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Synthesis of fluorescent lactosylceramide stereoisomers

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Abstract

The intracellular distribution of synthetic glycosphingolipids (GSLs) bearing a fluorophore can be monitored in living cells by fluorescence microscopy. We reported previously that variation in the length of the long-chain base and in the structure of the carbohydrate-containing polar head group of (2*S*,3*R*) (or D-*erythro*-)-β-lactosylceramide (LacCer) did not alter the mechanism of endocytic uptake from the plasma membrane of various mammalian cell types [Singh, R.D., Puri, V., Valiyaveettil, J.T., Marks, D.L., Bittman, R., Pagano, R.E., 2003. Selective caveolin-1-dependent endocytosis of glycosphingolipids. Mol. Biol. Cell 14, 3254–3265]. To extend our examination of the molecular features in LacCer that are responsible for its uptake by the caveolar-requiring endocytic pathway, we have synthesized the three unnatural stereoisomers [(2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)] of dipyrromethene difluoride (BODIPY^{TM})-LacCer. These analogues will be used to probe the role of stereochemistry in the long-chain base of LacCer in the mechanism of endocytic uptake.

Keywords: Fluorescent lipid analogues; Glycosphingolipids; Lipid synthesis

1. Introduction

A boron dipyrromethene difluoride (BODIPY^{TM}) (Johnson et al., 1991) fluorophore linked to the long-chain base of naturally occurring (2*S*,3*R*)-β-lactosylceramide (LacCer) via the ω end of a *N*-pentanoyl moiety (compound **a** in Fig. 1) has been used to examine the intracellular trafficking of this and other glycosphingolipids (GSLs) in normal and disease cell types (Pagano et al., 2000). This GSL was localized in lysosomes of a diseased cell type, but was observed at the Golgi complex in normal fibroblasts (Chen et al., 1998). (2*S*,3*R*)-C_5-BODIPY^{TM}-LacCer (which is available commercially) and a synthetic analogue bearing a maltosyl polar head group (2*S*,3*R*)-C_5-BODIPY^{TM}-MalCer, utilized the same caveolar-dependent endocytic pathway for uptake from the plasma membrane of different cells (Singh et al., 2003; Bittman, 2004). In contrast, BODIPY^{TM}-sphingomyelin utilizes both a clathrin-dependent and a caveolar-dependent pathway in approximately equal extents for internalization (Puri et al., 2001). To examine the role of stereochemistry at C2 and C3 of the sphingosine chain of LacCer in determining the mechanism of endocytosis, we have prepared the following unnatural stereoisomeric analogues: (2*R*,3*R*)-, (2*S*,3*S*)-, and (2*R*,3*S*)-BODIPY^{TM}-LacCer (compounds **b**–**d** in Fig. 1).

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

The sources of the chemicals were as follows: BODIPY^{TM}-C_5-*N*-hydroxysuccinimidoyl (NHS) ester,

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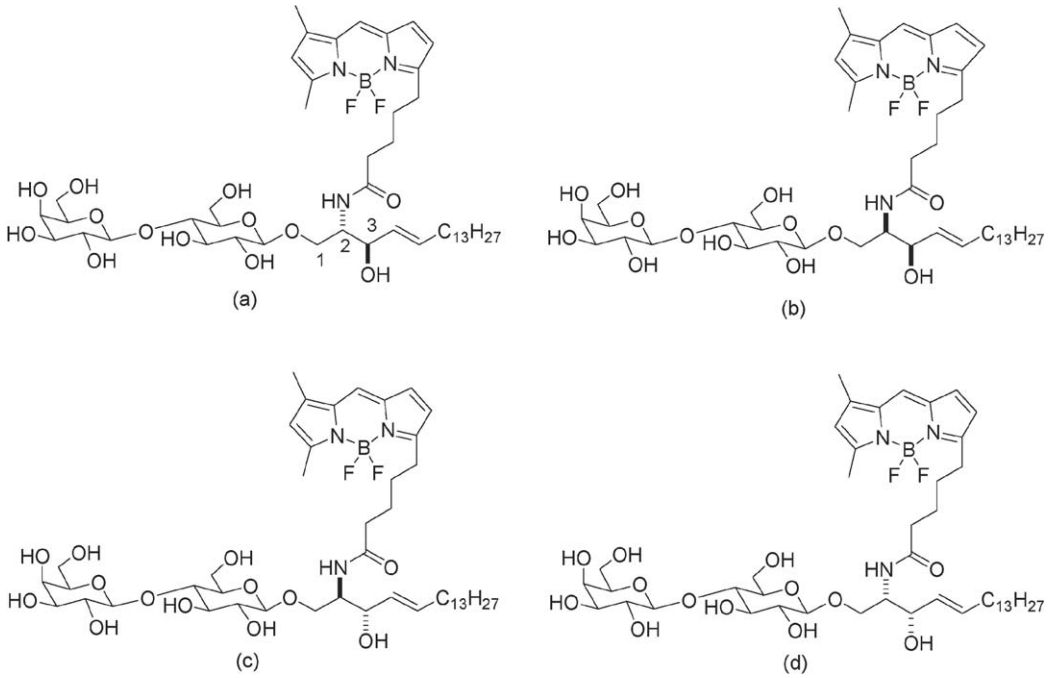


Fig. 1. Structures of (a) (2*S*,3*R*) (or D-*erythro*); (b) (2*R*,3*R*) (or D-*threo*); (c) (2*R*,3*S*) (or L-*erythro*); and (d) (2*S*,3*S*) (or L-*threo*)-BODIPY^{TM}-LacCer.

Invitrogen/Molecular Probes (Eugene, OR); *N*-Boc-D-serine and diisobutylaluminum hydride (DIBAL-H, a 20 wt.% solution in toluene), Acros (Morris Plains, NJ); L-*threo*-sphingosine, Avanti Polar Lipids (Alabaster, AL); 1-pentadecyne, *p*-toluenesulfonic acid monohydrate (*p*-TsOH), and sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al, a 70%, w/w solution in toluene), Alfa Aesar/Lancaster (Pelham, NH); β-D-lactosyl octaacetate, triphenylphosphine, trichloroacetonitrile, *tert*-butyldiphenylsilyl chloride (TBDPSCl), hydrazine acetate, benzoic anhydride, $BF_3{\cdot}OEt_2$, imidazole, 4-(dimethylamino)pyridine (DMAP), and $(n\text{-Bu})_4NF$ (TBAF), Sigma–Aldrich. Trifluoromethanesulfonyl azide (TfN_3) was prepared according to Vasella et al. (1991). Hepta-*O*-acetyllactosyl-1-trichloroacetimidate (compound **13**) was synthesized from per-*O*-acetyllactose as described (Amvam-Zollo and Sinay, 1986). Molecular sieves (300AW) were dried for 5 h at 150 °C and stored under vacuum over P_2O_5.

2.1.2. General methods

Air- and moisture-sensitive reactions were carried out under nitrogen in flame-dried glassware. THF and toluene were distilled from sodium/benzophenone and dichloromethane was distilled from calcium hydride prior to use. DMF was dried over calcium hydride. TLC was performed using aluminum-backed or glass-backed silica gel 60 F254 plates (0.25-mm thick), and the compounds were visualized by charring with 10% H_2SO_4 in EtOH or by UV light. Column chromatography was carried out with silica gel 60 (230–400 mesh) using the elution solvents indicated in the text. Suspended silica gel was removed by filtration through an Osmonics Cameo filter (Fisher Scientific, Pittsburgh, PA). The ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, and were referenced to the residual $CHCl_3$ at δ 7.24 (^1H) and the central line of $CDCl_3$ at δ 77.0 ppm (^{13}C). Optical rotations were measured on a digital polarimeter at room temperature in the solvents stated.

