Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Synthesis of a fluorescently tagged sialic acid analogue useful for live-cell imaging

Katsuhiko Suzuki, Atsuko Ohtake, Yukishige Ito* and Osamu Kanie*

Experimental Procedures

General Procedures

Thin-layer chromatography (TLC) was performed on Merck Art. 5715, Kieselgel 60 F_{254}/0.25 mm thickness plates. Preparative TLC was performed on Merck Art. 5745, Kieselgel 60 F_{254}/0.50 mm thickness plates. Visualization was accomplished with UV light and 1% $Ce(SO_4)_2$–1.5% $(NH_4)_6MoO_{24}\cdot 4H_2O$–10% H_2SO_4 solution or 0.5% ninhydrin *n*BuOH solution followed by heating. Silicagel column chromatography was conducted on Wakogel C–300 (Wako Pure Chemical Industries, Ltd., Osaka). Gel permeation chromatography was performed using Sephadex LH-20 or G-25 (Pharmacia Fine Chemicals). Optical rotation was measured in a 1.0 dm tube with a Horiba SPEA-200 polarimeter. Melting points were measured with Yanaco MP-S3 micro melting point apparatus. ^{1}H NMR (500 MHz) spectra were recorded with an AVANCE 500 spectrometer (Bruker Biospin Inc., Ettlingen) in deuterated solvent using $(CH_3)Si$ (0.00 ppm) or the solvent peak (HDO: 4.79 ppm or CD_3OD: 3.31 ppm) as the internal standard. ^{13}C NMR (125 MHz) spectra were recorded with an AVANCE 500 spectrometer (Bruker Biospin Inc.) in deuterated solvent using Me_4Si (0.00 ppm) or the solvent peak ($CDCl_3$; 77.0 ppm or CD_3OD; 49.0 ppm) as the internal standard. High resolution mass spectra (HRMS) were obtained on a LCMS−QIT−TOF coupled with ESI interface (quadrupole ion-trap TOF mass spectrometer with reflectron) (Shimadzu Corp., Kyoto) using sodium trifluoroacetate as an external standard for instrument adjustment. Fmoc-dPEG$_6^{TM}$ Acid was purchased from Quanta BioDesign, Ltd. (OH). BODIPY-FL SE^{TM} was purchased from Invitrogen (CA).

Antibodies

The following antibodies were used: Rabbit anti-TGN38 polyclonal antibody (Abnova Corp., Taipei City); Mouse anti-GM130 monoclonal antibody (BD Biosciences, CA); goat anti-mouse IgG conjugated with Alexa Fluor 488 (Invitrogen, CA); and donkey anti-rabbit IgG conjugated with Cy3 (Millipore Corp., MA).

Methyl (5-acetamido-4,7,8,9-tetra-O-benzyl-3,5-dideoxy-3-fluoro-D-erythro-L-manno-2-nonulopyranos)onate (**3**) and methyl (5-acetamido-4,7,8,9-tetra-O-benzyl-3,5-dideoxy-3-fluoro-D-erythro-L-gluco-2-nonulopyranos)onate (**4**)

Compound **3**: ^{1}H NMR ($CDCl_3$); δ 7.37−7.21 (m, 20H, Ph), 4.88 (dd, 1H, $J_{3,4}$ 2.1 Hz, $J_{3,F}$ 50.0 Hz, H-3), 4.73 (d, 1H, *J* 11.8 Hz, benzyl), 4.64 (d, 1H, *J* 11.8 Hz, benzyl), 4.64 (s, 2H, benzyl), 4.59 (s, 2H, benzyl), 4.55 (d, 1H, *J* 11.8 Hz, benzyl), 4.51 (d, 1H, *J* 10.9 Hz, H-6), 4.43 (d, 1H, *J* 11.8Hz, benzyl), 4.40 (ddd, 1H, $J_{4,5}$ 10.7 Hz, $J_{4,F}$ 28.9 Hz, H-4), 4.00 (dd, 1H, $J_{8,9a}$ 2.2 Hz, $J_{9a,9b}$ 10.8 Hz, H-9a), 3.93 (ddd, 1H, $J_{7,8}$ 6.6 Hz, $J_{8,9b}$ 4.5 Hz, H-8), 3.85-3.79 (m, 2H, H-5,7), 3.77 (s, 3H, OCH_3), 3.72 (dd, 1H, H-9b), 2.04 (s, 3H, NHAc); ^{13}C NMR ($CDCl_3$); δ 171.29, 170.55, 168.47, 138.61, 138.30, 138.08, 137.87, 129.38, 128.53, 128.46, 128.44, 128.24, 127.99, 127.97, 127.90, 127.76, 94.23 (d, *J* 24.7 Hz, C-2), 86.07 (d, *J* 184.1 Hz, C-3), 78.47 (C-8), 73.76 (C-7), 73.47 (d, *J* 17.5 Hz, C-4), 73.46, 72.70, 72.37, 71.69, 70.09 (C-6), 69.48 (C-9), 53.26 (OCH_3), 48.66 (C-5), 23.60 ($COCH_3$); ESIMS *m/z* calcd for $[C_{40}H_{44}FNO_9+Na]^+$; 724.3, found 724.3.

Compound **4**: ^{1}H NMR ($CDCl_3$); δ 7.38−7.27 (m, 20H, Ph), 4.89 (dd, 1H, $J_{3,4}$ 9.0 Hz, $J_{3,F}$ 49.7 Hz, H-3), 4.86 (d, 1H, *J* 11.8 Hz, benzyl), 4.77 (d, 1H, $J_{5,NH}$ 9.5Hz, N*H*), 4.67 (d, 1H, *J* 10.6 Hz, benzyl), 4.65 (d, 1H, *J* 11.3, Hz, benzyl), 4.58 (d, 1H, *J* 12.0 Hz, benzyl), 4.55 (d, 2H, *J* 11.4Hz, benzyl), 4.44 (d, 2H, *J* 11.0 Hz, benzyl), 4.32 (d, 1H, $J_{5,6}$ 10.9 Hz, H-6), 4.18 (q, 1H, $J_{4,5}$ 10.1 Hz, H-5), 3.96 (dt, 1H, $J_{4,F}$ 11.9 Hz, H-4), 3.85 (s, 3H, OCH_3), 3.78−3.69 (m, 3H, H-7,8,9a), 3.64 (dd, 1H, $J_{8,9b}$ 3.0 Hz, $J_{9a,9b}$ 10.6 Hz, H-9b), 2.05 (s, 3H, NHAc); ^{13}C NMR ($CDCl_3$): δ 169.92, 168.76, 138.11, 138.05, 138.03, 137.91, 129.04, 128.86, 128.58, 128.51, 128.44, 128.38, 128.35, 128.33, 128.29, 128.23, 128.17, 128.11, 128.02, 127.92, 127.82, 127.79, 127.74, 93.52 (d, *J* 21.3 Hz, C-2), 91.37 (d, *J* 191.94, C-3), 76.71, 74.38, 74.32, 74.19, 74.16, 73.44, 72.34, 70.15, 67.77, 54.04, 50.30 (d, *J* 7.5 Hz, C-5), 23.74; ESIMS *m/z* calcd for $[C_{40}H_{44}FNO_9+Na]^+$; 724.3, found 724.4.

