Supporting Information

Synthesis and evaluation of new trivalent ligands for hepatocyte targeting via the asialoglycoprotein receptor

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1. Experimental Section.

General: ^{1}H and ^{13}C NMR spectra were recorded on a BrukerAvance 400 spectrometer with operating frequencies of 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR in $CDCl_3$, D_2O or DMSO-d6. 2D NMR spectra were recorded at the Center for Collective Use "Chemistry" of the UIC UFRS RAS using a Bruker Avance III 500 MHz spectrometer (operating frequency 500.13 for ^{1}H and 125.47 MHz for ^{13}C NMR). Chemical shifts (δ) in ppm are reported as quoted relative to the residual signals of chloroform-*d* (7.26 for ^{1}H NMR and 77.0 for ^{13}C NMR), DMSO-*d6* (2.50 for ^{1}H NMR and 39.5, 118.3 for ^{13}C NMR) as internal references. The coupling constants (*J*) are given in Hertz. IR spectra were recorded on Thermo Scientific Nicolet iS5 FT-IR Spectrometer. ESI-HRMS spectra were measured with Thermo Scientific Orbitrap Elite. The silica gel used for column chromatography was MN Kieselgel 60 0.04-0.063 mm/230-400 mesh ASTM. TLC analysis was performed on Merck TLC Silica gel 60 F254 plates. Compounds were detected by $KMnO_4$ solution (1%) with subsequent heating at 100-120°C for 1-2 minutes. Organic chemicals with purity no less than 95% were used as purchased. Solvents were purified routinely and freshly distilled prior to use, or purchased in HPLC-quality. Cyanine5-COOH derivative was purchased from Lumiprobe, Russia.

Synthesis of compound 2

$FeCl_3$ (0.08 g, 0.5 mmol, 0.2 eq.) and $TMSN_3$ (0.5 ml, 3.8 mmol, 1.5 eq.) were added to a solution of D-galactosamine pentaacetate **1** (1.0 g, 2.5 mmol, 1 eq.) in CH_2Cl_2 (10 ml) and stirred in ambient conditions under argon atmosphere for 72 h. The mixture was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was separated, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude residue was used without chromatographic purification or purified by column chromatography on silica gel using mixture of CH_2Cl_2 - CH_3OH (10:1) as eluent.

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl azide (2). Yield: 0.89 g, 96%. m.p. 163-164 °C. R_f = 0.57 (CH_2Cl_2:CH_3OH 20:1). ν_{max} (cm^{-1}, KBr) 3258, 3087, 2111, 1749, 1647, 1572, 1373, 1261, 1231, 1089, 1061, 953. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.63 (d, 1 H, J = 8.5 Hz), 5.38 (d, 1 H, J = 2.5 Hz), 5.24 (dd, 1 H, J = 11.1, 2.8 Hz), 4.79 (d, 1 H, J = 9.2 Hz,), 4.16 (d, 2 H, J = 6.2 Hz), 4.09 – 3.97 (m, 2 H), 2.16 (s, 3 H), 2.06 (s, 3 H), 2.01 (s, 3 H), 1.99 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.5, 170.2, 88.6, 72.7, 69.7, 66.6, 61.5, 50.6, 23.2, 20.6. ESI-HRMS: m/z for $C_{14}H_{20}N_4O_8$ Calcd.: $[M+Cl]^-$ 407.0975; Found: $[M+Cl]^-$ 407.0977.

Synthesis of compound 3

Compound **2** (0.70 g, 1.88 mmol, 1 eq.) was treated with 0.1 M solution of MeONa in dry MeOH (10 ml) and stirred in ambient conditions for 5 h. Thereafter the resulting solution was treated with Dowex® 50WX8 (hydrogen form) under vigorous stirring until pH = 6 – 7. The resin was filtered out and washed with CH_3OH. The combined filtrate was evaporated to dryness under reduced pressure.

2-Acetamido-2-deoxy-β-D-galactopyranosyl azide (3). Yield: 0.46 g, 99% as a yellowish solid. m.p. 146-147 °C. R_f = 0.16 (CH_2Cl_2:CH_3OH 5:1). ν_{max} (cm^{-1}, KBr) 3334, 2098, 1649, 1556, 1249, 1151, 1111, 1068, 1020. ^{1}H NMR (400 MHz, D_2O): δ 4.66 (d, 1 H, J = 9.3 Hz), 3.97 (d, 1 H, J = 3.1 Hz), 3.95 – 3.88 (m, 1 H), 3.85 – 3.79 (m, 1 H), 3.79 – 3.74 (m, 3 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d6): δ 169.7, 89.0, 77.8, 71.0, 67.4, 60.5, 51.3, 23.0. ESI-HRMS: m/z for $C_8H_{14}N_4O_5$ Calcd.: $[M+Cl]^-$ 281.0658; Found: $[M+Cl]^-$ 281.0660.

Synthesis of compound 6

Tris(hydroxymethyl)aminomethane **5** (0.79 g, 6.5 mmol, 1 eq.) and Boc_2O (1.70 g, 7.8 mmol, 1.2 eq.) were added to a mixture of CH_3OH – H_2O (20 ml, 9:1) and stirred in ambient conditions for 72 h. After reaction completion, the mixture was evaporated to dryness under reduced pressure and resulted residue was recrystallized from hot EtOAc.

***tert*-Butyl (1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)carbamate (6)** Yield: 0.41 g, 61%, as a white solid. m.p. 144-145 °C. R_f = 0.28 (CH_2Cl_2:CH_3OH 20:1). ν_{max} (cm^{-1}, KBr) 3350, 1549, 1680, 1294, 1020. ^1H NMR (400 MHz, DMSO-d6): δ 5.77 (s, 1 H), 3.51 (d, 6 H, J = 5.7 Hz), 1.37 (s, 9 H). ^{13}C NMR (100 MHz, DMSO-d6): δ 155.4, 78.2, 60.8, 60.7, 28.6. ESI-HRMS m/z for $C_9H_{19}NO_5$ Calcd $[M+Cl]^-$ 256.0957, $[M+HCOO]^-$ 266.1245; Found $[M+Cl]^-$ 256.0957, $[M+HCOO]^-$ 266.1245.

Synthesis of compound 7

Compound **6** (0.22 g, 1 mmol, 1 eq.), 5-hexynoic acid (0.36 ml, 3.3 mmol, 3.3 eq.), EDC-Cl (0.63 g, 3.3 mmol, 3.3 eq.) DIPEA (0.56 ml, 3.3 mmol, 3.3 eq.) and DMAP (cat. amounts) were added to CH_2Cl_2 (20 ml) and stirred in ambient conditions for 24 h. The mixture was washed with brine and saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography on silica gel using a mixture of hexane - EtOAc (40:1) as the eluent.

2-((*tert*-butoxycarbonyl)amino)-2-((hex-5-ynoyloxy)methyl)propane-1,3-diyl bis(hex-5-ynoate) (7). Yield: 0.21 g, 42%. R_f = 0.66 (CH_2Cl_2:EtOAc 40:1). ν_{max} (cm^{-1}, KBr) 3309, 2964, 2925, 2852, 1703, 1259, 1093, 794. ^1H NMR (400 MHz, $CDCl_3$): δ 4.82 (s, 1 H), 4.33 (s, 6 H), 2.45 (t, 6 H, J = 7.4 Hz), 2.23 (m, 6 H), 1.96 (s, 3 H), 1.80 (m, 6 H), 1.39 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 154.3, 83.1, 69.5, 62.9, 56.9, 32.7, 28.4, 23.5, 17.9. ESI-HRMS m/z for $C_{27}H_{37}NO_8$ Calcd $[M+Na]^+$ 526.2411; Found $[M+Na]^+$ 526.2405.

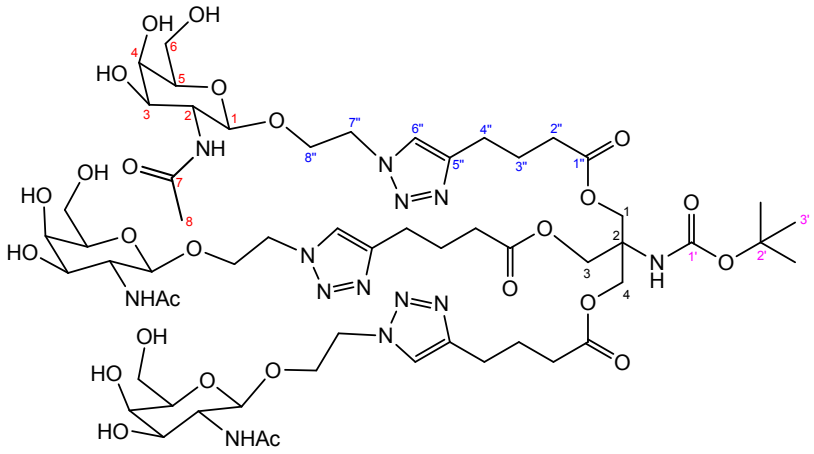
General procedure for the synthesis of compound 8 and 9

To the solution of compound **7** (0.13 g, 0.2 mmol) in dry DMF (5 ml) were added compound **3** or **4** (0.66 mmol, 3.3 eq.), CuI (0.02 g, 0.12 mmol, 0.6 eq.) and DIPEA (cat. amounts) and stirred in ambient conditions under argon atmosphere overnight. The precipitate was filtered off, washed with MeOH, and the combined filtrate was evaporated under reduced pressure and co-evaporated with toluene twice. The crude residue was purified by column chromatography on silica gel using a mixture of CH_2Cl_2 and MeOH (5:1) as the eluent.

Ligand (8). Yield: 0.13 g, 51%. R_f = 0.31 (CH_2Cl_2:CH_3OH 5:1). ν_{max} (cm^{-1}, KBr) 2923, 2854, 1737, 1660, 1462, 1377. ^1H NMR (400 MHz, DMSO-d6): δ 7.83 (s, 3 H), 7.73 (d, 3 H, J = 9.4 Hz), 6.95 (s, 1 H), 5.52 (d, 3 H, J = 9.9 Hz), 4.43 – 4.31 (m, 3 H), 4.20 (s, 6 H), 3.76 – 3.72 (m, 3 H), 3.68 – 3.60 (m, 6 H), 3.54 – 3.43 (m, 6 H), 2.57 (t, 6 H, J = 7.1 Hz), 2.28 (t, 6 H, J = 7.2 Hz), 1.81 – 1.74 (m, 6 H), 1.56 (s, 9 H), 1.30 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 169.8, 154.7, 146.2, 121.0, 87.0, 78.9, 71.6, 68.1, 61.9, 61.8, 56.7, 51.2, 49.0, 46.1, 32.9, 28.5, 24.5, 23.0. ESI-HRMS m/z for $C_{51}H_{79}N_{13}O_{23}$ Calcd $[M+Cl]^-$ 1276.5106, $[M+HCOO]^-$ 1286.5394; Found $[M+Cl]^-$ 1276.5112, $[M+HCOO]^-$ 1286.5393.

Ligand (9). Yield: 0.12 g, 44%. R_f = 0.19 (CH_2Cl_2:CH_3OH = 5:1). ν_{max} (cm^{-1}, ZnSe) 3311, 1556, 1408, 1049. ESI-HRMS m/z for $C_{57}H_{91}N_{13}O_{26}$ Calcd $[M+2H]^{2+}$ 687.8172; Found $[M+2H]^{2+}$ 687.8188.

№	Compound 9	
	NMR ^{13}C, DMSO-*d6*	NMR ^{1}H, DMSO-*d6*
1(3, 4)	61.71	4.24 (s, 6H, H-1(3,4));
2	59.73	-
NHBoc	-	8.41 (br.s, 1H);
1'	164.65	-
2'	78.43	-
3'	28.06	1.34 (s, 9H, H-3');
1''	172.21	-
2''	32.85	2.37 (t, 6H, ^{3}J = 7.6, H-2'');
3''	24.23	1.84 (pent, 6H, ^{3}J = 7.6, H-3'');
4''	24.34	2.61 (t, 6H, ^{3}J = 7.6, H-4'');
5''	145.87	-
6''	122.42	7.72 (s, 3H, H-6'');
7''	49.33	4.41 (ddd, 3H, ^{2}J = 14.6, $^{3}J_{10A\text{-}11A}$ = 7.1, $^{3}J_{7A\text{-}8B}$ = 4.3, H_A-7''); 4.48 (ddd, 3H, ^{2}J = 14.6, $^{3}J_{11B\text{-}10B}$ = 6.0, $^{3}J_{7A\text{-}8B}$ = 4.3, H_B-7'');
8''	66.34	3.76 (ddd, 3H, ^{2}J = 11.0, $^{3}J_{8A\text{-}7A}$ = 7.1, $^{3}J_{8A\text{-}7B}$ = 4.3, H_A-8''); 4.03 (ddd, 3H, ^{2}J = 11.0, $^{3}J_{8B\text{-}7B}$ = 6.0, $^{3}J_{8B\text{-}7A}$ = 4.3, H_B-8'');
1 (GalNAc)	101.10	4.27 (d, 3H, $^{3}J_{1\text{-}2}$ = 8.2, GalNAc_H-1);
2 (GalNAc)	51.70	3.74 (m, 3H, GalNAc_H-2);
3 (GalNAc)	71.34	3.41 (m, 3H, GalNAc_H-3); 4.63 (br.s, 6H, GalNAc_HO-3(6));
4 (GalNAc)	67.46	3.65 (m, 3H, GalNAc_H-4); 4.54 (d, 3H, ^{3}J = 4.0, GalNAc_HO-4);
5 (GalNAc)	75.44	3.33 (m, 3H, GalNAc_H-5);
6 (GalNAc)	60.47	3.50 (dd, 3H, ^{2}J = 10.8, ^{3}J = 5.9, GalNAc_H_A-6); 3.54 (dd, 3H, ^{2}J = 10.8, ^{3}J = 5.9, GalNAc_H_B-6);
NH (GalNAc)	-	7.59 (d, 3H, ^{3}J = 9.0);
7 (GalNAc)	169.54	-
8 (GalNAc)	23.01	1.76 (s, 9H, GalNAc_H-8);



Synthesis of compound 10

TFA (1.5 ml) was added portion-wise to the solution of compound **7** (0.46 g, 0.9 mmol) in dry DCM (15 ml) under stirring at 0°C. The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was evaporated to dryness under reduced pressure with $CHCl_3$ (5 x 20 ml).

2-amino-2-((hex-5-ynoyloxy)methyl)propane-1,3-diyl bis(hex-5-ynoate) (10)

Yield: 0.44 g, 92%. R_f = 0.50 (CH_2Cl_2:MeOH = 20:1). ν_{max} (cm^{-1}, ZnSe) 3267, 2918, 1739, 1680, 1601, 1537, 1469, 1315, 1153. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.31 (s, 6 H), 2.52 (t, J = 7.4 Hz, 6 H), 2.24 (td, J = 6.8, 2.6 Hz, 6 H), 1.98 (t, J = 2.7 Hz, 3 H), 1.82 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.5, 83.0, 69.5, 61.5, 57.3, 32.3, 23.2, 17.7. ESI-HRMS m/z for $C_{22}H_{29}NO_6$ Calcd $[M+H]^+$ 404.2068; Found $[M+H]^+$ 404.2071.

Synthesis of compound 11

To the solution of cyanine5 carboxylic acid (0.02 g, 0.04 mmol, 1 eq.) in dry DMF (5 ml) were added compound **10** (0.01 g, 0.02 mmol, 0.5 eq.), HATU (0.015 g, 0.04 mmol, 1 eq.) and DIPEA (cat. amounts) and stirred in ambient conditions under argon atmosphere overnight. The crude residue was purified by reversed-phase chromatography using C18 column and mixture of H_2O and CH_3CN (1:3) as the eluent.