2.2. Synthesis

2.2.1. *N*-[(1,1-Dimethylethoxy)carbonyl]-D-serine methyl ester (**2**)

To a cold solution of *N*-Boc-D-serine (compound **1** in Scheme 1, 3.0 g, 14.6 mmol) in DMF (20 ml) was added potassium carbonate (2.28 g, 16.5 mmol). After the mixture was stirred for 10 min in an ice-water bath, methyl iodide (1.88 ml, 4.26 g, 30 mmol) was added to the white suspension, and stirring was continued at 0 °C for 30 min, whereupon the mixture solidified. The reaction mixture was warmed to room temperature and stirred for an additional hour. The reaction mixture was filtered by suction and the filtrate was partitioned between EtOAc (30 ml) and water (30 ml). The organic phase was washed with brine (2 × 30 ml), dried (Na_2SO_4), filtered, and con-

centrated to give 2.76 g (86%) of compound **2** as a pale amber oil, which was used without further purification.

2.2.2. 3-(1,1-Dimethylethyl) 4-methyl-(R)-2,2-dimethyl-3,4-oxazolidinedicarboxylate (3)

To a 250 ml round-bottomed flask were added a solution of compound **2** (2.76 g, 12.5 mmol) in benzene (75 ml), 2,2-dimethoxypropane (DMP, 2.61 g, 25 mmol), and *p*-TsOH (33 mg, 0.18 mmol). The colorless solution was heated under reflux for 1 h, then slowly distilled until a volume of 65 ml was collected over 30 min. The cooled, amber solution was partitioned between saturated $NaHCO_3$ solution (20 ml) and Et_2O (2 × 50 ml). The organic layer was washed with brine (20 ml), then dried (Na_2SO_4), filtered, and concentrated to give crude product **3** as an amber oil. The material was vacuum distilled to give 2.68 g (80%) of compound **3** as a pale yellow liquid, bp 101–102 °C (2 mm Hg); ^{1}H NMR ($CDCl_3$) δ 4.48–4.37 (m, 1H), 4.18–4.12 (m, 1H), 1.67–1.64 (m, 3H), 1.53–1.41 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 171.4, 151.2, 95.1, 80.4, 66.3, 59.3, 52.4, 28.4, 28.3, 27.3, 26.0, 25.2, 25.0, 24.4.

2.2.3. 1,1-Dimethylethyl (R)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (4)

A solution of compound **3** (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-H 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 N HCl with swirling over 15 min, and the aqueous mixture was extracted with EtOAc (3 × 50 ml). The combined organic layers were washed with brine (50 ml), dried (Na_2SO_4), filtered, and concentrated to give the crude product as a colorless oil. The material was vacuum distilled to give 1.72 g (75%) of compound **4** as a colorless liquid, bp 83–88 °C (1.0–1.4 mm Hg).

2.2.4. tert-Butyl (4R,1′R)-2,2-dimethyl-4-(1′-hydroxyhexadec-2′-ynyl)oxazolidione-3-carboxylate (5)

n-Butyllithium (2.5 M in hexane, 2.0 ml, 5.0 mmol) was added dropwise to a solution of 1-pentadecyne (832 mg, 4.0 mmol) in dry Et_2O (20 ml) at −20 °C (see Scheme 2). After the white suspension was stirred at −20 °C for 1 h, anhydrous $ZnBr_2$ (1.2 g, 5.0 mmol) was added at 0 °C, with stirring for 1 h at 0 °C and 1 h at room temperature. A solution of compound **4** (690 mg, 3.0 mmol) in dry Et_2O (10 ml) was added dropwise at −78 °C. The reaction mixture was allowed to warm to room temperature overnight. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (20 ml) at −20 °C. After dilution with water (20 ml), the aqueous layer was separated and extracted with Et_2O (2 × 20 ml). The combined organic layers were washed with brine (10 ml), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 814 mg (62%) of compound **5**; R_f 0.45 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 4.37–4.36 (m, 1H), 2.46–2.45 (m, 1H), 1.89–1.88 (m, 1H), 1.74–1.68 (m, 2H), 1.47–1.45 (m, 2H), 1.40–1.26 (m, 37H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 82.7, 70.4, 60.0, 35.3, 29.5, 27.3, 27.2, 27.16, 27.12, 27.0, 26.9, 22.6, 20.3, 11.7.

2.2.5. tert-Butyl (1R,2R)-N-[2-hydroxy-1-(hydroxymethyl)-3-heptadecynyl]-carbamate (6)

To a solution of 0.70 g (1.60 mmol) of compound **5** in 10 ml of MeOH was added 0.50 g of Amberlyst 15 resin. After the heterogeneous mixture was stirred at room temperature for 48 h, the mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification by chromatography (elution with hexane/EtOAc 1:1) gave 480 mg (75%) of compound **6** as a white solid; R_f 0.52 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.18 (m, 1H), 4.60 (s, 1H), 3.83–3.77 (m, 3H), 3.35 (s, 1H), 2.91 (s, 1H), 2.22–2.18 (m, 2H), 1.52–1.30 (m, 31H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 156.4, 87.4, 80.0, 78.1, 63.6, 62.9, 55.9, 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 28.9, 28.6, 28.3, 28.1, 22.7, 18.7, 14.1.

2.2.6. tert-Butyl (3E,1R,2R)-N-[2-hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-carbamate (7)

To a solution of 440 mg (1.0 mmol) of compound **6** in dry Et_2O (20 ml) was added dropwise 3.0 ml (10.5 mmol) of Red-Al (a 3.5 M solution in toluene) at 0 °C under nitrogen. After the reaction mixture was stirred at room temperature for 24 h, the reaction was quenched by the slow addition of 3 ml of MeOH at 0 °C. The product was extracted with EtOAc (3 × 20 ml), and the combined organic layers were washed with brine (10 ml), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 264 mg (60%) of compound **7** as a white solid; R_f 0.38 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.75 (m, 1H), 5.53 (m, 1H), 5.18 (m, 1H), 4.33 (s, 1H), 3.80–3.55 (m, 3H), 2.71 (s, 2H), 2.05 (m, 2H), 1.45–1.05 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 156.6, 134.0, 129.0, 79.8, 73.5, 64.4, 55.5, 32.3, 31.9, 29.69, 29.66, 29.6, 29.5, 29.4, 29.2, 29.1, 28.4, 22.7, 14.1.

*2.2.7. D-threo-Sphingosine (**8**)*

A solution of 240 mg (0.60 mmol) of compound **7** in 5 ml of 1 M HCl and 5 ml of THF was heated at 70 °C with stirring for 8 h under nitrogen. The reaction mixture was cooled to room temperature and neutralized with saturated aqueous $NaHCO_3$ solution (5 ml). The product was extracted with EtOAc (3 × 20 ml), and the combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 140 mg (78%) of compound **8** as a white powder, which was used without further purification.

