Chemical Probes That Selectively Recognize the Earliest Aβ Oligomers in Complex Mixtures

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Supplemental Information

Materials and experimental protocols

Abbreviations Amyloid-β (Aβ), phosphate-buffered saline (PBS), tris-buffered saline (TBS), DIC, HOBt, dimethylformamide (DMF), dimethylsulfoxide (DMSO), methylene chloride (DCM) , bovine serum albumin (BSA), horseradish-peroxidase (HRP), trifluoroacetic acid (TFA), cerebrospinal fluid (CSF).

Materials: Aβ (1-40) was obtained from Anaspec (San Jose, CA) and Aβ (1-42) from EZBiolab (Westfield, IN). Fmoc-protected amino acids, biotin, and Wang resin were purchased from Anaspec. Unless otherwise noted, all solvents were purchased from Fisher. DIC and HOBt were purchased from Fluka (Milwaukee, WI) and GenScript (Piscataway, NJ), respectively. Microwave-assisted peptide synthesis was performed on a Biotage Initiator EXP using the times and temperatures described. All NMR data were collected and analyzed on a Varian 600 MHz system using VnmrJ™ version 2.2 revision C. Mass spectrometry data were obtained on a Micromass LCT time-of-flight mass spectrometer in the ES+ mode. All HPLC runs were performed on a Beckman-Coulter HPLC system measuring at 254nm with a Waters Spherisorb 10μm ODS2 4.6X250mm analytical column at a flow rate of 1 mL/min (acetonitrile:water gradient containing 0.1% trifluoroacetic acid). Cerebrospinal fluid was collected by the University of Michigan Hospitals. Samples were pooled from six non-coded donors. An IRB notification (HUM00033486) of "not regulated" status was received on 9/2/2009.

Aβ preparation and crosslinking Aβ (1-40) and Aβ (1-42) were prepared as previously described (Reinke and Gestwicki, *Chem Biol Drug Des*, 2007). Briefly, 1 mg was suspended in hexafluoroisopropanol (Fluka), aliquoted, dried down to a film under nitrogen, and stored at -30 °C. Immediately prior to the start of an experiment, 0.1 mg was suspended in PBS pH 7.2 (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) containing 1% DMSO to a final concentration of 25 μM, vortexed for 15 seconds and sonicated for 30 seconds. Aβ (1-40) was cross-linked as described (Bitan and Teplow, *Acc Chem Res*, 2004). Briefly, ammonium persulfate (1 mM) and Tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate (Ru(II)) (50 μM) were added to Aβ (final concentration 22.5 μM). The mixture was mixed by pipetting and exposed to UV light for 1 second. Excess Ru(II) was then removed using ~ 5 mg Isolute SI-Thiol (EC) resin (Biotage) per 200 μL Aβ mixture until the solution was no longer yellow. The sample was then gently centrifuged

(5,000 rpm) for 30 seconds to pellet the resin, and the supernatant (22.5 μM Aβ) was used in the gel electrophoresis experiments.

Native gel-electrophoresis, silver staining, and Western blot with 6E10 Varying amounts (0.4 – 2.0 μg) of crosslinked Aβ (1-40) or non-crosslinked (1-42) were separated by native gel gradient electrophoresis (10-20% tric-tricine, Invitrogen, Carlsbad, CA) using non-denaturing, non-reducing loading buffer (300 mM tris-HCl, 8% glycerol, 0.01% bromophenol blue). Gels were either stained using the ProteoSilver Silver Stain Kit (Sigma) according to the manufacturer's protocol, or transferred (1 hour, 180 milliamps, 4 °C) to 0.2 μm Protran nitrocellulose (Whatman, LOCATION). The membrane was blocked with 3% BSA in TBS-T (140 mM sodium chloride, 25 mM tris, 0.1% Tween-20) for 90 minutes at room temperature. The membrane was washed (3 X 5 minutes) with TBS-T and incubated overnight in 1:2000 6E10 (Covance, Dedham, MA) containing 3% BSA in TBS-T at 4 °C. The membrane was then washed again (3 X 5 minutes), incubated for one hour with HRP-conjugated goat anti-mouse IgG (Abcam, Cambridge, MA) in TBS-T, washed (3 X 5 minutes) and developed using the Western Lightning Plus-ECL kit according to the manufacturer's protocol (PerkinElmer, Waltham, MA).

Western blot with biotinylated KLVFF peptides Native gel-electrophoresis with Aβ (1-40) and Aβ (1-42) and transfer to nitrocellulose were performed as described. Ponceau staining was performed to ensure proper transfer to the membrane. Membranes were then spatially separated in order to probe the samples with individual peptides. The membrane was placed in a petri dish (100 mm X 15 mm) and 10 mL of 2 μM KLVFF peptide (0.5% BSA in TBS-T) was added. Note that the peptide and BSA were mixed immediately prior to the start of the experiment. The membrane was incubated with the peptide for 1 hour followed by TBS-T washes (10 X 5 minutes each), and incubation with 1 μg/mL streptavidin-HRP (Pierce, Rockfod, IL) for 20 minutes. Following a final TBS-T wash step (12 X 5 minutes each), the membrane was developed using the Western Lightning Plus-ECL kit according to the manufacturer's protocol (PerkinElmer, Waltham, MA). Each step was performed on a rocker at room temperature. All blots were exposed for 3, 10, 30 and 60 seconds.

Molecular dynamics simulations: The AMBER 10[1] package was used to perform the unrestrained all-atom Molecular Dynamics (MD) simulations. Coordinates of Aβ (16-42)-fibril were obtained from PDB[2] (PDB: 2BEG[3]), and the first frame of the NMR ensembles was used in the system construction. Because the N-termini of Aβ (16-42)-fibril are unresolved, an acetyl capping group was added to each Aβ (16-42)-fibril chain. The synthetic KLVFF-based ligand was constructed and placed on the Aβ chains using Molecular Operating System (MOE)[4] and PyMOL.[5,6] The initial placement of the KLVFF-based ligand was guided by the position of the KLVFF in the adjacent monomer. Parameters for the linker and biotin groups were generated using AM1-BCC charges and the ANTECHAMBER module of AMBER[7], while the protein was parameterized using the FF99SB force field.[8] Counter ions were added to neutralize both the dimer (3 Na+) and trimer (5 Na+) systems. Boxes of 3931 and 3986 TIP4P water were used to solvate the systems, respectively.

Following assembly of the bound ligand-Aβ system, MD simulations were run in the NPT ensemble and the SHAKE algorithm[9] was used to constrain all bonds to hydrogen atoms. A 1-fs time step was used along with a 10 Å cut-off for non-bonded interactions and particle mesh Ewald for long-distance electrostatics. For both systems, hydrogen atoms were first minimized, followed by the side chains and then all atoms. The system were then equilibrated by gradually heating the water molecules from 10 to 310 K over 50 ps, followed by water equilibrium for 100 ps at 310 K with protein restrained. This was followed by a full-system heating from 10 to 310 K over 30 ps and a full system equilibration with unrestrained protein for 200 ps. The production phase was run for 4 ns at 310 K. To measure the

distance between the two Aβ peptide "ends" over the MD trajectory, the distance between the carbonyl carbons of Lys7 was generated using PTRAJ module of AMBER. To estimate this distance in the tetramer complex, we have added the difference between the medians of the dimer and trimer simulation (approximately 4.8 Å) to the median of the trimer simulation (19.3 Å).

Peptide synthesis, ^{1}H NMR, ^{13}C NMR, and MS characterization, and experimental data:

Microwave-assisted solid-phase Fmoc-peptide synthesis was performed generally as described.[10,11] Briefly, for each peptide, 1 eq of Wang resin (1mmol/g) was activated with 6 eq each of DIC and HOBt for 30 minutes stirring in DMF at room temperature, after which 0.6 eq DMAP and 10 eq Fmoc-Phe were added. The reaction mixture was then irradiated for 60 minutes at 60 °C (pre-stirring 30 seconds). The coupling efficiency was measured by filtering and washing (DMF, methanol, and DCM) 1-2 mg of resin, to which 3 mL of 20% piperidine in DMF was added in a quartz cuvette. The absorbance at 290 nm (A_{290}) was then measured after a 5 minute incubation and inverting the cuvette several times. The percentage of coupled resin was then calculated using the following equation: % coupled = [(A_{290}) / (mass of resin (mg)]*100%. The coupling efficiency for each peptide was > 70%. The resin was then washed with DMF, methanol, and DCM. The Fmoc group was then removed (20% piperidine in DMF) by irradiating the mixture for 3 minutes at 60 °C, and then resin was again washed as described.
The peptides were subsequently built using 3 eq of each Fmoc-protected amino acid, and 5 eq each of DIC and HOBt. The reagents were combined in DMF and, after 5 minutes of stirring, were irradiated for 8-15 minutes at 60 °C, depending upon the amino acid. Coupling efficiency was evaluated using a standard Kaiser test. The resin was washed and the Fmoc group was removed as already described. These steps were also followed for the addition of biotin on the N-terminus of each peptide, except for Fmoc deprotection.

Following the addition of biotin, the peptides were cleaved from resin in 20-30% TFA in DCM, stirring at room temperature for 45 minutes. The resin was then filtered and washed with DCM. The biotinylated product was then precipitated from the resulting filtrate by H_2O addition, filtered, and dried under vacuum. Yields for each peptide are listed below.

HO-Phe-Phe-Val-Leu-Lys-Ala-biotin (**KLVFF-biotin**): ^{1}H NMR (600 MHz, DMSO) δ 8.24 (d, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 7.1 Hz, 1H), 7.99 (t, *J* = 7.6 Hz, 2H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.78 (s, 3H), 7.69 (d, *J* = 9.0 Hz, 1H), 7.32 – 7.17 (m, 10H), 6.48 (s, 1H), 6.45 (s, 1H), 4.59 (td, *J* = 9.0, 4.5 Hz, 1H), 4.49 (td, *J* = 8.1, 5.6 Hz, 1H), 4.38 – 4.31 (m, 2H), 4.31 – 4.24 (m, 2H), 4.15 (ddd, *J* = 15.8, 8.3, 5.7 Hz, 2H), 3.17 – 3.06 (m, 2H), 2.98 (ddd, *J* = 22.3, 14.0, 6.3 Hz, 2H), 2.86 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.78 (t, *J* = 11.6 Hz, 3H), 2.62 (d, *J* = 12.4 32Hz, 1H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.90 (td, *J* = 13.6, 6.8 Hz, 1H), 1.71 – 1.62 (m, 2H), 1.61 – 1.49 (m, 8H), 1.44 (dd, *J* = 23.7, 9.0 Hz, 1H), 1.33 (dd, *J* = 11.3, 7.2 Hz, 4H), 1.22 (d, *J* = 7.1 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 6.5 Hz, 3H), 0.74 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (600 MHz, DMSO) δ 172.58, 172.49, 172.18, 171.56, 171.24, 170.87, 170.40, 162.80, 158.55, 158.33, 158.11, 157.89, 143.02, 137.58, 137.29, 129.06, 128.17, 127.95, 126.43, 126.15, 117.56, 115.59, 60.99, 59.26, 57.45, 55.35, 53.37, 52.10, 50.98, 48.24, 40.49, 38.72, 37.55, 36.75, 34.82, 31.21, 30.69, 28.06, 27.98, 26.57, 25.18, 24.09, 23.10, 22.11, 21.57, 19.14, 17.91, 17.90. M/Z expected for $C_{48}H_{72}N_9O_9S^+$ 950.5; observed [M + H] 950.3 (100%). Yield 32%. Purity 92.4%.

