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Chemoenzymatic stereoconvergent synthesis of 3-*O*-benzoyl azidosphingosine

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Abstract—The synthesis of 3-*O*-benzoyl azidosphingosine **1** through a stereoconvergent approach is described. Nucleophilic addition of the Grignard reagent of 1-pentadecyne to cyclohexylidene-D-glyceraldehyde results in a mixture of diastereoisomeric propargylic alcohols. Subsequent enzymatic separation of these diastereoisomers, mediated by lipase from *Candida antarctica*, Mitsunobu inversion on the wrong diastereoisomer and extremely efficient introduction of azide using a chloromesylate leaving group affords the title compound in 30% overall yield.

1. Introduction

Glycosphingolipids are important membrane components of all eukaryotic cells, as they are involved in molecular recognition phenomena such as cell–cell interaction and interaction between the cell and other biological agents.[1]

Most natural glycosphingolipids contain the basic amino alcohol D-*erythro*-sphingosine [(2*S*,3*R*,4*E*)-2-amino-3-hydroxyoctadec-4-en-1-ol], which is linked to a fatty acid chain through an amide bond (ceramide unit) and to a hydrophilic carbohydrate portion through a glycosidic linkage.

The synthesis of glycosphingolipids involves the linkage among these three fragments and it has been observed that the coupling of the preformed ceramide unit to the carbohydrate part usually gives lower yield than the glycosylation of a sphingosine precursor, such as azidosphingosine,[2] followed by azido group reduction and *N*-acylation with suitable fatty acid derivative to generate the desired ceramide moiety. In particular 3-*O*-benzoyl azidosphingosine has been employed successfully in the so called azidosphingosine glycosylation procedure,[2,3] as a versatile synthon for the preparation of many glycosphingolipid derivatives. As a part of our project toward the synthesis of sulfated glycosphingolipid antigens we became interested in the development of a new synthetic approach to 3-*O*-benzoyl azidosphingosine **1** (Fig. 1).

Syntheses of azidosphingosine have been reported by two main approaches: the chiral pool approach and the asymmetric induction approach.[1] We focused our attention on the first one, planning a stereoselective nucleophilic addition to a protected D-glyceraldehyde, derived from D-mannitol.

2. Results and discussion

Nucleophilic additions to 2,3-*O*-alkylidene-D-glyceraldehyde for the stereoselective synthesis of chiral alcohols are reported to proceed with moderate stereoselectivity and the resulting diastereoisomeric

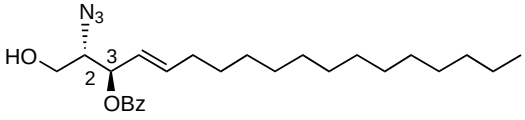

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Figure 1.

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alcohols are not easily separable through conventional procedures.[4] Among the commonly used acetals, the cyclohexylidene acetal has been exploited as a protecting group for D-glyceraldehyde as an alternative to the isopropylidene analogue, as it gives better results in terms of stability and usually allows more efficient separation of the mixture of diastereoisomeric alcohols generated from the addition reaction.[4,5] Following these considerations 2,3-*O*-cyclohexylidene-D-glyceraldehyde appeared a suitable chiral building block for the initial task of construction of the C_{18} precursor of D-*erythro*-azidosphingosine. 2,3-*O*-Cyclohexylidene-D-glyceraldehyde **2** was easily synthesized by periodate cleavage of 1,2:5,6-di-*O*-cyclohexylidene-D-mannitol according to a literature procedure.[5]

Treatment of aldehyde **2** with preformed Grignard reagent from 1-pentadecyne furnished the mixture of diastereoisomeric propargylic alcohols **3** and **4** (Scheme 1) in a *syn*/*anti* ratio of 4:6 and 90% yield.

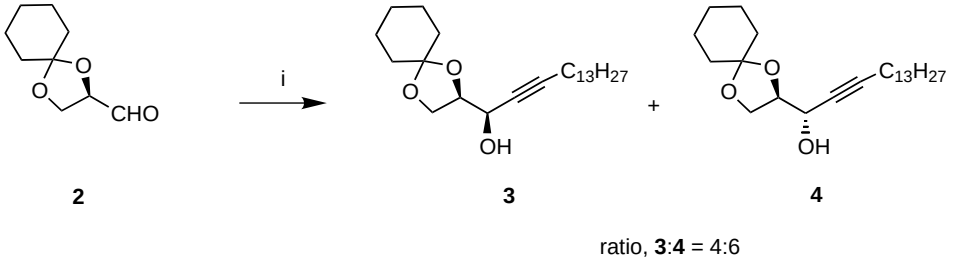
The diastereomeric ratio was established by ^{1}H NMR analysis of the mixture of compounds **3** and **4**. Their *syn* and *anti* configurations were assigned by comparing the ^{1}H NMR resonance of the *H*-COH protons with those for analogous propargylic alcohols reported in literature.[6,7,†]