Compound (11)

Yield: 0.02 g, 90%. R_f = 0.32 (CH_2Cl_2:MeOH = 20:1). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.40 (t, J = 13.1 Hz, 2 H), 7.57 (d, J = 7.4 Hz, 2 H), 7.46 – 7.35 (m, 4 H), 7.27 (t, J = 7.3 Hz, 2 H), 7.16 (s, 1 H), 6.70 (t, J = 12.4 Hz, 1 H), 6.41 (dd, J = 26.2, 13.8 Hz, 2 H), 4.46 (s, 6 H), 4.18 (t, J = 7.5 Hz, 2 H), 3.71 (s, 3 H), 2.47 (t, J = 7.4 Hz, 6 H), 2.37 (t, J = 2.6 Hz, 3 H), 2.27 – 2.19 (m, 9 H), 1.79 (p, J = 7.2 Hz, 6 H), 1.72 (s, 12 H), 1.56 – 1.47 (m, 2 H). ESI-HRMS m/z for $C_{54}H_{66}N_3O_7^+$ Calcd M^+ 868.4895; Found M^+ 868.4893.

Synthesis of compound 12

To the solution of compound **11** (0.01 g, 0.01 mmol, 1 eq.) in dry DMF (5 ml) were added compound **3** (0.01 g, 0.06 mmol, 4 eq.), CuI (cat. amounts) and DIPEA (cat. amounts) and stirred in ambient conditions under argon atmosphere overnight. The crude residue was purified by reversed-phase chromatography using C18 column and mixture of H_2O and CH_3CN (1:1) as the eluent.

Conjugate (12)

Yield: 0.005 g, 22%. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.20 (t, J = 12.9 Hz, 2 H), 7.99 (s, 3 H), 7.46 (d, J = 7.3 Hz, 2 H), 7.42 – 7.34 (m, 2 H), 7.30 – 7.16 (m, 4 H), 6.66 – 6.48 (m, 1 H), 6.33 – 6.19 (m, 2 H), 5.64 (d, J = 9.8 Hz, 3 H), 4.60 (s, 2 H), 4.48 (t, J = 10.2 Hz, 3 H), 4.39 (s, 6 H), 4.11 – 4.01 (m, 2 H), 3.97 (d, J = 2.9 Hz, 3 H), 3.86 – 3.70 (m, 12 H), 3.59 (s, 3 H), 2.68 (t, J = 7.3 Hz, 6 H), 2.33 (t, J = 7.3 Hz, 6 H), 2.19 (t, J = 7.0 Hz, 3 H), 1.90 (t, J = 7.3 Hz, 6 H), 1.73 (s, 9 H), 1.68 (s, 12 H), 1.35

– 1.22 (m, 3 H). ESI-HRMS m/z for $C_{78}H_{108}N_{15}O_{22}^{+}$ Calcd M^{+} 1606.7788, $[M^{+}+Na]^{2+}$ 814.8840, $[M^{+}+K]^{2+}$ 822.8710; Found M^{+} 1606.7790, $[M^{+}+Na]^{2+}$ 814.8850, $[M^{+}+K]^{2+}$ 822.8742.

2. Cell cultures.

Human liver cancer cell lines Huh7 and HepG2, prostate cancer cell line PC3 and umbilical vein endothelial cells EA.hy926 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), 2 mM L-glutamine (Gibco), 100 U/ml of penicillin and 100 μg/ml of streptomycin (Gibco) with the exception of EA.hy926, which were cultured in the same media but with addition of HT-supplement (hypoxanthine (100 μM) and thymidine (16 μM), Gibco).

Liver progenitor cell line HepaRG was purchased from Biopredic International (France).[1] During 4-weeks of cultivation HepaRG differentiate into mixed population with equal proportions of fully differentiated hepatocytes and primitive biliary cells. For differentiation process, HepaRG were cultivated for the first two weeks in the medium composed of Williams E (Gibco) supplemented with 2 mM Glutamax (Gibco), 10% FBS (Gibco), 100 U/ml of penicillin, 100 μg/ml of streptomycin (Gibco), 4 μg/ml of human insulin (Sigma Aldrich) and 50 μM of hydrocortisone hemisuccinate (Sigma Aldrich). The medium was replaced every 2-3 days. After two weeks cell cultures were shifted to the same medium supplemented with 1.7% of DMSO (Sigma-Aldrich) followed by next two weeks cultivation with regular renewal of the medium.

All cells were cultured at 37ºC in a humidified incubator supplied with 5% CO_2.

3. Cytotoxicity measurements.

The measurements of PC3, Huh7 and HepG2 cell cultures were carried out using the standard MTT method.[2] All the manipulations described below were performed using JANUS G3 Automated Workstation. 3 000 cells per well for PC3, 8 000 cells per well for Huh7 and 25 000 cells per well for HepG2 were plated out in 140 μl of media in 96-well plates. Cells were incubated at 37 ^oC in the 5% CO_2 incubator for the first 16 h without treating. Then 11 μl of test substance media-DMSO solutions were added to the cells (the final DMSO concentrations (v/v) in the media were 0.5% or less) and cells were treated for 72 h (triplicate each test substance concentration). The same dilutions of DMSO (Sigma-Aldrich) were used as controls. After that, the MTT reagent (Dia-M) was added to cells up to the final concentration of 0.5 mg/ml (10x stock solution in PBS was used) and cells were incubated for 1.5 h at 37 ºC in the 5% CO_2 incubator. The MTT solution was discarded and 140 μl of DMSO was added. The plates were swayed on a shaker (90 rpm) for 15 min to solubilize the formazan. The absorbance was measured using a microplate reader (VICTOR X5 Plate Reader) at a wavelength of 555 nm (in order to measure the formazan concentration).

Measurements for EA.hy926 and HepaRG were performed in 96-well plates using MTS assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega).[3] EA.hy926 were plated in amount of 7 000 cells per well and tested compounds were added after 48 h of incubation. Fully differentiated HepaRG cells after 28 days of cultivation were used in amount of 70 000 – 80 000 cells per well. To start experiment serially diluted amounts of test substances in 10 μl of media-DMSO solutions were added to 90 μl of freshly renewed media (triplicate each test substance concentration). The same dilutions of DMSO (Sigma-Aldrich) were used as controls. After 72 h of incubation the culture medium was removed and 100 μl of fresh medium with 20 μl of MTS reagent (CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay, Promega) were added. The absorbance was measured after 2 h (for HepaRG) or 4 h (for EA.hy926) incubation in the darkness using a microplate reader (Varioscan LUX microplate reader) at a wavelength of 490 nm.

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[1] A. Guillouzo, *et al*. *Chemico-Biological Interactions*, 2007, 168, 66–73.
[2] T. Mosmann, *J. Immunol. Methods*, 1983, 65, 55-63.
[3] R. S. Gieni, *et al*. *J. Immunol. Methods*, 1995, 187, 85-93.

The results were used to construct a four-parameter dose-response logistic curve and to estimate IC_{50} value by RStudio software using “drc” and “drexplorer” packages. Each drug concentration was examined in three independent experiments.

4. Surface plasmon resonance (SPR).

Equipment Biacore X100 device (Biacore AB, Uppsala, Sweden), CM5 chips (Biacore AB, Uppsala, Sweden), high-accuracy weighting machine Ohaus Explorer Pro (Switzerland), pH-meter “Econics-expert” (Russia), air displacement micropipettes, pipette tips (Eppendorf), chemical glassware.

Protein immobilization The experiment was carried out on a Biacore X100 machine (Biacore AB, Uppsala, Sweden) using CM5 chip, which consists of a gold surface to which a carboxymethylated dextran layer was bound. The surface of the chip consists of two flow cells. The first one was covered by immobilized ASGP-R, the second one was left blank for reference. Tris-base buffer solution (150 mM NaCl, 50 mM $CaCl_2$, 50 mM Tris, pH 7.4) was used as a running buffer. Both chip’s cells were activated by exposing them to a mixture of 0.05 M N-hydroxysuccinimide (NHS) and 0.2 M N-ethyl-N-dimethylaminopropyl-carbodiimide (EDC) for 7 min, the mixture was injected at a flow rate of 10 μL/min. The surface of the flow cell 1 was immobilized with rabbit liver ASGP-R in 50 mM Tris-HCl (pH 7.0) buffer solution, the surface of the flow cell 2 was not modified. Protein was injected for 15 minutes at a flow rate of 10 μL/min. The unreacted sites of both immobilized flow cells were blocked with 0.1 M ethanolamine (pH 8.5). The amount of immobilized rabbit liver ASGP-Rs was 2000 resonance units (RU).

Buffer composition Running buffer was composed of 150 mM NaCl, 50 mM $CaCl_2$, 50 mM Tris, pH 7.4. Buffer solution for protein keeping consisted of 50 mM Tris-HCl, pH 7.0. Regeneration solution was composed of 20 mM EDTA, pH 8.0. All solutions were filtered and deoxygenated.

Sample preparation Compounds were serially diluted in a running buffer (150 mM NaCl, 50 mM $CaCl_2$, 50 mM Tris, pH 7.4). DMSO was used in the case of low-solubility compounds. Ligands were prepared in concentrations 10^{-2} M to $5*10^{-11}$ M

Analysis procedure Compound solutions were injected at a flow rate of 20 μL/min at 25°C for 180 s followed by 60 s dissociation. The regeneration of the sensor chip was obtained by injection of 20 μL of 20 mM EDTA.

Data analysis Each sensorgram was analyzed via a global fitting procedure using BIAevaluation 3.0 software: the kinetic analysis of sensorgrams for the interaction of carbohydrates with the immobilized rabbit liver ASGP-Rs was based on the rate equation:

$$dR/dt = k_aCR_{max} - (k_aC+k_d)R_t, \text{ where}$$

dR/dt is the rate of change in the SPR signal (in RU, resonance units) due to each carbohydrate interaction with immobilized rabbit liver ASGP-Rs at time t second s;

k_a and k_d are the association and dissociation rate constants, respectively;

C is the concentration of each carbohydrate;

R_{max} is the maximum carbohydrate binding capacity to rabbit liver ASGP-Rs in resonance units.

The affinity constants were obtained from the ratio of k_a/k_d.

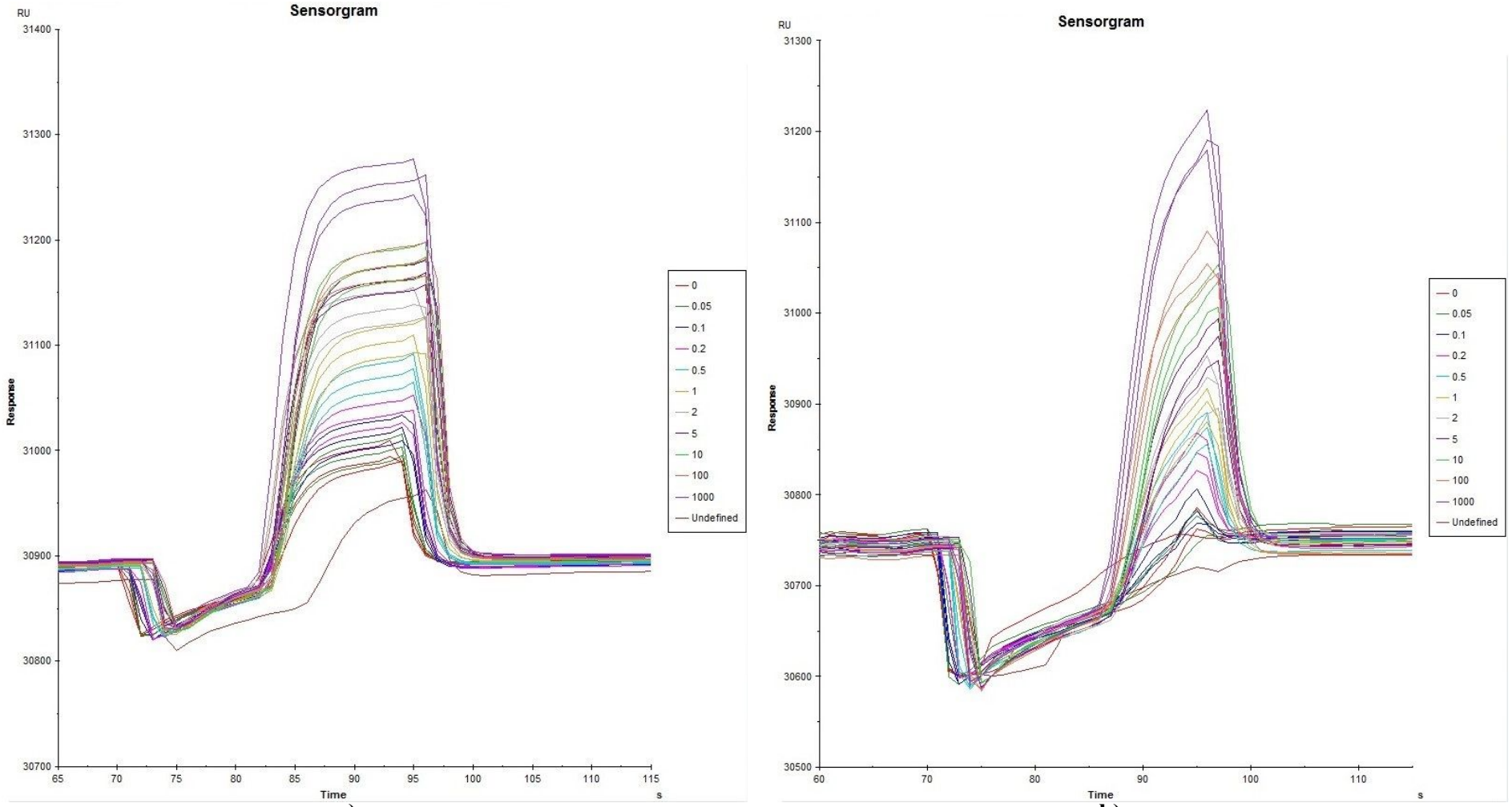


Figure S1. Raw sensogram showing binding of **8** (a) and **9** (b) ligands to asyaloglycoprotein receptor. A solutions of corresponding compound in various concentracions (color-coded, nM) were injected over a sensor chip containing ASGPR immobilized on flow cell at a flow rate of 20 μL/min at 25 °C for 30 s followed by 30 s dissociation. The regeneration of the sensor chip was obtained by injection of 20 μL of 20 mM EDTA.

5. Molecular docking studies

Receptor model preparation The crystallographic coordinates of ASGP-R protein (PDB: 5JPV)[4] were obtained from the Protein Data Bank (www.rcsb.org). The model was prepared by removing all the solvent, adding hydrogen atoms, and minimizing the energy in the presence of the bound ligand using the MacroModel package in the Maestro software (Schrodinger Inc.). Because the ligands are rather large, the energy grid was generated within a cubic box of dimensions 36 × 36 × 36 Å centered on the carbohydrate-binding site. The developed docking model was validated by redocking of the crystallographic ligand.

Ligands preparation Ligands were designed using ChemDraw software, appropriately ionized at pH 7.4 ± 1.0 and minimized with an MMFF94 force field by the LigPrep tool embedded in Maestro software (Schrodinger Inc.), retaining specified chiralities and without tautomers.

Molecular docking Molecular docking was performed using the Glide standard precision (SP) mode with default settings for all parameters and no constraints applied. The docking results were visualized using UCSF Chimera software.[5]

6. Hydrophobicity analysis

The RDKit chemoinformatics tool[6] was used for calculation of logP and TPSA values in order to analyze the hydrophobicity. Ligands **A** and **B** (Figure 1) were selected as comparison molecules. The calculation results are presented in Table 1.

