Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 6518–6526

Fluorescence modification of Gb3 oligosaccharide and rapid synthesis of oligosaccharide moieties using fluorous protective group

Tsuyoshi Miura,[a,b,*] Satoshi Tsujino,[a] Ai Satoh,[a] Kohtaro Goto,[a] Mamoru Mizuno,[a] Midori Noguchi,[a] Tetsuya Kajimoto,[c] Manabu Node,[c] Yasuoki Murakami,[b] Nobuyuki Imai[b] and Toshiyuki Inazu[a,d,*]

[a]*The Noguchi Institute, 1-8-1 Kaga, Itabashi-ku, Tokyo 173-0003, Japan*
[b]*Faculty of Pharmaceutical Sciences, Chiba Institute of Science, 15-8 Shiomi-cho, Choshi, Chiba 288-0025, Japan*
[c]*Kyoto Pharmaceutical University, 1 Shichono-cho, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan*
[d]*Department of Applied Chemistry, School of Engineering, and Institute of Glycotechnology, Tokai University, Kitakaname 1117, Hiratsuka, Kanagawa 259-1292, Japan*

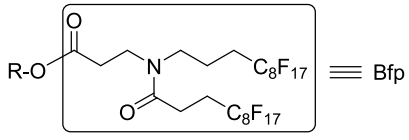
Received 24 March 2005; revised 30 April 2005; accepted 2 May 2005

Available online 23 May 2005

Abstract—The use of the bisfluorous chain-type propanoyl (Bfp) group as a fluorous protective group made it possible to rapidly synthesize the Gb2 and Gb3 oligosaccharide derivatives by a simple fluorous-organic extraction purification. Furthermore, the fluorescence-labeled Gb2 and Gb3 oligosaccharides were prepared as a potential Vero Toxins detecting reagent.

1. Introduction

Vero toxins (VTs) are the Shiga-like toxins produced by *Escherichia coli* O-157:H-7.[1] VTs cause serious clinical complications in humans. VTs initially produce diarrhea in human beings, then further progresses to the Hemolytic Uremic Syndrome, renal failure, and finally death. In order to suppress the infection progress by *E. coli* O-157:H-7 to a minimum, the development of an accurate and rapid detecting method of VTs is essential. VTs are AB_5 toxins with an enzymatically active A-subunit and a cell binding B-subunit. The B-subunit mainly recognizes a galactobiosyl α-(1→4)-linkage in the glycolipid Gb2 [Galα-(1→4)Gal-Cer] and Gb3 [Galα-(1→4)Galβ-(1→4)Glc-Cer], and VTs induce the carbohydrate-mediated internalization into the host cell.[2] A carbohydrate–lectin interaction in the solution phase was sensitively detected by fluorescence polarization using a fluorescence-labeled carbohydrate.[3] Recently, we reported that fluorescence polarization detected the carbohydrate–lectin interaction using the fluorescence-labeled oligosaccharides derived from glycosyl amino acids.[4] To develop a method sensitively detecting VTs, we attempted the synthesis of fluorescence-labeled Gb2 and Gb3 oligosaccharides, which were substituted with simple lipophilic fluorescence groups, such as a dansyl or fluorescein group, for the ceramide moieties of the glycolipids.



1a R = H
1b R = Me

The synthesis of the Gb2 and Gb3 oligosaccharides have already been accomplished by several groups.[5] However, each synthetic method requires much time and cost due to the purification procedure, such as column chromatography in multisteps. The heavy fluorous technique using a fluorous protecting group developed by Curran et al. is an excellent methodology to resolve these problems.[6] A highly fluorinated compound, in which a fluorous protecting group is introduced, is readily separated from nonfluorinated compounds by a simple fluorous-organic phase separation without column chromatography. Several fluorous protecting groups were developed for the fluorous synthesis.[7] We also have reported the fluorous oligosaccharide synthesis using the Bfp (bisfluorous chain-type propanoyl) group as a

* Corresponding authors. Tel.: +81 479304612; fax: +81 479304610 (T.M.); e-mail: tmiura@cis.ac.jp

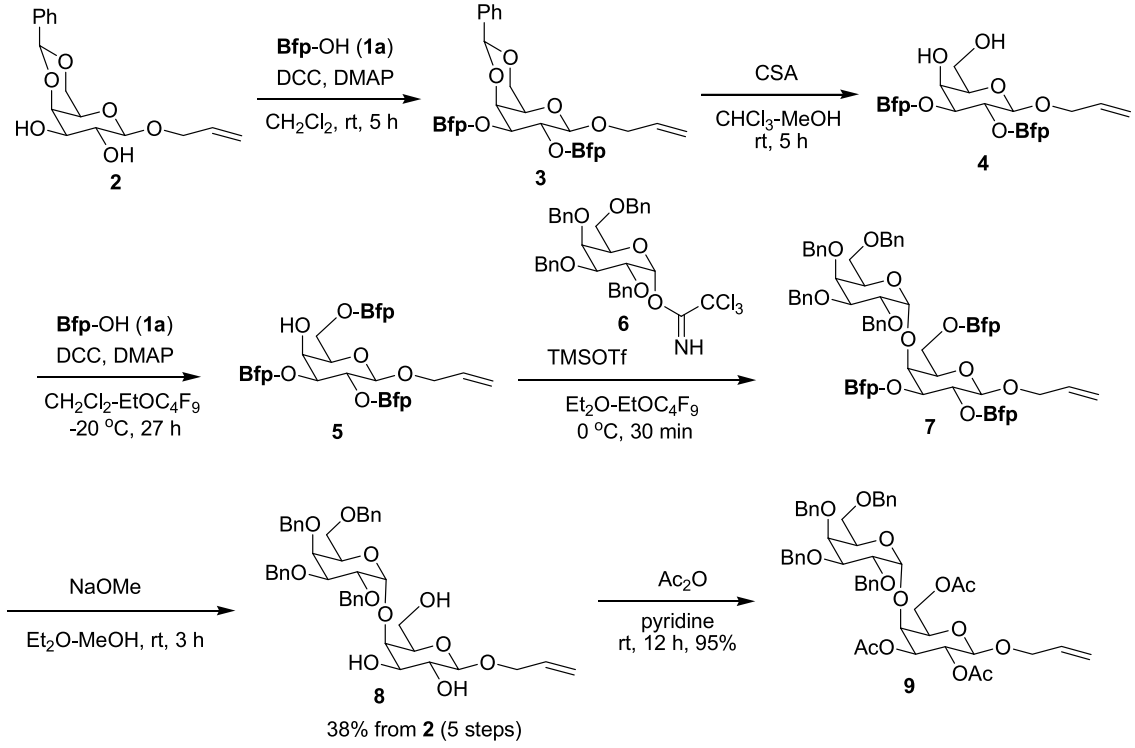
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.05.001

novel fluorous protective group,[8] the rapid synthesis of the Gb2 and Gb3 oligosaccharides using the Bfp group in a preliminary communication,[9] and the synthesis of oligosaccharides and peptides using the fluorous supports.[10] Herein, we would like to describe the full details of the rapid synthesis of the Gb2 and Gb3 oligosaccharides using the Bfp group as a fluorous protective group and the fluorescence modification of the Gb2 and Gb3 oligosaccharides.

