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Experimental section

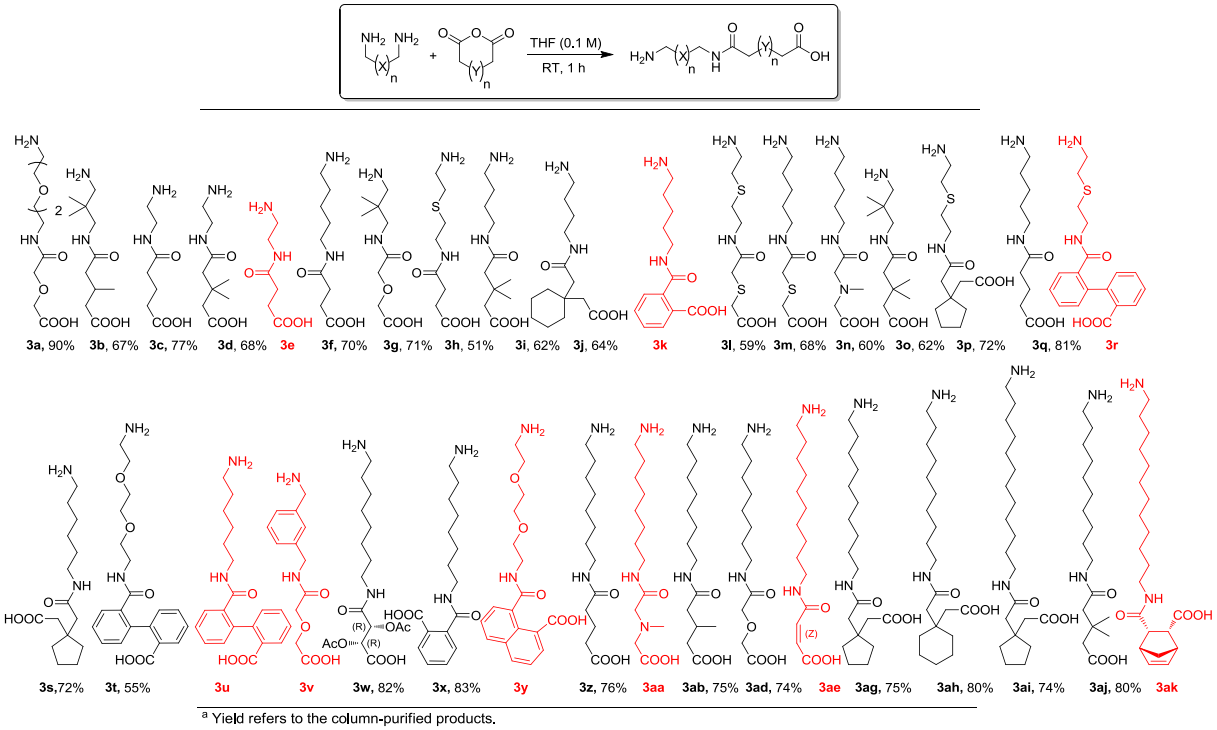
General information:

Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Avance 500 spectrometer (^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz)). Chemical shifts for ^{1}H NMR were reported as δ values and coupling constants were in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, d = doublet, t = triplet, dd = double doublet, m = multiplet, bs = broad singlet. Chemical shifts for ^{13}C NMR reported in ppm relative to the solvent peak. Thin layer chromatography was performed on Fluka precoated silica gel plates (0.20 mm thick, particle size 25 µm). Flash chromatography was performed on a Teledyne ISCO Combiflash Rf, using RediSep Rf Normal-phase Silica Flash Columns (Silica Gel 60 Å, 230 - 400 mesh). Reagents were available from commercial suppliers and used without any purification unless otherwise noted. All isocyanides were made in house by either performing the Hoffman or Ugi procedure. Other reagents were purchased from Sigma Aldrich, ABCR, Acros and AK Scientific and were used without further purification. Mass spectra were measured on a Waters Investigator Supercritical Fluid Chromatograph with a 3100 MS Detector (ESI) using a solvent system of methanol and CO_2 on a Viridis silica gel column (4.6 × 250 mm, 5 µm particle size) and reported as (m/z). High resolution mass spectra (HRMS) were recorded using a LTQ-Orbitrap-XL (Thermo Fisher Scientific; ESI pos. mode) at a resolution of 60000@m/z400. Electrospray ionization mass spectra (ESI-MS) were recorded on a Waters Investigator Semi-prep 15 SFC-MS instrument. Yields given refer to chromatographically purified and spectroscopically pure compounds unless otherwise stated.

Library of α,ω-amino acids:

SI_Table 1. Synthesized examples of α,ω-amino acids with yields. The red marked examples were not isolated but used directly in the following ring closure reaction.[a]

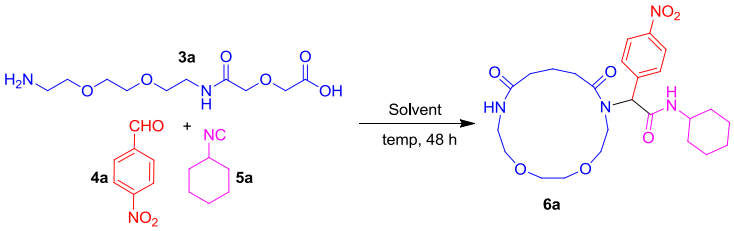
We optimized the direct α,ω-amino carboxylic acid formation from unprotected diamines. We used 1,2-bis(2-aminoethoxy)ethane with diglycolic anhydride in the ring opening reaction. At 1M concertation in THF we observed considerable amounts of bisacylated product formation, which could be suppressed by performing the reaction under dilute conditions (0.1 M). Performing the ring opening reaction in THF (0.1 M) and slowly dropping the anhydride to the diamine solution we isolated the product 14-amino-5-oxo-3,9,12-trioxa-6-azatetradecan-1-oic acid **3a** in 90% yield. Other apolar aprotic solvents (toluene, hexane and Et_2O) and polar aprotic solvents (THF, DCM, $CHCl_3$ and EtOAc) gave less or no yields.



[a] Yield refers to the column-purified products.

Optimization for Macrocyclization:

SI_Table 2: we optimized the conditions for the synthesis of macrocycles by reacting the α,ω-amino carboxylic acids together with oxo components and isocyanides in an Ugi-reaction. 14-amino-5-oxo-3,9,12-trioxa-6-azatetradecan-1-oic acid **3a** together with 4-nitrobenzaldehyde and cyclohexyl isocyanide was investigated as our model system. The Ugi-reaction with 4-nitrobenzaldehyde and cyclohexyl isocyanide in 1.0M solution of MeOH after 48h furnished the expected 15-membered macrocycle **6a** in 30% yield without any oligomerization byproducts. Nonpolar and polar aprotic solvents did not produce any product at all due to the low starting material solubility. Moreover, these are uncommon solvents for the Ugi reaction which is best performed in protic polar solvent. The same reaction was performed in different dilutions of methanol and it was found that highly dilute 0.01M equimolar mixture gave the 15-membered macrocycle **6a** in good yield (55%). Although trifluoroethanol (57% yield) was slightly superior to MeOH we chose MeOH for further scope and limitation studies due to the higher pricing of TFE.



S.No	Solvent (M)	temp	Yield (%)[a]
1.	MeOH (1.0 M)	RT	30 %
2.	MeOH (0.25 M)	RT	30 %
3.	MeOH (0.1 M)	RT	38 %
4.	MeOH (0.02 M)	RT	47 %
5.	MeOH (0.01M)	RT	**55 %**
6.	MeOH:H_2O (0.01M) (3:1)	RT	33 %
7.	MeOH (0.01M)	60 °C	48 %
8.	TFE (0.01M)	RT	**57 %**
9.	Glycerol:MEG (0.01M) (3:1)	RT	21 %

[a] Yield refers to the column-purified products.
MEG = Monoethylene Glycol

Whitty guidelines comparison:

SI_Table 3: Comparison of the Whitty criteria of synthesized 38 artificial macrocycles (**6a-6ak** and **6am**) with the Whitty dataset of 19 natural product macrocycles. Green filling denotes criteria overlap whereas yellow filling closeness to the criterion.

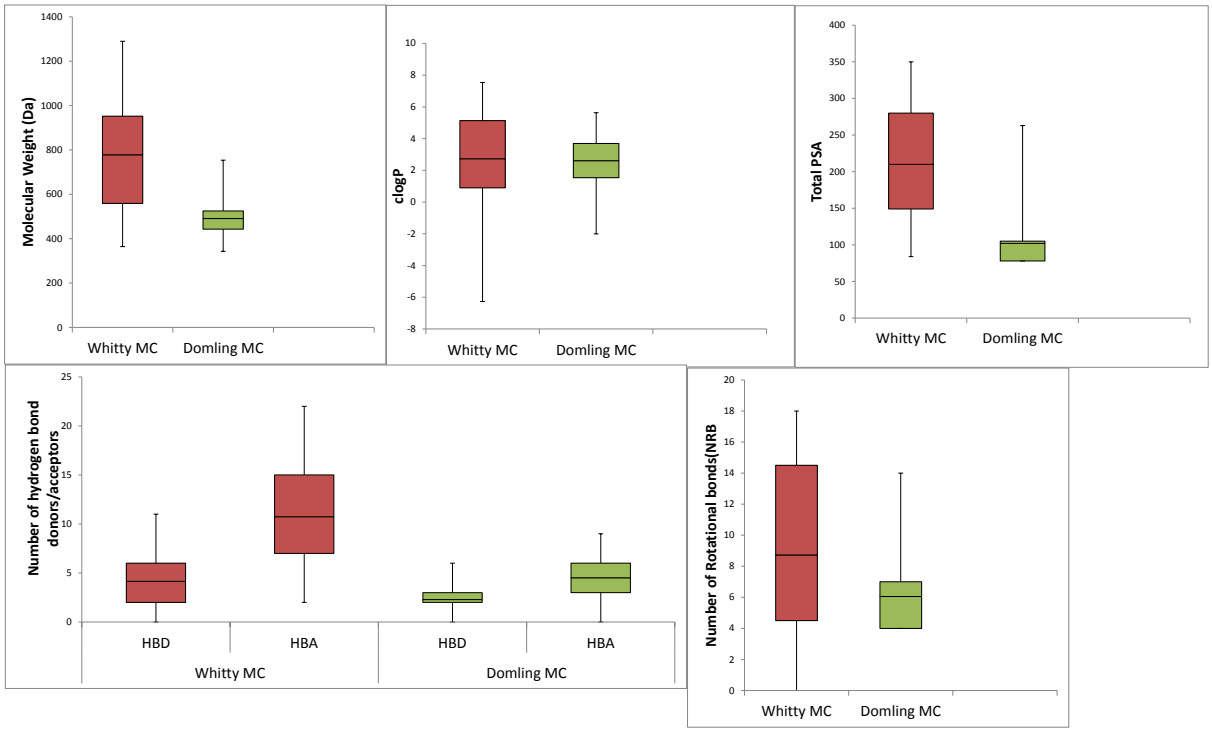
Property	Observed range **(Whitty)**	Our MC in range
Ring size (*R*)	14–38	8-19
Number of substituents	4.4 (3–8)	1-3
Large substituents (≥5 HA)**b**	1.9 (1–3)	1-3
Small substituents (2–4 HA)**b**	2.4 (1–6)	0-1
Proportion of HA that are in substituents	47% (40–59%)	53% (32-81%)
Number of peripheral groups	5–12	2-5
Polar/nonpolar balance, substituents	~30/70	~35/65
Polar/nonpolar balance, peripheral groups	~60/40	70/30
Degrees of unsaturation in ring	~0.4*R* – 4 (± 3)	~0.5*R*–4(±4)
N:O ratio	0.25:1 (0–0.4:1)	0.75:1
Chiral centers	15 (9–18)	0-6

SI_Table 4. MW, cLogP, PSA, HBDs, HBAs and N_{RB}value comparative table**:** the dataset of Whitty´s 19 unique MCs and our 38 MCs (**6a-6ak** and **6am**)

Property[a]	Whitty 19 MCs (*avg*)	Our 38 MCs (*avg*)
MW	778	491
clogP	2.73	2.61
PSA[b]	210 Å^2	102 Å^2
HBDs[c]	4.8	2.2
HBAs[d]	10.6	4.3
N_{RB}value	8.7	6.0

[a]Calculator plug-ins (Instant JChem version 16.5.2.0) were used for structure property prediction and average values are presented here. [b] Polar surface area. [c] Number of hydrogen bond donors. [d] Number of hydrogen bond acceptors.

MW, cLogP, PSA, HBDs, HBAs and NRBvalue whisker plots: The dataset of Whitty´s 19 unique MCs and our 38 MCs (**6a**-**6ak** and **6am**)



Different properties of dataset and mean values were calculated by using Instant JChem (version 16.5.2.0). Whitty´s 19 macrocycles included in the test set are Cyclosporin, Sanglifehrin A, Rapamycin, FK506, Scyptolin, GE2270A, Nodularin R, Arylomycin, Argadin, Argifin, Pectenotoxin-2, Kabiramide C, Reidispongiolide A, Latrunculin B, Soraphen A, Geldanamycin, Pochoxime A, Macbecin, Radicicol and the properties are compared with our 38 MCs **6a**-**6ak** and **6am**

General Experimental Procedures:

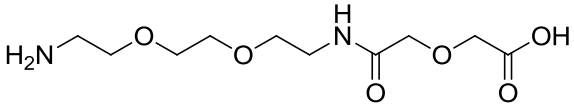
Procedure A: General procedure for synthesis of α,ω-amino carboxylic acids: Diamine **1** (1.0 mmol) was dissolved in THF (6 mL), then a solution of anhydride **2** (1.0 mmol) in THF (4 mL) was added dropwise during 30 mins. The reaction mixture was further stirred for 1h. Solvents were removed under vacuum. The crude mixture was purified by flash column chromatography using CH_2Cl_2:MeOH (1:9) to afford the product **3**.

Procedure B: General procedure for Ugi-macrocyclization: In an oven-dried 100mL round bottom flask equipped with a magnetic stir bar, the α,ω-amino carboxylic acid 3 (1.0 mmol, 1 equiv.) was disolved in methanol (10.0 ml). Aldehyde 4 (1.0 mmol, 1.0 equiv.) was added and the solution was stirred for 30 min at rt, then isocyanide 5 (1.0 mmol, 1 equiv.) was added to the reaction mixture and the reaction mixture was diluted with methanol to 0.01M (+90 mL) and further stirred for 48 h. After completion of the reaction, as monitored by LC – MS, the

reaction mixture was dried under reduced pressure via rotary evaporation and the residue was purified using flash chromatography (CH_2Cl_2: MeOH 9:1) to afford the product 6.

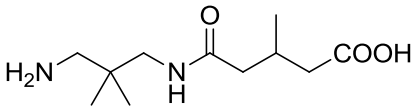
Procedure C: General procedure for one-pot macrocyclization: To the stirred solution of diamine **1** (1.0 mmol, 1.0 equiv.) in THF (6 mL) was added solution of anhydride **2** (1.0 mmol in 4mL THF) dropwise at 25 ^oC for 30 mins with dropping funnel. As soon as finished the addition of anhydride the reaction mixture was stirred for another 30 mins. Then after THF was removed under vacuum and refilled with methanol (0.01M) followed by addition of aldehyde/ketone and isocyanide at 25 ^oC stirred for 48 h. The reaction mixture was evaporated on rotavapor. The crude mixture obtained was purified by flash column chromatography using CH_2Cl_2: MeOH 9:1 to afford the macrocycle **6**.

14-amino-5-oxo-3,9,12-trioxa-6-azatetradecan-1-oic acid 3a: The product was obtained as



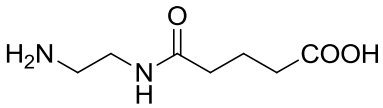
a colorless oil (77%, 0.134g); ^1H NMR (500 MHz, D_2O) δ 4.00 (s, 2H), 3.94 (s, 2H), 3.66 (t, J = 5.1 Hz, 2H), 3.61 (s, 4H), 3.58 (t, J = 4.8 Hz, 2H), 3.38 (t, J = 5.2 Hz, 2H), 3.12 (t, J = 5.3 Hz, 2H); ^{13}C NMR (126 MHz, D_2O) δ 177.0, 172.6, 70.1, 69.5, 69.5, 69.4, 68.7, 66.4, 39.1, 38.4.

5-((3-amino-2,2-dimethylpropyl)amino)-3-methyl-5-oxopentanoic acid 3b: The product



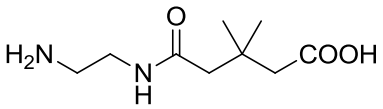
was obtained as a white solid (67%, 0.158g); ^1H NMR (500 MHz, Methanol-d_4) δ 3.04 – 2.91 (m, 2H), 2.61 – 2.52 (m, 2H), 2.30 – 2.15 (m, 2H), 2.12 – 2.02 (m, 1H), 2.02 – 1.91 (m, 2H), 0.90 (s, 9H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 178.0, 174.1, 44.4, 44.0, 43.3, 41.1, 33.1, 27.5, 21.0, 17.8.

5-((2-aminoethyl)amino)-5-oxopentanoic acid 3c: The product was obtained as a white



solid (77%, 0.134g); ^1H NMR (500 MHz, D_2O) δ 3.49 (t, J = 5.9 Hz, 2H), 3.13 (t, J = 6.2 Hz, 2H), 2.28 (t, J = 7.5 Hz, 3H), 2.22 (t, J = 7.3 Hz, 3H), 1.83 (p, J = 7.2 Hz, 2H). ^{13}C NMR (126 MHz, D_2O) δ 181.7, 177.3, 39.1, 36.7, 35.9, 35.1, 21.6.

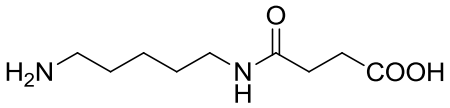
5-((2-aminoethyl)amino)-3,3-dimethyl-5-oxopentanoic acid 3d: The product was obtained



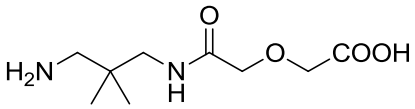
as a white solid (68%, 0.137g); ^1H NMR (500 MHz, D_2O) δ 3.50 (t, J = 6.1 Hz, 2H), 3.15 (t, J = 6.1 Hz, 2H), 2.29 (s, 2H), 2.17 (s, 2H), 1.05 (s, 6H). ^{13}C NMR (126 MHz, D_2O) δ 181.0,

176.0, 49.7, 47.5, 39.1, 36.6, 32.7, 27.5.

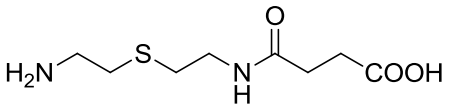
4-((5-aminopentyl)amino)-4-oxobutanoic acid 3f: The product was obtained as a white solid (70%, 0.141g). ^{1}H NMR (500 MHz, D_2O) δ 3.08 (t, *J* = 6.7 Hz, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.36 – 2.33 (m, 4H), 1.62 – 1.50 (m, 2H), 1.49 – 1.37 (m, 2H), 1.31 – 1.22 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 180.5, 175.6, 39.3, 38.8, 32.7, 32.2, 27.7, 26.3, 22.8.



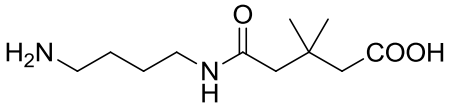
2-(2-((3-amino-2,2-dimethylpropyl)amino)-2-oxoethoxy)acetic acid 3g: The product was obtained as a white solid (71%, 0.154g); ^{1}H NMR (500 MHz, D_2O) δ 4.02 (s, 2H), 3.91 (s, 2H), 3.09 (s, 2H), 2.70 (s, 2H), 0.92 (s, 6H); ^{13}C NMR (126 MHz, D_2O) δ 177.3, 173.5, 70.4, 69.4, 46.3, 45.5, 34.4, 22.3.



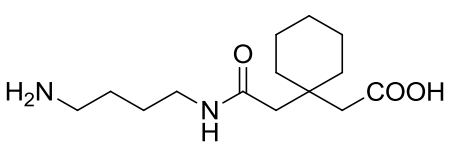
4-((2-((2-aminoethyl)thio)ethyl)amino)-4-oxobutanoic acid 3h: The product was obtained as a white solid (51%, 0.112g); ^{1}H NMR (500 MHz, D_2O) δ 3.28 (t, *J* = 6.6 Hz, 2H), 3.08 (t, *J* = 6.7 Hz, 2H), 2.74 (t, *J* = 6.7 Hz, 2H), 2.59 (t, *J* = 6.6 Hz, 2H), 2.34 (s, 4H); ^{13}C NMR (126 MHz, D_2O) δ 180.8, 176.0, 38.5, 38.3, 32.8, 32.2, 30.2, 28.1.



5-((4-aminobutyl)amino)-3,3-dimethyl-5-oxopentanoic acid 3i: The product was obtained as a white solid (62%, 0.142g); ^{1}H NMR (500 MHz, D_2O) δ 3.12 (t, *J* = 6.7 Hz, 2H), 2.89 (t, *J* = 7.4 Hz, 2H), 2.14 (s, 2H), 2.07 (s, 2H), 1.64 – 1.53 (m, 2H), 1.53 – 1.46 (m, 2H), 0.95 (s, 6H); ^{13}C NMR (126 MHz, D_2O) δ 180.8, 174.8, 49.6, 47.7, 39.0, 38.4, 32.7, 27.4, 27.4, 25.5, 24.3.



2-(1-(2-((4-aminobutyl)amino)-2-oxoethyl)cyclohexyl)acetic acid 3j: The product was obtained as a white solid (64%, 0.172g); ^{1}H NMR (500 MHz, D_2O) δ 3.12 (t, *J* = 6.7 Hz, 2H), 2.88 (t, *J* = 7.3 Hz, 2H), 2.24 (s, 2H), 2.16 (s, 2H), 1.65 – 1.54 (m, 2H), 1.53 – 1.43 (m, 2H), 1.41 – 1.34 (m, 4H), 1.34 – 1.23 (m, 6H); ^{13}C NMR (126 MHz, D_2O) δ 181.3, 175.0, 45.9, 44.2, 39.0, 38.3, 35.9, 35.9, 25.5, 24.3, 21.3.



2-((2-((2-((2-aminoethyl)thio)ethyl)amino)-2-oxoethyl)thio)acetic acid 3l: The product was obtained as a white solid (59%, 0.148g); ^{1}H NMR (500 MHz, D_2O) δ 3.36 (t, *J* = 6.5 Hz, 2H), 3.22 (s, 2H), 3.21 (s, 2H), 3.14 (t, *J* = 6.6 Hz, 2H), 2.79 (t, *J* = 6.6 Hz, 2H), 2.67 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (126 MHz, D_2O) δ 176.7, 172.4, 38.6, 38.3, 36.9, 35.5, 30.2, 28.2.

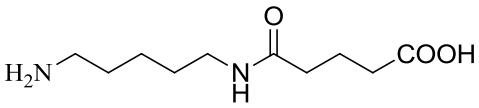
2-((2-((5-aminopentyl)amino)-2-oxoethyl)thio)acetic acid 3m: The product was obtained as a white solid (68%, 0.159g); ^{1}H NMR (500 MHz, D_2O) δ 3.18 (s, 2H), 3.15 (s, 2H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.89 (t, *J* = 7.5 Hz, 2H), 1.62 – 1.52 (m, 2H), 1.51 – 1.42 (m, 2H), 1.33 – 1.24 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 176.7, 172.0, 39.3(2xC), 36.9, 36.0, 27.7, 26.3, 22.9.

2-((2-((5-aminopentyl)amino)-2-oxoethyl)(methyl)amino)aceticacid 3n: The product was obtained as a yellow oil (60%, 0.138g); ^{1}H NMR (500 MHz, D_2O) δ 3.13 (t, *J* = 6.7 Hz, 2H), 3.03 (s, 2H), 2.97 (s, 2H), 2.91 – 2.81 (m, 2H), 2.18 (s, 3H), 1.61 – 1.50 (m, 2H), 1.50 – 1.41 (m, 2H), 1.33 – 1.20 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 178.7, 173.5, 61.3, 60.3, 42.5, 39.3, 38.6, 27.7, 26.3, 22.9.

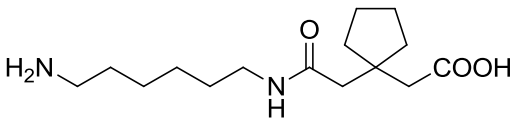
5-((3-amino-2,2-dimethylpropyl)amino)-3,3-dimethyl-5-oxopentanoic acid 3o: The product was obtained as a white solid (62%, 0.151g); ^{1}H NMR (500 MHz, D_2O) δ 3.03 (s, 2H), 2.69 (s, 2H), 2.20 (s, 2H), 2.10 (s, 2H), 0.96 (s, 6H), 0.91 (s, 6H). ^{13}C NMR (126 MHz, D_2O) δ 180.7, 175.8, 49.6, 47.6, 46.4, 46.0, 34.2, 32.7, 27.5, 22.4.

2-(1-(2-((2-((2-aminoethyl)thio)ethyl)amino)-2-oxoethyl)cyclopentyl)acetic acid 3p: The product was obtained as a brown oil (72%, 0.207g); ^{1}H NMR (500 MHz, D_2O) δ 3.33 (t, *J* = 6.5 Hz, 2H), 3.12 (t, *J* = 6.7 Hz, 2H), 2.77 (t, *J* = 6.7 Hz, 2H), 2.65 (t, *J* = 6.5 Hz, 2H), 2.28 (s, 2H), 2.22 (s, 2H), 1.58 – 1.52 (m, 4H), 1.48 – 1.42 (m, 4H); ^{13}C NMR (126 MHz, D_2O) δ 181.1, 175.4, 45.8, 44.3, 44.0, 38.4, 38.2, 37.4(2xC), 30.3, 28.1, 23.3 (2xC).

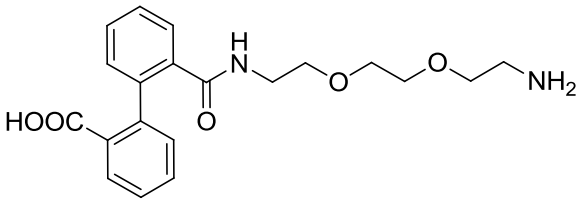
5-((5-aminopentyl)amino)-5-oxopentanoic acid 3q: The product was obtained as a yellow solid (81%, 0.174g); ^{1}H NMR (500 MHz, D_2O) δ 3.18 (t, *J* = 6.8 Hz, 2H), 2.98 (t, *J* = 7.3 Hz, 2H), 2.27 – 2.18 (m, 4H), 1.85 – 1.77 (m, 2H), 1.70 – 1.61 (m, 2H), 1.58 – 1.49 (m, 2H), 1.43 – 1.33 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 181.6, 176.2, 39.3, 38.8, 35.9, 35.3, 27.7, 26.3, 22.9, 22.1.



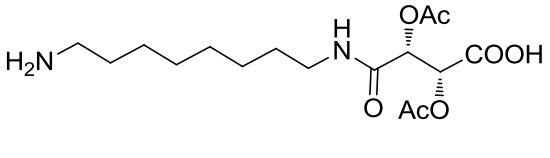
2-(1-(2-((6-aminohexyl)amino)-2-oxoethyl)cyclopentyl)acetic acid 3s: The product was obtained as a white solid (72%, 0.204g); ^{1}H NMR (500 MHz, Methanol-d_4) δ 3.10 – 3.02 (m, 2H), 2.81 – 2.72 (m, 2H), 2.23 (s, 2H), 2.12 (s, 2H), 1.63 – 1.49 (m, 6H), 1.49 – 1.36 (m, 6H), 1.36 – 1.23 (m, 4H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 177.1, 171.3, 43.8, 42.1, 42.0, 36.7, 35.7, 26.3, 26.2, 24.6, 23.6, 23.0, 21.2.



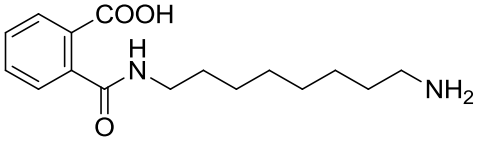
2'-((2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamoyl)-[1,1'-biphenyl]-2-carboxylic acid 3t: The product was obtained as a colorless oil (55%, 0.204g); ^{1}H NMR (500 MHz, D_2O) δ 7.55 – 7.51 (m, 1H), 7.46 (dd, *J* = 7.2, 1.8 Hz, 1H), 7.41 – 7.37 (m, 2H), 7.37 – 7.33 (m, 1H), 7.32 – 7.28 (m, 1H), 7.13 – 7.09 (m, 1H), 7.01 – 6.96 (m, 1H), 3.60 (t, *J* = 5.1 Hz, 2H), 3.49 (t, *J* = 4.4 Hz, 2H), 3.36 – 3.27 (m, 2H), 3.24 – 3.14 (m, 2H), 3.11 – 3.00 (m, 3H), 2.97 – 2.78 (m, 1H).; ^{13}C NMR (126 MHz, D_2O) δ 176.7, 172.3, 139.7, 138.3, 134.4, 130.3, 129.3, 129.1, 127.8, 127.7, 127.4, 127.2, 69.4, 69.4, 68.6, 66.3, 39.0, 39.0.



(2*R*,3*R*)-2,3-diacetoxy-4-((8-aminooctyl)amino)-4-oxobutanoic acid 3w: The product was obtained as a white solid (82%, 0.295g); ^{1}H NMR (500 MHz, D_2O) δ 5.43 (d, *J* = 2.5 Hz, 1H), 5.24 (d, *J* = 2.4 Hz, 1H), 3.19 – 3.04 (m, 2H), 2.89 (t, *J* = 7.6 Hz, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 1.62 – 1.48 (m, 2H), 1.47 – 1.34 (m, 2H), 1.32 – 1.10 (m, 8H); ^{13}C NMR (126 MHz, D_2O) δ 172.7, 172.4, 171.9, 168.6, 73.4, 73.4, 39.5, 39.1, 28.2, 28.0, 27.9, 26.6, 25.6, 25.4, 20.1, 20.0.

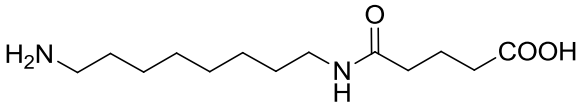


2-((8-aminooctyl)carbamoyl)benzoic acid 3x: The product was obtained as a white solid (83%, 0.239g); ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.47 (d, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.31

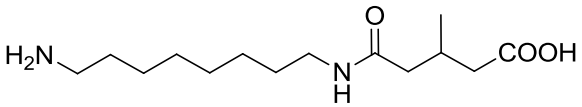


(t, *J* = 7.3 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 3.23 (t, *J* = 6.9 Hz, 2H), 2.78 (t, *J* = 7.4 Hz, 2H), 1.56 – 1.46 (m, 4H), 1.33 – 1.25 (m, 8H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 173.6, 169.2, 137.6, 132.5, 127.9, 126.4, 126.2, 126.1, 37.8, 37.7, 27.1, 26.6, 26.6, 25.3, 24.4, 23.9.

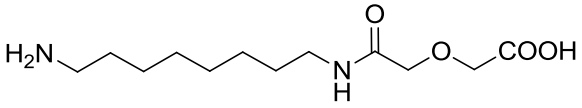
5-((8-aminooctyl)amino)-5-oxopentanoic acid 3z: The product was obtained as a white solid (76%, 0.193g); ^{1}H NMR (500 MHz, D_2O) δ 3.06 (t, *J* = 6.7 Hz, 2H), 2.86 (t, *J* = 7.6 Hz, 2H), 2.12 (t, *J* = 7.5 Hz, 2H), 2.07 (t, *J* = 7.6 Hz, 2H), 1.77 – 1.66 (m, 2H), 1.61 – 1.48 (m, 2H), 1.46 – 1.34 (m, 2H), 1.29 – 1.15 (m, 8H); ^{13}C NMR (126 MHz, D_2O) δ 182.4, 176.1, 39.4, 39.2, 36.7, 35.5, 28.1, 28.0, 27.9, 26.6, 25.7, 25.4, 22.6.



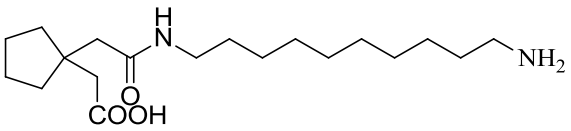
5-((8-aminooctyl)amino)-3-methyl-5-oxopentanoic acid 3ab: The product was obtained as a white solid (75%, 0.204g); ^{1}H NMR (500 MHz, Methanol-d_4) δ 2.98 – 2.84 (m, 2H), 2.67 – 2.59 (m, 2H), 2.10 – 1.96 (m, 2H), 1.91 (dd, *J* = 13.9, 6.3 Hz, 1H), 1.83 – 1.69 (m, 2H), 1.46 – 1.33 (m, 2H), 1.31 – 1.19 (m, 2H), 1.18 – 1.03 (m, 8H), 0.71 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 178.1, 172.1, 43.2, 41.5, 37.6, 37.1, 27.5, 27.3, 27.0, 26.9, 25.5, 24.7, 24.2, 17.3.



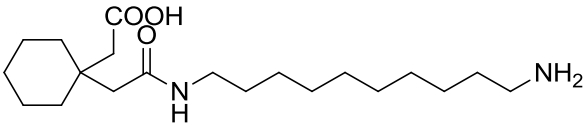
2-(2-((8-aminooctyl)amino)-2-oxoethoxy)acetic acid 3ad: The product was obtained as a colorless oil (74%, 0.192g); ^{1}H NMR (500 MHz, Methanol-d_4) δ 3.92 (s, 2H), 3.89 (s, 2H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.84 (d, *J* = 8.3 Hz, 2H), 1.58 (p, *J* = 7.3 Hz, 2H), 1.52 – 1.43 (m, 2H), 1.34 – 1.25 (m, 8H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 173.8, 169.3, 68.7, 68.3, 37.6, 36.9, 27.3, 27.0, 27.0, 25.5, 24.8, 24.3.



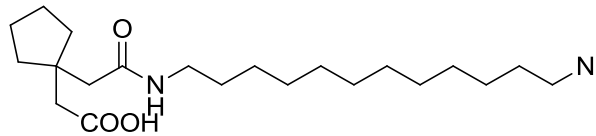
2-(1-(2-((10-aminodecyl)amino)-2-oxoethyl)cyclopentyl)acetic acid 3ag: The product was obtained as a white solid (75%, 0.255g); ^{1}H NMR (500 MHz, D_2O) δ 3.24 (t, *J* = 6.6 Hz, 2H), 3.03 (t, *J* = 8.4 Hz, 2H), 2.40 (s, 2H), 2.32 (s, 2H), 1.75 – 1.65 (m, 6H), 1.65 – 1.52 (m, 6H), 1.45 – 1.31 (m, 12H); ^{13}C NMR (126 MHz, D_2O) δ 181.4, 175.0, 46.4, 44.4, 44.0, 39.5, 39.1, 37.2, 28.3, 28.2, 28.1, 28.0, 26.6, 26.0, 25.4, 23.4.



2-(1-(2-((10-aminodecyl)amino)-2-oxoethyl)cyclohexyl)acetic acid 3ah: The product was obtained as a white solid (80%, 0.327g); ^{1}H NMR (500 MHz, Methanol-d4) δ 3.21 (t, J = 6.9, 4.0 Hz, 2H), 2.99 – 2.88 (m, 2H), 2.41 (s, 2H), 2.33 (s, 2H), 1.78 – 1.63 (m, 2H), 1.62 – 1.50 (m, 6H), 1.50 – 1.31 (m, 18H). ^{13}C NMR (126 MHz, Methanol-d4) δ 178.7, 173.2, 45.6, 43.8, 39.3, 38.8, 36.6, 35.7, 29.1, 28.9, 28.8, 28.7, 28.6, 27.1, 26.6, 26.0, 25.8, 21.4.

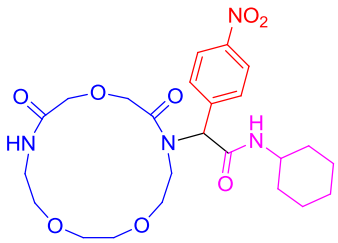


2-(1-(2-((12-aminododecyl)amino)-2-oxoethyl)cyclopentyl)acetic acid 3ai: The product was obtained as a white solid (74%, 0.272g); ^{1}H NMR (500 MHz, Methanol-d_4) δ 3.19 (t, *J* = 6.1 Hz, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 2.36 (s, 2H), 2.26 (s, 2H), 1.76 – 1.62 (m, 6H), 1.61 – 1.55 (m, 4H), 1.54 – 1.47 (m, 2H), 1.43 – 1.29 (m, 16H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 177.8, 172.0, 44.6, 42.8, 42.7, 37.8, 37.2, 36.3, 27.5, 27.3, 27.3, 27.2, 27.0, 25.6, 25.0, 24.4, 22.0.

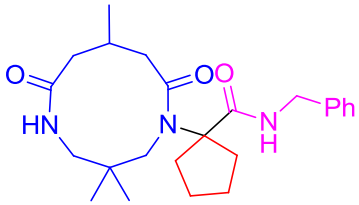


5-((10-aminodecyl)amino)-3,3-dimethyl-5-oxopentanoic acid 3aj: The product was obtained as a white solid (80%, 0.251g); 1H NMR (500 MHz, D_2O) δ 3.17 (t, *J* = 6.7 Hz, 2H), 2.97 (t, *J* = 7.4 Hz, 2H), 2.24 (s, 2H), 2.18 (s, 2H), 1.72 – 1.60 (m, 2H), 1.55 – 1.43 (m, 2H), 1.36 – 1.25 (m, 12H), 1.05 (s, 6H); ^{13}C NMR (126 MHz, D_2O) δ 180.8, 174.5, 49.8, 48.8, 47.7, 39.5, 39.2, 32.8, 28.4, 28.3, 28.3, 28.2, 28.1, 27.5, 26.7, 26.1, 25.5.

N-cyclohexyl-2-(8,12-dioxo-1,4,10-trioxa-7,13-diazacyclopentadecan-7-yl)-2-(4-nitrophenyl)acetamide 6a: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as a white solid (55%, 0.278g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 – 8.13 (m, 3H), 7.72 (t, *J* = 5.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 6.01 (s, 1H), 4.66 – 4.50 (m, 2H), 3.99 (s, 2H), 3.66 – 3.56 (m, 1H), 3.56 – 3.41 (m, 6H), 3.34 – 3.27 (m, 4H), 3.27 – 3.20 (m, 1H), 3.15 – 3.04 (m, 1H), 1.84 – 1.73 (m, 2H), 1.73 – 1.62 (m, 2H), 1.59 – 1.50 (m, 1H), 1.34 – 1.22 (m, 2H), 1.22 – 1.07 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.7, 169.6, 167.4, 147.3, 145.4, 130.1, 123.8, 69.8, 69.6, 69.5, 69.5, 68.3, 67.8, 61.0, 48.4, 45.6, 38.3, 32.6, 25.6, 25.0. HRMS (ESI) m/z calculated for $C_{24}H_{35}N_4O_8$ $[M+H]^+$: 507.2449; found $[M+H]^+$: 507.2447.



N-benzyl-1-(3,3,8-trimethyl-6,10-dioxo-1,5-diazecan-1-yl)cyclopentanecarboxamide 6b:



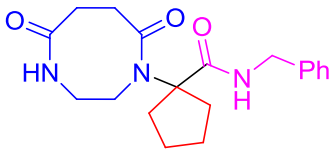
Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as colorless oil (38%, 0.157g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (br s, 1H), 7.33 – 7.30 (m, 4H), 7.29 – 7.26 (m, 1H), 6.94 (t, *J* = 5.5 Hz, 1H), 4.71 – 4.55 (m, 2H), 4.39 (dd, *J* = 14.5, 5.4 Hz, 1H), 3.62 (dd, *J* = 12.3, 5.8 Hz, 1H), 2.64 (d, *J* = 13.0 Hz, 1H), 2.62 – 2.54 (m, 1H), 2.49 (d, *J* = 14.3 Hz, 1H), 2.34 – 2.28 (m, 1H), 2.05 – 1.94 (m, 5H), 1.93 – 1.86 (m, 3H), 1.82 – 1.75 (m, 1H), 1.69 – 1.57 (m, 2H), 1.28 (s, 3H), 0.87 (s, 3H), 0.75 (d, *J* = 6.9 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 177.6, 176.0, 174.3, 138.1, 128.8, 128.0, 127.6, 73.6, 52.3, 50.3, 44.3, 44.0, 43.9, 40.9, 38.5, 34.5, 33.1, 28.3, 25.8, 24.4, 23.3. HRMS (ESI) m/z calculated for $C_{24}H_{36}N_3O_3$ $[M+H]^+$: 414.2751; found $[M+H]^+$: 414.2746.

N-benzyl-1-(5,9-dioxo-1,4-diazonan-1-yl)cyclopentanecarboxamide 6c: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi-solid (27%, 0.096g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.33 (m, 4H), 7.32 – 7.29 (m, 1H), 7.27 – 7.22 (m, 1H), 6.48 (t, *J* = 5.7 Hz, 1H), 4.54 – 4.40 (m, 2H), 3.99 – 3.89 (m, 1H), 3.88 – 3.78 (m, 1H), 3.25 (dd, *J* = 15.8, 4.4 Hz, 1H), 2.97 – 2.87 (m, 1H), 2.69 – 2.59 (m, 1H), 2.45 – 2.38 (m, 1H), 2.32 – 2.18 (m, 3H), 2.11 – 2.05 (m, 2H), 2.03 – 1.96 (m, 1H), 1.96 – 1.88 (m, 1H), 1.86 – 1.79 (m, 1H), 1.78 – 1.65 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.8, 175.8, 175.8, 138.2, 128.7, 127.6, 127.5, 73.3, 44.3, 43.9, 38.8, 36.8, 35.4, 33.7, 29.2, 23.8, 23.6, 22.9; HRMS (ESI) m/z calculated for $C_{20}H_{28}N_3O_3$ $[M+H]^+$: 358.2125; found $[M+H]^+$: 358,2125.

N-(4-chlorophenyl)-2-cyclopropyl-2-(3,3-dimethyl-6,9-dioxo-1,5-diazonan-1-yl) acetamide 6d: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as a white solid (59%, 0.230g). ^{1}H NMR (500 MHz, $CDCl_3$, rotamers were observed) δ 8.94 (s, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 2H), 6.64 (dd, *J* = 10.9, 3.5 Hz, 1H), 3.72 (dd, *J* = 15.0, 11.4 Hz, 1H), 3.63 (d, *J* = 15.5 Hz, 1H), 2.83 (t, *J* = 12.0 Hz, 2H), 2.75 – 2.69 (m, 3H), 2.64 – 2.57 (m, 1H), 1.96 – 1.86 (m, 1H), 1.08 (s, 3H), 0.97 – 0.93 (m, 1H), 0.92 (s, 3H), 0.89 – 0.83 (m, 1H), 0.78 – 0.71 (m, 1H), 0.58 – 0.52 (m, 1H), 0.52 – 0.46 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$, rotamers were observed) δ 175.9, 174.0, 168.2, 136.4, 129.3, 128.9, 121.6,

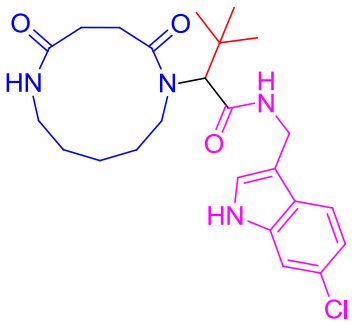
72.2, 57.9, 48.9, 39.7, 31.3, 28.9, 24.8, 24.0, 10.7, 4.4, 3.9. HRMS (ESI) m/z calculated for $C_{20}H_{27}ClN_3O_3$ $[M+H]^+$: 392.1733; found $[M+H]^+$: 392.1734.

N-benzyl-1-(5,8-dioxo-1,4-diazocan-1-yl)cyclopentanecarboxamide 6e: Prepared



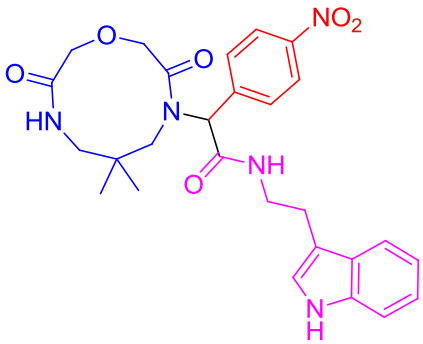
according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (50%, 0.171g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, *J* = 5.5 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.29 – 7.25 (m, 3H), 6.26 (t, *J* = 5.6 Hz, 1H), 4.41 (d, *J* = 5.8 Hz, 2H), 3.68 (t, *J* = 6.1 Hz, 2H), 3.35 (q, *J* = 6.0 Hz, 2H), 2.79 (t, *J* = 7.2 Hz, 2H), 2.69 – 2.63 (m, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 1.83 – 1.76 (m, 2H), 1.74 – 1.65 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.7, 174.6, 172.8, 138.4, 128.7, 127.7, 127.3, 73.3, 46.7, 43.7, 42.2, 36.0, 33.7, 31.2, 22.3. HRMS (ESI) m/z calculated for $C_{19}H_{26}N_3O_3$ $[M+H]^+$: 344.1968; found $[M+H]^+$: 344,1969.

N-((6-chloro-1H-indol-3-yl)methyl)-2-(2,5-dioxo-1,6-diazacycloundecan-1-yl)-3,3-



dimethylbutanamide 6f: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow semisolid (51%, 234g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.08 (s, 1H), 7.89 (d, *J* = 9.4 Hz, 1H), 7.63 (d, *J* = 5.8 Hz, 1H), 7.46 (s, 1H), 7.40 (s, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 4.57 (dd, *J* = 14.5, 4.2 Hz, 1H), 4.30 – 4.17 (m, 2H), 4.18 – 4.04 (m, 1H), 3.68 (d, *J* = 11.5 Hz, 1H), 3.54 – 3.39 (m, 2H), 3.23 – 3.14 (m, 2H), 2.57– 2.53 (m, 1H), 2.25 – 2.12 (m, 1H), 2.02 – 1.91 (m, 1H), 1.68 – 1.57 (m, 1H), 1.35 – 1.11 (m, 4H), 0.94 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.5, 171.9, 168.9, 137.1, 126.3, 125.9, 125.8, 120.5, 119.3, 112.4, 111.4, 67.7, 44.9, 39.0, 37.9, 36.2, 34.3, 30.4, 28.4, 27.8, 27.3, 21.2. HRMS (ESI) m/z calculated for $C_{24}H_{34}ClN_4O_3$; found:461.2316; found $[M+H]^+$: 461.23157.

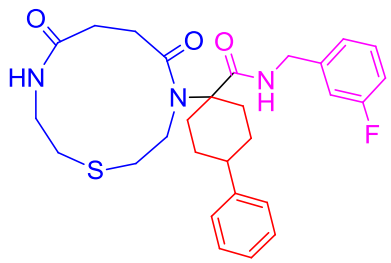
N-(2-(1H-indol-3-yl)ethyl)-2-(6,6-dimethyl-3,9-dioxo-1,4,8-oxadiazecan-4-yl)-2-(4-



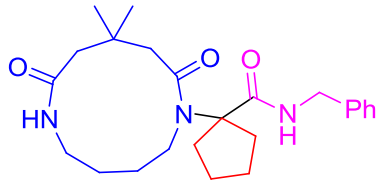
nitrophenyl)acetamide 6g: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi solid (50%, 0.260g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H), 8.15 – 8.11 (m, 2H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.45 – 7.41 (m, 2H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.24 – 7.20 (m, 2H), 7.17 – 7.12 (m, 2H), 7.01 (d, *J* = 2.1 Hz, 1H), 4.33 (s, 4H), 4.12 (s, 1H), 3.73 – 3.66 (m, 2H), 3.66 – 3.62 (m, 2H), 3.06 – 3.00 (m, 2H), 2.22 (d, *J* = 11.8 Hz, 1H),

2.11 (d, *J* = 11.9 Hz, 1H), 0.80 (s, 3H), 0.75 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9, 170.1, 147.5, 146.6, 136.4, 128.2, 127.4, 123.8, 122.2, 122.1, 119.5, 118.7, 112.6, 111.3, 67.8, 67.7, 57.0, 44.0, 39.3, 36.4, 25.0, 24.5, 24.2. HRMS (ESI) m/z calculated for $C_{27}H_{32}N_5O_6$ $[M+H]^+$: 522.23499; found $[M+H]^+$: 522.23486.

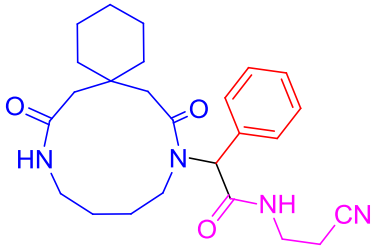
1-(5,8-dioxo-1-thia-4,9-diazacycloundecan-4-yl)-N-(3-fluorobenzyl)-4-phenyl cyclohexanecarboxamide 6h: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow semisolid (33%, 0.175g), ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.80 (t, *J* = 5.5 Hz, 1H), 7.27 (t, *J* = 7.4 Hz, 3H), 7.17 (t, *J* = 7.3 Hz, 1H), 7.12 (d, *J* = 7.4 Hz, 2H), 7.08 (d, *J* = 7.7 Hz, 1H), 7.06 – 7.01 (m, 1H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.03 (br s, 1H), 4.75 – 4.60 (m, 1H), 4.25 – 4.12 (m, 1H), 3.78 (br s, 1H), 3.56 – 3.48 (m, 2H), 3.24 – 3.13 (m, 1H), 3.12 – 3.00 (m, 1H), 2.87 – 2.70 (m, 4H), 2.65 – 2.57 (m, 2H), 2.56 – 2.49 (m, 1H), 2.47 – 2.40 (m, 1H), 2.24 – 2.09 (m, 2H), 2.01 – 1.85 (m, 2H), 1.84 – 1.67 (m, 2H), 1.45 – 1.34 (m, 1H), 1.27 – 1.15 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.8, 175.4, 173.0, 162.92 (d, *J* =245.7 Hz), 145.8, 141.5 (d, *J* = 7.0 Hz), 130.1 (d, *J* = 8.0 Hz), 128.5, 126.5, 126.4, 123.0, 114.3 (d, *J* = 22.1 Hz), 114.0 (d, *J* =21.1 Hz), 64.4, 46.0, 42.8, 39.8, 34.6, 34.2, 34.0, 32.8, 31.4, 30.5, 29.6, 29.0. HRMS (ESI) m/z calculated for $C_{28}H_{35}FN_3O_3S$ $[M+H]^+$:534.21973; found $[M+H]^+$: 534.21979.



N-benzyl-1-(9,9-dimethyl-7,11-dioxo-1,6-diazacycloundecan-1-yl)cyclopentanecarboxamide 6i: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (31% yield, 0.128g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (t, *J* = 6.3 Hz, 1H), 7.43 (d, *J* = 7.2 Hz, 2H), 7.37 (t, *J* = 8.4, 6.7 Hz, 2H), 7.32 – 7.29 (m, 1H), 6.14 (t, *J* = 5.8 Hz, 1H), 4.74 – 4.57 (m, 1H), 4.50 – 4.35 (m, 1H), 3.96 (s, 1H), 3.68 – 3.45 (m, 1H), 3.37 – 3.22 (m, 1H), 2.84 – 2.58 (m, 3H), 2.40 – 2.27 (m, 1H), 2.26 – 2.14 (m, 1H), 1.99 – 1.82 (m, 6H), 1.82 – 1.62 (m, 6H), 1.40 (s, 3H), 1.21 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.3, 173.6, 171.6, 138.3, 128.7, 127.7, 127.4, 73.2, 45.9, 44.8, 44.0, 42.1, 40.1, 36.0, 35.8, 34.8, 30.9, 30.0, 29.4, 27.6, 24.3, 23.6; HRMS (ESI) m/z calculated for $C_{24}H_{35}N_3NaO_3$ $[M+Na]^+$: 436.2571; found $[M+Na]^+$: 436.2570.



N-(2-cyanoethyl)-2-(8,15-dioxo-9,14-diazaspiro[5.10]hexadecan-9-yl)-2-phenylacetamide

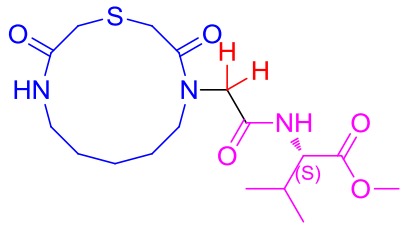


6j: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi-solid (25%, 0.109g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 – 8.12 (m, 1H), 7.48 – 7.40 (m, 3H), 7.39 – 7.34 (m, 2H), 5.86 (t, *J* = 6.3 Hz, 1H), 4.56 (s, 1H), 4.01 – 3.91 (m, 1H), 3.70 – 3.60 (m, 1H), 3.57 – 3.42 (m, 2H), 3.06 – 2.95 (m, 1H), 2.81 – 2.67 (m, 2H), 2.63 – 2.53 (m, 1H), 2.49 – 2.41 (m, 1H), 2.34 – 2.27 (m, 1H), 2.24 (d, *J* = 12.4 Hz, 1H), 2.13 (d, *J* = 12.4 Hz, 1H), 2.00 (br s, 1H), 1.93 – 1.80 (m, 5H), 1.79 – 1.72 (m, 1H), 1.68 – 1.56 (m, 2H), 1.54 – 1.38 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 171.5, 171.4, 134.0, 129.6, 129.4, 129.2, 117.9, 66.4, 48.7, 43.3, 39.9, 38.1, 37.9, 37.2, 36.3, 27.4, 27.2, 26.2, 21.9, 21.8, 17.6; HRMS (ESI) m/z calculated for $C_{25}H_{35}N_4O_3$ $[M+H]^+$: 439.2704; found $[M+H]^+$: 439.2701.

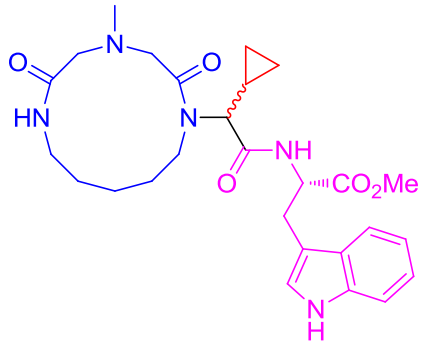
methyl 4-(2-(1,9-dioxo-4,5,6,7,8,9-hexahydro-1H-benzo[c][1,6]diazacycloundecin-2(3H)-yl)-3-methylbutanamido)butanoate 6k: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (42%, 0.181g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.51 – 7.42 (m, 2H), 7.28 (dd, *J* = 7.4, 1.4 Hz, 1H), 6.53 (d, *J* = 7.2 Hz, 1H), 6.51 – 6.46 (m, 1H), 4.03 – 3.96 (m, 1H), 3.92 – 3.83 (m, 1H), 3.61 (s, 3H), 3.46 – 3.30 (m, 2H), 3.14 (d, *J* = 10.5, 1H), 3.09 – 2.97 (m, 1H), 2.90 – 2.79 (m, 1H), 2.39 – 2.34 (m, 1H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.92 – 1.81 (m, 3H), 1.68 – 1.52 (m, 3H), 1.51 – 1.39 (m, 1H), 1.21 – 1.13 (m, 1H), 0.82 (d, *J* = 6.9 Hz, 3H), 0.64 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 172.8, 169.6, 168.6, 134.8, 134.2, 130.8, 129.7, 128.7, 128.2, 68.5, 51.8, 42.1, 40.3, 39.3, 31.7, 29.0, 28.5, 26.6, 24.5, 20.9, 19.6, 19.0. HRMS (ESI) m/z calculated for $C_{23}H_{34}N_3O_3$ $[M+H]^+$: 432.2493; found $[M+H]^+$: 432.2493.

2-(3,11-dioxo-1,7-dithia-4,10-diazacyclododecan-4-yl)-2-(3-hydroxyphenyl)-N-phenethylacetamide 6l: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow solid (72%, 0.350g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.55 (t, *J* = 5.7 Hz, 1H), 8.25 (t, *J* = 5.5 Hz, 1H), 7.31 – 7.23 (m, 2H), 7.22 – 7.17 (m, 3H), 7.15 (t, *J* = 7.9 Hz, 1H), 6.73 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.66 (s, 1H), 6.58 (d, *J* = 7.7 Hz, 1H), 5.91 (s, 1H), 3.57 – 3.45 (m, 4H), 3.42 – 3.35 (m, 2H), 3.30 – 3.22 (m, 2H), 3.13 (s, 2H), 2.80 – 2.62 (m, 4H), 2.60 – 2.53 (m, 1H), 2.06 – 1.95 (m, 1H); ^{13}C NMR (126

MHz, DMSO-d_6) δ 169.3, 169.0, 168.8, 157.9, 139.8, 137.8, 130.1, 129.1, 128.8, 126.6, 120.1, 116.4, 115.6, 60.4, 46.8, 43.2, 40.8, 35.6, 35.4, 33.4, 30.8, 30.0. HRMS (ESI) m/z calculated for $C_{24}H_{30}N_3O_4S_2$ $[M+H]^+$: 488.16653; found $[M+H]^+$: 488.16675.

methyl 2-(2-(3,11-dioxo-1-thia-4,10-diazacyclododecan-4-yl)acetamido)-3-methyl butanoate 6m: 

Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (42%, 0.162g). ^{1}H NMR (500 MHz, Chloroform-d, rotamers were observed) δ 6.60 (t, *J* = 8.4 Hz, 1H), 4.61 – 4.55 (m, 1H), 4.55 – 4.44 (m, 1H), 4.38 – 4.18 (m, 1H), 4.04 – 3.85 (m, 1H), 3.74 (s, 3H), 3.70 – 3.59 (m, 1H), 3.55 – 3.39 (m, 2H), 3.35 – 3.27 (m, 2H), 2.91 (t, *J* = 12.4 Hz, 1H), 2.69 – 2.56 (m, 1H), 2.26 – 2.15 (m, 1H), 1.92 – 1.82 (m, 2H), 1.64 – 1.53 (m, 2H), 1.46 – 1.34 (m, 2H), 1.21 – 1.10 (m, 1H), 0.97 – 0.89 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$, rotamers were observed) δ 173.1, 172.5, 171.2, 168.2, 57.7, 52.1, 47.9, 40.6, 38.3, 37.3, 35.7, 30.7, 26.7, 24.6, 19.2, 17.9. HRMS (ESI) m/z calculated for $C_{17}H_{30}N_3O_5S$ $[M+H]^+$: 388.19034; found $[M+H]^+$: 388.19025.

