

## SUPPLEMENTARY INFORMATION

### Design, Synthesis, and Initial Immunological Evaluation of Glycoconjugates based on Saponin Adjuvants and the Tn antigen

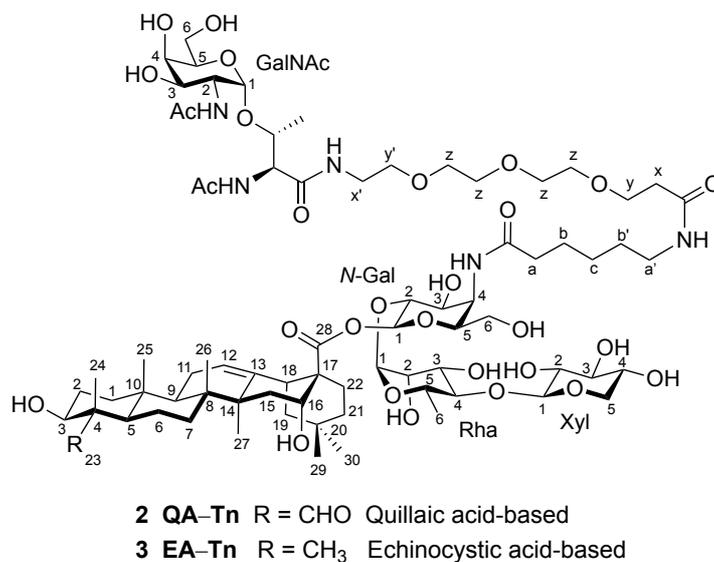
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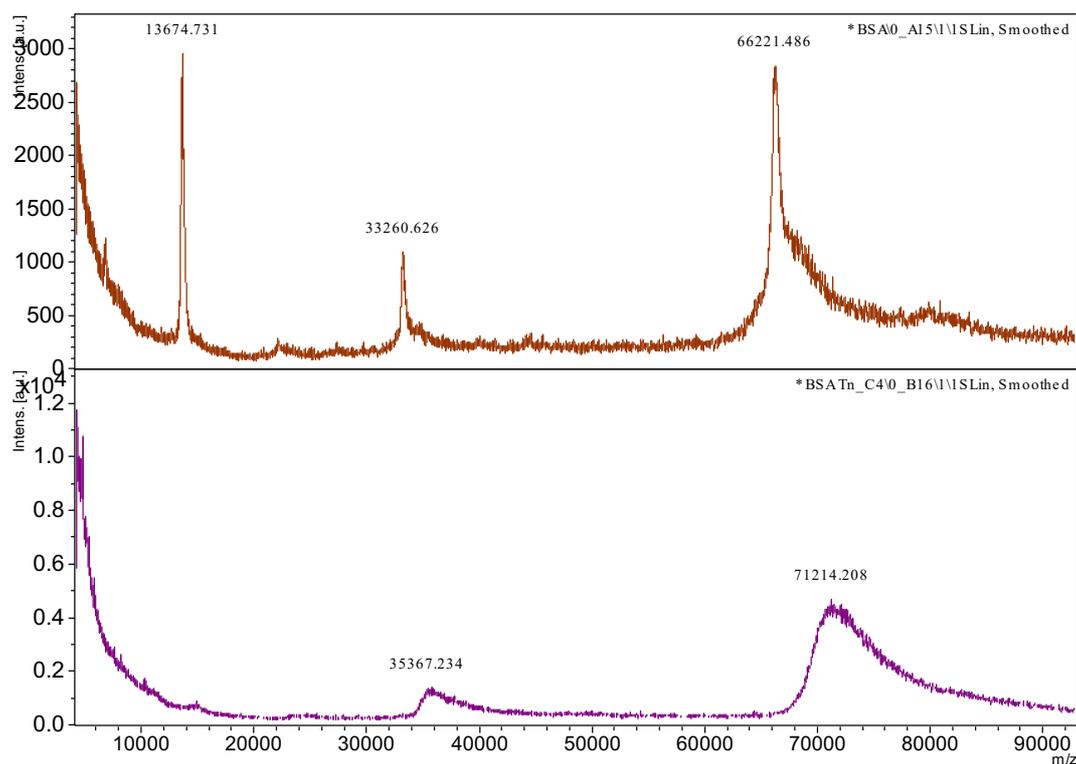
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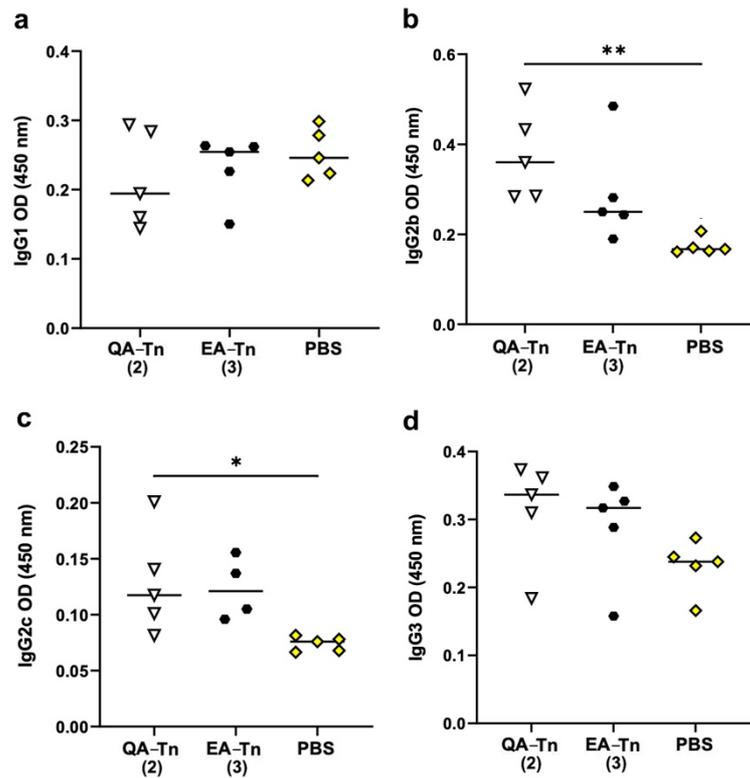
## I. SUPPLEMENTARY FIGURES



**Figure S1.** Identification codes used in the assignment of the different positions within the saponin-Tn conjugate structures.



**Figure S2.** MALDI-TOF analysis of BSA protein (top) and (b) BSA-(Tn)<sub>9</sub> conjugate (bottom).



**Figure S3.** Induced anti-Tn IgG subtype levels (OD) of (a) IgG1, (b) IgG2b, (c) IgG2c, and IgG3 antibodies on end-point day 42 after first immunization, as detected by ELISA using a 1/50 serum dilution. Horizontal bars indicate mean values. Statistical significance versus negative control group (PBS only) was determined using two-sided unpaired Student's t-test with \*  $p \leq 0.05$  and \*\*  $p \leq 0.01$ .

## **II. GENERAL INFORMATION**

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<sub>2</sub>. 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. Analytical thin-layer chromatography (TLC) was performed using aluminum-backed sheets pre-coated with 230–400 mesh silica gel 60 containing fluorescent indicator (F254). Preparative TLC (Analtech Uniplates) was performed using glass-backed sheets pre-coated with 500-micron silica gel containing fluorescent indicator (F254). TLC plates were visualized under UV light (254 nm) and by staining with cerium ammonium molybdate (CAM) or 5% sulfuric acid in ethanol solutions. Bovine Serum Albumin (BSA) was purchased from Fisher Scientific

**Nuclear magnetic resonance (NMR).** <sup>1</sup>H, APT <sup>13</sup>C, COSY and HSQC spectra were recorded on a Bruker Avance III instrument (<sup>1</sup>H NMR at 600 MHz and APT <sup>13</sup>C NMR at 151 MHz) and Bruker AVANCE NEO spectrometer (<sup>1</sup>H NMR at 400 MHz and APT <sup>13</sup>C NMR at 101 MHz), equipped with a SmartProbe, and operating under TopSpin 4.1.1. Chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent (CDCl<sub>3</sub>: δ 7.26 for <sup>1</sup>H NMR, δ 77.00 for <sup>13</sup>C NMR; methanol-*d*<sub>4</sub>: δ 3.31 for <sup>1</sup>H NMR, δ 49.15 for <sup>13</sup>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 LC-MS.** All reverse-phase RP-HPLC analyses/purifications were carried out on a Waters 1525 binary gradient system (Solv. A = 0.05% TFA in H<sub>2</sub>O; Solv. B = 0.05% TFA in CH<sub>3</sub>CN) equipped with a Waters 2998 photodiode array detector (PDA), and combined with a low-resolution single quadrupole (SQD2, Waters Corporation) mass spectrometer. Absorbances were monitored at wavelengths of 190–600 nm.

**HR-ESI-MS.** High resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on a Waters LCT Premier XE (Waters, Milford, MA, USA) in W-optics positive ionization scan mode. Mass spectrometry parameters were optimized to achieve the best signal-to-noise ratio: capillary voltage 1 kV, sample cone voltage 100 V, desolvation gas flow 600 Lh<sup>-1</sup>, cone gas flow 50 Lh<sup>-1</sup>, desolvation temperature 350 °C, source temperature 150 °C. The instrument was calibrated over the range m/z 200–2000 before measurement using a standard NaI solution (1 μM). In order to minimize the accuracy in the measurements, Leucine-Enkephalin was used as lockmass reference [2M+Na], m/z 1111,5459. Data analysis was performed with Masslynx software version 4.1 (Waters, Milford, MA, USA). Characterization by MS was corroborated after comparing the experimental isotopic pattern with the theoretical one.

**MALDI-TOF-HR-MS.** High resolution MALDI-TOF mass spectra analyses were performed on an UltrafleXtreme III MALDI-time-of-flight (TOF) mass spectrometer equipped

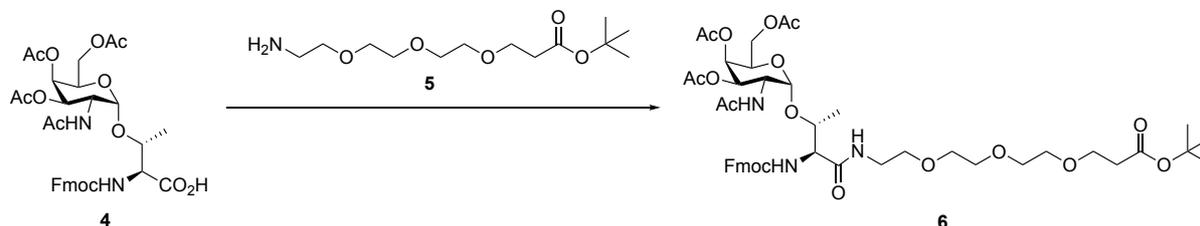
with a pulsed Nd:YAG laser (355 nm) and controlled by FlexControl 3.3 software (Bruker Daltonics, Bremen, Germany). The acquisitions were carried out in positive reflector ion mode with pulse duration of 50 ns. Laser intensity was set marginally above the threshold of ionization to avoid fragmentation. The  $m/z$  range was chosen according to the mass of the sample. The acquired data was processed using the mMass software.

Samples of BSA and BSA–Tn conjugate were desalted using ZipTip® C4 micro-columns (Millipore) (2  $\mu$ L sample) with elution using 0.5  $\mu$ L SA (sinapinic acid, 10 mg/ml in [70:30] acetonitrile:trifluoroacetic acid 0.1%) matrix onto a GroundSteel massive 384 target (Bruker Daltonics). Their MALDI-TOF-HR-MS analyses were carried out on an Autoflex III Smartbeam MALDI-TOF spectrometer (Bruker Daltonics), which was used in linear mode with the following settings: 5.000–93.000 Th window, linear positive mode, ion source 1: 20 kV, ion source 2: 18.5 kV, lens: 9 kV, pulsed ion extraction of 120 ns, high gating ion suppression up to 1000 Mr. Mass calibration was performed externally with protein 1 standard calibration mixture (Bruker Daltonics) in the same range as the samples. Data acquisition was performed using FlexControl 3.0 software (Bruker Daltonics), and peak peaking and subsequent spectra analysis was performed using FlexAnalysis 3.0 software (Bruker Daltonics).

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### III. CHEMICAL SYNTHESIS

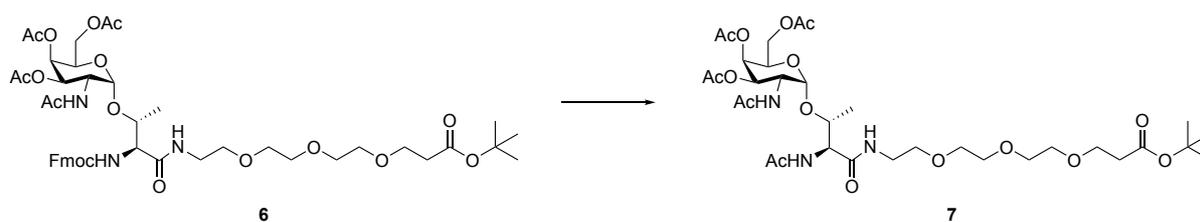
#### A. SYNTHESIS OF SPACER-CONTAINING TN ANTIGEN



#### Protected Fmoc-Tn(Thr)-TEG<sub>3</sub> *tert*-butyl ester (6).

To a solution of Tn-Thr acid **4**<sup>[1,2]</sup> (47 mg, 70.2  $\mu$ mol) in DCM (0.9 mL), HATU (29.4 mg, 77.3  $\mu$ mol, 1.1 equiv) and the amino-TEG<sub>3</sub>-*t*-butyl ester linker **5** (22.07  $\mu$ L, 84.3  $\mu$ mol, 1.2 equiv) were added. The mixture was cold to 0° and 2,4,6-collidine (10.2  $\mu$ L, 77.3  $\mu$ mol, 1.1 equiv) was added. After stirring for 1 h at 0°, the reaction was allowed to warm to r.t. and 1 h later TLC (95:5 DCM/methanol) confirmed consumption of starting material. The resulting crude was concentrated and dried under vacuum, and the residue was purified by silica gel column chromatography (DCM to 97:3 DCM/methanol) affording the Tn(Thr)-TEG<sub>3</sub> *tert*-butyl spacer **6** (53.9 mg, 82% yield) as a glassy solid.

