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Fluoro-glycosyl acridinones are ultra-sensitive active site titrating agents for retaining *β*-glycosidases

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Contents

Materials and methods

All fine chemicals were obtained from commercial suppliers (Sigma-Aldrich® and Fisher Scientific®). Methanol was distilled over Mg and MeCN was distilled over CaH_2. Deionized water was prepared using a Millipore-Directed QTM 5 Ultrapure Water System. TLC was performed on Merck pre-coated 0.2 mm aluminum-backed sheets of Silica Gel $60F_{254}$. TLC plates were stained in 10% sulfuric acid in EtOH or 10% ammonium molybdate in 2 M H_2SO_4, followed by development by heating. Flash column chromatography was performed using 230-400 mesh Silicycle® silica gel. ^1H and ^{13}C NMR spectra were acquired on a 300 or 400 MHz Bruker® spectrometer. Mass spectra were obtained using a Waters® ZQ Mass Detector equipped with ESCI ion source. LacZ was obtained from Sigma-Aldrich® and human GCase was obtained from Genzyme® as the recombinant drug Cerezyme®. *T. reesei* EG-I cellulase was a generous gift from the Iogen® corporation. Abg, Bhx and Cex were expressed in-house according to established procedures.[1-3]

Synthesis and characterization

General procedure for the synthesis of glycosyl bromides

The per-*O*-acetylated 2-deoxy-2-fluoro-sugar[4] was dissolved in DCM (1 ml/mmol sugar) under argon and cooled in an ice bath. Hydrogen bromide in AcOH (33% w/w, 0.5 ml/mmol sugar) was added drop-wise and the solution was stirred at r.t. Upon completion of the reaction (as determined by TLC), the mixture was diluted with DCM and washed with sat. $NaHCO_3$ until the aqueous layer was basic, followed by brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and the solvent evaporated under reduced pressure. These products were used immediately in further syntheses.

General procedure for Koenigs-Knorr reactions

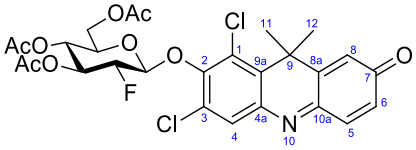
Glycosyl bromide in dry MeCN (2.0 ml/mmol glycosyl bromide) was added to a well-stirred suspension of DDAO (1.0 mol eqv.), 2,6-lutidine (2.0 mol eqv.), Ag_2O (2.0 mol eqv.) and $CaSO_4$ (300 mg/mmol glycosyl bromide) in MeCN (10 ml/mmol glycosyl bromide) under argon. The reaction was protected from light and stirred at r.t. overnight. The reaction was filtered through diatomaceous earth, washed with sat. $NaHCO_3$ and brine, dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The products were purified by flash chromatography and/or recrystallized to homogeneity.

General procedure for deacetylation

The per-*O*-acetylated glycoside was dissolved in dry MeOH (1–2 ml MeOH per 10 mg sugar) and cooled to 0°C. Sodium methoxide in MeOH (380 mM) was added drop-wise to give a final concentration of 20 mM. The reaction was stirred at r.t. Upon completion (as determined by TLC), the reaction was neutralized with acid resin (Amberlite® IR-120, H^+), filtered and concentrated under reduced pressure. Purification was carried out by flash chromatography on silica gel or recrystallization (MeOH) to homogeneity.

Synthetic chemistry experimental information

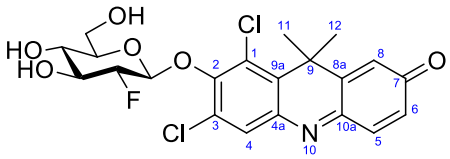
9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-*β*-D-glucopyranoside **(8)**



1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-fluoro-D-glucose[5] (448 mg, 1.20 mmol), was treated according to the general procedure for the synthesis of glycosyl bromides. The product was coupled to DDAO according to the general procedure for Koenigs-Knorr reactions. The crude product was purified by iterative flash chromatography (Me_2CO/hexanes, 1:9–3:17 then hexanes/DCM/EtOAc, 2:2:1). This material was recrystallized (Me_2CO/hexanes) to give glucoside **8** as yellow needles (230 mg, 32%). **HRMS** mass calculated for $C_{27}H_{26}Cl_2FNNaO_9$: 620.0866; found: 620.0880 $[M+Na]^+$. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.78 (s, 3 H, $CH_{3(Ar)}$) 1.80 (s, 3 H, $CH_{3(Ar)}$) 2.01 (s, 3 H, C(O)CH_3) 2.04 (s, 3 H, C(O)CH_3) 2.12 (s, 3 H, C(O)CH_3) 3.72 (ddd, 1 H, J_{H5-H4} 10.0 J_{H5-H6} 4.7 $J_{H5-H6'}$ 2.6 Hz, H5) 4.11 (dd, 1 H, $J_{H6'-H6}$ 12.2 $J_{H6'-H5}$ 2.6 Hz, H6') 4.20 (dd, 1 H, $J_{H6-H6'}$ 12.2 J_{H6-H5} 4.7 Hz, H6) 4.74 (ddd, 1 H, J_{H2-F} 50.7 J_{H2-H3} 9.0 J_{H2-H1} 7.6 Hz, H2) 5.17 (dd, 1 H, J_{H4-H3} 9.7 J_{H4-H5} 10.0 Hz, H4) 5.38 (dd, 1 H, J_{H1-H2} 7.6, J_{H1-F2} 2.4 Hz, H1) 5.42 (ddd, 1 H, J_{H3-F2} 14.6 J_{H3-H2} 9.0 J_{H3-H4} 9.7 Hz, H3) 6.67 (d, 1 H, $J_{H8(Ar)-H6(Ar)}$ 1.8 Hz, $H8_{(Ar)}$) 6.67 (dd, 1 H, $J_{H6(Ar)-H5(Ar)}$10.2 $J_{H6(Ar)-H8(Ar)}$ 1.8 Hz, $H6_{(Ar)}$) 7.36 (d, 1 H, $J_{H5(Ar)-H6(Ar)}$ 10.2 Hz, $H5_{(Ar)}$) 7.75 (s, 1 H, $H4_{(Ar)}$) **^{19}F NMR** (282 MHz, $CDCl_3$) δ ppm -199.25 (ddd, J_{F2-H2} 50.7 J_{F2-H3} 14.6 J_{F2-H1} 2.4 Hz, F2) **^{13}C NMR** (101 MHz, $CDCl_3$) δ ppm 20.86 (C(O)CH_3), 20.93 (C(O)CH_3), 20.97 (C(O)CH_3), 29.10 ($CH_{3(Ar)}$), 29.16 ($CH_{3(Ar)}$), 38.41 ($C9_{(Ar)}$), 61.72 (C6), 68.33 (d, J_{C4-F2} 6.9 Hz, C4) 72.56 (C5), 72.86 (d, J_{C3-F2} 19.9 Hz, C3), 90.04 (d, J_{C2-F2} 194.7 Hz, C2), 100.69 (d, J_{C1-F2} 23.0 Hz, C1),

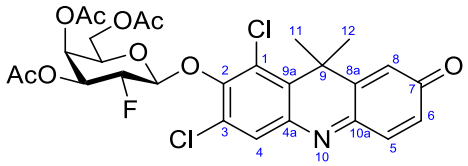
128.54, 129.19 ($C8_{(Ar)}$), 130.48 ($C6_{(Ar)}$), 132.67 ($C4_{(Ar)}$), 132.91, 133.59, 141.05, 141.20 ($C5_{(Ar)}$), 148.55, 149.81 ($C2_{(Ar)}$), 153.50, 169.77 ($C(O)CH_3$), 170.31 ($C(O)CH_3$), 170.62 ($C(O)CH_3$), 187.54 ($C{=}O_{(Ar)}$).

9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 2-deoxy-2-fluoro-*β*-D-glucopyranoside (DDAO-2FGlc) **(1)**



