Glycans meet sphingolipids: structure-based design of glycan containing analogues of a sphingosine kinase inhibitor

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SUPPORTING INFORMATION

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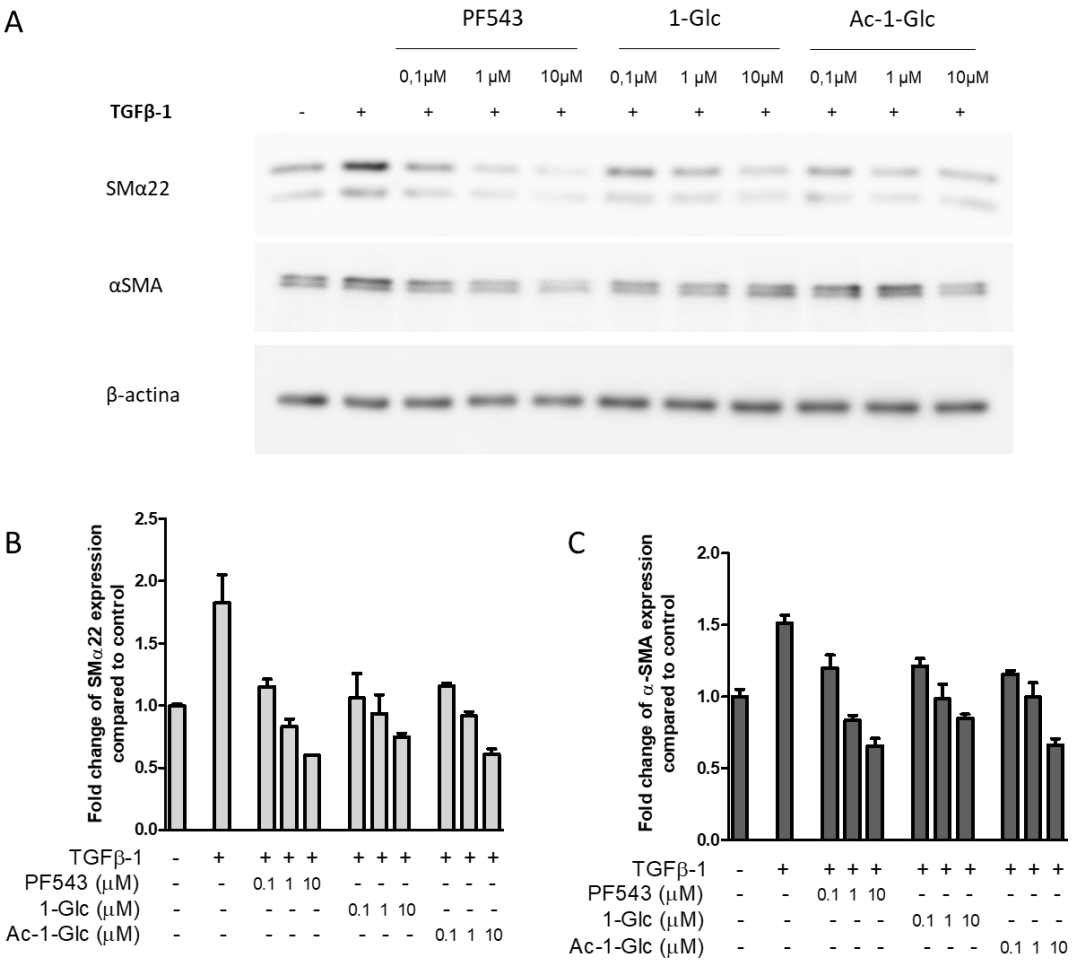


Figure S1. Dose dependence of **1-Glc** and **Ac-1-Glc** compared to the parental inhibitor **PF-543** on TGFβ1-induced fibrosis of murine myoblasts. C2C12 myoblasts were seeded to 80% confluence and treated with 5 ng/mL TGFβ1 after 120 min incubation with different concentrations of **PF-543** or **1-Glc** or **Ac-1-Glc** (0.1, 1, 10 μM). A. The expression of protein fibrosis markers, has been evaluated by western blot analysis, using specific anti-αSMA and anti-SMα22 antibodies. A. Representative blot is shown among three independent experiments with analogous results. B. and C. Densitometric analysis was performed of three independent experiments and data, normalized to β-actin expression, were reported as mean ± SD of fold change above control untreated.

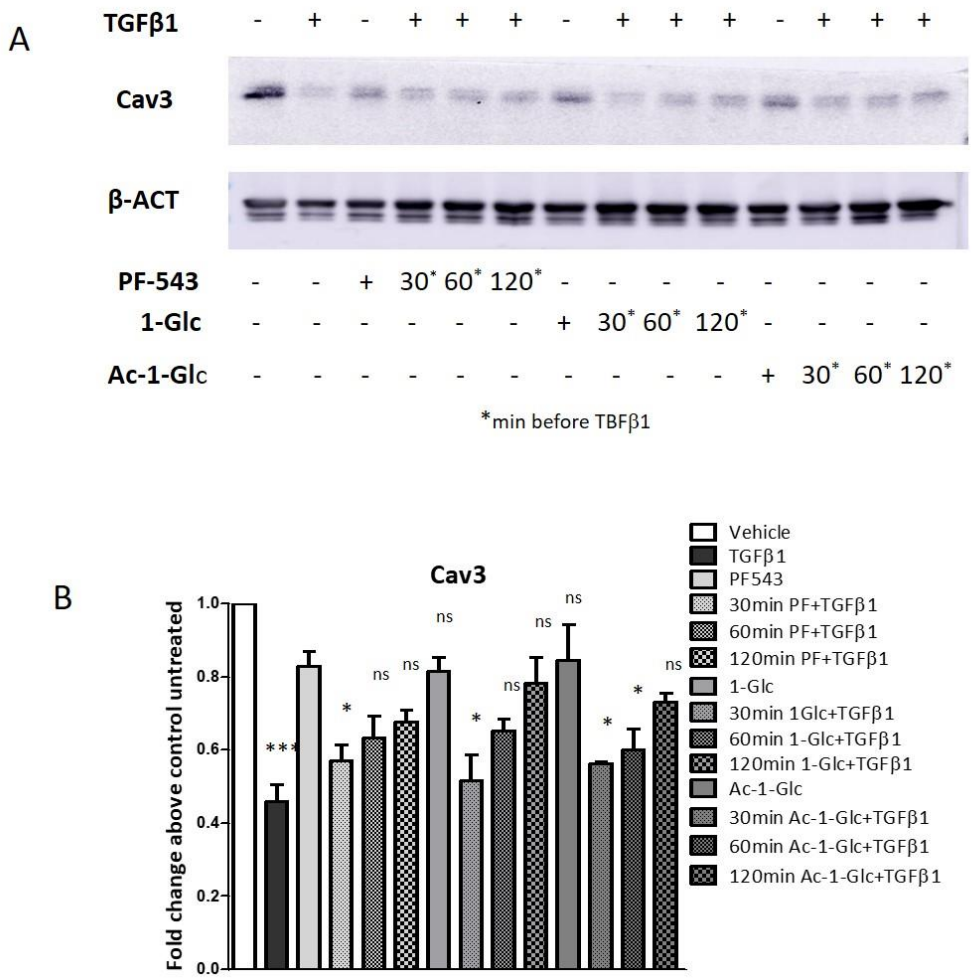
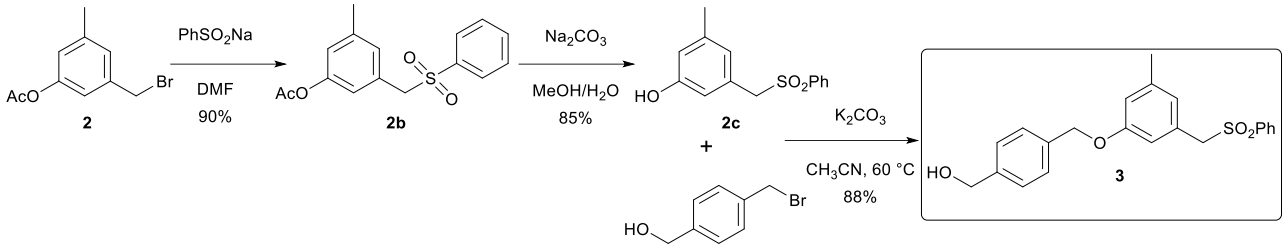


Figure S2. Effect of glycoconjugates **1-Glc** and **Ac-1-Glc** compared to the parental inhibitor **PF-543** in TGFβ1-induced myogenic marker expression in murine myoblasts. C2C12 myoblasts were seeded to 80% confluence and challenged with 5.0 ng/mL TGFβ1 for 48 h, 30, 60 or 120 min after being treated or not with **PF-543**, **1-Glc** and **Ac-1-Glc**. The expression of caveolin-3 (Cav3) which correlates with skeletal muscle differentiation has been evaluated by western blot analysis, using specific anti-Cav3 antibody. **A.** Representative blot is shown among three independent experiments with analogous results. **B.** Densitometric analysis of three independent experiments was performed and data, normalized to β-actin expression, were reported as mean ± SD of fold change above control untreated. The effect of TGFβ1 on Cav-3 expression was significant by one-way ANOVA followed by Bonferroni *post-hoc* test, ***P<0.001. The effect of pharmacological inhibition of SK1 on TGFβ1-induced Cav-3 expression was significant by two-way ANOVA followed by Bonferroni *post-hoc* test, * P<0.05. ns: not significant.

Materials and methods

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethanol (3)



Benzene sulfinic acid ($PhSO_2Na$, 0.16 g, 1.00 mmol) was added to a solution of commercially available 3-(bromomethyl)-5-methylphenyl acetate **2** (0.22 g, 0.91 mmol) in DMF (4.5 mL). The reaction was stirred for 1h at room temperature, then diluted with DCM (25 mL) and washed with water and brine. The organic phase was dried and concentrated. Purification of the crude by flash-chromatography (hexane/EtOAc, 75:25) gave compound **2b** (0.25 g, 90%). ^{1}H NMR ($CDCl_3$): δ = 7.70-7.68 (m, 2H, arom.), 7.66-7.62 (m, 1H, arom.), 7.53-7.48 (m, 2H, arom.), 6.90 (*br* s, 1H, arom.), 6.78 (*br* s, 1H, arom.), 6.68 (*br* s, 1H, arom.), 4.28 (s, 2H, CH_2SO_2), 2.29 (s, 3H, CH_3), 2.27 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ =169.2 (C=O), 150.6, 139.9, 137.9, 133.7, 129.3-128.7 (6C), 122.7, 121.0, 62.5 (CH_2SO_2), 21.1 (CH_3), 21.0 (CH_3).

A solution of sodium bicarbonate (0.16 g, 1.52 mmol) was added to a solution of compound **2b** (0.23 g, 0.76 mmol) in methanol (6 mL). The resulting milky solution was stirred at room temperature for 24h, then diluted with water (5 mL). Methanol was evaporated under reduced pressure, and the residue was acidified to pH = 1-2 by addition of HCl 1N. The mixture was extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with brine (3 x 30 mL), dried, and concentrated to give compound **2c** (0.17 g, 85%) as a white oil, which was reacted in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ = 7.72-7.69 (m, 2H, arom.), 7.65-7.59 (m, 1H, arom.), 7.52-7.45 (m, 2H, arom.), 6.64 (*br* s, 1H, arom.), 6.51 (*br* s, 1H, arom.), 6.38 (*br* s, 1H, arom.), 5.99 (*br* s, 1H, OH), 4.24 (s, 2H, CH_2SO_2), 2.18 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ = 156.0, 140.0, 137.9, 133.8, 128.9-128.6 (5C),124.0, 116.8, 114.9, 62.8 (CH_2SO_2), 21.1 (CH_3).

A mixture of compound **2** (0.16 g, 0.61 mmol), [4-(bromomethyl)phenyl]methanol (0.13 g, 0.67 mmol) and potassium carbonate (0.25 g, 1.83 mmol) in acetonitrile (7 mL) was stirred at 60 °C for 2h. The reaction was diluted with brine (30 mL) and washed with ethyl acetate (3 x 20 mL). The combined organics were dried and concentrated under reduced pressure. The residue was purified by

flash-chromatography (hexane/AcOEt, from 6:4 to 1:1) to give compound **3** (0.20 g, 88%) as an amorphous white solid. ^{1}H NMR ($CDCl_3$): δ = 7.70-7.67 (m, 2H, arom.), 7.66-7.62 (m, 1H, arom.), 7.52-7.47 (m, 2H, arom.), 7.43-7.38 (m, 4H, arom.), 6.77 (*br* s, 1H, arom.), 6.53 (*br* s, 1H, arom.), 6.49 (*br* s, 1H, arom.), 4.95 (s, 2H, CH_2SO_2),4.74 (s, 2H, CH_2O), 4.25 (s, 2H, CH_2O), 2.24 (s, 3H, CH_3), 1.68 (*br*s, 1H, OH). ^{13}C NMR ($CDCl_3$): δ = 158.7, 140.7, 139.8, 138.0, 136.2, 133.6, 129.0-128.7 (7C), 127.7, 127.2, 124.5, 116.5, 113.9, 69.7 (CH_2SO_2), 65.1, 62.9, 21.3 (CH_3). HRMS (ESI): *m/z* calcd for $C_{22}H_{21}O_4S$ 381.1160 $[M-H]^-$, found 381.1159.

