 132.13, 137.03.

Exact mass (ESI-MS) calculated for $C_{27}H_{49}ON_2$ $[M+H]^+$: 417.3845, found: 417.3844.

Ceramides 2.41a-c:

Applying an identical procedure as described for the reduction of amides **2.38a**, **2.38b** and **2.38e** quantitatively produced crude *E*-alkene amines **2.40a-c**, which were used without further purification.

An identical procedure as for the acylation of amines **2.37a-d** and **2.42** afforded ceramides **2.41a** (84%), **2.41b** (78%) and **2.41c** (71%) as colourless oils.

Sample data for **2.41a**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.84 (t, 3H, *J* = 6.70 Hz, -C*H*$_3$ acyl), 1.09 – 1.30 (m, 24H, acyl *H*), 1.36 – 1.48 (m, 2H, -COCH$_2$-C*H*$_2$-$C_{13}H_{27}$), 2.04 (t, 2H, *J* = 7.47 Hz, -CO-C*H*$_2$-$C_{14}H_{29}$), 2.21 – 2.42 (m, 5H, -C(1)*H*$_a$ and C*H*$_2$-N-C*H*$_2$ morpholine), 2.54 – 2.64 (m, 1H, -C(1)*H*$_b$), 3.48 – 3.54 (m, 4H, C*H*$_2$-O-C*H*$_2$ morpholine), 4.00 – 4.12 (m, 1H, -C(2)*H*), 4.24 – 4.32 (m, 1H, -C(3)*H*), 5.21 (d; 1H, *J* = 4.69 Hz, -C(3)O*H*) 6.23 (dd, 1H, *J* = 4.98 and 15.83 Hz, -C(4)*H*), 6.47 – 6.56 (dd, 1H, *J* = 1.18 and 15.83 Hz, -C(5)*H*), 7.11 – 7.37 (m, 5H, arom. *H*), 7.46 (d, 1H, *J* = 9.09 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 14.02, 22.16, 25.61, 28.55, 28.77, 29.91, 28.99, 29.09, 35.49, 49.86, 53.56, 58.63, 66.29, 70.71, 126.18, 127.07, 128.16, 128.19, 128.45, 128.81, 131.29, 136.99, 172.02.

Exact mass (ESI-MS) calculated for $C_{31}H_{53}O_3N_2$ $[M+H]^+$: 501.4056, found: 501.4058.

Sample data for **2.41b**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (app. t, 3H, *J* = 6.78 Hz, -C*H*$_3$ acyl), 1.09 – 1.30 (m, 32H, acyl chain and C*H*$_2$-C*H*$_2$-C*H*$_2$ piperidine), 2.04 (t, 2H, *J* = 7.03 Hz, -CO-C*H*$_2$-$C_{14}H_{29}$), 2.16 – 2.25 (dd, 1H, *J* = 4.69 and 12.02 Hz, -C(1)*H*$_a$), 2.25 – 2.38 (m, 4H, -C*H*$_2$-N-C*H*$_2$ piperidine), 2.42 – 2.50 (m, 1H, -C(1)*H*$_b$), 4.00 – 4.10 (m, 1H, -C(2)*H*), 4.24 – 4.30 (m, 1H, -C(3)*H*), 5.20 – 5.40 (br s, 1H, -C(3)O*H*), 6.23 (dd, 1H, *J* = 4.99 and 15.83 Hz, -C(4)*H*), 6.51 (dd, 1H, *J* = 1.17 and 15.83 Hz, -C(5)*H*), 7.05 – 7.38 (m, 5H, arom. *H*), 7.43 (d, 1H, *J* = 8.79 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.94, 22.09, 23.99, 25.54, 25.68, 28.49, 28.70, 28.85, 28.93, 28.99, 29.03, 31.29, 31.79, 35.46, 50.01, 54.31, 58.92, 70.91, 126.12, 127.06, 128.16, 128.21, 128.46, 128.78, 131.39, 137.05, 171.91.

Exact mass (ESI-MS) calculated for $C_{32}H_{55}O_2N_2$ $[M+H]^+$: 499.4263, found: 499.4258.

Sample data for **2.41c**:

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.79 – 0.88 (app. t, 3H, -C*H*$_3$ acyl), 1.09 – 1.30 (m, 24H, acyl chain), 1.34 - 1.48 (m, 2H, -$COCH_2$-C*H*$_2$-$C_{13}H_{27}$), 1.58 – 1.69 (m, 4H, C*H*$_2$-C*H*$_2$ pyrrolidine), 2.04 (dt, 2H, *J* = 3.52 and 7.04 Hz, -CO-C*H*$_2$-$C_{14}H_{29}$), 2.33 (dd, 1H, *J* = 7.62 and 12.02 Hz, -C(1)*H*$_a$), 2.38 – 2.46 (m, 4H, C*H*$_2$-N-C*H*$_2$ pyrrolidine), 2.65 (dd, 1H, *J* = 6.45 and 12.02 Hz, -C(1)*H*$_b$), 3.96 – 4.07 (m, 1H, -C(2)*H*), 4.26 – 4.33 (m, 1H, -C(3)*H*), 6.24 (dd, 1H, *J* = 4.98 and 15.83 Hz, -C(4)*H*), 6.52 (dd, 1H, *J* = 1.18 and 15.84 Hz, -C(5)*H*), 7.10 – 7.38 (m, 5H, arom.), 7.45 (d, 1H, *J* = 8.80 Hz, -N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.94, 22.08, 23.15, 25.52, 28.48, 28.69, 28.83, 28.91, 28.99, 29.02, 31.28, 35.44, 51.77, 53.75, 55.85, 70.73, 126.11, 127.05, 128.16, 128.73, 128.45, 128.73, 131.37, 137.04, 171.97.

Exact mass (ESI-MS) calculated for $C_{31}H_{53}O_2N_2$ $[M+H]^+$: 485.4107, found: 485.4109

Oxazolidinone 2.44:

To a cooled solution (0°C) of **2.38e** (64 mg, 149 μmol) and TEA (83 μL, 596 μmol, 4 eq.) in anhydrous CH_2Cl_2 (10 mL), triphosgene (46.5 mg, 156 μmol, 1.05 eq.) dissolved in CH_2Cl_2 (1 mL) was added dropwise and the resulting solution was stirred for 1 h at room temperature. After removal of the solvent under reduced pressure,

the mixture was purified by column chromatography (EtOAc) affording **2.44** (60 mg, 88%) as a white solid.

Sample data for **2.44**:

^{1}H NMR (500 MHz; DMSO-*d6*) δ: 0.85 (t, 3H, *J* = 7.00 Hz, -C*H*$_3$ acyl), 1.1 – 1.3 (m, 24H, acyl *H*), 1.48 (m, 2H, -COCH$_2$-C*H*$_2$-C$_{13}$H$_{27}$), 2.16 (dt, 1H, *J* = 7.33 and 13.91 Hz, -CO-C*H*$_a$-C$_{14}$H$_{29}$), 2.20 (dt, 1H, *J* = 7.32 and 13.91 Hz, -CO-C*H*$_b$-C$_{14}$H$_{29}$), 3.19 (ddd, 1H, *J* = 2.19, 6.71 and 11.60 Hz -C(4)*H*$_a$), 3.40 (ddd, 1H, *J* = 2.07, 5.13 and 11.60 Hz, -C(4)*H*$_b$), 4.36 (dddd, 1H, *J* = 3.66, 5.13, 6.59, and 7.81 Hz, -C(5)*H*), 5.41 (d, 1H, *J* = 3.42 Hz, -C(6)*H*), 7.40 (obsolete, -C(3)N*H*), 7.35 – 7.50 (m, 5H, arom. *H*), 8.33 (d, 1H, *J* = 7.82 Hz, -C(3)N*H*).

^{13}C NMR (125 MHz; DMSO-*d6*) δ: 13.99, 22.17, 25.53, 28.67, 28.79, 28.94, 28.96, 29.11, 31.37, 35.18, 41.99, 43.13, 68.12, 83.86, 86.81, 121.32, 128.67, 129.27, 131.72, 151.08, 172.98.

Exact mass (ESI-MS) calculated for $C_{28}H_{43}N_2O_3$ $[M+H]^+$: 455.3274 found: 455.3275.

In vitro analysis of GlcCer synthase

In vitro GlcCer synthase analysis in rat Golgi membranes was performed using *N*-[6-[(7-nitrobenzo-2-oxa-1,3-diazol-4-yl)amino]hexanoyl]D-*erythro*-sphingosine (C6-NBD-D-*erythro*-Cer). C6-D-*erythro*-NBD ceramide was synthesized by *N*-acylation of sphingosine using the NHS-ester of NBD-hexanoic acid (Molecular Probes)[16].

A Golgi fraction was isolated from rat liver by the method of Dominguez et al. [17], as follows. Rat liver was homogenized in ice-cold 0.25 M sucrose, 50 mM Tris-HCl, pH 7.4, 25 mM KCl, 5 mM $MgCl_2$, 4.5 mM $CaCl_2$ (STKCM buffer) using a motorized Potter-Elvehjem homogenizer. After centrifugation at 400g for 10 min, supernatants were adjusted to 0.2 M sucrose in STKCM buffer and underlayed beneath a discontinuous gradient of 0.9 and 0.4 M sucrose in STKCM buffer. After centrifugation at 83000 g_{av} for 3 h in a SW 32 rotor at 4oC, Golgi fractions were collected at the 0.4/0.9 M sucrose interface.

The in vitro reaction mixture contained a rat liver Golgi fraction (1.6 μg of protein), UDP-glucose (5 mM), C6-NBD-D-*erythro*-ceramide(5 μM), $MnCl_2$ (5 mM), and protease inhibitors in a total volume of 1 ml of TK buffer (50 mM Tris-HCl, 25 mM KCl, pH 7.4). The reactions were allowed to proceed for 20 min at 37oC, and were

terminated by addition of 3 ml of chloroform/methanol (1:2 v/v). Lipids were extracted [18] and separated by thin-layer chromatography using chloroform/methanol/9.8 mM $CaCl_2$ (60:35:8, v/v/v) as the developing solvent. C_6-NBD-sphingolipids were identified using authentic standards. C_6-NBD-fluorescence was quantified by Quantity One software after exposing the TLC plates using a Fluor-S Max spectrometer (BioRad).

In vivo analysis of GlcCer synthase activity

Human embryonic kidney HEK-293 cells were grown to 80-90% confluency. C6-NBD-D-erythro-Ceramide was added directly to the culture dishes, together with 10, 25, and 50 μM of inhibitors. Cells were incubated for 3 hours at 37oC in a 5% CO2 incubator. At the end of the incubation, dishes were washed with PBS, and cells removed by scraping with a rubber policeman into ice-cold water. Lipids were extracted[18], separated and quantified as above.

^{3}H-serine labeling

Dishes, containing HEK-293 and COS-7 cells, were incubated for 1 hour with 50 mM of compounds **2.43** and **2.41c**, followed by addition of L-[3-^{3}H]-serine (Amersham) (30 μCi in 3 ml medium) for another 3 hours. At the end of the incubation, dishes were washed with PBS, cells removed by scraping with a rubber policeman into ice-cold water, and lipids extracted[18]. Phospholipids were degraded by mild alkaline hydrolysis with methanolic NaOH (100mM) for 2 h at 37oC. Lipid extracts were desalted by Sephadex G-25 (superfine, Sigma)[19], and separated by TLC using chloroform/methanol/9.8 mM $CaCl_2$ (60:35:8, v/v/v) as the developing solvent. Sphingolipids were visualized by spraying with cupric sulfate, followed by brief charring. Ceramide, glucosylceramide, lactosylceramide, and sphingomyelin were identified using authentic standards. Corresponding bands were scraped from the TLC plates, radioactivity was recovered from silica in 1 ml of methanol, followed by addition of Ultima Gold scintillation cocktail. Radioactivity was determined by liquid scintillation counting.

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8 EXPERIMENTAL PART

General

IUPAC names were generated with Chemdraw Ultra 8.0 (Chemoffice 2004, Cambridge Soft, Cambridge, USA).

All reactions were carried out under inert (N_2) atmosphere. Precoated Macherey-Nagel (Düren, Germany) silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and/or revealed by sulphuric acid-anisaldehyde spray or phosphomolybdic acid spray. Column chromatography was performed on ICN silica gel (63-200 μM, ICN, Asse Relegem, Belgium). NMR spectra were obtained with a Varian Mercury 300 spectrometer (Varian, Palo Alto, California, USA). Chemical shifts are given in parts per million (δ relative to residual solvent peak) and coupling constants are expressed in Hz. Abbreviations used are: s = singlet, d = doublet, t = triplet, m = multiplet, br s = broad signal. All signals assigned to amino and hydroxyl groups were exchangeable with D_2O. Numbering for ^1H assignment is based on the IUPAC name of the compounds unless stated otherwise. Structural assignment was confirmed with COSY, HMQC and/or NOEDIF/NOESY if necessary. Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Most chemicals were obtained from Sigma-Aldrich (Bornem, Belgium) or Acros Organics (Geel, Belgium) and were used without further purification. Anhydrous THF was obtained by distillation from $LiAlH_4$.

(*R*)-tert-butyl 4-((*R*)-1-hydroxy-3-phenylprop-2-ynyl)-2,2-dimethyloxazolidine-3-carboxylate (2.1)

To a stirred and cooled (0°C) solution of lithium phenylacetylide (42.7 mL of a 1M solution in THF, 42.7 mmol, 2 eq.) in anhydrous Et_2O (200 mL), $ZnBr_2$ (10.11 g, 44.88 mmol, 2.1 eq.) was added, and the mixture was stirred for 1 h at 0°C and 1 h at room temperature and was subsequently cooled –78°C. D-Garner's aldehyde (4.90 g, 21.37 mmol) was dissolved in anhydrous Et_2O (25 mL), the resulting solution

cooled to -78°C and added dropwise to the above solution. The mixture was allowed to reach room temperature overnight and after cooling to 0°C treated with sat. NH_4Cl (50 mL). After separation of the phases, the aqueous layer was extracted with Et_2O (2 x 100 mL) and the combined organic phase was dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography (hexanes:EtOAc 9:1 → 85:15) affording **2.1** (3.125 g; 44%) and a mixture of **2.1** and **2.2** (2.014 g, 28%), both as a yellow oil.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.46 - 1.49 (m, 12H, -CH_3 and *tert*-butyl), 1.50 (s, 3H, s, -CH_3), 3.85 - 4.06 (m, 2H, -C(4)*H* and –C(5)H_a), 4.14 (dd, 1H, *J* = 2.35 and 9.09 Hz, –C(5)H_b), 4.82 - 4.92 (m, 1H, -C(4)C*H*OH), 5.88 (2 x d, 1H, *J* = 5.60 and 9.09 Hz, -C(4)CHO*H*), 7.35 - 7.40 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.78, 27.59, 60.68, 61.11, 63.65, 78.93, 83.84, 89.59, 93.34, 122.12, 127.87, 128.06, 131.02, 151.44.

Exact mass (ESI-MS) calculated for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1862, found: 332.1864.

***tert*-Butyl (2*R*,3*R*)-1,3-dihydroxy-5-phenylpent-4-yn-2-ylcarbamate (2.3)**

To a solution of **2.2** (5.91 g, 17.83 mmol) in MeOH (70 mL), *p*-TsOH (339 mg, 1.783 mmol, 0.1 eq.) was added, and the resulting solution was stirred for 36 h at room temperature. TEA (3 mL) was added to the cooled (0°C) solution, and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (100 mL) and the resulting solution extracted with sat. $NaHCO_3$ (2 x 25 mL) and brine (25 mL). After drying over $MgSO_4$, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (hexanes:EtOAc 3:2) yielding **2.3** (3.64 g, 70%) as a white foam.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.38 (s, 9H, *tert*-butyl), 3.25 – 3.68 (m, 3H, -C(1)H_2 and -C(2)*H*), 4.56 (dd, 1H, *J* = 3.82 and 6.75 Hz, -C(3)*H*), 4.64 (t, 1H, *J* = 5.57 Hz, -C(1)O*H*), 5.47 (d, 1H, *J* = 6.45 Hz, -C(3)O*H*), 6.36 (d, 1H, *J* = 8.50 Hz, -N*H*), 7.32 - 7.42 (m, 5H, arom. *H*).

Exact mass (ESI-MS) calculated for $C_{16}H_{22}NO_4$ $[M+H^+]$: 292.1549, found: 292.1545.

***tert*-Butyl (*E*,2*R* 3*R*)-1,3-dihydroxy-5-phenylpent-4-en-2-ylcarbamate (2.4)**

To a solution of **2.3** (3.45 g, 11.84 mmol) in anhydrous Et_2O (25 mL) at -78°C, Red-Al (42.67 mL of a 3.33M solution in toluene, 142 mmol, 12 eq.) was added dropwise and

the resulting mixture was allowed to reach room temperature overnight. After diluting the mixture with Et_2O (50 mL), saturated disodium tartrate solution (50 mL) was added dropwise at 0°C and the resulting mixture was stirred for 1 h at room temperature. After separation of the phases, the aqueous layer was extracted with Et_2O (3 x 50 mL) and the combined organic phase was washed with brine (25 mL). After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 1:1) yielding **2.4** (3.046 g, 88%) which proved to be pure enough for further manipulations.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.30 – 1.40 (m, 9H, *tert*-butyl), 3.31 – 3.60 (m, 3H, --C(1)H_2 and -C(2)*H*), 4.31 – 4.39 (m, 1H, -C(3)*H*), 4.52 (br. s, 1H, -C(1)O*H*), 4.99 (d, 1H, *J* = 5.65 Hz, -C(3)O*H*), 6.16 (d, 1H, *J* = 8.67 Hz, -N*H*), 6.26 (dd, 1H, *J* = 5.86 and 15.82 Hz, -C(4)*H*), 6.64 (d, 1H, J = 16.13 Hz, -C(5)*H*), 7.16 - 7.39 (m, 5H, arom. H).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 28.24, 55.54, 64.07, 73.32, 79.89, 126.56, 127.81, 128.44, 131.62, 136.42, 156.41.

Exact mass (ESI-MS) calculated for $C_{16}H_{24}NO_4$ $[M+H]^+$: 294.1705 found: 294.1707.

***N*-((*E*,2*R*,3*R*)-1,3-dihydroxy-5-phenylpent-4-en-2-yl)palmitamide (2.6)**

A) A solution of **2.4** (400 mg, 1.36 mmol) in a dioxane/1N HCl mixture (1:1; 30 mL) was heated to reflux for 30' and subsequently evaporated to dryness. EtOAc (25 mL) and K_2CO_3 (10 mL) were added to the residue and the aqueous phase was extracted with EtOAc (5 x 25 mL). After drying over $MgSO_4$, the solvent was removed under reduced pressure yielding the crude amine (190 mg, 72%). A small sample was purified by column chromatography (CH_2Cl_2:MeOH/6N NH_3 in MeOH 90:10:1) for identification purposes.

^{1}H NMR (300 MHz; DMSO-*d6* + D_2O) δ: 2.87 (td, 1H, *J* = 4.72 and 6.23 Hz, -C(2)*H*), 3.55 (dd, 1H, *J* = 6.24 and 10.92 Hz, -C(1)H_a), 3.70 (dd, 1H, *J* = 4.72 and 11.05Hz, -C(1)H_b), 4.24 (t, 1H, *J* = 6.24 Hz, -C(3)*H*), 6.31 (dd, 1H, *J* = 6.88 and 15.88 Hz, -C(4)*H*), 6.64 (d, 1H, *J* = 15.98 Hz, -C(5)*H*), 7.15 – 7.40 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 57.61, 62.33, 72.32, 126.35, 127.47, 128.81, 129.91, 131.12, 136.97.

Exact mass (ESI-MS) calculated for $C_{11}H_{16}NO_2$ $[M+H]^+$: 194.1181 found: 194.1183.

B) The crude amine (738 mg, 3.82 mmol) was dissolved in a mixture of THF (30 mL) and 50% NaOAc (30 mL). Palmitoyl chloride (1.134 mL, 0.98 eq., 3.743 mmol) was added dropwise over 30' and the reaction mixture was subsequently stirred for 2 h at room temperature. EtOAc (25 mL) was added and the phases were separated. The aqueous layer was extracted twice with EtOAc (25 mL) and the combined organic phase was dried over $MgSO_4$. After removal of the solvent under reduced pressure, the mixture was purified by column chromatography (hexanes:EtOAc 3:1) yielding **2.6** (962 mg, 58%) as a slightly yellow solid.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.84 (t, 3H, *J* = 6.91 Hz, -C*H*$_3$ alkyl), 1.14 – 1.22 (m, 24H, alkyl), 1.34 – 1.48 (m, 2H, -COCH$_2$C*H*$_2$ alkyl), 1.97 – 2.14 (m, 2H, -COC*H*$_2$ alkyl), 3.28 – 3.36 (m, 1H, -C(1)*H*$_a$), 3.51 (ddd, 1H, *J* = 6.06, 6.19 and 10.42 Hz, -C(1)*H*$_b$), 3.84 (ddd, 1H, *J* = 3.12, 6.87 and 9.29 Hz, -C(2)*H*), 4.37 (ddd, 1H, *J* = 5.12, 5.36 and 6.87 Hz, -C(3)*H*), 4.58 (t, 1H, *J* = 5.52 Hz, -C(1)O*H*), 5.07 (d, 1H, *J* = 5.13 Hz, -C(3)O*H*), 6.21 (dd, 1H, *J* = 5.38 and 15.94 Hz, -C(4)*H*), 6.51 (d, 1H, *J* = 16.06 Hz, -C(5)*H*), 7.13 – 7.35 (m, 6H, arom. *H* and –C(2)N*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.89, 22.03, 25.46, 28.52, 28.63, 28.80, 28.84, 28.98, 31.23, 35.43, 54.92, 60.19, 69.24, 126.06, 127.03, 128.41, 128.68, 131.60, 136.97, 172.26.

Exact mass (ESI-MS) calculated for $C_{27}H_{46}NO_3$ $[M+H]^+$: 432.3478 found: 432.3479.

((4*R*,5*R*)-4,5-dihydro-2-pentadecyl-5-styryloxazol-4-yl)methanol (2.7)

To a cooled (0°C) solution of **2.6** (51.3 mg, 0.118 mmol) and TEA (11 μL, 0.118 mmol, 1 eq.) in CH_2Cl_2 (5 mL), MsCl (9 μL, 0.118 mmol, 1 eq.) was added and the reaction was stirred at room temperature for 16 h. The solvent was subsequently removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2:MeOH:6N NH_3 in MeOH 95:5:0.25) yielding the oxazoline **2.7** (19 mg, 38%) as a slightly yellow wax.

^{1}H NMR(300 MHz; $CDCl_3$-*d1*) δ: 0.88 (t, 3H, *J* = 6.63 Hz, -C*H*$_3$ alkyl), 1.19 – 1.45 (m, 24H, alkyl), 1.60 – 1.70 (m, 2H, -N=CCH$_2$C*H*$_2$ alkyl), 2.27 (t, 2H, *J* = 7.49 Hz, -N=CC*H*$_2$ alkyl), 3.54 – 3.72 (m, 2H, C(1)*H*$_2$), 3.89 (dd, 1H, *J* = 5.47 and 9.62 Hz, -C(4')*H*), 4.97 (t, 1H, *J* = 6.95 Hz, C(5')*H*), 6.34 (dd, 1H, *J* = 6.99 and 15.89 Hz, -C(5')C*H*=CHPh), 6.51 (d, 1H, *J* = 15.90 Hz, -C(5')CH=C*H*Ph), 7.13 – 7.35 (m, 5H, arom. H).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 13.62, 22.54, 26.12, 27.93, 28.42, 29.58, 29.67, 63.72, 74.62, 82.18, 126.77, 127.11, 128.37, 128.78, 131.26, 136.84, 166.93.

Exact mass (ESI-MS) calculated for $C_{27}H_{44}NO_2$ $[M+H]^+$: 414.3372 found: 414.3377.

(*R*/*S*) ethyl 1-((*R*)-1-phenylethyl)aziridine-2-carboxylate (2.8 and 2.9)

To a solution of ethyl 2,3-dibromopropanoate (52.57 g, 0.202 mol) and TEA (56.4 mL, 0.404 mol, 2 eq.) in anhydrous toluene (150 mL), D-(+)-α-methylbenzylamine (25 g, 0.206 mol, 1.02 eq.) was added at 0°C and the reaction mixture was subsequently heated at reflux for 3 h. After cooling to room temperature, the organic phase was extracted with 0.5N HCl (3 x 100 mL), sat. $NaHCO_3$ (150 mL) and brine (150 mL) and subsequently dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 3:1) yielding **2.8** (8.56 g, 19%) and **2.9** (7.44 g, 17%) and a mixture of **2.8** and **2.9** (26.54 g, 60%).

2.8:

$[\alpha]^D_{25}$= + 63.30 (*c* 0.85, $CHCl_3$)

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.30 (t, 3H, *J* = 7.32 Hz, -OCH_2CH_3), 1.48 (d, 3H, *J* = 6.45 Hz, -C(1')CH_3), 1.60 (dd, 1H, *J* = 1.05 and 6.41 Hz, -C(3)H_a), 2.13 (dd, 1H, *J* = 0.88 and 3.14 Hz, -C(3)H_b), 2.20 (dd, 1H, *J* = 3.19 and 6.40 Hz, -C(2)*H*), 2.54 (q, 1H, *J* = 6.55 Hz, -C(1')*H*), 4.23 (2 x q, 2H, *J* = 7.14 Hz, -OCH_2CH_3), 7.22 – 7.42 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 14.16, 23.13, 33.95, 38.13, 61.09, 69.87, 126.84, 127.21, 128.29, 143.37, 170.87.