Compound **8** (53 mg, 0.089 mmol) was treated according to the general procedure for deacetylation. The crude product was purified by flash chromatography (EtOAc/hexanes, 3:2–4:1) to give polyol **1** as a yellow powder (38 mg, 91%). **HRMS** mass calculated for $C_{21}H_{20}Cl_2FNNaO_6$: 494.0549; found: 494.0562 $[M+Na]^+$. **^{1}H NMR** (400 MHz, CD_3OD) δ ppm 1.83 (s, 6 H, 2 × CH_3) 3.34 (m, H5) 3.44 (t, 1 H, $J_{H4\text{-}H3}$=$J_{H4\text{-}H5}$ 9.3 Hz, H, H4) 3.65 (dd, 1 H, $J_{H6\text{-}H6'}$ 12.0 $J_{H6\text{-}H5}$ 5.8 Hz, H6) 3.70 (ddd, 1 H, $J_{H3\text{-}F2}$ 16.0 $J_{H3\text{-}H2}$ 8.9 $J_{H3\text{-}H4}$ 9.3 Hz, H3) 3.80 (dd, 1 H, $J_{H6'\text{-}H6}$ 12.0 $J_{H6'\text{-}H5}$ 2.0 Hz, H6') 4.40 (ddd, 1 H, $J_{H2\text{-}F2}$ 51.4 $J_{H2\text{-}H3}$ 8.9 $J_{H2\text{-}H1}$ 7.8 Hz, H2) 5.41 (dd, 1 H, $J_{H1\text{-}H2}$ 7.8 $J_{H1\text{-}F2}$ 2.1 Hz, H1) 6.67 (dd, 1 H, $J_{H6(Ar)\text{-}H5(Ar)}$ 9.9 $J_{H6(Ar)\text{-}H8(Ar)}$ 1.8 Hz, $H6_{(Ar)}$) 6.79 (d, 1 H, $J_{H8(Ar)\text{-}H6(Ar)}$ 1.8 Hz, $H8_{(Ar)}$) 7.43 (d, 1 H, $J_{H5(Ar)\text{-}H6(Ar)}$ 9.9 Hz, $H5_{(Ar)}$) 7.77 (s, 1 H, $H4_{(Ar)}$) **^{19}F NMR** (282 MHz, CD_3OD) δ ppm -200.52 (ddd, $J_{F2\text{-}H2}$ 51.4 $J_{F2\text{-}H3}$ 16.0 $J_{F2\text{-}H1}$ 2.1 Hz, 93 F) **^{13}C NMR** (101 MHz, CD_3OD) δ ppm 29.09 ($CH_{3(Ar)}$) 29.20 ($CH_{3(Ar)}$) 39.60 ($C9_{(Ar)}$) 62.52 (C6) 71.19 (d, $J_{C4\text{-}F2}$ 7.7 Hz, C4) 76.35 (d, $J_{C3\text{-}F2}$ 17.6 Hz, C3) 78.94 (C5) 94.25 (d, $J_{C2\text{-}F2}$ 187.8 Hz, C2) 102.39 (d, $J_{C1\text{-}F2}$ 26.1 Hz, C1) 129.68, 129.99 ($C8_{(Ar)}$) 131.60, 133.30 ($C6_{(Ar)}$) 133.63 ($C4_{(Ar)}$) 134.97, 141.86, 142.48 ($C5_{(Ar)}$) 150.46, 151.74 ($C2_{(Ar)}$) 154.43, 189.14 ($C{=}O_{(Ar)}$).

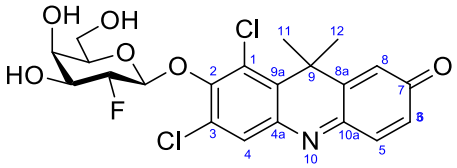
9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-*β*-D-galactopyranoside **(11)**



1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-fluoro-D-galactose[6] (430 mg, 1.23 mmol), was treated according to the general procedure for the synthesis of glycosyl bromides. The product was coupled to DDAO according to the general procedure for Koenigs-Knorr reactions. The crude product was purified by flash chromatography (EtOAc/hexanes, 3:7–1:1). This material was recrystallized

(Me_2CO/Et_2O/hexanes) to give galactoside **11** as yellow needles (130 mg, 18%). **HRMS** mass calculated for $C_{27}H_{26}Cl_2FNNaO_9$ 620.0866; found 620.0859 $[M+Na]^+$. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.80 (s, 3 H, C$H_{3(Ar)}$) 1.82 (s, 3 H, C$H_{3(Ar)}$) 1.98 (s, 3 H, C(O)CH_3) 2.10 (s, 3 H, C(O)CH_3) 2.21 (s, 3 H, C(O)CH_3) 3.94 (td, 1 H, $J_{H5\text{-}H6}$=$J_{H5\text{-}H6'}$ 7.0 $J_{H5\text{-}H4}$ 2.7 Hz, H5) 4.12 (m, 2 H, $J_{H6\text{-}H6'}$=$J_{H6'\text{-}H6}$ 10.9, H6 H6') 4.93 (ddd, 1 H, $J_{H2\text{-}F2}$ 51.4 $J_{H2\text{-}H3}$ 9.7 $J_{H2\text{-}H1}$ 7.5 Hz, H2) 5.22 (ddd, 1 H, $J_{H3\text{-}F2}$ 13.1 $J_{H3\text{-}H2}$ 9.7 $J_{H3\text{-}H4}$ 3.5 Hz, H3) 5.36 (dd, 1 H, $J_{H1\text{-}H2}$ 7.5 $J_{H1\text{-}F2}$ 3.2 Hz, H1) 5.46 (dd, 1 H, $J_{H4\text{-}H3}$ 3.5 $J_{H4\text{-}H5}$ 2.7 Hz, H4) 6.69 (d, 1 H, $J_{H8(Ar)\text{-}H6(Ar)}$ 1.8 Hz, H8(Ar)) 6.69 (dd, 1 H, $J_{H6(Ar)\text{-}H5(Ar)}$ 9.9 $J_{H6(Ar)\text{-}H8(Ar)}$ 1.8 Hz, H6$_{(Ar)}$) 7.38 (d, 1 H, $J_{H5(Ar)\text{-}H6(Ar)}$ 9.9 Hz, H5$_{(Ar)}$) 7.77 (s, 1 H, H4$_{(Ar)}$) **^{19}F NMR** (282 MHz, $CDCl_3$) δ ppm -206.63 (ddd, $J_{F2\text{-}H2}$ 51.4 $J_{F2\text{-}H3}$ 13.1 $J_{F2\text{-}H1}$ 3.2 Hz, 1 F) **^{13}C NMR** (101 MHz, $CDCl_3$) δ ppm 20.57 (3 C, 3 × C(O)CH_3) 28.79 ($CH_{3(Ar)}$) 28.86 ($CH_{3(Ar)}$) 38.09 (C9$_{(Ar)}$) 60.58 (C6) 67.27 (d, $J_{C4\text{-}F2}$ 8.4 Hz, C4) 70.80 (d, $J_{C3\text{-}F2}$ 18.3 Hz, C3) 71.28 (C5) 88.14 (d, $J_{C2\text{-}F2}$ 190.4 Hz, C2) 100.94 (d, $J_{C1\text{-}F2}$ 22.9 Hz, C1) 128.31, 128.88 (C8$_{(Ar)}$) 130.14, 132.35 (C5$_{(Ar)}$) 132.62 (C6$_{(Ar)}$) 133.24, 140.70, 140.90 (C4$_{(Ar)}$) 148.25, 149.67 (C2$_{(Ar)}$) 153.16, 169.91 (C(O)CH_3) 170.02 (C(O)CH_3) 170.22 (C(O)CH_3) 187.26 (C=O$_{(Ar)}$).

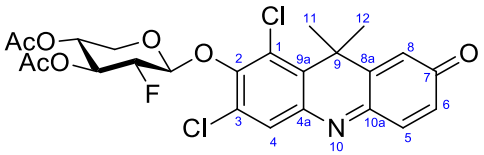
9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 2-deoxy-2-fluoro-β-D-galactopyranoside (DDAO-2FGal) **(2)**



Compound **11** (93 mg, 0.15 mmol) was treated according to the general procedure for deacetylation. The crude product was purified by flash chromatography (EtOAc/hexanes, 4:1) to give polyol **2** as a yellow powder (61 mg, 84%). **HRMS** mass calculated for $C_{21}H_{20}Cl_2FNNaO_6$: 494.0549; found: 494.0547 $[M+Na]^+$. **^{1}H NMR** (400 MHz, 30% $CDCl_3$ 70% CD_3OD) δ ppm 1.82 (s, 3 H, C$H_{3(Ar)}$) 1.82 (s, 3 H, C$H_{3(Ar)}$) 3.55 (m, 1 H, $J_{H5\text{-}H6}$ 6.7 $J_{H5\text{-}H6'}$ 5.9, H5) 3.69 (dd, 1 H, $J_{H6'\text{-}H6}$ 11.1 $J_{H6'\text{-}H5}$ 5.9 Hz, H6') 3.77 (dd, 1 H, $J_{H6\text{-}H6'}$ 11.1 $J_{H6'\text{-}H5}$ 6.7 Hz, H6) 3.83 (ddd, 1 H, $J_{H3\text{-}F2}$ 14.3 $J_{H3\text{-}H2}$ 9.2 $J_{H3\text{-}H4}$ 3.2 Hz, H3) 3.98 (m, 1 H, $J_{H4\text{-}H3}$ 3.2 Hz, H4) 4.77 (ddd, 1 H, $J_{H2\text{-}F2}$ 52.2 $J_{H2\text{-}H3}$ 9.2 $J_{H2\text{-}H1}$ 7.4 Hz, H2) 5.30 (dd, 1 H, $J_{H1\text{-}H2}$ 7.4 $J_{H1\text{-}F2}$ 3.0 Hz, H1) 6.67 (dd, 1 H, $J_{H6(Ar)\text{-}H5(Ar)}$ 9.7 $J_{H6(Ar)\text{-}H8(Ar)}$ 1.9 Hz, H6$_{(Ar)}$) 6.73 (d, 1 H, $J_{H8(Ar)\text{-}H6(Ar)}$ 1.9 Hz, H8$_{(Ar)}$) 7.41 (d, 1 H, $J_{H5(Ar)\text{-}H6(Ar)}$ 9.7 Hz, H5$_{(Ar)}$) 7.74 (s, 1 H, H4$_{(Ar)}$) **^{19}F NMR** (282 MHz, 30% $CDCl_3$ 70% CD_3OD) δ ppm -207.88 (ddd, $J_{F2\text{-}H2}$ 52.2 $J_{F2\text{-}H3}$ 14.3 $J_{F2\text{-}H1}$ 3.0 Hz, F2) **^{13}C NMR** (101 MHz, 30% $CDCl_3$ 70% CD_3OD) δ ppm 29.13 ($CH_{3(Ar)}$) 29.19 ($CH_{3(Ar)}$) 39.14 (C9$_{(Ar)}$) 61.30 (C6) 70.00 (d, $J_{C4\text{-}F2}$ 8.4 Hz, C4) 72.85 (d, $J_{C3\text{-}F2}$ 17.6 Hz, C3) 76.84 (C5) 92.93 (d, $J_{C2\text{-}F2}$ 179.4 Hz, C2)

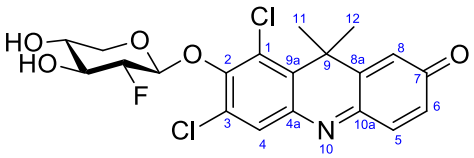
102.58 (d, $J_{C1\text{-}F2}$ 27.6 Hz, C1) 129.30 ($C8_{(Ar)}$) 129.53, 131.26, 132.91 ($C6_{(Ar)}$) 133.36 ($C4_{(Ar)}$) 134.3, 141.20, 142.07 ($C5_{(Ar)}$) 150.02, 151.55 ($C2_{(Ar)}$) 153.75, 188.74 ($C{=}O_{(Ar)}$).