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethyl 2,3,4,6-tetra-O-pivaloyl-β-D-glucopyranoside (6a)

Compound **3** (0.027 g, 0.070 mmol), tetrapivaloyl-D-glucopyranosyl trichloroacetimidate **4** (0.090 g, 0.14 mmol), and 4Å molecular sieves (0.075 g) were diluted in DCM (2 mL). The suspension was cooled at 0 °C, then trimethylsilyl trifluoromethanesulfonate (0.1 M solution in DCM, 0.14 mL) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt, 7:3). After 0.5 h the reaction was quenched by the addition of TEA, diluted with DCM, and filtered over a Celite pad. After evaporation of the solvent, then crude was purified by flash chromatography (hexane/AcOEt, 8:2) to give compound **6a** (0.059 g, 95 %) as an oil. $[\alpha]_D^{20}$ = -15.3 (*c* = 1 in chloroform). ^{1}H NMR ($CDCl_3$): δ = 7.70-7.68 (m, 2H, arom.), 7.68-7.62 (m, 1H, arom.), 7.53-7.45 (m, 2H, arom.), 7.39-7.34 (m, 2H, arom.), 7.34-7.29 (m, 2H, arom.), 6.76 (*br* s, 1H, arom.), 6.54 (*br* s, 1H, arom.), 6.49 (*br* s, 1H, arom.), 5.32 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.18-5.11 (m, 2H, H-2 and H-4), 4.94 (s, 2H, CH_2SO_2), 4.89 (d, 1H, J = 12.0 Hz, OC*Ha*Hb), 4.63 (d, 1H, J = 12.0 Hz, OCHa*Hb*), 4.60 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.28-4.25 (m, 3H, $PhCH_2O$ and H-6a), 4.10 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b),3.76-3.73 (m, 1H, H-5), 2.24 (s, 3H, CH_3), 1.27 (s, 9H, CMe_3), 1.17 (s, 9H, CMe_3), 1.14 (s, 9H, CMe_3), 1.13 (s, 9H, CMe_3). ^{13}C NMR ($CDCl_3$): δ = 178.1 (C=O), 177.2 (C=O), 176.6 (C=O), 176.5 (C=O), 158.7, 139.8, 138.0, 136.6, 136.3, 133.7, 129.0, 128.9 (2C), 128.7 (2C), 128.2 (2C), 127.5 (2C), 124.5, 116.5, 113.9, 99.3 (C-1), 72.4 (C-5), 72.3 (C-3), 71.1 (C-2), 70.1 (OCH_2), 69.6 (CH_2SO_2), 68.1 (C-4), 62.9 ($PhCH_2O$), 62.0 (C-6), 38.9-38.7 (4C), 27.2-26.6 (12C, CH_3), 21.3 (CH_3). HRMS (ESI): *m/z* calcd for $C_{48}H_{63}O_{13}S$ 879.3989 $[M-H]^-$, found 879.3986.

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethyl 2,3,4,6-tetra-O-pivaloyl-β-D-galactopyranoside (6b)

Compound **3** (0.029 g, 0.075 mmol), tetrapivaloyl-D-galactopyranosyl trichloroacetimidate **5** (0.10 g, 0.15 mmol), and 4Å molecular sieves (0.080 g) were diluted in DCM (1.5 mL). The suspension was cooled at 0 °C, then trimethylsilyl trifluoromethanesulfonate (0.1 M solution in DCM, 0.15 mL) was

added dropwise. The reaction was monitored by TLC (hexane/AcOEt, 7:3). After 0.5 h the reaction was quenched by the addition of TEA, diluted with DCM, and filtered over a Celite pad. After evaporation of the solvent, then crude was purified by flash chromatography (hexane/AcOEt, 8:2) to give compound **6b** (0.064 g, 97 %) as an oil. $[\alpha]_D^{20}$ = -14.1 (*c* = 1 in chloroform). ^{1}H NMR ($CDCl_3$): δ = 7.72-7.67 (m, 2H, arom.), 7.66-7.61 (m, 1H, arom.), 7.52-7.46 (m, 2H, arom.), 7.40-7.35 (m, 2H, arom.), 7.35-7.31 (m, 2H, arom.), 6.77 (*br* s, 1H, arom.), 6.54 (*br* s, 1H, arom.), 6.50 (*br* s, 1H, arom.),5.44 (*br*d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 5.33 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 5.12 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.95 (s, 2H, CH_2SO_2), 4.91 (d, 1H, J = 11.9 Hz, OC*Ha*Hb), 4.65 (d, 1H, J = 11.9 Hz, OCHa*Hb*), 4.62 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.27-4.22 (m, 3H, $PhCH_2O$ and H-6a), 4.10 (dd, 1H, $J_{5,6b}$ = 7.0 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6b), 3.99 (dd, 1H, $J_{5,6a}$ = 6.8 Hz, $J_{5,6b}$ = 7.0 Hz, H-5), 2.24 (s, 3H, CH_3), 1.30 (s, 9H, CMe_3), 1.23 (s, 9H, CMe_3), 1.16 (s, 9H, CMe_3), 1.14 (s, 9H, CMe_3). ^{13}C NMR ($CDCl_3$): δ = 177.9 (C=O), 177.4 (C=O), 177.0 (C=O), 176.8 (C=O), 158.7, 139.8, 138.0, 136.6, 136.4, 133.7, 129.0, 128.9 (2C), 128.7 (2C), 128.2 (2C), 127.4 (2C), 124.6, 116.5, 113.9, 99.9 (C-1), 71.1 (C-5), 71.0 (C-3), 70.2 (OCH_2), 69.6 (CH_2SO_2), 68.7 (C-2), 66.8 (C-4), 62.9 ($PhCH_2O$), 61.3 (C-6), 39.1-38.8 (4C), 27.2-27.1 (12C, CH_3), 21.3 (CH_3). HRMS (ESI): *m/z* calcd for $C_{48}H_{64}O_{13}NaS$ 903.3965 $[M+Na]^+$, found 903.3954.

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (6c)

A solution of commercially available D-glucosamine pentaacetate **7** (0.040 g, 0.10 mmol), compound **3** (0.0.20 g, 0.052 mmol) and $Yb(OTf)_3$ (0.012 g, 0.019 mmol) in dichloromethane (2 mL) was heated at 80 °C for 5 h. The solution was allowed to cool to room temperature, diluted with DCM (15 mL) and then washed with water (3 x 10 mL). The organic layer was dried and concentrated under reduced pressure. The crude was purified by flash chromatography (hexane/AcOEt, from 1:1 to 2:8) to give compound **6c** (0.025 g, 69 %) as an amorphous solid. $[\alpha]_D^{20}$ = -22.9 (*c* = 1 in chloroform). ^{1}H NMR ($CDCl_3$): δ = 7.68-7.61 (m, 3H, arom.), 7.50-7.46 (m, 2H, arom.), 7.39-7.36 (m, 2H, arom.), 7.35-7.32 (m, 2H, arom.), 6.77 (*br* s, 1H, arom.), 6.57 (*br* s, 1H, arom.), 6.44 (*br* s, 1H, arom.), 5.60 (d, 1H, $J_{2,NH}$ = 9.0 Hz, NH), 5.09 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 5.09 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 4.98 (s, 2H, CH_2SO_2), 4.91 (d, 1H, J = 12.3 Hz, OC*Ha*Hb), 4.66-4.60 (m, 2H, H-1 and OCHa*Hb*), 4.27 (dd, 1H, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.24 (s, 2H, $PhCH_2O$), 4.17 (dd, 1H, $J_{5,6b}$ = 2.4 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6b), 4.01 (ddd, 1H, $J_{1,2}$ = 8.5 Hz, $J_{2,3}$ = 10.4 Hz, $J_{2,NH}$ = 9.0 Hz, H-2), 3.65-3.61 (m, 1H, H-5), 2.24 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.91 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ = 170.8 (C=O), 170.7 (C=O), 170.2 (C=O), 169.4 (C=O), 158.6, 139.8, 138.1, 136.7, 136.6, 133.7, 128.9-128.3 (5C), 128.3 (2C), 127.5 (2C), 124.6, 116.8,

113.8, 99.4 (C-1), 72.5 (C-3), 71.8 (C-5), 70.2 OCH_2), 69.6 (CH_2SO_2), 68.6 (C-4), 62.9 (C-6), 62.1 ($PhCH_2O$), 54.4 (C-2), 23.3 (CH_3), 21.3 (CH_3), 20.8 (CH_3), 20.6 (CH_3), 20.5 (CH_3). HRMS (ESI): *m/z* calcd for $C_{36}H_{41}NO_{12}NaS$ 734.2247 $[M+Na]^+$, found 734.2255.

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (Ac-1-Glc)

Compound **Ac-1-Glc** was obtained by initial depivaloylation of **6a** (0.025 g, 0.028 mmol) followed by acetylation of the crude in Ac_2O/Py 1:2 (1.2 mL). The reaction was quenched by addition of methanol, and the solvent removed under reduced pressure. Purification of the crude gave pure compound **Ac-1-Glc** (0.015 g, 74%) as an oil. $[\alpha]_D^{20}$ = -28.5 (*c* = 0.75 in chloroform). ^{1}H NMR ($CDCl_3$): δ = 7.71-7.68 (m, 2H, arom.), 7.65-7.62 (m, 1H, arom.), 7.52-7.47 (m, 2H, arom.), 7.40-7.36 (m, 2H, arom.), 7.35-7.30 (m, 2H, arom.), 6.77 (*br* s, 1H, arom.), 6.55 (*br* s, 1H, arom.), 6.49 (*br* s, 1H, arom.), 5.20 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3), 5.14 (dd, 1H, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 9.7 Hz, H-4), 5.09 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{1,2}$ = 7.9 Hz, H-2), 4.97-4.91 (m, 3H, CH_2SO_2 and OC*Ha*Hb), 4.66 (d, 1H, J = 12.3 Hz, OCHa*Hb*), 4.59 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.30 (dd, 1H, $J_{5,6a}$ = 4.7 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.25 (s, 2H, $PhCH_2O$), 4.19 (dd, 1H, $J_{5,6b}$ = 2.4 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6b), 3.73-3.68 (m, 1H, H-5), 2.24 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ = 170.7 (C=O), 170.3 (C=O), 169.4 (C=O), 169.3 (C=O), 158.7, 139.8, 138.1, 136.6, 136.5, 133.6, 129.1-127.6 (9C), 124.5, 116.5, 113.8, 99.4 (C-1), 72.8 (C-3), 71.9 (C-5), 71.3 (C-2), 70.4 (sugarOCH_2), 69.6 (CH_2SO_2), 68.4 (C-4), 62.9 ($PhCH_2O$), 61.9 (C-6), 21.3 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3). HRMS (ESI): *m/z* calcd for $C_{36}H_{40}O_{13}NaS$ 735.2087 $[M+Na]^+$, found 735.2084.

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethyl β-D-galactopyranoside (1-Gal)

Compound **6b** (0.050 g, 0.057 mmol) was deprotected as described for the synthesis of **1-Glc** to give, after flash chromatography (DCM/MeOH, 9:1), compound **1-Gal** (0.025 g, 80%) as an amorphous solid. $[\alpha]_D^{20}$ = -17.3 (*c* = 1 in chloroform). ^{1}H NMR ($CDCl_3$/CD_3OD, 2:1): δ = 7.69-7.60 (m, 3H, arom.), 7.53-7.46 (m, 2H, arom.), 7.45-7.38 (m, 2H, arom.), 7.38-7.31 (m, 2H, arom.), 6.74 (*br* s, 1H, arom.), 6.49 (*br* s, 1H, arom.), 6.46 (*br* s, 1H, arom.), 4.97-4.87 (m, 3H, OC*Ha*Hb and CH_2SO_2), 4.67 (d, 1H, J = 11.9 Hz, OCHa*Hb*), 4.33 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.27 (s, 2H, $PhCH_2O$), 3.90 (*br*d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 3.86-3.75 (m, 2H, 2 H-6), 3.61 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 3.53-3.46 (m, 2H, H-3,5), 2.20 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$/CD_3OD, 2:1): δ = 158.6, 139.7, 137.6, 137.2, 136.3, 133.9, 128.9-127.3 (9C), 124.4, 116.4, 113.9, 102.4 (C-1), 74.9, 73.5, 71.3 (C-2), 70.4

(OCH_2), 69.7 (CH_2SO_2), 68.8 (C-4), 62.6 ($PhCH_2O$), 61.2 (C-6), 20.9 (CH_3). HRMS (ESI): *m/z* calcd for $C_{28}H_{32}O_9NaS$ 567.1665 $[M+Na]^+$, found 567.1669.

Synthesis of 4-[(3-Methyl-5-(phenylsulfonylmethyl)-phenoxy)methyl]-phenylmethyl 2-acetamido-2-deoxy-β-D-glucopyranoside (1-GlcNAc)

To a stirred solution of compound **6c** (0.23 g, 0.032 mmol) in DCM (1 mL) sodium methoxide in methanol (0.1 M solution, 0.25 mL) was added. The reaction was stirred at room temperature for 3h, then neutralized with an ion exchange resin (Dowex 50 × 8, H^+ form), filtered and concentrated. The crude product was subjected to flash chromatography (DCM/MeOH, 9:1) to give pure **1-GlcNAc** (0.015 g, 78%) as an amorphous white solid. $[\alpha]_D^{20}$ = -21.3 (*c* = 0.5 in methanol). ^{1}H NMR ($CDCl_3/CD_3OD$, 2:1): δ = 7.69-7.59 (m, 3H, arom.), 7.53-7.48 (m, 2H, arom.), 7.38-7.31 (m, 4H, arom.), 6.75 (*br* s, 1H, arom.), 6.52 (*br* s, 1H, arom.), 6.46 (*br* s, 1H, arom.), 4.93 (s, 2H, CH_2SO_2), 4.89 (d, 1H, J = 12.3 Hz, OC*Ha*Hb), 4.62 (d, 1H, J = 12.3 Hz, OCHa*Hb*), 4.46 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.31 (s, 2H, $PhCH_2O$), 3.90 (dd, 1H, $J_{5,6a}$ = 2.6 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.76-3.70 (m, 2H, H-2, 6b), 3.45-3.36 (m, 2H, H-3,4), 3.29-3.24 (m, 1H, H-5), 2.21 (s, 3H, CH_3), 1.97 (s, 3H, CH_3CO). ^{13}C NMR ($CDCl_3/CD_3OD$, 2:1): δ = 172.46 (C=O), 158.6, 139.7, 137.6, 137.4, 136.4, 133.8, 128.9-127.3 (9C), 124.4, 116.4, 113.9, 100.3 (C-1), 76.3 (C-5), 74.7 (C-3), 70.9 (C-4), 70.1 (OCH_2), 69.5 (CH_2SO_2), 62.4 ($PhCH_2O$), 61.6 (C-6), 56.0 (C-2), 22.3 (CH_3), 20.7 (CH_3). HRMS (ESI): *m/z* calcd for $C_{30}H_{35}NO_9NaS$ 608.1930 $[M+Na]^+$, found 608.1929.

