

Supporting Information

Expedient Synthesis of O-Glycosylated amino acids

Felicity J. Frank, Rebecca A. Lawson and Tom E. McAllister*

Chemistry, School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU.

Supporting Information

Cost of commercial Fmoc-amino acids	4
General experimental information	7
Fmoc-Thr-OMe 6	8
<i>Typical procedure for Glycosylation</i>	9
Fmoc-Thr(GalNAc(Ac) ₃ -α-D]-OMe α7	10
Fmoc-Thr(GalNAc(Ac) ₃ -β-D]-OMe β7	11
Fmoc-Ser(GalNAc(Ac) ₃ -α-D]-OMe α8	12
Fmoc-Ser(GalNAc(Ac) ₃ -β-D]-OMe β8	13
Fmoc-Thr(GlcNAc(Ac) ₃ -α-D]-OMe α9	14
Fmoc-Thr(GlcNAc(Ac) ₃ -β-D]-OMe β9	15
Fmoc-Ser(GlcNAc(Ac) ₃ -β-D]-OMe β10	16
<i>Typical procedure for demethylation with Lil.</i>	17
Fmoc-Thr(GalNAc(Ac) ₃ -α-D]-OH α1	17
Fmoc-Thr(GalNAc(Ac) ₃ -β-D]-OH β1	18
Fmoc-Ser(GalNAc(Ac) ₃ -α-D]-OH α2	19
Fmoc-Ser(GalNAc(Ac) ₃ -β-D]-OH β2	20
Fmoc-Thr(GlcNAc(Ac) ₃ -β-D]-OH β12	21
Fmoc-Ser(GlcNAc(Ac) ₃ -β-D]-OH β13	21
Anomerisation time course experiment	22
Calculation of Synthesis cost	24
NMR Spectra	28

Fmoc Thr-OMe 6	28
Fmoc-Thr(GalNAc(Ac)3- α -D]-OMe α 7	30
Fmoc-Thr(GalNAc(Ac)3- β -D]-OMe β 7	32
Fmoc-Ser(GalNAc(Ac)3- α -D]-OMe α 8	34
Fmoc-Ser(GalNAc(Ac)3- β -D]-OMe β 8	36
Fmoc-Thr(GlcNAc(Ac)3- α -D]-OMe α 9	38
Fmoc-Thr(GlcNAc(Ac)3- β -D]-OMe β 9	40
Fmoc-Ser(GlcNAc(Ac)3- β -D]-OMe β 10	42
Fmoc-Thr(GalNAc(Ac)3- α -D]-OH α 1	44
Fmoc-Thr(GalNAc(Ac)3- β -D]-OH β 1	46
Fmoc-Ser(GalNAc(Ac)3- α -D]-OH α 2	48
Fmoc-Ser(GalNAc(Ac)3- β -D]-OH β 2	50
References	52

Cost of commercial Fmoc-amino acids

Name; CAS	Supplier	Amount/g	Price/£	£/g	rMM	mmol	£/mmol	Link
Fmoc-Thr(GalNAc(Ac)3- α -D]-OH; 116783-35-8	Sigma Aldrich	0.1	770	7700	670.237	0.149	5160.825	772437-100MG
	Doug Discovery	0.1	477	4770	670.237	0.149	3197.030	F567280
	BLD Pharm	0.25	1049	4196	670.237	0.373	2812.314	BD131423
Fmoc-Thr(PO(OBzl)OH)-OH; 175291-56-2	Key Organics	5	462	92.4	511.467	9.776	47.260	AS-75165
	Doug Discovery	5	222	44.4	511.467	9.776	22.709	M03392
	BLD Pharm	5	234	46.8	511.467	9.776	23.937	175291-56-2
Fmoc-Thr(tBu)-OH; 71989-35-0	Doug Discovery	100	84	0.84	397.471	251.591	0.334	M03389
	Biosynth	100	108.45	1.0845	397.471	251.591	0.431	FF15778
	Key Organics	100	90	0.9	397.471	251.591	0.358	AS-14178
Fmoc-Ser(GalNAc(Ac)3- α -D]-OH; 120173-57-1	Sigma Aldrich	0.1	755	7550	656.22	0.152	4954.461	772445-100MG
	Key Organics	0.25	552	2208	656.22	0.381	1448.934	BS-49043
	BLD Pharma	0.25	556	2224	656.22	0.381	1459.433	BD131411
Fmoc-Ser(PO(OBzl)OH)-OH; 158171-14-3	Biosynth	5	350	70	497.43	10.052	34.820	FF47773
	Doug Discovery	5	141	28.2	497.43	10.052	14.028	M03387
	BLD Pharm	5	149	29.8	497.43	10.052	14.823	158171-14-3
Fmoc-Ser(tBu)-OH; 71989-33-8	Doug Discovery	100	53	0.53	383.444	260.794	0.203	M03382
	BLD Pharm	100	61	0.61	383.444	260.794	0.234	BD8607
	Key Organics	100	120	1.2	383.444	260.794	0.460	DS-13762

Table S1: Commercial pricing of amino acids. Correct as of 27th March 2025. The lowest prices we could identify, as listed on the websites for each compound from 3 separate suppliers are shown; cheaper options may be available. The cheapest identified is shown in bold for each compound and was used for price comparison.

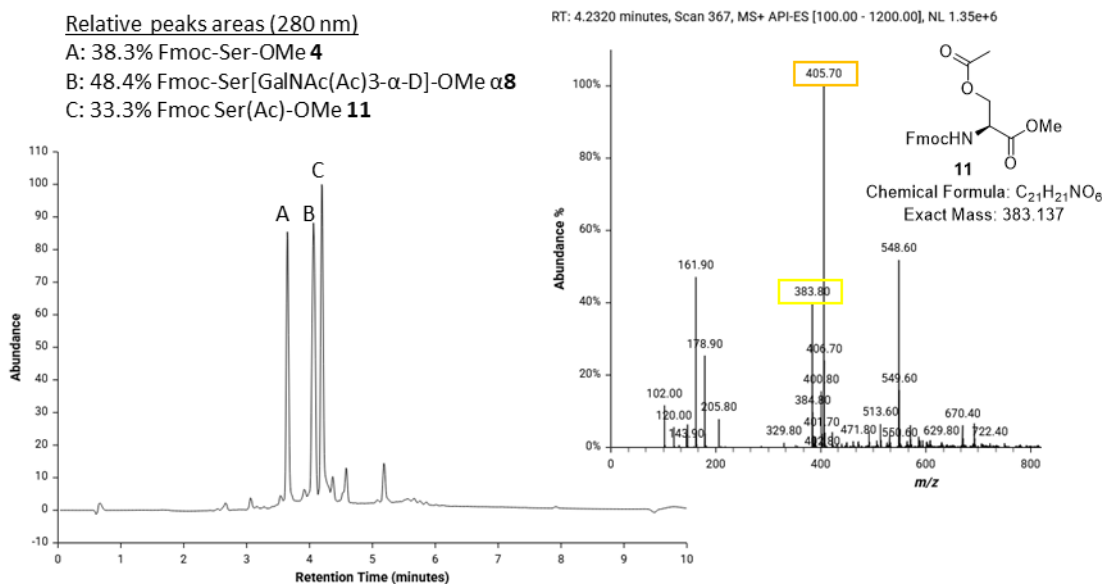


Figure S1. LCMS analysis of the reaction between 1 equiv. GalNAc donor **̢3**, 1 equiv. $Cu(OTf)_2$ and 1 equiv. Fmoc-Ser-OMe **4** (table 1, entry 7) after 10 h (compound numbering as in main text). UV absorbance at 280 nm with calculated relative areas for the peaks labelled A-C. Inset: Mass spectrometry results for peak C; $[M+H]^+$ highlighted in yellow and $[M+Na]^+$ highlighted in orange.

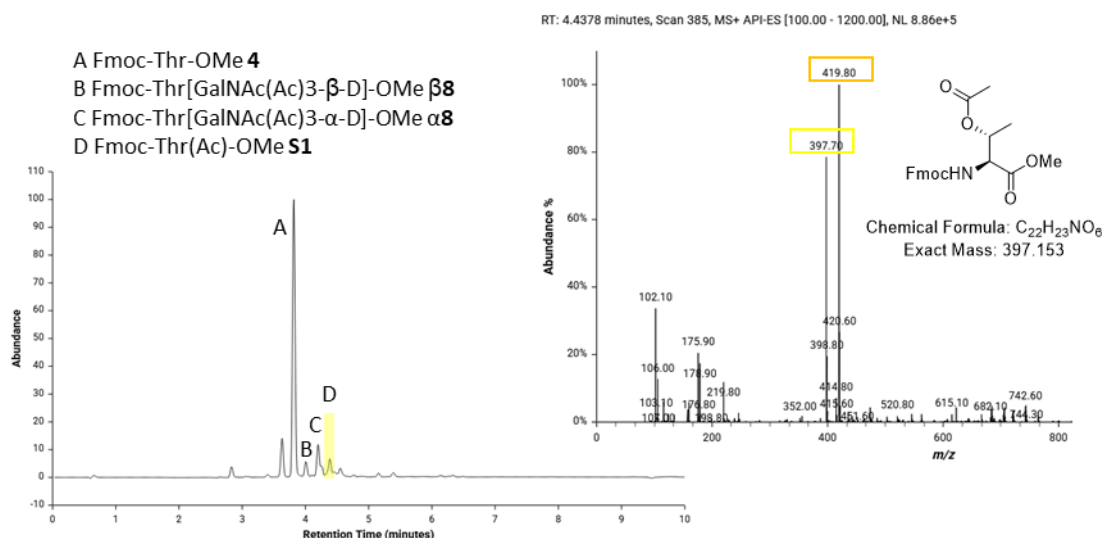


Figure S2. LCMS analysis of the reaction between 1 equiv. GalNAc donor **̢3**, 1 equiv. $Cu(OTf)_2$ and 5 equiv. Fmoc-Thr-OMe **6** (table 1, entry 15) after 16 h (compound numbering as in main text). UV absorbance at 280 nm with main peaks labelled. Inset: Mass spectrometry results for peak D corresponding to Fmoc-Thr(Ac)-OMe; $[M+H]^+$ highlighted in yellow and $[M+Na]^+$ highlighted in orange.

RT: 2.4688 minutes, Scan 213, MS+ MM-ES [100.00 - 1200.00], NL 9.13e+4

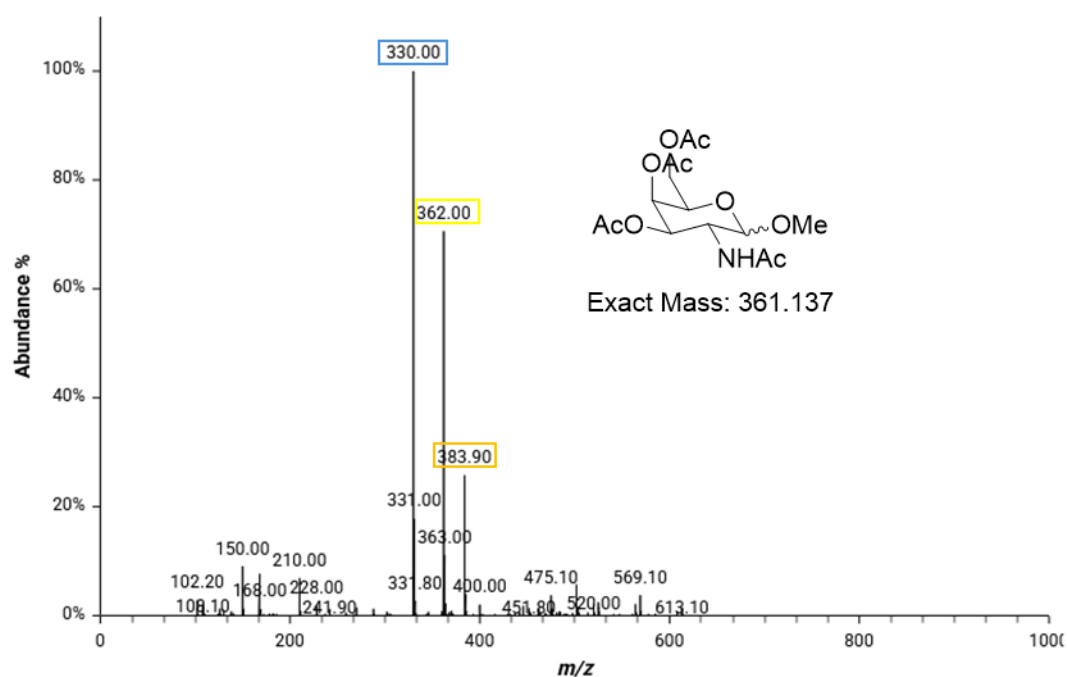
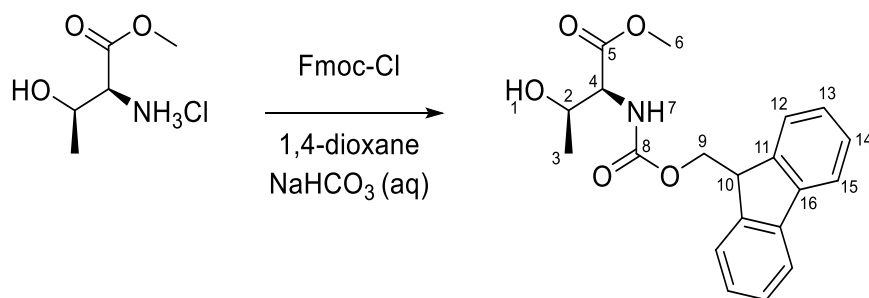


Figure S3. Mass spectrum showing formation of methyl 2-amino-2-deoxy- α/β -D-galactopyranoside from quenching an aliquot from the anomerisation reaction with methanol. 362.00 m/z $[M+H]^+$ highlighted in yellow, 383.90 m/z $[M+Na]^+$ highlighted in orange and the oxocarbenium ion/dioxalenium ion arising from neutral loss of methanol 330 m/z highlighted in blue.

General experimental information

^1H and ^{13}C NMR spectra were recorded directly with Bruker Advance III HD 700 MHz, a Jeol Lambda 500 MHz, Jeol ECS-400 MHz or Bruker Avance 300 MHz. LCMS data was obtained from samples either diluted with MeCN or MeOH using an Agilent Infinity 1290 II UPLC using a Raptor C₁₈ LC column (2.7 μm particle size, 100 \times 3.0 mm) coupled with Agilent MSD-XT. Each LCMS run used a solvent composition of MeCN:water with 0.1% (v/v) formic acid, from 5 to 95% MeCN over 10 mins. HRMS data was obtained from samples diluted in MeCN using a Waters G2-XS_QToF. IR spectra were obtained as neat samples using a Varian 800 FT-IR Scimitar Series spectrometer scanning from 4000-600 cm^{-1} . Chemicals were purchased from Sigma Aldrich, Doug discovery (Fluorochem) or ThermoFisher Scientific and used without further purification.

Fmoc-Thr-OMe 6



To a 100 mL rbf was added Thr-OMe HCl (2 g, 11.79 mmol) and aqueous NaHCO₃ (2.4 M, 20 mL, 47.6 mmol, 4 equiv.) and stirred for 1 min. 1,4-dioxane (6.06 mL) and Fmoc Cl (3.05 g, 11.79 mmol, 1 equiv.) was added and the reaction mixture was stirred vigorously for 1 hour. The reaction mixture was poured over water (10 mL), extracted with EtOAc (2 × 10 mL) and the combined organic extracts washed with brine (1 × 20 mL) and water (1 × 20 mL). The organic later was dried over MgSO₄, filtered and solvent removed under reduced pressure to give Fmoc-Thr-OMe as a white solid (4.17g, 99%). The crude Fmoc Thr-OMe taken forward without further purification due to high purity shown in ¹H NMR.

Data matched literature reports.¹

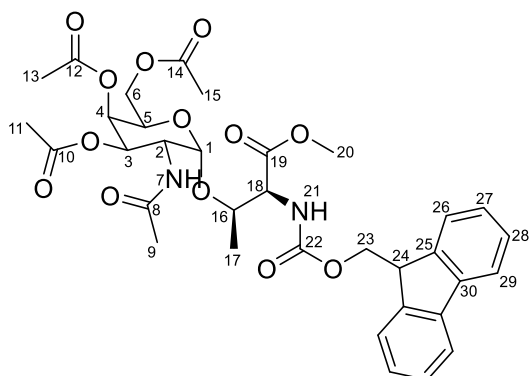
¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, *J* = 7.5 Hz, 2H, H¹⁵), 7.62 (t, *J* = 6.2 Hz, 2H, H¹²), 7.41 (t, *J* = 7.5 Hz, 2H, H¹⁴), 7.32 (t, *J* = 7.5 Hz, 2H, H¹³), 5.57 (s, 1H, H⁷), 4.43 (d, *J* = 7.1 Hz, 2H, H⁹), 4.36 (t, *J* = 7.0 Hz, 2H, H^{2,4}), 4.25 (t, *J* = 7.0 Hz, 1H, H¹⁰), 3.79 (s, 3H, H⁶), 1.89 (s, 1H, H¹), 1.26 (d, *J* = 6.2 Hz, 3H, H³). **¹³C NMR** (101 MHz, CDCl₃) δ 171.8 (C⁵), 156.9 (C⁸), 144.0/143.8 (C^{16/16}), 141.5 (C¹¹), 127.9 (C¹⁴), 127.2 (C¹³), 125.2 (C¹²), 120.2 (C¹⁵), 68.1 (C²), 67.4 (C⁹), 59.1 (C⁴), 52.8 (C⁶), 47.3 (C¹⁰), 20.0 (C³).

Typical procedure for Glycosylation

To a 2-neck 50 mL rbf, under a flow nitrogen gas, was added donor (*N*-acetyl- β -D-galactosamine tetraacetate β or *N*-acetyl- β -D-glucosamine tetraacetate, 100 mg, 0.258 mmol), Cu(OTf)₂ (93 mg, 0.258 mmol, 1 equiv. relative to donor), DCE (5 mL) and acceptor (Fmoc-Thr-OMe, 458 mg or Fmoc-Ser-OMe 440 mg, 1.29 mmol, 5 equiv. relative to donor). The reaction mixture was degassed three times and stirred at reflux (1.6 to 16 hours depending on reaction). The reaction mixture was cooled, diluted with dichloromethane (10 mL) and washed with water (3 \times 15 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed under reduced pressure to give a brown oil. The crude reaction mixture was purified by silica gel column chromatography (3:1 EtOAc:Hexane) to afford glycosylated product.

Variations on this procedure were conducted as outlined in Table 1.

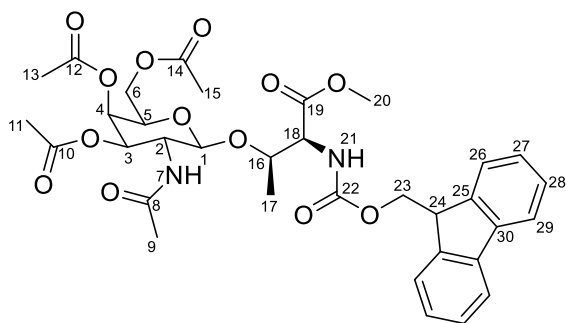
Fmoc-Thr(GalNAc(Ac)₃- α -D]-OMe α 7



Followed typical glycosylation procedure, in which the donor was *N*-acetylgalactosamine tetra acetate and acceptor was Fmoc-Thr-OMe (458 mg, 1.29 mmol, 5 equiv.) and reacted for 16 hours. Fmoc-Thr(GalNAc(Ac)₃- β -D]-OMe (β , 35 mg, 20%) and Fmoc-Thr(GalNAc(Ac)₃- α -D]-OMe (α , 69 mg, 39%) were each isolated as a light brown oil. Characterisation data below corresponds to α 7.