Methyl (5-acetamido-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranos)onate (**5**)

m.p. 131−133 ˚C; $[\alpha]_D^{26}$ −14.5° (*c* 1.02, H_2O); ^{1}H NMR (CD_3OD); δ 4.83 (dd, 1H, $J_{3,4}$ 2.2 Hz, $J_{3,F}$ 49.7 Hz, H-3), 4.23 (t, 1H, $J_{4,5} = J_{5,6}$ 10.6 Hz, H-5), 4.15−4.05 (m, 2H, H-4,6), 3.85−3.79 (m, 2H, H-8,9a), 3.81 (s, 3H, OCH_3), 3.67 (dd, 1H, $J_{8,9b}$ 3.8 Hz, $J_{9a,9b}$ 11.3 Hz, H-9b), 3.51 (d, 1H, $J_{7,8}$ 9.3 Hz, H-7), 2.03 (s, 3H, NHAc); (data in agreement with ref. 12)

Methyl (2,4,7,8,9-penta-O-acetyl-5-azido-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranos)onate (**7**)

$[\alpha]_D^{26}$ −42.1° (*c* 0.63, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.51 (dd, 1H, $J_{6,7}$ 1.2 Hz, $J_{7,8}$ 6.7 Hz, H-7), 6.26−5.23 (m, 1H, H-8), 5.21 (ddd, 1H, $J_{3,4}$ 2.5 Hz, $J_{4,5}$ 9.5 Hz, $J_{4,F}$ 27.3 Hz, H-4), 4.97 (dd, 1H, $J_{3,F}$ 48.9 Hz, H-3), 4.47 (dd, 1H, $J_{8,9a}$ 2.3 Hz, $J_{9a,9b}$ 12.6 Hz, H-9a), 4.23 (dd, 1H, $J_{8,9b}$ 5.5 Hz, H-9b), 3.83 (s, 3H, OCH_3), 3.73-3.66 (m, 2H, H-5,6), 2.21, 2.21, 2.16, 2.06, 2.05 (each s, 15H, OAc); HRMS (ESI) *m/z* calcd for $[C_{20}H_{26}FN_3O_{13}+Na]^+$; 558.1342, found 558.1333.

Methyl (4,7,8,9-tetra-O-acetyl-5-azido-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranos)onate (**8**)
$[\alpha]_D^{26}$ +13.2° (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.52 (dd, 1H, $J_{6,7}$ 1.2 Hz, $J_{7,8}$ 7.2 Hz, H-7), 5.38 (ddd, 1H, $J_{8,9a}$ 2.0 Hz, $J_{8,9b}$ 5.7 Hz, H-8), 5.27 (ddd, 1H, $J_{3,4}$ 2.3 Hz, $J_{4,5}$ 10.1 Hz, $J_{4,F}$ 27.6 Hz, H-4), 4.97 (dd, 1H, $J_{3,F}$ 49.8 Hz, H-3), 4.72 (br s, 1H, O*H*), 4.57 (dd, 1H, $J_{9a,9b}$ 12.6 Hz, H-9a), 4.19 (dd, 1H, H-9b), 3.87 (s, 3H, OCH_3), 3.85 (d, 1H, $J_{5,6}$ 10.4 Hz, H-6), 3.64 (t, 1H, H-5), 2.20, 2.19, 2.11, 2.07 (each s, 12H, OAc); HRMS (ESI) *m/z* calcd for $[C_{18}H_{24}FN_3O_{12}+Na]^+$; 516.1236, found 516.1227.

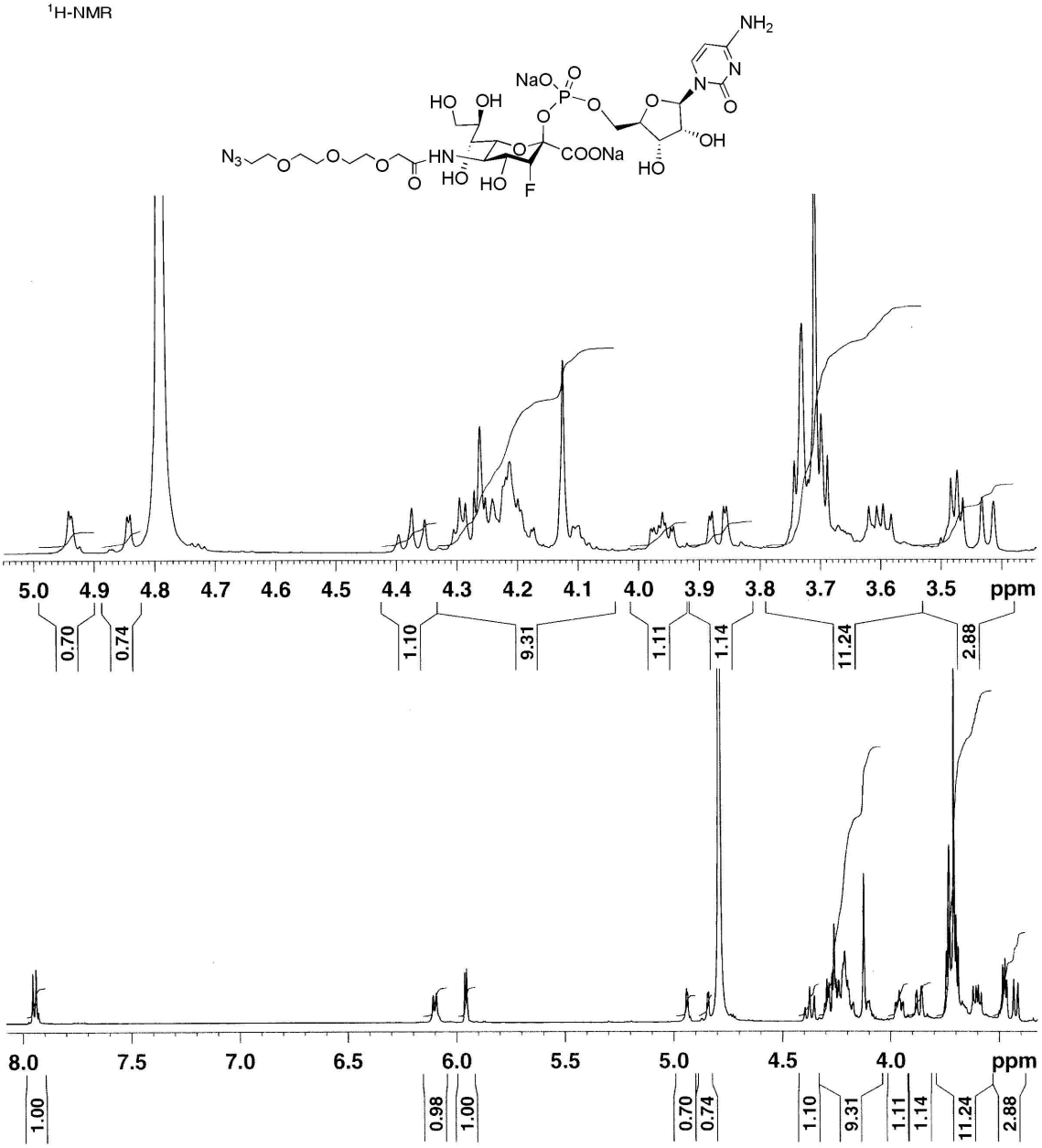
Methyl (5-amino-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranos)onate (**9**)
^{1}H NMR ($CDCl_3$); δ 5.51−5.46 (m, 2H, H-7,8), 5.09 (ddd, 1H, $J_{3,4}$ 2.3 Hz, $J_{4,5}$ 10.6 Hz, $J_{4,F}$ 27.9 Hz, H-4), 4.92 (dd, 1H, $J_{3,F}$ 49.9 Hz, H-3), 4.56 (dd, 1H, $J_{8,9a}$ 1.6 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.24 (dd, 1H, $J_{8,9b}$ 4.9 Hz, H-9b), 3.87 (s, 3H, OCH_3), 2.98 (t, 1H, H-5), 2.17, 2.17, 2.11, 2.06 (each s, 12H, OAc).

