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Note

Synthesis of fluorescent alkyl lactoside derivatives

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ABSTRACT

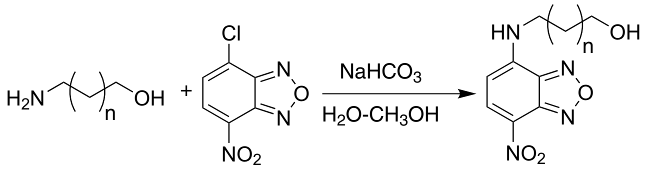
Fluorescent-labeled alkyl lactoside (NBD-alkyl lactoside) derivatives were synthesized by the reaction of NBD alkyl alcohol with heptaacetyllactosyl trichloroacetimidate in the presence of $BF_3 \cdot Et_2O$.

Glycosphingolipids (GSLs) are composed of lipophilic ceramide and a hydrophilic sugar moiety. They play an important role in biological processes as crucial components of cellular membranes.[1] The use of chemically synthesized GSLs is needed to study their biological function; however, because of their complex chemical structure it is difficult to synthesize each of them. To avoid this obstacle, much attention has been paid to biocombinatorial synthesis of GSL analogues,[2] taking advantage of the biosynthetic pathway of cells in which simple synthetic compounds are fed to a variety of cell lines to obtain complex GSL analogues.

Most GSLs contain lactosylceramide as their basic structure.[3] In biocombinatorial syntheses, alkyl and *N*-acylaminoethyl lactoside derivatives, lactoside primers, were taken up and glycosylated by the appropriate cells. Glycosylated lactoside derivatives were accumulated in cells or released from cell membranes into medium. Whether the modified lactoside derivatives stay inside or go outside the cells depends on the length of the alkyl chain used; thus several lactoside primers need to be synthesized with different alkyl chain lengths to obtain suitable compounds for biocombinatorial studies. To make the most of biocombinatorial synthesis, it is important that how and where cells recognize lactoside primers and modify them is revealed. If the path where lactoside primers come from and where they go could be determined by the use of appropriate chemical tools such as fluorescent primers, we would be better able to understand details of the glycosylation process. In this study, we report the synthesis of fluorescent-labeled alkyl lactoside mimics to visualize the translocation process of lactoside primers.

We used the 7-alkylamino-substituted 4-nitro-2,1,3-benzoxadiazole (NBD) group as a fluorescent moiety from a synthetic viewpoint as well as for its bright green fluorescence. A fluorescent group should not have any harmful effect on the biological process, such as uptake from medium or sugar modification at the Golgi compartment. Yamagata and co-workers reported that azido glycoside, in which the azido group is attached at the end of the alkyl chain, worked well in a lactoside primer study.[4] With this result in mind, we designed fluorescent alkyl lactoside derivatives having an NBD group at the end of the alkyl chain.

The synthetic approach is shown in Schemes 1 and 2. NBD-alcohols were prepared by reacting aminoalcohol $H_2N(CH_2)_nOH$ ($n = 6$, 10, and 12) with NBD-Cl. Protected NBD-alkyl lactosides were synthesized by the reaction of NBD-alcohols with heptaacetyllactosyl trichloroacetimidate in the presence of $BF_3 \cdot Et_2O$. Trichloroacetimidate activation is known to give β-glycosides, which is suitable for study of glycoside primers.[5] Deprotection of the acetyl group with sodium methoxide afforded target NBD-alkyl lactosides.



1a: n=4
1b: n=8
1c: n=10

2

3a: n=4
3b: n=8
3c: n=10

Scheme 1. Synthesis of NBD alkyl alcohols **3**.

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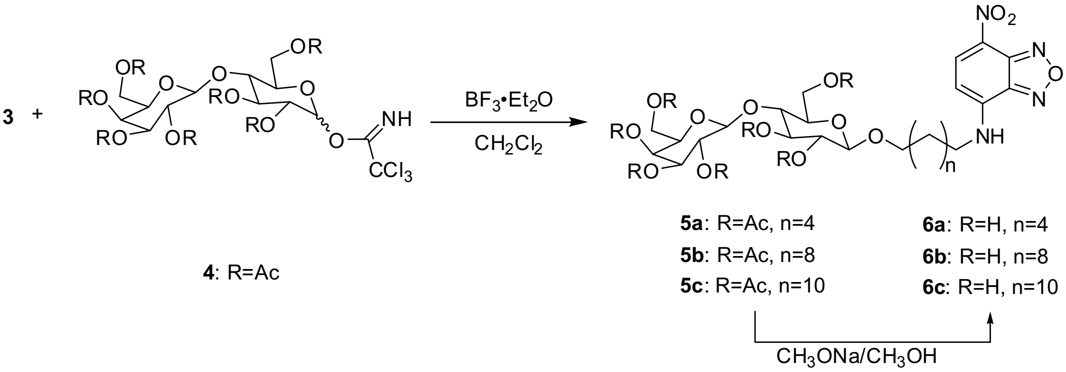