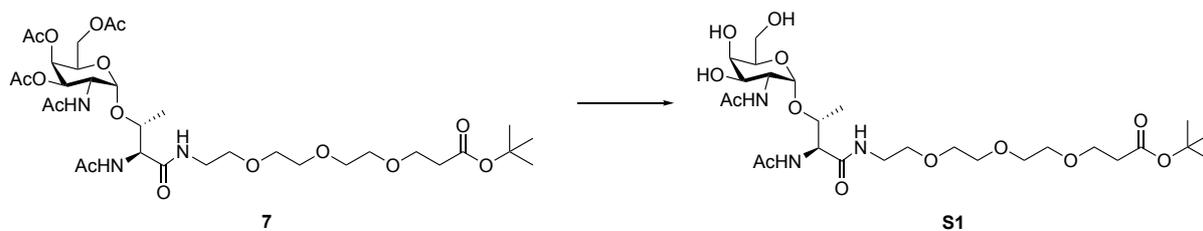
**TLC:**  $R_f$  0.40 (DCM); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.74 (d,  $J$  = 7.5 Hz, 2H, Ar-H Fmoc), 7.65–7.59 (m, 3H, Ar-H Fmoc, NH, NHCO [Thr-TEG]), 7.42–7.34 (m, 2H, Ar-H Fmoc), 7.33–7.26 (m, 2H, Ar-H Fmoc), 6.77 (dd,  $J$  = 9.7, 3.0 Hz, 1H, NH, NHAc), 6.28 (d,  $J$  = 8.8 Hz, 1H, NH, NHFmoc), 5.35 (d,  $J$  = 2.2 Hz, 1H, H-4), 5.05 (dd,  $J$  = 11.3, 3.2 Hz, 1H, H-3), 4.86 (d,  $J$  = 3.6 Hz, 1H, H-1), 4.53–4.44 (m, 2H, H-2, CHHa Fmoc), 4.41–4.33 (m, 1H, CHHb Fmoc), 4.30–4.12 (m, 4H, CH Fmoc, CH $\alpha$  NHFmoc, H-5, CH $\alpha$  CH<sub>3</sub> [Thr]), 4.11–3.98 (m, 2H, H-6a,b), 3.65 (t,  $J$  = 6.4 Hz, 2H, CH<sub>2(y)</sub> TEG), 3.61–3.52 (m, 8H, 4  $\times$  CH<sub>2(z)</sub> TEG), 3.52–3.42 (m, 2H, CH<sub>2(y')</sub> TEG), 3.42–3.36 (m, 2H, CH<sub>2(x')</sub> TEG), 2.45 (t,  $J$  = 6.4 Hz, 2H, CH<sub>2(x)</sub> TEG), 2.13 (s, 3H, CH<sub>3</sub> [AcO C-4]), 1.99 (s, 3H, CH<sub>3</sub> [AcO C-6]), 1.97 (s, 3H, CH<sub>3</sub> [NHAc C-2 GalNAc]), 1.96 (s, 3H, CH<sub>3</sub> [AcO C-3]), 1.40 (s, 9H, 3  $\times$  (CH<sub>3</sub>) [<sup>t</sup>Bu]), 1.25 (d,  $J$  = 6.3 Hz, 3H, CH<sub>3</sub> [Thr]). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta$  171.2 (CO<sub>2</sub><sup>t</sup>Bu), 171.1 (CONH [C-2 GalNAc]), 171.0 (CO [AcO C-3]), 170.62 (CO [AcO C-4]), 170.57 (CO [AcO C-6]), 170.5 (NHCO [Thr-TEG]), 157.0 (CO [Fmoc]), 143.8, 141.3 (C quatern [Fmoc]), 127.8, 127.2, 125.2, 125.1, 120.04, 120.02 (Ar Fmoc), 100.3 (C-1), 80.9 (C quatern [<sup>t</sup>Bu]), 78.2 (CH $\alpha$  CH<sub>3</sub> [Thr]), 70.5, 70.4, 70.2, 70.1 (4  $\times$  CH<sub>2(z)</sub> TEG), 69.5 (CH<sub>2(y')</sub> TEG), 68.9 (C-3), 67.5 (C-4), 67.23 (CH<sub>2</sub> Fmoc), 67.20 (C-5), 66.8 (CH<sub>2(y)</sub> TEG), 62.2 (C-6), 58.7 (CH $\alpha$  NHFmoc), 47.5 (C-2), 47.2 (CH Fmoc), 39.5 (CH<sub>2(x')</sub> TEG), 36.2 (CH<sub>2(x)</sub> TEG), 28.1 (3  $\times$  (CH<sub>3</sub>) [<sup>t</sup>Bu]), 22.9 (AcNH [C-2 GalNAc]), 20.8 (CH<sub>3</sub> [AcO C-3]), 20.7 (CH<sub>3</sub> [AcO C-4]), 20.6 (CH<sub>3</sub> [AcO C-6]), 18.1 (CH<sub>3</sub> [Thr]). **HRMS (MALDI)**  $m/z$ : Calcd for [C<sub>46</sub>H<sub>63</sub>N<sub>3</sub>O<sub>17</sub>Na]<sup>+</sup> [M+Na]<sup>+</sup> 952.4048, found 952.4033



### Protected AcHN-Tn(Thr)-TEG<sub>3</sub> *tert*-butyl ester (7).

Piperidine (240  $\mu$ L, 0.04 equiv) was added dropwise to a solution of Fmoc-protected Tn(Thr) moiety **6** (53.4 mg, 57.4  $\mu$ mol) in DMF (1.0 mL). After 45 min, TLC (EtOAc) revealed consumption of starting material and solvent was removed under vacuum. Upon further azeotropic drying with toluene, the crude residue was dissolved in pyridine (900  $\mu$ L) and acetic anhydride (300  $\mu$ L) was added dropwise. When 2 h later TLC (EtOAc) indicated full conversion of the intermediate, the reaction mixture was concentrated to dryness and further dried with toluene. Purification by silica gel column chromatography (DCM/methanol 97.5:2.5 to 96.5:3.5), afforded AcHN-Tn(Thr)-TEG<sub>3</sub> spacer **7** (40.2 mg, 93% yield) as a white solid.

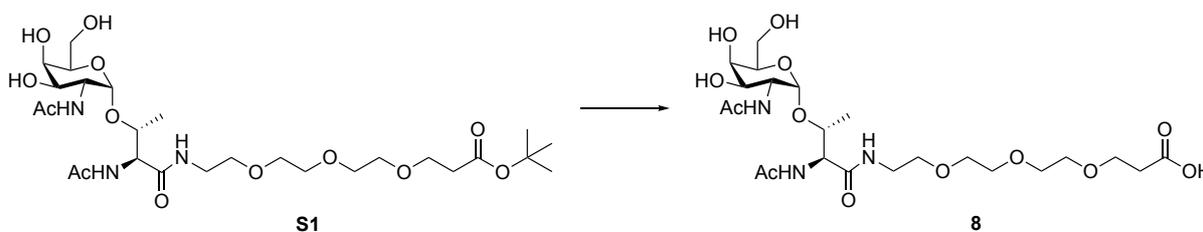
**TLC:**  $R_f$  0.75 (9:1 DCM/MeOH); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.32 (t,  $J$  = 5.4 Hz, 1H, NH, NHCO [Thr-TEG]), 6.66 (d,  $J$  = 8.9 Hz, 1H, NH, NHAc [Thr]), 6.59 (d,  $J$  = 9.5 Hz, 1H, NH, NHAc [C-2 GalNAc]), 5.34 (d,  $J$  = 3.6 Hz, 1H, H-4), 5.05 (dd,  $J$  = 11.3, 3.2 Hz, 1H, H-3), 4.88 (d,  $J$  = 3.6 Hz, 1H, H-1), 4.60 (dd,  $J$  = 9.1, 2.4 Hz, 1H, CH $\alpha$  NHAc [Thr]), 4.57 – 4.49 (m, 1H, H-2), 4.23 (t,  $J$  = 6.5 Hz, 1H, H-5), 4.16 (qd,  $J$  = 6.3, 2.3 Hz, 1H, CH $\alpha$  CH<sub>3</sub> [Thr]), 4.11 – 3.98 (m, 2H, H-6a,b), 3.69 (t,  $J$  = 6.3 Hz, 2H, CH<sub>2(y)</sub> TEG), 3.63–3.55 (m, 8H, 4  $\times$  CH<sub>2(z)</sub> TEG), 3.55–3.44 (m, 2H, CH<sub>2(y')</sub> TEG), 3.44–3.37 (m, 2H, CH<sub>2(x')</sub> TEG), 2.48 (t,  $J$  = 6.3 Hz, 2H, CH<sub>2(x)</sub> TEG), 2.13 (s, 3H, CH<sub>3</sub> [AcO C-4]), 2.09 (s, 3H, CH<sub>3</sub> [NHAc Thr]), 1.99 (s, 3H, CH<sub>3</sub> [AcO C-6]), 1.99 (s, 3H, CH<sub>3</sub> [NHAc C-2 GalNAc]), 1.96 (s, 3H, CH<sub>3</sub> [AcO C-3]), 1.42 (s, 9H, 3  $\times$  (CH<sub>3</sub>) [<sup>t</sup>Bu]), 1.25 (d,  $J$  = 6.3 Hz, 3H, CH<sub>3</sub> [Thr]). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta$  171.2 (CO<sub>2</sub><sup>t</sup>Bu), 171.0 (CO [AcO C-3]), 170.8 (CONH [NHAc Thr]), 170.78 (CONH [C-2 GalNAc]), 170.5 (CO [AcO C-4 & C-6]), 170.4 (NHCO [Thr-TEG]), 100.5 (C-1), 80.9 (C quatern [<sup>t</sup>Bu]), 78.4 (CH $\alpha$  CH<sub>3</sub> [Thr]), 70.6, 70.5, 70.4, 70.3 (4  $\times$  CH<sub>2(z)</sub> TEG), 69.5 (CH<sub>2(y')</sub> TEG), 69.1 (C-3), 67.5 (C-4), 67.3 (C-5), 66.9 (CH<sub>2(y)</sub> TEG), 62.3 (C-6), 56.6 (CH $\alpha$  NHAc [Thr]), 47.5 (C-2), 39.5 (CH<sub>2(x')</sub> TEG), 36.3 (CH<sub>2(x)</sub> TEG), 28.2 (3  $\times$  (CH<sub>3</sub>) [<sup>t</sup>Bu]), 23.3 (AcNH [Thr]), 23.1 (AcNH [C-2 Gal]), 20.88 (CH<sub>3</sub> [AcO C-3]), 20.86 (CH<sub>3</sub> [AcO C-4]), 20.7 (CH<sub>3</sub> [AcO C-6]), 18.3 (CH<sub>3</sub> [Thr]). **HRMS (MALDI)**  $m/z$ : Calcd for [C<sub>33</sub>H<sub>55</sub>N<sub>3</sub>O<sub>16</sub>Na]<sup>+</sup> [M+Na]<sup>+</sup> 772.3473, found 772.3416.



### AcHN-Tn(Thr)-TEG<sub>3</sub> *tert*-butyl ester (**S1**).

To a suspension of **7** (33 mg, 44  $\mu$ mol) in MeOH (1.0 mL), hydrazine (50  $\mu$ L) was added, resulting in a clear solution. The reaction was monitored by HPLC and after 90 min toluene was added. The mixture was concentrated and the residue was purified by silica gel chromatography using a Büchi Pure C-850 automated system (DCM/MeOH 90:10 to 82:18), providing the deacetylated Tn(Thr)-TEG<sub>3</sub> *tert*-butyl ester **S1** (37.2 mg, 84% yield) as a glassy solid.

**TLC:**  $R_f$  0.22 (9:1 DCM/MeOH); **<sup>1</sup>H NMR** (600 MHz, methanol-*d*<sub>4</sub>):  $\delta$  4.85 (d,  $J$  = 3.7 Hz, 1H, H-1), 4.52 (d,  $J$  = 2.6 Hz, 1H,  $CH\alpha$  NHAc [Thr]), 4.26–4.19 (m, 2H, H-2,  $CH\alpha$  CH<sub>3</sub> [Thr]), 3.91–3.86 (m, 2H, H-4, H-5), 3.75 (dd,  $J$  = 11.0, 3.2 Hz, 1H, H-3), 3.73–3.68 (m, 4H, H-6a,b,  $CH_{2(y)}$  TEG), 3.66–3.57 (m, 8H, 4  $\times$   $CH_{2(z)}$  TEG), 3.55 – 3.50 (m, 2H,  $CH_{2(y)}$  TEG), 3.50 – 3.45 (m, 1H,  $CHHa_{(x)}$  TEG), 3.31 – 3.27 (m, 1H,  $CHHb_{(x)}$  TEG), 2.48 (t,  $J$  = 6.2 Hz, 2H,  $CH_{2(x)}$  TEG), 2.09 (s, 3H, CH<sub>3</sub> [NHAc Thr]), 2.07 (s, 3H, CH<sub>3</sub> [NHAc C-2 GalNAc]), 1.45 (s, 9H, 3  $\times$  (CH<sub>3</sub>) [<sup>t</sup>Bu]), 1.27 (d,  $J$  = 6.4 Hz, 3H, CH<sub>3</sub> [Thr]). **<sup>13</sup>C NMR** (151 MHz, methanol-*d*<sub>4</sub>):  $\delta$  174.1 (CONH [C-2 GalNAc]), 173.6 (CONH [NHAc Thr]), 172.8 (CO<sub>2</sub><sup>t</sup>Bu), 172.3 (NHCO [Thr-TEG]), 101.0 (C-1), 81.7 (C quatern [<sup>t</sup>Bu]), 77.7 ( $CH\alpha$  CH<sub>3</sub> [Thr]), 72.9 (C-5), 71.53, 71.47, 71.4, 71.3 (4  $\times$   $CH_{2(z)}$  TEG), 70.40 (C-3), 70.38 ( $CH_{2(y)}$  TEG), 70.26 (C-4), 67.9 ( $CH_{2(y)}$  TEG), 62.7 (C-6), 58.4 ( $CH\alpha$  NHAc [Thr]), 51.4 (C-2), 40.5 ( $CH_{2(x)}$  TEG), 37.2 ( $CH_{2(x)}$  TEG), 28.4 (3  $\times$  (CH<sub>3</sub>) [<sup>t</sup>Bu]), 23.2 (AcNH [C-2 Gal]), 22.5 (AcNH [Thr]), 19.2 (CH<sub>3</sub> [Thr]). **HRMS (ESI)**  $m/z$ : Calcd for [C<sub>27</sub>H<sub>49</sub>N<sub>3</sub>O<sub>13</sub>Na]<sup>+</sup> [M+Na]<sup>+</sup> 646.3154, found 646.3128.