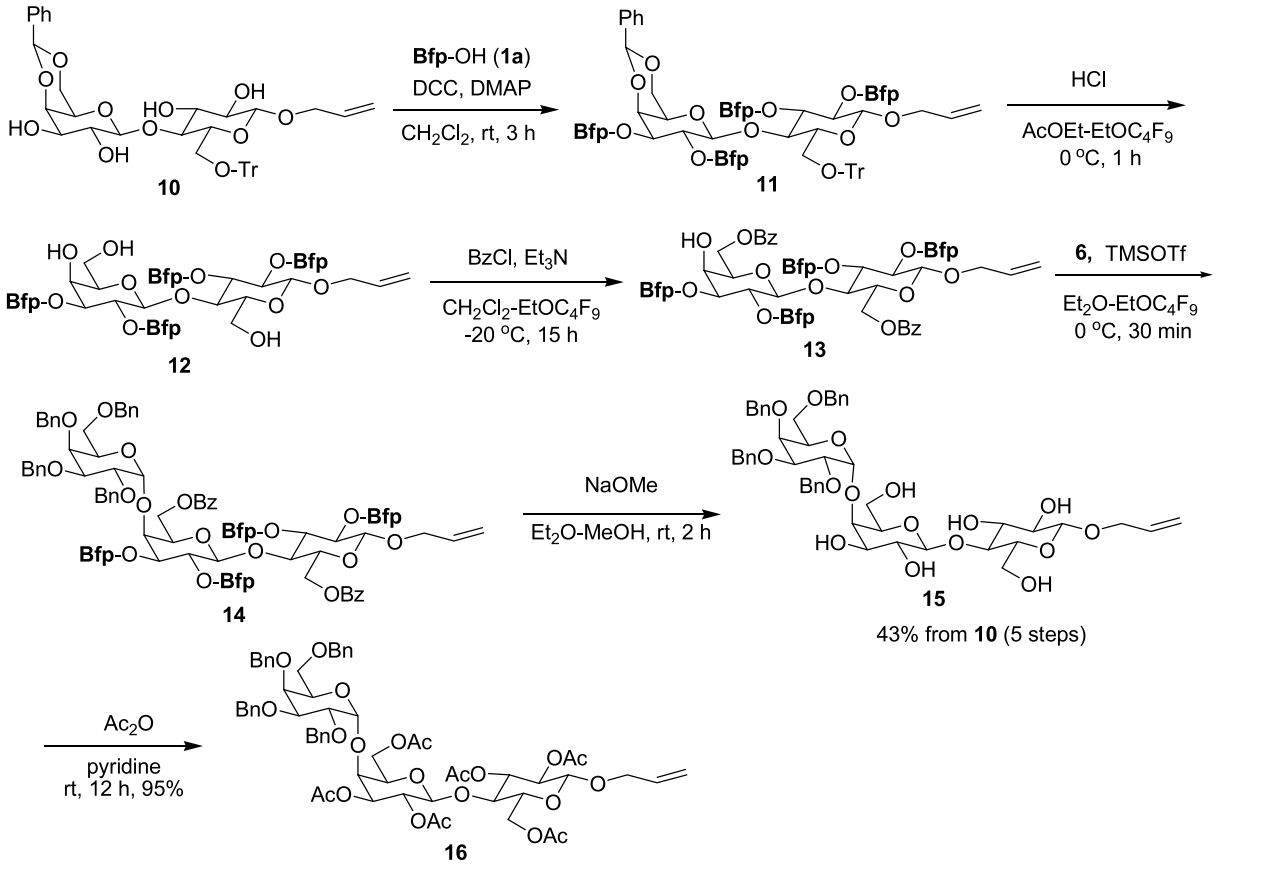
2. Results and discussion

We first synthesized the galabiose derivative **8** as shown in Scheme 1. The Bfp-OH (**1a**)[8] was attached to the hydroxyl functions of the galactose derivative **2** using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(*N*,*N*-dimethylamino) pyridine (DMAP) to give the fluorous compound **3**.[11] The benzylidene group of **3** was removed by treatment with camphorsulfonic acid (CSA) in MeOH–$CHCl_3$ to afford the corresponding product **4**.[11] The Bfp group was selectively introduced into the primary hydroxyl function of **4** using DCC and DMAP at −20 °C to give the fluorous glycosyl acceptor **5**.[11] The fluorous disaccharide **7**[11] was obtained by the reaction of **5** with the glycosyl donor **6** (6.1 equiv) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in Et_2O–$EtOC_4F_9$.[12] The α-selectivity of the glycosylation reaction was very high and no β-isomer could be detected. The fluorous intermediates **3**, **4**, **5**, and **7** were each extracted with the fluorous solvent FC-72[13] by partitioning the product mixture between FC-72 and an organic solvent, such as toluene or methanol. No further purification, such as silica-gel column chromatography, was carried out. The Bfp group of **7** was easily removed by treatment with sodium methoxide in MeOH–Et_2O to afford the crude **8**, which was extracted with MeOH by partitioning the mixture between FC-72 and MeOH. The methyl ester of Bfp (Bfp-OMe, **1b**) was extracted from the FC-72 layer and treated with aqueous NaOH to recover **1a**, which is able to be reused as a fluorous protective reagent. Finally, the pure galabiose derivative **8** was obtained from the single silica gel column chromatographic purification step in a 38% overall yield from **2** (five steps). The disaccharide **8** was converted to the acetate **9** by treatment with acetic anhydride in pyridine for identification of the structure.

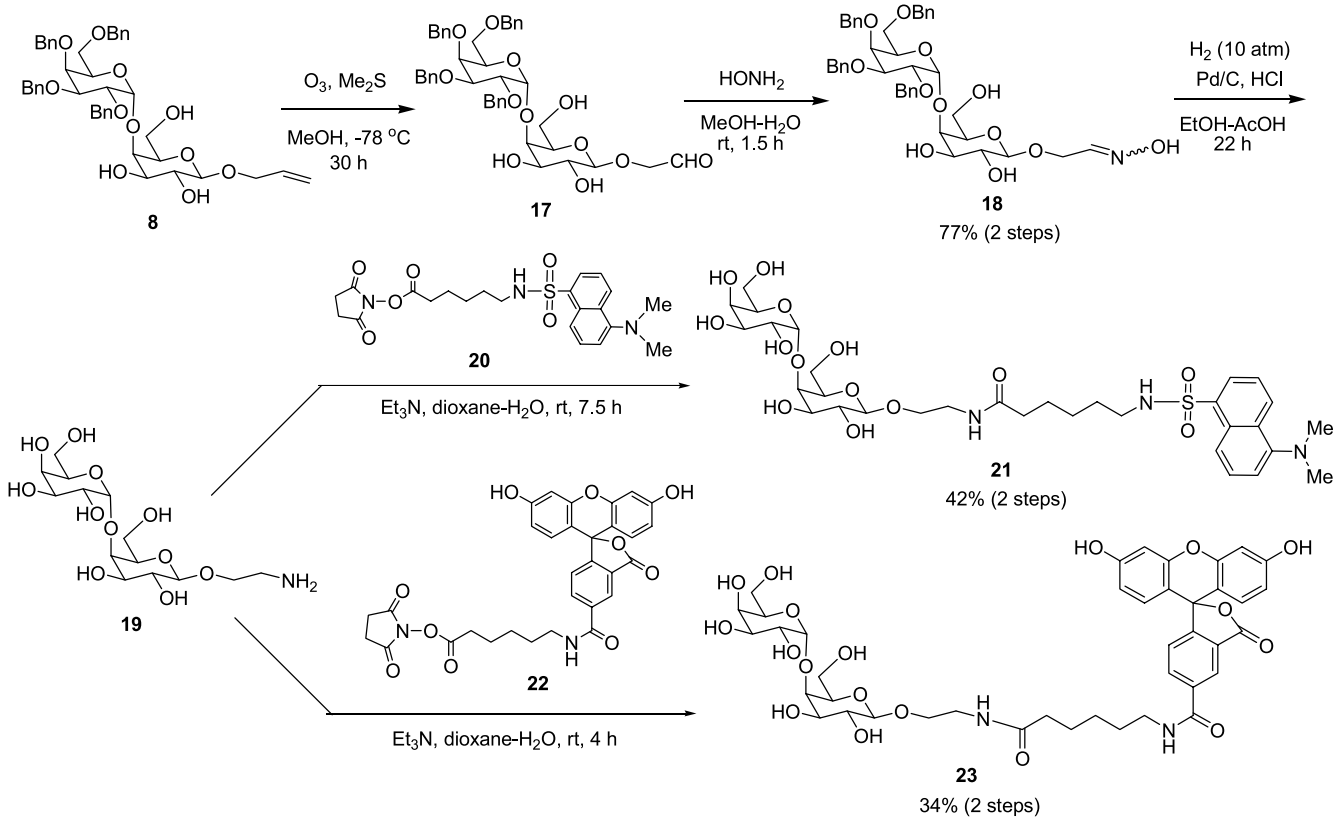
Next, we synthesized the Gb3 trisaccharide **15** as described in Scheme 2. The Bfp group was introduced to the four hydroxyl functions of the lactose derivative **10** using DCC and DMAP to give the fluorous compound **11**.[11] The benzylidene group and trityl (Tr) group of **11** were deprotected by treatment with HCl in AcOEt–$EtOC_4F_9$ to afford **12**.[11] The benzoyl (Bz) group was selectively introduced to the two primary hydroxyl functions of **12** to obtain the fluorous glycosyl acceptor **13**.[11] The reaction of the fluorous glycosyl acceptor **13** with the glycosyl donor **6** (5 equiv) in the presence of TMSOTf in ether–$EtOC_4F_9$ selectively afforded only the α-linked fluorous trisaccharide **14**.[11] The fluorous intermediates **11**, **12**, **13**, and **14** were extracted with FC-72 by partitioning the product mixture between FC-72 and an organic solvent (toluene or methanol), and were purified enough without silica gel column chromatography. The Bfp group of **14** was removed by treatment with sodium methoxide in MeOH–ether to afford **15**, which was extracted with MeOH by partitioning the crude residue between FC-72 and MeOH. Finally, the pure trisaccharide **15** was obtained by only one silica gel column chromatography purification during the final step in a 43% overall yield from **10** (five steps). The trisaccharide **15** was converted to **16** by treatment with acetic anhydride in pyridine for identification of the structure.