Computational Methods

The high resolution crystal structure of SK1 complex with **PF-543** inhibitor from PDB ID: 4V24 was used for the docking calculations.[1] Residues 93–449 from chain A were retained after removing all alternative conformation B atoms. Polar hydrogen atoms were added using AutoDockTools v1.5, which was also used to define a search space of 26×32×32 Å in the substrate-binding channel. The initial conformation of each compound was calculated using OpenEye OMEGA v3.2,[2] and docking calculations were carried out using AutoDock VINA v1.1 by setting the exhaustiveness level to 100.[3] Validation of the docking protocol was performed by re-docking of **PF-543**, which displayed the best-ranked conformation at root-mean-square deviation of 1.2 Å with respect to the crystallographic structure. The preferred conformation for the designed glycoconjugates was selected as the pose with the lowest binding energy that displayed the lowest variability with respect to the hydrophobic core of **PF-543** (PDB files of the complexes shown in Fig. 2 are provided as Supporting Info). Visual investigation of the complexes and rendering of the figures was carried out using VMD v1.9.[4]

Cell culture

Murine C2C12 myoblasts were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100U/mL penicillin and 100 µg/mL streptomycin at 37°C in 5% CO_2.
For the experiments, cells were seeded into six-well plates at a 80% confluence. Cell culture were shifted to DMEM without serum containing 0,1% BSA and treated with PF (10 µM) and PF analogue (all 10µM) 30min, 1h and 2h before transforming growth factor beta incubation (TGFβ1, 5 ng/mL).

Western blot analysis

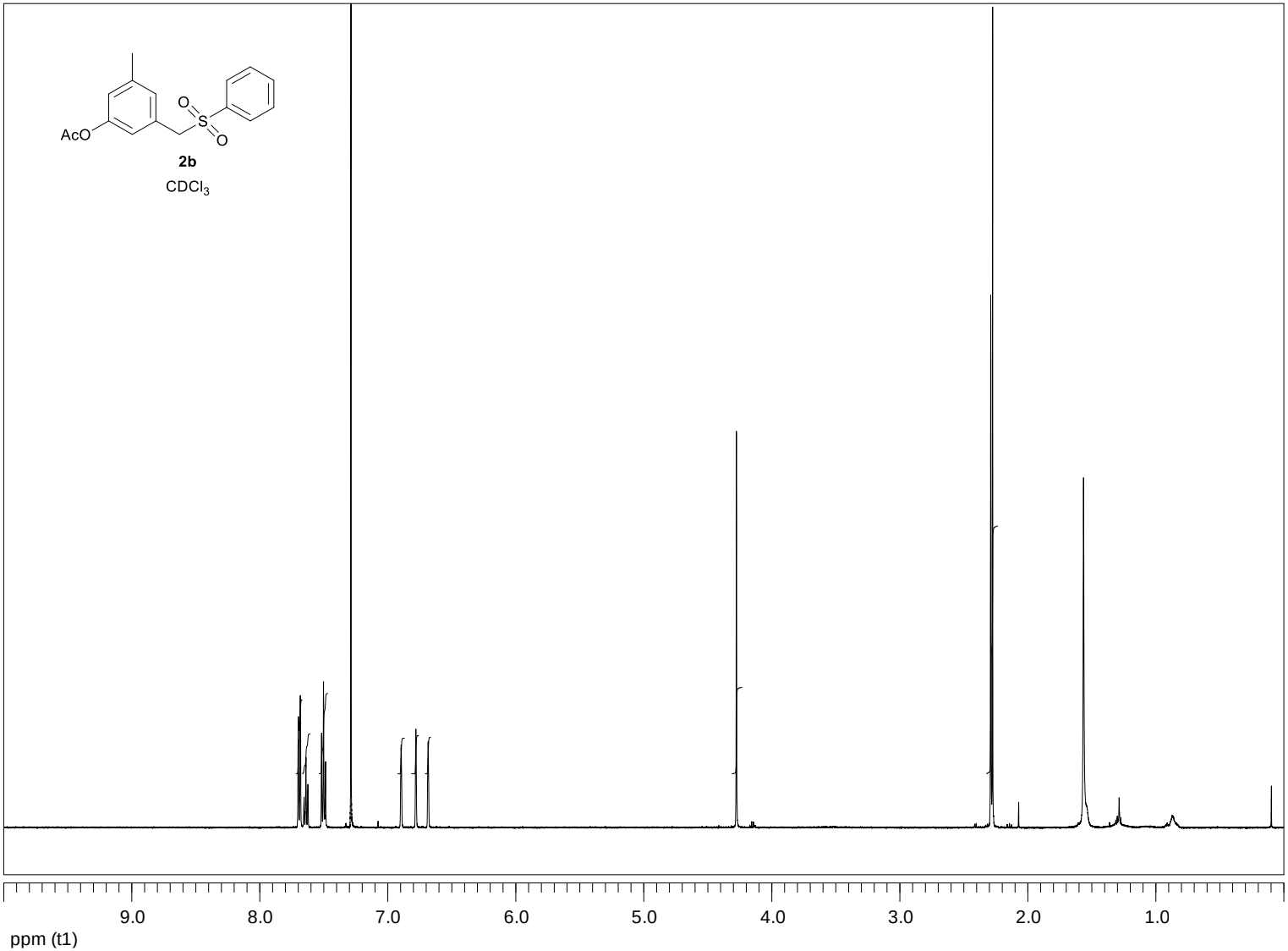
The expression of fibrosis markers anti-αSMA antibody (SC-53015, Santa Cruz Biotecnology, Texas, USA), anti-caveolin3 antibody (#610420, BD Transduction Laboratories NJ USA) and anti-SMα22 antibody (Transgelin, MA5-11547, Everest Biotech, Oxfordshire, UK) were evaluated on total cell lysates after 48 h PF and PF analogues challenge. Proteins were resolved by SDS–PAGE, transblotted to PVDF membranes and immunopositive bands visualized by enhanced chemiluminescence.

Immunostaining and Fluorescence Microscopy

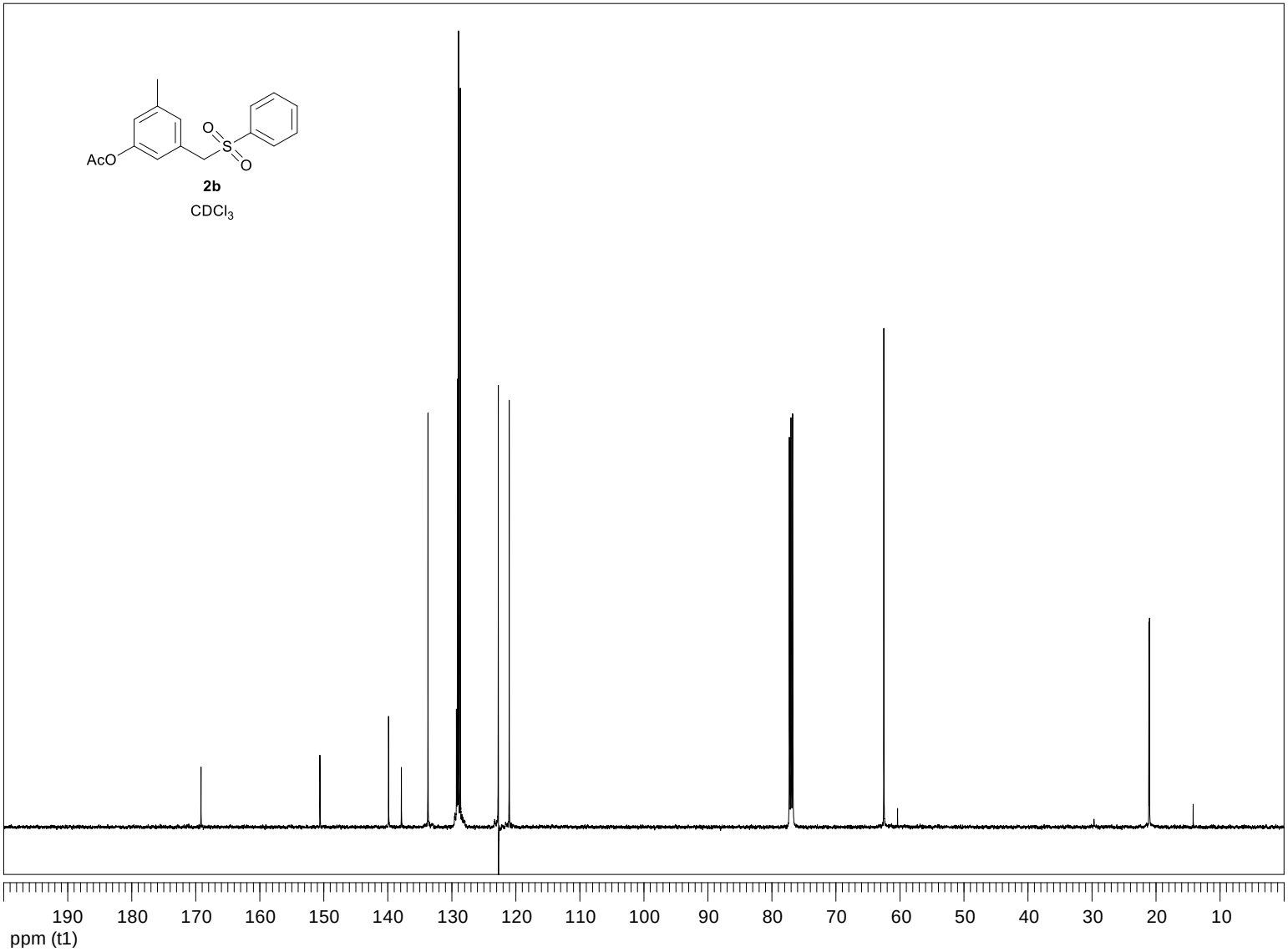
Cells were seeded on microscope slides and at confluence were treated with PF-543 and its glycoconjugate analogues for 30 min and 2 h before TGFβ1 5 ng/mL. After 48h of incubation, cells were fixed with 2% paraformaldehyde in PBS, permeabilized in 0.1% Triton X-100-PBS for 30 min and blocked in 3% BSA for 1 h. Cells were then incubated with anti-α-SMA antibody 1:100 for 2 h and fluorescein-conjugated anti-mouse secondary antibody for 1 h. To stain F-actin filaments, the specimen was incubated with TRITC-phalloidin for 30min. Images were taken using a Leica SP5 laser scanning confocal microscope (63× objective).

Statistical analysis

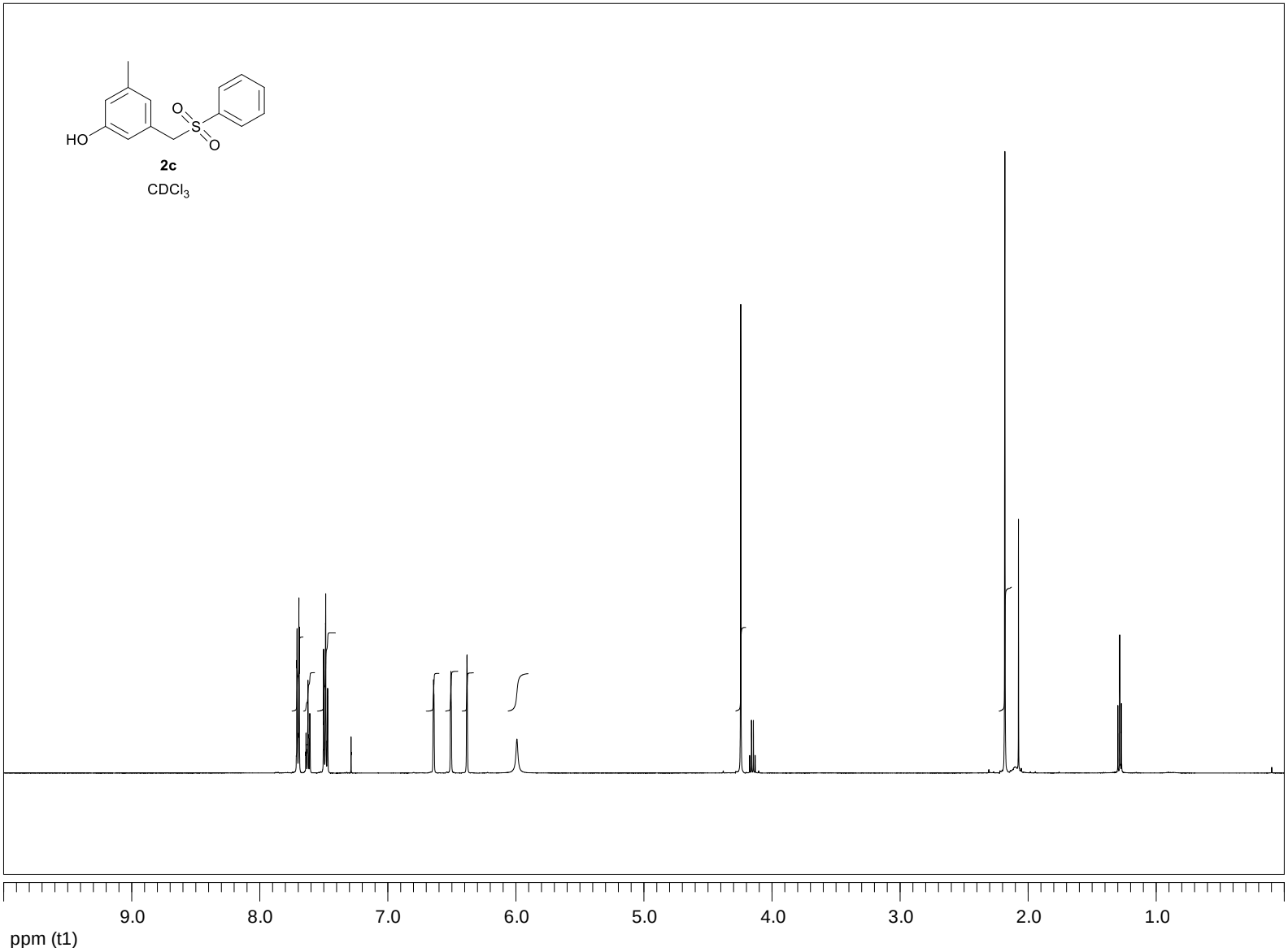
ImageJ software was used to perform densitometric analysis of the Western Blot bands. Graphical representations were obtained by GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). Statistical analysis was performed using ANOVA test.