Exact mass (ESI-MS) calculated for $C_{13}H_{18}NO_2$ $[M+H]^+$: 220.1338, found: 220.1339.

2.9:

$[\alpha]^D_{25}$ = + 42.45° (*c* 0.79, $CHCl_3$)

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.21 (t, 3H, *J* = 7.14 Hz, -OCH_2CH_3), 1.46 (d, 3H, *J* = 6.58 Hz, -C(1')CH_3), 1.78 (d, 1H, *J* = 6.75 Hz, -C(3)H_a), 2.05 (dd, 1H, *J* = 3.07 and 6.53 Hz, -C(2)*H*), 2.33 (d, 1H, *J* = 2.83 Hz, -C(3)H_b), 2.57 (q, 1H, *J* = 6.56 Hz, -C*H*CH_3), 4.14 (q, 2H, *J* = 7.04 Hz, -OCH_2CH_3), 7.22 – 7.38 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 14.02, 23.46, 34.77, 37.01, 60.83, 69.57, 126.32, 127.00, 128.30, 143.65, 170.53.

Exact mass (ESI-MS) calculated for $C_{13}H_{18}NO_2$ $[M+H]^+$: 220.1338, found: 220.1339.

((*R*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)methanol (2.10).

To a solution of **2.8** (8.56 g, 39 mmol) in anhydrous THF (100 mL), $LiAlH_4$ (1.11 g, 29.3 mmol, 1.5 eq.) was added at 0°C and the mixture was stirred overnight at room temperature. After cooling back to 0°C, EtOH was added until the formation of H_2-gas ceased, followed by saturated $NaHCO_3$ (150 mL) and EtOAc (150 mL). The aqueous layer was extracted with EtOAc (2 x 100 mL) and the combined organic phase was dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography (hexanes:EtOAc 2:3) yielding **2.10** (6.675 g, 97%) as a colourless oil.

$[\alpha]^D_{25}$ = + 74.47 (c = 1.0; $CHCl_3$)

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.38 (d, 1H, *J* = 6.06 Hz, -C(3')H_a), 1.46 (d, 3H, *J* = 6.15 Hz, -CHCH_3), 1.66 - 176 (m, 1H, -C(3')H_b), 1.80 – 1.90 (m, 1H, -C(2')*H*), 2.54 (q, 1H, *J* = 6.15 Hz, -C*H*CH_3), 3.46 (dd, 1H, *J* = 5.38 and 11.09 Hz, -C(1)H_a), 3.89 (d, 1H, *J* = 11.00 Hz, -C(1)H_b), 7.22 – 7.40 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 23.46, 30.65, 40.32, 62.75, 69.05, 126.72, 127.12, 128.30, 143.68.

Exact mass (ESI-MS) calculated for $C_{11}H_{16}NO$ $[M+H]^+$: 178.1232, found: 178.1238.

((*S*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)methanol (2.11).

To a solution of **2.9** (120 mg, 0.547 mmol) in anhydrous THF (1 mL), $LiAlH_4$ (16 mg, 0.421 mmol, 1.5 eq.) was added at 0°C and the mixture was stirred overnight at room temperature. After cooling back to 0°C, two drops of ethanol were added, followed by sat. $NaHCO_3$ (5 mL) and EtOAc (5 mL). The aqueous layer was extracted with EtOAc (2 x 5 mL) and the combined organic phase was dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography (hexanes:EtOAc 2:3) yielding **2.11** (92 mg, 95%).

$[\alpha]^D_{25}$= + 53.74 (c = 1.0; $CHCl_3$)

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.43 (d, 3H, *J* = 6.74 Hz, -CHCH_3), 1.50 (d, 1H, *J* = 6.45 Hz, -C(3')H_a), 1.64 – 1.72 (m, 1H, -C(3')H_b), 1.92 (d, 1H, *J* = 3.42 Hz, -C(2')*H*), 2.54 (q, 1H, *J* = 6.45 Hz, -C*H*CH_3), 3.34 (dd, 1H, *J* = 4.98 and 11.43 Hz, -C(1)H_a), 3.60 (dd, 1H, *J* = 3.52 and 11.43 Hz, -C(1)H_b), 7.22 – 7.38 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 22.66, 31.21, 39.88, 62.52, 69.23, 126.73, 127.34, 128.20, 143.92.

Exact mass (ESI-MS) calculated for $C_{11}H_{16}NO$ $[M+H]^+$: 178.1232, found: 178.1238.

(*R*)-1-((*R*)-1-phenylethyl)aziridine-2-carbaldehyde (2.12)

To a solution of oxalylchloride (28.2 mL of a 2M solution in CH_2Cl_2, 56.4 mmol, 1.5 eq.) at -78°C, a solution of DMSO (8 mL, 113 mmol, 3 eq.) in CH_2Cl_2 (10 mL), was added dropwise over 30' while the temperature raised to -70°C. When the solution had reached -60°C, a solution of **2.10** (6.675 g, 37.66 mmol) in CH_2Cl_2 (60 mL), was added dropwise over 60' while the temperature raised to -55°C. When the solution had reached -45°C, a solution of DIPEA (37.3 mL, 226 mmol, 6 eq.) in CH_2Cl_2 (10 mL) was added over 5' and the resulting solution was stirred for 30' at 0°C. The organic phase was subsequently extracted with ice-cold 0.1N HCL (200 mL) and the aqueous phase was back-extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was subsequently washed with phosphate buffer (3 x 100 mL, pH 7.6) and dried over Na_2SO_4. After removal of all volatiles *in vacuo*, the residue was purified by column chromatography (hexanes:EtOAc 85:15) yielding **2.12** (4.765 g, 72%) as a dark yellow oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.44 (d, 3H, *J* = 6.74 Hz, -CHC*H*$_3$), 1.81 (d, 1H, *J* = 6.75 Hz, -C(3)*H*$_a$), 2.14 – 2.26 (m, 2H, -C(3)*H*$_b$ and C(2)*H*), 2.61 (q, 1H, *J* = 6.75 Hz, -C*H*CH_3), 7.22 – 7.40 (m, 5H, arom. *H*), 8.96 (d, 1H, *J* = 6.45 Hz, aldehyde *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 22.96, 32.16, 45.11, 69.00, 126.62, 127.43, 128.48, 143.41, 200.05.

Exact mass (ESI-MS) calculated for $C_{11}H_{14}NO$ $[M+H]^+$: 176,1075, found: 176,1077.

(*R*/*S*)-3-phenyl-1-((*R*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)prop-2-yn-1-ol (2.13 and 2.14)

Method A: LiCl (17.15 g, 404 mmol, 15 eq.) was suspended in anhydrous THF (50 mL) and lithium phenylacetylide (54 mL of a 1M solution in THF, 54 mmol, 2 eq.) was added to the cooled (-78°C) solution. After stirring for 30', a solution of **2.12** (4.725 g, 26.96 mmol) in anhydrous THF (25 mL) was added dropwise over 30' and the resulting reaction mixture was allowed to warm to room temperature overnight. Saturated NH_4Cl (50 mL) was slowly added and the aqueous layer was extracted

with Et_2O (3 x 50 mL) and the combined organic layer was washed with brine (25 mL), dried over $MgSO_4$ and concentrated *in vacuo*. A mixture of erythro- and threo-alkynols was obtained, which was separated by flash chromatography (hexanes:EtOAc 4:1) affording **2.13** (4.30 g, 57%), **2.14** (728 mg, 10%) and a mixture of **2.13** and **2.14** (1.253 g, 17%).

Method B: LiBr (2.28 g, 26.31 mmol, 15 eq.) was added to a solution of **2.16** (483 mg, 1.75 mmol) in anhydrous THF (10 mL) and the resulting suspension was stirred for 30' at room temperature and subsequently cooled to -78°C, followed by slow addition of L-Selectride (3.5 mL of a 1M solution in THF, 3.5 mmol, 2 eq.). After stirring for 1 h at -78°C, TLC indicated complete consumption of the starting material. The reaction was quenched by slow addition of EtOH (2 mL) followed by addition of sat. NH_4Cl (10 mL). After separation of the phases, the aqueous layer was extracted with EtOAc (2 x 10 mL). Drying over $MgSO_4$, followed by removal of all volatiles under reduced pressure afforded mixture of both diastereomers which was separated by flash chromatography (hexanes:EtOAc 4:1) furnishing **2.13** (253 mg, 52%), **2.14** (44 mg, 9%) and a mixture of **2.13** and **2.14** (73 mg, 15%)

2.13:

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.42 (d, 1H, *J* = 6.34 Hz, -C(3')H_a), 1.55 (d, 3H, *J* = 6.45 Hz, -CHCH_3), 1.86 (d, 1H, *J* = 3.39 Hz, -C(3')H_b), 2.07 (td, 1H, *J* = 3.45 and 6.64 Hz, -C(2')*H*), 2.65 (q, 1H, *J* = 6.45 Hz, -C*H*CH_3), 3.46 (br s, 1H, -C(1)O*H*), 4.49 (d, 1H, *J* = 2.28 Hz, -C(1)*H*), 7.22 – 7.51 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 23.63, 30.32, 43.72, 61.07, 68.62, 84.51, 89.03, 122.53, 126.70, 127.09, 128.24, 128.33, 128.40, 131.71, 144.04.

Exact mass (ESI-MS) calculated for $C_{19}H_{20}NO$ $[M+H]^+$: 278.1545, found: 278.1543.

2.14:

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.47 (d, 1H, *J* = 6.23 Hz, -C(3')H_a), 1.48 (d, 1H, *J* = 6.58 Hz, -CHCH_3), 1.98 (d, 1H, *J* = 3.40 Hz, , -C(3')H_b), 2.10 (ddd, 1H, *J* = 3.43, 4.67 and 6.24 Hz, -C(2')*H*), 2.67 (q, 1H, *J* = 6.53 Hz, -C*H*CH_3), 2.98 (br s, 1H, -C(1)O*H*), 4.77 (d, 1H, *J* = 4.62 Hz, -C(1)*H*), 7.25 – 7.50 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 23.48, 30.68, 42.98, 61.70, 68.75, 85.33, 87.64, 122.47, 126.69, 127.16, 128.24, 128.37, 128.45, 131.76, 143.99.

Exact mass (ESI-MS) calculated for $C_{19}H_{20}NO$ $[M+H]^+$: 278.1545, found: 278.1543.

3-phenyl-1-((*R*)-1-((*R*)-1-phenylethyl)aziridin-2-yl)prop-2-yn-1-one (2.16)

Dess-Martin periodinane (14.5 mL of a 0.467 M solution in CH_2Cl_2, 6.77 mmol, 1.5 eq.) was added to a solution of **2.13** and **2.14** (1.253 g, 4.517 mmol) in CH_2Cl_2 (20 mL) and the resulting solution was stirred until TLC indicated complete consumption of the starting material (2 h). $NaHCO_3$ (20 mL) was added and after separation of both phases, the organic layer was washed with brine (2 x 20 mL). After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 9:1 → 85:15) affording ketone **2.15** (1.02 g, 82%) as a dark brown oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.49 (d, 3H, *J* = 6.54 Hz, -CHC*H*$_3$), 1.78 (d, 1H, *J* = 6.16 Hz, -C(3')*H*$_a$), 2.34 – 2.39 (m, 1H, -C(3')*H*$_b$), 2.51 (dd, 1H, *J* = 2.81 and 6.33 Hz, -C(2')*H*), 2.64 (q, 1H, *J* = 6.48 Hz, -C*H*CH_3), 7.24 – 7.64 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 16.43, 28.73, 39.31, 62.71, 79.28, 85.74, 119.81, 120.31, 121.40, 121.61, 123.82, 126.26, 136.69, 178.80.

Exact mass (ESI-MS) calculated for $C_{19}H_{18}NO$ $[M+H]^+$: 276.1383, found: 276.1387.

(3*R*,4*R*)-4-((*R*)-1-phenylethylamino)-5-azido-1-phenylpent-1-yn-3-ol (2.17)

To a solution of **2.13** (122 mg, 0.440 mmol) in CH_2Cl_2 (5 mL), $TMSN_3$ (173 μL, 1.32 mmol, 3 eq.) was added. The mixture was stirred for 16 h at room temperature and subsequently treated with 1N HCl (5 mL) and stirring was continued for 1 h. Sat. K_2CO_3 (25 mL) and EtOAc (50 mL) were added and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic phase was dried over Na_2SO_4 and removed under reduced pressure. Flash chromatography (hexanes:EtOAc 4:1) provided **2.17** (141 mg, quant.) as a white solid.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.42 (d, 3H, *J* = 6.45 Hz, -CHC*H*$_3$), 2.93 (m, 1H, -C(4)*H*), 3.32 (dd, 1H, *J* = 4.98 and 12.60 Hz, -C(5)*H*$_a$), 3.46 (dd, 1H, *J* = 5.28 and 12.31 Hz, -C(5)*H*$_b$), 4.08 (q, 1H, *J* = 6.45 Hz, -C*H*CH_3), 4.43 (d, 1H, *J* = 5.87 Hz, -C(3)*H*), 7.24 – 7.50 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 24.28, 51.84, 56.80, 59.41, 62.13, 86.04, 87.76, 122.26, 126.46, 127.45, 128.34, 128.62, 128.71, 131.71, 145.04.

Exact mass (ESI-MS) calculated for $C_{19}H_{21}N_4O$ $[M+H]^+$: 321.1715, found: 321.1717.

N-((2*R*,3*R*)-1-azido-3-hydroxy-5-phenylpent-4-yn-2-yl)-*N*-((*R*)-1-phenylethyl) palmitamide (2.19)

To a cooled solution (0°C) of **2.17** (637 mg, 1.99 mmol) and DIPEA (1.02 mL, 5.97 mmol, 3 eq.) in anhydrous CH_2Cl_2 (10 mL), palmitoyl chloride (584 μL, 1.93 mmol, 0.97 eq.) was added dropwise and the resulting solution was stirred overnight at room temperature. Sat. $NaHCO_3$ (10 mL) was added and after separation of the phases, the organic layer was washed with $NaHCO_3$ (10 mL) and brine (10 mL). After drying over $MgSO_4$ and removal of all volatiles under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 97:3) yielding **2.19** (862 mg, 78%) as a colourless oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 0.88 (t, 3H, *J* = 6.51 Hz, -*CH*$_3$ acyl), 1.20 – 1.35 (m, 24H, acyl *H*), 1.38 (d, 3H, *J* = 6.49 Hz, -CHC*H*$_3$), 1.55 – 1.70 (m, 3H, -$COCH_2CH_2$) and –C(3)O*H*), 2.35 (t, 2H, *J* = 7.63 Hz, -COC*H*$_2$), 2.89 (td, 1H, *J* = 4.83 and 6.77 Hz, -C(2)*H*), 3.32 (dd, 1H, *J* = 4.93 and 12.60 Hz, -C(1)*H*$_a$), 3.46 (dd, 1H, *J* = 7.18 and 12.56 Hz, -C(1)*H*$_b$), 4.13 (q, 1H, *J* = 6.42 Hz, -C*H*CH_3), 5.86 (d, 1H, *J* = 4.66 Hz, -C(3)*H*), 7.24 – 7.50 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 14.64, 22.78, 26.06, 25.57, 28.94, 29.30, 29.39, 29.53, 29.60, 29.68, 29.71, 32.00, 34.06, 52.33, 55.96, 58.00, 64.82, 85.29, 86.94, 121.98, 127.36, 127.55, 129.35, 129.86, 132.34, 146.22, 172.50.

Exact mass (ESI-MS) calculated for $C_{35}H_{51}N_4O_2$ $[M+H]^+$: 559.4012, found: 559.4017.

(*R*)-methyl 2-(dibenzylamino)-3-hydroxypropanoate (2.21)

To a solution of D-serine methyl ester hydrochloride (25.13 g, 161.5 mmol) in MeCN (400 mL), potassium carbonate (111.6 g, 0.808 mol, 5 eq.) was added, followed by benzyl bromide (69.1 g, 0.404 mol, 2.5 eq.). After stirring for 24 h at room temperature, water (500 mL) was added and after separation of both phases, the aqueous phase was extracted with EtOAc (3 × 500 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The

resulting residue was purified by column chromatography (hexanes:EtOAc 4:1 → 65:35) affording the title compound (42.01 g, 87%) as a colourless oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 2.58 (br.s, 1H, -C(3)O*H*), 3.59 (m, 1H, -C(2)*H*), 3.68 (d, 2H, *J* = 14.31 Hz, benzyl -CH_2), 3.71 - 3.82 (m, 2H, -C(3)H_2), 3.83 (s, 3H, -OCH_3), 3.94 (d, 2H, *J* = 14.31 Hz, benzyl -CH_2), 7.18 - 7.39 (m, 10 H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 51.23, 55.00, 59.29, 61.61, 127.27, 128.40, 128.90, 138.56, 171.09.

Exact mass (ESI-MS) calculated for $C_{18}H_{22}NO_3$ $[M+H]^+$: 300.1600, found: 300.1594.

Methyl (2*R*)-3-(*tert*-butyldiphenylsiloxy)-2-(dibenzylamino)-propanoate (2.22)

To a solution of ester **2.21** (15.0 g, 50.11 mmol) and imidazole (13.65 g, 0.2 mol, 4 eq.) in anhydrous DMF (100 mL), TBDPSCl (41.3 g, 0.15 mol, 3 eq.) was added at 0°C and the mixture was stirred for 5 h at room temperature. After removal of the solvent *in vacuo,* EtOAc (300 mL) was added to the residue and the organic layer was washed with brine (100 mL) and subsequently dried over $MgSO_4$. After removal of all volatiles *in vacuo*, the residue was purified by column chromatography (hexanes:EtOAc 95:5) yielding the title compound (26.9 g, 100%) as a viscous colourless oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.05 (s, 9H, *tert*-butyl), 3.68 (t, 1H, *J* = 6.18 Hz, -C(2)*H*), 3.76 (s, 3H, -OCH_3), 3.78 (d, 2H, *J* = 14.10 Hz, benzyl -CH_2), 3.99 (dd, 1H, *J* = 6.18 and 10.22 Hz, -C(3)H_a), 4.02 (d, 2H, *J* = 14.10 Hz, benzyl -CH_2), 4.07 (dd, 1H, *J* = 6.18 and 10.22 Hz, -C(3)H_b), 7.18 - 7.65 (m, 20H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 18.99, 26.50, 51.08, 55.25, 62.78, 63.12, 126.79, 127.27, 128.41, 128.90, 129.51, 133.05, 134.47, 139.59, 171.12.

Exact mass (ESI-MS) calculated for $C_{34}H_{40}NO_3Si$ $[M+H]^+$: 538,2777, found: 538,2772.

(*3R,4R*)-4-(dibenzylamino)-5-(*tert*-butyldiphenylsiloxy)-1-phenylpent-1-yn-3-ol (2.24)

To a solution of **2.22** (4.643 g, 8.63 mmol) in anhydrous toluene (75 mL), DiBAlH (17.27 mL of a 1M solution in toluene, 17.27 mmol, 2 eq.) was added dropwise over 60'. The mixture was stirred for 3 h at -78°C and subsequently quenched by slow addition of EtOH until the evolution of H_2-gas ceased. After addition of celite (40 g)

and subsequent filtration of the reaction mixture, all volatiles were removed under reduced pressure affording crude aldehyde **2.23**, which was used without further purification.

Anhydrous $ZnBr_2$ (2.53 g, 11.22 mmol, 1.3 eq.) was added to a cooled (0°C) solution of lithium phenylacetylide (10.4 mL of a 1M solution in THF, 10.4 mmol, 1.2 eq.) in anhydrous Et_2O (200 mL) and the resulting mixture was stirred for 2 h at room temperature. A cooled (-78°C) solution of crude **2.23** in anhydrous Et_2O (20 mL) was subsequently added at -78°C over 60' and the resulting mixture was allowed to reach room temperature overnight. The reaction was quenched by slow addition of sat. NH_4Cl (50 mL). After separation of both phases, the aqueous layer was extracted with Et_2O (2 x 100 mL) and the combined organic layers were washed with 0.5N HCl (100 mL) and brine (100 mL) and subsequently dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 93:7) yielding *threo*-**2.24** (3.36 g, 64%) and an impure fraction (262 mg, 5%), both as slightly yellow oils.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.03 (s, 9H, *tert*-butyl), 3.04 (dd, 1H, *J* = 6.15 and 10.95 Hz, -C(4)*H*), 3.86 (d, 2H, *J* = 14.36 Hz, benzyl -CH_2), 3.97 - 4.07 (m, 4H, benzyl -CH_2 and -C(5)H_2), 4.80 (1H, d, *J* = 6.16 Hz, -C(3)*H*), 5.57 (br s, 1H, -C(3)O*H*), 7.17 – 7.72 (m, 25H, arom. H)

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 18.67, 26.59, 54.59, 61.19, 62.15, 63.20, 84.51, 91.13, 109.26, 122.29, 126.61, 127.44, 127.78, 127.99, 128.36, 128.55, 129.81, 131.01, 132.68, 132.75, 134.43, 135.06, 135.12, 140.36.

Exact mass (ESI-MS) calculated for $C_{41}H_{44}NO_2Si$ $[M+H]^+$: 610.3141, found: 610.3148.

(*2R,3R*)-2-(dibenzylamino)-5-phenylpent-4-yne-1,3-diol (2.25)

To a solution of **2.24** (3.362 g, 5.51 mmol) in THF (50 mL), TBAF (11 mL of a solution 1M in THF, 11 mmol, 2 eq.) was added and the reaction mixture was stirred at room temperature until TLC indicated the disappearance of the starting material (30'). After removal of the solvent under reduced pressure, EtOAc (100 mL) and water (100 mL) were added and after separation of both phases, the aqueous layer was extracted with EtOAc (2 x 50 mL) and the combined organic phase was dried over $MgSO_4$. After removal of all volatiles under reduced pressure, the residue was

purified by column chromatography (hexanes:EtOAc 7:3) yielding the title compound (1.717 g, 84%) as a colourless oil.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 2.84 (dd, 1H, *J* = 5.86 and 11.72 Hz, -C(2)*H*), 3.81 (m, 4H, benzyl -CH_2 and -C(1)H_2), 4.01 (d, 2H, *J* = 13.78 Hz, benzyl -CH_2), 4.45 (br s, 1H, -C(1)O*H*), 4.71 (m, 1H, -C(3)*H*), 5.44 (br s, 1H, -C(3)O*H*), 7.14 – 7.45 (m, 15H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 54.59, 58.33, 61.46, 63.15, 84.29, 91.56, 122.57, 126.58, 127.98, 128.34, 128.55, 128.63, 131.05, 140.55.

Exact mass (ESI-MS) calculated for $C_{25}H_{26}NO_2$ $[M+H]^+$: 372.1964, found: 372.1967.

(4*R*,5*R*)-*N*,*N*-dibenzyl-2,2-dimethyl-4-(2-phenylethynyl)-1,3-dioxan-5-amine (2.27).

To a solution of **2.25** (37.2 mg, 0.1 mmol) in 2,2-dimethoxypropane/DMF (1:1; 3 mL), PPTS (cat.) was added and the mixture was stirred for 2 h at room temperature. The solvent was subsequently removed under reduced pressure and column chromatography (hexanes:EtOAc 96:4) of the residue yielded **2.27** (35 mg, 85%) as a colourless oil.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.33 (s, 3H, -CH_3 isopropylidene), 1.42 (s, 3H, -CH_3 isopropylidene), 2.57 - 2.64 (m, 1H, -C(5)*H*), 3.69 (d, 2H, *J* = 14.36 Hz, benzyl -CH_2), 3.96 (dd, 1H, *J* = 3.81 and 12.60 Hz, - C(6)H_a), 4.20 (dd, 1H, *J* = 2.06 and 12.59 Hz, - C(6)H_b), 4.23 (d, 2H, *J* = 14.07 Hz, benzyl -CH_2), 5.29 (d, 1H, *J* = 4.11 Hz, -C(4)*H*), 7.12 - 7.25 (m, 6H, arom. *H*), 7.42 - 7.58 (m, 9H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 19.58, 28.52, 52.04, 54.94, 58.11, 64.78, 85.82, 88.01, 98.73, 122.28, 126.65, 128.00, 128.28, 128.72, 128.83, 131.10, 141.84.

Exact mass (ESI-MS) calculated for $C_{28}H_{30}N_2O$ $[M+H]^+$: 412.2277, found: 412.2274.

Mesylation of 2.25:

To an ice-cold solution of **2.21** (1.1 g, 3.67 mmol), 4-DMAP (5 mg; cat.) and DIPEA (1.82 mL, 11.02 mmol, 3 eq.) in CH_2Cl_2 (20 mL), MsCl (427 μL, 5.51 mmol, 1.5 eq.) was added and the resulting mixture was stirred at room temperature. After 20', TLC indicated the formation of a single new product with a higher Rf whereas after 80', a more polar product appeared on TLC. A small aliquot was withdrawn from the reaction mixture and purified by column chromatography (hexanes:EtOAc 8:2 → 7:3)

affording two products which were identified as **2.26** and **2.28** (4:1). The reaction mixture was stirred for 15 h at room temperature and after removal of the solvent under reduced pressure, the residue was purified by column chromatography yielding **2.26** (624 mg, 45%) and **2.28** (476 mg, 46%).