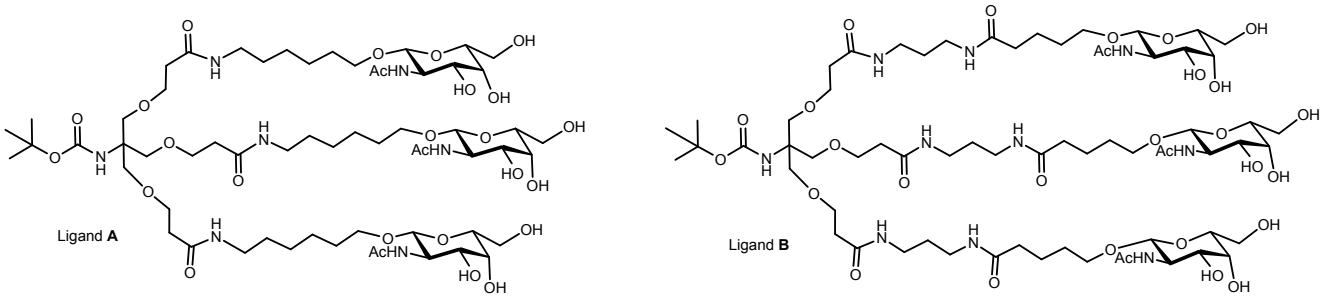


Figure S2. Comparison ligands **A** and **B** for calculations of Hydrophilic-hydrophobic balance.

7. Spatial geometry calculations

Visualization of fully extended conformations and measurement of distances and angles was carried out in the UCSF Chimera software. Maximal spacer length was measured as the distance between the anomeric center of the glycosidic residue and the branching point. Also, the angle between the two linkers was obtained. Thereafter the distance between sugars was calculated by applying the cosine rule to triangles.

[4] C. A. Sanhueza et al., JACS, 2017, 139, 3528.
[5] E. F. Pettersen et al., J. Comput. Chem., 2004, 25, 1605.
[6] RDKit: Open-source cheminformatics; http://www.rdkit.org

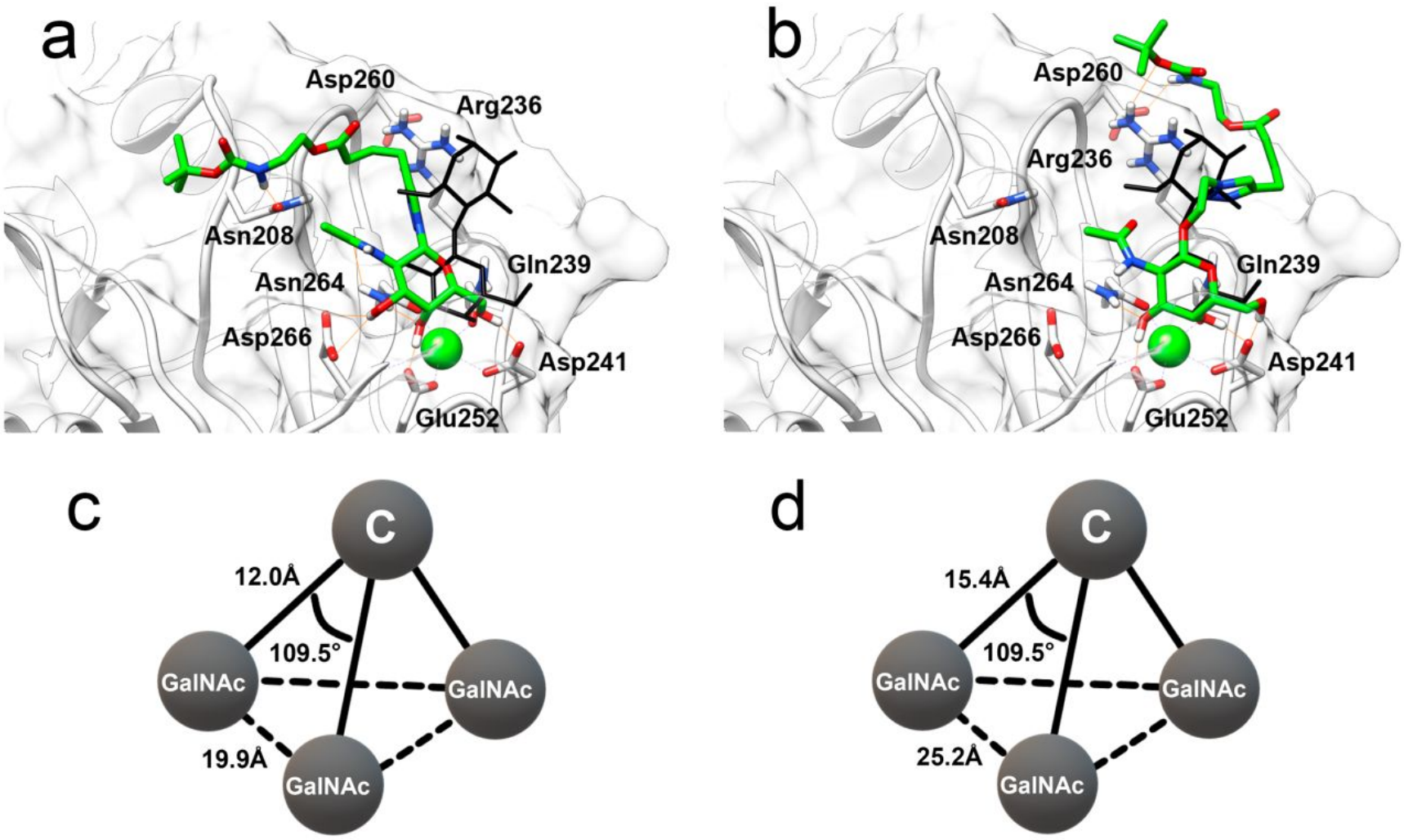


Figure S3. The mode of interaction between ligands **8** (a) and **9** (b) and the receptor (reference ligand is shown in black) and the spatial geometry of the molecules **8** (c) and **9** (d).

8. *In vitro* cellular uptake studies (live cell microscopy) of compound 12

HepG2 and PC3 cells, obtained from ATCC, were cultured in DMEM/F12 (Gibco) and RPMI 1640 (Gibco) medium, respectively, supplemented with 10% FBS (Gibco), 1X GlutaMax (Gibco) and 1X Pen-Strep 10000 U/mL (Gibco). For microscopy, HepG2 and PC3 cells were plated at a density of 65000 cells/ cm2 and 40000 cells/cm2 respectively in 35 mm glass-bottom dishes in supplemented medium. After approximately 20 hours under normal culture conditions, the cells were incubated with 5 uM of compound **12** in phenol red free medium supplemented with 1% FBS, 1X GlutaMax and 1X Pen-Strep for 60 minutes at 37°C. Ligand addition was accomplished by adding 30 µL of the desired compound (the equivalent volume of PBS was added as a blank control). After incubation, cells were washed three times with pre-warmed PBS (Gibco). Cells were imaged using EVOS FL Cell Imaging System (Thermo Fisher Scientific; Cy5 light cube Ex 628/40 nm, Em 692/40 nm). Images were processed with ImageJ (Fiji) software[7] to determine the accumulation of **12** in HepG2 and PC3 cultures by total cell fluorescence intensity.

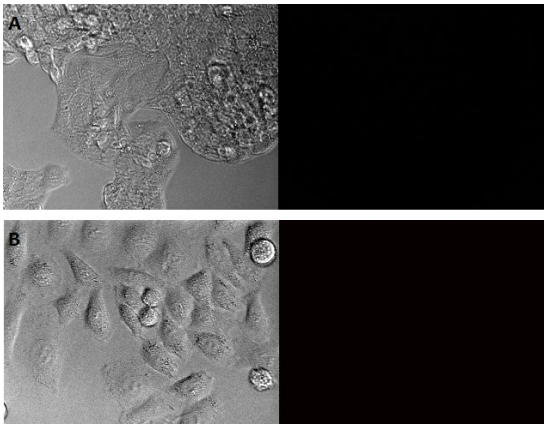


Figure S4. Representative brightfield (left) and fluorescence (right) microscopy images of untreated cells as controls: (A) HepG2 cells; (B) PC3 cells.

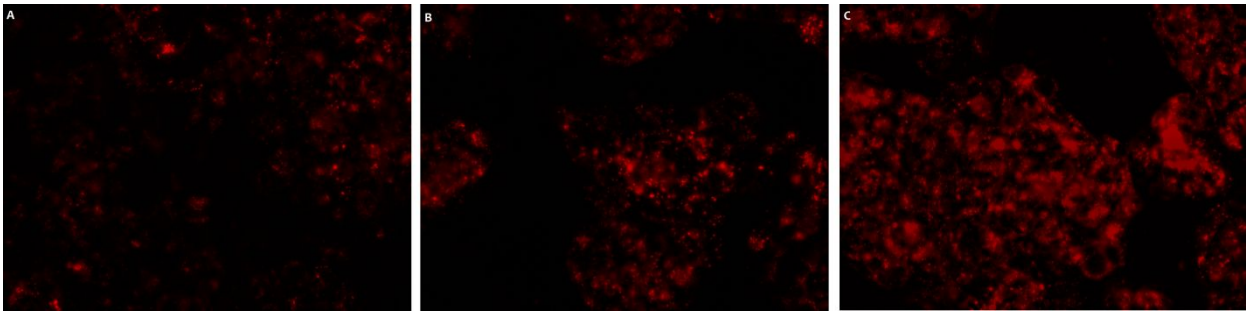


Figure S5. Fluorescence microscopy images of dose-dependent accumulation in HepG2 cells treated with **12** for 1 hour: (A) 1 µM; (B) 10 µM; (C) 50 µM.

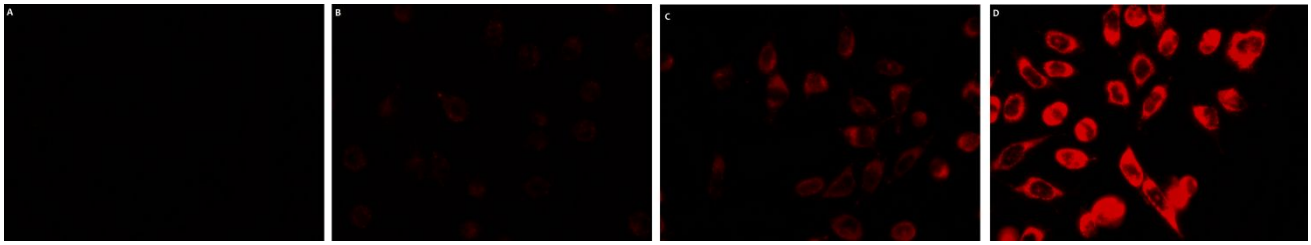


Figure S6. Fluorescence microscopy images of dose-dependent accumulation in PC3 cells treated with **12** for 1 hour: (A) 1 µM; (B) 10 µM; (C) 50 µM; (D) 100 µM.

[7] Schindelin, J. et al., Nature methods, 2012, 9(7), 676.

