Synthesis of Analogs of Sphingophospholipids, Glycolipids, and Plasmalogen

by

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Abstract

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Ravi Shankar Lankalapalli

Research Adviser: Professor Robert Bittman

This dissertation presents the asymmetric syntheses of naturally occurring sphingoid bases and novel sphingolipid analogs, including (a) an unnatural sphingomyelin with a Δ^4-cis double bond in the long-chain base (cis-SM), (b) L-*threo*-β-glucosyl and galactosyl-ceramides, (c) a photoactivatable analog of β-galactosyl sphingosine (psychosine), and (d) caged sphingosine 1-phosphate and ceramide 1-phosphate analogs. Also included in this dissertation is a novel synthesis of an unnatural analog of the glycerophospholipid plasmalogen with a trans-*O*-vinyl ether linkage at the *sn*-1 position of the glycerol backbone.

Chapter 1 presents the first synthesis of (2*S*,3*R*,4*Z*)-*N*-palmitoylsphingomyelin (cis-SM). The Cu^{2+}-mediated oxidation of 1-palmitoyl-2-linoleoylphosphatidylcholine in liposomes was inhibited more effectively by cis-SM than by the natural trans-SM.

Chapter 2 presents a short synthesis of the (*E*)-*O*-vinyl analog of plasmalogen (1-*O*-(1'-(*E*)-octadecenyl)-2-*O*-oleoyl-*sn*-glycero-3-phosphocholine). To elucidate the role of the double bond in natural plasmalogen in protecting lipids from oxidative damage we synthesized this unnatural analog, which possesses a trans-vinyl ether linkage. The key step of this synthesis is the iridium-catalyzed isomerization of an allylic ether to a trans-vinyl ether.

Chapter 3 presents the synthesis of L-*threo*-β-glucosyl- and galactosyl-ceramides with various N-acyl chains and also a sulfatide analog of β-galactosylceramide. These analogs may be promising as new anti-inflammatory glycolipids to treat liver diseases and also may serve as agents for blocking raft formation in living cells. A Koenigs-Knorr reaction and the Schmidt trichloroacetimidate method were used for the glycosylation reactions.

Chapter 4 presents the synthesis of the first photoactivatable analog of psychosine. This probe, which contains a benzophenone linked to the long-chain base, may be a useful tool in the identification of receptors of psychosine. A cross-metathesis and a microwave-assisted S_N2 reaction are the key steps of this synthesis.

Chapter 5 presents the first synthesis of coumarin- and a novel 3-bromo-4-hydroxynitrophenyl benzyl-caged sphingosine and ceramide phosphoesters. These caged compounds may have the facility to enter the cytosol of cultured cells and, upon irradiation with long wavelength ultraviolet light, could evoke an intracellular response which will be helpful in finding the intracellular receptors for sphingosine 1-phosphate and ceramide 1-phosphate. Phosphoramidite chemistry was applied for coupling the cages to a protected sphingosine analog. A one-pot removal of the protective groups afforded the final product in this synthesis.

Dedicated to those who made this thesis possible:

My parents, my wife and my son

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Abbreviations

Å	angstrom
ε	extinction coefficient
Ac	acetyl
AAPH	2,2'-azo-bis(2-amidinopropane) dihydrochloride
C1P	ceramide 1-phosphate
calcd	calculated
BHNPB	3-bromo-4-hydroxynitrophenyl benzyl
Boc	*tert*-butoxycarbonyl
BP	benzophenone
Bz	benzoyl
DBU	1,5-diazabicyclo[5.4.0]undec-5-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DEPT	distortionless enhancement by polarization transfer
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMA	dimethyl acetal
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
HMPA	hexamethylphosphoramide
HRMS	high-resolution mass spectrum

HWE	Horner-Wadsworth-Emmons
LRMS	low-resolution mass spectrum
LUVs	large unilamellar vesicles
MOM	methoxymethyl
MS	molecular sieves
NBS	*N*-bromosuccinimide
NKT cells	natural killer T cells
NMR	nuclear magnetic resonance
PC	phosphatidylcholine
2PE	2-photon excitation
rt	room temperature
S1P	sphingosine 1-phosphate
SM	sphingomyelin
SPC	sphingosylphosphorylcholine
TBAF	tetra-*n*-butylammonium fluoride
THF	tetrahydrofuran
TBDPS	*tert*-butyldiphenylsilyl
TLC	thin-layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tris	tris(hydroxymethyl)aminomethane
Ts	*p*-toluenesulfonyl
Red-Al	sodium bis(2-methoxyethoxy)aluminium hydride
UV	ultraviolet

Chapter 1

Synthesis of (2*S*,3*R*,4*Z*)-sphingomyelin (cis-SM) with a Δ^4 cis-double bond and the effect of double bond geometry on the antioxidant function of sphingomyelin

Abstract

The first synthesis of (2*S*,3*R*,4*Z*)-sphingomyelin (cis-SM) with a Δ^4- cis double bond is described. The key step involves the regioselective phosphorylation of unnatural sphingosine. Substitution of the natural trans double bond in SM with a cis double bond (cis-SM) surprisingly enhanced its ability to inhibit Cu^{2+}-mediated 16:0/18:2-phosphatidylcholine (PC) oxidation by about 8 fold. However, when the free radical initiator AAPH (2,2'-azo-bis(2-amidinopropane) dihydrochloride) was used as the oxidant, cis-SM increased the lag phase but had no effect on the rate of PC oxidation relative to trans-SM.

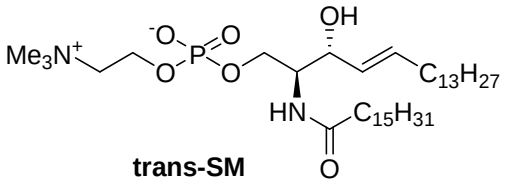
Introduction

SM (see Chart 1 for structure) is an abundant phospholipid in the cell membranes and lipoproteins of all vertebrate animals, and is an essential component of membrane rafts, the microdomains of cell membranes that play a critical role in cell signaling and other cellular processes.[1,2] SM is concentrated in the plasma membrane of cells, specifically in the exofacial leaflet that faces the environment. Recent epidemiologic studies show that increased plasma levels of SM are associated with an increase in risk of atherosclerosis,[3,4] but the underlying mechanisms are largely unknown. Part of the reason for this is that the normal physiological functions of SM in cells and lipoproteins are not well understood. The physiological function of SM in lipoproteins, where it is the second most abundant phospholipid, is not established. It was

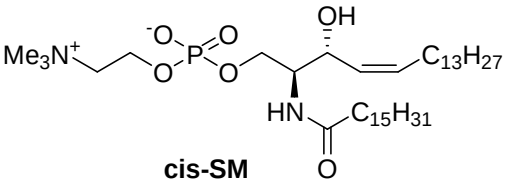
also demonstrated that SM inhibits the oxidation of unsaturated PC and cholesterol in lipoproteins and cell membranes.[5,6] However, the possible mechanism for this antioxidant function is not clear.

A unique structural feature of SM (and other sphingolipids) is the presence of a trans double bond in its sphingosine backbone, in contrast to the other naturally occurring membrane phospholipids which have cis unsaturation in their fatty acyl groups. In sphingolipid biosynthesis, the trans double bond is introduced into the long chain base of ceramide by the stereospecific dihydroceramide desaturase. The lack of this enzyme leads to cell cycle arrest in cultured cells,[7] and to growth retardation and death in experimental animals.[8] Therefore, it is likely that the trans double bond in SM has unique functions that are not fulfilled by the more common cis unsaturation found in glycerolipids. As a trans double bond tends to be less reactive chemically than a cis double bond, it may also be less susceptible to free-radical reactions. Unlike the cis double bond found in natural fatty acids which are in the interior of the bilayer, the trans double bond of SM is located at the C4 of its sphingosine backbone, and resides near the interfacial region, in the vicinity of the hydrogen bond forming C3-OH and C2-NHCO groups. It is, therefore, of interest to determine whether the trans double bond of the sphingosine backbone plays a role in the antioxidant effects of SM. Previous studies on oxidative modification of low-density lipoproteins used Cu^{2+} or the extensively studied water-soluble peroxyl radical generator AAPH.[9] This chapter describes the synthesis of an unnatural analog of SM containing a cis double bond at the C4 position of the sphingosine backbone (Chart 1 and compound **7**, Scheme 2A), and the elucidation of its effect on the oxidation of PC induced by Cu^{2+} and AAPH.

Chart 1. Structures of trans-SM and cis-SM



trans-SM

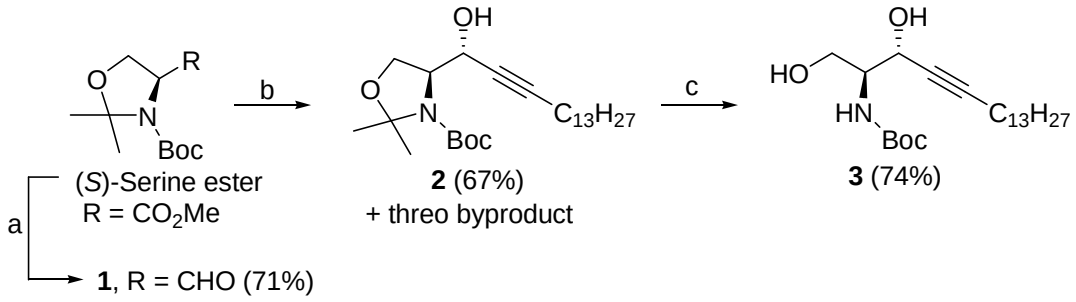


cis-SM

Results and Discussion

Synthesis of 1,3-diol 3 from (*S*)-Garner aldehyde 1 (Scheme 1). (*S*)-Garner aldehyde (**1**) was prepared from DIBAL reduction of (*S*)-serine methyl ester. Reaction of aldehyde **1** with the lithium salt of 1-pentadecyne in HMPA/THF (nonchelating conditions)[10] at -78 °C provided a mixture of erythro and threo propargylic diastereomers that were separated by flash chromatography (elution with EtOAc/hexane 1:3); the ratio of D-erythro to L-threo product was ~20:1.[10] The erythro diastereomer **2** was treated with Amberlyst-15 resin in methanol, affording *N*-Boc-protected diol **3**.

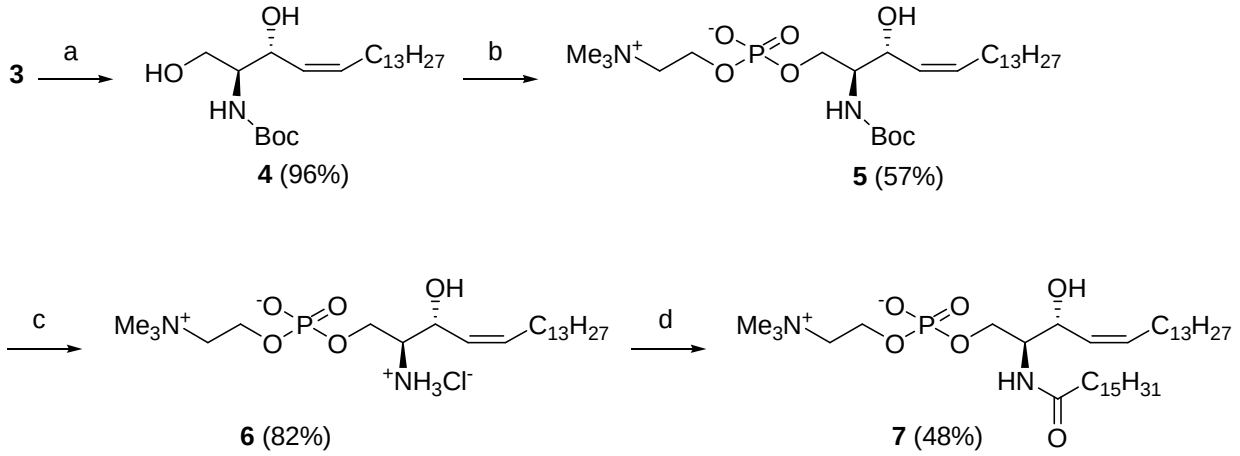
Scheme 1. Synthesis of 1,3-diol 3 from (*S*)-Garner aldehyde



Reagents and conditions: (a) DIBAL in toluene, -78 °C, 2 h; (b) 1-pentadecyne, *n*-BuLi, HMPA, THF, -78 °C; (c) Amberlyst-15, MeOH, 2.5 d.

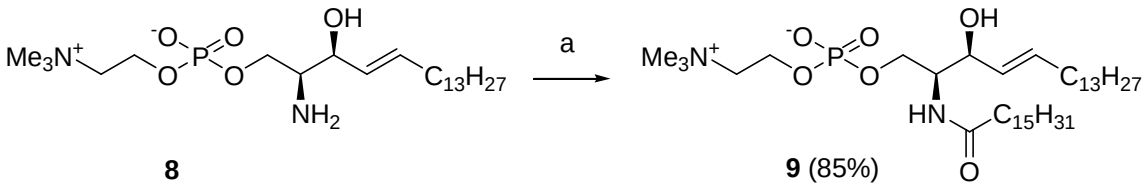
Preparation of D-*erythro*-(2*S*,3*R*,4*Z*)-*N*-palmitoyl-SM (cis-SM) 7 and L-*threo*-(2*S*,3*S*,4*E*)-*N*-palmitoyl-SM (L-*threo*-SM) 9 (Scheme 2). An attempt to partially reduce the triple bond by reaction with hydrogen in the presence of Lindlar catalyst[11] in ethyl acetate to afford a cis-double bond by stirring the reaction mixture for 1 h led to complete reduction of the triple bond. However, when the reaction mixture was stirred for 25 min, the product obtained was *cis*-*N*-Boc-D-*erythro*-sphingosine (**4**). The phosphocholine group was incorporated by initially treating ethylene chlorophosphite with bromine at -78 °C in CH_2Cl_2, followed by treating the resulting intermediate with alcohol **4**. This method is a slight variation of a previous method in which the alcohol initially was treated with a phosphitylation reagent (ethylene chlorophosphite) followed by oxidation of the resulting cyclic monophosphite with Br_2.[12] Azido analogs of sphingosine can also be monophosphitylated using this procedure. The final step involved quaternization using aqueous trimethylamine in CH_3CN/*i*-PrOH/$CHCl_3$ (1.5:1.5:0.9, v/v/v), affording *N*-Boc-D-*erythro*-4,5-*cis*-sphingosylphosphorylcholine (**5**) as a white powder. Deprotection of the *N*-Boc group with 3 M HCl/THF (1:1) at 70 °C provided lyso-4,5-cis-SM **6**. *N*-Acylation of amine **6** with *p*-nitrophenyl palmitate in dry DMF/CH_2Cl_2 (5:2, v/v) in the presence of anhydrous K_2CO_3 (as described previously)[13] afforded (2*S*,3*R*,4*Z*)-*N*-palmitoyl-SM (cis-SM, **7**). LC/MS analysis of this compound showed the presence of ~10% of dihydro-SM, which is apparently formed because of over-reduction of alkyne **3**. Also, (2*S*,3*S*,4*E*)-*N*-palmitoyl-SM [L-*threo*-SM, **9**] was prepared by *N*-acylation of L-*threo*-lyso-SM **8** (purchased from Matreya) with *p*-nitrophenyl palmitate.

Scheme 2A. Synthesis of cis-SM (7)



Reagents and conditions: (a) H_2, Lindlar catalyst, EtOAc, 25 min; (b) (i) ethylene chlorophosphite, Br_2, -78 °C, CH_2Cl_2, 1 h, (ii) **4**, *N*,*N*-diisopropylethylamine (5.0 equiv), 2 h at -78 °C, then rt, overnight, (iii) aqueous Me_3N, CH_3CN/*i*-PrOH/$CHCl_3$ (1.5:1.5:0.9), 24 h; (c) 3 M HCl/THF (1:1), 70 °C, overnight; (d) *p*-nitrophenyl palmitate, K_2CO_3, DMF/CH_2Cl_2 (5:2), 1 d.

Scheme 2B. Synthesis of L-*threo*-SM (9)

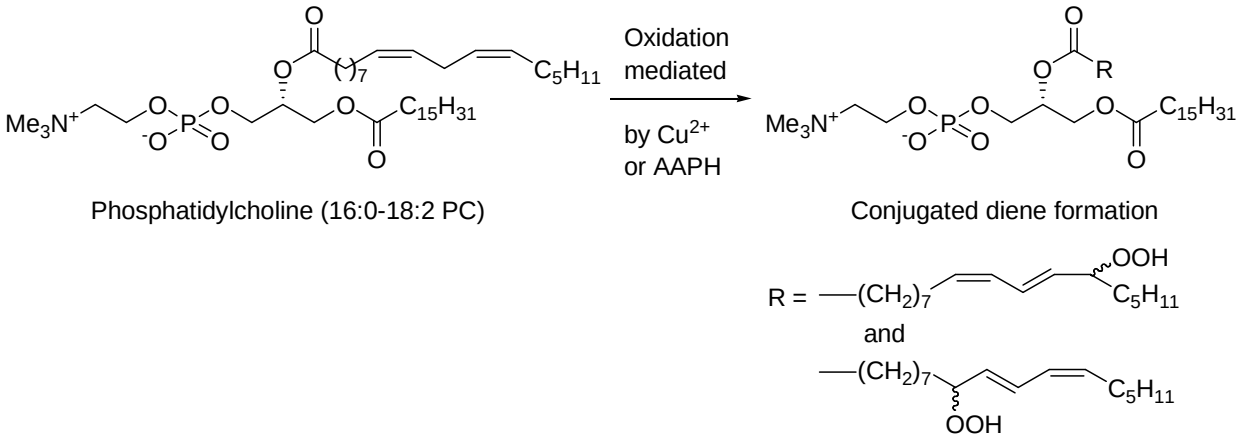


Reagents and conditions: (a) *p*-nitrophenyl palmitate, K_2CO_3, DMF/CH_2Cl_2 (5:2), 1 d.

Assay of PC peroxidation. The following experiments were carried out in the laboratory of Professor P. V. Subbaiah (University of Illinois at Chicago School of Medicine).[14] Liposomes (large unilamellar vesicles, LUVs) containing 16:0/18:2-PC (1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine) and various SM analogs (molar ratio of PC:SM, 5:1) were prepared by extrusion through polycarbonate filters. Briefly, chloroform solutions of PC (2 μmol), with or without SM (0.4 μmol), were added to a glass tube, and the solvent was evaporated under

nitrogen. The lipids were dissolved in 0.3 mL of ethanol, and the solvent was again evaporated under nitrogen. To the dried lipid film was added 1 mL of Tris buffer (20 mM Tris, pH 7.4), and the suspension was dispersed by vortexing for 1 min. The sample was flushed with nitrogen, incubated in the dark at 40 °C for 20 min, and passed through a 0.1 μm Nucleopore membrane (Whatman) 11 times using a Mini-extruder apparatus (Avanti Polar Lipids), while maintaining the temperature of the syringe at 40 °C with the help of a heating block. The liposomes were stored under nitrogen at 4 °C in the dark, and were used for oxidation studies within 10 days of the preparation. The ratios of PC and SM in the recovered LUVs were estimated by lipid phosphorus analysis[15] after TLC separation of lipids, and were found to be same as in the starting material. For the oxidation studies, 120 μL of the liposome preparation (240 nmol of PC) was treated with 50 μM $CuCl_2$ in 10 mM Tris, 150 mM NaCl, pH 7.4, in a final volume of 3.0 mL, in the cuvette of a spectrophotometer. The reactions were carried out at 37 °C for up to 600 min in a temperature-controlled spectrophotometer (Shimadzu UV-1601), and the readings at 234 nm (conjugated diene formation, Figure 1) were recorded continuously at 5-min intervals. The rates of oxidation were calculated from the slopes in the linear range, after subtraction of the corresponding blank values (samples incubated in the absence of Cu^{2+}). In some experiments, the oxidation was carried out in presence of the thermolabile free-radical generator AAPH (0.1 mM) instead of $CuCl_2$.

Figure 1. Postulated peroxidation reaction of 16:0/18:2-PC mediated by Cu^{2+} or AAPH.



SM unsaturation and inhibition of PC oxidation. The following experiments were carried out in the laboratory of Professor P. V. Subbaiah. To determine the effect of unsaturation in the long chain base, three SM analogs (dihydro-SM, cis-SM, and trans-SM) were incorporated into liposomes containing 16:0/18:2-PC at PC:SM molar ratios of 5:1. The oxidation of PC was measured in the presence of 50 μM Cu^{2+} at 37 °C. As shown in Figure 2, the presence of natural trans-SM (egg SM) increased the lag phase by 30 min and inhibited the rate of PC oxidation by about 20%, based on the linear oxidation rates. However, the incorporation of cis-SM increased the lag phase by 120 min, and inhibited the rate of oxidation by about 90%. The rate of PC oxidation was about 8 times higher in the presence of trans-SM relative to that in liposomes prepared with cis-SM. Dihydro-SM inhibited the oxidation by about 45%. Based on the total conjugated diene concentration at 300 min, the inhibition in the extent of PC oxidation was 31% in the presence of trans-SM, 36% in the presence of dihydro-SM, and 87% in the presence of cis-SM. Increasing the Cu^{2+} concentration by two fold did not significantly affect the rate of PC oxidation in the presence of cis-SM, but increasing it four fold (to 200 μM) markedly increased the rate (Figure 3). The effect of increasing the Cu^{2+} was less noticeable in the presence of trans-SM. When the thermolabile free-radical generator AAPH was used as the oxidizing agent, the

protection by cis-SM was less remarkable (Figure 4). These results suggest that the inhibitory effect of cis-SM was selective for the Cu^{2+}-mediated oxidation.

Figure 2. Effect of SM analogs on the oxidation of 16:0/18:2-PC in the presence of Cu^{2+}.

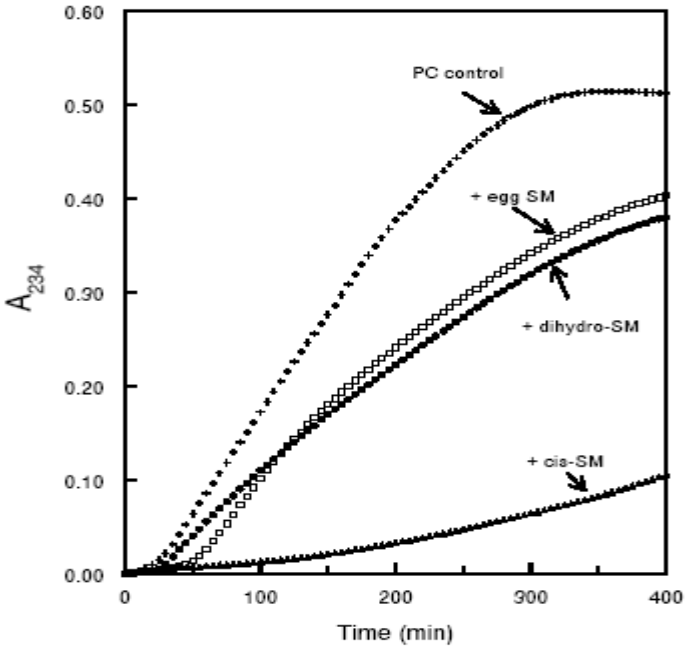


Figure 3. Effect of Cu^{2+} concentration on the inhibition of PC oxidation by SM analogs.

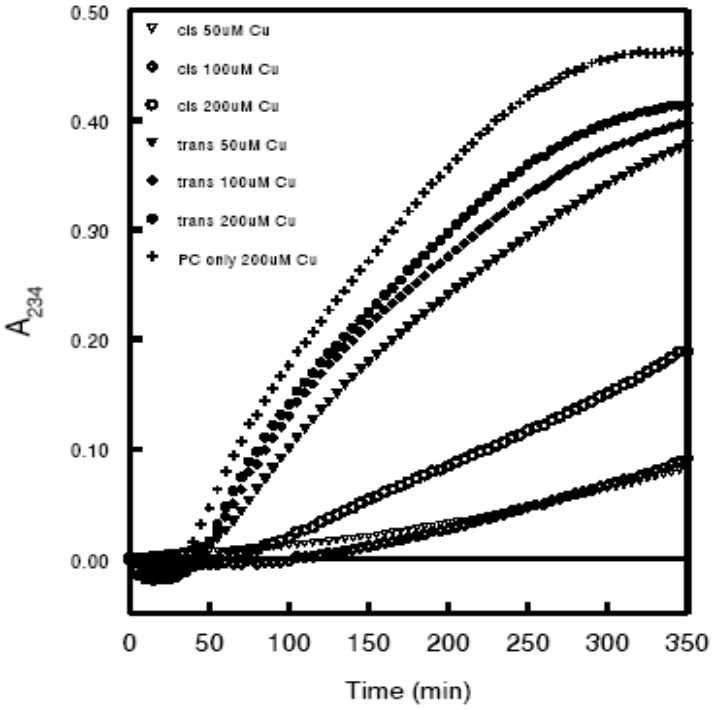
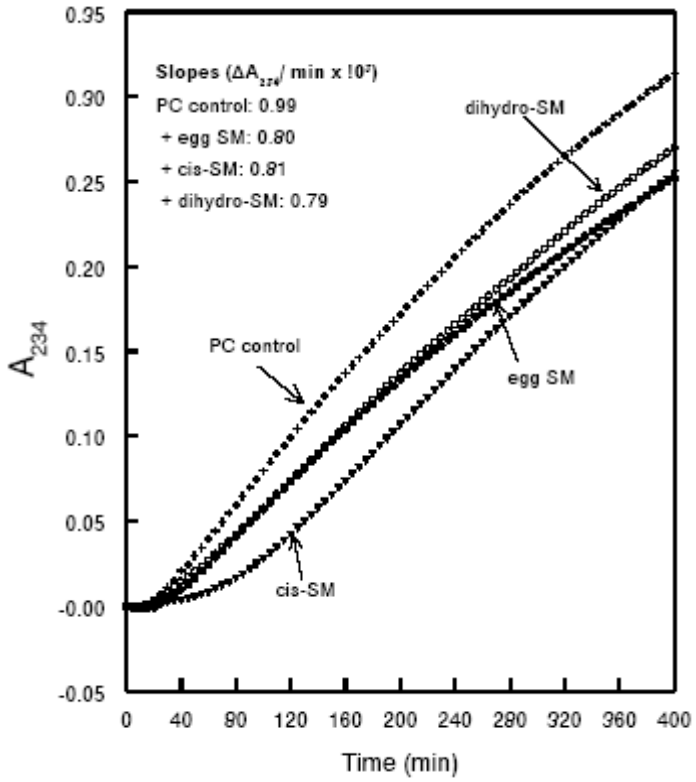


Figure 4. Effect of SM unsaturation on AAPH-mediated oxidation of PC.



Binding studies using NMR. The interaction of the cis and trans isomers of SM with Cu^{2+} was also studied using NMR spectroscopy. The effect of Cu^{2+} ($CuSO_4 \cdot 5H_2O$) on the chemical shifts of the vinylic protons of cis- and trans-SM was determined by NMR in CD_3OD at a SM:Cu^{2+} ratio of 1.0. A small downfield shift of the signal was observed in the case of cis-SM (from 5.375 to 5.395 ppm for the proton at C4, and from 5.517 to 5.537 ppm for the proton at C5) but not in the case of trans-SM (results not shown). However, these differences were not large enough to explain the large difference in the antioxidant effects of the two isomers. Furthermore, there was no significant difference in the T_1 relaxation times of the vinylic protons of the cis- and trans-SM isomers in the presence of Cu^{2+}. At a SM:Cu^{2+} ratio of 1.0, the percentage difference of relaxation times was found to be 94% and 82% for the downfield proton

of trans-SM and cis-SM, respectively. There was no difference in the T_1 values for the upfield protons of cis-SM/Cu^{2+} vs. trans-SM/Cu^{2+}. Furthermore, T_1 relaxation experiments conducted on model compounds (*cis*-2-pentene and *trans*-2-pentene) in the presence of Cu^{2+} gave rise to no significant differences. These results appear to rule out chelation of Cu^{2+} as a contributing factor for the aforementioned results.

Conclusions

A convenient method, based on a previously reported procedure for regioselective phosphorylation of sphingosine derivatives, has been demonstrated for the first synthesis of cis-SM. Replacing the trans double bond with a cis double bond resulted in up to an eight-fold increase in the antioxidant activity of SM in the presence of Cu^{2+} as the oxidizing agent. However, the effect of the cis double bond was less evident when AAPH was used as the oxidizing agent. NMR studies, did not indicate a significant chelation of Cu^{2+} by either cis- or trans-SM. These results suggest that the double bond geometry at the C4 position in the sphingosine base plays an important role in the inhibition of PC oxidation brought about by Cu^{2+}.

Experimental Section

General Information. The solvents were dried as follows. THF and Et_2O were heated at reflux over sodium benzophenone ketyl. Diisopropylethylamine and CH_2Cl_2 were distilled over calcium hydride, benzene was distilled over sodium metal, and methanol was heated at reflux over magnesium. Lindlar catalyst was purchased from Aldrich. Silica gel 60 F254 aluminum TLC plates of 0.2-mm thickness were used to monitor the reactions, with short wavelength ultraviolet light to visualize the spots and by charring the TLC plate after spraying with 15%

sulfuric acid. Phosphorus-containing compounds were detected with a molybdic acid spray. Flash chromatography was carried out with silica gel 60 (230-400 ASTM mesh). ^{1}H NMR spectra were recorded at 400 MHz, and chemical shifts are given in parts per million. ^{13}C NMR and ^{31}P NMR spectra were recorded at 100 MHz and 162 MHz, respectively. HRMS analysis was performed using the electrospray ionization technique.

(*S*)-*tert*-Butyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (1). A solution of *N*-Boc (*S*)-serine methyl ester (12.0 g, 46.3 mmol) in toluene (100 mL) was cooled to -78 °C under nitrogen. To the cooled solution was slowly added DIBAL (83 mL, a 1.5 M solution in toluene). The reaction mixture was stirred for 2 h at -78 °C, and the reaction was quenched by slowly adding 100 mL of cold MeOH. The resulting white emulsion was slowly poured into 200 mL of ice-cold 1 M HCl with swirling over 20 min, and the aqueous mixture was extracted with EtOAc (3 x 120 mL). The combined organic layers were washed with brine (150 mL), dried (Na_2SO_4), and concentrated to give the crude product as a colorless oil which was vacuum distilled to provide aldehyde **1** (7.5 g, 71%) as a colorless liquid, bp 115-125 °C (9.0 mm Hg).

(*S*)-*tert*-Butyl-4-((*R*)-1'-hydroxyhexadec-2'-ynyl)-2,2-dimethyloxazolidine-3-carboxylate (2). To a solution of 1-pentadecyne (3.25 g, 15.6 mmol) in dry THF (100 mL) was added *n*-BuLi (2.5 M in hexane, 3.6 mL, 17.4 mmol) at -40 °C under N_2. The mixture was stirred for 2 h before HMPA (6.05 mL, 34.8 mmol) was added at -78 °C. After the mixture was stirred for 45 min, a solution of (*S*)-Garner aldehyde **1**[16] (2.74 g, 12.0 mmol) in dry THF (20 mL) was added slowly. The solution was stirred at -78 °C for 2.5 h, and the reaction was quenched with aqueous saturated NH_4Cl solution (100 mL). The mixture was extracted with Et_2O (3 x 70 mL), and the combined organic phases were washed with brine (100 mL) and dried ($MgSO_4$). The crude oil was purified by flash chromatography (EtOAc/hexane 1:3) to afford **2** (3.5 g, 67%) as a

colorless oil: R_f 0.39 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.6 Hz), 1.25-1.61 (m, 37H), 2.21 (m, 2H), 3.91 (s, 1H), 4.13 (m, 2H), 4.52 (m, 1H), 4.70 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.8, 22.7, 25.4, 25.7, 28.4, 28.6, 28.9, 29.1, 29.3, 29.5, 29.6, 29.7, 31.9, 62.8, 64.2, 65.1, 77.9, 81.2, 86.6, 94.9, 154.1. Threo-by product: R_f 0.33 (EtOAc/hexane 1:3).