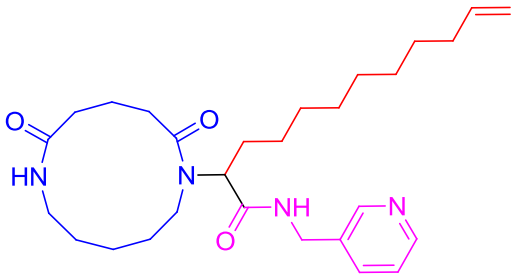
methyl 2-(2-cyclopropyl-2-(4-methyl-2,6-dioxo-1,4,7-triazacyclododecan-1-yl)acetamido)-3-(1H-indol-3-yl)propanoate 6n: 

Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow semisolid (35%, 0.178g). ^{1}H NMR (500 MHz, $CDCl_3$, 1:1 diastereomeric ratio) δ 8.70 (s, 1H), 8.57 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 2H), 7.52 – 7.46 (m, 1H), 7.40 – 7.29 (m, 3H), 7.22 – 7.13 (m, 3H), 7.14 – 7.05 (m, 5H), 4.96 – 4.87 (m, 2H), 3.74 (s, 3H), 3.69 (s, 3H), 3.49 (s, 1H), 3.47 – 3.31 (m, 7H), 3.27 – 3.18 (m, 2H), 3.17 – 3.06 (m, 2H), 3.08 – 2.93 (m, 7H), 2.43 (s, 3H), 2.39 (s, 3H), 2.26 – 2.07 (m, 3H), 1.83 – 1.61 (m, 3H), 1.62 – 1.34 (m, 11H), 0.69 – 0.49 (m, 4H), 0.32 – 0.13 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$, 1:1 diastereomeric ratio) δ 172.5, 172.6, 170.6, 170.6, 170.5, 170.3, 170.0, 169.8, 136.2, 136.2, 127.5, 127.5, 123.5, 123.1, 122.1, 122.0, 119.4, 119.4, 118.5, 111.4, 111.3, 109.7, 109.4, 64.0, 63.9, 63.1, 62.4, 58.9, 58.4, 52.7, 52.4, 52.3, 45.8, 45.6, 44.2, 44.1, 39.6, 39.6, 27.6, 27.5, 27.3, 26.2, 26.0, 24.6, 24.2, 9.8, 9.3, 5.9, 5.7, 3.5, 3.5. HRMS (ESI) m/z calculated for $C_{27}H_{38}N_5O_5$ $[M+H]^+$: 512.2867; found $[M+H]^+$: 512.2867.

N-([1,1'-biphenyl]-4-ylmethyl)-1-(3,3,8,8-tetramethyl-6,10-dioxo-1,5-diazecan-1-yl)cyclopentanecarboxamide 6o: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (37%, 0.186g). ^{1}H NMR (500 MHz, Methanol-d4) δ 7.64 – 7.55 (m, 4H), 7.53 – 7.47 (m, 2H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.39 – 7.30 (m, 1H), 4.59 (dd, *J* = 14.5, 2.7 Hz, 1H), 4.54 – 4.40 (m, 2H), 3.50 (dd, *J* = 12.9, 2.7 Hz, 1H), 2.74 – 2.59 (m, 3H), 2.55 – 2.44 (m, 1H), 2.34 (dd, *J* = 12.1, 2.6 Hz, 1H), 2.22 (dd, *J* = 14.3, 2.7 Hz, 1H), 2.12 – 2.01 (m, 1H), 2.01 – 1.89 (m, 3H), 1.89 – 1.82 (m, 1H), 1.74 – 1.53 (m, 3H), 1.36 (s, 3H), 1.33 (s, 3H), 0.94 (s, 3H), 0.56 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d4) δ 176.2, 175.2, 172.4, 139.1, 138.8, 136.3, 126.9, 126.9, 125.4, 125.3, 125.0, 72.7, 50.4, 48.3, 45.0, 44.5, 41.8, 38.8, 36.8, 35.8, 32.8, 32.1, 25.5, 25.4, 22.8, 22.3. HRMS (ESI) m/z calculated for $C_{31}H_{42}N_3O_3$ $[M+H]^+$: 504.3221; found $[M+H]^+$: 504.3221.

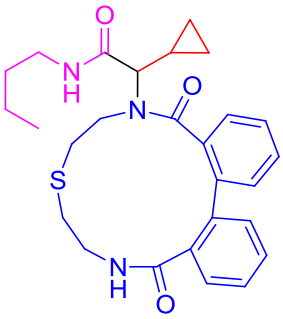
***tert*-butyl (3-((2-chloro-4-fluoro-3-methylbenzyl)amino)-2-(7,15-dioxo-11-thia-8,14-diazaspiro[4.11]hexadecan-8-yl)-3-oxopropyl)carbamate 6p:** Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow semisolid (26%, 0.159g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (br s, 1H), 7.18 (dd, *J* = 8.4, 6.1 Hz, 1H), 6.94 (t, *J* = 8.7 Hz, 1H), 5.95 (s, 1H), 5.20 (s, 1H), 4.79 (dd, *J* = 14.0, 5.9 Hz, 1H), 4.55(dd, *J* = 14.1, 3.3 Hz, 1H), 4.05 (s, 1H), 3.81 – 3.58 (m, 2H), 3.55 – 3.48 (m, 1H), 3.47 – 3.39 (m, 1H), 3.01 (s, 1H), 2.92 – 2.82 (m, 2H), 2.76 – 2.70 (m, 2H), 2.35 (s, 3H), 2.26 (d, *J* = 12.6, 1H), 2.19 (d, *J* = 12.6 Hz, 1H), 2.10 – 2.01 (m, 1H), 1.92 – 1.82 (m, 2H), 1.71 – 1.62 (m, 3H), 1.62 – 1.54 (m, 2H), 1.49 – 1.44 (m, 3H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.1, 172.4, 169.7, 159.7 (d, *J* =247.8 Hz), 156.1, 135.3 (d, *J* = 5.0Hz), 132.6 (d, *J* = 3.6Hz), 130.8 (d, *J* = 9.0 Hz), 123.4 (d, *J* = 17.7 Hz), 113.8 (d, *J* = 22.5 Hz), 79.9, 59.6, 46.5, 44.4, 43.6, 41.2, 39.5, 39.1, 38.7, 38.0, 35.3, 35.3, 34.2, 32.7, 28.3, 23.9, 23.6, 20.1. HRMS (ESI) m/z calculated for $C_{29}H_{43}ClFN_4O_5S$ $[M+H]^+$: 613.2622; found $[M+H]^+$: 613.2622.

2-(2,6-dioxo-1,7-diazacyclododecan-1-yl)-N-(pyridin-3-ylmethyl)dodec-11-enamide 6q:

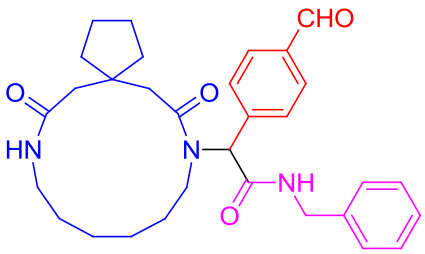


Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow semisolid (40%, 0.193g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 – 8.40 (m, 2H), 7.85 (s, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.28 – 7.24 (m, 1H), 7.01 – 6.88 (m, 1H), 5.85 – 5.68 (m, 1H), 4.98 – 4.86 (m, 2H), 4.70 – 4.56 (m, 1H), 4.57 – 4.44 (m, 1H), 4.26 – 4.11 (m, 1H), 3.53 (s, 1H), 3.25 (t, *J* = 6.8 Hz, 2H), 2.91 (s, 1H), 2.53 – 2.44 (m, 1H), 2.32 – 2.23 (m, 1H), 2.21 – 2.15 (m, 1H), 2.14 – 2.09 (m, 1H), 2.02 – 1.98 (m, 2H), 1.96 – 1.88 (m, 2H), 1.71 (s, 1H), 1.54 – 1.46 (m, 1H), 1.45 – 1.39 (m, 2H), 1.38 – 1.29 (m, 3H), 1.29 – 1.15 (m, 11H), 1.11 – 1.01 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.5, 172.8, 171.6, 161.2, 148.7, 148.2, 139.2, 136.2, 123.7, 114.2, 58.7, 44.4, 40.6, 39.7, 34.7, 33.8, 29.7, 29.4, 29.3, 29.2, 29.1, 28.9, 27.8, 26.6, 26.3, 22.3, 21.6. HRMS (ESI) m/z calculated for $C_{28}H_{45}N_4O_3$ $[M+H]^+$: 485.34842; found $[M+H]^+$: 485.34836.

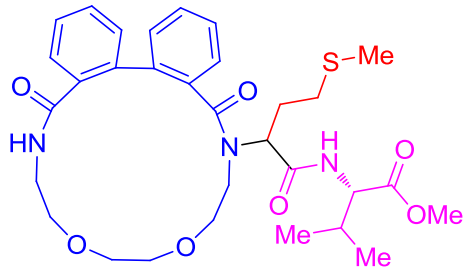
N-butyl-2-cyclopropyl-2-(5,13-dioxo-7,8,10,11,12,13-hexahydrodibenzo[f,h][1,4,11]thia diazacyclotridecin-6(5H)-yl)acetamide 6r:



Prepared according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as yellow semisolid (75%, 0.359g); ^{1}H NMR (500 MHz, $CDCl_3$, 1:1 ratio of atropisomers) δ 7.81 – 7.76 (m, 1H), 7.75 – 7.69 (m, 1H), 7.67 – 7.63 (m, 1H), 7.60 – 7.55 (m, 1H), 7.53 – 7.42 (m, 9H), 7.42 – 7.31 (m, 3H), 6.67 – 6.60 (m, 1H), 6.27 (t, *J* = 5.4 Hz, 1H), 6.17 (dd, *J* = 7.4, 4.3 Hz, 1H), 6.02 (t, *J* = 6.0 Hz, 1H), 3.92 – 3.82 (m, 2H), 3.80 – 3.66 (m, 2H), 3.63 – 3.40 (m, 3H), 3.38 – 3.20 (m, 3H), 3.18 – 2.98 (m, 3H), 2.84 – 2.68 (m, 3H), 2.66 – 2.41 (m, 2H), 2.15 (td, *J* = 13.1, 5.0 Hz, 1H), 2.03 – 1.91 (m, 1H), 1.64 – 1.53 (m, 1H), 1.50 – 1.40 (m, 3H), 1.38 – 1.28 (m, 4H), 1.28 – 1.17 (m, 4H), 0.93 (t, *J* = 7.2 Hz, 6H), 0.86 – 0.77 (m, 1H), 0.72 – 0.56 (m, 2H), 0.44 – 0.32 (m, 2H), 0.30 – 0.20 (m, 2H), -0.20 – -0.30 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$, 1:1 ratio of atropisomers) δ 171.5, 171.4, 170.4, 170.3, 168.3, 167.7, 137.2, 137.1, 137.0, 135.7, 135.5, 135.4, 135.0, 134.5, 132.7, 132.7, 132.3, 131.5, 129.7, 129.4, 128.9, 128.7, 128.3, 128.2, 128.0, 127.9, 127.4, 127.4, 63.9, 62.7, 48.9, 47.6, 42.6, 41.4, 39.1, 33.6, 31.4, 31.3, 31.3, 31.0, 30.6, 20.1, 20.0, 13.8, 13.7, 10.0, 9.3, 6.3, 6.0, 3.0, 2.9. HRMS (ESI) m/z calculated for $C_{27}H_{34}N_3O_3S$ $[M+H]^+$: 480.23136; found $[M+H]^+$: 480.23126.

N-benzyl-2-(7,16-dioxo-8,15-diazaspiro[4.12]heptadecan-8-yl)-2-(4-formylphenyl)acetamide 6s: 

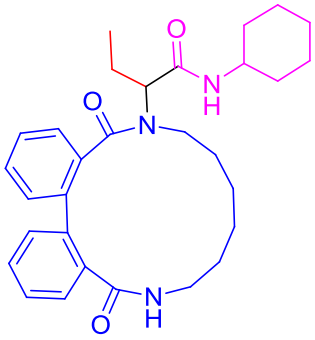
Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as colorless oil (43%,); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 7.88 (d, *J* = 8.2 Hz, 2H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.32 – 7.28 (m, 4H), 7.28 – 7.22 (m, 1H), 7.11 (t, *J* = 5.8 Hz, 1H), 6.12 (t, *J* = 5.9 Hz, 1H), 5.01 (s, 1H), 4.56 (dd, *J* = 15.0, 6.0 Hz, 1H), 4.40 (dd, *J* = 15.0, 5.6 Hz, 1H), 3.69 – 3.51 (m, 2H), 3.08 – 2.91 (m, 2H), 2.87 (d, *J* = 16.3 Hz, 1H), 2.50 (d, *J* = 12.9 Hz, 1H), 2.35 (d, *J* = 16.2 Hz, 1H), 2.26 – 2.15 (m, 1H), 2.06 (d, *J* = 12.8 Hz, 1H), 1.99 – 1.87 (m, 2H), 1.87 – 1.80 (m, 2H), 1.75 – 1.58 (m, 6H), 1.54 – 1.42 (m, 4H), 1.41 – 1.29 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.4, 173.0, 172.6, 169.2, 141.9, 137.7, 136.4, 130.4, 130.0, 128.6, 127.6, 127.5, 65.1, 45.7, 45.0, 44.1, 43.9, 41.0, 39.1, 38.0, 26.0, 25.8, 24.8, 24.3, 23.6, 22.3; HRMS (ESI) m/z calculated for $C_{31}H_{40}N_3O_4$ $[M+H]^+$: 518.3013; found $[M+H]^+$: 518.3012.

(2*S*)-methyl 2-(2-(5,16-dioxo-7,8,10,11,13,14,15,16-octahydrodibenzo[i,k][1,4,7,14]dioxadiazacyclohexadecin-6(5H)-yl)-4-(methylthio)butanamido)-3-methylbutanoate 6t: 

Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (42%, 0.251g); ^{1}H NMR (500 MHz, $CDCl_3$, mixture of 1:1:1:1 ratio of diastereomers and atropisomers) δ 9.21 (d, *J* = 8.8 Hz, 1H), 9.02 (d, *J* = 9.0 Hz, 1H), 8.97 (d, *J* = 8.2 Hz, 1H), 8.89 (d, *J* = 8.4 Hz, 1H), 7.90 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.75 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.74 – 7.69 (m, 2H), 7.69 – 7.64 (m, 2H), 7.59 – 7.50 (m, 4H), 7.50 – 7.41 (m, 10H), 7.41 – 7.35 (m, 3H), 7.27 – 7.23 (m, 1H), 7.20 – 7.16 (m, 2H), 4.93 (dd, *J* = 10.6, 3.4 Hz, 1H), 4.87 (dd, *J* = 11.0, 3.2 Hz, 1H), 4.77 (dd, *J* = 8.8, 6.0 Hz, 1H), 4.65 (dd, *J* = 8.4, 6.3 Hz, 1H), 4.62 – 4.55 (m, 2H), 4.54 – 4.45 (m, 1H), 4.04 (s, 1H), 3.83 (s, 3H), 3.78 (s, 6H), 3.65 – 3.59 (m, 3H), 3.59 – 3.50 (m, 6H), 3.44 – 3.32 (m, 5H), 3.30 – 3.22 (m, 4H), 3.22 – 3.15 (m, 3H), 3.09 – 2.95 (m, 3H), 2.86 – 2.76 (m, 3H), 2.65 – 2.54 (m, 3H), 2.52 – 2.44 (m, 2H), 2.43 – 2.34 (m, 2H), 2.33 – 2.23 (m, 4H), 2.09 (s, 6H), 1.96 (s, 3H), 1.75 (s, 2H), 1.12 (d, *J* = 6.9 Hz, 3H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H), 1.00 – 0.97 (m, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$, mixture of 1:1:1:1 ratio of diastereomers and atropisomers) δ 173.9, 173.4, 172.7, 172.5, 172.4, 171.1, 170.4, 170.1, 169.3, 143.7, 143.5, 139.0, 138.7, 137.6, 137.5, 136.3, 136.1,

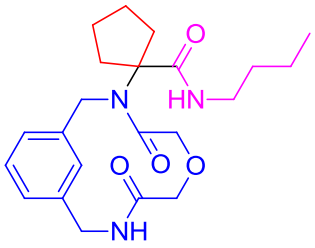
132.0, 131.6, 131.2, 131.2, 130.8, 129.8, 128.9, 128.8, 128.8, 128.4, 128.2, 127.9, 127.9, 127.9, 127.8, 127.7, 127.1, 127.1, 127.0, 72.6, 71.8, 71.6, 71.1, 70.7, 70.5, 69.9, 69.8, 68.3, 65.7, 65.4, 63.6, 63.5, 58.6, 58.4, 57.9, 57.5, 57.2, 52.1, 52.1, 52.0, 44.4, 44.0, 39.6, 38.9, 38.8, 31.3, 31.1, 31.1, 30.8, 30.4, 29.9, 29.8, 26.9, 19.4, 19.3, 19.0, 18.8, 18.2, 17.8, 15.3, 15.2, 15.0; HRMS (ESI) m/z calculated for $C_{31}H_{42}N_3O_7S$ $[M+H]^+$: 600.2738; found $[M+H]^+$: 600.2738.

N-cyclohexyl-2-(5,14-dioxo-7,8,9,10,11,12,13,14-octahydrodibenzo[c,e][1,8]diazacyclotetradecin-6(5H)-yl)butanamide 6u: Prepared

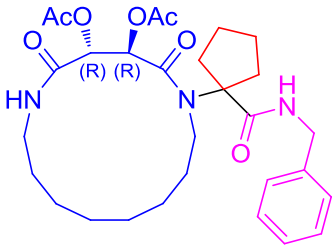


according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (41%, 0.200g); ^{1}H NMR (500 MHz, $CDCl_3$, 1:1 ratio of atropisomers) δ 8.01 – 7.95 (m, 1H), 7.87 – 7.83 (m, 1H), 7.58 – 7.54 (m, 1H), 7.52 – 7.48 (m, 4H), 7.48 – 7.46 (m, 1H), 7.45 (d, *J* = 2.2 Hz, 1H), 7.44 – 7.40 (m, 4H), 7.40 – 7.36 (m, 2H), 7.36 – 7.31 (m, 1H), 6.68 (d, *J* = 8.4 Hz, 1H), 6.09 (d, *J* = 6.3 Hz, 1H), 5.81 (t, *J* = 5.6 Hz, 1H), 5.58 (t, *J* = 5.7 Hz, 1H), 4.71 – 4.63 (m, 1H), 4.52 – 4.43 (m, 1H), 3.72 – 3.62 (m, 1H), 3.55 – 3.43 (m, 1H), 3.42 – 3.32 (m, 1H), 3.28 – 3.01 (m, 5H), 2.94 – 2.83 (m, 1H), 2.08 (s, 1H), 2.04 – 1.95 (m, 1H), 1.85 – 1.75 (m, 3H), 1.74 – 1.62 (m, 6H), 1.62 – 1.52 (m, 5H), 1.52 – 1.45 (m, 1H), 1.43 – 1.03 (m, 20H), 0.89 (t, *J* = 7.4 Hz, 3H), 0.71 – 0.62 (m, 1H), 0.53 – 0.48 (m, 1H), 0.43 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$ 1:1 ratio of atropisomers) δ 172.1, 171.9, 170.3, 169.9, 167.6, 167.3, 137.5, 137.2, 137.0, 136.9, 136.4, 135.3, 134.2, 133.5, 133.1, 132.4, 132.0, 131.4, 130.1, 129.8, 129.5, 128.9, 128.9, 128.7, 128.7, 128.4, 128.2, 128.1, 127.8, 59.7, 59.1, 47.9, 47.7, 46.1, 45.1, 39.1, 38.8, 32.8, 32.8, 32.5, 32.5, 27.2, 27.2, 26.8, 26.6, 25.5, 25.4, 24.9, 24.7, 24.6, 24.0, 23.5, 23.2, 22.7, 21.0, 20.4, 10.8, 9.8; HRMS (ESI) m/z calculated for $C_{30}H_{40}N_3O_3$ $[M+H]^+$: 490.3064; found $[M+H]^+$: 490.3065.

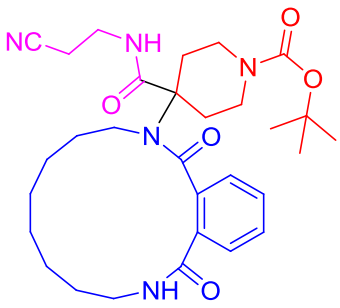
N-butyl-1-{4,8-dioxo-6-oxa-3,9-diazabicyclo[9.3.1]pentadeca-1(14),11(15),12-trien-3-yl}cyclopentane-1-carboxamide 6v: Prepared according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi solid (50%, 0.200g). ^{1}H NMR (500 MHz, CDCl3) δ 7.49 (s, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.11 (t, *J* = 6.7 Hz, 1H), 6.96 (d, *J* = 7.5 Hz, 1H), 6.84 – 6.77 (m, 1H), 4.41 (s, 3H), 3.99 (s, 1H), 3.93 (s, 2H), 3.43 (s, 2H), 3.25 – 3.18 (m, 2H), 2.69 – 2.59 (m, 1H), 1.80 (s, 2H), 1.60 (s, 3H), 1.51 – 1.40 (m, 2H), 1.37 – 1.25 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 172.9, 171.3, 140.9, 136.7, 129.1, 126.7, 123.4, 74.2, 71.6,



69.5, 49.3, 43.9, 39.5, 36.0, 31.5, 22.8, 20.1, 13.8. HRMS (ESI) m/z calculated for $C_{22}H_{32}N_3O_4$ $[M+H]^+$: 402.2387; found $[M+H]^+$: 402.2386.

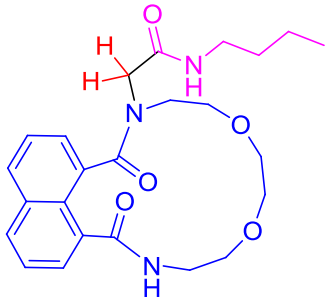
(3R,4R)-1-(1-(benzylcarbamoyl)cyclopentyl)-2,5-dioxo-1,6-diazacyclotetradecane-3,4-diyl diacetate 6w: 

Prepared according to procedure **B** and purified by column chromatography using DCM/MeOH (85:15) isolated as colorless oil (26%, 0.141g); ^{1}H NMR (500 MHz, Chloroform-d) δ 7.31 – 7.27 (m, 8H), 7.24 – 7.18 (m, 2H), 6.91 – 6.83 (m, 2H), 6.46 – 6.36 (m, 2H), 5.70 (dd, *J* = 7.8, 3.4 Hz, 2H), 5.62 (t, *J* = 3.8 Hz, 2H), 4.45 – 4.36 (m, 2H), 4.36 – 4.29 (m, 2H), 3.49 – 3.30 (m, 5H), 3.25 – 3.16 (m, 3H), 2.60 (s, 2H), 2.45 – 2.34 (m, 2H), 2.17 (s, 6H), 1.99 (s, 6H), 1.95 – 1.87 (m, 4H), 1.85 – 1.78 (m, 10H), 1.71 – 1.65 (m, 4H), 1.56 – 1.50 (m, 4H), 1.40 – 1.32 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.4, 174.4, 170.7, 170.6, 169.4, 169.4, 167.5, 167.4, 166.2, 165.9, 139.2, 139.2, 128.3, 127.6, 127.5, 126.8, 126.7, 73.6, 70.9, 70.7, 70.7, 70.5, 46.0, 45.8, 43.6, 39.4, 39.2, 36.2, 36.2, 36.1, 30.6, 29.8, 29.4, 29.1, 29.0, 28.3, 28.0, 26.8, 26.2, 26.1, 26.0, 24.6, 24.6, 24.5, 24.5, 20.7, 20.4, 20.3; HRMS (ESI) m/z calculated for $C_{29}H_{42}N_3O_7$ $[M+H]^+$: 544.3017; found $[M+H]^+$: 544.3016.

tert-butyl 4-((2-cyanoethyl)carbamoyl)-4-(1,12-dioxo-3,4,5,6,7,8,9,10,11,12-decahydrobenzo[c][1,6]diazacyclotetradecin-2(1H)-yl)piperidine-1-carboxylate 6x: 

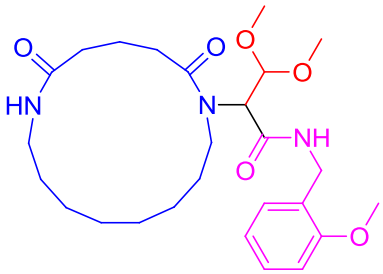
Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi-solid (32%, 0.177g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.67 (d, *J* = 8.1, 1.1 Hz, 1H), 7.56 (t, *J* = 7.5, 1.1 Hz, 1H), 7.45 (t, *J* = 7.7, 1.3 Hz, 1H), 7.19 (d, *J* = 7.6, 1.3 Hz, 1H), 6.49 (s, 1H), 4.20 – 4.07 (m, 1H), 3.95 – 3.85 (m, 1H), 3.69 – 3.50 (m, 3H), 3.42 (s, 1H), 3.17 – 3.08 (m, 1H), 3.08 – 3.01 (m, 1H), 2.93 (t, *J* = 13.6 Hz, 1H), 2.84 – 2.75 (m, 1H), 2.72 – 2.64 (m, 1H), 2.64 – 2.55 (m, 1H), 2.25 – 2.17 (m, 1H), 2.10 – 1.99 (m, 2H), 1.83 – 1.74 (m, 1H), 1.72 – 1.65 (m, 1H), 1.65 – 1.56 (m, 1H), 1.48 (s, 9H), 1.44 – 1.08 (m, 9H), 0.90 – 0.77 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.2, 172.7, 165.7, 154.9, 138.7, 132.3, 129.0, 127.9, 126.0, 118.6, 79.7, 63.6, 60.4, 48.0, 41.8, 40.5, 39.4, 36.5, 36.3, 32.9, 31.3, 28.7, 28.5, 26.5, 26.0, 23.2, 21.8, 20.4, 17.1; HRMS (ESI) m/z calculated for $C_{30}H_{43}N_5NaO_5$ $[M+H]^+$: 576.3156; found $[M+H]^+$: 576.3155.

N-butyl-2-(1,12-dioxo-3,4,6,7,9,10,11,12-octahydronaphtho[1,8-ij][1,4,7,13]dioxadiazacyclopentadecin-2(1H)-yl)acetamide 6y:



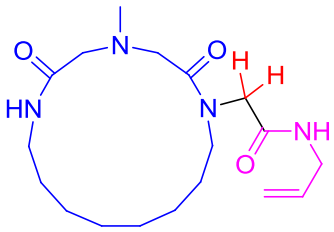
Prepared according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as colorless oil (22%, 0.097g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (d, *J* = 7.2 Hz, 2H), 8.23 (d, *J* = 8.2 Hz, 2H), 7.77 (t, *J* = 7.4 Hz, 2H), 4.45 (t, *J* = 6.1 Hz, 2H), 3.82 (t, *J* = 6.1 Hz, 2H), 3.75 – 3.66 (m, 5H), 3.63 – 3.54 (m, 3H), 3.48 (s, 2H), 3.28 – 3.19 (m, 2H), 1.55 – 1.39 (m, 2H), 1.30 (s, 2H), 0.88 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.4, 164.2, 134.1, 131.6, 131.3, 128.2, 127.0, 122.5, 70.1, 69.4, 68.8, 68.0, 53.0, 52.6, 49.0, 39.1, 31.6, 20.0, 13.8; HRMS (ESI) m/z calculated for $C_{24}H_{32}N_3O_5$ $[M+H]^+$: 442.2342; found $[M+H]^+$:442.2336.

2-(2,6-dioxo-1,7-diazacyclopentadecan-1-yl)-3,3-dimethoxy-N-(2-methoxybenzyl)propanamide 6z:



Prepared according to procedure A and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (46%, 0.226g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.23 (m, 2H), 6.95 – 6.88 (m, 2H), 5.45 (s, 1H), 5.07 (d, *J* = 7.9 Hz, 1H), 4.63 (s, 1H), 4.49 (dd, *J* = 14.6, 6.5 Hz, 1H), 4.38 (dd, *J* = 14.6, 5.4 Hz, 1H), 3.90 (s, 3H), 3.46 (s, 3H), 3.42 – 3.34 (m, 1H), 3.32 (s, 3H), 3.31 – 3.26 (m, 1H), 3.26 – 3.20 (m, 2H), 2.44 – 2.35 (m, 1H), 2.34 – 2.27 (m, 1H), 2.24 – 2.14 (m, 2H), 2.10 – 1.98 (m, 2H), 1.61 – 1.50 (m, 2H), 1.50 – 1.41 (m, 1H), 1.41 – 1.18 (m, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.3, 172.6, 168.3, 157.6, 129.5, 128.6, 126.4, 120.5, 110.2, 101.5, 55.6, 55.3, 53.2, 47.7, 39.3, 38.1, 35.3, 31.6, 28.4, 27.3, 26.0, 24.6, 23.9, 20.4; HRMS (ESI) m/z calculated for $C_{26}H_{41}N_3NaO_6$ $[M+Na]^+$: 514.2888; found $[M+Na]^+$: 514.2884.

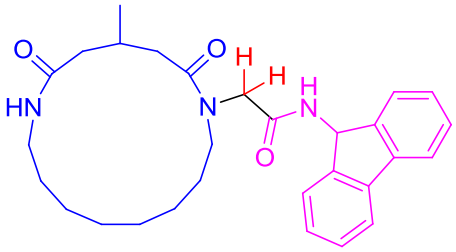
N-allyl-2-(4-methyl-2,6-dioxo-1,4,7-triazacyclopentadecan-1-yl)acetamide 6aa:



Prepared according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (41%, 0.144g); ^{1}H NMR (500 MHz, $CDCl_3$, rotamers were obsorved) δ 7.76 (s, 1H), 6.91 (t, *J* = 6.1 Hz, 1H), 6.70 (t, *J* = 5.6 Hz, 1H), 6.60 – 6.45 (m, 1H), 5.90 – 5.73 (m, 2H), 5.23 – 5.07 (m, 4H), 3.98 (s, 2H), 3.97 (s, 2H), 3.95 – 3.89 (m, 2H), 3.88 – 3.81 (m, 2H), 3.61 – 3.51 (m, 2H), 3.41 (s, 2H), 3.40 – 3.33 (m, 4H), 3.33 – 3.28 (m, 2H), 3.25 (s, 2H), 3.21 (s, 4H), 2.52 (s, 3H), 2.44 (s, 3H), 1.67 – 1.53 (m, 6H), 1.53 – 1.45 (m, 2H), 1.45 – 1.29 (m, 13H), 1.24 – 1.14 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$, rotamers were obsorved) δ 171.1, 170.8, 170.4, 169.8, 169.0,

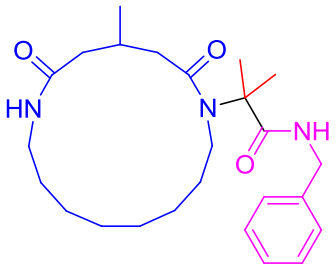
167.7, 133.8, 133.6, 117.0, 116.3, 61.2, 60.8, 59.3, 58.1, 51.0, 50.0, 49.7, 45.5, 44.7, 44.2, 42.0, 41.8, 38.6, 37.2, 27.7, 27.3, 26.7, 26.2, 25.9, 25.9, 25.3, 24.8, 23.9, 23.3, 23.1, 23.0; HRMS (ESI) m/z calculated for $C_{18}H_{33}N_4O_3$ $[M+H]^+$: 353.2547; found $[M+H]^+$: 353.2545.

N-(9H-fluoren-9-yl)-2-(4-methyl-2,6-dioxo-1,7-diazacyclopentadecan-1-yl)acetamide



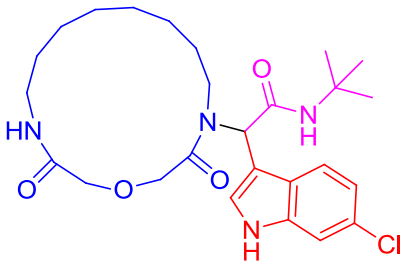
6ab: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (27%, 0.128g); ^{1}H NMR (500 MHz, $CDCl_3$+Methanol-d_4) δ 7.70 – 7.54 (m, 2H), 7.53 – 7.40 (m, 2H), 7.39 – 7.26 (m, 3H), 7.26 – 7.16 (m, 2H), 6.19 – 6.03 (m, 1H), 5.34 – 5.17 (m, 1H), 3.99 (h, *J* = 6.5, 5.1 Hz, 2H), 3.42 – 3.23 (m, 3H), 3.23 – 3.09 (m, 2H), 2.93 – 2.74 (m, 1H), 2.42 – 2.26 (m, 2H), 2.26 – 2.05 (m, 3H), 1.97 – 1.75 (m, 1H), 1.42 – 1.36 (m, 2H), 1.32 – 1.12 (m, 8H), 1.00 – 0.88 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$+Methanol-d_4) δ 172.9, 172.4, 170.6, 144.0, 140.6, 128.4, 127.5, 124.9, 119.7, 119.7, 54.6, 50.8, 49.9, 42.5, 41.2, 38.5, 37.9, 37.5, 28.7, 27.5, 26.1, 26.1, 25.1, 23.7, 23.7, 20.5; HRMS (ESI) m/z calculated for $C_{29}H_{38}N_3O_3$ $[M+H]^+$: 476.2908; found $[M+H]^+$: 476.2907.

N-benzyl-2-methyl-2-(4-methyl-2,6-dioxo-1,7-diazacyclopentadecan-1-yl)propanamide



6ac: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (32%, 0.137g); ^{1}H NMR (500 MHz, Chloroform-d) δ 7.29 (s, 1H), 7.27 – 7.22 (m, 4H), 7.20 – 7.15 (m, 1H), 4.46 – 4.15 (m, 2H), 3.63 – 3.53 (m, 1H), 3.31 – 3.27 (m, 1H), 3.24 – 3.16 (m, 1H), 3.16 – 3.07 (m, 1H), 2.91 (dt, *J* = 13.8, 4.5 Hz, 1H), 2.44 – 2.32 (m, 1H), 2.32 – 2.18 (m, 2H), 2.09 (dd, *J* = 15.8, 7.9 Hz, 1H), 1.88 (dd, *J* = 13.4, 7.9 Hz, 1H), 1.80 – 1.64 (m, 1H), 1.52 – 1.46 (m, 3H), 1.45 (s, 3H), 1.42 (s, 3H), 1.41 – 1.38 (m, 2H), 1.33 – 1.17 (m, 5H), 1.01 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, Chloroform-d) δ 176.0, 172.4, 172.2, 139.0, 128.3, 127.6, 126.9, 62.0, 44.4, 43.4, 42.5, 39.5, 37.2, 29.4, 28.8, 27.4, 26.1, 25.0, 25.0, 24.0, 23.6, 23.2, 20.6; HRMS (ESI) m/z calculated for $C_{25}H_{40}N_3O_3$ $[M+H]^+$: 452.2884; found $[M+H]^+$: 452.2882.

N-(tert-butyl)-2-(6-chloro-1H-indol-3-yl)-2-(3,14-dioxo-1-oxa-4,13-diazacyclopentadecan-4-yl)acetamide



6ad: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (15%, 0.076g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1H),

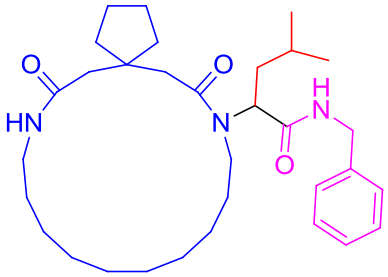
7.70 (d, *J* = 2.5 Hz, 1H), 7.46 – 7.38 (m, 2H), 7.14 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.51 – 6.39 (m, 1H), 6.21 (s, 1H), 6.06 (s, 1H), 4.44 – 4.27 (m, 2H), 4.17 (s, 2H), 3.44 – 3.32 (m, 2H), 3.26 – 3.15 (m, 1H), 3.13 – 3.01 (m, 1H), 1.69 – 1.56 (m, 7H), 1.36 (s, 11H), 1.26 – 1.21 (m, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.9, 168.8, 168.6, 136.1, 128.8, 126.6, 125.5, 121.2, 119.4, 111.4, 109.5, 70.9, 70.1, 54.4, 51.5, 45.6, 37.8, 28.6, 28.2, 27.2, 25.5, 25.2, 23.7, 23.1; HRMS (ESI) m/z calculated for $C_{26}H_{38}ClN_4O_4$ $[M+H]^+$: 504.2576; found $[M+H]^+$: 505.2576.

(Z)-N-(2-(1H-indol-3-yl)ethyl)-1-(2,5-dioxo-1,6-diazacyclohexadec-3-en-1-yl)cyclopentanecarboxamide 6ae: Prepared according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (47%, 0.237g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.54 – 8.50 (m, 1H), 8.24 – 8.20 (m, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.14 – 6.98 (m, 2H), 6.93 (dd, *J* = 11.0, 3.9 Hz, 1H), 6.54 (d, *J* = 11.8 Hz, 1H), 6.00 (d, *J* = 11.9 Hz, 1H), 3.74 – 3.67 (m, 1H), 3.48 – 3.43 (m, 2H), 3.18 – 3.15 (m, 2H), 2.98 – 2.83 (m, 3H), 2.75 – 2.66 (m, 1H), 2.04 – 1.94 (m, 1H), 1.94 – 1.84 (m, 1H), 1.82 – 1.72 (m, 2H), 1.67 – 1.51 (m, 5H), 1.37 – 1.30 (m, 2H), 1.29 – 1.21 (m, 6H), 1.18 – 1.10 (m, 3H), 1.08 – 1.03 (m, 1H), 0.98 – 0.89 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 179.7, 173.3, 169.2, 142.2, 141.4, 132.5, 128.5, 127.5, 126.0, 123.3, 123.2, 117.4, 116.6, 84.3, 76.8, 51.7, 45.5, 43.1, 42.3, 40.8, 33.8, 31.5, 31.4, 30.9, 30.8, 30.5, 29.8, 29.7, 29.4, 28.4. HRMS (ESI) m/z calculated for $C_{30}H_{43}N_4O_3$ $[M+H]^+$: 507.3329; found $[M+H]^+$: 507.3330.

2-(5,16-dioxo-7,8,10,11,13,14,15,16-octahydrodibenzo[i,k][1,4,7,14]dioxadiazacyclohexadecin-6(5H)-yl)-N-phenethylpentanamide 6af: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (37%, 0.206g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.06 (d, *J* = 8.7 Hz, 1H), 8.77 (t, *J* = 5.6 Hz, 1H), 7.63 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.46 – 7.40 (m, 3H), 7.38 – 7.32 (m, 4H), 7.31 – 7.25 (m, 4H), 7.23 – 7.19 (m, 1H), 7.02 (dd, *J* = 7.6, 1.2 Hz, 1H), 4.61 (dd, *J* = 11.7, 3.2 Hz, 1H), 4.47 – 4.35 (m, 1H), 3.84 – 3.73 (m, 1H), 3.71 – 3.58 (m, 2H), 3.53 – 3.46 (m, 1H), 3.44 – 3.38 (m, 3H), 3.20 – 3.15 (m, 1H), 3.11 – 3.03 (m, 1H), 2.99 – 2.92 (m, 5H), 2.31 – 2.21 (m, 1H), 2.21 – 2.14 (m, 1H), 1.62 – 1.49 (m, 1H), 1.30 – 1.13 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 170.5, 169.3, 143.6, 139.3, 138.9, 137.5, 131.9, 131.3, 131.1, 128.6, 128.6, 128.5, 127.9, 127.7, 127.3,

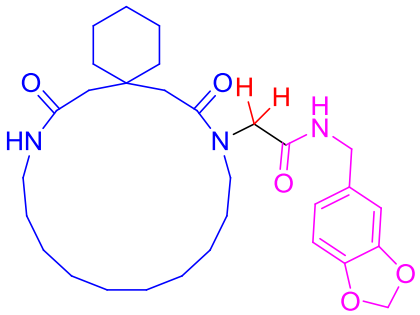
126.8, 126.5, 72.4, 70.7, 69.8, 65.8, 65.2, 43.9, 40.6, 38.7, 35.3, 32.2, 20.3, 13.9; HRMS (ESI) m/z calculated for $C_{33}H_{39}N_3NaO_5$ $[M+Na]^+$: 580.2782; found $[M+H]^+$: 580.5779.

N-benzyl-2-(7,20-dioxo-8,19-diazaspiro[4.16]henicosan-8-yl)-4-methylpentanamide 6ag:

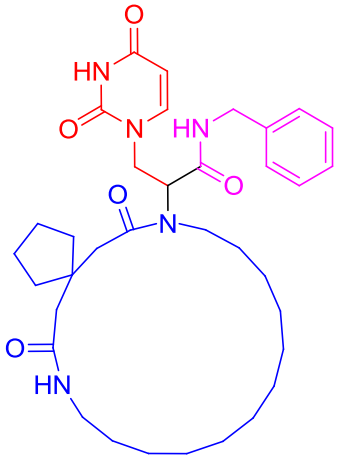


Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (47%, 0.246g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (t, *J* = 5.9 Hz, 1H), 7.30 (d, *J* = 7.3 Hz, 2H), 7.21 (t, *J* = 7.5 Hz, 2H), 7.16 – 7.12 (m, 1H), 5.95 – 5.81 (m, 1H), 4.41 (dd, *J* = 14.5, 6.3 Hz, 1H), 4.23 (dd, *J* = 14.5, 5.7 Hz, 1H), 3.35 – 3.15 (m, 2H), 2.98 – 2.76 (m, 2H), 2.59 – 2.51 (m, 1H), 2.48 (d, *J* = 16.1 Hz, 1H), 2.23 (d, *J* = 16.1 Hz, 1H), 2.08 – 2.01 (m, 1H), 1.84 – 1.75 (m, 1H), 1.67 – 1.56 (m, 8H), 1.50 – 1.41 (m, 3H), 1.40 – 1.34 (m, 2H), 1.33 – 1.28 (m, 2H), 1.28 – 1.12 (m, 12H), 0.90 (d, *J* = 3.0 Hz, 3H), 0.88 (d, *J* = 2.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6, 172.3, 172.0, 139.5, 128.1, 126.6, 55.7, 46.2, 44.0, 43.5, 41.3, 39.6, 39.5, 38.9, 36.7, 28.8, 27.0, 26.8, 26.7, 25.9, 25.3, 24.9, 24.7, 24.4, 24.1, 23.8, 23.4, 21.8. HRMS (ESI) m/z calculated for $C_{32}H_{52}N_3O_3$ $[M+H]^+$: 526.4003; found $[M+H]^+$: 526.4002.

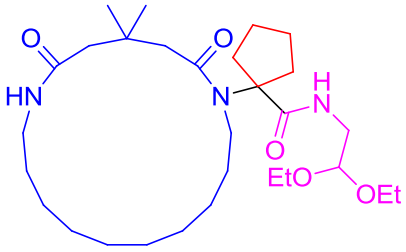
N-(benzo[d][1,3]dioxol-5-ylmethyl)-2-(8,21-dioxo-9,20-diazaspiro[5.16]docosan-9-yl)acetamide 6ah:



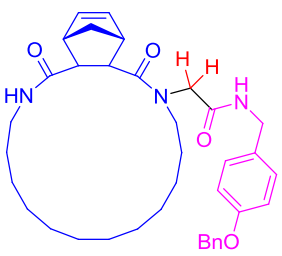
Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (47%, 0.247g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (t, *J* = 5.6 Hz, 1H), 6.84 (d, *J* = 1.3 Hz, 1H), 6.81 – 6.72 (m, 1H), 6.66 (d, *J* = 7.9 Hz, 1H), 5.86 (s, 2H), 5.81 (t, *J* = 6.1 Hz, 1H), 4.26 (d, *J* = 5.9 Hz, 2H), 4.07 – 3.99 (m, 2H), 3.25 – 3.12 (m, 2H), 3.14 – 2.99 (m, 2H), 2.40 (s, 2H), 2.27 (s, 2H), 1.79 (s, 1H), 1.64 – 1.56 (m, 2H), 1.53 – 1.35 (m, 12H), 1.34 – 1.16 (m, 12H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 171.9, 169.5, 147.4, 146.4, 133.3, 121.4, 109.0, 107.9, 100.8, 51.8, 51.6, 43.2, 43.0, 39.5, 39.0, 37.6, 35.7, 28.4, 27.0, 26.9, 26.6, 26.1, 24.7, 24.6, 24.3, 23.8, 21.7. HRMS (ESI) m/z calculated for $C_{30}H_{46}N_3O_5$ $[M+H]^+$: 528.3432; found $[M+H]^+$: 528.34314.

N-benzyl-3-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(7,22-dioxo-8,21-diazaspiro[4.18]tricosan-8-yl)propanamide 6ai: 

Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (29%, 0.180g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.38 (t, *J* = 6.0 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.30 – 7.21 (m, 5H), 7.20 – 7.15 (m, 1H), 5.64 (dd, *J* = 8.0, 2.1 Hz, 1H), 5.57 (dd, *J* = 7.9, 4.4 Hz, 1H), 4.61 (dd, *J* = 13.9, 7.1 Hz, 1H), 4.41 (dd, *J* = 14.7, 6.4 Hz, 1H), 4.28 (dd, *J* = 14.8, 5.6 Hz, 1H), 4.19 – 4.13 (m, 1H), 4.01 – 3.92 (m, 1H), 3.45 – 3.33 (m, 2H), 3.32 – 3.22 (m, 1H), 2.75 – 2.64 (m, 2H), 2.42 (d, *J* = 12.9 Hz, 1H), 2.13 – 2.06 (m, 2H), 2.02 – 1.88 (m, 1H), 1.85 – 1.75 (m, 1H), 1.67 – 1.49 (m, 8H), 1.46 – 1.40 (m, 2H), 1.35 – 1.26 (m, 14H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 172.2, 170.2, 164.3, 151.3, 147.2, 139.0, 128.1, 127.9, 126.7, 101.0, 61.6, 51.2, 50.7, 43.5, 43.3, 43.0, 41.0, 39.5, 39.4, 39.0, 28.3, 27.5, 27.4, 27.1, 26.9, 26.8, 26.6, 26.0, 25.6, 24.8, 24.2, 23.5; HRMS (ESI) m/z calculated for $C_{35}H_{52}N_5O_5$ $[M+H]^+$: 622.3963; found $[M+H]^+$: 622.3963.

N-(2,2-diethoxyethyl)-1-(4,4-dimethyl-2,6-dioxo-1,7-diazacycloheptadecan-1-yl)cyclopentanecarboxamide 6aj: 

Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as white solid (39%, 0.203g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 5.96 (s, 1H), 4.62 (t, *J* = 5.6 Hz, 1H), 3.77 – 3.63 (m, 2H), 3.61 – 3.49 (m, 2H), 3.28 – 3.16 (m, 6H), 2.49 (s, 2H), 2.32 (s, 4H), 1.88 – 1.73 (m, 6H), 1.70 – 1.59 (m, 2H), 1.52 – 1.46 (m, 2H), 1.44 – 1.38 (m, 4H), 1.37 – 1.29 (m, 8H), 1.21 (t, *J* = 7.1 Hz, 6H), 1.15 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.8, 172.6, 172.1, 100.5, 72.5, 61.7, 46.2, 45.7, 42.9, 41.8, 39.0, 36.8, 33.6, 30.0, 29.0, 28.8, 27.1, 26.8, 25.7, 25.4, 25.0, 24.8, 24.4, 15.4. HRMS (ESI) m/z calculated for $C_{29}H_{54}N_3O_5$ $[M+H]^+$: 546.38909; found $[M+H]^+$: 546.38928.

N-(4-(benzyloxy)benzyl)-2-((17S,20R)-1,16-dioxo-3,4,5,6,7,8,9,10,11,12,13,14,15,16,16a,17,20,20a-octadecahydro-17,20-methanobenzo[c][1,6]diazacyclooctadecin-2(1H)-yl)acetamide 6ak: 

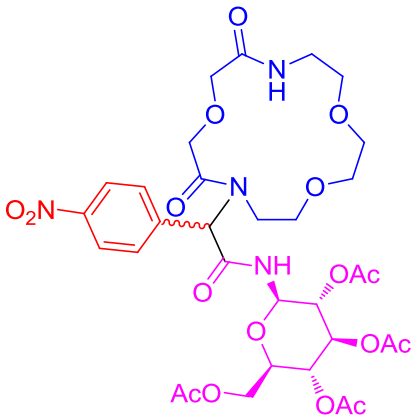
Prepared according to procedure **C** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi-solid (36%, 0.215g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.39 (m, 2H), 7.39 – 7.34 (m, 2H), 7.34 – 7.31 (m, 1H), 7.26 (t, *J* = 5.9 Hz, 1H), 7.17 – 7.11 (m, 2H),

6.93 – 6.88 (m, 2H), 6.08 (dt, *J* = 3.9, 1.8 Hz, 1H), 5.02 (s, 2H), 4.33 (d, *J* = 5.8 Hz, 2H), 3.36 (qt, *J* = 3.4, 2.0 Hz, 1H), 3.34 – 3.27 (m, 1H), 3.23 – 3.18 (m, 1H), 3.16 (s, 2H), 2.57 – 2.43 (m, 1H), 1.72 (dt, *J* = 8.7, 1.8 Hz, 1H), 1.57 – 1.49 (m, 1H), 1.42 (td, *J* = 16.0, 15.1, 8.5 Hz, 3H), 1.31 – 1.12 (m, 12H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.8, 170.2, 158.2, 136.9, 134.4, 130.5, 129.0, 128.6, 128.0, 127.4, 115.1, 70.0, 59.1, 56.4, 52.2, 45.7, 44.9, 42.7, 38.5, 29.5, 29.5, 29.5, 29.5, 29.1, 27.8, 27.4, 27.3, 26.9; HRMS (ESI) m/z calculated for $C_{37}H_{50}N_3O_4$ $[M+H]^+$: 600.3796; found $[M+H]^+$: 600.3782.

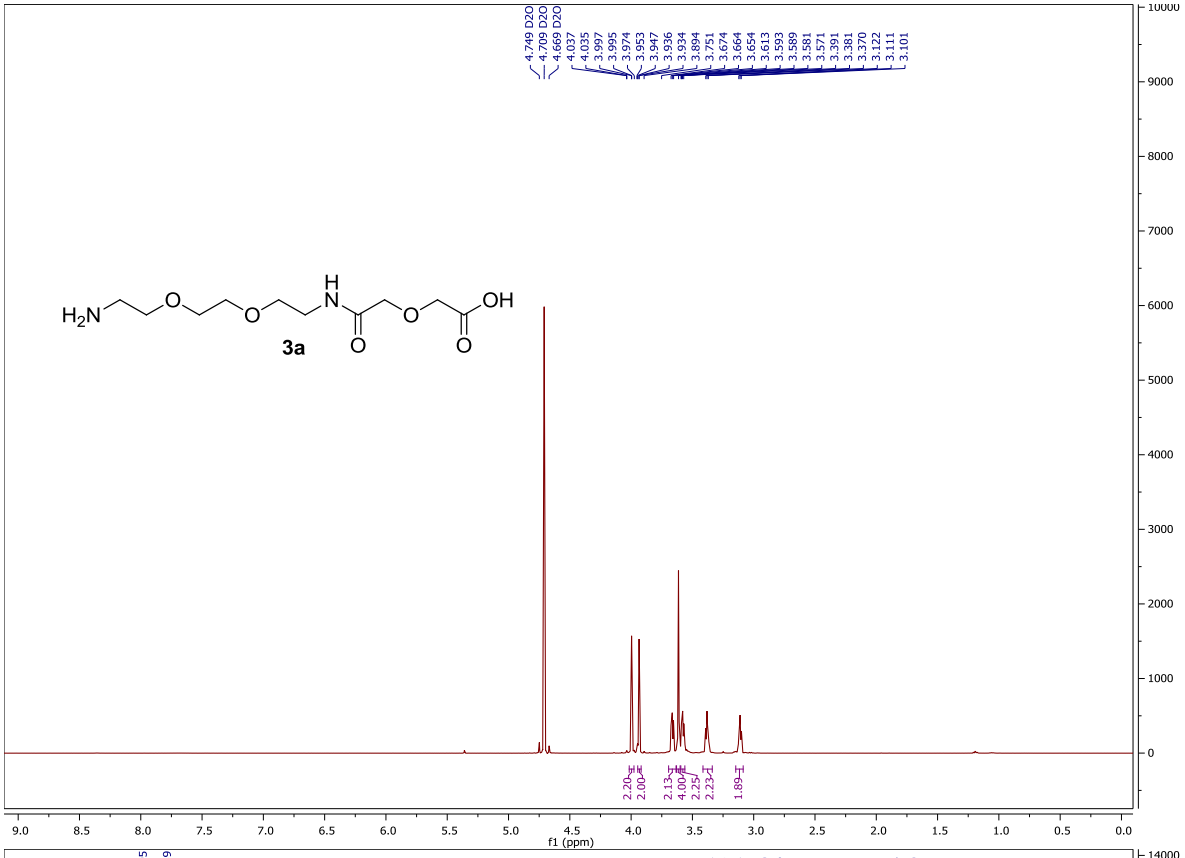
N-benzyl-3-((4R,5S,6S,7R,9R,11E,13E,15R,16R)-6-(((2R,3R,4R,5S,6R)-5-(((2S,5S,6S)-4,5-dihydroxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-16-ethyl-4-hydroxy-15-((((2R,3R,4R,5R,6R)-5-hydroxy-3,4-dimethoxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)-5,9,13-trimethyl-2,10-dioxooxacyclohexadeca-11,13-dien-7-yl)-2-(8,12-dioxo-1,4,10-trioxa-7,13-diazacyclopentadecan-7-yl)propanamide 6al: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as solid (53%, 0.677g); ^{1}H NMR (500 MHz, Chloroform-d, major diastereomer) δ 7.86 – 7.72 (m, 1H), 7.31 – 7.27 (m, 3H), 7.25 – 7.18 (m, 2H), 7.10 (t, *J* = 5.7 Hz, 1H), 6.25 (d, *J* = 15.4 Hz, 1H), 5.90 (d, *J* = 10.5 Hz, 1H), 4.95 – 4.88 (m, 2H), 4.60 (d, *J* = 14.3 Hz, 1H), 4.54 (d, *J* = 7.7 Hz, 1H), 4.49 (dd, *J* = 14.4, 6.5 Hz, 1H), 4.44 (d, *J* = 14.3 Hz, 1H), 4.27 – 4.15 (m, 4H), 4.04 – 3.93 (m, 4H), 3.76 – 3.72 (m, 1H), 3.59 (s, 4H), 3.57 – 3.50 (m, 4H), 3.45 (s, 4H), 3.42 – 3.36 (m, 3H), 3.34 – 3.24 (m, 5H), 3.20 – 3.12 (m, 4H), 3.02 (dd, *J* = 7.9, 2.9 Hz, 1H), 2.96 – 2.88 (m, 3H), 2.79 – 2.71 (m, 1H), 2.50 – 2.45 (m, 2H), 2.44 (s, 6H), 2.43 – 2.37 (m, 2H), 2.35 – 2.21 (m, 3H), 2.06 – 1.93 (m, 2H), 1.88 – 1.80 (m, 2H), 1.71 (dd, *J* = 14.3, 3.9 Hz, 1H), 1.65 – 1.49 (m, 4H), 1.25 (dd, *J* = 11.3, 5.6 Hz, 6H), 1.21 (s, 3H), 1.19 (d, *J* = 6.8 Hz, 3H), 0.99 (d, *J* = 5.5 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.91 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$, major diastereomer) δ 204.0, 173.7, 171.7, 170.4, 148.0, 143.2, 138.2, 134.6, 128.5, 128.3, 127.6, 127.3, 117.9, 102.7, 101.2, 96.5, 81.8, 79.9, 77.3, 75.0, 74.9, 73.0, 72.7, 71.9, 70.8, 70.5, 69.9, 69.7, 69.7, 69.4, 69.1, 68.6, 68.3, 67.5, 66.7,

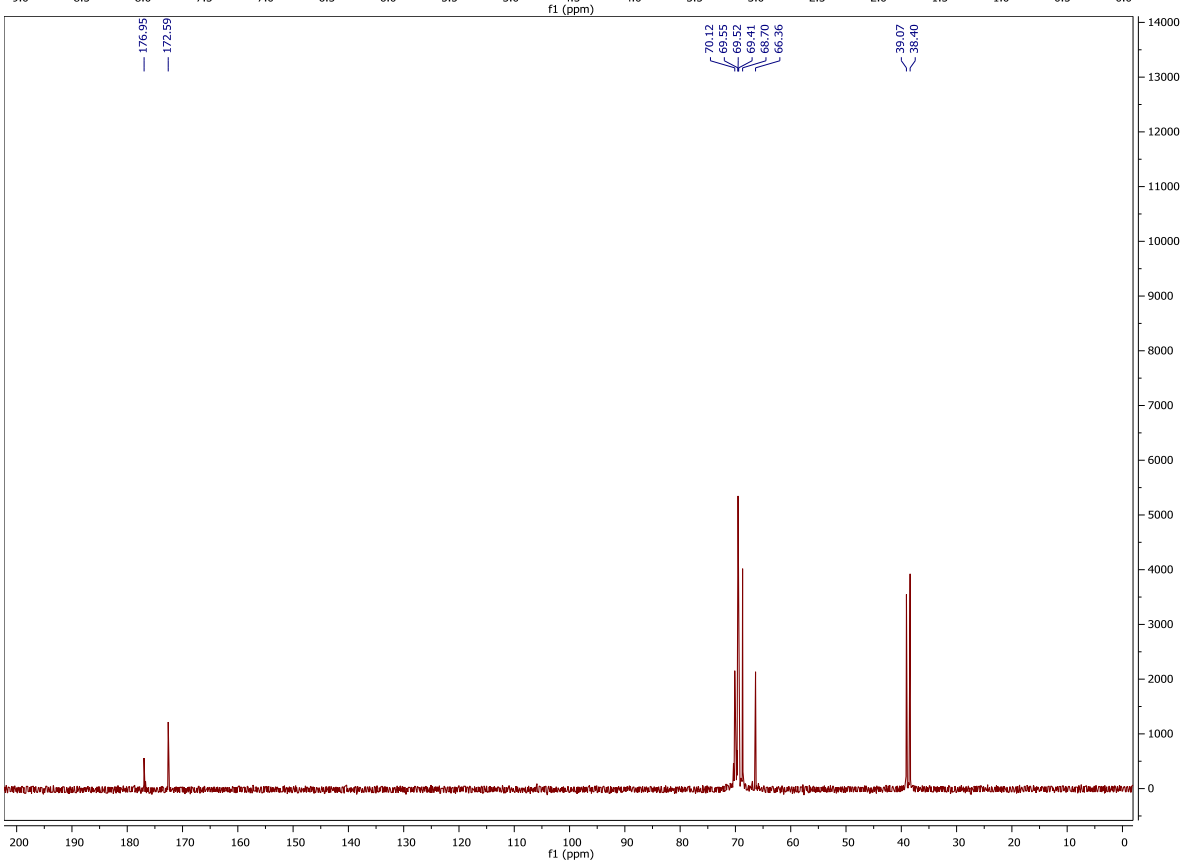
65.9, 61.8, 59.6, 53.5, 45.1, 45.1, 43.9, 41.9, 41.1, 40.8, 40.1, 38.7, 33.1, 32.6, 25.4, 25.1, 18.3, 18.2, 17.8, 17.6, 12.9, 9.6, 9.3; HRMS (ESI) m/z calculated for $C_{64}H_{103}N_4O_{22}$ $[M+H]^+$: 1279.7058; found $[M+H]^+$: 1279.7065.