Retention time (min)	Area	% Area
24.53	693389	4.2
26.42	15267340	92.4
29.58	565001	3.4

HO-Phe-Phe-Val-Leu-Lys-Ahx-Lys-Leu-Val-Phe-Phe-Ala-biotin (**d7**): ^{1}H NMR (600 MHz, DMSO) δ 8.26 (d, *J* = 7.5 Hz, 1H), 8.14 (s, 1H), 8.07 – 7.92 (m, 6H), 7.86 (d, *J* = 28.8 Hz, 3H), 7.74 (s, 6H), 7.67 (d, *J* = 9.3 Hz, 1H), 7.48 – 7.06 (m, 20H), 6.79 (d, *J* = 38.5 Hz, 1H), 6.43 (d, *J* = 21.7 Hz, 1H), 4.65 (s, 1H), 4.60 (s, 1H), 4.49 (d, *J* = 5.5 Hz, 2H), 4.34 (s, 3H), 4.29 (s, 2H), 4.23 (s, 3H), 4.15 (d, *J* = 6.9 Hz, 2H), 3.38 (d, *J* = 12.8 Hz, 1H), 3.07 (dd, *J* = 20.6, 15.3 Hz, 5H), 3.00 – 2.90 (m, 3H), 2.85 (dd, *J* = 22.2, 10.0 Hz, 2H), 2.81 – 2.71 (m, 6H), 2.61 (d, *J* = 12.2 Hz, 1H), 2.19 – 2.06 (m, 4H), 2.00 (dd, *J* = 13.9, 7.3 Hz, 1H), 1.90 (dd, *J* = 13.6, 6.7 Hz, 1H), 1.80 – 1.71 (m, 1H), 1.64 (s, 4H), 1.57 – 1.45 (m, 13H), 1.40 (s, 4H), 1.33 – 1.21 (m, 7H), 1.16 (s, 1H), 1.13 – 1.11 (m, 1H), 0.88 (ddd, *J* = 33.7, 22.8, 6.5 Hz, 18H), 0.75 (t, *J* = 5.9 Hz, 6H). ^{13}C NMR (600 MHz, DMSO) δ 172.56, 172.11, 172.07, 171.55, 170.91, 170.85, 170.80, 170.72, 170.69, 170.39, 162.73, 158.06, 157.84, 143.02, 137.54, 137.26, 129.17, 129.13, 129.05, 128.15, 128.00, 127.92, 126.40, 126.13, 117.91, 115.93, 60.92, 59.19, 58.54, 57.72, 57.35, 55.67, 55.27, 53.64, 53.33, 52.69, 52.23, 51.95, 51.07, 50.95, 48.07, 40.46, 38.70, 38.36, 37.55, 36.72, 35.08, 31.53, 31.34, 30.73, 30.55, 28.80, 27.99, 27.94, 26.62, 26.59, 26.00, 24.99, 24.13, 24.06, 23.07, 23.04, 22.21, 22.17, 21.59, 21.54, 19.13, 18.21, 17.87, 17.83. M/Z expected for $C_{89}H_{134}N_{16}O_{15}S^{2+}$: (Z = 1) 1698.99, (Z =2) 850.0; observed [M + H] 1697.9 (1%), [(M + H)/2] 849.4 (100%). Yield 11%. Purity 90.9%.

Retention time (min)	Area	% Area
49.22	1038265	9.0
58.82	10522303	90.9
63.33	10734	0.1

HO-Phe-Phe-Val-Leu-Lys-Ahx-Ahx-Lys-Leu-Val-Phe-Phe-Ala-biotin (**d13**): ^{1}H NMR (600 MHz, DMSO) δ 8.24 (d, *J* = 7.7 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.07 – 7.94 (m, 5H), 7.86 (dd, *J* = 23.2, 7.5 Hz, 3H), 7.77 (s, 8H), 7.69 – 7.62 (m, 1H), 7.32 – 7.12 (m, 20H), 6.46 (s, 2H), 4.69 – 4.44 (m, 5H), 4.37 – 4.26 (m, 4H), 4.22 (dd, *J* = 16.3, 9.0 Hz, 3H), 4.17 – 4.10 (m, 2H), 3.13 – 2.91 (m, 10H), 2.88 – 2.82 (m, 2H), 2.81 – 2.72 (m, 6H), 2.61 (d, *J* = 12.4 Hz, 1H), 2.15 – 2.09 (m, 3H), 2.06 (t, *J* = 7.4 Hz, 2H), 2.00 (dd, *J* = 13.5, 6.9 Hz, 1H), 1.90 (dd, *J* = 13.6, 6.8 Hz, 1H), 1.68 – 1.59 (m, 4H), 1.50 (s, 16H), 1.43 – 1.37 (m, 6H), 1.35 – 1.20 (m, 11H), 1.12 (d, *J* = 7.0 Hz, 2H), 0.93 – 0.82 (m, 18H), 0.74 (t, *J* = 5.6 Hz, 6H). ^{13}C NMR (600 MHz, DMSO) δ 172.57, 172.13, 171.99, 171.79, 171.56, 170.94, 170.86, 170.79, 170.72, 170.43, 162.77, 158.35, 158.12, 143.01, 137.55, 137.27, 129.20, 129.15, 129.06, 128.16, 128.00, 127.92, 126.42, 126.14, 117.46, 115.49, 60.95, 59.22, 57.73, 57.40, 55.29, 53.62, 53.35, 52.26, 52.00, 51.10, 50.95, 48.12, 40.47, 38.72, 38.69, 38.39, 38.33, 37.55, 37.27, 36.75, 35.35, 35.11, 34.83, 31.59, 31.37, 30.73, 30.58, 29.00, 28.79, 28.03, 27.94, 26.61, 26.12, 26.05, 25.05, 24.15, 24.08, 23.07, 23.03, 22.23, 22.17, 21.60, 21.56, 19.14, 18.20, 17.89, 17.87. M/Z expected for $C_{95}H_{145}N_{17}O_{16}S^{2+}$: (Z = 1) 1812.08, (Z =2) 906.0; observed [M + H] 1811.9 (1%), [(M + H)/2] 906.4 (100%). Yield 23%. Purity 95.4%.

Retention time (min)	Area	% Area
32.35	2155200	4.6
34.9	45102305	95.4

HO-Phe-Phe-Val-Leu-Lys-Ahx-Ahx-Axh-Lys-Leu-Val-Phe-Phe-Ala-biotin (**d19**): ^{1}H NMR (400 MHz, DMSO) δ 8.27 – 8.02 (m, 6H), 8.01 – 7.93 (m, 2H), 7.83 (ddd, *J* = 29.4, 12.1, 6.7 Hz, 10H), 7.62 (d, *J* = 9.5 Hz, 1H), 7.35 – 7.10 (m, 20H), 6.45 (d, *J* = 15.0 Hz, 2H), 4.69 – 4.61 (m, 1H), 4.58 (dd, *J* = 13.4, 9.2 Hz, 1H), 4.51 – 4.44 (m, 1H), 4.40 – 4.29 (m, 3H), 4.29 – 4.18 (m, 4H), 4.18 – 4.05 (m, 3H), 3.15 – 2.90 (m, 12H), 2.89 – 2.81 (m, 2H), 2.75 (dd, *J* = 17.9, 10.4 Hz, 6H), 2.61 (d, *J* = 12.4 Hz, 1H), 2.18 – 2.09 (m, 4H), 2.02 (dt, *J* = 13.7, 7.7 Hz, 6H), 1.62 (dd, *J* = 13.9, 7.0 Hz, 5H), 1.58 – 1.44 (m, 19H), 1.40 (dt, *J* = 14.6, 7.2 Hz, 8H), 1.34

– 1.15 (m, 12H), 1.12 (d, *J* = 7.1 Hz, 2H), 0.88 (ddd, *J* = 19.0, 14.8, 6.4 Hz, 18H), 0.71 (dt, *J* = 23.5, 11.9 Hz, 6H). ^{13}C NMR (600 MHz, DMSO) δ 172.63, 172.11, 171.97, 171.79, 171.77, 171.55, 170.91, 170.78, 170.72, 170.69, 170.47, 162.74, 157.95, 157.74, 143.00, 137.56, 137.46, 129.18, 129.14, 128.96, 127.99, 127.91, 126.23, 126.16, 118.36, 116.34, 60.93, 59.20, 57.69, 55.28, 53.65, 53.59, 52.25, 51.09, 48.06, 40.45, 40.05, 38.67, 38.30, 37.28, 36.67, 35.35, 35.09, 3963.4.82, 31.56, 30.73, 30.55, 28.98, 28.78, 28.01, 27.93, 26.60, 26.14, 26.04, 25.06, 25.04, 24.14, 23.12, 23.05, 22.15, 21.54, 21.37, 19.15, 19.04, 18.20, 17.85. M/Z expected for $C_{101}H_{156}N_{18}O_{17}S^{2+}$: (Z = 1) 1925.16, (Z =2) 963.2; observed [M + H] 1924.9 (1%), [(M + H)/2] 962.9 (100%). Yield 18%. Purity 92.7%.

Retention time (min)	Area	% Area
33.12	720021	4.9
34.9	13626546	92.7
39.4	360138	2.4

HO-Phe-Phe-Val-Leu-Lys-Ahx-Ahx-Ahx-Ahx-Lys-Leu-Val-Phe-Phe-Ala-biotin (**d24**): ^{1}H NMR (600 MHz, DMSO) δ 8.39 (d, *J* = 8.5 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.06 – 8.00 (m, 1H), 7.96 (d, *J* = 8.9 Hz, 3H), 7.88 – 7.80 (m, 3H), 7.76 (t, *J* = 5.4 Hz, 3H), 7.66 (s, 8H), 7.33 – 7.12 (m, 20H), 6.43 (d, *J* = 20.9 Hz, 2H), 4.62 (d, *J* = 32.8 Hz, 2H), 4.48 (s, 2H), 4.33 (d, *J* = 5.6 Hz, 3H), 4.28 (d, *J* = 6.3 Hz, 1H), 4.25 – 4.18 (m, 3H), 4.14 (s, 2H), 3.11 (d, *J* = 10.3 Hz, 2H), 3.03 (dd, *J* = 12.9, 6.7 Hz, 10H), 2.96 (s, 2H), 2.85 (dd, *J* = 21.3, 9.1 Hz, 3H), 2.77 (s, 6H), 2.65 (s, 1H), 2.61 (d, *J* = 12.5 Hz, 1H), 2.58 (s, 1H), 2.13 (d, *J* = 7.5 Hz, 3H), 2.06 (t, *J* = 7.5 Hz, 6H), 2.00 (s, 1H), 1.90 (d, *J* = 6.6 Hz, 1H), 1.64 (s, 4H), 1.49 (dd, *J* = 14.7, 7.4 Hz, 20H), 1.40 (dd, *J* = 14.6, 7.3 Hz, 10H), 1.31 (s, 5H), 1.27 – 1.20 (m, 9H), 1.12 (d, *J* = 7.1 Hz, 2H), 0.94 – 0.82 (m, 18H), 0.78 – 0.70 (m, 6H). ^{13}C NMR (600 MHz, DMSO) δ 172.75, 172.11, 171.79, 171.76, 171.54, 170.89, 170.68, 170.55, 170.32, 162.73, 157.63, 142.98, 137.34, 129.19, 128.13, 127.91, 126.19, 63.72, 60.93, 59.12, 57.70, 55.25, 53.27, 52.19, 51.09, 40.42, 38.30, 35.36, 35.10, 31.55, 28.98, 28.79, 28.01, 26.61, 26.14, 26.06, 25.06, 24.13, 24.06, 23.05, 22.16, 19.14, 18.19, 17.92, 17.83. M/Z expected for $C_{107}H_{167}N_{19}O_{18}S^{2+}$: (Z = 1) 2038.3, (Z =2) 1019.1; observed [M + H] 2036.2 (1%), [(M + H)/2] 1019.5 (68%). Yield 11%. Purity 93.1%.