***tert*-Butyl (2*S*,3*R*)-1,3-Dihydroxyoctadec-4-yn-2-yl-carbamate (3).** To a solution of **2** (2.5 g, 5.71 mmol) in 30 mL of MeOH was added Amberlyst 15 resin (2.0 g). After the heterogeneous mixture was stirred at rt for 2.5 days, the mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification by chromatography (EtOAc/hexane 1:1) provided **3** (1.7 g, 74%) as a white solid: R_f 0.17 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.25 (m, 22H), 1.45 (s, 9H), 2.19 (m, 2H), 3.52 (br s, 1H), 3.73 (m, 2H), 3.99 (m, 1H), 4.59 (m, 1H), 5.46 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.7, 22.6, 28.3, 28.5, 28.9, 29.1, 29.3, 29.5, 29.6, 29.7, 31.9, 55.5, 60.4, 62.3, 64.1, 77.9, 79.9, 87.7, 156.3.

***tert*-Butyl (2*S*,3*R*,4*Z*)-1,3-Dihydroxyoctadec-4-en-2-yl-carbamate (4).** A solution of **3** (678 mg, 1.76 mmol) in dry EtOAc (40 mL) was degassed with H_2 for 15 min. To this solution was added Lindlar catalyst (335 mg, ~ 5% palladium on calcium carbonate; poisoned with lead), and the mixture was stirred under H_2 atmosphere for 25 min. The reaction mixture was filtered on a Celite layer (washed with EtOAc) and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:3) to afford **4** (680 mg, 96%) as a white powder: R_f 0.16 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.27 (m, 22H), 1.45 (s, 9H), 2.04 (m, 2H), 2.6 (br s, 1H) 2.65 (br s, 1H), 3.52 (m, 1H), 3.98 (m, 2H), 4.60 (m, 1H), 5.37-5.72 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.9, 27.8, 28.3, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.8, 32.3, 34.4, 55.7, 62.6, 69.5, 74.6, 79.7, 128.6, 134.3, 156.2.

(2*S*,3*R*,4*Z*)-1-*O*-[2'-Trimethylaminoethyl(hydroxy)phosphoryl]-2-*N*-(*tert*-butoxy-carbonylamido)-3-hydroxyoctadec-4-ene (5). To a solution of ethylene chlorophosphite (0.45 mL, 5.1 mmol) in CH_2Cl_2 (70 mL) was added Br_2 (0.26 mL, 5.1 mmol) at -78 °C. After the reaction mixture had stirred for 1 h, diisopropylethylamine (1.5 mL, 8.5 mmol) was added. A solution of **4** (680 mg, 1.70 mmol) in CH_2Cl_2 (30 mL) was added to the reaction mixture. The reaction mixture was stirred for 2 h at -78 °C, and then warmed to rt. After the starting material disappeared, the yellow mixture was concentrated and dried (Na_2SO_4) to provide a brown residue that was dissolved in 39 mL of CH_3CN/*i*-PrOH/$CHCl_3$ (1.5:1.5:0.9). After 40% aqueous Me_3N (39 mL) was added, the reaction mixture was stirred for 24 h at rt. The solvents were evaporated and the residue was purified by chromatography ($CHCl_3$/MeOH/H_2O 65:35:8) to afford **5** (550 mg, 57%) as a white solid: R_f 0.18 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.25 (m, 31H), 2.13 (m, 2H), 2.59 (br s, 1H), 3.22 (s, 9H), 3.64-3.90 (m, 4H), 4.28 (m, 4H), 5.18 (m, 1H), 5.55 (t, 1H, J = 10.8 Hz), 5.70 (dt, 1H, J = 7.5, 10.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 22.7, 27.7, 28.5, 29.2, 29.3, 29.5, 29.6, 29.7, 29.8, 31.9, 54.3, 59.4, 60.5, 65.7, 66.2, 73.9, 78.8, 126.1, 136.8, 159.8; ^{31}P NMR ($CDCl_3$) δ -0.37; HRMS $(M+H)^+$ $C_{28}H_{58}N_2O_7P$ calcd for m/z 565.3982, found 565.3968. We also observed ~20% of the dihydro product because of over-reduction in the third step; HRMS $(M+H)^+$ $C_{28}H_{60}N_2O_7P$ calcd for m/z 567.4138, found 567.4102.

(2*S*,3*R*,4*Z*)-1-*O*-[2'-Trimethylaminoethyl(hydroxy)phosphoryl]-2-amino-3-hydroxy-octadec-4-ene (6). A solution of **5** (250 mg, 0.44 mmol) in THF (15 mL) and 3 M HCl (15 mL) was heated overnight at 70 °C. The solvent was evaporated and the residue was dried under vacuum and lyophilized from benzene to afford **6** (180 mg, 82%) as the hydrochloride salt, which was used without purification in the next step.

(2*S*,3*R*,4*Z*)-*N*-Palmitoylsphingomyelin (7). A solution of **6** (180 mg, 0.36 mmol), *p*-nitrophenyl palmitate (272 mg, 0.72 mmol), and anhydrous K_2CO_3 (75 mg, 0.54 mmol) was suspended in a solution of anhydrous DMF (20 mL) and CH_2Cl_2 (8 mL). After the reaction mixture had stirred for 1 day, the mixture was concentrated under high vacuum. The compound was dissolved in 20 mL of $CHCl_3$/MeOH/H_2O (65:35:8) and concentrated under vacuum. Purification by chromatography ($CHCl_3$/MeOH/H_2O 65:35:8), followed by removal of suspended silica gel by filtration through a Cameo filter, afforded **7** (120 mg, 48%) as a white solid: R_f 0.34 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR ($CDCl_3$/CD_3OD 20:1) δ 0.85 (t, 6H, *J* = 6.8 Hz), 1.25 (m, 46H), 1.64 (m, 2H), 2.03-2.18 (m, 4H), 3.23 (s, 9H), 3.67 (m, 4H), 3.84 (m, 1H), 4.04 (m, 1H), 4.29 (m, 2H), 5.55 (t, 1H, *J* = 10.8 Hz), 5.72 (dt, 1H, *J* = 7.3, 10.8 Hz), 7.21 (d, 1H, *J* = 9.2 Hz); ^{13}C NMR ($CDCl_3$/CD_3OD 20:1) δ 17.9, 26.5, 29.7, 29.8, 30.9, 31.6, 32.4, 33.0, 33.2, 33.3, 33.4, 33.5, 33.6, 35.7, 37.5, 38.7, 40.5, 58.0, 58.2, 62.9, 63.0, 68.5, 68.8, 70.2, 73.9, 133.1, 137.6, 177.8; ^{31}P NMR ($CDCl_3$/CD_3OD 20:1) δ -0.28; LRMS $(M+H)^+$ $C_{39}H_{80}N_2O_6P$ calcd for m/z 703.6, found 703.5. In addition, the peak at m/z 705.5 (~10%) indicated the presence of the dihydro product.

(2*S*,3*S*,4*E*)-*N*-Palmitoylsphingomyelin (9). L-*threo*-Sphingosylphosphocholine (**8**, 10 mg, 0.020 mmol, purchased from Matreya) was converted into SM by following the procedure used to prepare compound **7**. Purification by chromatography (elution with $CHCl_3$/MeOH/H_2O 65:35:8), followed by removal of suspended silica gel by filtration through a Cameo filter, afforded compound **9** (12 mg, 85%) as a white solid: R_f 0.34 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR ($CDCl_3$/CD_3OD 20:1) δ 0.86 (t, 6H, *J* = 6.8 Hz), 1.14 (m, 46H), 1.59 (m, 2H), 2.02 (m, 2H), 2.15 (t, 2H, *J* = 7.6 Hz), 3.24 (s, 9H), 3.66 (m, 2H), 3.78-3.98 (m, 3H), 4.28 (m, 2H), 4.42 (m, 1H), 5.41 (dd, 1H, *J* = 5.3, 15.4 Hz), 5.74 (dt, 1H, *J* = 6.8, 15.4 Hz), 6.72 (d, 1H, *J* = 8.4 Hz);

^{13}C NMR ($CDCl_3/CD_3OD$ 20:1) δ 14.0, 22.6, 25.8, 25.9, 27.0, 28.5, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.4, 34.8, 36.6, 53.5, 54.4, 63.5, 65.0, 68.5, 76.7, 128.8, 132.5, 172.0; ^{31}P NMR ($CDCl_3/CD_3OD$ 20:1) δ 0.02; LRMS $(M+H)^+$ $C_{39}H_{80}N_2O_6P$ calcd for m/z 703.6, found 703.5. A small amount (~10%) of dihydro SM was found in the final preparation because of the presence of dihydro-sphingosylphosphorylcholine in the starting material.

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Chapter 2

Enantioselective synthesis of the (*E*)-*O*-vinyl analog of plasmalogen (1-*O*-(1'-(*E*)-octadecenyl)-2-*O*-oleoyl-*sn*-glycero-3-phosphocholine)

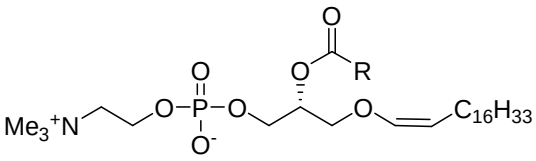
Abstract

In order to probe the protective role of the stereochemistry of the double bond in the ether linkage of plasmalogen in lipid peroxidation we synthesized an unnatural analog of plasmenylphosphocholine bearing a trans *O*-vinyl linkage at the *sn*-1 position. The key step in the synthesis involves enol ether formation with the desired E stereoselectivity via double bond migration of an *O*-allyl ether.

Introduction

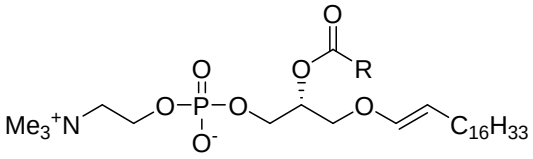
In order to elucidate the role of the cis- (or Z)-*O*-vinyl linkage of plasmalogen (Chart 1) in protecting polyunsaturated lipids from oxidative degradation, we synthesized an unnatural analog of plasmalogen in which the enol ether double bond at the *sn*-1 position of the phospholipid has the E configuration. A previous attempt to prepare the unnatural analog of plasmalogen experienced a poor yield.[1] In the present study, we prepared the unnatural analog in good yield, and used it to compare some of its properties with those of natural plasmalogen. Previous reports of the formation of trans 1-*O*-alkenyl glycerol were based on elimination reactions with poor stereoselectivity.[2] Our approach involves a transition metal catalyzed isomerization of an allylic ether formed by Lewis acid mediated opening of a silylated glycidyl derivative.

Chart 1. Structures of natural and unnatural plasmalogen



Plasmalogen

R = aliphatic chain

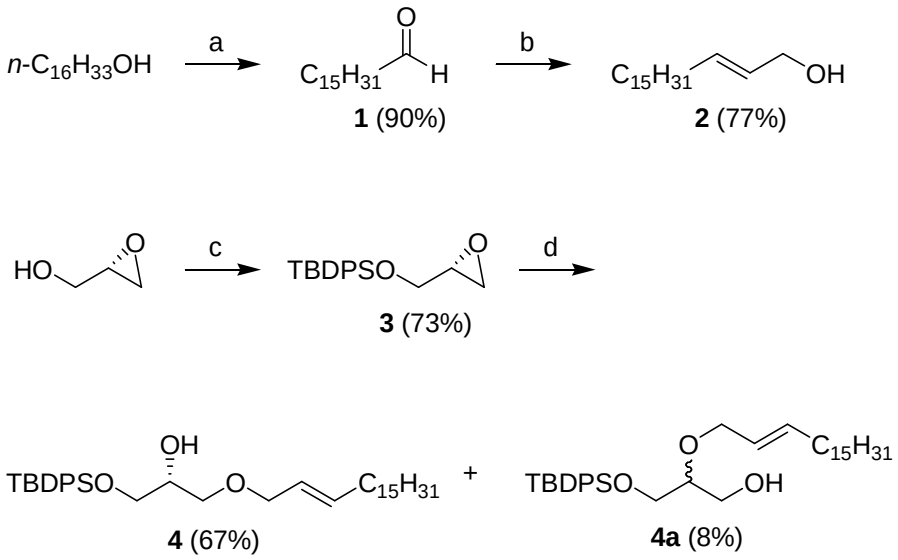


Unnatural plasmalogen

Results and Discussion

Synthesis of allyl ether 4 from glycidol (Scheme 1). (*S*)-Glycidol and 1-hexadecanol were chosen as the starting materials for the synthesis of the unnatural analog of plasmalogen (**7**, Scheme 1). The hydroxy group of (*S*)-glycidol was protected as a TBDPS ether. Dess-Martin periodinane (DMP) oxidation of 1-hexadecanol provided aldehyde **1**, and Horner-Wadsworth-Emmons reaction followed by reduction of the resulting α,β-unsaturated ester with DIBAL provided (*E)*-octadec-2-en-1-ol (**2**).[3] A regioselective ring-opening reaction of epoxide **3** with alcohol **2** using $BF_3 \cdot OEt_2$ as the mediator provided a 8:1 ratio of ether **4** (from C3 attack) to the undesired regioisomer **4a** (from C2 attack).

Scheme 1. Synthesis of allyl ether 4

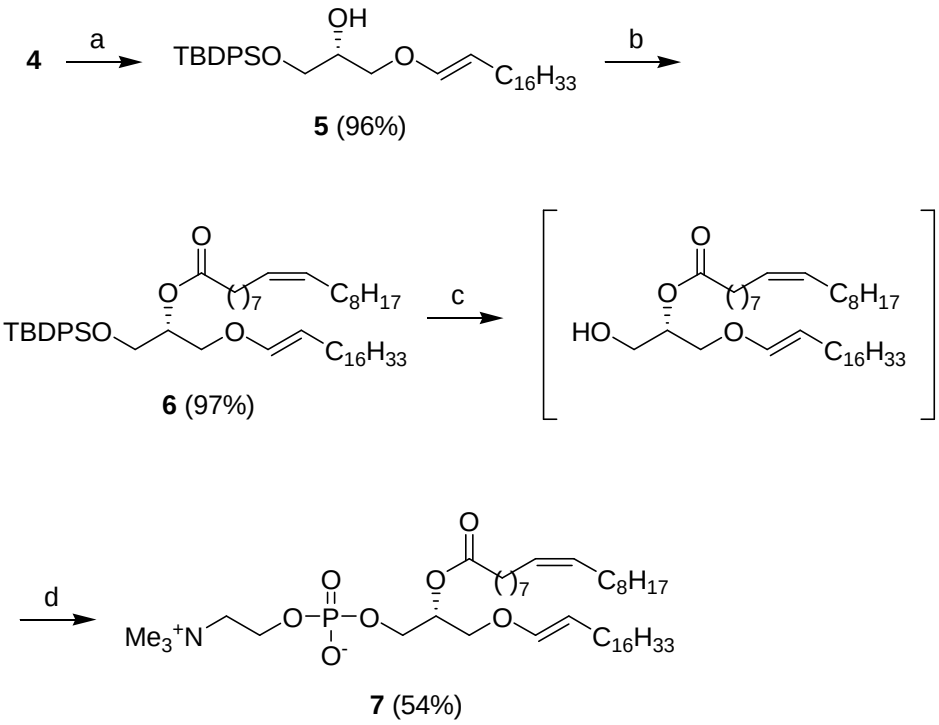


Reagents and conditions: (a) DMP, $NaHCO_3$, CH_2Cl_2, 1 h, 0 °C; (b) (i) $(i\text{-PrO})_2P(O)CH_2CO_2Et$, *t*-BuOK, THF, -78 °C, (ii) 1.2 M DIBAL, Et_2O, 0 °C; (c) TBDPSCl, imidazole, CH_2Cl_2; (d) **2**, $BF_3{\cdot}OEt_2$ (0.1 M), CH_2Cl_2, 0 °C to rt, overnight.

Synthesis of unnatural plasmalogen 7 (Scheme 2). Initially compound **4** was condensed with oleic acid and the resulting ester was used in an attempted double bond isomerization with *s*-BuLi and *t*-BuOK. Since the reaction resulted in a complex mixture, in the next attempt of isomerization compound **4** was used without esterification. Isomerization of the double bond was investigated by using transition metal complexes as catalysts.[4] After an initial attempt of isomerization of compound **4** using $Fe(CO)_5$ in NaOH resulted in a complex mixture again, we realized that the molecule is unstable to basic conditions. An attempt using $RhCl_3{\cdot}3H_2O$ as a catalyst resulted in no reaction, but the use of (1,5-cyclooctadiene)-bis(methyldiphenylphosphine)iridium (I) hexafluorophosphate ($Ir(COD)[PCH_3Ph_2]_2)PF_6$) activated by hydrogen resulted in stereoselective isomerization of allylic ether **4** to enol ether **5** with the desired E double bond as the only product. The coupling constant of 12.6 Hz for the peaks at

δ 4.77 and 6.24 ppm in the proton NMR spectrum of **5** is indicative of the desired E isomer, which was obtained in 96% yield. Secondary alcohol **5** was esterified with oleic acid using DCC in the presence of DMAP, affording ester **6**. The final steps in the synthesis of **7** were deprotection of the silyl ether in compound **6** and installation of the phosphocholine head group at the *sn*-3 position. The latter reaction was accomplished without accompanying acyl migration by opening of a cyclic phosphorylated intermediate with trimethylamine in a pressure tube.[5]

Scheme 2. Synthesis of unnatural plasmalogen 7



Reagents and conditions: (a) Ir{(COD)[PCH_3Ph_2]$_2$}PF_6, THF, rt, 2 h; (b) DCC, DMAP (cat.), CH_2Cl_2, oleic acid (2 equiv.), rt; (c) imidazole, TBAF, THF, -23 °C; (d) (i) 2-chloro-2-oxo-1,3,2-dioxaphospholane, pyridine, C_6H_6, 5 °C, 12 h, (ii) NMe_3, MeCN/C_6H_6 (3:1), 70 °C, pressure tube, 30 h.

Experimental Section

General Information. Acetonitrile and pyridine were distilled over calcium hydride. The solvents were dried and the analyses were performed according to the general information described on page 10.

(*E*)-Octadec-2-en-1-ol (2). To a solution of DMP reagent (4.5 g, 10.7 mmol) and $NaHCO_3$ (2.08 g, 24.7 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added 1-hexadecanol (2.0 g, 8.3 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was concentrated after stirring for 1 h. The residue was purified by flash chromatography (CH_2Cl_2) to afford hexadecanal (**1**, 1.8 g, 90%). A solution of diisopropyl (ethoxycarbonylmethyl)phosphonate (2.75 mL, 11.6 mmol) and *t*-BuOK (1.26 g, 11.2 mmol) in THF (25 mL) was stirred at 0 °C for 2 h, and then was cooled to -78 °C. A solution of hexadecanal (1.78 g, 7.4 mmol) in THF (10 mL) was added, and the reaction mixture was stirred for 2 h at the same temperature. The reaction was quenched with saturated aqueous NH_4Cl solution (30 mL) and the product was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were washed with brine (25 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:20) to afford ethyl (*E*)-octadec-2-enoate (1.8 g, 78%). To a solution of the unsaturated ester (1.8 g, 5.8 mmol) at 0 °C in Et_2O (30 mL) was added dropwise DIBAL (14.5 mL, 17.4 mmol, a 1.2 M solution in toluene). After the reaction mixture had stirred for 0.5 h, 1 M HCl (60 mL) was added at 0 °C, and the product was extracted with CH_2Cl_2 (2 x 65 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:9) to afford allylic alcohol **2** (1.2 g, 77%); R_f 0.28 (EtOAc/hexane 1:9). The NMR spectra were in agreement with the reported spectra.[3]

(*R*)-(*tert*-Butyldiphenylsiloxy)methyl Oxirane (3). To a solution of imidazole (4.63 g, 68.0 mmol) in CH_2Cl_2 (40 mL) was added TBDPSCl (9.7 mL, 37.0 mmol). After the reaction had stirred for 30 min at rt, (*S*)-glycidol (2.3 g, 30.9 mmol) was added, and the mixture was stirred overnight. The reaction was quenched with saturated aqueous NH_4Cl solution (40 mL), and the organic layer was separated. The product was extracted with CH_2Cl_2 (2 x 25 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography ($CHCl_3$) to afford **3** (7.0 g, 73%) as a colorless liquid; $[\alpha]^{25}_D$ +2.40° (*c* 1.9, $CHCl_3$); R_f 0.49 ($CHCl_3$); (lit. $[\alpha]^{25}_D$ +2.40° (*c* 9.07, $CHCl_3$),[6a] $[\alpha]^{25}_D$ +2.3° (*c* 2.0, $CHCl_3$)[6b]). The NMR spectra were in agreement with the reported spectra.[6]

(*R*)-1-(*tert*-Butyldiphenylsilyloxy)-3-(octadec-2'-(*E*)-enyloxy)propan-2-ol (4). To a solution of **2** (836 mg, 3.7 mmol) and **3** (973 mg, 3.1 mmol) in CH_2Cl_2 (25 mL) was added $BF_3{\cdot}OEt_2$ (3.7 mL, a 0.1 M solution in CH_2Cl_2) dropwise at 0 °C under N_2. After the reaction mixture was warmed to rt overnight, the solvent was removed under reduced pressure to give a residue that was purified by chromatography (EtOAc/hexane 1:9) to afford the allyl ether **4** (1.2 g, 67%); $[\alpha]^{25}_D$ +0.50° (*c* 2.8, $CHCl_3$); R_f 0.32 (EtOAc/hexane 1:9); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.04 (s, 9H), 1.26 (m, 26H), 2.02 (q, 2H, *J* = 6.82 Hz), 2.57 (OH, d, 1H, *J* = 4.0 Hz), 3.50 (m, 2H), 3.71 (d, 2H, *J* = 5.3 Hz), 3.81 (m, 1H), 3.93 (d, 2H, *J* = 6.8 Hz), 5.53 (dt, 1H, *J* = 6.8, 14.6 Hz), 5.65 (dt, 1H, *J* = 6.8, 14.9 Hz), 7.40 (m, 6H), 7.67 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 12.2, 17.3, 20.7, 24.8, 27.1, 27.3, 27.4, 27.5, 27.6, 27.7, 27.7, 29.9, 30.4, 62.8, 68.6, 68.8, 70.2, 123.9, 125.8, 127.8, 131.2, 133.2, 133.6; HRMS $[M+Na]^+$ $C_{37}H_{60}O_3SiNa$ calcd for *m/z* 603.4209, found 603.4213.

(*R*)-3-(*tert*-Butyldiphenylsilyloxy)-2-(octadec-2'-(*E*)-enyloxy)propan-1-ol (4a). Purification by chromatography (elution with a gradient of EtOAc/hexane, 1:9, 1:6, 1:3) afforded

150 mg (8%) of regioisomer **4a**; R_f 0.30 (EtOAc/hexane 1:9); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.04 (s, 9H), 1.26 (m, 26H), 2.02 (q, 2H, *J* = 6.82 Hz), 2.08 (OH, two doublets, 1H, *J* = 5.6 Hz), 3.50 (m, 1H), 3.65 (dd, 2H, *J* = 6.3, 10.8 Hz), 3.78 (m, 2H), 3.93 (dd, 1H, *J* = 6.3, 11.6 Hz), 4.02 (dd, 1H, *J* = 5.8, 11.8 Hz), 5.53 (dt, 1H, *J* = 6.8, 14.6 Hz), 5.65 (dt, 1H, *J* = 6.8, 14.9 Hz), 7.40 (m, 6H), 7.67 (m, 4H); HRMS $[M+Na]^+$ $C_{37}H_{60}O_3SiNa$ calcd for *m/z* 603.4209, found 603.4217.

(*R*)-1-(*tert*-Butyldiphenylsilyloxy)-3-(octadec-1'-(*E*)-enyloxy)propan-2-ol (5). A solution of allylic ether **4** (700 mg, 1.2 mmol) in THF (10 mL) was degassed under N_2. Catalyst $Ir\{(COD)[PCH_3Ph_2]_2\}PF_6$ (2 mg, 0.0024 mmol, 0.2 mol %) was added, and the solution was degassed again under N_2 at rt. A stream of H_2 was passed through the solution for 5 min to activate the catalyst, and the reaction mixture was stirred under N_2 for 2 h. The reaction mixture was concentrated and the residue was purified by chromatography (EtOAc/hexane 1:9) to afford enol ether **5** (665 mg, 96%); $[\alpha]^{25}_D$ +3.85° (*c* 1.8, $CHCl_3$); R_f 0.55 (EtOAc/hexane 1:9); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.06 (s, 9H), 1.25 (m, 28H), 1.90 (q, 2H, *J* = 6.6 Hz), 2.49 (d, 1H, *J* = 5.6 Hz), 3.71 (m, 4H), 3.93 (sextet, 1H, *J* = 5.6 Hz), 4.77 (dt, 1H, *J* = 7.3, 12.6 Hz), 6.24 (d, 1H, *J* = 12.6 Hz), 7.41 (m, 6H), 7.64 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.2, 19.3, 22.7, 26.9, 27.7, 29.0, 29.4, 29.5, 29.7, 29.8, 30.7, 31.9, 64.6, 69.5, 70.3, 104.8, 127.8, 129.8, 133.0, 135.6, 145.8; HRMS $[M+Na]^+$ $C_{37}H_{60}O_3SiNa$ calcd for *m/z* 603.4209, found 603.4206.

(*R*)-1-(*tert*-Butyldiphenylsilyloxy)-3-(octadec-1'-(*E*)-enyloxy)propan-2-oleate (6): A mixture of **5** (632 mg, 1.0 mmol), DCC (674 mg, 3.0 mmol), oleic acid (615 mg, 2.0 mmol), and DMAP (66 mg, 0.5 mmol) in CH_2Cl_2 (25 mL) was stirred overnight at rt. The mixture was filtered through a pad of Celite and concentrated. The residue was purified by chromatography (EtOAc/hexane 1:9) to afford **6** (820 mg, 97%); $[\alpha]^{25}_D$ +2.79° (*c* 1.6, $CHCl_3$); R_f 0.8

(EtOAc/hexane 1:9); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, *J* = 6.6 Hz), 1.04 (s, 9H), 1.25 (m, 48H), 1.58 (m, 2H), 1.87 (q, 2H, *J* = 6.8 Hz), 2.01 (q, 4H, *J* = 6.0 Hz), 2.29 (m, 2H), 3.79 (d, 2H, *J* = 5.3 Hz), 3.85 (t, 2H, *J* = 4.5 Hz), 4.76 (dt, 1H, *J* = 7.3, 12.9 Hz), 5.14 (p, 1H, *J* = 5.0 Hz), 5.34 (m, 2H), 6.18 (d, 1H, *J* = 12.9Hz), 7.39 (m, 6H), 7.66 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.1, 19.2, 22.7, 24.9, 26.7, 27.2, 27.7, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 30.7, 31.9, 32.0, 34.4, 62.2, 67.0, 72.2, 104.8, 127.7, 129.7, 130.0, 133.1, 135.5, 145.7, 173.2; HRMS $[M+Na]^+$ $C_{55}H_{92}O_4SiNa$ calcd for *m/z* 867.6663, found 867.6659.

1-*O*-(1'-(*E*)-Octadecenyl)-2-*O*-oleoyl-*sn*-glycero-3-phosphocholine (7): A solution of compound **6** (300 mg, 0.35 mmol) and imidazole (169 mg, 2.5 mmol) in THF (10 mL) was treated with TBAF (2.1 mL, 2.1 mmol, 1.0 M solution in THF) at -23 °C. The reaction mixture was stirred for 13 h, and then was passed through a silica gel pad and washed with cold hexane/Et_2O (1:1). The solvent was evaporated and the residue was lyophilized from benzene. To a solution of the resulting white solid in benzene (10 mL) were added pyridine (80 μL, 0.9 mmol) and 2-chloro-2-oxo-1,3,2-dioxaphospholane (85 μL, 0.9 mmol) at 5 °C. After the reaction mixture was stirred at the same temperature for 12 h, the solution was frozen at 0 °C and the solvent was evaporated under vacuum. The resulting white solid was dissolved in benzene (2 mL) and MeCN (6 mL), and the solution was transferred to a pressure tube. After the solution was cooled to -10 °C, NMe_3 (6 mL, 70 mmol) was collected in the tube. The reaction mixture was stirred in the sealed pressure tube at 70 °C for 30 h, and then cooled to 0 °C and loaded onto a silica gel column. Purification by chromatography (elution with a gradient of $CHCl_3$/MeOH/H_2O, 100:0:0, 80:20:0, 65:25:4) provided **7** (145 mg, 54%) as a white wax after filtration of a chloroform solution of **7** through a Cameo/Osmonics Teflon syringe filter, 0.45 μm (Fisher Scientific), to remove suspended silica and lyophilization from benzene; $[\alpha]^{25}_{D}$ -3.10° (*c*

2.1, $CHCl_3$/MeOH 4:1); R_f 0.35 ($CHCl_3$/MeOH/H_2O 65:25:4); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, *J* = 6.8 Hz), 1.28 (m, 48H), 1.58 (m, 2H), 1.87 (q, 2H, *J* = 7.0 Hz), 2.00 (q, 4H, *J* = 6.8 Hz), 2.30 (q, 2H, *J* = 7.6 Hz), 3.34 (s, 9H), 3.77 (m, 4H), 3.94 (m, 2H), 4.26 (m, 2H), 4.70 (dt, 1H, *J* = 7.3, 14.4 Hz), 5.14 (p, 1H, *J* = 5.8 Hz), 5.32 (m, 2H), 6.18 (t, 1H, *J* = 12.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.9, 27.1, 27.2, 27.7, 29.0, 29.1, 29.2, 29.3, 29.5, 29.5, 29.6, 29.7, 30.7, 31.9, 34.1, 34.3, 44.9, 54.4, 59.8, 62.8, 66.0, 67.1, 68.3, 72.1, 105.1, 129.6, 129.7, 145.5, 145.6, 173.4; ^{31}P NMR ($CDCl_3$) δ -1.1; HRMS $[M+H]^+$ $C_{44}H_{87}NO_7P$ calcd for *m/z* 772.6220, found 772.6217.

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Chapter 3

Synthesis of L-*threo*-β-glucosyl- and galactosyl-ceramide analogs

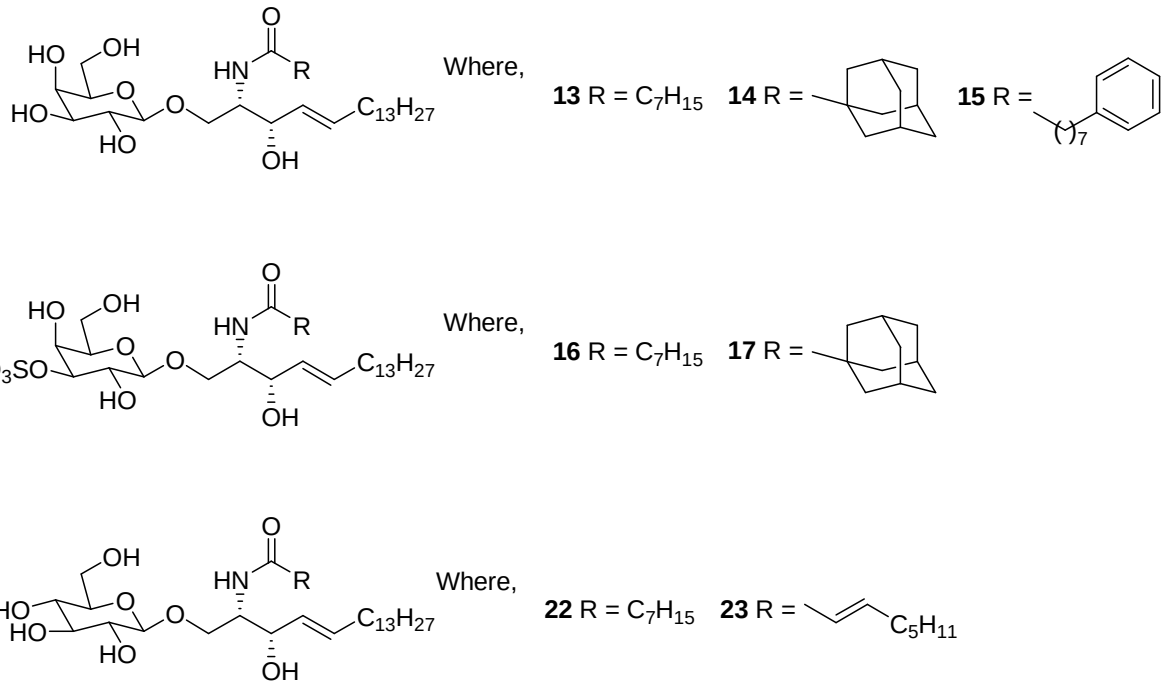
Abstract

The synthesis of L-*threo*-β-glucosyl- and galactosyl-ceramide analogs is reported. A Koenigs-Knorr reaction and the Schmidt trichloroacetimidate method were used for the glycosylation reactions.