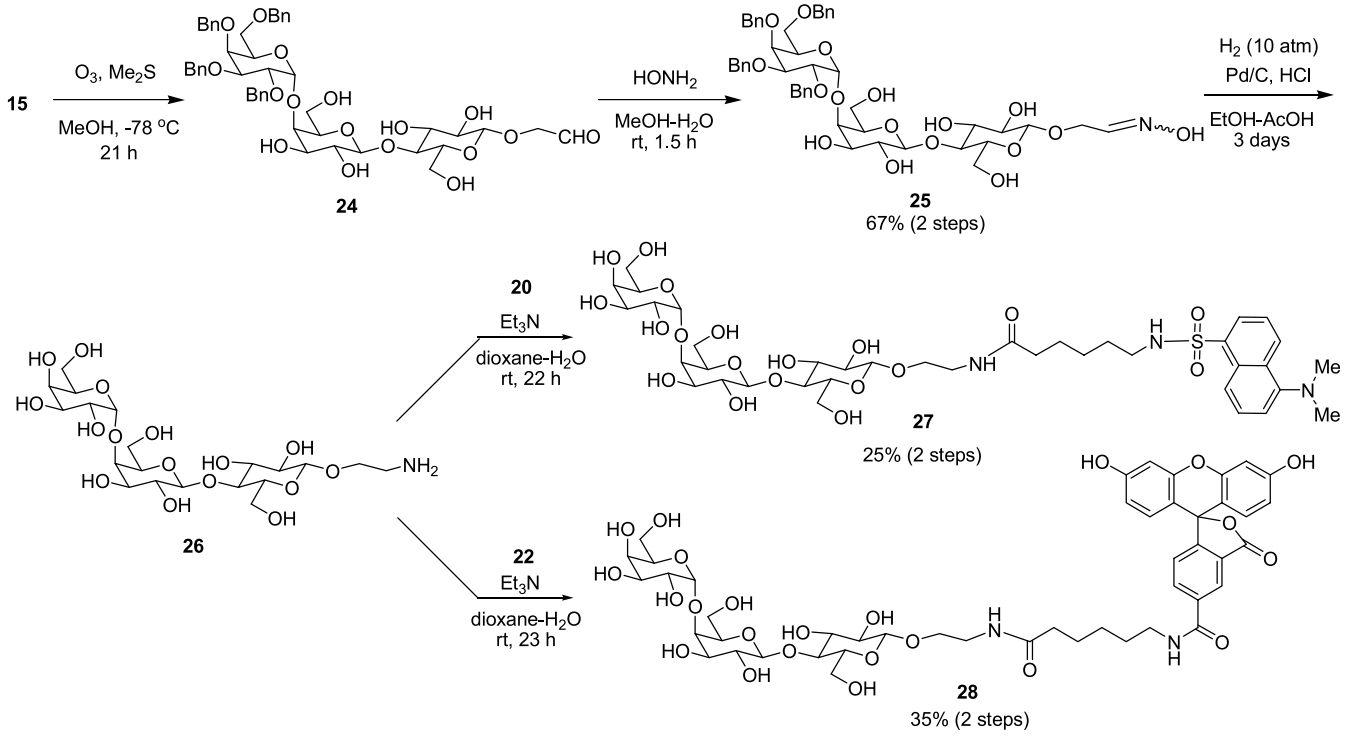
Scheme 1. Synthesis of Gb2 oligosaccharide using fluorous protecting gorup **Bfp**.



Scheme 2. Synthesis of Gb3 oligosaccharide using fluorous protecting group **Bfp**.



Scheme 3. Intorduction of fluorescein groups to Gb2 oligosaccharide.



Scheme 4. Introduction of fluorescein groups to Gb3 oligosaccharide.

We then selected the dansyl and fluorescein groups as the fluorescence group, and introduced them to the Gb2 oligosaccharide as indicated in Scheme 3. The disaccharide **8** was oxidized by ozonolysis, followed by condensation with hydroxylamine to afford the corresponding imine **18** as an inseparable mixture of *syn* and *anti* isomers in 77% yield (two steps). The imine **18** was hydrogenated over Pd/C to give the mixture of the amine **19** and the protected ones, which was coupled with **20** to afford the desired Gb2 oligosaccharide **21** which possessed the dansyl group as the fluorescence group in 42% yield (two steps). The amine **19** was also reacted with **22** to give the Gb2 oligosaccharide **23** with a fluorescein group in a 34% yield (two steps) (Scheme 4).

Finally, we synthesized the Gb3 oligosaccharide **27** and **28** by the similar procedure. The trisaccharide **15** was oxidized by ozone to give the aldehyde **24**, which was coupled with hydroxylamine to afford the corresponding compound **25** as an inseparable mixture of *syn* and *anti* isomers in 67% yield (two steps). Compound **25** was reduced by catalytic hydrogenation over Pd/C to give the mixture of the amine **26** and the protected ones, which was coupled with **20** to afford the desired Gb3 oligosaccharide **27** with a dansyl group in 25% yield (two steps). The amine **26** was also reacted with **22** to give the Gb3 oligosaccharide **28** with a fluorescein group in 35% yield (two steps).

3. Conclusion

In conclusion, we succeeded in synthesizing the Gb2 and Gb3 oligosaccharides containing fluorescence groups. The use of the Bfp group as a fluorous protecting group made it possible to rapidly synthesize the Gb2 and Gb3 oligosaccharide derivatives by a fluorous-organic extraction purification. The fluorous oligosaccharide synthesis can be carried out on a large scale due to the liquid phase synthesis. As each synthetic intermediate containing the Bfp group was monitored by TLC, NMR, and MS, the reaction conditions for each synthetic step could be rapidly optimized. The fluorous intermediates could also be purified by silica gel column chromatography when required purification. After optimization of the reaction conditions in each step, the synthesis in multisteps was accomplished by a fluorous-organic partition purification without column chromatography. The only final product, from which the Bfp groups were removed, was purified by column chromatography on silica gel. Therefore, the fluorous oligosaccharide synthesis using the Bfp group is an excellent strategic alternative to solid phase oligosaccharide synthesis, and removes some of the disadvantages of the solid phase method. The detection assay of VTs using the fluorescence-labeled Gb2 and Gb3 oligosaccharides is now in progress.

4. Experimental

4.1. General

The ^{1}H NMR spectra were recorded using JEOL JNM-EX-400 (400 MHz) and JEOL JNM-ECA-600 (600 MHz) spectrometers. The Mass spectra (MS) of the compounds with a high molecular weight were recorded using a MALDI-TOF-MS (Voyager-DE STR) spectrometer. The high-resolution MS (HRMS) were recorded on an ESI-TOF-MS (Mariner™) spectrometer. Part of the products was isolated by column chromatography on silica gel (Kanto Chemical, silica gel 60N, spherical, neutral, 40–50 μm). The fluorous solvent FC-72 and Novec HFE-7200 were

purchased from 3 M Tokyo. Bfp-OH (**1a**)[8] is commercially available from Kokusan Chemical.

4.1.1. Compound 3. DMAP (3.27 g, 26.8 mmol) and DCC (9.91 g, 48.1 mmol) were added to a solution of **2**[14] (2.50 g, 8.12 mmol) and **1a**[8] (19.0 g, 18.6 mmol) in dried CH_2Cl_2 (200 mL). After stirring for 5 h at rt, MeOH (100 mL) was added to the reaction mixture. The mixture was stirred for 30 min at rt, and the CH_2Cl_2 was evaporated. The reaction mixture was extracted three times with FC-72. The FC-72 layers were combined and evaporated to give a crude **3** (21.1 g). The crude **3** was used in the next step without further purification. The authentic sample was obtained as a colorless amorphous solid by purifying part of the sample (silica gel column chromatography with a 2:1 mixture of hexane and AcOEt). $R_f=0.19$ (hexane–AcOEt=2:1); $[\alpha]_D^{26}$ +12.8 (*c* 1.27, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 1.74–2.08 (8H, m), 2.44–2.68 (12H, m), 3.14–3.69 (9H, m), 4.09 (2H, m), 4.37 (3H, m), 4.58 (1H, m), 4.95 (1H, m), 5.19 (1H, d, $J=10.8$ Hz), 5.27 (1H, d, $J=17.2$ Hz), 5.41 (1H, t, $J=7.6$ Hz), 5.50 (1H, s), 5.85 (1H, m), 7.37 (3H, m), 7.51 (2H, m); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 2342.5. Calcd for $C_{66}H_{46}F_{68}N_2O_{10}Na$ $[M+Na]^+$ 2341.2. Found: *m/z* $[M+K]^+$ 2359.3. Calcd for $C_{66}H_{46}F_{68}N_2O_{10}K$ $[M+K]^+$ 2357.2.