9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 3,4-di-*O*-acetyl-2-deoxy-2-fluoro-*β*-D-xylopyranoside **(13)**



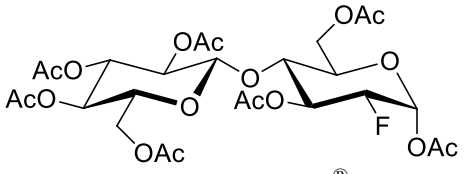
1,3,4-Tri-*O*-acetyl-2-deoxy-2-fluoro-D-xylose[7] (430 mg, 1.61 mmol), was treated according to the general procedure for the synthesis of glycosyl bromides. The product was coupled to DDAO according to the general procedure for Koenigs-Knorr reactions. The crude product was purified by flash chromatography (EtOAc/hexanes, 3:7–1:1). This material was recrystallized (EtOAc/hexanes) to give xyloside **13** as a yellow powder (211 mg, 31%). **HRMS** mass calculated for $C_{24}H_{23}Cl_2FNNaO_7$: 548.0655; found: 548.0654 $[M+Na]^+$. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.84 (s, 6 H, 2 × CH_3) 2.13 (s, 3 H, C(O)CH_3) 2.20 (s, 3 H, C(O)CH_3) 3.62 (dd, 1 H, $J_{H5ax\text{-}H5eq}$ 12.56 $J_{H5ax\text{-}H4}$ 6.02 Hz, $H5_{ax}$) 4.47 (dd, 1 H, $J_{H5eq\text{-}H5ax}$ 12.56 $J_{H5eq\text{-}H4eq}$ 4.19 Hz, $H5_{eq}$) 4.89 (ddd, 1 H, $J_{H2\text{-}F2}$ 47.97 $J_{H2\text{-}H3}$ 7.31 $J_{H2\text{-}H1}$ 5.03 Hz, H2) 5.07 (td, 1 H, $J_{H4\text{-}H3}$=$J_{H4\text{-}H5ax}$ 6.28, $J_{H4\text{-}H5eq}$ 4.34 Hz, H4) 5.40 (dt, $J_{H3\text{-}F2}$ 12.79 $J_{H3\text{-}H2}$=$J_{H3\text{-}H4}$ 7.00 Hz, 1 H) 5.55 (dd, 1 H, $J_{H1\text{-}F2}$ 8.15 $J_{H1\text{-}H2}$ 4.95 Hz, H1) 6.71 (d, 1 H, $J_{H8(Ar)\text{-}H6(Ar)}$ 1.68 Hz, $H8_{(Ar)}$) 6.71 (dd, 1 H, $J_{H6(Ar)\text{-}H5(Ar)}$ 10.51 $J_{H6(Ar)\text{-}H8(Ar)}$ 1.83 Hz, $H6_{(Ar)}$) 7.40 (d, 1 H, $J_{H5(Ar)\text{-}H6(Ar)}$ 10.20 Hz, $H5_{(Ar)}$) 7.81 (s, 1 H, $H4_{(Ar)}$) **^{19}F NMR** (282 MHz, $CDCl_3$) δ ppm –196.96 (ddd, $J_{F2\text{-}H2}$ 48.43 $J_{F2\text{-}H3}$ 13.82 $J_{F2\text{-}H1}$ 7.54 Hz, F2) **^{13}C NMR** (101 MHz, $CDCl_3$) δ ppm 20.92 ($CH_{3\ (Ar)}$) 28.92 (C(O)CH_3) 29.04 (C(O)CH_3) 38.25 ($C9_{(Ar)}$) 62.62 (C5) 68.46 (d, $J_{C4\text{-}F2}$ 4.60 Hz, C4) 69.74 (d, $J_{C3\text{-}F2}$ 24.54 Hz, C3) 87.86 (d, $J_{C2\text{-}F2}$ 183.26 Hz, C2) 101.06 (d, $J_{C1\text{-}F2}$ 29.14 Hz, C1) 128.30, 129.00 ($C8_{(Ar)}$) 130.00, 132.47 ($C6_{(Ar)}$) 132.85 ($C4_{(Ar)}$) 133.51, 140.75, 141.04 ($C5_{(Ar)}$) 148.37, 150.48 ($C2_{(Ar)}$) 153.23, 169.83 (C(O)CH_3) 170.19 (C(O)CH_3) 187.39 ($C{=}O_{(Ar)}$).

9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 2-deoxy-2-fluoro-*β*-D-xylopyranoside (DDAO-2FXyl) (**3**)



Compound **13** (88 mg, 0.17 mmol) was treated according to the general procedure for deacetylation. The crude product was purified by flash chromatography (EtOAc/hexanes, 4:1) to give polyol **3** as a yellow powder (78 mg, 99%). **HRMS** mass calculated for $C_{20}H_{18}Cl_3FNO_5$: 476.0235; found: 476.0237 $[M+Cl]^-$. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.81 (s, 3 H, $CH_{3(Ar)}$) 1.81 (s, 3 H, $CH_{3(Ar)}$) 2.48 (br. s., 1 H, OH) 2.75 (br. s., 1 H, OH) 3.43 (dd, 1 H, $J_{H5ax-H5eq}$ 11.9 $J_{H5ax-H4}$ 7.5 Hz, $H5_{ax}$) 3.88 (td, 1 H, J_{H4-H3}= $J_{H4-H5ax}$7.5 $J_{H4-H5eq}$ 4.6 Hz, H4) 3.94 (dt, 1 H, J_{H3-F2} 13.5 J_{H3-H2}=J_{H3-H4} 7.5 Hz, H3) 4.28 (dd, 1 H, $J_{H5eq-H5ax}$ 11.9 $J_{H5eq-H4}$ 4.6 Hz, $H5_{eq}$) 4.71 (ddd, 1 H, J_{H2-F2} 49.6 J_{H2-H3} 7.5 J_{H2-H1} 6.1 Hz, H2) 5.42 (dd, 1 H, J_{H1-H2} 6.1 J_{H1-F2} 4.6 Hz, H1) 6.68 (d, 1 H, $J_{H8(Ar)-H6(Ar)}$ 1.7 Hz, $H8_{(Ar)}$) 6.69 (dd, 1 H, $J_{H6(Ar)-H5(Ar)}$ 9.9 $J_{H6(Ar)-H8(Ar)}$ 1.7 Hz, $H6_{(Ar)}$) 7.37 (d, 1 H, $J_{H5(Ar)-H6(Ar)}$ 9.9 Hz, $H5_{(Ar)}$) 7.78 (s, 1 H, $H4_{(Ar)}$) **^{19}F NMR** (282 MHz, $CDCl_3$) δ ppm -200.66 (ddd, J_{F2-H2} 49.6 J_{F2-H3} 13.5 J_{F2-H1} 4.6 Hz, 1 F) **^{13}C NMR** (101 MHz, $CDCl_3$) δ ppm 28.93 ($CH_{3\ (Ar)}$) 29.04 ($CH_{3\ (Ar)}$) 38.26 ($C9_{(Ar)}$) 65.22 (C5) 69.12 (d, J_{C4-F2} 5.3 Hz, C4) 73.69 (d, J_{C3-F2} 19.1 Hz, C3) 91.05 (d, J_{C2-F2} 186.3 Hz, C2) 101.32 (d, J_{C1-F2} 26.0 Hz, C1) 128.28, 129.00 ($C8_{(Ar)}$) 130.09, 132.46 ($C4_{(Ar)}$) 132.84, 133.46, 140.75, 141.07 ($C5_{(Ar)}$) 148.42, 150.32 ($C2_{(Ar)}$), 153.20, 187.44 ($C{=}O_{(Ar)}$).