Introduction

In certain liver cell injuries induced by concanavalin A, α-galactosylceramide, salmonella infection, and lipopolysaccharides are associated with activation of natural killer T (NKT) cells.[1a] β-Glucosylceramide from soy inhibits the activation of NKT cell lymphocytes in the presence of dendritic cells in vivo.[1] L-*threo*-β-Lactosylceramide (L-t-LacCer) with an *N*-octanoyl chain interferes with raft formation in the plasma membrane of living cells.[2] The inability to form rafts leads to the blockade of many cell signaling events. For example, L-t-LacCer inhibits simian virus (SV) 40 binding to live CV1 monkey kidney cells, as detected using an anti-SV40 monoclonal antibody.[3] Therefore, a series of L-threo-glycosphingolipids was synthesized, since such compounds may be promising as new anti-inflammatory glycolipids to treat liver diseases and also may serve as agents for blocking raft formation in living cells. Short-chain N-acyl analogs are preferred in many clinical applications over the corresponding long-chain analogs for reasons involving ease of administration of compounds having appreciable water solubility. Chart 1 shows the structures of the L-*threo*-β-galactosylceramides (**13**, **14**, **15**), their sulfatide analogs (**16**, **17**), and L-*threo*-β-glucosylceramides (**22**, **23**) that were synthesized with a short N-acyl chain.

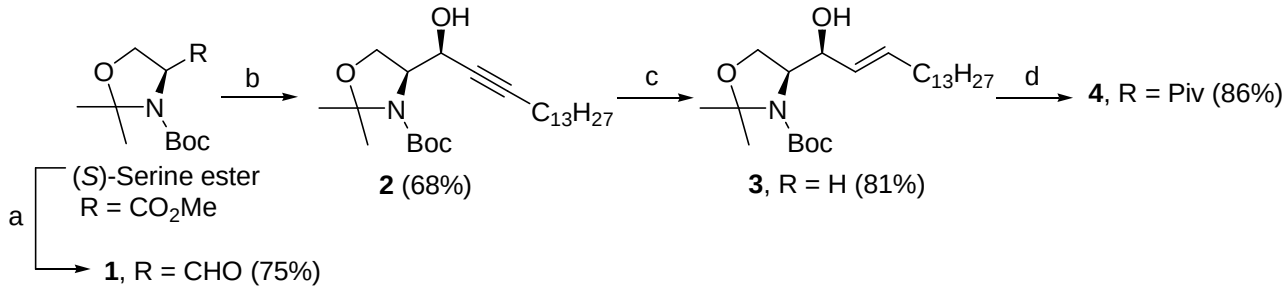
Chart 1. Structures of L-*threo*-glycolipids



Results and Discussion

Synthesis of L-*threo*-sphingosine (Scheme 1). (*S*)-Garner aldehyde **1** was treated with 1-pentadecyne and *n*-butyllithium in the presence of zinc bromide in diethyl ether to afford threo-propargyl alcohol **2** (Scheme 1).[4] The erythro byproduct was not observed during this reaction. A chelation-controlled mechanism has been proposed for the threo selectivity,[4] which is in contrast to the conditions used for producing erythro selectivity (page 3). Reduction of alkyne **2** using Red-Al afforded trans-alkene **3**, and pivaloylation afforded compound **4**.

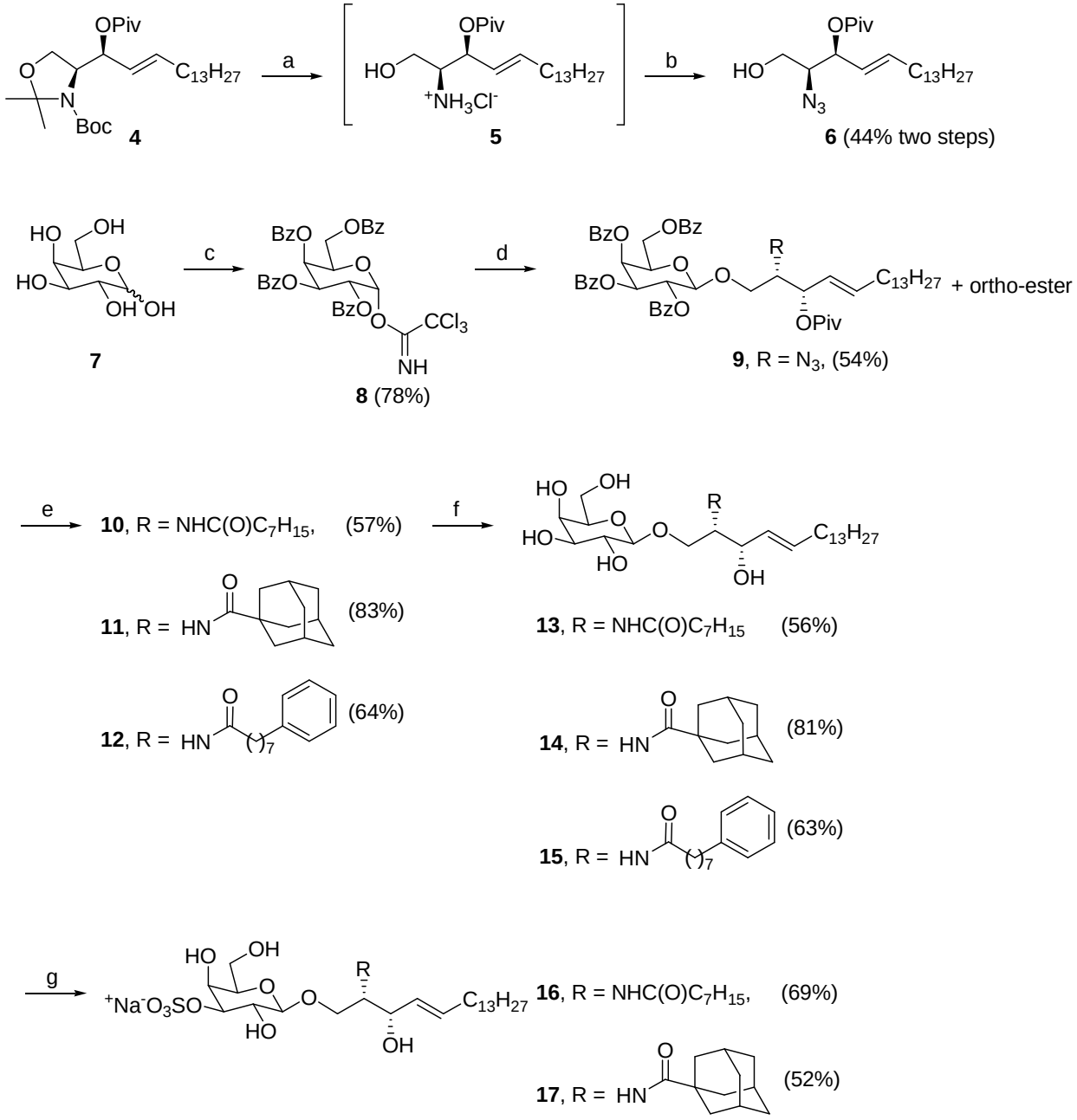
Scheme 1. Synthesis of the L-*threo*-sphingosine backbone



Reagents and conditions: (a) DIBAL in toluene, -78 °C, 2 h; (b) 1-pentadecyne, *n*-BuLi, $ZnBr_2$, Et_2O, -78 °C to rt; (c) Red-Al, THF, -78 °C to rt; (d) *t*-BuCOCl, DMAP (cat.), Et_3N, CH_2Cl_2.

Synthesis of L-*threo*-β-galactosyl-ceramide analogs (Scheme 2). The azido analog of sphingosine (**6**, Scheme 2) was prepared by removal of the oxazolidine and Boc groups using 2 M HCl in ethanol followed by diazo transfer on the resulting amine of **5**.[5] During this step, 1-*O*-pivaloylated and trimethylacetamide byproducts were also formed. After the hydroxy groups of D-galactose (**7**) were protected as benzoate esters, the perbenzoylated sugar was converted into galactosyl donor **8** by a reported procedure.[6] The reaction of trichloroacetimidate **8** and aglycone **6** in the presence of $BF_3 \cdot Et_2O$[7] provided the desired β-anomer **9** together with an ortho ester byproduct (~ 18%); no α-anomer was observed. Reduction of azide **9** with PPh_3 in benzene and water provided the amine, which was *N*-acylated with the requisite acyl chloride in the presence of Hunig's base to provide amides **10** and **11**. For the preparation of compound **12**, a *p*-nitrophenyl ester was used for the *N*-acylation reaction. When an acyl chloride was used, the reaction was completed in few minutes whereas the reaction with the *p*-nitrophenyl ester required two days. Global deprotection of the hydroxy groups using NaOMe afforded the L-*threo*-galactosyl analogs **13**, **14**, and **15**. The sulfatide analogs **16** and **17** were prepared by a dibutyltin oxide promoted selective sulfation using $Me_3N \cdot SO_3$,[8] as the sulfating agent.

Scheme 2. Synthesis of L-*threo*-β-galactosyl analogs

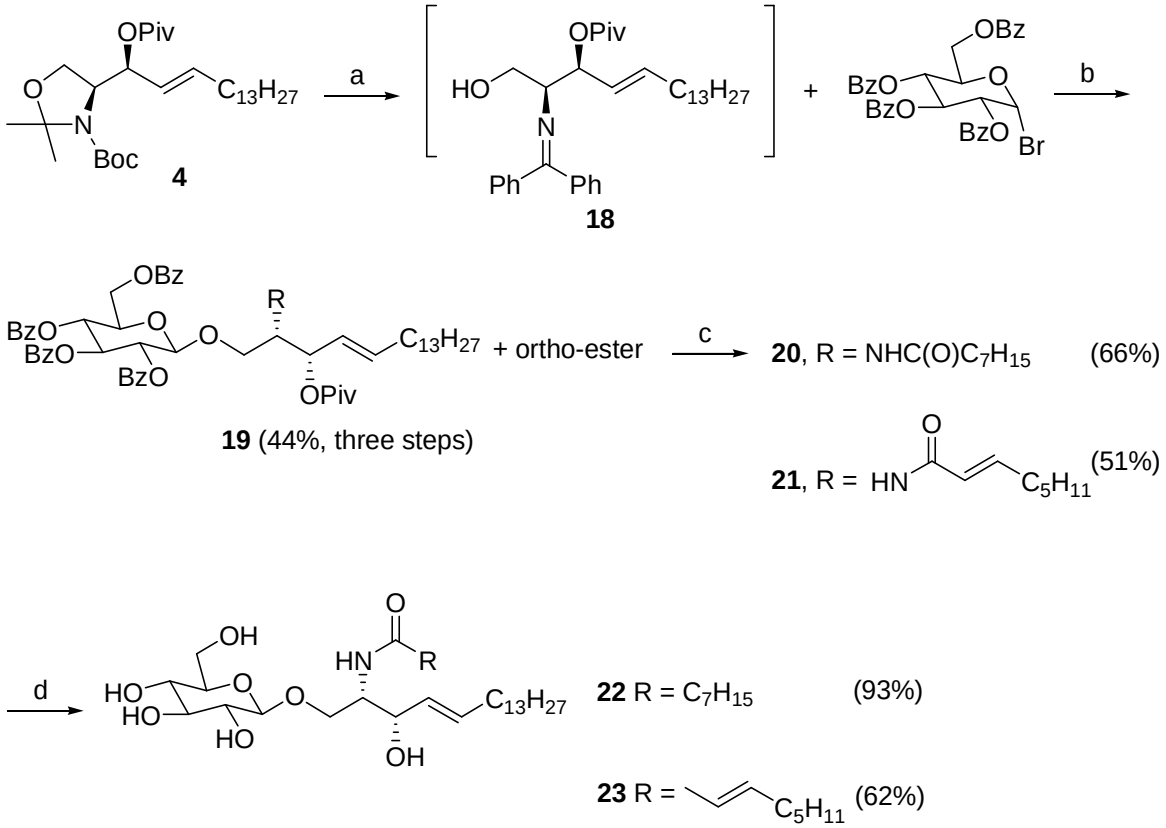


Reagents and conditions: (a) 2 M HCl, EtOH, 70 °C, 4 h; (b) (i) K_2CO_3, 1 mol% $CuSO_4$, (ii) TfN_3 in CH_2Cl_2, MeOH; (c) (i) BzCl, pyridine, (ii) 33% HBr in AcOH, Ac_2O, CH_2Cl_2, (iii) Ag_2CO_3, acetone/water (4:1), (iv) Cl_3CCN, DBU, CH_2Cl_2, 0 °C; (d) **6**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, 0 °C; (e) (i) PPh_3, benzene, H_2O, (ii) for **10** and **11**: *i*-Pr_2NEt, RCOCl, CH_2Cl_2, for **12**: *p*-

nitrophenyl 8-phenyloctanoate, DMAP, THF; (f) NaOMe, MeOH/THF (5:1), 2 d, rt; (g) (i) Bu_2SnO, MeOH, reflux, 2 h, (ii) $Me_3N{\cdot}SO_3$, THF, 6 h, rt, Dowex 50X8, Na^+.

Synthesis of L-*threo*-β-glucosyl-ceramide analogs (Scheme 3). To avoid the formation of the ortho ester byproduct in the glycosidation reaction, the Koenigs-Knorr method was adopted to prepare the β-glucosyl analogs. To overcome the ester migration aforementioned for the azide, a Schiff's base was used as the aglycone equivalent.[9] The amine salt **5**, produced from deprotection of **4**, was treated with benzophenone imine to provide aglycone **18** (Scheme 3). To avoid hydrolysis during chromatography glycosyl acceptor **18** was used in the next step without purification. β-Selective glycosylation of *N*-diphenylmethylene-protected intermediate **18** was achieved using α-bromoglucosyl tetrabenzoate as the donor and silver triflate as the promoter,[10] providing β-glycosylated product **19** without detection of ortho ester or α-anomer byproducts. The Schiff base of **19** was hydrolyzed using trifluoroacetic acid, and neutralization followed by *N*-acylation using acyl chlorides afforded amides **20** and **21**, respectively. Deprotection of the benzoate ester groups using sodium methoxide provided the final products, L-*threo*-glucosyl analogs **22** and **23**.

Scheme 3. Synthesis of L-*threo*-β-glucosyl analogs



Reagents and conditions: (a) (i) 2 M HCl, EtOH, 70 °C, 3.5 h, (ii) $Ph_2C{=}NH$, CH_2Cl_2, rt, overnight; (b) AgOTf, CH_2Cl_2, 4 Å MS, 0 °C to rt; (c) (i) TFA, THF/CH_2Cl_2 (1:1), H_2O, (ii) *i*-Pr_2NEt, RCOCl, CH_2Cl_2; (d) NaOMe, MeOH/THF (5:1), 2 d, rt.

Experimental Section

General Information. Molecular sieves were dried in an oven. α-D-Glucopyranosyl bromide tetrabenzoate and 1-adamantanecarbonyl chloride was purchased from Aldrich. The solvents were dried and the analyses were performed according to the general information described on page 10.

(*S*)-*tert*-Butyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (1). A solution of *N*-Boc *S*-serine methyl ester (2.68 g, 10 mmol) in toluene (25 mL) was cooled to -78 °C under

nitrogen. To the cooled solution was slowly added a solution of 1.5 M DIBAL in toluene (12 mL, 18 mmol). The reaction mixture was stirred for 2 h at -78 °C, and was then quenched by slowly adding 5 mL of cold MeOH. The resulting white emulsion was slowly poured into 50 mL of ice-cold 1 M HCl with swirling over 15 min, and the aqueous mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and concentrated to give the crude product as a colorless oil. The oil was vacuum distilled to provide 1.72 g (75%) of compound **1** as a colorless liquid, bp 115-125 °C (9.0 mm Hg).

(*S*)-*tert*-Butyl-4-((*S*)-1'-hydroxyhexadec-2'-ynyl)-2,2-dimethyloxazolidine-3-carboxylate (2). To a solution of 1-pentadecyne (1.1 g, 5.3 mmol) in dry Et_2O (20 mL) was added *n*-butyllithium (2.5 M in hexane, 2.6 mL, 6.6 mmol) dropwise at -40 °C under N_2. After the white suspension was stirred at -40 °C for 1 h, anhydrous $ZnBr_2$ (1.5 g, 6.6 mmol) was added at 0 °C, and the reaction mixture was stirred for 1 h at 0 °C and 1 h at rt. A solution of (*S*)-Garner aldehyde (**1**, 913 mg, 4.0 mmol) in dry Et_2O (10 mL) was added dropwise at -78 °C. The reaction mixture was allowed to warm to rt overnight. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (20 mL). After the reaction mixture was diluted with water (20 mL), the aqueous layer was separated and extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford compound **2** (1.2 g, 68%): $[\alpha]^{25}_D$ -32.4° (*c* 1.3, $CHCl_3$); (lit. $[\alpha]^{25}_D$ -32.4° (*c* 1.3, $CHCl_3$));[4] R_f 0.55 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.4 Hz), 1.33 (m, 22H), 1.49 (s, 9H), 1.57 (s, 6H), 2.19 (m, 2H), 4.03 (dd, 2H, *J* = 6.3, 9.6 Hz), 4.11 (m, 1H), 4.49 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.7, 21.0,

22.5, 22.8, 24.3, 26.3, 27.2, 28.3, 28.5, 28.8, 29.1, 29.3, 29.6, 29.7, 31.9, 62.6, 65.4, 66.5, 78.4, 81.6, 86.5, 94.6, 155.1.

(*S*)-*tert*-Butyl-4-((*S*)-1'-hydroxyhexadec-2'(*E*)-enyl)-2,2-dimethyloxazolidine-3-carboxylate (3). Alcohol **2** (1.2 g, 2.7 mmol) in dry THF (25 mL) was cooled to -78 °C. RedAl (1.5 mL of a 3.3 M solution in toluene, 4.9 mmol) was added dropwise via a syringe. The reaction mixture was stirred at -78 °C for 1 h and then was allowed to warm to rt overnight. After the reaction was quenched by slow addition of water (25 mL), the product was extracted with Et_2O (3 x 25 mL). The organic phase was dried (Na_2SO_4) and concentrated. Purification by flash chromatography (EtOAc/hexane 1:4) afforded allylic alcohol **3** (970 mg, 81%) as a colorless oil: $[\alpha]^{25}_D$ -35.3° (*c* 1.1, $CHCl_3$); (lit. $[\alpha]^{25}_D$ -37.8° (*c* 0.84, $CHCl_3$));[11] R_f 0.34 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.4 Hz), 1.33 (m, 22H), 1.49 (s, 9H), 1.57 (s, 6H), 2.05 (m, 2H), 3.89 (m, 2H), 3.95 (m, 1H), 4.16 (m, 1H), 5.41 (dd, 1H, *J* = 7.3, 14.4 Hz), 5.73 (dt, 1H, *J* = 6.5, 14.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.7, 22.6, 24.3, 25.7, 27.1, 28.3, 28.8, 29.0, 29.1, 29.2, 29.3, 29.4, 29.6, 31.9, 32.3, 62.1, 64.3, 76.3, 81.4, 94.3, 129.5, 135.3, 155.2.

(*S*)-*tert*-Butyl-4-((*S*)-1'-*O*-pivaloyl-hexadec-2'(*E*)-enyl)-2,2-dimethyloxazolidine-3-carboxylate (4). A solution of compound **3** (966 mg, 2.2 mmol), pivaloyl chloride (0.4 mL, 2.9 mmol), DMAP (54 mg, 0.4 mmol), and Et_3N (1.1 mL, 7.7 mmol) in CH_2Cl_2 (40 mL) was stirred at 0 °C for 1 h and then was allowed to warm to rt overnight. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and the product was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (2 x 20 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:4) to afford compound **4** (1.0 g, 86%) as a colorless oil: $[\alpha]^{25}_D$ -21.4° (*c* 1.2, $CHCl_3$); R_f 0.89 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.4 Hz),

1.20 (s, 9H), 1.33 (m, 22H), 1.49 (s, 9H), 1.59 (s, 6H), 2.09 (m, 2H), 3.92 (m, 2H), 3.98 (m, 1H), 5.42 (dd, 1H, *J* = 6.0, 15.2 Hz), 5.48 (m, 1H), 5.62 (dt, 1H, *J* = 6.4, 14.4 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 24.5, 25.2, 25.7, 26.5, 26.9, 27.0, 27.1, 28.3, 28.4, 28.7, 29.4, 29.6, 31.9, 32.3, 34.6, 38.7, 58.2, 63.9, 73.4, 80.2, 94.5, 123.5, 137.3, 151.8, 177.0; HRMS $[M+Na]^+$ $C_{31}H_{57}NO_5Na$ calcd for *m/z* 546.4134, found 546.4131.

(2*S*,3*S*,4*E*)-3-*O*-Pivaloyl-2-azido-sphingosine (6). To a solution of compound **4** (2.8 g, 5.5 mmol) in EtOH (16 mL) was added 2 M HCl (4 mL), and the reaction mixture was stirred at 70 °C. The starting material was completely consumed after 4 h. The reaction was quenched with H_2O (30 mL) and the product was extracted with $CHCl_3$/MeOH 7:1 (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford the crude amine salt **5**. Trifluoromethanesulfonyl (triflyl) azide (TfN_3) was prepared immediately prior to the reaction.[5] To a solution of the amine salt **5** (2.3 g, 5.5 mmol) in H_2O (25 mL) were added K_2CO_3 (760 mg, 5.5 mmol) and $CuSO_4 \cdot 5H_2O$ (14 mg, 0.055 mmol). After TfN_3 in CH_2Cl_2 (25 mL) was added in one portion to the reaction mixture, MeOH (83 mL) was added slowly along the walls of the reaction flask. After 5 h (when TLC indicated completion of the reaction), the solution was concentrated and purified by chromatography (elution with EtOAc/hexane 1:2) to afford **6** (980 mg, 44%, two steps): $[\alpha]^{25}_D$ -9.1° (*c* 1.0, $CHCl_3$); R_f 0.75 (EtOAc/hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.30 (m, 31H), 2.04 (q, 2H, *J* = 7.3 Hz), 2.22 (t, 1H, *J* = 6.6 Hz), 3.57 (m, 1H), 3.65 (m, 2H), 5.36 (dd, 1H, *J* = 5.8, 6.8 Hz), 5.45 (m, 1H), 5.84 (dt, 1H, *J* = 6.8, 14.9 Hz); ^{13}C NMR ($CDCl_3$) δ 12.1, 20.7, 25.0, 26.7, 27.0, 27.3, 27.4, 27.5, 27.6, 27.7, 29.9, 30.2, 37.0, 59.6, 64.0, 71.7, 122.1, 135.5, 175.9.

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl Trichloroacetimidate (8). To D-galactose **7** (1.8 g, 10.0 mmol) in pyridine (10 mL) was added benzoyl chloride (6.2 mL, 53

mmol). The mixture was stirred overnight, quenched with H_2O (30 mL), extracted with CH_2Cl_2 (2 x 30 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:2) to afford perbenzoylated D-galactose; ^{1}H NMR ($CDCl_3$) δ 4.67-4.85 (m, 3H), 5.82-5.92 (m, 2H), 6.31 (m, 1H), 6.86 (d, 1H, *J* = 4.8 Hz), 7.10-8.18 (m, 25H). To a solution of the protected galactose (6.2 g, 8.8 mmol) in CH_2Cl_2 (3 mL) were added HBr (57.2 mmol, 10 mL of a 33% solution in AcOH) and Ac_2O (0.2 mL, 2.2 mmol). After the reaction mixture was stirred for 2 h at rt, the mixture was diluted with CH_2Cl_2 (30 mL) and cold water (20 mL). Saturated aqueous $NaHCO_3$ solution (30 mL) was added slowly, and the product was extracted with CH_2Cl_2 (2 x 30 mL), dried (Na_2SO_4), and concentrated. To a solution of the crude D-galactosyl bromide in acetone/water (4:1, 20 mL) was added Ag_2CO_3 (7.4 g, 27.0 mmol). After the mixture was stirred for 3 h at rt, the mixture was filtered on a Celite pad and the filtrate was concentrated. To a solution of the resulting hemiacetal (3.0 g, 5.0 mmol) and Cl_3CCN (3.5 mL, 35 mmol) in CH_2Cl_2 (15 mL) at 0° C was added DBU (0.9 mL, 6.0 mmol) under N_2 with continued stirring at this temperature for 3 h. The mixture was concentrated and purified by flash chromatography (elution with $CHCl_3$) to provide α-D-galactosyl trichloroacetimidate **8** (2.8 g, 76%) as a crystalline solid: R_f 0.28 (EtOAc/hexane 1:2); the NMR spectra are in agreement with the reported spectra.[12]

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-galactopyranosy1)-2-azido-3-*O*-pivaloyl-4-octadecene (9). A mixture of galactosyl donor **8** (1.6 g, 2.1 mmol) and aglycone **6** (580 mg, 1.4 mmol) was lyophilized from benzene. The mixture was dissolved in dry CH_2Cl_2 (8 mL), and 4 Å molecular sieves (500 mg) were added under N_2. After the reaction mixture had stirred at 0 °C for 1 h, a solution of $BF_3{\cdot}Et_2O$ (5 mL of a 0.1 M solution in CH_2Cl_2, 0.5 mmol) was added dropwise, and stirring was continued at 0° C. After 1.5 h, the reaction mixture was

diluted with CH_2Cl_2 (8 mL), and a few drops of triethylamine were added. The solution was filtered through a Celite pad and concentrated. The residue was purified by chromatography (elution with EtOAc/hexane 1:4) to provide compound **9** (742 mg, 54%): $[\alpha]^{25}_D$ +51.3° (*c* 3.7, $CHCl_3$); R_f 0.70 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.16 (s, 9H), 1.25 (m, 22H), 1.97 (q, 2H, *J* = 6.8 Hz), 3.55 (q, 1H, *J* = 5.0 Hz), 3.81 (dd, 1H, *J* = 4.8, 10.8 Hz), 4.02 (dd, 1H, *J* = 6.6, 10.6 Hz), 4.35 (t, 1H, *J* = 6.8 Hz), 4.44 (dd, 1H, *J* = 6.8, 11.4 Hz), 4.70 (dd, 1H, *J* = 6.6, 11.4 Hz), 4.89 (d, 1H, *J* = 7.8 Hz), 5.26 (dd, 1H, *J* = 5.6, 7.1 Hz), 5.38 (m, 1H), 5.60 (dd, 1H, *J* = 3.5, 10.6 Hz), 5.70 (dd, 1H, *J* = 6.8, 14.9 Hz), 5.82 (dd, 1H, *J* = 8.1, 10.6 Hz), 6.01 (d, 1H, *J* = 3.5 Hz), 7.22-8.11 (m, 22H); ^{13}C NMR ($CDCl_3$) δ 14.2, 18.6, 22.7, 26.9, 27.0, 28.2, 28.7, 28.8, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.2, 38.8, 61.9, 63.4, 64.6, 68.0, 68.1, 69.6, 71.5, 71.7, 73.2, 74.2, 88.7, 101.5, 124.0, 128.3-120.0, 133.2-133.6, 137.2, 165.1, 165.5, 165.6, 166.0, 176.5, 176.8.

Ortho-ester byproduct (250 mg, 18%): R_f 0.71 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.16 (s, 9H), 1.25 (m, 22H), 1.99 (q, 2H, *J* = 7.0 Hz), 3.69 (m, 2H), 3.85 (m, 1H), 4.74 (m, 3H), 5.37 (m, 3H), 5.49 (m, 1H), 5.67 (d, 1H, *J* = 5.0 Hz), 5.81 (dt, 1H, *J* = 6.8, 14.6 Hz), 6.08 (m, 1H), 7.22-8.11 (m, 22H); ^{13}C DEPT-45 NMR ($CDCl_3$) δ 14.1, 22.7, 27.0, 28.7, 29.0, 29.3, 29.4, 29.6, 29.7, 31.9, 32.2, 63.4, 63.8, 66.7, 70.3, 73.4, 77.4, 81.9, 82.1, 105.7, 124.0, 128.4-128.5, 129.7-130.0, 133.1-133.5, 137.7.

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-galactopyranosy1)-2-[*N*-octanoylamido]-3-*O*-pivaloyl-4-octadecene (10). To a solution of azide **9** (80 mg, 0.080 mmol) in benzene (5 mL) was added triphenylphosphine (48 mg, 0.18 mmol). After the reaction mixture was stirred for 2 h at rt, water (50 μL) was added and stirring was continued until TLC indicated complete consumption of the starting material (24 h). The solvent was evaporated under reduced

pressure and the residue was dried as an azeotrope with toluene (10 mL). To the resulting amine in CH_2Cl_2 (8 mL) were added octanoyl chloride (16 mg, 0.090 mmol) and *i*-Pr_2NEt (28 μL, 0.16 mmol), with stirring for 4 h. The reaction was quenched with water (10 mL) and the product was extracted with CH_2Cl_2 (2 x 20 mL), dried (Na_2SO_4), concentrated, and purified by chromatography (elution with EtOAc/hexane 1:4) to provide compound **10** (50 mg, 57%): R_f 0.20 (EtOAc/hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, *J* = 6.8 Hz), 1.14 (s, 9H), 1.19-1.37 (m, 32H), 1.67 (m, 2H), 1.96 (q, 2H, *J* = 6.8 Hz), 2.35 (t, 1H, *J* = 7.6 Hz), 3.58 (dd, 1H, *J* = 4.5, 9.6 Hz), 4.06 (dd, 1H, *J* = 3.0, 9.8 Hz), 4.28 (m, 2H), 4.42 (dd, 1H, *J* = 6.8, 11.1 Hz), 4.63 (dd, 1H, *J* = 6.3, 11.1 Hz), 4.77 (d, 1H, *J* = 8.0 Hz), 5.40 (m, 2H), 5.61 (m, 2H), 5.75 (m, 2H), 6.0 (d, 1H, *J* = 3.5 Hz), 7.23-7.57 (m, 12H), 7.78 (m, 2H), 7.92 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 24.7, 25.4, 27.1, 28.8, 28.9, 29.0, 29.1, 29.3, 29.4, 29.6, 29.7, 31.6, 31.7, 31.9, 32.2, 36.4, 38.8, 51.1, 61.9, 68.0, 68.6, 69.9, 71.3, 71.4, 73.3, 101.5, 124.5, 128.3-129.3, 129.7, 130.0, 133.3, 133.4, 133.6, 136.8, 165.3, 165.4, 166.0, 172.7, 177.4.