Retention time (min)	Area	% Area
33.3	16047611	93.1
36.4	326682	1.9
38.9	854220	5

Supplemental References

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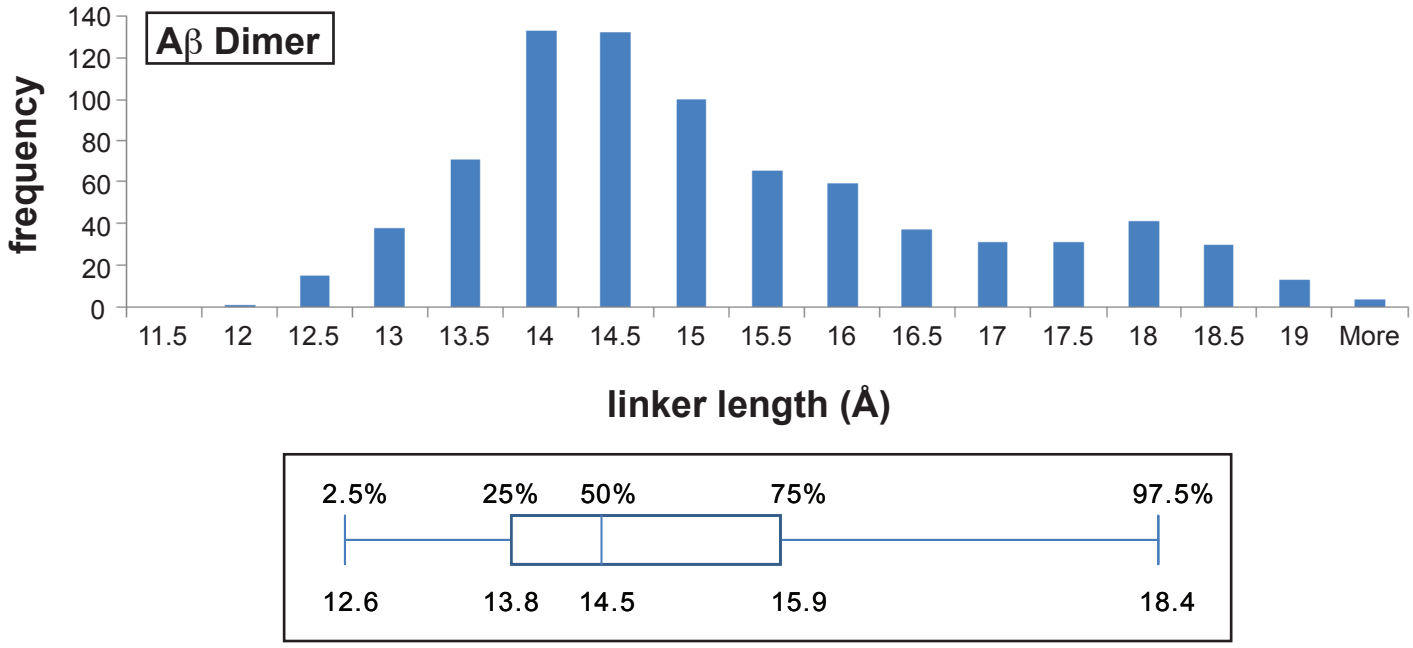
(a)



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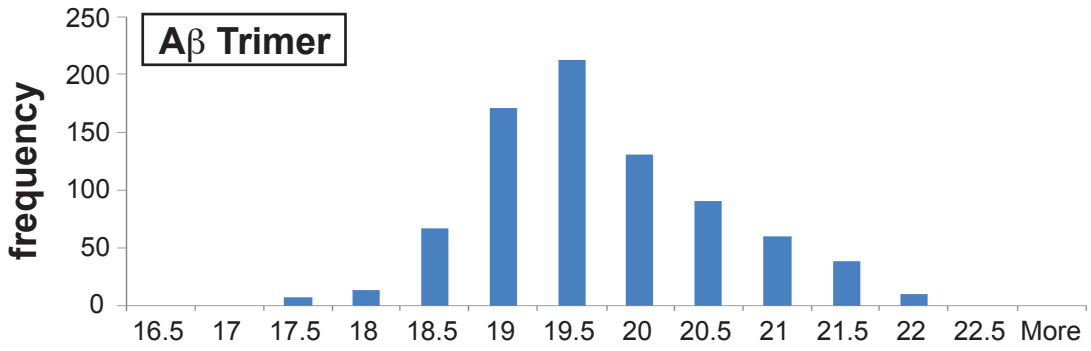
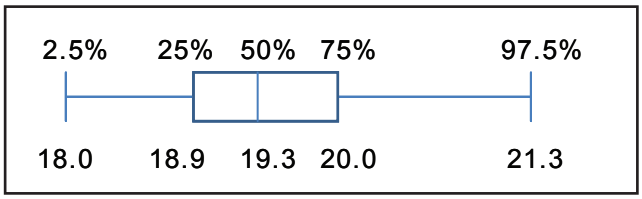
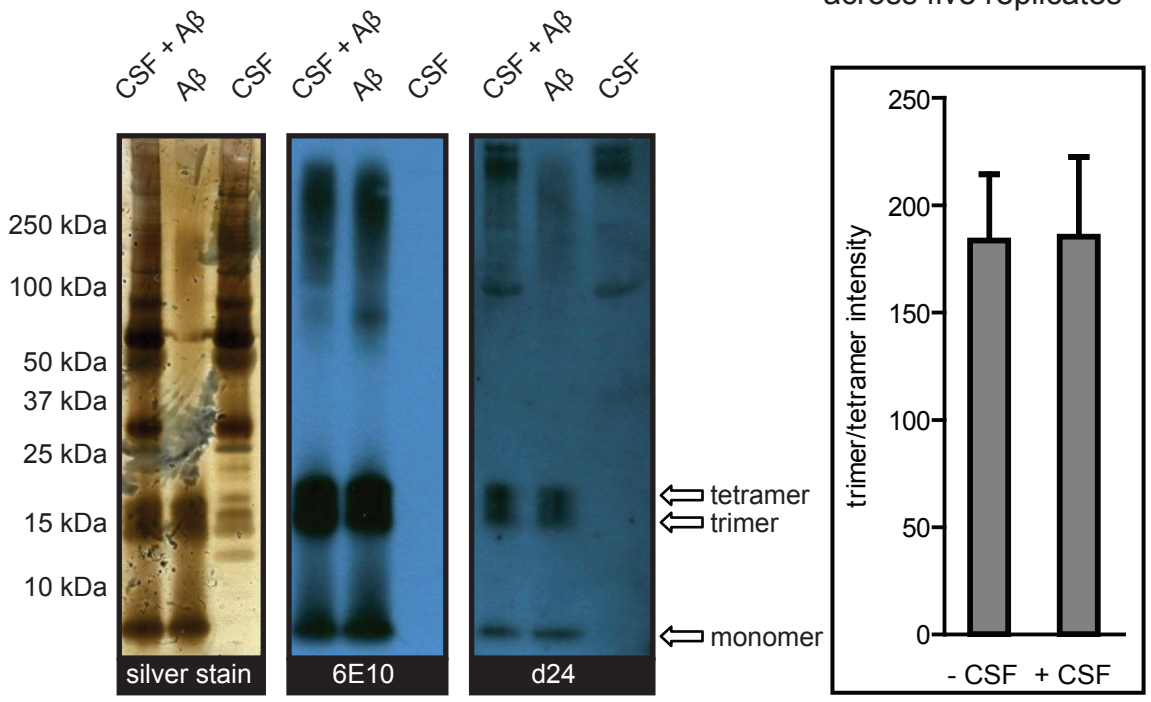




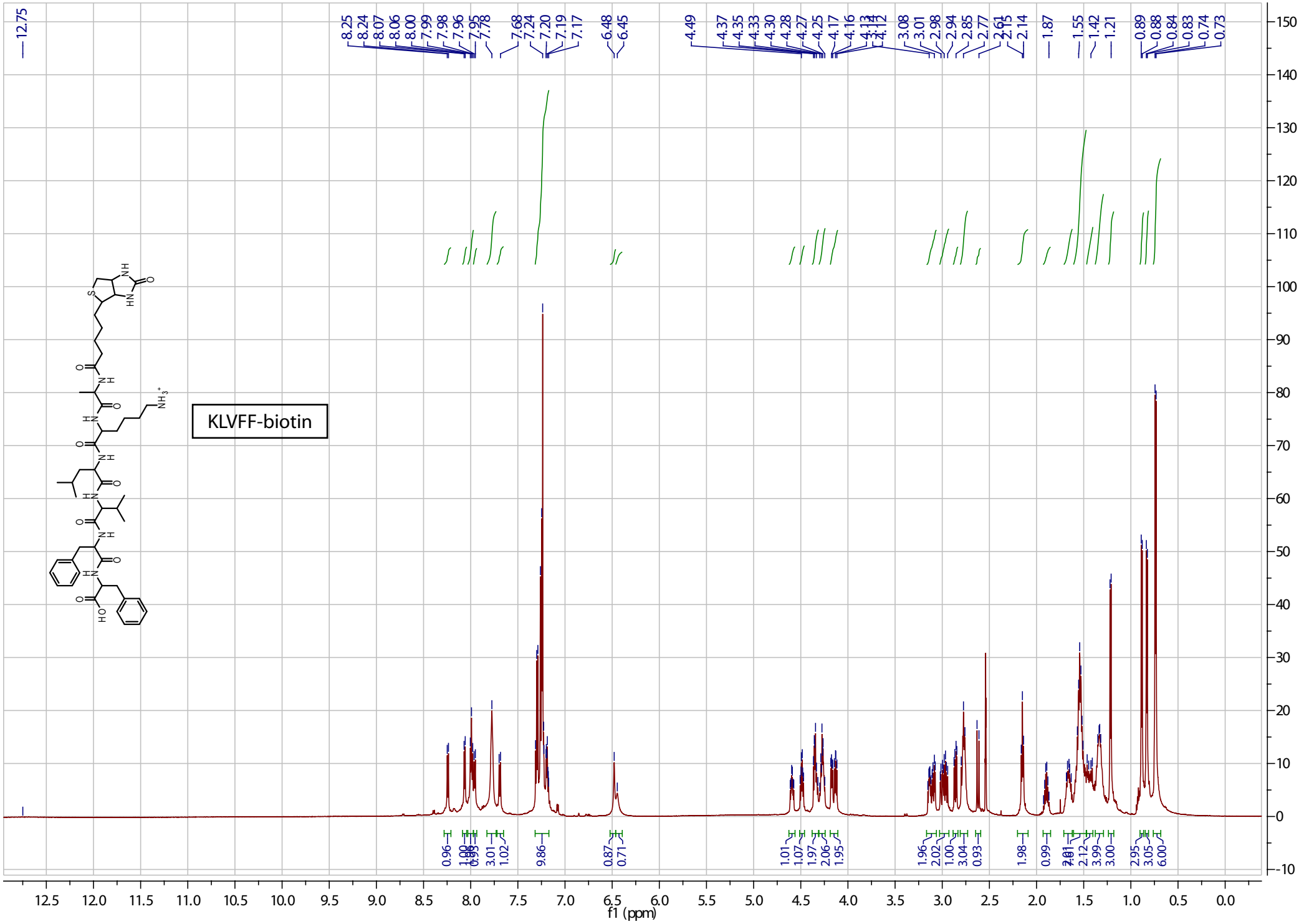
Supplemental Figure 1. Molecular dynamics simulations estimate the distribution of distances across the Aβ (a) dimer and (b) trimer. Distances were measured between Lys16 residues and the frequency of each distance is plotted. The dimer simulation yielded a broad range of distances, with the major median at 14.0-14.5 Å, and a second minor population around 17.5-18.5 Å. This large variation is expected because the core of the dimer is known to be less stable. For a trimer, a narrower range of distances was observed, corresponding to a distance of 19-20 Å. Note that these values are only approximate and that these structures are expected to sample a wide range of distances.