*2.2.8. (2R,3R,4E)-2-Azido-octadec-4-ene-1,3-diol (**9**)*

Dichloromethane (10 ml) and DMAP (150 mg, 1.23 mmol) were added to compound **8** (120 mg, 0.40 mmol), followed by dropwise addition of TfN_3 in CH_2Cl_2 (0.4 M solution, 10 ml, 4.0 mmol) (see Scheme 3). The reaction mixture was stirred at room temperature for 24 h, and then concentrated under reduced pressure. The residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 60 mg (46%) of azido diol **9**; R_f 0.60 (hexane/EtOAc 1:1); $[\alpha]_D^{25}$ −3.05° (*c* 2.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.78 (m, 1H), 5.52 (m, 1H), 4.21 (m, 1H), 3.80 (m, 1H), 3.72 (m, 1H), 2.42 (s, 2H), 2.07 (m, 2H), 1.45–1.18 (m, 22H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 135.5, 128.2, 73.5, 67.6, 62.9, 32.3, 31.9, 29.7, 29.6, 29.5, 29.47, 29.36, 29.2, 29.1, 28.9, 22.7, 14.1.

*2.2.9. (2R,3R,4E)-2-Azido-1-(tert-butyldiphenylsilanyloxy)-octadec-4-en-3-ol (**10**)*

A solution of TBDPSCl (50 mg, 0.18 mmol) and imidazole (25 mg, 0.36 mmol) in 10 ml of CH_2Cl_2 was stirred at room temperature for 1 h. A solution of compound **9** (55 mg, 0.167 mmol) in 5 ml of CH_2Cl_2 was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 68 mg (91%) of compound **10**; R_f 0.57 (hexane/EtOAc; 4:1); $[\alpha]_D^{25}$ −11.92° (*c* 2.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68–7.66 (m, 4H), 7.45–7.34 (m, 6H), 5.70 (m, 1H), 5.40 (m, 1H), 4.12 (m, 1H), 3.80 (m, 2H), 3.41 (m, 1H), 2.16 (s, 1H), 1.99 (m, 2H), 1.40–1.01 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 136.1, 135.63, 135.59, 135.2, 132.9, 132.8, 129.9, 129.7, 129.5, 128.3, 127.8, 127.7, 127.6, 127.3, 72.3, 67.9, 64.6, 32.3, 31.9, 29.70, 29.68, 29.6, 29.5, 29.4, 29.2, 29.1, 28.9, 28.4, 26.8, 22.7, 19.2, 14.1.

*2.2.10. (1′R,1R,2E)-Benzoic acid 1-[1′-azido-2′-(tert-butyldiphenylsilyloxy)-ethyl]-hexadec-2-enyl ester (**11**)*

To a solution of compound **10** (65 mg, 0.115 mmol) in 10 ml of dry CH_2Cl_2 was added DMAP (50 mg, 0.40 mmol), followed by the dropwise addition of a solution of benzoic anhydride (45 mg, 0.20 mmol) in 5 ml of CH_2Cl_2 at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 19:1) to give 70 mg (92%) of compound **11**; R_f 0.80 (hexane/EtOAc 4:1); $[\alpha]_D^{25}$ −4.82° (*c* 3.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.00 (m, 2H), 7.68–7.60 (m, 4H), 7.55 (m, 1H), 7.45–7.28 (m, 8H), 5.87 (m, 1H), 5.63 (m, 1H), 5.43 (m, 1H), 4.12 (m, 1H), 3.80 (m, 2H), 3.62 (m, 1H), 2.00 (m, 2H), 1.40–1.01 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.3, 137.7, 135.9, 133.3, 133.2, 133.1, 132.9, 132.8, 74.1, 65.9, 63.3, 32.3, 32.0, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 28.9, 28.7, 26.9, 26.7, 22.7, 19.2, 14.1.

*2.2.11. (1′R,1R,2E)-Benzoic acid 1-(1′-azido-2′-hydroxyethyl)hexadec-2-enyl ester (**12**)*

To a solution of compound **11** (68 mg, 0.10 mmol) and 50 mg (0.72 mmol) of imidazole in 5 ml of dry THF was added TBAF (0.2 ml, 0.20 mmol, a 1 M solution in THF) at −23 °C. The reaction mixture was stirred at −23 °C for 3 h, and was then quickly passed (to minimize benzoyl migration) through a silica gel column that was prewashed with cold elution solvent (elution with hexane/EtOAc 4:1) to give 26 mg (60%) of compound **12**; R_f 0.35 (hexane/EtOAc 4:1); $[\alpha]_D^{25}$ −8.34° (*c* 0.42, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 8.08 (m, 2H), 7.72–7.37 (m, 3H), 5.98 (m, 1H), 5.65 (m, 1H), 5.56 (m, 1H), 3.70 (m, 3H), 2.17 (m, 1H), 2.06 (m, 2H), 1.53–1.24 (m, 22H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.8, 138.1, 134.8, 133.4, 129.9, 129.6, 128.5, 127.7, 124.0, 74.7, 66.2, 61.7, 32.3, 31.9, 29.7, 29.6, 29.42, 29.37, 29.2, 28.7, 26.6, 22.7, 14.1.

*2.2.12. (1′R,1R,2E)-Benzoic acid 1-[1′-azido-2′-(β-hepta-O-acetyllactosyl)-ethyl]-hexadec-2-enyl ester (**14**)*

A mixture of 53 mg (0.068 mmol) of trichloroacetimidate **13** (see Scheme 4), 25 mg (0.058 mmol) of compound **12**, 200 mg of molecular sieves 300AW, and 5 ml of CH_2Cl_2 was stirred at room temperature for 1 h. A solution of $BF_3{\cdot}Et_2O$ (40 μl, 0.32 mmol) in 5 ml of CH_2Cl_2 was added, and the reaction mixture was stirred

overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 40 mg (66%) of compound **14**; R_f 0.55 (hexane/EtOAc 1:1); $[\alpha]_D^{25}$ −13.1° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.07 (m, 2H), 7.62–7.41 (m, 3H), 5.88 (m, 1H), 5.60 (m, 1H), 5.48 (m, 1H), 5.35 (m, 1H), 5.24–5.08 (m, 2H), 4.93 (m, 2H), 4.50 (m, 3H), 4.11 (m, 4H), 3.86 (m, 3H), 3.62 (m, 2H), 2.30–1.90 (m, 21H), 1.80–1.00 (m, 24H), 0.89 (t, 3H, J = 6.8 Hz).

2.2.13. (2R,3R,4E)-2-Azido-1-(β-hepta-O-acetyllactosyl)-octadec-4-en-3-ol (**15**)

A solution of 6 mg (0.26 mmol) of sodium in 1 ml of MeOH was added to 38 mg (0.036 mmol) of compound **14**. The reaction mixture was stirred for 6 h, the solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with MeOH/$CHCl_3$ 3:7) to give 16 mg (68%) of compound **15**; R_f 0.40 (MeOH/$CHCl_3$ 3:7); $[\alpha]_D^{25}$ −10.6° (*c* 0.80, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 5.58–5.30 (m, 2H), 5.03 (m, 1H), 4.15–2.95 (m, 17H), 1.00 (m, 24H), 0.89 (t, 3H, J = 6.8 Hz); HRMS (ESI) calcd for $C_{30}H_{55}N_3O_{12}Na$ $(M+Na)^+$ *m/z* 672.3683, found 672.3666.