R_f: 0.19 (2:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3329.2 (N-H, w, broad), 3072.3 – 2965.7 (C-H, w), 1753.1 (C=O, s), 1662.1 (C=C, m). **HRMS**: (ESI)⁺ calcd for C₃₄H₄₀N₂O₁₃ [M+H]⁺: 685.2603, found 685.2598. **¹H NMR** (400 MHz, CDCl₃) δ 7.78 (d, J = 7.5 Hz, 2H, H²⁹), 7.64 (dd, J = 7.5, 2.9 Hz, 2H, H²⁶), 7.41 (td, J = 7.5, 2.7 Hz, 2H, H²⁸), 7.34 (ddd, J = 10.2, 5.1, 2.5 Hz, 2H, H²⁷), 5.82 (d, J = 9.7 Hz, 1H, H⁷), 5.63 (d, J = 9.6 Hz, 1H, H²¹), 5.38 (d, J = 3.2 Hz, 1H, H⁴), 5.09 (dd, J = 11.4, 3.2 Hz, 1H, H³), 4.87 (d, J = 3.7 Hz, 1H, H¹), 4.55 (td, J = 10.5, 3.6 Hz, 1H, H²), 4.49 – 4.39 (m, 3H, H^{18,23}), 4.31 – 4.24 (m, 2H, H^{16,24}), 4.21 (t, J = 6.4 Hz, 1H, H⁵), 4.14 – 4.03 (m, 2H, H⁶), 3.74 (s, 3H, H²⁰), 2.16 (s, 3H, H^{11/13/15}), 2.03 (s, 3H, H^{11/13/15}), 2.00 (s, 3H, H^{11/13/15}), 1.99 (s, 3H, H⁹), 1.32 (d, J = 6.4 Hz, 3H, H¹⁷). **¹³C NMR** (101 MHz, CDCl₃) δ 171.5 (C¹⁹), 171.1 (C¹⁰), 170.5 (C^{8/12}), 170.5 (C^{8/12}), 170.4 (C¹⁴), 156.7 (C²²), 143.9/143.8 (C^{30/30}), 141.5 (C²⁵), 127.9 (C²⁸), 127.3 (C²⁷), 125.2/125.2 (C^{26/26}), 120.2/120.2 (C^{29/29}), 100.1 (C¹), 68.5 (C³), 67.5 (C⁴), 67.5 (C⁵), 67.4 (C²³), 62.2 (C⁶), 58.6 (C¹⁸), 52.8 (C²⁰), 47.7 (C²), 47.3 (C²⁴), 23.3 (C⁹), 20.9 (C^{11/13/15}), 20.9 (C^{11/13/15}), 20.8 (C^{11/13/15}), 18.3 (C¹⁷).

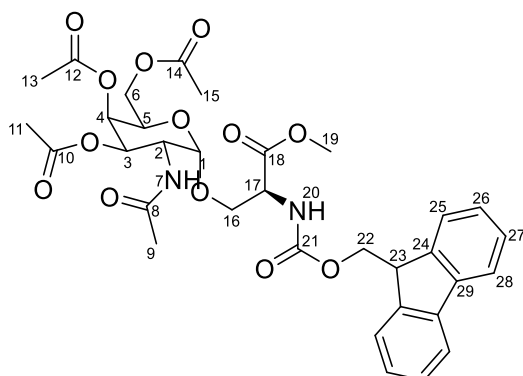
Fmoc-Thr(GalNAc(Ac)₃-β-D]-OMe β7



Followed typical glycosylation procedure, in which the donor was *N*-acetylgalactosamine tetra acetate and acceptor was Fmoc Thr-OMe (458 mg, 1.29 mmol, 5 equiv.) and reacted for 1 hour 40 mins. Fmoc-Thr(GalNAc(Ac)₃-β-D]-OMe was isolated (142 mg, 82%) as a white solid.

R_f: 0.07 (2:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3331.3 (N-H, w, broad), 3092.3 – 2954.4 (C-H, w), 1745.0 (C=O, s), 1665.7 (C=C, m). **HRMS**: (ESI)⁺ calcd for C₃₄H₄₀N₂O₁₃ [M+H]⁺: 685.2603, found 685.2599. **¹H NMR** (300 MHz, CDCl₃) δ 7.76 (d, *J* = 7.4 Hz, 2H, H²⁹), 7.66 (t, *J* = 6.5 Hz, 2H, H²⁶), 7.40 (t, *J* = 6.8 Hz, 2H, H²⁸), 7.32 (td, *J* = 7.4, 1.3 Hz, 2H, H²⁷), 5.76 (d, *J* = 9.0 Hz, 1H, H²¹), 5.57 (d, *J* = 8.4 Hz, 1H, H⁷), 5.34 (d, *J* = 3.0 Hz, 1H, H⁴), 5.28 (dd, *J* = 10.3, 4.2 Hz, 1H, H³), 4.70 (d, *J* = 8.3 Hz, 1H, H¹), 4.43 (td, *J* = 10.8, 7.1 Hz, 3H, H^{16,23}), 4.37 – 4.31 (m, 1H, H¹⁸), 4.26 (t, *J* = 7.2 Hz, 1H, H²⁴), 4.09 (d, *J* = 6.7 Hz, 2H, H⁶), 3.93 – 3.79 (m, 2H, H^{2,5}), 3.75 (s, 3H, H²⁰), 2.13 (s, 3H, H¹³), 2.05 (s, 3H, H¹¹), 2.00 (s, 3H, H¹⁵), 1.95 (s, 3H, H⁹), 1.20 (d, *J* = 6.3 Hz, 3H, H¹⁷). **¹³C NMR** (75 MHz, CDCl₃) δ 170.8 (C¹⁹), 170.6 (C¹⁴), 170.6 (C^{9,10}), 170.4 (C¹²), 156.9 (C²²), 144.1/143.9 (C^{30/30'}), 141.4 (C²⁵), 127.8 (C²⁸), 127.2/127.2 (C²⁷), 125.4/125.3 (C²⁶), 120.1 (C²⁹), 99.5 (C¹), 75.0 (C¹⁶), 70.6 (C⁵), 69.6 (C³), 67.3 (C²³), 66.7 (C⁴), 61.3 (C⁶), 58.7 (C¹⁸), 52.7 (C²⁰), 52.0 (C²), 47.3 (C²⁴), 23.6 (C⁹), 20.8 (C^{11/13/15}), 20.8 (C^{11/13/15}), 17.4 (C¹⁷).

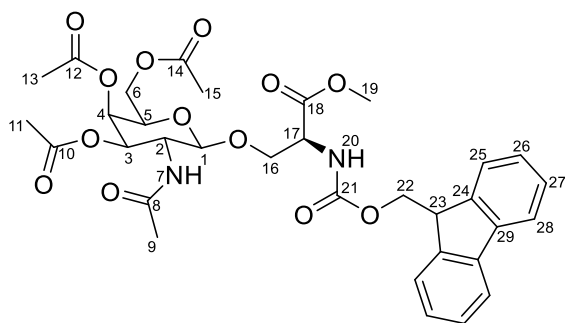
Fmoc-Ser(GalNAc(Ac)₃- α -D]-OMe α 8



Followed typical glycosylation procedure, in which the donor was *N*-acetylgalactosamine tetra acetate and acceptor was Fmoc Ser-OMe (88 mg, 0.258 mmol, 1 equiv.) and reacted for 10 hours. Fmoc-Ser(GalNAc(Ac)₃- α -D]-OMe was isolated (20 mg, 12%) as a light brown oil.

R_f: 0.22 (2:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3329.8 (N-H, w, broad), 3062.1 – 2899.2 (C-H, w), 1755.2 (C=O, s), 1666.6 (C=C, m). **HRMS**: (ESI)⁺ calcd for C₃₃H₃₈N₂O₁₃ [M+H]⁺: 671.2447, found 671.2443. **¹H NMR** (400 MHz, CDCl₃) δ 7.77 (d, J = 7.5 Hz, 2H, H²⁸), 7.62 (d, J = 7.6 Hz, 2H, H²⁵), 7.41 (t, J = 7.4 Hz, 2H, H²⁶), 7.33 (t, J = 7.4 Hz, 2H, H²⁷), 5.82 (d, J = 8.2 Hz, 1H, H²⁰), 5.67 (d, J = 9.7 Hz, 1H, H⁷), 5.37 (d, J = 3.2 Hz, 1H, H⁴), 5.10 (dd, J = 11.8, 3.1 Hz, 1H, H³), 4.84 (d, J = 3.6 Hz, 1H, H¹), 4.62 – 4.51 (m, 2H, H^{2,17}), 4.44 (d, J = 7.1 Hz, 2H, H²²), 4.25 (t, J = 6.9 Hz, 1H, H²³), 4.15 – 4.01 (m, 3H, H^{5,6}), 4.00 – 3.89 (m, 2H, H¹⁶), 3.79 (s, 3H, H¹⁹), 2.16 (s, 3H, H¹³), 2.00 (s, 6H, H^{11,15}), 1.97 (s, 3H, H⁹). **¹³C NMR** (176 MHz, CDCl₃) δ 171.1 (C¹⁰), 170.6 (C^{14,18}), 170.4 (C¹²), 170.3 (C⁸), 156.0 (C²¹), 143.8 (C²⁹), 141.5 (C²⁴), 128.0/128.0 (C^{27/27}), 127.3/127.3 (C^{26/26}), 125.2 (C²⁵), 120.2 (C²⁸), 99.3 (C¹), 69.8 (C²²), 68.4 (C³), 67.5 (C²²), 67.3 (C⁴), 62.0 (C⁶), 54.5 (C¹⁷), 53.0 (C¹⁹), 47.8 (C²), 47.2 (C²³), 23.4 (C⁹), 20.9 (C^{11/13/15}), 20.9 (C^{11/13/15}), 20.8 (C^{11/13/15}).

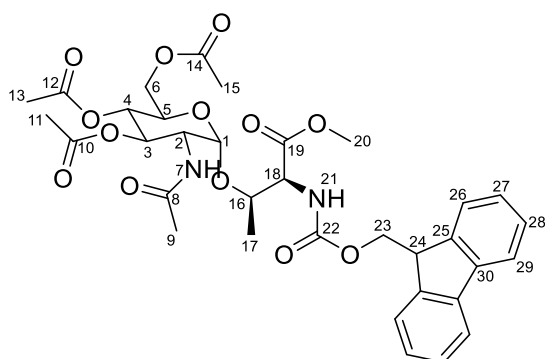
Fmoc-Ser(GalNAc(Ac)₃-β-D]-OMe β8



Followed typical glycosylation procedure, in which the donor was *N*-acetylgalactosamine tetra acetate and acceptor was Fmoc Ser-OMe (440 mg, 1.29 mmol, 5 equiv.) and reacted for 1 hour 40 mins. Fmoc-Ser(GalNAc(Ac)₃-β-D]-OMe was isolated (114 mg, 66%) as a white solid.

R_f: 0.10 (2:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3317.8 (N-H, m, broad), 3018.0 – 2955.0 (C-H, w), 1741.7 (C=O, s), 1662.2 (C=C, s). **HRMS**: (ESI)⁺ calcd for C₃₃H₃₈N₂O₁₃ [M+H]⁺: 671.2447, found 671.2443. **¹H NMR** (300 MHz, CDCl₃) δ 7.77 (d, J = 8.5 Hz, 2H, H²⁸), 7.64 (d, J = 7.4 Hz, 2H, H²⁵), 7.40 (td, J = 7.5, 1.3 Hz, 2H, H²⁷), 7.32 (tt, J = 7.4, 1.5 Hz, 2H, H²⁶), 5.79 (d, J = 8.2 Hz, 1H, H²⁰), 5.55 (d, J = 8.6 Hz, 1H, H⁷), 5.33 (dd, J = 3.4, 1.2 Hz, 1H, H⁴), 5.18 (dd, J = 11.3, 3.4 Hz, 1H, H³), 4.61 (d, J = 8.4 Hz, 1H, H¹), 4.55 – 4.38 (m, 3H, H^{17,22}), 4.26 – 4.17 (m, 2H, H^{16,23}), 4.11 (d, J = 6.5 Hz, 2H, H⁶), 3.96 (dt, J = 11.3, 8.5 Hz, 1H, H²), 3.89 – 3.82 (m, 2H, H^{5,23}), 3.76 (s, 3H, H¹⁹), 2.13 (s, 3H, H¹³), 2.03 (s, 3H, H¹⁵), 2.00 (s, 3H, H¹¹), 1.85 (s, 3H, H⁹). **¹³C NMR** (75 MHz, CDCl₃) δ 170.9 (C⁸), 170.7 (C¹⁰), 170.6 (C¹⁴), 170.3 (C^{12,18}), 156.2 (C²¹), 143.9/143.8 (C^{29/29'}), 141.4/141.4 (C^{24/24'}), 127.9 (C²⁷), 127.3/127.3 (C²⁶), 125.2 (C²⁵), 120.2/120.1 (C^{28/28'}), 101.5 (C¹), 70.9 (C⁵), 70.0 (C³), 69.0 (C¹⁶), 66.9 (C²²), 66.7 (C⁴), 61.5 (C⁶), 54.3 (C¹⁷), 52.9 (C¹⁹), 51.2 (C²), 47.3 (C²³), 23.4 (C⁹), 20.8 (C^{11/13/15}), 20.8 (C^{11/13/15}).

Fmoc-Thr(GlcNAc(Ac)₃- α -D]-OMe α 9

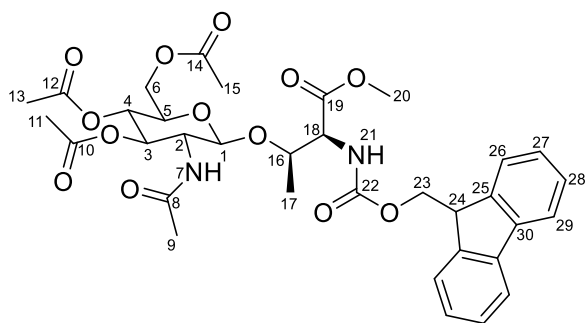


Followed typical glycosylation procedure, in which the donor was *N*-acetylglucosamine tetra acetate and acceptor was Fmoc Thr-OMe (458 mg, 1.29 mmol, 5 equiv.) and reacted for 16 hours. Fmoc-Thr(GlcNAc(Ac)₃- β -D]-OMe (β , 53 mg, 29%) and Fmoc-Thr(GlcNAc(Ac)₃- α -D]-OMe (α , 18 mg, 10%) were each isolated as a light brown oil.

Characterisation data below corresponds to α 10.

R_f: 0.23 (2:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3321.9 (N-H, w, broad), 3066.5 – 2955.3 (C-H, w), 1743.7 (C=O, s), 1663.6 (C=C, m). **HRMS**: (ESI)⁺ calcd for C₃₄H₄₀N₂O₁₃ [M+H]⁺: 685.2603, found 685.2599. **¹H NMR** (400 MHz, CDCl₃) δ 7.78 (d, J = 7.7 Hz, 2H, H²⁹), 7.66 (t, J = 6.1 Hz, 2H, H²⁶), 7.47 – 7.38 (m, 2H, H²⁸), 7.38 – 7.30 (m, 2H, H²⁷), 5.88 (d, J = 9.6 Hz, 1H, H⁷), 5.61 (d, J = 9.5 Hz, 1H, H²¹), 5.17 (t, J = 10.0 Hz, 1H, H³), 5.09 (t, J = 9.7 Hz, 1H, H⁴), 4.84 (d, J = 3.8 Hz, 1H, H¹), 4.50 – 4.41 (m, 3H, H^{18,23}), 4.37 – 4.25 (m, 3H, H^{2,16,24}), 4.21 (dd, J = 12.3, 5.1 Hz, 1H, H⁶), 4.15 – 4.06 (m, 1H, H⁶)K, 4.05 – 3.95 (m, 1H, H⁵), 3.75 (s, 3H, H²⁰), 2.08 (s, 3H, H^{11/13/15}), 2.05 (s, 3H, H^{11/13/15}), 2.03 (s, 3H, H^{11/13/15}), 2.00 (s, 3H, H⁹), 1.32 (d, J = 6.4 Hz, 3H, H¹⁷). **¹³C NMR** (101 MHz, CDCl₃) δ 171.5 (C¹⁹), 171.4 (C^{8/10/14}), 170.7 (C^{8/10/14}), 170.5 (C^{8/10/14}), 169.4 (C¹²), 156.7 (C²²), 143.8 (C³⁰), 141.5 (C²⁵), 128.0 (C²⁸), 127.3 (C²⁷), 125.3 (C²⁶), 120.2 (C²⁹), 99.6 (C¹), 71.3 (C³), 68.5 (C⁵), 68.4 (C⁴), 67.6 (C²³), 62.2 (C⁶), 58.6 (C¹⁸), 52.8 (C²⁰), 51.8 (C²), 47.3 (C²⁴), 23.2 (C⁹), 20.9 (C^{11/13/15}), 20.8 (C^{11/13/15}), 20.8 (C^{11/13/15}), 18.4 (C¹⁷).

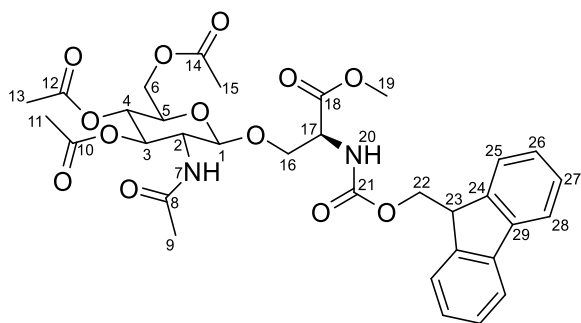
Fmoc-Thr(GlcNAc(Ac)₃-β-D]-OMe β9



Followed typical glycosylation procedure, in which the donor was *N*-acetylglucosamine tetra acetate and acceptor was Fmoc Thr-OMe (458 mg, 1.29 mmol, 5 equiv.) and reacted for 1 hour 40 mins. Fmoc-Thr(GlcNAc(Ac)₃-β-D]-OMe was isolated (100 mg, 57%) as a white solid.

R_f: 0.10 (2:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3326.0 (N-H, w, broad), 2971.1 (C-H, w), 1743.7 – 1727.6 (C=O, s), 1659.8 (C=C, m). **HRMS**: (ESI)⁺ calcd for C₃₄H₄₀N₂O₁₃ [M+H]⁺: 685.2603, found 685.2599. **¹H NMR** (300 MHz, CDCl₃) δ 7.76 (d, J = 7.4 Hz, 2H, H²⁹), 7.69 – 7.56 (m, 2H, H²⁶), 7.39 (t, J = 7.4 Hz, 2H, H²⁸), 7.31 (tt, J = 7.7, 1.9 Hz, 2H, H²⁷), 5.78 (d, J = 9.0 Hz, 1H, H²¹), 5.74 (d, J = 8.4 Hz, 1H, H⁷), 5.36 – 5.27 (m, 1H, H³) 5.03 (t, J = 9.6 Hz, 1H, H⁴), 4.72 (d, J = 8.3 Hz, 1H, H¹), 4.52 – 4.32 (m, 4H, H^{16,18,23}), 4.30 – 4.17 (m, 2H, H^{6,24}), 4.15 – 4.04 (m, 1H, H⁶), 3.73 (s, 3H, H²⁰), 3.71 – 3.61 (m, 2H, H^{2,5}), 2.06 (s, 3H, H¹⁵), 2.03 (s, 3H, H¹¹), 2.01 (s, 3H, H¹³), 1.93 (s, 3H, H⁹), 1.19 (d, J = 6.2 Hz, 3H, H¹⁷). **¹³C NMR** (75 MHz, CDCl₃) δ 171.0 (C¹⁰), 170.8 (C^{14/19}), 170.8 (C^{14/19}), 170.5 (C⁸), 169.5 (C¹²), 156.9 (C²²), 144.0/143.9 (C^{30/30'}), 141.4/141.4 (C^{25/25'}), 127.9/127.8 (C^{28/28'}), 127.2/127.2 (C^{27/27'}), 125.3/125.2 (C^{26/26'}), 120.1/120.1 (C^{29/29'}), 98.9 (C¹), 74.8 (C¹⁶), 72.0 (C³), 71.7 (C⁵), 68.7 (C⁴), 67.3 (C²³), 62.1 (C⁶), 58.7 (C¹⁸), 55.2 (C²⁰), 52.6 (C²), 47.3/47.2 (C^{24/24'}), 23.4 (C⁹), 20.8 (C^{11/13/15}), 20.7 (C^{11/13/15}), 17.1 (C¹⁷).

