Supporting Information

Synthesis and Screening of Alpha-Xylosides in Human Glioblastoma Cells

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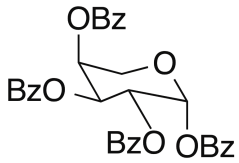
1. Materials

All reactions were done under dry and nitrogen atmosphere unless mentioned otherwise. Anhydrous chemicals and solvents were purchased from commercial suppliers— Dichloromethane (CAS 75-09-2, Sigma-Aldrich), Benzoyl chloride (CAS 98-88-4, Sigma-Aldrich), Pyridine (CAS 110-86-1, Sigma-Aldrich), Tin (IV) chloride (CAS 7646-78-8, Sigma-Aldrich), azido-trimethylsilane (CAS 4648-54-8, Sigma-Aldrich), (diethylamino)sulfur trifluoride (CAS 38078-09-0, Sigma-Aldrich), Sodium methoxide (CAS 124-41-4, Sigma-Aldrich), Methanol (CAS 67-56-1, Sigma-Aldrich), Cyclopentylacetylene (CAS 930-51-8, Sigma-Aldrich), 1-ethynyl-4-nitrobenzene (CAS 937-31-5, Sigma-Aldrich), phenylacetylene (CAS 536-74-3, Sigma-Aldrich), Amberlite IR120 hydrogen form (ACROS Organics, CAS 78922-04-0). Column chromatography was done with manual column chromatography using silica gel 60 (36-71 μm, Alfa Aesar) as solid phase. Some purification is done with SilicaFlash R60 gel (20-45 μm, SiliCycle Inc.) as mentioned in the Table 1. Thin layer chromatography (TLC) was conducted on pre-coated polyester sheets (40 x 80 mm) from Machery-Nagel (POLYGRAM® SIL G/UV254) with 0.2 mm silica gel 60 with fluorescent indicator. For visualization, a UV light source (254 nm and 366 nm) was used. The liquid chromatography-low resolution mass spectrometry was conducted in Waters Acquity UPLC BEH 17 um, 2.1 X 50 mm column using water/acetonitrile with 0.1% formic acid mobile phase. Instrument is Waters Acquity FTN-H Sample manager equipped with Acquity Quarternary solvent manager, Acquity Photo Diode Array detector, Acquity QDa mass detector & Acquity Evaporative Light Scattering detector. Nuclear magnetic resonance spectroscopy (NMR) was measured on a Bruker Avance III HD 400 equipped with a (^{1}H/^{13}C/^{19}F) probe head. Chemical shifts (δ) are given in ppm, coupling constants J in Hertz (Hz) and the multiplicities of the signals are designated as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, and m = multiplet. Reference for ^{1}H, ^{13}C is tetramethylsilane (TMS). The solvent signal of $CDCl_3$ and CD_3OD were calibrated on 7.26 ppm and 3.34 ppm for ^{1}H-NMR and 77.0 ppm and 49.15 ppm for ^{13}C-NMR spectra. Notre Dame Mass Spectrometry & Proteomics Facility recorded high-resolution mass spectra (HRMS). MTT assay was carried out using CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega, #G4100). U-251 MG cell line human, purchased from Sigma (catalog 09063001). U-87 MG (ATCC® HTB-14™) was purchased from the ATCC.

2. Synthesis of 4-deoxy-4-fluoro-xylose azides

Synthesis of 1,2,3,4-tetrabenzoyl-L-arabinose (**2**)

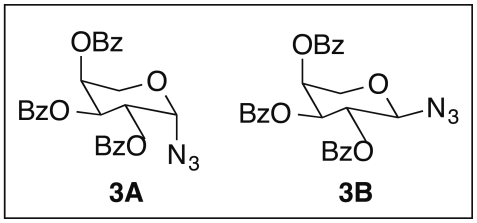


2

L-(+)-arabinose (10 g, 66.6 mmol) was charged into a 1 L round bottom flask (RB flask) and dried under vacuum for 30 min followed by three vacuum-nitrogen flushes. Dry pyridine (250 mL) was added to above flask resulting a heterogeneous mixture. Temperature of the mixture as brought to -20 °C by immerging the RB flask in dry ice/acetonitrile bath for 15 min. Benzoyl chloride (31 mL, 266 mmol) was added dropwise. Reaction was left in the dry ice bath for 16 hours (temperature changes from -20 °C to room temperature in this period). Rotavap-dry ice/acetone trap was used to evaporate off pyridine. The crude reaction was stirred vigorously in ethyl acetate (300 mL) and water (300 mL) and then transferred to a separatory funnel. The organic layer was washed with 1N HCl (5X 100 mL), saturated $NaHCO_3$ (2X 100 mL), brine (2X

100 mL), dried over anhydrous Na_2SO_4, evaporated off through rotavap yielding crude grey solid of ~20 g. The crude reaction mixture was used in the next step without purification.

Synthesis of 1-azido-2,3,4-tribenzoyl-L-arabinose (**3A, 3B**)

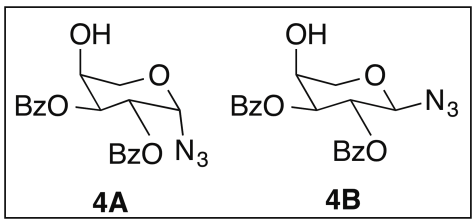


To a solution of **2** (10.3 g, 17.65 mmol) in dry DCM (200 mL) was added azido-trimethylsilane (3.5 mL, 26.47 mmol) dropwise, followed by tin (IV) chloride (1.03 mL, 8.82 mmol) addition. The reaction was stirred at room temperature for 16 hours. TLC analysis showed two products of R_f values- 0.66 (α-isomer) and 0.54 (β-isomer) in 7:3 hexane:ethyl acetate (H:E) thin layer chromatography (TLC) solvent. DCM was evaporated off and crude reaction was dissolved in ethyl acetate (250 mL). The organic layer was washed with saturated $NaHCO_3$ (5X 50 mL), brine (2X 50 mL), dried over anhydrous Na_2SO_4 and evaporated off through rotavap. Finally, the α- and β- isomers were separated through silica gel column chromatography in H:E (9:1). This yielded 3.67 g of **3A** (42.6% overall yield) and 3.44 g of **3B** (40% overall yield).

Compound **3A**
^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.13 – 8.06 (m, 2H), 8.04 – 7.97 (m, 2H), 7.89 – 7.82 (m, 2H), 7.60 (d, *J* = 7.4 Hz, 1H), 7.57 – 7.37 (m, 6H), 7.33 – 7.24 (m, 2H), 5.88 (d, *J* = 3.8 Hz, 1H), 5.84 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.82 – 5.72 (m, 2H), 4.36 (dd, *J* = 13.4, 1.6 Hz, 1H), 4.11 (dd, *J* = 13.3, 2.2 Hz, 1H).
^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.82, 165.67, 165.46, 133.72, 133.55, 133.36, 129.96, 129.89, 129.74, 129.40, 129.02, 128.69, 128.62, 128.58, 128.38, 87.64, 69.54, 68.54, 67.61, 62.61.
HRMS (ESI): Calcd.for $C_{26}H_{21}N_3NaO_7$ $[M+Na]^+$ 510.1272, found 510.1268

Compound **3B**
^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.11 – 8.04 (m, 2H), 8.04 – 7.97 (m, 2H), 7.92 – 7.85 (m, 2H), 7.65 – 7.38 (m, 7H), 7.36 – 7.24 (m, 3H), 5.76 – 5.67 (m, 2H), 5.61 (dd, *J* = 9.3, 3.4 Hz, 1H), 4.95 (d, *J* = 7.6 Hz, 1H), 4.41 (dd, *J* = 13.1, 3.3 Hz, 1H), 4.02 (dd, *J* = 13.2, 1.8 Hz, 1H).
^{13}CNMR ($CDCl_3$, 400 MHz): 165.62, 165.51, 165.21, 133.63, 133.60, 133.49, 129.91, 129.84, 129.24, 128.84, 128.78, 128.63, 128.53, 128.45, 88.66, 70.71, 69.22, 68.40, 65.38.
HRMS (ESI): Calcd.for $C_{26}H_{21}N_3NaO_7$ $[M+Na]^+$ 510.1272, found 510.1271

Synthesis of 1-azido-2,3-dibenzoyl-4-hydroxy-L-arabinose (**4A, 4B**)



Compounds **3A** (3.45 g, 7.07 mmol) and sodium methoxide (1.15 g, 21.23 mmol) were charged into a 100 mL RB flask equipped with a magnetic stir bar. A heterogeneous mixture was formed after addition of methanol (25 mL). The mixture was stirred vigorously for 16 hours. pH of the reaction was kept at ~9 during this period. The reaction was diluted with methanol (20 mL) and pH was brought down to ~7 by adding Amberlite IR120 hydrogen form. As soon as pH reached 7, the solutions were filtered immediately through glass wool. Methanol was evaporated off yielding crude product. Methyl benzoate, a side product of this reaction was removed by washing

the crude product with hexane (5x 10 mL) with sonication for 10 min. This resulted in 1.2 g of α- (100% yield) and 1.38 g of β- (100% yield).

The crude α- (or β-) compound (1.2 g, 7.07 mmol) was dissolved in dry pyridine (30 mL) and temperature was brought to -40 ^{0}C by using dry ice/acetonitrile bath. Benzoyl chloride (1.5 mL, 12.7 mmol) was added *very* slowly and let the reaction stir for 16 hours. Pyridine was evaporated and crude reaction was dissolved in ethyl acetate (50 mL). The organic layer was washed with 1N HCl (3X 25 mL), saturated $NaHCO_3$ (2X 25 mL), brine (2X 50 mL), dried over anhydrous Na_2SO_4 and evaporated off through rotavap. The dibenzoylated compound (**4A**, R_f= 0.31 for α-; **4B**, R_f= 0.25 for β- in H:E 7:3 TLC solvent mixture) was purified through silica gel column chromatography using H:E (4:1 for α- and 3:1 for β-) yielding 1.91 g of **4A** (18% yield) and 1.22 g of **4B** (12.3% yield).

NOTE: The side products of this reaction were recycled through sodium methoxide treatment. Next, di-benzoylation step was carried out.

Compound **4A**

^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.02 – 7.95 (m, 4H), 7.54 (tt, *J* = 7.4, 1.4 Hz, 2H), 7.39 (ddd, *J* = 9.1, 7.1, 1.7 Hz, 4H), 5.80 – 5.70 (m, 2H), 5.61 (dd, *J* = 9.9, 3.1 Hz, 1H), 4.36 (q, *J* = 2.4 Hz, 1H), 4.21 (dd, *J* = 12.7, 1.7 Hz, 1H), 3.97 (dd, *J* = 12.7, 2.5 Hz, 1H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.78, 165.64, 133.62, 129.93, 129.89, 129.82, 129.04, 128.81, 128.57, 128.55, 87.68, 70.25, 68.10, 67.71, 64.33.

HRMS (ESI): Calcd.for $C_{19}H_{17}N_3NaO_6$ [M+Na]$^{+}$ 406.1010, found 406.1009

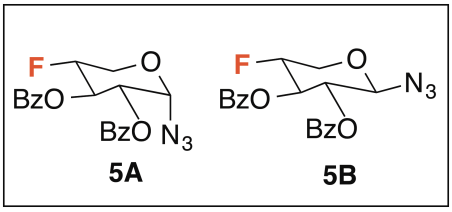
Compound **4B**

^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.97 (ddt, *J* = 8.4, 4.5, 1.9 Hz, 4H), 7.55 – 7.41 (m, 2H), 7.40 – 7.26 (m, 4H), 5.68 (ddd, *J* = 9.1, 7.5, 2.5 Hz, 1H), 5.40 (dd, *J* = 9.1, 3.2 Hz, 1H), 4.90 (dd, *J* = 7.5, 2.5 Hz, 1H), 4.37 (dt, *J* = 5.2, 2.4 Hz, 1H), 4.21 (dt, *J* = 12.7, 3.2 Hz, 1H), 3.86 (dd, *J* = 12.7, 2.0 Hz, 1H), 3.25 (s, 1H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 166.03, 165.40, 133.60, 133.56, 129.94, 129.88, 128.96, 128.89, 128.57, 128.51, 88.57, 73.33, 69.22, 67.32, 66.60.

HRMS (ESI): Calcd.for $C_{19}H_{17}N_3NaO_6$ [M+Na]$^{+}$ 406.1010, found 406.1000

Synthesis of 1-azido-2,3-dibenzoyl-4-deoxy-4-fluoro-L-arabinose (**5A, 5B**)



Compound **4A** (0.55 g, 1.43 mmol) was dissolved in dry DCM (20 mL) under N_2 atmosphere. Temperature of the reaction was brought to -40 ^{0}C by immerging the RB flask in dry ice/acetonitrile bath. Diethylaminosulfurtrifluoride (DAST,) was added slowly to the above solution resulting a pale yellow reaction mixture. The reaction was left at -40 ^{0}C and let warm slowly in 16 h. The reaction was then quenched with methanol (5 mL). The organic layer was washed with saturated $NaHCO_3$ (2X 25 mL), brine (2X 25 mL), dried over anhydrous Na_2SO_4 and evaporated off through rotavap. The crude product was subjected to column chromatography in silica gel using H:E (9:1 for both α- and β-) yielding 200 mg of **5A** (36.3% yield) and 246 mg of **5B** (44.74% yield)

Compound **5A**

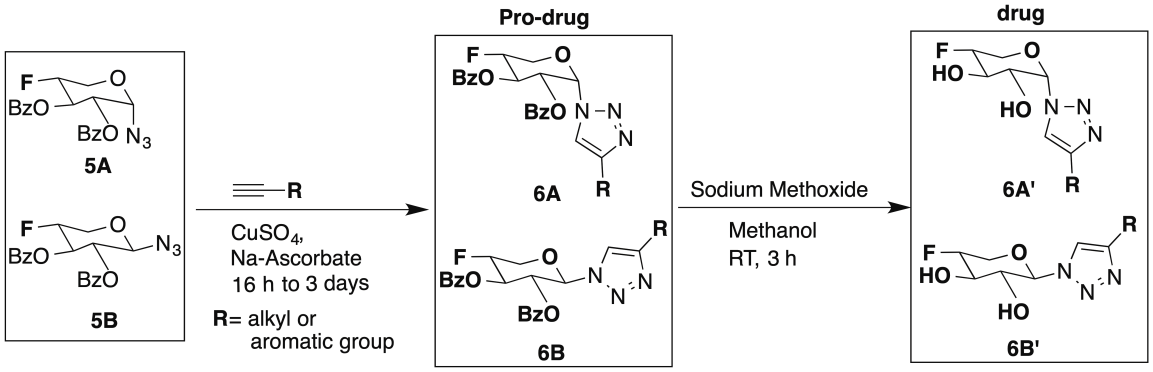
^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.00 (ddd, *J* = 8.4, 4.7, 1.4 Hz, 4H), 7.54 (dddt, *J* = 6.6, 5.0, 3.1, 1.6 Hz, 2H), 7.40 (d, *J* = 15.3 Hz, 2H), 5.90 (ddd, *J* = 12.9, 9.6, 8.6 Hz, 1H), 5.69 (t, *J* = 3.7 Hz, 1H), 5.21 (ddd, *J* = 9.5, 4.1, 0.9 Hz, 1H), 4.79 (dtd, *J* = 49.6, 9.2, 6.4 Hz, 1H), 4.16 – 4.06 (m, 2H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.62, 165.38, 133.79, 133.48, 130.04, 129.83, 129.09, 128.59, 128.47, 128.42, 87.14, 86.36, 85.29, 70.34, 70.26, 70.17, 69.96, 61.23, 60.96.

^{19}FNMR (CDCl3, 400 MHz): δ -198.24.
HRMS (ESI): Calcd.for $C_{19}H_{16}FN_3NaO_5$ $[M+Na]^+$ 408.0966, found 408.0969

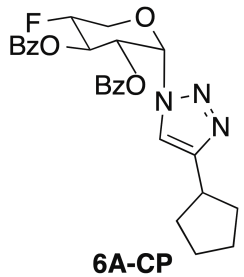
Compound **5B**
^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.07 – 7.96 (m, 4H), 7.60 – 7.51 (m, 2H), 7.43 (td, *J* = 7.7, 6.7, 4.4 Hz, 4H), 5.66 (dt, *J* = 11.9, 7.2 Hz, 1H), 5.25 (q, *J* = 7.2, 6.8 Hz, 1H), 5.04 (t, *J* = 4.5 Hz, 1H), 4.81 (dtd, *J* = 48.1, 7.4, 4.4 Hz, 1H), 4.40 (td, *J* = 12.7, 4.5 Hz, 1H), 3.85 (dt, *J* = 12.1, 7.4 Hz, 1H).
^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.23, 165.06, 133.67, 133.63, 129.99, 129.94, 128.82, 128.70, 128.53, 88.10, 86.52, 84.68, 70.82, 70.60, 69.40, 69.35, 63.59, 63.33
^{19}FNMR (CDCl3, 400 MHz): δ -198.14.
HRMS (ESI): Calcd.for $C_{19}H_{16}FN_3NaO_5$ $[M+Na]^+$ 408.0966, found 408.0975

3. General Procedure for Copper (I) Click Chemistry



3.1. Prodrug Synthesis via Copper (I) click reaction - Compound **5A** (or **5B**) (50 mg, 0.13 mmol) and an alkyne (0.194 mmol) were charged into a 50 mL RB flask equipped with a stir bar. A cocktail of acetone/water/THF (1:1:1, 3 mL) was added and stirred vigorously. Freshly prepared 1M sodium ascorbate (52 μL, 0.052 mmol) was added to the above reaction. After 5 min, freshly prepared an aqueous solution of 1M copper sulfate (26 μL, 0.026 mmol) was added. The reaction mixture was stirred at room temperature from 16 hours to 3 days depending upon the rate of the reaction, which was monitored through TLC. (**Table 1**) The organic compound was extracted with ethyl acetate (3X 5 mL), washed with brine (2X 5 mL), dried over anhydrous Na_2SO_4 and evaporated in rotavap. The crude reaction was purified through column chromatography (or preparatory TLC).

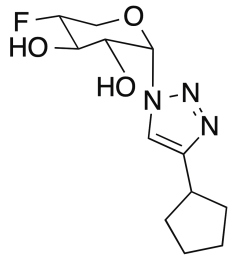
3.2. Deprotection of Prodrug- Compound **6A/6B** (0.062 mmol) was treated with sodium methoxide (0.156 mmol) in methanol (5 mL) in a glass vial for 3 h at room temperature. pH of the reaction was brought to neutral by adding Amberlite IR-120 hydrogen form and immediately filtered through glass wool. Methanol was evaporated through rotavap and the crude reaction was washed with cold hexane (7X 10 mL) or TLC showed complete removal of methyl benzoate side product. In some cases, the desired product was purified through either column chromatography. (**Table 1**)



6A-CP

^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.12 – 8.05 (m, 4H), 7.99 – 7.92 (m, 4H), 7.67 – 7.54 (m, 4H), 7.53 – 7.42 (m, 7H), 7.42 (d, *J* = 7.7 Hz, 3H), 6.45 (d, *J* = 3.3 Hz, 2H), 6.15 (dt, *J* = 10.1, 5.3 Hz, 2H), 5.59 (dd, *J* = 5.6, 3.3 Hz, 2H), 4.90 (td, *J* = 4.9, 3.0 Hz, 1H), 4.78 (td, *J* = 4.9, 3.0 Hz, 1H), 4.50 – 4.38 (m, 2H), 4.26 (ddd, *J* = 25.7, 13.0, 3.1 Hz, 2H), 3.11 (p, *J* = 7.8 Hz, 2H), 2.02 – 1.90 (m, 4H), 1.58 (ddt, *J* = 6.2, 4.1, 2.4 Hz, 7H), 1.56 – 1.43 (m, 3H).
^{13}CNMR ($CDCl_3$, 400 MHz): δ 164.85, 164.41, 152.72, 133.91, 133.83, 129.95, 129.92, 128.72, 128.68, 128.62, 128.43, 120.00, 85.28, 83.44, 82.94, 77.40, 77.09, 76.77, 68.52, 68.49, 68.34, 68.07, 65.65, 65.41, 36.46, 32.93, 25.00, 24.97.
^{19}FNMR ($CDCl_3$, 400 MHz): δ -194.99.

HRMS (ESI): Calcd.for $C_{26}H_{27}FN_3O_5$ $[M+H]^+$ 480.1929, found 480.1930



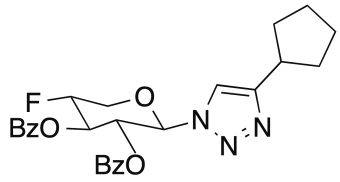
6A'-CP

^{1}HNMR (CD_3OD, 400 MHz): δ 7.96 (s, 1H), 6.10 (d, *J* = 3.0 Hz, 1H), 4.61 – 4.54 (m, 1H), 4.45 (t, *J* = 4.2 Hz, 0H), 4.35 (dt, *J* = 10.3, 5.1 Hz, 1H), 4.17 (td, *J* = 12.2, 11.5, 3.8 Hz, 1H), 4.11 (d, *J* = 3.8 Hz, 1H), 3.92 (dd, *J* = 5.2, 3.2 Hz, 1H), 3.19 (p, *J* = 7.9 Hz, 1H), 2.17 – 2.06 (m, 2H), 1.87 – 1.63 (m, 7H)

^{13}CNMR (CD_3OD, 400 MHz): δ 121.52, 88.87, 87.09, 85.01, 69.83, 69.80, 69.25, 69.03, 65.42, 65.19, 36.45, 32.76, 24.67.

^{19}FNMR (CD_3OD, 400 MHz): δ -194.70.

HRMS (ESI): Calcd.for $C_{12}H_{19}FN_3O_3$ $[M+H]^+$ 272.1405, found 272.1408



6B-CP

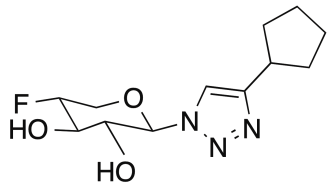
^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.99 – 7.92 (m, 2H), 7.80 – 7.73 (m, 2H), 7.59 – 7.35 (m, 5H), 7.35 – 7.24 (m, 1H), 6.03 (d, *J* = 8.6 Hz, 1H), 5.96 – 5.81 (m, 2H), 5.11 – 4.88 (m, 1H), 4.50 – 4.37

(m, 1H), 3.93 (ddd, J = 11.9, 10.0, 4.5 Hz, 1H), 3.15 (p, J = 8.0 Hz, 1H), 2.09 – 1.97 (m, 1H), 1.77 – 1.68 (m, 1H), 1.71 – 1.64 (m, 0H), 1.65 (s, 1H), 1.67 – 1.55 (m, 1H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.35, 164.82, 153.49, 133.71, 133.69, 133.61, 130.01, 129.88, 129.80, 129.77, 128.70, 128.63, 128.52, 128.49, 128.44, 127.98, 117.97, 87.27, 86.19, 85.41, 73.19, 72.99, 70.01, 69.93, 65.85, 65.57, 36.60, 33.06, 32.92, 25.02, 25.00.

^{19}FNMR ($CDCl_3$, 400 MHz): δ -200.33.

HRMS (ESI): Calcd.for $C_{26}H_{27}FN_3O_5$ $[M+H]^+$ 480.1929, found 480.1933



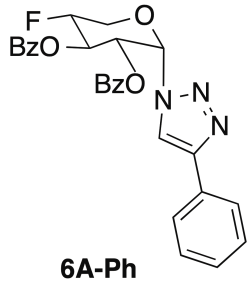
6B'-CP

^{1}HNMR (CD_3OD, 400 MHz): δ 7.97 – 7.92 (m, 1H), 5.55 (d, J = 9.1 Hz, 1H), 4.68 – 4.45 (m, 1H), 4.22 (dd, J = 11.3, 5.7 Hz, 1H), 3.99 (td, J = 9.2, 0.8 Hz, 1H), 3.80 (ddd, J = 15.3, 9.3, 8.6 Hz, 1H), 3.70 (ddd, J = 11.4, 10.3, 3.8 Hz, 1H), 3.26 – 3.13 (m, 1H), 2.18 – 2.07 (m, 2H), 1.85 – 1.77 (m, 1H), 1.81 – 1.65 (m, 4H).

^{13}CNMR (CD_3OD, 400 MHz): δ 174.45, 119.92, 89.57, 88.34, 88.32, 87.77, 75.37, 75.19, 71.89, 71.80, 65.38, 65.08, 36.47, 32.73, 32.72, 24.67.

^{19}FNMR (CD_3OD, 400 MHz): δ -202.44.

HRMS (ESI): Calcd.for $C_{12}H_{19}FN_3O_3$ $[M+H]^+$ 272.1405, found 272.1403



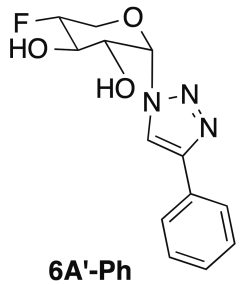
6A-Ph

^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.13 – 8.05 (m, 2H), 8.01 – 7.92 (m, 2H), 7.71 – 7.59 (m, 2H), 7.58 – 7.27 (m, 6H), 6.52 (dd, J = 3.5, 1.1 Hz, 1H), 6.22 (dt, J = 10.2, 5.4 Hz, 1H), 5.64 (dd, J = 5.8, 3.4 Hz, 1H), 4.86 (dtd, J = 46.2, 5.2, 3.2 Hz, 1H), 4.52 – 4.08 (m, 2H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.01, 164.45, 147.73, 133.94, 133.89, 129.99, 129.97, 128.90, 128.83, 128.71, 128.69, 128.63, 128.60, 128.51, 128.39, 128.33, 125.82, 125.78, 120.00, 85.36, 83.51, 83.17, 68.59, 68.56, 68.38, 68.12, 65.56, 65.32, 29.73.

^{19}FNMR ($CDCl_3$, 400 MHz): δ -194.95.

HRMS (ESI): Calcd.for $C_{27}H_{23}FN_3O_5$ $[M+H]+$ 488.1616, found 488.1611



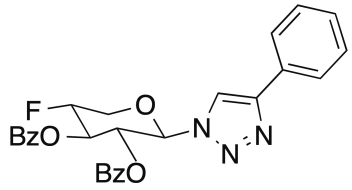
6A'-Ph

^{1}HNMR (CD_3OD, 400 MHz): δ 8.52 (s, 1H), 7.89 – 7.81 (m, 2H), 7.50 – 7.42 (m, 2H), 7.44 (d, *J* = 1.7 Hz, 1H), 7.45 – 7.32 (m, 1H), 6.20 (d, *J* = 3.0 Hz, 1H), 4.65 – 4.45 (m, 1H), 4.38 (dt, *J* = 10.1, 5.0 Hz, 1H), 4.29 – 4.15 (m, 2H), 3.99 (dd, *J* = 5.3, 3.0 Hz, 1H).

^{13}CNMR (CD_3OD, 400 MHz): δ 146.86, 130.20, 129.15, 128.62, 128.15, 128.07, 128.02, 125.42, 125.40, 125.32, 121.44, 88.84, 87.05, 85.27, 69.80, 69.78, 69.34, 69.16, 68.93, 68.87, 65.59, 65.36, 64.85.

^{19}FNMR (CD_3OD, 400 MHz): δ -194.69.

HRMS (ESI): Calcd.for $C_{13}H_{15}FN_3O_3$ $[M+H]^+$ 280.1092, found 280.1091



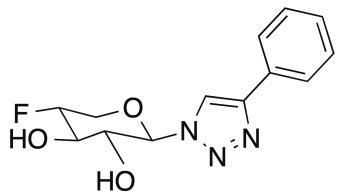
6B-Ph

^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.16 – 7.93 (m, 3H), 7.89 – 7.77 (m, 4H), 7.58 – 7.21 (m, 9H), 6.16 (d, *J* = 8.5 Hz, 1H), 6.02 – 5.89 (m, 2H), 5.20 – 4.88 (m, 1H), 4.52 (ddt, *J* = 13.4, 5.7, 2.7 Hz, 1H), 4.12 – 3.76 (m, 1H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.37, 164.84, 148.40, 133.77, 133.65, 130.03, 129.97, 129.90, 129.85, 128.95, 128.88, 128.67, 128.66, 128.53, 128.51, 128.50, 128.45, 127.89, 125.92, 117.92, 87.21, 86.39, 86.38, 85.35, 77.38, 77.06, 76.74, 73.13, 72.93, 69.99, 69.91, 65.88, 65.60.

^{19}FNMR ($CDCl_3$, 400 MHz): δ -200.19.

HRMS (ESI): Calcd.for $C_{27}H_{23}FN_3O_5$ [M+H]+ 488.1616, found 488.1615



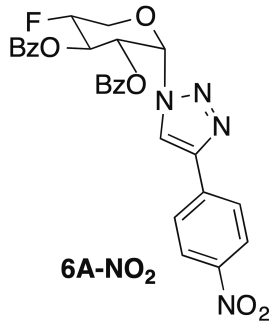
6B'-Ph

^{1}HNMR (CD_3OD, 400 MHz): δ 8.56 (s, 1H), 7.90 – 7.82 (m, 2H), 7.50 – 7.42 (m, 3H), 7.42 – 7.33 (m, 1H), 5.66 (d, *J* = 9.1 Hz, 1H), 4.61 (dddd, *J* = 50.0, 10.3, 8.6, 5.7 Hz, 1H), 4.27 (dd, *J* = 11.3, 5.7 Hz, 1H), 4.06 (td, *J* = 9.2, 0.8 Hz, 1H), 3.91 – 3.65 (m, 2H).

^{13}CNMR (CD_3OD, 400 MHz): δ 147.57, 130.12, 128.62, 128.11, 127.80, 125.35, 119.95, 89.58, 88.55, 87.78, 75.33, 75.15, 72.03, 71.94, 65.47, 65.17.

^{19}FNMR (CD_3OD, 400 MHz): δ -202.46.