The stereochemistry was later confirmed by chemical correlation when the *syn* derivative **3** was transformed into the known 3-*O*-benzoyloctadec-4-en-1,2,3-triol.[8] The low stereoselectivity of the addition, together with the almost impossible separation of the mixture of propargylic alcohols **3** and **4** by column chromatography prompted us to investigate an enzymatic approach for their separation. In fact it is known that lipases are useful tools for synthetic applications due to their ability to catalyze transesterification reactions in organic solvents with high regio- and stereoselectivities. For example, racemic mixtures of propargylic and allylic alcohols were resolved with excellent enantioselectivities via enzymatic transesterification promoted by various lipases in organic solvent.[9]

Treatment of the 4:6 diastereoisomeric mixture of **3** and **4** with lipase from *Candida antarctica* (LCA) and vinyl acetate in cyclohexane furnished, after chromatographic separation, the *anti* acetyl derivative **4a** (57% yield), while the *syn* alcohol **3** was recovered as unreacted starting material (38% yield); both compounds were found to be diastereoisomerically pure by ^{1}H and ^{13}C NMR analysis (Scheme 2). The presence of the *syn* acetylated derivative was never observed. The acylation reaction was reproducible up to at least 8 g scale and the enzyme was recycled up to three times without any significant loss of activity.

Following a convergent synthetic approach, the undesired *anti*-**4a** was recycled through Zemplèn deacetylation followed by Mitsunobu inversion with acetic acid[10] that furnished the acetyl derivative **3a**, with the desired *syn* configuration of hydroxyl groups at positions 2 and 3, giving an overall yield from **4a** to **3a** of 72%. The *trans*-allylic alcohol **5** was obtained in quantitative yield from **3** and **3a**, which were reacted independently with $LiAlH_4$ in THF. In this way the lack of stereoselectivity of the addition step was overcome since both the diastereoisomeric alcohols **3** and **4** were converted into **5**. The crude **5** was then subjected to conventional benzoylation to yield the fully protected derivative **6**; the yield of the two step reduction–benzoylation process was 80% starting from either **3** or **3a**. Benzoyl derivative **6** was then subjected to acidic deketalization. Attempts to perform the cleavage of the cyclohexylidene acetal with acid catalysis using PTSA or pyridinium tosylate gave mainly benzoyl group migration on position 1. To avoid this side reaction deketalization of **6** was carried out with aqueous trifluoroacetic acid giving compound **7** in quantitative yield, a small amount of which was purified and confirmed to be the known 3-*O*-benzoyloctadec-4-en-1,2,3-triol.[8] However, during the synthesis, compound **7** was used in the next step without purification, since it was observed that column chromatography on silica gel also promotes partial benzoyl group migration on position 1.

The synthesis of **1** was accomplished employing a series of high-yielding protecting group manipulations in order to introduce nitrogen at position 2 with the