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-galactopyranosy1)-2-[*N*-adamantanoylamido]-3-*O*-pivaloyl-4-octadecene (11). This compound was prepared by using the procedure described for compound **10**. 1-Adamantanecarbonyl chloride (117.5 mg, 0.6 mmol) was used as the acylation agent. Purification by chromatography (elution with EtOAc/hexane 1:3) afforded compound **11** (280 mg, 83%): $[\alpha]^{25}_D$ +43.8° (*c* 1.9, $CHCl_3$); R_f 0.4 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, *J* = 6.6 Hz), 1.17 (s, 9H), 1.21-1.29 (m, 22H), 1.47-1.98 (m, 15H), 2.01 (q, 2H, *J* = 6.8 Hz), 3.62 (dd, 1H, *J* = 4.8, 10.1 Hz), 4.05 (dd, 1H, *J* = 3.0, 9.8 Hz), 4.27 (m, 2H), 4.40 (dd, 1H, *J* = 6.8, 11.4 Hz), 4.62 (dd, 1H, *J* = 6.6, 11.4 Hz), 4.82 (d, 1H, *J* = 8.0 Hz), 5.41 (m, 2H), 5.61 (dd, 1H, *J* = 3.5, 10.6 Hz), 5.70 (dd, 1H, *J* = 7.1, 14.4 Hz), 5.79 (dd, 1H, *J* = 8.1, 10.4 Hz), 5.91 (d, 1H, *J* = 8.8 Hz), 6.0 (d, 1H, *J* = 3.3 Hz), 7.23-

7.57 (m, 12H), 7.78 (m, 2H), 7.92 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 27.1, 27.8, 27.9, 28.9, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 36.3, 36.4, 38.6, 38.8, 39.0, 40.5, 51.1, 62.0, 68.0, 68.3, 69.8, 71.3, 71.7, 73.2, 101.2, 124.6, 128.3-129.9, 133.3, 133.6, 136.5, 165.1, 165.5, 165.9, 177.4, 177.5.

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-galactopyranosy1)-2-[*N*-8"-phenyloctanoylamido]-3-*O*-pivaloyl-4-octadecene (12). The intermediate amine was prepared from the azide by using the procedure described for compound **10**. To the resulting amine in THF (8 mL) were added *p*-nitrophenyl 8-phenyloctanoate (62 mg, 0.18 mmol) and DMAP (22.4 mg, 0.18 mmol), with stirring for 2 days. After the reaction was quenched with water (30 mL), the product was extracted with Et_2O (3 x 20 mL), dried (Na_2SO_4), concentrated, and purified by chromatography (elution with EtOAc/hexane 1:2) to provide compound **12** (90 mg, 64%): $[\alpha]^{25}_D$ +47.5° (*c* 0.40, $CHCl_3$); R_f 0.30 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.15 (s, 9H), 1.18-1.33 (m, 36H), 1.59 (m, 2H), 1.97 (q, 2H, *J* = 6.8 Hz), 2.60 (t, 2H, *J* = 7.8 Hz), 3.60 (dd, 1H, *J* = 4.5, 9.6 Hz), 4.08 (dd, 1H, *J* = 2.8, 9.6 Hz), 4.30 (m, 2H), 4.41 (dd, 1H, *J* = 6.8, 11.4 Hz), 4.63 (dd, 1H, *J* = 6.6, 11.4 Hz), 4.75 (d, 1H, *J* = 8.0 Hz), 5.36 (m, 2H), 5.62 (m, 2H), 5.73 (m, 2H), 6.0 (d, 1H, *J* = 3.6 Hz), 7.15-7.62 (m, 17H), 7.78 (m, 2H), 7.96 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 25.3, 27.0, 27.1, 27.2, 28.9, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 35.9, 38.8, 51.1, 61.9, 68.0, 68.6, 69.9, 71.3, 71.4, 73.4, 101.5, 124.6, 128.3-129.8, 133.3, 133.4, 133.6, 136.8, 142.8, 165.3, 165.5, 165.9, 172.5, 177.2.

(2*S*,3*S*,4*E*)-1-*O*-(β-D-Galactopyranosy1)-2-[*N*-octanoylamido]-4-octadecen-3-ol (13). Sodium metal (30 mg) was dissolved in dry MeOH (5 mL) and the resulting solution was added to a solution of compound **10** (50 mg, 0.050 mmol) in dry MeOH/THF (5:1, 6 mL). After the reaction mixture was stirred for two days at rt, Dowex 50W-X8 resin (prewashed thoroughly

with MeOH) was added to neutralize the reaction mixture, which was filtered, concentrated, and purified by chromatography ($CHCl_3$/MeOH 10:1) to afford compound **13** (15 mg, 56%) as a white powder: $[\alpha]^{25}_D$ -11.4° (*c* 0.37, $CHCl_3$/MeOH 4:1); R_f 0.25 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, *J* = 6.8 Hz), 1.26 (m, 32H), 1.60 (m, 2H), 2.02 (q, 2H, *J* = 7.3 Hz), 3.40 (m, 1H), 3.52 (m, 2H), 3.66 (m, 1H), 3.78 (m, 1H), 3.86 (m, 2H), 3.93 (m, 1H), 4.0 (m, 1H), 4.24 (m, 1H), 4.34 (m, 1H), 5.42 (dd, 1H, *J* = 7.8, 14.9 Hz), 5.75 (dt, 1H, *J* = 6.8, 14.9 Hz), 6.63 (m, 1H); HRMS $[M+H]^+$ $C_{32}H_{62}NO_8$ calcd for *m/z* 588.4475, found 588.4470.

(2*S*,3*S*,4*E*)-1-*O*-(β-D-Galactopyranosy1)-2-[*N*-adamantanoylamido]-4-octadecen-3-ol (14). This compound was prepared by using the procedure described for compound **13**. Purification by chromatography ($CHCl_3$/MeOH 10:1) afforded compound **14** (35 mg, 81%) as a white powder: $[\alpha]^{25}_D$ -16.2° (*c* 0.63, $CHCl_3$/MeOH 4:1); R_f 0.30 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.8 Hz), 1.25 (m, 22H), 1.67 (q, 2H, *J* = 12.4 Hz), 1.82 (s, 10H), 2.0 (m, 5H), 3.37 (m, 1H), 3.49 (m, 2H), 3.66 (m, 2H), 3.75 (dd, 1H, *J* = 4.8, 12.4 Hz), 3.81 (dd, 1H, *J* = 6.3, 11.6 Hz), 3.89 (m, 1H), 4.06 (m, 1H), 4.22 (d, 1H, *J* = 8.0 Hz), 4.38 (m, 1H), 5.40 (dd, 1H, *J* = 5.3, 14.9 Hz), 5.75 (dt, 1H, *J* = 6.8, 14.9 Hz), 6.43 (d, 1H, *J* = 7.8 Hz); HRMS $[M+H]^+$ $C_{35}H_{62}NO_8$ calcd for *m/z* 624.4475, found 624.4468.

(2*S*,3*S*,4*E*)-1-*O*-(β-D-Galactopyranosy1)-2-[*N*-8'-phenyloctanoylamido]-4-octadecen-3-ol (15). This compound was prepared by using the procedure described for compound **13**. Purification by chromatography ($CHCl_3$/MeOH 10:1) afforded compound **15** (30 mg, 63%) as a white powder: $[\alpha]^{25}_D$ -7.9° (*c* 1.0, $CHCl_3$/MeOH 4:1); R_f 0.25 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.3 Hz), 1.31 (m, 32H), 1.62 (m, 2H), 2.0 (q, 2H, *J* = 7.1 Hz), 2.19 (t, 2H, *J* = 7.6 Hz) 2.60 (t, 2H, *J* = 7.6 Hz), 2.96 (br s, 5H), 3.52 (m, 2H), 3.60 (m, 1H) 3.68 (dd, 1H, *J* = 6.8, 10.1 Hz), 3.75 (dd, 1H, *J* = 4.8, 11.9 Hz), 3.81 (dd, 1H, *J* = 6.3, 11.9 Hz), 3.91 (m,

2H), 4.09 (m, 1H), 4.22 (d, 1H, *J* = 8.0 Hz), 4.38 (m, 1H), 5.41 (dd, 1H, *J* = 5.8, 15.4 Hz), 5.71 (dt, 1H, *J* = 6.6, 14.4 Hz), 6.77 (d, 1H, *J* = 8.8 Hz), 7.18-7.30 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 25.8, 29.1, 29.2, 29.3, 29.5, 29.6, 29.7, 31.4, 31.8, 35.9, 36.6, 53.1, 61.6, 69.0, 69.3, 70.2, 71.1, 73.4, 74.7, 103.7, 125.5, 128.2-132.9, 142.7, 174.8; HRMS $[M+Na]^+$ $C_{38}H_{65}NO_8Na$ calcd for *m/z* 686.4608, found 686.4624.

(2*S*,3*S*,4*E*)-1-*O*-[3'-O-(Sodiumoxysulfonyl)-β-D-galactopyranosy1-]-2-[*N*-octanoylamido]-4-octadecen-3-ol (16). Compound **13** (3.2 mg, 5.0 μmol) and Bu_2SnO (2.0 mg, 8.0 μmol) were stirred in MeOH (2.5 mL) at reflux under N_2 for 2 h. The solvent was evaporated under reduced pressure, and the dibutylstannylene complex was treated with $Me_3N{\cdot}SO_3$ (1.5 mg, 10.0 μmol) in THF (5 mL) for 6 h at rt. The solvents were removed under reduced pressure and the residue was dissolved in $CHCl_3$/MeOH 1:1 (3 mL) and loaded onto a cation-exchange resin column (Dowex 50X8, Na^+ form). The mixture was eluted with $CHCl_3$/MeOH 1:1, concentrated in vacuo, and purified by chromatography ($CHCl_3$/MeOH 5:1) to afford compound **16** (2.5 mg, 69%) as a white powder: R_f 0.55 ($CHCl_3$/MeOH 2:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, *J* = 6.8 Hz), 1.21 (m, 32H), 1.55 (m, 2H), 2.15 (m, 2H), 3.68 (m, 3H), 3.72 (m, 2H) 3.85 (m, 1H), 4.05 (m, 1H), 4.28 (m, 1H), 4.32 (m, 1H), 4.41 (m, 1H), 4.46 (d, 1H, *J* = 8.0 Hz), 5.12 (m, 1H), 5.38 (dd, 1H, *J* = 5.6, 14.9 Hz), 5.73 (dt, 1H, *J* = 6.6, 14.9 Hz); HRMS $[M-H]^-$ $C_{32}H_{60}NO_{11}S$ calcd for *m/z* 666.3887, found 666.3889.

(2*S*,3*S*,4*E*)-1-*O*-[3'-O-(Sodiumoxysulfonyl)-β-D-galactopyranosy1]-2-[*N*-adamantanoylamido]-4-octadecen-3-ol (17). This compound was prepared by using the procedure described for compound **16**. Purification by chromatography ($CHCl_3$/MeOH 5:1) afforded compound **17** (12 mg, 52%) as a white powder: $[\alpha]^{25}_D$ -8.8° (*c* 0.31, $CHCl_3$/MeOH 1:1); R_f 0.23 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, *J* = 6.3 Hz), 1.24 (m, 22H), 1.68 (q,

2H, *J* = 12.8 Hz), 1.79 (s, 10H), 2.0 (m, 5H), 3.72 (m, 8H), 4.07 (m, 1H), 4.46 (m, 5H), 5.37 (m, 1H), 5.73 (dt, 1H, *J* = 6.6, 14.9 Hz), 6.35 (m, 1H); HRMS $[M-H]^-$ $C_{35}H_{60}NO_{11}S$ calcd for *m/z* 702.3887, found 702.3893.

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-glucopyranosy1)-2-[*N*-(diphenylmethylene)amino]-3-*O*-pivaloyl-4-octadecene (19). To a solution of compound **4** (59 mg, 0.11 mmol) in EtOH (8 mL) was added 2 M HCl (2 mL), and the reaction mixture was stirred at 70 °C. The starting material was completely consumed after 3.5 h. The reaction was quenched with H_2O (20 mL) and the product was extracted with $CHCl_3$/MeOH 7:1 (2 x 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford the crude amine salt **5**. A solution of the crude amine salt and Ph_2C=NH (19 µL, 0.11 mmol) in CH_2Cl_2 (5 mL) was stirred at rt overnight under N_2. After TLC showed full conversion, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ solution (10 mL) and H_2O (5 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated to afford compound **18**. α-D-Glucopyranosyl bromide tetrabenzoate (72 mg, 0.10 mmol) and alcohol **18** (46 mg, 0.080 mmol) were placed in a flame-dried flask in CH_2Cl_2 (10 mL) along with powdered 4 Å molecular sieves under N_2 and cooled to 0 °C. AgOTf (30 mg, 0.12 mmol) was added in portions over 45 min. The reaction mixture was protected from the light and slowly warmed to rt and stirred for 5 h. The reaction mixture was quenched by the addition of *i*-Pr_2NEt (1 mL, 6 mmol). After the mixture had stirred for 10 min, the solution was filtered through a Celite pad to remove silver salts, washed with saturated aqueous Na_2CO_3 solution (5 mL), and the product was extracted with CH_2Cl_2 (2 x 10 mL). The organic layers were dried (Na_2SO_4), concentrated, and purified by chromatography (EtOAc/hexane 1:3) to afford compound **19** (41 mg, 44% overall yield for three steps): $[\alpha]^{25}_D$ -3.6° (*c* 0.6, $CHCl_3$); R_f 0.40 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$)

δ 0.89 (t, 3H, *J* = 6.8 Hz), 1.24 (s, 9H), 1.27 (m, 22H), 1.89 (m, 2H), 3.74 (dd, 2H, *J* = 4.0, 10.8 Hz), 4.03 (m, 2H), 4.39 (dd, 1H, *J* = 4.8, 11.8 Hz), 4.43 (dd, 1H, *J* = 3.8, 12.3 Hz), 4.94 (d, 1H, *J* = 7.8 Hz), 5.21 (m, 1H), 5.38 (t, 1H, *J* = 7.1 Hz), 5.51 (dd, 1H, *J* = 7.8, 9.7 Hz), 5.56 (m, 1H), 5.61 (t, 1H, *J* = 9.8 Hz), 5.85 (t, 1H, *J* = 9.4 Hz), 7.10-7.57 (m, 22H), 7.81 (m, 2H), 7.87 (m, 2H), 7.98 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.0, 22.9, 27.1, 28.8, 28.9, 291, 29.3, 29.4, 29.6, 29.7, 31.9, 32.4, 60.4, 63.4, 64.7, 69.4, 69.7, 70.6, 72.1, 73.2, 74.5, 101.1, 125.3-129.9, 133.0-133.3, 136.0, 165.1, 165.6, 166.0, 169.4, 171.2, 177.3; HRMS $[M+H]^+$ $C_{70}H_{80}NO_{12}$ calcd for *m/z* 1126.5681, found 1126.5678.

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-glucopyranosy1)-2-[*N*-octanoylamido]-3-*O*-pivaloyl-4-octadecene (20). To a solution of compound **19** (20 mg, 0.017 mmol) in THF/CH_2Cl_2 1:l (4 mL) were added trifluoroacetic acid (100 µL) and one drop of water. The reaction mixture was stirred at rt until TLC indicated that the hydrolysis was complete (~ 4 h). The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ solution (10 mL). The product was extracted with CH_2Cl_2 (3 x 10 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:3), which afforded the amine: R_f 0.30 (EtOAc). After the amine was dried by lyophilization from benzene, a solution of *i*-Pr_2NEt (6 µL, 0.034 mmol) in CH_2Cl_2 (5 mL) was added, followed by octanoyl chloride (3 mg, 0.019 mmol). After the reaction mixture was stirred for 1.5 h, TLC indicated complete consumption of the starting material. The reaction was quenched by addition of water (10 mL) and the product was extracted with CH_2Cl_2 (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated, and purified by chromatography (EtOAc/hexane 1:2) to afford compound **20** (12 mg, 66% over two steps): R_f 0.50 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, *J* = 6.8 Hz), 1.12 (s, 9H), 1.15-1.37 (m, 32H), 1.73 (m, 2H), 1.93 (m, 2H), 3.57 (dd, 1H, *J* = 4.3,

9.8 Hz), 4.04 (dd, 1H, *J* = 2.5, 9.6 Hz), 4.13 (m, 1H), 4.23 (m, 1H), 4.49 (dd, 1H, *J* = 5.0, 12.4 Hz), 4.62 (dd, 1H, *J* = 3.3, 12.4 Hz), 4.79 (d, 1H, *J* = 8.0 Hz), 5.33 (m, 2H), 5.51 (dd, 1H, *J* = 7.8, 9.8 Hz), 5.60 (d, 1H, *J* = 9.4 Hz), 5.68 (t, 1H, *J* = 9.6 Hz), 5.73 (m, 1H), 5.91 (t, 1H, *J* = 9.6 Hz), 7.26-7.57 (m, 12H), 7.83 (m, 2H), 7.89 (m, 2H), 8.00 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 12.0, 20.4, 20.5, 23.2, 24.9, 26.2, 26.6, 26.8, 26.9, 27.0, 27.2, 27.3, 27.5, 27.6, 29.5, 29.8, 30.0, 34.2, 34.5, 36.6, 48.9, 61.0, 66.4, 67.3, 69.9, 70.1, 70.4, 71.1, 74.5, 99.1, 122.2, 126.2-127.7, 131.0-131.3, 135.0, 162.9, 163.0, 163.6, 163.9, 170.5, 175.3.

(2*S*,3*S*,4*E*)-1-*O*-(2',3',4',6'-Tetra-*O*-benzoyl-β-glucopyranosy1)-2-[*N*-2''-*E*-octenoylamido]-3-*O*-pivaloyl-4-octadecene (21). This compound was prepared by using the procedure described for compound **20**. (*E*)-Oct-2-enoyl chloride was used as the acylation agent. Purification by chromatography (EtOAc/hexane 1:2) afforded compound **21** (5 mg, 51%, over two steps): R_f 0.48 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, *J* = 6.8 Hz), 1.10 (s, 9H), 1.12-1.38 (m, 28 H), 1.90 (m, 2H), 1.98 (m, 2H), 3.59 (dd, 1H, *J* = 4.3, 9.8 Hz), 4.09 (dd, 1H, *J* = 2.5, 9.8 Hz), 4.13 (m, 1H), 4.31 (m, 1H), 4.49 (dd, 1H, *J* = 5.0, 12.4 Hz), 4.61 (dd, 1H, *J* = 3.0, 12.1 Hz), 4.79 (d, 1H, *J* = 8.0 Hz), 5.28 (m, 1H), 5.37 (m, 2H), 5.53 (dd, 1H, *J* = 8.3, 10.1 Hz), 5.62 (d, 1H, *J* = 9.1 Hz), 5.70 (t, 1H, *J* = 9.8 Hz), 5.78 (m, 1H), 5.94 (t, 1H, *J* = 9.8 Hz), 6.67 (dt, 1H, *J* = 6.8, 14.4 Hz), 7.26-7.56 (m, 12H), 7.84 (m, 2H), 7.90 (m, 2H), 8.00 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.4, 27.6, 27.9, 28.4, 28.7, 29.1, 29.4, 29.6, 29.7, 31.4, 32.3, 36.6, 38.8, 51.5, 62.1, 63.0, 68.0, 69.5, 72.2, 72.6, 76.7, 101.4, 123.0, 124.4, 128.3-129.9, 133.2-131.4, 137.5, 152.4, 165.1, 165.5, 165.7, 166.0, 171.0, 177.6.

(2*S*,3*S*,4*E*)-1-*O*-(β-D-Glucopyranosy1)-2-[*N*-octanoylamido]-octadec-4-en-3-ol (22). This compound was prepared by using the procedure described for compound **13**. Purification by chromatography ($CHCl_3$/MeOH 10:1) afforded compound **22** (6 mg, 93%) as a white powder:

$[\alpha]^{25}_{D}$ -18.0° (*c* 0.2, $CHCl_3$/MeOH 1:1); R_f 0.48 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, *J* = 6.8 Hz), 1.25 (m, 30H), 1.60 (m, 2H), 2.02 (q, 2H, *J* = 6.6 Hz), 2.19 (t, 2H, *J* = 8.8 Hz), 3.30 (m, 2H), 3.44 (m, 2H), 3.66 (dd, 1H, *J* = 6.0, 9.6 Hz), 3.76 (dd, 1H, *J* = 4.8, 11.9 Hz), 3.85 (m, 1H), 3.95 (dd, 1H, *J* = 5.8, 9.6 Hz), 4.30 (d, 1H, *J* = 8.0 Hz), 4.32 (m, 1H), 5.42 (dd, 1H, *J* = 5.6, 15.1 Hz), 5.75 (dt, 1H, *J* = 6.6, 14.4 Hz), 6.64 (d, 1H, *J* = 9.1 Hz); HRMS $[M+H]^+$ $C_{32}H_{62}NO_8$ calcd for *m/z* 588.4475, found 588.4493.

(2*S*,3*S*,4*E*)-1-*O*-(β-D-Glucopyranosy1)-2-[*N*-2'(*E*)-octenoylamido]-octadec-4-en-3-ol (23). This compound was prepared by using the procedure described for compound **13**. Purification by chromatography ($CHCl_3$/MeOH 10:1) afforded compound **23** (1.0 mg, 62%) as a white powder: R_f 0.44 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, *J* = 6.8 Hz), 1.48 (m, 28H), 1.60 (m, 2H), 2.00 (m, 2H), 2.18 (m, 2H), 2.38 (m, 2H), 3.30 (m, 2H), 3.44 (m, 2H), 3.68 (m, 2H), 3.77 (dd, 1H, *J* = 4.8, 12.1 Hz), 3.87 (dd, 1H, *J* = 3.0, 12.3 Hz), 3.98 (dd, 1H, *J* = 6.0, 10.1 Hz), 4.12 (m, 1H), 4.29 (d, 1H, *J* = 8.0 Hz), 4.31 (m, 1H), 5.45 (m, 1H), 5.76 (dt, 1H, *J* = 6.8, 14.4 Hz), 5.83 (d, 1H, *J* = 15.2 Hz), 6.87 (dt, 1H, *J* = 6.8, 14.4 Hz); HRMS $[M+H]^+$ $C_{32}H_{60}NO_8$ calcd for *m/z* 586.4319, found 586.4313.

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Chapter 4

Synthesis of a Photoactivatable Analog of Psychosine

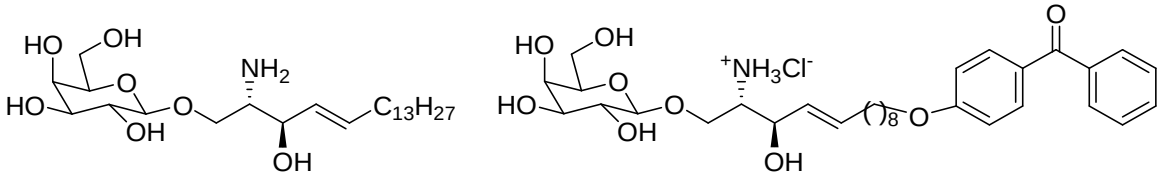
Abstract

The synthesis of a benzophenone-linked psychosine analog is reported. The benzophenone moiety was attached to the aliphatic chain of psychosine by an ether linkage using a Mitsunobu reaction and also by an S_N2 reaction under microwave conditions. Also, cross-metathesis was employed to couple the aliphatic chain to the galactosyl moiety.

Introduction

Psychosine (Chart 1) is the β-galactosyl derivative of D-*erythro*-sphingosine. Psychosine inhibits cytokinesis (cell division),[1] and accumulates in the brains of patients afflicted by the neurological disorder known as Krabbe's disease.[2] TDAG8, a G protein-coupled receptor, was postulated to be a receptor for psychosine,[3] but reports indicated that TDAG8 is a proton receptor.[4] Thus, knowledge about the psychosine receptor is at a very preliminary stage. Since photoactivatable probes can be used to identify lipid-binding proteins,[5] we synthesized a benzophenone-linked analog of psychosine (**1**, Chart 1) to be used to identify its receptor in intact cells.

Chart 1. Structure of photoactivatable analog of psychosine

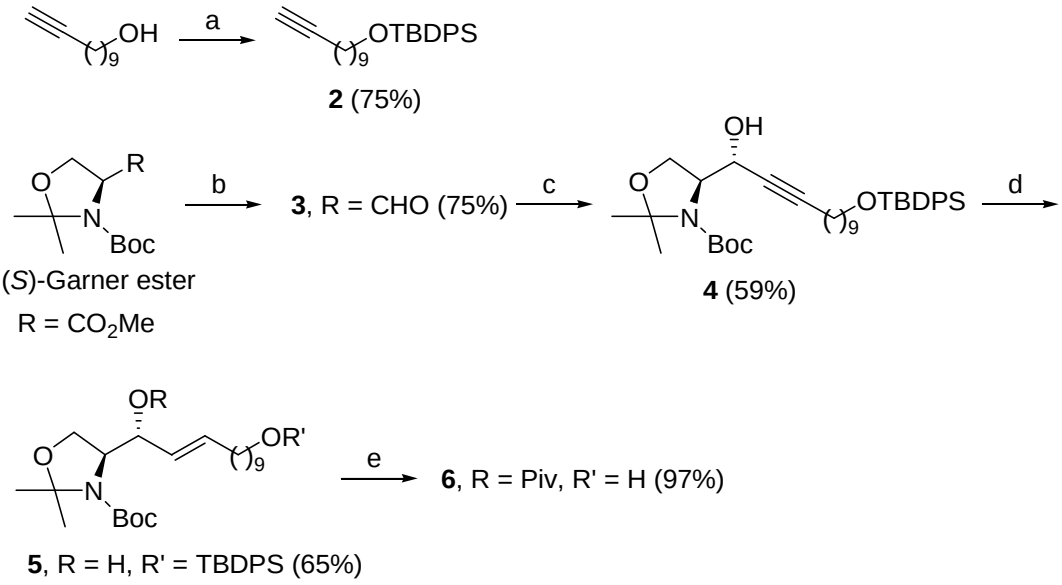


Psychosine **Benzophenone analog of psychosine, 1**

Results and Discussion

Synthesis of an ω-hydroxy analog of sphingosine (Scheme 1). The synthesis of a benzophenone analog of psychosine with an ether linkage to the long-chain base started from TBDPS-protected 10-undecyn-1-ol (**2**, Scheme 1). Compound **2** was initially treated with *n*-butyllithium at -78 °C and then was allowed to react with (*S*)-Garner aldehyde (**3**) at -78 °C in the presence of a stoichiometric amount of HMPA to afford the addition product **4** along with threo-adduct, which was separated by chromatography.[6] The triple bond was reduced using Red-Al to afford (*E*)-allylic alcohol **5**. The pivaloyl group was selected for the protection of the secondary alcohol because it is less likely to undergo ester migration than other esters (*cf.* acetyl, benzoyl) from C-3 to C-1 under basic conditions.[7] Silyl deprotection of the hydroxy group using TBAF afforded primary alcohol **6**.

Scheme 1. Synthesis of ω-hydroxy analog of sphingosine

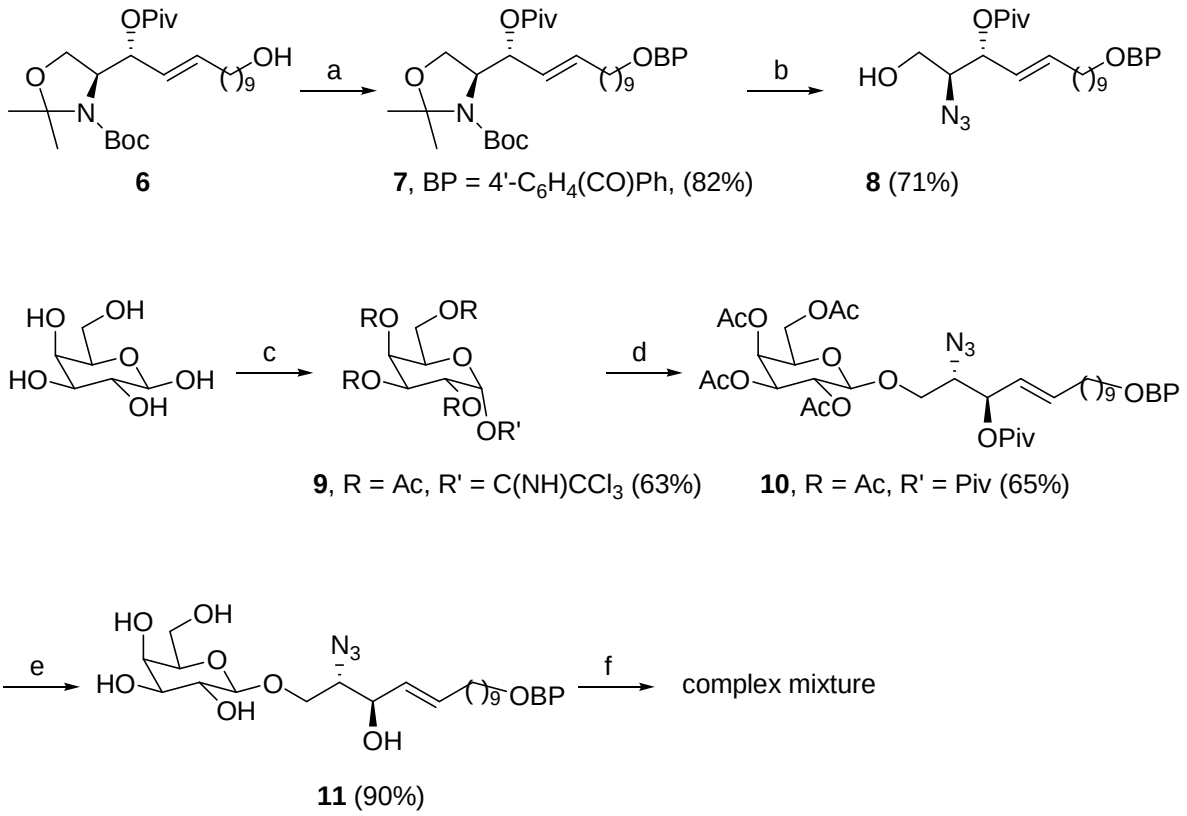


Reagents and conditions: (a) TBDPSCl, imidazole (2 equiv.), CH_2Cl_2; (b) DIBAL in toluene, -78 °C, 2 h; (c) **2**, *n*-BuLi, HMPA, THF, -78 °C, 5.5 h; (d) Red-Al, THF, -78°C to rt, overnight; (e) (i) *t*-BuC(O)Cl, DMAP/Et_3N, CH_2Cl_2, (ii) TBAF, THF.

Attempted synthesis of a photoactivatable psychosine (Scheme 2). A Mitsunobu reaction was used to install 4-hydroxybenzophenone at the terminus of the long-chain base to provide product **7** (Scheme 2).[8] After the oxazolidine and Boc groups were removed under acidic conditions, by a diazotransfer reaction[9] afforded azide **8**. We planned to perform the glycosylation reaction using Schmidt's protocol.[10] The galactosyl donor **9** was prepared from D-galactose,[11] and was then coupled to aglycone **8** in the presence of $BF_3 \cdot OEt_2$ as the promoter to afford glycosylated product **10**. When TMSOTf was used as the promoter the major product was an acetylated product of alcohol compound **8**. Global deprotection of esters of compound **10** using NaOMe in MeOH afforded the azido analog of photoactivatable psychosine **11**. We attempted to reduce azide **11** under Staudinger's conditions to afford the final product. After observation of a polar spot in TLC (indicating completion of azide reduction to amine), the

mixture was concentrated and purified by chromatography. Unfortunately, the product after isolation decomposed within one hour as observed by TLC. Also, the NMR spectra of the azide reduction were inconclusive.

