Chemoenzymatic synthesis of 3-deoxy-3-fluoro-L-fucose and its enzymatic incorporation into glycoconjugates

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Supporting Information

1 3-Deoxy-3-fluorofucose synthesis

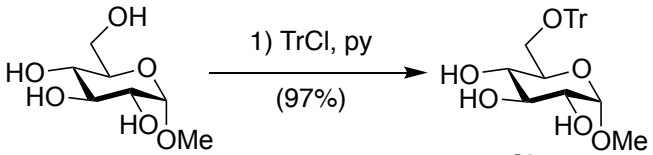
1.1 Materials for chemical reactions

Chemical reagents were obtained from commercial sources and used without further purification, unless stated otherwise. All air/moisture sensitive reactions were carried out under inert atmosphere (Ar) in flame-dried glassware. Anhydrous bottles of THF (tetrahydrofuran), toluene, CH_2Cl_2 and Et_3N, bought from commercial sources, were used for the reactions. When appropriate, other reagents and solvents were purified by standard techniques. Reactions were monitored by TLC (MERCK Kieselgel 60 F254, aluminium sheet), visualised under UV light (254 nm), and by staining with $KMnO_4$ (10% aq.) or sugar dip (0.3% (w/v) of N-(1-naphthyl)ethylenediamine and 5% (v/v) conc. H_2SO_4 in methanol). Column chromatography were performed on silica gel (MERCK Geduran 60 Å, particle size 40-63 µm) or using a biotage® isolera™ prime with biotage® SNAP KP-Sil columns. All reported solvent mixtures are volume measures.

^1H, ^{13}C and ^{19}F spectra were recorded in $CDCl_3$, methanol-d_4 or D_2O using a BRUKER AV400 (400, 101 and 376 MHz respectively) and AV500 (500, 125 and 470 MHz respectively) spectrometers. ^1H and ^{13}C chemical shifts (δ) are quoted in ppm relative to residual solvent peaks as appropriate. ^{19}F spectra were externally referenced to $CFCl_3$. The coupling constants (*J*) were recorded in Hertz (Hz). The coupling constants have not been averaged. Fourier-transform infrared (FT-IR) spectra are reported in wavenumbers (cm^{-1}) and were recorded as neat films on a Thermo Scientific Nicolet iS5 spectrometer using neat samples (solid or liquid). Electrospray mass spectra were obtained from a Waters 2700 sample manager ESI, and recorded in m/z (abundance). HRMS was obtained from a Bruker APEX III FT-ICR-MS. Samples were run in HPLC methanol or MeCN. Optical rotations were recorded on an Optical Activity POLAAR 2001 at 589 nm.

1.2 Experimental procedures

1.2.1 Methyl 6-*O*-trityl-α-D-glucopyranose SI1

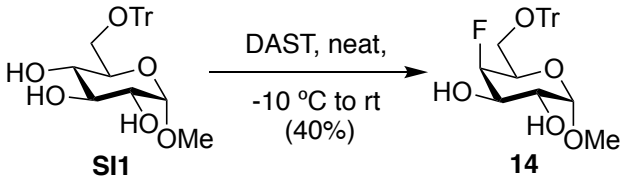


13 **SI1**

To a solution of **13** (10.0 g, 51.4 mmol) in anhydrous pyridine (80 mL) was added TrCl (15.6 g, 56.0 mmol). The reaction was then heated at 70 °C for 3.5 h. The RM was then diluted with EtOAc (200 mL), washed with HCl 2 M (5 × 90 mL), brine (2 ×100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give desired product **SI1** as a white solid (21.8 g, 50.0 mmol, 97% - calculated taking traces of pyridine and EtOAc into account). **^1H NMR** (400 MHz, $CDCl_3$) δ 7.36-7.23 (15H, m), 4.73 (1H, d, *J* 3.8 Hz), 3.67 (1H, d, *J* 9.2 Hz), 3.65 (1H, dd, *J* 9.6, 3.8 Hz), 3.49 (1H, t, *J* 2.6 Hz), 3.45 (1H, d, *J* 9.4 Hz),

3.39 (3H, s), 3.35 (2H, dd, *J* 7.5, 4.6 Hz) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.6, 128.6, 127.9, 127.2, 99.0, 87.1, 74.7, 72.2, 71.8, 69.8, 64.0, 55.3 ppm. Data consistent with the literature.[1]

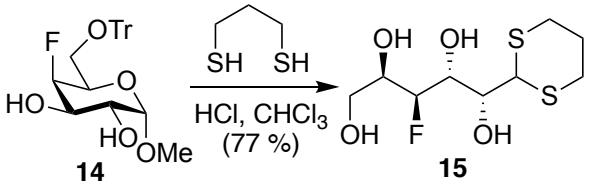
1.2.2 Methyl 4-deoxy-4-fluoro-6-*O*-trityl-α-D-glucopyranose 14



SI1 **14**

To neat DAST (24 ml, 182 mmol) at -10 °C was added **SI1** (7.0 g, 16.0 mmol) portionwise over 20 min. The RM was then allowed to reach RT. After 48 h the RM was diluted with CH_2Cl_2 (60 mL) and cooled at 0 °C. A solution of $NaHCO_3$ sat. solution (20 mL) was added dropwise until bubbling ceased. After an additional 30 min at RT, $NaHCO_3$ sat. solution (150 mL) was added, the aqueous phase was then separated, extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were then washed with $NaHCO_3$ sat. solution (3 × 100 mL), H_2O (2 × 100 mL), dried over $MgSO_4$, filtered, concentrated and then purified by column chromatography (silica, 10 to 80% acetone in hexane) to give first compound **14** as an off-white solid (2.23 g, 5.10 mmol, 32%), a second non pure fraction was re-purified by column chromatography (silica, 40-50% acetone in hexanes) also giving **14** as an off-white solid (600 mg, 1.37 mmol, 9%). Combining the two pure fractions gave a total of 2.83 g (6.5 mmol, 40%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45 (5H, m), 7.28 (10H, m), 4.93 (1H, dd, *J* 50.3, 2.0 Hz), 4.81 (1H, d, *J* 3.0 Hz), 3.81 (2H, dd, *J* 13.7, 7.0 Hz), 3.73 (1H, t, *J* 6.7 Hz) 3.42 (3H, s,) 3.39 (1H, m) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.7, 128.6, 127.9, 127.1, 99.2, 89.2 (d, *J* 181.2 Hz), 87.0 Hz, 70.2 (d, *J* 18.3 Hz), 69.9 (d, *J* 2.2 Hz), 68.6 (d, *J* 18.3 Hz), 61.6 (d, *J* 5.1 Hz), 55.6 ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -221.4 (1F, dt, *J* 50.3, 30.3 Hz) ppm; **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) δ -221.4 (1F, s) ppm. Data consistent with the literature.[2]

1.2.3 4-Deoxy-4-fluoro-D-galactose 1,3-propylidene dithioacetal 15

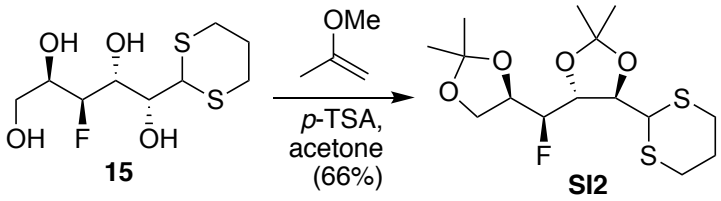


14 **15**

Adapted from a procedure described by Redlich and Kölln.[3] To a solution of **14** (1.00 g, 2.28 mmol) in $CHCl_3$ (2.89 mL) and conc. HCl (2.89 mL, 37.6 mmol) was added 1,3-propanedithiol (0.346 mL, 3.42 mmol). After 16 h the RM was diluted with toluene and acetone then concentrated (co evaporation with toluene). Purified by column chromatography (silica, MeOH/CH_2Cl_2 5/95 to 1/9) to give the desired compound **15** as an off-white solid (477 mg, 1.75 mmol, 77%). **R_f** 0.38 (MeOH/EtOAc, 1:9); **mp** 110-112 ºC (MeOH); **$[\alpha]_D^{23}$** -10.2 (*c* 1, MeOH); **IR** 3382 (br), 2934 (br), 1422 (m), 1093 (s), 1033 (s), 663 (m)

cm^{-1}; **^{1}H NMR** (500 MHz, CD_3OD) δ 4.62 (1H, ddd, *J* 46.0, 9.4, 1.1 Hz, H-4), 4.39 (1H, ddd, *J* 9.4, 3.7, 1.1 Hz, H-3), 4.14 (1H, d, *J* 9.8 Hz, H-1), 3.94 (1H, ddd, *J* 9.7, 2.9, 1.1 Hz, H-2), 3.94 (1H, dtd, *J* 29.8, 6.9, 1.0 Hz, H-5), 3.66 (2H, dd, *J* 7.1, 0.7 Hz, H-6), 2.95 (1H, ddd, *J* 14.2, 7.7, 3.0 Hz, SC$\underline{H}_2$), 2.90 (1H, ddd, *J* 14.1, 7.6, 2.9 Hz, SC$\underline{H}_2$), 2.77 (1 H, dt, *J* 9.2, 3.1 Hz, SC$\underline{H}_2$), 2.74 (1H, dt, *J* 9.2, 3.1 Hz, SC$\underline{H}_2$), 2.06 (1H, dtt, *J* 13.8, 7.6, 2.9 Hz, $SCH_2C\underline{H}_2$), 1.92 (1H, dtt, *J* 13.7, 9.2, 3.0 Hz, $SCH_2C\underline{H}_2$) ppm; **^{1}H{^{19}F} NMR** (500 MHz, CD_3OD) δ 4.62 (1H, dd, *J* 9.4, 1.3 Hz, H-4), 4.39 (1H, dd, *J* 9.4, 1.1 Hz, H-3), 4.14 (1H, d, *J* 9.8 Hz, H-1), 3.94 (1H, dd, *J* 9.8, 1.1 Hz, H-2), 3.93 (1H, td, *J* 6.8, 1.1 Hz, H-5), 3.66 (2H, d, *J* 7.1 Hz, H-6), 2.95 (1H, ddd, *J* 14.3, 7.7, 3.0 Hz, SC$\underline{H}_2$), 2.90 (1H, ddd, *J* 14.1, 7.5, 2.8 Hz, SC$\underline{H}_2$), 2.77 (1H, dt, *J* 9.2, 3.2 Hz, SC$\underline{H}_2$), 2.74 (1H, dt, *J* 9.0, 3.0 Hz, SC$\underline{H}_2$), 2.06 (1H, dtt, *J* 13.8, 7.6, 2.9 Hz, $SCH_2C\underline{H}_2$), 1.92 (1H, dtt, *J* 13.9, 9.0, 2.8 Hz, $SCH_2C\underline{H}_2$) ppm; **^{13}C NMR** (126 MHz, CD_3OD) δ 91.6 (d, *J* 176.2 Hz, C-4), 71.0 (d, *J* 11.4 Hz, C-5), 70.9 (d, *J* 4.1 Hz, C-2), 67.9 (d, *J* 26.2 Hz, C-3), 63.7 (d, *J* 5.7 Hz, C-6), 49.3 (s, C-1), 29.0 (s, S$\underline{C}H_2$), 28.7 (s, S$\underline{C}H_2$), 27.2 (s, $SCH_2\underline{C}H_2$) ppm; **^{19}F NMR** (471 MHz, CD_3OD) δ -213.5 (1F, ddt, 46.0, 29.8 , 3.0 Hz, F-4) ppm; **^{19}F{^{1}H} NMR** (471 MHz, CD_3OD) δ -213.5 (1F, s, F-4) ppm; **MS (ESI+)** (m/z) 295 $[M+Na]^+$; **HRMS** (ESI+) for $C_9H_{17}NaFO_4S_2$ calcd. 295.0450, found: 294.0438

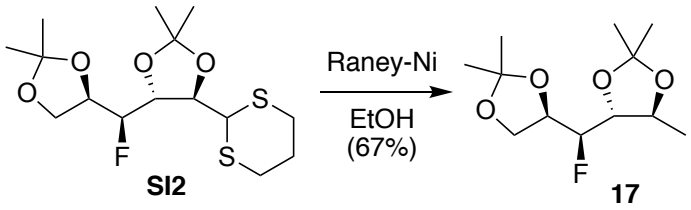
1.2.4 2,3:5,6-Di-*O*-isopropylidene-4-deoxy-4-fluoro-D-galactose 1,3-propylidene dithioacetal SI2



Adapted from a protocol described by Mann.[4] Thioacetal **15** (2.36 g, 8.67 mmol), *p*-TSA (20 mg, 0.11 mmol), and Na_2SO_4 (90 mg, 0.63 mmol) were added to a flask, which was filled with argon. Acetone (35 mL) was then added followed by 2-methoxypropene (2 mL, 20.88 mmol), adding an additional 0.5 ml of 2-methoxypropene after 2, 3 and 4 h. The RM was then stirred at RT for and additional 15 h. The RM was neutralised with K_2CO_3, then filtered, concentrated and then purified by column chromatography (silica, 15-25% EtOAc in petroleum ether) to give **SI2** as a yellow oil (2.01 g, 5.70 mmol, 66%). **R_f** 0.20 (acetone/hexane, 2:8); **$[\alpha]_D^{23}$** -29.8 (*c* 1, $CHCl_3$); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.48 – 4.27 (4H, m, H-2, H-3, H-4, H-5), 4.16 (1H, d, *J* 3.8 Hz, H-1), 4.11 (1H, ddd, *J* 8.4, 6.9, 1.3 Hz, H-6), 3.96 (1H, dd, *J* 8.5, 6.8 Hz, H-6'), 3.03 (1H, br ddd, *J* 14.4, 7.4, 3.0 Hz, SC$\underline{H}_2$), 3.02 (1H, br ddd, *J* 14.4, 7.3, 3.0 Hz, SC$\underline{H}_2$), 2.82 (1H, ddd, *J* 14.0, 9.8, 2.9 Hz, SC$\underline{H}_2$), 2.77 (1H, ddd, *J* 14.0, 9.7, 2.9 Hz, SC$\underline{H}_2$), 2.09 (1H, dtt, *J* 14.0, 6.9, 2.9 Hz, $SCH_2C\underline{H}_2$), 2.00 (1H, dtt, *J* 14.1, 9.8, 3.1 Hz, $SCH_2C\underline{H}_2$), 1.50 (3H, s, $C(C\underline{H}_3)_2$), 1.44 (3H, s, $C(C\underline{H}_3)_2$), 1.40 (3H, s, $C(C\underline{H}_3)_2$), 1.39 (3H, d, *J* 0.5 Hz, $C(C\underline{H}_3)_2$) ppm; **^{1}H{^{19}F} NMR** (500 MHz, $CDCl_3$) δ 4.46 (1H, dd, *J* 6.1, 3.7 Hz, H-2), 4.40 (1H, dd, *J* 7.3, 3.6 Hz, H-4), 4.35 (1H, dd, *J* 7.3, 6.2 Hz, H-3), 4.31 (1H, td, *J* 6.8, 3.7 Hz, H-5), 4.16 (1H, d, *J* 3.6 Hz, H-1), 4.11 (1H, dd, *J* 8.5, 6.8 Hz, H-6), 3.96

(1H, dd, *J* 8.5, 6.6 Hz, H-6'), 3.03 (1H, ddd, *J* 14.5, 7.7, 3.0 Hz, SC$\underline{H}_2$), 3.02 (1H, br ddd, *J* 14.5, 7.5, 3.0 Hz, SC$\underline{H}_2$), 2.82 (1H, ddd, *J* 13.9, 9.8, 2.8 Hz, SC$\underline{H}_2$), 2.77 (1H, ddd, *J* 13.9, 9.8, 2.8 Hz, SC$\underline{H}_2$), 2.09 (1H, dtt, *J* 13.9, 6.8, 2.8 Hz, $SCH_2C\underline{H}_2$), 2.00 (1H, dtt, *J* 13.9, 9.6, 3.0 Hz, $SCH_2C\underline{H}_2$), 1.50 (3H, s, C(C$\underline{H}_3$)$_2$), 1.44 (3H, s, C(C$\underline{H}_3$)$_2$), 1.40 (3H, s, C(C$\underline{H}_3$)$_2$), 1.39 (3H, s, C(C$\underline{H}_3$)$_2$) ppm; **^{13}C NMR** (126 MHz, $CDCl_3$) δ 111.3 (s, $\underline{C}(CH_3)_2$), 110.1 (s, $\underline{C}(CH_3)_2$), 92.6 (d, *J* 181.9 Hz, C-4), 83.1 (d, *J* 1.0 Hz, C-2), 76.5 (d, *J* 27.7 Hz, C-3), 75.2 (d, *J* 17.6 Hz, C-5), 65.2 (d, *J* 6.7 Hz, C-6), 47.7 (s, C-1), 29.6 (s, S$\underline{C}H_2$), 29.1 (s, S$\underline{C}H_2$), 27.4 (s, C($\underline{C}H_3$)$_2$), 27.4 (s, C($\underline{C}H_3$)$_2$), 26.2 (s, C($\underline{C}H_3$)$_2$), 25.9 (s, C($\underline{C}H_3$)$_2$), 25.7 (s, $SCH_2\underline{C}H_2$) ppm; **^{19}F NMR** (471 MHz, $CDCl_3$) δ -204.4 – -204.7 (1F, m, F-4) ppm; **^{19}F{^{1}H} NMR** (471 MHz, $CDCl_3$) δ -204.5 (1F, s, F-4) ppm; **HRMS** (ESI+) for $C_{15}H_{25}NaFO_4S_2$ calcd. 375.1076, found: 375.1078.

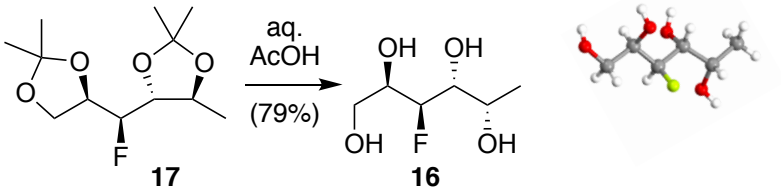
1.2.5 1,2:4,5-Di-*O*-isopropylidene-3-deoxy-3-fluoro-L-fucitol 17



SI2 **17**

To a solution of **SI2** (2.75 g, 7.8 mmol) in EtOH (100 mL) was added Raney Ni (75 mL) (the Raney Ni was prewashed in the measuring cylinder with EtOH 3 times before use). The reaction mixture was heated at reflux for 15 h then filtered on celite, washed with EtOH (150 mL), CH_2Cl_2 (3 × 150 mL), concentrated *in vacuo* and then purified by column chromatography (silica, 10% acetone in hexanes) to give **17** as a colourless oil (1.30 g, 5.22 mmol, 67%); **$[\alpha]_D^{23}$** -3.5 (*c* 1, $CHCl_3$); **IR** 2987 (s), 2937 (br), 1380 (s), 1371 (s), 1217 (s), 1065 (s) 857 (s) cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.31 (1H, dtd, *J* 22.4, 6.6, 3.9 Hz, H-2), 4.30 (1H, ddd, *J* 48.3, 7.9, 3.9 Hz, H-3), 4.17 (1H, dqd, *J* 7.7, 6.1, 6.1, 0.7 Hz, H-5), 4.09 (1H, ddd, *J* 8.5, 6.5, 1.3 Hz, H-1), 3.94 (1H, dd, *J* 8.5, 6.6 Hz, H-1'), 3.73 (1H, td, *J* 7.7, 5.7 Hz, H-4), 1.43 (3H, s, CH_3-b), 1.42 (3H, s, CH_3-d), 1.39 (3H, d, *J* 0.6 Hz, CH_3-b'), 1.38 (3H, dd, *J* 6.0, 0.8 Hz, H-6), 1.36 (3H, s, CH_3-d) ppm; **^{1}H{^{19}F} NMR** (500 MHz, $CDCl_3$) δ 4.35 – 4.25 (2H, m, H-2 + H3), 4.17 (1H, dq, *J* 7.5, 6.0 Hz, H-5), 4.09 (1H, dd, *J* 8.6, 6.6 Hz, H-1), 3.94 (1H, dd, *J* 8.5, 6.6 Hz, H-1'), 3.73 (1H, t, *J* 7.7 Hz, H-4), 1.43 (3H, s, CH_3-b), 1.42 (3H, s, CH_3-d), 1.39 (3H, s, CH_3-b'), 1.38 (3H, d, *J* 6.0 Hz, H-6), 1.36 (3H, s, CH_3-d) ppm; **^{13}C NMR** (126 MHz, $CDCl_3$) δ 110.0 (s, C-a), 109.5 (s, C-c), 93.0 (d, *J* 179.5 Hz, C-3), 79.4 (d, *J* 29.3 Hz, C-4), 76.4 (s, C-5), 75.4 (d, *J* 17.4 Hz, C-2), 65.2 (d, *J* 6.7 Hz, C-1), 27.5 (s, $\underline{C}H_3$-d), 26.9 (s, $\underline{C}H_3$-d), 26.2 (d, *J* 1.2 Hz, $\underline{C}H_3$-b), 25.7 (s, $\underline{C}H_3$-b), 19.0 (d, *J* 2.1 Hz, C-6) ppm; **^{19}F NMR** (471 MHz, $CDCl_3$) δ -205.4 (1F, ddd, *J* 48.3, 22.4, 5.6 Hz, F-3) ppm; **^{19}F{^{1}H} NMR** (471 MHz, $CDCl_3$) δ -205.4 (1F, s, F-3) ppm.

1.2.6 3-Deoxy-3-fluoro-L-fucitol 16



17 **16**

Following a protocol described by Kozikowski.[5] The protected 3-deoxy-3-fluorofucitol **17** (510 mg, 2.05 mmol) was dissolved in a mixture of THF (6.1 mL), H_2O (6.0 mL) and AcOH (12.1 mL). The solution was then heated at 80 °C for 19 h then concentrated and purified by column chromatography (silica, 10% MeOH in CH_2Cl_2) to give **16** as a white solid (274 mg, 1.62 mmol, 79%). **R_f** 0.26 (MeOH/EtOAc, 1:9); **mp** 110-111 ºC (MeOH); **$[\alpha]_D^{24}$** -14.3 (*c* 1, MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.54 (1H, ddd, *J* 46.1, 9.2, 1.4 Hz, H-3), 3.97 – 3.92 (1H, m, H-5), 3.94 (1H, dtd, *J* 29.4, 7.0, 1.4 Hz, H-2), 3.66 (1H, ddd, *J* 9.1, 4.5, 2.0 Hz, H-4), 3.65 (2H, dd, *J* 6.9, 1.0 Hz, H-1), 1.25 (3H, d, *J* 6.2 Hz, H-6) ppm; **^{1}H{^{19}F} NMR** (500 MHz, CD_3OD) δ 4.54 (1H, dd, *J* 9.1, 1.3 Hz, H-3), 3.98 – 3.90 (2H, m, H-2 + H-5), 3.66 (1H, dd, *J* 9.1, 1.9 Hz, H-4), 3.65 (2H, br dd, *J* 7.0, 1.1 Hz, H-1), 1.25 (3H, d, *J* 6.6 Hz, H-6) ppm; **^{13}C NMR** (126 MHz, CD_3OD) δ 91.7 (d, *J* 175.7 Hz, C-3), 72.4 (d, *J* 25.7 Hz, C-4), 71.2 (d, *J* 18.1 Hz, C-2), 67.0 (d, *J* 2.6 Hz, C-5), 63.7 (d, *J* 5.7 Hz, C-1), 20.0 (d, *J* 1.2 Hz, C-6) ppm; **^{19}F NMR** (471 MHz, CD_3OD) δ -213.2 (1F, ddt, *J* 46.1, 29.4, 3.6 Hz, F-3) ppm; **^{19}F{^{1}H} NMR** (471 MHz, CD_3OD) δ -213.2 (1F, s, F-3) ppm; **HRMS** (ESI+) for $C_6H_{13}FNaO_4$ calcd. 191.0695, found 191.0690.

1.3 GOase mediated oxidation to 3

1.3.1 Materials for enzymatic reaction

Galactose Oxidase F_2 was engineered as previously described.[6] Horse radish peroxidase (HRP) type VI and catalase were purchased from Sigma Aldrich.

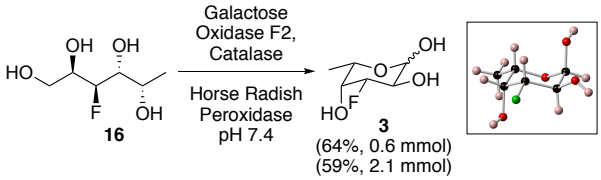
1.3.2 Expression and purification of Galactose Oxidase:

E.coli BL21 StarTM (DE3) harbouring the GOase F_2 plasmid were grown at 37 °C at 200 rpm in 5 mL LB medium supplemented with 5 µL kanamycin (30 mg/mL). 500 µL of the overnight culture was used to inoculate 250 mL of prepared auto induction medium as previously described.[7, 8] Cells were grown at 26 °C, 250 rpm for 60 h. Following growth, cells were harvested and lysed. Cell lysate was then dialysed overnight against 50 mM 'NP' buffer (50 mM NaPi 300 mM NaCl). Protein purification was carried out using 5 mL Strep-Tactin Superflow Plus Cartridges(Qiagen), eluting with 5 mM pH 8 'NPD' buffer (5 mM Desthiobiotin, 50 mM NaH_2PO_4, 300 mM NaCl) as previously described.[7, 8] Following purification, the protein solution was dialysed overnight against pH 7.4 50 mM NaPi buffer supplemented with $CuSO_4$. Following copper loading the protein solution was dialysed against copper free pH 7.4 50 mM NaPi overnight. Protein was then concentrated and quantified using a BCA protein assay kit (ThermoFisher).

1.3.3 Activity assay

Specific activities of purified GOase F_2 was measured against 3-deoxy-3-fluoro-fucitol using the ABTS-HRP assay previously described.[6] 10 µl of GOase F_2 was diluted in 90 µl of 'reaction mix' which contained 0.23 mg/mL HRP and 0.4 mg/mL ABTS 100 mM pH 7.4 NaPi buffer. The reaction was initiated with the addition of substrate (final substrate concentration 25 mM). Production of the reduced ABTS was measured using a Tecan infinite 200pro plate reader at 420 nm and 30 °C for 10 minutes. Measurements were made in triplicate.

1.3.4 3-Deoxy-3-fluoro-L-fucose 3F-fucose 3



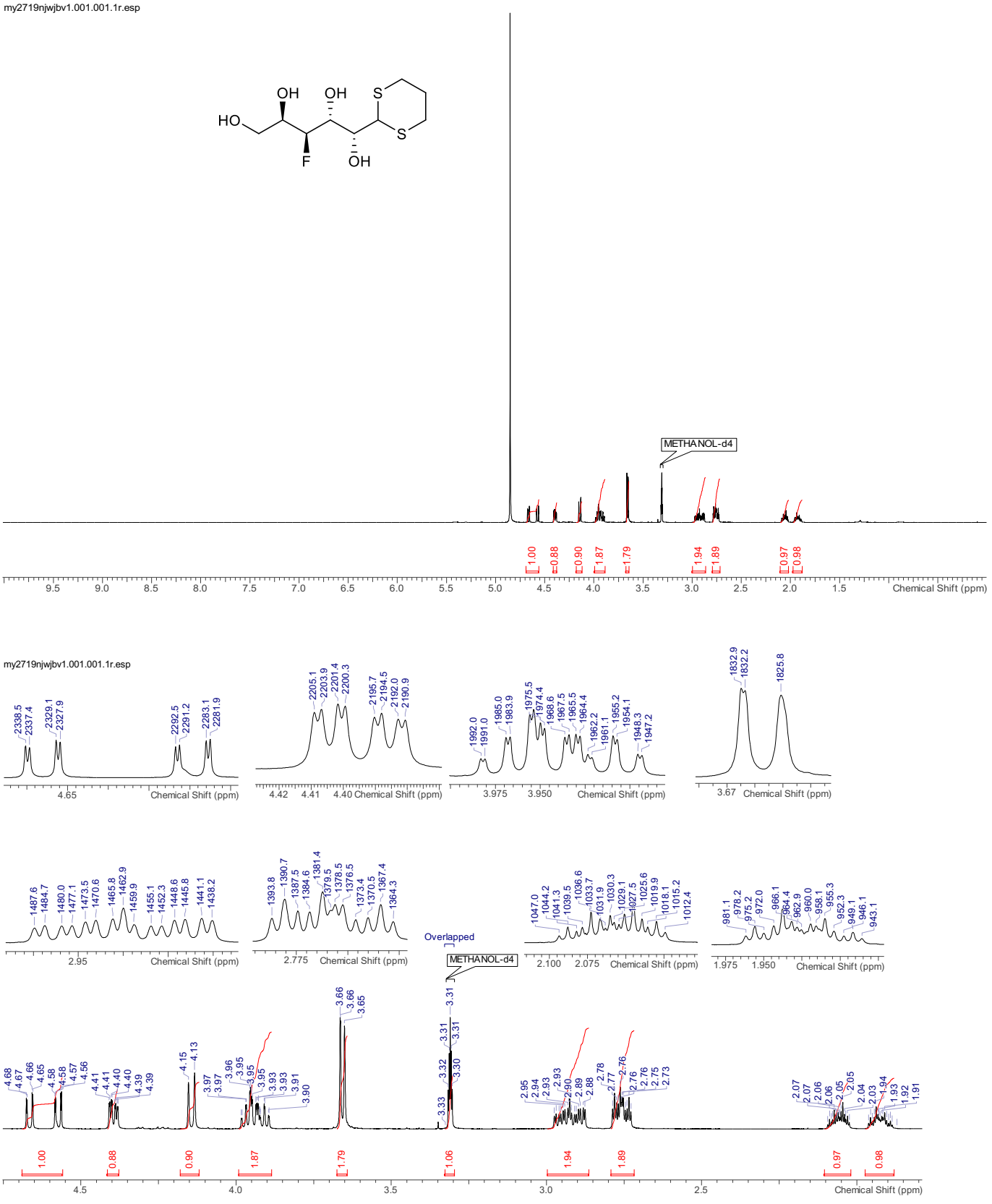
All buffers were vortexed prior to mixing to ensure saturation with oxygen. The biotransformation was performed in a 1L conical flask at a total volume of 100 mL to ensure sufficient aeration of the biotransformation. 1 $mg.mL^{-1}$ GOase F_2, 0.1 $mg.mL^{-1}$ HRP and catalase were prepared in the flask. 350 mg of 3-deoxy-3-fluorofucitol **16** (350 mg, 2.08 mmol), suspended in 100 mM pH 7.4 NaPi, was then added to the reaction to give a final volume of 100 mL. The flask was incubated at 25 °C with shaking at 250 rpm and left for 16 h. ^{19}F-NMR showed the biotransformation reached ~90% conversion, to push the reaction to completion a further 10 mg of purified F_2 was added to the reaction mix and left for a further 8 h. ^{19}F-NMR analysis at this point showed the 3F-Fucitol **37** had been fully converted. Protein was then removed using a vivaspin 20 (10 kDa MWCO) and the remaining sugar component dried *in vacuo*. The crude residue was then purified by column chromatography (silica, 10% MeOH in DCM) to give 3-deoxy-3-fluorofucose **3** as a white solid (204 mg, 1.23 mmol, 59%), $\mathbf{R_f}$ 0.44 (MeOH/EtOAc, 1:9); **mp** 115-116 ºC (MeOH); $\mathbf{[\alpha]_D^{30}}$ -87.9 (*c* 0.5, MeOH); **IR** 3588 (s), 3301 (br), 2933 (br), 1355 (m), 1165 (s), 1036 (s), 1000 (s), 808 (s), 670 (s), 663 (s) cm^{-1}; **^{1}H NMR** (500 MHz, D_2O) (ratio α:β, 5:3) δ 5.22 (1H,br t, *J* 4.7 Hz, H-1α), 4.73 (1H, dddd, *J* 49.2, 10.1, 3.5, 0.3 Hz, H-3α), 4.56 (1H, d, *J* 7.9 Hz, H-1β), 4.53 (1H, ddd, *J* 48.3, 9.7, 3.7 Hz, H-3β), 4.19 (1H, br q, *J* 6.6 Hz, H-5α), 4.05 (1H, ddd, *J* 7.4, 3.6, 1.1 Hz, H-4α), 3.77 (1H, qdd, *J* 6.5, 6.5, 6.5, 1.7, 1.1 Hz, H-5β), 3.69 (1H, ddd, *J* 12.8, 9.7, 7.7 Hz, H-2β), 1.23 (3H, dd, *J* 6.5, 0.6 Hz, H-6β), 1.20 (3H, dd, *J* 6.6, 0.7 Hz, H-6α) ppm; **^{1}H{^{19}F} NMR** (500 MHz, D_2O) δ 5.22 (1H, d, *J* 4.1 Hz, H-1α), 4.73 (1H, dd, *J* 10.3, 3.6 Hz, H-3α), 4.56 (1H, d, *J* 7.9 Hz, H-1β), 4.53 (1H, dd, *J* 9.6, 3.6 Hz, H-3β), 4.19 (1H, qdd, *J* 6.6, 1.1, 0.6 Hz, H-5α), 4.05 (1H, dd, *J* 3.6, 1.1 Hz, H-4α), 4.00 (1H, dd, *J* 10.3, 4.3 Hz, H-2α), 3.99 (1H, dd, *J* 3.6, 0.9 Hz, H-4β), 3.77 (1H, br qd, *J* 6.6, 0.9 Hz, H-5β) 3.69 (1H, dd, *J* 9.6, 7.9 Hz, H-2β), 1.23 (3H, d, *J* 6.4 Hz, H-6β), 1.20 (3H, d, *J* 6.6 Hz, H-6α) ppm; **^{13}C NMR** (126 MHz, D_2O) δ 95.5 (d, *J* 12.2 Hz, C-1β), 93.3 (d, *J* 182.6 Hz, C-3β), 92.3 (d, *J* 10.5 Hz, C-

1α), 91.0 (d, *J* 181.0 Hz, C-3α), 70.2 (d, *J* 17.9 Hz, C-2β), 70.0 (d, *J* 16.0 Hz, C-4α), 69.6 (d, *J* 8.3 Hz, C-5β), 69.5 (d, *J* 15.5 Hz, C-4α), 66.6 (d, *J* 17.6 Hz, C-2α), 65.7 (d, *J* 6.7 Hz, C-5α), 15.2 (s, C-6β), 15.2 (s, C-6α) ppm; **^{19}F NMR** (471MHz, D_2O) δ -198.6 (1F, ddd, *J* 48.1, 12.6, 5.2 Hz, F-3β), -202.3 (1F, dm, *J* 49.0, F-3α) ppm; **^{19}F{^{1}H} NMR** (471 MHz, D_2O) δ -198.6 (1F, s, F-3β) -202.3 (1F, s, F-3α) ppm; **HRMS (ESI)** for $C_6H_{11}FNaO_4$ (M + Na)+ calcd 189.0539, found 189.0537.