To a solution of **2.26** (624 mg, 1.65 mmol) in anhydrous DMF (10 mL), morpholine (1.44 mL, 16.55 mmol, 10 eq.) was added and the resulting solution was heated at 50°C. After 1 h, TLC analysis indicated the complete consumption of the starting material and the formation of a single new product. Removal of the solvent under reduced pressure, followed by column chromatography (hexanes:EtOAc 7:3) rendered **2.28** (465 mg, 100%) as a white solid.

(*R*)-2-(methoxycarbonyl)-2-(dibenzylamino)ethyl methanesulfonate (2.26)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 3.12 (s, 3H, mesyl -CH_3), 3.59 (d, 2H, *J* = 13.78 Hz, benzyl -CH_2), 3.60 - 3.66 (m, 1H, -C(2)*H*), 3.72 (s, 3H, -OCH_3), 3.81 (d, 2H, *J* = 13.78 Hz, benzyl -CH_2), 4.42 (dd, 1H, *J* = 7.03 and 10.55 Hz, -C(1)H_a), 4.51 (dd, 1H, *J* = 6.45 and 10.56 Hz, -C(1)H_b), 7.19 - 7.40 (m, 10H, arom. *H*).

^{13}C NMR (75 MHz; DMSO) δ: 36.54, 51.75, 54.36, 59.80, 67.45, 127.21, 128.34, 128.54, 138.79, 169.74.

Exact mass (ESI-MS) calculated for $C_{19}H_{24}NO_5S$ $[M+H]^+$: 378.1375, found: 378.1379.

(*R*)-methyl 2-benzyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (2.28)

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 2.90 (dd, 1H, *J* = 5.57 and 13.19 Hz, -C(4)H_a), 3.20 (dd, 1H, *J* = 9.38 and 13.19 Hz, -C(4)H_b), 3.57 (d, 2H, *J* = 13.48 Hz, benzyl -CH_2), 3.68 (d, 2H, *J* = 13.48 Hz, benzyl -CH_2), 3.70 (s, 3H, -OCH_3), 4.18 (dd, 1H, *J* = 5.86 and 9.38 Hz, -C(3)*H*), 7.22 - 7.42 (m, 9H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 52.73, 53.94, 54.98, 57.82, 58.78, 127.24, 127.28, 128.29, 128.33, 128.82, 128.94, 138.45, 169.55.

Exact mass (ESI-MS) calculated for $C_{18}H_{20}NO_2$ $[M+H]^+$: 282.1494, found: 282.1493.

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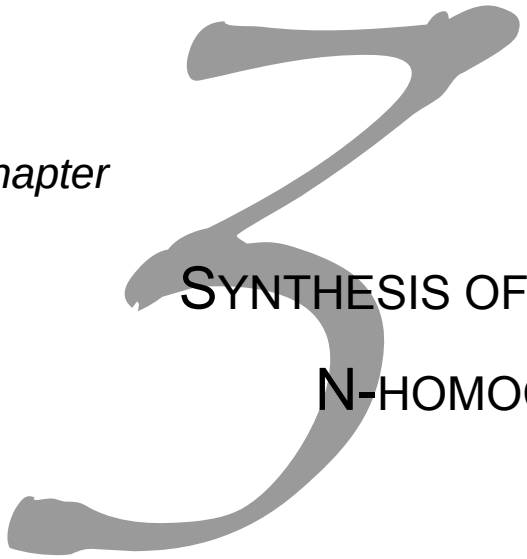
Chapter



SYNTHESIS OF A NOVEL CLASS OF N-HOMOCERAMIDES

10 D-GALACTOSE AS SOURCE OF CHIRALITY FOR THE SYNTHESIS OF N-HOMOCERAMIDES

10.1. INTRODUCTION - RETROSYNTHESIS

As chiral templates for the synthesis of biologically active compounds, sugars offer inherent advantages including extensive stereodiversity, high degree of functionality and, most importantly, high optical purity. It is therefore logical that sugars have extensively been exploited for the synthesis of SLs.[1] A typical synthetic protocol comprises the introduction of the amino group by activation of a sugar hydroxyl group followed by substitution with an appropriate N-nucleophile and the oxidative removal of unnecessary carbon units, thereby providing a suitable aldehyde intermediate for introduction of the aliphatic moiety.

A particularly interesting method for the synthesis of ceramides, as first reported by Schmidt *et al.,*[2] relies on the stereochemical and structural features of D-galactose. An optimized version of this synthetic strategy was later proposed by Duclos Jr.[3] The main advantages of this method over other reported strategies include the relatively limited number of synthetic steps and the ease of up-scaling, which allows the synthesis of large amounts of key intermediates. While the reported synthetic strategy aims at the synthesis of natural ceramides, thereby introducing the amino-group through substitution of the activated C2-hydroxyl function by an azide, introduction of an extra-methylene spacer for our envisioned compounds could be accomplished by simple replacement of the azide by a cyanide nucleophile (Figure 10.1). Indeed, nitriles have shown to be flexible functional groups for introduction of branching since judicious selection of derivatizing conditions allows straightforward access to the corresponding aldehydes,[4] carboxylic acids[5] or primary amines.[6] The resulting intermediate nitrile could then simply be elaborated to the desired N-homoceramides in 3 steps, including reduction of the nitrile to the corresponding primary amine, acylation and final benzylidene deprotection.

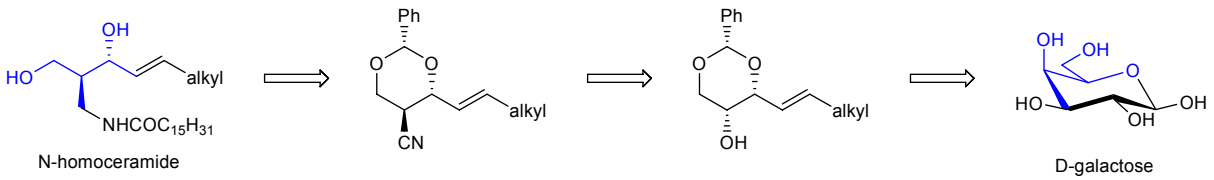


Figure 10.1: Retrosynthetic scheme for the synthesis of N-homoceramides starting from D-galactose. The sphingoid backbone is depicted in blue.

10.2. SYNTHESIS

Treatment of D-galactose with benzaldehyde in the presence of anhydrous $ZnCl_2$ afforded 4,6-*O*-benzylidene-α/β-D-galactose **3.1** (54%), which was oxidatively cleaved with sodium metaperiodate to give access to threose **3.2** (Figure 10.2).

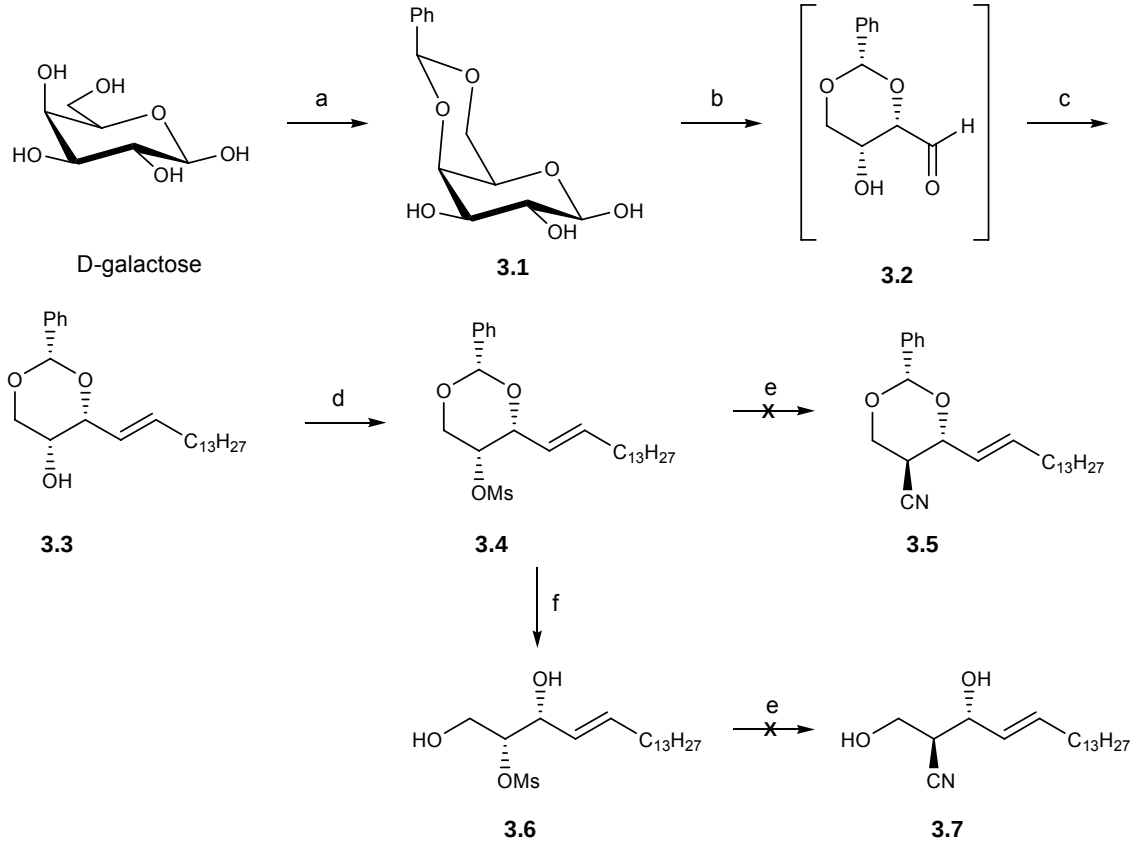


Figure 10.2: a) D-galactose, benzaldehyde, $ZnCl_2$, rt, 14 h (54%); b) $NaIO_4$, phosphate buffer pH 7.8, 1N NaOH, rt, 30' (100%); c) *i.* PPh_3, tetradecylbromide, 170°C, 60 h (76%); *ii.* bromobenzene, Li, tetradecylphosphoniumbromide, toluene:THF (9:2), -30°C, 3.5 h (46%); d) MsCl, TEA, THF, 0°C, 1 h (70%); e) see Table 10.1; f) *p*-TsOH, THF:MeOH (1:1), rt, 8 h (100%).

Schlosser-type modification of the Wittig reaction by treatment of the crude threose **3.2** with lithium and tetradecylphosphoniumbromide, prepared from bromobenzene and Li, afforded almost exclusively *E*-alkene **3.3** (*E*:*Z* >95:5; $^3J_{4,5}$ = 15.84 Hz), albeit in very modest yield (46%). Activation of the secondary alcohol in **3.3** is preferably achieved by mesylation since other leaving groups, such as the tosylate and triflate, have been reported to produce complex reaction mixtures.[7] Hence, the intermediate mesylate **3.4** was easily prepared in the presence of excess TEA in CH_2Cl_2 (70%).

Surprisingly, treatment of mesylate **3.4** with different cyanides failed to produce the desired nitrile **3.5** under various reaction conditions (Table 10.1). As lower reaction temperatures resulted in full recuperation of the starting material, higher temperatures gave rise to a gamut of products. These complex reaction mixtures might originate from double bond isomerisation and benzylidene deprotection, as previously described.[7] Since it had been shown that introduction of an azide on C2 of the sphingoid backbone proceeded more smoothly on the benzylidene deprotected intermediate thereby avoiding side products resulting from debenzylidenation, we decided to submit **3.4** to acid-catalyzed deprotection. Hence, 1,3-diol **3.6** was obtained quantitatively upon treatment of **3.4** with a catalytical amount of *p*-TsOH in MeOH:THF. Unfortunately, an identical reaction profile as for mesylate **3.4** was observed upon treatment of **3.6** with different cyanides. These results necessitated us to explore alternative routes for the synthesis of 2-cyano-1,3-diols.

Table 10.1: Tested reaction conditions for displacement of the mesyl group in **3.4** and **3.6** by CN^-.

Solvent	CN^- source (eq.)	T (°C)	Time (h)	Result
DMSO	NaCN (15)[4]	95	168	Decomposition
DMSO	NaCN (15) + 18-crown-6 (cat)	50	96	No reaction
DMSO	Bu_4NCN (2)[8]	50 → 90	96 + 24	Decomposition
DMSO	KCN (10)[9]	95	96	Decomposition
DMSO	Bu_4NCN (2) / NaCN[10]	95	96	Decomposition
MeCN	KCN (10) + 18-crown-6 (cat)	Reflux	48	No reaction
EtOH/H_2O (3:1)	KCN (6)[11]	Reflux	96	No reaction

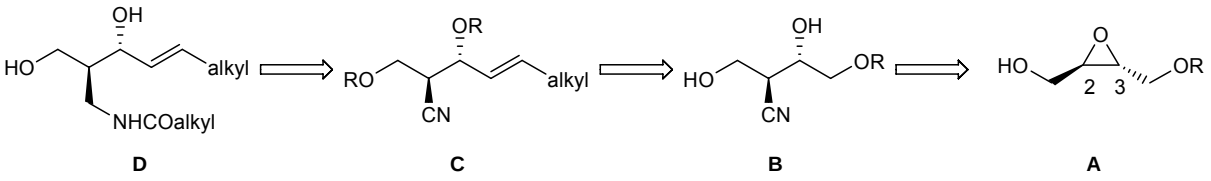
11 ACCESS TO N-HOMOCERAMIDES THROUGH REGIOSELECTIVE EPOXIDE OPENING

11.1. SYNTHESIS VIA A C4-O-BENZYL PROTECTED 2,3-EPOXY ALCOHOL

11.1.1 INTRODUCTION - RETROSYNTHESIS

In view of the flexiblility of the cyano-group for homologation, we investigated alternative methods for the synthesis of a 2-cyano-1,3-diol scaffold. A literature survey indicated that 2-cyano-1,3-diols have been accessed only through nucleophilic 2,3-epoxy alcohol opening.[12] However, a majority of literature reports have dealt with selective C3- rather than the C2-opening, required for our envisioned compounds.

Recently, Sasaki *et al.*[12g] reported a convenient procedure to access 2-cyano-1,3-diols by C2 regioselective substitution (up to 92:8) of various 2,3-epoxy alcohols (Scheme 11.1; **A**). Most interestingly, the epoxide-opening products (**B**) possess the required *erythro* stereochemistry for construction of the homosphingoid backbone. Hence, starting from the 2-cyano-1,3-diol, the *E*-alkene intermediate (**C**) could be obtained in 4 steps involving protection of the primary and secondary alcohol, oxidation of the deprotected C4 alcohol to the corresponding aldehyde and Wittig olefination. Reduction of the nitrile to the primary amine followed by acylation and final deprotection of the alcohols would afford the desired N-homoceramides (**D**).

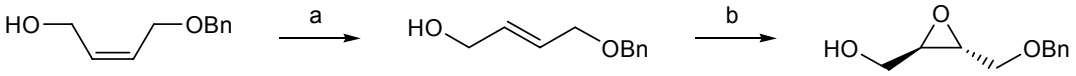


Scheme 11.1: Retrosynthetic scheme for the synthesis of N-homoceramides by regioselective epoxide opening.

11.1.2 SYNTHESIS

Commercially available *Z*-(benzyloxy)but-2-en-1-ol served as starting material and was isomerised to its *E*-isomer **3.7** in a two step sequence entailing oxidation of

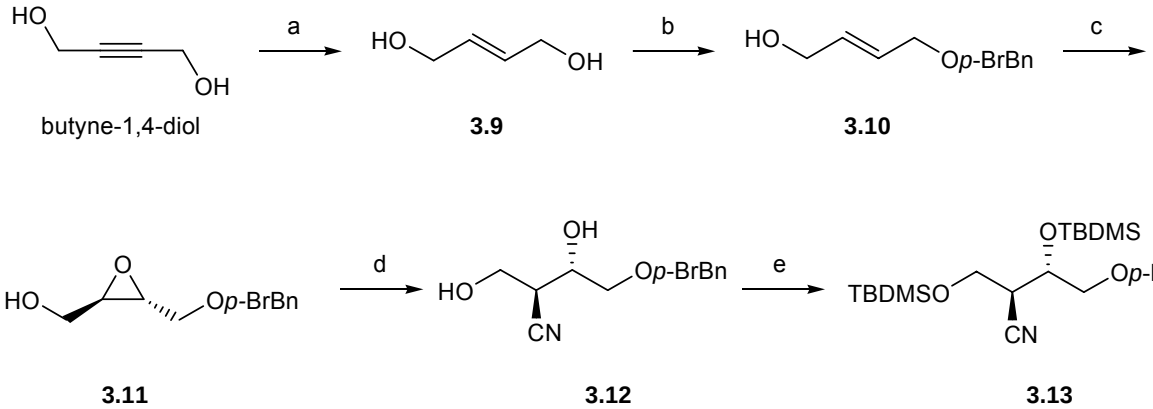
the primary alcohol with PCC and subsequent reduction of the crude aldehyde with DiBAlH (61%). Sharpless epoxidation[13] using standard conditions gave access to epoxide **3.8** in good yield (80%). Although the optical purity of epoxide **3.8** ($[\alpha]^{D}_{25}$= +19.6° in $CHCl_3$) was consistent with literature reports[14] ($[\alpha]^{D}_{25}$= +19.6°; ee = 94%), this lower ee value might compromise the outcome of biological assays. Since epoxide **3.8** prevails as an oil, improvement of the enantiomeric excess by crystallisation would necessitate its conversion to a crystallisable ester. Subsequent recrystallisation and hydrolysis of the ester would afford enantiomerically pure **3.8**.



Z-4-(benzyloxy)but-2-en-1-ol **3.7** **3.8**

Scheme 11.2: a) *i.* PCC, celite, CH_2Cl_2, RT, 18 h; *ii.* DiBalH, Et_2O, -78°C to RT, 90' (61%); b) $Ti(OiPr)_4$, D-(-)-DET, TBHP, CH_2Cl_2 , -20°C, overnight (80%).

However, it seemed more convenient at this point to adjust the synthetic route to allow recrystallisation without the involvement of extra synthetic steps. To this aim, we opted to switch the benzyl-protecting group for a *p*-bromobenzyl-protecting group.[15] Since the *p*-bromobenzyl analogue of the starting material was not commercially available, an alternative synthetic approach had to be pursued (Scheme 11.3).

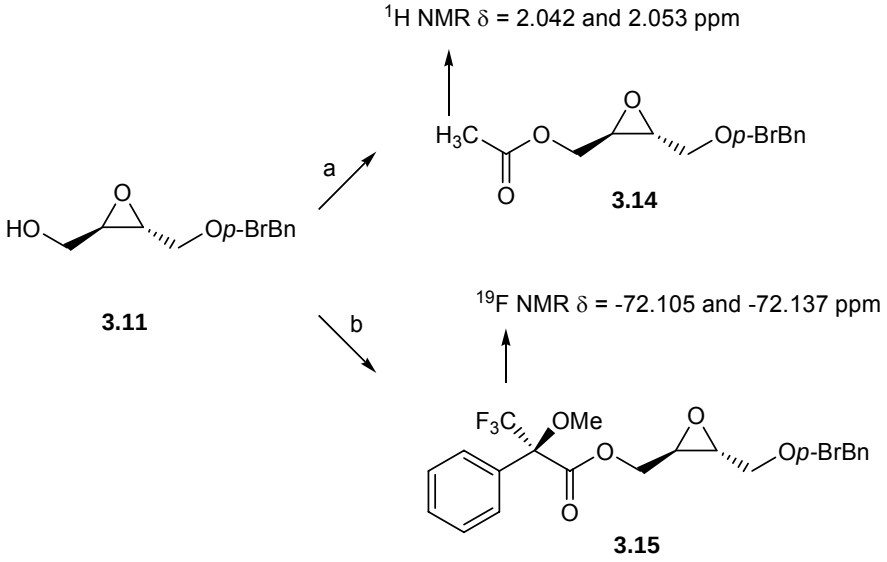


butyne-1,4-diol **3.9** **3.10**

3.11 **3.12** **3.13**

Scheme 11.3: a) $LiAlH_4$, THF, 0°C to reflux, 3.5 h (80%); b) Ag_2O, *p*-bromobenzyl bromide, CH_2Cl_2, RT, 15 h (81%); c) $Ti(OiPr)_4$, D-(-)-DET, TBHP, CH_2Cl_2 , -20°C, overnight (98%; 61% after recrystallisation); d) NaCN, $B(OEt)_3$, DMF, 70°C, 15 h (35%); e) TBDMSCl, imidazole, DMF, 0°C to RT, overnight (93%).

Hence, reduction of butyne-1,4-diol with $LiAlH_4$ to *E*-alkene **3.9** (80%) and subsequent Ag_2O mediated chemoselective monobenzylation with *p*-bromobenzyl bromide afforded alcohol **3.10** in good yield (81%). This method proved to be superior over standard conditions (4-bromobenzyl bromide, NaH, THF) for monobenzylation of 1,4-diols.[16] Asymmetric epoxidation rendered epoxide **3.11** in excellent yield (98%). Recrystallisation from hexanes/Et_2O afforded a first crop of epoxide **3.11** (61%). Repeating of this procedure produced a second crop (24%) of **3.11**, whereas the resulting filtrate was purified by flash chromatography gave a third crop, which was used as a reference for the determination of the enantiomeric excess (ee). Determination of the ee by use of a chiral shift reagent (europium(III) tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorate]) failed since no resolution of the signals could be observed. In order to circumvent this issue, epoxide **3.11** was converted to its acetate ester **3.14**, which was expected to exhibit distinct ^{1}H NMR signals for the acetate group upon treatment with the chiral shift reagent. Although splitting of NMR signals could be observed, integration was non-conclusive since no baseline separation of the signals could be obtained (Scheme 11.4).



Scheme 11.4: a) Ac_2O, 4-DMAP, CH_2Cl_2, RT, 2 h (100%); b) (+)-Mosher's chloride, DIPEA, 4-DMAP, CH_2Cl_2, RT, 1 h (95%).

Hence, we choze to prepare the corresponding Mosher esters by treatment of **3.11** with (+)-Mosher's[17] chloride in the presence of DIPEA and 4-DMAP. Unfortunately, both ^{1}H NMR and ^{19}F NMR spectra of **3.15** failed to produce baseline

separation of relevant signals. Since comparison of the measured optical rotations to literature data was likewise nonconclusive, the different crops of **3.11** were analyzed by chiral HPLC (Table 11.1). Based on these findings, the first recrystallisation crop was considered pure enough for further use.

Table 11.1: Optical rotations and enantiomeric excess of the different batches of **3.11**.

	$[\alpha]^{D}_{20}$ (c = 1 in $CHCl_3$)[a]	ee determined by chiral HPLC
1st crop	+ 21.78°	98%
2nd crop	+ 21.34°	95%
Filtrate	+ 8.81°	41%

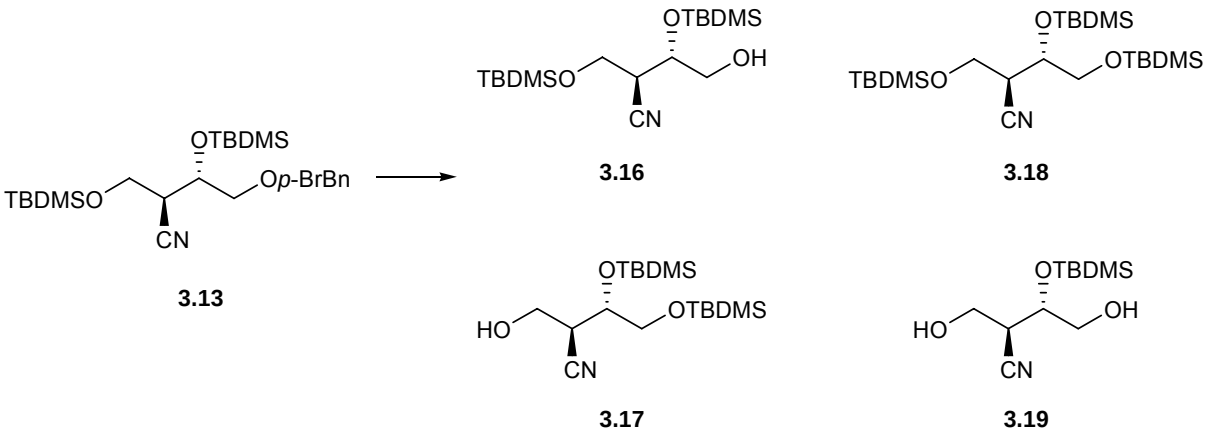
[a] Reported $[\alpha]^{D}_{20}$: +17° (c = 1.5 in $CHCl_3$)[18]

-

Treatment of epoxide **3.11** (50 mmol scale) with sodium cyanide and triethyl borate in DMF afforded, after a tedious work-up, crude **3.12** as a 1:9 mixture of 1,2- and 1,3-diols. Removal of the 1,2-diol by oxidative cleavage with sodium metaperiodate, followed by flash chromatography produced 1,3-diol **3.12** in disappointing yield (35%) compared to reported yields (83-96%; 0.3 mmol scale).[12g] However, apart from the 1,2- and 1,3-diols, a substantial amount (37%) of 4-bromobenzyl alcohol could be isolated from the reaction mixture after repeated chromatographic purification. Hence, debenzylation, which presumably occurs through boron mediated nucleophilic attack of cyanide on C4 of the epoxide ring-opening products, might account for the observed low yield. Although the corresponding C4-substituted nitrile could not be isolated, it is highly likely that it had been removed from the reaction mixture during aqueous work-up. Moreover, up-scaling of cyanide mediated epoxide ring opening reactions had previously proved to be troublesome.[12e]

Protection of 1,3-diol **3.12** with TBDMSCl in DMF afforded silyl ether **3.13** in very good yield (93%). Pd/C mediated removal of the *p*-bromobenzyl moiety unexpectedly gave rise to a complex reaction mixture (Scheme 11.5). The desired *p*-bromobenzyl deprotected product **3.16** could not be isolated in pure form but co-eluted with **3.17** (1:1.1), which resulted from intra –and/or intermolecular silyl migration. Apart from **3.17**, two other compounds resulting from intermolecular silyl migration could be isolated. Whereas **3.18** comprised a fully silyl protected 1,2,4-diol backbone, **3.19** resulted from desilylation of the primary alcohol of **3.13** (Table 11.2).

