Bodipy-VAD-Fmk, a useful tool to study Yeast Peptide *N*-Glycanase activity

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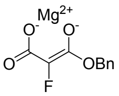
Abbreviations

CDI	1,1'-carbodiimidazole
DIC	*N*,*N*'-diisopropylcarbodiimide
DiPEA	*N*,*N*'-diisopropylethylamine
HCTU	2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HMPB	4-hydroxymethyl-3-methoxyphenoxybutyric acid
MBHA	4-methylbenzhydrylamine
TBS-Cl	tert-butyldimethylsilyl chloride
TF_2O	trifluoromethanesulfonic anhydride
TTBP	tert-butylpyrimidine

General Procedures:

All reagents were commercial grade and were used as received unless stated otherwise. Z-VAD(OMe)-Fmk was purchased from Biomol, international LP. Diethyl ether (Et_2O), ethyl acetate (EtOAc), light petroleum ether (PE) and toluene (Tol) were purchased from Riedel-de Haën. Acetonitrile (MeCN), dichloroethane, dichloromethane (CH_2Cl_2), dimethylformamide (DMF), methanol (MeOH), *N*-methylpyrrolidone (NMP), pyridine (pyr), tetrahydrofuran (THF) were obtained from Biosolve. THF was distilled over $LiAlH_4$ before use. Dichloromethane was boiled under reflux over CaH_2 for 2h and distilled prior to use. *n*-Butanol (*n*-BuOH) was refluxed over sodium for 2h, distilled and stored over 4Å MS. Trifluoromethanesulfonic anhydride (Tf_2O) was distilled from P_2O_5. Molecular sieves 4Å were flame dried before use. All reactions were performed under an inert atmosphere of Argon unless stated otherwise. Solvents used for flash chromatography were of pro analysi quality. Flash chromatography was performed on Screening Devices silica gel 60 (0.04 – 0.063 mm). TLC-analysis was conducted on DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254 nm) were applicable and by spraying with 20% sulphuric acid in ethanol followed by charring at ~150°C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/l) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10g/l) in 10% sulfuric acid in water followed by charring at ~150°C. ^1H and ^{13}C NMR spectra were recorded on a Brüker DMX-400 (400/100 MHz), a Brüker AV-400 (400/100 MHz), Brüker AV-500 (500/125 MHz) and a Brüker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to the chloroform residual solvent peak or tetramethylsilane as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded with a LTQ Orbitrap (Thermo Finnigan). LC/MS analysis was performed on a Jasco HPLC-system (detection simultaneously at 214 nm and 254 nm) equipped with an analytical Alltima C_{18} column (Alltech, 4.6 mmD × 250 mmL, 5μ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a Perkin Elmer Sciex API 165 mass instrument. For RP-HPLC purifications a BioCAD "Vision" automated HPLC system (PerSeptive Biosystems, inc.) equipped with a semi-preparative Alltima C_{18} column was used. The applied buffers were A: H_2O, B: MeCN and C: 1.0 % aq. TFA. Optical rotations were measured on a Propol automatic polarimeter (sodium D line, λ = 589 nm). FT-IR-spectra were recorded on a Paragon-PE 1000.

Magnesium enolate of monobenzyl-fluoromalonate (31)

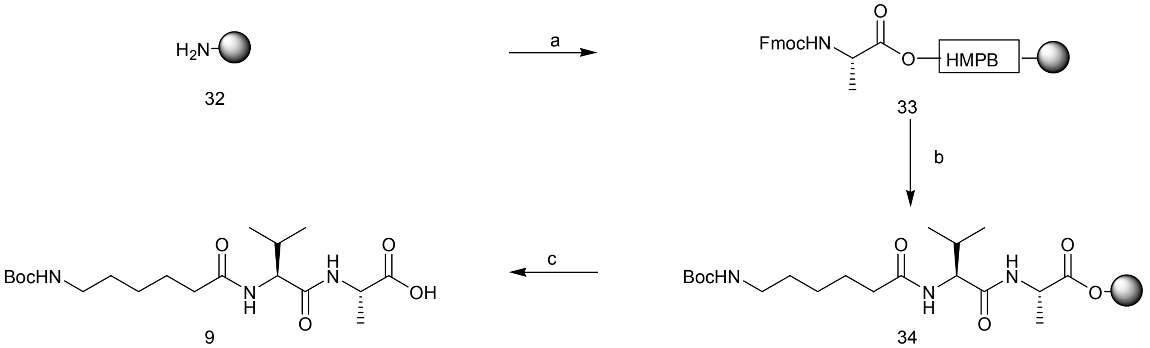


Monobenzyl-fluoromalonate (1.33 g, 6.25 mmol)[1] was dissolved in THF (2 mL/mmol) and cooled to 0°C before isopropylmagnesium chloride (2M in THF, 6.25 mL, 2 equiv.) was added. The white suspension was stirred for 1h and subsequently used for the next reaction.

Synthesis of peptide 9:

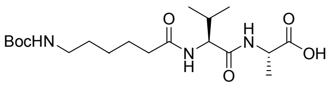
Peptide **9** was synthesized employing standard solid phase peptide synthesis. MBHA resin **32** was functionalized with a HMPB-linker before being loaded with Fmoc-Ala-OH. The resulting resin **33** was elongated furnishing resin-bound peptide **34**. Cleavage from the resin gave peptide **9** (Scheme 1).

Scheme 1.



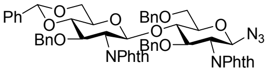
Reagents and conditions: (a) i) HMPB, HCTU, DiPEA, 3h; ii) Fmoc-Ala-OH, DIC, DMAP, CH_2Cl_2, 2h; (b) deprotection: piperidine/NMP (1/4, v/v); condensation: Fmoc-Val-OH or Boc-Ahx-OH, HCTU, DiPEA, NMP; (c) TFA/CH_2Cl_2 (1/99, v/v);

Boc-Ahx-Val-Ala-OH (9)



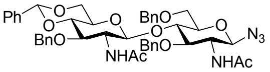
MBHA resin **32** (0.555 g, 0.46 mmol, 0.9 mmol/g) was solvated with NMP, before being reacted with HMPB (0.361 g, 1.5 mmol, 3 equiv.) in the presence of HCTU (0.62 g, 1.5 mmol, 3 equiv.) and diisproylethylamine (0.532 mL, 3 mmol, 6 equiv.). The resin was shaken for 3h, after which it was filtered and washed with NMP (3× 5 mL) and CH_2Cl_2 (3× 5 mL). Next, the resin was coevaporated twice with dichloroethane and condensed with Fmoc-Ala-OH (429 mg, 1.38 mmol, 3 equiv.) under the agency of diisppropylcarbodiimide (0.236 mL, 1.52 mmol, 3.3 equiv.) and DMAP (3 mg, 0.023 mmol, 0.05 equiv.) in CH_2Cl_2 for 2h. The resin was filtered, washed with CH_2Cl_2 (3× 5 mL) and subjected to a second condensation sequence. The obtained resin **33** was elongated by two cycles of Fmoc-solid phase synthesis. The consecutive steps of the cycles are as follows: (i) deprotection: piperidine in NMP (1/4, v/v, 15 min), (ii) wash with NMP (3× 5mL), (iii) condensation: Fmoc-Val-OH (1.82 mmol, 4 equiv.) or Boc-Ahx-OH (1.82 mmol, 4 equiv.) was dissolved in NMP (7 mL). HCTU (0.753 g, 1.82 mmol, 4 equiv.) and diisopropylethylamine (0.643 mL, 3.64 mmol, 8 equiv.) were added. The resulting mixture was transferred to the reaction vessel and shaken for 90 min. (iv) Wash with NMP (3× 5 mL) and CH_2Cl_2 (3× 5 mL). Peptide **34** was liberated from the resin by treatment with TFA/CH_2Cl_2 (1/99, v/v, 4× 2 min). Subsequent addition of toluene followed by concentration *in vacuo* furnished crude peptide **9** (quant, 0.185 g, 0.46 mmol) which was directly used for the condensation with **8**. LC/MS: R_t 5.32 min; linear gradient 10→90% B in 13.5 min; ESI/MS: m/z = 402.2 $(M+H)^+$, 302.2 $(M-Boc+H)^+$.

***O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (13)**[2]



Known donor **11** (1.36 g, 2.3 mmol, 1.1 equiv.), diphenylsulfoxide (0.512 g, 2.53 mmol, 1.3 equiv.) and TTBP (1.43 g, 5.75 mmol, 2.7 equiv.) were coevaporated thrice with toluene and dissolved in anhydrous CH_2Cl_2 (25 mL). Activated 4Å MS were added and the solution was stirred for 30 min before being cooled to -60°C. Tf_2O (0.406 mL, 2.415 mmol, 1.15 equiv.) was added. After 15 min stirring at -60°C, acceptor **12** (1.095 g, 2.13 mmol, 1 equiv.) was added in CH_2Cl_2 (5 mL). The temperature was raised to 0°C over 4h, after which the reaction was quenched with Et_3N, diluted with EtOAc, washed with $NaHCO_3$ (sat. aq.), brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (Tol→7.5% EtOAc/Tol) furnishing title compound **13** in 85% (1.79 g, 1.82 mmol) as a colorless oil. ^1H NMR (600 MHz, $CDCl_3$) δ ppm 7.92-7.48 (m, 9H, H Arom), 7.45-7.40 (m, 2H, H Arom), 7.34-7.18 (m, 9H, H Arom), 7.00-6.79 (m, 11H, H Arom), 5.44 (s, 1H, CHPh), 5.32 (d, J = 8.4 Hz, 1H, H-1'), 5.10 (d, J = 9.4 Hz, 1H, H-1), 4.74 (d, J = 12.4 Hz, 1H, CH_2 Bn), 4.71 (d, J = 12.3 Hz, 1H, CH_2 Bn), 4.45-4.34 (m, 5H, 4× CH_2 Bn,), 4.19-4.10 (m, 4H), 3.97 (t, J = 9.7, 9.7 Hz, 1H, H-2), 3.64 (t, J = 9.1, 9.1 Hz, 1H), 3.49-3.43 (m, 2H), 3.36-3.27 (m, 3H). ^{13}C NMR (150MHz, $CDCl_3$) δ ppm 168.08 (C=O phth), 168.06 (C=O phth), 167.50 (C=O phth), 167.46 (C=O phth), 138.28 (C_q Bn or Ph), 137.97 (C_q Bn or Ph), 137.83 (C_q Bn or Ph), 137.28 (C_q Bn or Ph), 134.03 (CH phth), 134.01 (CH phth), 133.99 (CH phth), 133.86 (CH phth), 131.43 (C_q phth), 128.96-125.95 (CH Arom), 123.32 (CH phth), 123.30 (CH phth), 123.27 (CH phth), 123.24 (CH phth), 101.18 (CHPh), 97.65 (C-1'), 85.46 (C-1), 83.10, 76.68, 76.50, 75.67, 74.45 (CH_2 Bn), 74.42, 74.08 (CH_2 Bn), 72.76 (CH_2 Bn), 68.65 (C-6 or C-6'), 67.50 (C-6 or C-6'), 65.74, 56.46 (C-2'), 55.08 (C-2). FT-IR: ν_{max}(neat)/cm^{-1} 2870.0, 2114.6, 1992.1, 1776.3, 1710.2, 1615.4, 1496.6, 1468.8, 1454.6, 1385.6, 1310.8, 1254.2, 1197.2, 1173.5, 1145.5, 1067.9, 1027.5, 996.3, 969.0, 874.1, 793.9, 738.8, 718.7, 696.0, 661.3. $[\alpha]_D^{23}$ + 17° (c = 0.43, $CHCl_3$). HRMS: ($M+Na^+$) calcd for $C_{56}H_{49}N_5O_{12}Na$ 1006.32699, found 1006.32767.

***O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (14)**

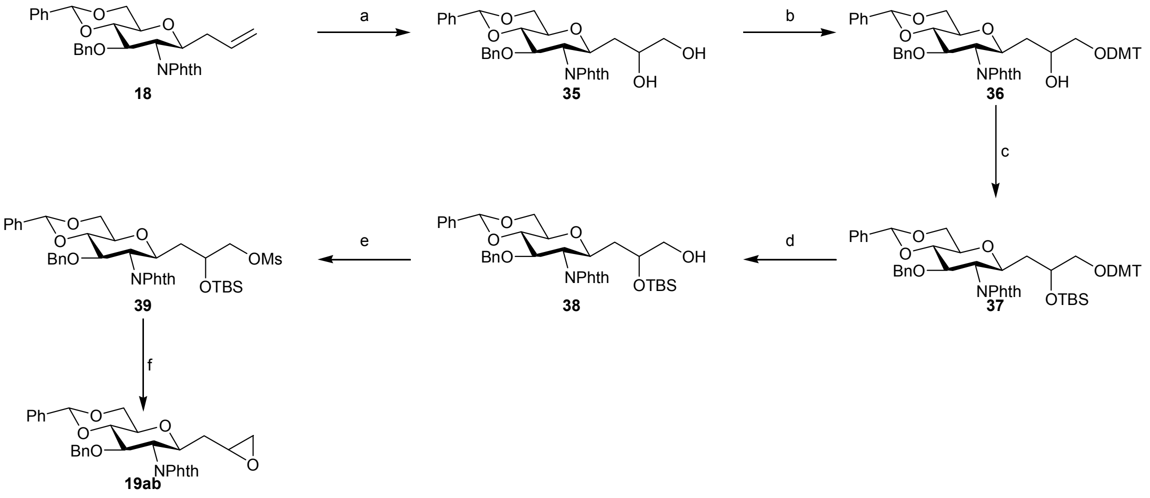


Disaccharide **13** (1.79 g, 1.82 mmol) was dissolved in n-BuOH/ethylenediamine (10/1 v/v, 40 mL) followed by stirring overnight at 90°C. The reaction mixture was concentrated *in vacuo*, coevaporated with toluene, redissolved in pyridine (10 mL) and cooled to 0°C. Subsequently, acetic anhydride (2 mL) was added. After 5h stirring, the solution was concentrated, redissolved in CH_2Cl_2, extracted with 1M HCl, dried (Na_2SO_4) and concentrated. Purification over silica gel column chromatography (CH_2Cl_2→2% MeOH/CH_2Cl_2) gave title compound **14** in 81% (1.19 g, 1.48 mmol) as a white solid. ^{1}H NMR (600 MHz, DMSO) δ ppm 8.10 (d, *J* = 8.5 Hz, 1H, H Arom), 8.07 (d, *J* = 9.2 Hz, 1H, H Arom), 7.43-7.25 (m, 20H, H Arom), 5.67 (s, 1H, CHPh), 4.82 (d, *J* = 11.0 Hz, 1H, CH_2 Bn), 4.75-4.70 (m, 2H, CH_2 Bn, H-1'), 4.67-4.51 (m, 5H), 4.03 (dd, *J* = 10.0, 4.6 Hz, 1H), 3.86-3.78 (m, 2H), 3.76-3.66 (m, 5H), 3.63 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.60-3.52 (m, 2H), 3.18-3.12 (m, 2H), 1.84 (s, 3H, CH_3 NHAc), 1.83 (s, 3H, CH_3 NHAc). ^{13}C NMR (150MHz, DMSO) δ ppm 169.28 (C=O NHAc), 169.19 (C=O NHAc), 138.83 (C_q Bn or Ph), 138.63 (C_q Bn or Ph), 138.43 (C_q Bn or Ph), 137.47 (C_q Bn or Ph), 128.66-125.87 (CH Arom), 100.76 (C-1'), 99.92 (CHPh), 87.78, 80.79, 80.07, 78.37, 76.17, 75.43, 73.26 (CH_2 Bn), 73.15 (CH_2 Bn), 71.87 (CH_2 Bn), 68.14 (C-6 or C-6'), 67.64 (C-6 or C-6'), 65.49, 55.23 (C-2 or C-2'), 53.50(C-2 or C-2'), 22.87 (CH_3 NHAc), 22.70 (CH_3 NHAc). FT-IR: ν_{max}(neat)/cm^{-1} 3273.7, 2874.0, 2118.5, 1717.7, 1655.0, 1545.8, 1497.9, 1453.7, 1370.5, 1323.4, 1255.2, 1173.6, 1143.8, 1071.2, 1027.6, 1015.1, 960.5, 917.4, 747.4, 694.2. $[\alpha]_D^{23}$ - 15° (c = 0.25, $CHCl_3$). HRMS: (M+H$^+$) calcd for $C_{44}H_{50}N_5O_{10}$ 808.35522, found 808.35582.

Synthesis of diastereomerically pure 19a and 19b:

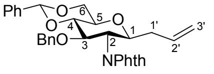
Epoxides **19ab** were synthesized from protected **18**. For analytic purposes diastereomerically pure epoxides **19a** and **19b** were synthesized. Epoxides **19a** and **19b** were synthesized as follows. Alkene **18** was dihydroxylated. The resulting mixture of diols **35a** and **35b** was separated by silica gel chromatography. Selective protection of the primary alcohol with 4,4'-dimethoxytrityl chloride followed by protection the of remaining secondary alcohol using TBS-Cl and imidazole gave protected **37a** and **37b**. Deprotection of the primary alcohol by treatment with dichloroacetic acid followed by mesylation furnished **39a** and **39b**. The resulting silyl mesylate was converted to diastereomerically pure epoxides **19a** and **19b** under the agency of TBAF in THF.

Scheme 2.



Reagents and conditions: (a) K_2OsO_4, NMO, THF/H_2O (6/1, v/v), 16h; (b) DMTrCl, Et_3N, CH_2Cl_2, 3h, **a**: 97%, **b**: 94%; (c) TBS-Cl, Et_3N, imidazole, DMF, 16h, **a**: 90%, **b**: 85%; (d) 2% dichloroacetic acid in CH_2Cl_2, TES, 1h, **a**: 73%, **b**: 82%; (e) MsCl, Et_3N, DMAP, CH_2Cl_2, 16h, **a**: 89%, **b**: 93%; (f) TBAF (1M in THF), THF, 2h, **a**: 48%, **b**: 67%.

3-*C*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-propene (18)

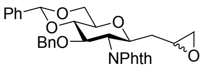


Known acetylated allyl glucosamine **17** (12.6 g, 27.4 mmol) was dissolved in MeOH. Amberlite IR-120 H^+ was added until pH 3. The reaction mixture was refluxed overnight after which TLC-analysis showed complete conversion of the starting material to a lower running spot. Subsequently, the solution was filtered, coevaporated thrice with anhydrous toluene and dissolved in MeCN. Benzaldehyde dimethylacetal (5.06 ml, 33.6 mmol, 1.2 equiv.) and pTsOH (521 mg, 2.74 mmol, 0.1 equiv.) were added. After 4h stirring, the reaction was quenched with Et_3N (5 mL) and concentrated *in vacuo*. Purification by silica gel column chromatography (5% EtOAc/PE→25% EtOAc/PE) gave the benzylidene protected glucosamine (10.57 g, 25 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.88-7.68 (m, 4H, H Arom), 7.51-7.32 (m, 5H, H Arom), 5.74 (tdd, *J* = 17.1, 10.2, 6.9, 6.9 Hz, 1H, H-2'), 5.55 (s, 1H, CHPh), 4.95 (ddd, *J* = 17.2, 3.0, 1.4 Hz, 1H, H-3a'), 4.90 (ddd, *J* = 10.3, 2.9, 1.4 Hz, 1H, H-3b'), 4.62 (dd, *J* = 10.2, 9.1 Hz, 1H, H-3), 4.37-4.31 (m, 2H, H_1, H-6a), 4.14 (t, *J* = 10.2, 10.2 Hz, 1H, H-2), 3.73 (dd, *J* = 10.3, 9.9 Hz, 1H, H-6b), 3.60 (dt, *J* = 9.9, 9.5, 5.1 Hz, 1H, H-5), 3.52 (dd, *J* = 9.5, 9.1 Hz, 1H, H-4), 2.73 (s, 1H, OH), 2.28-2.24 (m, 2H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 168.26 (C=O phth), 168.03 (C=O phth), 137.06 (C_q Ph), 134.13 (CH phth), 134.09 (CH phth), 133.01 (C-2'), 131.59 (C_q phth), 131.43 (C_q phth), 129.21-126.25 (CH Arom), 123.63 (CH phth), 123.24 (CH phth), 117.36 (C-3'), 101.78 (CHPh), 82.62 (C-4), 75.17 (C-1), 70.05 (C-5), 69.04 (C-3), 68.75 (C-6), 56.36 (C-2), 36.79 (C-1'). FT-IR: v_{max}(neat)/cm^{-1} 3311.9, 2865.7, 1768.1, 1709.7, 1662.1, 1651.9, 1472.0, 1456.7, 1440.8, 1385.8, 1359.0, 1336.7, 1251.2, 1220.5, 1122.3, 1090.2, 1056.1, 1040.2, 997.9, 968.3, 915.5, 881.6, 795.5, 770.2, 723.2, 701.8, 679.0, 662.3. $[\alpha]_D^{23}$ +4° (c = 1.00, $CHCl_3$). HRMS: (M+H$^+$) calcd for $C_{24}H_{24}NO_6$ 422.15981, found 422.15865.

Next, the resulting 3-OH (10.96 g, 26 mmol) was protected. Hence, it was coevaporated thrice with dry toluene before being dissolved in DMF (125 mL). Subsequently, benzylbromide (9.3 ml, 78 mmol, 3 equiv.) and TBAI (1.92 g, 5.2 mmol, 0.2 equiv.) were added and the reaction was cooled to 0°C. Sodium hydride, 60% in mineral oil, (1.14 g, 28.6 mmol, 1.1 equiv.) was added portionwise over 2h. TLC analysis showed complete consumption of the starting material after 4h of additional stirring. The reaction mixture was poured into NH_4Cl (sat. aq.), extracted with EtOAc, washed with 1M NaS_2O_3, brine, dried (Na_2SO_4) and concentrated. Crystallization from EtOAc/PE furnished benzyl protected **18** (68%, 9.01 g, 17.6 mmol,). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.84-7.63 (m, 4H, H Arom), 7.55-7.35 (m, 5H, H Arom), 7.00-6.85 (m, 5H, H Arom), 5.71 (dddd, *J* = 17.1, 10.2, 6.9, 6.9 Hz, 1H, H-2'), 5.62 (s, 1H, CHPh), 4.93 (ddd, *J* = 17.2, 3.0, 1.4 Hz, 1H, H-3a'), 4.89 (ddd, *J* = 10.3, 2.7, 1.2 Hz, 1H, H-3b'), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.51 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.45 (dd, *J* = 10.0, 9.0 Hz, 1H, H-3), 4.39 (dd, *J* = 10.4, 4.9 Hz, 1H, H-6a), 4.32 (td, *J* = 10.5, 5.6, 5.6 Hz, 1H, H-1), 4.14 (t, *J* = 10.2, 10.2 Hz, 1H, H-2), 3.78 (t, *J* = 10.3, 10.3

Hz, 1H, H-6b), 3.77 (t, *J* = 9.1, 9.1 Hz, 1H, H-4), 3.65 (dt, *J* = 9.9, 9.8, 4.9 Hz, 1H, H-5), 2.22 (tdd, *J* = 7.0, 5.7, 1.3, 1.3 Hz, 1H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.85 (C=O phth), 167.77 (C=O phth), 138.02 (C_q Bn or Ph), 137.44 (C_q Bn or Ph), 133.89 (CH phth), 133.81 (CH phth), 133.02 (C-2'), 131.55 (C_q phth), 131.48 (C_q phth), 128.91-126.02 (CH Arom), 123.36 (CH phth), 123.27 (CH phth), 117.37 (C-3'), 101.19 (CHPh), 83.62 (C-4), 75.24 (C-1 or C-3), 75.21 (C-1 or C-3), 74.04 (CH_2 Bn), 70.19 (C-5), 68.89 (C-6), 55.61 (C-2), 36.97 (C-1'). FT-IR: v_{max}(neat)/cm^{-1} 2854.5, 1783.6, 1713.3, 1497.9, 1458.1, 1430.4, 1409.9, 1382.1, 1363.7, 1301.9, 1207.8, 1170.9, 1143.2, 1118.9, 1099.0, 1066.7, 1050.5, 1012.1, 1001.7, 964.4, 919.8, 875.9, 857.9, 792.1, 764.0, 752.2, 718.6, 700.5, 693.9, 679.9, 663.9. $[\alpha]_D^{23}$ +73.2° (c = 1.00, $CHCl_3$). HRMS: (M+H$^+$) calcd for $C_{31}H_{30}NO_6$ 512.20676, found 512.20665.

(2R/S)-3-*C*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1,2-epoxypropane (19)



Compound **18** (7.67 g, 15 mmol) was dissolved in dichloromethane (150 mL). After the addition of m-chloroperoxybenzoic acid (8.51 g, 34.5 mmol, 2.3 equiv.) the reaction mixture was refluxed for 4h. Subsequently, the reaction was diluted with EtOAc before being washed with aqueous 1M NaS_2O_3, $NaHCO_3$ (sat. aq.) and brine. The organic layer was dried (Na_2SO_4) and concentrated. Silica gel column chromatography (20% EtOAc/PE→30% EtOAc/PE) gave a 2:3 mixture of diastereomers **19a** and **19b** of epoxide **19** in 88% (6.96 g, 13.2 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.85-7.80 (m, 1H, H Arom), 7.75-7.67 (m, 2H, H Arom), 7.65-7.62 (m, 1H, H Arom), 7.55-7.51 (m, 2H, H Arom), 7.43-7.35 (m, 3H, H Arom), 7.01-6.84 (m, 5H, H Arom), 5.63 (s, 1H, CHPh), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.53-4.48 (m, 2H), 4.47-4.42 (m, 1H), 4.42-4.37 (m, 1H), 4.17 (t, *J* = 10.3, 10.3 Hz, 0.4H, H-2), 4.12 (t, *J* = 10.2, 10.2 Hz, 0.6H, H-2), 3.83-3.75 (m, 2H), 3.73-3.65 (m, 1H), 3.06-2.99 (m, 1H, H-2'), 2.71 (dd, *J* = 4.7, 4.2 Hz, 0.6H, H-3a'), 2.65 (dd, *J* = 4.9, 4.1 Hz, 0.4H, H-3a'), 2.37-2.33 (m, 1H, H-3b'), 1.85 (ddd, *J* = 15.1, 8.7, 5.5 Hz, 0.4H, H-1a'), 1.77 (ddd, *J* = 14.6, 8.7, 4.0 Hz, 0.6H, H-1a'), 1.50 (ddd, *J* = 14.9, 5.8, 3.0 Hz, 0.4H, H=1b'), 1.43 (ddd, *J* = 14.7, 7.5, 3.2 Hz, 0.6H, H-1b'). ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 167.93 (C=O phth), 167.71 (C=O phth), 167.64 (C=O phth), 167.61 (C=O phth), 137.92 (C_q Bn or Ph), 137.86 (C_q Bn or Ph), 137.35 (C_q Bn or Ph), 137.34 (C_q Bn or Ph), 134.02 (CH phth), 133.91

(CH phth), 133.89 (CH phth), 131.48 (C_q phth), 131.36 (C_q phth), 128.94-126.00 (CH Arom), 123.47 (CH phth), 123.39 (CH phth), 123.32 (CH phth), 101.20 (CHPh), 83.56 (C-4), 83.49 (C-4), 75.04 (C-3), 74.09 (CH_2 Bn), 74.06 (CH_2 Bn), 74.04 (C-1), 73.80 (C-1), 70.32 (C-5), 70.11 (C-5), 68.81 (C-6), 55.91 (C-2), 55.64 (C-2), 49.09 (C-2'), 48.75 (C-2'), 47.36 (C-3'), 46.33 (C-3'), 35.99 (C-1'), 35.15 (C-1'). FT-IR: ν_{max}(neat)/cm^{-1} 2941.8, 2853.7, 1781.9, 1710.2, 1496.0, 1467.8, 1411.5, 1381.3, 1305.2, 1292.0, 1256.1, 1208.5, 1170.7, 1140.2, 1100.1, 1087.6, 1067.2, 1047.3, 1012.4, 1000.4, 965.3, 944.4, 910.2, 870.4, 838.6, 825.2, 796.5, 763.4, 752.4, 717.9, 700.0, 693.6, 658.6. HRMS: (M+H$^+$) calcd for $C_{31}H_{30}NO_7$ 528.20168, found 528.20148.

C-Allylglucosamine **18** (9.01 g, 17.6 mmol) was dissolved in 160 mL THF/H_2O (6/1 v/v), treated with K_2OsO_4 (130 mg, 0.352 mmol, 0.02 equiv.) in the presence of 4-methylmorpholino-*N*-oxide (5.2 g, 44 mmol, 2.5 equiv.). TLC analysis showed complete conversion to lower running spot after overnight stirring. The solution was diluted with EtOAc, washed with 1M HCl, 1M $Na_2S_2O_3$, brine, dried ($MgSO_4$) and concentrated under reduced pressure. Purification over column chromathography (2% EtOH/CH_2Cl_2) yielded higher running diastereomer **35a** (1.72 g, 3.1 mmol), lower running diastereomer **35b** (4.09 g, 7.5 mmol) and a mixture of alcohols **35a** and **35b** (3.49 g, 6.4 mmol) furnishing **35** in 97% total yield. Diol **35ab** was coevaporated thrice with toluene before being dissolved in anhydrous CH_2Cl_2 under Argon atm. The reaction mixture was cooled to 0°C, Et_3N (1.5 equiv.) and 4,4'-dimethoxytritylchloride (1.1 equiv.) were added. After 3h stirring, the reaction was quenched with $NaHCO_3$ (sat. aq.) and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. Silica gel chromathography (10% EtOAc/PE(1%Et_3N)→30% EtOAc/PE(1%Et_3N)) furnished **36a** (2.62 g, 3.1 mmol, 97%) and **36b** (5.96 g, 7.0 mmol, 94%). Primary protected **36ab** was dissolved in DMF, after which it was reacted with tert-butyldimethylsilyl chloride (2 equiv.) in the presence of Et_3N (2 equiv.) and imidazole (6 equiv.). After overnight stirring, an additional portion of tert-butyldimethylsilyl chloride (0.5 equiv.) was added followed by 2h additional stirring. Next, the solution was diluted with Et_2O, washed with $NaHCO_3$ (sat. aq.), brine, dried ($MgSO_4$) and concentrated. Column chromatography (10% EtOAc/PE(1%Et_3N)→20% EtOAc/PE(1%Et_3N)) gave **37a** (2.70 g, 2.8 mmol, 90%) and **37b** (5.81 g, 6.0 mmol, 85%).

Dimethoxytrityl protected **37ab** was treated with 2% dichloroacetic acid/CH_2Cl_2 (10mL/mmol) in the presence of triethylsilane (5 equiv.). After 1h, TLC analysis showed complete consumption of the starting material. The reaction was quenched with MeOH, extracted with $NaHCO_3$ (sat. aq.), dried (Na_2SO_4) and concentrated. Silica gel chromatography (5% EtOAc/PE(1%Et_3N)→40% EtOAc/PE(1%Et_3N)) afforded primary alcohol **38a** in 73% (1.35 g, 2.05 mmol) and **38b** in 82% (3.23 g, 4.9 mmol). Alcohol **38ab** was coevaporated with toluene before being dissolved in anhydrous dichloromethane. Subsequently, the solution was cooled to 0°C, reacted with methanesulfonyl chloride (2.5 equiv) under the agency of Et_3N (2.5 equiv.) and DMAP (0.1 equiv.). After stirring overnight, the reaction was diluted with EtOAc, washed with $NaHCO_3$ (sat. aq.), brine, dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (5% EtOAc/PE→30% EtOAc/PE) yielded mesylate **39a** (1.31 g, 1.78 mmol, 89%) and **39b** (3.359 g, 4.6 mmol, 93%). Mesylate **39ab** was dissolved in THF. TBAF (1M in THF, 2.2 equiv.) was added, stirred for 2h, poured into $NaHCO_3$ (sat. aq.), extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated under reduced. The residue was purified by silica gel chromatography affording epoxides **19a** (1.12 g, 2.1 mmol, 48%) and **19b** (1.63 g, 3.10 mmol, 67%).

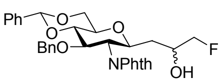
19a

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.85-7.82 (m, 1H, H Arom), 7.76-7.68 (m, 2H, H Arom), 7.66-7.63 (m, 1H, H Arom), 7.56-7.52 (m, 2H, H Arom), 7.43-7.35 (m, 3H, H Arom), 6.99-6.85 (m, 5H, H Arom), 5.64 (s, 1H, CHPh), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.52 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.47-4.38 (m, 3H, H-1, H-3, H-6a), 4.18 (t, *J* = 10.2, 10.2 Hz, 1H, H-2), 3.83-3.78 (m, 2H, H-4, H-6b), 3.68 (dt, *J* = 10.0, 9.9, 4.9 Hz, 1H, H-5), 3.04-3.00 (m, 1H, H-2'), 2.66 (t, *J* = 4.4, 4.4 Hz, 1H, H-3a'), 2.35 (dd, *J* = 4.9, 2.7 Hz, 1H, H-3b'), 1.86 (ddd, *J* = 14.4, 8.4, 5.3 Hz, 1H, H-1a'), 1.51 (ddd, *J* = 14.8, 5.8, 3.1 Hz, 1H, H-1b'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.68 (C=O, phth), 167.60 (C=O phth), 137.86 (C_q Bn or Ph), 137.33 (C_q Bn or Ph), 133.99 (CH phth), 133.88 (CH phth), 131.36 (C_q phth), 131.34 (C_q phth), 128.89-125.95 (CH Arom), 123.43 (CH phth), 123.27 (CH phth), 101.18 (CHPh), 83.46 (C-4), 75.07 (C-3), 74.03 (C-1), 74.01 (CH_2Bn), 70.31 (C-5), 68.79 (C-6), 55.63 (C-2), 49.03 (C-2'), 46.29 (C-3'), 35.13 (C-1'). FT-IR: ν_{max}(neat)/cm^{-1} 2877.9, 1775.7, 1710.0, 1613.3, 1495.5, 1468.5, 1453.5, 1382.9, 1301.9, 1172.5, 1089.7, 996.2, 916.6, 874.1, 836.4, 795.2, 749.9, 719.8, 697.0, 659.1, 647.2. $[\alpha]_D^{23}$ +59° (c = 1.11, $CHCl_3$). HRMS: ($M+H^+$) calcd for $C_{31}H_{30}NO_7$ 528.20168, found 528.20003.

19b

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.84-7.61 (m, 4H, H Arom), 7.55-7.34 (m, 5H, H Arom), 6.99-6.84 (m, 5H, H Arom), 5.63 (s, 1H, CHPh), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.50 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.50 (dd, *J* = 9.8, 9.0 Hz, 1H, H-3), 4.45 (ddd, *J* = 10.4, 8.6, 3.2 Hz, 1H, H-1), 4.39 (dd, *J* = 10.3, 4.7 Hz, 1H, H-6a), 4.12 (t, *J* = 10.2, 10.2 Hz, 1H, H-2), 3.81-3.75 (m, 2H, H-4, H-6b), 3.69 (dt, *J* = 9.7, 9.6, 4.7 Hz, 1H, H-5), 3.03 (dtd, *J* = 6.9, 4.0, 4.0, 2.6 Hz, 1H, H-2'), 2.70 (dd, *J* = 5.0, 4.0 Hz, 1H, H-3a'), 2.35 (dd, *J* = 5.0, 2.6 Hz, 1H, H-3a'), 1.77 (ddd, *J* = 14.6, 8.6, 4.0 Hz, 1H, H-1a'), 1.44 (ddd, *J* = 14.6, 6.9, 3.2 Hz, 1H, H-1b'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.85 (C=O phth), 167.56 (C=O phth), 137.89 (C_q Bn or Ph), 137.34 (C_q Bn or Ph), 133.85 (CH phth), 133.83 (CH phth), 131.46 (C_q phth), 131.34 (C_q phth), 128.86-125.95 (CH Arom), 123.32 (CH phth), 123.26 (CH phth), 101.15 (CHPh), 83.51 (C-4), 75.03 (C-3), 74.03 (CH_2Bn), 73.78 (C-1), 70.09 (C-5), 68.76 (C-6), 55.88 (C-2), 48.68 (C-2'), 47.27 (C-3'), 35.95 (C-1'). FT-IR: v_{max}(neat)/cm^{-1} 2853.6, 1781.6, 1710.1, 1467.7, 1431.9, 1410.6, 1380.8, 1292.0, 1256.0, 1208.5, 1170.2, 1141.2, 1118.4, 1100.2, 1066.6, 1048.0, 1012.1, 1000.3, 964.2, 944.5, 913.7, 870.1, 826.0, 797.2, 762.8, 752.5, 719.7, 700.1, 693.3, 658.6. $[\alpha]_D^{23}$ +55° (c = 1.00, $CHCl_3$). HRMS: ($M+H^+$) calcd for $C_{31}H_{30}NO_7$ 528.20168, found 528.20149.