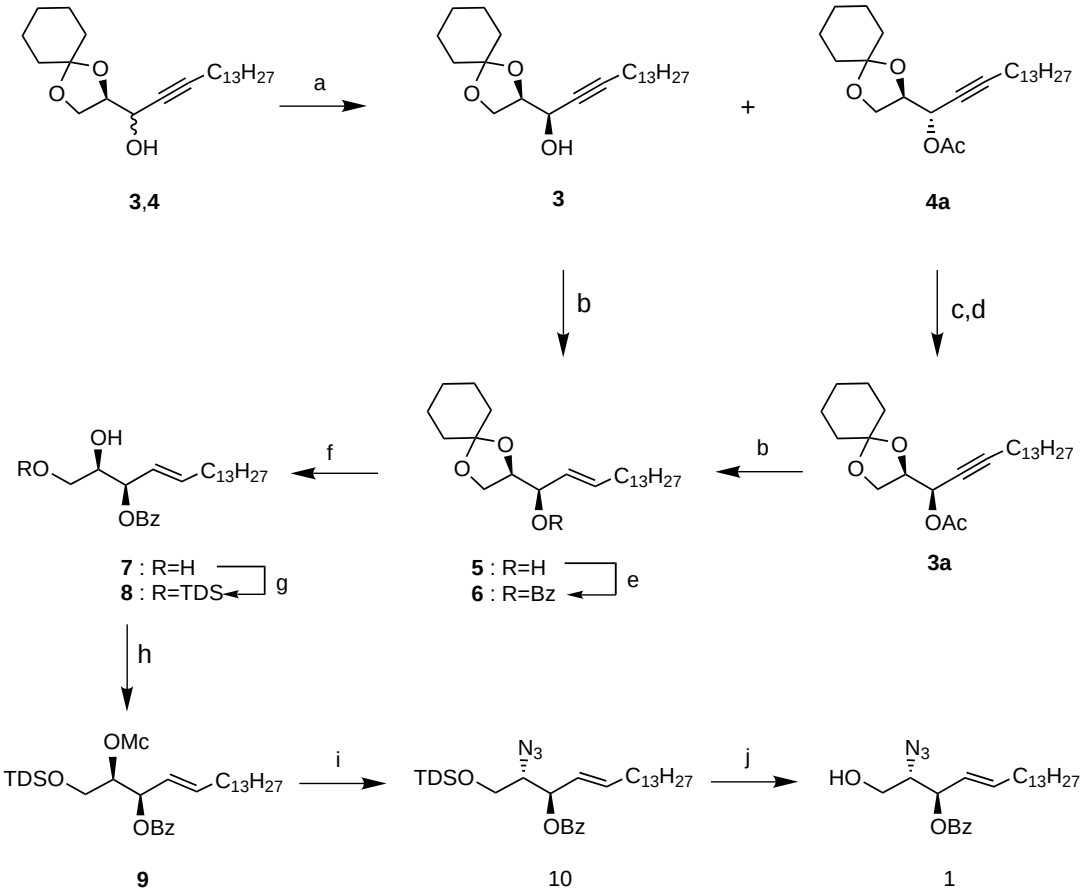
Scheme 1. (i) 1-Pentadecynyl magnesium bromide, Et_2O, THF, –40°C.

† During the assignment of the *syn*/*anti* configuration of compounds **3** and **4** it was found that the ^{1}H NMR spectra of the related compounds described in Ref. 6 (compounds **7b** and **8b** in Table 3 of page 107) had been exchanged.

desired (*S*)-configuration, by means of nucleophilic substitution with azide. A selective protecting orthogonal to benzoate for the primary hydroxyl group at position 3 is required to allow selectively deblocking for glycosylation. Although thexyldimethylsilyl ether protection fulfills these requirements it could hamper the leaving group displacement at position 2 by the N_3^- nucleophile as was recently observed with a TBS ether.[11] The proposed solution to this problem had been the change of the protecting group at position 1 before the substitution reaction.[11] Possible alternatives to avoid extra steps would be the enhancement either of the nucleophilicity of the N_3^- nucleophile or of the leaving group ability.

Selective 1-*O*-silylation was performed on the crude **7** according to a procedure by Ohlsson and Magnusson,[12] giving the TDS ether **8** with a yield of 80% from compound **6**. Hydroxyl group at position 2 is usually activated for nucleophilic displacement as the mesylate derivative, but the nucleophilic substitution carried out with NaN_3 requires harsh conditions and the use of crown ether.[12] The use of the good N_3^- donor, tetrabutylammonium azide, was expected to be a good alternative to the above procedure; however when applied to the mesylate derivative, obtained from **8** according to literature,[12] this method gave only a disappointing 56% yield. The attention turned to a more active leaving group, such as chloromesylate, which was demonstrated to give better results in comparison with mesylate and triflate, in the inversion of secondary alcohols with different nucleophiles, including NaN_3.[13,14] Therefore, chloromesylate **9** was obtained from compound **8** by treatment with chloromethanesulfonyl chloride in pyridine in 86% yield.[15] Chloromesylate **9** was then treated with NaN_3 in DMF at 85°C without crown ether affording a very satisfactory 85% yield of the fully protected azidosphingosine **10** in only 2 h. The choice of chloromesylate as the leaving group allowed us to avoid protecting group manipulation at position 1.[11]

To prevent benzoyl group migration, hydrolysis of the thexyldimethylsilyl ether of compound **10** under mild conditions was carried out with 2% HF solution in CH_3CN/THF,[16] affording the target compound **1** in 85% yield.