1.4 Copies of NMR spectra

1.4.1 4-Deoxy-4-fluoro-D-galactose 1,3-propylidene dithioacetal 15

1.4.1.1 ^{1}H NMR (500 MHz, CD_3OD)



1.4.1.2 $^{1}H\{^{19}F\}$ NMR (500 MHz, CD_3OD)

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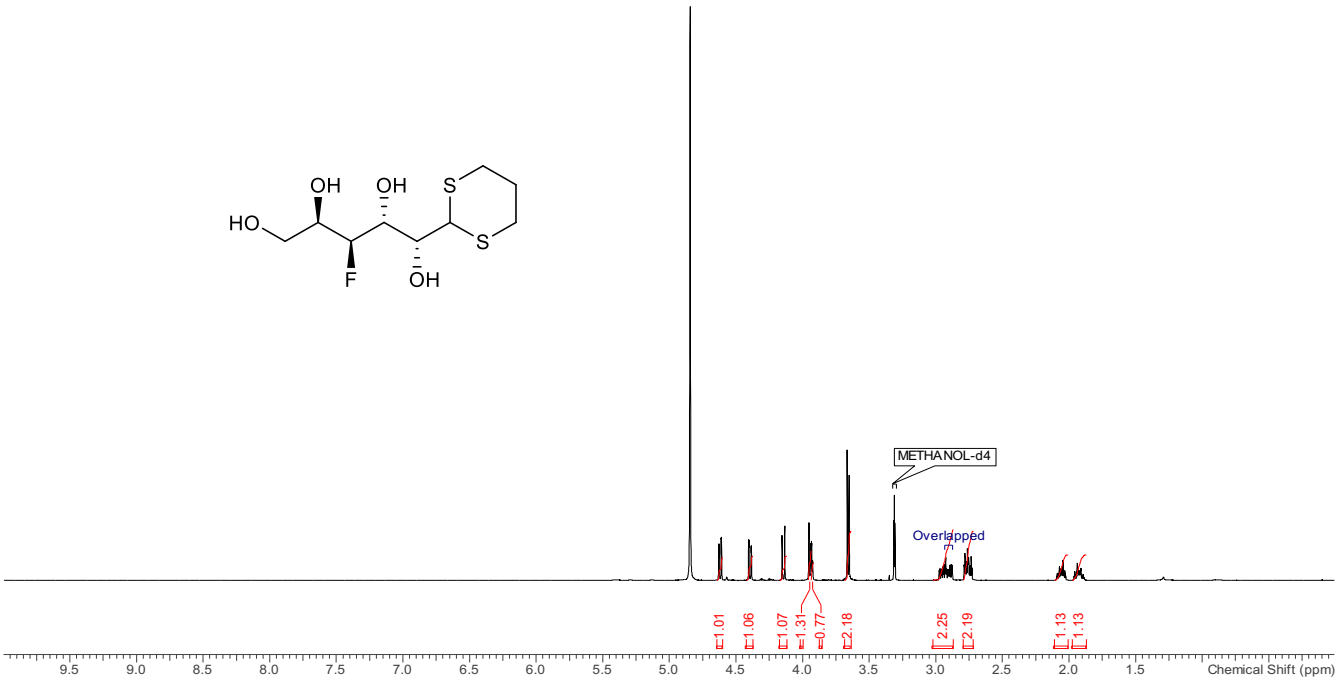

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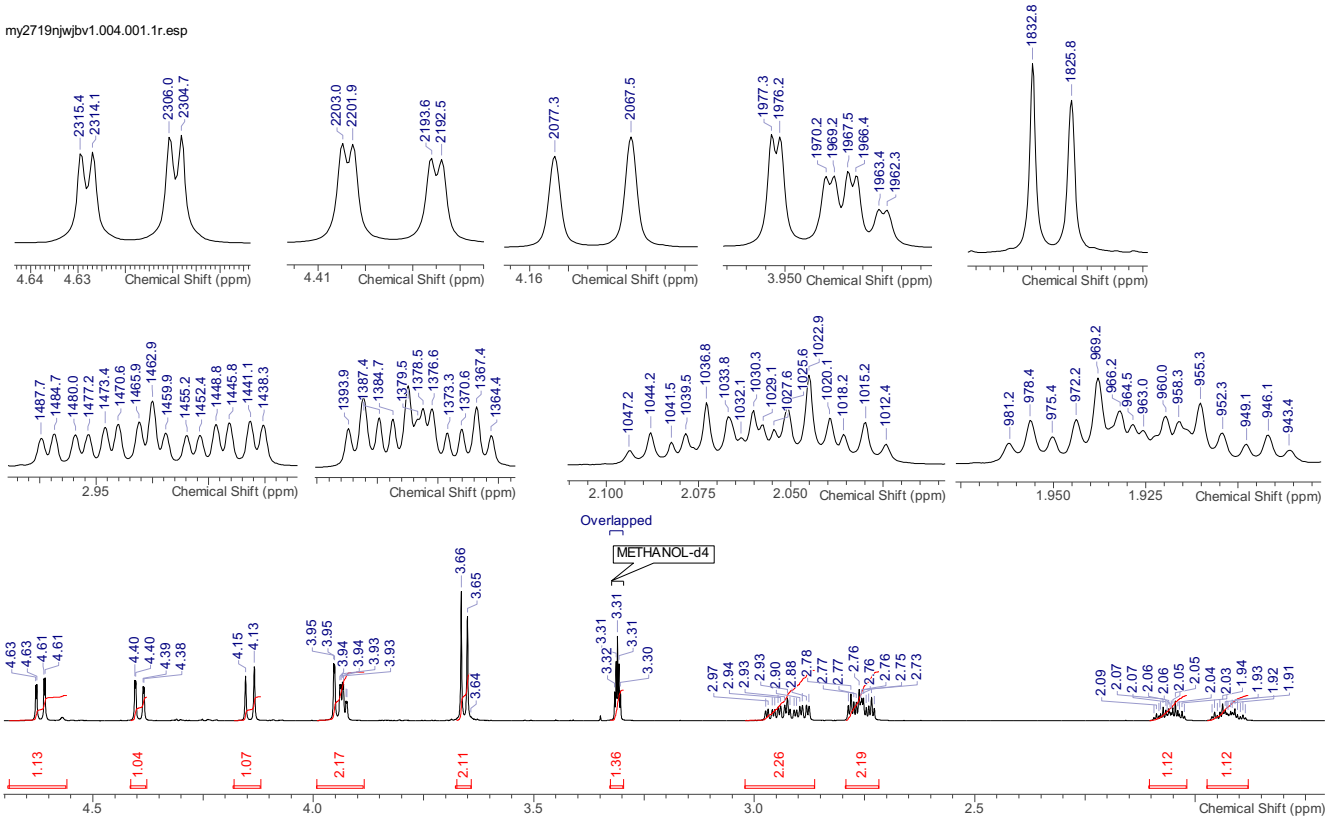
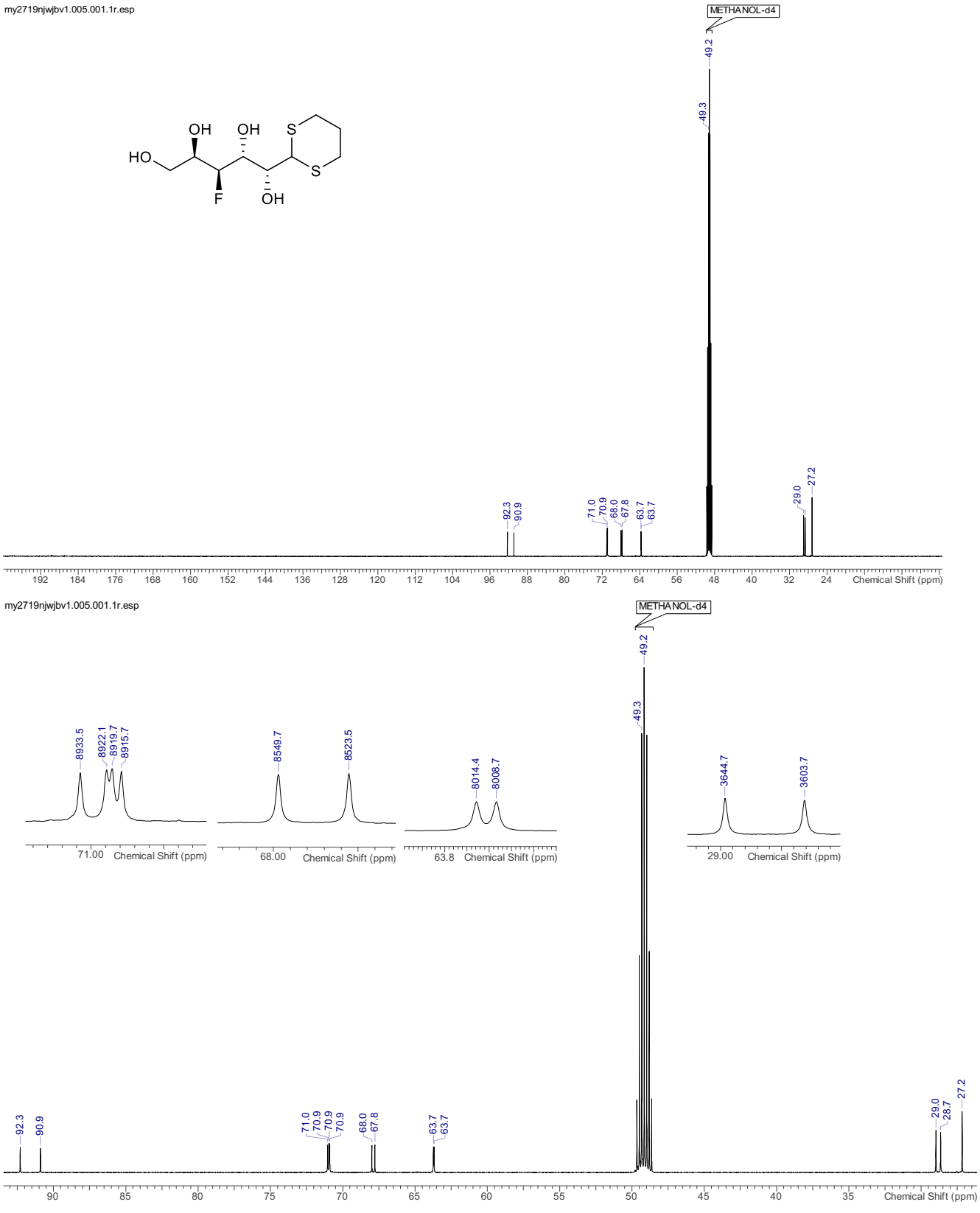


1.4.1.3 ^{13}C NMR (126 MHz, CD_3OD)



1.4.1.4 ^{19}F NMR (471 MHz, CD3OD)

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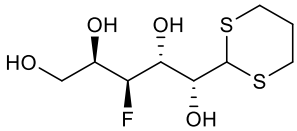
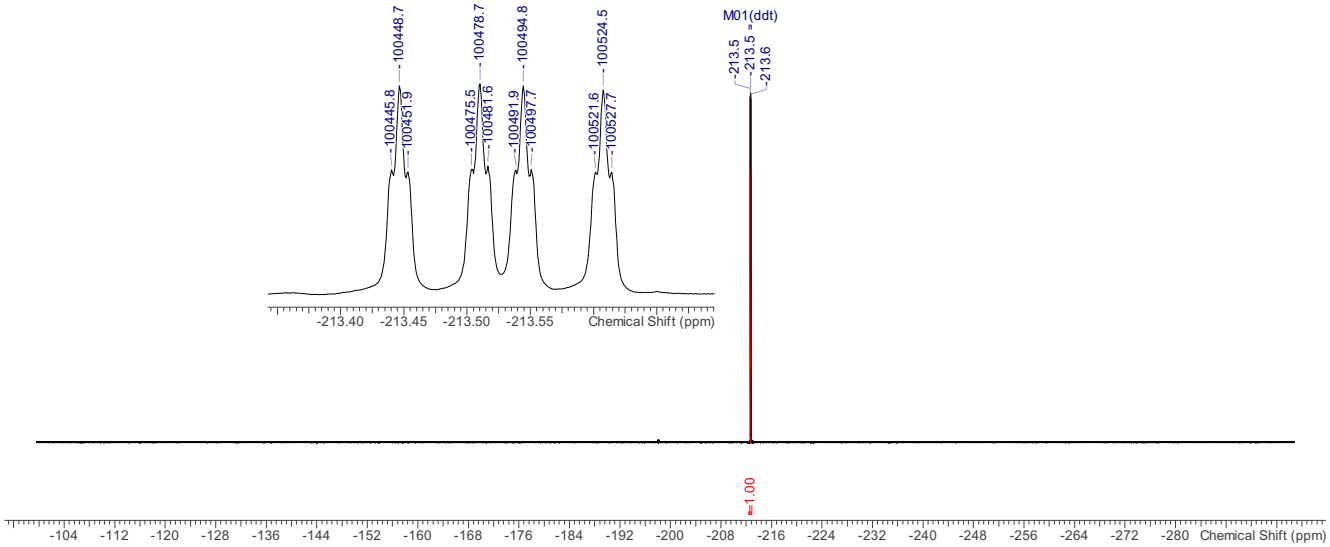
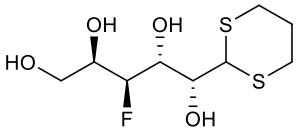
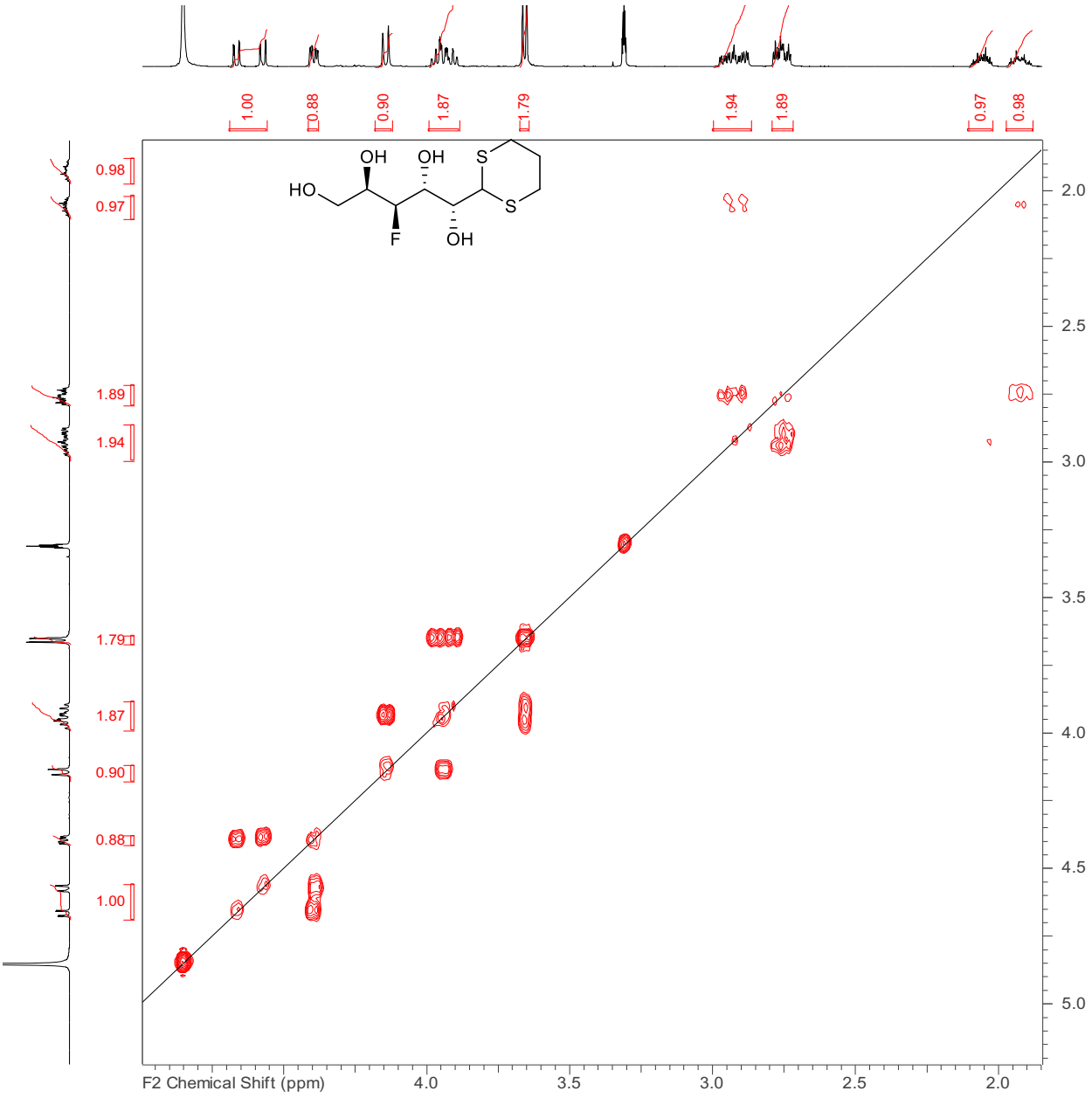




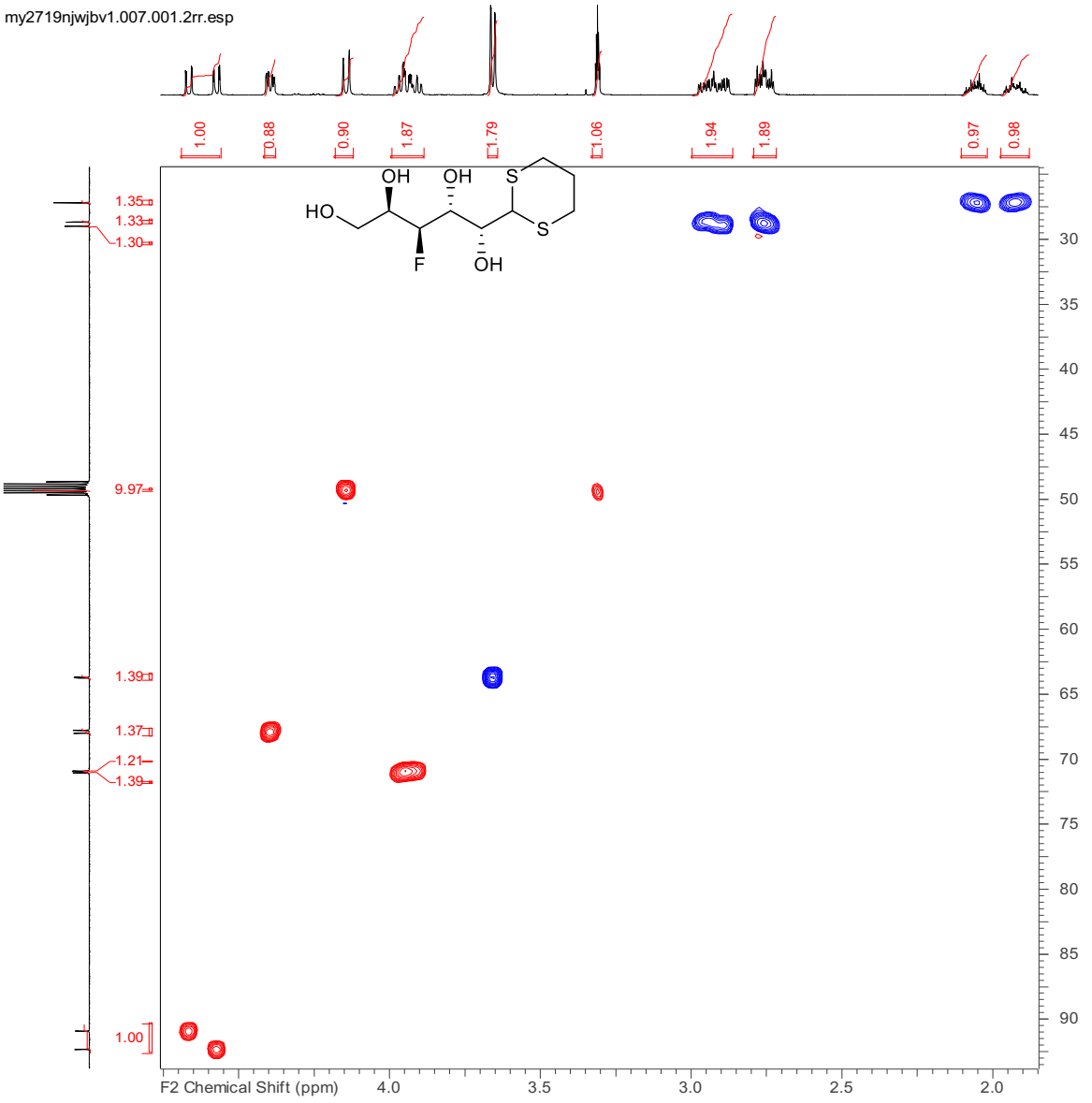
1.4.1.5 ^{19}F{^{1}H} NMR (471 MHz, CD_3OD)



1.4.1.6 ^{1}H-^{1}H COSY (500 MHz, CD_3OD)



1.4.1.7 ^{1}H-^{13}C HSQC (500 MHz, CD_3OD)

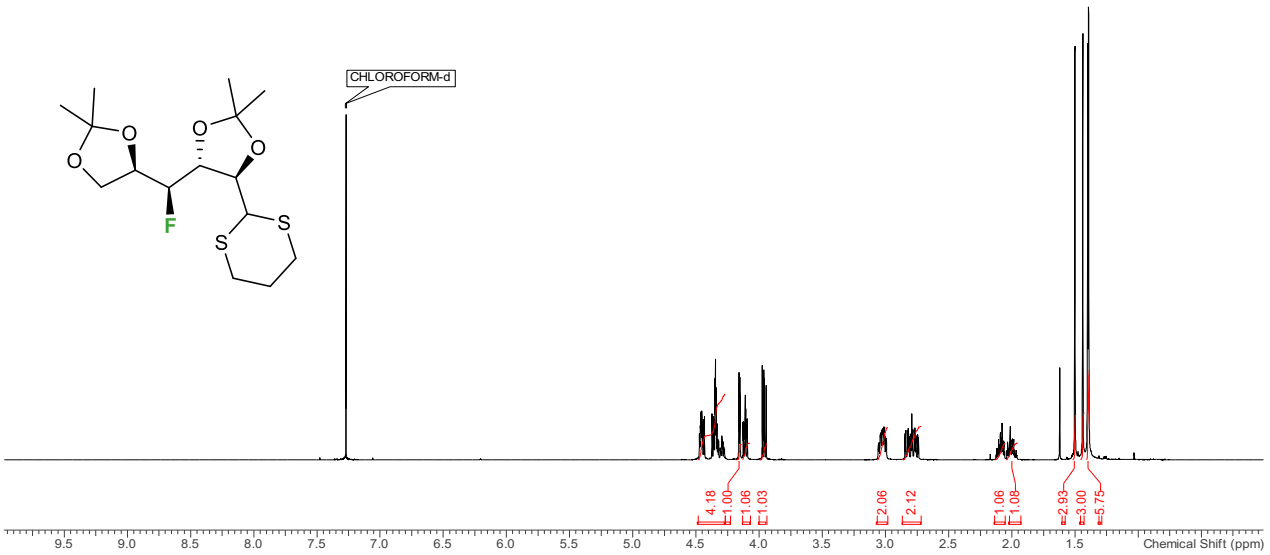


1.4.1.8 ^{1}H-^{13}C HMBC (500 MHz, CD_3OD)

1.4.2 2,3:5,6-Di-*O*-isopropylidene-4-deoxy-4-fluoro-D-galactose 1,3-propylidene dithioacetal SI2

1.4.2.1 ^{1}H NMR (500 MHz, CDCl3)

my2519njwjbv1.001.001.1r.esp



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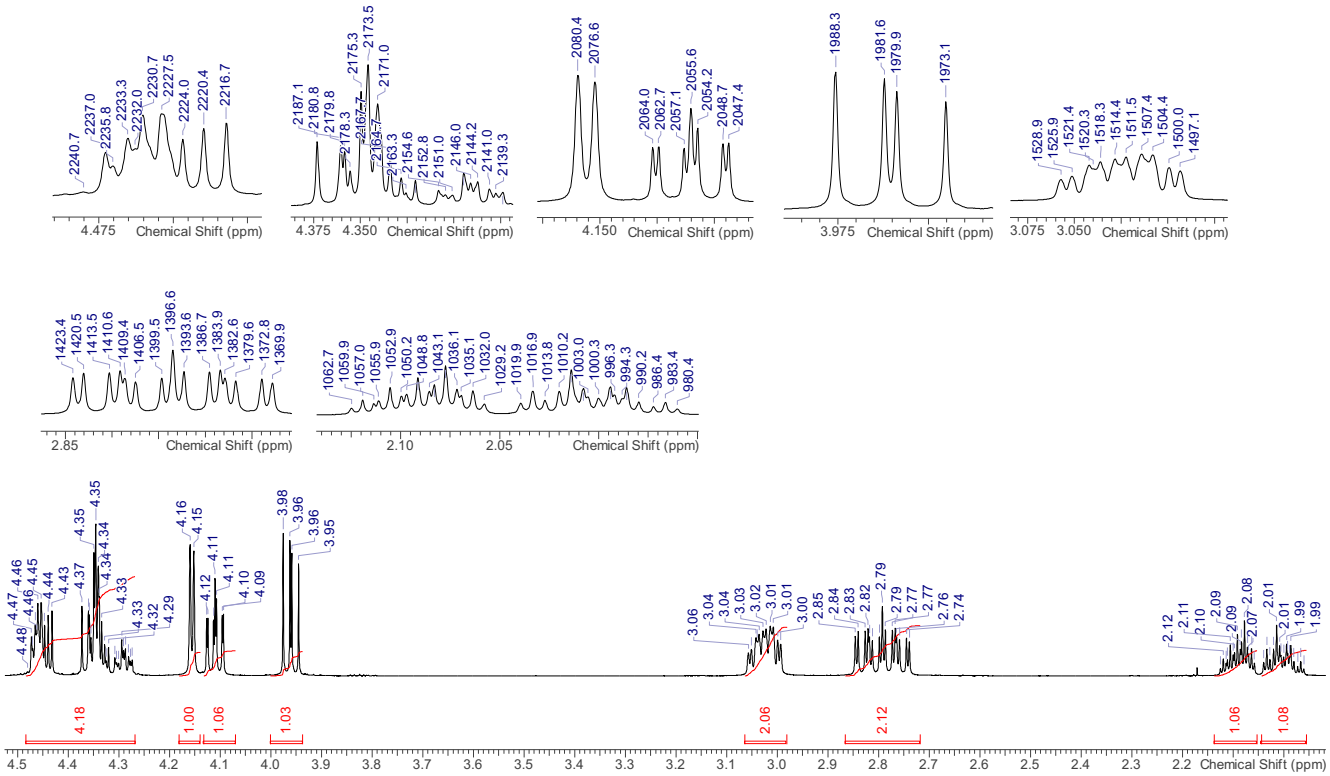
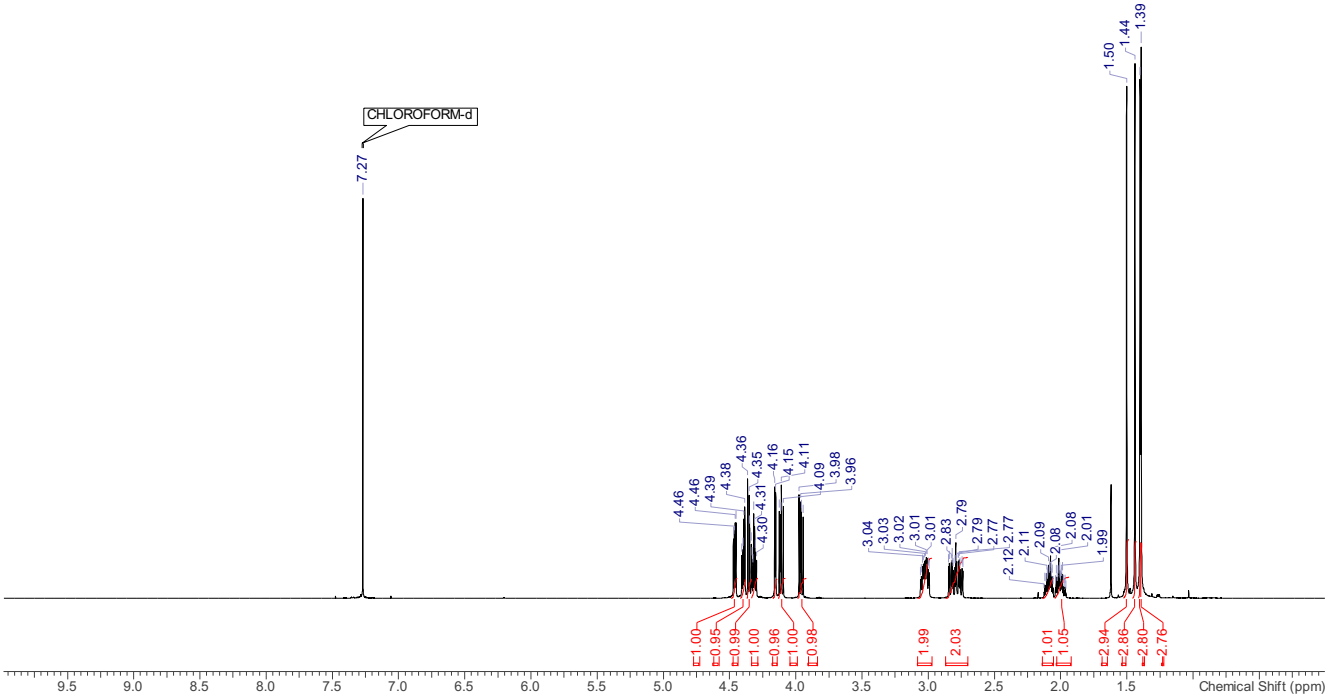


1.4.2.2 $^{1}H\{^{19}F\}$ NMR (500 MHz, $CDCl_3$)

my2519njwjbv1.004.001.1r



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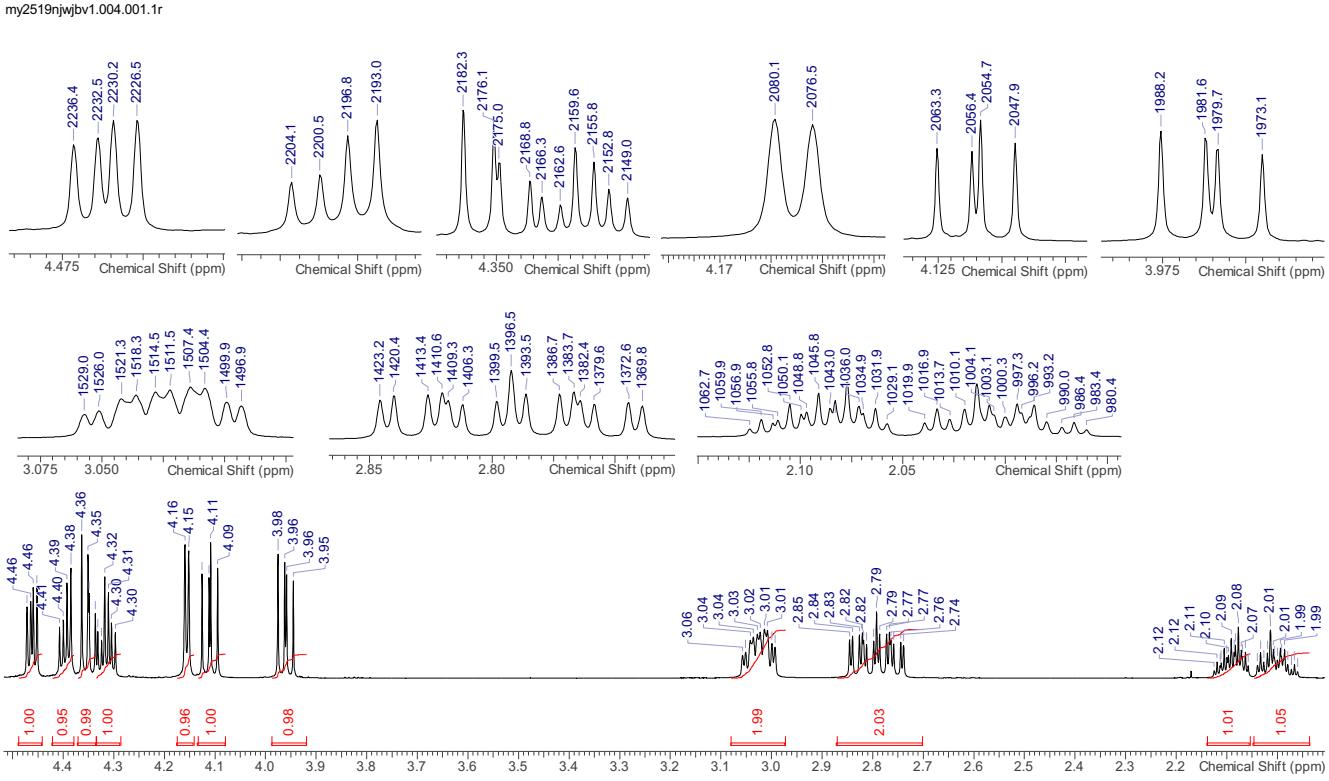
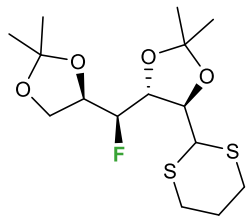
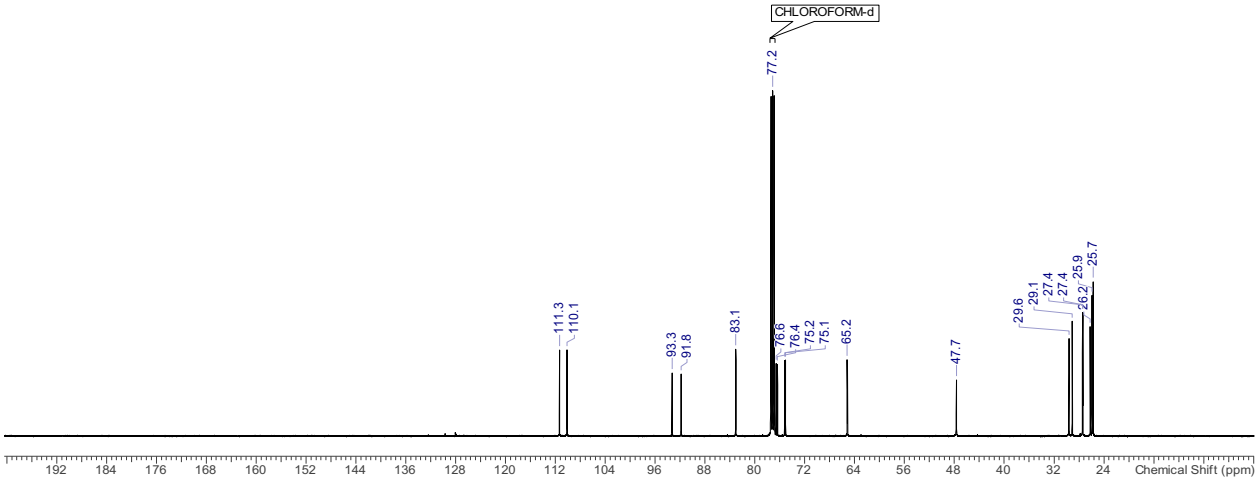
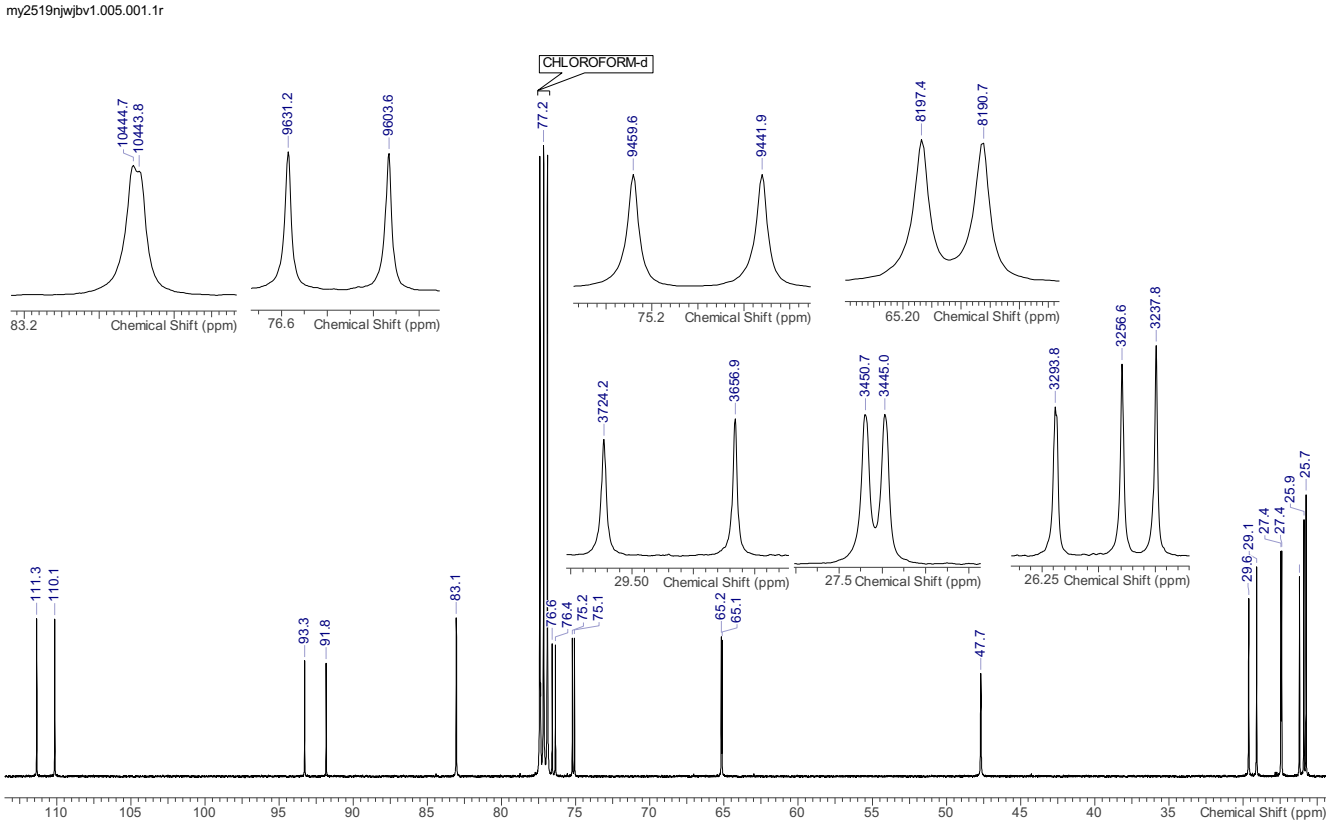


