Synthesis of Fluorine-Containing Core-2 Tetrasaccharides

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Abstract: Synthesis of core-2 branched tetrasaccharides **1**–**3**, in which a fluorine atom was substituted at the 3 or 4-position of galactose residues is described. Glycosyl imidates **13**, and **19** were prepared and used to provide novel glycosyl disaccharide donors **15** and **21**, respectively. Coupling of acceptor **7** with glycosyl bromide **6** provides a disaccharide that was further converted into disaccharide acceptor **8**. The coupling of acceptor **14** with donor **13**, and acceptor **20** with donor **19** provided disaccharides that were converted to disaccharide donors **15** and **21**, respectively. Regioselective glycosylation of acceptors **8**, and **16** with donors **9**, **15**, and **21** provided tetrasaccharides **10**, **17**, and **22** respectively, which were systematically deprotected to targets **1**–**3**.

Key words: tetrasaccharides, galactose residues, core-2 O-linked glycoproteins

The natural mucin ligands, and various tumor-associated core-2 O-linked glycoproteins are known to contain the Galβ1→4GlcNAcβ1→6Galβ1→3GalNHAcα-1 structure (Scheme 1).[1] These core-2 branched oligosaccharides are important in many functional biological processes.[2] However, there is little known about the detailed physiologic roles of these sialylated and sulfated oligosaccharides associated with cancers and inflammations. Tremendous efforts have been devoted to understanding the structure and function of O-glycans in binding with L, P, and E selectins, which were demonstrated by developing a variety of synthetic methodologies including glycosylation formation, and protection-deprotection strategies by various research groups.[3] We have become interested in fluorinated core-2 branched oligosaccharide analogues due to: (a) the small size of fluorine is comparable to that of a hydroxyl group;[4] (b) the fluorine atom in a C-F bond is believed to be capable of forming hydrogen bonds with hydrogen bond donors but not hydrogen bond acceptors; (c) substitution of 3-or 4-hydroxyl group from galactose residue can further terminate chain elongation of Galβ1→4GlcNAcβ1→6Galβ1→3GalNHAcα-1 structure, which is believed to be a possible glycosylation site for α(2,3)-sialyltransferase, 3-*O*-sulfotransferase and α(1,4)-GlcNAc transferase. Blocking the C-3, or C-4 position of the pertinent galactose residue will provide an opportunity to investigate and understand the biosynthetic mechanism of core-2 branched oligosaccharides, and may provide useful information in the search of inhibitors for these glycotransferases. Moreover, these modified analogs when per, or partially acetylated may have the ability to prime core-2 oligosaccharide glycan synthesis and thus inhibit biosynthesis of natural ligands for L and P selectins.[5] Herein, we have described effective syntheses of fluorinated core-2 branched oligosaccharides **1**–**3**. Core-2 tetrasaccharides **1**–**3** and the related building blocks are shown in Scheme 1. There are two galactose residues in each core-2 branched tetrasaccharide. Synthesis of these tetrasaccharides was thereby divided into synthetic targets **1**, of which the fluorine was located at the 3-position of down-chain galactose residue and targets **2**, **3** of which fluorine was located at 3, or 4-position of up-chain galactose residue. In order to synthesize tetrasaccharide **1**, the fluorinated bromide donor **6** was necessary. The fluorinated compound **4** was prepared according to a known method in good yield.[6] Compound **4** was acetolysized with a catalytic amount of concentrated sulfuric acid in acetic anhydride in an ice-bath for 2 h. The bromide donor **6** was obtained via treatment of compound **5** with HBr–HOAc (33%) at room temperature for 2 h (Scheme 2).[7] The disaccharide acceptor **8** was then prepared in two steps in a poor yield (28%) using a published methodology.[8] The regioselective glycosylation of acceptor **8** with disaccharide donor **9** at low temperature provided tetrasaccharide **10** as the only glycosylated product in a good yield (65%). The stereochemistry and linkage of trisaccharide **10** was established with a combination of two-dimensional NMR experiments including 2D DQF-COSY, TOCSY, and ROESY. Target **1** was obtained by systematic deprotection of compound **10** in three steps: 1) removal of Phth group from C-2 of sugar residue; 2) complete acetylation; 3) removal of acetyl group in good yield (45%). Synthesis of compound **2** is outlined in Scheme 3. Fluorine-containing disaccharide donor **15** was essential to produce compound **2**. Construction of the β(1→4) linkage of disaccharide donor **15** was due to regioselective coupling at the 4-hydroxyl of acceptor **14** with imidate donor **13**. An attempt to couple acceptor **14** with imidate donor **13**, at –45 °C to –40 °C, resulted in a mixture of the β(1→4) and β(1→3) linked disaccharides, which made separation by column chromatography difficult. However, when the reaction temperature was lowered to –65 °C to –70 °C, only the β(1→4) linked disaccharide was obtained as a glycosylation product. This product was then treated with dry pyridine and anhydrous acetic anhydride to provide disaccharide donor **15** in two steps, and in good yield (63%). Regioselective glycosylation of the 6-hydroxyl group of disaccharide acceptor **16** with donor **15** at low

