Exploiting Uniformly ^{13}C-Labeled Carbohydrates for Probing Carbohydrate-Protein Interactions by NMR Spectroscopy

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Supporting information

Table S1. Linewidths ($\nu_{1/2}$ in Hz) and intensities (*I*) of free Man_3 and CV-N-bound Man_3 resonances. Linewidths were measured in the ^1H dimension of CT-HSQC spectra at 600 MHz. Intensities were measured as volume integrals, normalized to H1 of the reducing-end sugar mannose.

			H1	H2	H3	H4	H5	H6a, H6b
Man''	Free	$\nu_{1/2}$	13	13	*	*	*	*
		I	0,70	0,49	*	0,52	*	*
	Bound	$\nu_{1/2}$	17	19	20	24	24	*
		I	0,74	0,52	0,55	0,66	0,68	*
Man'	Free	$\nu_{1/2}$	13	13	18	*	*	*
		I	0,95	0,77	0,68	*	*	*
	Bound	$\nu_{1/2}$	18	19	30	*	25	*
		I	0,74	0,54	0,47	*	0,77	*
Man	Free	$\nu_{1/2}$	12	13	*	*	19	*
		I	1,00	0,78	*	*	0,79	*
	Bound	$\nu_{1/2}$	16	19	30	*	27	28, *
		I	1,00	0,46	0,21	*	0,40	0,40, *

*Overlap prevented accurate determination.

Table S2. Complete list of intramolecular distances (Å) within Man_3, calculated from NOE build-up curves for free and bound Man_3, as well as the corresponding distances from the X-ray structure with Man-9 (PDB accession code 3GXZ).

			Free Man_3		Bound Man_3		
			r	std dev	r	std dev	3GXZ
Intra-residue	1	2	2.59	0.007	2.54	0.02	2.44
	1	*O*CH_3	2.94	0.004	2.71	0.02	
	2	3			2.39	0.01	2.52
	3	5			2.62	0.02	2.65
	4	6a			2.72	0.02	2.40/2.83
	5	6b			2.46	0.01	2.56
	1'	2'	2.50	0.001	2.57	0.02	2.52
	2'	3'	2.37	0.009	2.52	0.03	2.38
	3'	4'			3.00	0.03	3.06
	4'	5'			2.90	0.03	3.04
	4'	6'a			2.77	0.04	2.43/3.11
	4'	6'b			2.79	0.05	2.43/3.11
	5'	6'a			2.75	0.02	2.47/3.06
	1''	2''	2.53[a]	0.002	2.59	0.03	2.71
	2''	3''	2.44	0.006	2.55	0.02	2.47
	3''	4''			2.73	0.02	3.04
	3''	5''			2.62	0.02	3.25
	4''	5''			2.83	0.03	2.76
	4''	6''a			2.64	0.02	2.30/2.73
	4''	6''b			2.92	0.06	2.30/2.73
	5''	6''a			2.38	0.02	2.33
	5''	6''b			2.68	0.02	3.01
Inter-residue	1	1'	3.26	0.008	2.87	0.04	3.07
	1	5'	2.59	0.005	2.95	0.07	3.37
	1	6'b			3.10	0.04	4.73
	1'	2	2.21	0.001	2.12	0.01	2.10
	1'	1''	3.29	0.014	>3.0[b]		2.24
	1'	2''	3.93	0.051			4.39
	1'	5''	2.47	0.002	2.44	0.02	3.32
	1'	6''a			3.02	0.07	3.83
	2'	4	3.29	0.009			3.64
	1''	2'	2.22	0.002	2.30	0.01	2.60
	2''	4'	3.38	0.009			4.33

[a]Reference distance. See Table 2.

[b]Contribution of spin diffusion prevented accurate determination.

Table S3. Complete list of NOE-derived intermolecular distances in the CV-N/Man_3 complex.

CV-N	Man_3	r(exp)	std dev	r(3GXZ)
G2 NH	2''	2.82	0.02	2.44
K3 Hβb	4'	2.40	0.01	2.61
K3 Hβb	3''	3.06	0.03	3.01
K3 Hδ	3''	2.90	0.02	2.28
Q6 Hβa	4'	3.50	0.02	3.56
Q6 Hβa	6'	2.77	0.03	2.60/3.35
Q6 Hβb	6'	3.03	0.03	2.59/3.77
T7 Hγ	1	4.50	0.09	4.54
T7 Hγ	4'	4.01	0.06	3.98
T7 Hγ	5'	2.98	0.01	2.35
T7 Hγ	6'	2.67	0.03	1.82
E23 Hβb	6	2.29	0.01	2.40/3.09
T25 Hα	5	2.33	0.02	2.03
T25 Hγ	3	3.18	0.02	2.57
T25 Hγ	5	3.29	0.02	3.25
A92 Hβ	2'	3.44	0.02	3.46
A92 Hβ	1''	3.35	0.04	3.87
A92 Hβ	2''	2.99	0.03	2.57
N93 NH	2'	3.16	0.02	3.08
N93 NH	3'	2.58	0.01	2.66
N93 Hβa	2'	3.27	0.04	3.55
N93 Hβa	3'	2.32	0.01	1.86
N93 δNHa	4	2.97	0.01	3.26
N93 δNHa	6	2.52	0.05	2.56/2.69
N93 δNHb	4	3.12	0.02	3.4
N93 δNHb	6	2.58	0.05	2.55/3.46
N93 δNHb	3'	3.02	0.03	3.14
I94 Hδ	1'	4.42	0.04	4.44
I94 Hδ	2'	3.34	0.03	2.82
I94 Hδ	3'	4.87	0.04	4.82
I94 Hδ	1''	3.45	0.04	3.54
D95 NH	3	3.16	0.02	3.69
D95 NH	4	3.43	0.04	3.17

Spin diffusion and/or std. dev. >0.1

CV-N	Man_3	r(exp)	std dev	r(3GXZ)
K3 Hβa	4'	2.63	0.02	3.37
K3 Hβa	3''	3.62	0.07	4.56
K3 Hγa	3''	4.59	0.16	4.5
K3 Hδ	2''	3.12	0.04	4.06
Q6 Hγb	6'	3.86	0.02	4.44/4.94
E23 NH	6	3.41	0.03	4.13
E23 Hβb	5	4.16	0.05	4.62
E23 Hβa	6	2.57	0.03	3.83/4.82
E23 Hγb	6	2.46	0.03	4.52/4.53
T25 NH	5	4.88	0.18	4.39
T25 NH	6	3.95	0.15	4.46
T25 Hγ	2	4.25	0.04	5.07
A92 Hβ	3'	3.96	0.07	4.54
N93 NH	4'	3.55	0.05	4.27
N93 NH	1''	4.11	0.11	5.15
N93 Hβa	4	3.16	0.05	3.76
N93 Hβb	3'	2.80	0.04	3.64
N93 δNHa	5	3.45	0.06	5.40/4.84
N93 δNHa	3'	3.64	0.08	4.43
N93 δNHb	5	3.68	0.07	4.84
D95 NH	2	3.67	0.04	4.96
G96 NH	4	3.72	0.04	4.17
G96 NH	6	3.56	0.03	4.55/5.23

Table S4. Parameter settings for all NMR experiments.

Sample	Fig.	Experiment	Pulse sequence	B_0 (T)	NS	Dim.	SW (ppm)	TD	AQ (ms)	T (°C)	Comments	Exp. time
^{13}C-Man$_3$ + ^{15}N-CV-N	1, 2b, 3a[a]	^{1}H,^{13}C-CT-HSQC	hsqcctetgpsisp	14.1	4	F2-F1	8×60	1k×256	106×14	25	2T = 22 ms	28 min
^{13}C-Man$_3$ + ^{15}N-CV-N	-	2D ^{1}H,^{13}C-CT-HSQC-TOCSY	---[b]	14.1	32	F2-F1	15×120	2k×256	114×7	25	τ_{mix} = 20 ms, 2T = 22 ms	4 h
^{13}C-Man$_3$ + ^{15}N-CV-N	S1	2D HC(C)H-TOCSY	hcchdigp3d	14.1	32	F3-F2	15×140	2k×256	114×6	2	τ_{mix} = 11.1 ms	5 h
Man$_3$ (unlabeled)	2c	1D DPFGSE ^{1}H,^{1}H-T-ROESY	selrogp.2	14.1	128	F1	6	16k	2,277	25	τ_{mix} = 50-350 ms	23 min/exp.
^{13}C-Man$_3$ + ^{15}N-CV-N	2d, 3b, 4b	2D ^{1}H,^{13}C-HSQC-NOESY	hsqcetgpnosp	21.1	32	F2-F1	14×140	2k×256	81×4	20	τ_{mix} = 10-120 ms	3.7 h/exp.
^{13}C-Man$_3$ + ^{15}N-CV-N	-	3D NOESY-^{1}H,^{13}C-HSQC	na_c6noesyhsqcgp3d	14.1	32	F3-F2-F1	10×24×10	1k×24×128	85×3×11	20	τ_{mix} = 80 ms	41.5 h
^{13}C-Man$_3$ + ^{15}N-CV-N	3a	2D ^{13}C-filt. NOESY-^{1}H,^{13}C-HSQC	noesyhsqcgpwgx13d	21.1	32	F3-F2	8×60	1k×256	71×9	20	τ_{mix} = 80 ms	4 h
^{13}C-Man$_3$ + ^{15}N-CV-N	4a, S2	2D CNH-NOESY[c]	noesycngp3d	14.1	32	F3-F2	15×35	2k×256	114×60	25	τ_{mix} = 20 or 60 ms	4 h
^{13}C-Man$_3$ + ^{15}N-CV-N	4a, S2	^{1}H,^{15}N-HSQC	fhsqcf3gpph	14.1	8	F2-F1	15×35	2k×256	114×60	25		57 min
^{13}C-Man$_3$ + ^{13}C,^{15}N-CV-N	-	3D HNCACB	hncacbgpwg3d	14.1	48	F3-F2-F1	14×33×60	1k×44×100	61×11×6	20		69 h
^{13}C-Man$_3$ + ^{13}C,^{15}N-CV-N	-	3D CBCA(CO)NH	cbcaconhgpwg3d	14.1	32	F3-F2-F1	14×33×60	1k×36×100	61×9×6	20		38.5 h
^{13}C-Man$_3$ + ^{13}C,^{15}N-CV-N	-	3D HC(C)H-TOCSY	hcchdigp3d	14.1	16	F3-F2-F1	14×114×14	1k×96×100	61×3×6	20	τ_{mix} = 11.1 ms	70 h
^{13}C-Man$_3$ + ^{13}C,^{15}N-CV-N	-	3D NOESY-^{1}H,^{13}C/^{15}N-HSQC	noesyhsqcgpsismsp3d	16.4	32	F3-F2-F1	14×110×14	1k×80×100	61×3×6	20	τ_{mix} = 80 ms	91 h
^{13}C-Man$_3$ + ^{13}C,^{15}N-CV-N	-	^{1}H,^{13}C-CT-HSQC	hsqcctetgpsisp	18.8	4	F2-F1	13×130	2k×512	98×10	20	2T = 26.6 ms	1 h

[a]The HSQC spectra in Figures 2b and 3a were recorded on a 900 MHz spectrometer (21.1 T).

[b]Essentially identical to a ^{1}H,^{13}C-HSQC-TOCSY (hsqcdietgpsisp.2) experiment with addition of a constant-time period in t1.

[c]Equivalent to a ^{1}H,^{13}C-HSQC-NOESY-^{1}H,^{15}N-HSQC experiment.

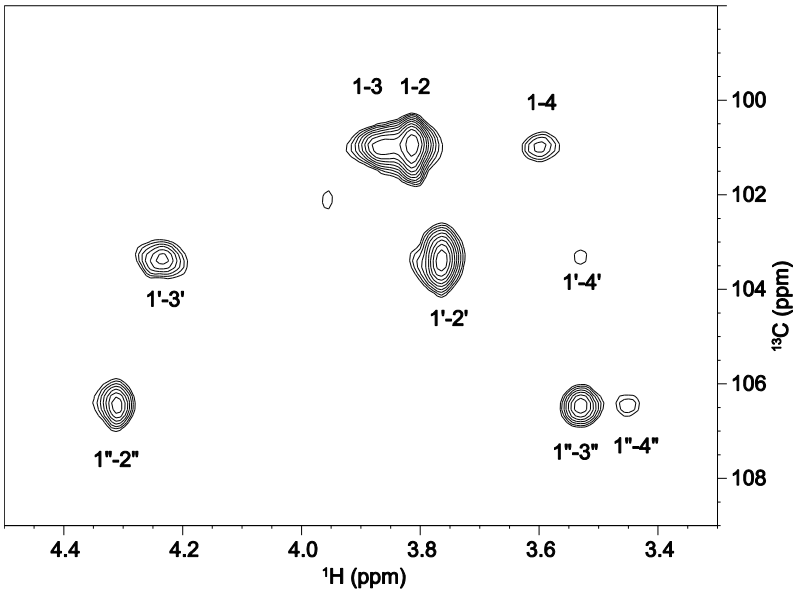


Figure S1. Selected region of the 2D version of the HC(C)H-TOCSY spectrum of the CV-N-bound Man_3. Cross peaks between the anomeric carbons and protons at the 2, 3 and 4 positions are labeled. The C-C spin-lock time was 11.1 ms.

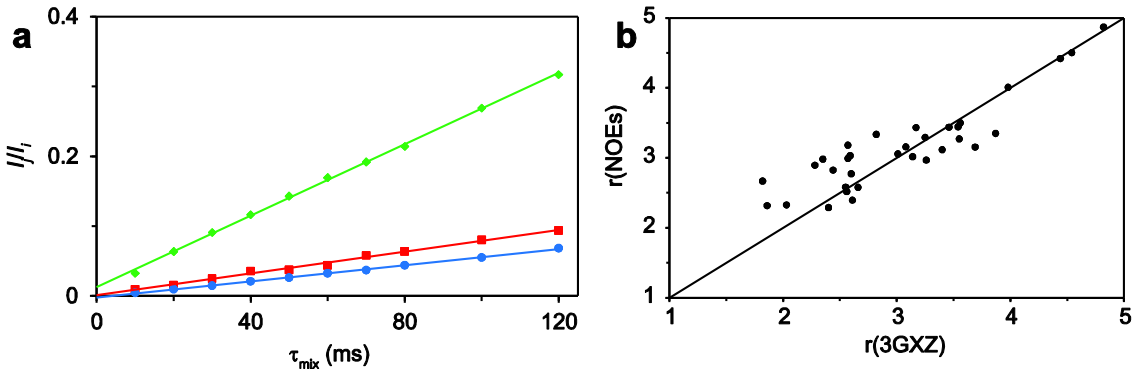


Figure S2. (a) Plot of I_j/I_i *versus* τ_{mix}, where I_j is the intensity of the intermolecular NOE peak and I_i is the intensity of the Man_3 diagonal peak. Plots are shown for H3′-N93 Hβa (green diamonds), H2″-G2 NH (red squares), and H5′-T7 Hγ (blue circles). Cross-relaxation rates were obtained from the slopes of the lines. (b) Correlation plot showing distances calculated from NOEs *versus* distances extracted from the X-ray structure with Man-9/CV-N (PDB file 3GXZ). The slope of the black line is 1. A correlation with $r^2 = 0.68$ was obtained.

