Synthesis of the tumor associative α-aminooxy disaccharide of the TF antigen and its conjugation to a polysaccharide immune stimulant

Jean Paul Bourgault, Kevin R. Trabbic, Mengchao Shi, and Peter R. Andreana*

Electronic Supplementary Information (ESI)

Table of contents

Experimental

General: All reagents and solvents were purchased from commercial sources and used without further purification. Compounds were purified using flash chromatography with Whatman Purasil 60 Å 230-400 mesh silica gel, with the exception of **2** which was purified with Biorad P2 gel. Thin layer chromatography was conducted with Dynamic absorbents inc. silica gel TLC 250 μm w/h F-254, catalog no. 84111. Proton and carbon NMR spectra were recorded using Mercury 400, Varian 400, Varian 500 and Bruker Avance III 600 spectrometers. The residual $CHCl_3$ was referenced to 7.26 and 77 ppm in proton and carbon spectra respectively, the residual HDO was referenced to 4.79 with spectra taken in D_2O. Mass spectral data were taken on a Waters ESI-MS, model LCT premier XE. PS A1 was isolated and purified as in De Silva, R. A.; Wang, Q.; Chidley, T.; Appulage, D. K.; Andreana, P. R., *J. Am. Chem. Soc.* **2009,** *131,* 9622-9623.

*Phenyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-1-thiol-D-galactopyranoside (**5**).* 3.22 g (8.62 mmol) of compound **4** was co-evaporated 3 times with toluene and dried under high vacuum for 2 hours. The dried compound was then dissolved in 48 mL of anhydrous DCM and then cooled to 0 ^oC. Next 1.32 mL (12.9 mmol) of thiophenol and 5.32 mL (43.1 mmol) of BF_3OEt_2 were added and the reaction was allowed to stir overnight while warming to room temperature. This was followed by dilution of the mixture with 50 mL DCM, and then pouring this diluted reaction mixture into 240 mL of water. The organic layer was then separated, washed with sat $NaHCO_3$, dried over $MgSO_4$, and concentrated to an oil. The crude product was then purified on silica gel with 2:7 EA/Hex to provide **5** (2.75 g, 75% yield) as a syrup. Data for **5**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (m, 1H_{Ar}), 7.49 (m, 2H_{Ar}), 7.32 (m, 5H_{Ar}), 5.68 (d, J = 5.6 Hz, 1H, $H_{1\alpha}$), 5.46 (d, J = 3.2 Hz, 1H, $H_{4\alpha}$), 5.33 (d, J = 2.4 Hz, 1H, $H_{4\beta}$), 5.16 (dd, J_1 = 3.2 Hz, J_2 = 11.2 Hz, 1H, $H_{3\alpha}$), 4.85 (dd, J_1 = 3.2 Hz, J_2 = 10.4 Hz, 1H, $H_{3\beta}$), 4.74 (t, J = 6.4 Hz, 1H, $H_{5\alpha}$), 4.51, (d, J = 10.0 Hz, 1H, $H_{1\beta}$), 4.30 (dd, J_1 = 5.6 Hz, J_2 = 11.2 Hz, 1H, $H_{2\alpha}$), 4.06 - 4.18 (m, 4H, $H_{6,6'\alpha,}$ $H_{6,6'\beta}$), 3.87 (t, J = 6.6 Hz, 1H, $H_{5\beta}$), 3.63 (t, J = 10.0 Hz, 1H, $H_{2\beta}$), 2.14 (s, 3H Ac_α), 2.07 (s, 3H Ac_β), 2.05 (s, 3H Ac_α), 2.02 (s, 3H Ac_β), 2.01 (s, 3H Ac_β), 1.96 (s, 3H Ac_α); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 169.84, 169.80, 169.6, 169.5, 133.5, 132.5, 132.4, 129.0, 128.9, 128.5, 127.9, 86.8, 86.3, 74.3, 72.9, 70.0, 67.42, 67.35, 66.4, 61.6, 61.5, 59.2, 58.0, 20.6, 20.5; ESI HRMS: $[M+Na]^+$ calcd for $C_{18}H_{21}N_3NaO_7S$ 446.0998, found 466.0995.

Phenyl 2-azido-4,6-O-benzylidene-2-deoxy-1-thio-D-galactopyranoside (**6**). Sodium metal (0.023 g0 was washed with hexanes and allowed to air dry briefly. The sodium was then dissolved in 10 mL of anhydrous MeOH to prepare a 0.1 M NaOMe solution.

Next 2.75 g (6.49 mmol) of **5** was dissolved in a separate round bottom with 8 mL of anhydrous MeOH. Then 6.5 mL of the 0.1 M NaOMe solution was added dropwise to the solution of **5**. When the reaction was judged to be completed by TLC 1:2 EA/Hex, Dowex 50W x 8-100 ion exchange resin was added portion wise until the pH reached 7. The resin was then filtered off and the solution was concentrated to a thick oil. Next the crude triol was co-evaporated 3 times with toluene and dried under high vacuum for 1 hour. The dried sugar was then dissolved in 68 mL of anhydrous acetonitrile. After the triol was completely dissolved, 0.125 g (0.649 mmol) of *p*-TSA was added. When the reaction was judged to be complete by TLC 1:1 EA/Hex, 2.1 mL of NEt_3 was added and the reaction mixture was concentrated. The crude oil was then purified on silica gel with EA/Hex 1:4 - 1:1 to provide **6α** and **6β** (2.38 g, 95%, combined yield) as syrups, α/β 1.7:1. Data for **6α** and **6β**. **6α**, 1H NMR (400 MHz, $CDCl_3$): δ 7.48 – 7.52 (m, $4H_{Ar}$) 7.39 – 7.41 (m, $3H_{Ar}$) 7.26-7.33 (m, $3H_{Ar}$), 5.76 (d, J = 5.6 Hz, 1H, H_1), 5.61 (s, 1H, $H_{C\underline{H}Ph}$), 4.33 (d, J = 2.4 Hz, 1H, H_4), 4.24 (m, 2H, H_6, H_5), 4.20 (dd, J_1 = 5.6 Hz, J_2 = 10.4 Hz 1H, H_2), 4.12 (m, 1H, $H_{6'}$), 4.02 (dt, J = 3.6 Hz, J2 = 10.4 Hz, 1H, H_3), 2.58 (m, 1H, H_{OH}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.1, 133.4, 130.8, 129.1, 128.9, 128.1, 127.1, 126.0, 100.9, 87.0, 74.8, 69.1, 68.8, 63.4, 60.8; ^1H NMR (400 MHz, $CDCl_3$): **6β**, δ 7.45 (d, *J* = 7.2 Hz, 1H, H_{Ar}), 7.29-7.43 (m, $8H_{Ar}$), 5.52 (s, 1H, $H_{C\underline{H}Ph}$), 4.40 (m, 2H, H_1, H_6), 4.15 (d, *J* = 3.2 Hz, 1H, H_4), 4.01 (d, *J* = 12.0 Hz, 1H, $H_{6'}$), 3.62 (dt, J_1 = 3.6 Hz, J_2 = 9.3 Hz, 1H, H_3), 3.54 (t, *J* = 9.8 Hz, 1H, H_2), 3.48 (s, 1H, H_5), 2.62 (d, *J* = 9.6 Hz, 1H, $H_{O\underline{H}}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.3, 134.1, 130.3, 129.4, 128.9, 128.4, 128.2, 126.4, 101.3, 85.0, 74.3, 73.0, 69.8, 69.1, 62.0; ESI HRMS: $[M + H]^+$ calcd for $C_{19}H_{20}N_3O_4S$ 386.1175, found 386.1174.

Phenyl 2-azido-4,6-O-benzylidene-3-O-t-butyldiphenyl silyl-2-deoxy-1-thio-α-D-galactopyranoside (**7**). 1.01 g (2.62 mmol) **6α** and 0.392 g (5.76 mmol) imidazole were dissolved in a minimal amount of DMF at room temperature then 0.74 mL (2.88 mmol) TBDPSCl was added slowly. After 4 hours an additional 0.27 mL (1.05 mmol) of TBDPSCl was added to the reaction mixture. At the 5 hour point an additional 0.34 mL (1.32 mmol) of the silyl chloride was added. The reaction was allowed to stir 3 more hours, then was quenched with 50 mL of water. The reaction mixture was extracted 3 times with 50 mL ethyl acetate, the combined organic layers were washed with sat. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated. The resulting residue was then purified on silica gel utilizing pure toluene as the eluent, to provide **7** (1.29 g, 79%) as a colorless oil. Data for **7**. ^1H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, *J* = 5.6 Hz, $4H_{Ar}$), 7.40 – 7.56 (m, $11H_{Ar}$), 7.20 – 7.35 (m, $5H_{Ar}$), 5.80 (d, *J* = 4.8 Hz, 1H, H_1), 5.06 (s, 1H, $H_{C\underline{H}Ph}$), 4.52 (dd, J_1 = 4.8 Hz, J_2 = 10.8 Hz, 1H, H_2), 4.13 (dd, J_1 = 3.6 Hz, J_2 = 10.4 Hz, 1H, H_3), 4.05 (d, *J* = 12.0 Hz, 1H, H_6), 3.83 (s, 1H, H_5), 3.72 (d, *J* = 12.8 Hz, 1H, $H_{6'}$), 3.43 (d, *J* = 3.2 Hz, 1H, H_4), 1.13 (s, $9H_{t\text{-butyl}}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.6, 135.8, 134.2, 133.9, 132.5, 130.9, 130.1, 130.0, 129.0, 128.9, 128.1, 128.0, 127.6, 127.1, 126.0, 100.4, 87.6, 74.9, 71.4, 69.1, 63.6, 61.7, 26.8, 19.3; ESI HRMS: [M + Na]$^+$ calcd for $C_{35}H_{37}N_3NaO_4SSi$ 646.2172, found 646.2173.

Succinimidyl 1-amino-2-azido-4,6-O-benzylidene-3-O-t-butyldiphenylsilyl-2-deoxy-α-D-galactopyranoside (**8**). 0.100 g (0.160 mmol) **7**, 0.020 g (0.176 mmol) of NHS, 2.91 mL DCM were stirred with 300 mg of flame dried 4 Å molecular sieves for 1 hour. Then 0.072 g (0.320 mmol) NIS was added and the reaction mixture was cooled to -40 ^{o}C. Next 0.006 mL (0.0642 mmol) of TfOH was added and the mixture was allowed to stir for 30 minutes. The reaction was stopped by the addition of 3 mL of sat $NaHCO_3$ and then diluted with 3 mL of DCM. The mixture was then filtered through a pad of celite and washed with 50 mL of 10% $Na_2S_2O_3$, water, and brine. The resulting organic layer was dried over $MgSO_4$, filtered and concentrated to provide an oil which was purified on silica gel using EA/Hex 1:3 to provide **8** (0.072 g, 73% yield, α/β 2:1) as a syrup. Data for **8α**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 – 7.78 (m, $4H_{Ar}$), 7.30 – 7.53 (m, $9H_{Ar}$), 7.30 (t, *J* = 7.2 Hz, $2H_{Ar}$), 6.18 (d, *J* = 7.2 Hz, 1H, H_1), 5.02 (s, 1H, $H_{C\underline{H}Ph}$), 4.88 (dd, J_1 = 3.6 Hz, J_2 = 10.0 Hz, 1H, H_3), 4.47 (dd, J_1 = 7.2 Hz, J_2 = 10.4 Hz, 1H, H_2), 4.00 (dd, = 1.6 Hz, J_2 = 12.4 Hz, 1H, H_6), 3.81 (s, 1H, H_5) 3.59 (m, 1H, $H_{6'}$), 3.51 (d, *J* = 3.2 Hz, 1H, H_4), 2.72 (s, $4H_{suc}$), 1.11 (s, $9H_{t\text{-butyl}}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 137.7, 135.8, 134.3, 133.1, 129.9, 129.8, 128.8, 128.1, 127.8, 127.6, 126.0, 118.9, 100.3, 76.6, 74.7, 71.2, 69.3, 68.6, 60.5, 28.1, 26.9, 19.3; ESI HRMS: [M + Na]$^+$ calcd for $C_{33}H_{36}N_4NaO_6Si$ 635.2302, found 635.2314.

Succinimidyl 2-azido-4,6-O-benzylidene-3-O-t-butyldiphenylsilyl-2-deoxy-α-D-galactopyranoside (**9**). 0.100 g (0.160 mmol) of compound **7** and 0.074 g (0.641 mmol) of *N*-hydroxy succinimide (NHS) were dissolved in a minimal amount of anhydrous acetonitrile then 5 mL of anhydrous toluene was added and the solution was evaporated to dryness. This was repeated 3 times and the mixture was left under high vacuum overnight. To the dried reagents were added 300 mg of flame dried 4 Å molecular sieves and 2.9 mL of anhydrous DCM. This mixture was then allowed to stir at room temperature for 1 hr. Next 0.040 g (0.176 mmol) of NIS was added and the reaction mixture was cooled to -40 ^{o}C. Subsequently 0.003 mL (0.0321 mmol) of TfOH was added. After 3 hrs thioglycoside still remained in the reaction mixture, therefore an additional 0.040 g (0.176 mmol) NIS was added followed by 0.003 mL (0.0321 mmol) TfOH. The reaction was stopped after 4.5 hrs by the addition of 3 mL of sat $NaHCO_3$ and then the reaction was diluted with 3 mL of DCM. The biphasic mixture was then filtered through a pad of celite and washed with 50 mL of 10% $Na_2S_2O_3$, water, and brine. The resulting organic layer was dried over $MgSO_4$, filtered, and concentrated to an oil which was purified on silica gel using EA/Hex 1:3 to provide **9** (0.075 g, 75%) as a syrup. Data for **9**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 7.6 Hz, 4H_{Ar}), 7.34 – 7.50 (m, 9H_{Ar}), 7.30 (t, *J* = 8.4 Hz, 2H_{Ar}), 5.54 (d, *J* = 3.6 Hz, 1H, H_1), 5.00 (s, 1H, $H_{C\underline{H}Ph}$), 4.37 (dd, J_1 = 3.6 Hz, J_2 = 11.2 Hz, 1H, H_3), 4.23 (s, 1H, H_5), 4.18 (dd, J_1 = 4.0 Hz, J_2 = 10.4 Hz, 1H, H_2), 3.98 (d, *J* = 12.4 Hz, 1H, H_6), 3.65 (d, *J* = 12.8 Hz, 1H, $H_{6'}$), 3.47 (d, *J* = 3.2 Hz, 1H, H_4), 2.67 (s, 4H, H_{NHS}), 1.10 (s, 9H, $H_{t\text{-butyl}}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 137.6, 135.8, 135.7, 134.1, 132.4, 130.0, 129.9, 128.8, 128.1, 127.9, 127.6, 125.9, 103.1, 100.2, 74.6, 69.4, 68.7, 64.7, 60.0, 26.8, 25.3, 19.2; ESI HRMS: [M + Na]$^+$ calcd for $C_{33}H_{36}N_4NaO_7Si$ 651.2251, found 651.2247.

Phenyl 2-azido-4,6-O-benzylidene-3-O-chloroacetyl-2-deoxy-1-thiol-α-D-galactopyranoside (**11α**). 2.23 g (5.79 mmol) of **6α** was dissolved in 30 mL of anhydrous DCM and 1 mL of pyridine this mixture was then cooled to 0 °C. Next 0.553 mL (0.695 mmol) of chloroacetyl chloride was added dropwise and the reaction was allowed to warm to r.t. over 2 hours. Subsequently 30 mL of water was added to the reaction mixture and the organic layer was then separated and washed with 3M HCl, sat $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated. This residue was purified with EA/Hex 1:5 to provide **11α** (2.25 g, 84% yield) as a white solid. Data for **11α**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.51 (m, 4HAr), 7.37-7.41 (m, 3HAr), 7.28-7.34 (m, 3HAr), 5.82 (d, J = 5.2 Hz, 1H, H_1), 5.56 (s, 1H, $_{C\underline{H}Ph}$), 5.18 (dd, J_1 = 3.6 Hz, J_2 = 11.2 Hz, 1H, H_3), 4.60 (dd, J_1 = 5.2 Hz, J_2 = 10.8 Hz, 1H, H_2), 4.57 (m, 1H, H_4), 4.27 (s, 1H, H_5), 4.23 (dd, J_1 = 1.2 Hz, J_2 = 12.8 Hz, 1H, H_6), 4.17 (s, 2H_{CH_2Cl}), 4.10 (dd, J_1 = 1.6 Hz, J_2 = 12.8 Hz, 1H, $H_{6'}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 137.2, 133.0, 131.3, 129.1, 129.2, 128.2, 127.7, 126.1, 100.8, 87.1, 73.2, 72.7, 69.0, 63.3, 57.8, 40.6; ESI HRMS: [M + Na]$^+$ calcd for $C_{21}H_{20}ClN_3NaO_5S$ 484.0710, found 484.0710.

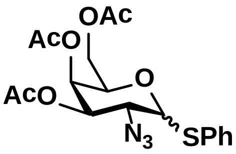
Succinimidyl 2-azido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (**10**). 1.06 g (2.30 mmol) of compound **11α** or **11β** 1.06 g (9.20 mmol) of *N*-hydroxy succinimide (NHS) were dissolved in a minimal amount of anhydrous acetonitrile then 10 mL of anhydrous toluene was added and the solution was evaporated to dryness. This was repeated 3 times and the mixture was left under high vacuum overnight. To the dried reagents were added 3 g of flame dried 4 Å molecular sieves and 28 mL of anhydrous DCM. This mixture was then allowed to stir at room temperature for 1 hr. Next 1.04 g (4.60 mmol) of NIS was added and the reaction mixture was cooled to -40 °C. Subsequently 19 drops of TMSOTf was added. After 3 hours the reaction was stopped by the addition of 30 mL of sat $NaHCO_3$. The biphasic mixture was then filtered through a pad of celite and washed with 30 mL of 10% $Na_2S_2O_3$, water, and brine. The resulting organic layer was dried over $MgSO_4$, filtered, and concentrated to an oil. Next the crude oil was dissolved in 90 mL of 5:1 EtOH:py then 0.574 g (7.55 mmol) thiourea was added and this mixture was allowed to stir for 1 hr at r.t. The reaction mixture was concentrated and co-evaporated with toluene 2 times. The residue was extracted 3 times with DCM. The combined organic layers were washed with sat. $NaHCO_3$, water, dried over $MgSO_4$, filtered and concentrated. The crude product was purified on silica gel with EA:Hex 3:2 to provide **10** (0.642 g, 65% over 2 steps) as a white solid. Data for **10**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 – 7. 50 (m, $2H_{Ar}$), 7.37 – 7.40 (m, $3H_{Ar}$), 5.58 (s, $1H_{C\underline{H}Ph}$), 5.54 (d, J = 3.5 Hz, 1H, H_1), 4.66 (s, 1H, H_5), 4.37 (d, J = 3.5 Hz, 1H, H_4), 4.26 (dt, J_1 = 3.5 Hz, J_2 = 10.5 Hz 1H, H_3), 4.20 (dd, J_1 = 1.5 Hz, J_2 = 13.0 Hz 1H, H_6), 4.08 (dd, J_1 = 1.5 Hz, J_2 = 13.0 Hz 1H, $H_{6'}$), 3.85 (dd, J_1 = 3.5 Hz, J_2 = 11.0 Hz 1H, H_2) 2.73 (s, $4H_{NHS}$), 2.67 (d, J = 10.0 Hz, 1H $H_{O\underline{H}}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 137.2, 129.4, 128.3, 126.1, 103.0, 101.2, 75.0, 68.9, 67.2, 64.8, 59.7, 25.4; ESI HRMS: $[M + Na]^+$ calcd for $C_{17}H_{18}N_4NaO_7$ 413.1073, found 413.1073.

Succinimidyl [2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]-(1,3)-2-azido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (**13**). 0.109 g (0.279 mmol) of acceptor **10** and 0.391 g (0.794 mmol) of trichloroacetimidate donor **12** were co-evaporated with anhydrous toluene 3 times and then left overnight under high vacuum. These dried starting materials were then transferred by canulla with 2.25 mL of anhydrous DCM onto 220 mg of flame dried AW-300 molecular sieves and stirred at r.t. for 30 minutes. Next the reaction mixture was cooled to 0 ^{o}C then 1 drop of TMSOTf was added and the reaction was monitored by TLC (2:1 EA/Hex). The reaction was stopped after 1 hour by the addition of 3 mL of sat $NaHCO_3$. This mixture was filtered through a pad of celite, the organic layer was separated and washed with water, brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified with silica gel chromatography using 2:1 EA:Hex to provide **13** (0.113 g, 56%) as a white solid. Data for **13**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 - 7.53 (m, $2H_{Ar}$), 7.34 – 7.40 (m, $3H_{Ar}$), 5.57 (m, 2H, $H_{C\underline{H}Ph}$, H_{1GalN_3}), 5.41 (d, J = 3.2 Hz, 1H, H_{4Gal}), 5.30 (dd, J_1 = 8.0 Hz, J_2 = 10.4 Hz, 1H, H_{2Gal}), 5.03 (dd, J_1 = 3.2 Hz, J_2 = 10.4 Hz, 1H, H_{3Gal}), 4.79 (d, J = 8.0 Hz, 1H, H_{1Gal}), 4.58 (s, 1H, H_{5GalN_3}), 4.47 (d, J = 2.4 Hz, 1H, H_{4GalN_3}), 4.10 – 4.22 (m, 5H, H_{3GalN_3}, $H_{6/6'Gal}$, H_{6GalN_3}, H_{2GalN_3}), 4.07 (d, J = 12.8 Hz, 1H, $H_{6'GalN_3}$), 3.94 (t, J = 6.4 Hz, 1H, H_{5Gal}), 2.76 (s, $4H_{NHS}$), 2.17 (s, $3H_{Ac}$), 2.06 (s, $6H_{Ac}$), 1.98 (s, $3H_{Ac}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.2, 170.0, 169.3, 137.4, 128.9, 128.1, 126.0, 102.9, 102.3, 100.5, 75.3, 75.1, 71.0, 68.7, 68.5, 67.0, 65.2, 61.5, 57.9, 25.4, 20.7, 20.5; ESI HRMS: $[M + Na]^+$ calcd for $C_{31}H_{36}N_4NaO_{16}$ found 743.2024, 743.2018.