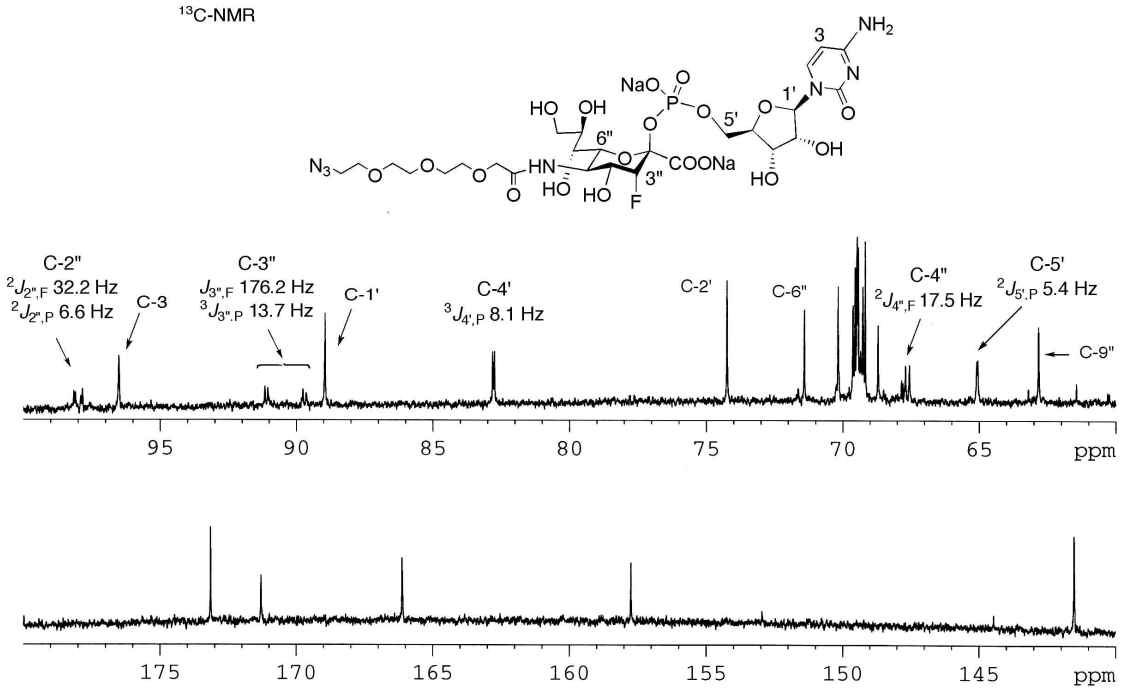
Methyl
(5-(2-(2-(2-Azido-ethoxy)-ethoxy)ethoxy)-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranos)onate (**11**)
$[\alpha]_D^{26}$ +22.1° (*c* 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$); δ 7.20 (d, 1H, $J_{5,NH}$ 10.1 Hz, N*H*), 5.42 (dd, 1H, $J_{6,7}$ 2.0 Hz, $J_{7,8}$ 4.4 Hz, H-7), 5.34 (ddd, 1H, $J_{3,4}$ 2.2 Hz, $J_{4,5}$ 10.9 Hz, $J_{4,F}$ 27.4 Hz, H-4), 5.28 (ddd, 1H, $J_{8,9a}$ 2.3 Hz, $J_{8,9b}$ 8.0 Hz, H-8), 4.99 (dd, 1H, $J_{3,F}$ 49.7 Hz, H-3), 4.84 (dd, 1H, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.50 (q, 1H, $J_{5,6}$ 10.3 Hz, H-5), 4.39 (br d, 1H, H-6), 4.11 (dd, 1H, H-9b), 3.91 {q, 2H, *J* 16.3 Hz, OCH_2C(=O)}, 3.86 (s, 3H, OCH_3), 3.76−3.65 (m, 10H, OCH_2), 3.51-3.42 (m, 2H, CH_2N_3), 2.16, 2.08, 2.06, 2.04 (each s, 12H, OAc); HRMS (ESI) *m/z* calcd for $[C_{26}H_{39}FN_4O_{16}+Na]^+$; 705.2237, found 705.2235.

Methyl
(5-(2-(2-(2-Azido-ethoxy)-ethoxy)ethoxy)-acetamido-4,7,8,9-tetra-O-acetyl-2-(N-acetyl-2',3'-di-O-acetyl-cytidin-5'-O-cyamoethylphosphoryl)-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranos)onate (**13**)
^{1}H NMR ($CDCl_3$); δ 9.16 (s, 1H, N*H*Ac), 7.82 {d, 1H, $J_{5",NH}$ 9.9 Hz, C(=O)N*H*}, 7.68 (d, 1H, $J_{2,3}$ 7.6 Hz, H-2), 7.46 (d, 1H, H-3), 5.73 (dd, 1H, *J* 6.3 Hz, *J* 3.9 Hz, H-2'), 5.69−5.65 (m, 2H, H-1',3'), 5.64 (br t, 1H, *J* 2.5 Hz, H-7"), 5.41 (ddd, 1H, $J_{3",4"}$ 1.8 Hz, $J_{4",5"}$ 11.1 Hz, $J_{4",F}$ 27.2 Hz, H-4"), 5.28−5.26 (m, 1H, H-8"), 5.09 (dd, 1H, $J_{3",F}$ 48.8 Hz, H-3"), 4.77−4.75 (m, 2H, H-6",9"a), 4.67 (q, 1H, $J_{5",6"}$ 10.5 Hz, H-5"), 4.55−4.45 (m, 2H, H-5'), 4.40−4.30 (m, 3H, H-4',CH_2CH$_2$CN), 4.22 (dd, 1H, $J_{8",9"b}$ 8.4 Hz, $J_{9"a,9"b}$ 12.2 Hz, H-9"b), 3.98 {d, 2H, *J* 3.6 Hz, CH_2C(=O)NH}, 3.92 (s, 3H, OCH_3), 3.70−3.60 (m, 10H, OCH_2), 3.39 (q, 2H, *J* 5.0 Hz, CH_2N_3), 2.79 (t, 2H, *J* 6.0 Hz, CH$_2$CH_2CN), 2.23, 2.17, 2.13, 2.12, 2.07, 1.99, 1.92 (each s, 21H, OAc).

5-(2-(2-(2-Azido-ethoxy)-ethoxy)ethoxy)-acetamido-2-(cytidin-5'-O-phosphoryl)-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranosonic acid bis sodium salt (**14**)
Extensive NMR analysis was carried out for compound **14** in order to determine the anomeric configuration of the newly introduced phosphoryl linkage.
^{1}H-NMR (D_2O); δ 7.95 (d, 1H, $J_{2,3}$ 7.6 Hz, H-2), 6.10 (d, 1H, H-3), 5.96 (d, 1H, $J_{1',2'}$ 4.4 Hz, H-1'), 4.89 (dd, 1H, $J_{3",4"}$ 2.2 Hz, $J_{3",F}$ 48.4 Hz, H-3"), 4.38 (t, 1H, $J_{4",5"} = J_{5",6"}$ 10.6 Hz, H-5"), 4.31-4.17 (m, 7H, H-2',3',4',5'a,5'b,4",6"), 4.13 {s, 2H, CH_2C(=O)NH}, 3.96 (ddd, 1H, $J_{7",8"}$ 9.7 Hz, $J_{8",9"a}$ 2.4 Hz, $J_{8",9"b}$ 6.6 Hz, H-8"), 3.87 (dd, 1H, $J_{9"a,9"b}$ 11.9 Hz, H-9"a), 3.74-3.69 (m, 10H, OCH_2), 3.60 (dd, 1H, H-9"b), 3.47 (t, 2H, *J* 4.9 Hz, CH_2N_3), 3.42 (d, 1H, H-7"). ^{13}C-NMR (D_2O); δ 173.15, 171.29, 166.12, 157.74, 141.53, 97.99 (dd, $^2J_{2",F}$ 32.2 Hz, $^2J_{2",P}$ 6.6 Hz, C-2"), 96.52 (C-3), 90.40 (dd, $J_{3",F}$ 176.2 Hz, $^3J_{3",P}$ 13.7 Hz, C-3"), 88.95 (C-1'), 82.78 (d, $^3J_{4',P}$ 8.1 Hz, C-4'), 74.24 (C-2'), 71.42 (C-6"), 70.17, 69.63, 69.55, 69.47, 69.43, 69.26, 69.18, 68.70, 67.62 (d, $^2J_{4",F}$ 17.5 Hz, C-4"), 65.07 (d, $^2J_{5',P}$ 5.4 Hz, C-5'), 62.83 (C-9"), 50.06 (CH_2N$_3$), 46.59 (C-5"). HRMS (ESI) *m/z* calcd for $[C_{26}H_{39}FN_7O_{19}PNa_3]^+$; 872.1710, found 872.1702.