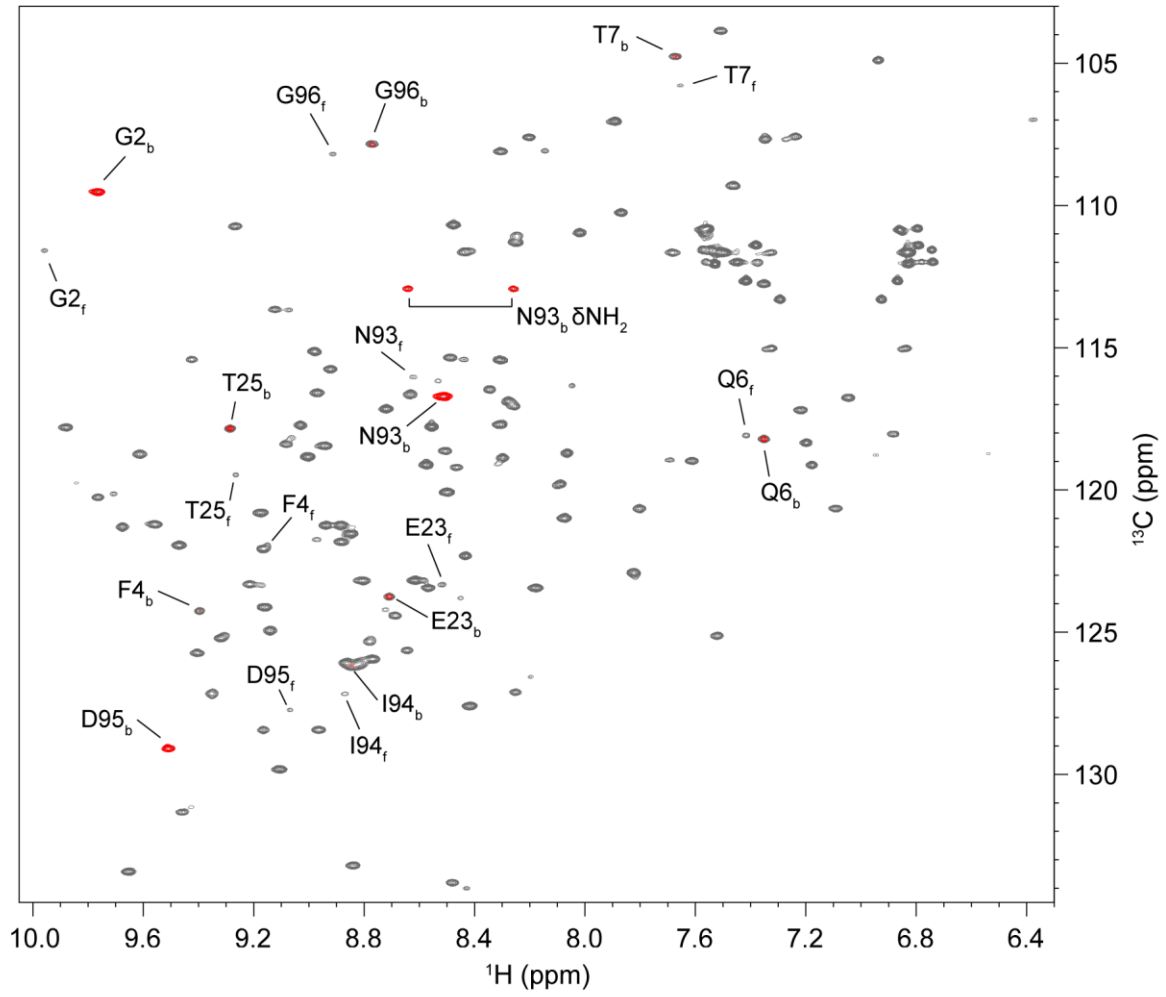


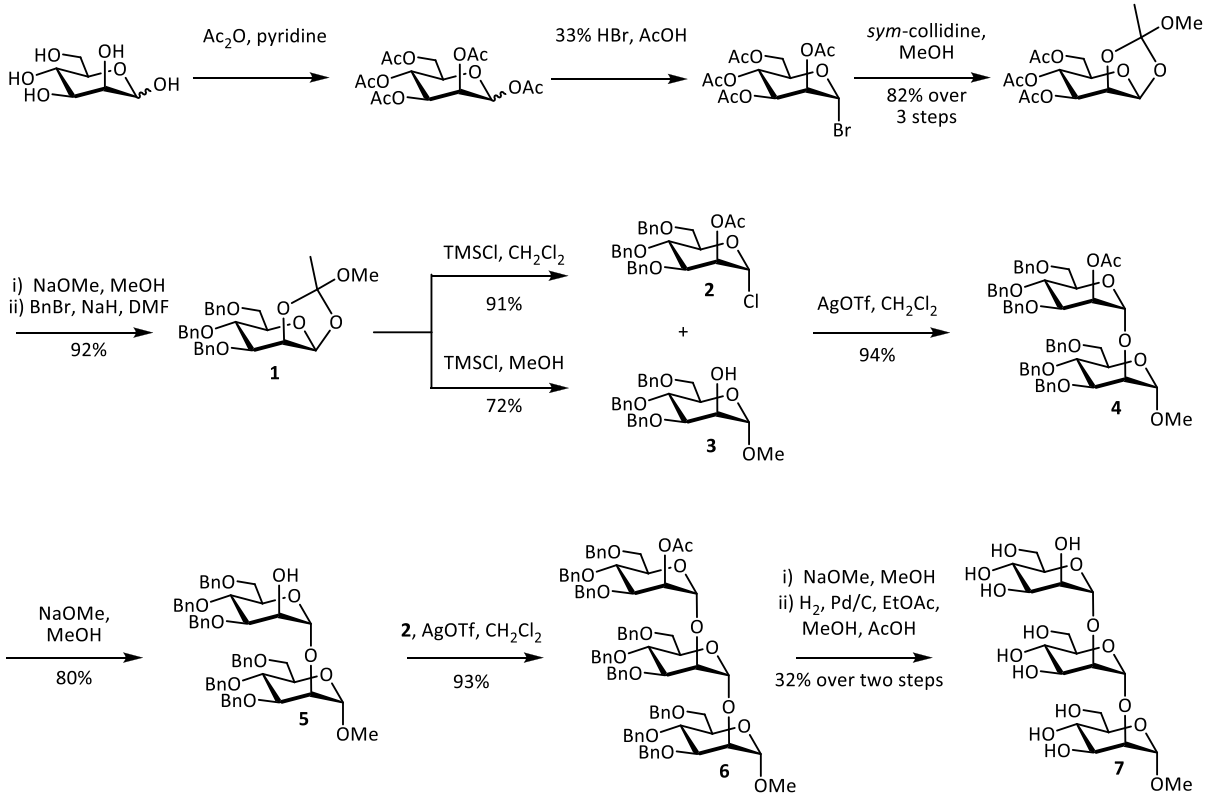
Figure S3. Superposition of the $^{1}H,^{15}N$ 2D version of a CNH-NOESY spectrum (red, 60 ms mixing time) and the $^{1}H,^{15}N$-HSQC spectrum (grey) of the CV-N/Man_3 complex. NOEs between ^{13}C-attached sugar ring protons and ^{15}N amide/amino protons are colored red. Direct NOEs as well as relayed NOEs (due to spin diffusion) are observed. Selected amide resonances of free ($_f$) and Man_3-bound ($_b$) protein are labeled by amino acid name and number.

Experimental for the synthesis of ^{13}C-labeled mannosides

General Experimental:

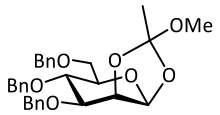
The D-[U-$^{13}C_6$]Mannose was purchased from Omicron Biochemicals, while all other reagents and solvents were purchased from Sigma Aldrich, Fisher Scientific and Alfa Aesar. Thin layer chromatography was performed using Merck silica gel 60F_{254} glass plates and visualised under UV and/or with an 8% H_2SO_4 stain. Column chromatography was carried out using Davisil high-purity grade silica gel, 60Å pore size, 40-63 µm particle size. HPLC was carried out using a Gilson-system on a C18 RP-phase column (VP 250/21 Nucleosil 100-5 C18, Machery Nagel). NMR coupled and ^{13}C decoupled ^1H NMR spectra were recorded in $CDCl_3$ or D_2O at 25°C on Varian instruments (300, 500 and 600 MHz for ^1H and 126 and 151 MHz for ^{13}C) and were calibrated with respect to tetramethylsilane. The un-labeled compounds were synthesised in parallel with the labeled compounds **1-7** and in cases where ^{13}C decoupled ^1H NMR spectra were difficult to obtain, the spectra of the un-labeled compounds were used to report the ^1H chemical shifts.[1]

Synthetic Scheme:



[1] Asterisk (*) indicates that the ^1H chemical shifts of the un-labeled compounds were reported.

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxy-ethylidene)-β-D-[$^{13}C_6$]mannopyranose (**1**).[1,2]



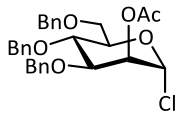
D-[$^{13}C_6$]Mannose (1.04 g, 5.58 mmoles) was dissolved in pyridine (11.3 mL) and acetic anhydride (10.6 mL) and stirred at r.t. for 15 hrs. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 1M HCl (50 mL). The aqueous layer was washed with CH_2Cl_2 (3 x 100 mL) and the combined organic fractions were dried over $MgSO_4$ and concentrated.

The crude per acetylated D-[$^{13}C_6$]mannose (2.37 g, 5.98 mmoles) was dissolved in anhydrous CH_2Cl_2 (3 mL) under N_2 and cooled to 0°C. To this 33% HBr in acetic acid (2.62 mL, 14.95 mmoles) was added dropwise and this was stirred at 0°C for 15 minutes and then at r.t. for 17 hrs. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with aq. Na_2CO_3 (50 mL). The aqueous layer was then washed with CH_2Cl_2 (3 x 100 mL) and the combined organic fractions were dried over $MgSO_4$ and concentrated.

The crude bromosugar (2.34 g, 5.07 mmoles) was then dissolved in anhydrous CH_2Cl_2 (10 mL) and anhydrous MeOH (8 mL) and to this *sym*-collidine (1.6 mL) was added. This was stirred at 40°C for 16 hrs, after which time the reaction mixture was concentrated and precipitated from pentane and diethyl ether to yield the crude acetylated orthoester (1.68 g, 82% over three steps).

The acetylated orthoester (1.60 g, 4.35 mmoles) was dissolved in CH_2Cl_2 (1 mL) and MeOH (10 mL) and to this 1M NaOMe (0.44 mL, 0.44 mmoles) was added and stirred at r.t. for 30 mins. The reaction mixture was then concentrated and dried. The crude triol (1.2 g, 4.93 mmoles) was dissolved in anhydrous DMF (15 mL) under N_2 and cooled to 0°C. NaH (1.03 g, 25.88 mmoles) was added over 15 mins and then left to stir at this temperature for a further 15 mins. BnBr (2.93 mL, 24.65) was then added and left to stir at r.t. for a total of 15 hrs. The reaction mixture was quenched with MeOH (5 mL), concentrated and purified using flash silica gel chromatography (9.75:0.25 cyclohexane/Et_3N to 9:0.75:0.25 cyclohexane/EtOAc/Et_3N) to yield compound **1** (2.06 g, 92%, exo/endo = 97:3). NMR of exo product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 – 7.16 (m, 15H), 5.35 (d, *J* = 2.5 Hz, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.78 (s, 2H), 4.60 (d, *J* = 11.3 Hz, 2H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.40 (dd, *J* = 4.0, 2.5 Hz, 1H), 3.92 (t, *J* = 9.3 Hz, 1H), 3.79 – 3.66 (m, 3H), 3.48 – 3.36 (m, 1H), 3.28 (s, 3H), 1.73 (s, 3H).* ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.2 (d, *J* = 2.7 Hz) (x2), 137.8 (d, *J* = 2.1 Hz), 128.5, 128.4, 128.3, 128.0, 128.0, 127.8, 127.5, 127.5, 97.6 (dd, *J* = 34.5, 3.8 Hz), 79.5 – 78.5 (m), 77.6 – 76.5 (m), 75.2, 74.7 – 73.6 (m), 73.3, 72.4, 69.5 – 68.3 (m), 49.8, 24.4. NMR data agrees with published values.[2]

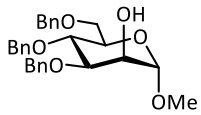
2-*O*-acetyl-3,4,6-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl chloride (**2**).[3]



Compound **1** (230 mg, 0.45 mmoles) was dissolved in anhydrous CH_2Cl_2 (3.5 mL) under N_2. To this TMSCl (0.11 mL, 0.85 mmoles) was added and stirred at 45°C for 2 hrs. The reaction mixture was concentrated and dried to yield compound **3** (210 mg, 91%). This was used in the following step without any further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.24 (m, 13H), 7.18 – 7.14 (m, 2H),

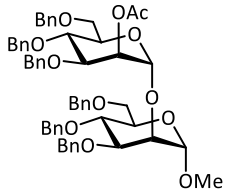
6.06 (bs, 1H), 5.46 (bs, 1H), 4.86 (d, *J* = 10.7 Hz, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.66 (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 11.2 Hz, 1H), 4.50 (dd, *J* = 11.4, 4.5 Hz, 2H), 4.25 (bd, *J* = 8.7 Hz, 1H), 4.08 – 4.02 (m, 1H), 3.98 (t, *J* = 10.2 Hz, 1H), 3.83 (dd, *J* = 11.2, 3.8 Hz, 1H), 3.69 (dd, *J* = 11.26, 1.76 Hz, 1H), 2.16 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 138.1 (d, *J* = 3.4 Hz), 137.9 (d, *J* = 2.4 Hz), 137.5 (d, *J* = 2.9 Hz), 128.5 (x2), 128.4 (x3), 128.1 (x2), 128.0 (x2), 127.9 (x2), 127.8 (x2), 127.7 (x2), 90.3 (dd, *J* = 45.1, 2.9 Hz), 77.3 – 76.2 (m), 75.4, 74.7 – 73.9 (m), 73.8 – 73.1 (m), 73.5, 72.2, 70.9 (dd, *J* = 44.9, 38.8 Hz), 68.3 – 67.6 (m), 20.9. NMR data agrees with published values.[3,4]

Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (**3**).[1]



Compound **1** (263 mg, 0.51 mmoles) was dissolved in anhydrous MeOH (2.2 mL) under N_2. To this TMSCl (0.11 mL, 8.524 mmoles) was added and stirred at r.t. for 15 hours. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with aq. $NaHCO_3$. The aqueous layer was then washed with CH_2Cl_2 (3 x 100 mL) and the combined organic fractions were dried over $MgSO_4$, concentrated and purified using flash silica gel chromatography (8:2 cyclohexane/EtOAc) to yield compound **2** (173 mg, 72%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.23 (m, 13H), 7.17 (dd, *J* = 7.5, 1.9 Hz, 2H), 4.82 (d, *J* = 10.9 Hz, 1H), 4.79 (bs, 1H), 4.72 – 4.64 (m, 2H), 4.66 (d, *J* = 1.6 Hz, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.50 (d, *J* = 10.9 Hz, 1H), 4.02 (bs, 1H), 3.89 – 3.80 (m, 2H), 3.78 – 3.68 (m, 3H), 3.36 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.3 (d, *J* = 3.4 Hz), 138.2 (d, *J* = 2.3 Hz), 137.9 (d, *J* = 2.7 Hz), 128.5 (x2), 128.3 (x3), 127.9, 127.8 (x2), 127.8 (x3), 127.8 (x2), 127.6, 127.6, 100.3 (d, *J* = 48.6 Hz), 80.4 – 79.5 (m), 75.0, 74.2 (t, *J* = 41.4 Hz), 73.5, 72.0, 70.9 (ddt, *J* = 43.2, 40.8, 2.3 Hz), 68.9 (dt, *J* = 44.9, 3.2 Hz), 68.2 (dd, *J* = 48.5, 35.7 Hz), 54.9 (dd, *J* = 4.0, 2.0 Hz). NMR data agrees with published values.[3]

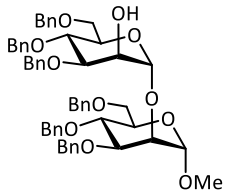
Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (**4**).