HRMS (ESI): Calcd.for $C_{13}H_{15}FN_3O_3$ $[M+H]^+$ 280.1092, found 280.1089



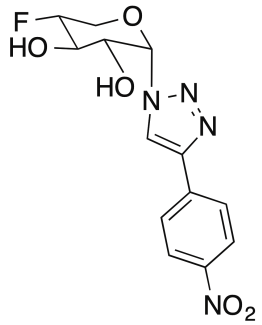
6A-NO_2

^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.25 – 8.17 (m, 2H), 8.14 – 8.06 (m, 3H), 8.00 – 7.93 (m, 2H), 7.85 – 7.78 (m, 2H), 7.69 – 7.46 (m, 4H), 7.51 – 7.38 (m, 2H), 6.55 (d, *J* = 3.1 Hz, 1H), 6.12 (dt, *J* = 9.5, 5.0 Hz, 1H), 5.65 (dd, *J* = 5.2, 3.1 Hz, 1H), 4.87 (dtd, *J* = 45.7, 4.6, 2.8 Hz, 1H), 4.46 (tdd, *J* = 11.7, 4.4, 1.1 Hz, 1H), 4.35 (dd, *J* = 13.2, 2.9 Hz, 1H), 4.28 (dd, *J* = 13.2, 2.9 Hz, 1H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 164.78, 164.35, 147.48, 145.58, 136.21, 134.09, 134.05, 129.98, 129.94, 128.77, 128.45, 128.24, 126.22, 124.27, 121.36, 84.93, 83.53, 83.09, 68.26, 68.24, 67.92, 67.65, 66.06, 65.82.

^{19}FNMR ($CDCl_3$, 400 MHz): δ -194.64.

HRMS (ESI): Calcd.for $C_{27}H_{22}FN_4O_7$ $[M+H]^+$ 533.1467, found 533.1468



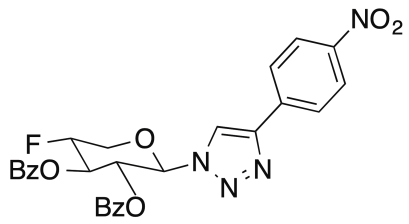
6A'-NO_2

^{1}HNMR (CD_3OD, 400 MHz): δ 8.74 (s, 1H), 8.41 – 8.25 (m, 2H), 8.21 – 8.05 (m, 2H), 6.23 (d, *J* = 2.9 Hz, 1H), 4.72 – 4.44 (m, 1H), 4.36 (dt, *J* = 9.8, 4.9 Hz, 1H), 4.29 – 4.12 (m, 2H), 3.99 (dd, *J* = 5.2, 3.0 Hz, 1H).

^{13}CNMR (CD_3OD, 400 MHz): δ 147.40, 144.74, 136.76, 132.85, 129.14, 128.15, 127.69, 125.97, 123.91, 123.19, 88.78, 85.41, 72.06, 69.69, 69.00, 68.78, 65.71, 65.47.

^{19}FNMR (CD_3OD, 400 MHz): δ -194.64.

HRMS (ESI): Calcd.for $C_{13}H_{14}FN_4O_5$ $[M+H]+$ 325.0943, found 325.0943



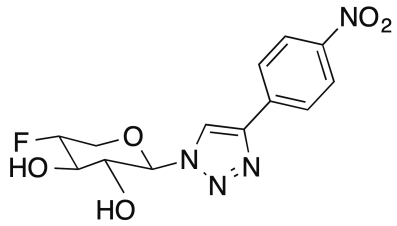
6B-NO_2

^{1}HNMR ($CDCl_3$, 400 MHz): δ 8.35 – 8.28 (m, 2H), 8.25 (s, 1H), 8.05 – 7.94 (m, 4H), 7.87 – 7.77 (m, 2H), 7.60 – 7.46 (m, 2H), 7.45 – 7.30 (m, 4H), 6.16 (d, *J* = 8.5 Hz, 1H), 6.07 – 5.88 (m, 2H), 5.07 (dddd, *J* = 49.1, 9.8, 8.1, 5.6 Hz, 1H), 4.55 (ddt, *J* = 10.6, 5.0, 2.5 Hz, 1H), 4.02 (ddd, *J* = 12.0, 9.9, 4.7 Hz, 1H).

^{13}CNMR ($CDCl_3$, 400 MHz): δ 165.31, 164.91, 147.61, 146.22, 136.15, 133.94, 133.73, 129.90, 129.88, 128.56, 128.53, 127.71, 126.43, 124.40, 124.34, 119.53, 87.07, 86.54, 85.20, 72.89, 72.68, 70.12, 70.04, 66.01, 65.73.

^{19}FNMR ($CDCl_3$, 400 MHz): δ -200.24.

HRMS (ESI): Calcd.for $C_{27}H_{22}FN_4O_7$ $[M+H]^+$ 533.1467, found 533.1474



6B'-NO_2

^{1}HNMR (CD_3OD, 400 MHz): δ 8.78 (s, 1H), 8.37 – 8.30 (m, 2H), 8.15 – 8.09 (m, 2H), 5.69 (d, *J* = 9.1 Hz, 1H), 4.73 – 4.48 (m, 1H), 4.28 (dd, *J* = 11.3, 5.7 Hz, 1H), 4.10 – 4.00 (m, 1H), 3.92 – 3.72 (m, 2H).

^{13}CNMR (CD_3OD, 400 MHz): δ 147.47, 145.45, 136.59, 125.98, 123.91, 121.75, 89.57, 88.60, 87.77, 75.28, 75.10, 72.11, 72.01, 65.51, 65.21.

^{19}FNMR (CD_3OD, 400 MHz): δ -202.50.

HRMS (ESI): Calcd.for $C_{13}H_{14}FN_4O_5$ [M+H]+ 325.0943, found 325.0940

4. MTT Assay

On day 1, U251 cells were plated in a 96-well plate (2,000 cells/well/100 μL) and incubated overnight. On day 2, media was aspirated and added the compounds (prodrug or drug) of interest dissolved in the fresh media. The cells were incubated for days 1, 2, 3, 4.

Added 15 μL/well of MTT Dye solution and incubated at 37 °C for 4 h. Added 100 μL/well of STOP solution. Incubated again at 37 °C for 1 h. Transferred 100 μL/well into new 96-well plate after mixing several times by pipetting. Absorbance of the solution was measured at 570 nm and background at 650 nm.

The data: X axis= concentrations, Y axis= corrected absorbance (absorbance at 570 nm - background at 650 nm) are plotted in Prism software (version 8.4.3). Statistical analysis was performed using Microsoft excel and Prism 8 software (version 8.4.3). Data were analyzed using an unpaired two-tailed Student's t-test. All graphs are depicted with error bars corresponding to the standard error of the mean.

5. IC_{50} Plots

The concentrations were expressed in logarithm scale, normalized, and plotted in Prism software (version 8.4.3). The data were fitted with nonlinear regression (curve fit) followed by log(inhibitor) v. response- variable slope (four parameters).

6. Molecular Modeling

Protein structure of XYLT-1 (pdb id: 6ej7) enzyme was initially subjected to a protein preparation step using Protein Preparation Wizard (Schrodinger Inc). In this step, missing residues and loops in the protein structures were build and optimized, protonation states of titratable residues were adjusted, and the resulting structures were subsequently energy minimized such that heavy atoms did not move beyond 0.3Å from their starting positions. We also retained co-crystallized inhibitor in the active site. We replaced these inhibitors with our presumed inhibitors prior to docking. Docking consists of two steps: grid preparation and docking. We used the coordinates of the co-crystallized inhibitor to define the center of the docking grid. Structures of our presumed inhibitors were prepared using Edit/Built panel of Maestro software (Schrodinger Inc), energy minimized using LigPrep software (v.5.40749, Schrodinger Inc), and subsequently docked to the active site of XYLT-1 using Glide docking software (v8.7, Schrodinger Inc). We docked the ligands using extra precision (XP) docking scoring function followed by molecular-mechanics energy function-based rescoring (PRIME MM-GBSA, v3.0) to identify docking poses and relative ranks.

7. Action of Compound 6A-NO_2 on MTT dye via LCMS

We have performed LCMS experiments to show that our lead compounds— 6A-NO_2 and 6A'-NO_2 cannot reduce MTT dye (Figure S6).

Methods

MTT dye (5 μL) was added to DMSO (35 μL) in a 96-well plate

Results and Discussion

In this study, we used LCMS to investigate if our lead inhibitors (6A-NO_2 and 6A'-NO_2) could reduce MTT dye to tetrazolium salts and thus gave false positive in MTT assay. We mimicked the MTT assay experiments and incubated the dye with difference concentrations (2 μM, 20 μM, and 200 μM) of the lead compounds.

We compared the LCMS data of a) MTT dye only, b) and c) MTT dye incubated with lead compounds (15 min at room temperature and 4 h at 37 °C) (Figure S6). Our study concludes that our compounds do not reduce MTT dye, confirming fidelity of the MTT assay.

8. FIGURES

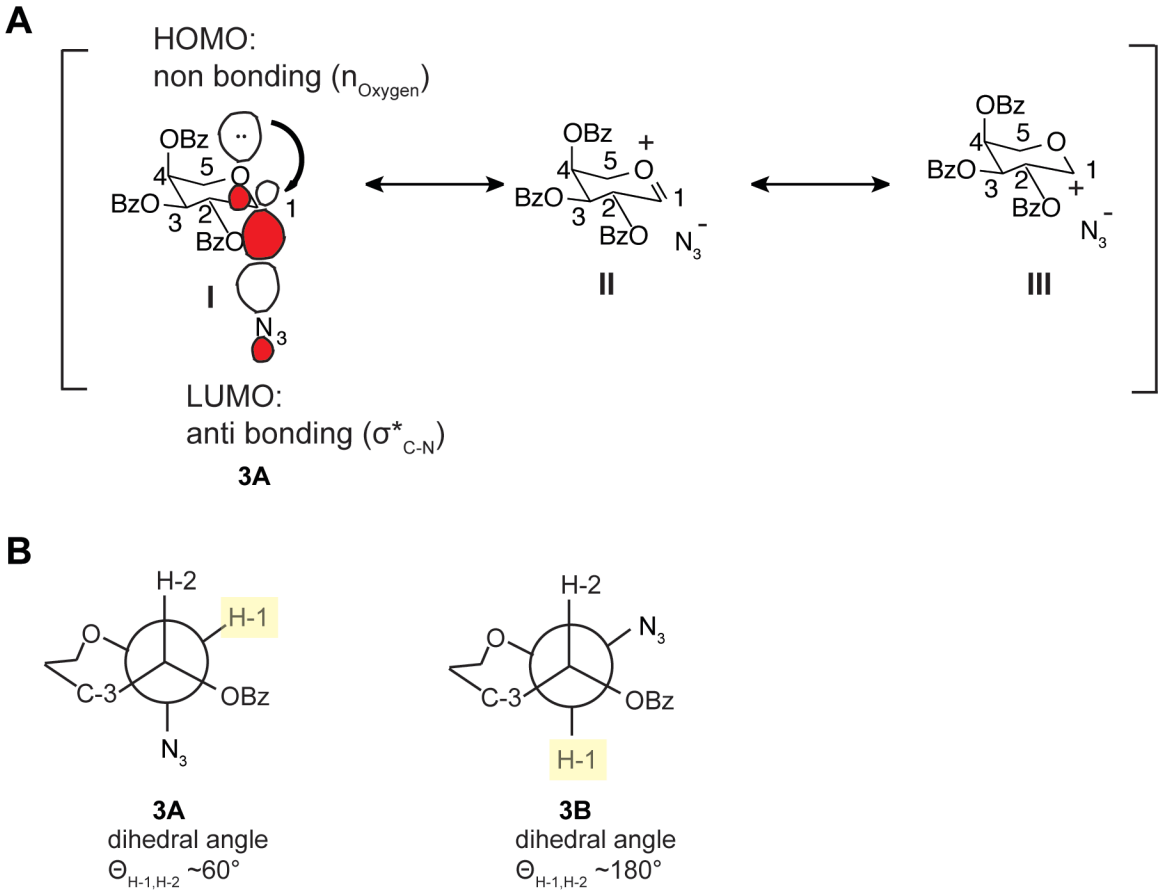


Figure S1. Chemical shift difference of the xylose residue in compounds 3A and 3B. A) The downfield shift of the anomeric proton, H-1 in the α-azide (compound **3A**) can be explained by the reduced electron density in the carbon C-1 center due to the hyperconjugation resonance in the axial conformation (anomeric effect). The positively charged C-1 in the resonance structure III contribute to the downfield shift of H-1. **B**) The Newman projections of **3A** and **3B** looking down through C-2 and C-1 carbon centers show the dihedral angles, θ between H-1 and H-2 (~60° for **3A** and ~180° for **3B**). These angles determine the spin-spin splitting constant, ^3J between H-1 and H-2 through Karplus relationship.

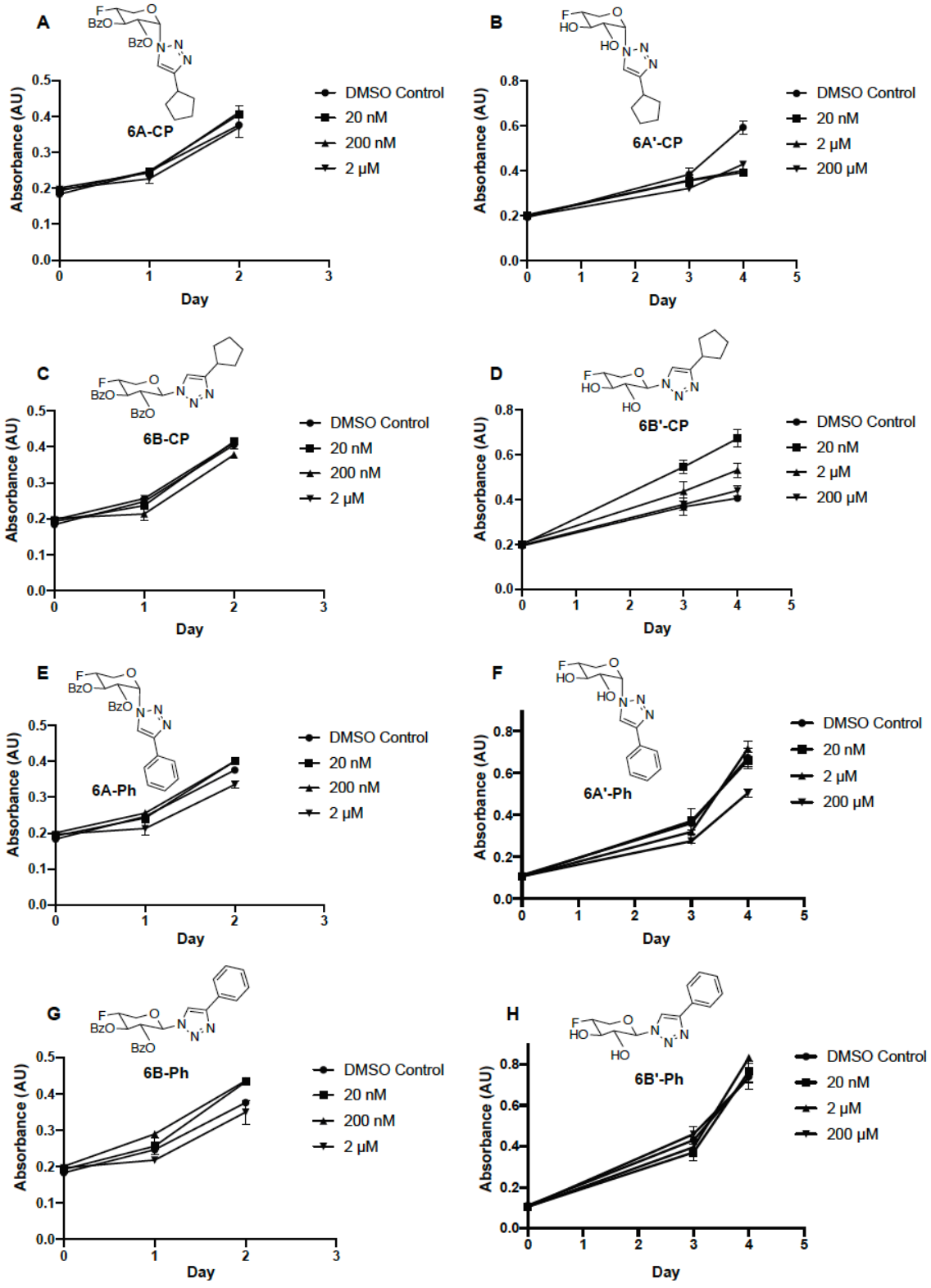


Figure S2. MTT assay of the prodrugs and drugs show concentration and time (day) dependent cell toxicity in U251 cells. The X-axis represents time (day) and the Y-axis shows absorbance (arbitrary unit) that were normalized, and plotted in Prism software (version 8.4.3).

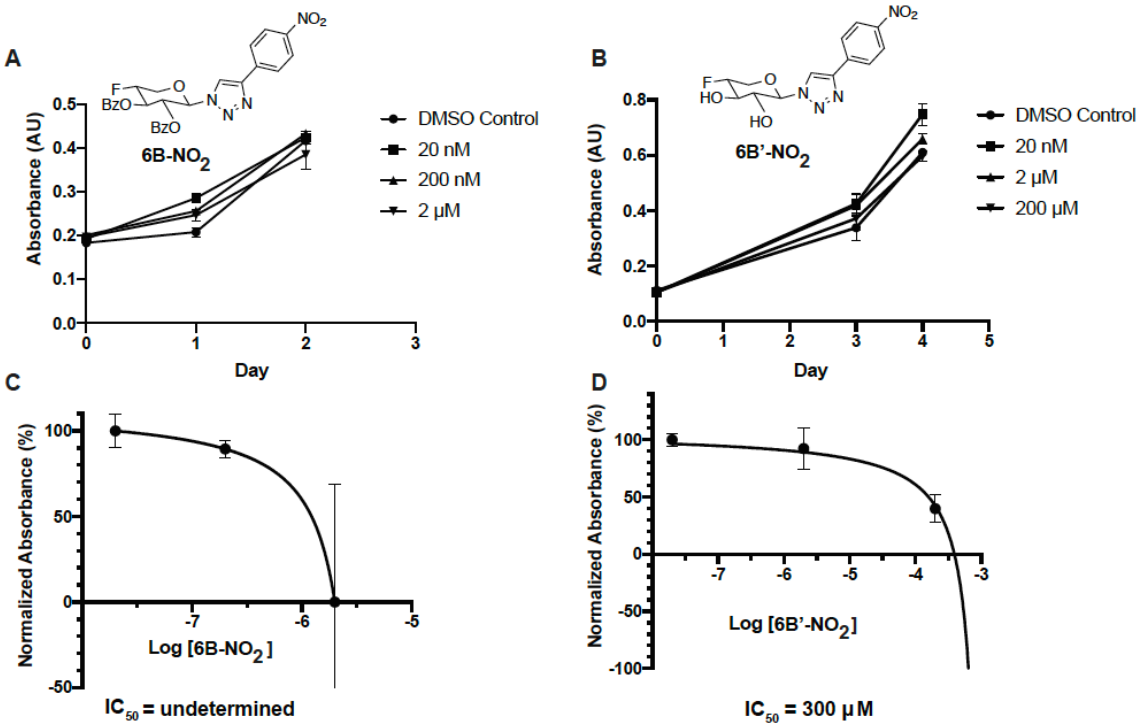


Figure S3. MTT assay and IC_{50} of 6B-NO_2 and 6B'-NO_2 in U251 cells A, B) Compound 6A-NO_2, a prodrug and its hydrophilic drug, compound **6B'-NO_2** showed concentration and time (day) dependent cell toxicity. **C, D**) The plotting of concentration and normalized absorbance furnished the IC_{50} values of the compounds. The concentrations were expressed in logarithm scale, normalized, and plotted in Prism software (version 8.4.3). The data were fitted with nonlinear regression (curve fit) followed by log(inhibitor) v. response- variable slope (four parameters).

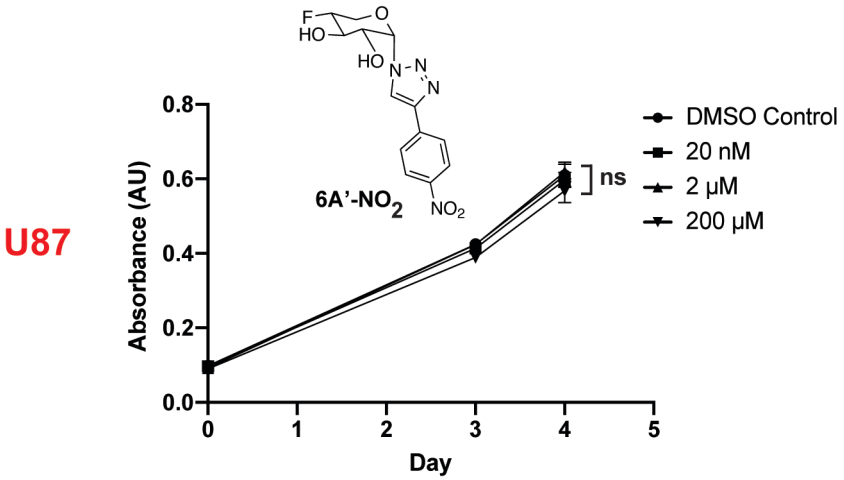


Figure S4. MTT assay of 6A'-NO_2 in U87 cells. Hydrophilic drug treatment in U87 cells showed only 7% reduction in the absorbance on day 4 with 200 µM (*P* = not significant, unpaired t-test for day 4).

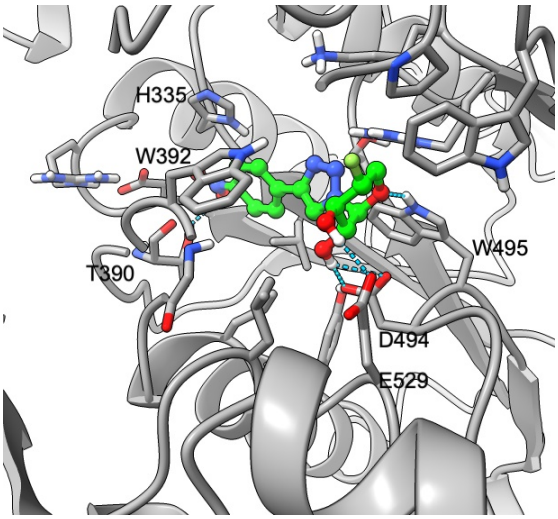


Figure S5. Computational docking poses of 6A'-NO_2 in the active site of XYLT-1 (pdb 6ej7). Residues that form hydrogen bond to inhibitor are labeled as dotted line. Carbon atoms of protein atoms are shown in gray color. Carbon atoms of inhibitors are shown in green color. Hydrogen, nitrogen, oxygen and fluorine atoms are shown in white, blue, red and light-green colors. Bonds of protein residues are shown in tube representation and inhibitors are shown in ball-and-stick representation. Hydrogen bond between inhibitor and protein atoms are shown as blue dashed lines. XYLT-1 (pdb id: 6ej7) enzymes was subjected to a protein preparation step using Protein Preparation Wizard (Schrodinger Inc). Docking consists of two steps: grid preparation and docking. We used the coordinates of the co-crystallized inhibitor to define the center of the docking grid. Structures of our presumed inhibitors were prepared using Edit/Built panel of Maestro software (Schrodinger Inc), energy minimized using LigPrep software (v.5.40749, Schrodinger Inc), and subsequently docked to the active sites of XYLT-1 and β-GALT-7 using Glide docking software (v8.7, Schrodinger Inc).

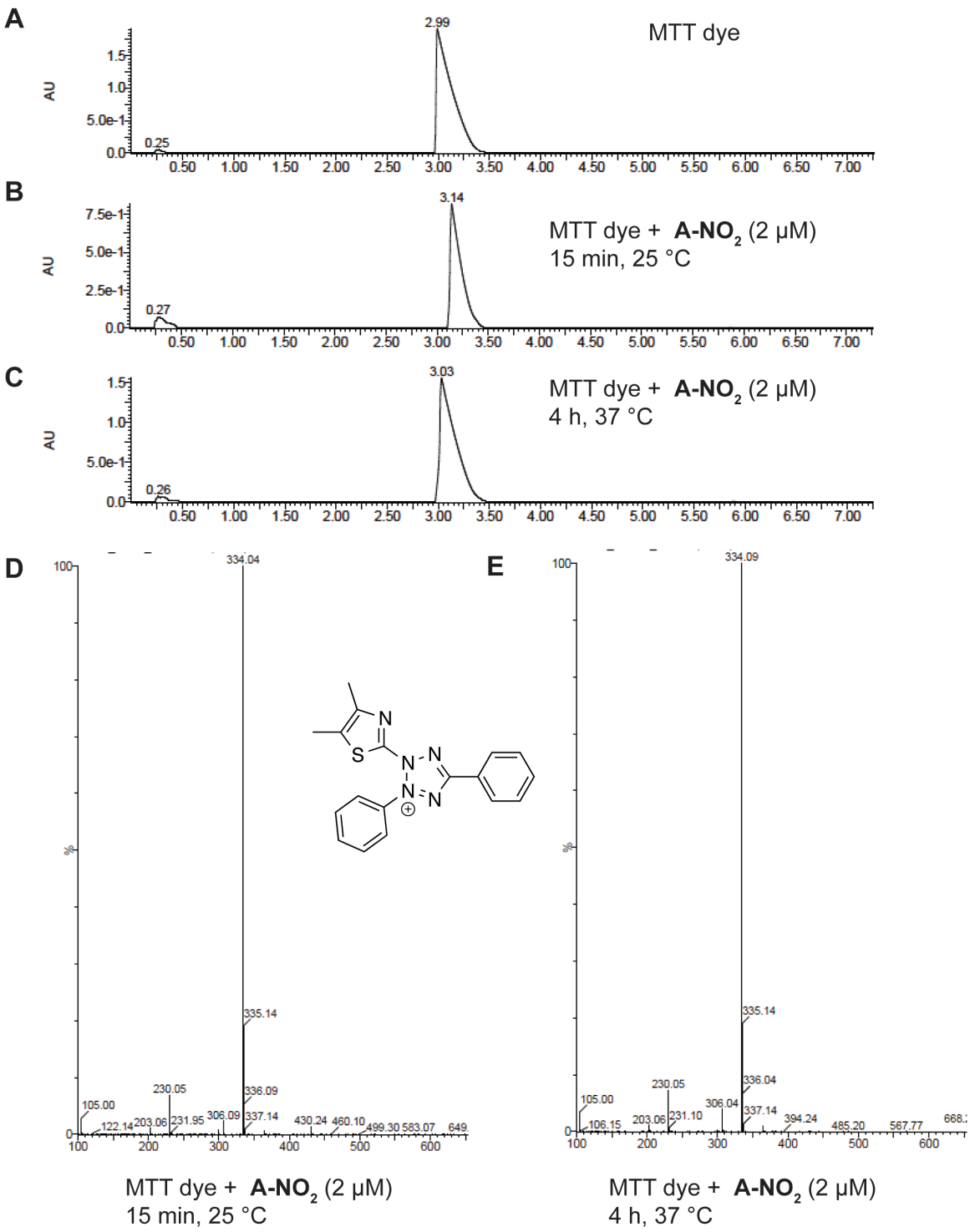
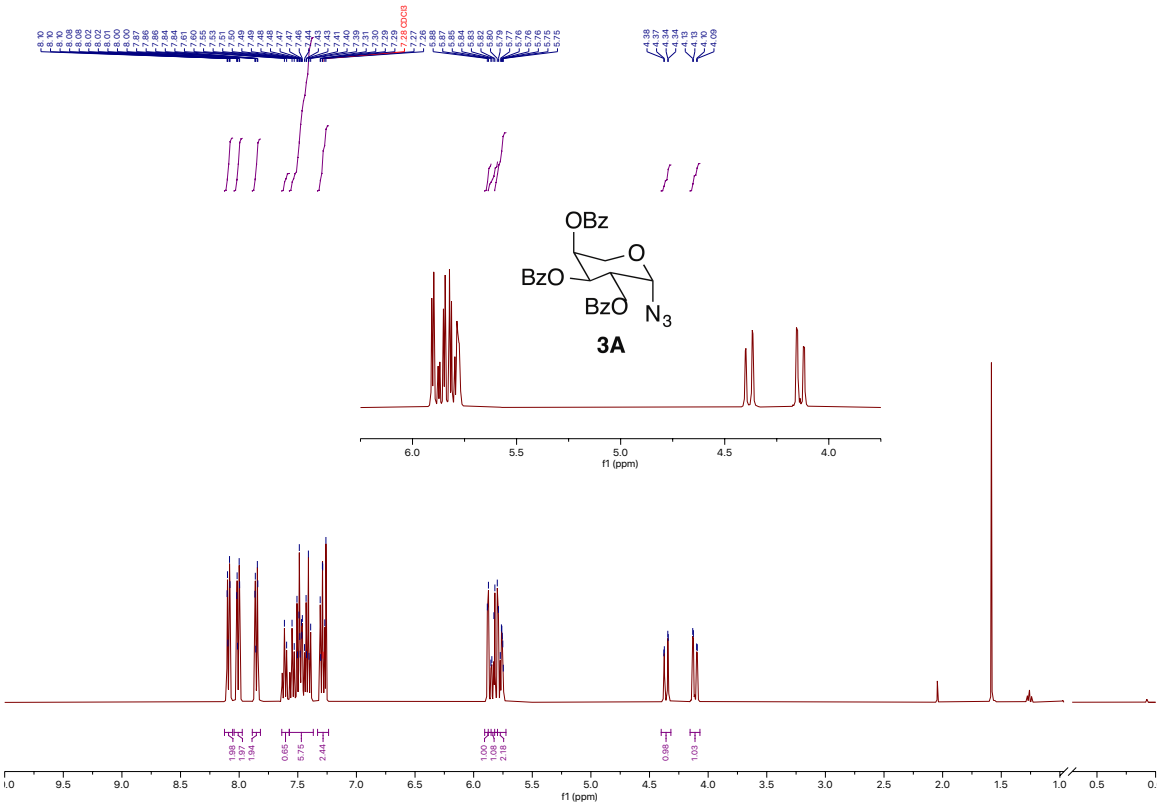
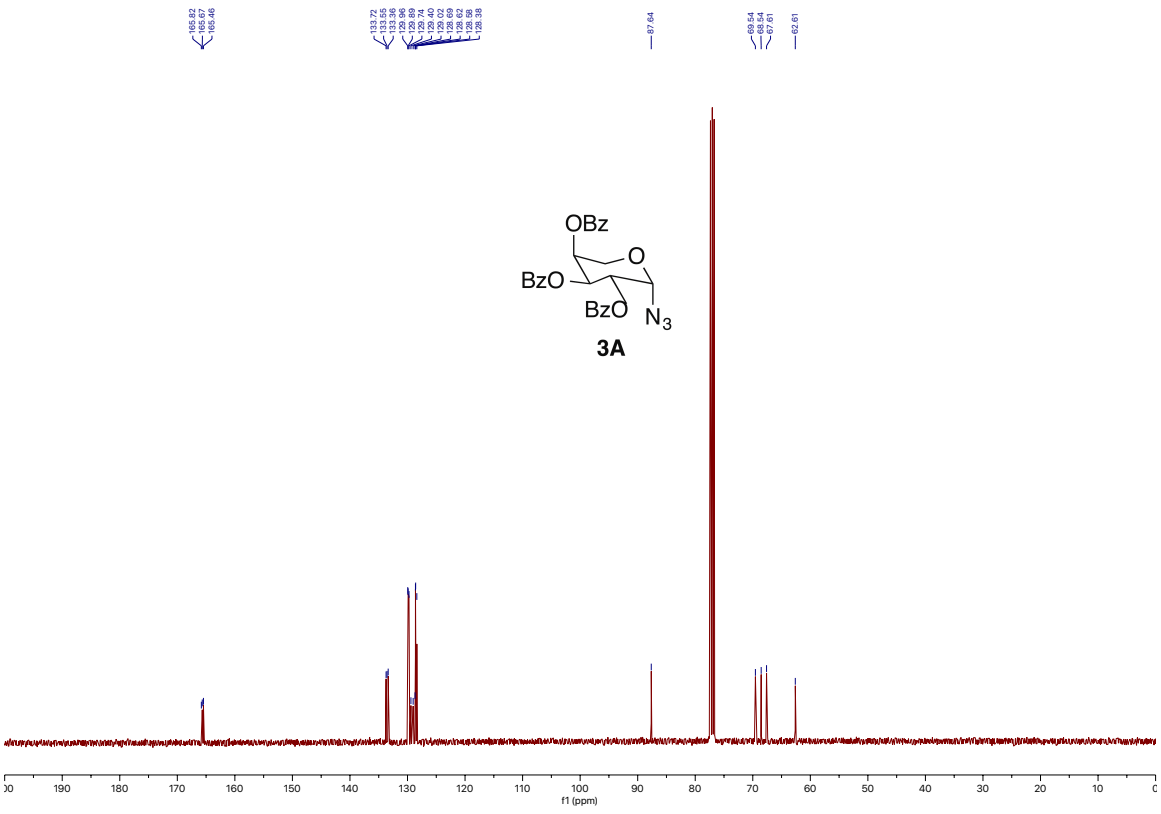
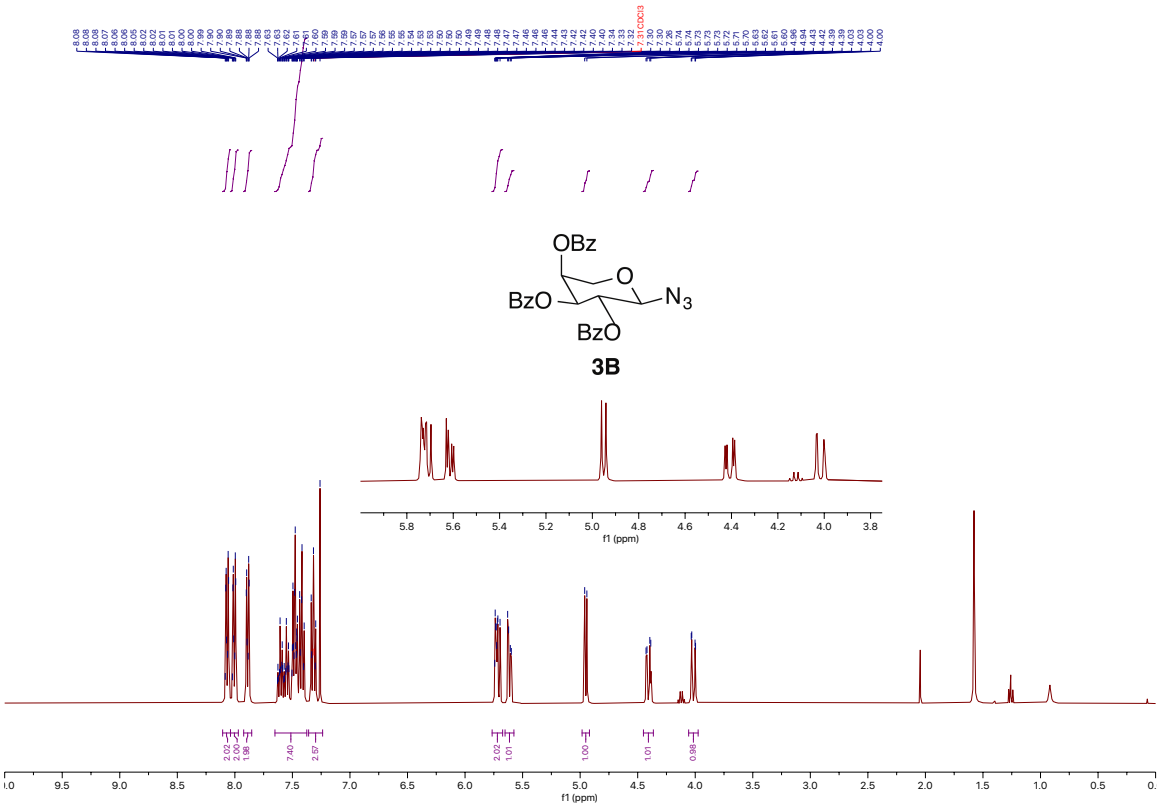