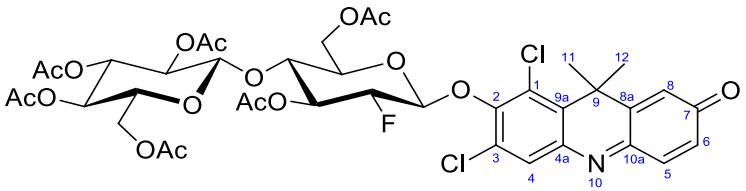
1,3,6,2',3',4',6'-Hepta-*O*-acetyl-2-deoxy-2-fluoro-*α*-cellobiose **(15)**



Per-*O*-acetyl cellobial[8] **14** (6.7 g, 12 mmol) and Selectfluor® (6.0 g, 18 mmol, 1.5 eqv.) were dissolved in $MeNO_2$ (100 ml) and water (20 ml). The reaction was warmed to ambient temperature and stirred for 72 h. The mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ followed by brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The mixture (4 g) was dissolved in pyridine (25 ml) and Ac_2O (120 ml) and stirred at r.t. overnight. The mixture was evaporated under reduced pressure, diluted with EtOAc and washed with 1N HCl, water, sat. $NaHCO_3$ then brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and the solvent evaporated under reduced pressure. Iterative recrystallization ($CHCl_3$/Et_2O) gave the

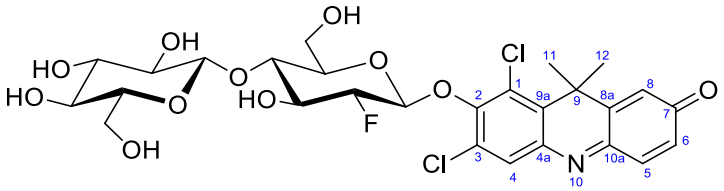
fluorosugar **15** (250 mg, 12% yield). ^{1}H and ^{19}F NMR spectral data were commensurate with those previously reported.[9]

9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 3,6,2',3',4',6'-hexa-*O*-acetyl-2-deoxy-2-fluoro-*β*-cellobioside **(16)**



Per-*O*-acetyl-2-deoxy-2-fluoro-cellobiose **15** (100 mg, 0.16 mmol), was treated according to the general procedure for the synthesis of glycosyl bromides. The product was coupled to DDAO according to the general procedure for Koenigs-Knorr reactions. The crude product was purified by flash chromatography (EtOAc/DCM/MePh, 1:2:2) to give cellobioside **16** as a yellow powder (12 mg, 8%). **HRMS:** mass calculated for $C_{39}H_{42}Cl_2FNNaO_{17}$: 908.1712; found: 908.1707, $[M+Na]^+$. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.79 (s, 3 H, $CH_{3(Ar)}$) 1.80 (s, 3 H, $CH_{3(Ar)}$) 1.99 (s, 3 H, C(O)CH_3) 2.00 (s, 3 H, C(O)CH_3) 2.02 (s, 3 H, C(O)CH_3) 2.06 (s, 3 H, C(O)CH_3) 2.11 (s, 3 H, C(O)CH_3) 2.14 (s, 3 H, C(O)CH_3) 3.61 (ddd, $J_{H5\text{-}H4}$ 9.7 $J_{H5\text{-}H6a}$ 4.8 $J_{H5\text{-}H6b}$ 2.1 Hz, 1 H, H5) 3.69 (ddd, $J_{H5'\text{-}H4'}$ 9.9 $J_{H5'\text{-}H6'a}$ 4.2 $J_{H5'\text{-}H6'b}$ 2.0 Hz, 1 H, H5') 3.88 (dd, $J_{H4\text{-}H3}$ 9.0 $J_{H4\text{-}H5}$ 9.7 Hz, 1 H, H4) 4.07 (dd, $J_{H6a\text{-}H6b}$ 12.1 $J_{H6a\text{-}H5}$ 4.8 Hz, 1 H, H6a) 4.08 (d, $J_{H6'b\text{-}H6'a}$ 12.4 $J_{H6'b\text{-}H5'}$ 2.0 Hz, 1 H, H6'b) 4.39 (dd, $J_{H6'a\text{-}H6'b}$ 12.4 $J_{H6'a\text{-}H5'}$ 4.2 Hz, 1 H, H6'a) 4.51 (dd, $J_{H6b\text{-}H6a}$ 12.1 $J_{H6b\text{-}H5}$ 2.1 Hz, 1 H, H6b) 4.55 (d, $J_{H1'\text{-}H2'}$ 7.9 Hz, 1 H, H1') 4.66 (ddd, $J_{H2\text{-}F2}$ 50.2 $J_{H2\text{-}H3}$ 9.0 $J_{H2\text{-}H1}$ 7.4 Hz, 1 H, H2) 4.94 (dd, $J_{H2'\text{-}H3'}$ 9.2 $J_{H2'\text{-}H1'}$ 7.9 Hz, 1 H, H2') 5.09 (dd, $J_{H4'\text{-}H3'}$ 9.2 $J_{H4'\text{-}H5'}$ 9.9 Hz, 1 H, H4') 5.16 (t, $J_{H3'\text{-}H2'}$=$J_{H3'\text{-}H4'}$ 9.2 Hz, 1 H, H3') 5.35 (dd, $J_{H1\text{-}H2}$ 7.4 $J_{H1\text{-}F2}$ 2.2 Hz, 1 H, H1) 5.40 (dt, $J_{H3\text{-}F2}$ 14.7 $J_{H3\text{-}H2}$=$J_{H3\text{-}H4}$ 9.0 Hz, 1 H, H3) 6.68 (d, $J_{H8(Ar)\text{-}H6(Ar)}$ 1.8 Hz, 1 H, $H8_{(Ar)}$) 6.68 (dd, $J_{H6(Ar)\text{-}H5(Ar)}$ 10.0 $J_{H6(Ar)\text{-}H8(Ar)}$ 1.8 Hz, 1 H, $H6_{(Ar)}$) 7.36 (d, $J_{H5(Ar)\text{-}H6(Ar)}$ 10.0 Hz, 1 H, $H5_{(Ar)}$) 7.75 (s, 1 H, $H4_{(Ar)}$) **^{19}F NMR** (282 MHz, $CDCl_3$) δ ppm -198.87 (ddd, $J_{F2\text{-}H2}$ 50.2 $J_{F2\text{-}H3}$ 14.7 $J_{F2\text{-}H1}$ 2.2 Hz, F2) **^{13}C NMR** (101 MHz, $CDCl_3$) δ ppm 20.47 (C(O)CH$_3$) 20.51 (2 × C(O)CH$_3$) 20.58 (C(O)CH$_3$) 20.65 (C(O)CH$_3$) 20.72 (C(O)CH$_3$) 28.77 ($CH_{3(Ar)}$) 28.82 ($CH_{3(Ar)}$) 38.05 ($C9_{(Ar)}$) 60.88 (C6) 61.58 (C6') 67.73 (C4') 71.53 (C2') 72.05 (C5') 71.99 (d, $J_{C3\text{-}F2}$ 19.1 Hz, C3) 72.79 (C3') 73.18 (C5) 75.84 (d, $J_{C4\text{-}F2}$ 6.8 Hz, C4) 89.86 (d, $J_{C2\text{-}F2}$ 193.5 Hz, C2) 100.27 (d, $J_{C1\text{-}F2}$ 23.7 Hz, C1) 100.71 (C1') 128.85 ($C6_{(Ar)}$) 129.01, 130.07 ($C8_{(Ar)}$) 132.34 ($C4_{(Ar)}$) 132.56, 133.25, 140.69 ($C6_{(Ar)}$) 140.88, 148.22, 149.47 ($C2_{(Ar)}$) 153.15, 168.85 (C(O)CH$_3$) 169.25 (C(O)CH$_3$) 169.49 (C(O)CH$_3$) 169.87 (C(O)CH$_3$) 170.22 (C(O)CH$_3$) 170.46 (C(O)CH$_3$) 187.24 ($C{=}O_{(Ar)}$).

9*H*-(1,3-Dichloro-9,9-dimethylacridin-7-on-2-yl) 2-deoxy-2-fluoro-*β*-D-cellobioside (DDAO-2FCel) **(4)**



Compound **16** (40 mg, 0.46 mmol) was treated according to the general procedure for deacetylation. The crude product was recrystallized (MeOH) to give polyol **4** as a yellow powder (15 mg, 53%). **HRMS:** mass calculated for $C_{27}H_{30}Cl_2FNNaO_{11}$: 656.1078; found: 656.1072 $[M+Na]^+$. **^{1}H NMR** (400 MHz, CD_3OD) δ ppm 1.84 (s, 3 H, $CH_{3(Ar)}$) 1.85 (s, 3 H, $CH_{3(Ar)}$) 3.37 - 3.94 (m, 11 H, H3-H6 and H2'-H6') 4.47 (d, $J_{H1'-H2'}$ 7.7 Hz, 1 H, H1') 4.48 (ddd, J_{H2-F2} 51.3 J_{H2-H3} 8.8 J_{H2-H1} 7.6 Hz, 1 H, H2) 5.45 (dd, J_{H1-H2} 7.6 J_{H1-F2} 2.2 Hz, 1 H, H1) 6.69 (dd, $J_{H6(Ar)-H5(Ar)}$ 9.7 $J_{H6(Ar)-H8(Ar)}$ 1.8 Hz, 1 H, $H6_{(Ar)}$) 6.81 (d, $J_{H8(Ar)-H6(Ar)}$ 1.8 Hz, 1 H, $H8_{(Ar)}$) 7.45 (d, $J_{H5(Ar)-H6(Ar)}$ 9.7 Hz, 1 H, $H5_{(Ar)}$) 7.80 (s, 1 H, $H4_{(Ar)}$) **^{19}F NMR** (282 MHz, CD_3OD) δ ppm -198.74 (ddd, J_{F2-H2} 51.3 J_{F2-H3} 13.19 J_{F2-H1} 2.2 Hz, F2) **^{13}C NMR** (101 MHz, CD_3OD) δ ppm 29.08 ($CH_{3(Ar)}$) 29.19 ($CH_{3(Ar)}$) 39.60 ($C9_{(Ar)}$) 55.04, 61.69, 62.61, 71.55, 74.85 (d, J_{C4-F2}17.5 Hz, C4) 75.03, 77.38, 78.14 (d, J_{C3-F2} 30.5 Hz, C3) 79.96, 93.86 (d, J_{C2-F2} 188.9 Hz, C2) 102.33 (d, J_{C1-F2} 22.9 Hz, C1) 104.66 (C1') 129.65, 130.00 ($C8_{(Ar)}$) 131.59, 133.31($C6_{(Ar)}$) 133.63 ($C4_{(Ar)}$) 134.98, 141.92, 142.48 ($C5_{(Ar)}$) 150.42, 151.70, 154.49, 189.12 ($C{=}O_{(Ar)}$).

