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Note

A short and practical route to 3-*O*-benzoyl azidosphingosine

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Abstract

A short and practical route to 3-*O*-benzoyl azidosphingosine from D-xylose is described. The synthesis avoids the use of expensive and hazardous chemicals (i.e. mercury salts), and it is reproducible up to at least a 20 g scale. Furthermore, the synthesis proceeds to 3-*O*-benzoyl azidosphingosine with a minimum of protection group manipulation, by exploiting a regioselective protection of the primary HO-1 with thexyldimethylsilyl chloride.

Keywords: Glycolipid; Azidosphingosine; Synthesis

Glycosphingolipids are ubiquitous components of cell membranes, where they function as receptors for proteins, antibodies, and other biomolecules, for example during the initial phase of infection by pathogens.[1] An important component of the glycosphingolipids is D-*erythro*-sphingosine. 3-*O*-Benzoyl azidosphingosine (**1**) is the preferable glycosyl acceptor in the preparation of these glycolipids, since it minimizes the problem with inferior yield of byproducts that generally accompanies glycosylations of suitably protected ceramide or sphingosine derivatives.[2–4] A number of syntheses of azidosphingosine have been published.[3,5–9] In our study, a synthesis involving a Grignard reagent for the carbon–carbon bond-formation[8] has been highly reproducible, and thus, suitable for large scale preparation. However, the published procedure uses mercury salts and involves three protection and deprotection steps for the transformation of azidosphingosine into **1**. We herein describe a modified synthesis that avoids expensive chemicals, the use of mercury salts and is reproducible on up to at least a 20 g scale. Furthermore it proceeds with a minimum protection groups manipulations to 3-*O*-benzoyl azidosphingosine (**1**).

The synthesis of **1** starts with the conversion of D-xylose to dithioethyl protected xylose,[10,11] which without purification was converted to **2**[12] with 2,2-dimethoxypropane and a catalytic amount *p*-TsOH (Scheme 1). Compound **2** was purified by dry column chromatography[13] giving 90 g in three 400 mL portions of solvent. Deprotection of the dithioacetal **2** was accomplished using I_2 and $NaHCO_3$ in ace-

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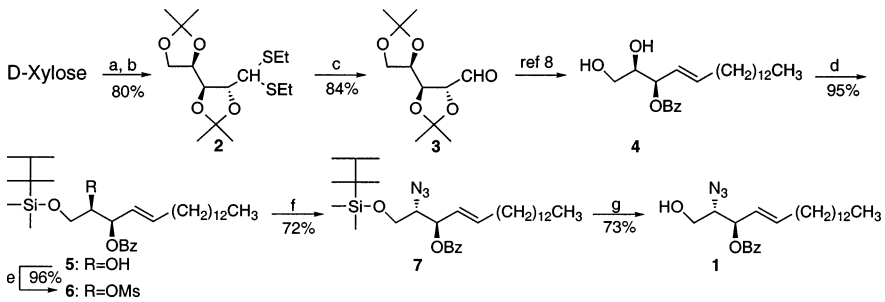

Scheme 1. (a) Aq HCl, EtSH.[10,11] (b) 2,2-Dimethoxypropane, *p*-TsOH. (c) I_2, $NaHCO_3$, acetone, H_2O. (d) $ThexMe_2SiCl$, pyridine. (e) MsCl, pyridine. (f) NaN_3, 18-crown-6, DMF, 95 °C. (g) 1% aq HCl in EtOH.

tone–water[14] giving a yield of **3** equivalent to that reported using $HgO–HgCl_2$.[11] Compound **4** was prepared from **3** according to the reported procedure[8] and then selectively silylated in high yield with thexyldimethylsilylchloride in pyridine at room temperature to furnish **5**. Other protection groups and conditions (for example TIPS–ether, TBDMS–ether and chloroacetate) were evaluated, but they showed lower regioselectivity, gave benzoyl migration, or lower yields in the following steps. Treating compound **5** with methanesulfonyl chloride in pyridine at room temperature gave the mesylate **6**. Attempts were made to use trifluoromethanesulfonate as a leaving group, but this turned out to be unstable. Substitution of the mesylate with NaN_3 using 18-crown-6 as catalyst in DMF at 95°C for 4 days furnished the fully protected azidosphingosine in 72% yield. Basic cleavage (F^-) of the silyl protection group gave benzoyl migration as a major side reaction. Fortunately, mild acidic hydrolysis[15] furnished the 3-*O*-benzoylprotected azidosphingosine (**1**) in good yield after purification on a chromatotron.