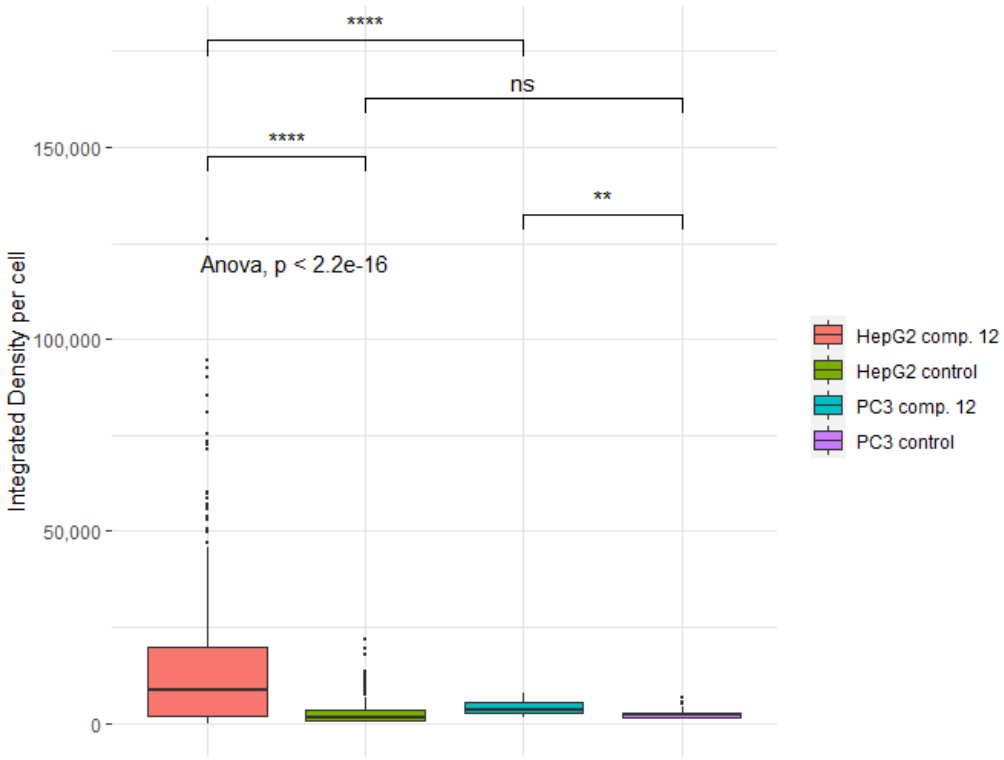
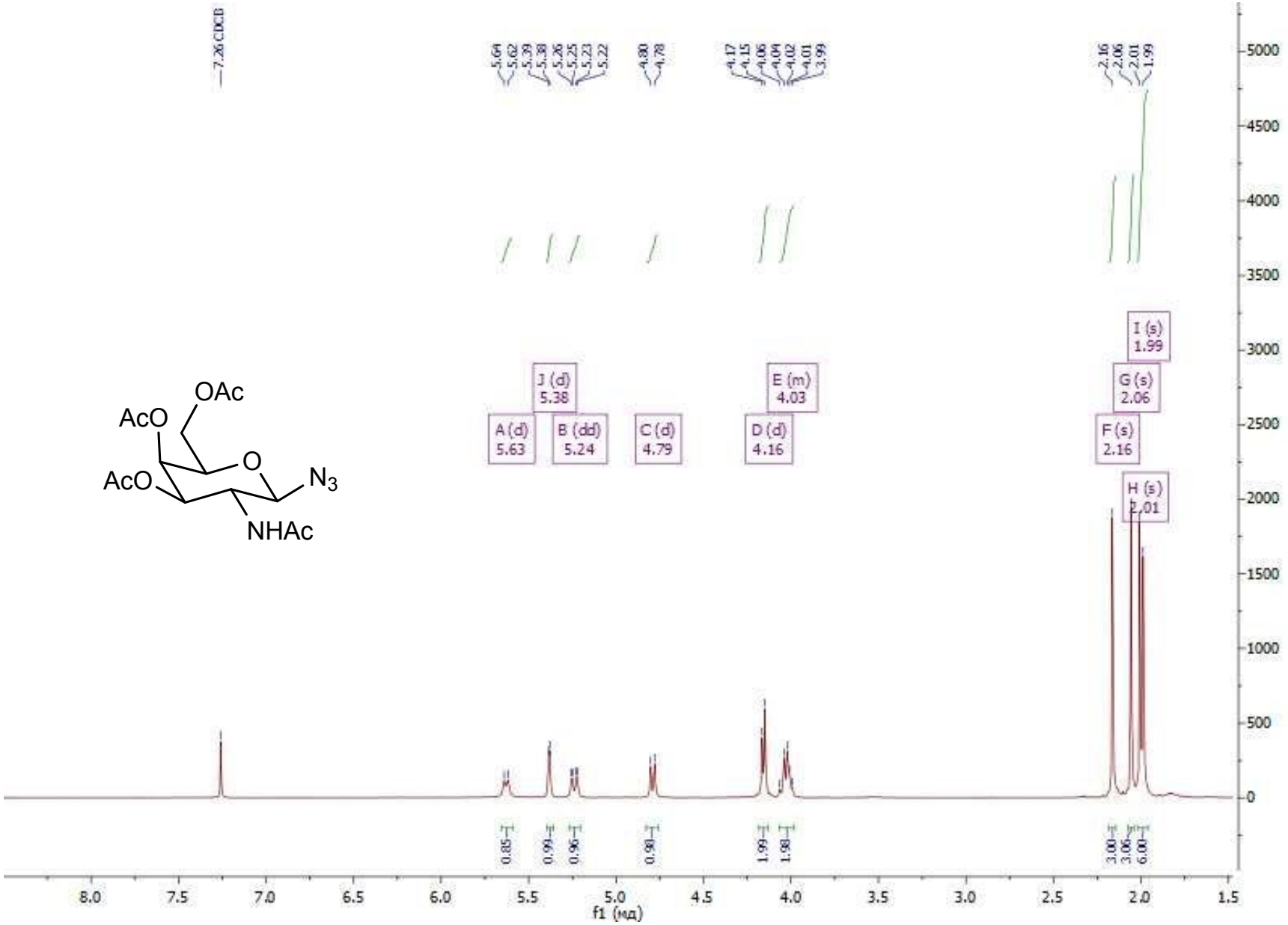
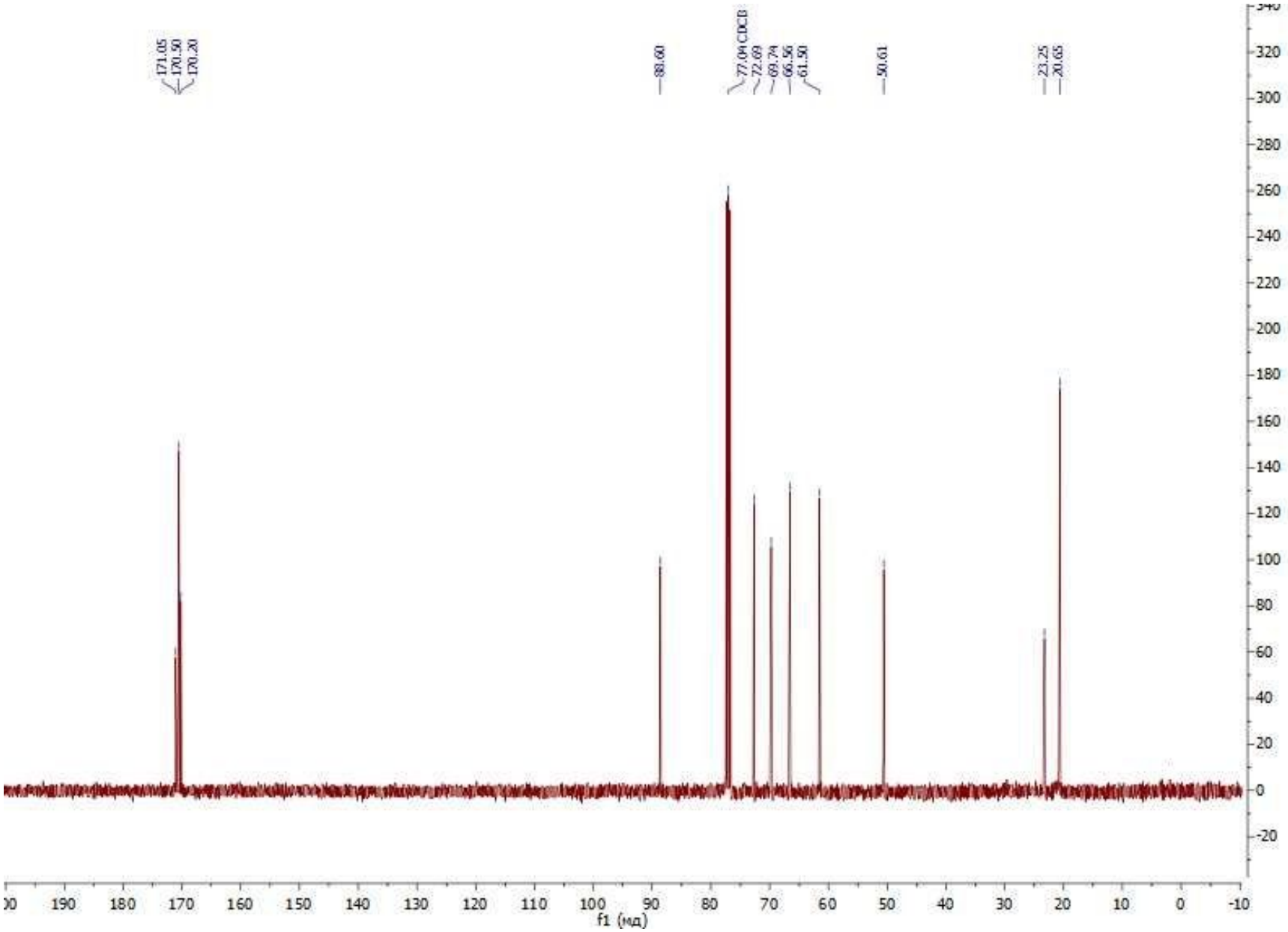


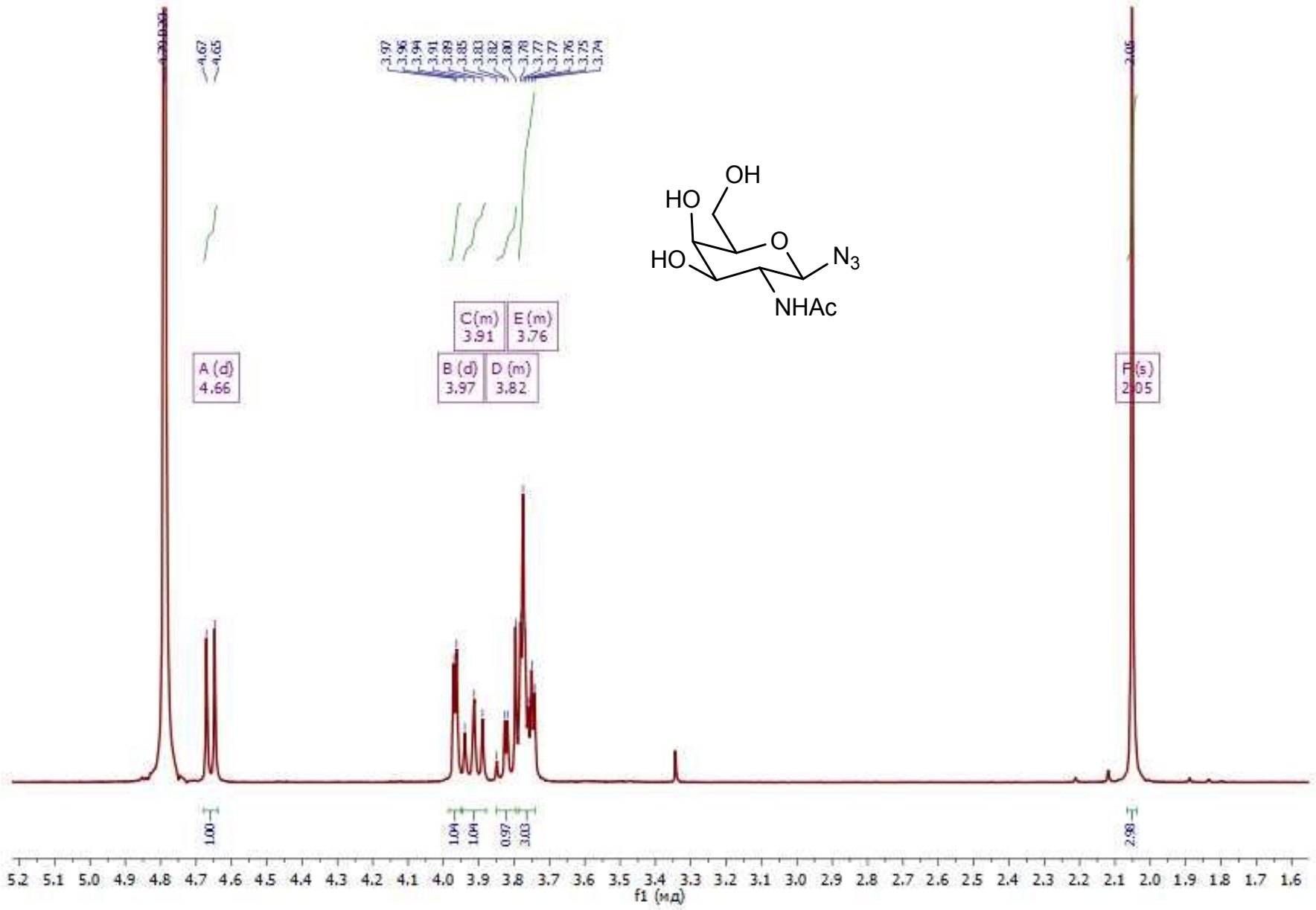
Figure S7. Cell fluorescence intensity of untreated HepG2 and PC3 cells and cells treated with **12** (5 µM) for 1 hour. ** p<0.01, **** p<0.0001, ns - non-significant.