Kinetic analyses

Enzyme kinetic experiments were performed as described below using the conditions outlined in Table S1. Extinction coefficients for all aglycones, except those for DDAO, were obtained from Kempton and Withers.[10] All experiments were performed in buffer with DMSO content (v/v) not exceeding 5%.

Table S1: Reaction conditions used in enzymatic assays.

enzyme	substrate for indirect inactivation assays*	buffer	pH	T (°C)
Abg	*β*GlcPNP	50 mM NaP_i	6.8	37
GCase	*β*GlcDNP	50 mM citrate	5	30
LacZ	*β*GalONP	50 mM NaP_i, 1 mM $MgCl_2$	6.8	37
Bhx	*β*XylPNP	50 mM NaP_i	6.8	37
EG-I	--	50 mM citrate	5	30
Cex	--	50 mM NaP_i	6.8	37

* *β*GlcPNP = 4-nitrophenyl *β*-D-glucopyranoside, *β*GlcDNP = 2,4-dinitrophenyl *β*-D-glucopyranoside, *β*GalONP = 2-nitrophenyl *β*-D-galactopyranoside, *β*XylPNP = 4-nitrophenyl *β*-D-xylopyranoside

General procedure for indirect inactivation assays

Varying concentrations of the inactivator compound were incubated with enzyme in the buffer and at the temperature listed in Table S1. At appropriate time-points, aliquots of this enzyme-inactivator reaction mixture were removed and diluted into a cuvette containing the appropriate substrate at a high concentration (7–15 × K_m) that had been pre-incubated under the same buffer and temperature conditions (Table S1), such that the final volume was 200 µl. The residual intial rate of substrate hydrolysis for each time-point was determined by monitoring the change in absorbance of the sample at an appropriate wavelength with respect to time. Each time point was plotted against its rate and the data set then fit to a pseudo-first order decay equation to obtain the rate constant of inactivation (k_{obs}) at that inactivator concentration. The resulting k_{obs} values were plotted against the corresponding inactivator concentration and fit to Equations 1 or 2 to obtain the kinetic parameters k_i and K_i.

$$\text{Equation 1:} \quad k_{obs} = \frac{k_i[I\text{-X}]}{K_i + [I\text{-X}]}$$

For reactions at inactivator concentrations well below the K_i value, a k_i/K_i was obtained by fit to:

$$\text{Equation 2:} \quad k_{obs} = \frac{k_i[I\text{-X}]}{K_i}$$

General procedure for direct inactivation assays

Varying concentrations of the inactivator were pre-incubated in the buffer and at the temperature listed in Table S1. The inactivation reaction was initiated by the addition of enzyme. DDAO release was monitored continuously by observing the solutions' absorbance at 600 nm. The inactivation parameters (k_i and K_i) were obtained either by fitting the initial rates of inactivation, or the observed rate constants of inactivation (k_{obs}), to a Michaelis-Menten type equation (Equation 1). For the latter, the k_{obs} values were obtained by fitting the derivative of the absorbance vs. time plots to an exponential decay curve using GraFit 7.0.

General procedure for direct inactivation assays by stopped flow

Experiments were performed on an Applied Photophysics SX20 stopped flow machine coupled with a water bath. Two syringes were filled with 800 µl of 0.64 µM Abg or inactivator **1** (2.58 µM to 137 µM) and incubated at 37°C for 5 minutes. DDAO release was monitored by absorbance at 600 nm, and the resulting curves were averaged and fit with the accompanying software (Pro-Data SX Viewer) using an equation for a single exponential to obtain the observed rate constant of inactivation (k_{obs}). Inactivation kinetic parameters k_i and K_i were obtained by fitting a plot of k_{obs} vs. inactivator concentration to Equation 1.

pK_a Determinations

pK_a determinations for DDAO were performed in buffer containing 50 mM citrate, 50 mM sodium phosphate and 50 mM Tris. Buffer pH was adjusted using NaOH or HCl. Solutions (1000 µl) of DDAO (2 µM) were incubated at 37°C for 5 min. The fluorescence response at 600 V was recorded and plotted as a function of pH. This data set was fit to a pH titration curve using GraFit 5.0.

Extinction coefficient determinations

DDAO (11–15 mg) was dissolved in DMSO (1.0 ml), diluted 100-fold into DMSO, then further diluted 20-fold into 50 mM sodium phosphate buffer (pH 6.8) to give a final concentration of 17–24 µM DDAO. Further dilutions into buffer were made (10, 20, 50 and 100-fold) and, after equilibration at 37°C for 5 min, their absorbance at 600 nm were measured. These values were plotted as a function of DDAO concentration to give a good linear correlation ($R^2 > 0.97$ in all cases). The final extinction coefficient value was determined using Beer's law and is an average of seven independent replicates.

Active site titrations of Abg

Solutions of Abg in sodium phosphate buffer at pH 6.8 were equilibrated at 37°C for 5 min. A baseline absorbance (600 nm) or fluorescence (λ_{ex} = 600 nm, λ_{em} = 656 nm) was measured. Absorbance at 700 nm was also measured as an internal control. Active site titrant DDAO-2FGlc **1** was added to a final concentration of 70 μM. The change in absorbance or fluorescence response was monitored. The final change in absorbance was calculated as follows:

$$(A_{600} - A_{700})_{final} - (A_{600} - A_{700})_{initial}$$

The change in DDAO concentration was then calculated by Beer's Law ($A = \varepsilon bc$) where ε = 32100 $M^{-1}cm^{-1}$, b = 1 cm.

DDAO release by fluorescence was quantified using an appropriate calibration curve for DDAO in this buffer system.

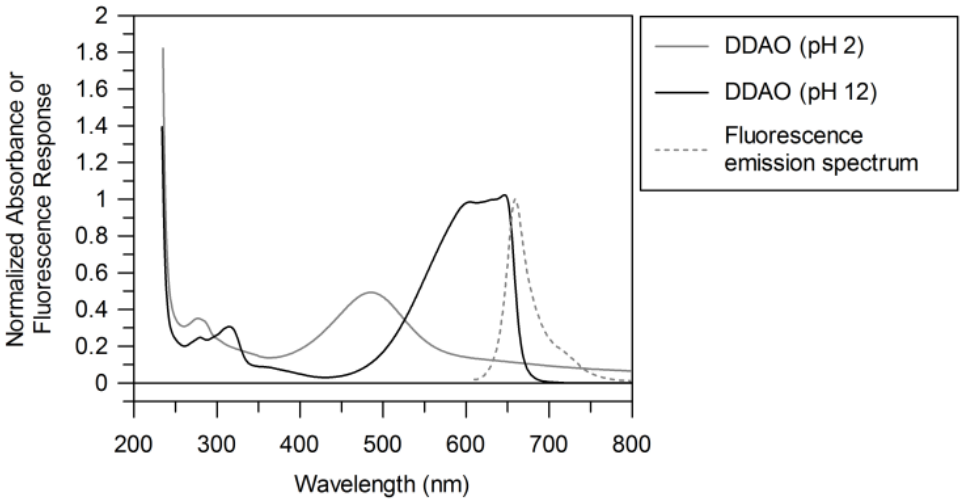


Figure S1: Absorbance spectra of protonated (pH 2) and deprotonated (pH 12) DDAO and a fluorescence emission spectrum (λ_{ex} = 600 nm).