^{1}H-NMR

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C-3
C-3"
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$^3J_{3",P}$ 13.7 Hz
C-1'
C-4'
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C-2'
C-6"
C-4"
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C-5'
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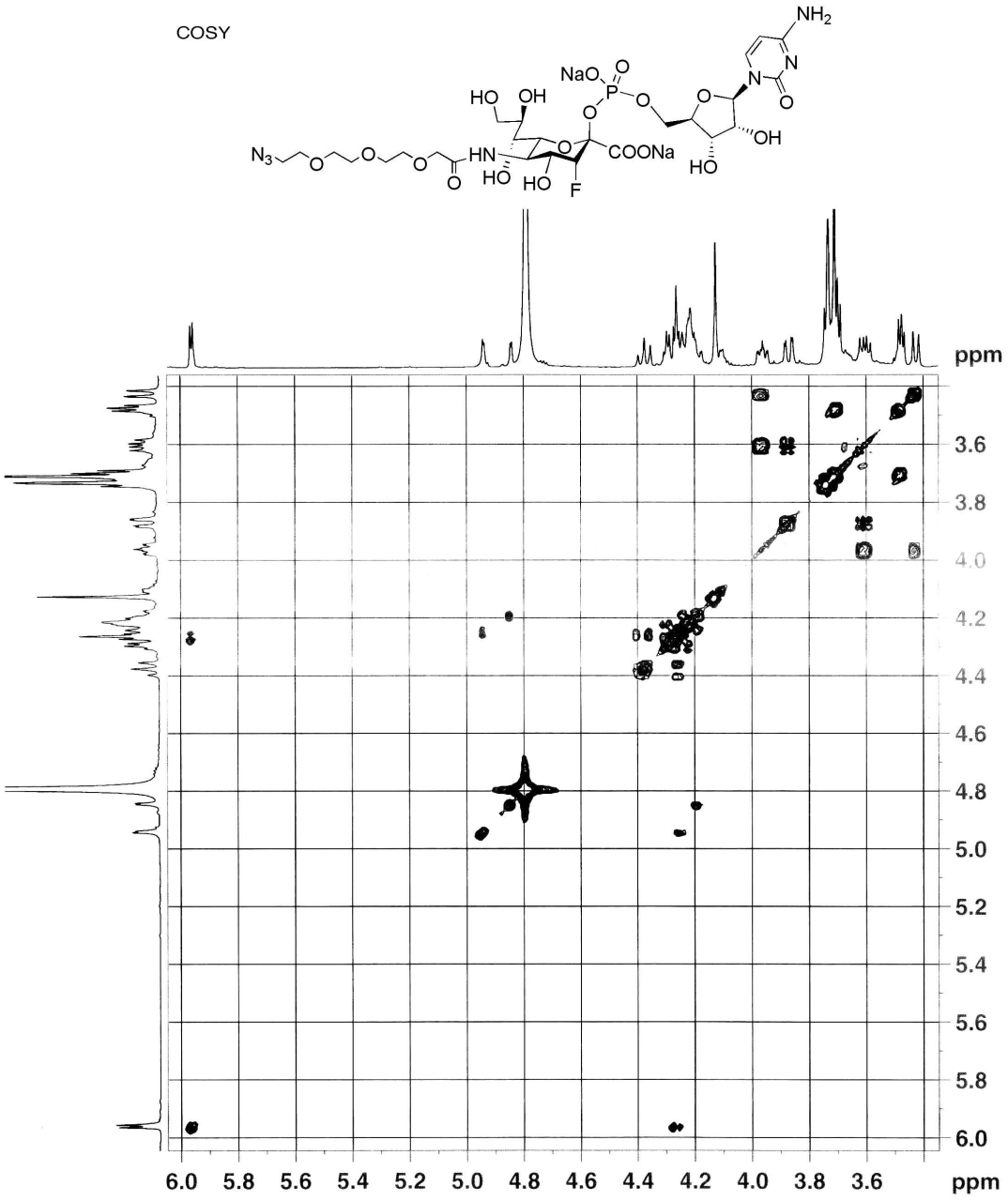
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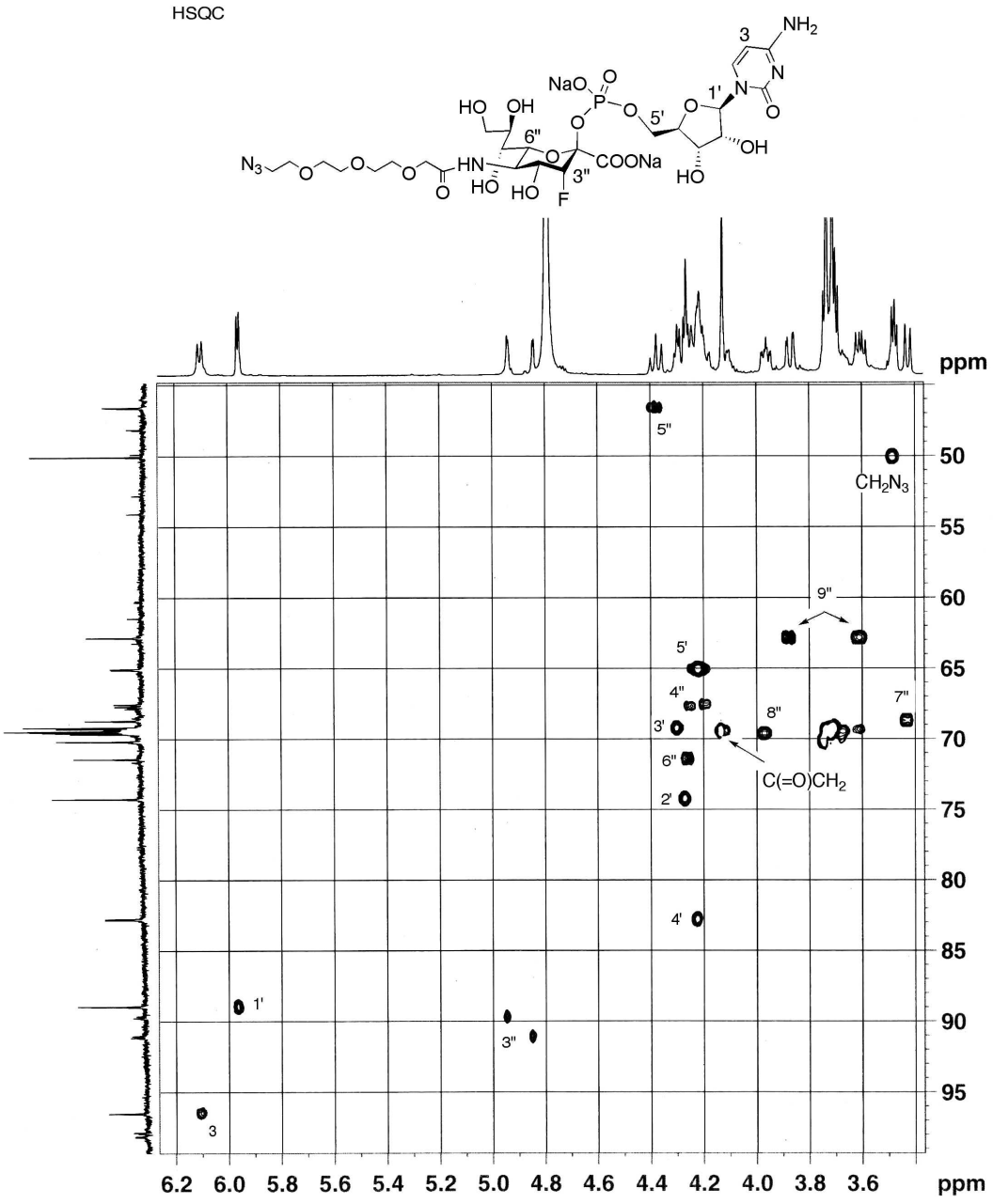
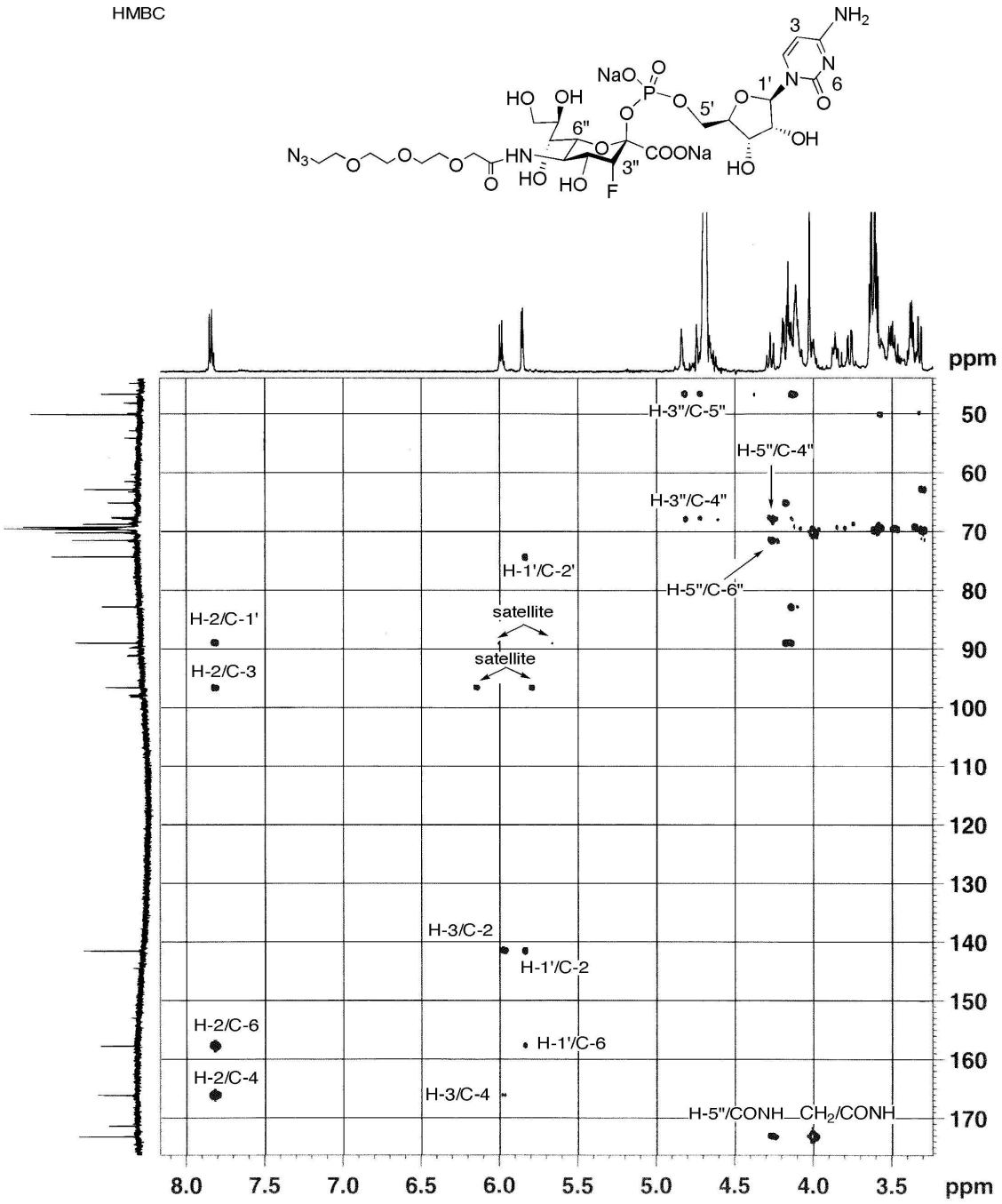