Procedure adapted from Bundle *et al*.[5] Compounds **2** (100 mg, 0.212 mmoles) and **3** (210 mg, 0.407 mmoles) were dried on the Schlenck line overnight then dissolved in CH_2Cl_2 (3 mL) under N_2, cooled to -15°C and stirred with activated 4Å molecular sieves (300 mg) for 45 mins. In a separate round bottom flask the AgOTf (256 mg, 0.996 mmoles) was dissolved in toluene (1 mL) and CH_2Cl_2 (1 mL) at r.t. under N_2 and stirred with activated 4Å molecular sieves (80 mg) for the same amount of time. This was then added dropwise to the mixture of compound **2** and **3** and this was left to slowly warm to r.t. over 4 hrs. The reaction mixture was then quenched with Et_3N (0.3 mL), filtered through Celite, concentrated and purified using flash silica gel chromatography (8:2 cyclohexane/EtOAc) to yield compound **4** (191 mg, 94%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.21 (m, 26H), 7.21 – 7.16 (m, 4H), 5.53

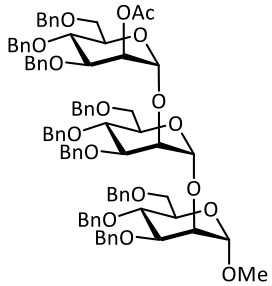
(bs, 1H), 5.07 (bs, 1H), 4.84 (dd, *J* = 10.8, 5.7 Hz, 2H), 4.77 (s, 1H), 4.67 (s, 3H), 4.65 (d, *J* = 2.9 Hz, 2H), 4.55 (dd, *J* = 11.6, 6.6 Hz, 2H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.46 (d, *J* = 10.8 Hz, 1H), 4.40 (d, *J* = 10.9 Hz, 1H), 4.03 – 3.92 (m, 3H), 3.92 – 3.86 (m, 1H), 3.86 – 3.79 (m, 2H), 3.80 – 3.72 (m, 2H), 3.73 – 3.66 (m, 3H), 3.25 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.5 (x2), 138.4, 138.4, 138.2, 138.0, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.6, 127.5, 127.5 (x2), 127.4, 127.4, 99.8 (d, *J* = 17.4 Hz), 99.4 (d, *J* = 17.9 Hz), 80.1 – 79.3 (m), 78.1 (dd, *J* = 41.6, 37.8 Hz), 75.1 (x2), 74.56 (td, *J* = 41.3, 28.6 Hz), 73.5, 73.3, 71.9 (x2), 71.7 (td, *J* = 42.5, 41.7, 19.1 Hz) (x3), 69.6 – 68.8 (m) (x2), 68.8 – 68.2 (m) (x3), 54.6, 21.1. NMR data agrees with published values.[3,6]

Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]manno-pyranoside (**5**)



Compound **4** (185 mg, 0.195 mmoles) was dissolved in CH_2Cl_2 (1 mL) and MeOH (5.5 mL) and to this 1M NaOMe (78 µL, 0.078 mmoles) was added and stirred at r.t. for 4 hrs. Amberlite (IR 120 H+ form) resin (30 mg) was added and stirred for 30 mins. The reaction mixture was then filtered, concentrated and purified using flash silica gel chromatography (100% toluene to 9:1 toluene/acetone) to yield compound **5** (143 mg, 80%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 – 7.22 (m, 26H), 7.19 (m, 4H), 5.14 (bs, 1H), 4.83 (dd, *J* = 13.8, 9.6 Hz, 2H), 4.80 (bs, 1H), 4.71 – 4.66 (m, 2H), 4.66 – 4.61 (m, 2H), 4.59 (d, *J* = 11.3 Hz, 1H), 4.55 (s, 2H), 4.55 – 4.47 (m, 3H), 4.12 (t, *J* = 3.3 Hz, 1H), 4.03 (s, 1H), 3.94 – 3.81 (m, 6H), 3.74 – 3.68 (m, 4H), 3.24 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.6, 138.5 (d, *J* = 3.4 Hz), 138.4 (d, *J* = 3.4 Hz), 138.3 (d, *J* = 2.8 Hz), 138.2, 137.9 (d, *J* = 2.8 Hz), 128.5, 128.4, 128.3 (x2), 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 101.1 (d, *J* = 48.6 Hz), 99.8 (d, *J* = 47.9 Hz), 80.3 – 79.4 (m) (x2), 74.6 (dt, *J* = 50.3, 41.3 Hz) (x3), 73.4, 73.3, 71.6 (ddd, *J* = 44.5, 41.0, 23.6 Hz) (x2), 72.2, 72.1, 69.2 (ddt, *J* = 44.9, 21.1, 3.3 Hz) (x2), 68.5 (ddd, *J* = 48.6, 35.7, 3.4 Hz), 54.7 (dd, *J* = 4.0, 2.0 Hz). NMR data agrees with published values.[3,6,7]

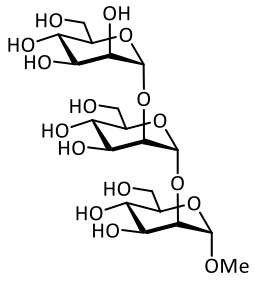
Methyl 2-O-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (**6**).



Compounds **2** (83.5 mg, 0.162 mmoles) and **5** (70 mg, 0.077 mmoles) were dried on the Schlenck line overnight, then dissolved in CH_2Cl_2 (3 mL) under N_2, cooled to -15°C and stirred with activated 4Å

molecular sieves (250 mg) for 45 mins. In a separate round bottom flask the AgOTf (157 mg, 0.611 mmoles) was dissolved in toluene (1 mL) and CH_2Cl_2 (1 mL) at r.t. under N_2 and stirred with activated 4Å molecular sieves (50 mg) for the same amount of time. This was then added dropwise to the mixture of compound **2** and **5** and this was left to slowly warm to r.t. over 4.5 hrs. The reaction mixture was then quenched with Et_3N (0.3 mL), filtered through Celite, concentrated and purified using flash silica gel chromatography (8:2 cyclohexane/EtOAc) to yield compound **6** (100 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.09 (m, 45H), 5.53 (bs, 1H), 5.19 (bs, 1H), 5.04 (bs, 1H), 4.88 – 4.77 (m, 3H), 4.75 – 4.36 (m, 14H), 4.31 (d, *J* = 12.1 Hz, 1H), 4.13 – 4.05 (m, 2H), 4.01 – 3.59 (m, 15H), 3.52 (d, *J* = 10.6 Hz, 1H), 3.22 (s, 3H), 2.12 (s, 3H).* ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 138.6, 137.5, 138.4 (x2), 138.3, 138.1, 128.3, 128.3, 128.2, 128.1, 127.9, 127.8 (x2), 127.7, 127.6, 127.5, 127.4 (x2), 100.6 (d, *J* = 48.0 Hz), 99.7 (d, *J* = 46.7 Hz), 99.4 (d, *J* = 44.4 Hz), 79.4 (t, *J* = 39.5 Hz) 79.7 (t, *J* = 39.7 Hz), 78.1 (t, *J* = 39.9 Hz), 75.5 – 73.4 (m) (x4), 75.3, 75.0 (x2), 72.8 – 71.1 (m) (x3), 73.3 (x3), 72.1, 71.8 (x2), 69.4 (dd, *J* = 44.7, 21.0 Hz) (x3), 69.1 – 68.2 (m) (x2), 54.6, 21.1. NMR data agrees with published values.[8]

Methyl α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranoside (**7, Man$_3$**).



Compound **6** (100 mg, 0.072 mmoles) was dissolved in MeOH (2 mL) and to this 1M NaOMe (100 µL, 0.100 mmoles) was added and stirred at r.t. for 3 hrs. Amberlite (IR 120 H+ form) resin (20 mg) was then added and stirred for 30 mins. The reaction mixture was then filtered, concentrated and purified using flash silica gel chromatography (8:2 cyclohexane/EtOAc) to yield the benzylated trimannoside (58 mg, 60%).

The benzylated trimannoside (50 mg, 0.037 mmoles) was dissolved in EtOAc (0.8 mL) and to this AcOH (0.2 mL) and a suspension of Pd/C (10% wt) (36 mg, 0.033 mmoles) in anhydrous MeOH (2 mL) was added. The round bottom flask was purged with hydrogen and left to stir under a balloon of H_2 at r.t. for 16 hrs. The reaction mixture was filtered through Celite, concentrated to yield crude compound **7** (21 mg, 54%). A portion of this was then purified using reverse phase HPLC. ^{1}H NMR (500 MHz, D_2O) δ 5.18 (bs, 1H), 4.93 (bs, 1H), 4.87 (bs, 1H), 3.99 (bs, 1H), 3.95 (bs, 1H), 3.87 – 3.69 (m, 7H), 3.67 – 3.58 (m, 5H), 3.58 – 3.44 (m, 4H), 3.28 (s, 3H).* ^{13}C NMR (126 MHz, D_2O) δ 102.2 (d, *J* = 45.1 Hz), 100.6 (d, *J* = 47.6 Hz), 99.2 (d, *J* = 47.0 Hz), 79.2 – 77.9 (m) (x2), 72.8 (dt, *J* = 85.2, 41.6 Hz) (x3), 70.8 – 69.2 (m) (x4), 66.9 (tt, *J* = 39.2, 14.4 Hz) (x3), 61.9 – 60.6 (m) (x3), 54.7. NMR data agrees with published values.[8,9]

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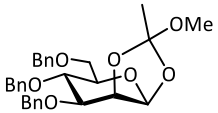
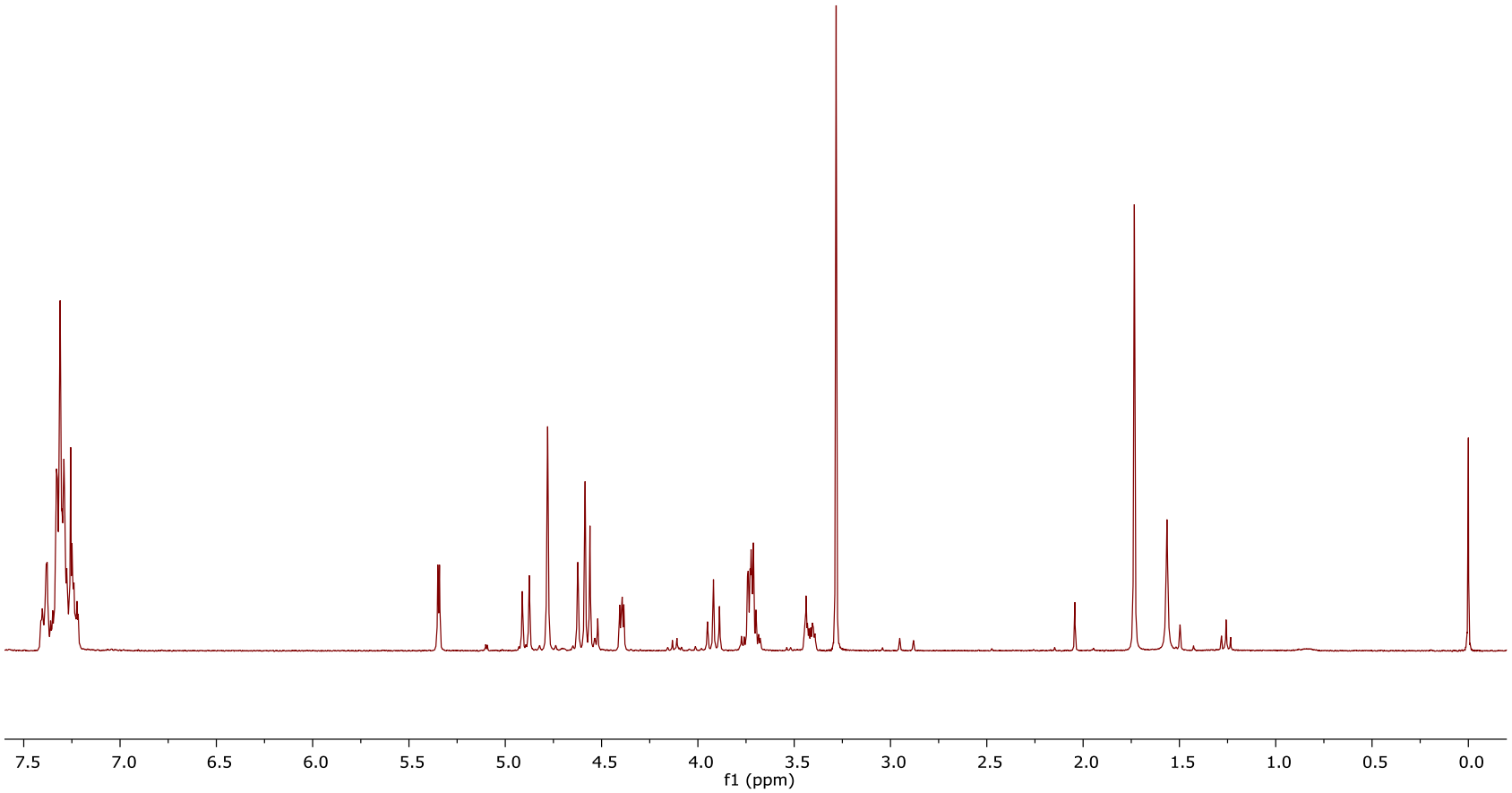
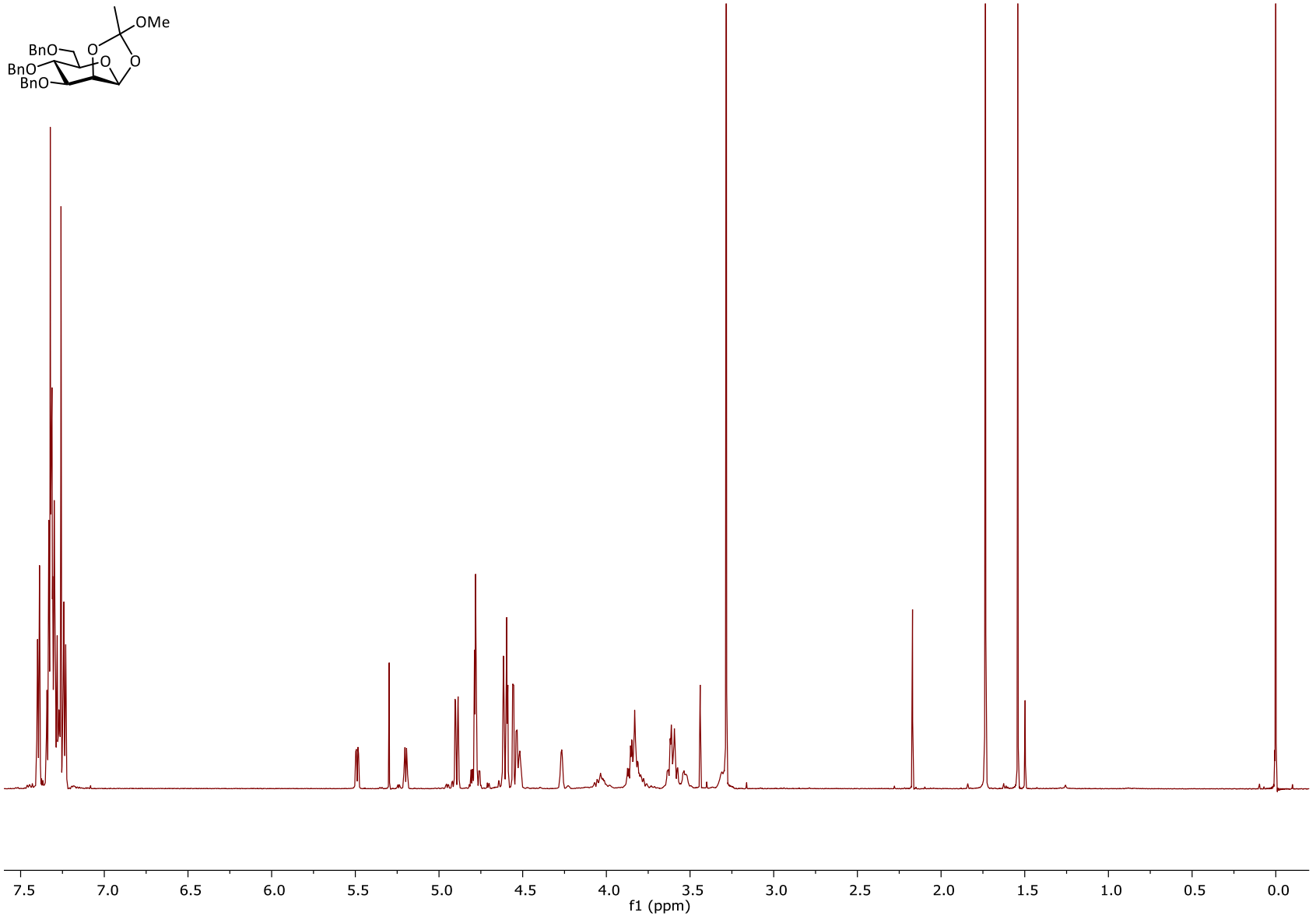
^{1}H NMR spectrum of 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxy-ethylidene)-β-D-mannopyranose (1).*