4.1.2. Compound 4. CSA (1.63 g, 7.0 mmol) was added to a solution of the crude **3** (10.7 g) in $CHCl_3$ (80 mL)–MeOH (40 mL). After stirring for 5 h at rt, toluene (100 mL) and saturated aqueous $NaHCO_3$ (100 mL) were added to the reaction mixture. The mixture was stirred for 30 min at rt, and the organic solvents ($CHCl_3$ and MeOH) were evaporated. The reaction mixture was then extracted three times with FC-72. The FC-72 layers were combined, dried over anhydrous Na_2SO_4, and evaporated to afford a crude **4** (9.54 g). The crude **4** was used in the next step without further purification. The authentic sample was obtained as a colorless amorphous solid by purifying part of the crude **4** (silica gel column chromatography, eluent; hexane–AcOEt=1:1). $R_f=0.30$ (hexane–AcOEt=1:1); $[\alpha]_D^{26}$ −1.1 (*c* 1.25, FC-72); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.87$ (4H, m), 2.09 (4H, m), 2.58 (14H, m), 3.38–3.68 (8H, m), 3.78–4.18 (4H, m), 4.33 (2H, m), 4.52 (1H, d, $J=8.1$ Hz), 4.78 (1H, m), 5.17 (1H, d, $J=10.3$ Hz), 5.25 (1H, d, $J=$ 17.3 Hz), 5.34 (1H, m), 5.85 (1H, m); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 2251.6. Calcd for $C_{59}H_{42}F_{68}N_2O_{10}$-Na $[M+Na]^+$ 2253.2. Found: *m/z* $[M+K]^+$ 2267.8. Calcd for $C_{59}H_{42}F_{68}N_2O_{10}K$ $[M+K]^+$ 2269.1.

4.1.3. Compound 5. DMAP (243 mg, 2.00 mmol) and DCC (264 mg, 1.28 mmol) were added to a solution of the crude **4** (0.89 g) and **1a** (436 mg, 0.43 mmol) in CH_2Cl_2 (20 mL)–$EtOC_4F_9$ (20 mL) at −20 °C. After stirring for 27 h at −20 °C, MeOH (6 mL) and toluene (50 mL) were added to the reaction mixture. The mixture was stirred for 30 min at rt, and then the CH_2Cl_2 was evaporated. The mixture was extracted three times with FC-72. The FC-72 layers were combined and evaporated to give a crude **5** (1.26 g). The crude **5** was used in the next step without further purification. The authentic sample was obtained as a colorless amorphous solid by purifying part of the crude **5** (silica gel column chromatography, eluent: hexane–AcOEt=3:2). $R_f=0.32$ (hexane–AcOEt=3:2); $[\alpha]_D^{26}$ −7.1 (*c* 3.02, FC-72); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.87$ (6H, m), 2.10 (6H, m), 2.57 (19H, m), 3.43–3.98 (13H, m), 4.10 (2H, m), 4.34 (3H, m), 4.49 (1H, m), 4.75 (1H, m), 5.26 (3H, m), 5.82 (1H, m); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 3257.5. Calcd for $C_{84}H_{55}F_{102}N_3O_{10}Na$ $[M+Na]^+$ 3258.2. Found: *m/z* $[M+K]^+$ 3274.2. Calcd for $C_{84}H_{55}F_{102}N_3O_{10}K$ $[M+K]^+$ 3274.2.

4.1.4. Compound 7. Molecular sieves 4A powder (3.3 g) was added to a solution of the crude **5** (1.26 g) and **6** (1.63 g, 2.38 mmol) in anhydrous ether (40 mL)–$EtOC_4F_9$ (20 mL) under an argon atmosphere. After stirring for 3 h at rt, TMSOTf (210 μL, 1.16 mmol) was added at 0 °C to the reaction mixture. The mixture was stirred for 30 min at 0 °C, and filtered on Celite. The filtrate was added to saturated aqueous $NaHCO_3$ and extracted three times with AcOEt. The AcOEt layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was dissolved in MeOH and FC-72, and extracted three times with FC-72. The FC-72 layers were combined and evaporated to afford a crude **7** (1.28 g). The crude **7** was used in the next step without further purification. The authentic sample was obtained as a colorless amorphous solid by purifying part of the crude **7** (silica gel column chromatography, eluent: hexane–AcOEt=2:1). $R_f=0.37$ (hexane–AcOEt=2:1); $[\alpha]_D^{26}$ +11.5 (*c* 2.33, FC-72); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.87$ (6H, m), 2.04 (6H, m), 2.54 (18H, m), 3.21–3.71 (15H, m), 4.06 (5H, m), 4.27–4.55 (8H, m), 4.67–4.94 (7H, m), 5.18 (1H, d, $J=10.5$ Hz), 5.27 (2H, m), 5.84 (1H, m), 7.27 (20H, m); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 3781.5. Calcd for $C_{118}H_{89}F_{102}N_3$-$O_{17}Na$ $[M+Na]^+$ 3780.5. Found: *m/z* $[M+K]^+$ 3797.4. Calcd for $C_{118}H_{89}F_{102}N_3O_{17}K$ $[M+K]^+$ 3796.4.

4.1.5. Compound 8. A 28% solution of sodium methoxide in MeOH (140 μL) was added to a solution of the crude **7** (1.28 g) in ether (15 mL)–MeOH (15 mL). After stirring for 3 h at rt, the reaction mixture was neutralized with Amberlite IR-120 (H^+ form), and filtered. The filtrate was evaporated. The residue was dissolved in MeOH and FC-72, and extracted three times with FC-72. The methanol layer was evaporated to give a crude **8**. The FC-72 layers were combined and evaporated to afford the pure compound **1b**.[8b] The crude **8** was purified by column chromatography on silica gel with a 1:2 mixture of hexane and AcOEt to give the pure **8** (114 mg, 38% in five steps from **2**) as colorless crystals. Mp 68–70 °C; $[\alpha]_D^{26}$ +33.1 (*c* 1.45, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=2.10$ (2H, brs), 3.27 (1H, dd, $J=9.3$, 3.4 Hz), 3.37 (1H, m), 3.45 (1H, dd, $J=10.0$, 7.6 Hz), 3.56 (1H, t, $J=9.0$ Hz), 3.70 (4H, m), 3.90 (1H, brs), 3.94 (1H, brs), 4.01 (1H, dd, $J=10.0$, 2.4 Hz), 4.13 (3H, m), 4.24 (1H, d, $J=7.3$ Hz), 4.35 (1H, m), 4.37 (1H, d, $J=11.2$ Hz), 4.45 (1H, d, $J=11.2$ Hz), 4.53 (1H, d, $J=$ 11.5 Hz), 4.72 (1H, d, $J=12.7$ Hz), 4.77 (2H, s), 4.82 (1H, d, $J=3.4$ Hz), 4.91 (2H, d, $J=11.5$ Hz), 5.21 (1H, d, $J=$ 10.3 Hz), 5.32 (1H, d, $J=17.3$ Hz), 5.94 (1H, m), 7.30 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=60.35$, 69.58, 70.40, 71.36, 72.10, 72.68, 73.47, 73.72, 74.09, 74.35, 74.61, 74.95, 75.69, 79.07, 80.70, 100.85, 102.03, 117.83, 127.22, 127.65, 127.70, 127.82, 127.98, 128.03, 128.19, 128.32, 128.39, 128.43, 128.58, 133.66, 136.86, 137.31, 137.84, 137.86; HRMS (ESI-TOF): calcd for $C_{43}H_{50}O_{11}Na$

$(M+Na)^+$: 765.3245. Found: 765.3220. Anal. Calcd for $C_{43}H_{50}O_{11}H_2O$: C, 67.88; H, 6.89. Found: C, 67.55; H, 6.85.