Scheme 2. Attempted synthesis of a photoactivatable psychosine

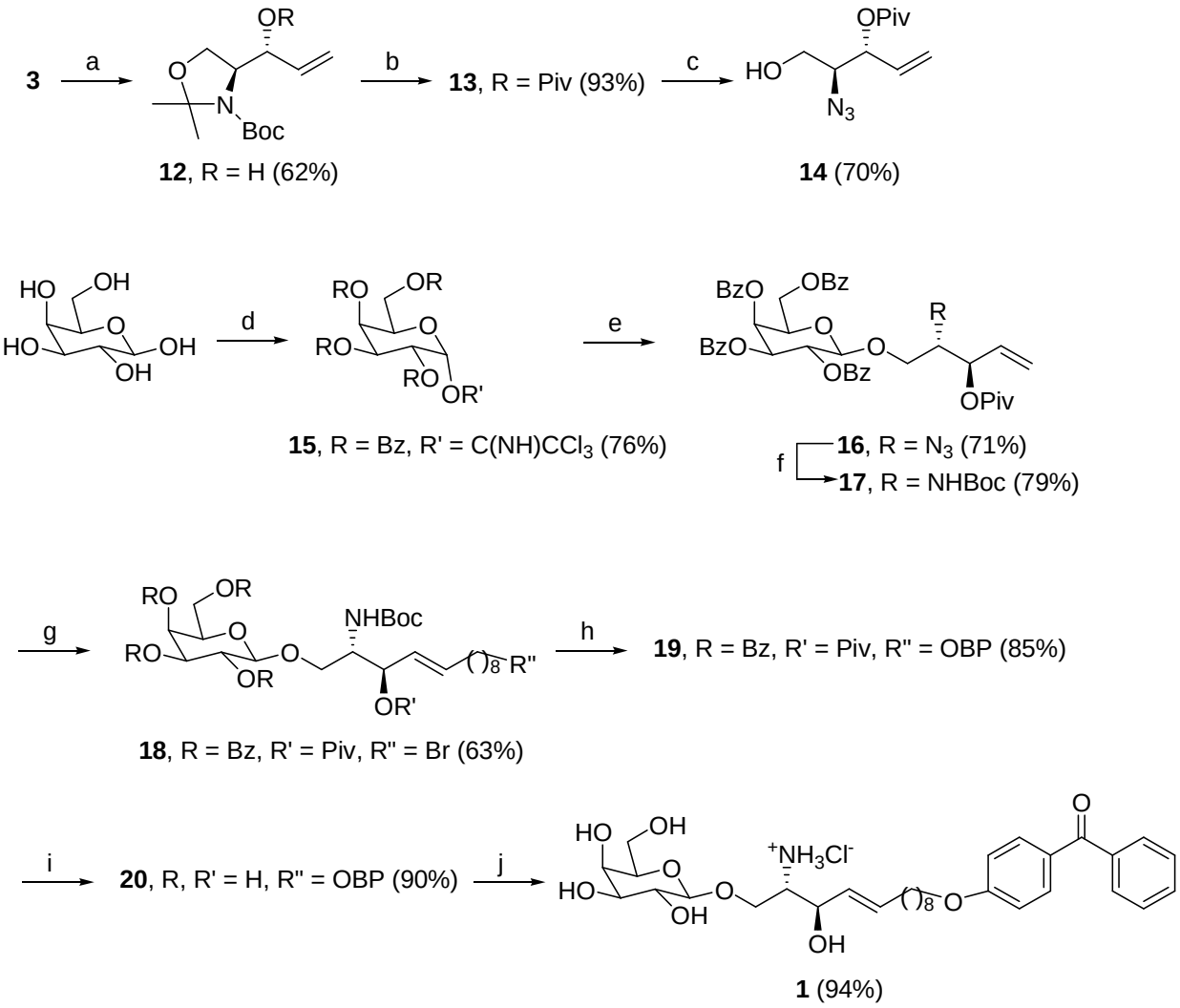


Reagents and conditions: (a) Ph_3P, DIAD, CH_2Cl_2, 4-hydroxybenzophenone, 0 °C to rt, overnight; (b) (i) 2 M HCl, EtOH, 70 °C, 3 h, (ii) TfN_3 in CH_2Cl_2, K_2CO_3, 1 mol% $CuSO_4$, MeOH, H_2O, 5 h; (c) Ref. 11; (d) **8**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å, MS, 4 h; (e) NaOMe in MeOH, THF, 2 d; (f) Ph_3P, THF/H_2O (9:1), overnight.

Synthesis of photoactivatable psychosine analog 1 (Scheme 3). In the next attempt, we used the Boc group in the final stage in order to make an amine salt. Recently, cross metathesis has been used for preparing the sphingosine backbone;[12] this approach was adopted in our next attempt. Also, instead of a Mitsunobu reaction, an S_N2 reaction to link the benzophenone to

compound **18** was attempted. Allyl alcohol **12** (Scheme 3) was prepared from (*S*)-Garner aldehyde (**3**) by the addition of vinylmagnesium bromide following a known procedure.[13] The secondary alcohol **12** was protected with a pivaloyl group, and the resulting compound **13** was subjected to a diazotransfer reaction to prepare azide **14**. The glycosylation reaction was carried out by using a similar procedure to that in the previous attempt. Perbenzoylated galactosyl trichloroacetimidate (**15**) was prepared as described previously.[14] The glycosylated product **16** was prepared from aglycone **14** using Schmidt's protocol. Cross metathesis of 10-bromo-1-decene (4 equiv.) and azide **16** using Grubb's second generation catalyst resulted in the formation of undesired products. However, the N-Boc derivative **17** prepared from azide **16** reacted with 10-bromo-1-decene, affording metathesis product **18** after refluxing in CH_2Cl_2 for 16 h. S_N2 reaction of bromide **18** with 4-hydroxybenzophenone in DMF using K_2CO_3 as base was sluggish even at temperatures above 100 °C. However, when microwave conditions were used[15] for this substitution reaction in DMF, compound **19** was obtained in 85% yield in 1.5 h. Deprotection of the benzoyl and pivaloyl ester groups using NaOMe in MeOH provided compound **20**. The final step was the deprotection of the carbamate group of **20** using 1 M HCl in 90% AcOH to afford benzophenone-linked psychosine **1** without any byproducts resulting from cleavage of the sugar in acidic conditions.

Scheme 3. Synthesis of photoactivatable psychosine analog 1



Reagents and conditions: (a) CH_2=CHMgBr, THF, -78 °C, 2 h; (b) *t*-BuC(O)Cl, DMAP/Et_3N, CH_2Cl_2; (c) (i) 2 M HCl/EtOH (1:4), 70-80 °C, 3 h, (ii) TfN_3 in CH_2Cl_2, K_2CO_3, 1 mol% $CuSO_4$, MeOH, H_2O; (d) Ref. 14; (e) **14**, $BF_3 \cdot Et_2O$, CH_2Cl_2, 4 Å MS; (f) (i) Ph_3P, C_6H_6, H_2O (1 drop), (ii) $(Boc)_2O$, Et_3N, CH_2Cl_2; (g) 10-bromo-1-decene (4 equiv.), Grubb's second generation catalyst (30 mol%), CH_2Cl_2, reflux, 16 h; (h) 4-hydroxybenzophenone, K_2CO_3, DMF, microwave, 120 °C, 1.5 h; (i) NaOMe in MeOH, THF; (j) 1 M HCl in 90% AcOH, CH_2Cl_2, rt, 35 min.

Experimental Section

General Information. Triethylamine and DMF were distilled over calcium hydride. The solvents were dried and the analyses were performed according to the general information described on page 10.

***tert*-Butyldiphenyl(undec-10-ynyloxy)silane (2).** To a solution of imidazole (2.0 g, 28.8 mmol) in CH_2Cl_2 (30 mL) was added TBDPSCl (3.8 mL, 14.4 mmol) with stirring for 30 min. To the resulting mixture was added undec-10-ynol (2.0 g, 12 mmol), and the mixture was stirred overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (30 mL) and the organic layer was separated. The product was extracted with CH_2Cl_2 (2 x 25 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (hexane/EtOAc 20:1) to afford **2** (3.6 g, 75%) as a colorless liquid: R_f 0.49 (hexane/EtOAc 20:1); ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H), 1.26 (s, 6H), 1.37 (m, 4H), 1.52 (m, 4H), 1.91 (t, 1H, J = 2.8 Hz), 2.18 (m, 2H), 3.67 (t, 2H, J = 6.8 Hz), 7.39 (m, 6H), 7.69 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 18.5, 19.3, 25.8, 26.9, 28.6, 28.8, 29.1, 29.4, 29.5, 32.6, 64.0, 68.2, 84.8, 127.6, 129.6, 134.2, 135.6.

(*S*)-*tert*-Butyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (3). Refer to page 31.

(*S*)-*tert*-Butyl-4-((*R*)-1'-hydroxydodec-2'-yn-12'-*tert*-butyldiphenylsilanyloxy)-2,2-dimethyloxazolidine-3-carboxylate (4). To a solution of alkyne **2** (2.3 g, 5.6 mmol) in dry THF (30 mL) was added *n*-BuLi (2.5 M in hexane, 2.52 mL, 6.3 mmol) at -78 °C under N_2. The mixture was stirred for 2 h before HMPA (2.2 mL, 12.6 mmol) was added at -78 °C. After the mixture had stirred for 45 min, a solution of (*S*)-Garner aldehyde **3** (995 mg, 4.34 mmol) in 10 mL of dry THF was added slowly. The solution was stirred at -78 °C for 2.5 h and then was quenched with aqueous saturated NH_4Cl solution (30 mL). The mixture was extracted with Et_2O (3 x 30 mL), and the combined organic phases were washed with brine (30 mL), dried ($MgSO_4$),

and concentrated. The crude oil was purified by flash chromatography (EtOAc/hexane 1:3) to afford **4** (1.6 g, 59%) as a colorless oil: $[\alpha]^{25}_{D}$-22.9° (*c* 3.0, $CHCl_3$); R_f 0.39 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 9H), 1.25-1.61 (m, 29H), 2.19 (m, 2H), 3.65 (t, 2H, *J* = 6.4 Hz), 3.91 (br s, 1H), 4.07 (m, 1H), 4.13 (m, 1H), 4.52 (m, 1H), 4.71 (m, 1H), 7.39 (m, 6H), 7.67 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 18.8, 19.2, 25.4, 25.7, 26.0, 26.9, 28.0, 28.3, 28.4, 28.6, 28.9, 29.1, 29.4, 29.5, 32.6, 62.8, 64.0, 64.1, 65.0, 77.9, 81.2, 86.6, 94.9, 127.5, 129.4, 134.2, 135.5, 154.1.

(*S*)-*tert*-Butyl-4-((*R*)-1'-hydroxydodec-2'*E*-en-12'-*tert*-butyldiphenylsilanyloxy)-2,2-dimethyloxazolidine-3-carboxylate (5). Alcohol **4** (930 mg, 1.5 mmol) in dry THF (25 mL) was cooled to -78 °C and RedAl (590 mL, 2.9 mmol) was added dropwise via syringe. The reaction mixture was stirred at -78 °C for 1 h and then allowed to warm to rt overnight. The reaction was quenched by slow addition of water (25 mL) and the product was extracted with Et_2O (3 x 25 mL). The organic phase was dried (Na_2SO_4) and evaporated. Purification by chromatography (EtOAc/hexane 1:4) afforded (*E*)-allylic alcohol **5** (620 mg, 65%) as a colorless oil: $[\alpha]^{25}_{D}$-13.9° (*c* 2.2, $CHCl_3$); R_f 0.28 (EtOAc/hexane 1:4); ^{1}H NMR ($CDCl_3$) δ 1.04 (s, 9H), 1.20-1.58 (m, 29H), 2.05 (q, 2H, *J* = 6.8 Hz), 3.65 (t, 2H, *J* = 6.4 Hz), 3.84 (br s, 1H), 4.00 (m, 1H), 4.12 (m, 1H), 4.21 (m, 2H), 5.47 (dd, 1H, *J* = 5.6, 15.2 Hz), 5.73 (dt, 1H, *J* = 6.8, 14.8 Hz), 7.40 (m, 6H), 7.66 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 19.3, 24.7, 25.8, 26.3, 26.9, 28.4, 29.2, 29.3, 29.4, 29.5, 29.6, 32.4, 32.6, 62.3, 64.0, 64.9, 74.0, 81.0, 94.4, 127.6, 128.2, 129.5, 133.3, 134.2, 135.6, 154.2.

(*S*)-*tert*-Butyl-4-((*R*)-1'-*O*-pivaloyl-2'*E*-en-12'-hydroxy)-2,2-dimethyloxazolidine-3-carboxylate (6). A solution of compound **5** (1.4 g, 2.2 mmol), pivaloyl chloride (0.68 mL, 5.5 mmol), DMAP (53 mg, 0.4 mmol), and Et_3N (1.5 mL) in CH_2Cl_2 (15 mL) was stirred at 0 °C for 1 h, and then allowed to warm to rt overnight. The reaction mixture was quenched with saturated

aqueous $NaHCO_3$ solution (5 mL) and the product was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:4) to afford pivaloylated product (1.58 g, 88%) as a colorless oil: R_f 0.55 (EtOAc/hexane 1:4). To a solution of pivaloylated product (1.5 g, 2.0 mmol) in THF (30 mL) was added TBAF (1.0 M in THF, 4.1 mL, 4.1 mmol). The reaction mixture was stirred at rt until the starting material was completely consumed. The reaction was quenched with water and extracted with Et_2O (2 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:3) to afford compound **6** (940 mg, 97%) as a colorless oil: $[\alpha]^{25}_D$ -27.0° (*c* 0.83, $CHCl_3$); R_f 0.18 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.20-1.35 (m, 23H), 1.48 (s, 9H), 1.50-1.56 (m, 6H), 2.04 (m, 2H), 3.63 (t, 2H, *J* = 6.8 Hz), 3.91 (m, 2H), 4.13 (m, 1H), 5.27 (dd, 1H, *J* = 6.9, 15.3 Hz), 5.50 (m, 1H), 5.73 (m, 1H), 7.38 (m, 6H), 7.75 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 14.2, 19.0, 23.2, 24.5, 25.7, 26.5, 27.0, 27.2, 28.4, 28.7, 29.0, 29.3, 29.4, 29.5, 29.7, 32.8, 38.9, 59.6, 63.0, 73.4, 74.1, 80.3, 94.5, 127.7, 134.8, 152.6, 177.2.

(*S*)-*tert*-Butyl-4-((*R*)-1'-*O*-pivaloyl-2'*E*-en-12'-*O*-(4'-benzoylphenyl)-2,2-dimethyloxazolidine-3-carboxylate (7). To a solution of DIAD (158 µL, 0.80 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added a solution of Ph_3P (223 mg, 0.85 mmol) in dry CH_2Cl_2 (5 mL). After the mixture was stirred for 10 min, a solution of 4-hydroxybenzophenone (159 mg, 0.80 mmol) in dry CH_2Cl_2 (5 mL) was added over a period of 20 min. The reaction mixture was stirred for another 10 min, and a solution of alcohol **6** (242 mg, 0.5 mmol) in dry CH_2Cl_2 (10 mL) was added over a period of 20 min. After the mixture was stirred for 10 min at 0 °C, the ice bath was removed, and the mixture was stirred at rt overnight. When TLC indicated the disappearance of **6**, the solution was concentrated and the resulting residue was purified by

chromatography (elution with EtOAc/hexane, 1:6) to afford **7** (272 mg, 82%) : $[\alpha]^{25}_{D}$-16.9° (*c* 3.9, $CHCl_3$); R_f 0.55 (EtOAc/hexane, 1:3); ^{1}H NMR ($CDCl_3$) δ 1.20-1.34 (m, 21H), 1.48 (s, 9H), 1.52-1.56 (m, 6H), 1.81 (m, 2H), 1.97 (m, 2H), 3.91 (m, 2H), 4.03 (t, 2H, *J* = 6.5 Hz), 4.09 (m, 1H), 5.28 (dd, 1H, *J* = 6.9, 15.3 Hz), 5.48 (m, 1H), 5.72 (m, 1H), 6.93 (m, 2H), 7.45-7.83 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 20.7, 22.0, 23.6, 23.9, 24.7, 25.9, 26.3, 26.5, 26.6, 26.7, 26.9, 27.0, 27.1, 29.8, 29.9, 36.5, 57.2, 61.6, 65.8, 71.7, 77.8, 92.0, 111.6, 123.1, 125.7, 127.3, 127.4, 129.4, 130.1, 133.0, 135.9, 149.4, 150.2, 160.4, 174.8, 193.2.

(2*S*,3*R*)-3-*O*-Pivaloyl-2-Azido-14-*O*-(4'-benzoylphenyl)-(4*E*)-tetradecene (8). A solution of **7** (79 mg, 0.12 mmol) in 2 M HCl/EtOH (1:4, 10 mL) was stirred at 70°C. After 3 h, the solution was concentrated and water (10 mL) was added. The amine salt was extracted with $CHCl_3$/MeOH (7:1), dried (Na_2SO_4), concentrated, and the crude product was used in the next step without purification. Trifluoromethanesulfonyl (triflyl) azide was prepared fresh prior to the reaction.[9] To the amine salt in H_2O (1.5 mL), K_2CO_3 (16.5 mg, 0.12 mmol) and $CuSO_4 \cdot H_2O$ (0.3 mg, 1.2 μmol) were added. After TfN_3 in CH_2Cl_2 (1.5 mL) was added at once to the reaction mixture, MeOH (5 mL) was added slowly along the walls of the reaction flask. After 5 h, when TLC indicated completion of the reaction, the solution was concentrated and purified by chromatography (elution with EtOAc/hexane, 1:3) to afford **8** (47 mg, 71%): $[\alpha]^{25}_{D}$-32.7° (*c* 0.8, $CHCl_3$); R_f 0.65 (EtOAc/hexane, 1:1); ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.25-1.48 (m, 12H), 1.81 (m, 2H), 2.07 (m, 2H), 2.12 (br s, 1H), 3.53 (m, 1H), 3.65 (m, 2H), 4.04 (t, 2H, *J* = 6.5 Hz), 5.34 (dd, 1H, *J* = 4.9, 7.7 Hz), 5.48 (dd, 1H, *J* = 7.8, 15.3 Hz), 5.84 (dt, 1H, *J* = 6.8, 15.0 Hz), 6.96 (m, 2H), 7.45-7.83 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 20.7, 22.7, 24.0, 25.1, 26.4, 27.0, 27.4, 27.7, 29.9, 36.9, 59.9, 64.2, 66.3, 71.8, 112.0, 121.6, 126.2, 127.7, 127.9, 129.9, 130.6, 136.1, 136.4, 160.9, 175.4, 193.7.

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl Trichloroacetimidate (9).[10,11] D-(+)-Galactose (3.0 g, 16.7 mmol) was dissolved in acetic anhydride (25 mL), and a mixture of $HClO_4$ (2 mL) in Ac_2O (5 mL) was added dropwise . After the reaction mixture had stirred overnight at rt an additional 0.1 mL of $HClO_4$ was added, and the solution was stored in the refrigerator. The separated crystalline material was filtered and dried under vacuum. The penta-*O*-acetylpyranose product was dissolved in DMF (10 mL), and hydrazine acetate (1.6 g, 16.7 mmol) was added under Ar at 60° C. When TLC (hexanes/EtOAc 1:1) showed the disappearance of the starting material, the mixture was diluted with EtOAc, washed with 5% aqueous NaCl solution (2 × 20 mL) and H_2O (25 mL), dried (Na_2SO_4), and concentrated. A solution of the crude product was reacted with trichloroacetonitrile (17 mL, 166.5 mmol) and DBU (5 mL, 33.3 mmol) in CH_2Cl_2 (30 mL) for 60 min at 0° C. When TLC (hexanes/EtOAc 2:1) showed the conversion to be complete, the solution was concentrated and purified by flash chromatography (EtOAc/hexane, 1:3) to afford **9** (5.2 g, 63%), R_f 0.18 (EtOAc/hexane, 1:2.5); ^{1}H NMR ($CDCl_3$): δ 1.99 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.18 (s, 3H), 4.08 (dd, 1H, *J* = 6.7, 11.3 Hz), 4.17 (dd, 1H, *J* = 6.6, 11.3 Hz), 4.45 (m, 1H), 5.36 (dd, 1H, *J* = 10.8, 3.4 Hz), 5.43 (dd, 1H, *J* = 10.8, 3.0 Hz), 5.56 (dd, 1H, *J* = 3.0, 1.4 Hz), 6.61 (d, 1H, *J* = 3.4 Hz), 8.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.7, 20.8, 61.1, 66.4, 67.3, 67.4, 68.8, 93.4, 160.8, 169.7, 169.9, 170.0, 170.3. The NMR spectrum is in agreement with that reported in the literature.[10,11]

(2*S*,3*R*,4*E*)-2-Azido-3-*O*-pivaloyl-14-*O*-(4''-benzoylphenyl)-1-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-galactopyranosy1)-4-tetradecene (10). A mixture of galactosyl donor **9** (57.8 mg, 0.117 mmol), and aglycone **8 (**43 mg, 0.078 mmol) in dry CH_2Cl_2 (5 mL) was stirred in the presence of 4Å molecular sieves at rt for 1 h. A solution of $BF_3{\cdot}Et_2O$ (0.1 M, 0.23 mL, 0.023 mmol) in dry CH_2Cl_2 was added slowly to the reaction mixture. After 4 h, TLC indicated the

completion of reaction. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (15 mL) and the product was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 1:4) to afford compound **10** (46 g, 65%): $[\alpha]^{25}_D$ -11.66° (*c* 0.54, $CHCl_3$); R_f 0.40 (EtOAc/hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H), 1.26-1.45 (m, 12H), 1.81 (m, 2H), 1.99 (s, 3H), 2.05 (s, 3H), 2.07 (m, 2H), 2.10 (s, 3H), 2.16 (s, 3H), 3.49 (dd, 1H, *J* = 5.8, 10.3 Hz), 3.75 (m, 1H), 3.85 (dd, 1H, *J* = 6.8, 10.3 Hz), 3.91 (t, 1H, *J* = 6.8 Hz), 4.04 (t, 2H, *J* = 6.5 Hz), 4.15 (m, 2H), 4.47 (d, 1H, *J* = 8.0 Hz), 5.00 (dd, 1H, *J* = 3.5, 10.3 Hz), 5.22 (dd, 1H, *J* = 8.1, 10.3 Hz), 5.30 (dd, 1H, *J* = 4.3, 8.1 Hz), 5.40 (d, 1H, *J* = 3.5 Hz), 5.46 (m, 1H), 5.84 (dt, 1H, *J* = 6.8, 14.4 Hz), 6.96 (m, 2H), 7.46-7.83 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7, 20.8, 22.7, 26.0, 27.0, 28.8, 29.0, 29.1, 29.3, 29.5, 32.3, 38.9, 61.1, 63.4, 66.9, 68.2, 68.5, 70.8, 70.9, 73.8, 100.9, 114.0, 122.9, 128.1, 129.7, 129.8, 131.8, 132.5, 138.3, 138.4, 162.8, 169.3, 170.2, 170.3, 170.4, 176.9, 195.6; HRMS $[M+Na]^+$ $C_{46}H_{61}N_3O_{14}Na$ calcd for *m/z* 902.4051, found 902.4028.

(2*S*,3*R*,4*E*)-2-Azido-3-hydroxy-14-*O*-(4''-benzoylphenyl)-1-*O*-β-galactopyranosy1-4-tetradecene (11). Sodium metal (5 mg) was dissolved in dry MeOH (3 mL) and added to a solution of **10** (18 mg, 0.020 mmol) in dry MeOH/THF (5:1, 6 mL). The reaction mixture was stirred for two days at rt. Dowex 50W-X8 resin (prewashed thoroughly with MeOH) was added to neutralize the reaction mixture. The reaction mixture was filtered, concentrated, and purified by chromatography ($CHCl_3$/MeOH 10:1) to afford compound **11** (11.4 mg, 90%): $[\alpha]^{25}_D$ -7.36° (*c* 0.31, $CHCl_3$/MeOH 1:1); R_f 0.42 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) 1.31-1.48 (m, 12H), 1.84 (m, 2H), 2.08 (q, 2H, *J* = 6.8 Hz), 2.36 (br s, 1H), 3.44 (m, 1H), 3.55 (m, 2H), 3.64 (m, 1H), 3.79 (dd, 1H, *J* = 4.5, 8.2 Hz), 3.82 (m, 1H), 3.91 (dd, 1H, *J* = 6.3, 12.0 Hz), 3.98 (m, 1H), 4.04

(t, 2H, *J* = 6.5 Hz), 4.14 (dd, 1H, *J* = 4.5, 10.6 Hz), 4.28 (m, 2H), 5.50 (dd, 1H, *J* = 7.6, 15.4 Hz), 5.84 (dt, 1H, *J* = 6.8, 14.4 Hz), 6.96 (m, 2H), 7.46-7.82 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 23.9, 26.9, 27.0, 27.1, 27.3, 27.4, 27.5, 27.7, 30.3, 59.9, 63.2, 66.3, 67.1, 69.1, 71.2, 72.6, 74.7, 101.2, 112.0, 126.2, 127.7, 129.9, 130.6, 133.6, 136.2, 160.9, 194.0; HRMS $[M+Na]^+$ $C_{33}H_{45}N_3O_9Na$ calcd for *m/z* 650.3053, found 650.3030.

(*S*)-*tert*-Butyl-4-((*R*)-1-hydroxyallyl)-2,2-dimethyloxazolidine-3-carboxylate (12). To a -78 °C solution of (*S*)-Garner aldehyde (**3**, 7.0 g, 30.5 mmol) in dry THF (50 mL) under N_2 was slowly added vinylmagnesium bromide (92 mL, 92 mmol, a 1 M solution in THF) via cannula. After the cloudy yellow solution was stirred for 2 h at -78 °C, it was warmed to 0 °C, and the reaction mixture was quenched with saturated aqueous NH_4Cl solution (60 mL). The product was extracted with Et_2O (2 x 60 mL). The combined organic layers were washed with brine (60 mL), dried (Na_2SO_4), and concentrated. The mixture of erythro and threo diastereomers were separated by column chromatography by gravity (hexane/EtOAc 5:1) to afford **12** (4.8 g, 62%) and its C3-epimer (0.8 g, 10.2%). Compound **12**: $[\alpha]^{25}_D$ -37.7° (*c* 1.8, $CHCl_3$); R_f 0.26 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3H), 1.50 (s, 9H), 1.58 (s, 3H), 3.91 (m, 2H), 3.98 (m, 1H), 4.25 (m, 1H), 4.39 (m, 1H), 5.24 (m, 1H), 5.38 (m, 1H), 5.85 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.3, 28.3, 61.8, 64.4, 73.3, 81.4, 94.4, 116.1, 117.8, 137.6, 155.0; HRMS $[M+Na]^+$ $C_{13}H_{23}NO_4Na$ calcd for *m/z* 280.1525, found 280.1519.

***tert*-Butyl-(2*S*,3*R*)-1-hydroxy-3-(pivaloyl)pent-4-en-2-yl-carbamate (13).** To a solution of allylic alcohol **12** (2.7 g, 10.5 mmol) in CH_2Cl_2 (25 mL) were added Et_3N (4.4 mL, 31.5 mmol), PivCl (2 mL, 15.8 mmol), and DMAP (250 mg, 2.1 mmol) at 0 °C. After 10 min, the cooling bath was removed, and the reaction mixture was stirred overnight at rt. The reaction mixture was diluted with saturated aqueous NH_4Cl solution (60 mL) and the product was

extracted with CH_2Cl_2 (2 x 50 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, purified by vacuum distillation, and the fraction boiling at 120-125 °C (4 mm of Hg) was collected to afford pivaloyl-protected alcohol **13** (3.35 g, 93%): R_f 0.45 (EtOAc/hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 1.46-1.58 (m, 15H), 3.96 (m, 2H), 4.14 (m, 1H), 5.20 (m, 1H), 5.28 (m, 1H), 5.57 (m, 1H), 5.75 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 26.4, 27.2, 28.3, 38.8, 59.3, 63.6, 73.2, 80.3, 93.9, 117.4, 134.0, 151.8, 177.2, 185.0.

(2*S*,3*R*,4*E*)-2-Azido-3-*O*-pivaloyl-pent-4-ene (14). Azido compound **14** was prepared from pivalate **13** (760 mg, 2.22 mmol) according to the procedure used to prepare compound **8**, affording azide **14** (352 mg, 70%): $[\alpha]^{25}_D$ -29.36° (*c* 3.7, $CHCl_3$); R_f 0.31 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 3.05 (br s, 1H), 3.57 (m, 1H), 3.71 (m, 2H), 5.31-5.42 (m, 3H), 5.86 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 27.0, 39.0, 61.4, 65.6, 73.4, 119.2, 131.8, 177.4.

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl Trichloroacetimidate (15). Refer to page 34.

(2*S*,3*R*,4*E*)-2-Azido-3-*O*-pivaloyl-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-galactopyranosy1)-4-pentene (16). The glycosylation reaction of azide **14** (285 mg, 1.25 mmol) and galactosyl donor **15** (1.4 g, 1.87 mmol) was performed according to the procedure used for preparing compound **10**, affording product **16** (720 mg, 71%): $[\alpha]^{25}_D$ +77.22° (*c* 0.86, $CHCl_3$); R_f 0.19 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.19 (s, 9H), 3.70 (dd, 1H, *J* = 5.5, 10.4 Hz), 3.82 (m, 1H), 4.00 (dd, 1H, *J* = 7.3, 10.4 Hz), 4.34 (m, 1H), 4.43 (m, 1H), 4.68 (dd, 1H, *J* = 6.6, 11.2 Hz), 4.88 (d, 1H, *J* = 7.8 Hz), 5.19 (m, 2H), 5.35 (m, 1H), 5.60 (dd, 1H, *J* = 3.7, 10.6 Hz), 5.73 (m, 1H), 5.81 (dd, 1H, *J* = 8.0, 10.4 Hz), 6.00 (d, 1H, *J* = 3.5 Hz), 7.21-8.03 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 27.1, 38.9, 61.9, 63.1, 67.9, 69.5, 71.6, 73.1, 73.7, 101.2, 119.1, 119.9, 128.3-130.0, 131.5, 132.5-133.6, 165.1-166.0, 176.8.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-*O*-pivaloyl-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-galactopyranosy1)-4-pentene (17). To a solution of azide **16** (290 mg, 0.36 mmol) in 10 mL of benzene with 1 drop of H_2O was added PPh_3 (236 mg, 0.9 mmol). The reaction mixture was stirred overnight. After TLC indicated complete conversion of the azide to the amine, the reaction mixture was concentrated under vacuum. To a solution of the residue of the amine in CH_2Cl_2 (5 mL) were added Boc_2O (196 mg, 0.9 mmol) and Et_3N (150 μL, 1.0 mmol). After the reaction mixture was stirred overnight, saturated aqueous NH_4Cl solution (10 mL) was added and the product was extracted with CH_2Cl_2 (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated, and purified by chromatography (hexane/EtOAc 6:1) to afford Boc-protected compound **17** (250 mg, 79%): $[\alpha]^{25}_D$ +62.4° (*c* 1.14, $CHCl_3$); R_f 0.18 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H), 1.32 (s, 9H), 3.67 (dd, 1H, *J* = 4.3, 9.8 Hz), 4.00 (m, 1H), 4.13 (dd, 1H, *J* = 3.3, 9.8 Hz), 4.34 (m, 1H), 4.41 (dd, 1H, *J* = 6.7, 11.4 Hz), 4.63 (dd, 1H, *J* = 6.6, 11.2 Hz), 4.80 (m, 1H), 4.83 (d, 1H, *J* = 7.8 Hz), 5.20 (m, 2H), 5.35 (t, 1H, *J* = 6.2 Hz), 5.63 (dd, 1H, *J* = 3.4, 10.4 Hz), 5.76 (m, 2H), 6.00 (d, 1H, *J* = 3.4 Hz), 7.21-8.12 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 27.1, 28.3, 38.8, 52.2, 62.0, 67.9, 69.9, 71.5, 73.4, 79.4, 101.4, 118.3, 128.3-130.0, 133.3, 133.6, 155.3, 165.2-166.0, 176.9.