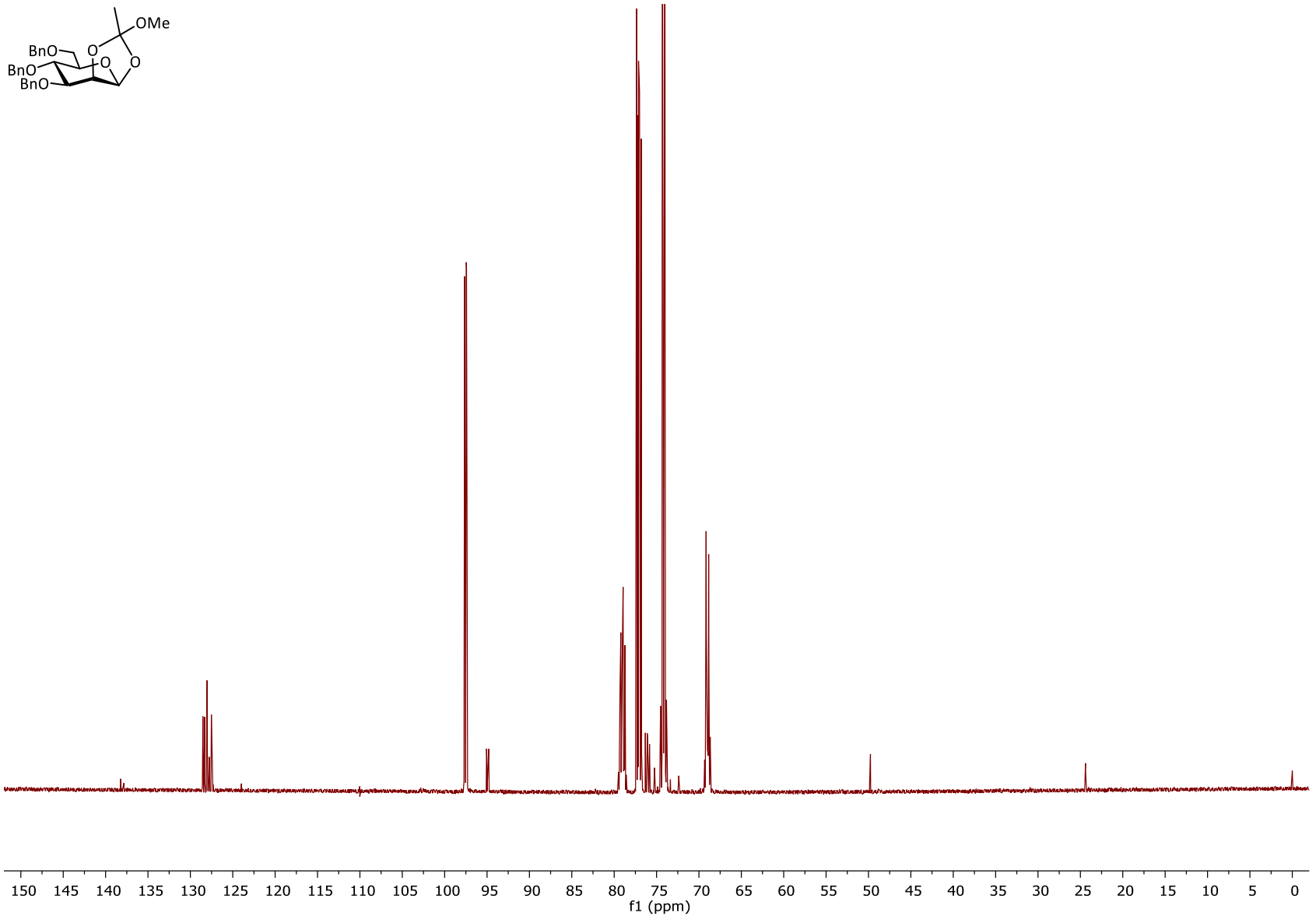




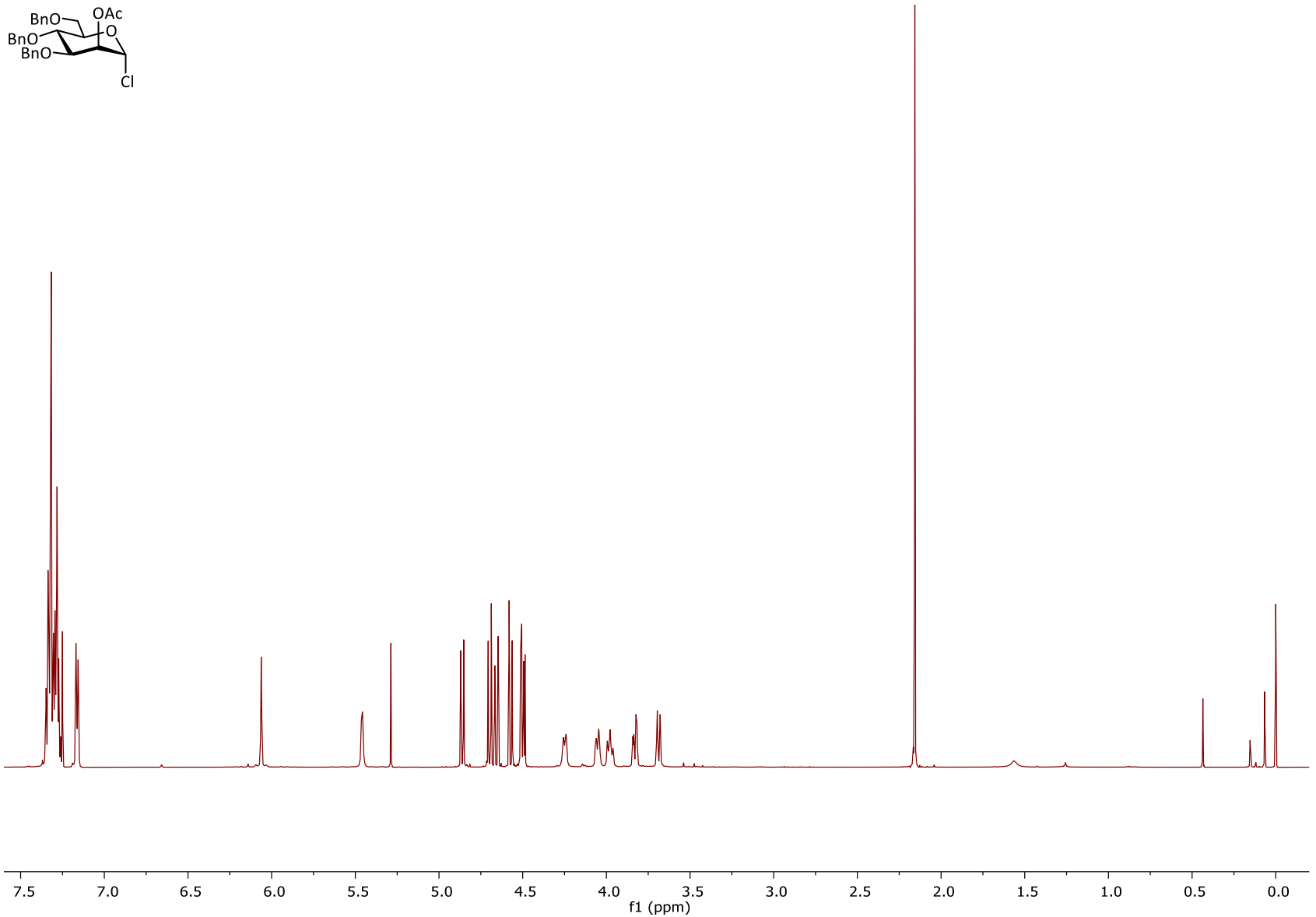
^{1}H NMR spectrum of 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxy-ethylidene)-β-D-[$^{13}C_6$]mannopyranose (1).



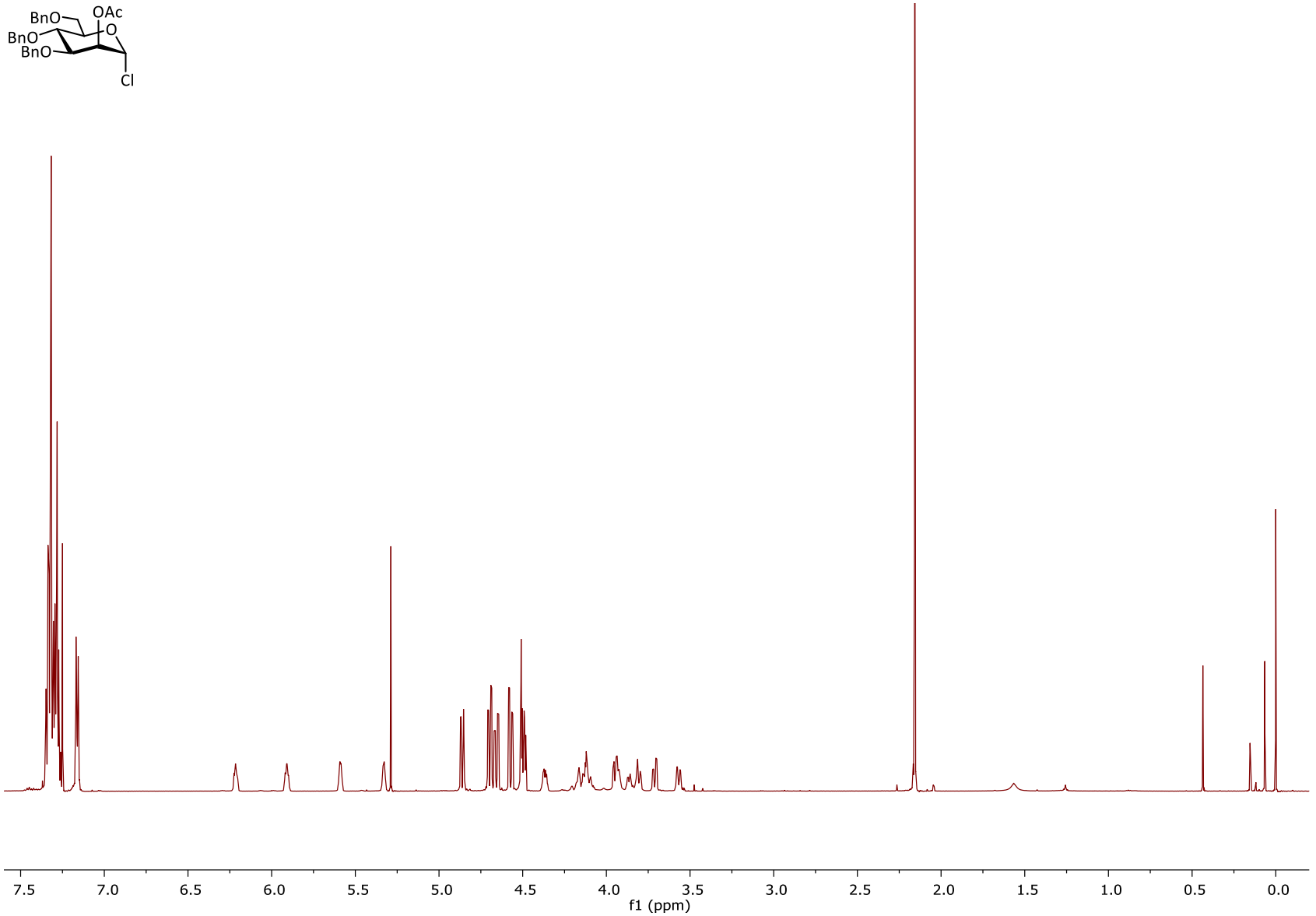
^{13}C NMR spectrum of 3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-methoxy-ethylidene)-β-D-[$^{13}C_6$]mannopyranose (1).



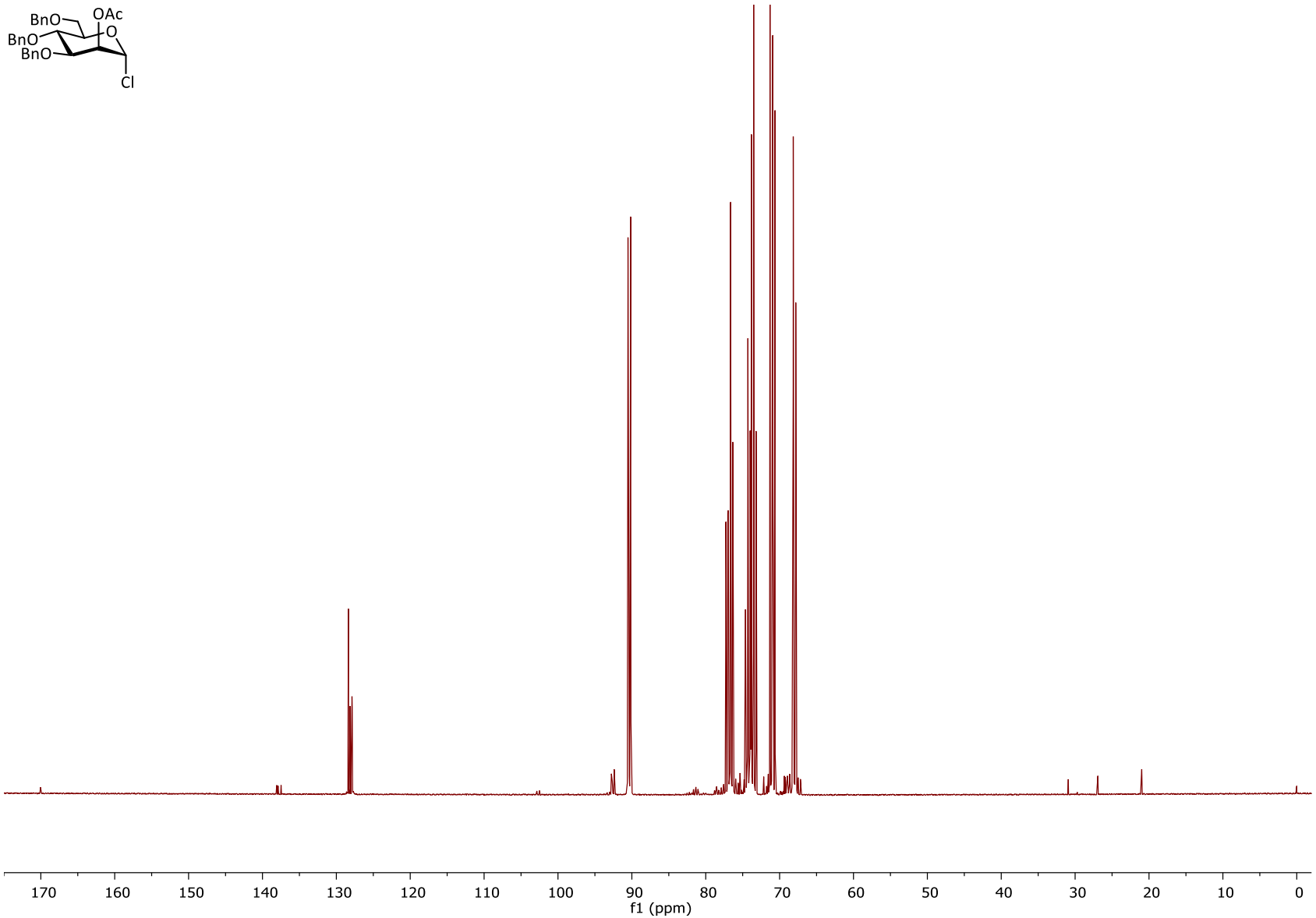
^{1}H NMR (^{13}C decoupled) spectrum of 2-*O*-acetyl-3,4,6-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl chloride (2).



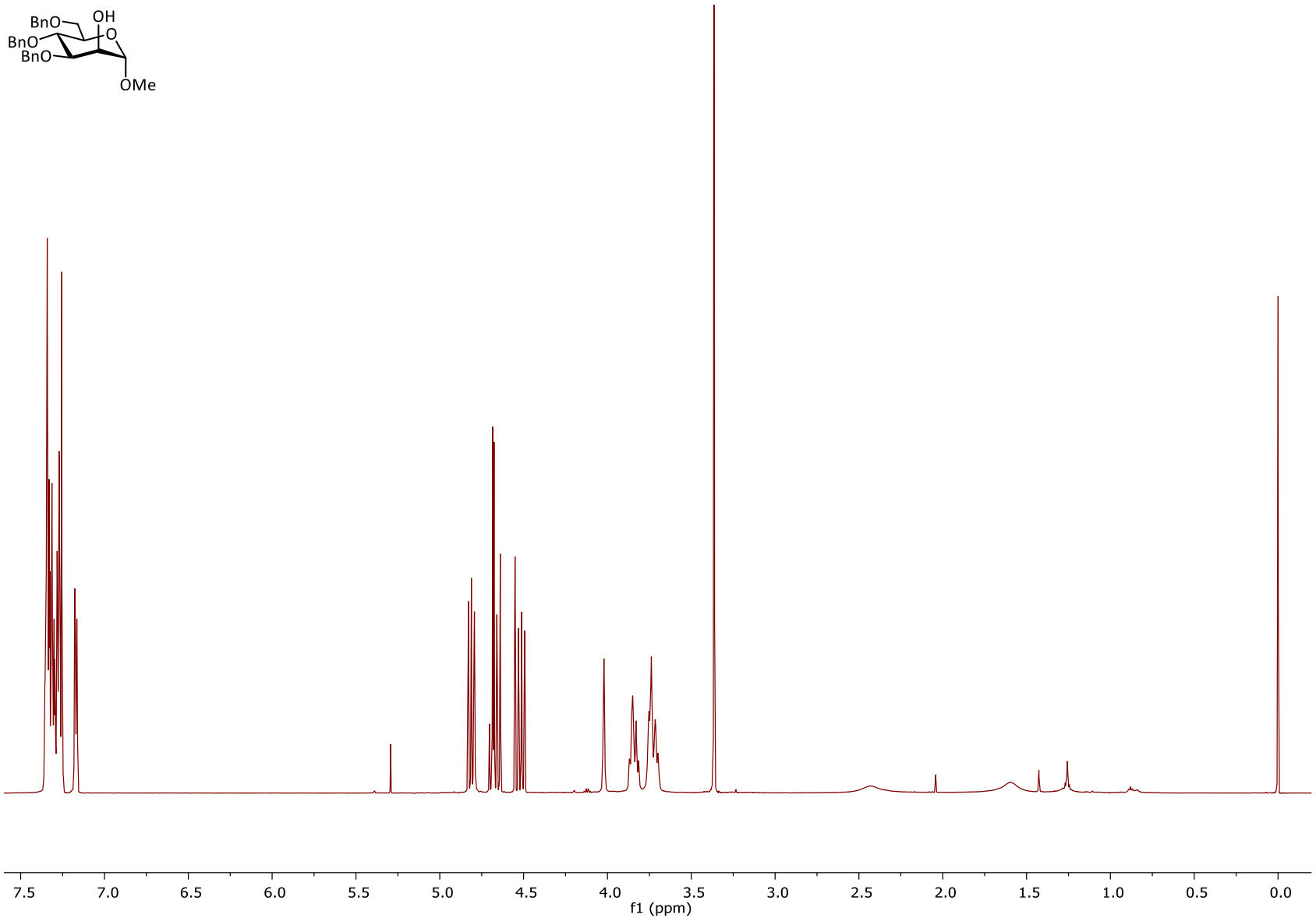
^{1}H NMR spectrum of 2-*O*-acetyl-3,4,6-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl chloride (2).