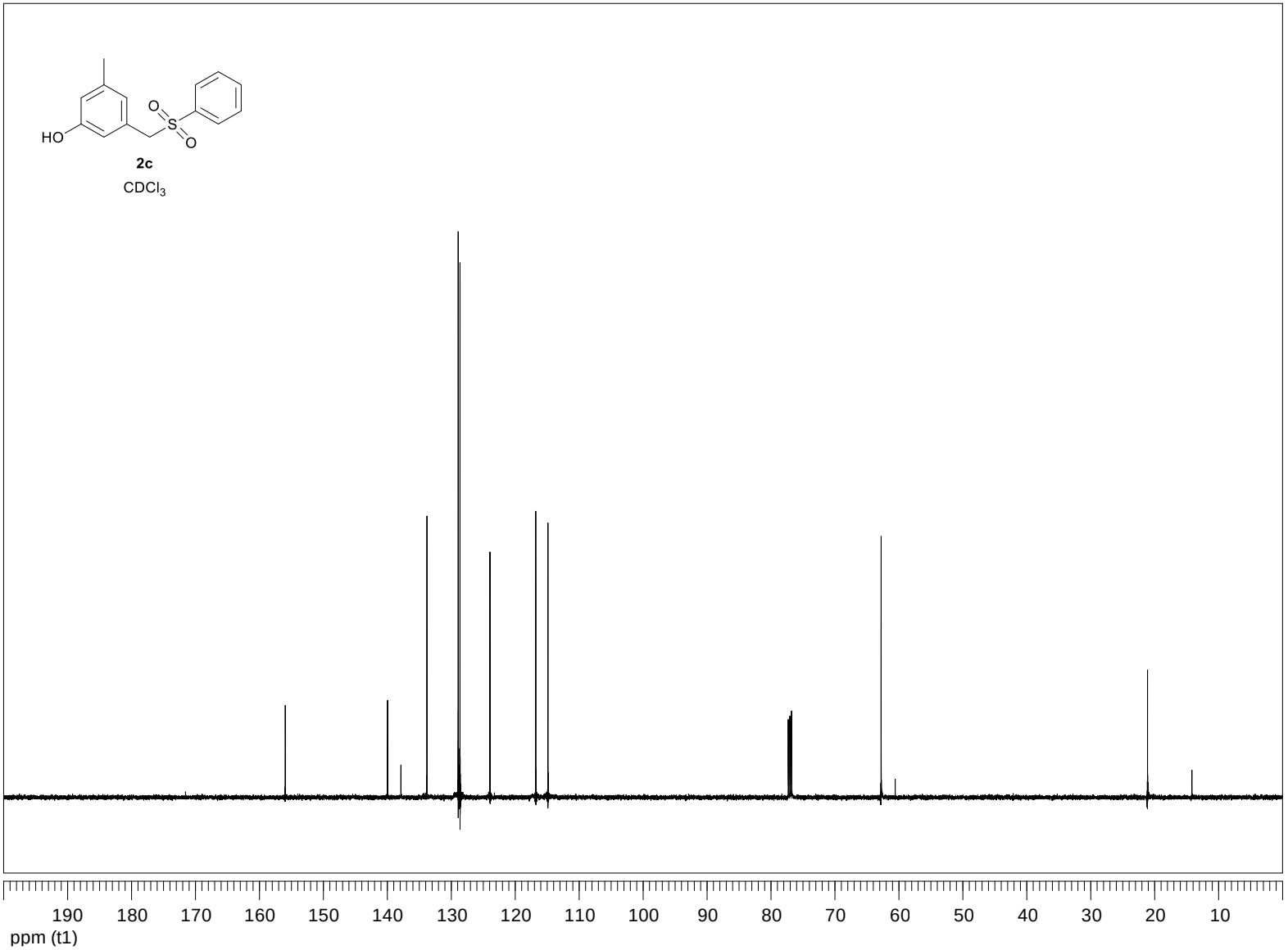
^{1}H NMR spectrum of **2b** in $CDCl_3$.



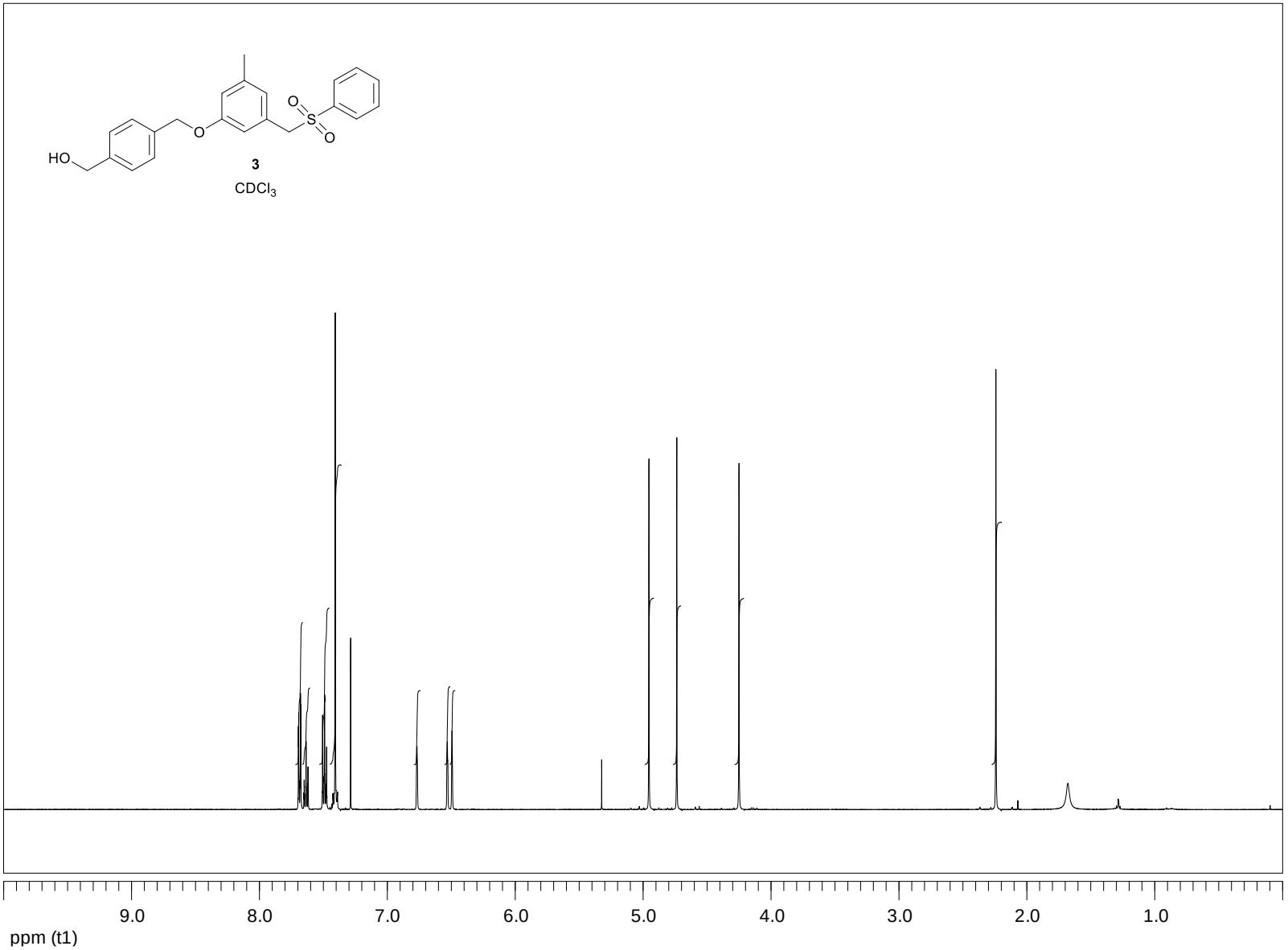
^{13}C NMR spectrum of **2b** in $CDCl_3$.



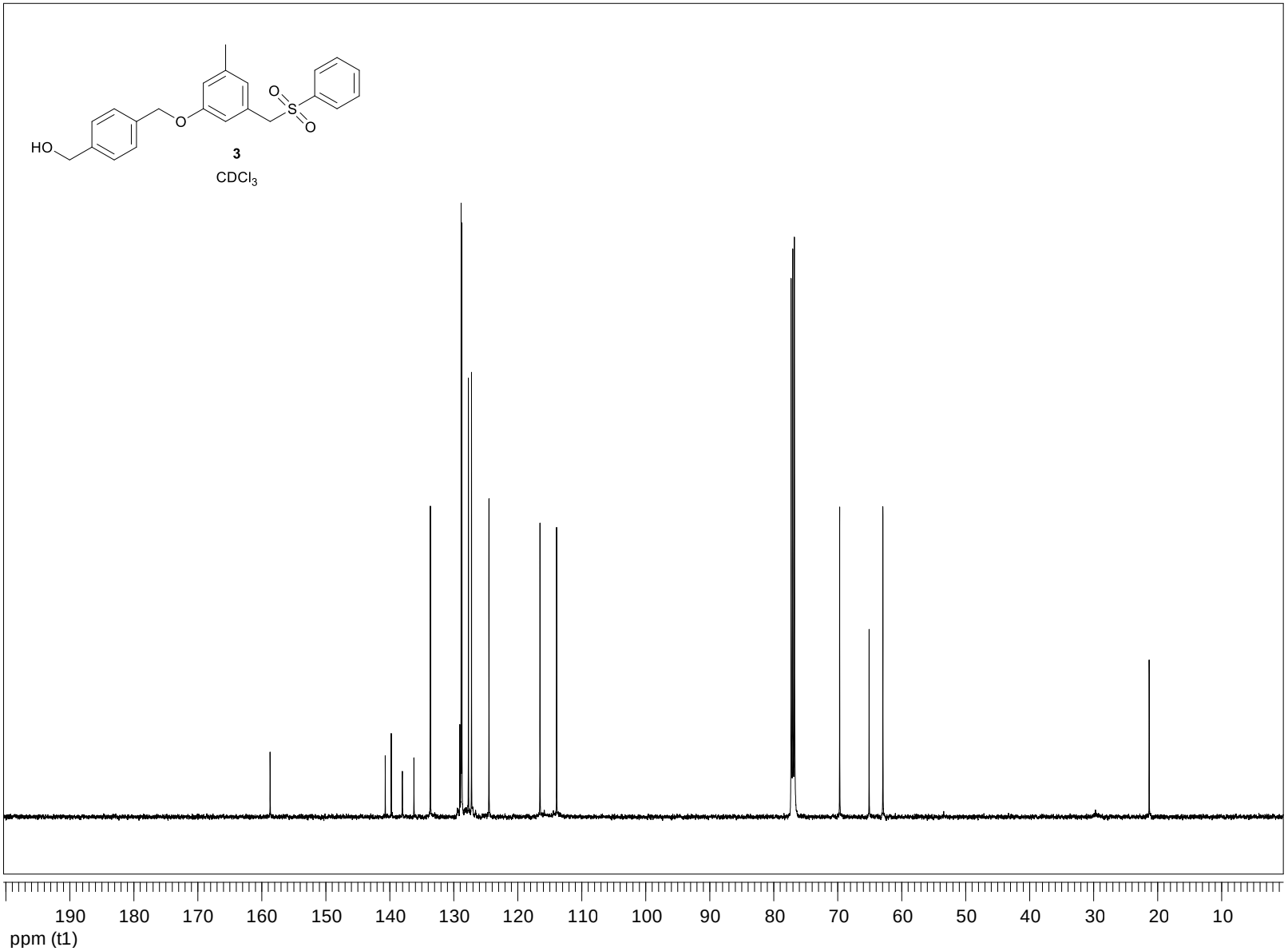
^{1}H NMR spectrum of **2c** in $CDCl_3$.