References

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DDAO-2FGlc

1H

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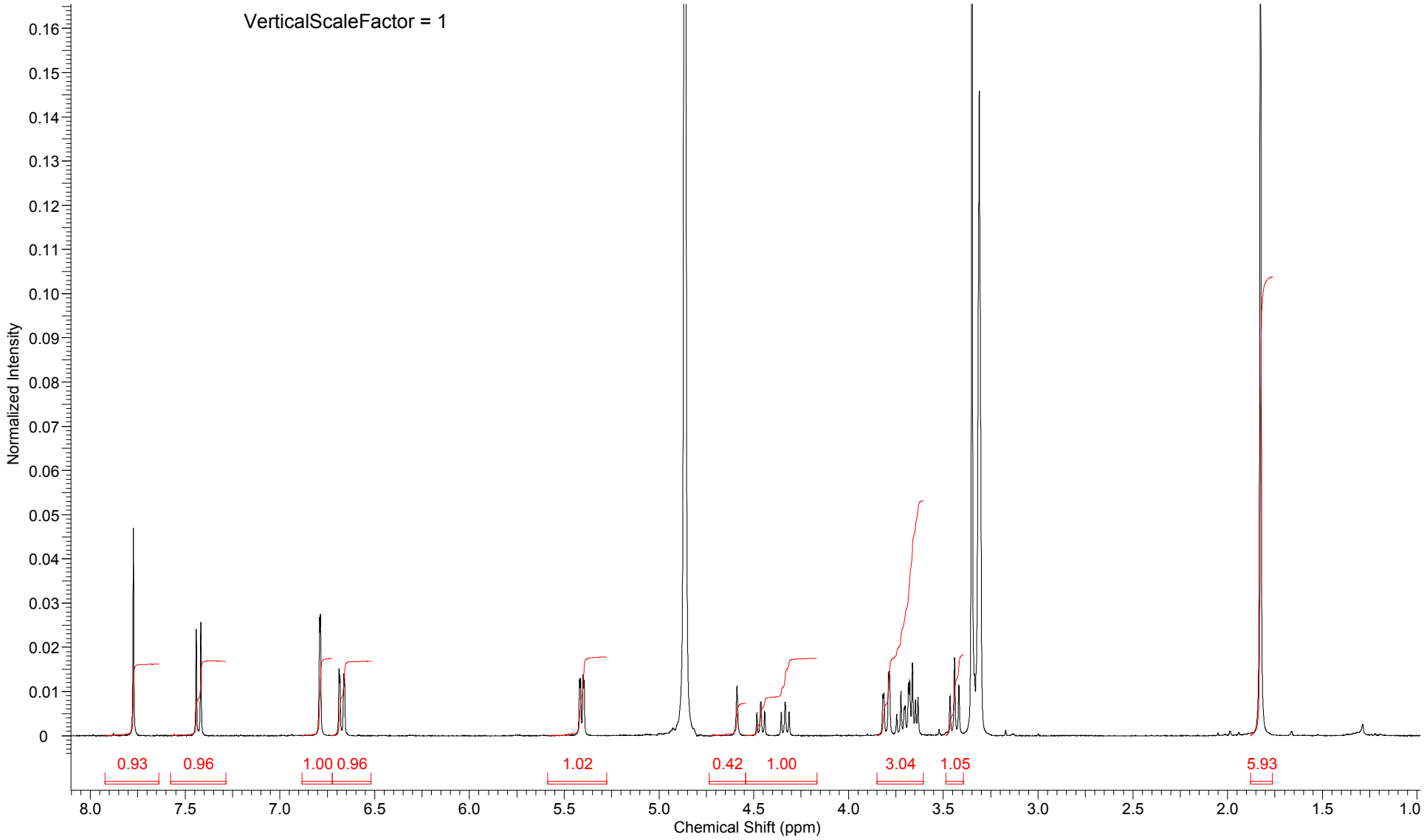

DDAO-2FGlc

13C

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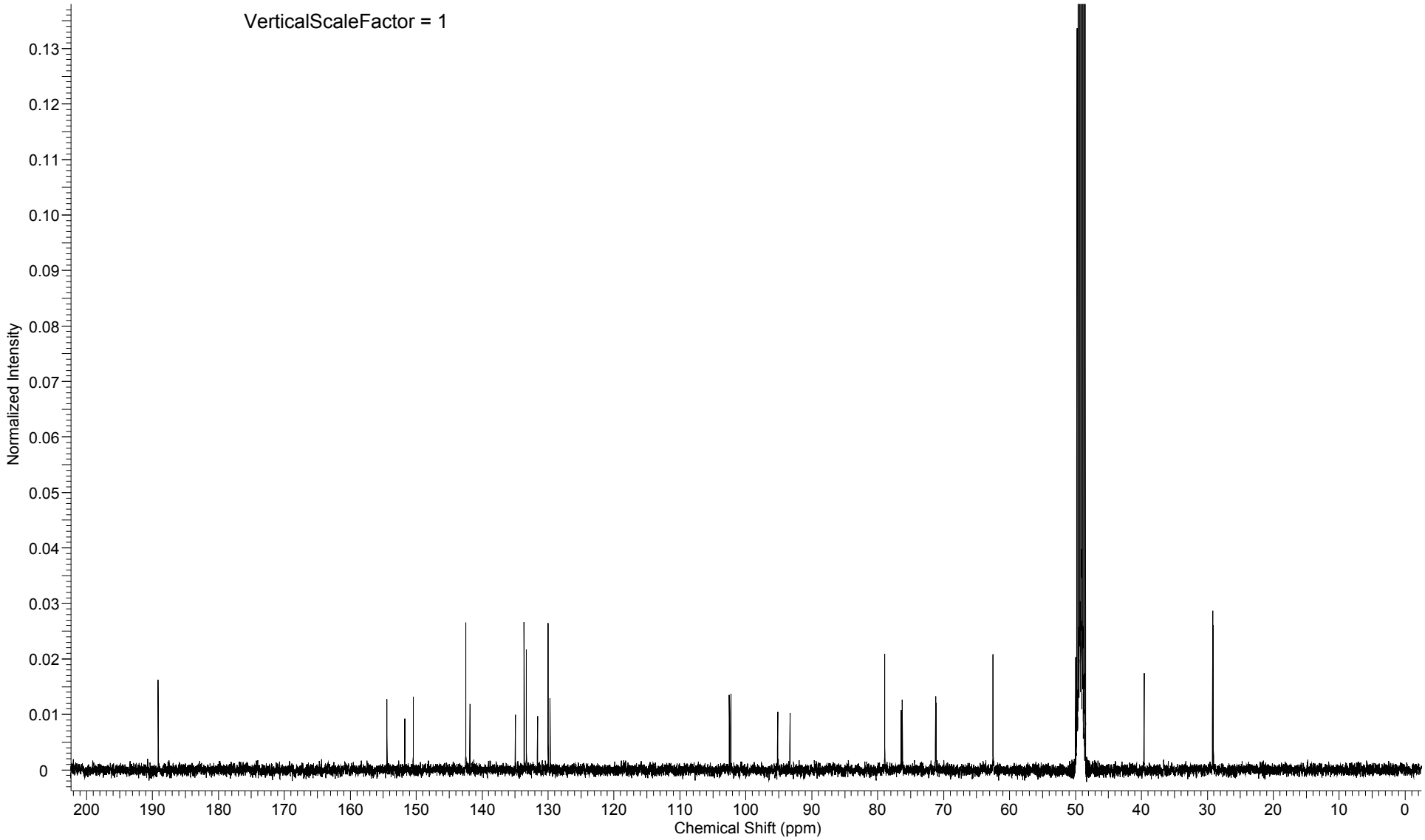

DDAO-2FGal

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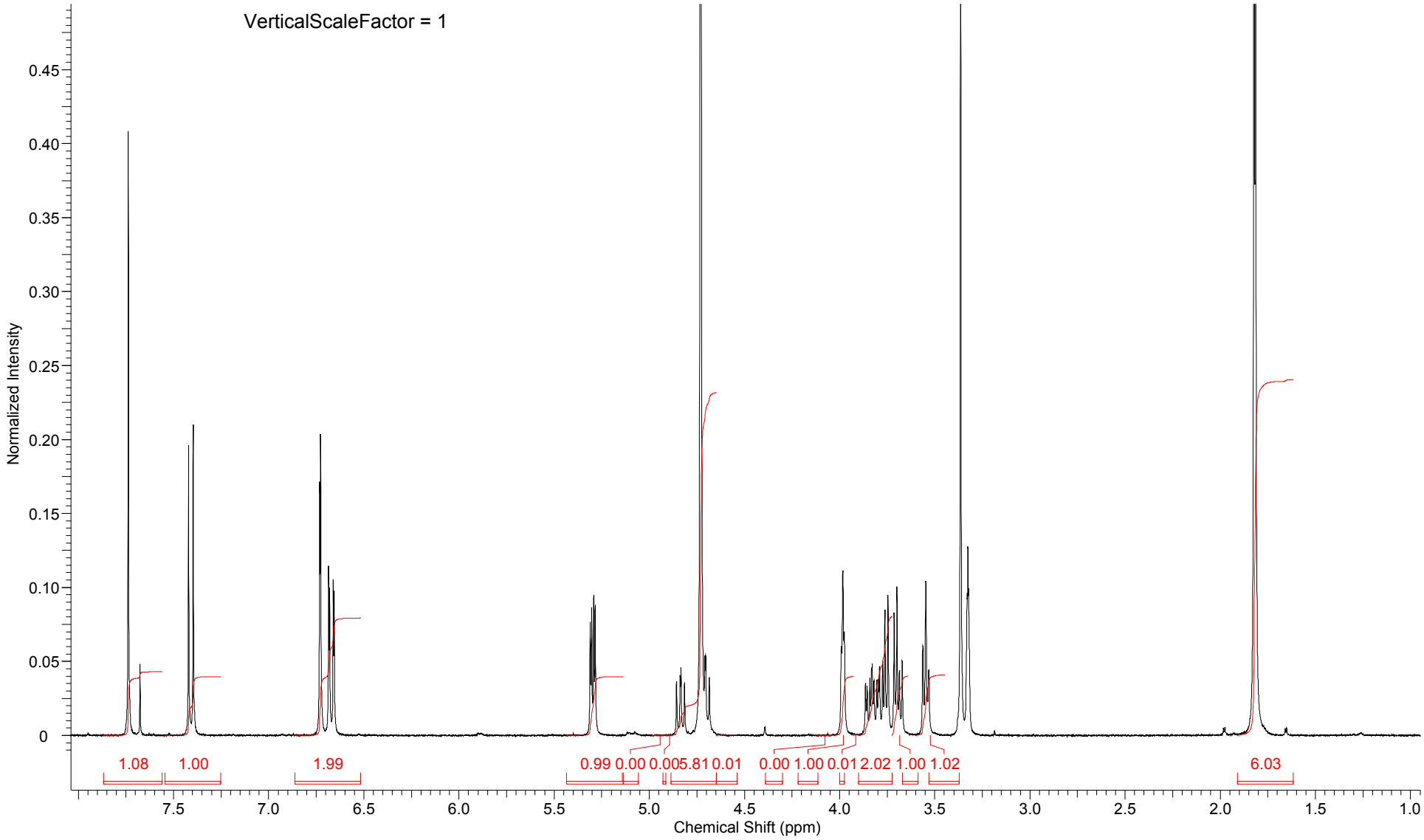