10-Bromo-1-decene. A solution of 9-decen-1-ol (5.0 g, 32.4 mmol) and triphenylphosphine (11.0 g, 42.1 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C. *N*-Bromosuccinimide (7.5 g, 42.1 mmol) was added in small portions over a period of 1 h at 0 °C. The mixture was filtered through a silica gel pad, washed with CH_2Cl_2 (50 mL), concentrated, and purified by vacuum distillation. The product boiling at 30-35 °C (2 mm Hg) was collected, providing 10-bromo-1-decene (5.8 g, 83%): R_f 0.71 (EtOAc/hexane 1:5); ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 10H), 1.85 (p, 2H, *J* = 7.2 Hz), 2.03 (q, 2H, *J* = 7.0 Hz), 3.40 (t, 2H, *J* = 7.0

Hz), 4.96 (m, 2H), 5.81 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.2, 28.8, 28.9, 29.0, 29.2, 29.3, 32.8, 33.6, 33.8, 34.0, 114.2, 138.9.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-*O*-pivaloyl-13-bromo-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-galactopyranosy1)-4-tridecene (18). To a solution of alkene **17** (185 mg, 0.21 mmol) in CH_2Cl_2 (8 mL) was added 10-bromo-1-decene (183 mg, 0.84 mmol) and benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexyl-phosphine)ruthenium catalyst (53.6 mg, 0.063 mmol) at rt under N_2. After the reaction mixture was stirred for 16 h under reflux, the solvent was removed and the product was purified by chromatography (hexane/EtOAc 4:1) to afford compound **18** (142 mg, 63%): $[\alpha]^{25}_D$ +41.5° (*c* 0.67, $CHCl_3$); R_f 0.68 (EtOAc/hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 1.17-1.39 (m, 28H), 1.83 (p, 2H, *J* = 7.0 Hz), 1.94 (q, 2H, *J* = 6.9 Hz), 3.39 (t, 2H, *J* = 6.9 Hz), 3.67 (dd, 1H, *J* = 3.9, 9.8 Hz), 3.97 (m, 1H), 4.13 (dd, 1H, *J* = 3.3, 10.0 Hz), 4.34 (m, 1H), 4.39 (dd, 1H, *J* = 6.8, 11.0 Hz), 4.63 (dd, 1H, *J* = 6.2, 11.0 Hz), 4.76 (m, 1H), 4.80 (d, 1H, *J* = 8.0 Hz), 5.25 (m, 1H), 5.34 (dd, 1H, *J* = 7.5, 15.7 Hz), 5.62 (dd, 1H, *J* = 3.6, 10.4 Hz), 5.68 (m, 1H), 5.74 (dd, 1H, *J* = 8.0, 10.5 Hz), 5.98 (d, 1H, *J* = 2.8 Hz), 7.21-8.12 (m, 20H); ^{13}C NMR ($CDCl_3$) δ 27.1, 28.2, 28.3, 28.7, 28.8, 29.0, 29.2, 32.8, 34.0, 38.8, 52.2, 61.9, 67.9, 69.9, 71.5, 73.3, 79.3, 101.4, 124.9, 128.3-130.0, 133.3, 133.6, 136.5, 155.3, 165.3-166.0, 176.9; HRMS $[M+Na]^+$ $C_{57}H_{68}BrNO_{14}Na$ calcd for *m/z* 1092.3720, found 1092.3723.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-*O*-pivaloyl-13-*O*-(4''-benzoylphenyl)-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-galactopyranosy1)-4-tridecene (19). A solution of bromide **18** (35 mg, 0.033 mmol), 4-hydroxybenzophenone (10 mg, 0.049 mmol), and K_2CO_3 (7.0 mg, 0.049 mmol) in DMF (3.5 mL) was heated in a Smith Creator microwave at 120 °C for 1.5 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution (10 mL) and the product

was extracted with EtOAc (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated, and purified by chromatography (hexane/EtOAc 4:1) to afford benzophenone-linked product **19** (33 mg, 85%): $[\alpha]^{25}_D$ +46.71° (*c* 1.55, $CHCl_3$); R_f 0.48 (EtOAc/hexane 1:2); ^{1}H NMR ($CDCl_3$) δ 1.16-1.48 (m, 28H), 1.81 (m, 2H), 1.97 (q, 2H, *J* = 7.2 Hz), 3.68 (dd, 1H, *J* = 4.0, 9.5 Hz), 4.00 (m, 1H), 4.04 (t, 2H, *J* = 7.2 Hz), 4.14 (m, 1H), 4.35 (t, 1H, *J* = 6.8 Hz), 4.42 (dd, 1H, *J* = 6.8, 11.2 Hz), 4.66 (dd, 1H, *J* = 7.5, 12.3 Hz), 4.80 (m, 1H), 4.83 (d, 1H, *J* = 8.0 Hz), 5.28 (m, 1H), 5.38 (dd, 1H, *J* = 7.5, 15.0 Hz), 5.65 (dd, 1H, *J* = 3.4, 10.2 Hz), 5.72 (m, 1H), 5.78 (dd, 1H, *J* = 7.8, 10.2 Hz), 6.01 (d, 1H, *J* = 3.0 Hz), 6.97 (d, 2H, *J* = 9.2 Hz), 7.25-8.13 (m, 27H); ^{13}C NMR ($CDCl_3$) δ 26.0, 27.1, 28.3, 28.3, 28.8, 29.0, 29.1, 29.3, 29.4, 29.6, 32.2, 38.8, 52.3, 61.9, 64.8, 68.0, 68.3, 69.9, 71.3, 71.6, 73.4, 79.3, 101.4, 114.0, 126.2, 127.9-133.6, 136.6, 138.4, 155.3, 162.9, 165.3-166.0, 176.9, 195.6; HRMS $[M+Na]^+$ $C_{70}H_{77}NO_{16}Na$ calcd for *m/z* 1210.5140, found 1210.5141.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-hydroxy-13-*O*-(4'-benzoylphenyl)-1-*O*-β-galactopyranosy1-4-tridecene (20). Compound **20** (30 mg, 0.025 mmol) was deesterified according to the procedure used for compound **11**, affording 15.6 mg of **20** (90%): $[\alpha]^{25}_D$ -1.05° (*c* 0.76, $CHCl_3$/MeOH 3:1); R_f 0.4 ($CHCl_3$/MeOH 3:1); ^{1}H NMR ($CDCl_3$/CD_3OD 20:1) δ 1.27-1.47 (m, 19H), 1.80 (p, 2H, *J* = 6.6 Hz), 2.01 (q, 2H, *J* = 6.4 Hz), 3.55 (m, 1H), 3.62 (m, 2H), 3.70 (m, 2H), 3.82 (m, 2H), 3.91 (m, 1H), 4.02 (t, 2H, *J* = 6.4 Hz), 4.05 (m, 1H), 4.16 (m, 1H), 4.28 (d, 1H, *J* = 7.8 Hz), 5.48 (dd, 1H, *J* = 6.9, 15.2 Hz), 5.73 (dt, 1H, *J* = 6.7, 14.0 Hz), 6.94 (d, 2H, *J* = 8.8 Hz), 7.37-8.07 (m, 7H); ^{13}C NMR ($CDCl_3$/CD_3OD 20:1) δ 26.0, 28.4, 29.1, 29.2, 29.3, 29.4, 32.4, 54.6, 61.3, 64.8, 67.9, 68.2, 68.9, 69.4, 71.0, 72.5, 73.3, 74.6, 79.6, 103.8, 114.0, 126.2, 127.5-132.6, 138.2, 156.3, 162.9, 195.8; HRMS $[M+Na]^+$ $C_{37}H_{53}NO_{11}Na$ calcd for *m/z* 710.3516, found 710.3515.

(2*S*,3*R*,4*E*)-2-Amino-3-hydroxy-13-*O*-(4'-benzoylphenyl)-1-*O*-β-galactopyranosy1-4-tridecene (1). A mixture of compound **20** (12 mg, 0.017 mmol) and 1 M HC1 in 90% aqueous AcOH (50 μL) was stirred at rt in CH_2Cl_2 (10 mL) for 35 min. After concentration, the residue was diluted with MeOH (5 mL) and concentrated to afford the hydrochloride salt of benzophenone-linked psychosine **1** (10.2 mg, 94%): $[\alpha]^{25}_D$ -6.22° (*c* 0.45, $CHCl_3$/MeOH 4:1); R_f 0.08 ($CHCl_3$/MeOH 3:1); ^{1}H NMR ($CDCl_3$/CD_3OD 20:1) δ 1.24-1.39 (m, 10H), 1.72 (p, 2H, *J* = 6.6 Hz), 2.01 (q, 2H, *J* = 6.9 Hz), 3.27 (m, 1H), 3.35 (m, 1H), 3.48 (m, 2H), 3.66 (m, 2H), 3.74 (m, 1H), 3.81 (m, 1H), 3.87 (m, 1H), 3.95 (t, 2H, *J* = 6.6 Hz), 4.21 (m, 1H), 4.37 (m, 1H), 5.34 (dd, 1H, *J* = 5.6, 15.3 Hz), 5.73 (dt, 1H, *J* = 6.6, 14.3 Hz), 6.86 (d, 2H, *J* = 8.8 Hz), 7.35-7.96 (m, 7H); ^{13}C NMR ($CDCl_3$/CD_3OD 20:1) δ 21.8, 29.8, 32.8, 32.9, 33.1, 33.2, 33.5, 36.2, 61.6, 65.5, 69.5, 72.2, 73.0, 73.4, 74.9, 76.9, 78.9, 106.9, 117.9, 130.2, 132.1, 133.5, 133.6, 135.9, 136.6, 139.4, 142.0, 166.9, 200.3; HRMS $[M+Na]^+$ $C_{32}H_{45}NO_9Na$ calcd for *m/z* 610.2992, found 610.2997.

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Chapter 5

Part A: Synthesis of coumarin-caged sphingosine 1-phosphate and ceramide 1-phosphate analogs

Abstract

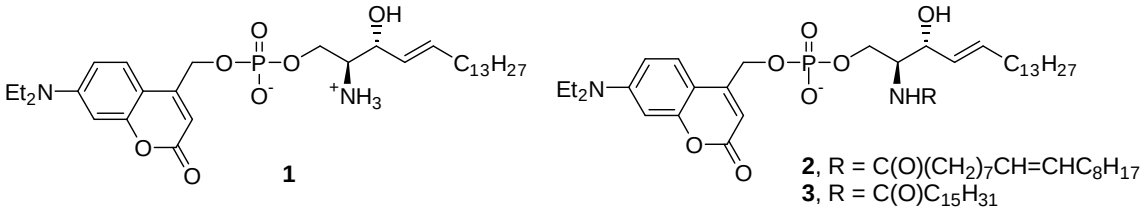
The synthesis of coumarin-caged sphingosine 1-phosphate (S1P) and ceramide 1-phosphate is reported via phosphoramidite chemistry. The sphingosine backbone was prepared using cross-metathesis. Coumarin-caged S1P in EtOH exhibited strong absorption at a wavelength of 376 nm in UV-visible spectra. Qualitative measurement of coumarin-caged S1P uncaging efficiency in 50% aqueous EtOH, pH 7.4, showed 100% uncaging after 60 min upon irradiation using a wavelength of 365 nm.

Introduction

For the analysis of biological phenomena induced by cell-impermeable molecules, "caged compounds" have been synthesized that are cell permeable.[1] In caged compounds, a photolabile group is employed to temporarily mask the ionic groups in the bioactive molecule. Caged compounds are biologically inert, but upon photolysis they release compounds with bioactivity. Typical photolabile groups used in caged compounds are 2-nitrobenzyl and its derivatives, coumarin methyl esters, bromohydroxyquinoline, nitrodibenzofuran, etc.[2] The parent bioactive molecule is generated in the cytosol on photoexcitation, usually at wavelengths >360 nm to avoid photodecomposition of endogenous biomolecules such as proteins and nucleic acids. Previously, nitrobenzyl-caged sphingosine analogs were synthesized to study the role of sphingosine in signal transduction pathways.[2b,c] The nitrobenzyl cages photolyze relatively slowly and display rather low photoefficiencies. In many recent applications, the coumarin

chromophore has been used because of its high molar absorptivity at λ >350 nm.[3] Part A of this chapter reports the first syntheses of coumarin-caged S1P (**1**, Chart 1) and ceramide 1-phosphate (C1P) analogs bearing a *N*-oleoyl or a *N*-palmitoyl chain (**2**, **3**). The syntheses started from (*S*)-Garner aldehyde and 4-methyl-7-(diethylamino)coumarin. The phosphate ester was introduced by phosphoramidite chemistry.

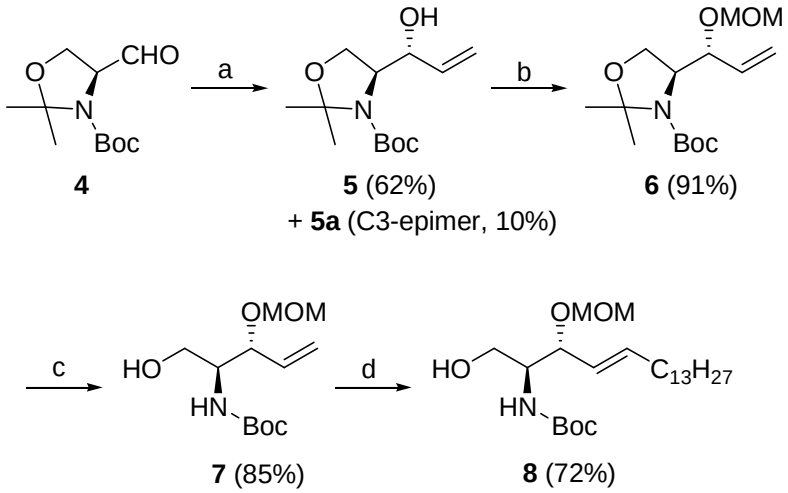
Chart 1. Structures of coumarin-caged S1P (1) and C1P (2, 3)



Results and Discussion

Synthesis of sphingosine backbone (Scheme 1). The coumarin-caged sphingosine 1-phosphate and ceramide 1-phosphate analogs were prepared from (*S*)-Garner aldehyde **4** (Scheme 1), which was treated with vinylmagnesium bromide at -78 °C to provide a mixture of erythro allyl alcohol **5** and its C3-epimer (6:1); the compounds in the mixture was separated using chromatography (hexane/EtOAc 5:1).[4] After the allylic hydroxyl group of **5** was protected as a MOM ether, the oxazolidine group of **6** was selectively deprotected using 1 M HCl at rt to afford *N*-Boc alcohol **7**. An *E*-selective cross metathesis of alcohol **7** with 1-pentadecene using Grubb's second generation catalyst (benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexyl-phosphine)ruthenium) afforded the protected D-*erythro*-sphingosine **8**; this approach was used recently to prepare the sphingosine backbone.[5]

Scheme 1. Synthesis of sphingosine backbone



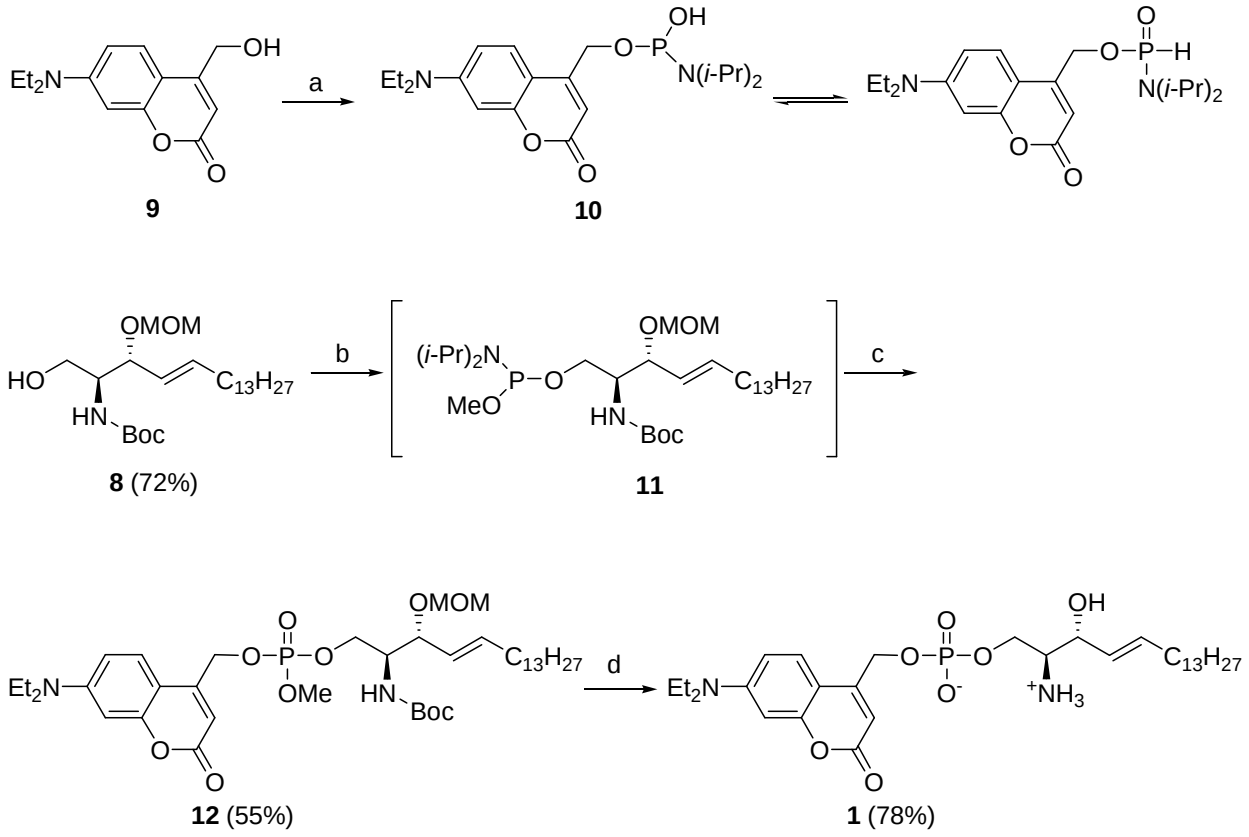
Reagents and conditions: (a) CH_2=CHMgBr, THF, -78 °C, 2 h; (b) MOMCl (2 equiv.), DIPEA (2.5 equiv.), CH_2Cl_2, 0 °C to rt; (c) 1 M HCl/THF (1:5), rt; (d) 1-pentadecene (4 equiv.), CH_2Cl_2, Grubb's second generation catalyst (3 mol%), reflux, 4 h.

Synthesis of coumarin-caged sphingosine 1-phosphate (Scheme 2). Diethylamino-4-hydroxymethylcoumarin (**9**) was prepared from 4-methyl-7-diethylaminocoumarin by a known procedure.[3d] Initially, a bis-coumarin diisopropylamino phosphitylation reagent was planned but when coumarin **9** was treated with diisopropylphosphoramidous dichloride the mono-substituted product **10** was formed predominantly. Compound **10** tautomerizes to the stable pentavalent state, as evidenced by the ^{31}P NMR signal at δ 14.8 ppm, which is not suitable for performing phosphoramidite chemistry. Since a bis-coumarin adduct was not formed, presumably because of severe steric constraints, we proceeded to prepare a mono-coumarin caged sphingosine derivative.

The protected sphingosine **8** was treated with *N*,*N*-diisopropylmethylphosphonamidic chloride in the presence of Hunig's base to provide phosphoramidite **11**, which reacted with coumarin **9** in the presence of 1*H*-tetrazole to provide a phosphite intermediate. The phosphite

was oxidized with *tert*-butyl hydroperoxide in the same pot to afford phosphate **12**. An initial attempt to deprotect both the Boc and MOM groups with trifluoroacetic acid resulted in deprotection of the Boc group in 1 h; however, only 50% deprotection of the MOM group was achieved even after stirring for 3 days at rt. An initial attempt to deprotect the methyl ester of the phosphate using TMSBr in CH_2Cl_2 resulted in the loss of the coumarin moiety from the lipid as observed by TLC. Reaction of compound **12** with 1 M HCl at 60 °C for 1 h resulted in no deprotection. Fortunately, we found that when **12** was heated in 6 M HCl/THF (2:1) at 60 °C the Boc and MOM groups were removed and the methyl phosphate ester was also hydrolyzed in a single step after 6 h to provide the target mono-coumarin caged sphingosine 1-phosphate (**1**).

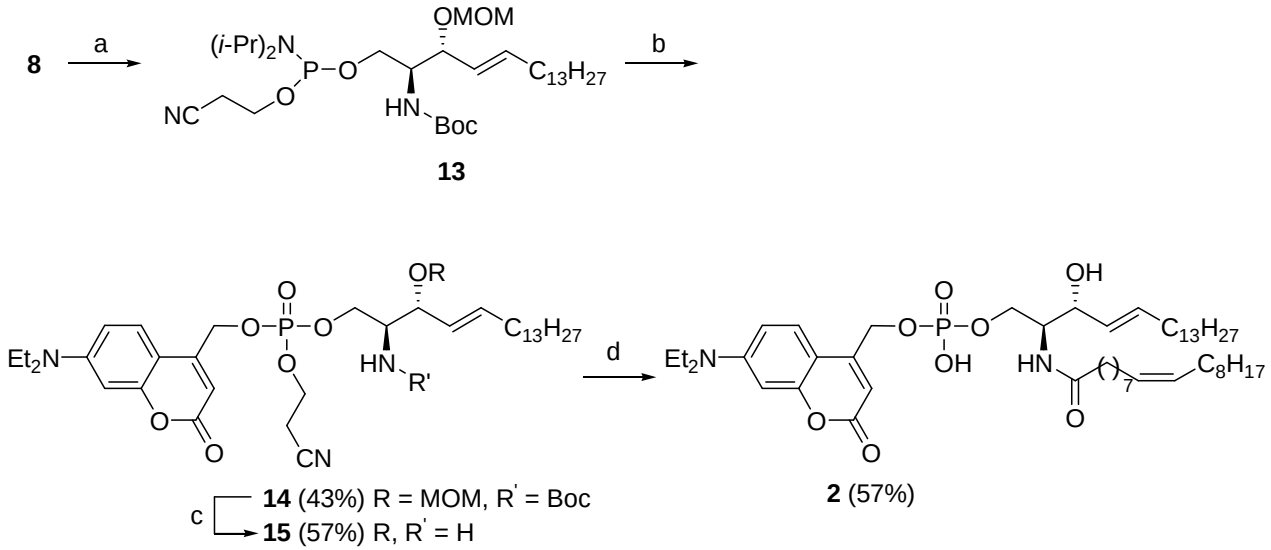
Scheme 2. Synthesis of coumarin-caged sphingosine 1-phosphate analog 1



Reagents and conditions: (a) (*i*-Pr)$_2$NPCl$_2$ (0.6 equiv.), Et$_3$N (1.2 equiv.), THF, 0 °C, 1 h; (b) (*i*-Pr)$_2$NP(OMe)Cl (2 equiv.), DIPEA (4 equiv.), THF, 0 °C, 30 min; (c) (i) **9** (2.9 equiv.), 1*H*-tetrazole (5 equiv.), CH$_3$CN, rt, 3 h, (ii) 1 M *t*-BuOOH, toluene, 0 °C to rt, 4 h; (d) 6 M HCl/THF (2:1), 60 °C, 6 h.

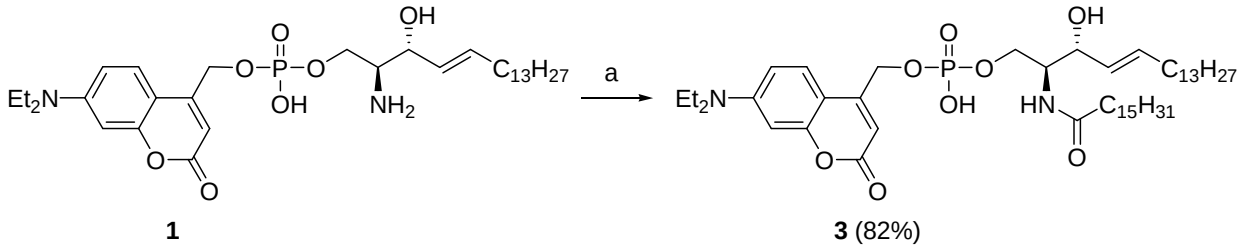
Synthesis of coumarin-caged ceramide 1-phosphate (Scheme 3). To prepare the ceramide 1-phosphate caged derivative (**2**) the protected sphingosine **8** was treated with cyanoethyl diisopropylchlorophosphoroamidite in the presence of Hunig's base to provide phosphoramidite **13**, which upon coupling with coumarin **9** using 1*H*-tetrazole, followed by oxidation, afforded phosphate **14**. The Boc and MOM groups were deprotected using 6 M HCl/THF (2:1) to provide sphingosine 1-phosphate analog **15**. Reaction of **15** with 4-nitrophenyl oleate[6] was carried out by initially neutralizing the amine with potassium carbonate, which also deprotected the cyanoethoxy group. When THF/H$_2$O (9:1) was used as the solvent, at least 4 days were required for completion of the amide formation, but with DMF/CH$_2$Cl$_2$ (5:2)[7] as the solvent mixture the coupling occurred overnight to afford the target mono-coumarin caged ceramide 1-phosphate (**2**). To prepare the palmitoyl amide, sphingosine 1-phosphate (**1**) was used as the starting material instead; compound **1** was treated with *p*-nitrophenyl palmitate in DMF/CH$_2$Cl$_2$ (5:2) for 2 days, affording ceramide analog **3** (Scheme 3B).

Scheme 3a. Synthesis of coumarin-caged ceramide 1-phosphate analog 2 from 8



Reagents and conditions: (a) $(i\text{-Pr})_2NP(OCH_2CH_2CN)Cl$, DIPEA, THF, 0 °C, 30 min; (b) (i) **9**, 1*H*-tetrazole, CH_3CN, rt, 3 h, (ii) 1 M *t*-BuOOH, toluene, 0 °C to rt, 4 h; (c) 6 M HCl/THF (2:1), 60 °C, 6 h; (d) *p*-nitrophenyl oleate, K_2CO_3, DMF/CH_2Cl_2 (5:2), overnight.

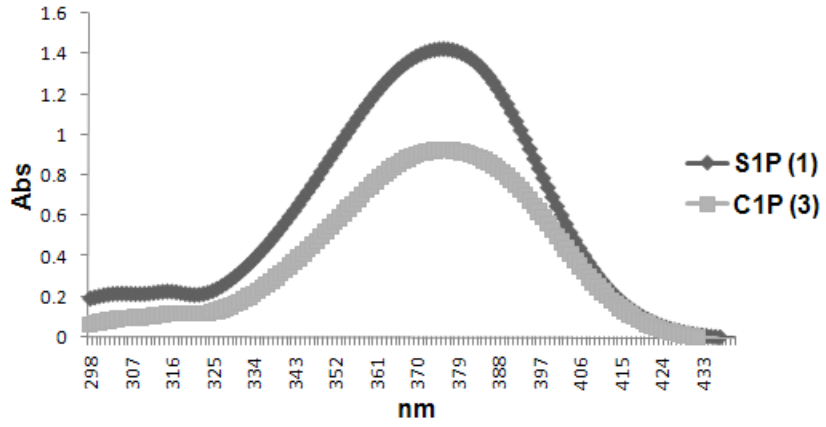
Scheme 3b. Synthesis of coumarin-caged ceramide 1-phosphate analog 3 from 1



Reagents and conditions: (a) *p*-nitrophenyl palmitate, K_2CO_3, DMF/CH_2Cl_2 (5:2), 2 d.

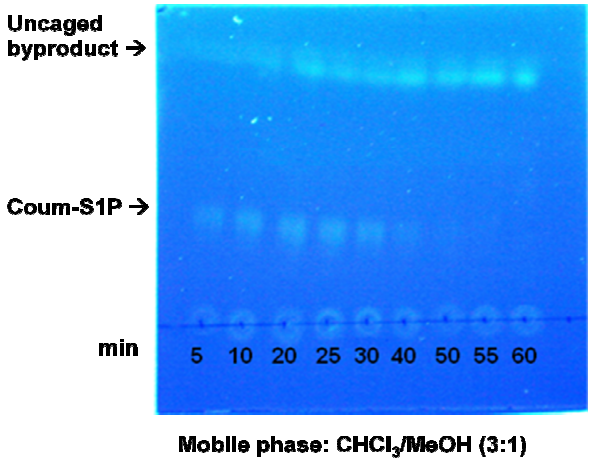
Absorption spectra of coumarin-caged S1P and C1P analogs. Figure 1 shows the UV-visible spectra of compounds **1** and **3**. Coumarin-caged S1P **1** shows a λ_{max} at 376 nm (ϵ = 15,792 $M^{-1}cm^{-1}$) in EtOH. Coumarin-caged C1P **3** shows a λ_{max} at 376 nm (ϵ = 15,466 $M^{-1}cm^{-1}$) in CH_2Cl_2.

Figure 1. Absorption spectra of coumarin-caged S1P (**1**) in EtOH and C1P (**3**) in CH_2Cl_2.



Qualitative measurement of uncaging efficiency A 100 µM solution of S1P **1** in 50% aqueous EtOH and 50% 10 mM Tris, pH 7.35, was placed in a quartz cuvette and placed at a distance of 1 cm from a conventional UV lamp (Entela UVGL-25 UV lamp). The compound was irradiated using longer wavelength (365 nm) and after a certain time, as indicated in the TLC (Figure 2), an aliquot was applied to a TLC plate, which was developed using $CHCl_3$/MeOH 3:1. Photodecomposition of 100% coumarin-caged S1P took place around 60 min.

Figure 2. Qualitative measurement of uncaging efficiency of coumarin-caged S1P (**1**) in 50% aqueous EtOH, pH 7.4.



Experimental Section

General Information. Acetonitrile was distilled over calcium hydride. Diisopropylphosphoramidous dichloride, *N*,*N*-diisopropylmethylphosphonamidic chloride and 2-cyanoethyl diisopropylchlorophosphoroamidite were purchased from Aldrich. The solvents were dried and the analyses were performed according to the general information described on page 10.

(*S*)-*tert*-Butyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (4). Refer to page 11.

(*S*)-*tert*-Butyl-4-((*R*)-1-hydroxyallyl)-2,2-dimethyloxazolidine-3-carboxylate (5). To a -78 °C solution of (*S*)-Garner aldehyde (**4**, 7.0 g, 30.5 mmol) in dry THF (50 mL) under N_2 was slowly added vinylmagnesium bromide (92 mL, 92 mmol, a 1 M solution in THF) via cannula. After the cloudy yellow solution was stirred for 2 h at -78 °C, it was warmed to 0 °C, and the reaction was quenched with saturated aqueous NH_4Cl solution (60 mL). The product was extracted with Et_2O (2 x 60 mL). The combined organic layers were washed with brine (60 mL), dried (Na_2SO_4), and concentrated. The mixture of erythro and threo diastereomers was separated by column chromatography by gravity (hexane/EtOAc 5:1) to afford **5** (4.8 g, 62%) and its C3-epimer (0.8 g, 10.2%). Compound **5**: $[\alpha]^{25}_D$ -37.7° (*c* 1.8, $CHCl_3$); R_f 0.26 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3H), 1.50 (s, 9H), 1.58 (s, 3H), 3.91 (m, 2H), 3.98 (m, 1H), 4.25 (m, 1H), 4.39 (m, 1H), 5.24 (m, 1H), 5.38 (m, 1H), 5.85 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.3, 28.3, 61.8, 64.4, 73.3, 81.4, 94.4, 116.1, 117.8, 137.6, 155.0; HRMS $[M+Na]^+$ $C_{13}H_{23}NO_4Na$ calcd for *m/z* 280.1525, found 280.1519.