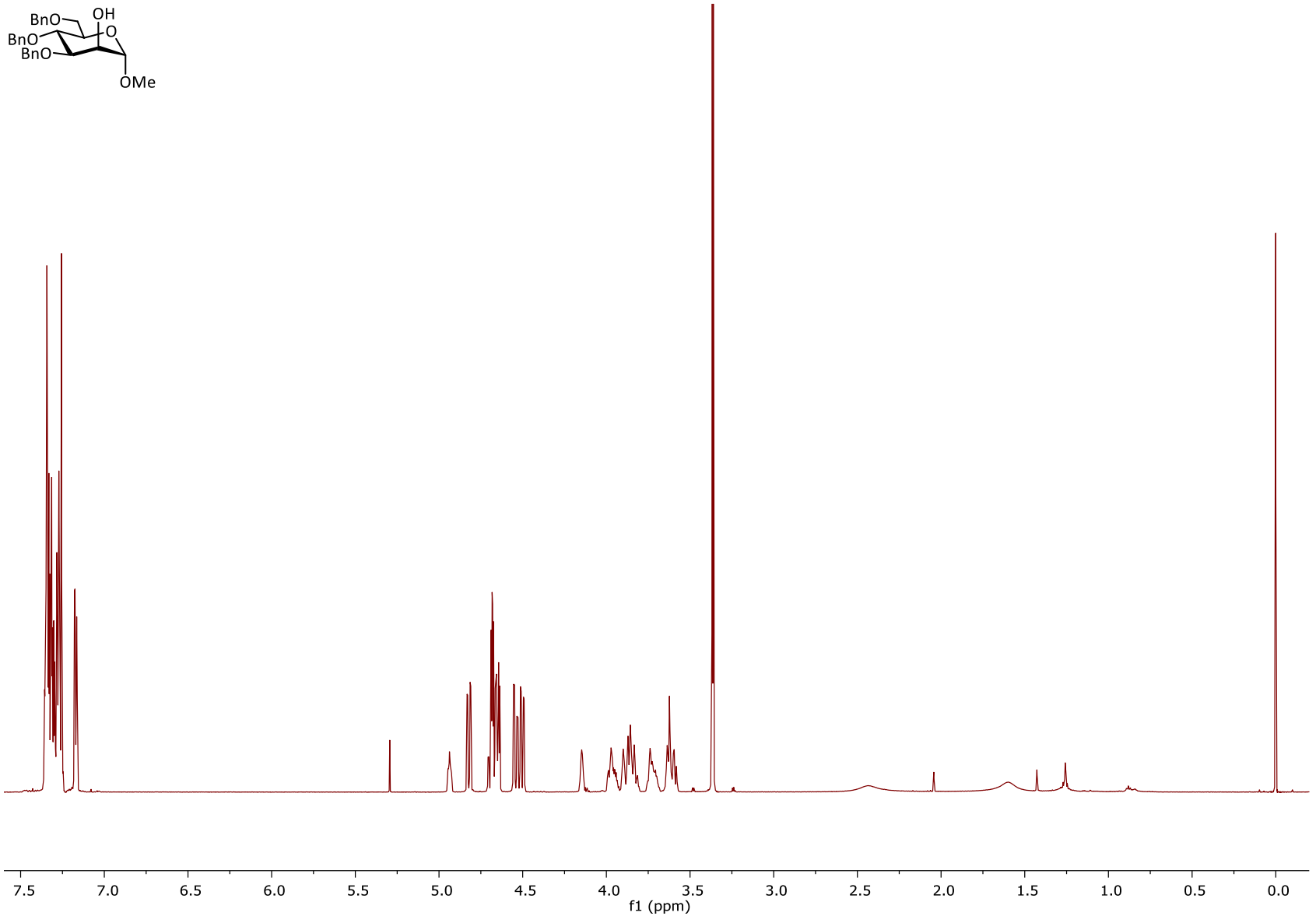
^{13}C NMR spectrum of 2-*O*-acetyl-3,4,6-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl chloride (2).



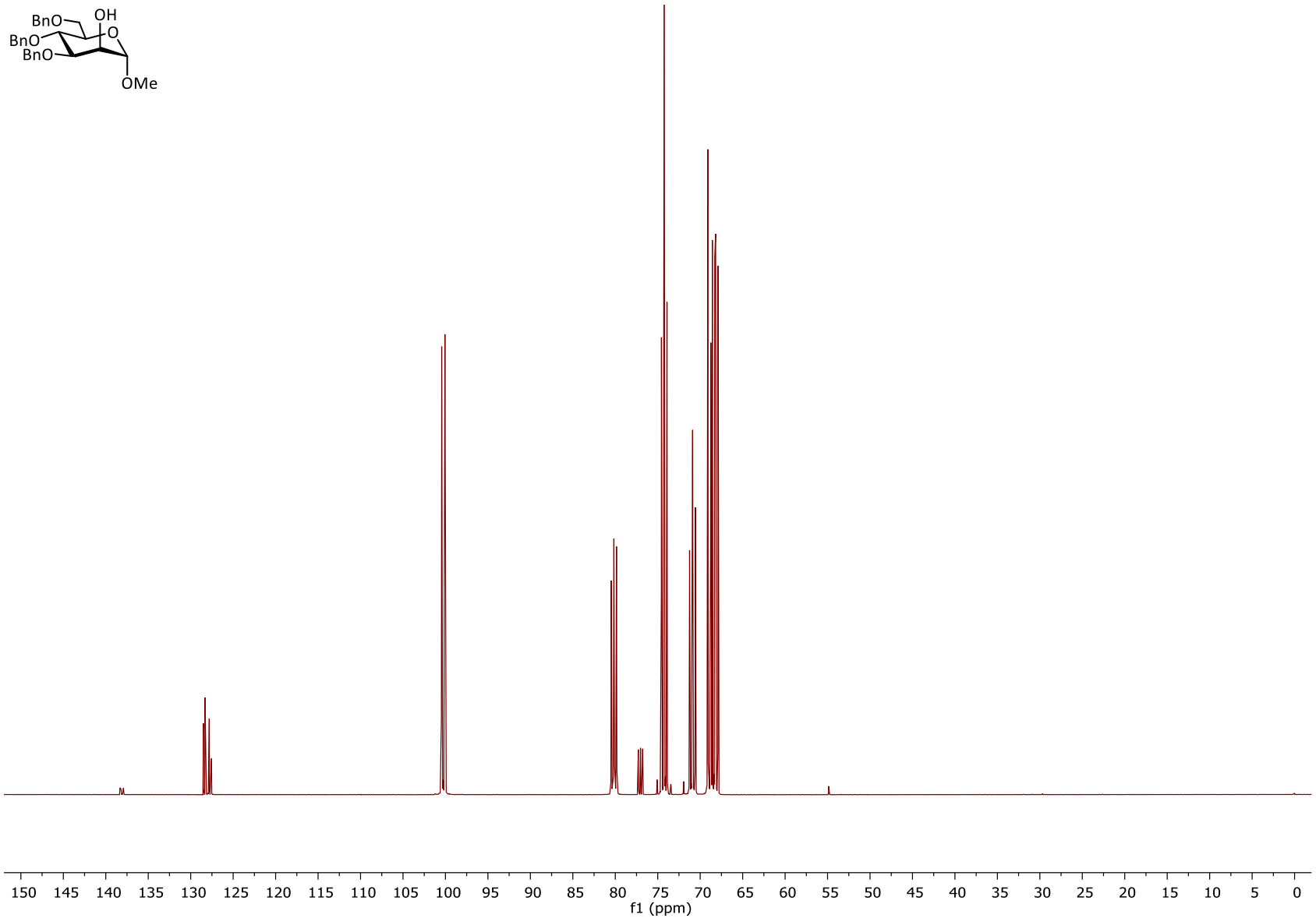
^{1}H NMR (^{13}C decoupled) spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (3).



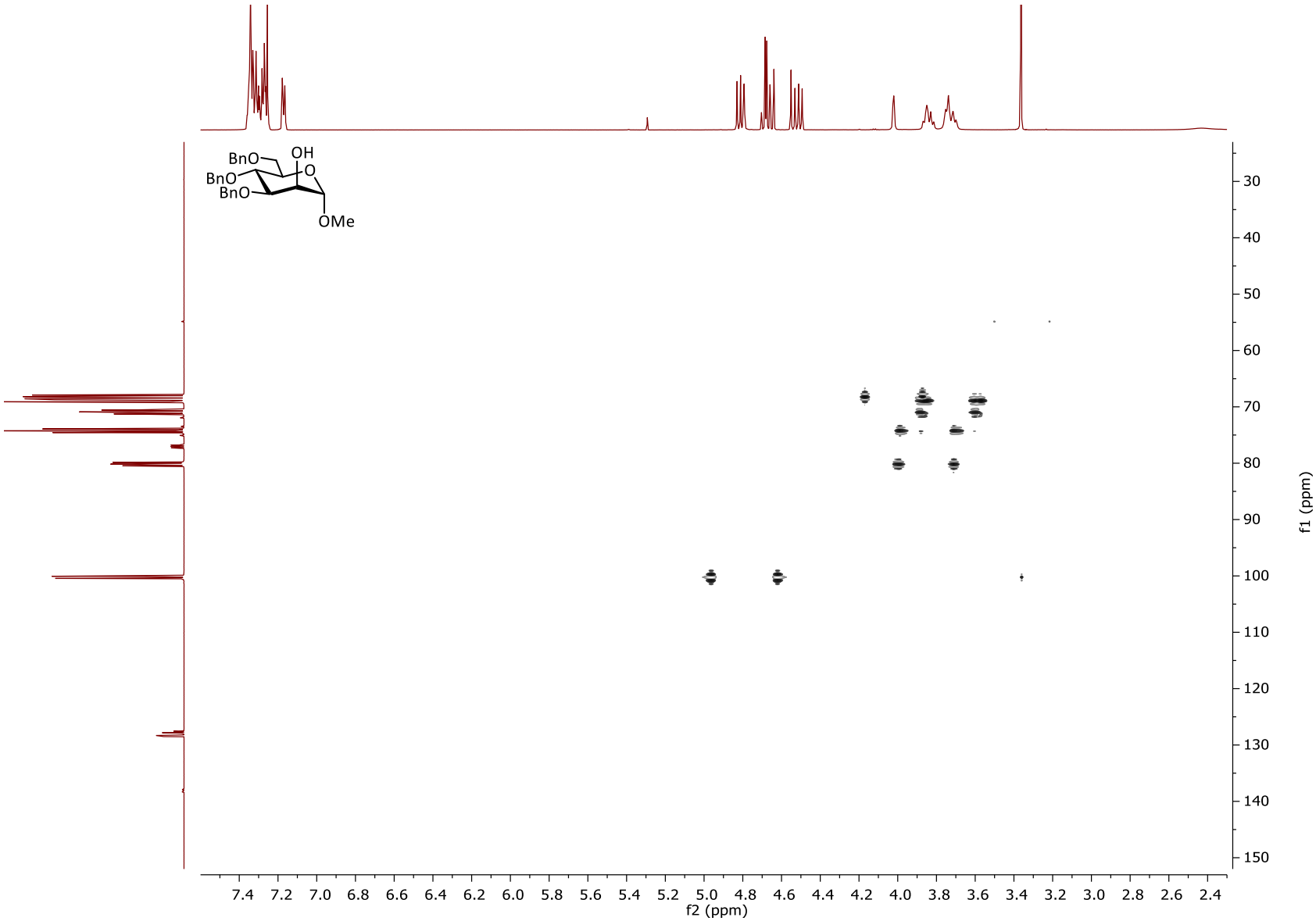
^{1}H NMR spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (3).



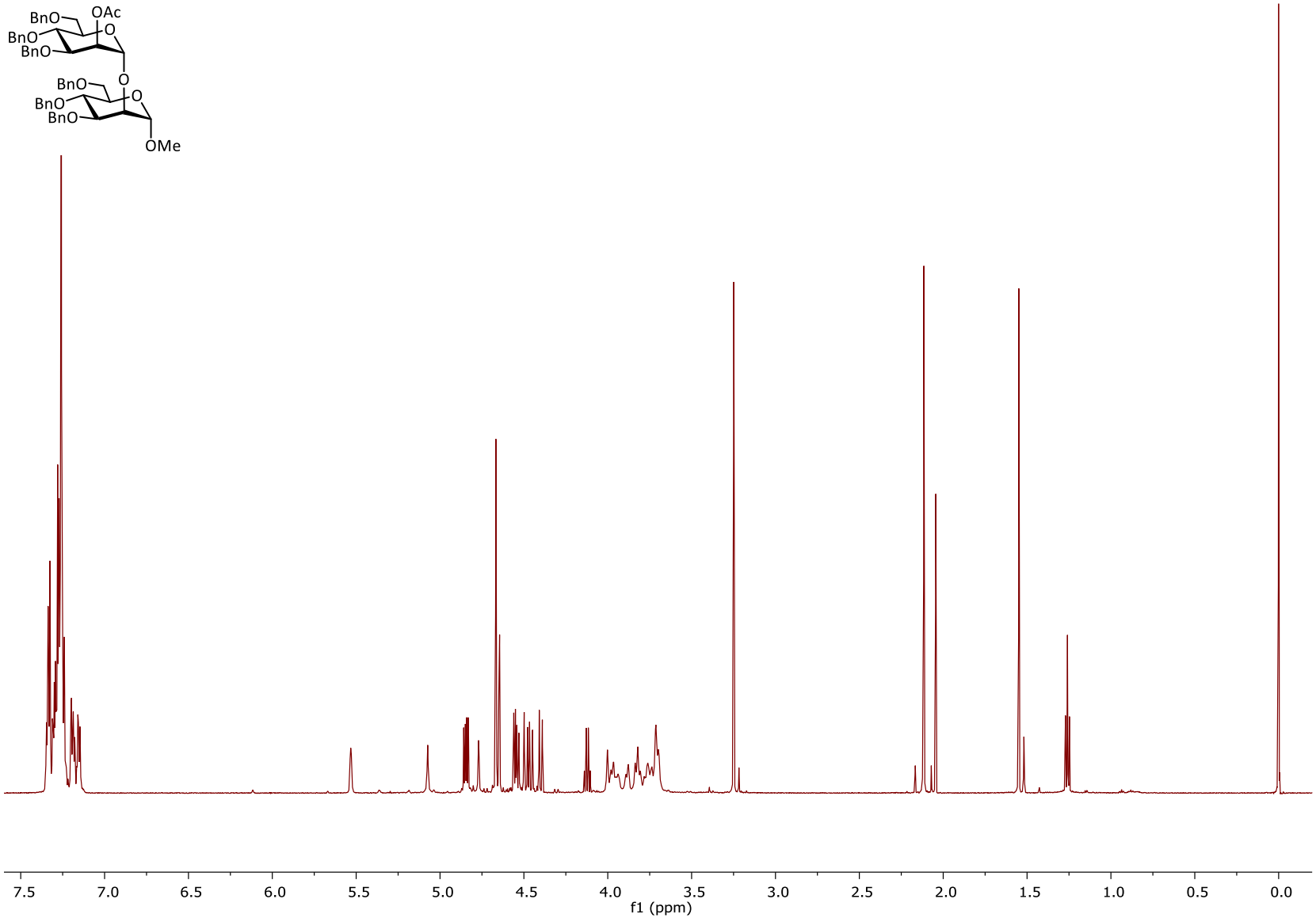
^{13}C NMR spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (3).