(2R/S)-3-*C*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-fluoro-2-hydroxypropane (20)



Epoxide **19** (264 mg, 0.5 mmol) and TBA·H_2F_3 (425 mg, 1.51 mmol, 3 equiv.) were suspended in toluene (0.5 mL/mmol), after which the reaction mixture was heated in the microwave to 180°C for 20 min. The resulting oil was diluted with EtOAc, washed with $NaHCO_3$ (sat. aq.), brine, dried ($MgSO_4$) and concentrated. Silica gel column chromatography purification (20% EtOAc/PE→30% EtOAc/PE) furnished fluorohydrin **20** in 84% (226 mg, 0.41 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.86-7.62 (m, 4H, H Arom), 7.57-7.35 (m, 5H, H Arom), 7.00-6.85 (m, 5H, H Arom), 5.64 (s, 1H, CHPh), 4.83-4.79 (m, 1H), 4.56-4.44 (m, 3H), 4.41-4.36 (m, 1H), 4.32-4.27 (m, 1H), 4.24-3.98 (m, 3H), 3.85-3.67 (m, 3H), 3.01 (s, 1H, OH), 2.51 (s, 1H, OH), 1.74-1.50 (m, 2H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.88 (C=O phth), 167.73 (C=O phth), 167.63 (C=O phth), 167.54 (C=O phth), 137.87 (C_q Bn or Ph), 137.74 (C_q Bn or Ph), 137.29 (C_q Bn or Ph), 137.18 (C_q Bn or Ph), 134.02 (CH phth), 133.93 (CH phth), 133.90 (CH phth), 133.87 (CH phth), 131.41 (C_q phth), 131.29 (C_q phth), 131.25 (C_q phth), 128.92-125.94 (CH Arom), 123.46 (CH phth), 123.37 (CH phth), 123.34 (CH phth), 101.18 (CHPh), 101.15 (CHPh), 86.65 (d, *J* = 169.4 Hz, C-3'), 85.76 (d, *J* = 170.0 Hz, C-3'), 83.46, 83.16, 75.32, 75.00, 74.03, 72.85, 70.26, 70.04, 68.83 (C-2'), 68.68 (C-6), 68.50 (C-6), 66.62 (d, *J* = 19.5 Hz, C-2'), 55.79 (C-2), 55.71 (C-2), 34.42 (d, *J* = 6.5 Hz, C-1'). FT-IR: ν_{max}(neat)/cm^{-1} 2871.1, 1775.6, 1709.9, 1615.4, 1496.4, 1455.4, 1385.5, 1173.2, 1087.1, 999.7, 963.0, 874.4, 750.3, 720.4, 696.8, 660.5. HRMS: (M+H$^+$) calcd for $C_{31}H_{31}FNO_7$ 548.20791, found 548.20764.

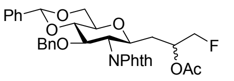
20a

Diastereomerically pure epoxide **19a** (1.12 g, 2.1 mmol) was transformed to the fluorohydrin as previously depicted furnishing **20a** as a colorless oil in 62% (0.719 g, 1.31 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.87-7.62 (m, 4H, H Arom), 7.55-7.36 (m, 5H, H Arom), 6.99-6.85 (m, 5H, H Arom), 5.63 (s, 1H, CHPh), 4.79 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.50 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.50-4.46 (m, 1H, H-1), 4.45 (dd, *J* = 9.9, 9.1 Hz, 1H, H-3), 4.38 (dd, *J* = 10.2, 4.6 Hz, 1H, H-6a), 4.26 (dd, *J* = 47.3, 4.8 Hz, 2H, H-3'), 4.17 (t, *J* = 10.2, 10.2 Hz, 1H, H-2), 4.08-3.98 (m, 1H, H-2'), 3.83-3.76 (m, 2H, H-4, H-6b), 3.71 (ddd, *J* = 10.1, 9.3, 4.6 Hz, 1H, H-5), 2.89 (s, 1H, OH), 1.69-1.62 (m, 2H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.80 (C=O phth), 167.72 (C=O phth), 137.83 (C_q Bn or Ph), 137.22 (C_q Bn or Ph), 134.11 (CH phth), 134.02 (CH phth), 131.40 (C_q phth), 131.36 (C_q phth), 129.04-126.02 (CH Arom), 123.56 (CH phth), 123.45 (CH phth), 101.32 (CHPh), 85.83 (d, *J* = 170 Hz, C-3'), 83.28 (C-4), 75.63 (C-1), 74.81 (C-3), 74.15 (CH_2 Bn), 70.38 (C-5), 69.00 (d, *J* = 20.4 Hz, C-2'), 68.62 (C-6), 55.89 (C-2), 34.54 (d, *J* = 5.9 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 3476.0, 2877.9, 1775.2, 1709.8, 1612.2, 1496.5, 1454.7, 1384.9, 1172.6, 1091.4, 1001.1, 962.5, 873.9, 750.7, 720.5, 697.1, 659.7. $[\alpha]_D^{23}$ +57° (c = 0.27, $CHCl_3$). HRMS: ($M+H^+$) calcd for $C_{31}H_{31}FNO_7$ 548.20791, found 548.20612.

20b

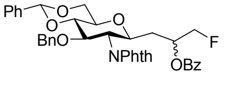
Diastereomerically pure epoxide **19b** (1.63 g, 3 mmol) was regioselectively opened as described for the diastereomeric mixture. Fluorohydrin **20b** was obtained in 77% (1.25 g, 2.3 mmol) ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.84-7.63 (m, 4H, H Arom), 7.54-7.36 (m, 5H, H Arom), 6.99-6.85 (m, 5H, H Arom), 5.63 (s, 1H, CHPh), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.54-4.48 (m, 3H, CH_2 Bn, H-1, H-3), 4.38 (dd, *J* = 10.3, 4.5 Hz, 1H, H-6a), 4.34 (ddd, *J* =47.3, 9.2, 3.0 Hz, 1H, H-3a'), 4.18 (ddd, *J* = 47.3, 9.4, 6.3 Hz, 1H, H-3b'), 4.16-4.05 (m, 2H, H-2, H-2'), 3.78 (m, 2H, H-4, H-6b), 3.69 (ddd, *J* = 10.2, 9.3, 4.7 Hz, 1H, H-5), 2.22 (s, 1H, OH), 1.54 (dd, *J* = 6.4, 5.6 Hz, 1H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.94 (C=O phth), 167.61 (C=O phth), 137.95 (C_q Bn or Ph), 137.34 (C_q Bn or Ph), 134.00 (CH phth), 133.97 (CH phth), 131.52 (C_q phth), 131.36 (C_q phth), 128.99-126.03 (CH Arom), 123.47 (CH phth), 123.45 (CH phth), 101.29 (CH Ph), 86.70 (d, *J* = 169.3 Hz, C-3'), 83.57 (C-4), 75.06 (C-1), 74.15 (CH_2Bn), 73.00 (C-3), 70.17 9 (C-5), 68.79 (C-6), 66.83 (d, *J* = 19.6 Hz, C-2'), 55.76 (C-2), 34.46 (d, *J* = 6.6 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 2876.0, 1775.3, 1709.9, 1496.4, 1455.1, 1385.4, 1172.8, 1086.1, 998.1, 963.9, 874.6, 750.4, 720.4, 696.9, 668.0, 660.1. $[\alpha]_D^{23}$ +23° (c = 1.00, $CHCl_3$). HRMS: ($M+H^+$) calcd for $C_{31}H_{31}FNO_7$ 548.20791, found 548.20765.

(2R/S)-3-*C*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-fluoro-2-acetoxypropane (21)



Fluorohydrin **20** (4.737 g, 8.7 mmol) was dissolved in pyridine (100 mL), cooled to 0°C, before acetic anhydride (33 mL) was added. After stirring overnight, the reaction was quenched with MeOH, concentrated, diluted with EtOAc, washed with 1M HCl, $NaHCO_3$ (sat. aq.), brine, dried ($MgSO_4$) and concentrated under reduced pressure. Purification over silica gel chromatography (Tol→10% EtOAc/Tol) yielded acetylated fluorohydrin **21** (96%, 4.93 g, 8.35 mmol). ^1H NMR (500 MHz, $CDCl_3$) δ ppm 7.86-7.82 (m, 1H, H Arom), 7.76-7.62 (m, 3H, H Arom), 7.55-7.51 (m, 2H, H Arom), 7.43-7.35 (m, 3H, H Arom), 6.99-6.85 (m, 5H, H Arom), 5.62 (s, 1H, CHPh), 5.28-5.10 (m, 1H, H-2'), 4.79 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.54-4.32 (m, 5H), 4.28-4.21 (m, 1H), 4.14-4.07 (m, 1H), 3.80-3.72 (m, 2H), 3.68-3.58 (m, 1H, H-5), 2.02-2.00 (m, 3H, CH_3 Ac), 1.86-1.62 (m, 2H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 170.22 (C=O Ac), 170.05 (C=O Ac), 167.84 (C=O phth), 167.72 (C=O phth), 167.57 (C=O phth), 167.51 (C=O phth), 137.84 (C_q Bn or Ph), 137.79 (C_q Bn or Ph), 137.36 (C_q Bn or Ph), 137.30 (C_q Bn or Ph), 133.99 (CH phth), 133.93 (CH phth), 131.45 (C_q phth), 131.36 (C_q phth), 131.33 (C_q phth), 128.93-125.98 (CH Arom), 123.45 (CH phth), 123.33 (CH phth), 123.28 (CH phth), 101.22 (CHPh), 83.85 (d, *J* = 173.8 Hz, C-3'), 83.50, 83.38, 82.95 (d, *J* = 173.3 Hz, C-3'), 74.91, 74.82, 74.03, 73.98, 72.58, 72.31, 70.21, 70.13, 69.62 (d, *J* = 19.4 Hz, C-2'), 68.73 (d, *J* = 19.2 Hz, C-2'), 68.69 (C-6), 55.77 (C-2), 55.70 (C-2), 32.64 (d, *J* = 5.9 Hz, C-1'), 31.72 (d, *J* = 6.5 Hz, C-1'), 20.91 (CH_3 OAc), 20.82 (CH_3 OAc). FT-IR: ν_{max}(neat)/cm^{-1} 2877.0, 1775.7, 1738.4, 1710.3, 1613.2, 1495.9, 1468.9, 1454.2, 1427.7, 1383.5, 1371.9, 1233.0, 1172.5, 1097.8, 1073.6, 1013.0, 962.1, 916.5, 873.8, 819.0, 795.4, 750.6, 738.4, 720.9, 697.6, 660.2, 646.3, 619.8. HRMS: (M+H$^+$) calcd for $C_{33}H_{33}FNO_8$ 590.21847, found 590.21671.

(2R/S)-3-*C*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-fluoro-2-benzoyloxypropane (22)



After coevaporation with anhydrous toluene, fluorohydrin **20** (0.826 g, 1.5 mmol) was dissolved in pyridine (7 mL), cooled to 0°C, treated with benzoylchloride (0.435 mL 3.75 mmol, 2.5 equiv.) and catalytic DMAP. TLC analysis showed complete conversion of the starting material to a higher running spot, after overnight stirring. The reaction mixture was concentrated, redissolved in EtOAc and washed with 1M HCl, $NaHCO_3$ (sat. aq.) and brine. The organic layer was dried ($MgSO_4$), concentrated and applied to silica gel column chromathography (5% EtOAc/PE→20%

EtOAc/PE) affording benzoyl protected **22** in 92% (0.895 g, 1.4 mmol) as a colorless oil. ^{1}H NMR (600 MHz, CDCl$_3$) δ ppm 7.98-7.93 (m, 2H, H Arom), 7.87-7.80 (m, 1H, H Arom), 7.77-7.62 (m, 3H, H Arom), 7.59-7.47 (m, 4H, H Arom), 7.44-7.33 (m, 5H, H Arom), 6.96-6.83 (m, 5H, H Arom), 5.60-5.56 (m, 1H, CHPh), 5.48-5.37 (m, 1H, H-2'), 4.80-4.75 (m, 1H, CH$_2$ Bn), 4.65-4.53 (m, 1H), 4.51-4.36 (m, 4H), 4.29 (dd, *J* = 10.5, 4.9 Hz, 1H), 4.18-4.09 (m, 1H), 3.79-3.67 (m, 2H), 3.65-3.53 (m, 2H), 2.01-1.75 (m, 2H, H-1'). ^{13}C NMR (150 MHz, CDCl$_3$) δ ppm 167.91 (C=O phth), 167.83 (C=O phth), 167.52 (C=O phth), 167.48 (C=O phth), 165.80 (C=O Bz), 165.62 (C=O Bz), 137.87 (C$_q$ Bn or Ph), 137.83 (C$_q$ Bn or Ph), 137.34 (C$_q$ Bn or Ph), 137.32 (C$_q$ Bn or Ph), 134.02-133.09 (CH Arom), 131.46 (C$_q$ phth), 131.39 (C$_q$ phth), 131.33 (C$_q$ phth), 131.31 (C$_q$ phth), 129.76-123.35 (CH Arom), 101.22 (CHPh), 83.86 (d, *J* = 174.1 Hz, C-3'), 83.51, 83.36, 83.01 (d, *J* = 173.9 Hz, C-3'), 74.90, 74.87, 74.05 (CH$_2$ Bn), 74.01 (CH$_2$ Bn), 73.13, 72.42, 70.39 (d, *J* = 19.6 Hz, C-2'), 70.11, 69.39 (d, *J* = 19.3 Hz, C-2'), 68.66 (C-6), 68.60 (C-6), 55.88 (C-2), 55.81 (C-2), 32.59 (d, *J* = 5.7 Hz, C-1'), 32.11 (d, *J* = 6.1 Hz,C-1'). FT-IR: v_{max}(neat)/cm^{-1} 1775.4, 1710.1, 1452.4, 1383.5, 1267.2, 1175.1, 1096.0, 1013.4, 873.6, 750.7, 712.2, 697.0, 646.1.

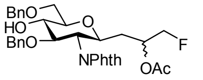
22a

Fluorohydrin **20a** (0.719 g, 1.31 mmol) was benzoylated as described, giving title compound **22a** in 91% (0.778 g, 1.19 mmol) as a colorless oil. ^{1}H NMR (500 MHz, CDCl$_3$) δ ppm 8.01-7.95 (m, 2H, H Arom), 7.84-7.81 (m, 1H, H Arom), 7.74-7.65 (m, 2H, H Arom), 7.58-7.49 (m, 4H, H Arom), 7.44-7.36 (m, 5H, H Arom), 6.99-6.82 (m, 5H, H Arom), 5.58 (s, 1H, CHPh), 5.43 (dddd, *J* = 22.6, 10.4, 6.7, 4.1 Hz, 1H, H-2'), 4.78 (d, *J* = 12.3 Hz, 1H, CH$_2$ Bn), 4.60-4.47 (m, 4H, H-1, H-3', CH$_2$ Bn), 4.44 (dd, *J* = 9.9, 8.9 Hz, 1H, H-3), 4.16-4.10 (m, 2H, H-2, H-6a), 3.75 (t, *J* = 8.9, 8.9 Hz, 1H, H-4), 3.67-3.59 (m, 2H, H-5, H-6b), 1.98-1.86 (m, 2H, H-1'). ^{13}C NMR (125 MHz, CDCl$_3$) δ ppm 167.85 (C=O phth), 167.56 (C=O phth), 165.65 (C=O Bz), 137.88 (C$_q$ Bn or Ph), 137.36 (C$_q$ Bn or Ph), 133.98 (CH phth), 133.92 (CH phth), 133.11 (CH Arom), 131.38 (C$_q$ phth), 131.35 (C$_q$ phth), 130.13 (C$_q$ Bz), 129.81-126.03 (CH Arom), 123.47 (CH phth), 123.37 (CH phth), 101.24 (CHPh), 83.02 (d, *J* = 174.0 Hz, C-3'), 82.34 (C-4), 74.94 (C-3), 74.08 (CH$_2$ Bn), 73.18 (C-1), 70.40 (d, *J* = 19.6 Hz, C-2'), 70.15 (C-5), 68.63 (C-6), 55.93 (C-2), 32.14 (d, *J* = 6.2 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 1776.3, 1713.8, 1699.9, 1455.3, 1385.6, 1270.0, 1096.0, 1014.5, 873.9, 750.9, 697.3. $[\alpha]_D^{23}$ +48° (c = 0.57, $CHCl_3$). HRMS: (M+Na^+) calcd for $C_{38}H_{34}FNO_8Na$ 674.21607, found 674.21425.

22b

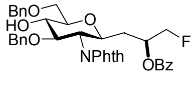
Fluorohydrin **20b** (1.25 g, 2.3 mmol) was converted to benzoyl protected fluorohydrin **22b** as depicted for **22** giving benzoyl protected **22b** as a colorless oil in 92% yield (1.39 g, 2.13 mmol). ^1H NMR (500 MHz, $CDCl_3$) δ ppm 7.99-7.92 (m, 2H, H Arom), 7.87-7.85 (m, 1H, H Arom), 7.77-7.70 (m, 2H, H Arom), 7.65-7.63 (m, 1H, H Arom), 7.58-7.54 (m, 1H, H Arom), 7.44-7.34 (m, 5H, H Arom), 6.98-6.84 (m, 5H, H Arom), 5.60 (s, 1H, CHPh), 5.45 (dddd, *J* = 23.9, 9.5, 6.9, 3.4 Hz, 1H, H-2') 4.78 (d, *J* = 12.3 Hz, 1H, CH_2 Bn), 4.67-4.37 (m, 5H, H-1, H-3, H-3', CH_2 Bn), 4.30 (dd, *J* = 10.4, 4.9 Hz, 1H, H-6a), 4.16 (t, *J* = 10.2, 10.2 Hz, 1H, H-2), 3.78 (t, *J* = 9.1, 9.1 Hz, 1H, H-4), 3.71 (t, *J* = 10.3, 10.3 Hz, 1H, H-6b), 3.57 (dt, *J* = 9.8, 9.8, 4.9 Hz, 1H, H-5), 1.94 (ddd, *J* = 14.8, 9.5, 2.0 Hz,, 1H, H-1a'), 1.81 (ddd, *J* = 14.8, 10.0, 3.4 Hz, 1H, H-1b'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.90 (C=O phth), 167.49 (C=O phth), 165.80 (C=O Bz), 137.90 (C_q Bn or Ph), 137.36 (C_q Bn or Ph), 134.01 (CH phth), 133.93 (CH phth), 133.13 (CH Arom), 131.48 (C_q phth), 131.41 (C_q phth), 129.67-126.01 (CH Arom), 123.50 (CH phth), 123.37 (CH phth), 101.24 (CHPh), 83.86 (d, *J* = 174.1 Hz, C-3'), 83.51 (C-4), 74.95 (C-3), 74.02 (CH_2 Bn), 72.45 (C-1), 70.14 (C-5), 69.41 (d, *J* = 19.3 Hz, C-2'), 68.67 (C-6), 55.83 (C-2), 32.61 (d, *J* = 5.9 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 2875.5, 1775.9, 1710.2, 1602.6, 1495.9, 1452.4, 1384.8, 1315.0, 1266.3, 1174.9, 1097.3, 1069.9, 1026.2, 1001.8, 962.8, 873.7, 750.9, 712.2, 697.2, 659.5. $[\alpha]_D^{23}$ +198° (c = 1.00, $CHCl_3$). HRMS: (M+Na^+) calcd for $C_{38}H_{34}FNO_8Na$ 674.21607, found 674.21594.

(2R/S)-3-*C*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-fluoro-2-acetoxypropane (23)



Acetyl protected fluorohydrin **21** (4.83 g, 8.18 mmol) was coevaporated thrice with toluene before being dissolved in freshly distilled dichloromethane. Activated 4Å MS and triethylsilane (4.36 ml, 27 mmol, 3.3 equiv.) were added and the reaction mixture was cooled to -78°C. Subsequently, trifluoromethanesulfonic acid (2.17 ml, 24.54 mmol, 3 equiv.) was added and the reaction was stirred for 45 min at -78°C. The reaction was quenched by addition of MeOH (5 ml) and Et_3N (5 ml), heated to room temperature, extracted with $NaHCO_3$ (sat. aq.), brine, dried ($MgSO_4$) and concentrated. Purification by silicagel chromathography (10% EtOAc/PE→40% EtOAc/PE) gave building block **23** in 84% (4.52 g, 6.9 mmol) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.81-7.76 (m, 1H, H Arom), 7.71-7.63 (m, 3H, H Arom), 7.38-7.28 (m, 5H, H Arom), 7.03-6.99 (m, 2H, H Arom), 6.94-6.90 (m, 3H, H Arom), 5.30-5.10 (m, 1H, H-2'), 4.80-4.73 (m, 1H, CH_2 Bn), 4.62 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.59-4.00 (m, 8H), 3.87-3.69 (m, 3H), 3.62-3.52 (m, 1H, H-5), 3.19-3.10 (m, 1H, OH), 2.02-1.97 (m, 3H, CH_3 Ac), 1.84-1.60 (m, 2H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 170.19 (C=O Ac), 170.03 (C=O Ac), 167.82 (C=O phth), 167.78 (C=O phth), 167.70 (C=O phth), 138.07 (C_q Bn), 137.98 (C_q Bn), 137.55 (C_q Bn), 133.84 (CH phth), 133.80 (CH phth), 131.28 (C_q phth), 131.18 (C_q phth), 128.32-127.19 (CH Arom), 123.27 (CH phth), 123.19 (CH phth), 123.11 (CH phth), 83.82 (d, *J* = 173.6 Hz, C-3'), 82.99 (d, *J* = 172.6 Hz, C-3'), 79.30, 79.11, 77.68, 77.43, 74.21, 73.92, 73.53, 73.49, 71.54, 71.50, 70.28, 69.86 (d, *J* = 19.3 Hz, C-2'), 68.93 (d, *J* = 19.0 Hz, C-2'), 55.41 (C-2), 55.24 (C-2), 32.28 (d, *J* = 5.9 Hz, C-1'), 31.38 (d, *J* = 6.6 Hz, C-1') 20.79 (CH_3 OAc), 20.73 (CH_3 OAc). FT-IR: ν_{max}(neat)/cm^{-1} 3475.9, 2871.9, 1774.1, 1738.5, 1709.8, 1496.8, 1454.1, 1384.0, 1233.9, 1074.2, 1026.1, 962.6, 874.3, 736.6, 720.3, 697.6. HRMS: (M+H$^+$) calcd for $C_{33}H_{35}FNO_8$ 592.23412, found 592.23417.

(2R/S)-3-*C*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-1-fluoro-2-benzoyloxypropane (24)



Benzoyl protected fluorohydrin **22** (0.895 g, 1.4 mmol) was converted to acceptor **24** as described for acetylated **23**. Silica gel purification (10% EtOAc/PE→30% EtOAc/PE) furnished **24** (80%, 0.730 g, 1.11 mmol) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 7.99-7.92 (m, 2H, H Arom), 7.84-7.77 (m, 1H, H Arom), 7.74-7.62 (m, 3H, H Arom), 7.58-7.53 (m, 1H, H Arom), 7.45-7.27 (m, 7H, H Arom), 7.03-6.89 (m, 5H, H Arom), 5.50-5.38 (m, 1H, H-2'), 4.78-4.70 (m, 1H, CH_2 Bn), 4.67-4.34 (m, 5H, CH_2 Bn, H-3'), 4.33-4.27 (m, 1H), 4.25-4.20 (m, 1H), 4.10-4.05 (m, 1H), 3.87-3.80 (m, 1H), 3.78-3.74 (m, 1H), 3.70-3.66 (m, 1H), 3.61-3.47 (m, 1H), 2.98-2.83 (m, 1H, OH), 1.99-1.69 (m, 2H, H-1'). ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 167.97 (C=O phth), 167.84 (C=O phth), 167.79

(C=O phth), 165.82 (C=O Bz), 165.61 (C=O Bz), 138.19 (C_q Bn), 137.55 (C_q Bn), 133.98 (CH phth), 133.92 (CH phth), 133.10 (CH phth), 131.48 (C_q phth), 131.42 (C_q phth), 129.67-127.33 (CH Arom), 123.46 (CH phth), 123.34 (CH phth), 83.96 (d, *J* = 172.5 Hz, C-3'), 83.10 (d, *J* = 172.5 Hz, C-3'), 82.53, 79.23, 77.20, 74.59, 74.32 (CH_2 Bn), 73.65 (CH_2 Bn), 72.21, 70.54 (C-6), 69.64 (d, *J* = 19.5 Hz, C-2'), 55.53 (C-2), 55.50 (C-2), 32.37 (d, *J* = 6.0 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 3479.8, 3031.9, 2873.5, 1775.5, 1709.9, 1700.0, 1602.3, 1495.8, 1469.0, 1452.6, 1385.1, 1315.8, 1267.1, 1207.6, 1176.8, 1070.2, 1025.7, 964.5, 874.3, 821.1, 736.7, 712.2, 697.4, 647.6. HRMS: (M+H$^+$) calcd for $C_{38}H_{37}FNO_8$ 654.24977, found 654.24998.

24a

The benzylidene of diastereomerically pure **22a** (0.713 g, 1.1 mmol) was regioselectively opened as described for **23**. After silica gel purification (10% EtOAc/PE→30% EtOAc/PE) acceptor **24a** (79%, 0.570 g, 0.87 mmol) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.99-7.94 (m, 2H, H Arom), 7.81-7.51 (m, 4H, H Arom), 7.43-7.28 (m, 8H, H Arom), 7.04-6.91 (m, 5H, H Arom), 5.42 (dddd, *J* = 22.4, 12.0, 6.2, 3.2 Hz, 1H, H-2'), 4.73 (d, *J* = 12.2 Hz, 1H, CH_2 Bn), 4.56 (ddd, *J* = 47.6, 10.6, 5.0 Hz, 1H, H-3a'), 4.54 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.54 (ddd, *J* = 47.6, 10.6, 3.2 Hz, 1H, H-3b'), 4.51 (d, *J* = 12.2 Hz, 1H, CH_2 Bn) 4.46 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.39 (td, *J* = 10.4, 6.0, 6.0 Hz, 1H, H-1), 4.25 (dd, *J* = 10.3, 8.6 Hz, 1H, H-3), 4.06 (t, *J* = 10.3, 10.3 Hz, 1H, H-2), 3.80 (t, *J* = 8.6, 8.6 Hz, 1H, H-4), 3.71-3.66 (m, 1H, H-6a), 3.61-3.57 (m, 2H, H-4, H-6b), 2.87 (s, 1H), 1.89 (t, *J* = 6.0, 6.0 Hz, 1H, H-1'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.87(C=O phth), 165.62 (C=O Bz), 138.17 (C_q Bn), 137.55 (C_q Bn), 133.94 (CH phth), 133.88 (CH phth), 133.01 (CH Arom), 131.44 (C_q phth), 131.34 (C_q phth), 129.89 (C_q Bz), 129.71-127.36 (CH Arom), 123.44 (CH phth), 123.29 (CH phth), 83.12 (d, *J* = 173.43 Hz, C-3'), 79.26 (C-3), 77.28 (C-5), 74.54 (C-4), 74.35 (CH_2 Bn), 73.67 (CH_2 Bn), 72.26 (C-1), 70.64 (C-6), 70.61 (d, *J* = 19.56 Hz, C-2'), 55.52 (C-2), 31.91 (d, *J* = 6.33 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 1702.1, 1383.9, 1269.7, 1070.9, 711.0. $[\alpha]_D^{23}$ +39° (c = 0.67, $CHCl_3$). HRMS: (M+H$^+$) calcd for $C_{38}H_{37}FNO_8$ 654.24977, found 654.24831.

24b

Protected **22b** (1.32 g, 2.03 mmol) was converted to acceptor **24b** as depicted for **23**. Silica gel column chromatography gave title compound **24b** (85%, 1.13 g, 1.73 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.99-7.94 (m, 2H, H Arom), 7.84-7.64 (m, 4H, H Arom), 7.59-7.40 (m, 3H, H Arom), 7.39-7.29 (m, 5H, H Arom), 7.03-6.92 (m, 5H, H Arom), 5.46 (qdd, *J* = 24.0, 9.8, 4.2, 3.7, 2.7 Hz, 1H, H-2'), 4.76 (d, *J* = 12.2 Hz, 1H, CH_2 Bn), 4.61 (ddd, *J* = 48.1, 10.4, 2.7 Hz, 1H, H-3a'), 4.60 (d, *J* = 12.2 Hz, 1H, CH_2 Bn), 4.53 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.51 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.39 (ddd, *J* = 46.8, 10.4, 4.2 Hz, 1H, H-3b'), 4.31 (dt, *J* = 10.2, 10.2, 2.1 Hz, 1H, H-1), 4.24 (dd, *J* = 10.3, 8.5 Hz, 1H, H-3), 4.09 (t, *J* = 10.3, 10.2 Hz, 1H, H-2), 3.85 (dd, *J* = 9.3, 8.5 Hz, 1H, H-4), 3.77 (dd, *J* = 10.1, 4.3 Hz, 1H, H-6a), 3.69 (dd, *J* = 10.1, 5.1 Hz, 1H, H-6b), 3.51 (td, *J* = 9.4, 5.1, 4.3 Hz, 1H, H-5), 2.89 (s, 1H), 1.92-1.85 (m, 1H, H-1a'), 1.78 (ddd, *J* = 14.5, 10.2, 3.7 Hz, 1H, H-1b'). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm 167.94 (C=O phth), 167.78 (C=O phth), 165.80 (C=O Bz), 138.21 (C_q Bn), 137.56 (C_q Bn), 133.96 (CH phth), 133.90 (CH phth), 133.08 (CH Arom), 131.49 (C_q phth), 131.44 (C_q phth), 129.72 (C_q Bz), 129.67-127.31 (CH Arom), 123.45 (CH phth), 123.32 (CH phth), 83.95 (d, *J* = 173.9 Hz, C-3'), 79.25 (C-3), 77.18 (C-5), 74.62 (C-4), 74.31 (CH_2 Bn), 73.69 (CH_2 Bn), 71.72 (C-1), 70.54 (C-6), 69.65 (d, *J* = 19.1 Hz, C-2'), 55.54 (C-2), 32.38 (d, *J* = 5.8 Hz, C-1'). FT-IR: v_{max}(neat)/cm^{-1} 3474.9, 2923.0, 1775.3, 1709.9, 1699.9, 1602.4, 1496.1, 1452.7, 1385.4, 1266.9, 1176.8, 1070.2, 1025.7, 874.3, 819.8, 737.0, 712.1, 697.5, 667.9, 646.3. $[\alpha]_D^{23}$ +63° (c = 1.24, $CHCl_3$). HRMS: (M+H$^+$) calcd for $C_{38}H_{37}FNO_8$ 654.24977, found 654.24836.

E. coli cel extracts overexpressing YPng1 and YPng(C191A) were labeled with β-VAD-Fmk **1**. The labeling was visualized in the wet gel slabs with the Thyphoon imager. Next, the total protein amount was visualized by silver staining (Figure 1).

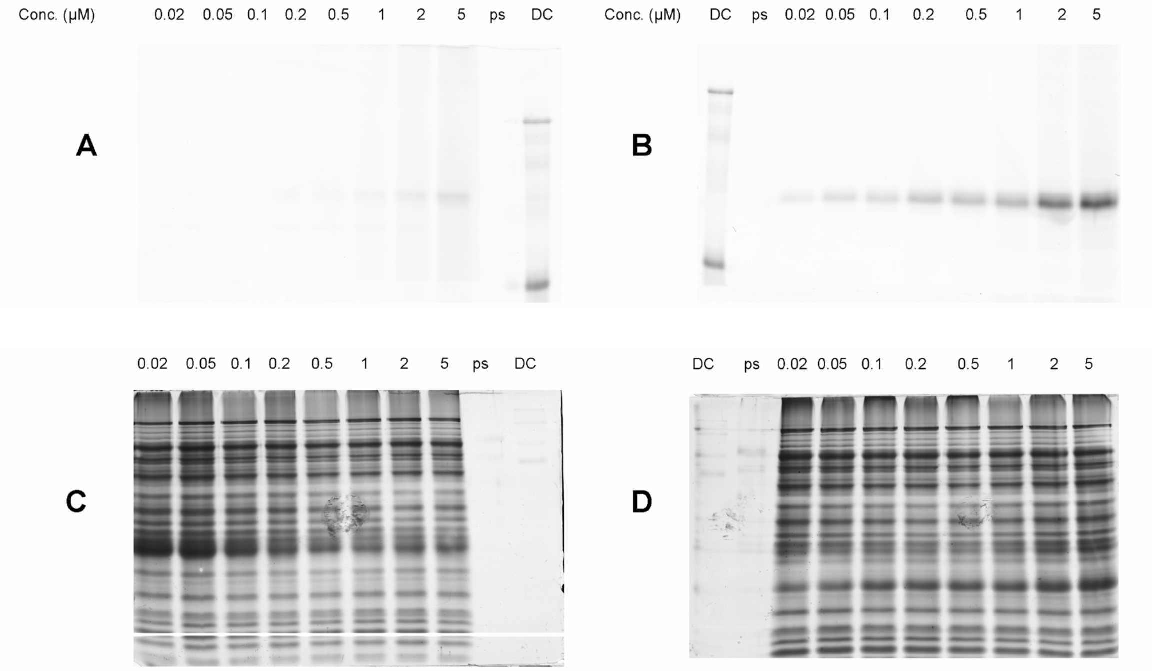


Figure 1. Labeling of YPng1 and YPng(C191A) with β-VAD-Fmk **1** in crude *E. coli* cell extracts. Fluorescent read-out of (A) YPng(C191A) (1mg/mL) and (B) YPng1 (1mg/mL). Silver staining of the same gels, (C) YPng(C191A), (D) YPng1.

The results of the competition experiments were quantified with Imagequant and the results were plotted in Graphpad. The resulting inhibitor-respons curves are given in figure 2.