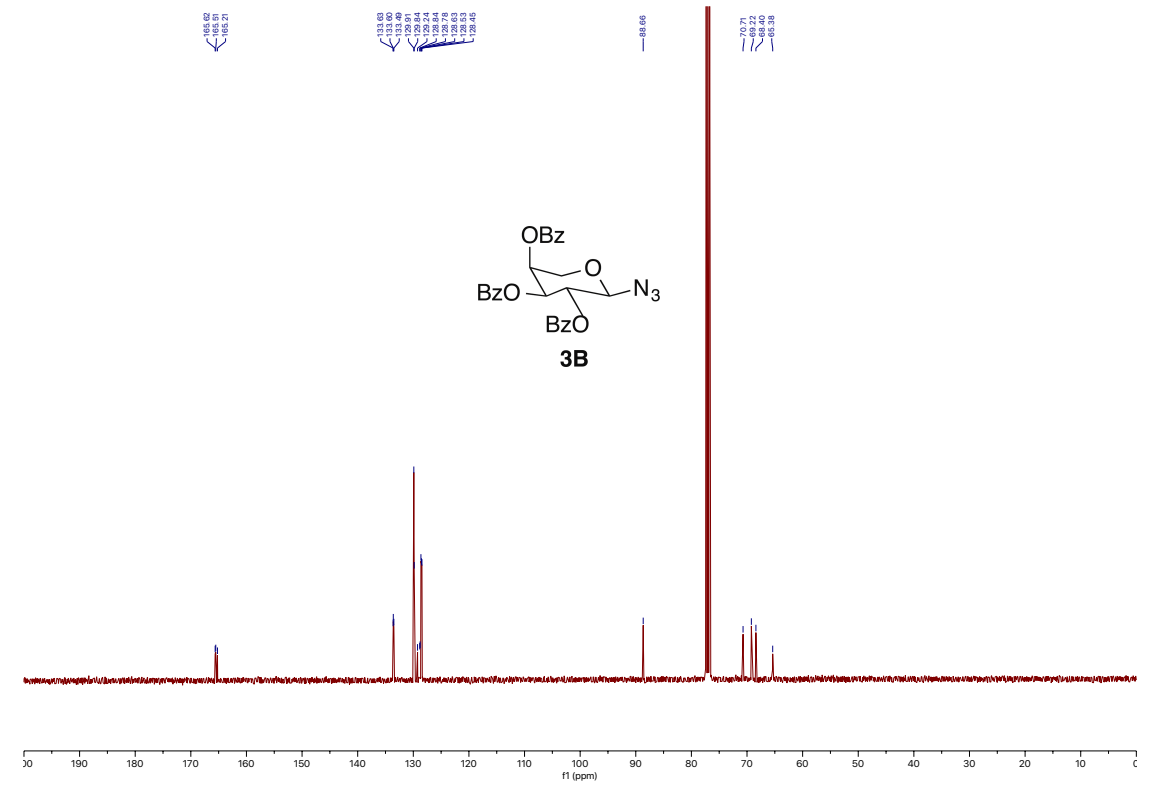
Figure S6. LCMS show the fidelity of MTT dye in presence of lead inhibitor (6A-NO_2). LCMS of **A)** MTT dye alone, **B)** MTT dye and A-NO_2 (2 µM), 15 min after incubation, **C)** MTT dye and A-NO_2 (2 µM), 4 h after incubation at 37 °C, **D)** and **E)** are the corresponding mass spectrometry pooled out of B) and C). [M^+]= 334.04

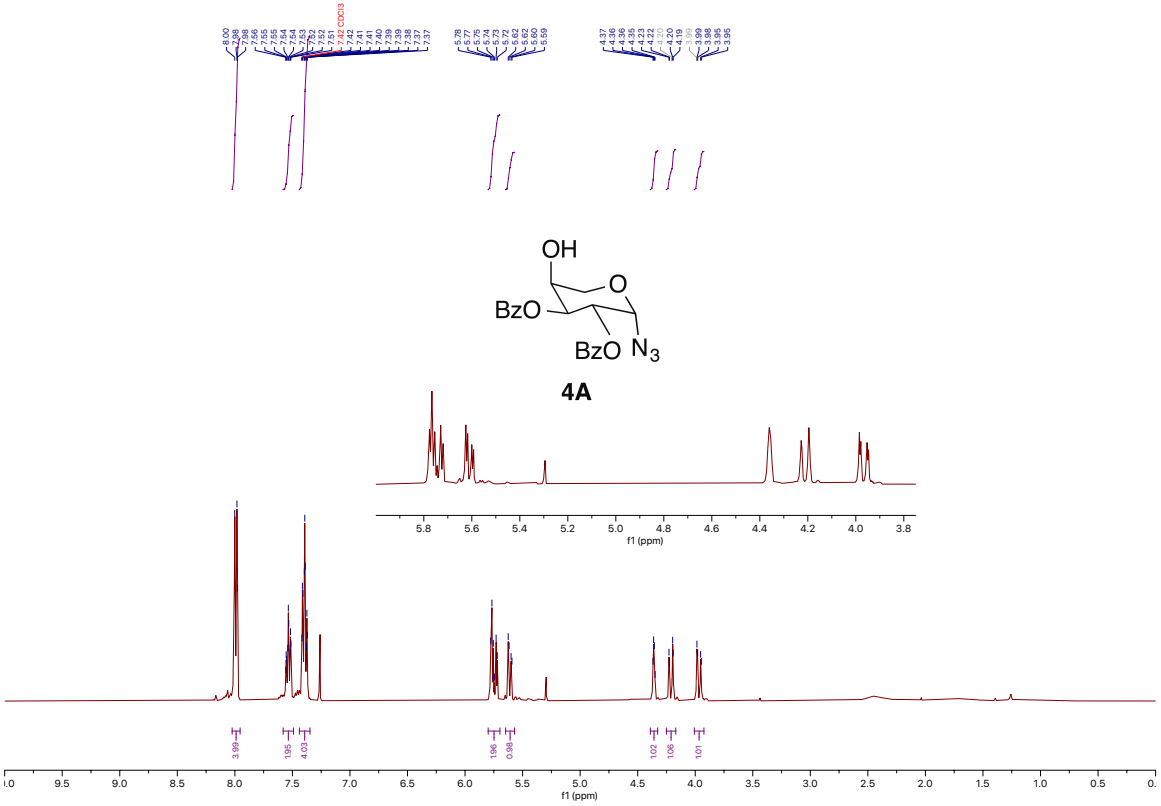
9. ^{1}H, ^{13}C Spectra, and ^{19}F of Synthetic Compounds

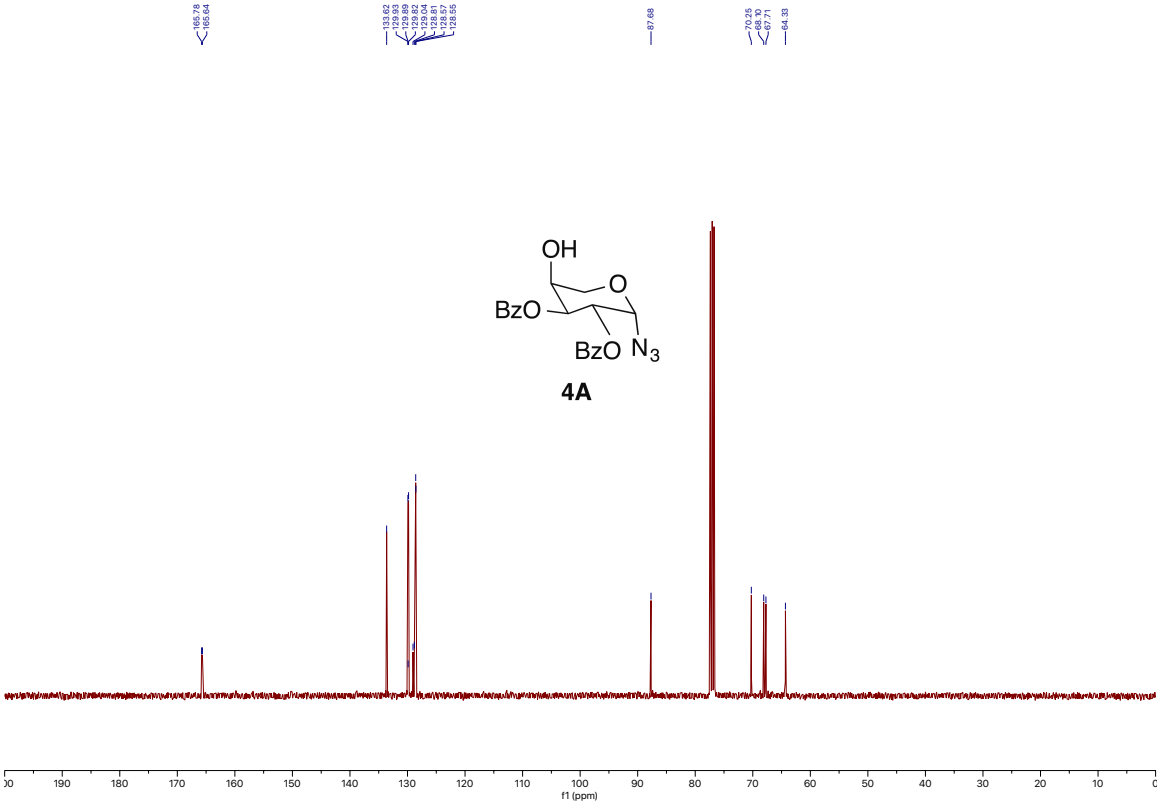


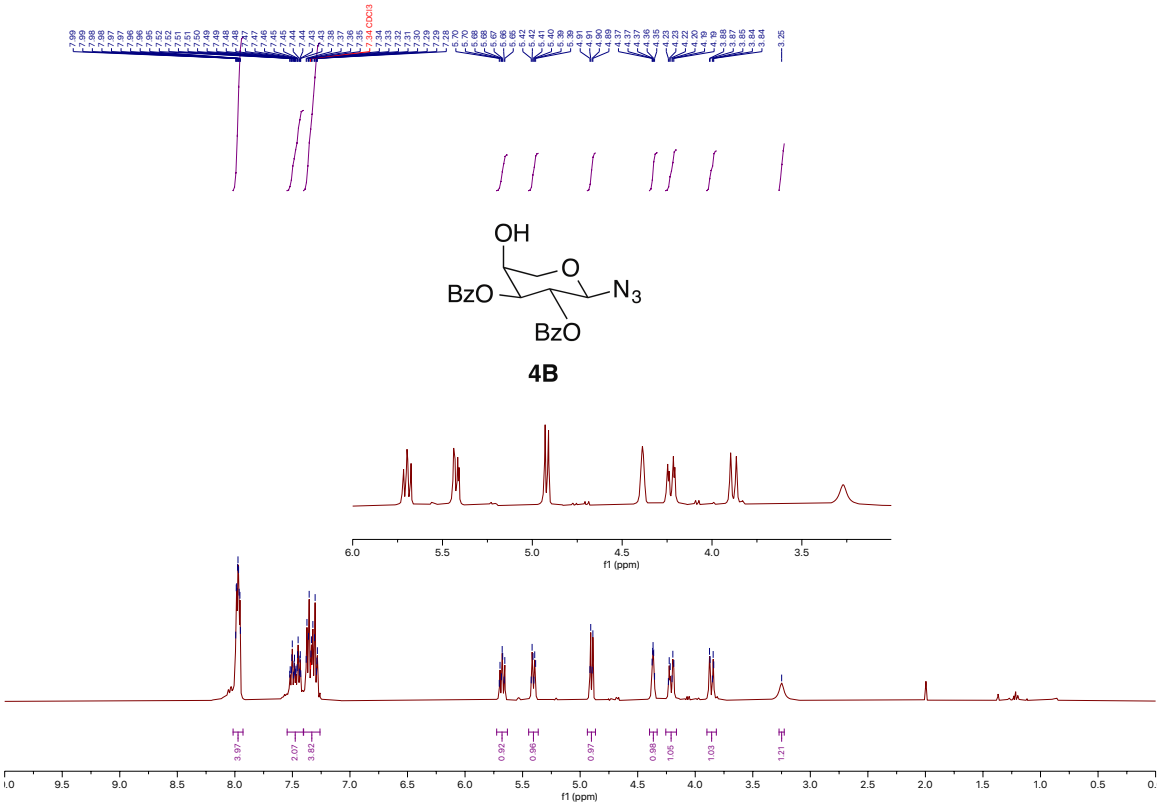


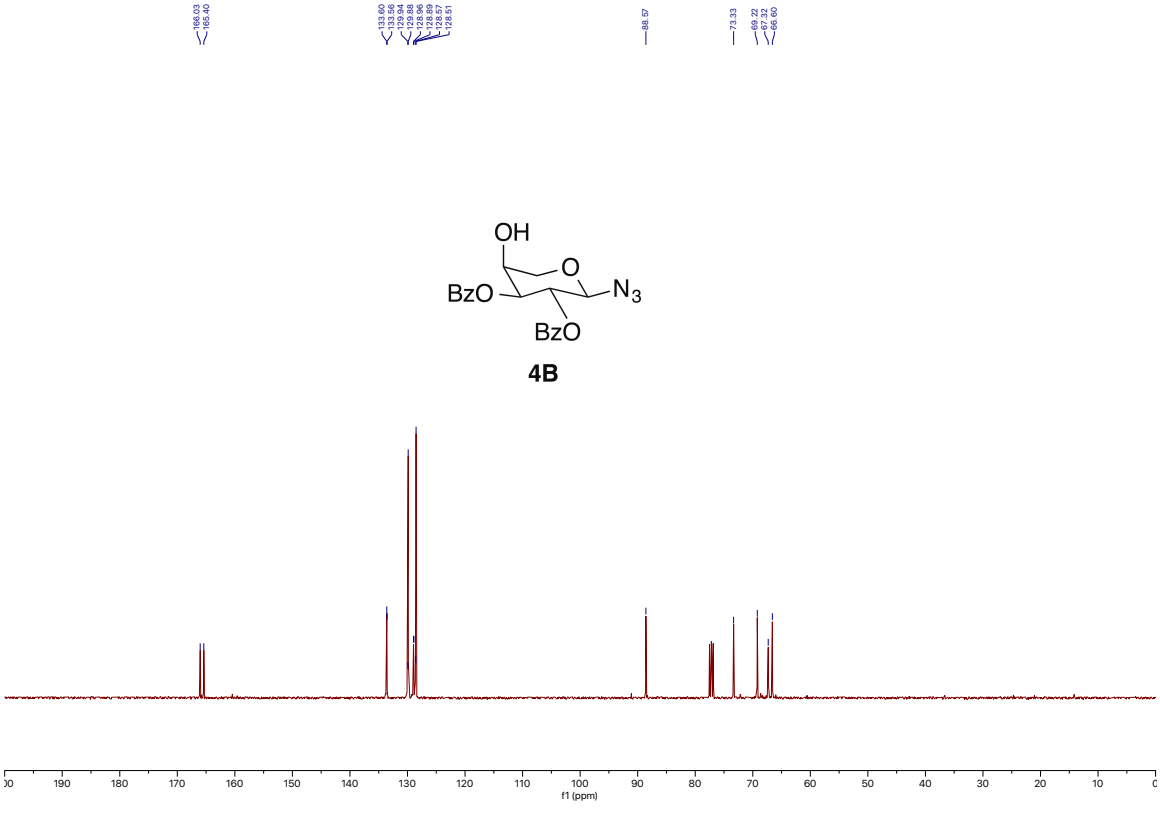

OBz
O
BzO
N3
BzO
3B
f1 (ppm)

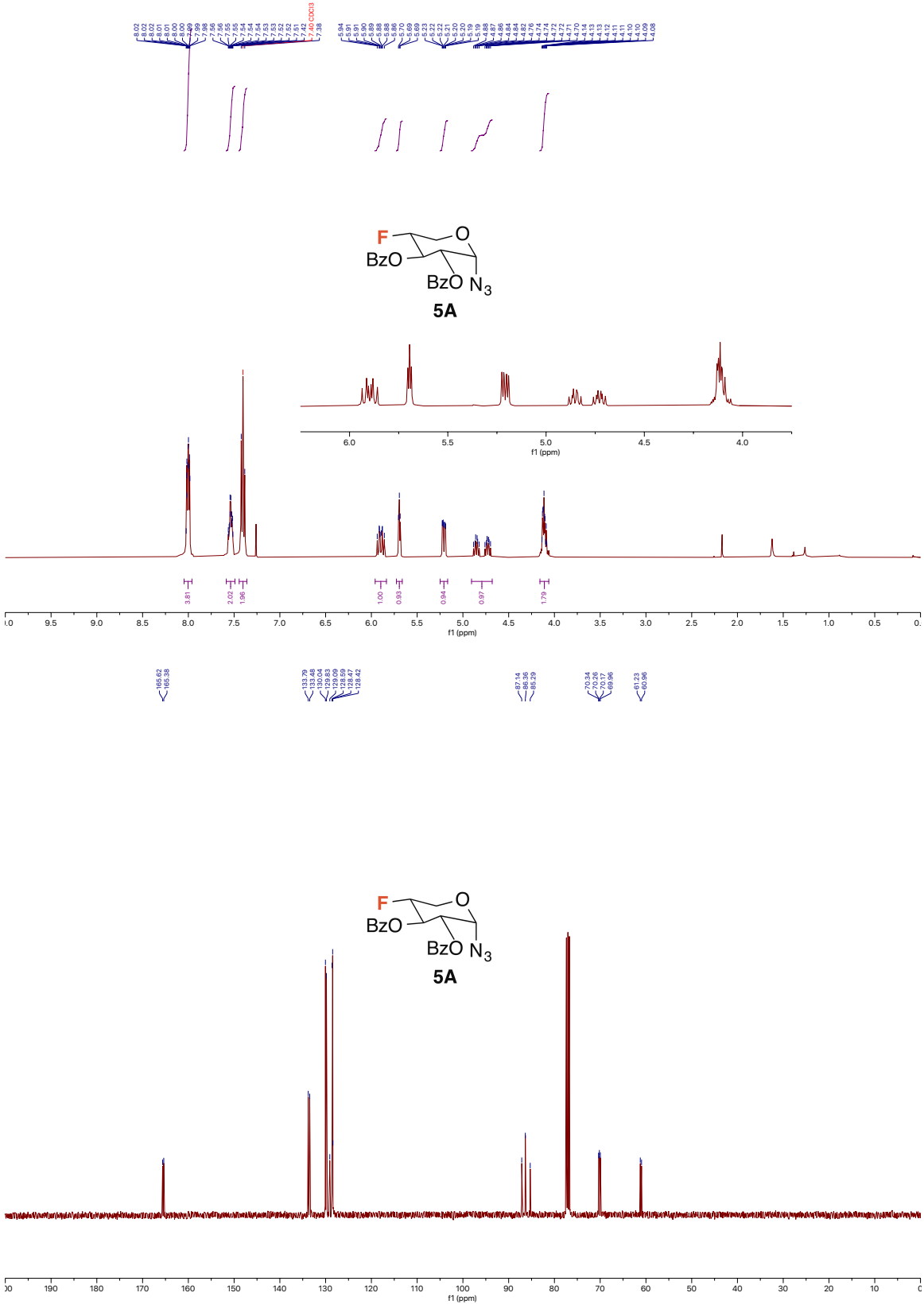

OBz
O
BzO
N3
BzO
3B
f1 (ppm)

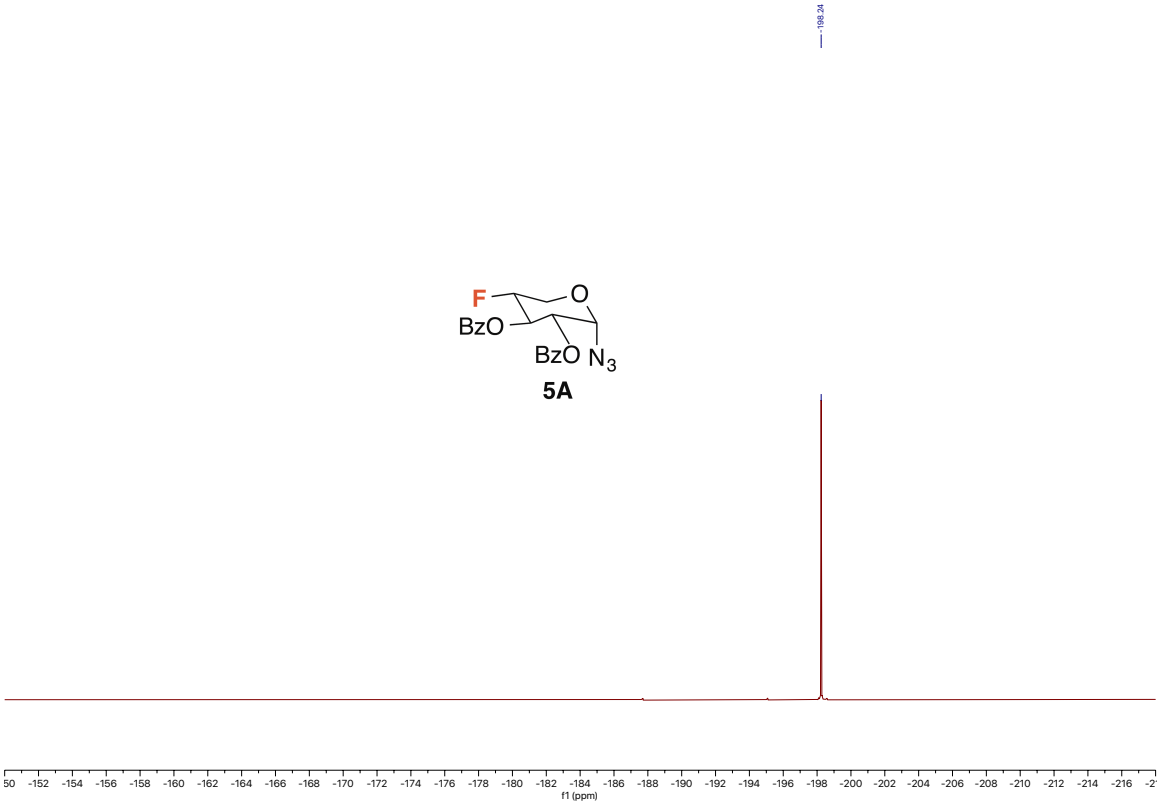

OH
O
BzO
BzO
N3
4A
f1 (ppm)


OH
O
BzO
BzO
N3
4A
f1 (ppm)


OH
O
BzO
N3
BzO
4B
f1 (ppm)


OH
O
BzO
N3
BzO
4B
f1 (ppm)