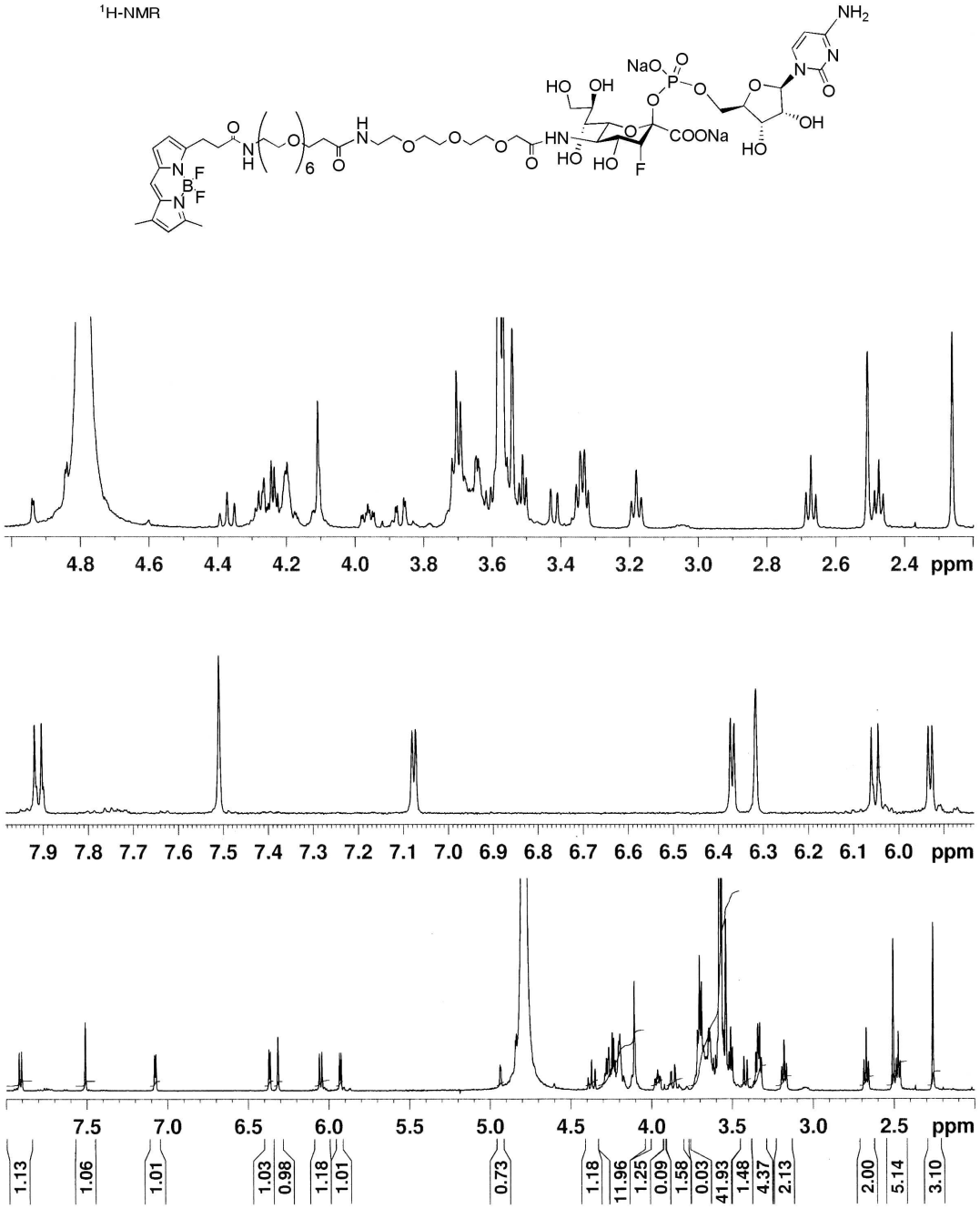


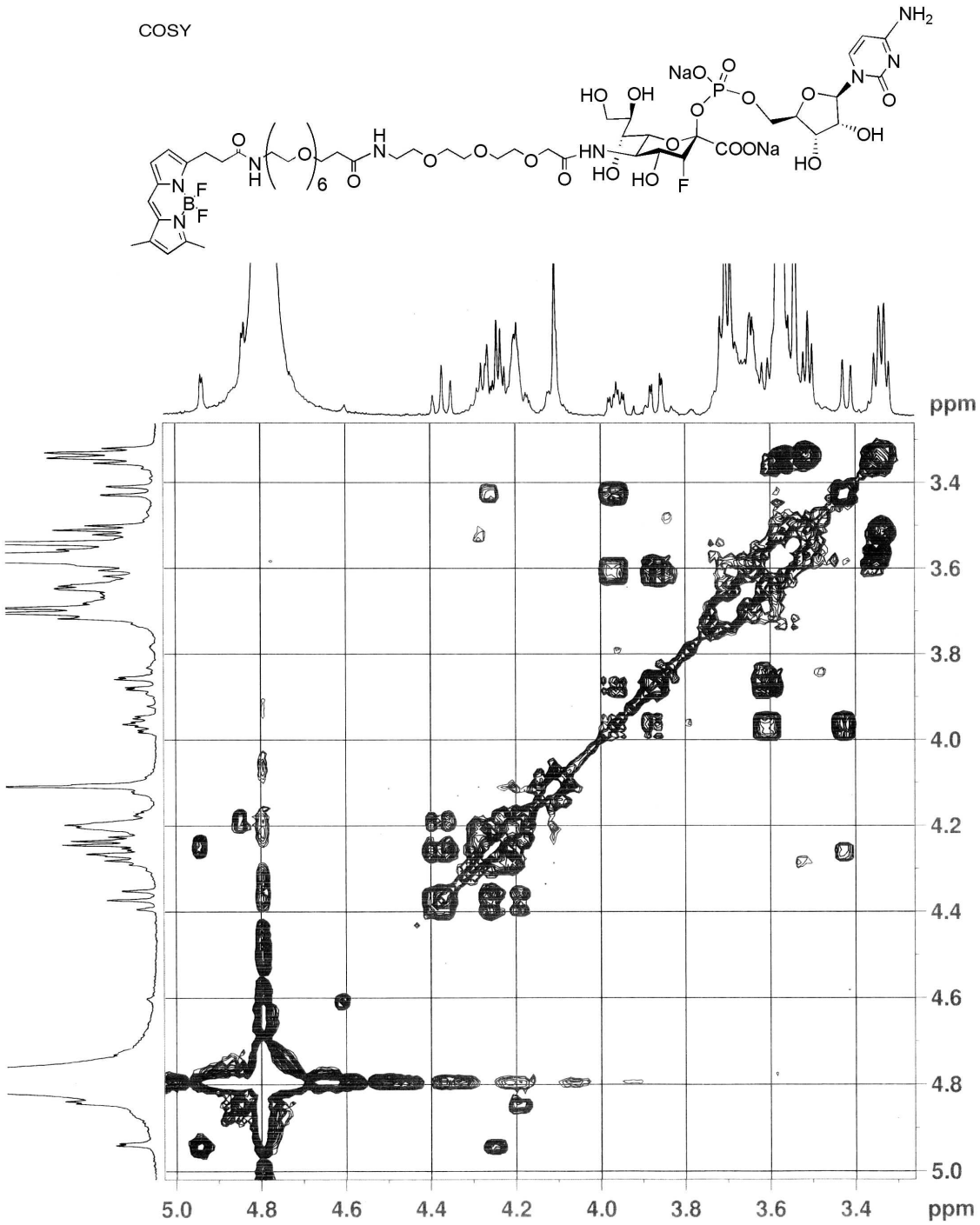
5-(2-(2-(2-Aminoethoxy)-ethoxy)ethoxy)-acetamido-2-(cytidin-5'-O-phosphoryl)-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranosonic acid bis sodium salt (**15**)
^{1}H NMR (D_2O); δ 7.93 (d, 1H, $J_{2,3}$ 7.6 Hz, H-2), 6.09 (d, 1H, H-3), 5.81 (d, 1H, $J_{1',2'}$ 4.3 Hz, H-1'), 4.89 (br d, 1H, $J_{3",F}$ 48.7 Hz, H-3"), 4.38 (t, 1H, $J_{4",5"} = J_{5",6"}$ 10.7 Hz, H-5"), 4.30−4.12 {m, 9H, H-2',3',4',5'a,5'b,4",6", CH_2C(=O)NH}, 3.96 (ddd, 1H, $J_{7",8"}$ 9.5 Hz, $J_{8",9"a}$ 2.6 Hz, $J_{8",9"b}$ 6.6 Hz, H-8"), 3.86 (dd, 1H, $J_{9"a,9"b}$ 11.9 Hz, H-9"a), 3.74-3.69 (m, 10H, OCH_2), 3.61 (dd, 1H, H-9"b), 3.43 (d, 1H, H-7"), 3.11 (t, 2H, *J* 4.7 Hz, CH_2NH_2).

Succinimidyl
3-{15-[2-(4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-aminoethoxy]-pentaethyleneglycoloxy}-propionate (BODIPY-dPEG$_6$ succinimide ester) (**16**)
^{1}H NMR ($CDCl_3$); δ 7.45 (s, 1H), 7.02 (d, 1H, *J* 4.0 Hz), 6.34 (d, 1H, *J* 4.0 Hz), 6.22 (s, 1H), 3.81 (t, 2H, *J* 6.1 Hz), 3.62−3.58 (m, 12H), 3.53 (t, 2H, *J* 5.4 Hz), 3.37 (t, 2H, *J* 5.4 Hz), 3.22 (t, 2H, *J* 7.7 Hz), 2.89 (t, 2H, *J* 6.1 Hz), 2.82 (s, 4H), 2.63 (t, 2H, *J* 7.6 Hz), 2.51 (t, 2H, *J* 6.4 Hz), 2.29 (s, 3H).