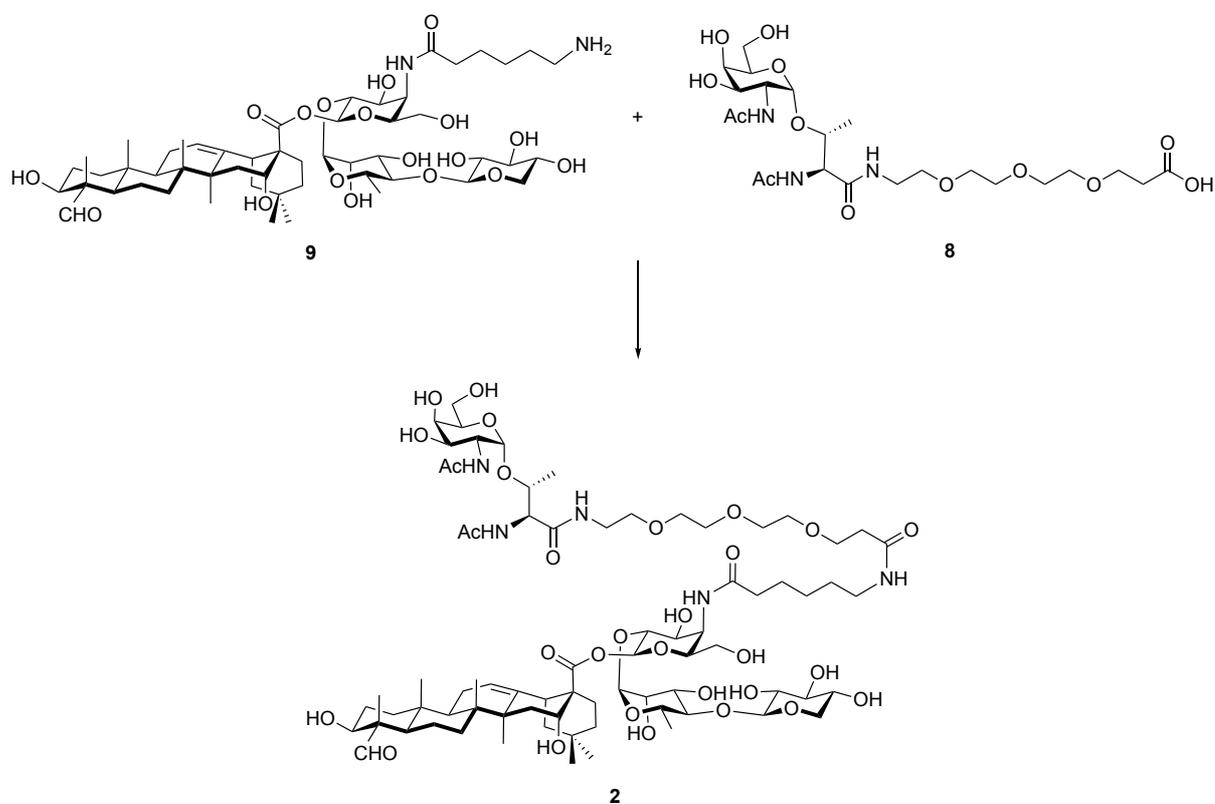
### AcHN-Tn(Thr)-TEG<sub>3</sub> acid (**8**).

*Tert*-butyl ester **S1** (26.5 mg, 42  $\mu$ mol) at 0 °C was dissolved in a precooled (0 °C) solution of TFA (0.8 mL). The reaction mixture was stirred at this temperature and its progress was monitored by HPLC. After 1 h, DCM was added and the contents were evaporated to dryness. The crude was purified by reverse phase (RP) chromatography using a Büchi Pure C-850 automated system [0–100% acetonitrile/water (0.05% TFA)], affording the deprotected Tn(Thr)-TEG<sub>3</sub> carboxylic acid **8** (20.5 mg, 86% yield) as a white solid.

**HPLC:**  $t_R$  = 13.56 min,  $\lambda_{max}$  = 194.52 nm; **<sup>1</sup>H NMR** (600 MHz, methanol-*d*<sub>4</sub>):  $\delta$  4.85 (d,  $J$  = 3.8 Hz, 1H, H-1), 4.52 (d,  $J$  = 2.6 Hz, 1H,  $CH\alpha$  NHAc [Thr]), 4.26 – 4.20 (m, 2H, H-2,  $CH\alpha$  CH<sub>3</sub> [Thr]), 3.91 – 3.87 (m, 2H, H-4, H-5), 3.77 – 3.70 (m, 5H, H-3, H-6a, H-6b,  $CH_{2(y)}$  TEG), 3.65 – 3.58 (m, 8H, 4  $\times$   $CH_{2(z)}$  TEG), 3.54 – 3.50 (m, 2H,  $CH_{2(y)}$  TEG), 3.50 – 3.45 (m, 1H,  $CHHa_{(x)}$

TEG), 3.32 – 3.28 (m, 1H, CHH<sub>b(x)</sub> TEG), 2.58 – 2.54 (m, 2H, CH<sub>2(x)</sub> TEG), 2.09 (s, 3H, CH<sub>3</sub> [NHAc Thr]), 2.07 (s, 3H, CH<sub>3</sub> [NHAc C-2 GalNAc]), 1.27 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub> [Thr]). <sup>13</sup>C NMR (151 MHz, methanol-*d*<sub>4</sub>): δ 175.4 (CO<sub>2</sub>H), 174.2 (CONH [C-2 GalNAc]), 173.7 (CONH [NHAc Thr]), 172.4 (NHCO [Thr-TEG]), 101.0 (C-1), 77.7 (CH<sub>α</sub> CH<sub>3</sub> [Thr]), 72.9 (C-5), 71.54, 71.45, 71.4, 71.3 (4 × CH<sub>2(z)</sub> TEG), 70.42 (C-3), 70.40 (CH<sub>2(y')</sub> TEG), 70.3 (C-4), 67.8 (CH<sub>2(y)</sub> TEG), 62.7 (C-6), 58.5 (CH<sub>α</sub> NHAc [Thr]), 51.4 (C-2), 40.5 (CH<sub>2(x)</sub> TEG), 35.8 (CH<sub>2(x)</sub> TEG), 23.2 (AcNH [C-2 Gal]), 22.5 (AcNH [Thr]), 19.2 (CH<sub>3</sub> [Thr]). **HRMS (ESI)** *m/z*: Calcd for [C<sub>23</sub>H<sub>41</sub>N<sub>3</sub>O<sub>13</sub>Na]<sup>+</sup> [M+Na]<sup>+</sup> 590.2485, found 590.2528.

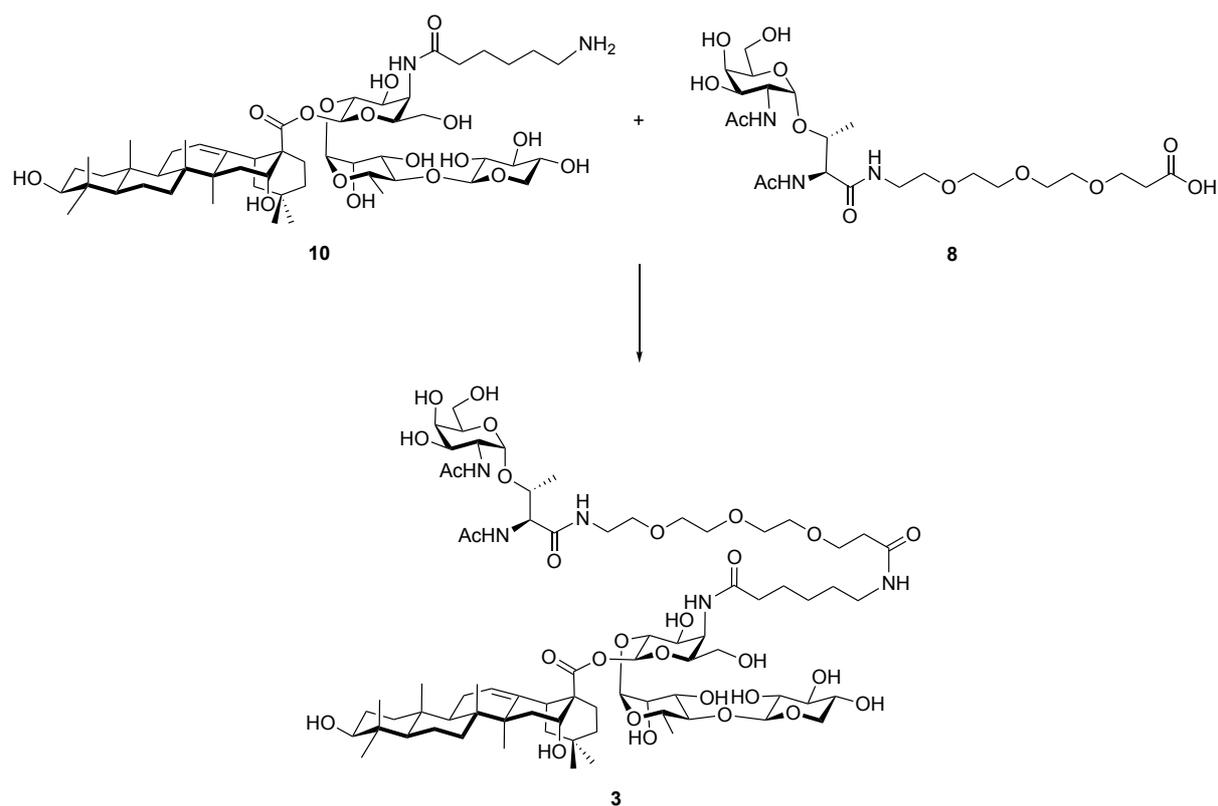
## B. SYNTHESIS OF SAPONIN-Tn GLYCOCONJUGATES



### Quillaic acid saponin-Tn conjugate [QA-Tn] (**2**).

To a stirred solution of Tn(Thr)-spacer acid **8** (3.9 mg, 6.9 μmol, 1.5 equiv), benzotriazol-1-yl-oxy]tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (3.8 mg, 7.3 μmol, 1.6 equiv) and quillaic acid saponin amine **9**<sup>[3]</sup> (4.8 mg, 4.6 μmol, 1.0 equiv) in DMF (1.6 mL), diisopropylethylamine (DIPEA) (2.4 μL, 13.8 μmol, 3.0 equiv) was added dropwise. The reaction mixture was stirred at r.t. for 1 h and then concentrated under high vacuum. The resulting residue was dissolved in 50% acetonitrile/water, filtered through 0.2 μm PTFE filter disk and purified by RP-HPLC on a XBridge prep BEH300 C18 column (5 μm, 19 × 250 mm) using a linear gradient of 20–100% acetonitrile/water (0.05% TFA) over 30 min at a flow rate of 17 mL/min. The fraction containing the major peak was collected and lyophilized to dryness, providing the desired quillaic acid saponin-Tn conjugate **2** (6.2 mg, 85% yield) as a white solid.

**HPLC:**  $t_R = 22.56$  min,  $\lambda_{max} = 193.5$  nm.  **$^1H$  NMR** (600 MHz, methanol- $d_4$ ):  $\delta$  9.31 (s, 1H, H-23 [CHO] QA), 8.16 (t,  $J = 5.6$  Hz, 1H, NH [CONH Thr–TEG]), 8.10 (d,  $J = 9.1$  Hz, 1H, NH [NHAc Thr]), 7.97 (d,  $J = 9.2$  Hz, 1H, NH [CONH C-4 N-Gal]), 7.44 (d,  $J = 9.2$  Hz, 1H, NH [NHAc C-2 GalNAc]), 5.39 (d,  $J = 1.8$  Hz, 1H, H-1 Rha), 5.34 (d,  $J = 7.8$  Hz, 1H, H-1 N-Gal), 5.31 (t,  $J = 3.7$  Hz, 1H, H-12 QA), 4.86 – 4.83 (m, 1H, H-1 GalNAc), 4.56–4.51 (m, 1H,  $CH\alpha$  NHAc [Thr]), 4.51 – 4.47 (m, 2H, including 4.50 [d,  $J = 7.6$  Hz, 1H, H-1 Xyl], & H-16 QA), 4.37 – 4.32 (m, 1H, H-4 N-Gal), 4.27 – 4.19 (m, 2H, H-2 GalNAc,  $CH\alpha$   $CH_3$  [Thr]), 3.97 – 3.87 (m, 5H, H-3 & H-2 N-Gal, H-2 Rha, H-4 & H-5 GalNAc), 3.87 – 3.78 (m, 3H, H-3 & H-5 Rha, H-5a Xyl), 3.78 – 3.68 (m, 7H, H-3 QA, H-6a,b & H-3 GalNAc,  $CH_{2(y)}$  TEG, H-5 N-Gal), 3.65 – 3.58 (m, 8H,  $4 \times CH_{2(z)}$  TEG), 3.58 – 3.45 (m, 6H, H-6a N-Gal,  $CHHa_{(x)}$  &  $CH_{2(y)}$  TEG, H-4 Xyl, H-4 Rha), 3.45 – 3.40 (m, 1H, H-6b N-Gal), 3.35 – 3.27 (m, 2H,  $CHHb_{(x)}$  TEG, H-3 Xyl), 3.24 – 3.16 (m, 4H,  $CH_{2(a)}$ NHCO acyl, H-5b & H-2 Xyl), 2.94 (dd,  $J = 14.3, 4.6$  Hz, 1H, H-18 QA), 2.44 (t,  $J = 6.2$  Hz, 2H,  $CH_{2(x)}$  TEG), 2.37 – 2.28 (m, 3H,  $CH_{2(a)}$ CONH acyl, H-19a QA), 2.09 (s, 3H,  $CH_3$  [NHAc Gal]), 2.07 (s, 3H,  $CH_3$  [NHAc Thr]), 2.00 – 1.89 (m, 4H, H-11a,b, H-22a & H-21a QA), 1.83 – 1.62 (m, 8H,  $CH_{2(b)}$  $CH_2$ CONH acyl, H-22b, H-9, H-1a, H-2a,b & H-15a QA), 1.60 – 1.43 (m, 5H,  $CH_{2(b)}$  $CH_2$ NHCO acyl, H-6a, H-7a & H-15b QA), 1.43 – 1.30 (m, 10H,  $CH_{2(c)}$  $CH_2CH_2$ CONH acyl, H-7b & H-5 QA), [1.41 s,  $CH_3$  C-27 QA], [1.33 d,  $J = 6.3$  Hz, 3H,  $CH_3$  Rha], 1.27 (d,  $J = 6.4$  Hz, 3H,  $CH_3$  Thr), 1.20 – 1.09 (m, 2H, H-21b & H-1b QA), 1.09 – 1.03 (m, 1H, H-19b QA), 1.02 (s, 3H,  $CH_3$  C-24 QA), 1.01 (s, 3H,  $CH_3$  C-25 QA), 0.95 (s, 3H,  $CH_3$  C-30 QA), 0.94 – 0.90 (m, 1H, H-6b), 0.88 (s, 3H,  $CH_3$  C-29 QA), 0.77 (s, 3H,  $CH_3$  C-26 QA).  **$^{13}C$  NMR** (151 MHz, methanol- $d_4$ ):  $\delta$  208.8 (CHO QA), 178.2 (CONH acyl [C-4 N-Gal]), 176.9 (CO [C-28] QA), 174.2 (CONH [C-2 GalNAc]), 173.8 (CONH [TEG–acyl]), 173.7 (CONH [NHAc Thr]), 172.4 (NHCO [Thr–TEG]), 144.8 (C-13 QA), 123.2 (C-12 QA), 107.0 (C-1 Xyl), 101.3 (C-1 Rha), 101.0 (C-1 GalNAc), 95.6 (C-1 N-Gal), 84.1 (C-4 Rha), 78.2 (C-3 Xyl), 77.7 ( $CH\alpha$   $CH_3$  [Thr]), 76.4 (C-5 N-Gal), 76.2 (C-2 Xyl), 74.9 (C-3 N-Gal), 74.6 (C-16 QA), 74.4 (C-2 N-Gal), 72.94 (C-5 GalNAc), 72.87 (C-3 QA), 72.2 (C-3 Rha), 71.9 (C-2 Rha), 71.54, 71.46, 71.32, 71.30 ( $4 \times CH_{2(z)}$  TEG), 71.1 (C-4 Xyl), 70.4 (C-3 GalNAc &  $CH_{2(y)}$  TEG), 70.3 (C-4 GalNAc), 68.9 (C-5 Rha), 68.3 ( $CH_{2(y)}$  TEG), 67.3 (C-5 Xyl), 62.7 (C-6 GalNAc), 61.7 (C-6 N-Gal), 58.5 ( $CH\alpha$  NHAc [Thr]), 56.8 (C-4 QA), 52.5 (C-4 N-Gal), 51.4 (C-2 GalNAc), 50.0 (C-17 QA), 48.9 (C-5 QA), 48.05 (C-19 QA), 48.02 (C-9 QA), 42.8 (C-14 QA), 42.4 (C-18 QA), 41.1 (C-8 QA), 40.5 ( $CH_{2(x)}$  TEG), 40.3 ( $CH_{2(a)}$ NHCO acyl), 39.5 (C-1 QA), 37.7 ( $CH_{2(x)}$  TEG), 37.0 (C-10 QA), 36.6 ( $CH_{2(a)}$ CONH acyl), 36.54 (C-21 QA), 36.53 (C-15 QA), 33.6 (C-7 QA), 33.4 ( $CH_3$  C-29 QA), 32.0 (C-22 QA), 31.3 (C-20 QA), 30.1 ( $CH_{2(b)}$  $CH_2$ NHCO acyl), 27.5 ( $CH_{2(c)}$  $CH_2CH_2$ CONH acyl), 27.2 ( $CH_3$  C-27 QA), 27.0 (C-2 QA), 26.8 ( $CH_{2(b)}$  $CH_2$ CONH acyl), 24.9 ( $CH_3$  C-30 QA), 24.5 (C-11 QA), 23.2 ( $CH_3$  [NHAc, Thr]), 22.6 ( $CH_3$  [NHAc, Gal]), 21.9 (C-6 QA), 19.2 ( $CH_3$  Thr), 18.4 ( $CH_3$  Rha), 17.8 ( $CH_3$  C-26 QA), 16.3 ( $CH_3$  C-25 QA), 9.5 ( $CH_3$  C-24 QA). **HRMS (MALDI)**  $m/z$ : Calcd for  $[C_{76}H_{125}N_5O_{30}Na]^+$   $[M+Na]^+$  1610.8300, found 1610.8349.