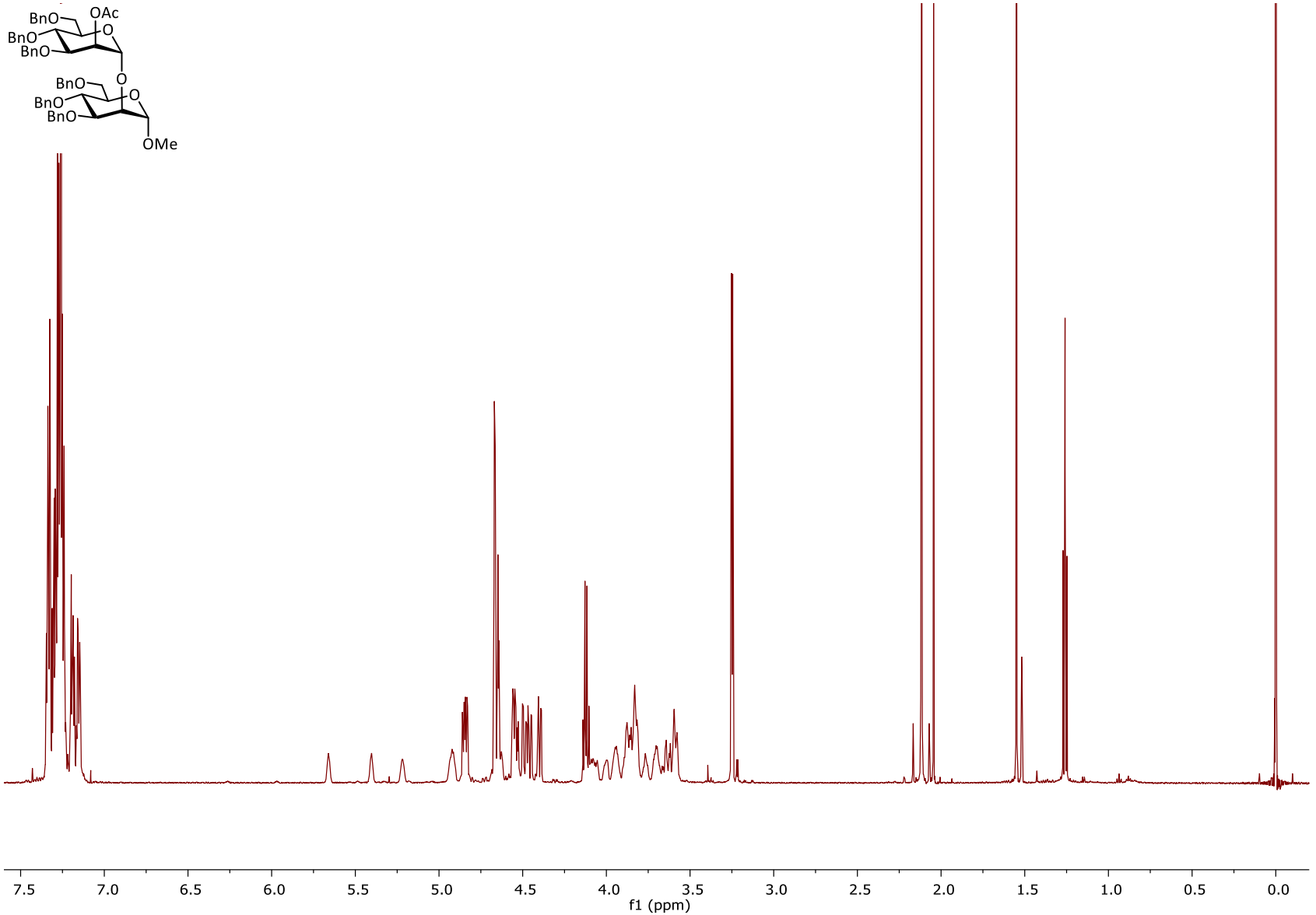
Coupled HSQC spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (3).



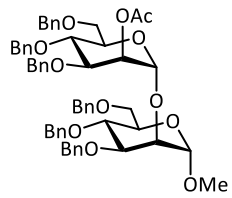
^{1}H NMR (^{13}C decoupled) spectrum of Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (4).

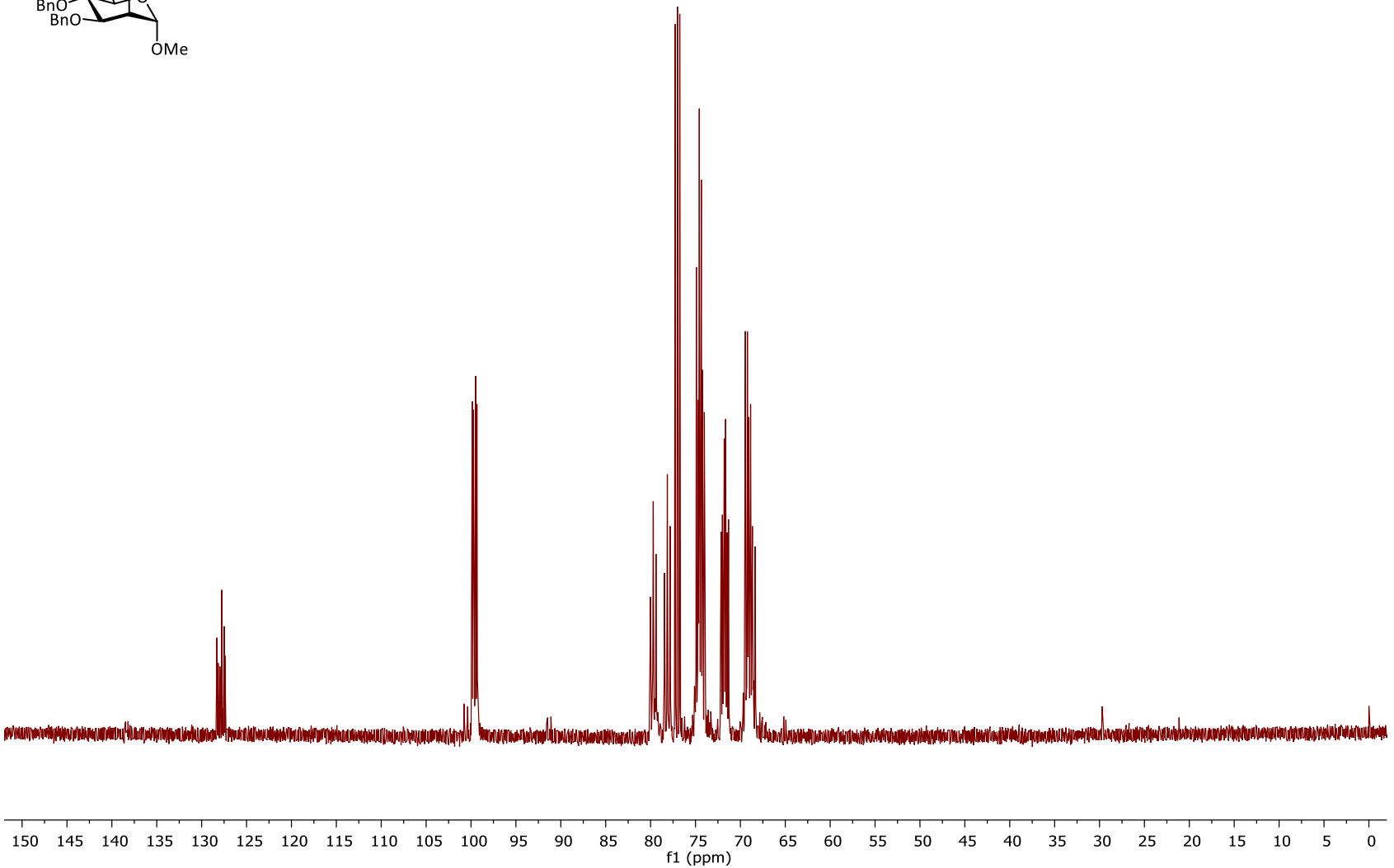


^{1}H NMR spectrum of Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (4).

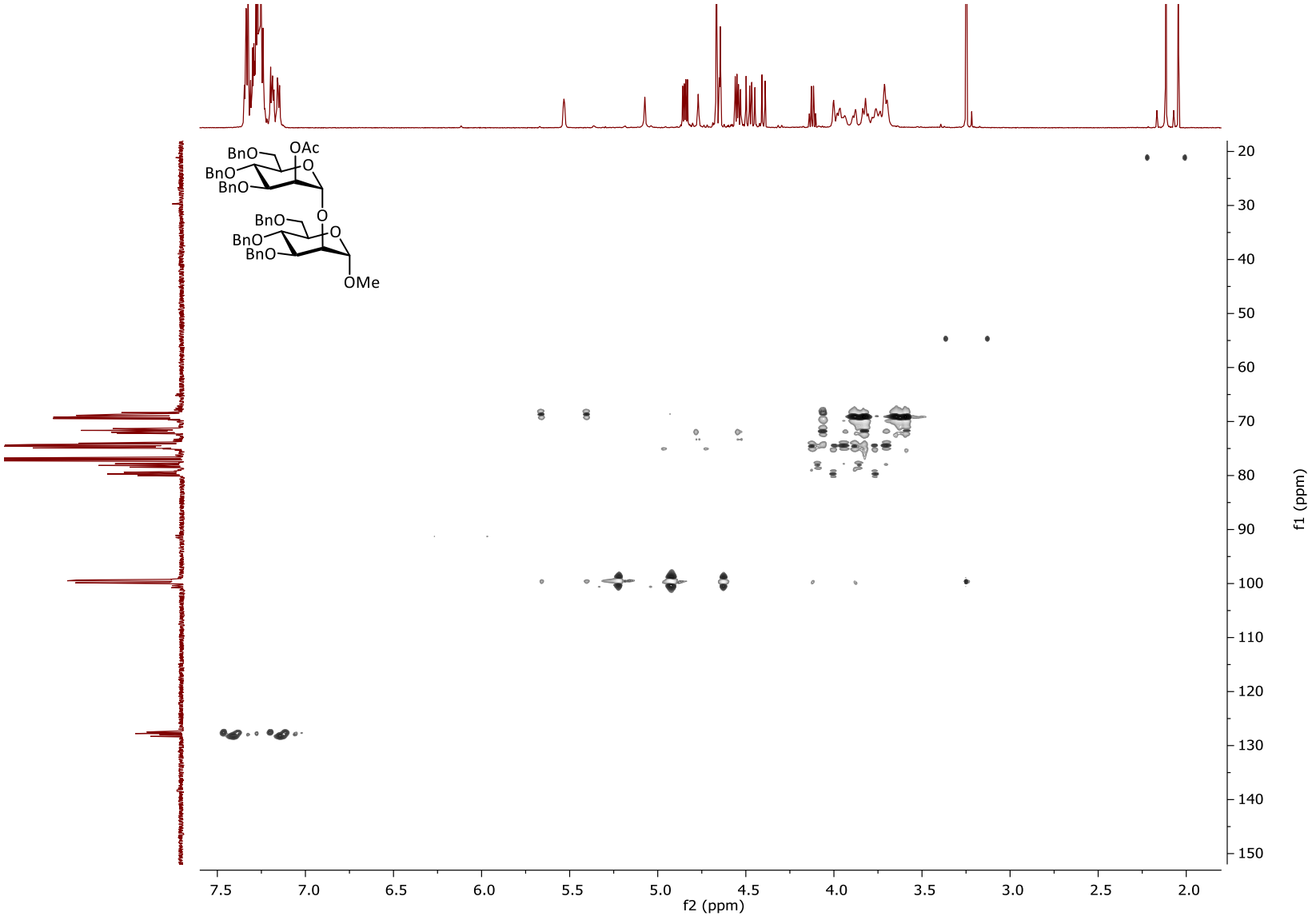


^{13}C NMR spectrum of Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (4).

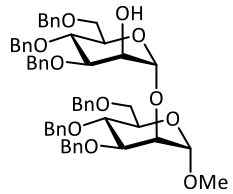


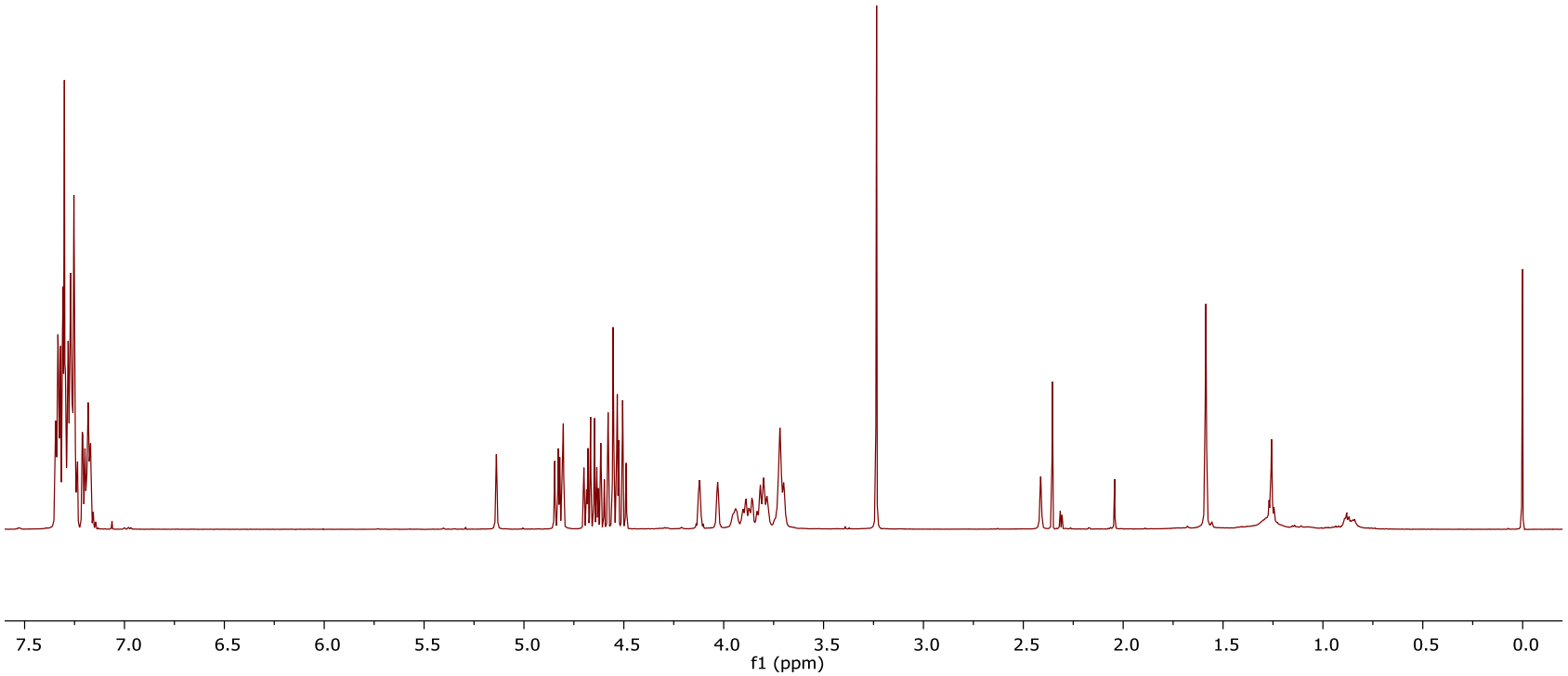


Coupled HSQC spectrum of Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]manno-pyranoside (4**).**