Fmoc-Ser(GlcNAc(Ac)₃-β-D]-OMe β10

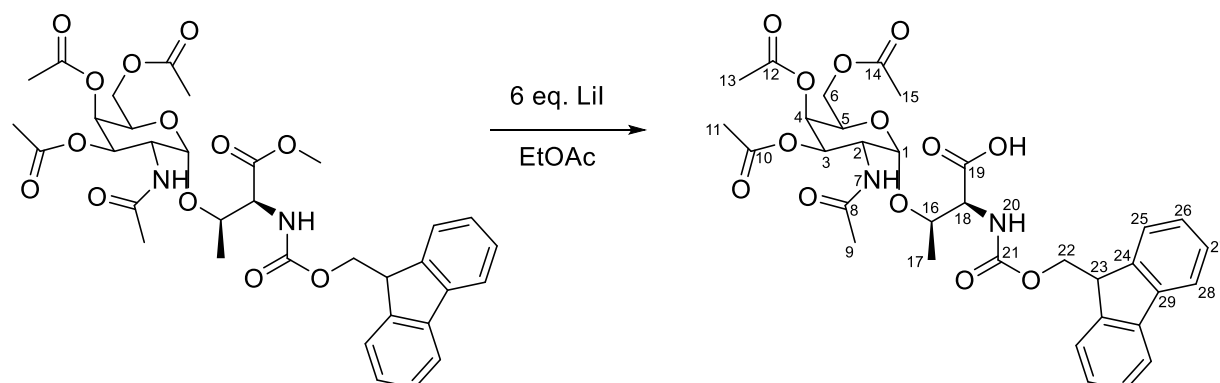


Followed typical glycosylation procedure, in which the donor was *N*-acetylglucosamine tetra acetate and acceptor was Fmoc Ser-OMe (440 mg, 1.29 mmol, 5 equiv.) and reacted for 1 hour 40 mins. Fmoc-Ser(GlcNAc(Ac)₃-β-D]-OMe was isolated (117 mg, 68%) as a white solid.

R_f: 0.18 (3:1 EtOAc:Hexane). **IR** (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3316.5 (N-H, m, broad), 2980.6 – 2890.5 (C-H, w), 1741.8 (C=O, s), 1665.1 (C=C, m). **HRMS**: (ESI)⁺ calcd for C₃₃H₃₈N₂O₁₃ [M+H]⁺: 671.2447, found 671.2443. **¹H NMR** (300 MHz, CDCl₃) δ 7.81 – 7.70 (m, 2H, H²⁸), 7.63 (d, J = 7.5 Hz, 2H, H²⁵), 7.39 (t, J = 7.4 Hz, 2H, H²⁷), 7.31 (td, J = 7.4, 1.3 Hz, 2H, H²⁶), 5.84 (d, J = 8.5 Hz, 1H, H²⁰), 5.78 (d, J = 8.6 Hz, 1H, H⁷), 5.23 (dd, J = 10.6, 9.3 Hz, 1H, H³), 5.03 (t, J = 9.6 Hz, 1H, H⁴), 4.63 (d, J = 8.2 Hz, 1H, H¹), 4.53 – 4.35 (m, 3H, H^{17,22}), 4.29 – 4.06 (m, 4H, H^{6,16,23}), 3.89 – 3.79 (m, 1H, H^{2,16}), 3.74 (s, 3H, H¹⁹), 3.66 (ddd, J = 10.0, 4.8, 2.5 Hz, 1H, H⁵), 2.06 (s, 3H, H¹⁵), 2.02 (s, 3H, H¹¹), 2.01 (s, 3H, H¹³), 1.84 (s, 3H, H⁹). **¹³C NMR** (75 MHz, CDCl₃) δ 171.0 (C¹⁰), 170.8 (C^{8/14}), 170.8 (C^{8/14}), 170.3 (C¹⁸), 169.5 (C¹²), 156.2 (C²¹), 143.8 (d, J = 9.6 Hz, C²⁹), 141.4 (d, J = 4.7 Hz, H²⁴), 127.9 (d, J = 3.1 Hz, H²⁷), 127.2 (d, J = 2.0 Hz, H²⁶), 125.2 (d, J = 2.1 Hz, H²⁵), 120.1 (d, J = 1.9 Hz, H²⁸), 100.9 (C¹), 72.3 (C³), 72.0 (C⁵), 70.0 (C¹⁶), 68.6 (C⁴), 67.0 (C²²), 62.1 (C⁶), 54.4 (C¹⁷), 54.3 (C²), 52.8 (C¹⁹), 47.3 (C²³), 23.2 (C⁹), 20.8 (C^{11/13/15}), 20.8 (C^{11/13/15}), 20.7 (C^{11/13/15}).

Typical procedure for demethylation with Lil.

Fmoc-Thr(GalNAc(Ac)₃- α -D]-OH α 1

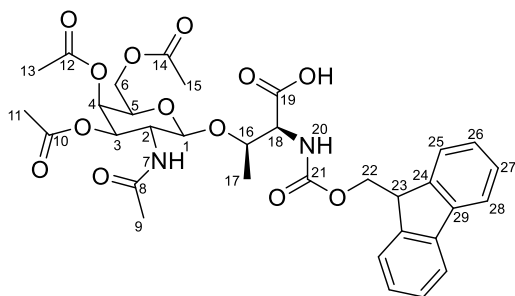


To a 2-neck 25 mL rbf, under nitrogen, was added Fmoc-Thr(GalNAc(Ac)₃- α -D]-OMe (281 mg, 0.410 mmol) and Lil (330 mg, 2.462 mmol). Dry EtOAc (4.1 mL, 10 mL/mmol) was added, degassed (x3) and stirred under reflux for 24 hours. The reaction mixture was diluted with EtOAc (5 mL), washed with 10% HCl (15mL) and sat. Na₂O₃S₃ (15 mL). The desired product was extracted from the organic layer with sat. NaHCO₃ (3 x15 mL) and then the aqueous layer was acidified with 10% HCl. The desired product was extracted from the aqueous layer with EtOAc (3 x15 mL), dried over MgSO₄, filtered and solvent removed under reduced pressure to give Fmoc-Thr(GalNAc(Ac)₃- α -D]-OH (120 mg, 44%) as a light brown oil.

Data matched literature reports.²

HRMS: (ESI)+ calcd for C₃₂H₃₆N₂O₁₃ [M+H]⁺: 657.2290, found 657.2285. **¹H NMR** (700 MHz, MeOD) δ 7.83 (d, J = 7.6 Hz, 2H, H²⁸), 7.70 (ddd, J = 12.5, 7.5, 1.0 Hz, 2H, H²⁵), 7.41 (dddd, J = 9.3, 4.5, 2.6, 0.9 Hz, 2H, H²⁷), 7.33 (qd, J = 7.5, 1.2 Hz, 2H, H²⁶), 5.41 (dd, J = 3.3, 1.2 Hz, 1H, H⁴), 5.08 (dd, J = 11.5, 3.3 Hz, 1H, H³), 4.95 (d, J = 3.9 Hz, 1H, H¹), 4.61 (dd, J = 10.8, 6.4 Hz, 1H, H²²), 4.48 (dd, J = 10.8, 6.2 Hz, 1H, H²²), 4.42 – 4.37 (m, 2H, H^{2,16}), 4.32 – 4.24 (m, 3H, H^{5,18,23}), 4.19 – 4.08 (m, 2H, H⁶), 2.15 (s, 3H, H^{11/13/15}), 2.05 (s, 3H, H^{11/13/15}), 1.96 (s, 3H, H^{11/13/15}), 1.95 (s, 3H, H⁹), 1.25 (d, J = 6.5 Hz, 3H, H¹⁷). **¹³C NMR** (176 MHz, MeOD) δ 173.6 (C⁸), 173.3 (C¹⁹), 172.1 (C^{10/12/14}), 172.1 (C^{10/12/14}), 172.0 (C^{10/12/14}), 159.2 (C²¹), 145.4/145.1 (C^{29/29}), 142.7/142.7 (C^{24/24}), 128.8 (C²⁷) 128.2 (C²⁶), 126.2/126.0 (C^{25/25}), 121.0/121.0 (C^{28/28}), 100.8 (C¹), 77.6 (C¹⁶), 69.7 (C³), 68.8 (C⁴), 68.2 (C⁵), 67.7 (C²²), 63.3 (C⁶), 59.8 (C¹⁸), 48.9 (C²³), 48.6 (C²), 22.9 (C⁹), 20.6 (C^{11/13/15}), 20.6 (C^{11/13/15}), 20.5 (C^{11/13/15}), 19.2 (C¹⁷).

Fmoc-Thr(GalNAc(Ac)₃-β-D]-OH β1

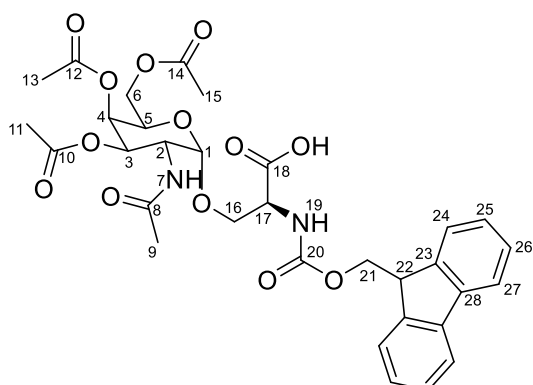


Followed demethylation procedure starting from Fmoc-Thr(GalNAc(Ac)₃-β-D]-OMe (200 mg, 0.292 mmol) and refluxed for 12 hours. Fmoc-Thr(GalNAc(Ac)₃-β-D]-OH was isolated (165 mg, 84%) as a colourless oil.

Data matched literature reports.²

HRMS: (ESI)+ calcd for C₃₃H₃₈N₂O₁₃ [M+H]⁺: 671.2447, found 671.2441. **¹H NMR** (700 MHz, MeOD) δ 7.82 (d, *J* = 7.6 Hz, 2H, H²⁸), 7.72 (dd, *J* = 12.5, 7.5 Hz, 2H, H²⁵), 7.41 (tdd, *J* = 7.5, 2.2, 1.3 Hz, 2H, H²⁷), 7.34 (tdd, *J* = 7.5, 2.0, 1.2 Hz, 2H, H²⁶), 5.35 (dd, *J* = 3.4, 1.2 Hz, 1H, H⁴), 5.09 (dd, *J* = 11.3, 3.4 Hz, 1H, H³), 4.61 (d, *J* = 8.5 Hz, 1H, H¹), 4.45 (qd, *J* = 6.4, 2.7 Hz, 1H, H¹⁶), 4.43 – 4.35 (m, 2H, H²²), 4.28 (t, *J* = 7.2 Hz, 1H, H²³), 4.25 (d, *J* = 2.7 Hz, 1H, H¹⁸), 4.18 (dd, *J* = 11.1, 7.6 Hz, 1H, H⁶), 4.12 (dd, *J* = 11.7, 5.6 Hz, 1H, H⁶), 4.06 (dd, *J* = 11.3, 8.4 Hz, 1H, H²), 4.01 (ddd, *J* = 7.5, 6.1, 1.2 Hz, 1H, H⁵), 2.12 (s, 3H, H^{13/15}), 2.04 (s, 3H, H^{13/15}), 1.97 (s, 3H, H¹¹), 1.96 (s, 3H, H⁹), 1.22 (d, *J* = 6.4 Hz, 3H, H¹⁷). **¹³C NMR** (176 MHz, MeOD) δ 174.0 (C⁸), 173.4 (C¹⁹), 172.2 (C^{12/14}), 172.1 (C^{12/14}), 171.7 (C¹⁰), 159.1 (C²¹), 145.3/145.1 (C^{29/29}), 142.6/142.6 (C^{24/24}), 128.8 (C²⁷), 128.2/128.2 (C^{26/26}), 126.4/126.3 (C^{25/25}), 120.9/120.9 (C^{28/28}), 101.7 (C¹), 76.8 (C¹⁶), 71.9 (C³), 71.6 (C⁵), 68.3 (C²²), 67.8 (C⁴), 62.2 (C⁶), 60.0 (C¹⁸), 51.6 (C²), 48.4 (C²³), 22.9 (C⁹), 20.6 (C^{11/13/15}), 20.5 (C^{11/13/15}), 18.0 (C¹⁷).

Fmoc-Ser(GalNAc(Ac)₃-α-D]-OH α2

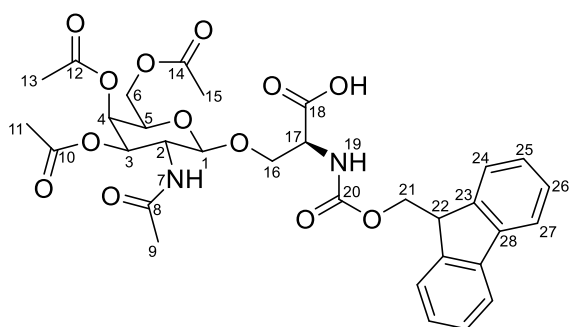


Followed demethylation procedure starting from Fmoc-Ser(GalNAc(Ac)₃-α-D]-OMe (73 mg, 0.109 mmol). Fmoc-Ser(GalNAc(Ac)₃-α-D]-OH was isolated (44 mg, 62%) as a light brown oil.

*Data matched literature reports.*³

HRMS: (ESI)+ calcd for C₃₂H₃₆N₂O₁₃ [M+H]⁺: 657.2290, found 657.2285. **¹H NMR** (700 MHz, MeOD) δ 7.81 (d, *J* = 7.5 Hz, 2H, H²⁷), 7.69 (dd, *J* = 11.2, 7.2 Hz, 2H, H²⁴), 7.40 (t, *J* = 7.5 Hz, 2H, H²⁶), 7.36 – 7.31 (m, 2H, H²⁵), 5.40 (dd, *J* = 3.3, 1.3 Hz, 1H, H⁴), 5.15 (dd, *J* = 11.5, 3.3 Hz, 1H, H³), 4.90 (d, *J* = 3.8 Hz, 1H, H¹), 4.52 – 4.38 (m, 4H, H^{2,17,21}), 4.25 (q, *J* = 6.5 Hz, 2H, H^{5,22}), 4.09 (dd, *J* = 16.3, 6.6 Hz, 1H, H⁶), 4.03 (dd, *J* = 11.2, 7.1 Hz, 1H, H⁶), 3.95 (t, *J* = 4.1 Hz, 2H, H¹⁶), 2.14 (s, 3H, H^{11/13/15}), 1.96 (s, 3H, H^{11/13/15}), 1.95 (s, 3H, H^{11/13/15}), 1.93 (s, 3H, H⁹). **¹³C NMR** (176 MHz, MeOD) δ 173.6 (C⁸), 173.2 (C¹⁸), 172.1 (C^{10/12/14}), 172.1 (C^{10/12/14}), 171.9 (C^{10/12/14}), 158.5 (C²⁰), 145.3/145.2 (C^{28/28'}), 142.7 (C²³), 128.8 (C²⁶), 128.2 (C²⁵), 126.2/126.1 (C^{24/24'}), 121.0 (C²⁷), 99.9 (C¹), 69.8 (C¹⁶), 69.6 (C³), 68.6 (C⁴), 68.2 (C⁵), 68.0 (C²¹), 63.0 (C⁶), 55.8 (C¹⁷), 48.9 (C²), 48.4 (C²²), 22.6 (C⁹), 20.7 (C^{11/13/15}), 20.5 (C^{11/13/15}), 20.5 (C^{11/13/15}).

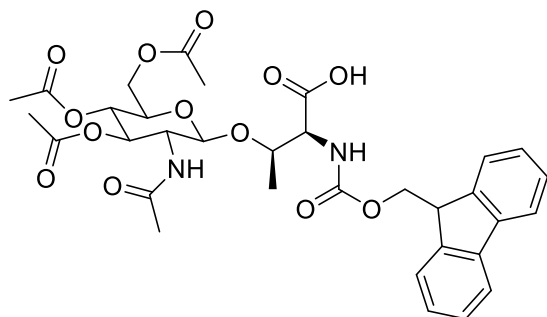
Fmoc-Ser(GalNAc(Ac)₃-β-D]-OH **β2**



Followed demethylation procedure starting from Fmoc-Ser(GalNAc(Ac)₃-β-D]-OMe (200 mg, 0.298 mmol) and refluxed for 4 hours. Fmoc-Ser(GalNAc(Ac)₃-β-D]-OH was isolated (167 mg, 85%) as a colourless oil.

IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3334.7 (N-H & O-H, m, broad), 2968.3 (C-H, w), 1741.4 (C=O, s), 1662.0 (C=C, s). **HRMS**: (ESI)⁺ calcd for C₃₂H₃₆N₂O₁₃ [M+H]⁺: 657.2290, found 657.2285. **¹H NMR** (700 MHz, MeOD) δ 7.80 (d, J = 7.5 Hz, 2H, H²⁷), 7.69 (t, J = 8.2 Hz, 2H, H²⁴), 7.40 (td, J = 7.4, 2.6 Hz, 2H, H²⁶), 7.33 (qd, J = 7.9, 1.1 Hz, 2H, H²⁵), 5.33 (d, J = 2.4 Hz, 1H, H⁴), 5.07 (dd, J = 11.3, 3.3 Hz, 1H, H³), 4.63 (d, J = 8.5 Hz, 1H, H¹), 4.44 (dd, J = 10.6, 6.8 Hz, 1H, H²¹), 4.39 (t, J = 4.7 Hz, 1H, H⁵), 4.33 (dd, J = 10.6, 7.0 Hz, 1H, H²¹), 4.25 (t, J = 6.8 Hz, 1H, H²²), 4.18 – 4.09 (m, 3H, H^{6,16}), 4.06 (dd, J = 11.2, 8.5 Hz, 1H, H²), 3.99 (t, J = 7.2 Hz, 1H, H¹⁷), 3.93 (dd, J = 10.5, 4.1 Hz, 1H, H⁶), 2.12 (s, 3H, H¹⁵), 2.01 (s, 3H, H¹³), 1.95 (s, 3H, H¹¹), 1.88 (s, 3H, H⁹). **¹³C NMR** (176 MHz, MeOD) δ 174.0 (C⁸), 173.0 (C¹⁸), 172.2 (C¹²), 172.2 (C¹⁴), 171.8 (C¹⁰), 158.4 (C²⁰), 145.3/145.2 (C^{28/28'}), 142.6/142.6 (C^{23/23'}), 128.8 (C²⁶), 128.2 (C²⁵), 126.3/126.2 (C^{24/24'}), 121.0 (C²⁷), 102.5 (C¹), 71.9 (C¹⁷), 71.9 (C³), 69.9 (C⁶), 68.1 (C⁴), 68.1 (C²¹), 62.6 (C¹⁶), 55.5 (C⁵), 51.5 (C²), 48.3 (C²²), 22.9 (C⁹), 20.6 (C^{11/13/15}), 20.5 (C^{11/13/15}), 20.5 (C^{11/13/15}).

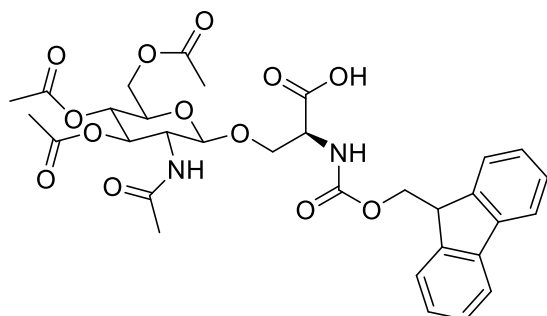
Fmoc-Thr(GlcNAc(Ac)₃-β-D]-OH β12



Followed demethylation procedure starting from Fmoc-Thr(GlcNAc(Ac)₃-β-D]-OMe (27 mg, 0.039 mmol). Fmoc-Thr(GlcNAc(Ac)₃-β-D]-OH was isolated (15 mg, 58%) as a colourless oil.