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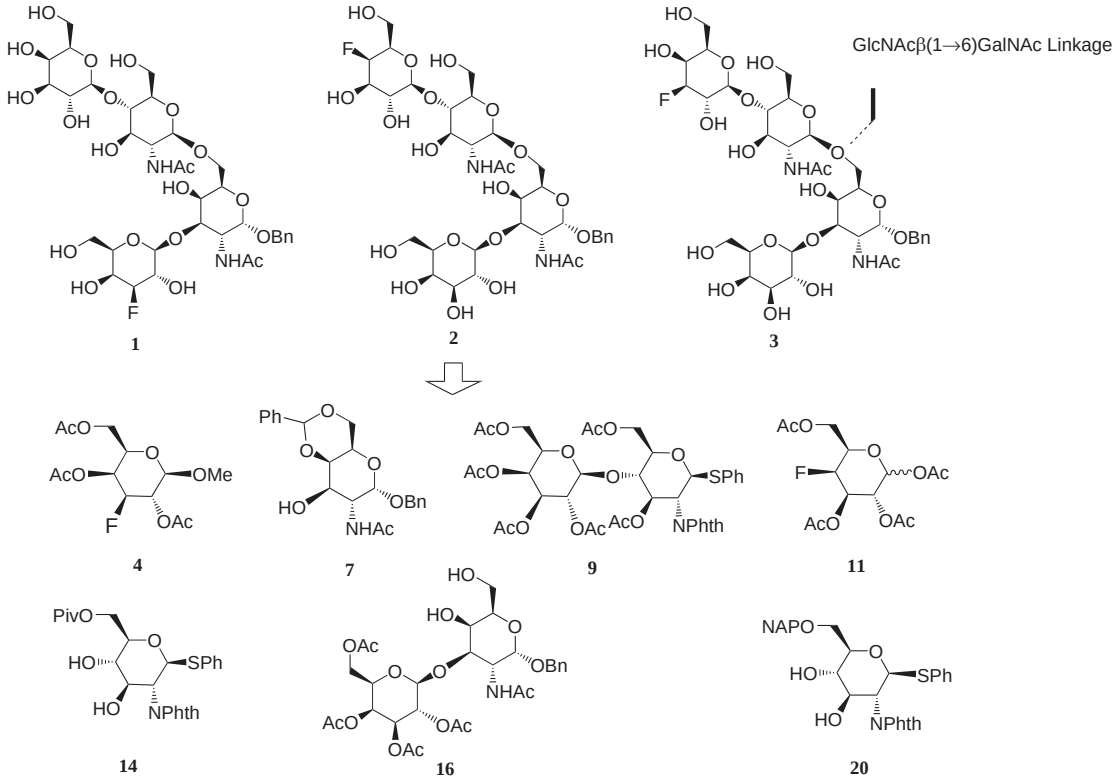