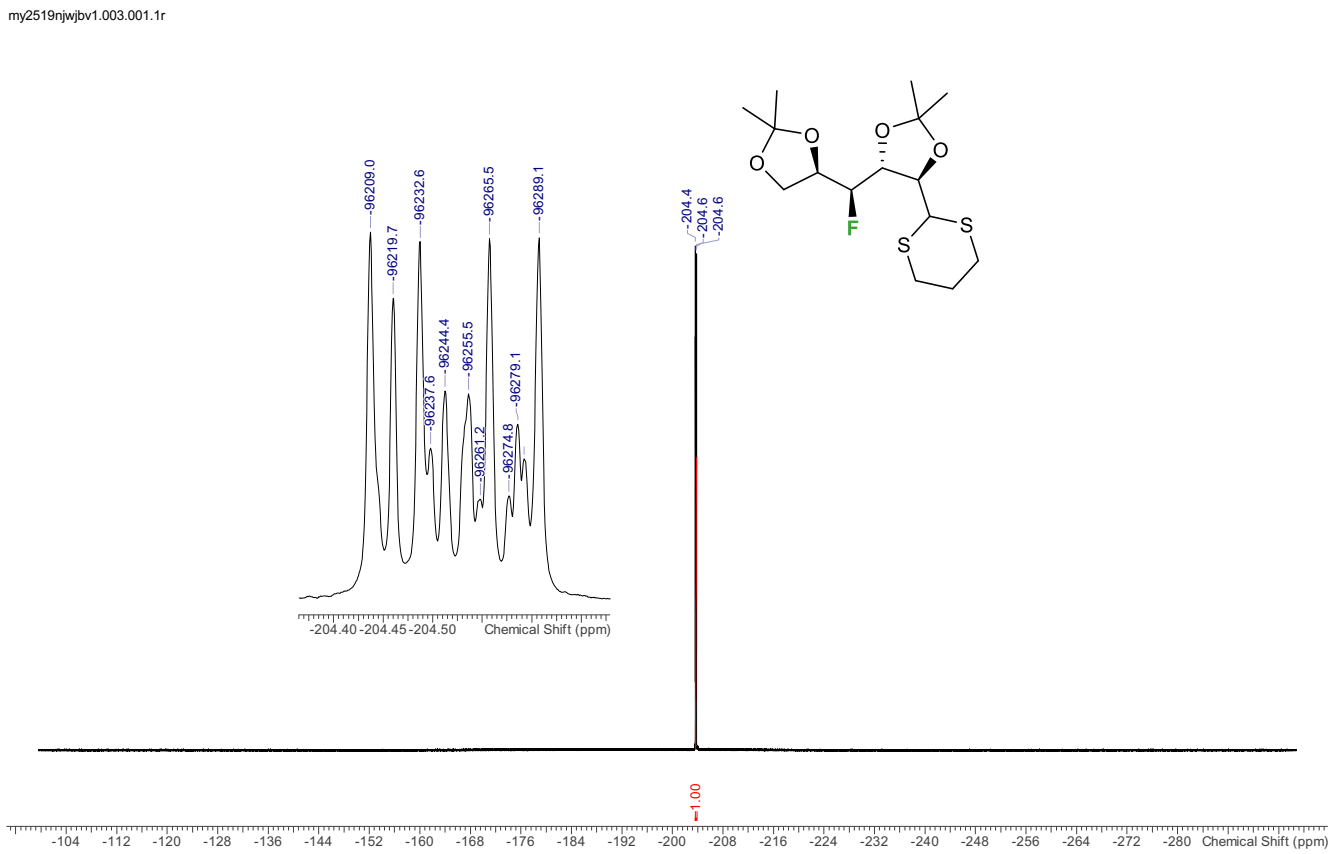
1.4.2.3 ^{13}C NMR (126 MHz, $CDCl_3$)



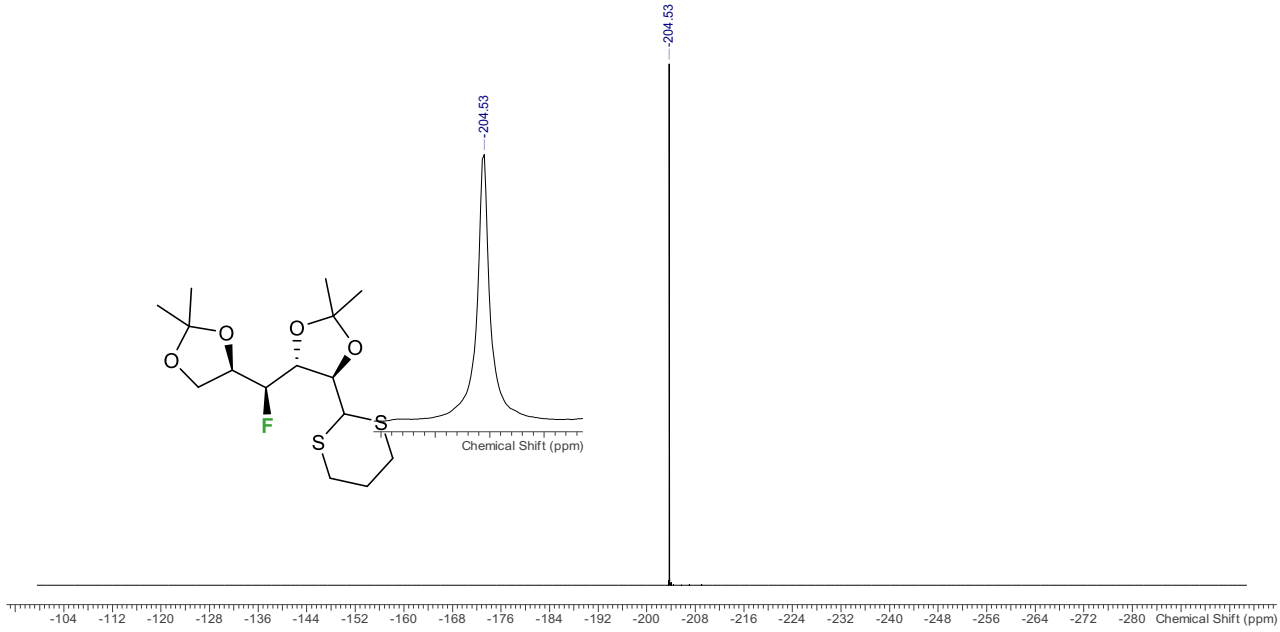




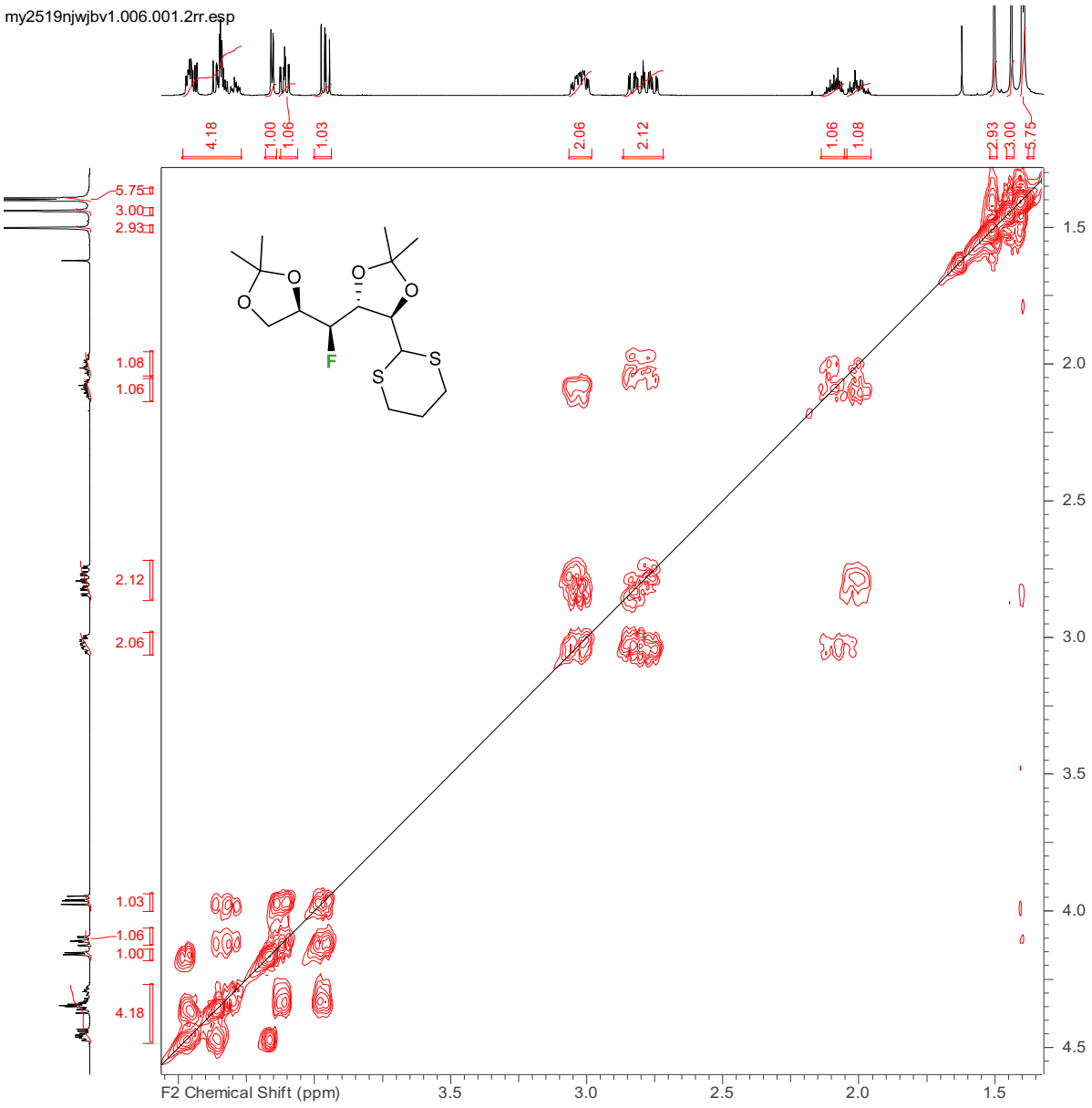
1.4.2.4 ^{19}F NMR (471 MHz, $CDCl_3$)



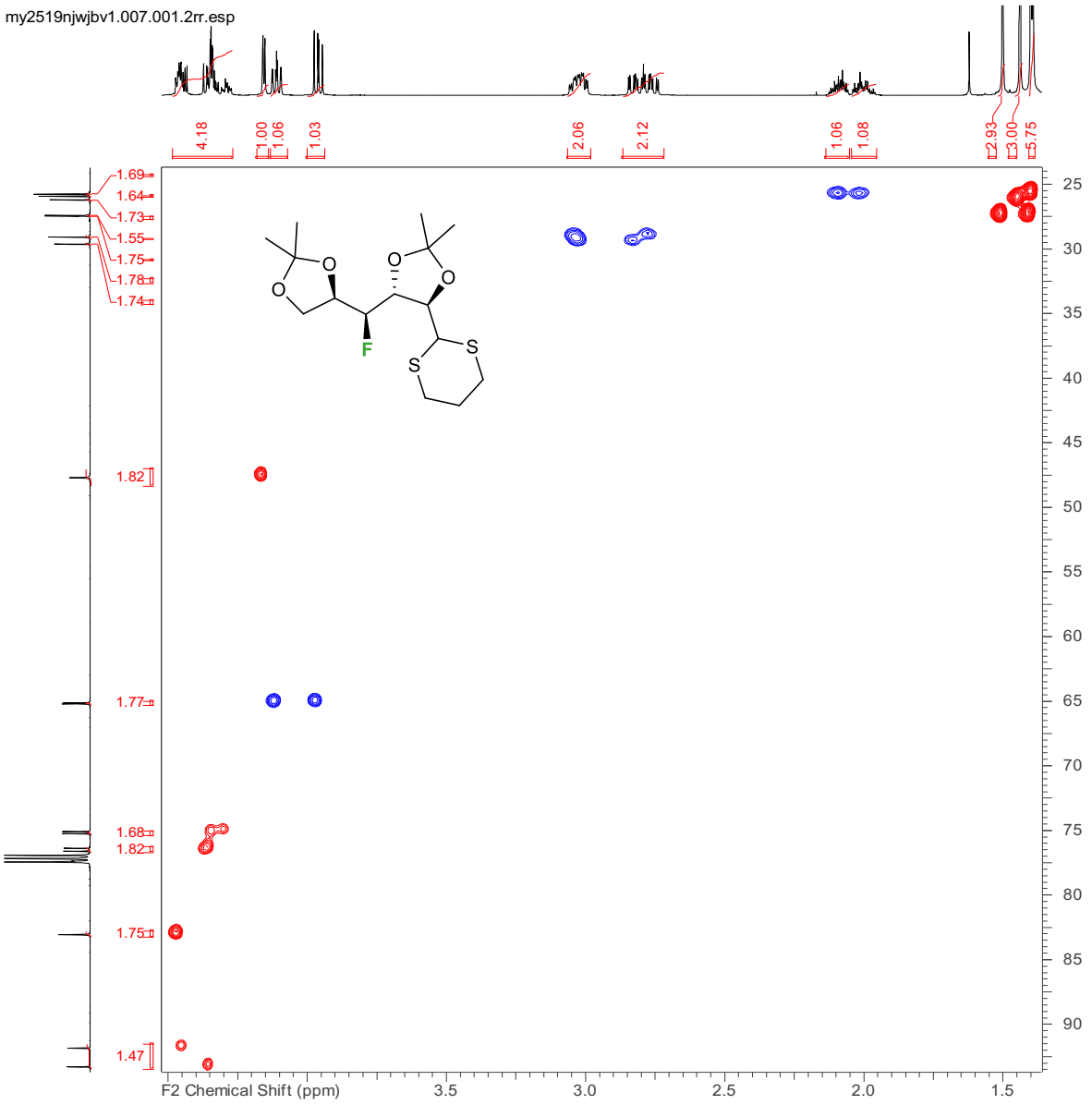
1.4.2.5 $^{19}F\{^{1}H\}$ NMR (471 MHz, $CDCl_3$)



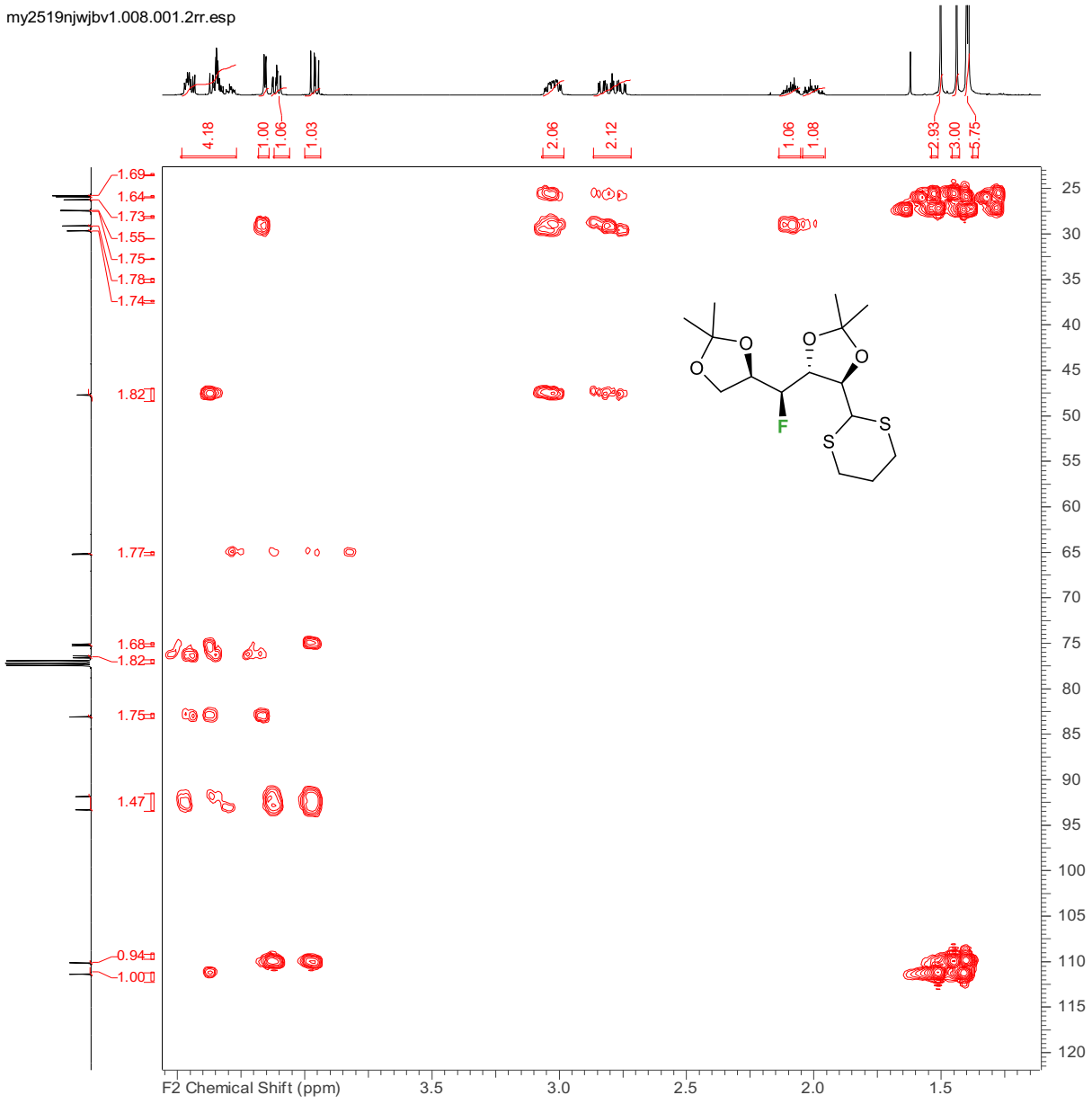
1.4.2.6 ^{1}H-^{1}H COSY (500 MHz, CDCl3)



1.4.2.7 ^{1}H-^{13}C HSQC (500 MHz, $CDCl_3$)

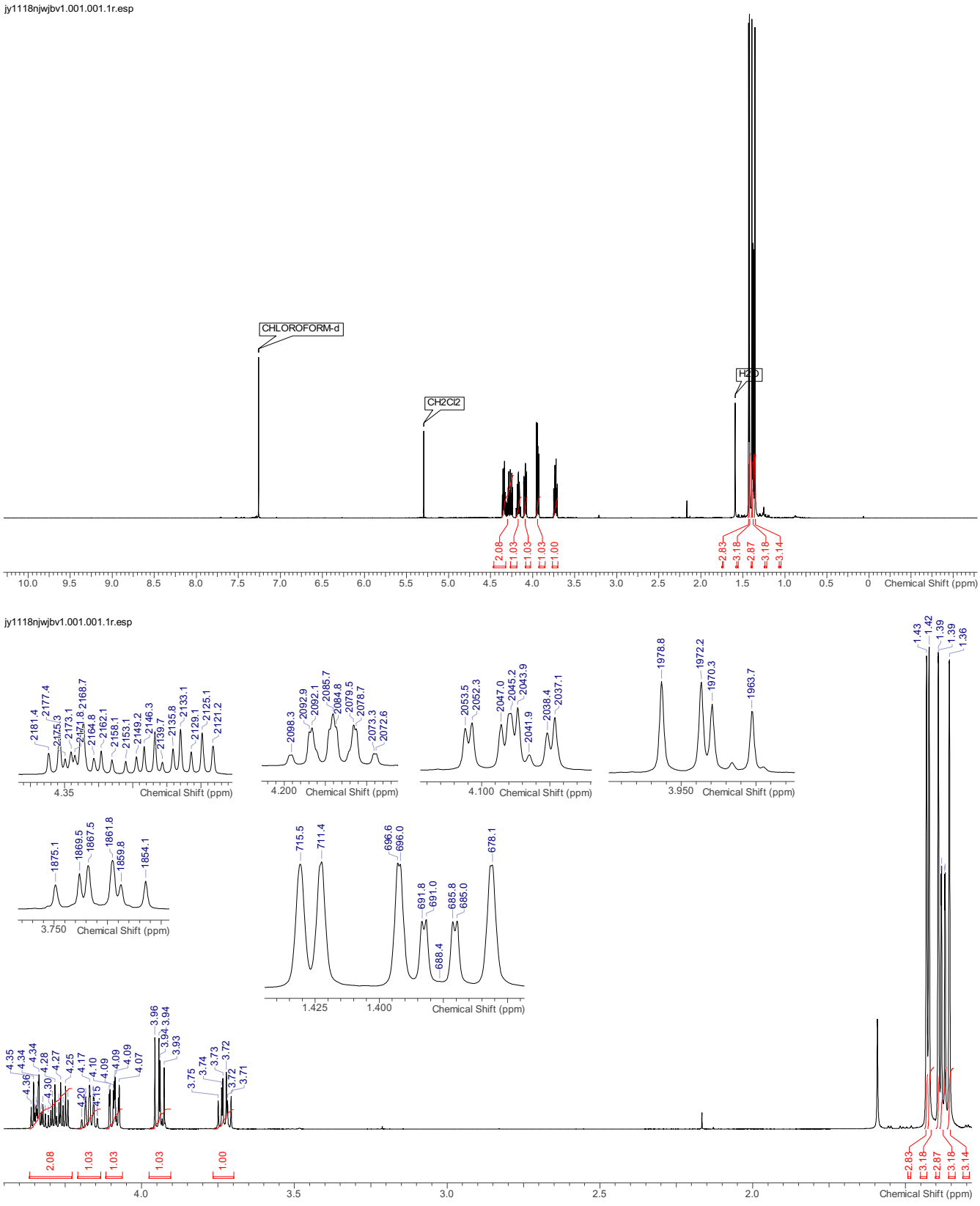


1.4.2.8 ^{1}H-^{13}C HMBC (500 MHz, CDCl3)

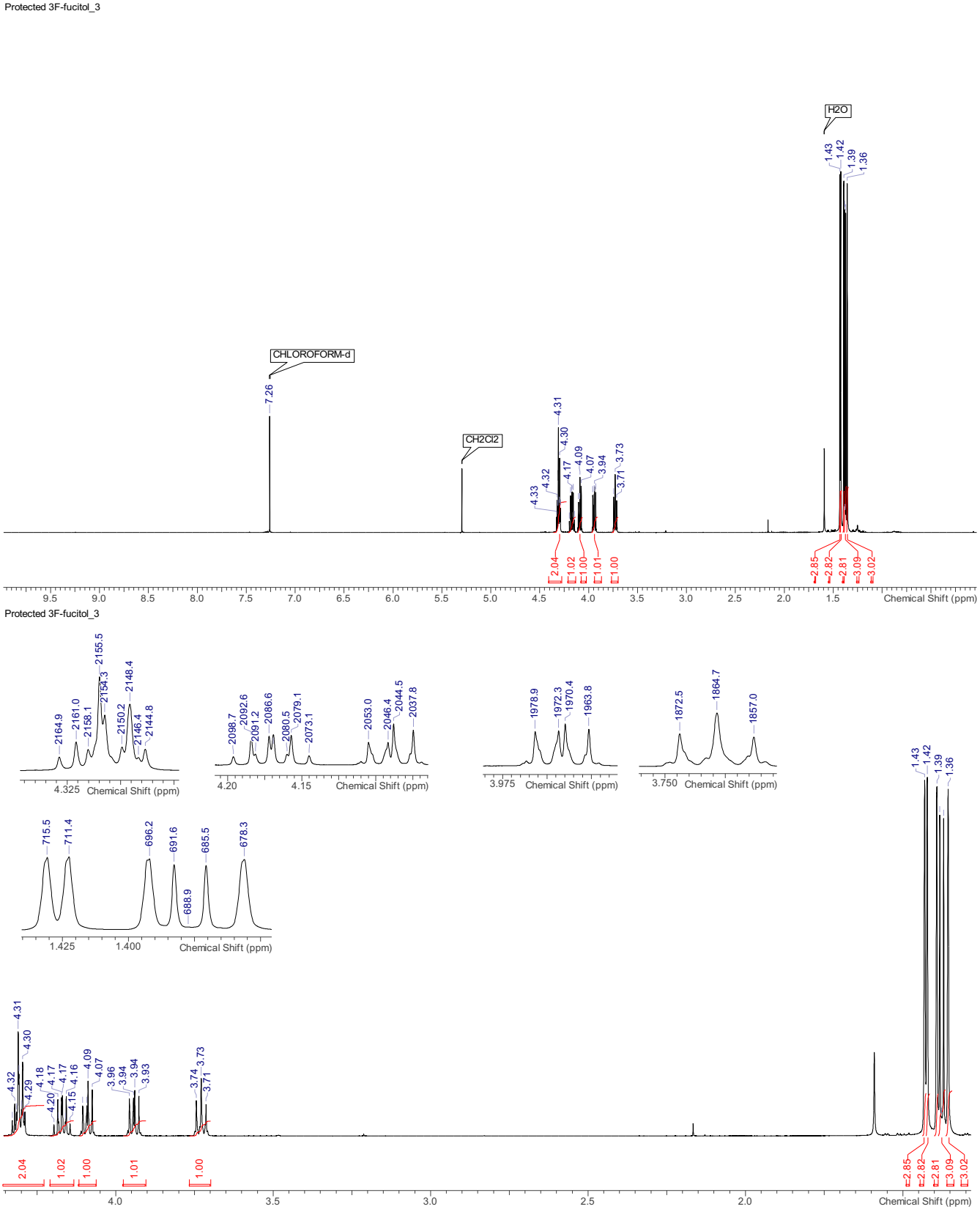


1.4.3 1,2:4,5-Di-*O*-isopropylidene-3-deoxy-3-fluoro-L-fucitol 17

1.4.3.1 ^{1}H NMR (500 MHz, $CDCl_3$)

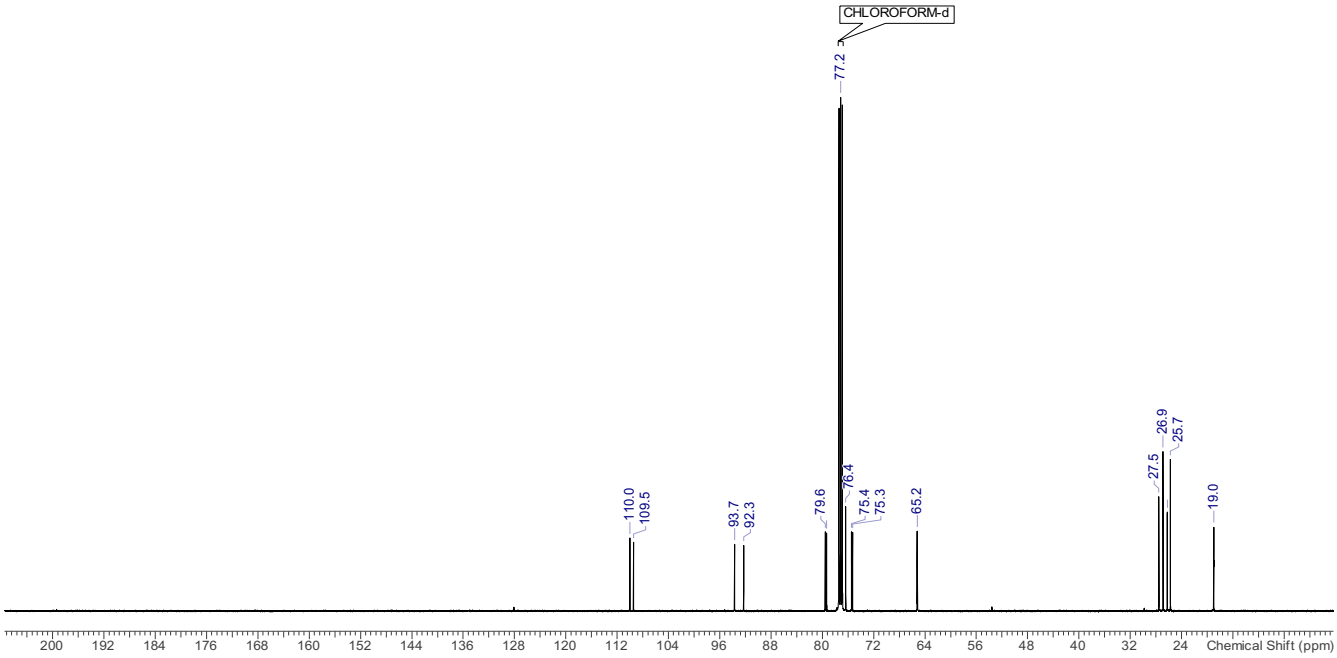


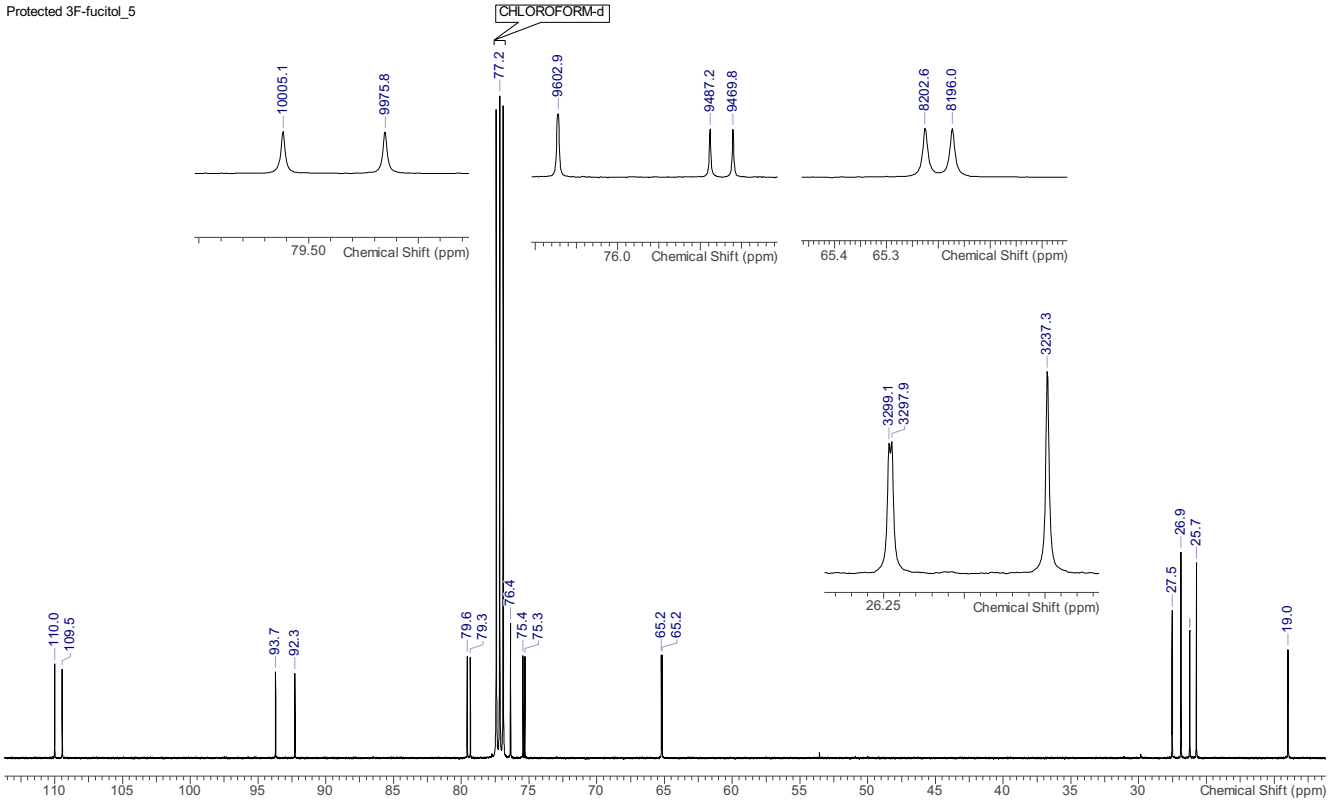
1.4.3.2 $^{1}H\{^{19}F\}$ NMR (500 MHz, $CDCl_3$)



1.4.3.3 ^{13}C NMR (126 MHz, $CDCl_3$)