Data matched literature reports.²

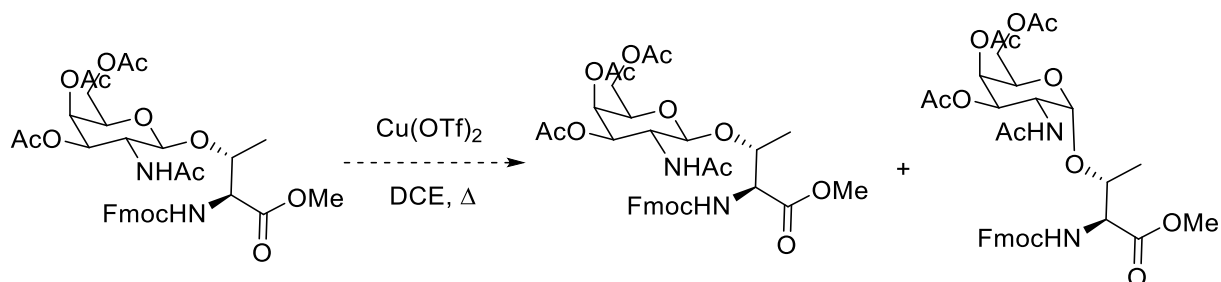
Fmoc-Ser(GlcNAc(Ac)₃-β-D]-OH β13



Followed demethylation procedure starting from Fmoc-Ser(GlcNAc(Ac)₃-β-D]-OMe (26 mg, 0.039 mmol). Fmoc-Ser(GlcNAc(Ac)₃-β-D]-OH was isolated (17 mg, 66%) as a colourless oil.

Data matched literature reports.⁴

Anomerisation time course experiment



To a 2-neck 25mL rbf, under nitrogen, was added Fmoc-Thr(GalNAc(Ac)₃-β-D]-OMe **β7** and Fmoc-Thr(GalNAc(Ac)₃-β-D]-OH **α7** (80 mg, 0.117 mmol, approx. 7:1 **β7**/**α7** ratio), Cu(OTf)₂ (40 mg, 0.117 mmol) and DCE (2.27 mL). The reaction mixture was degassed (x3) and heated to reflux. The reaction was initially monitored by LCMS, taking a timepoint every 30 mins, starting from t = 0 up to t = 330, after which the reaction was left overnight with a final timepoint at t = 1380 mins. Each LCMS sample was made up of 2.8 μL of crude reaction mixture and 1 mL of MeCN. The UV absorbance at 280 nm was used to quantify the species present based on peak area:

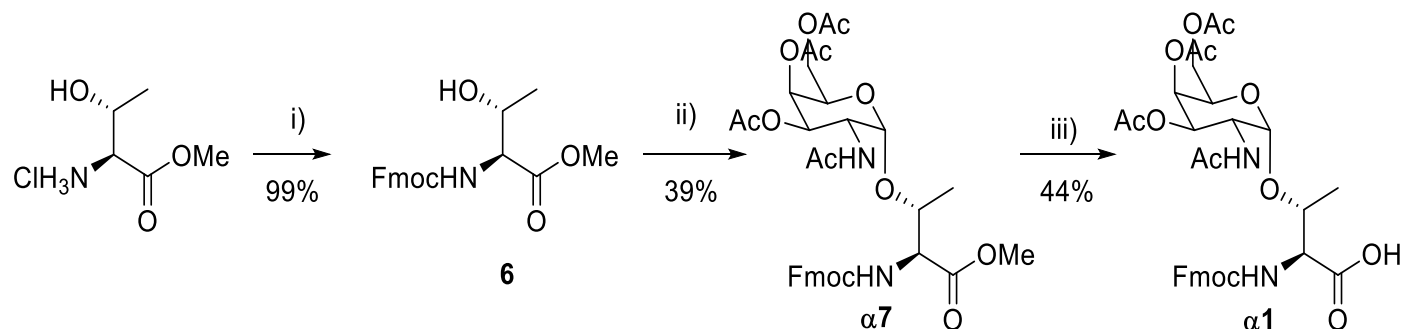
	Peak retention time (min)	Region for area calculation (min)
Fmoc-Thr-OMe 6	3.86	3.78-3.94
Fmoc-Thr(GalNAc(Ac) ₃ -β-D]-OMe β7	4.04	3.97-4.11
Fmoc-Thr(GalNAc(Ac) ₃ -α-D]-OMe α7	4.22	4.15-4.29

The areas for **6**, **β7** and **α7** were divided by the sum of the areas at each timepoint, to normalise between samples, and multiplied by 100 to calculate the percentage of each species in the reaction at the given time. These values are shown below (Table SX) and was plotted as a function of time to produce Figure 2 of the main text.

Time (min)	Percentage area		
	6	β7	α7
0	3.95	82.23	13.82
30	13.06	76.28	10.66
60	24.92	61.99	13.10
90	34.04	55.10	10.86
120	39.38	48.25	12.37

150	43.95	43.24	12.81
180	51.67	36.98	11.35
210	55.44	32.91	11.65
240	57.17	30.81	12.02
270	55.97	30.19	13.84
300	58.66	27.61	13.73
330	58.85	25.64	15.51
1380	48.20	11.17	40.63

Calculation of Synthesis cost



- i) $\text{Cl.H}_3\text{N-Thr-OMe}$ (1 equiv.), Fmoc-Cl (1 equiv.); 1,4-dioxane/20% $\text{NaHCO}_3(\text{aq})$ (1:2) 0.66 M
 ii) Fmoc-Thr-OMe (5 equiv.), GalNAc(Ac₃)-β-OAc (1 equiv.), $\text{Cu}(\text{OTf})_2$ (1 equiv.); 1,4-dichloroethane 51 mM
 iii) Fmoc-Thr(GalNAc(Ac)₃-α-D]-OMe (1 equiv.), Lil (6 equiv.); EtOAc 10 mM

To calculate the total cost of synthesis Fmoc-Thr(GalNAc(Ac)₃-α-D]-OH α1 the yields across steps i)-iii) were used to calculate the amount of each commercially available compound required to produce the target quantity of 100 mg.

As an example in the demethylation reaction, step iii); 100 mg of Fmoc-Thr(GalNAc(Ac)₃-α-D]-OH α1 (0.149 mmol) can be expected from a reaction with input of 243.1 mg Fmoc-Thr(GalNAc(Ac)₃-α-D]-OMe α7 (0.355 mmol) as the expected yield is 44%. A reaction on this scale would require:

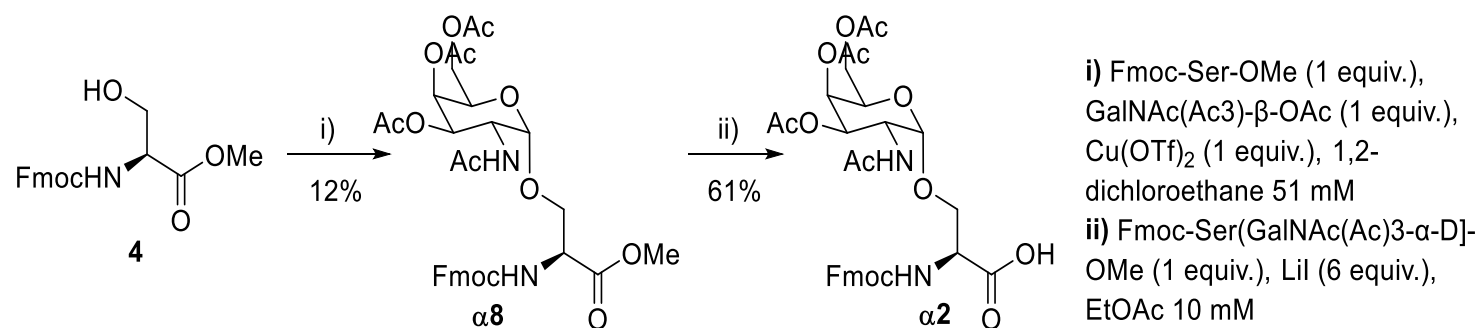
- Fmoc-Thr(GalNAc(Ac)₃-α-D]-OMe α7: 243.1 mg (0.355 mmol, 1 equiv.) produced from step ii). A similar calculation was used to determine the input reactants required to produce this quantity from step ii) and likewise for step i) to determine the quantity required of all commercial reagents.
- Lil: 285.3 mg (2.131 mmol, 6 equiv.) of Lil. This is purchased and would cost £0.229.
- EtOAc: 35.5 mL (10 mM reactant concentration based on α7). This is purchased and would cost £1.302.

The sum of the cost of the required reagents was calculated to give the total cost.

Step	Reagents	rMM	mass (mg)	mmol	Equiv.	cost (£)	yield(%)
	Fmoc-Thr(GalNAc(Ac) ₃ -α-D]-OH	670.24	100.0	0.149	N/A	Made in iii)	44
iii)	Fmoc-Thr(GalNAc(Ac) ₃ -α-D]-OMe	684.25	243.1	0.339	1	Made in ii)	39
	LiI	133.84	285.3	2.035	6	0.229	
ii)	GalNAc(Ac) ₃ -β-OAc	389.36	354.7	0.869	1	0.704	
	Cu(OTf) ₂	361.68	329.4	0.869	1	0.252	
	Fmoc-Thr-OMe	355.14	1617.4	4.347	5	Made in i)	99
i)	Cl.H3N-Thr-OMe	169.60	780.2	4.391	1	1.013	
	Fmoc-Cl	258.70	1190.1	4.391	1	0.727	
	Solvents		Volume (mL)	mmol	concentration (mM)	cost (£)	
iii)	EtOAc		33.9	0.339	10	1.302	
ii)	1,2-Dichloroethane		17.0	0.869	51	1.454	
i)	1,4-dioxane		2.2	4.391	2000*	0.425	
					Total cost (£):	6.105	

Table S2: Cost calculation for Fmoc-Thr(GalNAc(Ac)₃-α-D]-OH **α1**. *reaction uses mixed solvent system, this would be the concentration with 1,4-dioxane only

Cheapest commercial alternative: BLD Pharm BD131423, 250 mg for £1049 (Table S1). 100 mg cost = £419.60. Our cost is 1.46% of this.



Step	Reagents	rMM	mass (mg)	mmol	Equiv.	cost (£)	yield(%)
	Fmoc-Ser(GalNAc(Ac) ₃ -α-D]-OH	656.22	100.0	0.149	N/A	Made in ii)	61
ii)	Fmoc-Ser(GalNAc(Ac) ₃ -α-D]-OMe	670.24	167.4	0.250	1	Made in i)	12
	Lil	133.84	200.6	1.499	6	0.169	
i)	GalNAc(Ac ₃)-β-OAc	389.36	810.6	2.082	1	1.686	
	Cu(OTf) ₂	361.68	329.4	2.082	1	0.602	
	Fmoc-Ser-OMe	355.14	1617.4	2.082	1	0.739	
	Solvents		Volume (mL)	mmol	concentration (mM)	cost (£)	
ii)	EtOAc		24.9	0.250	10	0.959	
i)	1,2-Dichloroethane		40.8	2.082	51	3.482	
	Total cost (£):					7.637	

Table S3: Cost calculation for Fmoc-Ser(GalNAc(Ac)₃-α-D]-OH α2

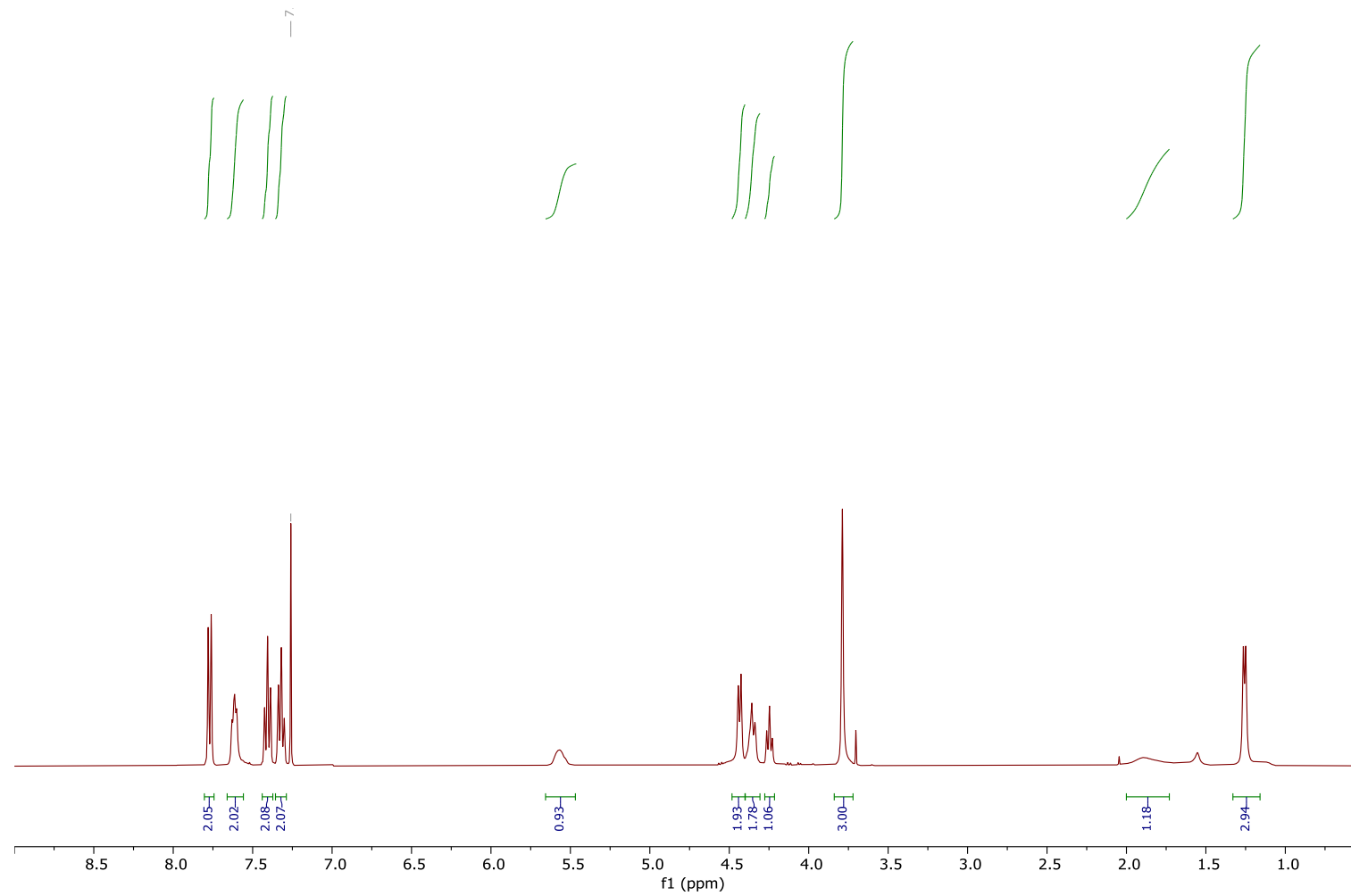
Cheapest commercial alternative: Key Organics BS-49043, 250 mg for £552 (Table S1). 100 mg cost = £220.80. Our cost is 3.46% of this.

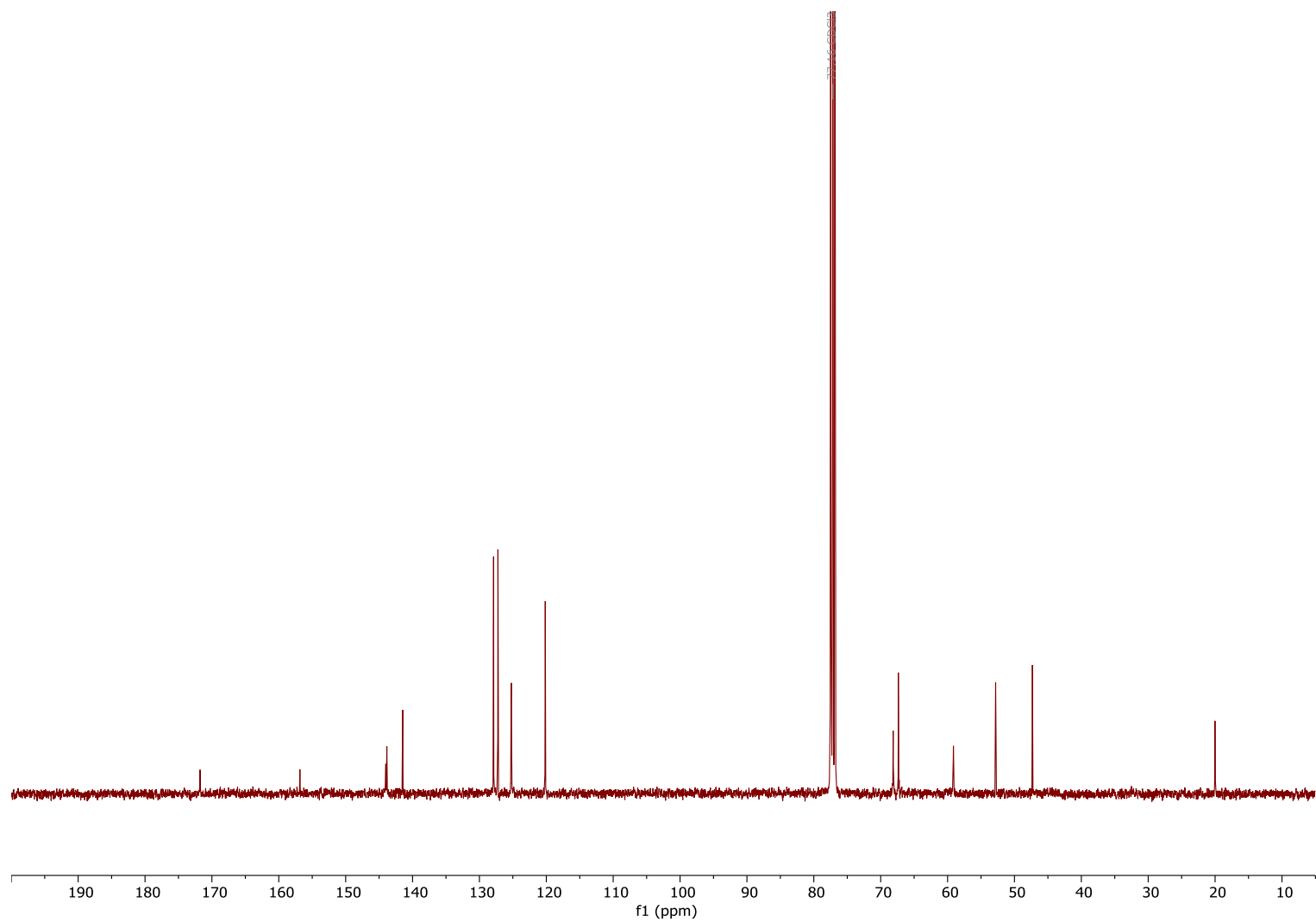
Reagents	CAS	Supplier	Amount (g)	Price/£	Link	Date
Fmoc-Ser-OMe	82911-78-2	Doug Discovery	25	25	F234541	07/03/2025
Cl.H3N-Thr-OMe	39994-75-7	Doug Discovery	25	34	M02985	07/03/2025
Fmoc-Cl	28920-43-6	Doug Discovery	25	16	F022072	07/03/2025
GalNAc(Ac3)- β -OAc	3006-60-8	Doug Discovery	25	52	F238299	27/03/2025
GlcNAc(Ac3)- β -OAc	7772-79-4	Doug Discovery	25	26	F239393	27/03/2025
Cu(OTf) ₂	34946-82-2	Doug Discovery	25	20	F012761	07/03/2025
Lil	10377-51-2	Doug Discovery	25	21	F493928	07/03/2025
Solvents	CAS	Supplier	Amount (L)	Price/£	Link	Date
1,2-Dichloroethane	107-06-2	Sigma Aldrich	1	85.3	284505	07/03/2025
EtOAc	141-78-6	Sigma Aldrich	2.5	96	33211m	07/03/2025
1,4-dioxane	123-91-1	Doug Discovery	0.1034	20	F044719	07/03/2025

Table S4: Cost of reagents used.