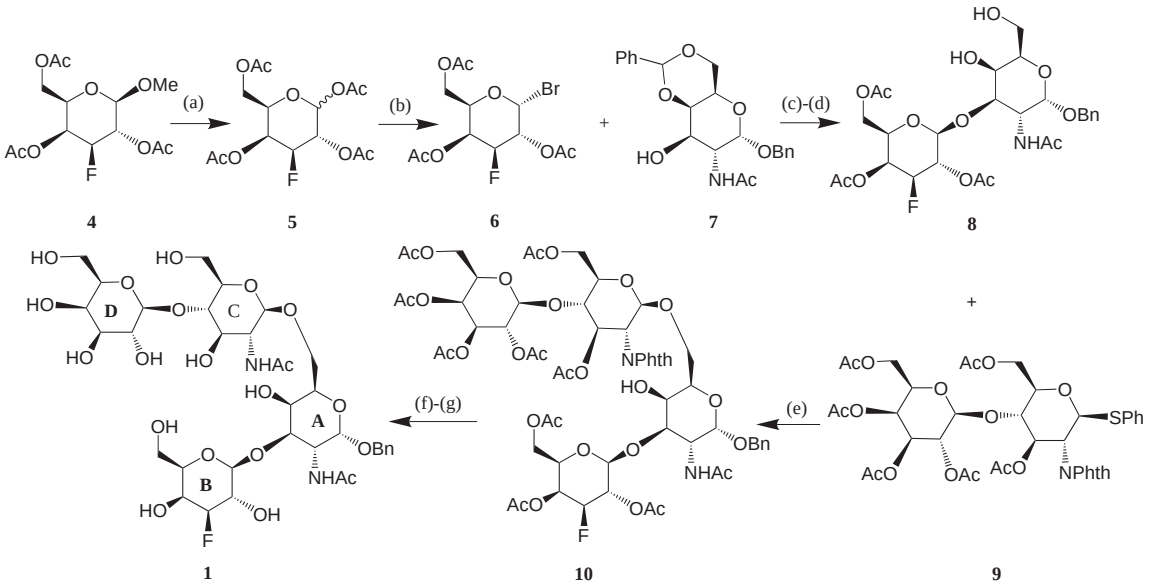
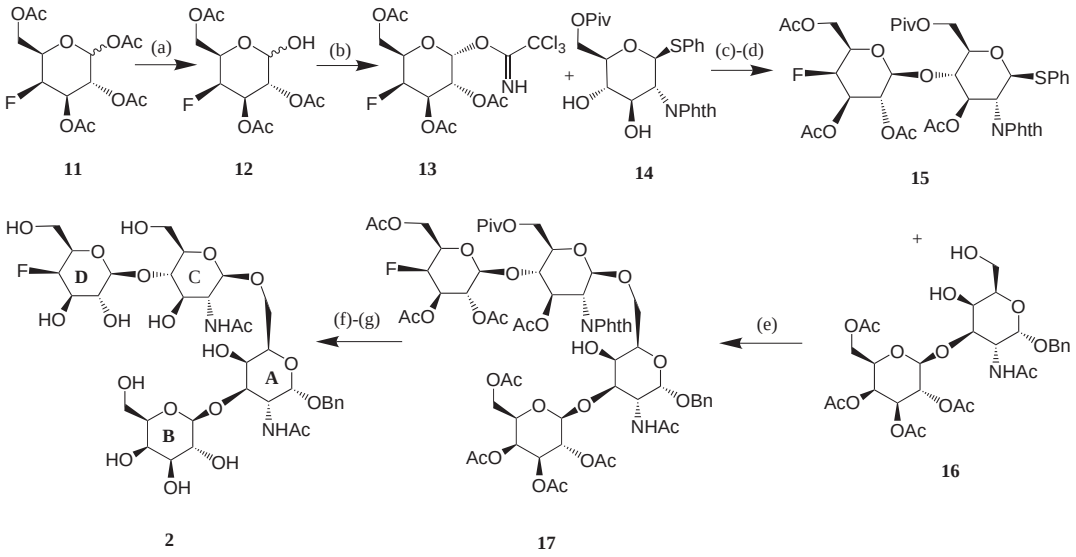


