SUPPLEMENTARY INFORMATION

Exploiting structure-activity relationships of QS-21 in the design and synthesis of streamlined saponin vaccine adjuvants

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I. SUPPLEMENTARY TABLE S1 AND FIGURES S1-S10

Table S1. ^{1}H and ^{13}C δ chemical shifts assignments for saponins 3-8. Data obtained from HSQC spectra (600 MHz, methanol-d_4, 298 K) (see figure S2-left for identification codes and chemical structure).

	3 QA(O) (R^1=CHO, R^2=OH)		**4** EA(O) (R^1=CH_3, R^2=OH)		**5** OA(O) (R^1=CH_3, R^2=H)		**6** QA(S) (R^1=CHO, R^2=OH)		**7** EA(S) (R^1=CH_3, R^2=OH)		**8** OA(S) (R^1=CH_3, R^2=H)	
	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)
H1	1.13, 1.73	39.5	1.65, 1.0	39.93	1.63, 0.99	41.1	1.7, 1.13	39.4	1.64, 1.01	39.9	1.63, 0.99	39.7
H2	1.69	26.9	1.63, 1.58	27.8	1.63, 1.56	29.0	1.69	26.9	1.64, 1.56	27.9	1.57	27.7
H3	3.77	72.8	3.16, 3.15	79.6	3.14	80.9	3.75	72.2	3.14	79.6	3.13	79.6
H4	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H5	1.34	48.7	0.75	56.7	0.74	57.9	1.33	48.8	0.74	56.7	0.74	56.7
H6	0.92, 1.50	21.8	1.58, 1.39	19.5	1.60, 1.41	20.7	1.52, 0.92	21.7	1.58, 1.40	19.5	1.59, 1.42	19.6
H7	1.35, 1.58	33.6	1.51, 1.42	35.5	1.48, 1.43	35.3	1.53, 1.32	33.4	1.50, 1.39	34.0	1.44, 1.38	33.7
H8	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H9	1.76	47.9	1.64	48.06	1.57	50.2	1.76	47.8	1.63	48.1	1.58	48.9
H10	-	nd	-	nd	-	nd	-	nd	-	nd	-	nd
H11	1.93	24.4	1.89	24.4	1.89	25.7	1.93	24.3	1.89	24.4	1.89	24.5
H12	5.30	123.0	5.30	123.5	5.24	124.8	5.33	124.0	5.32	124.2	5.26	124.3
H13	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H14	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H15	1.45, 1.69	36.4	1.70, 1.46	36.48	2.06, 1.62	25.1	1.78, 1.36	35.8	1.78, 1.38	35.7	2.15, 1.67	24.8
H16	4.48	74.6	4.49	74.7	1.62, 1.21	30.3	4.48	75.6	4.49	75.7	1.73, 1.10	28.4
H17	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H18	2.94	42.1	2.95, 2.93	42.4	2.81	44.0	2.97, 1.08	42.8	2.97	42.9	2.87	43.1
H19	1.06, 2.32	47.9	2.29, 1.0	47.8	1.73, 1.14	48.4	2.35	48.0	2.34, 1.07	48.1	1.77, 1.16	47.4
H20	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H21	1.17, 1.97	36.4	1.94, 1.17	36.5	1.40, 1.23	35.9	1.97, 1.2	36.5	1.96, 1.20	36.5	1.42, 1.26	34.8
H22	1.83, 1.92	31.9	1.91, 1.82	31.8	1.78, 1.57	34.2	1.82	33.1	1.80	33.0	1.75, 1.48	34.3
H23*	9.31	*nd*	0.99	28.6	0.98	30.0	9.31	*nd*	0.98	28.6	0.99	28.7
H24	1.02	9.4	0.79	16.5	0.78	17.5	1.02	9.4	0.78	16.3	0.79	16.2
H25	1.00	16.2	0.97	16.0	0.95	17.2	0.99	16.2	0.95	16	0.94	15.9
H26	0.78	17.7	0.77	17.75	0.79	19.0	0.76	17.9	0.75	18	0.76	17.8
H27	1.41	27.05	1.38	27.1	1.16	27.4	1.4	27.1	1.37	27.1	1.16	26.3
H28	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*	-	*nd*
H29	0.88	33.3	0.88	33.28	0.91	34.6	0.89	33.1	0.89	33.3	0.92	33.2
H30	0.96	24.8	0.96	24.9	0.93	25.3	0.98	25.1	0.97	25.2	0.95	24.1

Gal1	5.34	95.4	5.35	95.4	5.40	96.6	4.89	82.7	4.88	82.8	4.94	82.6
Gal2	3.94-3.91[#]	74.4-74.5[§]	3.93-3.92[¥]	74.7	3.95	73.0/75.9[‡]	3.87	76.3	3.92	75.4	3.91	75.6
Gal3	3.94-3.91[#]	74.4-74.5[§]	3.93-3.92[¥]	74.7	3.95	73.0/75.9[‡]	3.91	75.3	3.88	76.2	3.89	76.1
Gal4	4.32	52.4	4.33	52.5	4.32	53.7	4.36	52.5	4.36	52.5	4.37	52.5
Gal5	3.69	76.1	3.69	76.1	3.69	77.4	3.65	79.7	3.65	79.6	3.68	79.5
Gal6	3.41, 3.51	61.6	3.51, 3.41	61.6	3.51, 3.40	62.8	3.44, 3.35	61.7	3.45, 3.34	61.6	3.46, 3.36	61.6
Rha1	5.38	101.1	5.38	101.3	5,40	102,6	5.23	102.6	5.22	102.5	5.21	102.6
Rha2	3.92	71.9	3.93	71.7	3,84	73,3	3.93	71.9	3.94	72	3.95	71.9
Rha3	3.82	72.0	3.83	72.2	3.95	75.9	3.77	72.7	3.75	72.2	3.76	72.2
Rha4	3.55	84.0	3.56	84.0	3.51	85.9	3.53	84.2	3.53	84.3	3.52	84.3
Rha5	3.80	68.5	3.81	68.8	3.82	69.9	4.02	69.1	4.03	69.3	4.06	69.1
Rha6	1.33	18.2	1.34	18.2	1.30	19.4	1.32	18.0	1.33	18	1.32	18.0
Xyl1	4.49	106.7	4.49	106.9	4.43	108.6	4.44	107.0	4.45	107.2	4.43	107.1
Xyl2	3.21	76.0	3.25	76.1	3.21	77.4	3.2	75.9	3.22	76.2	3.21	76.1
Xyl3	3.32	78.0	3.33	78.1	3.31	79.5	3.3	78.2	3.31	78.1	3.31	78.2
Xyl4	3.46	70.9	3.47	71.0	3.45	72.2	3.45	70.9	3.46	71	3.45	70.9
Xyl5	3.20, 3.85	67.3	3.87, 3.86	67.3	3.84, 3.18	68.5	3.83, 3.18	67.2	3.85, 3.19	67.3	3.8, 3.18	67.1
a	2.33	36.6	2.33	36.7	2.33	37.9	2.33	36.8	2.34	36.7	2.31	36.8
a'	2.28	34.9	2.26	35.5	2.27	36.1	2.27	35.2	2.27	35.3	2.26	35.5
b	1.63	27.2	1.63	27.1	1.62	28.3	1.63	27.1	1.62	27.1	1.63	27.1
b'	1.60	26.0	1.60	26.4	1.59	27.2	1.6	26.1	1.59	26.1	1.6	26.3
c	1.32-1.37	30.3	1.32	30.3	1.32	31.6	1.33	30.3	1.33	30.3	1.33	30.3

*CH_3 or CHO, [#,§,¥,‡] These assignments are interchangeable. *nd* not determined data from HSQC experiment.

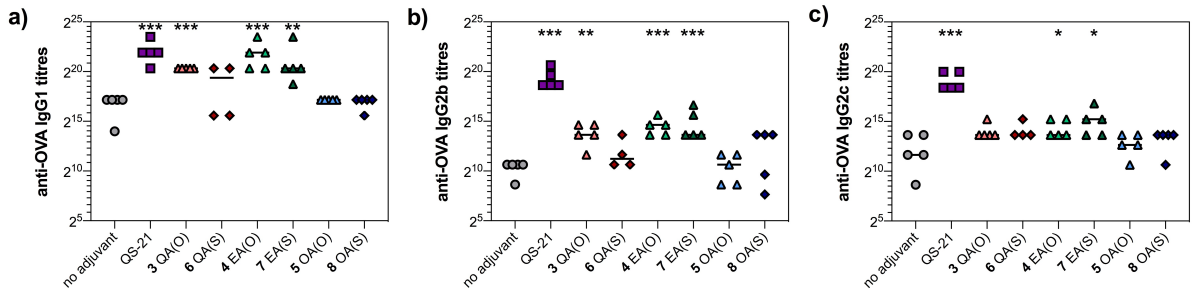


Figure S1. Immunological evaluation in mice of saponins 3-8 with OVA antigen. Induced anti-OVA IgG subype titres of (a) IgG1, (b) IgG2b, and (c) IgG2c antibodies on day 46 after first immunization. Data points correspond to five mice per group and horizontal bars indicate median titres. Statistical significance compared to 'no-adjuvant' control group using a two-tailed unpaired Student's *t*-test with a 95% confidence interval (CI). * = $p \leq 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

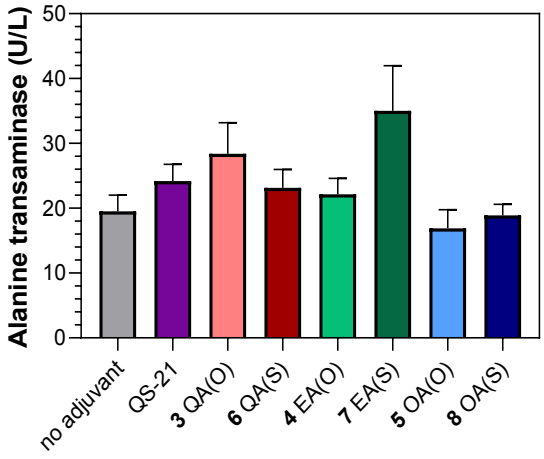


Figure S2. Toxicity assessment based on hepatic alanine transaminase (ALT) levels in mouse sera on day 46 after first vaccination, indicating non-toxicity of the saponin variants. Data are presented as mean ± SEM of 5 mice per group. Each immunization group was compared to 'no-adjuvant' control using a two-tailed unpaired Student's *t*-test with a 95% confidence interval (CI). No statistically significant differences were found, indicating non-toxicity of the saponin variants.

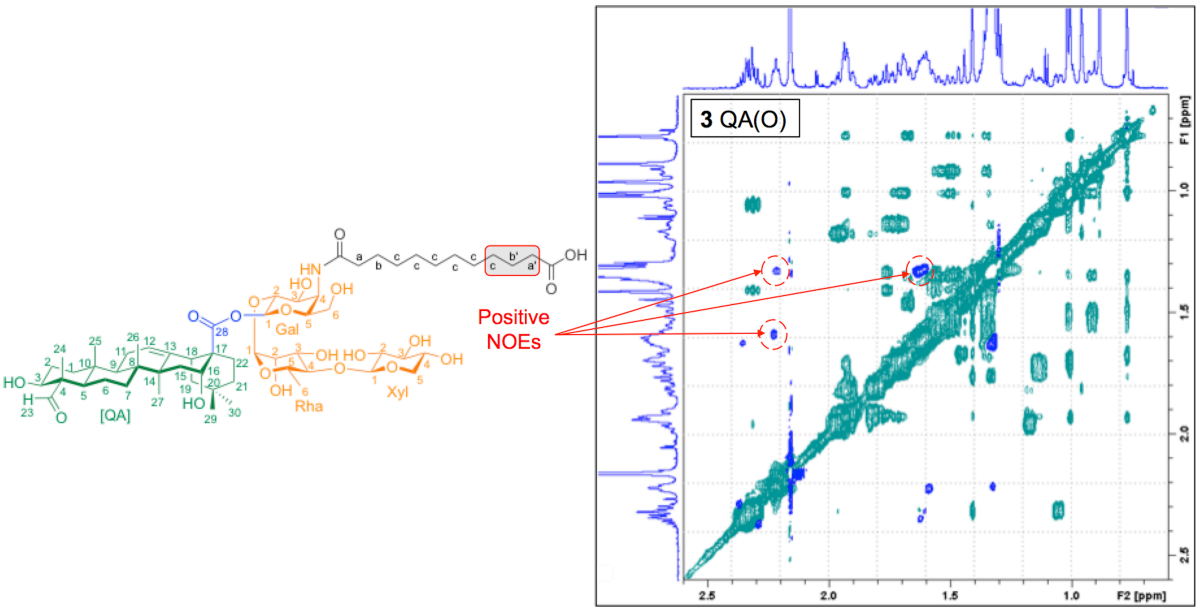


Figure S3. Identification codes for the different positions of saponin variants (left). Representative 2D NOESY spectrum of quillaic acid ester variant 3 QA(O), showing positive NOEs for the distal acyl chain methylene protons (right). 2D NOESY experiments were run in methanol-d_4 at 288 K at 600 MHz with mixing time of 400 ms.

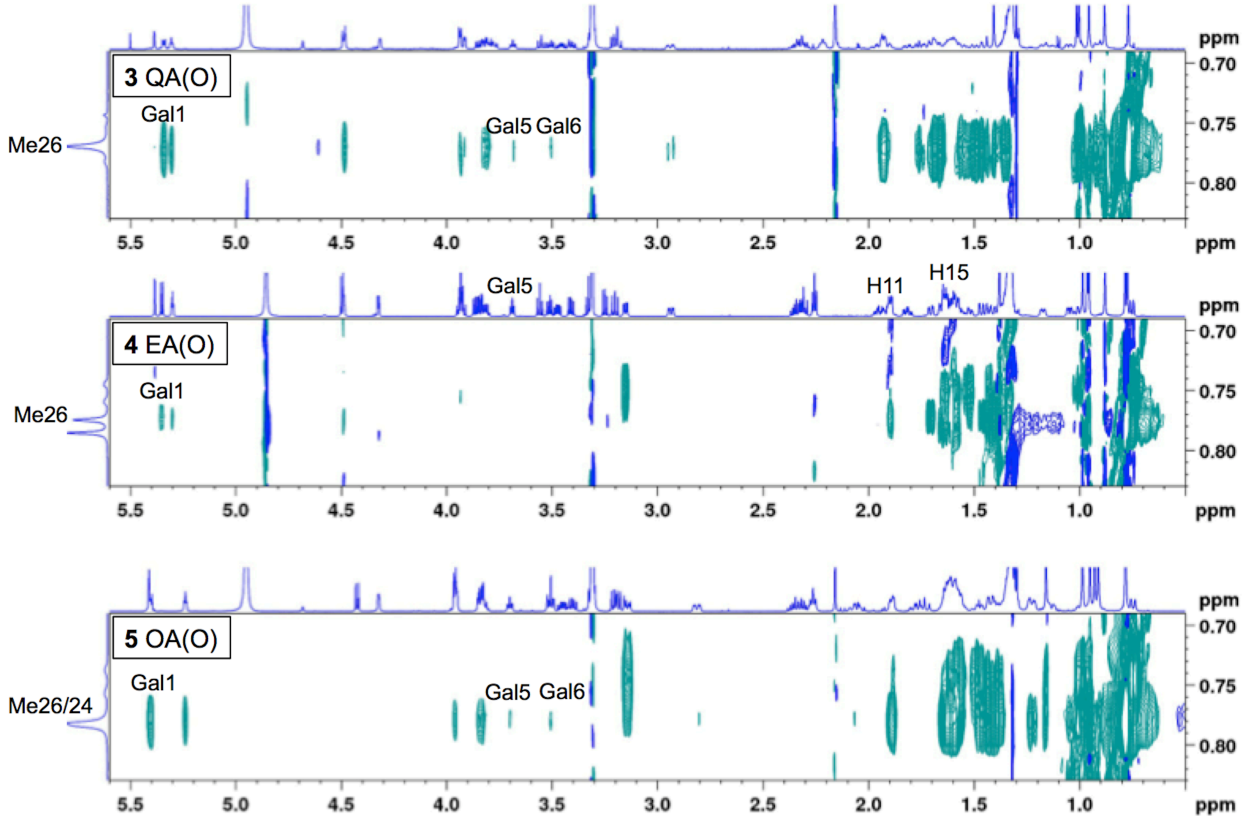


Figure S4. Sections of 2D NOESY spectra for saponin ester variants 3 QA(O), 4 EA(O), and 5 OA(O), showing main NOE cross-peaks between Gal(H1)–triterpene(Me26), Gal(H5)–triterpene(Me26), and Gal(C6)–triterpene(Me26) protons. 2D NOESY experiments were run in methanol-d_4 at 288 K at 600 MHz with mixing time of 400 ms, except for **4** EA(O), which were run at 298 K at 800 MHz.

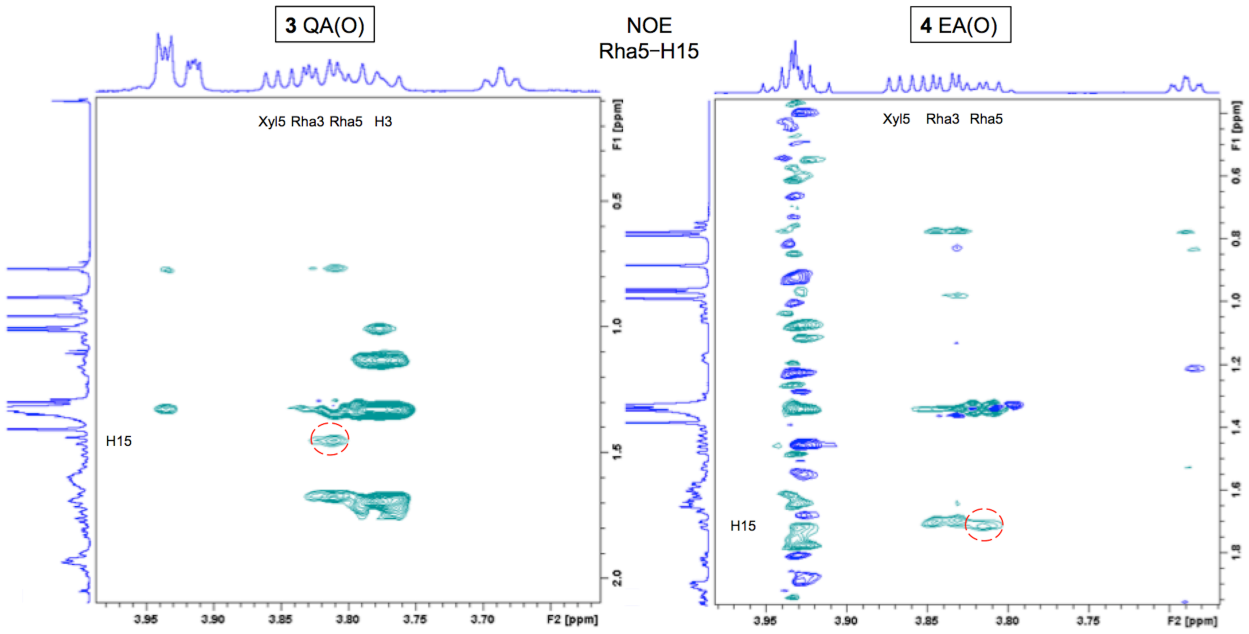


Figure S5. Details of 2D NOESY spectra showing crosspeaks between Rha5–H15 protons for saponin ester variants 3 QA(O) and 4 EA(O). 2D NOESY experiments were run in methanol with a 400 ms mixing time; at 288 K and 600 MHz for **3** QA(O) and at 298 K and 800 MHz for **4** EA(O).

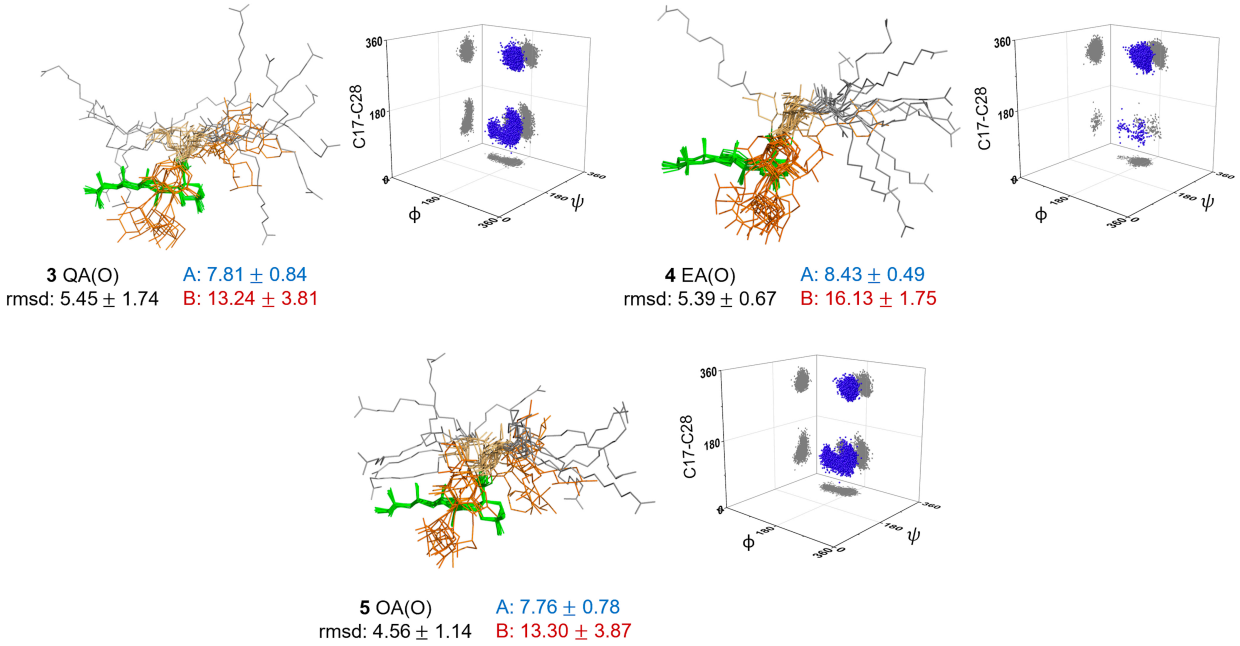


Figure S6. Structural ensembles obtained from unrestrained 0.5 µs molecular dynamics simulations of ester variants 3-5 in explicit methanol. The rmsd (Å) for heavy atoms relative to average structure, key distances (Å), and three-dimensional plots of torsion angle distributions (C17–C28, ϕ and ψ) around the central glycosidic linkage are shown (see main text for definitions). Saponin structural domains are color-coded: triterpene (green),

linear trisaccharide (orange), acyl chain (grey). In the three-dimensional plots, torsion angle distributions are shown in blue and projections onto each plane are shown in grey. For clarity of presentation, the axes have been shifted to minimize the number of datapoints appearing on the 360º→0º radial transition (see Section V for details).

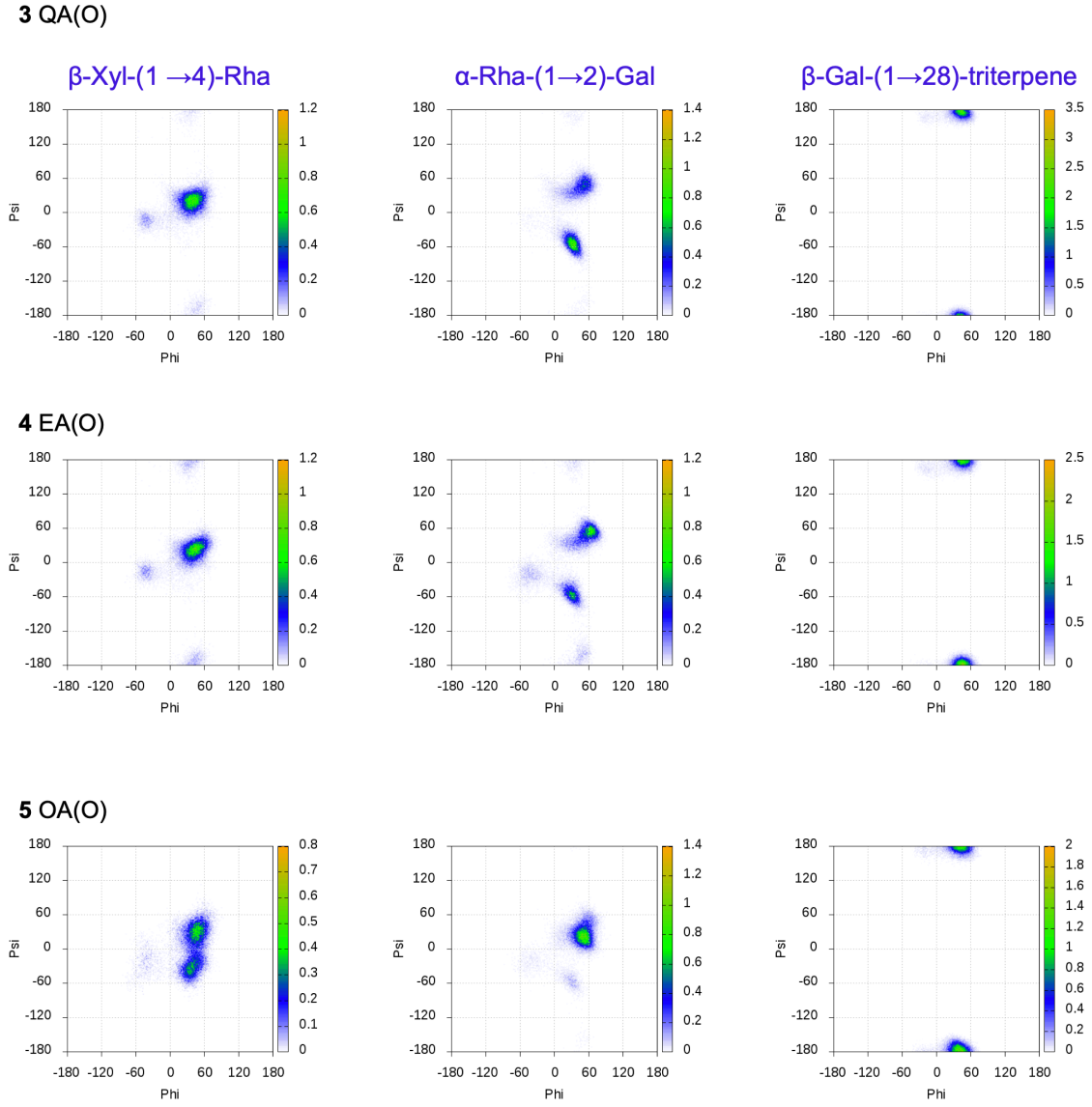


Figure S7. Torsional angle distributions of glycosidic linkages for ester variants 3-5 in water. The dihedral angles ϕ and ψ were obtained from 0.5 μs unrestrained molecular dynamics simulations in explicit water. See Section V for angle definitions.

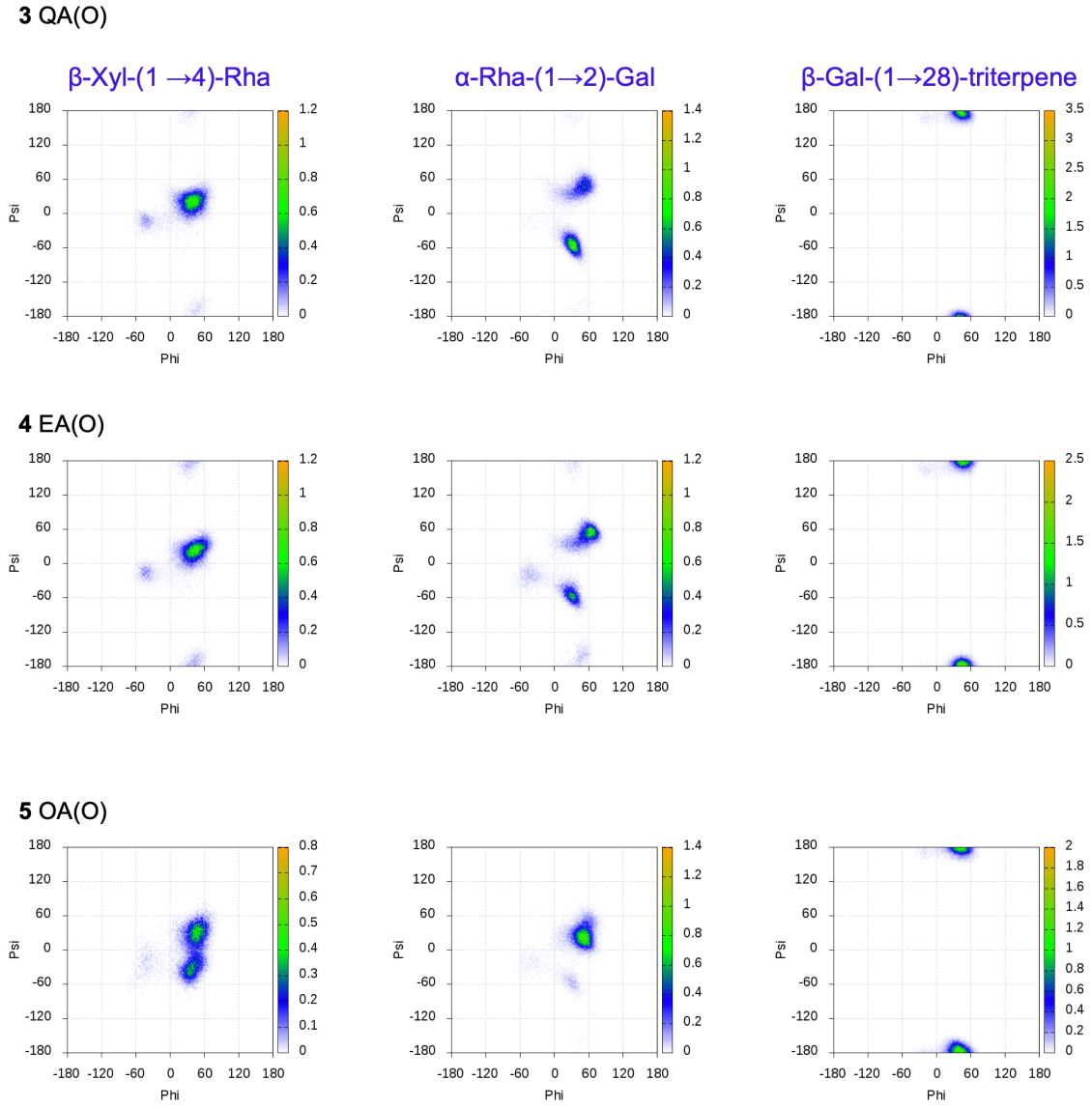


Figure S8. Torsional angle distributions of glycosidic linkages for ester variants 3-5 in methanol. The dihedral angles ϕ and ψ were obtained from 0.5 μs unrestrained molecular dynamics simulations in explicit methanol. See Section V for angle definitions.

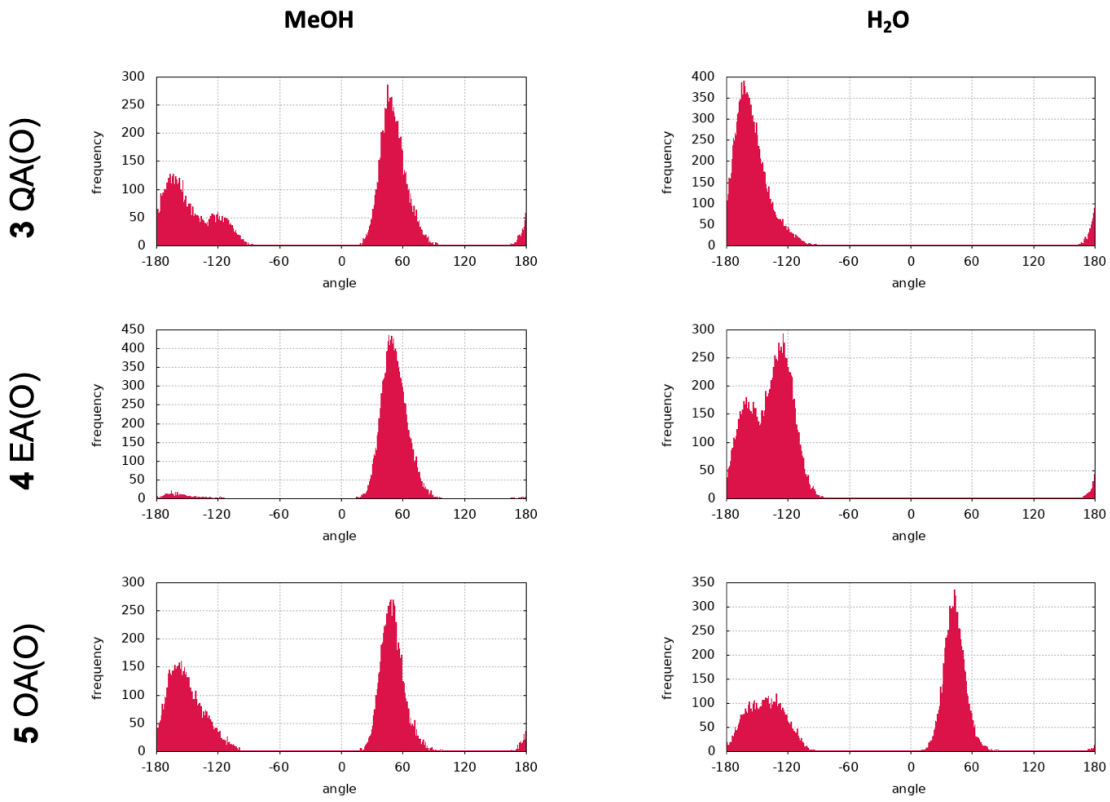


Figure S9. Comparison between the dihedral angle distribution C17–C28 in methanol and water for variants 3-5 derived from 0.5 µs molecular dynamics simulations.

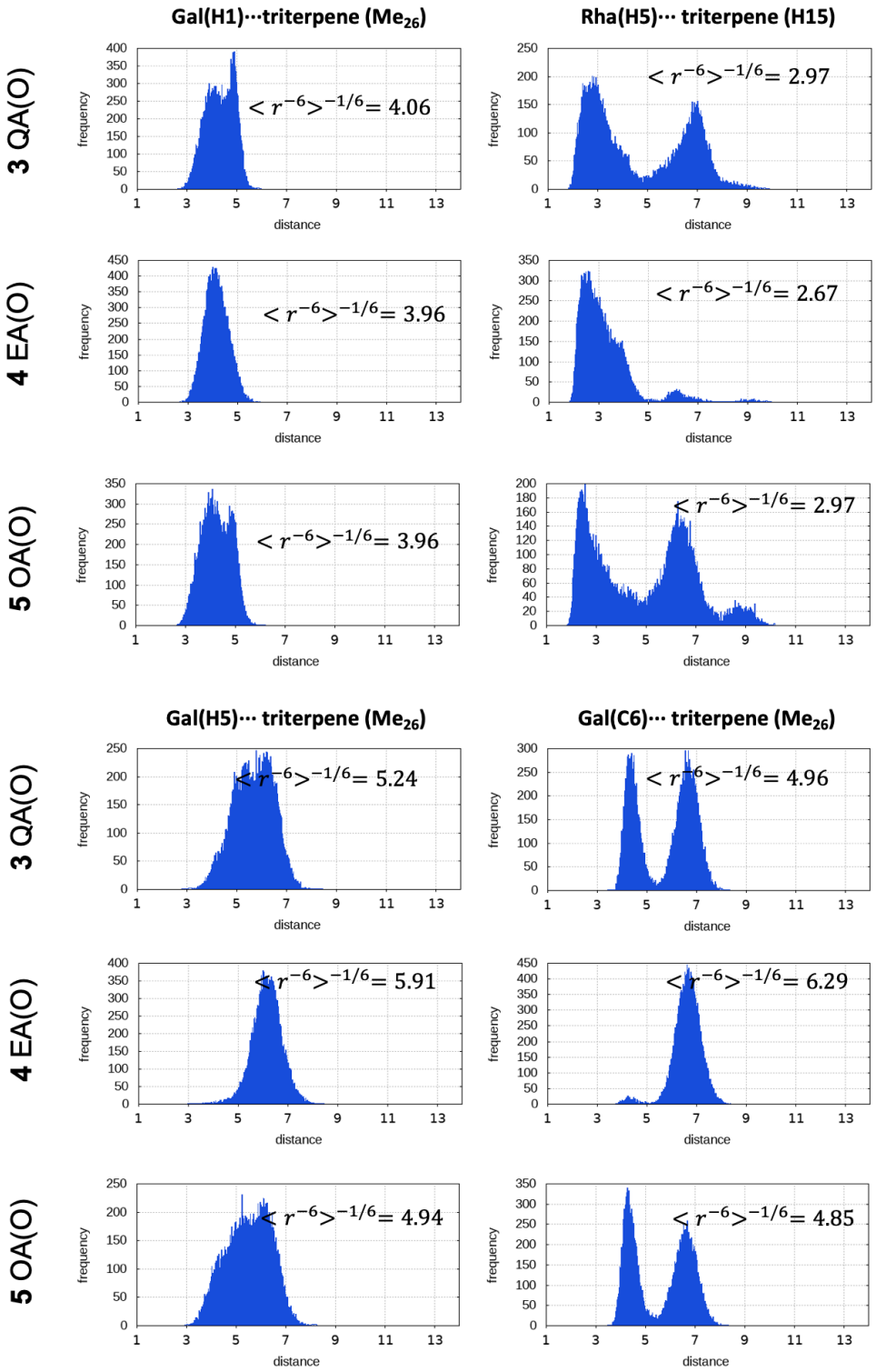


Figure S10. Relevant H–H distance distribution for the conformational analysis in methanol obtained for variants 3-5 from 0.5 µs molecular dynamics simulations in methanol. Distances are given in Å. *Note*: for Gal(C6)–triterpene(Me26), the calculated distance is referred to the C6 atom instead of to H-6a,b proton pair.

II. GENERAL INFORMATION

Materials and Methods. All commercially available materials were used without further purification except boron trifluoride diethyl etherate and trifluoromethanesulfonic anhydride, which were distilled from calcium hydride and phosphorus pentoxide, respectively, at 1 atm under N_2. All manipulations with air-sensitive reagents and chemical reactions were carried out under a dry argon atmosphere using standard Schlenk techniques. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then dried under vacuum. Organic solutions were concentrated under reduced pressure by rotary evaporation below 40 °C. Column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography (TLC) was performed using aluminum-backed sheets precoated with 230–400 mesh silica gel 60 containing fluorescent indicator (F254). TLC plates were visualized under UV light (254 nm) and by staininig with cerium ammonium molybdenate (CAM), phosphomolybidic acid (PMA), or 5% sulfuric acid in ethanol solutions.

^{1}H, APT ^{13}C, COSY and HSQC nuclear magnetic resonance. ^{1}H, APT ^{13}C, COSY and HSQC spectra were recorded on a Bruker Avance III instrument (^{1}H NMR at 600 MHz and APT ^{13}C NMR at 151 MHz). Chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent ($CDCl_3$: δ 7.26 for ^{1}H NMR, δ 77.00 for ^{13}C NMR; methanol-d_4: δ 3.31 for ^{1}H NMR, δ 49.15 for ^{13}C NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment.

RP-HPLC purification and UPLC/ESI-TOF-HRMS. All reverse-phase RP-HPLC purifications and analyses were carried out on a Waters 1525 binary gradient HPLC system equipped with a Waters 2998 photodiode array detector (PDA) and an SQD2 mass spectrometer, and absorbances were monitored at wavelengths of 210–600 nm.

High resolution mass spectra (UPLC/ESI-TOF-HRMS) were recorded on a Waters Acquity UPLC system (Acquity BEH C18 column, 100 × 2.1 mm, 1.7 μm) equipped with a PDA detector and coupled to a Waters LCT XE time-of-flight (TOF) mass spectrometry detector with electrospray ionization source (ESI).

III. GENERAL EXPERIMENTAL PROCEDURES

A. ACYLATION OF SAPONIN AMINE WITH CARBOXYLIC ACID CHAIN

Et_3N (40.0 equiv) was added to a stirred solution of acid chain **23**[1] (10.0 equiv) in dry THF (3-9 mL). The solution was cooled to 0 °C, EtOCOCl (7.0 equiv) was added at 0 °C via syringe and the resulting mixture was stirred at 0 °C for 3 h under inert atmosphere. A solution of saponin amine (esters **17-19**,[2] thioesters **20-22**) (1.0 equiv) in dry THF (3-9 mL) was added via cannula at 0 °C and the mixture was stirred at 0 °C for 4 h until consumption of the starting material. At this point, the reaction mixture was quenched with H_2O (1.0 mL) and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate 10:0 to 8:2 v/v) to afford a mixture of acid **23** and the fully protected saponin (**24-29**) as a transparent oil. This mixture was further purified by another silica gel chromatography (toluene/ethyl acetate 100:0 to 97:3 containing 0.5% of Et_3N, v/v) to provide the fully protected saponin ester (**24-26**) and thioester (**27-29**) as a transparent film.

B. GLOBAL DEPROTECTION VIA HYDROGENOLYSIS AND ACID HYDROLYSIS

To a stirred solution of fully protected saponin (esters **24-26**, thioesters **27-29**) (1.0 equiv) in THF/EtOH (1:1 v/v, 3-6 mL), 10% (dry basis) Pd/C wet Degussa type E101 NE/W (5.0 equiv) was added. The reaction mixture was stirred at room temperature under H_2 atmosphere (1 atm, balloon) for 16 h for compounds **24-26**, and for 20 min for thioesters **27-29**. The suspension was filtered through 0.45 μm PTFE filter disk, washed with MeOH (50 mL) and concentrated under reduced pressure. The residue was dissolved in a precooled (0 °C) solution of TFA/H_2O (3:1 v/v, 2-5 mL), stirred at 0 °C for 1 h and then concentrated under reduced pressure at room temperature.

The residue was dissolved in MeOH (final concentration = 2 mg/mL) and purified by RP-HPLC (0.5 mL per injection) on a XBridge semi-prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of ~40–95% acetonitrile/water (0.05% TFA) over 20 min at a flow rate of 5 mL/min. The fraction containing the major peak was collected and lyophilized to dryness to afford final saponin ester (**3-5**) and thioester (**6-8**) as a white solid.