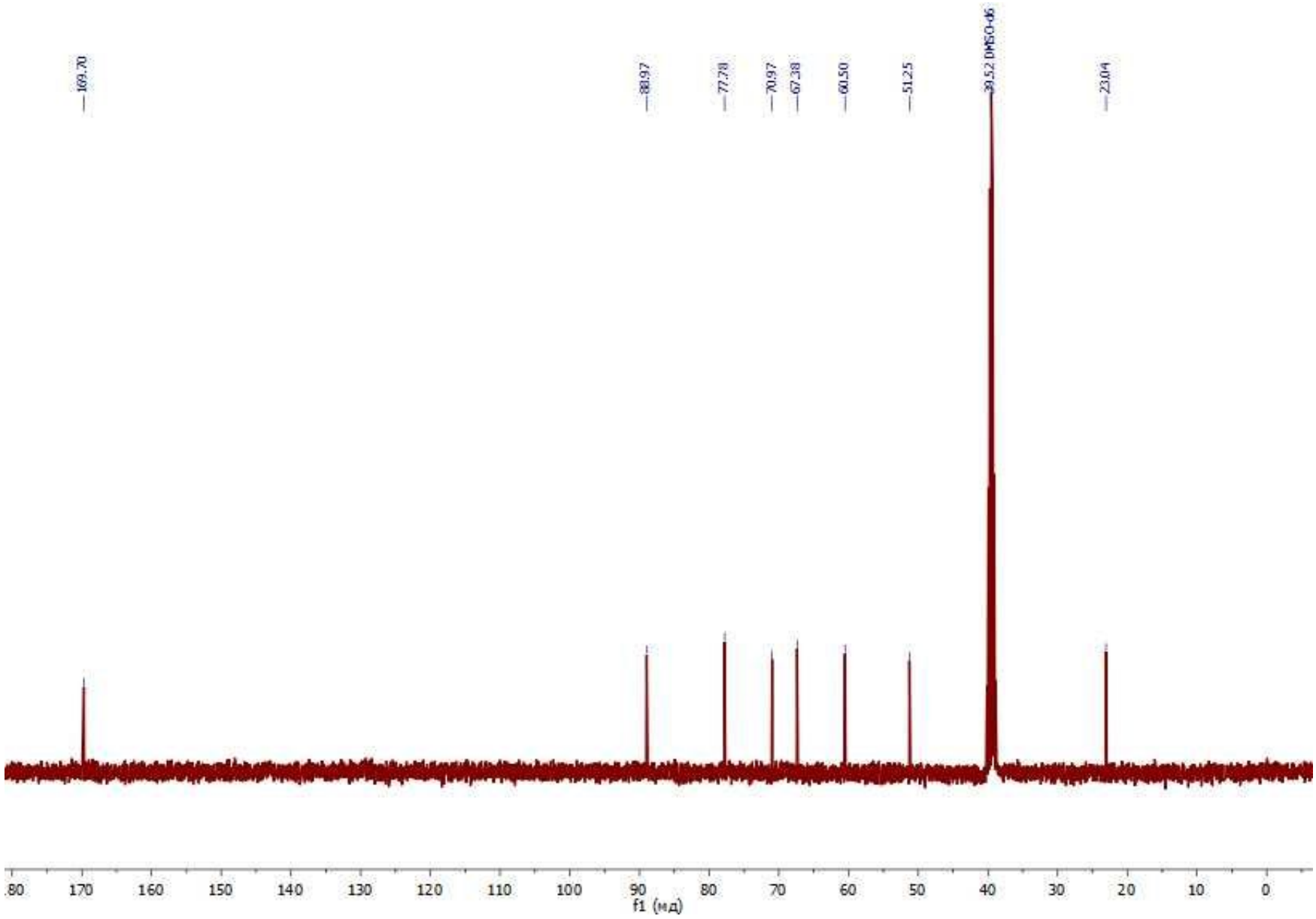
^{1}H NMR of Compound 2



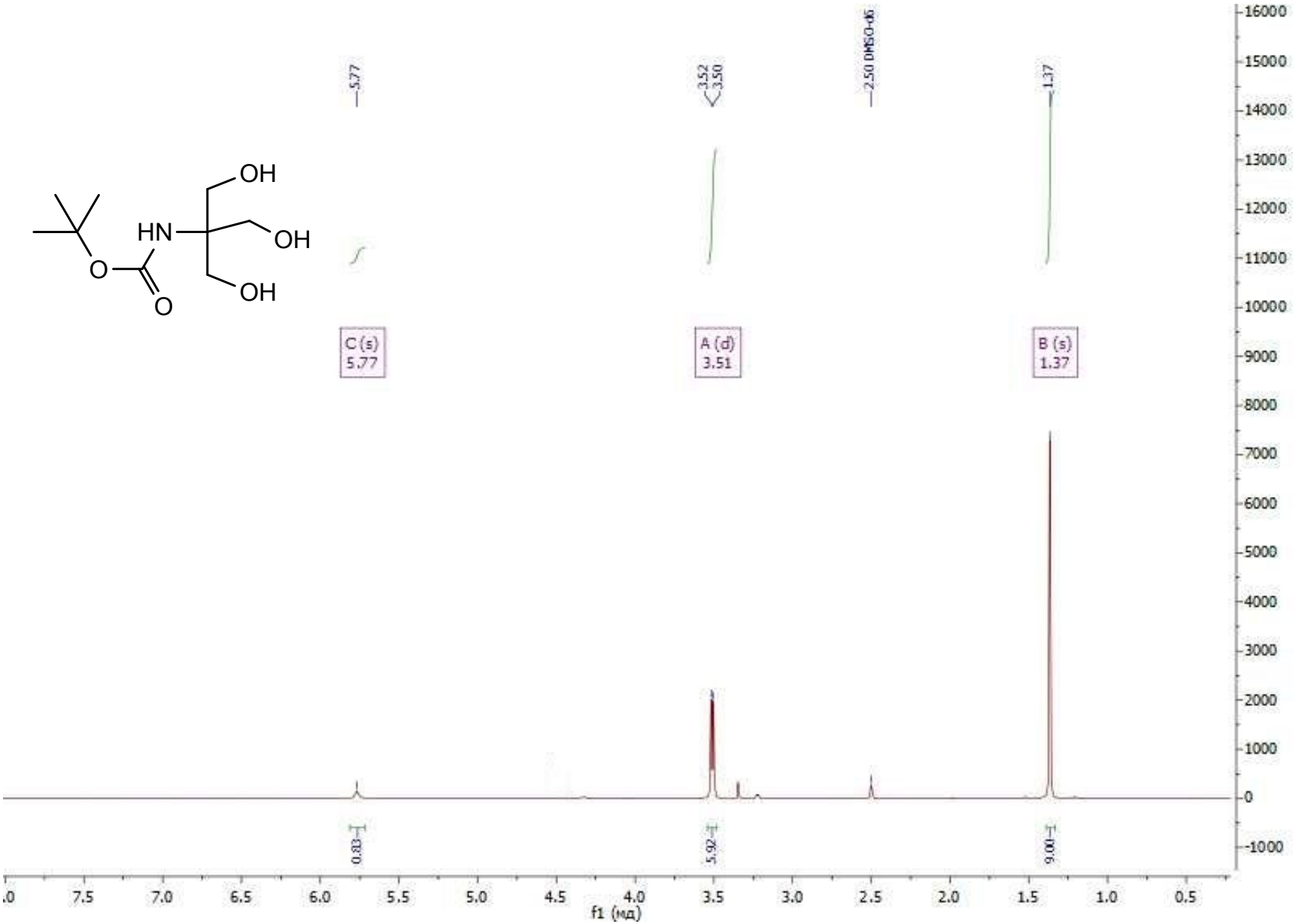
^{13}C NMR of Compound 2



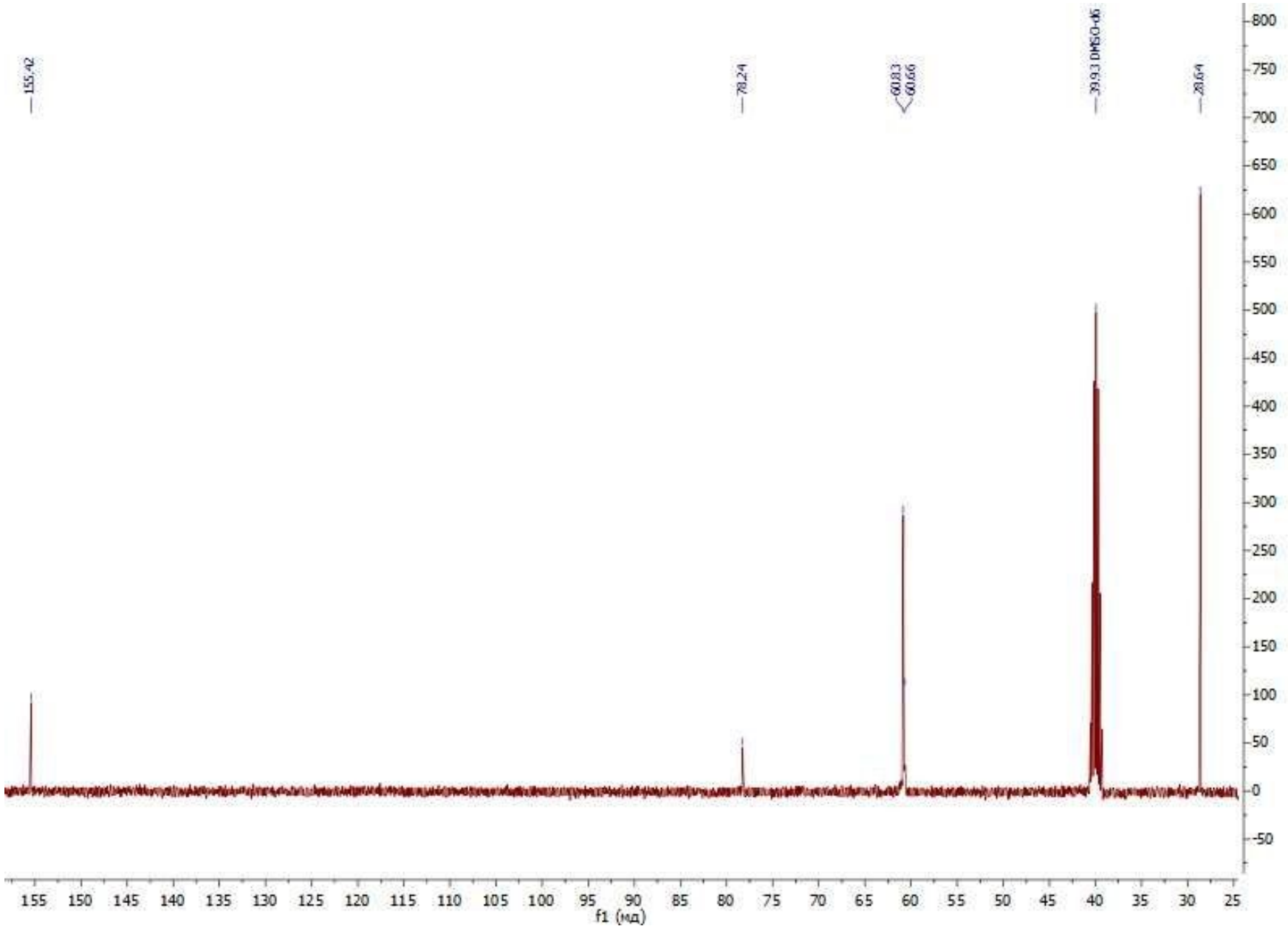
^{1}H NMR of Compound 3



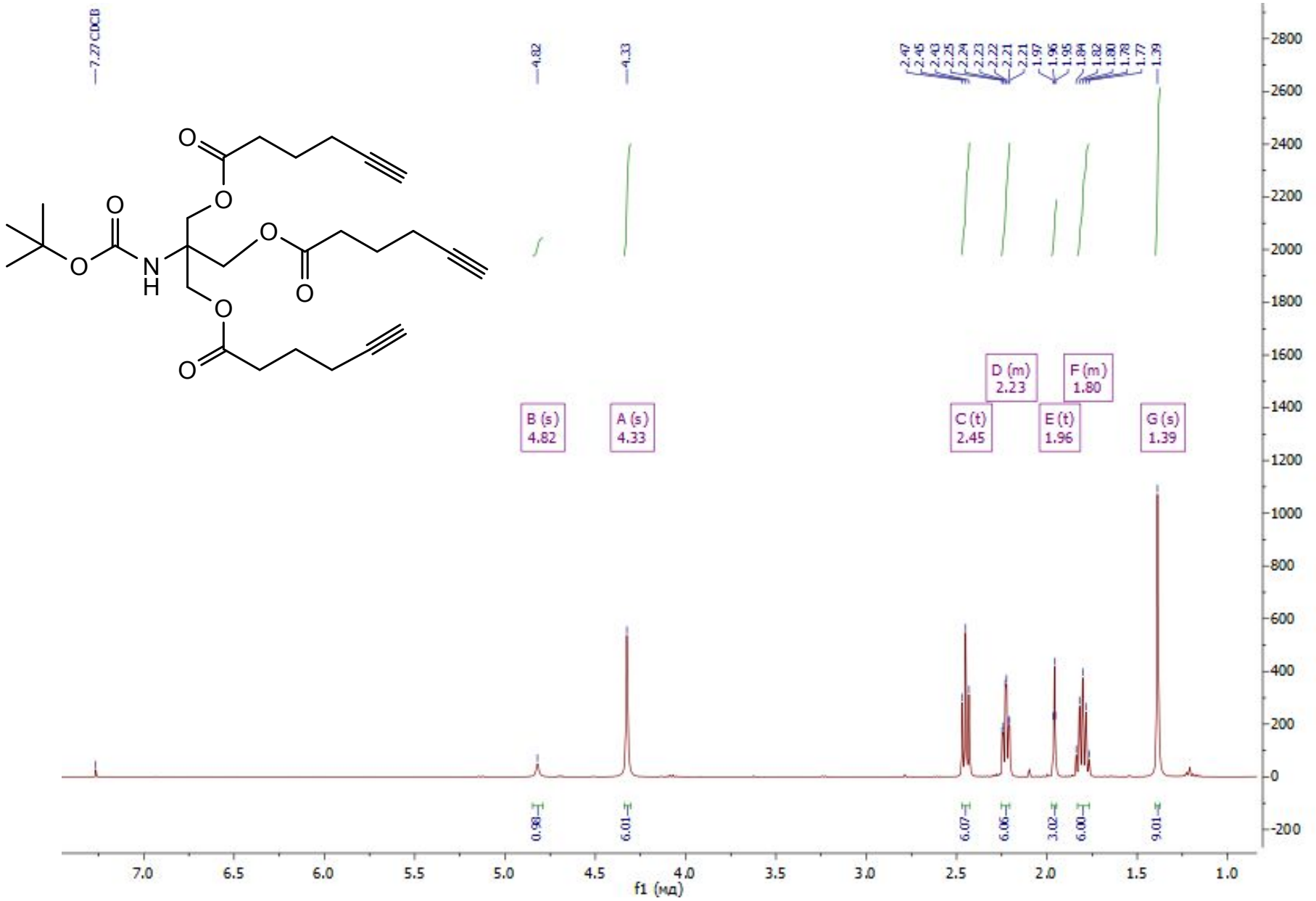
^{13}C NMR of Compound 3



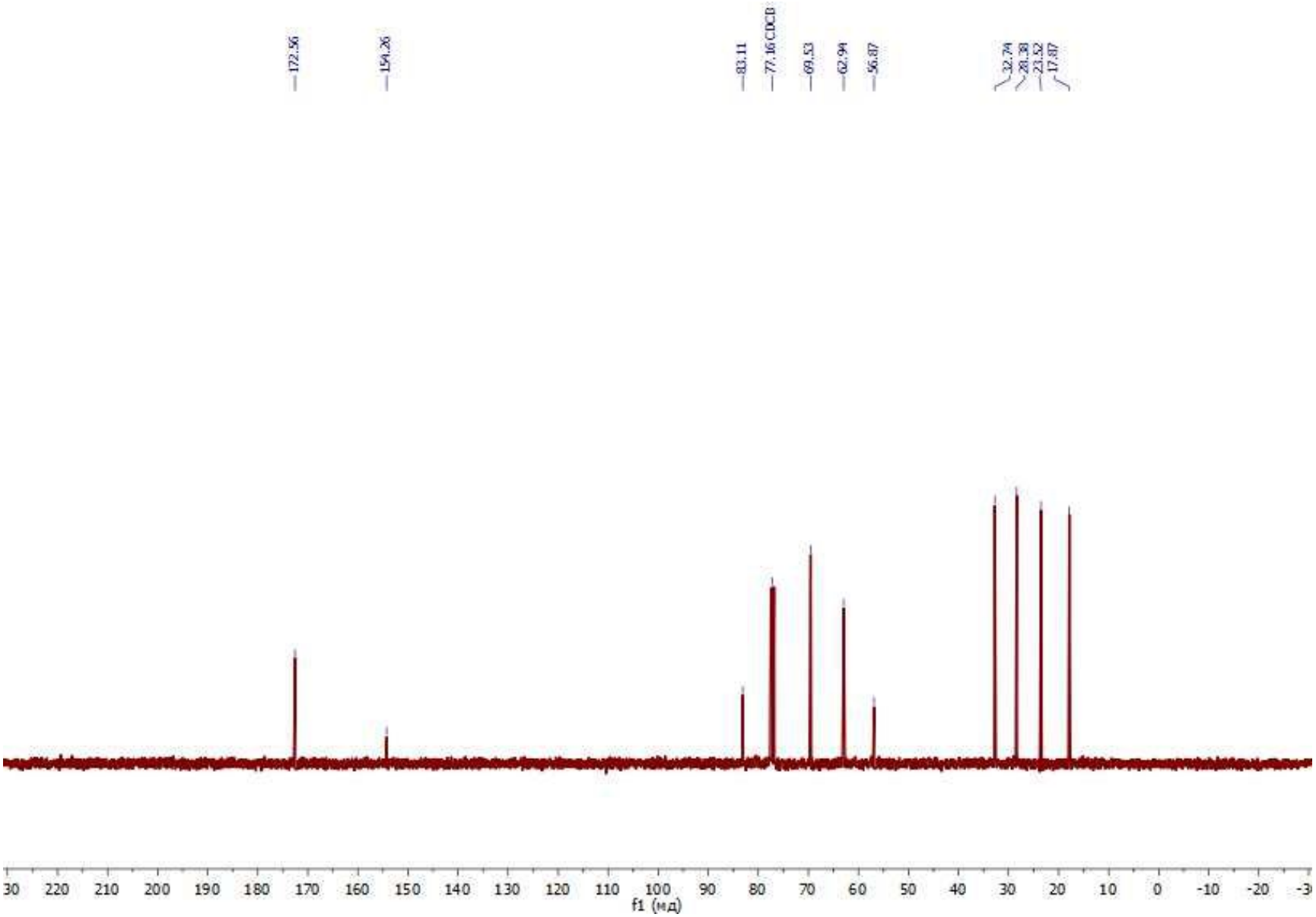
[1]H NMR of Compound 6