Scheme 1 Fluorine containing core 2 oligosaccharides **1**–**3** and related building blocks

temperature provided compound **17** in a high yield (76%). The structure of tetrasaccharide **17** was confirmed by a variety of 2D NMR experiments. Target compound **2** was obtained by a similar procedure used for the deprotection of compound **10** to compound **1** in three steps in a reasonable yield (39%). Synthesis of target oligosaccharide **3** is shown in Scheme 4. Fluorinated imidate donor **19**[9] was prepared in low yield (32%). Regioselective glycosylation of the 4-hydroxyl group of acceptor **20** with imidate donor **19** was achieved by a glycosylation procedure established in our laboratory. This method provided the β(1→4) linkage for disaccharide **21**. Coupling of disaccharide accep-



Scheme 2 a) Ac_2O–H_2SO_4, 0–5 °C, 2 h, 58%, b) HBr–HOAc, r.t., 65%, c) $Hg(CN)_2$/CH_3NO_2–benzene, 65 °C, 12 h, d) 60% HOAc, 65 °C to 70 °C, 1.5 h, 28% in two steps, e) NIS–TfOH/CH_2Cl_2, –45 °C to –40 °C, 65%, f) i) NH_2-NH_2·H_2O–CH_3OH (1:5), 90 °C, 6 h, ii) Ac_2O–pyridine (1:1), DMAP, r.t., 12 h, g) 1 M CH_3ONa–CH_3OH/CH_3OH–H_2O, r.t., 12 h, 45% in three steps.



Scheme 3 a) NH_2NH_2·HOAc/DMF, 50 °C, 2 h, b) CCl_3CN/DBU, CH_2Cl_2, 0 °C to 25 °C, 2 h, 80% in two steps, c) TMSOTf/CH_2Cl_2, 4 Å MS, –65 °C to –70 °C, 2 h, 41%, d) Ac_2O–pyridine (1:1), DMAP, r.t., 12 h, 63%, e) NIS–TfOH/CH_2Cl_2, 4 Å MS, –65 °C to –60 °C, 2 h, 76%, f) Ac_2O–pyridine (1:1), DMAP, r.t.,12 h, 45%, g) (i) NH_2-NH_2·H_2O–CH_3OH (1:5), 90 °C, 6 h, (ii) Ac_2O–pyridine (1:1), DMAP, r.t, 12 h, (iii) 1 M CH_3ONa–CH_3OH/CH_3OH–H_2O, r.t., 12 h, 39% in three steps.

tor **16** with disaccharide donor **21** afforded the desired β(1→6) linkage of tetrasaccharide **22** in a reasonable yield (52%). Target oligosaccharide **3** was obtained by complete deprotection of compound **22** in four steps in a reasonable yield.

In summary, fluorine core-2 oligosaccharide analogues **1**–**3**[10] having the fluorine atom located at different positions on the galactose residue were synthesized by a convergent method. Biochemical investigation of fluorinated carbohydrates-enzyme interactions and fluorinated carbohydrate-selectin interactions are underway.

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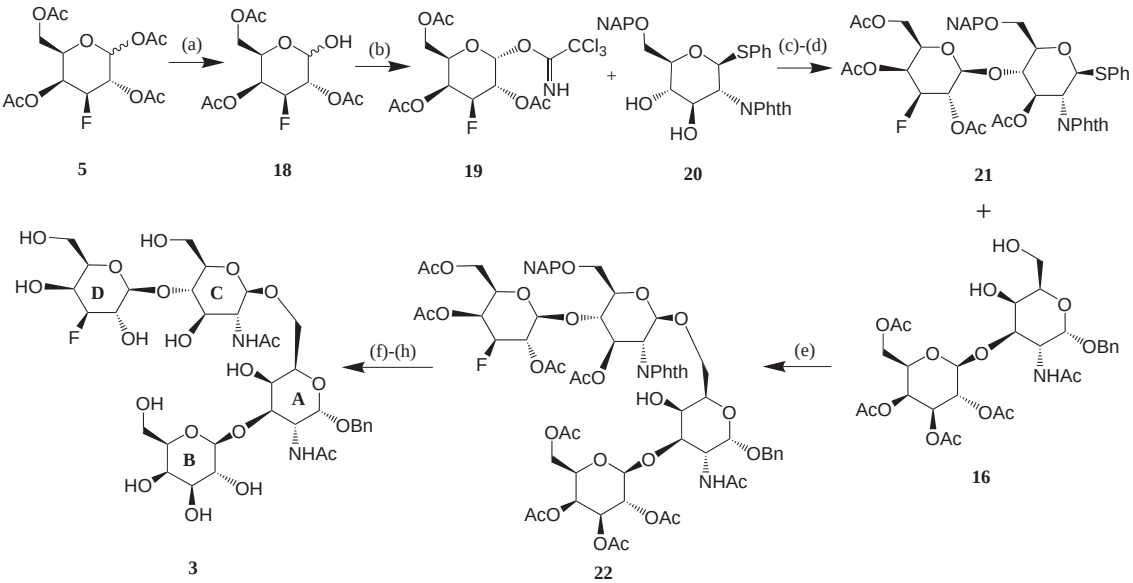