C. GLYCOSYLATION OF TRITERPENE ACYL CHLORIDE AND AZIDE REDUCTION

To a stirred solution of triterpene acid (**9-11**)[2] (1.0 equiv) in dry DCM (1.8 mL) under inert atmosphere pyridine (10 equiv) was added, and the solution was cooled at 0 °C. Thionyl chloride ($SOCl_2$) (2.0 equiv) was added at 0 °C, the reaction mixture was removed from the ice bath and stirred for 3 h at room temperature. The solvent was removed under a stream of nitrogen and then concentrated under reduced pressure. The residue was redissolved in toluene, filtered over a celite pad, and then concentrated under reduced pressure to give crude acyl chloride (**12-14**), which was directly advanced to the glycosylation step without further purification.

To a stirred solution of triterpene acyl chloride (**12-14**) (1.5 equiv) and trisaccharide thiohemiacetal **16**[3] (1.0 equiv) in dry THF (3 mL), NaH (3.0 equiv) was added at 0 °C under inert atmosphere. After stirring for 2 h at room temperature, a second portion of NaH (2.0 equiv) was added. The suspension was stirred for 1 h at room temperature, then ice-cooled $NaHCO_3$ aqueous solution (30 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (20 mL), dried with

anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue (protected β-glycosyl thioester azide) was unstable under silica gel chromatography and was used immediately in the next reaction without further purification.

This crude residue was dissolved in anhydrous Et_3N (10 mL) and a solution of freshly prepared PhSeH (0.5 mmol, 20 equiv, see General Experimental Procedure D) in toluene/THF (1:1 v/v, 3.4 mL) was added via cannula. After being stirred for 3 h at 40 °C under inert atmosphere, the resulting yellow mixture was concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel (toluene/ethyl acetate 10:0 to 8:2 containing 1% of Et_3N, v/v) to provide thioester amine (**20-22**) as a glassy film.

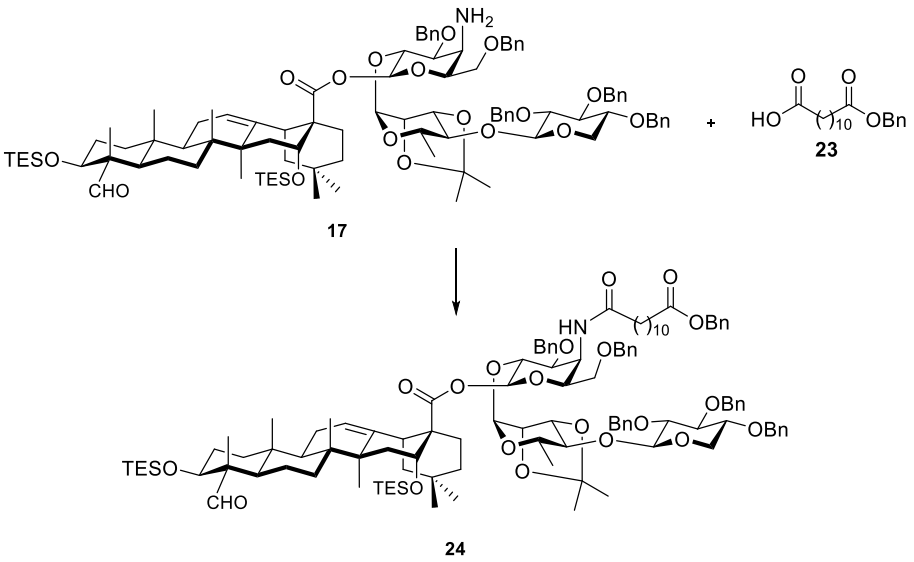
D. Preparation of Phenylselenol Solution

A stirred solution of diphenyl diselenide $(PhSe)_2$ (72 mg, 0.23 mmol, 10 equiv) in THF (1.6 mL) was treated with H_3PO_4 50% aqueous solution (273 µL, 2.53 mmol, 110 equiv) and the mixture was stirred at 40 °C for 1 h under inert atmosphere. Toluene (1.5 mL) and H_2O (1.5 mL) were added via syringe and the mixture was stirred vigorously; the aqueous layer was removed with a syringe, and the organic phase was dried with $MgSO_4$, providing a freshly prepared solution of phenylselenol (PhSeH).

IV. SYNTHESIS OF SAPONIN VARIANTS

A. SYNTHESIS OF ESTER VARIANTS

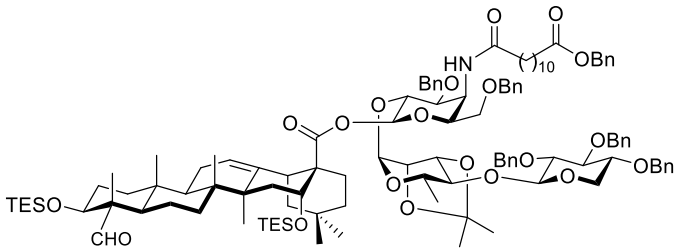
1. SYNTHESIS OF QUILLAIC ACID VARIANT 3 [QA(O)]



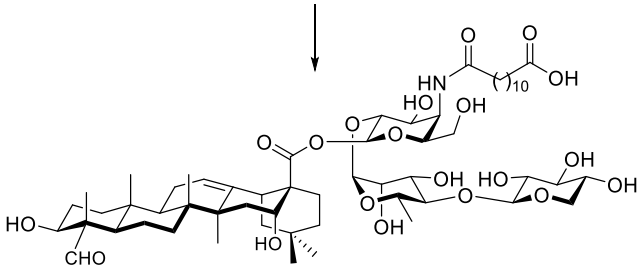
Fully protected quillaic acid saponin ester (24) [MG-I-073]. Following General Experimental Procedure A, saponin amine **17**[2] (18.0 mg, 10.94 µmol, 1.0 equiv) in dry THF (3.3 mL) was acylated for 4 h at 0 ºC with acid **23**[1] (35.0 mg, 0.11 mmol, 10.0 equiv) in dry THF (3.3 mL) via activation with EtOCOCl (7.3 µL, 76.6 µmol, 7.0 equiv) and Et_3N (61 µL, 0.44 mmol, 40.0 equiv) to provide, after silica gel column chromatography purification, the fully protected quillaic acid saponin ester **24** (13.0 mg, 61% yield) as a transparent film.

TLC: R_f 0.55 (toluene/ethyl acetate 9:1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 9.31 (s, 1H, H-23 QA C*H*O), 7.39–7.17 (m, 30H, ArH), 5.65–5.57 (s, 1H, NH), 5.39 (d, *J* = 7.1 Hz, 1H, H-1 Gal), 5.30 (t, *J* = 3.7 Hz, 1H, H-12 QA), 5.21 (d, *J* = 1.7 Hz, 1H, H-1 Rha), 5.11 (s, 2H, CH_2Ph), 4.90 (d, *J*=11.1 Hz, 1H, CH_2Ph), 4.88–4.78 (m, 5H, H-1 Xyl, H-4 Gal, CH_2Ph), 4.72 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 11.1 Hz, 1H, CH_2Ph), 4.62 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.52–4.42 (m, 4H, H-16 QA, CH_2Ph), 4.15 (dd, *J*=7.5, 6.0 Hz, 1H, H-3 Rha), 4.11 (dd, *J* = 6.0, 1.8 Hz, 1H, H-2 Rha), 3.92 (dd, *J* = 11.7 Hz, 4.2 Hz, 1H, H-5a Xyl), 3.81–3.76 (m, 2H, H-5 Gal), 3.71–3.58 (m, 5H, H-2 Gal, H-4 Xyl, H-5 Rha, H-3 QA), 3.54–3.46 (m, 3H, H-6a,b Gal, H-4 Rha), 3.32–3.28 (m, 1H), 3.23–3.18 (m, 1H, H-5b Xyl), 2.88 (dd, *J* = 14.3, 4.4 Hz, 1H, H-18 QA), 1.45 (s, 3H, CH_3 isopr), 1.38 (s, 3H, CH_3 isopr), 1.25 (s, 3H, CH_3 QA), 1.15 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.05 (s, 3H, CH_3 QA), 0.97 (t, *J* = 7.9 Hz, 9H, CH_2CH_3), 0.93 (s, 3H, CH_3 QA), 0.91 (t, *J* = 7.9 Hz, CH_2CH_3, 12H, CH_3 QA), 0.87 (s, 3H, CH_3 QA), 0.73 (s, 3H, CH_3 QA), 0.65 (qd, *J* = 7.9, 3.6 Hz, 6H, C$H_2$$CH_3$), 0.52 (qd, *J*=7.9, 4.6 Hz, 6H, C$H_2$$CH_3$). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 207.41, 175.20, 173.69, 173.07, 143.74, 138.76, 138.64, 138.25, 137.67, 137.45, 136.12, 128.55, 128.45, 128.35, 128.33, 128.31, 128.29, 128.18, 127.98, 127.96, 127.85, 127.82, 127.78, 127.61, 127.55, 121.33, 109.41, 102.28, 97.83, 83.83, 82.02, 78.92, 78.37, 78.15, 77.96, 77.25, 77.04, 76.83, 76.11, 75.59, 75.38, 75.29, 74.75, 74.46, 73.51, 73.23, 72.90, 71.56, 69.11, 68.30, 66.53, 66.09, 63.78, 62.34, 62.31, 62.00, 56.00, 49.13, 47.77, 46.90, 46.47, 45.93, 41.57, 40.49, 39.84, 38.22, 36.96, 35.79, 35.23, 34.52, 34.34,

34.14, 34.05, 32.72, 32.36, 30.87, 30.47, 29.73, 29.54, 29.50, 29.48, 29.42, 29.34, 29.29, 29.24, 29.18, 27.62, 26.81, 26.34, 26.06, 25.79, 24.98, 24.31, 23.39, 22.62, 20.61, 17.74, 17.12, 15.90, 9.54, 7.16, 6.85, 5.07, 4.93. **HRMS (ESI)** m/z: Calcd for $C_{116}H_{163}NO_{20}Si_2$ $(M+Na)^+$ 1969.1205, found 1969.1210.



24

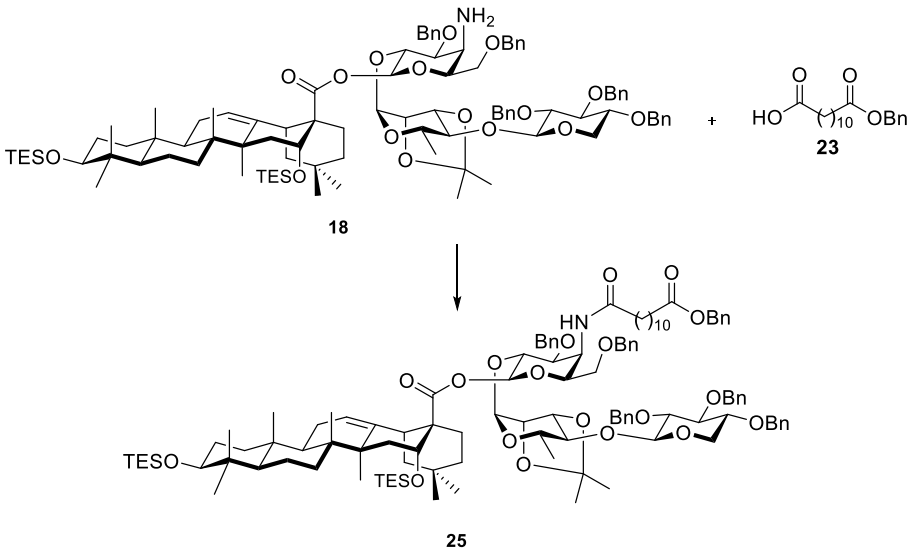


3

Quillaic acid saponin ester (3) [MG-II-003]. Following General Experimental Procedure B, fully protected variant **24** (12.0 mg, 5.91 μmol, 1.0 equiv) was subjected to hydrogenolysis (3.0 mL THF/EtOH, 63 mg Pd/C) for 16 h followed by acid hydrolysis (2.0 mL TFA/H_2O) at 0 ºC for 1 h to provide, after HPLC purification [30–80% acetonitrile/water (0.05% TFA)], quillaic acid saponin ester **3** (4.77 mg, 84% yield,) as a white solid.

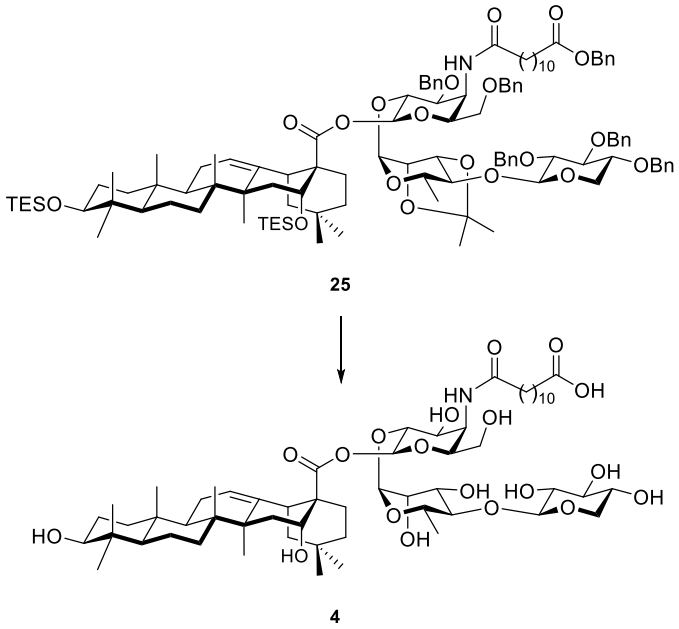
HPLC: t_R = 18.56 min, λ_{max} = 200 nm. **^{1}H NMR** (600 MHz, methanol-d_4) characteristic resonances: δ 9.31 (s, 1H, H-23 QA C*H*O), 5.38 (d, *J* = 1.8 Hz, 1H, H-1 Rha), 5.34 (d, *J* = 7.6 Hz, 1H, H-1 Gal), 5.31 (t, *J* = 3.3 Hz, 1H, H-12 QA), 4.50–4.47 (m, 2H, H-1 Xyl, H-16 QA), 4.33–4.31 (m, 1H, H-4 Gal), 3.96–3.90 (m, 3H, H-2 Gal, H-3 Gal, H-2 Rha), 3.87–3.75 (m, 4H, H-3 Rha, H-5 Rha, H-5a Xyl), 3.69 (td, *J* = 6.6, 1.7 Hz, 1H, H-5 Gal), 3.58–3.49 (m, 2H, H-4 Rha, H-6a Gal), 3.46 (ddd, *J* = 10.4, 8.9, 5.4 Hz, 1H, H-4 Xyl), 3.41 (dd, *J* = 11.5, 6.9 Hz, 1H, H-6b Gal), 3.34 (d, *J* = 11.8 Hz, 1H, H-3 Xyl), 3.20 (dt, *J* = 12.5, 9.3 Hz, 2H, H-2 Xyl, H-5b Xyl, H-3 QA), 2.94 (dd, *J* = 14.4, 4.6 Hz, 1H, H-18 QA), 1.41 (s, 3H, CH_3 QA), 1.33 (d, *J* = 5.8 Hz, 3H, CH_3 Rha), 1.02 (s, 3H, CH_3 QA), 1.01 (s, 3H, CH_3 QA), 0.96 (s, 3H, CH_3 QA), 0.88 (s, 3H, CH_3 QA), 0.78 (s, 3H, CH_3 QA). **^{13}C NMR** (151 MHz, methanol-d_4, based on HSQC data): δ 121.72, 105.51, 99.85, 94.05, 82.60, 76.76, 74.90, 74.70, 73.26, 73.20, 71.36, 70.78, 70.46, 69.64, 67.42, 65.86, 60.30, 51.11, 49.11, 47.86, 47.40, 46.59, 46.59, 40.97, 38.06, 35.85, 35.32, 35.09, 35.09, 32.19, 32.01, 30.60, 29.29, 29.22, 29.15, 25.80, 25.79, 25.64, 23.46, 23.11, 22.82, 20.54, 16.95, 16.32, 15.00, 8.05. **HRMS (ESI)** *m/z*: Calcd for $C_{59}H_{95}NO_{20}$ $(M+Na)^+$ 1160.6345, found 1160.6365.

2. SYNTHESIS OF ECHINOCYSTIC ACID VARIANT 4 [EA(O)]



Fully protected echinocystic acid saponin ester (25) [MG-I-072]. Following General Experimental Procedure A, saponin amine **18**[2] (49.0 mg, 30.9 μmol, 1.0 equiv) in dry THF (9.0 mL) was acylated for 4 h at 0 ºC with acid **23**[1] (96.0 mg, 0.30 mmol, 10.0 equiv) in dry THF (9.0 mL) via activation with EtOCOCl (20.0 μL, 0.21 mmol, 7.0 equiv) and Et_3N (167 μL, 1.20 mmol, 40.0 equiv) to provide, after silica gel column chromatography purification, the fully protected echinocystic acid saponin ester **25** (38.0 mg, 65% yield) as a transparent film.

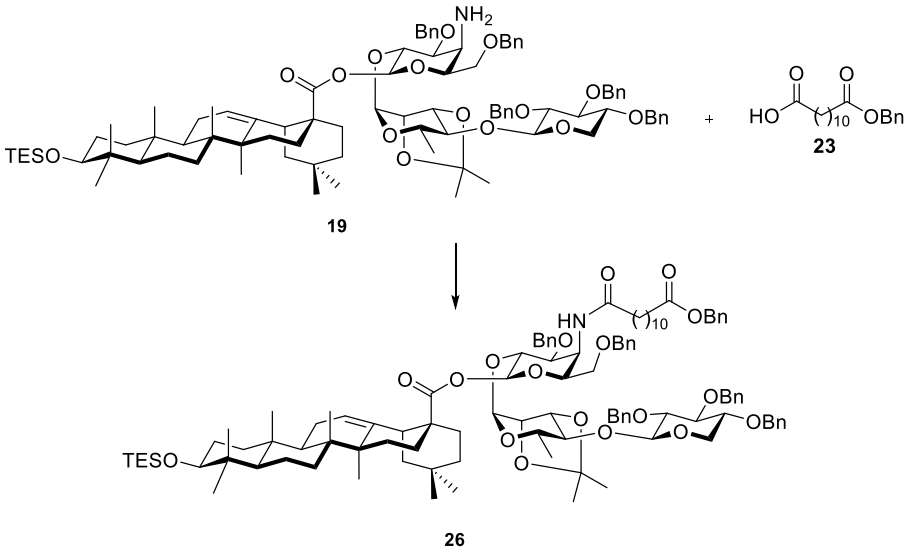
TLC: R_f 0.22 (hexane/ethyl acetate 4:1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 7.40–7.18 (m, 30H, ArH), 5.68–5.58 (br s, 1H, NH), 5.41 (d, *J* = 7.2 Hz, 1H, H-1 Gal), 5.33–5.29 (m, 1H, H-12 EA), 5.24 (d, *J* = 1.7 Hz, 1H, H-1 Rha), 5.12 (s, 2H, C*H*$_2$Ph), 4.92 (d, *J* = 11.1 Hz, 1H, C*H*$_2$Ph), 4.90–4.80 (m, 5H, H-1 Xyl, H-4 Gal, C*H*$_2$Ph), 4.73 (d, *J* = 11.7 Hz, 1H, C*H*$_2$Ph), 4.69 (d, *J* = 11.1 Hz, 1H, C*H*$_2$Ph), 4.63 (d, *J* = 11.7 Hz, 1H, C*H*$_2$Ph), 4.54–4.44 (m, 4H, H-16 EA, C*H*$_2$Ph), 4.17 (dd, *J* = 7.5, 6.0 Hz, 1H, H-3 Rha), 4.13 (dd, *J* = 6.0, 1.8 Hz, 1H, H-2 Rha), 3.94 (dd, *J* = 11.9, 3.9 Hz, 1H, H-5a Xyl), 3.78 (td, *J* = 6.3, 1.6 Hz, 1H, H-5 Gal), 3.73–3.59 (m, 5H, H-3 Gal, H-3 Xyl, H-4 Xyl, H-5 Rha), 3.54 (dd, *J* = 9.7, 7.5 Hz, 1H, H-4 Rha), 3.50 (d, *J* = 6.2 Hz, 2H, H-6a,b Gal), 3.34–3.30 (m, 1H, H-2 Xyl), 3.23–3.19 (m, 2H, H-5b Xyl, H-3 EA), 2.88 (dd, *J* = 14.2, 4.5 Hz, 1H, H-18 EA), 1.47 (s, 3H, CH_3 isopr), 1.37 (s, 3H, CH_3 isopr), 1.28 (s, 3H, CH_3 EA), 1.17 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 0.98 (q, *J* = 7.8 Hz, 18H, CH_2CH_3), 0.93 (s, 3H, CH_3 EA), 0.92 (s, 3H, CH_3 EA), 0.87 (s, 3H, CH_3 EA), 0.87 (s, 3H, CH_3 EA), 0.75 (s, 3H, CH_3 EA), 0.75 (s, 3H, CH_3 EA), 0.66 (qd, *J* = 7.9, 3.1 Hz, 6H, C*H*$_2$CH$_3$), 0.60 (qd, *J* = 8.0, 1.4 Hz, 6H, C*H*$_2$CH$_3$). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 175.34, 173.69, 173.13, 143.64, 138.76, 138.65, 138.25, 137.70, 137.48, 136.13, 128.55, 128.45, 128.36, 128.34, 128.32, 128.29, 128.18, 128.00, 127.96, 127.85, 127.81, 127.78, 127.60, 127.56, 121.71, 109.41, 102.27, 97.81, 83.86, 82.04, 79.45, 79.02, 78.39, 78.17, 77.96, 76.21, 75.61, 75.58, 75.48, 74.77, 73.54, 73.23, 72.91, 71.53, 68.28, 66.46, 66.08, 63.80, 55.32, 49.21, 41.50, 40.45, 39.55, 39.34, 38.61, 36.97, 36.88, 34.35, 33.03, 32.73, 30.92, 30.48, 29.73, 29.51, 29.49, 29.43, 29.33, 29.29, 29.18, 28.48, 27.73, 27.64, 26.31, 26.08, 25.81, 24.99, 24.33, 23.43, 18.54, 17.75, 17.18, 16.13, 15.75, 7.18, 7.09, 5.29, 4.96. **HRMS (ESI)** *m/z*: Calcd for $C_{116}H_{165}NO_{19}Si_2$ $(M+Na)^+$ 1955.1412, found 1955.1475.



Echinocystic acid saponin ester (4) [MG-II-006]. Following General Experimental Procedure B, fully protected variant **25** (19.0 mg, 9.85 μmol, 1.0 equiv) was subjected to hydrogenolysis (4.8 mL THF/EtOH, 104 mg Pd/C) for 16 h followed by acid hydrolysis (4.1 mL TFA/H_2O) at 0 ºC for 1 h to provide, after HPLC purification [40–95% acetonitrile/water (0.05% TFA)], echinocystic acid saponin ester **4** (5.46 mg, 49% yield) as a white solid.

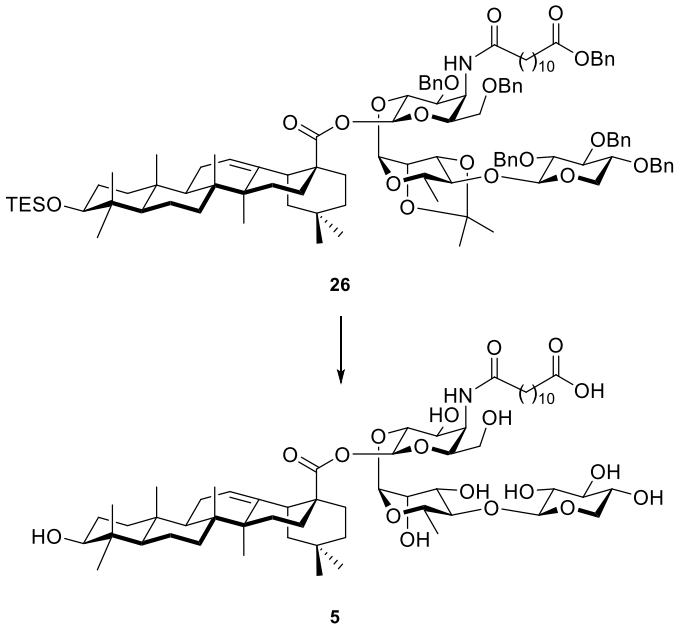
HPLC: t_R = 15.25 min, λ_{max} = 200 nm. **^{1}H NMR** (600 MHz, methanol-d_4) characteristic resonances: δ 5.39 (d, *J* = 1.8 Hz, 1H, H-1 Rha), 5.35 (d, *J* = 7.4 Hz, 1H, H-1 Gal), 5.30 (t, *J* = 3.7 Hz, 1H, H-12 EA), 4.51–4.48 (m, 2H, H-1 Xyl, H-16 EA), 4.33 (dd, *J* = 4.3, 1.7 Hz, 1H, H-4 Gal), 3.96–3.90 (m, 3H, H-3 Rha, H-2 Gal, H-3 Gal), 3.88–3.79 (m, 3H, H-3 Rha, H-5 Rha, H-5a Xyl), 3.69 (td, *J* = 6.6, 1.7 Hz, 1H, H-5 Gal), 3.58–3.45 (m, 3H, H-4 Rha, H-4 Xyl, H-6a Gal), 3.41 (dd, *J* = 11.6, 6.9 Hz, 1H, H-6b Gal), 3.35–3.31 (m, 1H, H-3 Xyl), 3.27–3.18 (m, 2H, H-2 Xyl, H-5b Xyl), 3.15 (dd, *J* = 11.4, 4.7 Hz, 1H, H-3 EA), 2.94 (dd, *J* = 14.5, 4.5 Hz, 1H, H-18 EA), 2.38–2.24 (m, 5H), 1.38 (s, 3H, CH_3 EA), 1.34 (d, *J* = 6.3 Hz, 3H, CH_3 Rha), 0.99 (s, 3H, CH_3 EA), 0.96 (s, 3H, CH_3 EA), 0.96 (s, 3H, CH_3 EA), 0.88 (s, 3H, CH_3 EA), 0.79 (s, 3H, CH_3 EA), 0.77 (s, 3H, CH_3 EA). **^{13}C NMR** (151 MHz, methanol-d_4, based on HSQC data): δ 124.56, 108.18, 102.46, 96.66, 85.33, 80.88, 79.27, 77.44, 77.26, 75.88, 75.86, 73.34, 73.03, 72.26, 70.01, 68.44, 62.85, 58.05,53.62, 50.35, 49.28, 49.13, 43.52, 41.13, 37.89, 37.67, 37.62, 36.48, 35.45, 34.50, 33.09, 31.55, 29.92, 29.01, 28.38, 28.29, 27.39, 26.04, 25.66, 20.68, 19.51, 18.95, 17.47, 17.31. **HRMS (ESI)** *m/z*: Calcd for $C_{59}H_{97}NO_{19}$ $(M+H)^+$ 1124.6733, found 1124.6732.

3. SYNTHESIS OF OLEANOLIC ACID VARIANT 5 [OA(O)]



Fully protected oleanolic acid saponin ester (26) [MG-I-065]. Following General Experimental Procedure A, saponin amine **19**[2] (23.0 mg, 15.3 μmol, 1.0 equiv) in dry THF (4.6 mL) was acylated for 4 h at 0 ºC with acid **23**[1] (50.0 mg, 0.15 mmol, 10.0 equiv) in dry THF (4.6 mL) via activation with EtOCOCl (10.2 μL, 0.11 mmol, 7.0 equiv) and Et_3N (85 μL, 0.61 mmol, 40.0 equiv) to provide, after silica gel column chromatography purification, the fully protected oleanolic acid saponin ester **26** (25.0 mg, 90% yield) as a transparent film.

TLC: R_f 0.25 (hexane/ethyl acetate, 4:1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 7.40–7.22 (m, 30H, ArH), 5.63 (d, *J* = 10.0 Hz, 1H, NH), 5.49 (s, 1H, H-1 Rha), 5.39 (d, *J* = 7.6 Hz, 1H, H-1 Gal), 5.25 (t, *J* = 3.7 Hz, 1H, H-12 OA), 5.12 (s, 2H, CH_2Ph), 4.92 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.89–4.77 (m, 5H, H-4 Gal, CH_2Ph), 4.71 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.63 (m, 2H, CH_2Ph), 4.52 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.49–4.43 (m, 2H, CH_2Ph), 4.17–4.11 (m, 2H, H-2 Rha, H-3 Rha), 3.95–3.91 (m, 1H, H-5a Xyl), 3.84–3.76 (m, 2H, H-5 Gal), 3.71 (dd, *J* = 9.9, 6.2 Hz, 1H, H-5 Rha), 3.63–3.59 (m, 2H, H-3 Xyl, H-4 Xyl), 3.54 (dd, *J* = 9.9, 7.3 Hz, 1H, H-4 Rha), 3.52–3.49 (m, 2H, H-6a,b Gal), 3.32 (dd, *J* = 9.1, 7.4 Hz, 1H, H-2 Xyl), 3.24–3.19 (m, 1H, H-5b Xyl), 3.17 (dd, *J* = 11.3, 4.5 Hz 1H, H-3 OA), 2.75 (dd, *J* = 13.8, 4.6 Hz, 1H, H-18 OA), 2.37–2.32 (m, 4H), 2.16 (td, *J* = 7.3, 5.3 Hz, 2H), 1.49 (s, 3H, CH_3 isopr), 1.33 (s, 3H, CH_3 isopr), 1.26 (s, 3H, CH_3 OA), 1.23 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.04 (s, 3H, CH_3 OA), 0.96 (t, *J* = 7.9 Hz, 9H, CH_2CH_3), 0.88 (s, 3H, CH_3 OA), 0.88 (s, 3H, CH_3 OA), 0.84 (s, 3H, CH_3 OA), 0.71 (s, 3H, CH_3 OA), 0.69 (s, 3H, CH_3 OA), 0.61–0.56 (m, 6H, CH_2CH_2). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 175.97, 173.72, 173.25, 143.52, 138.75, 138.44, 138.25, 137.78, 137.28, 136.13, 128.56, 128.54, 128.45, 128.42, 128.36, 128.35, 128.31, 128.30, 128.19, 127.97, 127.89, 127.85, 127.80, 127.76, 127.72, 127.56, 122.16, 109.24, 102.99, 97.52, 94.38, 83.79, 82.12, 79.63, 79.44, 78.98, 78.09, 77.93, 76.11, 75.62, 74.90, 73.49, 73.28, 73.15, 73.12, 71.22, 68.27, 66.11, 66.09, 65.33, 63.70, 55.22, 47.54, 46.66, 46.18, 46.06, 41.79, 41.31, 39.33, 39.28, 38.50, 36.96, 36.86, 34.35, 34.34, 33.95, 33.74, 33.01, 32.81, 31.68, 30.61, 29.73, 29.48, 29.46, 29.40, 29.34, 29.29, 29.27, 29.25, 29.21, 29.17, 29.11, 29.05, 28.48, 27.82, 27.69, 27.62, 26.28, 25.84, 25.57, 24.99, 24.95, 24.74, 23.61, 23.44, 23.35, 18.50, 17.84, 17.11, 16.06, 15.52, 7.09, 5.29. **HRMS (ESI)** *m/z*: Calcd for $C_{110}H_{151}NO_{18}Si$ $(M+Na)^+$ 1825.0631, found 1825.0574.

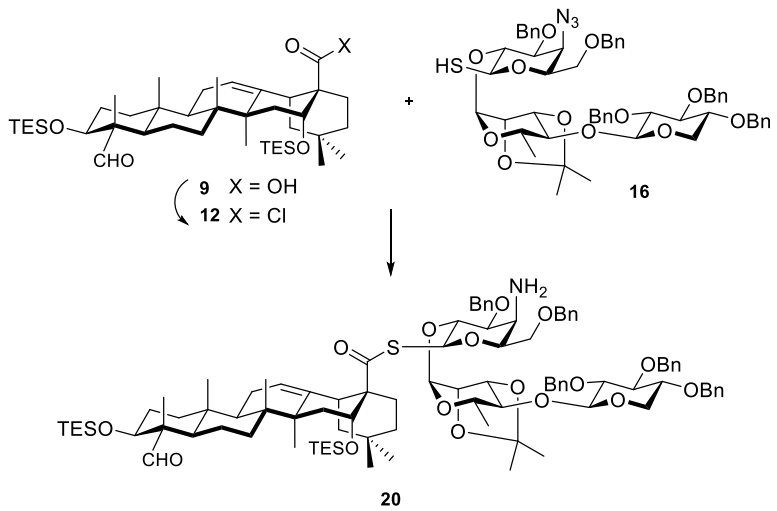


Oleanolic acid saponin ester (5) [MG-II-002]. Following General Experimental Procedure B, fully protected variant **26** (21.0 mg, 11.8 μmol, 1.0 equiv) was subjected to hydrogenolysis (6.0 mL THF/EtOH, 116 mg Pd/C) for 16 h followed by acid hydrolysis (4.9 mL TFA/H_2O) at 0º C for 1 h to provide, after HPLC purification [50–95% acetonitrile/water (0.05% TFA)], oleanolic acid saponin ester **5** (8.18 mg, 63% yield) as a white solid.

HPLC: t_R = 13.91 min, λ_{max} = 200 nm. **^{1}H NMR** (600 MHz, methanol-d_4) characteristic resonances: δ 5.42–5.39 (m, 2H, H-1 Rha, H-1 Gal), 5.26–5.23 (m, 1H, H-12 OA), 4.43 (d, *J* = 7.7 Hz, 1H, H-1 Xyl), 4.33 (q, *J* = 1.8 Hz, 1H, H-4 Gal), 3.98–3.93 (m, 3H, H-2 Gal, H-3 Gal, H-2 Rha), 3.87–3.79 (m, 3H, H-3 Rha, H-5 Rha, H-5a Xyl), 3.70 (td, *J* = 6.8, 1.8 Hz, 1H, H-5 Gal), 3.53–3.49 (m, 2H, H-4 Rha, H-6a Gal), 3.46 (ddd, *J* = 10.4, 8.9, 5.4 Hz, 1H, H-4 Xyl), 3.41 (dd, *J* = 11.6, 6.8 Hz, 1H, H-6b Gal), 3.35–3.33 (m, 1H, H-3 Xyl), 3.23–3.12 (m, 3H, H-2 Xyl, H-5b Xyl, H-3 OA), 2.82 (dd, *J* = 13.9, 4.6 Hz, 1H, H-18 OA), 2.39–2.24 (m, 4H), 1.92–1.87 (m, H-12a,b OA), 1.30 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.16 (s, 3H, CH_3 OA), 0.99 (s, 3H, CH_3 OA), 0.95 (s, 3H, CH_3 OA), 0.93 (s, 3H, CH_3 OA), 0.91 (s, 3H, CH_3 OA), 0.79 (s, 3H, CH_3 OA), 0.78 (s, 3H, CH_3 OA). **^{13}C NMR** (151 MHz, methanol-d_4, based on HSQC data): δ 124.79, 108.6, 102.61, 96.69, 86.01, 80.91, 79.42, 77.40, 77.38, 75.90, 73.39, 73.03, 72.19, 69.87, 68.39, 62.84, 57.93, 53.63, 50.43, 50.16, 48.46, 44.07, 41.04, 37.92, 36.09, 35.92, 35.42, 34.62, 34.16, 31.80, 31.52 30.23 29.93 29.93 28.98 28.49 27.31 27.28 25.72 25.36 25.25 25.06 20.71 19.41 18.94 17.48 17.21. **HRMS (ESI)** *m/z*: Calcd for $C_{59}H_{97}NO_{18}$ $(M+Na)^+$ 1130.6603, found 1130.6545.

B. SYNTHESIS OF THIOESTER VARIANTS

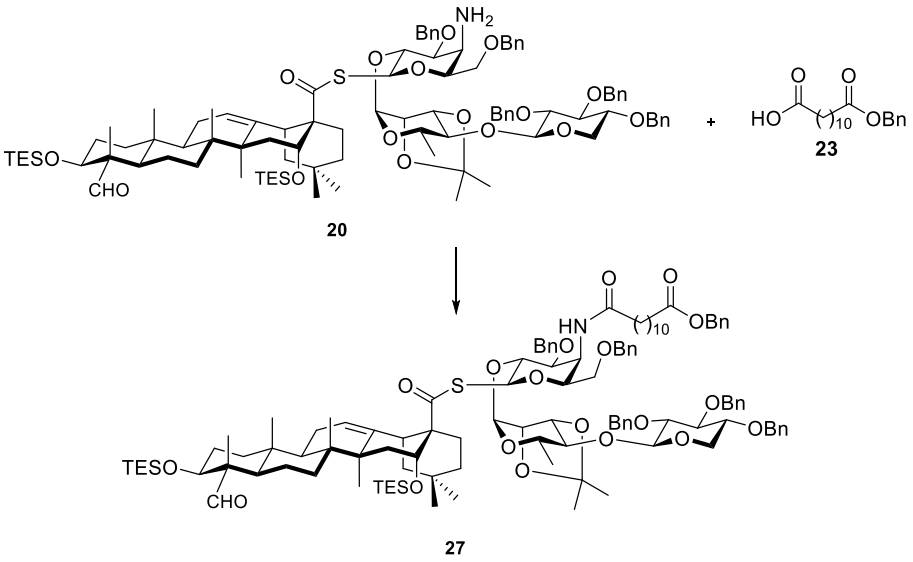
1. SYNTHESIS OF QUILLAIC ACID VARIANT 6 [QA(S)]



Protected quillaic acid thioester amine (20) [MG-I-105]. Following General Experimental Procedure C, acyl chloride **12** (29.0 mg, 39.4 μmol, 1.5 equiv) [obtained itself by activation of triterpene acid **9**[2] (40.0 mg, 55.9 μmol, 1.0 equiv) for 3 h in dry DCM (1.7 mL) with $SOCl_2$ (8.2 μL, 0.11 mmol, 2.0 equiv) and pyridine (45 μL, 0.56 mmol 10 equiv)] was reacted for 3 h with trisaccharide **16**[3] (26.0 mg, 26.3 μmol, 1.0 equiv) in dry THF (3.5 mL) in the presence of NaH (5.0 mg, 0.13 mmol, 5.0 equiv). Subsequent azide reduction for 3 h at 40 ºC with PhSeH (0.52 mmol, 20 equiv) in Et_3N (11.0 mL) provided the corresponding thioester amine **20** (25.0 mg, 57% overall yield) as a glassy film after silica gel column chromatography purification.

TLC: R_f 0.14 (toluene/ethyl acetate, 9:1 with 1% Et_3N, v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 9.29 (s, 1H, H-23 QA C*H*O), 7.36–7.26 (m, 25H, ArH), 5.55 (s, 1H, H-1 Rha), 5.33 (t, *J* = 3.8 Hz, 1H, H-12 QA), 4.91 (d, *J* = 7.6 Hz, 1H, H-1 Xyl), 4.90 (d, *J* = 10.4 Hz, 1H, H-1 Gal), 4.87–4.81 (m, 4H, CH_2Ph), 4.73 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.66 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.62 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.56 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.54–4.52 (m, 1H, H-16 QA), 4.50 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.49 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.15–4.10 (m, 2H, H-1 Rha), 4.01 (dd, *J* = 10.3, 8.9 Hz, 1H, H-2 Gal), 3.95 (dd, *J* = 11.6, 4.8 Hz, 1H, H-5a Xyl), 3.91 (dd, *J* = 10.1, 6.2 Hz, 1H, H-5 Rha), 3.78 (dd, *J* = 11.3, 4.6 Hz, 1H, H-4 Xyl), 3.74 (td, *J* = 6.3, 1.6 Hz, 1H, H-5 Gal), 3.66–3.58 (m, 5H, H-3 Xyl, H-6a Gal), 3.55 (dd, *J* = 8.9, 3.8 Hz, 1H, H-3 Gal), 3.52 (dd, *J* = 10.1, 5.9 Hz, 1H, H-6b Gal), 3.45–3.43 (m, 1H H-4 Gal), 3.30–3.26 (m, 1H H-2 Xyl), 3.22–3.18 (m, 1H, H-5b Xyl), 2.88 (dd, *J* = 13.6, 4.6 Hz, 1H, H-18 QA), 2.23 (t, *J* = 13.2 Hz, 1H, H-15a QA), 1.51 (s, 3H, CH_3 isopr), 1.33 (s, 3H, CH_3 isopr), 1.32 (s, 3H, CH_3), 1.31 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.26 (s, 3H, CH_3), 1.03 (s, 3H, CH_3 QA), 0.94 (t, *J* = 8.0 Hz, 9H, CH_2CH_3), 0.91 (t, *J* = 7.9 Hz, 9H, CH_2CH_3), 0.89 (s, 3H, CH_3 QA), 0.83 (s, 3H, CH_3 QA), 0.63 (s, 3H, CH_3 QA), 0.59 (qd, *J* = 7.9, 2.4 Hz, 6H, CH_2CH$_3$), 0.52 (qd, *J* = 7.9, 4.7 Hz, 6H, CH_2CH$_3$). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 207.59, 203.68, 142.51, 138.88, 138.63, 138.41, 138.22, 137.27, 128.63, 128.57, 128.50, 128.45, 128.29, 128.25, 128.22, 128.16, 127.91, 127.89, 127.82, 127.79, 127.77, 127.71, 123.60, 109.06, 102.59, 98.58, 84.35, 84.06, 82.66, 81.81, 78.36, 78.25, 78.22, 78.00, 76.40, 75.86, 75.70, 75.19, 73.64, 73.42, 73.34, 71.90, 70.87, 69.06, 65.40, 63.97, 48.94, 47.99, 46.99, 46.69, 41.64, 41.41,

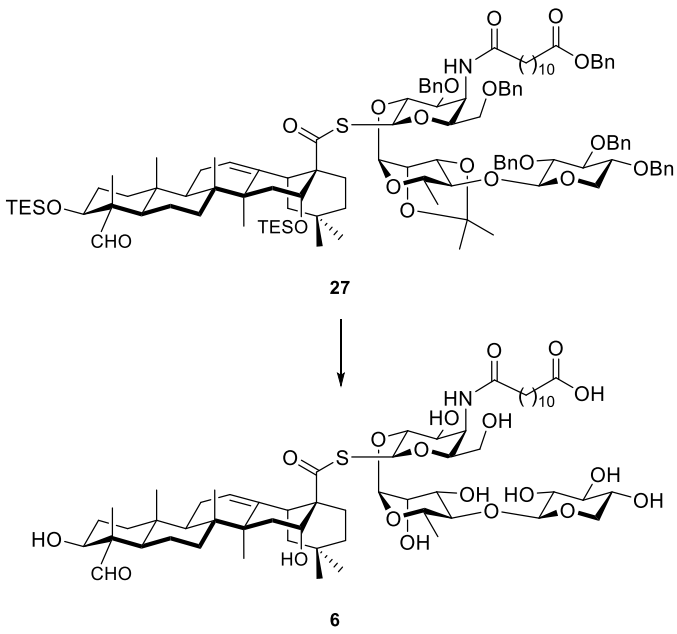
40.02, 38.32, 35.93, 35.26, 34.19, 32.69, 32.39, 32.30, 30.48, 29.84, 27.95, 26.95, 26.54, 26.52, 24.75, 23.59, 20.75, 17.76, 17.33, 15.84, 9.68, 8.40, 7.22, 6.95, 5.22, 5.05. **HRMS (ESI)** *m/z*: Calcd for Calcd for $C_{97}H_{137}NO_{16}SSi_2$ $(M+H)^+$ 1660.9275, found 1660.9341.