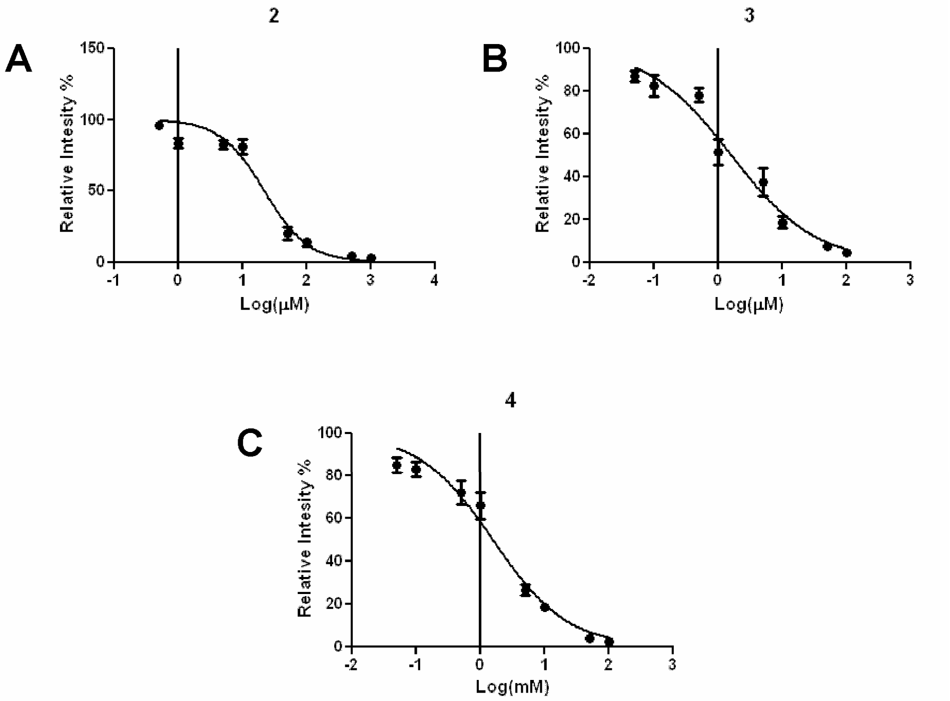


Figure 2. Dose-response curves for inhibitors **2-4**. (A) Z-VAD(OMe)-Fmk **2**; (B) haloacetamide **3**; (C) Epoxysuccinate inhibitor **4**.

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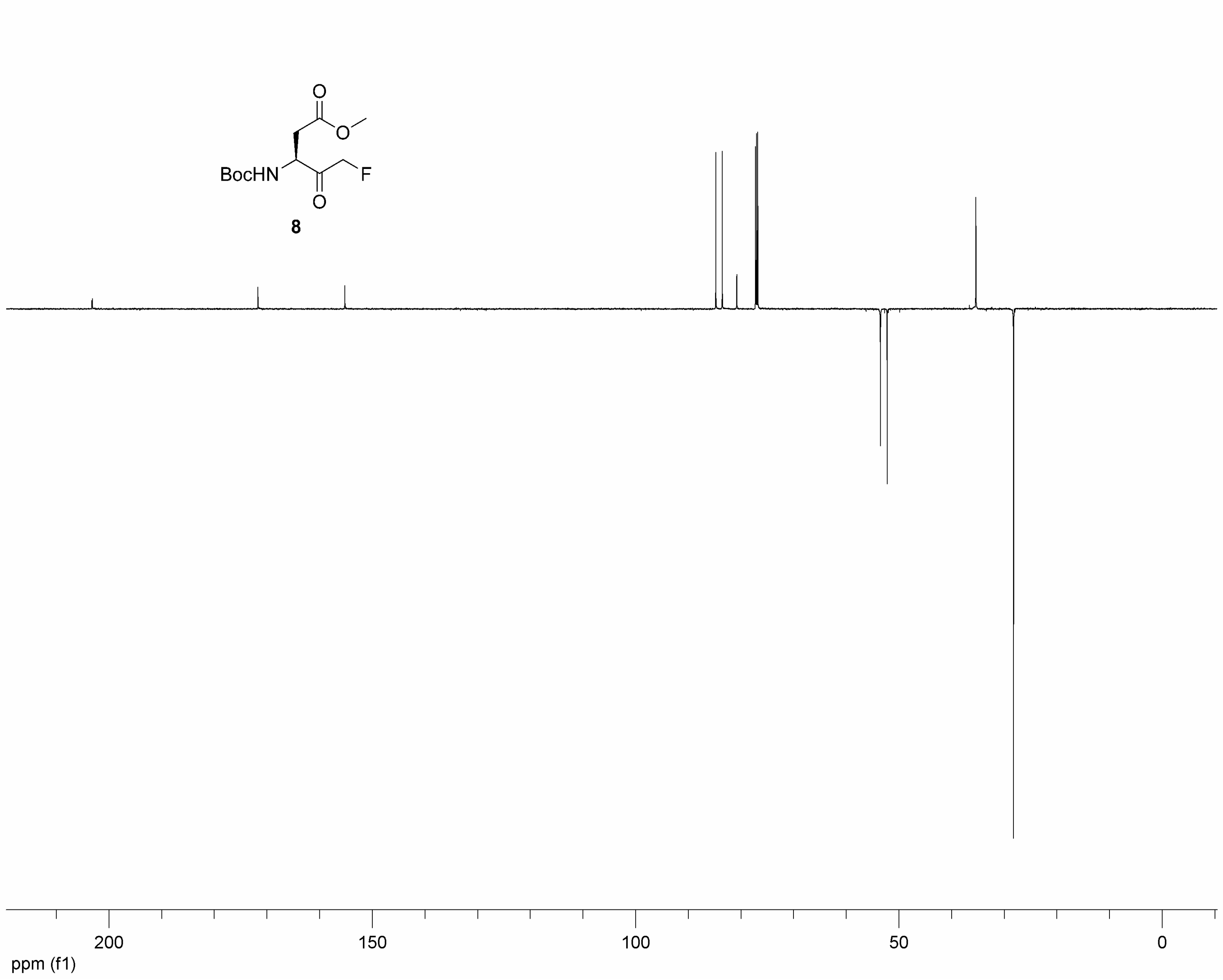
[1] T.S. Morris, S. Frormann, S. Shechosky, C. Lowe, M.S. Lall, V. Gauss-Müller, R.H. Purcell, S.U. Emerson, J.C. Vederas and B.A. Malcolm, *Bioorg. med. chem.*, 1997, **5**, 797.

[2] H. Hojo, J. Watabe, Y. Nakahara, Y. Nakahara, Y. Ito, K. Nabeshima and B.P. Toole, *Tetrahedron Lett.*, 2001, **42**, 3001.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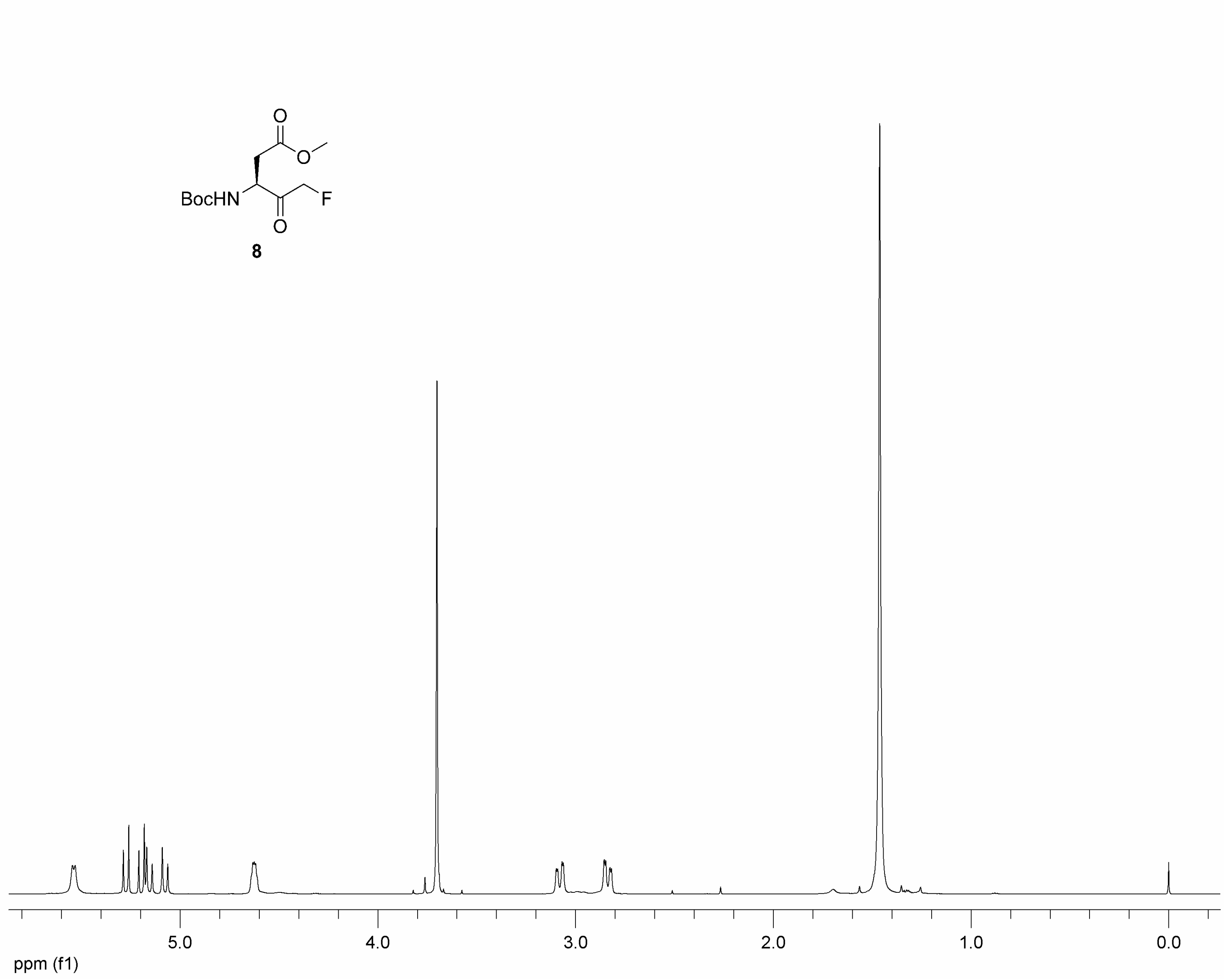
100
50
0
ppm (f1)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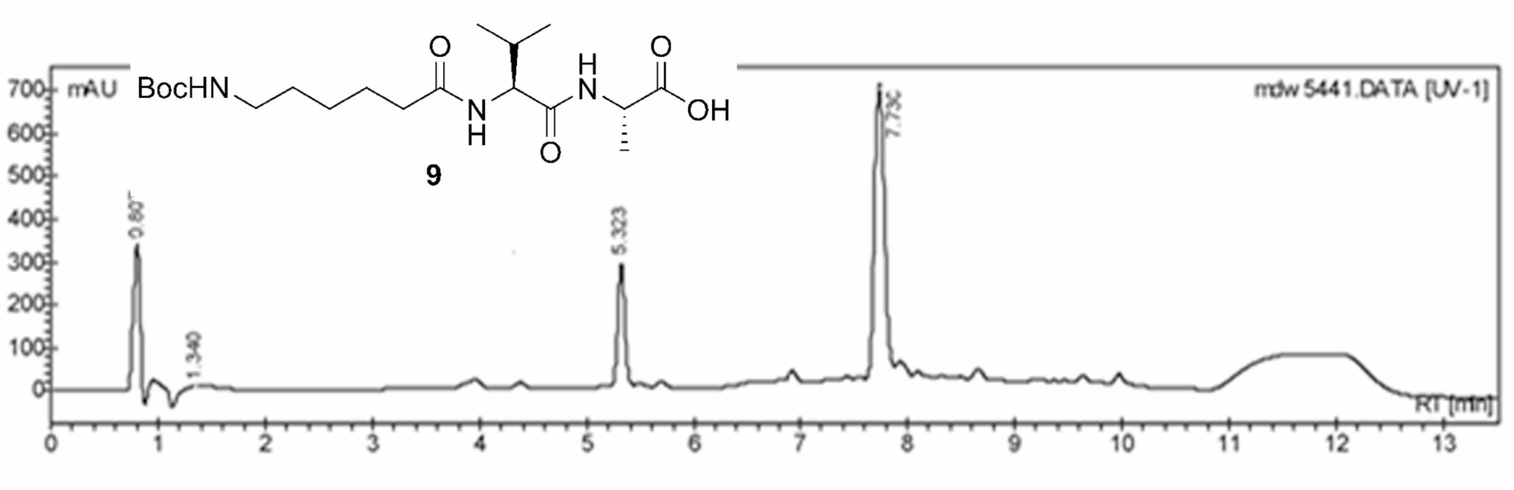
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H
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0.807
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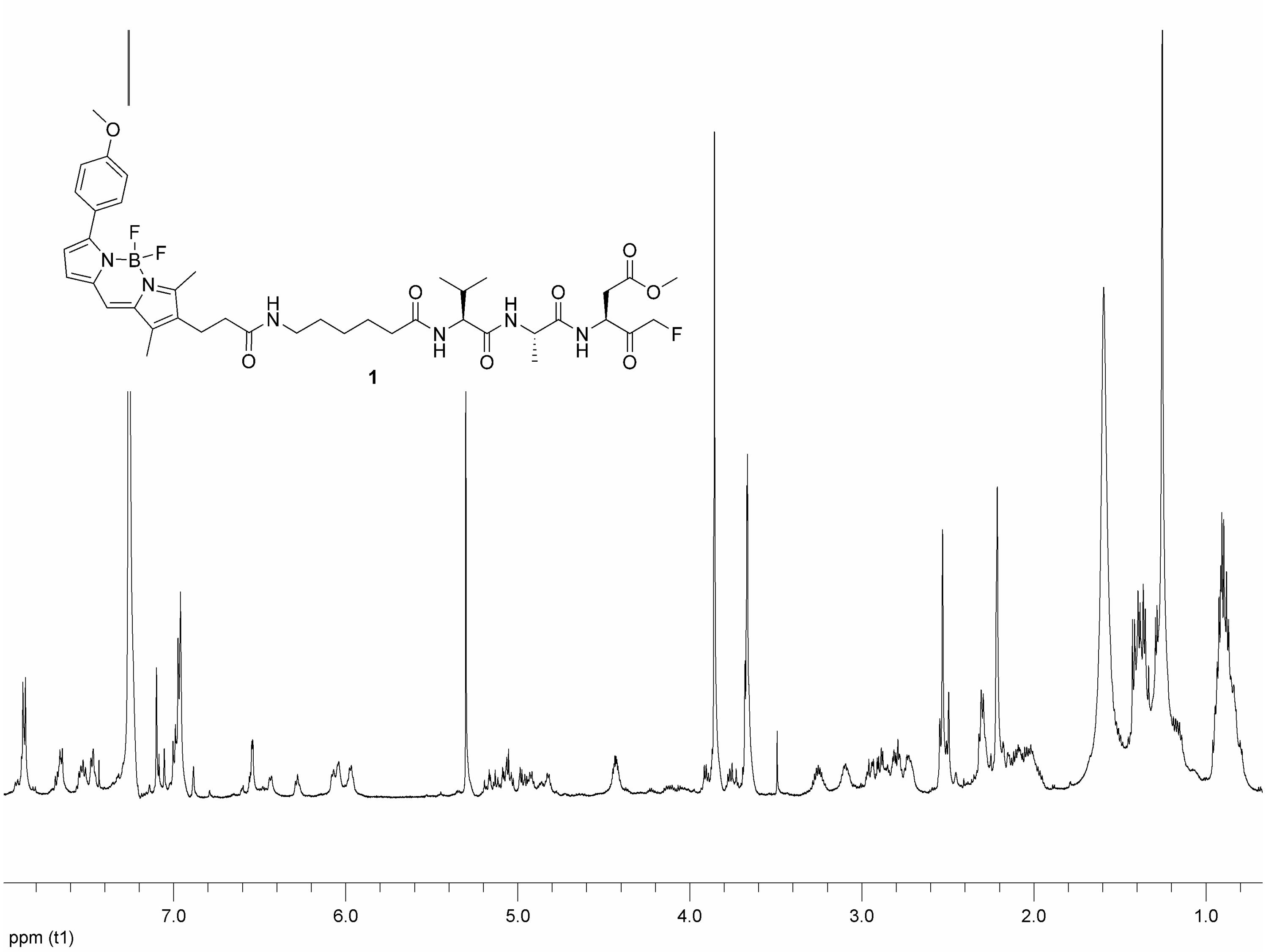
7.730
RT [min]
0
1
2
3
4
5
6
7
8
9
10
11
12
13



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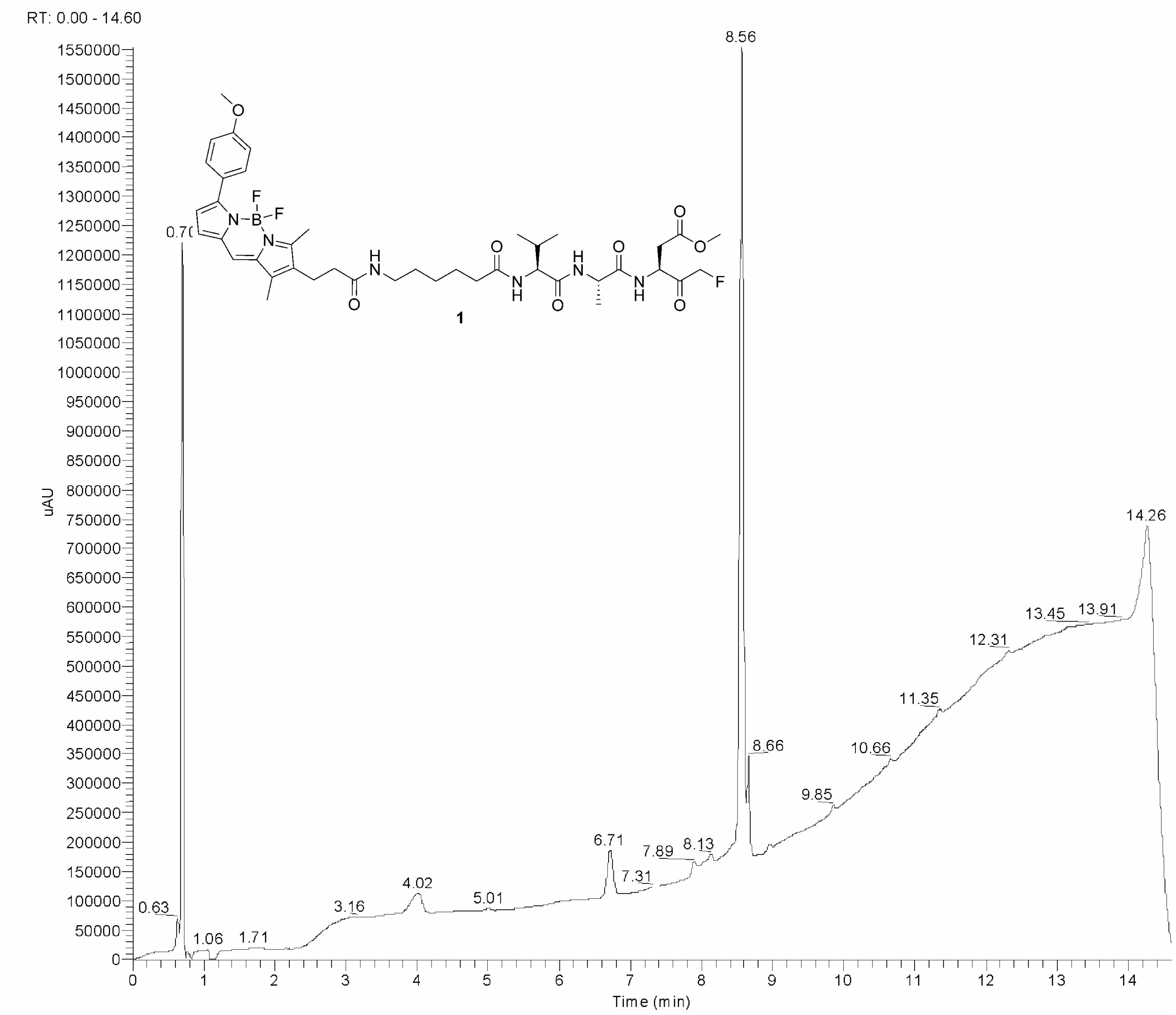
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400000
350000
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250000
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0.7
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1.71
3.16
4.02
5.01
6.71
7.31
7.89
8.13
8.56
8.66
9.85
10.66
11.35
12.31
13.45
13.91
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11
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13
14
Time (min)
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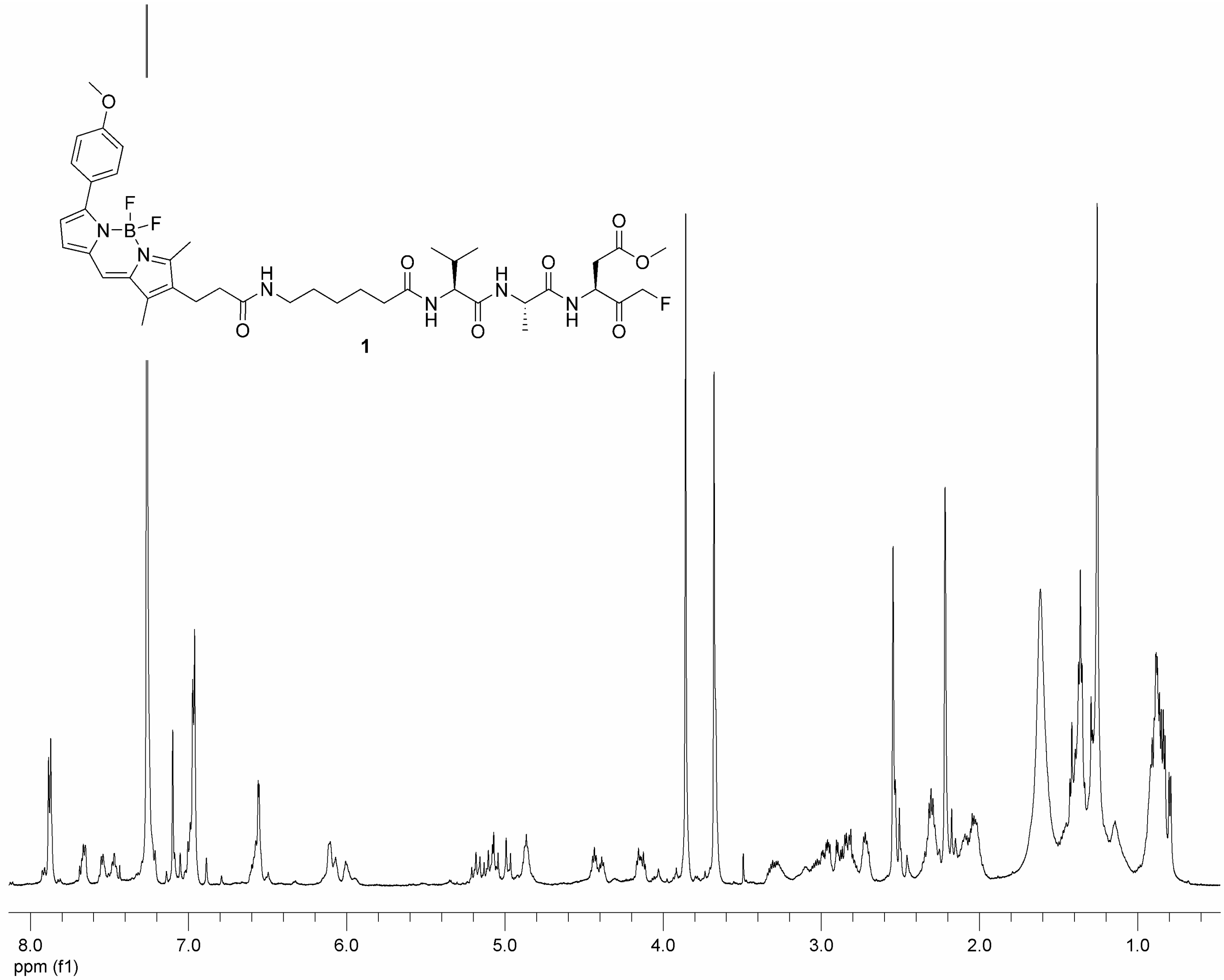

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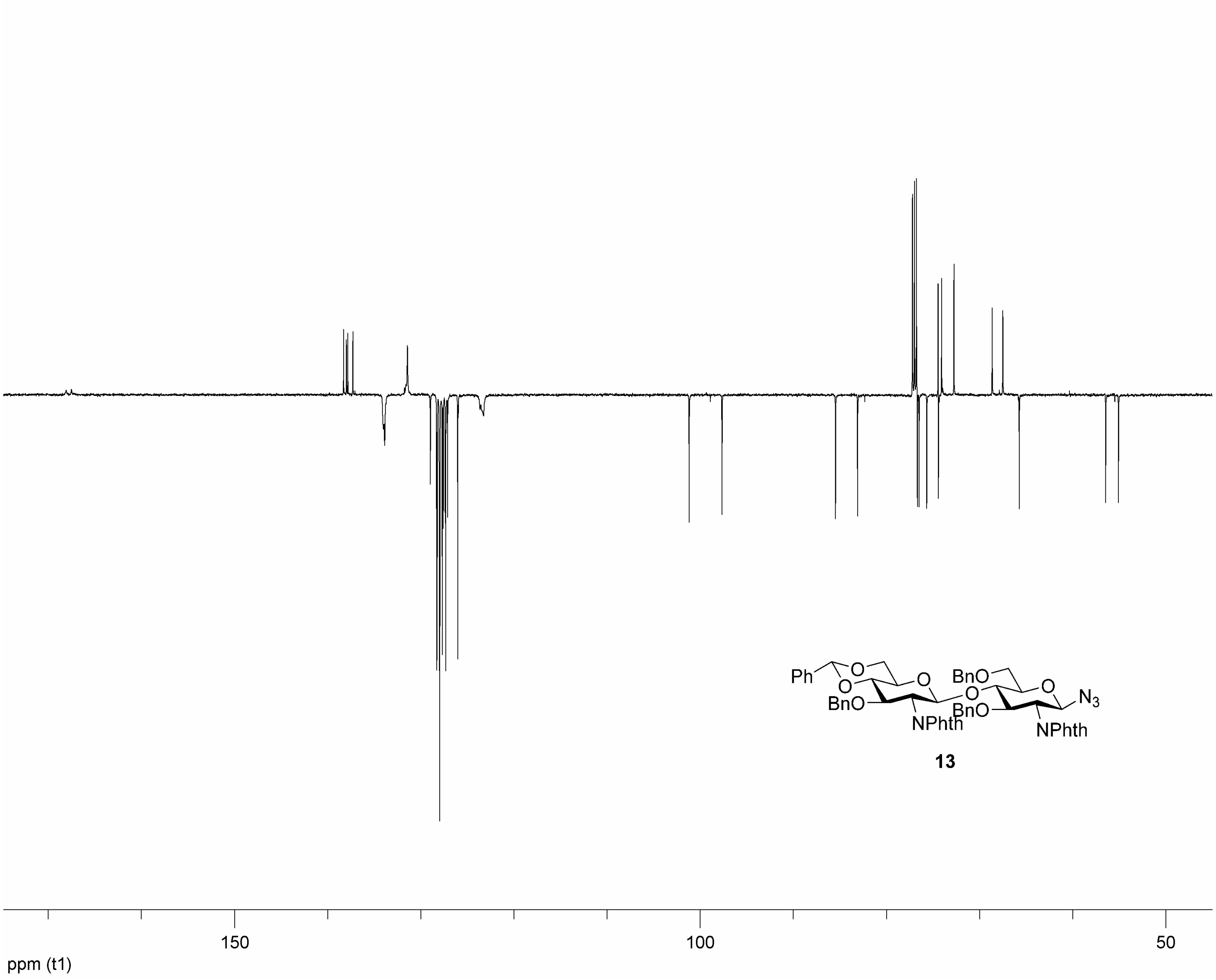

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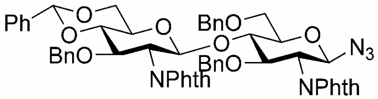
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N3
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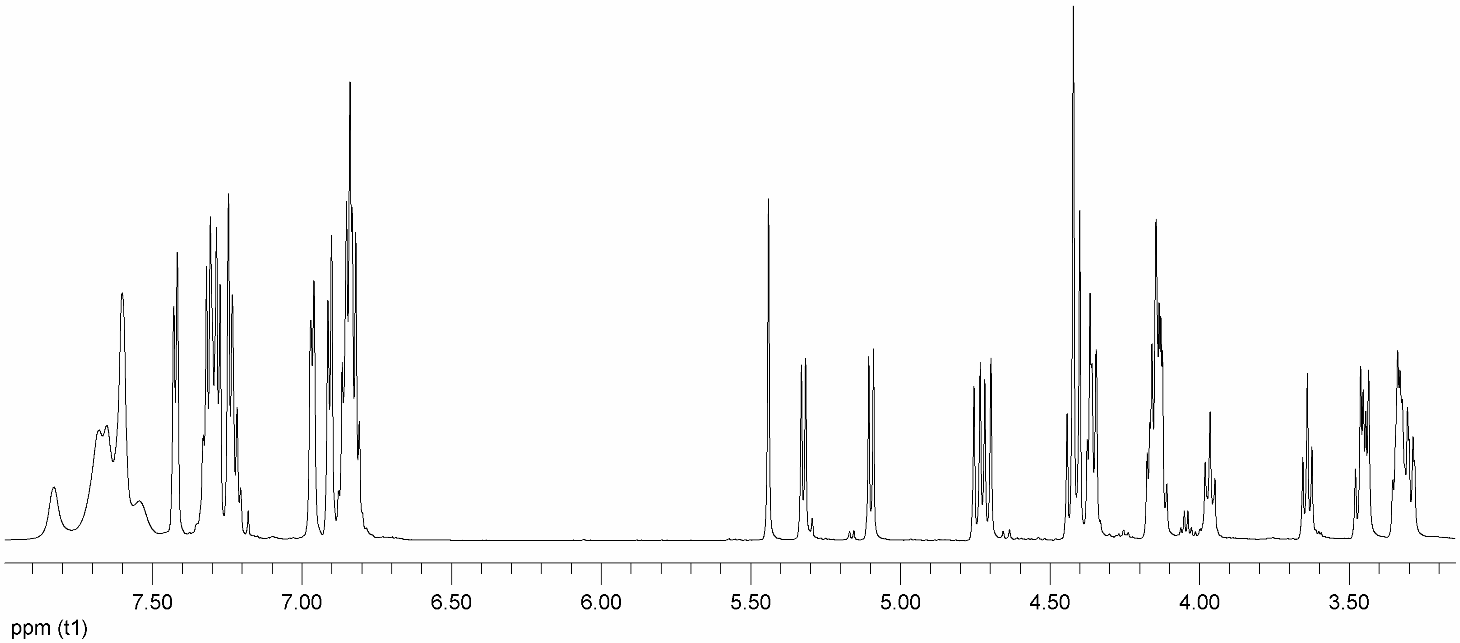
13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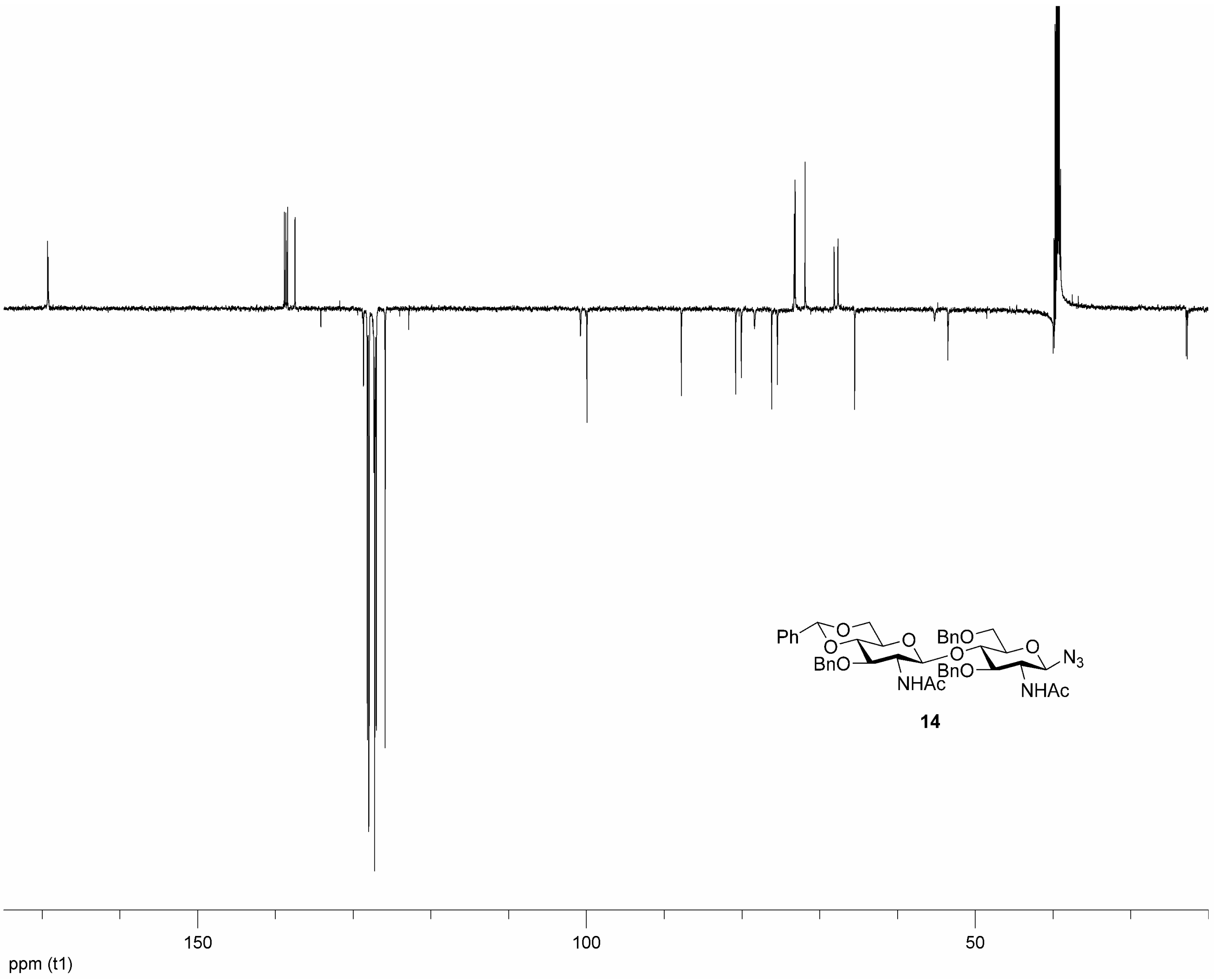
ppm (t1)