Scheme 2. Synthesis of NBD alkyl lactosides **6**.

In ^{1}H NMR spectra of NBD-alkyl lactosides, **6b** for instance, anomeric protons chemical shifts are found at 4.27 and 4.36 ppm in CD_3OD with coupling constants of 7.9 and 7.4 Hz, respectively. These values are characteristic for a β-lactoside linkage, which is a preferable anomer for the study of lactoside primers.

The NBD-alkyl lactosides **6a–c** synthesized here have absorption maxima at 335 and 467 nm in MeOH. They show emission maxima around 530 nm in MeOH with excitation at 467 nm. They are soluble in DMSO, and **6a**, with a short alkyl chain, is also soluble in water. Although **6b** and **6c** do not show very good solubility in water, they are still acceptable for biological experiments. In the preliminary results, the green fluorescence of the NBD group was observed in B16 cells after incubation with **6c**, suggesting that these NBD-alkyl lactosides are promising chemical tools for lactoside primer study.

In summary, we described the synthesis of NBD-alkyl lactosides with different alkyl chain lengths. These compounds should prove useful for visualizing the process of the modification of lactoside primers as well as for the biosynthesis of glycolipids.

1. Experimental

1.1. General methods

Melting points were determined on a Yanaco micro melting point apparatus. All melting points were uncorrected. Preparative gel permeation chromatography was performed by LC-908 (Japan Analytical Industry) with a JAIGEL GS-310 column with MeOH or JAIGEL 1H + 2H columns with $CHCl_3$ as solvent. Optical rotations were measured on a Jasco DIP-360 digital polarimeter at room temperature. UV–vis spectra were recorded on a HITACHI U-3010 spectrophotometer. Fluorescence emission spectra were measured on a HORIBA Spex Fluorolog 3 spectrometer. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$, DMSO-d_6 or CD_3OD with a JEOL JNM-GSX 270 or Bruker Avance 300 spectrometer using tetramethylsilane as an external standard. FAB mass spectral data were obtained on a JEOL JMS-600 mass spectrometer. Elemental analyses were performed by the Instrument Analysis Center of Faculty of Pharmaceutical Sciences, Toho University.

1.2. 7-(6-Hydroxyhexyl)amino-4-nitro-2,1,3-benzoxadiazole (3a)

To a soln of 6-amino-1-hexanol (310 mg, 2.57 mmol) in 0.3 M aq $NaHCO_3$ (10 mL) was added a soln of 4-chloro-7-nitro-2,1,3-benzoxadiazole (497 mg, 2.49 mmol) in MeOH (20 mL) and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under diminished pressure. The residue was purified by column chromatography (SiO_2/1:2 hexane–AcOEt) to afford **3a** (529 mg, 1.89 mmol, 77%) as red crystals; mp 99.0–100.5 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.36–1.87 (m, 8H), 3.50 (q, 2H, *J* 6.6 Hz), 3.70 (t, 2H, *J* 6.1 Hz), 6.17 (d, 1H, *J* 8.6 Hz), 6.37 (br s, 1H), 8.50 (d, 1H, *J* 8.6 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 25.2 (t, CH_2), 26.6 (t, CH_2), 28.3 (t, CH_2), 32.2 (t, CH_2), 43.9 (t, NCH_2), 62.7 (t, CH_2OH), 98.5 (d, C6 in NBD), 123.9 (s, C4 in NBD), 136.51 (d, C5 in NBD), 143.9 (s, 2C), 144.2 (s) (C7, C8, and C9 in NBD); FAB-MS: *m/z* 281 $[M+H]^+$. Anal. Calcd for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.17; H, 5.67; N, 19.77.