Fully protected quillaic acid saponin thioester (27) [MG-I-109]. Following General Experimental Procedure A, saponin amine **20** (25.0 mg, 15.1 μmol, 1.0 equiv) in dry THF (4.5 mL) was acylated with acid **23**[1] (48.0 mg, 0.15 mmol, 10.0 equiv) in dry THF (4.5 mL) via activation with EtOCOCl (10.0 μL, 0.11 mmol, 7.0 equiv) and Et_3N (84.0 μL, 0.60 mmol, 40.0 equiv) to provide, after purification by silica gel column chromatography, the fully protected quillaic acid saponin thioester **27** (19.0 mg, 65% yield) as a transparent film.

TLC: R_f 0.25 (hexane/ethyl acetate, 4:1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 9.29 (s, 1H, H-23 QA C*H*O), 7.39–7.24 (m, 30H, ArH), 5.85 (br s, 1H, NH), 5.50 (s, 1H, H-1 Rha), 5.32 (t, *J* = 3.8 Hz, 1H, H-12 QA), 5.12 (s, 2H, CH_2Ph), 4.92–4.80 (m, 5H, H-1 Gal, H-4 Gal, H-1 Xyl, CH_2Ph), 4.72 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.63 (d, *J* = 11.6 Hz, 2H, CH_2Ph), 4.53–4.50 (m, 2H, H-16 QA, CH_2Ph), 4.45–4.41 (m, 2H, CH_2Ph), 4.12 (dd, *J* = 7.4, 5.6 Hz, 1H, H-3 Rha), 4.09 (d, *J* = 5.7 Hz, 1H, H-2 Rha), 3.94 (dd, *J* = 11.5, 4.5 Hz, 1H, H-5a Xyl), 3.91 – 3.82 (m, 2H), 3.80–3.74 (m, 2H, H-5 Gal, H-5 Rha), 3.63–3.57 (m, 4H, H-3 Xyl, H-4 Xyl, H-4 Rha, H-3 QA), 3.40 (dd, *J* = 5.9, 3.3 Hz, 1H, H-6a,b Gal), 3.30–3.27 (m, 1H, H-2 Xyl), 3.22–3.17 (m, 1H, H-5b Xyl), 2.86 (dd, *J* = 13.8, 4.5 Hz, 1H, H-18 QA), 1.50 (s, 3H, CH_3 isopr), 1.35 (s, 3H, CH_3 isopr), 1.33 (s, 3H, CH_3 QA), 1.29 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.03 (s, 3H, CH_3 QA), 0.95 (t, *J* = 7.9 Hz, 9H, CH_2CH_3), 0.91 (s, 3H, CH_3 QA), 0.91 (t, *J* = 7.9 Hz, 9H, CH_2CH_3), 0.88 (s, 3H, CH_3 QA), 0.84 (s, 3H, CH_3 QA), 0.64–0.59 (m, 9H, including 0.62 [s, 3H, CH_3 QA], and C$H_2$$CH_3$), 0.52 (qd, *J* = 7.9, 4.6 Hz, 6H, C$H_2$$CH_3$). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 207.42, 202.96, 173.68, 173.29, 142.82, 138.75, 138.53, 138.28, 137.86, 137.22, 136.16, 128.73, 128.54, 128.44, 128.37, 128.33, 128.29, 128.16, 128.15, 128.10, 128.02, 127.81, 127.79, 127.76, 127.70, 127.67, 127.58, 122.83, 109.02, 102.44, 98.54, 83.92, 82.44, 81.91, 81.75, 78.18, 78.08, 78.06, 77.40, 76.15, 75.77, 75.71, 75.01, 73.59, 73.29, 73.21, 70.80, 68.81, 66.06, 65.28, 63.85, 57.78, 56.65, 55.92, 47.85, 46.91, 46.48, 46.23, 41.32, 41.22, 39.89, 38.18, 36.97, 35.79, 35.19, 34.35, 34.03, 32.53, 32.21, 30.33, 29.72, 29.54, 29.48, 29.45, 29.40, 29.29, 29.26, 29.23, 29.16, 27.81, 26.82, 26.43, 26.36, 25.91, 24.99, 24.48, 23.44, 20.59, 17.68,

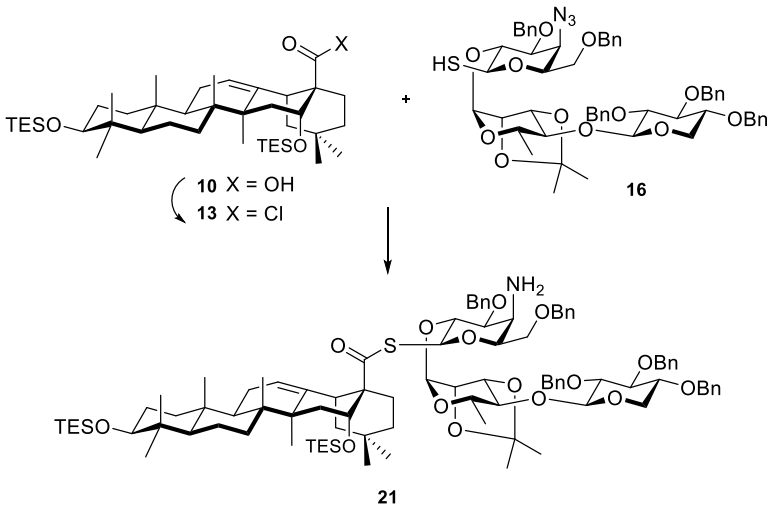
17.11, 15.74, 9.59, 7.09, 6.83, 5.09, 4.93. **HRMS (ESI)** *m/z*: Calcd for $C_{116}H_{163}NO_{19}SSi_2$ $(M+Na)^+$ 1985.0977, found 1985.1052.



Quillaic acid saponin thioester (6) [MG-II-075]. Following General Experimental Procedure B, fully protected variant **27** (15.0 mg, 7.64 μmol, 1.0 equiv) was subjected to hydrogenolysis (3.7 mL THF/EtOH, 81 mg Pd/C) for 20 min followed by acid hydrolysis (3.0 mL TFA/H_2O) at 0º C for 1 h to provide, after HPLC purification [30–95% acetonitrile/water (0.05% TFA)], quillaic acid saponin thioester **6** (4.81 mg, 46% yield) as a white solid.

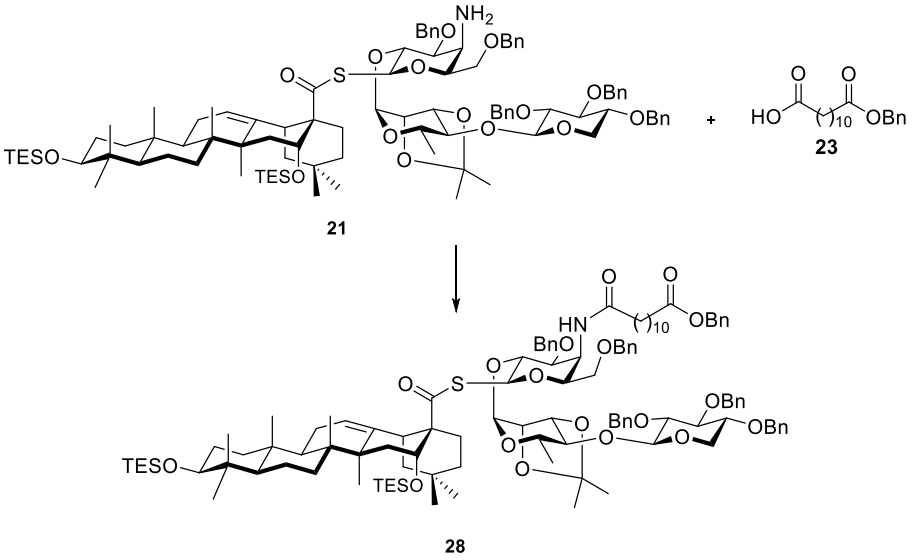
HPLC: t_R = 18.71 min, λ_{max} = 200 nm. **^{1}H NMR** (600 MHz, methanol-d_4) characteristic resonances: δ 9.31 (s, 1H, H-23 QA C*H*O), 5.34 (t, *J* = 3.7 Hz, 1H, H-12 QA), 5.23 (d, *J* = 1.8 Hz, 1H, H-1 Rha), 4.89 (d, *J* = 9.8 Hz, 1H, H-1 Gal), 4.50–4.48 (m, 1H, H-16 QA), 4.44 (d, *J* = 7.7 Hz, 1H, H-1 Xyl), 4.36 (dd, *J* = 4.5, 1.5 Hz, 1H, H-4 Gal), 4.05–3.99 (m, 1H, H-5 Rha), 3.94–3.86 (m, 3H, H-2 Rha, H-2 Gal, H-3 Gal), 3.83 (dd, *J* = 11.4, 5.4 Hz, 1H, H-5a Xyl), 3.79–3.74 (m, 2H, H-3 Rha), 3.67–3.64 (m, 1H, H-5 Gal), 3.53 (t, *J* = 9.5 Hz, 1H, H-4 Rha), 3.47–3.42 (m, 2H, H-4 Xyl, H-6a Gal), 3.34 (dd, *J* = 11.5, 7.0 Hz, 1H, H-6a Gal), 3.32–3.28 (m, 1H, H-3 Xyl), 3.21–3.15 (m, 2H, H-2 Xyl, H-5b Xyl), 2.98 (dd, *J* = 13.9, 4.5 Hz, 1H, H-18 QA), 1.40 (s, 3H, CH_3 QA), 1.38–1.28 (m, 22H including CH_3 Rha), 1.02 (s, 3H, CH_3 QA), 0.99 (s, 3H, CH_3 QA), 0.98 (s, 3H, CH_3 QA), 0.89 (s, 3H, CH_3 QA), 0.76 (s, 3H, CH_3 QA).**^{13}C NMR** (151 MHz, methanol-d_4, based on HSQC data): δ 123.78, 106.85, 102.35, 84.07, 82.59, 79.41, 77.98, 76.01, 75.80, 75.43, 75.07, 72.38, 72.18, 71.69, 70.76, 68.97, 66.96, 61.48, 52.39, 48.56, 47.75, 47.69, 42.63, 39.16, 36.52, 36.23, 35.61, 35.01, 33.13, 32.99, 32.97, 30.16, 26.93, 26.89, 26.69, 25.92, 21.59, 20.36, 17.87, 17.74, 16.01, 9.20. **HRMS (ESI)** *m/z*: Calcd for $C_{59}H_{95}NO_{19}S$ $(M+Na)^+$ 1176.6117, found 1176.6075.

2. SYNTHESIS OF ECHINOCYSTIC ACID VARIANT 7 [EA(S)]



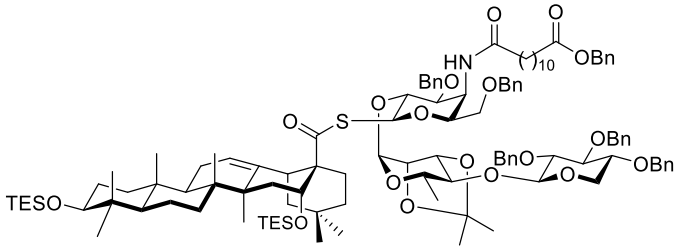
Protected echinocystic acid thioester amine (21) [MG-I-104]. Following General Experimental Procedure C, acyl chloride **13** (29.0 mg, 39.4 μmol, 1.5 equiv) [obtained itself by activation of triterpene acid **10**[2] (42.0 mg, 59.9 μmol, 1.0 equiv) for 3 h in dry DCM (1.8 mL) with $SOCl_2$ (8.7 μL, 0.12 mmol, 2.0 equiv) and pyridine (48 μL, 0.59 mmol 10 equiv)] was reacted for 3 h with trisaccharide **16**[3] (26.0 mg, 26.3 μmol, 1.0 equiv) in dry THF (3.5 mL) in the presence of NaH (5.0 mg, 0.13 mmol, 5.0 equiv). Subsequent azide reduction for 3 h at 40 ºC with PhSeH (0.52 mmol, 20 equiv) in Et_3N (11.0 mL) provided the corresponding thioester amine **21** (43.0 mg, 99% overall yield) as a glassy film after silica gel column chromatography purification.

TLC: R_f 0.14 (toluene/ethyl acetate, 9:1 with 1% Et_3N, v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 7.29–7.17 (m, 25H, ArH), 5.47 (s, 1H, H-1 Rha), 5.26 (t, *J* = 3.8 Hz, 1H, H-12 EA), 4.84 (d, *J* = 10.3 Hz, 1H, H-1 Gal), 4.83 (d, *J* = 7.6 Hz, 1H, H-1 Xyl), 4.82–4.73 (m, 4H, C*H*$_2$Ph), 4.64 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.60–4.54 (m, 3H, C*H*$_2$Ph), 4.50 (d, *J* = 11.8 Hz, 1H, C*H*$_2$Ph), 4.47–4.45 (m, 1H, H-16 EA), 4.41 (dd, *J* = 11.5, 4.4 Hz, 2H, C*H*$_2$Ph), 4.08–4.02 (m, 2H, H-2 Rha), 3.93 (dd, *J* = 10.3, 8.9 Hz, 1H, H-2 Gal), 3.87 (dd, *J* = 11.6, 4.7 Hz, 1H, H-5a Xyl), 3.83 (dd, *J* = 10.1, 6.1 Hz, 1H, H-5 Rha), 3.67 (td, *J* = 6.2, 1.6 Hz, 1H, H-5 Gal), 3.58–3.50 (m, 4H, H-3 Xyl, H-4 Xyl, H-4 Rha, H-6a Gal), 3.49–3.44 (m, 2H, H-3 Gal, H-6b Gal), 3.36 (dd, *J* = 3.8, 1.6 Hz, 1H, H-4 Gal), 3.22 (dd, *J* = 9.0, 7.6 Hz, 1H, H-2 Xyl), 3.16–3.08 (m, 2H, H-5b Xyl), 2.82 (dd, *J* = 13.7, 4.5 Hz, 1H, H-3 EA), 2.15 (t, *J* = 13.3 Hz, 1H, H-18a EA), 1.43 (s, 3H, CH_3 isopr), 1.25–1.23 (m, 6H, including 1.24 [d, 3H, *J* = 6.8 Hz, CH_3 Rha], and CH_3 isopr), 1.18 (s, 3H, CH_3 EA), 0.88 (td, *J* = 7.9, 7.0 Hz, 18H, CH_2CH_3), 0.84 (s, 3H, CH_3 EA), 0.80 (s, 3H, CH_3 EA), 0.77 (s, 3H, CH_3 EA), 0.76 (s, 3H, CH_3 EA), 0.64 (s, 3H, CH_3 EA), 0.56 (s, 3H, CH_3 EA), 0.55–0.48 (m, 9H, CH_2CH_3). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 203.36, 142.25, 138.79, 138.53, 138.28, 138.15, 137.19, 128.51, 128.45, 128.39, 128.32, 128.15, 128.14, 128.03, 128.01, 127.79, 127.76, 127.69, 127.65, 127.56, 123.83, 108.94, 102.66, 98.52, 84.26, 83.94, 82.55, 81.65, 79.55, 78.38, 78.26, 78.12, 77.94, 76.31, 75.71, 75.67, 75.08, 73.54, 73.17, 71.94, 70.75, 68.96, 65.28, 63.82, 56.43, 55.38, 48.83, 46.87, 46.64, 41.42, 41.23, 39.59, 39.33, 38.59, 36.90, 32.97, 32.58, 32.29, 30.36, 29.72, 29.55, 29.53, 29.44, 29.23, 29.11, 28.48, 27.85, 27.75, 26.42, 26.38, 24.61, 23.49, 18.62, 17.64, 17.24, 16.11, 15.51, 7.13, 7.07, 5.33, 4.95. **HRMS (ESI)** *m/z*: Calcd for $C_{97}H_{139}NO_{15}SSi_2$ $(M+H)^+$ 1646.9486, found 1646.9552.

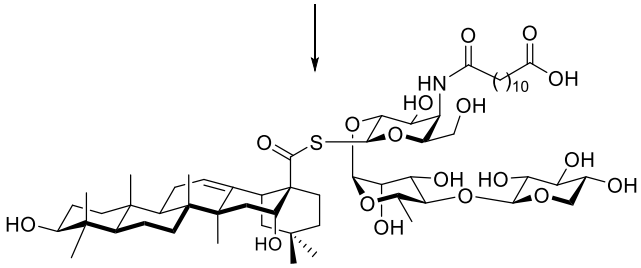


Fully protected echinocystic acid saponin thioester (28) [MG-I-108]. Following General Experimental Procedure A, saponin amine **21** (43.0 mg, 26.1 μmol, 1.0 equiv) in dry THF (7.9 mL) was acylated with acid **23**[1] (84.0 mg, 0.26 mmol, 10.0 equiv) in dry THF (7.9 mL) via activation with EtOCOCl (17.5 μL, 0.18 mmol, 7.0 equiv) and Et_3N (145 μL, 1.04 mmol, 40.0 equiv) to provide, after purification by silica gel column chromatography, the fully protected echinocystic acid saponin thioester **28** (36.0 mg, 71% yield) as a transparent film.

TLC: R_f 0.23 (hexane/ethyl acetate, 4:1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 7.42–7.26 (m, 30H, ArH), 5.86 (br s, 1H, NH), 5.53 (s, 1H, H-1 Rha), 5.35 (t, *J* = 3.7 Hz, 1H, H-12 EA), 5.14 (s, 2H, C*H*$_2$Ph), 4.95–4.82 (m, 7H, H-1 Gal, H-4 Gal, H-1 Xyl, C*H*$_2$Ph), 4.75 (d, *J* = 11.7 Hz, 1H, C*H*$_2$Ph), 4.68–4.64 (m, 2H, C*H*$_2$Ph), 4.58–4.54 (m, 2H, H-16 EA, C*H*$_2$Ph), 4.48–4.43 (m, 2H, C*H*$_2$Ph), 4.17–4.11 (m, 2H, H-2 Rha, H-3 Rha), 3.97 (dd, *J* = 11.7, 4.2 Hz, 1H, H-5a Xyl), 3.94–3.85 (m, 2H, H-5 Rha), 3.79 (t, *J* = 6.1 Hz, 1H, H-5 Gal), 3.67–3.59 (m, 4H, H-4 Rha), 3.46–3.42 (m, 2H, H-6a,b Gal), 3.35–3.31 (m, 1H), 3.25–3.19 (m, 2H, H-3 EA, H-5b Xyl), 2.90 (dd, *J* = 13.8, 4.4 Hz, 1H, H-18 EA), 1.53 (s, 3H, CH_3 isopr), 1.37 (s, 3H, CH_3 isopr), 1.35 (s, 3H, CH_3 EA), 1.33 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 0.99 (t, *J* = 8.0 Hz, 9H, $CH_2C\mathit{H}_3$), 0.99 (t, *J* = 8.0 Hz, 9H, CH_2CH_3), 0.93 (s, 3H, CH_3 EA), 0.91 (s, 3H, CH_3 EA), 0.87 (s, 6H, CH_3 EA), 0.75 (s, 3H, CH_3 EA), 0.69–0.59 (m, 15H, including 0.66 [s, 3H, CH_3 EA], and C*H*$_2$CH$_3$). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 202.83, 173.68, 173.31, 142.64, 138.77, 138.54, 138.27, 137.89, 137.25, 136.17, 128.73, 128.54, 128.44, 128.38, 128.32, 128.29, 128.16, 128.15, 128.12, 128.00, 127.79, 127.77, 127.75, 127.69, 127.66, 127.57, 123.27, 109.01, 102.57, 98.56, 83.93, 82.45, 81.87, 81.78, 79.48, 78.35, 78.10, 78.08, 77.43, 76.17, 75.88, 75.70, 75.02, 73.63, 73.18, 70.80, 68.81, 66.05, 65.28, 63.82, 58.35, 56.69, 55.34, 46.91, 46.55, 46.23, 41.25, 41.17, 39.59, 39.32, 38.57, 36.97, 36.88, 35.25, 34.36, 34.17, 32.90, 32.54, 32.30, 30.33, 29.48, 29.45, 29.41, 29.28, 29.25, 29.17, 28.46, 27.83, 27.73, 26.44, 26.34, 25.91, 25.00, 24.47, 23.47, 18.57, 17.68, 17.17, 16.11, 15.55, 7.12, 7.06, 5.31, 4.95. **HRMS (ESI)** *m/z*: Calcd for $C_{116}H_{165}NO_{18}SSi_2$ $(M+Na)^+$ 1971.1184, found 1971.1238.



28

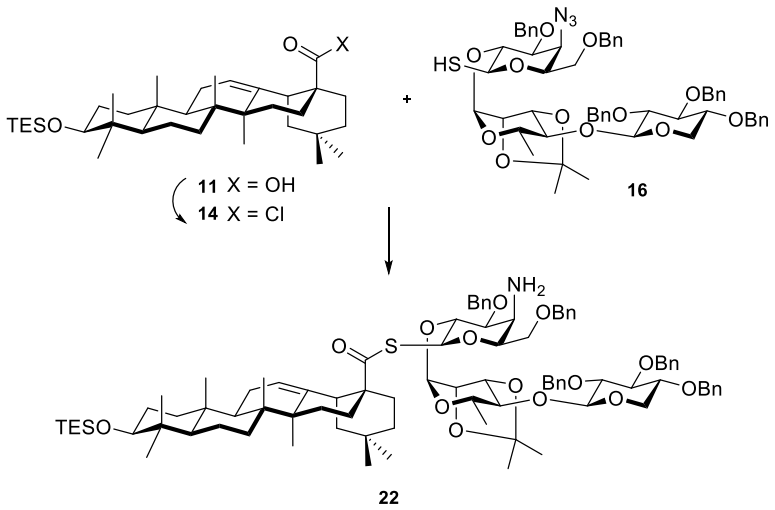


7

Echinocystic acid saponin thioester (7) [MG-II-072]. Following General Experimental Procedure B, fully protected variant **28** (27.0 mg, 13.9 μmol, 1.0 equiv) was subjected to hydrogenolysis (6.8 mL THF/EtOH, 140 mg Pd/C) for 20 min followed by acid hydrolysis (5.0 mL TFA/H_2O) at 0º C for 1 h to provide, after HPLC purification [40–95% acetonitrile/water (0.05% TFA)], echinocystic acid saponin thioester **7** (6.33 mg, 36% yield) as a white solid.

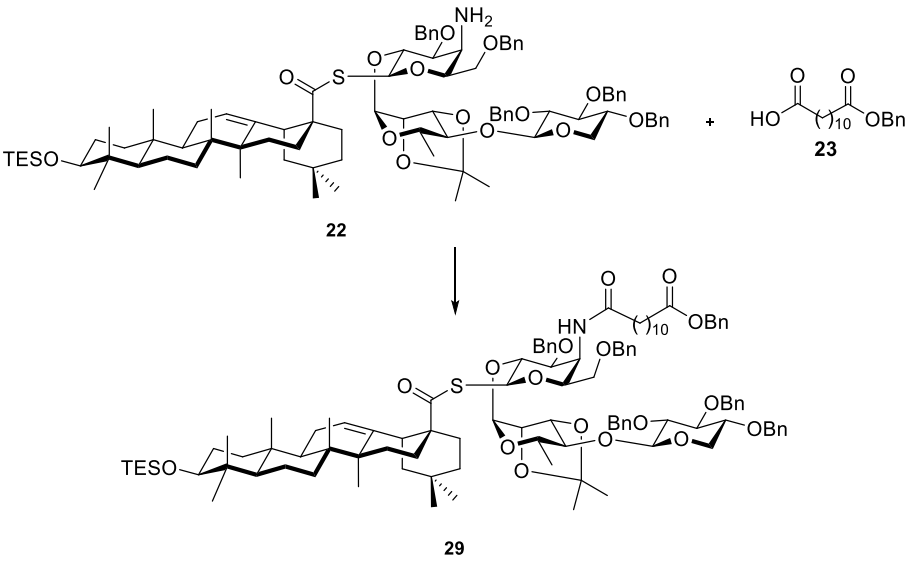
HPLC: t_R = 18.05 min, λ_{max} = 200 nm. **^{1}H NMR** (600 MHz, methanol-d_4) characteristic resonances: δ 5.32 (t, *J* = 3.8 Hz, 1H, H-12 EA), 5.22 (d, *J* = 1.8 Hz, 1H, H-1 Rha), 4.89 (d, *J* = 9.8 Hz, 1H, H-1 Gal), 4.50– 4.48 (m, 1H, H-16 EA), 4.45 (d, *J* = 7.7 Hz, 1H, H-1 Xyl), 4.36 (dd, *J* = 4.5, 1.5 Hz, 1H, H-4 Gal), 4.03 (dq, *J* = 9.7, 6.3 Hz, 1H, H-5 Rha), 3.95–3.86 (m, 3H, H-2 Rha, H-2 Gal, H-3 Gal), 3.84 (dd, *J* = 11.5, 5.4 Hz, 1H, H-5a Xyl), 3.76 (dd, *J* = 9.4, 3.2 Hz, 1H, H-3 Rha), 3.67–3.64 (m, 1H, H-5 Gal), 3.53 (t, *J* = 9.4 Hz, 1H, H-4 Rha), 3.49–3.43 (m, 2H, H-4 Xyl, H-6a Gal), 3.36–3.32 (m, 2H, H-3 Xyl, H-6b Gal), 3.25–3.13 (m, 3H, H-2 Xyl, H-3 EA, H-5b Xyl), 2.98 (dd, *J* = 13.9, 4.5 Hz, 1H, H-18 EA), 1.91–1.89 (m, 1H, H-11 EA), 1.42–1.30 (m, 20H including CH_3 Rha), 0.98 (s, 3H, CH_3 EA), 0.98 (s, 3H, CH_3 EA), 0.95 (s, 3H, CH_3 EA), 0.89 (s, 3H, CH_3 EA), 0.78 (s, 3H, CH_3 EA), 0.76 (s, 3H, CH_3 EA). **^{13}C NMR** (151 MHz, methanol-d_4, based on HSQC data): δ 124.15, 106.99, 102.49, 84.20, 82.65, 79.49, 79.46, 78.06, 76.10, 75.90, 75.57, 75.21, 72.20, 71.80, 70.90, 69.08, 67.05, 61.54, 56.66, 52.45, 49.00, 47.91, 42.73, 39.69, 36.62, 36.29, 35.73, 34.85, 33.95, 33.06, 33.03, 30.24, 28.55, 27.66, 27.06, 26.94, 25.95, 25.09, 24.34, 19.37, 17.96, 17.87, 16.16, 15.96. **HRMS (ESI)** *m/z*: Calcd for $C_{59}H_{97}NO_{18}S$ $(M+Na)^+$ 1162.6324, found 1162.6343.

3. SYNTHESIS OF OLEANOLIC ACID VARIANT 8 [OA(S)]



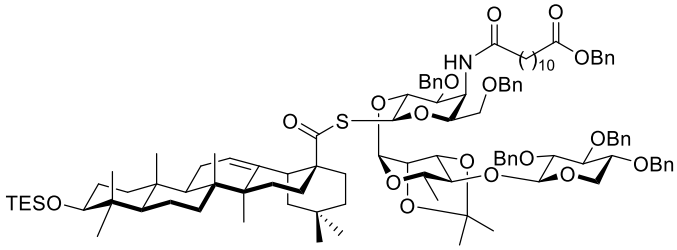
Protected oleanolic acid thioester amine (22) [MG-I-094]. Following General Experimental Procedure C, acyl chloride **14** (20.0 mg, 33.9 μmol, 1.5 equiv) [obtained itself by activation of triterpene acid **11**[2] (35.0 mg, 61.3 μmol, 1.0 equiv) for 3 h in dry DCM (1.8 mL) with $SOCl_2$ (8.9 μL, 0.12 mmol, 2.0 equiv) and pyridine (50 μL, 0.61 mmol 10 equiv)] was reacted for 3 h with trisaccharide **16**[3] (23.0 mg, 23.2 μmol, 1.0 equiv) in dry THF (2.6 mL) in the presence of NaH (5.0 mg, 0.13 mmol, 5.0 equiv). Subsequent azide reduction for 3 h at 40 ºC with PhSeH (0.46 mmol, 20 equiv) in Et_3N (10.0 mL) provided the corresponding thioester amine **22** (26.0 mg, 75% overall yield) as a glassy film after silica gel column chromatography purification.

TLC: R_f 0.14 (toluene/ethyl acetate, 7:3 with 1% Et_3N, v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 7.37–7.26 (m, 25H, ArH), 5.53 (s, 1H, H-1 Rha), 5.27 (t, *J* = 3.7 Hz, 1H, H-12 OA), 4.97 (d, *J* = 10.3 Hz, 1H, H-1 Gal), 4.91–4.87 (m, 2H, H-1 Xyl, C*H*$_2$Ph), 4.84 (d, *J* = 10.9 Hz, 1H, C*H*$_2$Ph), 4.81 (d, *J* = 10.9 Hz, 1H, C*H*$_2$Ph), 4.72 (d, *J* = 11.7 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J* = 11.3 Hz, 1H, C*H*$_2$Ph), 4.65–4.60 (m, 2H, C*H*$_2$Ph), 4.58 (d, *J* = 11.9 Hz, 1H, C*H*$_2$Ph), 4.53–4.50 (m, 2H, C*H*$_2$Ph), 4.16–4.12 (m, 2H, H-2 Rha), 4.00 (dd, *J* = 10.3, 8.9 Hz, 1H, H-2 Gal), 3.95 (dd, *J* = 11.5, 4.4 Hz, 1H, H-5a Xyl), 3.91 (dd, *J* = 10.1, 6.2 Hz, 1H, H-5 Rha), 3.77 (ddd, *J* = 7.2, 5.8, 1.6 Hz, 1H, H-5 Gal), 3.65 (dd, *J* = 10.0, 6.8 Hz, 1H, H-6a Gal), 3.62–3.56 (m, 6H, H-3 Gal, H-6b Gal, H-3 Xyl, H-4 Xyl, H-4 Rha), 3.46 (dd, *J* = 3.8, 1.6 Hz, 1H, H-4 Gal), 3.30 (dd, *J* = 9.1, 7.6 Hz, 1H, H-2 Xyl), 3.23–3.16 (m, 2H, H-3 OA, H-5b Xyl), 2.80 (dd, *J* = 13.2, 4.5 Hz, 1H, H-18 OA), 1.52 (s, 3H, CH_3 isopr), 1.33 (s, 3H, CH_3 isopr), 1.31 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.05 (s, 3H, CH_3 OA), 0.97 (t, *J* = 7.9 Hz, 9H, $CH_2C\mathit{H}_3$), 0.87 (s, 3H, CH_3 OA), 0.86 (s, 3H, CH_3 OA), 0.86 (s, 3H, CH_3 OA), 0.83 (s, 3H, CH_3 OA), 0.69 (s, 3H, CH_3 OA), 0.66 (s, 3H, CH_3 OA), 0.61–0.56 (m, 6H, C*H*$_2$CH$_3$). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 203.80, 142.74, 138.78, 138.50, 138.29, 138.12, 137.26, 128.50, 128.44, 128.39, 128.34, 128.29, 128.17, 128.10, 127.98, 127.81, 127.78, 127.74, 127.70, 127.67, 127.53, 123.50, 108.94, 102.79, 98.61, 84.21, 83.84, 82.41, 81.51, 79.51, 78.54, 78.31, 78.06, 77.72, 77.25, 77.04, 76.83, 76.37, 75.65, 74.93, 73.51, 73.13, 72.14, 70.76, 68.72, 65.29, 63.76, 55.29, 54.44, 48.72, 47.62, 46.30, 41.78, 41.52, 39.54, 39.30, 38.50, 36.89, 33.94, 33.08, 32.90, 32.74, 30.54, 29.72, 28.48, 27.85, 27.71, 27.11, 26.40, 25.85, 23.82, 23.72, 23.50, 18.68, 17.47, 17.15, 16.06, 15.38, 7.07, 5.32. **HRMS (ESI)** *m/z*: Calcd for $C_{91}H_{125}NO_{14}SSi$ $(M+H)^+$ 1516.8668, found 1516.8751.

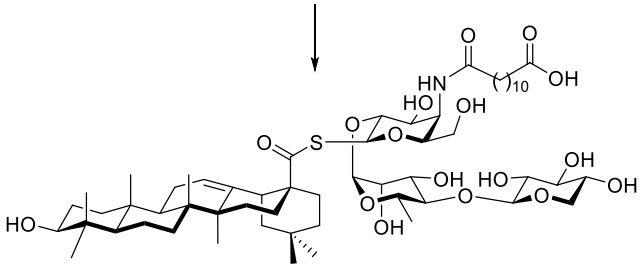


Fully protected oleanolic acid saponin thioester (29) [MG-I-107]. Following General Experimental Procedure A, saponin amine **22** (26.0 mg, 17.1 μmol, 1.0 equiv) in dry THF (5.2 mL) was acylated with acid **23**[1] (55.0 mg, 0.17 mmol, 10.0 equiv) in dry THF (7.9 mL) via activation with EtOCOCl (17.5 μL, 0.18 mmol, 7.0 equiv) and Et_3N (145 μL, 1.04 mmol, 40.0 equiv) to provide, after purification by silica gel column chromatography, the fully protected oleanolic acid saponin thioester **29** (24.0 mg, 77% yield) as a transparent film.

TLC: R_f 0.26 (hexane/ethyl acetate, 4:1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$) characteristic resonances: δ 7.40–7.24 (m, 30H, ArH), 5.89 (br s, 1H, NH), 5.49 (s, 1H, H-1 Rha), 5.27 (t, *J* = 3.7 Hz, 1H, H-12 OA), 5.12 (s, 2H, CH_2Ph), 4.93–4.79 (m, 7H, H-1 Gal, H-4 Gal, H-1 Xyl, CH_2Ph), 4.71 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.65–4.61 (m, 2H, CH_2Ph), 4.53 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.48–4.41 (m, 2H, CH_2Ph), 4.17–4.10 (m, 2H, H-2 Rha, H-3 Rha), 3.97–3.92 (m, 1H, H-5a Xyl), 3.92–3.82 (m, 2H, including 3.85 [dd, *J* = 10.1, 6.1 Hz, 1H, H-5 Rha], and H-3 Gal), 3.79 (t, *J* = 6.2 Hz, 1H, H-5 Gal), 3.65–3.56 (m, 4H, H-2 Gal, H-3 Xyl, H-4 Xyl, H-4 Rha), 3.47–3.40 (m, 2H, H-6a,b Gal), 3.32–3.28 (m, 1H, H-2 Xyl), 3.23–3.16 (m, 2H, H-3 OA, H-5b Xyl), 2.78 (dd, *J* = 13.5, 4.6 Hz, 1H, H-18 OA), 1.50 (s, 3H, CH_3 isopr), 1.35 (s, 3H, CH_3 isopr), 1.29 (d, *J* = 6.2 Hz, 3H, CH_3 Rha), 1.07 (s, 3H, CH_3 OA), 0.97 (t, *J* = 7.9 Hz, 9H, $CH_2C\mathit{H}_3$), 0.88–0.86 (m, 9H, CH_3 OA), 0.83 (s, 3H, CH_3 OA), 0.70 (s, 3H, CH_3 OA), 0.66 (s, 3H, CH_3 OA), 0.61–0.56 (m, 6H, CH_2CH_3). **^{13}C NMR** (151 MHz, $CDCl_3$) characteristic resonances: δ 203.72, 173.69, 173.32, 142.98, 138.77, 138.52, 138.28, 137.87, 137.31, 136.17, 128.69, 128.54, 128.43, 128.38, 128.33, 128.29, 128.28, 128.16, 128.15, 128.13, 127.98, 127.83, 127.78, 127.75, 127.74, 127.69, 127.67, 127.54, 123.11, 109.02, 102.64, 98.61, 83.84, 82.35, 81.78, 81.74, 79.46, 78.46, 78.13, 78.03, 76.22, 75.65, 74.91, 73.61, 73.14, 70.80, 68.62, 66.05, 65.28, 63.78, 55.27, 54.58, 47.57, 46.29, 46.20, 41.59, 41.56, 39.54, 39.31, 38.50, 36.98, 36.87, 34.36, 33.99, 33.07, 32.87, 32.69, 30.52, 29.48, 29.45, 29.39, 29.28, 29.25, 29.16, 28.46, 27.83, 27.70, 27.07, 26.43, 25.92, 25.82, 24.99, 23.64, 23.50, 18.63, 17.59, 17.16, 16.08, 15.44, 7.07, 5.31. **HRMS (ESI)** *m/z*: Calcd for $C_{110}H_{151}NO_{17}SSi$ $(M+Na)^+$ 1841.0370, found 1841.0415.



29



8

Oleanolic acid saponin thioester (8) [MG-II-002]. Following General Experimental Procedure B, fully protected variant **29** (24.0 mg, 13.2 μmol, 1.0 equiv) was subjected to hydrogenolysis (6.8 mL THF/EtOH, 140 mg Pd/C) for 20 min followed by acid hydrolysis (5.5 mL TFA/H_2O) at 0º C for 1 h to provide, after HPLC purification [40–95% acetonitrile/water (0.05% TFA)], oleanolic acid saponin thioester **8** (3.46 mg, 23% yield) as a white solid.

HPLC: t_R = 17.89 min, λ_{max} = 200 nm. **^{1}H NMR** (600 MHz, methanol-d_4) characteristic resonances: δ 5.26 (t, *J* = 3.7 Hz, 1H, H-12 OA), 5.21 (d, *J* = 1.7 Hz, 1H, H-1 Rha), 4.94 (d, *J* = 9.6 Hz, 1H, H-1 Gal), 4.43 (d, *J* = 7.7 Hz, 1H, H-1 Xyl), 4.38–4.36 (m, 1H, H-4 Gal), 4.08–4.03 (m, 1H, H-5 Rha), 3.95 (dd, *J* = 3.3, 1.8 Hz, 1H, H-2 Rha), 3.93–3.87 (m, 2H, H-2 Gal, H-3 Gal), 3.84 (dd, *J* = 11.5, 5.4 Hz, 1H, H-5a Xyl), 3.76 (dd, *J* = 9.5, 3.2 Hz, 1H, H-3 Rha), 3.69–3.66 (m, 1H, H-5 Gal), 3.52 (t, *J* = 9.5 Hz, 1H, H-4 Rha), 3.49–3.33 (m, 4H, H-3 Xyl, H-4 Xyl, H-6a Gal, H-6b Gal), 3.23–3.12 (m, 4H, H-2 Xyl, H-5b Xyl, H-3 OA), 2.87 (d, *J* = 11.9 Hz, 1H, H-18 OA), 1.90–1.87 (m, 2H, H-11 OA), 1.38–1.28 (m, 20H, including CH_3 Rha), 1.16 (s, 3H, CH_3 OA), 0.98 (s, 3H, CH_3 OA), 0.95 (s, 3H, CH_3 OA), 0.94 (s, 3H, CH_3 OA), 0.92 (s, 3H, CH_3 OA), 0.78 (s, 3H, CH_3 OA), 0.76 (s, 3H. CH_3 OA). **^{13}C NMR** (151 MHz, methanol-d_4, based on HSQC data): δ 124.14, 106.96, 102.45, 82.49, 79.45, 77.92, 75.85, 75.78, 75.78, 72.22, 71.68, 70.79, 69.09, 66.96, 61.49, 56.49, 52.43, 48.61, 47.07, 42.97, 39.61, 36.54, 35.21, 34.23, 33.05, 30.28, 28.47, 26.96, 26.11, 26.02, 24.76, 24.27, 24.01, 19.33, 17.79, 17.74, 16.10, 15.74. **HRMS (ESI)** *m/z*: Calcd for $C_{59}H_{97}NO_{17}S$ $(M+Na)^+$ 1146.6375, found 1146.6372.

V. IMMUNOLOGICAL EVALUATION IN A PRECLINICAL MOUSE VACCINATION MODEL

Animals. Animals were cared for and handled in compliance with the Guidelines for Accommodation and Care of Animals (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes) and internal guidelines. Mice were housed in ventilated cages and fed on a standard diet *ad libitum*. All the experimental procedures were approved by the appropriate local authorities. The CIC bioGUNE animal facility is fully accredited by AAALAC International.

Vaccination of mice. Groups of five mice (C57BL/6, female, 6-8 weeks old) were vaccinated subcutaneously three times every two weeks (days 0, 14, and 28) with OVA (20 μg/mouse, Sigma Chemical Co.) in phosphate-buffered saline (PBS, 100 μL) either alone (without adjuvant), with QS-21 (20 μg/mouse, Desert King Int.) or with the synthetic saponins (50 μg/mouse). To analyze the antibody responses over time, mice were bled via the facial vein at the indicated pre- (day –1) and post-vaccination time points (days 26 and 39), and by cardiac puncture at the experimental endpoint (day 46). Blood was collected in BD Microtainer® tubes (Clot Activator/SST™ Gel) and centrifuged at 7500*g* for 10 min, after which serum was harvested and stored at –20 °C until further analysis.