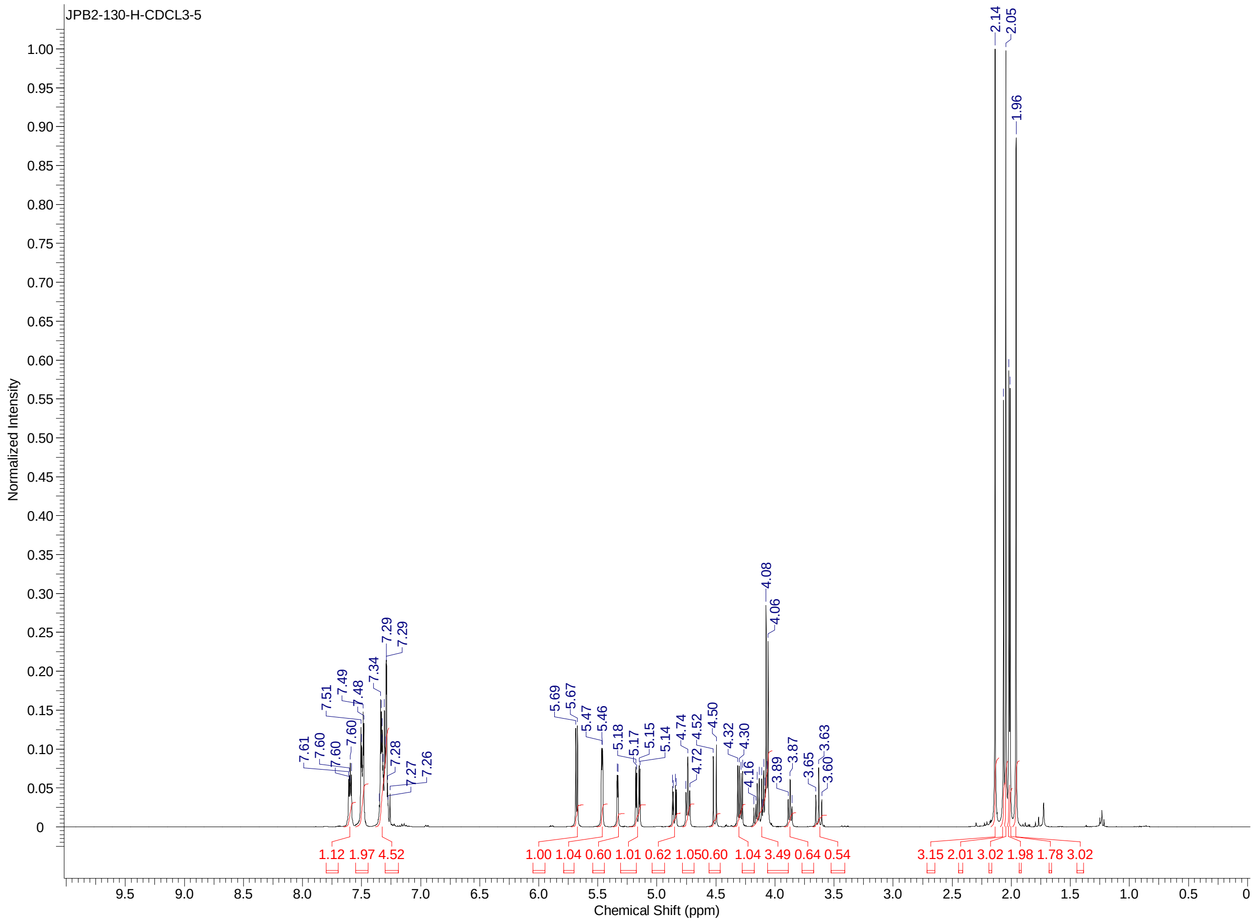
Succinimidyl [2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]-(1,3)-2-N-acetyl-2-deoxy-α-D-galactopyranoside (**14**). 0.110 g of **13** (0.153 mmol) was dissolved in 2.54 mL of 3:2:1 THF/AcOH/Ac_2O. This mixture then was cooled to 0 ^oC and 0.211 g of activated zinc dust was added. The reaction was then allowed to stir for 3.5 hours. Then an additional 0.050 g of zinc was added and the reaction was allowed to reach r.t. At 4.5 hours of reaction time, the reaction was diluted with DCM and this mixture was filtered through a pad of celite. The organic solution was then separated and washed with water, sat $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The crude residue obtained was then dissolved in 10 mL of 80% acetic acid and heated at 80 ^oC for 1.5 hours. Next the reaction mixture was concentrated and co-evaporated with toluene 3 times. The crude product was purified on silica gel using 15:1 DCM/MeOH to provide diol **14** (0.060 g, 61% over 2 steps) as a white solid. Data for **14**. MP 150-152 ^oC; $[\alpha]_D$ +94 (c 1.91, MeOH); ^1H NMR (400 MHz, $CDCl_3$): δ 6.39 (d, J = 8.8 Hz, $1H_{N\underline{H}Ac}$), 5.36 (d, J = 3.2 Hz, 1H, H_{4Gal}), 5.21 (d, J = 4.0 Hz, 1H, $H_{1GalNAc}$), 5.18 (m, 1H, H_{2Gal}, overlapping with $H_{1GalNAc}$), 5.02 (dd, J_1 = 3.6 Hz, J_2 = 10.4 Hz 1H, H_{3Gal}), 4.71 (m, 1H, $H_{2GalNAc}$, overlapping with H_{1Gal}), 4.70 (d, J = 7.6 Hz, 1H, H_{1Gal}), 4.56 (t, J = 4.4 Hz, 1H, $H_{5GalNAc}$), 4.24 (s, 1H, $H_{4GalNAc}$), 4.17 (dd, J_1 = 7.2 Hz, J_2 = 11.2 Hz, 1H, $H_{6'Gal}$), 4.09 (dd, J_1 = 6.4 Hz, J_2 = 13.6 Hz, 1H, $H_{6'Gal}$), 3.91 (m, 3H, H_{5Gal}, $H_{3GalNAc}$, $H_{6GalNAc}$), 3.81 (dd, = 4.0 Hz, J_2 = 11.2 Hz, 1H, $H_{6'Gal}$), 2.74 (s, $4H_{NHS}$), 2.15 (s, $3H_{Ac}$), 2.05 – 2.06 (m, $9H_{Ac}$), 1.97 (s, $3H_{Ac}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 170.6, 170.5, 170.2, 170.15, 169.5, 105.2, 101.6, 76.9, 71.7, 70.9, 70.5, 69.6, 68.5, 66.9, 62.9, 61.4, 47.1, 25.5, 23.2, 20.6, 20.5; ESI HRMS: $[M+H]^+$ calcd for $C_{26}H_{37}N_2O_{17}$ 649.2092 found 649.2103.

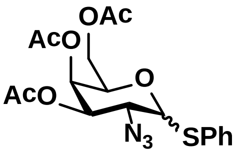
Aminooxy *[β-D-galactopyranosyl]-(1,3)-2-N-acetyl-2-deoxy-α-D-galactopyranoside* (**2**). Dissolved 0.060 g (0.0925 mmol) of **14** in 3 mL of 95% ethanol, then 0.090 mL (1.85 mmol) of hydrazine hydrate was added and the reaction was allowed to stir for 8 hrs. The reaction mixture was then concentrated to dryness. This residue was dissolved in a minimal amount of water and purified using P-2 biogel with water as the eluent (column 1.5 cm x 22.5 cm, collecting ~0.5 mL fractions), to provide **2** (0.024 g, 64%) as a white solid. Data for **2**. MP 186-187 ^oC; $[\alpha]_D$ +100 (c 0.67, H_2O); ^1H NMR (600 MHz, D_2O): δ 5.00 (d, J = 4.1 Hz, 1H, $H_{1GalNAc}$), 4.50 (d, J = 7.8 Hz, 1H, H_{1Gal}), 4.44 (dd, J_1 = 4.1 Hz, J_2 = 11.3 Hz, 1H, $H_{2GalNAc}$), 4.29 (d, J = 2.7 Hz, 1H, $H_{4GalNAc}$), 4.07 (dd, J_1 = 4.7 Hz, J_2 = 7.6 Hz, 1H, $H_{5GalNAc}$), 4.02 (dd, J_1 = 3.1 Hz, J_2 = 11.3 Hz, 1H, $H_{3GalNAc}$), 3.95 (d, J = 3.4 Hz, 1H, H_{4Gal}), 3.78 – 3.87 (m, 4H, $H_{6,6'GalNAc}$, $H_{6,6'Gal}$), 3.70 (dd, J_1 = 4.5 Hz, J_2 = 7.8 Hz, 1H, H_{5Gal}), 3.65 (dd, J_1 = 3.4 Hz, J_2 = 10.0 Hz, 1H, H_{3Gal}), 3.56 (dd, J_1 = 7.8 Hz, J_2 = 10.0 Hz, 1H, H_{2Gal}), 2.07 (s, $3H_{Ac}$); ^{13}C NMR (150 MHz, D_2O): δ 174.7, 104.7, 100.8, 77.0, 75.0, 72.6, 70.8, 70.6, 68.7, 68.6, 61.1, 61.0, 47.9, 22.0; ESI HRMS: $[M+Na]^+$ calcd for $C_{14}H_{26}N_2NaO_{11}$ 421.1434 found 421.1423.

TF-PS A1 (**16**). 0.001g (9.1 x 10^{-9} mol) of PS A1(**15**) was dissolved in 0.5 mL of 0.1 M NaOAc pH 5 buffer in a 1 dram amber vial. To this solution 55 μL of 0.01 M $NaIO_4$ (5.5 x 10^{-7} mol, 0.5 equiv. assuming PS A1 has an average MW of 110,000 g/mol and 120 repeating units/mol) was added and the reaction was allowed to stir for 90 min in the dark at r.t. Subsequently 2 mg of KCl was added and the mixture was allowed to stir for an additional 30 min. Finally 0.0007 g (1.8 x 10^{-6} mol, 3.2 equiv.) of α-TF was added and the reaction was left 18 hrs in the dark. Next the reaction mixture was dialyzed against distilled water for 48 hrs and lyophilized. This material was then further purified with DEAE sepharose (GE lifesciences), using 50 mM tris·HCl (pH 7.3) as the eluent. Fractions were then collected in 1 mL intervals analyzed by ^1H NMR, pooled, dialyzed, and lyophilized to provide the TF-PS A1 construct.

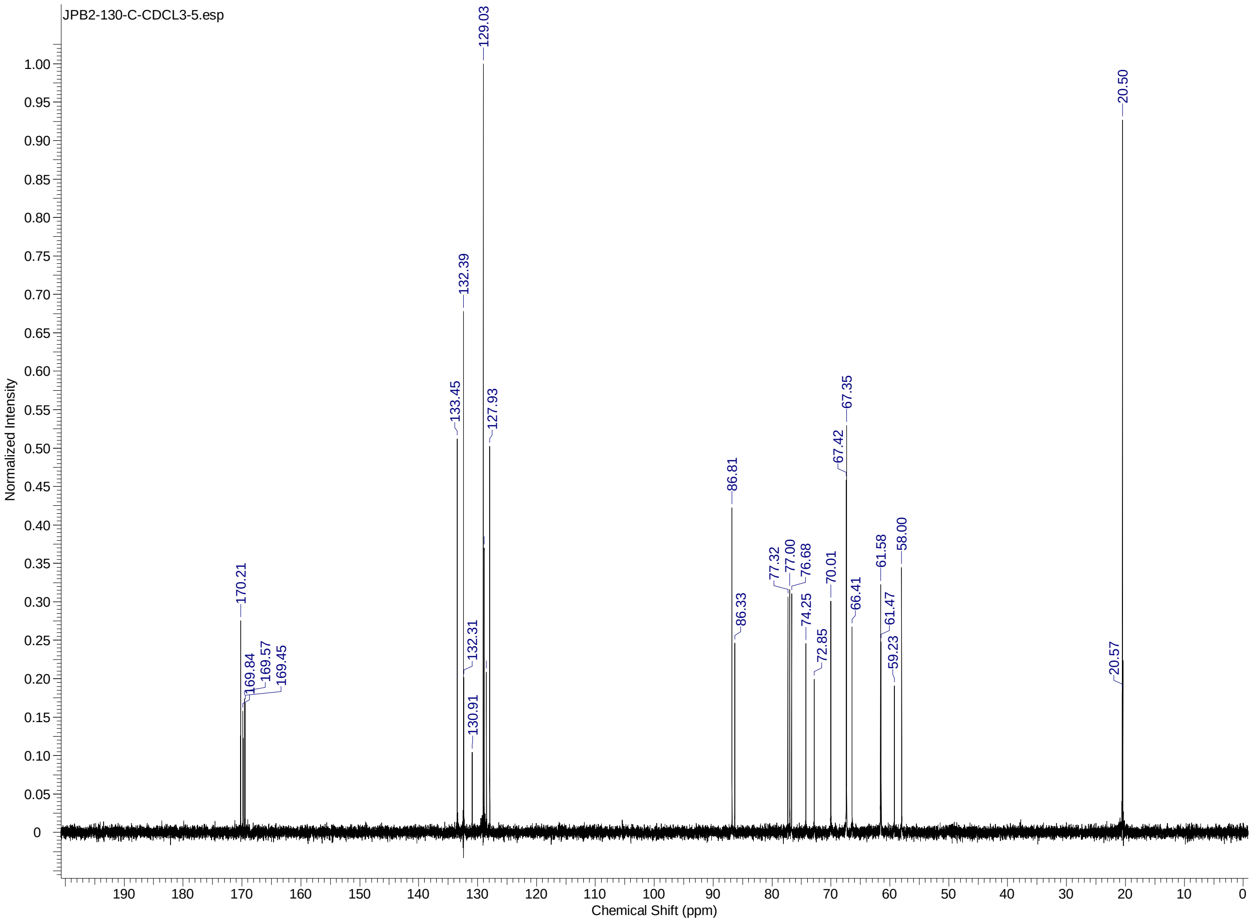

OAc
AcO
O
AcO
N3
SPh

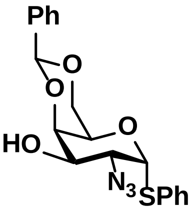
5


JPB2-130-H-CDCL3-5
Normalized Intensity
Chemical Shift (ppm)
7.61
7.60
7.60
7.60
7.51
7.49
7.48
7.34
7.29
7.29
7.28
7.27
7.26
5.69
5.67
5.47
5.46
5.18
5.17
5.15
5.14
4.74
4.72
4.52
4.50
4.32
4.30
4.16
4.08
4.06
3.89
3.87
3.65
3.63
3.60
2.14
2.05
1.96
1.12
1.97
4.52
1.00
1.04
0.60
1.01
0.62
1.05
0.60
1.04
3.49
0.64
0.54
3.15
2.01
3.02
1.98
1.78
3.02

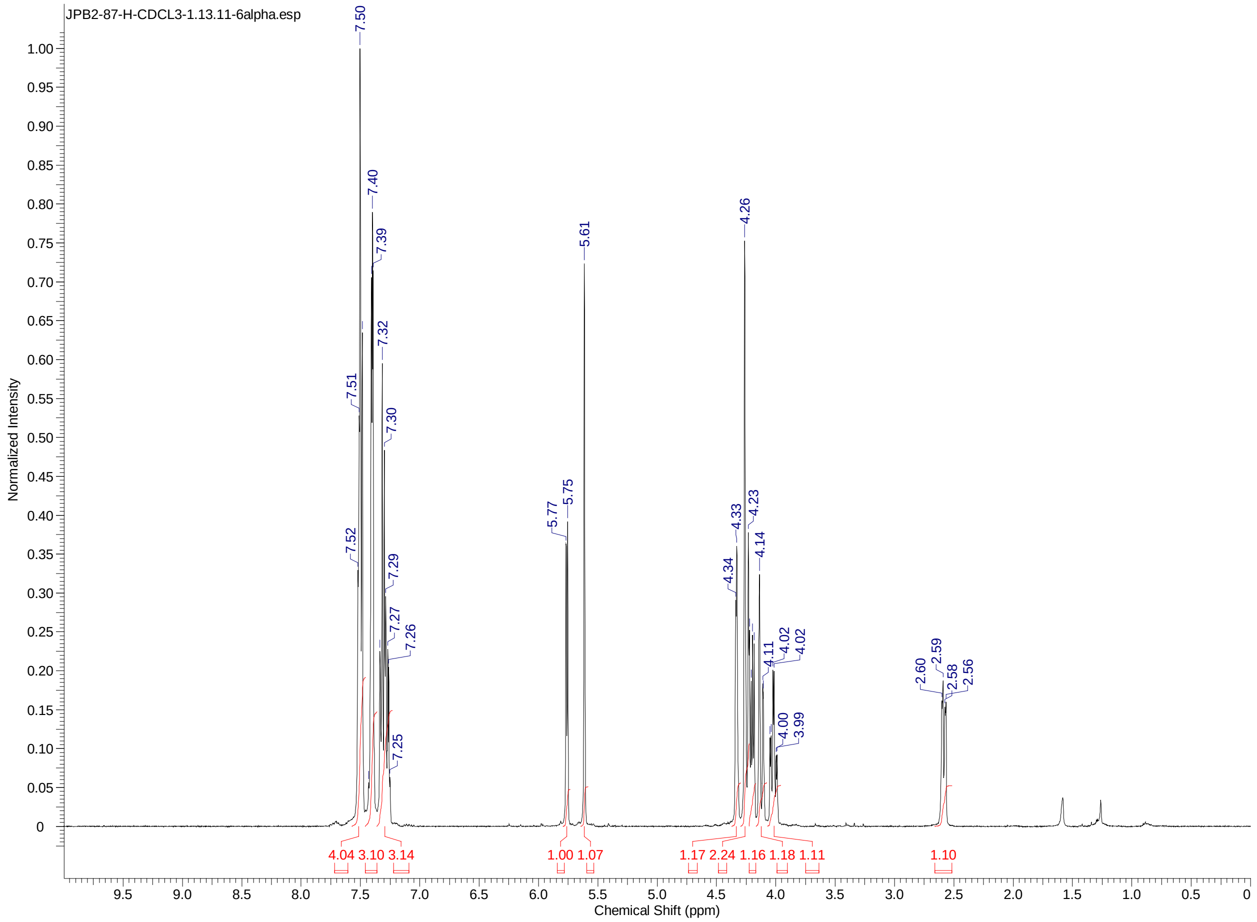

OAc
AcO
O
AcO
N3
SPh

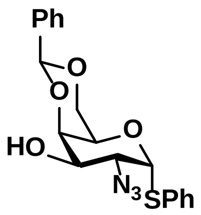
5


JPB2-130-C-CDCL3-5.esp
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
170.21
169.84
169.57
169.45
133.45
132.39
132.31
130.91
129.03
127.93
86.81
86.33
77.32
77.00
76.68
74.25
72.85
70.01
67.42
67.35
66.41
61.58
61.47
59.23
58.00
20.50
20.57
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

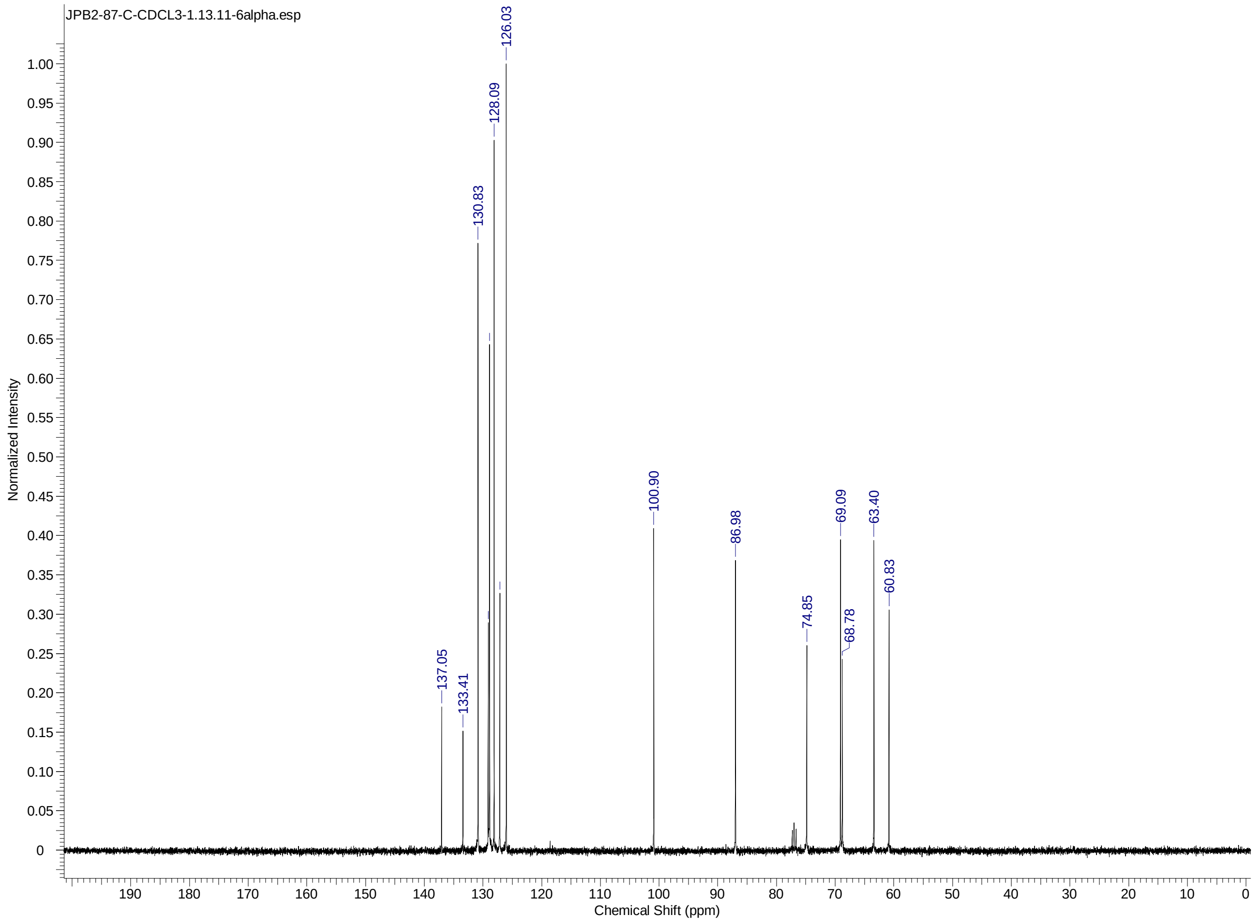

Ph
O
O
O
HO
N3
SPh

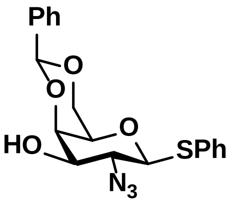
6α


JPB2-87-H-CDCL3-1.13.11-6alpha.esp
Normalized Intensity
Chemical Shift (ppm)
7.52
7.51
7.50
7.40
7.39
7.32
7.30
7.29
7.27
7.26
7.25
5.77
5.75
5.61
4.34
4.33
4.26
4.23
4.14
4.11
4.02
4.02
4.00
3.99
2.60
2.59
2.58
2.56
4.04
3.10
3.14
1.00
1.07
1.17
2.24
1.16
1.18
1.11
1.10