A literature survey indicated that desilylation and silyl migration under hydrogenating conditions had earlier been observed.[19]



Scheme 11.5: Pd/C, H_2, EtOH, RT, 4 h (**3.16** + **3.17**: 32%; **3.18**: 10%; **3.19**, 50%)

A recent study pointed out that the occurrence of silyl migration/deprotection depends mainly on the quality of the palladium catalyst.[19b] In an earlier paper, the same authors indicated that this issue could easily be overcome by use of encapsulated palladium catalysts (Pd EnCat[20]).[19a]

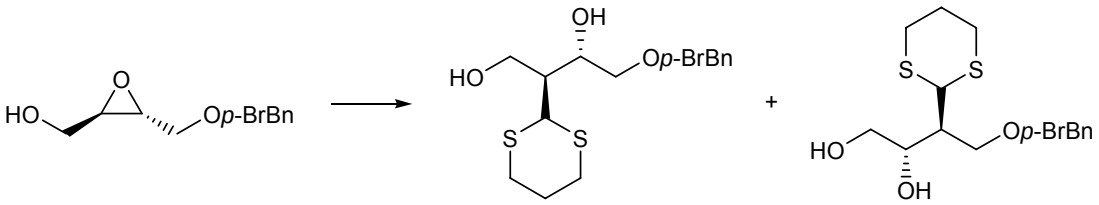
Table 11.2: Comparison of NMR data for **3.16**, **3.17**, **3.18** and **3.19**.

	3.16		**3.17**		**3.18**		**3.19**	
	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)
C(1)H_a	3.50-3.66	m	3.50–3.66	m	3.87	dd	3.54-3.70	m
C(1)H_b	3.50-3.66	m	3.50–3.66	m	3.93	dd	3.54-3.70	m
C(1)O*H*	-	-	5.16	t	-	-	5.17	dd
C(2)*H*	2.90	ddd	2.97	dt	3.09	m	2.96	dt
C(3)*H*	3.85-3.93	m	3.85-3.93	m	4.05	m	3.90	dt
C(4)H_a	3.75	dd	3.37	td	3.70	dd	3.40	dd
C(4)H_b	3.80	dd	3.45	td	3.78	dd	3.46	dd
C(4)O*H*	4.85	t	-	-	-	-	4.88	t

Nonetheless, it had become clear that our envisioned synthetic approach was hampered by too many obstacles, and since we were back at the point of epoxide opening, we investigated more efficient methods for introduction of branching on a 2,3-epoxy alcohol scaffold. Our attention was drawn to a report in which branching was achieved in high yield (90%) by C2 regioselective (C2:C3 9:1) opening of a 2,3-

epoxy alcohol with lithium 1,3-dithiane.[21] Indeed, 1,3-dithianes have shown to be invaluable for introduction of C_1 fragments in natural product syntheses.[22]

Hence, epoxide **3.11** was treated with lithium 1,3-dithiane in THF. Although the reaction proceeded in high yield (95%), it gave rise to low regioselectivity as 1,3-dithianes **3.20** and **3.21** were obtained in a 2:1 ratio (Scheme 11.6). Comparison of NMR data of **3.20** and **3.21** is presented in Table 11.3.



3.11 **3.20** **3.21**

Scheme 11.6: *n*BuLi, 1,3-dithiane, DMPU, THF, -78°C to -20°C, 1.5 h (95%)

Table 11.3: Comparison of NMR data of **3.20** and **3.21**

	3.20		**3.21**	
	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
C(1)H_a	3.62 (dd)	58.36	3.36 (app.t)	64.08
C(1)H_b	3.67 (dd)			
C(1)O*H*	4.51 (t)	-	4.55 (t)	-
C(2)*H*	1.86 – 1.96 (m)	48.08	3.79 (ddd)	69.84
C(2)O*H*	-	-	4.57 (d)	-
C(3)*H*	3.97- 4.06 (m)	68.19	1.94 – 2.11 (m)	45.46
C(3)O*H*	4.82 (d)	-	-	-
C(4)H_a	3.48 (dd)	73.15	3.58 (dd)	69.84
	3.52 (dd)		3.62 (dd)	

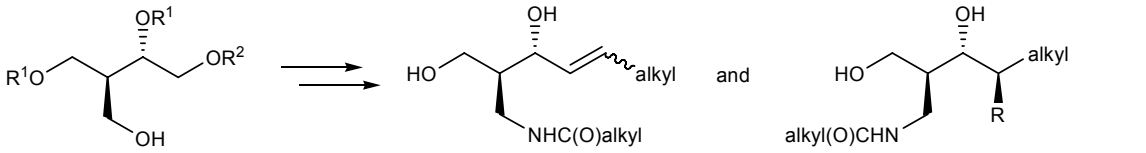
We conceived that higher regioselectivity could be achieved by replacement of the *p*-bromobenzyl moiety by a sterically more demanding protecting group suchas a trityl group. To this aim, an alternative synthetic strategy starting from D-isoascorbic acid was investigated.

11.2. ACCESS TO N-HOMOCERAMIDES STARTING FROM D-ISOASCORBIC ACID

SYNTHESIS OF A VERSATILE (S)-3-(HYDROXYMETHYL)BUTANE-1,2,4-TRIOL BUILDING BLOCK AND ITS APPLICATION FOR THE STEREOSELECTIVE SYNTHESIS OF A NOVEL CLASS OF HOMOCERAMIDES.

Ulrik Hillaert and Serge Van Calenbergh

Submitted to Angewandte Chemie International Edition



1

E- and *Z*-N-homoceramides

R = H: dihydro-N-homoceramide
R = OH: D-*ribo*-N-homophytoceramide

*A versatile (S)-3-(hydroxymethyl)butane-1,2,4-triol (**1**) building block has been synthesized starting from D-isoascorbic acid, a common food preservative. The key transformation in this approach was the introduction of branching through a high yield and fully regioselective epoxide opening. This flexible synthon has been elaborated to a new class of (dihydro)-N-homo(phyto)ceramides.*

The development and availability of reliable and efficient methods for the construction of chiral building blocks are crucial for the synthesis of many pharmaceutical agents and complex natural products. These chiral building blocks can be derived from the chiral pool or by chemical/enzymatic means from achiral or racemic starting material.

(*S*)-3-(Hydroxymethyl)butane-1,2,4-triol is a multivalent, flexible scaffold with defined stereochemical features which can be exploited by judicious selection of appropriate protecting groups. Some examples of the synthetic potential of this intermediate are summarized in Figure 1.

Indeed, sugar derivatives (*S*,*S*)-4-(hydroxymethyl)pyrrolidine-3-ol,[1a,b] the enantiomer of the common precursor of second-generation purine phosphorylase inhibitors[1c-e] and oxetanocin A, a known antibacterial, antitumoral and antiviral natural product,[2] are readily accessible through a limited number of steps (**A**). Moreover, inversed amide ceramide analogues (**B**) could provide useful biochemical tools for assessment of ceramide interaction with a myriad of clinically relevant enzymes. Finally, simple elaboration of the other primary alcohol (C(4)OH) to the amide part gives access to PDMP homologues[3a] (D-*threo*-1-phenyl-2-

aminodecanoyl-3-morpholinopropanol), an inhibitior of glucosyl ceramide synthase which is a potential target in the treatment of cancer (**C**). [3b,c]

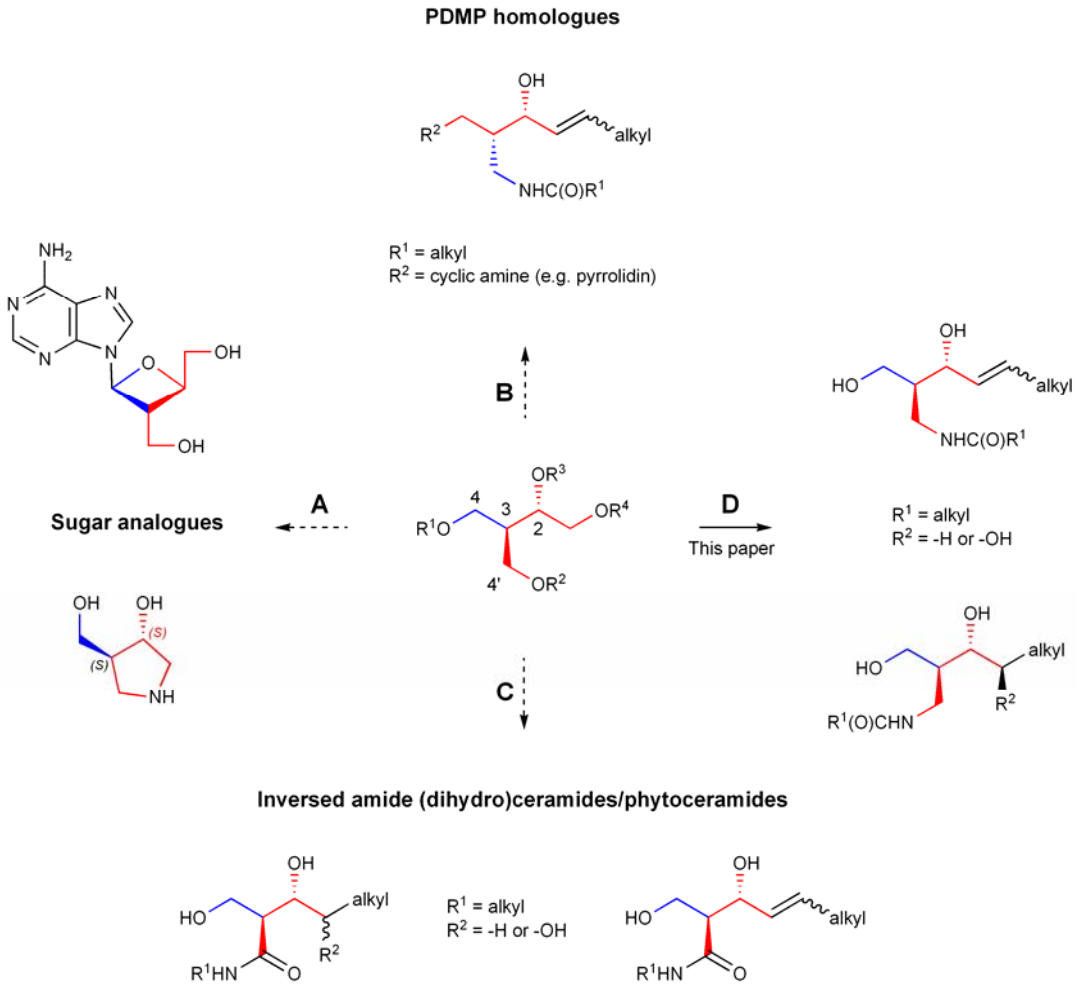


Figure 1. Synthetic potential of key intermediate (S)-3-(hydroxymethyl) butane-1,2,4-triol: A) (aza)sugar derivatives; B) inversed amide ceramides and phytoceramides; C) PDMP homologues; D) N-(dihydro)homo(phyto)ceramides

Here, we wish to demonstrate the usefulness of the (*S*)-3-(hydroxymethyl)butane-1,2,4-triol scaffold in preparing a novel class of homoceramide analogues (Figure 1; **D**), which contain an additional methylene group between the *N*-acyl chain and C2 (Figure 2; **2.22-24**). Interestingly, our procedure seemed also convenient for the synthesis of N-homophytoceramide (**2.25**), which can serve as key intermediate for the synthesis of α-galactosyl-N-homoceramide. This latter compound represents a homologue of α-galactosylceramide, a potentially useful agent for the treatment of autoimmune diseases.[4]

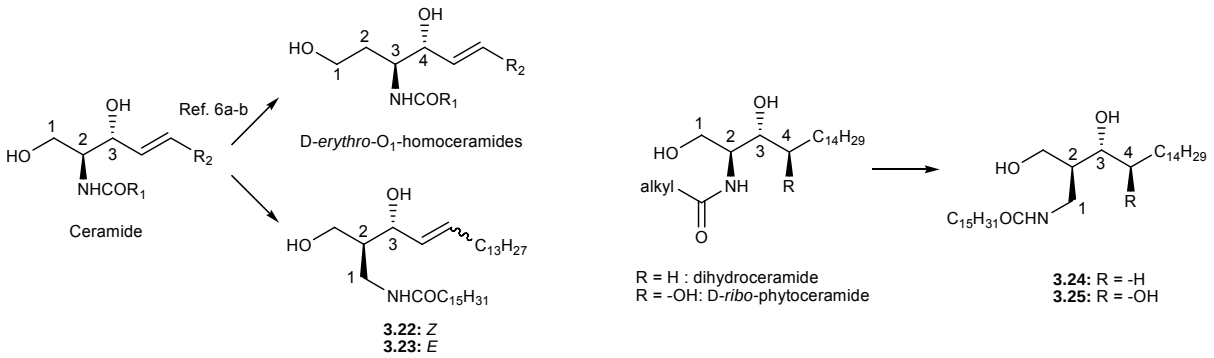
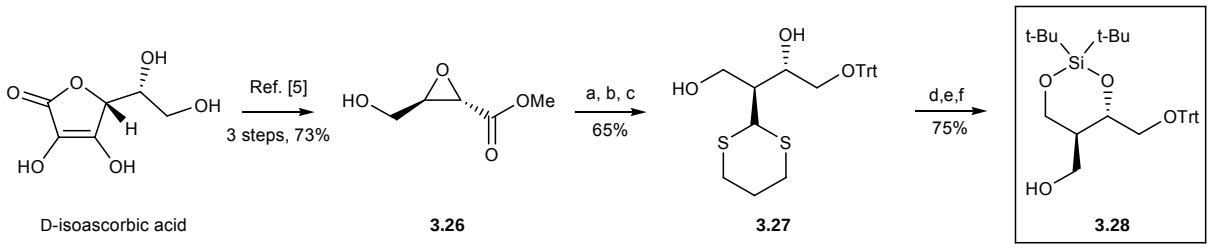


Figure 2. General stuctures of O_1-homoceramides and N-(dihydro)homo(phyto)ceramides (**3.22-3.25**).

Homologation is a classical tool in medicinal chemistry to alter biological properties of endogenous compounds. Salbutamol, for instance, a widely[5] used bronchodilator with agonistic properties for β_2-receptors, consists of a 4-hydroxy-3-hydroxymethylphenyl moiety instead of the catechol ring, which is present in (nor)adrenaline.

Recently, our group reported an expedient route for the synthesis of D-*erythro*-O_1-homoceramides[6a] (Figure 2). An alternative synthetic procedure for this class of non-natural ceramide analogues was later proposed by Ogino and coworkers.[6b,c] The authors found that several representatives exhibited considerable apoptotic activities. Recently, Schmidt and coworkers[7] presented the synthesis of O_1-homosphingosine-phosphonate starting from D-galactose.

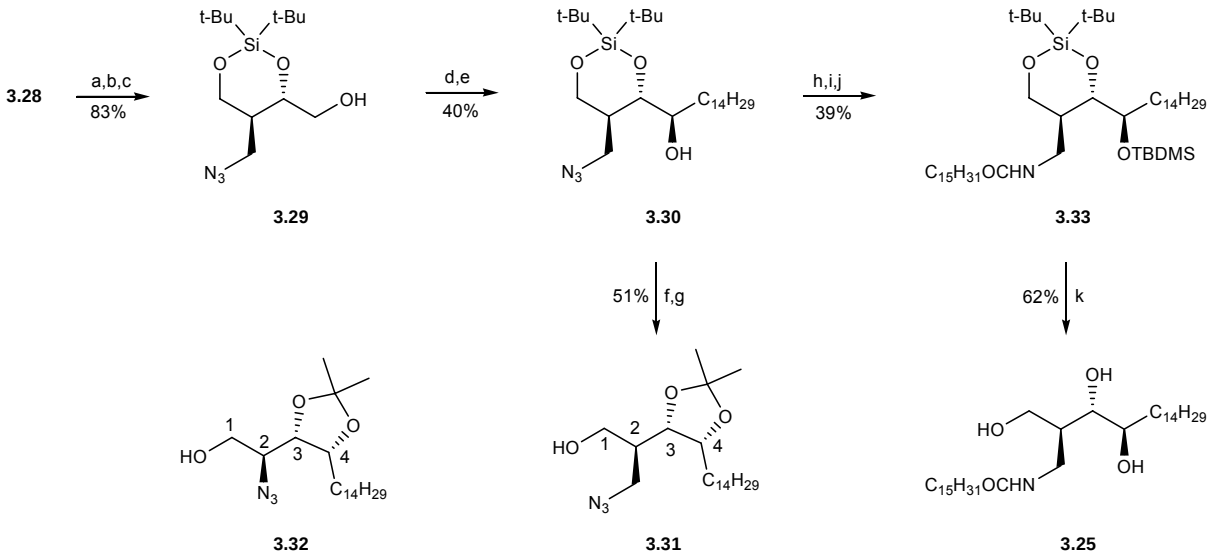


Scheme 1. **Conditions**: a) TrtCl, Hünig's base, CH_2Cl_2, 2.5 h at 0°C, then RT for 3 h (83%); b) $NaBH_4$, MeOH/THF (3:2), 0°C to RT, overnight (91%); c) 1,3-dithiane, *n*BuLi, THF, -20°C to -10°C, 27 h (86%); d) di-*tert*-butylsilyl ditriflate, pyridine, CH_2Cl_2, -78°C to -20°C, 1.5 h (100%); e) MeI, $CaCO_3$, MeCN:H_2O (8:1), reflux, 24 h (99% crude); f) $NaBH_4$, EtOH/THF (5:3), 0°C to RT, 5 h (86% from **3.27**).

Epoxide synthon **3.26** (Scheme 1), prepared from D-isoascorbic acid as previously described,[8] provided the stereochemical and structural features required for our synthetic approach. Since epoxide opening is often hampered by

regioselectivity issues involving the use of hazardous cyanide[1b] or additional synthetic steps implicated in allylic transformations[2b] (two common methylene sources), we opted to use 1,3-dithiane[9] to introduce branching.

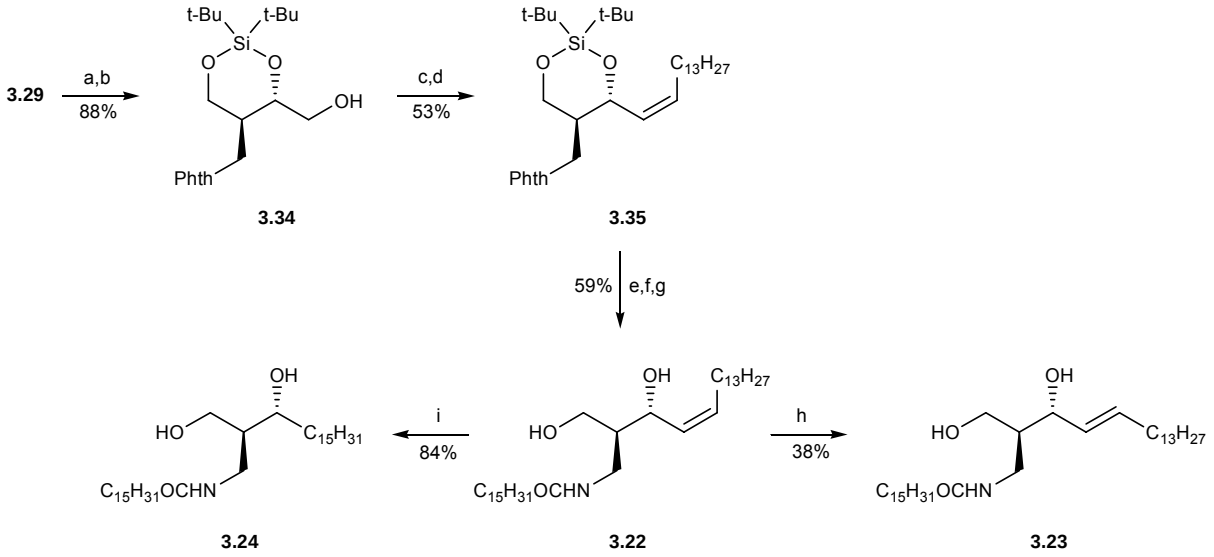
Hence, tritylation followed by reduction of the ester and subsequent epoxide opening with 2-lithio-1,3-dithiane[10] produced intermediate 1,3-diol **3.27** with complete regioselectivity (47% yield in six steps from D-isoascorbic acid). Protection of 1,3-diol **3.27** with di-*tert*-butylsilyl ditriflate followed by dithiane deprotection with MeI under alkaline conditions and final reduction of the unmasked aldehyde with $NaBH_4$ gave access to **3.28** (75% from **3.27**, 36% from D-isoascorbic acid in 9 steps), which represents a unique intermediate from which each of the primary alcohols can selectively be addressed for further modification.



Scheme 2. Conditions: a) MsCl, 2,6-lutidine, 4-DMAP, CH_2Cl_2, 0°C to RT, 21 h (100% crude); b) NaN_3, DMF, 70°C, 2h (86%); c) $ZnBr_2$, CH_2Cl_2/*i*PrOH (85:15), RT, 10 h (96%); d) Dess-Martin periodinane, CH_2Cl_2/pyridine (12:1), 0°C to RT, 4 h; e) tetradecylmagnesium chloride in THF, Et_2O, overnight (40% from **3.29**); f) TBAF, THF, RT, 6 h (62%); g) 2,2-dimethoxypropane, *p*-TsOH, RT, 14 h (83%); h) TBDMSCl, 4-DMAP, imidazole, DMF, 0°C then 50°C for 18 h (68%); i) PPh_3, THF, H_2O, 30 h; j) palmitoyl chloride, Hünig's base, CH_2Cl_2, 0°C, 0.5 h (58% over two steps); k) TBAF, THF, RT, 48 h (62%).

Access to D-*ribo*-N-homophytoceramide **3.25** is outlined in Scheme 2. Mesylation of intermediate **3.28** followed by azide introduction and trityl removal provided alcohol **3.29** in good yield (83%). Subsequent periodinane oxidation and addition of tetradecylmagnesium chloride to the thus formed aldehyde furnished protected azido-N-homophytosphingosine **3.30** (40%) as a single diastereomeric

form. Assignment of the *erythro* configuration was achieved by converting intermediate **3.30** to the 3,4-isopropylidene protected triol **3.31** in a two steps sequence entailing silyl deprotection and dioxolane formation (51%) and subsequent comparison of ^{1}H NMR data with natural D-*ribo*-azidophytosphingosine **3.32**.[11,12] Azide reduction under Staudinger conditions following TBDMS protection of the secondary alcohol in **3.30** and subsequent acylation of the primary amine with palmitoyl chloride afforded silyl protected intermediate **3.33** (39%). Final desilylation with TBAF furnished D-*ribo*-N-homophytoceramide **3.25** (62%).



Scheme 3. Conditions: a) see Scheme 2 (100%); b) *N*-ethoxycarbonyl phthalimide, Hünig's base, THF, 75°C, 2 h (88%); c) Dess-Martin periodinane, CH_2Cl_2, 0°C to RT, 3.5 h; d) PhLi, tetradecylphosphonium bromide, LiBr, Et_2O, THF, -78°C to RT, 5.5 h (53% from **3.34**); e) hydrazine, EtOH, 55°C, 3 h (100% crude); f) palmitoylchloride, Hünig's base, CH_2Cl_2, 0°C to RT, 1 h (65% from **3.35**); g) TBAF, THF, RT, 3 h (91%); h) diphenyldisulfide, hν, cyclohexane/dioxane (3:1), RT, 4 h (38%); i) Pd/C, EtOAc, RT, 48 h (84%).

Since the presence of azides in Wittig olefination has led to controversial results,[13] we opted to transform the azide to a phthalimide in a two step sequence involving reduction of **3.29** under Staudinger conditions followed by phthalimide protection of the thus formed primary amine, thereby yielding intermediate **3.34** in good yield (88%; Scheme 3). Subsequent oxidation of the primary alcohol with Dess-Martin periodinane yielded the intermediate aldehyde. Although reaction conditions specifically addressed the *E-isomer,* Schlosser-Wittig olefination surprisingly only yielded *Z*-isomer **3.35**. Hydrazine mediated phthalimide deprotection followed by

acylation with palmitoyl chloride and silyl deprotection with TBAF furnished *Z*-N-homoceramide **3.22** (59%). Photoinduced double bond isomerisation[12c] in the presence of diphenyl disulfide as sensitizer produced, after two recrystallisations, isomerically pure *E*-N-homoceramide **3.23** (38%). Finally, hydrogenation of the *Z*-double bond in **3.22** gave access to dihydro-N-homoceramide **3.24** (84%).

In summary, we have reported an expedient route towards a versatile (*S*)-3-(hydroxymethyl)butane-1,2,4-triol scaffold starting from D-ascorbic acid, a common food preservative. The key transformation in this approach was the introduction of branching through a high yield and fully regioselective 2-litio-1,3-dithiane epoxide opening. Based on this flexible synthon, we report the first synthesis of (dihydro)-N-homoceramides **3.22-24**. In addition, a fully stereoselective Grignard reaction gave access to D-*ribo*-N-homophytoceramide **3.25**, which will be utilised in a further study towards the elaboration of its α-galacosyl derivative.