2.2.14. C_5-BODIPY^{TM}-D-threo-LacCer (**16**)

BODIPY^{TM}-C_5-NHS (5 mg, 0.020 mmol), triphenylphosphine (6 mg, 0.023 mmol), 2.7 ml of THF, and 0.3 ml of water were added to 13 mg (0.020 mmol) of compound **15**. After the reaction, mixture was stirred overnight at room temperature, the solvents were removed under reduced pressure, and the residue was purified by chromatography (elution with MeOH/$CHCl_3$ 1:1) to give 7 mg (38%) of compound **16**; R_f 0.35 (MeOH/$CHCl_3$ 1:4); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.60 (m, 2H), 7.07 (s, 1H), 6.87 (s, 1H), 6.23 (m, 1H), 6.04 (m, 1H), 3.83–2.15 (m, 18H), 1.70–0.70 (m, 35H); LRMS (APCI, negative-ion mode) calcd for $C_{46}H_{74}BClF_2N_3O_{13}$ $(M+{}^{35}Cl)^-$ *m/z* 960.5, found 960.5; HRMS (EI) calcd for $C_{46}H_{75}N_3O_{13}F_2B$ (MH^+ of the boron-10 isotope) *m/z* 925.5397, found 925.5408.

2.2.15. tert-Butyl (4R,1′S)-2,2-dimethyl-4-(1′-hydroxyhexadec-2′-ynyl)oxazolidione-3-carboxylate (**17**)

n-Butyllithium (2.5 M in hexane, 2.0 ml, 5.0 mmol) was added dropwise to a solution of 1-pentadecyne (832 mg, 4.0 mmol) in dry THF (20 ml) at −20 °C (see Scheme 5). After the mixture was stirred at −20 °C for 2 h, HMPA (0.73 ml, 5.0 mmol) was added, followed by a solution of compound **4** (690 mg, 3.0 mmol) in dry THF (10 ml) at −78 °C. The reaction mixture was stirred for 1 h at −78 °C, allowed to warm to −20 °C within 2 h, and quenched by the addition of saturated aqueous NH_4Cl solution (20 ml). The mixture was diluted with water (20 ml), and the aqueous layer was separated and extracted with Et_2O (3 × 20 ml). The combined organic layers were washed with 0.5 N HCl (2 × 10 ml) and brine (10 ml), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 788 mg (60%) of compound **17**; R_f 0.48 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 4.74 (m, 1H), 4.51 (m, 1H), 4.10 (m, 2H), 3.90 (s, 1H), 2.19 (m, 2H), 1.65–1.45 (m, 15H), 1.40–1.20 (m, 22H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 152.1, 92.9, 84.6, 79.2, 75.9, 63.1, 62.1, 60.9, 29.9, 27.7, 27.6, 27.5, 27.4, 27.1, 26.9, 26.6, 26.4, 26.0, 23.8, 23.4, 21.0, 20.7,16.8, 12.1.

2.2.16. tert-Butyl (1R,2S)-N-[2-hydroxy-1-(hydroxymethyl)-3-heptadecynyl]-carbamate (**18**)

To a solution of 0.50 g (1.14 mmol) of compound **17** in 10 ml of MeOH was added 0.40 g of Amberlyst 15 resin, and the heterogeneous mixture was stirred at room temperature for 48 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification by chromatography (elution with hexane/EtOAc 1:1) afforded 329 mg (73%) of compound **18** as a white solid, which was used without further purification; R_f 0.55 (hexane/EtOAc 1:1).

2.2.17. tert-Butyl (3E,1R,2S)-N-[2-hydroxy-1-(hydroxymethyl)-3-heptadecenyl]-carbamate (**19**)

To a solution of 300 mg (0.76 mmol) of compound **18** in dry Et_2O (20 ml) was added dropwise 2.0 ml (7.0 mmol) of Red-Al (a 3.5 M solution in toluene) at 0 °C under nitrogen. After the reaction mixture was stirred at room temperature for 24 h, the reaction was quenched by the slow addition of 3 ml of MeOH at 0 °C. The product was extracted with EtOAc (3 × 20 ml), and the combined organic layers were washed with brine (10 ml), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 181 mg (60%) of compound **19** as a white solid; R_f 0.40 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.78 (m, 1H), 5.52 (m, 1H), 5.33 (m, 1H), 4.28 (m, 1H), 3.90 (m, 1H), 3.70 (m, 1H), 3.60 (s, 1H), 3.05 (s, 2H), 2.05 (m, 2H), 1.50–1.30 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 156.3, 134.1, 129.0, 79.8, 74.7, 62.61, 55.47, 32.3, 31.9, 31.7, 29.70, 29.67, 29.6, 29.5, 29.4, 29.3, 29.2, 28.4, 22.7, 14.1.

*2.2.18. L-erythro-Sphingosine (**20**)*

A solution of 160 mg (0.40 mmol) of compound **19** in 5 ml of 1 M HCl and 5 ml of THF was heated at 70 °C with stirring for 8 h under nitrogen. The reaction mixture was cooled to room temperature and neutralized with saturated aqueous $NaHCO_3$ solution (5 ml). The product was extracted with EtOAc (3 × 20 ml), and the combined organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated to give 90 mg (75%) compound **20** as a white powder, which was used without further purification.

*2.2.19. (2R,3S,4E)-2-Azido-octadec-4-ene-1,3-diol (**21**)*

The diazo transfer reaction was carried out as described for the synthesis of compound **9**. To compound **20** (80 mg, 0.27 mmol) were added CH_2Cl_2 (10 ml) and DMAP (100 mg, 0.82 mmol), followed by the dropwise addition of a solution of TfN_3 in CH_2Cl_2 (0.4 M solution, 7.0 ml, 2.8 mmol) (see Scheme 6), with stirring at room temperature for 24 h. Concentration gave a residue that was purified by chromatography (elution with hexane/EtOAc 1:1), affording 40 mg (46%) of azido diol **21**; R_f 0.50 (hexane/EtOAc 1:1); $[\alpha]_D^{25}$ +25.2° (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.80 (m, 1H), 5.53 (m, 1H), 4.25 (m, 1H), 3.78 (m, 2H), 3.50 (m, 1H), 2.23 (s, 2H), 2.08 (m, 2H), 1.40–1.20 (m, 22H), 0.88 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 136.0, 128.0, 73.8, 66.8, 62.6, 32.3, 31.9, 29.69, 29.66, 29.6, 29.5, 29.4, 29.2, 28.9, 22.7, 14.1.