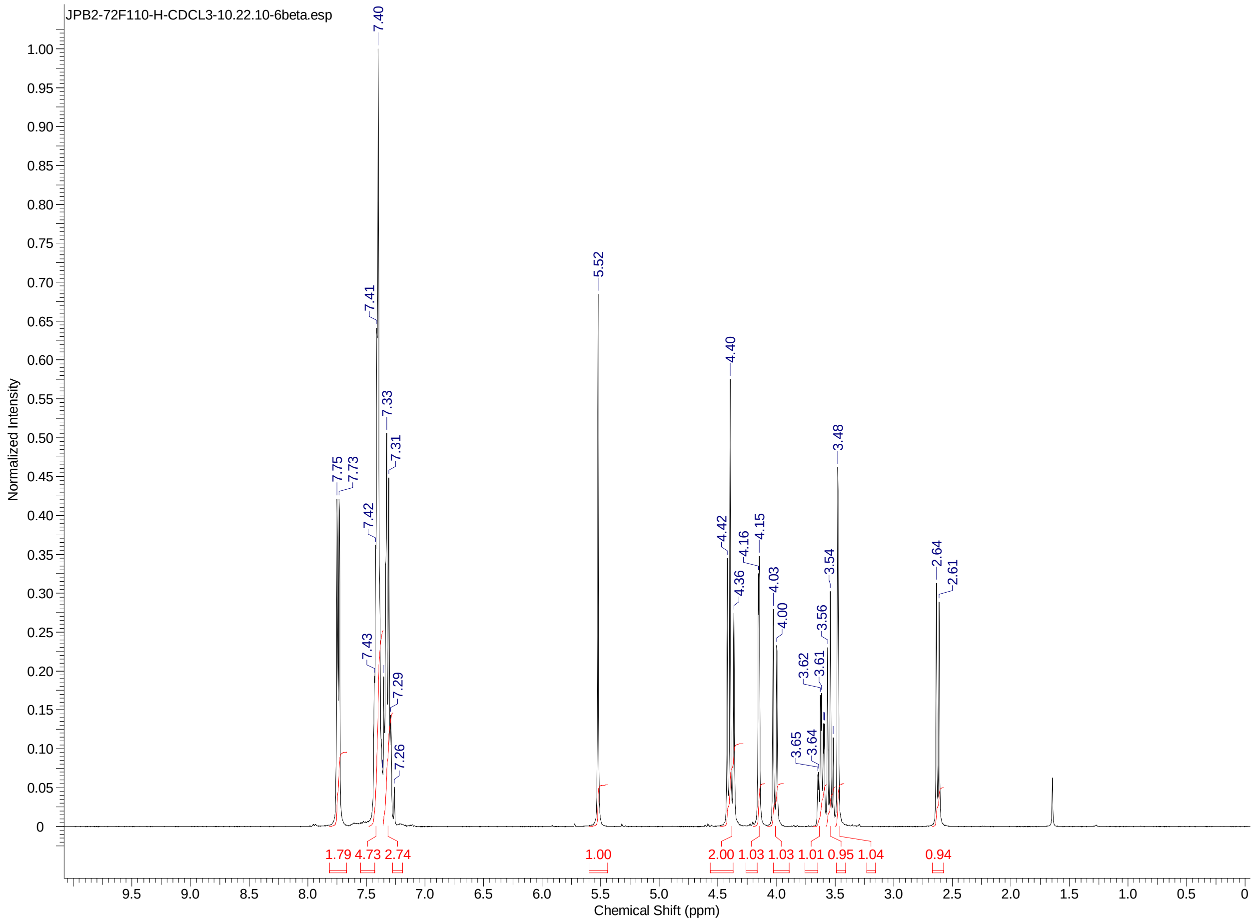

Ph
O
O
O
HO
N3
SPh

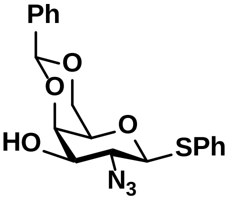
6α


JPB2-87-C-CDCL3-1.13.11-6alpha.esp
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
137.05
133.41
130.83
128.09
126.03
100.90
86.98
74.85
69.09
68.78
63.40
60.83
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

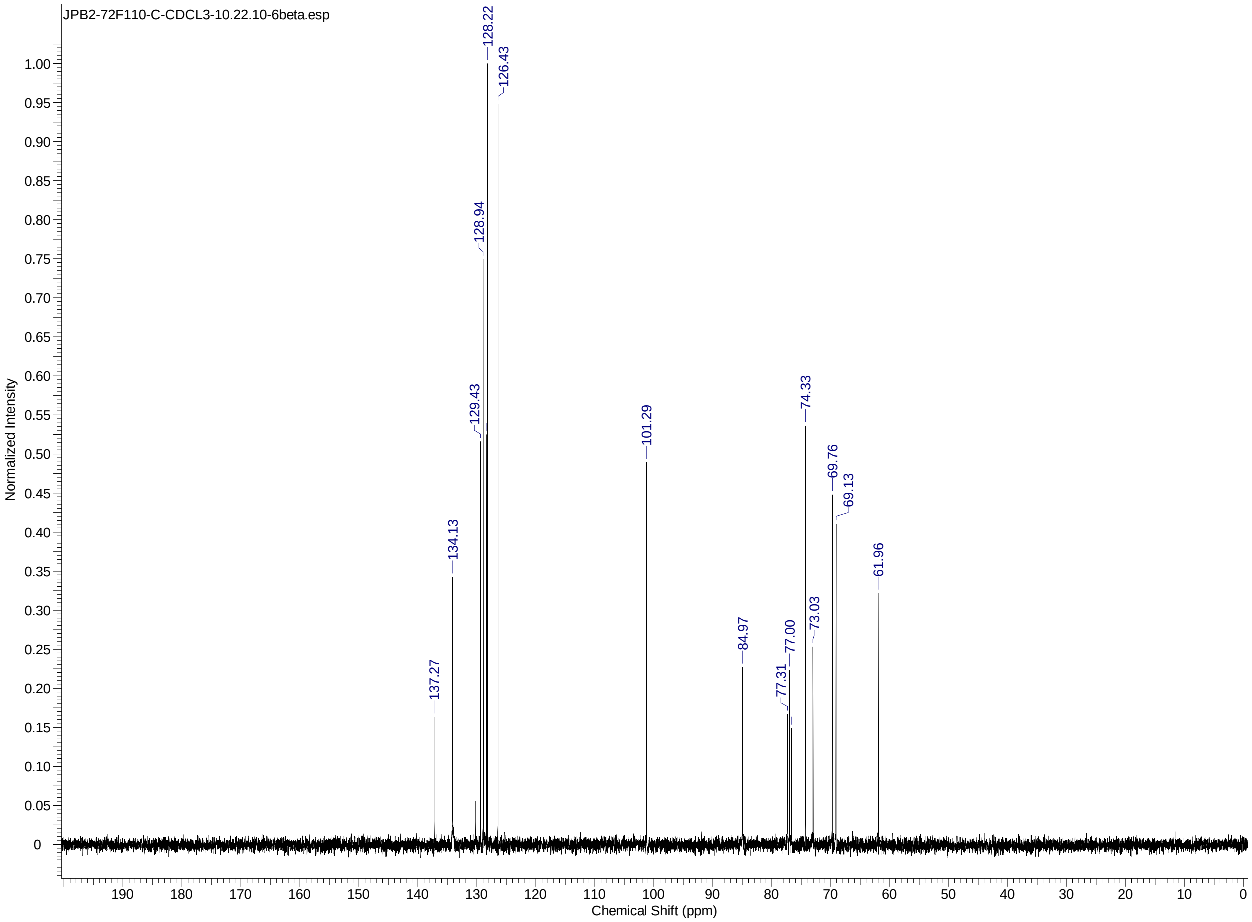

Ph
O
O
O
HO
SPh
N3

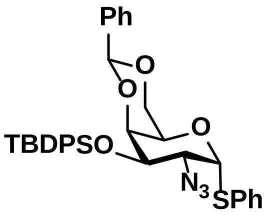
6β


JPB2-72F110-H-CDCL3-10.22.10-6beta.esp
Normalized Intensity
Chemical Shift (ppm)
7.75
7.73
7.43
7.42
7.41
7.40
7.33
7.31
7.29
7.26
5.52
4.42
4.40
4.36
4.16
4.15
4.03
4.00
3.65
3.64
3.62
3.61
3.56
3.54
3.48
2.64
2.61
1.79
4.73
2.74
1.00
2.00
1.03
1.03
1.01
0.95
1.04
0.94

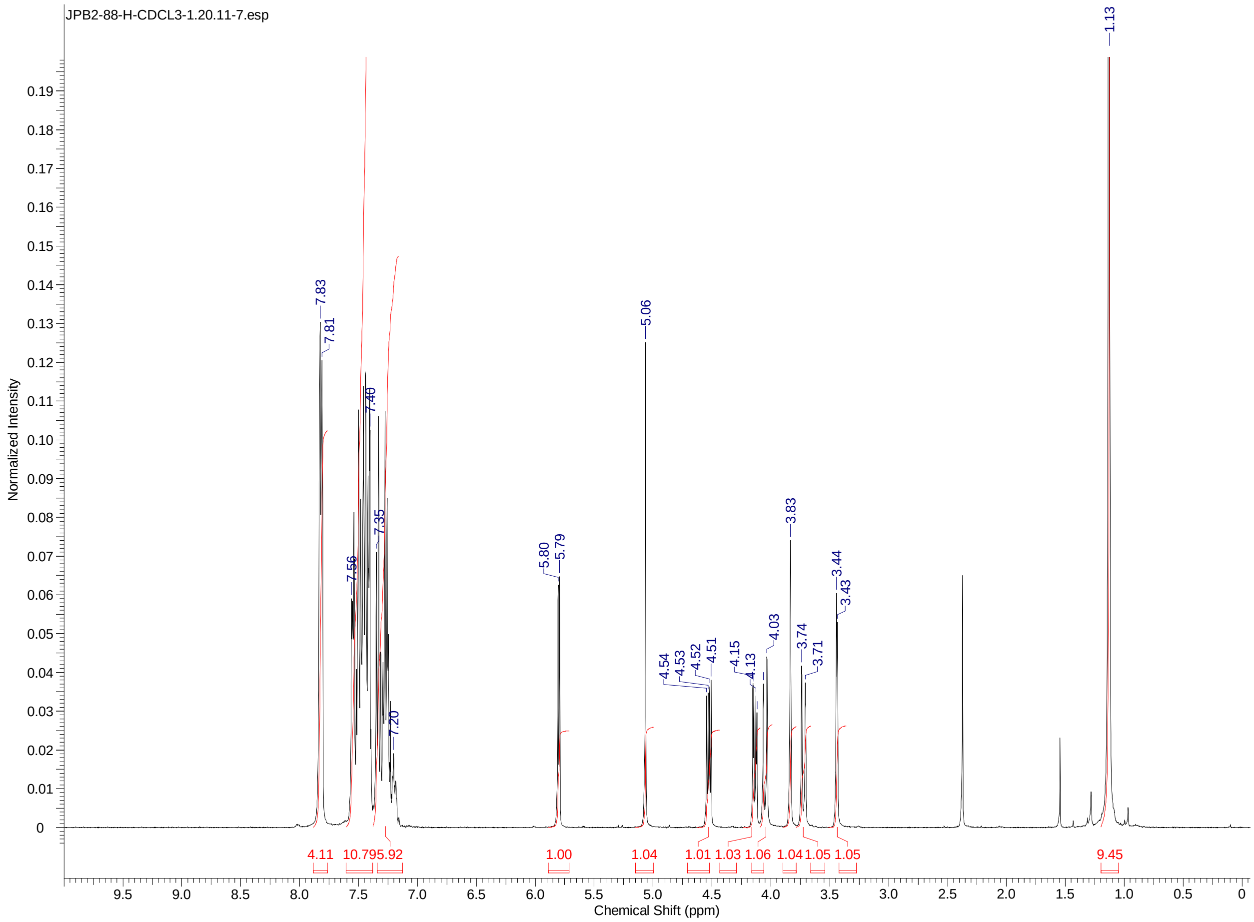

Ph
O
O
O
HO
SPh
N3

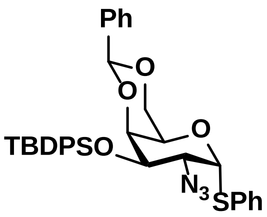
6β


JPB2-72F110-C-CDCL3-10.22.10-6beta.esp
Normalized Intensity
Chemical Shift (ppm)
128.22
126.43
128.94
129.43
134.13
137.27
101.29
84.97
77.31
77.00
73.03
74.33
69.76
69.13
61.96
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

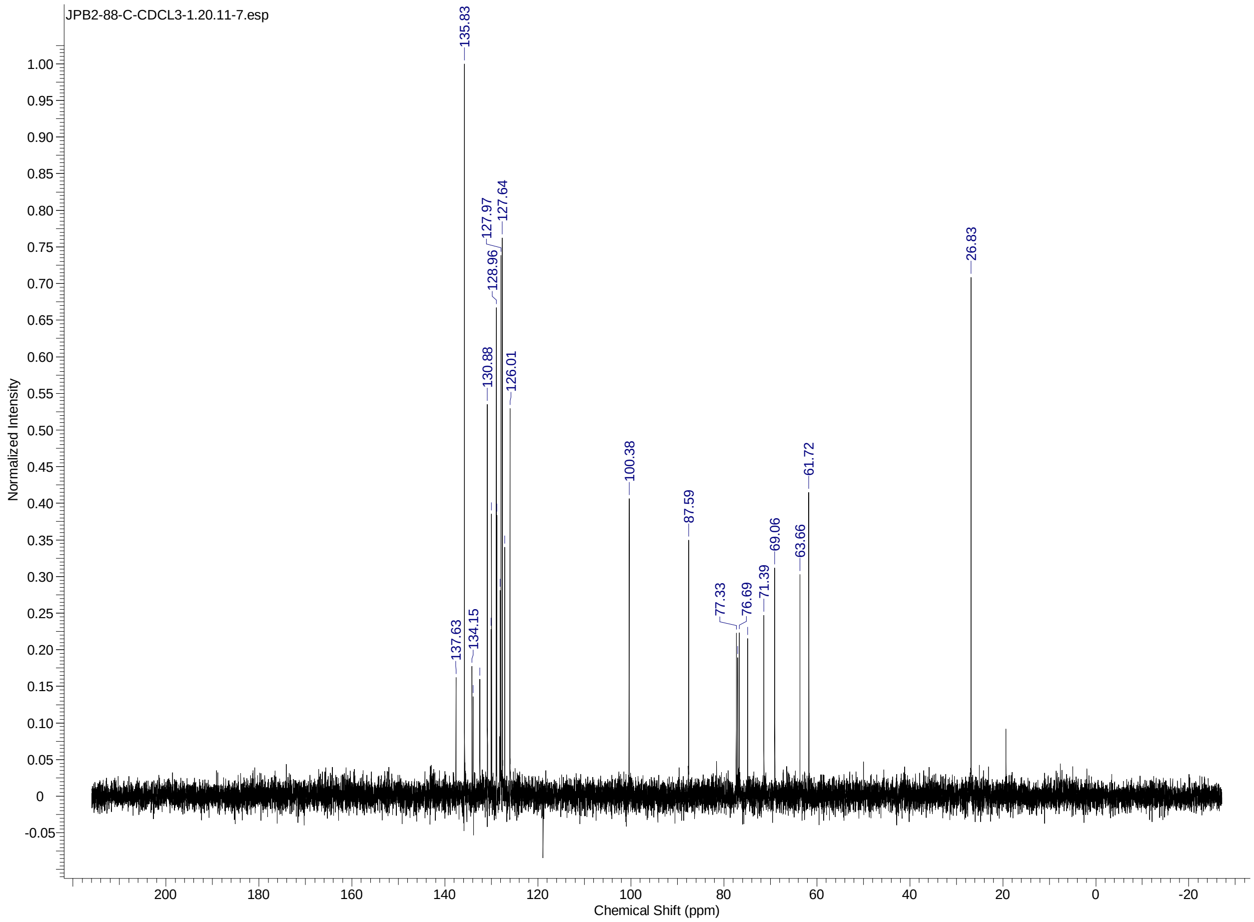

Ph
O
O
O
TBDPSO
N3
SPh

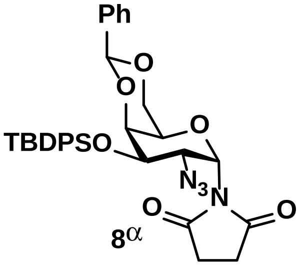
7


JPB2-88-H-CDCL3-1.20.11-7.esp
Normalized Intensity
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
7.83
7.81
7.56
7.40
7.35
7.20
5.80
5.79
5.06
4.54
4.53
4.52
4.51
4.15
4.13
4.03
3.83
3.74
3.71
3.44
3.43
1.13
4.11
10.79
5.92
1.00
1.04
1.01
1.03
1.06
1.04
1.05
1.05
9.45
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
0
Chemical Shift (ppm)

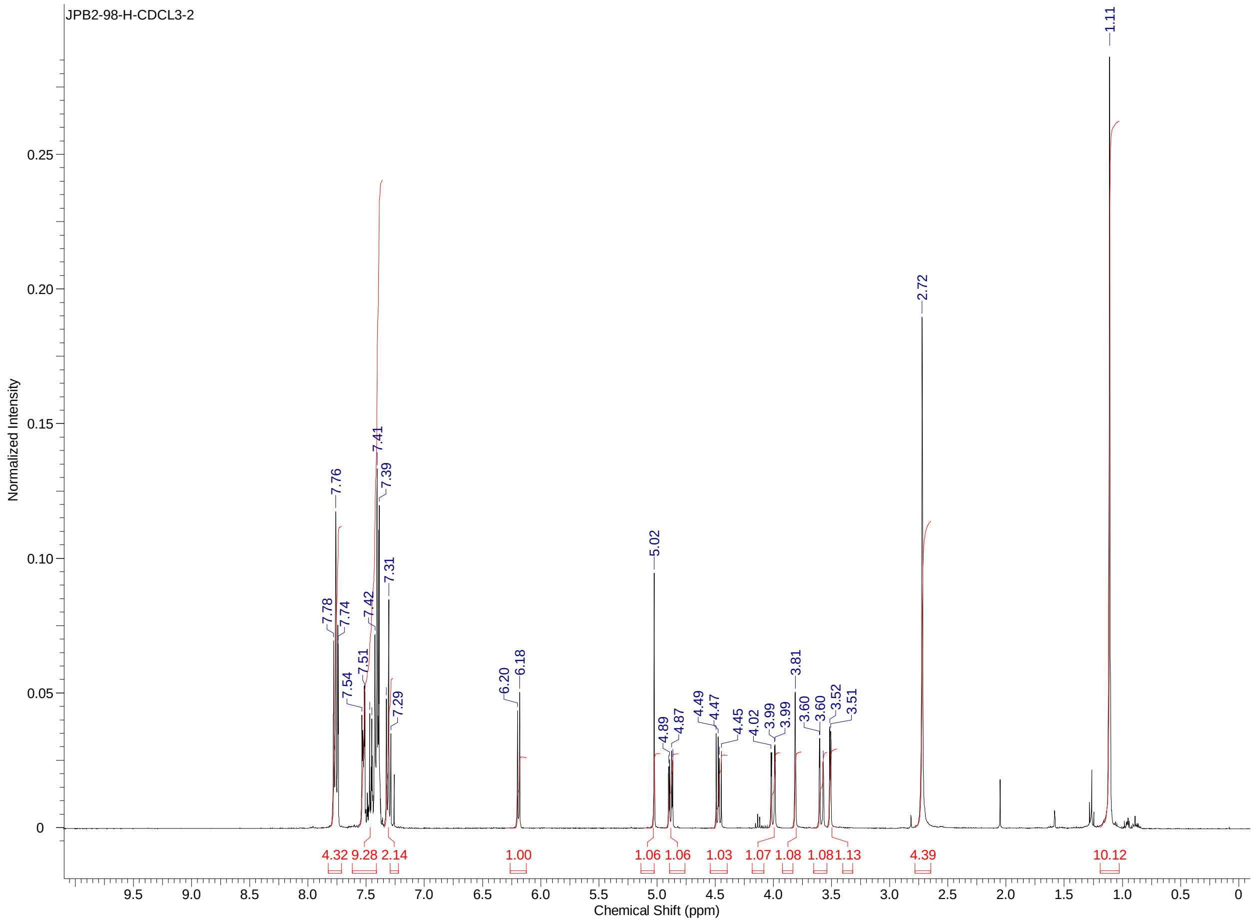

Ph
O
O
O
TBDPSO
N3
SPh

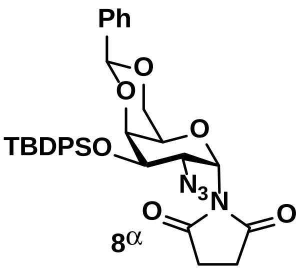
7


JPB2-88-C-CDCL3-1.20.11-7.esp
135.83
127.97
127.64
128.96
130.88
126.01
26.83
100.38
61.72
87.59
69.06
63.66
77.33
76.69
71.39
137.63
134.15
Normalized Intensity
Chemical Shift (ppm)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
200
180
160
140
120
100
80
60
40
20
0
-20

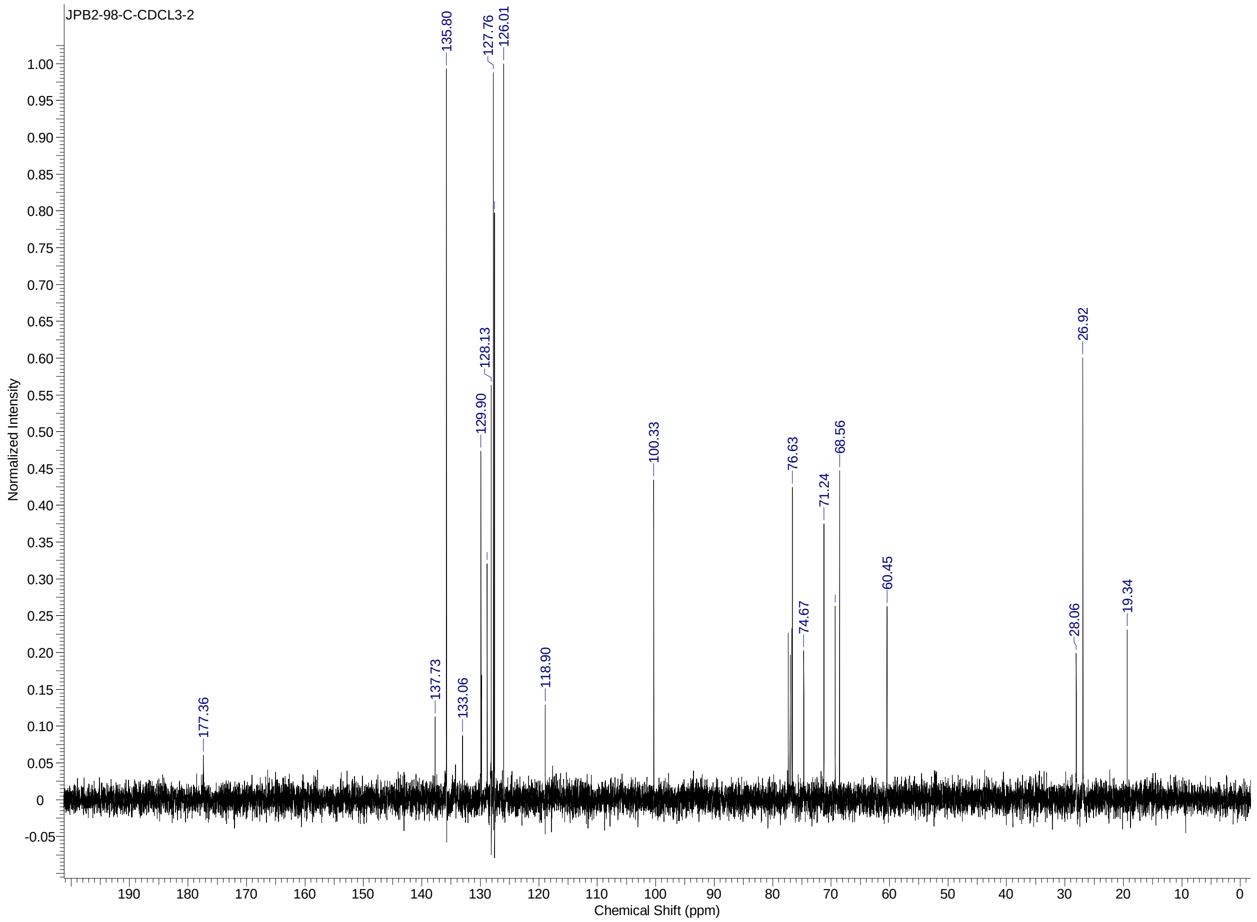

Ph
O
O
O
TBDPSO
N3
N
O
O

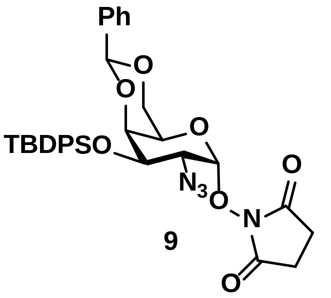
8α


JPB2-98-H-CDCL3-2
Normalized Intensity
0.25
0.20
0.15
0.10
0.05
0
7.78
7.76
7.74
7.54
7.51
7.42
7.41
7.39
7.31
7.29
6.20
6.18
5.02
4.89
4.87
4.49
4.47
4.45
4.02
3.99
3.99
3.81
3.60
3.60
3.52
3.51
2.72
1.11
4.32
9.28
2.14
1.00
1.06
1.06
1.03
1.07
1.08
1.08
1.13
4.39
10.12
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
0
Chemical Shift (ppm)