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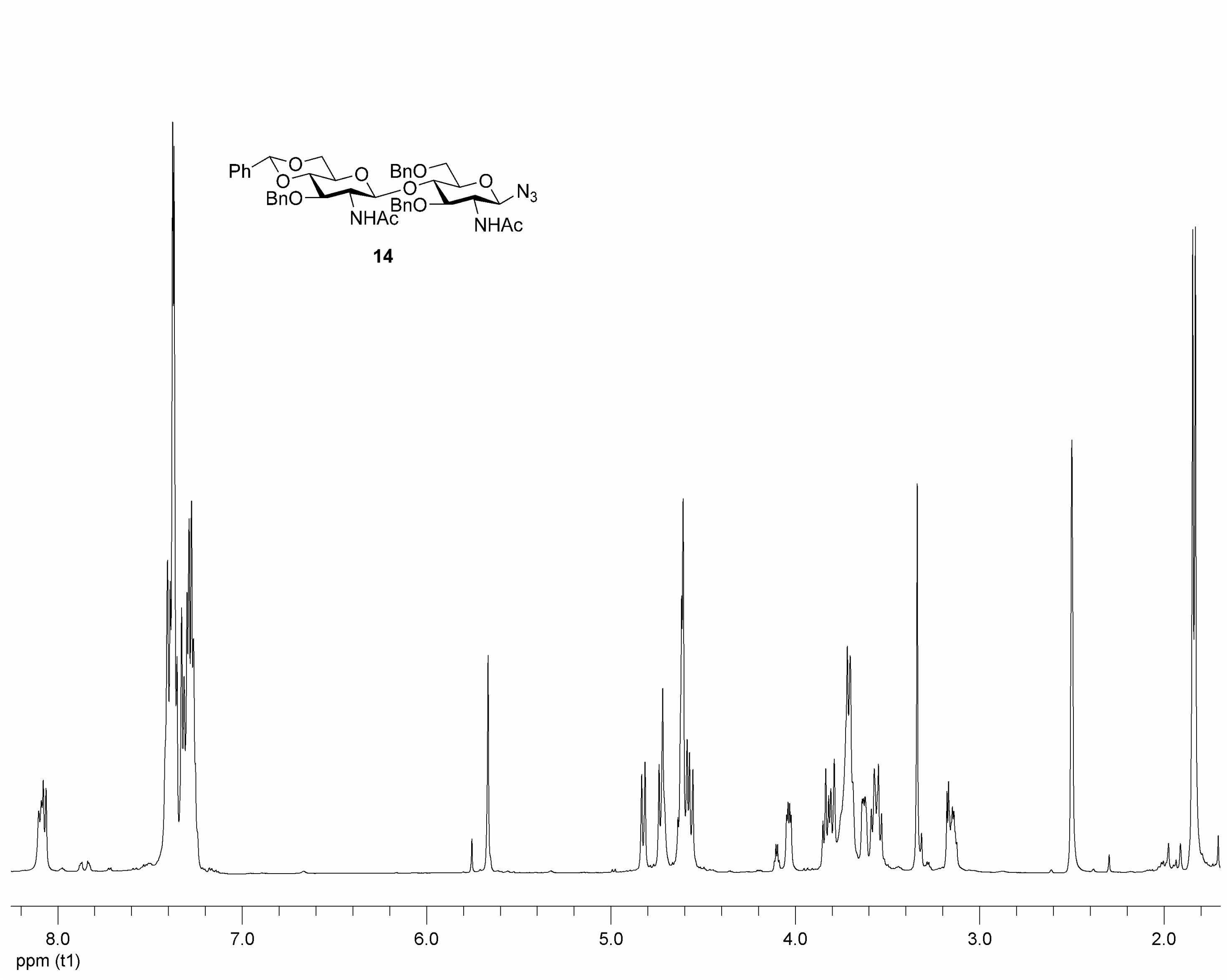

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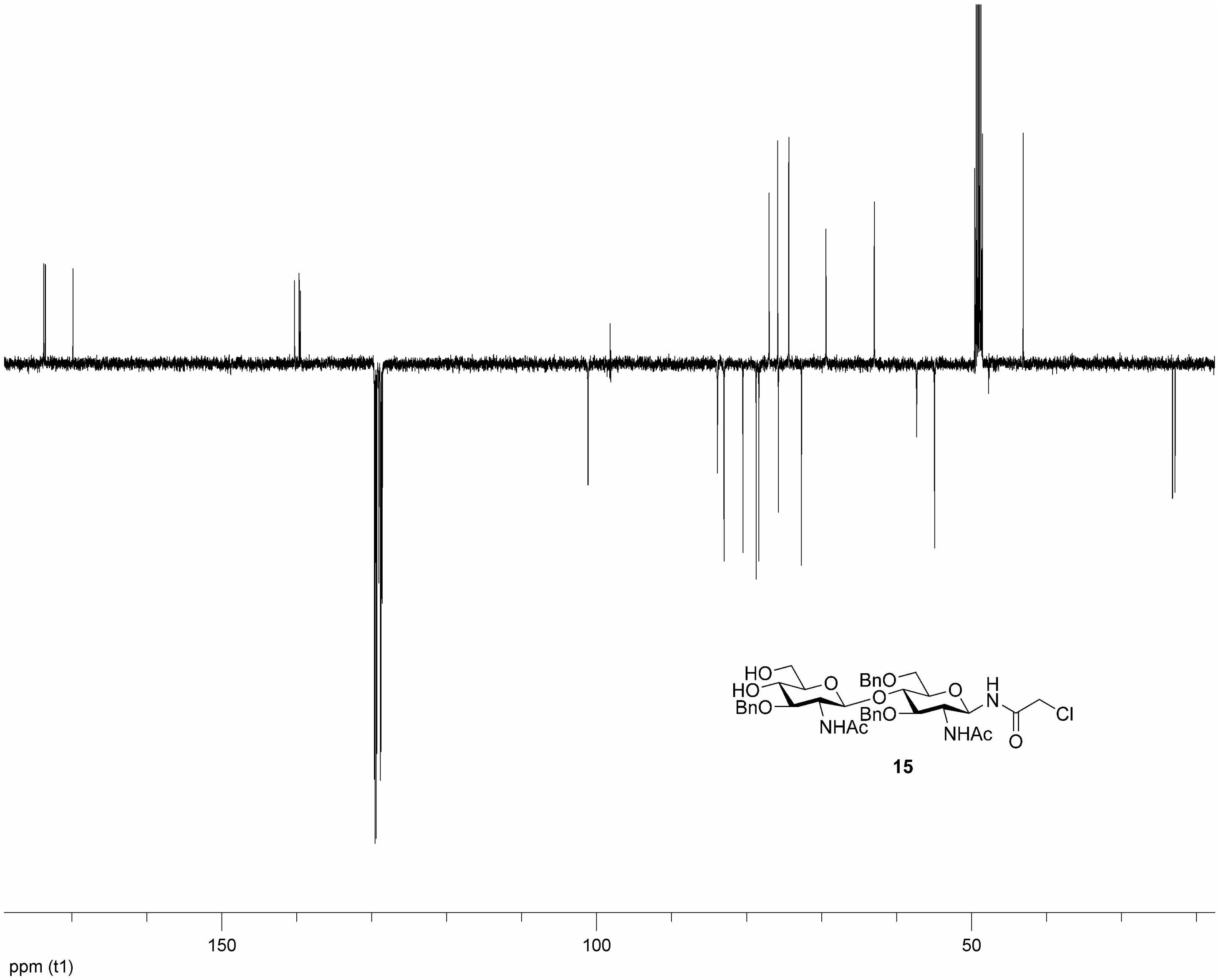

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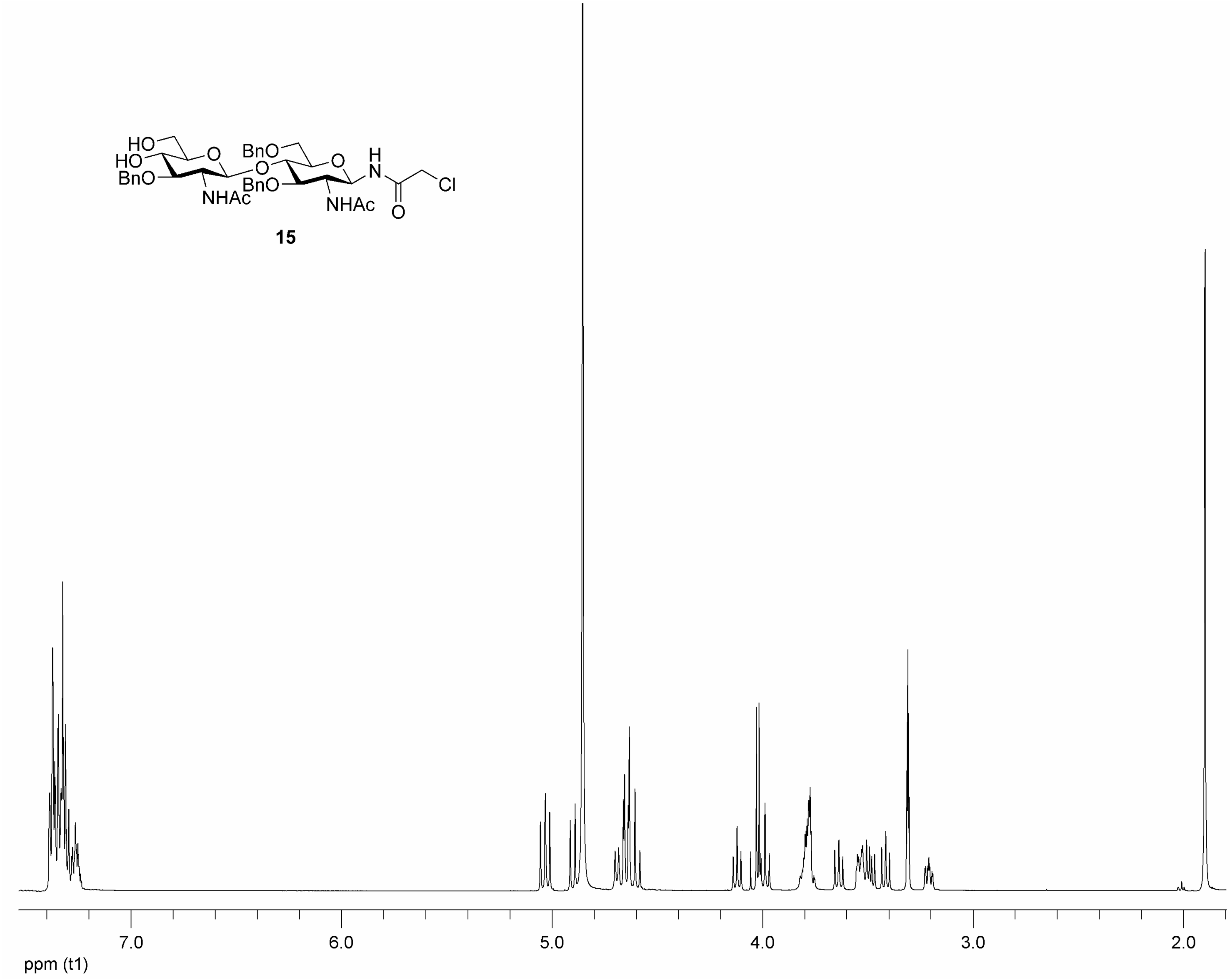
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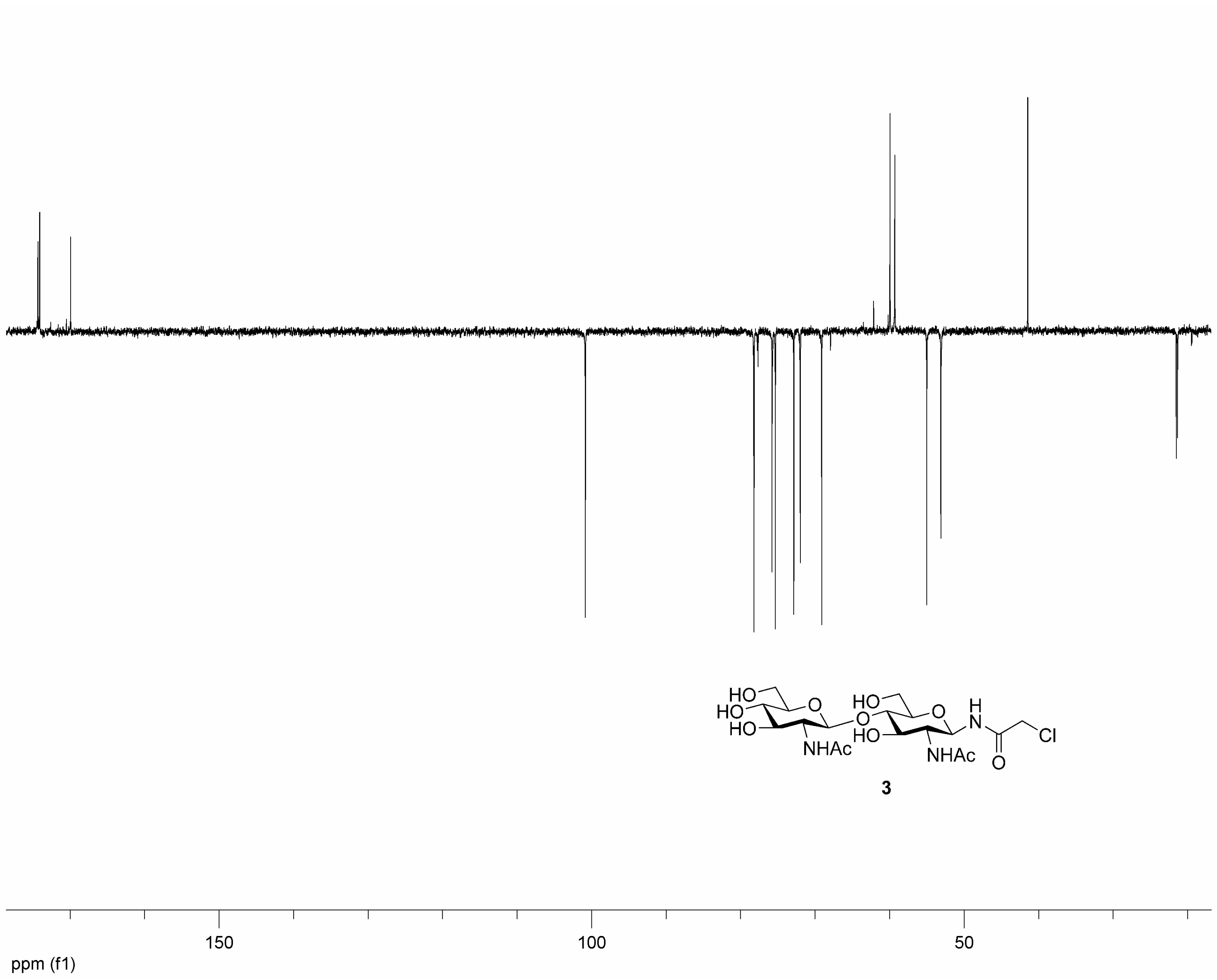
2.0
ppm (t1)



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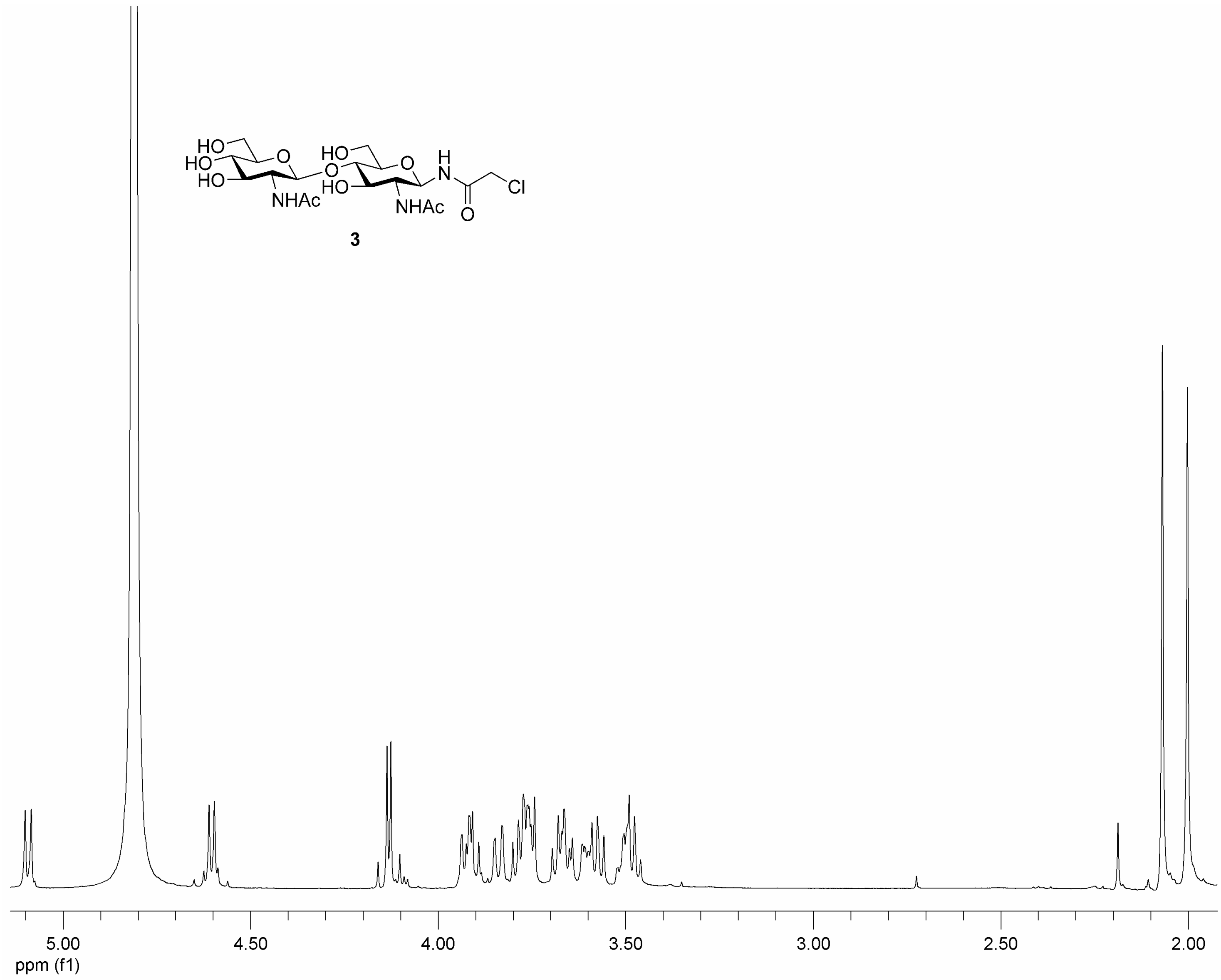
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Cl
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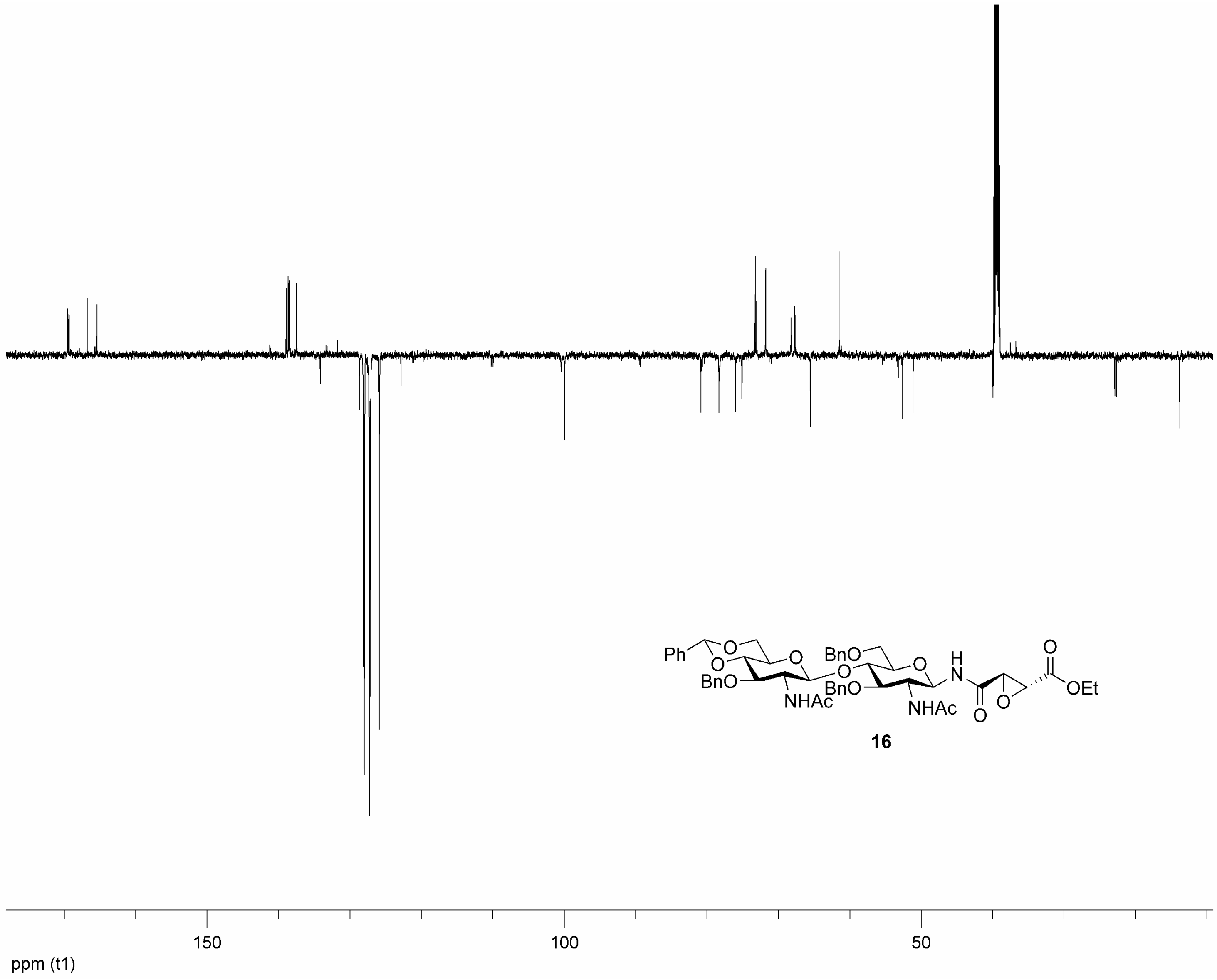
2.00
ppm (f1)



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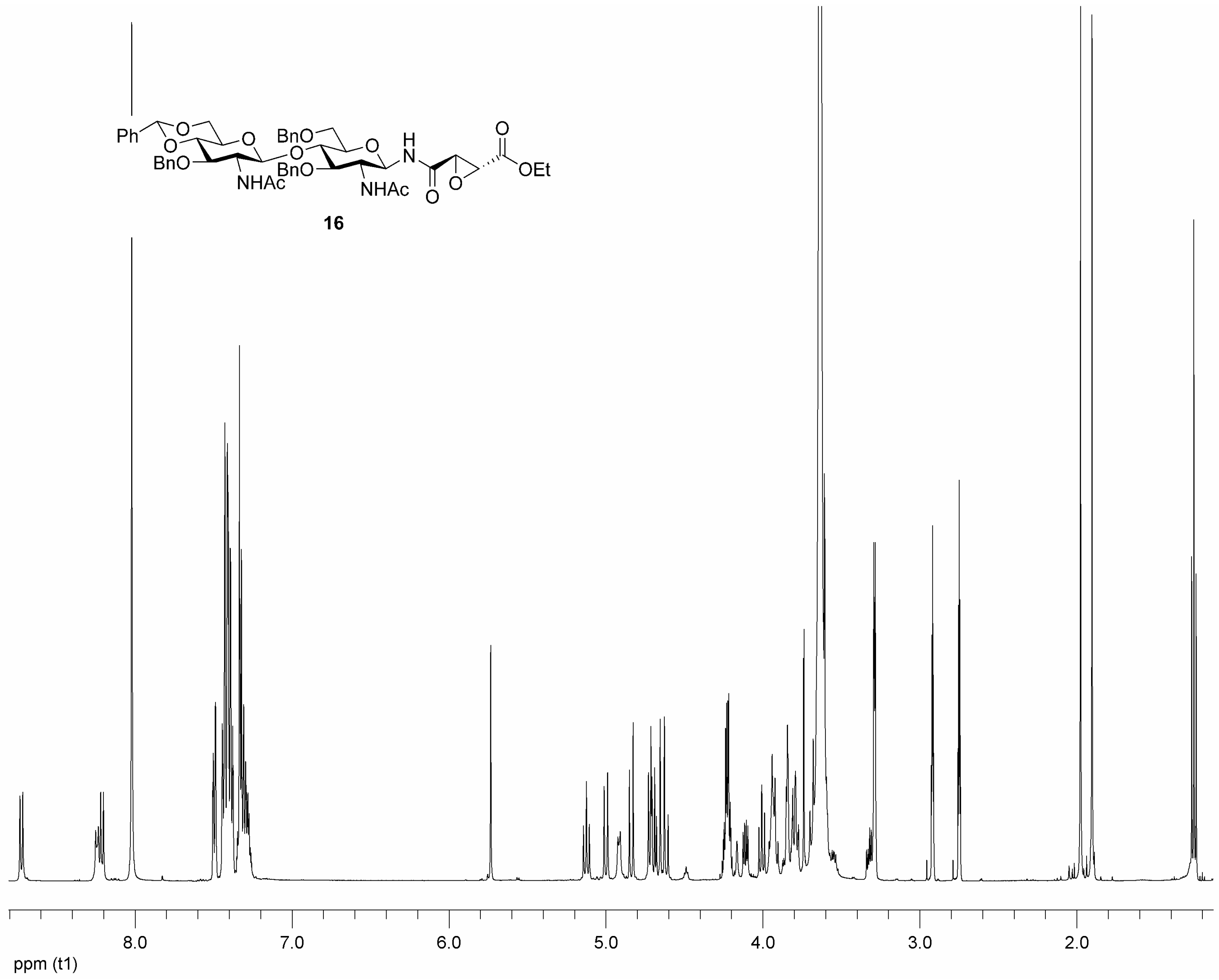
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NHAc
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N
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3.0
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ppm (t1)

16

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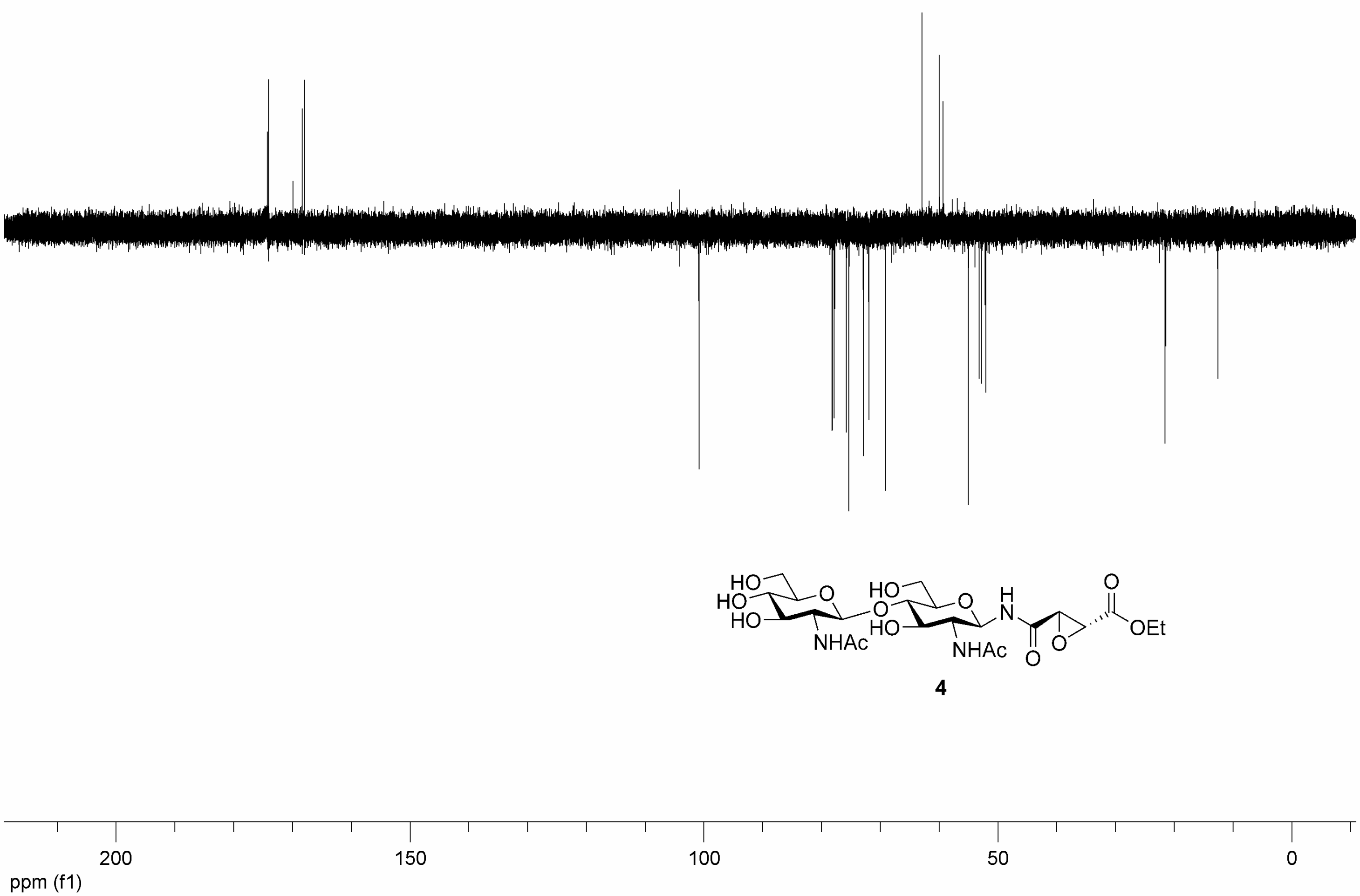

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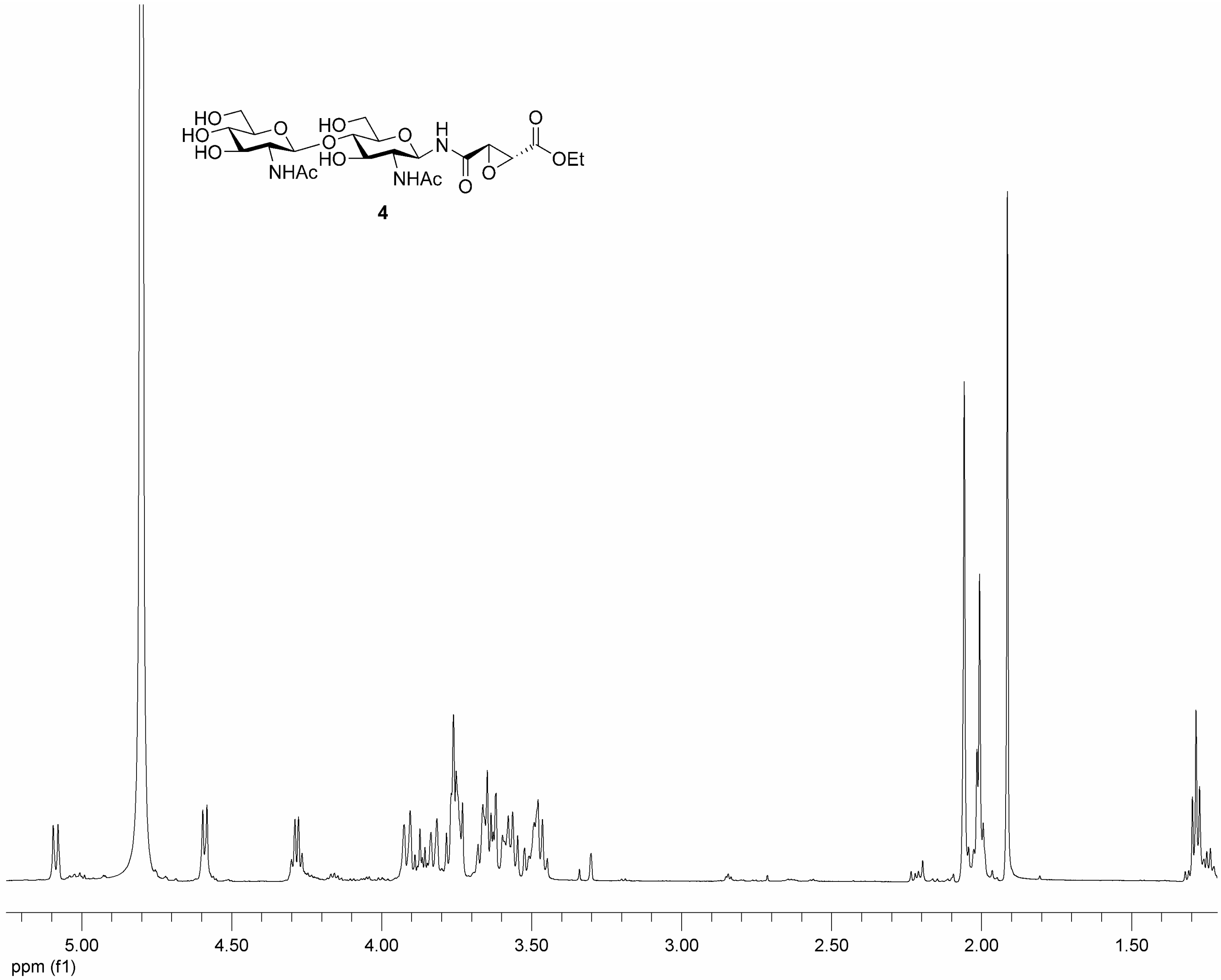

4

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NHAc
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H
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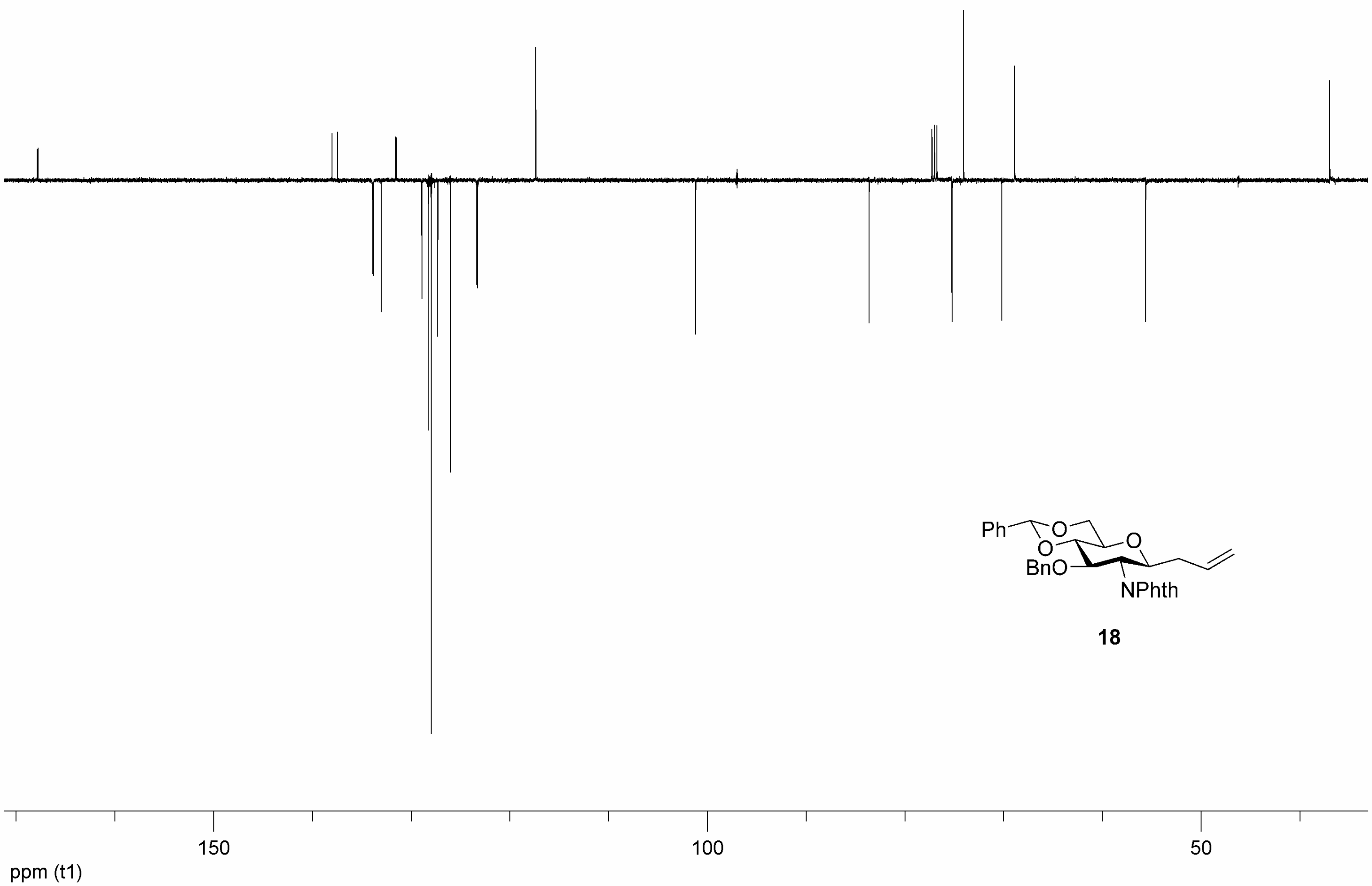
ppm (f1)