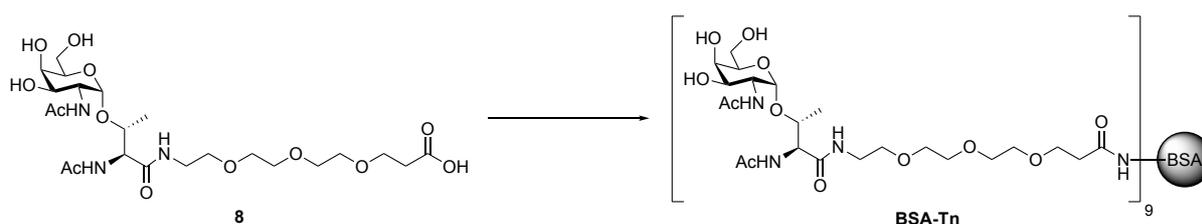
### Echinocystic acid saponin–Tn conjugate [EA–Tn] (3).

To a stirred solution of Tn(Thr) acid **8** (5.5 mg, 9.7  $\mu\text{mol}$ , 1.3 equiv), benzotriazol-1-yl-oxy]tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (5.85 mg, 11.2  $\mu\text{mol}$ , 1.5 equiv) and echinocystic acid saponin amine **10**<sup>[3]</sup> (7.69 mg, 7.5  $\mu\text{mol}$ , 1.0 equiv) in DMF (2.6 mL), diisopropylethylamine (DIPEA) (3.0  $\mu\text{L}$ , 17.7  $\mu\text{mol}$ , 2.3 equiv) was added dropwise. The reaction mixture was stirred at r.t. for 1 h and then concentrated under high vacuum. The resulting residue was dissolved in 50% acetonitrile/water, filtered through 0.2  $\mu\text{m}$  PTFE filter disk and purified by RP-HPLC on a XBridge prep BEH300 C18 column (5  $\mu\text{m}$ , 19  $\times$  150 mm) using a linear gradient of 20–100% acetonitrile/water (0.05% TFA) over 30 min at a flow rate of 17 mL/min. The fraction containing the major peak was collected and lyophilized to dryness, providing the desired echinocystic acid saponin–Tn conjugate **3** (10.0 mg, 84% yield) as a white solid.

**HPLC:**  $t_{\text{R}}$  = 20.12 min,  $\lambda_{\text{max}}$  = 194.5 nm. **<sup>1</sup>H NMR** (600 MHz, methanol-*d*<sub>4</sub>):  $\delta$  5.39 (d,  $J$  = 1.8 Hz, 1H, H-1 Rha), 5.35 (d,  $J$  = 7.8 Hz, 1H, H-1 N-Gal), 5.31 – 5.29 (m, 1H, H-12 EA), 4.86 – 4.85 (m, 1H, H-1 GalNAc), 4.53 (d,  $J$  = 2.6 Hz, 1H,  $\text{CH}\alpha$  NHAc [Thr]), 4.51 – 4.47 (m, 2H, including 4.49 [d,  $J$  = 7.6 Hz, 1H, H-1 Xyl], & H-16 EA), 4.34 (dd,  $J$  = 4.7, 1.6 Hz, 1H, H-4 N-Gal), 4.27 – 4.20 (m, 2H, H-2 GalNAc,  $\text{CH}\alpha$  CH<sub>3</sub> [Thr]), 3.97 – 3.78 (m, 8H, H-3 & H-2 NGal, H-2, H-3 & H-5 Rha, H-4 & H-5 GalNAc, H-5a Xyl), 3.77 – 3.68 (m, 6H, H-6a,b & H-3 GalNAc,  $\text{CH}_{2(y)}$  TEG, H-5 N-Gal), 3.67 – 3.56 (m, 9H, 4  $\times$   $\text{CH}_{2(z)}$  TEG, H-4 Rha), 3.55 – 3.45 (m, 5H H-6a N-Gal, H-4 Xyl,  $\text{CH}_{2(y)}$  &  $\text{CHH}\alpha(x)$  TEG), 3.45 – 3.40 (m, 1H, H-6b N-Gal), 3.36 – 3.28 (m, 2H,  $\text{CHH}\beta(x)$  TEG, H-3 Xyl), 3.28 – 3.22 (m, 1H, H-2 Xyl), 3.22 – 3.13 (m, 4H,  $\text{CH}_{2(a)}$  NHCO acyl, H-5b Xyl, H-3 EA), 2.93 (dd,  $J$  = 14.3, 4.5 Hz, 1H, H-18 EA), 2.47 – 2.42 (m, 2H,  $\text{CH}_{2(x)}$  TEG), 2.37 – 2.27 (m, 3H,  $\text{CH}_{2(a)}$  CONH acyl, H-19a EA), 2.09 (s, 3H, CH<sub>3</sub> [NHAc Gal]), 2.07 (s, 3H, CH<sub>3</sub> [NHAc Thr]), 2.00 – 1.86 (m, 4H, H-11a,b, H-22a, H-21a EA), 1.79 (td,  $J$  = 14.4, 13.6, 5.2 Hz, 1H, H-22b EA), 1.71 – 1.62 (m, 6H,  $\text{CH}_{2(b)}$  CH<sub>2</sub> CONH acyl,

H-15a, H-2a, H-9 & H-1a EA), 1.62 – 1.41 (m, 7H,  $\text{CH}_{2(b)}$ CH<sub>2</sub>NHCO acyl, H-6a, H-2b, H-7a,b & H-15b EA), 1.41 – 1.37 (m, 6H, H-6b EA,  $\text{CH}_{2(c)}$ CH<sub>2</sub>CH<sub>2</sub>CONH acyl, [1.38 s, CH<sub>3</sub> C-27 EA]), 1.34 (d,  $J = 6.2$  Hz, 3H, CH<sub>3</sub> Rha), 1.27 (d,  $J = 6.4$  Hz, 3H, CH<sub>3</sub> Thr), 1.20 – 1.14 (m, 1H, H-21b EA), 1.08 – 1.00 (m, 2H, H-19b, H-1b EA), 0.99 (s, 3H, CH<sub>3</sub> C-23 EA), 0.96 (s, 3H, CH<sub>3</sub> C-25 EA), 0.95 (s, 3H, CH<sub>3</sub> C-30 EA), 0.88 (s, 3H, CH<sub>3</sub> C-29 EA), 0.79 (s, 3H, CH<sub>3</sub> C-24 EA), 0.77 (s, 3H, CH<sub>3</sub> C-26 EA), 0.75 (d,  $J = 12.3$  Hz, 1H, H-5 EA). <sup>13</sup>C NMR (151 MHz, methanol-*d*<sub>4</sub>):  $\delta$  178.2 (CONH acyl [C-4 N-Gal]), 177.0 (CO [C-28] EA), 174.2 (CONH [C-2 GalNAc]), 173.8 (CONH [TEG-acyl]), 173.7 (CONH [NHAc Thr]), 172.4 (NHCO [Thr-TEG]), 144.7 (C-13 EA), 123.5 (C-12 EA), 107.1 (C-1 Xyl), 101.3 (C-1 Rha), 101.0 (C-1 GalNAc), 95.6 (C-1 N-Gal), 84.2 (C-4 Rha), 79.8 (C-3 EA), 78.2 (C-3 Xyl), 77.7 (CH $\alpha$  CH<sub>3</sub> [Thr]), 76.3 (C-5 N-Gal), 76.2 (C-2 Xyl), 74.9 (C-3 N-Gal), 74.7 (C-16 EA), 74.5 (C-2 N-Gal), 72.9 (C-5 GalNAc), 72.2 (C-3 Rha), 71.9 (C-2 Rha), 71.53, 71.46, 71.30, 71.29 (4  $\times$  CH<sub>2(z)</sub> TEG), 71.1 (C-4 Xyl), 70.41 (CH<sub>2(y)</sub> TEG), 70.39 (C-4 GalNAc), 70.3 (C-3 GalNAc), 68.9 (C-5 Rha), 68.3 (CH<sub>2(y)</sub> TEG), 67.3 (C-5 Xyl), 62.7 (C-6 GalNAc), 61.7 (C-6 N-Gal), 58.5 (CH $\alpha$  NHAc [Thr]), 56.9 (C-5 EA), 52.5 (C-4 N-Gal), 51.4 (C-2 GalNAc), 50.1 (C-17 EA), 48.15 (C-9 EA), 48.05 (C-19 EA), 42.7 (C-14 EA), 42.4 (C-18 EA), 40.8 (C-8 EA), 40.5 (CH<sub>2(x)</sub> TEG), 40.3 (CH<sub>2(a)</sub>NHCO acyl), 40.0 (C-1 EA), 39.9 (C-4 EA), 38.2 (C-10 EA), 37.7 (CH<sub>2(x)</sub> TEG), 36.6 (CH<sub>2(a)</sub>CONH acyl, C-21 & C-15 EA), 34.3 (C-7 EA), 33.4 (CH<sub>3</sub> C-29 EA), 32.0 (C-22 EA), 31.3 (C-20 EA), 30.1 (CH<sub>2(b)</sub>CH<sub>2</sub>NHCO acyl), 28.8 (CH<sub>3</sub> C-23 EA), 27.9 (C-2 EA), 27.5 (CH<sub>2(c)</sub>CH<sub>2</sub>CH<sub>2</sub>CONH acyl), 27.2 (CH<sub>3</sub> C-27 EA), 26.8 (CH<sub>2(b)</sub>CH<sub>2</sub>CONH acyl), 24.9 (CH<sub>3</sub> C-30 EA), 24.5 (C-11 EA), 23.2 (CH<sub>3</sub> [NHAc, Thr]), 22.6 (CH<sub>3</sub> [NHAc, Gal]), 19.6 (C-6 EA), 19.2 (CH<sub>3</sub> Thr), 18.4 (CH<sub>3</sub> Rha), 17.8 (CH<sub>3</sub> C-26 EA), 16.4 (CH<sub>3</sub> C-24 EA), 16.2 (CH<sub>3</sub> C-25 EA). **HRMS (MALDI)  $m/z$ :** Calcd for [C<sub>76</sub>H<sub>127</sub>N<sub>5</sub>O<sub>29</sub>Na]<sup>+</sup> [M+Na]<sup>+</sup> 1596,8505, found 1596,8461.

### C. SYNTHESIS OF BSA-Tn CONJUGATE



#### **BSA-Tn conjugate.**

Spacer-containing Tn antigen **8** (2.8 mg, 5.05  $\mu\text{mol}$ ), *N*-hydroxysuccinimide (NHS) (2.3 mg, 20.24  $\mu\text{mol}$ , 4.0 equiv) and dicyclohexylcarbodiimide (DCC) (2.08 mg, 10.1  $\mu\text{mol}$ , 2.0 equiv) were dissolved in DMF (0.4 mL). After stirring at r.t. for 15 h, the reaction mixture was concentrated and dried under vacuum. The residue was redissolved in DMF (50  $\mu\text{L}$ ) and a solution of BSA (8.83 mg, 0.13  $\mu\text{mol}$ , 0.025 equiv) in PBS (0.4 mL). The resulting mixture was stirred at r.t. for 48 h and then diluted with milliQ water containing 0.05% TFA (0.8 mL). Purification of the contents was carried out by RP-HPLC on a XBridge prep BEH300 C18 column (5  $\mu\text{m}$ , 19  $\times$  150 mm) using a linear gradient of 20–100% acetonitrile/water (0.05% TFA) over 30 min at a flow rate of 17 mL/min. The fraction containing the major peak was

collected and lyophilized to dryness to afford the BSA–Tn conjugate [**BSA–(Tn)<sub>9</sub>**] (6.67 mg, 65% yield based on recovered starting material) as a white solid. Subsequent MALDI analysis revealed a loading of ~9 copies of Tn moiety per BSA. **HRMS (MALDI) *m/z***: found 71214,208.

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#### **IV. IMMUNOLOGICAL EVALUATION**

**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 CIC bioGUNE guidelines. Mice were housed in standard cages with an automatic water system and fed on a standard diet *ad libitum*. Body weights were measured before and after immunization procedures and cage side observations were made daily. Mice were euthanized by CO<sub>2</sub> inhalation at the end of the study. 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 the vaccine glycoconjugate constructs (60 µg/mouse) in phosphate-buffered saline (PBS, 100 µl). An additional negative control group was vaccinated in a similar way with PBS. To analyze the antibody responses over time, mice were bled via submandibular vein at the indicated post-vaccination time point (day 21), and by cardiac puncture at the experimental endpoint (day 42). Blood was collected in BD Microtainer® tubes (Clot Activator/SST™ Gel) and centrifuged at 13000g for 10 min. Sera was harvested and stored at -20 °C until further analysis.