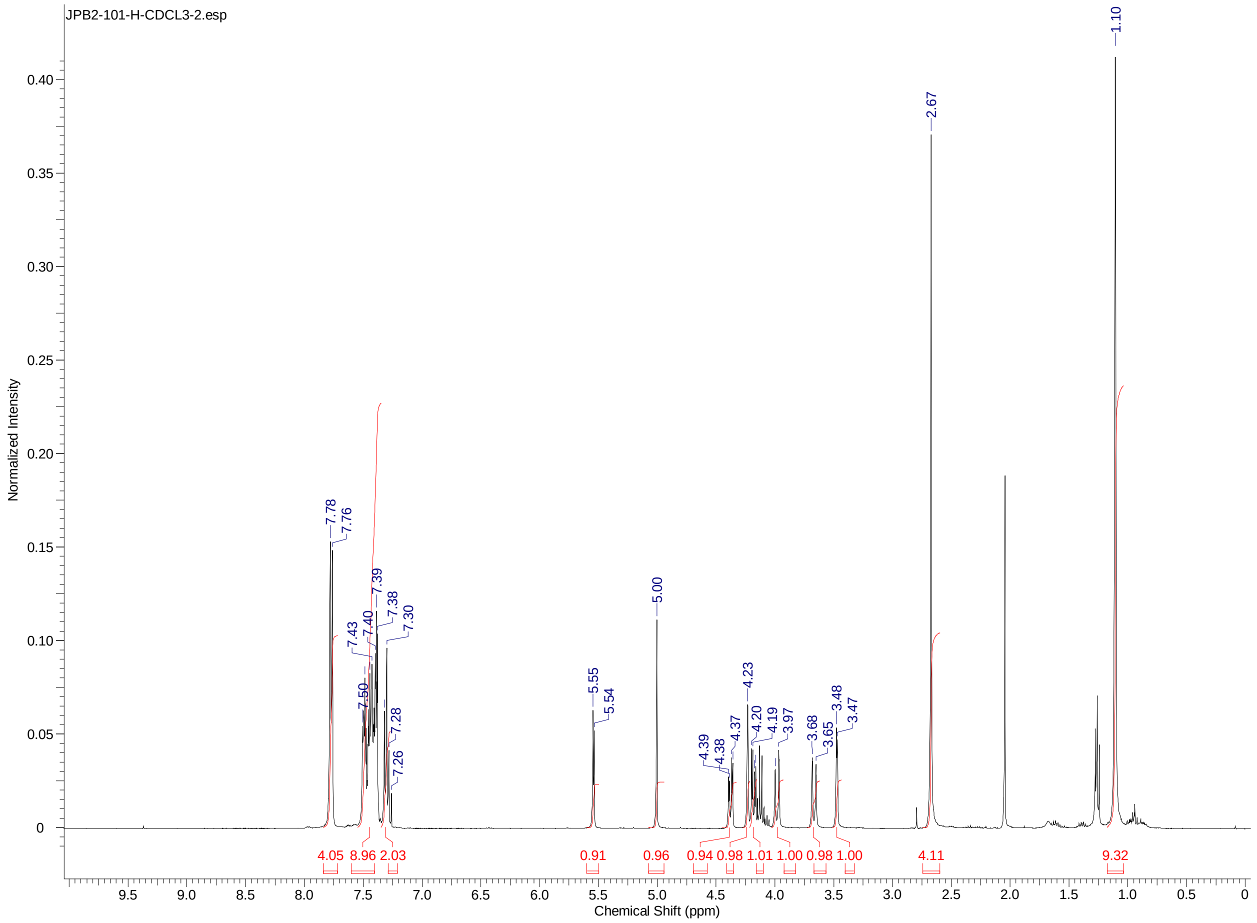

Ph
O
O
O
TBDPSO
N3
N
O
O

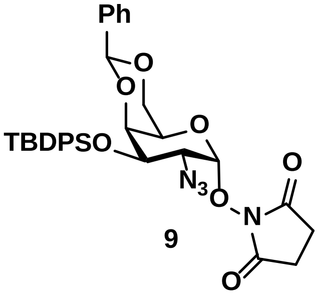
8α


JPB2-98-C-CDCL3-2
135.80
127.76
126.01
128.13
129.90
137.73
133.06
118.90
177.36
100.33
76.63
74.67
71.24
68.56
60.45
28.06
26.92
19.34
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

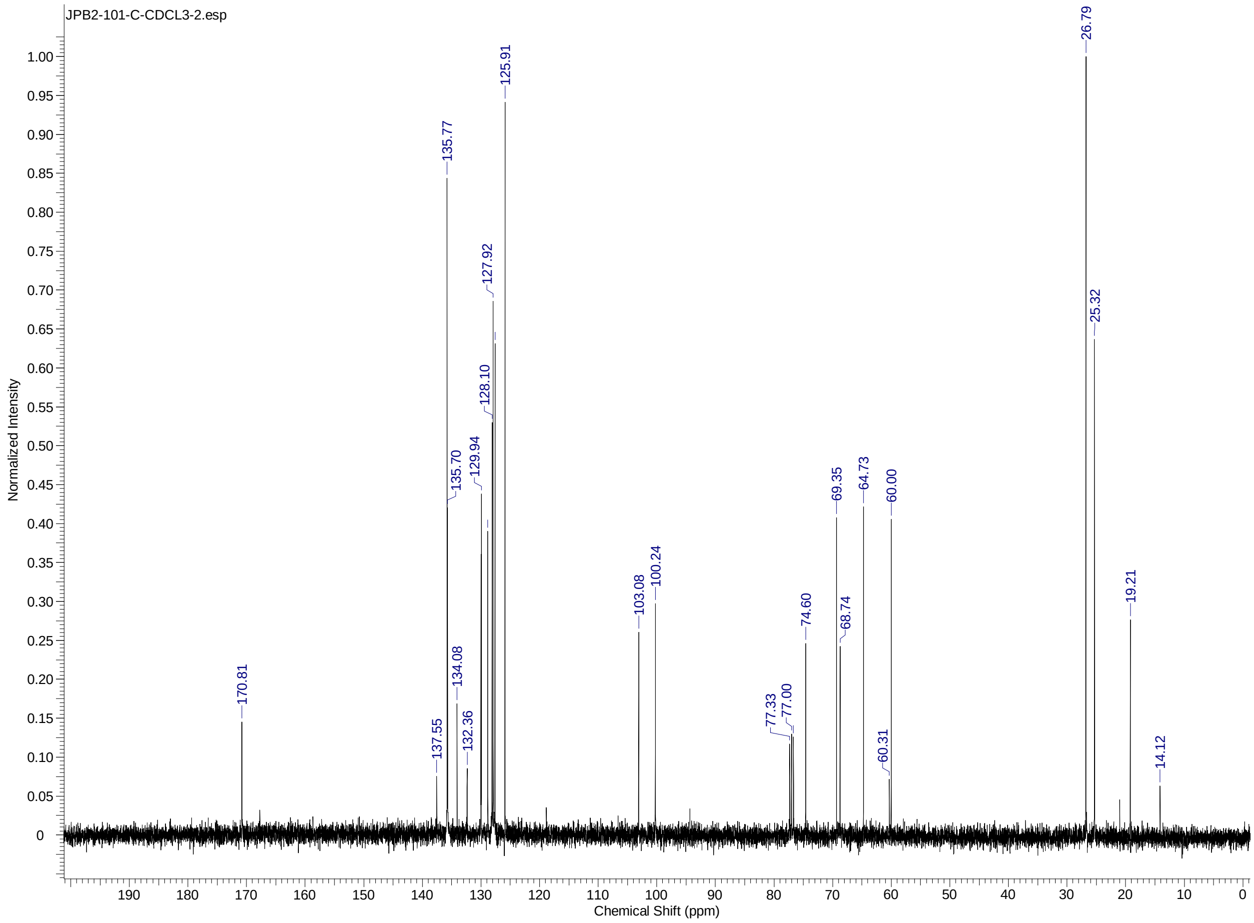

Ph
O
O
O
TBDPSO
N3
O
N
O
O

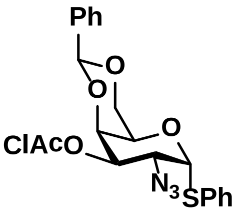
9


JPB2-101-H-CDCL3-2.esp
Normalized Intensity
Chemical Shift (ppm)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
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
0
7.78
7.76
7.50
7.43
7.40
7.39
7.38
7.30
7.28
7.26
5.55
5.54
5.00
4.39
4.38
4.37
4.23
4.20
4.19
3.97
3.68
3.65
3.48
3.47
2.67
1.10
4.05
8.96
2.03
0.91
0.96
0.94
0.98
1.01
1.00
0.98
1.00
4.11
9.32

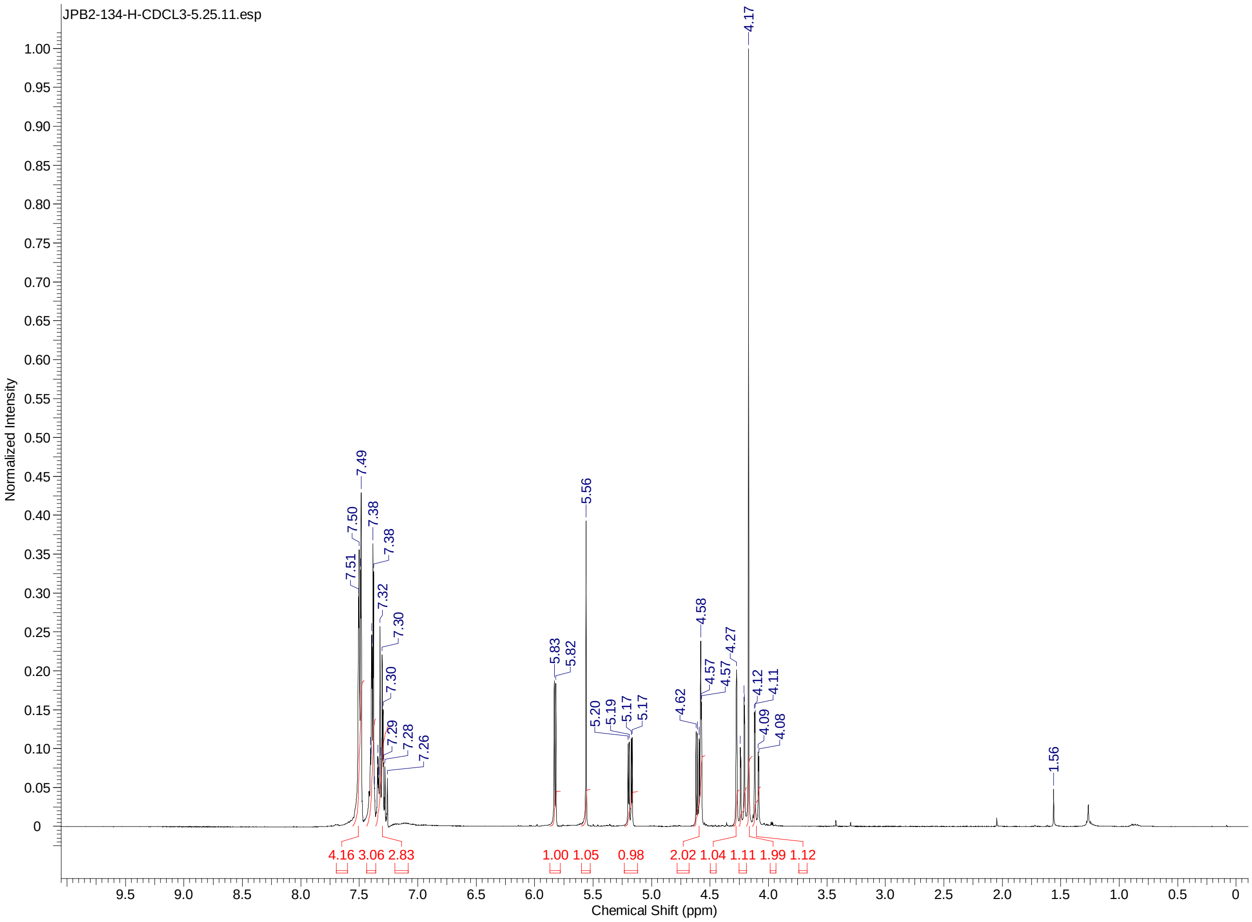

Ph
O
O
O
TBDPSO
N3
O
N
O
O

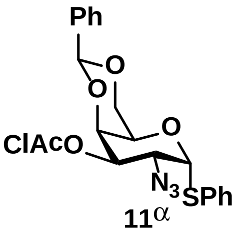
9


JPB2-101-C-CDCL3-2.esp
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
170.81
137.55
135.77
135.70
134.08
132.36
129.94
128.10
127.92
125.91
103.08
100.24
77.33
77.00
74.60
69.35
68.74
64.73
60.31
60.00
26.79
25.32
19.21
14.12
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

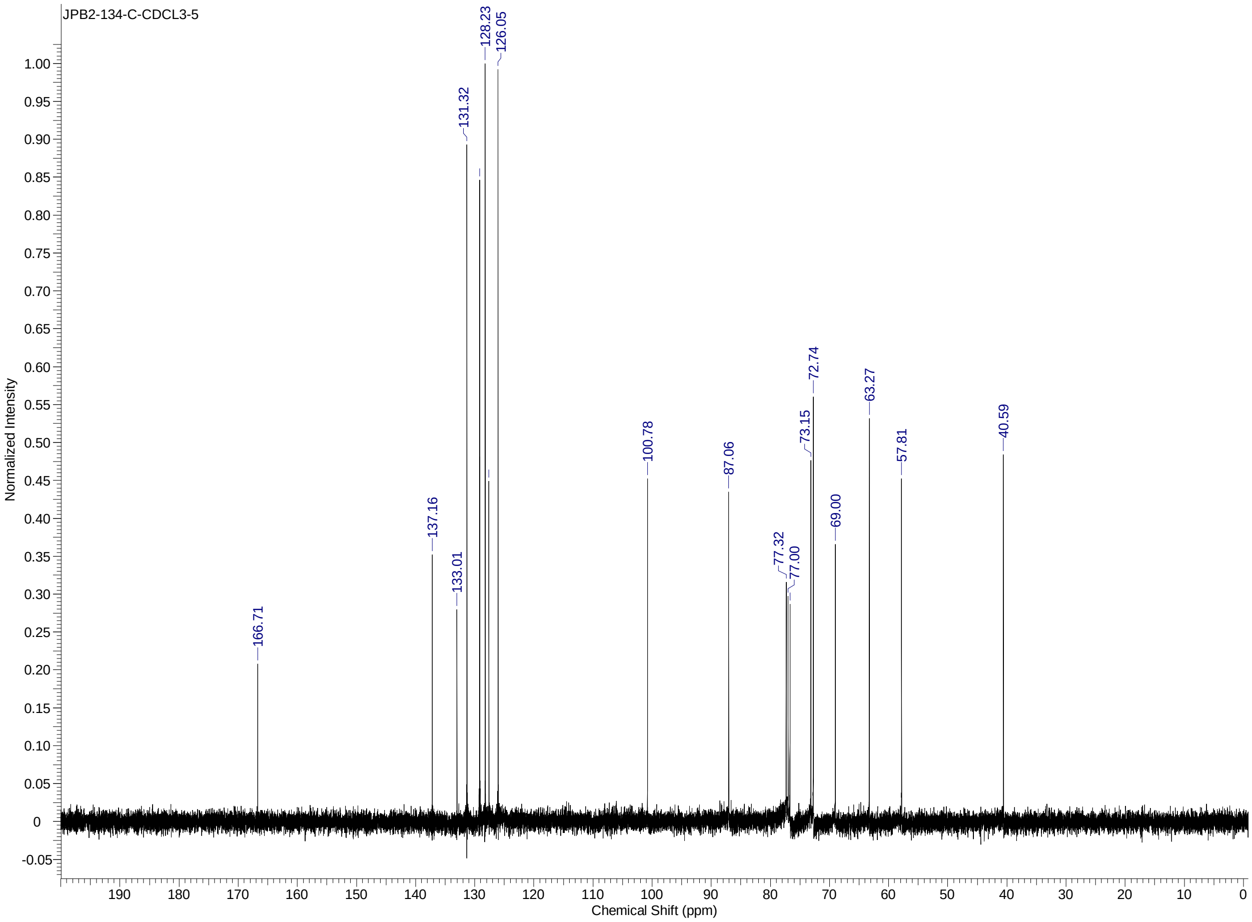

Ph
O
O
O
ClAcO
N3
SPh

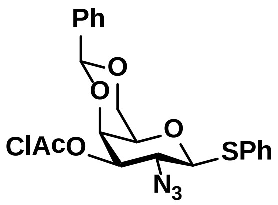
11α


JPB2-134-H-CDCL3-5.25.11.esp
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
7.51
7.50
7.49
7.38
7.38
7.32
7.30
7.30
7.29
7.28
7.26
5.83
5.82
5.56
5.20
5.19
5.17
5.17
4.62
4.58
4.57
4.57
4.27
4.17
4.12
4.11
4.09
4.08
1.56
4.16
3.06
2.83
1.00
1.05
0.98
2.02
1.04
1.11
1.99
1.12
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
0
Chemical Shift (ppm)

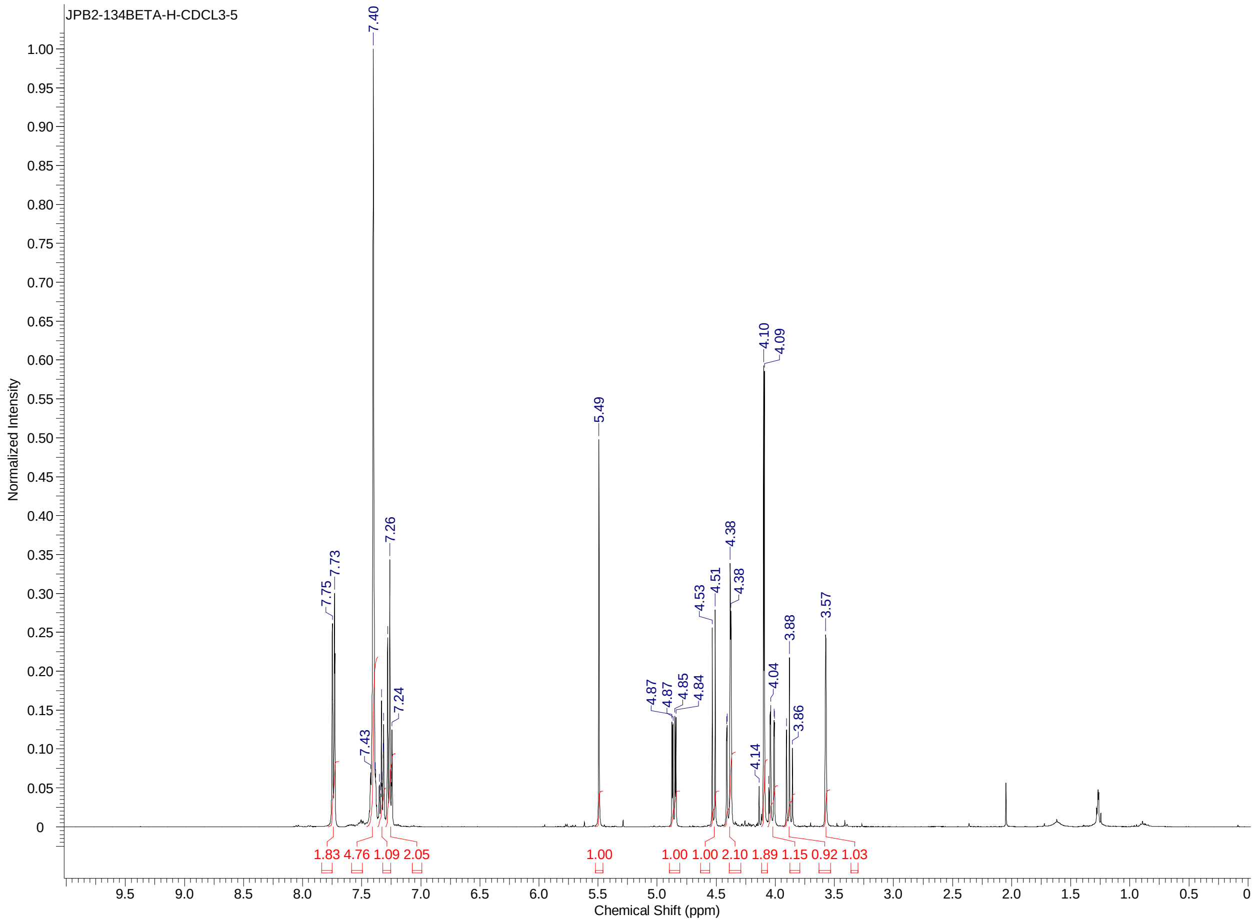

Ph
O
O
O
ClAcO
N_3
SPh
α

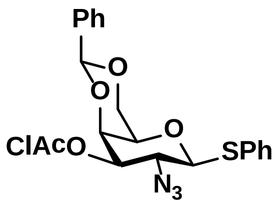
11α


JPB2-134-C-CDCL3-5
166.71
137.16
133.01
131.32
128.23
126.05
100.78
87.06
77.32
77.00
73.15
72.74
69.00
63.27
57.81
40.59
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