Scheme 2. (a) LCA, vinyl acetate, cyclohexane, 40°C, 95%; (b) $LiAlH_4$, THF, 40°C; (c) MeONa, MeOH; (d) AcOH, PPh_3, DIAD, pyridine, THF, 0°C then rt, 72% (from **4a**); (e) BzCl, pyridine, CH_2Cl_2, 80% (two steps either from **3** or **3a**); (f) 60% aq. trifluoroacetic acid, 0°C; (g) TDSCl (TDS = thexyldimethylsilyl), pyridine, 0°C then rt, 80% (from **6**); (h) McCl (Mc = chloromethanesulfonyl), pyridine, 0°C then rt, 86%; (i) NaN_3, DMF, 85°C, 85%; (j) 2% aq. HF, THF, CH_3CN, 85%.

3. Conclusions

In conclusion, we have developed a new synthesis of 3-*O*-benzoyl azidosphingosine following a chemoenzymatic stereoconvergent approach, which is based on an enzymatic resolution mediated by *C. antarctica* lipase, Mitsunobu inversion and very smooth introduction of azide through the use of chloromesylate as a leaving group, with an overall yield of 30% from this ten step synthesis; this work also confirms the effectiveness of chloromesylate as a leaving group.

4. Experimental

4.1. General methods

Dry solvents and liquid reagents were distilled prior to use: THF and diethyl ether were distilled from sodium, dichloromethane and pyridine were distilled from calcium hydride, DMF was dried over 4 Å molecular sieves; all reaction vessels, after being dried were kept under argon. Organic solutions were dried over anhydrous sodium sulfate, and the solvent was evaporated at reduced pressure below 40°C. TLC was performed on glass plates coated with silica gel 60 F-254 Merck, spots being developed with 5% sulfuric acid in methanol/water (1:1), or with phosphomolybdate based reagent. Silica gel Merck 60 (230–400 mesh) was used for flash chromatography.

Optical rotation measurements were obtained for $CHCl_3$ solutions with a 241 Perkin–Elmer polarimeter at 20°C. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions with a Bruker AM-500 spectrometer. Chemical shifts are given in ppm (δ), relative to $SiMe_4$ as internal standard; coupling constant (J)-values are given in Hz. Diastereoisomeric ratios were determined by integration of well-separated signals in ^1H NMR spectra. IR spectra were measured neat by a Perkin–Elmer 1420 Spectrophotometer (NaCl crystal windows). Mass spectrometry was performed on a Hewlett–Packard HP-5988-A spectrometer. Mass spectra were recorded by electronic impact (LC-EI) or chemical ionization (LC-CI) techniques as described in Ref. 17. 2,3-*O*-Cyclohexylidene-D-glyceradehyde **2** was prepared from 1,2:5,6-di-*O*-cyclohexylidene-D-mannitol according to a literature procedure[4] and subjected to azeotropic distillation with toluene just before the use. *C. antarctica* lipase SP 435L, immobilized on a macroporous acrylic resin (Novozym® 435, LCA, specific activity 9.5 PL units/mg solid), was a generous gift from Novo Nordisk A/S; 1-pentadecyne was purchased from Fluka; chloromethanesulfonyl chloride was purchased from Alfa Aesar.

4.2. Addition of 1-pentadecynyl magnesium bromide to 2,3-*O*-cyclohexylidene-D-glyceraldehyde 2

To a stirred solution of 1-pentadecyne (16.4 mL, 62.0 mmol) in THF (30 mL) at 0°C was added dropwise freshly prepared ethylmagnesium bromide (16.4 mL, 41.0 mmol, 2.5 M solution in diethyl ether). The solution was heated under reflux for 1 h, cooled to −40°C, and a solution of **2** (6.96 g, 41 mmol) in THF (30 mL) was added dropwise. The mixture was stirred at −40°C for 1 h and then at rt overnight. The reaction was quenched by addition of satd aq. NH_4Cl solution and extracted with diethyl ether (3×100 mL). The combined organic layers were washed with water and brine, dried and concentrated. The residue was purified by flash chromatography (hexane/ethyl acetate, 9:1), affording the mixture of diastereoisomeric propargylic alcohols **3** and **4** (20.4 g, 90%) in a *syn*:*anti* ratio of 4:6. The diastereoisomeric ratio was determined by the ^1H NMR through the integral ratio of the signals due to *H*-C-OH, which resonates at 4.48 (*anti* diastereoisomer) and 4.25 ppm (*syn* diastereoisomer). Full characterizations of compounds **3** and **4** are reported after enzymatic separation.