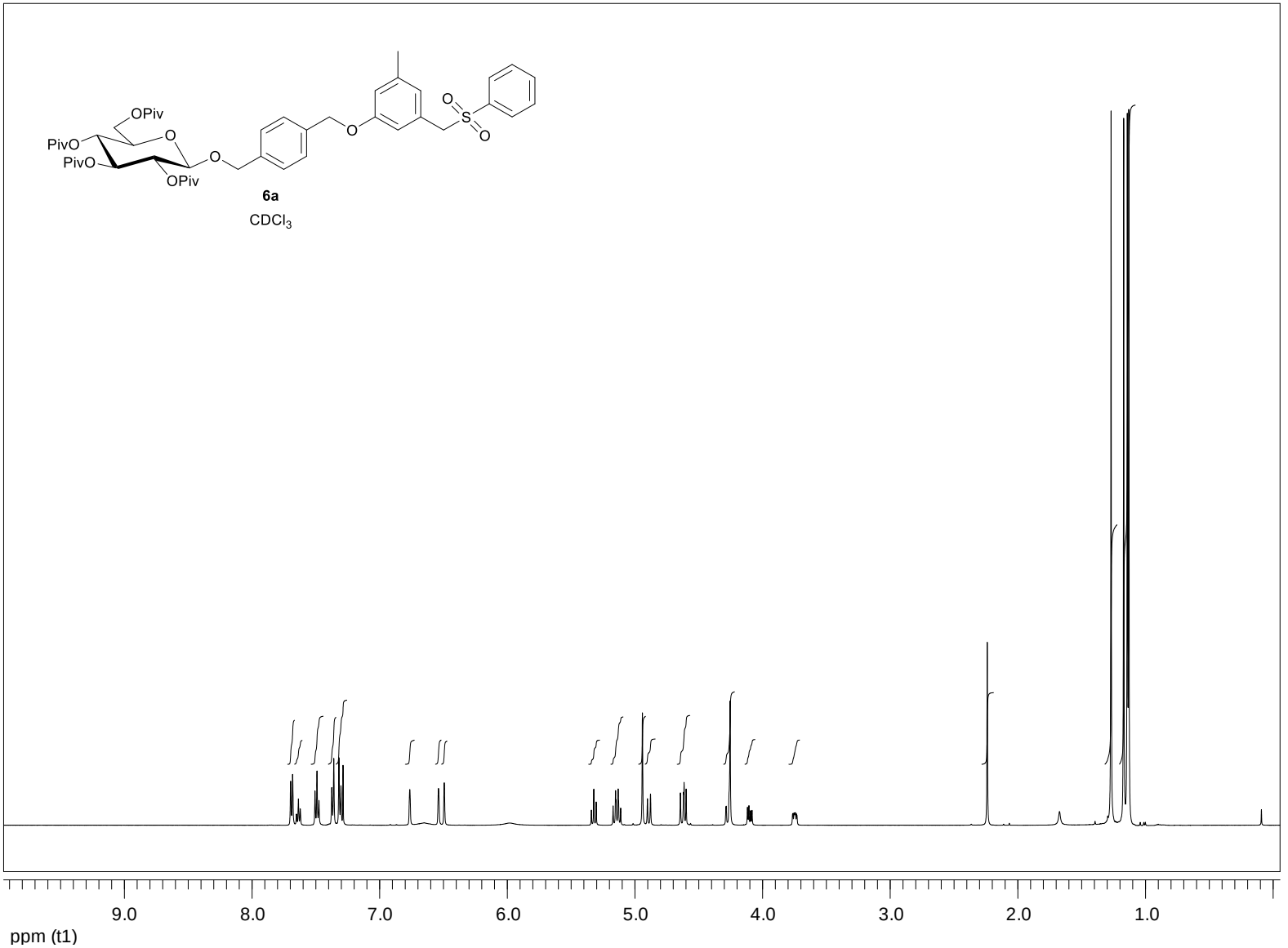
^{13}C NMR spectrum of **2c** in $CDCl_3$.



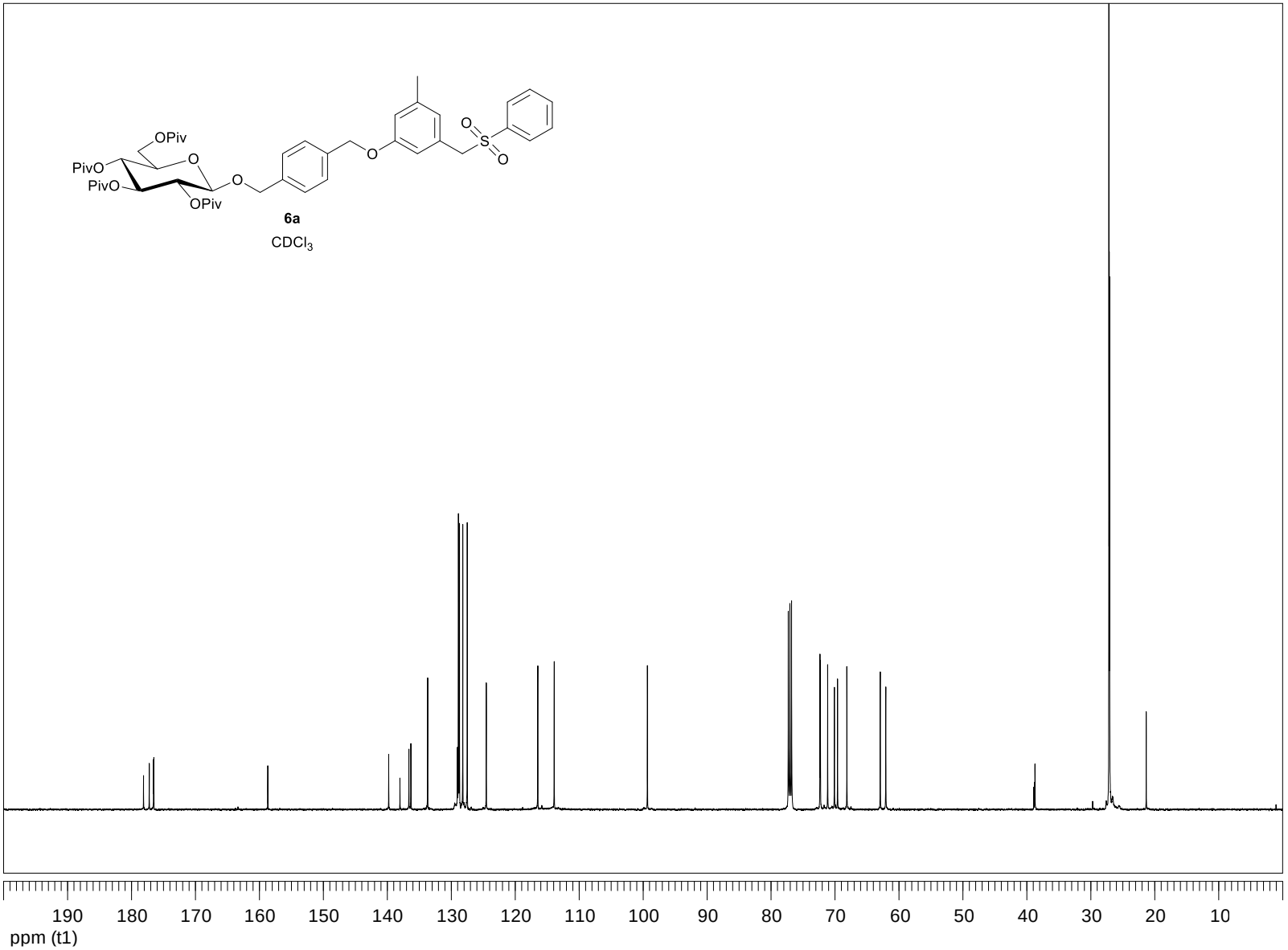
^{1}H NMR spectrum of **3** in $CDCl_3$.



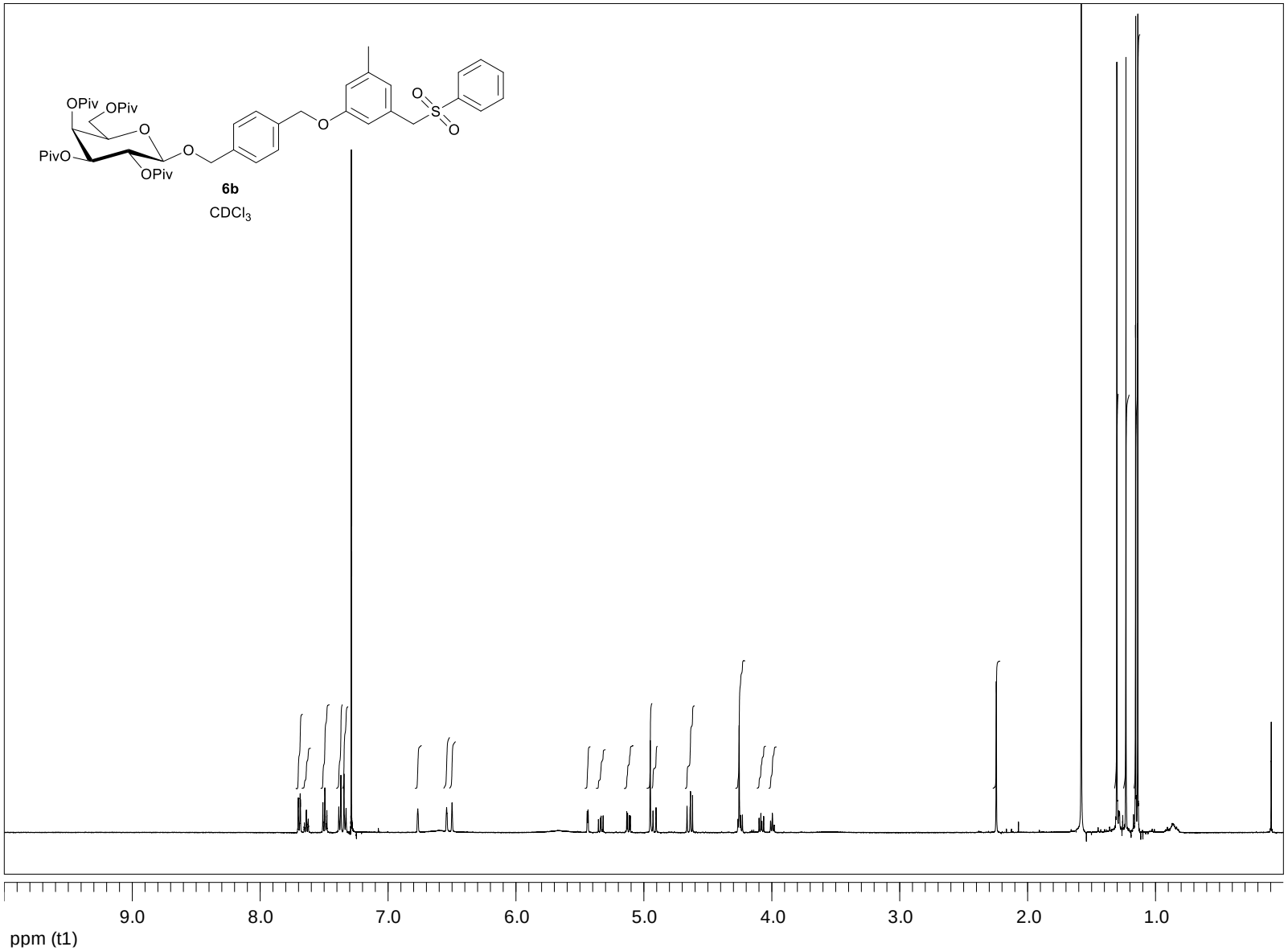
^{13}C NMR spectrum of **3** in $CDCl_3$.



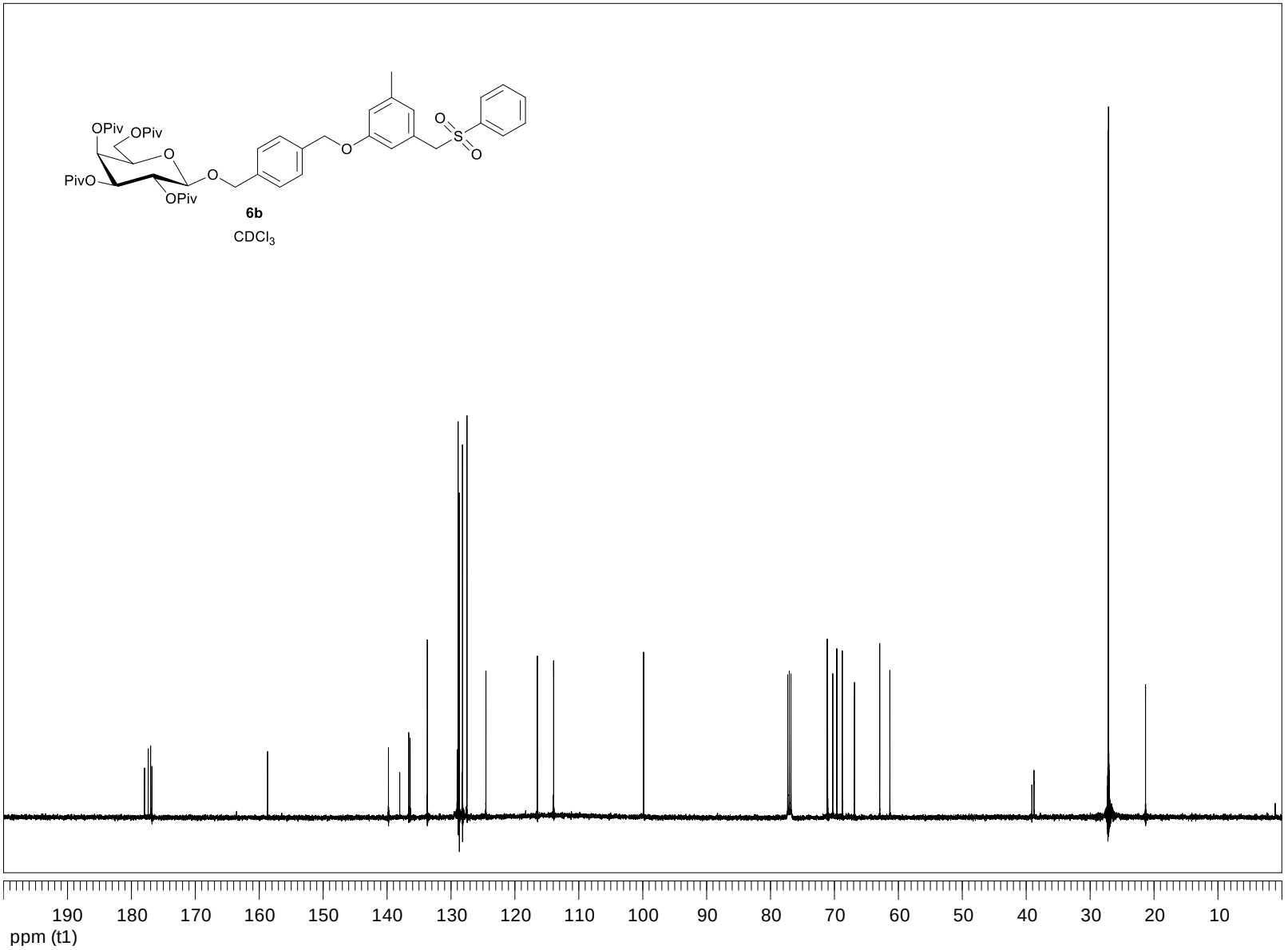
^{1}H NMR spectrum of **6a** in $CDCl_3$.