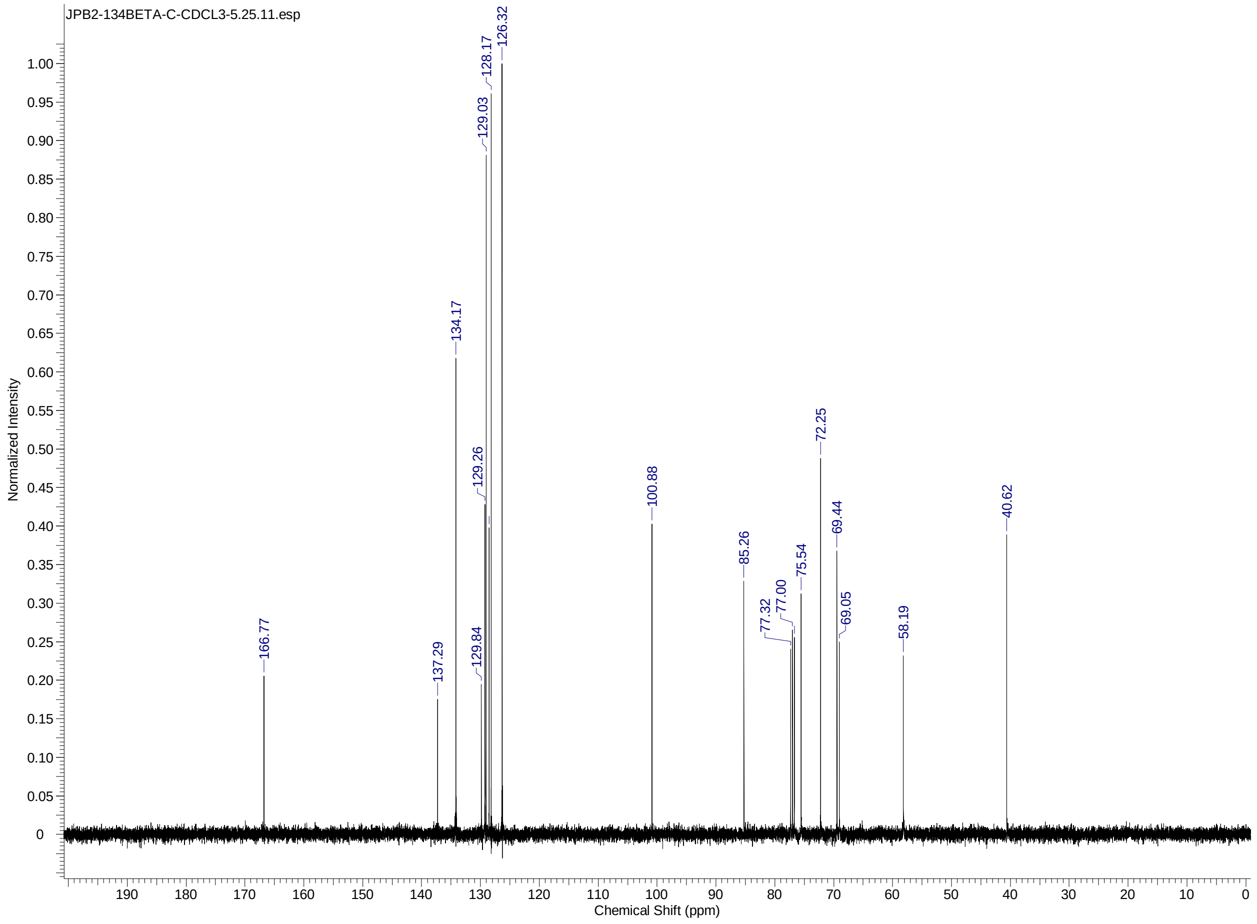

Ph
O
O
O
ClAcO
SPh
N_3

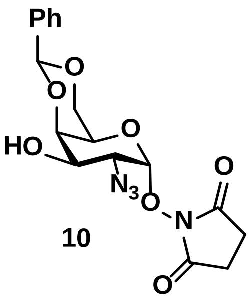
11β


JPB2-134BETA-H-CDCL3-5
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
7.75
7.73
7.43
7.40
7.26
7.24
5.49
4.87
4.87
4.85
4.84
4.53
4.51
4.38
4.38
4.14
4.10
4.09
4.04
3.88
3.86
3.57
1.83
4.76
1.09
2.05
1.00
1.00
1.00
2.10
1.89
1.15
0.92
1.03
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
0
Chemical Shift (ppm)

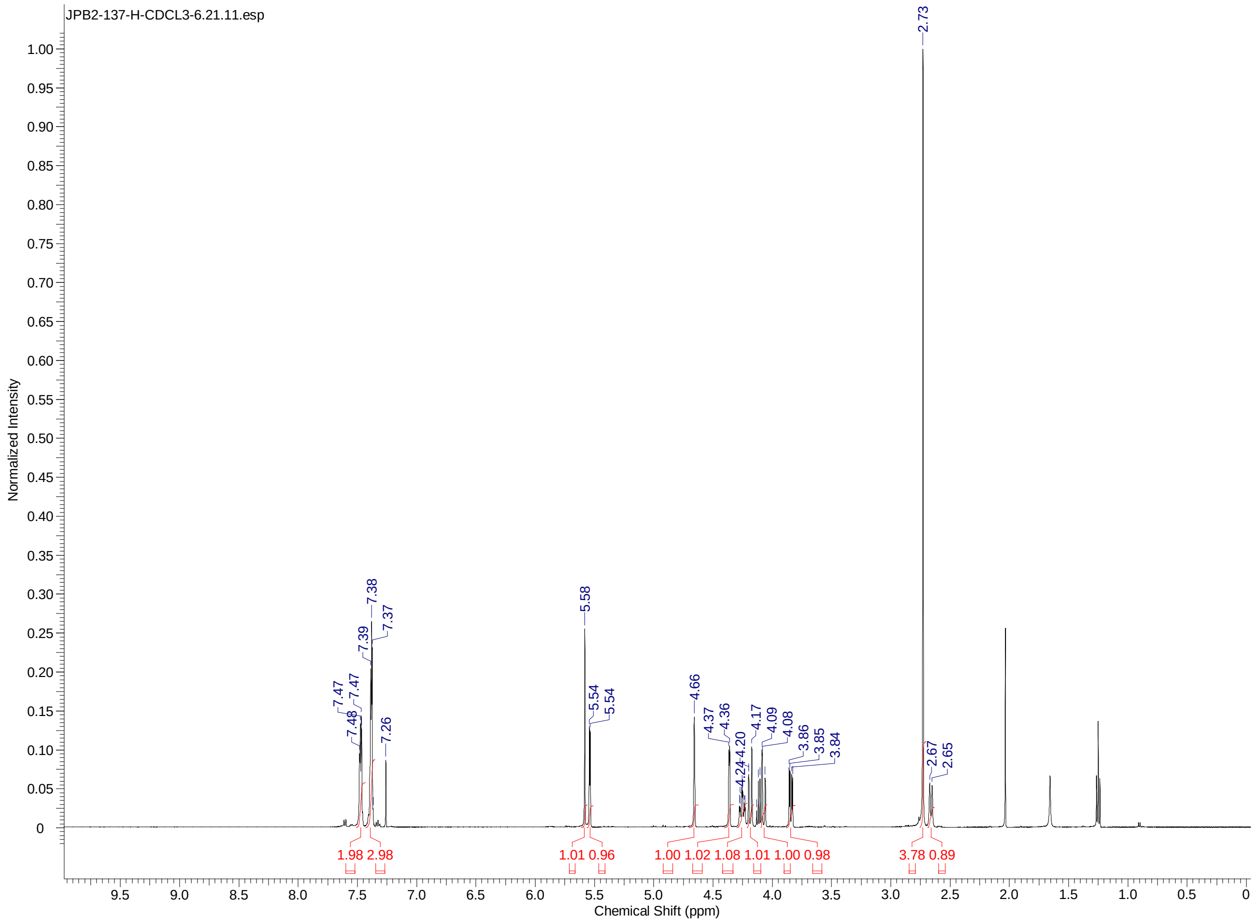

Ph
O
O
O
ClAcO
SPh
N3

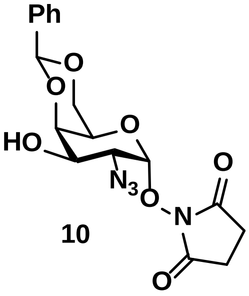
11β


JPB2-134BETA-C-CDCL3-5.25.11.esp
Normalized Intensity
Chemical Shift (ppm)
166.77
137.29
134.17
129.84
129.26
129.03
128.17
126.32
100.88
85.26
77.32
77.00
75.54
72.25
69.44
69.05
58.19
40.62

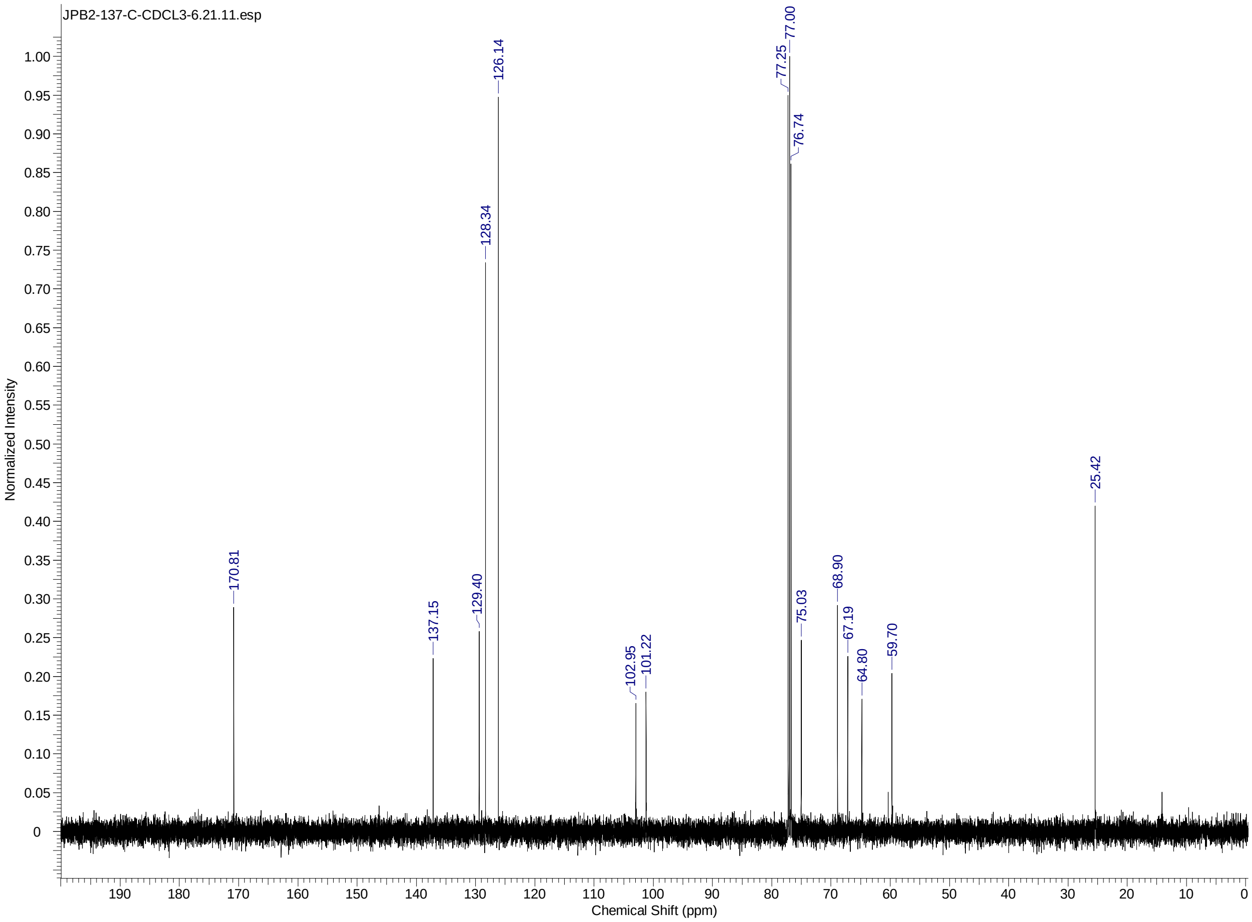

Ph
O
O
HO
O
N3
O
N
O
O

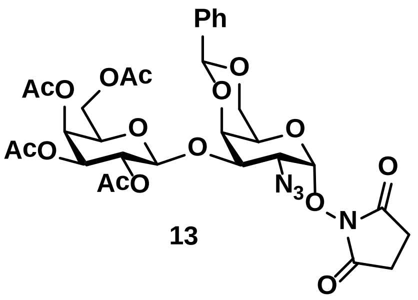
10


JPB2-137-H-CDCL3-6.21.11.esp
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
7.48
7.47
7.47
7.39
7.38
7.37
7.26
5.58
5.54
5.54
4.66
4.37
4.36
4.24
4.20
4.17
4.09
4.08
3.86
3.85
3.84
2.73
2.67
2.65
1.98
2.98
1.01
0.96
1.00
1.02
1.08
1.01
1.00
0.98
3.78
0.89
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
0
Chemical Shift (ppm)

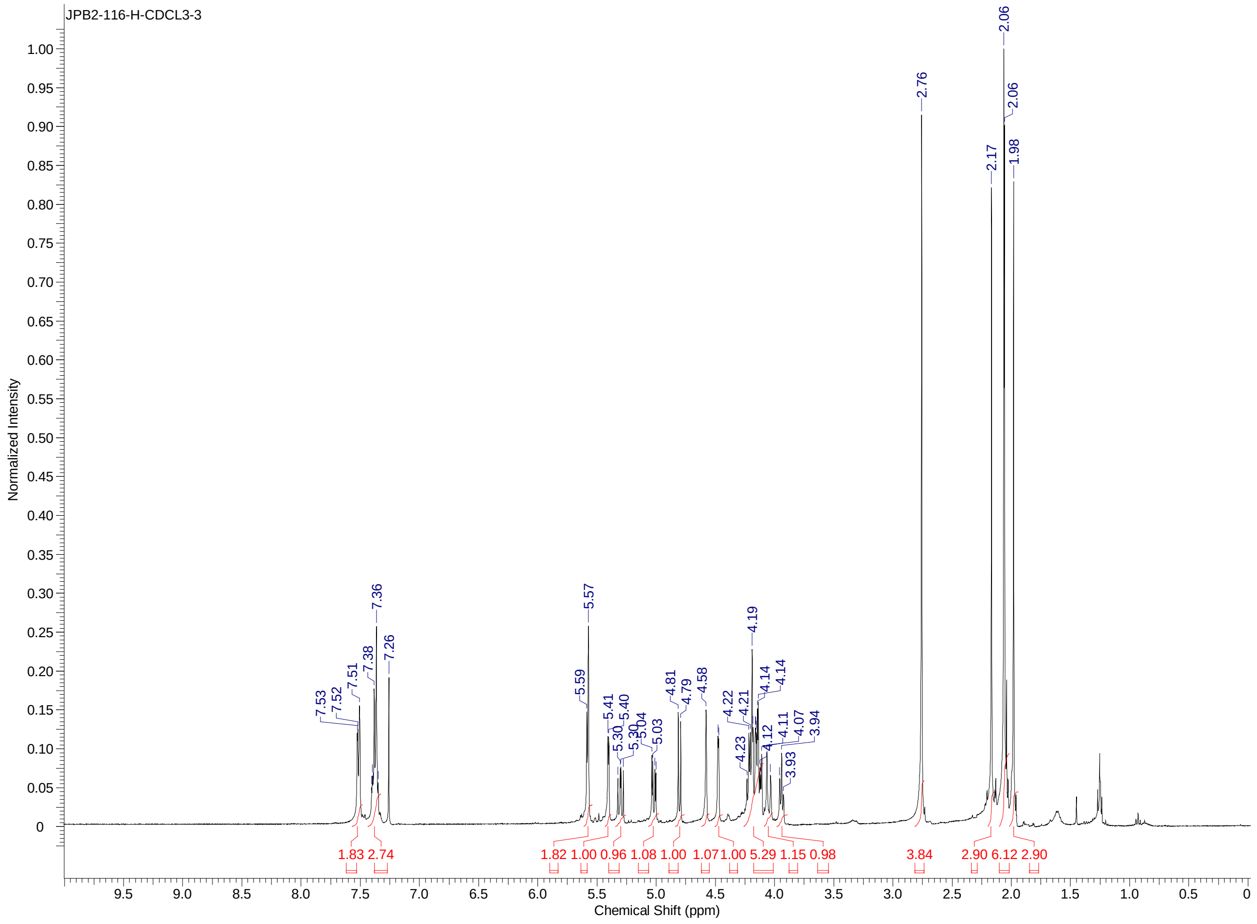

Ph
O
O
O
HO
N3
O
N
O
O

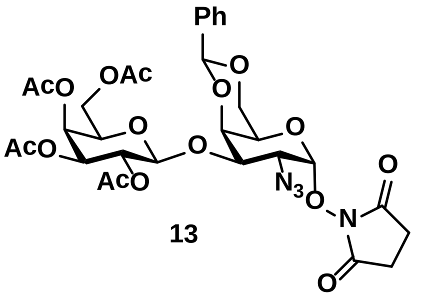
10


JPB2-137-C-CDCL3-6.21.11.esp
Normalized Intensity
Chemical Shift (ppm)
170.81
137.15
129.40
128.34
126.14
102.95
101.22
77.25
77.00
76.74
75.03
68.90
67.19
64.80
59.70
25.42

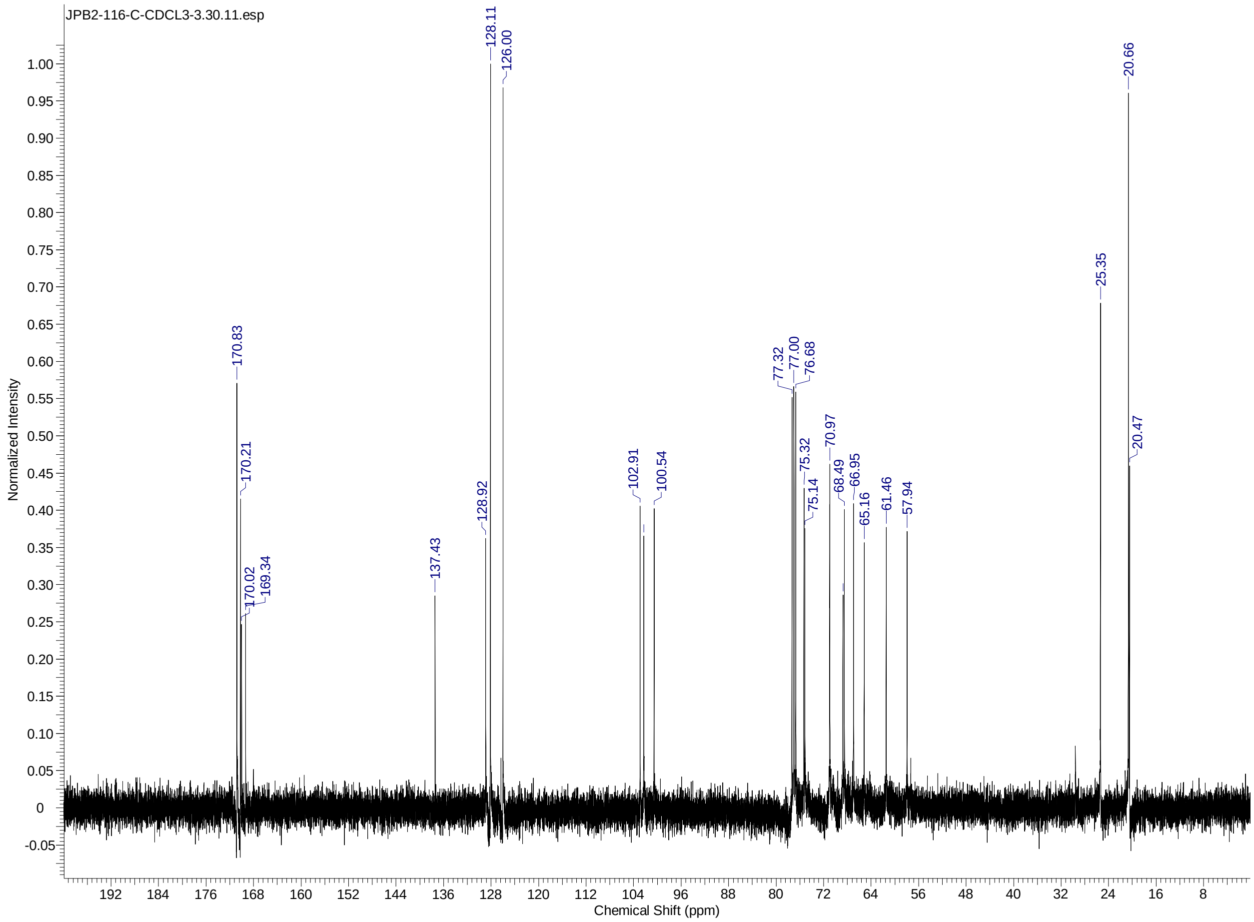

Ph
O
O
AcO
OAc
O
O
AcO
O
AcO
N3
O
N
O
O

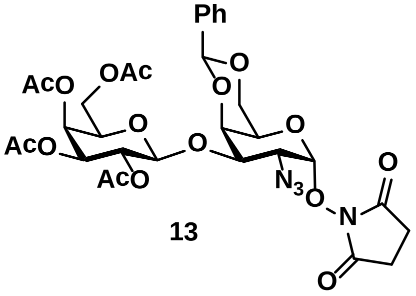
13


JPB2-116-H-CDCL3-3
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
7.53
7.52
7.51
7.38
7.36
7.26
5.59
5.57
5.41
5.40
5.30
5.30
5.04
5.03
4.81
4.79
4.58
4.23
4.22
4.21
4.19
4.14
4.14
4.12
4.11
4.07
3.94
3.93
2.76
2.17
2.06
2.06
1.98
1.83
2.74
1.82
1.00
0.96
1.08
1.00
1.07
1.00
5.29
1.15
0.98
3.84
2.90
6.12
2.90
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
0
Chemical Shift (ppm)