4.3. Enzymatic resolution of propargylic alcohols 3 and 4 to (2*R*,3*S*)-3-*O*-acetyl-1,2-*O*-cyclohexylidene-4-octadecyn-1,2,3-triol 4a and (2*R*,3*R*)-1,2-*O*-cyclohexylidene-4-octadecyn-1,2,3-triol 3

To a solution of **3** and **4** (1.45 g, 3.83 mmol) in cyclohexane (30 mL), lipase from *C. antarctica* (4.50 g) and vinyl acetate (1.41 mL, 15.3 mmol) were added. The mixture was shaken at 40°C for 8 h, at rt overnight and finally for 5 h at 40°C. The enzyme was filtered off and washed with cyclohexane. After evaporation of the solvent the residue was submitted to flash chromatography (petroleum ether/ethyl acetate, 9:1) affording the *anti* acetyl derivative **4a** (0.92 g, 57%) and the *syn* alcohol **3** (0.55 g, 38%) as unconverted starting material, both as colorless oils.

4.3.1. (2*R*,3*R*)-1,2-*O*-Cyclohexylidene-4-octadecyn-1,2,3-triol 3. $[\alpha]_D=+16.9$ (*c* 1); ^1H NMR δ 0.85 (t, 3H, $J=7.0$ Hz, CH_3), 1.20–1.65 (m, 32H, CH_2), 2.16 (td, 2H, $J=6.5$ Hz, $J=1.5$ Hz, =CH-CH_2), 2.39 (d, 1H, $J=3.5$ Hz, OH), 3.84 (dd, 1H, $J=8.5$ Hz, $J=5.0$ Hz, OCH_aH_b), 4.04 (dd, 1H, $J=8.5$ Hz, $J=6.5$ Hz, OCH$_a$H$_b$), 4.10 (m, 1H, OC*H*CH$_2$), 4.25 (m, 1H, C*H*OH); ^{13}C NMR δ 14.7, 19.3, 23.3, 24.4, 24.7, 25.7, 29.1, 29.5, 29.7, 30.0, 30.1–30.3 (5C), 32.6, 35.5, 37.3, 65.6, 66.6, 77.9, 79.6, 88.0, 111.6; IR: 3430, 2940, 2860, 1460, 1380, 1170, 1100, 1050, 940 cm^{-1}; MS (EI): *m*/*e* 378 [M$^+$]. Anal. calcd for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18. Found: C, 76.30; H, 11.02%.

4.3.2. (2*R*,3*S*)-3-*O*-Acetyl-1,2-*O*-cyclohexylidene-4-octadecyn-1,2,3-triol 4a. $[\alpha]_D=+57.5$ (*c* 0.68); ^1H NMR δ 0.86 (t, 3H, $J=7.0$ Hz, CH_3), 1.20–1.65 (m, 32H, CH_2), 2.09 (s, 3H, $COCH_3$), 2.17 (td, 2H, $J=7.0$ Hz, $J=2.0$ Hz, =CH-CH_2), 3.94 (dd, 1H, $J=8.5$ Hz, $J=6.5$ Hz, OCH_aH_b), 4.07 (dd, 1H, $J=8.5$ Hz, $J=6.5$ Hz, OCH$_a$H$_b$), 4.26 (td, 1H, $J=6.5$ Hz, $J=4.0$ Hz, OC*H*CH$_2$), 5.51 (m, 1H, C*H*OCOCH$_3$); ^{13}C NMR δ 14.7, 19.4, 21.6, 23.3, 24.4, 24.5, 25.8, 29.0–30.3 (9C), 32.6, 35.7, 36.5, 64.5, 65.9, 75.0, 77.1, 88.5, 111.6, 170.4; IR: 2980, 2900, 1770, 1460, 1380, 1250, 1170, 1060, 950 cm^{-1}; MS (EI): *m*/*e* 420 [M$^+$]. Anal. calcd for $C_{26}H_{44}O_4$: C, 74.24; H, 10.54. Found: C, 74.48; H, 10.21%.