1.3. 6-(4-Nitro-2,1,3-benzoxadiazole-7-yl)aminohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (5a)

To a soln of **3a** (105 mg, 0.37 mmol) and heptaacetyllactosyl trichloroacetimidate (332 mg, 0.43 mmol) in CH_2Cl_2 (10 mL) was added $BF_3{\cdot}EtO_2$ (48%, 120 μL, 0.46 mmol) under Ar atmosphere and the reaction mixture was stirred for 3 h at room temperature. To it was then added satd aq $NaHCO_3$ and the organic layer was washed with satd aq $NaHCO_3$, then brine. The organic layer was dried over anhyd $MgSO_4$, filtered, and the solvent was evaporated under diminished pressure. The residue was purified by column chromatography (SiO_2/ 1:1 to 2:3 hexane–EtOAc) and gel permeation chromatography (JAIGEL GS-310, MeOH) to afford **5a** (87 mg, 0.097 mmol, 26%) as red crystals; mp 86.0–87.5 °C; $[\alpha]_D$ −8.7 (*c* 1.0, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.38–1.73 (m, 8H), 1.88 (s, 3H), 1.96 (s, 3H), 1.98 (s, 9H), 2.04 (s, 3H), 2.07 (s, 3H), 3.40–3.54 (m, 4H), 3.70–3.87 (m, 3H), 3.98–4.08 (m, 3H), 4.36–4.47 (m, 3H), 4.71–5.14 (m, 4H), 5.28 (d, 1H, *J* 3.0 Hz), 6.12 (d, 1H, *J* 8.6 Hz), 6.82 (br s, 1H), 8.40 (d, 1H, *J* 8.6 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 20.3 (q, CH_3), 20.5 (q, CH_3, 3C), 20.58 (q, CH_3), 20.63 (q, CH_3), 20.7 (q, CH_3), 25.3 (t, CH_2), 26.3 (t, CH_2), 28.0 (t, CH_2), 28.9 (t, CH_2), 43.8 (t, NCH_2), 60.7 (t, C6 in sugar), 61.7 (t, C6 in sugar), 66.5 (d), 69.0 (d), 69.7 (t, OCH_2 in alkyl chain), 70.5 (d), 70.8 (d), 71.6 (d), 72.5 (d), 72.6 (d), 76.3 (d), 98.4 (d, C6 in NBD), 100.4 (d, C1 in sugar), 100.9 (d, C1 in sugar), 123.2 (s, C4 in NBD), 136.6 (d, C5 in NBD), 143.8 (s), 144.1 (s, 2C) (C7, C8, and C9 in NBD), 169.0 (s,CO), 169.5 (s, CO), 169.7 (s, CO), 169.9 (s, CO), 170.0 (s, CO), 170.2 (s, CO), 170.3 (s, CO); FABMS: *m/z* 921 $[M+Na]^+$. Anal. Calcd for $C_{38}H_{50}N_4O_{21}$: C, 50.78; H, 5.72; N, 6.23. Found: C, 50.65; H, 5.59; N, 5.83.

1.4. 6-(4-Nitro-2,1,3-benzoxadiazole-7-yl)aminohexyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6a)

To a soln of **5a** (87 mg, 0.097 mmol) in MeOH (5 mL) was added a soln of MeONa (33 mg, 0.61 mmol) in MeOH (2 mL) and the reaction mixture was stirred for 40 min at room temperature. It was then acidified by addition of DOWEX 50WX8 H^+ ion exchange resin, the resin was filtered, and the solvent was evaporated under diminished pressure to afford **6a** (46 mg, 0.076 mmol, 79%) as red crystals; mp 147.0–149.0 °C; $[\alpha]_D$ +5.0 (*c* 1.0, water); UV (MeOH) λ_{max} (ε) 335 (7650), 467 (19700); ^{1}H NMR (270 MHz, DMSO-d_6): δ 1.35–1.66 (m, 8H), 2.99–3.72 (m, 9H), 4.15–4.17 (m, 2H), 4.53–4.79 (m, 5H), 5.09 (br s, 2H), 6.35 (d, 1H, *J* 8.7 Hz), 8.45 (d, 1H, *J* 8.7 Hz), 9.50 (br s, 1H); ^{13}C NMR (67.5 MHz, DMSO-d_6): δ 25.3 (t, CH_2), 26.3 (t, CH_2), 27.7 (t, CH_2), 29.2 (t, CH_2), 43.4 (t, NCH_2), 60.5 (t, C6 in sugar), 60.6 (t, C6 in sugar), 68.2 (d), 68.7 (t, OCH_2 in alkyl chain), 70.6 (d), 73.2 (d), 73.3 (d), 74.8 (d), 75.1 (d), 75.6 (d), 80.9 (d), 99.1 (d, C6 in NBD), 102.6 (d, C1 in sugar), 103.9 (d, C1 in sugar), 120.5 (s, C4 in NBD), 138.0 (d, C5 in NBD), 144.2 (s), 144.4 (s), 145.2 (s) (C7, C8, and C9 in NBD); FABMS: *m/z* 627 [M+Na]$^+$, 605 [M+H]$^+$. Anal. Calcd for $C_{24}H_{36}N_4O_{14}\cdot H_2O$: C, 46.30; H, 6.15; N, 9.00. Found: C, 46.59; H, 5.81.; N, 8.21.