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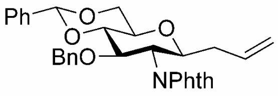

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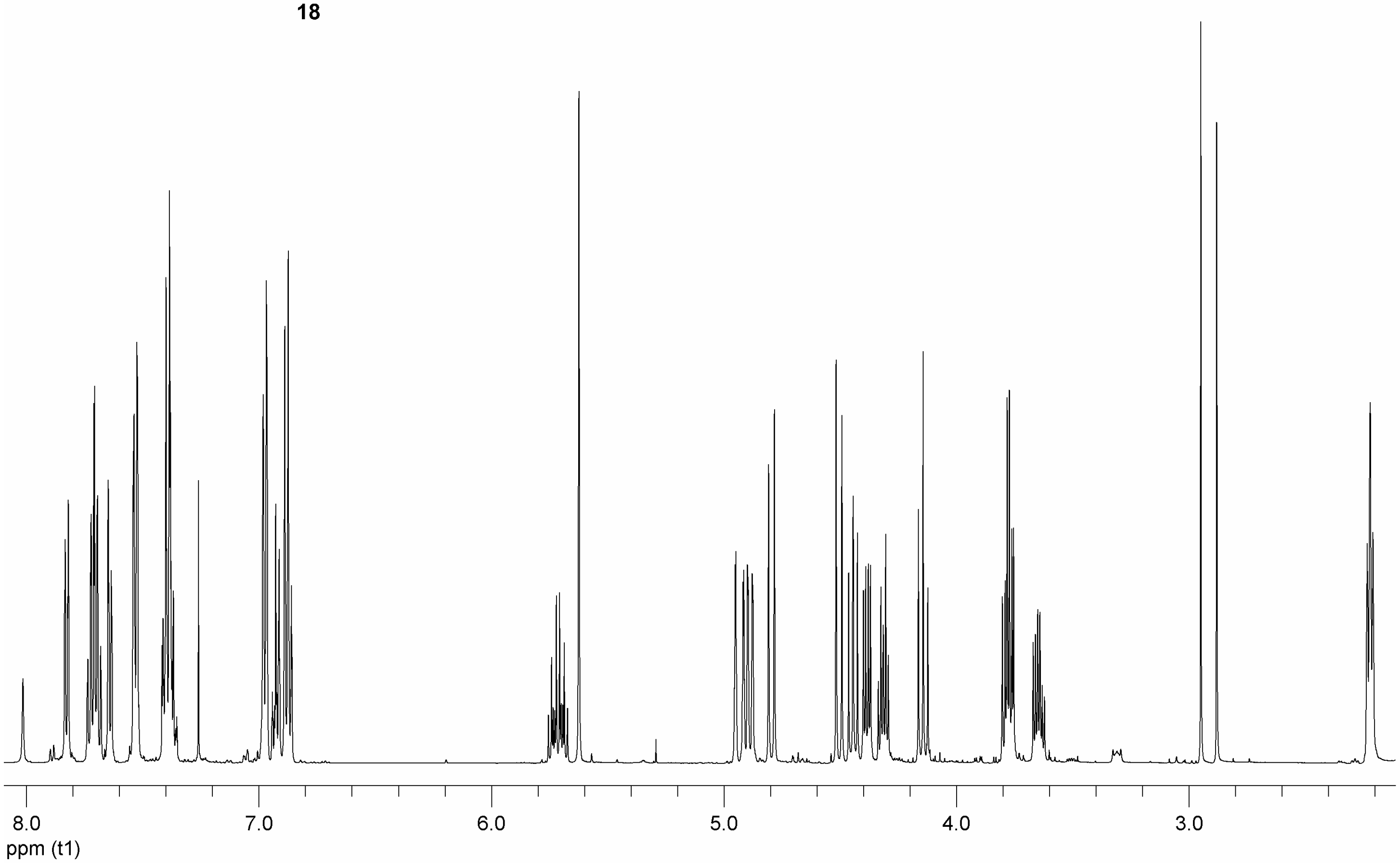

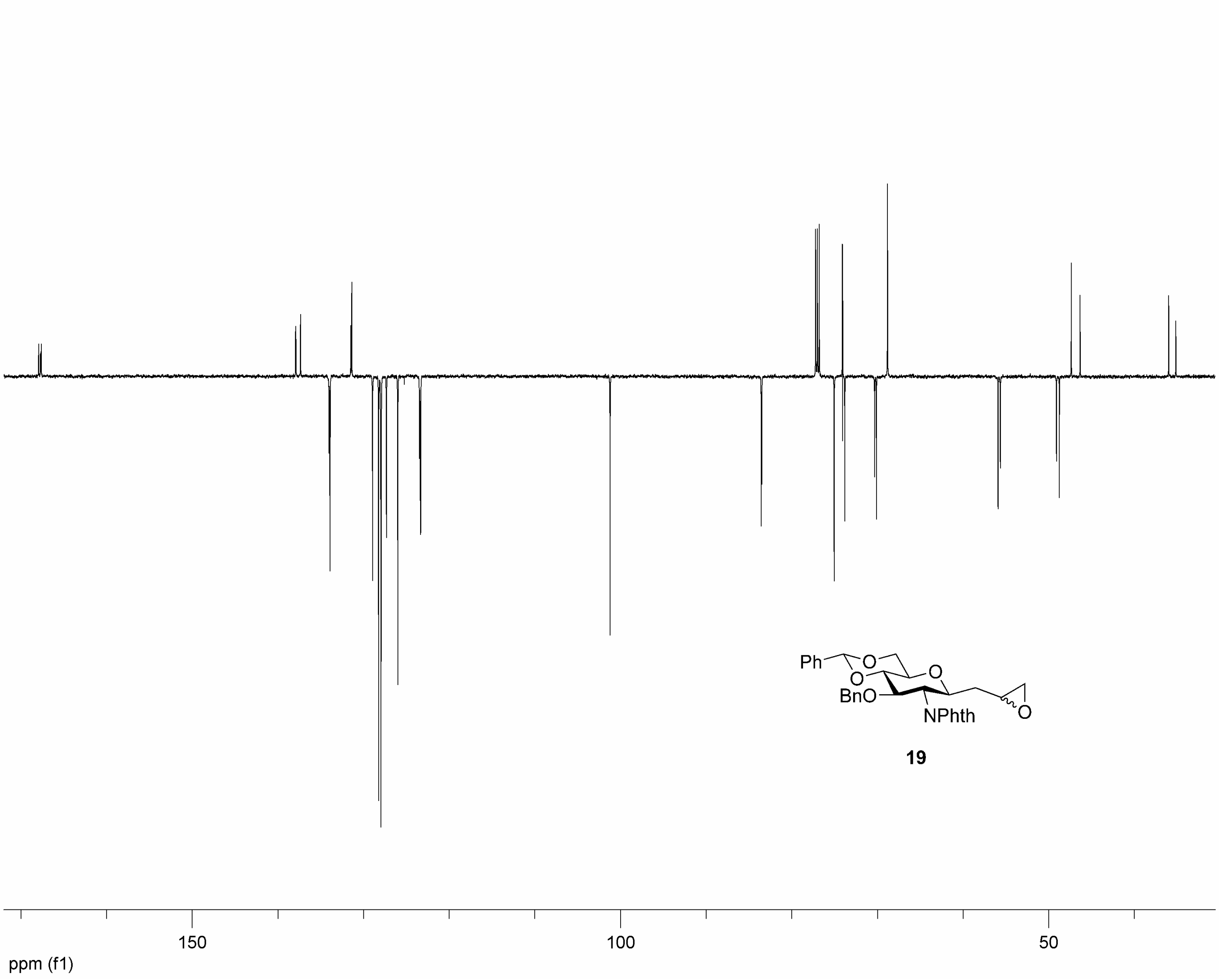

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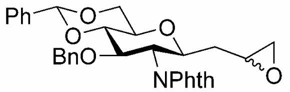

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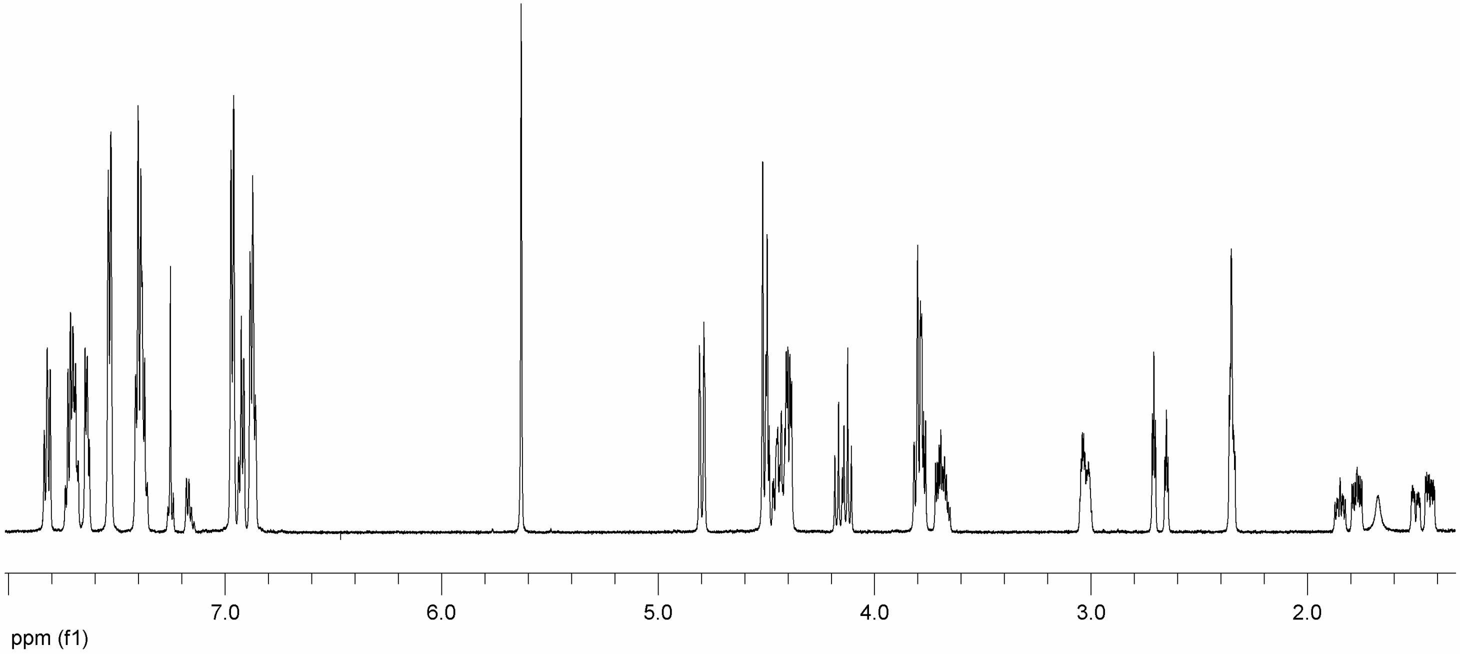

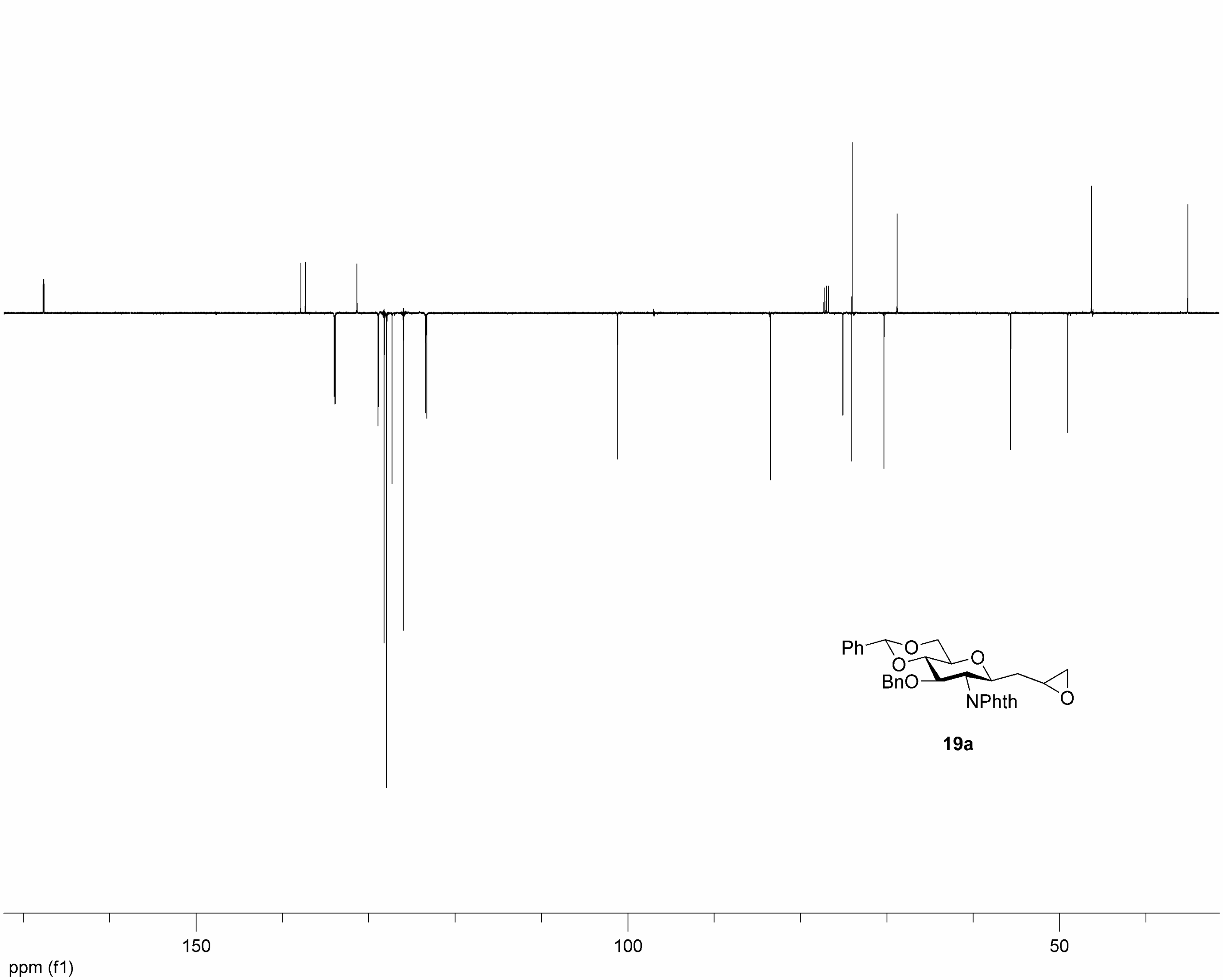
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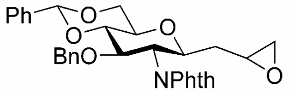
ppm (f1)



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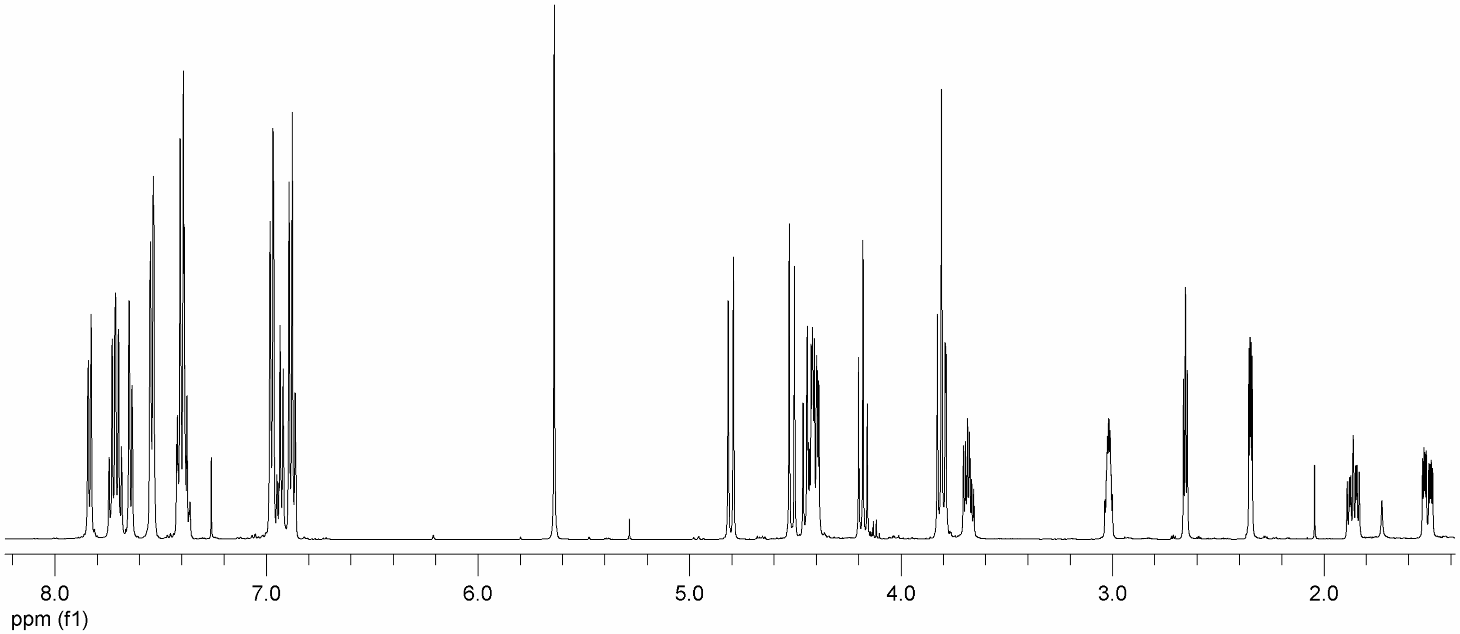

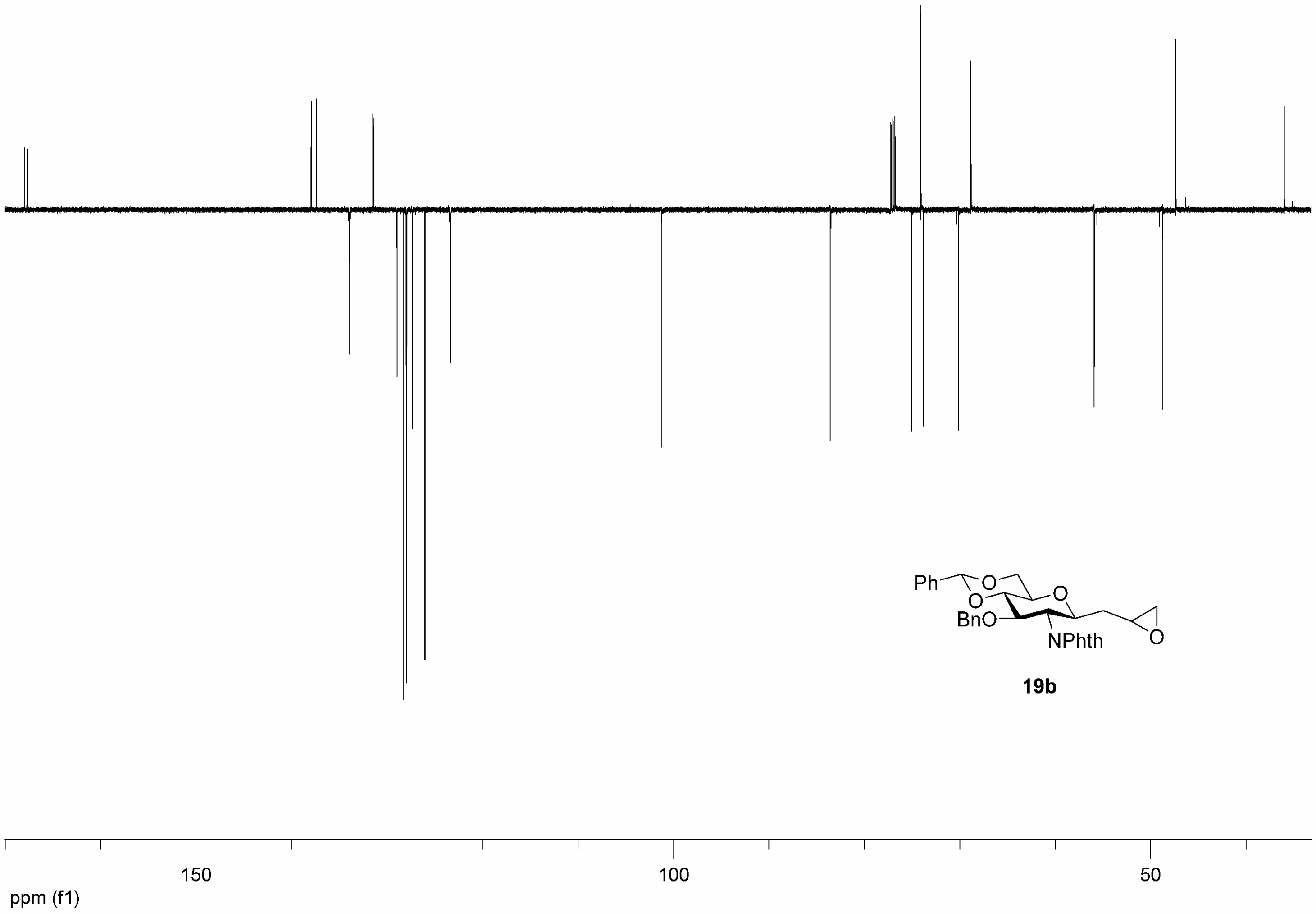

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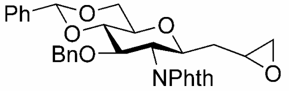

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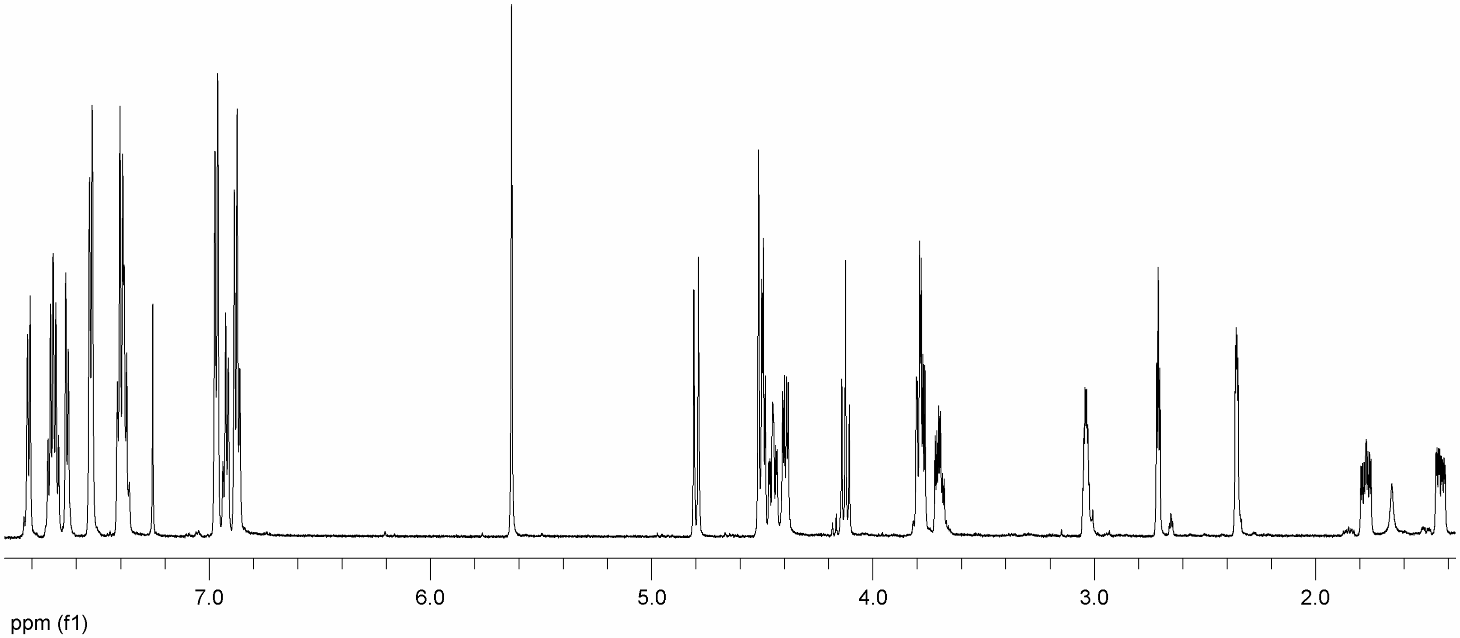

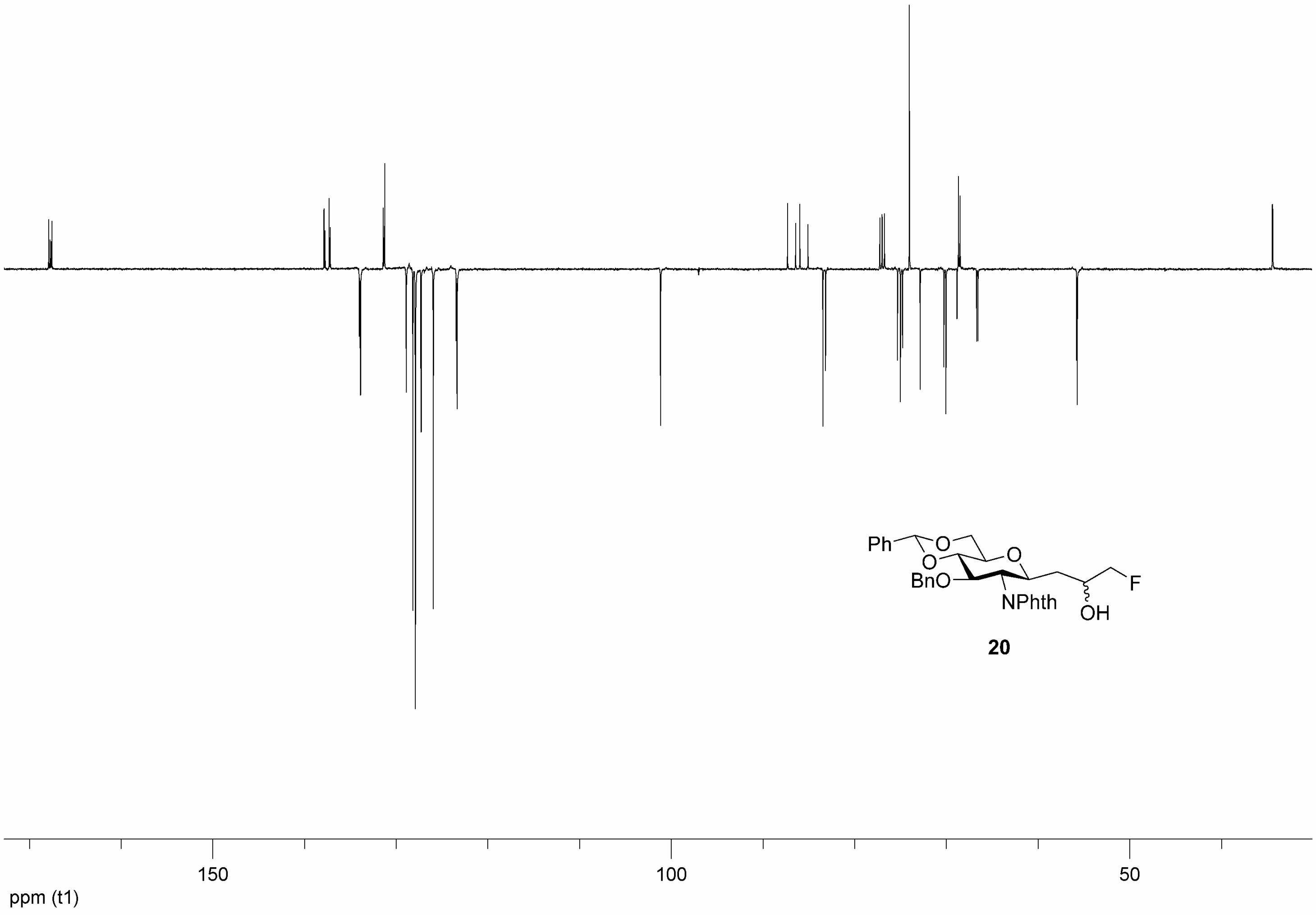

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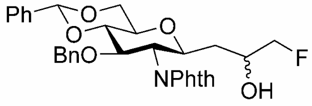

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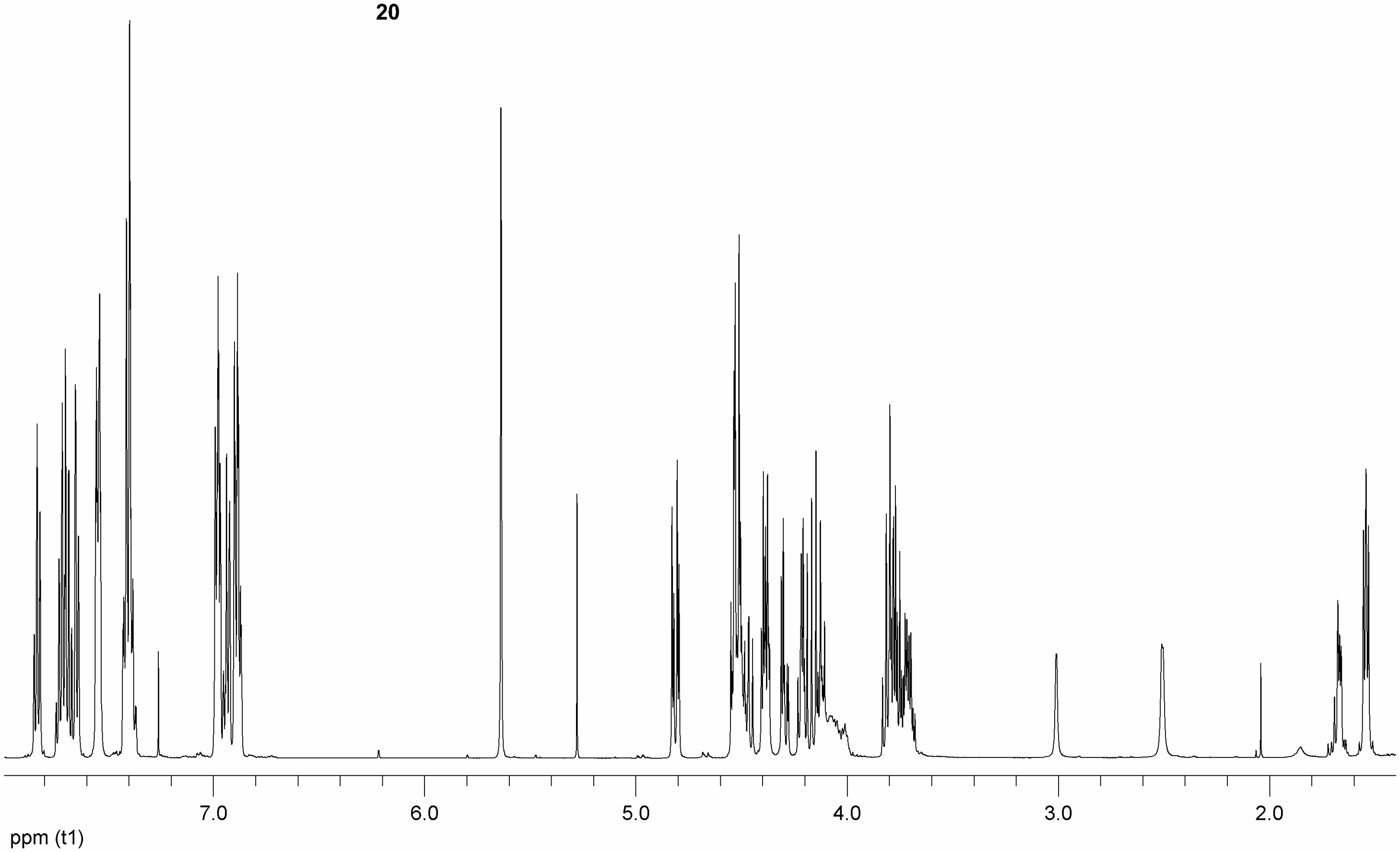

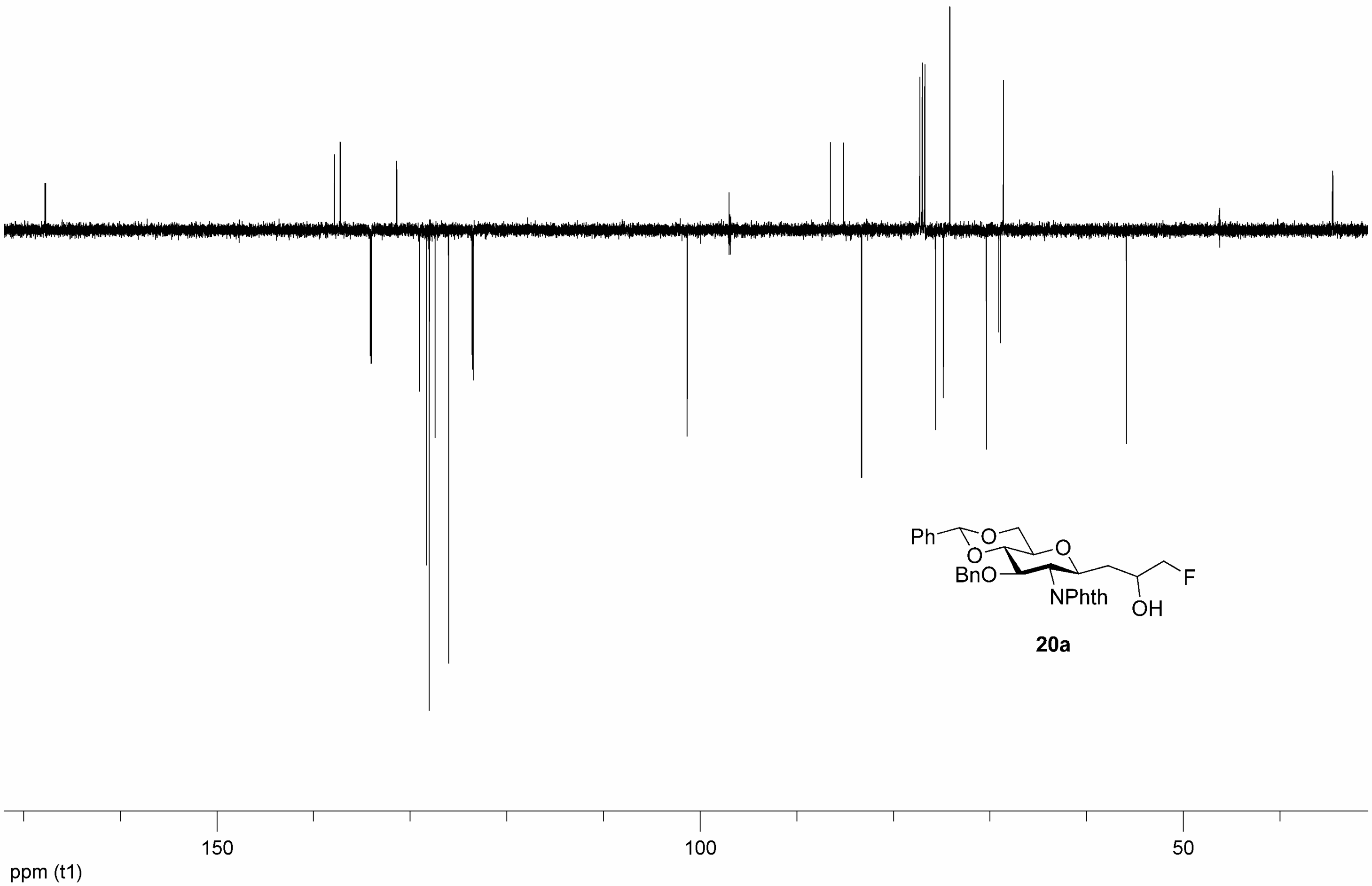

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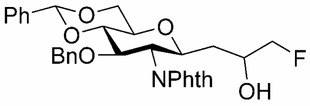