(a) **d24** binds Aβ (1-42) trimers and tetramers in the presence of CSF

(b) **d24** recognition of trimers and tetramers across five replicates



Supplemental Figure 2: **d24** recognizes Aβ trimers and tetramers in the presence of human CSF. (a) Experiments performed as in Figure 3C, except that non-cross-linked Aβ (1-42) was used instead of Aβ (1-40).These studies revealed that **d24** (5 μM) recognizes the trimer/tetramer bands (~12-17 kDa) in both the absence and presence of CSF. (b) The trimer/tetramer reactivity was quantified with and without CSF over five independent replicates. These data have been compiled across three separate preparations of **d24** and include reactivity against Aβ (1-40) and Aβ (1-42).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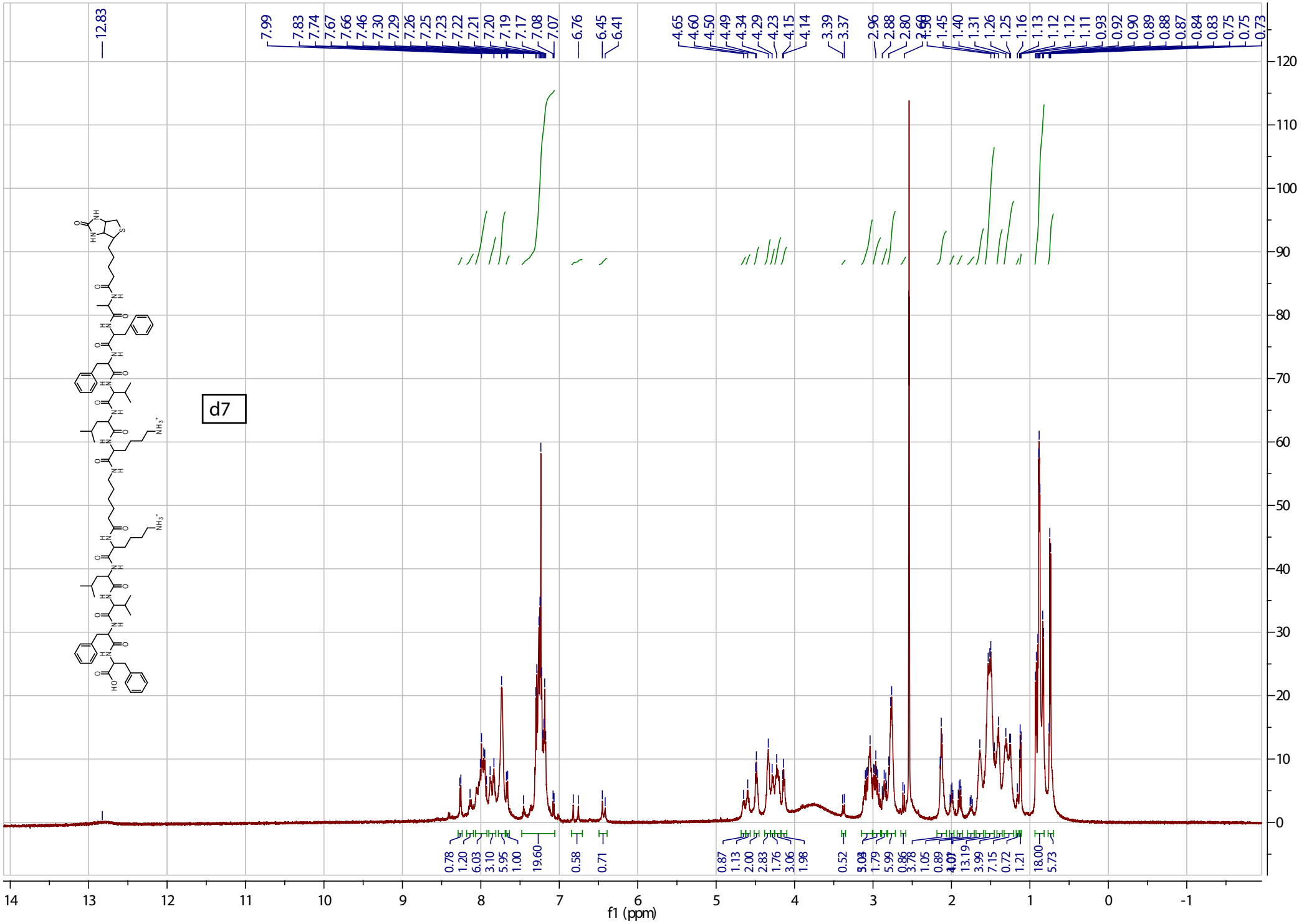
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7.19
7.17
7.08
7.07
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1.13
1.12
1.11
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0.92
0.90
0.89
0.88
0.87
0.84
0.83
0.75
0.75
0.73
d7
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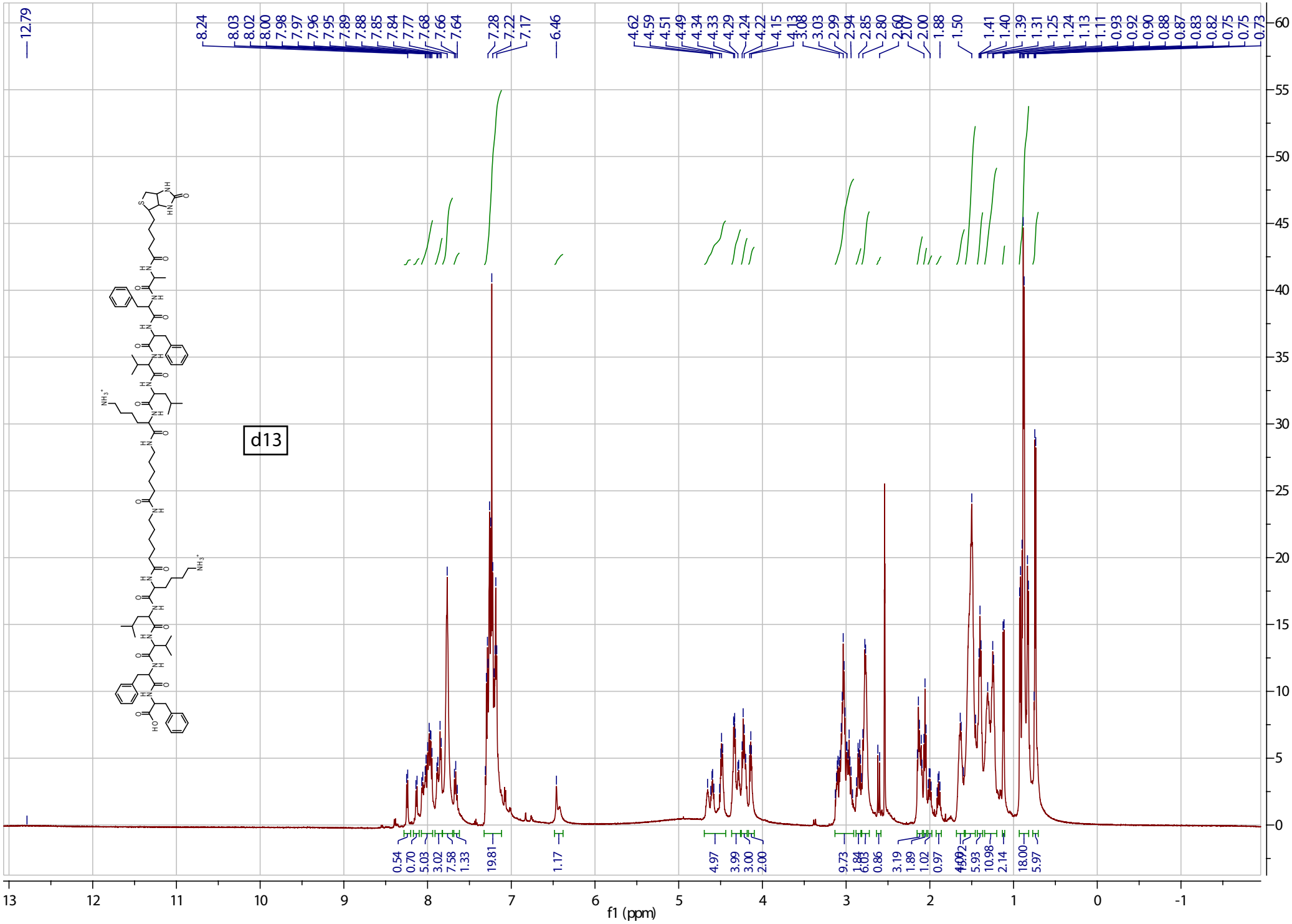