F
O
BzO
BzO
N3
5A
f1 (ppm)
f1 (ppm)
F
O
BzO
BzO
N3
5A
f1 (ppm)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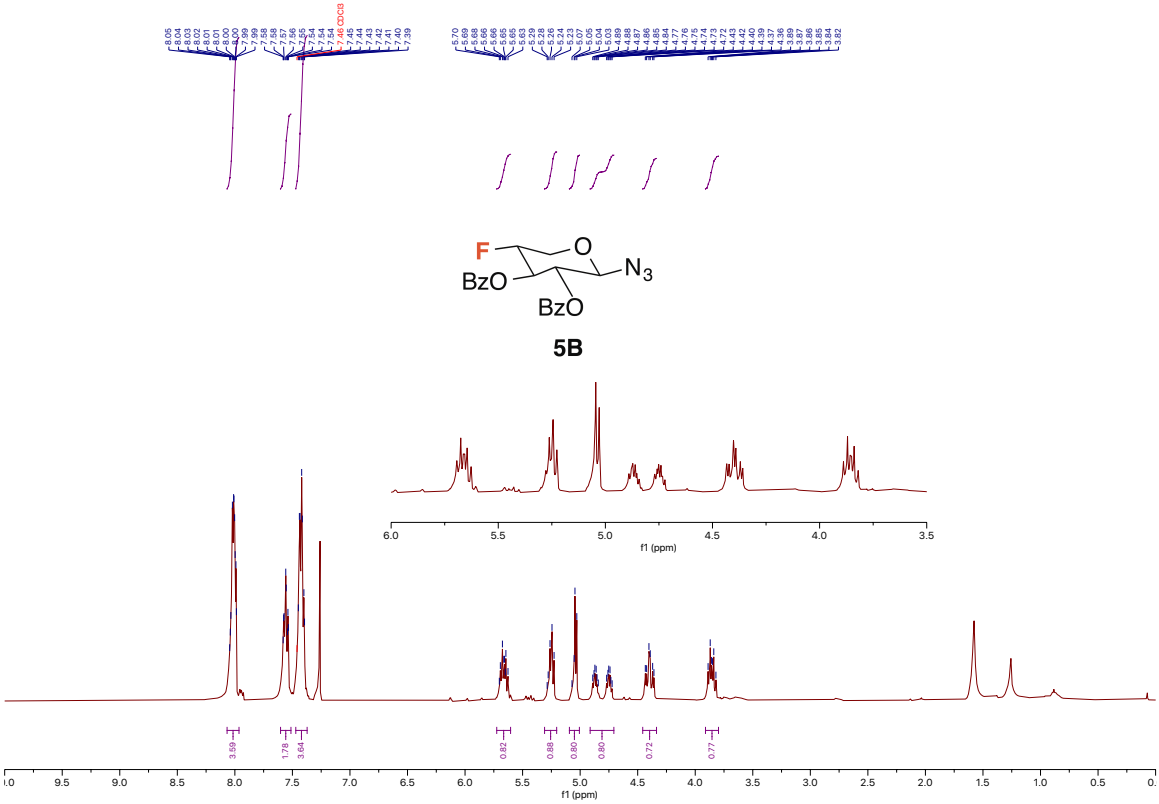
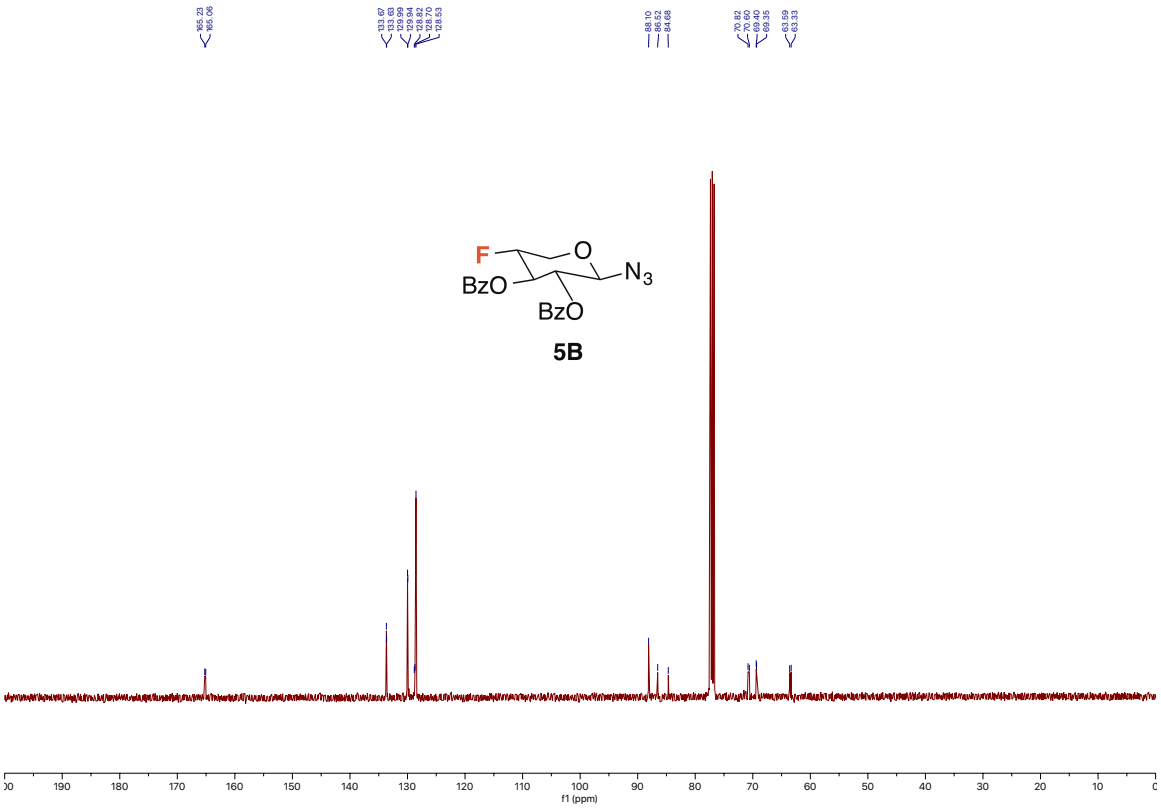
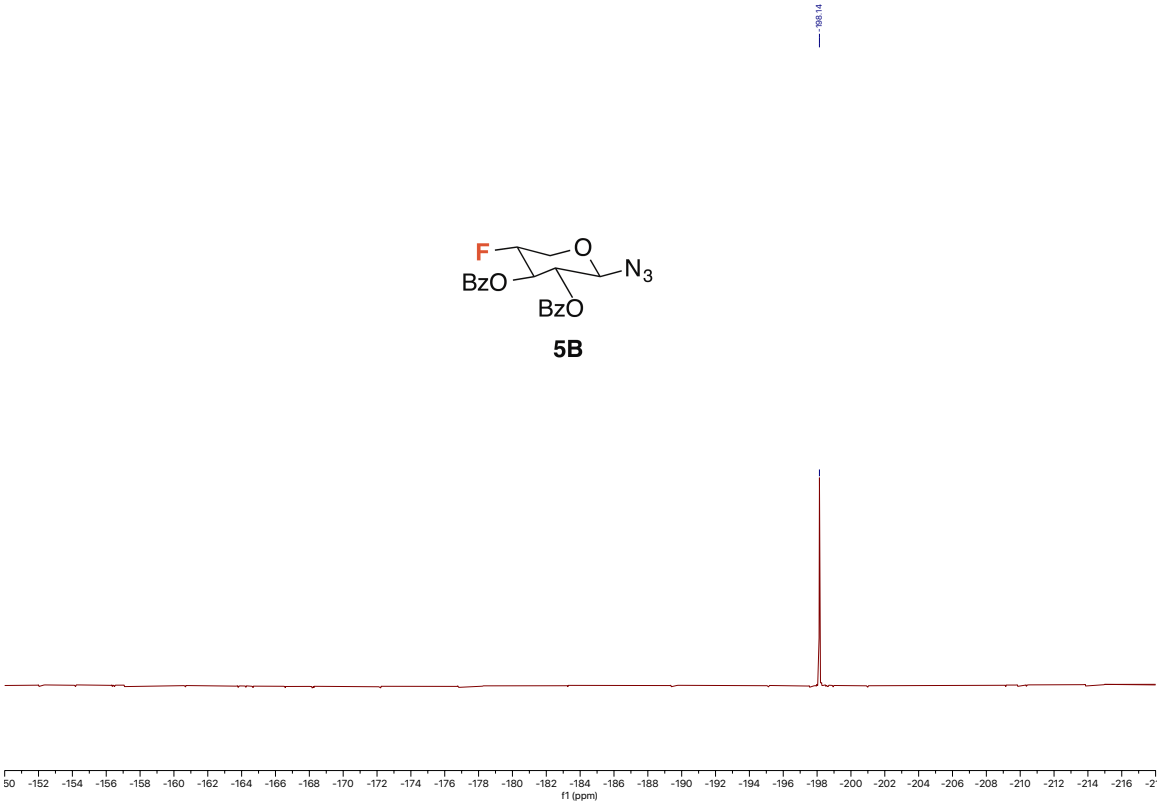
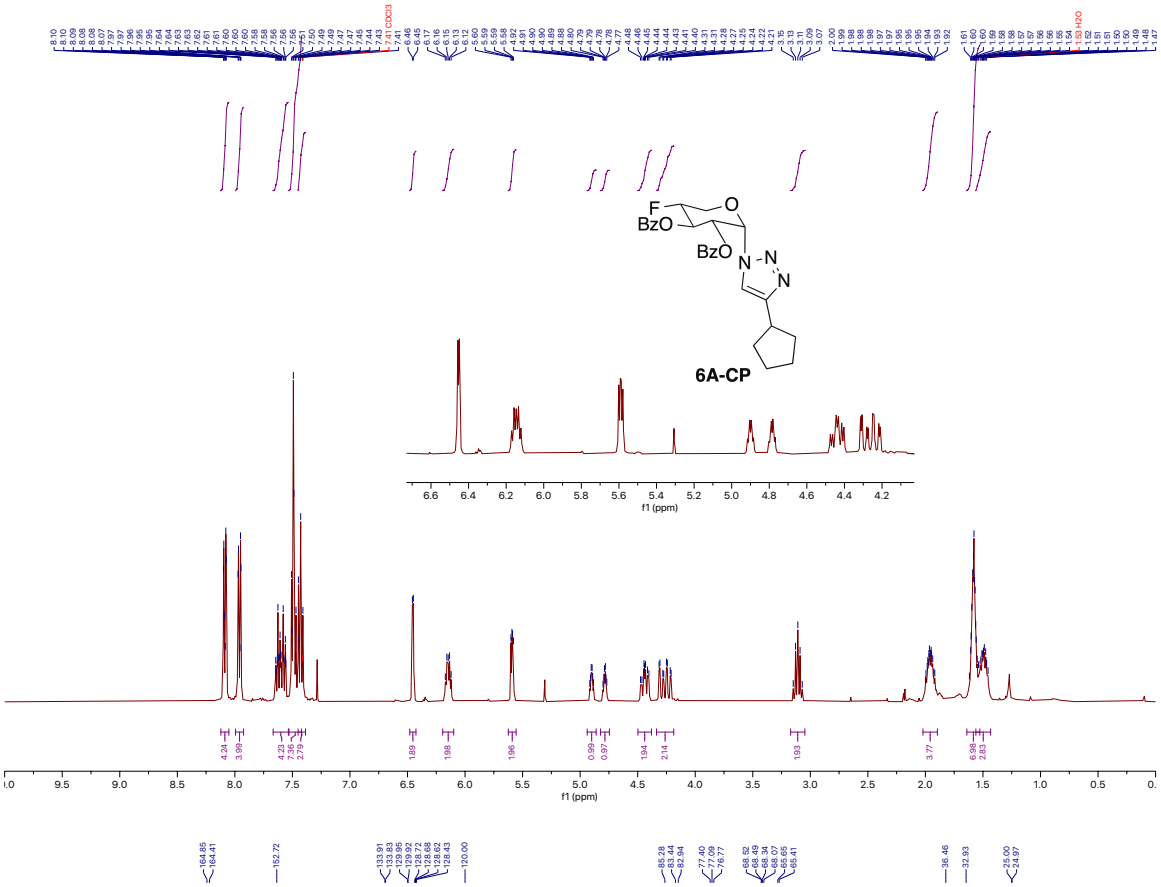
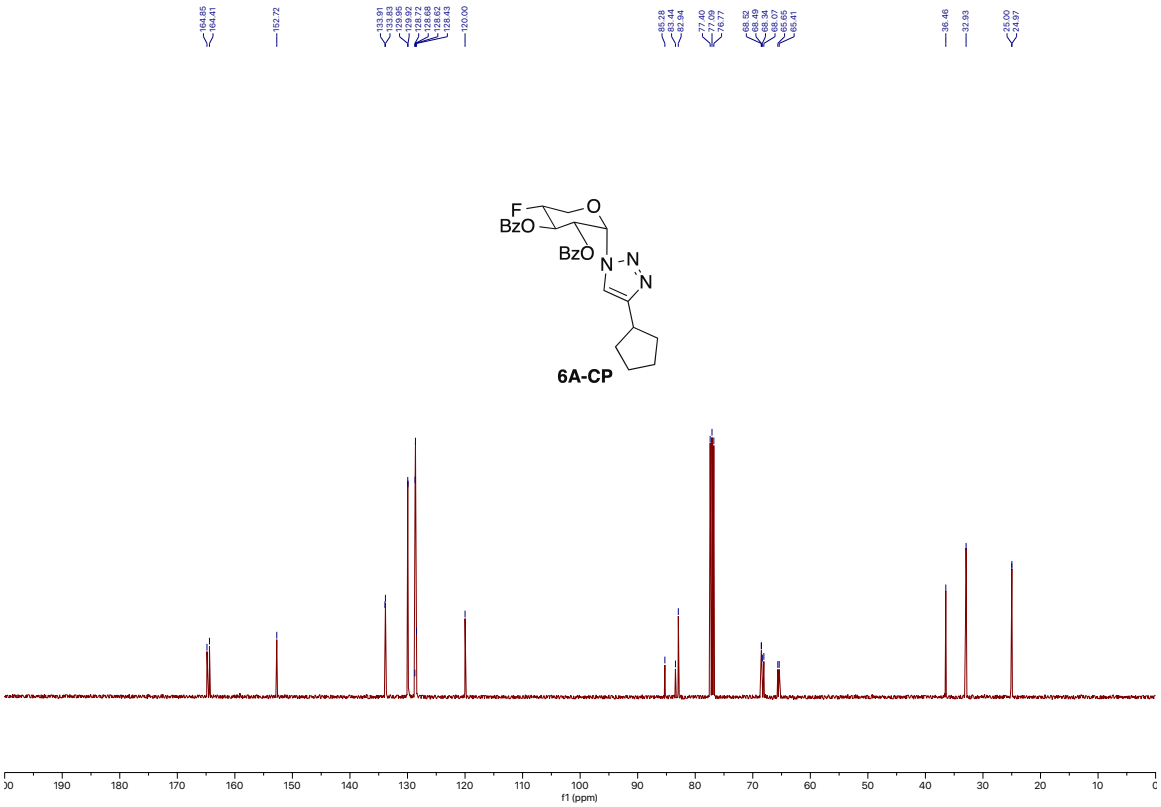
O
BzO
BzO
N3
5A
-198.24
f1 (ppm)

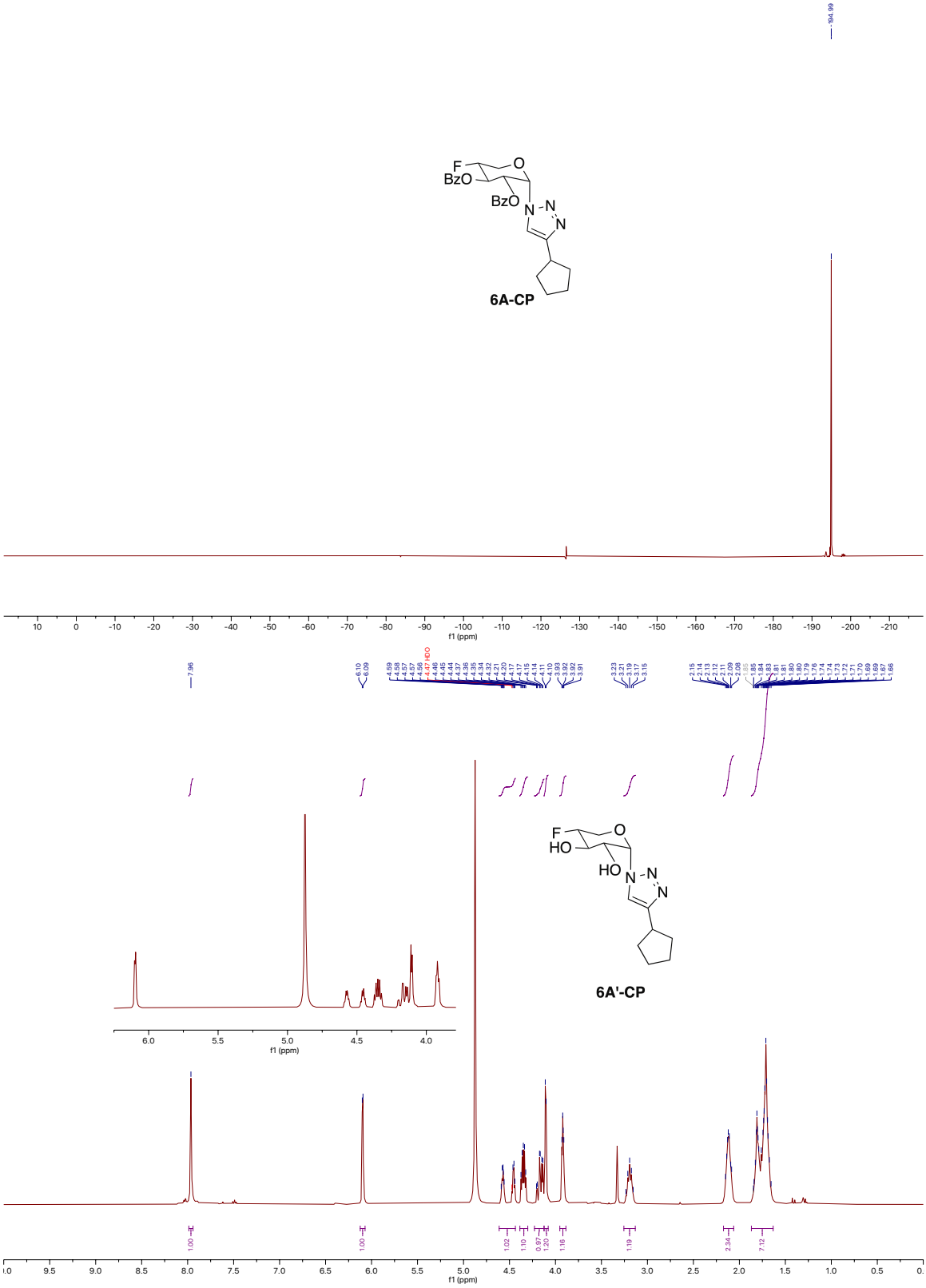

F
O
N3
BzO
BzO
5B
f1 (ppm)

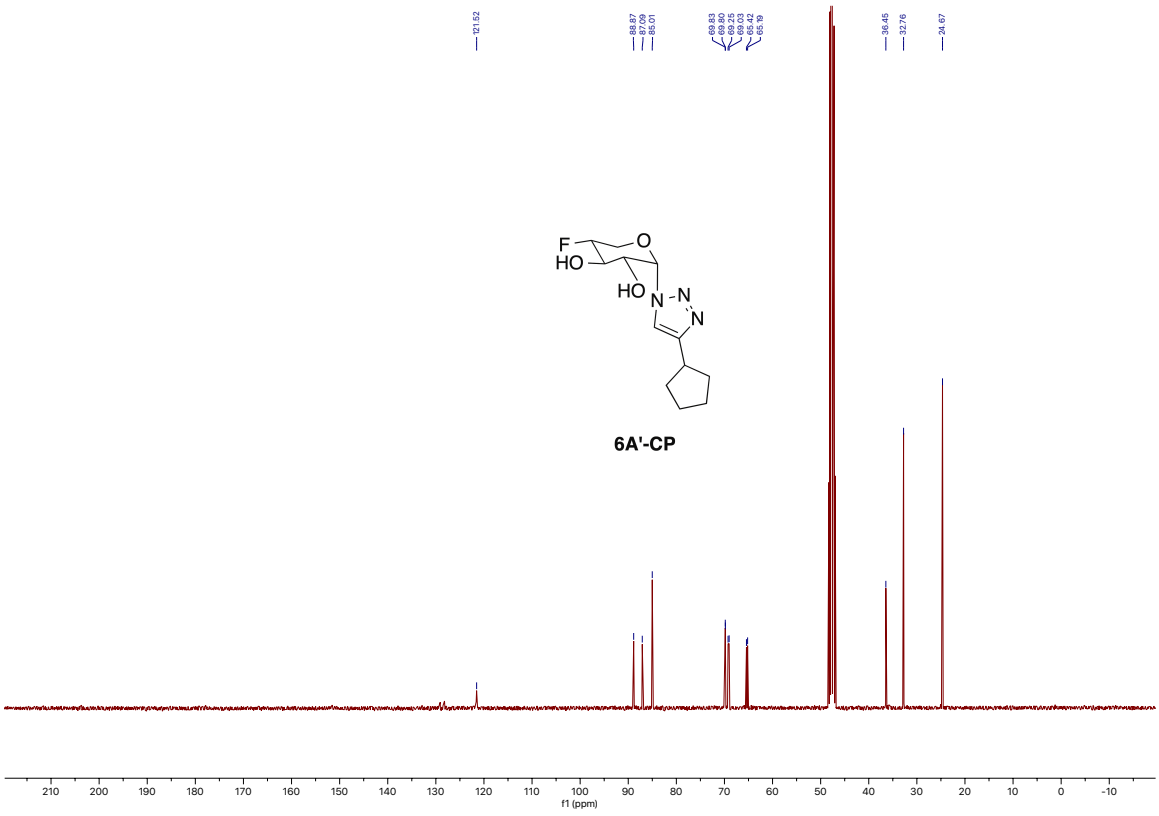

165.23
165.06
133.67
133.63
130.08
129.94
128.82
128.70
128.53
88.10
86.52
84.68
70.82
70.60
69.40
69.35
63.59
63.33
F
O
N3
BzO
BzO
5B
f1 (ppm)

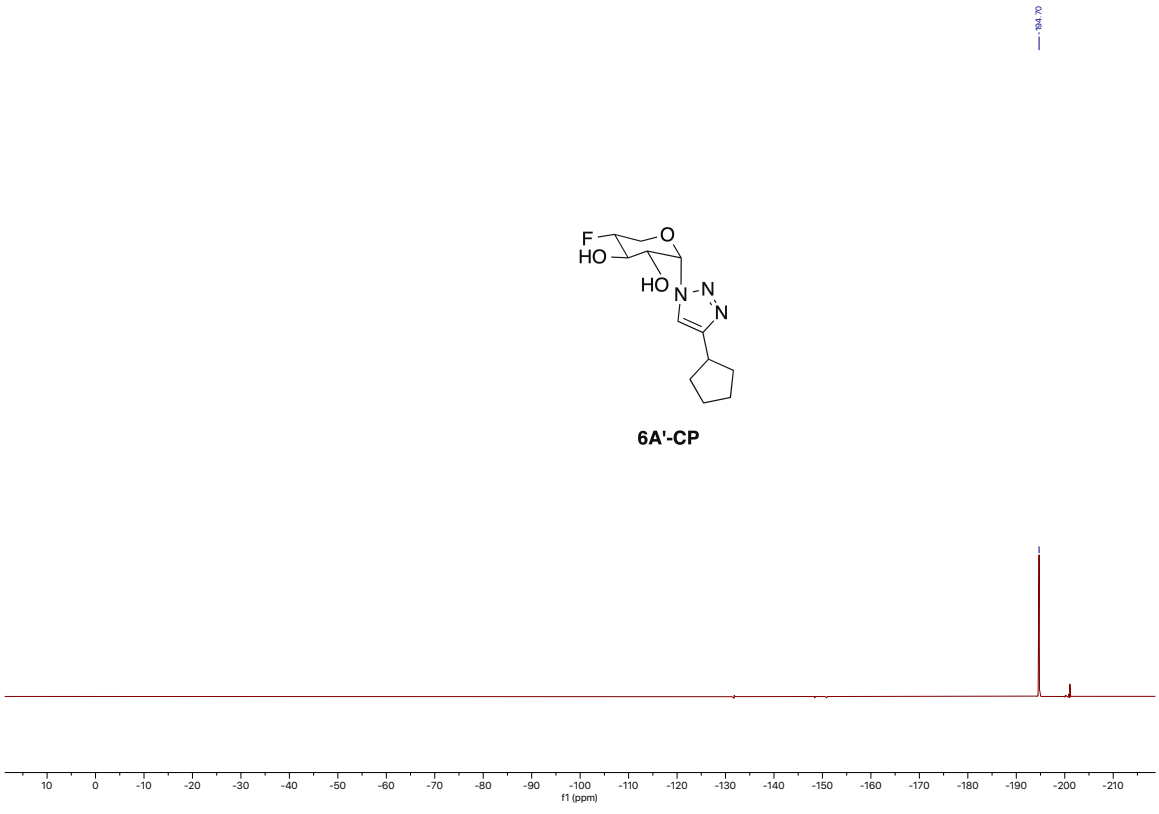

-198.14
F
O
N3
BzO
BzO
5B
f1 (ppm)

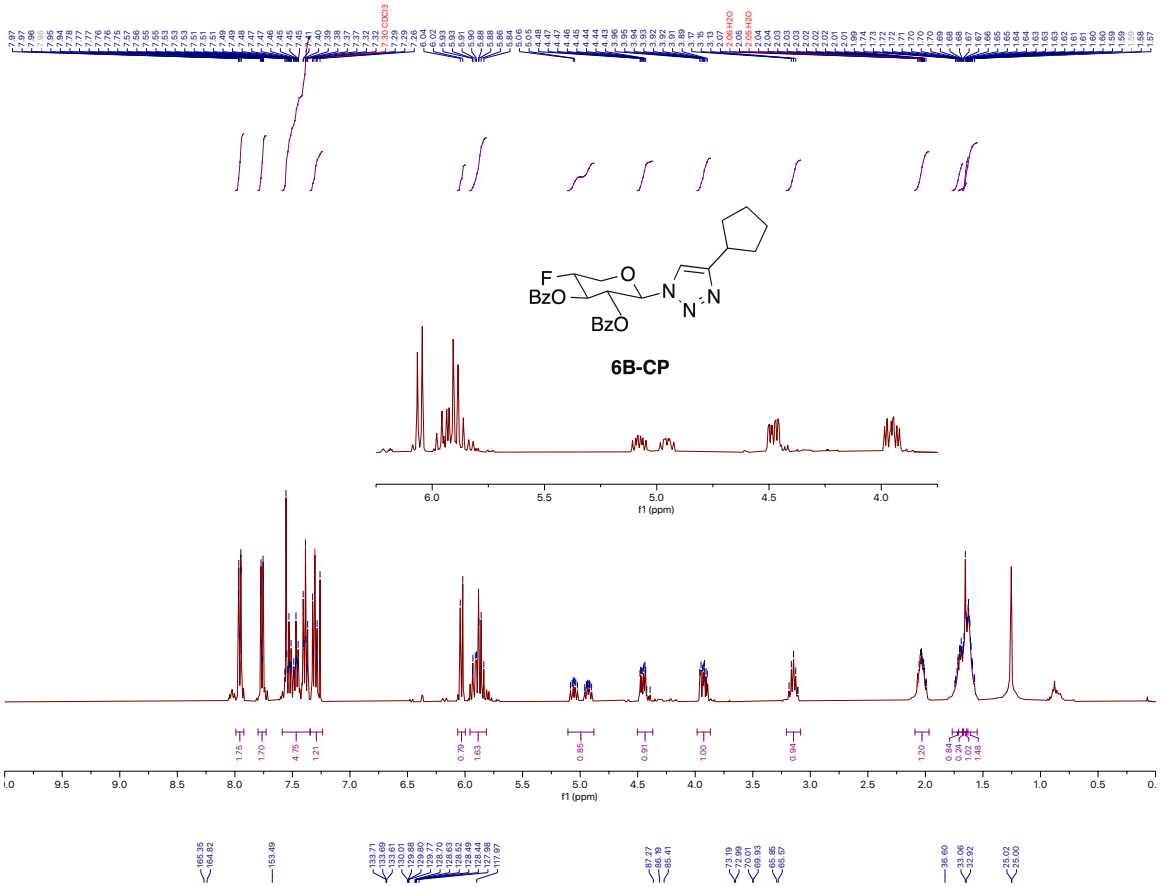

F
O
BzO
BzO
N
N
N
6A-CP
f1 (ppm)

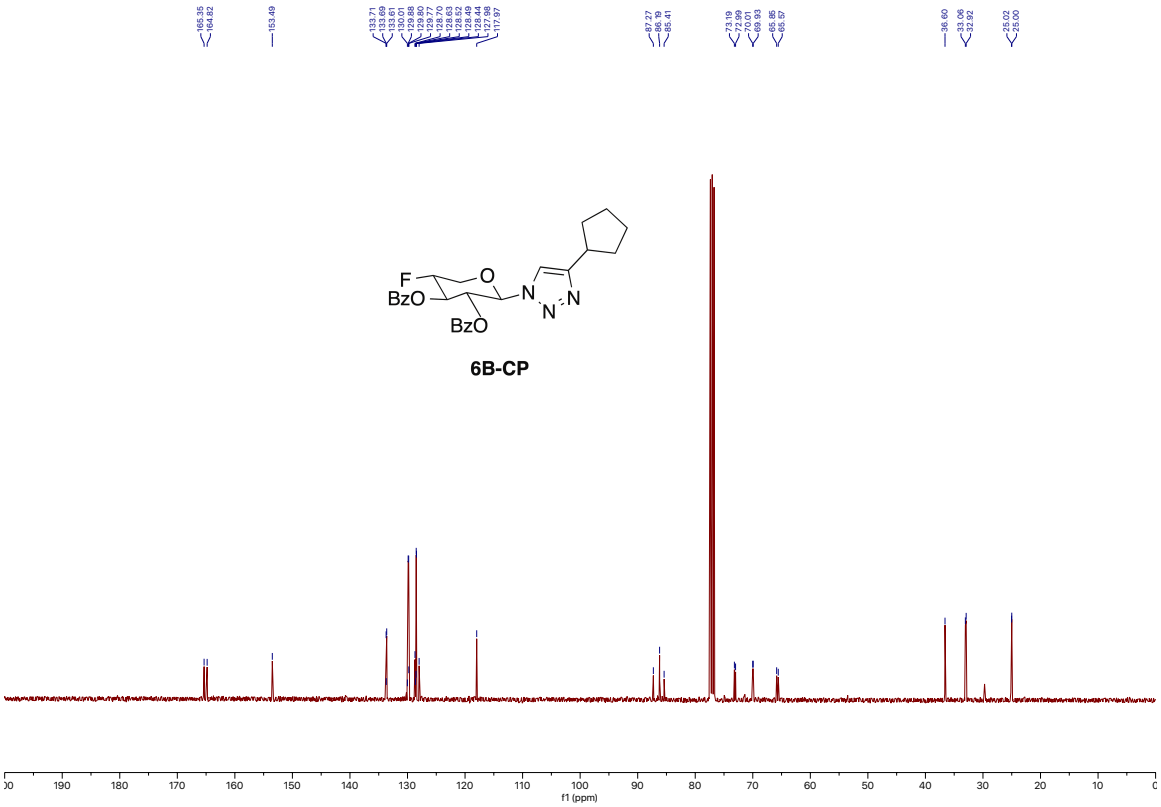

F
O
BzO
BzO
N
N
N
6A-CP
f1 (ppm)

