Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

for

Ratiometric Electrochemical Detection of β-Galactosidase

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General information:

Proton and carbon nuclear magnetic resonance (NMR) spectra were recorded on an Agilent Technologies 500 MHz spectrometer (^{1}H NMR at 500 MHz and ^{13}C NMR at 126 MHz). Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane and are referenced to residual protium in the solvent (^{1}H NMR: $CHCl_3$ at 7.26 ppm, CD_2HOD at 3.31 ppm, and C_6H_6 at 7.16 ppm). Chemical shifts for carbons are reported in parts per million downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent peak (^{13}C NMR: $CDCl_3$ at 77.0 ppm, MeOH at 49.1, and C_6H_6 at 128.14). NMR data are represented as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constants (Hz). IR spectra were recorded on a Perkin-Elmer 1600 FT IR spectrophotometer, with absorbencies quoted as ν in cm^{-1}. High resolution mass spectrometry was performed on a Bruker MaXis HD electrospray ionisation quadrupole time-of-flight (ESI-QTOF) mass spectrometer. Melting points were obtained on a OptiMelt MPA100 automated melting point system. Electrochemical analysis was performed on a Metrohm Autolab PGSTAT30 potentiostat. Analytical thin layer chromatography (TLC) were performed using aluminium-backed plates coated with Alugram® SIL G/UV254 purchased from Macherey-Nagel and visualised by UV light (254 nm) and/or Vanillin staining. Silica gel column chromatography was carried out using 60 Å, 200-400 mesh particle size silica gel purchased from Sigma-Aldrich.

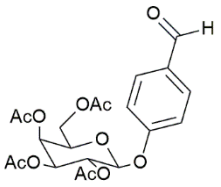
Materials:

All reactions were carried out under an atmosphere of nitrogen, in oven-dried glassware unless otherwise stated. Dichloromethane, tetrahydrofuran (THF) and toluene were dried and degassed by passing through anhydrous alumina columns using an Innovative Technology Inc. PS-400-7 solvent purification system and stored under an atmosphere of argon prior to use. D-galactose pentaacetate was purchased from Carbosynth. All other chemicals were purchased from Sigma-Aldrich. All other chemicals were used as received. β-galactosidase was purchased as a lyophilised solid from Sigma Aldrich and stored in a -20 °C freezer. Prior to use a stock solution of the enzyme was prepared using 50 mM Tris buffer (pH 9) and stored at 4 °C until immediate use.

Electrochemical analysis:

Electrochemical analysis was performed by applying a 10 µL sample to screen-printed electrochemical cell equipped with carbon working and counter electrodes and a silver (pseudo Ag/AgCl) reference electrode. The potential across the cell was powered by a Metrohm Autolab PGSTAT30 potentiostat controlled by a laptop running General Purpose Electrochemical System (GPES) software in differential pulse mode (modulation = 0.04 s, interval = 0.1 s, initial voltage = −400 mV, end voltage = 600 mV, step potential = 3 mV, modulation amplitude 49.95 mV). Post-scan, a baseline correction (moving average: peak width = 0.03) was performed. Peak integrals were obtained using the 'peak search' function and conversions calculated using the equation: $Conversion\ (\%) = \frac{(\int 3)}{(\int 3 + \int 1)} \times 100$

(Per-(*O*)-acetyl-β-D-galactopyranosyl)-4-oxybenzaldehyde



β-D-galactopyranose pentaacetate (3.90 g, 10 mmol, 1 eq.) was suspended in HBr (45 % in AcOH, 10 mL) and acetic acid (5 mL) was added. The reaction was stirred for 1 h after which DCM (20 mL) and ice (20 g) were added. The organic layer was separated and the aqueous later was extracted with DCM (2 x 30 mL). The combined organic layers were washed with water (3 x 30 mL), sat. $NaHCO_3$ (30 mL), brine (20 mL), then dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (DCM 19:1 MeOH (R_f = 0.69)) gave the brominated intermediate as a pale yellow oil (3.65 g, 89%).

A solution of the intermediate (3.65 g) in acetone (20 mL) was added dropwise to a solution of 4-hydroxybenzaldehyde (2.44 g, 20 mmol, 2 eq.) in NaOH (1 m, 20 mL) and the reaction was stirred for 20 h, after which DCM (20 mL) and water (20 mL) were added. The organic layer was separated and aqueous layer extracted with DCM (2 × 20 mL). The combined organic layers were washed with NaOH (2 × 30 mL), water (20 mL), brine (20 mL), then dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (DCM 19:1 MeOH (R_f = 0.41)) gave the title compound as a pale yellow oil. Trituration with ethanol gave the title compound as a white crystalline solid. (2.28 g, 50% over two steps).

Mp; 117–118 °C (lit.[1] 115–117 °C)

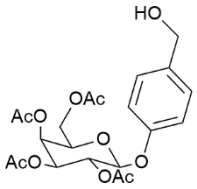
IR (solid cm^{-1}); 2984, 1743, 1692, 1600, 1584, 1508, 1368, 1208, 1159, 1041.

^{1}H NMR (500 MHz, $CDCl_3$); 9.93 (1 H, s), 7.90–7.74 (2 H, m), 7.13–7.05 (2 H, m), 5.52 (1 H, dd, *J* 10.4, 7.9), 5.47 (1 H, dd, *J* 3.5, 1.1), 5.17 (1 H, d, *J* 7.9), 5.13 (1 H, dd, J 10.4, 3.5), 4.23 (1 H, dd, *J* 11.1, 7.0), 4.16 (1 H, dd, *J* 11.1, 6.1), 4.12 (1 H, ddd, *J* 7.0, 6.1, 1.1), 2.18 (3 H, s), 2.06 (6 H, m), 2.02 (3 H, s).

^{13}C NMR (126 MHz, $CDCl_3$); 190.6, 170.2, 170.1, 170.0, 169.3, 161.3, 131.8, 131.8, 116.7, 98.6, 71.3, 70.7, 68.4, 66.7, 61.3, 20.7, 20.6, 20.6, 20.5.

Data in accordance with literature precedent.[1]

[4-(hydroxymethyl)phenyl]-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 5



[4-(hydroxymethyl)phenyl]-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (2.30 g, 5.59 mmol, 1 eq.) was added to $CHCl_3$ (100 mL) and *i*PrOH (40 mL) and then cooled to 0 °C. Sodium borohydride (0.423 g, 11.2 mmol, 2 eq.) was then added in one portion and the reaction allowed to warm to room temperature and stirred for 5 h, after which citric acid (100 mL, aq. 10%) was added. The organic layer was separated and the aqueous layer extracted with $CHCl_3$ (2 × 30 mL). The combined organic layers were washed with sat. $NaHCO_3$ (30 mL), water (30 mL), brine (30 mL) then dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (Pet. 40–60°C 1:1 EtOAc (R_f = 0.27)) gave the title compound as a colourless oil (1.74 g, 69%). Trituration with EtOH gave the title compound as a white crystalline solid.

Mp; 113–116 °C (lit.[1] 100–112 °C).

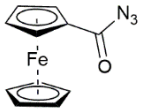
IR (solid cm^{-1}); 3558, 2924, 1732, 1520, 1371, 1213, 1061.

^{1}H NMR (500 MHz, $CDCl_3$); 7.33–7.29 (2 H, m), 7.02–6.98 (2 H, m), 5.49 (1 H, dd, *J* 10.5, 8.0), 5.46 (1 H, dd, *J* 3.4, 1.2), 5.11 (1 H, dd, *J* 10.5, 3.4), 5.03 (1 H, d, *J* 8.0), 4.65 (2 H, d, *J* 5.9), 4.23 (1 H, dd, *J* 11.4, 7.0), 4.16 (1 H, dd, *J* 11.4, 6.4), 4.08–4.04 (1 H, m), 2.18 (3 H, s), 2.07 (3 H, s), 2.06 (3 H, s), 2.01 (3 H, s), 1.60 (1 H, *t*, J 5.9).

^{13}C NMR (126 MHz, $CDCl_3$); 170.5, 156.7, 136.1, 128.7, 117.3, 100.0, 71.3, 71.1, 68.9, 67.1, 65.0, 61.6, 21.0, 20.9, 20.8.

Data in accordance with literature precedent.[1]

Ferrocenyl azide 6



Ferrocenecarboxaldehyde (2.00 g, 8.69 mmol, 1 eq.) was suspended in anhydrous DCM (20 mL) under N_2 and then cooled to 0 °C. Oxalyl chloride (1.49 mL, 17.33 mmol, 2 eq.) was then added dropwise followed by a drop of DMF. The reaction was allowed to warm to room temperature and stirred for 3 h, after which the solvent was removed in vacuo. The solid residue was taken up in anhydrous DCM (20 mL) and cooled to 0 °C. TBAB (30 mg, 0.09 mmol, 0.01 eq.) was added followed by NaN_3 (0.85 g, 13.07 mmol, 1.5 eq.) in water (4 mL) and the reaction was left to stir for 48 h after which the reaction was diluted with water (50 mL) and the layers separated. The aqueous layer was extracted with DCM (2 x 20 mL) and the combined organic layers were dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification *via* silica gel column chromatography (Pet. 40–60°C 1:1 DCM (R_f = 0.45)) gave the title compound as a red-orange crystalline solid (1.98 g, 89%).

Mp: 85-89 °C (lit.[2] 84-86 °C)

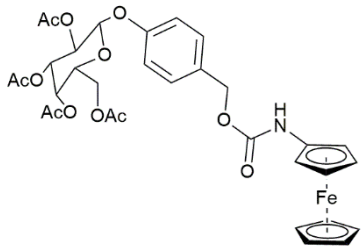
IR (solid cm^{-1}); 3108, 3079, 2148, 1670, 1453, 1372, 1206, 1184, 1054.

^{1}H NMR (500 MHz, C_6D_6); 4.74 (2 H, t, *J* 2.0), 4.02 (2 H, t, *J* 2.0), 3.91 (5 H, s).

^{13}C NMR (126 MHz, C_6D_6); 176.9, 73.3, 71.3, 71.0.

Data in accordance with literature precedent.[2]

4-((2,3,4,6-tetraacetyl-β-D-galactopyranosyl)oxy)benzyl (ferrocenyl)carbamate 7



Ferocenoyl azide (225 mg, 1 mmol, 1 eq.) was suspended in anhydrous toluene (2 mL) under argon. A solution of (per-(O)-acetyl-β-D-galactopyranosyl)-4-oxybenzyl alcohol (454 mg, 1 mmol, 1 eq.) in anhydrous toluene (2 mL) was added and the reaction refluxed for 2 h, after which the reaction was allowed to cool to room temperature and then the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (Pet. 40–60°C 2:1 EtOAc (R_f = 0.18)) gave the title compound as an orange oil (531 mg, 78%).

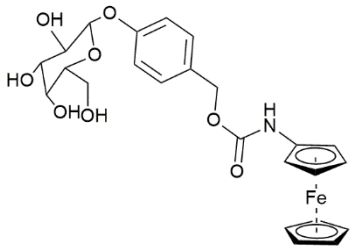
IR (film cm^{-1}); 3343, 2957, 1745, 1547, 1511, 1368, 1210, 1050.

^{1}H NMR (500 MHz, $CDCl_3$); 7.23 (2 H, d, *J* 8.2), 6.90 (2 H, d, *J* 8.2), 5.63 (1 H, s), 5.39 (1 H, dd, *J* 10.5, 8.0), 5.36 (1 H, d, *J* 3.4), 5.08–4.96 (3 H, m), 4.94 (1 H, d, *J* 7.9), 4.62 (2 H, s), 4.26–4.02 (8 H, m), 4.00–3.92 (1 H, m), 2.09 (3 H, s), 1.96 (6 H, s), 1.92 (3 H, s).

^{13}C NMR (126 MHz, $CDCl_3$); 170.3, 170.2, 170.1, 169.3, 156.9, 131.2, 129.9, 117.0, 99.6, 71.1, 70.8, 69.6, 68.6, 66.8, 66.4, 64.8, 61.3, 60.7, 20.7, 20.7, 20.6, 20.6.

HRMS (ESI); calc'd for $C_{32}H_{35}FeNO_{12}$ $[M]^+$: *m/z* 681.150, found 681.157.

4-((β-D-galactopyranosyl)oxy)benzyl (ferrocenyl)carbamate 1



4-((2,3,4,6-tetraacetyl-β-D-galactopyranosyl)oxy)benzyl (ferrocenyl)carbamate (531 mg, 0.78 mmol, 1 eq.) was suspended in MeOH (10 mL). Sodium methoxide (210 mg, 3.9 mmol, 5 eq.) in one portion and the reaction was stirred for 20 min, after which the reaction mixture was filtered. The filtrate was concentrated to give the title compound as an orange oil. Trituration with DCM gave the title compound as an orange crystalline solid. (0.239 g, 60 %).

Mp: 140-145 °C

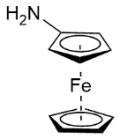
IR (solid cm^{-1}); 3285, 2996, 1770.