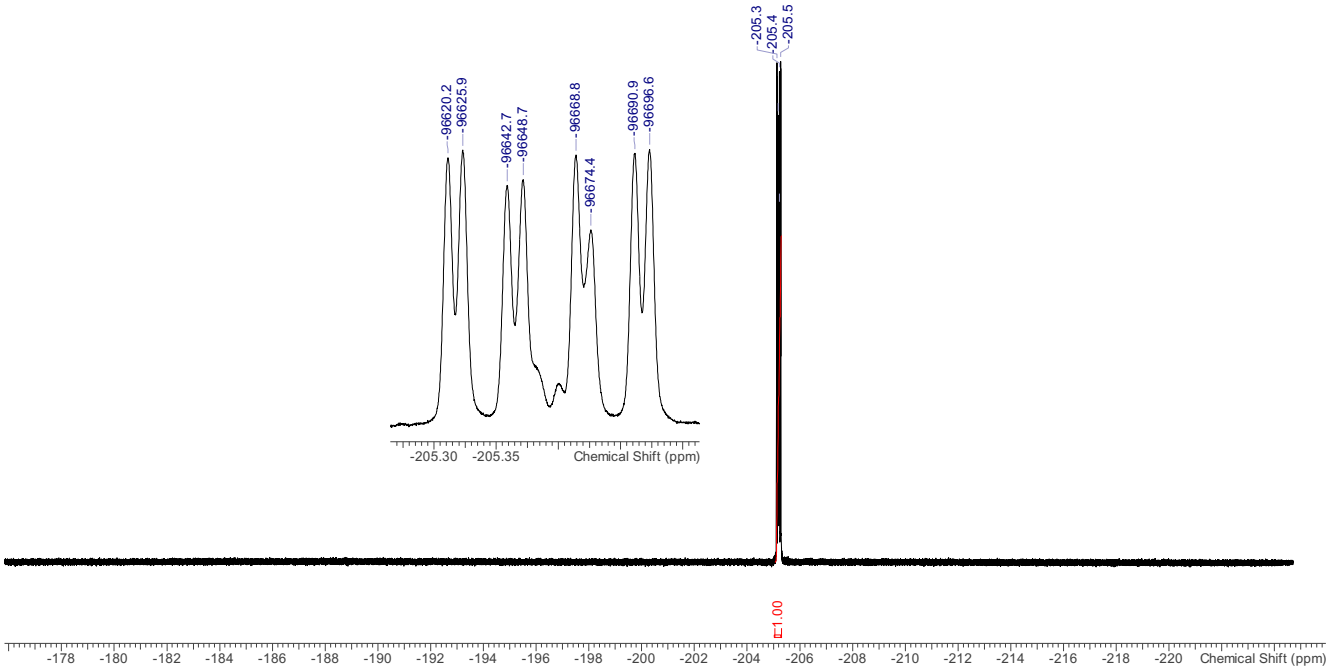
Protected 3F-fucitol_5





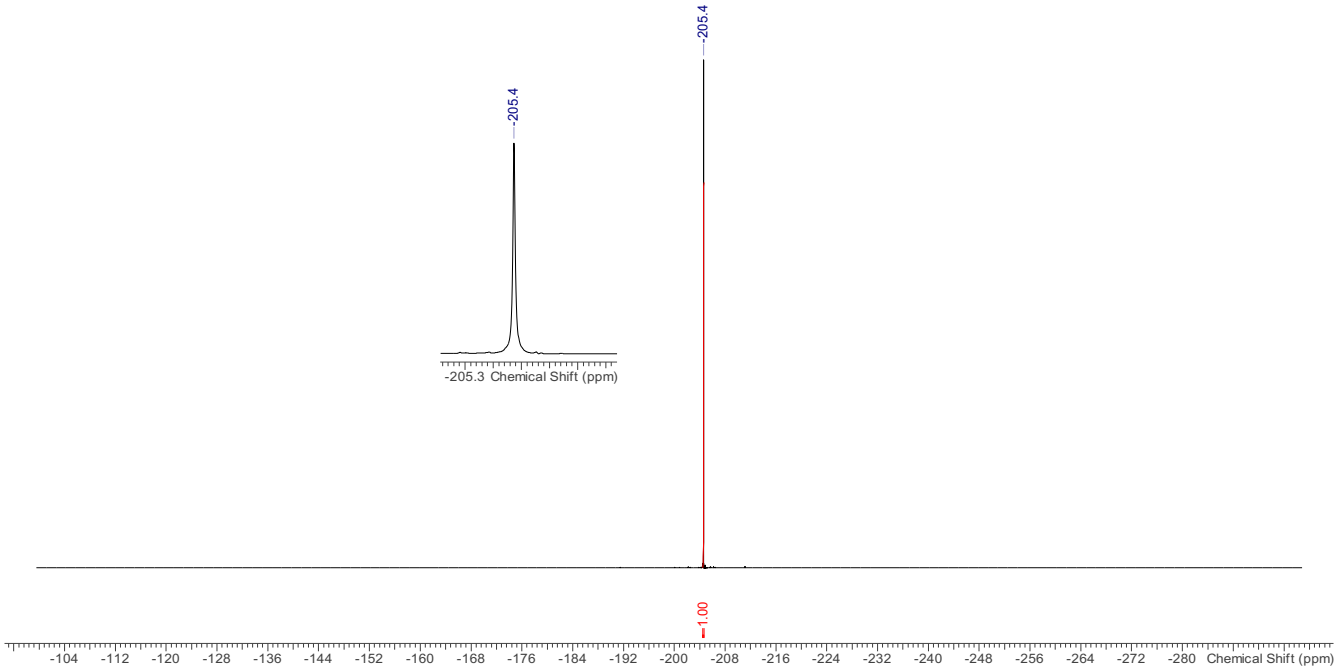
1.4.3.4 ^{19}F NMR (471 MHz, $CDCl_3$)

Protected 3F-fucitol_2

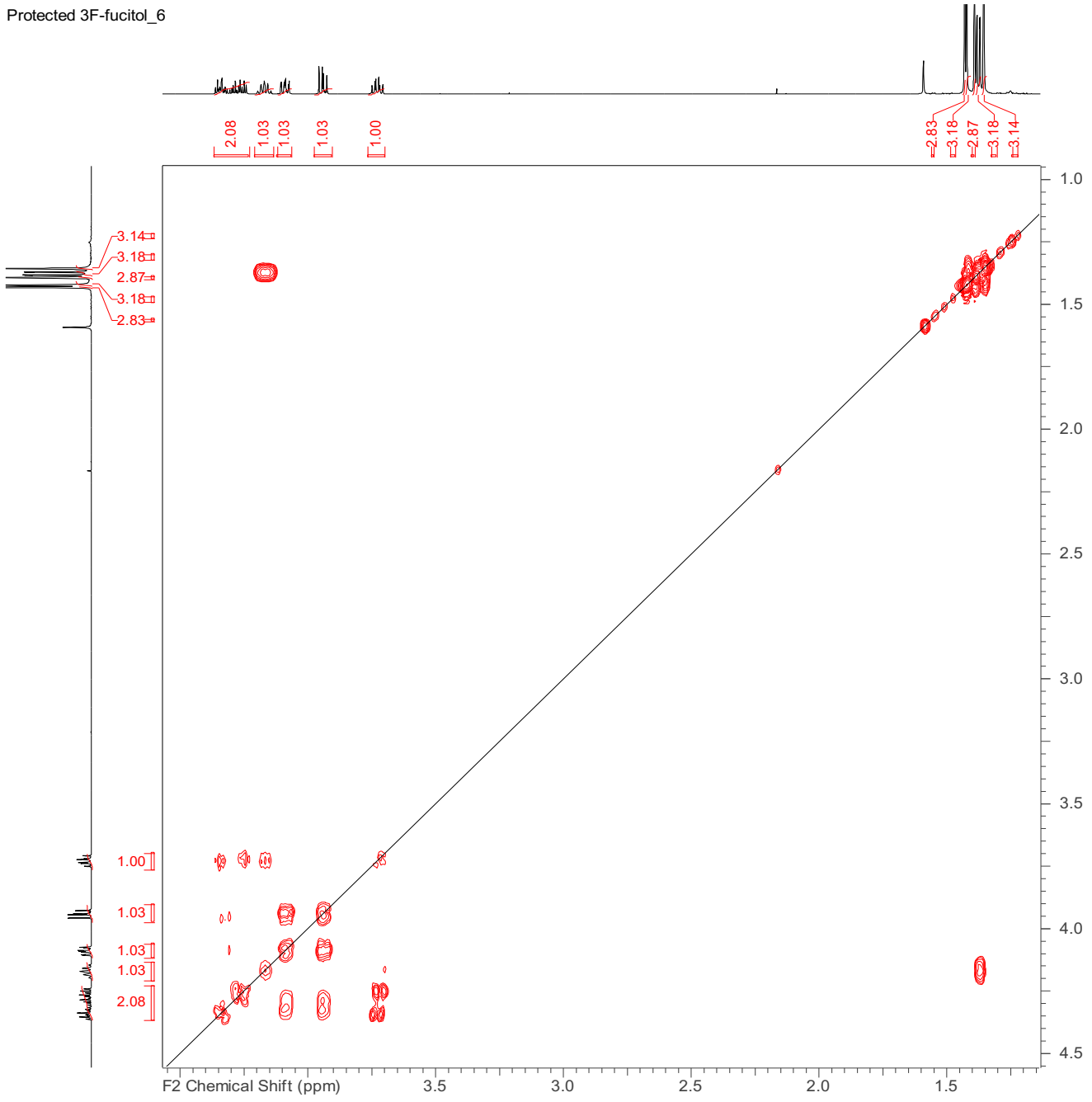


1.4.3.5 $^{19}F\{^{1}H\}$ NMR (471 MHz, $CDCl_3$)

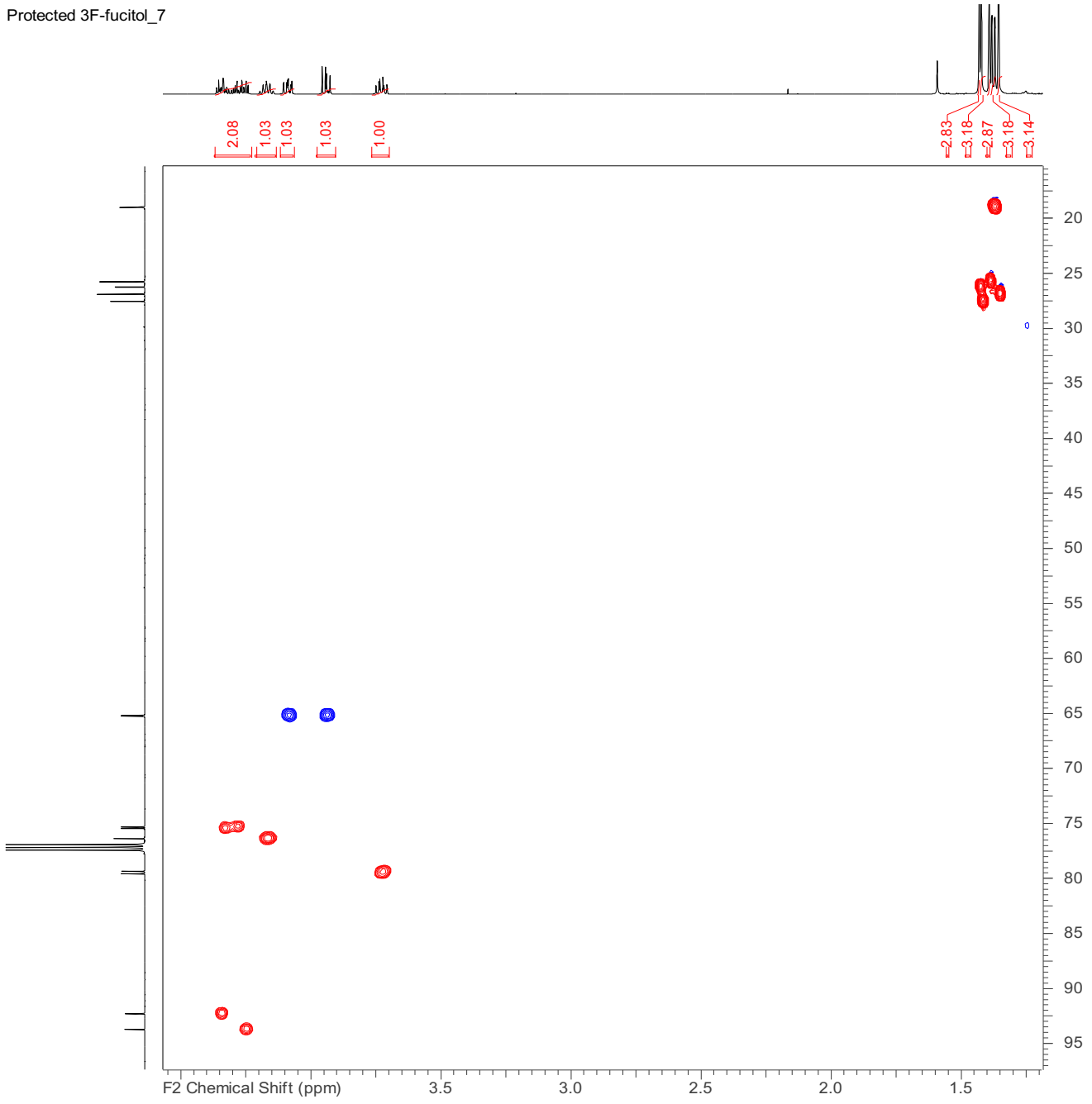
Protected 3F-fucitol



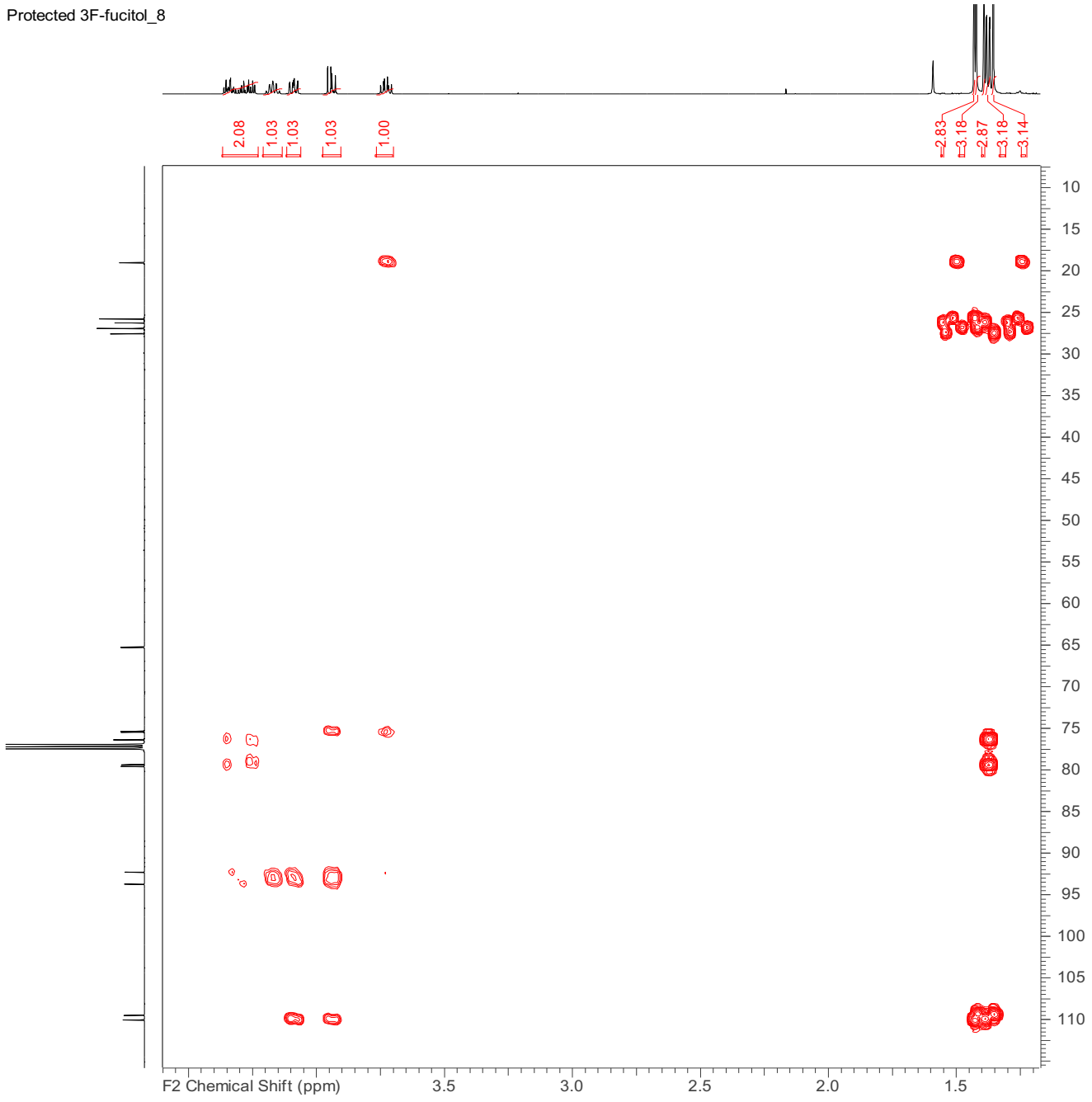
1.4.3.6 ^{1}H-^{1}H COSY (500 MHz, $CDCl_3$)



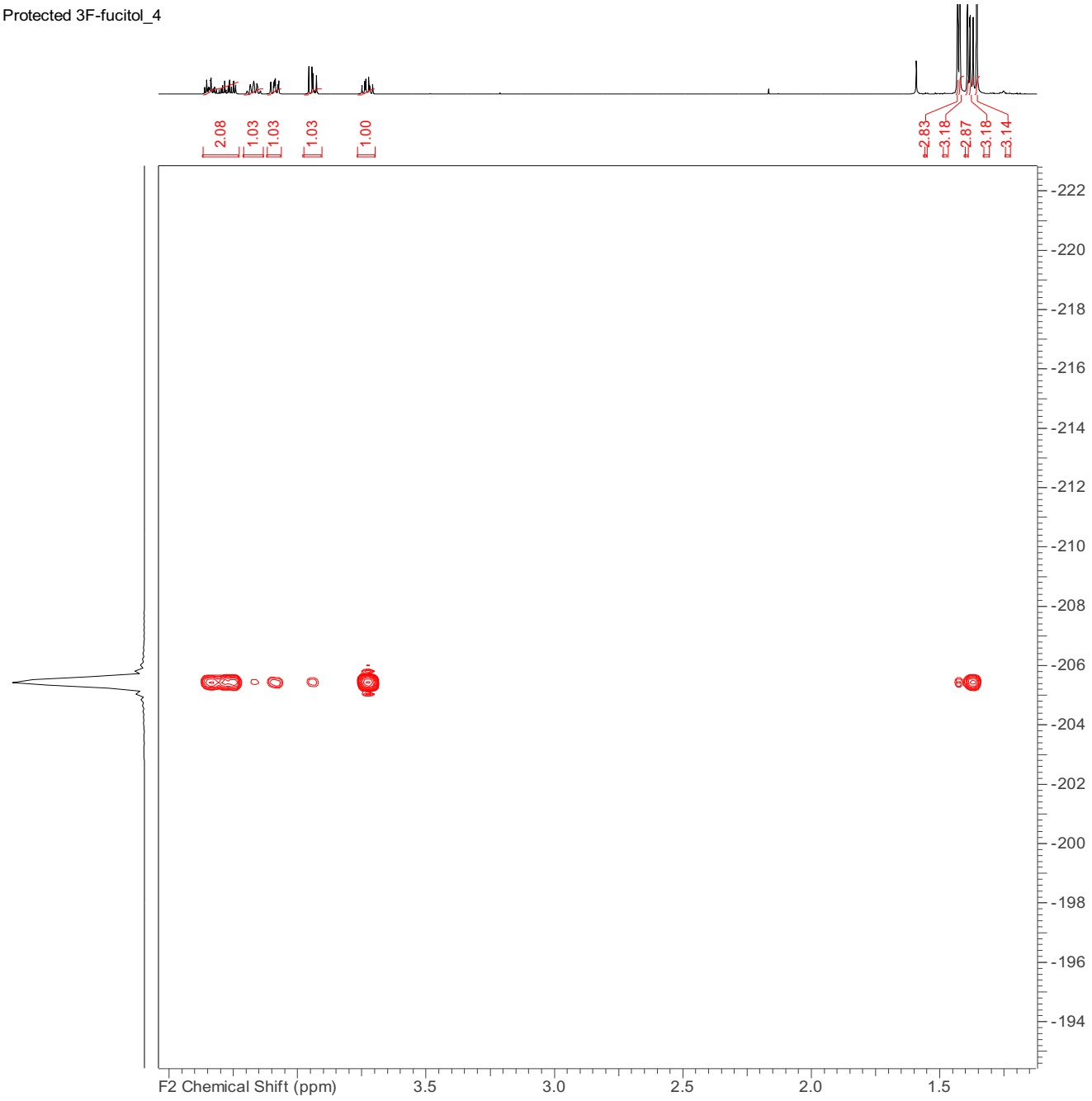
1.4.3.7 ^{1}H-^{13}C HSQC (500 MHz, $CDCl_3$)



1.4.3.8 ^{1}H-^{13}C HMBC (500 MHz, $CDCl_3$)

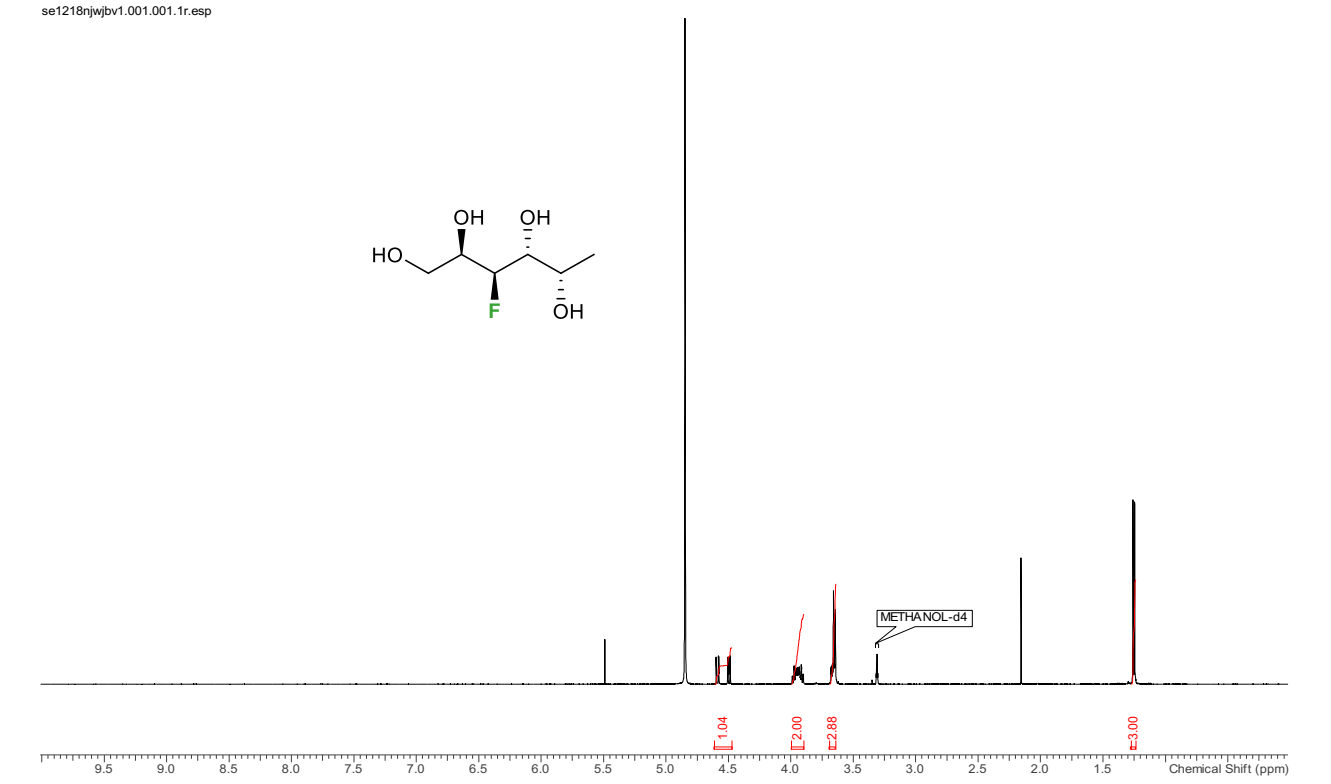


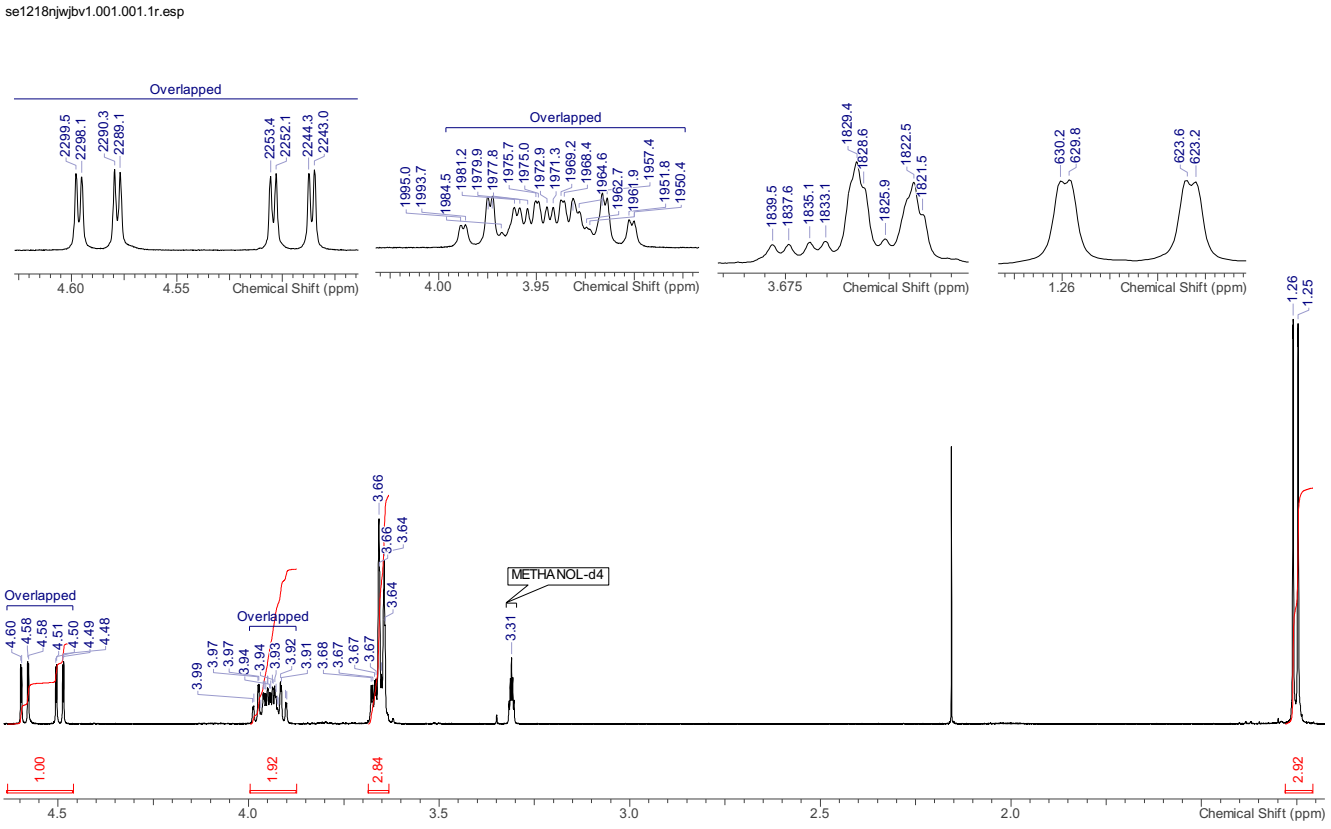
1.4.3.9 ^{1}H-^{19}F HMBC (500 MHz, $CDCl_3$)



1.4.4 3-Deoxy-3-fluoro-L-fucitol 16

1.4.4.1 ^{1}H NMR (500 MHz, CD_3OD)





1.4.4.2 $^{1}H\{^{19}F\}$ NMR (500 MHz, CD_3OD)

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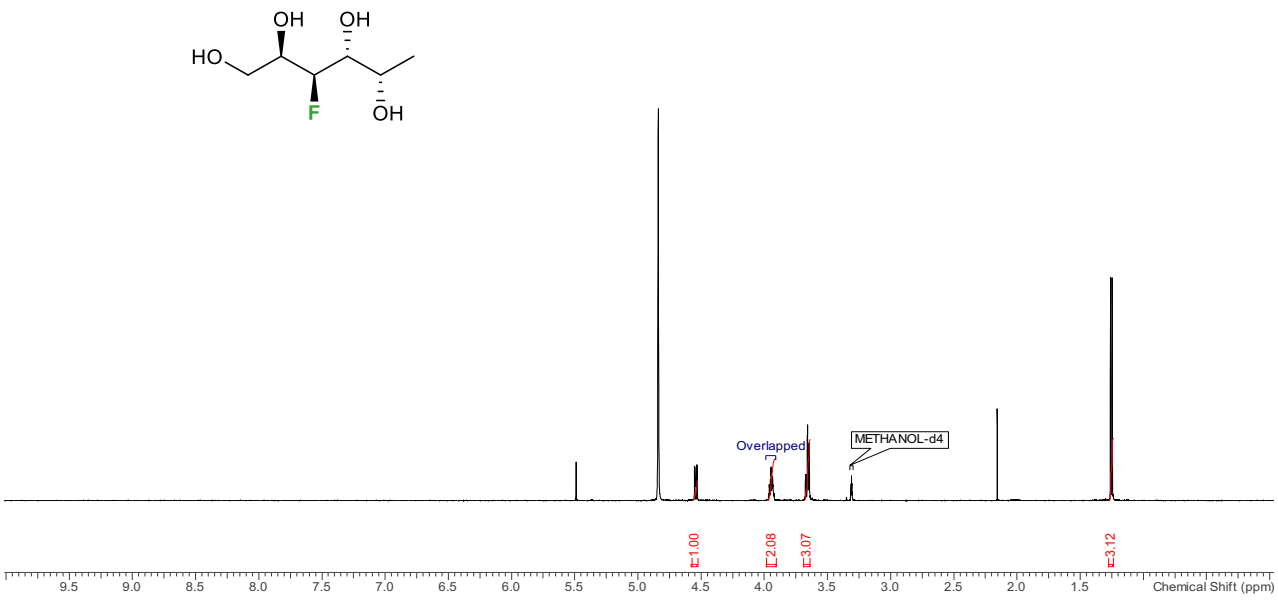

1.4.4.3 $^{1}H\{^{19}F\}$ NMR (500 MHz, CD_3OD)

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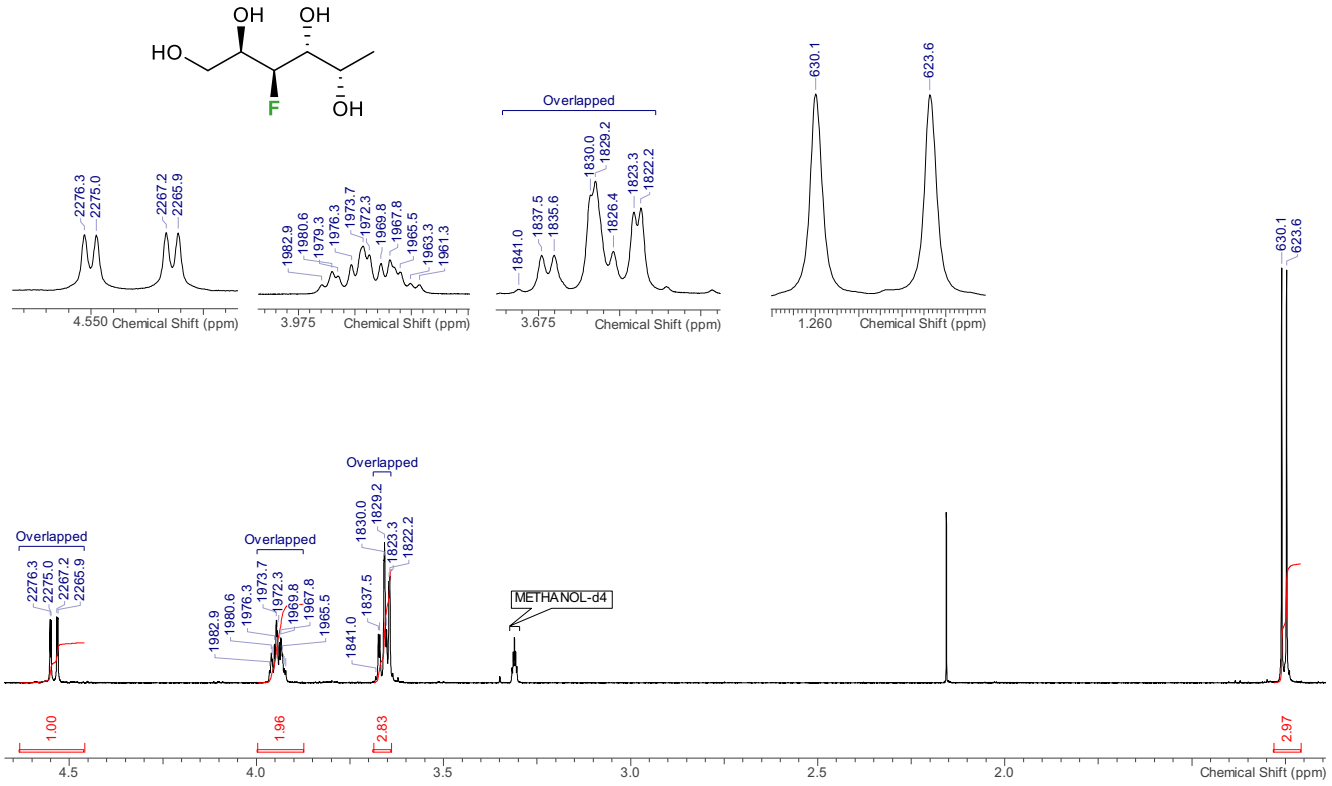
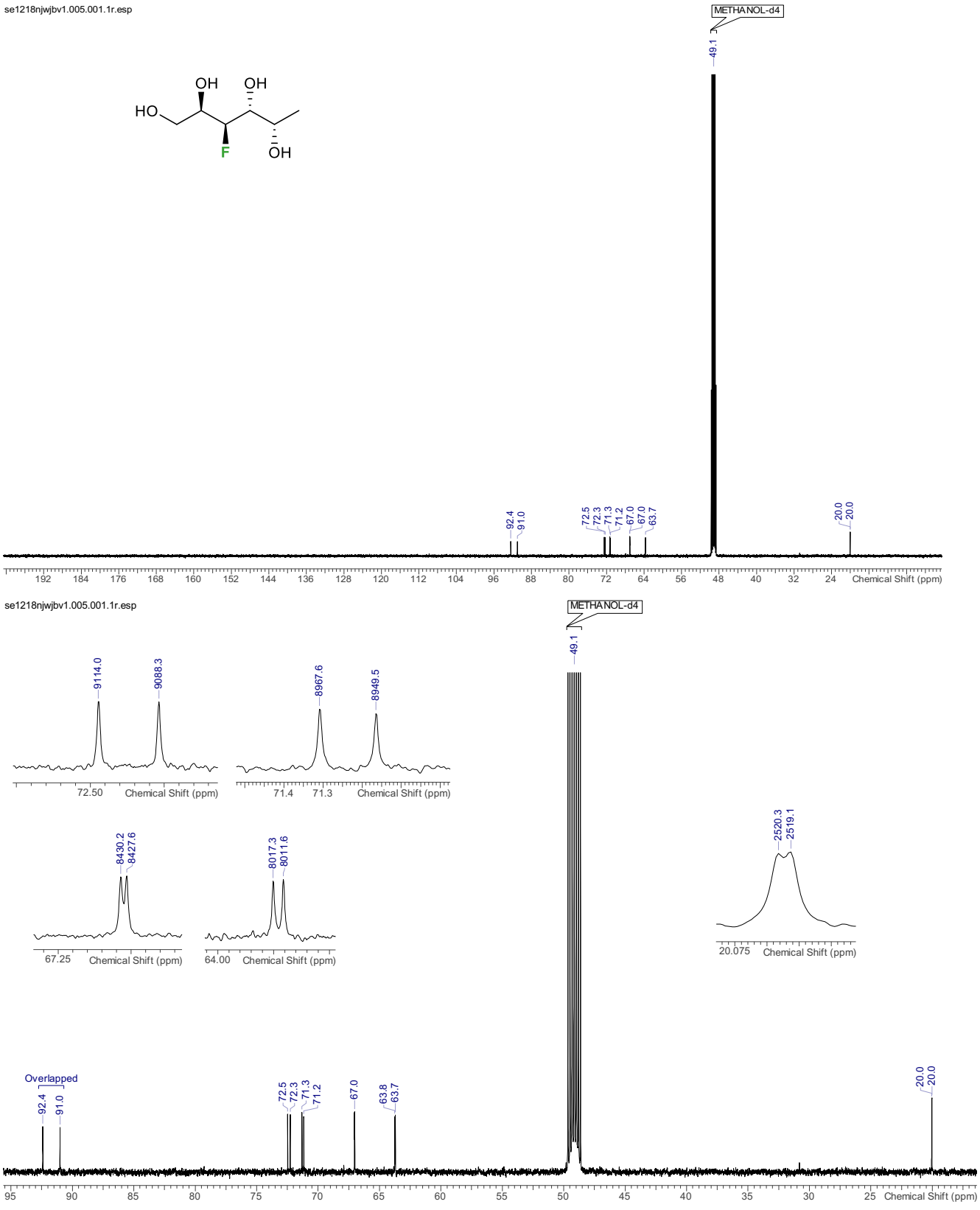
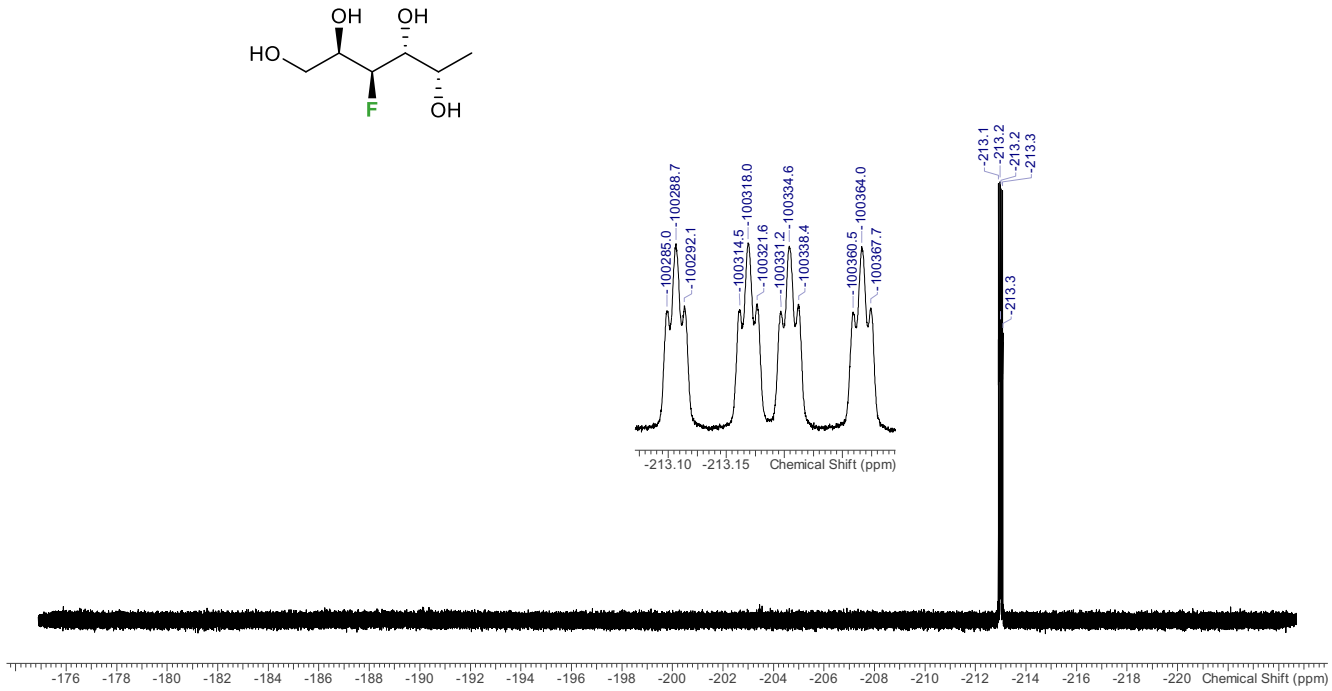