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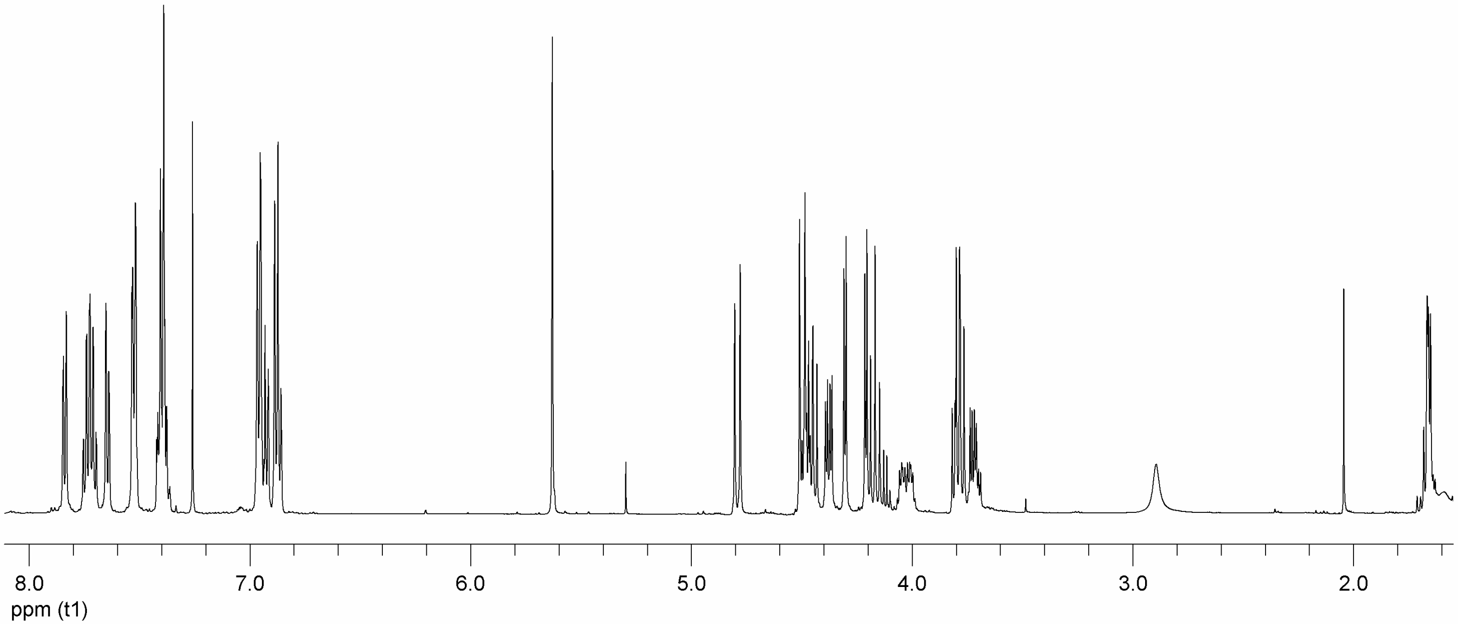

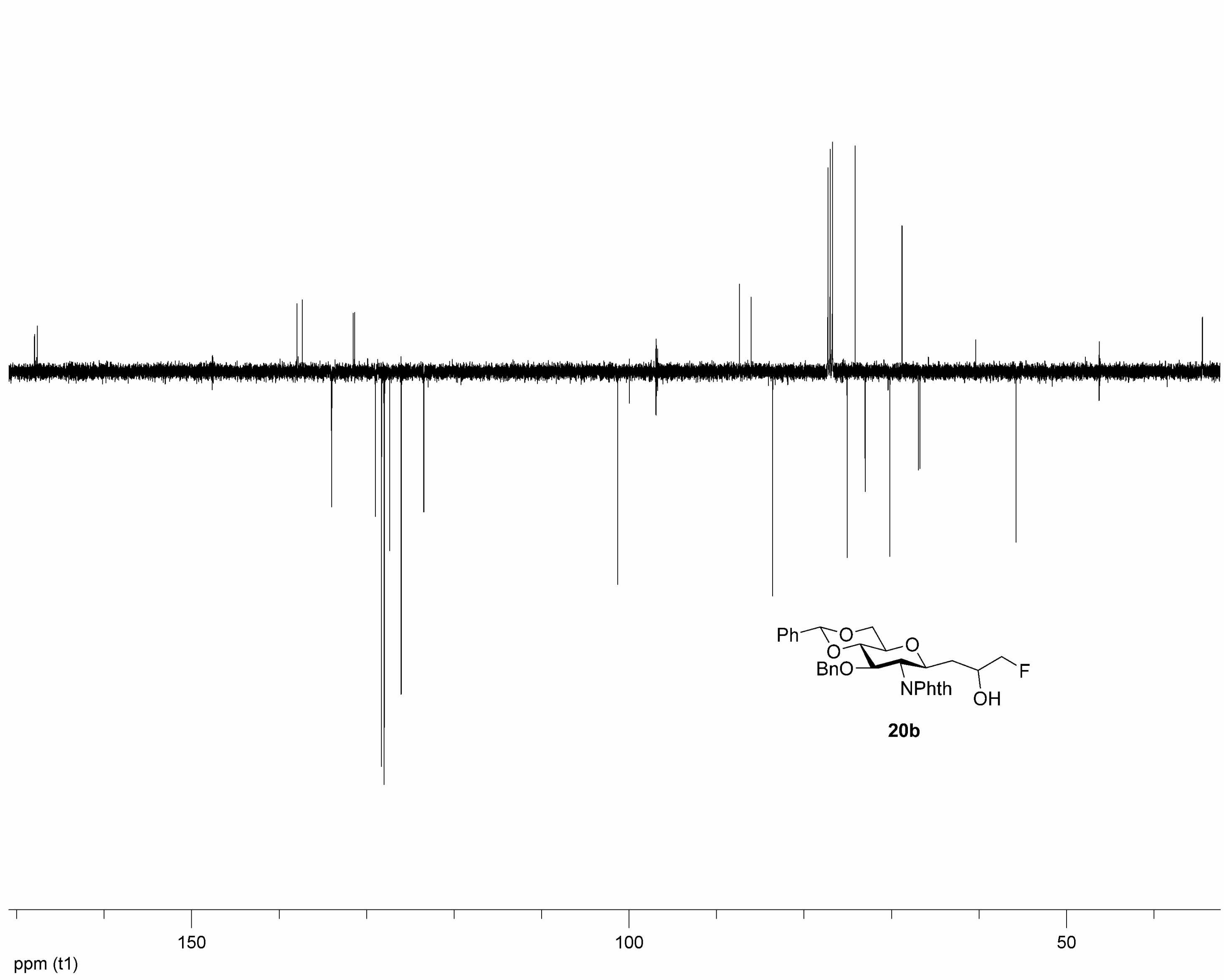

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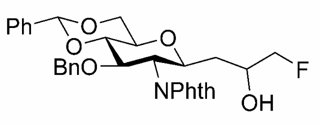

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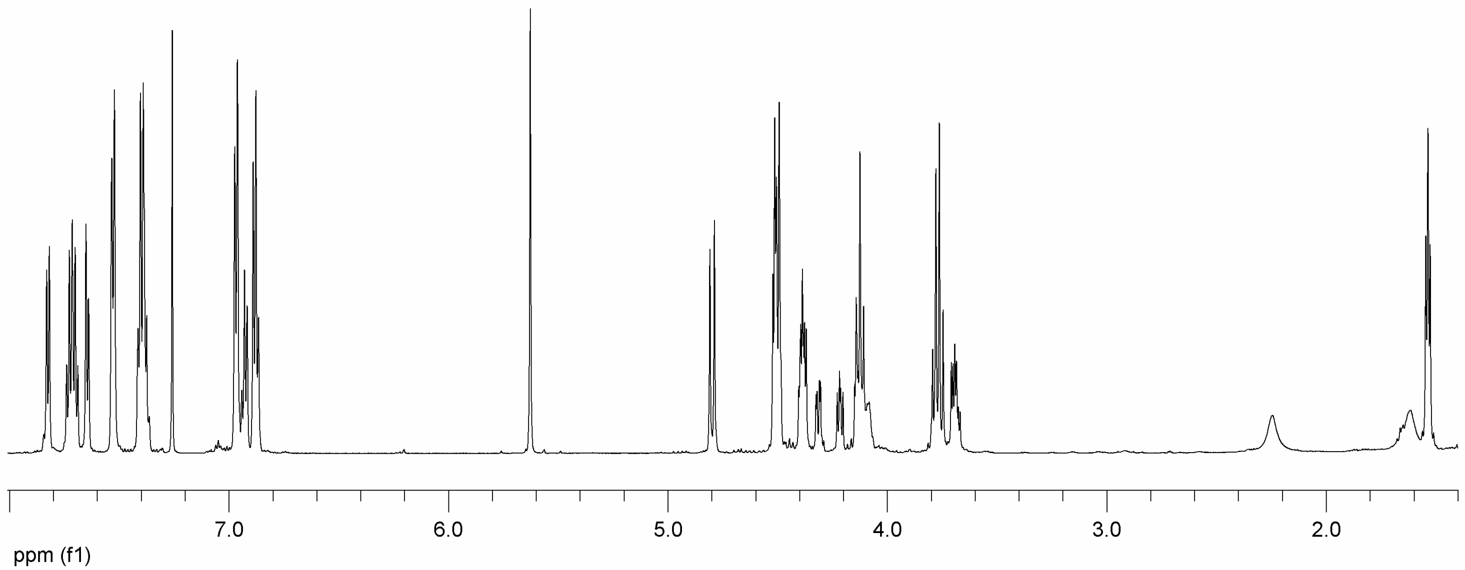

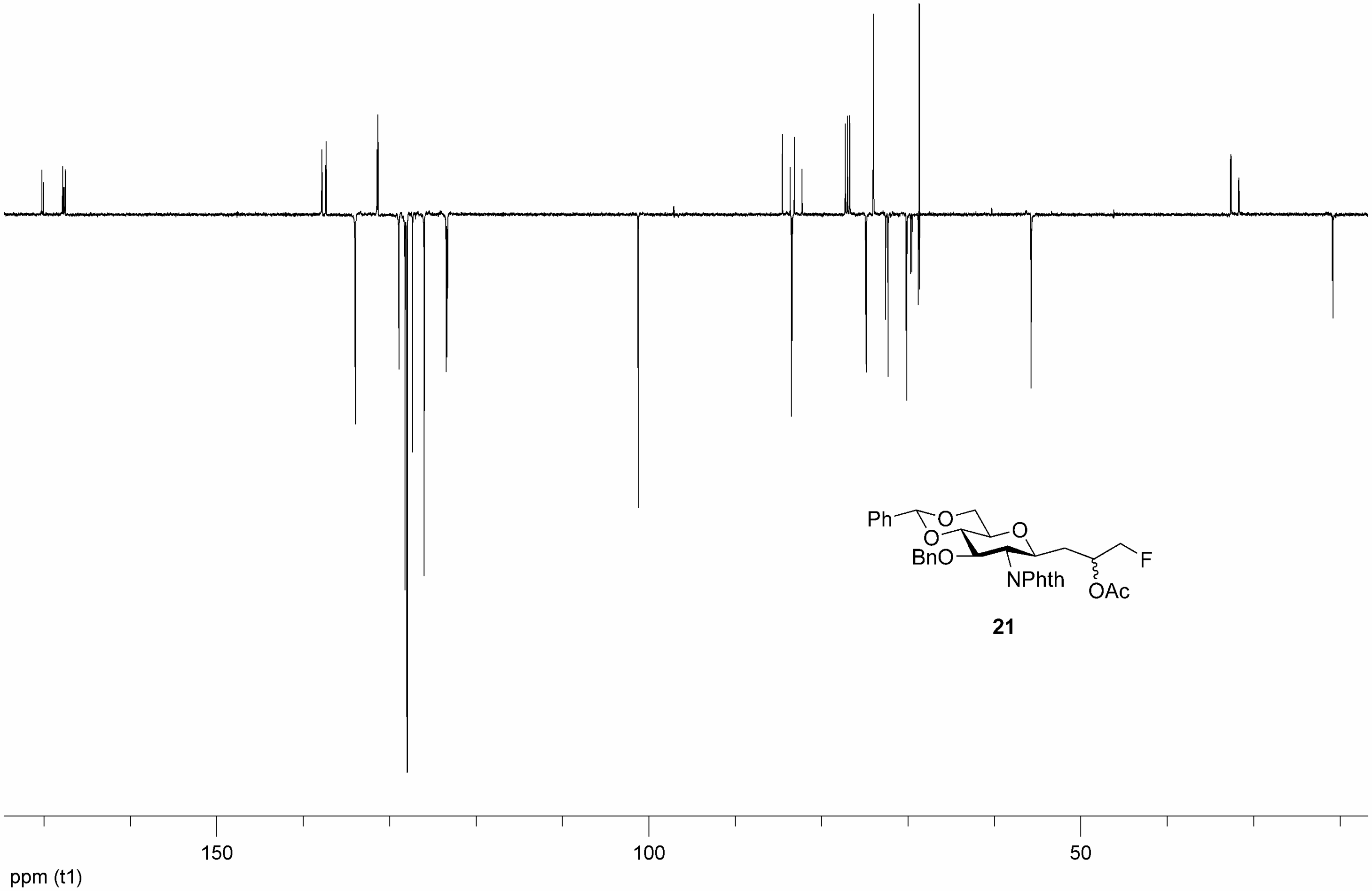
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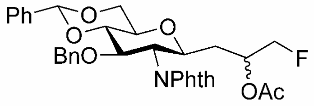

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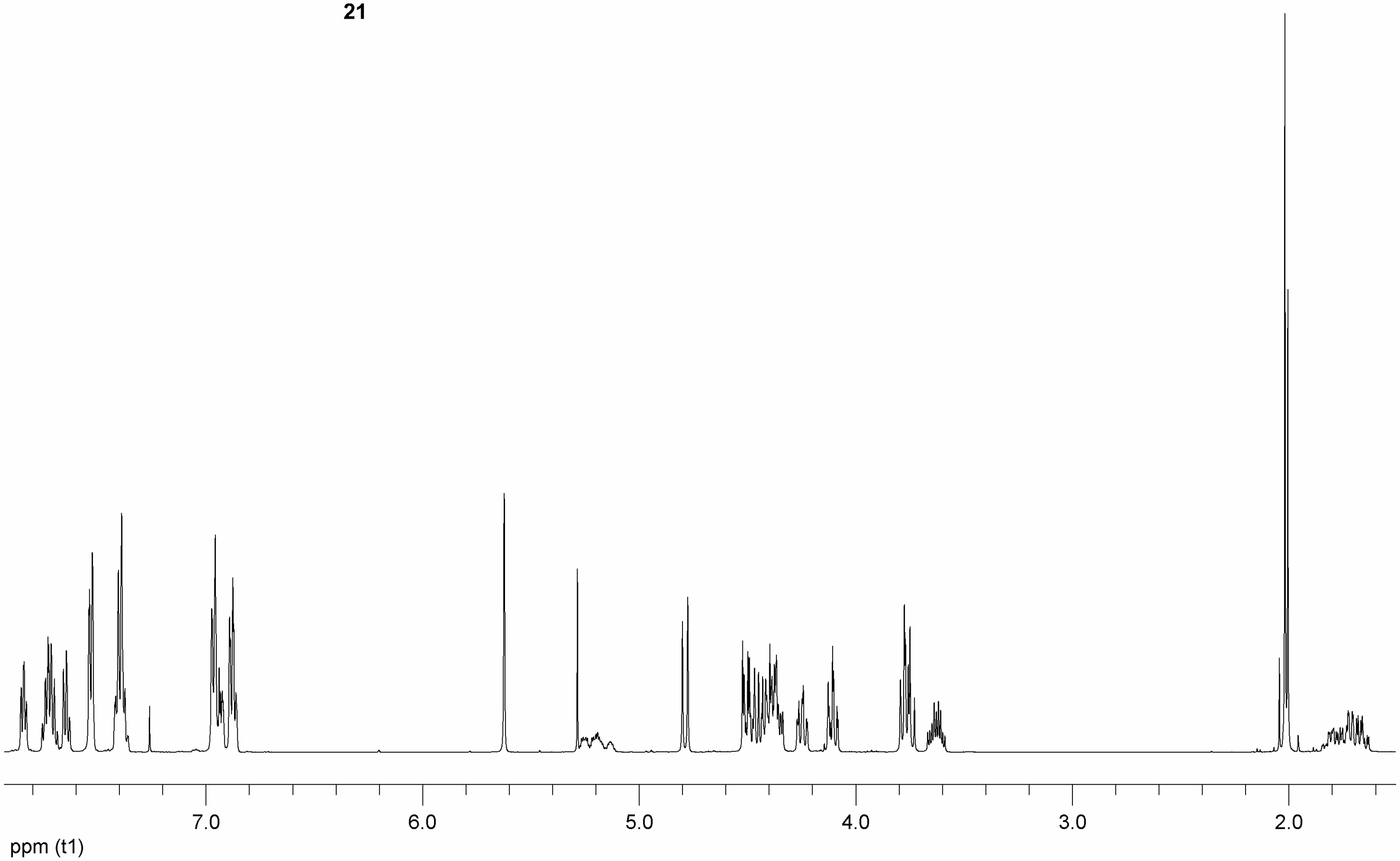

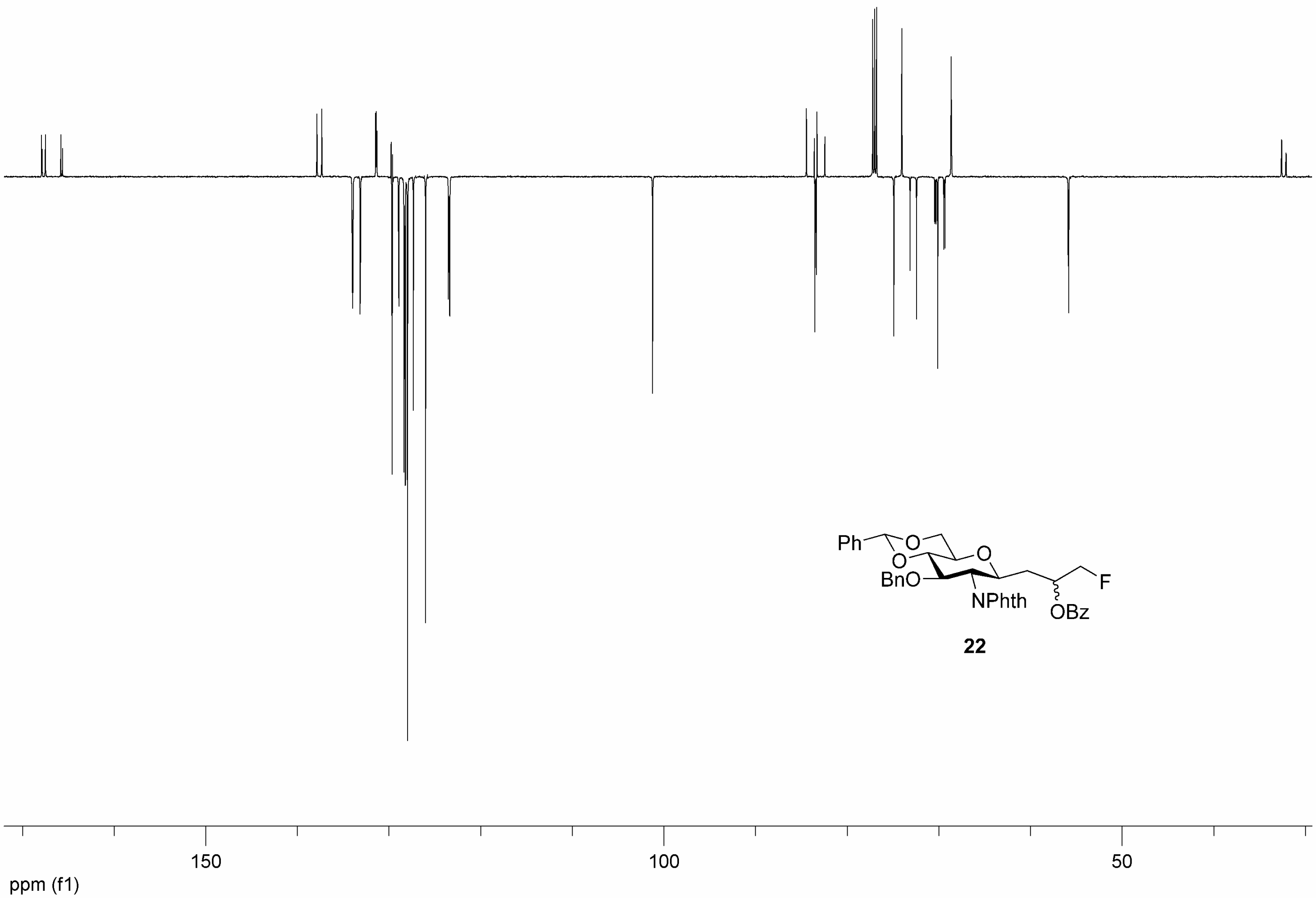

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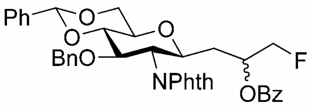

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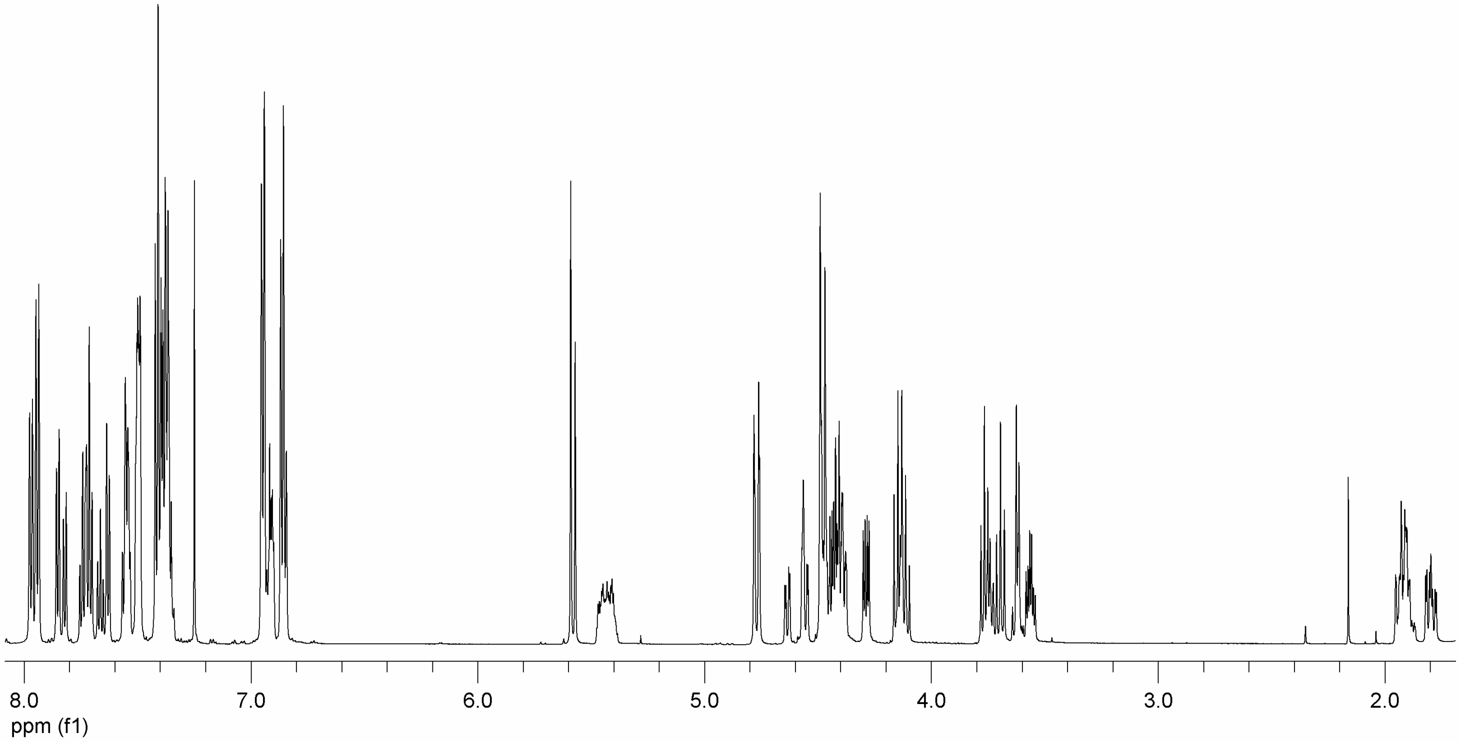

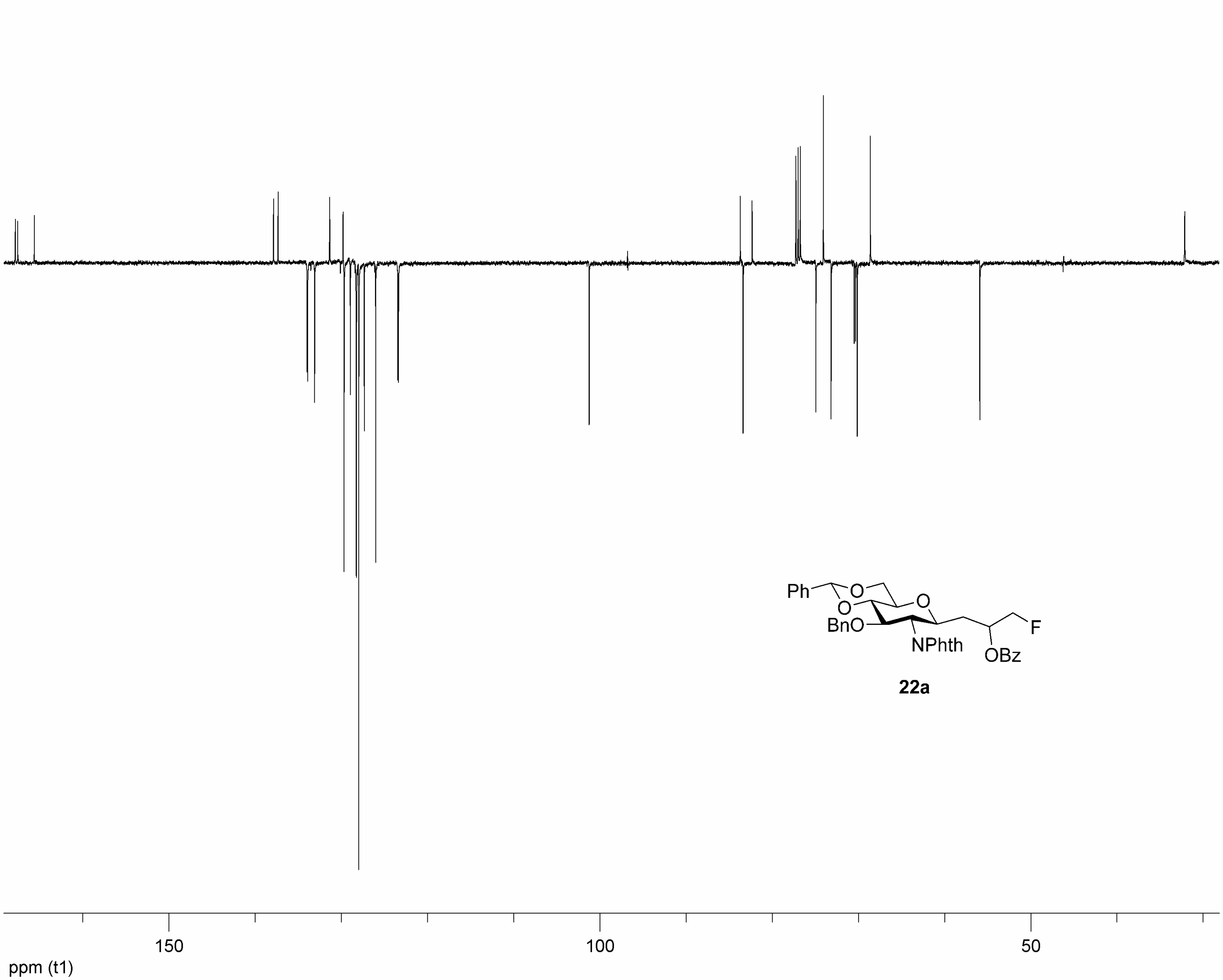

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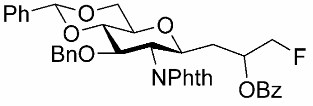

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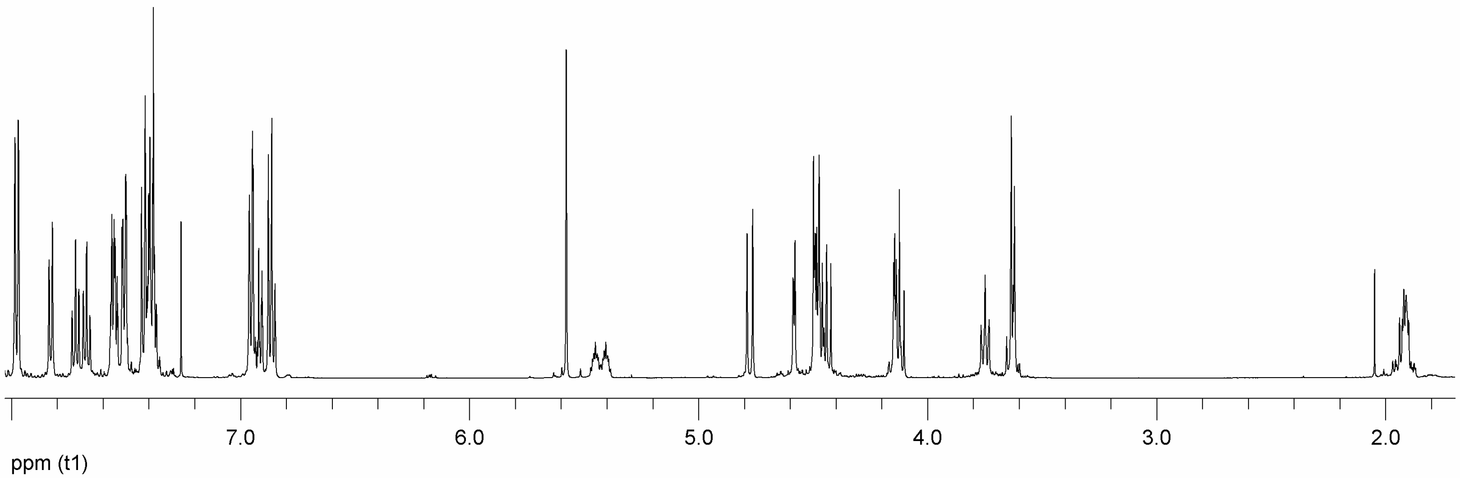
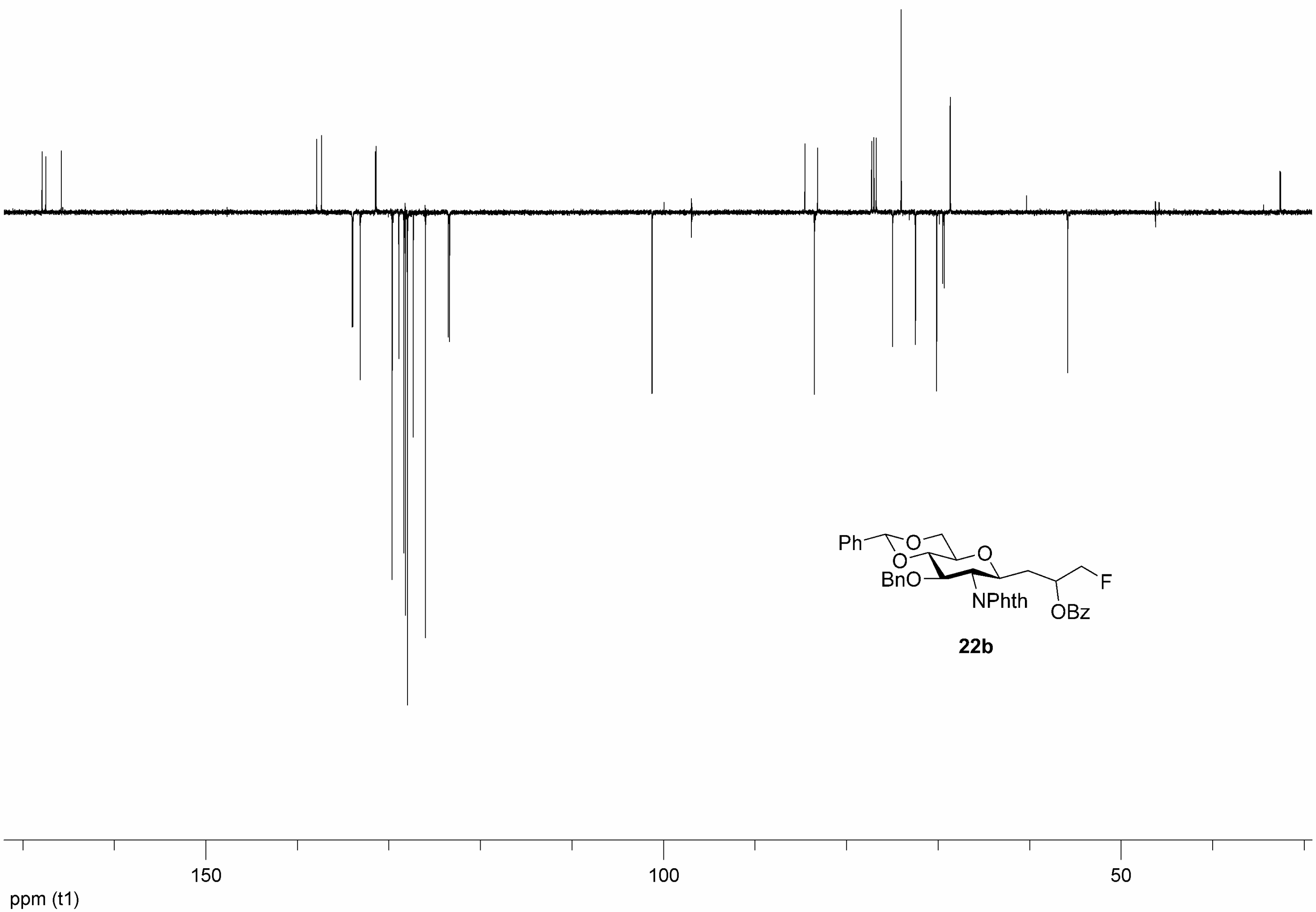
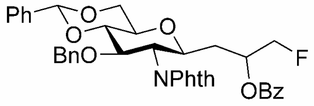
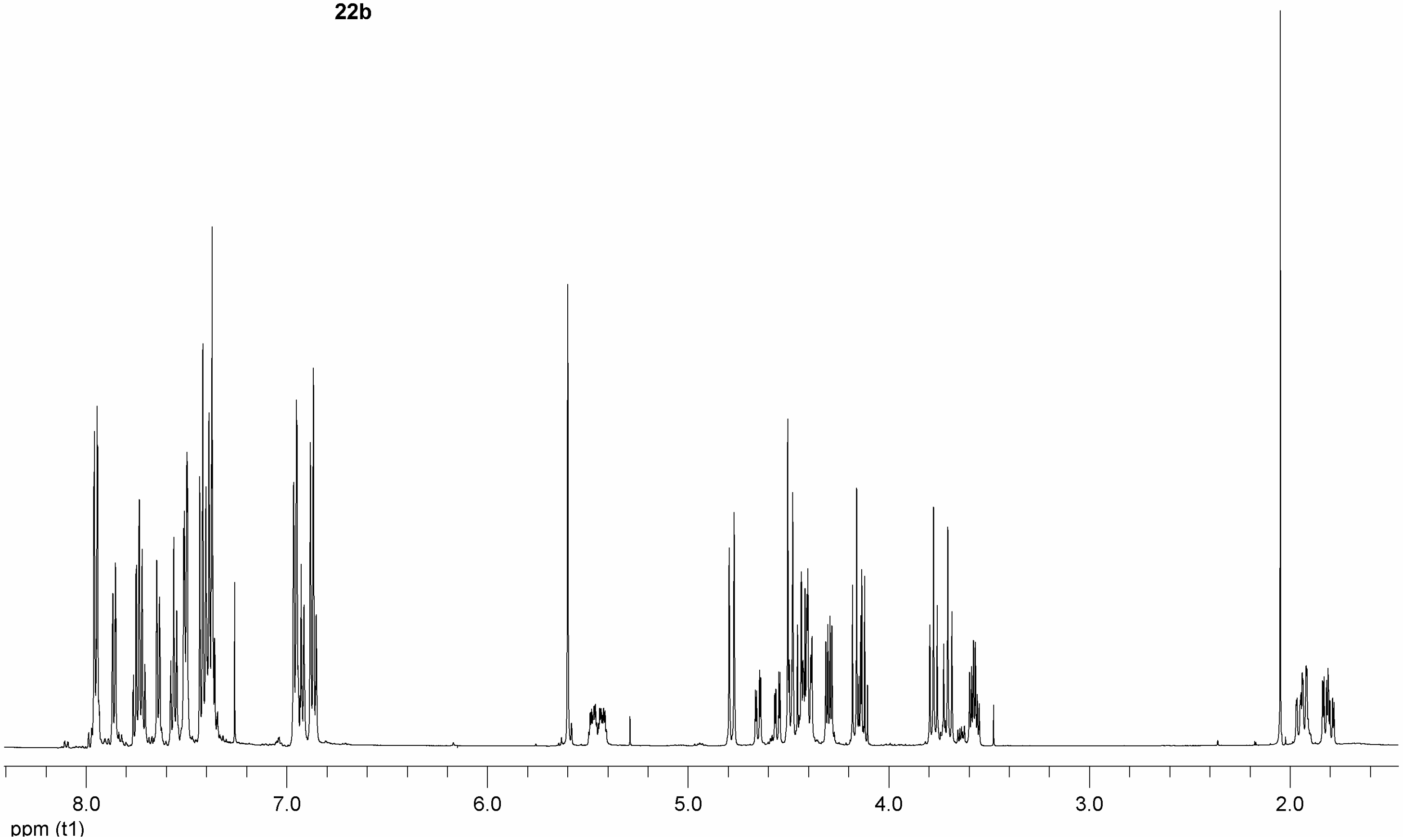