**Evaluation of immune response.** Analysis of the produced plasma antibodies specific against the Tn antigen were performed by an indirect enzyme-linked immunosorbent assay (ELISA). Briefly, Nunc MaxiSorp™ ELISA plates (Thermo Scientific) were coated with BSA-Tn conjugate 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 1 h at room temperature. After wash, goat anti-mouse total IgG, IgM (Jackson ImmunoResearch) or subclass-specific IgG1, IgG2b, IgG2c and IgG3 (SouthernBiotech) antibodies conjugated to horseradish peroxidase (HRP) were added to each well. IgG, IgM and IgG3 were diluted 1/1000, whereas IgG1 and IgG2b were added at 1/4000 and IgG2c at 1/10000 dilution, all in blocking buffer. After 1 h incubation at room temperature, KPL SureBlue reserve™ commercial solution (100 µl/well, SeraCare) containing 3,3',5,5'-tetramethylbenzidine (TMB) was added as peroxidase substrate. The reaction was stopped after 10 min incubation by adding 2 N H<sub>2</sub>SO<sub>4</sub> (100 µl/well). For absorbance measurements, optical density (OD) at 450 nm was immediately determined using a BioTek® Synergy HT multi-detection microplate reader.

**Toxicity assessment in mice.** As initial standard assessment of the potential toxicity of the constructs, in addition to visual inspection for potential signs of discomfort, animal weight was monitored before and after each immunization. The median percentage weight change in mice at 0-, 24-, 48- and 72-hours post-injections was determined to assess toxicity.

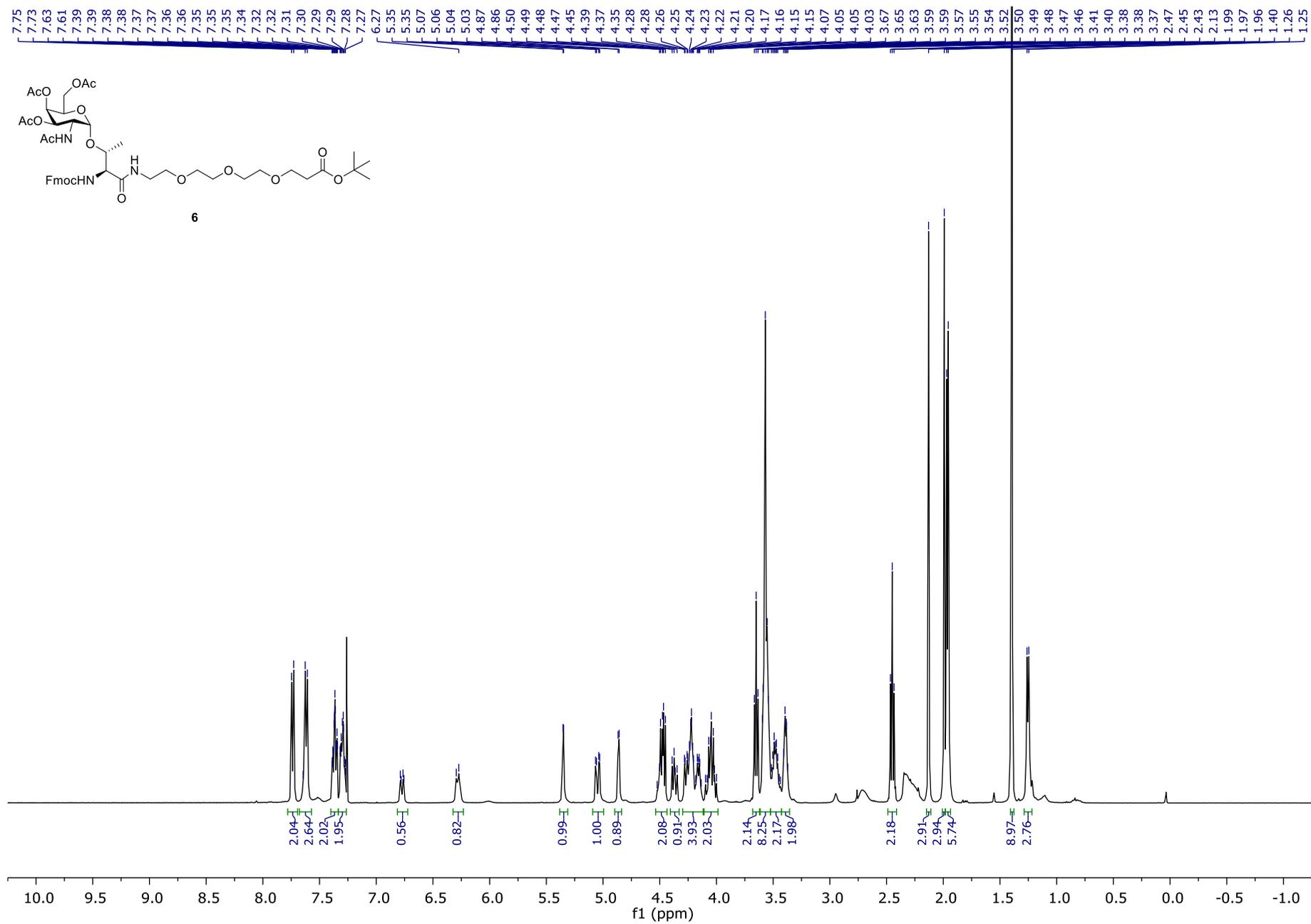
**Statistics.** Antibody levels data are presented as median of five mice. The statistical significance of the antibody response for each of the experimental groups compared to the PBS control was assessed using a two-tailed unpaired Student's t-test with a 95% confidence interval (CI) (GraphPad Prism, GraphPad Software, La Jolla, CA). *P* values of less than 0.05 were considered statistically significant.

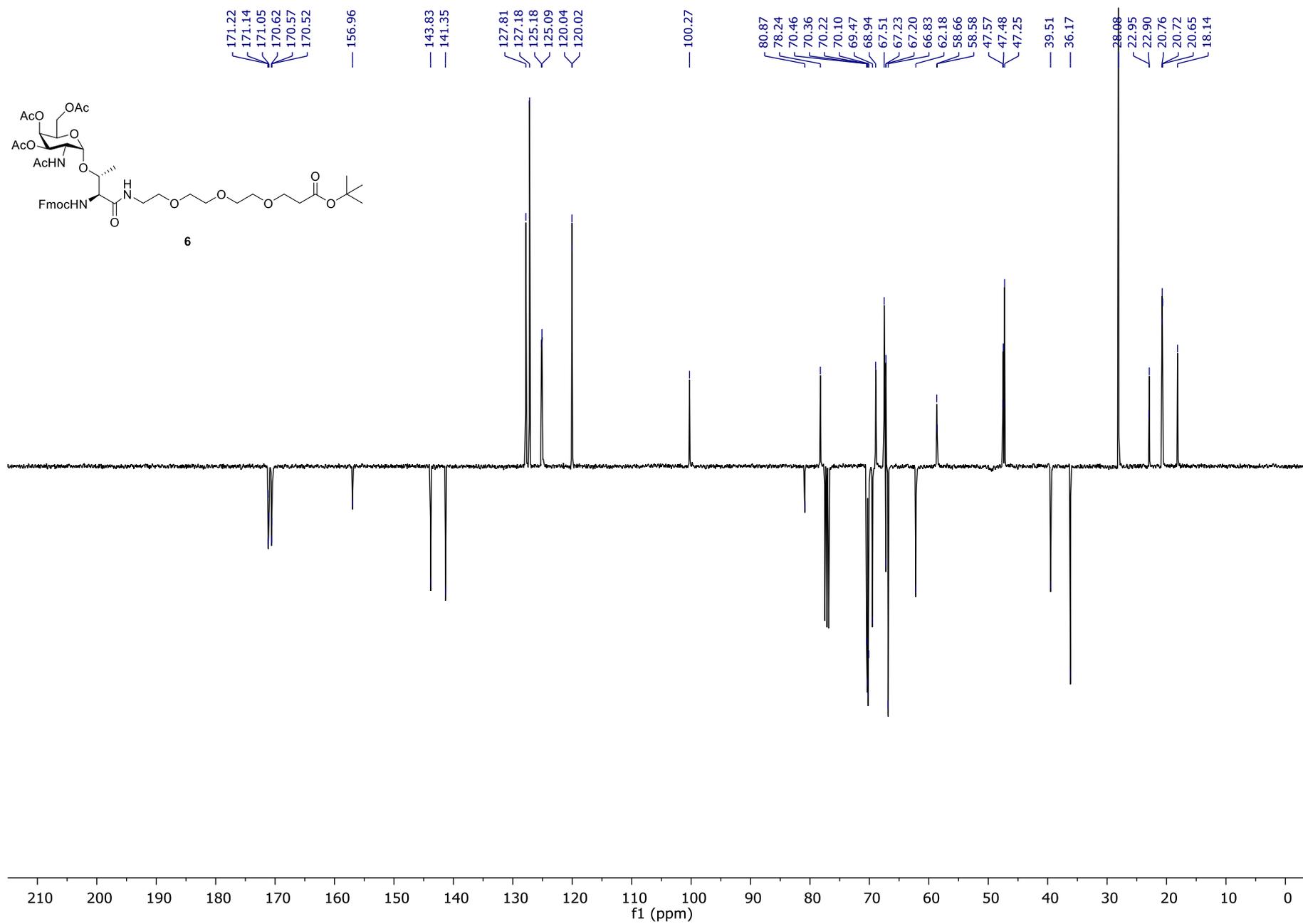
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**V. NMR CHARACTERIZATION: <sup>1</sup>H, APT <sup>13</sup>C, COSY, HSQC SPECTRA**

**A. SYNTHESIS OF SPACER-CONTAINING TN ANTIGEN**

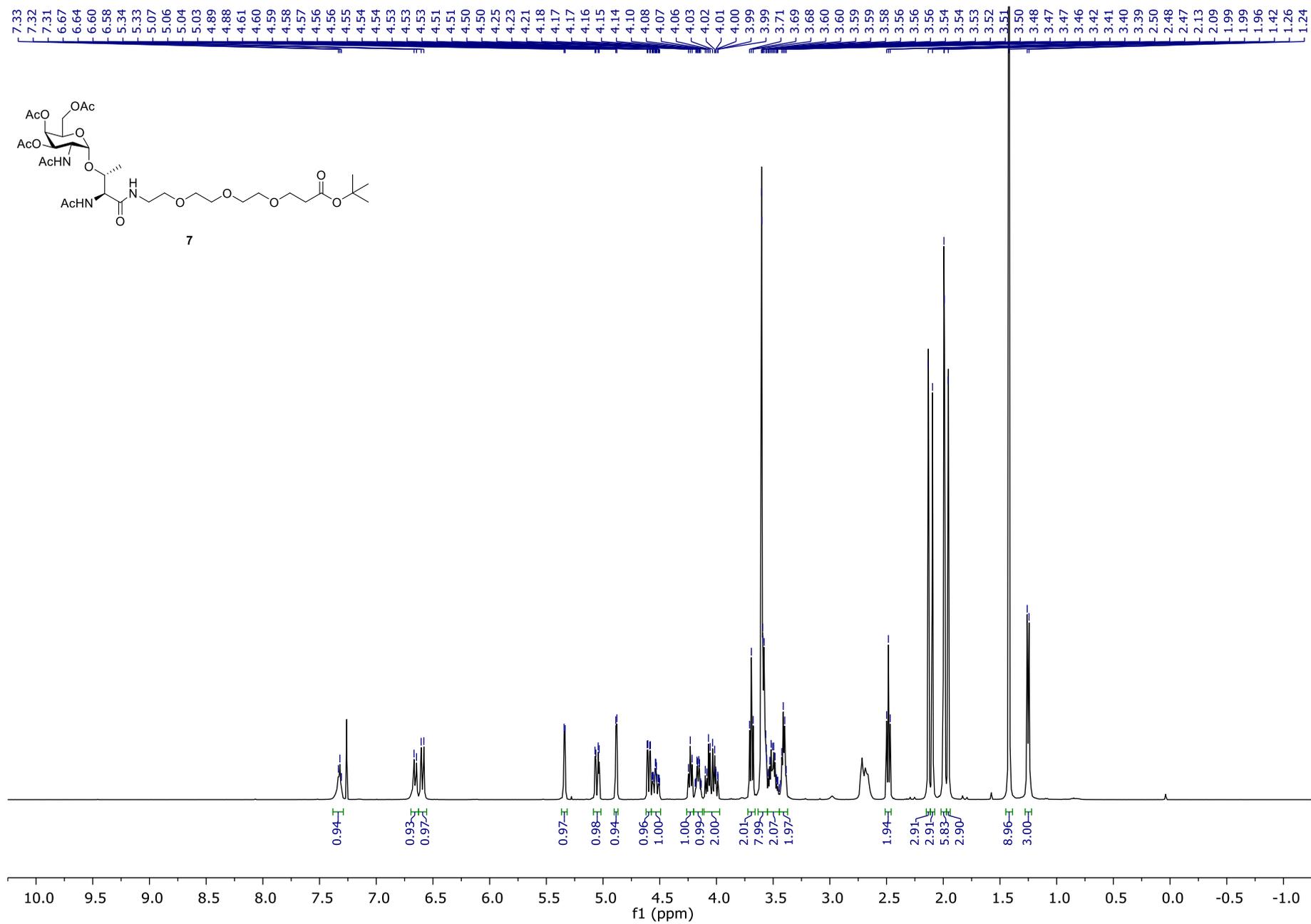
**B. SYNTHESIS OF SAPONIN- TN GLYCOCONJUGATES**