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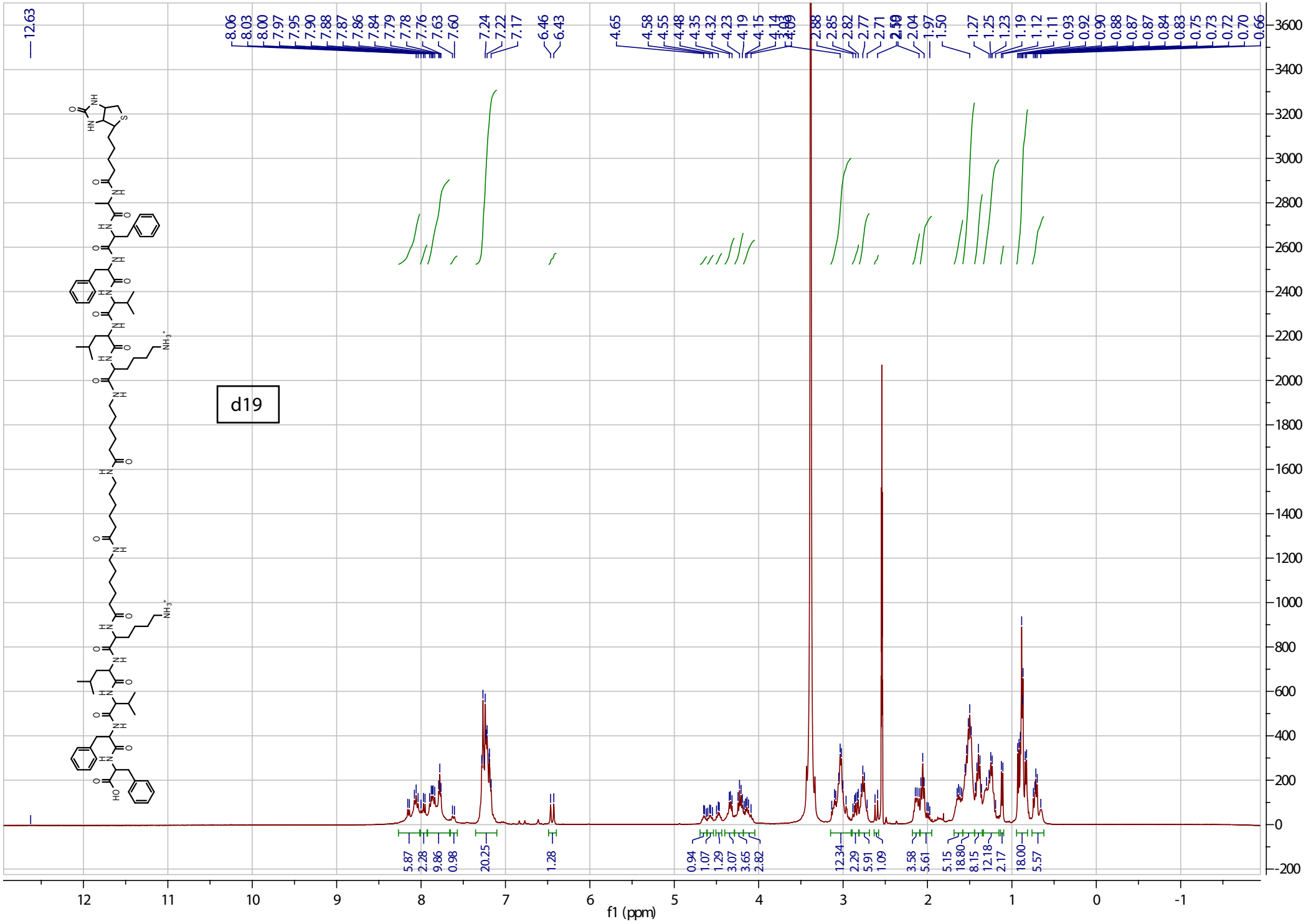
f1 (ppm)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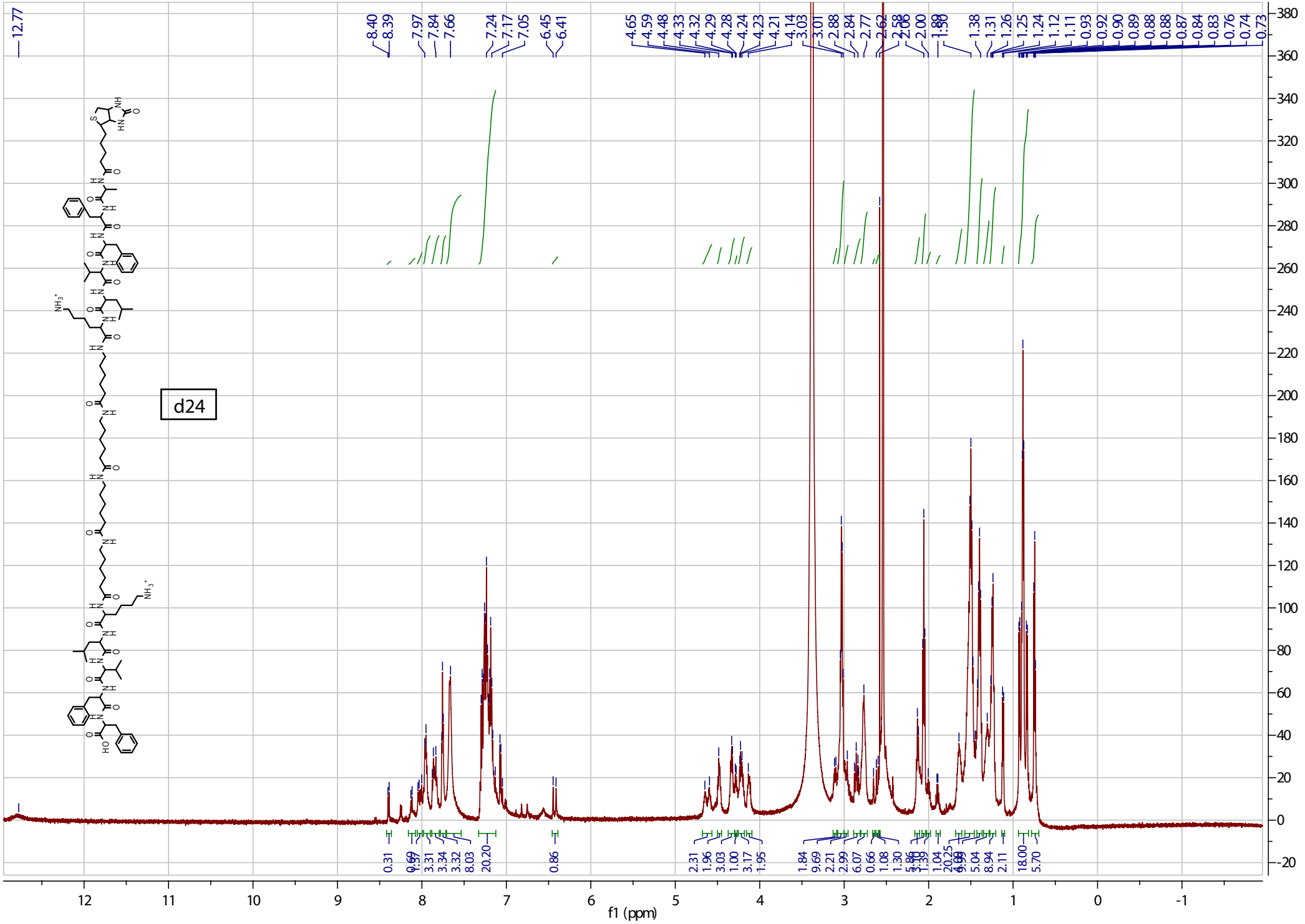
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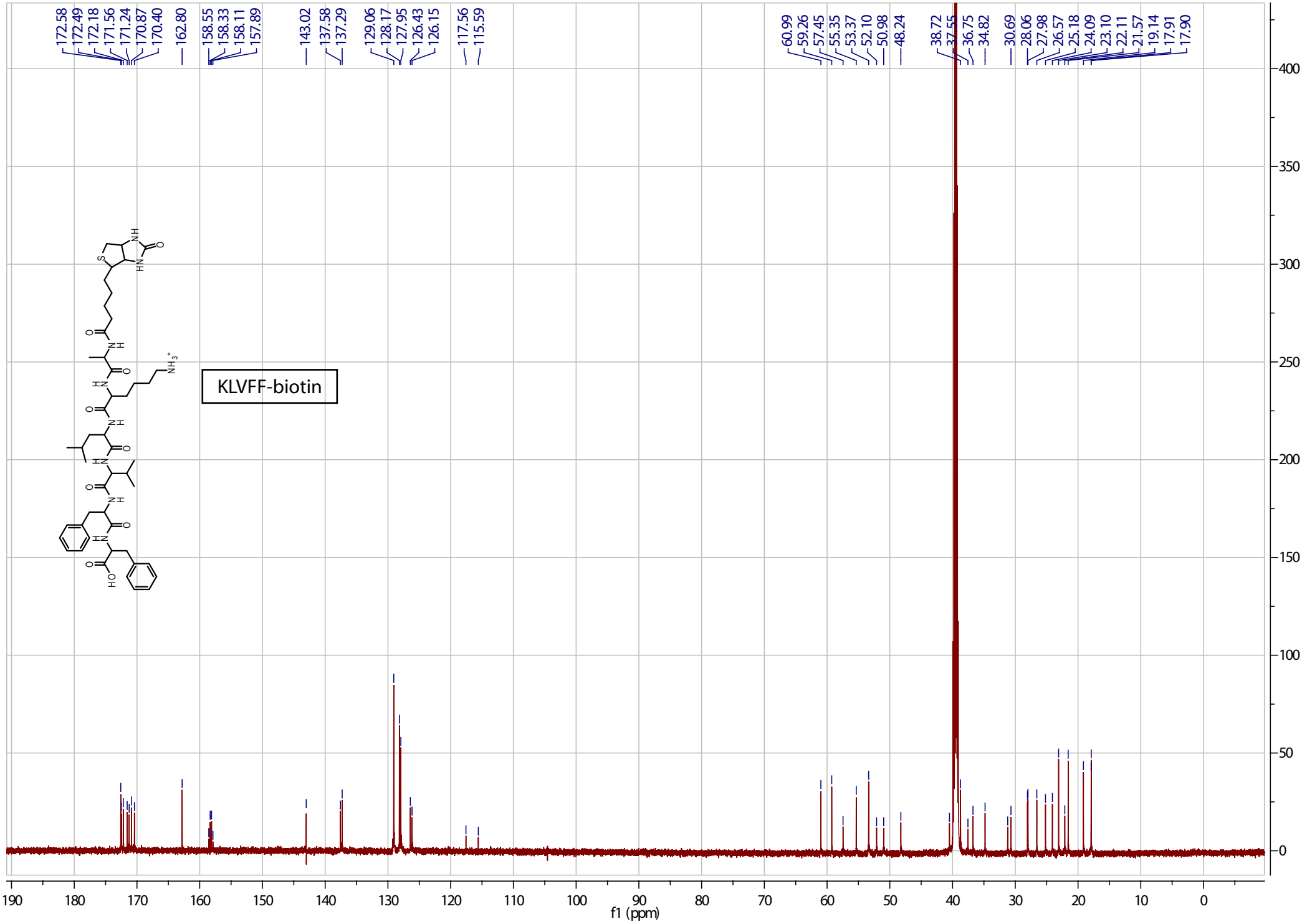
f1 (ppm)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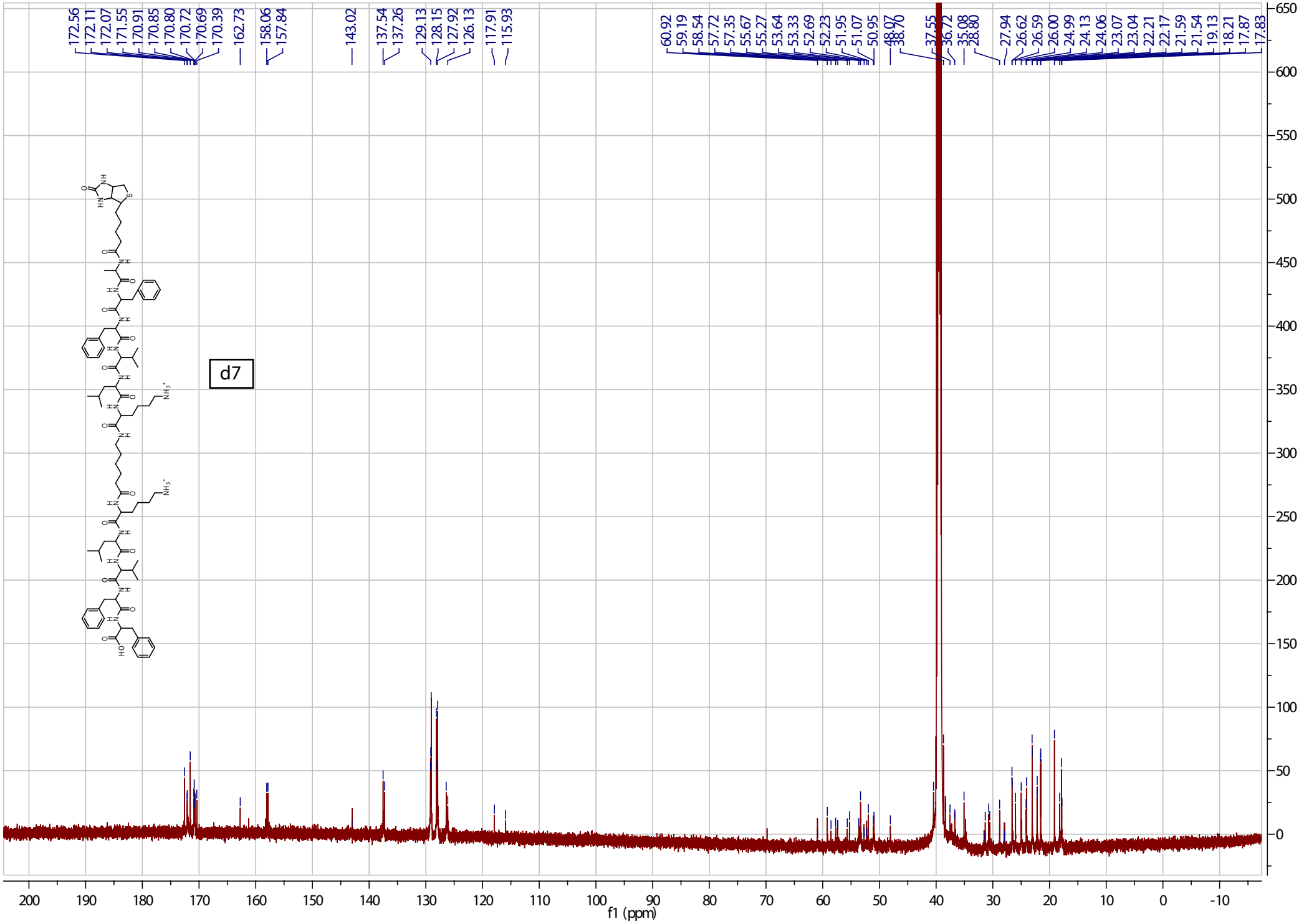
f1 (ppm)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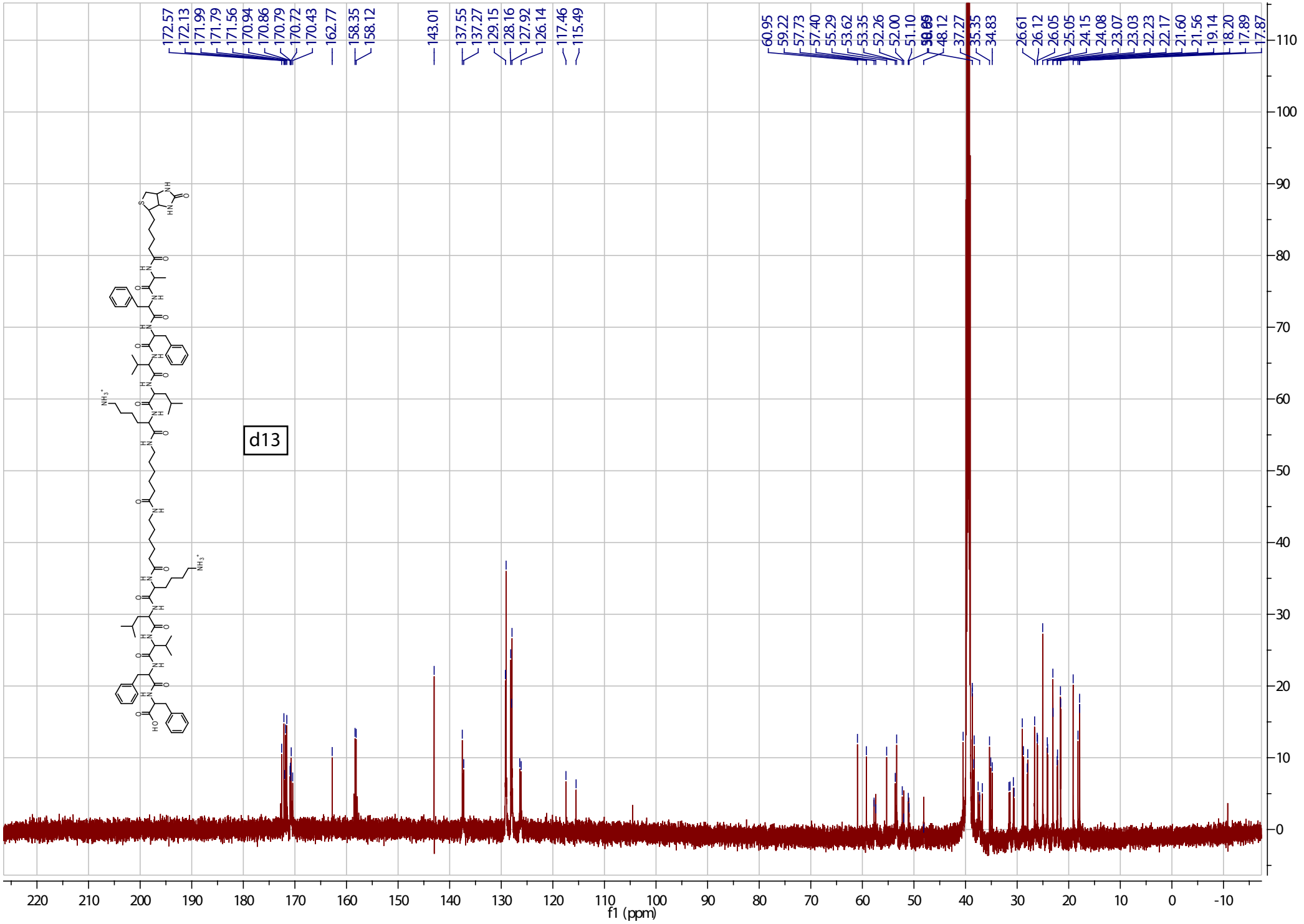
f1 (ppm)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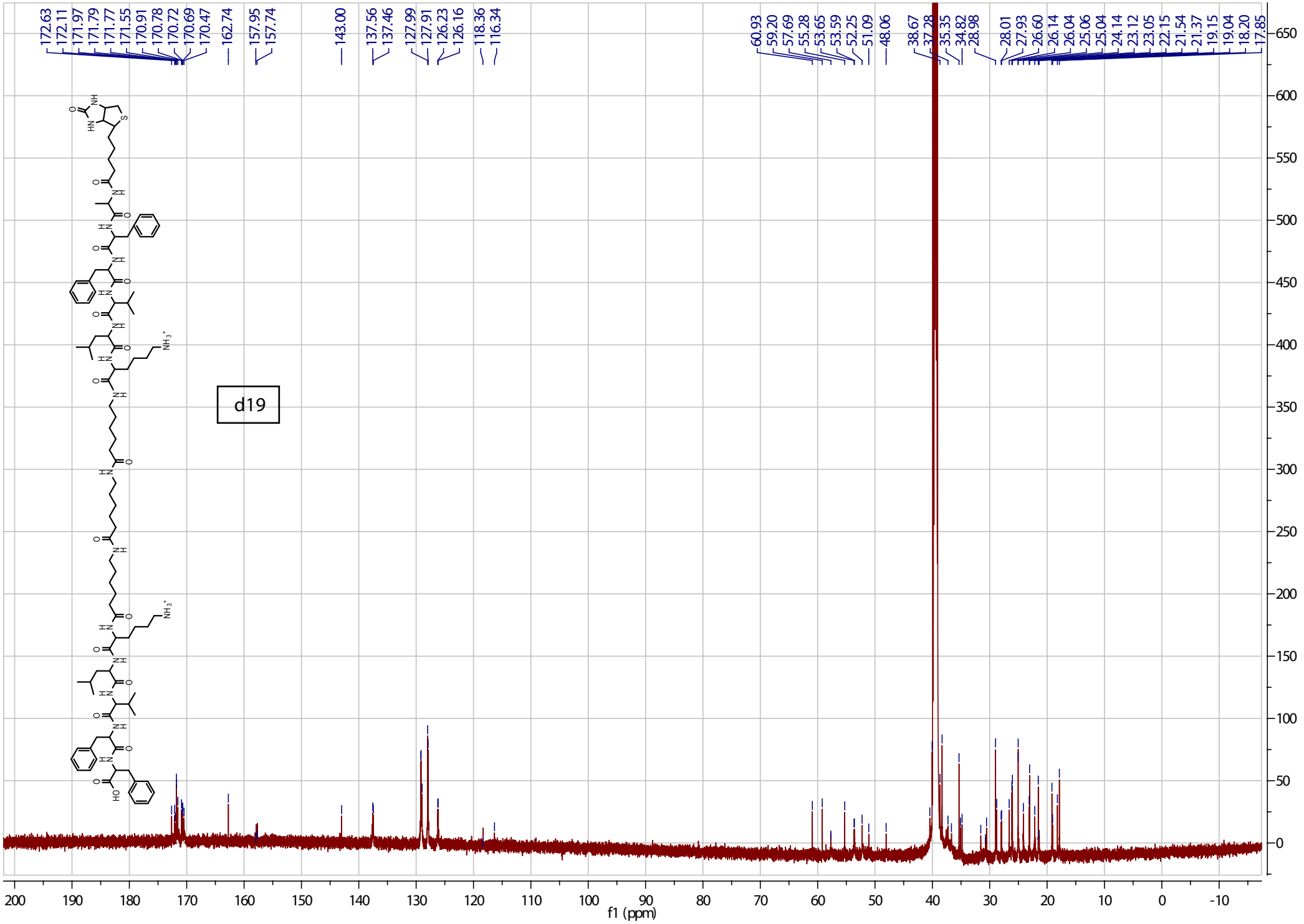
d13