^{1}H NMR (500 MHz, CD_3OD); 7.34 (2 H, d, *J* 8.2), 7.11 (2 H, d, *J* 8.2), 5.07 (2 H, s), 4.87 (1 H, d, *J* 7.9), 4.46 (2 H, s), 4.09 (5 H, s), 3.92 (2 H, s), 3.90 (1 H, dd, *J* 3.4, 1.0), 3.79 (1 H, dd, *J* 9.7, 7.9), 3.77–3.71 (2 H, m), 3.68 (1 H, ddd, *J* 6.8, 5.3, 1.1), 3.57 (1 H, dd, *J* 9.7, 3.4).

^{13}C NMR (126 MHz, CD_3OD); 170.32, 130.51, 117.78, 102.89, 76.96, 74.86, 72.28, 70.22, 69.96, 67.18, 65.03, 62.40, 61.55.

HRMS (ESI); calc'd for $C_{24}H_{27}FeNO_8$ $[M]^+$: *m/z* 513.109, found 513.114.

Aminoferrocene 3



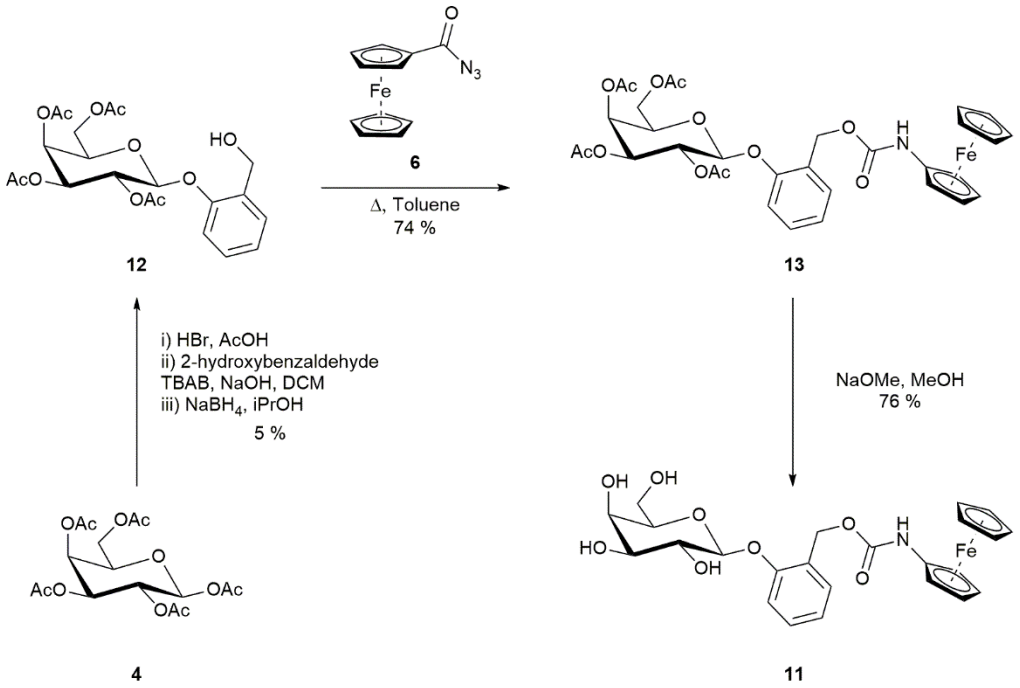
Synthesised according to a literature procedure.[3]

Mp: 149-152 °C (lit.[3] 151-153 °C)

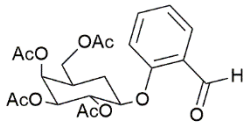
^{1}H NMR (500 MHz, $CDCl_3$); 4.11 (5 H, s), 4.01 (2 H, s), 3.86 (2 H, s), 2.56 (2 H, brs).

^{13}C NMR (126 MHz, $CDCl_3$); 68.9, 63.4, 58.7.

Synthesis of Substrate 11



Per-(O)-acetyl-β-D-galactopyranosyl)-2-oxybenzaldehyde



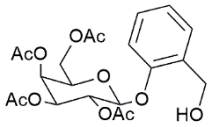
β-D-galactopyranose pentaacetate (780 mg, 2 mmol, 1 eq.) was suspended in HBr (45 % in AcOH, 2 mL) and acetic acid (1 mL) was added. The reaction was stirred for 2 h after which DCM (10 mL) and ice (10 g) were added. The organic layer was separated and the aqueous later was extracted with DCM (2 x 10 mL). The combined organic layers were washed with water (3 x 10 mL), sat. $NaHCO_3$ (10 mL), brine (10 mL), then dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (DCM 19:1 MeOH (R_f = 0.68)) gave the brominated intermediate as a pale yellow oil.

A solution of the intermediate in DCM (4 mL) was added dropwise to a solution of 2-hydroxybenzaldehyde (0.17 mL, 1.6 mmol, 0.8 eq.) and tetra-n-butylammonium bromide (516 mg, 1.6 mmol, 0.8 eq.) in NaOH (1 m, 4 mL) and the reaction was refluxed for 20 h, after which the reaction was cooled to room temperature. DCM (10 mL) and water (10 mL) were added. The organic layer was separated and aqueous layer extracted with DCM (2 × 20 mL). The combined organic layers were washed with HCl (1 m, 10 mL), water (10 mL), NaOH (1 m, 10 mL), brine (20 mL), then dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (Pet. 40-60 °C 2:1 EtOAc) gave the title compound as a colourless oil (325 mg, 26 %).

^{1}H NMR (500 MHz, $CDCl_3$); 10.36 (1 H, s), 7.86 (1 H, dd, *J* 7.5, 1.8), 7.56 (1 H, ddd, *J* 8.3, 7.5, 1.8), 7.18 (1 H, td, *J* 7.5, 1.0), 7.12 (1 H, dd, *J* 8.3, 1.0), 5.59 (1 H, dd, *J* 10.5, 7.9), 5.48 (1 H, dd, *J* 3.4, 1.1), 5.16 – 5.12 (2 H, m), 4.24 (1 H, dd, *J* 11.2, 6.9), 4.16 (1 H, dd, *J* 11.2, 6.2), 4.13 – 4.08 (1 H, m), 2.20 (3 H, s), 2.06 (6 H, s), 2.02 (3 H, s).

^{13}C NMR (126 MHz, $CDCl_3$); 189.2, 170.3, 170.1, 170.0, 169.3, 158.8, 135.6, 128.3, 126.2, 123.5, 115.8, 99.5, 71.3, 70.6, 68.4, 66.7, 61.2, 20.6, 20.6, 20.6, 20.5.

[2-(hydroxymethyl)phenyl]-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside 12

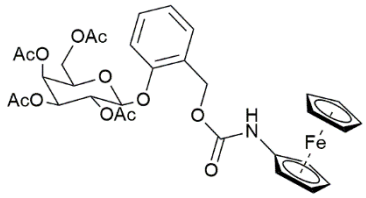


[2-(hydroxymethyl)phenyl]-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (289 mg, 0.64 mmol, 1 eq.) was suspended in *i*PrOH (20 mL) and heated to 50 °C and then cooled to room temperature. Sodium borohydride (24 mg, 0.64 mmol, 1 eq.) was then added in one portion and the reaction and stirred for 3 h, after which the reaction mixture was poured onto ice/water (10 mL) and pH adjusted to 6.5. The reaction mixture was extracted with $CHCl_3$ (3 × 10 mL) and he combined organic layers were washed with water (2 × 20 mL), brine (10 mL) then dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (Pet. 40–60 °C 1:1 EtOAc) gave the title compound as a colourless oil (92 mg, 21 %).

^{1}H NMR (500 MHz, $CDCl_3$); 7.33 (1 H, dd, *J* 7.4, 1.7), 7.30 – 7.25 (1 H, m), 7.08 (1 H, td, *J* 7.5, 1.0), 7.02 (1 H, dd, *J* 8.2, 1.0), 5.52 (1 H, dd, *J* 10.5, 7.9), 5.47 (1 H, dd, *J* 3.5, 1.1), 5.14 (1 H, dd, *J* 10.5, 3.5), 5.08 (1 H, d, *J* 7.9), 4.68 – 4.56 (2 H, m), 4.22 (1 H, dd, *J* 11.4, 7.2), 4.16 (1 H, dd, *J* 11.4, 6.0), 4.07 (1 H, ddd, *J* 7.2, 6.0, 1.1), 2.20 (3 H, s), 2.11 (3 H, s), 2.05 (3 H, s), 2.02 (3 H, s).

^{13}C NMR (126 MHz, $CDCl_3$); 170.3, 170.2, 170.0, 169.9, 154.8, 131.1, 129.6, 129.1, 123.6, 115.2, 99.8, 71.1, 70.6, 68.7, 66.8, 61.3, 61.2, 20.8, 20.6, 20.6, 20.5.

2-((2,3,4,6-tetraacetyl-β-d-galactopyranosyl)oxy)benzyl (ferrocenyl)carbamate 13



Ferocenoyl azide (45 mg, 0.2 mmol, 1 eq.) was suspended in anhydrous toluene (0.5 mL) under argon. A solution of (per-(O)-acetyl-β-D-galactopyranosyl-2-oxybenzyl alcohol (92 mg, 0.2 mmol, 1 eq.) in anhydrous toluene (0.5 mL) was added and the reaction refluxed for 2 h, after which the reaction was allowed to cool to room temperature and then the solvent was removed *in vacuo*. Purification *via* silica gel column chromatography (Pet. 40–60 °C 2:1 EtOAc) gave the title compound as an orange oil (101 mg, 74%).

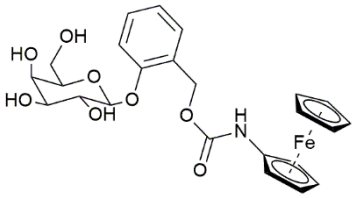
IR (film cm^{-1}); 3323, 2881, 1678, 1552, 1416.

^{1}H NMR (500 MHz, $CDCl_3$); 7.37 – 7.31 (1 H, m), 7.31 – 7.26 (1 H, m), 7.12 – 7.06 (2 H, m), 5.63 (2 H, brs), 5.53 (1 H, dd, *J* 10.3, 7.7), 5.46 (1 H, s), 5.20 (1 H, d, *J* 12.8), 5.15 – 5.01 (5 H, m), 4.41 (5 H, s), 4.25 (1 H, dd, *J* 11.4, 6.7), 4.16 (1 H, dd, *J* 11.4, 5.9), 4.06 (1 H, d, *J* 6.5), 2.19 (3 H, s), 2.11 (3 H, s), 2.06 (3 H, s), 2.02 (3 H, s).

^{13}C NMR (126 MHz, $CDCl_3$); 170.4, 170.2, 170.1, 169.5, 154.6, 129.5 – 129.3 (m), 126.5, 123.5, 115.7, 99.9, 71.1, 70.7, 68.6, 66.8, 61.4, 20.8, 20.7, 20.6, 20.6.

HRMS (ESI); calc'd for $C_{32}H_{36}FeNO_{12}$ $[M+H]^+$: *m/z* 682.158, found 682.166.

2-((β-D-galactopyranosyl)oxy)benzyl (ferrocenyl)carbamate 11



2-((2,3,4,6-tetraacetyl-β-D-galactopyranosyl)oxy)benzyl (ferrocenyl)carbamate (87 mg, 0.13 mmol, 1 eq.) was suspended in MeOH (1 mL). Sodium methoxide (3 mg, 0.64 mmol, 5 eq.) in one portion and the reaction was stirred for 1 h, after which the reaction mixture was filtered. The filtrate was concentrated to give an orange oil. Purification *via* silica gel column chromatography (DCM 9:1 MeOH (R_f = 0.38)) gave the title compound as an orange solid (50 mg, 76%).

Mp: 160-163 °C

IR (solid cm^{-1}); 3568, 3287, 2879, 1679, 1608, 1552, 1499.

^{1}H NMR (500 MHz, CD_3OD); 7.38 (1 H, d, *J* 7.6), 7.31 – 7.23 (2 H, m), 7.04 (1 H, t, *J* 7.4), 5.35 – 5.25 (2 H, m), 4.87 (1 H, d, *J* 7.8), 4.48 (2 H, s), 4.10 (5 H, s), 3.93 (2 H, t, *J* 2.0), 3.91 (1 H, dd, *J* 3.4, 1.0), 3.86 (1 H, dd, *J* 9.7, 7.8), 3.78 (2 H, qd, *J* 11.4, 6.0), 3.69 (1 H, ddd, *J* 6.8, 5.2, 1.0), 3.59 (1 H, dd, *J* 9.7, 3.4).

^{13}C NMR (126 MHz, CD_3OD) 156.9, 130.4, 129.9, 127.8, 123.4, 116.8, 103.7, 77.1, 74.9, 72.4, 70.2, 70.0, 65.1, 62.8, 62.5, 61.6.

HRMS (ESI); calc'd for $C_{24}H_{27}FeNO_8$ $[M]^+$: *m/z* 513.109, found 513.112.