4.1.6. Compound 9. Acetic anhydride (0.5 mL) was added to a solution of **8** (13.7 mg, 18.5 μmol) in pyridine (1.0 mL). After stirring for 15 h at rt, MeOH (10 mL) was added at 0 °C to the reaction mixture and evaporated. The residue was purified by column chromatography on silica gel with a 7:4 mixture of hexane and AcOEt to give the pure **9** (15.2 mg, 95%) as a colorless oil. $[\alpha]_D^{22}$ +32.9 (*c* 0.83, $CHCl_3$); ^1H NMR (400 MHz, CD_3OD): $\delta=1.90$ (3H, s), 2.00 (3H, s), 2.03 (3H, s), 3.47 (1H, dd, $J=8.8$, 5.1 Hz), 3.61 (1H, t, $J=8.8$ Hz), 3.86 (1H, t, $J=6.3$ Hz), 3.96 (1H, dd, $J=10.0$, 3.2 Hz), 4.08 (1H, d, $J=2.9$ Hz), 4.11 (3H, m), 4.30 (2H, m), 4.35 (1H, dd, $J=11.5$, 5.9 Hz), 4.43 (1H, dd, $J=11.5$, 7.1 Hz), 4.45 (1H, d, $J=12.0$ Hz), 4.49 (1H, d, $J=12.0$ Hz), 4.51 (1H, d, $J=11.2$ Hz), 4.61 (1H, d, $J=7.8$ Hz), 4.70, (1H, d, $J=11.7$ Hz), 4.76 (2H, s), 4.78 (1H, d, $J=11.7$ Hz), 4.79 (1H, d, $J=3.2$ Hz), 4.85 (1H, d, $J=11.2$ Hz), 4.96 (1H, dd, $J=10.5$, 2.9 Hz), 5.16 (1H, dd, $J=10.5$, 1.5 Hz), 5.23 (1H, dd, $J=10.5$, 7.8 Hz), 5.27 (1H, dd, $J=17.3$, 1.5 Hz), 5.89 (1H, m), 7.31 (20H, m); ^{13}C NMR (100 MHz, CD_3OD): $\delta=20.79$, 20.81, 20.94, 63.92, 69.04, 70.73, 70.85, 70.99, 73.59, 73.91, 73.95, 74.31, 74.59, 76.08, 76.35, 76.82, 77.27, 79.76, 101.35, 101.75, 117.27, 128.45, 128.50, 128.64, 128.84, 129.08, 129.10, 129.19, 129.28, 129.29, 135.12, 139.40, 139.74, 140.00, 140.06, 171.16, 171.88, 172.11; MALDI-TOF MS found: *m/z* $[M+Na]^+$ 889.9. Calcd for $C_{49}H_{56}O_{14}Na$ $[M+Na]^+$ 891.4. Found: *m/z* $[M+K]^+$ 905.8. Calcd for $C_{49}H_{56}O_{14}K$ $[M+K]^+$ 907.3.

4.1.7. Compound 10. Triphenylmethyl chloride (7.39 g, 26.5 mmol) was added at rt to a solution of allyl 4′,6′-*O*-benzylidene-β-lactoside[15] (4.13 g, 8.80 mmol) in pyridine (50 mL). After stirring for 24 h at 50 °C, MeOH (2 mL) was added to the reaction mixture. After cooling, the reaction mixture was evaporated. The residue was treated with water and extracted three times with AcOEt. The AcOEt layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica gel with a 20:1 mixture of $CHCl_3$ and MeOH to afforded the pure **10** (3.88 g, 62%) as a colorless powder. $[\alpha]_D^{23}$ −25.6 (*c* 1.04, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.68$ (1H, d, $J=3.4$ Hz), 2.38 (1H, d, $J=8.8$ Hz), 2.56 (1H, d, $J=2.0$ Hz), 3.30 (1H, dd, $J=10.5$, 4.4 Hz), 3.38 (1H, dd, $J=9.5$, 3.7 Hz), 3.42 (1H, s), 3.52 (2H, m), 3.63 (3H, m), 3.74 (1H, t, $J=9.0$ Hz), 4.04 (1H, d, $J=12.9$ Hz), 4.13 (1H, d, $J=3.2$ Hz), 4.17 (1H, d, $J=7.6$ Hz), 4.26 (3H, m), 4.43 (1H, d, $J=7.6$ Hz), 4.46 (1H, dd, $J=12.9$, 5.4 Hz), 5.27 (1H, d, $J=10.5$ Hz), 5.38 (1H, d, $J=17.1$ Hz), 5.50 (1H, s), 6.04 (1H, m), 7.28 (9H, m), 7.36 (3H, m), 7.47 (8H, m); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=62.40$, 66.64, 68.59, 69.96, 71.02, 72.29, 73.52, 73.83, 74.55, 74.65, 79.70, 86.38, 101.03, 102.60, 117.82, 125.99, 126.77, 127.49, 127.95, 128.45, 128.82, 133.53, 136.90, 143.43; HRMS (ESI-TOF): calcd for $C_{41}H_{44}O_{11}Na$ $(M+Na)^+$: 735.2776. Found: 735.2814.

4.1.8. Compound 11. DMAP (3.28 g, 26.8 mmol) and DCC (7.90 g, 38.3 mmol) were added to a solution of **10** (2.73 g, 3.83 mmol) and **1a** (16.5 g, 16.1 mmol) in anhydrous CH_2Cl_2 (150 mL). After stirring for 3 h at rt, MeOH (30 mL) was added to the reaction mixture. The mixture was stirred for 1 h at rt, toluene was added to the reaction mixture. The mixture was extracted three times with FC-72. The FC-72 layers were combined and evaporated to give a crude **11** (17.4 g). The crude **11** was used in the next step without further purification. The authentic sample was obtained by purifying part of the sample (silica gel column chromatography, eluent: hexane–AcOEt=2:1) as a colorless amorphous solid. $R_f=0.44$ (hexane–AcOEt=2:1); $[\alpha]_D^{23}$ −1.5 (*c* 1.05, AcOEt); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.70$–2.08 (16H, m), 2.34–2.72 (24H, m), 3.00–3.75 (20H, m), 4.00 (1H, m), 4.13–4.72 (8H, m), 4.96 (1H, m), 5.12 (2H, m), 5.21 (1H, d, $J=10.5$ Hz), 5.29 (1H, d, $J=17.3$ Hz), 5.44 (1H, m), 5.92 (1H, m), 7.27 (3H, m), 7.33 (9H, m), 7.42 (2H, m), 7.50 (6H, m); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 4753.6. Calcd for $C_{141}H_{96}F_{136}N_4O_{19}Na$ $[M+Na]^+$ 4755.4.

4.1.9. Compound 12. To a solution of the crude **11** (6.71 g) in $EtOC_4F_9$ (60 mL) were added 20 mL of a 4 M solution of hydrochloric acid in AcOEt and 1 mL of water were added at 0 °C. After stirring for 1 h at 0 °C, toluene and water were added to the reaction mixture. The reaction mixture was extracted three times with FC-72. The FC-72 layers were combined, dried over anhydrous Na_2SO_4, and evaporated to give a crude **12** (5.65 g). The crude **12** was used in the next step without further purification. The authentic sample was obtained by purifying part of the sample (silica gel column chromatography, eluent: $CHCl_3$–MeOH=30:1) as a colorless amorphous solid. Compound **12** is insoluble in all deuterium solvents, therefore, no NMR spectra could be obtained. $R_f=0.26$ ($CHCl_3$–MeOH=20:1); $[\alpha]_D^{23}$ +0.8 (*c* 0.93, FC-72); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 4423.2. Calcd for $C_{115}H_{78}F_{136}N_4O_{19}Na$ $[M+Na]^+$ 4425.3. Found: *m/z* $[M+K]^+$ 4440.3. Calcd for $C_{115}H_{78}F_{136}N_4O_{19}K$ $[M+K]^+$ 4441.3.

4.1.10. Compound 13. Benzoyl chloride (0.75 mL, 6.46 mmol) was added at −20 °C to a solution of the crude **12** (6.65 g) and triethylamine (5.4 mL, 38.5 mmol) in anhydrous CH_2Cl_2 (100 mL)–$EtOC_4F_9$ (100 mL). After stirring for 15 h at −20 °C, MeOH (50 mL) was added to the reaction mixture, and then the CH_2Cl_2 and $EtOC_4F_9$ were evaporated. MeOH was added to the mixture, and the resulting solution was extracted three times with FC-72. The FC-72 layers were combined and evaporated to give a crude **13** (6.01 g). The crude **13** was used in the next step without further purification. The authentic sample was obtained as a colorless amorphous solid by purifying part of the sample (silica gel column chromatography, eluent: hexane–AcOEt=1.8:1). $R_f=0.71$ ($CHCl_3$–MeOH=20:1); $[\alpha]_D^{23}$ 3.3 (*c* 1.13, AcOEt); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.85$ (8H, m), 2.06 (8H, m), 2.57 (25H, m), 3.38–3.75 (17H, m), 3.88 (2H, m), 4.07 (2H, m), 4.27 (2H, m), 4.41–4.98 (7H, m), 5.16 (4H, m), 5.80 (1H, m), 7.41–7.60 (6H, m), 8.00 (4H, m); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 4632.5. Calcd for $C_{129}H_{86}F_{136}N_4O_{21}Na$ $[M+Na]^+$ 4633.4. Found: *m/z* $[M+K]^+$ 4648.4. Calcd for $C_{129}H_{86}F_{136}N_4O_{21}K$ $[M+K]^+$ 4649.3.