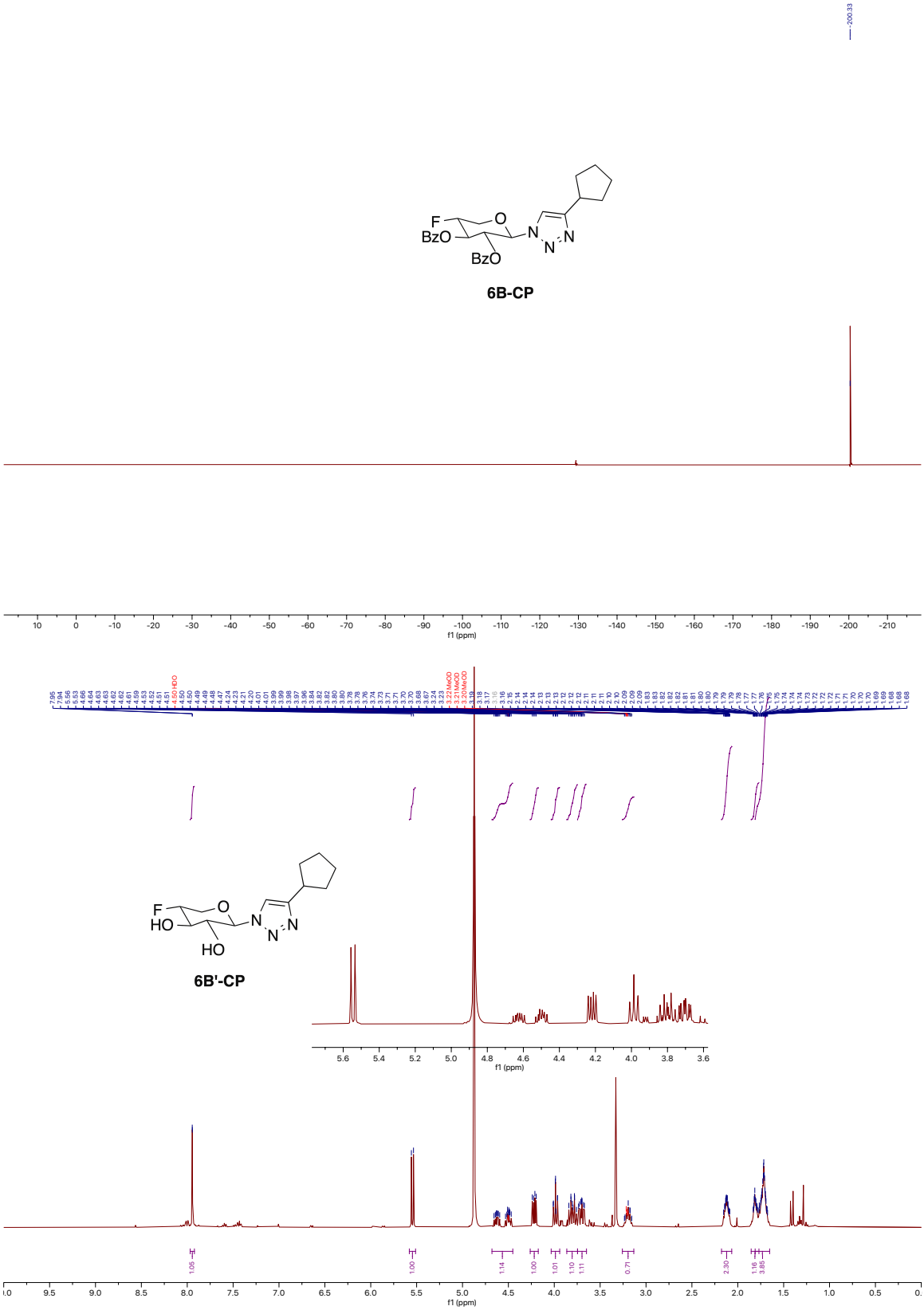

F
BzO
O
BzO
N
N
N
6A-CP
-194.99
f1 (ppm)
7.96
6.10
6.09
4.47 HDO
3.23
3.21
3.19
3.17
3.15
2.15
2.14
2.13
2.12
2.11
2.10
2.09
2.08
1.85
1.84
1.83
1.81
1.80
1.79
1.78
1.74
1.73
1.71
1.70
1.69
1.67
1.66
F
HO
O
HO
N
N
N
6A'-CP
f1 (ppm)
1.00
1.00
1.02
1.10
0.97
1.20
1.16
1.19
2.34
7.12
f1 (ppm)

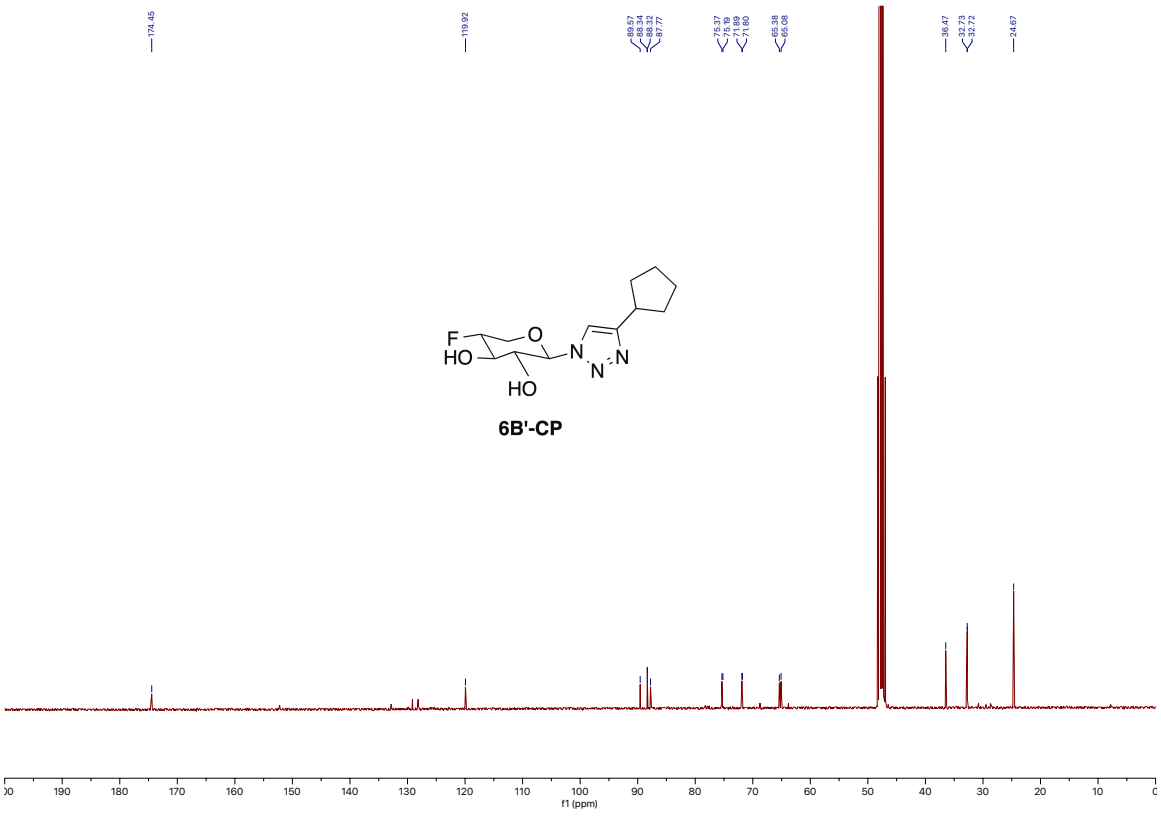

121.52
88.87
87.09
86.01
69.83
69.80
69.25
69.03
68.42
65.19
36.45
32.76
24.67
F
HO
O
HO
N
N
N
6A'-CP
f1 (ppm)

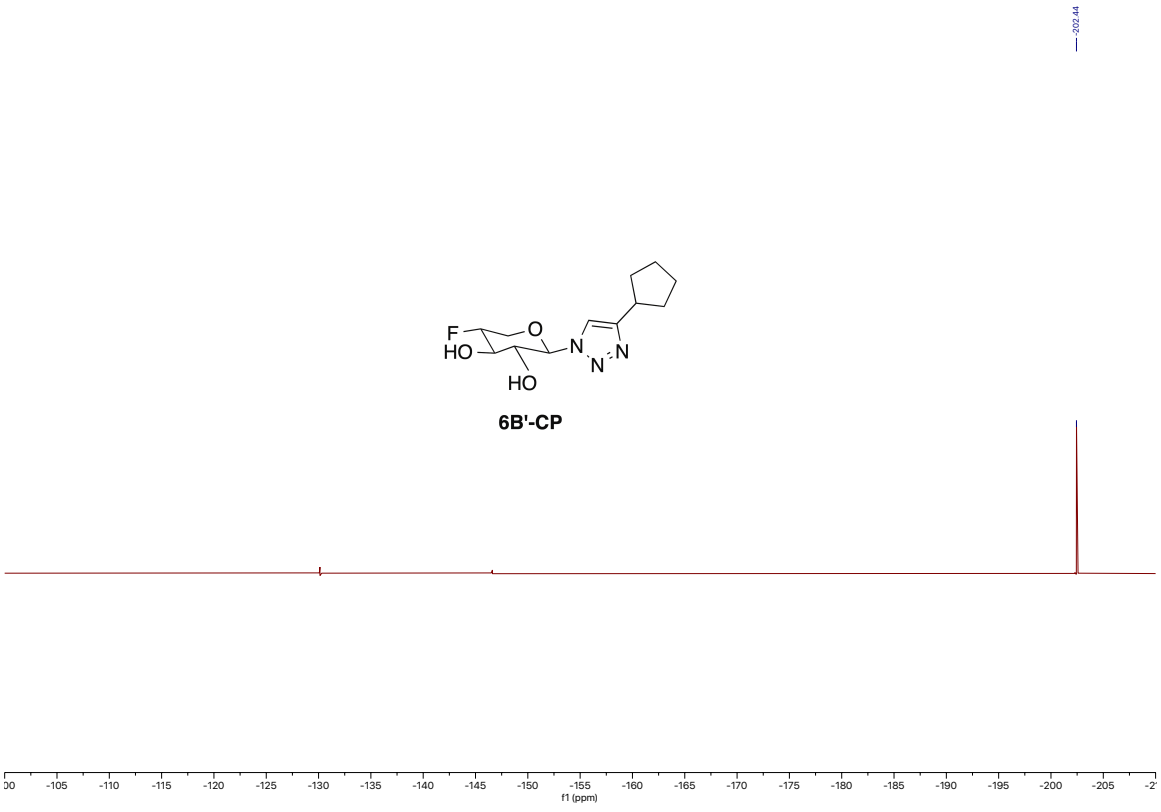

-194.70
F
HO
O
HO
N
N
N
6A'-CP
f1 (ppm)

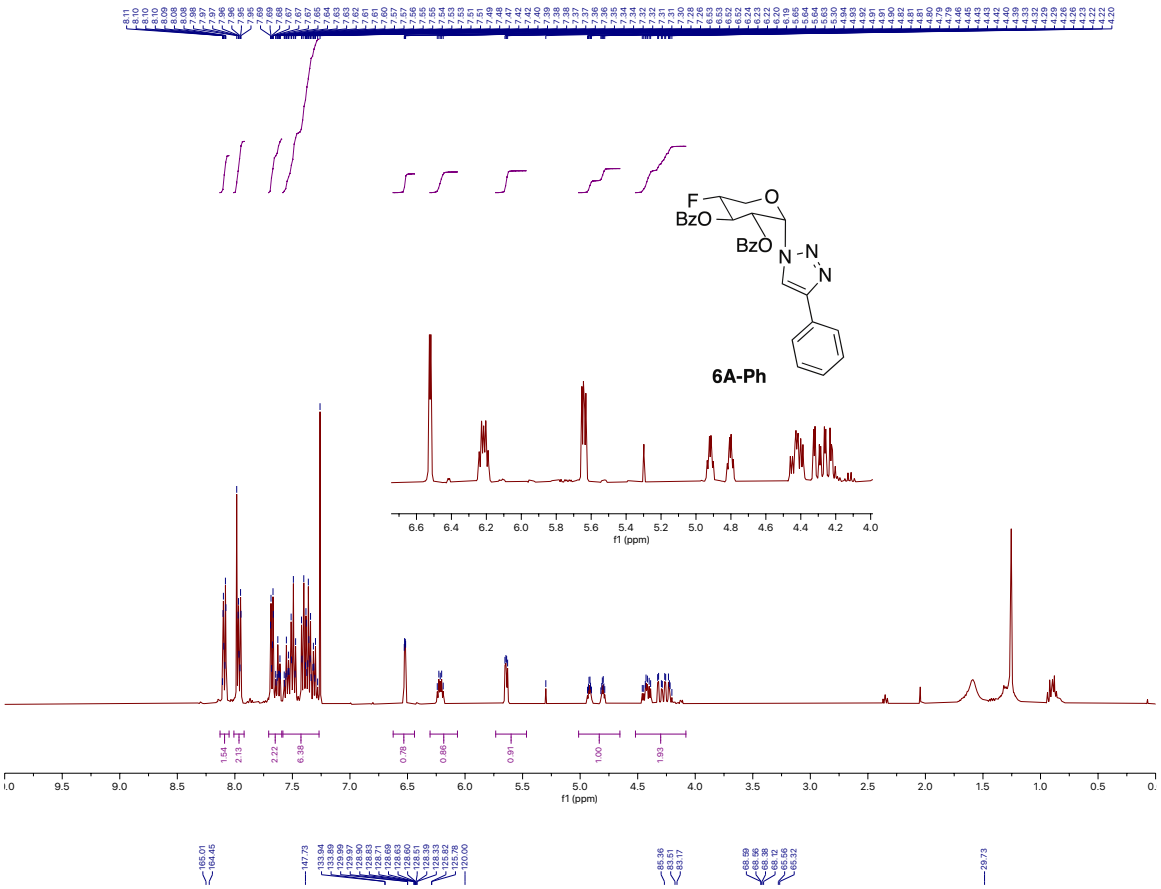

F
BzO
BzO
O
N
N
N
6B-CP
f1 (ppm)

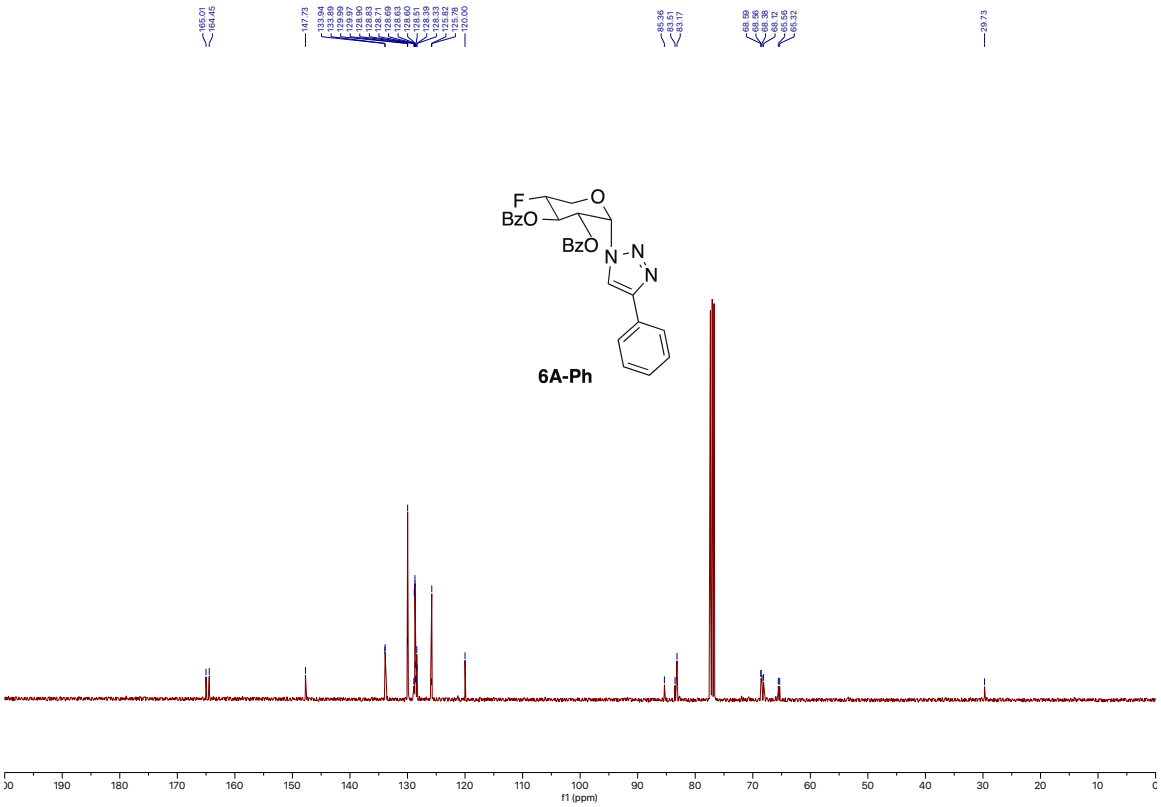

F
BzO
BzO
O
N
N
N
6B-CP
f1 (ppm)

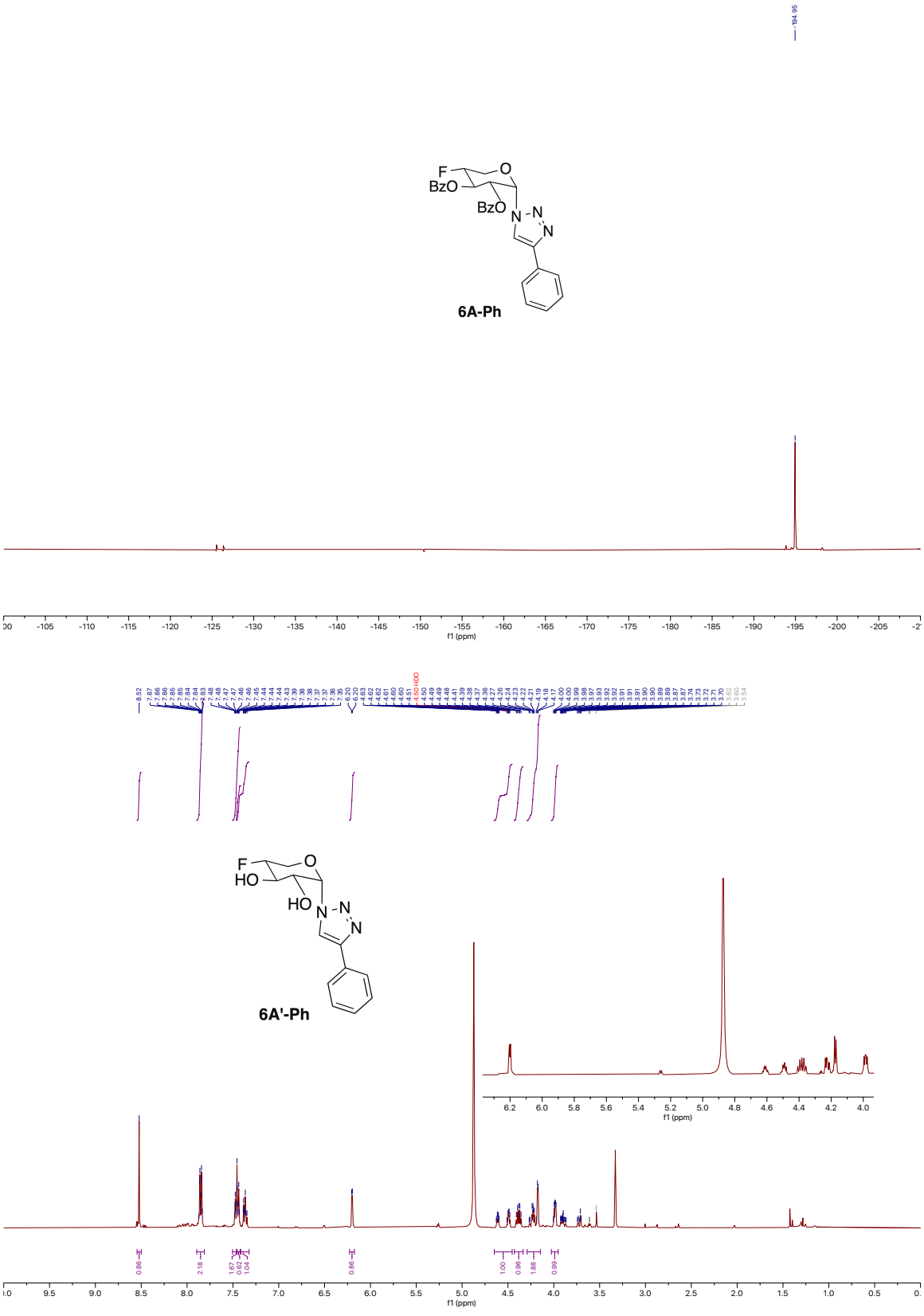

200.33
F
BzO
O
N
N
N
BzO
6B-CP
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)
4.50 HDO
3.22 MeOD
3.31 MeOD
F
HO
O
N
N
N
HO
6B'-CP
5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6
f1 (ppm)
1.05
1.00
1.14
1.00
1.01
1.10
1.11
0.71
2.30
1.16
3.85
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

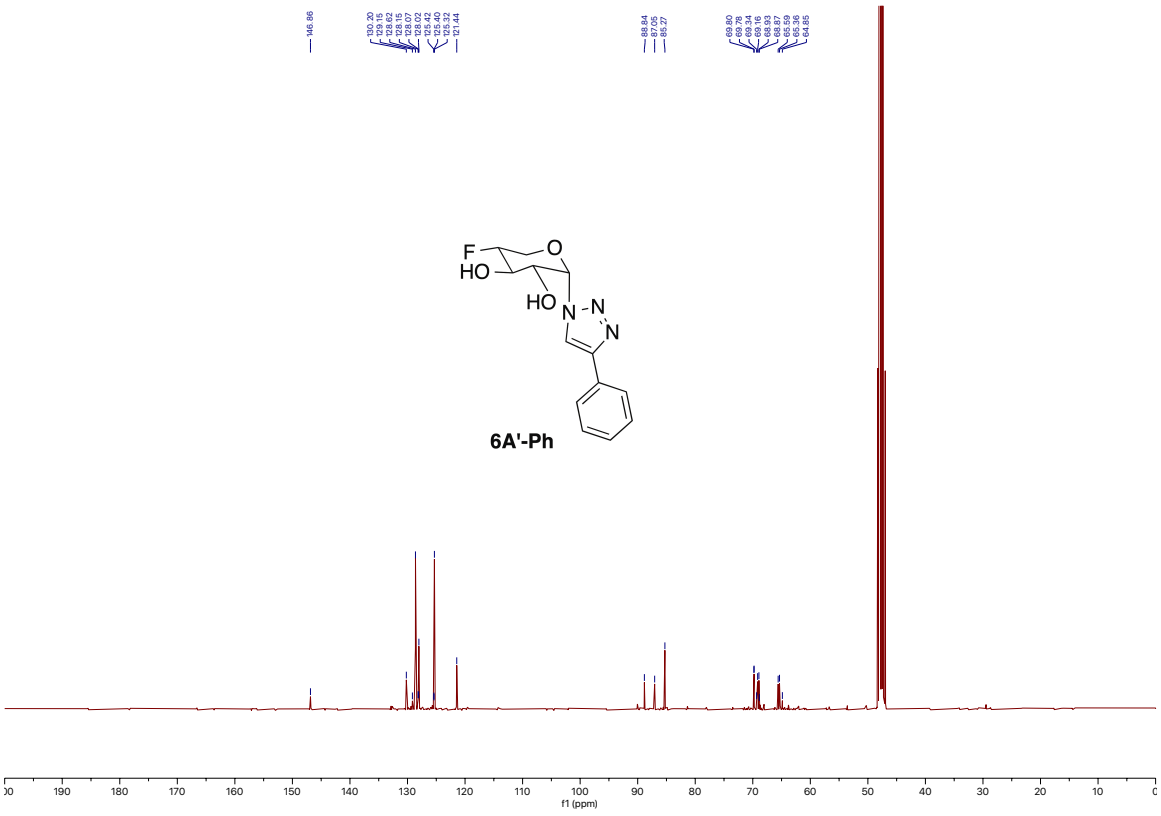

174.45
119.92
89.57
89.34
88.32
87.77
75.37
75.19
73.98
71.80
65.38
65.08
36.47
32.73
32.72
24.67
F
HO
HO
O
N
N
N
6B'-CP
f1 (ppm)

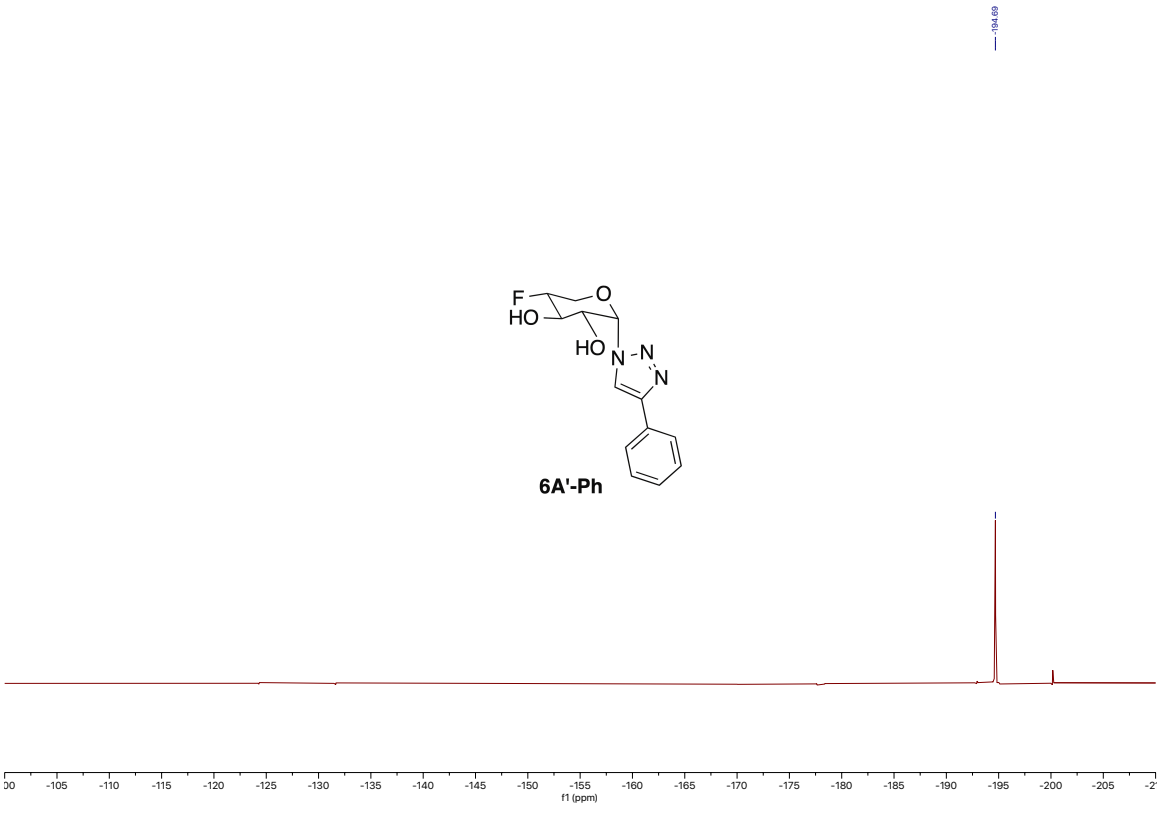

202.44
F
HO
HO
O
N
N
N
6B'-CP
f1 (ppm)

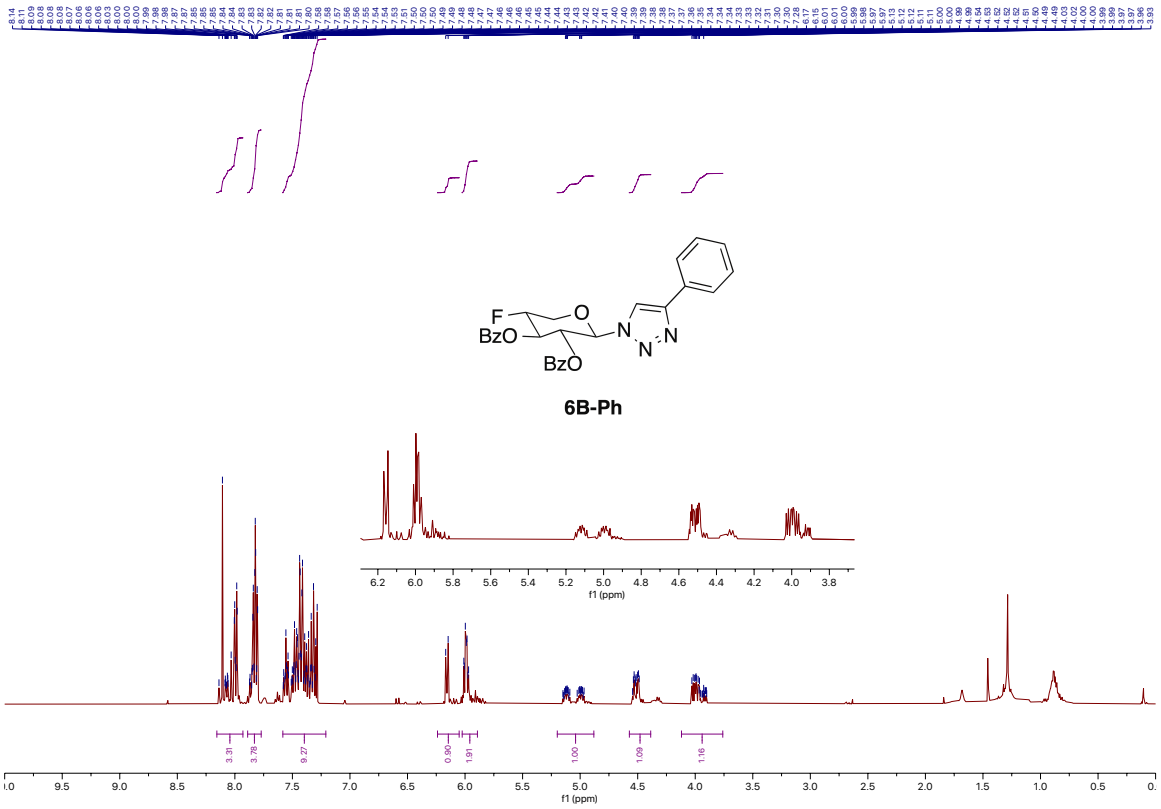

F
O
BzO
BzO
N
N
N
6A-Ph
f1 (ppm)