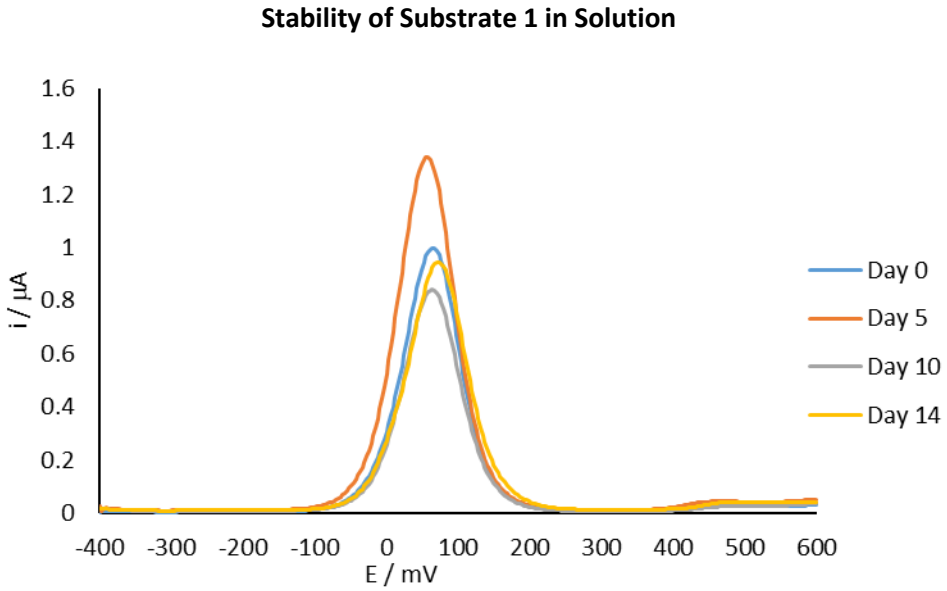


Figure **S1** – Differential pulse voltammogram obtained of substrate **1** (1 mM) after X days.

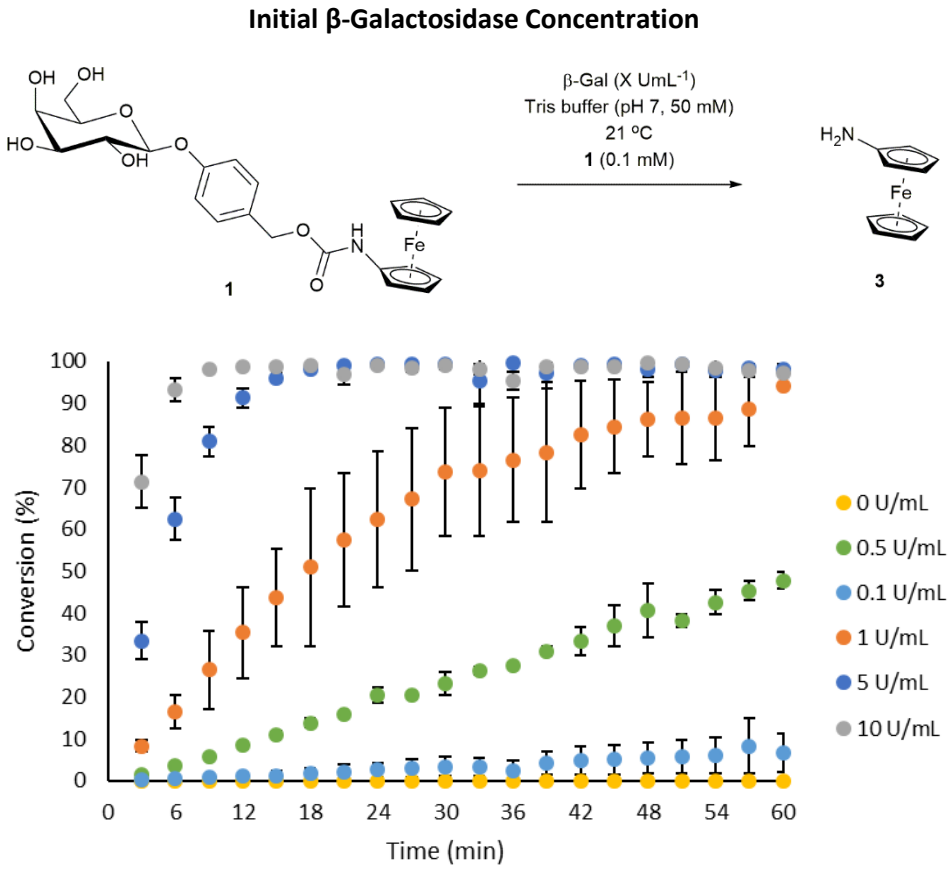


Figure **S2** - Conversion of the substrate **1** (0.1 mM) to the product after addition of β-Gal (X UmL^{-1}) at room temperature in tris buffer (pH 7, 50 mM). Error bars represent the standard deviation where n = 3.

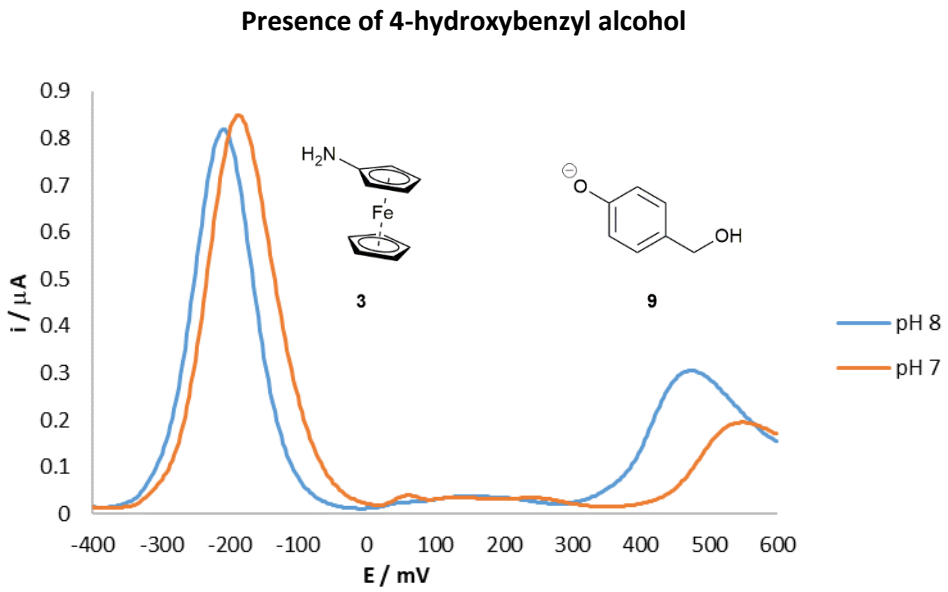


Figure **S3** - Differential pulse voltammogram obtained of substrate **1** (1 mM) after addition of β-Gal (1 UmL^{-1}) at room temperature in tris buffer (pH X, 50 mM) after 45 minutes.

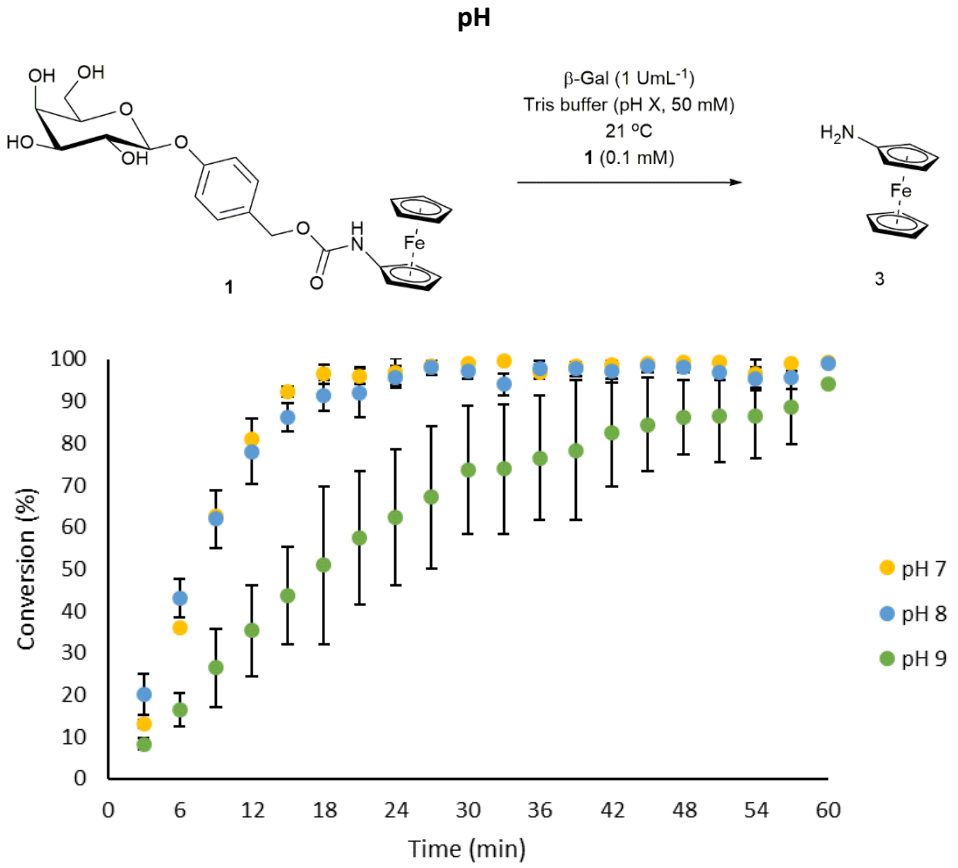


Figure **S4** - Conversion of the substrate 1 (0.1 mM) to the product after addition of β-Gal (1 UmL^{-1}) at room temperature in tris buffer (pH X, 50 mM). Error bars represent the standard deviation where n = 3.

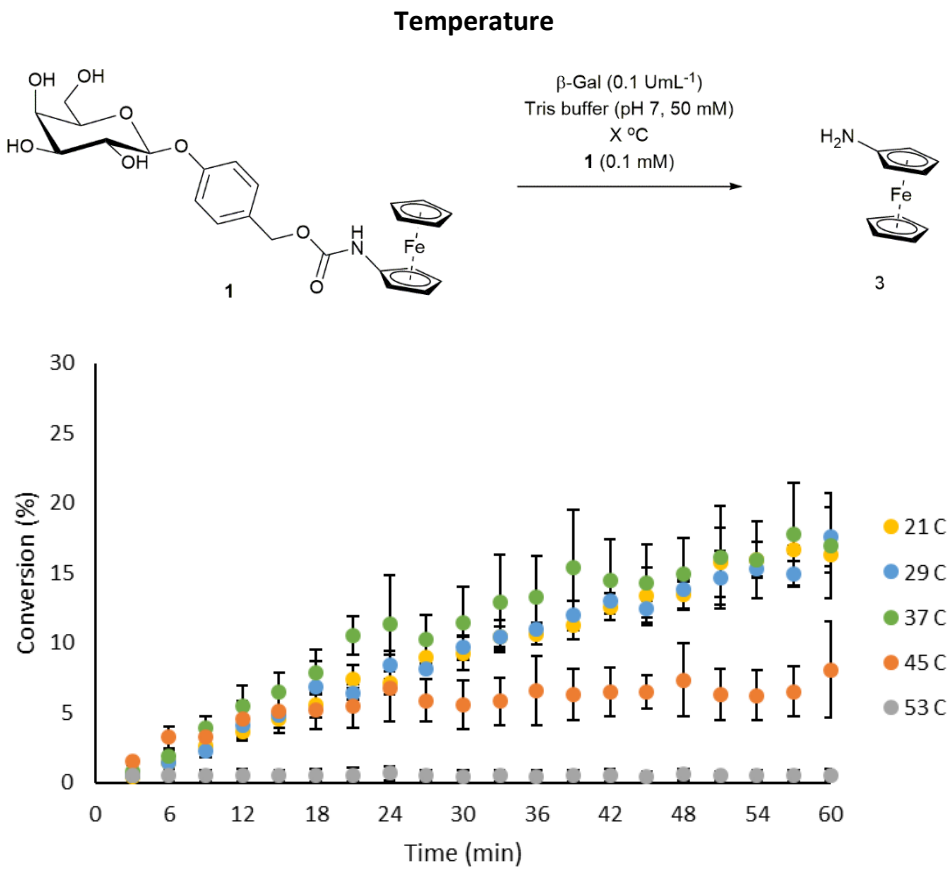


Figure **S5** - Conversion of the substrate 1 (0.1 mM) to the product after addition of β-Gal (0.1 UmL^{-1}) at varying temperatures (X °C) in tris buffer (pH 7, 50 mM). Error bars represent the standard deviation where n = 3.