f1 (ppm)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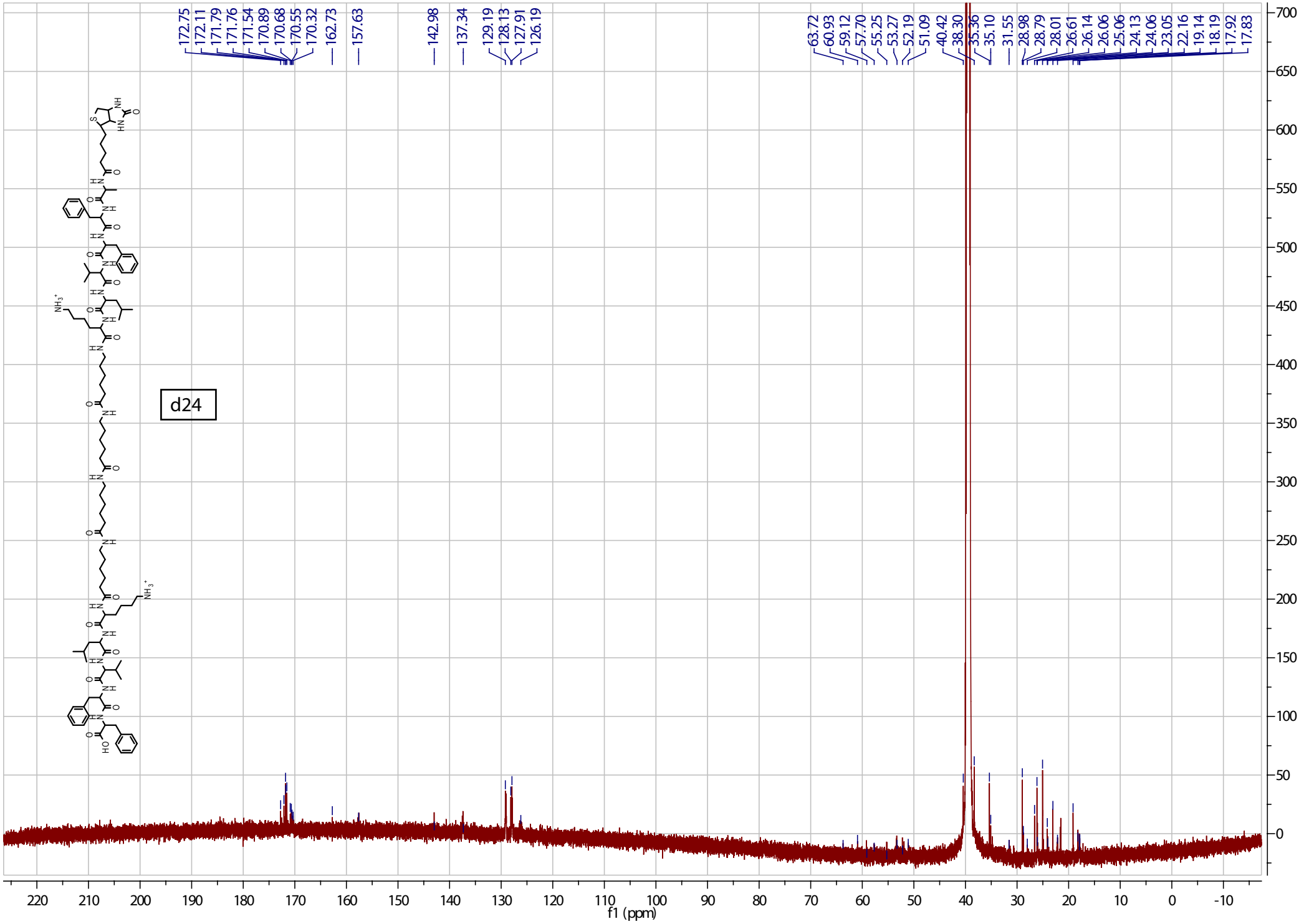
f1 (ppm)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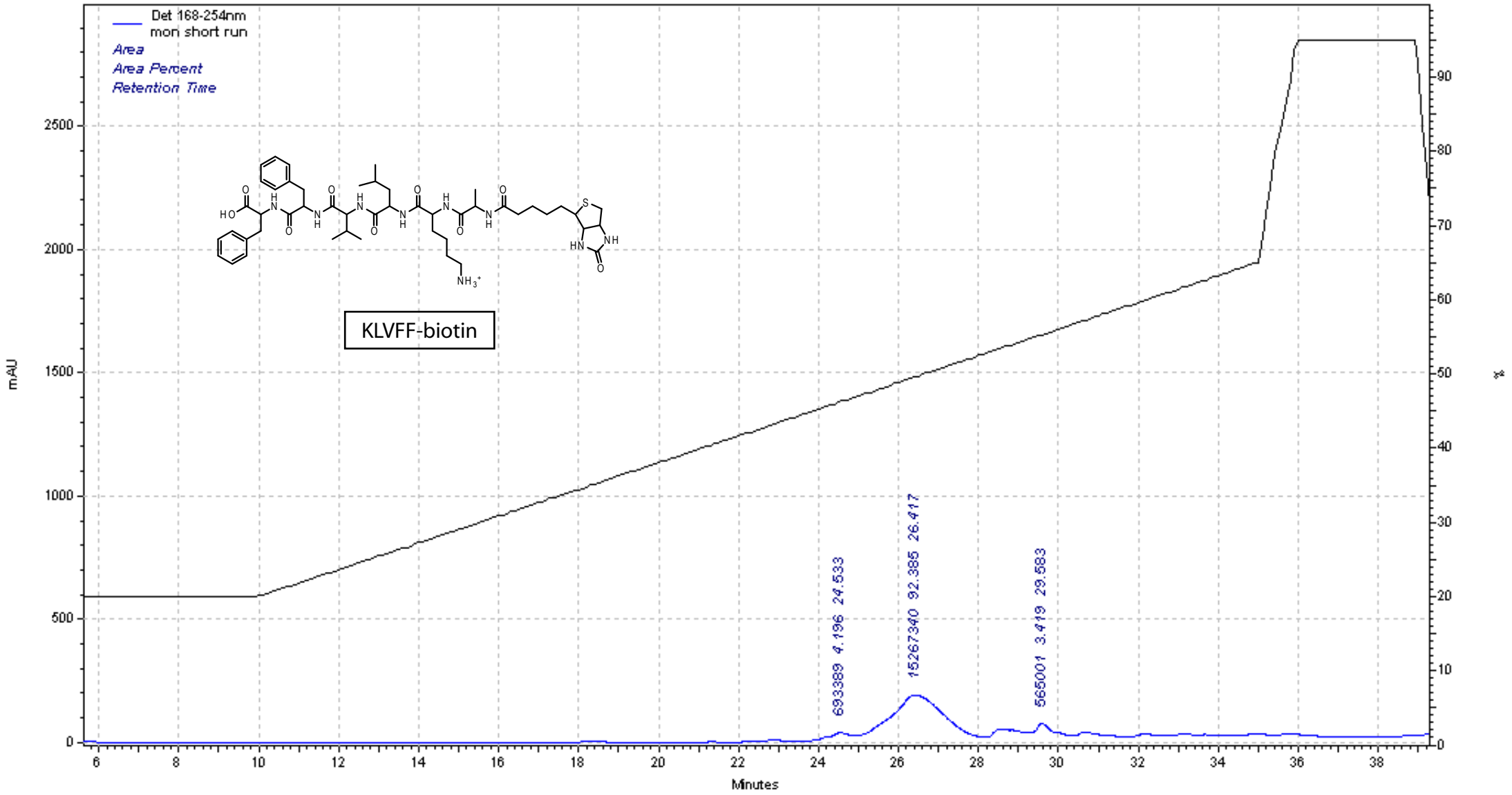
f1 (ppm)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565001 3.419 29.583
mAU
%
Minutes
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1000
1500
2000
2500
6
8
10
12
14
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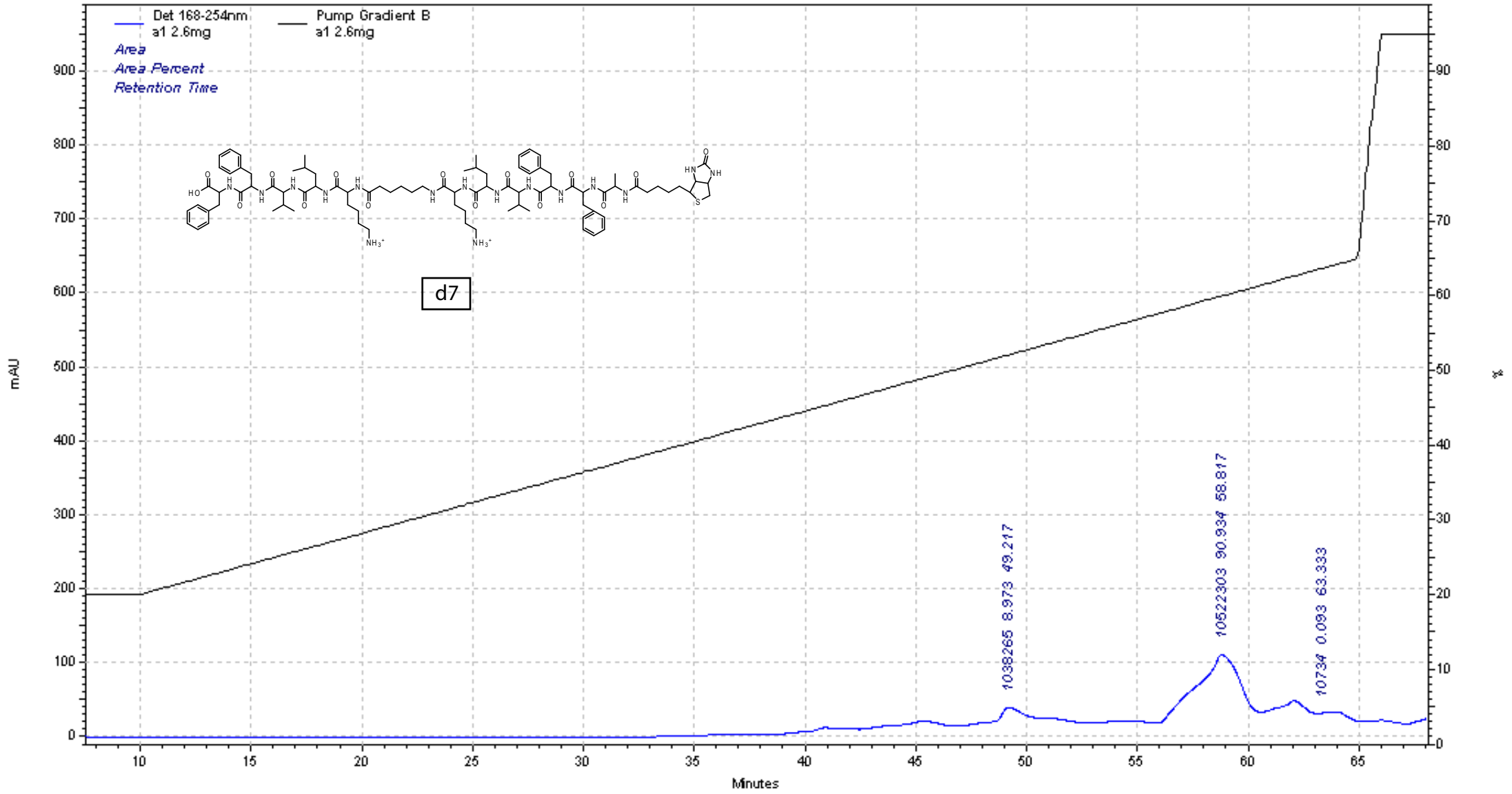
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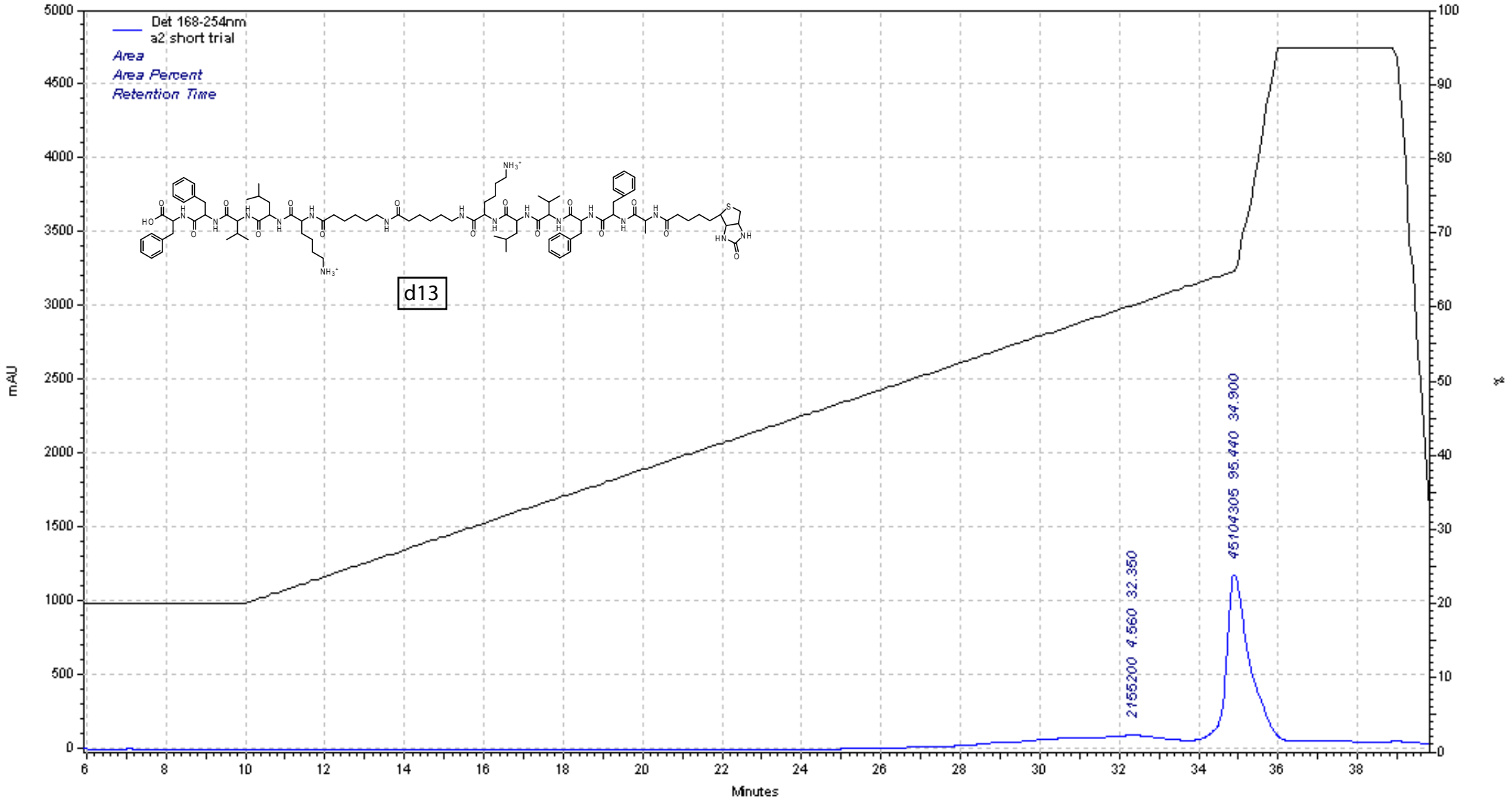
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%