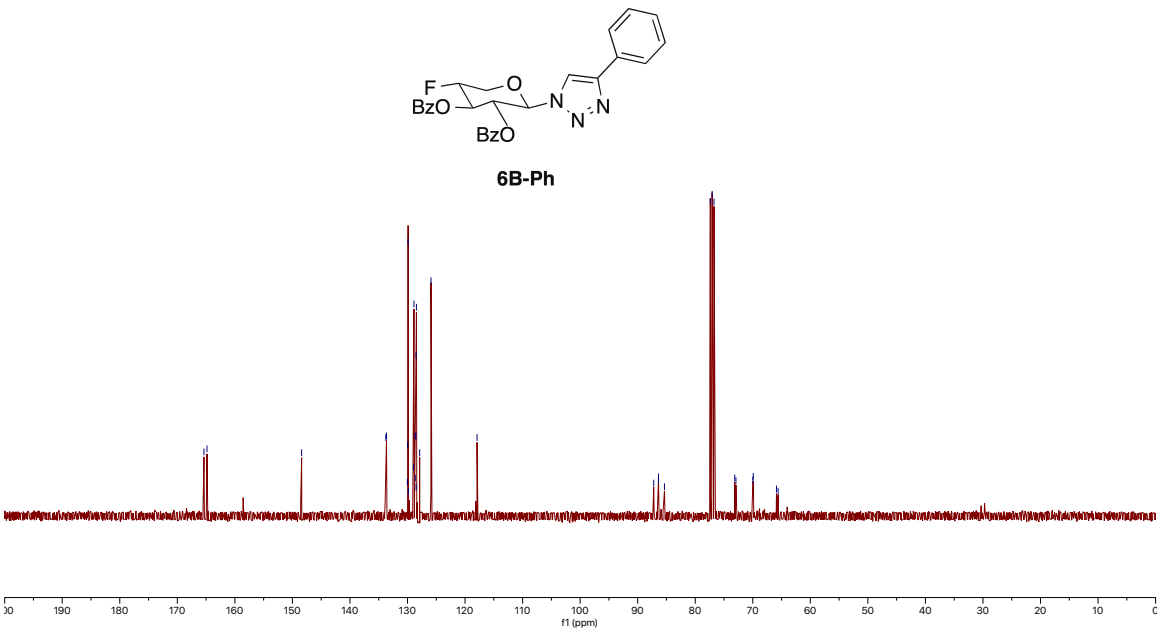

F
O
BzO
BzO
N
N
N
6A-Ph
f1 (ppm)

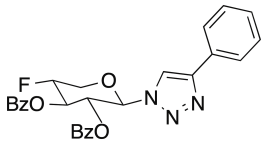

-194.95
F
BzO
BzO
N
N
N
6A-Ph
f1 (ppm)
F
HO
HO
N
N
N
6A'-Ph
f1 (ppm)
f1 (ppm)


146.86
130.20
129.15
128.65
128.15
128.07
128.02
126.42
125.40
125.32
121.44
88.84
87.95
85.27
69.80
69.78
69.34
69.16
68.93
68.87
65.59
65.36
64.98
F
HO
HO
O
N
N
N
6A'-Ph
f1 (ppm)

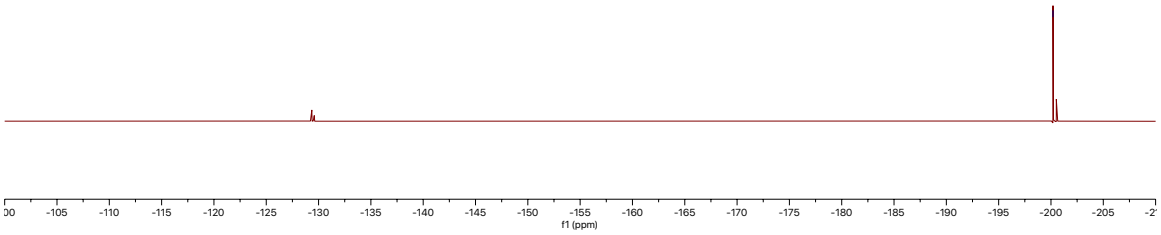

-194.69
F
HO
HO
O
N
N
N
6A'-Ph
f1 (ppm)

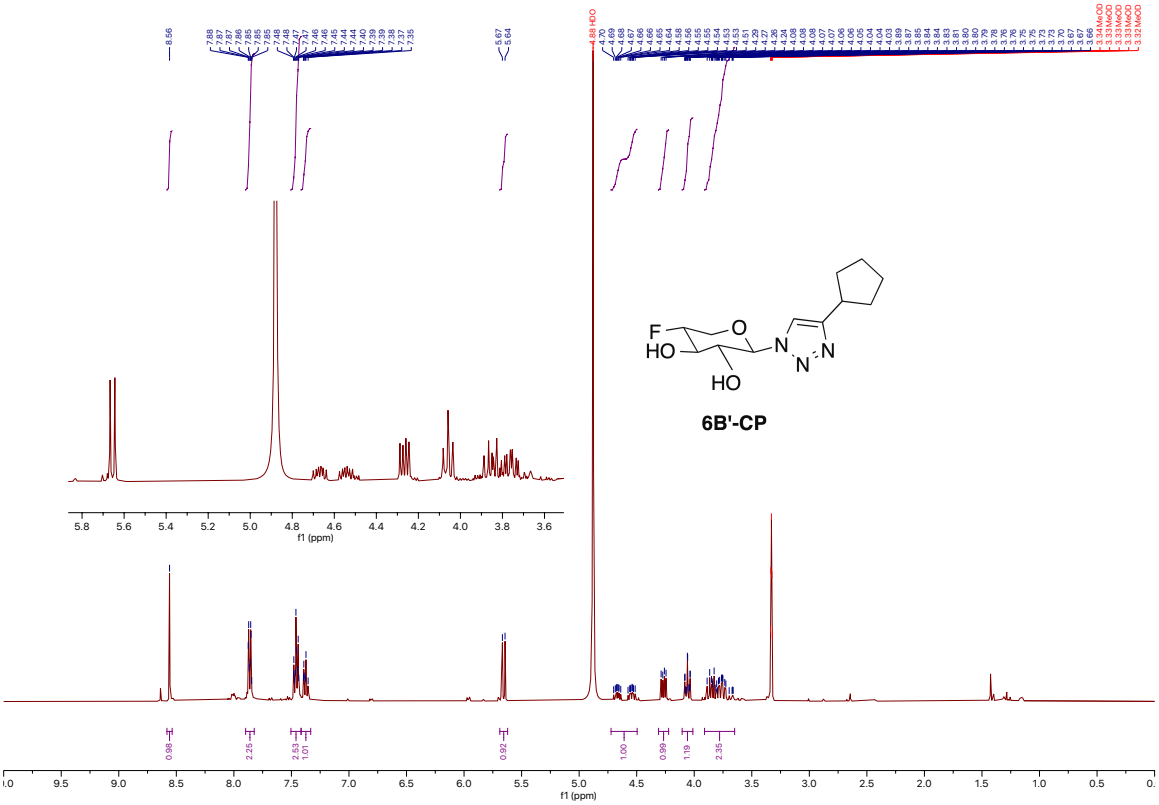

F
BzO
BzO
O
N
N
N
6B-Ph
f1 (ppm)

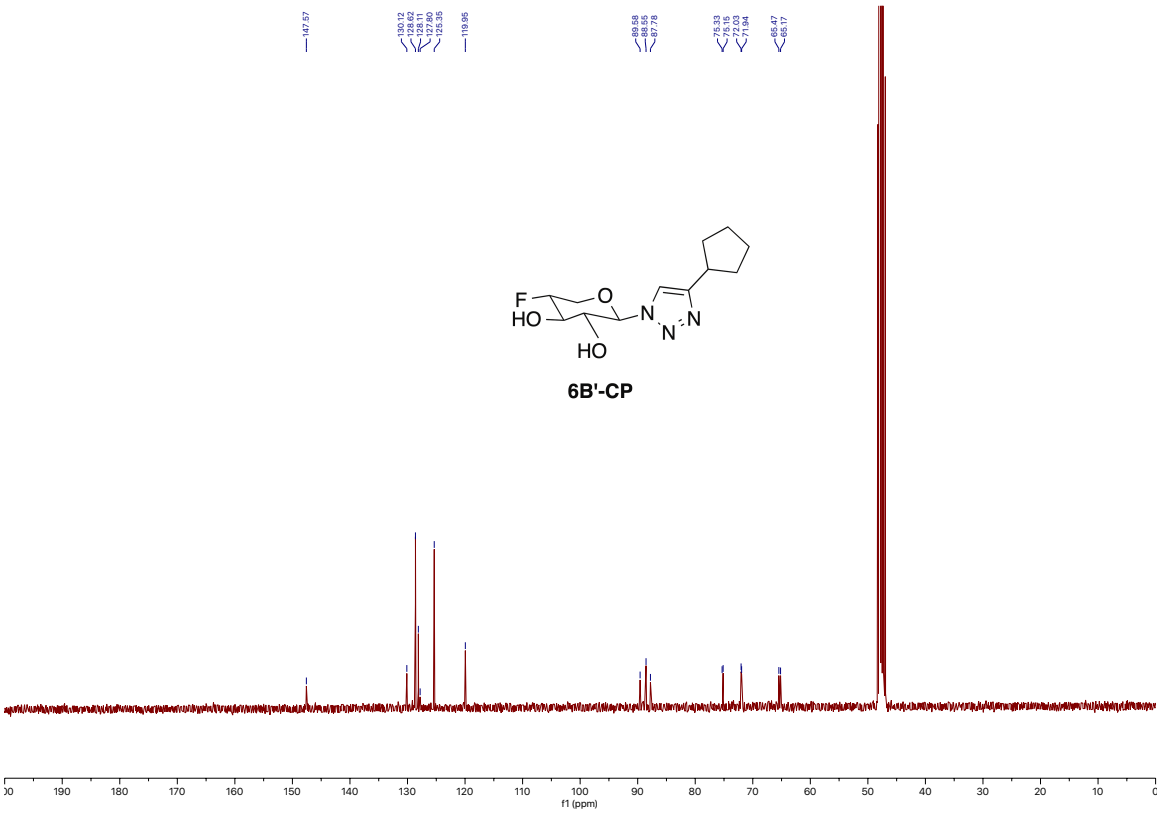

F
BzO
BzO
O
N
N
N
6B-Ph
f1 (ppm)

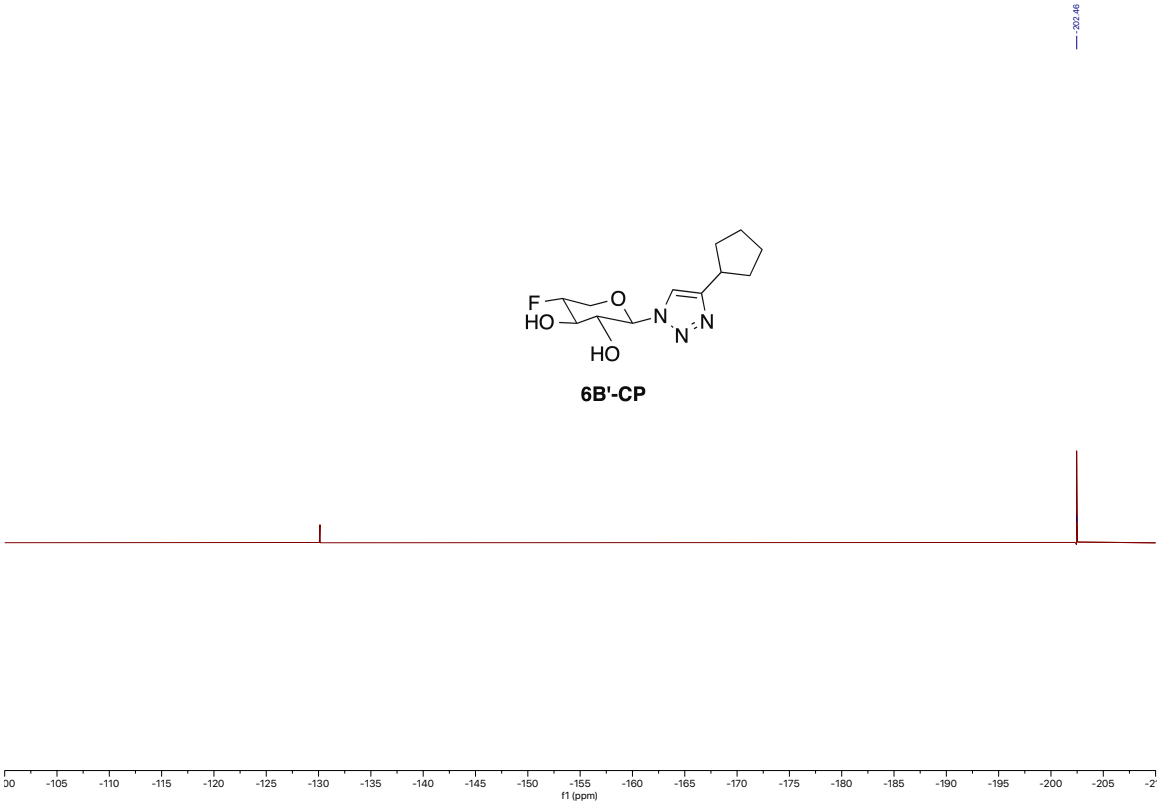

F
BzO
O
N
N
N
BzO

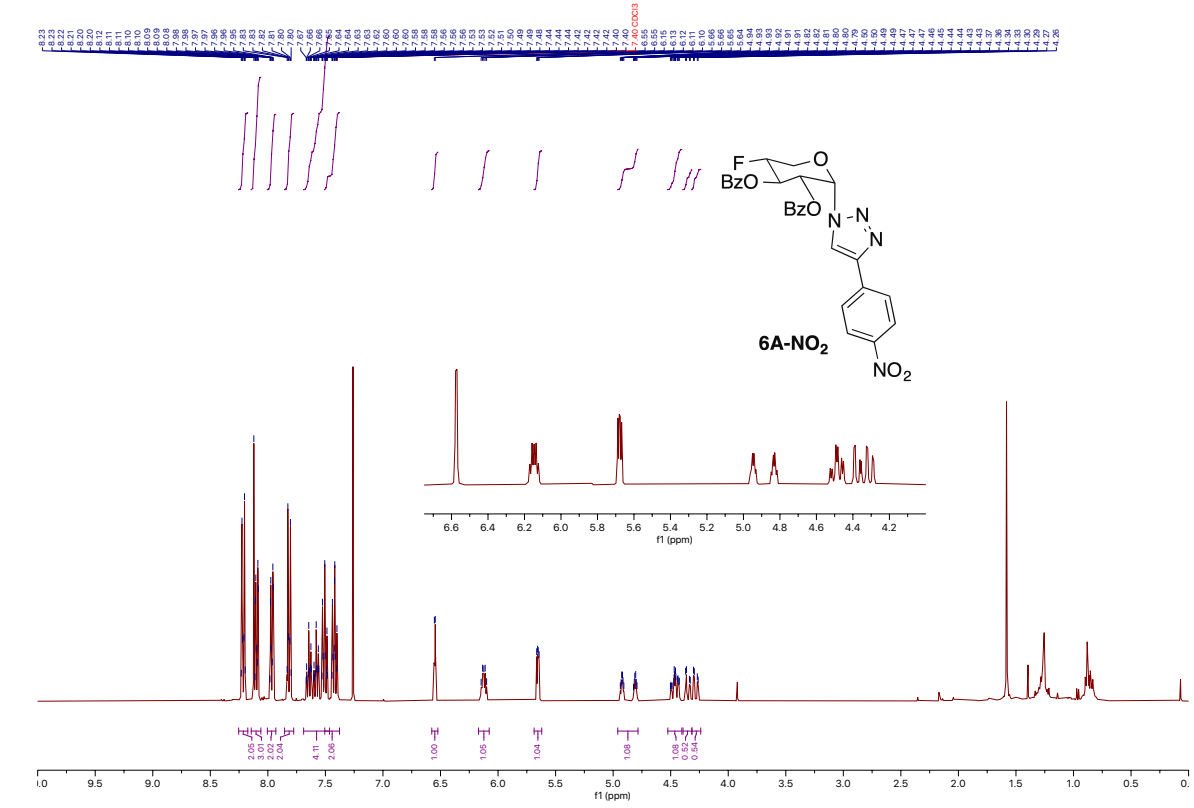
6B-Ph

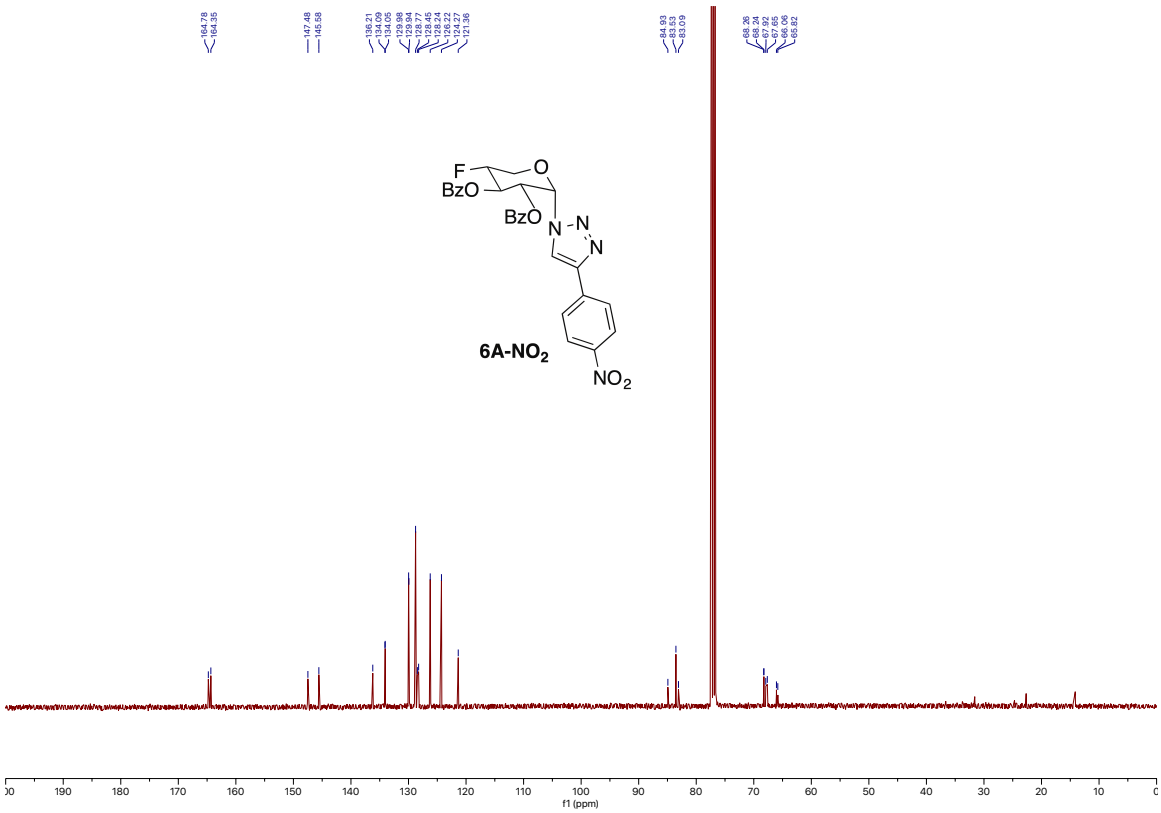

-200.19
f1 (ppm)

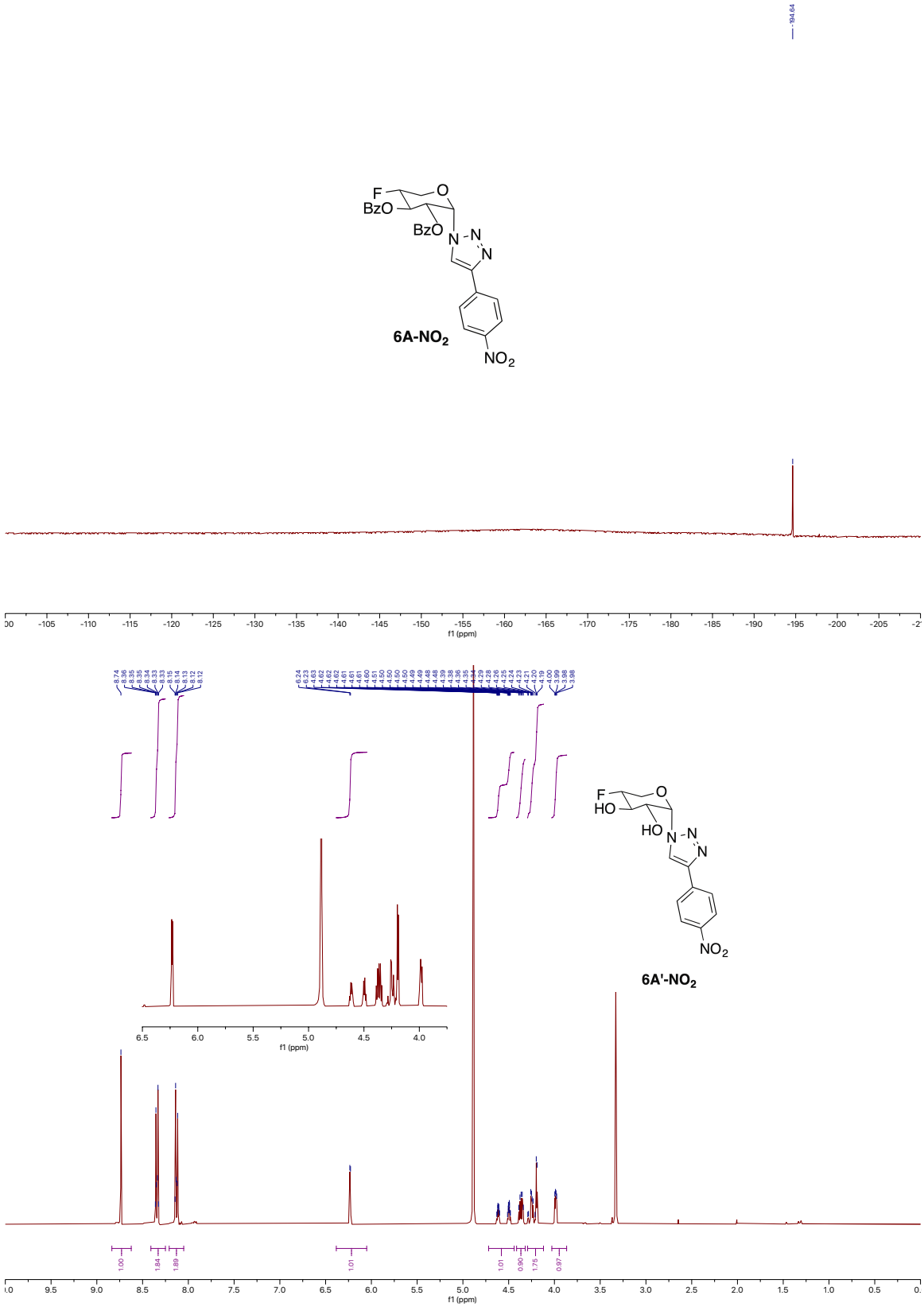

8.56
7.88
7.87
7.87
7.86
7.85
7.85
7.48
7.48
7.47
7.47
7.46
7.46
7.45
7.44
7.44
7.40
7.39
7.39
7.38
7.37
7.35
5.67
5.64
4.88 HDO
4.69
4.69
4.68
4.67
4.66
4.66
4.65
4.64
4.58
4.56
4.55
4.55
4.54
4.54
4.53
4.53
4.51
4.50
4.49
4.27
4.26
4.24
4.08
4.08
4.08
4.07
4.07
4.06
4.06
4.05
4.05
4.04
4.04
4.03
4.03
3.89
3.87
3.85
3.84
3.84
3.83
3.81
3.80
3.80
3.79
3.78
3.78
3.76
3.75
3.75
3.74
3.73
3.73
3.72
3.70
3.67
3.67
3.66
3.34MeOD
3.33MeOD
3.33MeOD
3.33MeOD
3.32MeOD
F
HO
O
N
N
N
HO
6B'-CP
0.98
2.25
2.51
1.01
0.92
1.00
0.99
1.19
2.35
f1 (ppm)

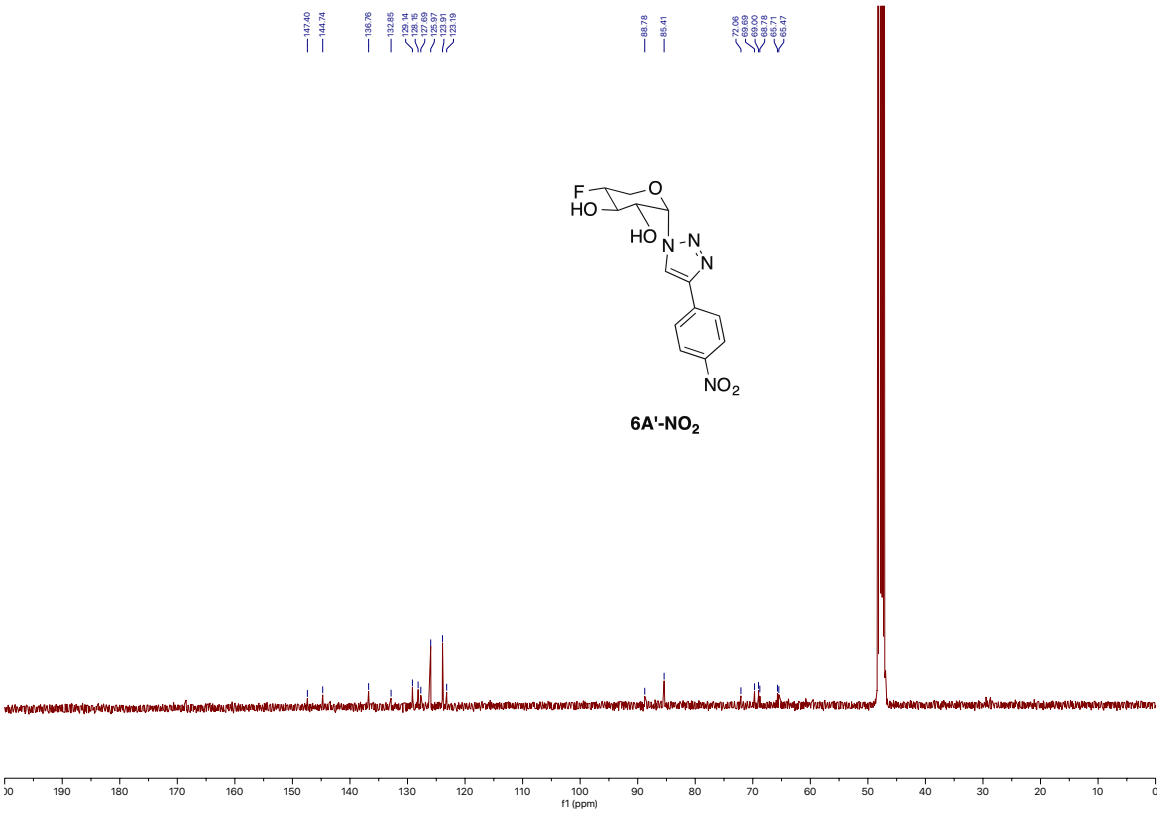

147.57
130.12
128.62
128.11
127.89
125.35
119.95
89.68
88.95
87.78
75.33
75.15
72.03
71.94
65.47
65.17
F
HO
O
N
N
N
HO
6B'-CP
f1 (ppm)