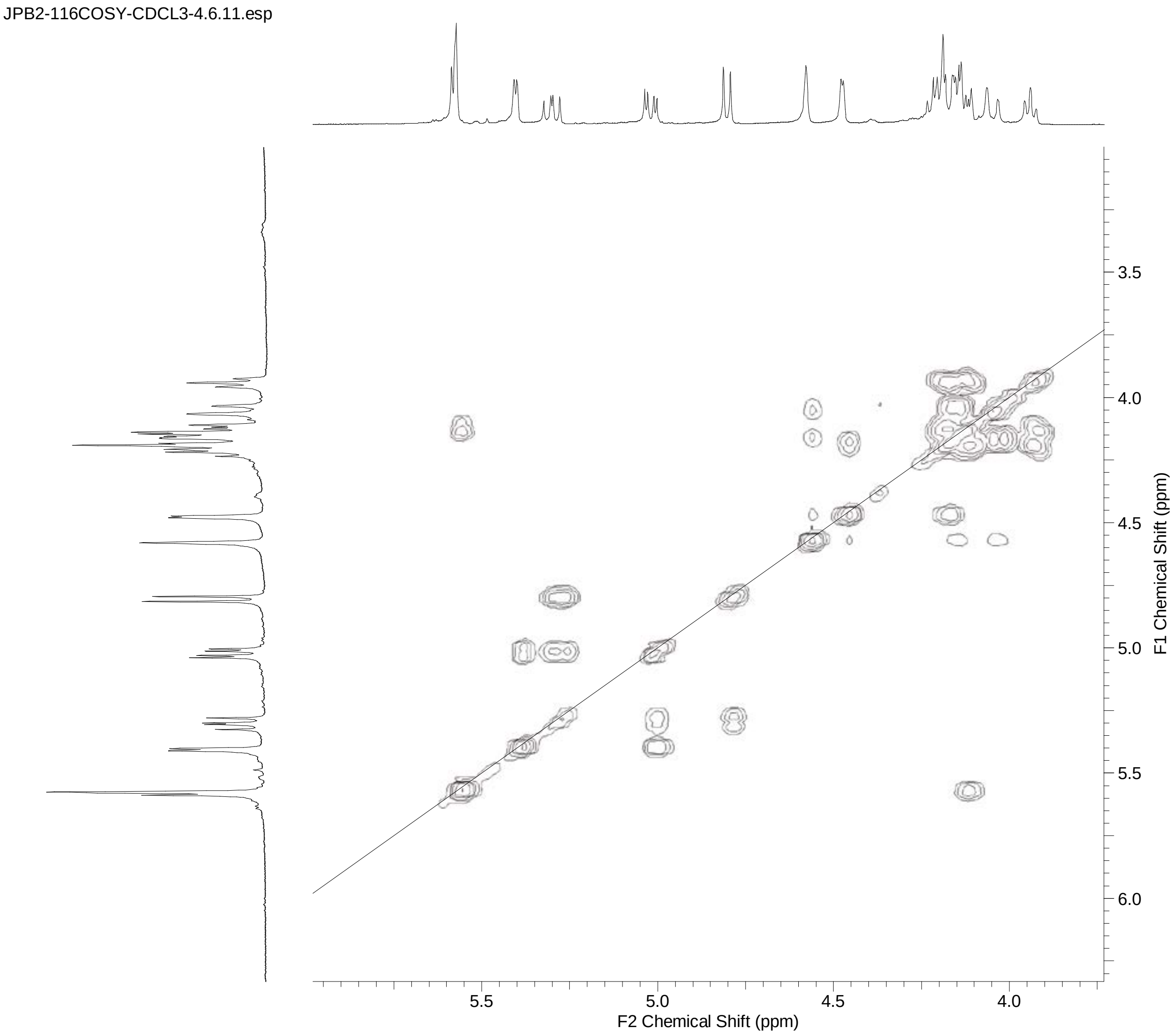

Ph
O
O
AcO
OAc
O
O
AcO
O
AcO
N3
O
N
O
O

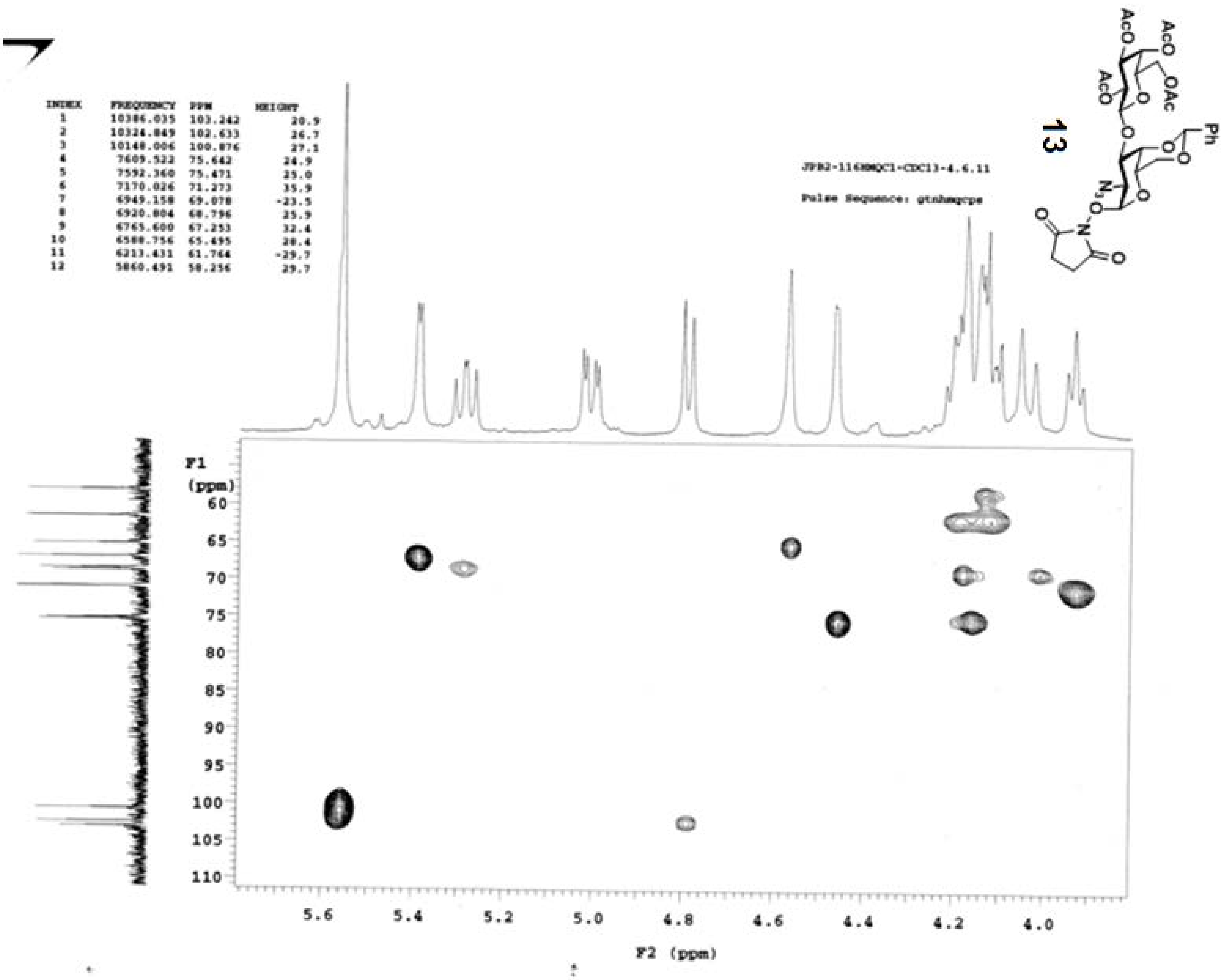
13


JPB2-116-C-CDCL3-3.30.11.esp
Normalized Intensity
Chemical Shift (ppm)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
170.83
170.21
170.02
169.34
137.43
128.92
128.11
126.00
102.91
100.54
77.32
77.00
76.68
75.32
75.14
70.97
68.49
66.95
65.16
61.46
57.94
25.35
20.66
20.47


Ph
O
O
AcO
OAc
O
AcO
O
O
O
AcO
N3
O
N
O
O

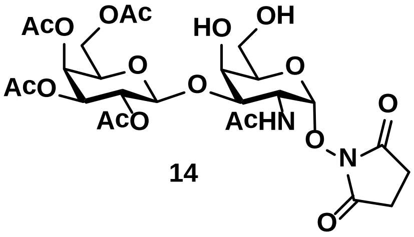
13


JPB2-116COSY-CDCL3-4.6.11.esp
3.5
4.0
4.5
5.0
5.5
6.0
F1 Chemical Shift (ppm)
5.5
5.0
4.5
4.0
F2 Chemical Shift (ppm)

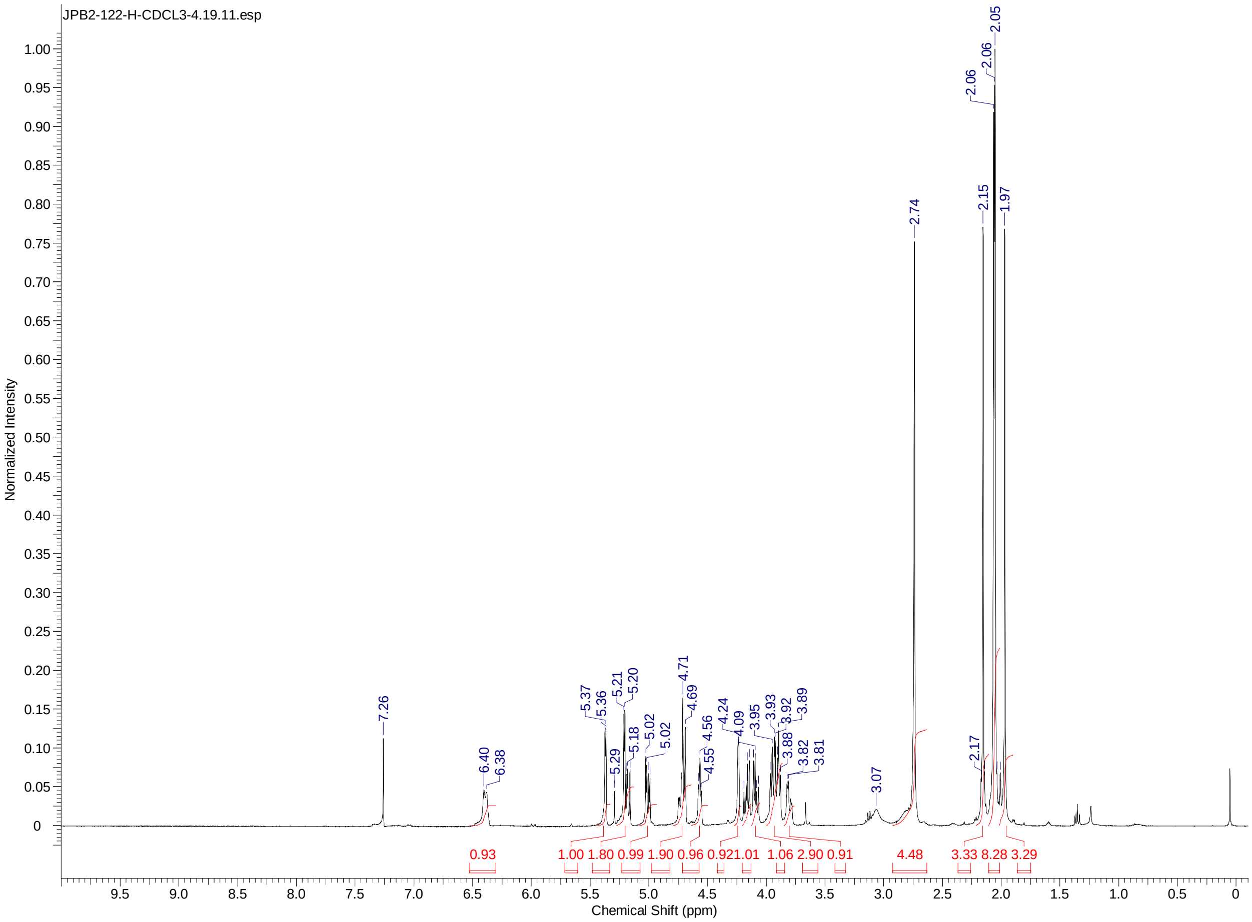


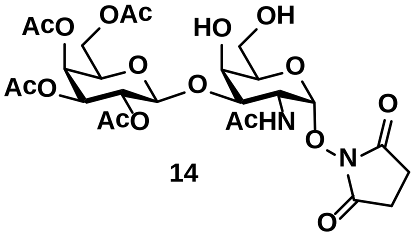
13

INDEX	FREQUENCY	PPM	HEIGHT
1	10386.035	103.242	20.9
2	10324.849	102.633	26.7
3	10148.006	100.876	27.1
4	7609.522	75.642	24.9
5	7592.360	75.471	25.0
6	7170.026	71.273	35.9
7	6949.158	69.078	-23.5
8	6920.804	68.796	25.9
9	6765.600	67.253	32.4
10	6588.756	65.495	28.4
11	6213.431	61.764	-29.7
12	5860.491	58.256	29.7

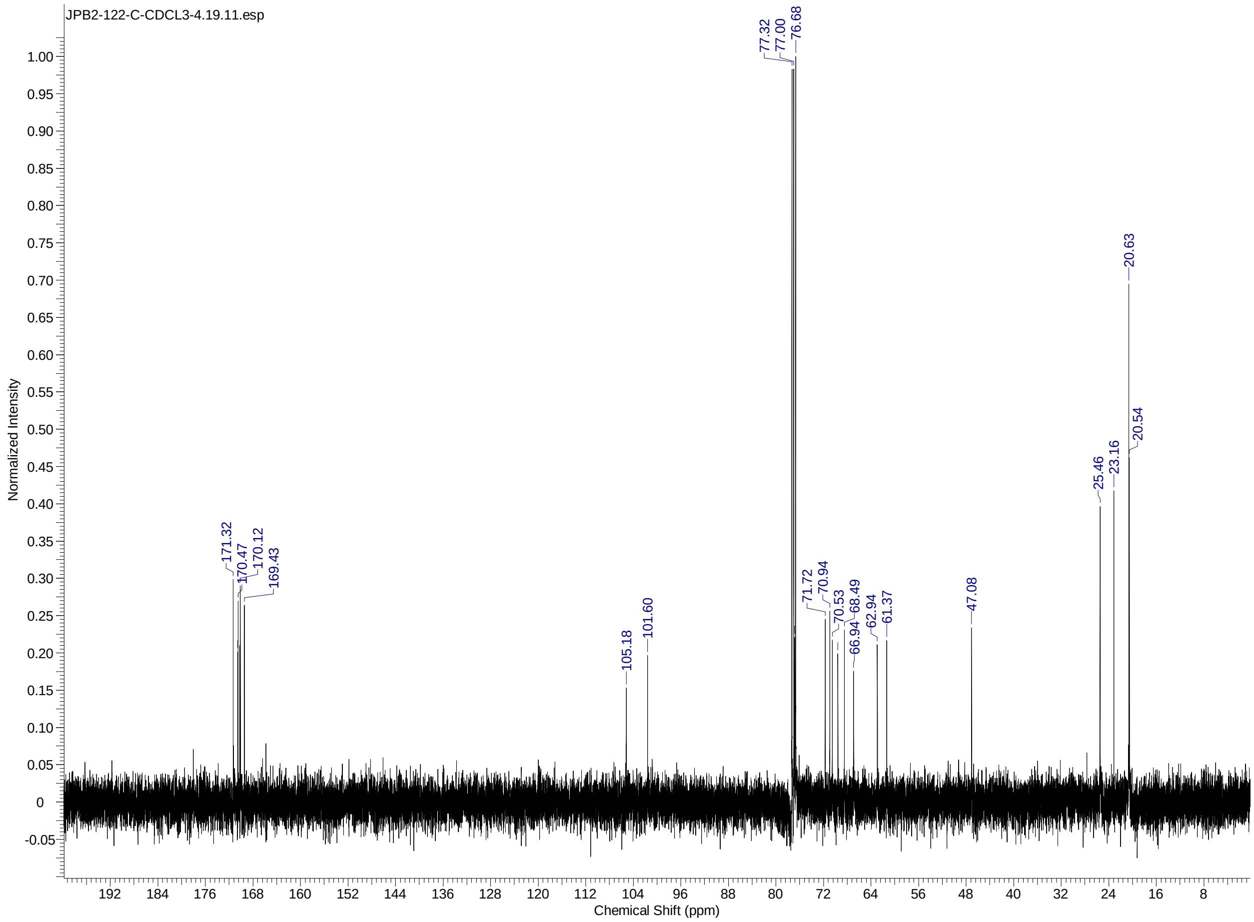

AcO
OAc
AcO
AcO
O
O
HO
OH
O
AcHN
O
N
O
O

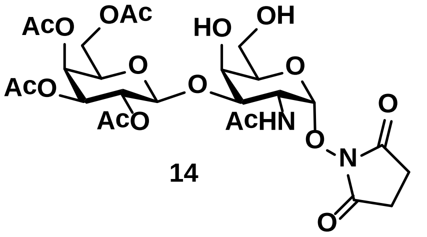
14


JPB2-122-H-CDCL3-4.19.11.esp
Normalized Intensity
Chemical Shift (ppm)
7.26
6.40
6.38
5.37
5.36
5.29
5.21
5.20
5.18
5.02
5.02
4.71
4.69
4.56
4.55
4.24
4.09
3.95
3.93
3.92
3.89
3.88
3.82
3.81
3.07
2.74
2.17
2.15
2.06
2.06
2.05
1.97
0.93
1.00
1.80
0.99
1.90
0.96
0.92
1.01
1.06
2.90
0.91
4.48
3.33
8.28
3.29

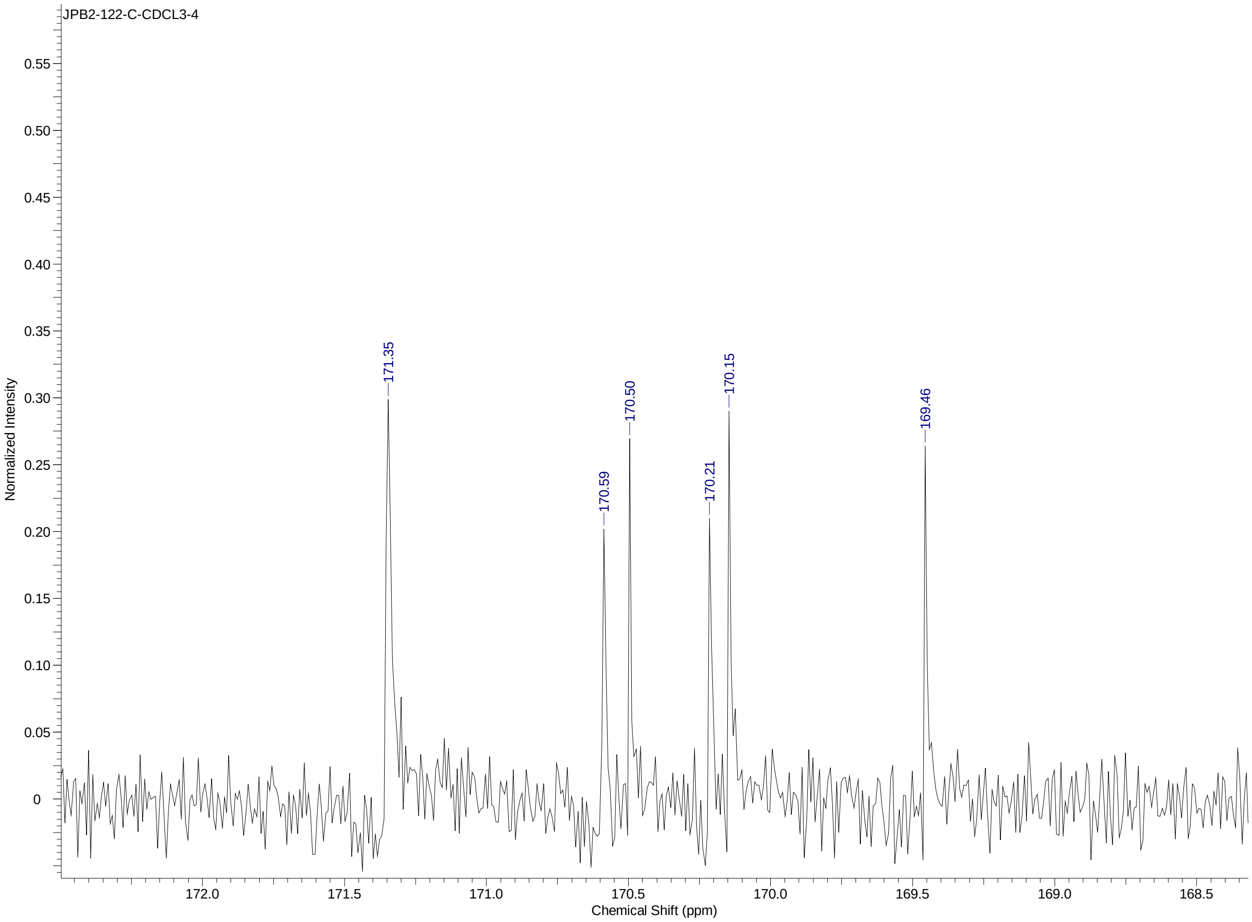


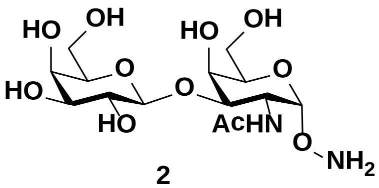
14


JPB2-122-C-CDCL3-4.19.11.esp
Normalized Intensity
Chemical Shift (ppm)
171.32
170.47
170.12
169.43
105.18
101.60
77.32
77.00
76.68
71.72
70.94
70.53
68.49
66.94
62.94
61.37
47.08
25.46
23.16
20.63
20.54

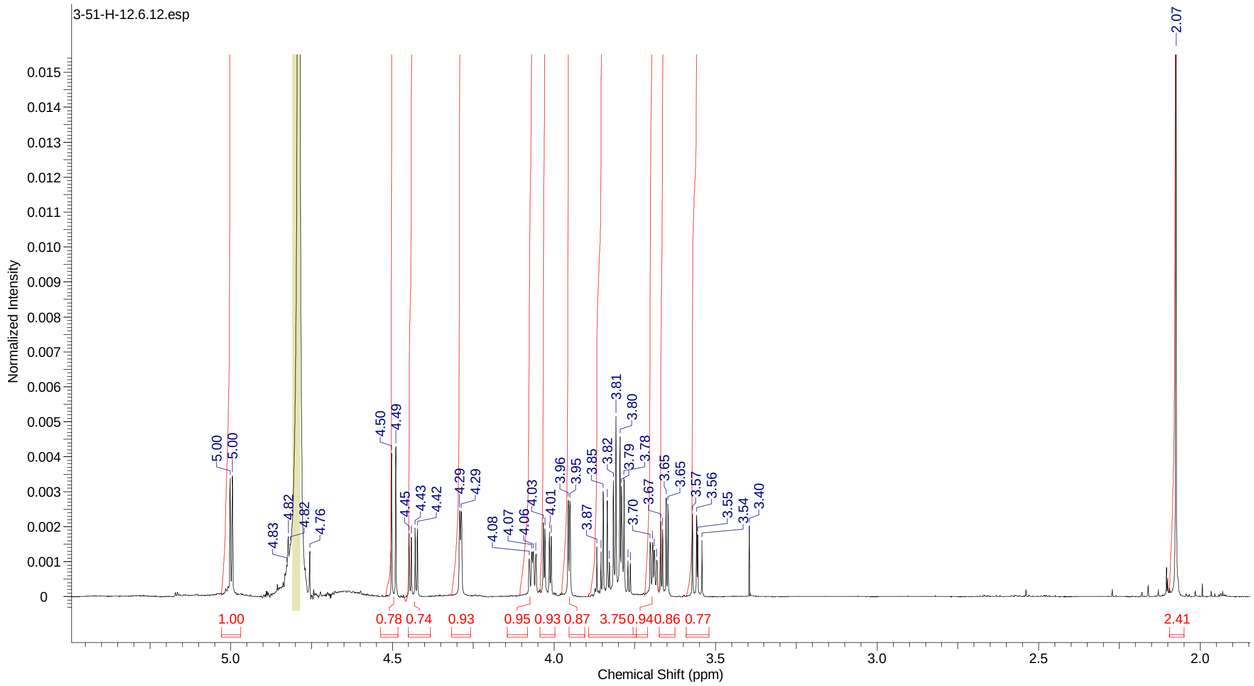

AcO
OAc
AcO
AcO
O
O
HO
OH
O
AcHN
O
N
O
O

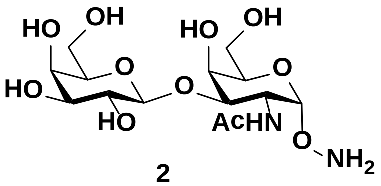
14


JPB2-122-C-CDCL3-4
Normalized Intensity
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
171.35
170.59
170.50
170.21
170.15
169.46
172.0
171.5
171.0
170.5
170.0
169.5
169.0
168.5
Chemical Shift (ppm)