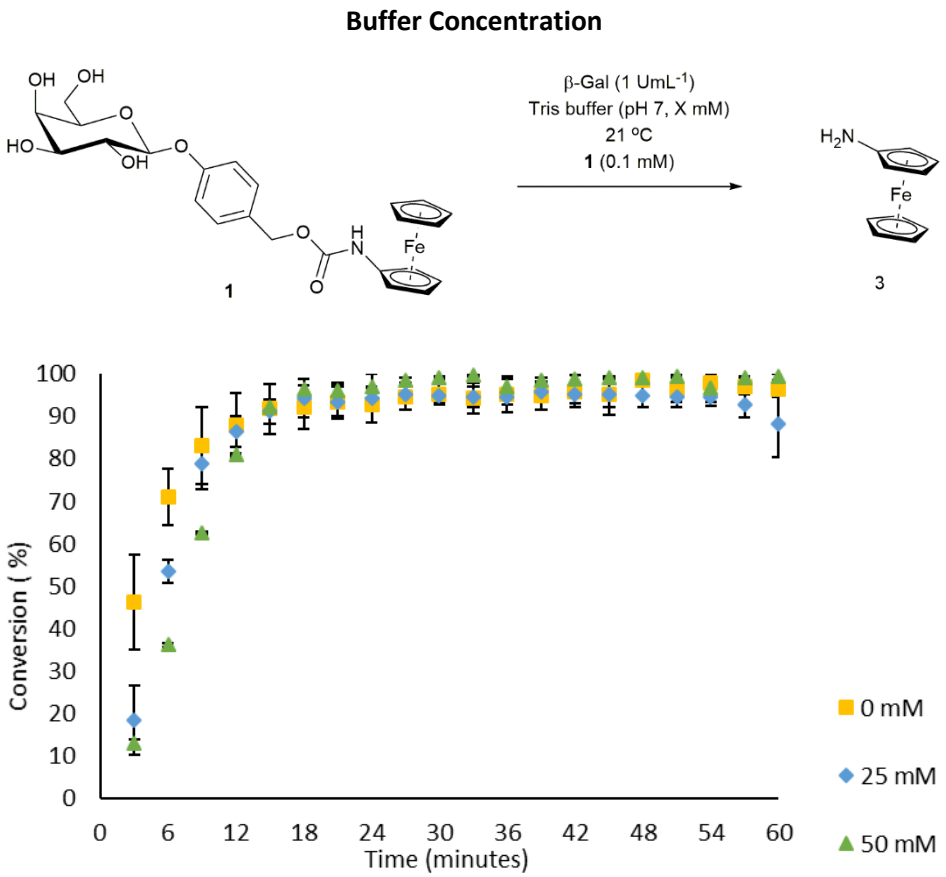


Figure S6 - Conversion of the substrate 1 (0.1 mM) to the product after addition of β-Gal (1 UmL-1) at room temperatures (21 °C) in vary concentrations of tris buffer (pH 7, X mM). Error bars represent the standard deviation where n = 3.

Kinetic Linear Transformation of Substrate 1

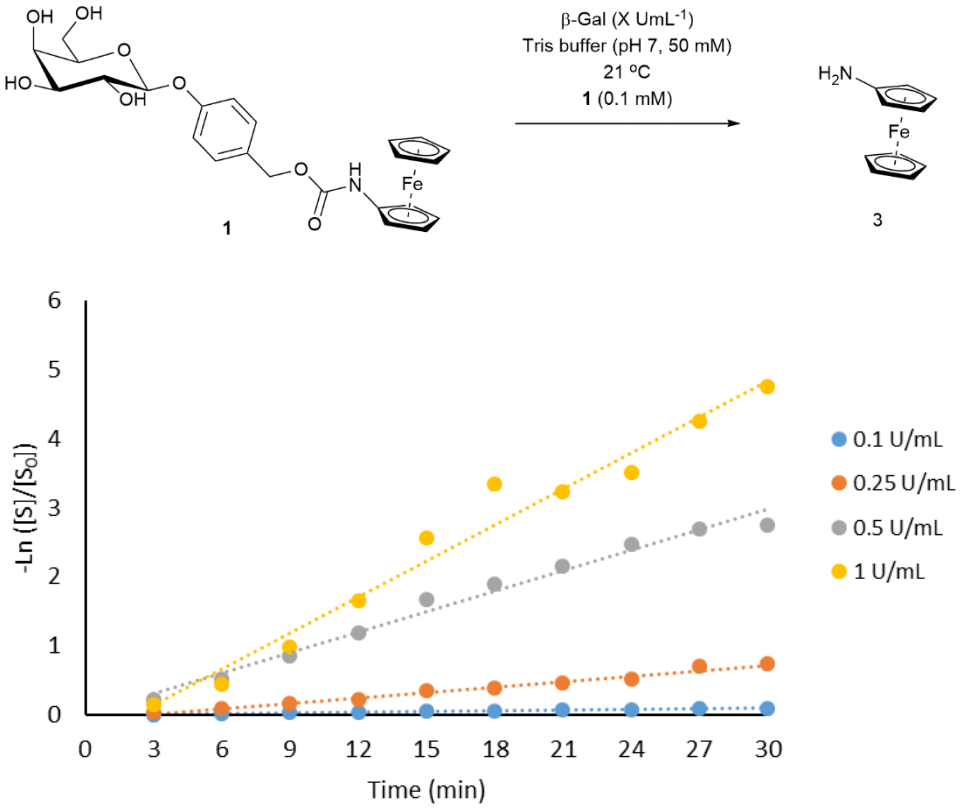
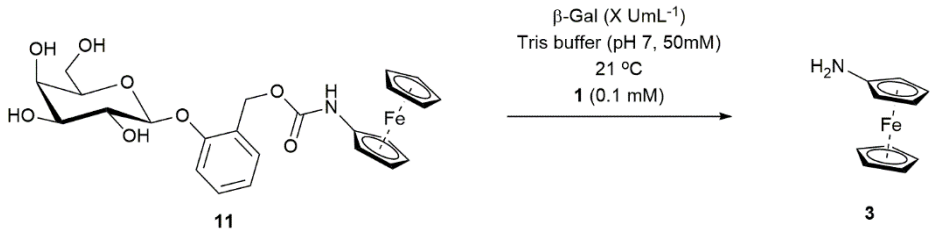


Figure **S7** – Kinetic linear transformation curves of substrate **1** (0.1 mM) at increasing concentration of β-Gal (X U mL^{-1}) at room temperature (21 °C) in tris buffer (pH 7, 50 mM).

Kinetic calculations

Pseudo-first order equation y = k x + C where: for 1 UmL^{-1} k = 0.1744 min^{-1} (2.91 x 10^{-3} s^{-1}), C = -0.3883; for 0.5 UmL^{-1} k = 0.0996 min^{-1} (1.66 x 10^{-3} s^{-1}), C = -0.0009; for 0.25 UmL^{-1} k = 0.0264 min^{-1} (0.44 x 10^{-3} s^{-1}), C = -0.0692; for 0.1 UmL^{-1} k = 0.0036 min^{-1} (0.06 x 10^{-3} s^{-1}), C = -0.0056.

Reactivity of Substrate 11



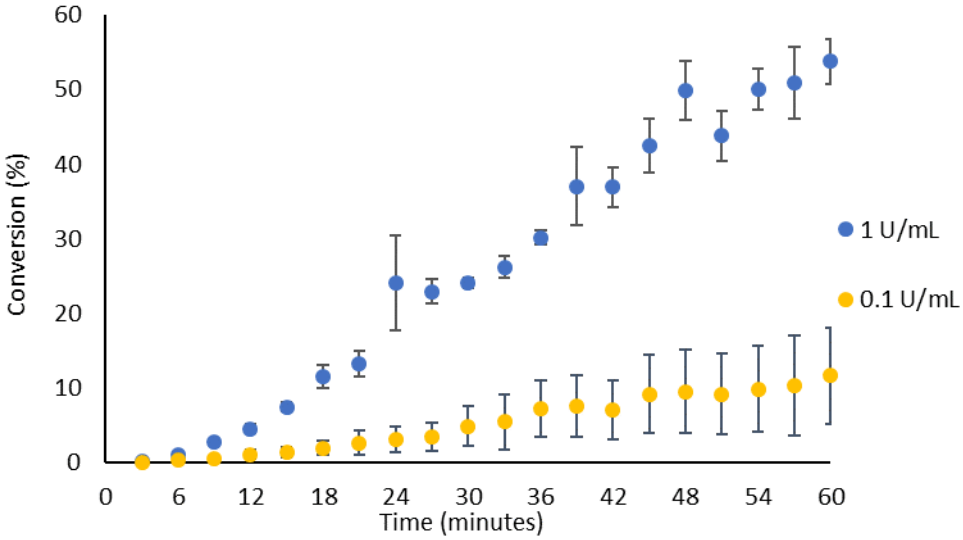


Figure **S8** - Conversion of the substrate **11** (0.1 mM) to the product after addition of varying concentrations of β-Gal (X UmL-1) at room temperatures (21 °C) in tris buffer (pH 7, 50 mM). Error bars represent the standard deviation where n = 3.

Kinetic Linear Transformation of Substrate 11

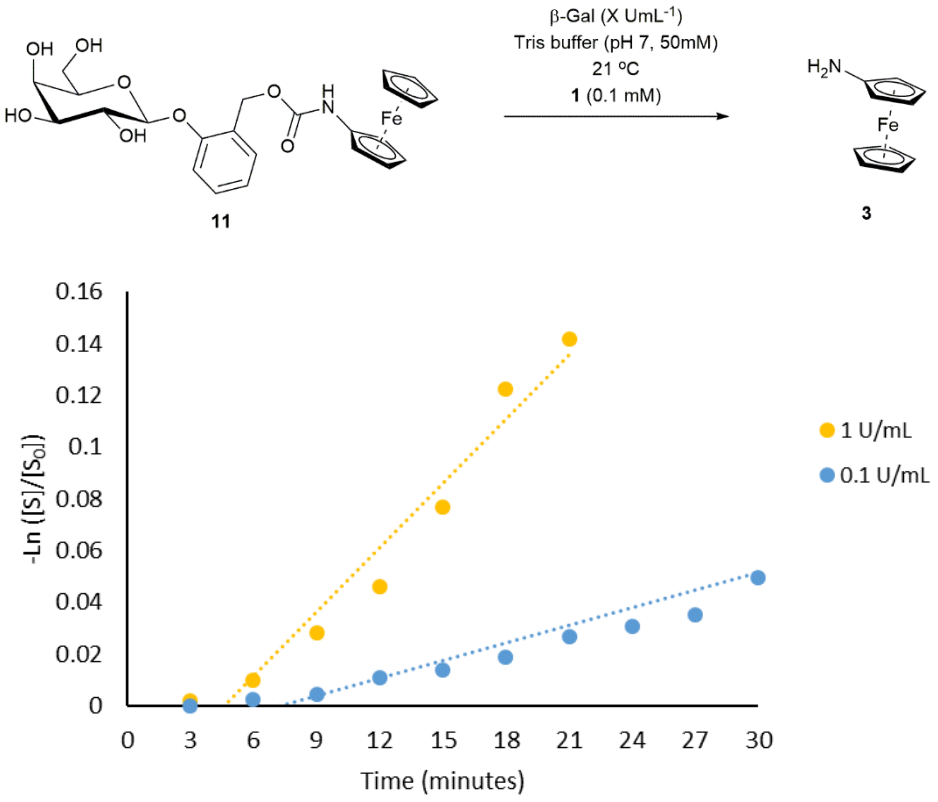


Figure **S9** – Kinetic linear transformation curves of substrate **11** (0.1 mM) at increasing concentration of β-Gal (X U mL^{-1}) at room temperature (21 °C) in tris buffer (pH 7, 50 mM).

Kinetic calculations

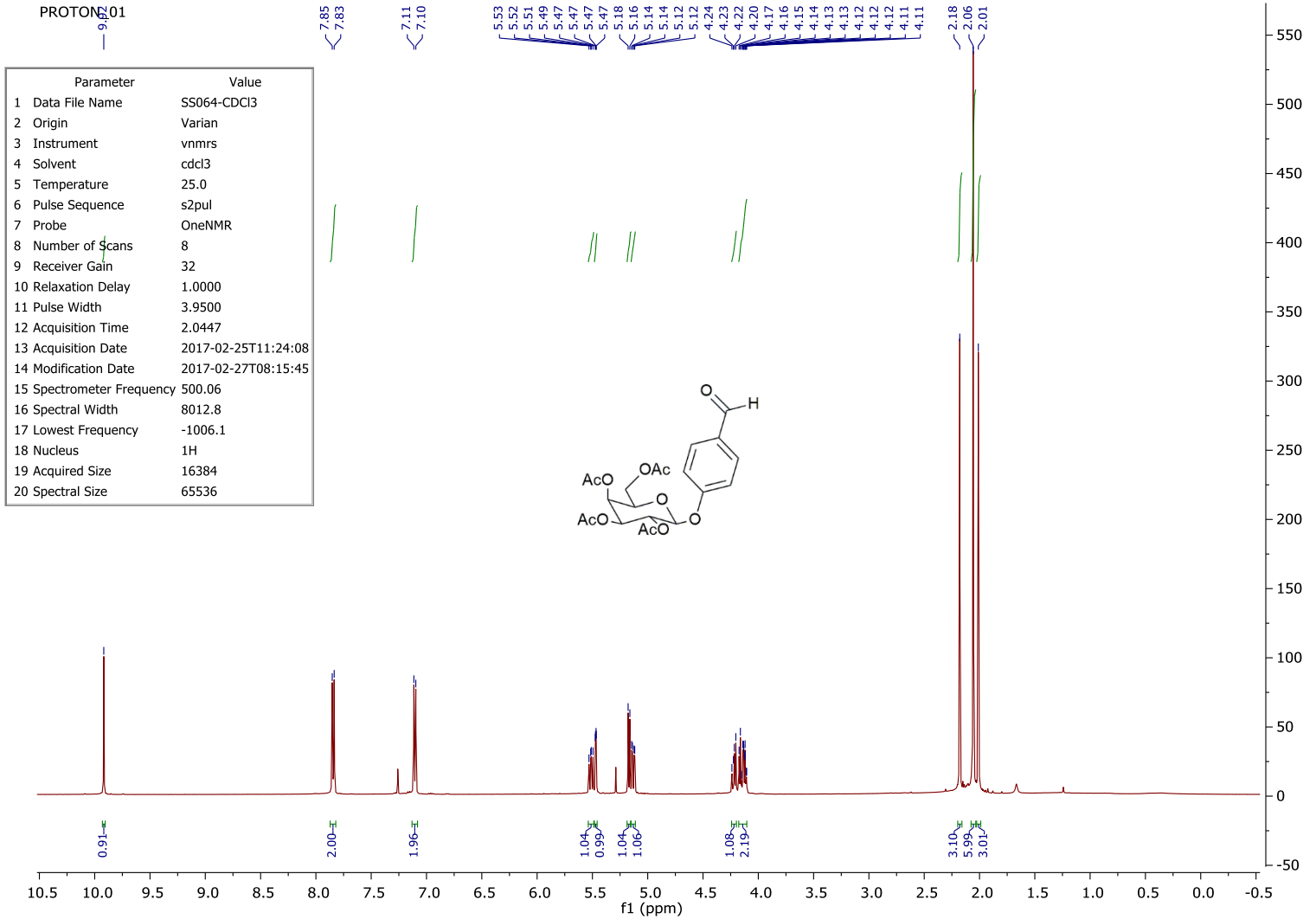
Pseudo-first order equation y = k x + C where: for 1 UmL^{-1} k = 0.0083 min^{-1} (0.14 x 10^{-3} s^{-1}), C = -0.0382; for 0.1 UmL^{-1} k = 0.0023 min^{-1} (0.04 x 10^{-3} s^{-1}), C = -0.0165.