1.5. 7-(10-Hydroxydecyl)amino-4-nitro-2,1,3-benzoxadiazole (3b)

To a soln of 10-amino-1-decanol (prepared by the reaction of phthalimide potassium salt 921 mg, 4.97 mmol with 10-bromo-1-decanol 1.001 g, 4.22 mmol) in 0.3 M aq $NaHCO_3$ (15 mL) was added a soln of 4-chloro-7-nitro-2,1,3-benzoxadiazole (860 mg, 4.31 mmol) in MeOH (25 mL) and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under diminished pressure. The residue was purified by column chromatography (SiO_2/2:1 hexane–EtOAc). Recrystallization from $CHCl_3$ gave **3b** (360 mg, 1.07 mmol, 20%) as red crystals; mp 100.0–101.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33–1.60 (m, 14H), 1.82 (quint., 2H, *J* 7.0 Hz), 3.49 (q, 2H, *J* 7.0 Hz), 3.65 (t, 2H, *J* 6.5 Hz), 6.18 (d, 1H, *J* 8.6 Hz), 6.27 (br s, 1H), 8.51 (d, 1H, *J* 8.6 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.7 (t, CH_2), 26.9 (t, CH_2), 28.5 (t, CH_2), 29.1 (t, CH_2), 29.3 (t, CH_2, 2C), 29.4 (t, CH_2), 32.7 (t, CH_2), 44.0 (t, NCH_2), 63.0 (t, CH_2OH), 99.5 (d, C6 in NBD), 124.0 (s, C4 in NBD), 136.5 (d, C5 in NBD), 143.8 (s), 143.9 (s), 144.2 (s) (C7, C8, and C9 in NBD); FABMS: *m/z* 337 [M+H]$^+$. Anal. Calcd for $C_{16}H_{24}N_4O_4$: C, 57.13; H, 7.19; N, 16.66. Found: C, 56.84; H, 7.08; N, 16.60.

1.6. 10-(4-Nitro-2,1,3-benzoxadiazole-7-yl)aminodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (5b)