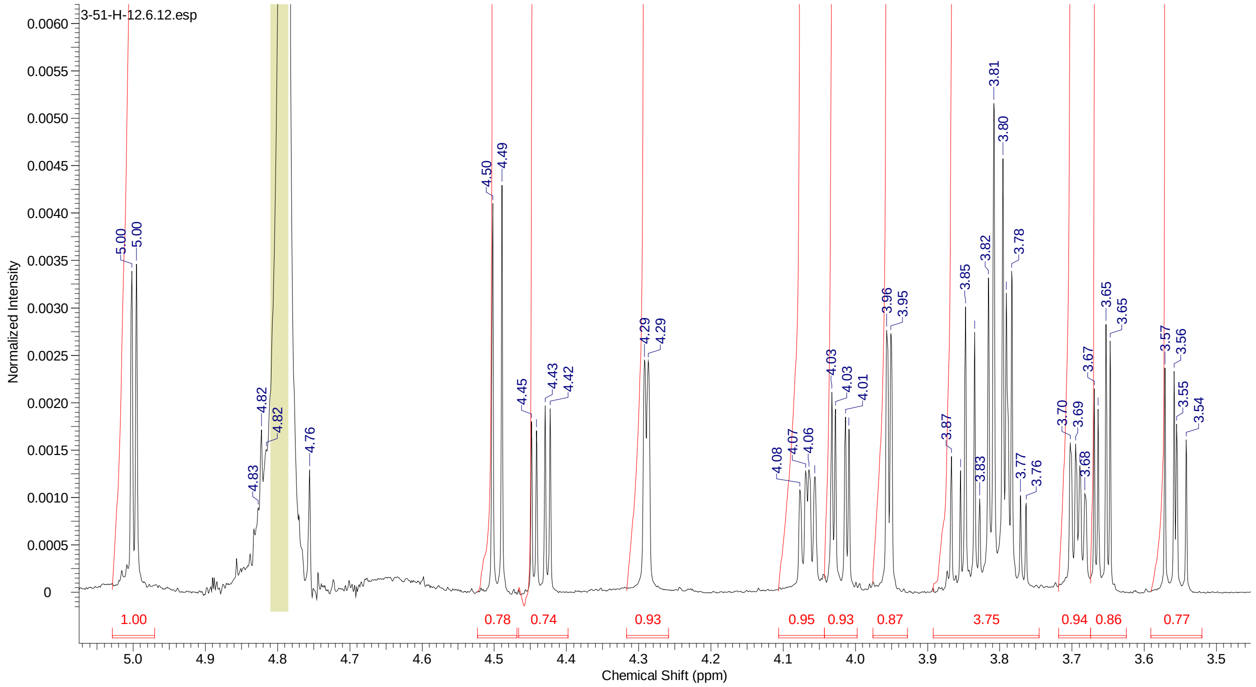

HO
OH
O
HO
HO
O
HO
OH
O
AcHN
O
NH2

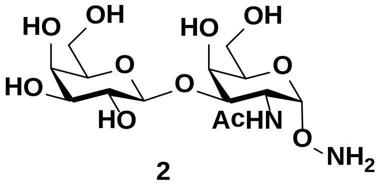
2


3-51-H-12.6.12.esp
Normalized Intensity
0.015
0.014
0.013
0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
5.00
5.00
4.83
4.82
4.82
4.76
4.50
4.49
4.45
4.43
4.42
4.29
4.29
4.08
4.07
4.06
4.03
4.01
3.96
3.95
3.87
3.85
3.82
3.81
3.80
3.79
3.78
3.70
3.67
3.65
3.65
3.57
3.56
3.55
3.54
3.40
2.07
1.00
0.78
0.74
0.93
0.95
0.93
0.87
3.75
0.94
0.86
0.77
2.41
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)

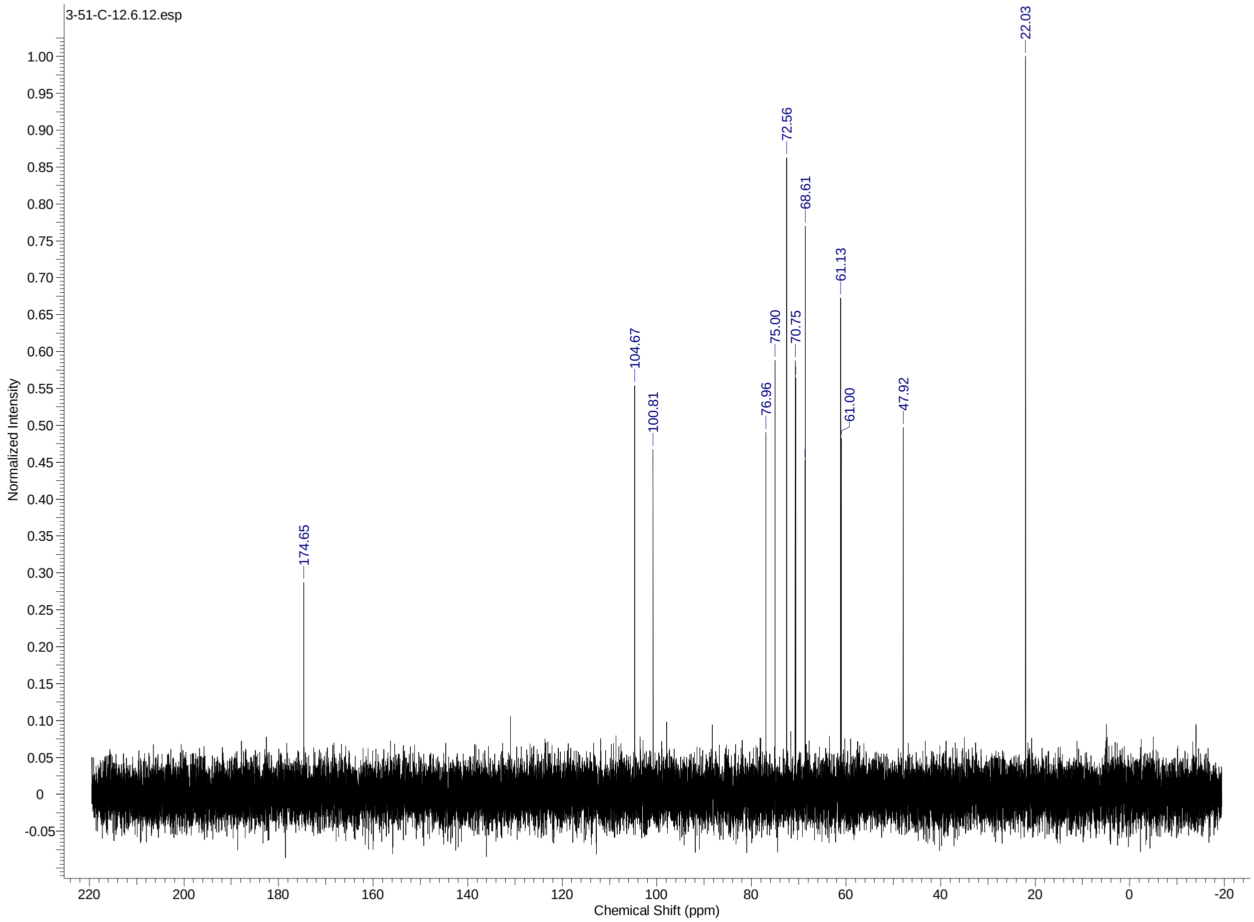

HO
OH
O
HO
HO
O
HO
OH
O
AcHN
O
NH2

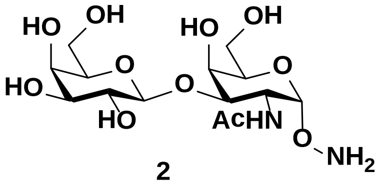
2


3-51-H-12.6.12.esp
Normalized Intensity
0.0060
0.0055
0.0050
0.0045
0.0040
0.0035
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0
5.00
5.00
4.83
4.82
4.82
4.76
4.50
4.49
4.45
4.43
4.42
4.29
4.29
4.08
4.07
4.06
4.03
4.03
4.01
3.96
3.95
3.87
3.85
3.83
3.82
3.81
3.80
3.78
3.77
3.76
3.70
3.69
3.68
3.67
3.65
3.65
3.57
3.56
3.55
3.54
1.00
0.78
0.74
0.93
0.95
0.93
0.87
3.75
0.94
0.86
0.77
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
Chemical Shift (ppm)

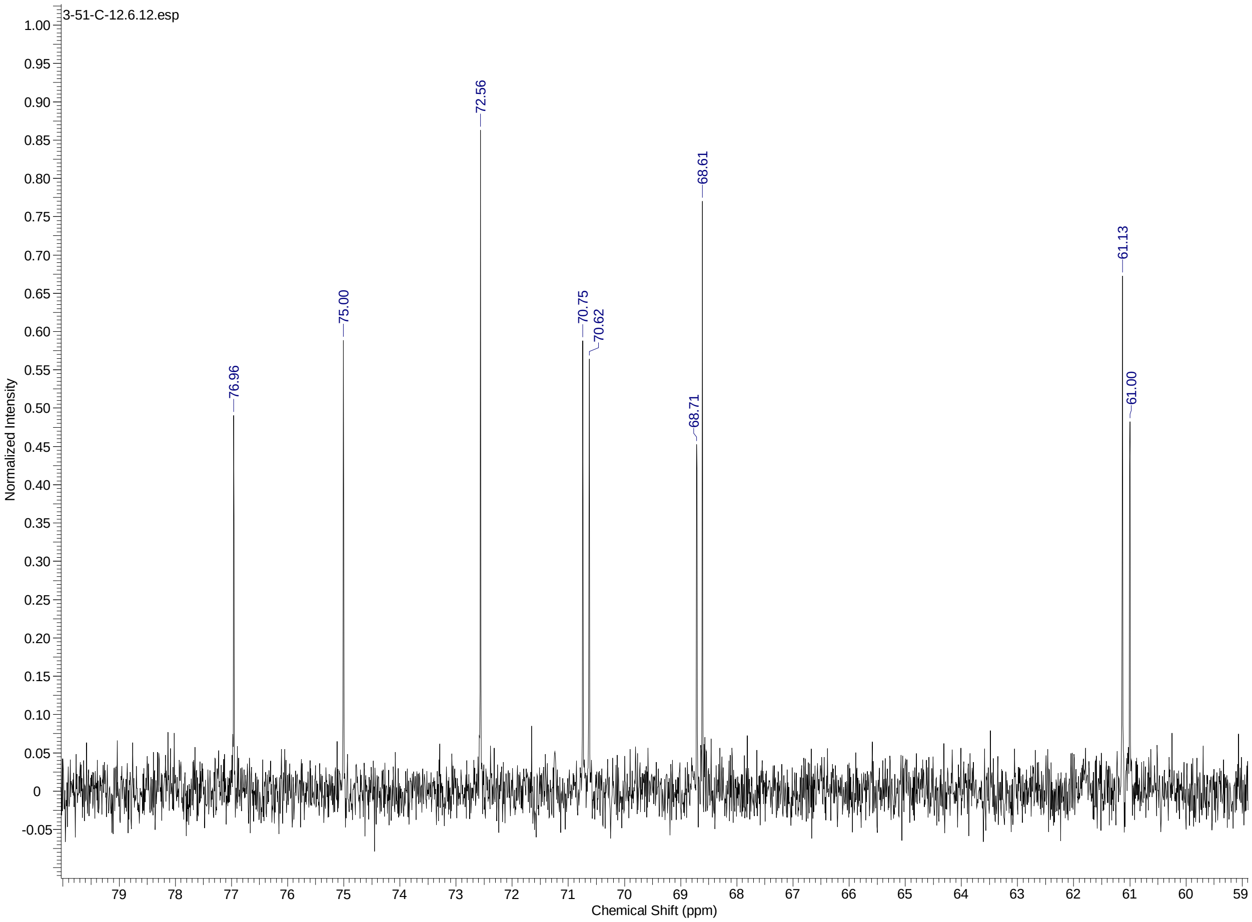

HO
OH
HO
OH
O
O
HO
O
O
HO
AcHN
O
NH2

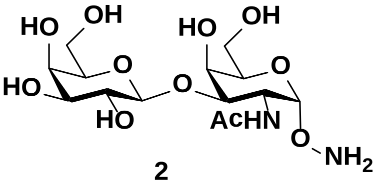
2


3-51-C-12.6.12.esp
22.03
72.56
68.61
61.13
75.00
70.75
104.67
76.96
100.81
61.00
47.92
174.65
Normalized Intensity
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

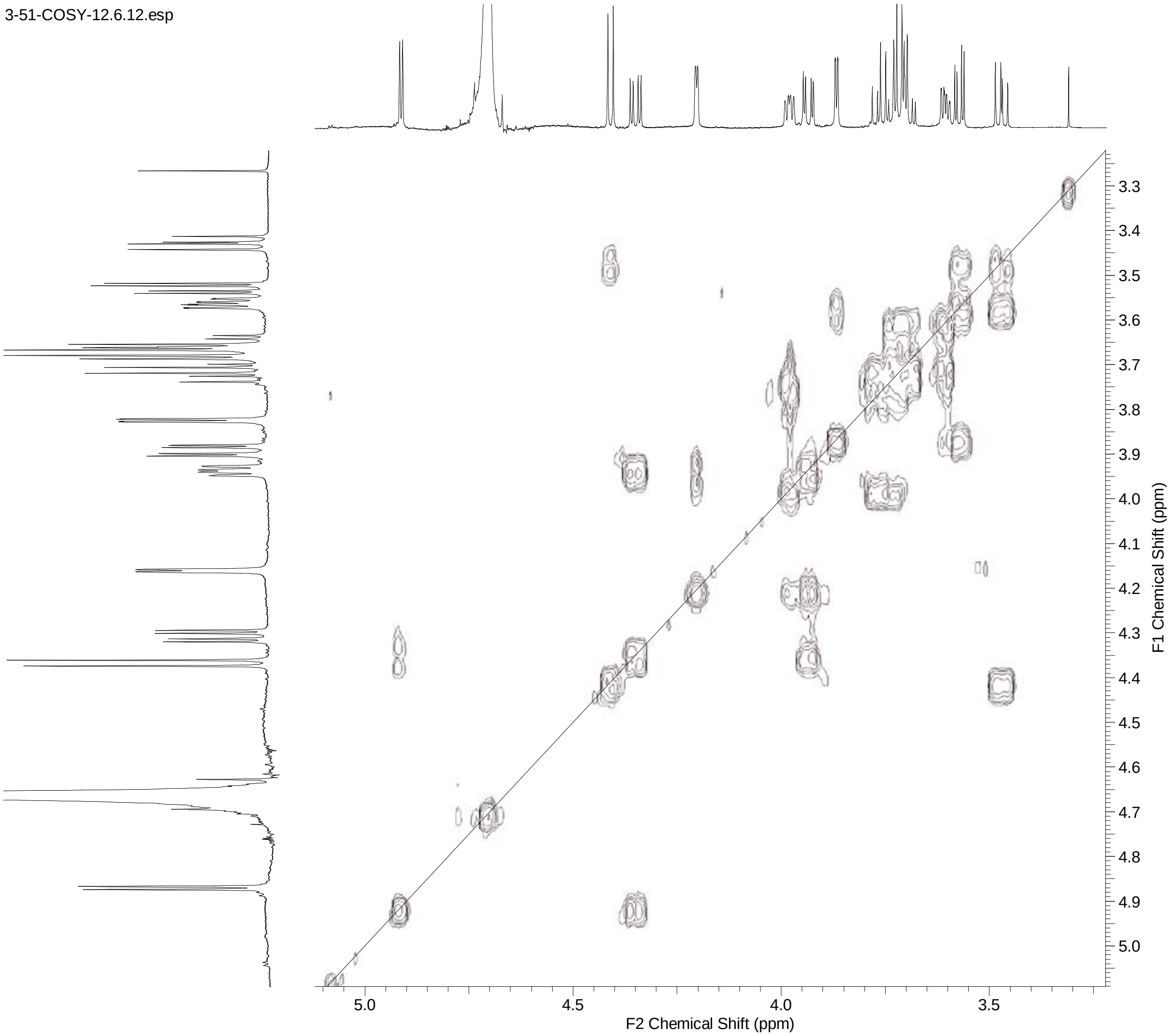

HO
OH
HO
OH
O
HO
O
O
HO
AcHN
O
NH2

2

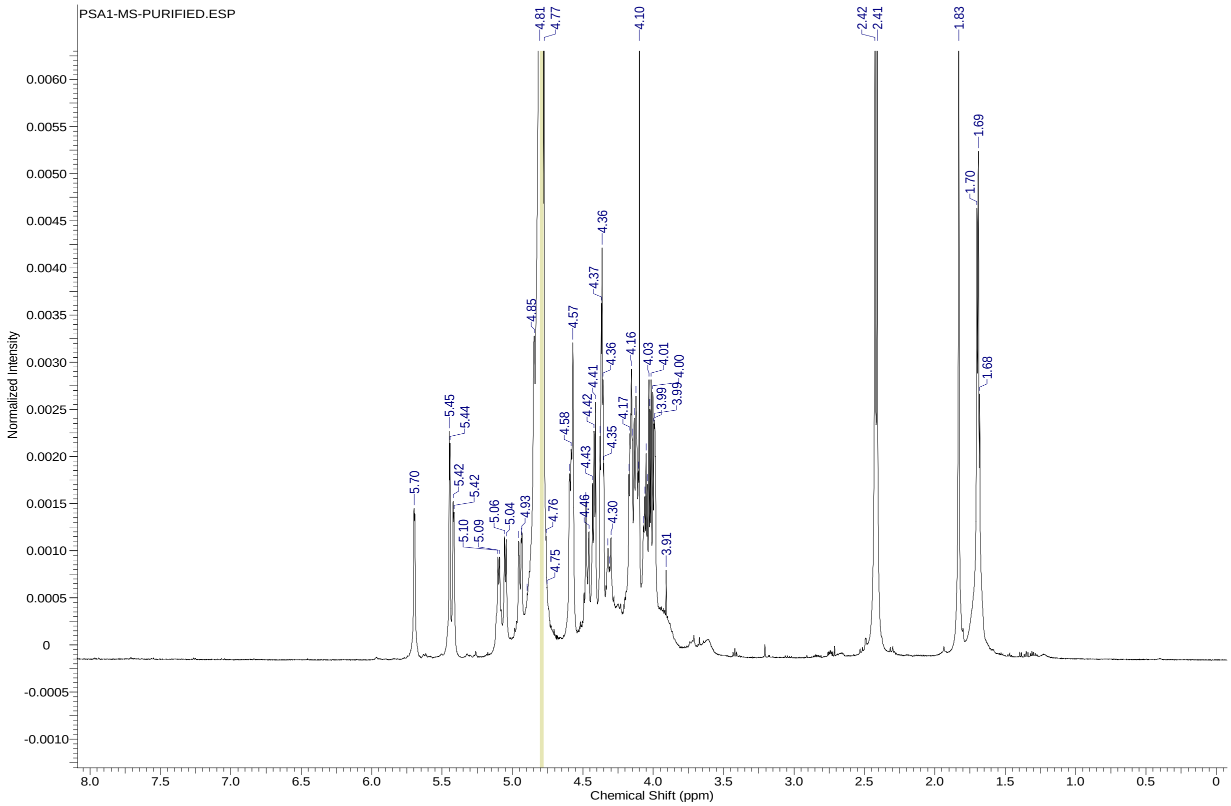

3-51-C-12.6.12.esp
Normalized Intensity
Chemical Shift (ppm)
76.96
75.00
72.56
70.75
70.62
68.71
68.61
61.13
61.00
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
-0.05
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59

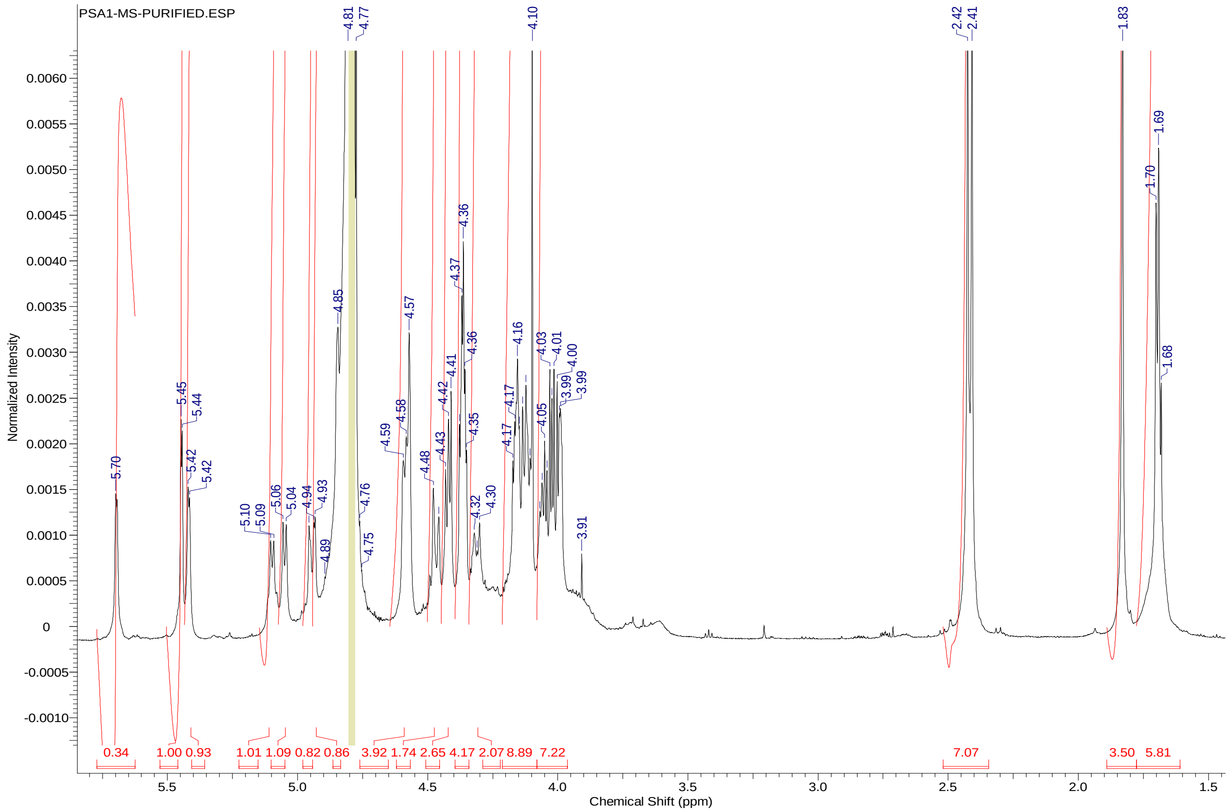

HO
OH
HO
OH
O
O
HO
O
HO
AcHN
O
NH2

2

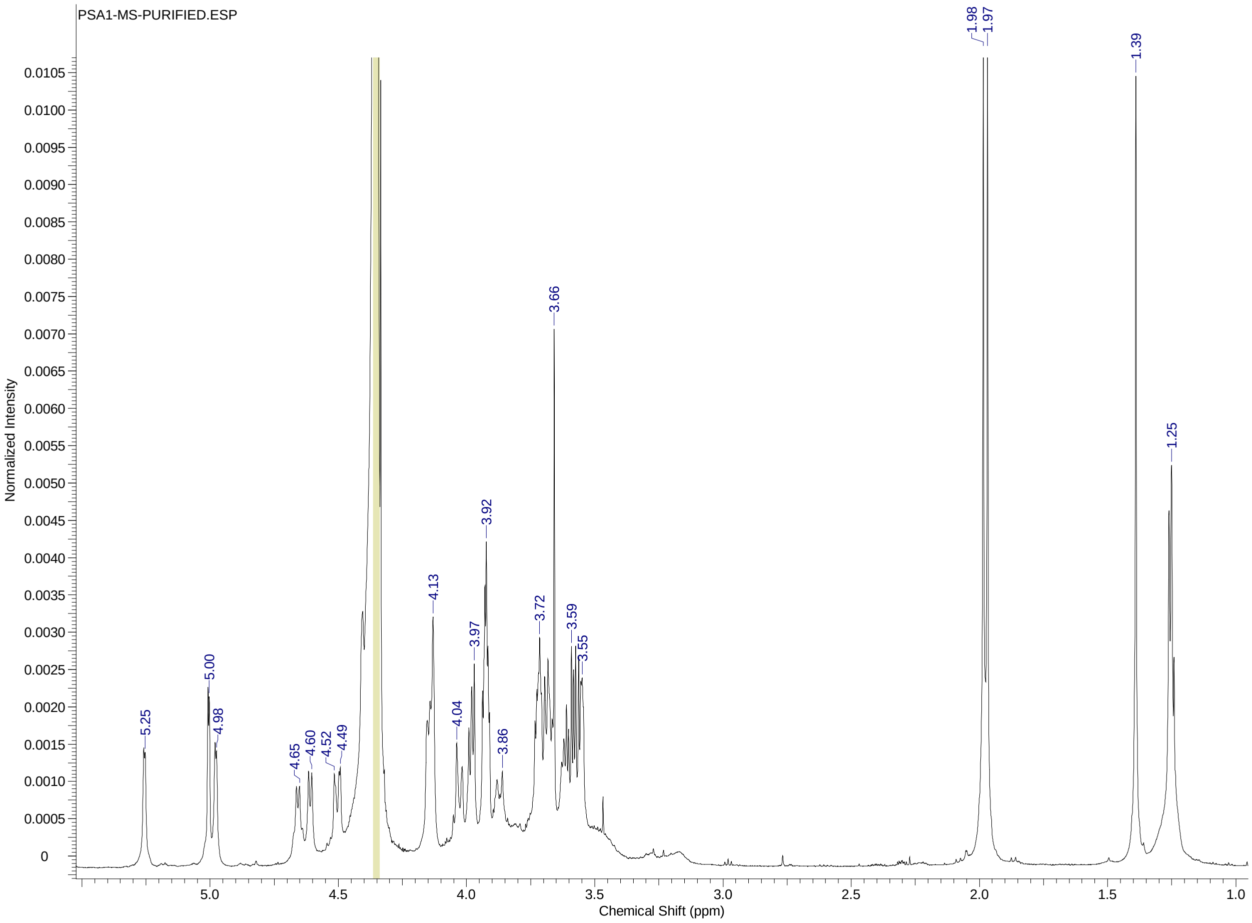

3-51-COSY-12.6.12.esp
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
F1 Chemical Shift (ppm)
5.0
4.5
4.0
3.5
F2 Chemical Shift (ppm)