***tert*-Butyl-(2*S*,3*R*)-1-hydroxy-3-(methoxymethoxy)pent-4-en-2-yl-carbamate (7).** To a solution of allylic alcohol **5** (900 mg, 3.5 mmol) in CH_2Cl_2 (25 mL) were added DIPEA (1.5 mL, 8.7 mmol) and MOMCl (0.56 mL, 7.0 mmol) at 0 °C. After 10 min, the cooling bath was

removed, and the reaction mixture was stirred overnight at rt. After TLC indicated the complete protection of the alcohol, the reaction mixture was diluted with saturated aqueous NH_4Cl solution (40 mL) and the product was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to afford the MOM-protected alcohol **6** (960 mg, 91%). Without further purification, compound **6** (960 mg, 3.2 mmol) was dissolved in THF (25 mL), and 1 M HCl (5 mL) was added. The reaction mixture was stirred at rt until the starting material disappeared (overnight). The acid was neutralized by washing with saturated aqueous $NaHCO_3$ (30 mL) and the product was extracted with CH_2Cl_2 (2 x 25 mL). The organic layer was purified by chromatography (hexane/EtOAc 3:1) to afford alcohol **7** (705 mg, 85%): R_f 0.20 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.21 (br s, 1H), 3.39 (s, 3H), 3.68 (m, 2H), 3.88 (dd, 1H, *J* = 4.2, 11.6 Hz), 4.27 (t, 1H, *J* = 5.0 Hz), 4.57 (d, 1H, *J* = 6.6 Hz), 4.65 (d, 1H, *J* = 6.6 Hz), 5.32 (m, 3H), 5.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.9, 54.6, 55.4, 62.0, 76.0, 78.3, 79.4, 94.0, 119.0, 134.7, 156.3; HRMS $[M+Na]^+$ $C_{12}H_{23}NO_5Na$ calcd for *m/z* 284.1474, found 284.1468.

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-hydroxy-3-(methoxymethoxy)octadec-4-en-2-yl-carbamate (8).** To a solution of alcohol **7** (230 mg, 0.88 mmol) in CH_2Cl_2 (8 mL) was added 1-pentadecene (741 mg, 3.52 mmol). The solution was degassed twice using N_2 (2 x 10 min). To the reaction mixture was added benzylidene[1,3-bis(2,4,6-trimethyl-phenyl)-2-imidazolidinylidene]dichloro-(tricyclohexylphosphine)ruthenium catalyst (22.4 mg, 0.026 mmol) at rt under N_2. After the reaction mixture was stirred for 4 h under reflux, the solvent was removed and the product was purified by chromatography (hexane/EtOAc 4:1) to afford **8** (280 mg, 72%): R_f 0.45 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 7.0 Hz), 1.25 (m, 24H), 1.44 (m, 9H), 2.06 (q, 2H, *J* = 7.7 Hz), 2.89 (d, 1H, *J* = 5.2 Hz), 3.38 (s, 3H), 3.67 (m, 2H), 3.94 (dt, 1H, *J* =

3.2, 11.1 Hz), 4.22 (dd, 1H, *J* = 5.0, 7.6 Hz), 4.53 (d, 1H, *J* = 6.6 Hz), 4.67 (d, 1H, *J* = 6.6 Hz), 5.26 (d, 1H, *J* = 7.6 Hz), 5.36 (dd, 1H, *J* = 8.0, 15.4 Hz), 5.75 (dt, 1H, *J* = 6.6, 14.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.4, 29.0, 29.1, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 54.9, 55.6, 62.3, 76.1, 78.3, 79.4, 93.8, 125.9, 136.9, 155.9; HRMS $[M+Na]^+$ $C_{25}H_{49}NO_5Na$ calcd for *m/z* 466.3508, found 466.3514.

7-Diethylaminocoumarin-4-yl-methoxy *N,N*-Diisopropylphosphonamidate (10). Diethylamino-4-hydroxymethylcoumarin (**9**) was prepared from 4-methyl-7-diethylaminocoumarin by a known procedure.[3d] To a solution of coumarin **9** (60 mg, 0.243 mmol) in THF (5 mL) was added Et_3N (41 μL, 0.29 mmol). Diisopropylphosphoramidous dichloride (27 μL, 0.145 mmol) was then added slowly to the reaction mixture at 0 °C under N_2. After the reaction mixture was stirred at 0 °C for 1 h, H_2O (10 mL) was added, and the product was extracted with EtOAc (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated, and the residue was purified by flash chromatography ($CHCl_3$/MeOH 9:1) to afford *H*-phosphonate **10** (50 mg, 55%): R_f 0.43 (EtOAc);^{1}H NMR ($CDCl_3$) δ 1.20 (t, 6H, *J* = 7.1 Hz), 1.29 (dd, 12H, *J* = 4.1, 6.8 Hz), 3.42 (q, 4H, *J* = 7.1 Hz), 3.56 (m, 1H), 5.14 (m, 2H), 6.20 (s, 1H), 6.26 (s, 1H), 6.51 (d, 1H, *J* = 2.5 Hz), 6.58 (dd, 1H, *J* = 2.6, 9.0 Hz), 7.29 (d, 1H, *J* = 9.0 Hz), 7.85 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.4, 23.0, 44.8, 45.4, 60.7, 97.7, 105.7, 105.9, 108.6, 124.2, 150.4, 150.6, 156.2, 162.0; ^{31}P NMR ($CDCl_3$) δ 14.8; HRMS $[M+H]^+$ $C_{20}H_{32}N_2O_4P$ calcd for *m/z* 395.2099, found 395.2099.

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-[7-diethylaminocoumarin-4-yl-methoxy(methoxy)phosphor-yloxy]-3-(methoxymethoxy)octadec-4-en-2-yl-carbamate (12):** To a solution of compound **8** (55 mg, 0.124 mmol) in THF (8 mL) was added DIPEA (86.4 μL, 0.49 mmol). Methyl diisopropylphosphoramidochloridite (48 μL, 0.24 mmol) was added dropwise to the reaction

mixture at 0 °C under N_2. After the reaction mixture was stirred at the same temperature for 30 min, H_2O (10 mL) was added, and the product was extracted with EtOAc (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated, and the residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford phosphoramidite **11**: R_f 0.68 (hexane/EtOAc 3:1); ^{31}P NMR ($CDCl_3$) δ 140.8. A mixture of coumarin **9** (71 mg, 0.28 mmol) and phosphoramidite **11** (58 mg, 0.096 mmol) was dried by lyophilization from benzene, and the residue was dissolved in CH_3CN (8 mL). The resulting solution was transferred by cannula to a solution of 1*H*-tetrazole (34 mg, 0.48 mmol) in CH_3CN (3 mL) at rt. After 3 h, the consumption of the phosphoramidite was complete as observed by TLC (hexane/EtOAc 3:1). To the intermediate phosphite, *t*-BuOOH (0.14 mL, a 1 M solution in toluene, 0.14 mmol) was added at 0 °C, and the reaction mixture was warmed to rt. After 4 h, TLC indicated the complete conversion of the phosphite to the corresponding phosphate. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ solution (15 mL). The organic layer was dried (Na_2SO_4), concentrated, and the residue was purified by chromatography (hexane/EtOAc 2:1) to afford phosphate **12** (52 mg, 55%): $[\alpha]^{25}_D$ -26.5° (*c* 0.6, $CHCl_3$); R_f 0.22 (EtOAc/hexane/Et_3N 48:50:2); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.20 (t, 6H, *J* = 7.1 Hz), 1.25 (m, 23 H), 1.41 (s, 9H), 2.03 (q, 2H, *J* = 6.5 Hz), 3.35 (s, 3H), 3.42 (q, 4H, *J* = 7.1 Hz), 3.84 (d, 3H, *J* = 10.5 Hz), 3.91 (br s, 1H), 4.09 (t, 1H, *J* = 7.2 Hz), 4.25 (m, 1H), 4.31 (m, 1H), 4.50 (d, 1H, *J* = 6.6 Hz), 4.69 (d, 1H, *J* = 6.6 Hz), 4.95 (t, 1H, *J* = 6.4 Hz), 5.20 (m, 2H), 5.29 (dd, 1H, *J* = 8.4, 15.0 Hz), 5.75 (dt, 1H, *J* = 5.9, 14.1 Hz), 6.21 (s, 1H), 6.50 (d, 1H, *J* = 2.4 Hz), 6.58 (dd, 1H, *J* = 2.5, 9.0 Hz), 7.29 (d, 1H, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 12.4, 14.1, 22.7, 28.3, 29.0, 29.2, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 44.7, 54.7, 54.8, 55.7, 64.6, 67.0, 76.2, 79.6, 93.5, 97.8, 105.5, 106.3,

108.7, 124.3, 125.6, 138.1, 149.1, 150.7, 155.4, 156.2, 161.7; ^{31}P NMR ($CDCl_3$) δ 0.43; HRMS $[M+Na]^+$ $C_{40}H_{67}N_2O_{10}PNa$ calcd for *m/z* 789.4431, found 789.4417.

(2*S*,3*R*,4*E*)-2-Amino-3-hydroxyoctadec-4-enyl (7-Diethylaminocoumarin-4-yl) methyl Phosphate (1). To a solution of phosphate **12** (25 mg, 0.032 mmol) in THF (1 mL) was added 6 M HCl (2 mL). The reaction mixture was stirred at 60 °C for 6 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and brine (10 mL). The product was extracted with CH_2Cl_2 (3 x 15 mL), dried (K_2CO_3), and purified by chromatography ($CHCl_3$/MeOH 6:1). After removal of suspended silica gel by filtration of a solution of **1** in $CHCl_3$ through a Cameo filter, compound **1** was obtained (15.5 mg, 78%): $[\alpha]^{25}_D$ +2.22° (*c* 0.6, $CHCl_3$/MeOH 1:1); R_f 0.70 ($CHCl_3$/MeOH/H_2O 65:35:8); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, *J* = 5.5 Hz), 1.23 (m, 26H), 1.48 (m, 2H), 1.98 (q, 2H, *J* = 5.9 Hz), 3.20-3.79 (m, 9H), 4.15 (m, 2H), 4.41 (m, 1H), 5.07 (m, 2H), 5.40 (dd, 1H, *J* = 5.4, 15.2 Hz), 5.81 (dt, 1H, *J* = 5.6, 13.4 Hz), 6.26 (s, 1H), 6.41 (s, 1H), 6.51 (d, 1H, *J* = 8.0 Hz), 7.29 (d, 1H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 12.3, 14.0, 22.6, 28.2, 29.0, 29.3, 29.4, 29.6, 31.8, 32.3, 44.6, 55.8, 62.4, 63.1, 69.6, 97.3, 104.7, 108.8, 124.4, 126.2, 135.5, 150.6, 155.8, 163.0; ^{31}P NMR ($CDCl_3$) δ -0.38; HRMS $[M+H]^+$ $C_{32}H_{54}N_2O_7P$ calcd for *m/z* 609.3669, found 609.3670; UV: λ_{max} 376 nm (ϵ = 15792) in EtOH (90 μM).

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-[7-diethylaminocoumarin-4-yl-methoxy(2'-cyanoethoxy)-phosphoryloxy]-3-(methoxymethoxy)-octadec-4-en-2-yl-carbamate (14).** Phosphoramidite **13**, which was prepared by treating alcohol **8** (31 mg, 70 μmol) with 2-cyanoethyl diisopropylchloro-phosphoroamidite (47 μL, 0.21 mmol) and DIPEA (37 μL, 0.21 mmol) in THF (5 mL), was treated with coumarin **9** according to the procedure used for compound **12**. The resulting phosphite was oxidized using *t*-BuOOH and purified by chromatography (hexane/EtOAc 2:1) to afford phosphate **14** (24 mg, 43%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* =

6.4 Hz), 1.18-1.43 (m, 39H), 2.09 (q, 2H, *J* = 6.6 Hz), 2.78 (m, 2H), 3.36 (s, 3H), 3.44 (q, 4H, *J* = 7.1 Hz), 3.90 (br s, 1H), 4.05-4.38 (m, 5H), 4.49 (d, 1H, *J* = 6.4 Hz), 4.69 (d, 1H, *J* = 6.6 Hz), 4.95 (d, 1H, *J* = 8.9 Hz), 5.23 (m, 2H), 5.30 (dd, 1H, *J* = 8.5, 15.0 Hz), 5.78 (dt, 1H, *J* = 6.6, 15.2 Hz), 6.18 (s, 1H), 6.51 (d, 1H, *J* = 2.5 Hz), 6.58 (dd, 1H, *J* = 2.5, 9.0 Hz), 7.29 (d, 1H, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 16.0, 19.6, 22.7, 28.3, 29.0, 29.2, 29.3, 29.4, 29.6, 29.7, 31.9, 32.3, 44.7, 53.8, 55.7, 61.7, 62.3, 64.6, 66.8, 76.2, 79.5, 93.6, 97.8, 105.4, 106.4, 108.7, 116.3, 124.3, 125.7, 138.0, 148.7, 150.8, 155.3, 156.3, 161.6; ^{31}P NMR ($CDCl_3$) δ -1.2; HRMS $[M+Na]^+$ $C_{42}H_{68}N_3O_{10}PNa$ calcd for *m/z* 828.4540, found 828.4538.

(2*S*,3*R*,4*E*)-2-Amino-3-hydroxyoctadec-4-enyl (7-Diethylamino-coumarin-4-yl) methyl 2'-Cyanoethyl Phosphate (15). The MOM and Boc groups of phosphate **14** (20 mg, 0.025 mmol) were removed according to the procedure used to prepare compound **1** to afford cyanoethyl-protected intermediate **15** (9.4 mg, 57%): R_f 0.28 ($CHCl_3$/MeOH 9:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.21 (t, 6H, *J* = 7.1 Hz), 1.25 (m, 23H), 1.80 (br s, 2H), 2.05 (q, 2H, *J* = 6.8 Hz), 2.81 (t, 2H, *J* = 6.1 Hz), 3.04 (br s, 1H), 3.42 (q, 4H, *J* = 7.1 Hz), 4.02 (t, 1H, *J* = 6.5 Hz), 4.15-4.38 (m, 5H), 5.25 (d, 2H, *J* = 7.4 Hz), 5.43 (dd, 1H, *J* = 7.3, 15.4 Hz), 5.78 (dt, 1H, *J* = 6.7, 14.7 Hz), 6.21 (s, 1H), 6.51 (d, 1H, *J* = 2.5 Hz), 6.59 (dd, 1H, *J* = 2.5, 9.0 Hz), 7.27 (d, 1H, *J* = 9.0 Hz); ^{31}P NMR ($CDCl_3$) δ -0.92.

(2*S*,3*R*,4*E*)-2-Oleamido-3-hydroxyoctadec-4-enyl (7-Diethylaminocoumarin-4-yl)methyl Phosphate (2). A solution of compound **15** (4.0 mg, 6.0 μmol), *p*-nitrophenyl oleate **16** (10 mg, 0.024 mmol), and anhydrous potassium carbonate (3.0 mg, 0.021 mmol) was suspended in a solution of anhydrous DMF (5 mL) and CH_2Cl_2 (2 mL). After the reaction mixture had stirred overnight, the mixture was concentrated under high vacuum. The product was purified by chromatography ($CHCl_3$/MeOH 9:1), followed by removal of suspended silica

gel by filtration of a solution of **2** in $CHCl_3$ through a Cameo filter, affording coumarin-caged ceramide 1-phosphate **2** (3.0 mg, 57%) as a pale yellow wax: R_f 0.79 ($CHCl_3$/MeOH 2:1); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, *J* = 6.5 Hz), 1.19 (t, 6H, *J* = 7.1 Hz), 1.21-1.59 (m, 46H), 1.98 (m, 4H), 2.16 (t, 2H, *J* = 7.6 Hz), 3.43 (m, 4H), 3.86 (m, 1H), 4.00 (m, 1H), 4.12 (m, 1H), 4.26 (m, 1H), 5.04 (m, 2H), 5.33 (m, 2H), 5.45 (dd, 1H, *J* = 7.4, 15.5 Hz), 5.71 (dt, 1H, *J* = 6.7, 14.5 Hz), 6.27 (s, 1H), 6.48 (d, 1H *J* = 2.2 Hz), 6.56 (dd, 1H, *J* = 2.2, 9.0 Hz), 7.28 (d, 1H, *J* = 9.0 Hz); HRMS $(M+H)^+$ $C_{50}H_{86}N_2O_8P$ calcd for *m/z* 873.6122, found 873.6125.

(2*S*,3*R*,4*E*)-2-Palmitamido-3-hydroxyoctadec-4-enyl (7-Diethylaminocoumarin-4-yl)methyl Phosphate (3). Compound **1** (7.0 mg, 0.010 mmol) was *N*-acylated according to the procedure used to prepare compound **2**, affording ceramide **3** (7.0 mg, 82%) as a yellow solid: R_f 0.60 ($CHCl_3$/MeOH 2:1); $[\alpha]^{25}_D$ +4.73° (*c* 0.27, $CHCl_3$:MeOH 1:1); R_f 0.62 ($CHCl_3$/MeOH 2:1); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, *J* = 6.5 Hz), 1.16-1.53 (m, H), 1.96 (q, 2H *J* = 6.9 Hz), 2.13 (t, 1H, *J* = 7.3 Hz), 3.38 (m, 4H), 3.88 (m, 1H), 4.01 (m, 1H), 4.12 (t, 1H, *J* = 7.2 Hz), 4.26 (m, 1H), 5.04 (m, 2H), 5.44 (dd, 1H, *J* = 7.2, 14.8 Hz), 5.70 (dt, 1H, *J* = 6.3, 14.1 Hz), 6.28 (s, 1H), 6.43 (s, 1H), 6.54 (d, 1H, *J* = 8.0 Hz), 7.27 (d, 1H, *J* = 8.3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.6, 22.9, 23.6, 25.8, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 30.2, 31.9, 32.4, 36.4, 38.6, 44.6, 68.2, 97.4, 105.8, 108.8, 128.7, 130.9, 132.2, 134.7, 150.6, 155.8, 163.3, 167.9, 174.2; ^{31}P NMR ($CDCl_3$) δ -0.83; HRMS $(M+H)^+$ $C_{48}H_{84}N_2O_8P$ calcd for *m/z* 847.5965, found 847.5973; UV: λ_{max} 376 nm (ϵ = 15466) in EtOH (60 μM).

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Chapter 5

Part B: Synthesis of 3-bromo-4-hydroxynitrophenyl benzyl-caged sphingosine 1-phosphate and ceramide 1-phosphate analogs

Abstract

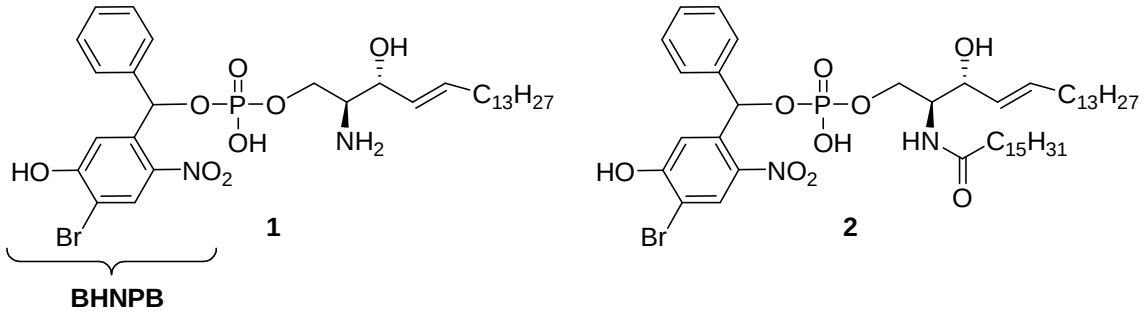
The synthesis of 3-bromo-4-hydroxynitrophenyl benzyl-caged sphingosine 1-phosphate (BHNPB-caged S1P) and ceramide 1-phosphate analogs is reported. BHNPB-caged S1P in 50% aqueous EtOH, pH 7.4, exhibited strong absorption at a wavelength of 406 nm in UV-visible spectra and was also stable in the dark. Qualitative measurement of BHNPB-caged S1P uncaging efficiency in 50% aqueous EtOH, pH 7.4, showed 100% uncaging after 65 min upon irradiation using a wavelength of 365 nm.

Introduction

The most prevalent cage employed to mask the activity of many bioactive molecules is the 2-nitrobenzyl group and its derivatives. Several modifications of the nitrobenzyl cage have been made to improve the kinetics of release and absorptivity of the molecule at longer wavelengths. [1,2] A bromohydroxyquinoline cage has been released by one-photon excitation as well as two-photon excitation (2PE).[3] Recently, 2PE has emerged as a useful method because it uses infrared wavelengths for photoexcitation.[4] In an attempt to increase the photolytic efficiency and sensitivity to 2PE, we sought to incorporate bromo and hydroxy groups into the classic nitrobenzyl cage along with a phenyl group at the benzylic position by synthesizing a 3-bromo-4-hydroxynitrophenyl benzyl alcohol (BHNPB, Chart 1). Incorporating the phenyl group at the benzylic position creates one more chiral center, which leads to a diastereomeric mixture of

caged products. Incorporation of a hydroxy group into the cage can also increase the solubility of the analog in water at physiological pH and enhance its molar absorptivity. We used this novel cage to prepare sphingosine 1-phosphate (S1P) and ceramide 1-phosphate (C1P) caged analogs (**1**, **2**, and **3**).

Chart 1. Structures of BHNPB-caged S1P (1) and C1P (2)

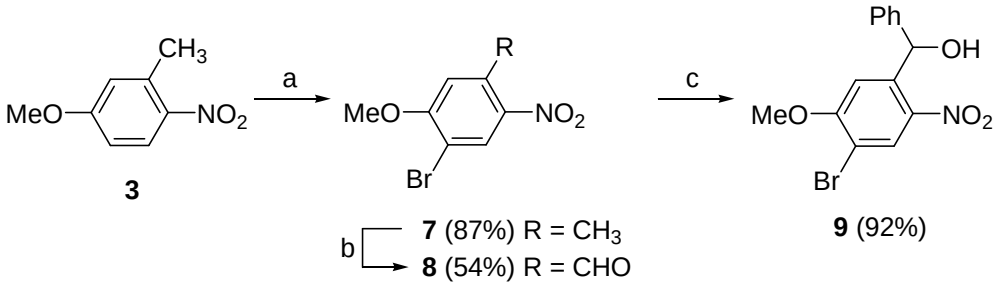


Results and Discussion

Synthesis of (4-bromo-5-methoxy-2-nitrophenyl)(phenyl)methanol 9 (Scheme 1). The BHNPB cage was prepared from 4-methoxy-2-methyl-1-nitrobenzene (**3**, Scheme 1). Oxidation of a benzylic carbon-containing substituent ortho to a nitro group was reported using DMF dimethyl acetal, followed by oxidation of the intermediate enamine with $NaIO_4$, to afford an aldehyde.[5] This procedure was adopted to oxidize compound **3** to aldehyde **4**.[6] Addition of PhMgBr to aldehyde **4** followed by attempted bromination of alcohol **5** with NBS resulted in undesired oxidation of the secondary alcohol, affording benzophenone **6** as evident from a peak at δ 193.2 ppm characteristic of a benzophenone carbonyl group (see page 96).[6] Therefore, we changed the strategy by heating nitrobenzene **3** at reflux in acetonitrile with NBS overnight (Scheme 1). However, after purification we still observed a substantial amount of the starting material along with the product. Hence, a pressure tube was used for this reaction; heating at 140 °C overnight resulted in a mixture of the desired brominated product **7** along with other

unseparable byproducts, but the starting material was completely consumed as observed by NMR. The byproducts were removed by chromatography after oxidation of the methyl group with $NaIO_4$ to afford aldehyde **8**. An addition reaction of aldehyde **8** with PhMgBr afforded the desired 3-bromo-4-methoxy-nitrophenyl benzyl alcohol (**9**). A reaction time of 30 min at -78 °C is critical for this addition reaction, as decomposition of compound **9** was observed after 30 min.

Scheme 1. Synthesis of (4-bromo-5-methoxy-2-nitrophenyl)(phenyl)methanol 9

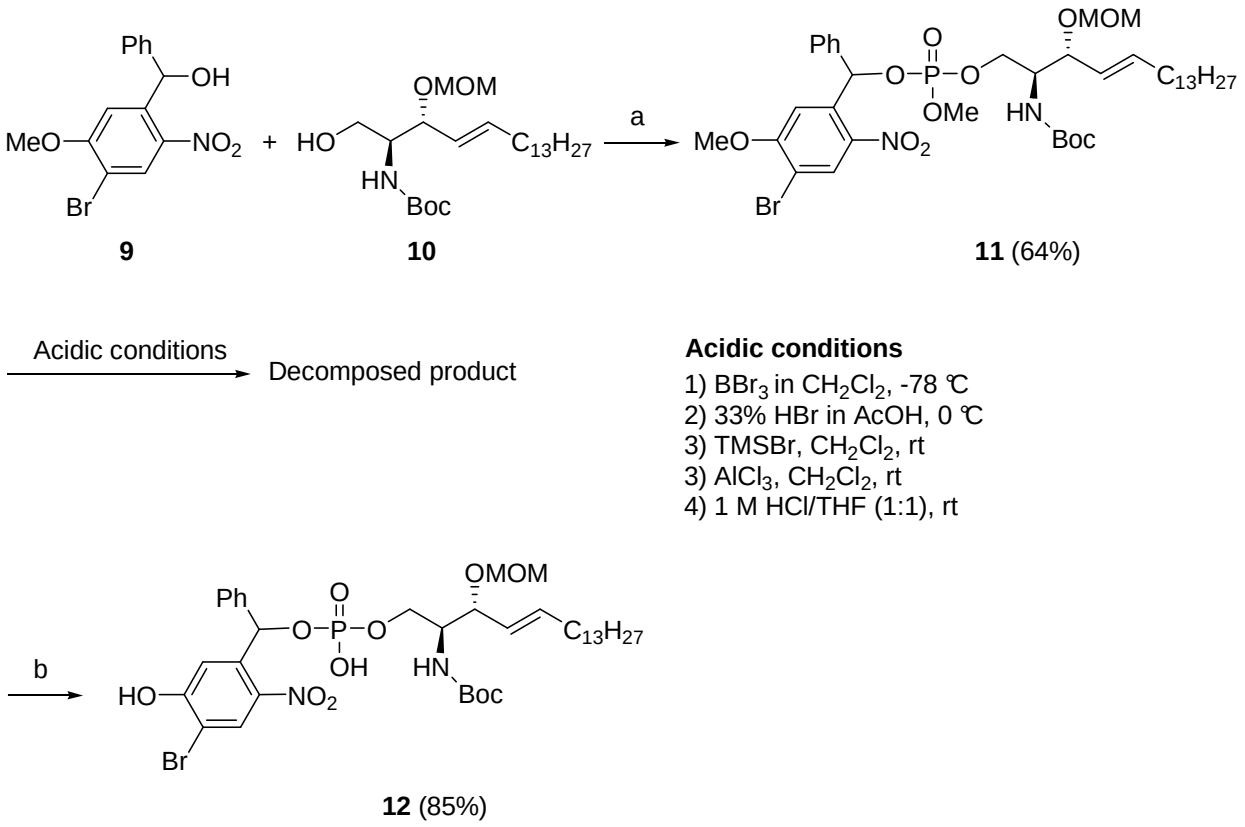


Reagents and conditions: (a) NBS, CH_3CN, pressure tube, 140 °C, overnight; (b) (i) DMF-DMA, DMF, 140 °C, 16 h, (ii) $NaIO_4$ (3.6 equiv.), H_2O/DMF (4:1), 0 °C to rt, 8 h; (c) PhMgBr, THF, -78 °C, 30 min.

Attempted synthesis of BHNPB-caged sphingosine 1-phosphate (Scheme 2). Phosphoramidite chemistry was applied to couple compound **10**, used in the coumarin project, with compound **9**. The phosphoramidite reaction afforded compound **11** in a good yield, but unfortunately the acidic conditions used for the attempted deprotection resulted in cleavage of the cage from the lipid moiety. However, the anisole methyl group and the methyl phosphate ester of compound **11** were deprotected in a single step using LiI (20 equiv.) in refluxing pyridine overnight, affording demethylated product **12** (R_f 0.63, $CHCl_3$/MeOH 3:1). Compound **12** was tested for stability under acidic conditions by dissolving the compound in 3 M HCl/MeOH/CH_2Cl_2 (1:2:1) and stirring overnight at rt. Fortunately, there was no observation of

decomposition of **12** but a new polar spot was observed by TLC (R_f 0.28, $CHCl_3$/MeOH 3:1), which could arise from Boc group deprotection along with unreacted **12**.

Scheme 2. Attempted synthesis of BHNPB-caged sphingosine 1-phosphate 12

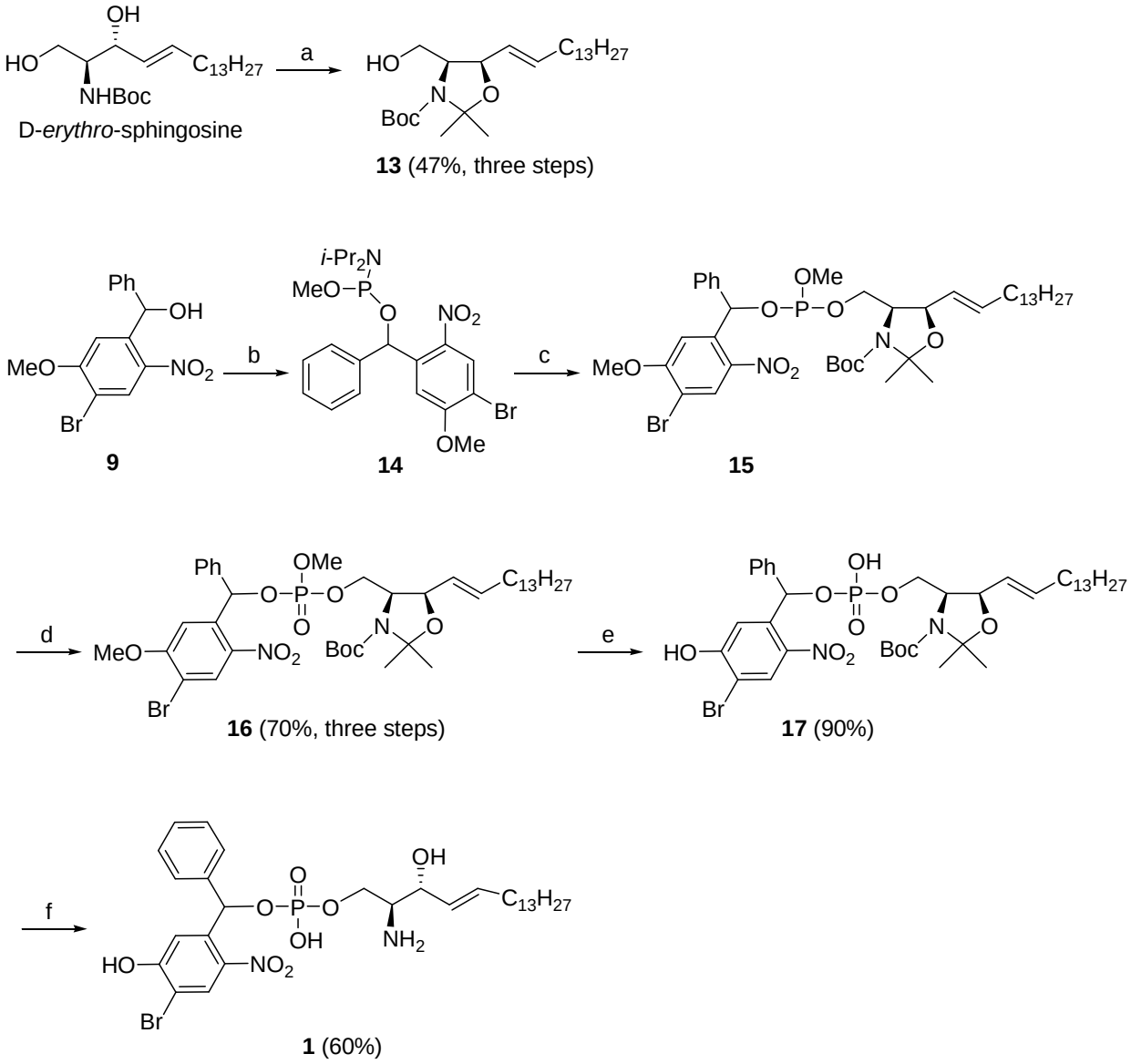


Reagents and conditions: (a) (i) $(i\text{-Pr})_2$NP(OMe)Cl (1.5 equiv.), DIPEA (2 equiv.), THF, rt, 3 h, (ii) **10** (2 equiv.), 1*H*-tetrazole (3 equiv.), CH_3CN, rt, 3 h, (iii) 1 M *t*-BuOOH, toluene, rt, 1 h; (b) LiI (20 equiv.), pyridine, reflux, overnight.