Evaluation of immune response augmentation. Analysis of the produced antibodies (antibody titers against OVA) was performed by an indirect enzyme-linked immunosorbent assay (ELISA). Briefly, ELISA plates (Thermo Scientific) were coated with OVA full length protein (Sigma-Aldrich Co.) at 0.05 μg/well in carbonate buffer (pH 9.5) and plates were incubated overnight at 4 °C. After washing the wells (PBS, 10 mM, containing 0.05% Tween 20), plates were blocked with 10% of fetal calf serum (FCS, Biowest) in PBS buffer for 1 h. Serial dilutions of mouse sera in blocking buffer (10% FCS in PBS buffer) were added to wells with appropriate controls and incubated for 2 h at room temperature. After wash, goat anti-mouse total IgG (Jackson ImmunoResearch) or subclass-specific IgG1, IgG2b and IgG2c (SouthernBiotech) antibodies conjugated to horseradish peroxidase (HRP) were added diluted 1/1000 in blocking buffer and incubated for 1 h at room temperature. KPL SureBlue reserve™ commercial solution (100 μL/well, SeraCare) containing 3,3',5,5'-tetramethylbenzidine (TMB) was added as peroxidase substrate and after incubation for 10 min, the reaction was stopped with 2N H_2SO_4 (50 μL/well). Absorbance (450 nm) was immediately measured using a BioTek® Synergy HT multi-detection microplate reader. Antibody endpoint titres were calculated using the method described by Frey *et al*, using the mean and the standard deviation of the pre-sera values.[4]

Toxicity assessment in mice. To assess the potential toxicity of the injected saponin constructs, the levels of hepatic alanine transaminase (ALT) in blood serum of immunized mice (1/5 dilution in RNase free water, HyClone) at the time of sacrifice (day 46) were determined using a Vitalab Selectra Junior biochemistry analyzer following the manufacturer's instructions.

Statistics. Antibody titers data are presented as median of five mice. The statistical significance of the antibody response for each of the experimental groups compared to the 'no-adjuvant' control was assessed using a two-tailed unpaired Student's *t*-test with a 95% confidence interval (CI) (GraphPad Prism, GraphPad Software, La Jolla, CA). P values of less than 0.05 were considered statistically significant. Toxicity data (hepatic alanine transaminase levels) are presented as mean ± standard error mean of five mice per group. The

statistical significance compared to the ‘no-adjuvant’ control was assessed using a two-tailed unpaired Student’s *t*-test with a 95% confidence interval (CI).

VI. CONFORMATIONAL ANALYSIS BY NMR AND MOLECULAR DYNAMICS SIMULATIONS

A. NMR EXPERIMENTS

NMR experiments. Synthetic saponin variants **3-8** (1-2 mg) were dissolved and analysed in methanol-d_4 (600 μL), using residual deuterated solvent signal as chemical shift reference. NMR spectra were recorded on a Bruker AVIII-600 MHz spectrometer equipped with a 5mm PATXI ^{1}H/D-^{13}C/^{15}N XYZ-GRD probe or on a AVIII-800 MHz spectrometer equipped with a 5mm CPTCI ^{1}H-^{13}C/^{15}N/D Z-GRD probe. For signal assignment, 2D COSY, TOCSY and edited-HSQC experiments were performed at 298 K, using standard Bruker pulse sequences. Normal acquisition parameters values were 1024-2048 points (F2) × 256-512 points (F1), for spectral width of 6-10 ppm in ^{1}H and 130-165 ppm for ^{13}C. Conformational analysis was performed based on 2D NOESY experiments, which were run at 288 K at 600 MHz for QA(O/S) and OA(O/S) variants (**3**, **5**, **6**, **8**), and at 298 K at 800 MHz for EA(O/S) variants (**4** and **7**). The change of temperature to 288 K was due to the NOE zero regimen found at 298 K at 600 MHz for the QA/OA samples. 2048 × 384-512 data points matrices were used for acquisition and experiments were recorded using 400 ms of mixing time.

B. MOLECULAR DYNAMICS (MD) SIMULATIONS

MD Simulations. Parameters for the substrates were generated with the antechamber module of AMBER 18[5] using a combination of GLYCAM06[6] parameters for the sugar units and the general Amber force field (GAFF)[7] for the rest of the molecule, with partial charges set to fit the electrostatic potential generated with HF/631G(d) by RESP.[8] The charges were calculated according to the Merz-Singh-Kollman scheme using Gaussian 09[9]. Each saponin molecule was immersed in a water box with a 10 Å buffer of TIP3P[10] water molecules or a 15 Å buffer of MeOH molecules. The systems were neutralized by adding explicit counterions (Na^+). A two-stage geometry optimization approach was performed. The first stage minimizes only the positions of solvent molecules and ions, and the second stage is an unrestrained minimization of all the atoms in the simulation cell. The systems were then heated by incrementing the temperature from 0 to 300 K under a constant pressure of 1 atm and periodic boundary conditions. Harmonic restraints of 30 kcal/mol were applied to the solute, and the Andersen temperature coupling scheme[11] was used to control and equalize the temperature. The time step was kept at 1 fs during the heating stages, allowing potential inhomogeneities to self-adjust. Water molecules were treated with the SHAKE algorithm such that the angle between the hydrogen atoms is kept fixed. Long-range electrostatic effects were modelled using the particle mesh-Ewald method.[12] An 8 Å cut-off was applied to Lennard-Jones interactions. Each system was equilibrated for 2 ns with a 2 fs timestep at a constant pressure (1 atm) and temperature of 300 K. Production trajectories were then run for additional 0.5 μs under the same simulation conditions.

Torsional angle definitions. For each (C1–O1–C^n) glycosidic linkage in the linear trisaccharide, ϕ and ψ dihedral angles were defined as follows:

$$\phi = H1–C1–O1–C^n$$
$$\psi = C1–O1–C^n–H^n$$

For the central glycosidic linkage connecting the bridging monosaccharide to the triterpene core, the ψ dihedral angles were defined based on heavy atoms as follows:

$$\psi = C1–O–C28–C17$$

For the C17–C28 bond, the dihedral angle was defined as follows:

$$C17–C28 = C16–C17–C28–O$$

To generate three-dimensional plots of torsional angle distributions (ϕ, ψ, C17–C28) about the central glycosidic linkage (Figures 3 and S5), each axis was shifted by a fixed value to minimize the number of datapoints appearing along the 360°→0° radial transition point, for clarity of presentation. Thus, the raw data (–180° to +180°) were shifted in Excel as follows:

$$\phi_{shift} = MOD (\phi + 480, 360)$$
$$\psi_{shift} = MOD (\psi + 480, 360)$$
$$C17–C28_{shift} = MOD (C17–C28 + 240, 360)$$

VII. NMR CHARACTERIZATION SPECTRA. ^{1}H, APT ^{13}C, COSY, HSQC

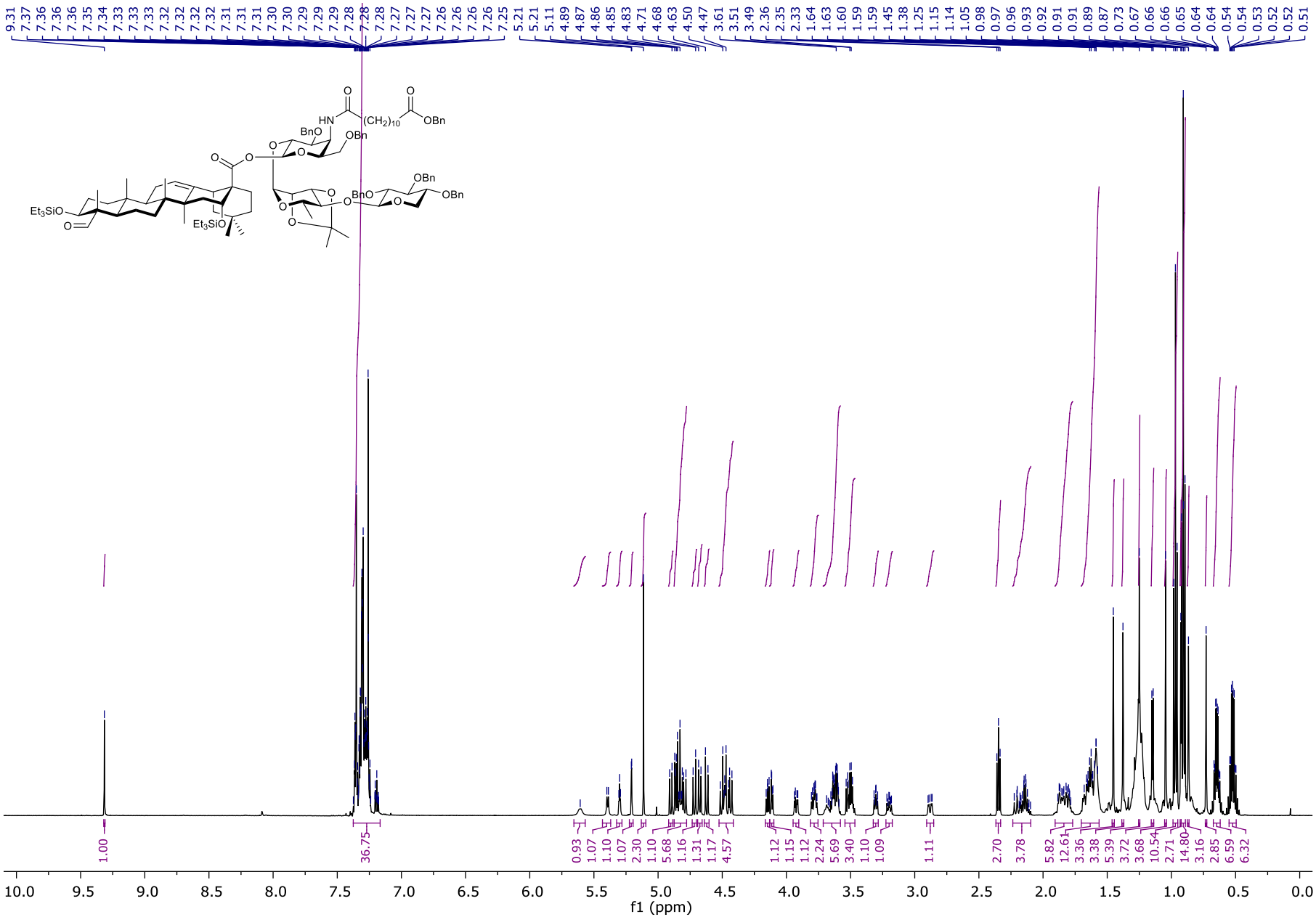
A. SYNTHESIS OF SAPONIN ESTER VARIANTS

1. SYNTHESIS OF QUILLAIC ACID VARIANT **3** QA(O) S34
2. SYNTHESIS OF ECHINOCYSTIC ACID VARIANT **4** EA(O) S41
3. SYNTHESIS OF OLEANOLIC ACID VARIANT **5** OA(O) S48

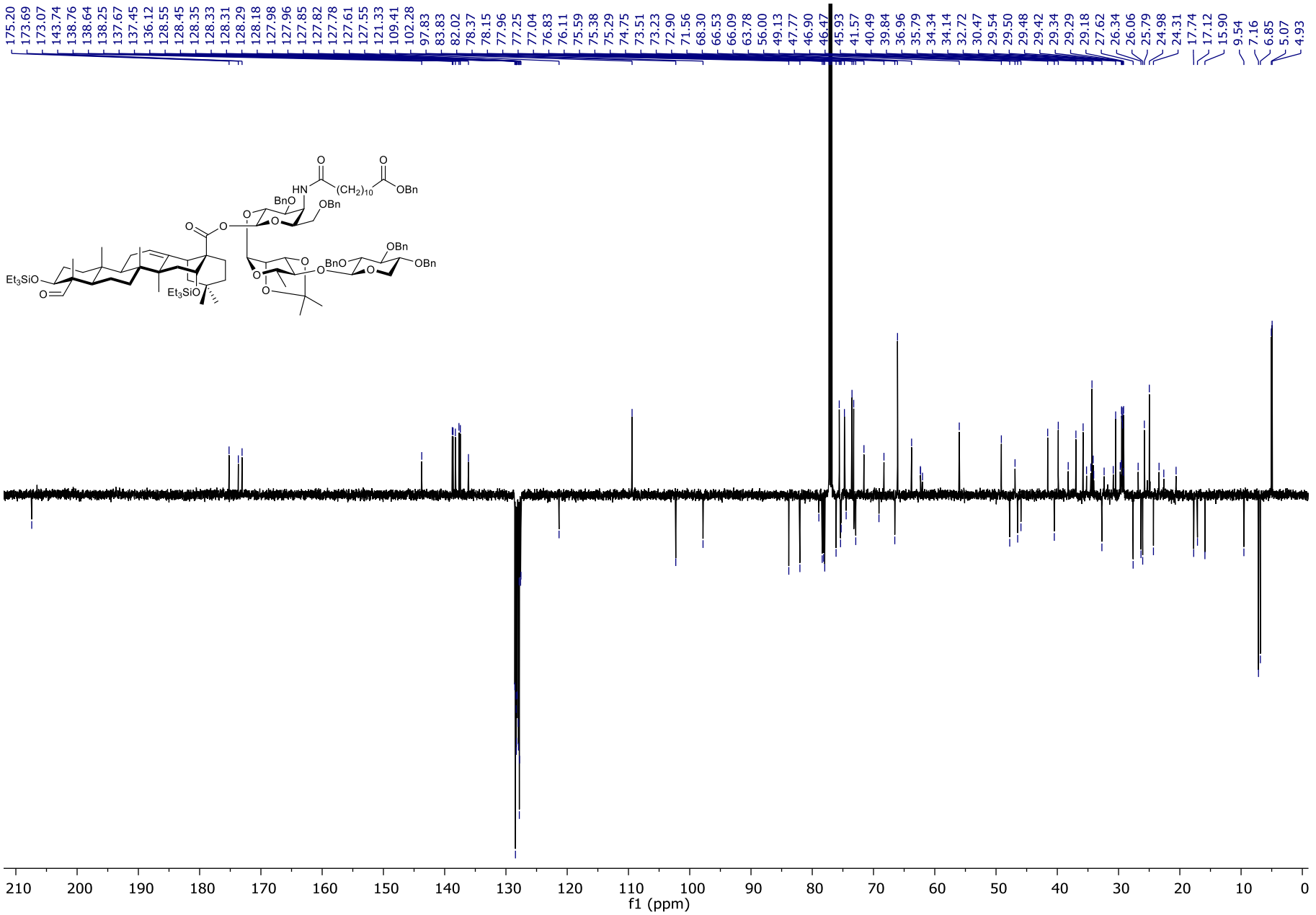
B. SYNTHESIS OF SAPONIN THIOESTER VARIANTS

1. SYNTHESIS OF QUILLAIC ACID VARIANT **6** QA(S) S55
2. SYNTHESIS OF ECHINOCYSTIC ACID VARIANT **7** EA(S) S66
3. SYNTHESIS OF OLEANOLIC ACID VARIANT **8** OA(S) S77

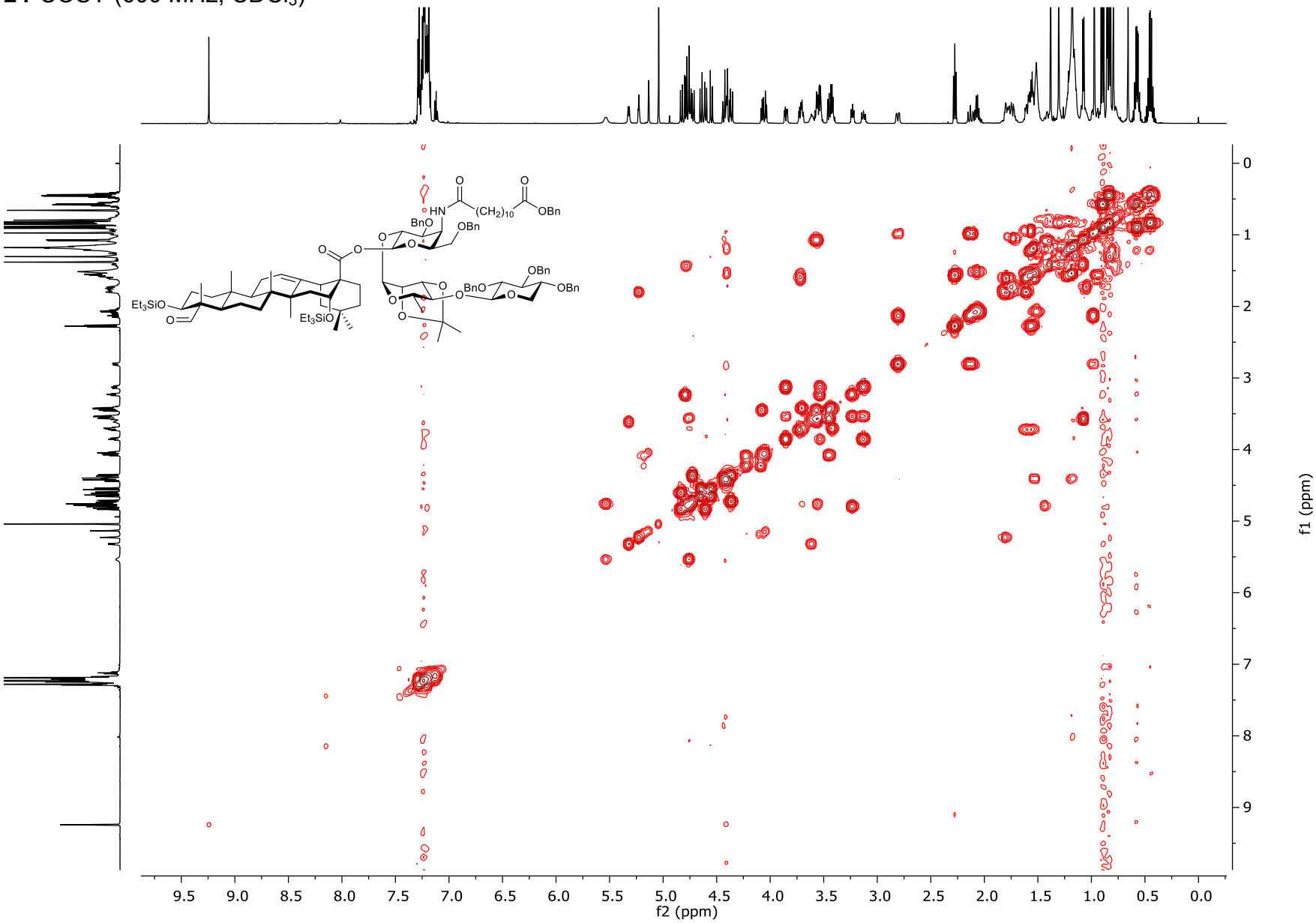
24 ^{1}H-NMR (600 MHz, $CDCl_3$)



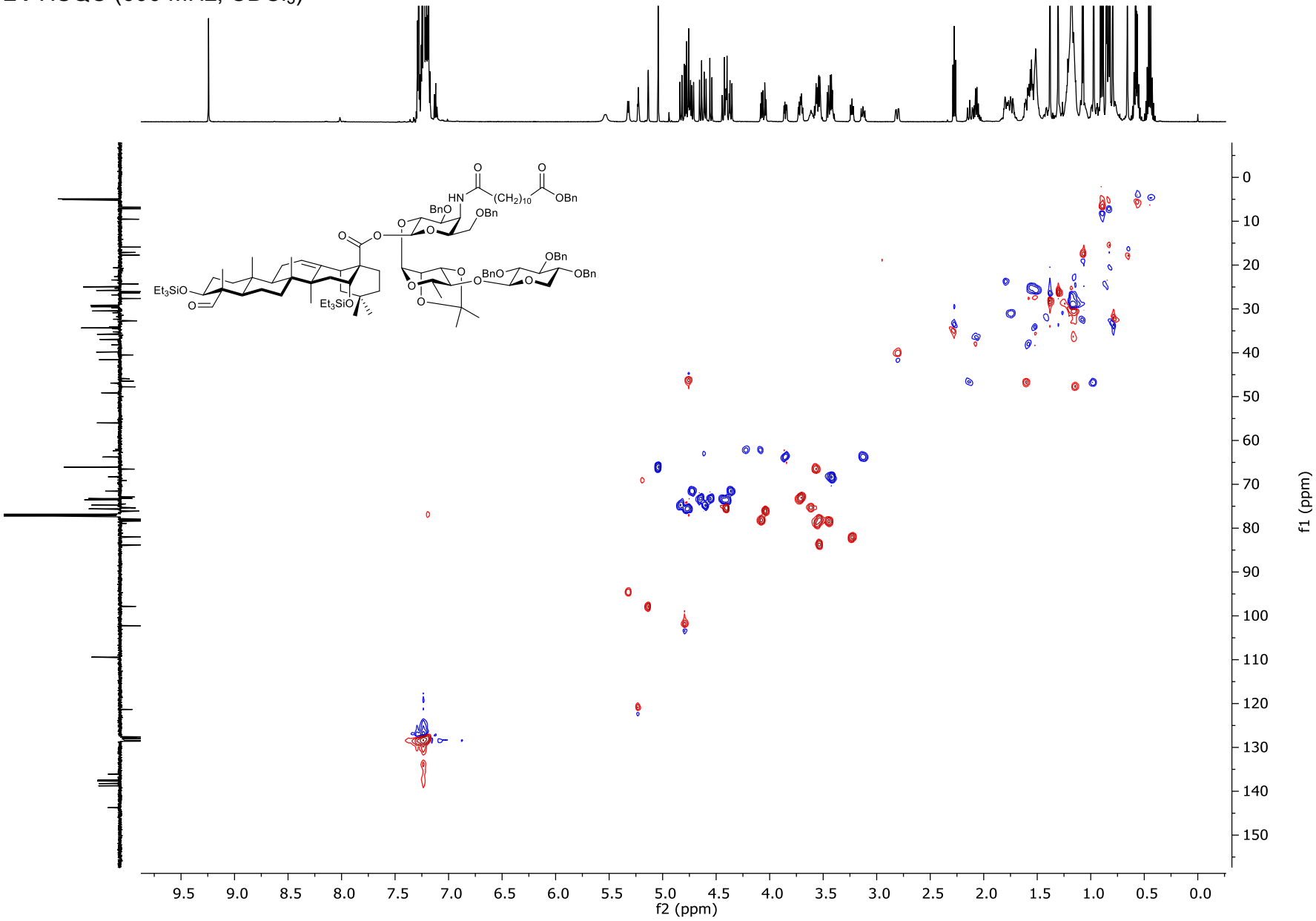
24 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



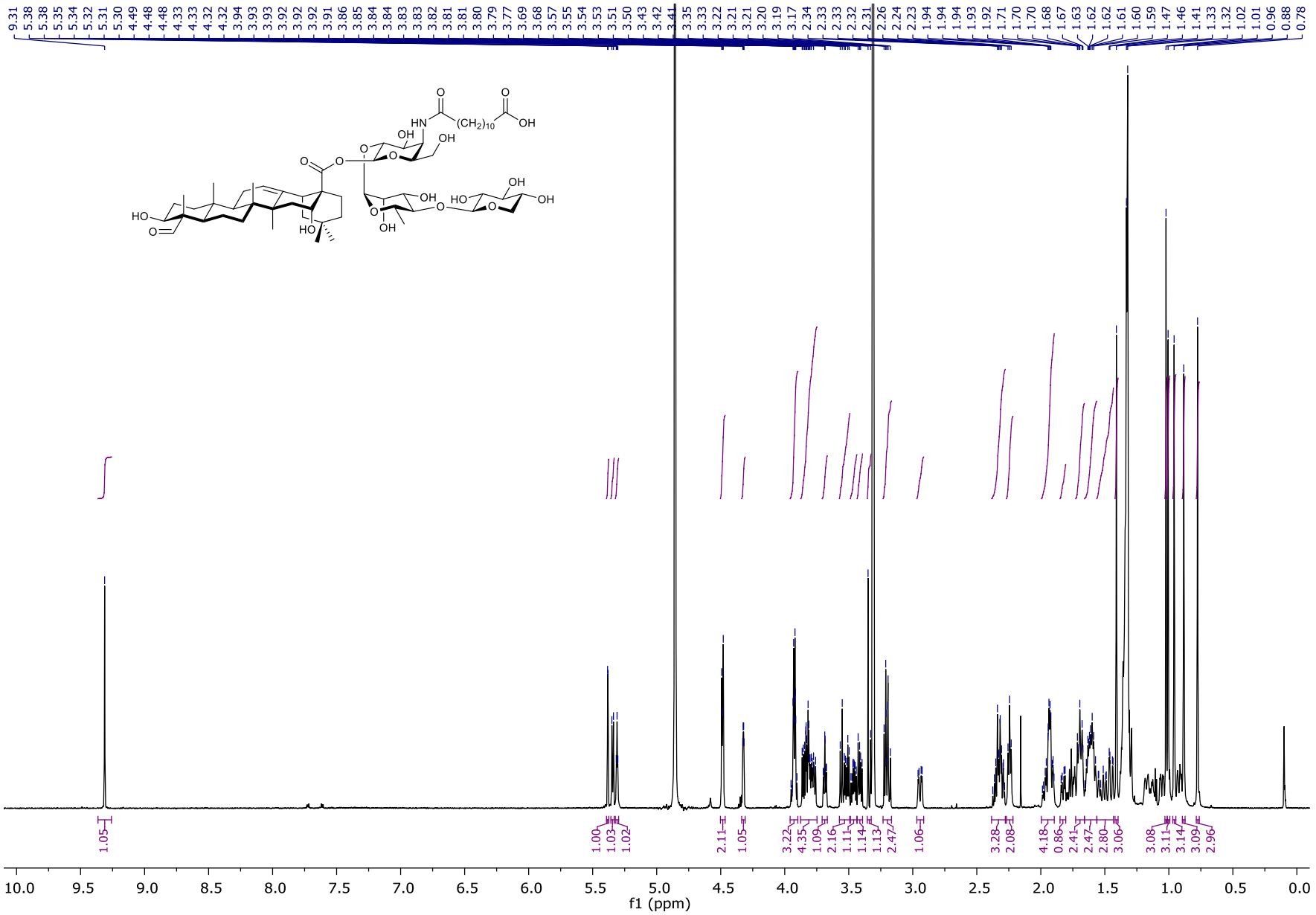
24 COSY (600 MHz, $CDCl_3$)



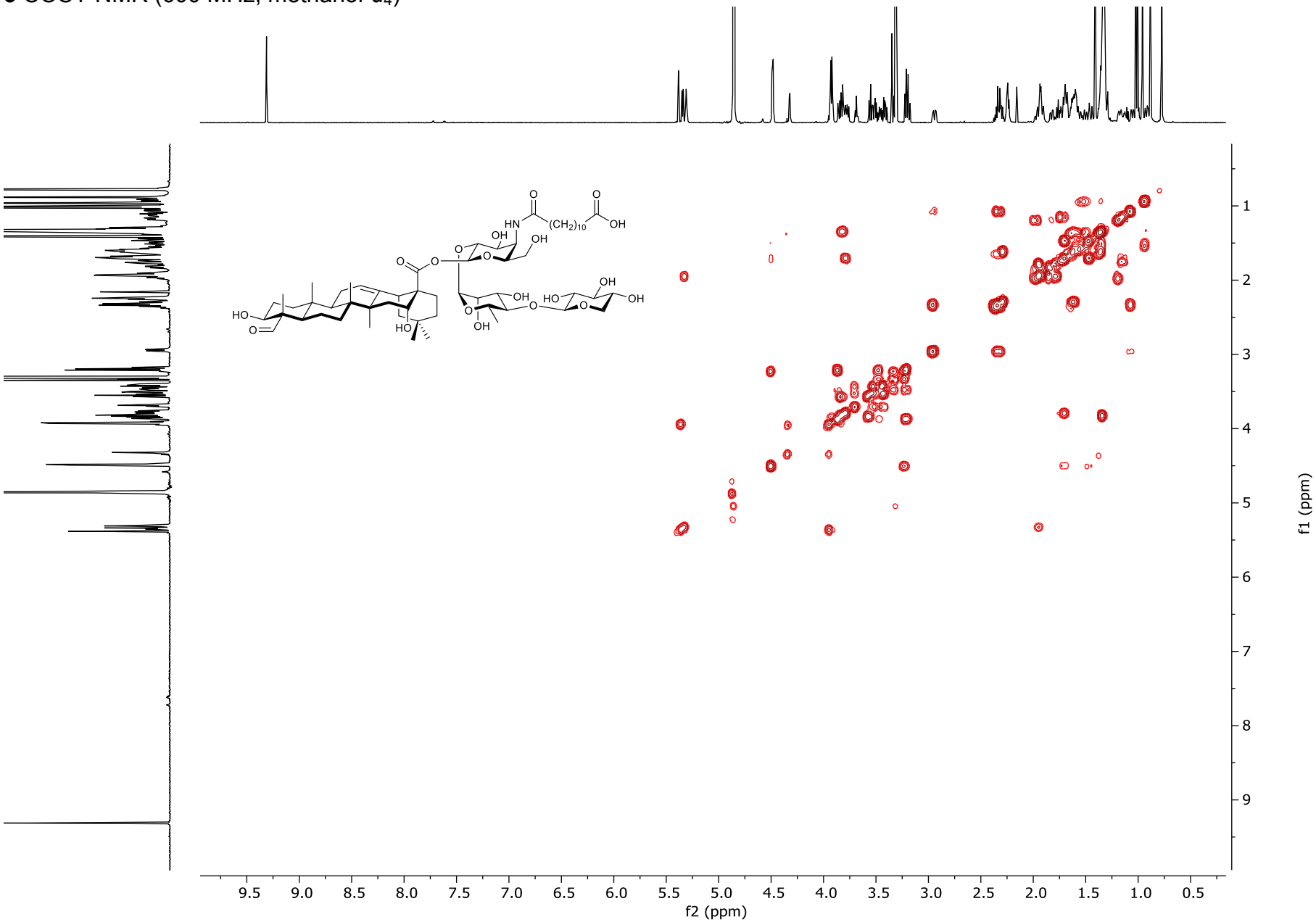
24 HSQC (600 MHz, $CDCl_3$)



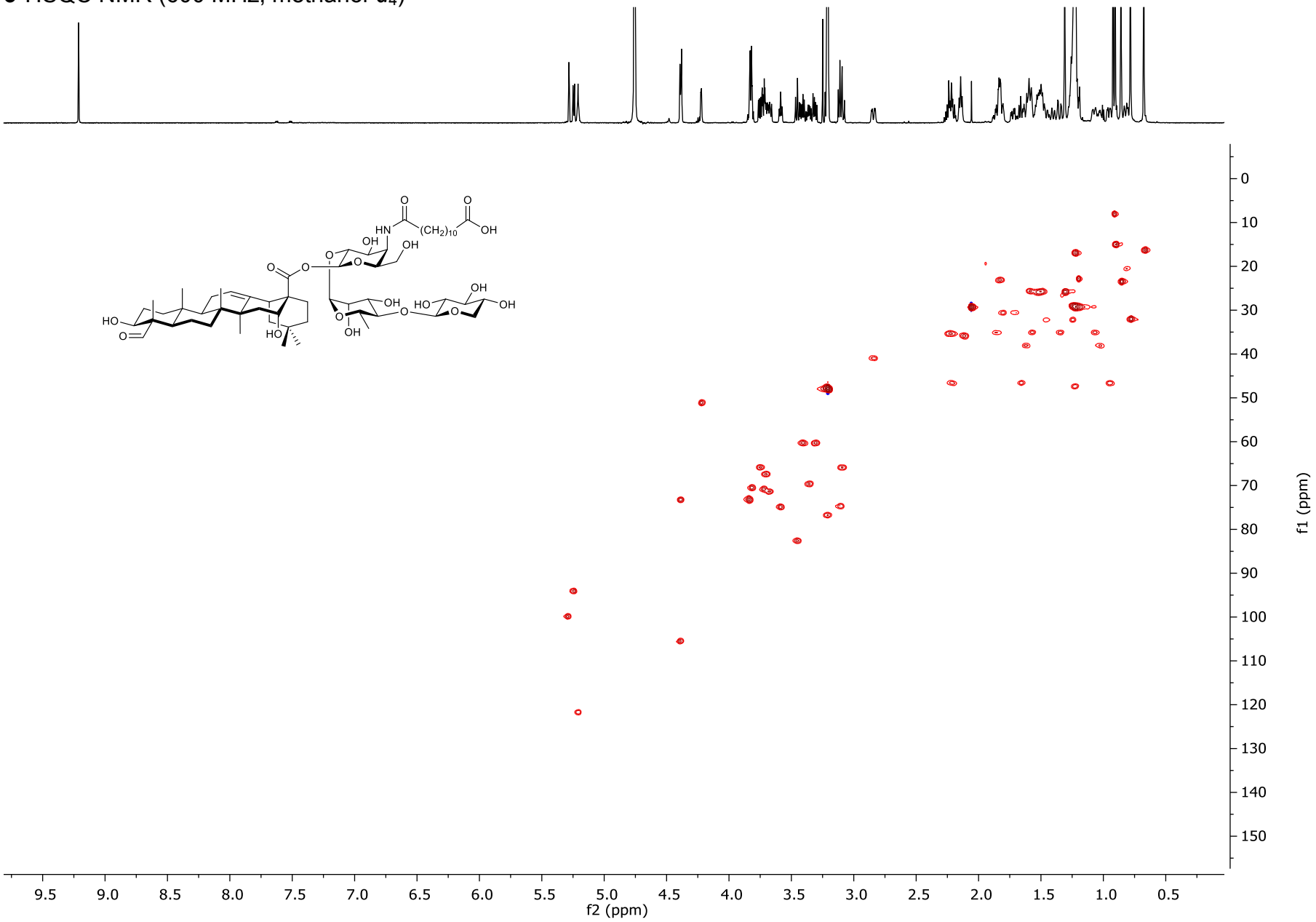
3 ^{1}H-NMR (600 MHz, methanol-d_4)



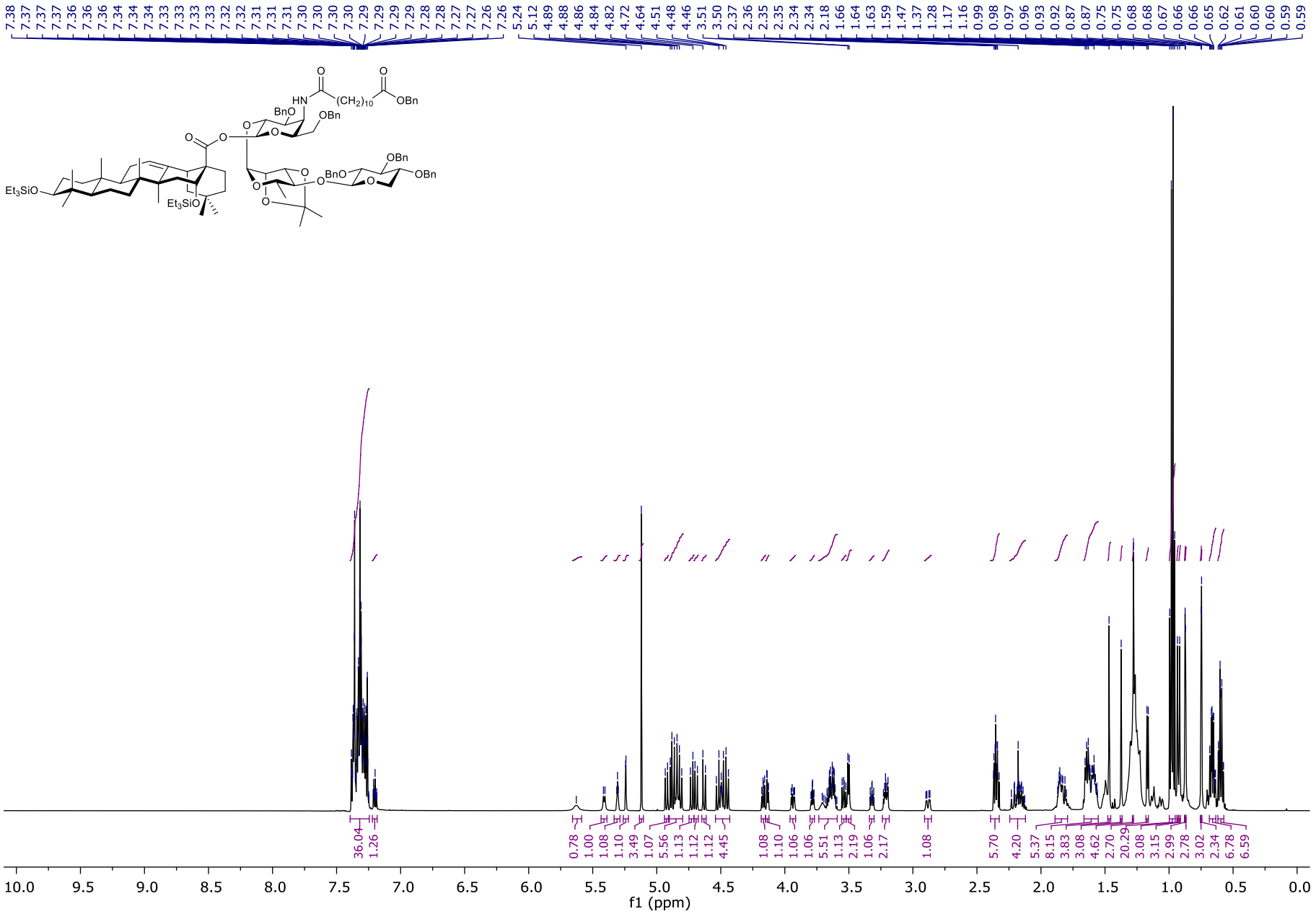
3 COSY NMR (600 MHz, methanol-d_4)



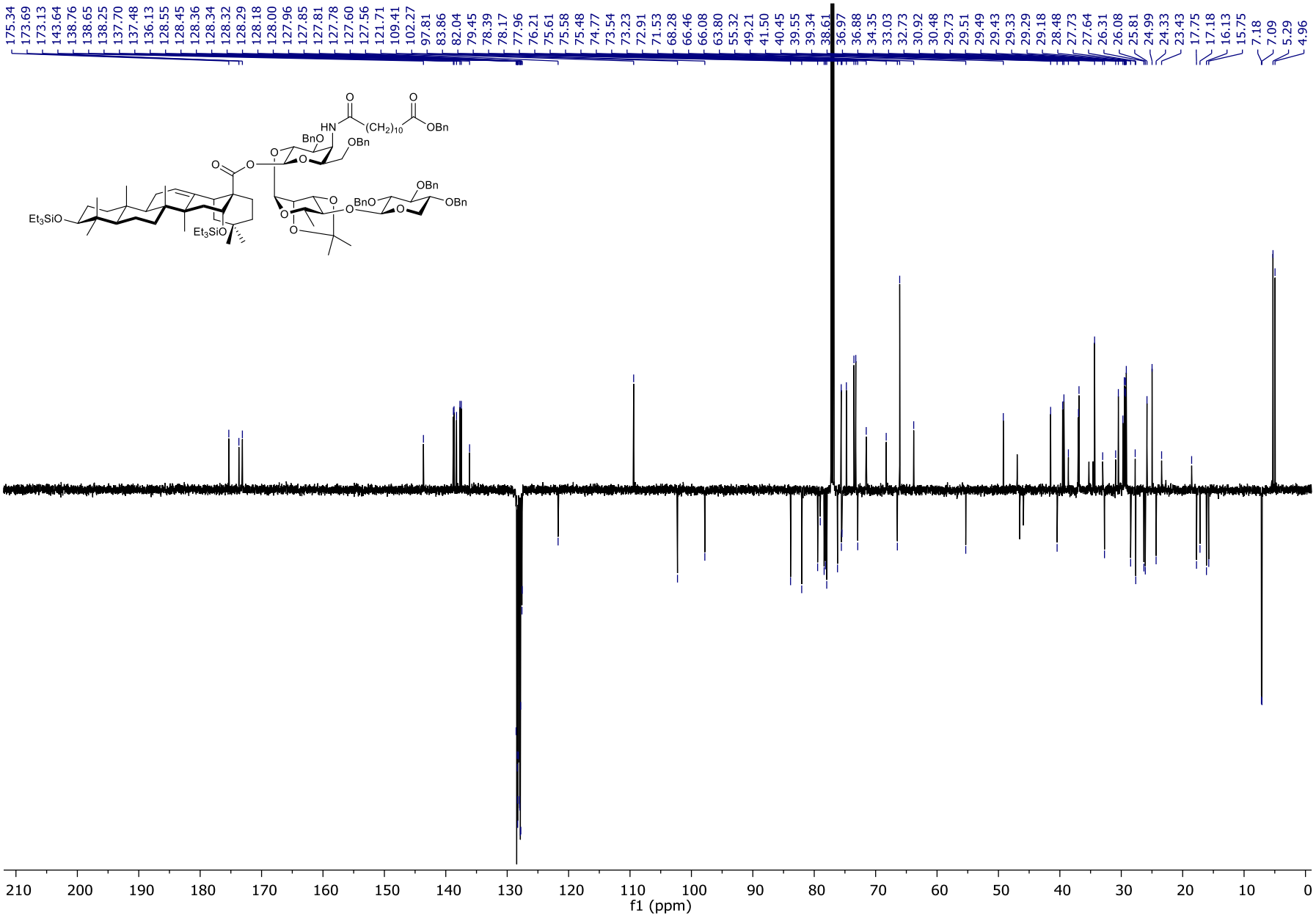
3 HSQC NMR (600 MHz, methanol-d_4)