Scheme 4 a) NH_2NH_2·HOAc/DMF, 50 °C, 2 h, b) CCl_3CN/DBU, CH_2Cl_2, 0°C to 25 °C, 32% in two steps, c) TMSOTf, CH_2Cl_2, 4 Å MS, –65 °C, 2 h, 43%, d) Ac_2O–pyridine (1:1), DMAP, r.t., 12 h, 76%,e) NIS–TfOH/CH_2Cl_2, 4 Å MS, –65 °C to –60 °C, 2 h, 52%, f) Ac_2O–pyridine (1:1), DMAP, r.t.,12 h, 45%, g) (i) NH_2-NH_2·H_2O–CH_3OH (1:5), 90 °C, 6 h, (ii) Ac_2O–pyridine (1:1), DMAP, r.t., 12 h, (h) 1 M CH_3ONa–CH_3OH/CH_3OH–H_2O, r.t., 12 h, 45% in three steps.

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(10) Physical data: Compound **1**: R_f = 0.5 (*i*-C_3H_9OH–HOAc–H_2O, 3:1:1); ^{1}H NMR (D_2O, DQF-COSY and ROESY, 400 MHz): δ = 7.50–7.20 (m, 5 H, ArH), 4.99 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H^A-1), 4.72 (d, 1 H, J_{gem} = 12.6 Hz, $PhCH_AO$, ABq), 4.61 (dq, 1 H, H^B-3), 4.58 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H^B-1), 4.53 (d, 1 H, J_{gem} = 12.4 Hz, $PhCH_BO$, ABq), 4.49 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H^C-1), 4.48 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H^D-1), 4.35 (dd, 1 H, H^A-2), 4.23 (d, 1 H, *J* = 2.8 Hz, H^A-4), 4.20 (dd, 1 H, H^B-4), 4.15 (dd, 1 H, H^A-5), 4.07 (dd, 1 H, H^A-6b), 4.04 (dd, 1 H, H^A-3), 4.01 (dd, 1 H), 3.94 (d, 1 H, *J* = 2.7 Hz, H^D-4), 3.90–3.50 (m, 15 H, H^B-2, H^D-2), 2.31 (s, 3 H, Ac), 2.21 (s, 3 H, Ac); ^{13}C NMR (D_2O, 100.6 MHz): δ = 175.76 (C=O), 175.52 (C=O), 138.24, 130.07, 129.96, 129.71, 105.12 (d, $^3J_{C\text{-}F}$ = 11.3 Hz), 104.21, 102.76, 97.62, 94.10 (d, $^1J_{C\text{-}F}$ = 183.4 Hz), 79.90, 78.48, 76.65, 76.06, 75.02, 74.96, 73.84, 73.80, 72.27, 70.91, 70.86, 70.77, 70.59 (d, $^2J_{C\text{-}F}$ = 18.6 Hz), 69.97 (d, $^2J_{C\text{-}F}$ = 20.4 Hz), 62.31, 61.94, 61.89, 61.40, 56.39, 49.83, 23.58 (NAc), 23.25 (NAc). ESIMS (negative mode) Calcd for $C_{35}H_{53}O_{20}N_2F$ (*m/z*)[M = 840]. Found: 839.5 $[M]^-$, 875.5 $[M + Cl]^-$. Compound **2**: ^{1}H NMR (D_2O, DQF-COSY, TOCSY and ROESY, 400 MHz): δ = 7.50–7.00 (m, 5 H, ArH), 5.00 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H^A-1), 4.87 (dd, 1 H, *J* = 2.8 Hz, $^2J_{H\text{-}F}$ = 52.0 Hz, H^D-4), 4.73 (d, 1 H, J_{gem} = 12.3 Hz, $PhCH_AO$, ABq), 4.59 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H^D-1), 4.58 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H^C-1), 4.53 (d, 1 H, J_{gem} = 