Method for the electrochemical detection of β-Galactosidase (optimised conditions)

A 10 mM stock solution of substrate **1** (5 mg) was prepared in DMSO (1 mL). A 100 μM stock solution of substrate **1** (10 μL) was prepared using 50 mM pH 7 tris buffer (990 μL). To 800 μL of buffer (50 mM pH 7 tris buffer) in a small screw top vial equipped with a small magnetic stirrer was added 100 μL of the stock solution of **1** then 100 μL buffered (50 mM pH 7 tris buffer) solution of β-galactosidase. Every 3 minutes for 60 minutes thereafter, a 10 μL sample was subjected to electrochemical analysis.

References

1 T. Chauvin, P. Durand, M. Bernier, H. Meudal, T-B. Doan, F. Noury, B. Badet, J-C. Beloeil and E. Tóth, *Angew. Chemie. Int. Ed.*, 2008, **47**, 4370.
2 D. C. D. Butler and C. J. Richards, *Organometallics*, 2002, **21**, 5433.
3 S. Goggins, E. A. Apsey, M. F. Mahon and C. G. Frost, *Org. Biomol. Chem.*, 2017, **15**, 2459.


PROTON_01
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7.83
7.11
7.10
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5.52
5.51
5.49
5.47
5.47
5.47
5.47
5.18
5.16
5.14
5.14
5.12
5.12
4.24
4.23
4.22
4.20
4.17
4.16
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4.12
4.12
4.12
4.11
4.11
2.18
2.06
2.01
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13 Acquisition Date 2017-02-25T11:24:08
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15 Spectrometer Frequency 500.06
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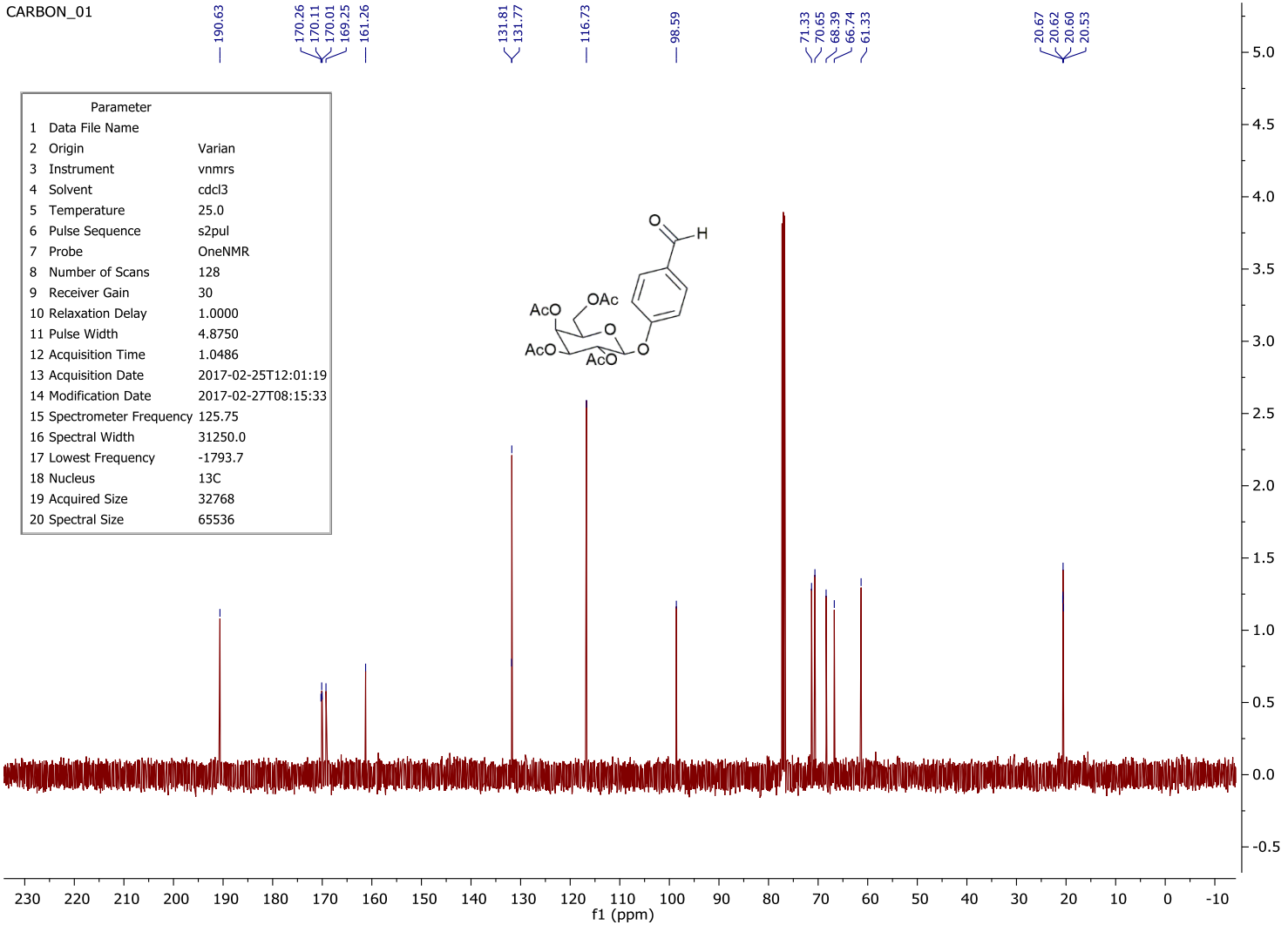
17 Lowest Frequency -1006.1
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 65536
O
H
AcO
OAc
O
AcO
AcO
O
0.91
2.00
1.96
1.04
0.99
1.04
1.06
1.08
2.19
3.10
5.99
3.01
f1 (ppm)

CARBON_01



Parameter	
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11 Pulse Width	4.8750
12 Acquisition Time	1.0486
13 Acquisition Date	2017-02-25T12:01:19
14 Modification Date	2017-02-27T08:15:33
15 Spectrometer Frequency	125.75
16 Spectral Width	31250.0
17 Lowest Frequency	-1793.7
18 Nucleus	13C
19 Acquired Size	32768
20 Spectral Size	65536

PROTON_01

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3	Instrument	vnmrs
4	Solvent	cdcl3
5	Temperature	25.0
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7	Probe	OneNMR
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10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
13	Acquisition Date	2017-03-08T07:43:00
14	Modification Date	2017-03-08T07:55:55
15	Spectrometer Frequency	500.06
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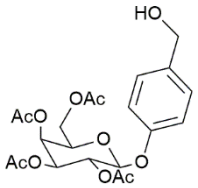
17	Lowest Frequency	-1006.6
18	Nucleus	1H
19	Acquired Size	16384
20	Spectral Size	262144



5

f1 (ppm)

CARBON_01

	Parameter	Value
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7	Probe	OneNMR
8	Number of Scans	128
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
13	Acquisition Date	2017-03-08T08:36:27
14	Modification Date	2017-03-08T09:55:24
15	Spectrometer Frequency	125.75
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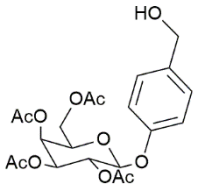
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536



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PROTON_01

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7	Probe	OneNMR
8	Number of Scans	8
9	Receiver Gain	36
10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
13	Acquisition Date	2017-06-15T10:42:01
14	Modification Date	2017-06-15T11:53:47
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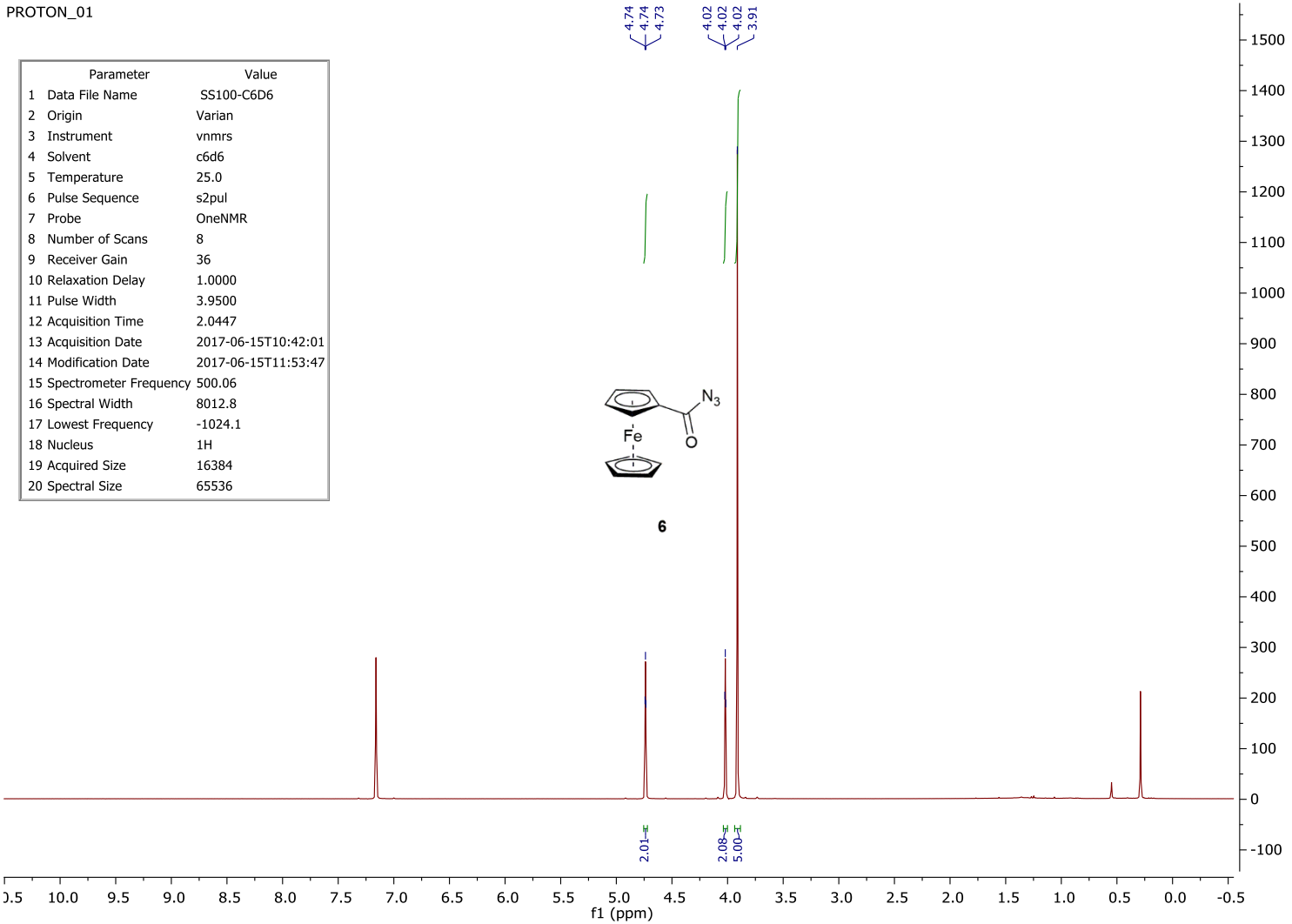
18	Nucleus	1H
19	Acquired Size	16384
20	Spectral Size	65536