1. Experimental

General methods.—NMR spectra were recorded with a 400 MHz instrument. ^1H NMR spectral assignments were made by COSY experiments. Concentrations were made using rotary evaporation with a bath temperature at or below 40°C. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Flash chromatography was performed on Grace Amicon Silica gel 60 (0.035–0.070 mm) and TLC was performed on Kieselgel 60 F_{254} plates (E. Merck). Dichloromethane was distilled from CaH_2. Pyridine was stored over 4 Å MS. DMF was distilled under reduced pressure.

*(2*S*,3*R*,4*E*)-2-Azido-3-benzoyloxy-octadec-4-ene-1-ol* (**1**).—To compound **7** (631 mg, 1.10 mmol) was added a 1% mixture of concd aq HCl in EtOH (19 mL) and the solution was stirred at rt overnight. A second aliquot of concd aq HCl (0.2 mL) was added and the resulting solution was stirred for 2 days. Evaporation under reduced pressure gave a crude that was purified as reported[7] to give **1** (347 mg, 73%). ^1H NMR and $[\alpha]_D^{25}$ were in agreement with reported data.[2,7]

2,3:4,5-Di-O-*isopropylidene-1,1-dithioethyl*-D-*xylose* (**2**).—Crude 1,1-dithioethyl-D-xylose[11] was dissolved in 2,2-dimethoxypropane (400 mL), a catalytic amount of *p*-toluenesulfonic acid was added, and the resulting mixture was stirred overnight. Aq NH_3 (25%) was added until the reaction mixture was neutral (moist pH paper), and the mixture was concentrated and flash chromatographed (dry column SiO_2 technique, 10:1 → 6:1 heptane–EtOAc gradient) to give **2** (90 g, 80%). ^1H NMR was in agreement with reported data.[16]

2,3:4,5-Di-O-*isopropylidene*-D-*xylose* (**3**).—To a solution of **2** (5.00 g, 14.9 mmol) in acetone (100 mL) and water (2.3 mL) at 0°C was added $NaHCO_3$ (5.5 g, 65.4 mmol) and I_2 (8.3 g, 32.7 mmol). The mixture was slowly warmed to rt and stirred overnight. A second portion of $NaHCO_3$ (0.62 g, 7.4 mmol) and I_2 (1.89 g, 7.4 mmol) was added. After 5 h, a satd aq solution of $Na_2S_2O_3$ was added until the reaction mixture was colorless. The mix-

ture was partly concentrated and the aq phase was saturated with NaCl and extracted with EtOAc (6 × 100 mL). The combined extracts were dried (Na_2SO_4), concentrated, and flash chromatographed (4:1 → 1:2 heptane–EtOAc gradient) to give **3** (3.12 g, 84%). ^{1}H NMR was in agreement with reported data.[17]

*(2*S*,3*R*,4*E*)-3-Benzoyloxy-1-thexyldimethylsilyloxy-octadec-4-ene-2-ol* (**5**).—To a solution of **4** (1.00 g, 2.47 mmol) in anhyd pyridine (14 mL) at −40°C was added thexyldimethylsilylchloride (0.580 mL, 2.97 mmol). The mixture was stirred overnight at rt, then diluted with CH_2Cl_2 (20 mL), and washed with satd aq $NaHCO_3$ (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 10 ml), and the organic phases were combined, dried (Na_2SO_4), concentrated, and flash chromatographed (10:1 heptane–EtOAc) to give **5** (1.28 g, 95%). $[\alpha]_D^{23}$ +12 (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.10–8.06 (m, 2 H, Ph), 7.59–7.54 (m, 1 H, Ph), 7.47–7.43 (m, 2 H, Ph), 5.95–5.88 (m, 1 H, =C*H*–CH_2), 5.61–5.52 (m, 2 H, =C*H*–C*H*OBz), 3.85–3.80 (m, 1 H, C*H*OH), 3.70 (ddd, 2 H, *J* 4.0, 6.2, 11.2 Hz, SiOCH_2), 2.51 (d, 1 H, *J* 6.3 Hz, OH), 2.10–2.04 (m, 2 H, =CH–CH_2), 1.64 (heptet, 1 H, *J* 6.9 Hz, C*H*Me_2), 1.40–1.23 (m, 22 H, CH_2), 0.91–0.87 (m, 15 H, (CH_3)$_2$CH, CH_3, (CH_3)$_2$C)), 0.12 (s, 6 H, (CH_3)$_2$Si). ^{13}C NMR ($CDCl_3$): δ 166.3, 137.6, 133.3, 130.9, 130.1, 128.7, 124.9, 76.3, 73.7, 63.7, 34.6, 32.8, 32.4, 30.1, 30.1, 30.0, 29.9, 29.8, 29.6, 29.3, 29.3, 25.6, 23.1, 20.7, 20.7, 19.0, 19.0, 14.6, −3.1. HRMS Calcd for $C_{33}H_{58}O_4SiNa$ (M + Na): *m*/*z* 569.4002; Found: *m*/*z* 569.4002.