To a soln of **3b** (253 mg, 0.75 mmol) and heptaacetyllactosyl trichloroacetimidate (655 mg, 0.84 mmol) in CH_2Cl_2 (10 mL) was added $BF_3\cdot EtO_2$ (48%, 230 μL, 0.88 mmol) at 0 °C under Ar atmosphere. The reaction mixture was warmed to room temperature and stirred for 10 h. To the reaction mixture was added satd aq $NaHCO_3$ and the aq layer was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and the solvent was evaporated under diminished pressure. The residue was purified by column chromatography (SiO_2/1:1 to 1:3 hexane–AcOEt), gel permeation liquid chromatography (JAIGEL 1H+2H, $CHCl_3$), and column chromatography (SiO_2/$CHCl_3$) to afford **5b** (149 mg, 0.156 mmol, 21%) as red crystals; mp 75.0–76.5 °C; $[\alpha]_D$ −1.8 (*c* 1.0, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.29–1.82 (m, 16H), 1.97 (s, 3H), 2.04 (s, 3H), 2.05 (s, 6H), 2.06 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 3.46–3.61 (m, 4H), 3.77–3.91 (m, 3H), 4.08–4.14 (m, 3H), 4.44–4.51 (m, 3H), 4.86–5.21 (m, 4H), 5.36 (br s, 1H), 6.18 (d, 1H, *J* 8.6 Hz), 6.45 (br s, 1H), 8.50 (d, 1H, *J* 8.6 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 20.3 (q, CH_3), 20.4 (q, CH_3, 2C), 20.5 (q, CH_3), 20.6 (q, CH_3), 20.7 (q, CH_3), 20.8 (q, CH_3), 25.5 (t, CH_2), 26.7 (t, CH_2), 28.2 (t, CH_2), 28.9 (t, CH_2), 29.05 (t, CH_2), 29.11 (t, CH_2), 43.9 (t, NCH_2), 60.7 (t, C6 in sugar), 61.9 (t, C6 in sugar), 66.5 (d), 68.9 (d), 69.9 (t, OCH_2 in alkyl chain), 70.4 (d), 70.8 (d), 71.5 (d), 72.4 (d), 72.6 (d), 76.1 (d), 98.4 (d, C6 in NBD), 100.4 (d, C1 in sugar), 100.8 (d, C1 in sugar), 123.1 (s, C4 in NBD), 136.6 (d, C5 in NBD), 143.8 (s), 144.1 (s, 2C) (C7, C8, and C9 in NBD), 169.0 (s, CO), 169.5 (s, CO), 169.7 (s, CO), 169.9 (s, CO), 170.0 (s, CO), 170.2 (s, CO), 170.3 (s, CO); FABMS: *m/z* 977 [M+Na]$^+$. Anal. Calcd for $C_{42}H_{58}N_4O_{21}$: C, 52.83; H, 6.12; N, 5.87. Found: C, 52.68; H, 6.06; N, 5.26.

1.7. 10-(4-Nitro-2,1,3-benzoxadiazole-7-yl)aminodecyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6b)

To a soln of **5b** (118 mg, 0.12 mmol) in MeOH (5 mL) was added MeONa (48 mg, 0.89 mmol) in MeOH (3 mL) and the reaction mixture was stirred for 50 min at room temperature. The reaction mixture was acidified by addition of DOWEX 50WX8 H^+ion exchange resin, the resin was filtered and the solvent was evaporated under diminished pressure. The residue was recrystallized from $CHCl_3$ to afford **6b** (44 mg, 0.067 mmol, 54%) as red crystals; mp 158.5–160.0 °C; $[\alpha]_D$ +5.0 (*c* 1.0, DMSO); UV (MeOH) λ_{max} (ε) 335 (7470), 467 (19000); ^{1}H NMR (270 MHz, DMSO-d_6): δ 1.26–1.67 (m, 16H), 3.27–3.76 (m, 9H), 4.15–4.21 (m, 2H), 4.51–4.84 (m, 5H), 5.10 (br s, 2H), 6.40 (d, 1H, *J* 8.9 Hz), 8.49 (d, 1H, *J* 8.9 Hz), 9.55 (br s, 1H); ^{1}H NMR (300 MHz, CD_3OD): δ 1.33–1.60 (m, 14H), 1.77 (t, 2H, *J* 7.0 Hz), 3.20–3.91 (m, 16H), 4.27 (d, 1H, *J* 7.9 Hz), 4.36 (d, 1H, *J* 7.4 Hz), 4.62 (s, 1H), 6.35 (d, 1H, *J* 8.8 Hz), 8.53 (d, 1H, *J* 8.8 Hz); ^{13}C NMR (67.5 MHz, DMSO-d_6): δ 25.6 (t, CH_2), 26.5 (t, CH_2), 27.8 (t, CH_2), 28.8 (t, CH_2), 29.0 (t, CH_2), 29.1 (t, CH_2, 2C), 29.3 (t, CH_2), 43.6 (t, NCH_2), 60.5 (t, C6 in sugar), 60.6 (t, C6 in sugar), 68.2 (d), 68.8 (t, OCH_2 in alkyl chain), 70.6 (d), 73.2 (d), 73.3 (d), 74.8 (d), 75.1 (d), 75.6 (d), 80.9 (d), 99.3 (d, C6 in NBD), 102.6 (d, C1 in sugar), 103.9 (d, C1 in sugar), 120.4 (s, C4 in NBD), 138.1 (d, C5 in NBD), 144.3 (s), 144.6 (s), 145.4 (s) (C7, C8, and C9 in NBD); FABMS: *m/z* 683 [M+Na]$^+$, 661 [M+H]$^+$. Anal. Calcd for $C_{28}H_{44}N_4O_{14}\cdot H_2O$: C, 49.55; H, 6.83; N, 8.26. Found: C, 49.65; H, 6.56; N, 7.93.