5-(2-(2-(2-BODIPY-dPEG$_6$-amidoethoxy)-ethoxy)ethoxy)-acetamido-2-(cytidin-5'-O-phosphoryl)-3,5-dideoxy-3-fluoro-β-D-erythro-L-manno-2-nonulopyranosonic acid bis sodium salt (**1**)

^{1}H-NMR (D_2O); 7.90 (d, 1H, $J_{2,3}$ 7.6 Hz, H-2), 7.50, 6.31 (each s, 2H, BODIPY), 7.07, 6.37 (each d, 2H, *J* 3.9 Hz, BODIPY), 6.05 (d, 1H, H-3), 5.93 (d, 1H, $J_{1',2'}$ 4.4 Hz, H-1'), 4.89 (dd, 1H, $J_{3'',4''}$ 2.2 Hz, $J_{3'',F}$ 48.4 Hz, H-3''), 4.37 (t, 1H, $J_{4'',5''} = J_{5'',6''}$ 10.6 Hz, H-5''), 4.31-4.05 {m, 9H, H-2',3',4',5'a,5'b,4'',6'', CH_2C(=O)NH}, 3.96 (ddd, 1H, $J_{7'',8''}$ 9.6 Hz, $J_{8'',9''a}$ 2.2 Hz, $J_{8'',9''b}$ 7.0 Hz, H-8''), 3.87 (dd, 1H, $J_{9''a,9''b}$ 11.9 Hz, H-9''a), 3.74-3.50 (m, 35H, H-9''b, OCH_2), 3.42 (d, 1H, H-7''), 3.34 (t, 2H, *J* 5.9 Hz, CH_2NH), 3.18 (t, 2H, *J* 6.9 Hz, BODIPY-C$H_2$$CH_2$), 2.67 (t, 2H, *J* 7.2 Hz, BODIPY-CH_2CH_2), 2.50, 2.26 (each s, 6H, BODIPY), 2.47 {t, 2H, *J* 6.2 Hz, CH_2CH_2C(=O)NH}. ^{13}C-NMR (D_2O); δ 90.4 (C-3''), 82.8 (C-4'), 74.1 (C-2'), 71.3 (C-6''), 67.5 (C-4''), 65.1 (C-5'), 62.8 (C-9''), 46.7 (C-5''). HRMS: $[M+3Na]^{3+}$ calcd for 500.4874, found 500.4864, $[M-2H]^{2-}$ calcd for 693.2581, found 693.2556.