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7	Probe	OneNMR
8	Number of Scans	128
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
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15	Spectrometer Frequency	125.75
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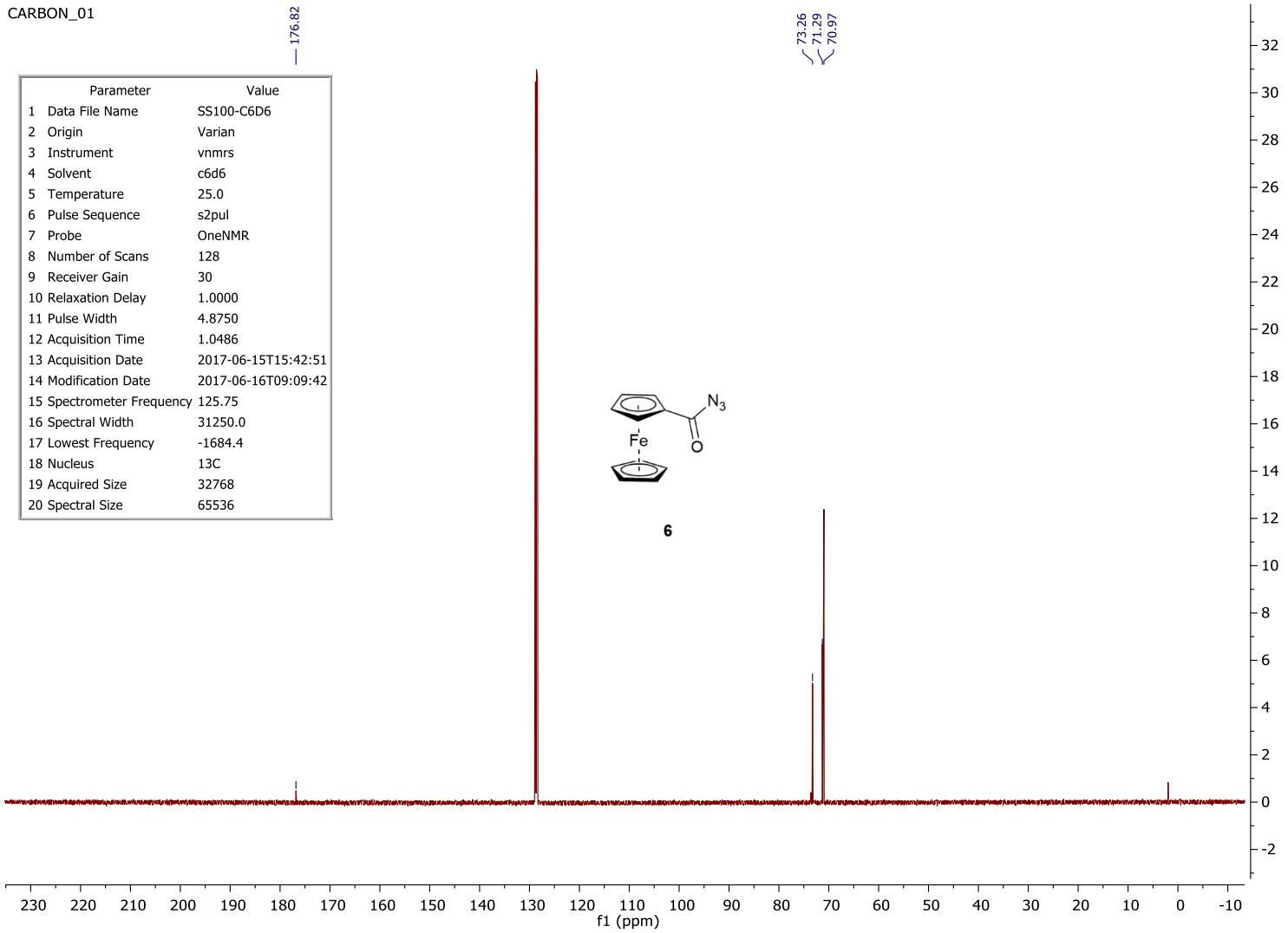
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536



PROTON_01

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3	Instrument	vnmrs
4	Solvent	cdcl3
5	Temperature	25.0
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7	Probe	OneNMR
8	Number of Scans	8
9	Receiver Gain	38
10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
13	Acquisition Date	2017-06-20T13:09:31
14	Modification Date	2017-06-20T13:40:14
15	Spectrometer Frequency	500.06
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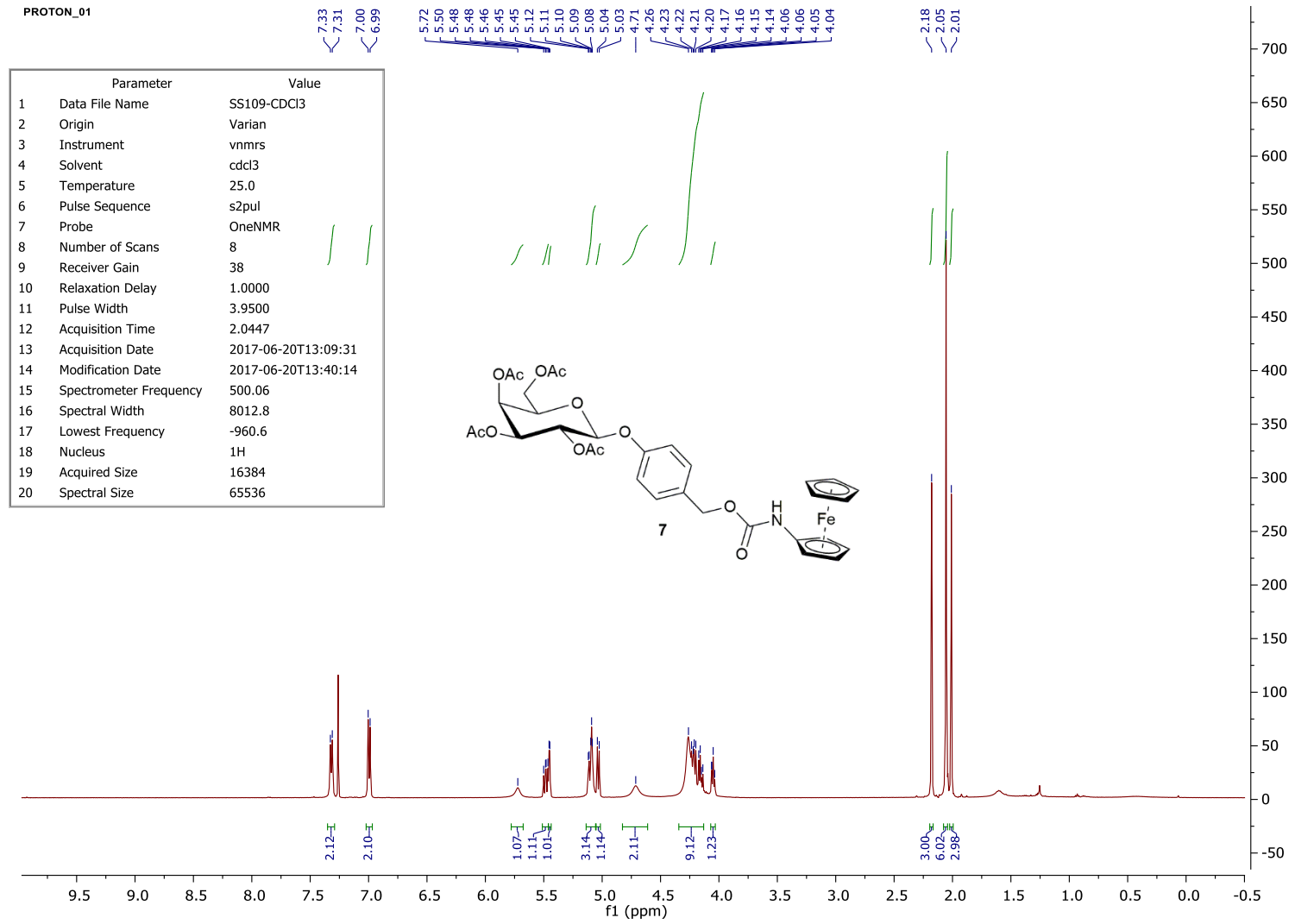
17	Lowest Frequency	-960.6
18	Nucleus	1H
19	Acquired Size	16384
20	Spectral Size	65536



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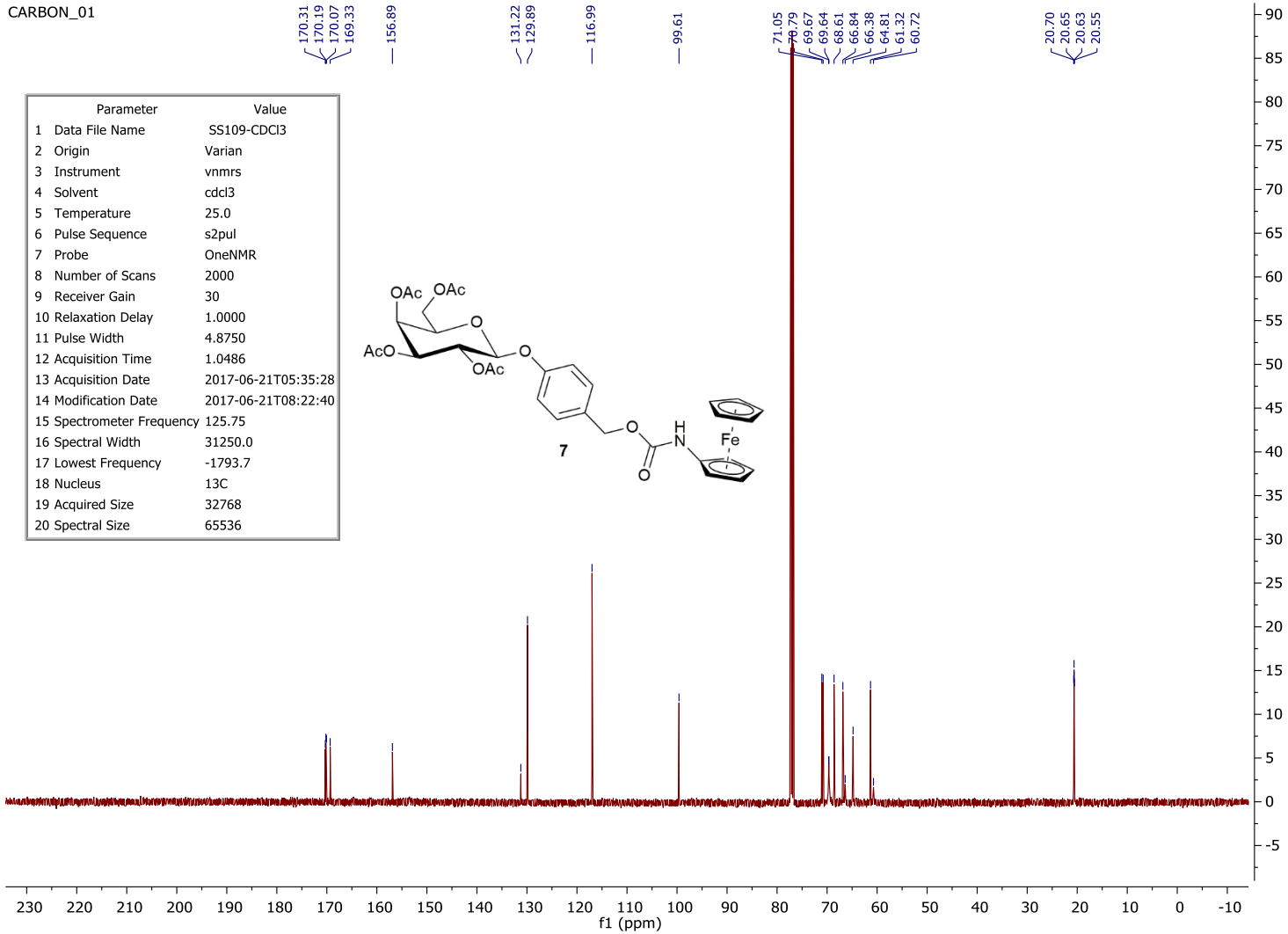

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5	Temperature	25.0
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7	Probe	OneNMR
8	Number of Scans	2000
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
13	Acquisition Date	2017-06-21T05:35:28
14	Modification Date	2017-06-21T08:22:40
15	Spectrometer Frequency	125.75
16	Spectral Width	31250.0
17	Lowest Frequency	-1793.7
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536