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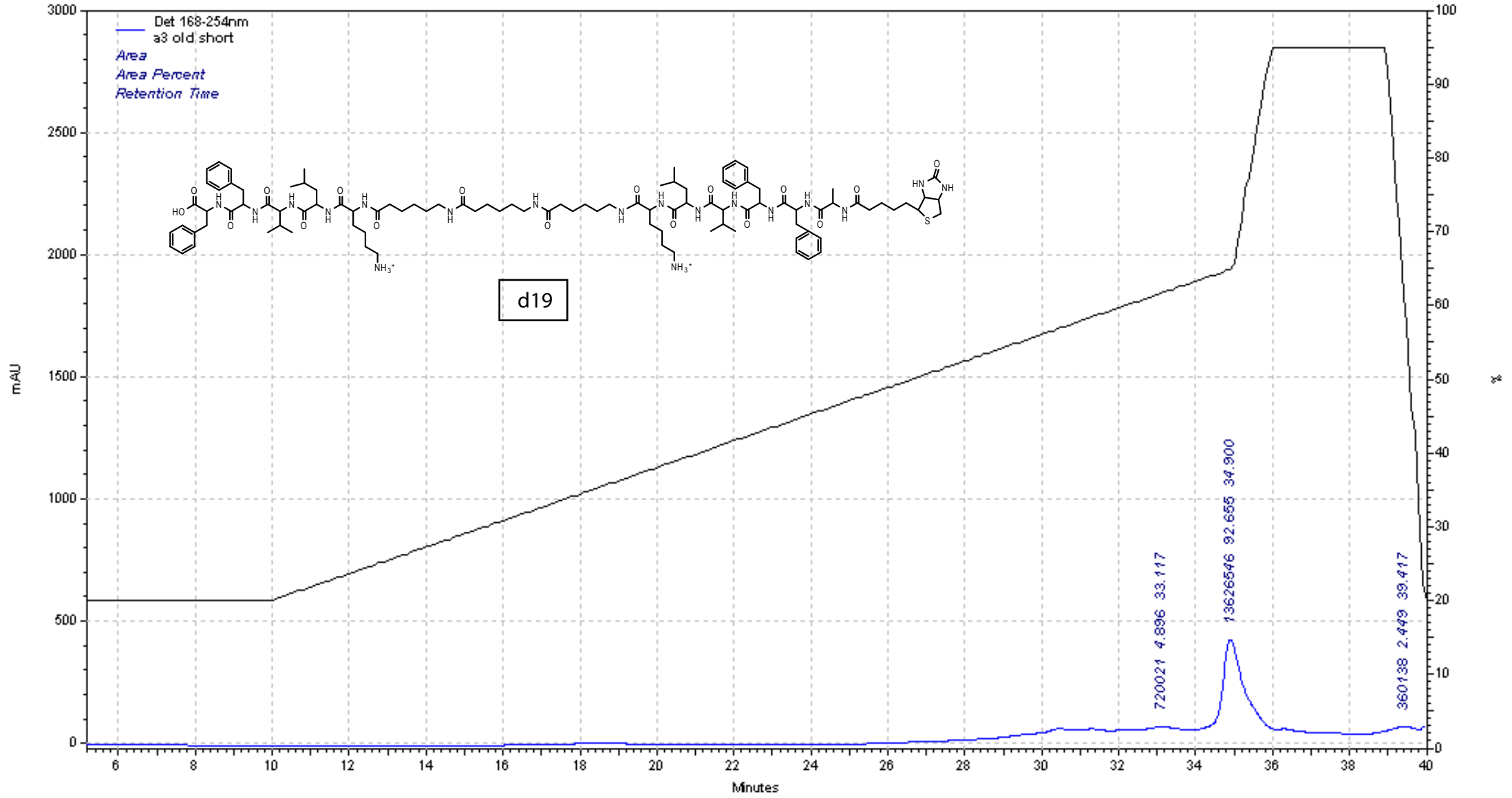
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mAU
%
Minutes
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2500
2000
1500
1000
500
0
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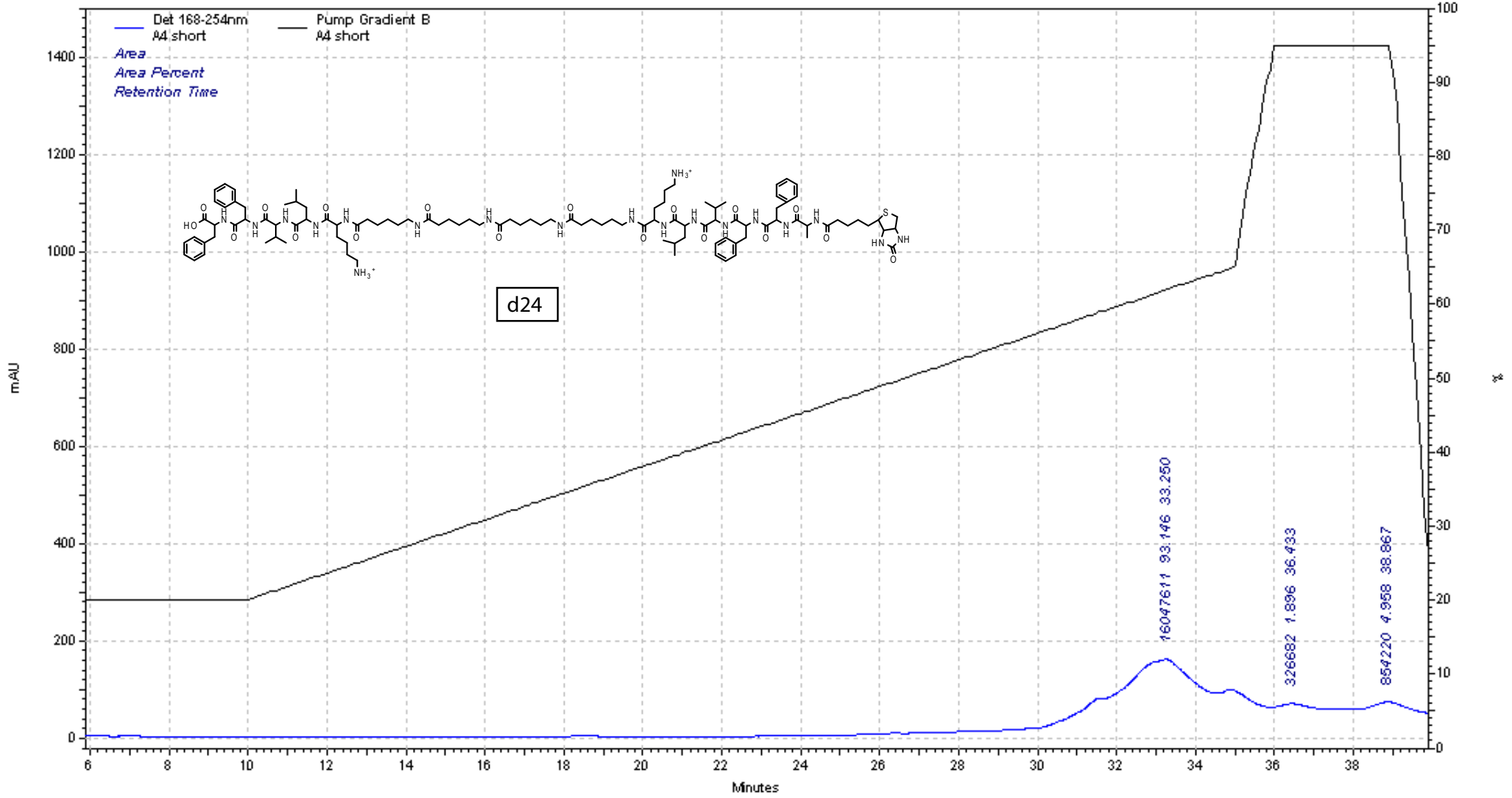
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A4 short
Pump Gradient B
A4 short
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Area Percent
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%
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