Synthesis of BHNPB-caged sphingosine 1-phosphate analog 1 (Scheme 3). Considering the stability of compound **12** under mild acidic conditions and anticipating the harsh conditions required to deprotect a MOM group, we decided to use a mild acid-sensitive isopropylidene protecting group. Thus we prepared compound **13**[1c] (Scheme 3) from D-*erythro*-sphingosine in four steps. After the amino group of sphingosine was protected as a carbamate, the primary alcohol was selectively protected as a TBDPS ether. Reaction of the resulting

intermediate with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-TsOH afforded a N,O-isopropylidene acetal, and deprotection of the silyl group using TBAF afforded protected-sphingosine **13**. Alcohol **9** reacted with *N*,*N*-diisopropylmethylphosphonamidic chloride, and the resulting intermediate **14** was purified by flash chromatography. Phosphoramidite **14** was coupled with alcohol **13** in the presence of 1*H*-tetrazole to afford phosphite **15**. Compound **15** was purified by flash chromatography to overcome purification problems after oxidation due to co-elution of the resulting phosphate with excess alcohol **13**. Phosphite **15** was oxidized with *tert*-butyl hydroperoxide to afford phosphate **16** in 70% over three steps. The product is a diastereomeric mixture because of the generation of two more chiral centers as evident from the ^{31}P NMR spectrum of **16** (which shows four peaks). By employing the deprotection strategy used in Scheme 2, phosphate **16** was converted to compound **17** using LiI in refluxing pyridine. Intermediate **17** was first treated with 3 M HCl/MeOH/CH_2Cl_2 (1:2:1) at rt overnight and then heated at reflux for an additional 2 h to afford BHNPB caged S1P **1**.

Scheme 3. Synthesis of BHNPB-caged sphingosine 1-phosphate analog 1

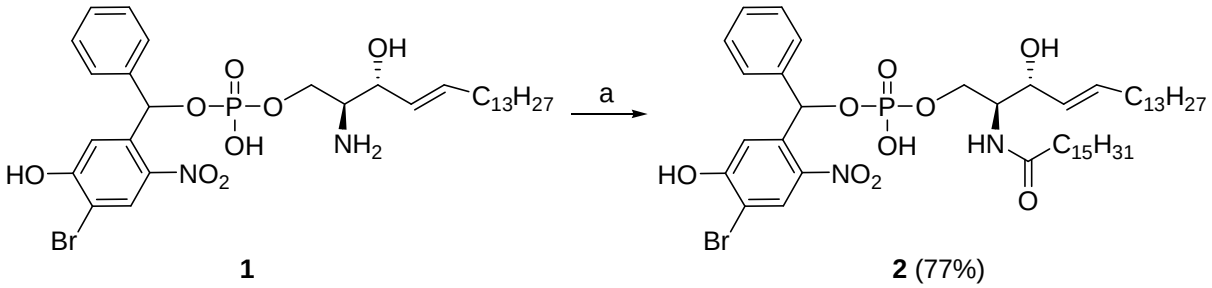


Reagents and conditions: (a) (i) Boc_2O, 1 N NaOH, EtOH/H_2O (2:1), (ii) TBDPSCl, imidazole (4 equiv.), CH_2Cl_2, 3 h, (iii) 2,2-dimethoxypropane, *p*-TsOH·H_2O (cat.), benzene, 30 min, (iv) TBAF, THF, 6 h, rt; (b) *i*-Pr_2NP(OMe)Cl, DIPEA, THF, rt, 3 h; (c) **13**, 1*H*-tetrazole, CH_3CN, rt, 3 h; (d) 1 M *t*-BuOOH in toluene, CH_3CN, rt, 1 h; (e) LiI (20 equiv.), pyridine, reflux, overnight; (f) 3 M HCl/MeOH/CH_2Cl_2 (1:2:1), rt, overnight and gentle reflux, (50-60 °C), 2 h.

Synthesis of BHNPB-caged ceramide 1-phosphate analog 2 from 1 (Scheme 4).

Compound **1** was treated with *p*-nitrophenyl palmitate in DMF/CH_2Cl_2 (5:2) using K_2CO_3 as base. Stirring the reaction mixture for 2 days afforded ceramide analog **2**.

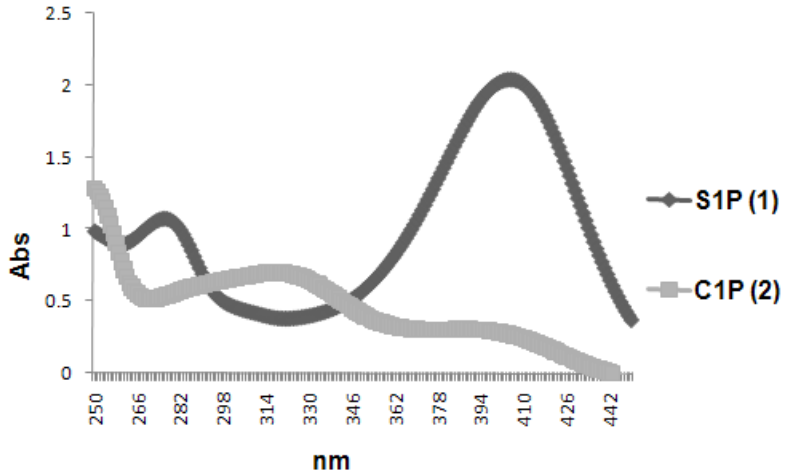
Scheme 4. Synthesis of BHNPB-caged ceramide 1-phosphate analog 2 from 1



Reagents and conditions: (a) *p*-nitrophenyl palmitate, K_2CO_3, DMF/CH_2Cl_2 (5:2), 2 d.

Absorption spectra of BHNPB-caged S1P and C1P analogs. Figure 1 shows the UV-visible spectra of compounds **1** and **2**. Caged S1P **1** absorbs at 406 nm (ϵ = 12000 $M^{-1}cm^{-1}$) and 278 nm (ϵ = 6294 $M^{-1}cm^{-1}$) in 50% aqueous EtOH, 50% 10 mM Tris, pH 7.35. Caged C1P **2** absorbs at 397 nm (ϵ = 1747 $M^{-1}cm^{-1}$) and 320 nm (ϵ = 4111 $M^{-1}cm^{-1}$) in EtOH.

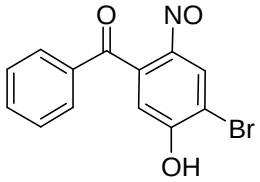
Figure 1. Absorption spectra of BHNPB-caged S1P (**1**) in 50% aqueous EtOH, pH 7.4, and C1P (**2**) in EtOH.



Stability of caged S1P in buffer in the dark. The stability of BHNPB-caged S1P (**1**) in 50% aqueous EtOH, 50% 10 mM Tris (170 μM), pH 7.35, was tested by withdrawing aliquots from the solution over a period of seven days. The analysis showed no change in the R_f value (0.35 $CHCl_3$/MeOH 3:1) of the product, and no new spot was observed.

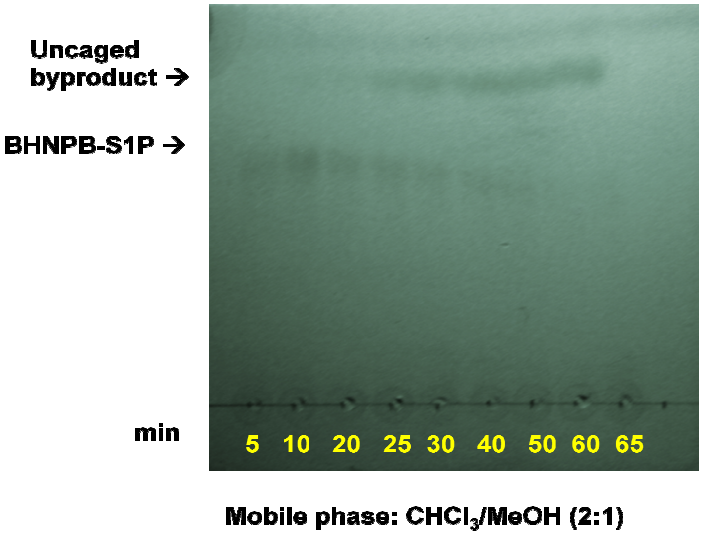
Qualitative measurement of uncaging efficiency. A 100 μM solution of BHNPB-caged S1P (**1**) in 50% aqueous EtOH and 50% 10 mM Tris, pH 7.35, was placed in a quartz cuvette and irradiated at 365 nm at a distance of 1 cm with a conventional UV lamp (Entela UVGL-25 UV lamp). Aliquots were withdrawn at various times and analyzed by TLC (developed using $CHCl_3$/MeOH 2:1). The mechanism of photolysis of 2-nitrobenzyl caged compounds was thoroughly studied and is known to generate 2-nitrosobenzaldehyde as the photo byproduct of the reaction.[7] Similarly, our BHNPB uncaging experiment is expected to generate a 2-nitrosobenzophenone derivative as the byproduct (Figure 2). After being photoirradiated for 65 min, the sample in the cuvette was concentrated and analyzed by HRMS. The result shows a strong peak at m/z 327.9596 (M+Na), which is the expected mass of the photo byproduct. Figure 3 shows that 100% photodecomposition of BHNPB-S1P took place after 65 min.

Figure 2. Uncaged photo-byproduct, 4-bromo-5-hydroxy-2-nitroso-benzophenone.



HRMS $[M+Na]^+$ $C_{13}H_8{}^{79}BrNO_3Na$ calcd for *m/z* 327.9585, found 327.9596

Figure 3. Qualitative measurement of uncaging efficiency of BHNPB-caged S1P (**1**) in 50% aqueous EtOH, pH 7.4.



Experimental Section

General Information. Pyridine was distilled over calcium hydride. The solvents were dried and the analyses were performed according to the general information described on pages 10 and 72.

5-Methoxy-2-nitrobenzaldehyde (4). To a stirred solution of 4-methoxy-2-methyl-1-nitrobenzene **3** (658 mg, 3.94 mmol) in dry DMF (5 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (DMF·DMA) (1.5 mL, 11.82 mmol). After the reaction mixture was heated at 140 °C for 16 h, the dark red solution was cooled to 0 °C and added rapidly to a stirred solution of $NaIO_4$ (3.0 g, 14.2 mmol) in H_2O/DMF (4:1, 10 mL) at 0 °C. After 8 h of stirring, the brown solution was filtered, rinsed the reaction flask with toluene/EtOAc (1:1, 30 mL) and again filtered. The filtrate was washed with saturated aqueous NaCl solution (45 mL) and extracted with EtOAc (2 x 25 mL). The organic phase was dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford **4** (660 mg, 92%): R_f

0.25 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3H), 7.16 (dd, 1H, *J* = 2.9, 9.0 Hz), 7.34 (d, 1H, *J* = 2.9 Hz), 8.19 (d, 1H, *J* = 9.0 Hz), 10.49 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.4, 113.3, 118.6, 127.3, 129.2, 134.4, 164.0, 188.6; HRMS $[M-H]^-$ $C_8H_6NO_4$ calcd for *m/z* 180.0296, found 180.0303

(5-Methoxy-2-nitrophenyl)(phenyl)methanol (5). To a solution of aldehyde **4** (90 mg, 0.497 mmol) in THF (10 mL) at -78 °C was added slowly a solution of PhMgBr (1 mL, a 1.0 M solution in THF, 1 mmol). After the reaction mixture was stirred at this temperature for 30 min, the reaction was quenched with 1 M HCl (15 mL). The product was extracted with Et_2O (2 x 20 mL). The organic layer was dried (Na_2SO_4), and concentrated, and the residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford **5** (120 mg, 93%): ^{1}H NMR ($CDCl_3$) δ 3.15 (br s, 1H), 3.90 (s, 3H), 6.51 (s, 1H), 6.89 (dd, 1H, *J* = 2.9, 9.0 Hz), 7.32 (m, 6H), 8.09 (d, 1H, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 55.9, 71.9, 113.0, 114.3, 127.1, 127.9, 128.5, 140.9, 141.5, 142.0, 163.7.

(5-Methoxy-2-nitrophenyl)benzophenone (6). *N*-Bromosuccinimide (45 mg, 0.25 mmol) was added to a solution of benzylic alcohol **5** (25.9 mg, 0.1 mmol) in CH_3CN (5 mL). After the reaction mixture was heated at reflux overnight, the reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (15 mL), and the product was extracted with Et_2O (2 x 15 mL), dried (Na_2SO_4), and purified by chromatography (hexane/EtOAc 3:1). Instead of the desired brominated product, we observed that the benzylic alcohol was oxidized to benzophenone **6** (20 mg, 78%): R_f 0.48 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H), 6.91 (d, 1H, *J* = 2.7 Hz), 7.10 (dd, 1H, *J* = 2.7, 9.2 Hz), 7.47 (m, 2H), 7.61 (m, 1H), 7.78 (m, 2H), 8.27 (d, 1H, *J* = 9.2 Hz); ^{13}C NMR ($CDCl_3$) δ 56.3, 113.3, 115.3, 127.0, 128.8, 129.1, 133.8, 135.8, 138.9, 164.2, 193.2.

1-Bromo-2-methoxy-4-methyl-5-nitrobenzene (7). To a solution of nitrobenzene **3** (2.2 g, 13.1 mmol) in CH_3CN (8 mL) in a pressure tube was added *N*-bromosuccinimide (8.2 g, 45.8 mmol). The tube was sealed and the contents were heated to 140 °C overnight on an oil bath. The reaction mixture turned brownish-red overnight. After the reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution (150 mL), the product was extracted with Et_2O (2 x 150 mL), dried (Na_2SO_4), and purified by chromatography (hexane/EtOAc 3:1) to afford brominated product **7** along with other byproducts that were not separable by chromatography (hexane/EtOAc 3:1). The product mixture (2.8 g, 87%) was used directly in the next step: R_f 0.29 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 2.66 (s, 3H), 3.98 (s, 3H), 6.76 (s, 1H), 8.31 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.7, 56.8, 108.9, 114.3, 125.2, 130.4, 136.3, 141.9, 159.3; HRMS [M-Br-CH_3-H]$^-$ $C_7H_6NO_3$ calcd for *m/z* 152.0348, found 152.0349.

4-Bromo-5-methoxy-2-nitrobenzaldehyde (8). Oxidation of bromide **7** (2.3 g, 9.3 mmol) was performed according to the procedure used to prepare compound **4**, affording aldehyde **8** (1.3 g, 54%) which was purified by chromatography (hexane/EtOAc 3:1): R_f 0.22 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 4.09 (s, 3H), 7.38 (s, 1H), 8.43 (s, 1H), 10.50 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 57.4, 110.5, 116.8, 130.3, 132.6, 142.3, 160.5, 187.7; HRMS [M-H]$^-$ $C_8H_5{}^{79}BrNO_4$ calcd for *m/z* 257.9402, found 257.9407.

(4-Bromo-5-methoxy-2-nitrophenyl)(phenyl)methanol (9). The addition of PhMgBr to aldehyde **8** (200 mg, 0.77 mmol) was performed according to the procedure used to prepare compound **5**, affording addition product **9** (240 mg, 92%): R_f 0.32 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 2.96 (br s, 1H), 3.96 (s, 3H), 6.52 (s, 1H), 7.27 (m, 5H), 7.37 (s, 1H), 8.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 57.0, 71.7, 110.5, 110.9, 127.1, 128.3, 128.7, 130.6, 140.6, 141.2, 160.0: HRMS [M+COOH]$^-$ $C_{15}H_{13}{}^{79}BrNO_6$ calcd for *m/z* 381.9926, found 381.9931.

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-[((4'-bromo-5'-methoxy-2'-nitrophenyl)(phenyl)methoxy) (methoxy)phosphoryloxy]-3-(methoxymethoxy)octadec-4-en-2-yl-carbamate (11):** To a solution of compound **9** (40 mg, 0.12 mmol) in THF (8 mL) was added diisopropylethylamine (41 μL, 0.24 mmol). Methyl diisopropyl phosphoramidochloridite (34 μL, 0.18 mmol) was then added slowly to the reaction mixture at rt under N_2. After the reaction mixture was stirred at the same temperature for 3 h, H_2O (8 mL) was added and the product was extracted with EtOAc (2 x 8 mL). The organic layer was dried (Na_2SO_4) and concentrated, and the product was purified by flash chromatography (hexane/EtOAc 3:1) to afford a phosphoramidite intermediate: R_f 0.55 (hexane/EtOAc 3:1). A mixture of the phosphoramidite intermediate (37 mg, 0.1 mmol) and protected sphingosine **10** (84 mg, 0.20 mmol) was dried by lyophilization from benzene, and the residue was dissolved in CH_3CN (5 mL). The resulting solution was transferred by cannula to a solution of 1*H*-tetrazole (20 mg, 0.30 mmol) in CH_3CN (3 mL) at rt. After 3 h, complete consumption of the phosphoramidite took place as observed by TLC (hexane/EtOAc 3:1). The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ solution (10 mL) and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford a phosphate intermediate: R_f 0.50 (hexane/EtOAc 3:1). To the intermediate phosphite was added *t*-BuOOH (0.2 mL, 1 M solution in toluene, 0.20 mmol) at rt. After 1 h, TLC indicated the complete conversion of the phosphite to the corresponding phosphate **11**. The reaction mixture was concentrated and purified by chromatography (hexane/EtOAc 3:1) to afford phosphate **11** (65 mg, 64%): R_f 0.18 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.6 Hz), 1.25 (m, 22 H), 1.41 (s, 9H), 2.03 (m, 2H), 3.30 (s, 3H), 3.61 (dd, 2H, *J* = 2.0, 11.3 Hz), 3.68 (dd, 1H, *J* = 4.9, 11.4 Hz), 3.79 (m, 1H), 3.93 (m, 2H), 4.06 (s, 3H), 4.18 (m, 1H), 4.43 (d, 1H, *J* = 6.6 Hz), 4.62 (d, 1H, *J* = 6.6

Hz), 4.74 (m, 1H), 5.20 (m, 1H), 5.66 (m, 1H), 7.24 (m, 1H), 7.34 (m, 5H), 7.46 (m, 1H), 8.36 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.3, 29.0, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 54.5, 55.7, 57.1, 66.6, 79.5, 93.5, 110.0, 111.3, 125.6, 127.8, 128.5, 128.7, 128.9, 129.0, 130.8, 137.7, 138.0, 139.7, 155.3, 160.2; ^{31}P NMR ($CDCl_3$) δ -0.62, -0.74, -0.86, -1.00; HRMS $[M+Na]^+$ $C_{40}H_{62}{}^{81}BrN_2O_{11}PNa$ calcd for *m/z* 881.3152, found 881.3152.

***tert*-Butyl-(2*S*,3*R*,4*E*)-1-[((4'-bromo-5'-hydroxy-2'-nitrophenyl)(phenyl)methoxy) (hydroxy)phosphoryloxy]-3-(methoxymethoxy)octadec-4-en-2-yl-carbamate (12):** A solution of phosphate **11** (15.5 mg, 0.02 mmol) and anhydrous LiI (53.5 mg, 0.40 mmol) in dry pyridine (2.5 mL) was heated at reflux overnight. The reaction was quenched with saturated aqueous NH_4Cl solution (10 mL) and the product was extracted with CH_2Cl_2 (3 x 10 mL), dried (Na_2SO_4), concentrated, and purified by chromatography ($CHCl_3$/MeOH 3:1) to afford di-demethylated compound **12** (14 mg, 85%): R_f 0.63 ($CHCl_3$/MeOH 3:1); HRMS $[M+H]^+$ $C_{38}H_{59}{}^{79}BrN_2O_{11}P$ calcd for *m/z* 829.3040, found 829.2864.

(4*S*,5*R*,1'*E*)-*tert*-Butyl-4-(hydroxymethyl)-2,2-dimethyl-5-(pentadec-1'-enyl)-oxazolidine-3-carboxylate (13).[1c] To a solution of D-*erythro*-sphingosine (400 mg, 1.3 mmol) in EtOH/H_2O (2:1, 9 mL) was added aqueous 1 N NaOH (3.9 mL, 3.9 mmol) and $(Boc)_2O$ (851 mg, 3.9 mmol). The reaction mixture was stirred at rt until the consumption of the starting material was observed by TLC (hexane/EtOAc 3:1): R_f 0.12. The reaction was quenched with saturated aqueous NH_4Cl solution (30 mL), and the product was extracted with EtOAc (2 x 25 mL), dried (Na_2SO_4), and concentrated. The resulting residue was purified by flash chromatography (hexane/EtOAc 3:1) to afford the *N*-Boc-sphingosine derivative (495 mg, 95%): R_f 0.22. The Boc derivative (180 mg, 0.45 mmol) was thoroughly dried in vacuum and then was dissolved in CH_2Cl_2 (10 mL). After imidazole (122 mg, 1.8 mmol) and TBDPSCl (236 μL, 0.90

mmol) were added, the reaction mixture was stirred for 3 h at rt, and then was concentrated. The residue was purified by flash chromatography (hexane/EtOAc 3:1): R_f 0.65. To a solution of the silylated primary alcohol in benzene (30 mL) were added 2,2-dimethoxypropane (220 μL, 1.8 mmol) and *p*-TsOH·H_2O (1 mg, 0.010 mmol). The reaction mixture was heated at reflux in a Dean-Stark trap for 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (15 mL) and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford the isopropylidene derivative: R_f 0.75. The isopropylidene intermediate was treated with TBAF (11.25 mL, 1 M solution in THF, 11.25 mmol) for 6 h at rt. The reaction mixture was diluted with H_2O (10 mL), and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford isopropylidene **13** (92 mg, 47%): R_f 0.51 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 6.8 Hz), 1.26 (m, 22H), 1.49 (s, 9H), 1.54 (s. 3H), 1.60 (s, 3H), 2.07 (q, 2H, *J* = 5.6 Hz), 3.50 (br s, 1H), 3.65 (m, 1H), 3.80 (m, 1H), 4.09 (m, 1H), 4.57 (m, 1H), 5.46 (dd, 1H, *J* = 7.5, 15.2 Hz), 5.88 (dt, 1H, *J* = 6.5, 13.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.7, 27.8, 28.4, 28.8, 29.2, 29.3, 29.4, 29.5, 29.7, 31.9, 62.0, 63.8, 81.2, 92.9, 123.2, 137.5, 154.4; HRMS $[M+Na]^+$ $C_{26}H_{49}NNaO_4$ calcd for *m/z* 462.3559, found 462.3554.

(4*S*,5*R*,1"*E*)-*tert*-Butyl-4-((((4'-bromo-5'-methoxy-2'-nitrophenyl)(phenyl)methoxy)(methoxy)phosphoryloxy)methyl)-2,2-dimethyl-5-(pentadec-1"-enyl)oxazolidine-3-carboxylate (16). To a solution of compound **9** (44 mg, 0.13 mmol) in THF (8 mL) was added DIPEA (54 μL, 0.31 mmol). Methyl diisopropyl phosphoramidochloridite (51 μL, 0.26 mmol) was then added slowly to the reaction mixture at rt under N_2. After the reaction mixture was stirred at the same temperature for 3 h, H_2O (8 mL) was added and the product was extracted

with EtOAc (2 x 8 mL). The organic layer was dried (Na_2SO_4), concentrated, and the product was purified by flash chromatography (hexane/EtOAc 3:1) to afford phosphoramidite **14**: R_f 0.65 (hexane/EtOAc 3:1). A mixture of phosphoramidite **14** (52 mg, 0.1 mmol) and protected sphingosine **13** (88 mg, 0.20 mmol) was dried by lyophilization from benzene, and the residue was dissolved in CH_3CN (5 mL). The resulting solution was transferred by cannula to a solution of 1*H*-tetrazole (21 mg, 0.30 mmol) in CH_3CN (3 mL) at rt. After 3 h, complete consumption of the phosphoramidite took place as observed by TLC (hexane/EtOAc 3:1). The reaction was quenched with saturated aqueous $NaHCO_3$ solution (10 mL), and the product was extracted with EtOAc (2 x 15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexane/EtOAc 3:1) to afford phosphite **15**: R_f 0.55. To the intermediate phosphite was added *t*-BuOOH (0.3 mL, 1 M solution in toluene) at rt. After 1 h, TLC indicated the complete conversion of the phosphite to the corresponding phosphate. The reaction mixture was concentrated and purified by chromatography (hexane/EtOAc 3:1) to afford phosphate **16** (60 mg, 70%): R_f 0.15 (EtOAc/hexane 1:3); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.7 Hz), 1.26 (m, 22H), 1.46 (s, 9H), 1.53 (s. 3H), 1.64 (s, 3H), 2.07 (m, 2H), 3.64 (m, 3H), 3.86 (m, 1H), 3.99 (m, 1H), 4.06 (m, 4H), 4.50 (m, 1H), 5.48 (m, 1H), 5.88 (m, 1H), 7.25 (m, 1H), 7.32 (m, 6H), 7.51 (m, 1H), 8.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 28.3, 29.0, 29.4, 29.5, 29.6, 29.7, 31.9, 32.5, 54.4, 57.1, 59.2, 80.3, 93.1, 110.0, 111.3, 122.8, 127.9, 128.0, 128.6, 129.0, 130.8, 137.9, 139.8, 160.1; ^{31}P NMR ($CDCl_3$) δ -1.60, -1.46, -1.31, -1.13; HRMS $[M+Na]^+$ $C_{41}H_{62}BrN_2O_{10}PNa$ calcd for *m/z*, 875.3223 found 875.3218.

(4*S*,5*R*,1"*E*)-*tert*-Butyl-4-((((4'-bromo-5'-hydroxy-2'-nitrophenyl)(phenyl)methoxy)(hydroxy)phosphoryloxy)methyl)-2,2-dimethyl-5-(pentadec-1"-enyl)oxazolidine-3-carboxylate (17). The anisole methyl group and the methyl phosphate ester of compound **16**

were deprotected according to the procedure used to prepare compound **12**, affording compound **17** (37 mg, 90%): R_f 0.60 ($CHCl_3$/MeOH 3:1); ^{1}H NMR (CD_3OD) δ 0.78 (t, 3H, *J* = 6.6 Hz), 1.18 (m, 31H), 1.38 (m, 6H), 1.90 (m, 2H), 3.79 (m, 3H), 4.40 (m, 1H), 5.42 (m, 1H), 5.69 (m, 1H), 6.99 (m, 1H), 7.23 (m, 6H), 7.53 (m, 1H), 8.20 (m, 1H); ^{31}P NMR (CD_3OD) δ -2.92, -2.03; HRMS $[M+Na]^+$ $C_{39}H_{58}{}^{81}BrN_2O_{10}PNa$ calcd for *m/z* 849.2889, found 849.2894.

(2*S*,3*R*,4*E*)-2-Amino-3-hydroxyoctadec-4-enyl-(4'-bromo-5'-hydroxy-2'-nitrophenyl)(phenyl)methyl Hydrogen Phosphate (1). A solution of phosphate **17** (25 mg, 0.030 mmol) in 3 M HCl/MeOH/ CH_2Cl_2 (1:2:1, 4 mL) was stirred at rt overnight. The reaction mixture was gently heated at reflux at 50-60 °C for 2 h. The reaction mixture was then diluted with CH_2Cl_2 (30 mL) and washed with brine (10 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography ($CHCl_3$/MeOH 6:1, 3:1), followed by removal of suspended silica gel by filtration of a solution of **1** in $CHCl_3$ through a Cameo filter, to afford BHNPB-caged S1P **1** (12 mg, 60%) as a yellow solid: R_f 0.35 ($CHCl_3$/MeOH 3:1); ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H, *J* = 7.0 Hz), 1.28 (m, 22H), 2.03 (m, 2H), 2.95 (m, 1H), 3.59 (m, 1H), 3.75 (m, 1H), 4.07 (m, 1H), 5.28 (dd, 1H, *J* = 6.5, 15.4 Hz), 5.70 (dt, 1H, *J* = 6.7, 14.7 Hz), 7.02 (m, 1H), 7.25 (m, 5H), 7.44 (m, 1H), 8.25 (m, 1H); ^{13}C DEPT-45 NMR (CD_3OD) δ 14.5, 23.7, 30.2, 30.4, 30.5, 30.6, 30.7, 30.8, 33.1, 33.3, 57.0, 63.3, 70.6, 76.4, 116.8, 127.8, 128.8, 129.0, 129.2, 129.3, 129.4, 131.8, 136.3, 136.8; ^{31}P NMR (CD_3OD) δ -1.41, -1.20; HRMS $[M-H]^-$ $C_{31}H_{45}BrN_2O_8P$ calcd for *m/z* 683.2096, found 683.2095; UV: λ_{max} 406 nm (ϵ = 12000 $M^{-1}cm^{-1}$), 278 nm (ϵ = 6294 $M^{-1}cm^{-1}$) in 50% aqueous EtOH, 50% 10 mM Tris, pH 7.35 (170 μM).

(4'-Bromo-5'-hydroxy-2'-nitrophenyl)(phenyl)methyl-(2*S*,3*R*,4*E*)-3-hydroxy-2-palmitamidooctadec-4-enyl Hydrogen Phosphate (2). A solution of compound **1** (1.0 mg, 1.5 μmol), *p*-nitrophenyl palmitate (1.7 mg, 4.5 μmol), and anhydrous potassium carbonate (1 mg,

4.5 μmol) was suspended in a solution of anhydrous DMF (1.25 mL) and CH_2Cl_2 (0.5 mL). After the reaction mixture had stirred for 2 days, the mixture was concentrated under high vacuum. The compound was purified by chromatography ($CHCl_3$/MeOH 6:1), followed by removal of suspended silica gel by filtration of a solution of compound **2** in $CHCl_3$ through a Cameo filter, affording BHNPB caged C1P **2** (1 mg, 77%): R_f 0.33 ($CHCl_3$/MeOH 6:1); ^{1}H NMR ($CDCl_3$/CD_3OD 30:1) δ 0.83 (t, 6H, *J* = 6.8 Hz), 1.25 (m, 46H), 1.92 (m, 2H), 2.10 (m, 2H), 3.58 (m, 1H), 3.83 (m, 1H), 3.92 (m, 2H), 4.0 (m, 1H), 5.34 (m, 1H), 5.62 (m, 1H), 7.02 (m, 1H), 7.23 (m, 5H), 7.50 (m, 1H), 8.29 (m, 1H); ^{13}C DEPT-45 NMR ($CDCl_3$/CD_3OD 30:1) δ 14.1, 19.8, 22.7, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 30.2, 31.9, 32.4, 36.5, 53.5, 57.0, 71.4, 74.4, 77.4, 110.8, 127.5, 127.7, 128.2, 128.4, 130.3, 135.0; ^{31}P NMR ($CDCl_3$/CD_3OD 30:1) δ -1.23, -1.11; HRMS $[M+Na]^+$ $C_{47}H_{76}BrN_2O_9PNa$ calcd for *m/z* 945.4369, found 945.4363; UV: λ_{max} 397 nm (ϵ = 1747 $M^{-1}cm^{-1}$), 320 nm (ϵ = 4111 $M^{-1}cm^{-1}$) in EtOH (170 μM).

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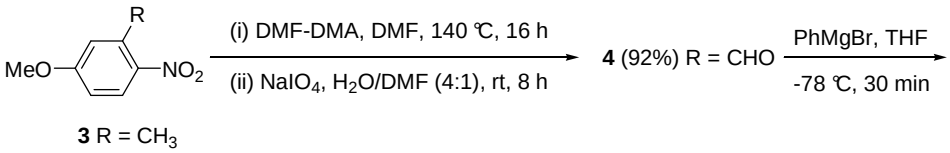

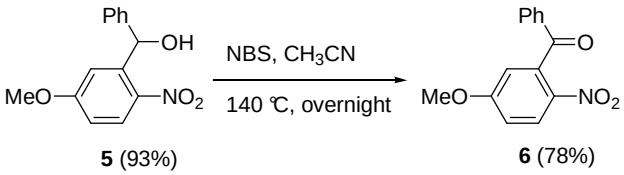

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Chapter 5B

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