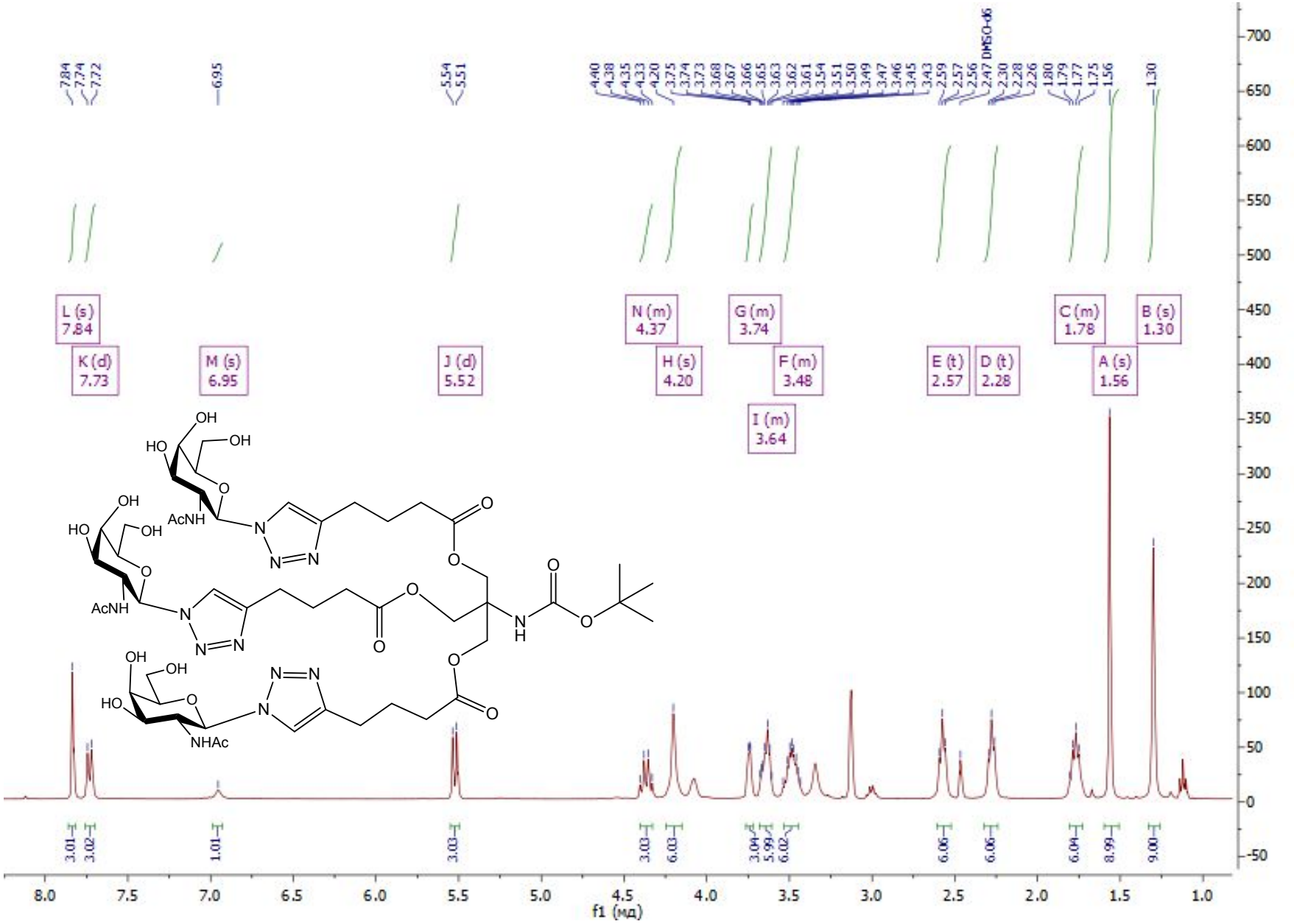
^{13}C NMR of Compound 6



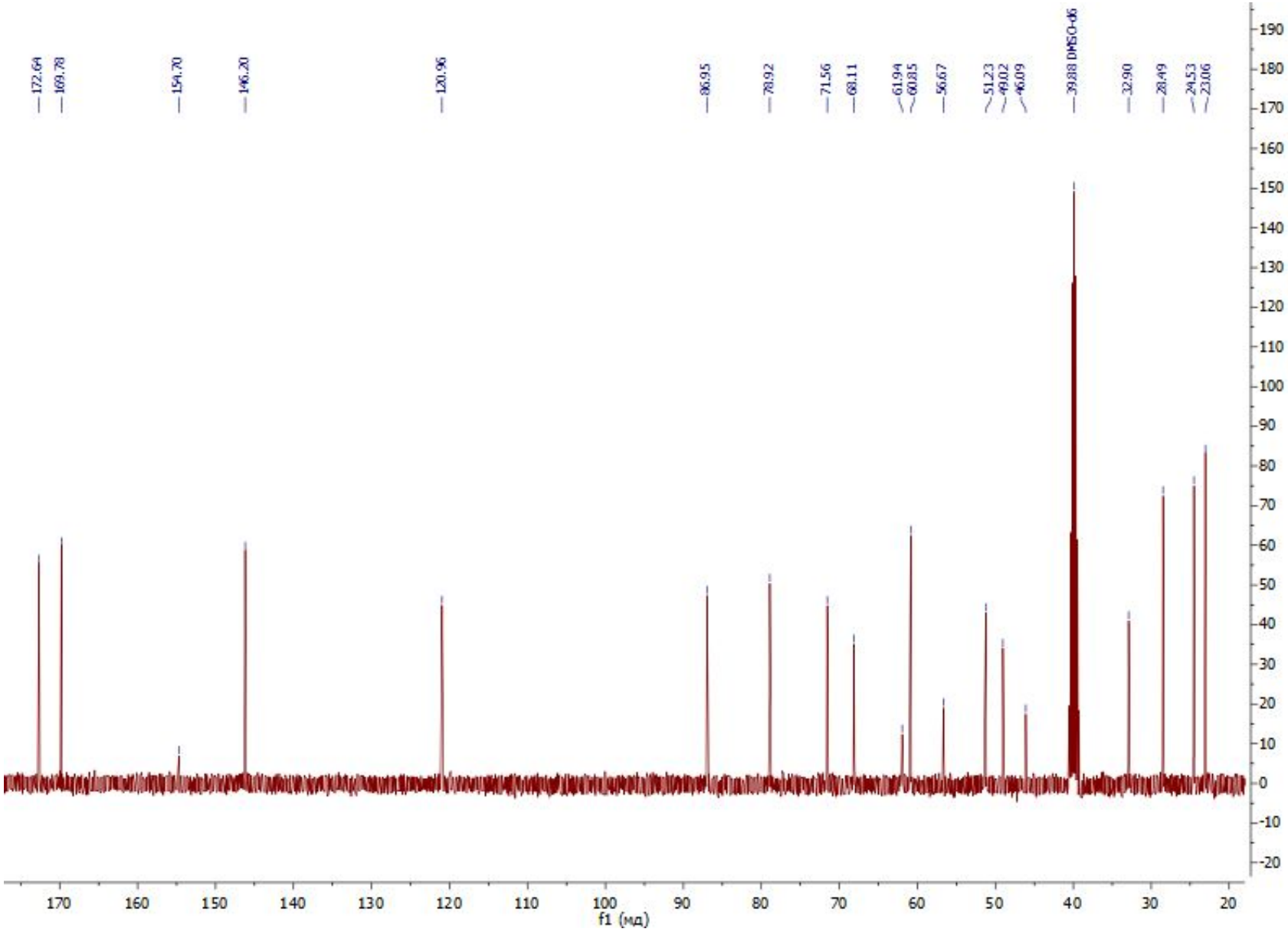
[1]H NMR of Compound 7



^{13}C NMR of Compound 7



^{1}H NMR of Compound 8



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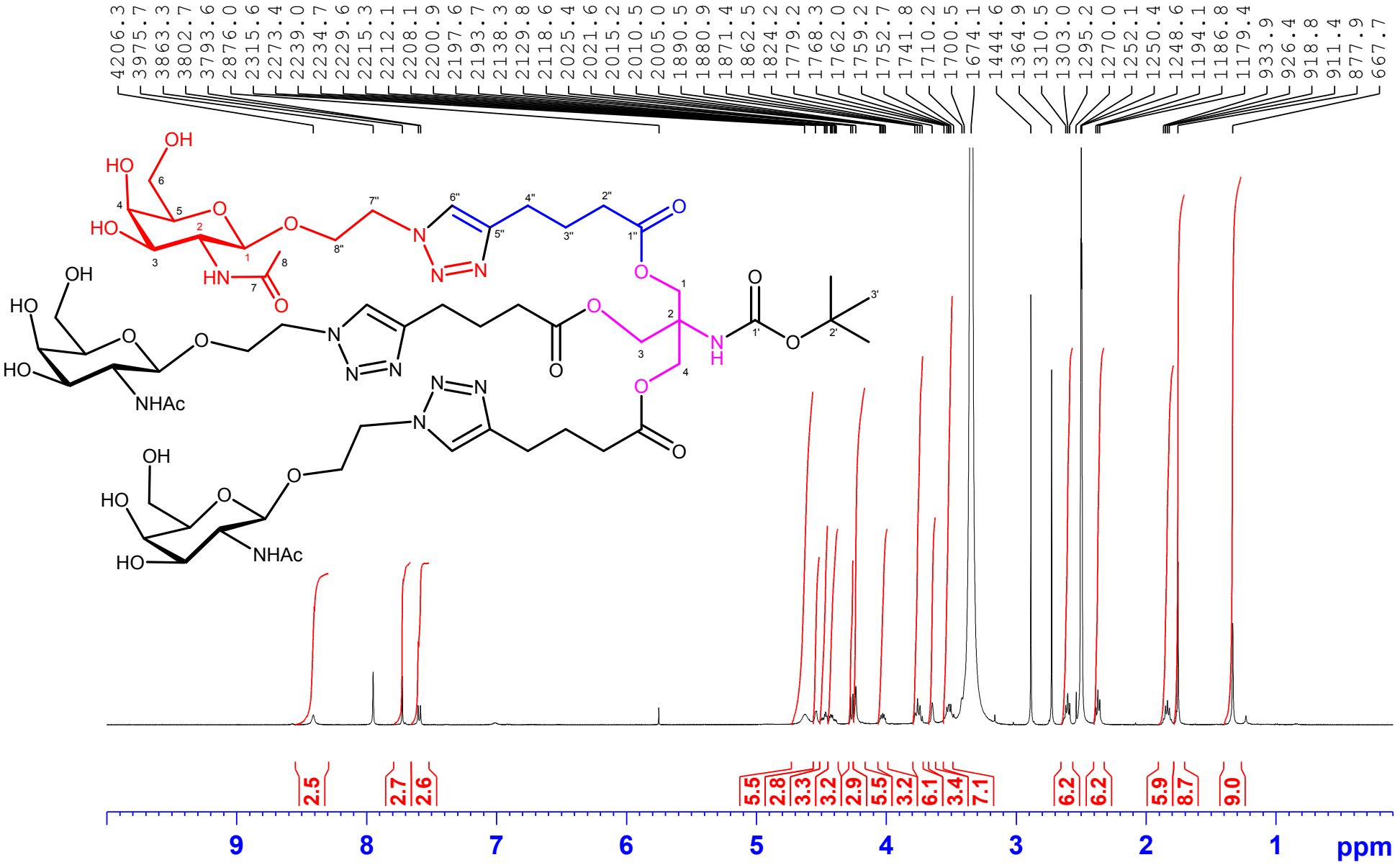
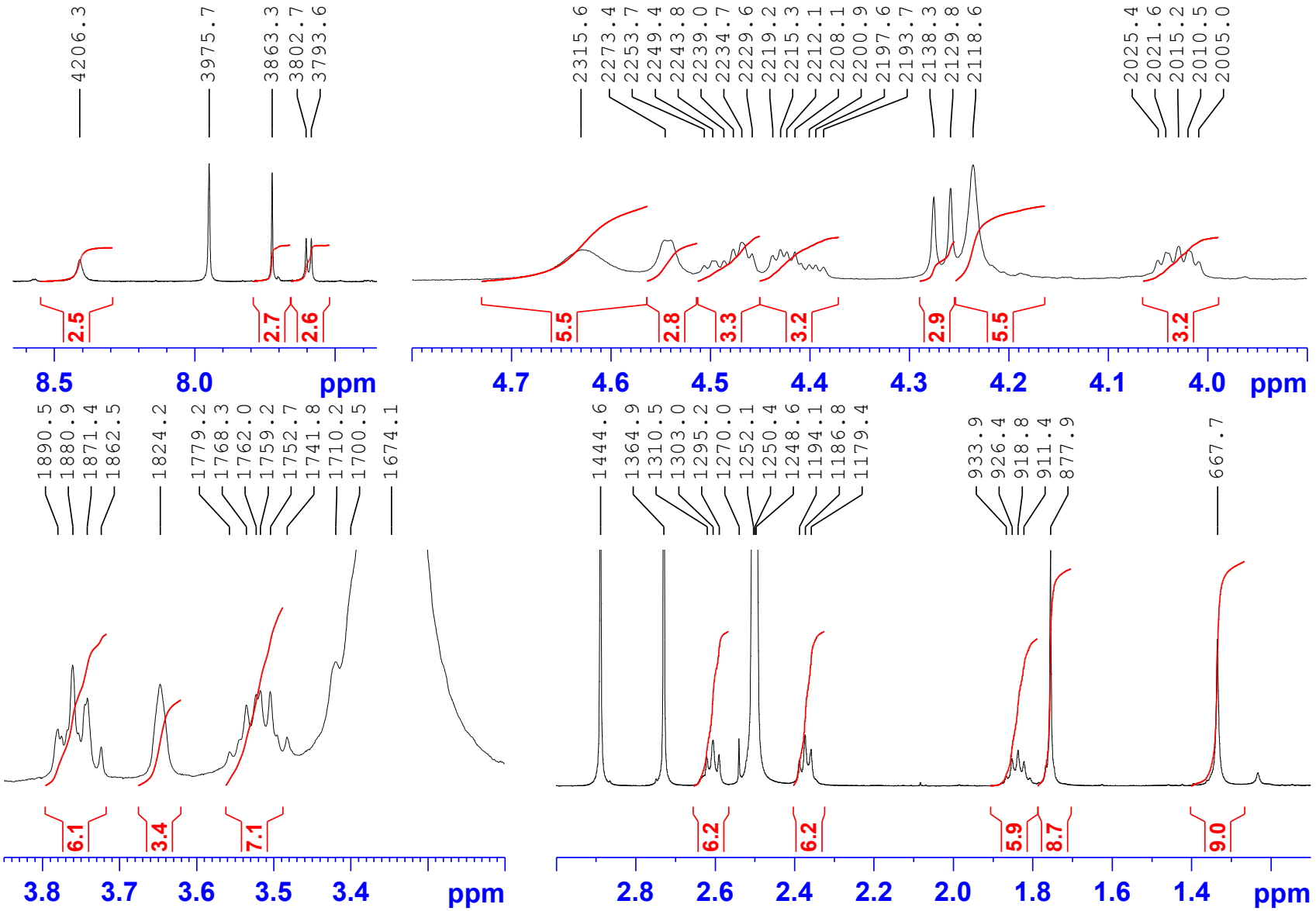
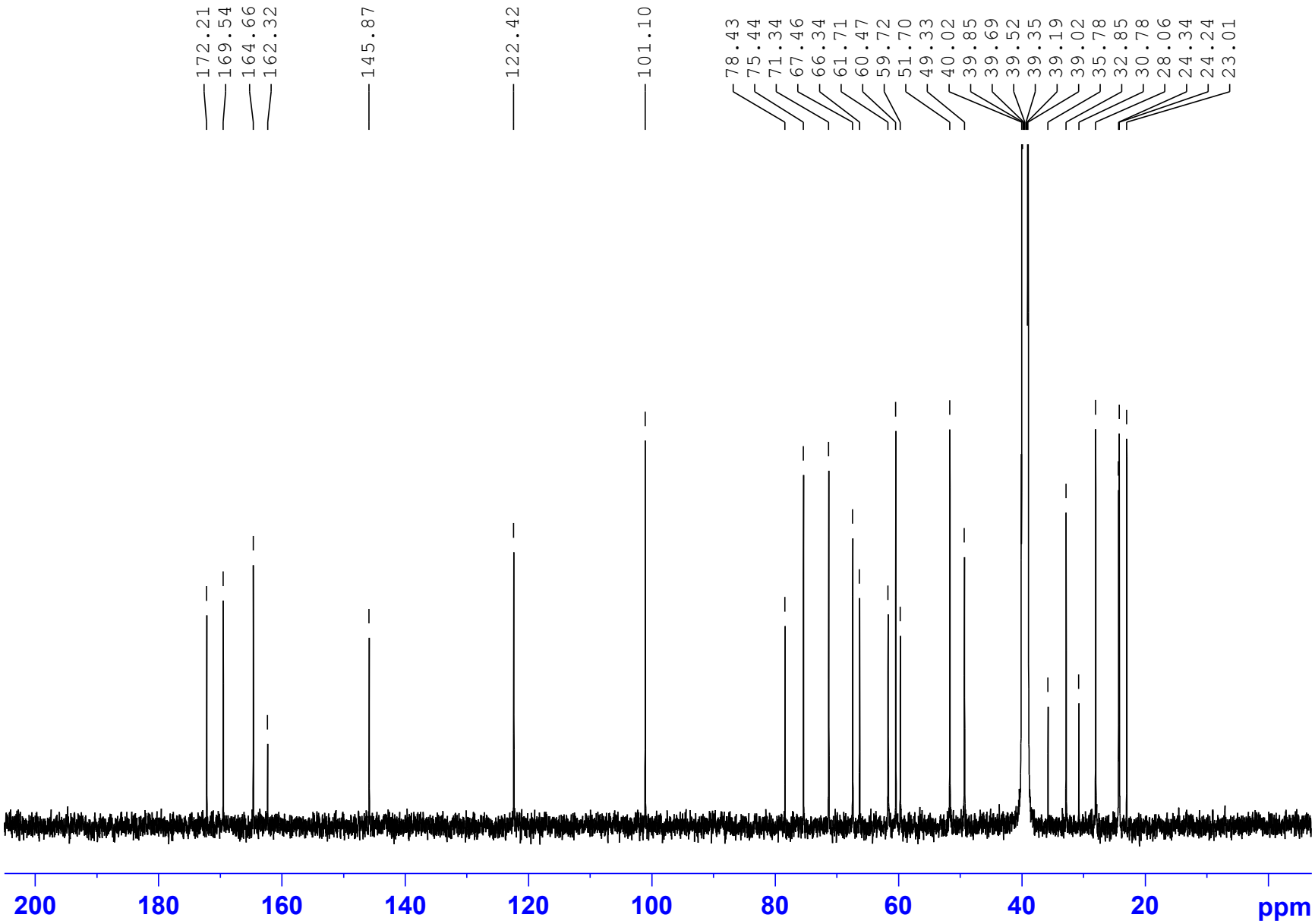