4.1.11. Compound 14. Molecular sieves 4A powder (4.0 g) was added to a solution of the crude **13** (1.85 g) and **6** (1.92 g, 2.81 mmol) in anhydrous ether (40 mL)–$EtOC_4F_9$

(40 mL) under an argon atmosphere. After stirring for further 3 h at rt, TMSOTf (145 μL, 0.81 mmol) was added at 0 °C to the reaction mixture. The mixture was stirred for 30 min at 0 °C, quenched with triethylamine (1 mL), and filtered on Celitection mixture. The filtrate was treated with saturated aqueous $NaHCO_3$, and extracted three times with AcOEt. The AcOEt layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was dissolved in MeOH and FC-72, and extracted three times with FC-72. The FC-72 layers were combined and evaporated to give a crude **14** (1.74 g). The crude **14** was used in the next step without further purification. The authentic sample was obtained by purifying part of the sample (silica gel column chromatography, eluent: hexane–AcOEt=5:2) as a colorless amorphous solid. R_f=0.60 (hexane–AcOEt=2:1); $[\alpha]_D^{23}$ +10.1 (*c* 1.09, AcOEt); ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.68–2.07 (m, 16H), 2.35–2.69 (m, 24H), 3.20–3.90 (m, 21H), 4.08 (m, 5H), 4.28 (m, 4H), 4.40 (m, 1H), 4.52 (m, 4H), 4.70–4.98 (m, 10H), 5.17 (m, 4H), 5.80 (m, 1H), 7.22 (m, 18H), 7.40 (m, 4H), 7.50 (m, 2H), 7.59 (m, 2H), 7.99 (m, 4H); MALDI-TOF MS found: *m/z* $[M+Na]^+$ 5157.5. Calcd for $C_{163}H_{120}F_{136}N_4O_{26}Na$ $[M+Na]^+$ 5155.6. Found: *m/z* $[M+K]^+$ 5173.2. Calcd for $C_{163}H_{120}F_{136}N_4O_{26}K$ $[M+K]^+$ 5171.6.

4.1.12. Compound 15. To a solution of the crude **14** (1.72 g, 0.36 mmol) in ether (30 mL)–MeOH (20 mL) was added 200 μL of a 28% solution of sodium methoxide in methanol. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 (H^+ form). After filtration, the filtrate was evaporated. The residue was dissolved in MeOH and FC-72, and extracted three times with FC-72. The FC-72 layers were combined and evaporated to afford the pure product **1b**. The methanol layer was evaporated to give a crude **15**. The crude **15** was purified by column chromatography on silica gel with a 15:1 mixture of $CHCl_3$ and MeOH to give the pure product **15** (174 mg, 43% in five steps from **10**) as a colorless amorphous solid. Mp 128–130 °C; $[\alpha]_D^{23}$ +25.0 (*c* 1.53, $CHCl_3$: MeOH=1:1); ^{1}H NMR (400 MHz, $CDCl_3$: CD_3OD=1:1): δ=3.37–3.62 (8H, m), 3.69 (2H, m), 3.81 (1H, m), 3.87 (2H, m), 3.98 (2H, m), 4.07 (1H, dd, *J*=10.3, 3.7 Hz), 4.17 (2H, m), 4.36 (1H, d, *J*=7.1 Hz), 4.39 (1H, m), 4.43 (1H, d, *J*=11.7 Hz), 4.51 (1H, d, *J*=11.7 Hz), 4.54 (1H, d, *J*=11.7 Hz), 4.72 (1H, d, *J*=11.7 Hz), 4.78 (2H, s), 4.85 (1H, d, *J*=11.7 Hz), 4.89 (1H, d, *J*=11.7 Hz), 4.91 (1H, d, *J*=3.7 Hz), 5.20 (1H, dd, *J*=10.3, 1.5 Hz), 5.34 (1H, dd, *J*=17.1, 1.5 Hz), 5.96 (1H, m), 7.33 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD=1:1): δ=61.34, 61.70, 69.46, 70.69, 71.33, 72.01, 73.14, 73.72, 73.92, 74.11, 74.73, 74.89, 75.12, 75.21, 75.37, 76.49, 79.04, 80.69, 81.34, 100.89, 102.18, 104.57, 117.68, 127.81, 127.96, 128.06, 128.14, 128.33, 128.53, 128.55, 128.57, 128.69, 128.71, 128.76, 134.21, 137.82, 137.88, 138.35, 138.50; HRMS (ESI-TOF): calcd for $C_{49}H_{60}O_{16}Na$ $(M+Na)^+$: 927.3774. Found: 927.3750. Anal. Calcd for $C_{49}H_{60}O_{16}1/2H_2O$: C, 64.39; H, 6.73. Found: C, 64.45; H, 6.58.

4.1.13. Compound 16. Acetic anhydride (1.5 mL) was added to a solution of **15** (37.3 mg, 41.2 μmol) in pyridine (3.0 mL). After stirring for 17 h at rt, MeOH (5 mL) was added at 0 °C to the reaction mixture and evaporated. The residue was purified by column chromatography on silica gel with a 1:1 mixture of hexane and AcOEt to give the pure product **16** (45.3 mg, 95%) as a colorless oil. $[\alpha]_D^{23}$ 33.5 (*c* 2.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.85 (3H, s), 1.87 (3H, s), 2.04 (9H, s), 2.12 (3H, s), 3.42 (1H, dd, *J*=8.8, 4.9 Hz), 3.60 (3H, m), 3.74 (1H, t, *J*=9.5 Hz), 3.97 (1H, d, *J*=2.9 Hz), 4.05 (4H, m), 4.15 (1H, brs), 4.28 (2H, m), 4.43 (6H, m), 4.50 (1H, d, *J*=7.8 Hz), 4.55 (1H, d, *J*=11.0 Hz), 4.66 (1H, d, *J*=11.5 Hz), 4.75 (2H, d, *J*=11.2 Hz), 4.76 (1H, d, *J*=4.2 Hz), 4.83 (2H, brs), 4.92 (2H, m), 5.14 (2H, m), 5.19 (1H, d, *J*=11.2 Hz), 5.25 (1H, d, *J*=17.3 Hz), 5.83 (1H, m), 7.24–7.43 (20H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.60, 20.73, 20.84, 20.88, 61.02, 61.90, 67.51, 69.49, 69.56, 69.88, 71.34, 72.24, 72.36, 72.47, 72.61, 72.73, 73.26, 74.15, 74.52, 74.88, 75.28, 75.87, 76.02, 79.01, 99.32, 100.92, 101.23, 117.44, 127.22, 127.29, 127.51, 127.56, 127.88, 127.97, 128.01, 128.11, 128.15, 128.18, 128.29, 133.19, 137.75, 138.96, 138.58, 138.64, 168.60, 169.40, 169.83, 170.19, 170.24, 170.52; MALDI-TOF MS found: *m/z* $[M+Na]^+$ 1179.3. Calcd for $C_{61}H_{72}O_{22}Na$ $[M+Na]^+$ 1179.4. Found: *m/z* $[M+K]^+$ 1195.8. Calcd for $C_{61}H_{72}O_{22}K$ $[M+K]^+$ 1195.4.

4.1.14. Compound 17. A solution of **8** (58.6 mg, 79 μmol) was treated with ozone at −78 °C by bubbling until the solution remained blue. The reaction mixture was evacuated with air for 5 min to remove the ozone, and then dimethyl sulfide (0.2 mL) was added at −78 °C to the reaction mixture. The resulting solution was allowed to warm to rt, and then evaporated to afford a crude **17** (60.4 mg). The crude **17** was used in the next step without further purification due to its lability.

4.1.15. Compound 18. Hydroxylamine hydrochloric acid salt (1.5 mg, 21.7 μmol) was added at rt to a solution of the crude **17** (10.5 mg, 14.1 μmol) in MeOH (15 mL)–H_2O (2 mL). After stirring for 1.5 h at rt, the MeOH was evaporated. The resulting mixture was treated with water and extracted three times with AcOEt. The AcOEt layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with a 20:1 mixture of $CHCl_3$ and MeOH to give an inseparable mixture of *E*- and *Z*-isomers **18** (8.2 mg, 77% in two steps) as a colorless amorphous solid.