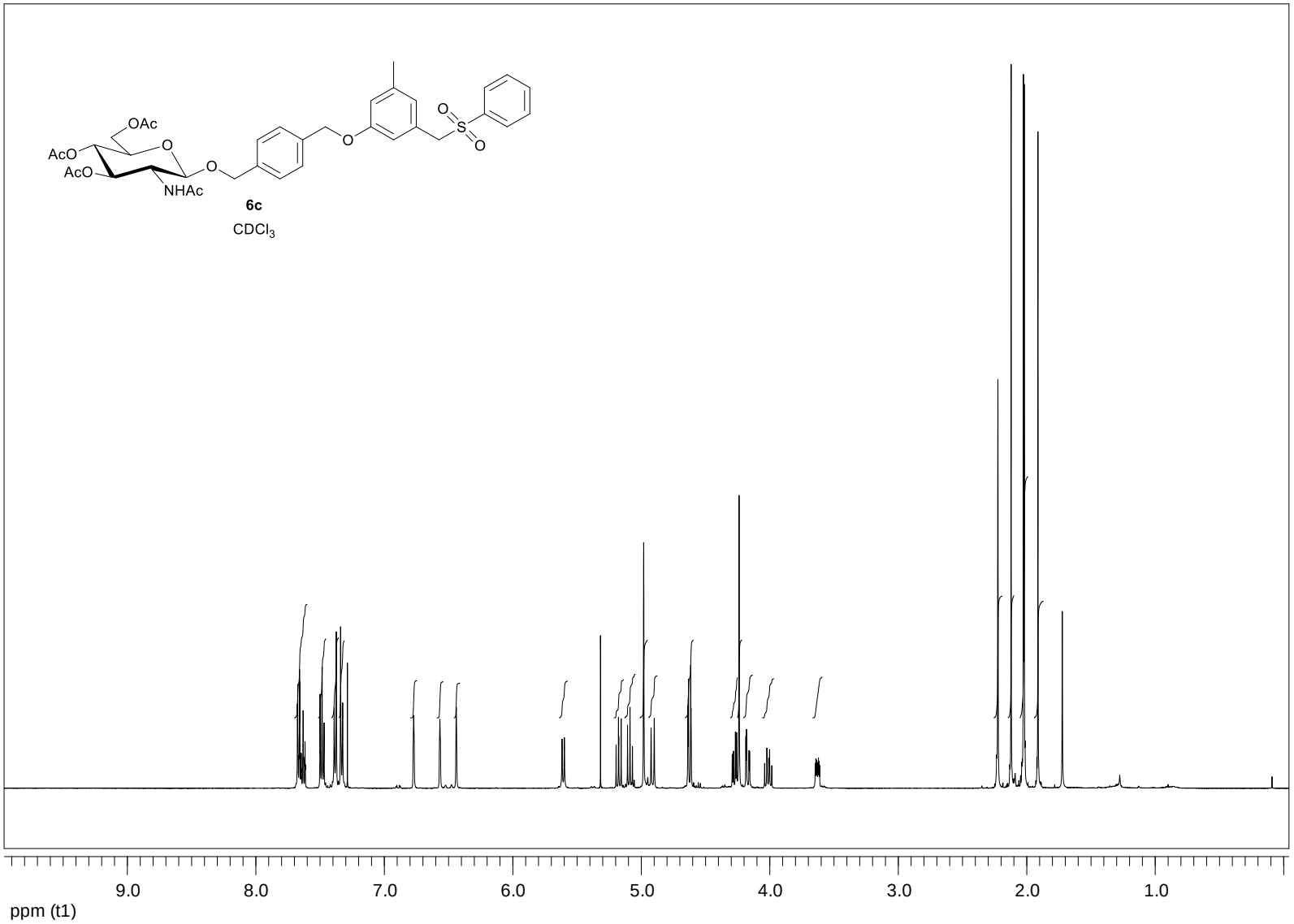
^{13}C NMR spectrum of **6a** in $CDCl_3$.



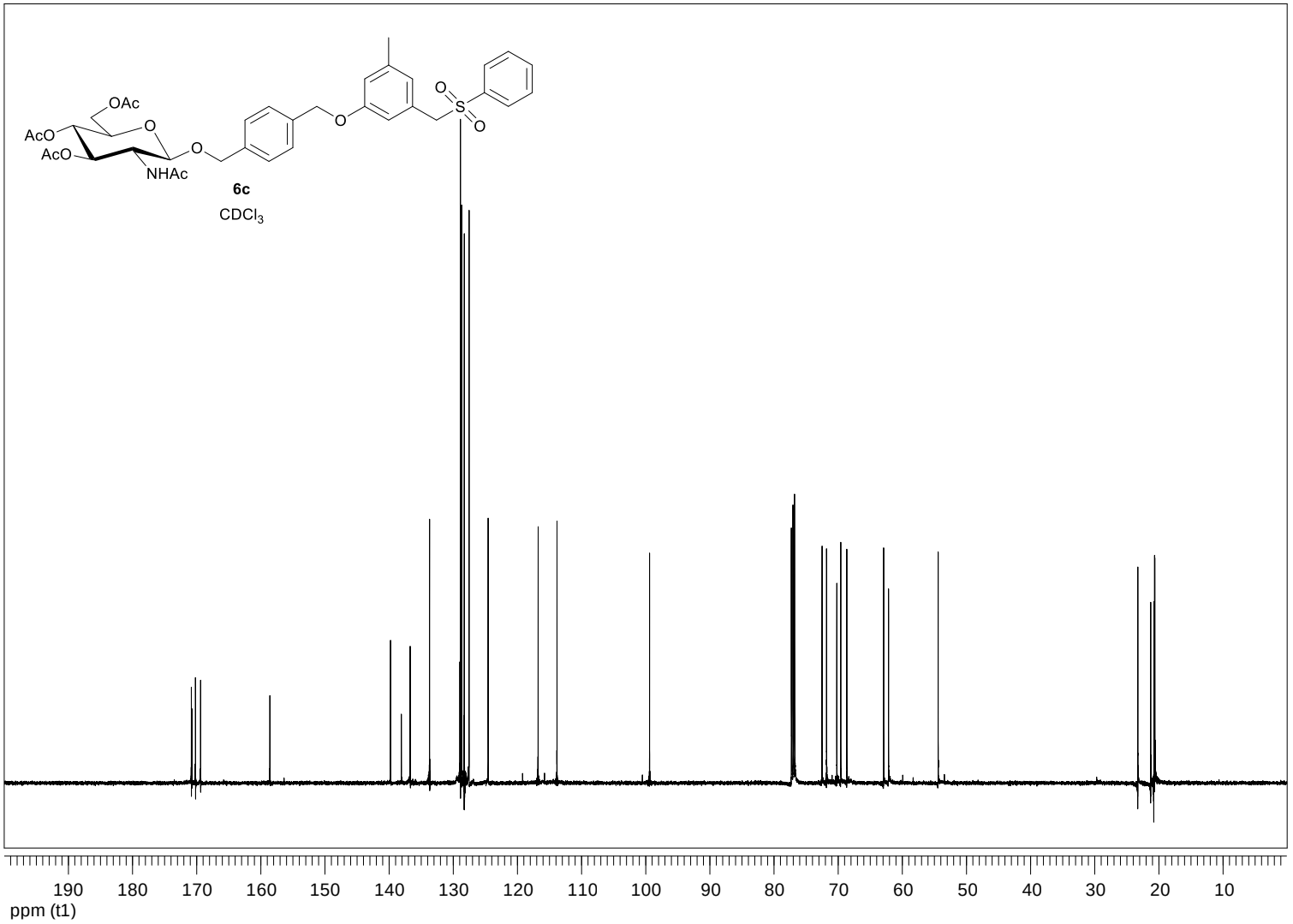
^{1}H NMR spectrum of **6b** in $CDCl_3$.



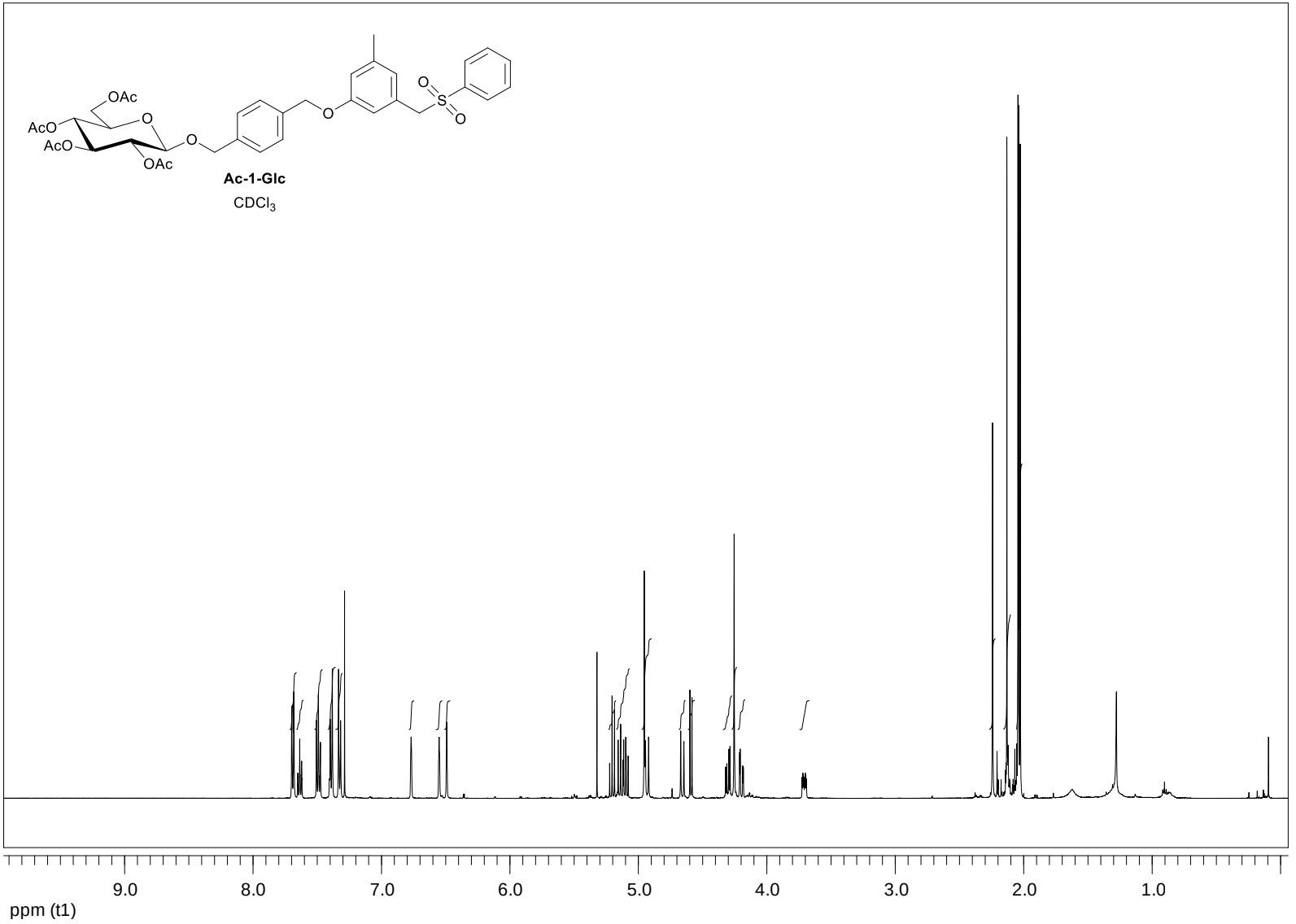
^{13}C NMR spectrum of **6b** in $CDCl_3$.