PROTON_01

	Parameter	Value
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2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cd3od
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	8
9	Receiver Gain	38
10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
13	Acquisition Date	2017-06-26T10:58:39
14	Modification Date	2017-06-26T12:25:10
15	Spectrometer Frequency	500.06
16	Spectral Width	8012.8
17	Lowest Frequency	-1003.5
18	Nucleus	1H
19	Acquired Size	16384
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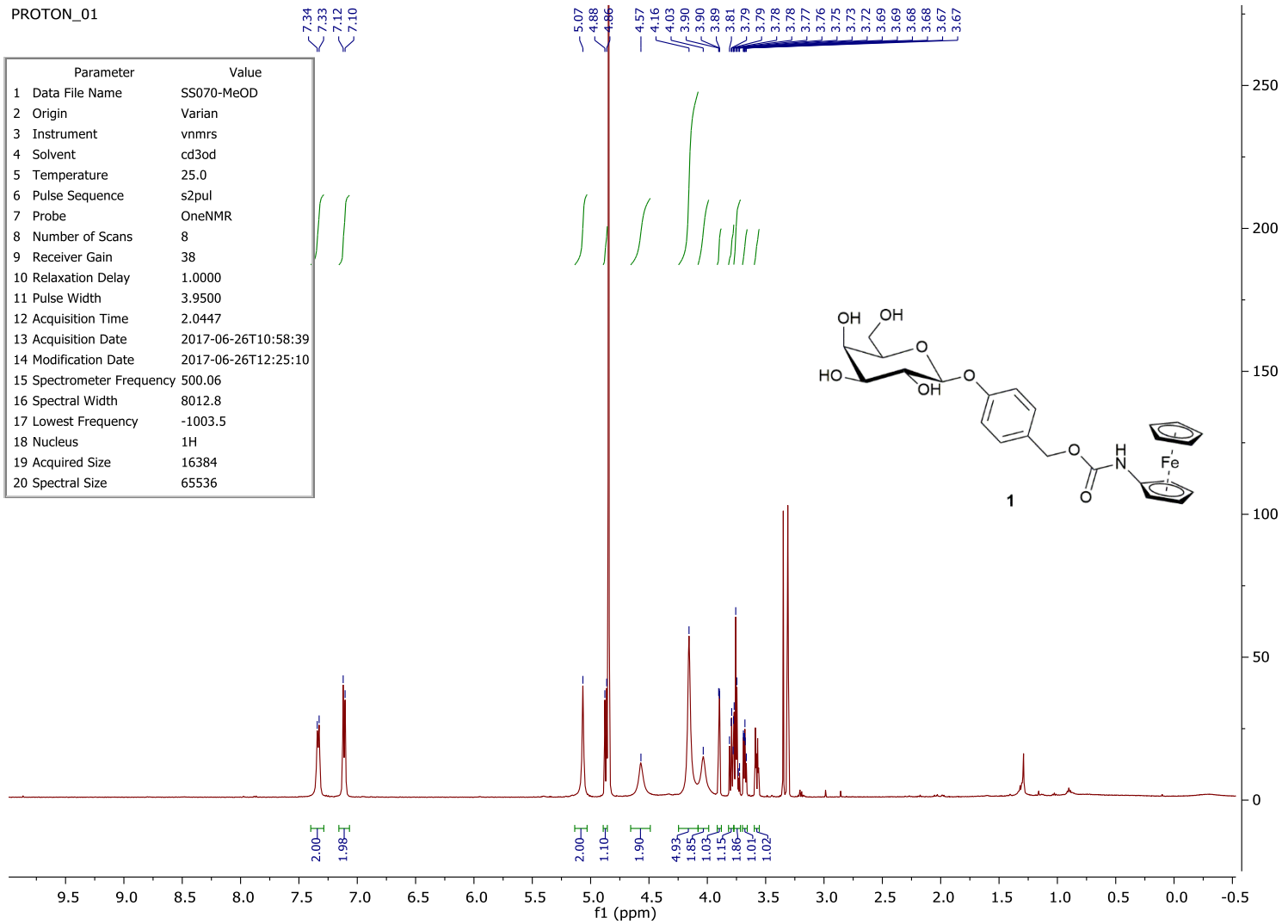

CARBON_02

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3	Instrument	vnmrs
4	Solvent	cd3od
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9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
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15	Spectrometer Frequency	125.75
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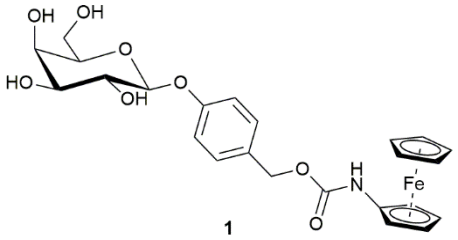
17	Lowest Frequency	-1613.7
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536



1

PROTON_01

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7	Probe	OneNMR
8	Number of Scans	8
9	Receiver Gain	40
10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
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15	Spectrometer Frequency	500.06
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19	Acquired Size	16384
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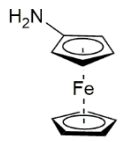

3

CARBON_01

	Parameter	Value
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4	Solvent	cdcl3
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7	Probe	OneNMR
8	Number of Scans	128
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
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19	Acquired Size	32768
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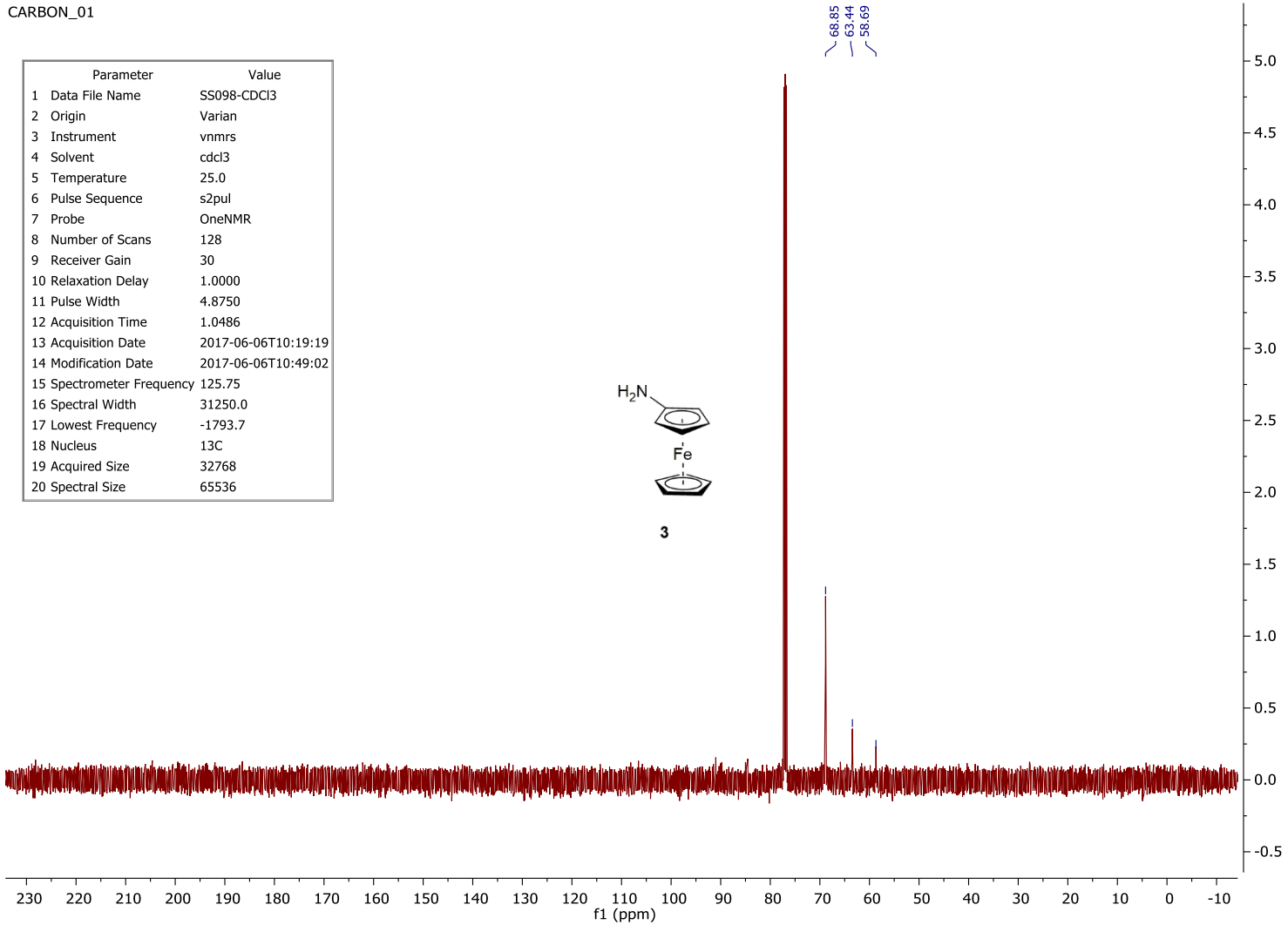