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[12] Both **11** ($^3J_{3,4}$ = 5.57 Hz) and **12** ($^3J_{3,4}$ = 5.38 Hz) exhibit a comparable vicinal coupling constant thereby indicating a *cis*-relationship of the ring substituents; standard sphingosine numbering was applied for clarity reasons.

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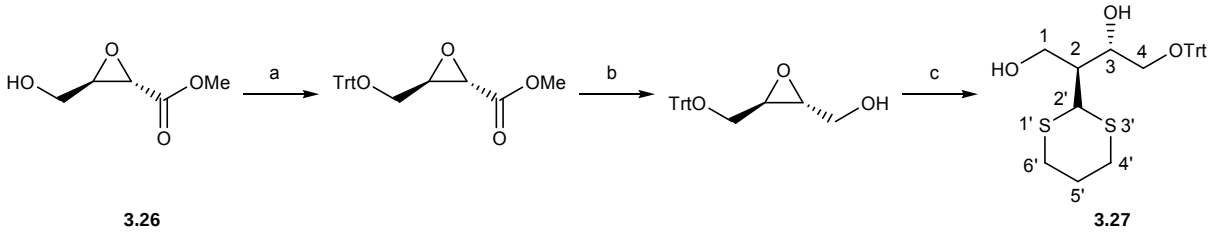
Supporting information:

General:

All reactions were carried out under inert (N_2) atmosphere. Precoated Macherey-Nagel (Düren, Germany) silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and/or revealed by sulphuric acid-anisaldehyde spray or phosphomolybdic acid spray. Column chromatography was performed on ICN silica gel (63-200 μM, ICN, Asse Relegem, Belgium). NMR spectra were obtained with a Varian Mercury 300 spectrometer (Varian, Palo Alto, California, USA). Chemical shifts are given in parts per million (δ) relative to residual solvent peak. All signals assigned to amino and hydroxyl groups were exchangeable with D_2O. Numbering for ^1H assignment is based on the IUPAC name of the compounds, exept for compound **3.31** where standard sphingolipid numbering was applied. Structural assignment was confirmed with COSY, HMQC and/or NOEDIF if necessary. Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Abbreviations: 4-DMAP = 4-dimethylaminopyridine; DMF = *N, N*-dimethylformamide; DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; MsCl = methanesulfonyl chloride; *i*-PrOH = isopropanol; RT = room temperature; THF = tetrahydrofuran; *p*-TsOH = *para*-toluenesulfonic acid; TBAF = tetrabutylammonium fluoride; TrtCl = tritylchloride; TBDMSCl = *tert*-butyldimethylsilyl chloride.

1,3-diol 3.27:



3.26 **3.27**

a) To an ice-cold solution of **3.26** (10.0 g, 75.69 mmol) and Hünig's base (39 mL, 0.228 mol, 3 eq.) in CH_2Cl_2 (300 mL), trityl chloride (22.16 g, 79.48 mmol, 1.05 eq.) in CH_2Cl_2 (150 mL) was added dropwise over 90'. After stirring for 1 h at 0°C and 3 h at RT, TLC indicated complete consumption of the starting material. Sat. $NaHCO_3$ (50 mL) was added and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic phase was dried over Na_2SO_4 and all volatiles were removed under reduced pressure. The resulting syrup was covered with EtOH (100 mL) and heated at 50°C until a clear solution was obtained. Overnight standing in a refrigerator (2°C) yielded trityl protected **3.26** (18.2 g, 63%) as white crystals. Flash chromatography of the residue (EtOAc:hexanes:TEA 30:70:1) yielded another crop of tritylated **3.26** (5.6 g, 20%) as a white solid.

^{1}H NMR (300 MHz, [D6]DMSO, 25°C): δ= 2.96 – 3.04 (m, 1H, -C(3)*H*), 3.31 – 3.38 (m ,2H, -C(2)*H* and –C(2)CH_a), 3.58 (d, 1H, *J* = 1.76 Hz, –C(2)CH_b), 3.65 (s, 3H, -OCH_3), 7.22 – 7.38 (m, 15H, arom. H).

^{13}C NMR (75 MHz, [D6]DMSO, 25°C): δ= 49.72, 52.21, 56.26, 62.59, 86.26, 127.19, 128.03, 128.15, 143.35, 168.80.

Exact mass (ESI-MS) calculated for $C_{24}H_{22}NaO_4$ $[M+Na]^+$: 397.1416, found: 397.1419.

b) To a cooled solution (0°C) of tritylated **3.26** (23.812 g, 63.59 mmol) in a mixture of MeOH/THF (3:2; 250 mL), $NaBH_4$ (2.646 g, 69.95 mmol, 1.1 eq.) was added in small portions and the mixture was stirred overnight at RT. The solvent was removed

under reduced pressure and the residue was dissolved in EtOAc (300 mL) and cooled to 0°C. Ice-cold 0.1N HCl was added under vigorous stirring until pH 2. After separation of both layers, the aqueous layer was extracted with EtOAc (2 x 100mL). The combined organic phase was extracted with $NaHCO_3$ (2 x 100 mL) and brine (100 mL) and subsequently dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was recrystallised from diisopropylether (100 mL) yielding the intermediate epoxide (20.083 g, 91%) as colourless needles.

^{1}H NMR (300 MHz, [D6]DMSO, 25°C): δ= 2.89 (dd, 1H, *J* = 5.55 and 10.86 Hz, -C(1)H_a), 2.93 – 2.97 (m, 1H, -C(3)*H*), 3.07 (dt, 1H, *J* = 2.36 and 5.54 Hz, -C(2)*H*), 3.25 (dd, 1H, *J* = 2.36 and 10.86 Hz, -C(1)H_b), 3.34 (dt, 1H, *J* = 5.87 and 12.56 Hz, -C(3)CH_a), 3.57 (ddd, 1H, *J* = 3.23, 5.57 and 12.28 Hz, -C(3)CH_b), 4.81 (t, 1H, *J* = 5.86 Hz, C(1)O*H*), 7.22 – 7.39 (m, 15H, arom. H).

^{13}C NMR (75 MHz, [D6]DMSO, 25°C): δ= 53.9, 55.8, 60.9, 63.9, 86.0, 127.1, 128.0, 128.2, 143.6.

Exact mass (ESI-MS) calculated for $C_{23}H_{22}NaO_3$ $[M+Na]^+$: 369.1467, found: 369.1470.

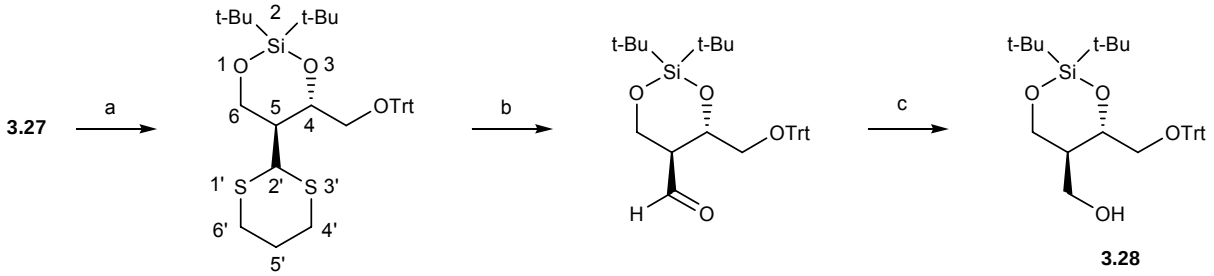
c) To a stirred solution of 1,3-dithiane (19.84 g, 0.165 mol, 5 eq.) in anhydrous THF (250 mL) at -20°C, *n*BuLi (100 mL of 1.6M solution in hexanes, 0.160 mol, 4.85 eq.) was added slowly over 30' and the resulting mixture was stirred for 2 h while the temperature reached -15°C. DMPU (41.3 mL, 0.33 mol, 10 eq.) was added in one portion followed by dropwise addition of the intermediate epoxide (11.43 g, 33.0 mmol) in anhydrous THF (100 mL) over 30'. The temperature was kept between -15°C and -10°C. Since the starting material and **3.27** have an identical Rf, a small aliquot was withdrawn from the reaction mixture after 24 h and partitioned between Et_2O and sat. NH_4Cl. The organic layer was separated, dried over Na_2SO_4 and all volatiles were removed in vacuo. ^{1}H NMR analysis indicated the absence of starting material and the presence of a new compound, besides unreacted 1,3-dithiane. The reaction mixture was subsequently treated with sat. NH_4Cl (200 mL) and Et_2O (300 mL). After separation of both phases, the aqueous layer was extracted with Et_2O (2 x 150 mL) and the combined organic phase was dried over Na_2SO_4. After removal of all volatiles, excess 1,3-dithiane was sublimed (1 mBar at 56°C). The resulting yellow syrup was purified by column chromatography (hexanes:EtOAc:TEA 30:70:0.1 → 40:60:0.1) yielding **3.27** (13.21 g, 86%) as a slightly yellow foam.

^{1}H NMR (300 MHz, [D6]DMSO, 25°C): δ= 1.50 – 1.70 (m, 1H, -C(5')H_a), 1.93 – 2.08 (m, 2H, -C(2)*H* and -C(5')H_b), 2.62 – 2.81 (m, 4H, -C(4')H_2 and –C(6')H_2), 2.94 – 3.06 (m, 2H, -C(4)H_2), 3.49 – 3.64 (m, 2H, -C(1)H_2), 4.02 – 4.12 (m, 1H, -C(3)*H*), 4.31 (d, 1H, *J* = 4.10 Hz, -C(2')*H*), 4.48 (t, 1H, *J* = 4.99 Hz, -C(1)O*H*), 4.93 (d, 1H, *J* = 5.57 Hz, -C(3)O*H*), 7.22 – 7.44 (m, 15H, arom. *H*).

^{13}C NMR (75 MHz, [D6]DMSO, 25°C): δ= 26.02, 30.23, 30.29, 47.84, 48.94, 58.28, 65.92, 68.64, 85.70, 126.95, 127.84, 128.34, 143.92.

Exact mass (ESI-MS) calculated for $C_{27}H_{30}NaO_3S_2$ $[M+Na]^+$: 489.1534, found: 489.1538.

Key intermediate 3.28:



a) To a cooled solution (-78°C) of **3.27** (12.045 g, 25.81 mmol) and pyridine (9 mL, 0.111 mol, 4.3 eq.) in anhydrous CH_2Cl_2 (500 mL), di-*tert*-butylsilyl ditriflate (11.369 g, 25.81 mmol, 1 eq.) dissolved in CH_2Cl_2 (100 mL) was added dropwise over 60'. The mixture was subsequently allowed to warm up and when it reached – 20°C, TLC indicated the disappearance of the starting material. Sat. $NaHCO_3$ (250 mL) was added and the resulting suspension was stirred for 20' while the temperature reached 20°C. After separation of the phases, the aqueous layer was extracted with CH_2Cl_2 (200 mL) and the combined organic phase was extracted with ice cold 0.1N HCl (2 x 100 mL), sat. $NaHCO_3$ (100 mL) and brine (100 mL). After drying over anhydrous Na_2SO_4 and removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc:TEA 95:5:0.1) yielding silyl protected **3.27** (15.67 g, quant.) as a white foam.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 1.05 (s, 9H, *tert*-butyl), 1.14 (s, 9H, *tert*-butyl), 1.62 – 1.73 (m, 1H, -C(5')H_a), 1.91 – 2.02 (m, 1H, -C(5')H_b), 2.25 (dt, 1H, *J* = 2.16 and 14.08 Hz, -C(4' or 6')H_a), 2.57 (dt, 1H, *J* = 3.61 and 14.09 Hz, -C(4' or 6')H_b), 2.71 – 2.84 (m, 3H, -C(4' or 6')H_a+H_b and -C(5)*H*), 3.44 (dd, 1H, *J* = 2.53 and 10.11 Hz, -C(4)CH_a), 3.55 (dd, 1H, *J* = 1.80 and 10.11 Hz, -C(4)CH_b), 3.93 (d, 1H, *J*

= 3.26 Hz, -C(2')*H*), 4.16 (t, 1H, *J* = 11.19 Hz, -C(6)H_a), 4.27 – 4.34 (m, 2H, -C(6)H_b and -C(4)*H*), 7.23 – 7.35 (m, 9H, arom. H), 7.54 - 7.58 (m, 6H, arom. H).
^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 20.04, 22.82, 26.11, 27.10, 27.58, 31.03, 32.00, 45.12, 48.43, 64.90, 66.35, 75.28, 85.96, 126.93, 127.79, 128.80, 143.96.
Exact mass (ESI-MS) calculated for $C_{35}H_{46}NaO_3S_2Si$ $[M+Na]^+$: 629.2555, found: 629.2553.

b) To a solution of silyl protected **3.27** (10.52 g, 17.33 mmol) and $CaCO_3$ (8.67 g, 86.66 mmol, 5 eq.) in a degassed (N_2) mixture of MeCN:H_2O (8:1, 173 mL), MeI (21.58 mL, 34.66 mmol, 2 eq.) was added in one portion and the resulting mixture was refluxed for 24 h. After reduction of the solvent in vacuo (~50 mL), ice-cold H_2O (100 mL) and EtOAc (150 mL) were added and the heterogeneous mixture was filtered. Both phases were separated and the aqueous layer was extracted with Et_2O (3 x 50 mL). The combined organic phase was dried over Na_2SO_4 and removed under reduced pressure. The resulting yellow syrup (8.9 g, 99%) was used without further purification. A small aliquot was purified by column chromatography (PE:Me_2CO 98:2) for analytical purposes.
^{1}H NMR (300 MHz, [D6]DMSO, 25°C): δ= 0.98 (s, 9H, *tert*-butyl), 0.99 (s, 9H, *tert*-butyl), 3.05 (dd, 1H, *J* = 4.27 and 10.05 Hz, -C(4)CH_a), 3.11 (ddt, 1H, *J* = 2.05, 5.06 and 9.57 Hz, -C(5)*H*), 3.19 (dd, 1H, *J* = 4.14 and 9.99 Hz, -C(4)CH_b), 4.04 – 4.15 (m, 2H, -C(6)CH_2), 4.43 (td, 1H, *J* = 4.12 and 9.49 Hz, -C(4)*H*), 7.21 – 7.44 (m, 15H, arom. H), 9.47 (d, 1H, *J* = 2.05 Hz, aldehyde –*H*).
^{13}C NMR (75 MHz, [D6]DMSO, 25°C): δ= 19.71, 22.08, 26.75, 27.17, 53.90, 62.67, 66.25, 72.97, 86.12, 127.08, 127.85, 128.18, 143.57, 200.73.
Exact mass (ESI-MS) calculated for $C_{32}H_{40}O_4SiNa$ $[M+Na]^+$: 539.2594, found: 539.2598.

c) The crude aldehyde (8.9 g, 17.16 mmol) was dissolved in EtOH:THF (5:3, 80 mL) and the resulting clear solution was cooled to 0°C in an ice-bath. $NaBH_4$ (649 mg, 17.16 mmol, 4 eq.) was added portionwise over 10' and the reaction mixture was stirred for 1 h at 0°C and 5 h at RT. Saturated $NaHCO_3$ (5 mL) was added and after stirring for 10', the solvent was reduced in vacuo (~40 mL). Et_2O (200 mL) was added and the heterogeneous mixture was filtered over celite. The filter was rinsed

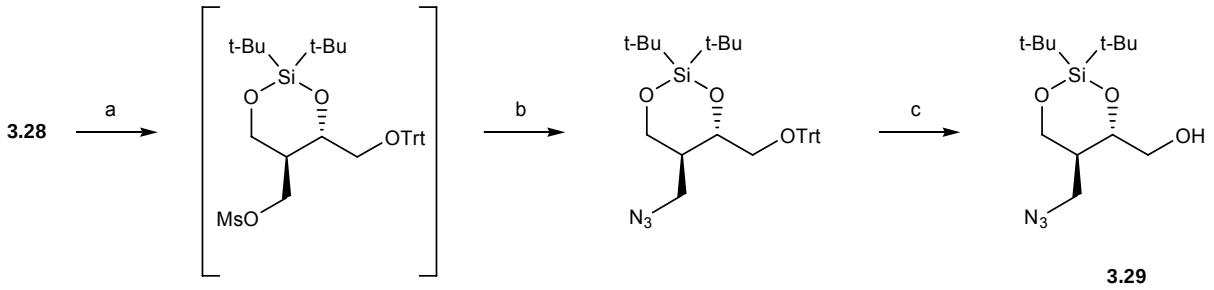
with Et_2O (3 x 50 mL) and after drying over Na_2SO_4, all volatiles were removed under reduced pressure. Flash chromatography (hexanes:EtOAc 85:15) of the residue yielded **3.28** (7.69 g, 86% over 2 steps from silyl protected **3.27**).

^{1}H NMR (300 MHz, [D6]DMSO, 25°C): δ= 1.00 (s, 9H, *tert*-butyl), 1.07 (s, 9H, *tert*-butyl), 2.20 – 2.31 (m, 1H, -C(5)*H*), 3.05 (dd, 1H, *J* = 3.63 and 9.99 Hz, -C(4)CH_a), 3.03 (dd, 1H, *J* = 6.02 and 11.02 Hz, -C(5)CH_a), 3.18 (dd, 1H, *J* = 3.15 and 11.02 Hz, -C(5)CH_b), 3.29 (dd, 1H, *J* = 1.44 and 9.89 Hz, -C(4)CH_b), 3.94 (app t, 1H, *J* = 11.17 Hz, -C(6)H_a), 4.04 – 4.13 (m, 2H, -C(4)*H* and -C(6)H_b), 7.19 – 7.34 (m, 9H, arom. *H*), 7.44 – 7.52 (m, 6H, arom. *H*).

^{13}C NMR (75 MHz, [D6]DMSO, 25°C): δ= 19.67, 22.36, 26.92, 27.39, 42.46, 58.61, 65.50, 67.13, 75.08, 85.36, 126.92, 127.71, 128.27, 143.97.

Exact mass (ESI-MS) calculated for $C_{32}H_{42}O_4SiNa$ $[M+Na]^+$: 541.2751, found: 541.2750.

Azide 3.29:



a) To a cold solution (0°C) of **3.28** (7.421 g, 14.30 mmol), 2,6-lutidine (3.33 mL, 28.61 mmol, 2 eq.) and 4-DMAP (cat.) in CH_2Cl_2 (100 mL), MsCl (1.66 mL, 21.46 mmol, 1.5 eq.) dissolved in CH_2Cl_2 (20 mL) was added dropwise over 30' and the resulting solution was stirred at RT for 21 h. Sat. $NaHCO_3$ (100 mL) and Et_2O (200 mL) were added and after separation of the phases, the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organic phase was washed with ice-cold 0.1N HCl (2 x 50 mL), sat. $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4 and evaporated yielding crude mesylated **3.28** (8.55 g, > 100%) which was used without further purification.

b) To a solution of crude mesylated **3.28** (8.55 g) in anhydrous DMF (120 mL), NaN_3 (3.72 g, 57.22 mmol, 4 eq.) was added and the resulting suspension was heated at 70°C until TLC indicated complete consumption of the starting material (2 h). After

removal of the solvent under reduced pressure, sat. $NaHCO_3$ (100 mL) and Et_2O (100 mL) were added and after separation of the phases, the aqueous layer was washed with Et_2O (2 x 50 mL). The combined organic phase was washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The residue was treated with EtOH (100 mL) and subsequently cooled to 0°C. After 20', the crystallised product was filtered and the filter was rinsed with ice-cold EtOH (2 x 10 mL) yielding the intermediate azide (6.04 g, 78%) as a white solid. The filtrate was evaporated in vacuo yielding a yellow syrup which was purified by column chromatography (hexanes:EtOAc 9:1) yielding an additional crop of the intermediate azide (640 mg, 8%) and detritylated product **3.29** (355 mg, 8%).

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 1.07 (s, 9H, *tert*-butyl), 1.15 (s, 9H, *tert*-butyl), 2.45 – 2.57 (m, 1H, -C(5)*H*), 2.93 (dd, 1H, *J* = 3.25 and 10.31 Hz, -C(4)CH_a), 3.03 (dd, 1H, *J* = 6.53 and 12.68 Hz, -C(5)CH_a), 3.15 (dd, 1H, *J* = 3.41 and 12.67 Hz, -C(5)CH_b), 3.29 (dd, 1H, *J* = 2.45 and 10.33 Hz, -C(4)CH_b), 3.97 (app t, 1H, *J* = 11.12 Hz, -C(6)H_a), 4.06 (dt, 1H, *J* = 2.83 and 9.85 Hz, -C(4)*H*), 4.13 (dd, 1H, *J* = 4.13 and 10.88 Hz, -C(6)H_b), 7.23 – 7.35 (m, 9H, arom. H), 7.52 – 7.56 (m, 6H, arom. H).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 20.07, 22.81, 27.08, 27.53, 40.42, 49.93, 65.78, 67.50, 75.34, 86.26, 127.02, 127.81, 128.66, 143.98.

Exact mass (ESI-MS) calculated for $C_{32}H_{41}N_3O_3SiNa$ $[M+Na]^+$: 566.2815, found: 566.2813.

c) A solution of anhydrous $ZnBr_2$ (45.03 g, 0.20 mol, 1M in 200 mL CH_2Cl_2:*i*PrOH 85:15) was added to the intermediate azide (6.68 g, 12.28 mmol) and the resulting yellow solution was stirred for 10 h at RT. Water (100 mL) was added and after separation of the phases, the aqueous layer was extracted with Et_2O (3 x 100 mL). The combined organic phase was washed with sat. $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL) and subsequently dried over Na_2SO_4. After removal of all volatiles under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 95:5 → 85:15) yielding **3.29** (3.21 g, 96%; 83% over 3 steps from **3.28**) as a colourless oil.

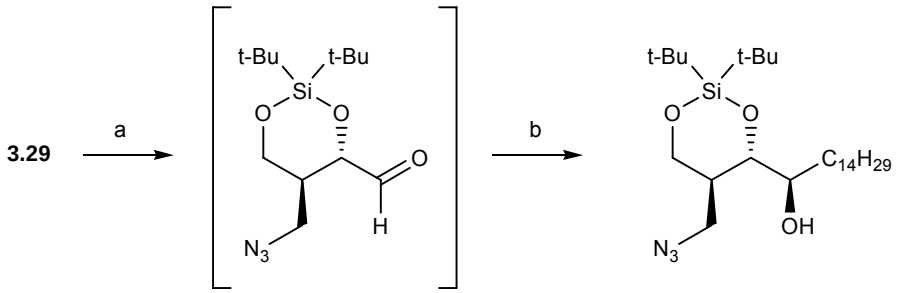
^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 1.00 (s, 9H, *tert*-butyl), 1.04 (s, 9H, *tert*-butyl), 2.12 (m, 1H, -C(5)*H*), 3.25 (dd, 1H, *J* = 4.27 and 11.55 Hz, -C(4)CH_a), 3.31

(dd, 1H, *J* = 3.30 and 11.54 Hz, -C(4)CH_b), 3.55 (dd, 1H, *J* = 5.96 and 11.38 Hz, -C(5)CH_a), 3.76 (dd, 1H, *J* = 3.05 and 11.36 Hz, -C(5)CH_b), 3.97 (app t, 1H, *J* = 11.06 Hz, -C(6)H_a), 4.00 - 4.06 (m, 1H, -C(4)*H*), 4.08 (dd, 1H, *J* = 4.51 and 10.97 Hz, -C(6)H_b).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 19.90, 22.68, 27.07, 27.38, 40.51, 49.79, 65.01, 66.84, 76.18.

Exact mass (ESI-MS) calculated for $C_{13}H_{28}N_3O_3Si$ $[M+H]^+$: 302.1900, found: 302.1908.

Intermediate protected azido homophytosphingosine 3.30:



3.30

a) Dess-Martin periodinane (9.324 g of a 15 wt% solution in CH_2Cl_2, 3.298 mmol, 2 eq.) was added to a solution of **3.29** (497 mg, 1.649 mmol) in CH_2Cl_2/pyridine (6:0.5, 32.5 mL) at 0°C and the resulting solution was stirred for 4.5 h at RT. Sat. $NaHCO_3/Na_2S_2O_3$ (5:1, 50 mL) was added and the mixture was stirred until a clear solution was obtained (1 h). After separation of the phases, the aqueous layer was extracted with Et_2O (2 x 50 mL) and the combined organic phase was dried over Na_2SO_4 yielding the crude aldehyde (502 mg, > 100%) which was used without further purification.

b) The crude aldehyde (502 mg) was dissolved in anhydrous Et_2O (20 mL) and cooled to -78°C. Tetradecylmagnesium chloride (3.3 mL of a 1M solution in THF, 3.3 mmol, 2 eq.) was added dropwise over 20' and the resulting brown solution was allowed to reach RT overnight. After cooling 0°C, H_2O (20 mL) was slowly added and the reaction mixture was stirred for an additional 10'. After separation of the phases, the aqueous layer was extracted with Et_2O (4 x 20 mL) and the combined organic phase was washed with ice-cold 0.1N HCl (20 mL) and sat. $NaHCO_3$ (20 mL) and subsequently dried over Na_2SO_4. After removal of all volatiles in vacuo, the

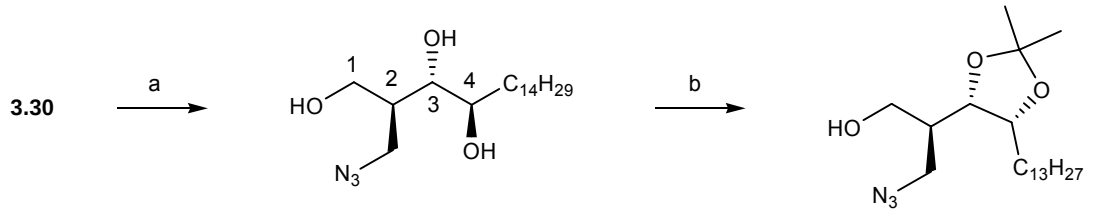
residue was purified by column chromatography (PE:Me_2CO 97:3) yielding **3.30** (327 mg, 40%) as a single diastereomer.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.81 (t, 3H, *J* = 6.69 Hz, alkyl –C*H*$_3$), 0.93 (s, 9H, *tert*-butyl), 0.97 (s, 9H, *tert*-butyl), 1.10 – 1.60 (m, 26H, alkyl *H*), 1.91 – 2.04 (m, 1H, -C(5)*H*), 3.16 (dd, 1H, *J* = 6.33 and 12.76 Hz, -C(5)C*H*$_a$), 3.31 (dd, 1H, *J* = 4.46 and 12.75 Hz, -C(5)C*H*$_b$), 3.46 – 3.52 (m, 1H, -C(4)C*H*(OH)), 3.89 (app t, 1H, *J* = 10.89 Hz, -C(6)*H*$_a$), 3.99 - 4.06 (m, 1H, -C(4)*H*), 4.01 (dd, 1H, *J* = 6.36 and 10.91 Hz, -C(6)*H*$_b$).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.11, 20.20, 22.68, 22.78, 25.65, 27.09, 27.21, 27.51, 29.35, 29.67, 30.58, 31.92, 41.19, 49.72, 67.40, 72.57, 78.92.