12.4 Hz, $PhCH_BO$, ABq), 4.46 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H^B-1), 4.34 (dd, 1 H, H^A-2), 4.25 (d, 1 H, *J* = 2.8 Hz, H^A-4), 4.16 (dd, 1 H, H^A-5), 4.10 (dd, 1 H, H^A-6b), 4.08 (dd, 1 H, H^A-3), 4.02 (dd, 1 H), 3.92–3.80 (m, 6 H, H^C-4), 3.81 (dd, 1 H, H^D-3), 3.80–3.50 (m, 10 H, H^D-2, H^A-6a, H^C-3, H^D-2, H^C-2), 2.31 (s, 3 H, Ac), 2.29 (s, 3 H, Ac); ^{13}C NMR (D_2O, 100.6 MHz): δ = 175.80 (C=O), 175.65 (C=O), 129.60, 129.40, 129.30, 105.90, 104.10, 102.85, 97.85, 79.80 (d, $^1J_{C\text{-}F}$ = 178.5 Hz), 76.10 (d, $^2J_{C\text{-}F}$ = 50.6 Hz), 73.90, 72.15 (d, $^2J_{C\text{-}F}$ = 50.9 Hz), 71.50, 71.23, 70.25, 70.00, 62.25, 61.23, 56.20, 49.95, 23.50 (NAc), 23.20 (NAc). ^{19}F NMR ($CDCl_3$, 376.4 MHz): δ = –172.95 ppm. ESIMS (negative mode) Calcd for $C_{35}H_{53}O_{20}N_2F$ (*m/z*) [M = 840]. Found: 839.6 $[M]^-$. Compound **3**: R_f = 0.5 (*i*-C_3H_9OH–HOAc–H_2O, 3:1:1). ^{1}H NMR (D_2O, DQF-COSY and ROESY, 400 MHz): δ = 7.50–7.20 (m, 5 H, ArH), 4.99 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H^A-1), 4.73 (d, 1 H, J_{gem} = 12.6 Hz, $PhCH_AO$, ABq), 4.61 (dq, 1 H, *J* = 2.8 Hz, *J* = 9.6 Hz, $^2J_{H\text{-}F}$ = 68.0 Hz, H^D-3), 4.58 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H^D-1), 4.55 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H^C-1), 4.52 (d, 1 H, J_{gem} = 12.5 Hz, $PhCH_BO$), 4.46 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H^B-1), 4.33 (dd, 1 H, H^A-2), 4.24 (d, 1 H, *J* = 2.8 Hz, H^A-4), 4.03 (d, 1 H, *J* = 3.2 Hz, H^D-4), 4.16 (m, 1 H, H^A-5), 4.07 (dd, 1 H, H^A-6b), 4.04 (dd, 1 H, H^A-3), 4.00 (dd, 1 H, H^C-3), 3.91 (d, 1 H, *J* = 2.8 Hz, H^B-4), 3.91–3.72 (m, 9 H), 3.64 (m, 2 H, H^C-5, H^B-3), 3.52 (dd, 1 H, H^B-2), 2.31 (s, 3 H, Ac), 2.28 (s, 3 H, Ac); ^{13}C NMR (D_2O, 100.6 MHz): δ = 175.77 (C=O), 175.54 (C=O), 138.22, 130.07, 129.95, 129.71, 105.89, 103.41 (d, $^3J_{C\text{-}F}$ = 12.7 Hz), 102.76, 97.58, 94.08 (d, $^1J_{C\text{-}F}$ = 183.9 Hz), 79.81, 78.26, 76.15 (d, $^2J_{C\text{-}F}$ = 17.8Hz), 75.32 (d, $^3J_{C\text{-}F}$ = 6.8 Hz), 73.79, 73.75, 71.93, 71.05, 70.99, 70.86, 70.73, 70.13, 69.89, 68.15, 67.97, 62.29, 61.99, 61.97, 61.32, 56.43, 49.87, 23.57 (NAc), 23.26 (NAc). ESIMS (negative mode) Calcd for $C_{35}H_{53}O_{20}N_2F$ (*m/z*) [M = 840]. Found: 839.5 $[M]^-$, 875.5 $[M + Cl]^-$.