1.8. 7-(12-Hydroxydodecyl)amino-4-nitro-2,1,3-benzoxadiazole (3c)

To a soln of 12-amino-1-dodecanol (prepared by the reaction of phthalimide potassium salt 940 mg, 5.07 mmol with 12-bromo-1-dodecanol 1.147 g, 4.32 mmol) in 0.3 M aq $NaHCO_3$ (10 mL) was added a soln of 4-chloro-7-nitro-2,1,3-benzoxadiazole (565 mg, 2.83 mmol) in MeOH (15 mL) and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under diminished pressure. The residue was purified by column chromatography (SiO_2/2:1 hexane–EtOAc). Recrystallization from $CHCl_3$ gave **3c** (393 mg, 1.08 mmol, 39%) as red crystals; mp 106.0–108.0 °C; ^{1}H NMR (270 MHz, DMSO-d_6): δ 1.20–1.65 (m, 20H), 3.13–3.43 (m, 4H), 4.31 (t, 1H, *J* 4.8 Hz), 6.37 (d, 1H, *J* 8.9 Hz), 8.47 (d, 1H, *J* 8.9 Hz), 9.53 (br s, 1H); ^{13}C NMR (67.5 MHz, DMSO-d_6): δ 25.5 (t, CH_2), 26.4 (t, CH_2), 27.6 (t, CH_2), 28.7 (t, CH_2), 28.97 (t, CH_2, 2C), 28.98 (t, CH_2), 29.02 (t, CH_2), 29.1 (t, CH_2), 32.5 (t, CH_2), 43.4 (t, NCH_2), 60.7 (t, CH_2OH), 99.1 (d, C6 in NBD), 120.5 (s, C4 in NBD), 138.0 (d, C5 in NBD), 144.2 (s), 144.4 (s), 145.2 (s) (C7, C8, and C9 in NBD); FABMS: *m/z* 365 [M+H]$^+$. Anal. Calcd for $C_{18}H_{28}N_4O_4$: C, 59.32; H, 7.74; N, 15.37. Found: C, 59.03; H, 7.63; N, 15.43.

1.9. 12-(4-Nitro-2,1,3-benzoxadiazole-7-yl)aminododecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (5c)

To a soln of **3c** (363 mg, 1.00 mmol) and heptaacetyllactosyl trichloroacetimidate (734 mg, 0.94 mmol) in CH_2Cl_2 (10 mL) was added $BF_3\cdot EtO_2$ (48%, 500 μL, 1.91 mmol) at 0 °C under Ar atmosphere. The reaction mixture was warmed to room temperature and stirred overnight. To the reaction mixture was added satd aq $NaHCO_3$ and the aq layer was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered and the solvent was evaporated under diminished pressure. The residue was purified by column chromatography (Sephadex LH-20/MeOH and SiO_2/1:1 hexane–EtOAc) to afford **5c** (183 mg, 0.19 mmol, 20%) as red crystals; mp 65.0–67.0 °C; $[\alpha]_D$ +2.6 (*c* 1.0, MeOH); ^1H NMR (270 MHz, $CDCl_3$): δ 1.27–1.57 (m, 20H), 1.97 (s, 3H), 2.03 (s, 3H), 2.05 (s, 6H), 2.06 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 3.48–3.58 (m, 4H), 3.76–3.90 (m, 3H), 4.08–4.13 (m, 3H), 4.43–4.50 (m, 3H), 4.85–5.23 (m, 4H), 5.35 (d, 1H, *J* 2.6 Hz), 6.18 (d, 1H, *J* 8.6 Hz), 6.26 (br s, 1H), 8.51 (d, 1H, *J* 8.6 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 20.4 (q, CH_3), 20.55 (q, CH_3, 3C), 20.62 (q, CH_3), 20.7 (q, CH_3), 20.8 (q, CH_3), 25.7 (t, CH_2), 26.9 (t, CH_2), 28.3 (t, CH_2), 29.1 (t, CH_2, 2C), 29.3 (t, CH_2), 29.4 (t, CH_2, 4C), 44.0 (t, NCH_2), 60.8 (t,C6 in sugar), 62.0 (t, C6 in sugar), 66.7 (d), 69.1 (d), 70.1 (t, OCH_2 in alkyl chain), 70.6 (d), 70.9 (d), 71.7 (d), 72.5 (d), 72.8 (d), 76.2 (d), 98.5 (d, N6 in NBD), 100.5 (d, C1 in sugar), 100.9 (d, C1 in sugar), 123.1 (s, N4 in NBD), 136.7 (d, N5 in NBD), 143.9 (s), 144.2 (s, 2C) (C7, C8, and C9 in NBD), 169.1 (s, CO), 169.5 (s, CO), 169.8 (s, CO), 170.0 (s, CO), 170.1 (s, CO), 170.3 (s, CO), 170.4 (s, CO); FABMS: *m/z* 1005 $[M+Na]^+$. Anal. Calcd for $C_{44}H_{62}N_4O_{21}\cdot0.5H_2O$: C, 53.27; H, 6.40; N, 5.65. Found: C, 53.16; H, 6.25; N, 5.02.