1.4.4.4 ^{13}C NMR (126 MHz, CD_3OD)

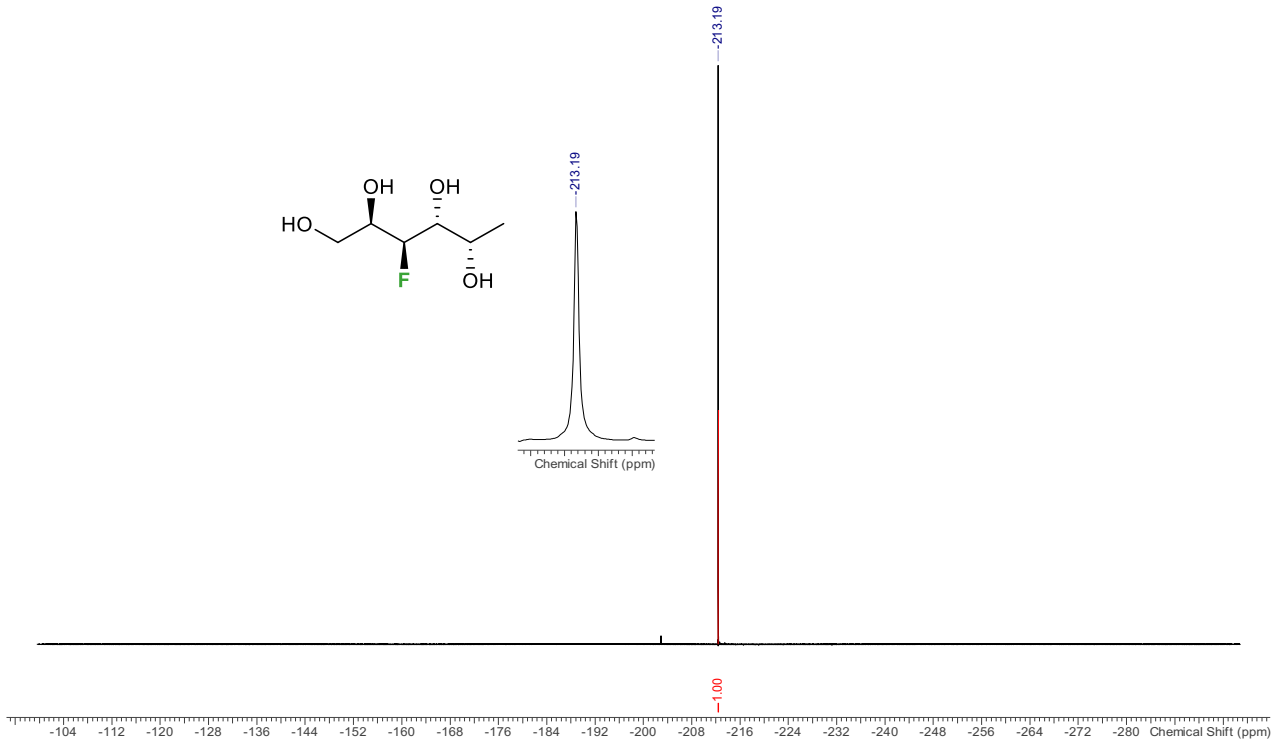


1.4.4.5 ^{19}F NMR (471 MHz, CD_3OD)

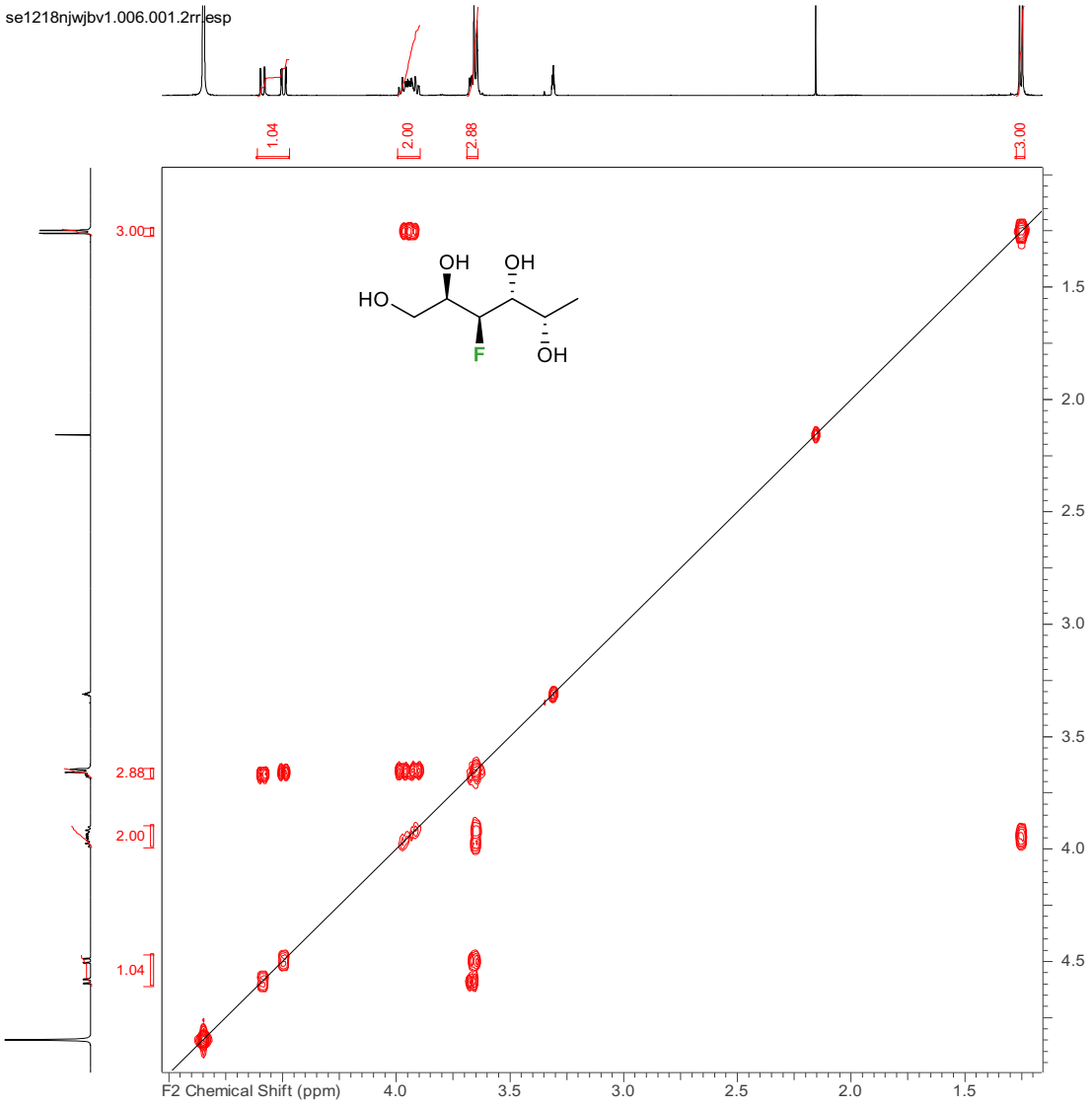
se1218njwjbv1.003.001.1r.esp



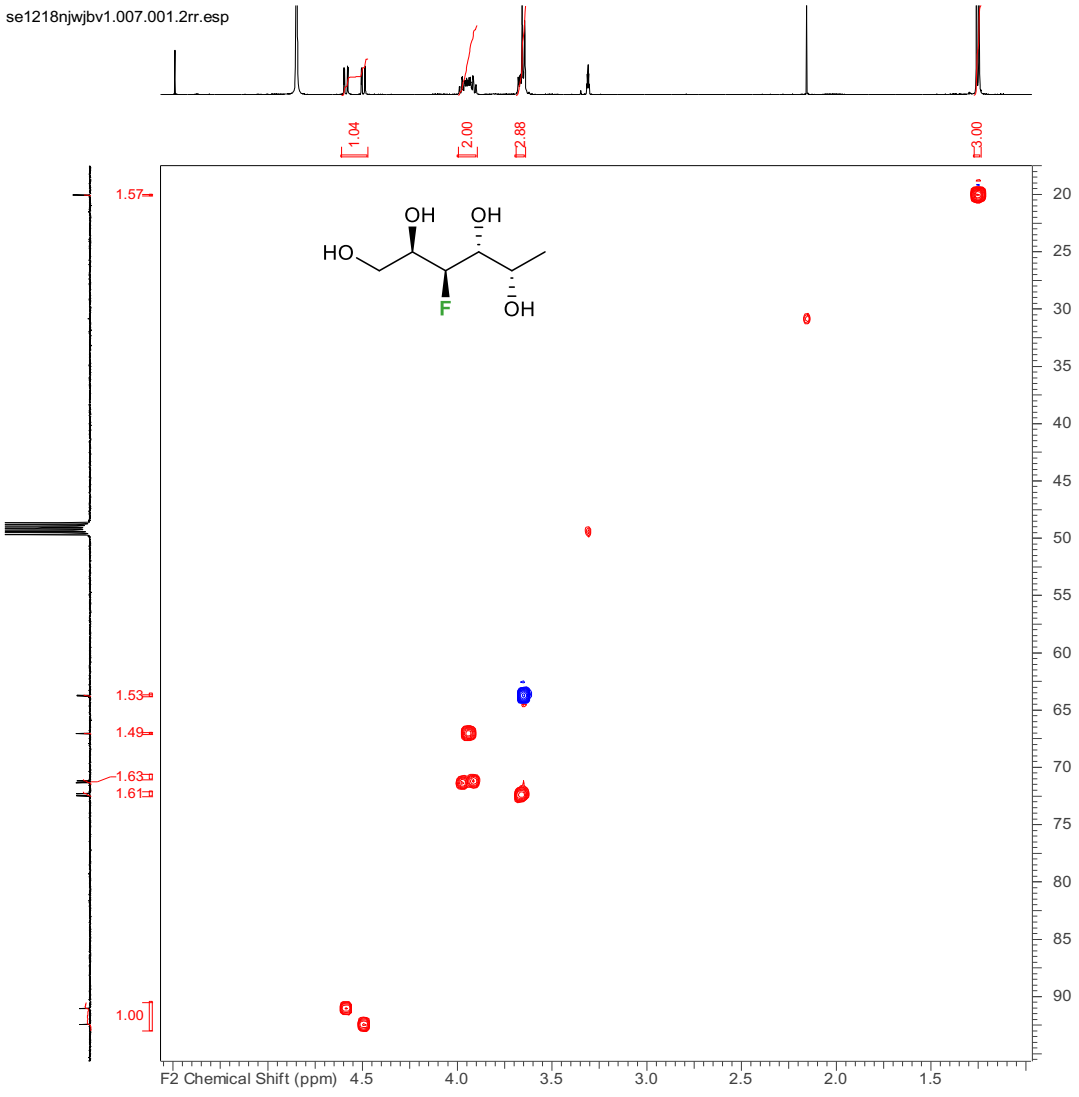
1.4.4.6 ^{19}F{^{1}H} NMR (471 MHz, CD_3OD)



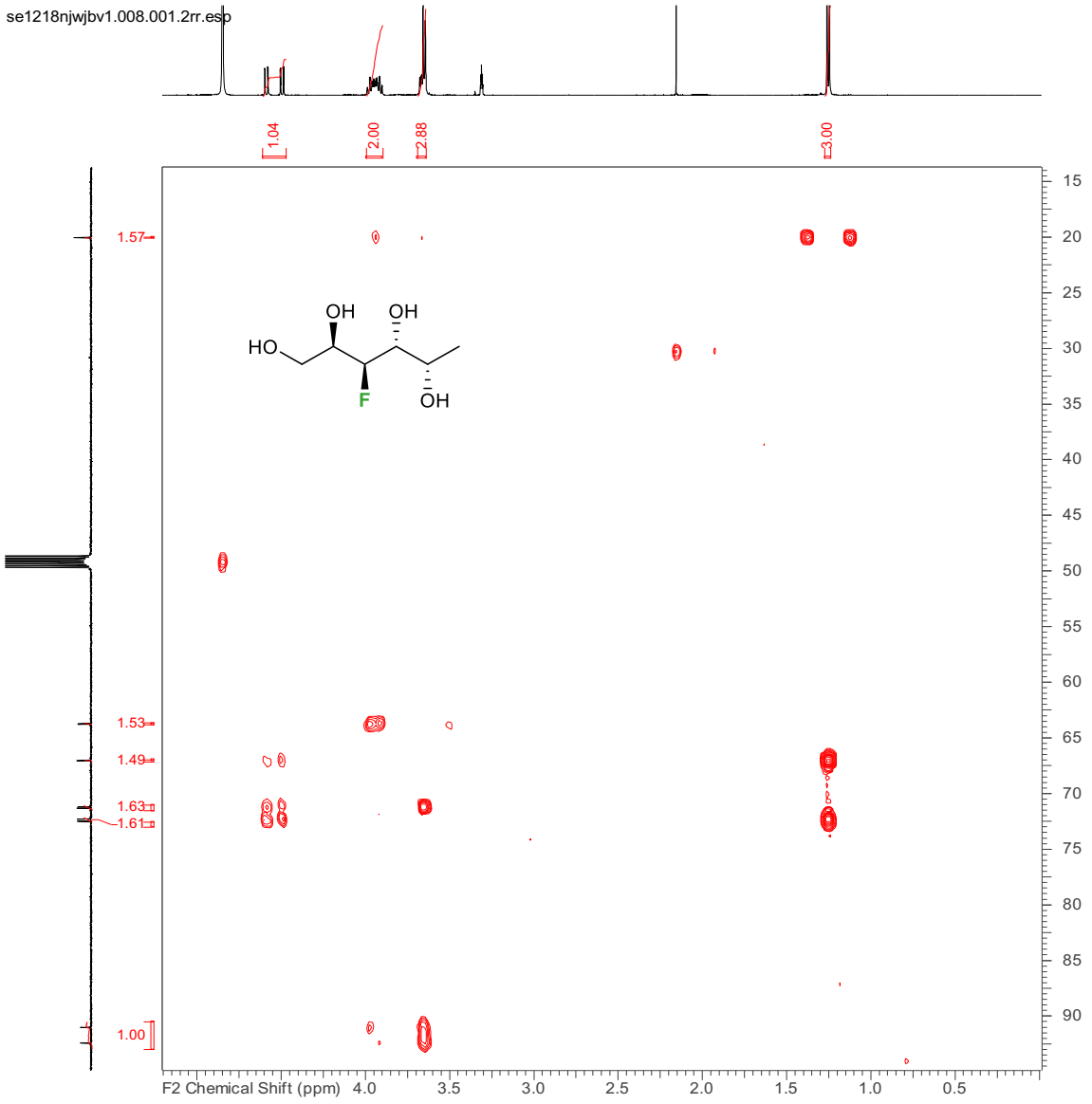
1.4.4.7 ^{1}H-^{1}H COSY (500 MHz, CD_3OD)



1.4.4.8 ^{1}H-^{13}C HSQC (500 MHz, CD_3OD)

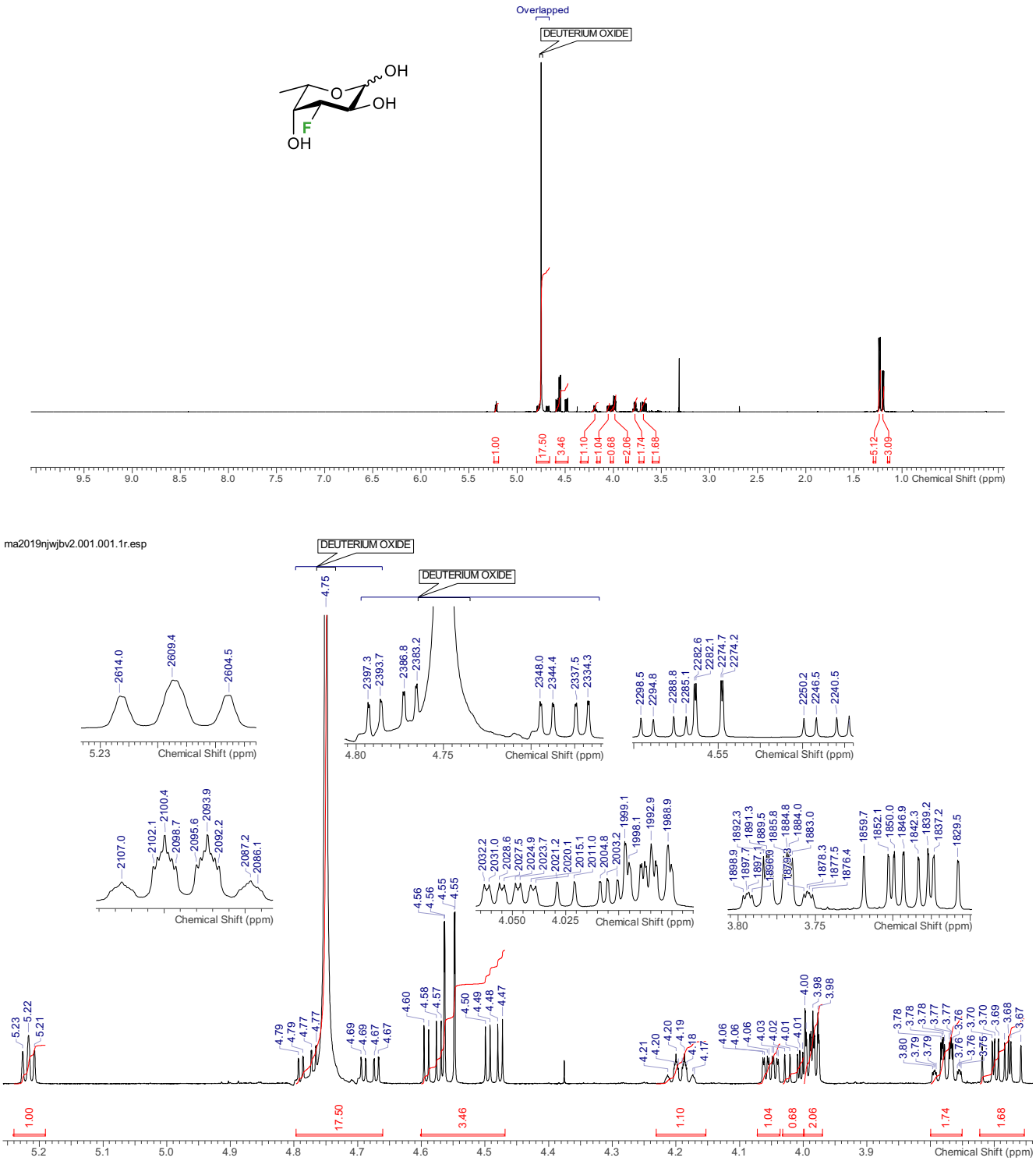


1.4.4.9 ^{1}H-^{13}C HMBC (500 MHz, CD_3OD)

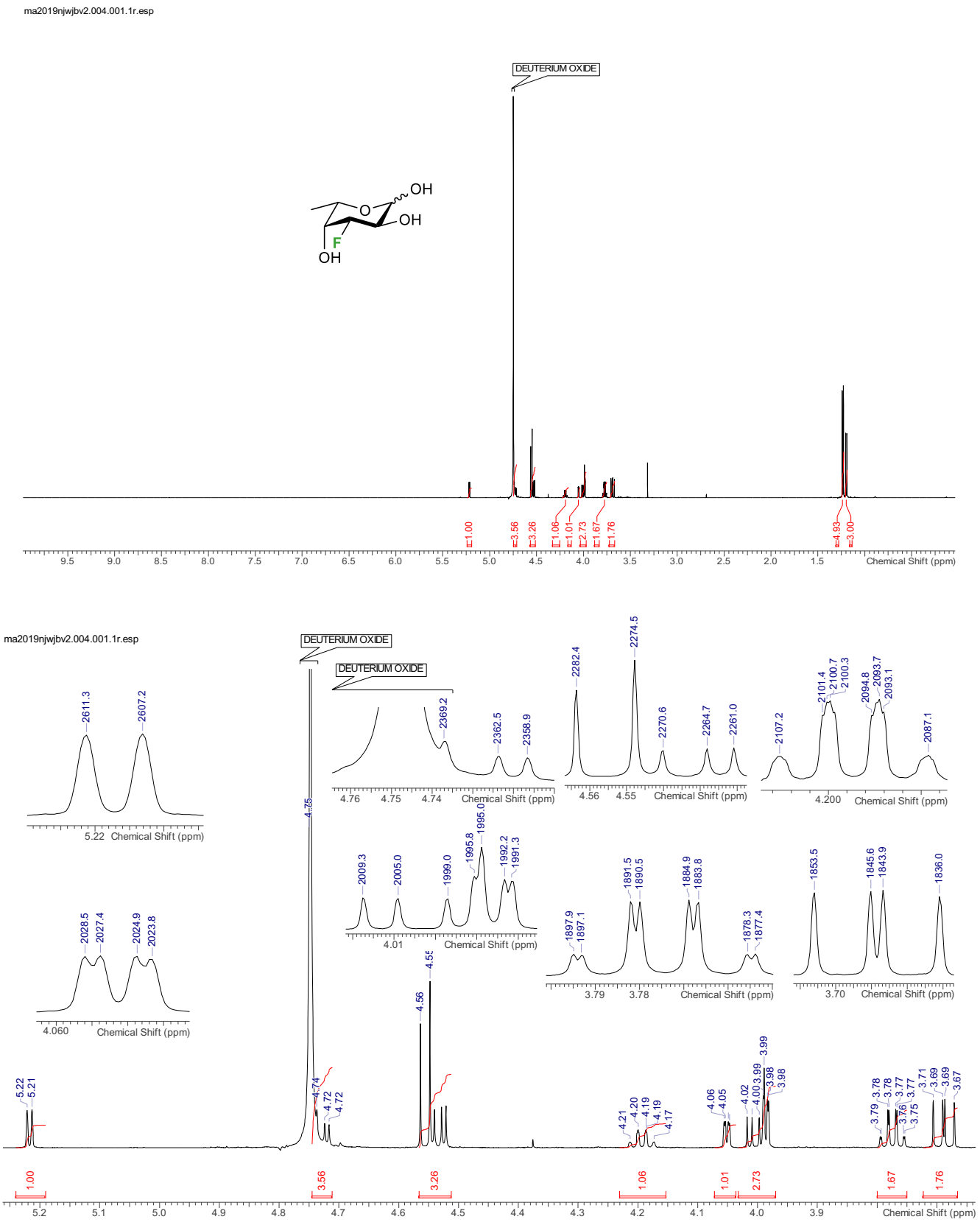


1.4.5 3-Deoxy-3-fluoro-L-fucose 3

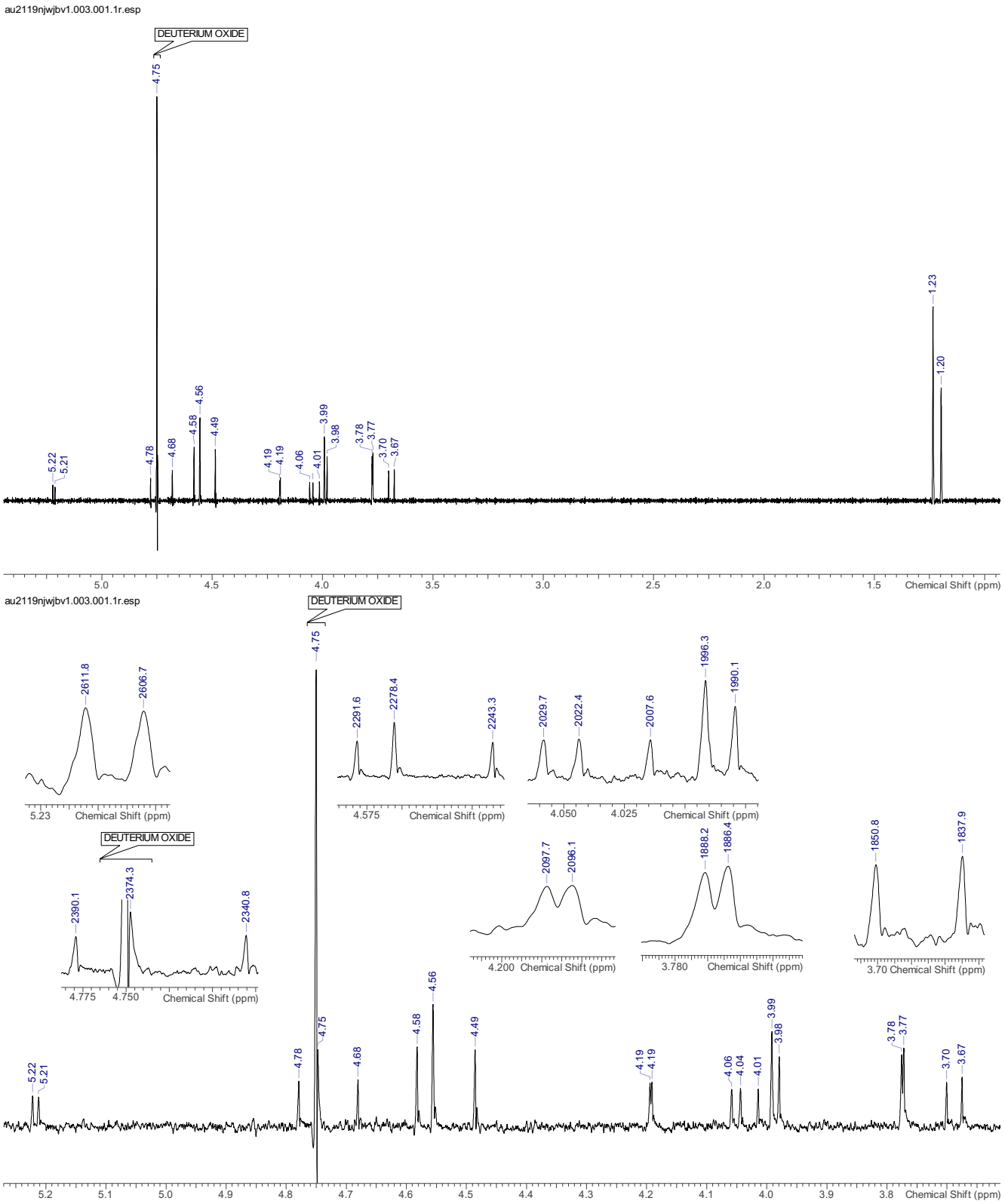
1.4.5.1 ^{1}H NMR (500 MHz, D_2O)



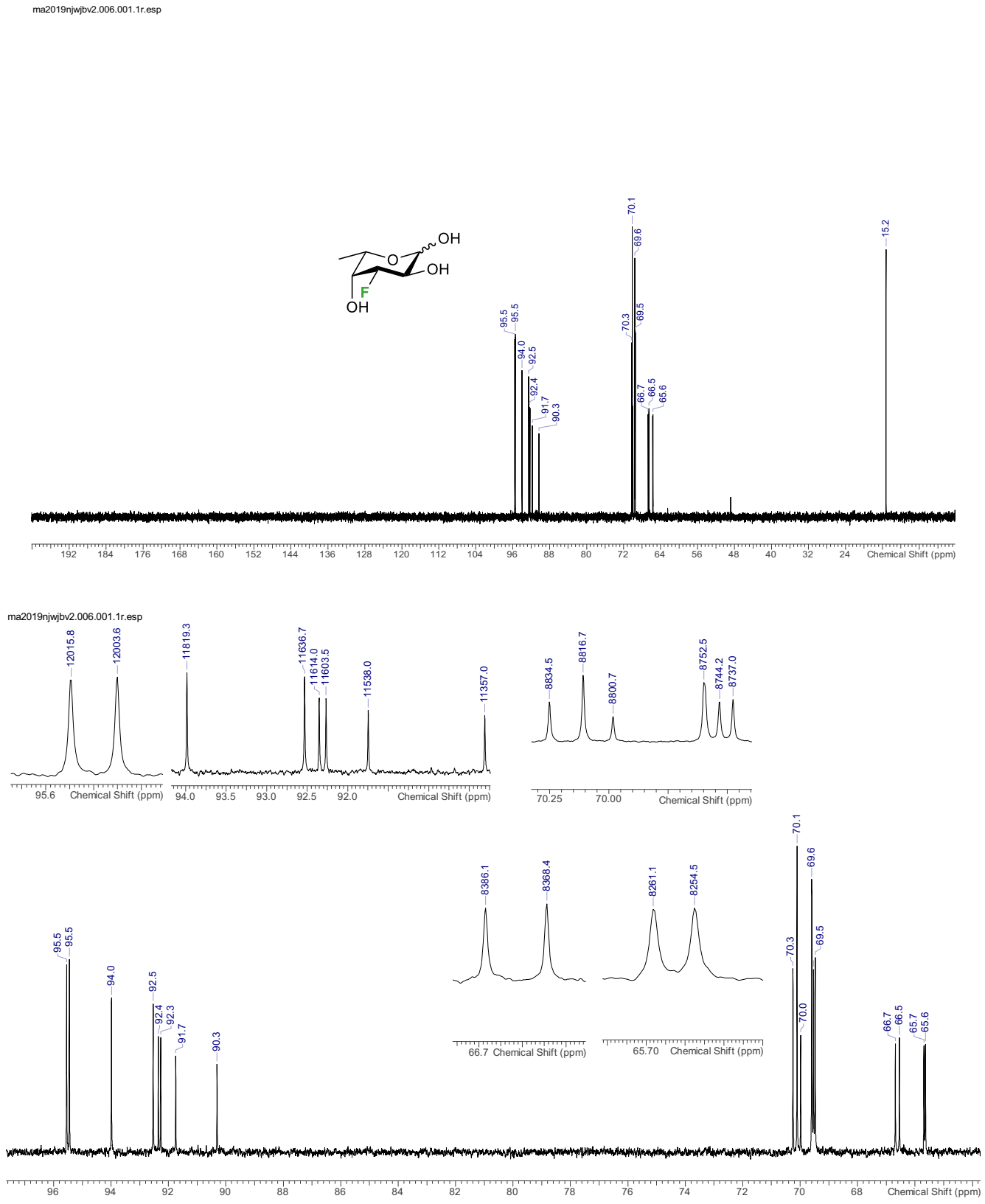
1.4.5.2 $^{1}H\{^{19}F\}$ NMR (500 MHz, D_2O)



1.4.5.3 ^{1}H-^{1}H PSYCHE (500 MHz, D_2O)

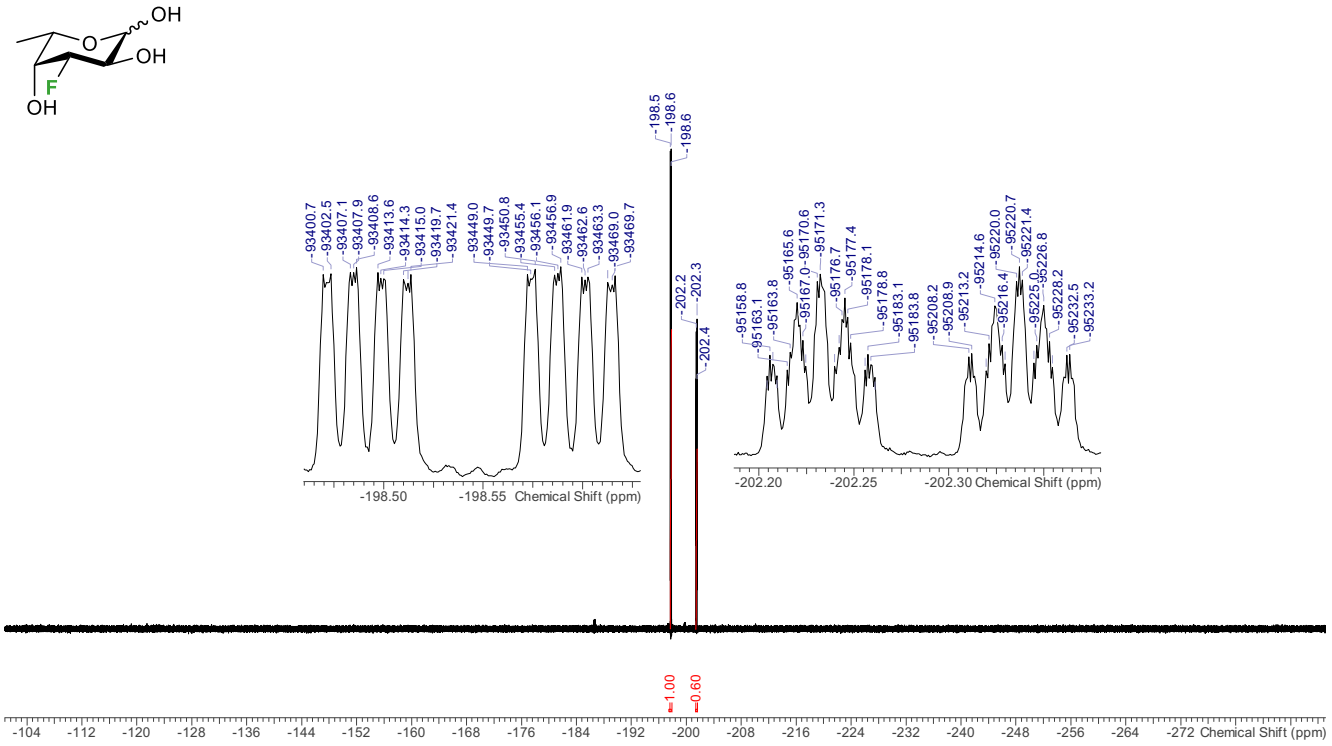


1.4.5.4 ^{13}C NMR (126 MHz, D_2O)