4.4. (2*R*,3*R*)-3-*O*-Acetyl-1,2-*O*-cyclohexylidene-4-octadecyn-1,2,3-triol 3a

A solution of MeONa in MeOH (0.2 mL, 0.1 M) was added to **4a** (0.84 g, 1.99 mmol) in anhydrous MeOH (5 mL), and the mixture was stirred for 1 h at rt. Subsequently it was neutralized with ion-exchange resin Dowex H^+ (50W-X8). The resin was filtered off and washed with MeOH. The filtrate was concentrated, affording crude **4** (0.74 g), which was used in the next step without further purification. A sample was purified by flash chromatography (hexane/ethyl acetate, 9:1) to afford (2*R*,3*S*)-1,2-*O*-cyclohexylidene-4-octadecyn-1,2,3-triol **4** as a colorless oil. $[\alpha]_D = +20.0$ (*c* 1); ^{1}H NMR δ 0.85 (t, 3H, $J = 7.0$ Hz, CH_3), 1.20–1.75 (m, 32H, CH_2), 2.14 (d, 1H, $J = 4.0$ Hz, OH), 2.18 (td, 2H, $J = 7.0$ Hz, $J = 2.0$ Hz, $=CHCH_2$), 4.03 (m, 2H, OCH_2), 4.19 (m, 1H, $OCHCH_2$), 4.48 (m, 1H, $CHOH$); ^{13}C NMR δ 14.8, 19.4, 23.4, 24.4, 24.6, 25.8, 29.1, 29.5, 29.8, 30.0, 30.2–30.3 (5C), 32.6, 35.4, 36.6, 63.1, 65.4, 77.7, 78.4, 88.0, 111.2; IR: 3450, 2960, 2890, 1450, 1370, 1170, 1120, 1040, 940 cm^{-1}; MS (EI): *m*/*e* 378 [M^+]. Anal. calcd for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18. Found: C, 76.35; H, 10.95%.

To a solution of the crude **4** (0.74 g) in anhydrous THF (24 mL) at 0°C were added PPh_3 (2.06 g, 7.85 mmol), glacial acetic acid (0.56 mL, 9.80 mmol) and pyridine (0.32 mL, 7.85 mmol). The mixture was cooled to −40°C and DIAD (1.50 mL, 7.85 mmol) was added dropwise. After being stirred at 0°C for 5 h the solution was taken up in ether (50 mL) and washed with satd aq. $NaHCO_3$, 5% aq. HCl solution, and brine. The organic layer was dried and concentrated. The residue was purified by flash chromatography, (hexane/ethyl acetate, 9:1) to give **3a** as a colorless oil (0.59 g, 72%). $[\alpha]_D = -25.8$ (*c* 1); ^{1}H NMR δ 0.85 (t, 3H, $J = 7.0$ Hz, CH_3), 1.20–1.65 (m, 32H, CH_2), 2.08 (s, 3H, $COCH_3$), 2.15 (dt, 2H, $J = 7.0$ Hz, $J = 2.0$ Hz, $=CHCH_2$), 3.94 (dd, 1H, $J = 9.0$ Hz, $J = 5.5$ Hz, OCH_aH_b), 4.06 (dd, 1H, $J = 9.0$ Hz, $J = 7.0$ Hz, OCH_aH_b), 4.19 (m, 1H, $OCHCH_2$), 5.38 (dt, 1H, $J = 7.5$ Hz, $CHOCOCH_3$); ^{13}C NMR δ 14.8, 19.3, 21.7, 23.4, 24.5, 24.6, 25.7, 29.0, 29.5, 29.8, 30.0, 30.2–30.3 (5C), 32.6, 35.7, 36.9, 66.7 (2C), 75.1, 77.0, 88.6, 111.9, 170.5; IR: 2960, 2880, 1750, 1460, 1380, 1240, 1170, 1110, 940 cm^{-1}; MS (EI): *m*/*e* 420 [M^+]. Anal. calcd for $C_{26}H_{44}O_4$: C, 74.24; H, 10.54. Found: C, 74.45; H, 10.32%.

4.5. (2*R*,3*R*,4*E*)-3-*O*-Benzoyl-1,2-*O*-cyclohexylidene-4-octadecen-1,2,3-triol 6

From 3a: To a stirred solution of **3a** (0.39 g, 0.93 mmol) in THF (8 mL) $LiAlH_4$ (0.08 g, 2.20 mmol) was added in small portions; the suspension was stirred at 40°C for 5 h. After destroying the excess of $LiAlH_4$ with 2-propanol, water (1 mL) and silica gel (2 g) were added. The mixture was stirred for 1 h at 0°C, then $MgSO_4$ was added, the insoluble material was removed by filtration through Celite and the filtrate was concentrated affording crude **5** (0.35 g).