*2.2.20. (2R,3S,4E)-2-Azido-1-(tert-butyldiphenylsilyloxy)-octadec-4-en-3-ol (**22**)*

The silylation reaction was carried out as described for the preparation of compound **10**. A solution of compound **21** (39 mg, 0.12 mmol) in 5 ml of CH_2Cl_2 was added to TBDPSCl (35 mg, 0.13 mmol) and imidazole (18 mg, 0.26 mmol) in 10 ml of CH_2Cl_2. The reaction mixture was stirred overnight, the solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 4:1) to give 56 mg (83%) of compound **22**; R_f 0.60 (hexane/EtOAc 4:1); $[\alpha]_D^{25}$ +10.7° (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.68–7.66 (m, 4H), 7.45–7.34 (m, 6H), 5.72 (m, 1H), 5.43 (m, 1H), 4.22 (m, 1H), 3.79 (m, 2H), 3.51 (m, 1H), 2.11 (s, 1H), 2.01 (m, 2H), 1.36–1.01 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 134.1, 134.0, 133.7, 133.6, 133.5, 131.9, 131.7, 131.2, 130.9, 128.0, 127.8, 127.6, 125.9, 125.8, 70.9, 65.0, 62.2, 50.3, 30.4, 30.0, 27.8, 27.7, 27.6, 27.5, 27.3, 27.1, 25.0, 24.9, 24.8, 20.8, 17.3, 17.2, 12.2.

*2.2.21. (1′R,1S,2E)-Benzoic acid 1-[1′-azido-2′-(tert-butyldiphenylsilanyloxy)-ethyl]-hexadec-2-enyl ester (**23**)*

Compound **23** was prepared by the method used to synthesize compound **11**. DMAP (40 mg, 0.32 mmol) was added to a solution of compound **22** (50 mg, 0.089 mmol) in 10 ml of dry CH_2Cl_2, followed by the dropwise addition of a solution of benzoic anhydride (34 mg, 0.15 mmol) in 5 ml of CH_2Cl_2 at 0 °C. After the reaction mixture was stirred overnight room temperature, concentration gave a residue that was purified by chromatography (elution with hexane/EtOAc 19:1), affording 53 mg (89%) of compound **23**; R_f 0.85 (hexane/EtOAc 4:1); $[\alpha]_D^{25}$ +11.6° (*c* 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.01 (m, 2H), 7.68–7.50 (m, 5H), 7.48–7.28 (m, 8H), 5.90 (m, 1H), 5.68 (m, 1H), 5.50 (m, 1H), 3.90–3.70 (m, 4H), 2.00 (m, 2H), 1.50–1.01 (m, 31H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.2, 135.6, 135.4, 133.1, 132.9, 132.7, 130.1, 129.8, 129.7. 128.4, 127.8, 123.2, 74.1, 65.9, 63.3, 31.9, 29.7, 29.6, 29.4, 19.1, 28.7, 26.7, 22.7, 19.2, 14.1.

*2.2.22. (1′R,1S,2E)-Benzoic acid 1-(1′-azido-2′-hydroxyethyl)hexadec-2-enyl ester (**24**)*

The desilylation reaction was carried out as described for the preparation of compound **11** (2 equiv of TBAF, ∼7 equiv of imidazole, dry THF, −23 °C). The reaction mixture was stirred at −23 °C for 3 h, and then was quickly passed through a silica gel column that was prewashed with cold elution solvent (elution with hexane/EtOAc 4:1) to give 19 mg (59%) of compound **24**; R_f 0.30 (hexane/EtOAc 4:1); $[\alpha]_D^{25}$ +38.2° (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 2H), 7.60–7.44 (m, 3H), 5.94 (m, 1H), 5.61 (m, 2H), 3.80 (m, 2H), 3.64 (m, 1H), 2.10 (m, 3H), 1.50–1.24 (m, 22H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 165.5, 138.9, 133.4, 129.8, 129.7, 128.5, 123.3, 74.6, 66.2, 62.0, 32.4, 31.9, 29.7, 29.6, 29.43, 29.37, 29.2, 28.7, 22.7, 14.1.

*2.2.23. (1′R,1S,2E)-Benzoic acid 1-[1′-azido-2′-(β-heptaacetyllactosyl)-ethyl]-hexadec-2-enyl ester (**25**)*

A mixture of 53 mg (0.068 mmol) of trichloroacetimidate **13**, 19 mg (0.044 mmol) of compound **24**, 100 mg of molecular sieves 300AW, and 5 ml of CH_2Cl_2 was stirred at room temperature for 1 h (see Scheme 7). Then a solution of $BF_3{\cdot}OEt_2$ (40 μl, 0.32 mmol) in 5 ml of CH_2Cl_2 was added, and the reaction mixture was stirred

overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 25 mg (54%) of compound **25**; R_f 0.50 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 7.97 (m, 2H), 7.56–7.38 (m, 3H), 5.80 (m, 1H), 5.63 (m, 1H), 5.50 (m, 1H), 5.40 (m, 1H), 5.28 (m, 1H), 5.10 (m, 2H), 4.89 (m, 2H), 4.45 (m, 3H), 4.05 (m, 4H), 3.80 (m, 3H), 3.55 (m, 2H), 2.10–1.86 (m, 21H), 1.35–1.12 (m, 24H), 0.80 (t, 3H, J = 6.8 Hz).

2.2.24. (2R,3R,4E)-2-Azido-1-(β-hepta-O-acetyllactosyl)-octadec-4-en-3-ol (**26**)

A solution of 5.0 mg (0.22 mmol) of sodium in 1 ml of MeOH was added to 21 mg (0.020 mmol) of compound **25**. After the reaction mixture was stirred for 6 h, the solvent was removed to give 8 mg (62%) of compound **26**. HRMS (ESI) calcd for $C_{30}H_{55}N_3O_{12}Na$ $(M + Na)^+$ *m/z* 672.3683, found 672.3682.

2.2.25. C_5-BODIPY^{TM}-L-erythro-LacCer (**27**)

A mixture of 8 mg (0.012 mmol) of compound **26**, BODIPY^{TM}-C_5-NHS (5.0 mg, 0.020 mmol), triphenylphosphine (6.0 mg, 0.023 mmol), 2.7 ml of THF, and 0.3 ml of water was stirred overnight at room temperature. Removal of the solvents gave a residue that was purified by chromatography (elution with MeOH/$CHCl_3$ 1:1), affording 4 mg (36%) of compound **27**; R_f 0.38 (MeOH/$CHCl_3$ 1:4); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.78–6.04 (m, 4H), 6.23 (m, 1H), 6.04 (m, 1H), 3.83–2.05 (m, 18H), 1.70–0.70 (m, 35H); LRMS (APCI, negative-ion mode) calcd for $C_{46}H_{74}BClF_2N_3O_{13}$ $(M + {}^{35}Cl)^-$ *m/z* 960.5, found 960.5; HRMS (EI) calcd for $C_{46}H_{75}N_3O_{13}F_2B$ (MH^+ of the boron-10 isotope) *m/z* 925.5397, found 925.5409.

2.2.26. 2-Azido-L-threo-sphingosine (**29**)

TfN_3 (1 ml, 0.4 mmol, a 0.4 M solution in CH_2Cl_2) was added dropwise to L-*threo*-sphingosine (compound **28**, 25 mg, 0.084 mmol) and DMAP (20 mg, 0.164 mmol) in 5 ml of CH_2Cl_2 (see Scheme 8). The reaction mixture was stirred at room temperature for 24 h, and then concentrated to give a residue that was purified by chromatography (elution with hexane/EtOAc 3:1), affording 26 mg (95%) of compound **29**; R_f 0.82 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.69 (dt, 1H, J = 10.8, 7.2 Hz), 5.48 (dd, 1H, J = 10.8, 8.8 Hz), 4.62 (m, 1H), 3.81 (m, 2H), 3.52 (m, 1H), 2.13 (m, 2H), 1.45–1.20 (m, 22H), 0.91 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 136.0, 127.5, 68.2, 66.9, 62.6, 31.9, 29.69, 29.66, 29.6, 29.5, 29.4, 29.3, 28.0, 22.7, 14.1.