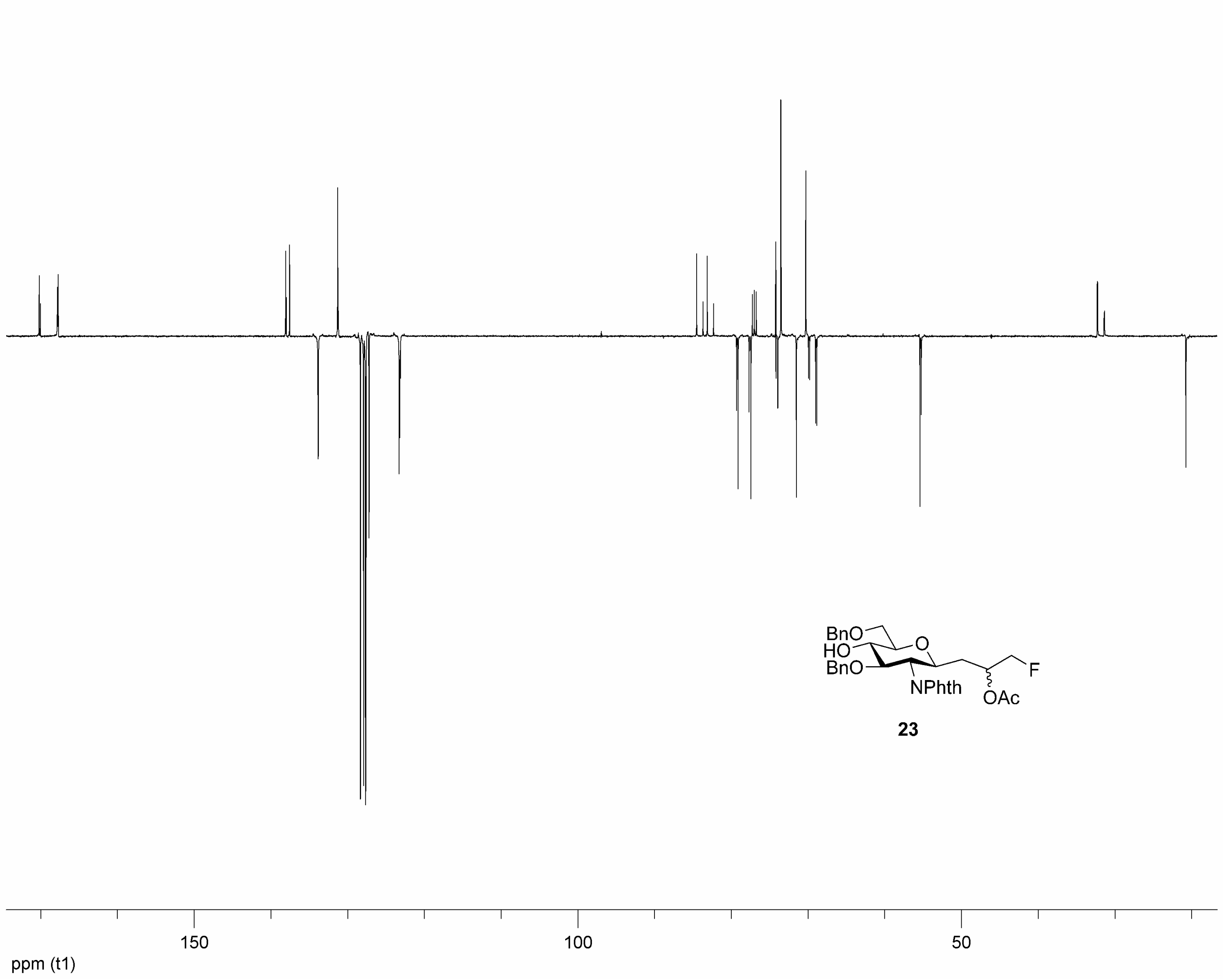


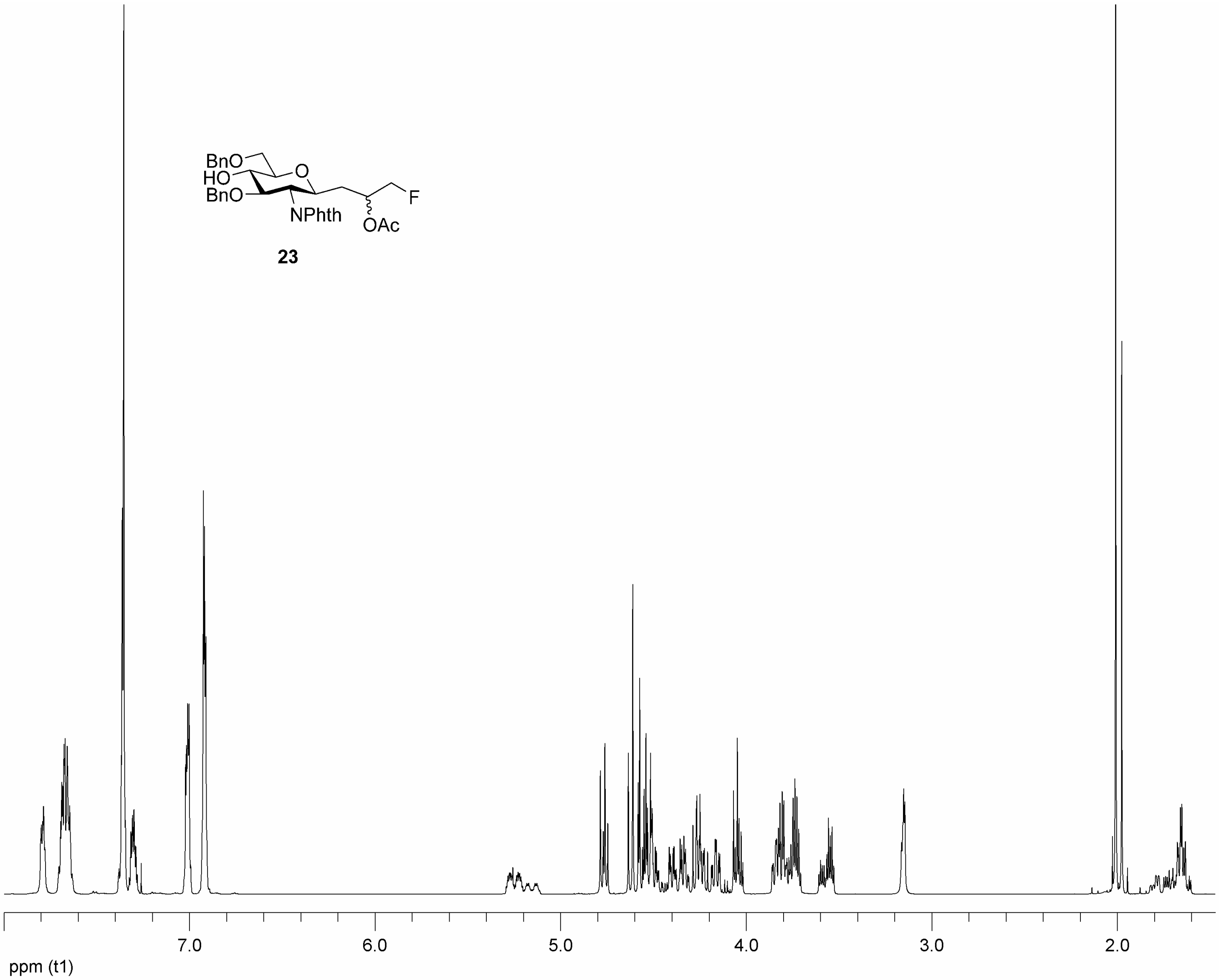
22b

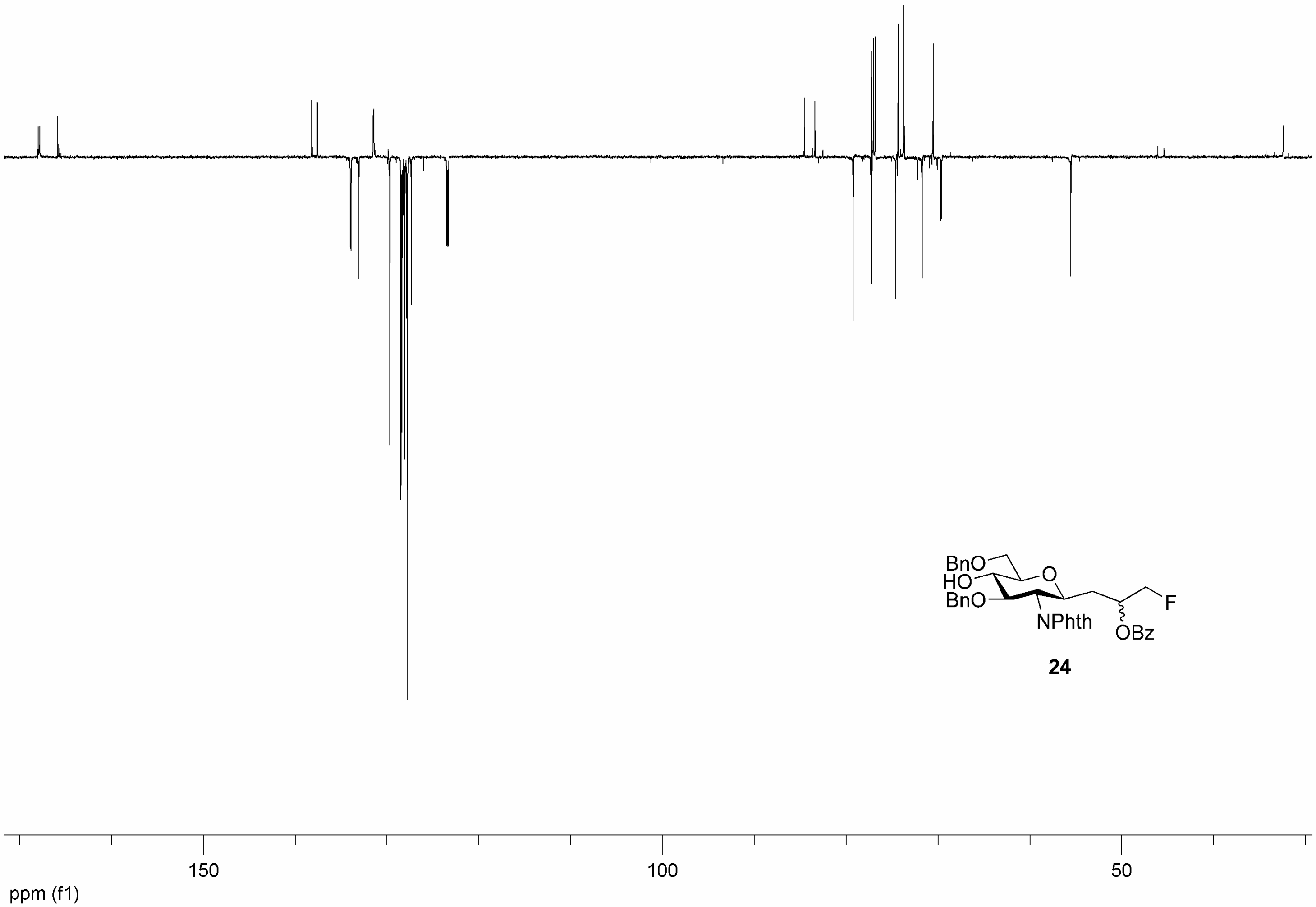


22b




BnO
HO
BnO
O
NPhth
F
OAc
23
150
100
50
ppm (t1)


BnO
HO
BnO
O
NPhth
OAc
F
23
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)



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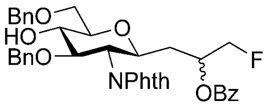

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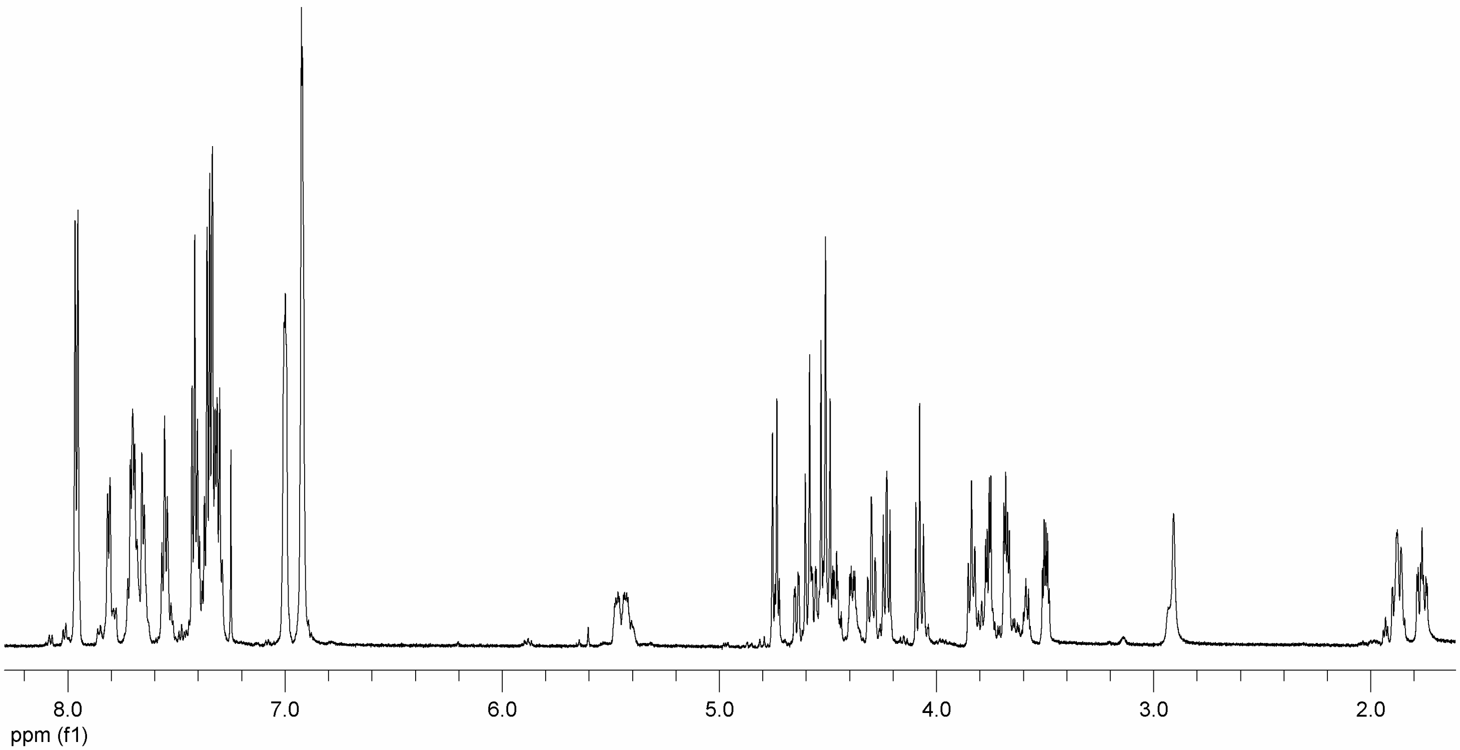
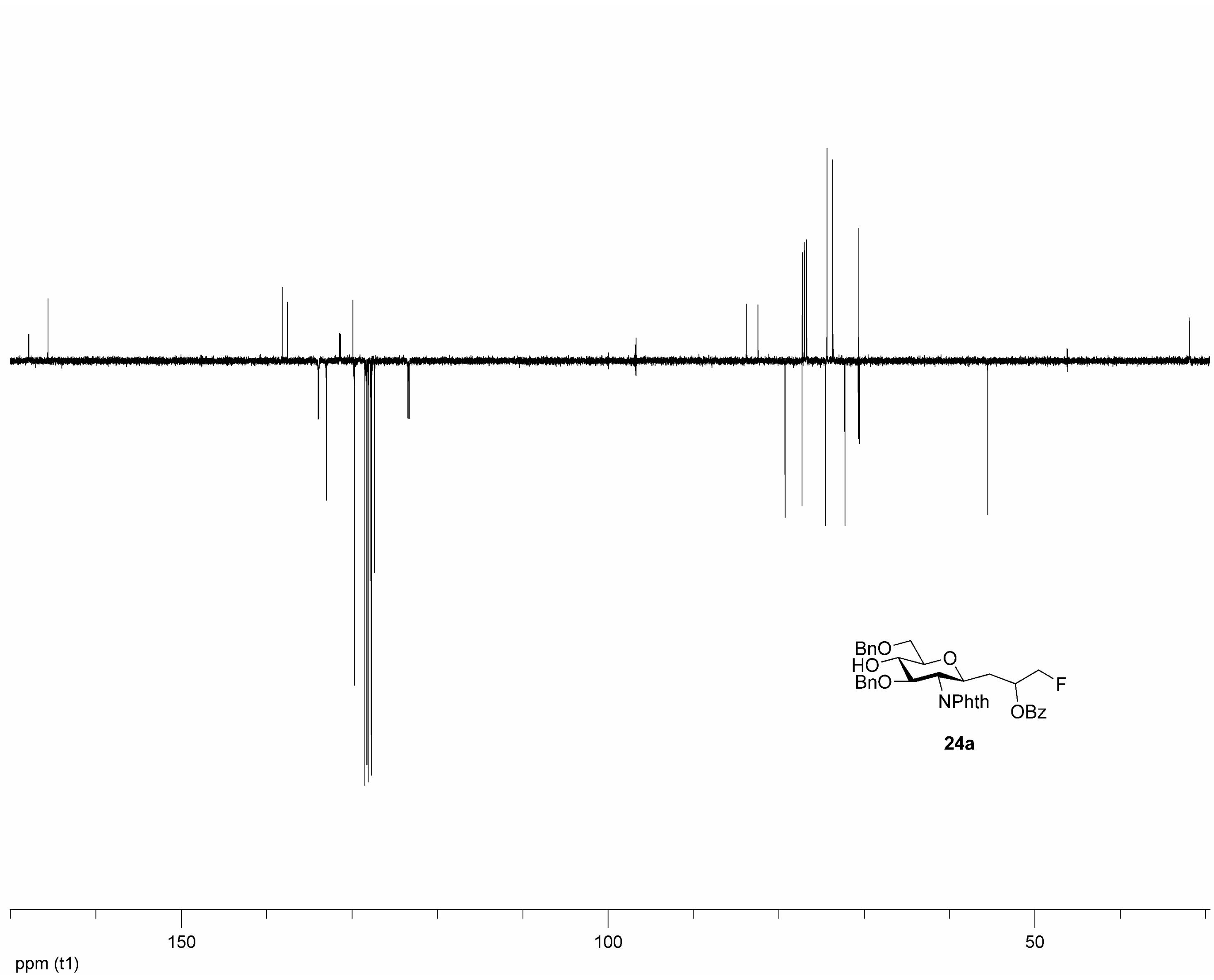
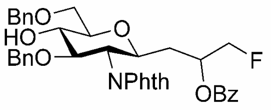




24a



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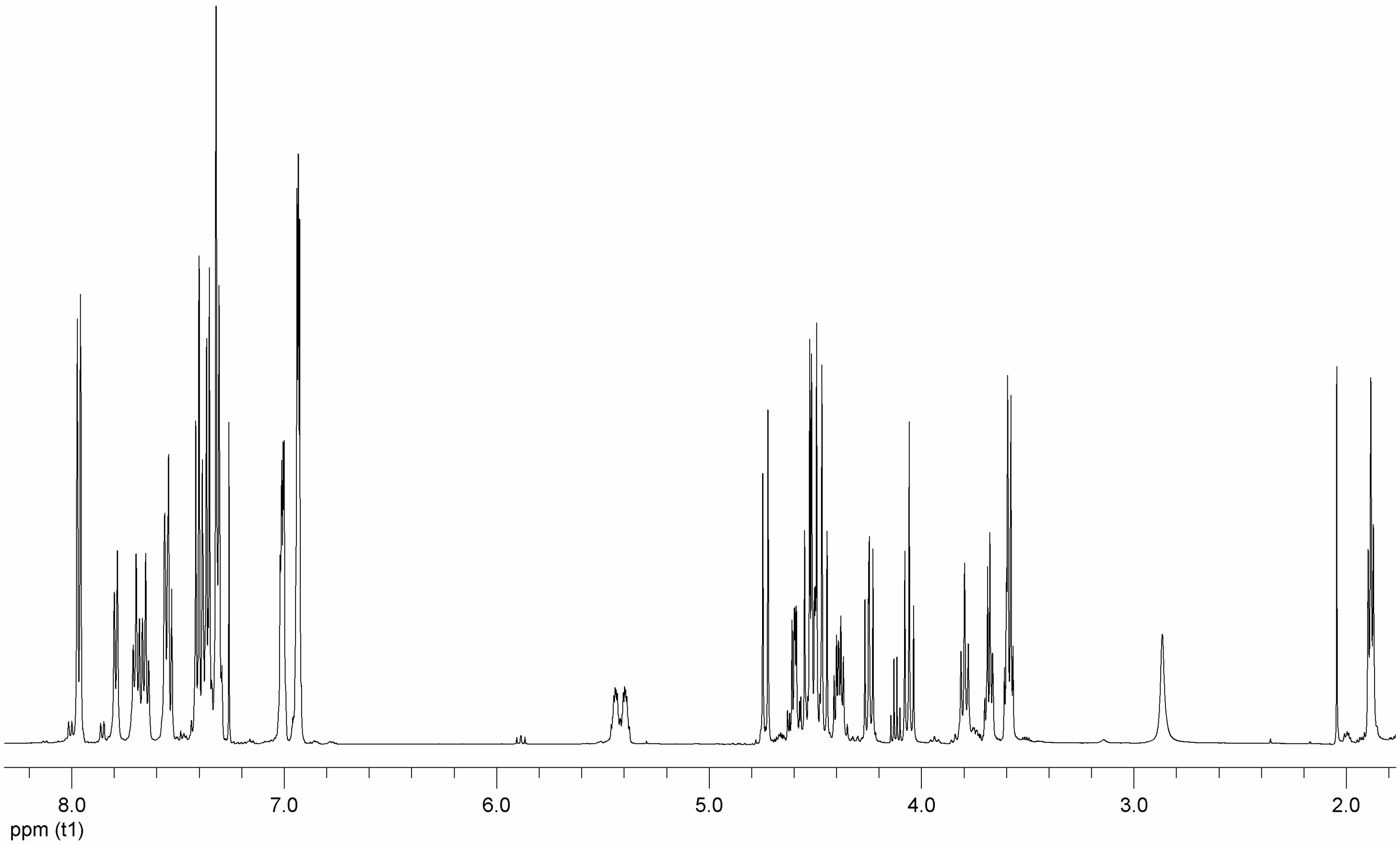
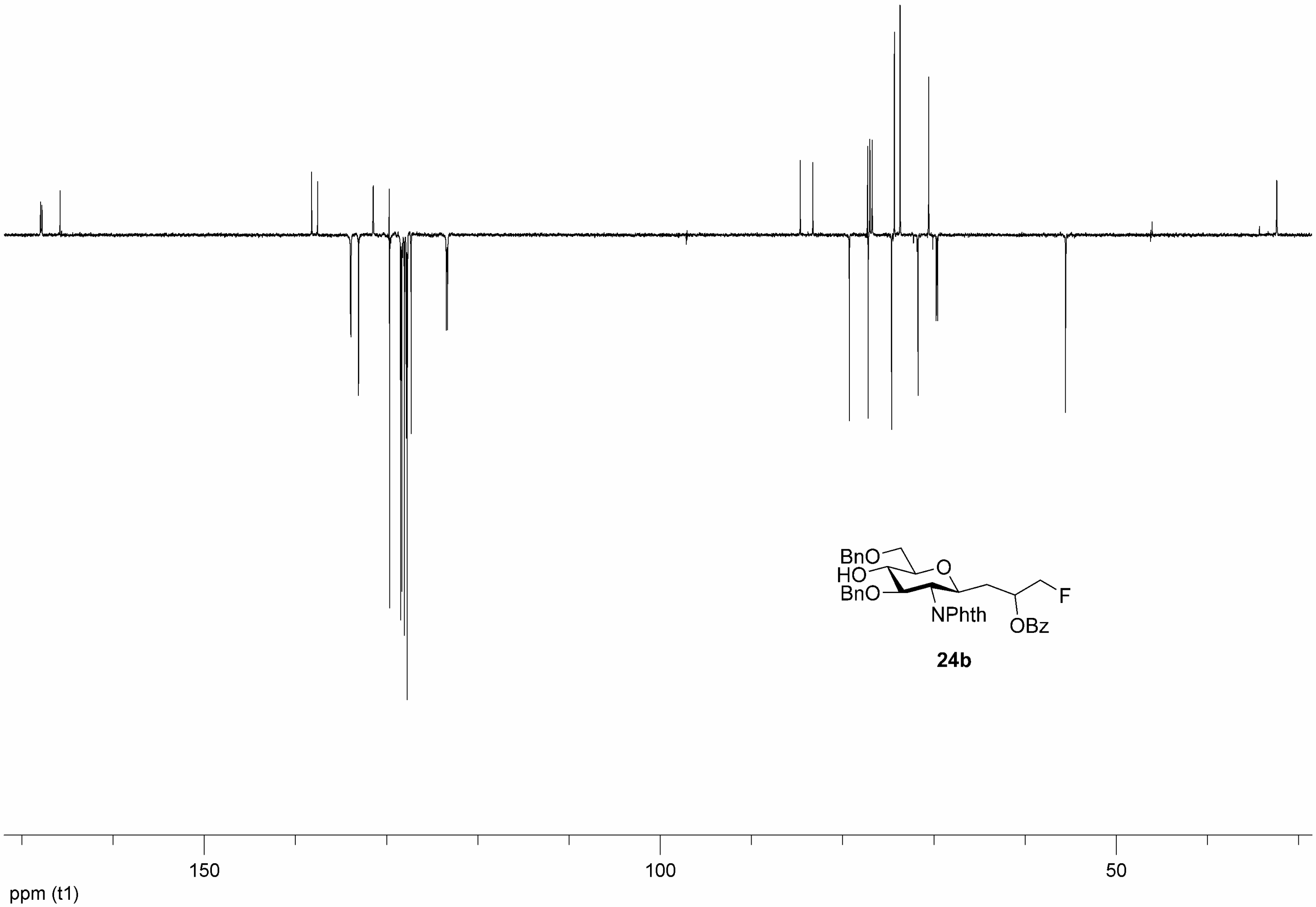
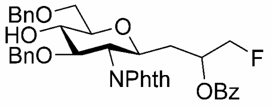
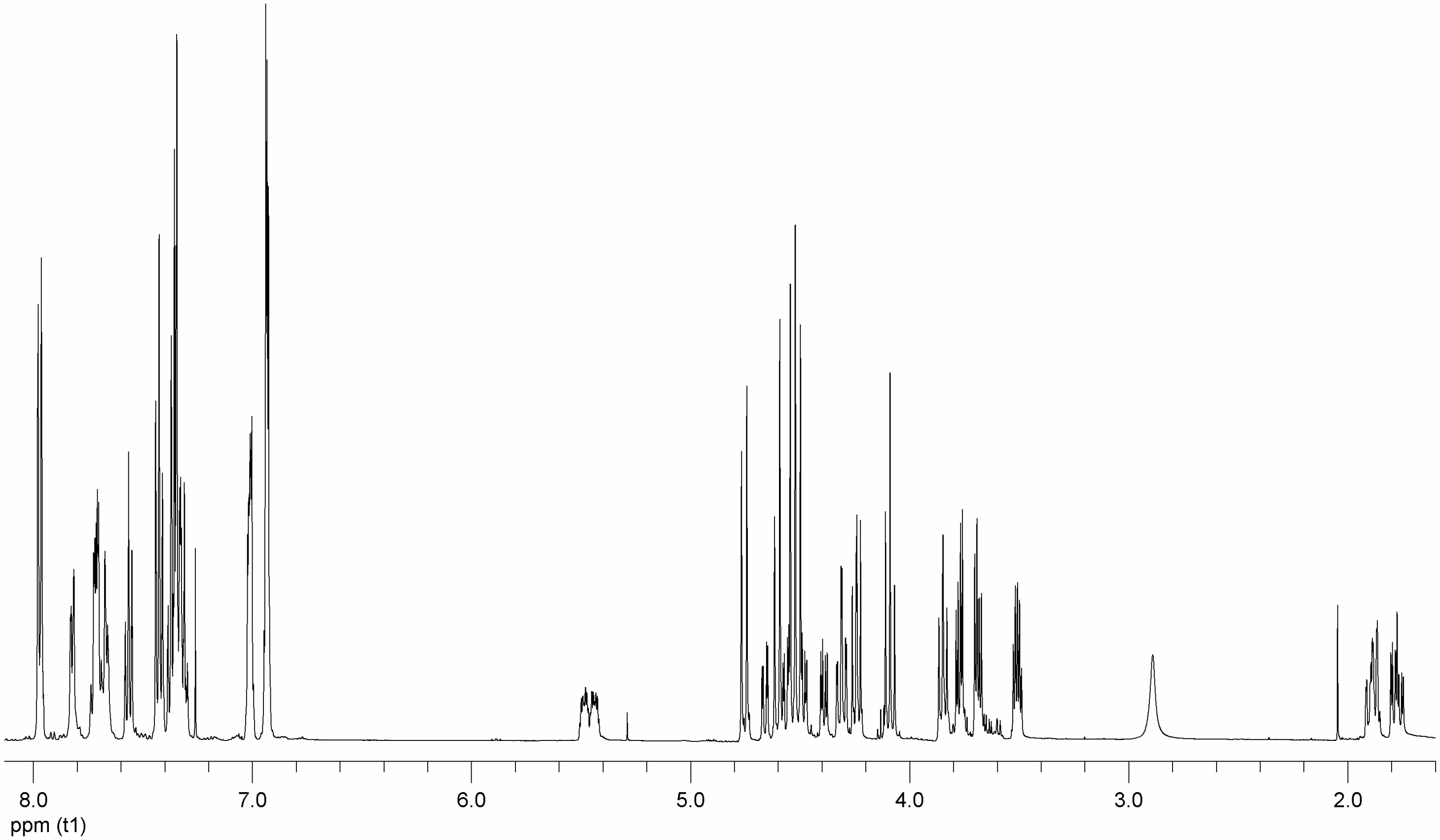
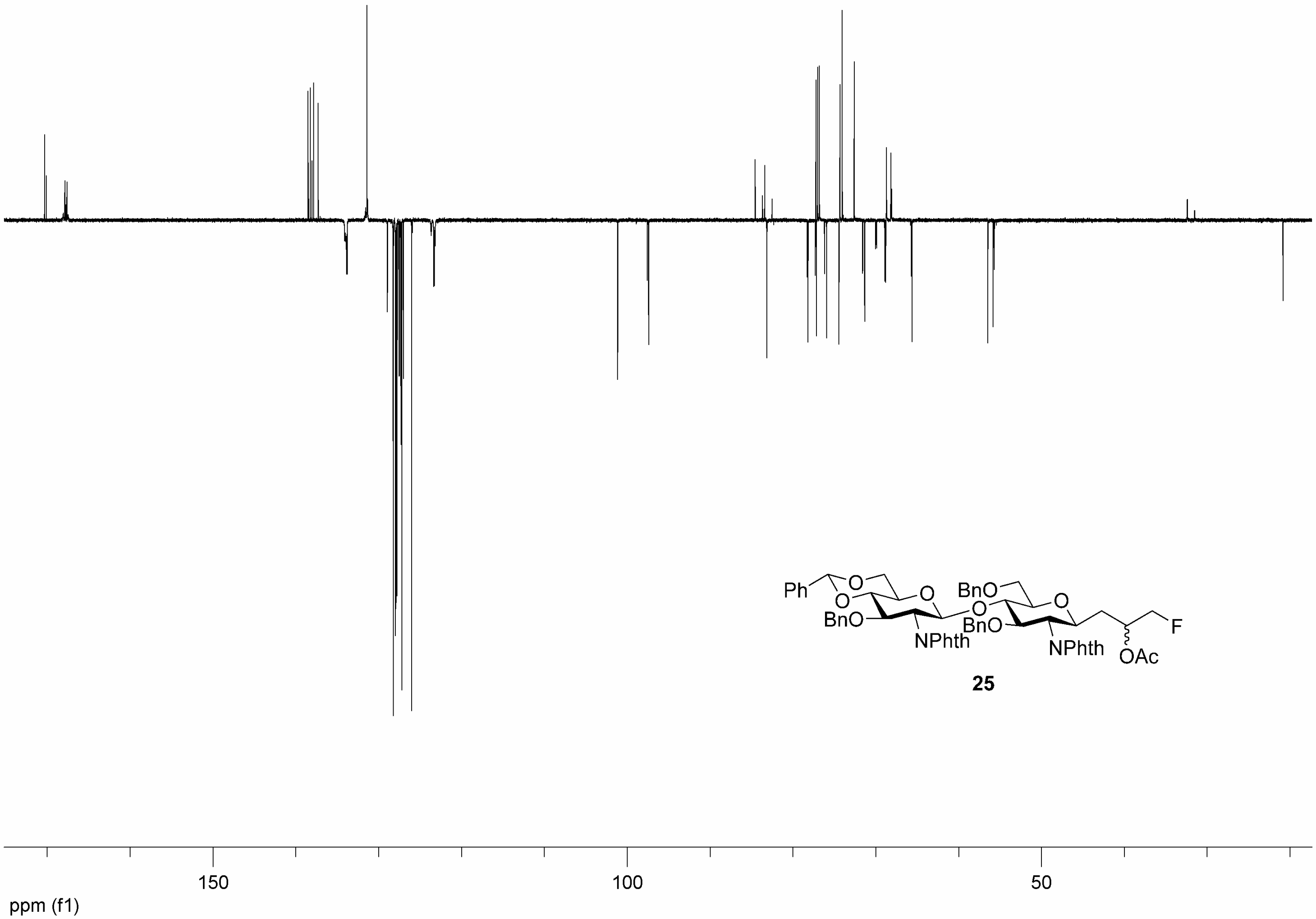