NMR Spectra

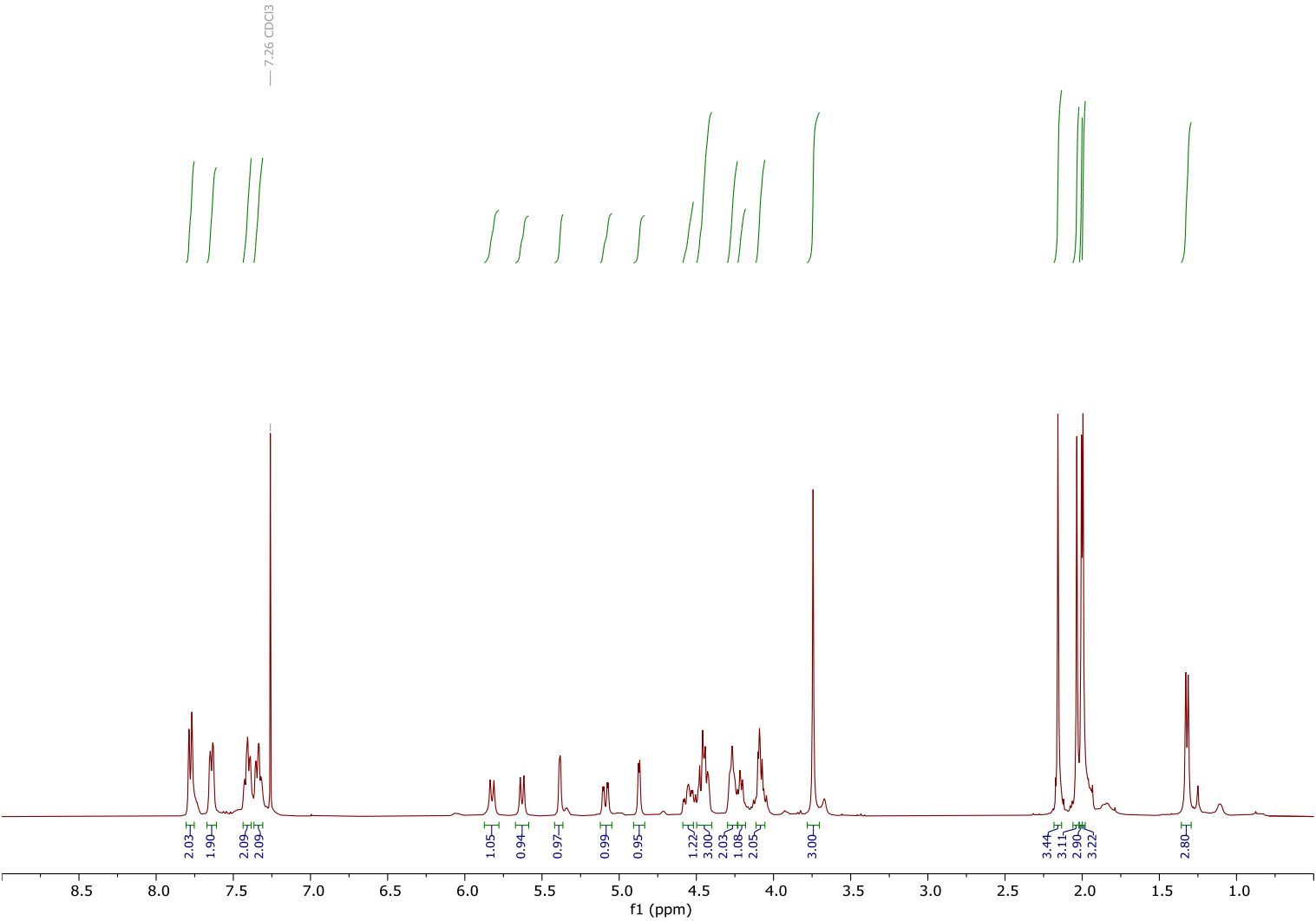
Fmoc Thr-OMe 6

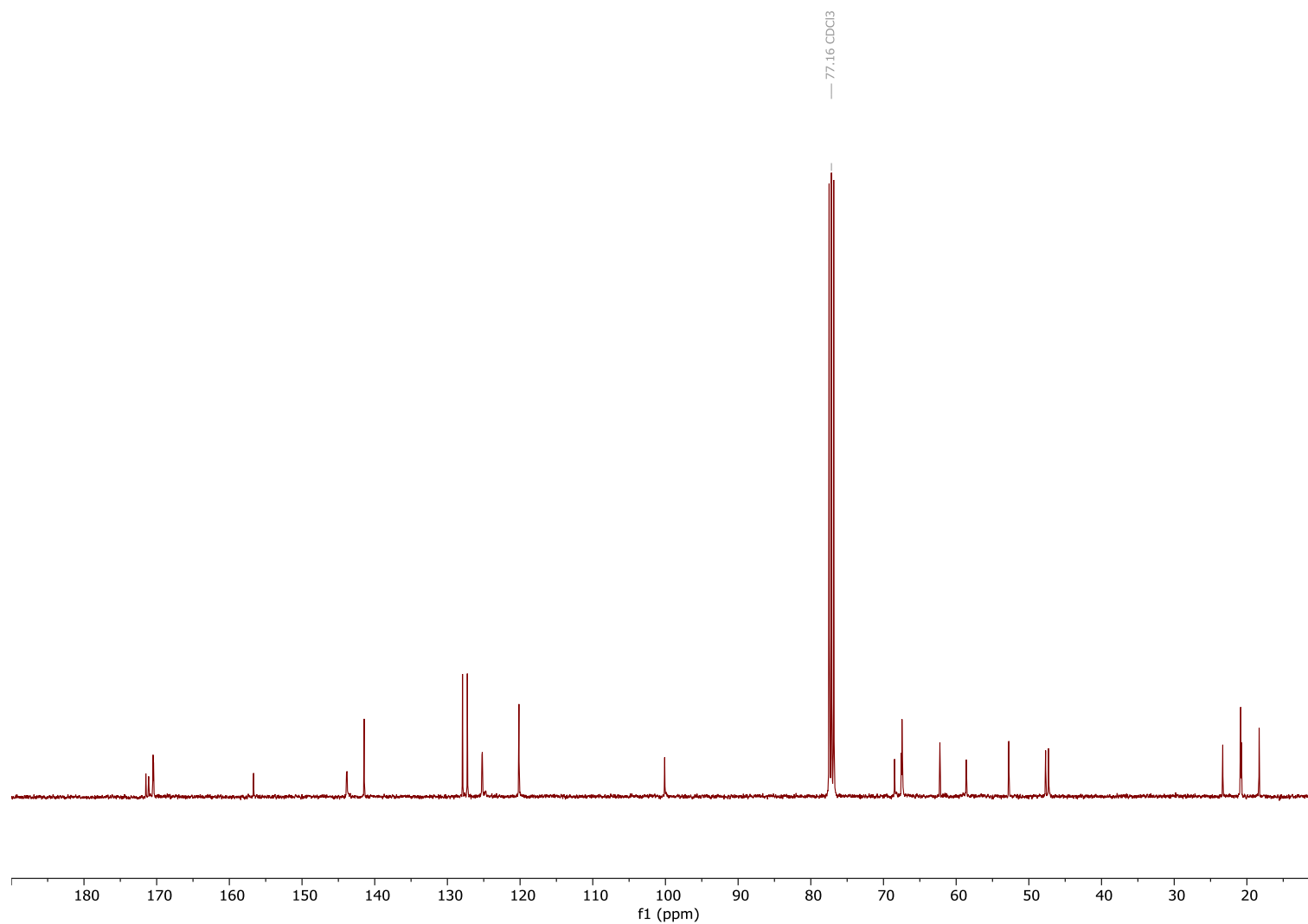




S29

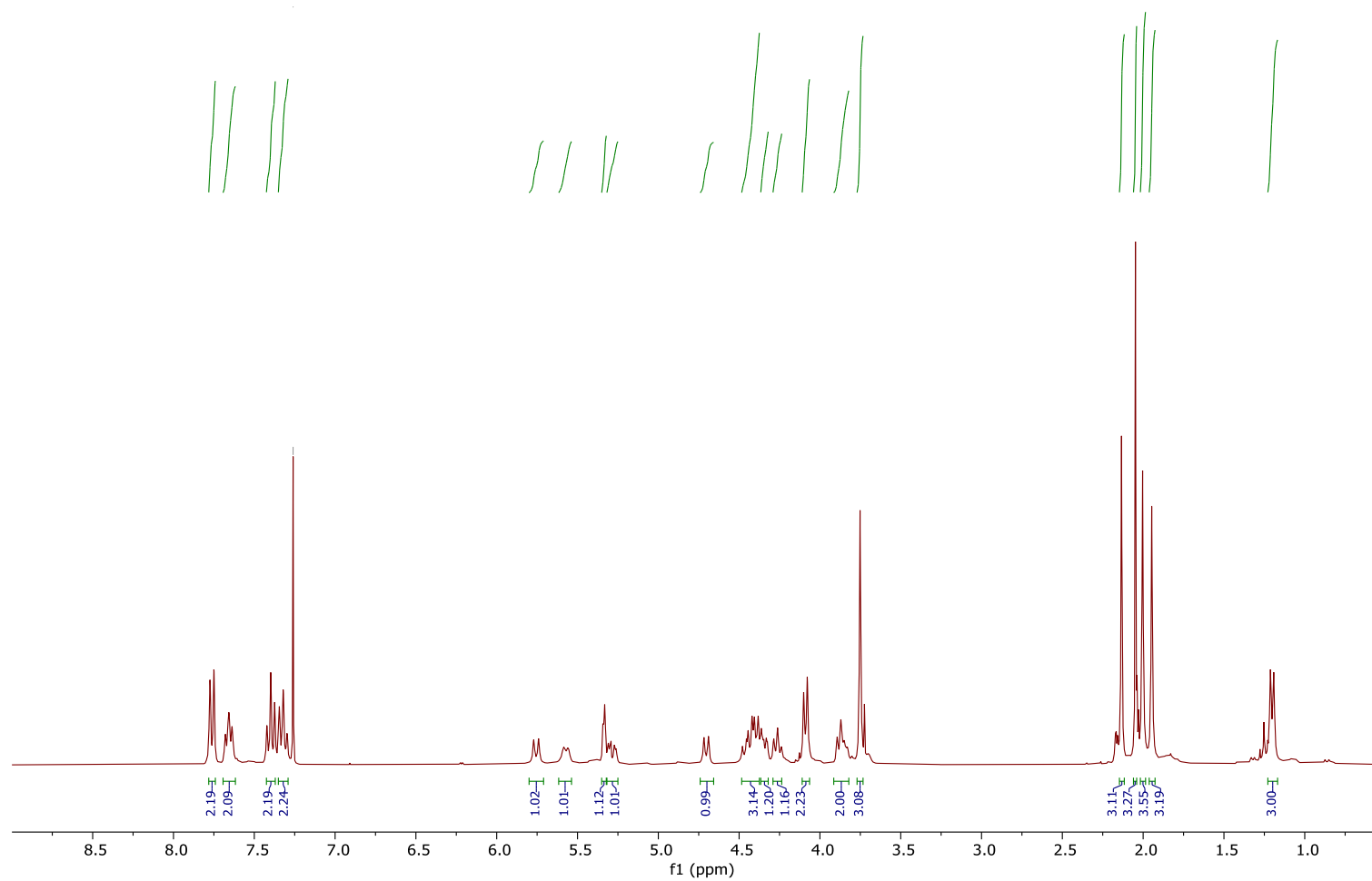
Fmoc-Thr(GalNAc(Ac)3-α-D]-OMe α7

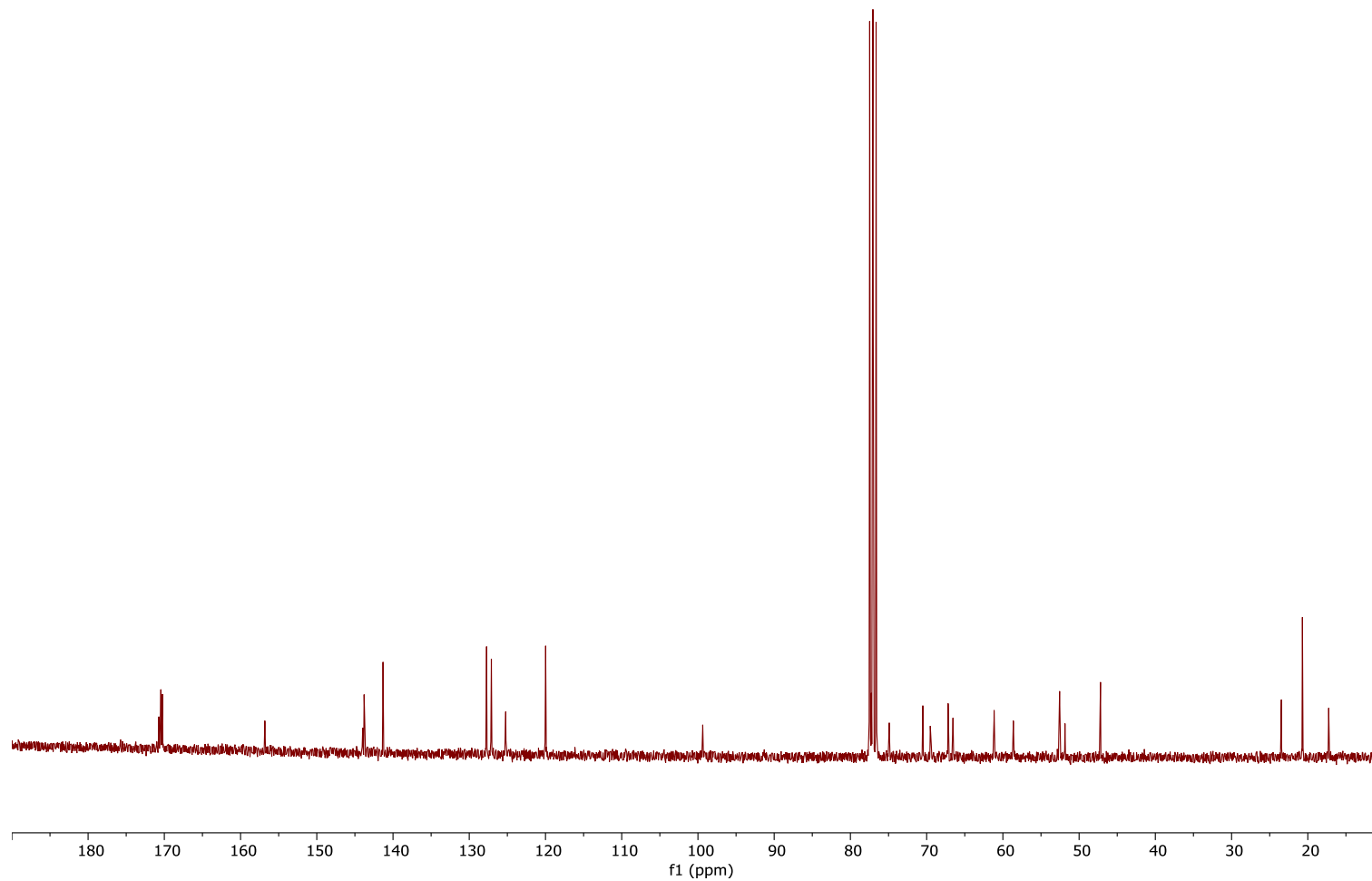




S31

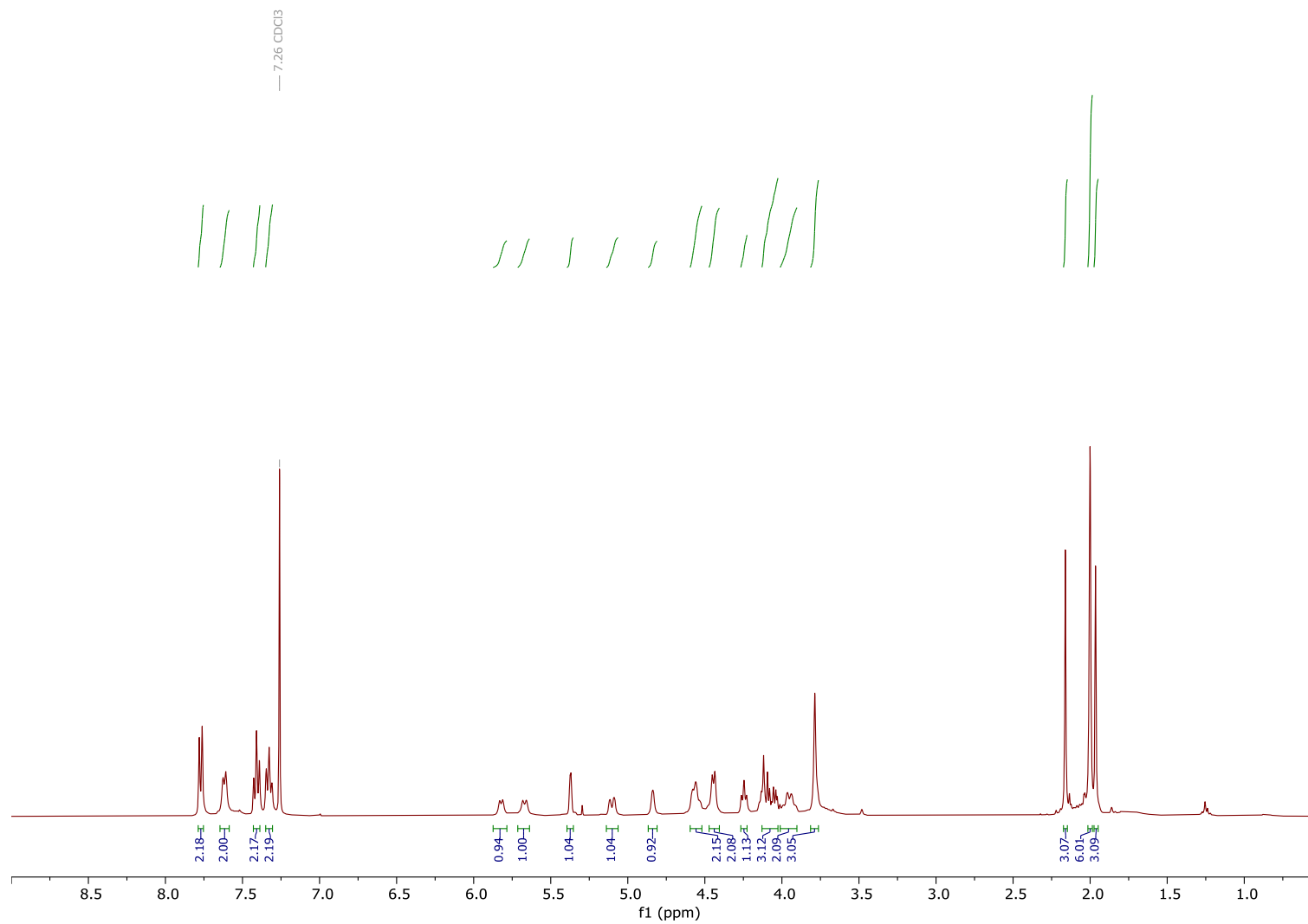
Fmoc-Thr(GalNAc(Ac)3-β-D]-OMe β7

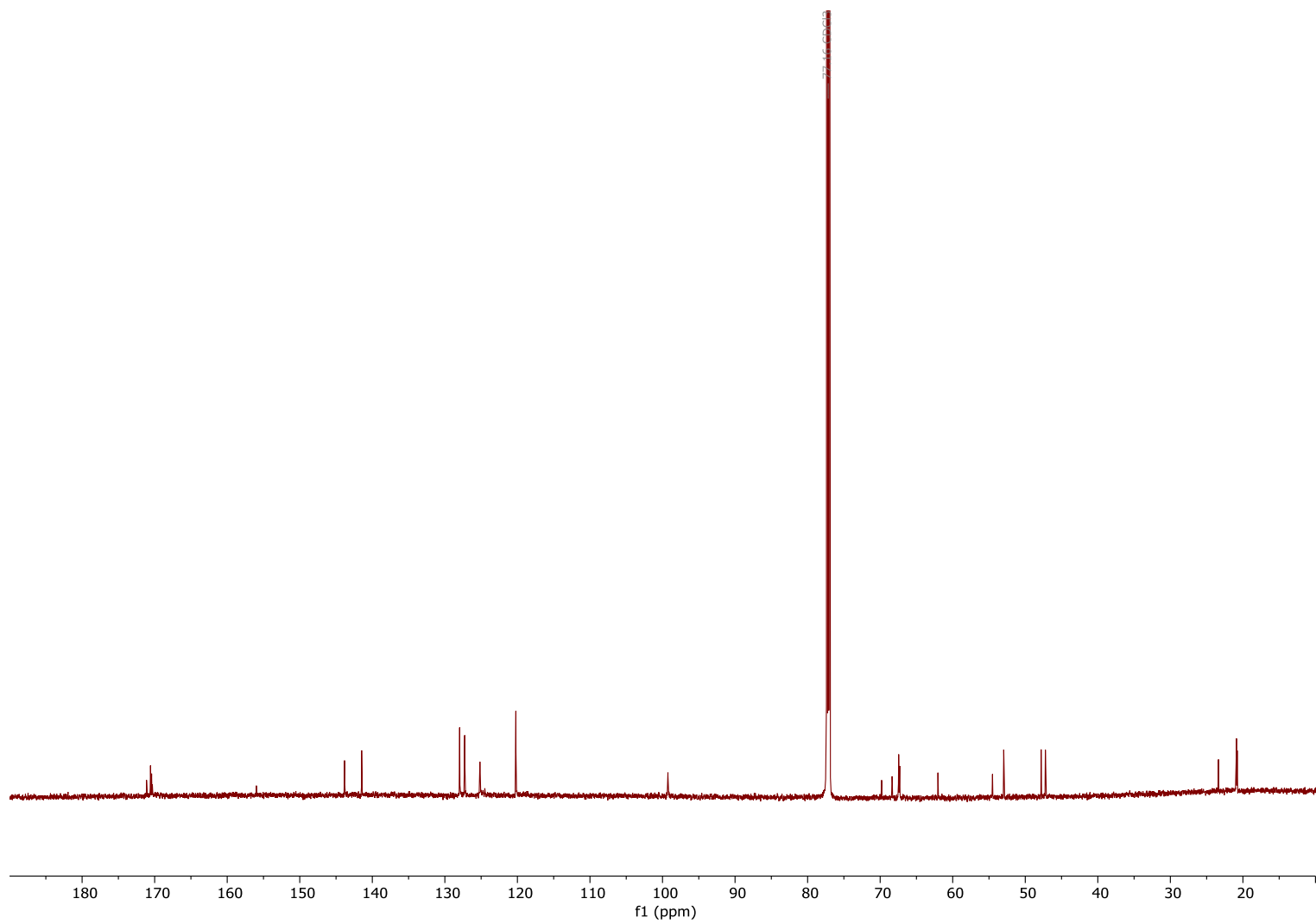




S33

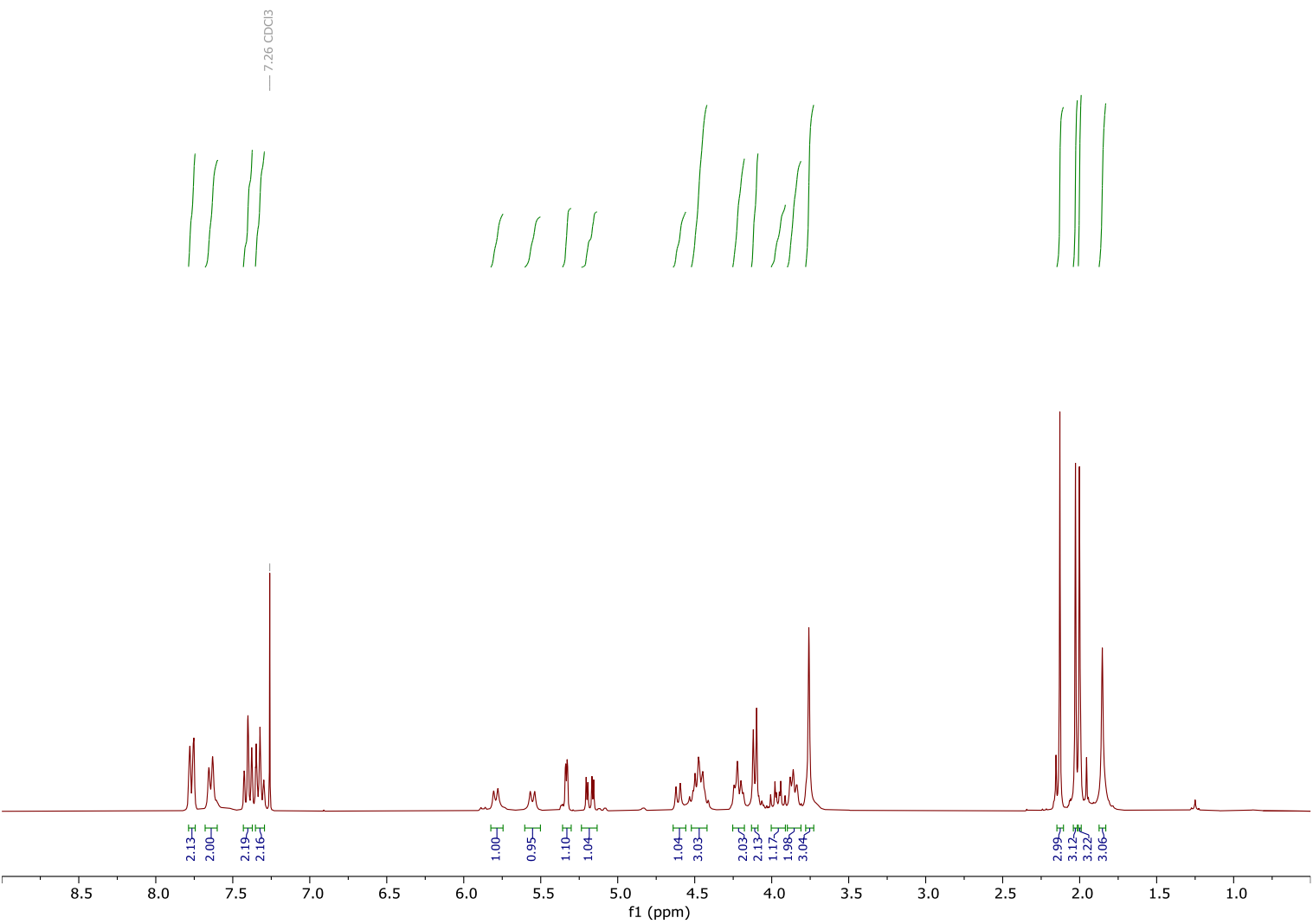
Fmoc-Ser(GalNAc(Ac)3- α -D]-OMe α 8

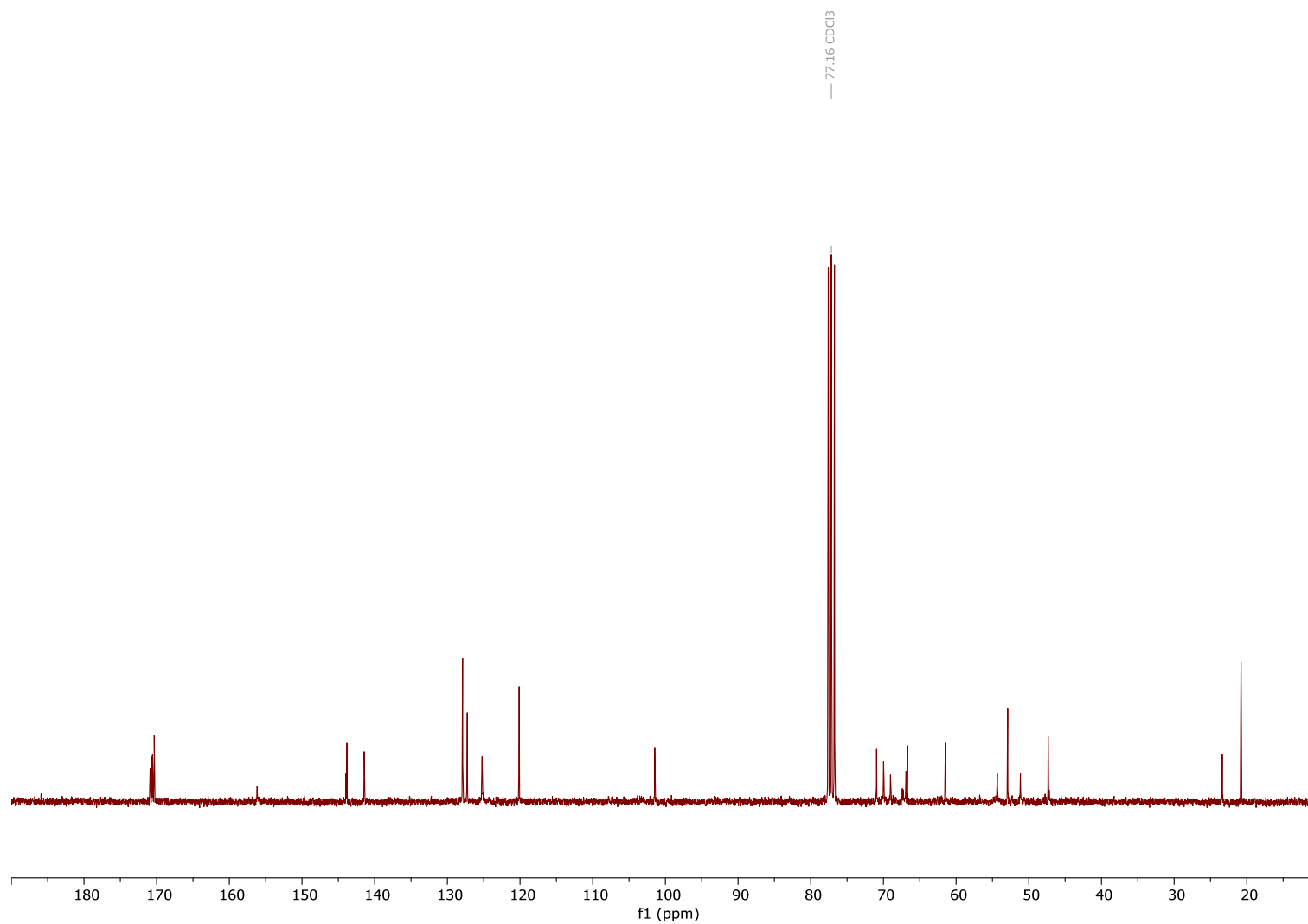




S35

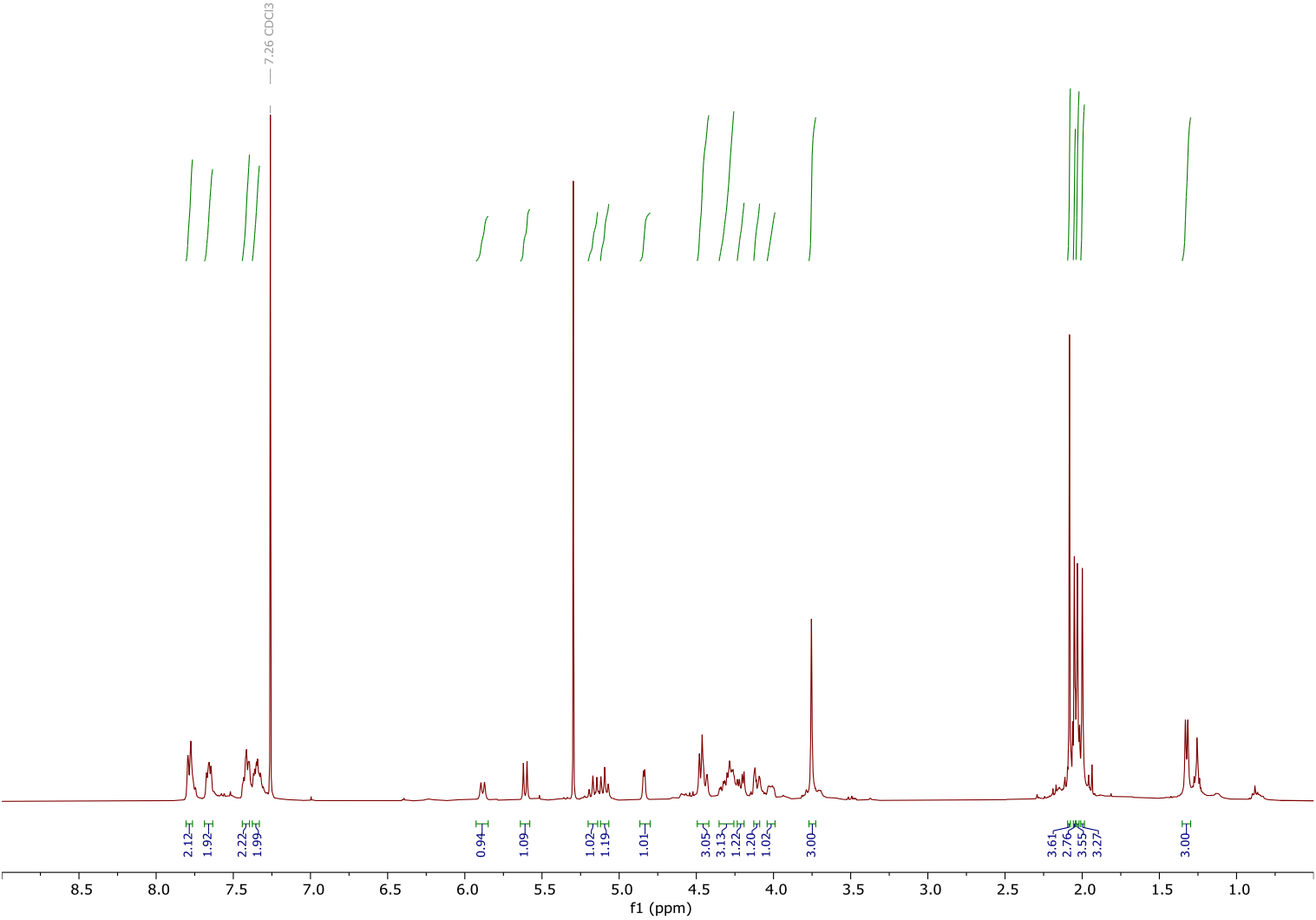
Fmoc-Ser(GalNAc(Ac)3-β-D]-OMe β8