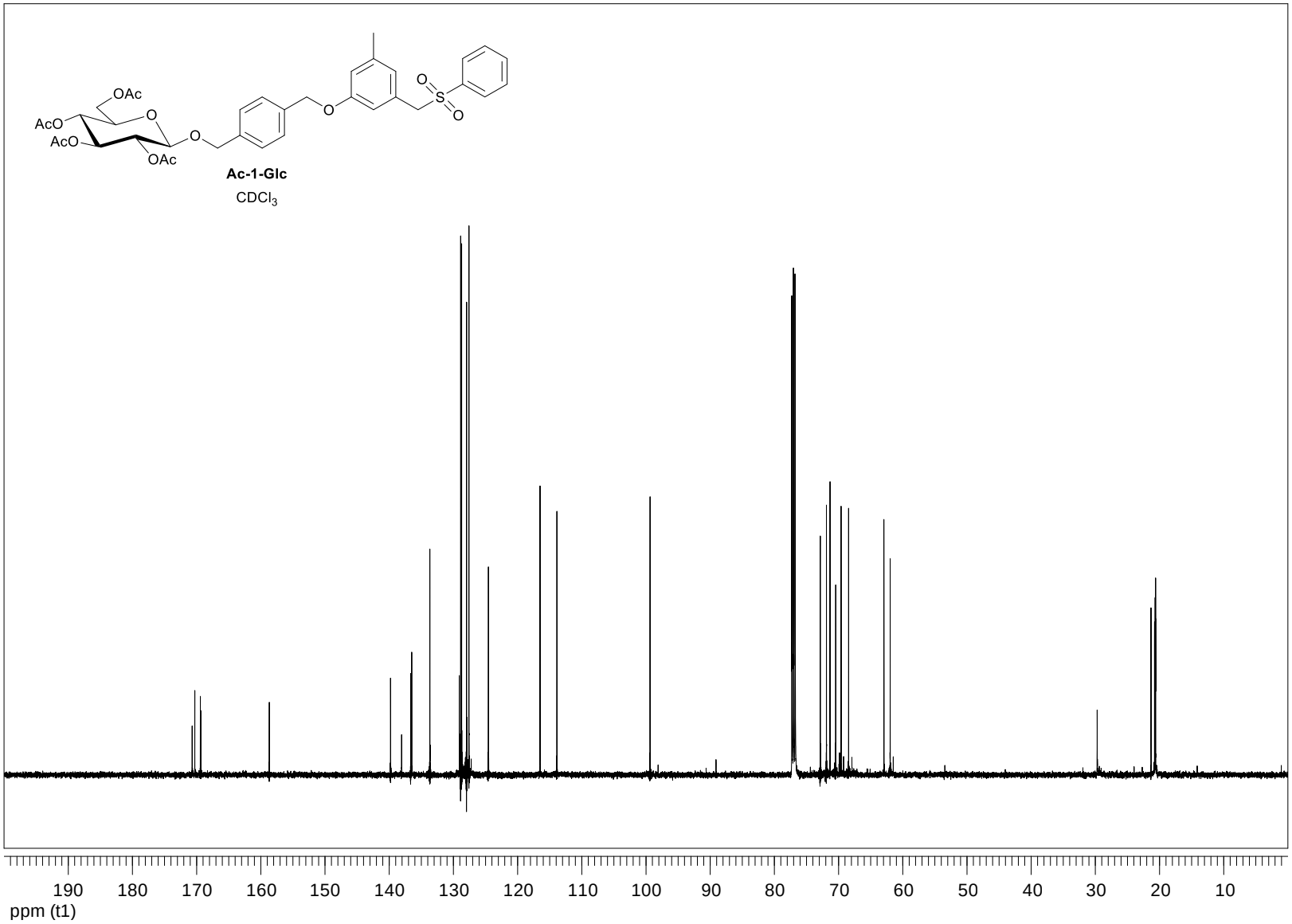
^{1}H NMR spectrum of **6c** in $CDCl_3$.



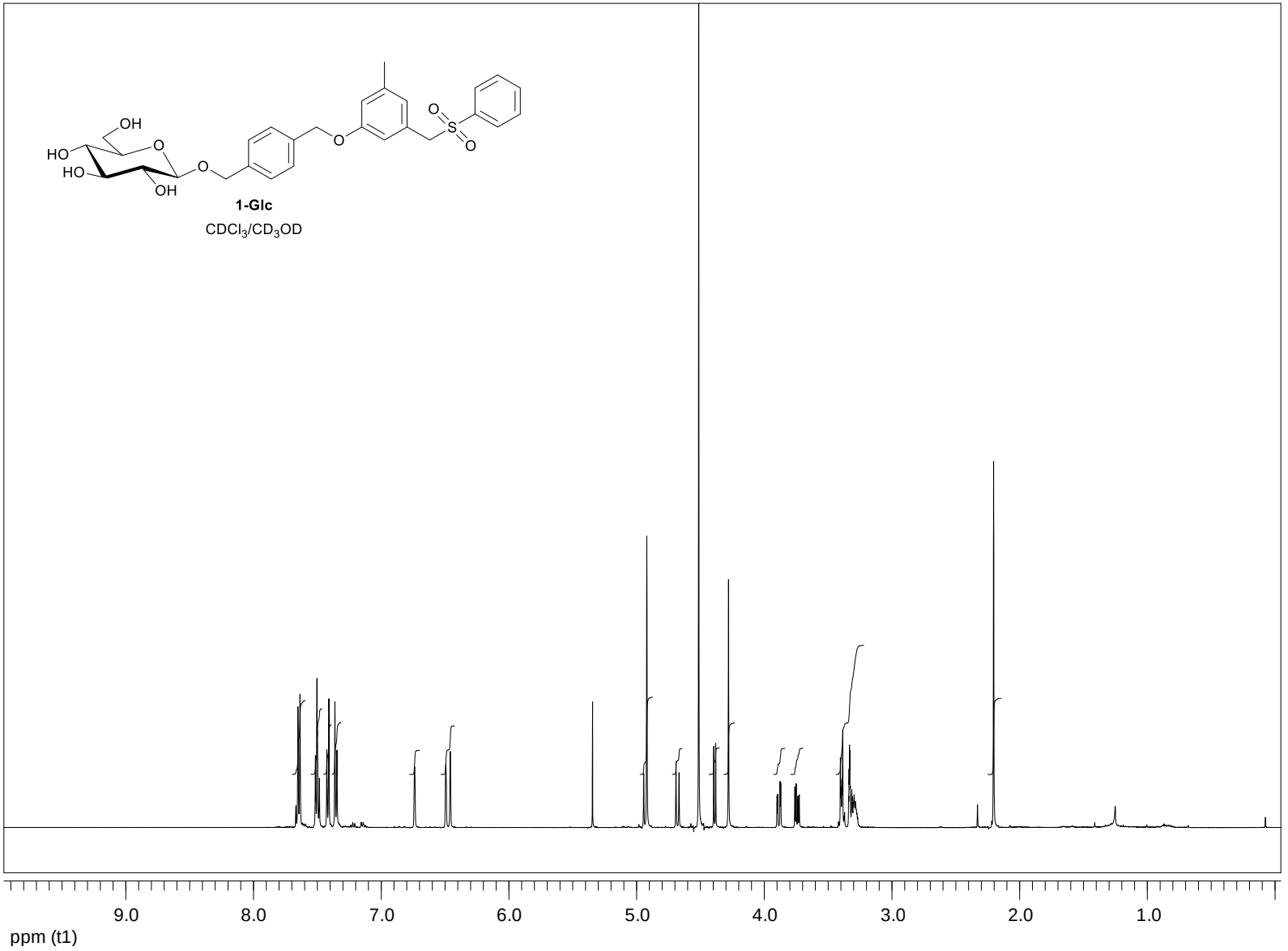
^{13}C NMR spectrum of **6c** in $CDCl_3$.



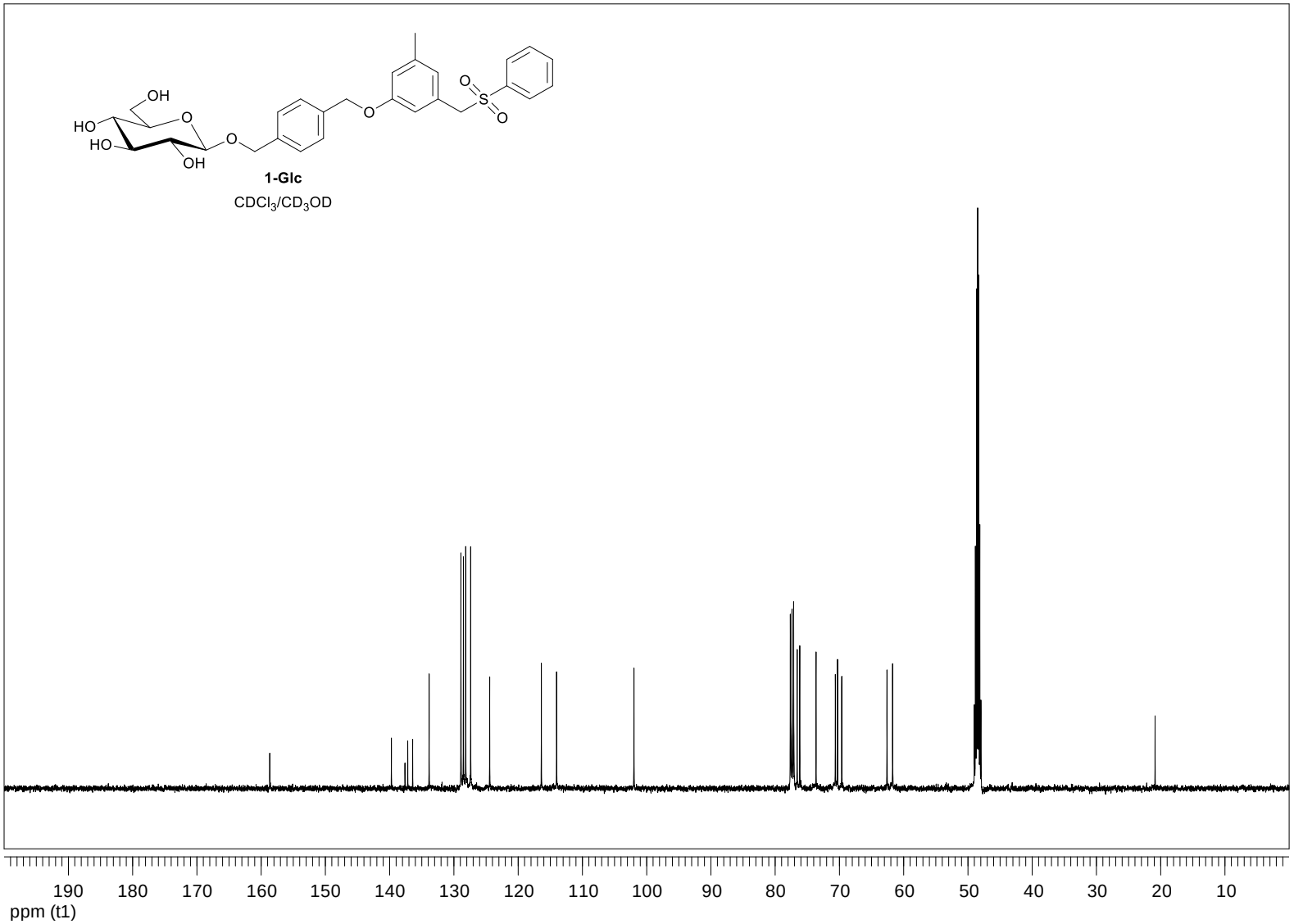
^{1}H NMR spectrum of **Ac-1-Glc** in $CDCl_3$.



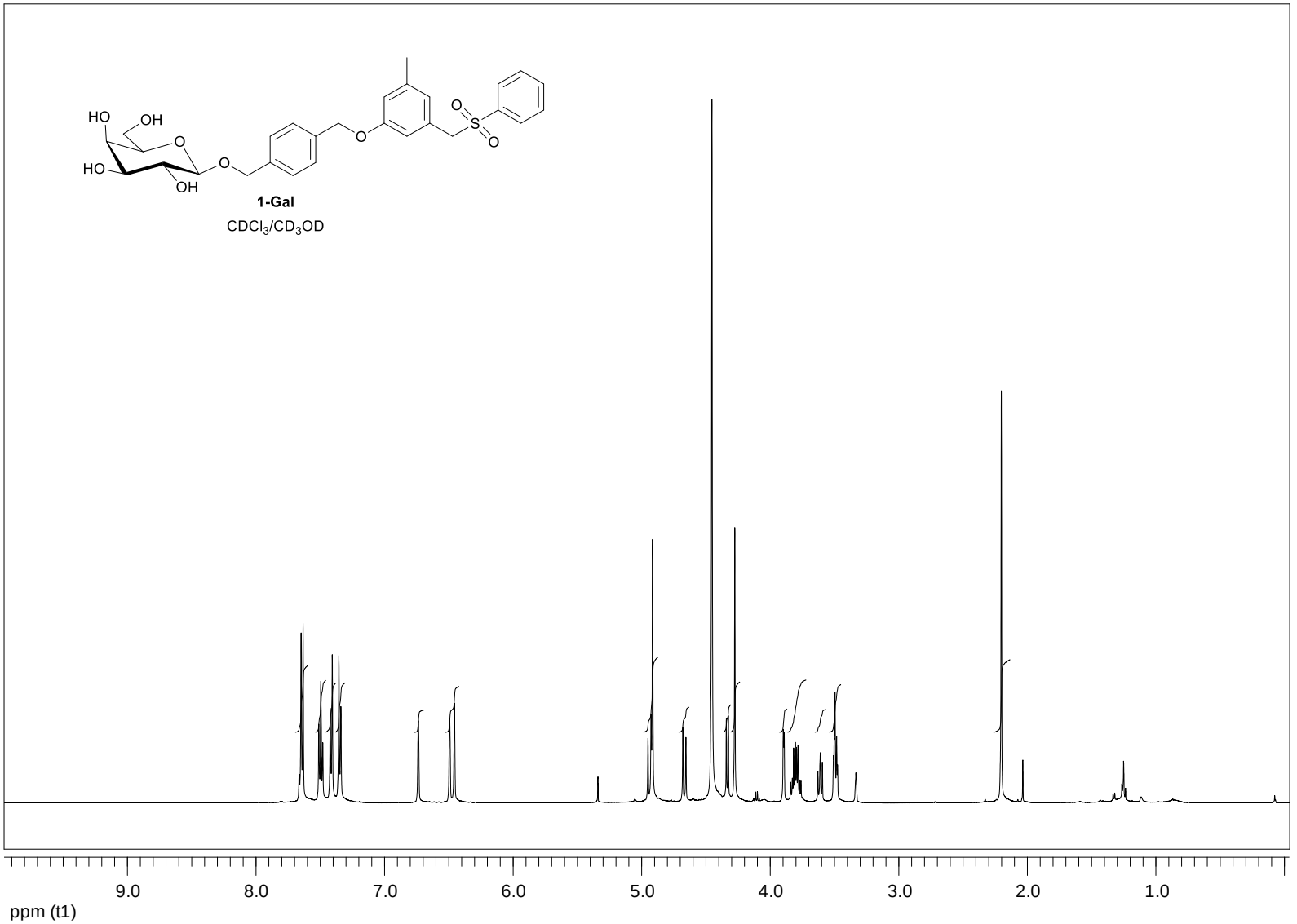
^{13}C NMR spectrum of **Ac-1-Glc** in $CDCl_3$.