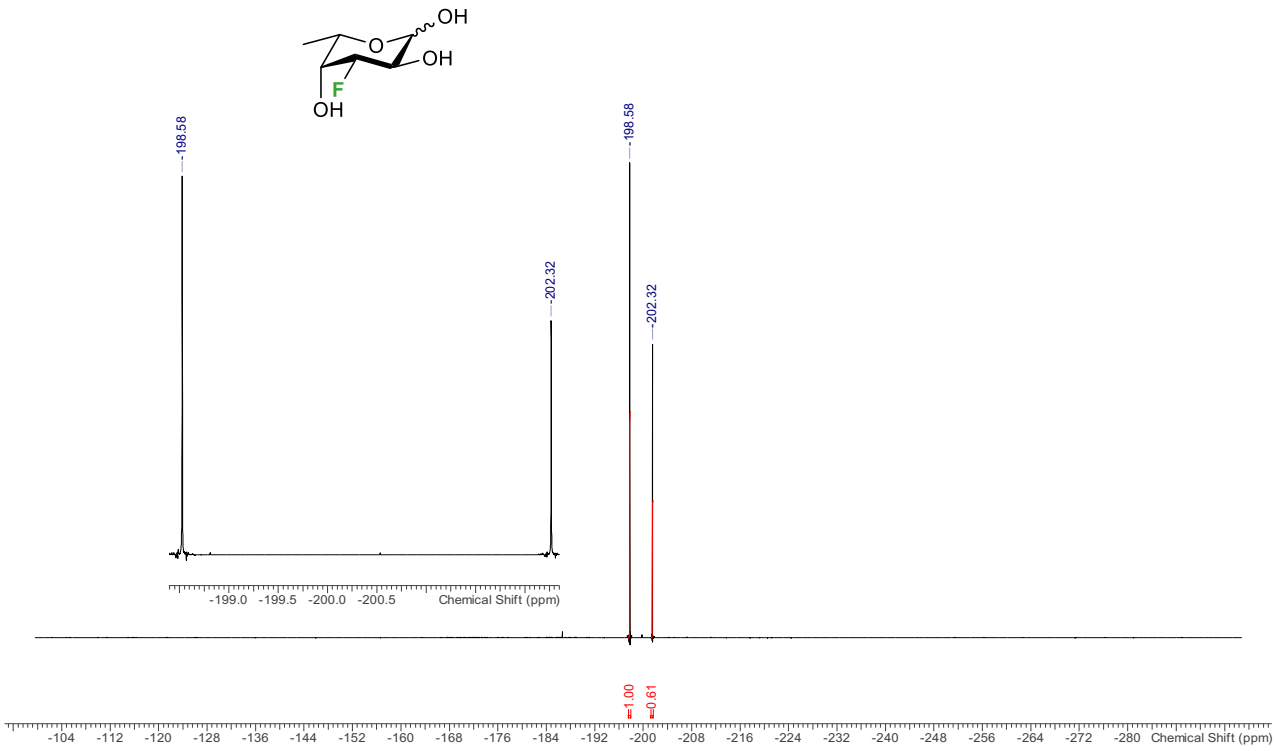
1.4.5.5 ^{19}F NMR (471 MHz, D_2O)

ma2019njwjbv2.003.001.1r.esp

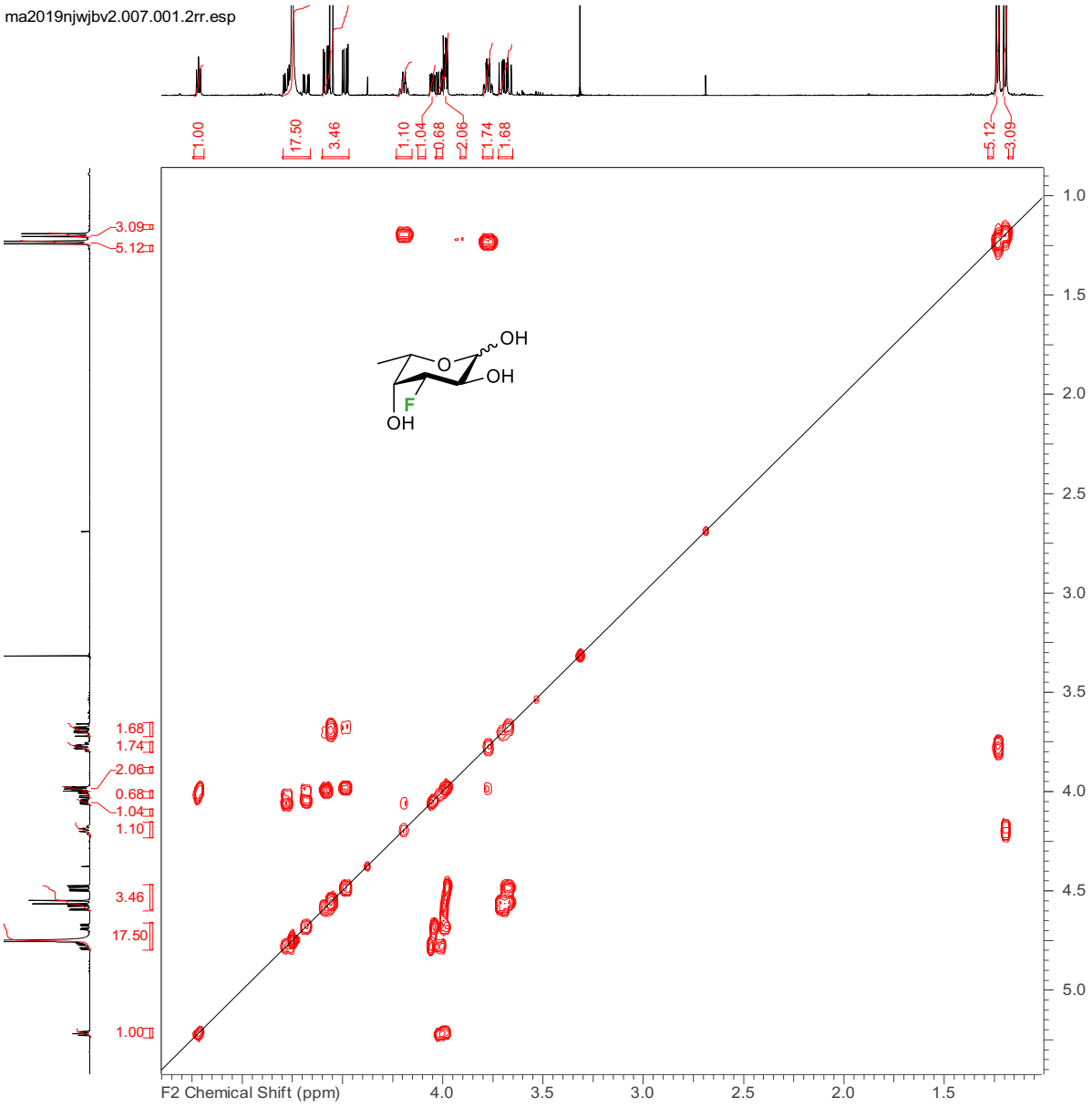


1.4.5.6 ^{19}F{^{1}H} NMR (471 MHz, D2O)

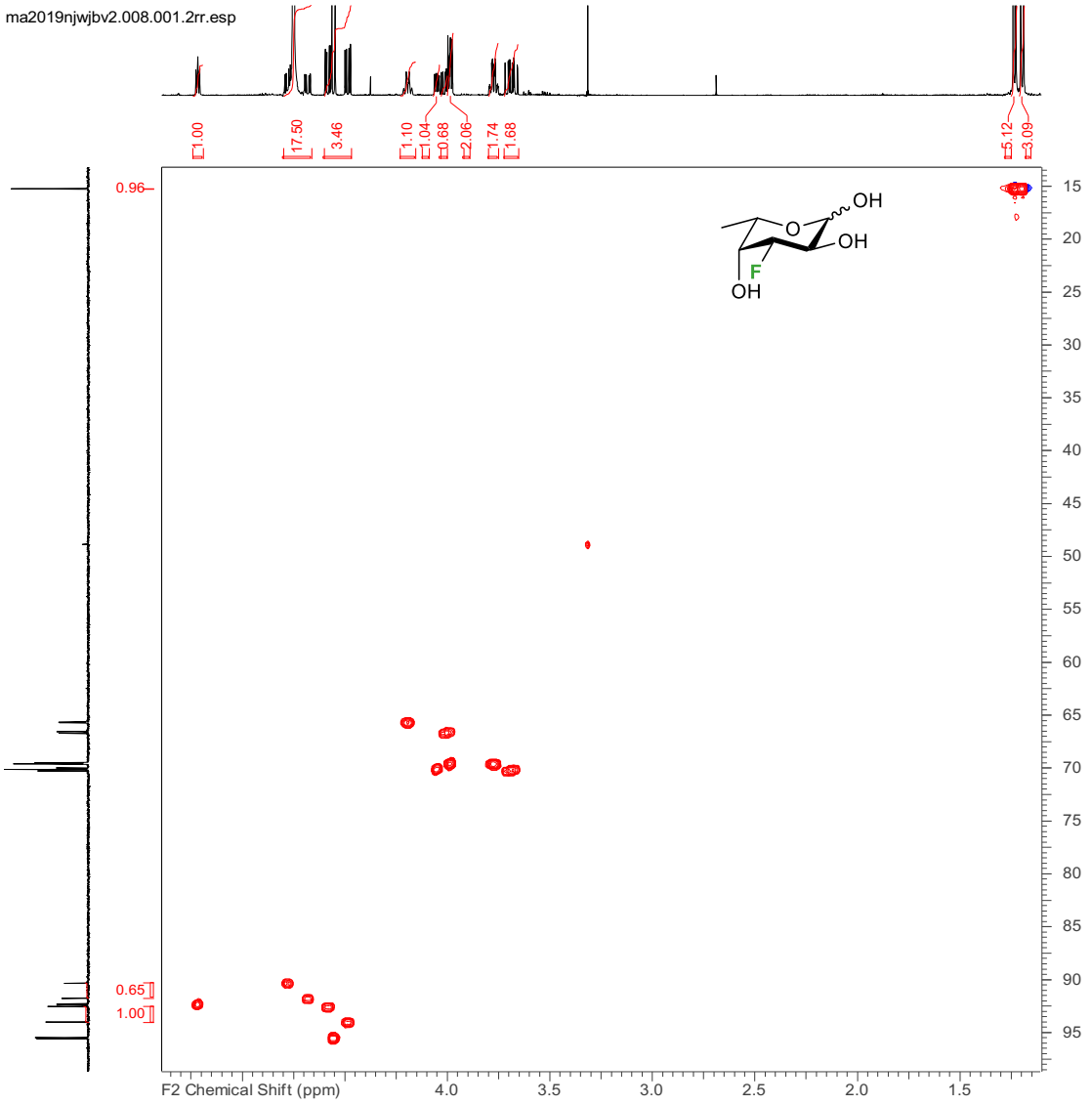
ma2019njwjbv2.002.001.1r.esp



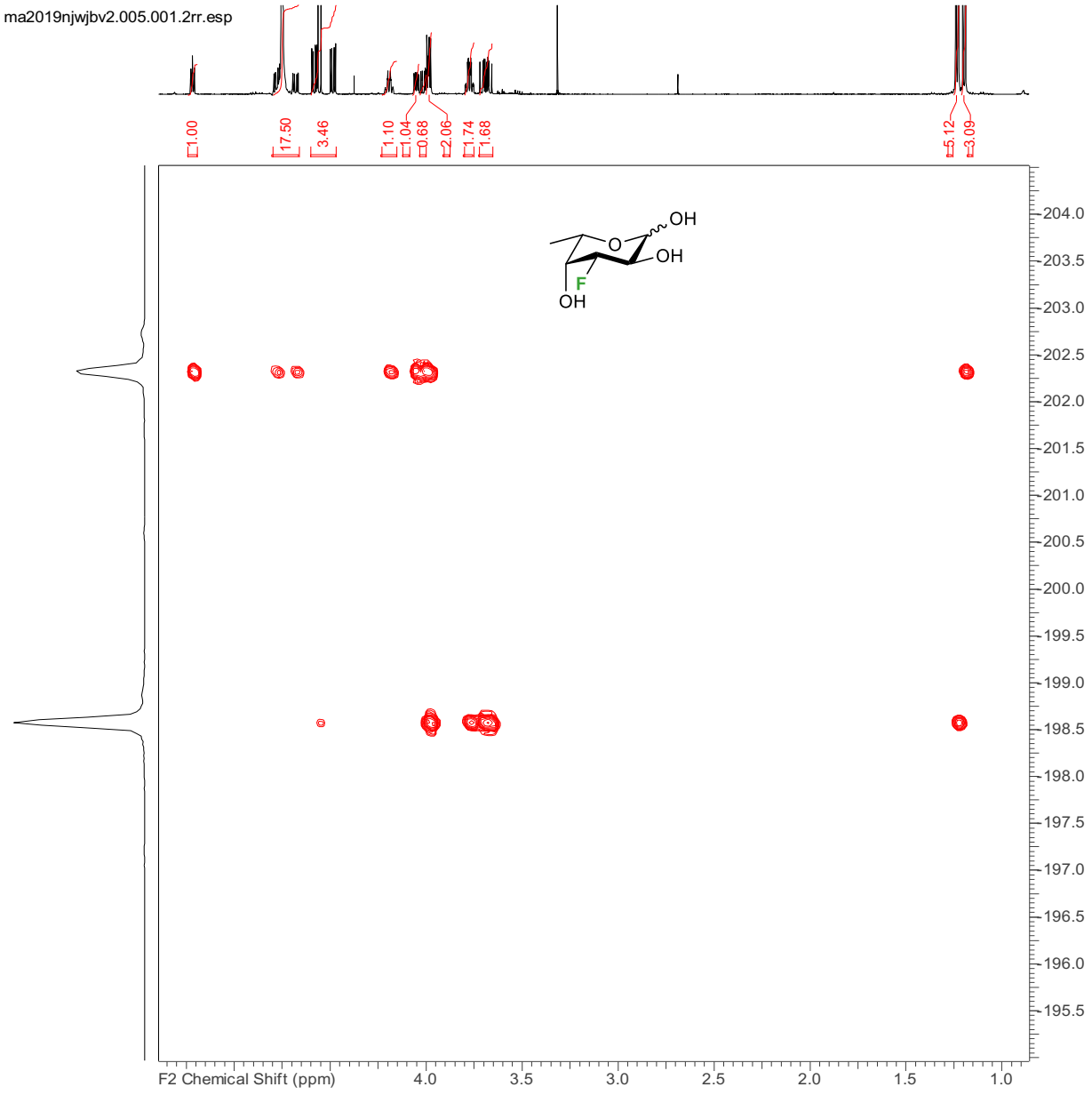
1.4.5.7 ^{1}H-^{1}H COSY (500 MHz, D_2O)



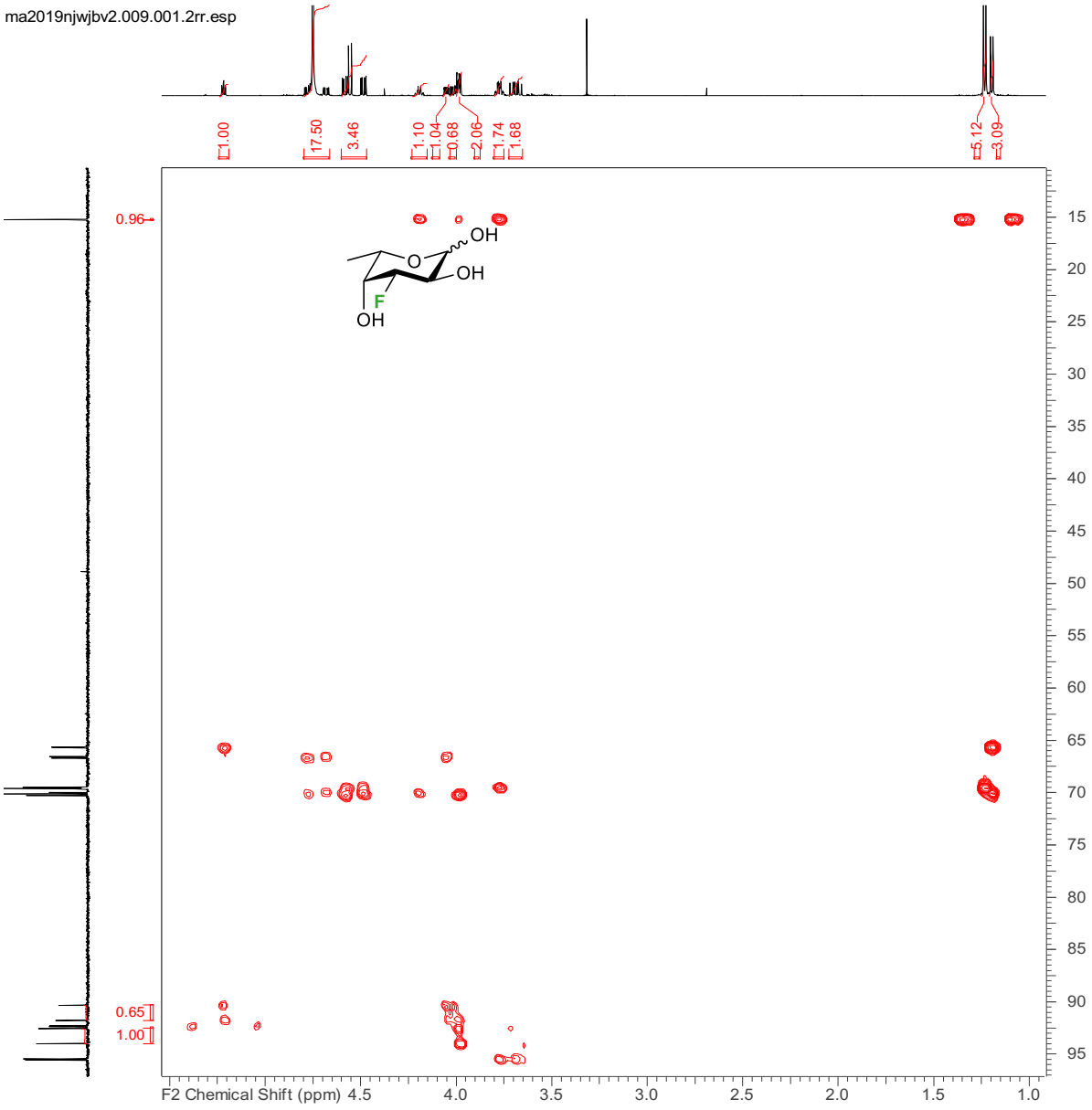
1.4.5.8 ^{1}H-^{13}C HSQC (500 MHz, D_2O)



1.4.5.9 ^{19}F-^{1}H HMBC (500 MHz, D_2O)



1.4.5.10 ^{1}H-^{13}C HMBC (500 MHz, D_2O)



1.5 X-ray crystallographic data of 3-Deoxy-3-fluoro-α-D-fucose 3 (CCDC 1922810)

Experimental. Single clear colourless prism-shaped crystals of **3** were recrystallised from methanol by slow evaporation. A suitable crystal 0.29×0.11×0.06 mm^3 was selected and mounted on a MITIGEN holder with silicon oil on an Rigaku AFC12 FRE-HF diffractometer. The crystal was kept at a steady T = 100(2) K during data collection. The structure was solved with the **ShelXT**[9] structure solution program using the Intrinsic Phasing solution method and by using **Olex2**[10] as the graphical interface. The model was refined with version 2016/6 of **ShelXL**[11] using Least Squares minimisation.

Crystal Data. $C_6H_{11}O_4F$, M_r = 166.15, monoclinic, $P2_1$ (No. 4), a = 8.1522(3) Å, b = 4.7390(2) Å, c = 9.7711(4) Å, β = 99.908(4)°, $\alpha = \gamma$ = 90°, V = 371.86(3) $Å^3$, T = 100(2) K, Z = 2, Z' = 1, μ(MoK_α) = 0.138, 5611 reflections measured, 1895 unique (R_{int} = 0.0251) which were used in all calculations. The final wR_2 was 0.0791 (all data) and R_1 was 0.0296 (I > 2(I)).

Compound	**3F-fucose**
Formula	$C_6H_{11}O_4F$
$D_{calc.}$/ g cm^{-3}	1.484
μ/mm^{-1}	0.138
Formula Weight	166.15
Colour	clear colourless
Shape	prism
Size/mm^3	0.29×0.11×0.06
T/K	100(2)
Crystal System	monoclinic
Flack Parameter	0.0(4)
Hooft Parameter	0.3(4)
Space Group	$P2_1$
a/Å	8.1522(3)
b/Å	4.7390(2)
c/Å	9.7711(4)
α/°	90
β/°	99.908(4)
γ/°	90
V/Å^3	371.86(3)
Z	2
Z'	1
Wavelength/Å	0.71073
Radiation type	MoK$_\alpha$
Θ_{min}/°	3.011
Θ_{max}/°	28.493
Measured Refl.	5611
Independent Refl.	1895
Reflections with I > 2(I)	1854
R_{int}	0.0251
Parameters	113
Restraints	1
Largest Peak	0.422
Deepest Hole	-0.165
GooF	1.075
wR_2 (all data)	0.0791

wR_2	0.0785
R_1 (all data)	0.0304
R_1	0.0296

Structure Quality Indicators

Reflections:	d min (Mo)	0.74	I/σ	39.7	Rint	2.51%	complete 100% (IUCr)	100%	
Refinement:	Shift	0.000	Max Peak	0.4	Min Peak	-0.2	GooF	1.075	Flack -.0(4)

A clear colourless prism-shaped crystal with dimensions 0.29×0.11×0.06 mm^3 was mounted on a MITIGEN holder with silicon oil. X-ray diffraction data were collected using a Rigaku AFC12 FRE-HF diffractometer equipped with an Oxford Cryosystems low-temperature device, operating at T = 100(2) K.

Data were measured using profile data from ω-scans of 1.0 ° per frame for 2.0 s using MoK$_\alpha$ radiation (Rotating-anode X-ray tube, 45.0 kV, 55.0 mA). The total number of runs and images was based on the strategy calculation from the program **CrysAlisPro** (Rigaku, V1.171.40.18b, 2018).The maximum resolution achieved was Θ = 28.493°.

Cell parameters were retrieved using the **CrysAlisPro** (Rigaku, V1.171.40.18b, 2018) software and refined using **CrysAlisPro** (Rigaku, V1.171.40.18b, 2018) on 4101 reflections, 73 % of the observed reflections. Data reduction was performed using the **CrysAlisPro** (Rigaku, V1.171.40.18b, 2018) software that corrects for Lorentz polarisation. The final completeness is 99.70 % out to 28.493° in Θ.

A multi-scan absorption correction was performed using CrysAlisPro 1.171.40.18b (Rigaku Oxford Diffraction, 2018) using spherical harmonics as implemented in SCALE3 ABSPACK.. The absorption coefficient μ of this material is 0.138 mm^{-1} at this wavelength (λ = 0.711Å) and the minimum and maximum transmissions are 0.591 and 1.000

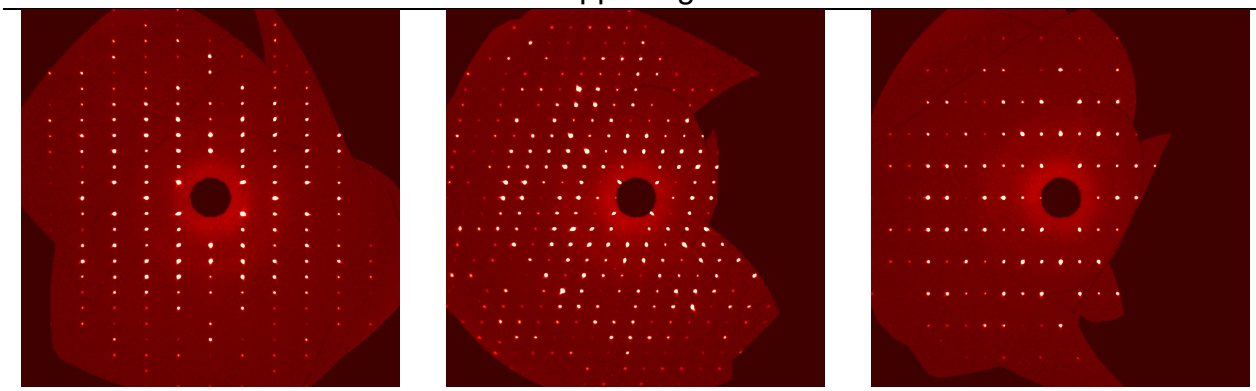
The structure was solved in the space group $P2_1$ (# 4) by Intrinsic Phasing using the **ShelXT**[9] structure solution program and refined by Least Squares using version 2016/6 of **ShelXL**.[11] All non-hydrogen atoms were refined anisotropically. Most hydrogen atom positions were calculated geometrically and refined using the riding model, but some hydrogen atoms were refined freely.

_exptl_absorpt_process_details: CrysAlisPro 1.171.40.18b (Rigaku Oxford Diffraction, 2018) using spherical harmonics as implemented in SCALE3 ABSPACK.

There is a single molecule in the asymmetric unit, which is represented by the reported sum formula. In other words: Z is 2 and Z' is 1.

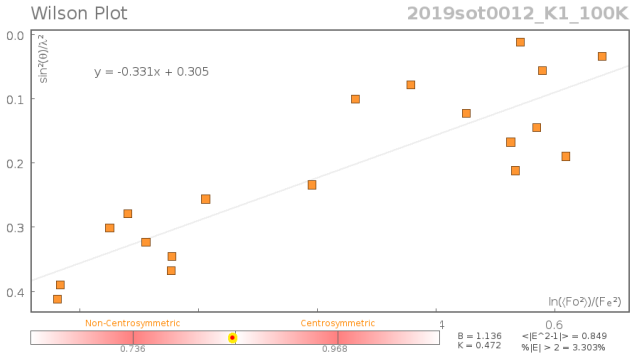
The Flack parameter was refined to 0.0(4). Determination of absolute structure using Bayesian statistics on Bijvoet differences using the Olex2 results in 0.3(4). Note: The Flack parameter is used to determine chirality of the crystal studied, the value should be near 0, a value of 1 means that the stereochemistry is wrong and the model should be inverted. A value of 0.5 means that the crystal consists of a racemic mixture of the two enantiomers.

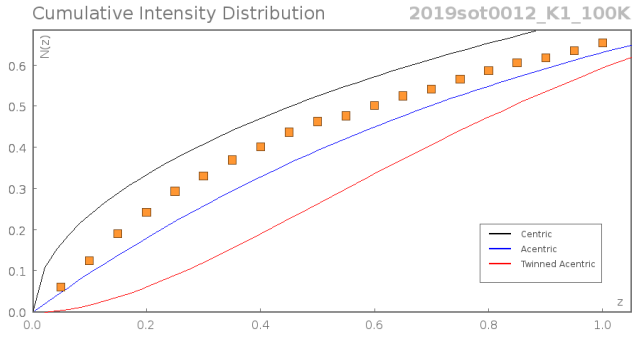
Generated precession images

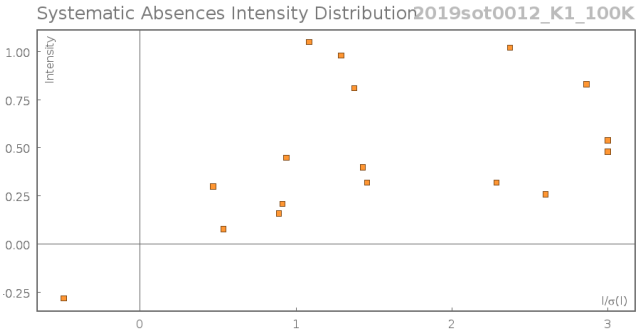


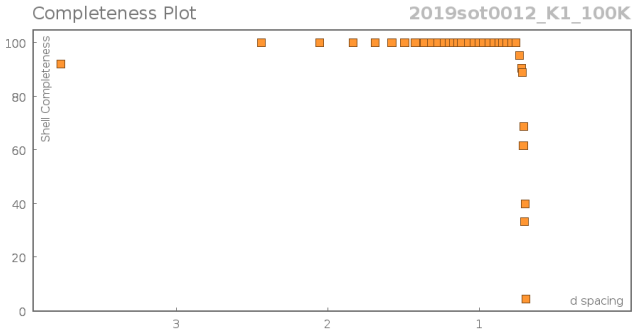
0kl h0l hk0

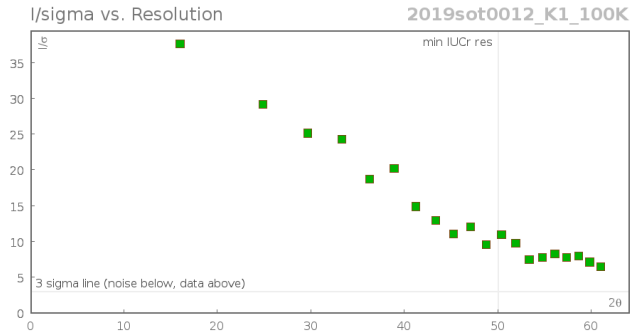
Data Plots: Diffraction Data



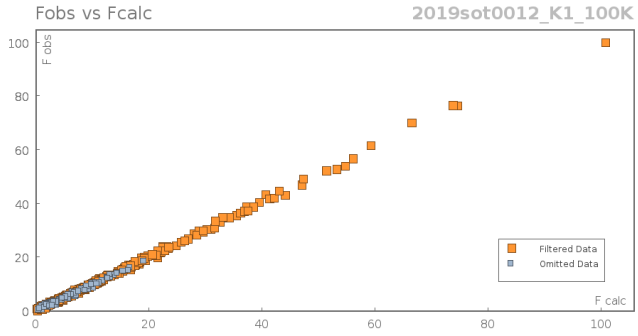


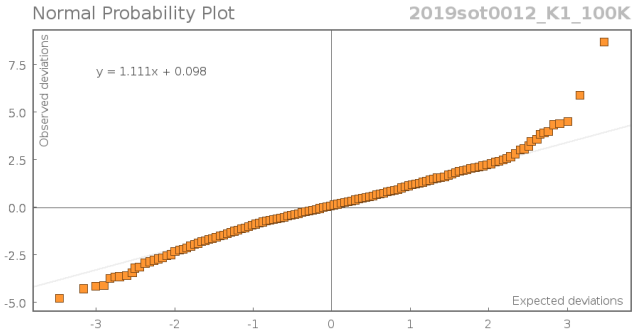






Data Plots: Refinement and Data





Reflection Statistics

Total reflections (after filtering)	5628	Unique reflections	1895
Completeness	0.999	Mean I/σ	27.14
hkl_{max} collected	(9, 6, 13)	hkl_{min} collected	(-11, -6, -14)
hkl_{max} used	(10, 6, 13)	hkl_{min} used	(-10, -6, 0)
Lim d_{max} collected	100.0	Lim d_{min} collected	0.74
d_{max} used	6.77	d_{min} used	0.74
Friedel pairs	998	Friedel pairs merged	0
Inconsistent equivalents	0	R_{int}	0.0251
R_{sigma}	0.0252	Intensity transformed	0
Omitted reflections	0	Omitted by user (OMIT hkl)	0
Multiplicity	(1247, 1008, 601, 203, 18)	Maximum multiplicity	10
Removed systematic absences	17	Filtered off (Shel/OMIT)	340

Images of the Crystal on the Diffractometer

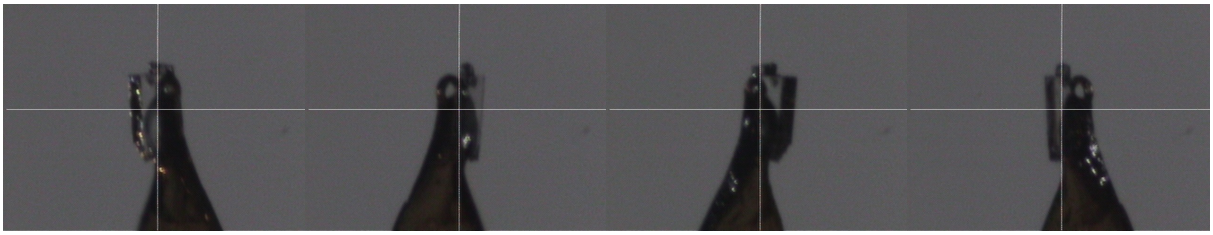


Table 1: Fractional Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Parameters (Å²×10³) for **2019sot0012_K1_100K**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
F1	-375.0(11)	4647(2)	1206.2(10)	15.1(2)
O1	4221.3(14)	4335(3)	3672.6(12)	12.5(3)
O2	2861.6(16)	7999(3)	4631.7(13)	15.6(3)
O3	-118.9(15)	5327(3)	4126.8(12)	14.2(3)
O4	2587.2(16)	1975(3)	1091.6(13)	14.7(3)
C1	2887.4(19)	5107(4)	4370.8(16)	11.7(3)
C2	1208.6(19)	4315(4)	3489.6(16)	10.9(3)
C3	1087(2)	5667(4)	2067.8(16)	10.5(3)
C4	2564.9(19)	4919(4)	1373.6(16)	11.6(3)
C5	4169(2)	5724(4)	2337.4(17)	12.8(3)
C6	5726(2)	4816(5)	1800.3(18)	20.1(4)

Table 2: Anisotropic Displacement Parameters (×10⁴) **2019sot0012_K1_100K**. The anisotropic displacement factor exponent takes the form: $-2\pi^2(h^2a^{*2} \times U_{11} + \ldots + 2hka^* \times b^* \times U_{12})$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
F1	9.7(4)	20.8(6)	13.3(4)	0.3(4)	-2.4(3)	-2.0(4)
O1	10.5(5)	17.7(6)	9.3(5)	1.5(5)	1.4(4)	3.2(5)
O2	13.0(6)	14.9(7)	17.7(6)	-4.3(5)	-0.7(4)	-0.1(5)
O3	11.6(5)	17.2(6)	15.4(5)	0.2(5)	6.6(4)	-0.7(5)
O4	14.5(6)	15.6(7)	13.0(6)	-3.5(5)	-0.6(5)	2.0(5)
C1	10.6(7)	15.0(8)	9.6(6)	-0.1(6)	1.6(5)	2.6(6)
C2	10.6(7)	12.4(8)	9.9(7)	0.3(6)	2.4(5)	-0.5(6)
C3	8.0(7)	12.9(8)	9.7(6)	-0.4(6)	-1.2(5)	-1.2(6)
C4	11.4(7)	13.9(9)	9.6(6)	1.9(6)	2.0(5)	0.8(6)
C5	11.1(7)	16.8(9)	10.4(7)	1.9(6)	1.1(6)	-0.2(6)
C6	11.2(7)	33.1(11)	16.7(8)	1.8(8)	4.3(6)	0.4(8)

Table 3: Bond Lengths in Å for **2019sot0012_K1_100K**.

Atom	Atom	Length/Å
F1	C3	1.4202(18)
O1	C1	1.4272(18)
O1	C5	1.4553(19)
O2	C1	1.395(2)
O3	C2	1.4215(18)
O4	C4	1.423(2)

Atom	Atom	Length/Å
C1	C2	1.533(2)
C2	C3	1.518(2)
C3	C4	1.523(2)
C4	C5	1.523(2)
C5	C6	1.517(2)

Table 4: Bond Angles in ° for **2019sot0012_K1_100K**.