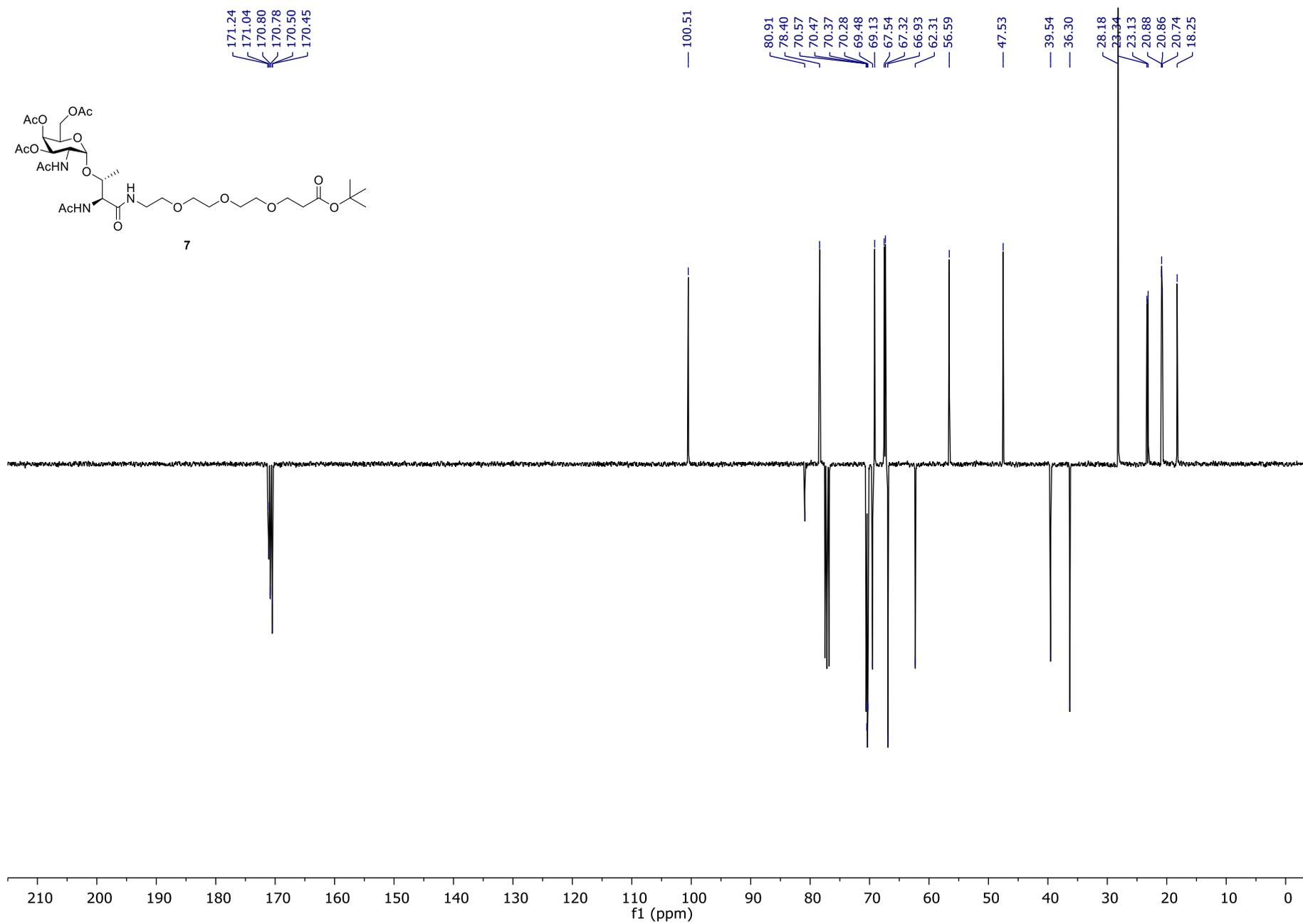
**6**  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )

**6** APT  $^{13}\text{C}$ -NMR (101 MHz,  $\text{CDCl}_3$ )





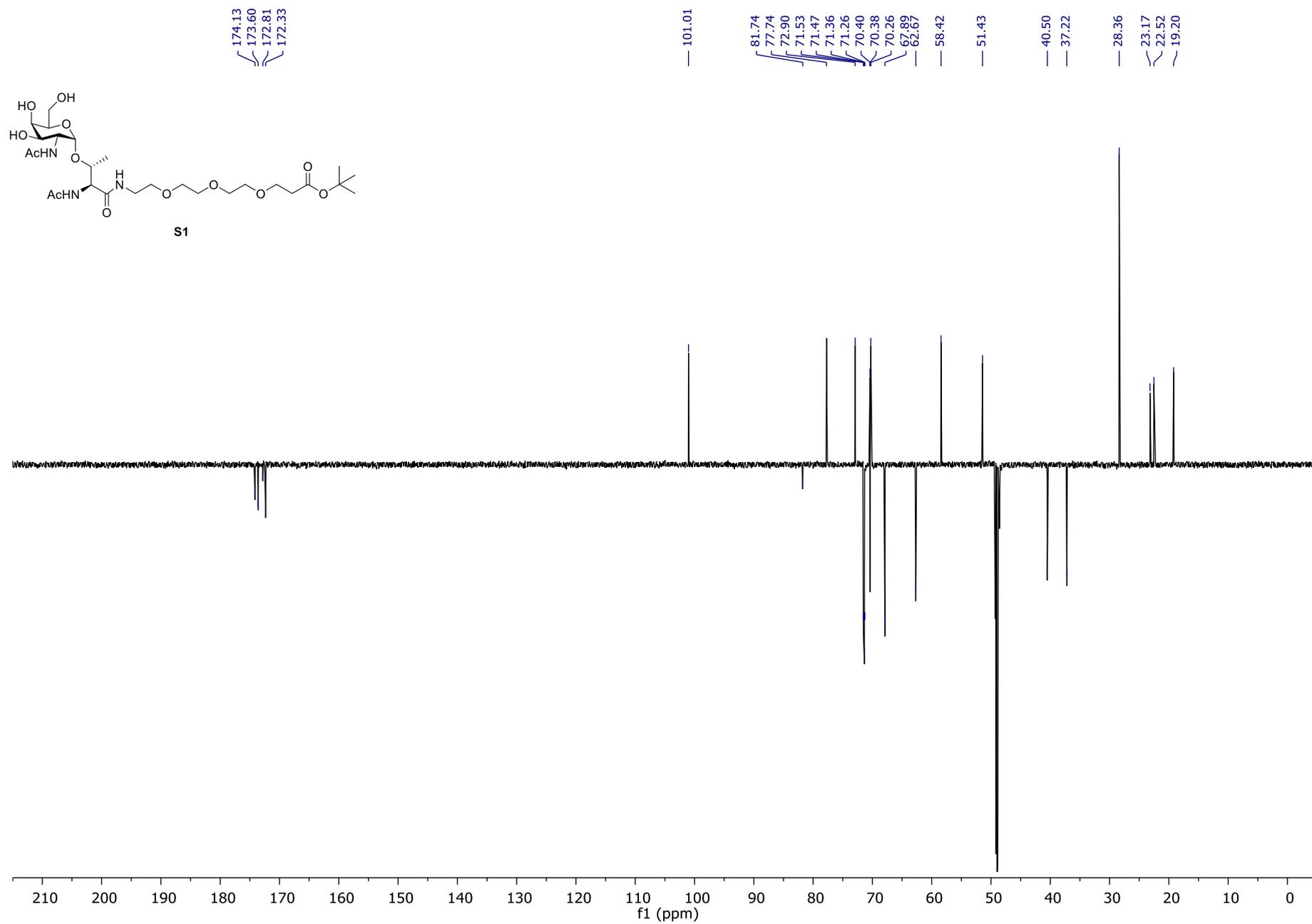
**7**  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )

**7** APT  $^{13}\text{C}$ -NMR (101 MHz,  $\text{CDCl}_3$ )



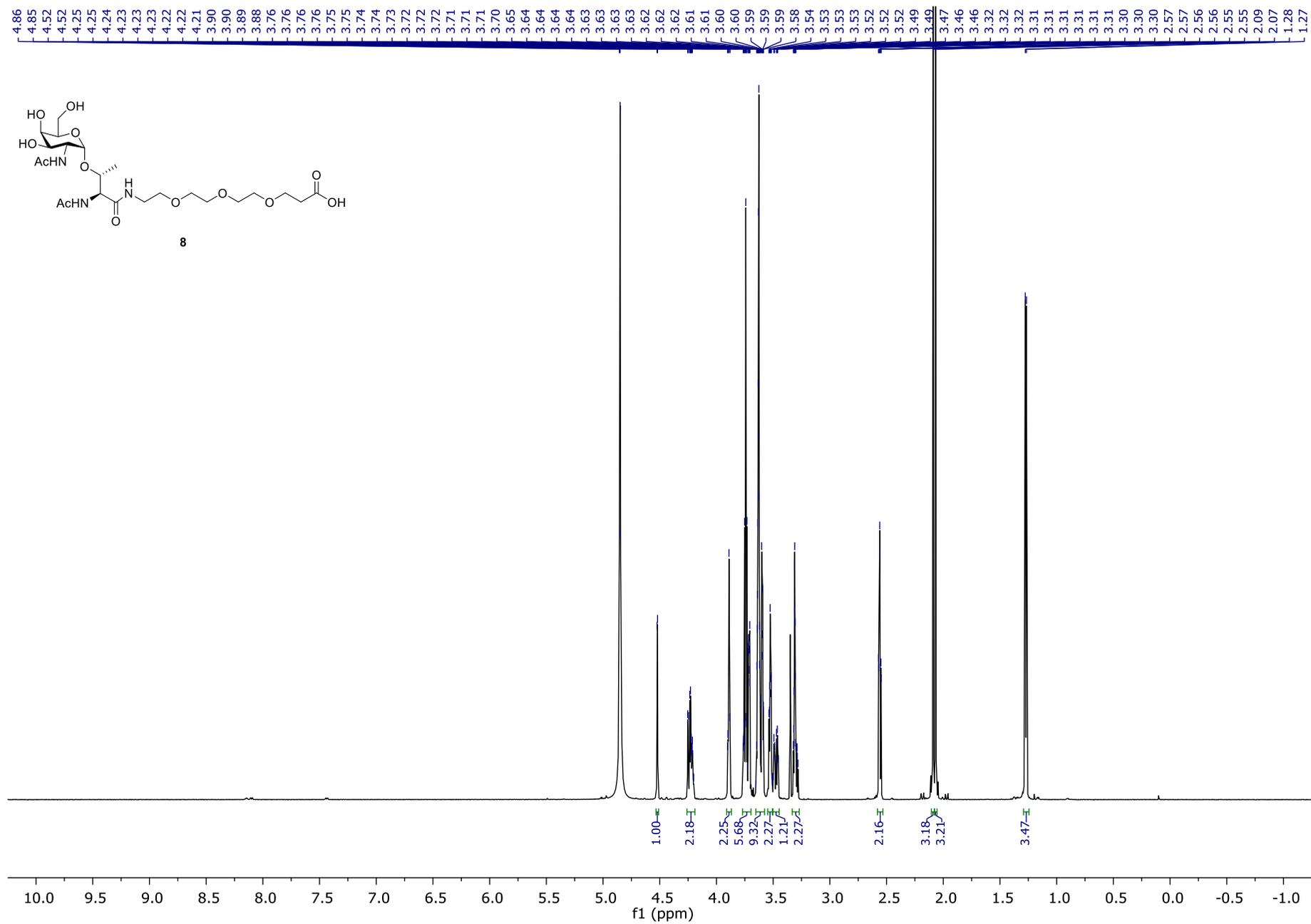


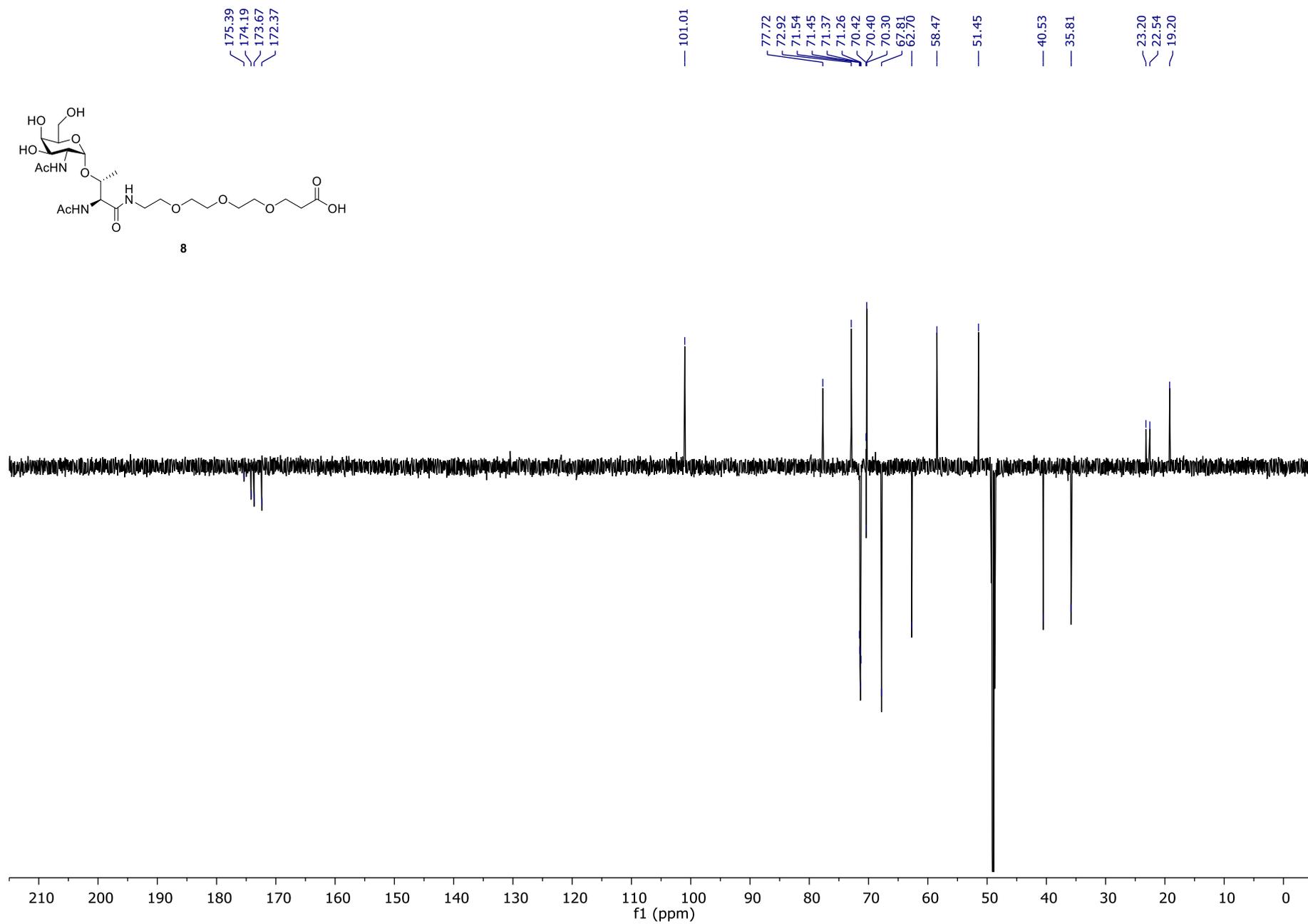


**S1** APT  $^{13}\text{C}$ -NMR (151 MHz, methanol- $d_4$ )

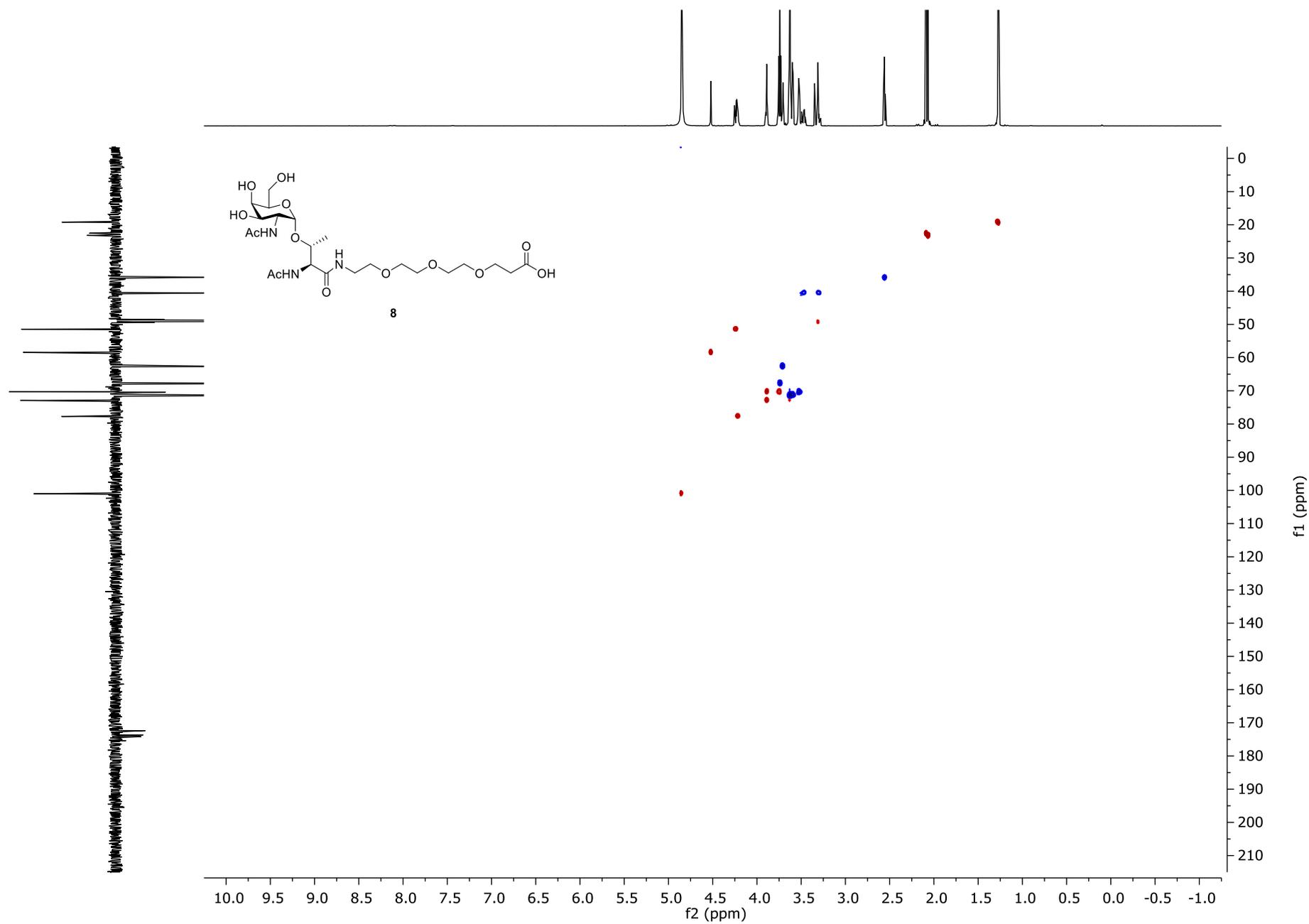


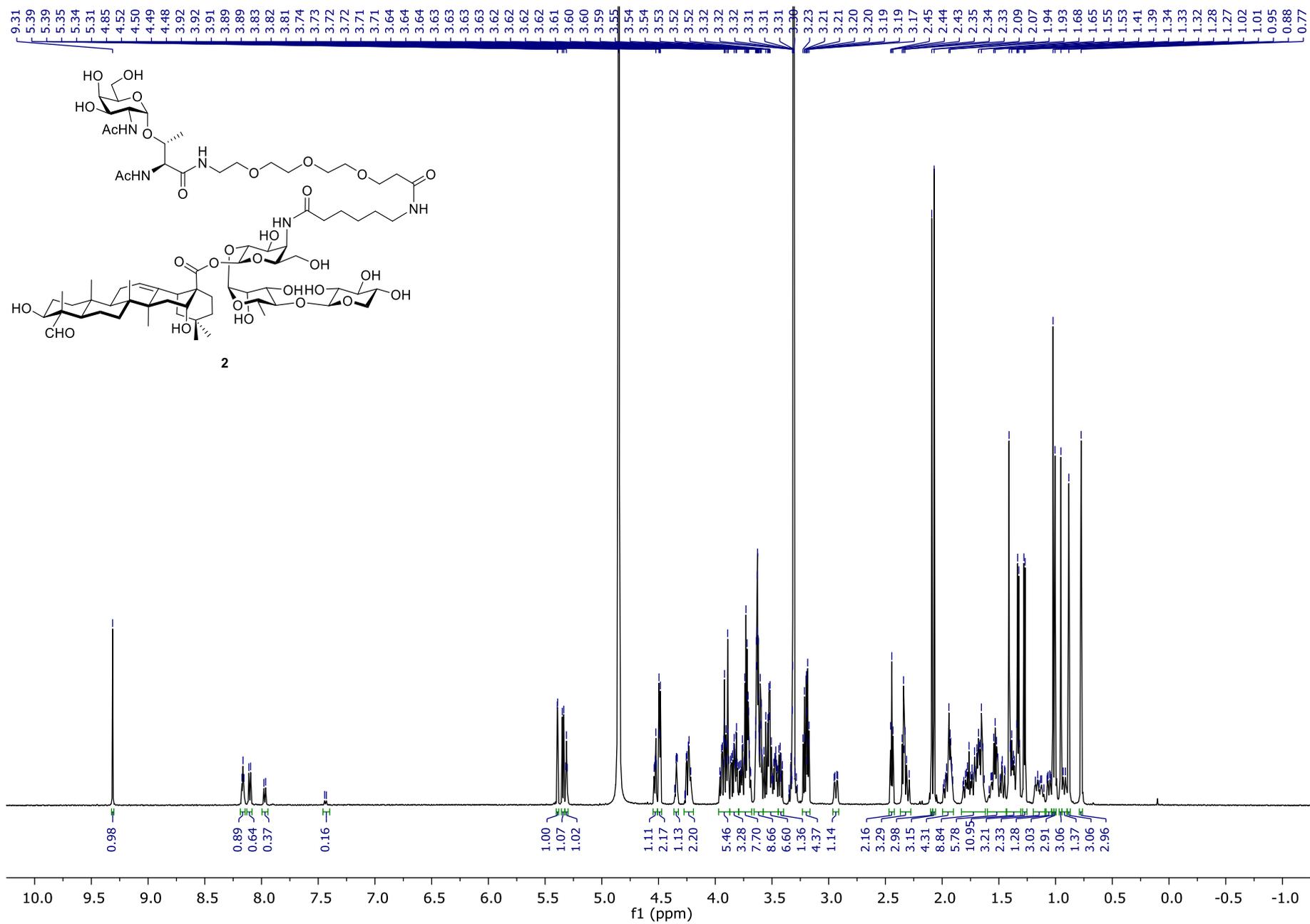


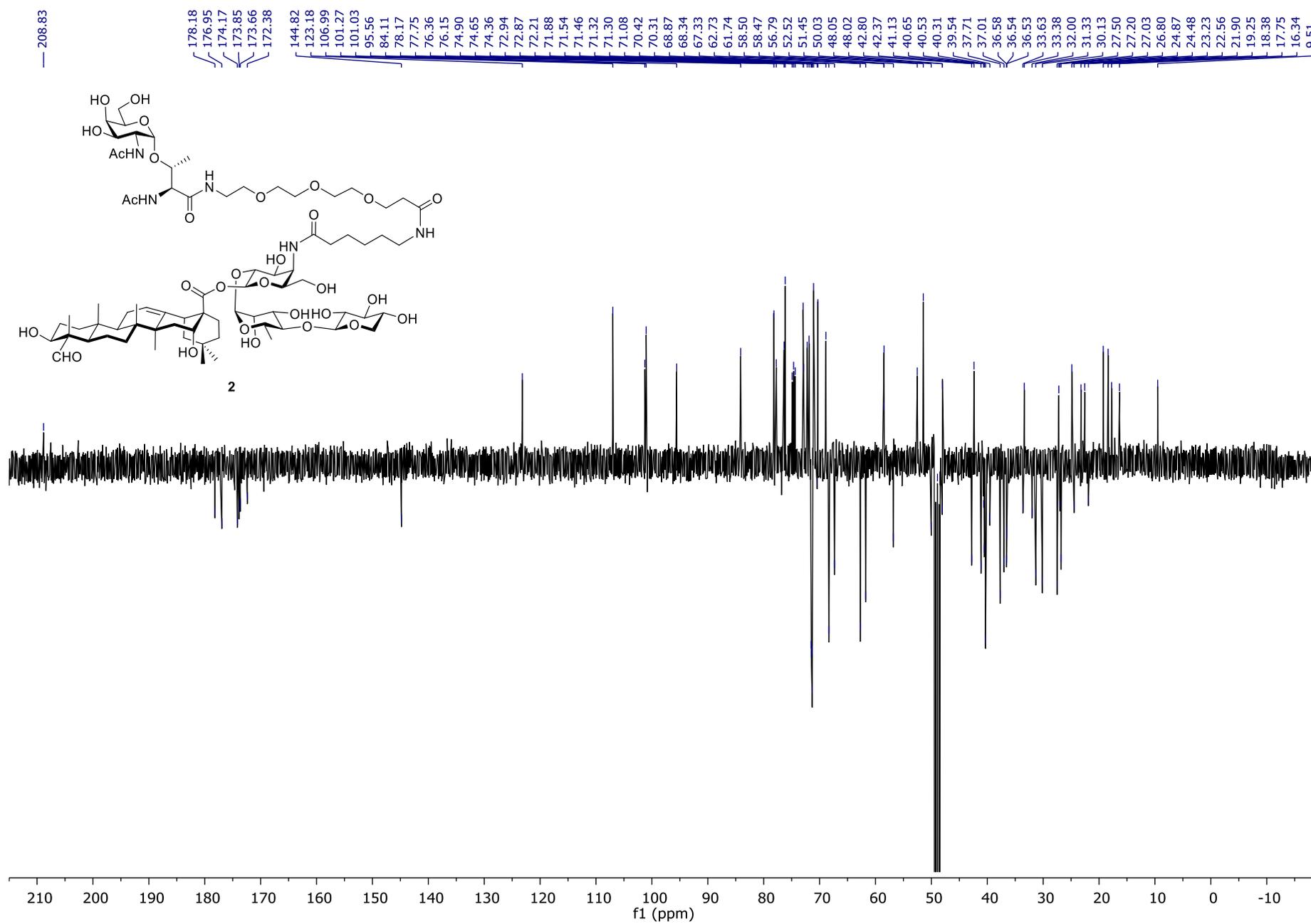
**8**  $^1\text{H-NMR}$  (600 MHz, methanol- $d_4$ )

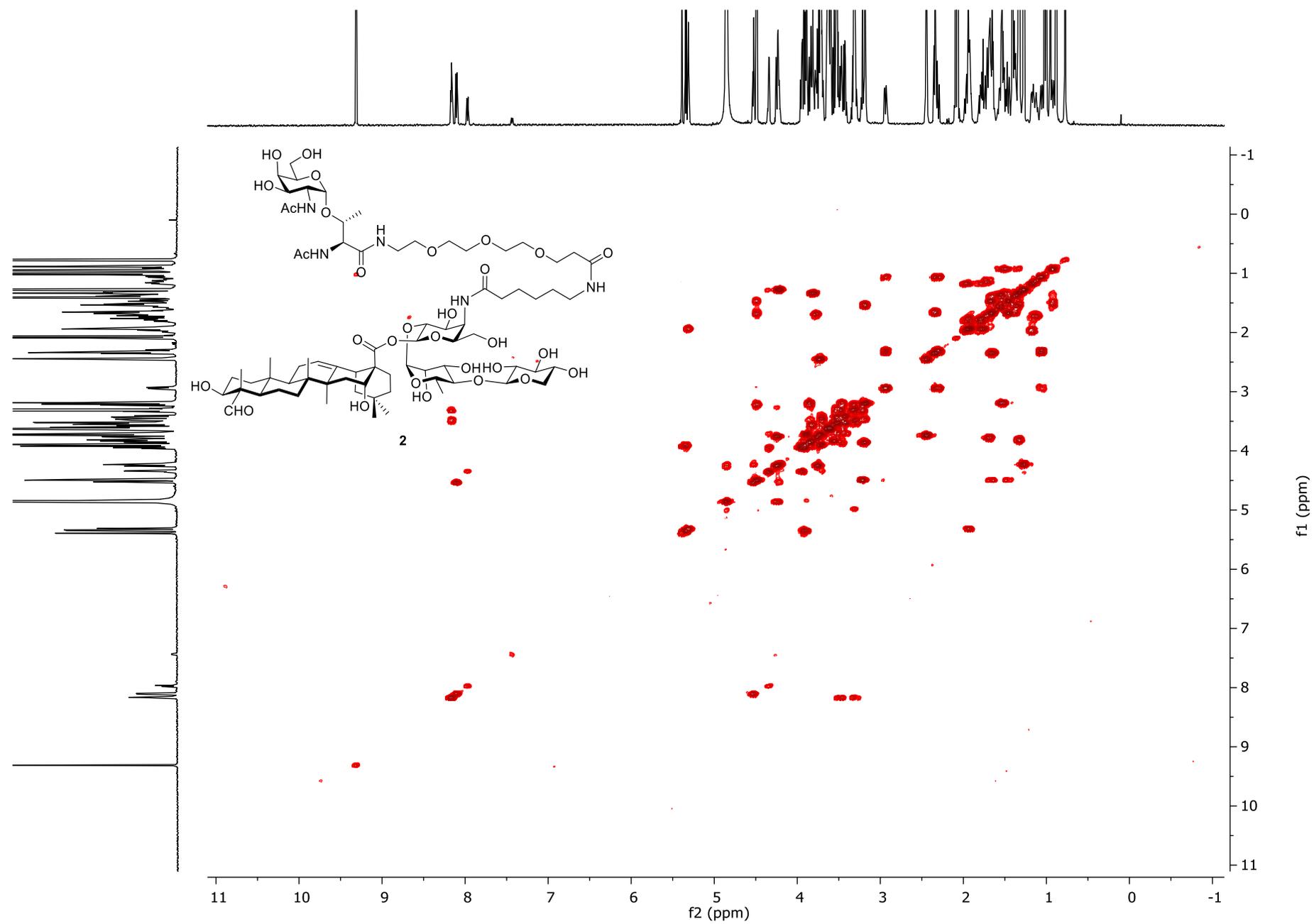
**8** APT  $^{13}\text{C}$ -NMR (151 MHz, methanol- $d_4$ )