Exact mass (ESI-MS) calculated for $C_{27}H_{56}N_3O_3Si$ $[M+H]^+$: 498.4091, found: 498.4093.

Dioxolane 3.31:



3.31

a) TBAF (2.17 mL of a 1M solution in THF, 2.17 mmol, 6 eq.) was added to a solution of **3.30** (180 mg, 0.361 mmol) in THF (2 mL) and after stirring for 6 h at RT, the solvent was removed under reduced pressure. Column chromatography (hexanes:EtOAc 3:7) of the residue yielded the intermediate azido homophytosphingosine (80 mg, 62%) as a colourless solid.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.88 (t, 3H, *J* = 6.70 Hz, alkyl –C*H*$_3$), 1.20 – 1.60 (m, 26H, alkyl *H*), 2.07 (dtd, 1H, *J* = 3.39, 6.63 and 9.87 Hz, -C(2)*H*), 2.85 – 3.15 (br. s, 3H, -C(1)O*H*, -C(3)O*H* and –C(4)O*H*), 3.45 – 3.54 (m, 2H, C(2)C*H*$_2$), 3.66 – 3.85 (m, 4H, -C(1)*H*$_2$, C(3)*H* and –C(4)*H*).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.09, 22.67, 25.89, 29.34, 29.60, 29.63, 29.67, 31.90, 32.81, 40.85, 51.90, 60.22, 73.61, 74.32.

Exact mass (ESI-MS) calculated for $C_{19}H_{40}N_3O_3$ $[M+H]^+$: 358.3070, found: 358.3070.

b) To a solution of the intermediate azido homophytosphingosine (80 mg, 0.224 mmol) in 2,2-dimethoxypropane (5 mL), *p*TsOH (4 mg, 22.4 μmol, 10 mol%) was

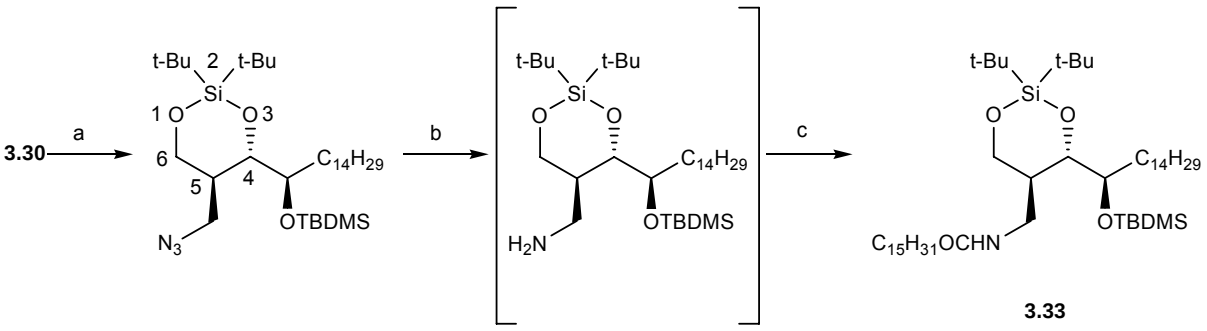
added and the resulting solution was stirred for 14 h at RT. After removal of the solvent *in vacuo*, the residue was dissolved in acetone (2 mL) and 1 N HCl (2 mL) and the resulting mixture was vigorously stirred for 1 h. EtOAc (20 mL) and water (5 mL) were added and after separation of the phases, the aqueous layer was washed with EtOAc (2 x 10 mL). After drying the combined organic phase over Na_2SO_4 and removal of all volatiles in vacuo, the residue was purified by column chromatography (hexanes:EtOAc 95:5) yielding **3.31** (72 mg, 83%) as a colourless oil.

^{1}H NMR (300 MHz, [D5]pyridine, 25°C): δ= 0.88 (t, 3H, *J* = 6.45 Hz, -C*H*$_3$ alkyl), 1.18 – 1.38 (m, 24H, alkyl *H*), 1.40 (s, 3H, -C*H*$_3$), 1.53 (s, 3H, -C*H*$_3$), 1.65 – 1.80 (m, 2H, -C(5)*H*$_2$), 2.22 – 2.34 (m, 1H, -C(2)*H*), 3.73 (dd, 1H, *J* = 5.57 and 12.31 Hz, -C(2)C*H*$_a$), 3.84 (dd, 1H, *J* = 6.15 and 12.31 Hz, -C(2)C*H*$_b$), 4.13 (dd, 1H, *J* = 6.74 and 10.56 Hz, -C(1)*H*$_a$), 4.25 – 4.34 (ddd, 1H, *J* = 2.94, 5.57 and 8.51 Hz, -C(4)*H*, partially resolved from -C(1)*H*$_b$), 4.32 (dd, 1H, *J* = 3.52 and 10.55 Hz, -C(1)*H*$_b$), 4.40 (dd, 1H, *J* = 5.57 and 8.50 Hz, -C(3)*H*), 6.36 (br. s, 1H, -C(1)O*H*)).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.10, 22.67, 25.66, 25.97, 27.89, 29.34, 29.54, 29.57, 29.64, 29.67, 30.05, 31.91, 40.18, 50.87, 62.48, 77.69, 77.81, 107.86.

Exact mass (ESI-MS) calculated for $C_{22}H_{43}N_3O_3Na$ $[M+H]^+$: 420.3202, found: 420.3209.

Intermediate 3.33:



a) To a cooled solution (0°C) of **3.30** (177 mg, 0.355 mmol), imidazole (145 mg, 2.133 mmol, 6 eq.) and 4-DMAP (cat.) in anhydrous DMF (5 mL), TBDMSCl (161 mg, 1.067 mmol, 3 eq.) was added portionwise and the resulting solution was heated at 50°C for 18 h. After removal of the solvent under reduced pressure, the residue was partitioned between Et_2O (20 mL) and sat. $NaHCO_3$ (20 mL) and the aqueous layer was extracted with Et_2O (2 x 20 mL). The combined organic phase was washed with

ice-cold 1N HCl (20 mL), sat. $NaHCO_3$ (20 mL) and brine (20 mL) and subsequently dried over Na_2SO_4. After removal of all volatiles in vacuo, the residue was purified by column chromatography (hexanes:Me_2CO 100:0.3) yielding silyl protected **3.30** (149 mg, 68%) as a colourless oil.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.03 (s, 6H, 2 x -C*H*$_3$), 0.80 (t, 3H, *J* = 6.76 Hz, alkyl –C*H*$_3$), 0.83 (s, 9H, *tert*-butyl), 0.92 (s, 9H, *tert*-butyl), 0.95 (s, 9H, *tert*-butyl), 1.15 – 1.40 (m, 25H, alkyl *H*), 1.59 – 1.65 (m, 1H, -C(4)CH(OH)C*H*$_b$), 1.88 – 2.00 (m, 1H, -C(5)*H*), 3.22 (dd, 1H, *J* = 7.09 and 12.57 Hz, -C(5)C*H*$_a$), 3.36 (dd, 1H, *J* = 9.94 and 12.56 Hz, -C(5)C*H*$_b$), 3.70 (dt, 1H, *J* = 3.86 and 6.62 Hz, -C(4)C*H*(OH)), 3.81 – 3.90 (m, 2H, -C(4)*H* and -C(6)*H*$_a$), 4.00 (dd, 1H, *J* = 4.19 and 11.11 Hz, -C(6)*H*$_b$);

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= -4.28, -4.25, 14.11, 18.07, 20.32, 22.62, 22.69, 24.86, 25.93, 27.22, 27.52, 29.36, 29.61, 29.66, 29.87, 32.93, 32.13, 41.73, 50.66, 66.81, 75.06, 78.05.

Exact mass (ESI-MS) calculated for $C_{33}H_{69}N_3O_3Si_2$ $[M+Na]^+$: 634.4775, found: 634.4780.

b) A solution of silyl protected **3.30** (147 mg, 240 μmol) and PPh_3 (126 mg, 480 μmol, 2 eq.) in anhydrous THF (4 mL) was stirred for 30' followed by addition of H_2O (250 μL) and the resulting reaction mixture was stirred for 29 h at RT. Removal of the solvent under reduced pressure produced the crude amine, which was used without further purification.

c) To a cooled solution (0°C) of the crude amine in CH_2Cl_2 (2 mL) and Hünig's base (209 μL, 1.2 mmol, 5 eq.), palmitoylchloride (147 μL, 480 μmol, 2 eq.) was added dropwise and the resulting mixture was stirred at 0°C until TLC indicated disappearance of the starting material (30'). Sat. $NaHCO_3$ (10 mL) was added and the aqueous layer was extracted with Et_2O (2 x 20 mL). After drying over Na_2SO_4 and removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 88:12) producing **3.33** (115 mg, 58% over two steps) as a colourless oil.

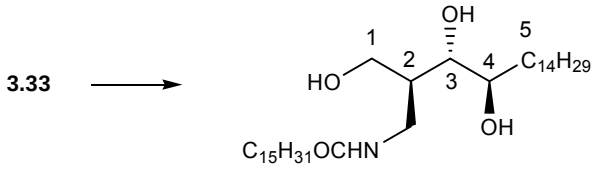
^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.00 (s, 3H, -C*H*$_3$), 0.01 (s, 3H, -C*H*$_3$), 0.78 (t, 6H, *J* = 6.74 Hz, alkyl and acyl –C*H*$_3$), 0.82 (s, 9H, *tert*-butyl), 0.90 (s, 9H,

tert-butyl), 0.92 (s, 9H, *tert*-butyl), 1.12 – 1.42 (m, 48H, alkyl and acyl *H*), 1.30 – 1.38 (m, 1H, C(4)CH(OSi)CH_a), 1.44 – 1.56 (m, 2H, -C(O)CH_2CH_2), 1.60 – 1.70 (m, 1H, -C(4)CH(OSi)CH_b), 1.84 – 1.96 (m, 1H, -C(5)*H*), 2.03 (t, 2H, *J* = 7.57 Hz, -C(O)CH_2), 3.02 (app. td, 1H, *J* = 6.15 and 13.96 Hz, -C(5)CH_a), 3.21 (app td, 1H, *J* = 5.37 and 13.98 Hz, -C(5)CH_b), 3.65 – 3.79 (m, 3H, -C(4)*H*, -C(4)C*H*(OSi) and -C(6)H_a), 3.79 (dd, 1H, *J* = 4.02 and 9.00 Hz, -C(6)H_b), 5.70 (t, 1H, *J* = 5.71 Hz, -N*H*).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= -4.17, -4.09, 14.11, 18.16, 20.22, 22.63, 22.68, 24.95, 25.83, 26.04, 27.19, 27.54, 29.36, 29.49, 29.66, 29.69, 29.90, 31.92, 32.63, 36.88, 38.10, 42.33, 67.44, 76.11, 78.54, 172.97.

Exact mass (ESI-MS) calculated for $C_{49}H_{102}NO_4Si_2$ $[M+H]^+$: 824.7347, found:824.7355.

N-homophytoceramide 3.25:



3.25

TBAF (597 μL of a 1M solution in THF (5% H_2O), 597 μmol, 6 eq.) was added to a solution of **3.33** (84 mg, 99.45 μmol) in THF (1 mL) and the resulting mixture was stirred for 48 h at RT. After removal of all volatiles in vacuo, the residue was partitioned between CH_2Cl_2 (10 mL) and sat. $NaHCO_3$ (10 mL) and the aqueous phase was washed with CH_2Cl_2 (5 x 10 mL). The combined organic phase was extracted with brine (10 mL) and after drying over Na_2SO_4 and removal of the solvent under reduced pressure, the residue was purified by flash chromatography (CH_2Cl_2:MeOH 97:3) yielding **3.25** (35 mg, 62%) as a white solid.

$[\alpha]_D^{20}$ = + 7.9° (*c*=0.42 in pyridine).

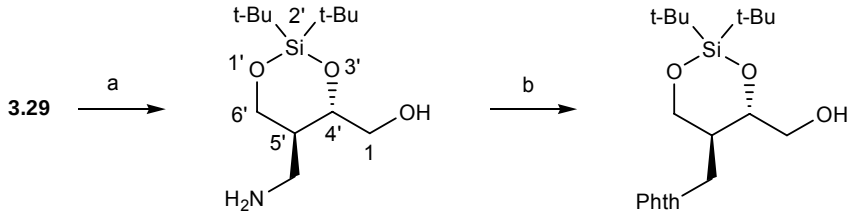
^{1}H NMR (300 MHz, [D5]pyridine, 25°C): δ= 0.88 (t, 6H, *J* = 6.54 Hz, alkyl and acyl –CH_3), 1.20 – 1.50 (m, 46H, alkyl and acyl *H*), 1.58 – 1.74 (m, 1H, -C(6)H_a), 1.76 – 2.00 (m, 4H, -C(6)H_b, -C(5)H_a and –C(O)CH_2CH_2), 2.16 – 2.31 (m, 1H, -C(5)H_b), 2.44 (t, 2H, *J* = 7.39 Hz, -C(O)CH_2), 2.77 – 2.90 (m, 1H, -C(2)*H*), 3.95 (td, 1H, *J* = 6.24 and 13.62 Hz, -C(1)H_a), 4.10 (dd, 1H, *J* = 6.84 and 13.78 Hz, -C(1)H_b), 4.15 – 4.34 (m, 3H, -C(2)CH_a, -C(3)*H* and –C(4)*H*), 4.31 (dd, 1H, *J* = 4.73 and 10.80 Hz, -

C(2)CH_b), 6.00 – 6.50 (m, 3H, -C(2)CH_2O*H*, -C(3)O*H* and –C(4)O*H*), 8.79 (t, 1H, *J* = 6.00 Hz, -N*H*).

^{13}C NMR (75 MHz, [D5]pyridine, 25°C): δ= 14.20, 22.84, 26.34, 26.47, 29.51, 29.60, 29.68, 29.77, 29.83, 29.88, 29.90, 29.93, 30.04, 30.28, 32.03, 34.81, 36.64, 39.91, 43.11, 60.37, 72.76, 74.94, 174.68.

Exact mass (ESI-MS) calculated for $C_{35}H_{72}NO_4$ $[M+H]^+$: 570.5461, found:570.5459.

Pthalimide 3.34:



3.34

a) To a cooled (0°C) solution of azide **3.29** (498 mg, 1.652 mmol) in anhydrous THF (10 mL), PPh_3 (867 mg, 3.304 mmol, 2 eq.) was added followed by water (1 mL) after stirring for 30'. The resulting reaction mixture was stirred for 26 h at RT. After removal of all volatiles in vacuo, the residue was purified by column chromatography (CH_2Cl_2:MeOH:6N NH_3 in MeOH 95:5:0.25) yielding the intermediate amine (454 mg, quant.) as a colourless oil.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.98 (s, 9H, *tert*-butyl), 1.01 (s, 9H, *tert*-butyl), 1.83 (ttd, 1H, *J* = 4.18, 8.30 and 9.69 Hz, -C(5')*H*), 2.50 – 2.70 (m, 5H, -C(1)O*H*, -C(5')CH_2NH_2 and -C(5')CH_2), 3.64 (app. d, 2H, *J* = 5.46 Hz, -C(1)H_2), 3.78 (app. t, 1H, *J* = 11.22 Hz, C(6')CH_a), 3.91 (td, 1H, *J* = 5.42 and 9.69 Hz, -C(4')*H*), 4.00 (dd, 1H, *J* = 4.30 Hz and 10.94 Hz, - C(6')CH_b).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 19.85, 22.65, 27.08, 27.36, 40.60, 45.94, 66.52, 67.53, 78.89.

Exact mass (ESI-MS) calculated for $C_{13}H_{30}NO_3Si$ $[M+H]^+$: 276.1995, found: 276.1997.

b) To a solution of the intermediate amine (421 mg, 1.528 mmol) and Hünig's base (0.532 mL, 3.057 mmol, 2 eq.) in anhydrous THF (20 mL), *N*-ethoxycarbonylphthalimide (352 mg, 1.605 mmol, 1.05 eq.) was added portionwise

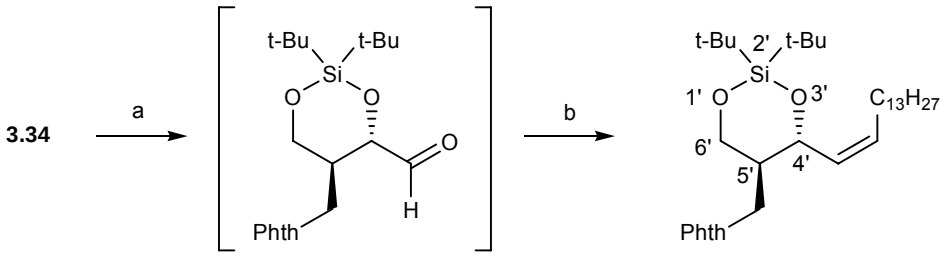
and the resulting solution was heated at 75°C for 2 h. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 82:18) yielding **3.34** (540 mg, 88%) as a colourless solid.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.96 (s, 9H, *tert*-butyl), 1.02 (s, 9H, *tert*-butyl), 2.32 (ttd, 1H, *J* = 4.38, 8.69 and 10.58 Hz, -C(5')*H*), 2.48 (dd, 1H, *J* = 4.21 and 8.71 Hz, -C(4')CH_2O*H*), 3.44 (dd, 1H, *J* = 8.48 and 14.20 Hz, -NCH_a), 3.61 (dd, 1H, *J* = 4.27 and 14.16 Hz, -NCH_b), 3.71 (ddd, 1H, *J* = 4.24, 6.74 and 11.20 Hz, -C(4')CH_a), 3.87 – 4.04 (m, 4H, -C(4')CH_b, -C(6')H_2 and -C(4')*H*), 7.69 – 7.78 (m, 2H, arom. *H*), 7.81 – 7.89 (m, 2H, arom. *H*.).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 19.85, 22.70, 27.12, 27.45, 36.14, 40.85, 65.58, 66.69, 77.13, 123.45, 131.92, 134.15, 168.23.

Exact mass (ESI-MS) calculated for $C_{21}H_{32}NO_5Si$ $[M+H]^+$: 406.2050, found: 406.2050.

***Z*-alkene intermediate 3.35:**



3.35

a) A solution of **3.34** (500 mg, 1.233 mmol) in CH_2Cl_2 (20 mL) was cooled in an ice-bath and Dess-Martin periodinane (5.26 mL of a 15% (*w/w*) in CH_2Cl_2, 2.466 mmol, 2 eq.) was added dropwise. The resulting solution was stirred for 3.5 h at RT and subsequently cooled again to 0°C. Sat. $NaHCO_3$:$Na_2S_2O_3$ (5:1, 50 mL) was added and the reaction was stirred until two clear phases were obtained (30'). After separation of the phases, the aqueous layer was extracted with Et_2O (3 x 50 mL) and the combined organic phase was washed with 0.1N HCl (3 x 10 mL) and brine (1 x 30 mL). Drying over Na_2SO_4 followed by removal of the solvent under reduced pressure yielded the intermediate aldehyde as a slightly yellow solid which was used without further purification.

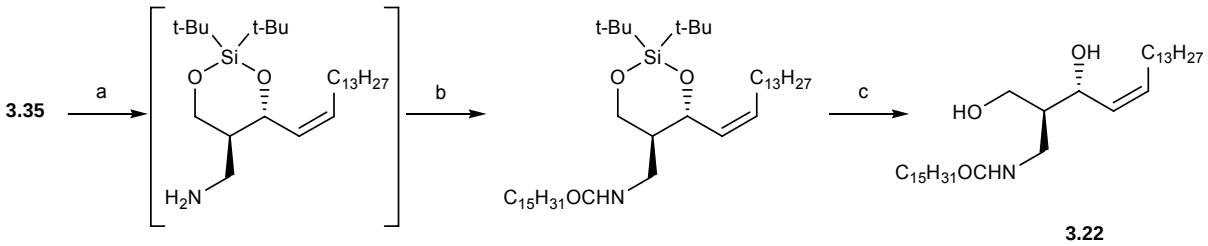
b) To a solution of PhLi (3.0 mL of a 1M solution in THF, 3.0 mmol, 2.42 eq.) in Et_2O (17 mL), LiBr (261 mg, 3 mmol, 2.42 eq.) was added under a N_2 atmosphere and the resulting brown mixture was added to a cooled solution (-78°C) of tetradecylphosphoniumbromide (1.646 g, 3.05 mmol, 2.46 eq) in anhydrous THF (20 mL). The reaction mixture was stirred for 1 h at -78°C and 1 h at RT. A cooled solution (-78°C) of the intermediate aldehyde was added dropwise the above prepared solution (20 mL). After stirring for 10' at – 78°C, another portion of the ylid solution (20 mL) was added dropwise to the reaction mixture. After stirring at – 78°C for an additional 30', the mixture was allowed to reach RT over 3 h and was subsequently cooled to 0°C. Sat. NH_4Cl (20 mL) was slowly added and the resulting slurry was stirred for 30' at RT. After addition of H_2O (50 mL) and Et_2O (50 mL), both phases were separated and the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organic layer was washed with brine (50 mL) and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (hexanes:EtOAc 98:2) yielding **3.35** (385 mg, 53%) as a colourless solid.

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.87 (t, 3H, *J* = 6.63 Hz, -CH_3 alkyl), 0.96 (s, 9H, *tert*-butyl), 1.04 (s, 9H, *tert*-butyl), 1.20 – 1.42 (m, 22H, alkyl), 1.96 – 2.38 (m, 3H, -C(5')*H* and allyl –CH_2), 3.31 (dd, 1H, *J* = 10.73 and 13.88 Hz, -NCH_a), 3.57 (dd, 1H, *J* = 4.29 and 13.90 Hz, -NCH_b), 3.89 (dd, 1H, *J* = 4.12 and 11.31 Hz, -C(6')H_a), 3.98 (app. t, 1H, *J* = 11.12 Hz, -C(6') H_b), 4.64 (app. t, 1H, *J* = 9.50 Hz, -C(4')*H*), 5.51 (app. t, *J* = 10.81 Hz, -C(4')C*H*=CH), 5.63 (dt, 1H, *J* = 7.25 and 10.93 Hz, -C(4')CH=C*H*), 7.67 – 7.77 (m, 2H, arom. *H*), 7.80 – 7.87 (m, 2H, arom. *H*).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.10, 19.75, 22.67, 22.72, 27.09, 27.47, 27.93, 29.31, 29.34, 29.51, 29.60, 29.65, 29.67, 31.90, 36.45, 43.59, 66.93, 72.44, 123.30, 130.43, 131.90, 133.88, 134.04, 168.18.

Exact mass (ESI-MS) calculated for $C_{35}H_{58}NO_4Si$ $[M+H]^+$: 584.4135, found: 584.4141.