Atom	Atom	Atom	Angle/°
C1	O1	C5	113.91(12)
O1	C1	C2	110.41(12)
O2	C1	O1	112.08(14)
O2	C1	C2	107.66(13)
O3	C2	C1	110.16(12)
O3	C2	C3	108.88(13)
C3	C2	C1	108.80(13)
F1	C3	C2	108.37(13)
F1	C3	C4	107.72(12)
C2	C3	C4	112.17(13)
O4	C4	C3	110.60(13)
O4	C4	C5	109.10(14)
C5	C4	C3	109.11(13)
O1	C5	C4	109.31(13)
O1	C5	C6	106.54(13)
C6	C5	C4	113.31(14)

Table 5: Hydrogen Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **2019sot0012_K1_100K**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
H2	3770(30)	8550(60)	5240(30)	28(7)
H3	-410(40)	4070(70)	4570(30)	35(8)
H4	1850(40)	1720(60)	440(30)	28(7)
H1	3016.02	4071.51	5274.53	14
H2A	1127.62	2217.32	3388.44	13
H3A	1013.91	7763.22	2158.34	13
H4A	2484.98	5992.01	483.26	14
H5	4195.33	7815.15	2477.44	15
H6A	5753.97	2753.27	1739.67	30
H6B	6712.43	5479.65	2437.14	30
H6C	5717.85	5629.72	876.97	30

Table 6: Hydrogen Bond information for **2019sot0012_K1_100K**.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/deg
O2	H2	O1[1]	0.90(3)	1.83(3)	2.7287(17)	172(3)
O4	H4	F1[2]	0.81(3)	2.08(3)	2.8489(16)	159(3)

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[1]1-x,1/2+y,1-z; [2]-x,-1/2+y,-z

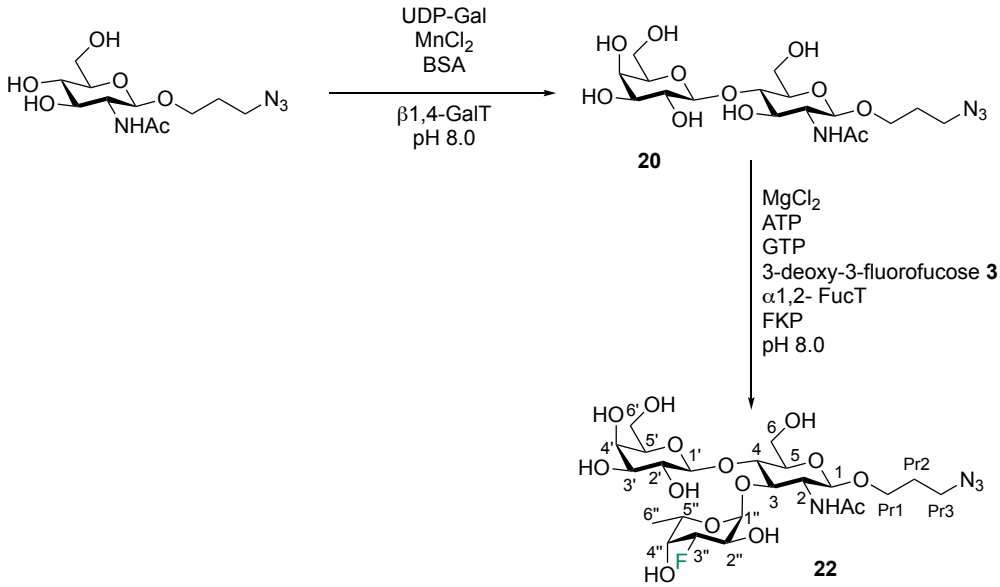
2 Enzymatic trisaccharide synthesis

2.1 Synthesis of lewis x analogue Gal(β1-4)(Fuc(α1,3))GlcNAc-$(CH_2)_3$-N_3 22

2.1.1 Enzyme sources

β-1,4-Galactosyltransferase 1 from *H. sapiens* was expressed in house from a clone provided by Tomasz Kamiński (Webb and Turnbull labs, University of Leeds). α-1,3/4-Fucosyltransferase from *H. pylori* and FKP from *B. fragilis* were provided by Prozomix.

2.1.2 Synthesis of Gal(β1-4)(Fuc(α1,3))GlcNAc-$(CH_2)_3$-N_3 22



GlcNAc-N_3[12] (10.4 mg, 10 mM), UDP-Gal (21.5 mg, 11 mM), $MnCl_2$ (4.0 mg, 10 mM), BSA (3.25 mg, 1 mg / ml), B4GalT1 *HS* (7.8 mg, 2.4 mg/ml), Tris buffer (39.3 mg, 100 mM, pH 8.0) in H_2O at a total volume of 3.25 ml were incubated overnight at 37 °C. To crude Gal(β1-4)GlcNAc-$(CH_2)_3$-N_3 **20** (16 mg, 4.2 mM) was added Tris, pH 8.0 (196 mg, 200 mM), $MgCl_2$ (7.71 mg, 10 mM), ATP (175 mg, 16 mM), GTP (87.7 mg, 8 mM), 3FFuc (10.7 mg, 8 mM), α1-3 FucT *HP* (4.8 mg, 0.59 mg/ml, 20 U) and FKP (2.51 mg, 0.31 mg/ml) in H2O at a total volume of 8.12 ml this was incubated overnight at 37 °C. The reaction mixture was passed through a 10K MWCO spin concentrator and the filtrate was dried under reduced pressure with 600 mg silica gel. The resulting powder was loaded into a 4 g empty dry load cartridge and connected to a 12 g flash silica cartridge. The separation was run on a biotage flash system (20:80 MeOH:EtOAc → 50:50 MeOH:EtOAc over 15 CV). The fractions containing the product were pooled, the solvent removed under reduced pressure and re-dissolved in water. The mixture was then finally purified by size exclusion on a 10/300 biogel P2 column and the fractions containing product pooled and freeze dried to give the product **22** as a white powder. (10.5 mg, 50%). **IR** 3299 (br), 2938 (br), 2100 (m), 1648 (m), 1066 (s), 1032 (s) cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 5.15 (1H, t, *J* 4.2 Hz, H-1''), 4.85 (1H, ddd, J 3.4, 10.3, 50.4 Hz, 1H, H-3''), 4.84 (1H, *J* 6.5 Hz,, H-5''), 4.51 (1H, d, *J* 7.8 Hz, H-1), 4.42 (1H, d, *J* 7.9 Hz, H-1''), 4.03 (1H, dd, *J* 8.2, 2.8 Hz, H-4''), 3.99 – 3.88 (m, 6H, H-2, H-2'', H-3, H-4, H-4', H-Pr1a), 3.82 (1H, dd, *J* 12.4, 4.8 Hz, H-6b), 3.75 – 3.65 (2H, m, H-6a', H-6b'), 3.65 – 3.60 (1H, m, H-Pr1b), 3.62 (1H, dd, *J* 9.4, 3.5 Hz, H-3'), 3.59 – 3.53 (2H, m,

H-5, H-5'), 3.44 (1H, dd, *J* 9.8, 7.8 Hz, H-2), 3.38 (2H, td, *J* 6.1, 4.8 Hz, H-Pr3), 2.05 (s, 3H, H-Ac), 1.85 (2H, quint, *J* 6.6 Hz, H-Pr2), 1.18 (3H, d, *J* 6.5 Hz, H-6'') ppm; ^{1}H-{^{19}F} NMR (500 MHz, D_2O) δ 5.15 (1H, d, *J* 4.2 Hz, H-1''), 4.85 (1H, dd, *J* 3.4, 10.3 Hz, 1H, H-3''), 4.84 (1H, *J* 6.5 Hz,, H-5''), 4.51 (1H, d, *J* 7.8 Hz, H-1), 4.42 (1H, d, *J* 7.9 Hz, H-1''), 4.03 (1H, d, *J* 2.8 Hz, H-4''), 3.99 – 3.88 (m, 6H, H-2, H-2'', H-3, H-4, H-4', H-Pr1a), 3.82 (1H, dd, *J* 12.4, 4.8 Hz, H-6b), 3.75 – 3.65 (2H, m, H-6a', H-6b'), 3.65 – 3.60 (1H, m, H-Pr1b), 3.62 (1H, dd, *J* 9.4, 3.5 Hz, H-3'), 3.59 – 3.53 (2H, m, H-5, H-5'), 3.44 (1H, dd, *J* 9.8, 7.8 Hz, H-2), 3.38 (2H, td, *J* 6.1, 4.8 Hz, H-Pr3), 2.05 (s, 3H, H-Ac), 1.85 (2H, quint, *J* 6.6 Hz, H-Pr2), 1.18 (3H, d, *J* 6.5 Hz, H-6'') ppm; ^{13}C NMR (126 MHz, D_2O) δ 174.2 (C=O), 101.8 (C-1'), 100.9 (C-1), 98.4 (d, *J* 10.7 Hz, C-1''), 90.8 (d, *J* 181.0 Hz, C-3''), 75.3 (C-5), 74.9 (C-5'), 74.5 (C-3), 73.3 (C-4), 72.4 (C-3'), 71.1 (C-2'), 70.2 (d, *J* 15.7 Hz, C-4''), 68.3 (C-4'), 67.2 (C-Pr1), 66.3 (C-5''), 66.2 (d, *J* 11.7 Hz, C-2''), 61.4 (C-6'), 59.7 (C-6), 55.8 (C-2), 47.7 (C-Pr3), 28.1 (C-Pr2), 22.2 (C-Ac), 15.0 (d, *J* 2.1 Hz, C-6'') ppm, **^{19}F NMR (471 MHz, D_2O)** δ -202.8 (1F, m, a coupling of *J* 50.4 Hz could be isolated, F-3''), **^{19}F-{^{1}H} NMR (471 MHz, D_2O)** δ -202.8 (1F, s, F-3''), HRMS: Found 637.2340 ([M+Na]$^{+}$), $C_{23}H_{39}FN_4O_{14}Na^{+}$ requires 637.2339.

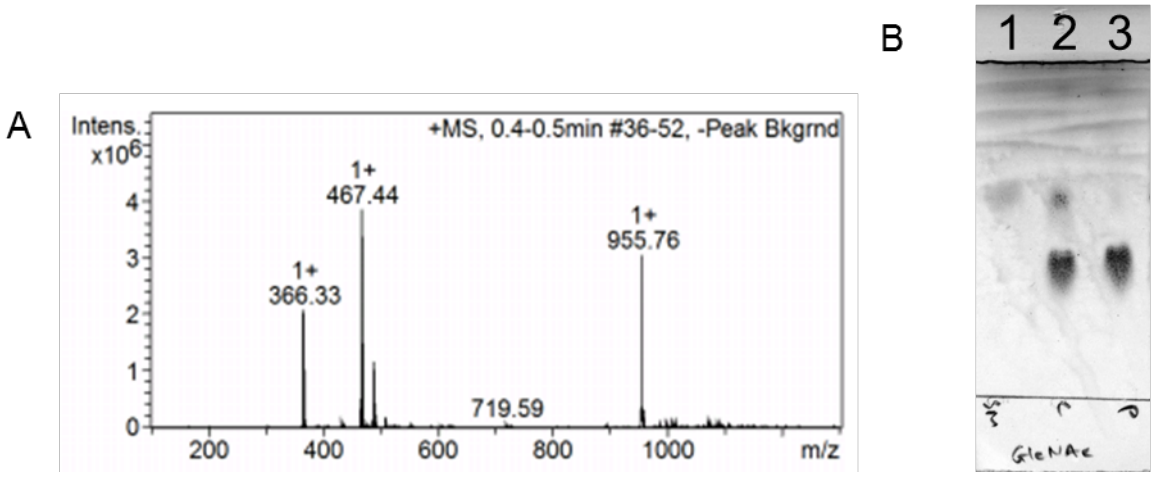
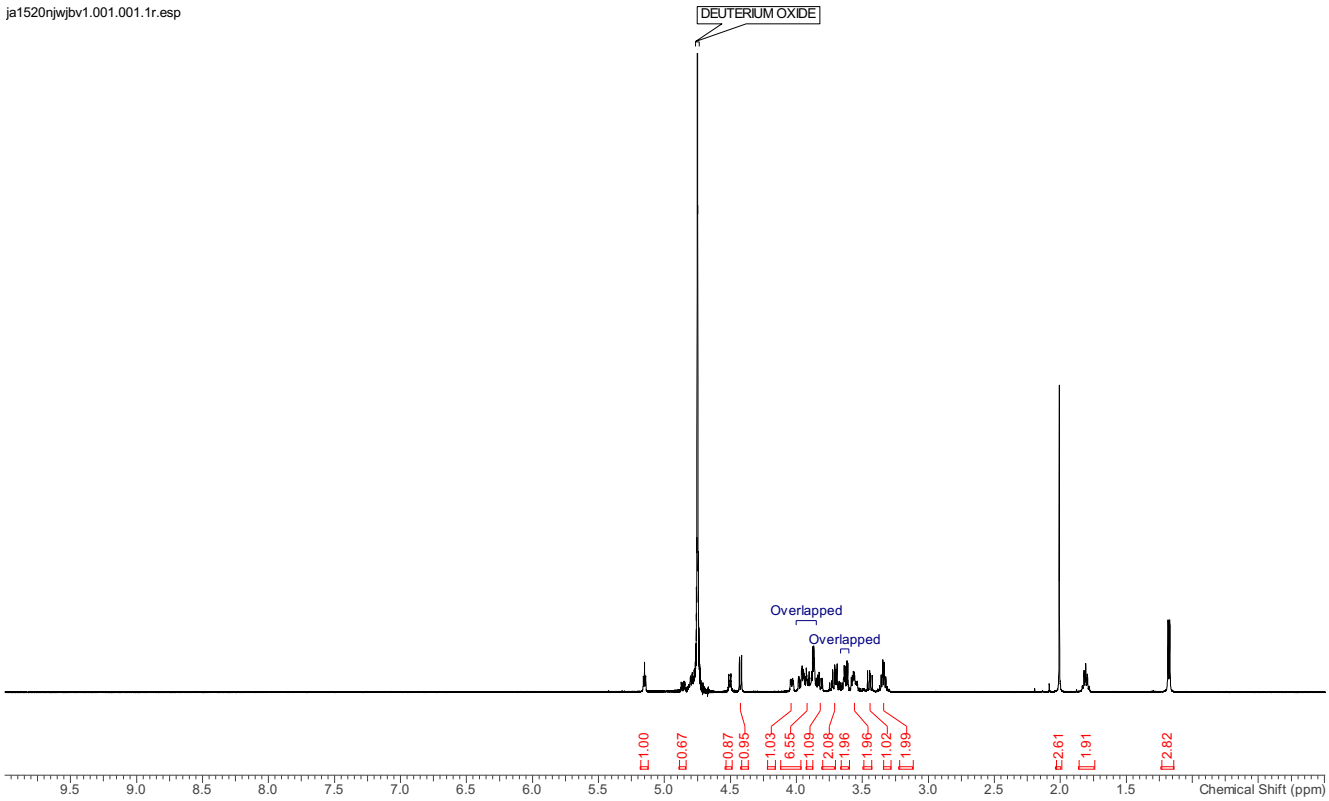
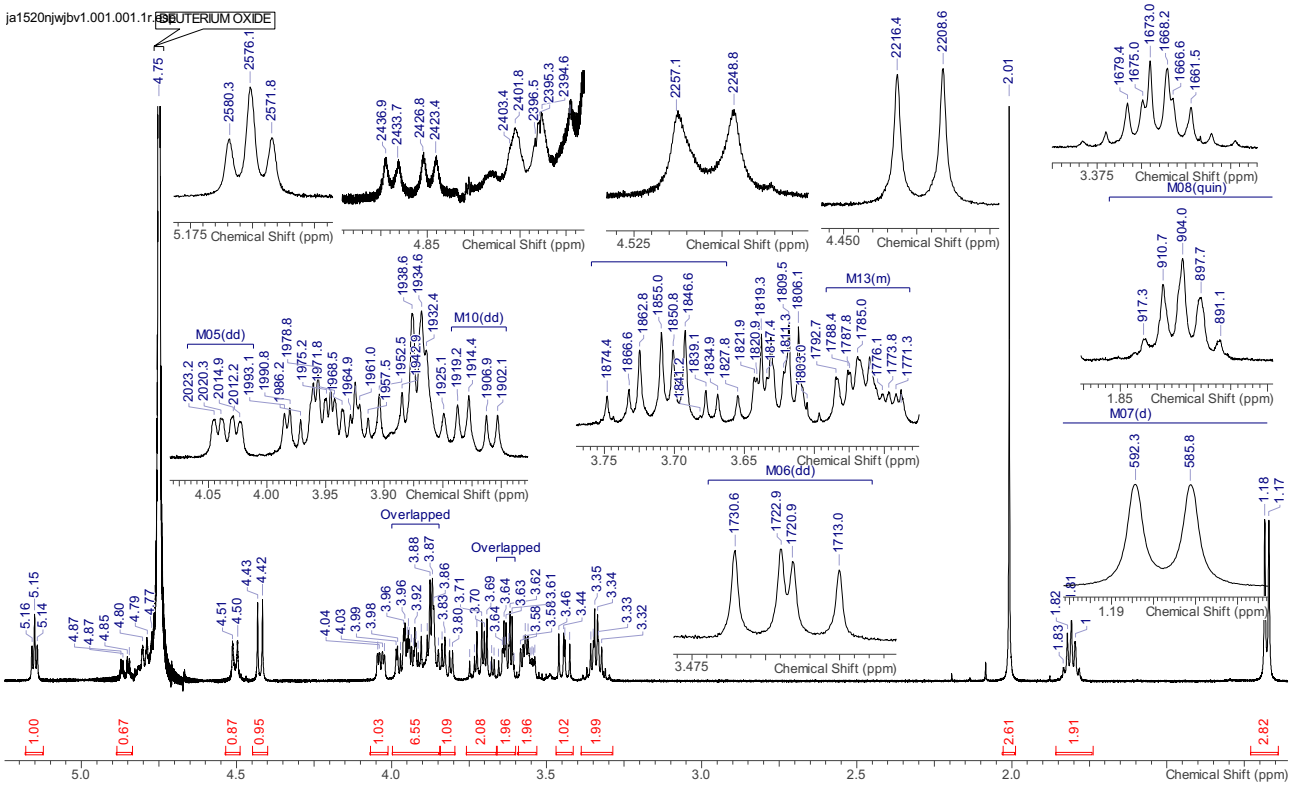


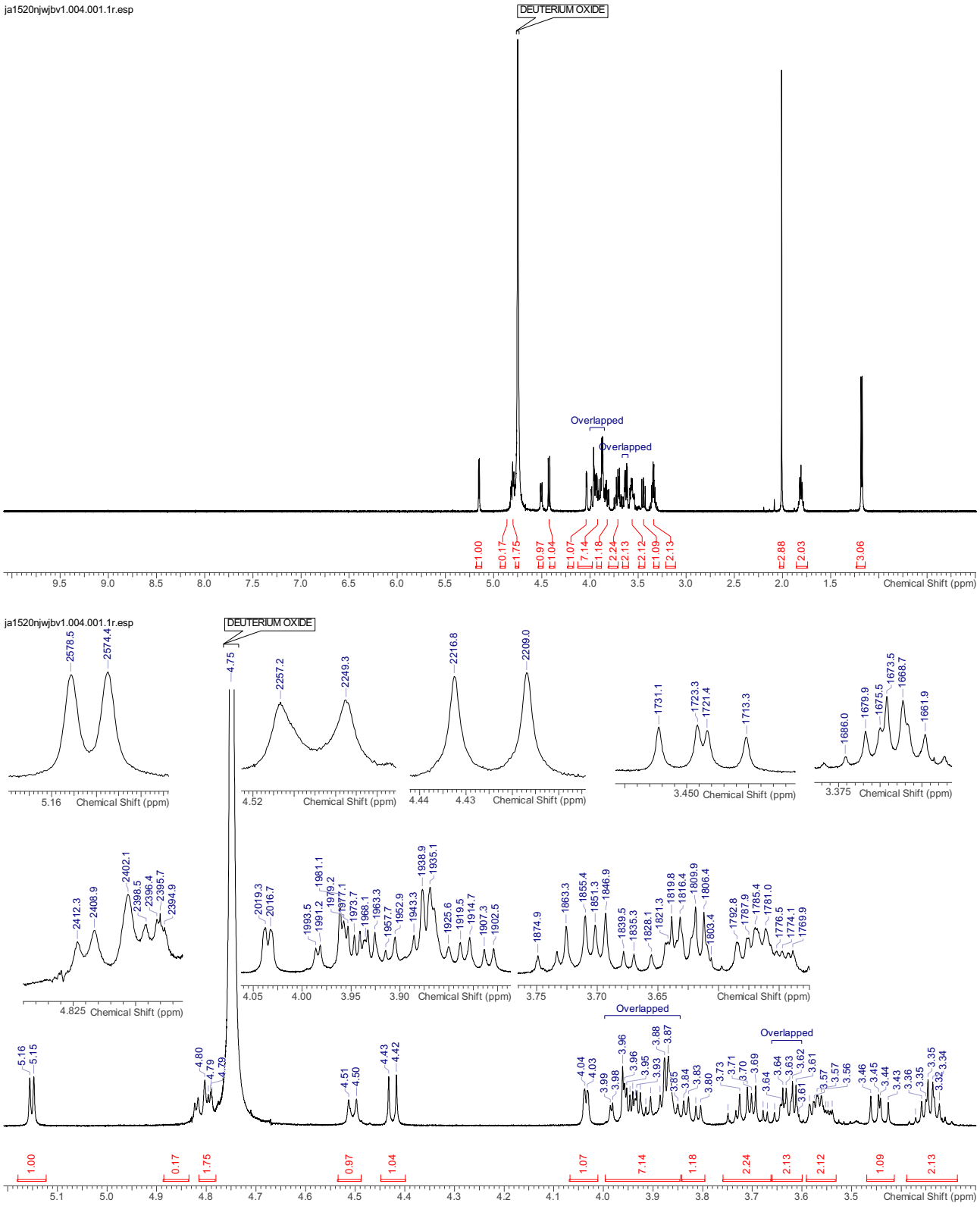
Figure S1: A) Mass spectrometry showing formation of intermediate 19 with an [M+H]$^{+}$ of 467.44. B) TLC of the formation of intermediate 19 with 1: starting material, 2: composite of starting material and reaction mixture and 3: reaction mixture.

2.1.3 ^{1}H NMR (500 MHz, D_2O)



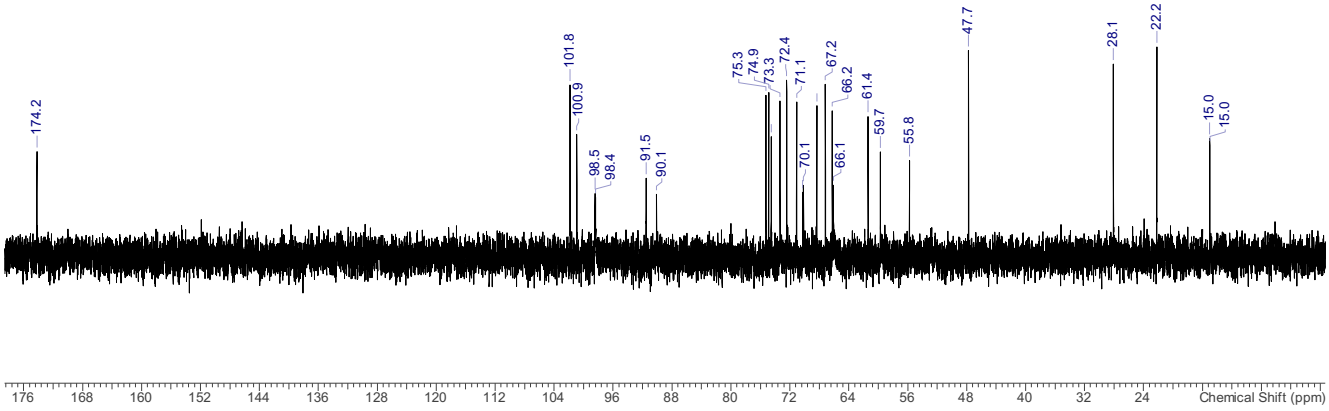


2.1.4 $^{1}H\{^{19}F\}$ NMR (500 MHz, D_2O)

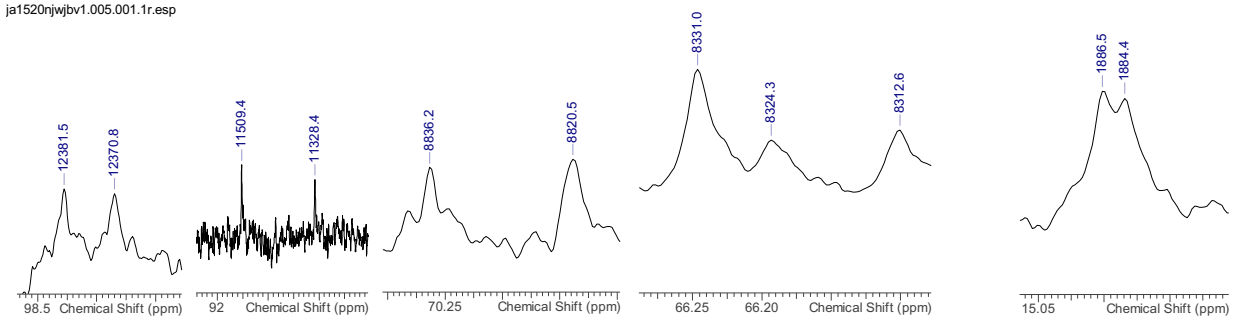


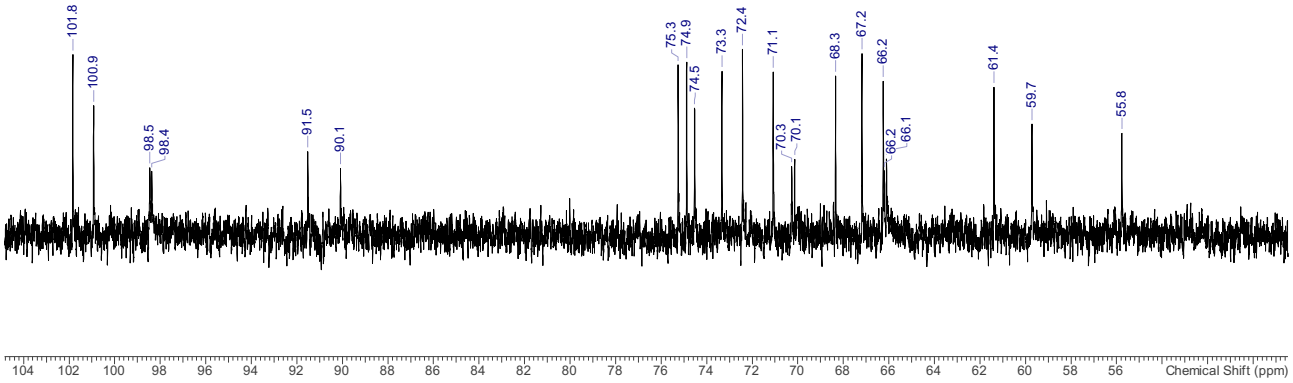
2.1.5 ^{13}C NMR (126 MHz, D_2O)

ja1520njwjbv1.005.001.1r.esp



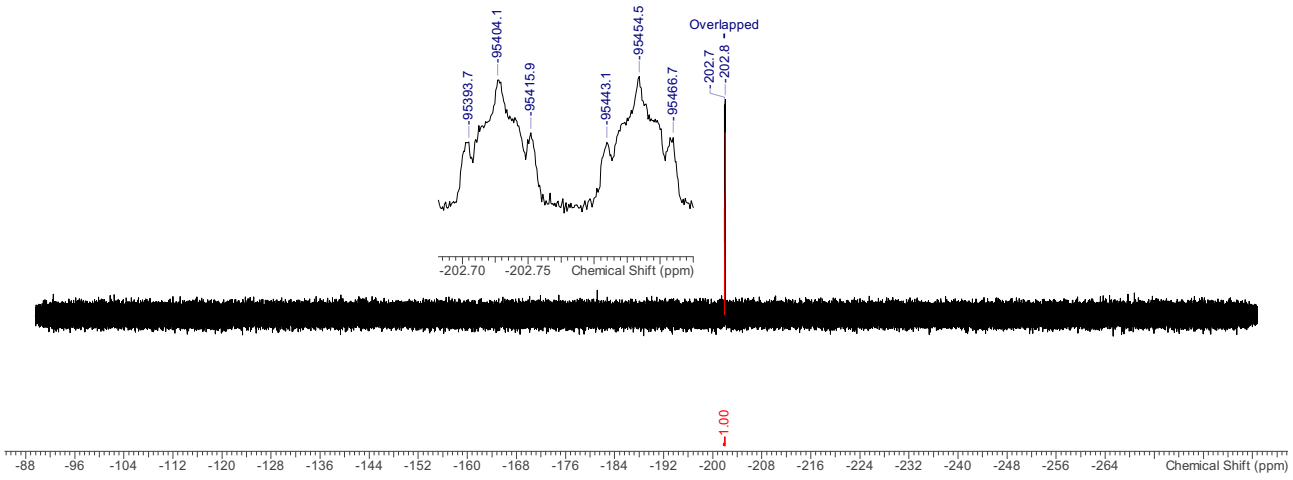
ja1520njwjbv1.005.001.1r.esp





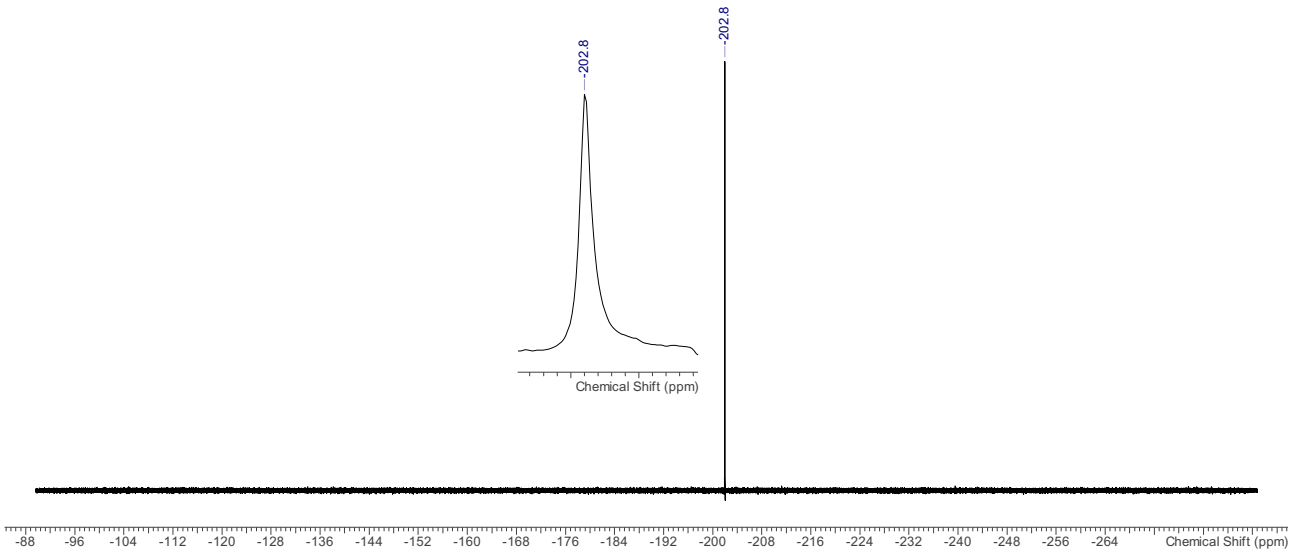
2.1.6 ^{19}F NMR (471 MHz, D_2O)

ja1520njwjbv1.003.001.1r.esp

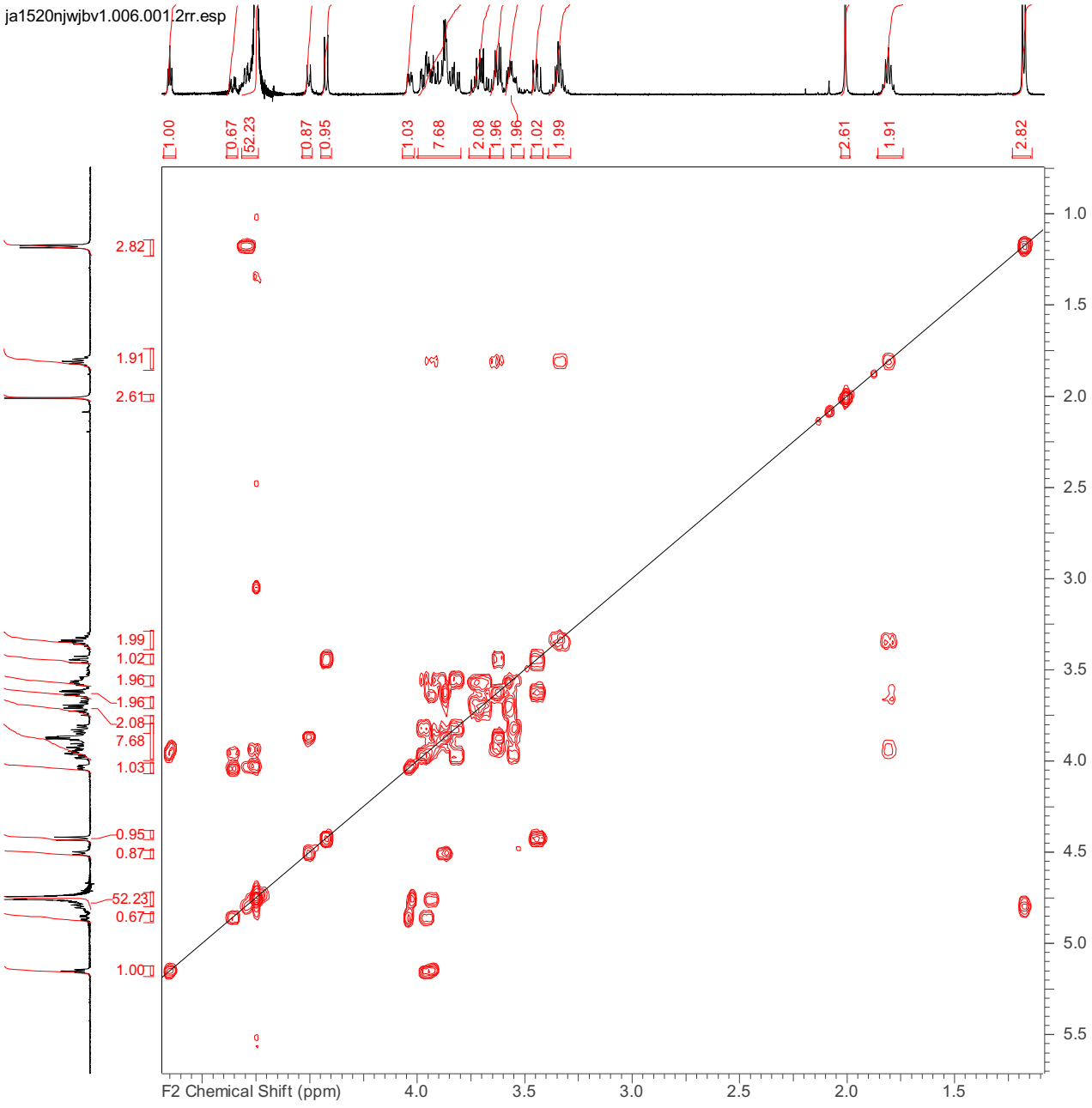


2.1.7 $^{19}F\{^{1}H\}$ NMR (471 MHz, D2O)