2.2.27. 2-Azido-1-(tert-butyldiphenylsilanyloxy)-L-threo-sphingosine (**30**)

Compound **30** was prepared by the method used to synthesize compound **10**. A solution of compound **29** (26 mg, 0.080 mmol) in 5 ml of CH_2Cl_2 was added to TBDPSCl (23 mg, 0.084 mmol) and imidazole (12 mg, 0.17 mmol) in 5 ml of CH_2Cl_2. The reaction mixture was stirred overnight, the solvent was removed, and the residue was purified by chromatography (elution with hexane/EtOAc from 9:1 to 4:1) to give 39 mg (91%) of compound **30**; R_f 0.77 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 7.71 (m, 4H), 7.46 (m, 6H), 5.64 (dt, 1H, J = 10.8, 7.2 Hz), 5.42 (dd, 1H, J = 10.8, 8.8 Hz), 4.61 (m, 1H), 3.84 (m, 2H), 3.56 (m, 1H), 2.01 (m, 2H), 1.61 (s, 1H), 1.40–1.06 (m, 31H), 0.91 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 135.8, 135.6, 134.6, 129.9, 129.1, 127.9, 127.5, 67.5, 66.0, 64.1, 32.0, 29.7, 29.4, 28.0, 26.9, 26.8, 22.7, 19.1, 14.1.

2.2.28. 2-Azido-3-benzoic acid-1-(tert-butyldiphenylsilanyloxy)-L-threo-sphingosine (**31**)

To a solution of compound **30** (38 mg, 0.067 mmol) in 5 ml of dry CH_2Cl_2 was added DMAP (25 mg, 0.20 mmol), followed by the dropwise addition of a solution of benzoic anhydride (18 mg, 0.080 mmol) in 5 ml of CH_2Cl_2 at 0 °C. The reaction mixture was stirred overnight, the solvent was removed, and the residue was purified by chromatography (elution with hexane, then with hexane/EtOAc 19:1) to give 38 mg (85%) of compound **31**; R_f 0.90 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 8.04 (m, 2H), 7.71 (m, 4H), 7.46 (m, 9H), 6.06 (m, 1H), 5.77 (dt, 1H, J = 10.8, 7.2 Hz), 5.50 (dd, 1H, J = 10.8, 8.8 Hz), 3.82 (m, 3H), 2.25 (m, 2H), 1.50–1.10 (m, 31H), 0.93 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 162.8, 135.8, 133.5, 133.23, 133.19, 133.0, 132.4, 132.0, 130.7, 130.5, 130.4, 128.1, 127.7, 127.52, 127.51, 127.4, 127.2, 127.1, 126.1, 125.5, 125.44, 125.35, 125.1, 120.5, 67.0, 63.72, 63.67, 61.1, 29.6, 27.4, 27.34, 27.28, 27.2, 27.09, 27.06, 27.0, 25.9, 24.7, 24.5, 24.4, 23.3, 20.4, 16.8, 11.8. HRMS (ESI) calcd for $C_{41}H_{57}N_3O_3SiNa$ $(M + Na)^+$ *m/z* 690.4067, found 690.4081.

2.2.29. 2-Azido-3-benzoic acid-L-threo-sphingosine (**32**)

TBAF (0.1 ml, 0.1 mmol, 1 M in THF) was added to a solution of compound **31** (35 mg, 0.051 mmol) and 25 mg (0.36 mmol) of imidazole in 5 ml of dry CH_2Cl_2 at −23 °C. After being stirred at −23 °C for 3 h, the reaction mixture was quickly passed through a silica gel column that had been prewashed with cold elution solvent. Elution with hexane/EtOAc 4:1) afforded 14 mg (63%)

of compound **32**; R_f 0.65 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 2H), 7.46 (m, 3H), 6.00 (m, 1H), 5.75 (dt, 1H, J = 10.8, 7.2 Hz), 5.52 (dd, 1H, J = 10.8, 8.8 Hz), 4.51 (m, 2H), 3.88 (m, 1H), 2.13 (m, 2H), 1.50–1.10 (m, 31H), 0.93 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 166.4, 165.6, 138.4, 136.5, 135.2, 134.8, 133.4, 129.9, 129.8, 129.7, 129.5, 128.53, 128.51, 127.7, 126.7, 122.8, 69.6, 67.3, 66.4, 65.1, 64.3, 61.9, 32.0, 29.71, 29.68, 29.6, 29.50, 29.48, 29.4, 29.3, 28.2, 28.1, 26.6, 22.7, 19.0, 14.2.

2.2.30. L-*threo*-C_5-BODIPY^{TM}-LacCer (**33**)

A mixture of 21 mg (0.027 mmol) of compound **32**, 12 mg (0.027 mmol) of trichloroacetimidate **13**, and 100 mg of molecular sieves 300AW in 5 ml of CH_2Cl_2 was stirred at room temperature for 1 h. A solution of $BF_3{\cdot}OEt_2$ (20 μl, 0.16 mmol) in 2 ml of CH_2Cl_2 was added, and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the residue was purified by chromatography (elution with $CHCl_3$, then with MeOH/$CHCl_3$ 1:9) to give 9 mg (30%) of the glycosylation product. Alkaline methanolysis of the acetate and benzoate ester functionalities was carried out by adding a solution of 2 mg (0.08 mmol) of sodium in 1 ml of dry MeOH, followed by stirring at room temperature for 6 h. Dowex 50W-X8 resin (prewashed with 50 ml of MeOH) was added to neutralize the reaction mixture. The reaction mixture was filtered and solvent was removed under vacuum. After BODIPY^{TM}-C_5-NHS (3 mg, 0.012 mmol), triphenylphosphine (4 mg, 0.016 mmol), 2.7 ml of THF, and 0.3 ml of water were added, the reaction mixture was stirred overnight at room temperature. The solvents were removed, and the residue was purified by chromatography (elution with MeOH/$CHCl_3$ from 1:9 to 1:4) to give 1.5 mg (38%) of compound **33**; R_f 0.45 (MeOH/$CHCl_3$ 1:4); ^{1}H NMR ($CDCl_3$): same as for compound **27**; HRMS (EI) calcd for $C_{46}H_{75}N_3O_{13}F_2B$ (MH^+ of the boron-10 isotope) m/z 925.5397, found 925.5416.

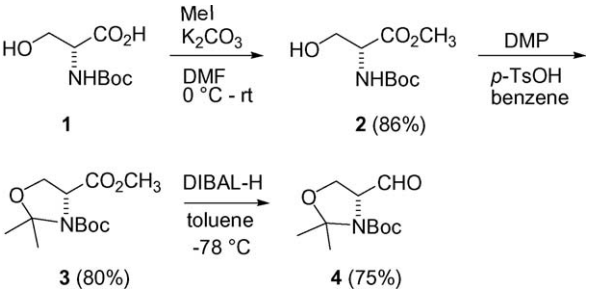
3. Results

3.1. Retrosynthetic plan

As shown in the retrosynthetic plan (Fig. 2), the preparation of the BODIPY^{TM}-LacCer stereoisomers consists of three building blocks: a 2-azido-3-benzoylsphingosine derivative composed of the desired configurations at C2 and C3, an activated BODIPY^{TM}-linked fatty acid, and an activated and protected lactosyl donor (hepta-*O*-acetyl-β-lactosyl-1-trichloroacetimidate) (Amvam-Zollo and Sinay, 1986).