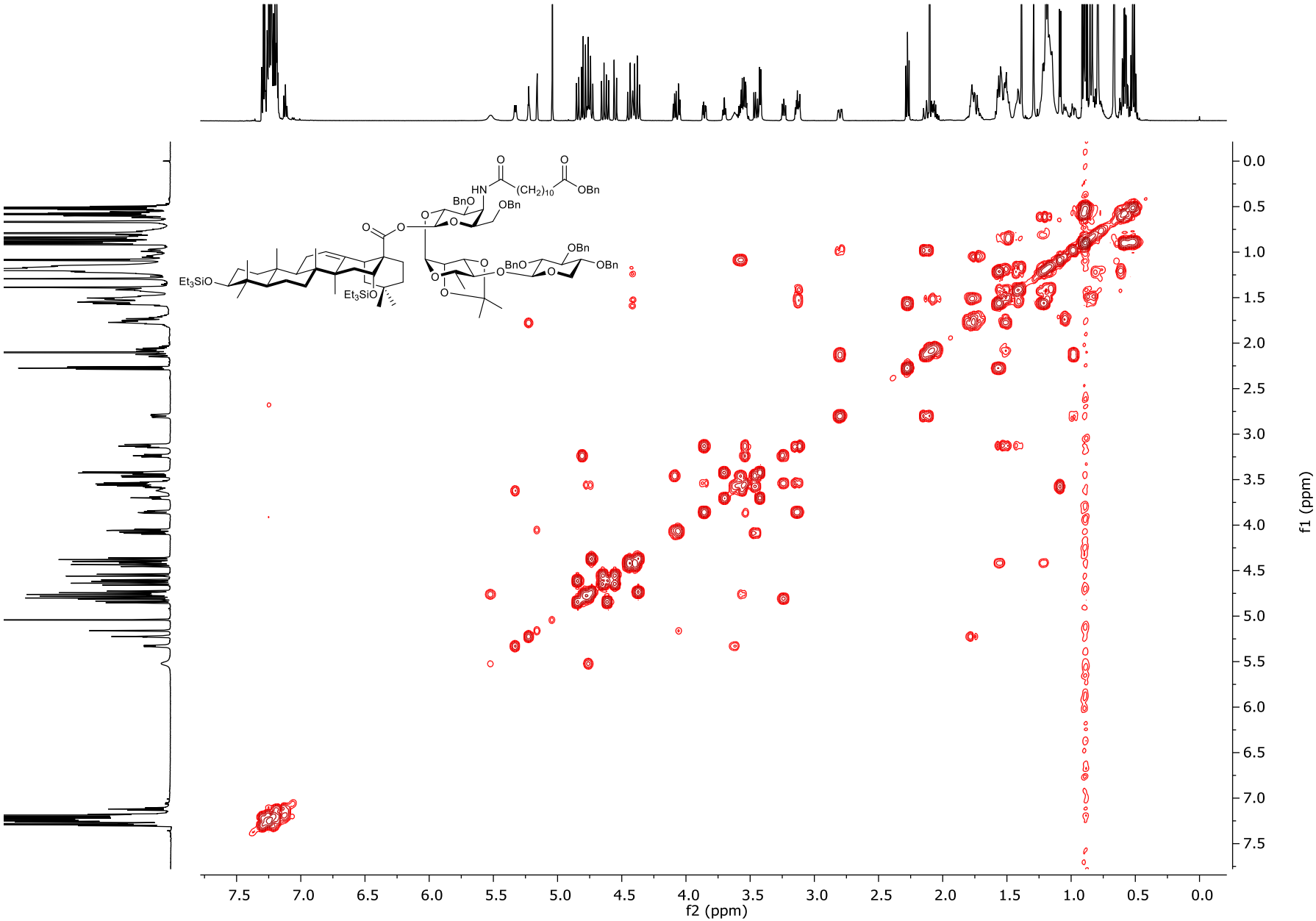
25 ^{1}H-NMR (600 MHz, $CDCl_3$)



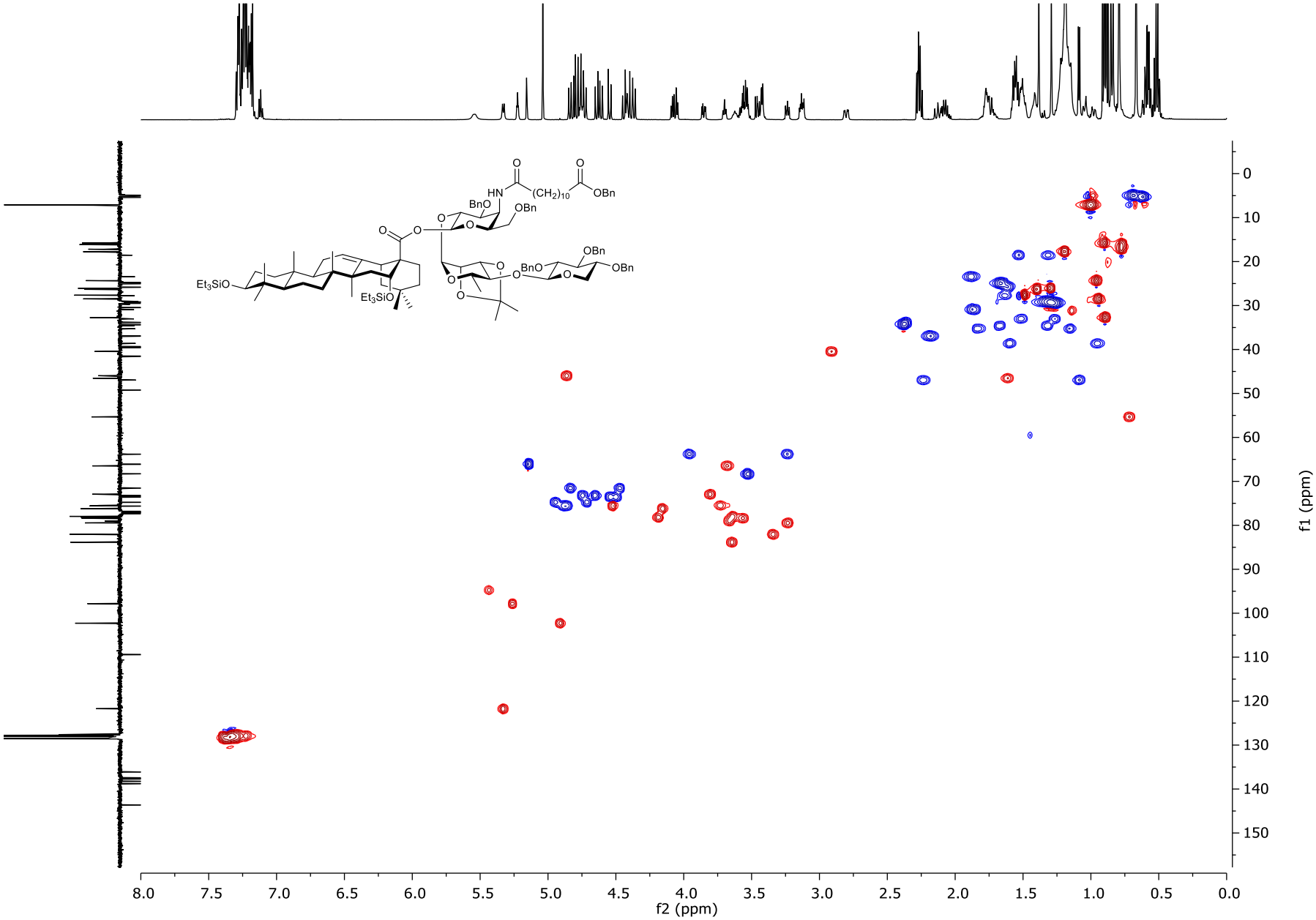
25 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



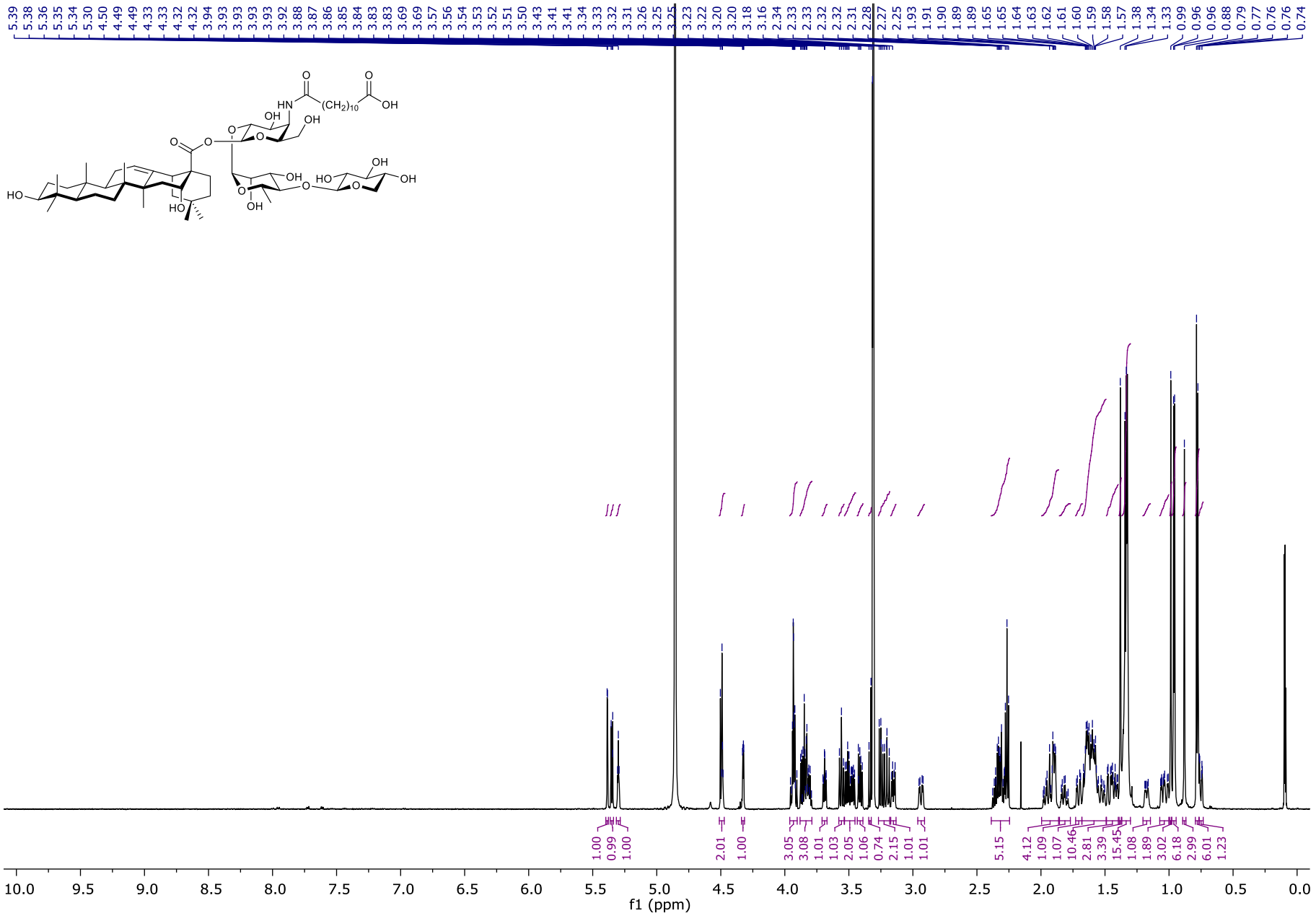
25 COSY (600 MHz, $CDCl_3$)



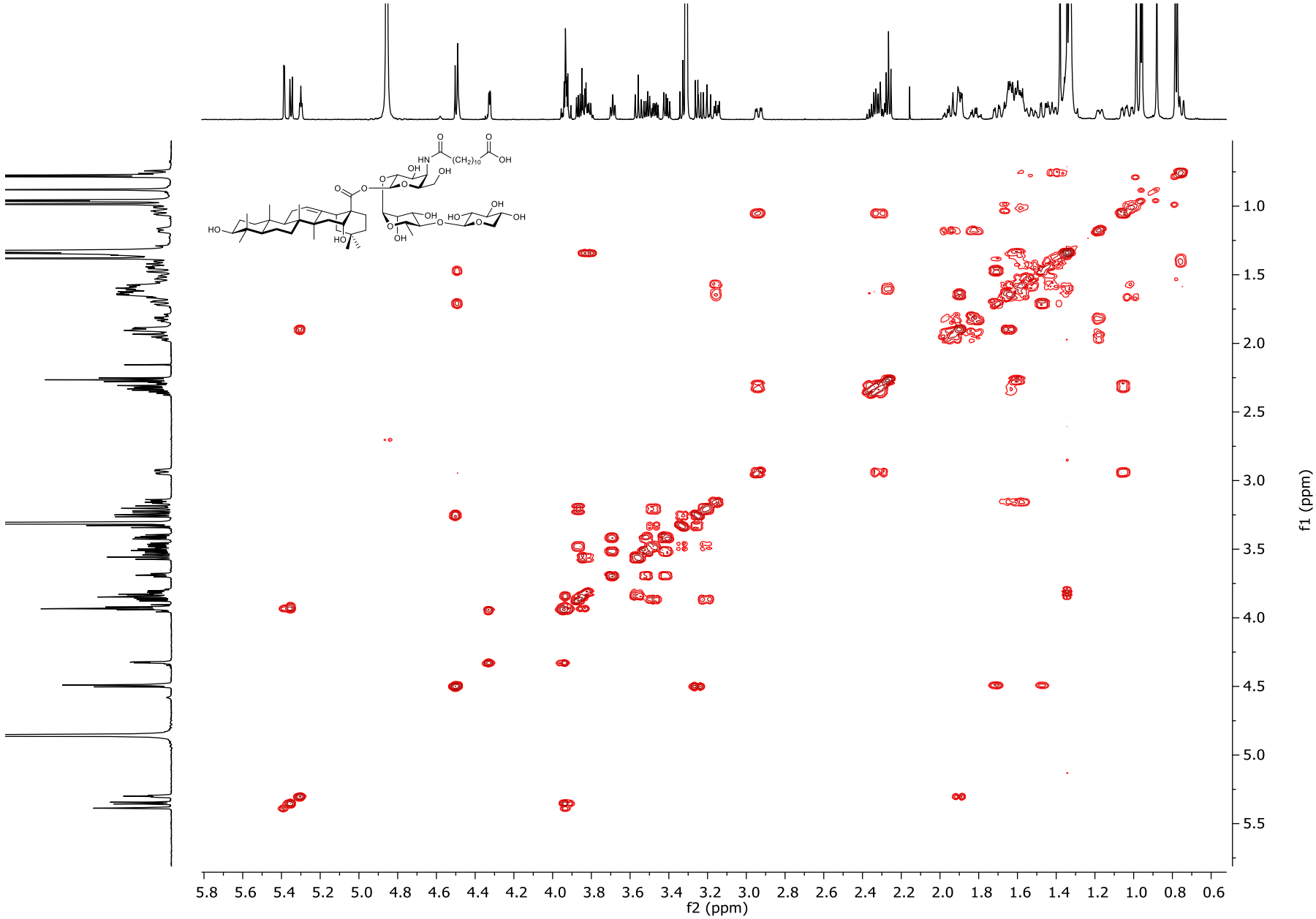
25 HSQC (600 MHz, $CDCl_3$)



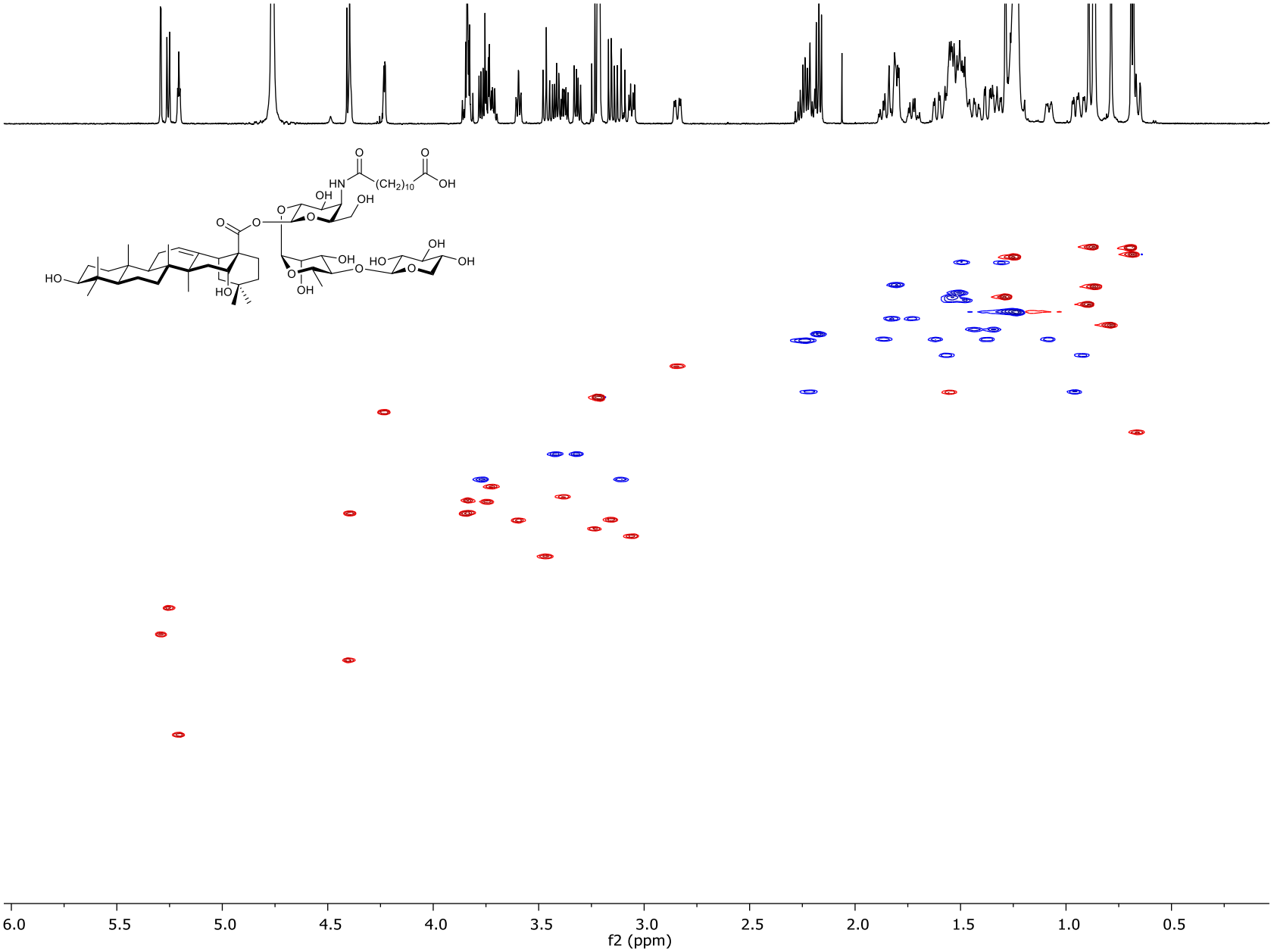
4 ^{1}H-NMR (600 MHz, methanol-d_4)



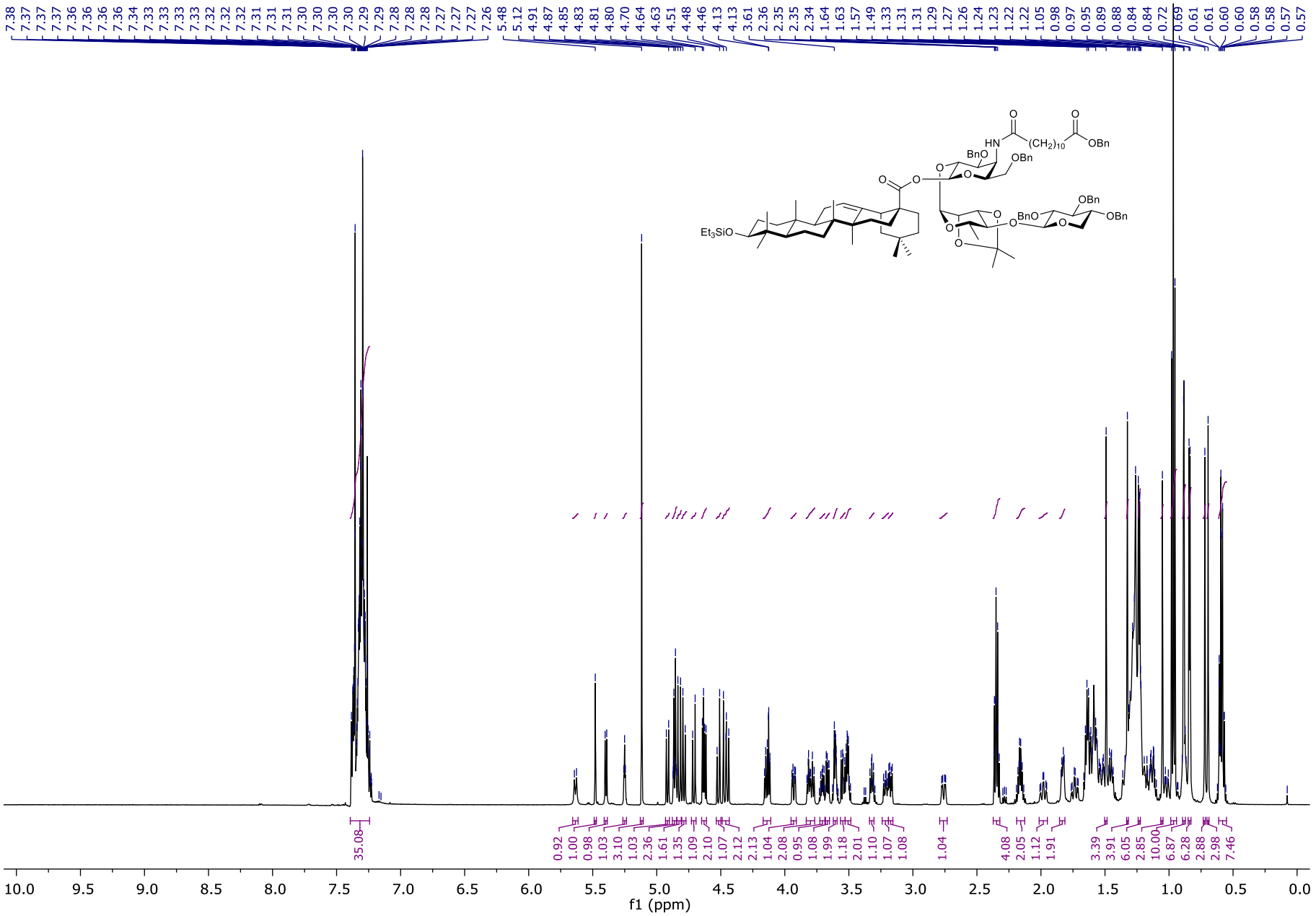
4 COSY (600 MHz, methanol-d_4)



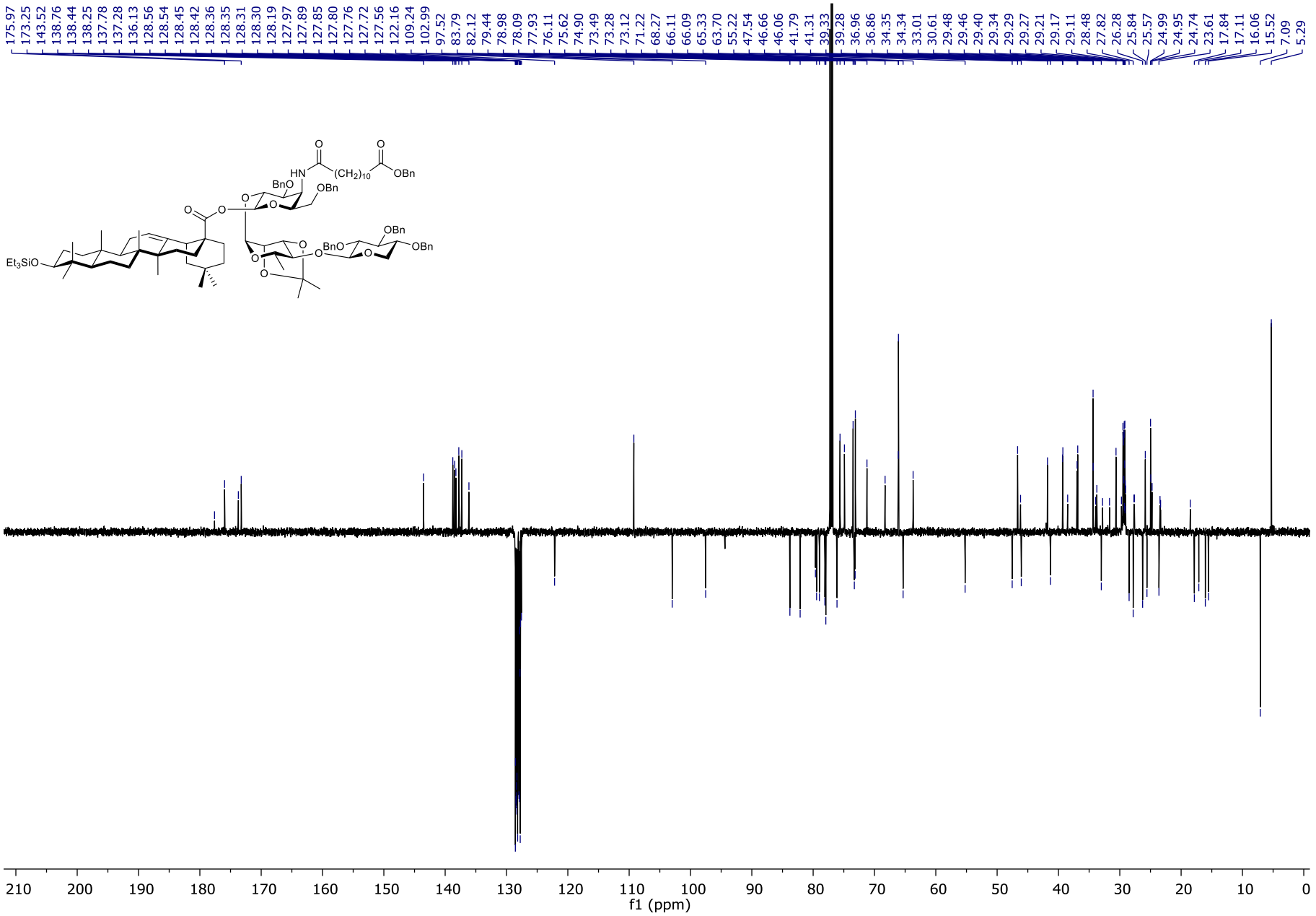
4 HSQC (600 MHz, methanol-d_4)



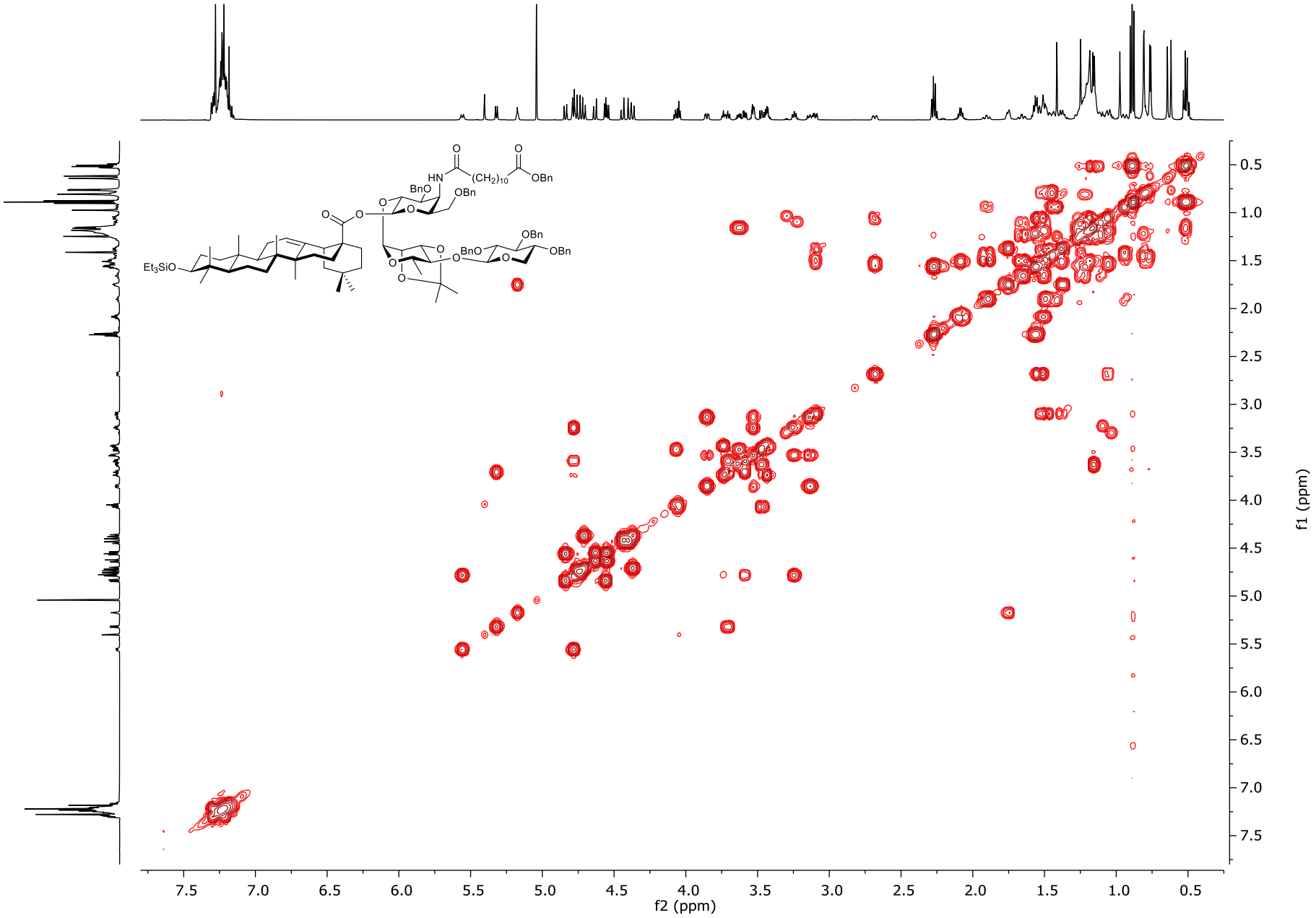
26 ^{1}H-NMR (600 MHz, $CDCl_3$)



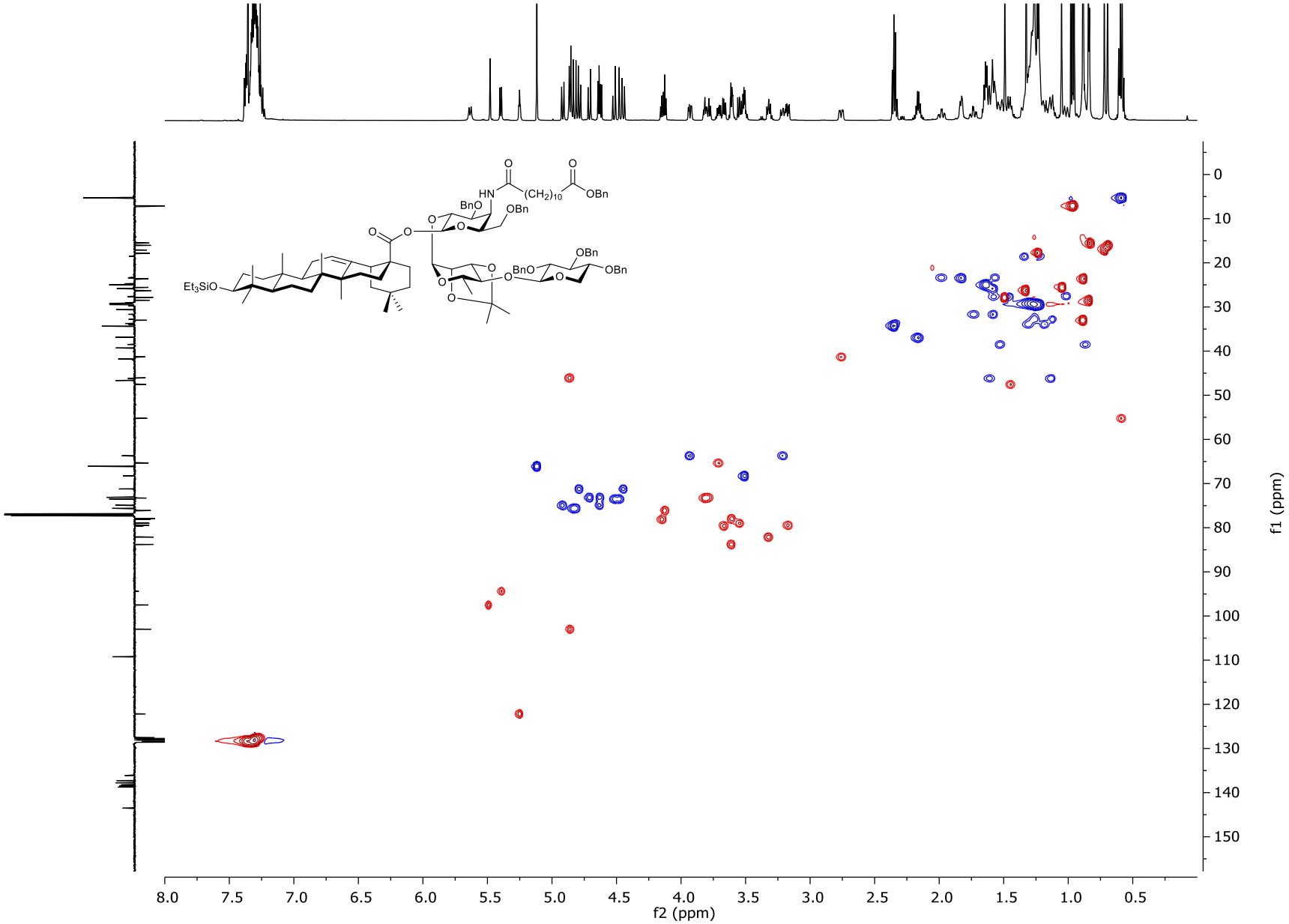
26 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



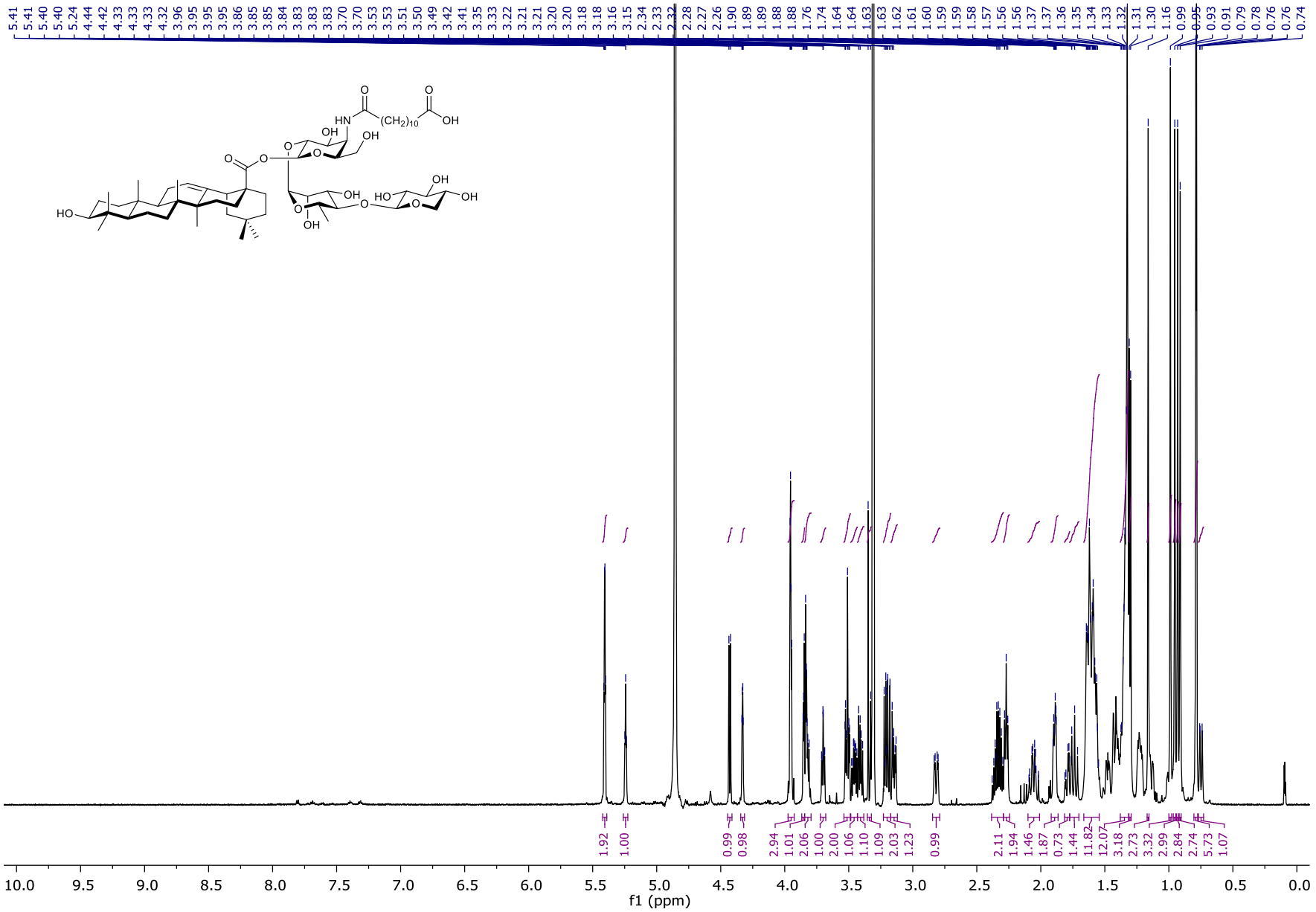
26 COSY (600 MHz, $CDCl_3$)



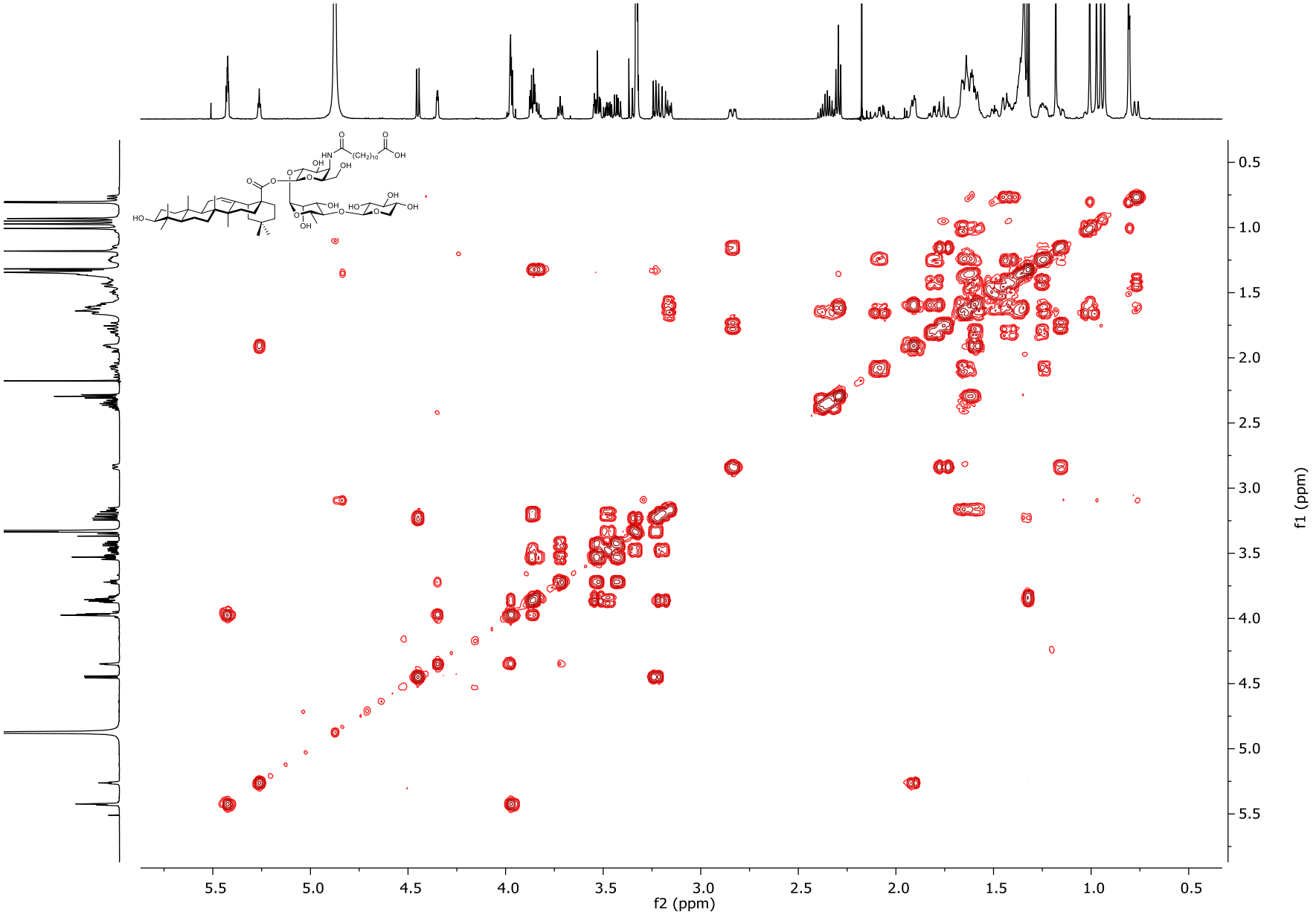
26 HSQC (600 MHz, $CDCl_3$)



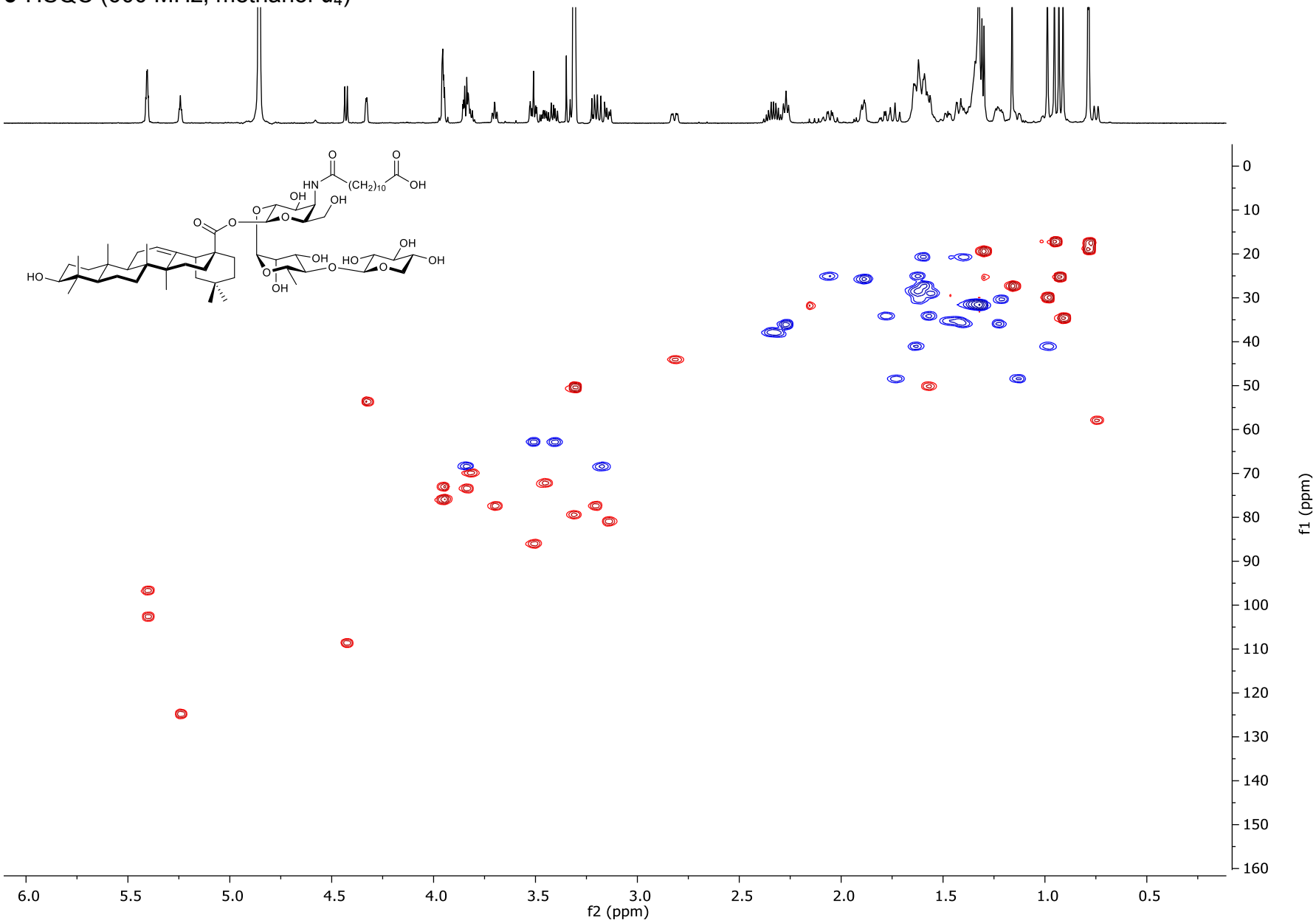
5 ^{1}H-NMR (600 MHz, methanol-d_4)