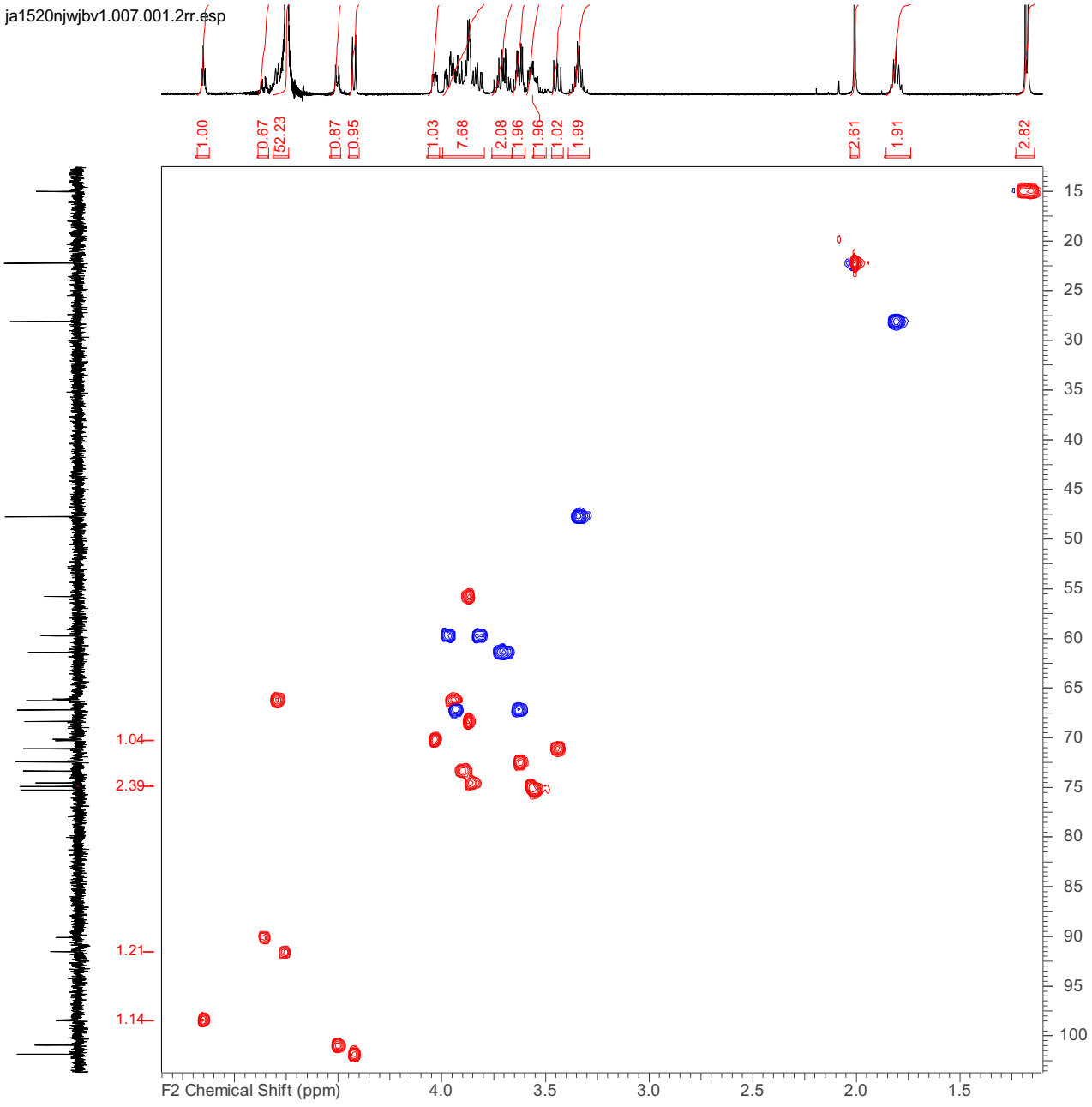
ja1520njwjbv1.002.001.1r.esp



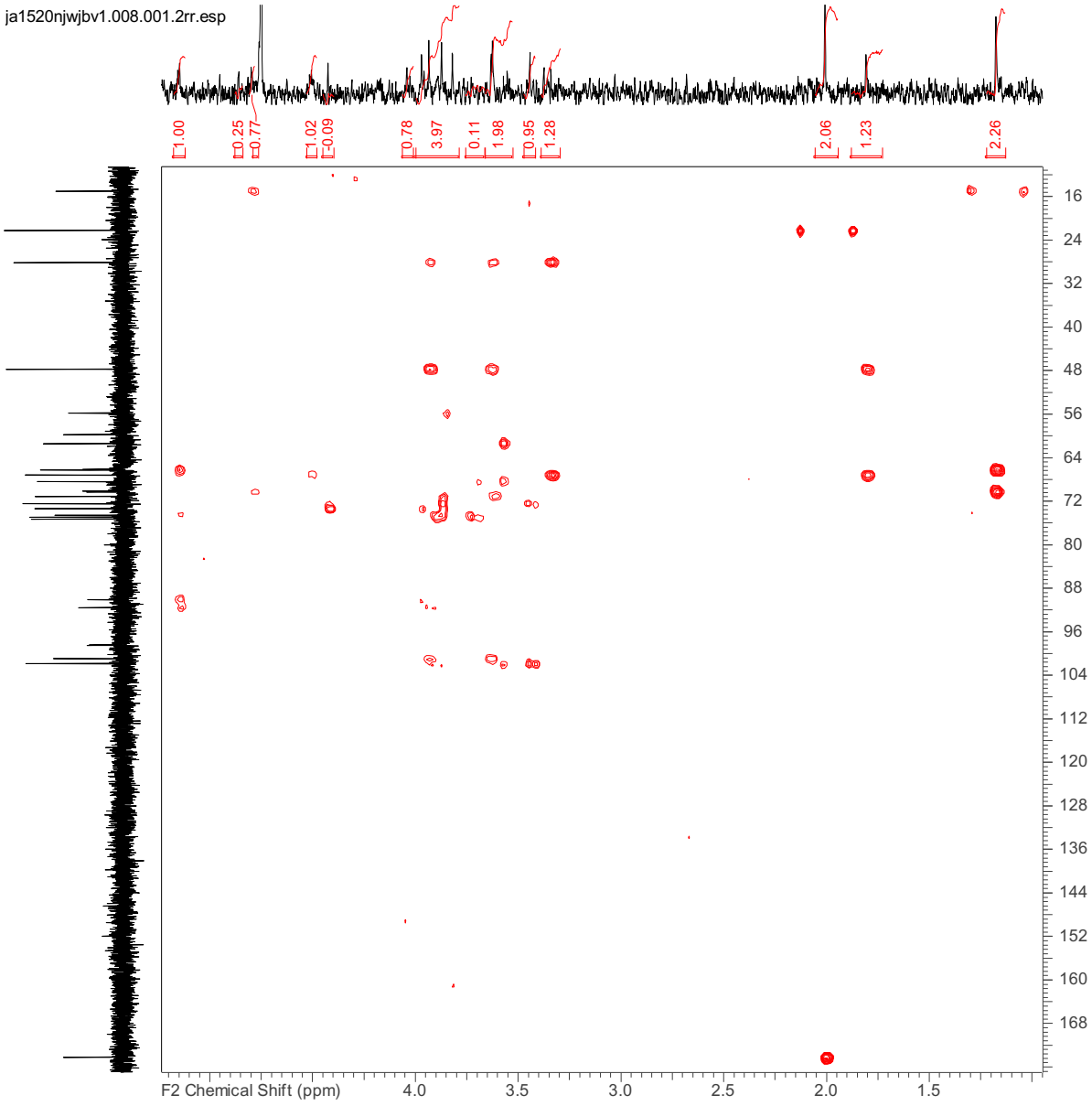
2.1.8 COSY ^{1}H-^{1}H (500 MHz, D_2O)



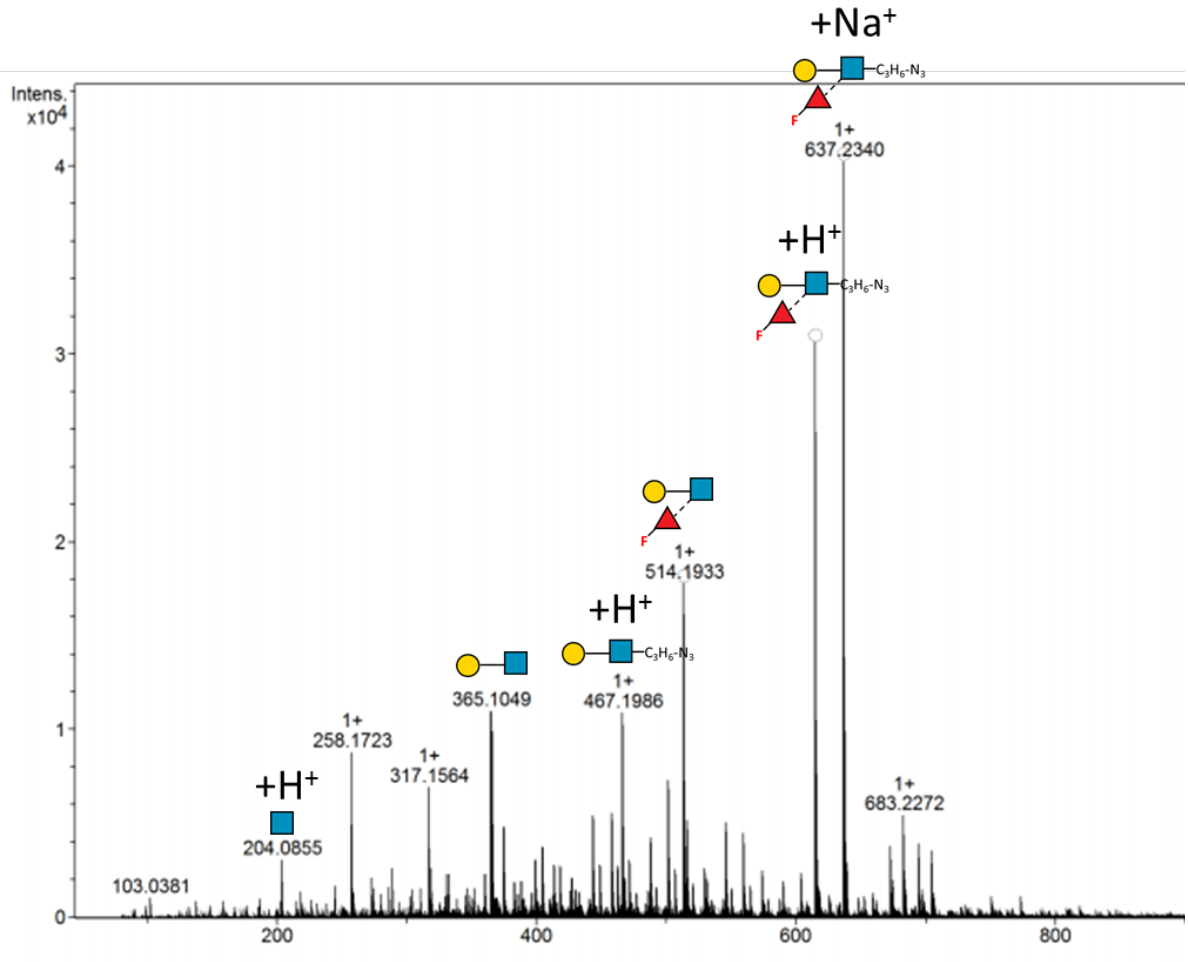
2.1.9 ^{1}H-^{13}C HSQC (500 MHz, D_2O)



2.1.9.1 ^{1}H-^{13}C HMBC (500 MHz, D_2O)



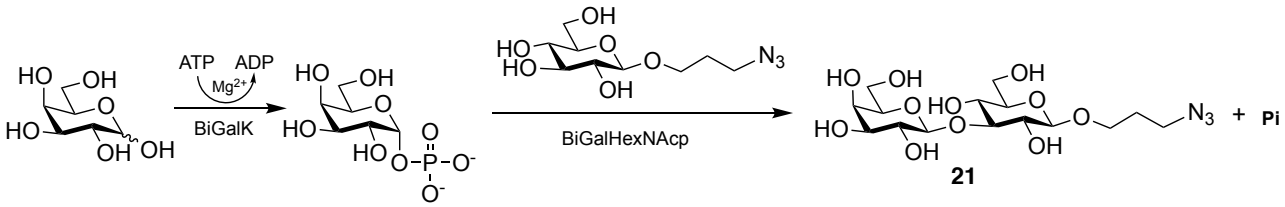
2.1.10 HRMS of Gal(β1-4)(3FFuc(α1,3))GlcNAc-N_3 22



The $[M+Na]^+$ product peak at 637.23 can be seen as well as fragment peaks at 514.19 and 467.19.

2.2 Synthesis of the Type 1 blood group antigen analogue 23

2.2.1 Enzymatic synthesis of the lacto-n-biose Galβ1,3GlcNAc-$(CH_2)_3$-N_3



2.2.1.1 Enzyme sources

BiGalHexNAcP was produced in house exactly as previously described.[13] BiGalK expression conditions were based on those described previously.[14] A plasmid encoding the GalK gene from *Bifidobacterium longum* subsp. *infantis* (GenBank accession number: CEF10481.1) cloned into the NdeI and HindIII restriction sites of pET22b, was ordered from Genscript. The plasmid was introduced into chemically competent *E. coli* BL21(DE3) cells by heat shock and selected on LB agar with ampicillin (100 µg/mL) at 37 °C for 16 h. Starter cultures were prepared by picking single

clones into LB with ampicillin (100 µg/mL) and grown at 37 °C for 8 h with shaking (180 rpm). 1 mL of starter culture was added to 1L of LB with ampicillin (100 µg/mL) and grown to an OD_{600nm} of 0.6 – 0.7 at 37 °C with shaking (180 rpm). Cultures were placed in an ice bath for 15 min. IPTG was added to a final concentration of 0.2 mM and the cultures were grown at 16 °C for 20 h with shaking (180 rpm). Cells were harvested by centrifugation (6000 x g, 6 °C, 10 min) and the pellet was resuspended in lysis buffer (30 mM Tris pH 8.0, 300 mM NaCl, 20 mM imidazole, 1 mg/mL lysozyme, protease inhibitor, benzonase 1 U/mL). The cells lysed by sonication on ice and the lysate was clarified by centrifugation (18 000 rpm, 4 °C, 50 min). The lysate was loaded onto a HisTrap FF column (GE Healthcare) pre-equilibrated with binding buffer (30 mM Tris pH 8.0, 300 mM NaCl, 20 mM imidazole). After washing the column with 20 column volumes of binding buffer, recombinant BiGalK with a C-terminal His_6-tag was eluted with elution buffer (30 mM Tris pH 8.0, 300 mM NaCl, 250 mM imidazole). The purity of BiGalK was assessed by SDS-PAGE (Fig. S2). The protein was dialysed into 30 mM Tris pH 8.0 buffer. The protein was stored in 10 % (v/v) glycerol at -80 °C. Typical yield was ~ 40 mg/L culture.

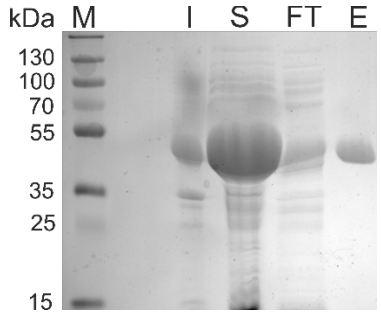
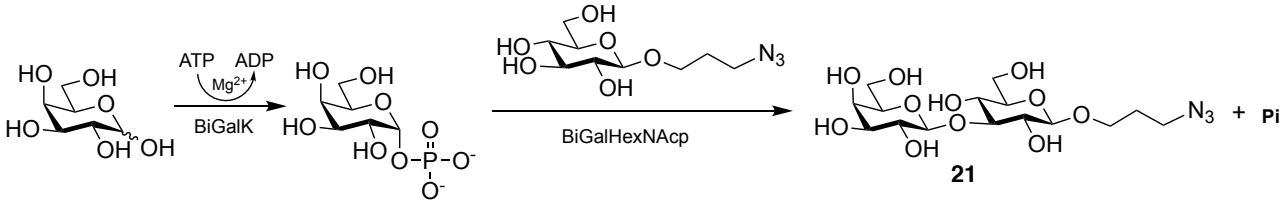


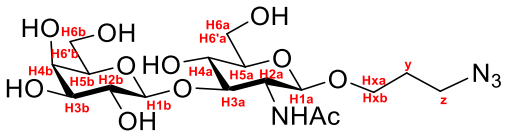
Fig. S2 SDS-PAGE analysis of BiGalK after Ni^{2+}-NTA affinity purification. 1: molecular weight marker, 2: insoluble fraction, 3: soluble fraction, 4: flow through, 5: elution fraction.

2.2.1.2 Synthesis of Galβ1,3GlcNAc-N_3 21



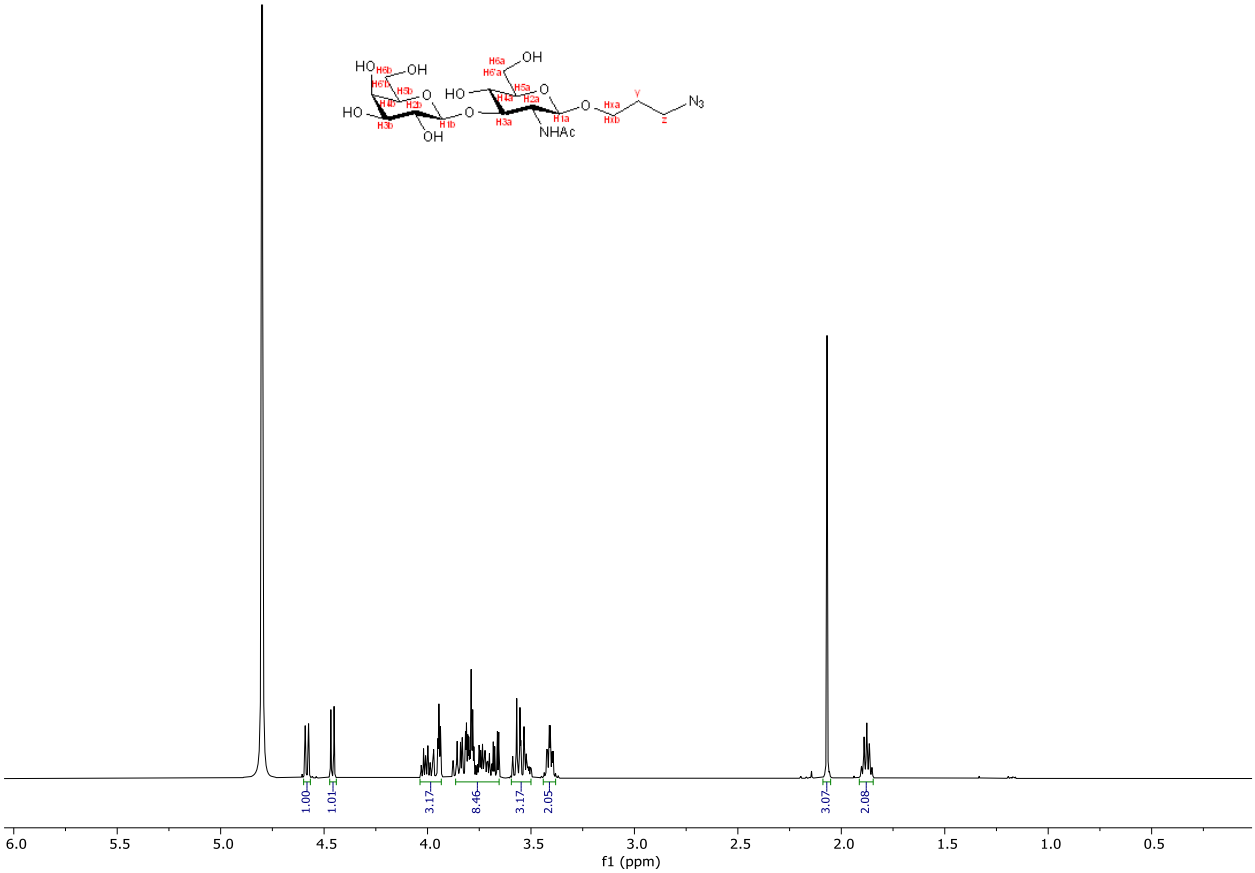
Reactions were assembled by adding GlcNAc-N_3 (77 mg), Gal (88.77 mg) and ATP (407 mg) in 4 mL of ddH_2O, to 1644 µL of 1 M Tris buffer (pH 6.5) and 328 µL of 1 M $MgCl_2$ in a 50 mL falcon tube. After adjusting the pH to 6.5, 10.7 mg of BiGalK and 7.8 mg of BiGalHexNAcP was added and the reaction was made up to 16.44 mL with ddH_2O. After incubation at 37 °C with shaking (120 rpm) for 90 h, the enzymes were removed by ultrafiltration (Vivaspin Protein Concentrator Spin Column 30000 MWCO). The reaction was lyophilised, resuspended in ddH_2O and purified by BioGel P2 column chromatography in H_2O. Fractions containing the desired product (**21**) were dried onto silica gel and further purified by flash chromatography (EtOAc: MeOH: H2O = 4:1:0.1).

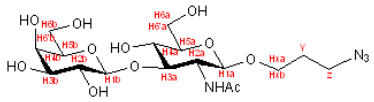
Fractions containing the desired product (**21**) were pooled and evaporated *in vacuo* (55.2 mg, 47 %). Characterisation data in agreement with the literature.[15]

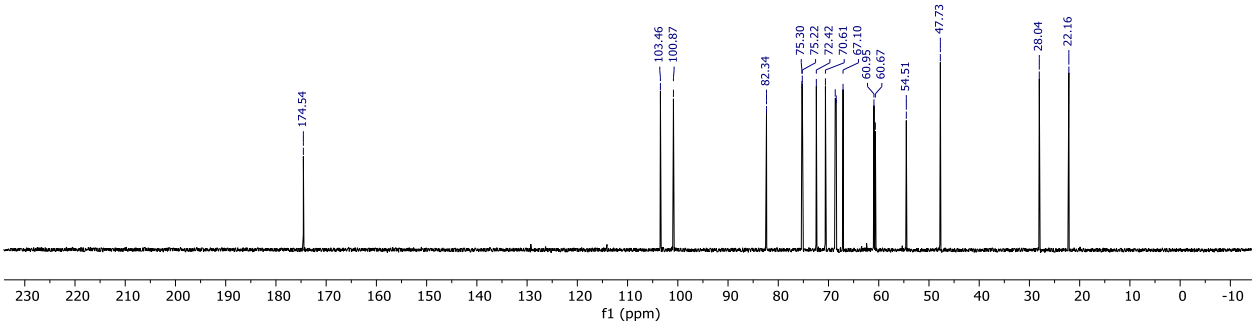


Appearance: white powder. **ATR-FTIR** V_{max} (thin film/cm^{-1}) 3305, 2881, 2410, 2098, 1948, 1614, 1424, 1372, 1300, 1116, 1077, 1077, 1029, 892, 779, 618, 551 cm^{-1}. **HRMS** (ESI) *m/z* calcd. for $C_{17}H_{31}N_4O_{11}$ (M + H) 467.1987 found 467.1986. **^{1}H NMR (500 MHz, D_2O)** δ 4.58 (d, *J* = 8.1 Hz, 1H, H-1b), 4.46 (d, *J* = 7.8 Hz, 1H, H-1a), 4.04 – 3.93 (m, 3H), 3.86 – 3.65 (m, 8H), 3.59 – 3.50 (m, 3H), 3.41 (td, *J* = 6.6, 2.4 Hz, 2H, H-z), 2.07 (s, 3H, NHCOC$\underline{H}_3$), 1.91 – 1.85 (m, 2H, H-y) ppm; **^{13}C NMR (125 MHz, D_2O)** δ 174.54 (C=O), 103.46 (C-1a), 100.87 (C-1b), 82.34, 75.30, 75.22, 72.42, 70.61, 68.66, 68.46, 67.10 (C-x), 60.95, 60.67, 54.51, 47.73 (C-z), 28.04 (C-y), 22.16 (NHCO$\underline{C}H_3$) ppm.

2.2.1.3 ^{1}H NMR and ^{13}C NMR spectra of Synthesis of Galβ1,3GlcNAc-N_3 21





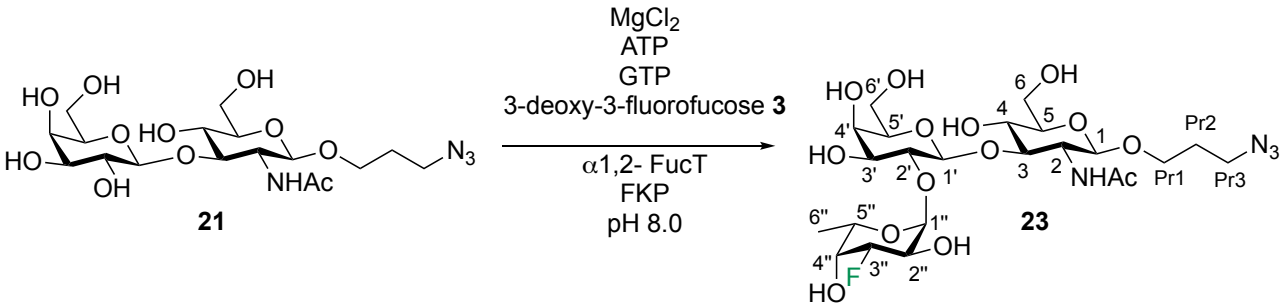


2.2.2 Enzymatic synthesis of Fuc(α1,2)Gal(β1-3)GlcNAc-$(CH^2)_3$-N_3 23

2.2.2.1 Enzyme source

α-1,2-Fucosyltransferase from *H. mustelae* was purchased from Chemily Glycoscience.

2.2.2.2 Synthesis of Fuc(α1,2)Gal(β1-3)GlcNAc-$(CH^2)_3$-N_3 23

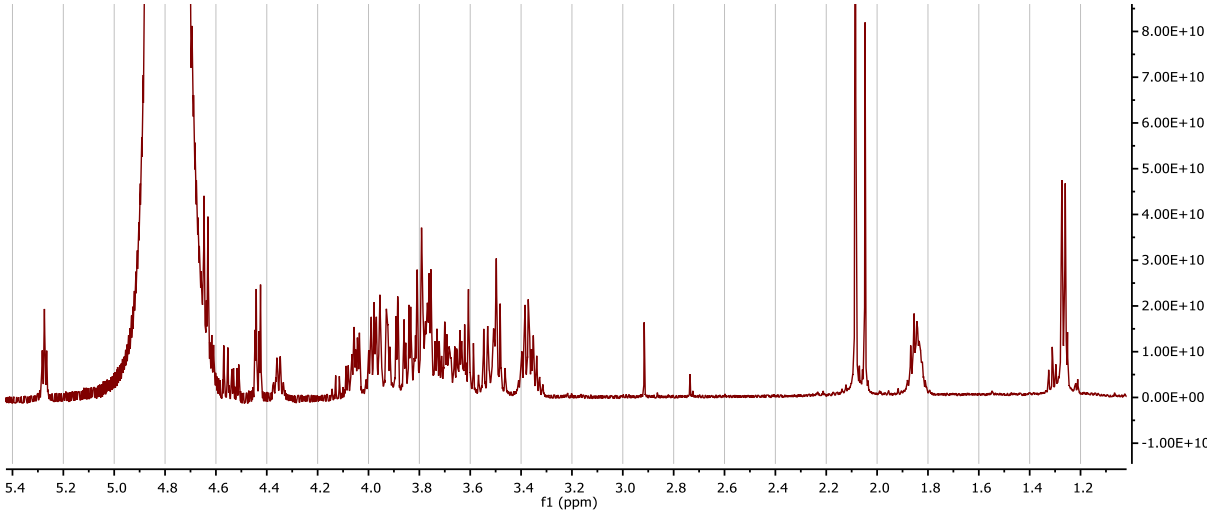


To Gal(β1-3)GlcNAc-$(CH_2)_3$-N_3 **21** (12 mg, 2.56 mM) was added Tris, pH 8.0 (242 mg, 200 mM), MgCl2 (9.5 mg, 10 mM), ATP (135 mg, 10 mM), GTP (67.5 mg, 5 mM), 3FFuc (4.98 mg, 3 mM), a1-2 FucT *HP* (0.4 U) and FKP (3.1 mg, 0.31 mg/ml) in H2O at a total volume of 8.12 ml was incubated overnight at 37 °C. As the reaction was not complete an additional aliquot of ATP (45 mg), GTP (45 mg), a1-2 FucT *HP* (0.5 U) and FKP (0.15 mg) was added and the reaction incubated overnight at 37 °C. Again the reaction was not completed and so ATP (45 mg), GTP (45 mg), a1-2 FucT *HP* (0.2 U) and FKP (0.15 mg) was added and incubated overnight at 37 °C. The reaction mixture was

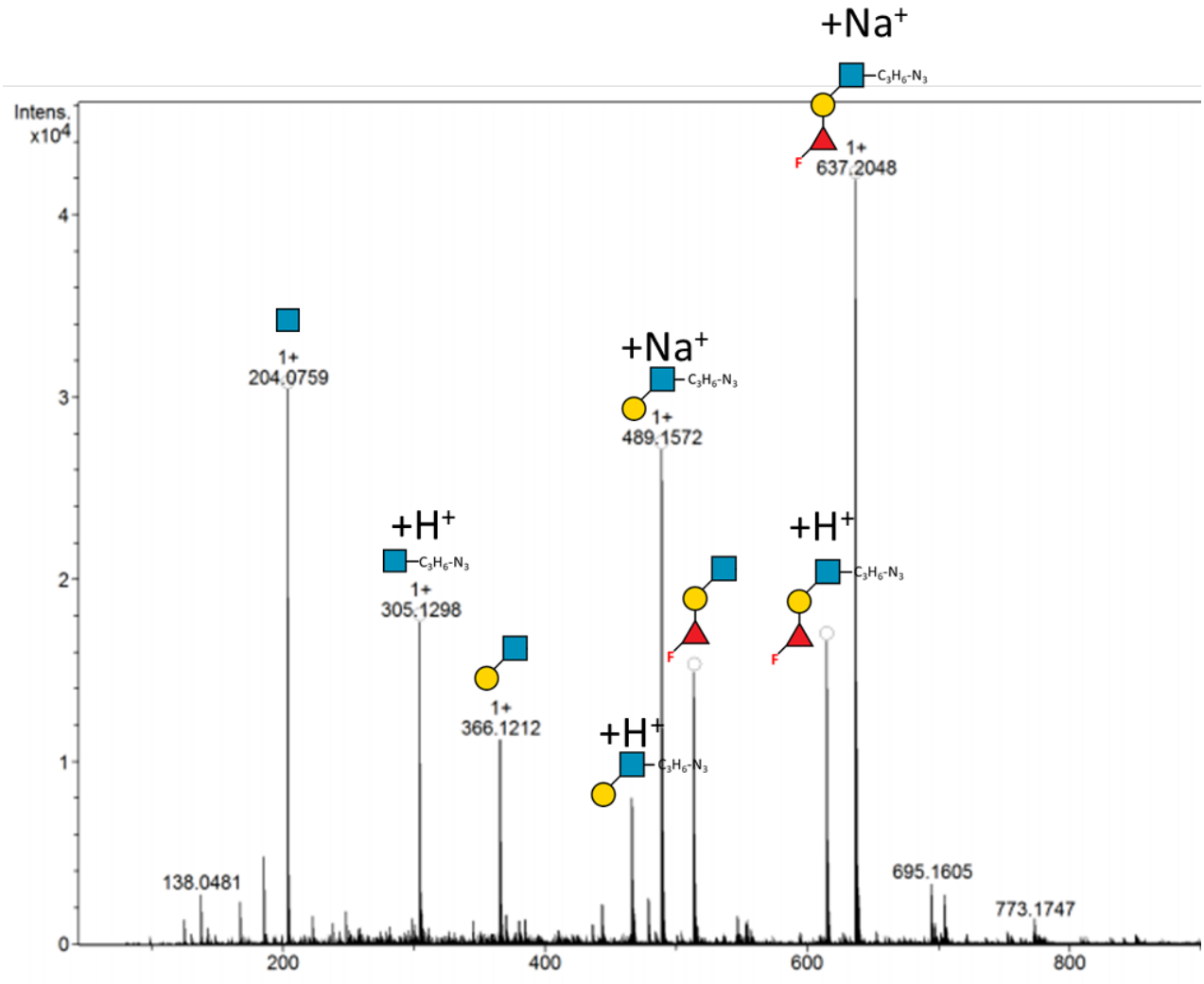
passed through a 10K MWCO spin concentrator and the filtrate was dried under reduced pressure with 600 mg silica gel. The resulting powder was loaded into a 4 g empty dry load cartridge and connected to a 12 g flash silica cartridge. The separation was run on a biotage flash system (20:80 MeOH:EtOAc → 50:50 MeOH:EtOAc over 15 CV). The fractions containing the product were pooled, the solvent removed under reduced pressure and re-dissolved in water. The mixture was then finally purified by size exclusion on a 10/300 biogel P2 column and the fractions containing product pooled and freeze dried to give the product as a white powder (1.3 mg, 8%). NMR analysis showed a 27% impurity of Gal(β1-3)GlcNAc-$(CH_2)_3$-N_3. **^{1}H NMR** (501 MHz, Deuterium Oxide) δ 5.27 (t, J = 4.4 Hz, 1H, H-1''), 4.64 (d, J = 7.7 Hz, 1H, H-1'), 4.57 (ddd, J = 49.9, 10.2, 3.5 Hz, 1H, H-3''), 4.44 (d, J = 8.1 Hz, 1H, H-1), 4.36 (q, J = 6.3 Hz, 1H, H-5''), 4.10 – 4.03 (m, 2H, H-2'', H-4''), 4.01 – 3.95 (m, 2H, H-3, H-Pr1a), 3.94 – 3.91 (m, 1H, H-6a), 3.89 (d, J = 3.3 Hz, 1H, H-4'), 3.85 (dd, J = 9.7, 3.3 Hz, 1H, H-3'), 3.83 – 3.74 (m, 4H, H-2, H-6a', H-6b, H-6b'), 3.74 – 3.63 (m, 2H, H-5', H-Pr1b), 3.60 (dd, J = 9.7, 7.7 Hz, 1H, H-2'), 3.43 – 3.30 (m, 2H, H-4, H-5), 3.41 – 3.31 (m, 2H, H-Pr3), 2.09 (s, 3H, H-Ac), 1.88 – 1.80 (m, 2H, H-Pr2), 1.27 (d, J = 6.6 Hz, 3H, H-6''). **^{13}C NMR** (126 MHz, D2O) δ 100.2 (C-1), 101.8 (C-1'), 99.4 (C-1''), 90.2 (C-3''), 77.2 (C-3), 76.5 (C-2'), 75.4 (C-5), 75.1 (C-5'), 73.5 (C-3'), 70.1 (C-4''), 69.2 (C-4'), 68.7 (C-4), 67.3 (C-Pr1), 66.6 (C-2''), 66.0 (C-5''), 61.0 (C-6'), 60.9 (C-6), 54.8 (C-2), 47.6 (C-Pr3), 28.0 (C-Pr2), 22.3 (C-Ac), 14.7 (C-6''). **^{19}F NMR** (471 MHz, D2O) δ -201.12. **HRMS**: Found 637.2048 ([M+Na]$^+$), $C_{23}H_{39}FN_4O_{14}Na^+$ requires 637.2339.

2.2.2.3 ^{1}H NMR (500 MHz, D_2O) of Purified 3FFuc(α1,2)Gal(β1-3)GlcNAc-$(CH_2)_3$-N_3 23

(mixture with disaccharide substrate **21**)



2.2.2.4 HRMS of Purified 3FFuc(α1,2)Gal(β1-3)GlcNAc-$(CH_2)_3$-N_3 23.



The trisaccharide **23** $[M+Na]^+$ peak at 637.23 is clear as well as the disaccharide **21** $[M+Na]^+$ peak at 489.15.

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