*(2*S*,3*R*,4*E*)-3-Benzoyloxy-2-methylsulfonyloxy-1-thexyldimethylsilyloxy-octadec-4-ene* (**6**).—To a solution of **5** (1.23 g, 2.25 mmol) in anhyd pyridine (20 mL) at 0°C was added methanesulfonyl chloride (0.261 mL, 3.38 mmol) and the mixture was stirred at rt overnight. The reaction was quenched by addition of MeOH (4 mL), and the mixture was co-concentrated with toluene. The residue was flash chromatographed (10:1 heptane–EtOAc) to give **6** (1.35 g, 96%). $[\alpha]_D^{23}$ −0.6 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.11–8.08 (m, 2 H, Ph), 7.61–7.56 (m, 1 H, Ph), 7.49–7.44 (m, 2 H, Ph), 6.04–5.95 (m, 1 H, =C*H*–CH_2), 5.76 (t, 1 H, *J* 7.4 Hz, C*H*OBz), 5.50 (dd, 1 H, *J* 7.6, 15.6 Hz, =C*H*–CHOBz), 4.85–4.81 (m, 1 H, C*H*OMs), 3.94 (dd, 1 H, *J* 3.4, 11.6 Hz, SiOCH_2), 3.86 (dd, 1 H, *J* 4.8, 11.4 Hz, SiOCH_2), 2.99 (s, 3 H, SAc), 2.10–2.04 (m, 2 H, =CH–CH_2), 1.64 (heptet, 1 H, *J* 6.9 Hz, C*H*Me_2), 1.40–1.23 (m, 22 H, CH_2), 0.91–0.87 (m, 15 H, (CH_3)$_2$CH, CH_3, (CH_3)$_2$C), 0.12 (s, 6 H, (CH_3)$_2$Si). ^{13}C NMR ($CDCl_3$): δ 165.6, 139.0, 133.6, 130.4, 130.2, 128.9, 123.4, 83.4, 73.6, 62.7, 39.2, 34.5, 32.8, 32.4, 32.3, 30.1, 30.1, 30.0, 29.9, 29.8, 29.6, 29.5, 29.1, 25.7, 23.1, 20.7, 20.6, 19.0, 18.9, 14.6, −3.1, −3.2. HRMS Calcd for $C_{34}H_{60}O_6SSiNa$ (M + Na): *m*/*z* 647.3778; Found: *m*/*z* 647.3771.

*(2*R*,3*R*,4*E*) - 2 - Azido - 3 - benzoyloxy - 1-thexyldimethylsilyloxy-octadec-4-ene* (**7**).—To a solution of **6** (3.70 g, 5.92 mmol) in freshly distilled DMF (50 mL) was added NaN_3 (3.9 g, 59 mmol) and a catalytic amount of 18-crown-6, and the mixture was stirred at 95°C for 4 days. The reaction mixture was poured into 1:1 Et_2O–water (400 mL) and the aqueous phase was extracted with Et_2O (3 × 80 mL). The organic phases were combined, washed with brine (1 × 80 mL), dried ($MgSO_4$), concentrated, and flash chromatographed (19:1 → 10:1 heptane–EtOAc gradient) to give **7** (2.44 g, 72%) and recovered starting material (0.61 g, 16%). $[\alpha]_D^{23}$ −17 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.09–8.06 (m, 2 H, Ph), 7.61–7.56 (m, 1 H, Ph), 7.49–7.44 (m, 2 H, Ph), 5.97–5.90 (m, 1 H, =C*H*–CH_2), 5.60–5.54 (m, 2 H, =C*H*–C*H*OBz), 3.80 (m, 1 H, C*H*N_3), 3.73–3.65 (m, 2 H, SiOCH_2), 2.11–2.06 (m, 2 H, =CH–CH_2), 1.64 (heptet, 1 H, *J* 6.9 Hz, C*H*Me_2), 1.40–1.23 (m, 22 H, CH_2), 0.91–0.87 (m, 15 H, (CH_3)$_2$CH, CH_3, (CH_3)$_2$C), 0.12 (s, 6 H, (CH_3)$_2$Si). ^{13}C NMR ($CDCl_3$): δ 165.7, 139.0, 133.5, 130.5, 130.2, 128.9, 123.6, 75.0, 66.3, 62.9, 34.5, 32.8, 32.4, 30.1, 30.1, 30.0, 29.9, 29.8, 29.6, 29.2, 25.5, 23.1, 20.6, 20.6, 18.9, 18.9, 14.6, −3.2, −3.2. HRMS Calcd for $C_{33}H_{57}N_3O_3SiNa$ (M + Na): *m*/*z* 594.4067; Found: *m*/*z* 594.4062.

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