BnO
HO
BnO
O
NPhth
OBz
F
24b
150
100
50
ppm (t1)



24b





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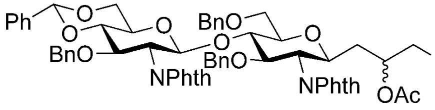

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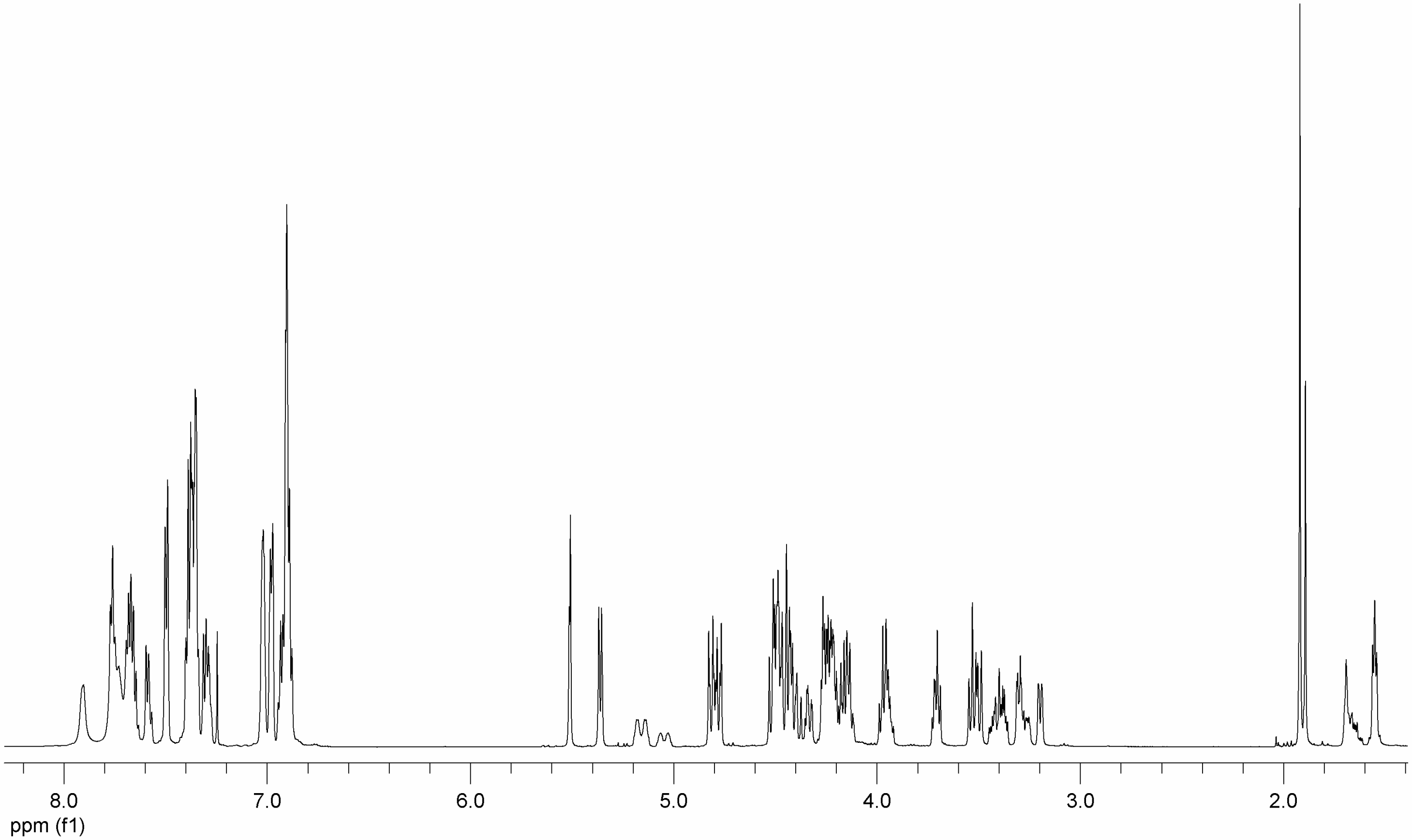

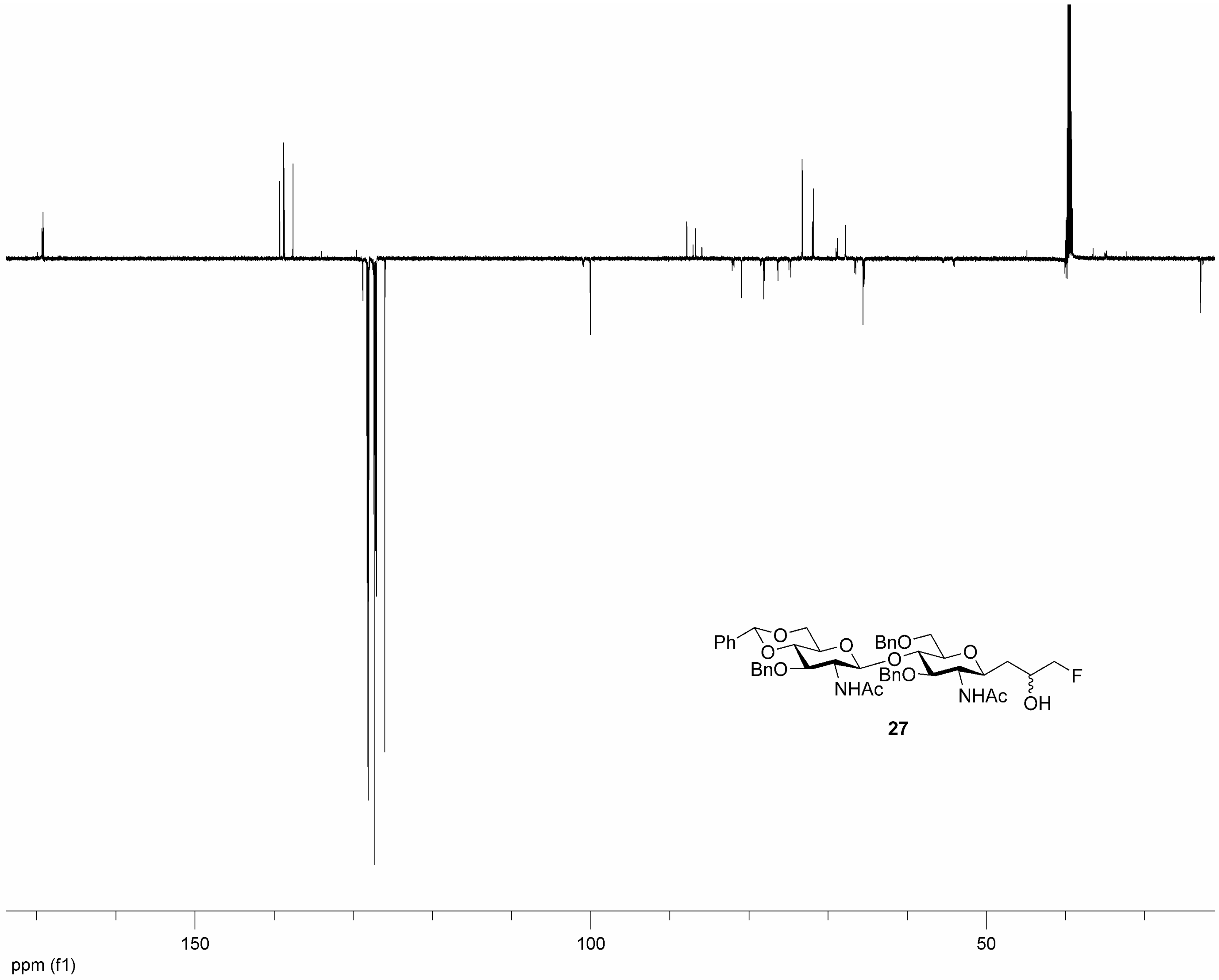

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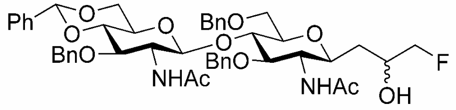

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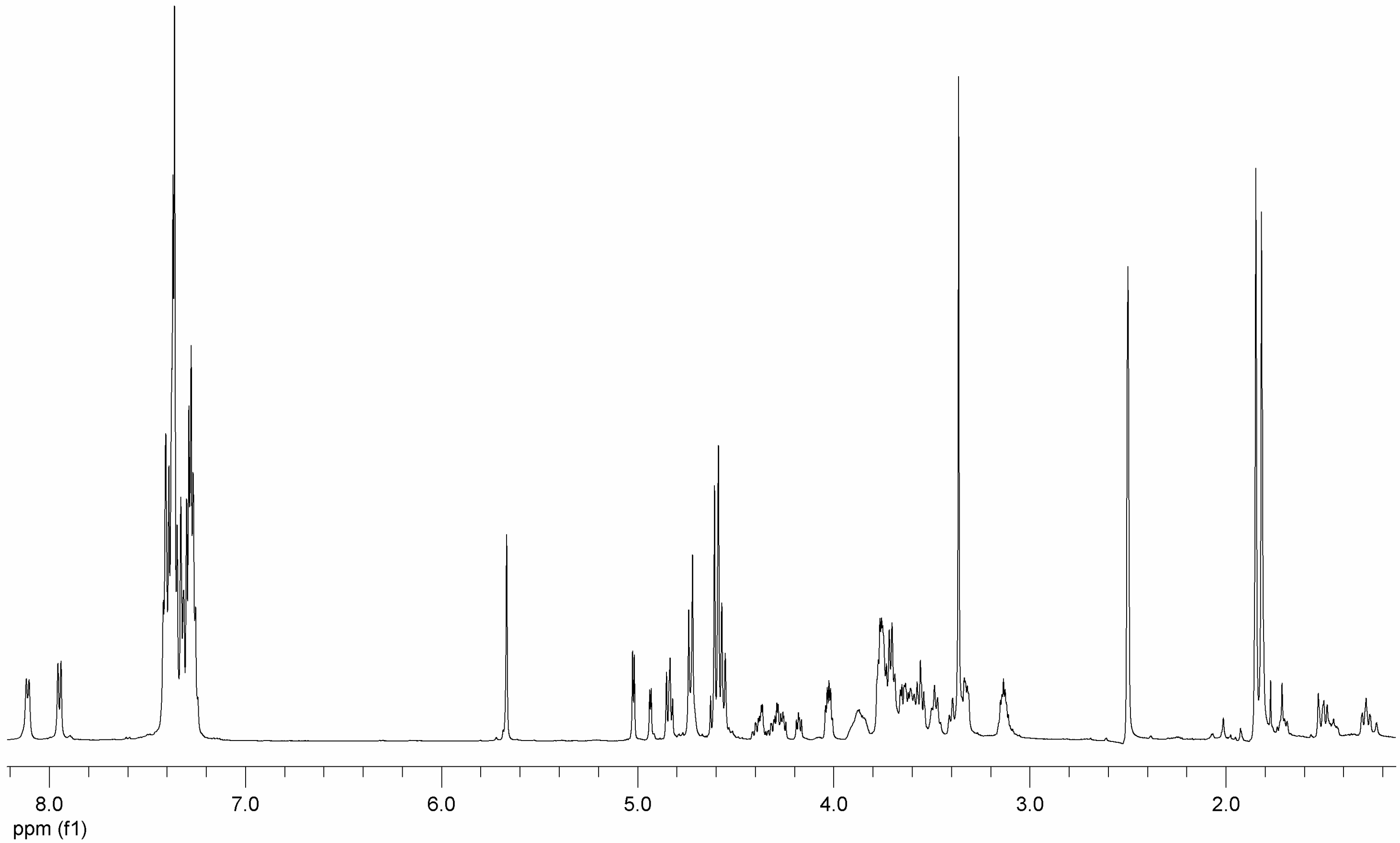

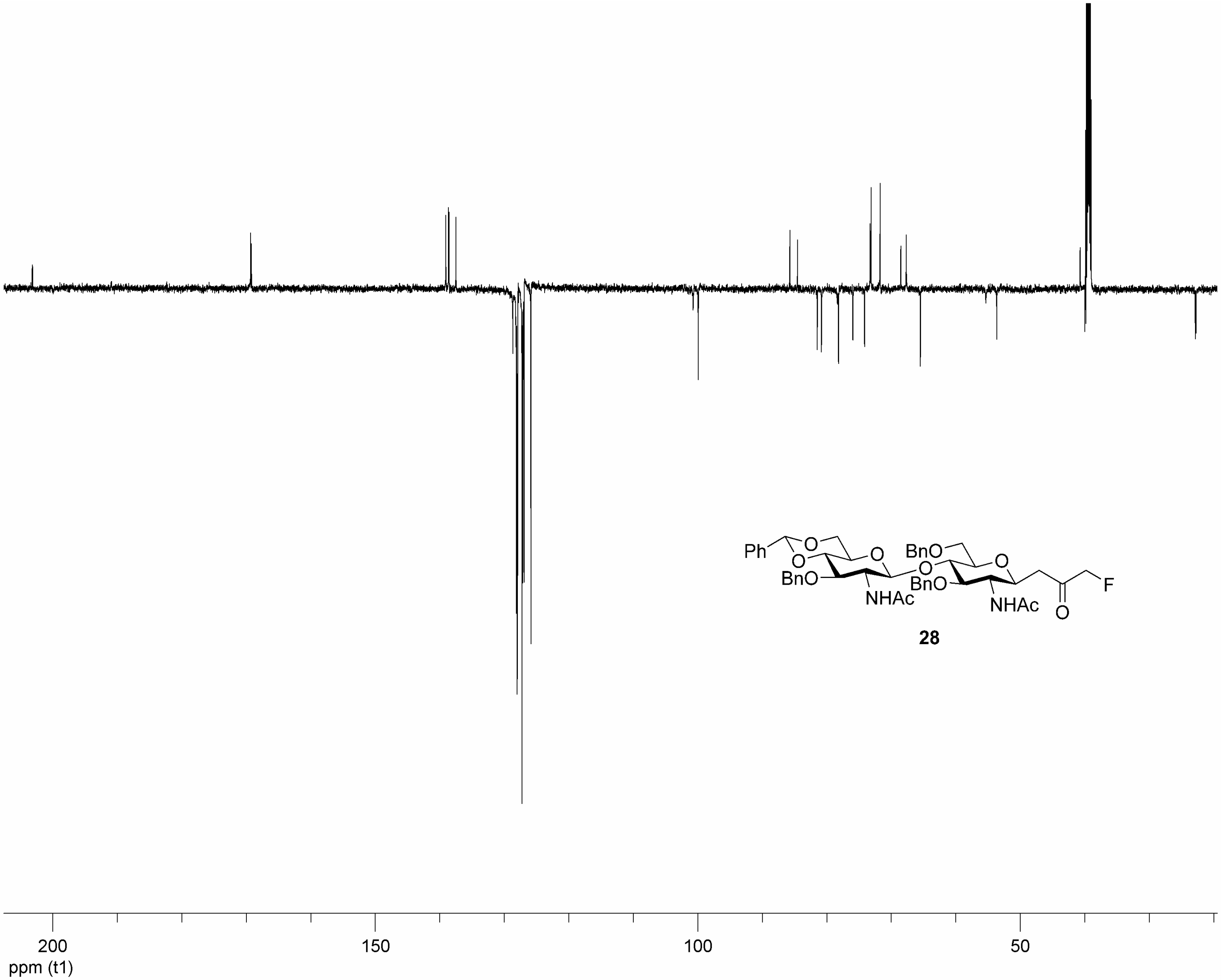

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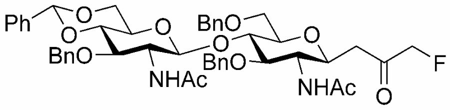

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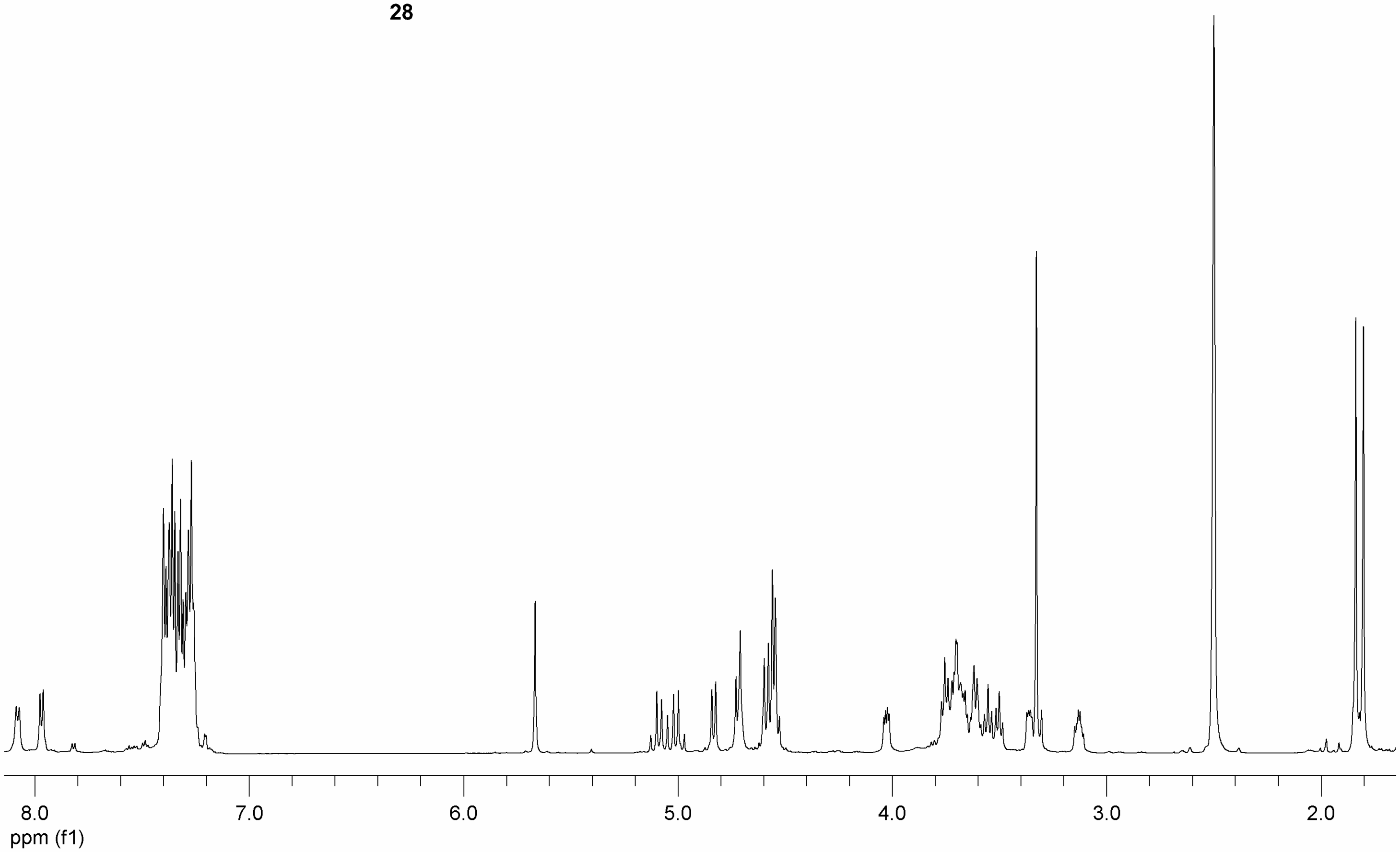

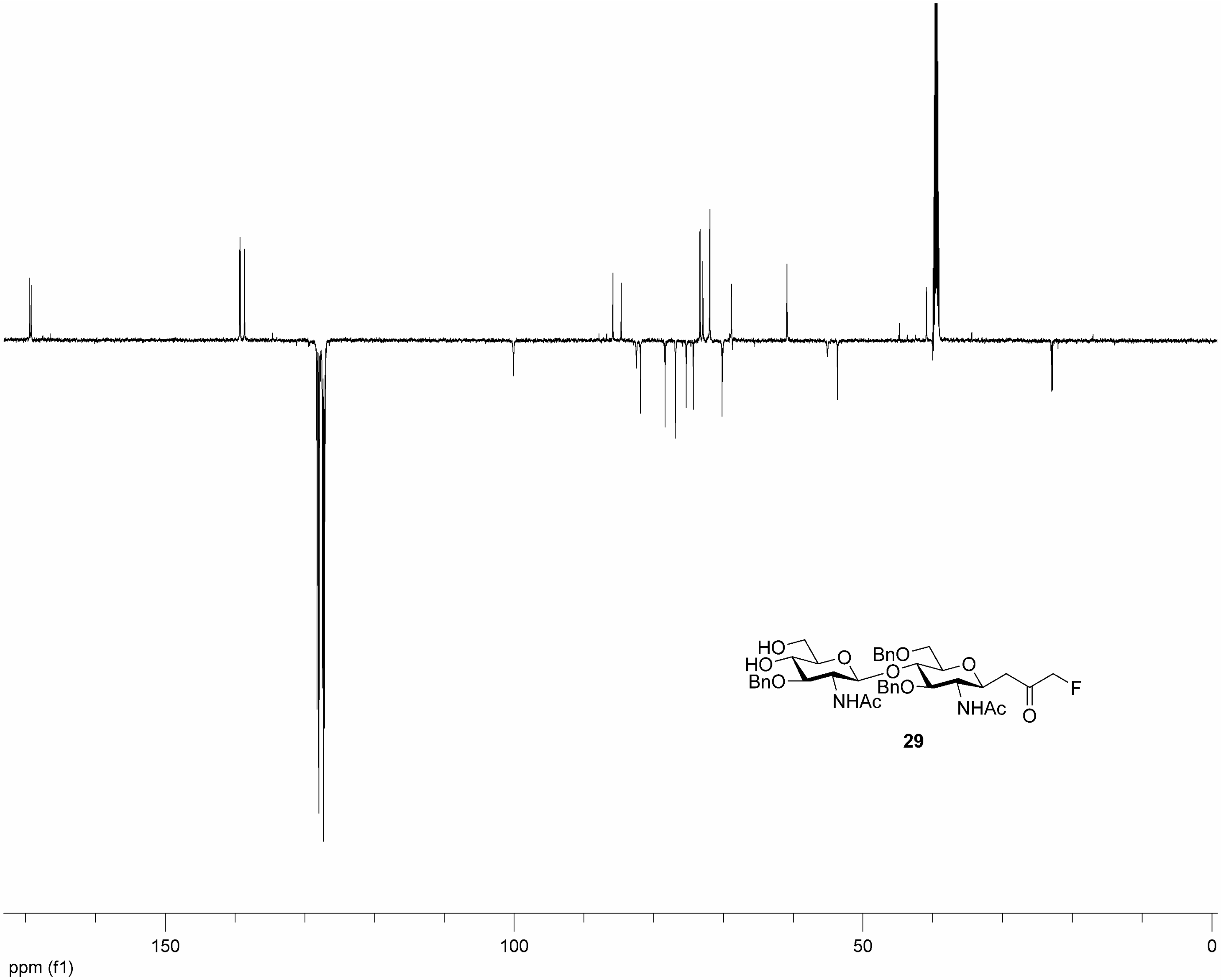

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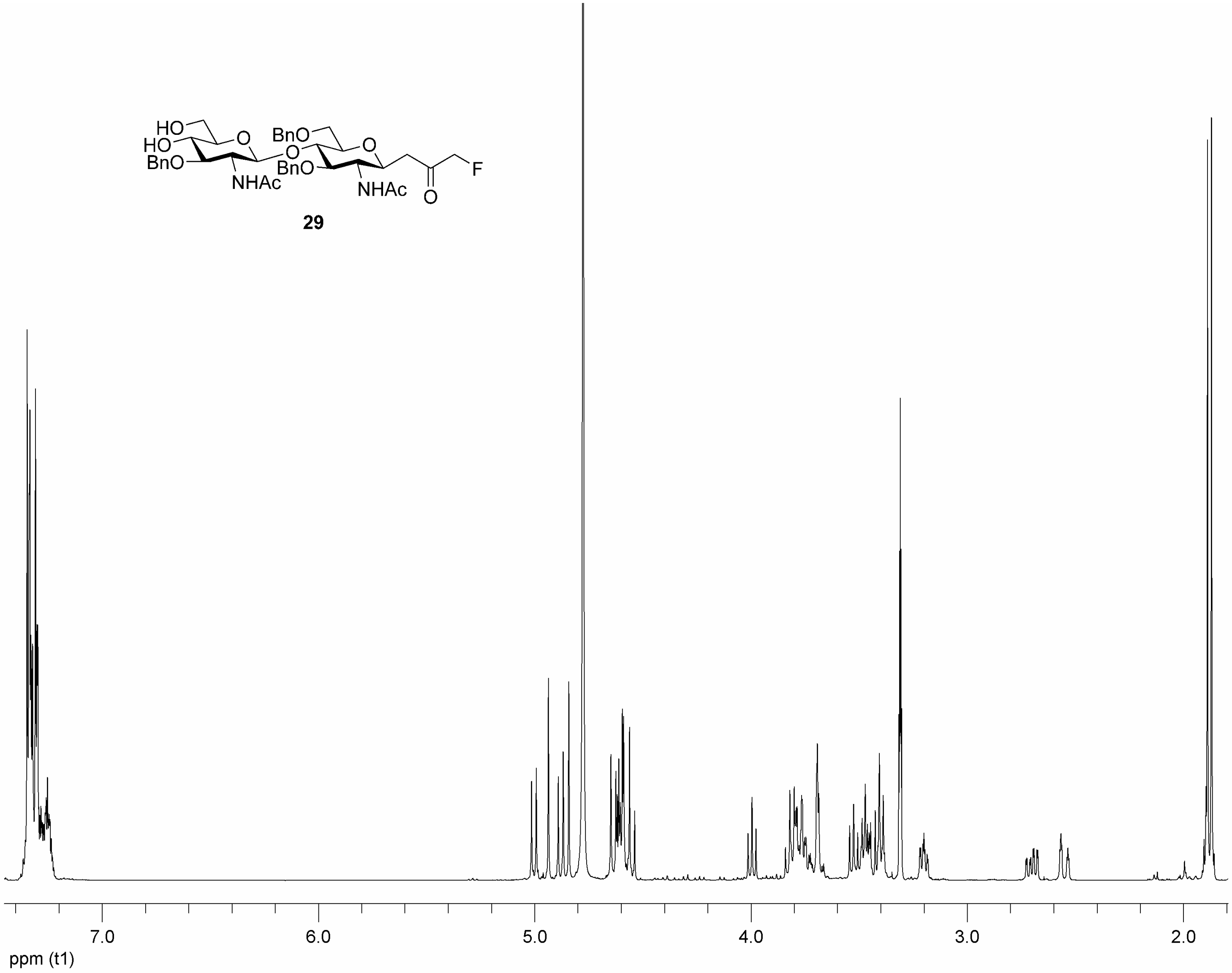

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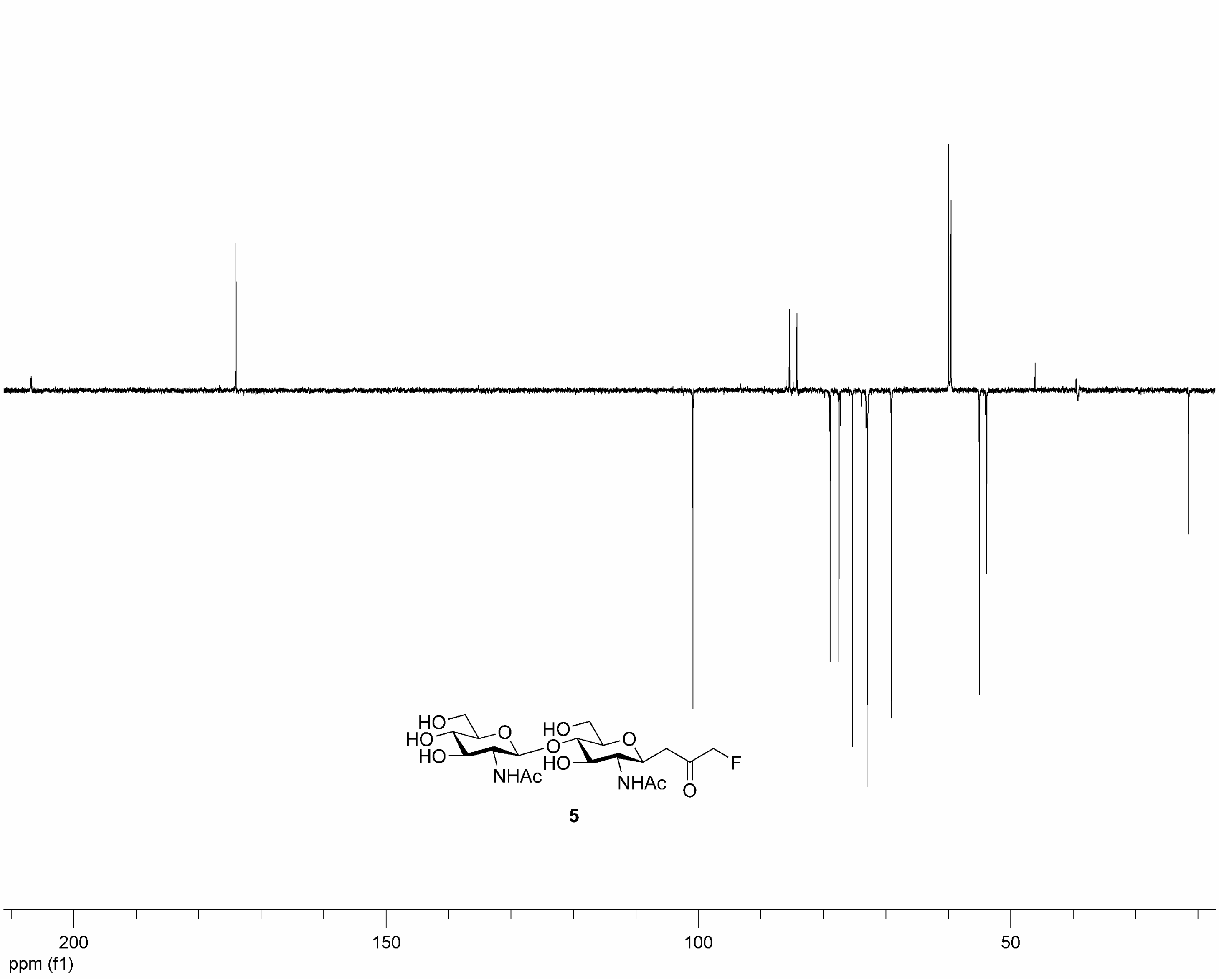
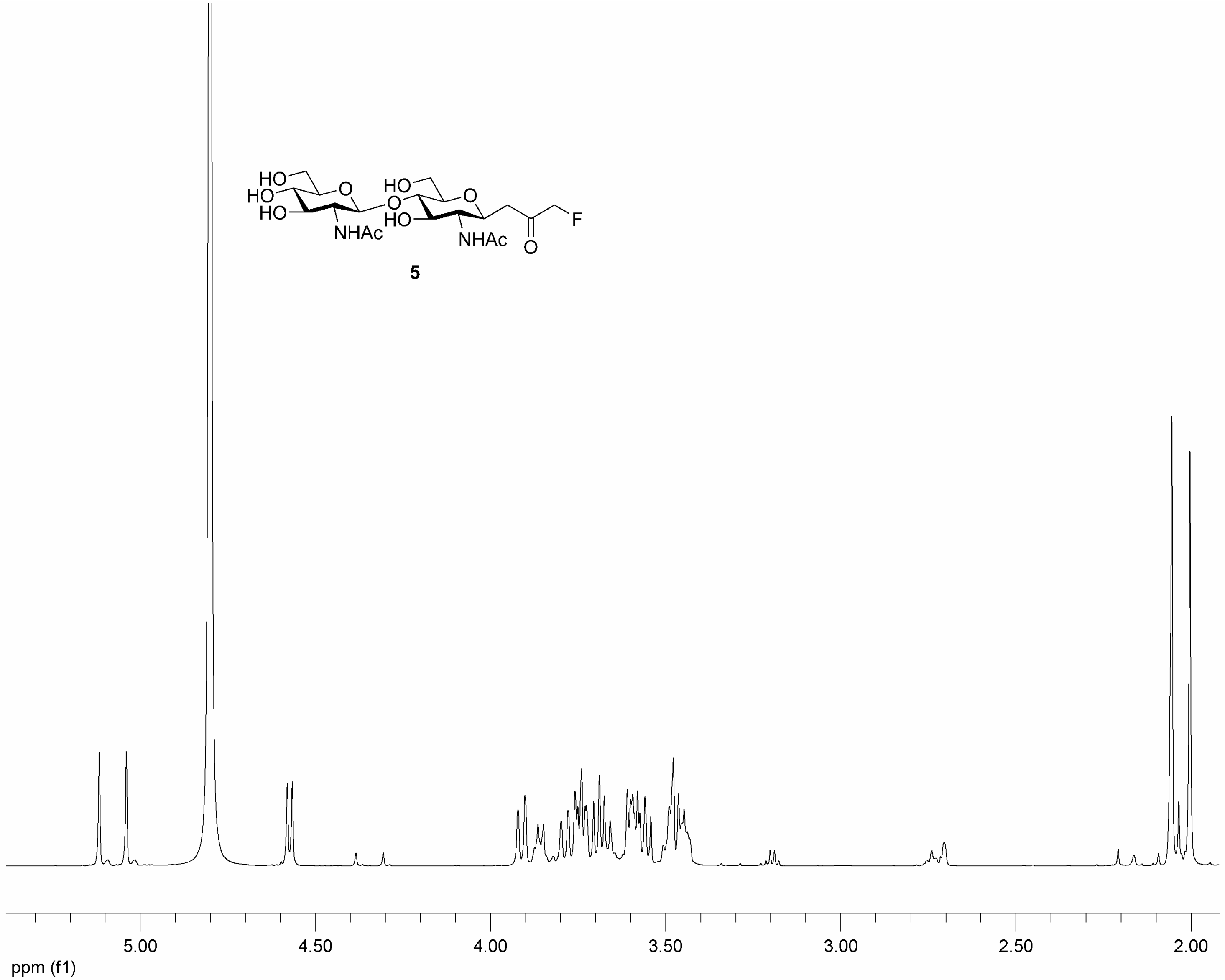

HO
HO
HO
O
NHAc
HO
O
HO
O
NHAc
O
F
5
200
150
100
50
ppm (f1)



5