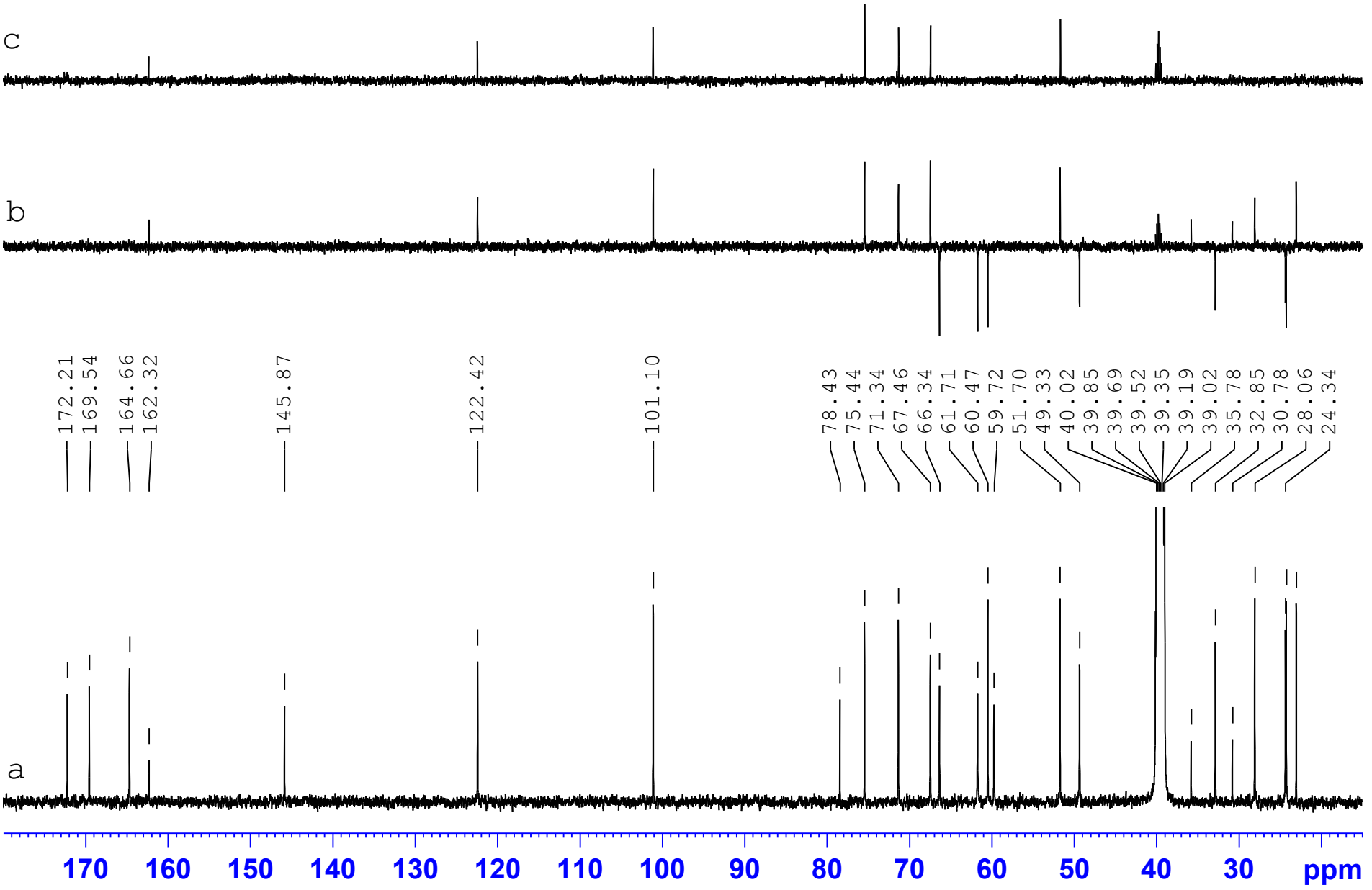
Complete ^{1}H NMR spectrum of Compound 9



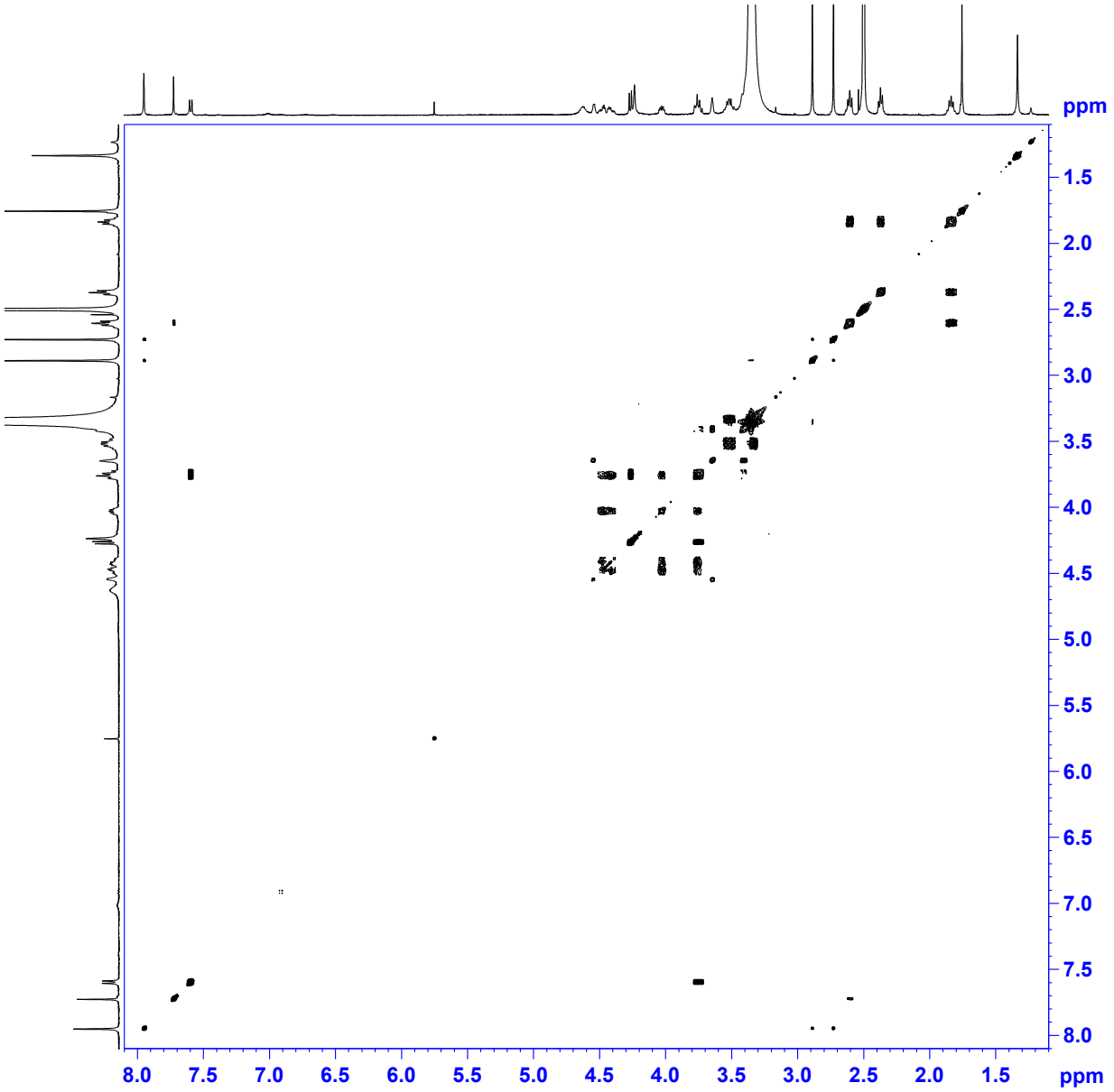
Expanded ^{1}H NMR spectrum of Compound 9



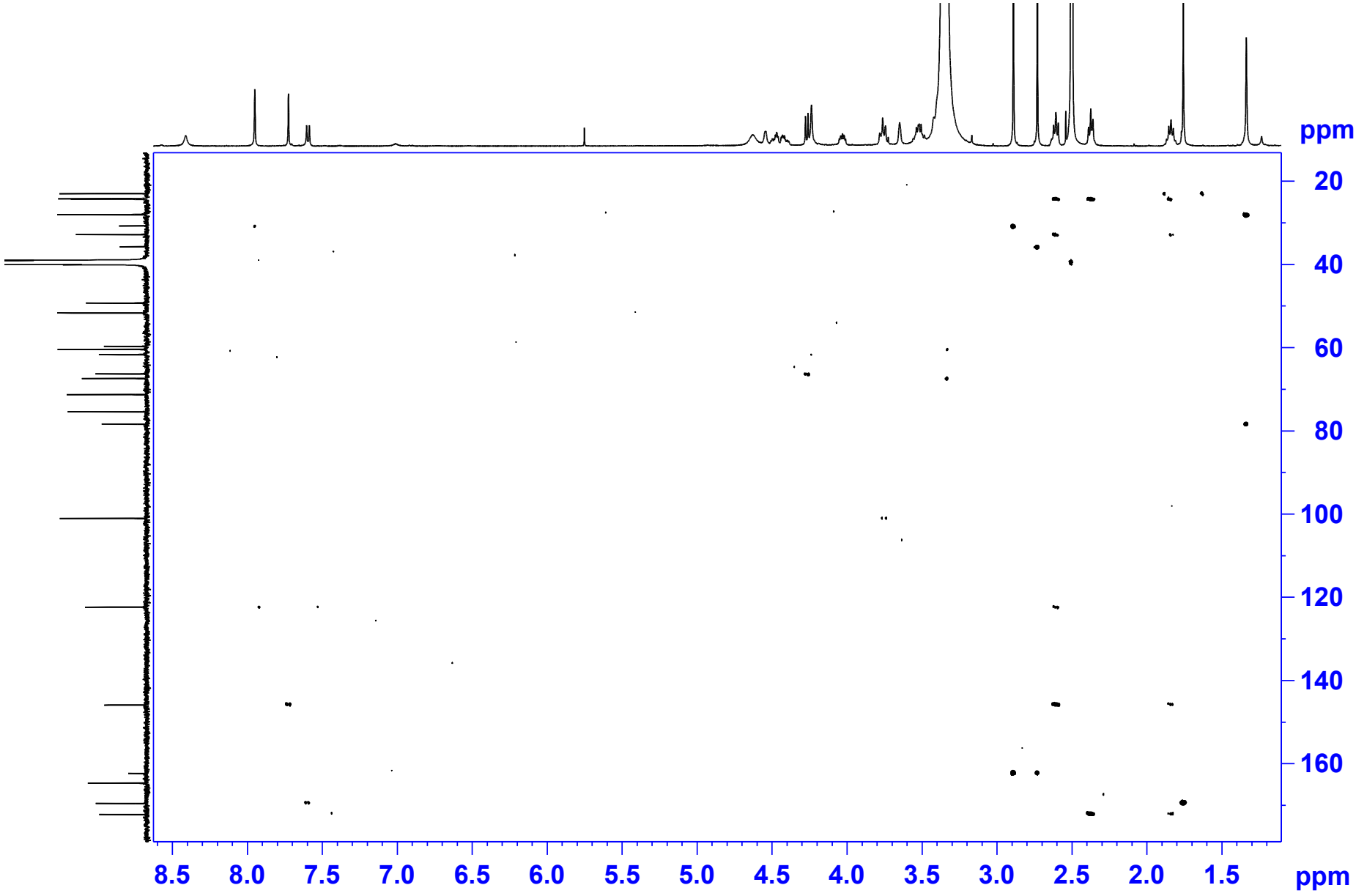
Complete $^{13}C\{^{1}H\}$ spectrum of Compound 9



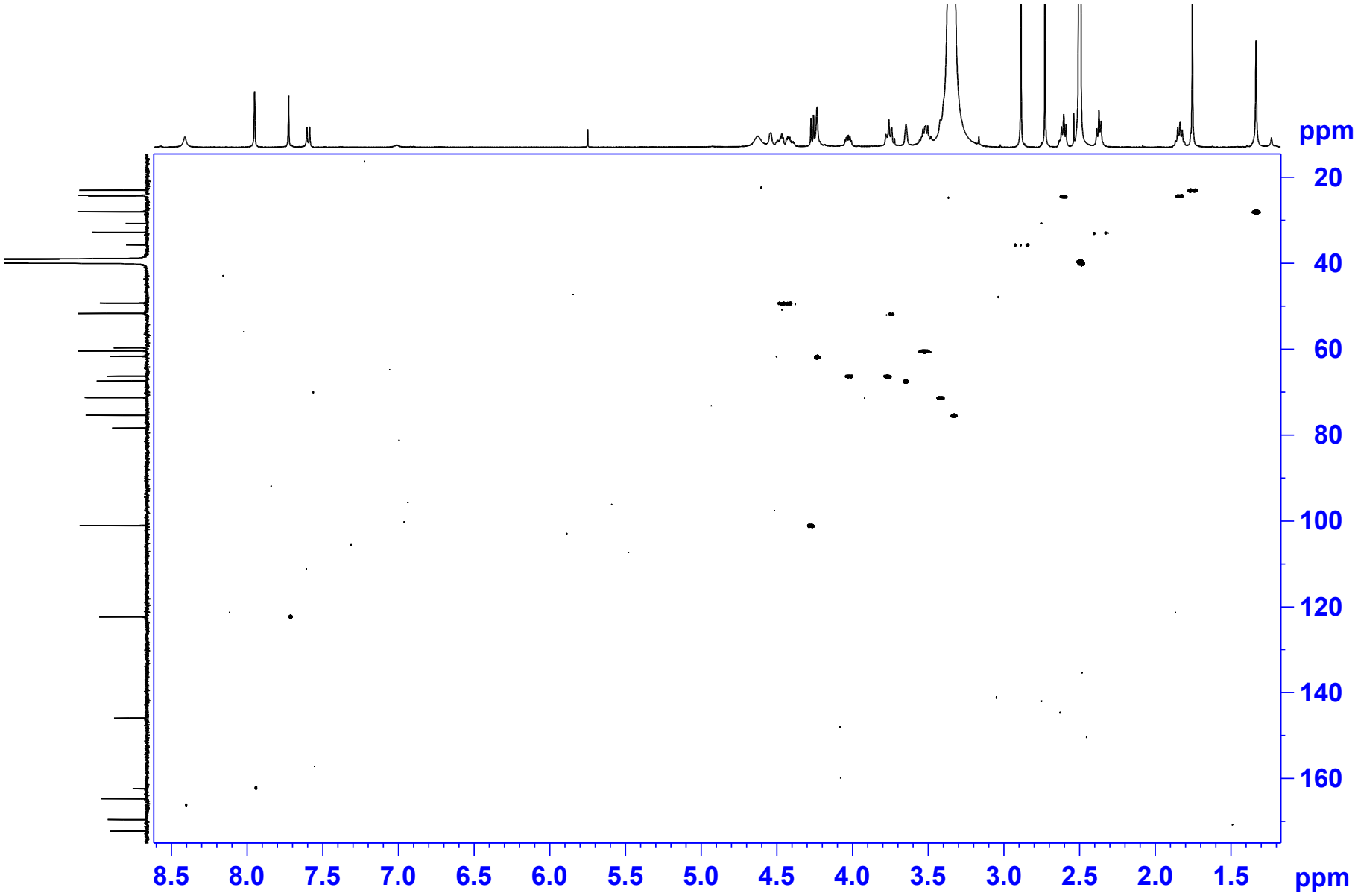
DEPT editing ^{13}C NMR spectrum of compound 9: a) $^{13}C\{^{1}H\}$ spectrum; b) DEPT-135; c) DEPT-90