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HO
HO
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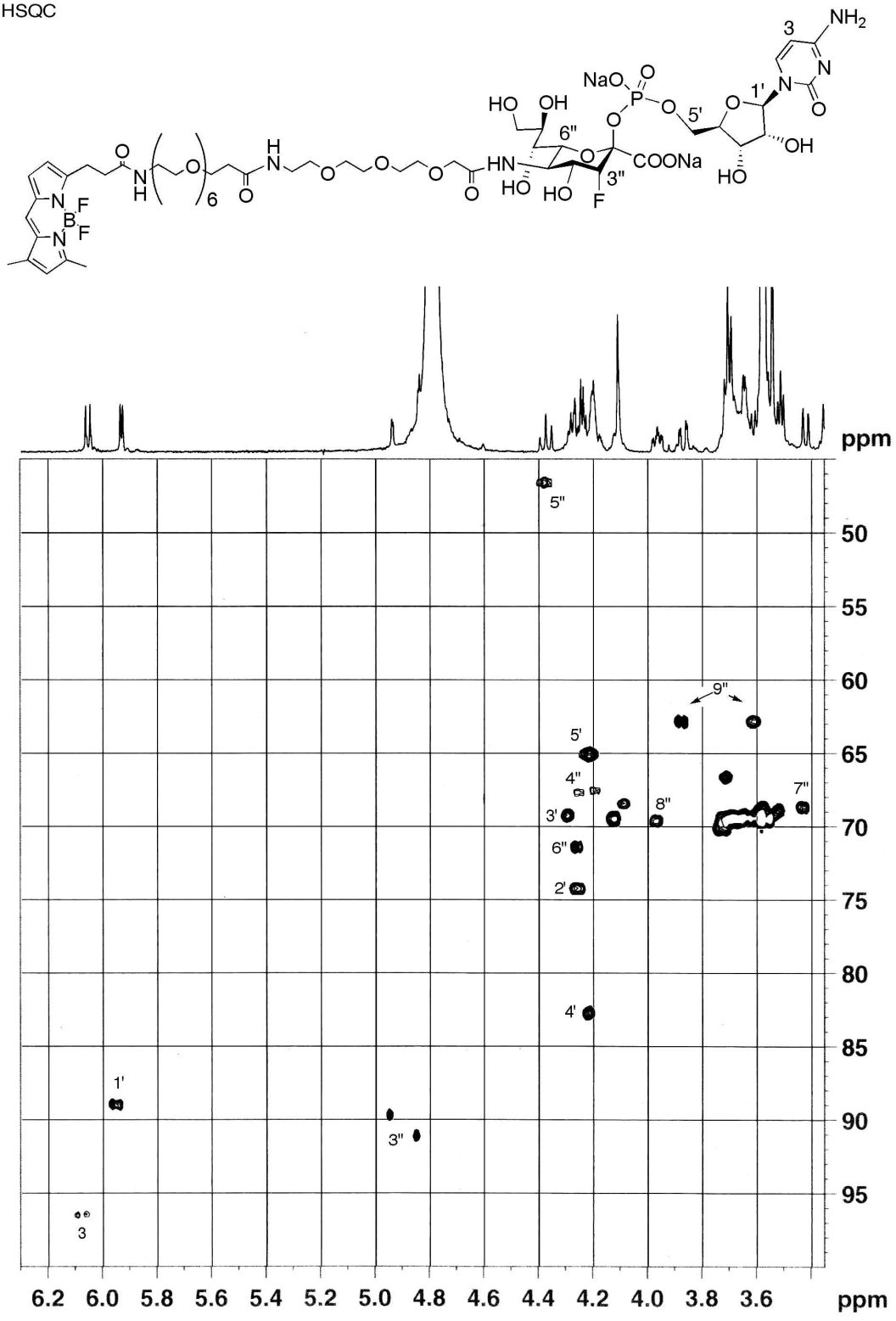
HSQC


NH2
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1'
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NaO
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O
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ppm

Confocal Microscopy

Laser-scanning confocal microscopy was performed using an Olympus FluoView 1000 confocal inverted microscope with an UPlanSApo oil-immersion objective lens (100×, NA = 1.40; Olympus). Laser lines from an argon laser with a wavelength of 488 nm and Helium-Neon laser with a wavelength of 543 nm were used for excitation. The excitation laser beams were passed through a dichroic mirror (DM488/543/633), and the fluorescence emission was separated with beam splitters (SDM560). The laser unit, confocal microscope, and detection units were connected to a computer and controlled using Olympus FluoView software (version 1.5).

Cell

PC-12D cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% horse serum, 5% fetal bovine serum, penicillin G (100 U/mL), streptomycin sulfate (100 μg/mL), and glutamine at 37 °C with 5% CO_2.

Stamporation

PC-12D cells (ca 2.0×10^4 cells/dish) were grown to 10−20% confluence on collagen-coated tissue culture dish (30 mm in diameter) at 37°C for ca 48h with 5% CO_2. A medium consisted of 25 mM HEPES, and N-2 Supplement (Invitrogen, CA) in DMEM (1.5 mL). To this culture dish, a solution of compound **1** (2.7 μmol) in Hanks' Balanced Salt Solution (HBSS) (0.09 mL) was added. On an inverted microscope (IX71, Olympus Corp., Tokyo) equipped with stamporation unit (SU100, Olympus Corp., Tokyo), compound **1** was introduced into a lumen at the edge of cells by stamporation. Cells were incubated at 37°C with 5% CO_2 for 5 h and observed by fluorescent microscopy (FluoView 1000; Olympus Corp., Tokyo).

Antibody Internalization

PC-12D cells (ca 2.0×10^4 cells/dish) were grown to 10−20% confluence on collagen-coated tissue culture chambers (Chamber Slide, Nunc Inc., Naperville) at 37°C for ca 48h with 5% CO_2. The cells were incubated with 4% PFA (paraformaldehyde) at room temperature for 15 min and washed in PBS (phosphate buffered saline) (5 min × 3). The fixed cells were then treated with primary antibodies (anti-TGN38 and anti-GM130 antibodies (1/200)) in the presence of 0.05 % (w/v) saponin (NACALAI TESQUE INC., Kyoto) and 5% FBS (Fetal Bovine Serum) for 5 min at room temperature. After washing with PBS (5 min × 3), secondary antibodies (1/200) were introduced in PBS over 45 min, and the cells were washed with PBS (5 min × 3). Experiments were performed in duplicate.

Preparation of Golgi vesicles.

Golgi-enriched membranes were isolated from rat liver (Sprague-Dawley rats) using a modification of the protocol of Leelavathi *et al*. [as described in *Cell Biology:* A Laboratory Handbook 3rd Edition, p33-39. Edited by Julio Celis. Elsevier Science (USA), San Diego, 2006.] Protein concentration of the obtained Golgi fraction was 209μg/mL.

Observation of extracted Golgi vesicles from rat liver.

To a Golgi vesicles solution 10 μL (prepared as above) were added CMP-3"F-Sia-BODIPY (**1**) 5 μL (1 μM in TKM buffer: pH 7.5, 10 mM Tris, 0.15M KCl, 1 mM $MgCl_2$) and an additive 5 μL (in TKM buffer solution). The mixture was observed by fluorescent microscopy (FluoView 1000; Olympus Corp., Tokyo). To compare the images, Triton X-100 (1%) and CMP-Sia (1 mM) were used as the additive compound. Dextran-BODIPY™ (1 μM, mw: ~10,000) was used for a negative control.

*Internalization of compound **1** into extracted Golgi vesicles.*

The Golgi-enriched membranes were observed by the differential interference contrast (DIC) image. (Figure 1S) The observed Golgi vesicles were disappeared when the extracts were treated by a detergent, Triton X-100. (Figure 2S) Under these conditions, accumulation of compound **1** was not observed. Accumulation of compound **1** into Golgi vesicles was observed. (Figure 3S) A series of fluorescent vesicles were due to the thermal liquid movement during the microscopic experiment using confocal microscopy. The same experiment was carried out in the presence of a native substrate of CST, CMP-sialic acid. (Figure 4S) The image clearly indicated the incorporation of **1** into Golgi vesicles was inhibited by CMP-Sia indicating that compound **1** was accepted as a substrate by CST and transported into Golgi vesicles. A control experiment was performed using BODIPY-tagged dextran, which is known not to interfere with cellular events. (Figure 5S) The image suggested that there was no interaction of BODIPY-based nonspecific accumulations.