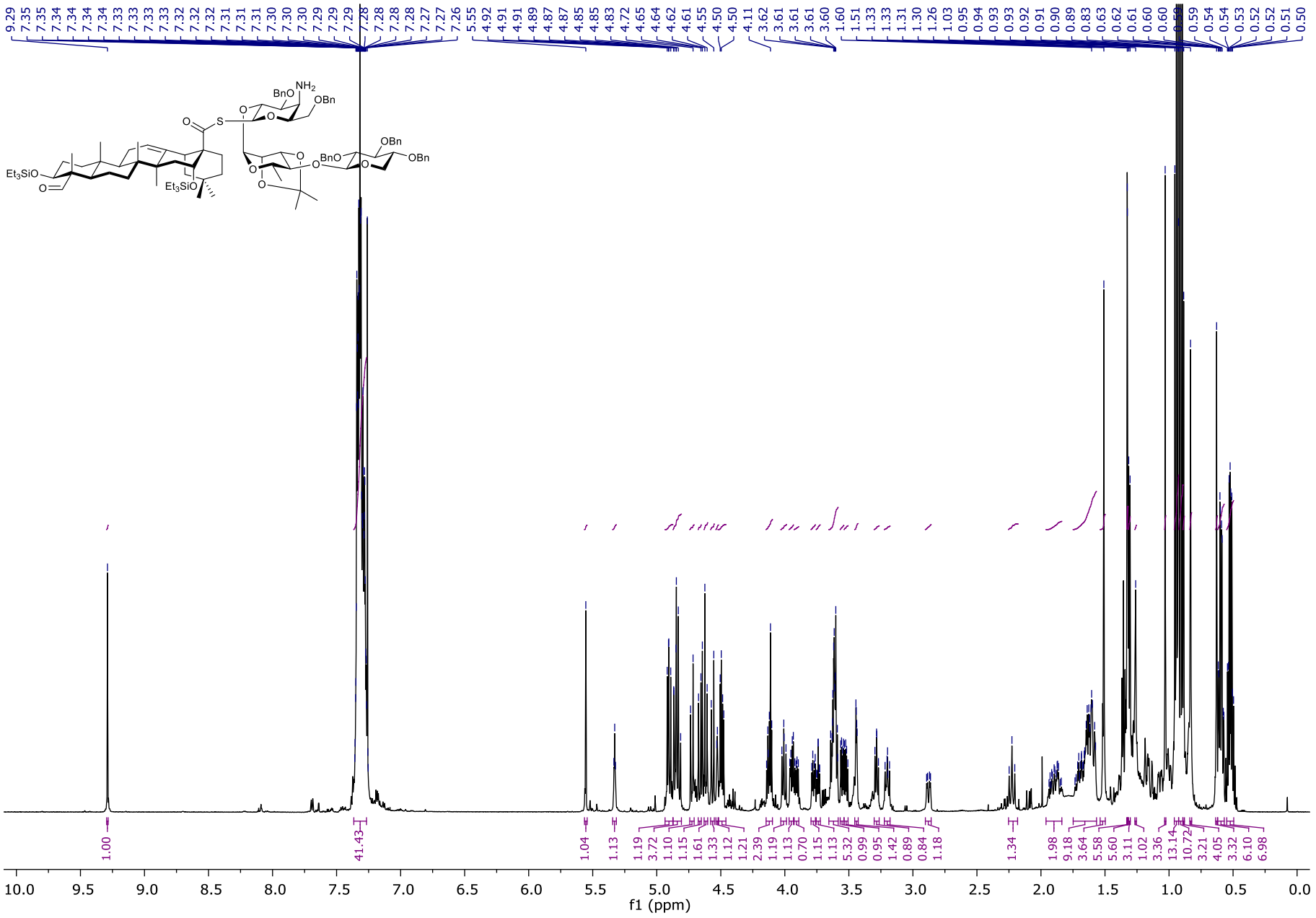
5 COSY (600 MHz, methanol-d_4)



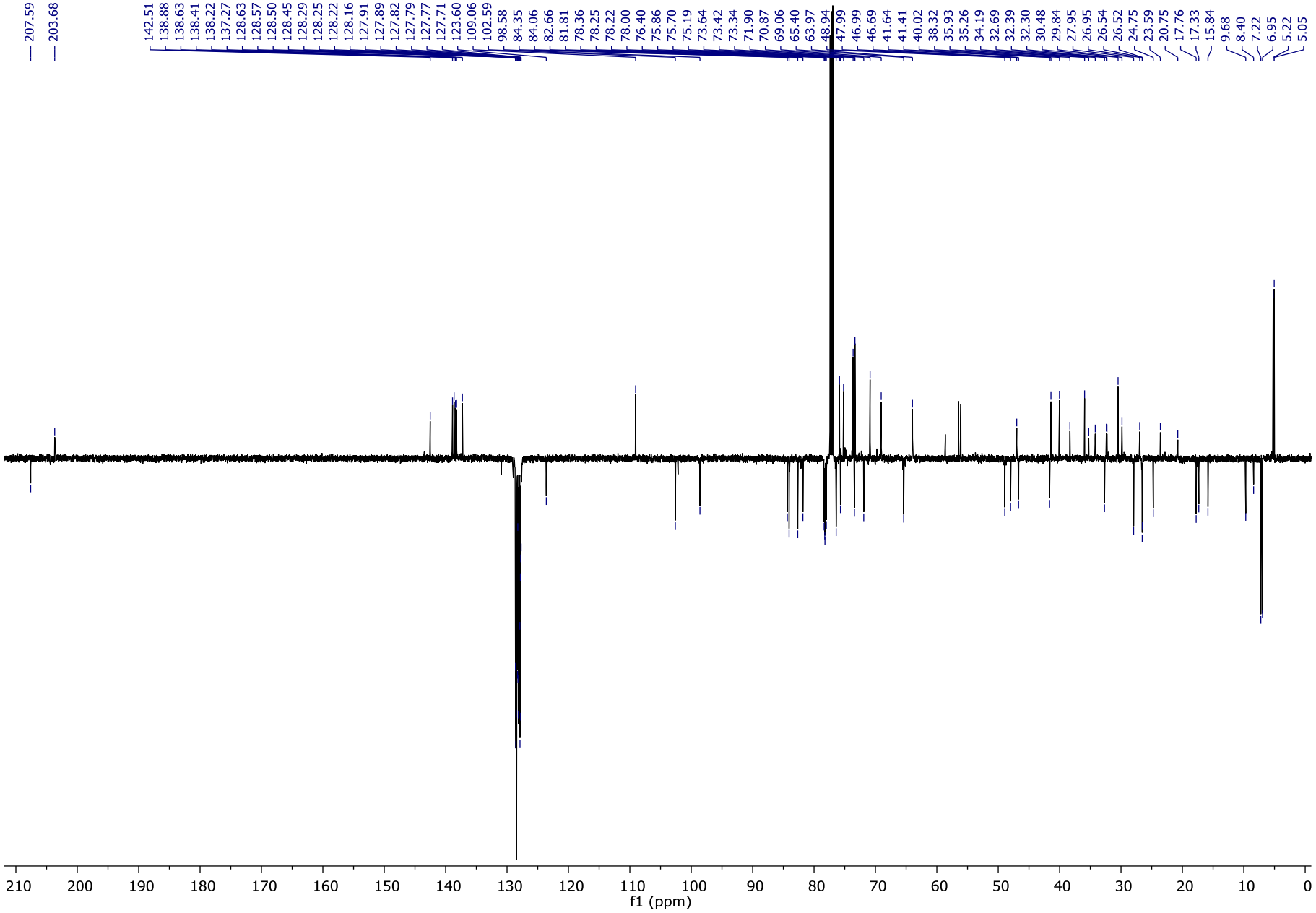
5 HSQC (600 MHz, methanol-d_4)



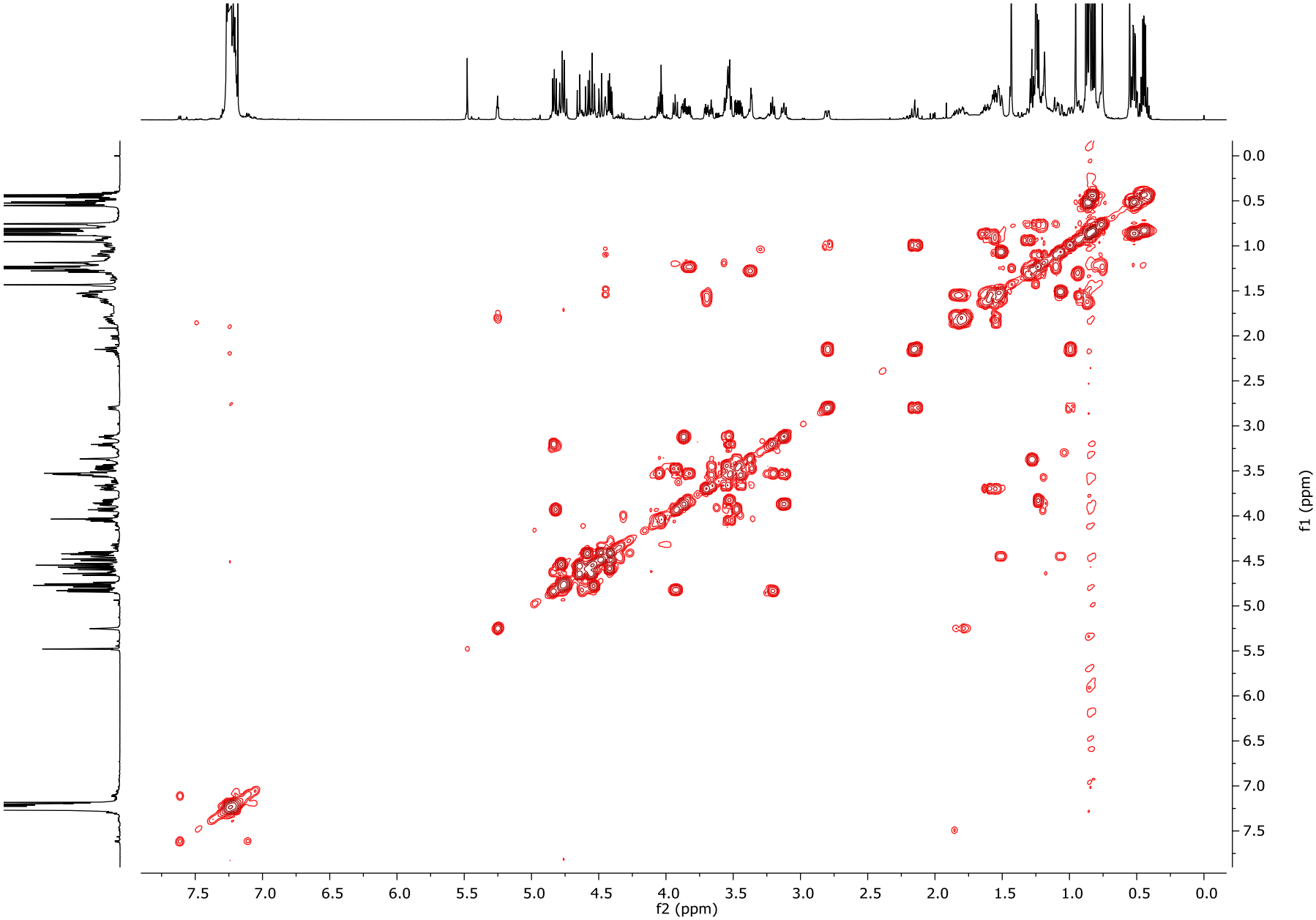
20 ^{1}H-NMR (600 MHz, $CDCl_3$)



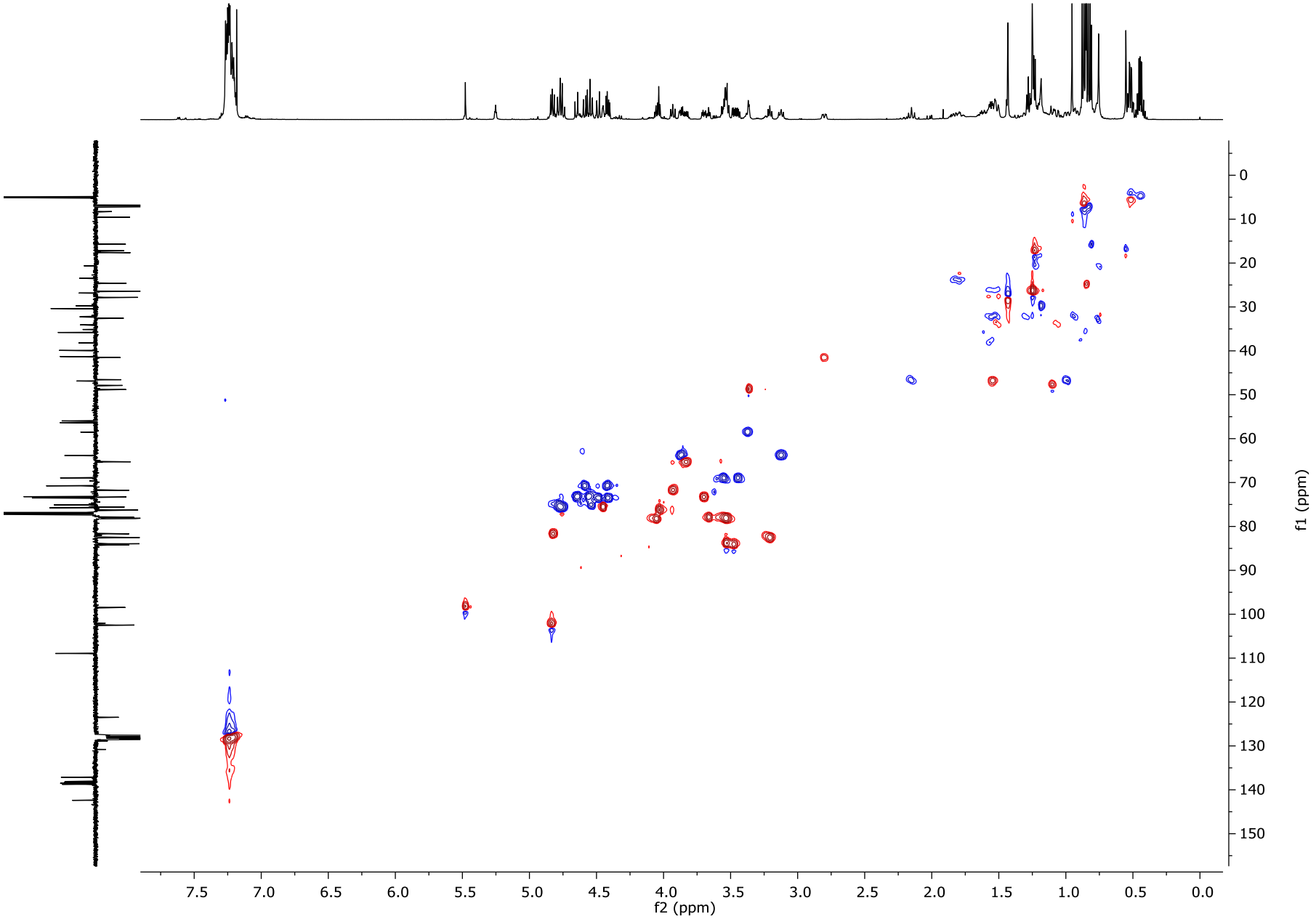
20 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



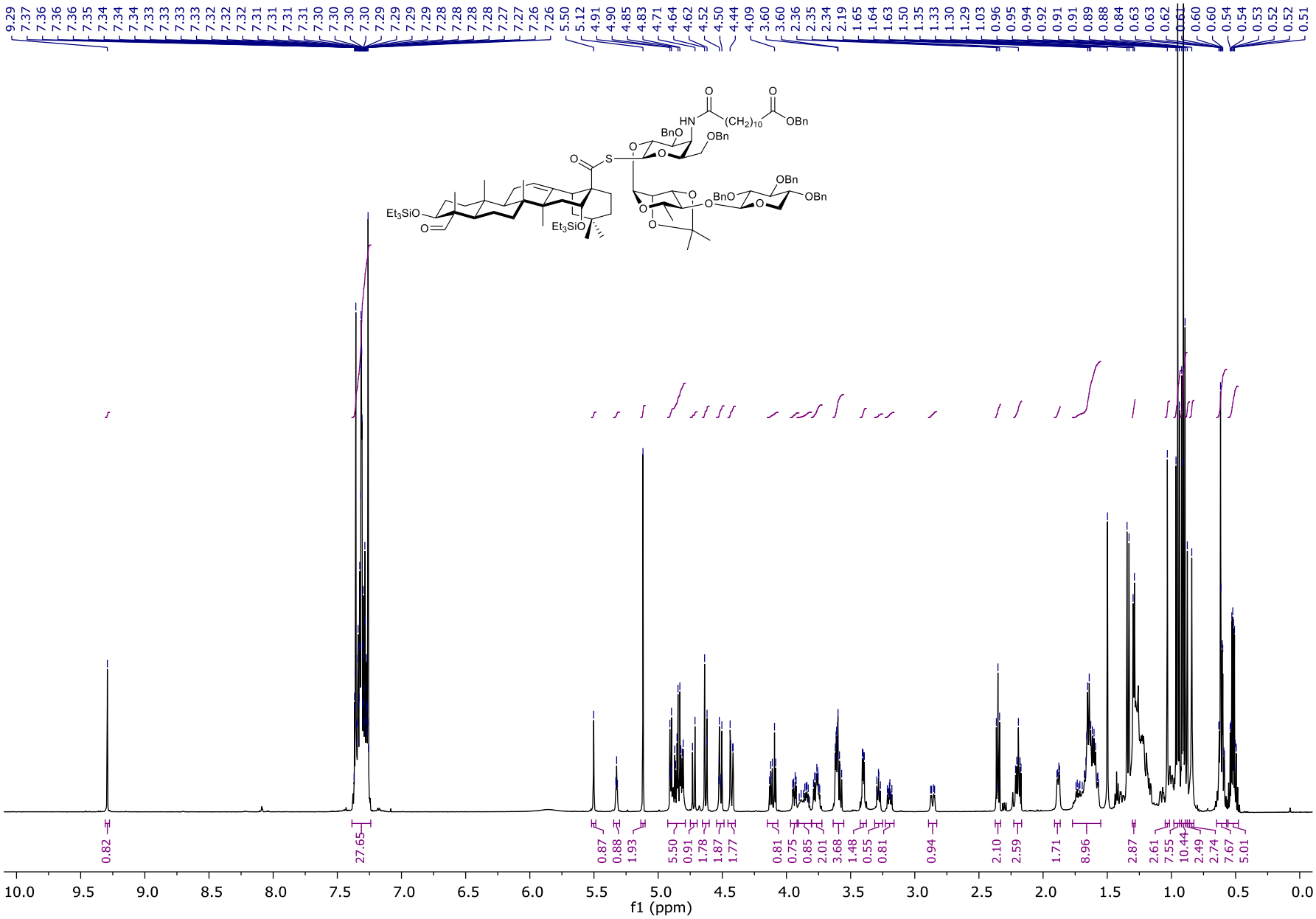
20 COSY (600 MHz, $CDCl_3$)



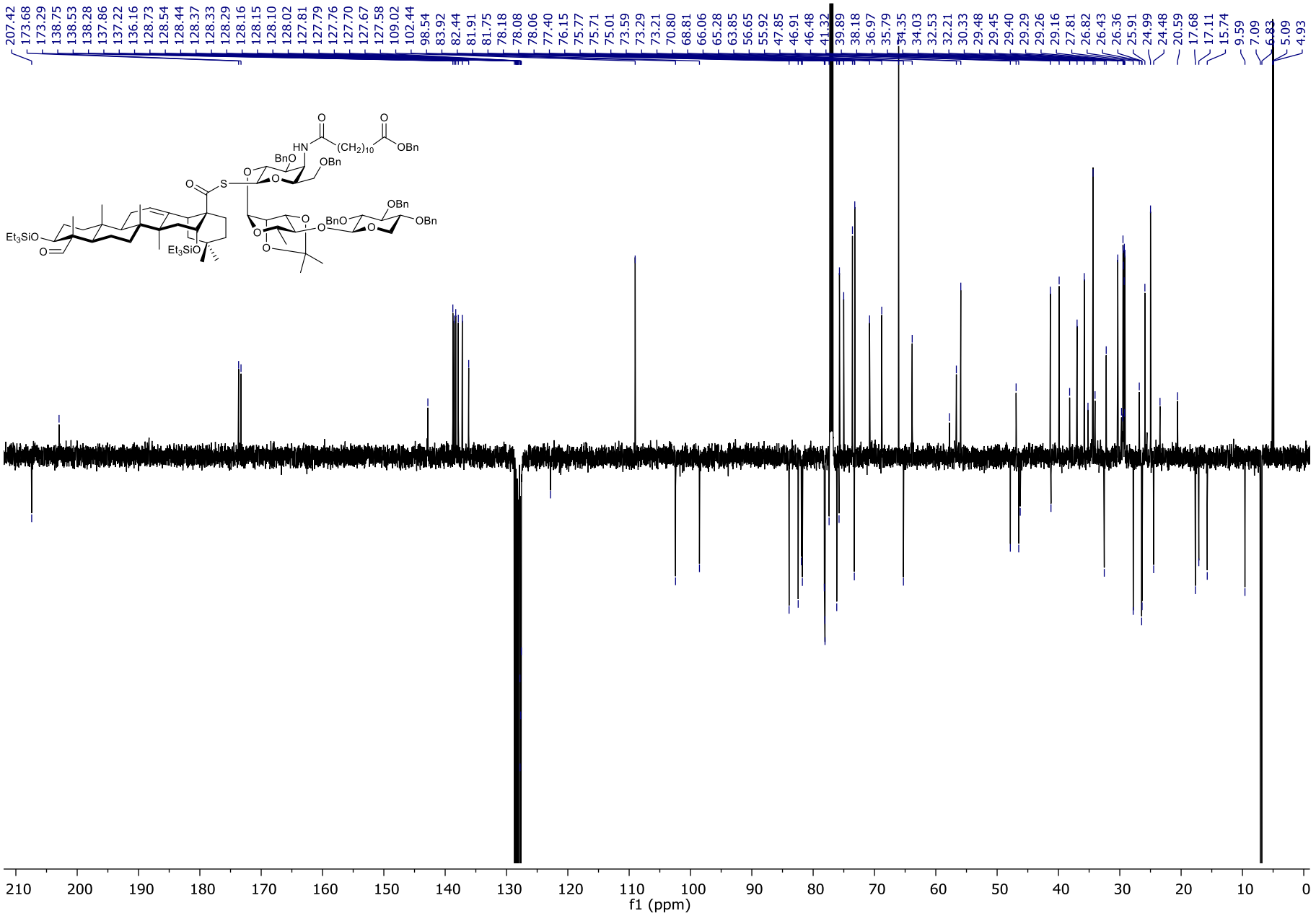
20 HSQC (600 MHz, $CDCl_3$)



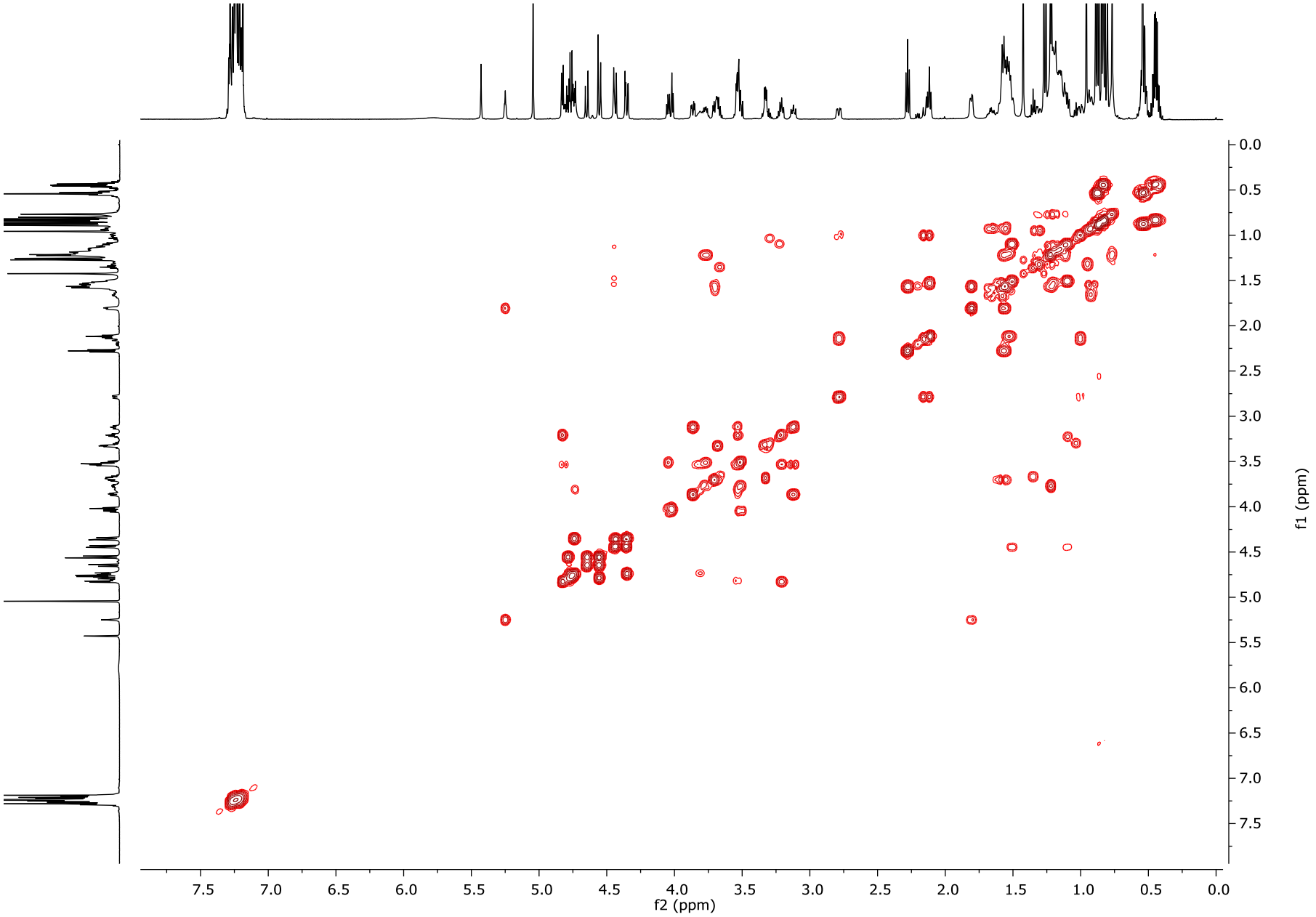
27 ^{1}H-NMR (600 MHz, $CDCl_3$)



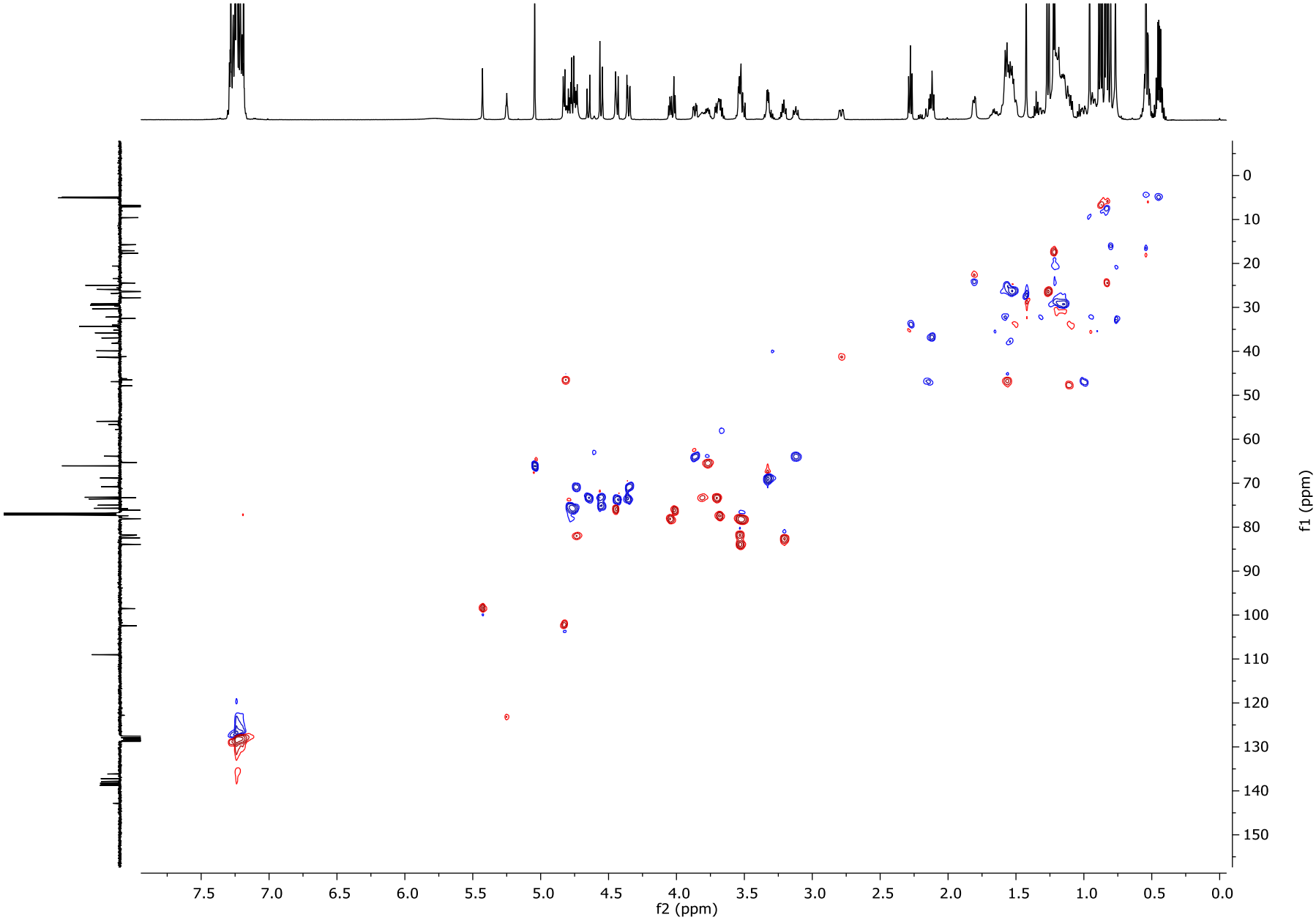
27 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



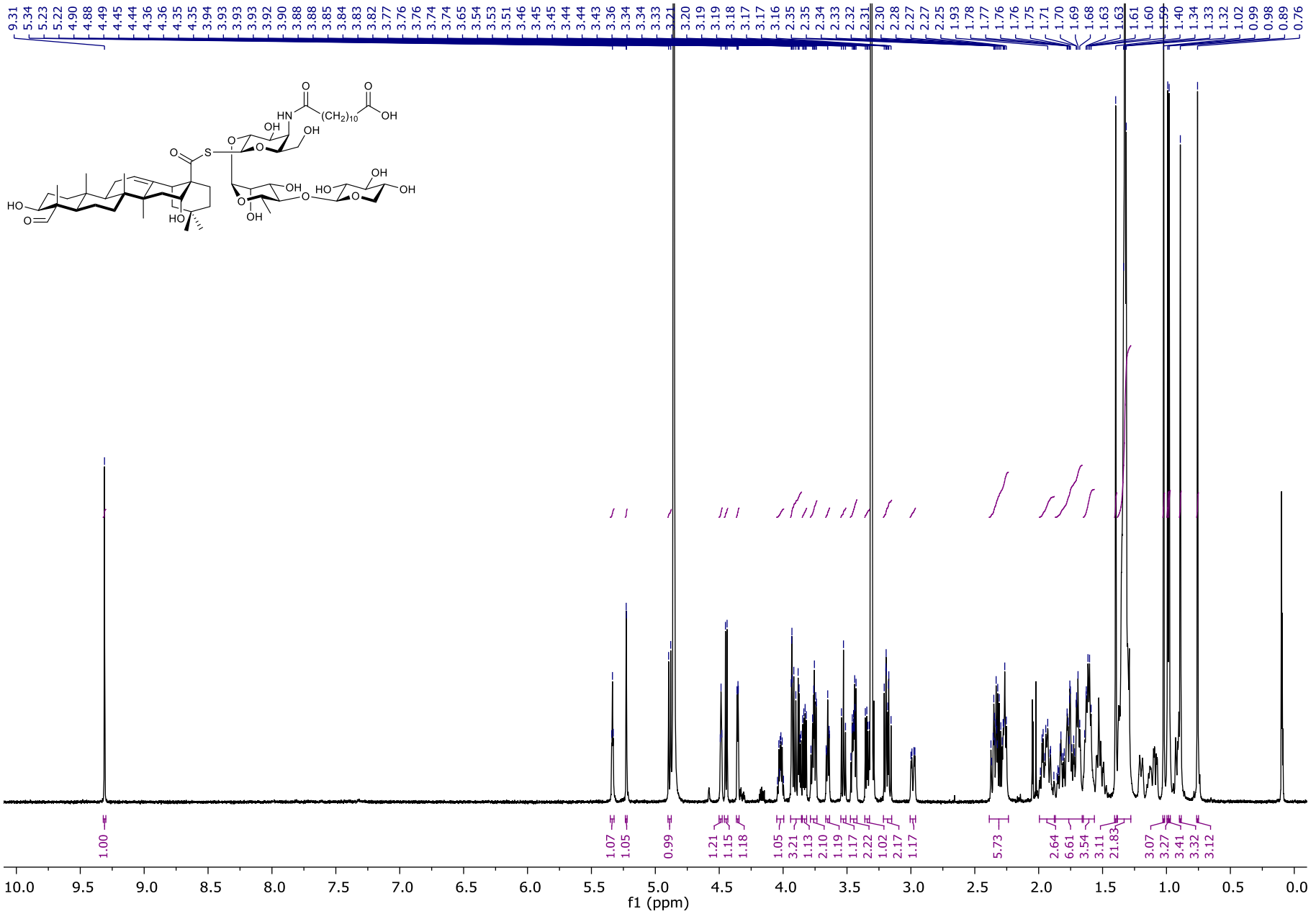
27 COSY (600 MHz, $CDCl_3$)



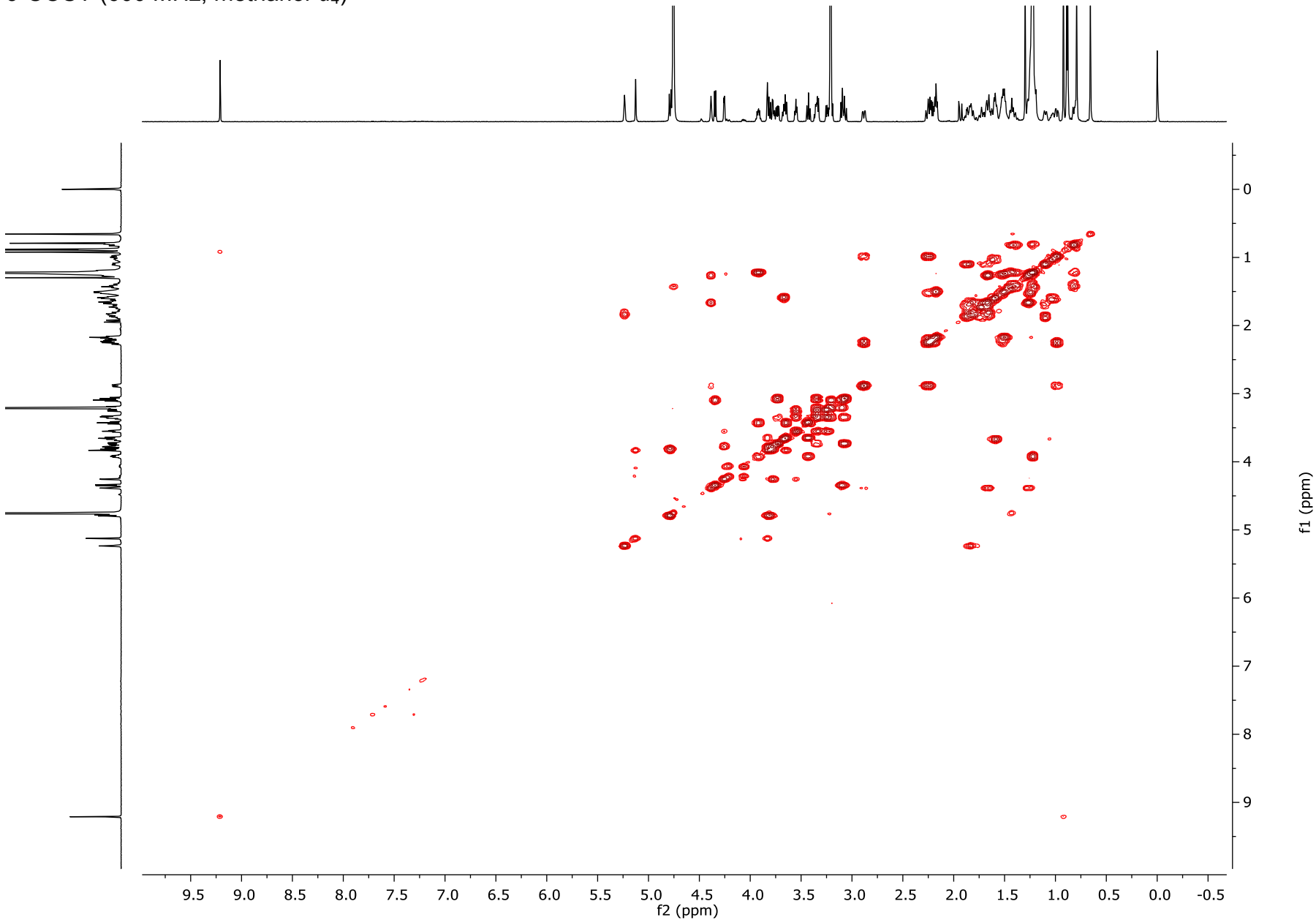
27 HSQC (600 MHz, $CDCl_3$)



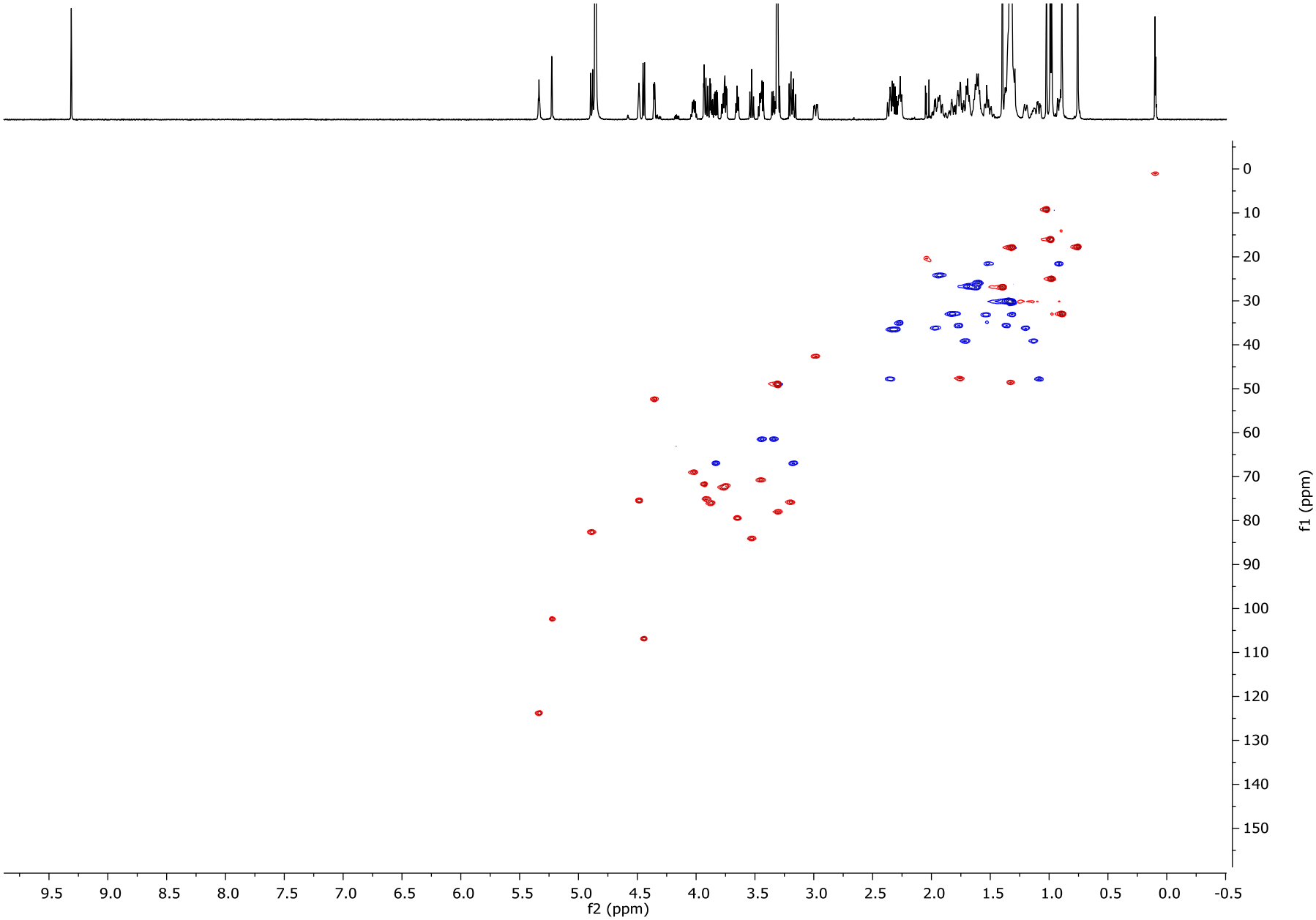
6 ^{1}H-NMR (600 MHz, methanol-d_4)