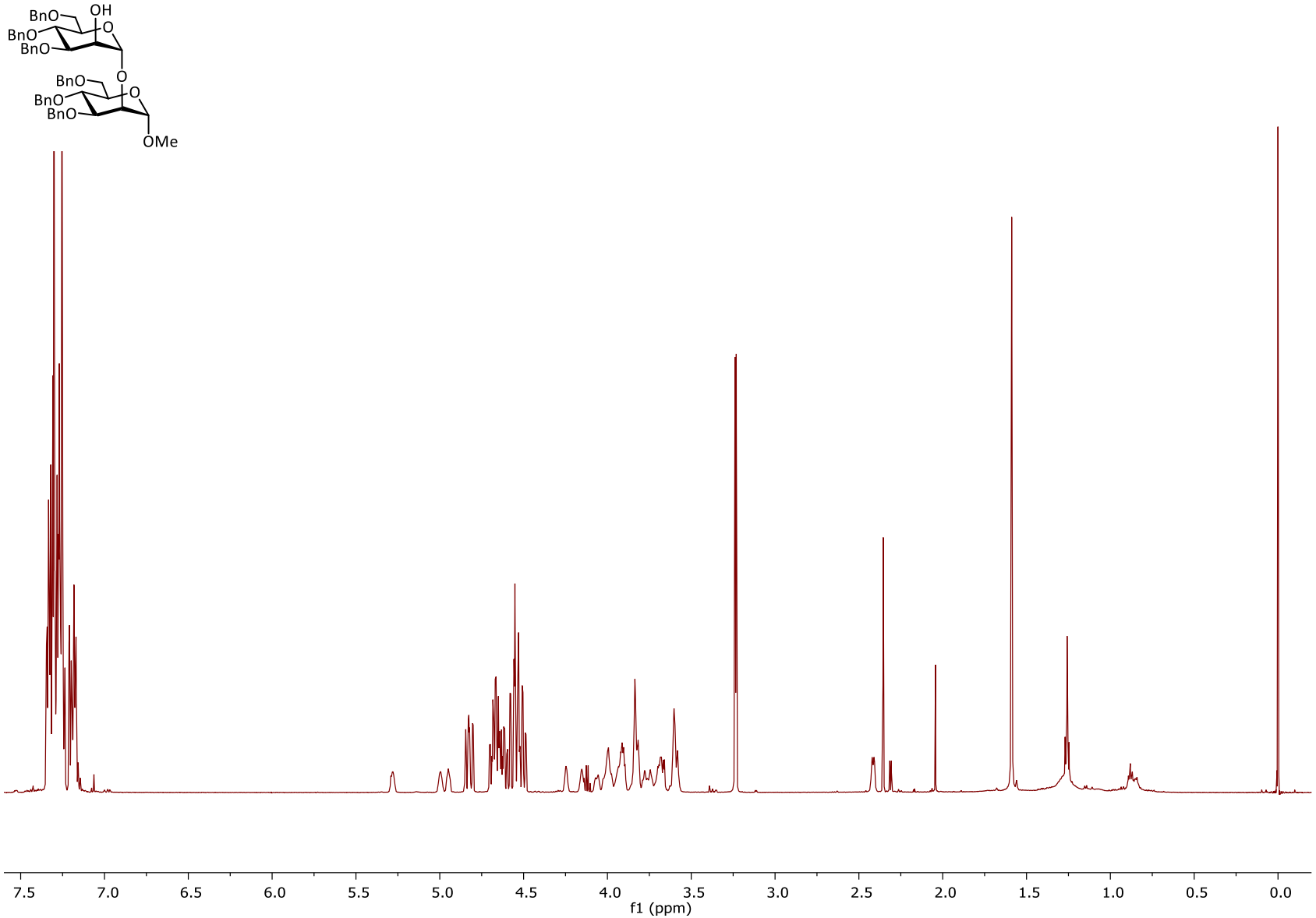


^{1}H NMR (^{13}C decoupled) spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]manno-pyranoside (5).

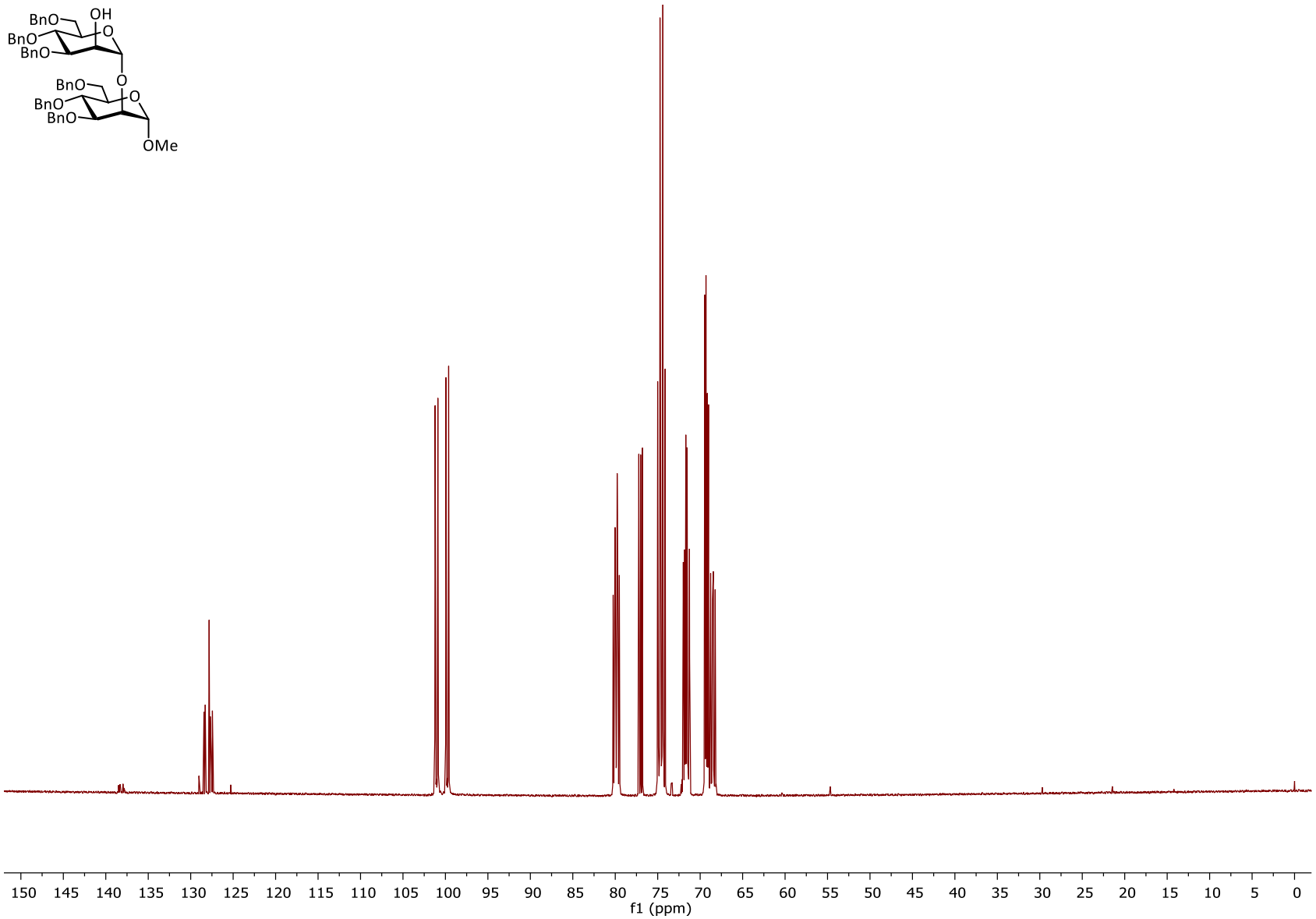




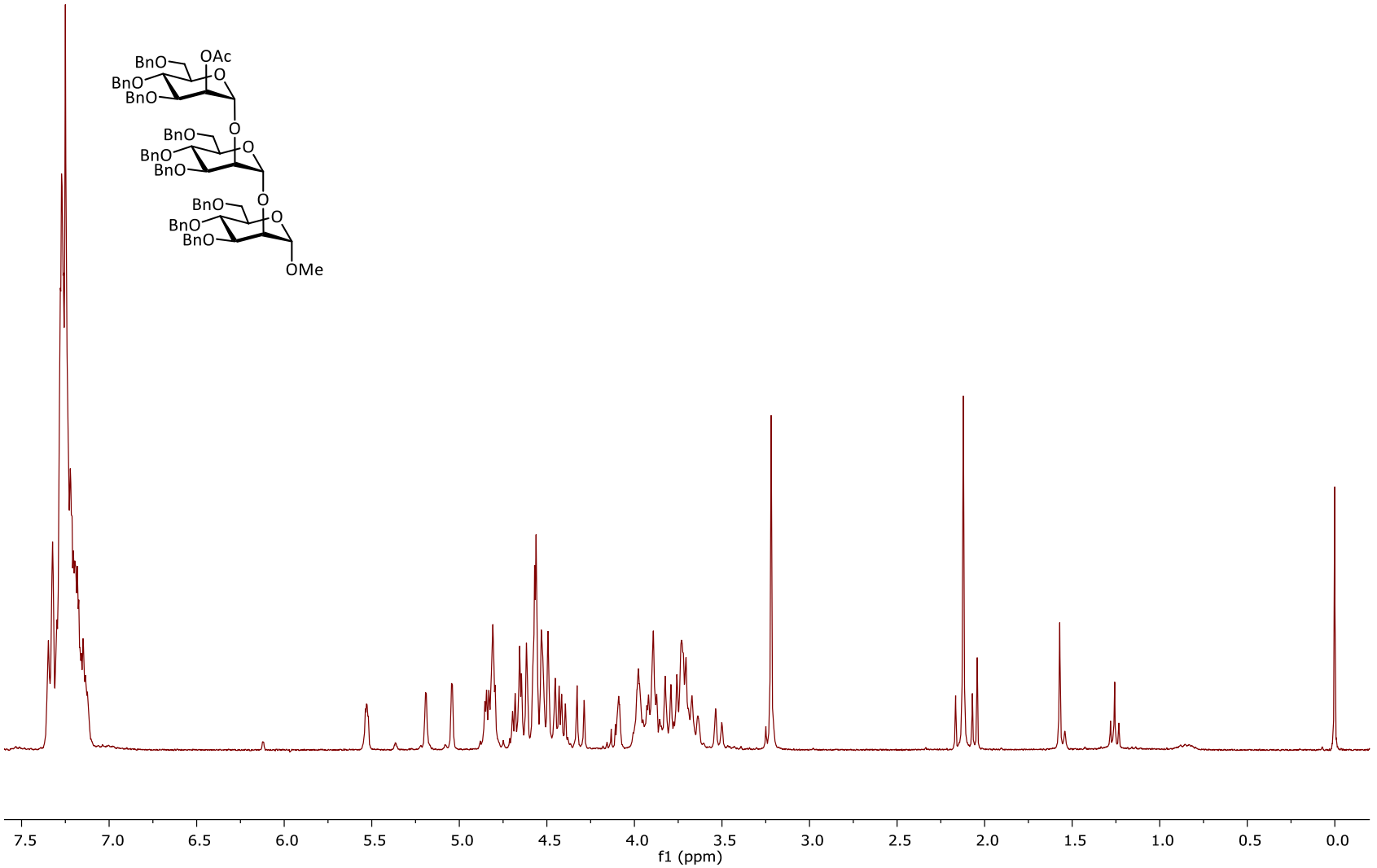
^{1}H NMR spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (5).



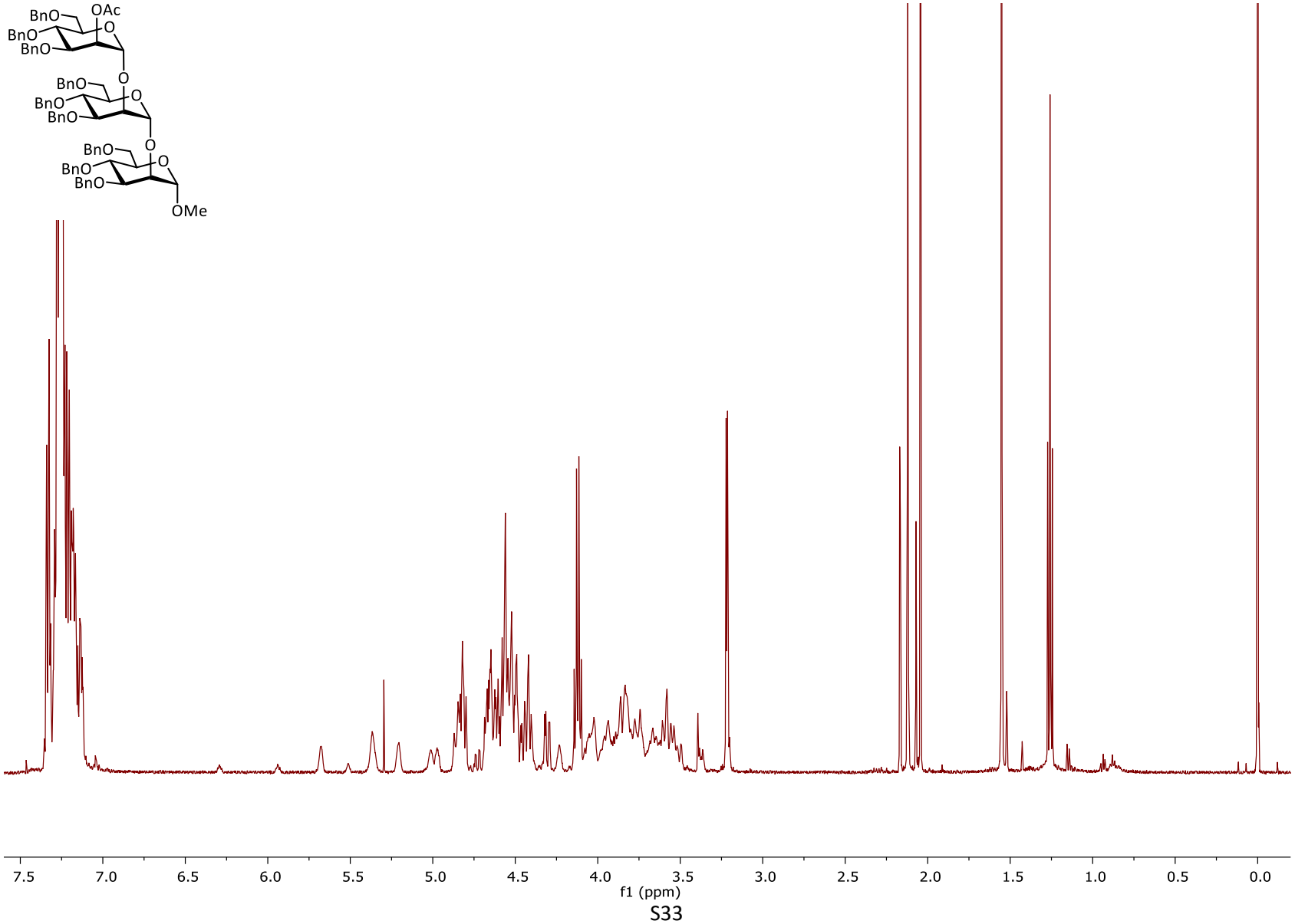
^{13}C NMR spectrum of Methyl 3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (5).



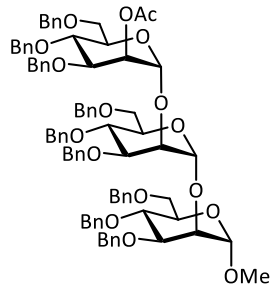
^{1}H NMR spectrum of Methyl 2-O-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (6).*

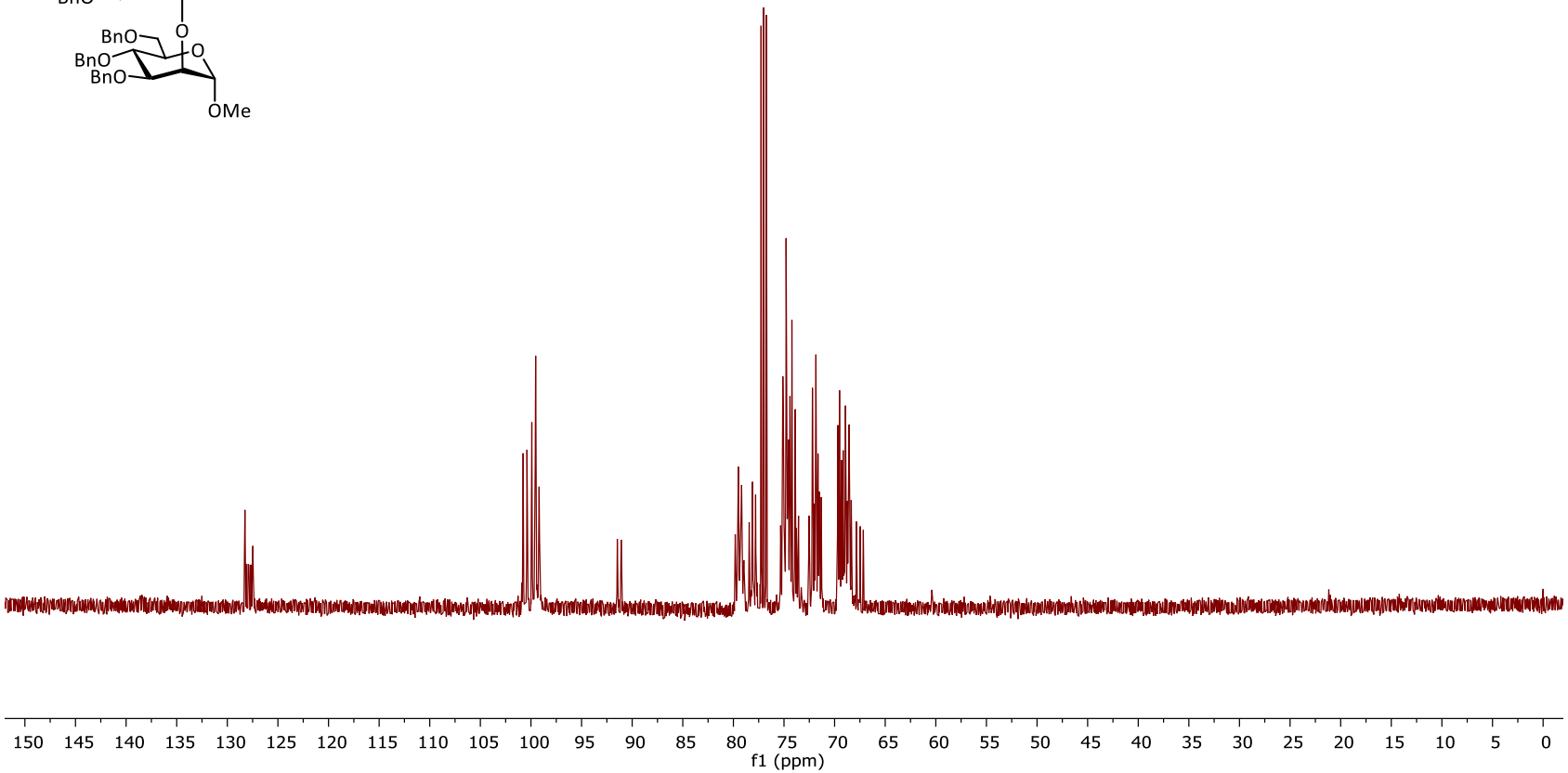


^{1}H NMR spectrum of Methyl 2-O-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (**6**).