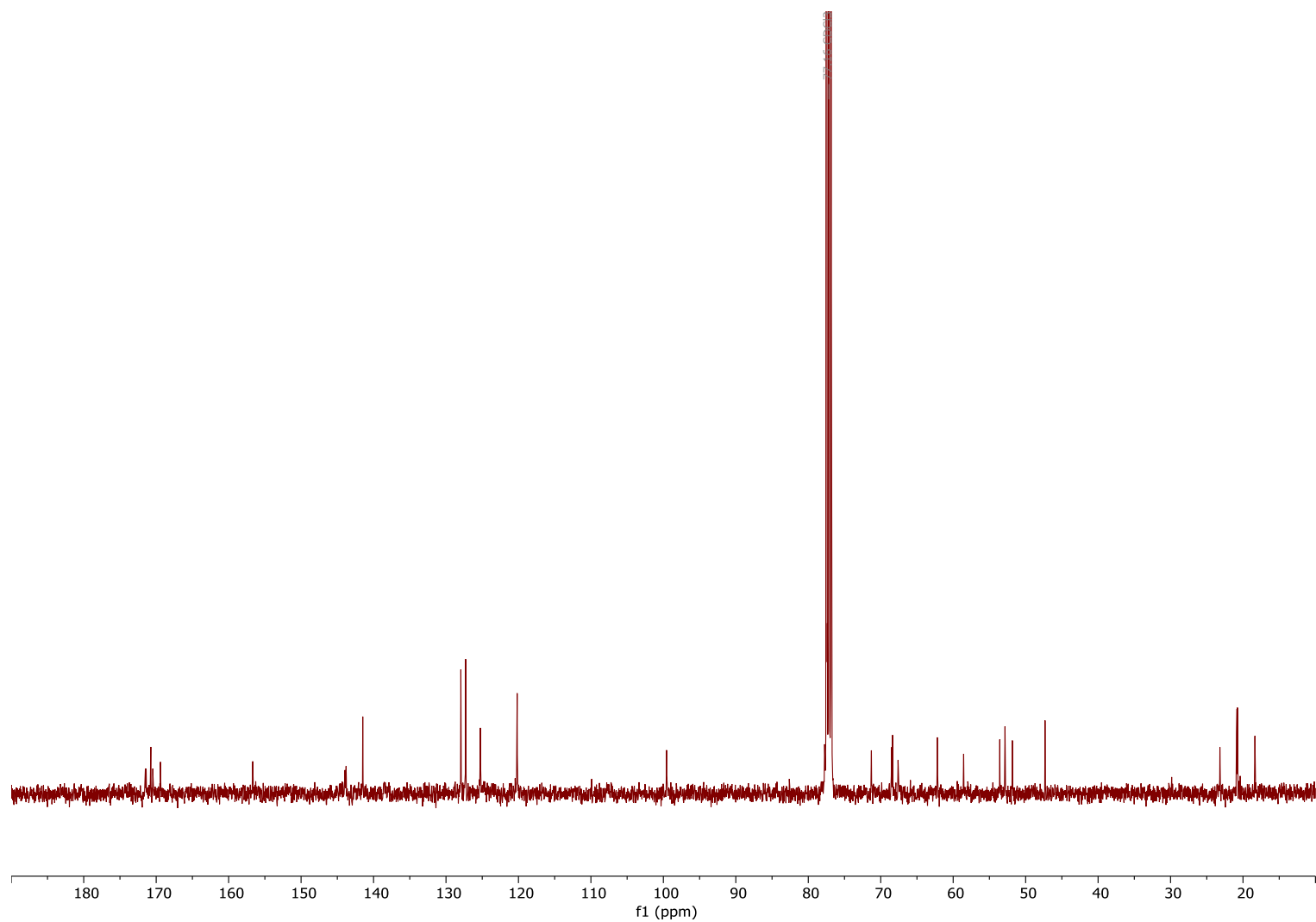




S37

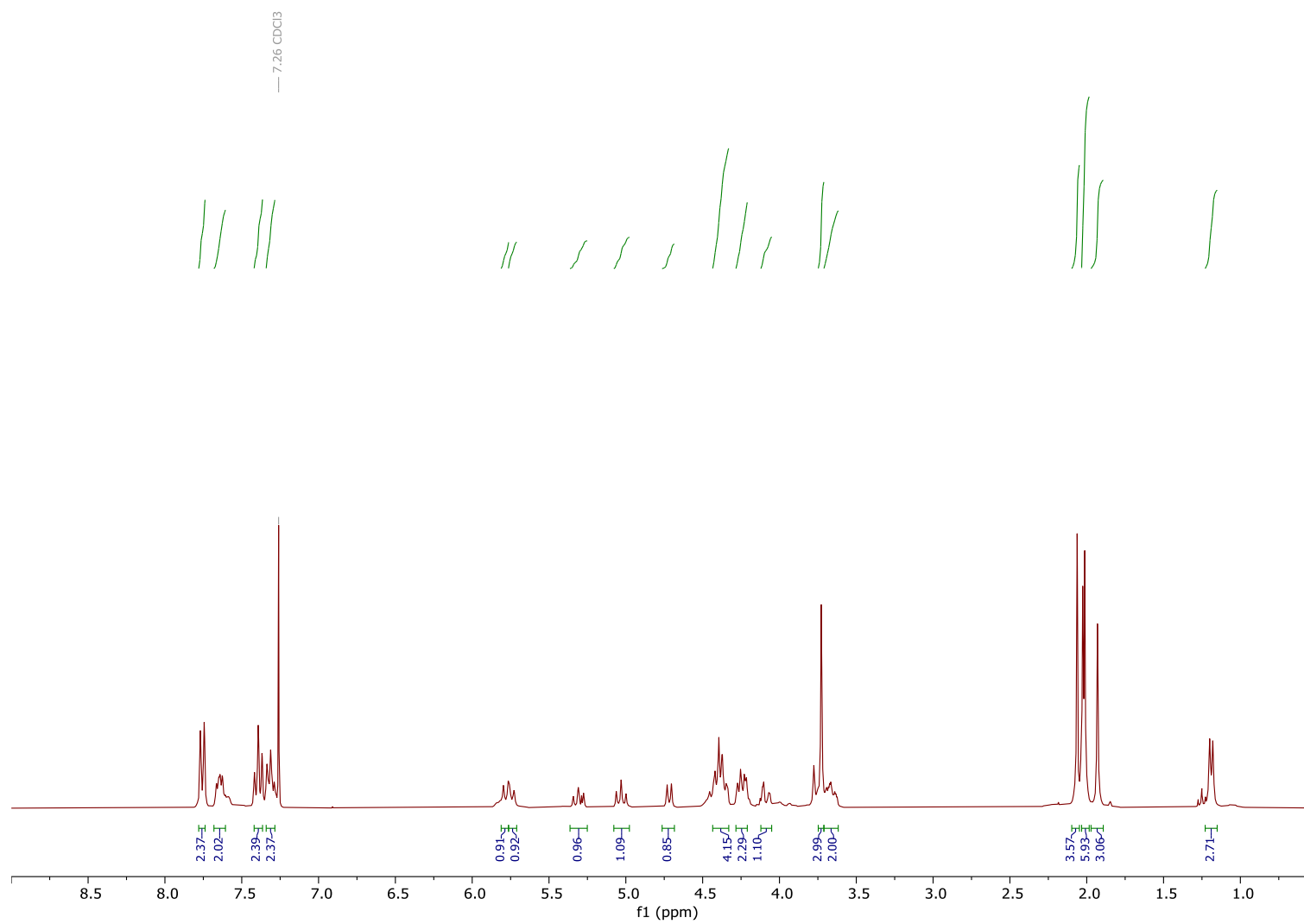
Fmoc-Thr(GlcNAc(Ac)3- α -D]-OMe α 9

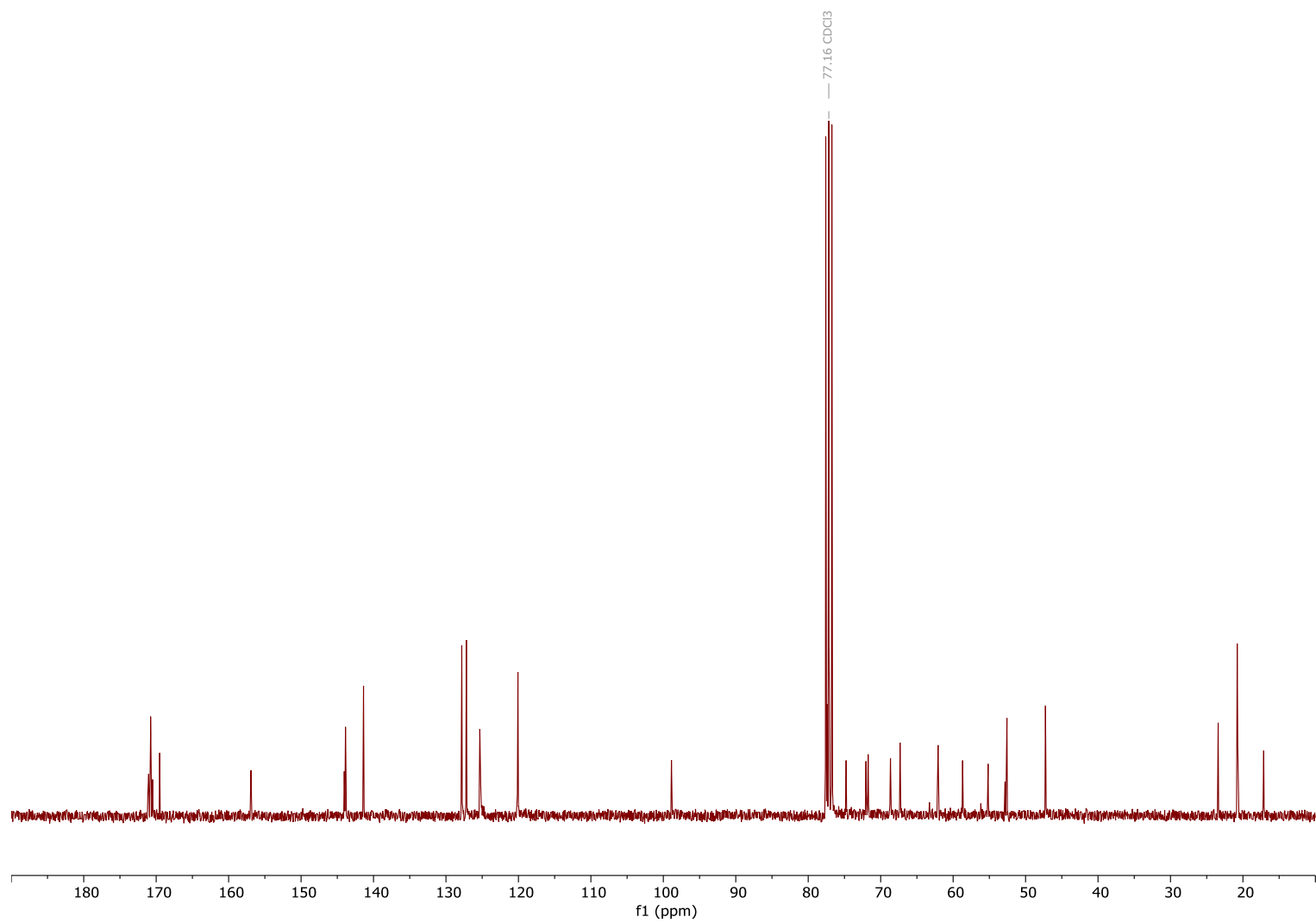




S39

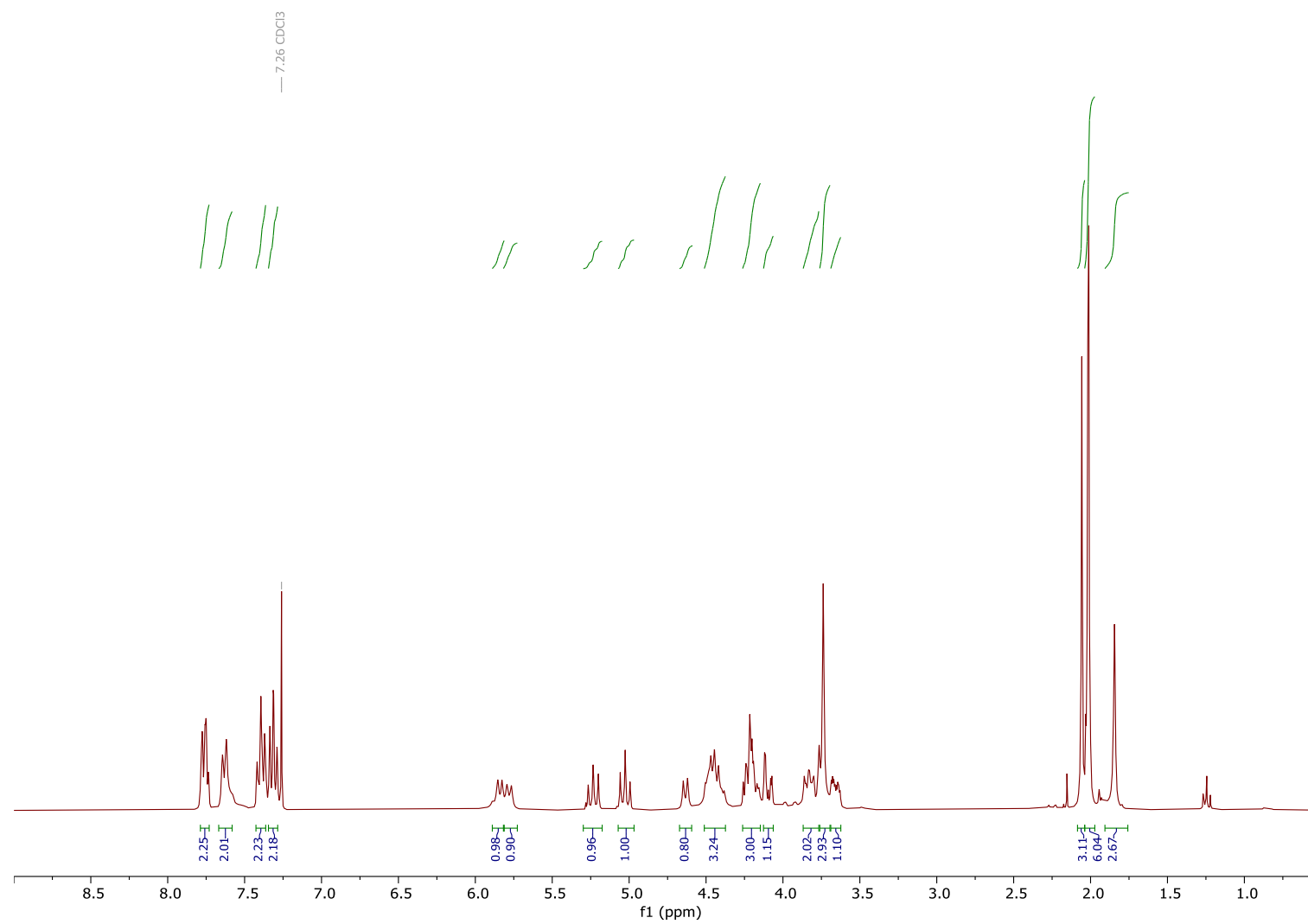
Fmoc-Thr(GlcNAc(Ac)3-β-D]-OMe β9

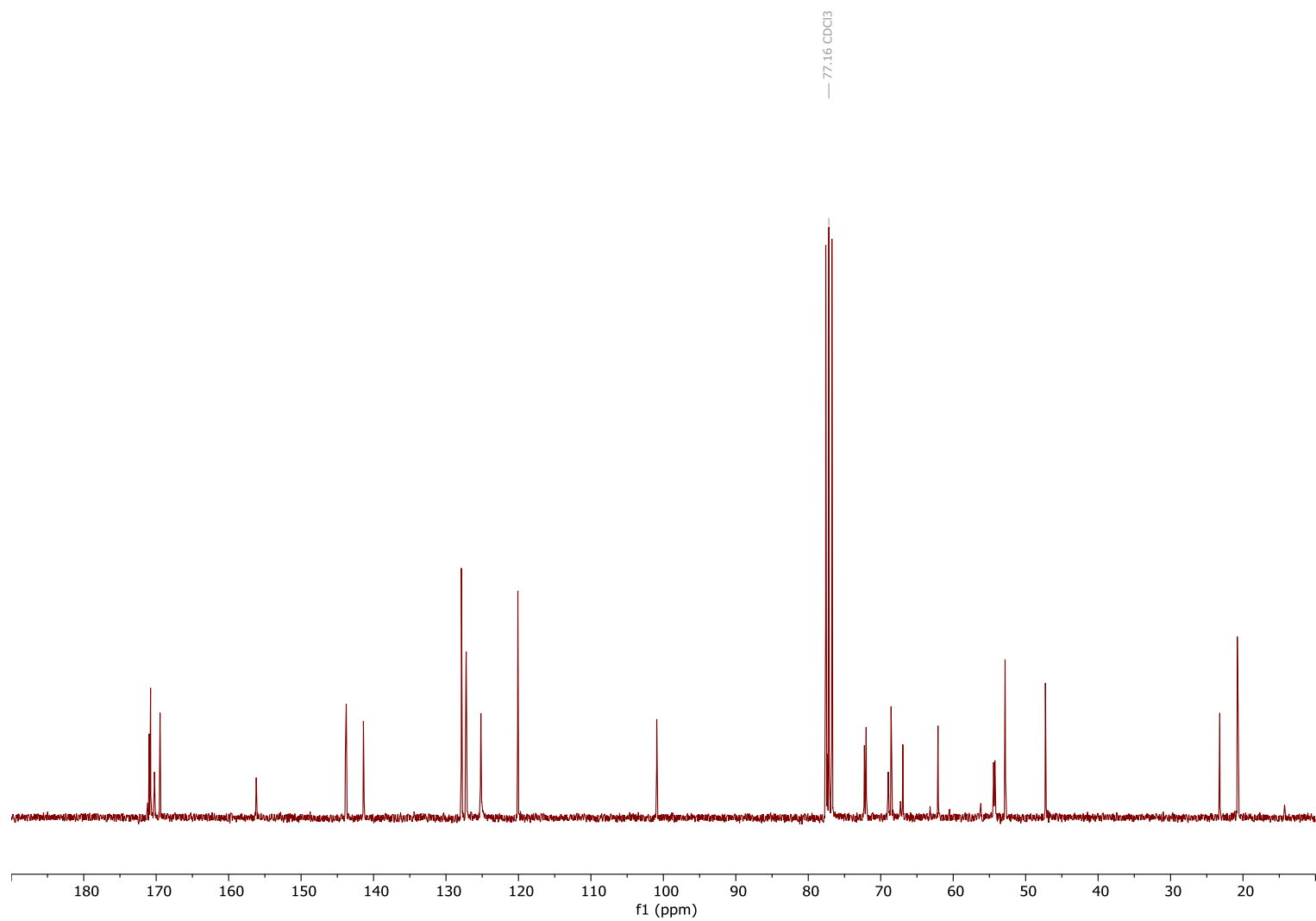




S41

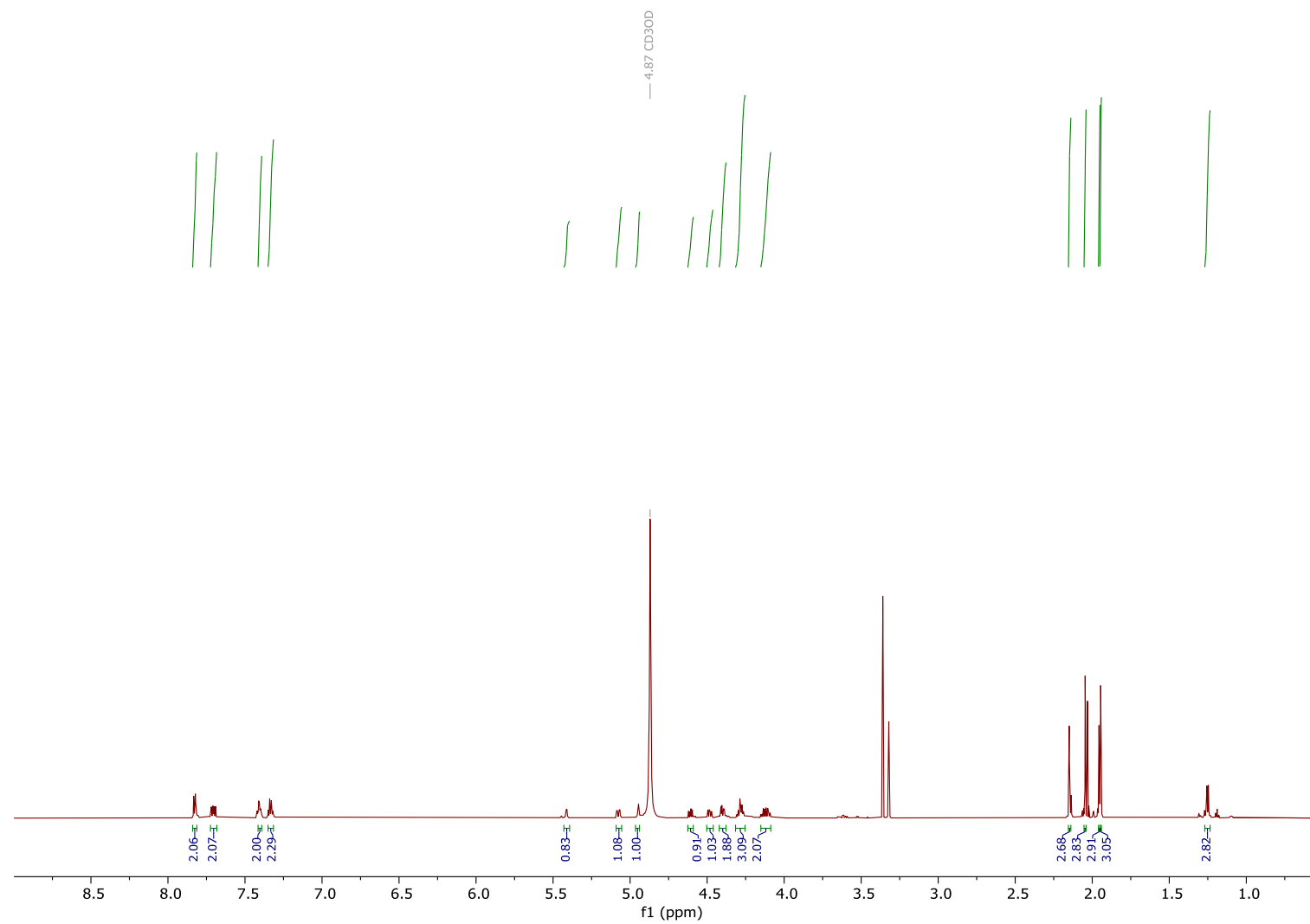
Fmoc-Ser(GlcNAc(Ac)3-β-D]-OMe β10

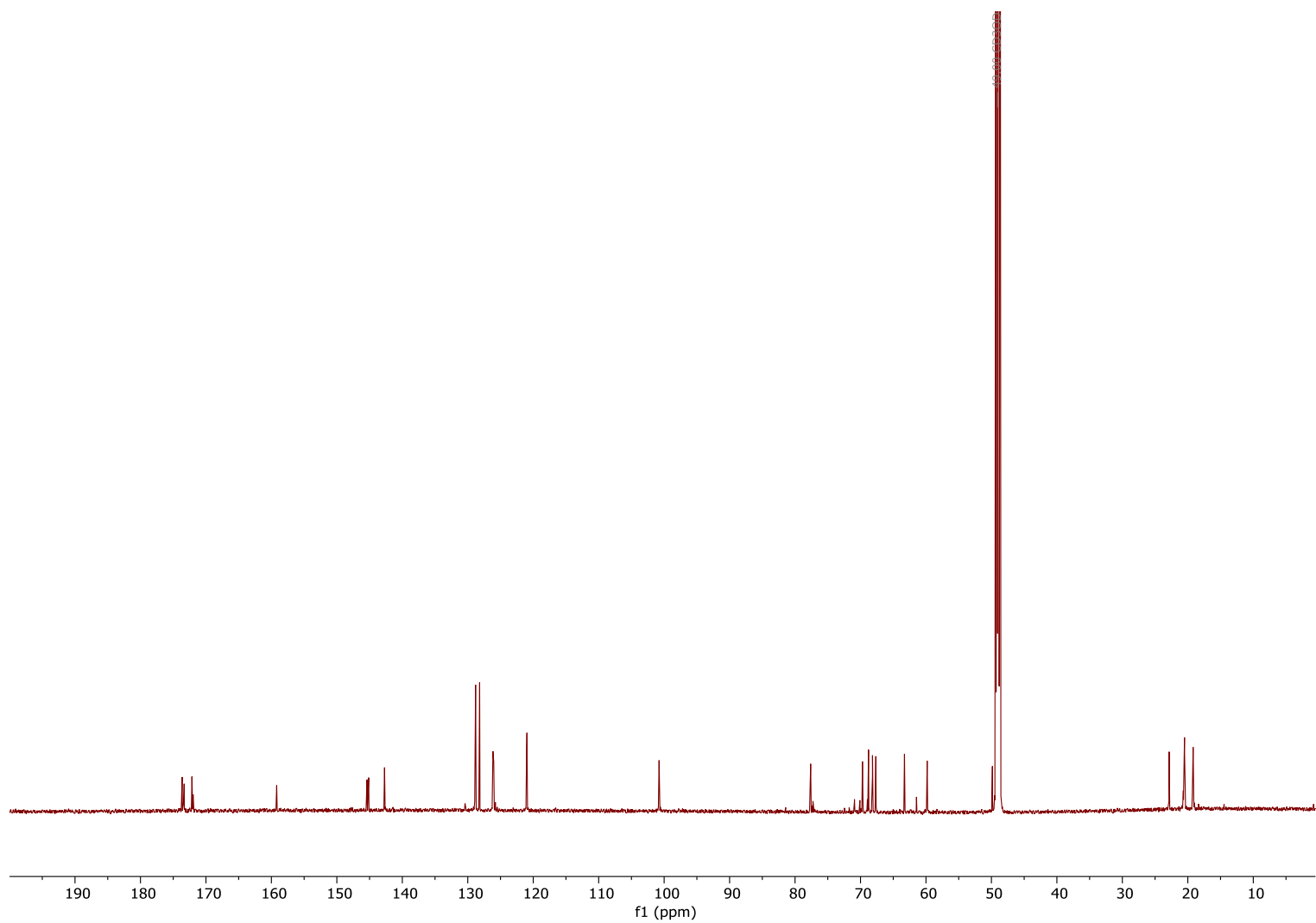




S43

Fmoc-Thr(GalNAc(Ac)3- α -D]-OH α 1

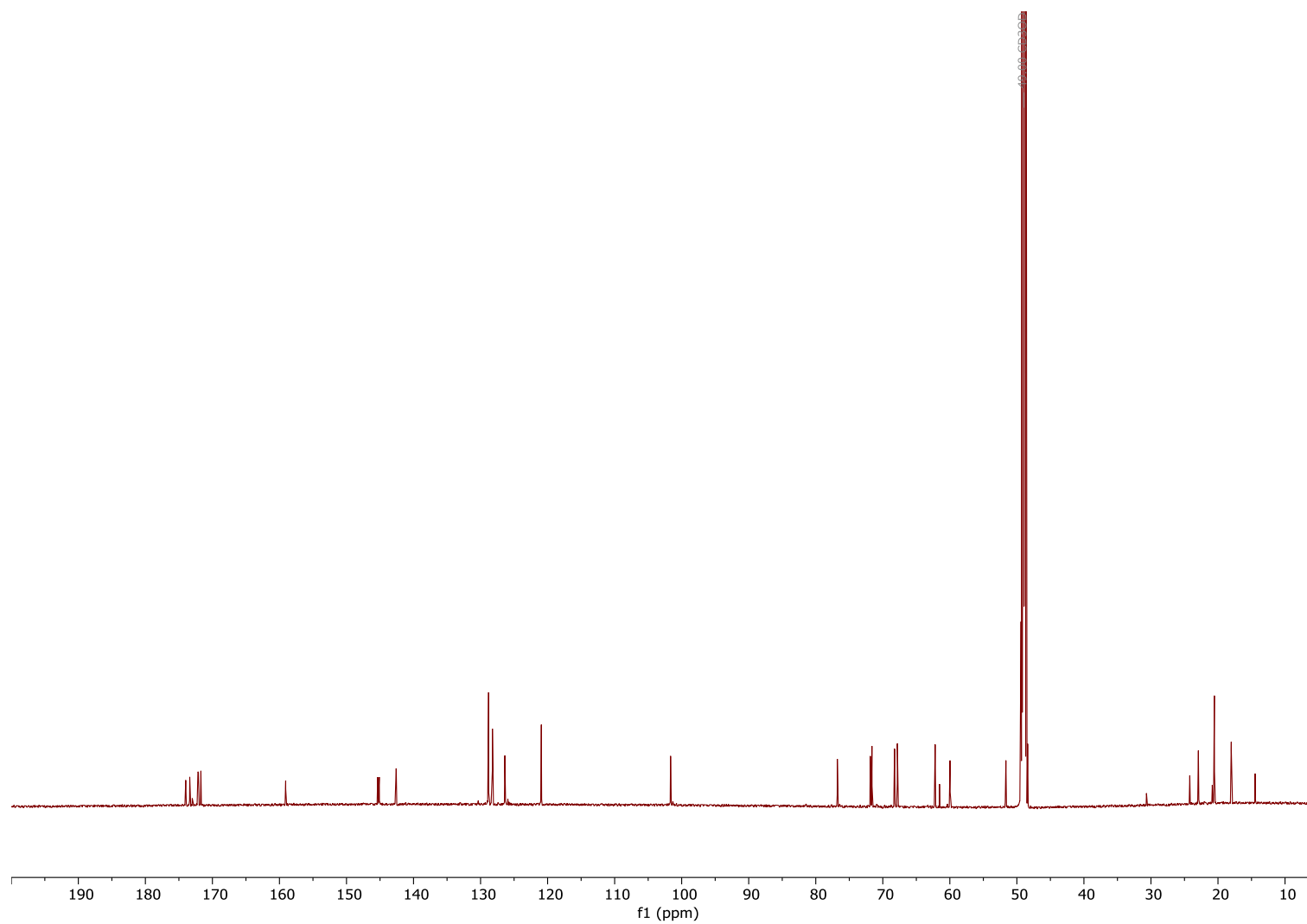




S45

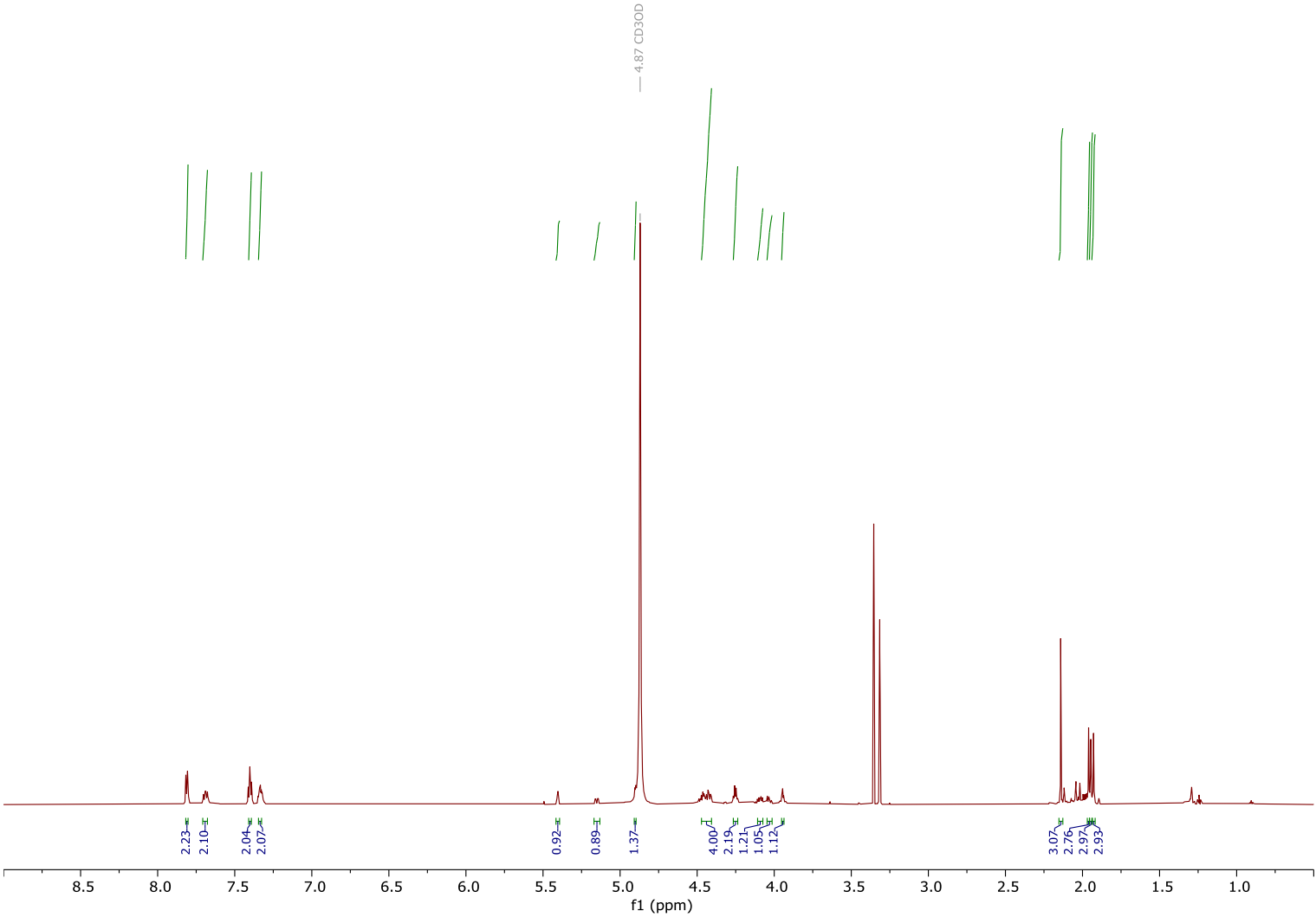