1.10. 12-(4-Nitro-2,1,3-benzoxadiazole-7-yl)aminododecyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6c)

To a soln of **5c** (183 mg, 0.19 mmol) in MeOH (5 mL) was added MeONa (69 mg, 1.28 mmol) in MeOH (3 mL) and the reaction mixture was stirred for 80 min at room temperature. It was then acidified by addition of DOWEX 50WX8 H^+ ion exchange resin, the resin was filtered and the solvent was evaporated under diminished pressure. The residue was washed with $CHCl_3$ and filtered to afford **6c** (82 mg, 0.12 mmol, 64%) as red crystals; mp 167.0–168.5 °C; $[\alpha]_D$ +7.5 (*c* 0.6, DMSO); UV (MeOH) λ_{max} (ε) 335 (6720), 467 (17000). ^1H NMR (270 MHz, DMSO-d_6): δ 1.22–1.66 (m, 20H), 2.97–3.74 (m, 9H), 4.13–4.19 (m, 2H), 4.51–4.79 (m, 5H), 5.07–5.09 (m, 2H), 6.39 (d, 1H, *J* 9.1 Hz), 8.49 (d, 1H, *J* 9.1 Hz), 9.54 (br s, 1H); ^{13}C NMR (67.5 MHz, DMSO-d_6): δ 25.5 (t, CH_2), 26.4 (t, CH_2), 27.6 (t, CH_2), 28.7 (t, CH_2), 29.0 (t, CH_2, 4C), 29.1 (t, CH_2), 29.3 (t, CH_2), 43.4 (t, NCH_2), 60.4 (t, C6 in sugar), 60.5 (t, C6 in sugar), 68.2 (d), 68.8 (t, OCH_2 in alkyl chain), 79.6 (d), 73.2 (d), 73.3 (d), 74.8 (d), 75.0 (d), 75.5 (d), 80.8 (d), 99.2 (d, C6 in NBD), 102.5 (d, C1 in sugar), 103.9 (d, C1 in sugar), 120.5 (s, C4 in NBD), 138.0 (d, C5 in NBD), 144.2 (s), 144.5 (s), 145.2 (s) (C7, C8, and C9 in NBD); FABMS: *m/z* 711 $[M+Na]^+$, 689 $[M+H]^+$. Anal. Calcd for $C_{30}H_{48}N_4O_{14}\cdot1.5H_2O$: C, 50.34; H, 7.18; N, 7.83. Found: C, 50.36; H, 6.98; N, 7.44.

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Supplementary data

Supplementary data (^1H and ^{13}C NMR spectra for compounds **6a**–**c**) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.05.008.

References

1. Degroote, S.; Wolthoorn, J.; Meer, G.. *Sem. Cell Devel. Biol.* **2004**, *15*, 375–387.
2. Sato, T.; Hatanaka, K.; Hashimoto, H.; Yamagata, T. *Trends Glycosci. Glycotechnol.* **2007**, *19*, 1–17.
3. Sandhoff, K.; Kolter, T. *Philos. Trans. R. Soc. London, Ser. B* **2003**, *358*, 847–861.
4. Kasuya, M. C. Z.; Wang, L. X.; Lee, Y. C.; Mitsuki, M.; Nakajima, H.; Miura, Y.; Sato, T.; Hatanaka, K.; Yamagata, S.; Yamagata, T. *Carbohydr. Res.* **2000**, *329*, 755–763.
5. Schmidt, R. R.; Jung, K.-H. Oligosaccharide Synthesis with Trichloroacetimidates. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 283–312.