From 3: Following the same procedure described above crude **5** (0.43 g) was obtained starting from **3** (0.46 g, 1.23 mmol). The two crudes were combined (0.78 g) and used in the next step without purification. A sample was purified by flash chromatography (hexane/ethyl acetate, 9:1) to afford (2*R*,3*R*,4*E*)-1,2-*O*-cyclohexylidene-4-octadecen-1,2,3-triol **5** as a colorless oil. $[\alpha]_D = -1.0$ (*c* 1); ^{1}H NMR δ 0.86 (t, 3H, $J = 6.5$ Hz, CH_3), 1.20–1.68 (m, 32H, CH_2), 2.02 (m, 2H, $=CHCH_2$), 2.34 (d, 1H, $J = 2.0$ Hz, OH), 3.69 (dd, 1H, $J = 8.0$ Hz, $J = 5.0$ Hz, $OCHCH_2$), 3.90–4.00 (m, 3H, $CHOH$, OCH_2), 5.35 (dd, 1H, $J = 15.0$ Hz, $J = 7.0$ Hz, $CH{=}CHCH_2$), 5.76 (m, 1H, $CH{=}CHCH_2$); ^{13}C NMR δ 14.7, 23.3, 24.4, 24.7, 25.8, 29.6–30.3 (9C), 32.6, 33.0, 35.6, 37.3, 66.3, 75.4, 79.4, 111.1, 128.4, 136.3; IR: 2900, 2840, 1450, 1360, 1270, 1150, 1100, 1040, 960 cm^{-1}; MS (CI): *m*/*e* 398 [$M+NH_4$]$^+$. Anal. calcd for $C_{24}H_{44}O_3$: C, 75.74; H, 11.65. Found: C, 75.48; H, 11.92%.

To a stirred solution of crude **5** (0.78 g) in dichloromethane (10 mL) pyridine (1.00 mL, 12.3 mmol) and benzoyl chloride (0.72 mL, 6.21 mmol) were added. The solution was stirred at rt for 2 h then water was added, after separation, the organic layer was washed with satd aq. $NaHCO_3$ solution (10 mL); then dried and concentrated. The residue was purified by flash chromatography, (petroleum ether/ethyl acetate, 99:1) to give **6** as a colorless oil (0.84 g, 80%). $[\alpha]_D = +16.7$ (*c* 1); ^{1}H NMR δ 0.87 (t, 3H, $J = 9.5$ Hz, CH_3), 1.20–1.68 (m, 32H, CH_2), 2.03 (m, 2H, $=CHCH_2$), 3.81 (dd, 1H, $J = 8.5$ Hz, $J = 6.0$ Hz, OCH_aH_b), 4.01 (dd, 1H, $J = 8.5$ Hz, $J = 6.5$ Hz, OCH_aH_b), 4.30 (m, 1H, $OCHCH_2$), 5.43–5.52 (m, 2H, $CH{=}CHCH_2$, $CHOCOPh$), 5.90 (dt, 1H, $J = 15.0$ Hz, 7.0 Hz, $CH{=}CHCH_2$), 7.42 (m, 2H, Ph), 7.53 (m, 1H, Ph), 8.04 (m, 2H, Ph); ^{13}C NMR δ 14.0, 22.6, 23.8 (2C), 25.0, 28.7–29.5 (9C), 31.8, 32.3, 34.9, 36.0, 65.4, 75.8, 76.3, 110.5, 123.8, 128.2 (2C), 129.6 (2C), 129.9, 132.8, 137.6, 165.6; IR: 2890, 2810, 1700, 1450, 1430, 1250, 1240, 1230, 1170, 1150, 1100, 1050, 1010, 950, 690 cm^{-1}; MS (EI), *m*/*e* 484 [M^+]. Anal. calcd for $C_{31}H_{48}O_4$: C, 76.82; H, 9.98. Found: C, 76.70; H, 9.92%.

4.6. (2*R*,3*R*,4*E*)-1-*O*-Thexyldimethylsilyl-3-*O*-benzoyl-4-octadecen-1,2,3-triol 8

Compound **6** (0.84 g, 1.73 mmol) was diluted with 60% aq. trifluoroacetic acid (6 mL) and stirred for 3 h at 0°C; satd aq. $NaHCO_3$ solution mixed with ice was added until neutralization and the suspension was extracted with ethyl acetate (3×50 mL), the combined organic layers were dried and concentrated. The crude **7** (0.82 g), was used in the next step without purification. A sample was purified by flash chromatography (petroleum ether/ethyl acetate, 8:2) to afford (2*R*,3*R*,4*E*)-3-*O*-benzoyl-4-octadecen-1,2,3-triol **7** as a colorless oil; ^{1}H NMR and $[\alpha]_D$ were in agreement with those reported in Ref. 8. ^{13}C NMR δ 14.8, 23.4, 29.5, 29.8, 30.1–30.3 (7C), 32.6, 33.0, 63.8, 74.3, 76.8, 124.6, 129.1 (2C), 130.4 (2C), 130.7, 133.9, 138.4, 166.9.