DDAO-2FGal

13C

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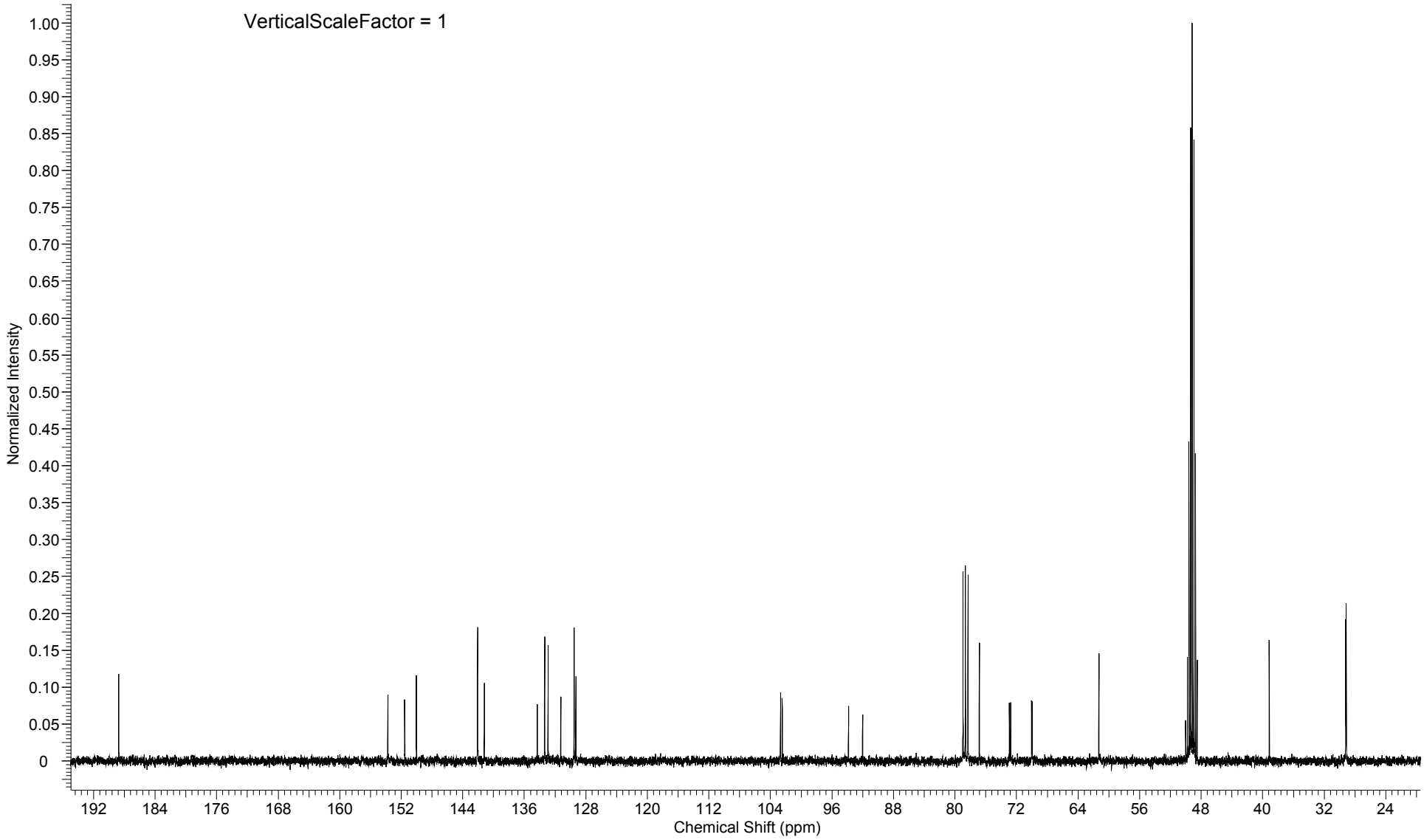

DDAO-2FXyl

JOB NO: 1H spectrum ref. to CDCl3 at 7.27 ppm

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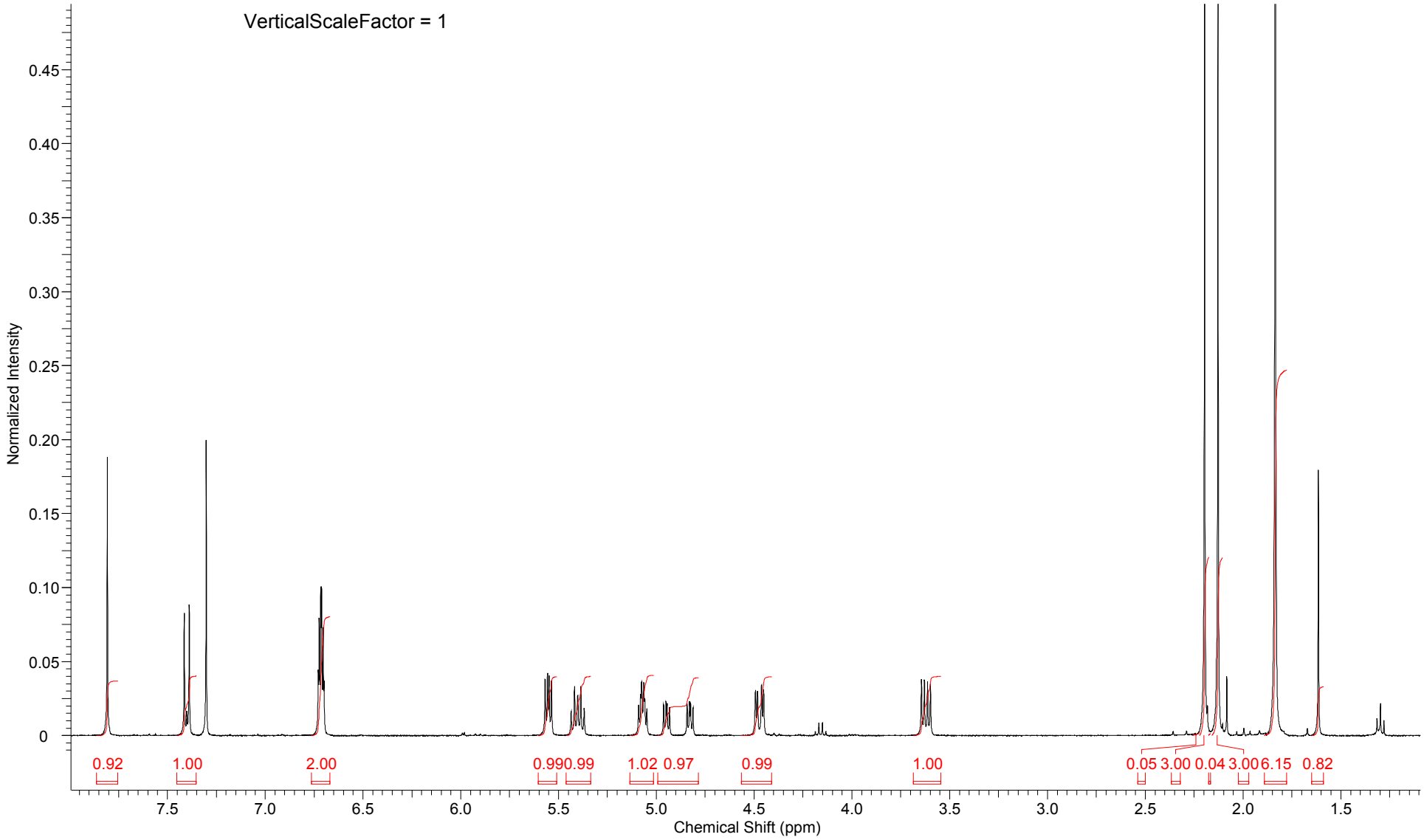
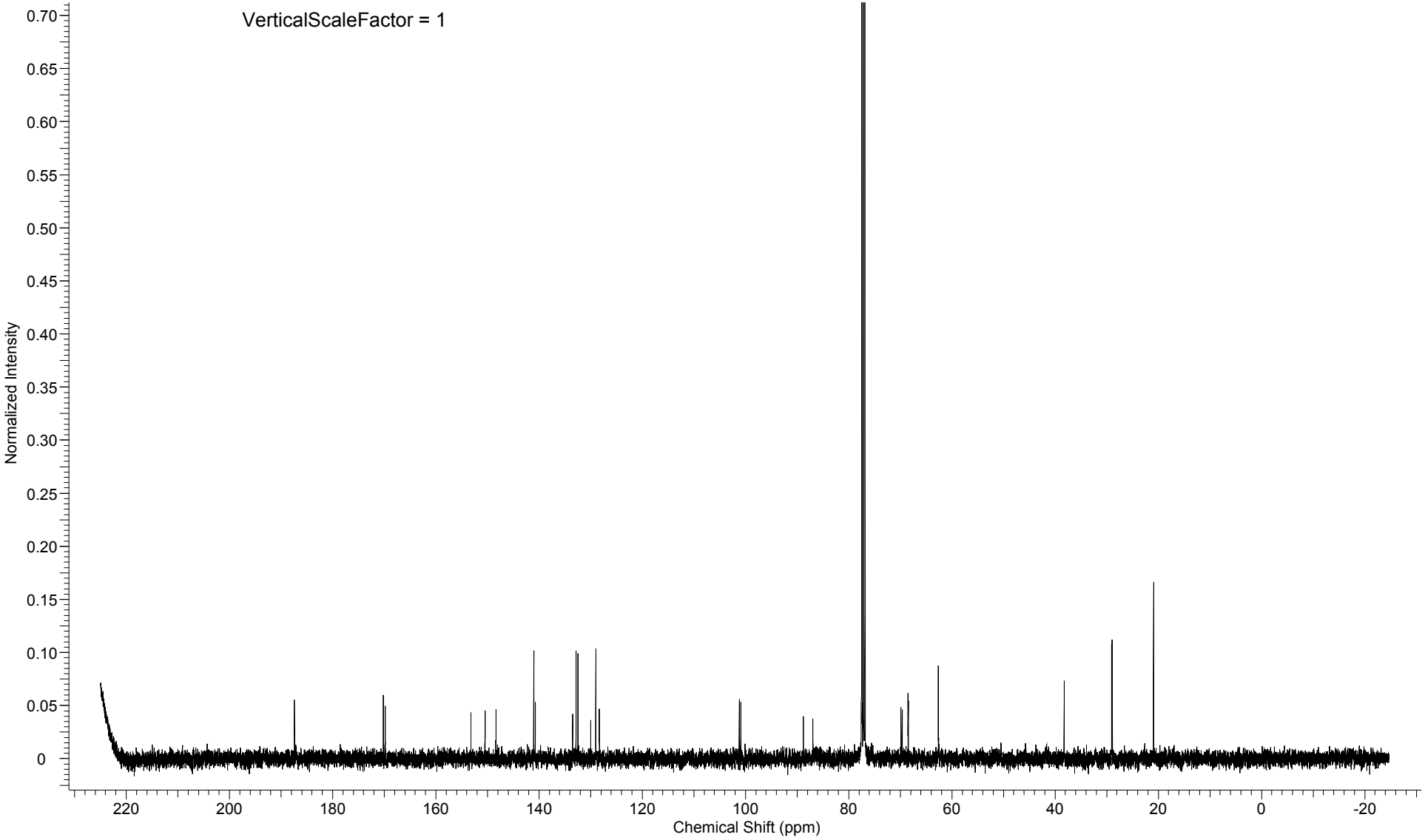
Receiver Gain	812.70	***SW(cyclical) (Hz)***	4990.02	***Solvent***	CHLOROFORM-d		
Spectrum Offset (Hz)	2395.7190	***Sweep Width (Hz)***	4989.87	***Temperature (degree C)***	25.160		