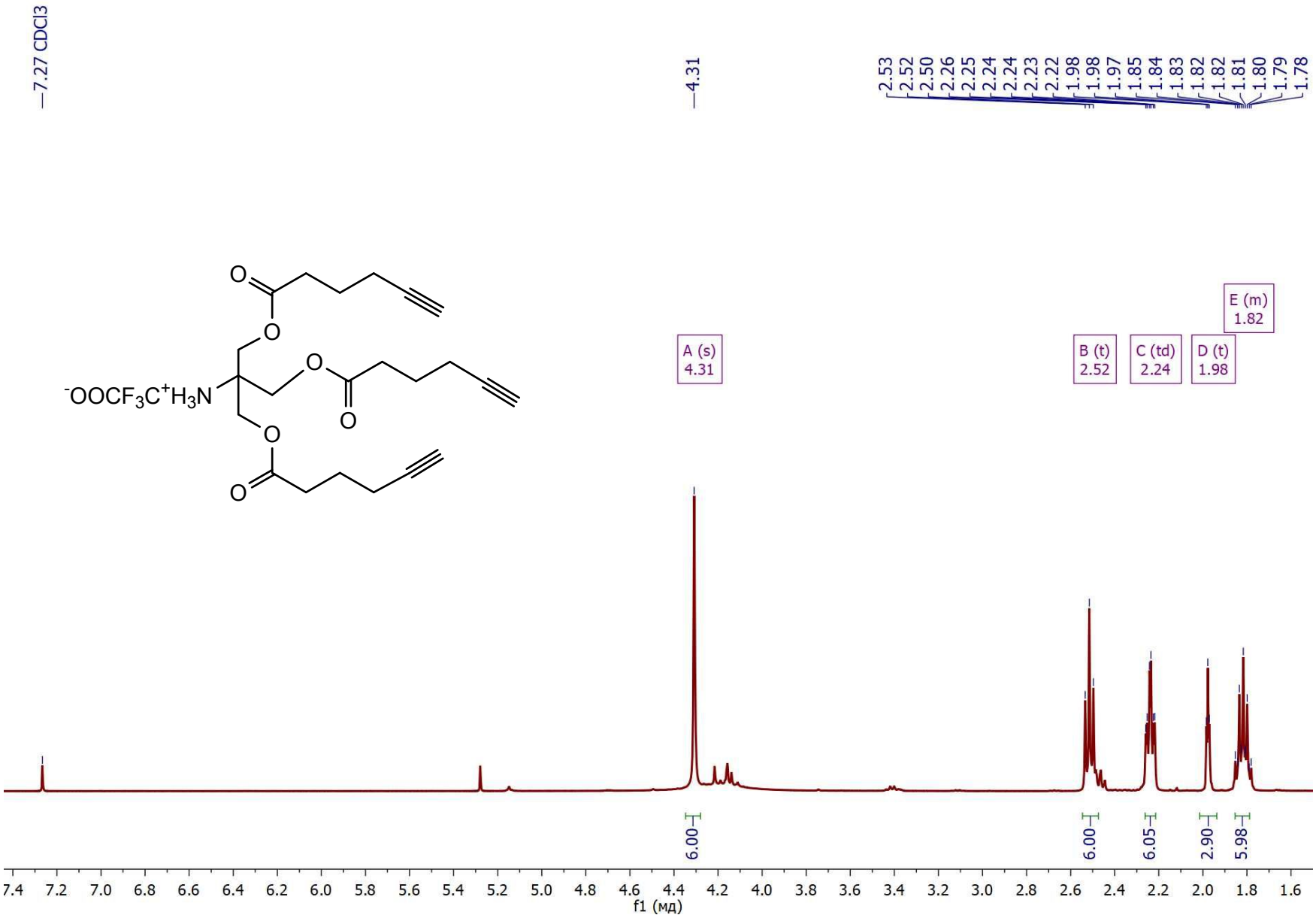
{^{1}H, ^{1}H} COSY spectrum of compound 9



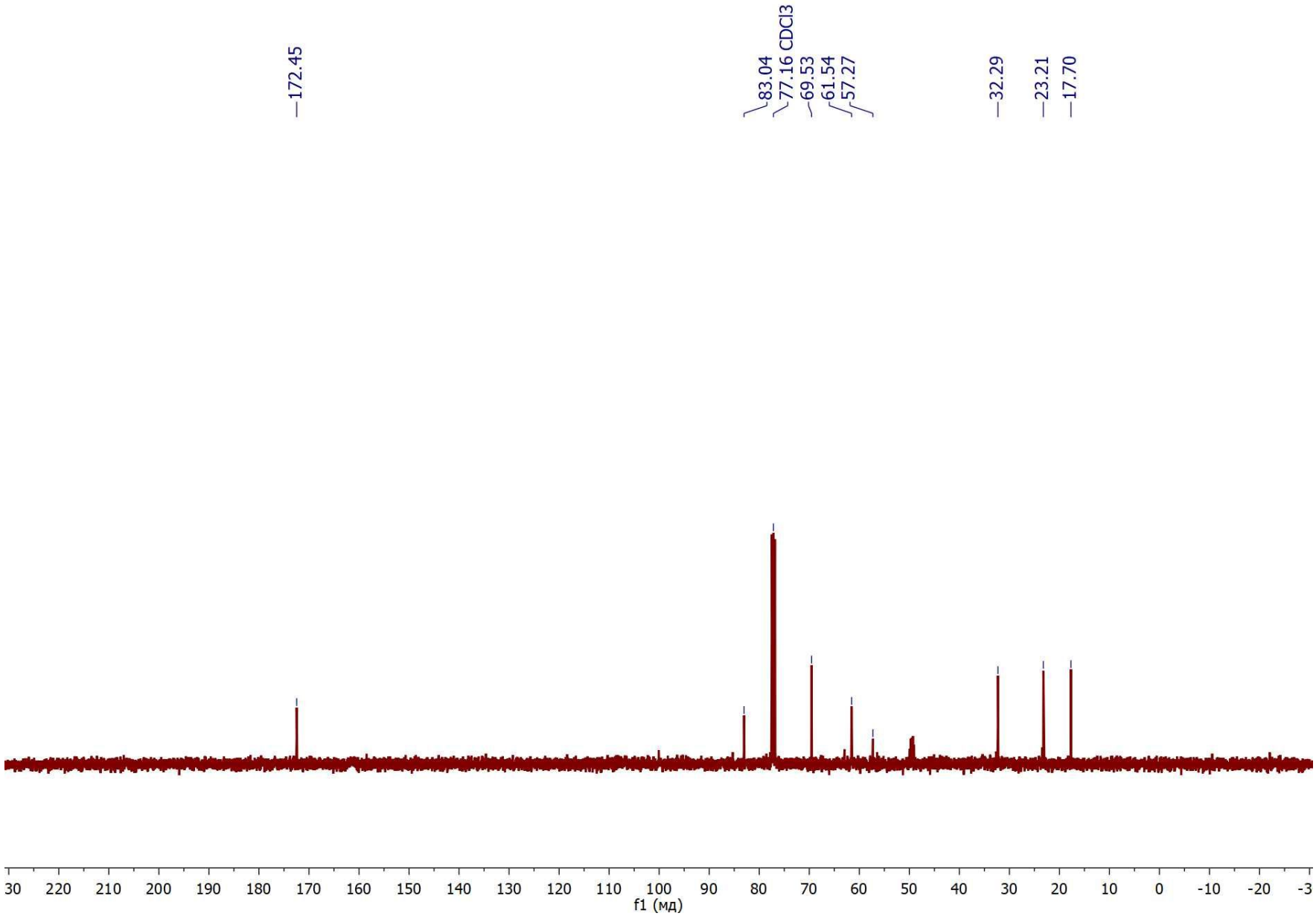
{^{1}H, ^{13}C} HMBC spectrum of compound 9



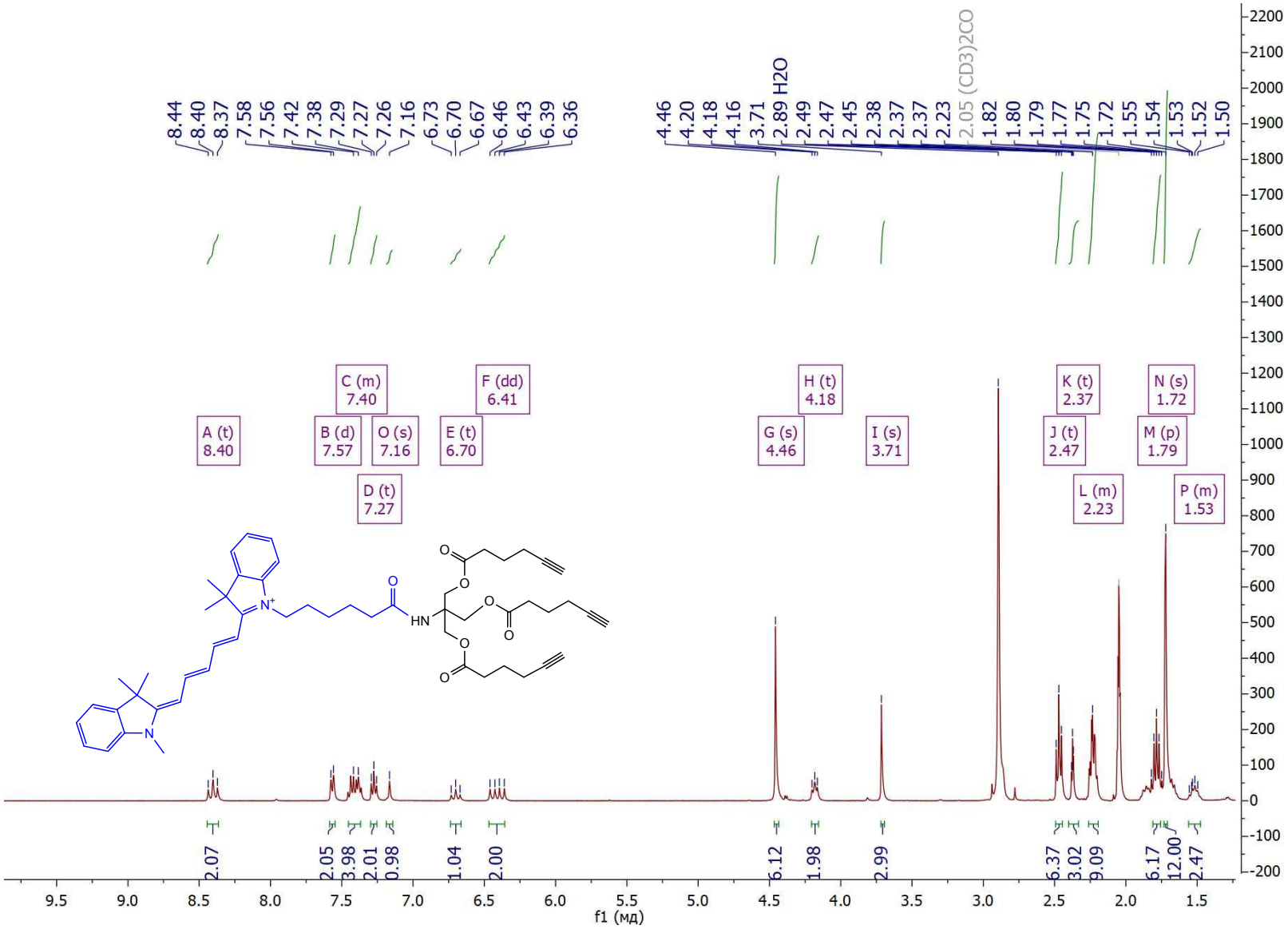
{^{1}H, ^{13}C} HSQC spectrum of compound 9



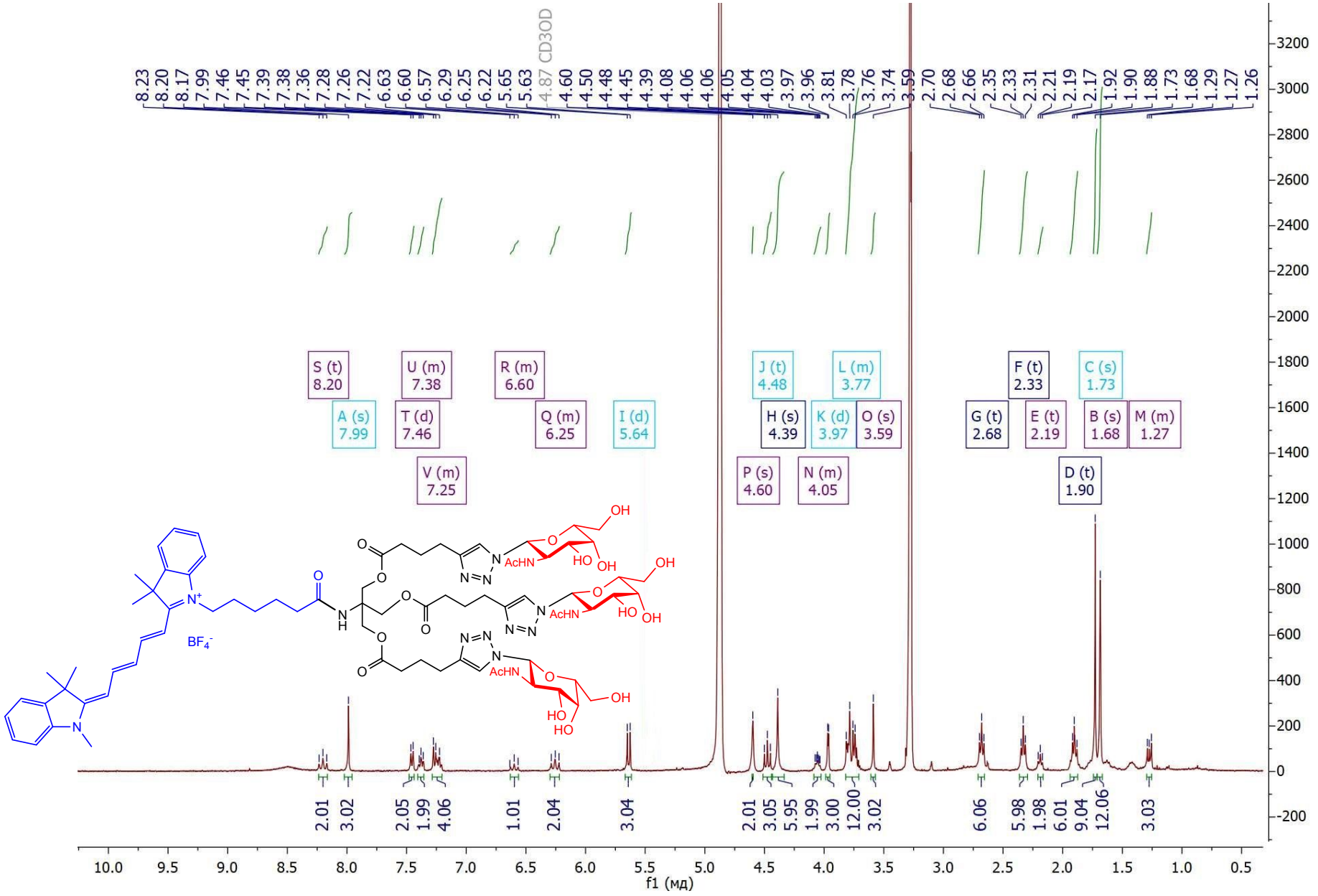
^{1}H NMR of Compound 10



^{13}C NMR of Compound 10



^{1}H NMR of Compound 11



^{1}H NMR of Compound 12