202.46
F
HO
O
N
N
N
HO
6B'-CP
f1 (ppm)

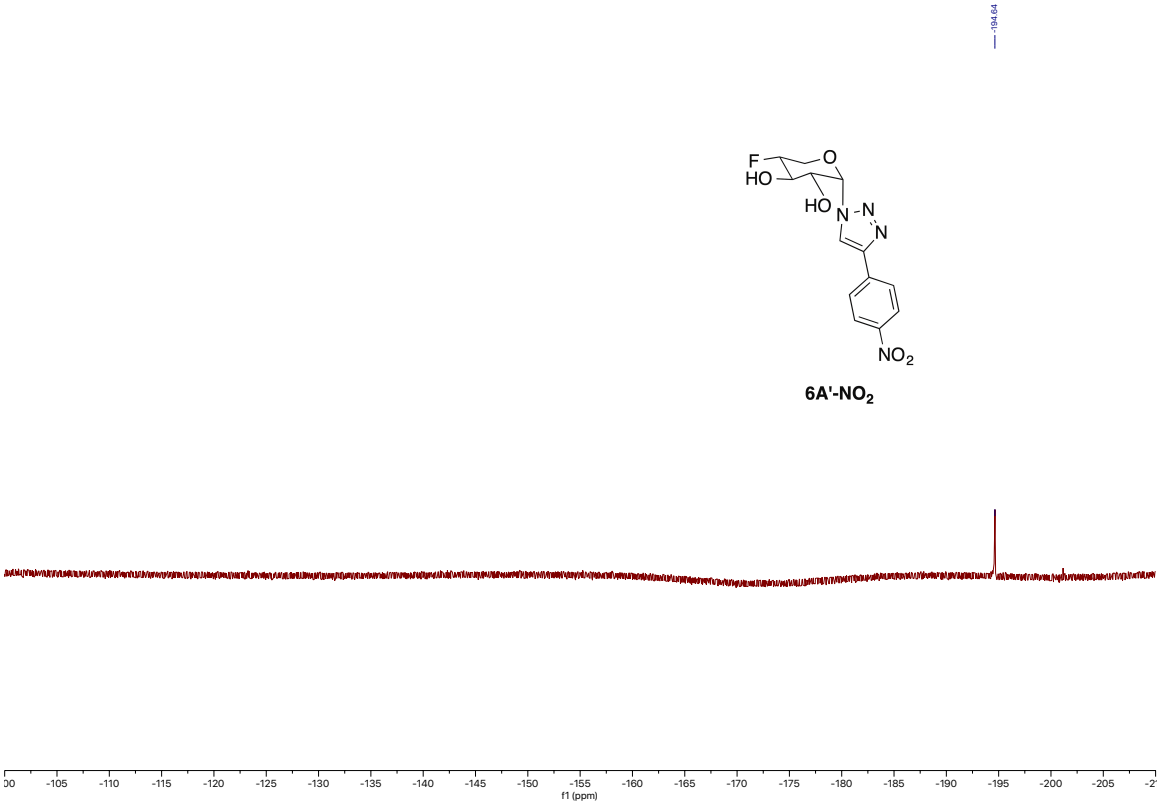

F
O
BzO
BzO
N
N
N
6A-NO2
NO2
f1 (ppm)


F
O
BzO
BzO
N
N
N
6A-NO2
NO2
f1 (ppm)

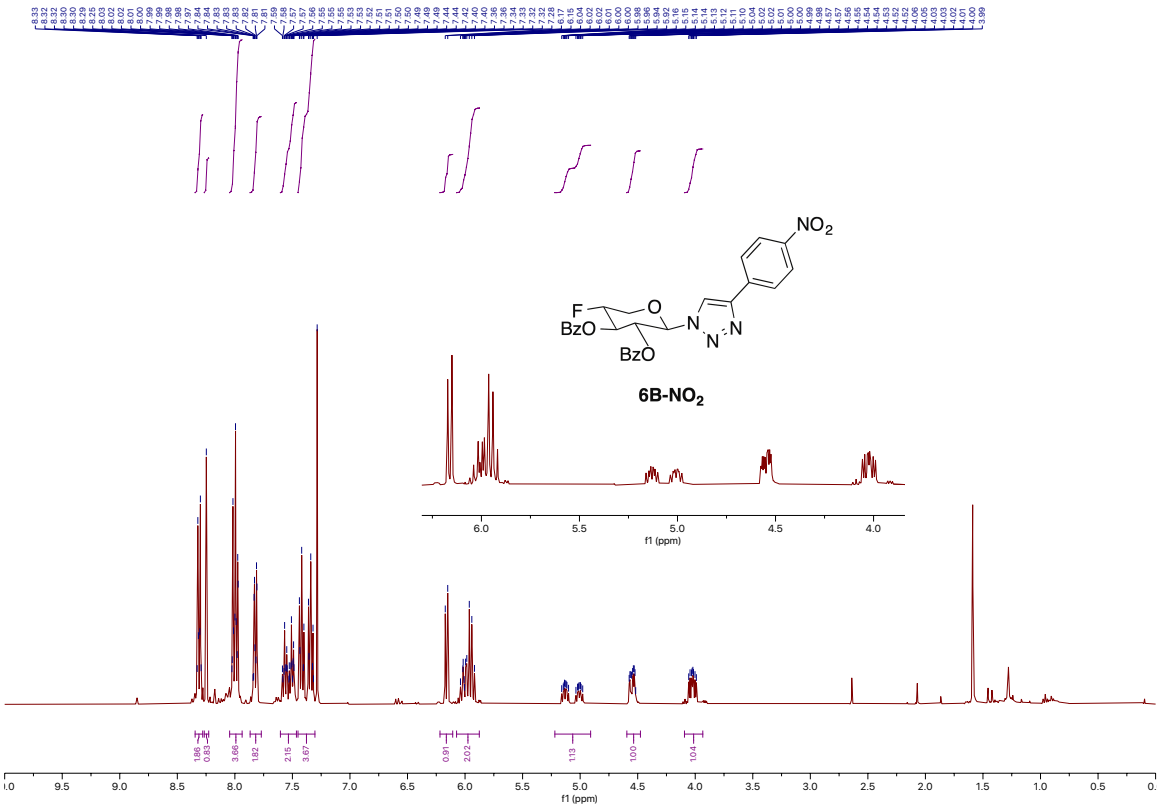

-194.64
F
O
BzO
BzO
N
N
N
6A-NO2
NO2
f1 (ppm)
F
O
HO
HO
N
N
N
6A'-NO2
NO2
f1 (ppm)
f1 (ppm)

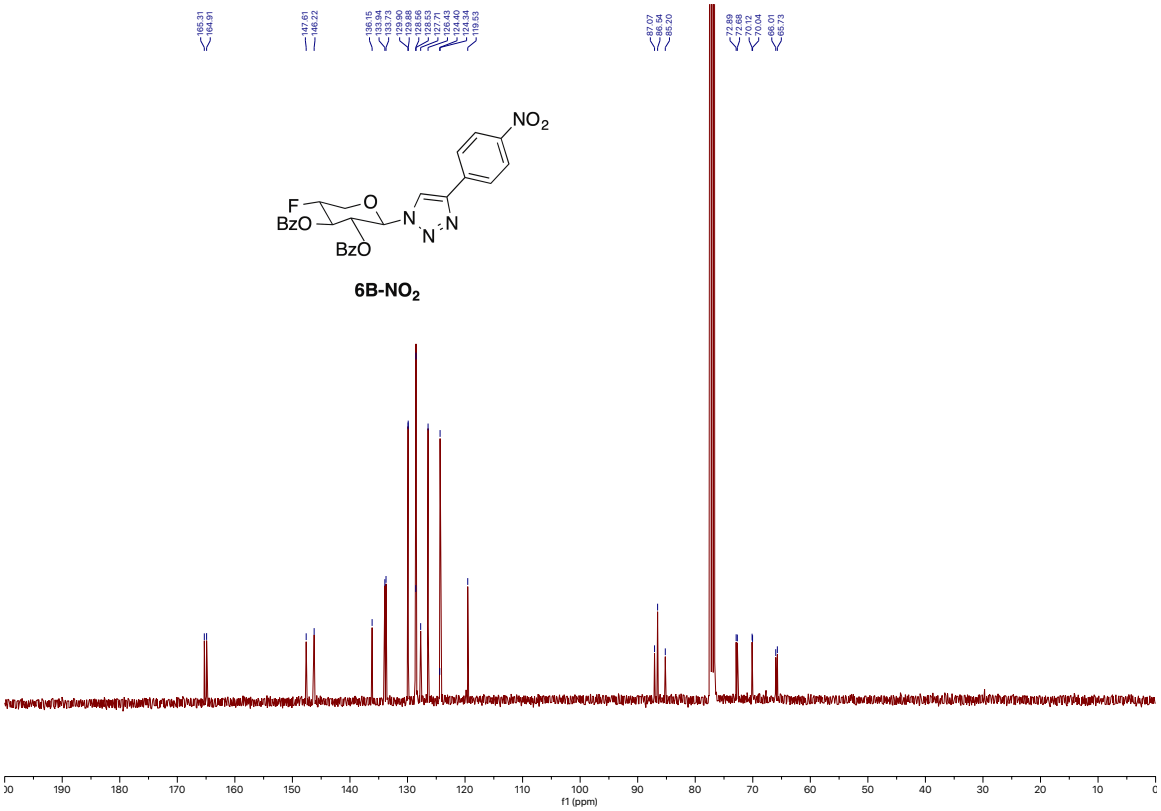

147.40
144.74
136.76
132.85
129.14
128.35
127.69
125.97
123.61
123.19
88.78
85.41
72.06
69.69
69.20
68.74
65.71
65.47
F
HO
HO
O
N
N
N
NO2
f1 (ppm)

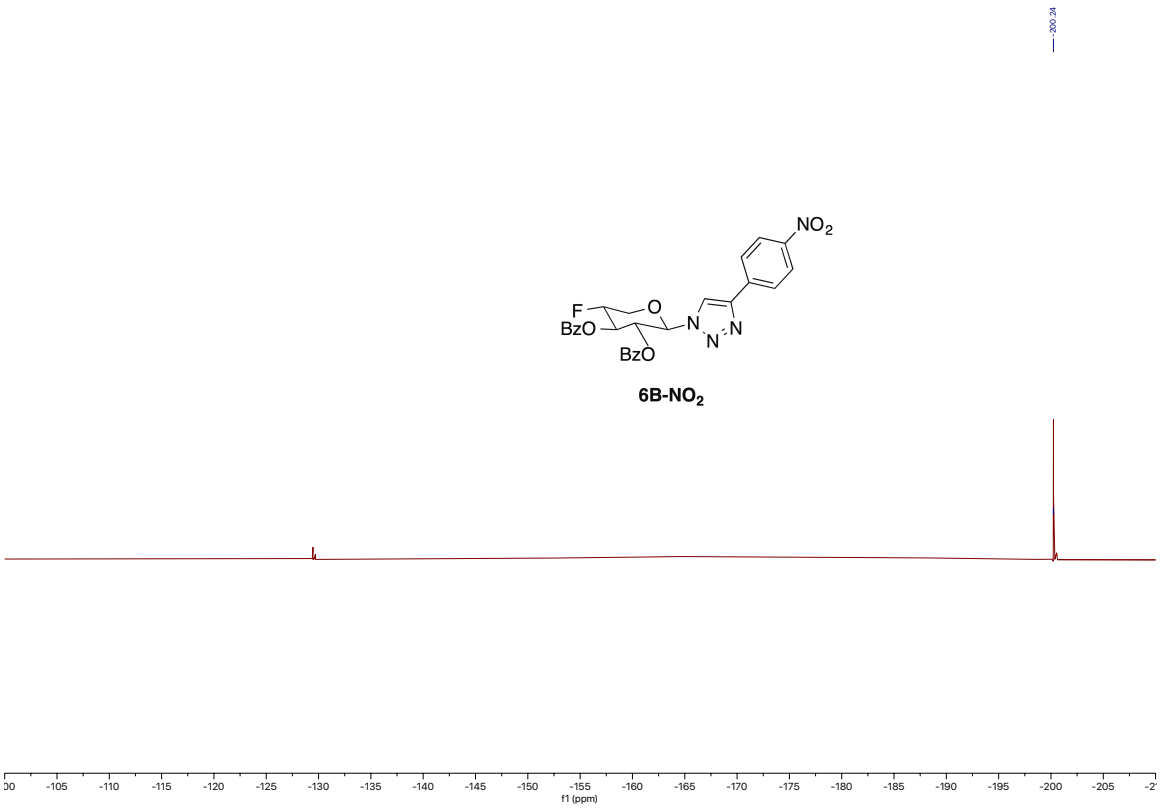
6A'-NO_2


-194.64
F
HO
HO
O
N
N
N
NO2
f1 (ppm)

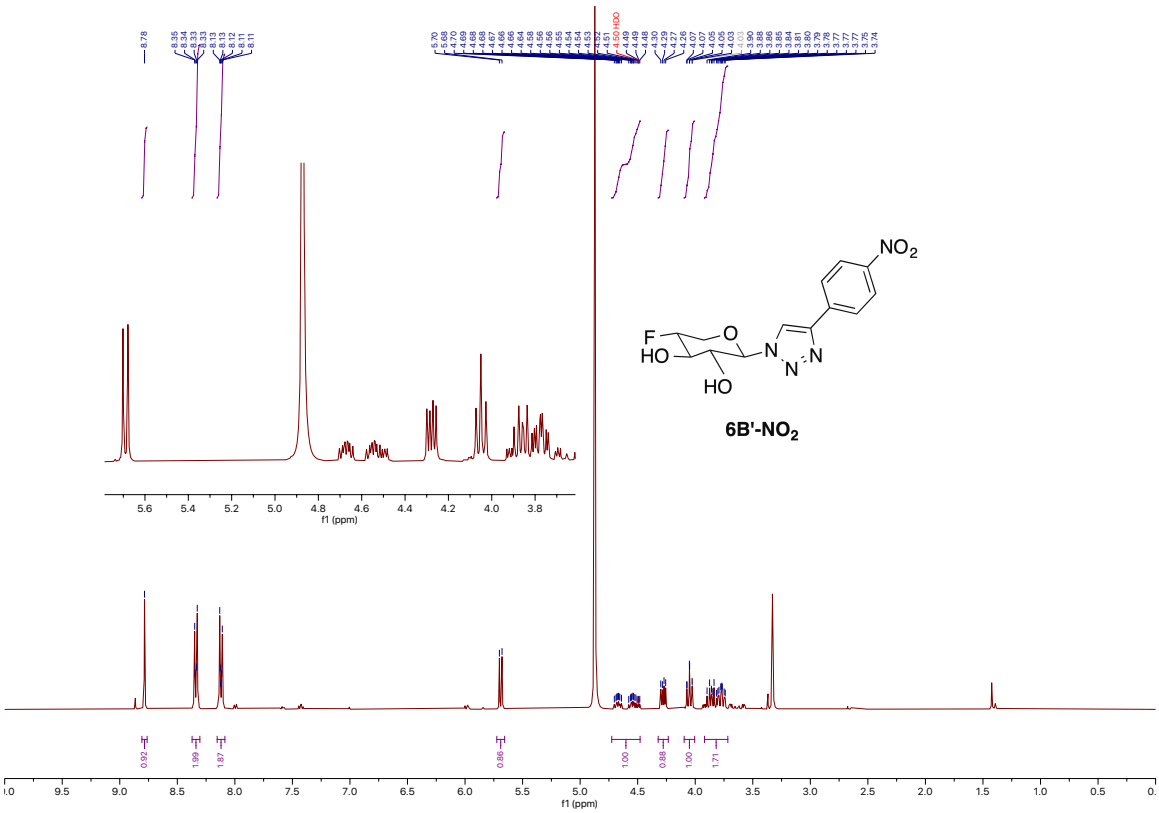
6A'-NO_2


NO2
F
O
BzO
BzO
N
N
N
6B-NO2
6.0
5.5
5.0
4.5
4.0
f1 (ppm)
1.86
0.83
3.66
1.82
2.15
3.67
0.91
2.02
1.13
1.00
1.04
f1 (ppm)

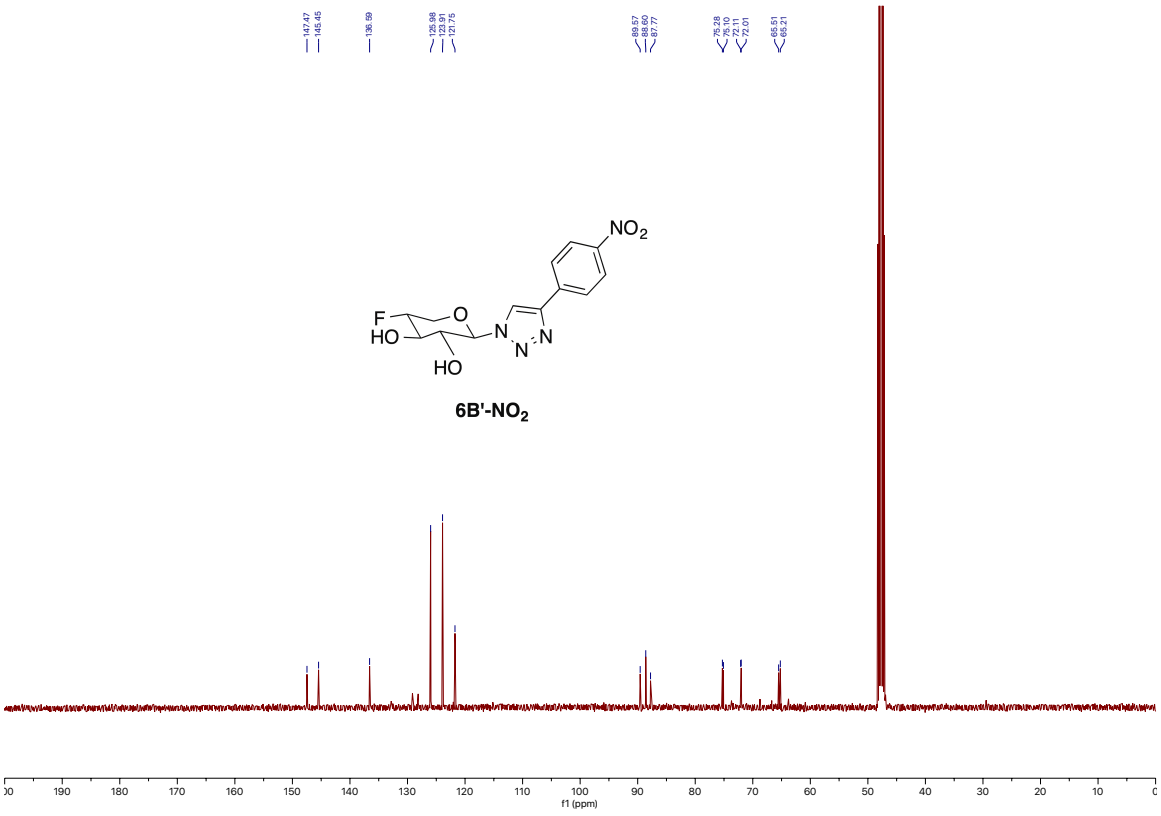

NO2
F
O
BzO
BzO
N
N
N
6B-NO2
f1 (ppm)


-200.24
NO2
F
O
BzO
N
N
N
BzO
f1 (ppm)

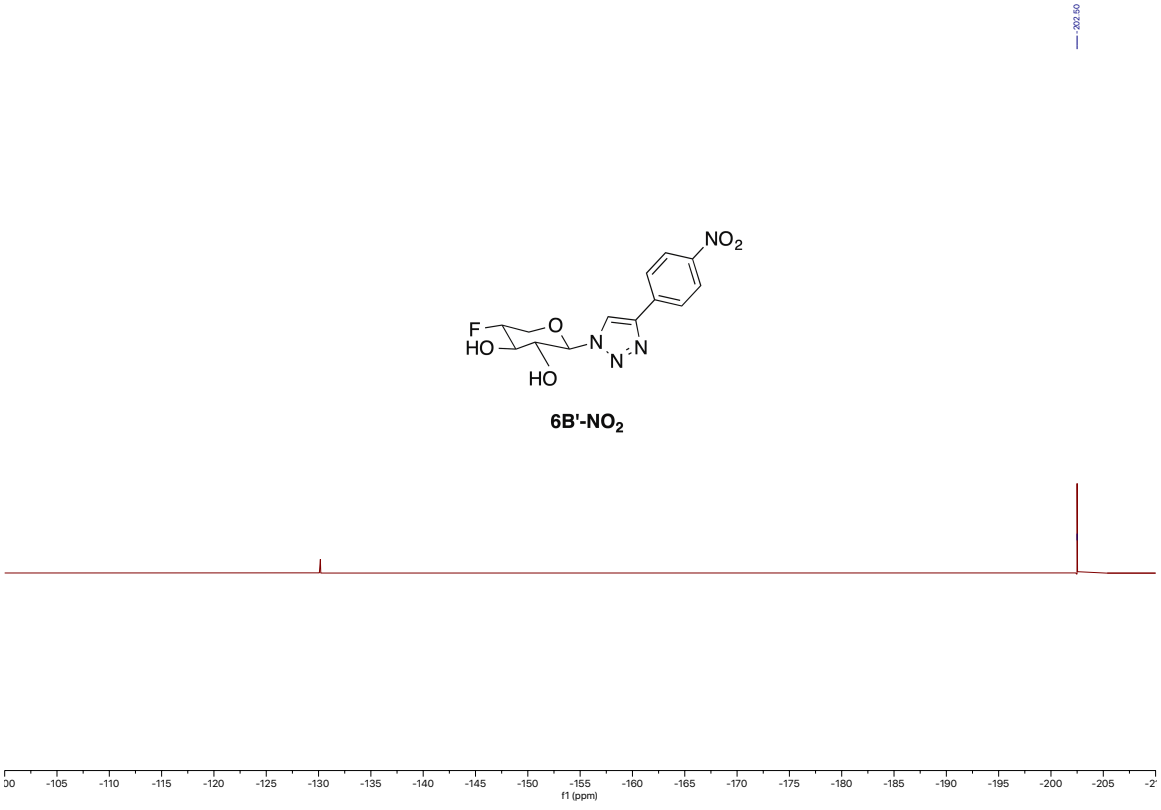
6B-NO$_2$


8.78
8.35
8.34
8.33
8.13
8.13
8.12
8.11
8.11
NO2
F
O
HO
HO
N
N
N
f1 (ppm)

6B'-NO$_2$

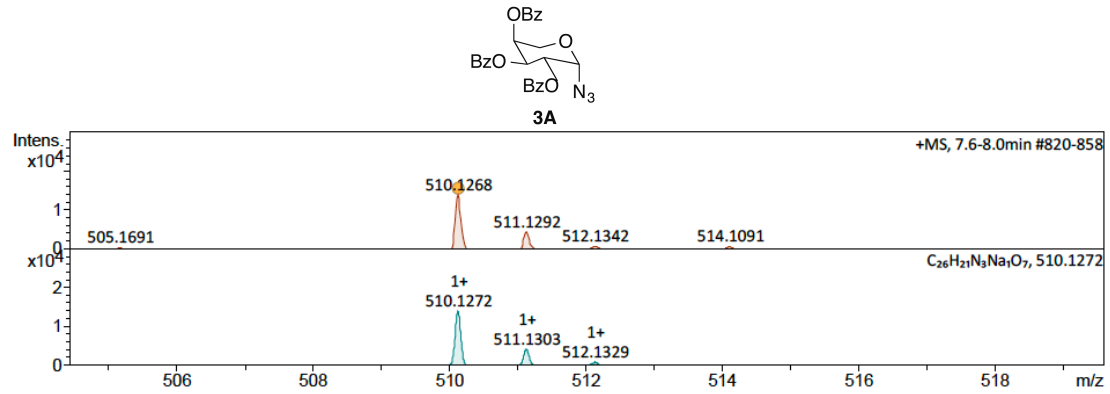

147.47
146.45
136.99
135.98
133.91
121.75
89.67
88.60
87.77
75.28
75.10
72.11
72.01
65.61
65.21
NO2
F
HO
HO
O
N
N
N
f1 (ppm)

6B'-NO_2

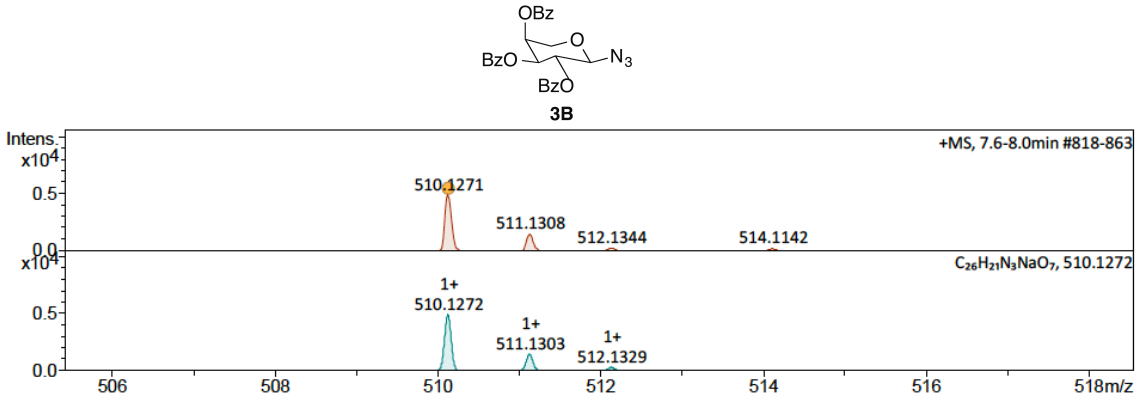

202.50
NO2
F
HO
HO
O
N
N
N
f1 (ppm)

6B'-NO_2

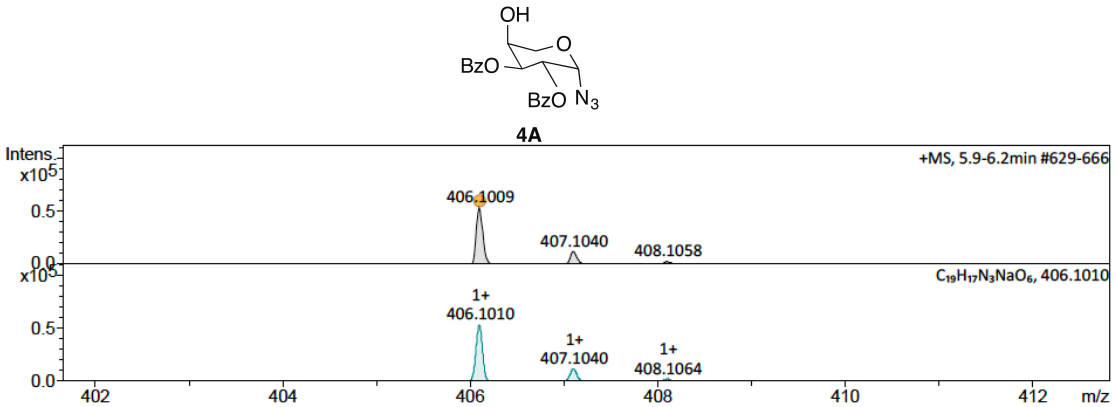
10. High Resolution Mass Spectrometry



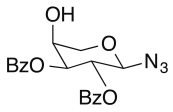
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
510.1268	1	C26H21N3NaO7	510.1272	0.6	6.9	1	100.00	17.5	even	ok



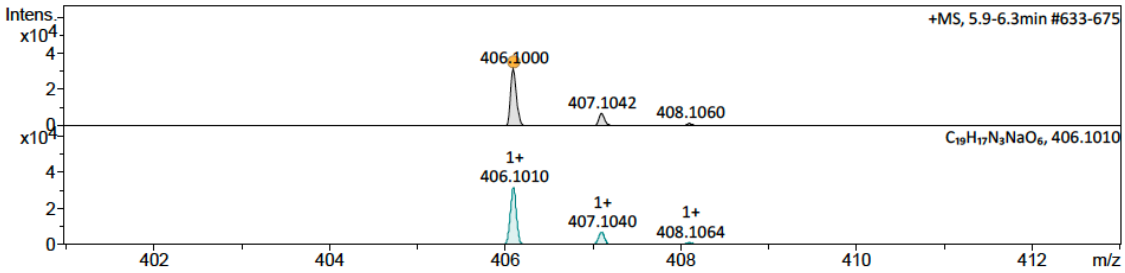
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
510.1271	1	C26H21N3NaO7	510.1272	0.2	6.6	1	100.00	17.5	even	ok



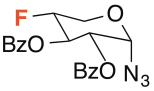
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
406.1009	1	C19H17N3NaO6	406.1010	0.2	5.0	1	100.00	12.5	even	ok



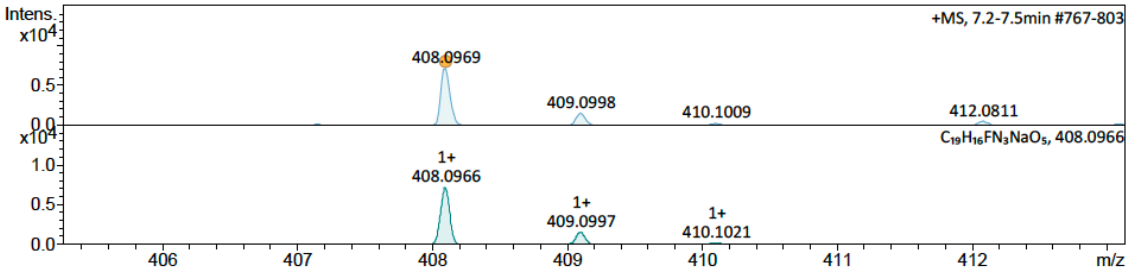
4B



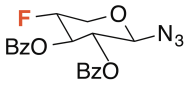
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
406.1000	1	C19H17N3NaO6	406.1010	2.3	5.7	1	100.00	12.5	even	ok