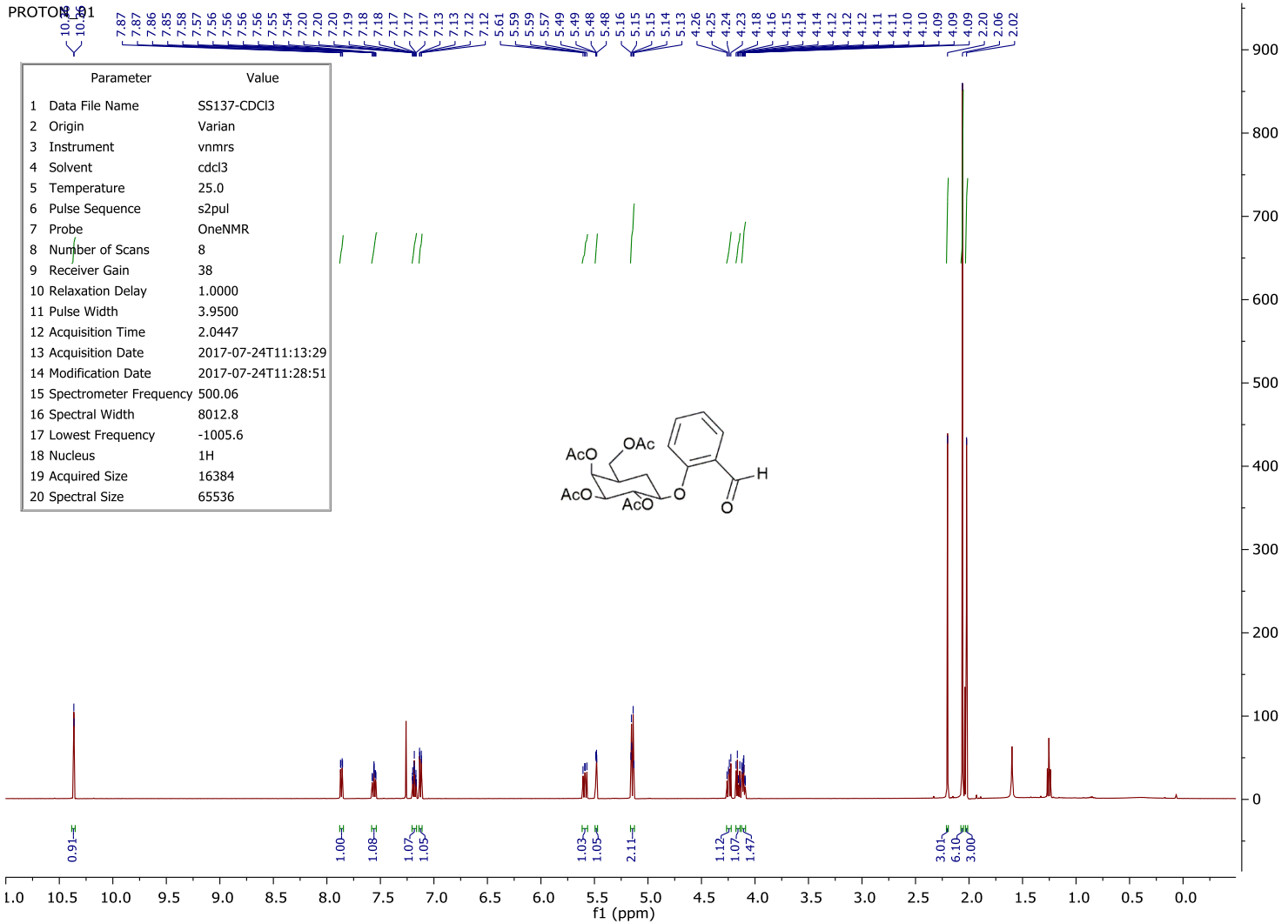
PROTON_01
10.31
10.31
7.87 7.87 7.86 7.85 7.58 7.57 7.56 7.56 7.56 7.56 7.55 7.54 7.20 7.20 7.20 7.19 7.18 7.18 7.17 7.17 7.17 7.13 7.13 7.12 7.12
5.61 5.59 5.59 5.57 5.49 5.49 5.48 5.48 5.16 5.15 5.15 5.14 5.13
4.26 4.25 4.24 4.23 4.18 4.16 4.15 4.14 4.14 4.12 4.12 4.12 4.11 4.11 4.10 4.10 4.09 4.09 4.09 2.20 2.06 2.02
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2 Origin Varian
3 Instrument vnmrs
4 Solvent cdcl3
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7 Probe OneNMR
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9 Receiver Gain 38
10 Relaxation Delay 1.0000
11 Pulse Width 3.9500
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15 Spectrometer Frequency 500.06
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17 Lowest Frequency -1005.6
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 65536
AcO
OAc
AcO
AcO
O
O
H
O
900
800
700
600
500
400
300
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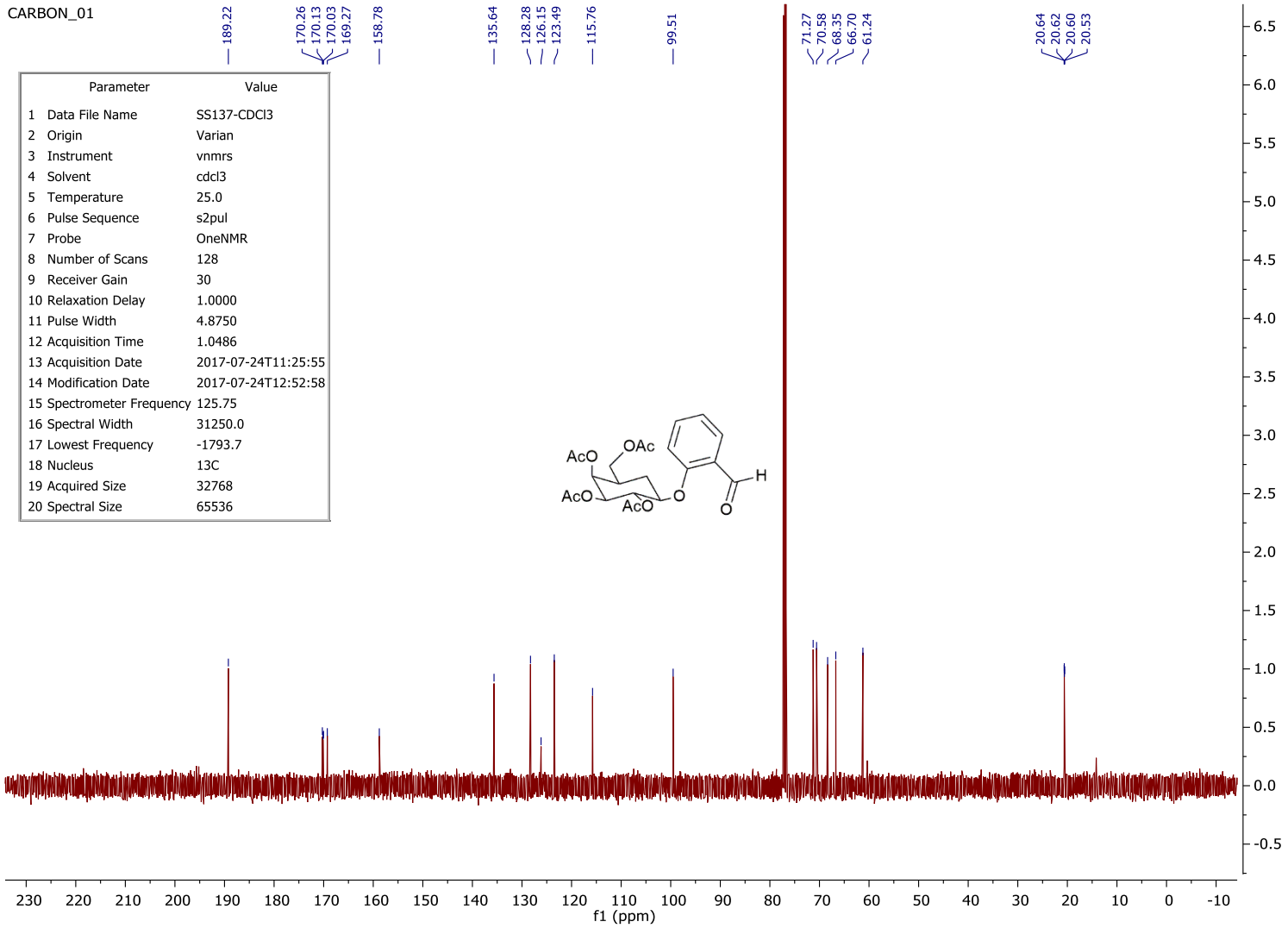
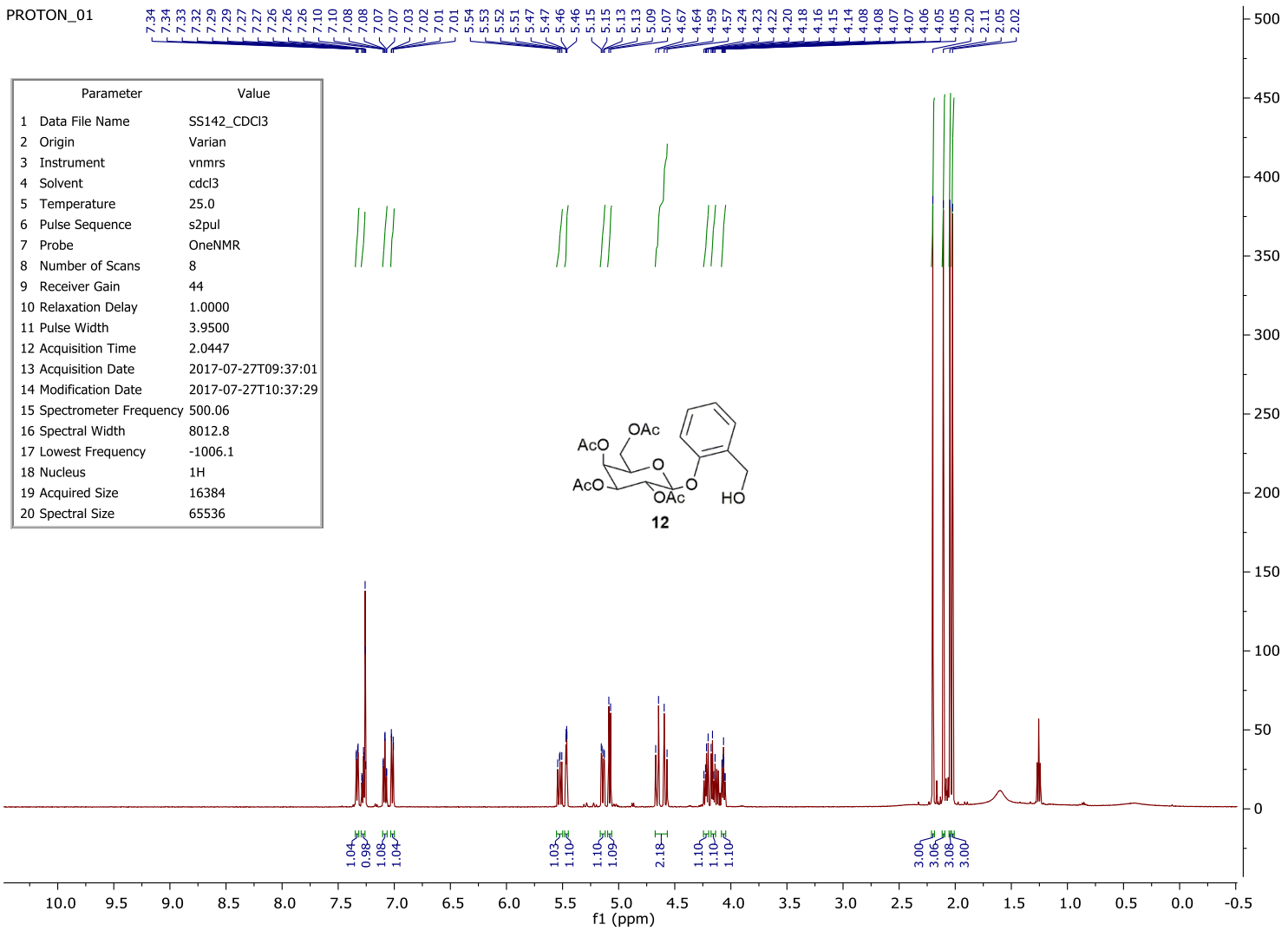
1.08
1.07
1.05
1.03
1.05
2.11
1.12
1.07
1.47
3.01
6.10
3.00
1.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

CARBON_01



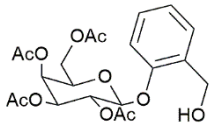
	Parameter	Value
1	Data File Name	SS137-CDCl3
2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cdcl3
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	128
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
13	Acquisition Date	2017-07-24T11:25:55
14	Modification Date	2017-07-24T12:52:58
15	Spectrometer Frequency	125.75
16	Spectral Width	31250.0
17	Lowest Frequency	-1793.7
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536

PROTON_01


7.34 7.34 7.33 7.32 7.29 7.29 7.27 7.27 7.26 7.26 7.26 7.10 7.10 7.08 7.08 7.07 7.07 7.03 7.02 7.01 7.01
5.54 5.53 5.52 5.51 5.47 5.47 5.46 5.46 5.15 5.15 5.13 5.13 5.09 5.07 4.67 4.64 4.59 4.57 4.24 4.23 4.22 4.20 4.18 4.16 4.15 4.14 4.08 4.08 4.07 4.07 4.06 4.05 4.05 2.20 2.11 2.05 2.02
Parameter Value
1 Data File Name SS142_CDCl3
2 Origin Varian
3 Instrument vnmrs
4 Solvent cdcl3
5 Temperature 25.0
6 Pulse Sequence s2pul
7 Probe OneNMR
8 Number of Scans 8
9 Receiver Gain 44
10 Relaxation Delay 1.0000
11 Pulse Width 3.9500
12 Acquisition Time 2.0447
13 Acquisition Date 2017-07-27T09:37:01
14 Modification Date 2017-07-27T10:37:29
15 Spectrometer Frequency 500.06
16 Spectral Width 8012.8
17 Lowest Frequency -1006.1
18 Nucleus 1H
19 Acquired Size 16384
20 Spectral Size 65536
AcO
OAc
AcO
O
O
OAc
HO
12
1.04 0.98 1.08 1.04 1.03 1.10 1.10 1.09 2.18 1.10 1.10 1.10 3.00 3.06 3.08 3.00
500 450 400 350 300 250 200 150 100 50 0
10.0 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 0.0 -0.5
f1 (ppm)

CARBON_01

	Parameter	Value
1	Data File Name	SS142_CDCl3
2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cdcl3
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	128
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
13	Acquisition Date	2017-07-27T10:53:20
14	Modification Date	2017-07-27T11:30:45
15	Spectrometer Frequency	125.75
16	Spectral Width	31250.0
17	Lowest Frequency	-1793.7
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536



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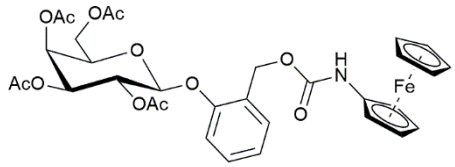
PROTON_01

	Parameter	Value
1	Data File Name	SS143-CDCl3
2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cdcl3
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	8
9	Receiver Gain	40
10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
13	Acquisition Date	2017-07-28T14:39:51
14	Modification Date	2017-07-31T08:00:04
15	Spectrometer Frequency	500.06
16	Spectral Width	8012.8
17	Lowest Frequency	-1006.1
18	Nucleus	1H
19	Acquired Size	16384
20	Spectral Size	65536



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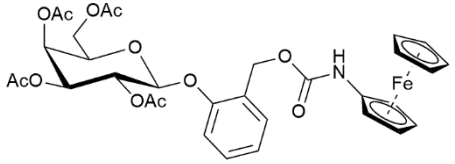
CARBON_01

	Parameter	Value
1	Data File Name	SS143-CDCl3
2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cdcl3
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	2000
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
13	Acquisition Date	2017-07-28T22:39:37
14	Modification Date	2017-07-31T07:59:54
15	Spectrometer Frequency	125.75
16	Spectral Width	31250.0
17	Lowest Frequency	-1793.7
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536



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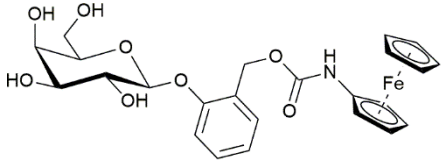
PROTON_01

	Parameter	Value
1	Data File Name	SS144-MeOD
2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cd3od
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	8
9	Receiver Gain	42
10	Relaxation Delay	1.0000
11	Pulse Width	3.9500
12	Acquisition Time	2.0447
13	Acquisition Date	2017-08-01T10:31:10
14	Modification Date	2017-08-01T11:07:18
15	Spectrometer Frequency	500.06
16	Spectral Width	8012.8
17	Lowest Frequency	-1006.0
18	Nucleus	1H
19	Acquired Size	16384
20	Spectral Size	65536



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f1 (ppm)

CARBON_01

	Parameter	Value
1	Data File Name	SS144-MeOD
2	Origin	Varian
3	Instrument	vnmrs
4	Solvent	cd3od
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Probe	OneNMR
8	Number of Scans	2000
9	Receiver Gain	30
10	Relaxation Delay	1.0000
11	Pulse Width	4.8750
12	Acquisition Time	1.0486
13	Acquisition Date	2017-08-02T05:24:01
14	Modification Date	2017-08-02T08:21:15
15	Spectrometer Frequency	125.75
16	Spectral Width	31250.0
17	Lowest Frequency	-1614.9
18	Nucleus	13C
19	Acquired Size	32768
20	Spectral Size	65536