***Z*-N-homoceramide 3.22:**



a) To a solution of **3.35** (373 mg, 0.639 mmol) in EtOH (15 mL) at 55°C, hydrazine (157 μL, 3.20 mmol, 5 eq.) was added and the solution was stirred for 3 h. After filtration of the formed white precipitate and rinsing of the filter, the solvent was removed in vacuo. The residue was covered with 1M K_2CO_3 (50 mL) and CH_2Cl_2 (50 mL) and after separation of the phases, the aqueous layer was extracted with CH_2Cl_2 (5 x 25 mL) until no product could be detected in the organic layer as judged by TLC. Removal of all volatiles under reduced pressure yielded the crude amine (325 mg, > 100%) which was used without further purification.

b) A cooled solution of the crude amine in CH_2Cl_2 (15 mL) was successively treated with Hünig's base (556 μL, 3.194 mmol, 5 eq.) and palmitoylchloride (234 μL, 0.766 mmol, 1.2 eq.). After stirring for 1 h at RT, the solvent was removed under reduced pressure and the residue was purified by column chromatography (hexanes:EtOAc 9:1) yielding the intermediate amide (289 mg, 65% from **3.35**) as a slightly yellow oil.
^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.87 (t, 6H, *J* = 6.70 Hz, -C*H*$_3$ alkyl and acyl), 0.98 (s, 9H, *tert*-butyl), 1.03 (s, 9H, *tert*-butyl), 1.15 – 1.45 (m, 46H, alkyl), 1.52 – 1.67 (m, 2H, -C(O)CH$_2$C*H*$_2$), 1.83 – 2.24 (m, 5H, -C(5')*H*, -C(O)C*H*$_2$ and allyl – C*H*$_2$), 2.95 (app. td, 1H, *J* = 4.71 and 14.02 Hz, -NC*H*$_a$), 3.57 (dd, 1H, *J* = 7.88 and 14.07 Hz, -NC*H*$_b$), 3.87 (app. t, 1H, *J* = 11.22 Hz, -C(6')*H*$_a$), 3.98 (dd, 1H, *J* = 4.09 and 11.21 Hz, -C(6')C*H*$_b$), 4.57 (app. t, 1H, *J* = 9.63 Hz, -C(4')*H*), 5.35 – 5.50 (m, 2H, -N*H* and -C(4')C*H*=CH), 5.63 (dt, 1H, *J* = 7.46 and 10.93 Hz, -C(4')CH=C*H*).
^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.10, 19.80, 22.678, 25.71, 27.12, 27.445, 27.95, 29.35, 29.51, 29.65, 29.68, 31.91, 36.81, 37.73, 45.38, 67.21, 72.68, 130.97, 133.44, 173.05.
Exact mass (ESI-MS) calculated for $C_{43}H_{86}NO_3Si$ $[M+H]^+$: 692.6377, found: 692.6372.

c) To a solution of the intermediate amide (255 mg, 0.368 mmol) in THF (10 mL), TBAF (1.1 mL of a 1M solution in THF, 1.10 mmol, 3 eq.) was added and the resulting

solution was stirred for 3 h at RT. After evaporation of all volatiles under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 1:3) yielding **3.22** (186 mg, 91%) as a colourless solid.

$[\alpha]_D^{20}$ = + 9.8° (*c*=0.45 in pyridine).

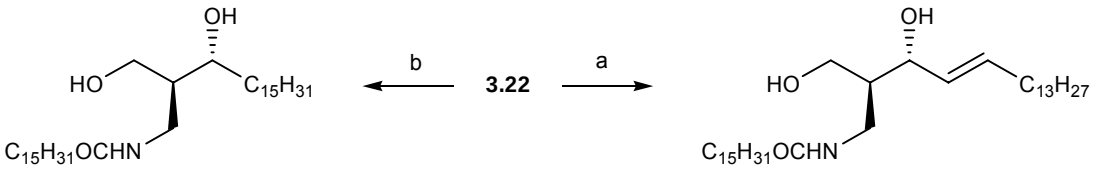
^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.81 (t, 6H, *J* = 6.71 Hz, -C*H*$_3$ alkyl and acyl), 1.15 – 1.45 (m, 46H, alkyl and acyl *H*), 1.50 – 1.63 (m, 3H, -C(2)*H* and -C(O)CH$_2$C*H*$_2$), 1.86 – 2.08 (m, 2H, allyl –C*H*$_2$), 2.14 (t, 2H, *J* = 7.60 Hz, -C(O)C*H*$_2$), 2.90 – 3.20 (m, 1H, -C(3)O*H*), 3.29 – 3.44 (m, 2H, -C(1)C*H*$_2$), 3.50 – 3.78 (m, 1H, -C(2)CH$_2$O*H*), 3.56 (dd, 1H, *J* = 3.61 and 11.69 Hz, -C(2)C*H*$_a$), 3.82 (dd, 1H, *J* = 4.18 and 11.63 Hz, -C(2)C*H*$_b$), 4.45 (dd, 1H, *J* = 5.72 and 7.36 Hz, -C(3)*H*), 5.40 – 5.52 (m, 2H, -C(4)*H* and –C(5)*H*), 5.96 (t, 1H, *J* = 6.39 Hz, -N*H*).

^{1}H NMR (300 MHz; [D5]pyridine, 25°C) δ: 0.88 (t, 6H, *J* = 6.42 Hz, -C*H*$_3$ alkyl and acyl), 1.15 – 1.35 (m, 46H, alkyl and acyl *H*), 1.80 – 1.91 (m, 2H, -C(O)CH$_2$C*H*$_2$), 2.13 – 2.42 (m, 3H, -C(2)*H* and allyl -C*H*$_2$), 2.45 (t, 2H, *J* = 7.44 Hz, -C(O)C*H*$_2$), 3.89 – 4.01 (m, 2H, -C(1)C*H*$_2$), 4.26 (dd, 1H, *J* = 4.59 and 10.86 Hz, -C(2)C*H*$_a$), 4.45 (dd, 1H, *J* = 5.05 and 10.94 Hz, -C(2)C*H*$_b$), 5.16 (dd, 1H, *J* = 6.61 and 8.51 Hz, -C(3)*H*), 5.61 (td, 1H, *J* = 7.50 and 11.01 Hz, –C(5)*H*), 5.80 – 6.20 (m, 1H, -C(3)O*H*), 5.95 (dd, 1H, *J* = 9.18 and 10.83 Hz, -C(4)*H*), 6.40 – 6.65 (m, 1H, -C(2)CH$_2$O*H*), 8.63 (t, 1H, *J* = 5.55 Hz, -N*H*).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.08, 22.66, 25.83, 27.78, 29.34, 29.52, 29.67, 31.90, 36.72, 38.46, 46.29, 60.22, 67.90, 130.37, 132.93, 174.94.

Exact mass (ESI-MS) calculated for $C_{35}H_{70}NO_3$ $[M+H]^+$: 552.5356, found: 552.5352.

E-N-homoceramide 3.23 and dihydro-N-homoceramide 3.24:



3.24 **3.23**

a) A solution of **3.22** (100 mg, 0.181 mmol) and diphenyl disulfide (8 mg, 36.25 μmol, 0.2 eq.) in cyclohexane:dioxane (3:1) was degassed for 30' with N_2 in a Pyrex tube and subsequently irradiated with a high pressure mercury lamp (300 W) for 60'. The slightly yellow solution was treated four times with diphenyl disulfide (4 x 8 mg, 4 x

36.25 μmol) and irradiated over a total period of 4 h (4 x 1 h). After removal of the solvent under reduced pressure, the residue was purified by column chromatography (PE:EtOAc:Me_2CO 70:15:15) yielding a mixture of **3.22** and **3.23** (80 mg, 80%, *E*/*Z* = 69/31 as determined by ^{1}H NMR) as a slightly yellow solid. The mixture was subsequently dissolved in diisopropylether (5 mL) and heated (55°C) until all solids dissolved. A white precipitate formed upon cooling to RT. Filtration of the suspension and rinsing of the filter with ice-cold diisopropylether (2 x 1 mL) produced a mixture of **3.22** and **3.23** (48 mg, 48%, *E*/*Z* = 93/7). Repeating of this procedure eventually gave isomerically pure **3.23** (38 mg, 38%, *E*/*Z* > 99.5/0.5).

$[\alpha]_D^{20}$ = + 6.6° (*c*=0.45 in pyridine).

^{1}H NMR (300 MHz, [D1]CDCl3, 25°C): δ= 0.81 (t, 6H, *J* = 6.62 Hz, -C*H*$_3$ alkyl and acyl), 1.15 – 1.45 (m, 46H, alkyl and acyl *H*), 1.50 – 1.66 (m, 3H, -C(2)*H* and -C(O)CH_2C*H*$_2$), 1.98 (m, 2H, allyl –C*H*$_2$), 2.14 (app. t, 2H, *J* = 7.58 Hz, -C(O)C*H*$_2$), 3.00 – 3.14 (br. s, 1H, -C(3)O*H*), 3.34 – 3.60 (m, 4H, -C(1)C*H*$_2$, -C(2)C*H*$_a$ and –C(2)CH_2O*H*), 3.74 – 3.87 (m, 1H, -C(2)C*H*$_b$), 4.07 – 4.17 (m, 1H, -C(3)*H*), 5.44 (dd, 1H, *J* = 6.16 and 15.38 Hz, -C(4)*H*), 5.67 (td, 1H, *J* = 6.48 and 15.36 Hz, -C(5)*H*), 5.84 (t, 1H, *J* = 6.26 Hz, -N*H*).

^{13}C NMR (75 MHz, [D1]CDCl3, 25°C): δ= 14.10, 22.68, 25.78, 29.25, 29.29, 29.35, 29.49, 29.65, 29.68, 31.91, 32.27, 36.71, 38.68, 45.72, 60.20, 73.12, 130.63, 132.84, 174.96.

Exact mass (ESI-MS) calculated for $C_{35}H_{70}NO_3$ $[M+H]^+$: 552.5356, found: 552.5352.

b) Pd/C (45 mg, 100 w%, 10% Pd on carbon) was added to a solution of **3.22** (45 mg, 81.53 μmol) in EtOAc (3 mL) and the resulting solution was stirred for 48 h. at RT. After filtration over celite, all volatiles were removed in vacuo yielding a slightly yellow solid. Diisopropylether (2 mL) was added to the above residue and the mixture was heated (55°C) until a clear solution was obtained. After cooling down to RT, the formed white precipitate was filtered and rinsed with ice-cold diisopropylether yielding **3.24** (38 mg, 84%).

$[\alpha]_D^{20}$ = + 7.2° (*c*=0.52 in pyridine).

^{1}H NMR (300 MHz, [D5]pyridine, 25°C): δ= 0.88 (t, 6H, *J* = 6.22 Hz, -C*H*$_3$ alkyl and acyl), 1.15 – 1.60 (m, 50H, alkyl and acyl *H*), 1.72 – 1.94 (m, 4H, -C(4)*H*$_2$ and -C(O)CH_2C*H*$_2$), 2.20 – 2.30 (m, 1H, -C(2)*H*), 2.45 (t, 2H, *J* = 7.55 Hz, -C(O)C*H*$_2$), 3.95

(app. td, 1H, *J* = 6.23 and 13.31 Hz, -C(1)H_a), 4.07 (app. td, 1H, *J* = 6.58 and 13.32 Hz, -C(1)H_b), 4.15 – 4.35 (m, 3H, -C(2)CH_2 and C(3)*H*), 8.67 (br. t, 1H, *J* = 5.57 Hz, -N*H*).

^{13}C NMR (75 MHz, [D5]pyridine, 25°C): δ= 14.15, 22.79, 26.31, 26.80, 29.46, 29.53, 29.61, 29.66, 29.72, 29.77, 29.83, 29.96, 30.02, 31.97, 35.28, 36.65, 39.49, 46.88, 60.85, 70.98, 174.48.

Exact mass (ESI-MS) calculated for $C_{35}H_{72}NO_3$ $[M+H]^+$: 554.5512, found: 554.5519.

12 EXPERIMENTAL PART

General

IUPAC names were generated with Chemdraw Ultra 8.0 (Chemoffice 2004, Cambridge Soft, Cambridge, USA).

All reactions were carried out under inert (N_2) atmosphere. Precoated Macherey-Nagel (Düren, Germany) silica gel F_{254} plates were used for TLC and spots were examined under UV light at 254 nm and/or revealed by sulphuric acid-anisaldehyde spray or phosphomolybdic acid spray. Column chromatography was performed on ICN silica gel (63-200 μM, ICN, Asse Relegem, Belgium). NMR spectra were obtained with a Varian Mercury 300 spectrometer (Varian, Palo Alto, California, USA). Chemical shifts are given in parts per million (δ relative to residual solvent peak) and coupling constants are expressed in Hz. Abbreviations used are: s = singlet, d = doublet, t = triplet, m = multiplet, br. s = broad signal. All signals assigned to amino and hydroxyl groups were exchangeable with D_2O. Numbering for ^1H assignment is based on the IUPAC name of the compounds unless stated otherwise. Structural assignment was confirmed with COSY, HMQC and/or NOEDIF/NOESY if necessary. Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof2, Micromass, Manchester, UK) equipped with a standard electrospray ionisation (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. HPLC determination of the ee of **3.11** was performed on an Agilent 1100 series HPLC equipped with a DAD and Chiracel OD-H column (4.6 x 250 mm; hexanes:EtOH 96:4; 1 mL/min).

Most chemicals were obtained from Sigma-Aldrich (Bornem, Belgium) or Acros Organics (Geel, Belgium) and were used without further purification. Anhydrous THF was obtained by distillation from $LiAlH_4$.

4,6-*O*-benzylidene-α/β-D-galactose (3.1)

A mixture of D-galactose (50.0 g, 0.278 mol), benzaldehyde (177 g, 1.67 mol, 6 eq.), and $ZnCl_2$ (38.6 g, 0.283 mol, 1.02 eq.) were vigorously mechanically shaken for 14 h. Water (100 mL) was added and after separation of both phases, the aqueous layer was extracted with hexanes (200 mL). The aqueous layer was subsequently

treated with Na_2CO_3 (300 mL of 15% solution) during which a white precipitate formed. After filtration of over celite, the solvent was removed under reduced pressure. The resulting white solid was recrystallized from EtOAc/EtOH affording **3.1** (40.3 g, 54%) as a 1.8:1 mixture of the α/β anomers.

^{1}H NMR (300 MHz; DMSO-*d6*) α-**3.1** δ: 3.59 (ddd, 1H, *J* = 3.44, 6.67 and 9.40 Hz, -C(2)*H*), 3.70-3.77 (m, 2H, -C(3)*H* and –C(5)*H*), 3.96 (d, 1H, *J* = 1.44 and 12.21 Hz, -C(6)H_a), 4.06 (d, 1H, *J* = 12.20 Hz, -C(6)H_b), 4.09 (d, 1H, *J* = 0.72 and 3.47 Hz, -C(4)*H*), 4.42 (d, 1H, *J* = 6.67 Hz, -C(2)O*H*), 4.60 (d, 1H, *J* = 6.24 Hz, -C(3)O*H*), 5.03 (app. t, 1H, *J* = 3.98 Hz, -C(1)*H*), 5.51 (s, 1H, -C*H*-Ph), 6.26 (d, 1H, *J* = 4.14 Hz, -C(1)O*H*), 7.30 – 7.50 (m, 5H, arom. *H*).

^{1}H NMR (300 MHz; DMSO-*d6*) β-**3.1** δ: 3.25 – 3.33 (m, 1H, -C(2)*H*), 3.37 - 3.35 (m, 2H, -C(3)*H* and –C(5)*H*), 3.98 – 4.10 (m, 3H, -C(4)*H* and –C(6)H_2), 4.31 (app. t, 1H, *J* = 7.22 Hz, -C(1)*H*), 4.77 (d, 1H, *J* = 5.92 Hz, -C(3)O*H*), 5.78 (d, 1H, *J* = 4.54 Hz, -C(2)O*H*), 5.53 (s, 1H, -C*H*-Ph), 6.56 (d, 1H, *J* = 6.99 Hz, -C(1)O*H*), 7.30 - 7.50 (m, 5 H, arom. *H*)

^{13}C NMR (75 MHz; DMSO-*d6*) α/β-**3.1** δ: 62.00, 65.77, 67.77, 68.44, 68.84, 68.93, 71.56, 72.06, 76.16, 76.73, 93.13, 97.26, 99.66, 99.70, 126.24, 126.27, 127.83, 127.86, 128.53, 138.72, 137.79.

Exact mass (ESI-MS) calculated for $C_{13}H_{17}O_6$ [M+H$^+$]: 257.0178, found: 257.0177

(2*R*,3*R*,4*E*)-1,3-benzylidene-4-octadecene-1,2,3-triol (3.3)

a) Tetradecyltriphenylphosphonium bromide

PPh_3 (27.54 g, 0.105 mol, 1 eq.) was suspended in tetradecylbromide (29.11 g, 0.105 mol) and the resulting mixture was heated for 60 h at 170°C. After cooling to room temperature, Me_2CO (100 mL) and Et_2O (100 mL) were added and the resulting suspension was heated at 40°C until all solids dissolved. The clear solution was placed in a refrigerator and allowed to crystallize overnight. The resulting crystalline solid was subsequently subject to recrystallisation producing tetradecyltriphenylphoshoniumbromide (43.07 g, 76%) as white shiny flakes.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.92 (t, 3H, *J* = 6.45 Hz, -CH_3), 1.15 – 1.35 (m, 20H, alkyl), 1.38 – 1.60 (m, 4H, -C(2)H_2 and –C(3)H_2), 3.50 – 3.64 (m, 2H, -C(1)H_2), 7.60 – 8.00 (m, 15H, arom. *H*)

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.98, 19.80, 20.46, 21.78, 22.12, 28.14, 28.73, 28.90, 29.00, 29.05, 29.71, 29.93, 31.31, 118.04, 119.17, 130.16, 130.32, 133.55, 133.68, 134.90.

^{31}P NMR (121 MHz; DMSO-*d6*) δ: 25.16.

*b) Threose **3.2***

A solution of **3.1** (13.19 g, 49.2 mmol) in phosphate buffer (0.067M, 300 mL, pH 7.8) was vigorously stirred while $NaIO_4$ (24.19 g, 0.113 mol, 2.3 eq.) was added portionwise. The pH of the reaction mixture was kept at 7.8 by dropwise addition of 1 N NaOH. After stirring for 30', the mixture was frozen and lyophilized. The solid residue was subsequently treated with anhydrous THF, filtered over celite and the resulting filtrate was concentrated under reduced pressure yielding crude **3.2** (10.24 g, 100%), which was used without further purification.

*c) E-alkene **3.3***

A cooled solution (- 30°C) of tetradecyltriphenylphosphoniumbromide (58.3 g, 0.108 mol, 2.5 eq.) in anhydrous toluene (450 mL) was treated over 1 h with phenyllithium (99.4 mL of a 1M solution in THF, 99.4 mmol, 2.3 eq.), obtained from bromobenzene (10.5 mL, 100 mmol) and lithium wire (694 mg, 100 mmol, 1 eq.). After stirring for 60' at -30°C, a solution of **3.2** (9.0 g, 43.22 mmol) in anhydrous THF (100 mL) was added dropwise over 45'. The reaction mixture was stirred for an additional 30' at -30 °C and subsequently quenched by addition of MeOH (25 mL) and H_2O (150 mL). After stirring for 3 h at room temperature, both layers were separated and the aqueous layer was extracted with Et_2O (4 x 100 mL) and subsequently dried over Na_2SO_4. Removal of the solvent followed by flash chromatography of the brown oily residue afforded **3.3** (7.81 g, 47 %) as a white solid.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (t, 3H, *J* = 6.45 Hz, -C*H*$_3$ alkyl), 1.14 – 1.36 (m, 22H, alkyl *H*), 1.98 (dt, 2H, *J* = 6.45 and 6.74 Hz, -C(6)*H*$_2$), 3.36 (m, 1H, -C(2)*H*), 3.96 (dd, 1H, *J* = 1.76 and 12.02 Hz, -C(1)*H*$_a$), 3.96 (dd, 1H, *J* = 1.76 and 12.02 Hz, -C(1)*H*$_b$), 4.32 (br. d, 1H, *J* = 5.86 Hz, -C(3)*H*), 4.77 (d, 1H, *J* = 6.75 Hz, -C(2)O*H*), 5.56 (s, 1H, -*CH*-Ph), 5.57 (dd, 1H, *J* = 6.45 and 15.83 Hz, -C(4)*H*), 5.69 (dt, 1H, *J* = 6.45 and 15.84 Hz, -C(5)*H*), 7.30 – 7.37 (m, 3H, arom. *H*), 7.40 – 7.48 (m, 2H, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 13.99, 22.15, 28.58, 28.68, 28.77, 28.96, 29.07, 29.11, 31.35, 31.87, 65.15, 72.09, 80.04, 100.22, 126.38, 127.83, 127.88, 128.56, 132.79, 138.86.

Exact mass (ESI-MS) calculated for $C_{25}H_{41}O_3$ $[M+H]^+$: 389.3056, found: 389.3057.

(2*R*,3*R*,4*E*)-1,3-benzylidene-2-methanesulfonyl-4-octadecene-1,2,3-triol (3.4)

To a cooled solution (0°C) of **3.4** (100 mg, 0.257 mmol) and TEA (179 μL, 1.29 mmol, 5 eq.) in anhydrous THF (5 mL), MsCl (40 μL, 0.515 mmol, 2 eq.) was added dropwise. After stirring for 1 h at 0°C, the crude mixture was concentrated and chromatographed (hexanes:EtOAc:TEA 70:30:0.1) yielding mesylate **3.4** (84 mg, 70 %) as a white solid.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.82 (t, 3H, *J* = 6.45 Hz, -CH_3 alkyl), 1.10 – 1.38 (m, 22H, alkyl *H*), 2.00 (dt, 2H, *J* = 6.45 and 6.74 Hz, -C(6)H_2), 3.16 (s, 3H, mesyl -CH_3), 4.19 (d, 1H, *J* = 12.90 Hz, -C(1)H_a), 4.25 (d, 1H, *J* = 12.90 Hz, -C(1)H_b), 4.60 – 4.67 (m, 2H, -C(3)*H* and –C(2)*H*), 5.50 (dd, 1H, *J* = 5.86 and 15.83 Hz, -C(4)*H*), 5.70 (s, 1H, -*CH*-Ph), 5.78 (dt, 1H, *J* = 6.45 and 15.83 Hz, -C(5)*H*), 7.31 – 7.44 (m, 5H, arom. *H*)

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 14.02, 22.16, 28.40, 28.64, 28.76, 28.95, 29.07, 29.10, 31.34, 31.68, 37.86, 69.05, 75.10, 77.34, 99.83, 125.59, 126.14, 128.11, 128.87, 134.27, 138.08.

Exact mass (ESI-MS) calculated for $C_{26}H_{43}O_5S$ $[M+H]^+$: 467.2831, found: 467.2829.

(2*R*,3*R*,4*E*)-2-methanesulfonyl-4-octadecene-1,2,3-triol (3.6)

A catalytical amount of *p*-TsOH was added to a solution of **3.4** (185 mg, 0.396 mmol) in THF:MeOH (1:1, 10 mL) and the resulting reaction mixture was stirred until TLC indicated complete consumption of the starting material (8 h). The solution was subsequently neutralized by addition of TEA (0.5 mL) and after removal of the solvent *in vacuo*, the resulting residue was purified by column chromatography (hexanes:EtOAc:TEA 65:35:1) yielding diol **3.6** (137 mg, 100%) as a slightly yellow oil.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.83 (t, 3H, *J* = 6.45 Hz, -CH_3 alkyl), 1.15 – 1.39 (m, 22H, alkyl *H*), 1.97 (dt, 2H, *J* = 6.45 and 6.75 Hz, -C(6)*H*), 3.12 (s, 3H, mesyl -CH_3), 3.48 (m, 1H, -C(1)H_a), 3.64 (ddd, 1H, *J* = 3.52, 4.98 and 12.02 Hz, -C(1)H_b),

4.14 (m, 1H, -C(3)*H*), 4.32 (ddd, 1H, *J* = 3.52, 5.57 and 6.74 Hz, -C(2)*H*), 4.99 (t, 1H, J = 5.42 Hz, -C(1)O*H*), 5.23 (d, 1H, *J* = 5.28 Hz, -C(3)O*H*), 5.41 (dd, 1H, *J* = 6.15 and 15.53 Hz, -C(4)*H*), 5.65 (dt, 1H, *J* = 6.45 and 15.54 Hz, -C(5)*H*).

Exact mass (ESI-MS) calculated for $C_{19}H_{39}O_5S$ $[M+H]^+$: 379.2518, found: 379.2518.

_E_-4-(benzyloxy)but-2-en-1-ol (3.7)

A solution of *E*-4-(benzyloxy)but-2-en-1-ol (5.0 g, 28.05 mmol) in anhydrous CH_2Cl_2 (10 mL) was added over 10 min to a suspension of PCC (9.07 g, 42.08 mmol, 1.5 eq.) and celite (28.05 g, 1 g/mmol) in anhydrous CH_2Cl_2 (100 mL), and the reaction mixture was stirred for 18 h at RT. Et_2O (250 mL) was added and after stirring for 30 min, the mixture was filtered. The filter cake was repeatedly rinsed with Et_2O (100 mL each time) and the combined filtrates were concentrated under reduced pressure to afford a brown oily mass which was suspended in Et_2O and passed through a small pad of silica gel to yield the aldehyde intermediate as a slightly yellow oil which was used without further purification. To a solution of this aldehyde in anhydrous Et_2O (50 mL), DiBAlH (34 mL of 1M in toluene, 34.0 mmol, 1.2 eq.) was added dropwise at -78°C over 10 min and after stirring for 15 min at -78°C, the mixture was allowed to reach room temperature over 1 h. The mixture was subsequently cooled to 0°C and MeOH was added dropwise until evolution of H_2 ceased. After addition of a saturated solution of disodiumtartrate (100 mL), the mixture was stirred for 10 min and subsequently transferred to a separatory funnel. The water phase was extracted with Et_2O (2 x 100 mL) and the combined organic phase was dried over $MgSO_4$. After removal of all volatiles under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 7:3) to afford **3.7** (3.067 g, 61%).

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 2.05 (br s, 1H, -C(1)O*H*), 4.01 – 4.20 (m, 4H, -C(1)H_2 and –C(4)H_2), 4.50 (s, 2H, benzyl *H*), 5.75 - 5.92 (m, 2H, -C(2)*H* and -C(3)*H*), 7.31 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 62.77, 70.02, 72.21, 127.57, 127.62, 127.68, 128.31, 132.25, 138.11.

Exact mass (ESI-MS) calculated for $C_{11}H_{15}O_2$ $[M+H]^+$: 179.1072, found: 179.1074.