¹H NMR spectrum (CDCl₃) of compound 10a. The x-axis represents the chemical shift in ppm, ranging from 0 to 8.5. The spectrum shows several peaks with corresponding integration values:

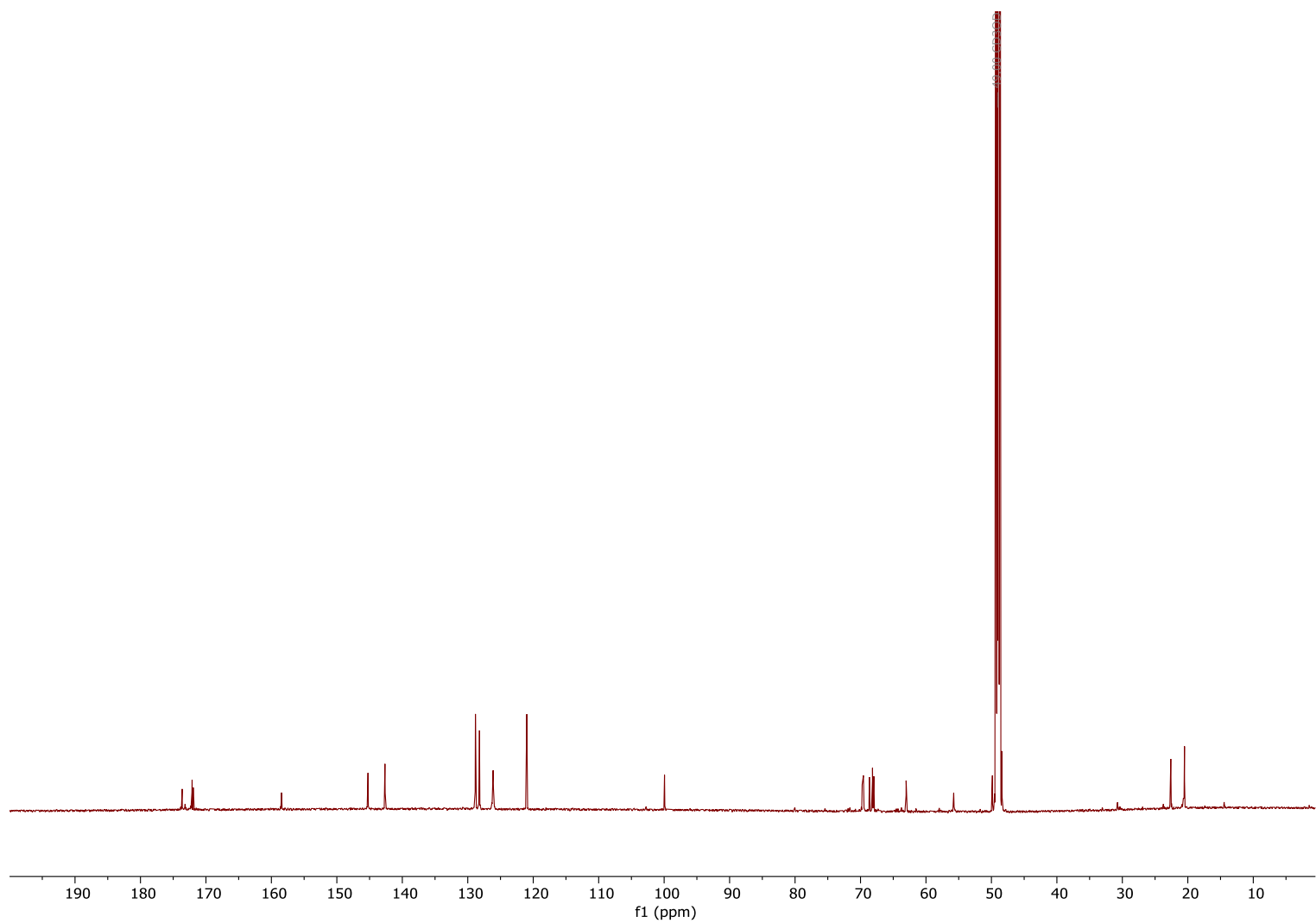
- Aromatic protons (7.2-7.8 ppm): Integration values 1.97, 1.95, 2.15, 2.12.
- Solvent peak (7.26 ppm): Integration value 1.00.
- Methoxy singlet (3.8 ppm): Integration value 1.09.
- Methine doublet (2.69 ppm): Integration value 1.01.
- Methyl singlet (2.0 ppm): Integration values 2.00, 1.05, 0.91, 1.03, 1.22, 0.93, 0.94.
- Integration values for the 2.0 ppm peak: 2.81, 2.79, 2.93, 2.98.