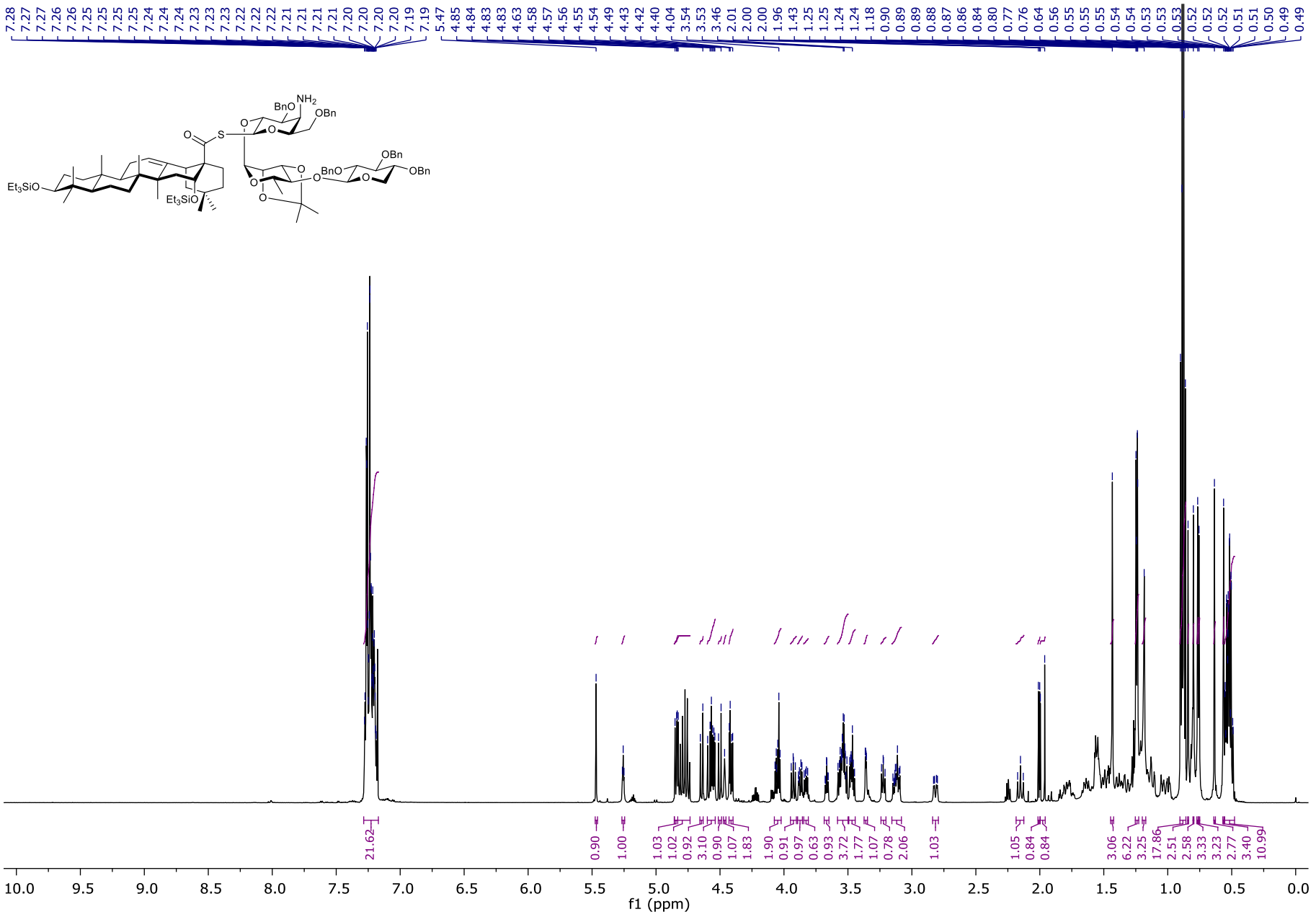
6 COSY (600 MHz, methanol-d_4)



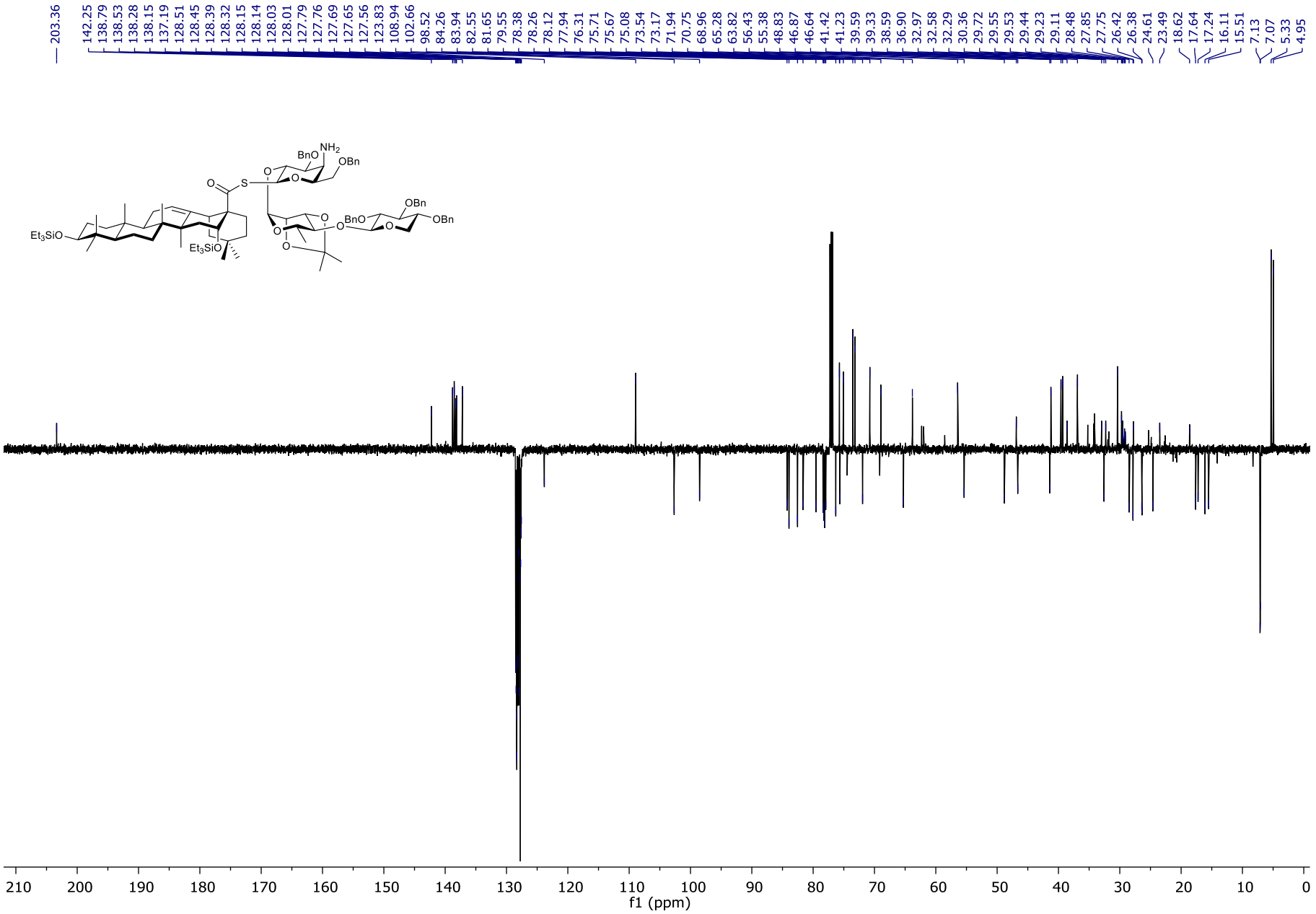
6 HSQC (600 MHz, methanol-d_4)



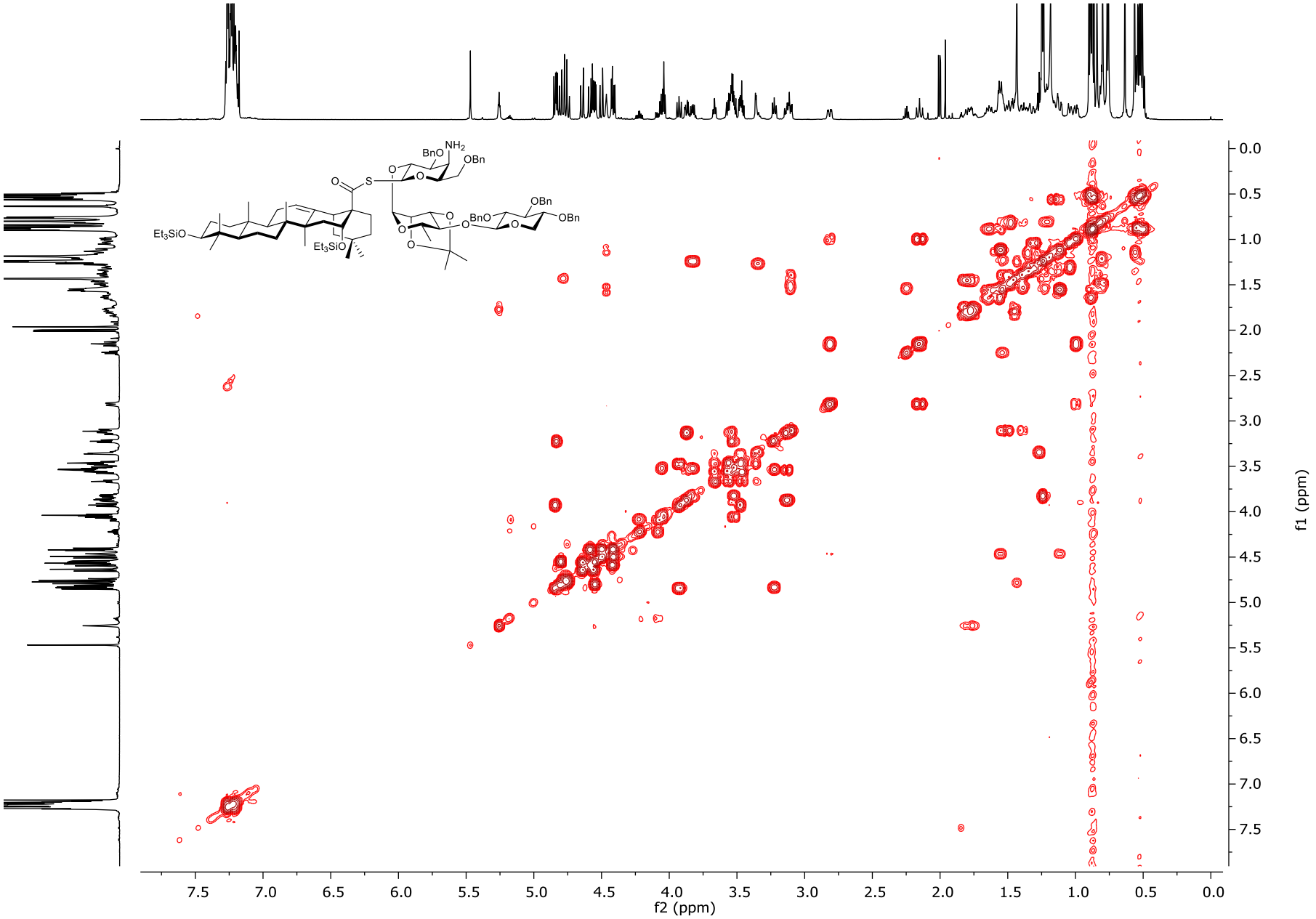
21 ^{1}H-NMR (600 MHz, $CDCl_3$)



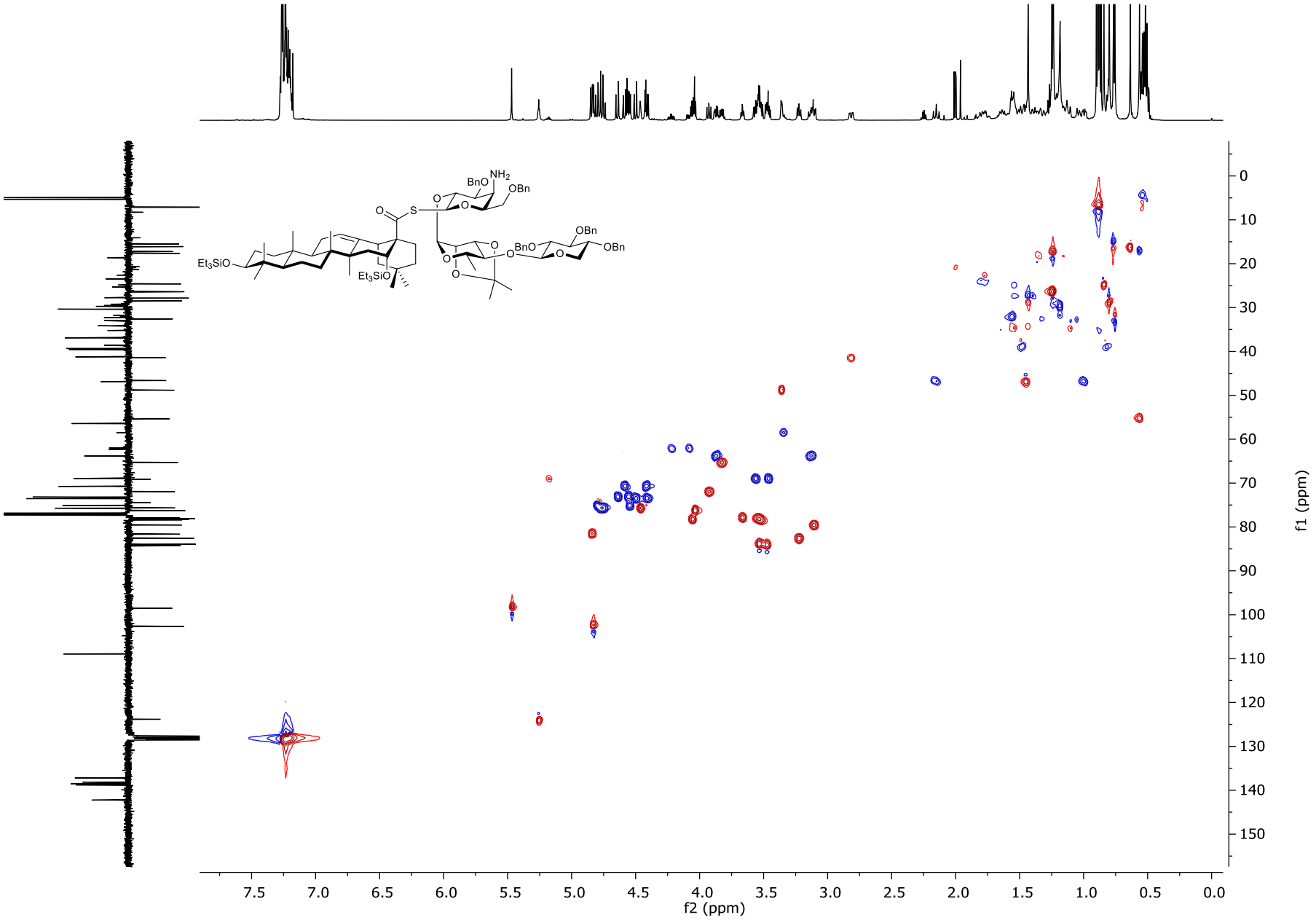
21 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



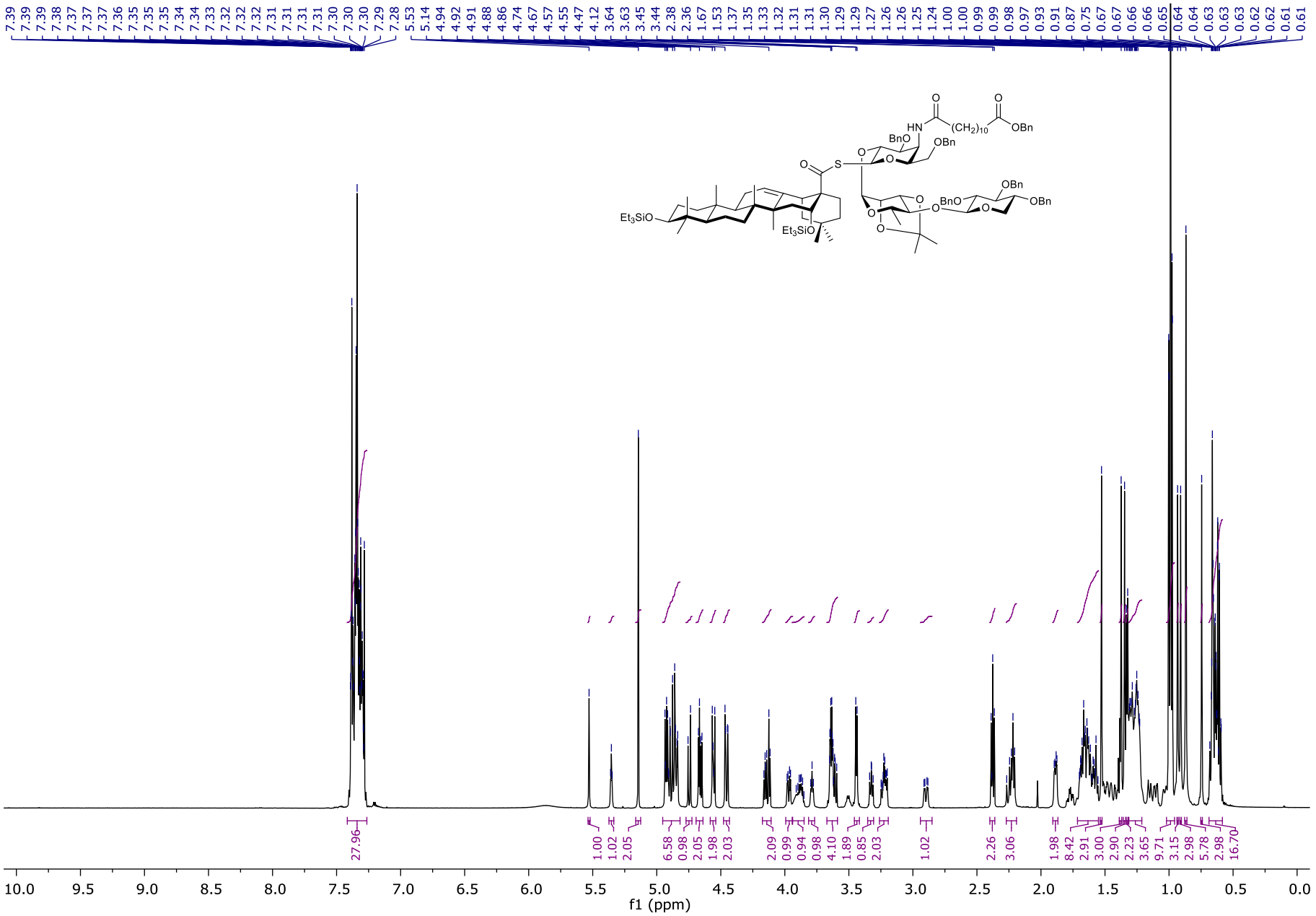
21 COSY (600 MHz, $CDCl_3$)



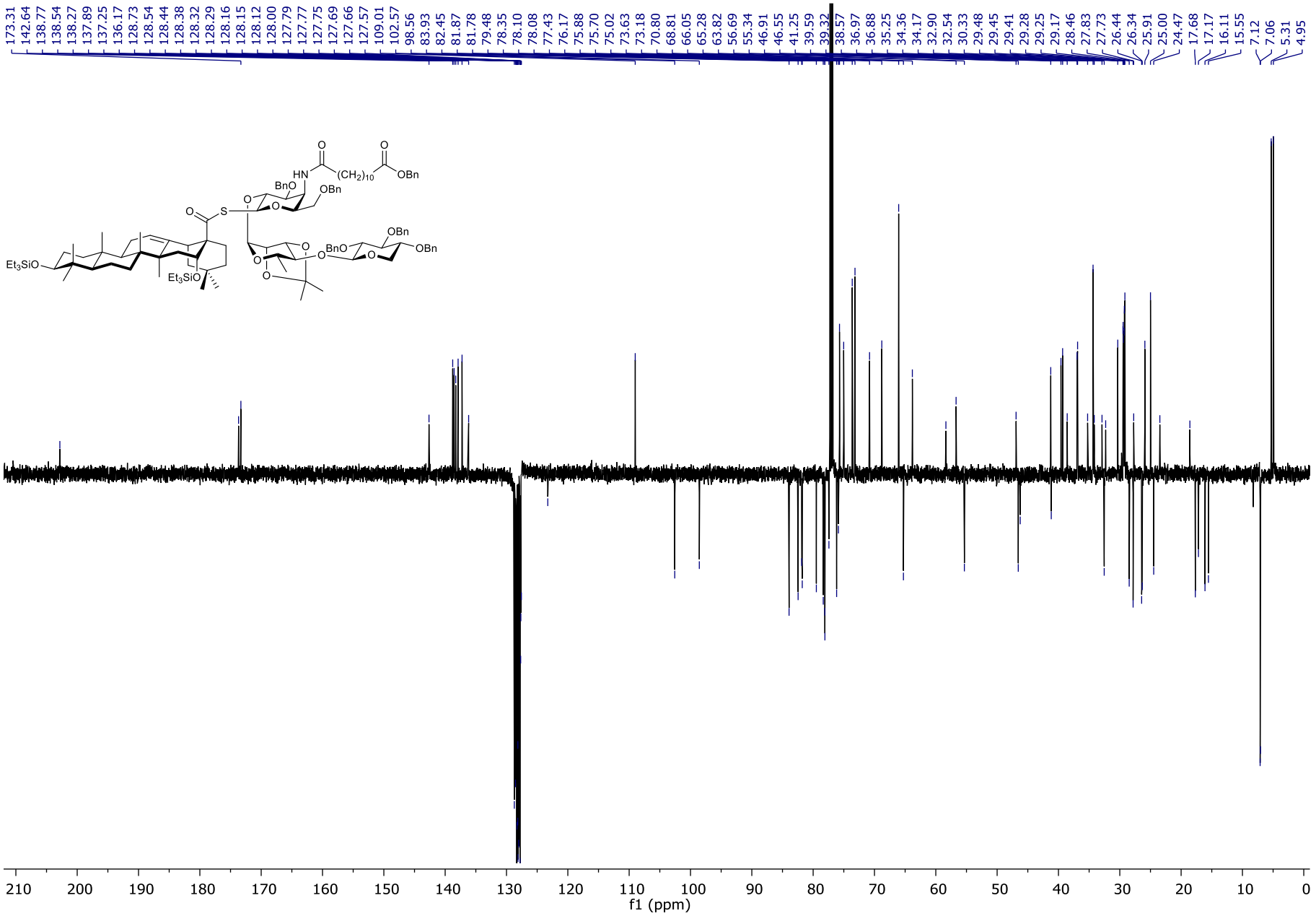
21 HSQC (600 MHz, $CDCl_3$)



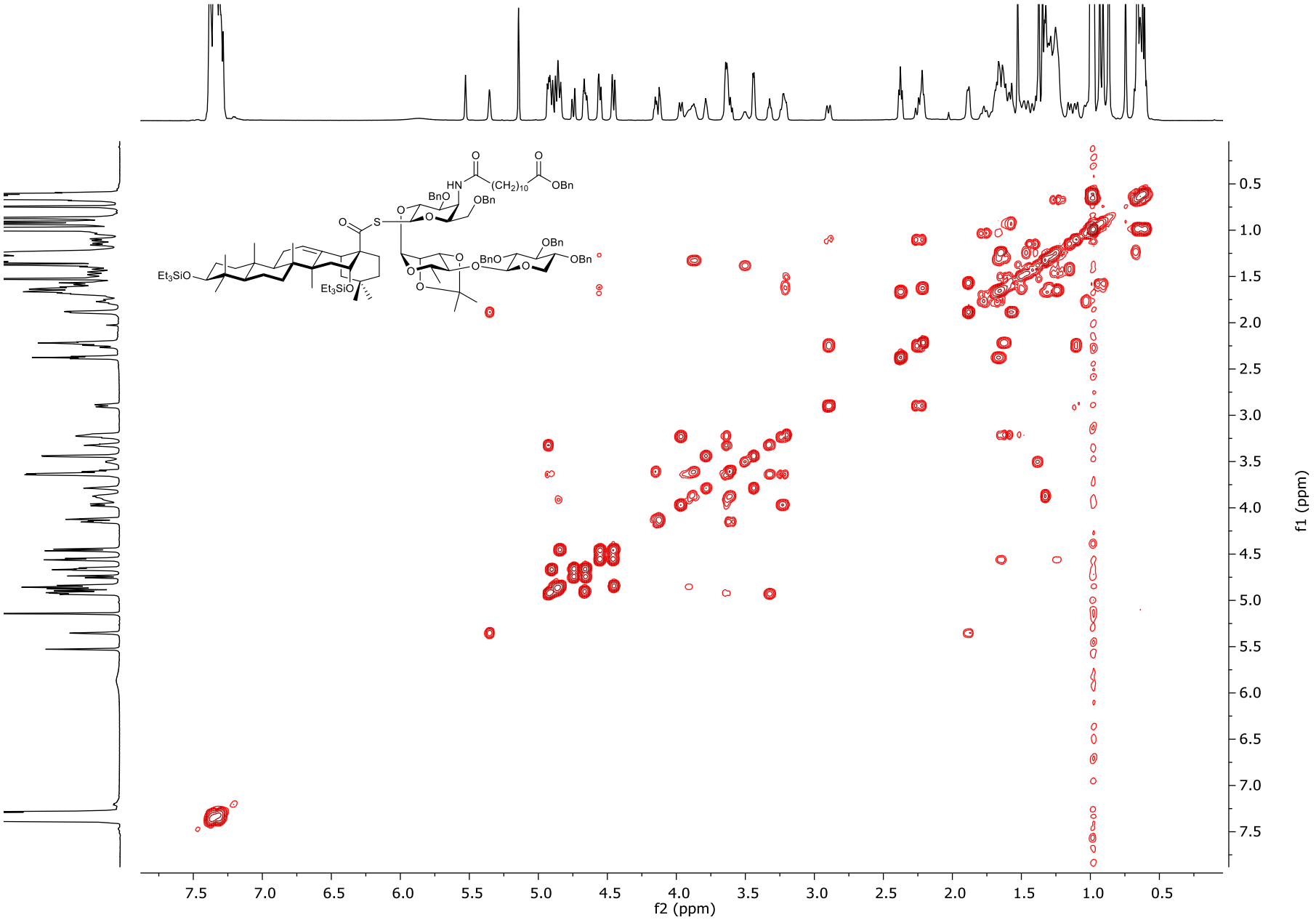
28 ^{1}H-NMR (600 MHz, $CDCl_3$)



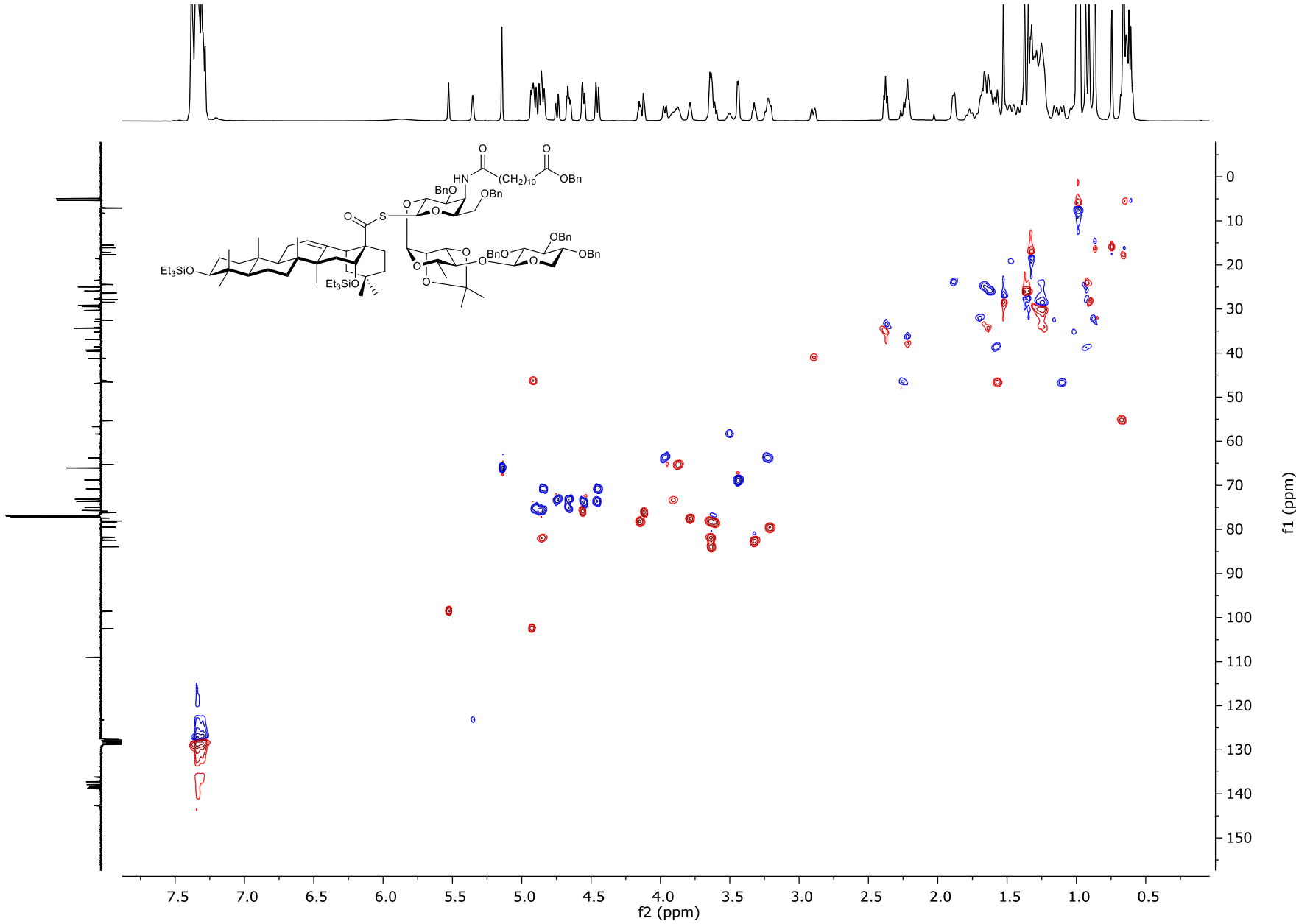
28 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



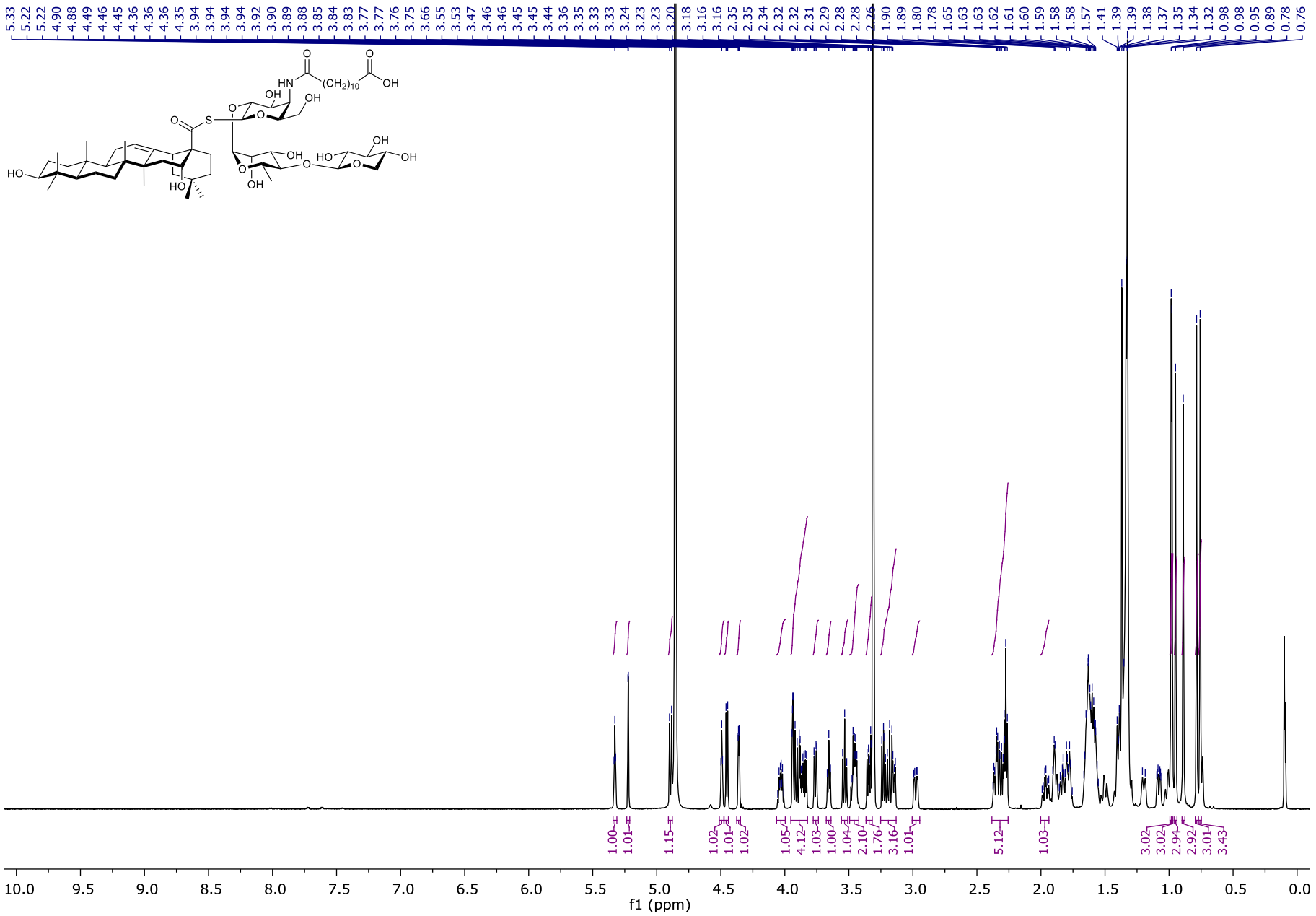
28 COSY (600 MHz, $CDCl_3$)



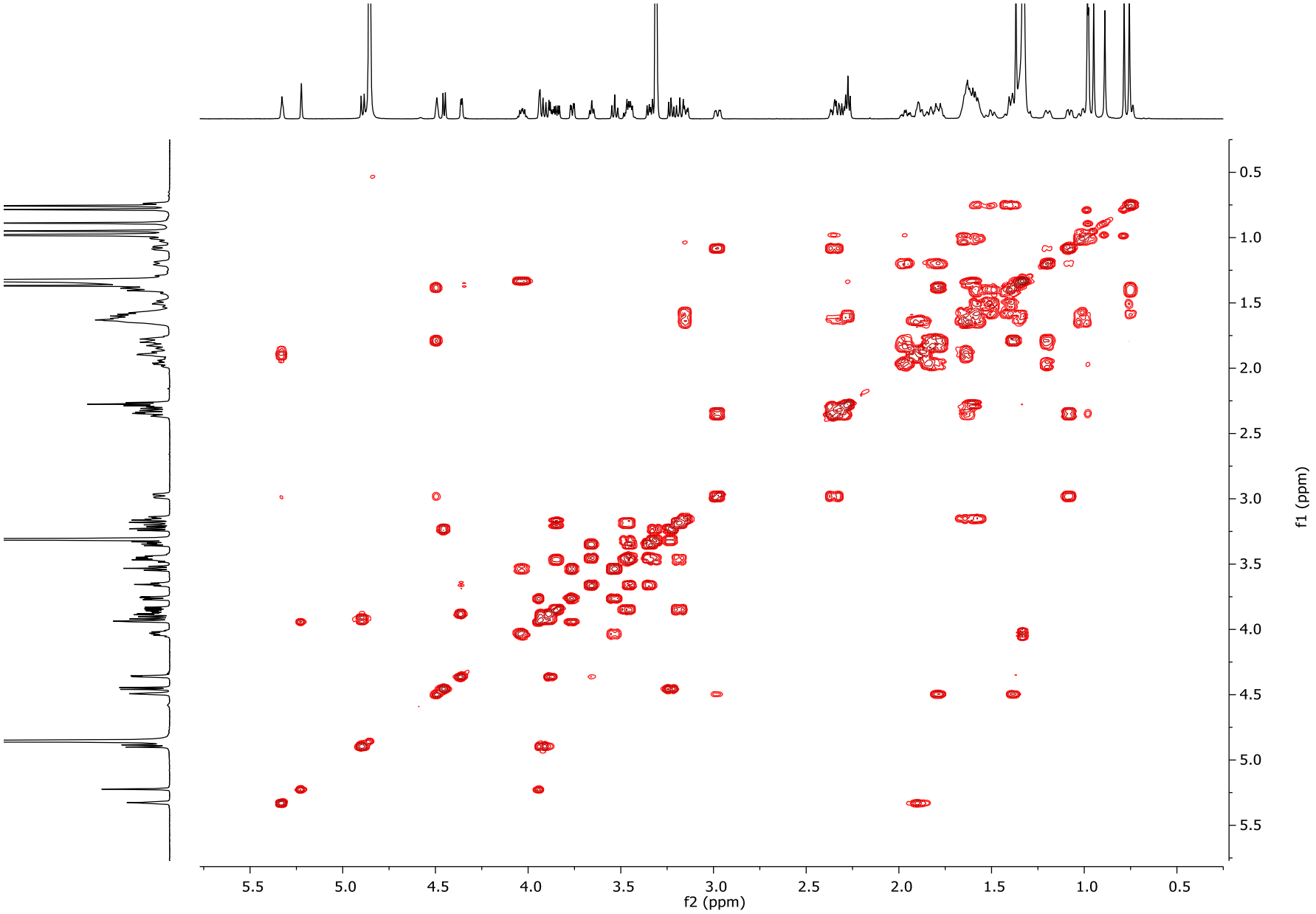
28 HSQC (600 MHz, $CDCl_3$)



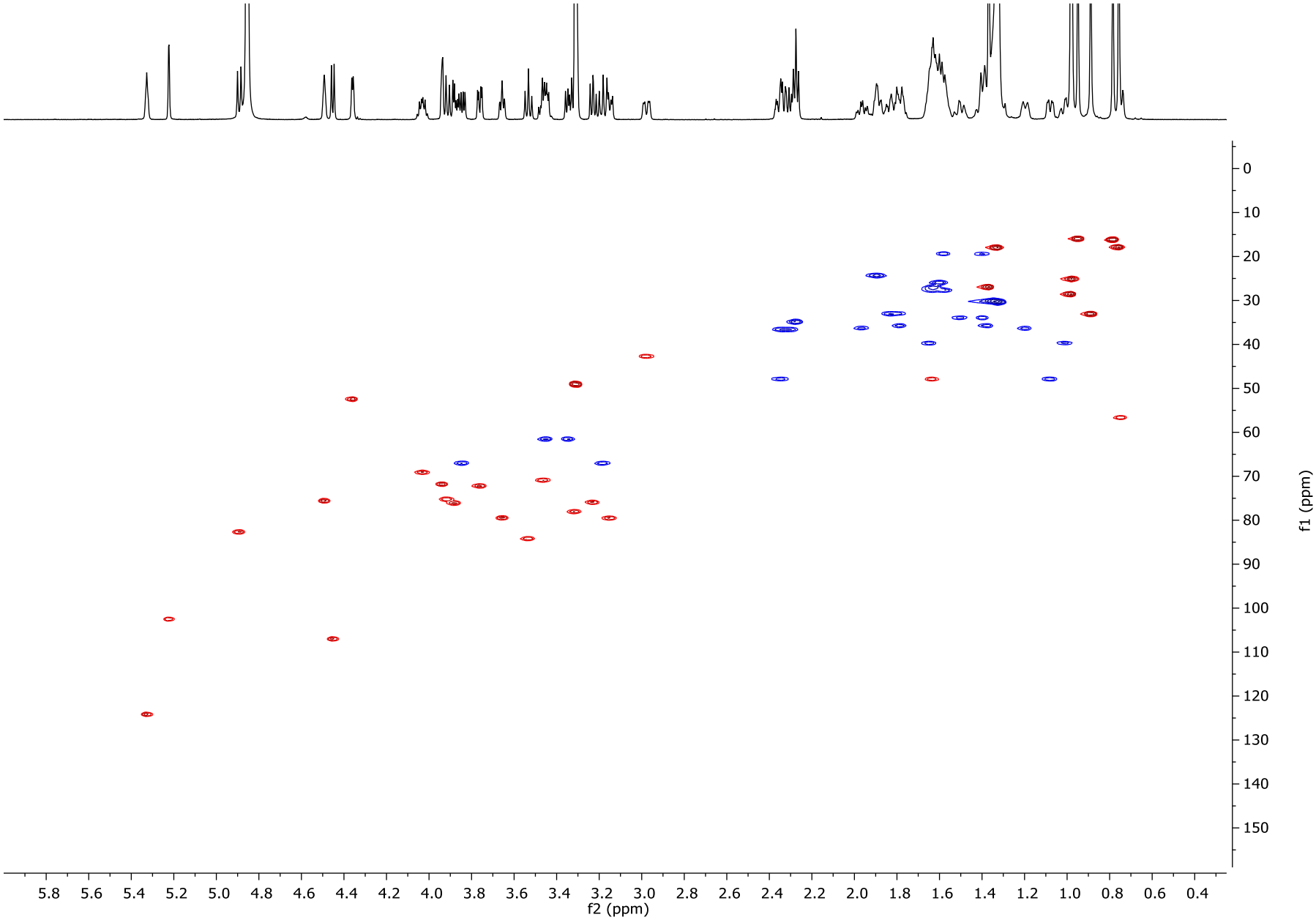
7 ^{1}H-NMR (600 MHz, methanol-d_4)