3.2. Synthesis of D-*threo* C_5-BODIPY^{TM}-LacCer analogue (**16**)

See Scheme 4.



Scheme 1. Synthesis of (*R*)-Garner aldehyde (**4**).

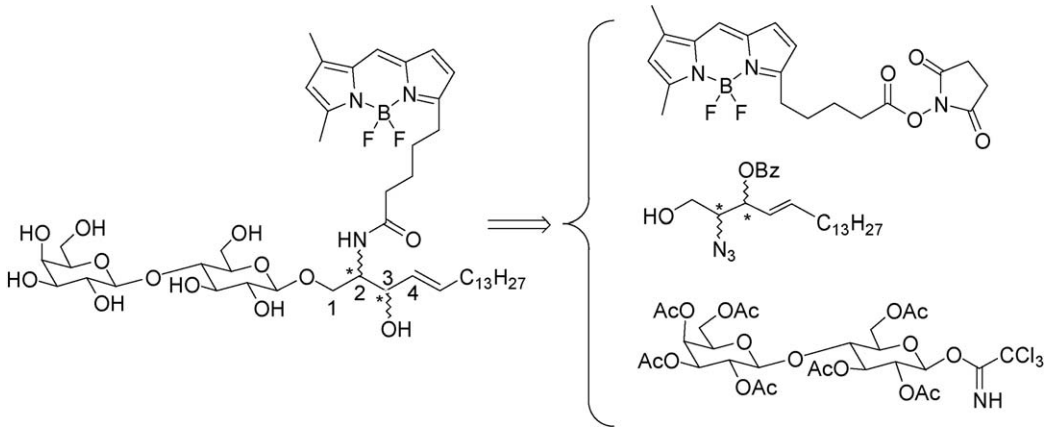
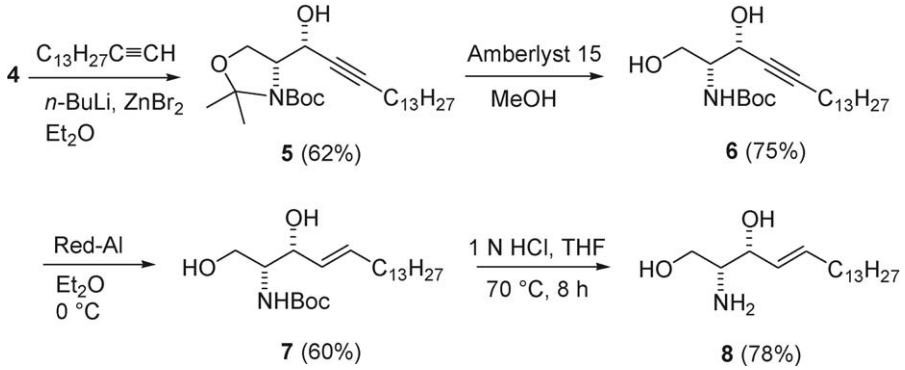
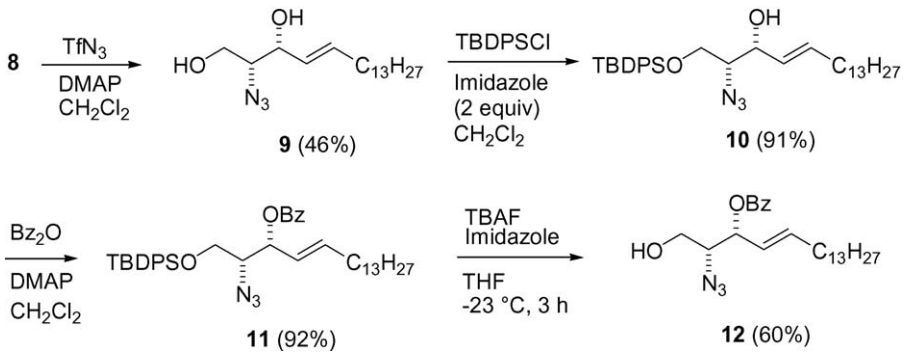


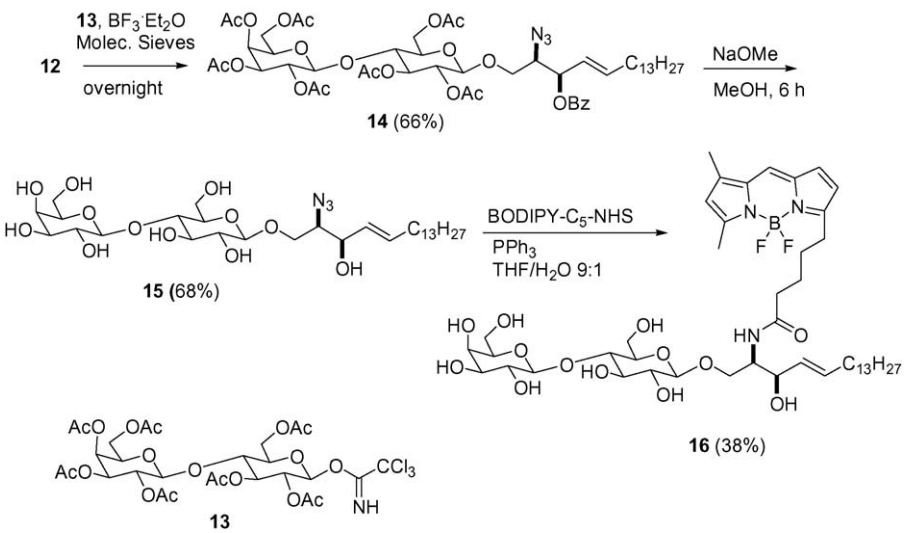
Fig. 2. Retrosynthetic plan for the preparation of D-*threo*- and L-*erythro*-C_5-BODIPY^{TM}-LacCer.



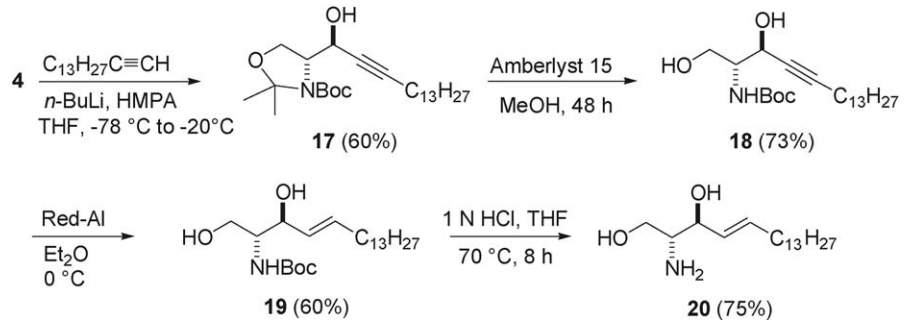
Scheme 2. Synthesis of (2*R*,3*R*)-sphingosine (**8**).