S47

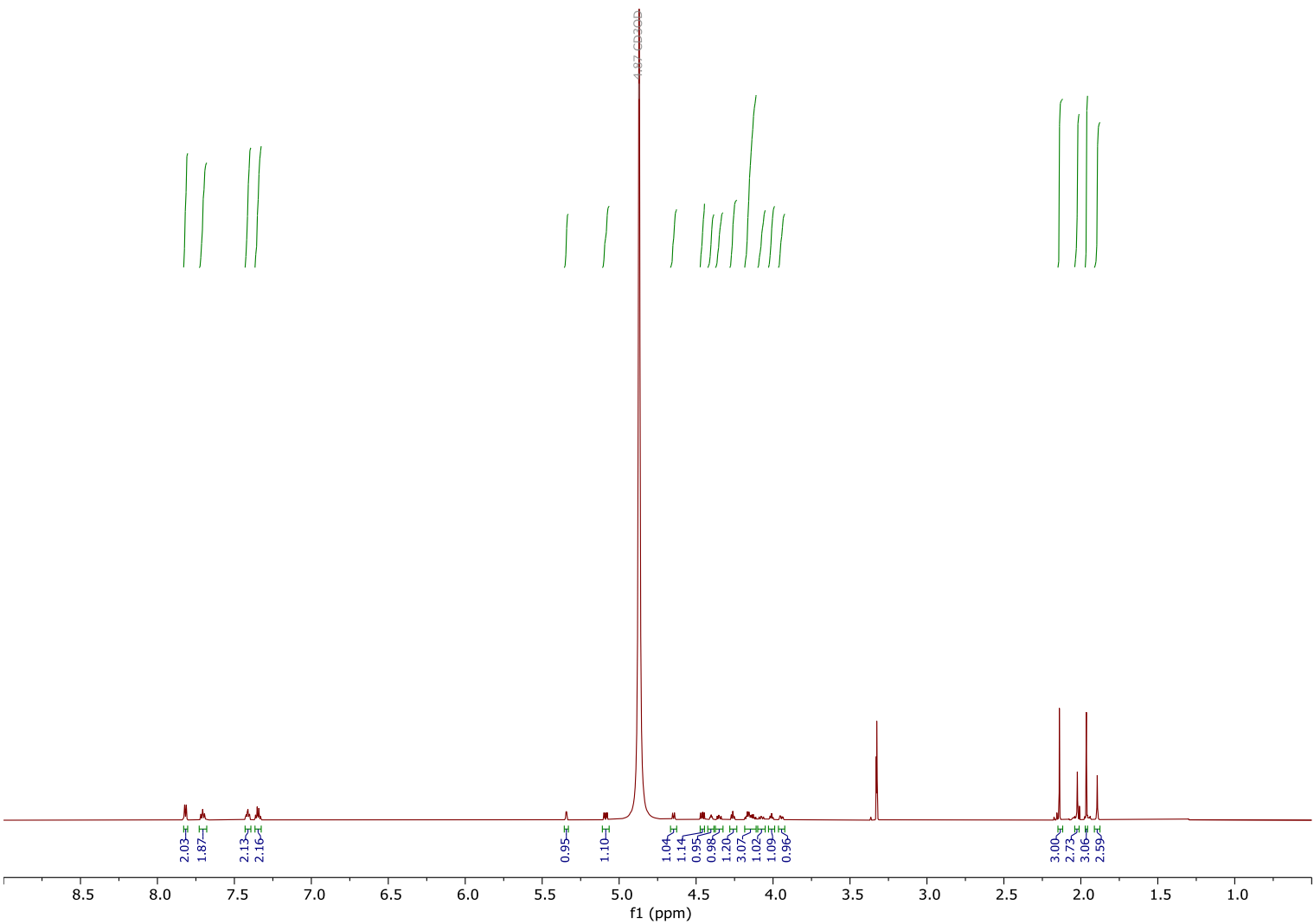
Fmoc-Ser(GalNAc(Ac)3- α -D]-OH α 2

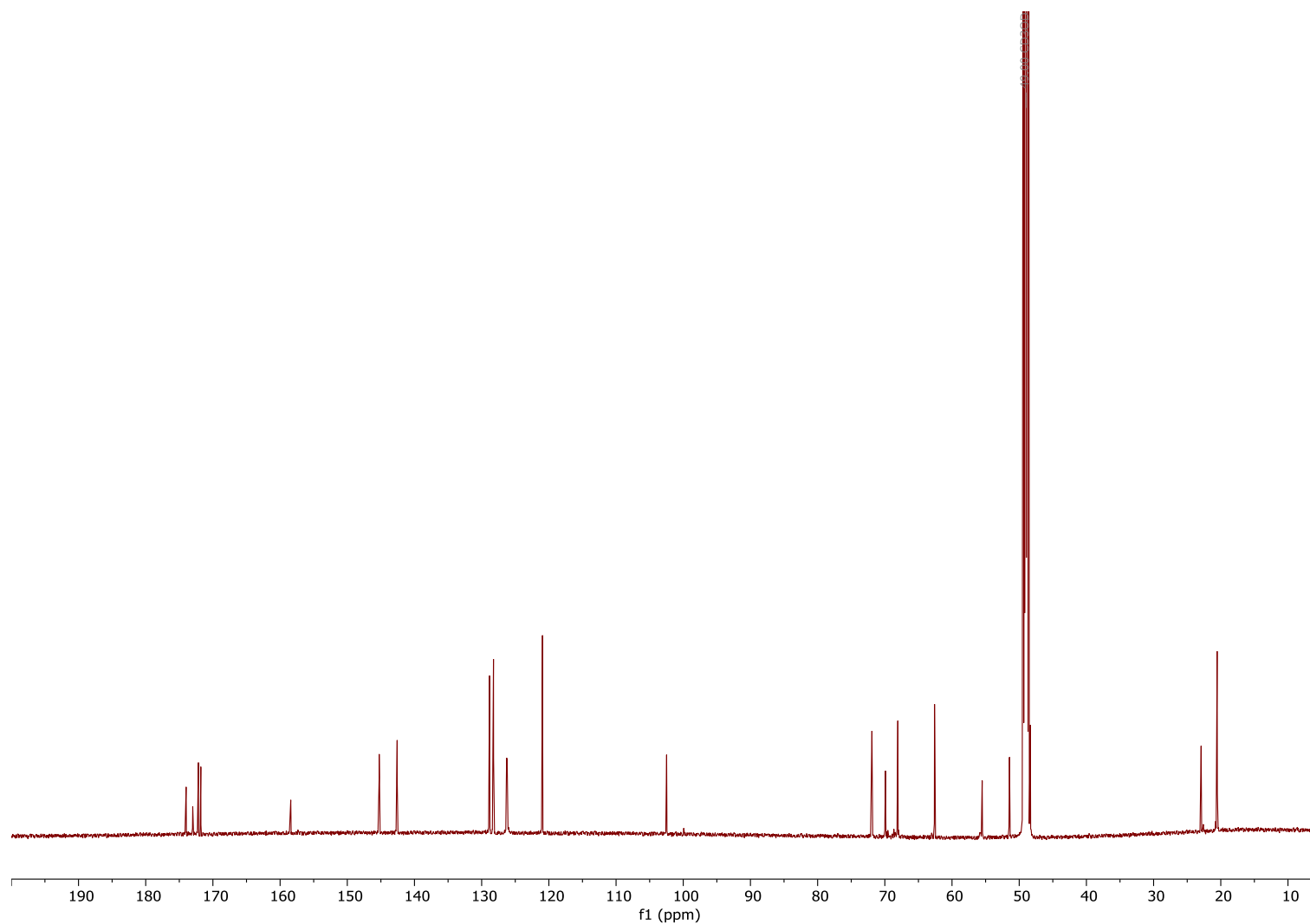




S49

Fmoc-Ser(GalNAc(Ac)3-β-D]-OH β2





S51

References

1. M. Hashimoto, M. Sugiura and S. Terashima, *Tetrahedron*, 2003, **59**, 3063-3087.
2. S. Talat, M. Thiruvikraman, S. Kumari and K. J. Kaur, *Glycoconj. J.*, 2011, **28**, 537-555.
3. E. T. Sletten, S. K. Ramadugu and H. M. Nguyen, *Carbohydr. Res.*, 2016, **435**, 195-207.
4. B. Yan, W. Li and C. P. R. Hackenberger, *Org. Biomol. Chem.*, 2021, **19**, 8014-8017.