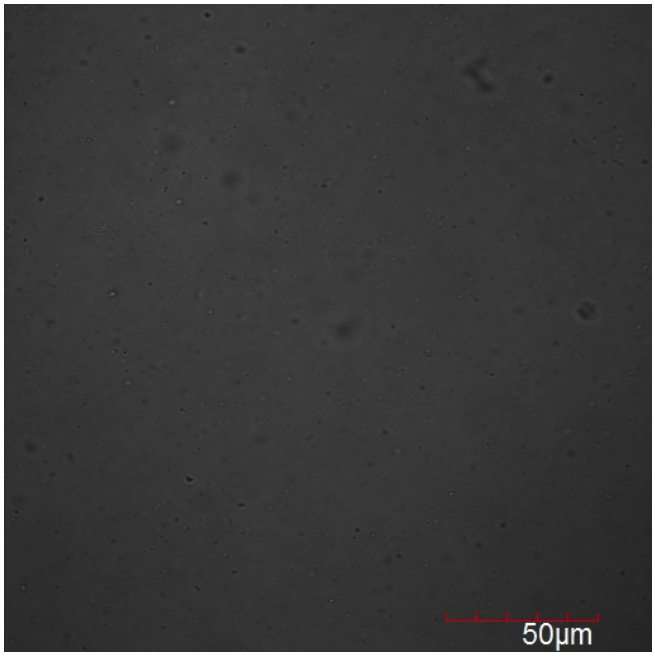

Figure 1S. The differential interference contrast (DIC) image of the vesicles. Area: 211 μm^2.

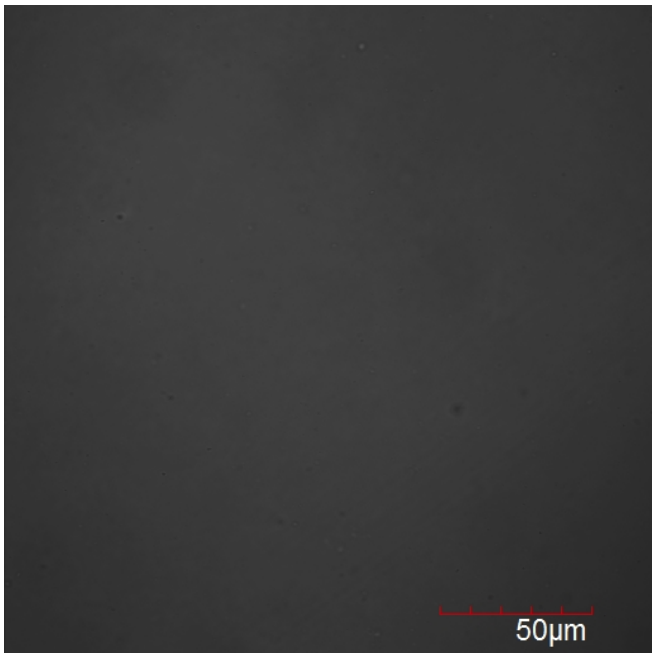

Figure 2S. The DIC image of the vesicles mixed with Triton X-100. (After 10 min).

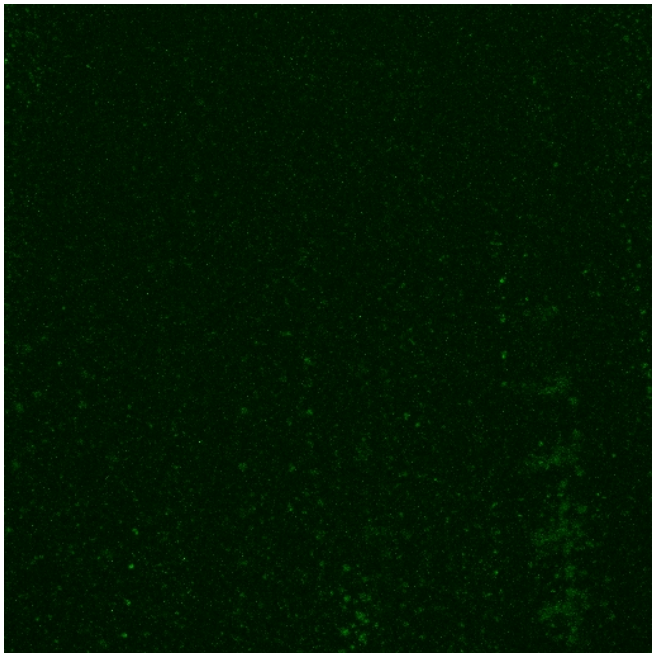

Figure 3S. Fluorescence image of the vesicles mixed with compound **1** and incubated for 5 h. Confocal surfaces (300 layers) were overlaid for depth of 0.12 mm.

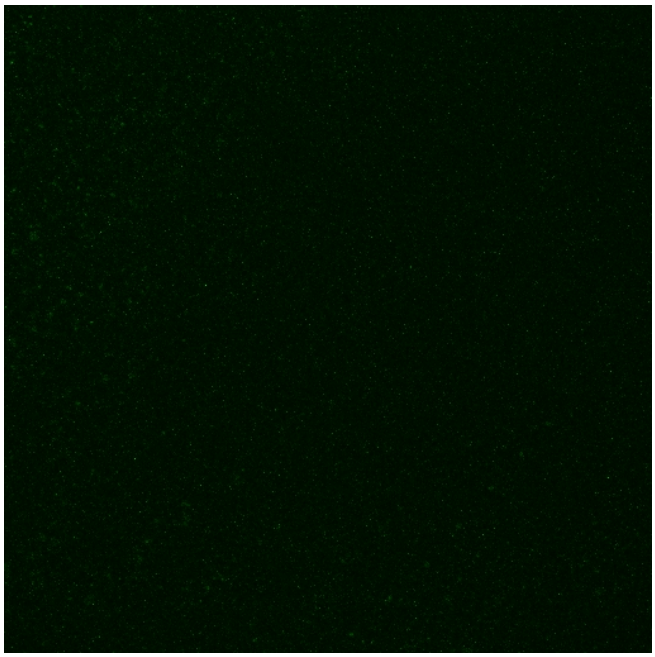

Figure 4S. Fluorescence image of the vesicles mixed with **1** and CMP-Sia. (After 5 h, depth 0.12 mm overlaid).

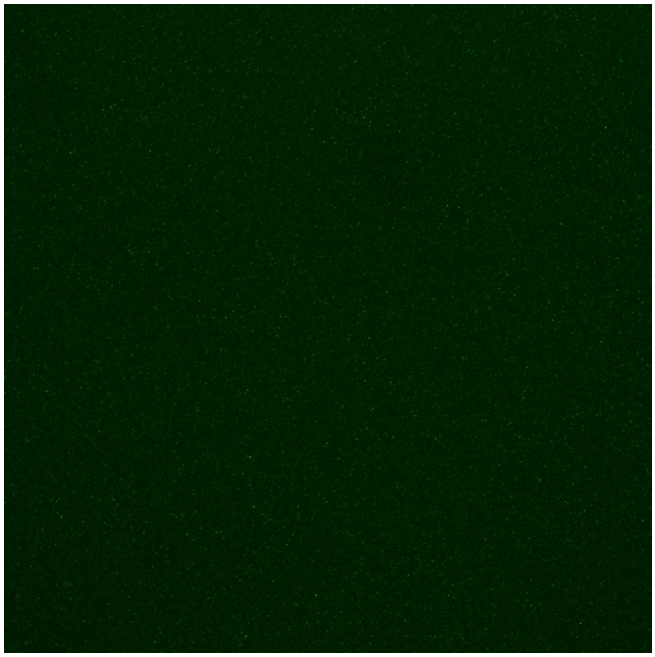

Figure 5S. Fluorescence image of the vesicles mixed with Dextran-BODIPY™. (After 5 h, depth 0.12 mm overlaid).