PS A1 ^{1}H NMR at 60 ^{o}C

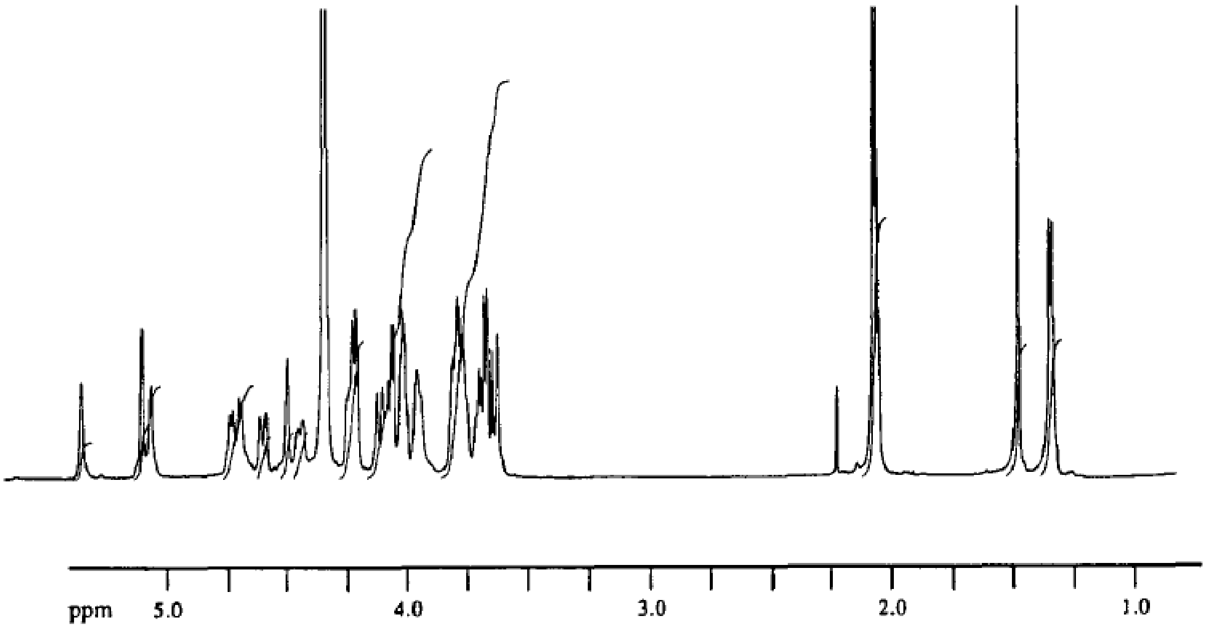




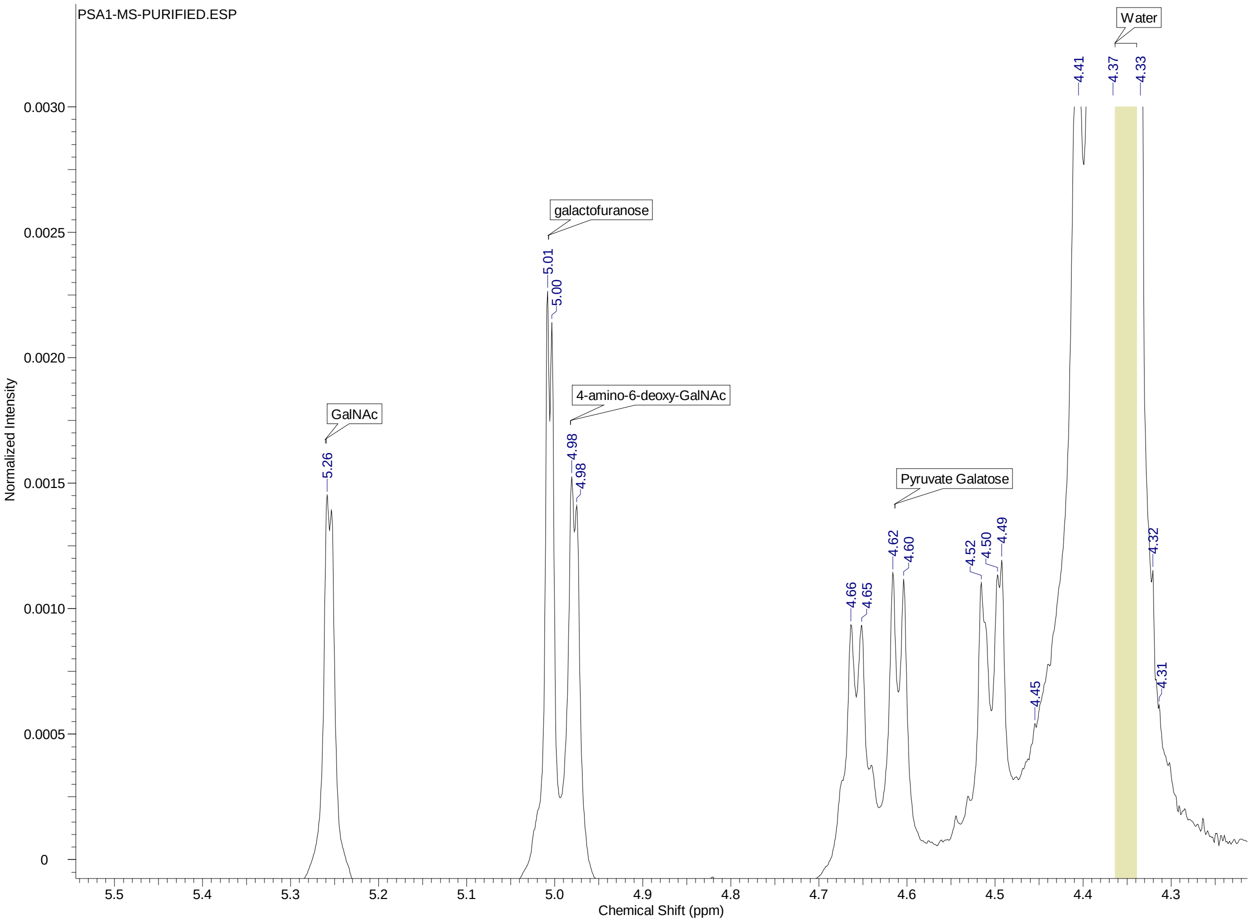
Expansion of isolated PS A1 (**15**), HDO referenced to 4.35 for comparison with literature data see S42 for previously published data



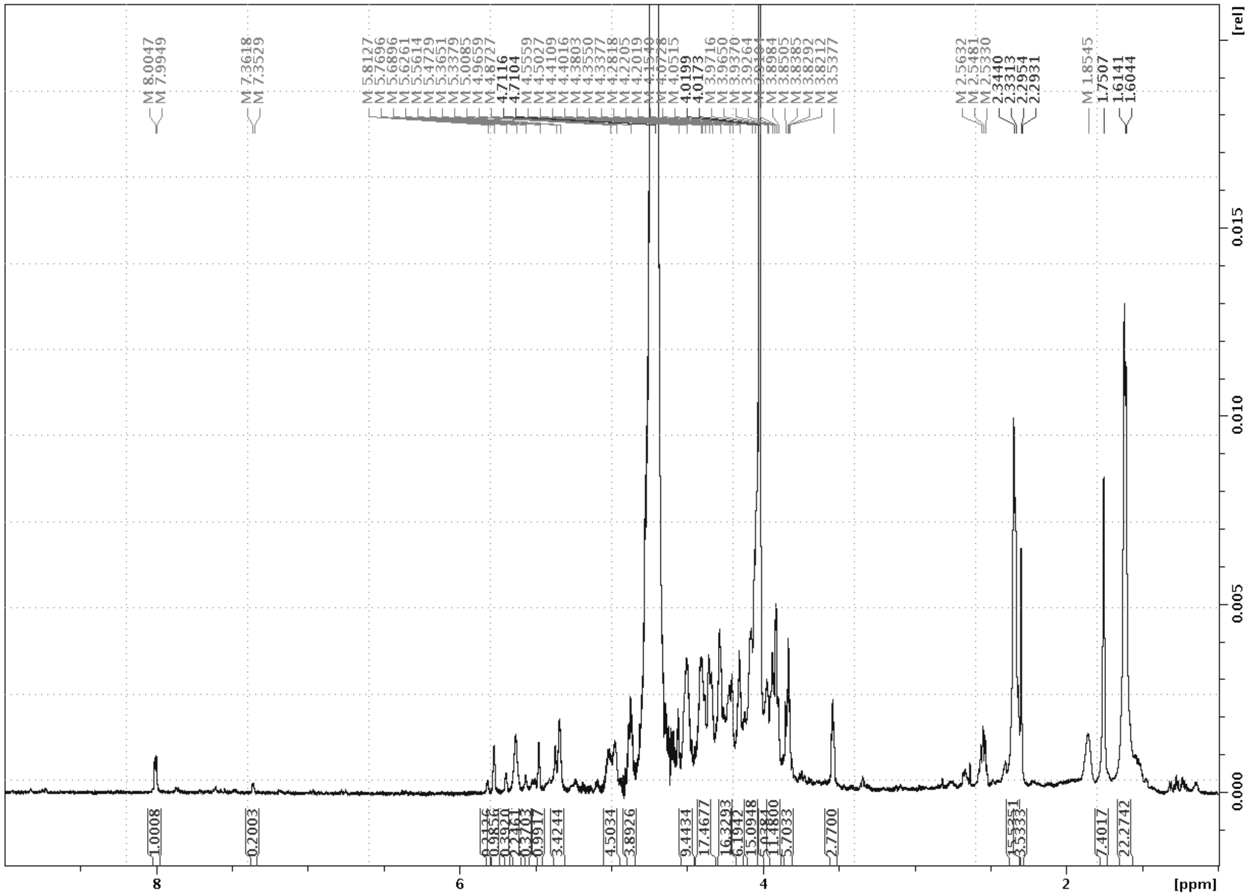
^{1}H NMR of PS A1 (**15**) from, Tzianabos, A. O.; Pantosti, A.; Baumann, H.; Brisson, J. R.; Jennings, H. J.; Kasper, D. L., *J. Biol. Chem.* **1992,** *267,* 18230-18235.



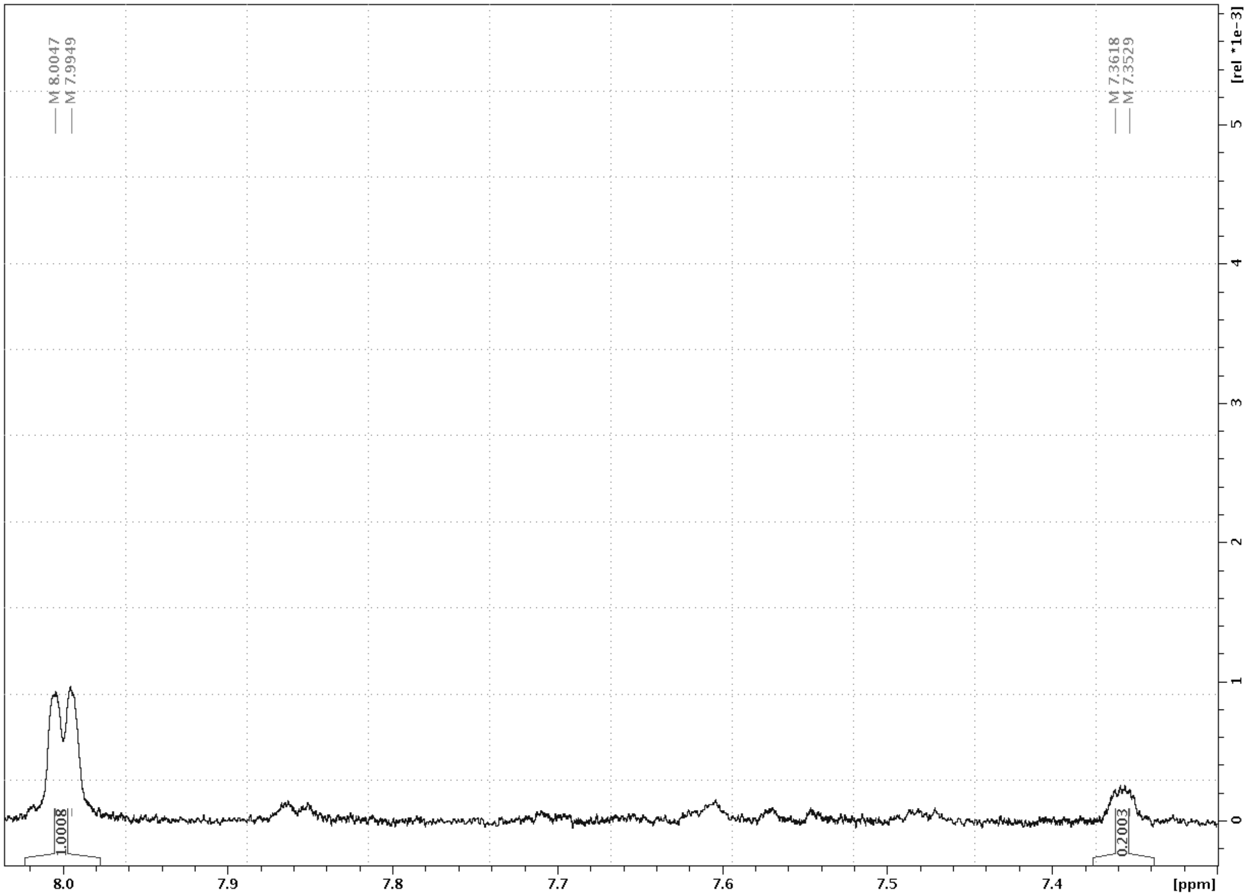
^{1}H NMR expansion 4.3-5.5 ppm of PS A1 (**15**) at 60 ^{o}C



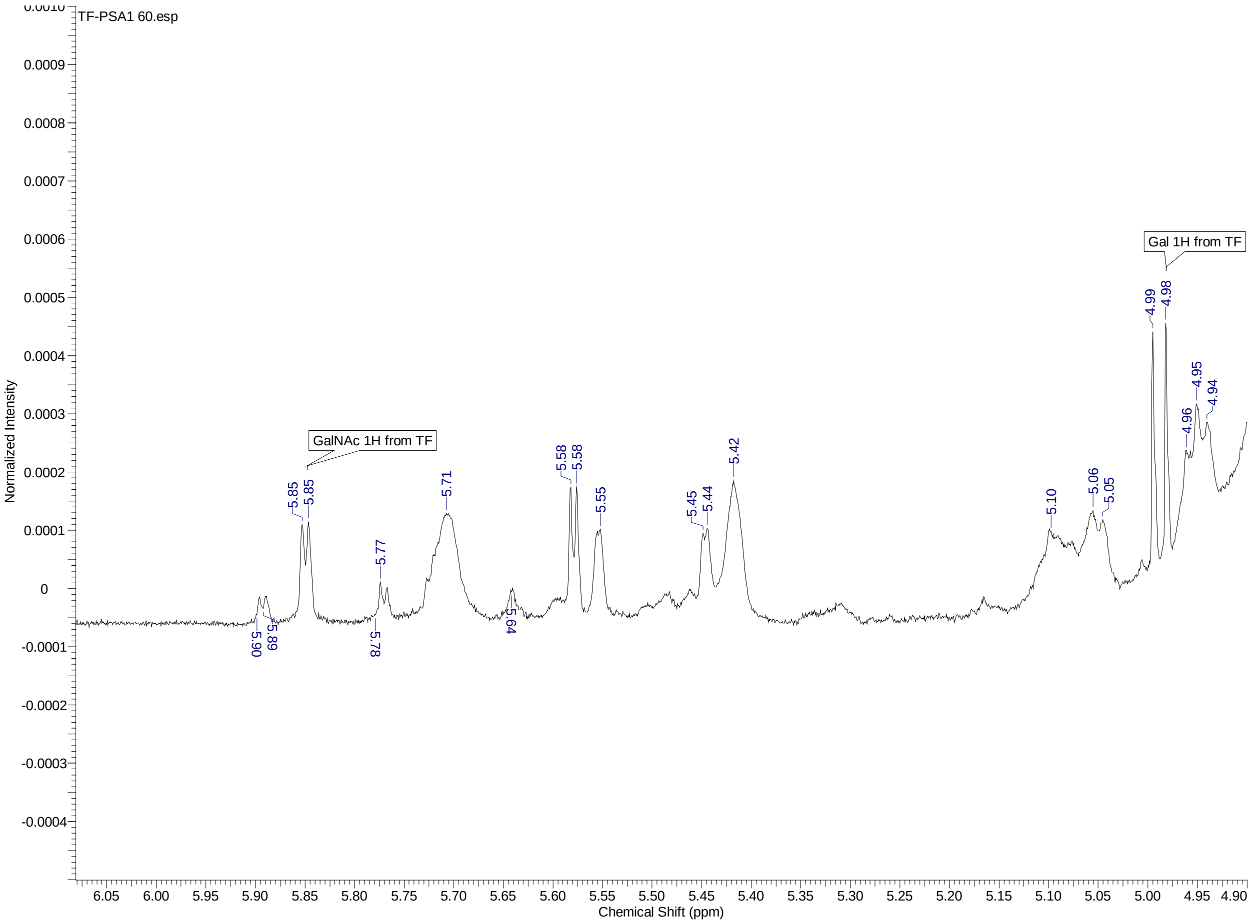
^{1}H NMR of TF-PS A1 (**16**) at room temperature



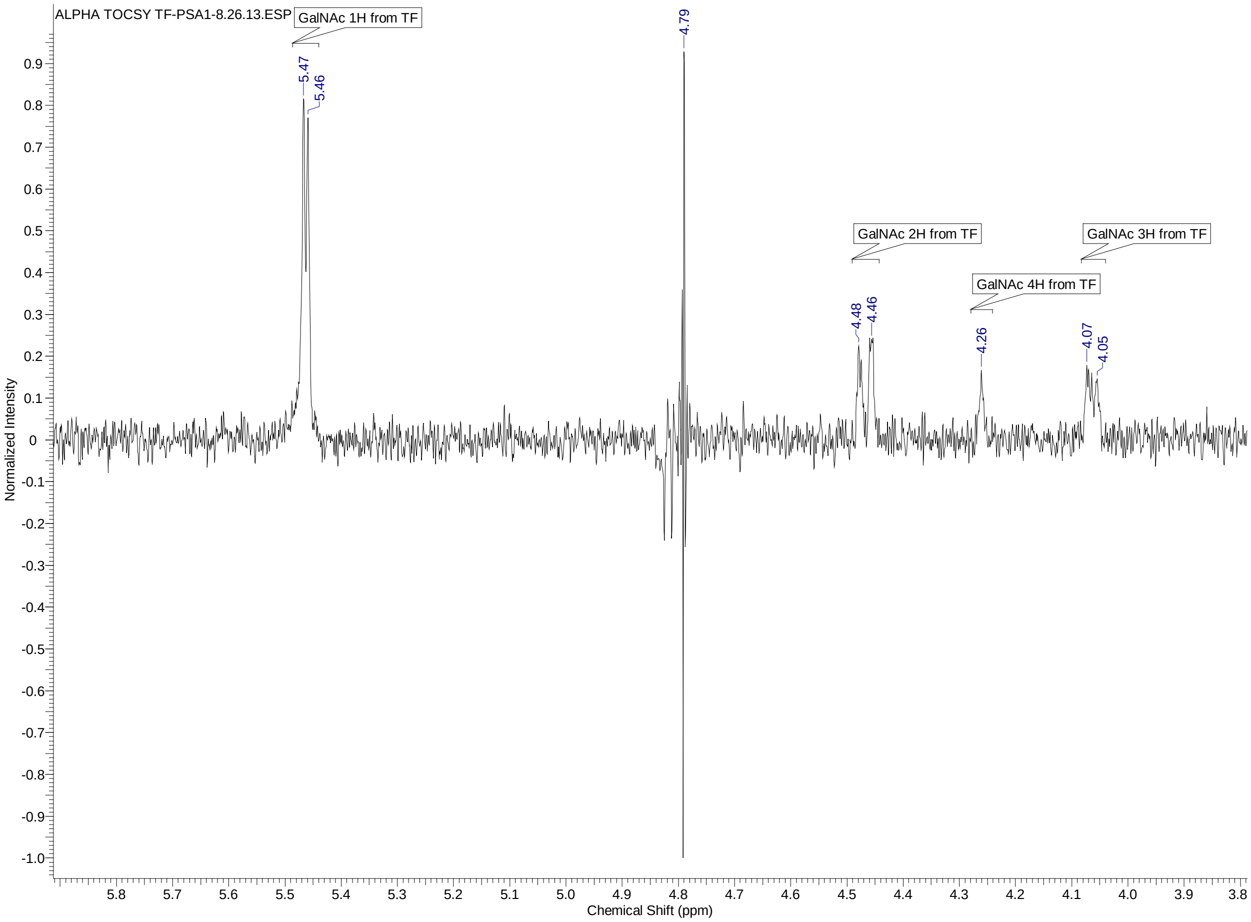
Expansion of TF-PS A1 ^{1}H NMR



Expansion of TF-PS A1 ^{1}H NMR at 60 ^{o}C



1D TOCSY TF-PSA1 (**16**) of doublet at 5.46, at r.t. with 120 ms mixing time



1D TOCSY TF-PSA1 (**16**) of doublet at 4.61, at r.t. with 120 ms mixing time