((2*R*,3*R*)-3-((benzyloxy)methyl)oxiran-2-yl)methanol (3.8)

A mixture of $Ti(OiPr)_4$ (1.52 mL, 5.13 mmol, 0.35 eq.), D-(-)-diethyltartrate (1.03 mL, 6.02 mmol, 0.30 eq.) and molecular sieves (17 g; 1 g/mmol) in CH_2Cl_2 (80 mL) was

cooled to -20°C (CCl_4-CO_2) and stirred for 30 min. After addition of TBHP (6.22 mL of 5.5M in nonane, 34.19 mmol, 2 eq.) and aging for 30 min, **3.7** (3.047 g, 17.1 mmol) in CH_2Cl_2 (6 mL) was added dropwise over 10 min. The resulting mixture was stirred for 2 h at -20°C before being placed in a -20°C freezer overnight. The reaction was quenched by dropwise addition of 30% NaOH in brine (20 mL) under vigorous stirring at -20°C and allowed to reach room temperature over 1 h. The resulting suspension was filtered over celite and the filtrate was transferred to a separatory funnel. After separation of both phases, the organic layer was washed with brine (2 x 30 mL) and subsequently dried over $MgSO_4$. After removal of all volatiles under reduced pressure, the resulting oil was purified by column chromatography (hexanes:EtOAc 3:2) yielding **3.8** (2.66 g, 80%) as a colourless oil.

$[\alpha]^{D}_{25}$= + 19.6° (*c*=1.0 in $CHCl_3$)

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.80 – 2.00 (br. s, 1H, -C(1)O*H*), 3.05 – 3.12 (m, 1H, C(2)*H*), 3.19 – 3.26 (m, 1H, C(3)*H*), 3.53 (dd, 1H, *J* = 5.28 and 11.44 Hz, C(4)H_a), 3.64 (dd, 1H, *J* = 4.10 and 12.61 Hz, C(1)H_a), 3.76 (dd, 1H, *J* = 2.64 and 11.43 Hz, C(4)H_b), 3.92 (dd, 1H, *J* = 2.34 and 12.61 Hz, C(1)H_b), 4.57(ABq, 2H, *J* = 12.02 Hz, benzyl *H*), 7.25 – 7.38 (m, 5H, arom. *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 54.25, 55.72, 61.15, 69.62, 73.34, 127.73, 127.78, 128.41, 137.78

Exact mass (ESI-MS) calculated for $C_{11}H_{15}O_3$ $[M+H]^+$: 195.1021, found: 195.1023.

E-butene-1,4-diol (3.9)

To a well-stirred solution of $LiAlH_4$ (56 mL of a 3.5 M solution in THF, 0.196 mol, 1.2 eq.) in anhydrous THF (700 mL), butyne-1,4-diol (14.0 g, 0.163 mol) in THF (250 mL) was added dropwise at 0°C over 30 min. The suspension was subsequently heated under reflux cooling until TLC indicated complete consumption of the starting material (3 h). After cooling the white suspension to 0°C, 3N NaOH (41 mL) was added slowly until evolution of H_2-gas ceased. After adjusting the pH of the reaction mixture to pH=8, celite (90 g) was added and the heterogeneous mixture was stirred for 10 min. After filtration, the solvent was reduced *in vacuo* (~100 mL), silica gel (130 g) was added and the remaining solvent was removed under reduced pressure. The free flowing product/silica gel mixture was then loaded on a column and flashed (hexanes:EtOAc 1:4) yielding *E*-butene-1,4-diol (11.445 g, 80%) as a colourless oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 1.60 (m, 2H, 2 x -O*H*), 4.14 – 4.18 (m, 4H, 2 x -CH_2), 5.86 – 5.91 (m, 2H, 2 x alkene *H*).

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: 62.93, 130.55.

Exact mass (ESI-MS) calculated for $C_4H_9O_2$ $[M+H]^+$: 89.0602, found: 89.0605.

***E*-4-(4-bromobenzyloxy)but-2-en-1-ol (3.10)**

To a solution of diol **3.9** (4.96 g, 56.30 mmol) in CH_2Cl_2 (200 mL), Ag_2O (13.05 g, 56.30 mmol, 1 eq.) and 4-bromobenzyl bromide (15.48 g, 91.93 mmol, 1.1 eq.) were added and the heterogeneous mixture was stirred for 15 h at room temperature. After addition of celite (40 g), the mixture was filtered and the solvent was removed under reduced pressure. The resulting oil was purified by column chromatography (hexanes:EtOAc 7:3 → 3:2) yielding the title compound (11.74 g, 81%) as a colourless oil.

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 3.92 – 3.98 (m, 4H, -C(1)H_2 and –C(4)H_2), 4.41 (s, 2H, benzyl H), 4.71 (t, 1H, *J* = 5.57 Hz, -C(1)O*H*), 5.64 – 5.83 (m, 2H, -C(2)*H* and –C(3)*H*), 7.26 (d, 2H, *J* = 8.80 Hz, arom. *H*), 7.51 (d, 2H, *J* = 8.21 Hz, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 60.84, 69.77, 70.22, 120.36, 125.52, 129.51, 131.13, 133.51, 138.01

Exact mass (ESI-MS) calculated for $C_{11}H_{14}O_2Br$ $[M+H]^+$: 257.0177, found: 257.0177

((2*R*,3*R*)-3-((4-bromobenzyloxy)methyl)oxiran-2-yl)methanol (3.11)

A mixture of Ti(O*i*Pr)$_4$ (4.72 mL, 20 mol%, 15.93 mmol), D-(-)-diethyltartrate (3.75 mL, 27.5 mol%, 21.9 mmol) and molecular sieves (6 g) in CH_2Cl_2 (400 mL) was cooled to -23°C (CCl_4-CO_2) and stirred for 30 min. After addition of THBP (28.96 mL of 5.5M in decane, 2 eq., 159.3 mmol) and additional stirring for 15 min, **3.10** (20.48 g, 79.65 mmol) in CH_2Cl_2 (200 mL) was added dropwise over 60 min at -20°C. The resulting mixture was stirred for 2 h at -20°C and then placed in a -20°C freezer overnight. The mixture was subsequently cooled in an ice-bath and 30% NaOH in brine (20 mL) was slowly added under vigorous stirring. The resulting suspension was filtered over celite and the resulting filtrate was extracted with brine (2 x 30 mL). After drying over $MgSO_4$ and removal of all volatiles under reduced pressure, the resulting oil was purified by column chromatography (hexanes:EtOAc 1:1) yielding **3.11** (21.32 g, 98%) as a white powder. The product was suspended in hexanes (100 mL) followed

by slow addition of Et_2O until a clear solution was obtained which was refrigerated (-20°C) for 3 h. The formed white needles were filtered, rinsed with hexane (2 x 20 mL) and subsequently dried *in vacuo* yielding enantiomerically pure **3.11** (14.2 g, 65%). Repeating of this procedure produced a second crop of **3.11** (4.78 g, 22%). The resulting filtrate was purified by column chromatography affording a third crop of **3.11** (1.96 g, 9%).

$[\alpha]^{D}_{25}$ = + 21.8° (*c*=1 in $CHCl_3$)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.89 (br. s, 1H, -C(1)O*H*), 3.06 – 3.13 (m, 1H, C(2)*H*), 3.21 – 3.27 (m, 1H, C(3)*H*), 3.49 (dd, 1H, *J* = 5.53 and 11.68 Hz, C(4)H_a), 3.65 (br. d, 1H, *J* = 12.60 Hz, C(1)H_a), 3.78 (dd, 1H, *J* = 2.77 and 11.68 Hz, C(4)H_b), 3.94 (br. d, 1H, *J* = 12.91 Hz, C(1)H_b), 4.52 (ABq, 2H, *J* = 12.29 Hz, benzyl *H*), 7.21 (d, 2H, *J* = 8.30 Hz, arom. *H*), 7.46 (d, 2H, *J* = 8.30 Hz, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 54.10, 55.54, 60.99, 69.67, 72.49, 121.63, 129.30, 131.52, 136.74

Exact mass (ESI-MS) calculated for $C_{11}H_{14}BrO_3Na$ $[M+Na]^+$: 294.9946, found: 294.9951

(2*S*,3*S*)-4-(4-bromobenzyloxy)-3-hydroxy-2-(hydroxymethyl)butanenitrile (3.12)

To a solution of **3.11** (13.5 g, 49.42 mmol) and NaCN (9.69 g, 198 mmol, 4 eq.) in anhydrous DMF (400 mL), $B(OEt)_3$ (25.23 mL, 148 mmol, 3 eq.) was added at room temperature and the resulting solution was heated at 70°C for 15 h. After cooling to 0°C, a saturated solution of $NaHCO_3$ (400 mL) was added and the mixture was stirred for 30 min. The resulting mixture was extracted with EtOAc (5 x 200 mL) and the combined organic phase was washed with water (100 mL) and brine (100 mL) and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was purified by column chromatography (hexanes:EtOAC 1:1) yielding a mixture of regioisomers (9:1) (7.587 g, 51%) and a faster eluting compound which was identified as 4-bromobenzyl alcohol (3.42 g, 37%) Treatment of the mixture of regioisomers with $NaIO_4$ (5.4 g, excess) in acetone/water (1:1; 300 mL) followed by column chromatography with an identical solvent system as mentioned above afforded the title compound as a slightly yellow oil (5.207 g, 35%).

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 2.87 – 2.93 (m, 2H, -C(2)*H*), 3.48 (dd, 1H, *J* = 5.57 and 10.26 Hz, -C(4)H_a), 3.54 (dd, 1H, *J* = 4.10 and 10.26 Hz, -C(4)H_b), 3.58 – 3.70

(m, 2H, -C(2)CH_2), 3.82 – 3.92 (m, 1H, -C(3)*H*), 4.48 (qAB, 2H, *J* = 12.31 Hz, benzyl H), 5.19 (br s., 1H, -C(2)CH_2*OH*), 5.48 (d, 1H, *J* = 5.28 Hz, - C(3)*OH*), 7.31 (d, 2H, *J* = 8.21 Hz, arom. *H*), 7.53 (d, 2H, *J* = 8.21 Hz, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 38.82, 58.10, 66.77, 71.60, 72.42, 120.42, 120.51, 129.64, 131.16, 137.79.

Exact mass (ESI-MS) calculated for $C_{12}H_{15}BrNO_3$ $[M+H]^+$: 300.0235, found: 300.0235.

(2*S*,3*S*)-4-(4-bromobenzyloxy)-3-*tert*-butyldimethylsilyloxy-2-(*tert*-butyldimethylsilyloxy methyl)butanenitrile (3.13)

To a solution of **3.12** (6.49 g, 21.62 mmol) and imidazole (8.83 g, 130 mmol, 6 eq.) in DMF (100 mL), TBDMSCl (9.78 g, 65 mmol, 3 eq.) was added at 0°C and the mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 93:7) affording nitrile **3.13** (10.60 g, 93%) as a colourless oil.

^{1}H NMR (300 MHz; $CDCl_3$-*d1*) δ: 0.06 (s, 3H, -SiCH_3), 0.08 (s, 3H, -SiCH_3), 0.09 (s, 3H, -SiCH_3), 0.10 (s, 3H, -SiCH_3), 0.85 (s, 9H, *tert*-butyl), 0.87 (s, 9H, *tert*-butyl), 3.00 (dd, 1H, *J* = 5.57 and 11.73 Hz, -C(2)*H*), 3.59 (dd, 2H, *J* = 1.47 and 4.98 Hz, -C(4)H_2), 3.86 (d, 2H, *J* = 5.57 Hz, -C(2)CH_2), 4.13 (dd, 1H, *J* = 4.69 and 10.85 Hz, -C(3)*H*), 4.59 (app. s, 2H, benzyl *H*), 7.28 – 7.38 (4H, m, arom. *H*)

^{13}C NMR (75 MHz; $CDCl_3$-*d1*) δ: -5.54, -5.41, -5.07, -4.48, 18.02, 18.22, 25.70, 25.80, 39.35, 59.66, 68.85, 72.65, 72.78, 119.35, 127.59, 129.30, 131.49, 136.79.

Exact mass (ESI-MS) calculated for $C_{24}H_{43}BrNO_3Si_2$ $[M+H]^+$: 528.1965, found: 528.1965.

((2*R*,3*R*)-3-((4-bromobenzyloxy)methyl)oxiran-2-yl)methyl acetate (3.14)

To a solution of **3.11** (40 mg, 0.146 mmol) and 4-DMAP (cat.) in CH_2Cl_2 (2 mL), Ac_2O (28 μL, 0.293 mmol, 2 eq.) was added and the resulting mixture was stirred at room temperature until TLC indicated complete consumption of the starting material. After removal of the solvent under reduced pressure, EtOAc (10 mL) was added to the residue which was subsequently washed with 0.1N HCl (10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL). After drying over $MgSO_4$, the solvent was removed *in*

vacuo and the resulting colourless oil (46 mg, 100%) showed to be pure enough for analytical purposes.

^{1}H NMR (300 MHz; C_6D_6-*d6*) δ: 1.56 (s, 3H, -COCH_3), 2.65 -2.68 (m, 1H, -C(2)*H*), 2.76 (ddd, 1H, *J* = 2.93, 5.28 and 5.57 Hz, -C(3)*H*), 2.97 (dd, 1H, *J* = 5.57 and 11.73 Hz, -C(4)H_a), 3.22 (dd, 1H, *J* = 2.93 and 11.73 Hz, -C(4)H_b), 3.62 (dd, 1H, *J* = 6.16 and 12.31 Hz, -C(1)H_a), 4.04 (ABq, 2H, *J* = 12.31 Hz, benzyl H), 4.09 (dd, 1H, *J* = 3.08 and 12.32 Hz, -C(1)H_b), 6.81 (d, 2H, *J* = 8.50 Hz, arom. *H*), 7.22 (d, 2H, *J* = 8.21 Hz, arom. *H*).

^{13}C NMR (75 MHz; C_6D_6-*d6*) δ: 20.19, 52.46, 54.65, 64.30, 69.74, 72.25, 121.73, 129.44, 131.70, 137.54, 169.93.

Exact mass (ESI-MS) calculated for $C_{13}H_{17}BrO_4$ $[M+H]^+$: 315.0232, found: 315.0232.

(2*R*)-((2*R*,3*R*)-3-((4-bromobenzyloxy)methyl)oxiran-2-yl)methyl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (3.15)

To a solution of **3.11** (40 mg, 0.146 mmol), diisopropylethylamine (48 μL, 0.293 mmol, 2 eq.) and 4-DMAP (cat.) in CH_2Cl_2 (2 mL), (+)-Mosher's chloride (41 μL, 0.220 mmol, 1.5 eq.) was added and the mixture was stirred for 1 h at room temperature. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexanes:EtOAc 8:2) producing Mosher ester **3.15** (68 mg, 95%) as a colourless oil.

^{1}H NMR (300 MHz; C_6D_6-*d6*) δ: 2.64 - 2.70 (m, 2H, -C(2')*H* and -C(3')*H*), 2.90 (dd, 1H, *J* = 5.28 and 11.73 Hz, -C(4')H_a), 3.13 (dd, 1H, *J* = 2.64 and 11.73 Hz, -C(4')H_b), 3.36 (s, 3H, -OCH_3), 3.62 (dd, 1H, *J* = 5.57 and 12.32 Hz, -C(1')H_a), 4.00 (ABq, 2H, *J* = 12.32 Hz, benzyl -CH_2), 4.17 (dd, 1H, *J* = 2.48 and 12.31 Hz, -C(1')H_b), 6.81 (d, 2H, *J* = 8.21 Hz, arom. *H*), 6.98 – 7.08 (m, 3H, arom. *H*), 7.21 (d, 2H, *J* = 8.51 Hz, arom. *H*), 7.63 (d, 2H, *J* = 7.63 Hz, arom. *H*).

^{13}C NMR (75 MHz; C_6D_6-*d6*) δ: 51.77, 54.58, 55.43, 65.10, 69.36, 72.29, 121.81, 127.73, 128.05, 128.38, 128.74, 129.43, 129.91, 131.73, 132.68, 137.41, 166.49.

^{19}F NMR (282 MHz; C_6D_6-*d6*) δ: -72.137, -72.105.

Hydrogenation of 3.13:

Pd (10% on carbon, 2.14 g, 2.01 mmol, 10 mol%) was added to a solution of **3.13** (10.6 g, 20.11 mmol) in EtOH (100 mL) and the resulting reaction mixture was stirred

under an H_2 atmosphere until TLC indicated complete consumption of the starting material. The reaction mixture was subsequently filtered over celite and after removal of all volatiles *in vacuo*, the residue was purified by column chromatography (hexanes:EtOAc 4:1) affording, a mixture of **3.16** and **3.17** (2.33 g, 32%), **3.18** (0.95 g, 10%) and **3.19** (2.47 g, 50%) as colourless oils.

(2*S*,3*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-2-(hydroxymethyl)butanenitrile (3.16) and (2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-((*tert*-butyldimethylsilyloxy)methyl)-4-hydroxybutanenitrile (3.17) (1:1.1)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.00 (s, 6H, 2 x -SiCH_3), 0.01 (s, 3H, -SiCH_3), 0.019 (s, 6H, 2 x -SiCH_3), 0.024 (s, 3H, -SiCH_3), 0.03 (s, 6H, 2 x -SiCH_3), 0.81 (s, 18H, 2 x *tert*-butyl), 0.822 (s, 9H, *tert*-butyl), 0.824 (s, 9H, *tert*-butyl), 2.90 (ddd, 1H, *J* = 5.13, 5.88 and 6.89 Hz, -C(2)*H* **3.16**), 2.97 (dt, 1H, *J* = 5.06 and 6.22 Hz, -C(2)*H* **3.17**), 3.37 (td, 1H, *J* = 5.53 and 11.27 Hz, -C(4)H_a **3.17**), 3.45 (td, 1H, *J* = 4.92 and 11.26 Hz, -C(4)H_b **3.17**), 3.50 – 3.66 (m, 4H, -C(1)H_2 **3.16** and -C(1)H_2 **3.17**), 3.75 (dd, 1H, *J* = 5.75 and 9.47 Hz, -C(4)H_a **3.16**), 3.80 (dd, 1H, *J* = 4.35 and 9.45 Hz, -C(4)H_b **3.16**), 3.85 – 3.93 (m, 2H, -C(3)*H* **3.16** & **3.17**), 4.85 (t, 1H, *J* = 5.28 Hz, -C(1)O*H* **3.16**), 5.16 (t, 1H, *J* = 5.26 Hz, -C(4)O*H* **3.17**).

Exact mass (ESI-MS) calculated for $C_{17}H_{38}NO_3Si_2$ $[M+H]^+$: 360.239, found: 360.2398.

(2*S*,3*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-2-((*tert*-butyldimethylsilyloxy)methyl) butanenitrile (3.18)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.12 (s, 6H, 2 x -SiCH_3), 0.13 (s, 3H, -SiCH_3), 0.140 (s, 3H, -SiCH_3), 0.146 (s, 3H, -SiCH_3), 0.15 (s, 3H, -SiCH_3), 0.93 (s, 9H, *tert*-butyl), 0.94 (s, 18H, 2 x *tert*-butyl), 3.09 (m, 1H, -C(2)*H*), 3.70 (dd, 1H, *J* = 4.99 and 11.14 Hz, -C(4)H_a), 3.78 (dd, 1H, *J* = 4.10 and 11.14 Hz, -C(4)H_b), 3.87 (dd, 1H, *J* = 6.16 and 10.27 Hz, -C(1)H_a), 3.93 (dd, 1H, *J* = 4.98 and 10.26 Hz, -C(1)H_b), 4.05 (m, 1H, -C(3)*H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: -5.57, -5.48, -5.37, -5.24, -5.02, -4.63, 17.90, 18.11, 18.22, 25.61, 25.72, 25.96, 38.68, 60.03, 63.84, 70.14, 119.22.

Exact mass (ESI-MS) calculated for $C_{23}H_{52}NO_3Si_3$ $[M+H]^+$: 474.3255, found: 474.3258.

(2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-2-(hydroxymethyl)butanenitrile (3.19)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 0.06 (s, 3H, -SiCH_3), 0.08 (s, 3H, -SiCH_3), 0.85 (s, 9H, *tert*-butyl), 2.96 (dt, 1H, *J* = 5.28 and 7.33 Hz, -C(2)*H*), 3.40 (dd, 1H, *J* = 5.57 and 11.14 Hz, -C(4)H_a), 3.46 (dd, 1H, *J* = 5.57 and 11.14 Hz, -C(4)H_b), 3.54 – 3.70 (m, 2H, -C(1)H_2), 3.90 (dt, 1H, *J* = 5.28 and 5.57 Hz, -C(3)*H*), 4.88 (t, 1H, *J* = 5.28 Hz, -C(1)O*H*), 5.17 (dd, 1H, *J* = 4.99 and 5.87 Hz, C(4)O*H*)

^{13}C NMR (75 MHz; DMSO-*d6*) δ: -5.18, -4.43, 17.79, 25.71, 38.91, 57.64, 63.21, 70.93, 120.55.

Exact mass (ESI-MS) calculated for $C_{11}H_{25}NO_3Si$ $[M+H]^+$: 246.1525, found: 246.1525.

Opening of 3.11 with 1,3-dithiane:

To a solution of 1,3-dithiane (6.26 g, 52.1 mmol, 5 eq.) in anhydrous THF (70 mL) at -78°C, *n*BuLi (26 mL of a 1.6M solution in hexanes, 41.67 mmol, 4 eq.) was added and the mixture was allowed to reach 0°C over 2 h. After cooling to -78°C, DMPU (12.6 mL, 104.2 mmol, 10 eq.) and **3.11** (2.846 g, 10.42 mmol) in anhydrous THF (20 mL) were added dropwise over 30 min. After stirring for 1 h at -78°C, TLC indicated complete consumption of the starting material. The mixture was subsequently allowed to reach -20°C and saturated NH_4Cl solution (50 mL) was added in one portion. After stirring for 30 min at room temperature, EtOAc (200 mL) was added and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 200 mL) and the combined organic fractions were dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was purified by column chromatography affording a mixture of **3.20** and **3.21** (3.88 g, 95% in a 2:1 ratio). A fraction of this mixture (250 mg) was purified by preparative HPLC (hexanes:EtOAc 1:1, isocratic at 35 mL/min) providing pure **3.20** (114 mg) and **3.21** (89 mg).

(2*S*,3*S*)-4-(4-bromobenzyloxy)-2-(1,3-dithian-2-yl)butane-1,3-diol (3.20)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.58 – 1.74 (m, 1H, -C(5')H_a), 1.86 – 1.96 (m, 1H, -C(2)*H*), 1.98 – 2.09 (m, 1H, -C(5')H_b), 2.73 – 2.93 (m, 4H, -C(4')H_2 and -C(6')H_2), 3.48 (dd, 1H, *J* = 6.45 and 9.97 Hz, -C(4)H_a), 3.52 (dd, 1H, *J* = 5.28 and 9.97 Hz, -

C(4)H_b), 3.62 (dd, 1H, *J* = 5.28 and 10.85 Hz, -C(1)H_a), 3.67 (dd, 1H, *J* = 5.28 and 10.85 Hz, -C(1)H_b), 3.97- 4.06 (m, 1H, -C(3)*H*), 4.42 (d, 1H, *J* = 4.69 Hz, -C(2')*H*), 4.48 (s, 2H, benzyl *H*), 4.51 (t, 1H, *J* = 5.28 Hz, -C(1)O*H*), 4.82 (d, 1H, *J* = 5.28 Hz, -C(3)O*H*), 7.31 (d, 2H, *J* = 8.50 Hz, arom. *H*), 7.54 (d, 2H, *J* = 8.50 Hz, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 25.99, 30.09, 30.19, 48.08, 48.43, 68.19, 71.18, 73.15, 120.33, 129.52, 131.06, 138.08.

Exact mass (ESI-MS) calculated for $C_{15}H_{22}BrO_3S_2$ $[M+H]^+$: 393,0194, found: 393,0192.

(2*S*,3*S*)-4-(4-bromobenzyloxy)-3-(1,3-dithian-2-yl)butane-1,2-diol (3.21)

^{1}H NMR (300 MHz; DMSO-*d6*) δ: 1.58 – 1.74 (m, 1H, -C(5')H_a), 1.94 – 2.11 (m, 2H, -C(3)*H* and -C(5')H_b), 2.73 – 2.94 (m, 4H, -C(4')H_2 and -C(6')H_2), 3.36 (app. t, 2H, *J* = 5.57 Hz, -C(1)H_2), 3.58 (dd, 1H, *J* = 6.45 and 9.97 Hz, -C(4)H_a), 3.62 (dd, 1H, *J* = 4.69 and 9.97 Hz, -C(4)H_b), 3.79 (ddd, 1H, *J* = 3.52, 5.87 and 9.68 Hz, -C(2)*H*), 4.37 (d, 1H, *J* = 4.98 Hz, -C(2')*H*), 4.41 (s, 2H, benzyl *H*), 4.55 (t, 1H, *J* = 5.57 Hz, -C(1)O*H*), 4.57 (d, 1H, *J* = 5.27 Hz, -C(2)O*H*), 7.31 (d, 2H, *J* = 8.50 Hz, arom. *H*), 7.54 (d, 2H, *J* = 8.50 Hz, arom. *H*).

^{13}C NMR (75 MHz; DMSO-*d6*) δ: 26.00, 30.13, 45.46, 49.43, 64.08, 67.31, 69.84, 71.22, 120.31, 129.51, 131.07, 138.09.

Exact mass (ESI-MS) calculated for $C_{15}H_{22}BrO_3S_2$ $[M+H]^+$: 393,0194, found: 393,0192.

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