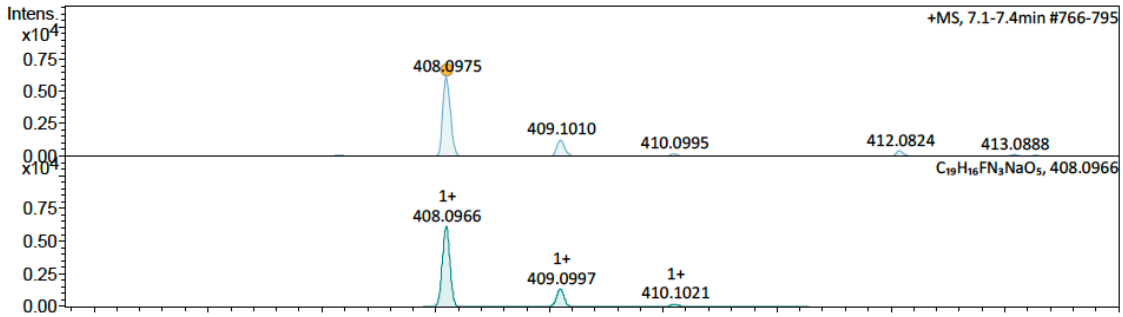
5A



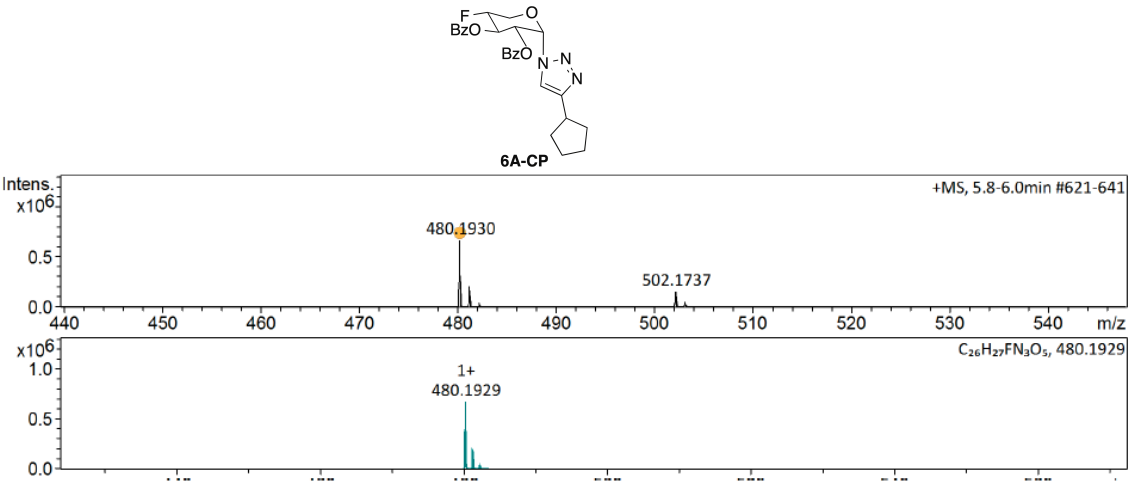
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
408.0969	1	C19H16FN3NaO5	408.0966	-0.7	3.8	1	100.00	12.5	even	ok



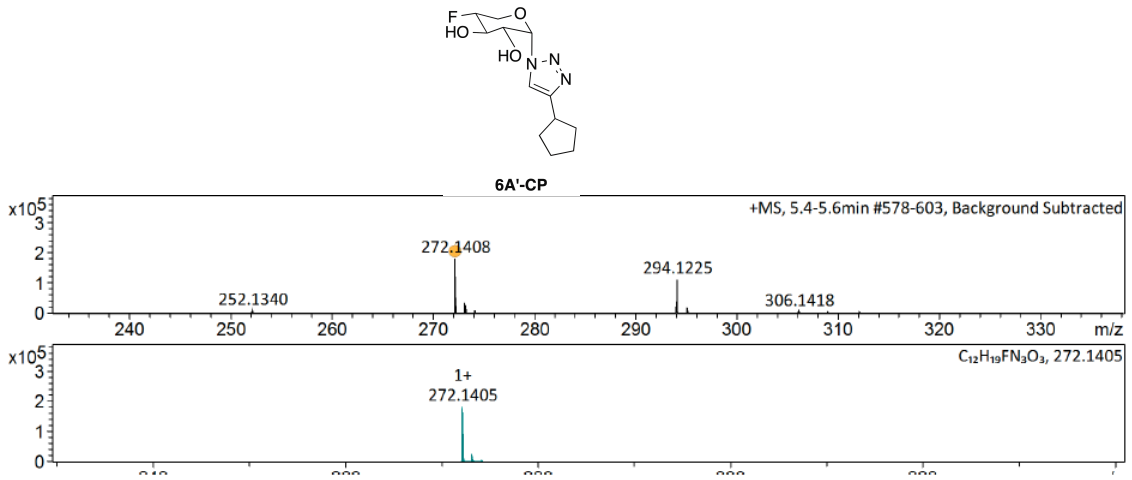
5B



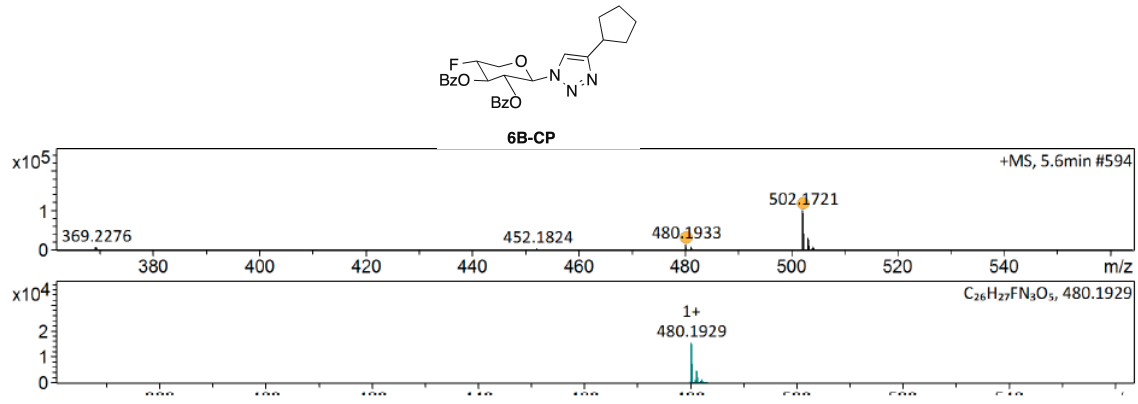
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
408.0975	1	C19H16FN3NaO5	408.0966	-2.3	4.9	1	100.00	12.5	even	ok



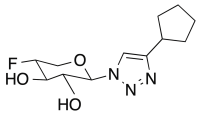
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
480.1930	1	C26H27FN3O5	480.1929	-0.2	4.8	2	100.00	14.5	even	ok



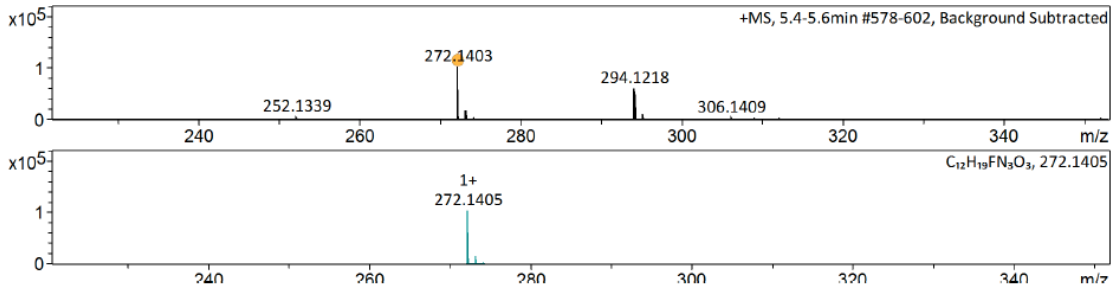
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
272.1408	1	C12H19FN3O3	272.1405	-1.3	19.8	3	60.89	4.5	even	ok



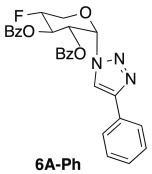
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
480.1933	1	C26H27FN3O5	480.1929	-0.8	34.9	2	84.00	14.5	even	ok
502.1721	1	C26H26FN3NaO5	502.1749	5.4	4.8	3	100.00	14.5	even	ok



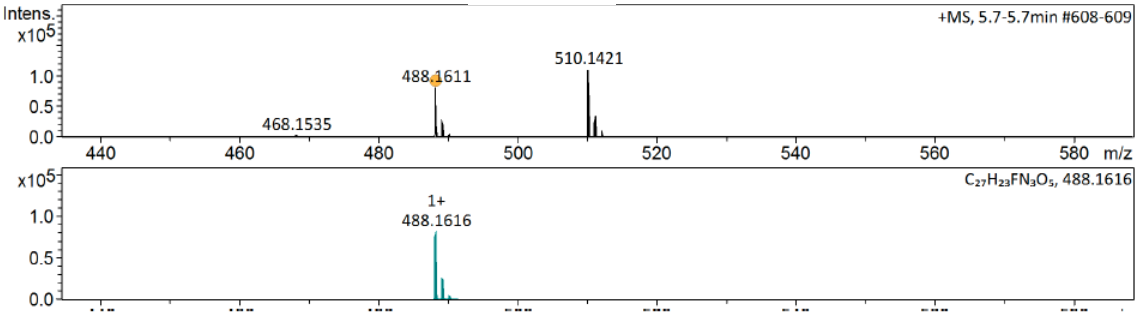
6B'-CP



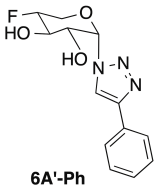
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
272.1403	1	C12H19FN3O3	272.1405	0.6	19.3	3	84.38	4.5	even	ok



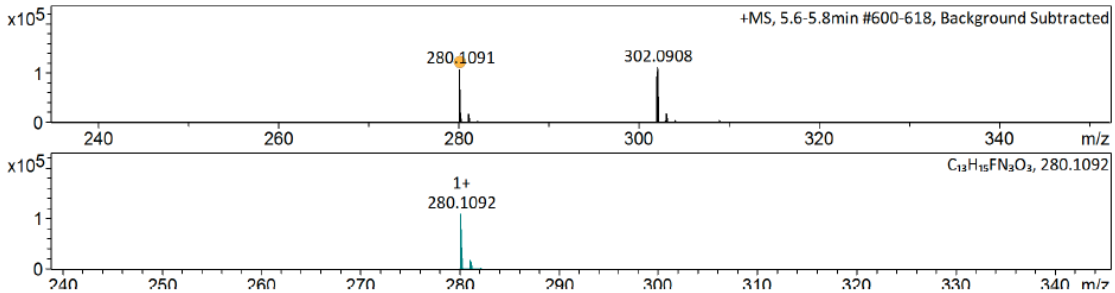
6A-Ph



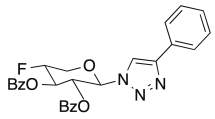
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
488.1611	1	C27H23FN3O5	488.1616	1.0	6.7	2	100.00	17.5	even	ok



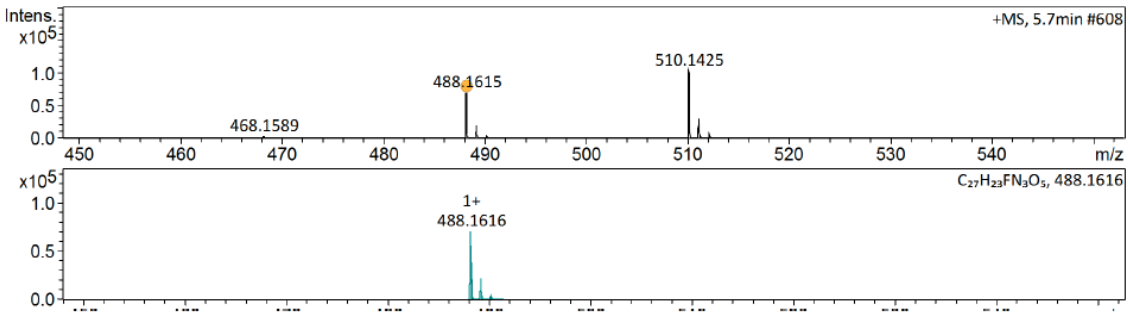
6A'-Ph



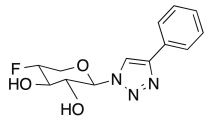
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
280.1091	1	C13H15FN3O3	280.1092	0.4	0.9	3	100.00	7.5	even	ok



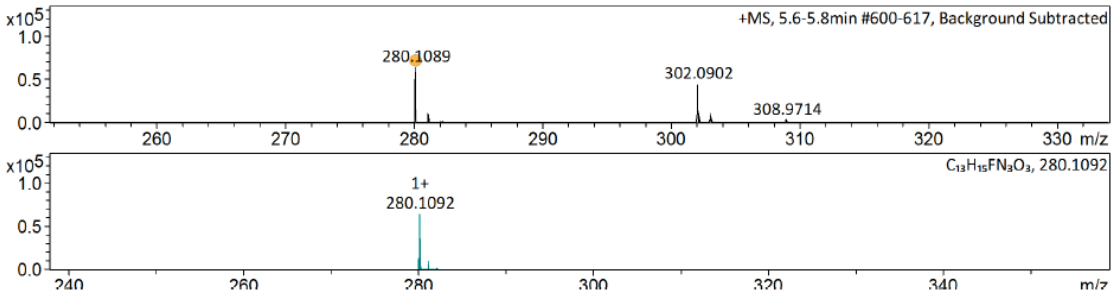
6B-Ph



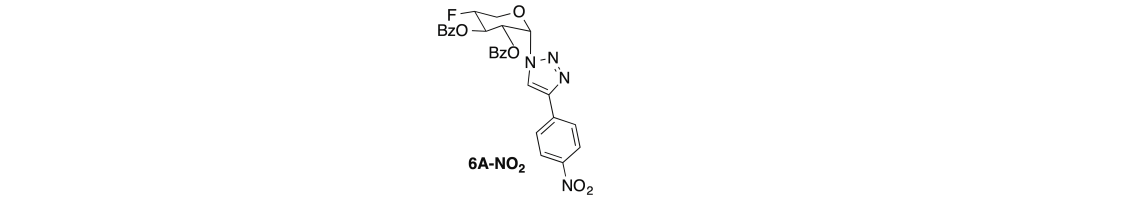
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
488.1615	1	C27H23FN3O5	488.1616	0.3	4.0	2	100.00	17.5	even	ok



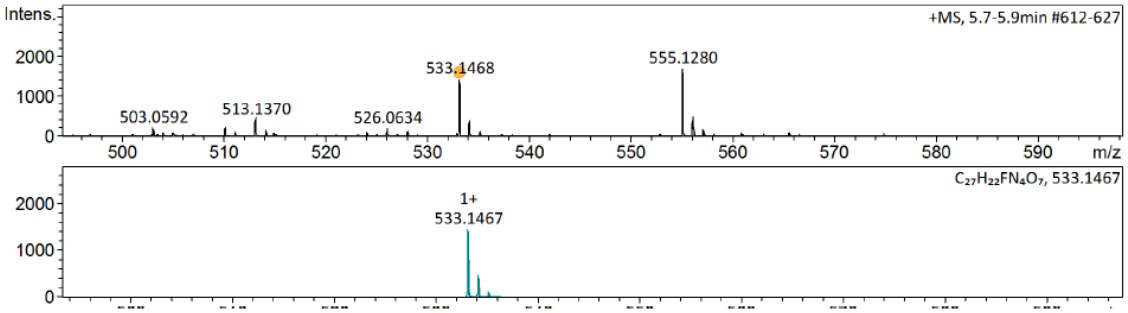
6B'-Ph



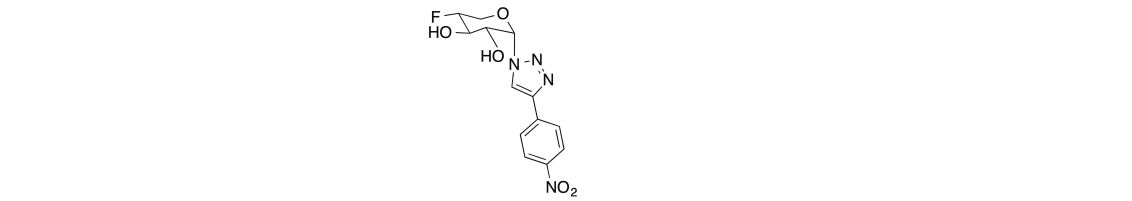
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
280.1089	1	C13H15FN3O3	280.1092	0.9	1.9	3	100.00	7.5	even	ok



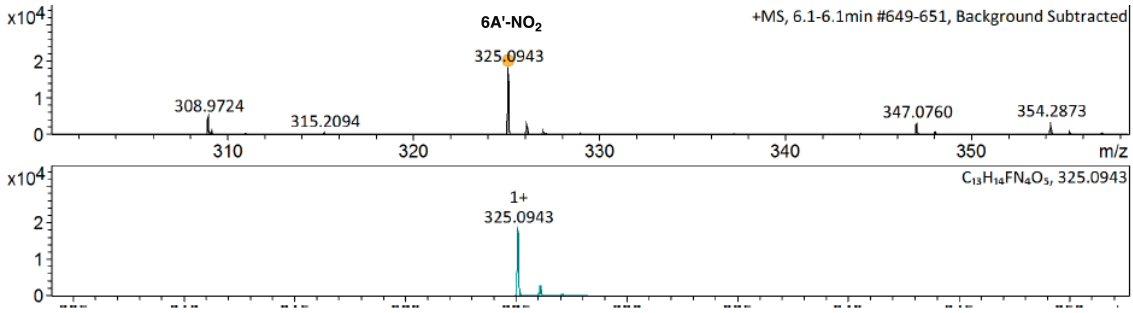
6A-NO_2



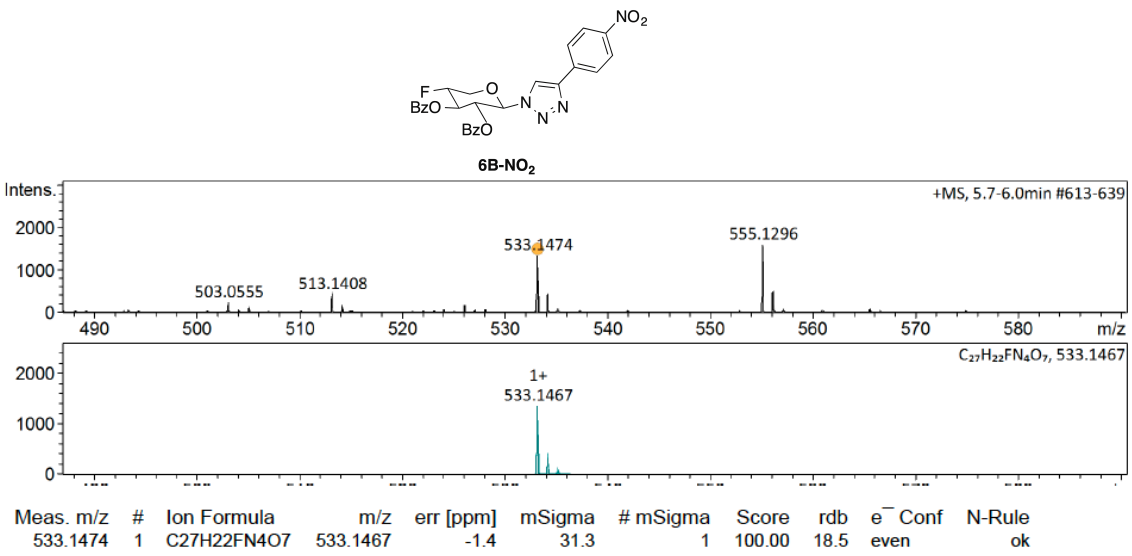
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
533.1468	1	C27H22FN4O7	533.1467	-0.1	40.9	1	100.00	18.5	even	ok



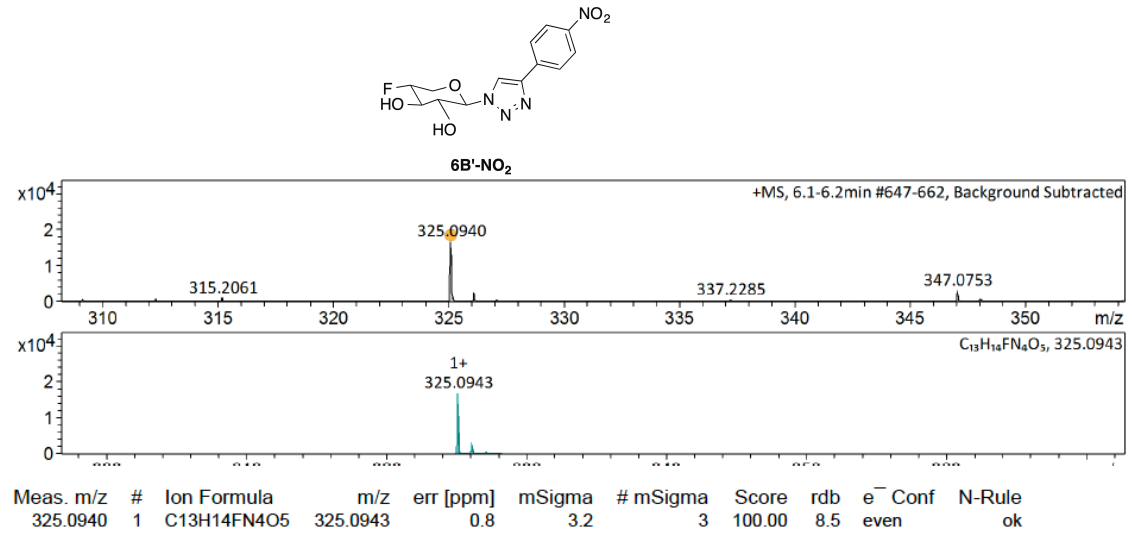
6A'-NO_2



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
325.0943	1	C13H14FN4O5	325.0943	0.1	7.0	3	97.38	8.5	even	ok



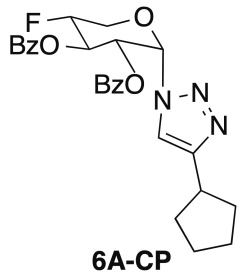
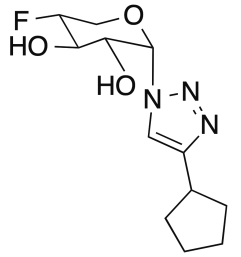
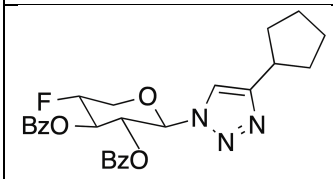
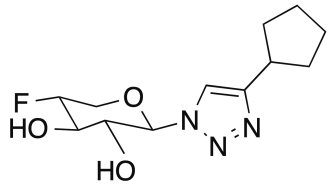
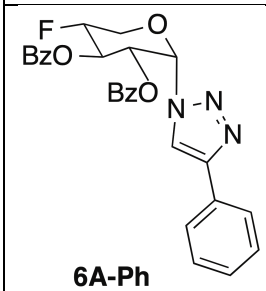
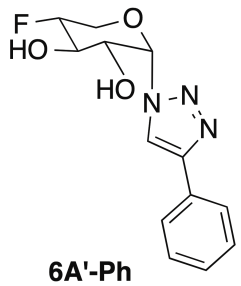
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
533.1474	1	C27H22FN4O7	533.1467	-1.4	31.3	1	100.00	18.5	even	ok

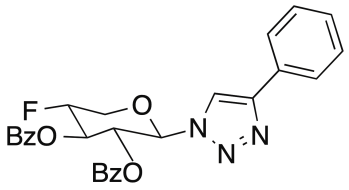
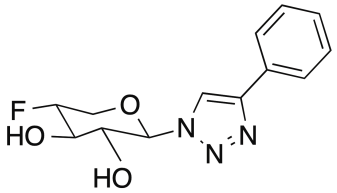
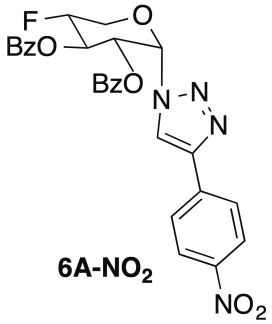
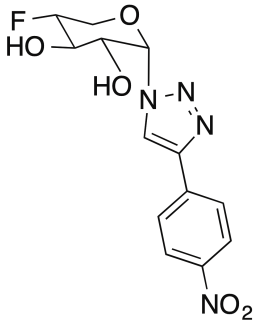
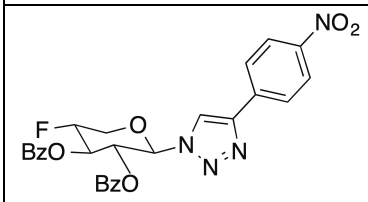


Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
325.0940	1	C13H14FN4O5	325.0943	0.8	3.2	3	100.00	8.5	even	ok

TABLE S1. Synthesis and purification conditions of prodrug and Drug

Compounds	Synthesis Condition	Purification Method	% Yield

 **6A-CP**	3 days, RT	silica gel (20-45 μm) hexane:ethyl acetate 4:1 to 7:3	34
 **6A'-CP**	4 hours	Hexane wash (7x)	99
 **6B-CP**	2 days, RT	silica gel (20-45 μm) hexane:ethyl acetate 4:1 to 7:3	59
 **6B'-CP**	4 hours	Hexane wash (7x)	99
 **6A-Ph**	3 days, RT	silica gel (20-45 μm) hexane:ethyl acetate 4:1 to 7:3	43
 **6A'-Ph**	4 hours, RT	Hexane wash (7x)	00

 **6B-Ph**	2 days, RT	silica gel (20-45 μm) hexane:ethyl acetate 4:1 to 7:3	50
 **6B'-Ph**	4 hours, RT	Hexane wash (7x)	100
 **6A-NO_2**	3-7 days, RT	silica gel (20-45 μm) hexane:ethyl acetate 4:1 to 7:3	25
 **6A'-NO_2**	4 hours, RT	silica gel (20-45 μm) hexane:ethyl acetate 1:1	95
 **6B-NO_2**	2 days, RT	silica gel (20-45 μm) hexane:ethyl acetate 4:1 to 7:3	58

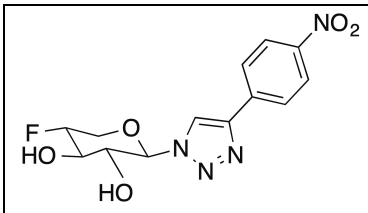
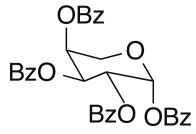
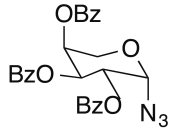
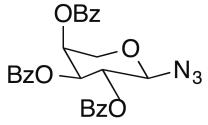
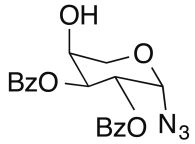
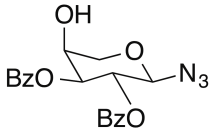
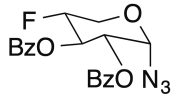
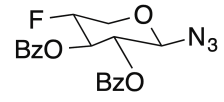
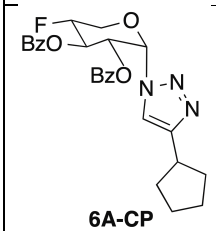
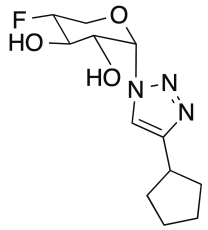
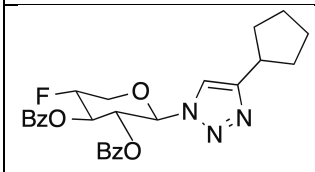
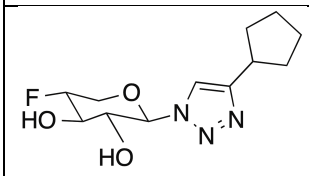
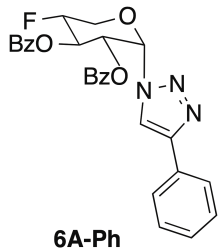
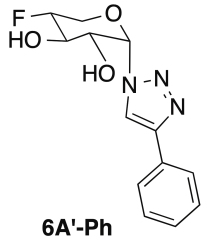
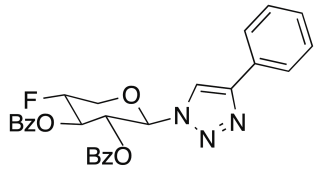
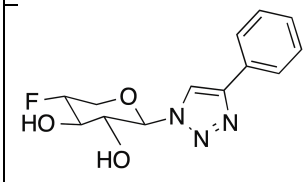
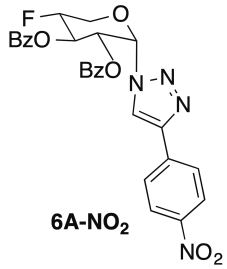
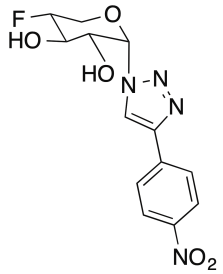
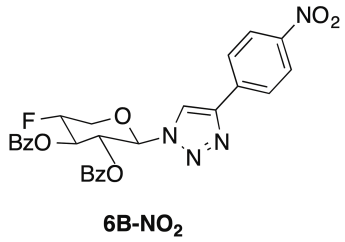
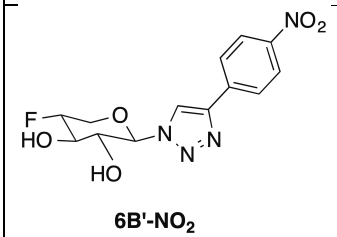
 **6B'-NO_2**	4 hours, RT	silica gel (20-45 μm) hexane:ethyl acetate 1:1	98

Table S2. TLC conditions and R_f values

Compounds	TLC Conditions Hexane : Ethyl Acetate	R_f values
 **2**	7:3	0.57
 **3A**	7:3	0.66
 **3B**	7:3	0.54[a]
 **4A**	7:3	0.51
 **4B**	7:3	0.44
 **5A**	4:1	0.72

 **5B**	4:1	0.64
 **6A-CP**	4:1 (two sequential runs)[b]	0.33
 **6A'-CP**	1:4	0.53
 **6B-CP**	4:1 (two sequential runs)[b]	0.32
 **6B'-CP**	1:4	0.53
 **6A-Ph**	4:1	0.28
 **6A'-Ph**	1:4	0.46

 **6B-Ph**	4:1	0.28
 **6B'-Ph**	1:4	0.46
 **6A-NO_2**	7:3	0.50
 **6A'-NO_2**	1:4	0.45
 **6B-NO_2**	7:3	0.50
 **6B'-NO_2**	1:4	0.45

[a] R_f value of compound **3B** overlaps with the starting material **2**. A quick LCMS showed a new compound, which after NMR studies confirmed as compound **3B**.
b TLC was run twice in hexane:ethyl acetate (4:1) to separate major product from the minor product (R_f = 0.4)