4.1.16. Compound 21. A solution of the mixture **18** (8.2 mg, 10.8 μmol) in EtOH (4 mL)–AcOH (1 mL) and hydrochloric acid in 1,4-dioxane (4 M, 5 μL) were added at rt to a suspension of 10% Pd/C (15 mg) in EtOH (3 mL). After stirring for 22 h under a hydrogen atmosphere (10 atm), the reaction mixture was filtered. The filtrate was evaporated to give the crude compound **19** (8.7 mg). To a solution of the crude **19** (8.7 mg) in 1,4-dioxane (2 mL)–H_2O (2 mL) were added **20** (20.3 mg, 43.9 μmol) and triethylamine (6.4 μL, 46 μmol) at rt. After stirring for 3.5 h at rt, the reaction mixture was dissolved in AcOEt and water, and extracted three times with water. The water layers were combined and evaporated to half to original volume. The water layer was poured into Diaion HP-20. The resin was washed with water, and then eluted with MeOH. The MeOH fractions were then collected and evaporated. The residue was purified by column chromatography on silica gel with a 7:3:0.4 mixture of $CHCl_3$, MeOH, and H_2O

to give the pure **21** (3.3 mg, 42% in two steps) as a green oil. $[\alpha]_D^{25}$ +47.1 (*c* 0.51, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ=1.16 (m, 2H), 1.34 (m, 4H), 2.01 (t, *J*=7.6 Hz, 2H), 2.81 (t, *J*=6.8 Hz, 2H), 2.86 (s, 6H), 3.42 (m, 1H), 3.48 (dd, *J*=10.3, 7.3 Hz, 1H), 3.54 (dd, *J*=10.0, 2.9 Hz, 1H), 3.61 (m, 3H), 3.67 (m, 2H), 3.75 (m, 4H), 3.89 (m, 2H), 3.97 (d, *J*=2.9 Hz, 1H), 4.25 (m, 1H), 4.27 (d, *J*=7.3 Hz, 1H), 4.94 (d, *J*=3.4 Hz, 1H), 7.25 (d, *J*=7.6 Hz, 1H), 7.56 (m, 2H), 8.16 (d, *J*=7.4 Hz, 1H), 8.33 (d, *J*=8.8 Hz, 1H), 8.54 (d, *J*=8.5 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ=26.32, 27.13, 30.32, 36.81, 40.47, 43.70, 45.83, 61.09, 62.62, 69.84, 70.68, 71.02, 71.32, 72.70, 72.82, 74.59, 76.16, 79.31, 102.57, 105.11, 116.33, 120.52, 124.24, 128.96, 130.05, 130.88, 130.98, 131.10, 137.06, 153.05, 175.95; HRMS (ESI-TOF): calcd for $C_{32}H_{50}N_3O_{14}S$ $(M+H)^+$: 732.3008. Found: 732.2991.

4.1.17. Compound 23. To a solution of the crude **19** (14.7 mg) in 1,4-dioxane (2 mL)–H_2O (2 mL) were added **22** (26.9 mg, 46 μmol) and triethylamine (10 μL, 72 μmol) at rt. After stirring for 4 h at rt, the reaction mixture was dissolved in AcOEt and water, and extracted three times with water. The water layers were combined and evaporated to half to original volume. The water layer was poured into Diaion HP-20. The resin was washed with water, and then eluted with MeOH. The MeOH fractions were then collected and evaporated. The residue was purified by column chromatography on silica gel with a 7:3.5:0.6 mixture of $CHCl_3$, MeOH, and H_2O to give the pure **23** (4.7 mg, 14% in two steps) as an orange oil. $[\alpha]_D^{25}$ +10.8 (*c* 0.13, MeOH–H_2O=4:1); ^{1}H NMR (400 MHz, CD_3OD): δ=1.35 (m, 2H), 1.58 (m, 4H), 2.15 (t, *J*=7.3 Hz, 2H), 3.33–3.43 (m, 4H), 3.46 (dd, *J*=10.3, 3.0 Hz, 1H), 3.52–3.75 (m, 9H), 3.81 (m, 2H), 3.89 (d, *J*=2.4 Hz, 1H), 4.17 (t, *J*=6.6 Hz, 1H), 4.20 (d, *J*=7.3 Hz, 1H), 4.86 (d, *J*=3.2 Hz, 1H), 6.48 (dd, *J*=9.1, 2.5 Hz, 2H), 6.58 (d, *J*=2.5 Hz, 2H), 6.66 (d, *J*=9.1 Hz, 2H), 7.21 (d, *J*=8.1 Hz, 1H), 8.04 (dd, *J*=8.1, 1.7 Hz, 1H), 8.33 (d, *J*=1.7 Hz, 1H); HRMS (ESI-TOF): calcd for $C_{41}H_{49}N_2O_{18}H$ $(M+H)^+$: 857.2975. Found: 857.2944.

4.1.18. Compound 24. A solution of **15** (46.2 mg, 51 μmol) was treated with ozone at −78 °C by bubbling until the solution remained blue. The reaction mixture was evacuated with air for 5 min to remove the ozone, and then dimethyl sulfide (0.2 mL) was added at −78 °C to the reaction mixture. The resulting solution was allowed to warm to rt, and then evaporated to afford a crude **24** (44.4 mg). The crude **24** was used in the next step without further purification due to its lability.

4.1.19. Compound 25. Hydroxylamine hydrochloric acid salt (4.9 mg, 71 μmol) was added at rt to a solution of **24** (44.4 mg, 49 μmol) in MeOH (60 mL)–H_2O (8 mL). After stirring for 2 h at rt, MeOH was evaporated. The reaction mixture was quenched with water, and extracted three times with AcOEt. The AcOEt layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with a 10:1 mixture of $CHCl_3$ and MeOH to give an inseparable mixture of *E*- and *Z*-isomers **25** (31.6 mg, 67% in two steps) as a colorless amorphous solid.

4.1.20. Compound 26. A solution of the mixture **25** (14.8 mg, 16 μmol) in EtOH (5 mL)–AcOH (1 mL) and hydrochloric acid in 1,4-dioxane (4 M, 5 μL) were added at rt to a suspension of 10% Pd/C (18 mg) in EtOH (5 mL). After stirring for 3 days under a hydrogen atmosphere (10 atm), the reaction mixture was filtered. The filtrate was evaporated to give a crude **26** (20.5 mg). The crude **26** was used in the next step without further purification.

4.1.21. Compound 27. To a solution of the crude **26** (20.5 mg) in 1,4-dioxane (3 mL)–H_2O (3 mL) were added **20** (19.6 mg, 42 μmol) and triethylamine (5 μL, 36 μmol) at rt. After stirring for 22 h at rt, the reaction mixture was dissolved in AcOEt and water, and extracted three times with water. The water layers were combined and evaporated to half to original volume. The water layer was poured into Diaion HP-20. The resin was washed with water, and then eluted with MeOH. The MeOH fractions were then collected and evaporated. The residue was purified by column chromatography on silica gel with a 7:3:0.4 mixture of $CHCl_3$, MeOH, and H_2O to give the pure **27** (3.3 mg, 25% in two steps) as a green oil. $[\alpha]_D^{25}$ +15.6 (*c* 0.22, MeOH–H_2O=4:1); ^{1}H NMR (400 MHz, CD_3OD–D_2O=4:1): δ=1.13 (m, 2H), 1.31 (m, 4H), 2.02 (t, *J*=7.6 Hz, 2H), 2.84 (t, *J*=7.1 Hz, 2H), 2.87 (s, 6H), 3.43 (m, 3H), 3.52–3.74 (m, 10H), 3.79–3.94 (m 6H), 3.99 (d, *J*=2.7 Hz, 1H), 4.28 (t, *J*=6.3 Hz, 1H), 4.35 (d, *J*=8.8 Hz, 1H), 4.44 (d, *J*=7.5 Hz, 1H), 7.31 (d, *J*=7.3 Hz, 1H), 7.62 (m, 2H), 8.17 (dd, *J*=7.3, 1.2 Hz, 1H), 8.30 (d, *J*=9.8 Hz, 1H), 8.53 (d, *J*=8.5 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD–D_2O=4:1): δ=26.11, 26.81, 29.97, 36.69, 40.38, 43.62, 45.90, 61.49, 62.22, 69.61, 70.22, 70.63, 70.90, 71.19, 72.50, 74.14, 74.50, 76.00, 76.25, 76.56, 79.33, 80.62, 102.24, 103.95, 104.99, 116.59, 120.38, 124.51, 129.31, 130.18, 130.68, 130.84, 131.12, 136.51, 152.83, 176.69; HRMS (ESI-TOF): calcd for $C_{37}H_{57}N_3O_{19}SH$ $(M+H)^+$: 894.3536. Found: 894.3524.