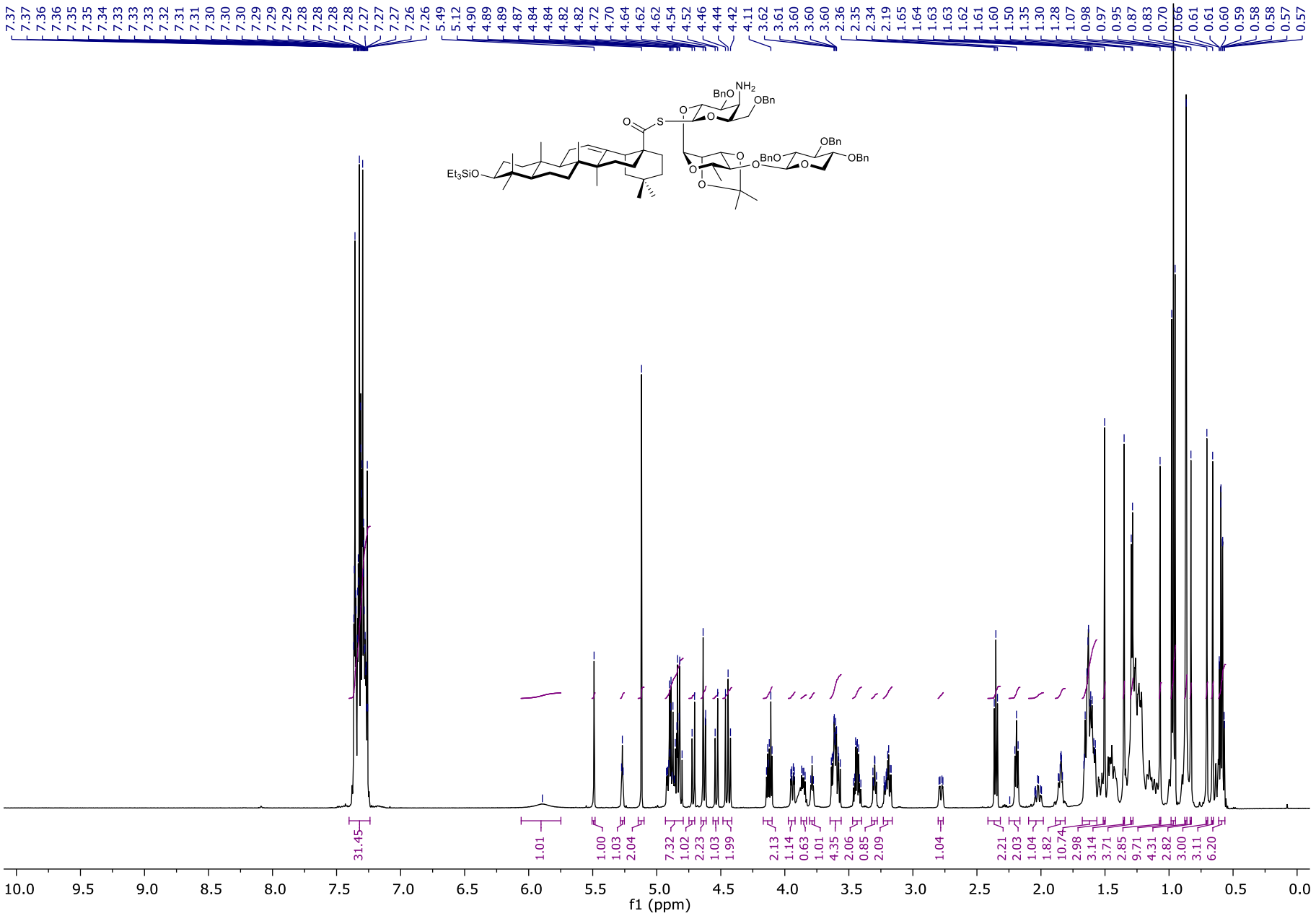
7 COSY (600 MHz, Methanol-d_4)



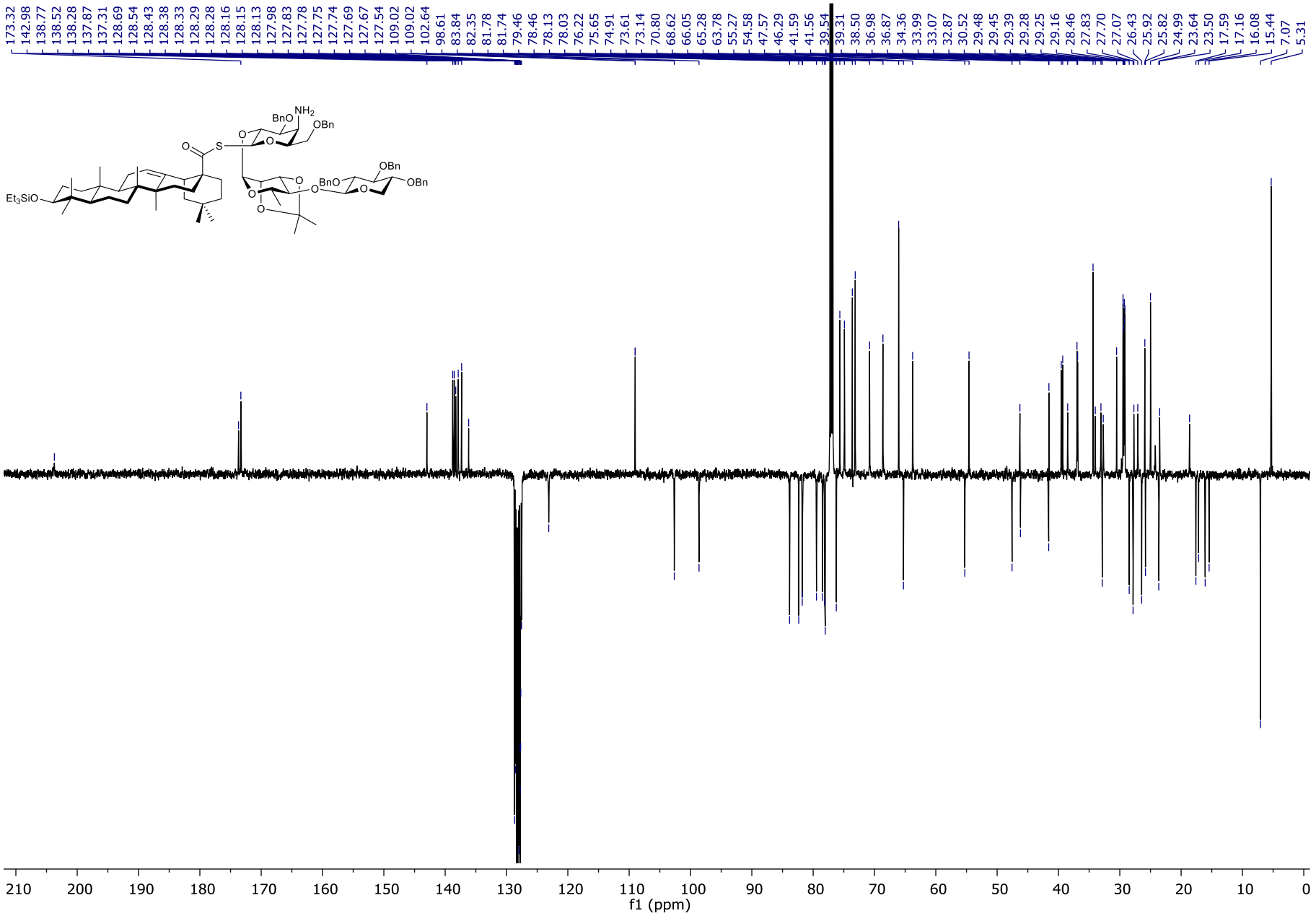
7 HSQC (600 MHz, methanol-d_4)



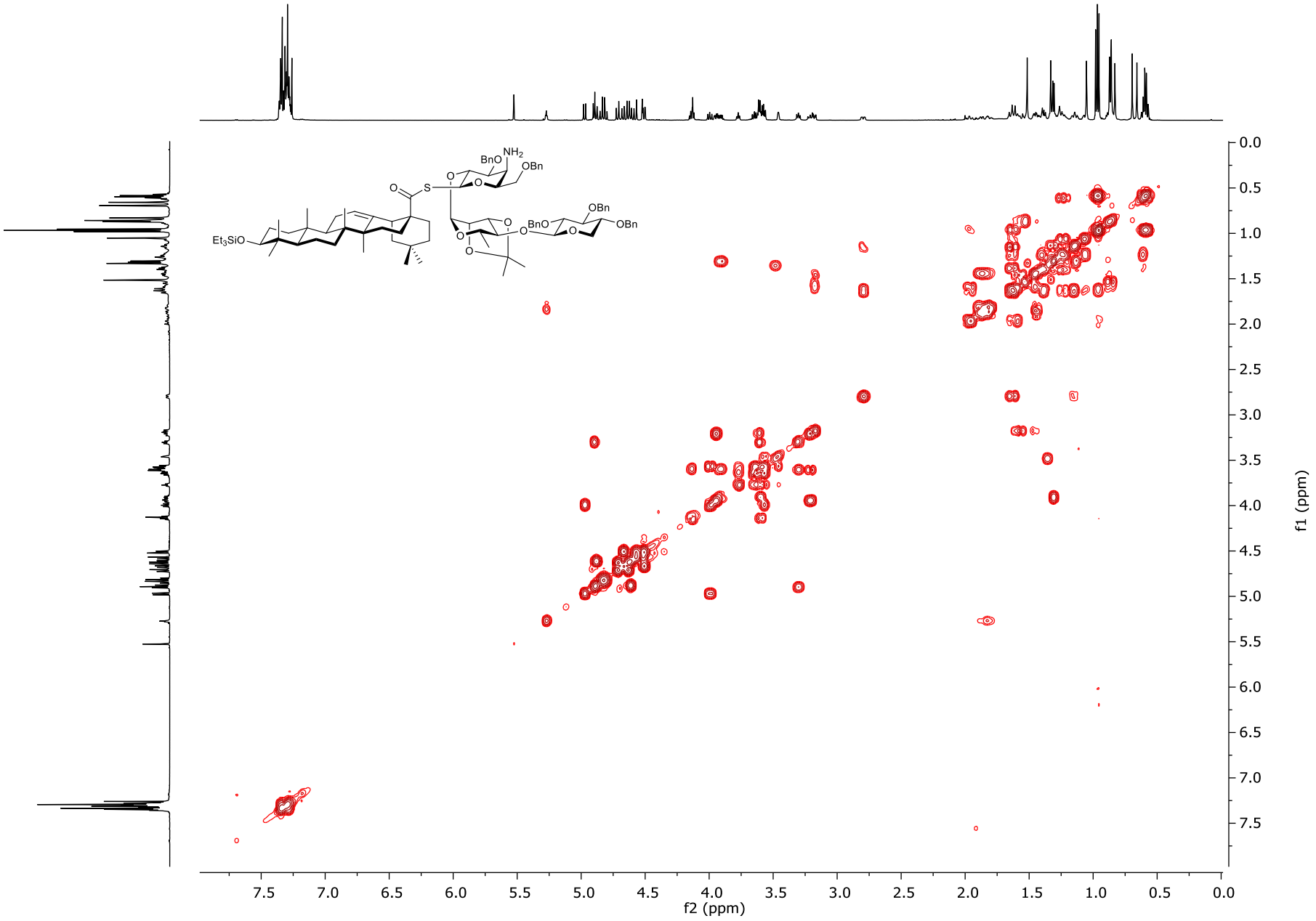
22 ^{1}H-NMR (600 MHz, $CDCl_3$)



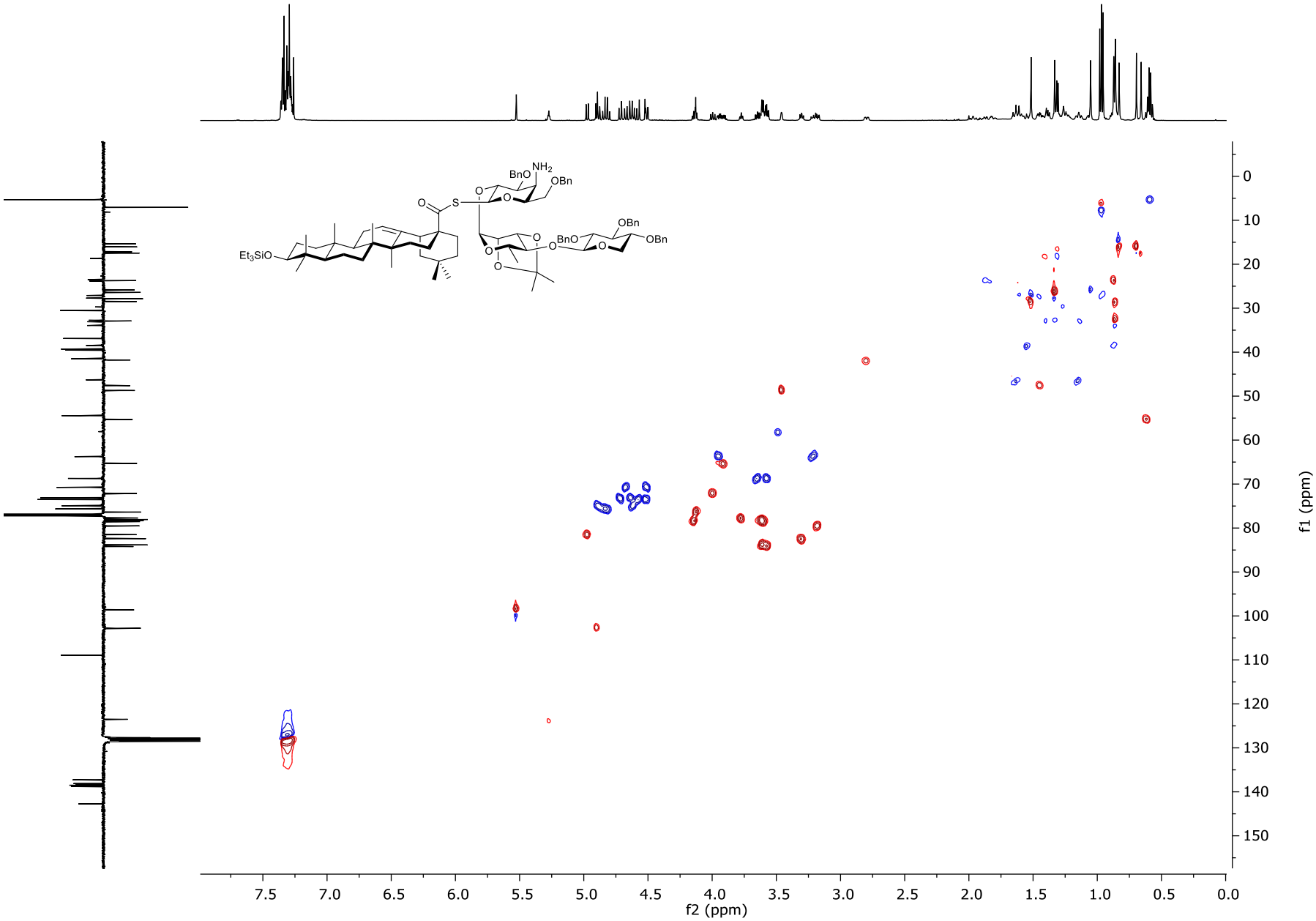
22 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



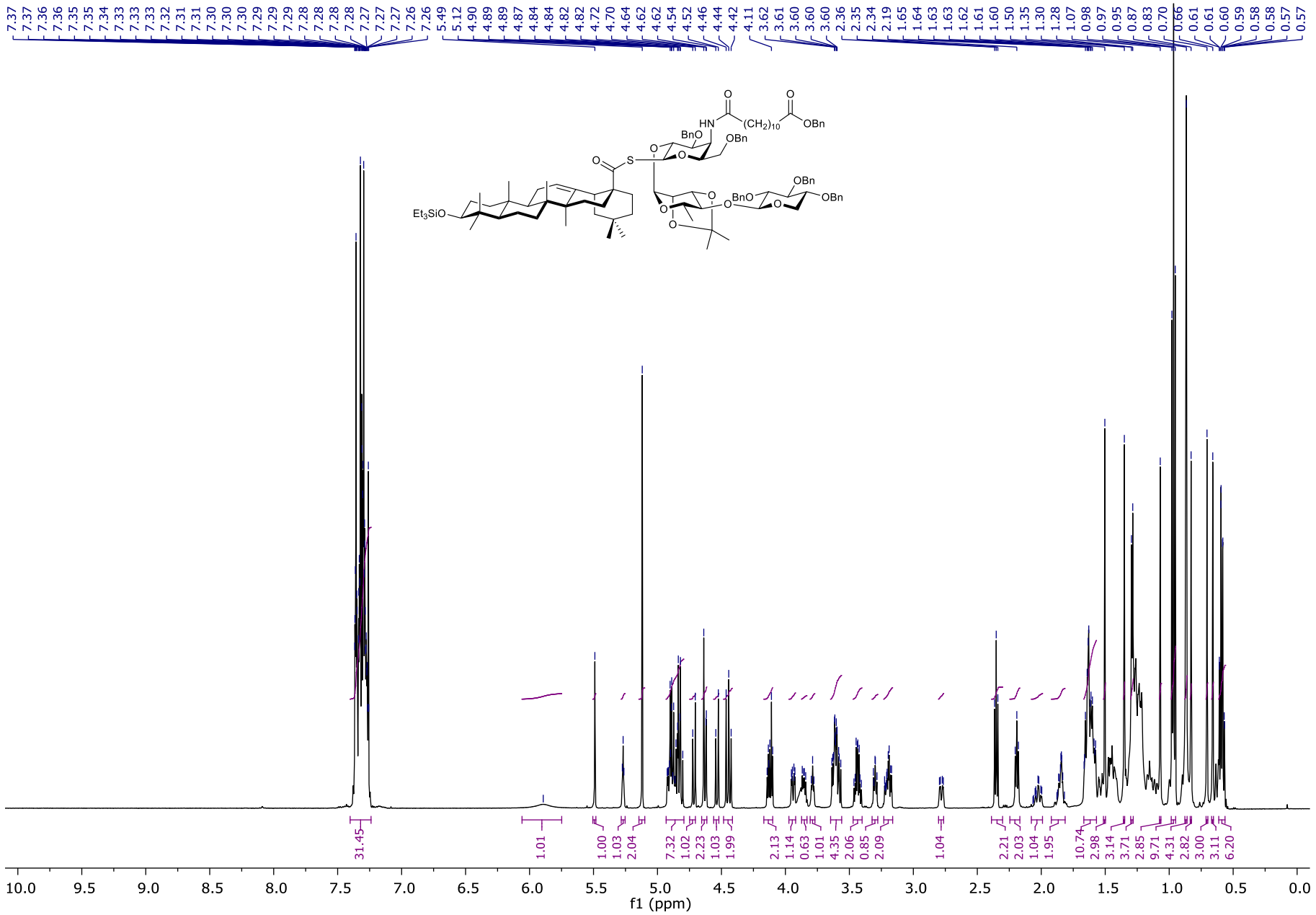
22 COSY (600 MHz, $CDCl_3$)



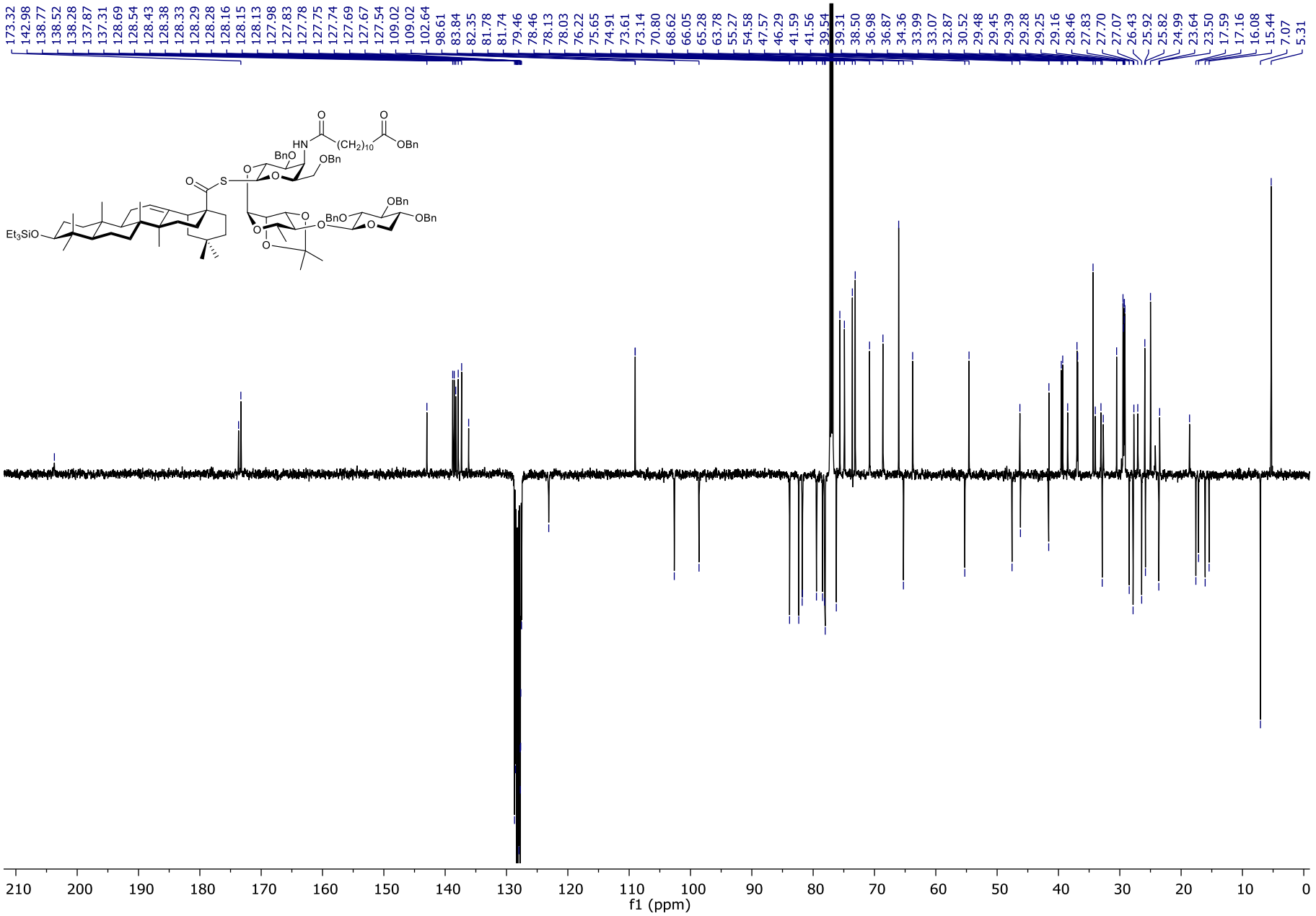
22 HSQC (600 MHz, $CDCl_3$)



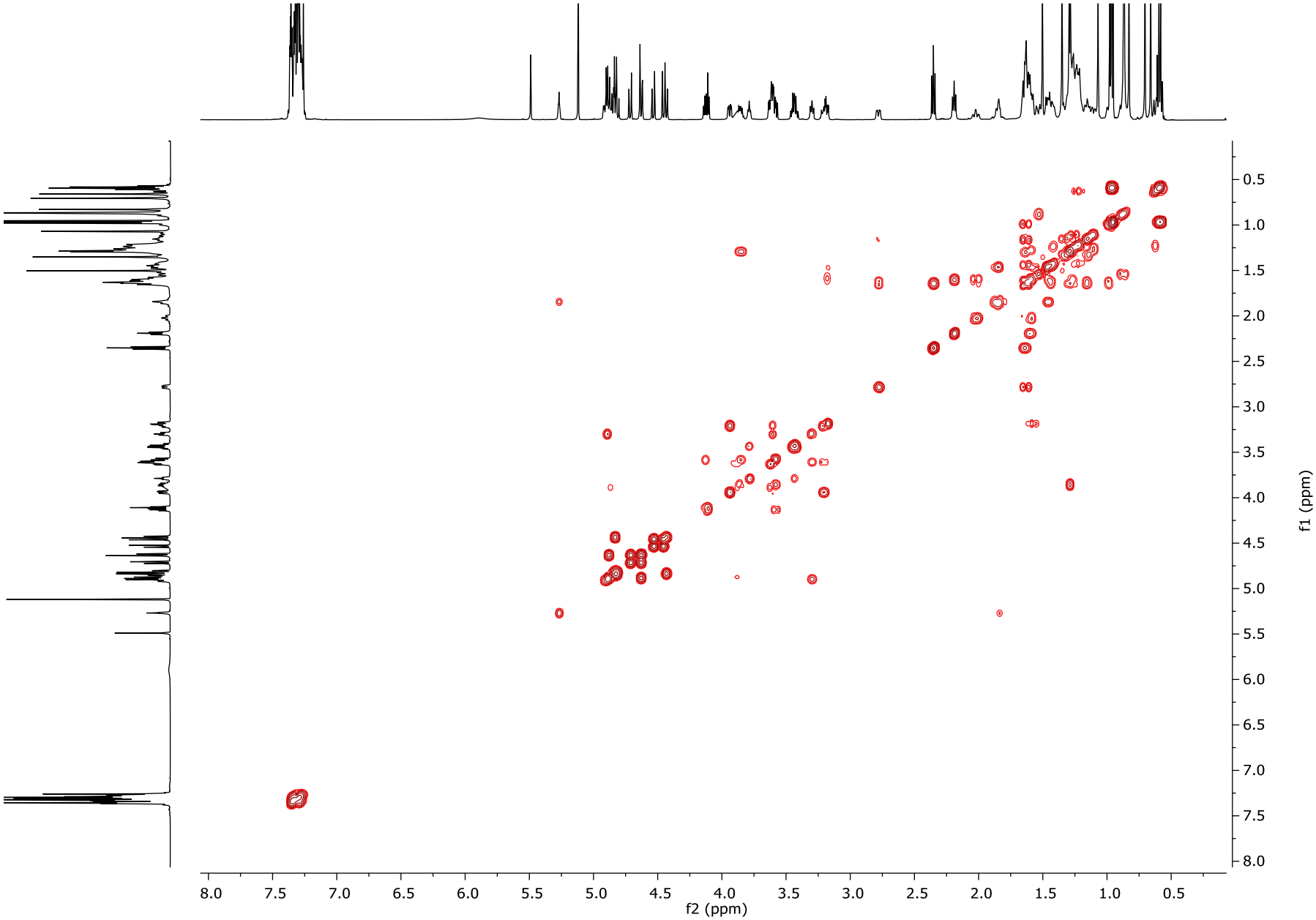
29 ^{1}H-NMR (600 MHz, $CDCl_3$)



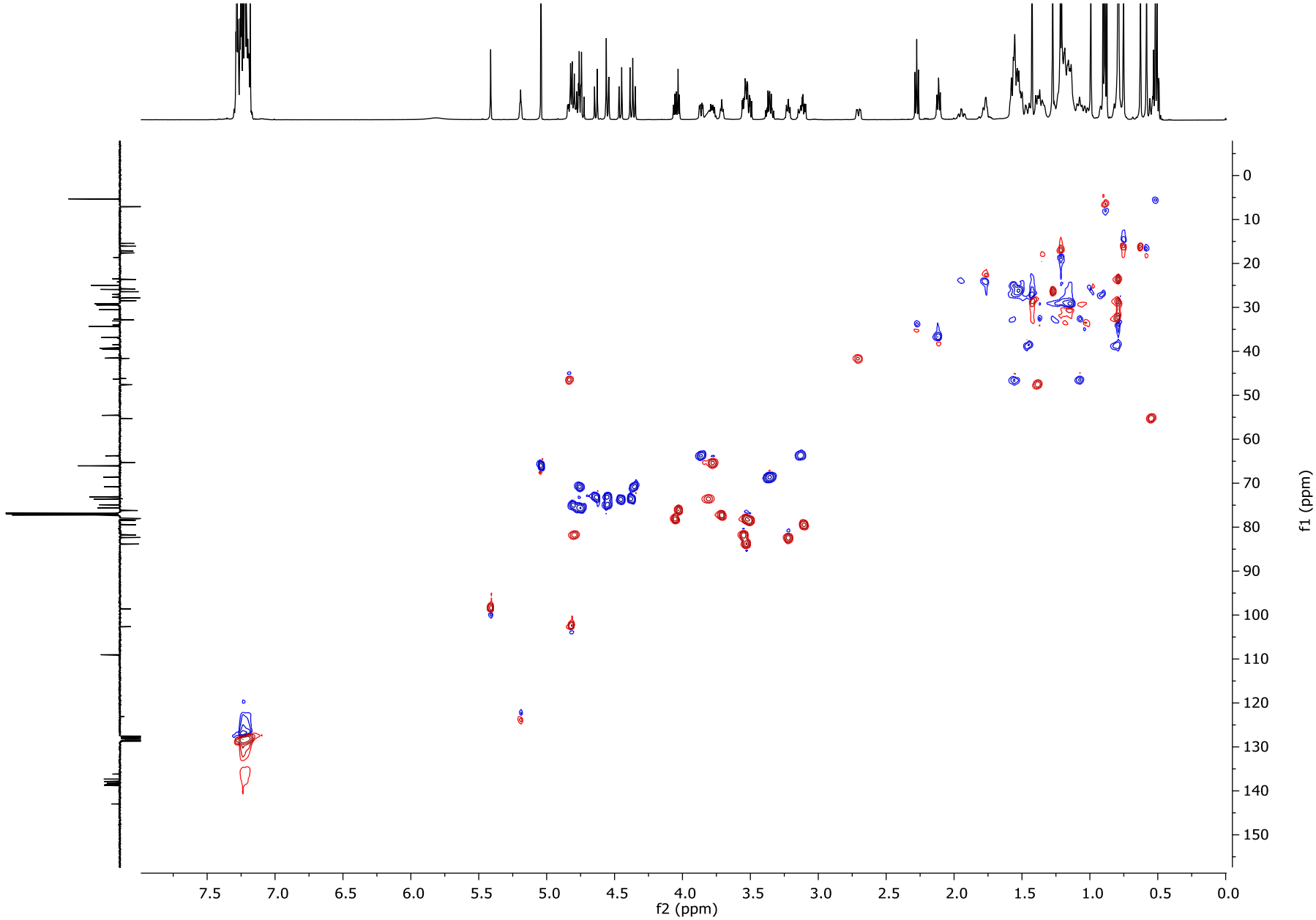
29 APT ^{13}C-NMR (151 MHz, $CDCl_3$)



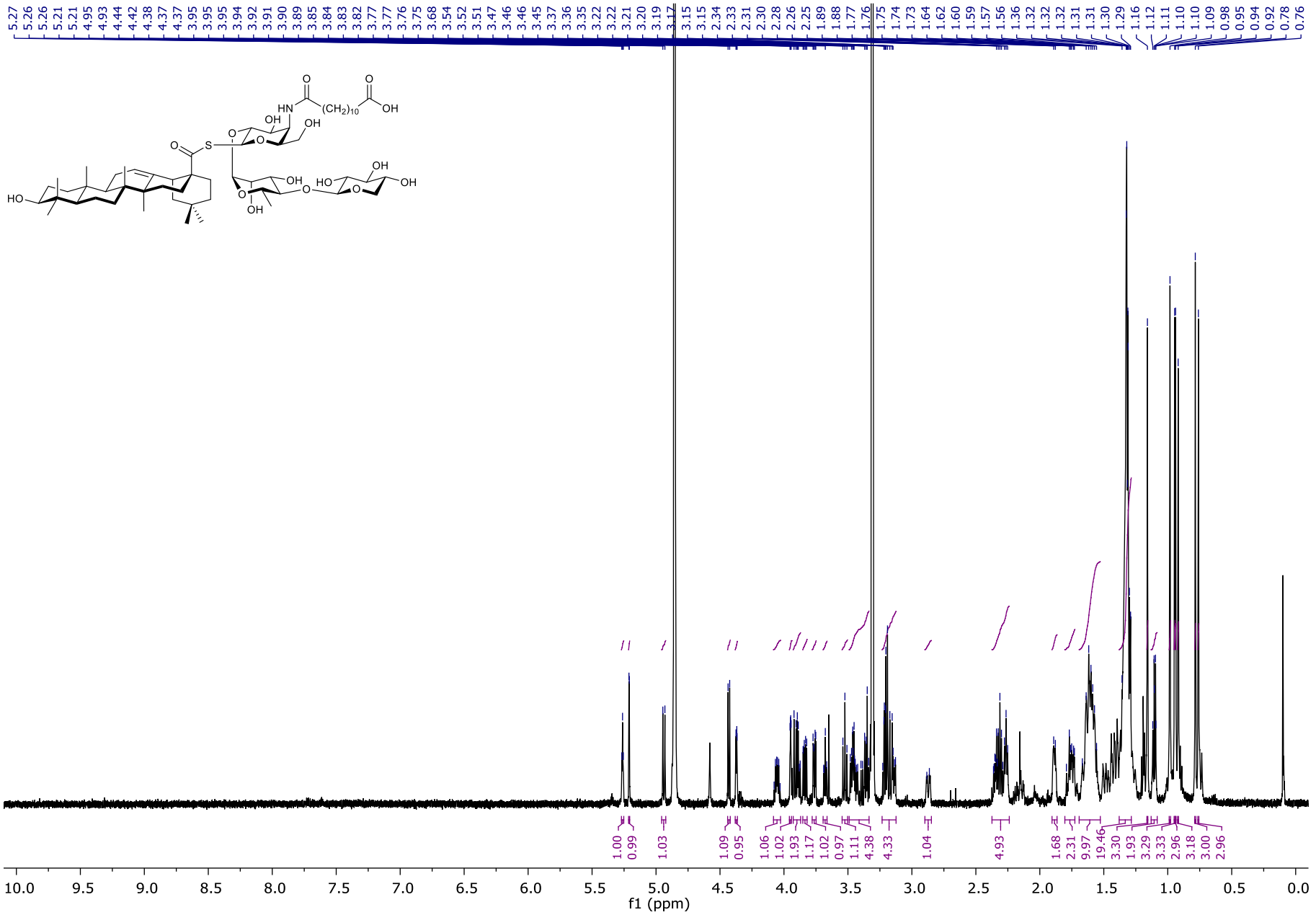
29 COSY (600 MHz, $CDCl_3$)



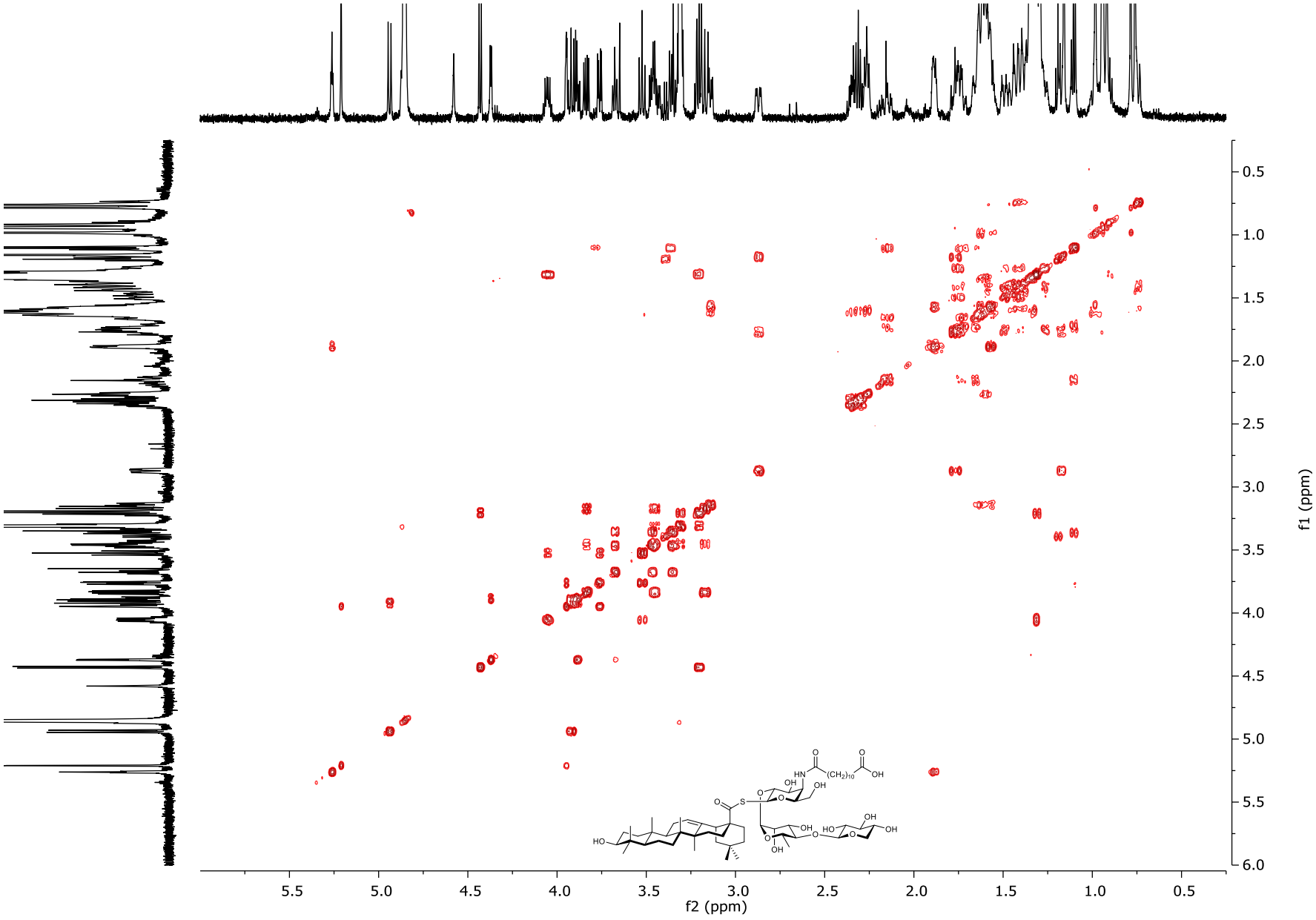
29 HSQC (600 MHz, $CDCl_3$)



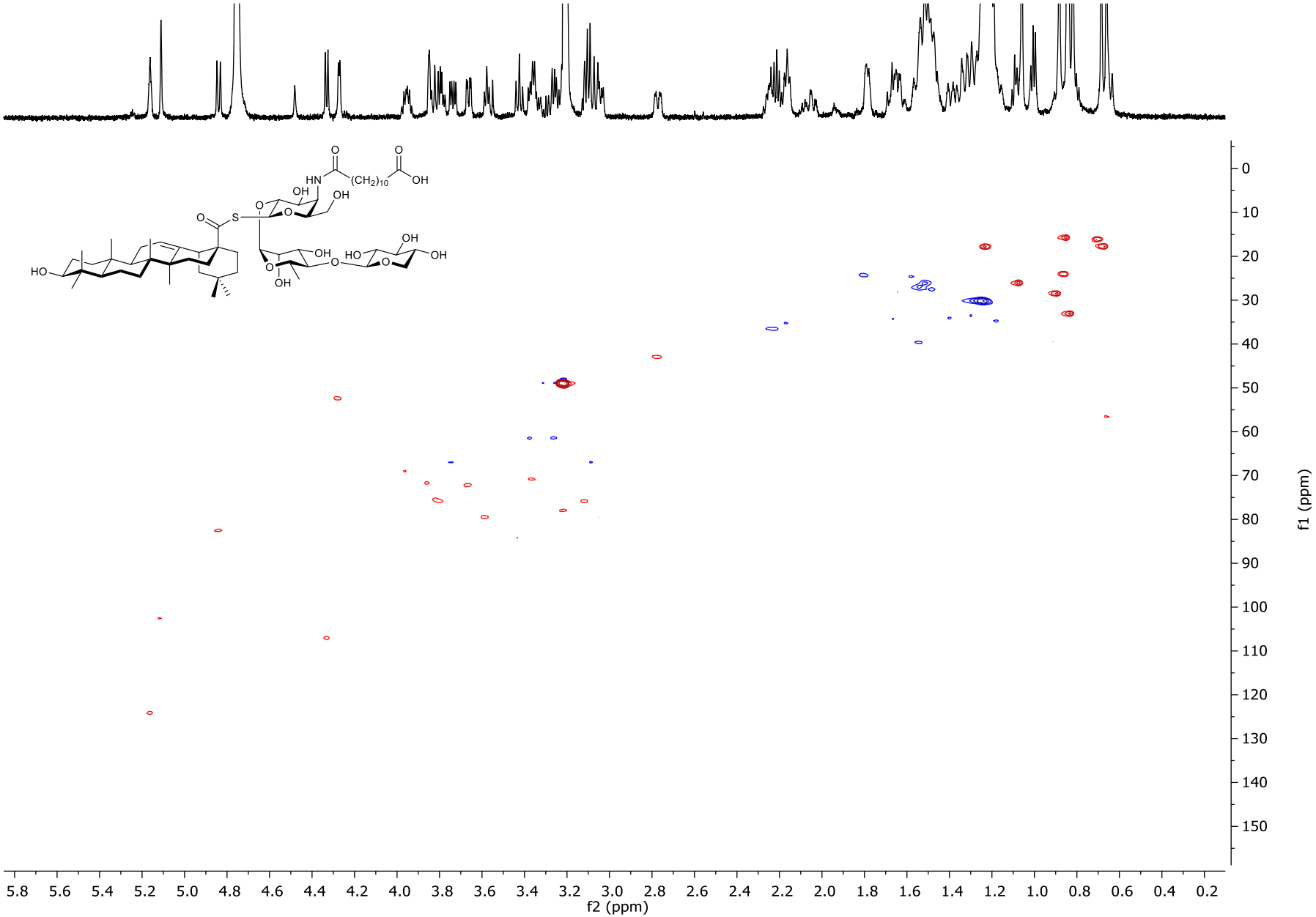
8 ^{1}H-NMR (600 MHz, methanol-d_4)



8 COSY (600 MHz, methanol-d_4)



8 HSQC (600 MHz, methanol-d_4)



VIII. SUPPLEMENTARY INFORMATION REFERENCES

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