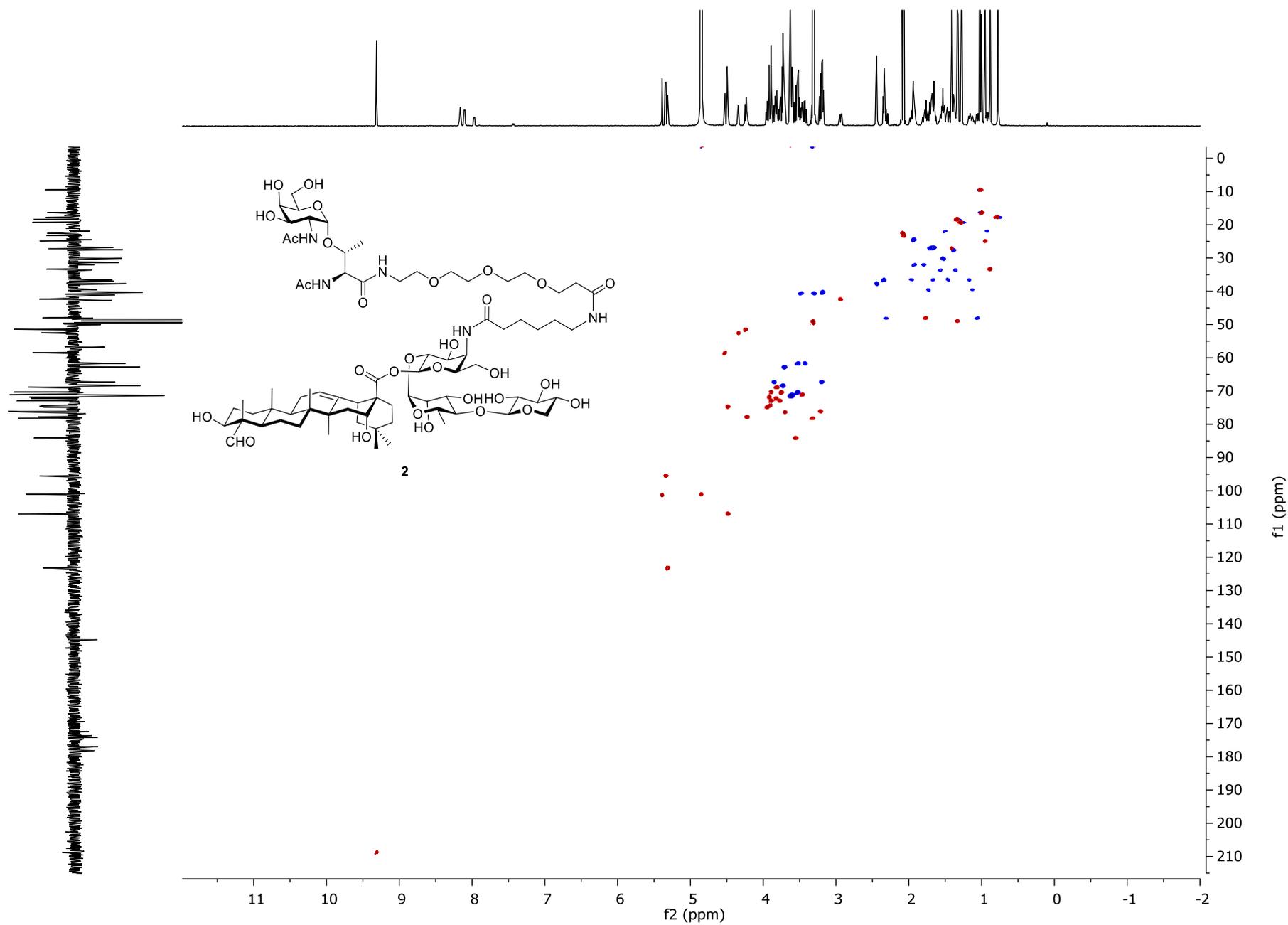


**8** HSQC (600 MHz, methanol-*d*<sub>4</sub>)

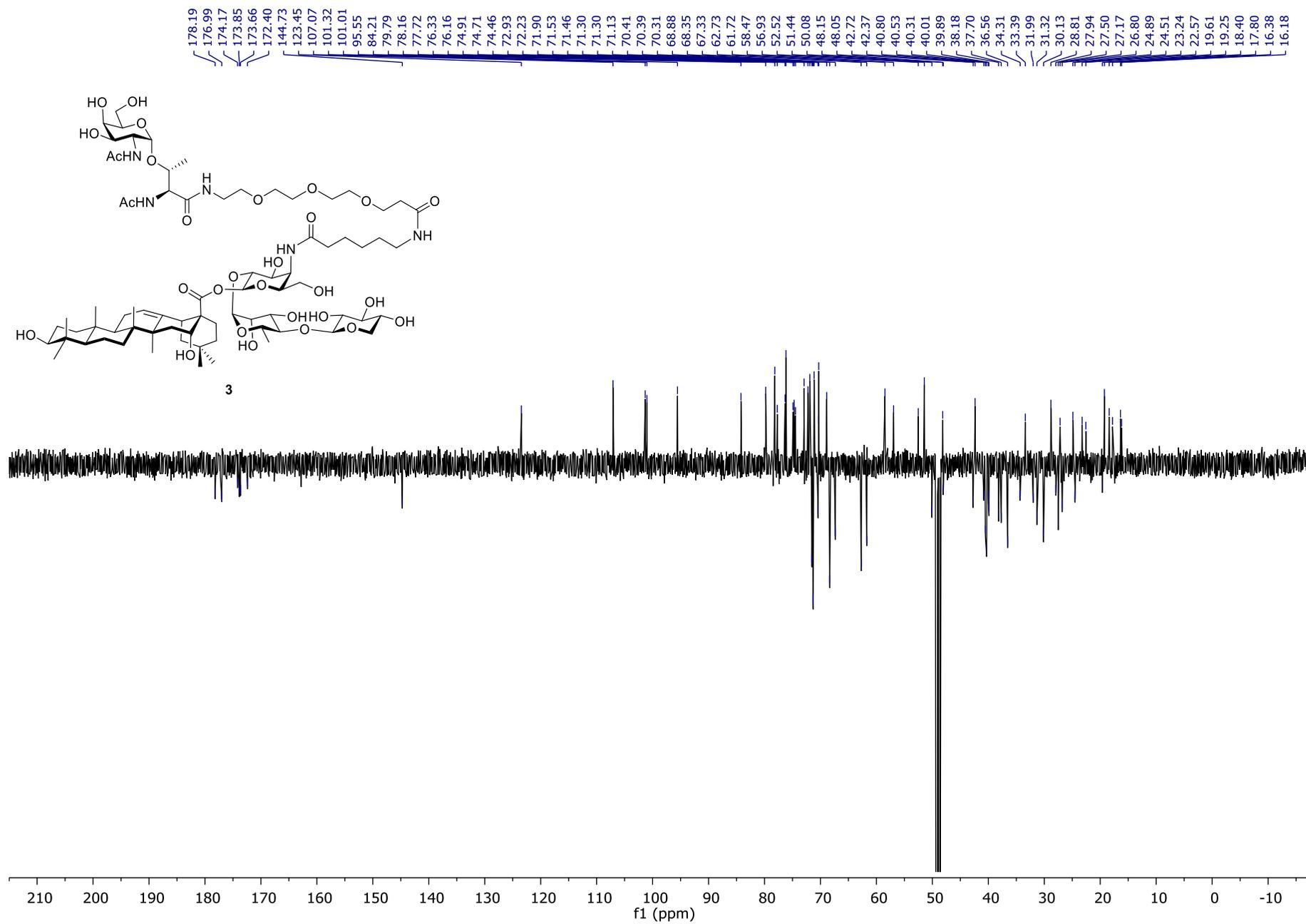
**2**  $^1\text{H-NMR}$  (600 MHz, methanol- $d_4$ )

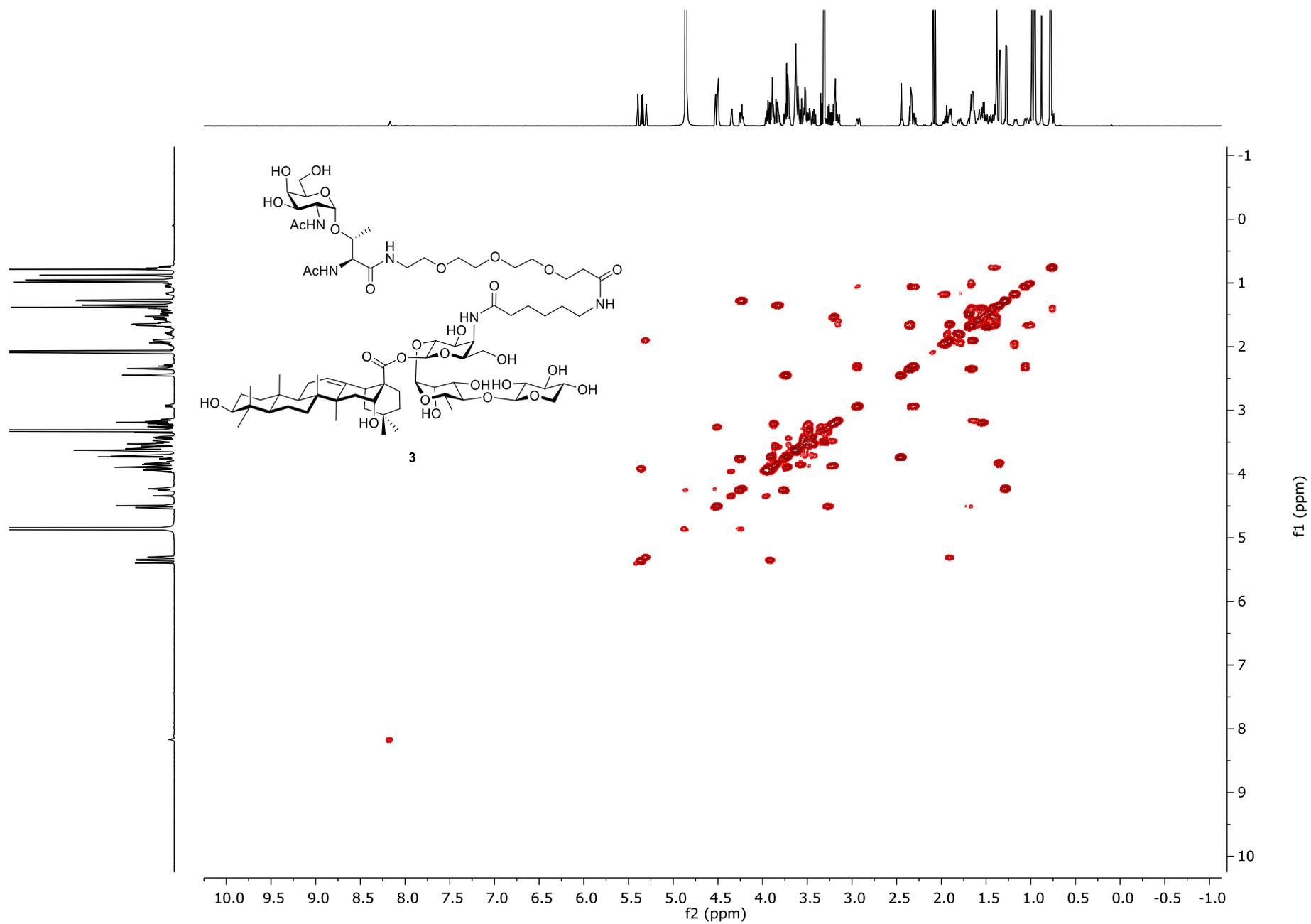
**2** APT  $^{13}\text{C}$ -NMR (151 MHz, methanol- $d_4$ )

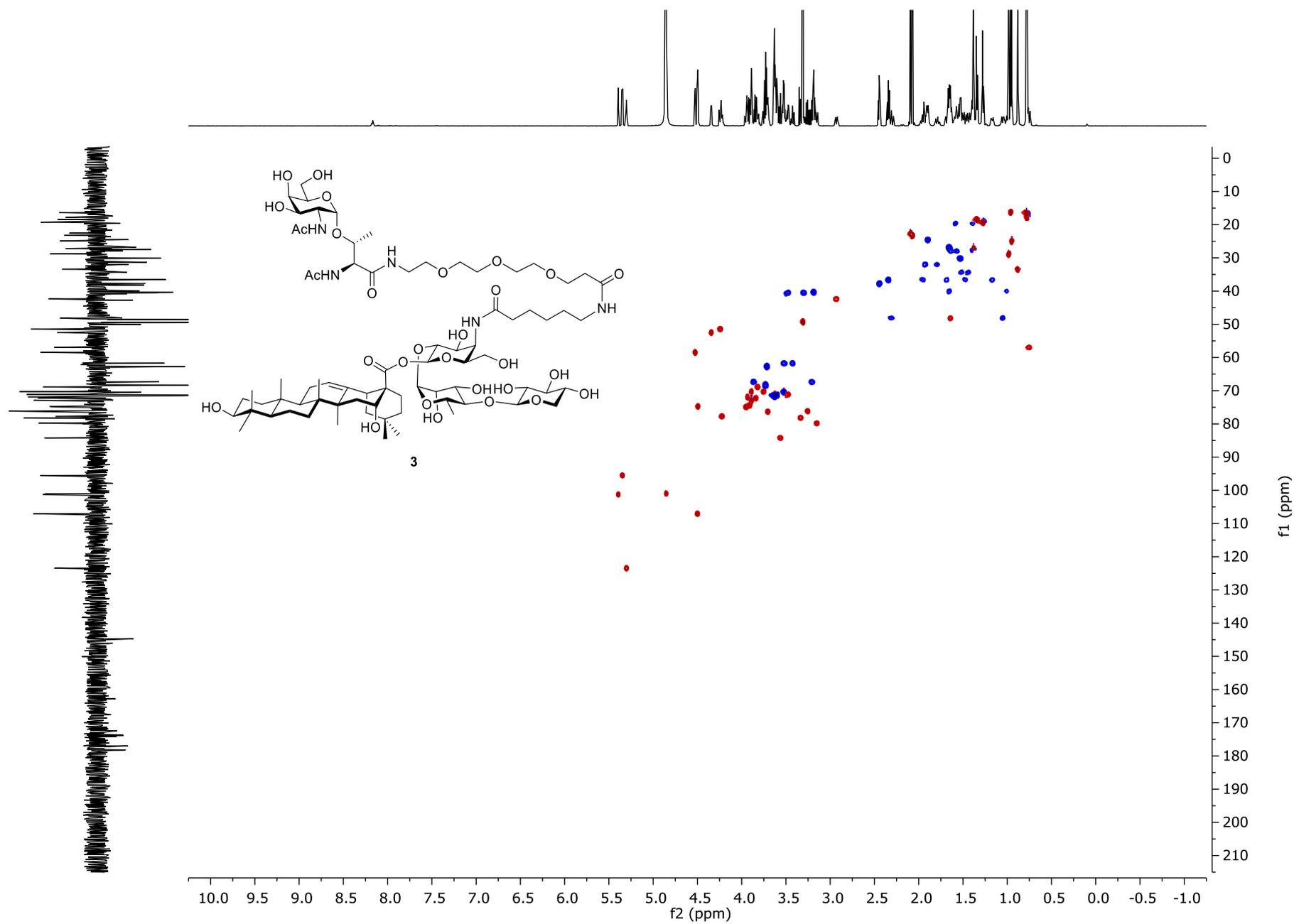
**2** COSY (600 MHz, methanol-*d*<sub>4</sub>)

**2** HSQC (600 MHz, methanol-*d*<sub>4</sub>)



**3** APT  $^{13}\text{C}$ -NMR (151 MHz, methanol- $d_4$ )

**3** COSY (600 MHz, methanol-*d*<sub>4</sub>)

**3** HSQC (600 MHz, methanol-*d*<sub>4</sub>)

**VI. SUPPLEMENTARY INFORMATION REFERENCES**

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- [2] Z. Wu, X. Guo and Z. Guo, *Chem. Commun.* 2010, **46**, 5773–5774.
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