4.1.22. Compound 28. To a solution of the crude compound **26** (9.4 mg) in 1,4-dioxane (3 mL)–H_2O (3 mL) were added **22** (10.0 mg, 17 μmol) and triethylamine (12 μL, 86 μmol) at rt. After stirring for 23 h at rt, the reaction mixture was dissolved in AcOEt and water, and extracted three times with water. The water layers were combined and evaporated to half to original volume. The water layer was poured into Diaion HP-20. The resin was washed with water, and then eluted with MeOH. The MeOH fractions were then collected and evaporated. The residue was purified by column chromatography on silica gel with a 6:4:0.9 mixture of $CHCl_3$, MeOH, and H_2O to give the pure **28** (6.1 mg, 35% in two steps) as an orange oil. $[\alpha]_D^{25}$ +26.8 (*c* 0.33, MeOH–H_2O=4:1); ^{1}H NMR (400 MHz, CD_3OD): δ=1.44 (m, 2H), 1.68 (m, 4H), 2.24 (t, *J*=7.3 Hz, 1H), 3.42–3.59 (m, 9H), 3.64–3.97 (m, 14H), 4.24 (t, *J*=6.3 Hz, 1H), 4.31 (d, *J*=8.6 Hz, 1H), 4.40 (m, 1H), 4.94 (d, *J*=3.9 Hz, 1H), 6.57 (d, *J*=8.8 Hz, 2H), 6.68 (s, 2H), 6.73 (d, *J*=8.8 Hz, 2H), 7.31 (d, *J*=8.1 Hz, 1H), 8.14 (d, *J*=8.1 Hz, 1H), 8.43 (s, 1H); ^{1}H NMR (400 MHz, CD_3OD–D_2O=4:1): δ=1.43 (m, 2H), 1.67 (m, 4H), 2.26 (t, *J*=7.3 Hz, 2H), 3.44 (m, 5H), 3.54–3.73 (m, 9H), 3.81–3.98 (m, 9H), 4.28 (t, *J*=5.9 Hz, 1H), 4.35 (d, *J*=7.8 Hz, 1H), 4.44 (d, *J*=7.3 Hz, 1H), 6.62 (d, *J*=8.7 Hz, 2H), 7.75 (m, 4H), 7.31 (d, *J*=8.1 Hz, 1H), 8.13 (d, *J*=8.1 Hz, 1H), 8.40 (s, 1H);

MALDI-TOF MS found: *m/z* $[M+H]^+$ 1019.9. Calcd for $C_{47}H_{59}N_2O_{23}$ $[M+H]^+$ 1019.4. Found: *m/z* $[M+Na]^+$ 1041.9. Calcd for $C_{47}H_{58}N_2O_{23}Na$ $[M+Na]^+$ 1041.3. Found: *m/z* $[M+K]^+$ 1057.7. Calcd for $C_{47}H_{58}N_2O_{23}K$ $[M+K]^+$ 1057.3.

Acknowledgements

This work was partly supported by a Grant-in-Aid for Young Scientists (B) (No. 16790026) from the Japan Society for the Promotion of Science and a grant for Hi-Tech Research from Tokai University. This work was performed through the Noguchi Fluorous Project by our institute.

References and notes

1. Naiki, M.; Marcus, D. M. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 1105.
2. (a) Lingwood, C. A.; Law, H.; Richardson, S.; Petric, M.; Brunton, J. L.; Grandis, S. D.; Karmali, M. *J. Biol. Chem.* **1987**, *262*, 8834. (b) Lindberg, A. A.; Brown, J. E.; Stromberg, N.; Westling-Ryd, M.; Schultz, J. E.; Karlsson, K. A. *J. Biol. Chem.* **1987**, *262*, 1779. (c) Arab, S.; Lingwood, C. A. *Glycoconjugate J.* **1996**, *13*, 159. (d) Karlsson, K. A. *Annu. Rev. Biochem.* **1989**, *58*, 309.
3. (a) Hirabayashi, J. *Trends Biotechnol.* **2003**, *21*, 141. (b) Mellet, C. O.; Fernandez, J. M. G. *ChemBioChem.* **2002**, *3*, 819.
4. Mizuno, M.; Noguchi, M.; Imai, M.; Motoyoshi, T.; Inazu, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 485.
5. (a) Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, H.; Yokoyama, S.; Ito, S.; Mori, H.; Kobayashi, K. *Org. Lett.* **2002**, *4*, 355. (b) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669. (c) Lundquist, J. J.; Debenham, S. D.; Toone, E. J. *J. Org. Chem.* **2000**, *65*, 8245. (d) Mylvaganam, M.; Lingwood, C. A. *Biochem. Biophys. Res. Commun.* **1999**, *257*, 391. (e) Ling, H.; Boodhoo, A.; Hazes, B.; Cummings, M. D.; Armstrong, G. D.; Brunton, J. L.; Read, R. J. *Biochemistry* **1998**, *37*, 1777.
6. (a) *Handbook of Fluorous Chemistry*; Curran, D. P., Horvath, I. T., Eds.; Wiley-VCH: Weinheim, 2004. (b) Curran, D. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 1174. (c) Curran, D. P. *Pure Appl. Chem.* **2000**, *72*, 1649 and references therein.
7. (a) Wipf, P.; Reeves, J. T. *Tetrahedron Lett.* **1999**, *40*, 5139. (b) Studer, A.; Curran, D. P. *Tetrahedron* **1997**, *53*, 6681. (c) Wipf, P.; Reeves, J. T.; Balachandran, R.; Giuliano, K. A.; Hamel, E.; Day, B. W. *J. Am. Chem. Soc.* **2000**, *122*, 9391. (d) Röver, S.; Wipf, P. *Tetrahedron Lett.* **1999**, *40*, 5667. (e) Wipf, P.; Reeves, J. T. *Tetrahedron Lett.* **1999**, *40*, 4649. (f) Curran, D. P.; Ferritto, R.; Hua, Y. *Tetrahedron Lett.* **1998**, *39*, 4937. (g) Curran, D. P.; Amatore, M.; Guthrie, D.; Campbell, M.; Go, E.; Luo, Z. *J. Org. Chem.* **2003**, *68*, 4643. (h) Schwinn, D.; Bannwarth, W. *Helv. Chim. Acta* **2002**, *85*, 255. (i) Filippov, D. V.; Zoelen, D. J.; Oldfield, S. P.; Marel, G. A.; Overkleeft, H. S.; Drijfhout, J. W.; Boom, J. H. *Tetrahedron Lett.* **2002**, *43*, 7809. (j) Pardo, J.; Cobas, A.; Guitlán, E.; Castedo, L. *Org. Lett.* **2001**, *3*, 3711. (k) Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261.
8. (a) Miura, T.; Hirose, Y.; Ohmae, M.; Inazu, T. *Org. Lett.* **2001**, *3*, 3947. (b) Miura, T.; Goto, K.; Waragai, H.; Matsumoto, H.; Hirose, Y.; Ohmae, M.; Ishida, H.-K.; Satoh, A.; Inazu, T. *J. Org. Chem.* **2004**, *69*, 5348. (c) Miura, T.; Satoh, A.; Goto, K.; Murakami, Y.; Imai, N.; Inazu, T. *Tetrahedron: Asymmetry* **2005**, *16*, 3.
9. Miura, T.; Inazu, T. *Tetrahedron Lett.* **2003**, *44*, 1819.
10. (a) Miura, T.; Goto, K.; Hosaka, D.; Inazu, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 2047. (b) Mizuno, M.; Goto, K.; Miura, T.; Hosaka, D.; Inazu, T. *Chem. Commun.* **2003**, 972. (c) Mizuno, M.; Goto, K.; Miura, T.; Matsuura, T.; Inazu, T. *Tetrahedron Lett.* **2004**, *45*, 972. (d) Goto, K.; Miura, T.; Hosaka, D.; Matsumoto, H.; Mizuno, M.; Ishida, H.-K.; Inazu, T. *Tetrahedron* **2004**, *60*, 8845. (e) Goto, K.; Miura, T.; Mizuno, M.; Takaki, H.; Imai, N.; Murakami, Y.; Inazu, T. *Synlett* **2004**, 2221.
11. Fluorous compounds **3**, **7**, **13**, and **14** were partitioned between FC-72 and methanol. Fluorous compounds **4**, **5**, **11**, and **12** were partitioned between FC-72 and toluene. All fluorous compounds were not detected by TLC from the organic layer after three extractions with FC-72. These results show that these compounds were quantitatively extracted with FC-72.
12. The fluorocarbon solvent ($EtOC_4F_9$, Novec™ HFE-7200) is commercially available.
13. The fluorocarbon solvent (FC-72, bp 56 °C, formally called Fluorinert™ FC-72) is commercially available and consists of perfluorohexane isomers (C_6F_{14}).
14. Yoshida, T.; Chiba, T.; Yokochi, T.; Onozaki, K.; Sugiyama, T.; Nakashina, I. *Carbohydr. Res.* **2001**, *335*, 167.
15. Dasgupta, F.; Anderson, L. *Carbohydr. Res.* **1994**, *264*, 155.