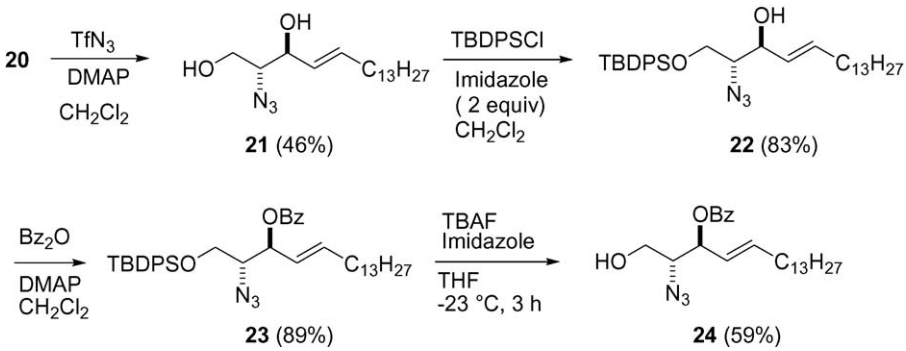
Scheme 3. Synthesis of (2*R*,3*R*)-2-azido-3-benzoylsphingosine (**12**).



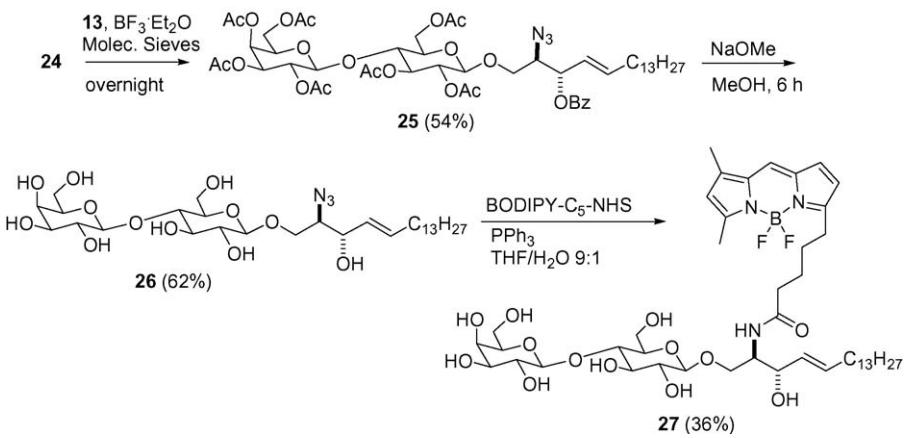
Scheme 4. Synthesis of (2*R*,3*R*)-C5-BODIPY^{TM}-LacCer (**16**).



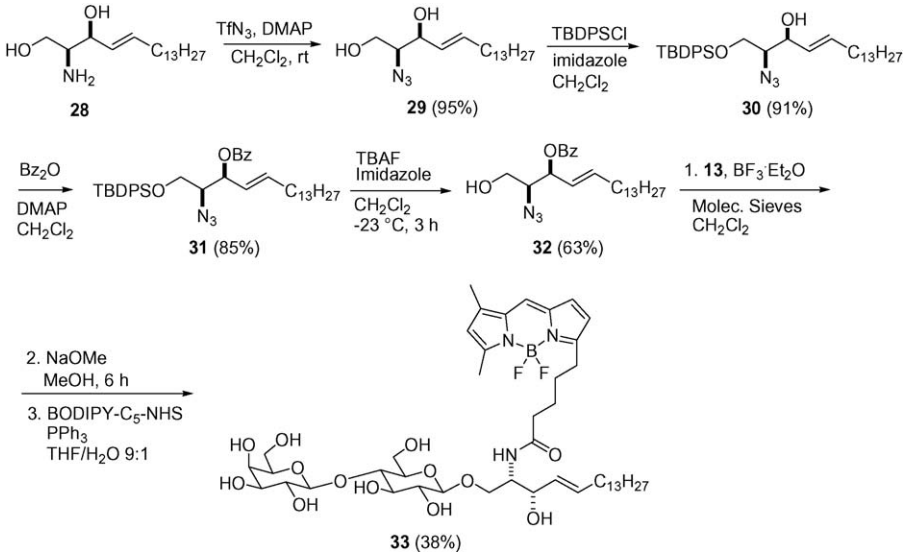
Scheme 5. Synthesis of (2*R*,3*S*)-sphingosine (**20**).



Scheme 6. Synthesis of (2*R*,3*S*)-2-azido-3-benzoylsphingosine (**24**).



Scheme 7. Synthesis of (2*R*,3*S*)-C_5-BODIPY^{TM}-LacCer (**27**).



Scheme 8. Synthesis of L-*threo*-C_5-BODIPY^{TM}-LacCer (**33**).

3.3. Synthesis of L-*erythro* C_5-BODIPY^{TM}-LacCer *analogue (**27**)*

See Scheme 7.

3.4. Synthesis of L-*threo* C_5-BODIPY^{TM}-LacCer *analogue (**33**)*

See Scheme 8.

4. Summary

The (2*R*,3*R*) (or D-*threo*, compound **8**) and (2*R*,3*S*) (or L-*erythro*, compound **20**) sphingosines were synthesized as outlined in Schemes 2 and 5, respectively, by the reaction of (*R*)-Garner aldehyde (compound **4**) (Garner et al., 1988; Garner and Park, 1987; Garner and Park, 1992) with lithium pentadecyne in the presence of zinc bromide in Et_2O or HMPA in THF (Herold, 1988), respectively. (*R*)-Garner aldehyde was prepared (see Scheme 1) from *N*-Boc-D-serine (**1**), which was converted to its methyl ester and then treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in benzene, followed by DIBAL-H reduction at −78 °C. The oxazolidine ring was opened with Amberlyst 15 resin, and Red-Al reduction (Van Overmeire et al., 1999) of the propargylic alcohol in Et_2O afforded (2*R*,3*R*)- and (2*R*,3*S*)-*N*-Boc-sphingosines (compounds **7** and **19**, respectively). The diazo transfer reaction afforded the stereoisomeric 2-azido sphingosine derivatives, compounds **9**, **21**, and **29**. Silylation of the primary hydroxy group was carried out in the presence of 2 equivalents of imidazole; after benzoylation of the secondary hydroxy group, the desilylation reaction was performed at −23 °C (Mattjus et al., 2002), followed by rapid elution through a cold silica gel column, to minimize benzoyl migration, furnishing the three stereoisomers of 2-azido-3-benzoylsphingosines: compounds **12** (Scheme 3), **24** (Scheme 6), and **32** (Scheme 8). After $BF_3{\cdot}OEt_2$-mediated lactosylation of the 2-azido-3-benzoylsphingosine stereoisomers with hepta-*O*-acetyllactosyl trichloroacetimidate in CH_2Cl_2 in the presence of molecular sieves, base-catalyzed deprotection afforded the β-lactosyl-2-azidosphingosines. Staudinger reduction of the azido group with triphenylphosphine in aqueous THF (Gololobov et al., 1981), followed by *N*-acylation with the *N*-hydroxysuccinimidoyl ester of BODIPY^{TM}-C_5 and purification by column chromatography on silica gel (elution with $CHCl_3$/MeOH 4:1, v/v), furnished the target unnatural BODIPY^{TM}-LacCer stereoisomers: compounds **16** (Scheme 4), **27** (Scheme 7), and **33** (Scheme 8). NMR spectroscopy and mass spectrometry confirmed the structures of these analogues.

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