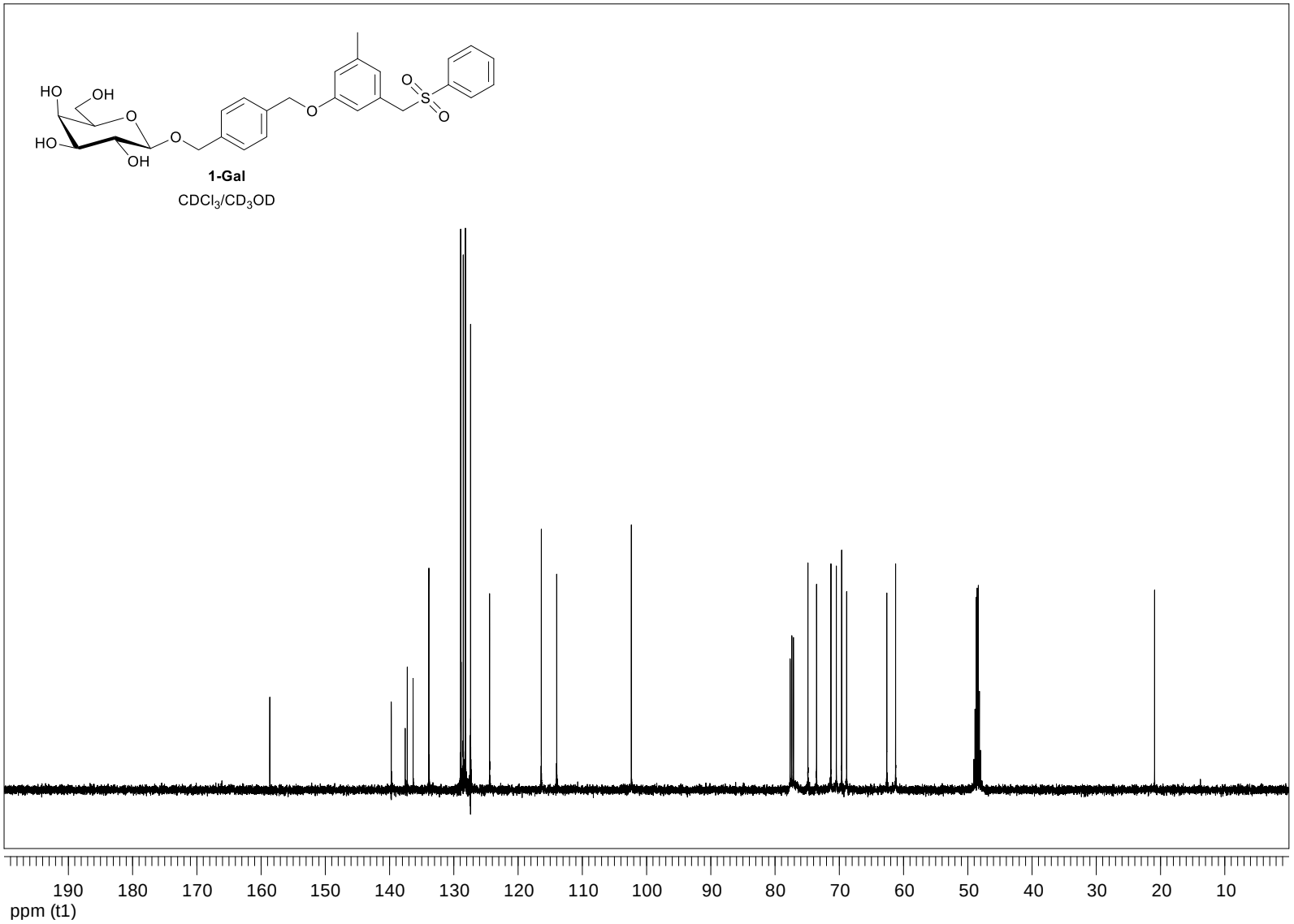
^{1}H NMR spectrum of **1-Glc** in $CDCl_3/CD_3OD$, 2:1



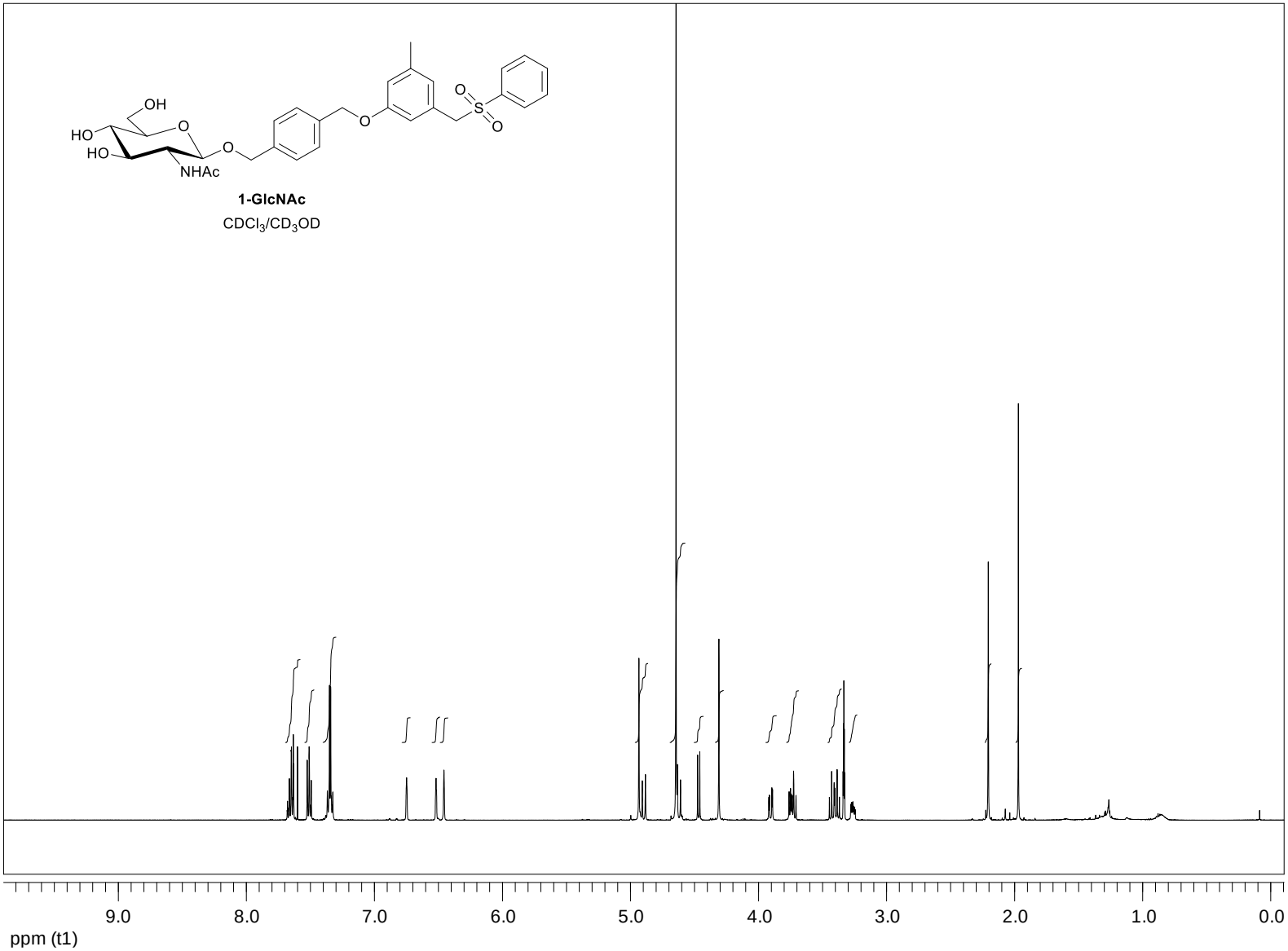
^{13}C NMR spectrum of **1-Glc** in $CDCl_3/CD_3OD$, 2:1



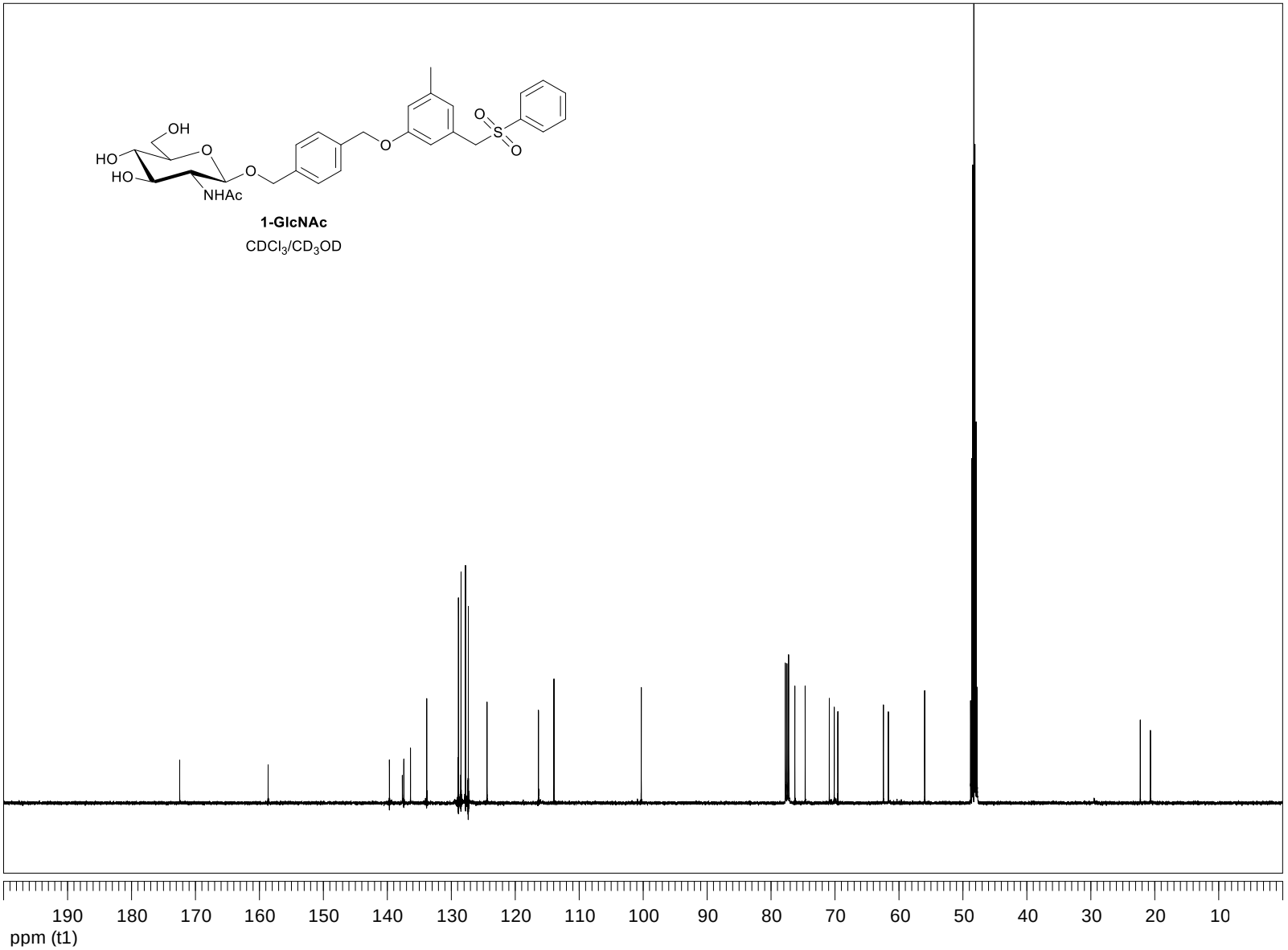
^{1}H NMR spectrum of **1-Gal** in $CDCl_3/CD_3OD$, 2:1



^{13}C NMR spectrum of **1-Gal** in $CDCl_3/CD_3OD$, 2:1



^{1}H NMR spectrum of **1-GlcNAc** in $CDCl_3/CD_3OD$, 2:1



^{13}C NMR spectrum of **1-GlcNAc** in $CDCl_3/CD_3OD$, 2:1

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1. Wang, J.; Knapp, S.; Pyne, N. J.; Pyne, S.; Elkins, J. M. Crystal Structure of Sphingosine Kinase 1 with PF-543. *ACS Med. Chem. Lett.* **2014**, *5 (12*), 1329-1333.

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