To a solution of the crude **7** (0.82 g) in pyridine (10 mL) at 0°C was added thexyldimethylsilyl chloride (0.51 mL, 2.60 mmol). The solution was stirred overnight at rt, then diluted with dichloromethane (30 mL) and washed with satd aq. $NaHCO_3$ solution (20 mL). The aq. layer was extracted with dichloromethane (3×20 mL) and the combined organic layers were dried and concentrated. The crude was submitted to flash chromatography (petroleum ether/ethyl acetate, 10:1) to give **8** (0.76 g, 80%) as a colorless oil. Physical data were in agreement with those reported in Ref. 11.

4.7. (2*R*,3*R*,4*E*)-3-*O*-Benzoyl-2-*O*-chloromethylsulfonyl-1-*O*-thexyldimethylsilyl-4-octadecen-1,2,3-triol 9

To a solution of **8** (0.46 g, 0.84 mmol) in pyridine (10 mL) at 0°C methanesulfonyl chloride was added (0.11 mL, 1.26 mmol). The solution was stirred for 2 h at rt, then it was diluted with water and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with 1 M aq. HCl solution and brine, dried and purified by flash chromatography (hexane/ethyl acetate, 10:1) to give **9** (0.48 g, 86%) as a colorless oil.

$[\alpha]_D = -1.6$ (*c* 1); ^{1}H NMR δ 0.09 (s, 3H, $(CH_3)_2Si$), 0.10 (s, 3H, $(CH_3)_2Si$), 0.82–0.94 (m, 15H, $5CH_3$), 1.20–1.40 (m, 22H, CH_2), 1.60 (heptet, 1H, $J=6.0$ Hz, $CH(CH_3)_2$), 2.04 (m, 2H, $CH{=}CHCH_2$), 3.86 (dd, 1H, $J=11.5$ Hz, $J=5.0$ Hz, OCH_aH_b), 3.94 (dd, 1H, $J=11.5$ Hz, $J=3.0$ Hz, OCH_aH_b), 4.55–4.60 (m, 2H, SO_2CH_2Cl), 4.86–4.93 (m, 1H, $OCHCH_2$), 5.46 (dd, 1H, $J=15.5$ Hz, $J=7.5$ Hz, $CH{=}CHCH_2$), 5.72 (t, 1H, $J=7.5$ Hz, CHOCOPh), 5.98 (dt, 1H, $J=15.5$ Hz, $J=6.5$ Hz, $CH{=}CHCH_2$), 7.45 (m, 2H, Ph), 7.55 (m, 1H, Ph), 8.10 (m, 2H, Ph); ^{13}C NMR δ –2.9 (2C), 14.8, 19.2 (2C), 20.9, 21.0, 23.4, 26.0, 29.3–30.3 (9C), 32.6, 33.0, 34.8, 54.9, 62.8, 73.6, 86.3, 123.4, 129.1 (2C), 130.5 (3C), 133.9, 139.7, 165.9; IR: 2910, 2890, 2820, 1700, 1450, 1370, 1240, 1160, 1080, 1000, 860, 810, 690 cm^{-1}; MS (CI), *m*/*e* 676 $[M+NH_4]^+$. Anal. calcd for $C_{34}H_{59}ClO_6SSi$: C, 61.93; H, 9.02. Found: C, 61.60; H, 9.31%.

4.8. (2*S*,3*R*,4*E*)-2-Azido-3-*O*-benzoyl-1-*O*-thexyldimethylsilyl-4-octadecen-1,2,3-triol 10

To a stirred solution of **9** (0.47 g, 0.71 mmol) in dry DMF (7 mL) NaN_3 (0.28 g, 4.26 mmol) was added, and the mixture was stirred at 85°C for 2 h. The reaction mixture was diluted with water and extracted with diethyl ether (3×10 mL). The organic layer was washed with satd brine, dried and purified by flash chromatography (petroleum ether/ethyl acetate, 10:0.2) to give **10** (0.34 g, 85%) as a colorless oil. Physical data were in agreement with those reported in Ref. 11.

4.9. (2*S*,3*R*,4*E*)-2-Azido-3-*O*-benzoyl-4-octadecen-1,2,3-triol 1

Compound **10** (0.12 g, 0.22 mmol) was dissolved in HF solution (2%, 4.4 mL), prepared by adding 40% aq. HF to a solution of CH_3CN:THF (9:1). The solution was stirred at rt overnight, then diluted with dichloromethane (100 mL) and washed with satd aq. $NaHCO_3$ solution (100 mL). The aq. layer was extracted with dichloromethane (3×30 mL) and the combined organic layers were dried. The crude was purified by flash chromatography (hexane/ethyl acetate, 10:1.5) to afford **1** as a colorless oil (0.08 g, 85%). Physical data were in agreement with those reported in Refs. 3 and 18.

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