DDAO-2FXyl

13C

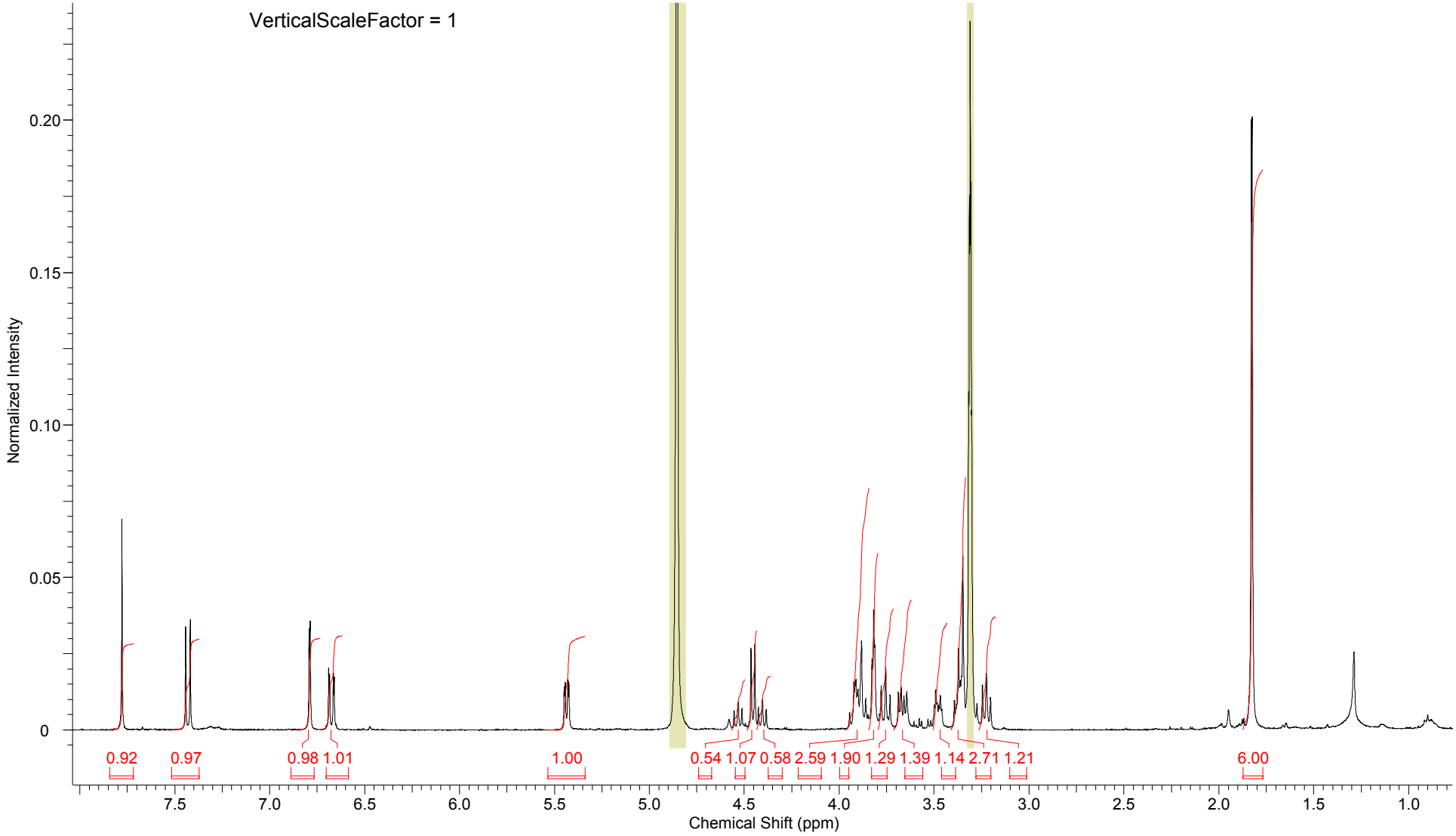
Acquisition Time (sec)	0.6521	***Comment***	13C	***Date***	05 Jul 2011 11:14:08		
Date Stamp	05 Jul 2011 11:14:08			***File Name***	C:\USERS\TANYA\DOCUMENTS\NMR\110705_001001r		
Frequency (MHz)	100.63	***Nucleus***	13C	***Number of Transients***	770	***Origin***	spect
Original Points Count	16384	***Owner***	root	***Points Count***	32768	***Pulse Sequence***	zgpg30
Receiver Gain	1024.00	***SW(cyclical) (Hz)***	25125.63	***Solvent***	CHLOROFORM-d		
Spectrum Offset (Hz)	10074.8779	***Sweep Width (Hz)***	25124.86	***Temperature (degree C)***	25.360		



DDAO-2FCel

JOB NO: 1H spectrum ref. to CDCl3 at 7.27 ppm

Acquisition Time (sec)	2.1998	***Comment***	JOB NO: 1H spectrum ref. to CDCl3 at 7.27 ppm			***Date***	23 Mar 2012 15:19:44
Date Stamp	23 Mar 2012 15:19:44						
File Name	C:\Users\Tanya\Documents\Withers Group\NMR\120323 2FCDY 400inv\1H regular\pdata\1\1r					***Frequency (MHz)***	400.13
Nucleus	1H	***Number of Transients***	49	***Origin***	av400	***Original Points Count***	10977
Owner	psm	***Points Count***	32768	***Pulse Sequence***	zg30	***Receiver Gain***	287.40
SW(cyclical) (Hz)	4990.02	***Solvent***	METHANOL-d4	***Spectrum Offset (Hz)***	2389.5759	***Spectrum Type***	STANDARD
Sweep Width (Hz)	4989.87	***Temperature (degree C)***	25.060				



DDAO-2FCel

JOB NO: 13C spectrum ref to CDCl3 at 77.23 ppm

Acquisition Time (sec)	0.6537	***Comment***	JOB NO: 13C spectrum ref to CDCl3 at 77.23 ppm			***Date***	28 Mar 2012 10:06:08
Date Stamp	28 Mar 2012 10:06:08			***File Name***	C:\Users\Tanya\Documents\Withers Group\NMR\120327 2FCDY_2\5\pdata\1\1r		
Frequency (MHz)	100.61	***Nucleus***	13C	***Number of Transients***	9835	***Origin***	av400
Original Points Count	16384	***Owner***	psm	***Points Count***	32768	***Pulse Sequence***	zgpg30
Receiver Gain	14596.50	***SW(cyclical) (Hz)***	25062.66	***Solvent***	CHLOROFORM-d		
Spectrum Offset (Hz)	10115.8955	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	25061.89	***Temperature (degree C)***	25.060