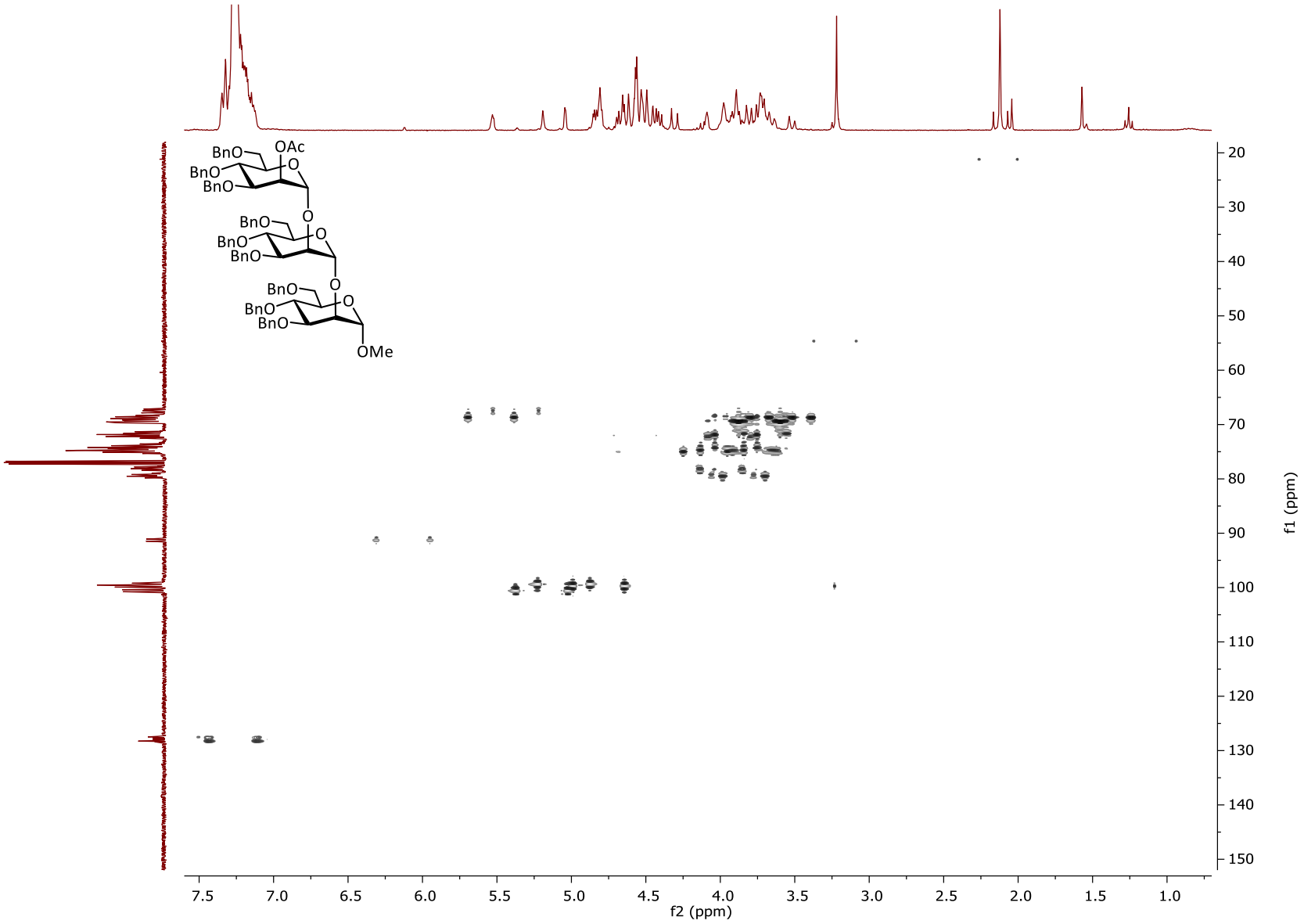


^{13}C NMR spectrum of Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (**6**).

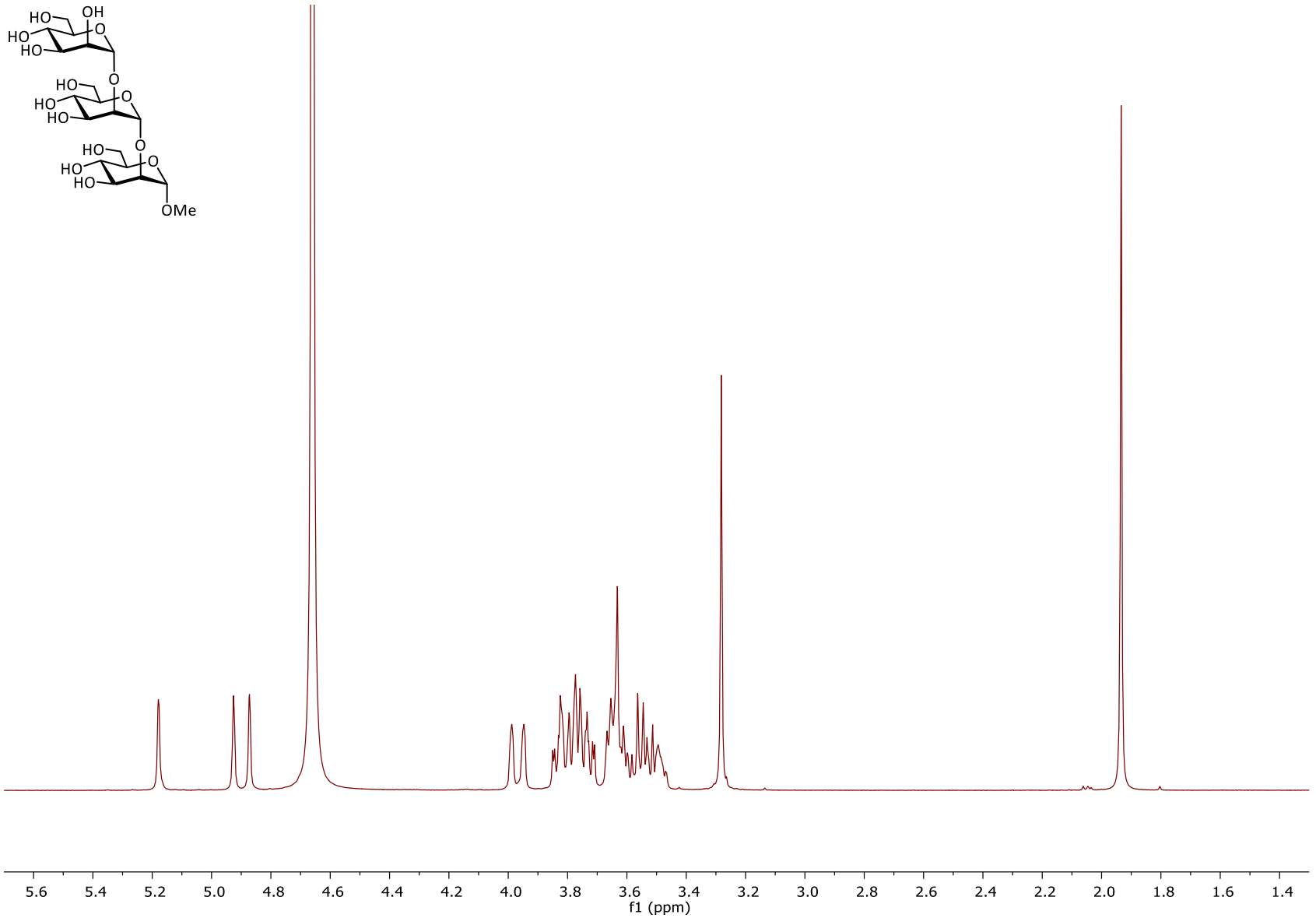




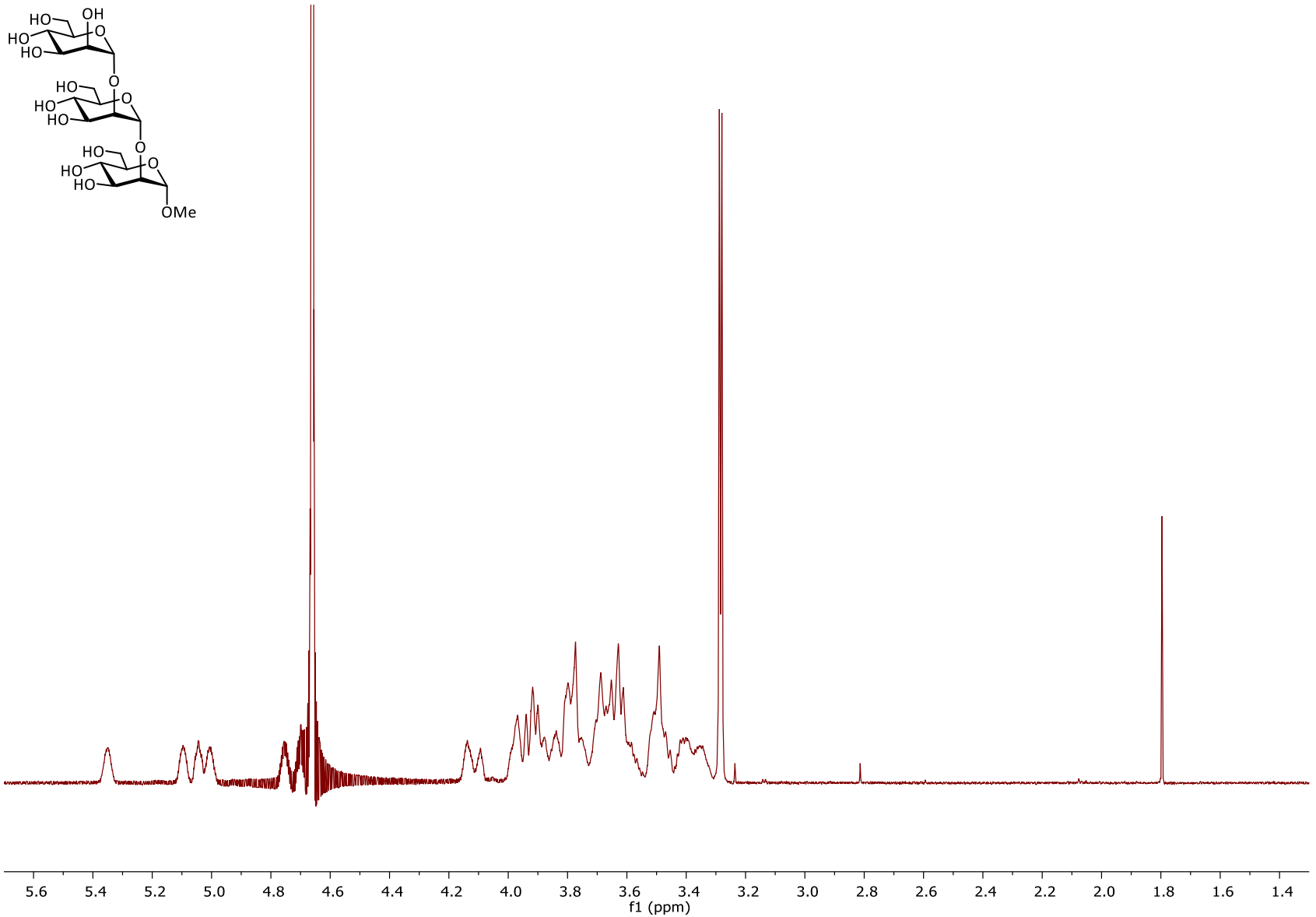
Coupled HSQC of Methyl 2-O-acetyl-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-[$^{13}C_6$]mannopyranoside (**6**).



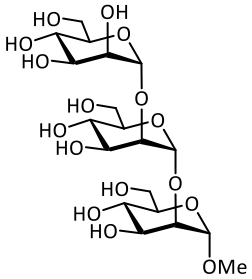
^{1}H NMR spectrum of Methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (7).*

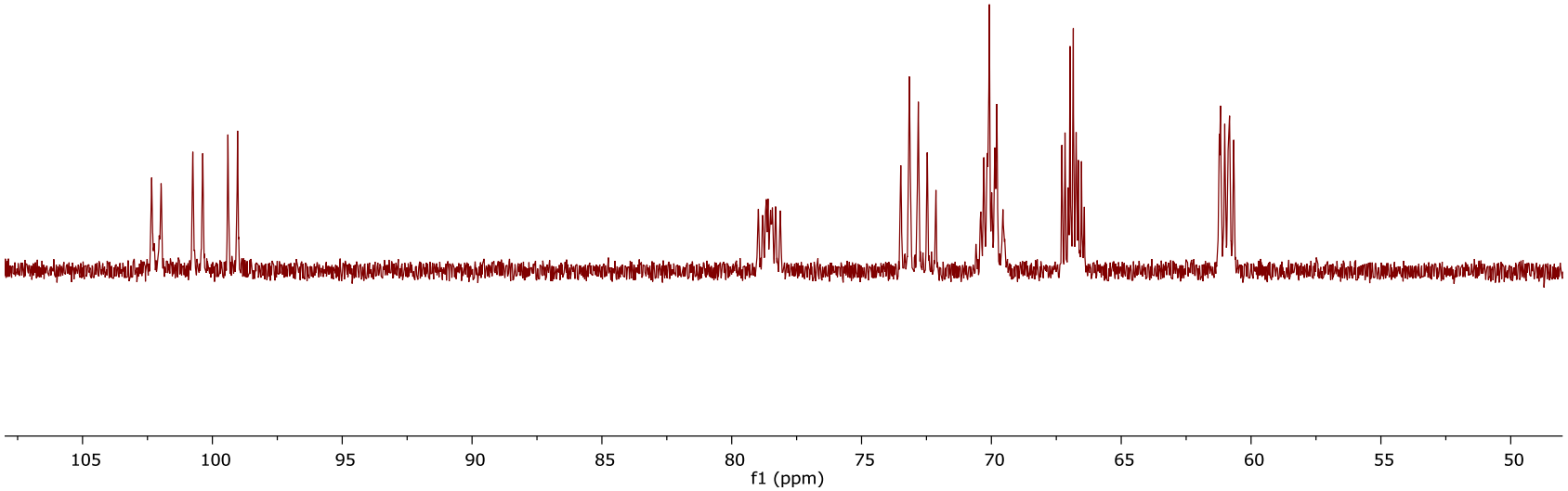


^{1}H NMR spectrum of Methyl α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranoside (7).



^{13}C NMR spectrum of Methyl α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranoside (7).





Coupled HSQC spectrum of Methyl α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranosyl-(1→2)-α-D-[$^{13}C_6$]mannopyranoside (7).