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(2-(8,12-dioxo-1,4,10-trioxa-7,13-diazacyclopentadecan-7-yl)-2-(4-nitrophenyl)acetamido)tetrahydro-2H-pyran-3,4,5-triyl triacetate 6am: Prepared according to procedure **B** and purified by column chromatography using CH_2Cl_2:MeOH isolated as semi-solid (22%, 0.166g); ^{1}H NMR (500 MHz, $CDCl_3$, **2:1 ratio of diastereomers, major isomer**) δ 8.25 (d, *J* = 8.6 Hz, 2H), 7.95 – 7.89 (m, 1H), 7.82 – 7.75 (m, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 6.21 (s, 1H), 5.54 – 5.45 (m, 2H), 5.29 – 5.22 (m, 2H), 4.91 (d, *J* = 14.4 Hz, 1H), 4.38 (d, *J* = 16.4 Hz, 1H), 4.27 – 4.22 (m, 1H), 4.16 – 4.09 (m, 2H), 3.95 (d, *J* = 16.2 Hz, 1H), 3.90 – 3.84 (m, 4H), 3.75 – 3.69 (m, 2H), 3.63 – 3.60 (m, 2H), 3.54 – 3.46 (m, 2H), 3.35 – 3.29 (m, 2H), 2.74 (d, *J* = 15.4 Hz, 1H), 2.20 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 170.4, 169.7, 169.7, 169.6, 167.7, 147.9, 141.0, 131.2, 124.1, 78.5, 72.7, 70.9, 70.7, 69.8, 69.6, 68.7, 67.5, 67.5, 67.3, 64.8, 61.8, 60.8, 43.8, 38.5, 20.9, 20.7, 20.6, 20.5; ^{1}H NMR (500 MHz, $CDCl_3$, **2:1 ratio of diastereomers, minor isomer**) δ 8.17 (d, *J* = 8.7 Hz, 2H), 7.95 – 7.89 (m, 1H), 7.82 – 7.75 (m, 1H), 7.62 (d, *J* = 8.7 Hz, 2H), 6.21 (s, 1H), 5.45 – 5.35 (m, 2H), 5.22 – 5.13 (m, 2H), 4.90 – 4.79 (m, 1H), 4.35 (d, *J* = 16.4 Hz, 1H), 4.22 – 4.17 (m, 1H), 4.09 – 4.05 (m, 2H), 3.95 (d, *J* = 16.2 Hz, 1H), 3.84 – 3.78 (m, 4H), 3.69 – 3.65 (m, 2H), 3.59 – 3.54 (m, 2H), 3.45 – 3.41 (m, 2H), 3.29 – 3.21 (m, 2H), 2.74 (d, *J* = 15.4 Hz, 1H), 2.23 (s, 3H), 2.13 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.5, 170.4, 169.7, 169.7, 169.4, 168.7, 147.6, 141.2, 130.9, 123.3, 78.2, 72.6, 71.2, 71.0, 70.0, 69.3, 68.9, 67.6, 67.5, 67.4, 67.2, 66.3, 61.8, 61.1, 47.9, 38.8, 21.1, 20.7, 20.6, 20.5; HRMS (ESI) m/z calculated for $C_{32}H_{43}N_4O_{17}$ $[M+H]^+$: 755.2618; found $[M+H]^+$: 755.2617.

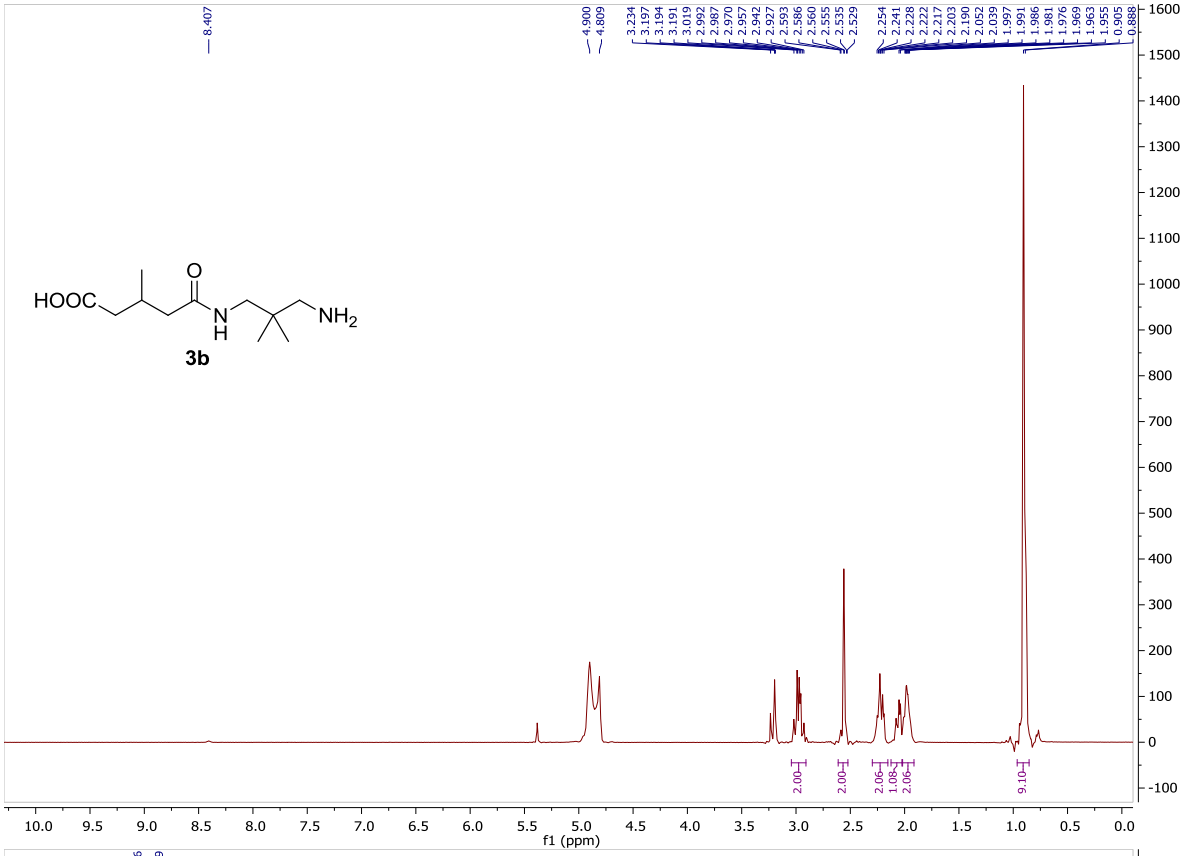


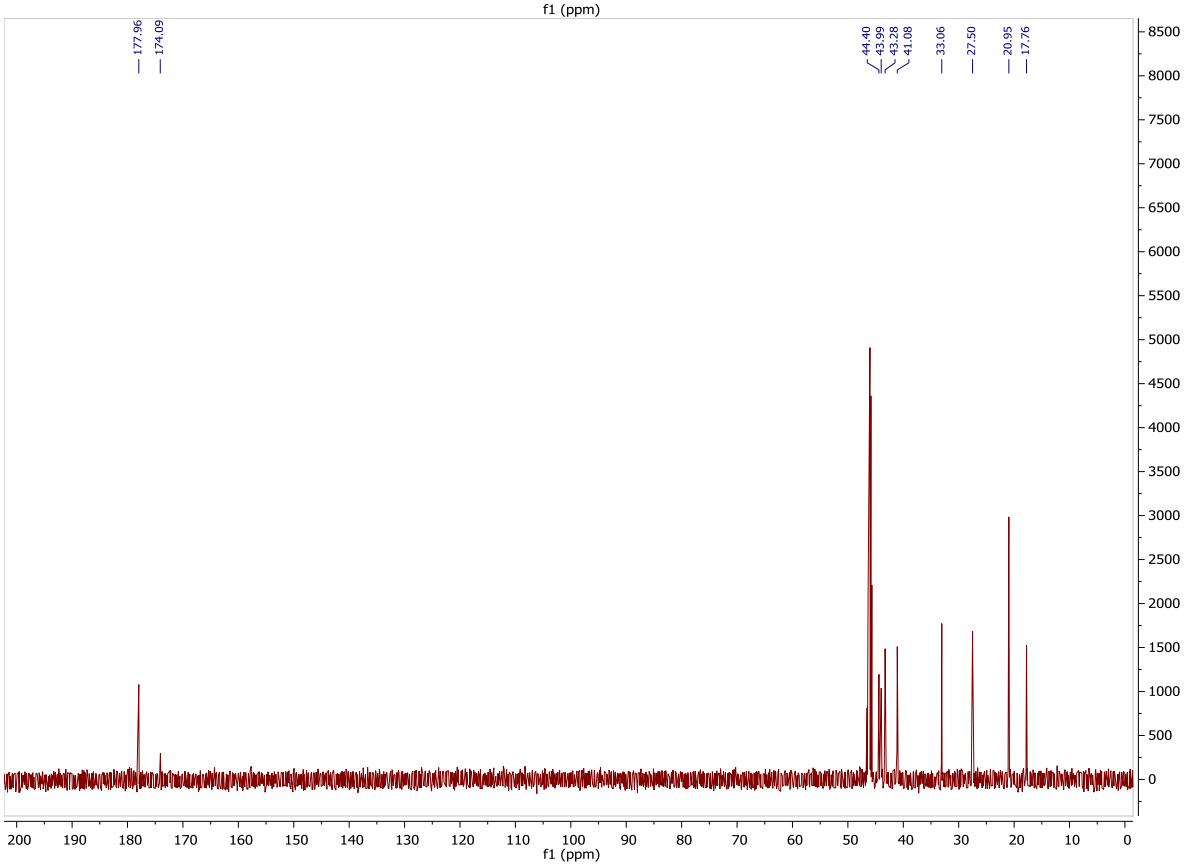
3a: 14-amino-5-oxo-3,9,12-trioxa-6-azatetradecan-1-oic acid.



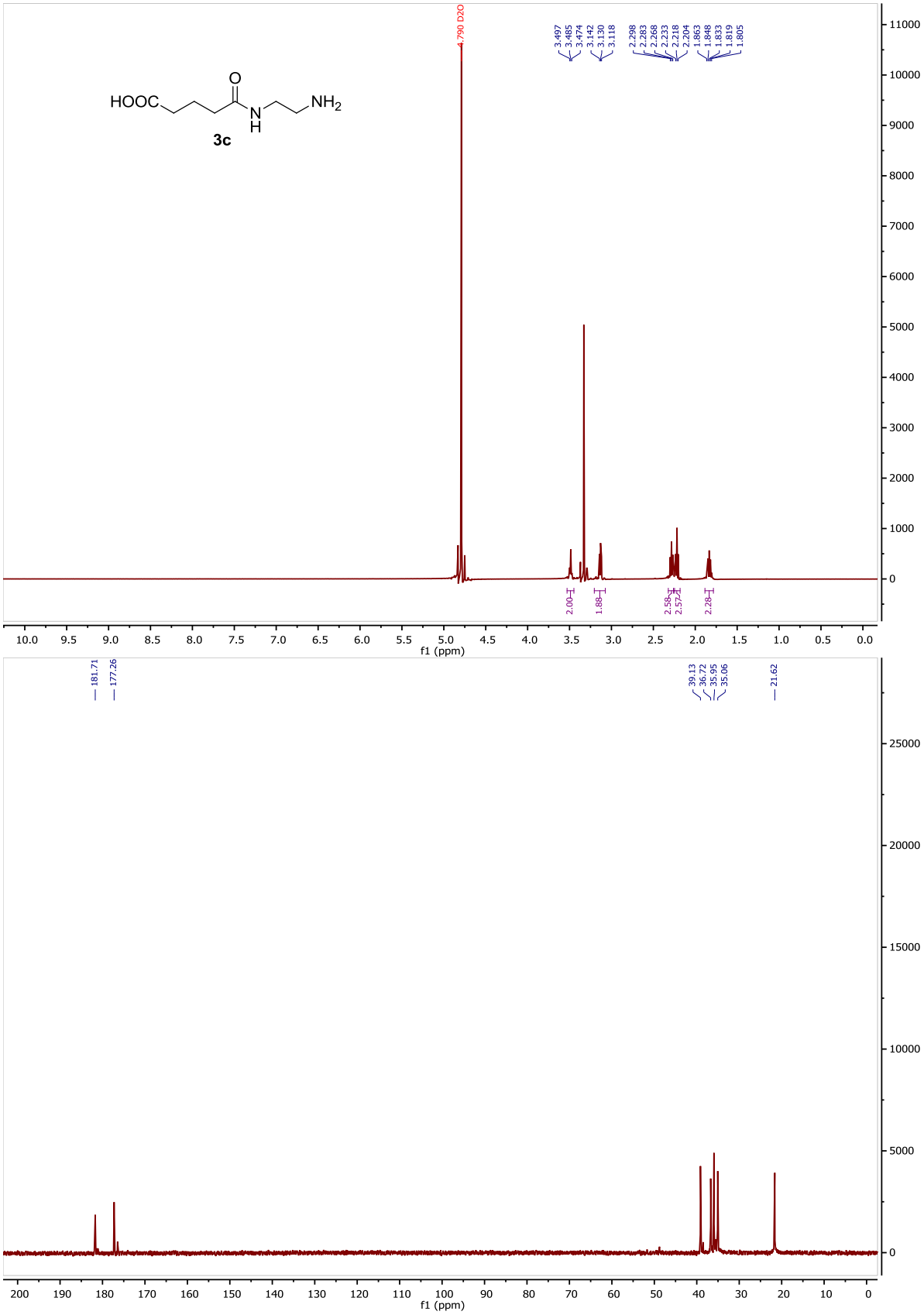


3b: 5-((3-amino-2,2-dimethylpropyl)amino)-3-methyl-5-oxopentanoic acid.

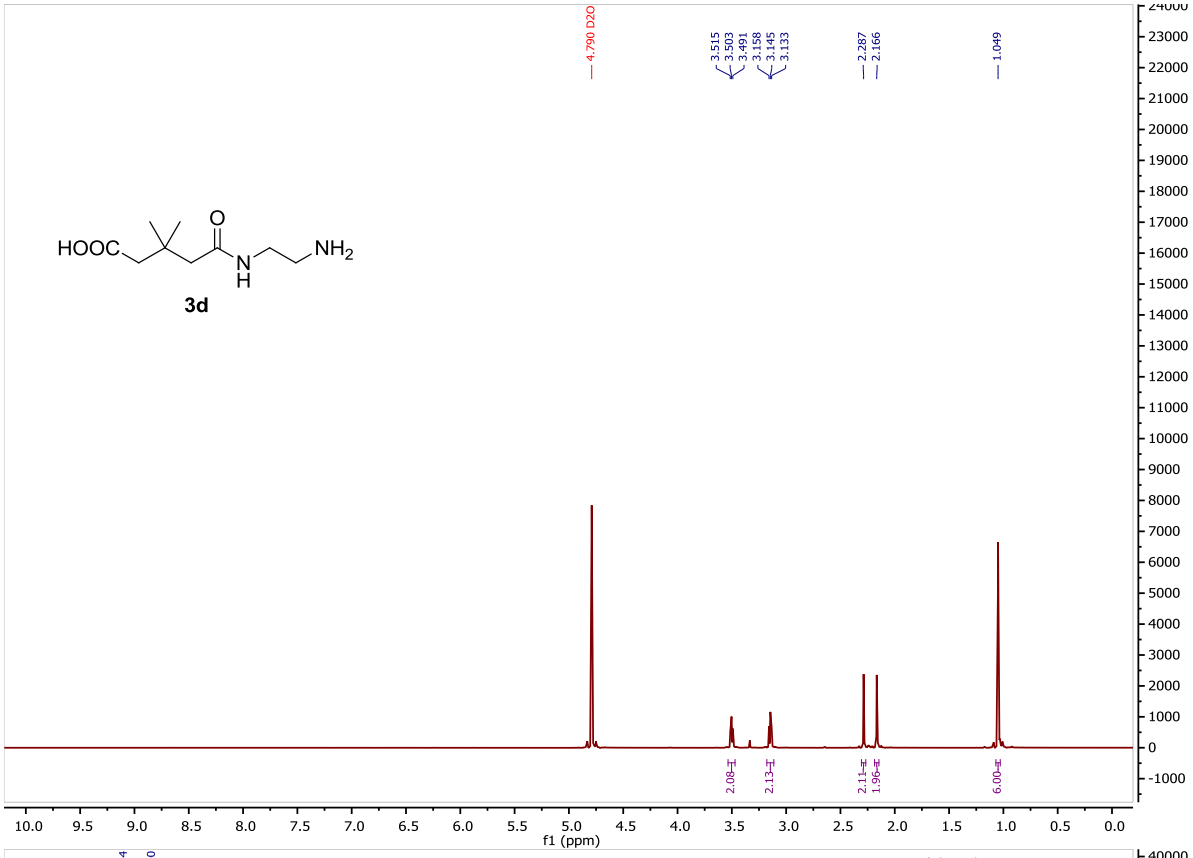


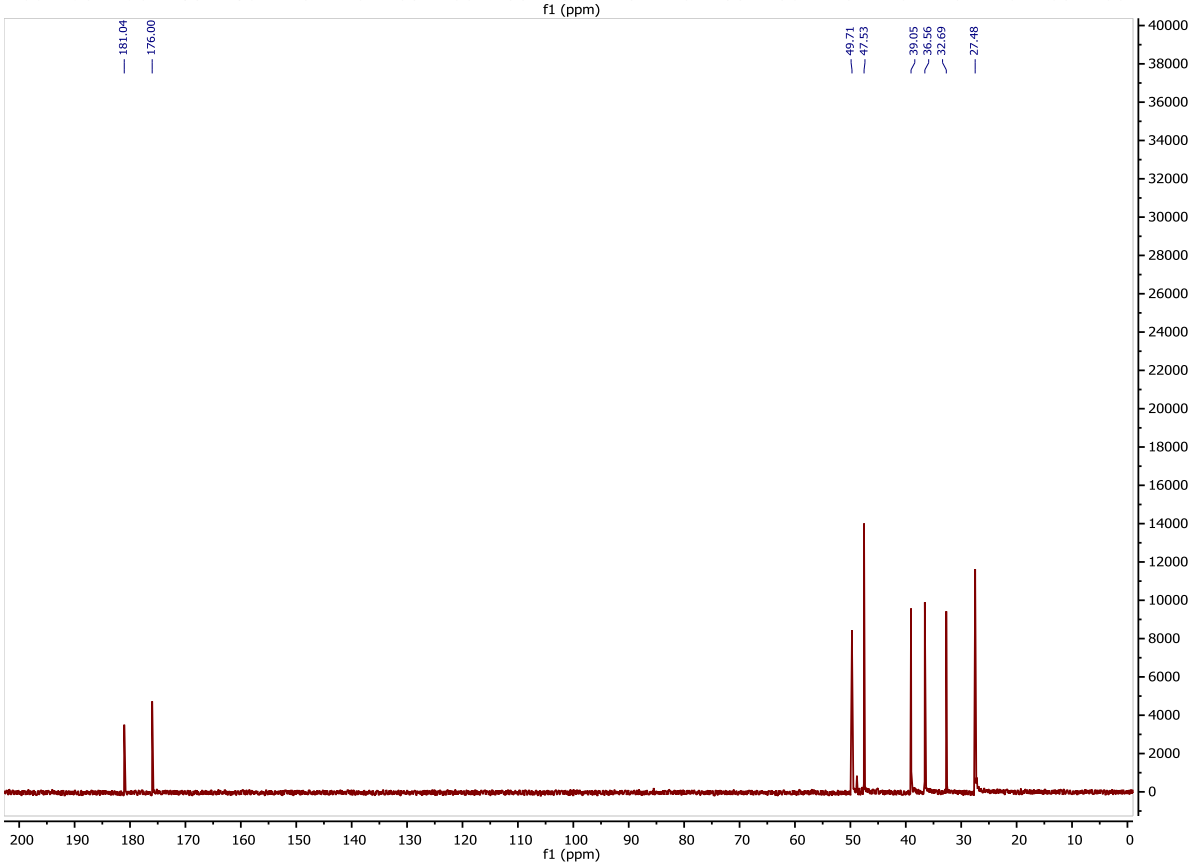


3c: 5-((2-aminoethyl)amino)-5-oxopentanoic acid.

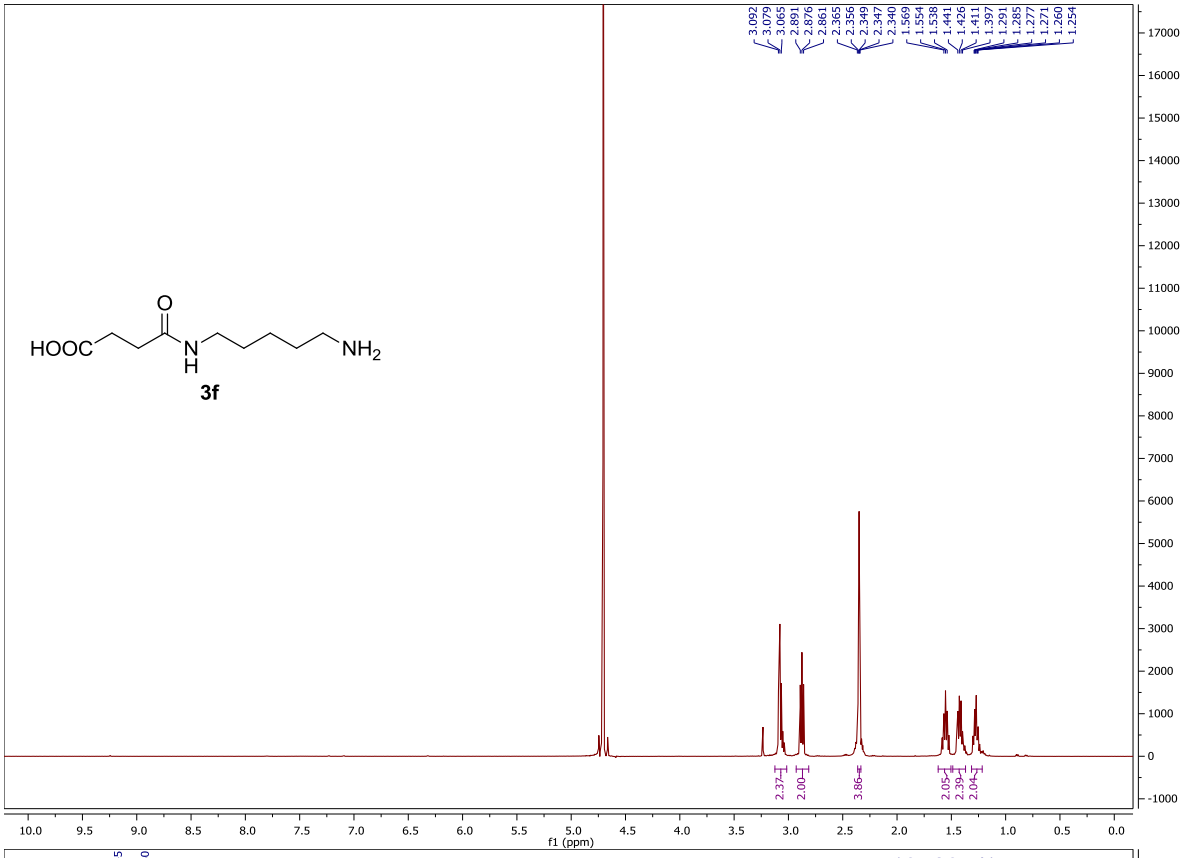


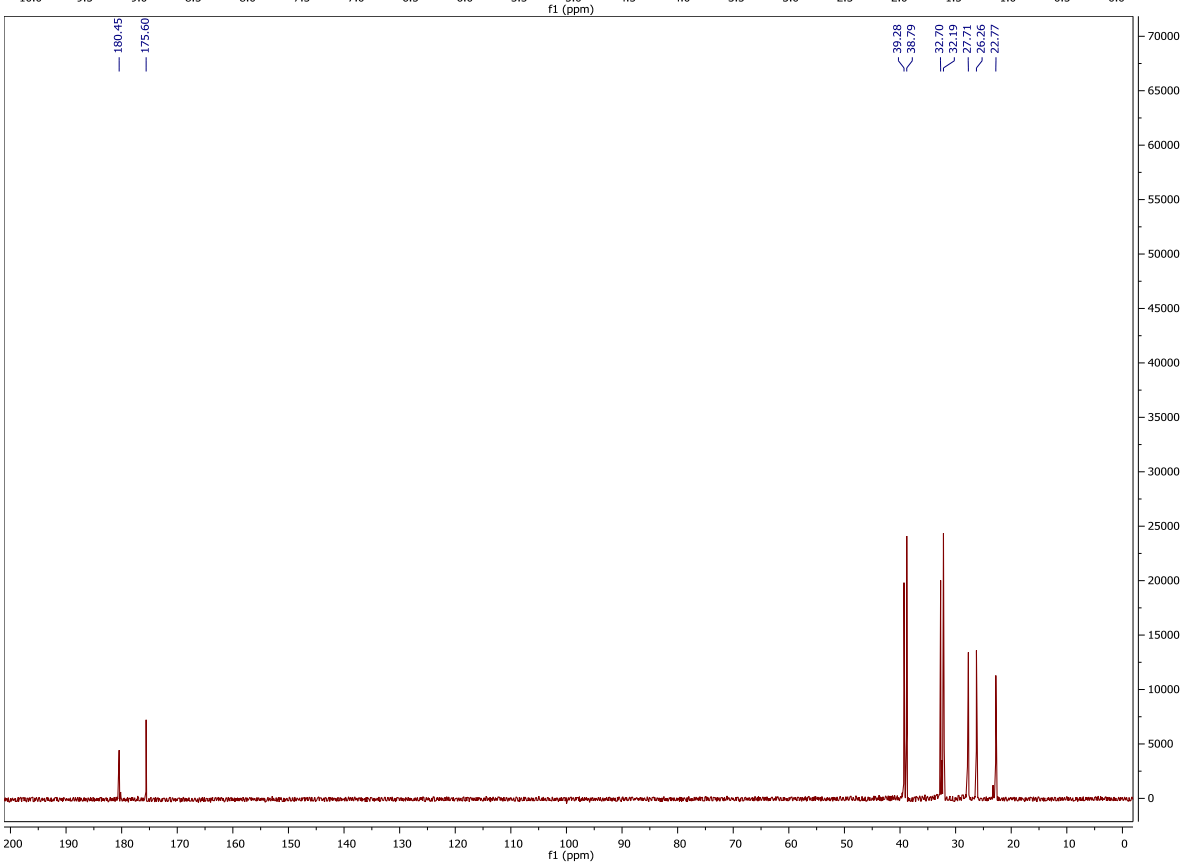
3d: 5-((2-aminoethyl)amino)-3,3-dimethyl-5-oxopentanoic acid.



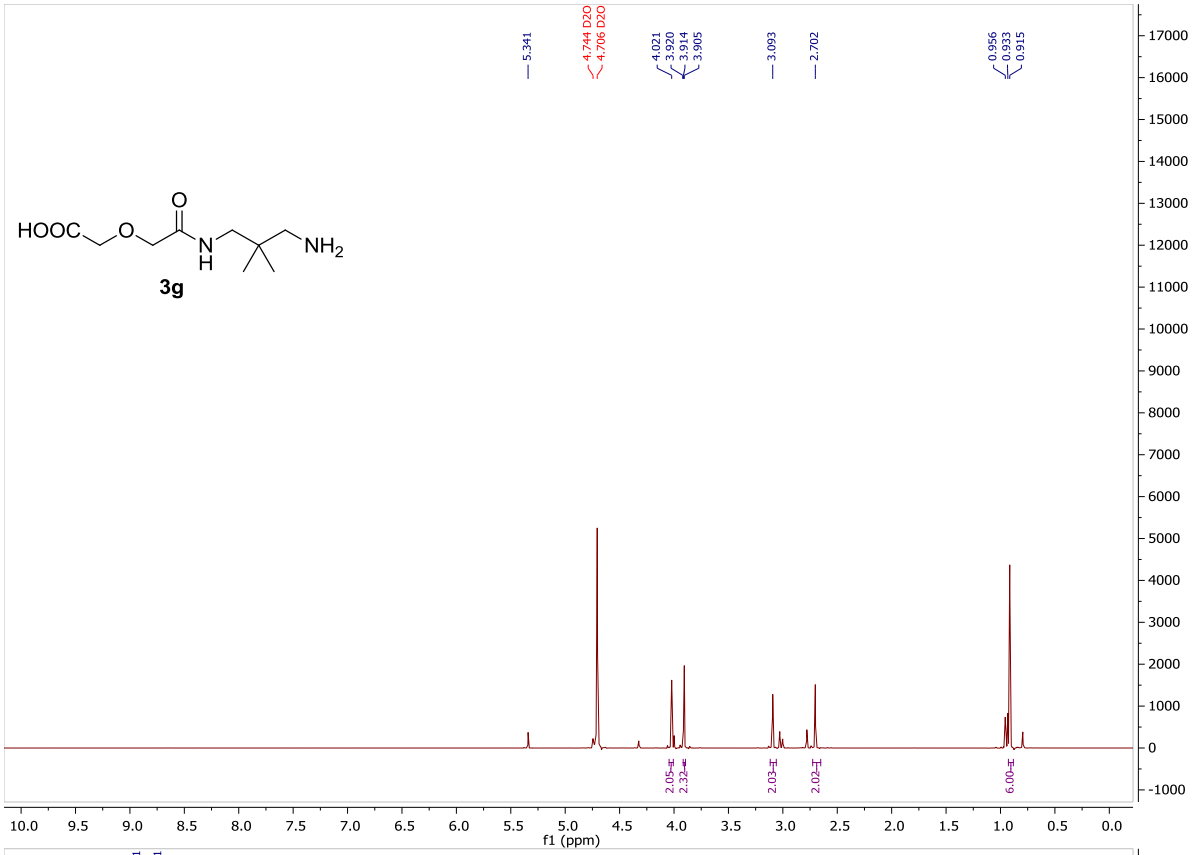


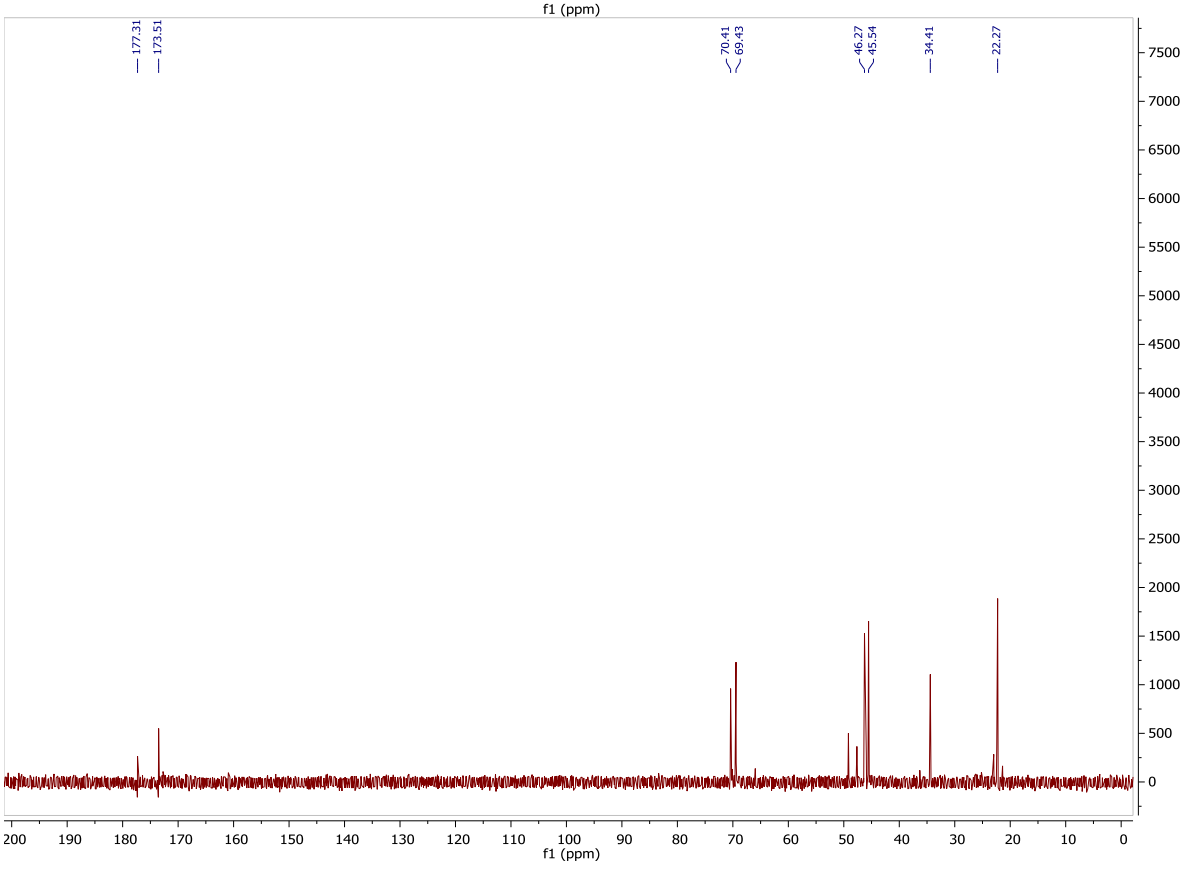
3f: **4-((5-aminopentyl)amino)-4-oxobutanoic acid.**



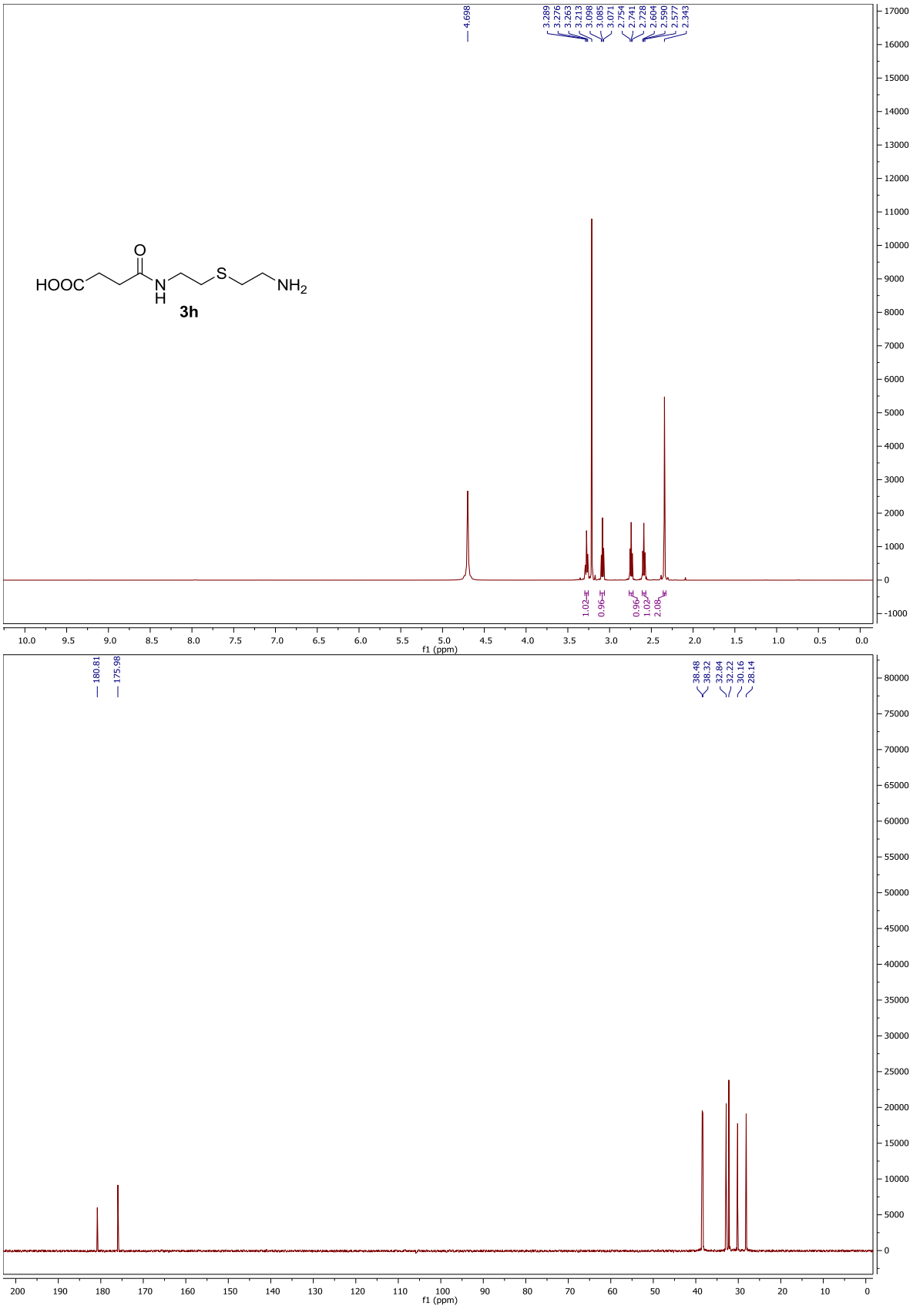


3g: **2-(2-((3-amino-2,2-dimethylpropyl)amino)-2-oxoethoxy)acetic acid.**



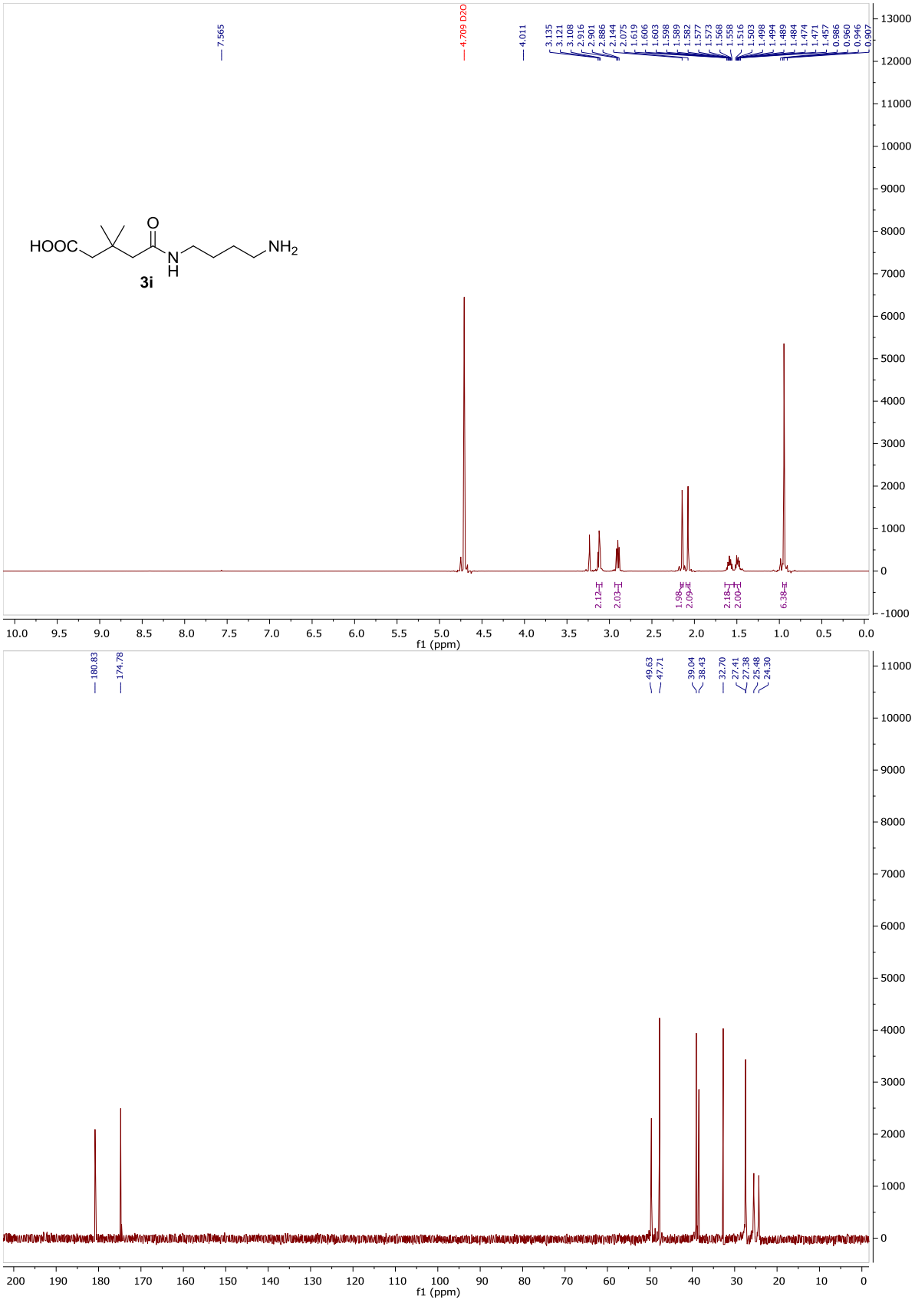


3h: **4-((2-((2-aminoethyl)thio)ethyl)amino)-4-oxobutanoic acid**:

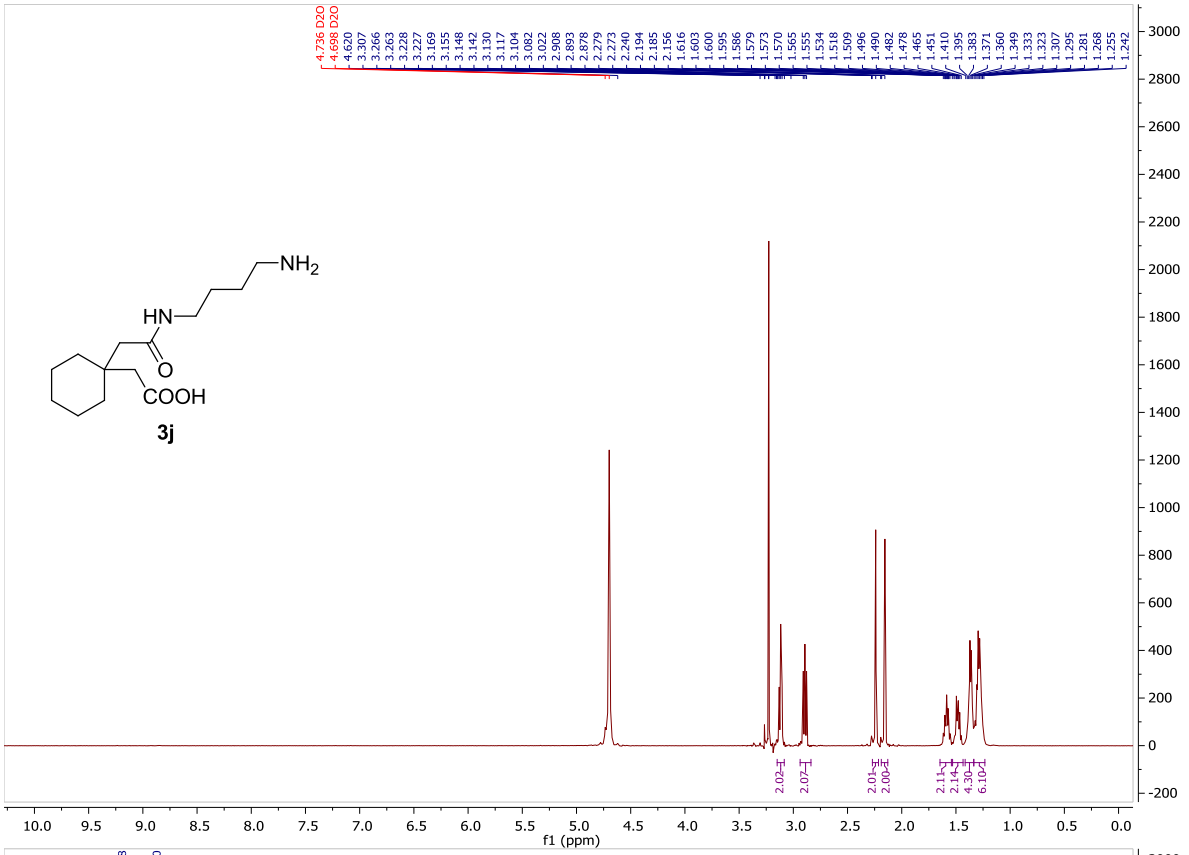


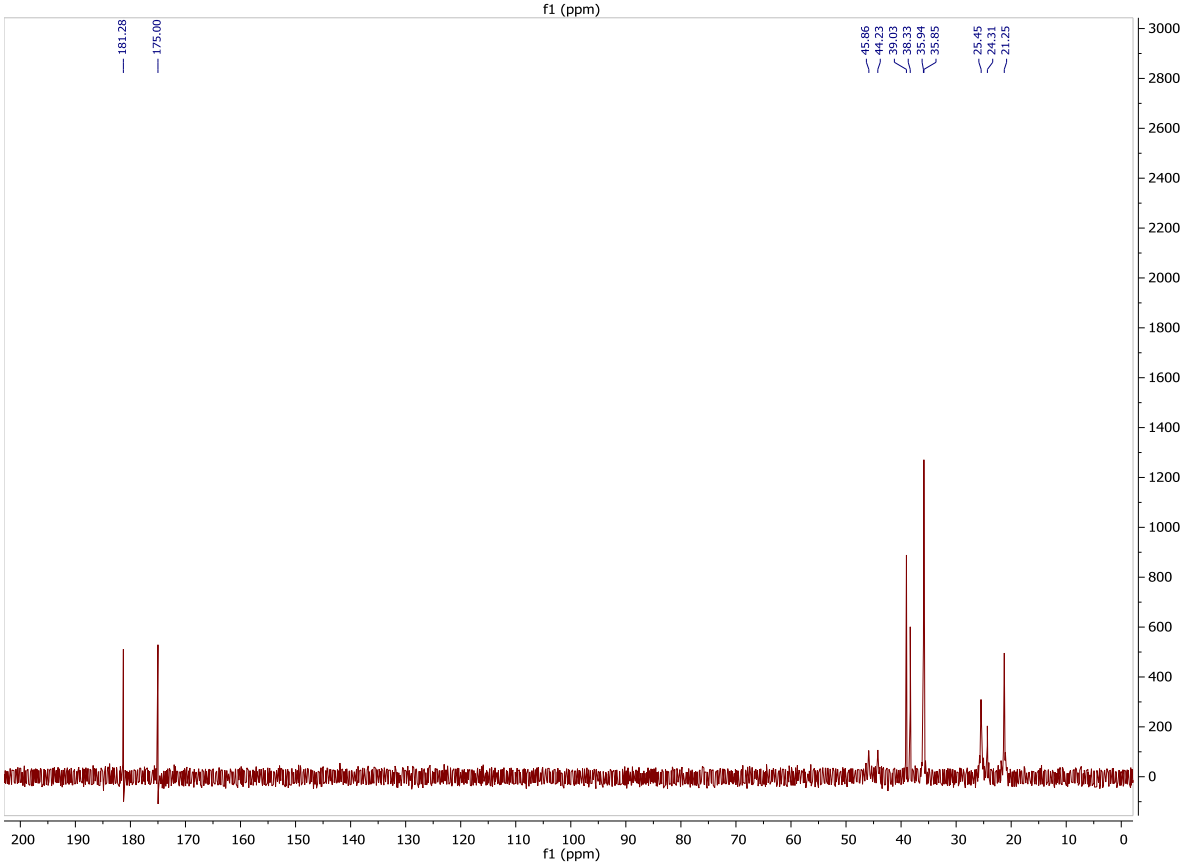
3h

3i: **5-((4-aminobutyl)amino)-3,3-dimethyl-5-oxopentanoic acid**:

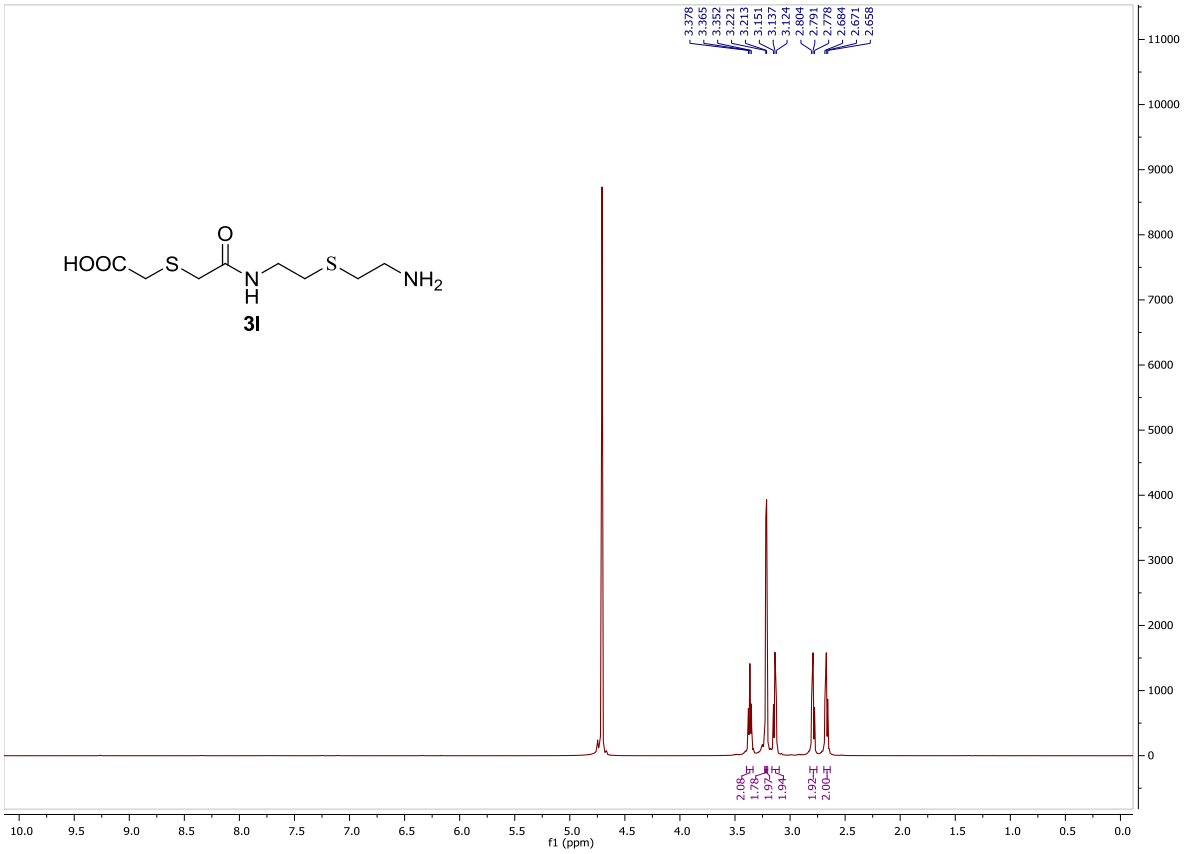


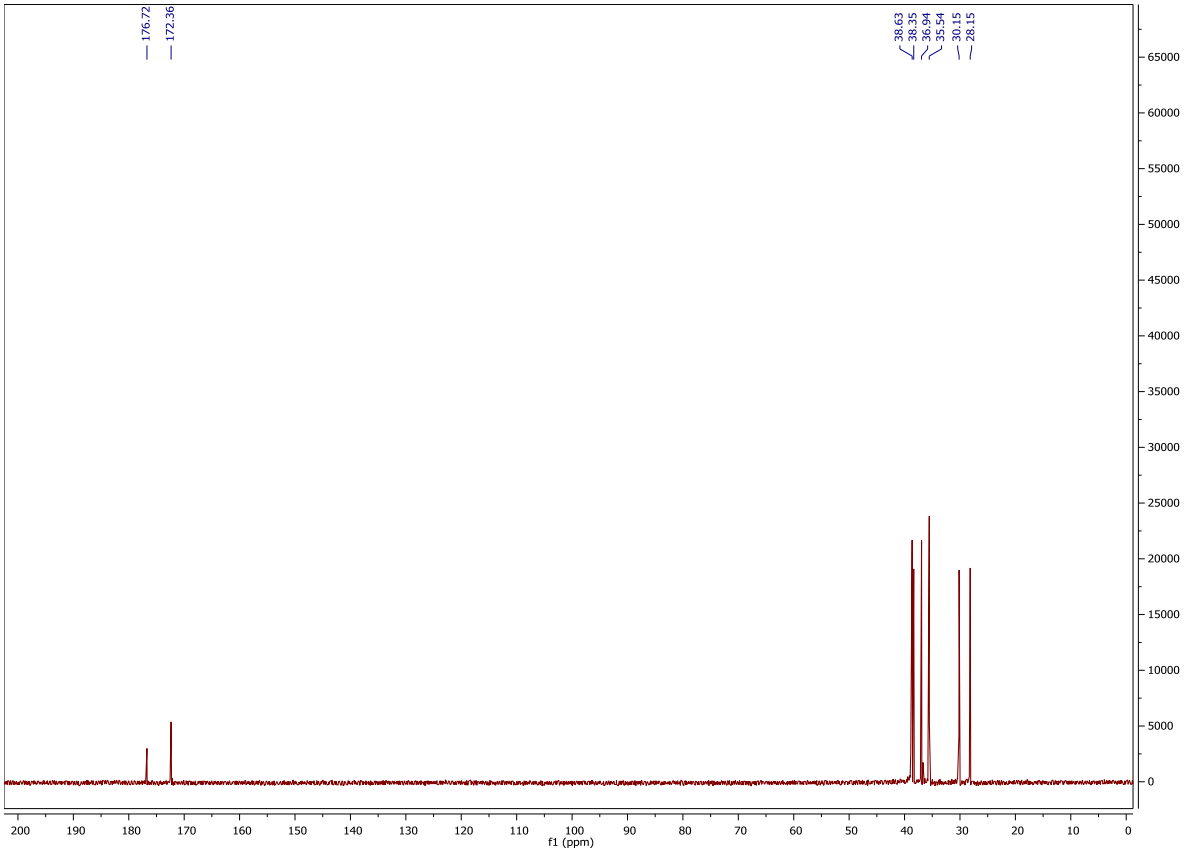
3j: **2-(1-(2-((4-aminobutyl)amino)-2-oxoethyl)cyclohexyl)acetic acid**:



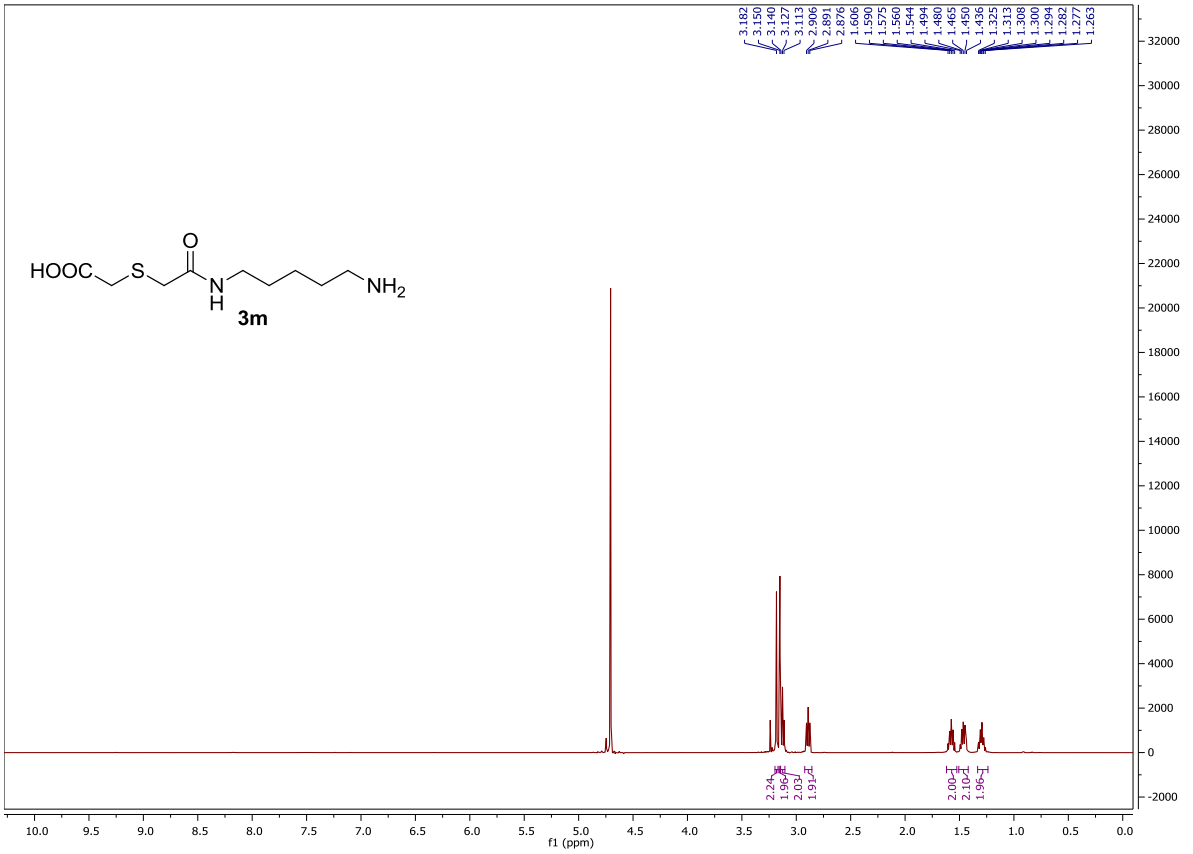


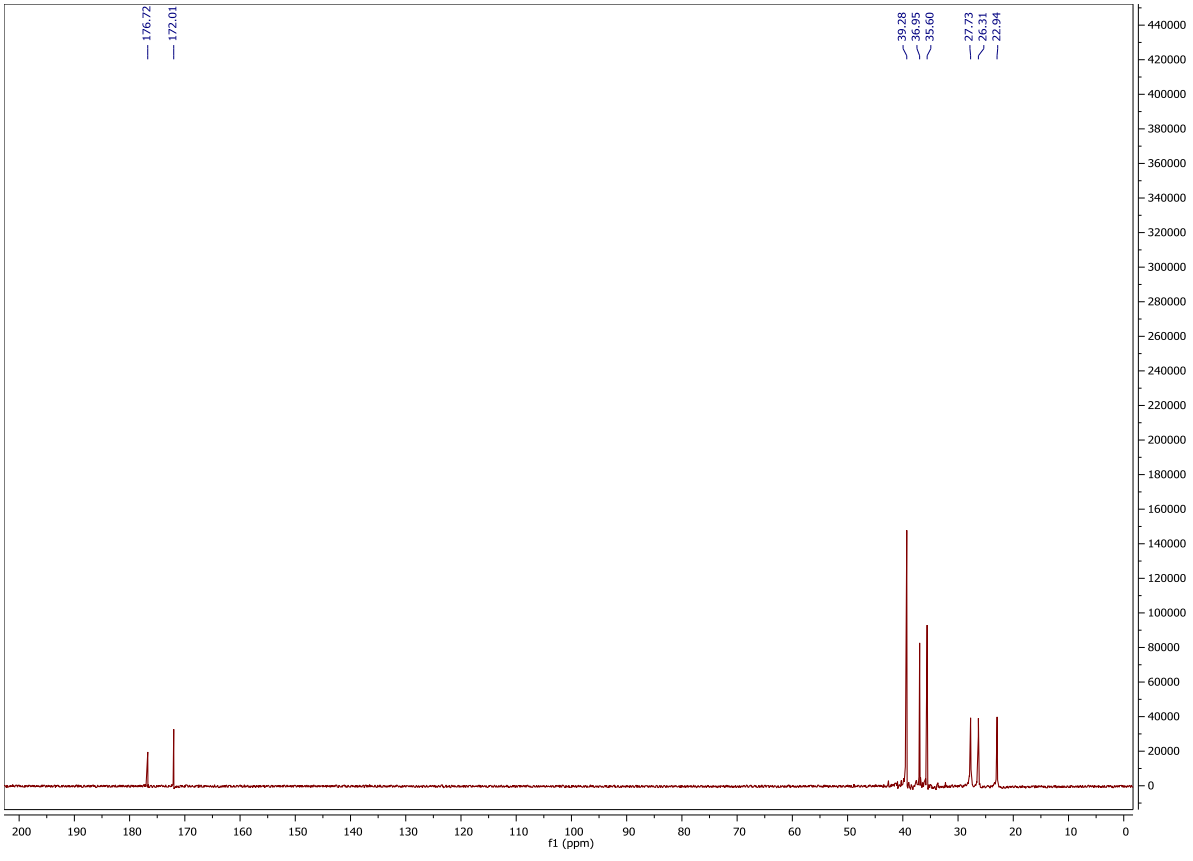
3l: **2-((2-((2-((2-aminoethyl)thio)ethyl)amino)-2-oxoethyl)thio)acetic acid**:



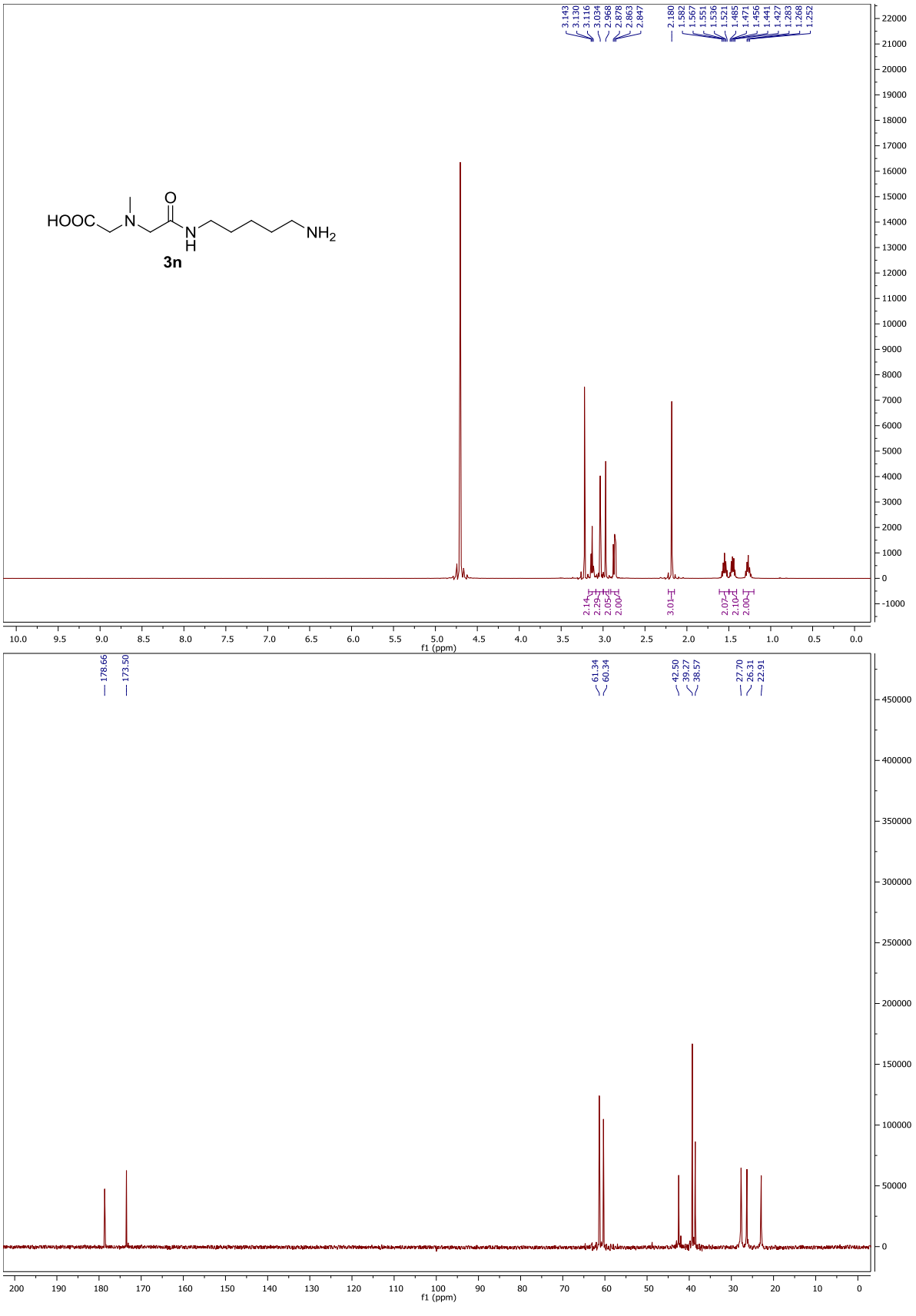


3m : 2-((2-((5-aminopentyl)amino)-2-oxoethyl)thio)acetic acid:

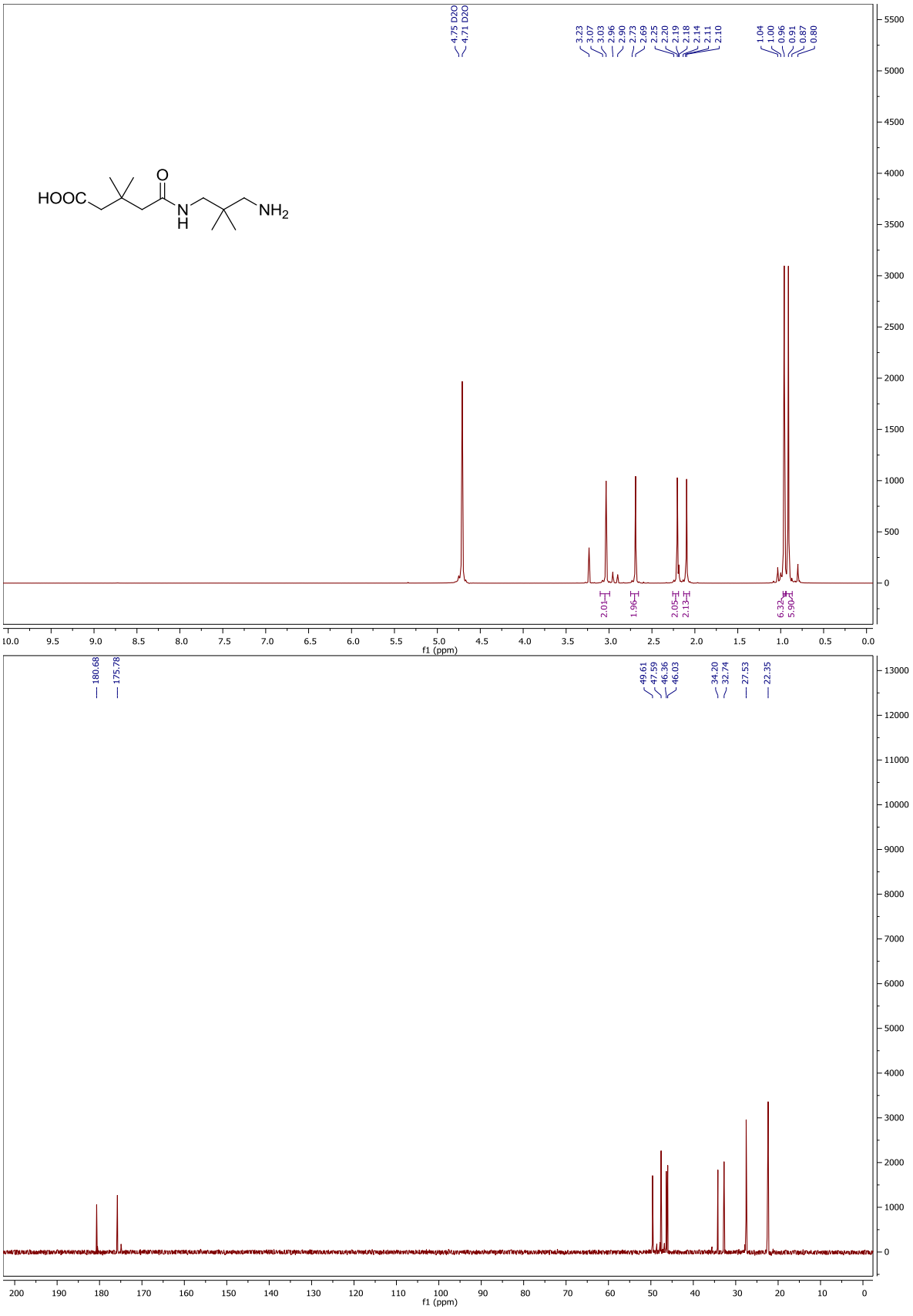




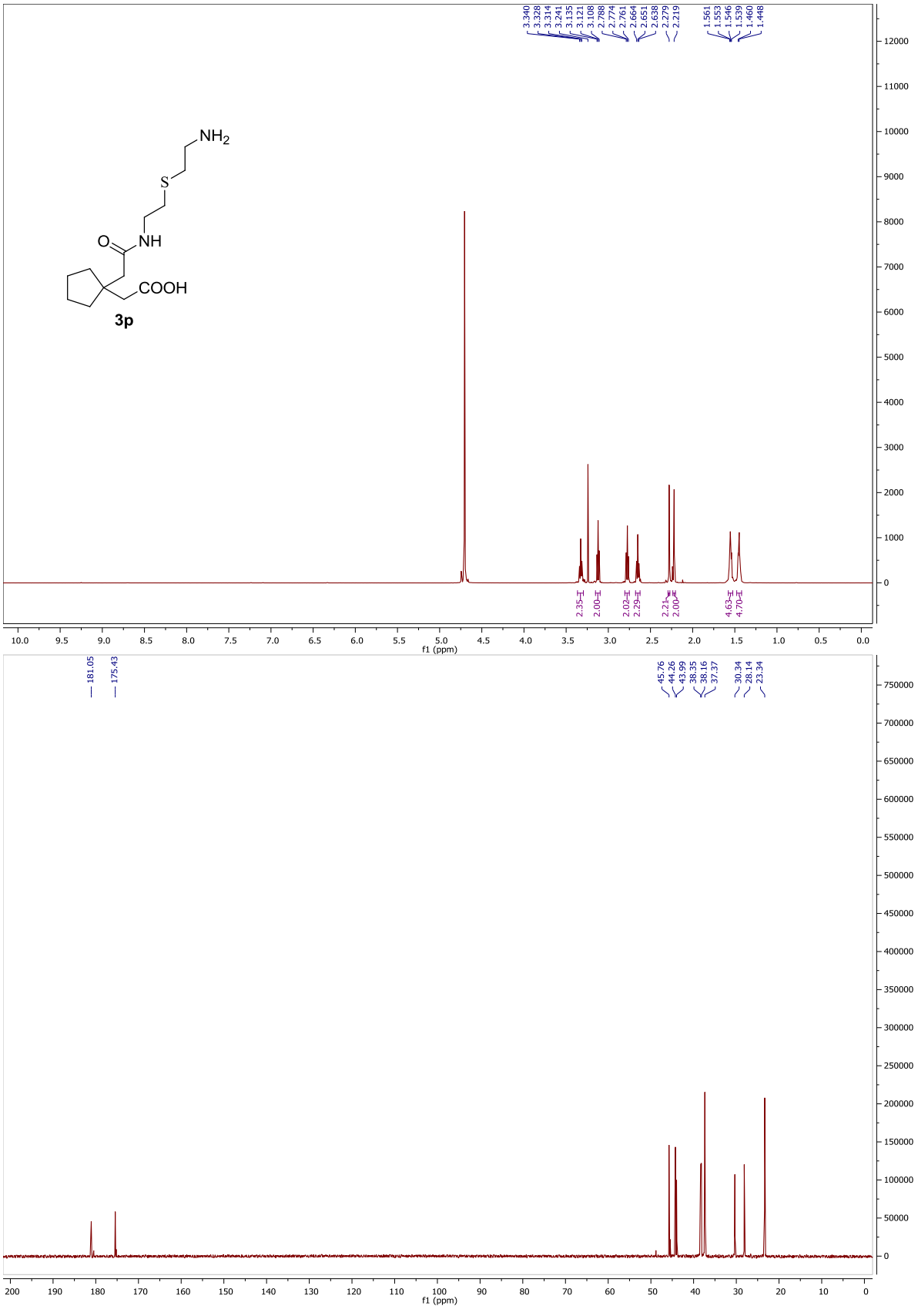
3n: **2-((2-((5-aminopentyl)amino)-2-oxoethyl)(methyl)amino)acetic acid**:



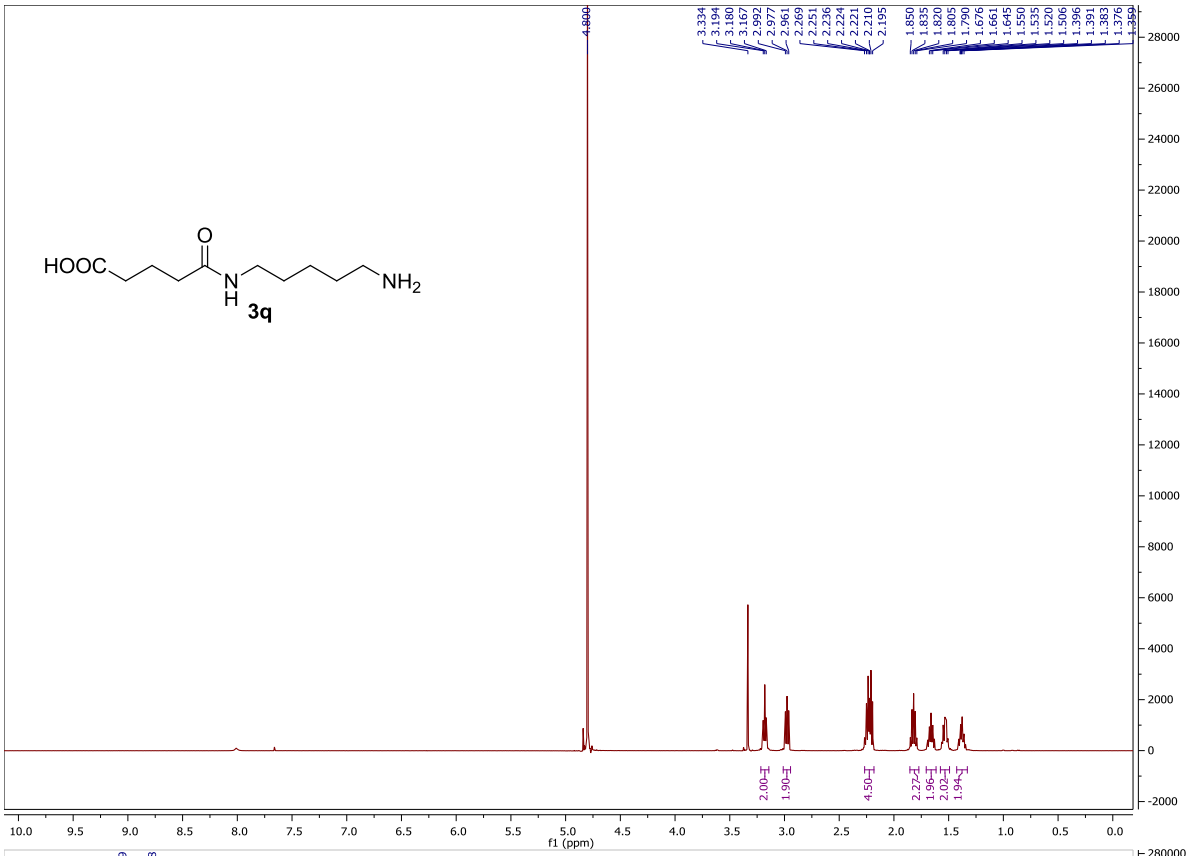
3o: **5-((3-amino-2,2-dimethylpropyl)amino)-3,3-dimethyl-5-oxopentanoic acid**:

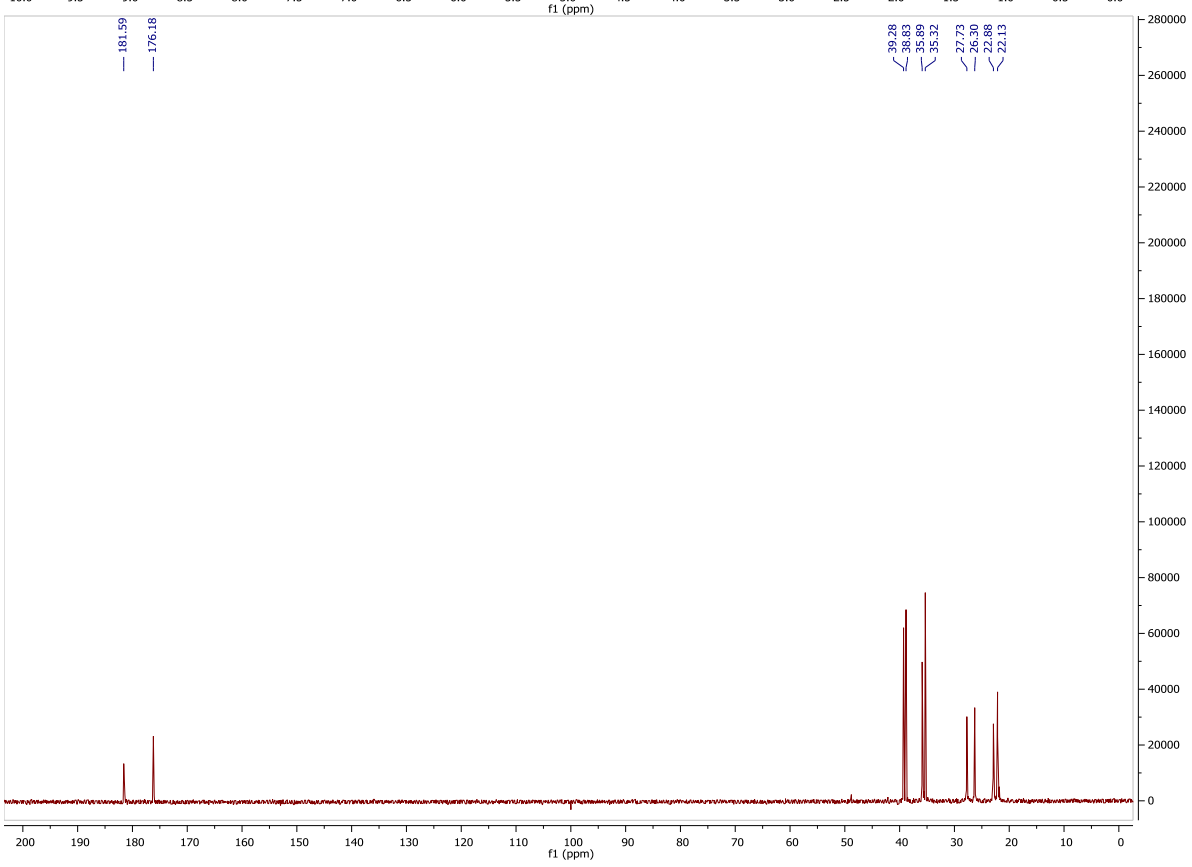


3p: 2-(1-(2-((2-((2-aminoethyl)thio)ethyl)amino)-2-oxoethyl)cyclopentyl)acetic acid:

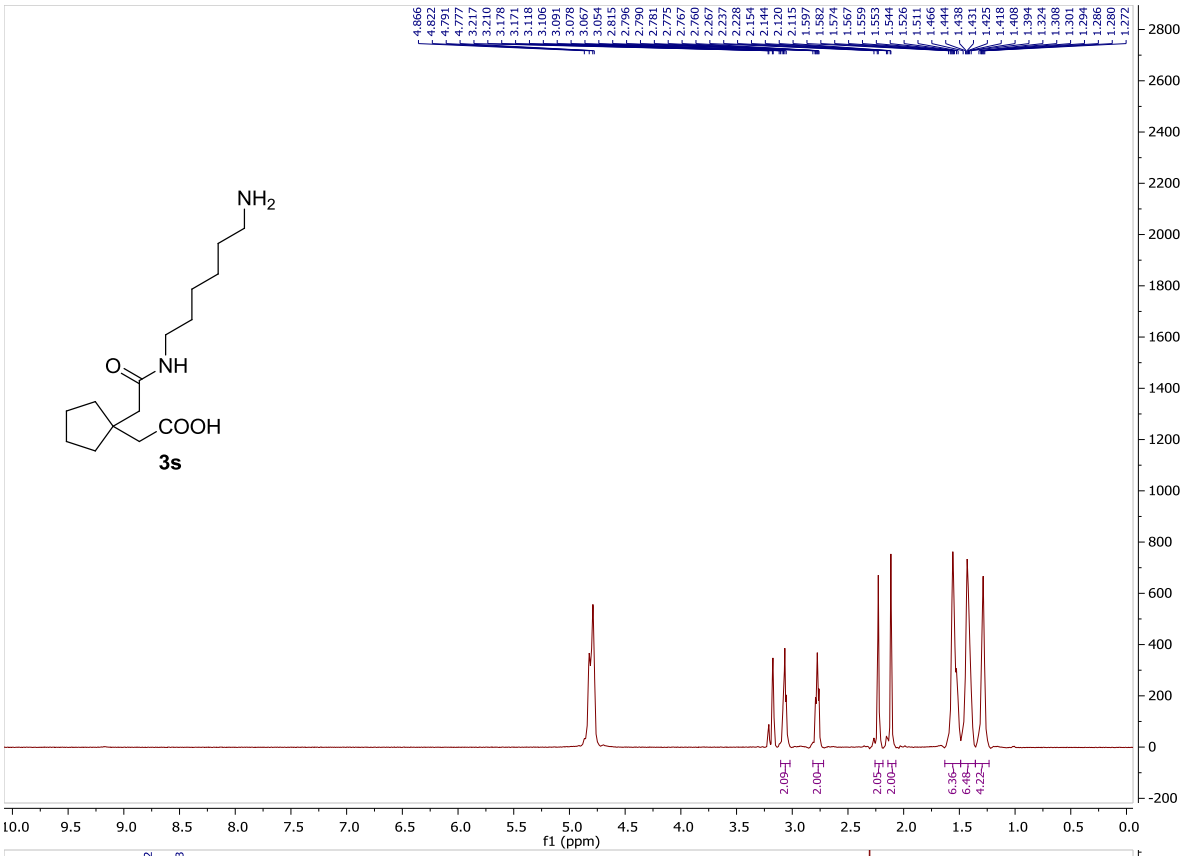


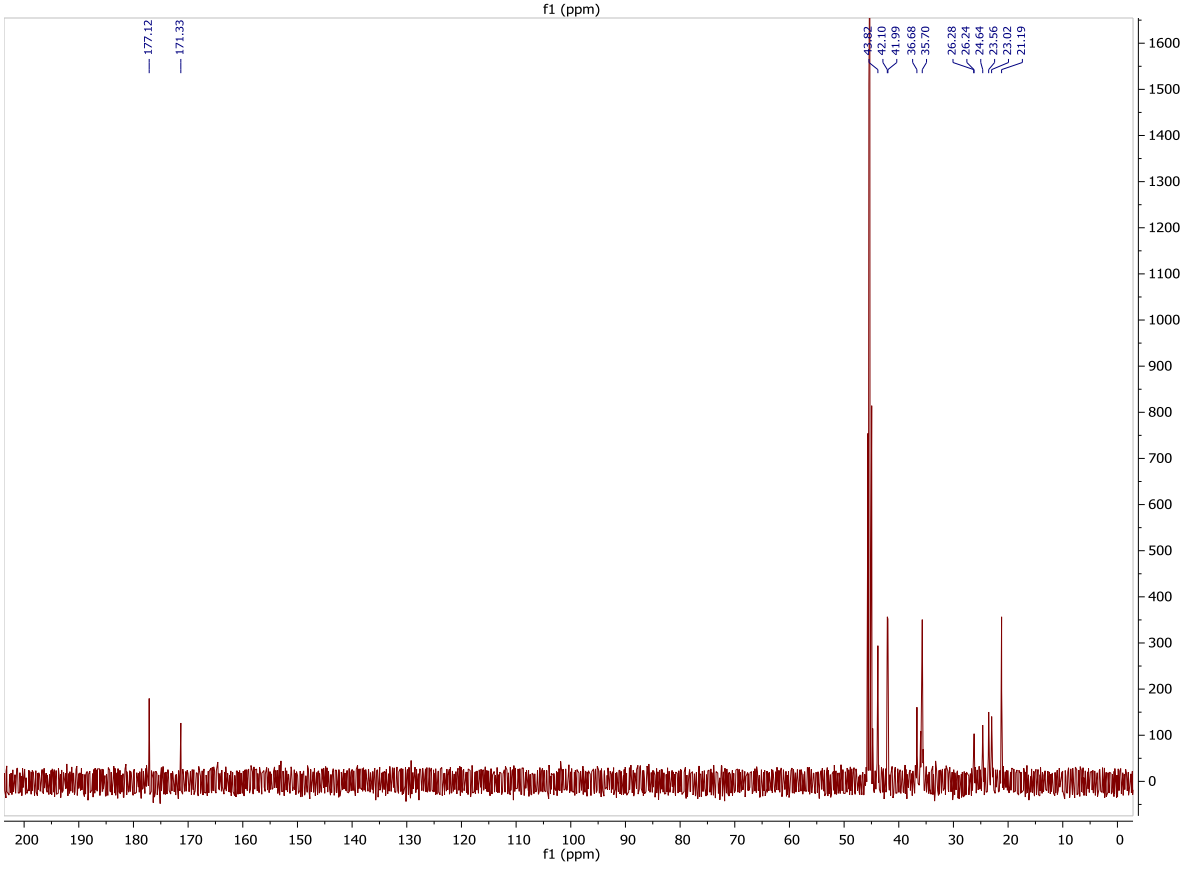
3q: 5-((5-aminopentyl)amino)-5-oxopentanoic acid:



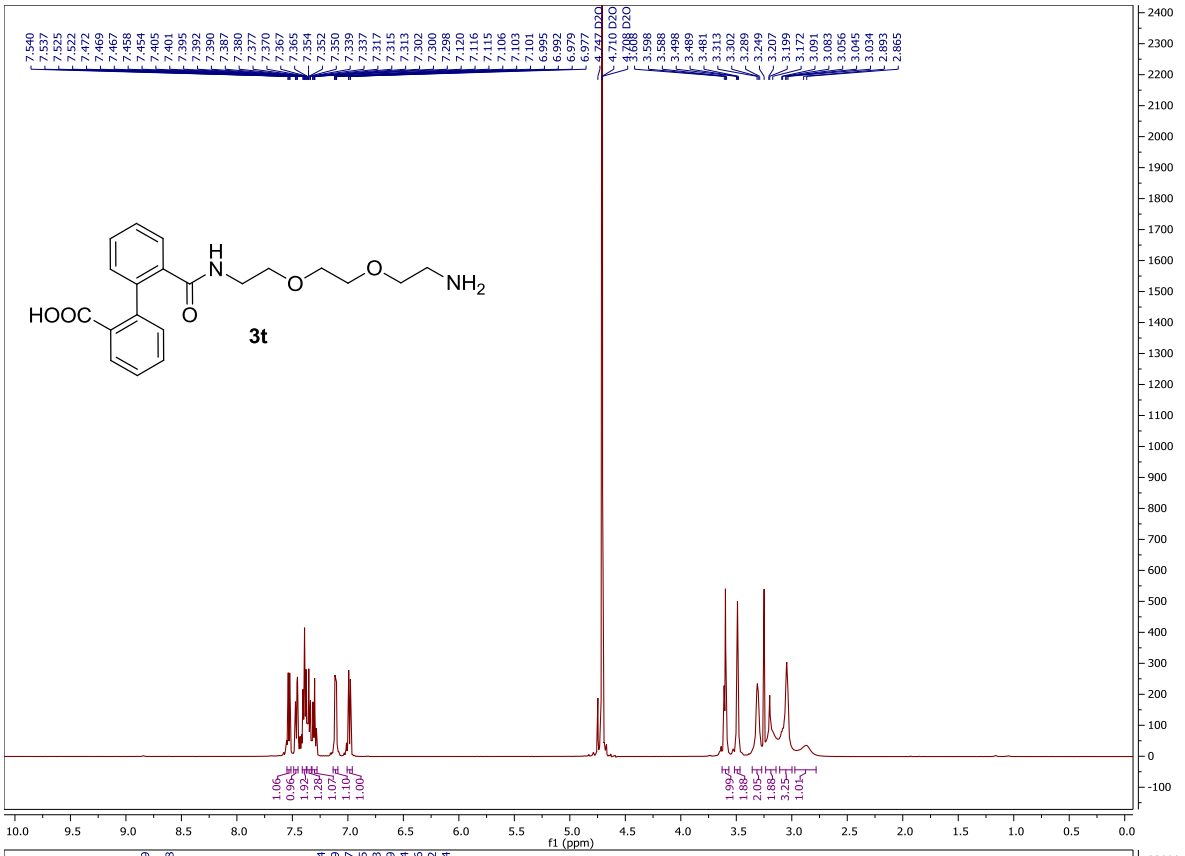


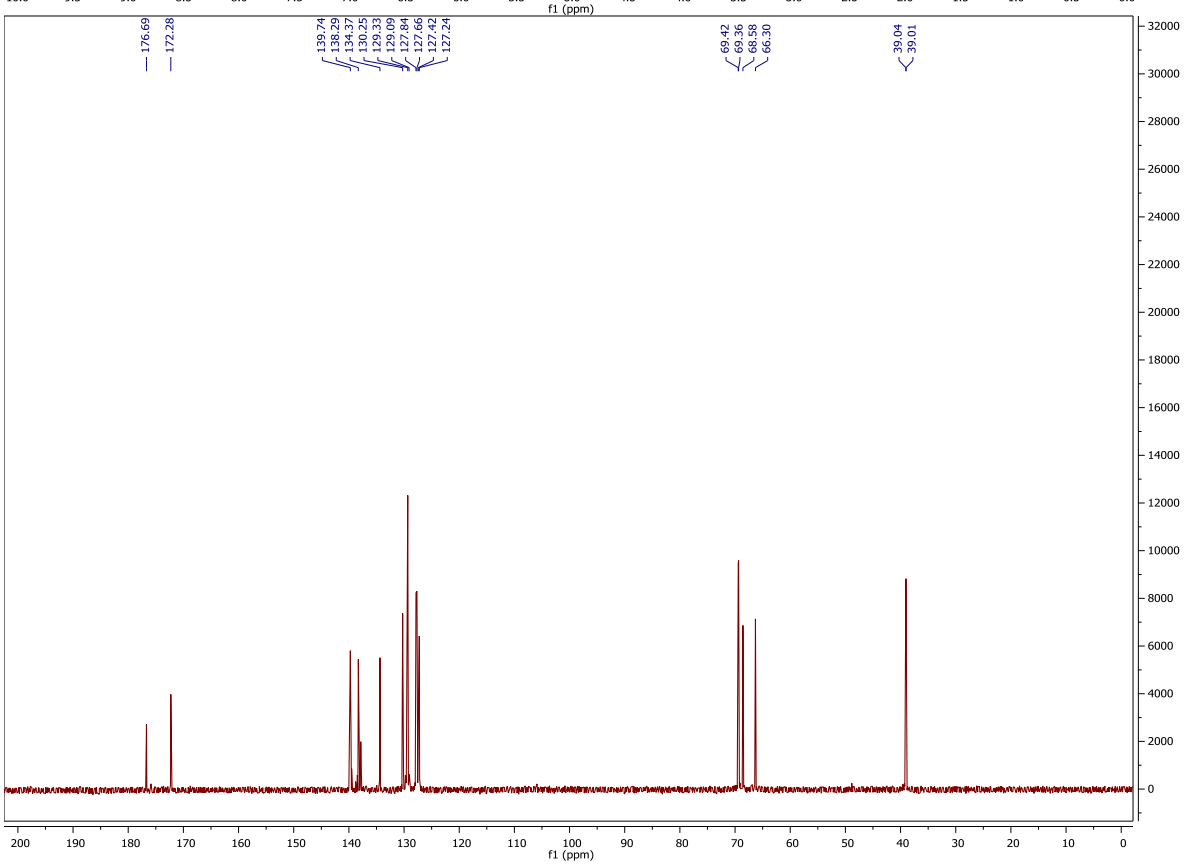
3s: 2-(1-(2-((6-aminohexyl)amino)-2-oxoethyl)cyclopentyl)acetic acid:



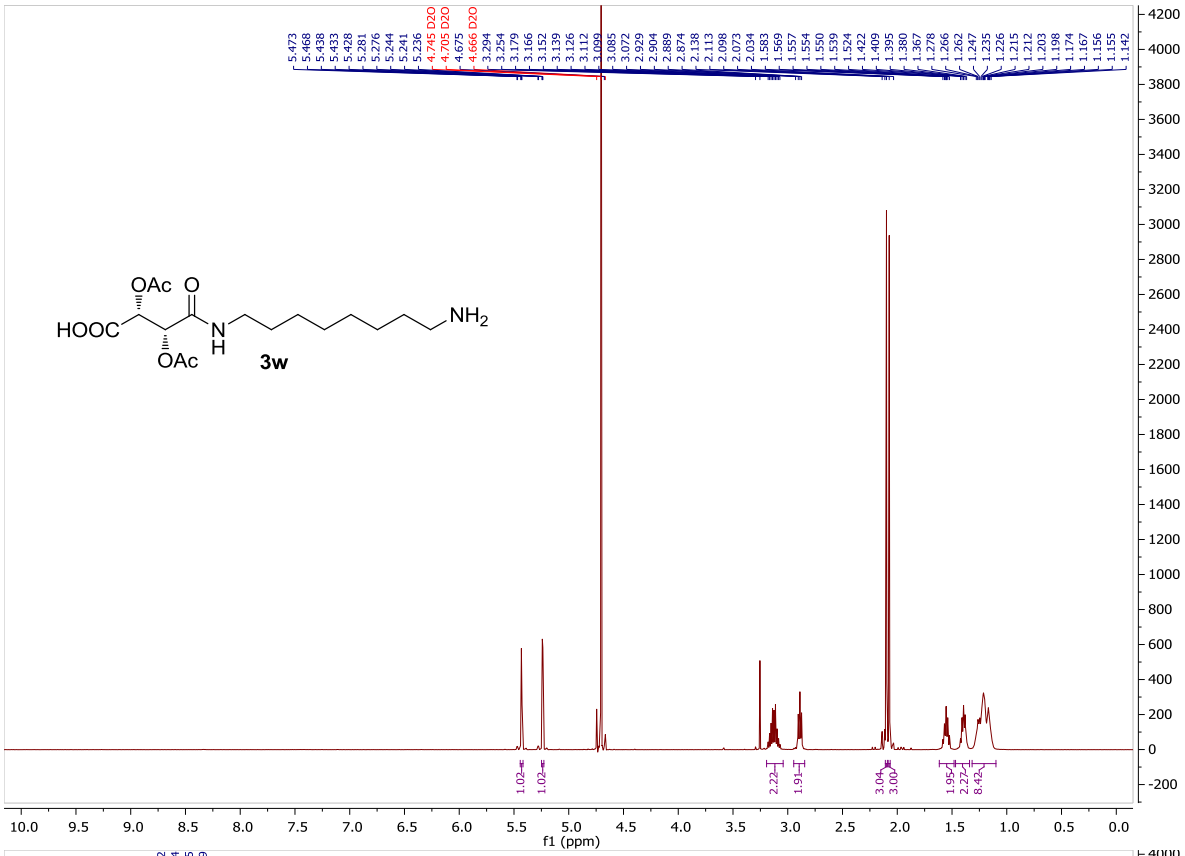


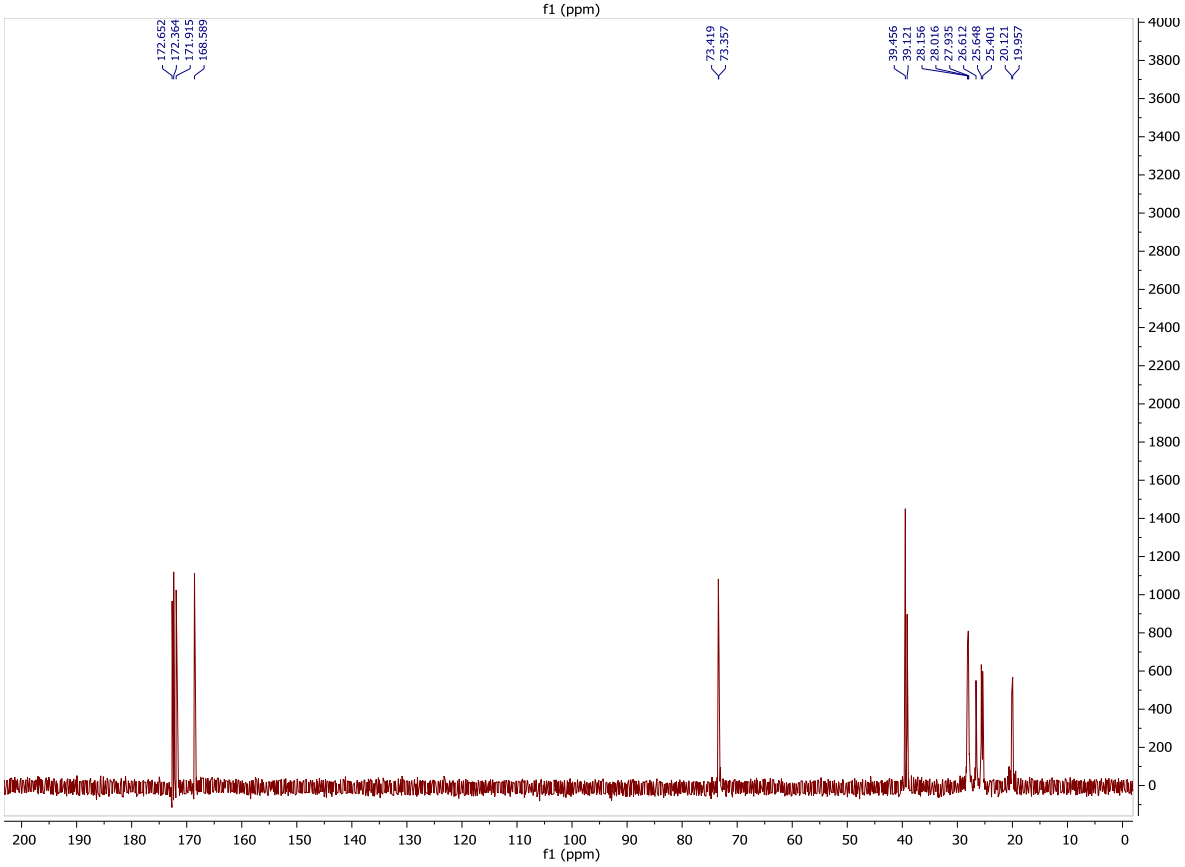
3t: 2'-((2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamoyl)-[1,1'-biphenyl]-2-carboxylic acid:



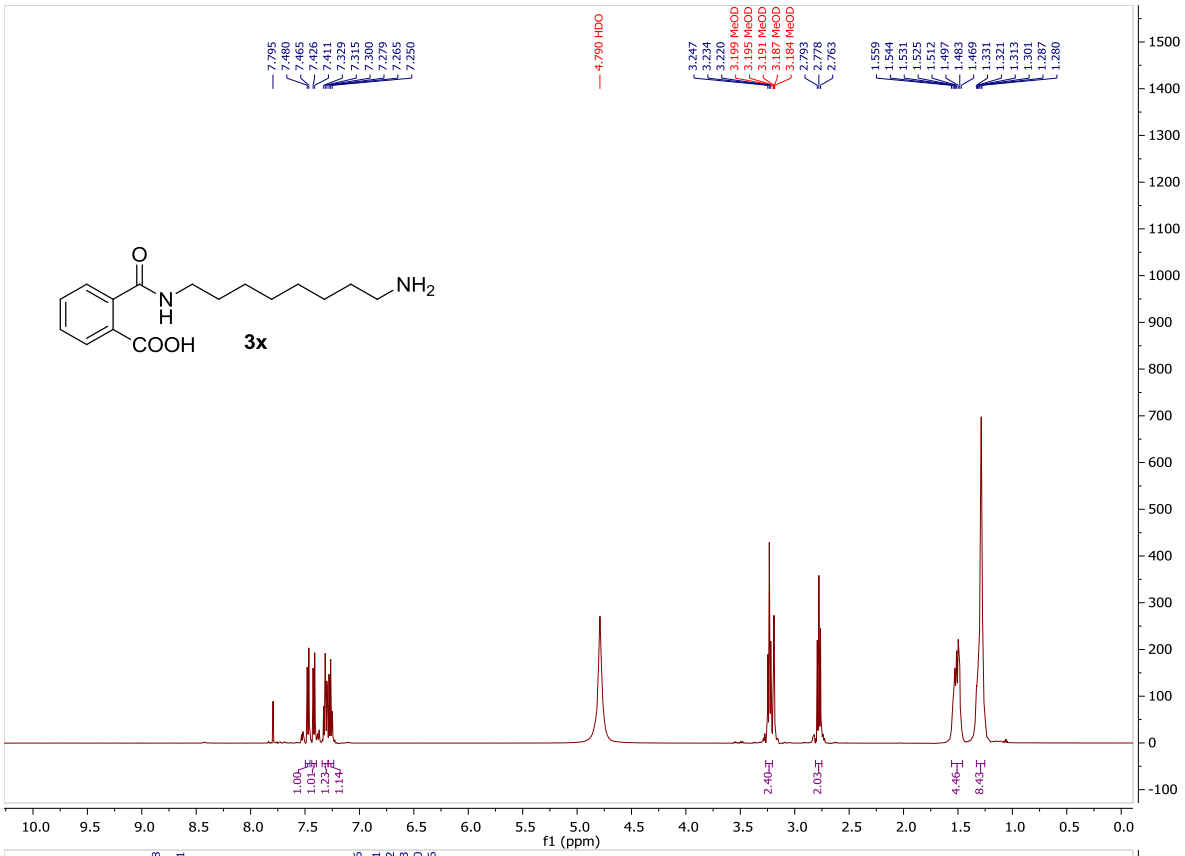


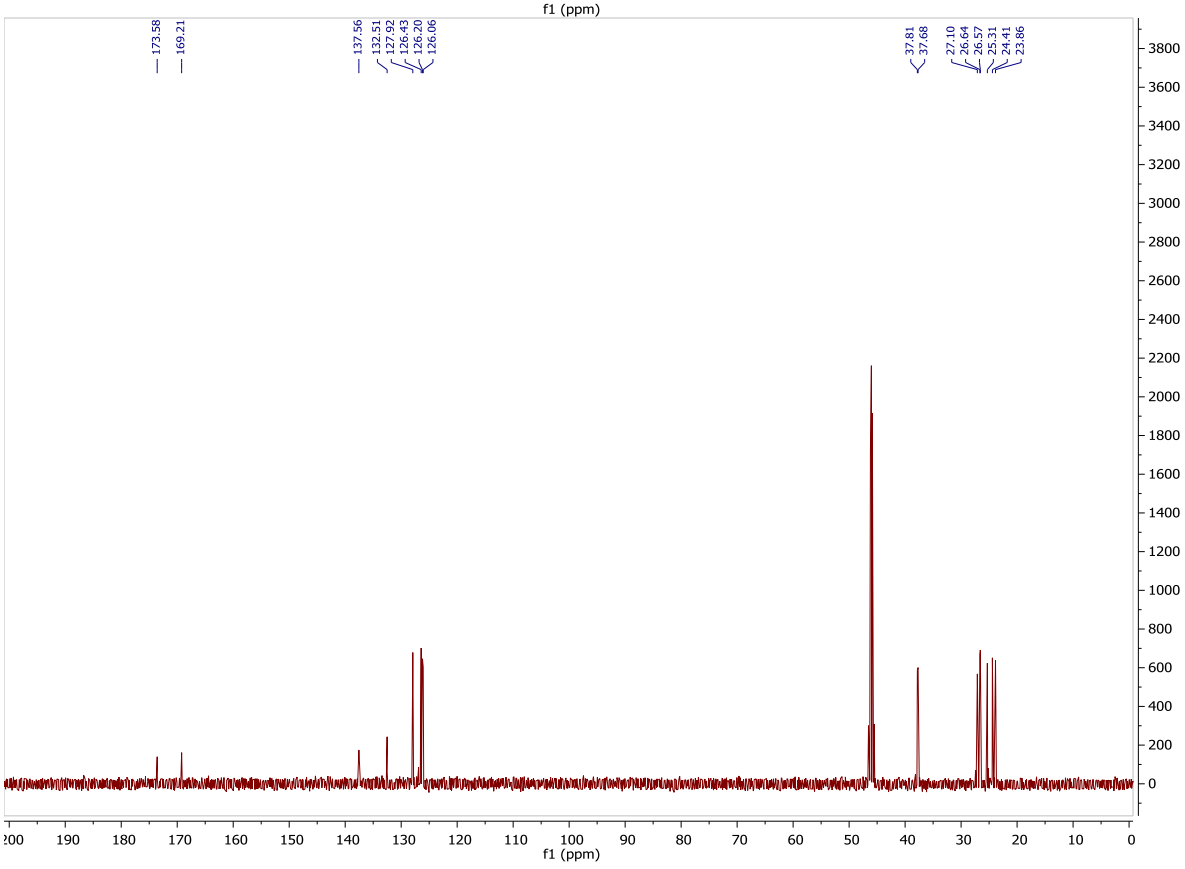
3w: (2*R*,3*R*)-2,3-diacetoxy-4-((8-aminooctyl)amino)-4-oxobutanoic acid:



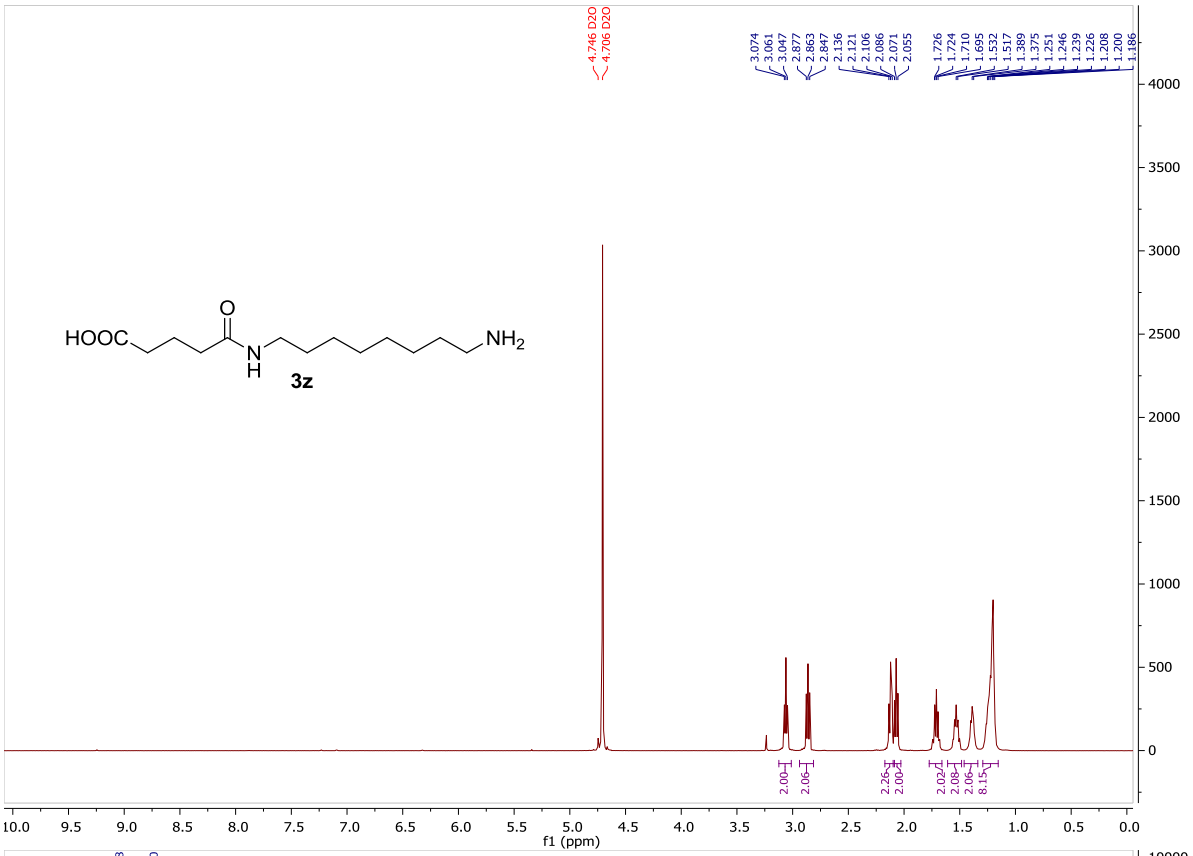


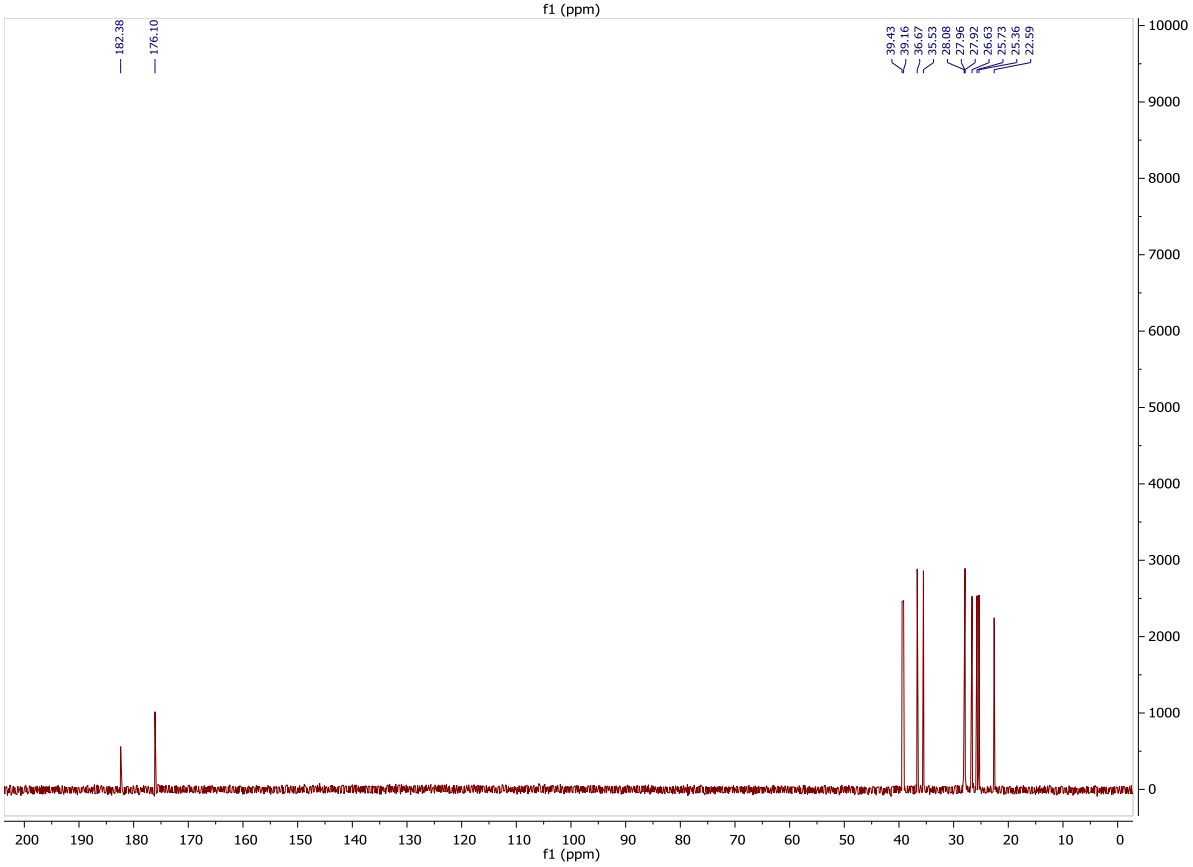
3x: 2-((8-aminooctyl)carbamoyl)benzoic acid:



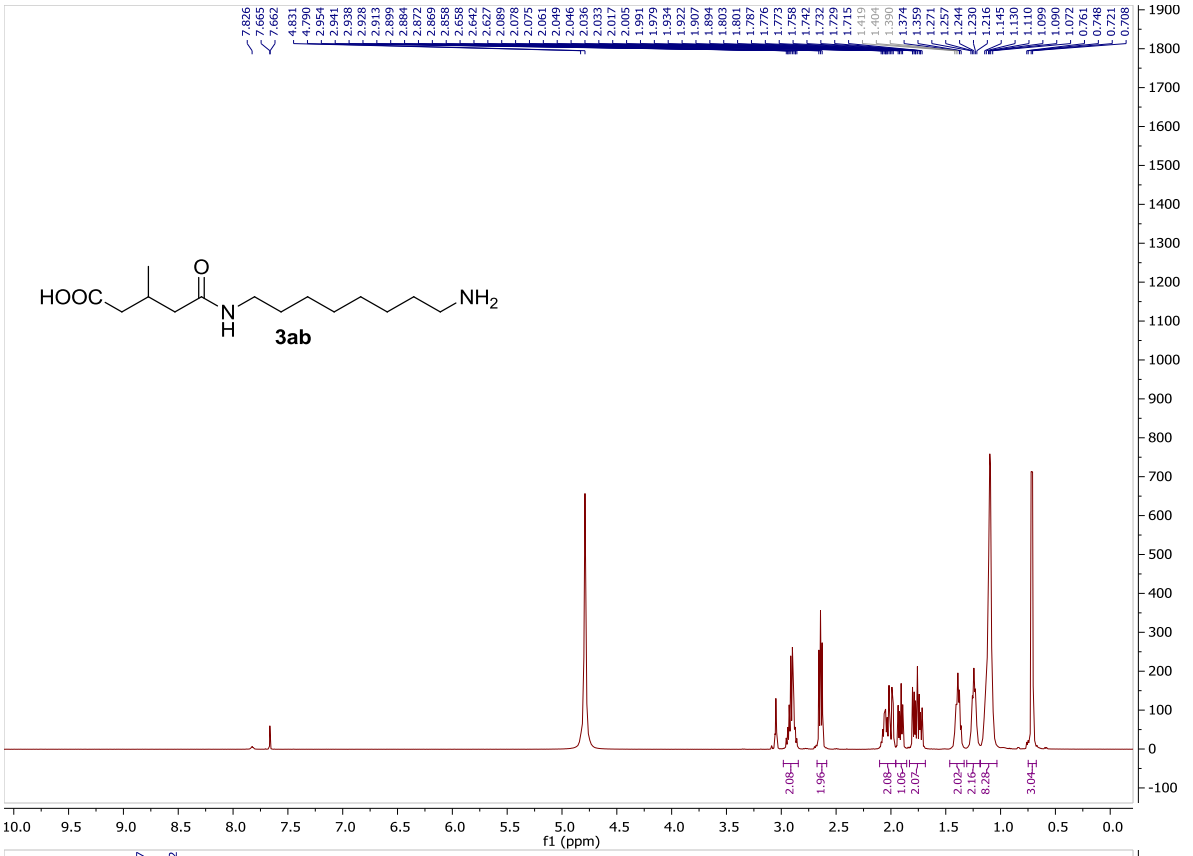


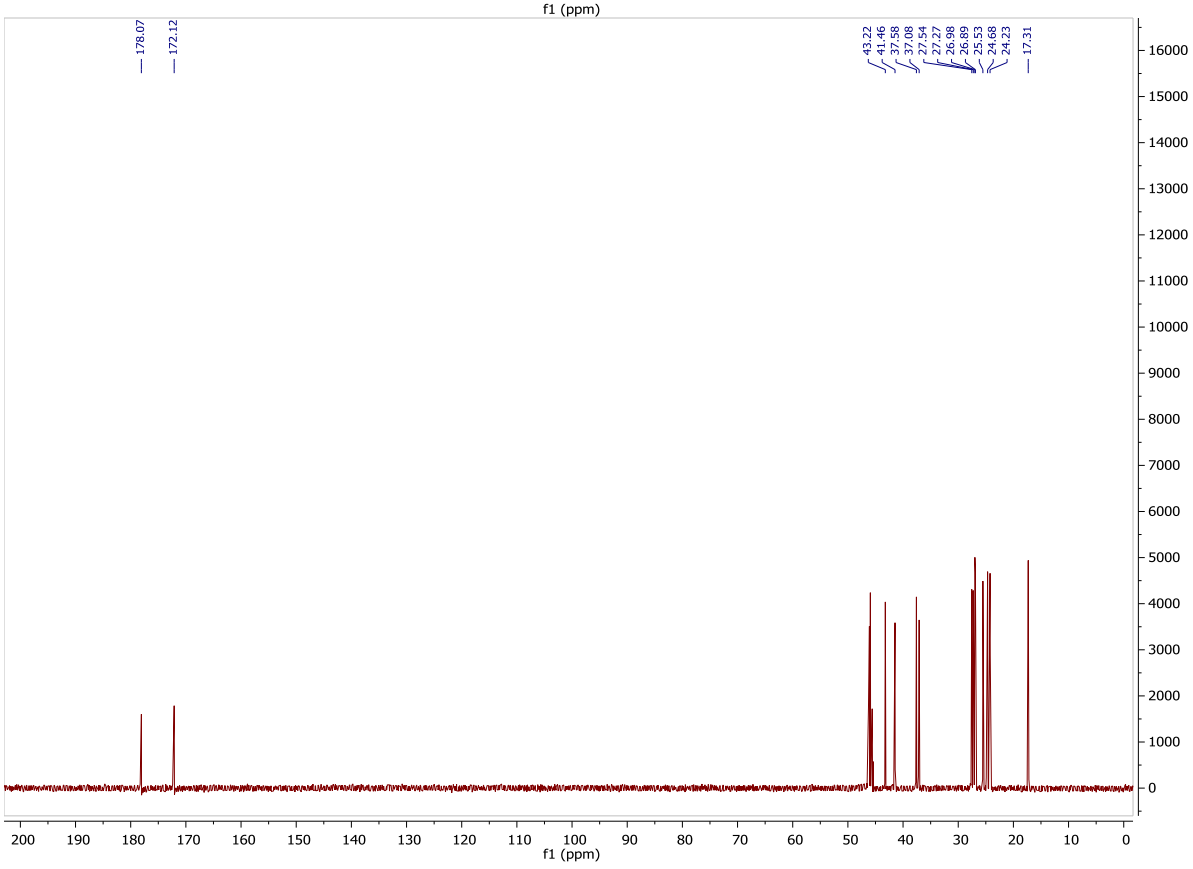
3z: 5-((8-aminooctyl)amino)-5-oxopentanoic acid:



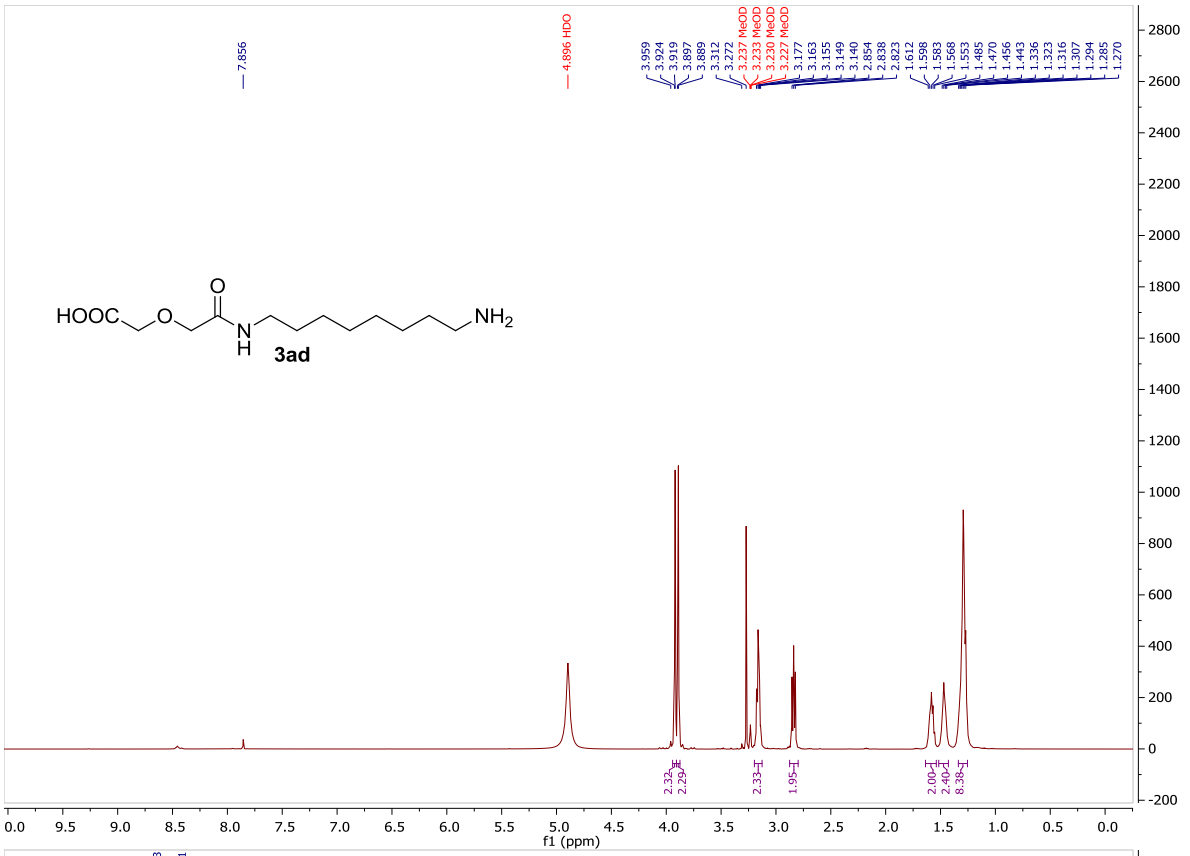


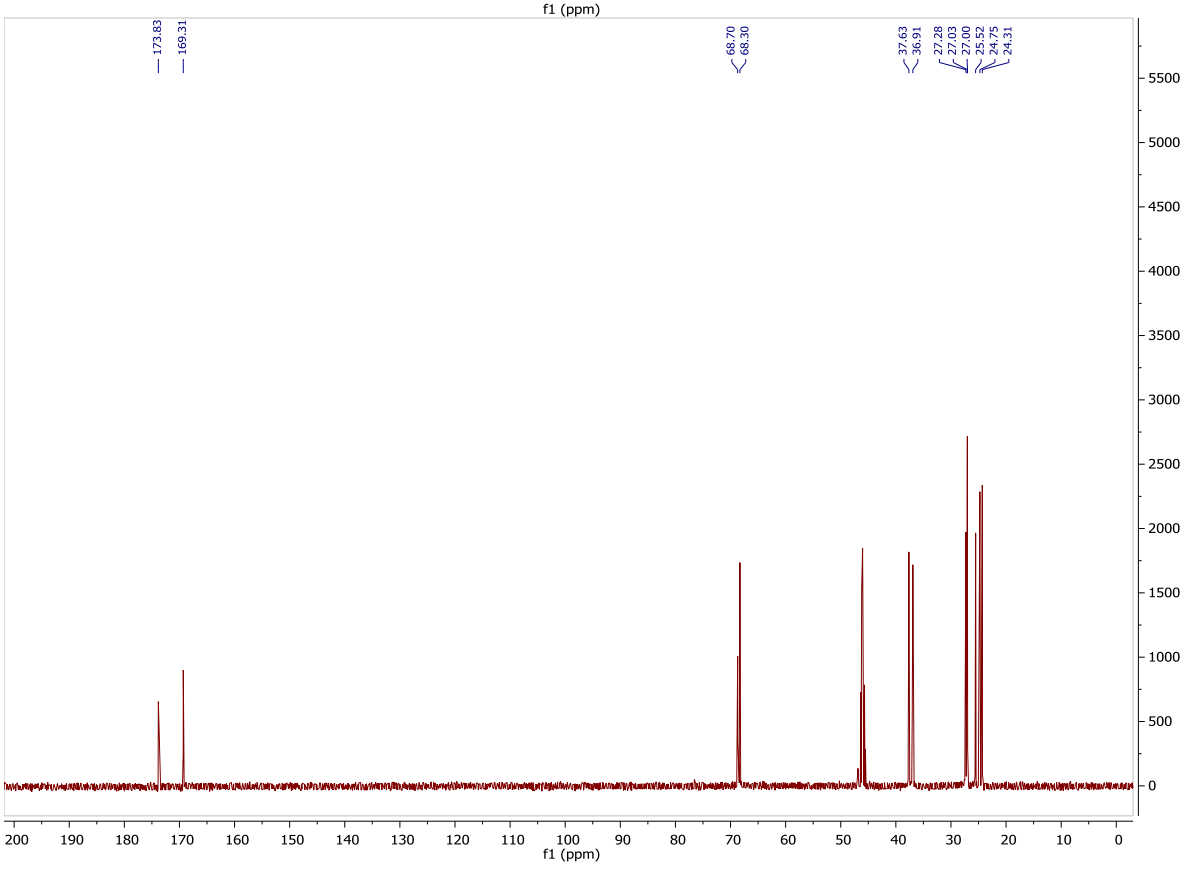
3ab: 5-((8-aminooctyl)amino)-3-methyl-5-oxopentanoic acid:



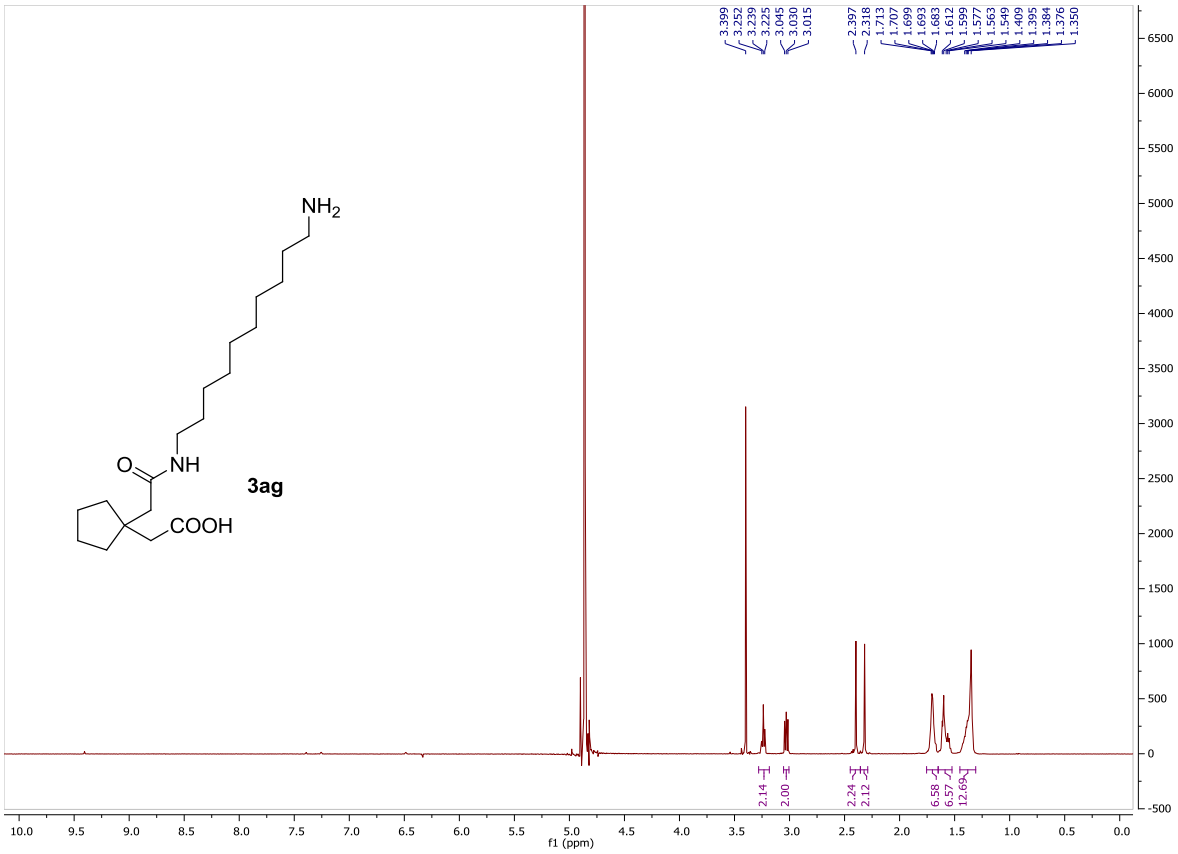


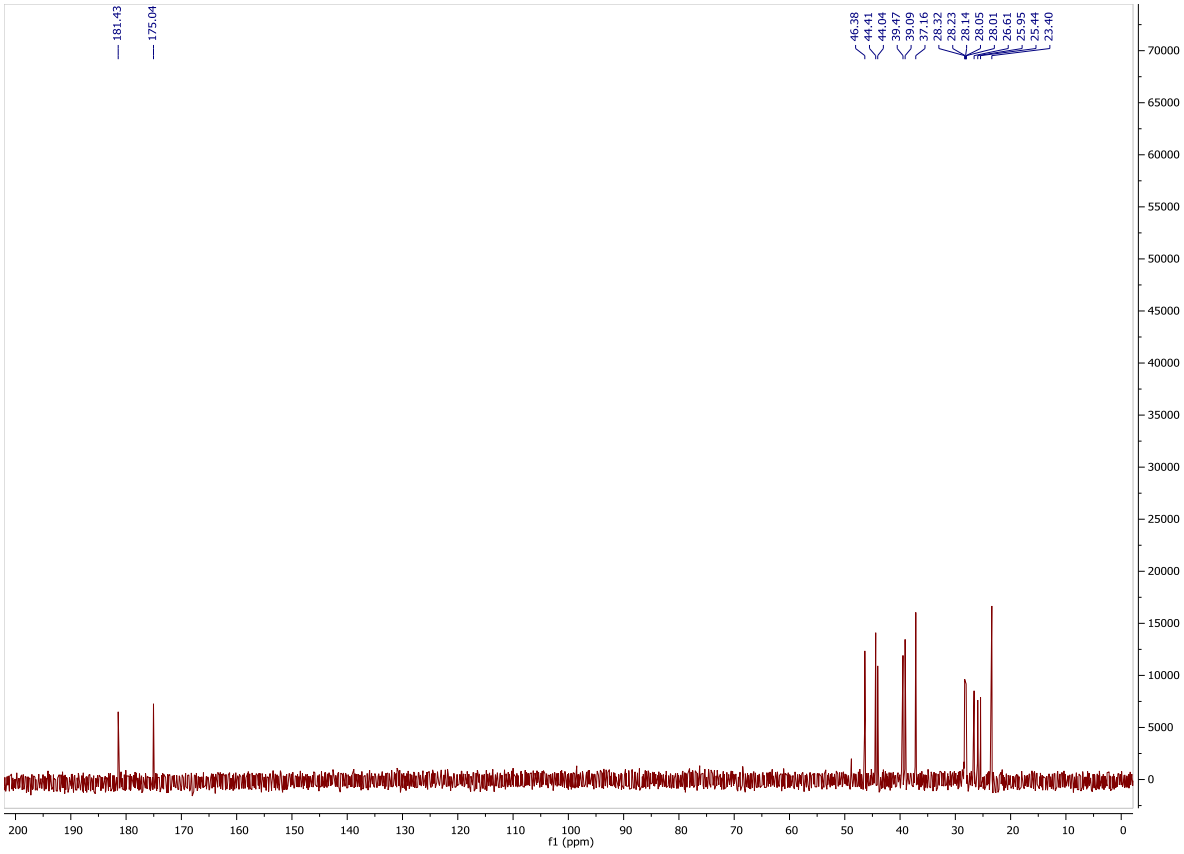
3ad: 2-(2-((8-aminooctyl)amino)-2-oxoethoxy)acetic acid:



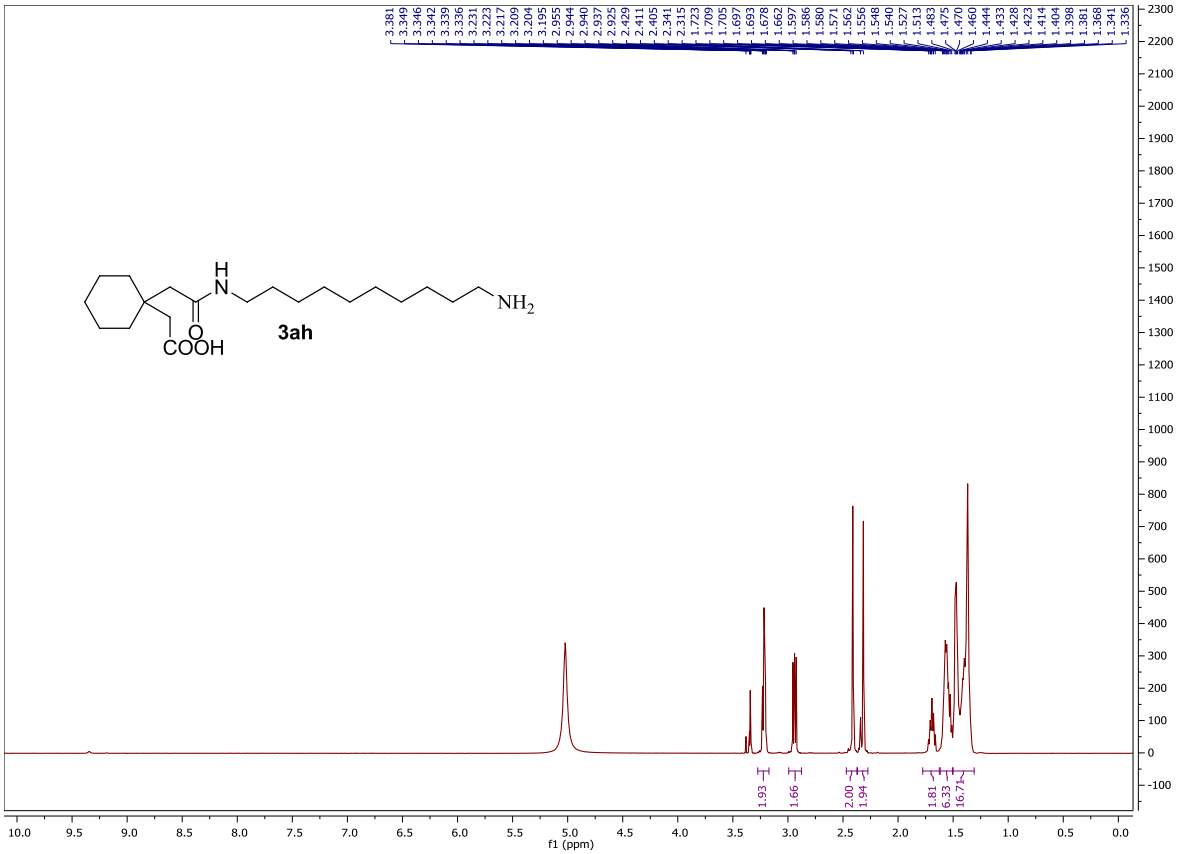


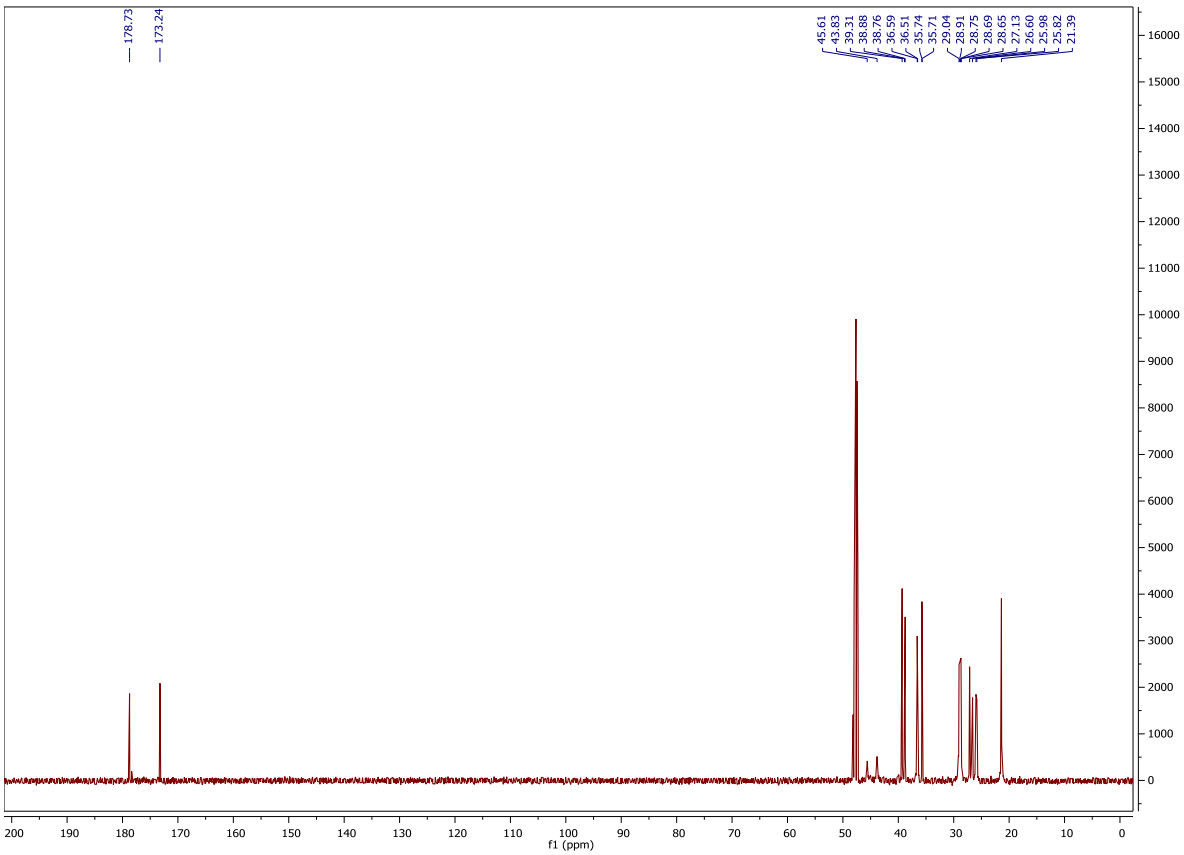
3ag: 2-(1-(2-((10-aminodecyl)amino)-2-oxoethyl)cyclopentyl)acetic acid:



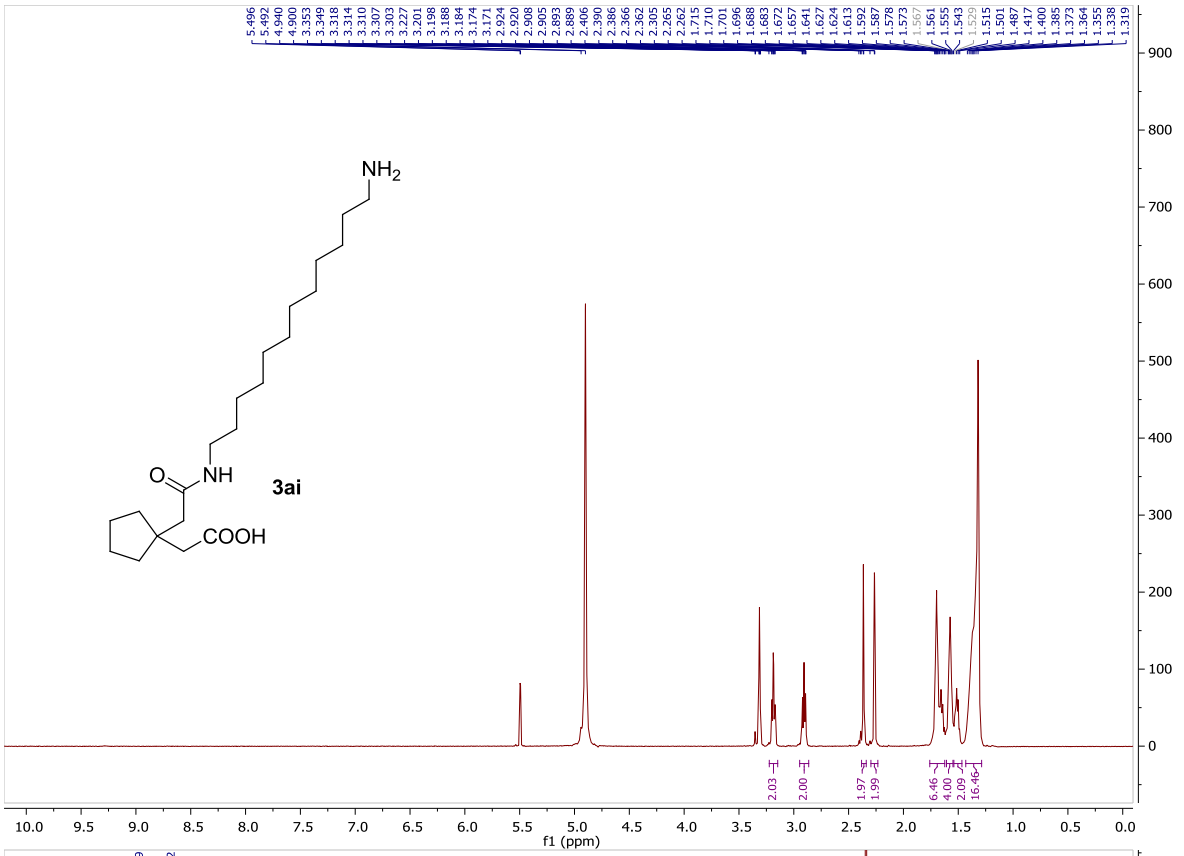


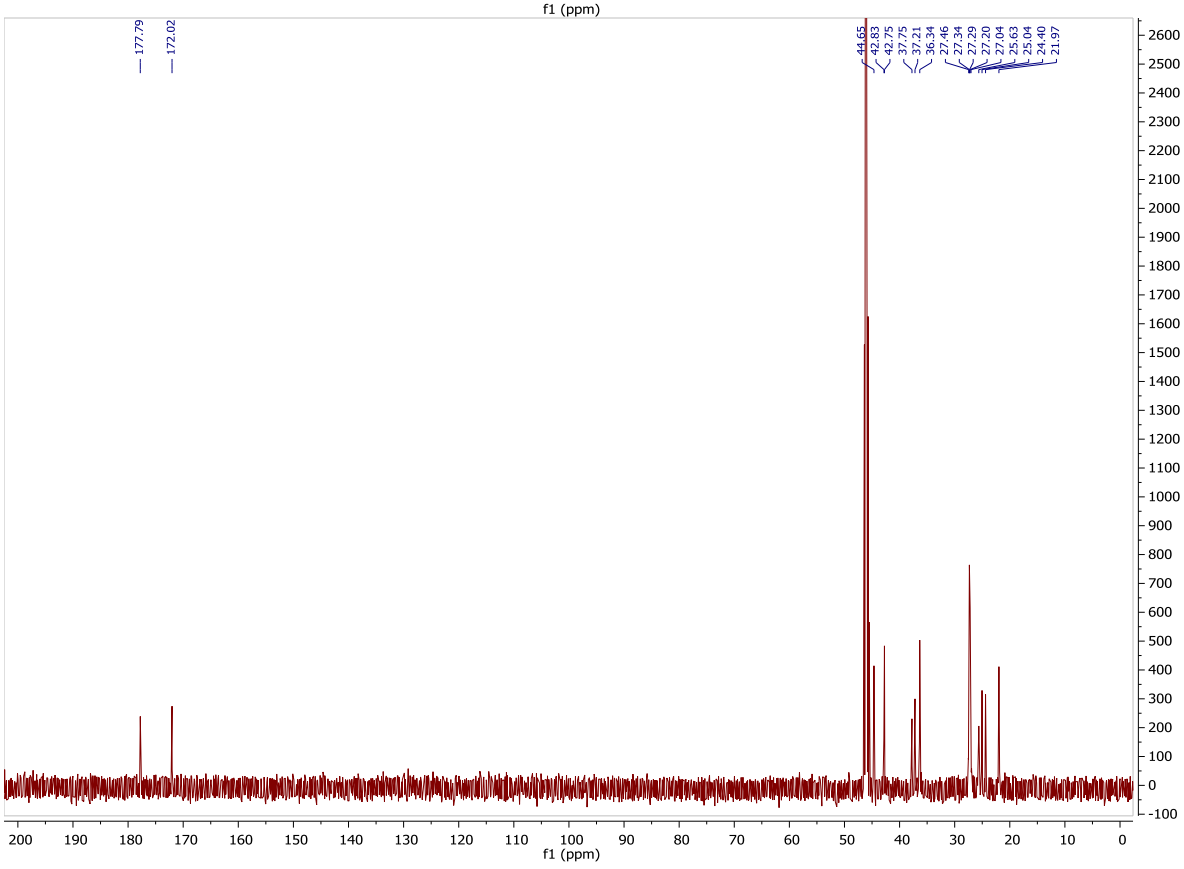
3ah: 2-(1-(2-((10-aminodecyl)amino)-2-oxoethyl)cyclohexyl)acetic acid:



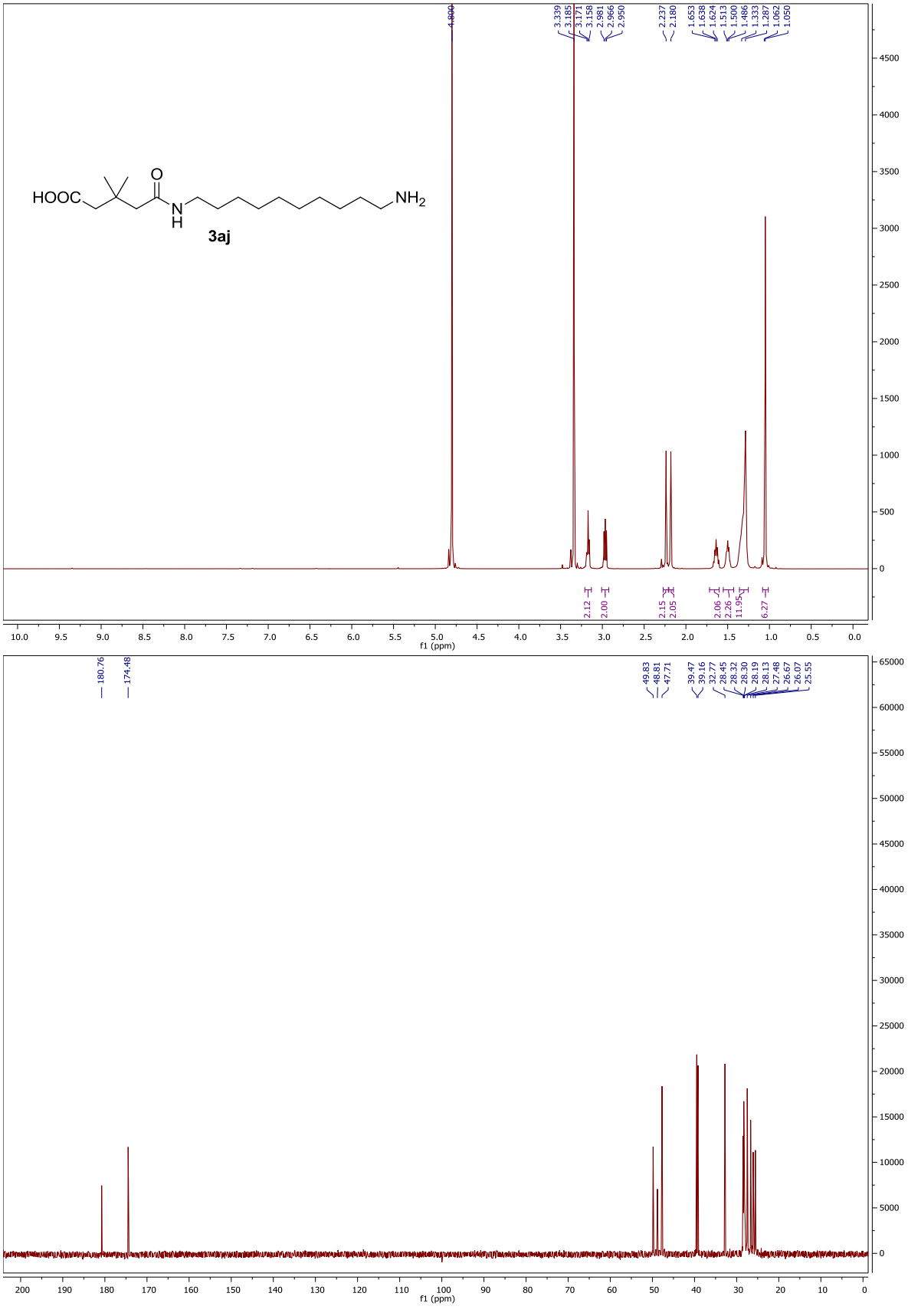


3ai: 2-(1-(2-((12-aminododecyl)amino)-2-oxoethyl)cyclopentyl)acetic acid:

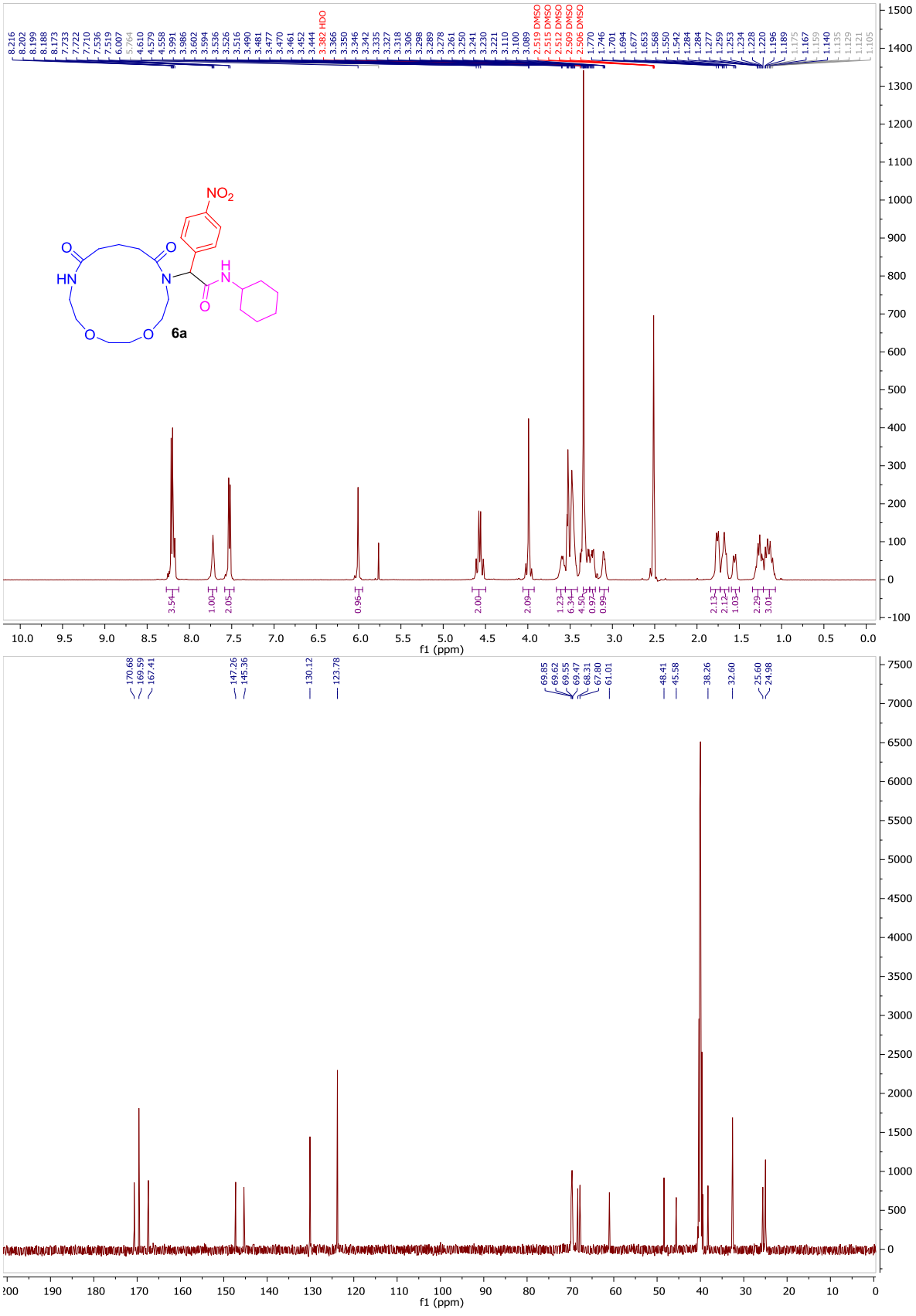




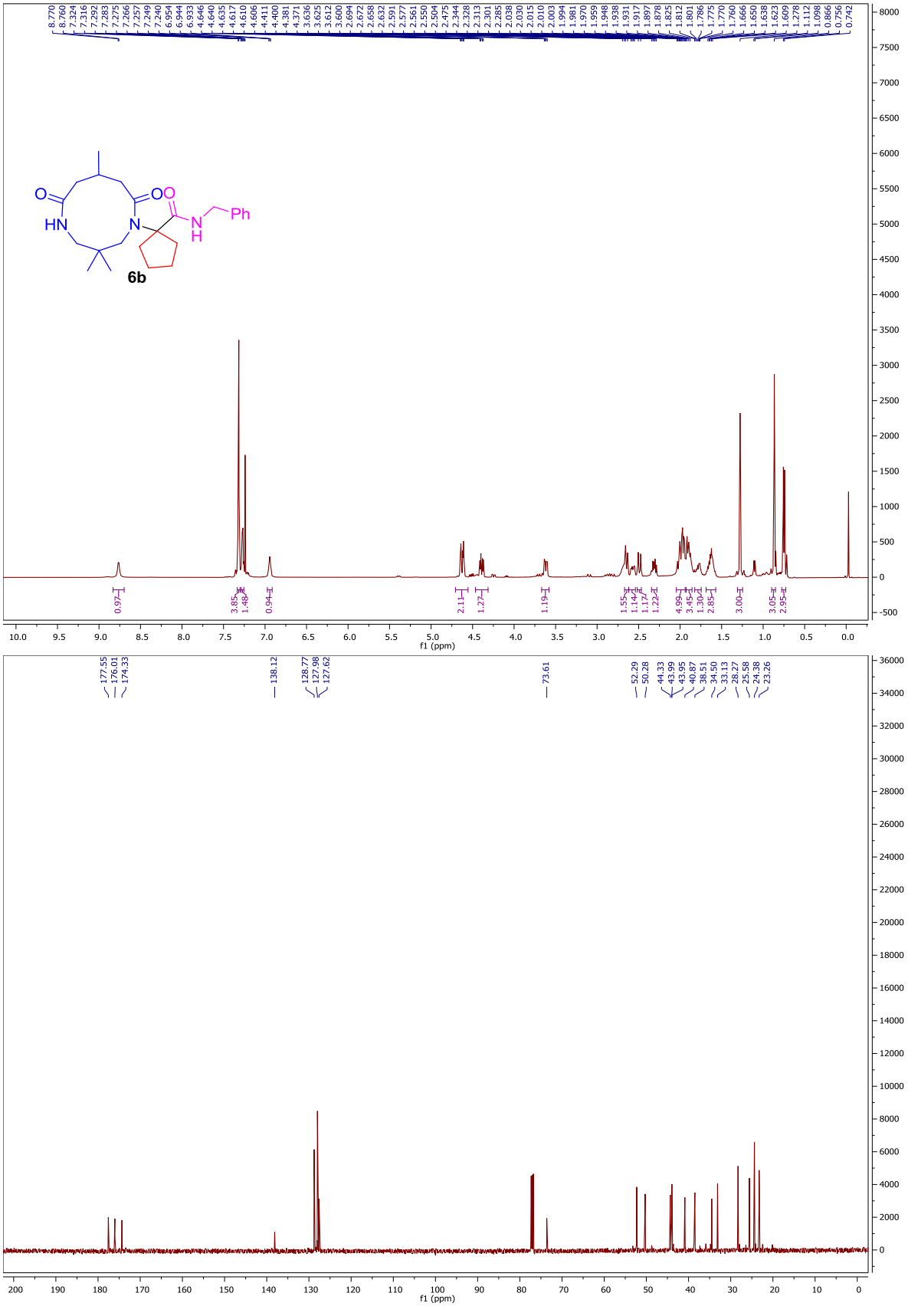
3aj: 5-((10-aminodecyl)amino)-3,3-dimethyl-5-oxopentanoic acid:



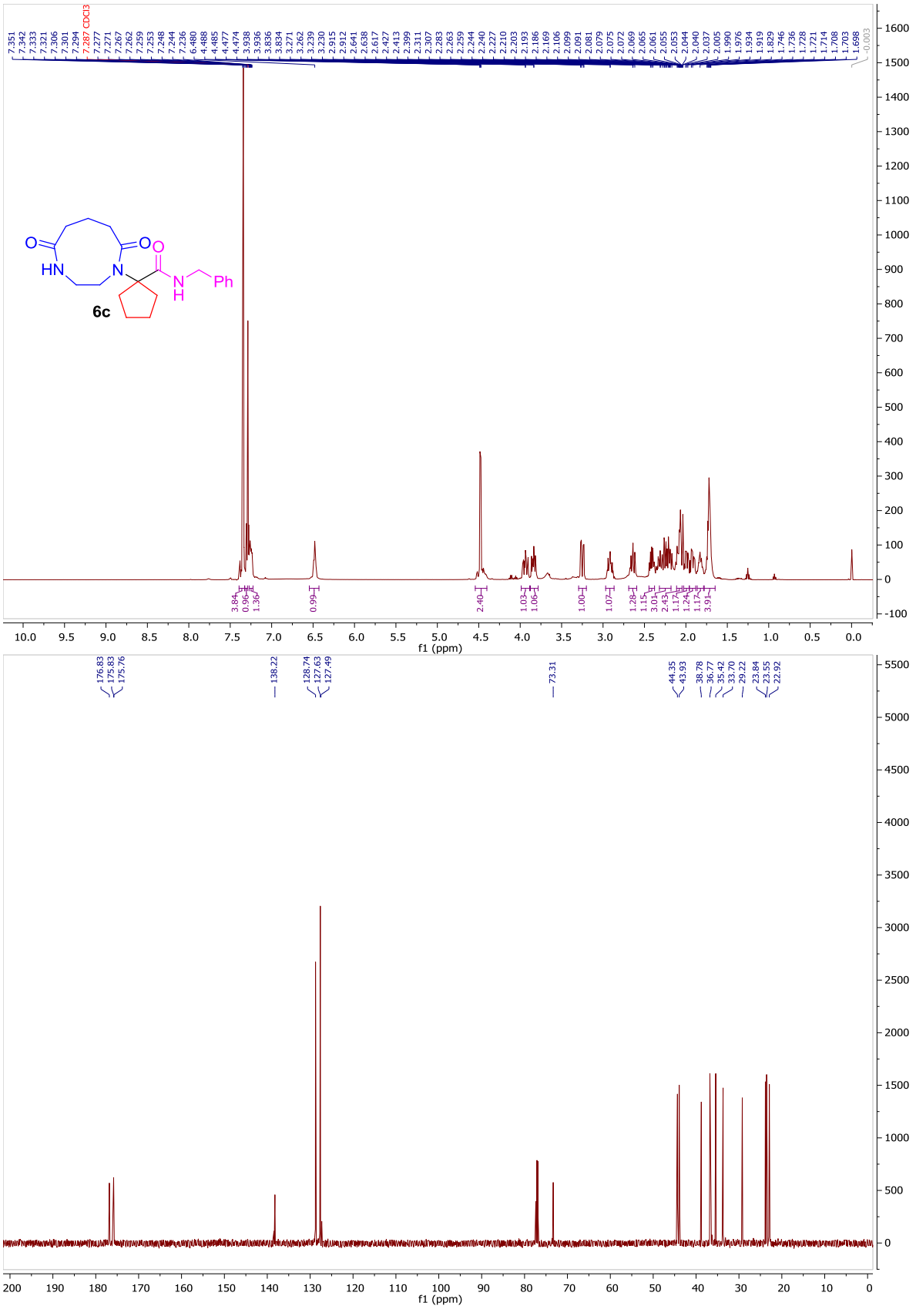
6a: *N*-cyclohexyl-2-(8,12-dioxo-1,4-dioxa-7,13-diazacyclopentadecan-7-yl)-2-(4-nitrophenyl)acetamide:



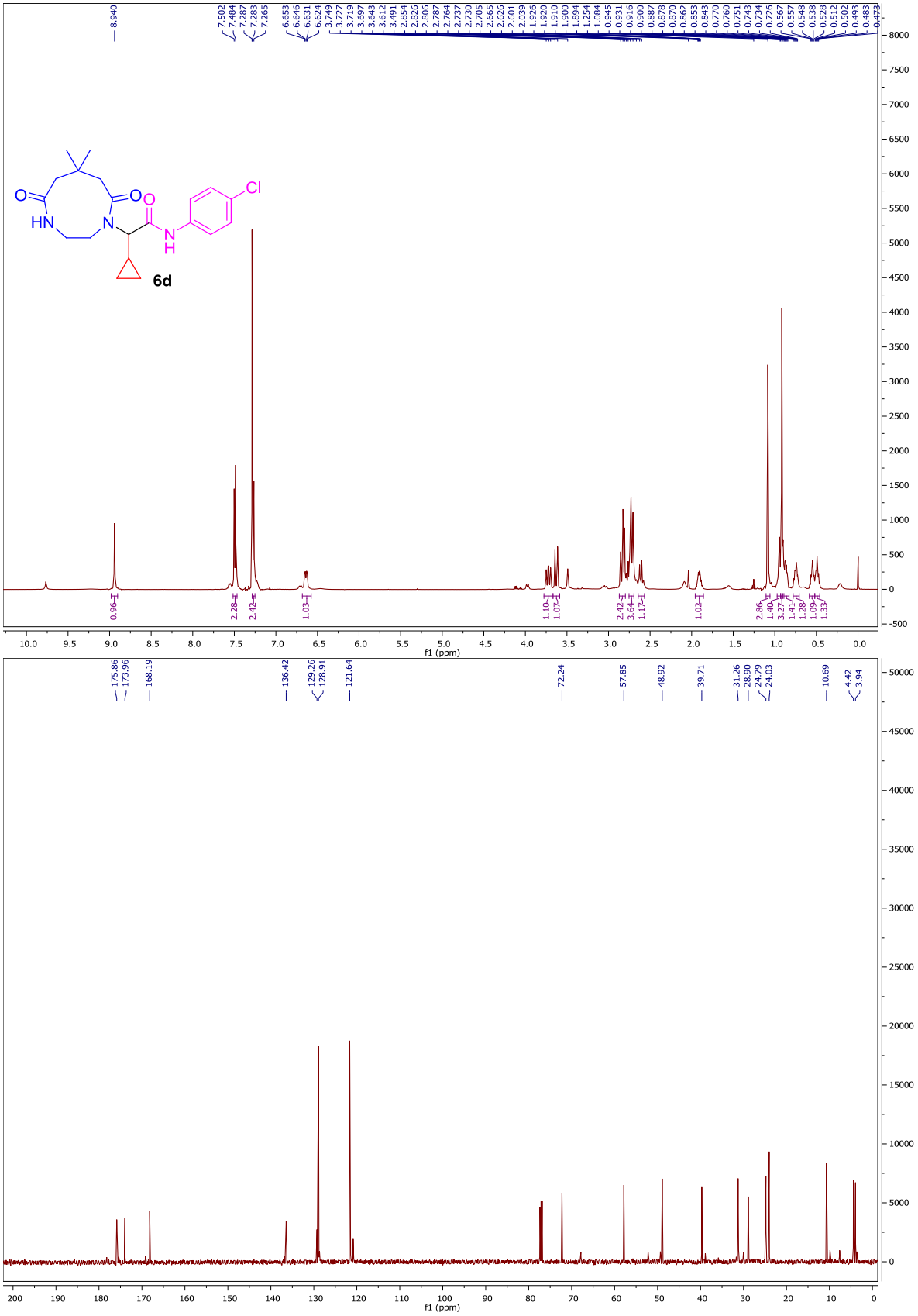
6b: N-benzyl-1-(3,3,8-trimethyl-6,10-dioxo-1,5-diazecan-1-yl)cyclopentanecarboxamide:



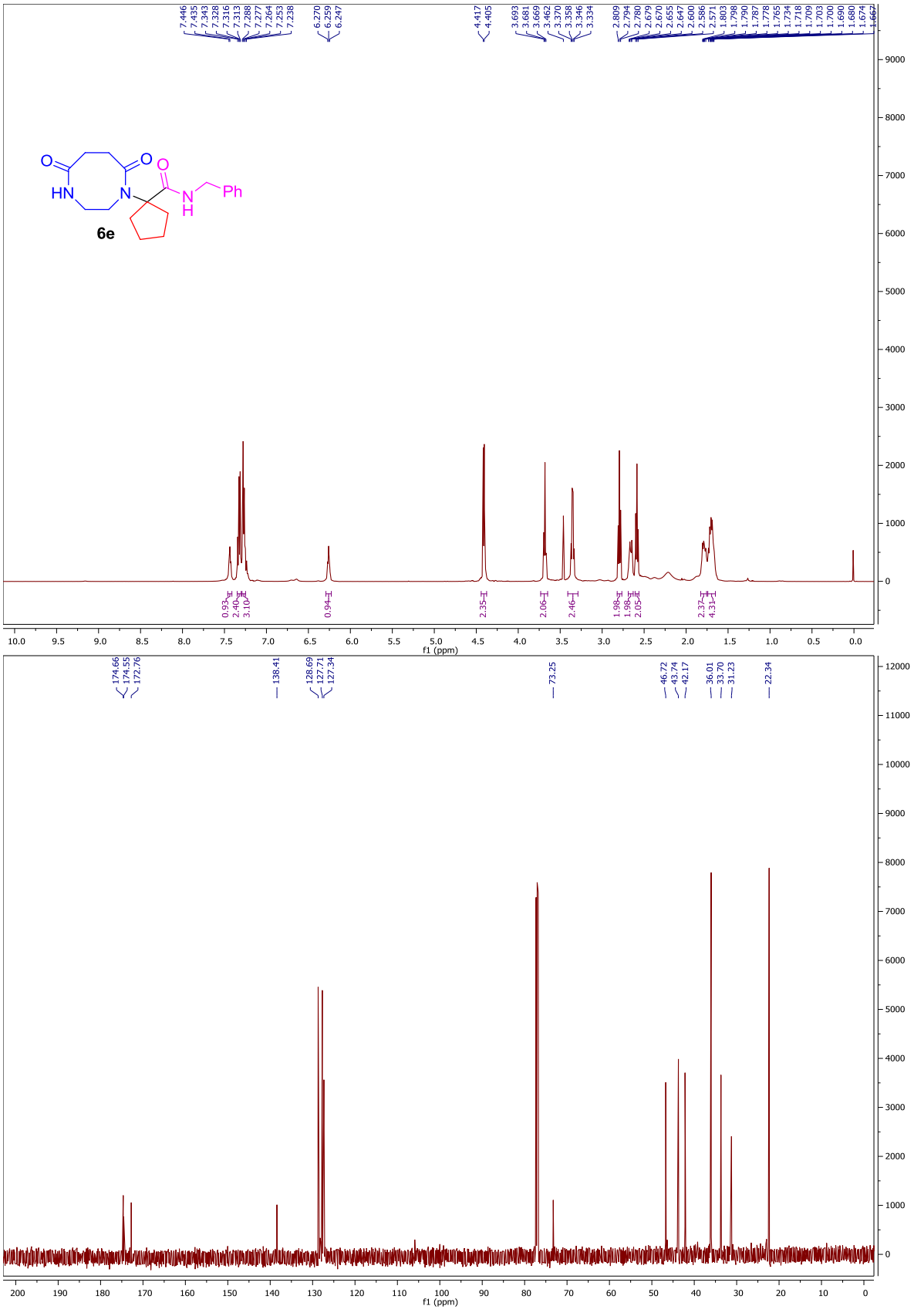
6c: *N*-benzyl-1-(5,9-dioxo-1,4-diazonan-1-yl)cyclopentanecarboxamide:



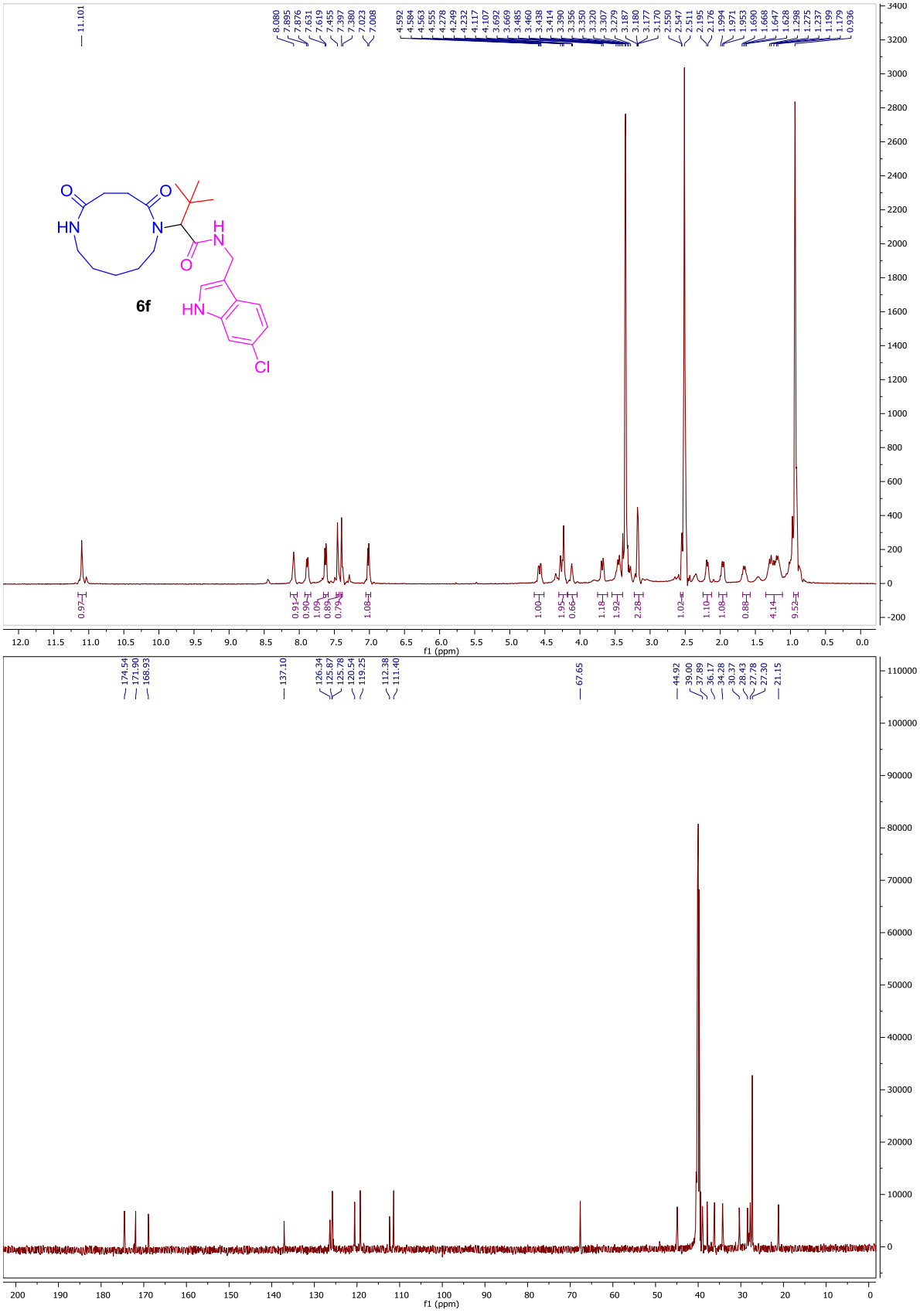
6d: *N*-(4-chlorophenyl)-2-cyclopropyl-2-(7,7-dimethyl-5,9-dioxo-1,4-diazonan-1-yl)acetamide:



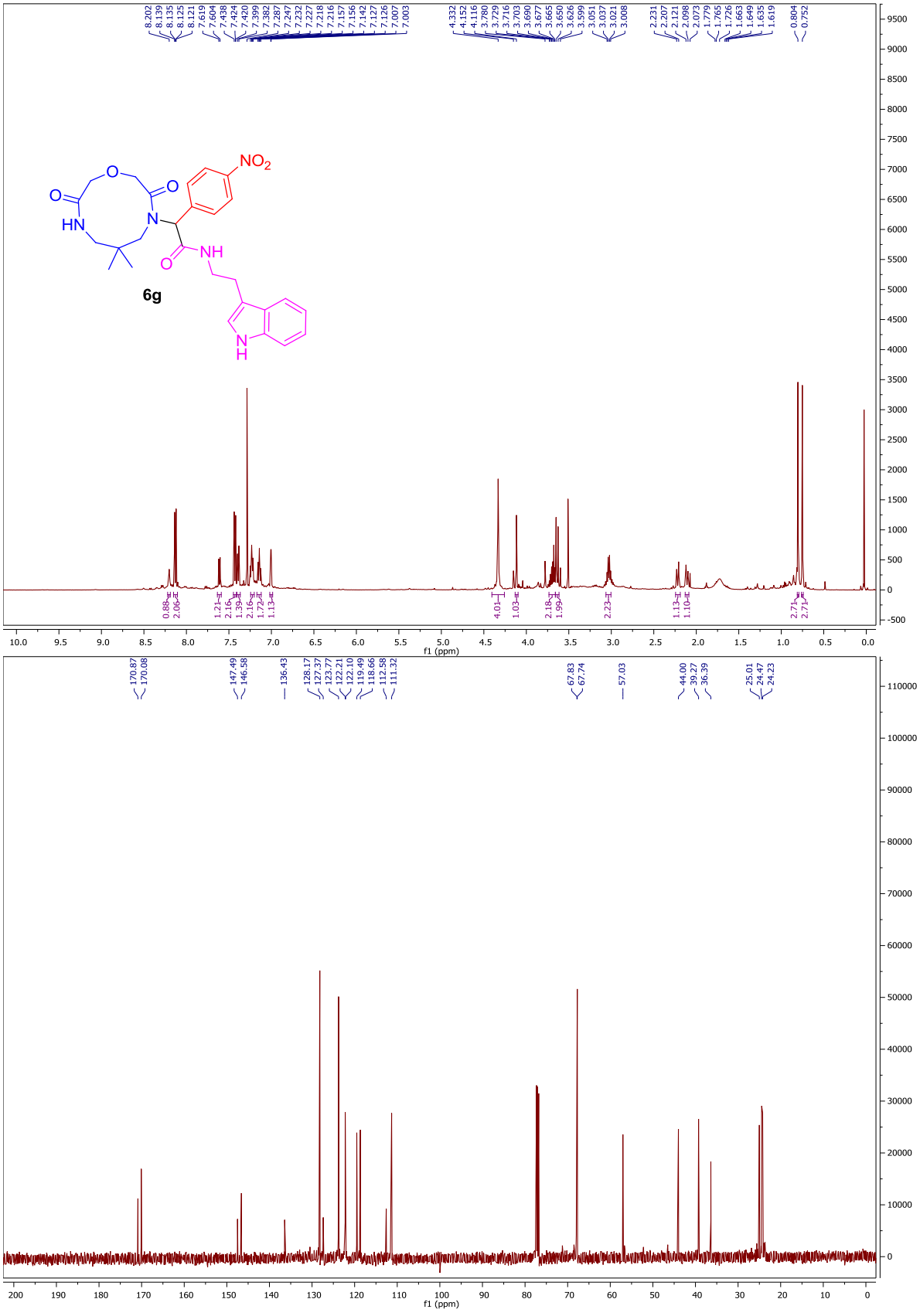
6e: *N*-benzyl-1-(5,8-dioxo-1,4-diazocan-1-yl)cyclopentanecarboxamide:



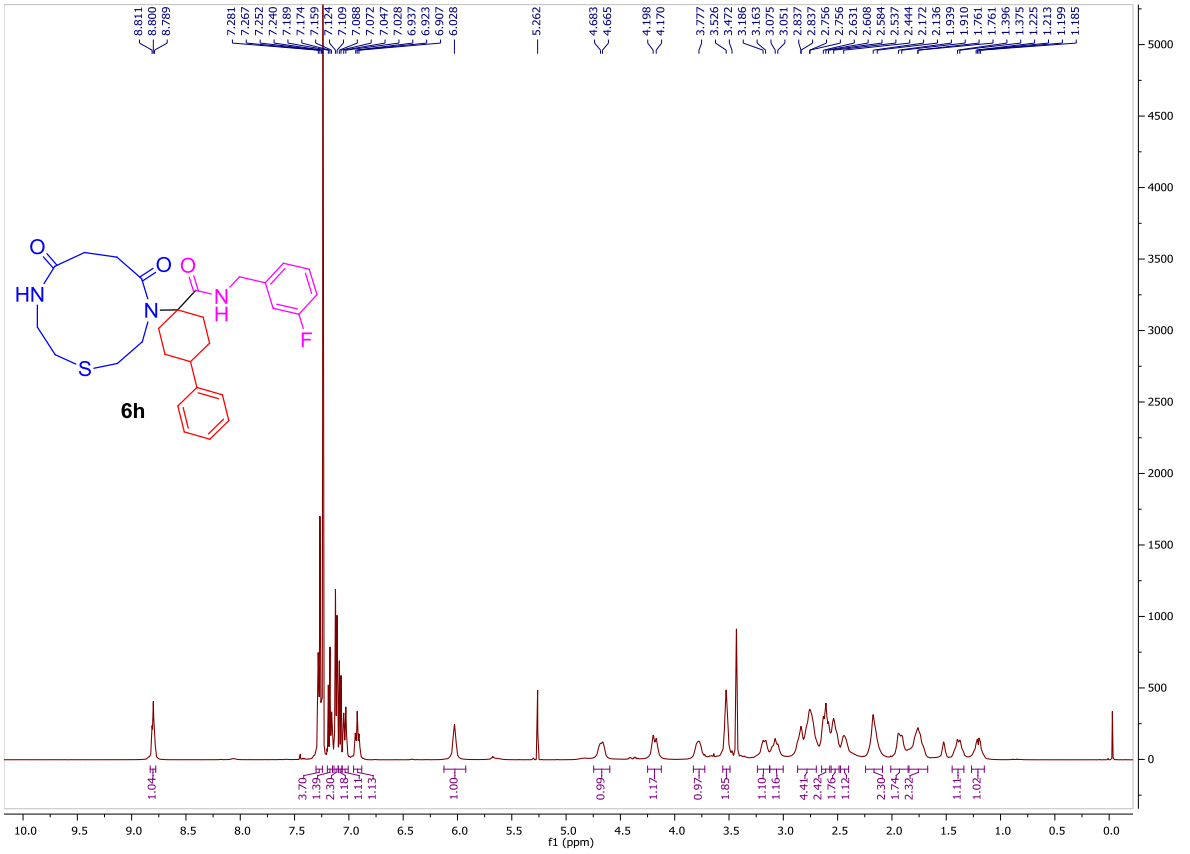
6f: *N*-((6-chloro-1H-indol-3-yl)methyl)-2-(2,5-dioxo-1,6-diazacycloundecan-1-yl)-3,3-dimethylbutanamide:

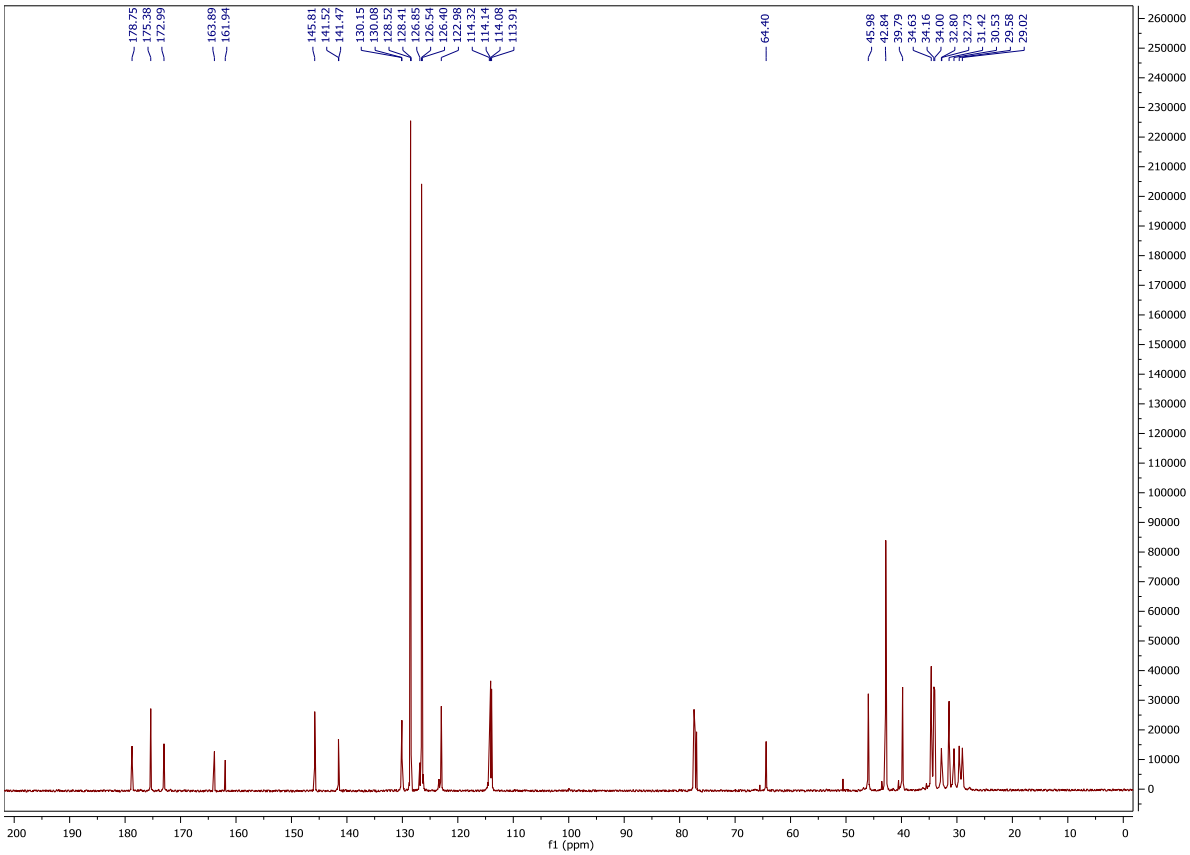


6g: *N*-(2-(1H-indol-3-yl)ethyl)-2-(6,6-dimethyl-3,9-dioxo-1,4,8-oxadiazecan-4-yl)-2-(4-nitrophenyl)acetamide:

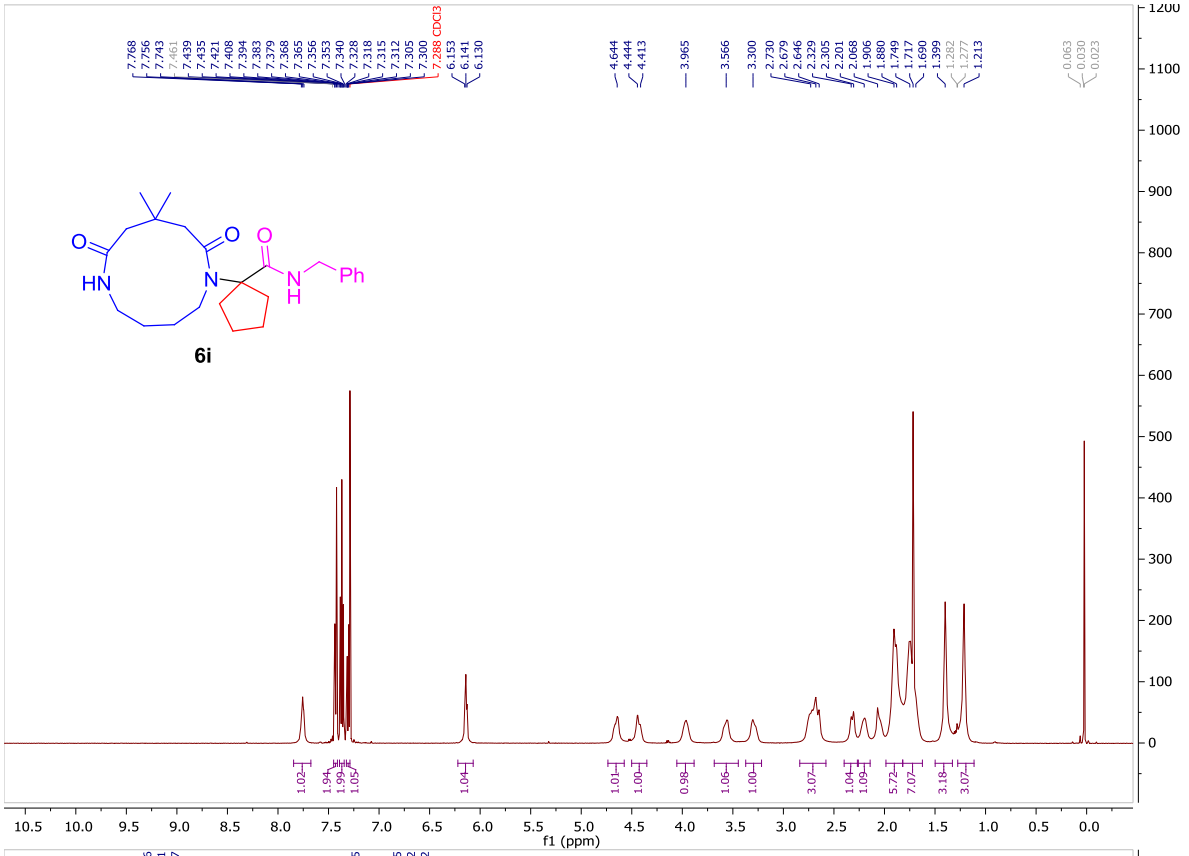


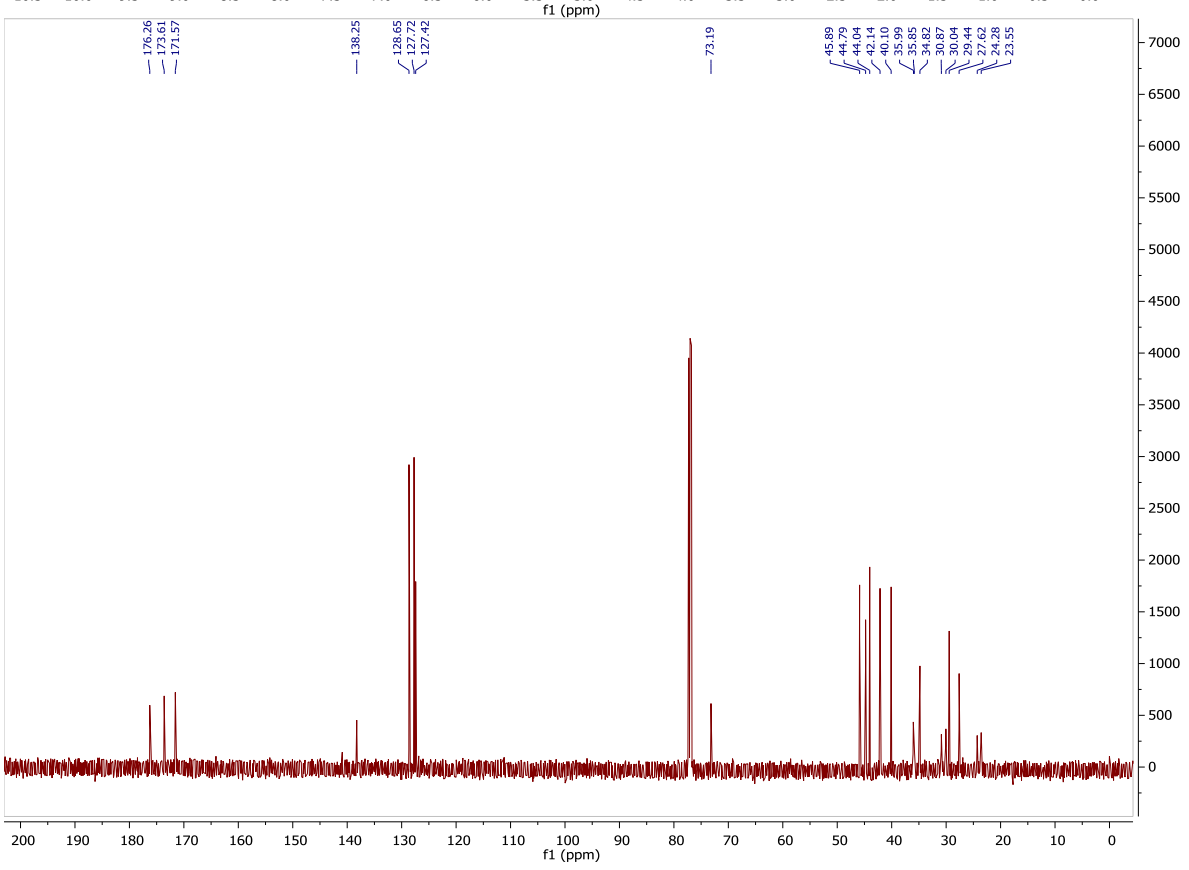
6h: 1-(5,8-dioxo-1-thia-4,9-diazacycloundecan-4-yl)-N-(3-fluorobenzyl)-4-phenylcyclohexanecarboxamide:



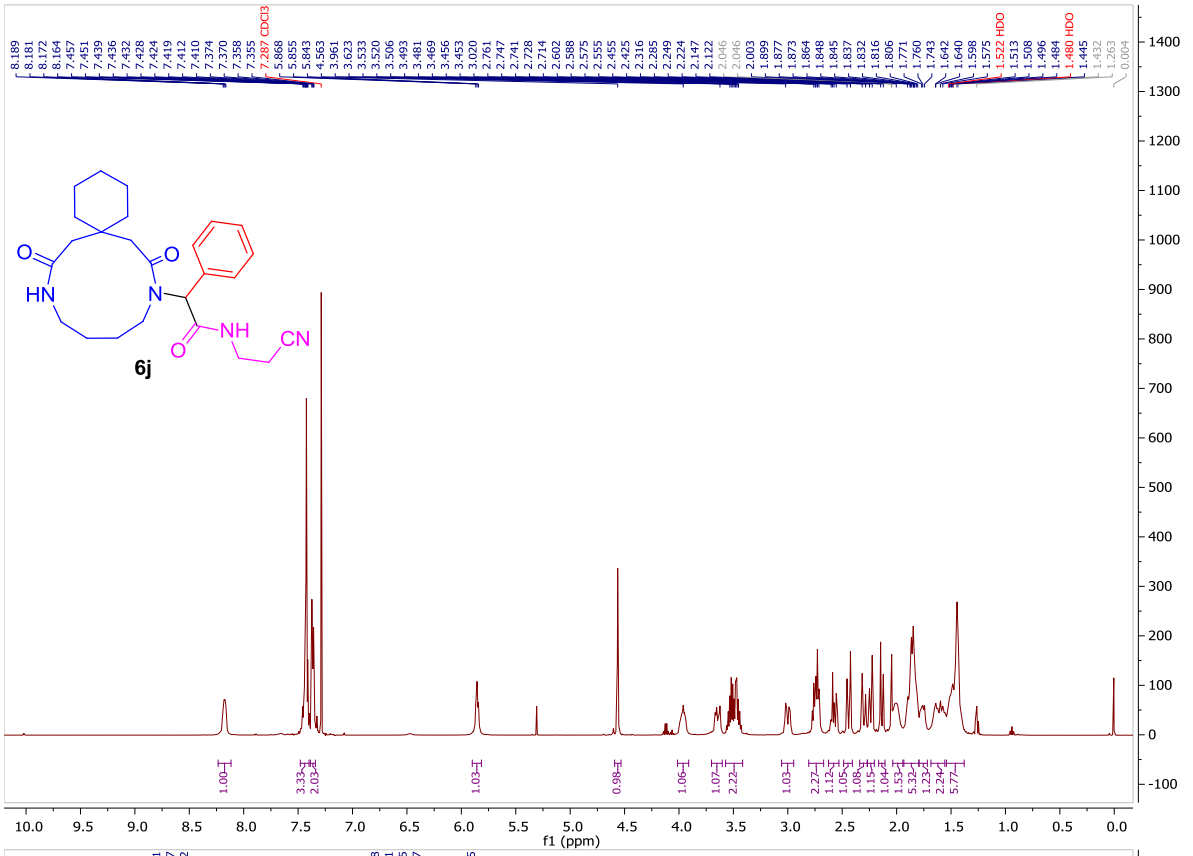


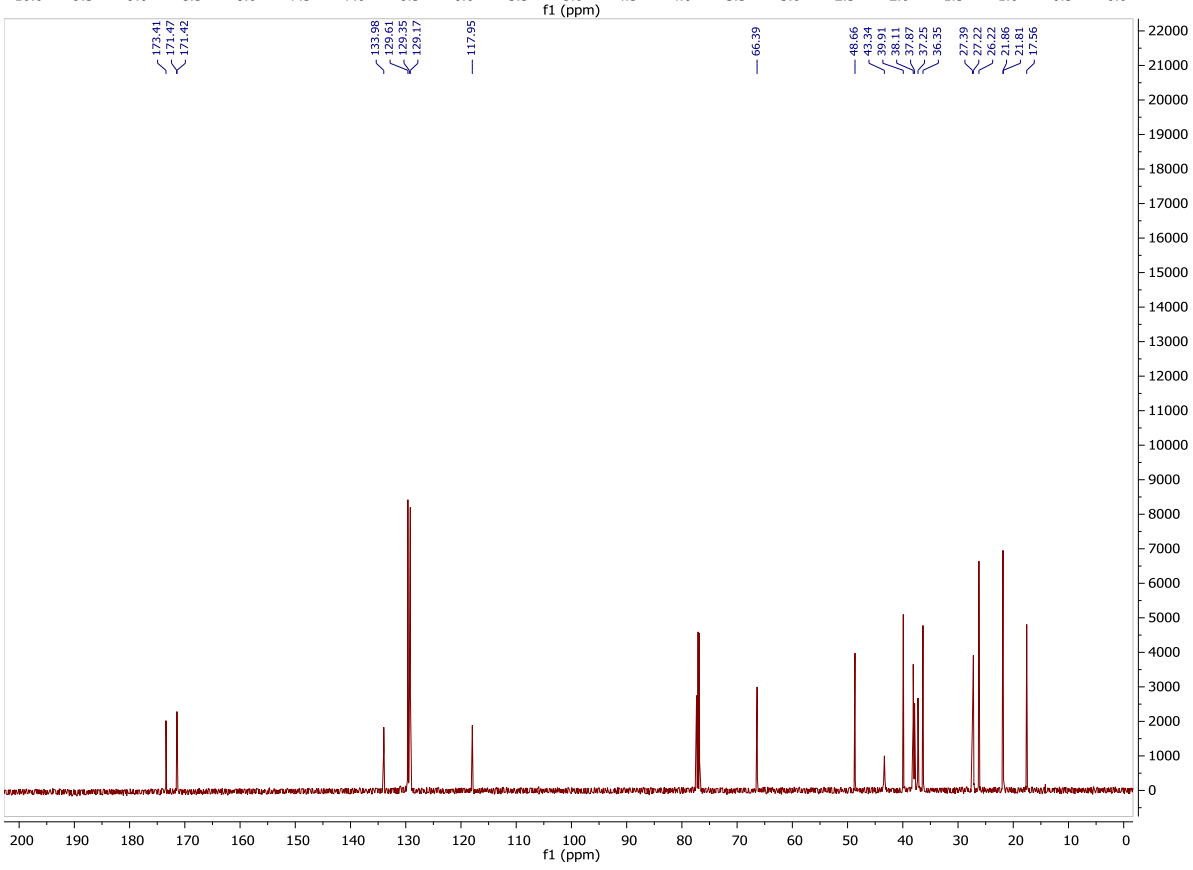
6i: N-benzyl-1-(9,9-dimethyl-7,11-dioxo-1,6-diazacycloundecan-1-yl)cyclopentanecarboxamide:



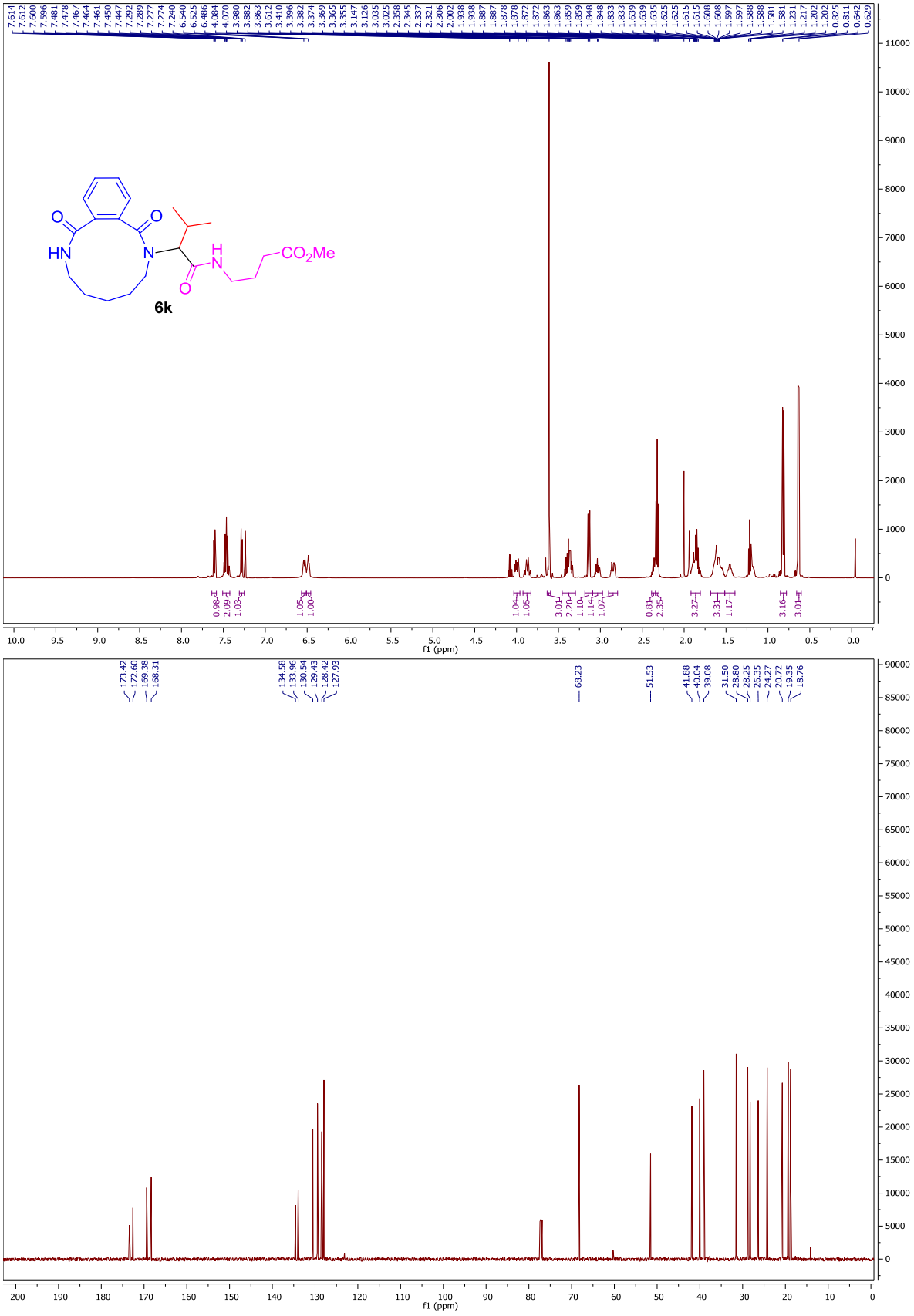


6j: N-(2-cyanoethyl)-2-(8,15-dioxo-9,14-diazaspiro[5.10]hexadecan-9-yl)-2-phenylacetamide:

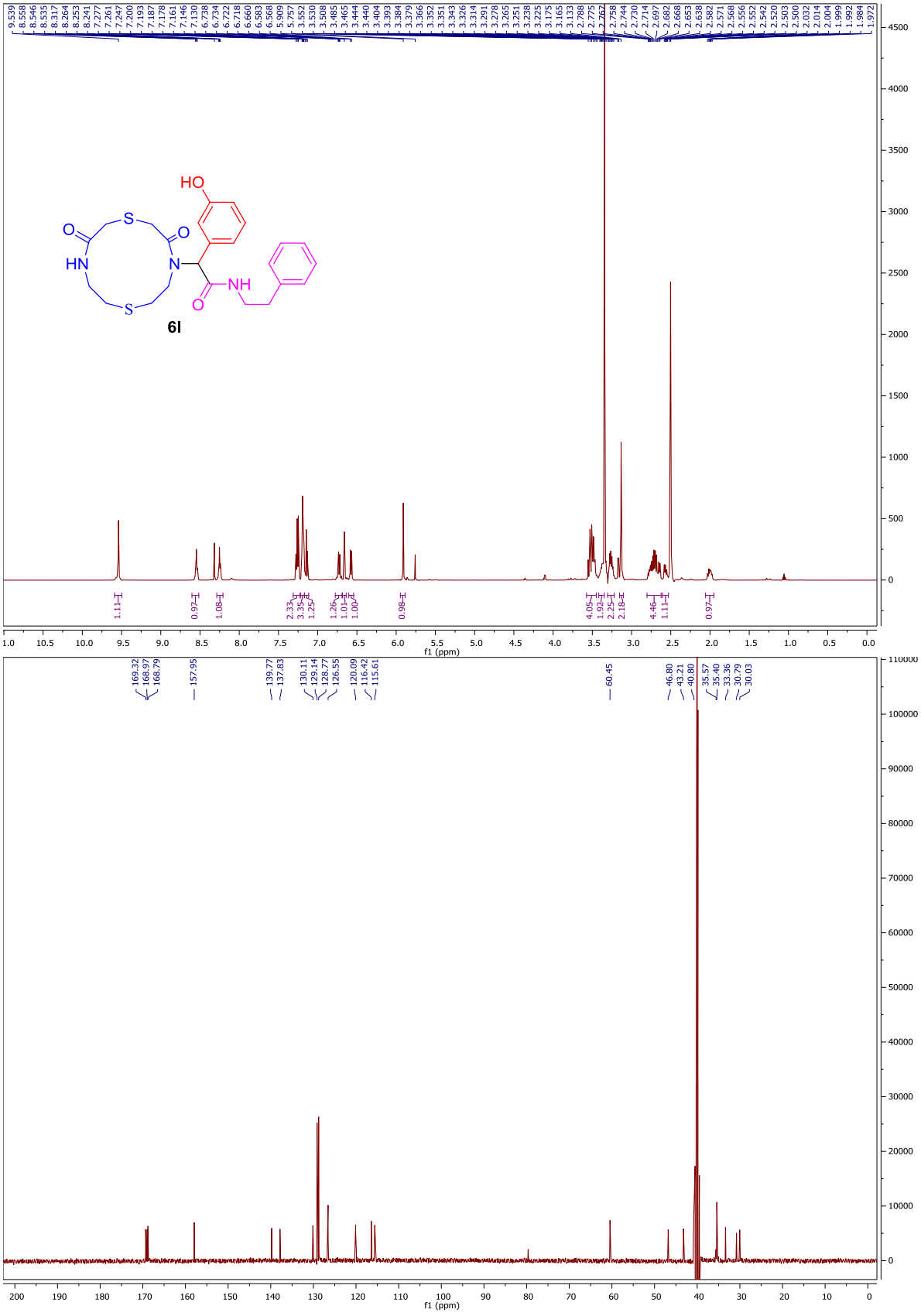




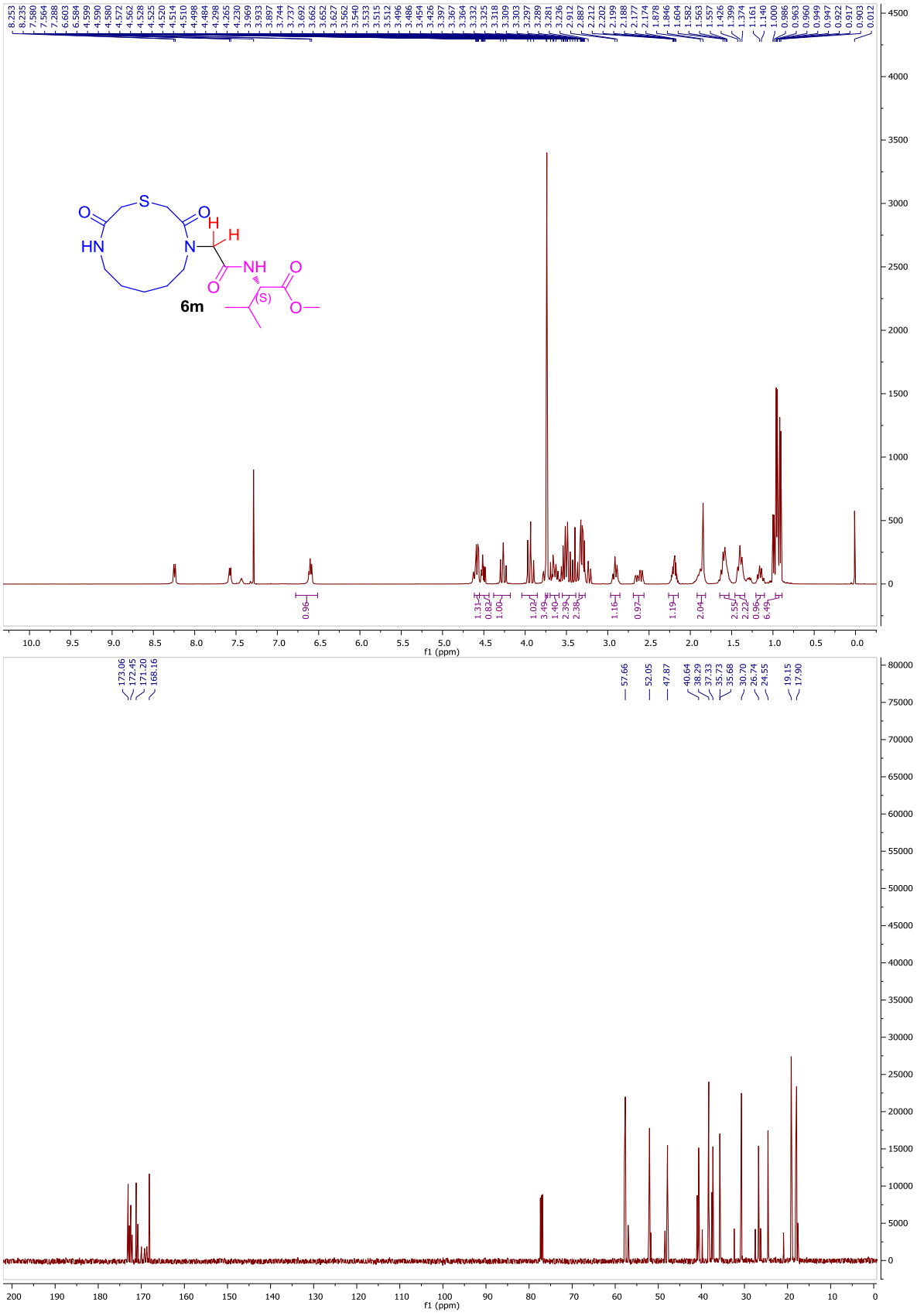
6k: methyl 4-(2-(1,9-dioxo-4,5,6,7,8,9-hexahydro-1H-benzo[c][1,6]diazacycloundecin-2(3H)-yl)-3-methylbutanamido)butanoate:



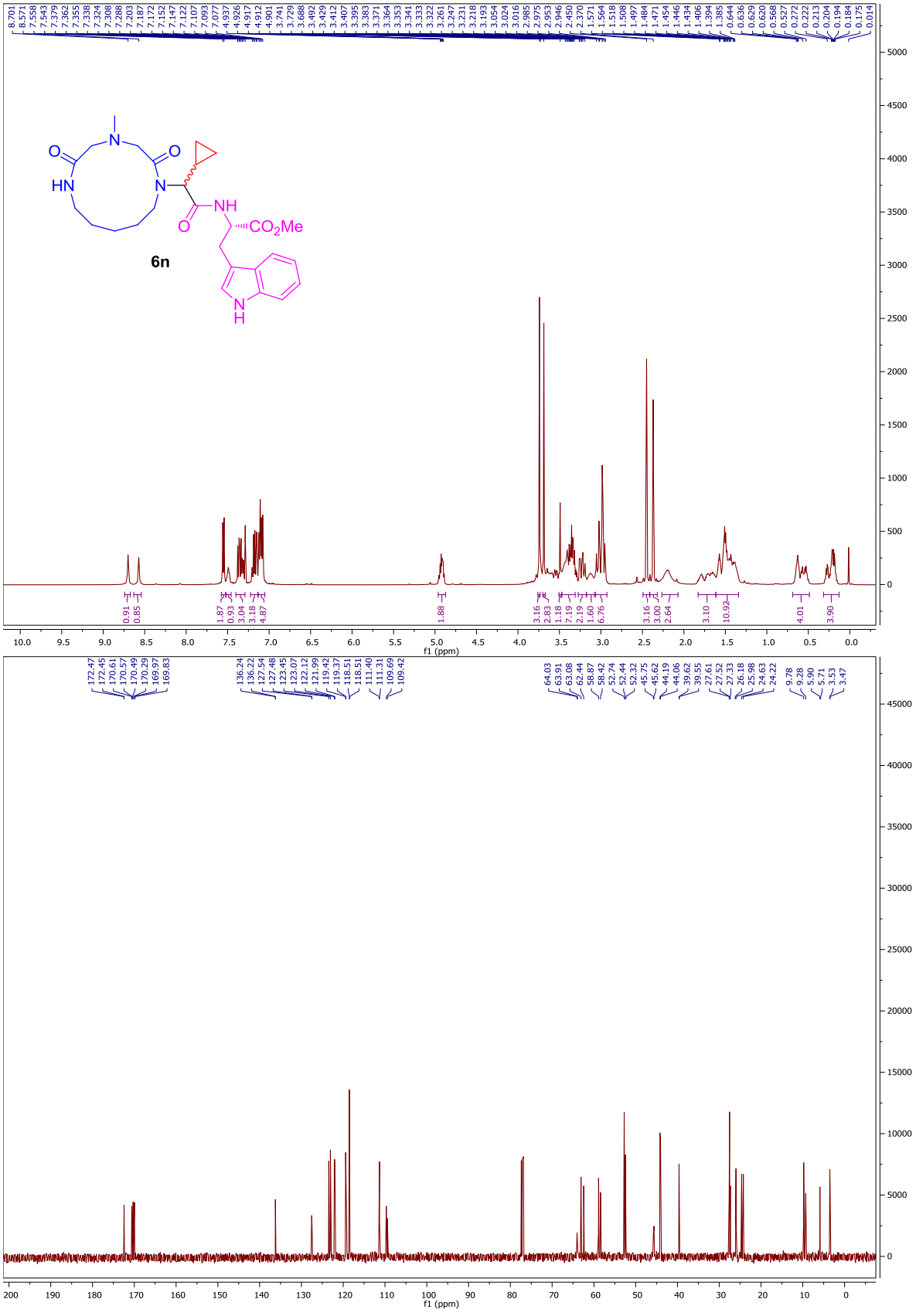
6l: 2-(3,11-dioxo-1,7-dithia-4,10-diazacyclododecan-4-yl)-2-(3-hydroxyphenyl)-N-phenethylacetamide:



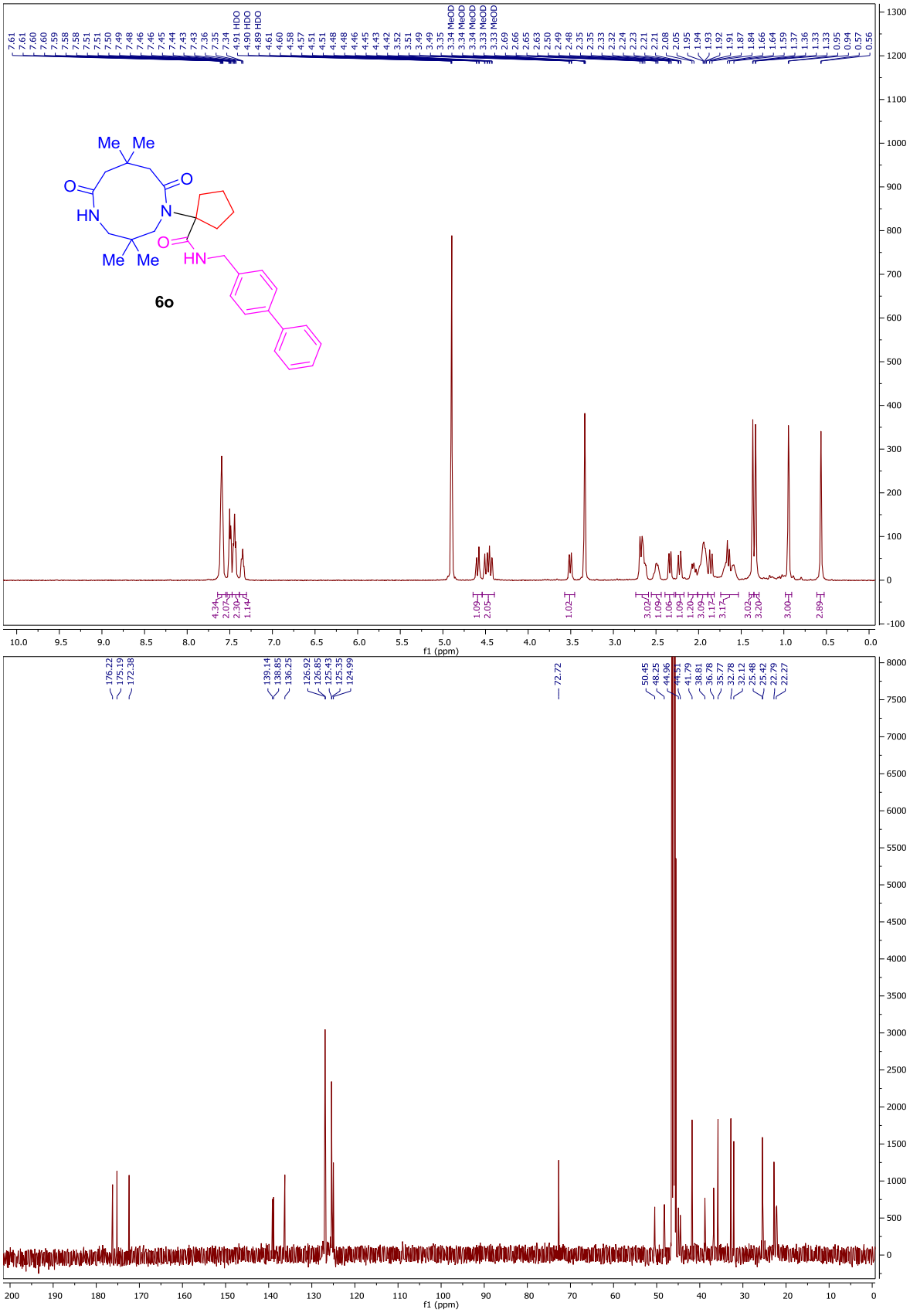
6m: (*S*)-methyl 2-(2-(3,11-dioxo-1-thia-4,10-diazacyclododecan-4-yl)acetamido)-3-methylbutanoate:



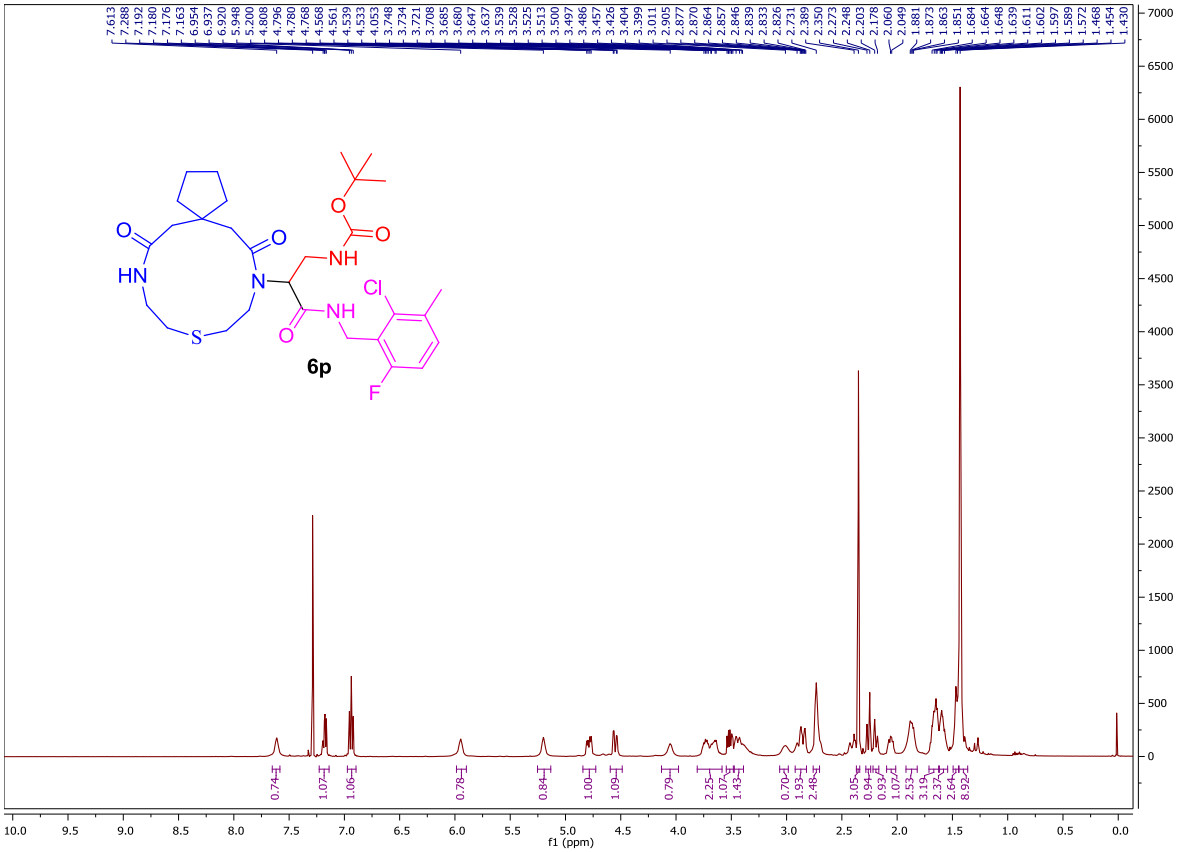
6n: (2S)-methyl 2-(2-cyclopropyl-2-(4-methyl-2,6-dioxo-1,4,7-triazacyclododecan-1-yl)acetamido)-3-(1H-indol-3-yl)propanoate:

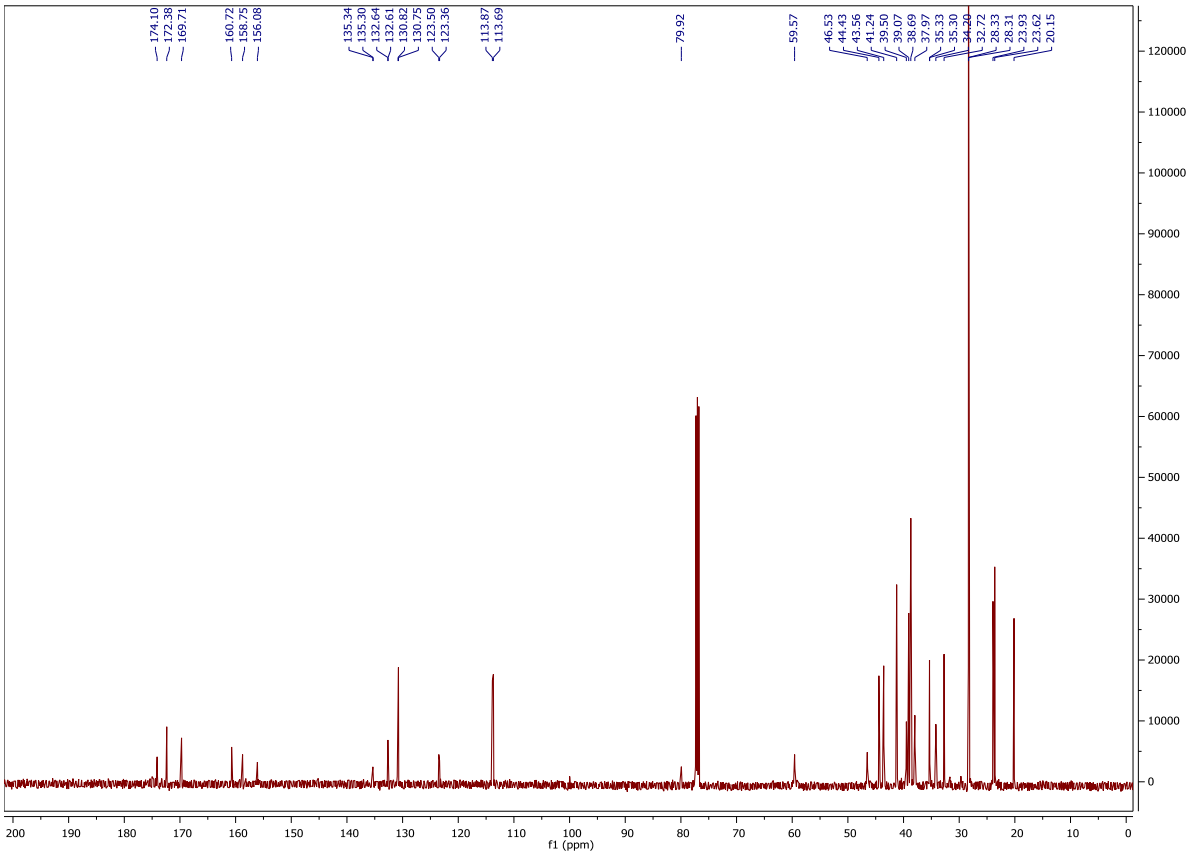


6o: N-([1,1'-biphenyl]-4-ylmethyl)-1-(3,3,8,8-tetramethyl-6,10-dioxo-1,5-diazecan-1-yl)cyclopentanecarboxamide:

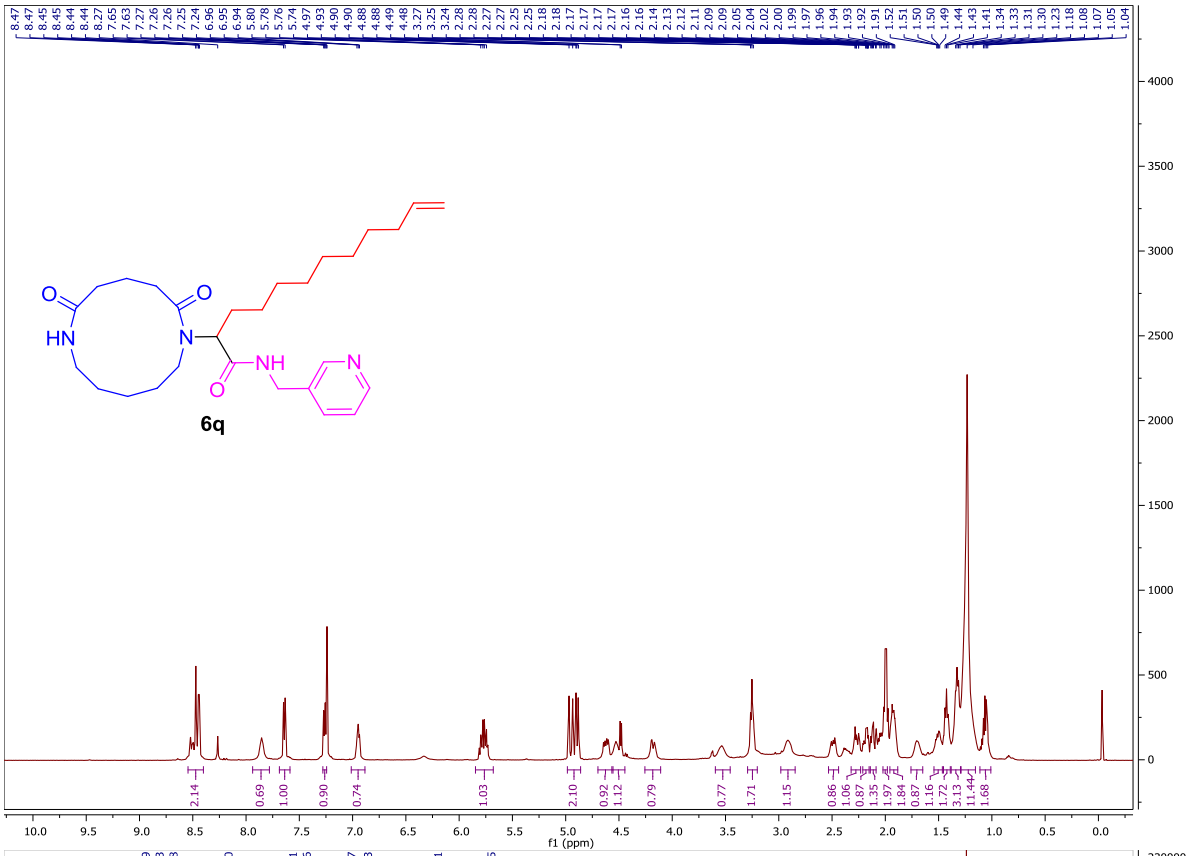


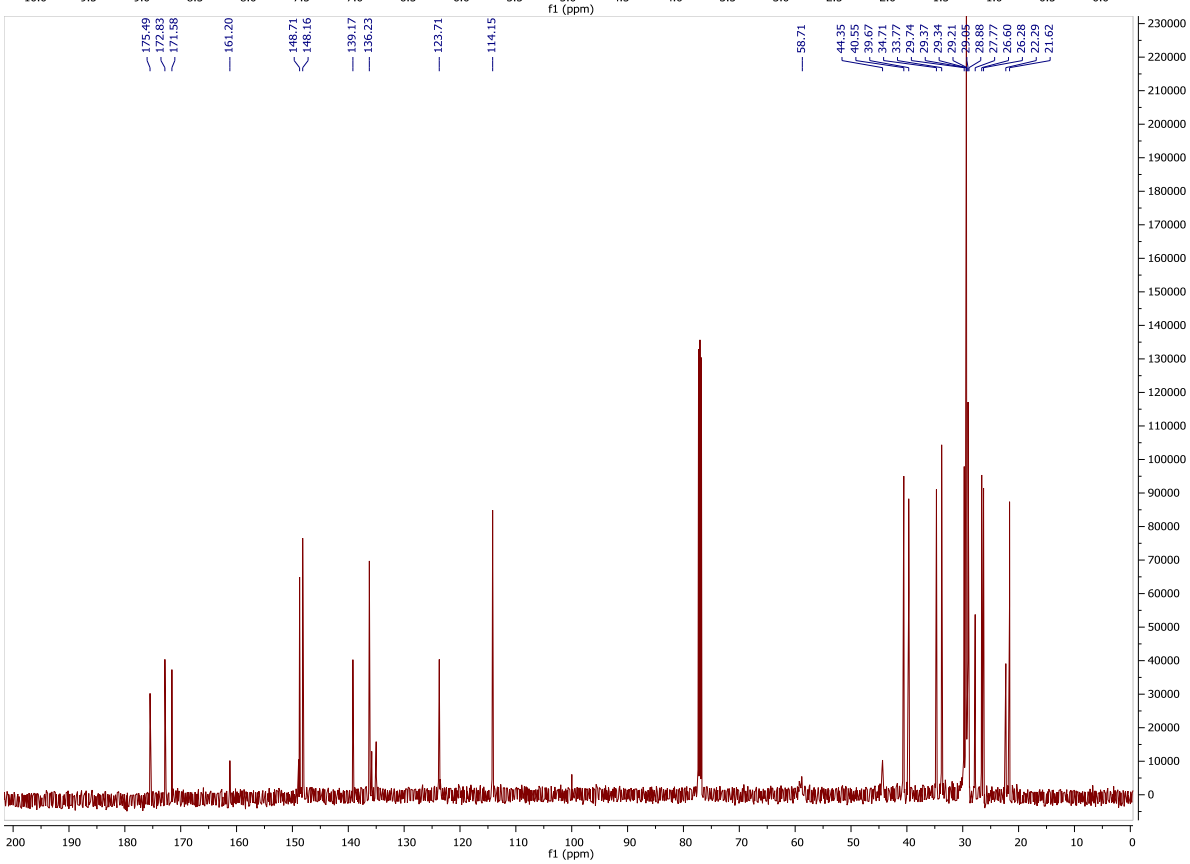
6p: tert-butyl (3-((2-chloro-6-fluoro-3-methylbenzyl)amino)-2-(7,15-dioxo-11-thia-8,14-diazaspiro[4.11]hexadecan-8-yl)-3-oxopropyl)carbamate:



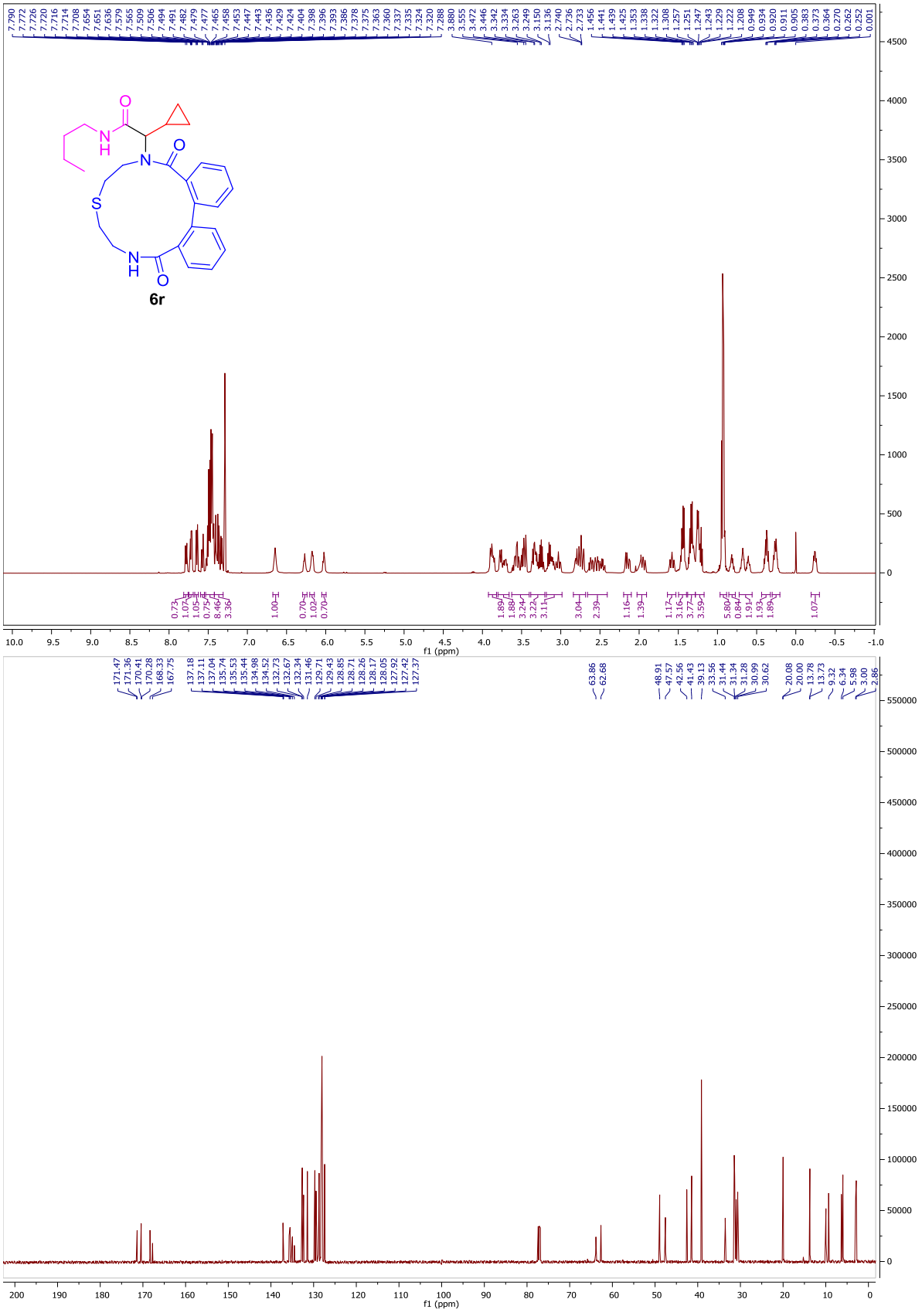


6q: 2-(2,6-dioxo-1,7-diazacyclododecan-1-yl)-N-(pyridin-3-ylmethyl)dodec-11-enamide:

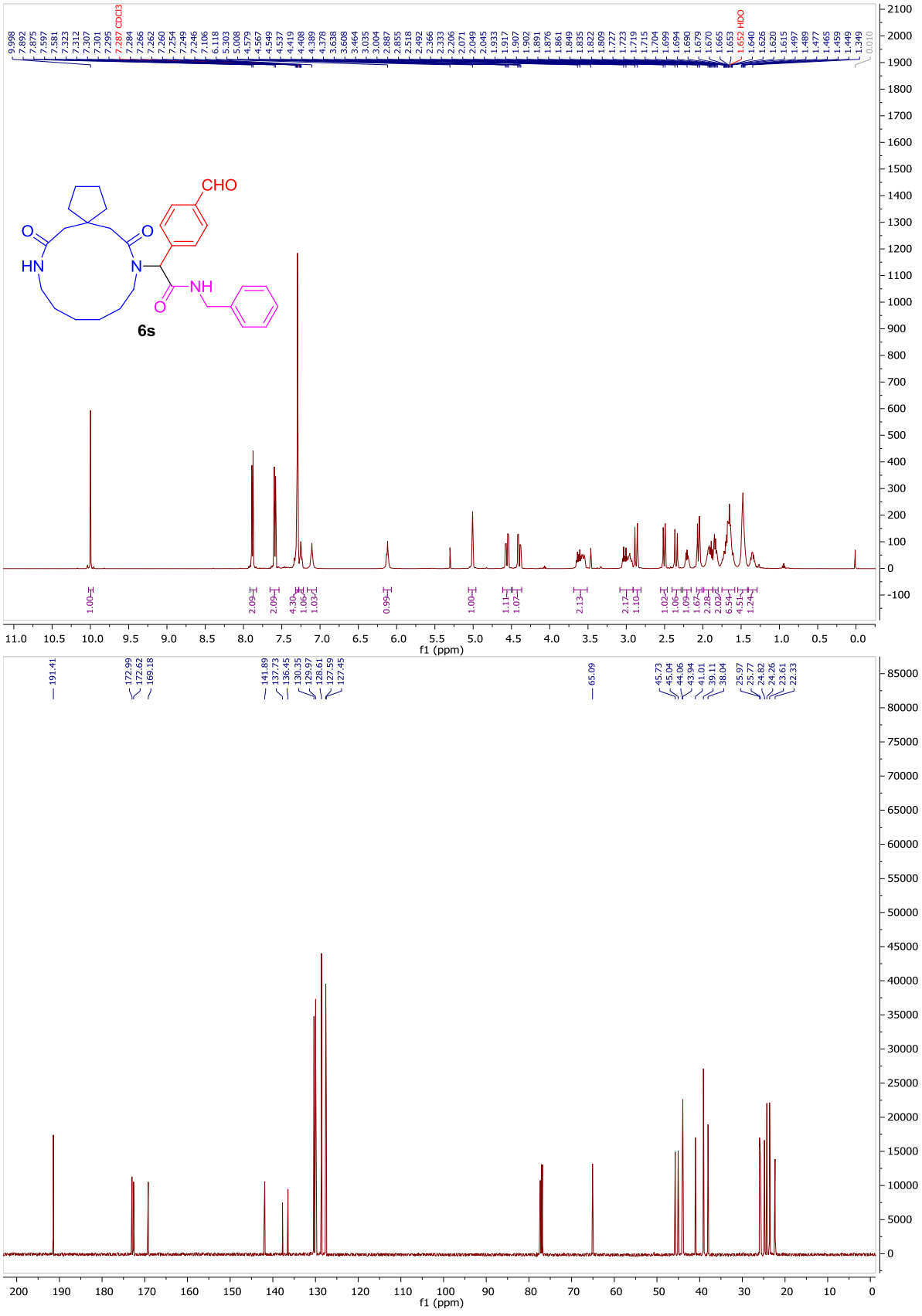




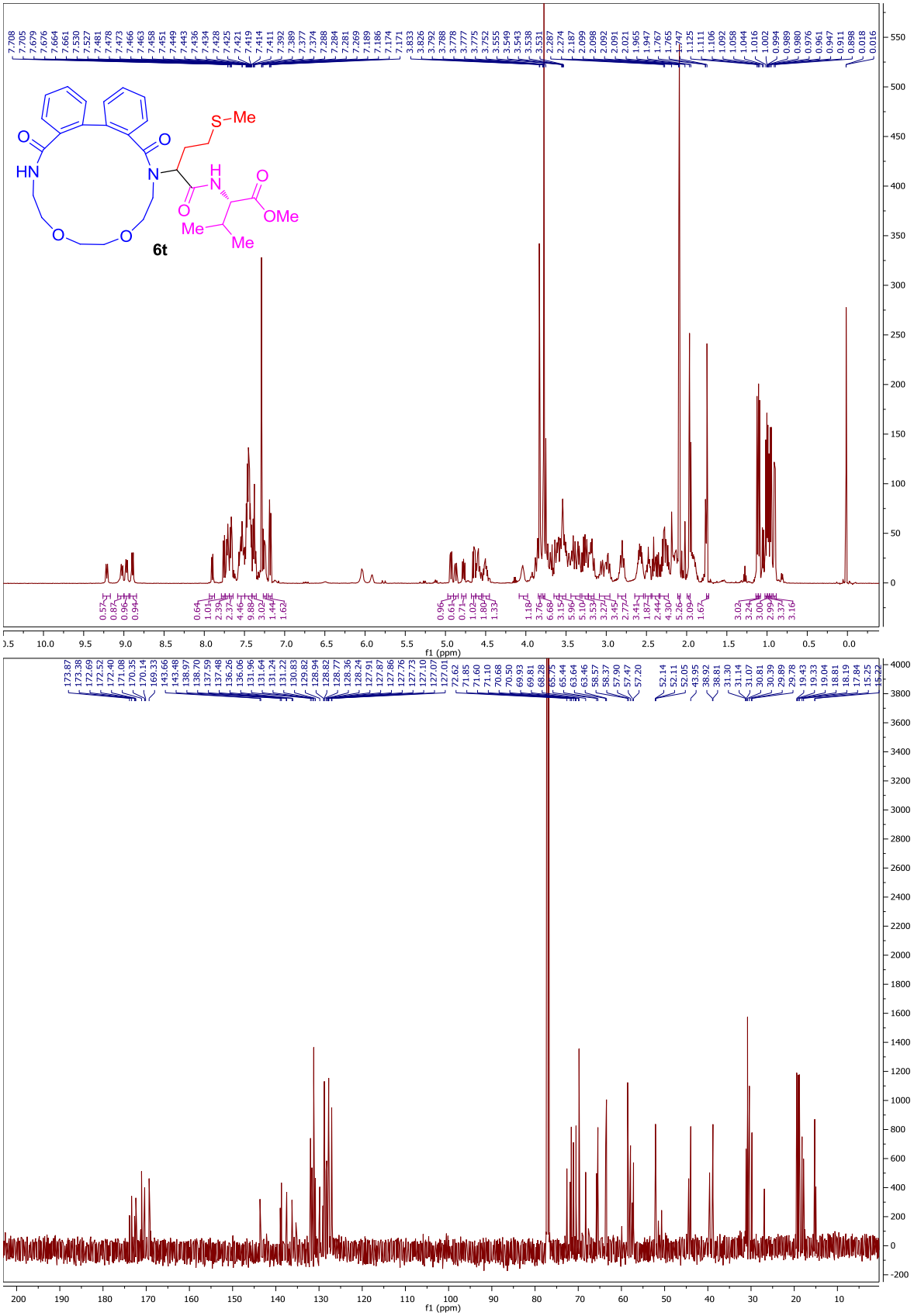
6r: N-butyl-2-cyclopropyl-2-(5,13-dioxo-7,8,10,11,12,13-hexahydrodibenzo[f,h][1,4,11]thiadiazacyclotridecin-6(5H)-yl)acetamide:



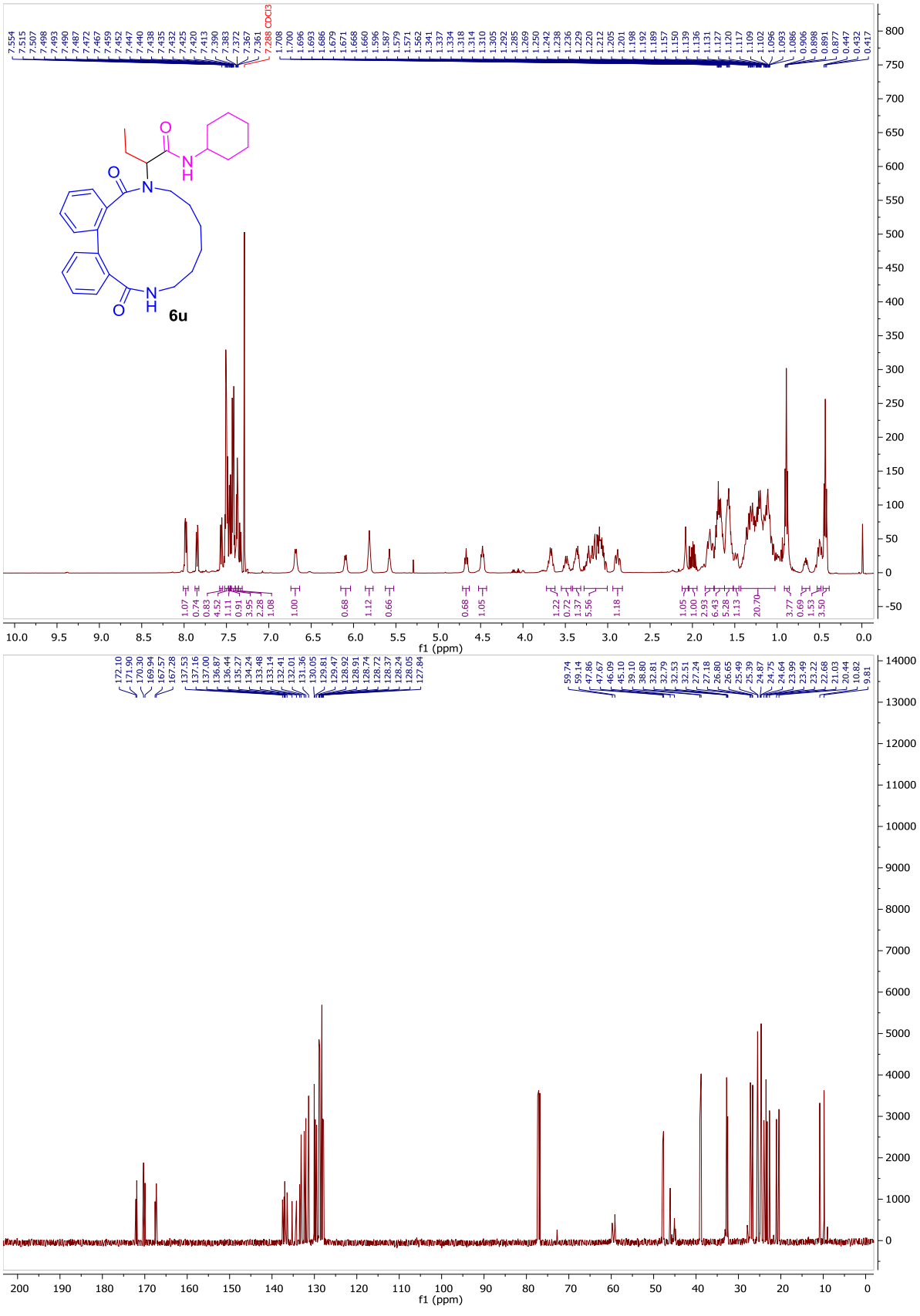
6s: N-benzyl-2-(7,16-dioxo-8,15-diazaspiro[4.12]heptadecan-8-yl)-2-(4-formylphenyl)acetamide:



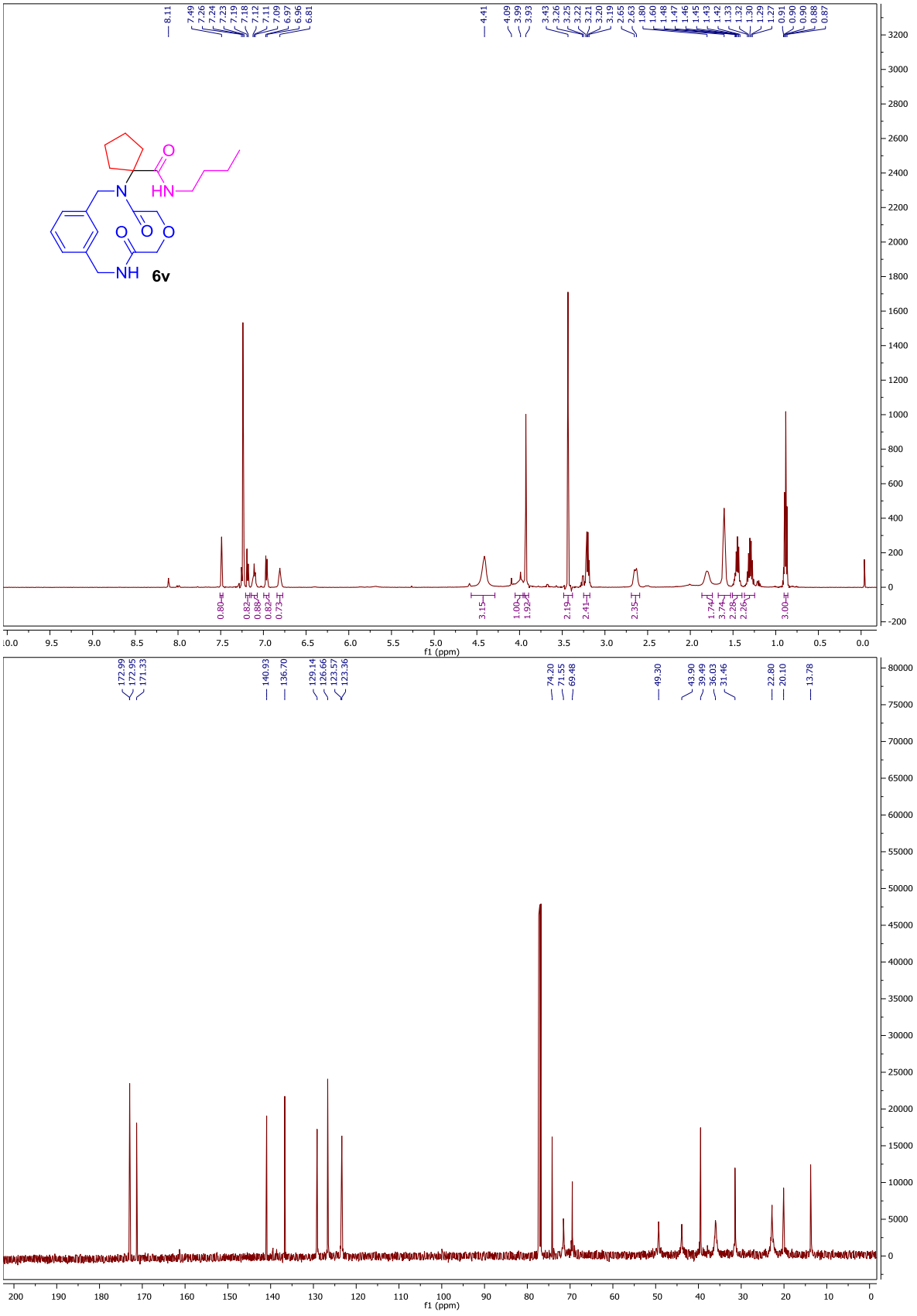
6t: methyl 2-(2-(5,16-dioxo-7,8,10,11,13,14,15,16-octahydrodibenzo[i,k][1,4,7,14]dioxadiazacyclohexadecin-6(5H)-yl)-4-(methylthio)butanamido)-3-methylbutanoate:



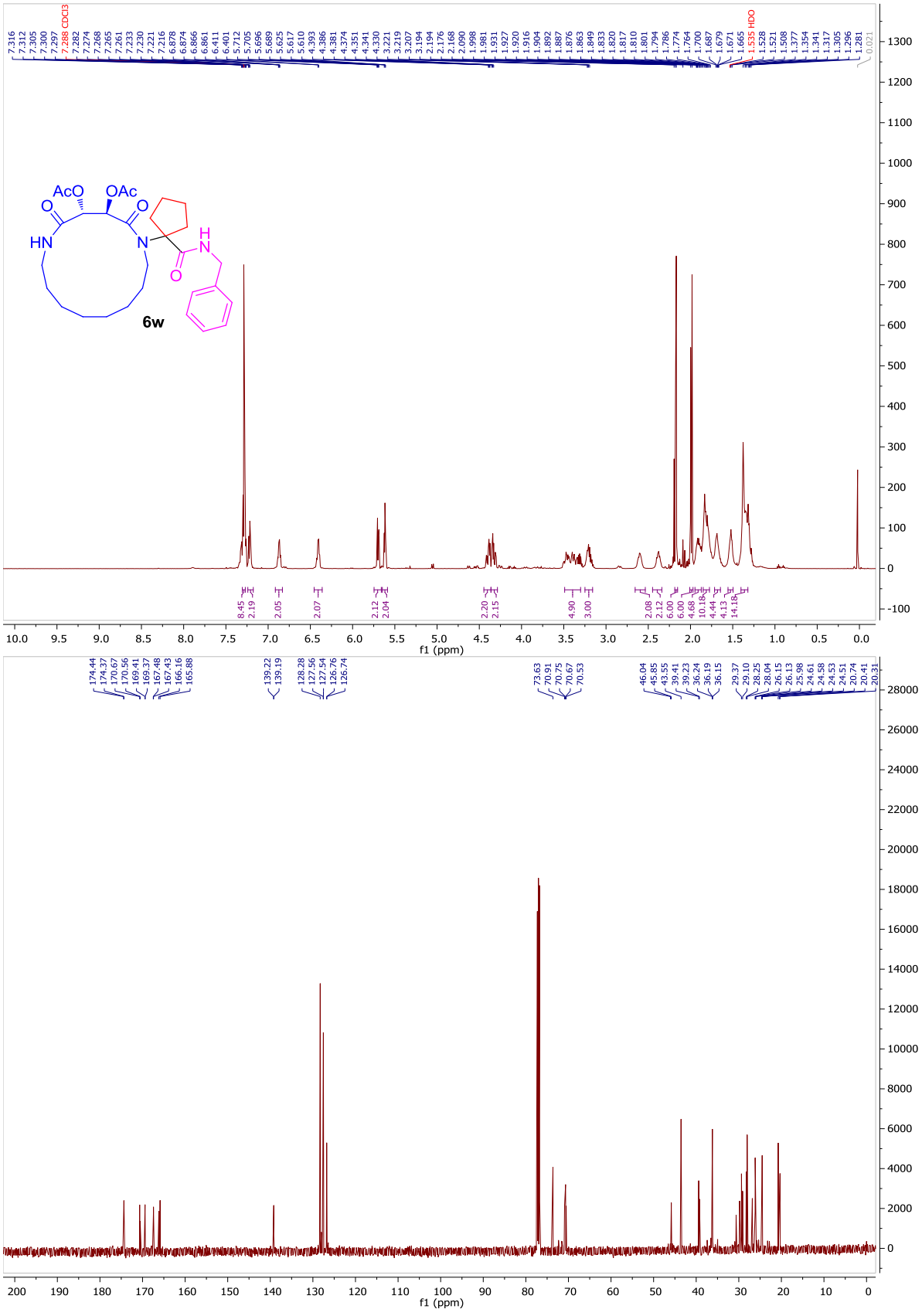
6u: N-cyclohexyl-2-(5,14-dioxo-7,8,9,10,11,12,13,14-octahydrodibenzo[c,e][1,8]diazacyclotetradecin-6(5H)-yl)butanamide:



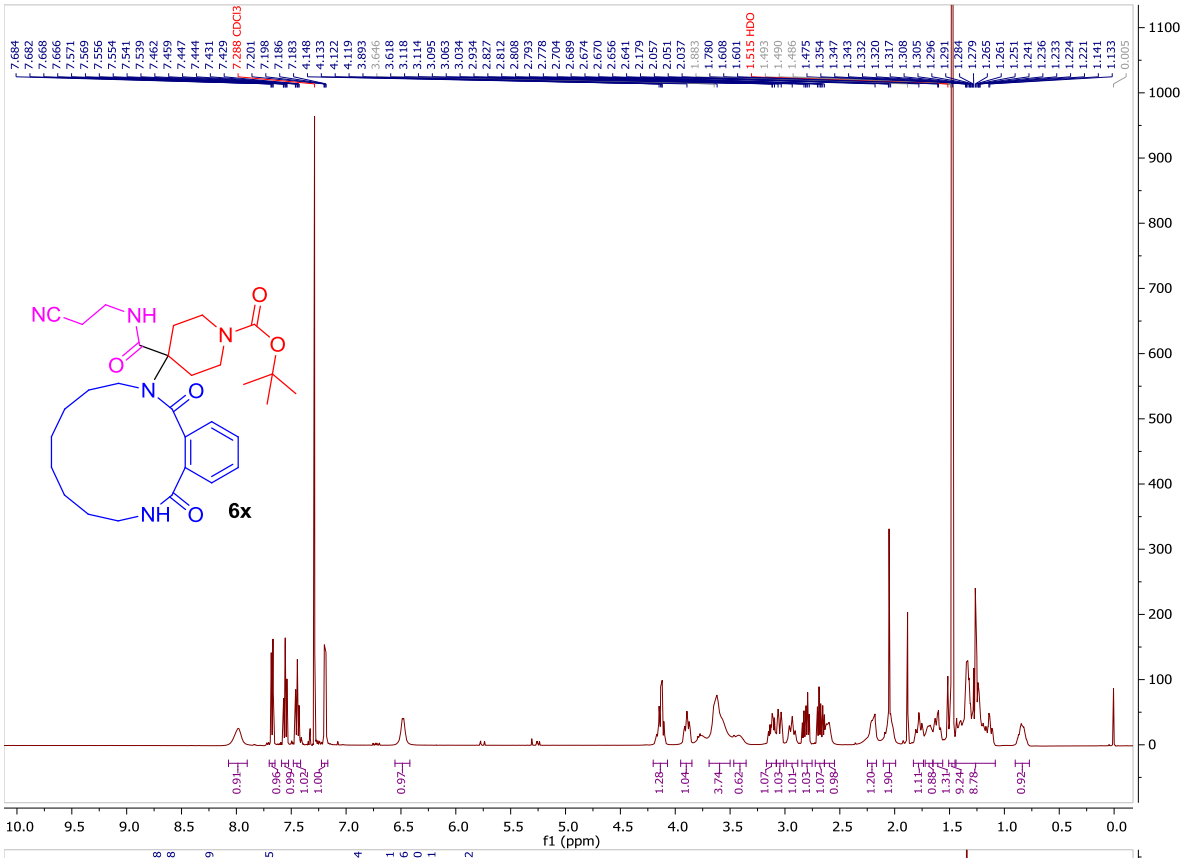
6v: N-butyl-1-{4,8-dioxo-6-oxa-3,9-diazabicyclo[9.3.1]pentadeca-1(14),11(15),12-trien-3-yl}cyclopentane-1-carboxamide:

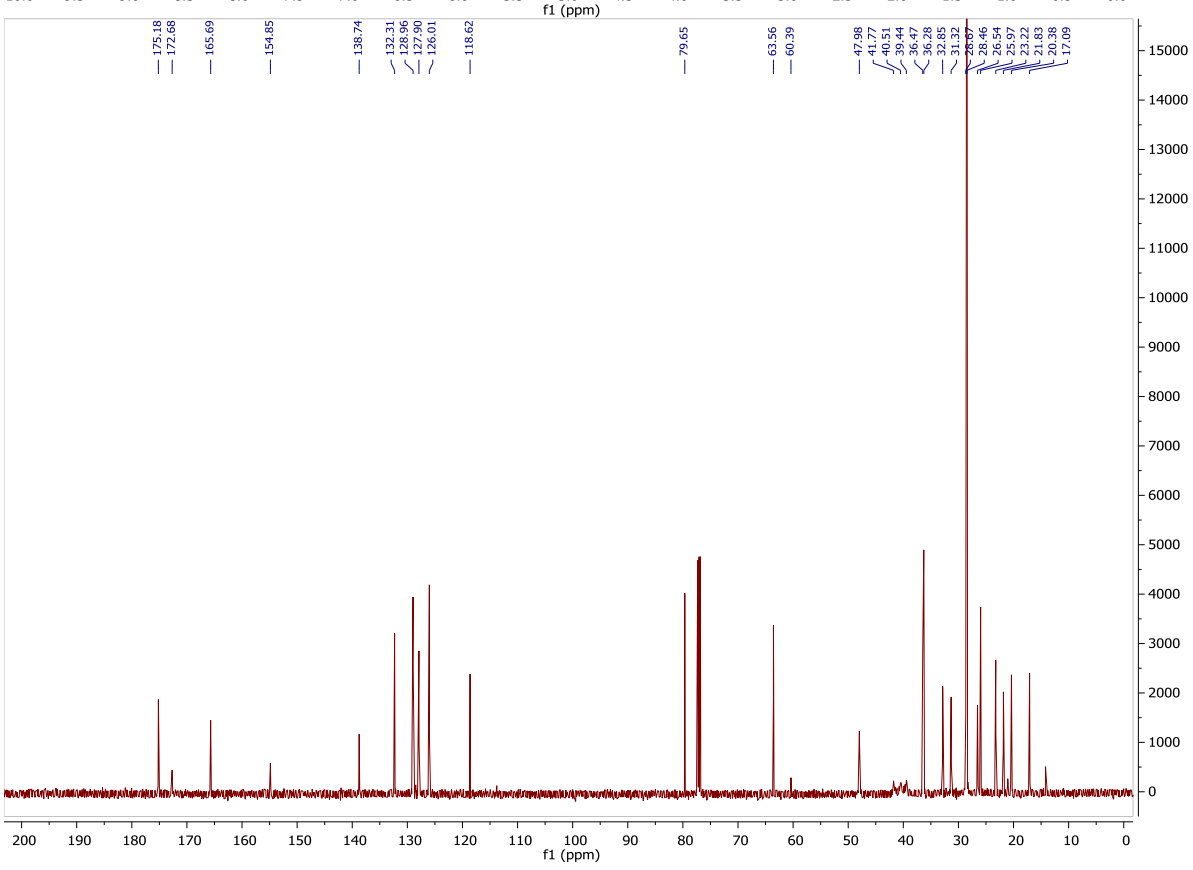


6w: (3*R*,4*R*)-1-(1-(benzylcarbamoyl)cyclopentyl)-2,5-dioxo-1,6-diazacyclotetradecane-3,4-diyl diacetate:

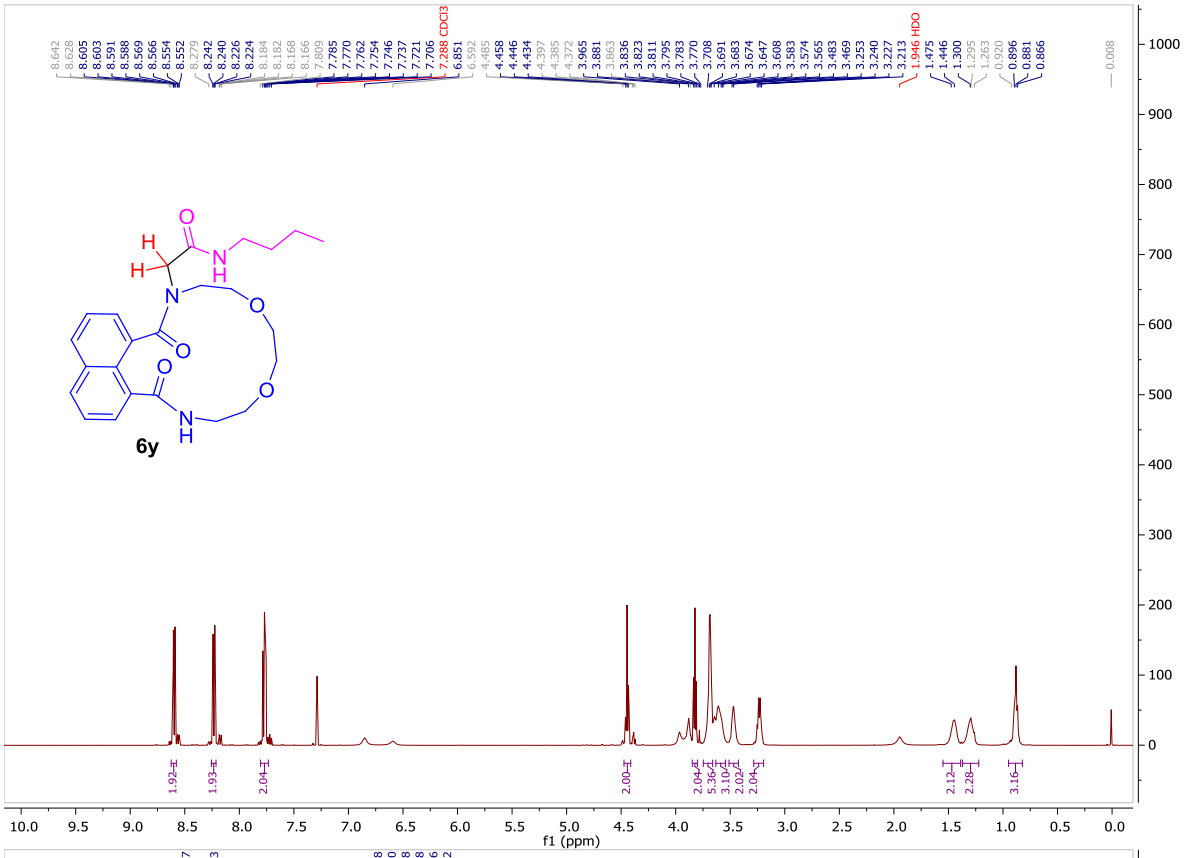


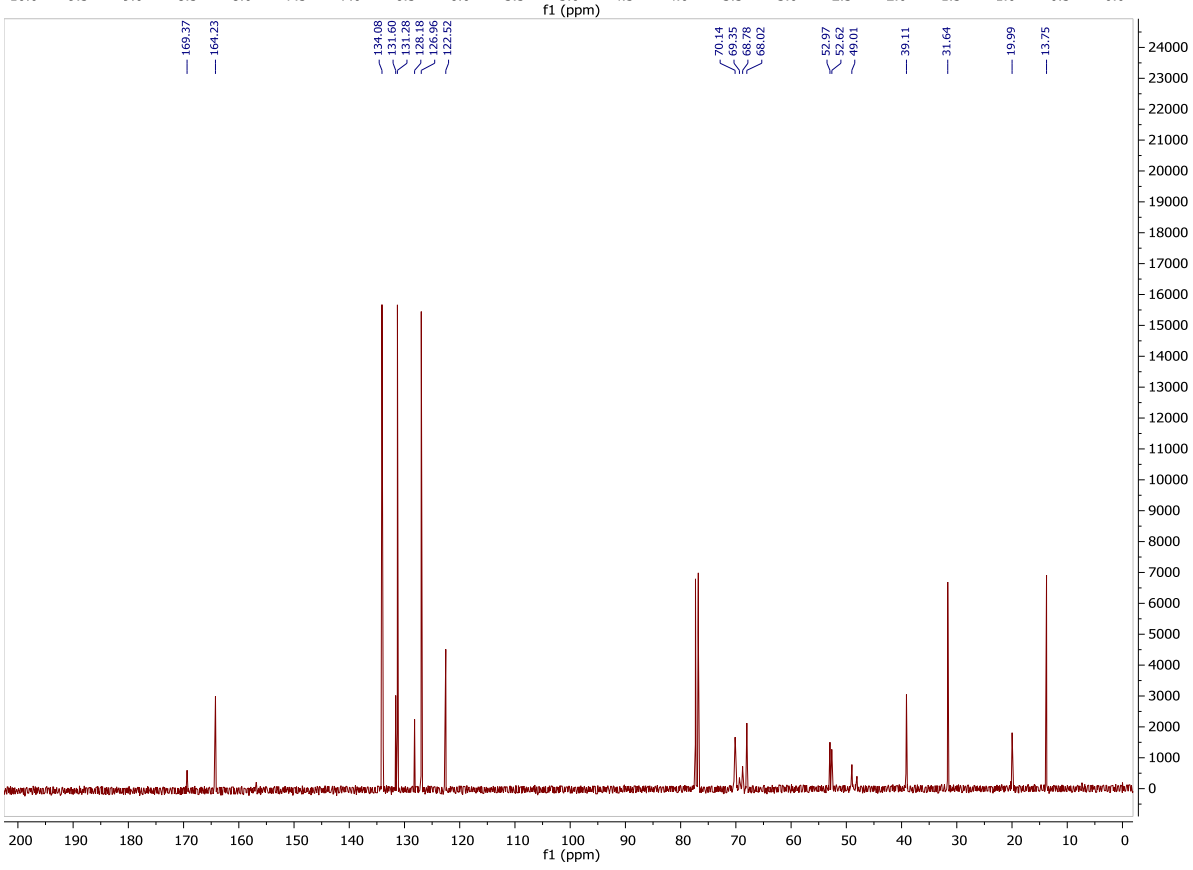
6x: tert-butyl 4-((2-cyanoethyl)carbamoyl)-4-(1,12-dioxo-3,4,5,6,7,8,9,10,11,12-decahydrobenzo[c][1,6]diazacyclotetradecin-2(1H)-yl)piperidine-1-carboxylate:



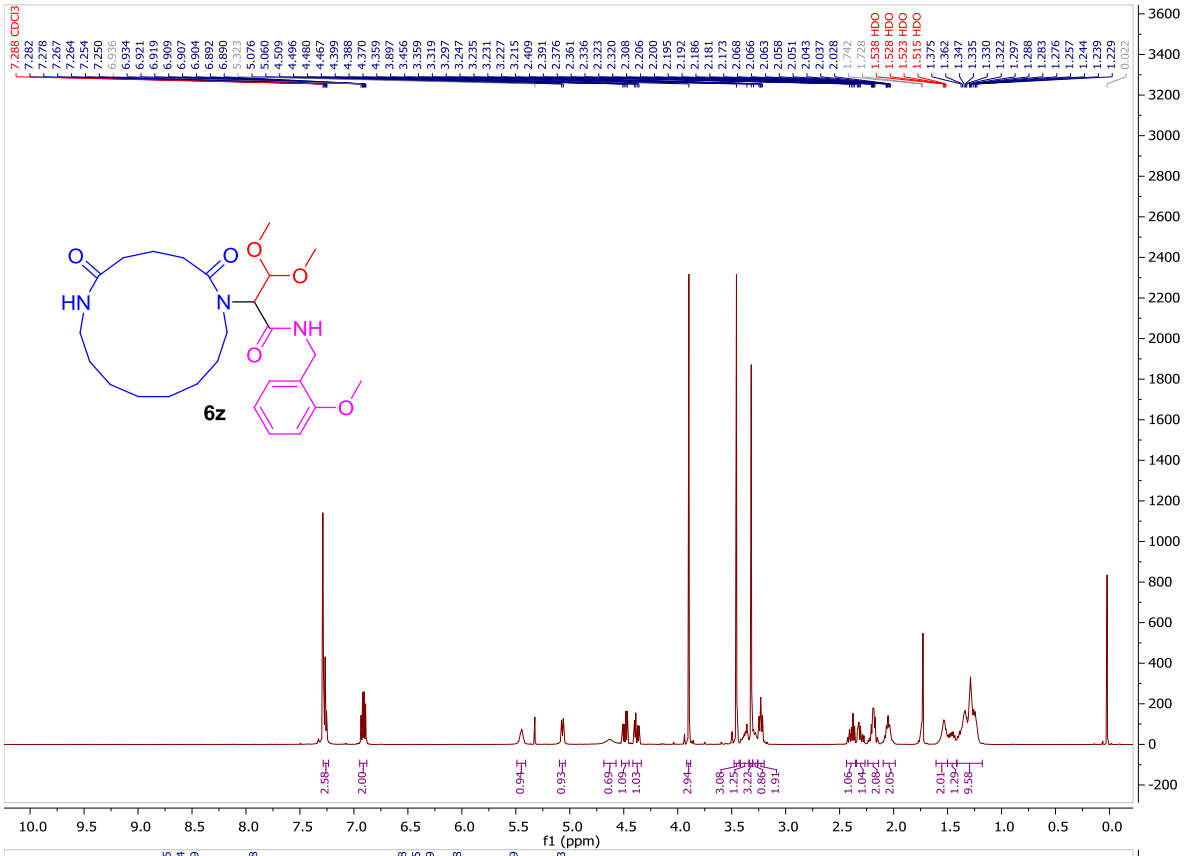


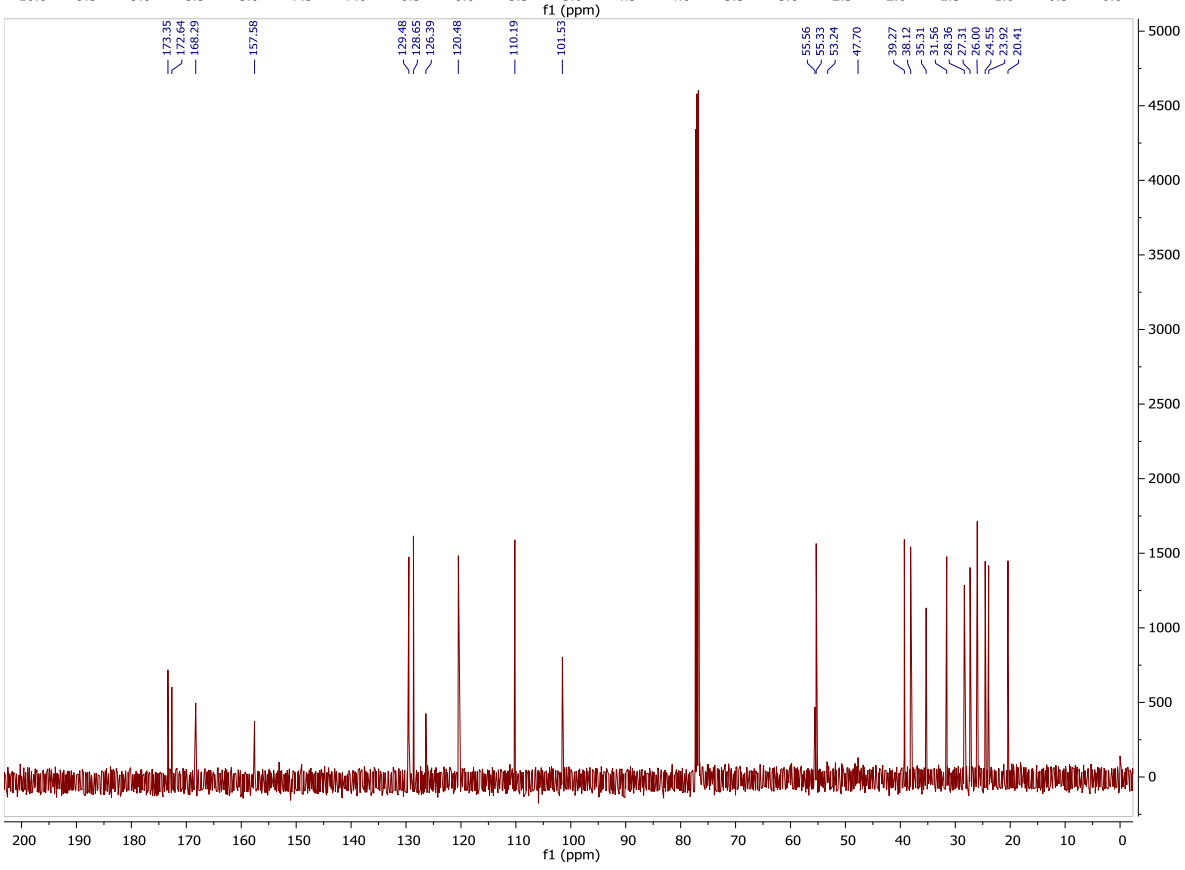
6y: N-butyl-2-(1,12-dioxo-3,4,6,7,9,10,11,12-octahydronaphtho[1,8-ij][1,4,7,13]dioxadiazacyclopentadecin-2(1H)-yl)acetamide:



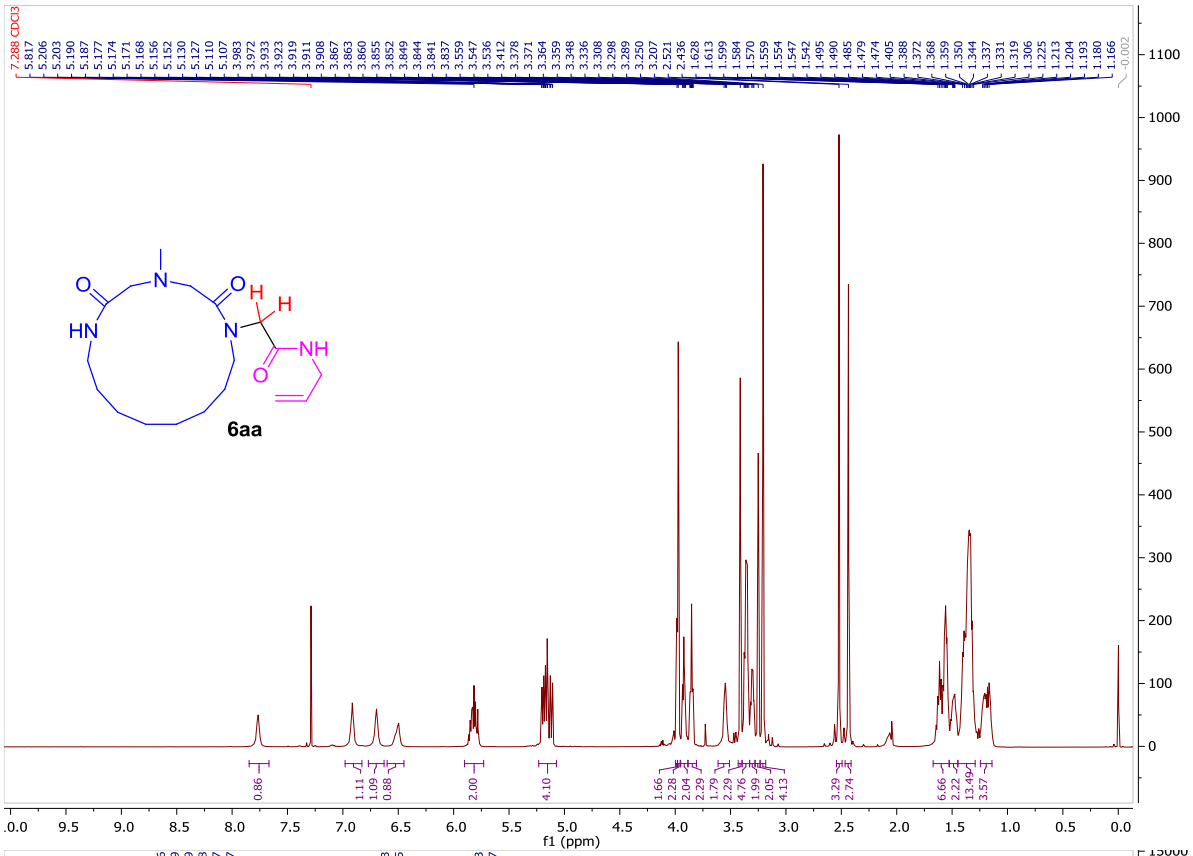


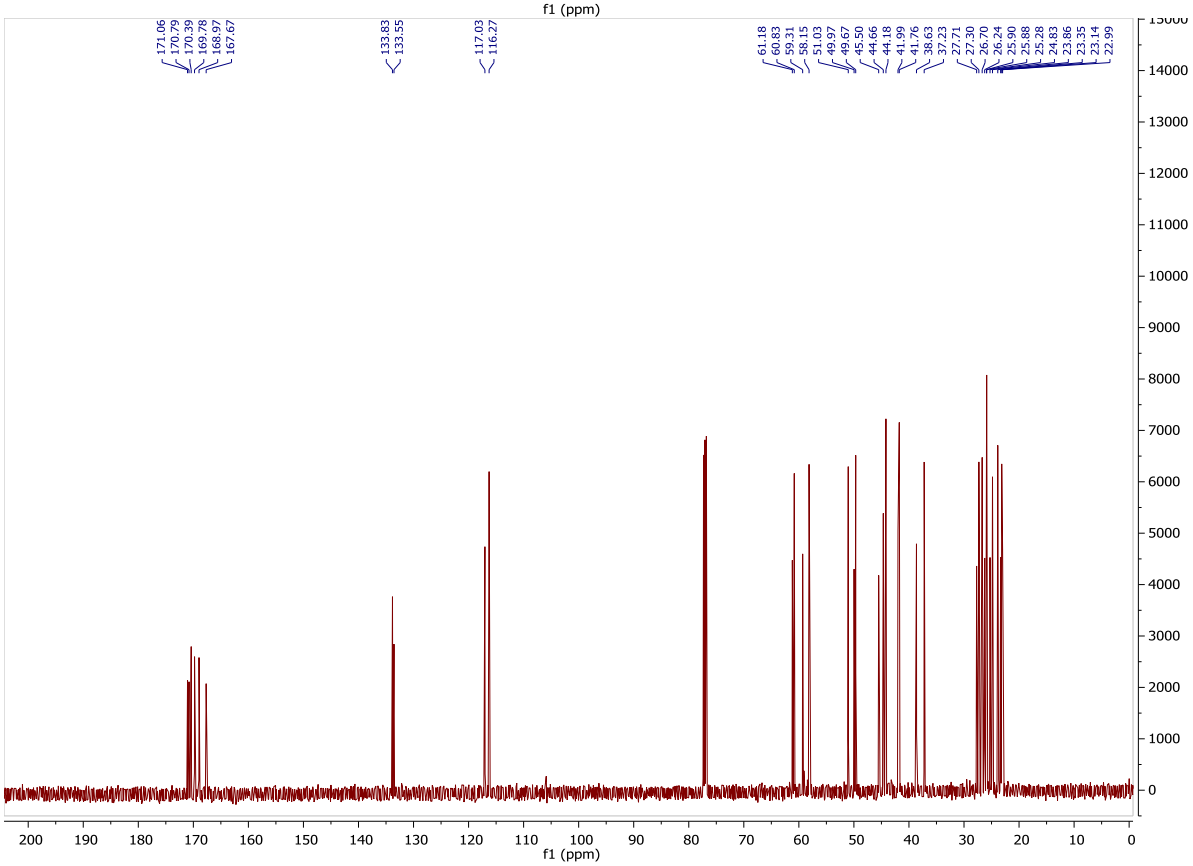
6z: 2-(2,6-dioxo-1,7-diazacyclopentadecan-1-yl)-3,3-dimethoxy-N-(2-methoxybenzyl)propanamide:



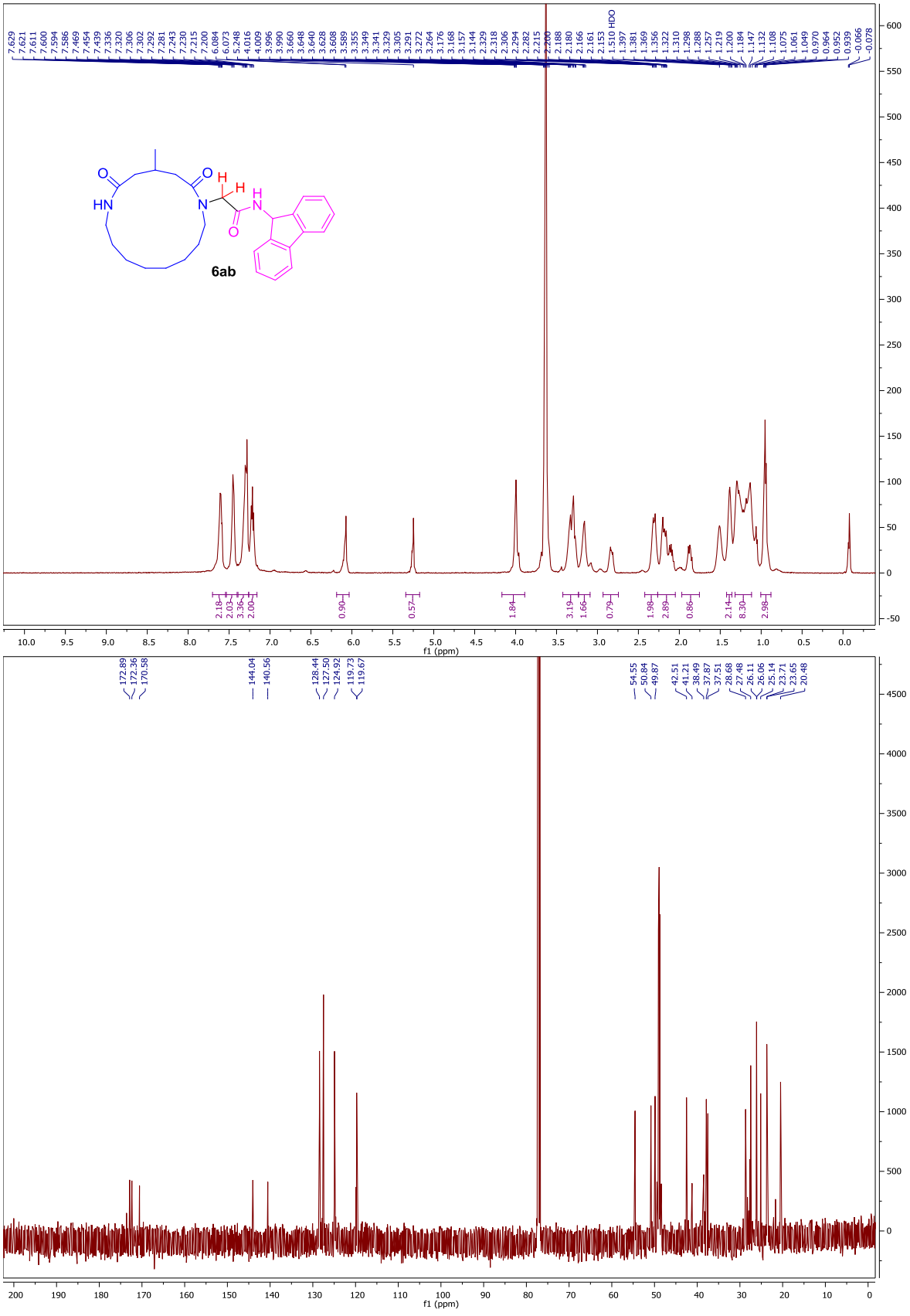


6aa: N-allyl-2-(4-methyl-2,6-dioxo-1,4,7-triazacyclopentadecan-1-yl)acetamide:

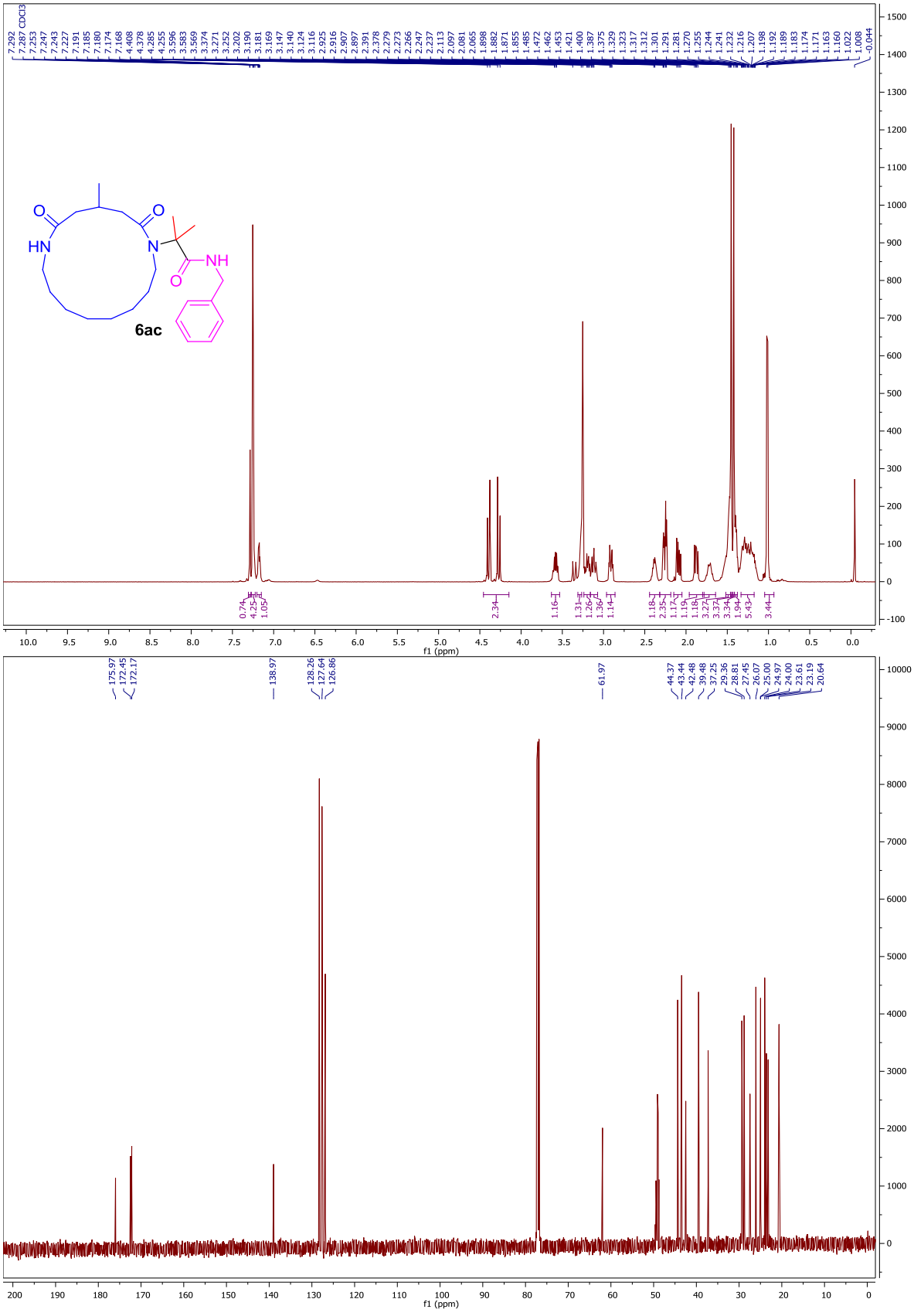




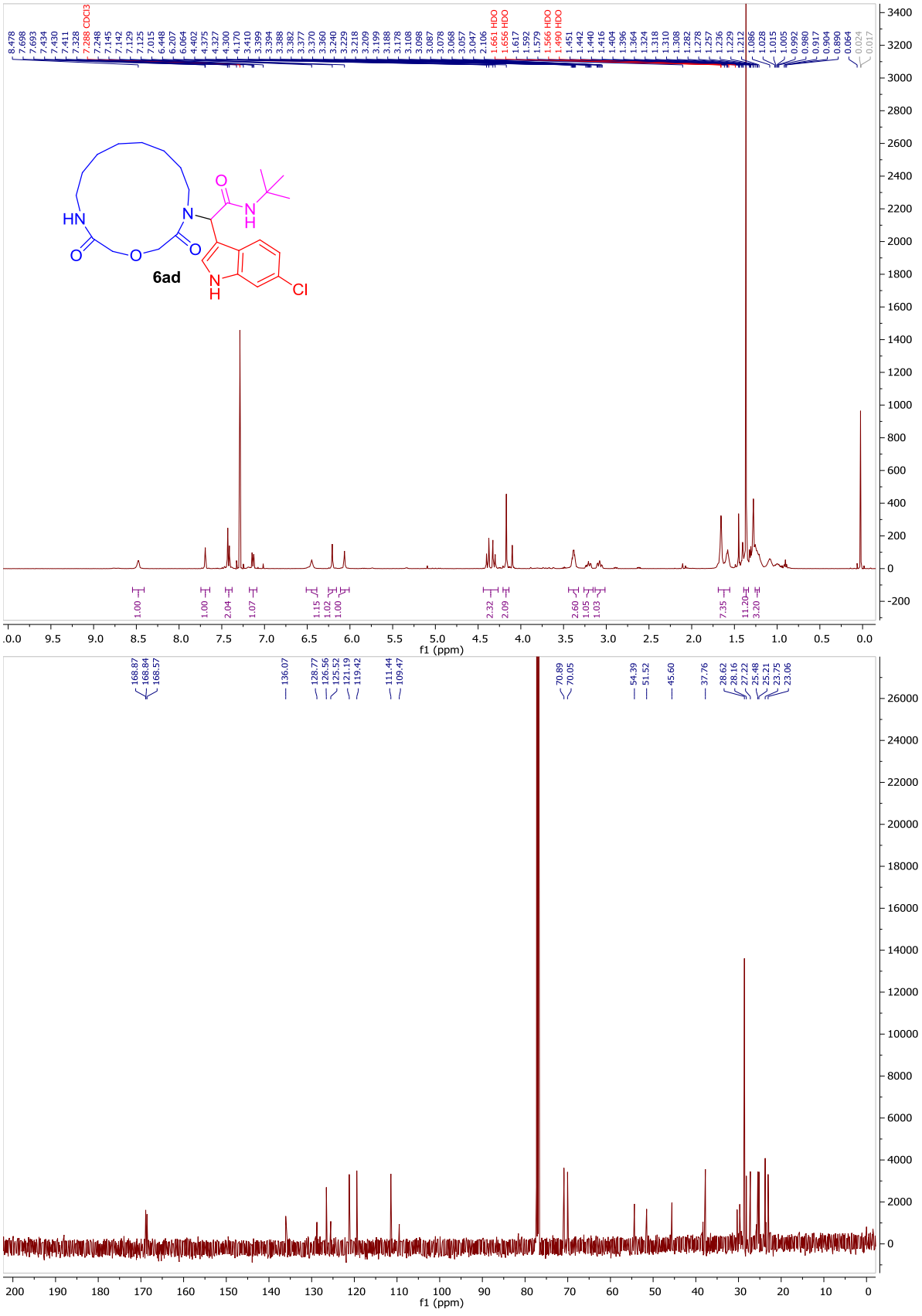
6ab: *N*-(9H-fluoren-9-yl)-2-(4-methyl-2,6-dioxo-1,7-diazacyclopentadecan-1-yl)acetamide:



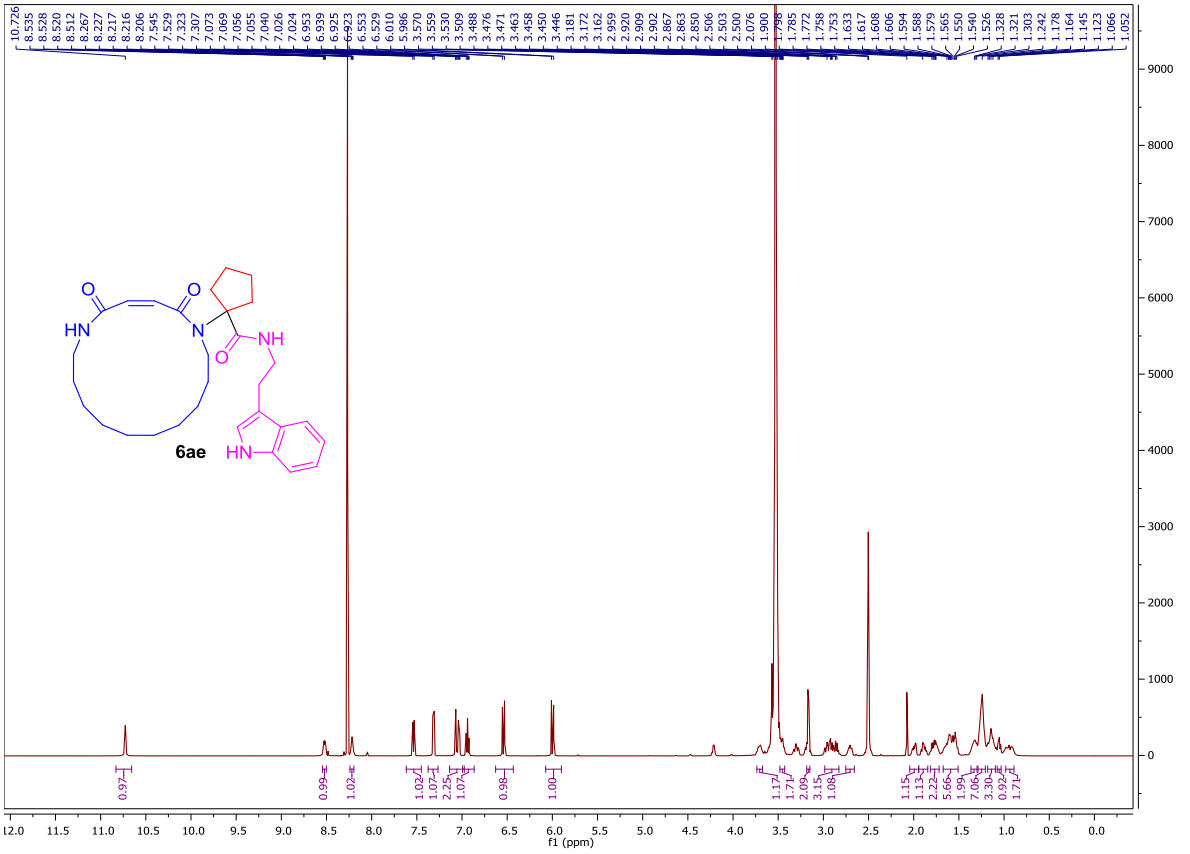
6ac: N-benzyl-2-methyl-2-(4-methyl-2,6-dioxo-1,7-diazacyclopentadecan-1-yl)propanamide:

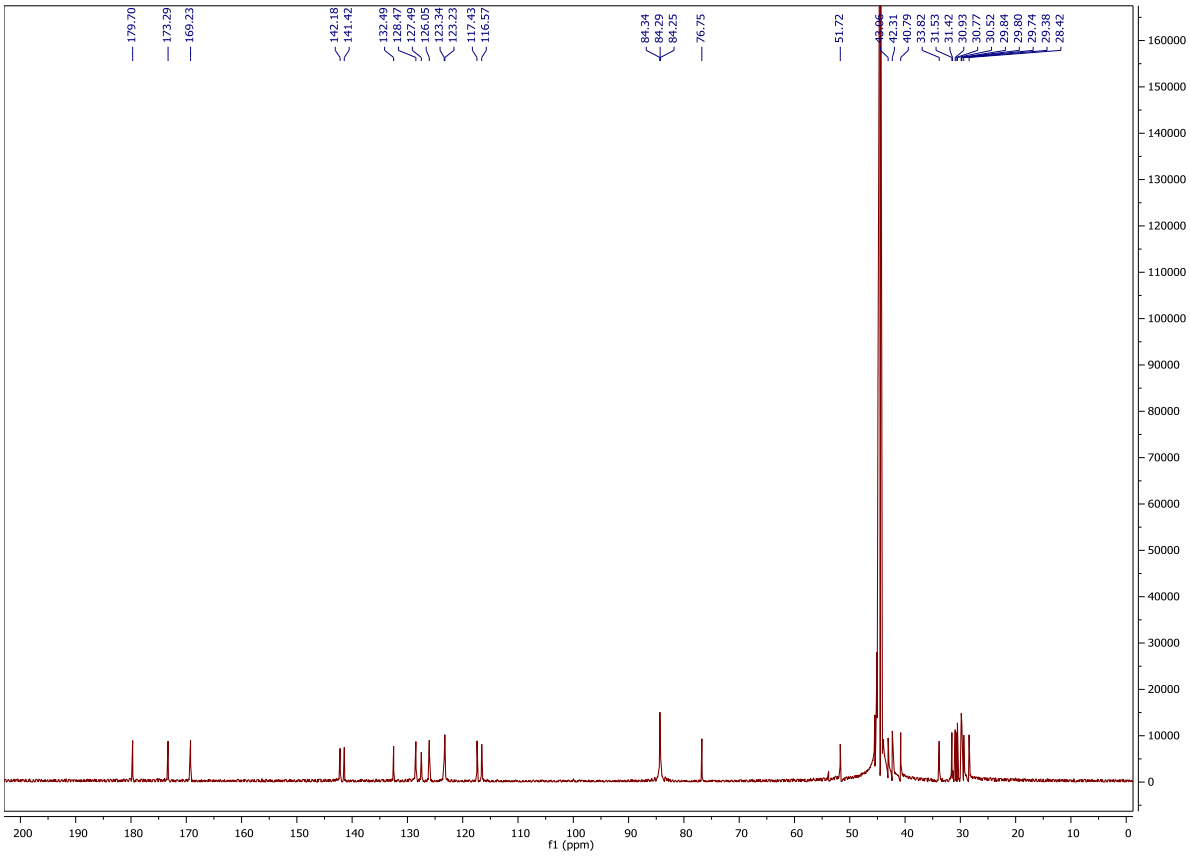


6ad: N-(tert-butyl)-2-(6-chloro-1H-indol-3-yl)-2-(3,14-dioxo-1-oxa-4,13-diazacyclopentadecan-4-yl)acetamide:

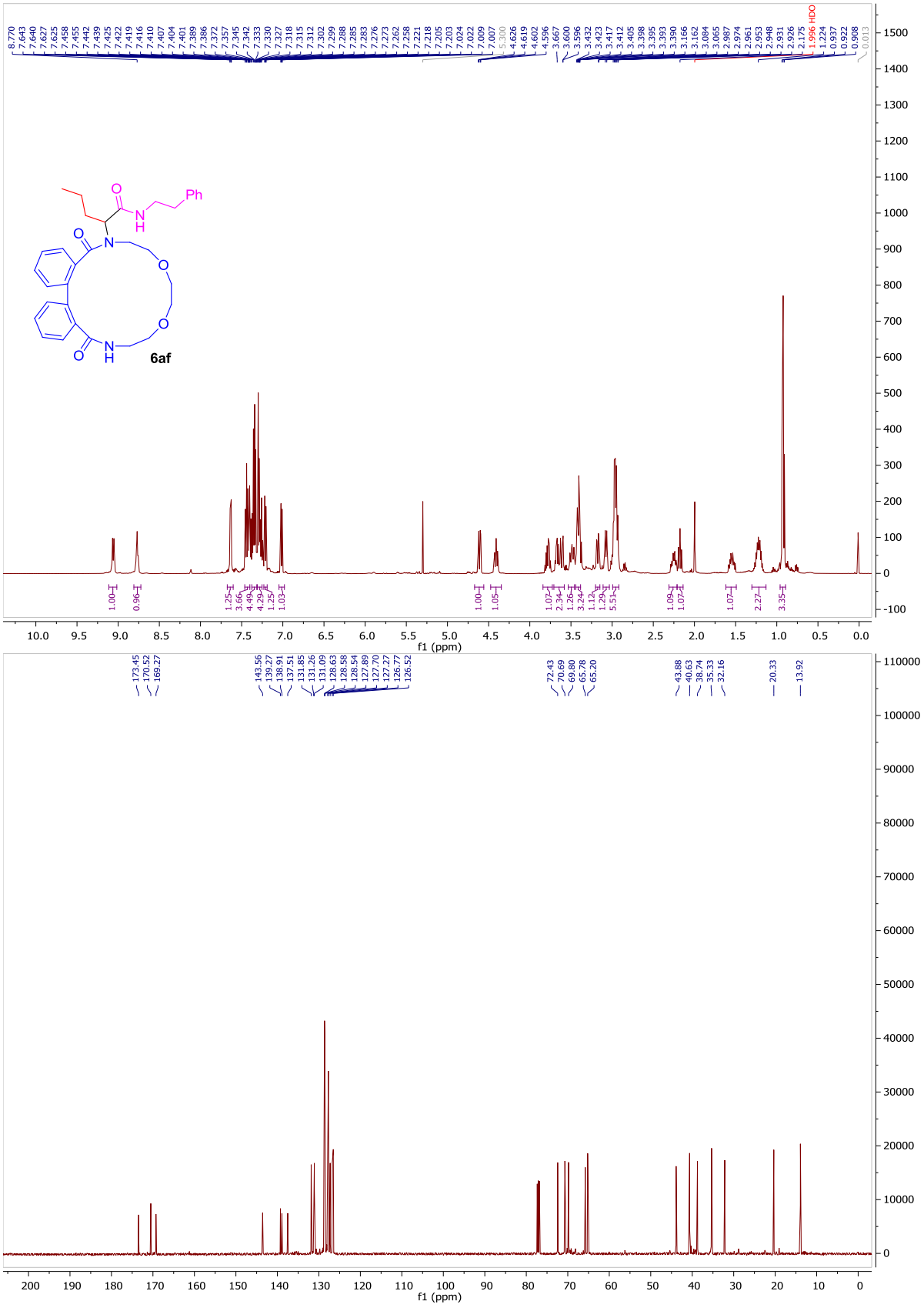


6ae: (Z)-N-(2-(1H-indol-3-yl)ethyl)-1-(2,5-dioxo-1,6-diazacyclohexadec-3-en-1-yl)cyclopentanecarboxamide:

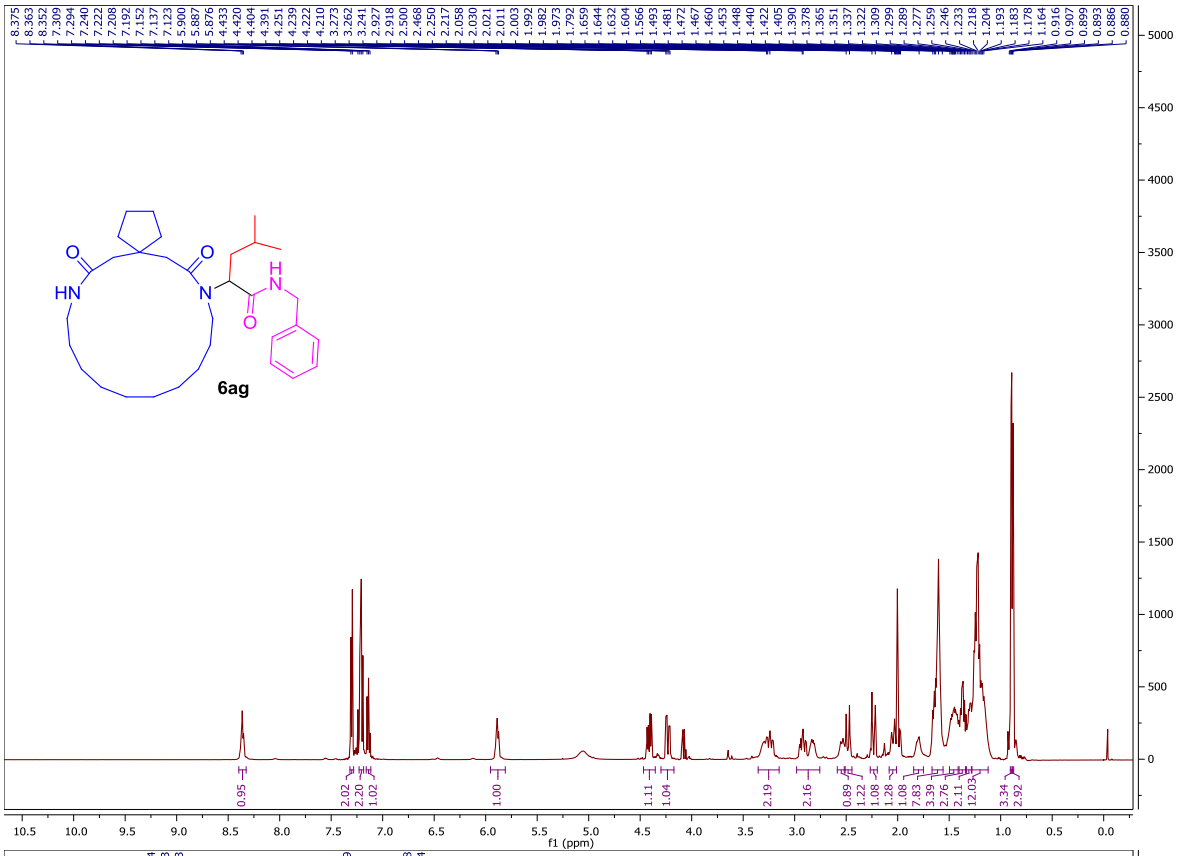


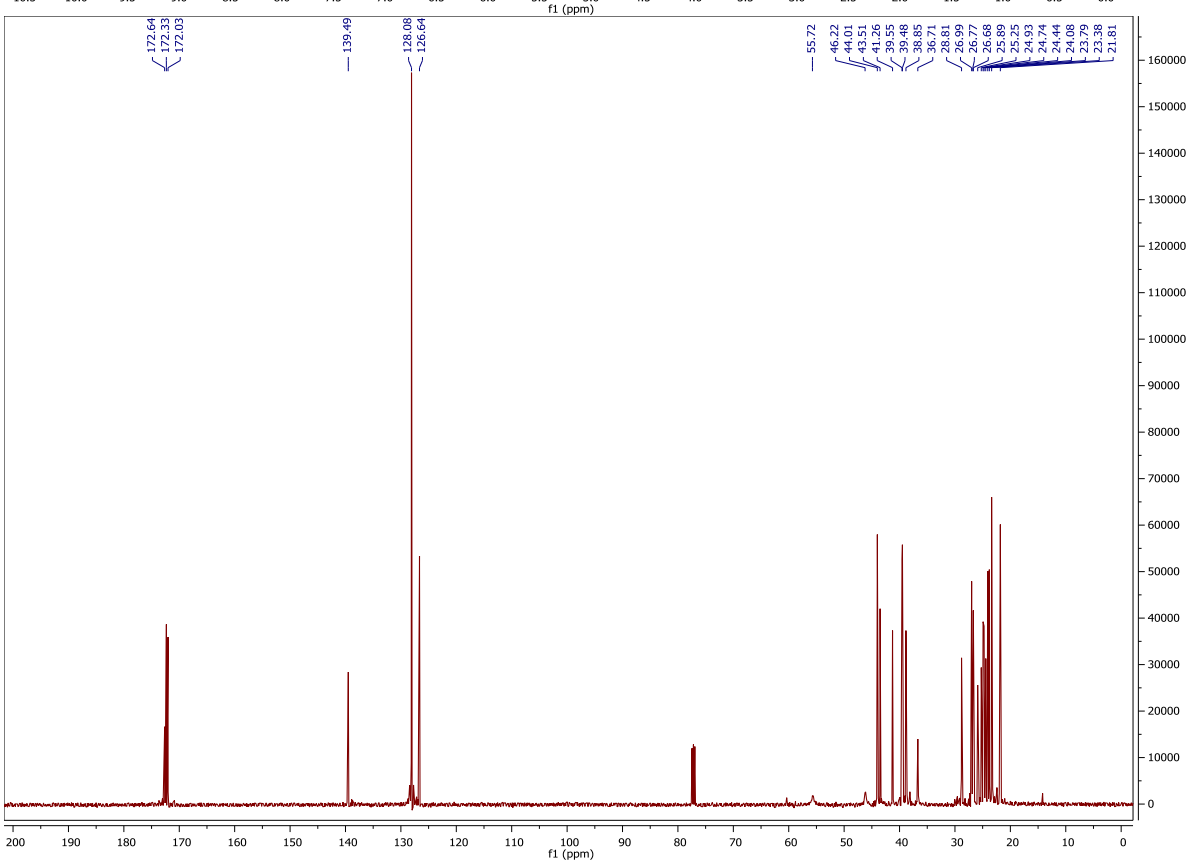


6af: 2-(5,16-dioxo-7,8,10,11,13,14,15,16-octahydrodibenzo[i,k][1,4,7,14]dioxadiazacyclohexadecin-6(5H)-yl)-N-phenethylpentanamide:

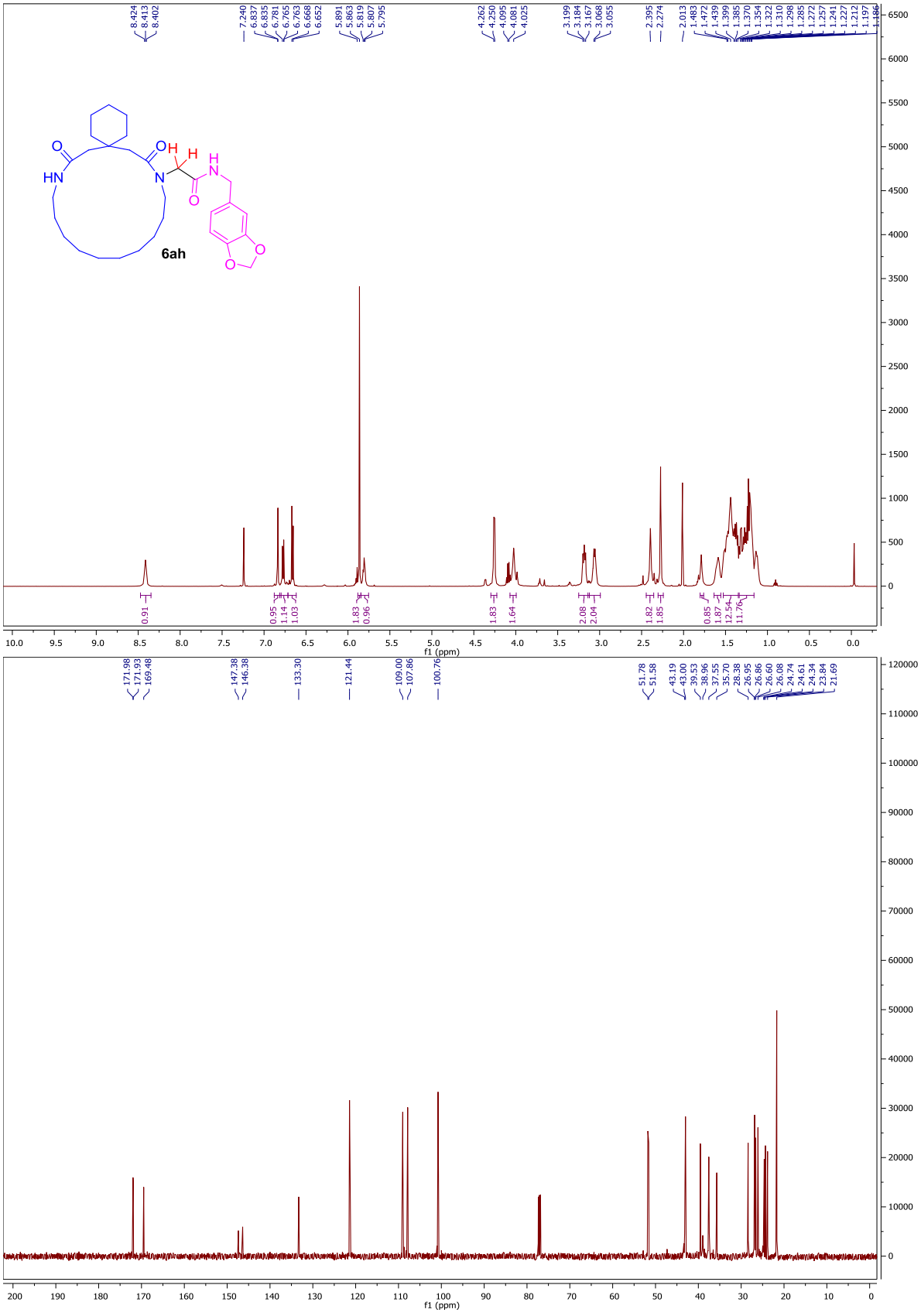


6ag: N-benzyl-2-(7,20-dioxo-8,19-diazaspiro[4.16]henicosan-8-yl)-4-methylpentanamide:

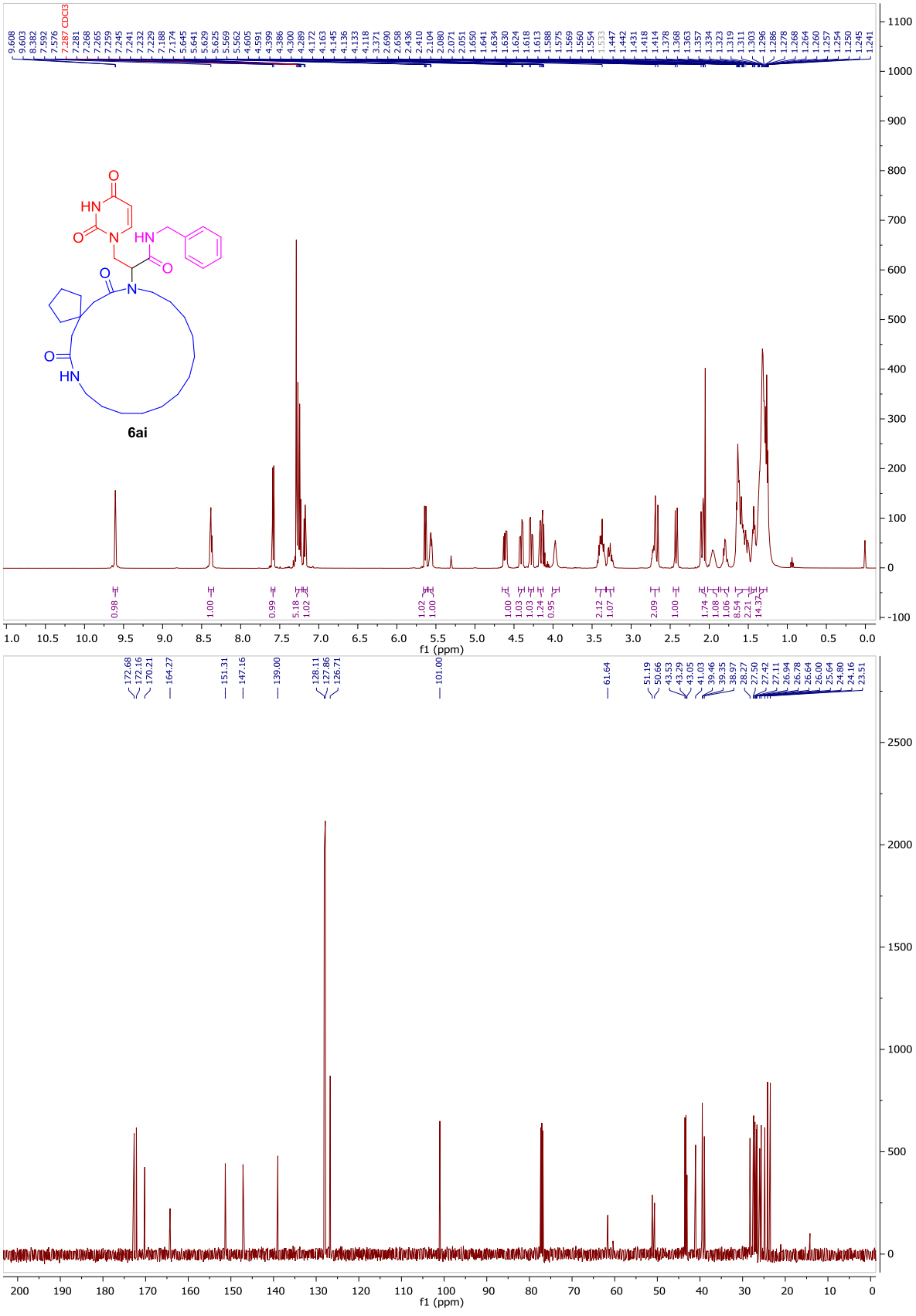




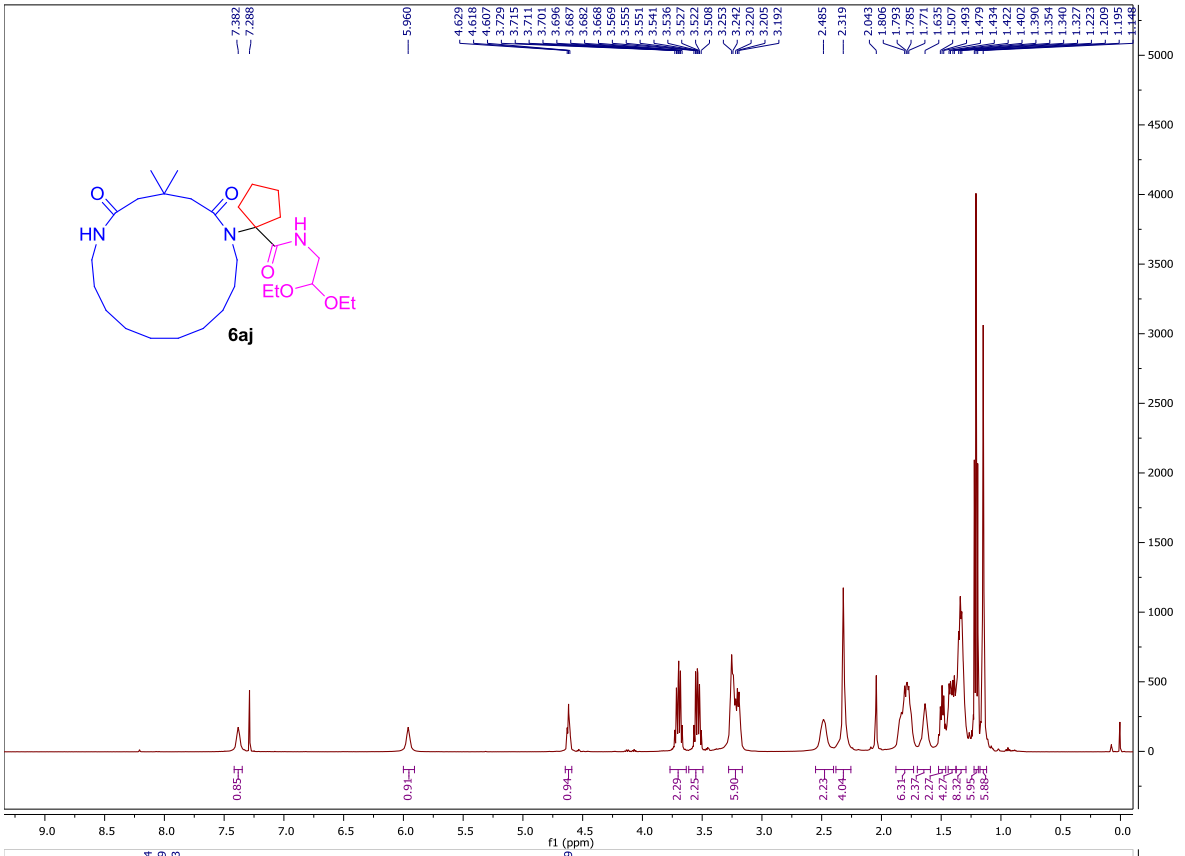
6ah: *N*-(benzo[d][1,3]dioxol-5-ylmethyl)-2-(8,21-dioxo-9,20-diazaspiro[5.16]docosan-9-yl)acetamide:

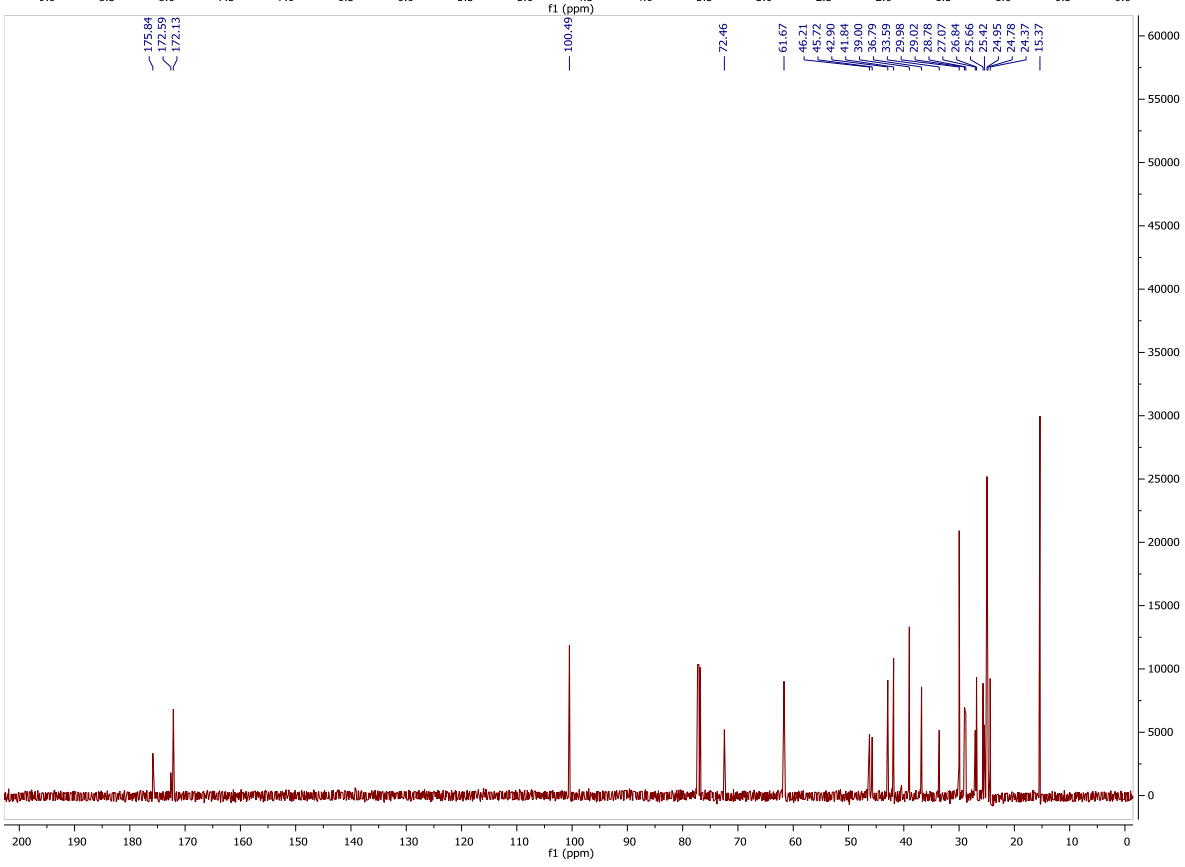


6ai: N-benzyl-3-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(7,22-dioxo-8,21-diazaspiro[4.18]tricosan-8-yl)propanamide:

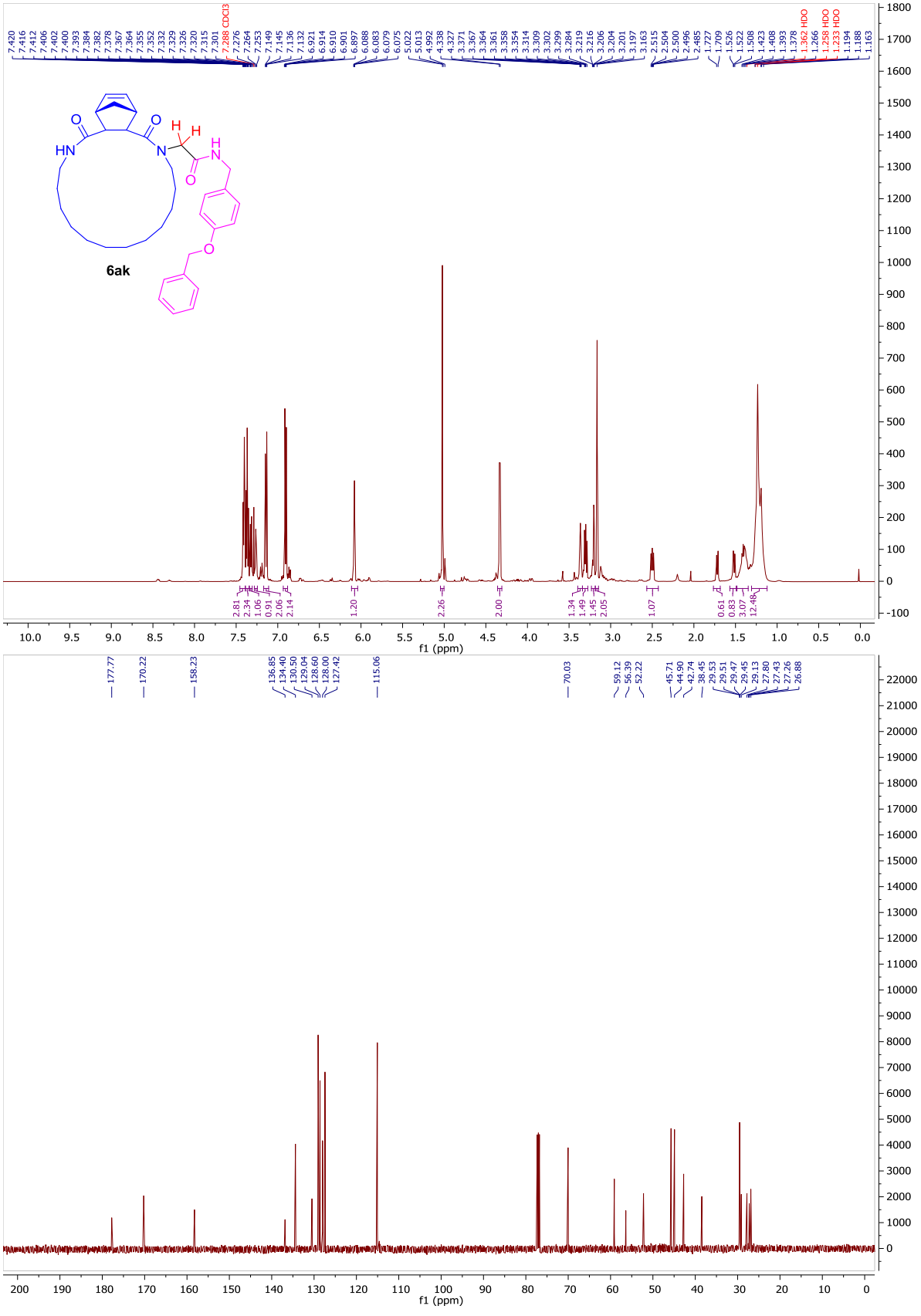


6aj: N-(2,2-diethoxyethyl)-1-(4,4-dimethyl-2,6-dioxo-1,7-diazacycloheptadecan-1-yl)cyclopentanecarboxamide:

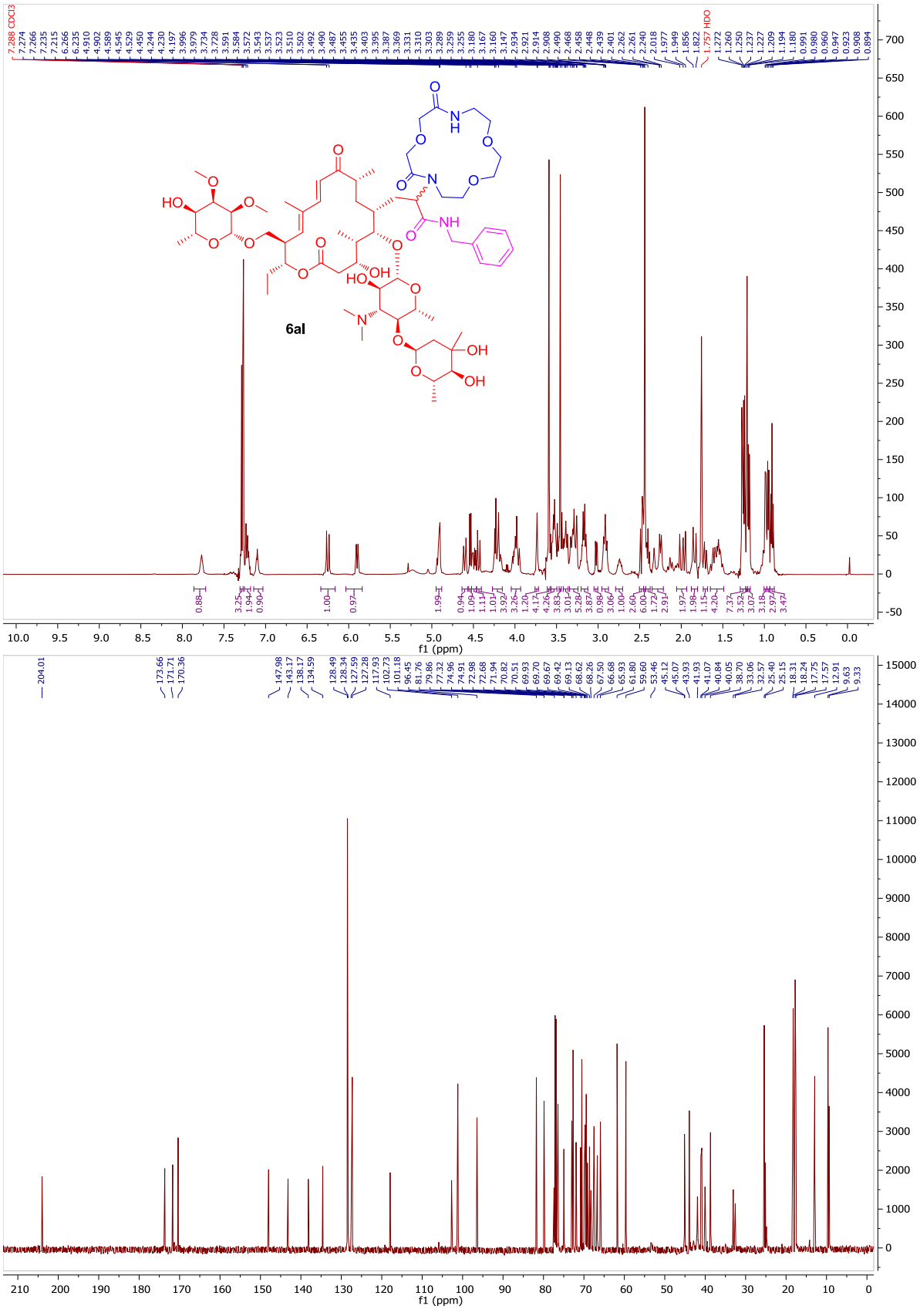




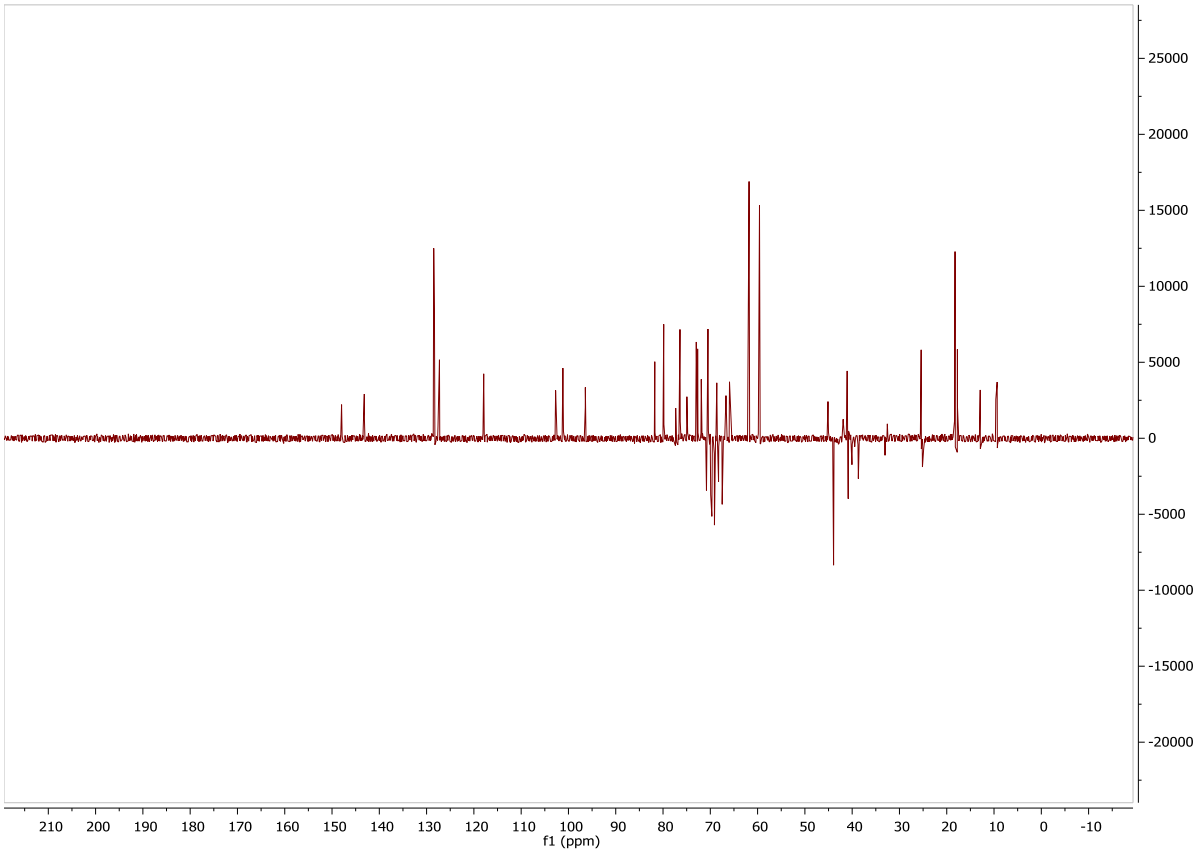
6ak: N-(4-(benzyloxy)benzyl)-2-((17S,20R)-1,16-dioxo-3,4,5,6,7,8,9,10,11,12,13,14,15,16,16a,17,20,20a-octadecahydro-17,20-methanobenzo[c][1,6]diazacyclooctadecin-2(1H)-yl)acetamide:



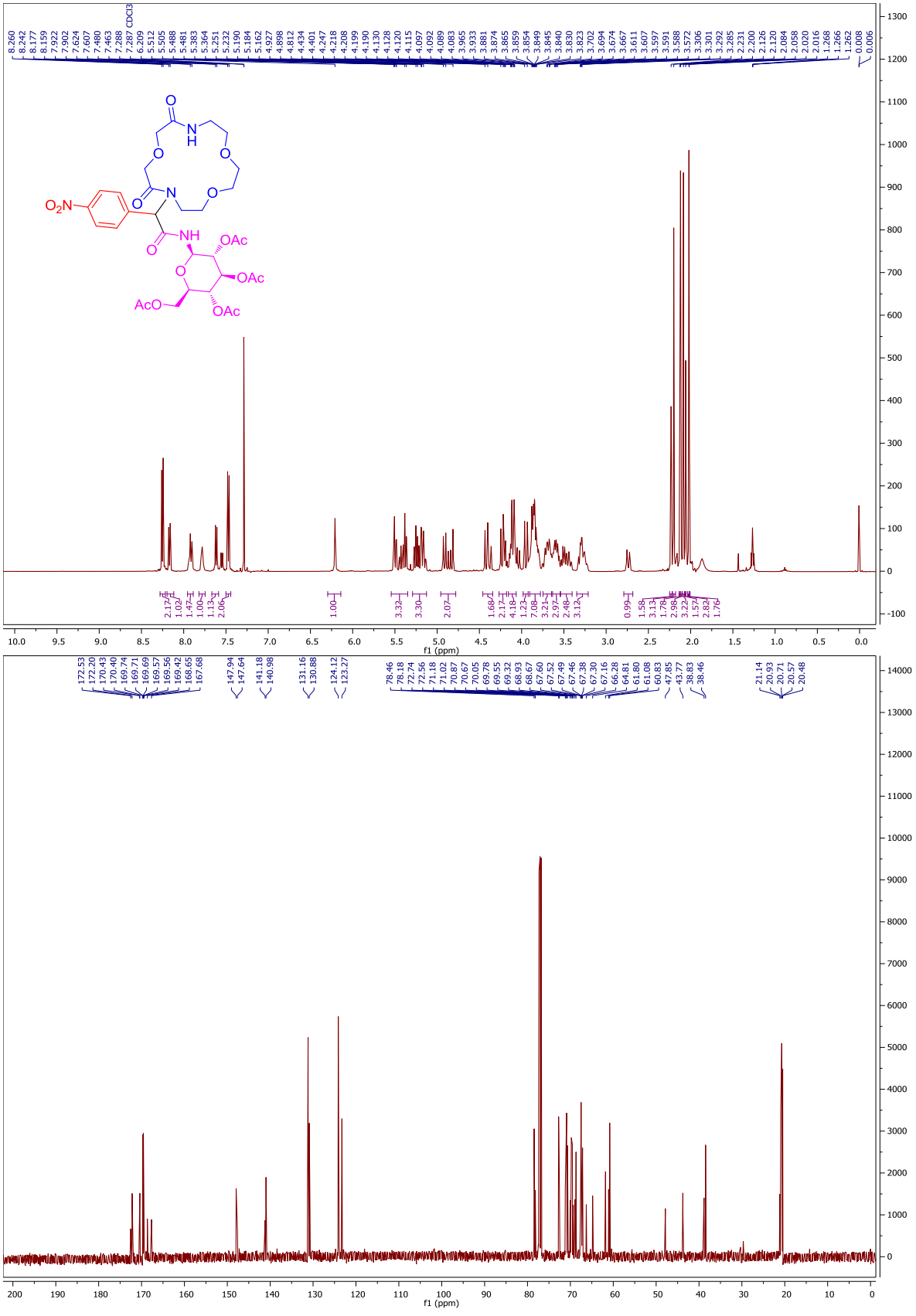
6al:



6al: **DEPT135**


25000
20000
15000
10000
5000
0
-5000
-10000
-15000
-20000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

6am: (2*R*,3*R*,4*S*,5*R*,6*R*)-2-(acetoxymethyl)-6-(2-(8,12-dioxo-1,4,10-trioxa-7,13-diazacyclopentadecan-7-yl)-2-(4-nitrophenyl)acetamido)tetrahydro-2H-pyran-3,4,5-triyl triacetate



Evaluation of inhibitory activity of 6ad compound towards Mdm2/MdmX:

1. Determination of dissociation constant of labeled peptide P2 and Mdm2/MdmX

New protein stock (constructs: Mdm2 1-118; MdmX 1-134) was thawed and the concentration was measured using Bradford method. Dissociation constant (K_d) of labeled peptide P2 – Mdm2/MdmX was determined for further evaluation of optimal protein concentration for inhibition constant determination. Peptide P2 was labeled with carboxyfluorescein and had the following sequence: LTFEHYWAQLTS. K_d was determined for each thawed new protein stock using fluorescence polarization (FP) assay.

FP assay was performed in duplicates on 96-well microplates (Corning NBS 3991) in final volume 100 µl (70 µl protein + 5 µl DMSO + 25 µl P2). Protein dilutions were made in FP buffer (10 mM Tris-HCL pH 8.0, 1 mM EDTA, 50 mM NaCl) to have to concentrations appropriate for K_d determination: 1,07 µM for Mdm2 and 5,36 µM for MdmX. First column of the Corning NBS 3991 microplate was filled with protein, rest of the columns were filled with FP buffer. Next, serial dilutions of the protein (range from 750 to 0,012 nM final concentration on the plate for Mdm2 and from 3750 to 0,10 nM for MdmX) were prepared in columns 2 -11, last column (12) contained only FP buffer. The highest protein concentration in first column (0,75 µM for Mdm2 and 3,75 µM for MdmX) corresponded to FP values of the peptide saturated with protein; whereas the FP buffer with no protein corresponded to FP values of the peptide alone. To all columns DMSO (Bioshop DMS555.500) previously distributed on Greiner PP 651201 microwell plate was added to protein dilutions on Corning NBS 3991 plate. After mixing and 15 min incubation at room temperature, 40 nM P2 peptide solution prepared in 15 ml Sarstedt Falcon and was added to every column (1-12) so that the final concentration of P2 peptide was 10 nM. FP measurements were made using BioTek Synergy H1 microplate reader. K_d was determined by fitting curve of *Equation 1.* to the experimental data.

Equation 1.

$$y = y_0 \frac{ax}{K_d + x}$$

where y_0: FP_{min}

a: $FP_{max} - FP_{min}$

x: protein concentration

y: FP value measured at the desired concentration.

2. Preparation of stock solutions

Compound **6ad** was dissolved in deuterated DMSO (Sigma Aldrich 175943-10G 08828EJ) to obtain 50 mM concentration. Provided compound was fully dissolved at room temperature at 50 mM and gave yellowish solutions.

3. Determination of the inhibition constants of the compound

Optimal protein concentration for the measurement (f_0=0.8) was calculated based on the determined K_d value (see **SI Table 5** point). FP assay was made using 96-well microplates (Corning NBS 3991) and final volumes of 100 µl (70 µl protein + 5 µl inhibitor + 25 µl P2).

Serial dilutions in DMSO (Bioshop DMS555.500) of the tested compound was prepared in duplicates on 96-well Greiner 651201 microplates (wells A2-H12). The dilutions ranged from 50 µM to 0.05 µM (final concentrations on the plate). Wells A1-F1 were filled with DMSO to obtain the P_{max}, P_{min} and P_{f0} values. Wells G1-H1 were controls filled with Nutlin 3 (Cayman Chemicals; control; final concentration 25 µM). Protein at the optimal concentration was prepared in 15 ml Sarstedt Falcon and added into A2-H12 and E1-H1 wells on Corning NBS 3991 96-well microplates. Wells A1-B1 contained protein at final concentrations of 0.75 µM for Mdm2 and 3.75 µM for MdmX in order to determine P_{max}, whereas wells C1-D1- FP buffer only (to determine P_{min}). Next, 5 µl of each inhibitor dilution was transferred from Greiner 651201 to Corning NBS plate, mixed and incubated for 15 minutes at room temperature. After incubation, 40 nM P2 peptide solution was added (to final 10 nM concentration of the P2) and FP measurements were made using BioTek Synergy H1 microplate reader. Inhibition constants (K_i) were determined by fitting curves into experimental values.

4. NMR measurement

Uniform ^{15}N isotope labeling was achieved by expression of the protein in the M9 minimal media containing $^{15}NH_4Cl$ as the sole nitrogen source. The final step of purification of Mdm2 (residues 1−118, chosen to enable interactions of N-terminal Mdm2 part) for NMR consisted of gel filtration into the NMR buffer (50 mM phosphate buffer at pH 7.4 containing 150 mM NaCl, 5 mM DTT). Then, 10% (v/v) D_2O was added to the samples to provide a lock signal. All the spectra were recorded at 300 K using a Bruker Avance III 600 MHz spectrometer.

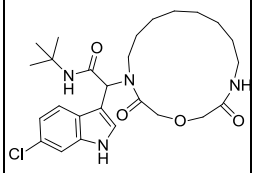
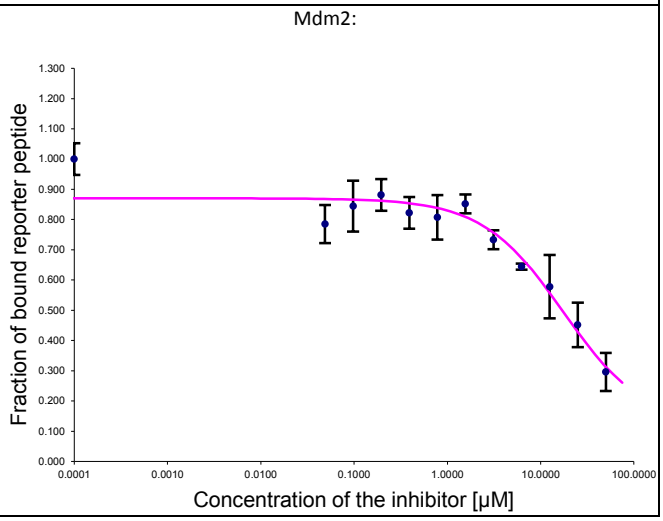
5. Results

The results of the assay of inhibitory activity of tested compound is presented in **SI Table 5**.

6. Summary

Inhibitory activities of **6ad** compound against Mdm2 and MdmX were evaluated using FP assay. **6ad** K_i: 2.25 µM (Mdm2)

SI_Table 5. Results of the evaluation of inhibitory activity of **6ad** compound towards Mdm2/MdmX.

Compound	Structure	Obtained solubility [mM]	K_i Mdm2 [µM]	K_i MdmX [µM]	Plot
6ad		50	2.25	not active	

Crystal structure determination:

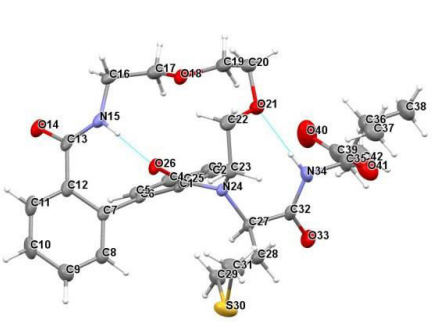
X-ray diffraction data for single crystals of compounds **6i, 6o, 6t, 6z** and **6ad** were collected using SuperNova (Rigaku - Oxford Diffraction) four circle diffractometer with a mirror monochromator and a microfocus MoKα radiation source (λ = 0.7107 Å) for **6i**, **6t** and **6ad** and CuKα radiation source (λ = 1.5418 Å) for **6o** and **6z**. Additionally, the diffractometer was equipped with a CryoJet HT cryostat system (Oxford Instruments) allowing low temperature experiments. Single crystals were mounted on Micro MountsTM. Intensities were collected at 120-130 K. The obtained data sets were processed with CrysAlisPro software [S1]. The phase problem was solved by direct methods using SHELXS [S2] or SUPERFLIP [S3]. Parameters of obtained models were refined by full-matrix least-squares on F^2 using SHELXL-2014/6 [S2]. Calculations were performed using WinGX integrated system (ver. 2013.2) [S4]. Figures were prepared with Mercury 3.5 software [S5].

All non-hydrogen atoms in the crystal structures of **6i, 6o, 6t, 6z** and **6ad** were refined anisotropically to ensure the convergence of the refinement process. All hydrogen atoms attached to carbon atoms were positioned with the idealised geometry and refined using the riding model with the isotropic displacement parameter $U_{iso}[H] = 1.2$ (or 1.5) $U_{eq}[C]$. The position of hydrogen atoms linked to the N atoms were found on the difference Fourier map and refined with no restrains on the isotropic displacement parameter. Crystal data and structure refinement results for compounds **6i, 6o, 6t, 6z** and **6ad** are shown in **SI_Table 6**. Molecular geometry of compounds **6i, 6o, 6t, 6z** and **6ad** observed in the crystal structures are shown in **SI_Figure 1**.

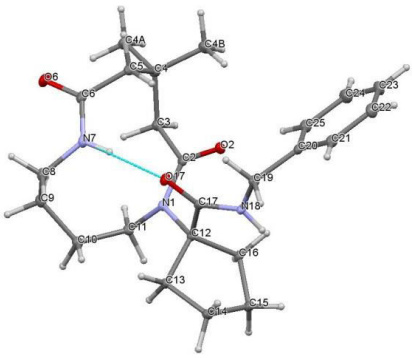
In the crystal structure of compound **6o** a partial, conformational disorder was observed for cyclopentane fragment. The two alternative conformations were modelled with 64% and 36% refined occupancies for components A and B, respectively. Additionally, voids observed in the crystal lattice are filled with disordered water molecule. Due to high disorder observed of the solvent, hydrogen atoms were not included into the final model.

In the crystal structure of compound **6z** a solvent accessible voids are observed (119 $Å^3$), however the Fourier difference map does not indicate solvent position in the crystal lattice.

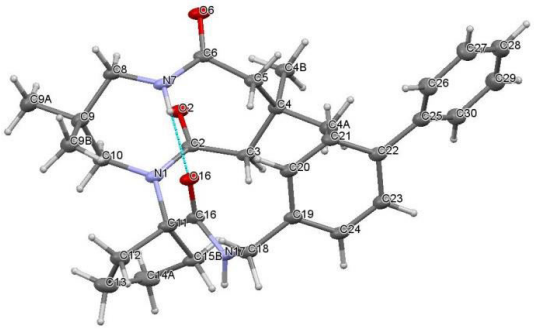
Crystallographic data for structures presented in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 1507159 (**6t**), CCDC 1508125 (**6i**), CCDC 1508127 (**6o**), CCDC 1508126 (**6z**) and CCDC 1547917 (**6ad**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).



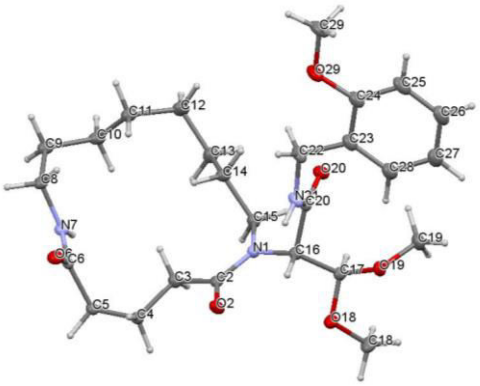
6t



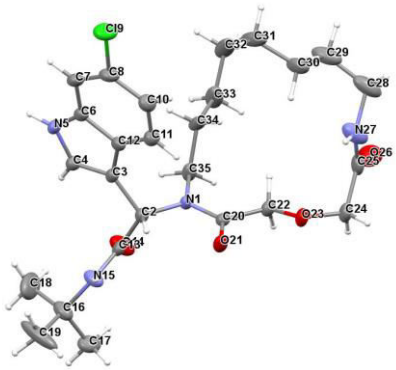
6i



6o



6z



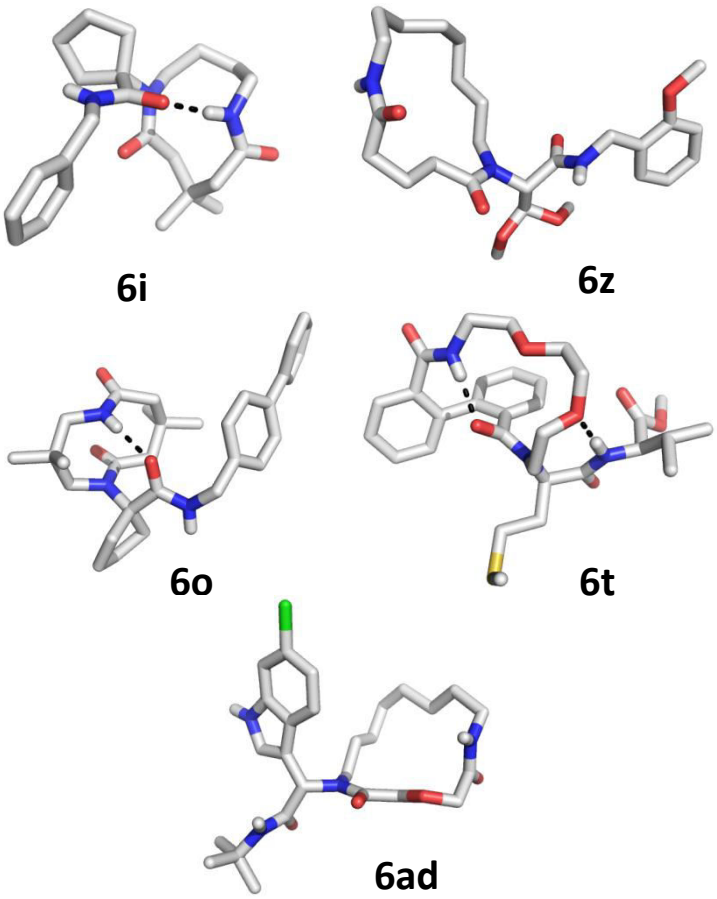
6ad

SI_Figure 1. Molecular geometry observed in the crystal structures of compounds **6i, 6o, 6t, 6z** and **6ad**, showing the atom labelling scheme. For the crystal structure of compound **6o** a conformational disorder is observed and only the more abundant conformer is shown here. The disordered solvent molecules in the asymmetric unit of **6o** was removed for clarity of this figure. For molecules **6i, 6o** and **6t**, the intramolecular hydrogen bond formation is shown with the light-blue, dashed line. Displacement ellipsoids of non-hydrogen atoms are drawn at the 30% probability level. H atoms are presented as small spheres with an arbitrary radius.

SI_Table 6. Crystal data and structure refinement results for compounds **6i, 6o, 6t, 6z** and **6ad**.

	6i	**6o**	**6t**	**6z**	**6ad**
Empirical moiety formula	C_{24} H_{35} N_3 O_3	C_{31} H_{41} N_3 O_3, H_2O	C_{31} H_{41} N_3 O_7 S	C_{26} H_{41} N_3 O_6	C_{26} H_{37} Cl N_4 O_4
Formula weight [g/mol]	413.55	519.61	599.73	491.62	505.05
Temperature [K]	130 (2)	130(2)	119.9 (10)	130(2) K	130 K
Wavelength [Å]	0.7107	1.5418	0.7107	1.5418	0.7107
Crystal system	Monoclinic	Orthorhombic	Monoclinic	Orthorhombic	Hexagonal
Space group	C*c*	P *c c n*	$P2_1/c$	P *b c a*	$P6_1$
Unite cell dimensions	a=14.0931(5) Å b=13.557(4) Å c=12.0951(5) Å α=90° β=108.895(4)° γ=90°	a=27.6390(8) Å b=17.7059(5) Å c= 12.3033(3) Å α=90° β= 90° γ=90°	a=11.5868(3) Å b=14.9472(4) Å c=17.5410(7) Å α= 90.0° β=91.091(3)° γ=90.0°	a=13.252(2) Å b=8.5410(8) Å c= 49.520(7) Å α=90° β= 90° γ=90°	a = 9.8322(2) Å b = 9.8322(2) Å c = 47.9529(10) Å α=90° β=90° γ=120°
Volume [$Å^3$]	2186.49(14)	6020.9(3)	3037.38(17)	5605.0(12)	4014.64(14)
Z	4	8	4	8	6
D_{calc} [Mg/m^3]	1.256	1.147	1.311	1.165	1.244
μ [mm^{-1}]	0.083	0.604	0.158	0.672	0.176
F(000)	896	2240	488	2128	1620
Crystal size [mm^3]	0.6 x 0.4 x 0.3	0.3 x 0.2 x 0.05	0.2 x 0.2 x 0.2	0.3 x 0.1 x 0.04	0.4 x 0.3 x 0.2
Θ range	2.14° to 25.8°	2.96° to 71.06°	2.94° to 28.66°	3.57° to 70.87°	2.93 to 28.63°
Index ranges	-17 ≤ h ≤ 17, -16 ≤ k ≤ 16, -14 ≤ l ≤ 14	-33 ≤ h ≤ 33, -21 ≤ k ≤ 19, -14 ≤ l ≤ 15	-12 ≤ h ≤ 15, -19 ≤ k ≤ 20, -23 ≤ l ≤ 23	-15 ≤ h ≤ 16, -6 ≤ k ≤ 10, -59 ≤ l ≤ 58	-15 ≤ h ≤ 14, -16 ≤ k ≤ 16, -26 ≤ l ≤ 28
Refl. collected	14589	88345	25560	33602	29066
Independent reflections	4030 [R(int)=0.0276]	5797 [R(int) = 0.0533]	7206 [R(int)=0.0577]	5244 [R(int) = 0.0504]	13803 [R(int) = 0.0588]
Completeness [%] to Θ	99.8 (Θ 25.2°)	100 (Θ 67.7°)	99.80 (Θ 26.31°)	99.6 (Θ 67.7°)	99.8 (Θ 26.3°)
Absorption correction	Multi-scan	Multi-scan	Multi-scan	Multi-scan	Multi-scan
Max. and min. transmission	0.638 and 1.000	0.592 to 1.000	0.664 and 1.000	0.845 to 1.000	0.733 and 1.000
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/ restraints/parameters	4030 / 2 / 282	5797 / 14 / 375	7206 / 0 / 391	5244 / 0 / 328	13803 / 0 / 759
GooF on F2	1.042	1.062	1.006	1.049	1.000
Final R indices [I>2sigma(I)]	R1= 0.0446, wR2= 0.1170	R1= 0.0590, wR2= 0.1572	R1= 0.0588, wR2= 0.1363	R1= 0.0467, wR2= 0.1189	R1= 0.0552, wR2= 0.1113
R indices (all data)	R1= 0.0446, wR2= 0.1171	R1= 0.0652, wR2= 0.1631	R1= 0.0897, wR2= 0.1551	R1= 0.0527, wR2= 0.1246	R1= 0.0794, wR2= 0.1279
$\Delta\rho_{max}$, $\Delta\rho_{min}$ [$e \cdot Å^{-3}$]	0.24 and -0.18	0.39 and -0.37	0.68 and -0.38	0.36 and -0.27	0.325 and -0.281

SI_Figure 2. 3D structures and intramolecular hydrogen bonding of several macrocycles **6i**, **6o**, **6t 6z** and **6ad** as indicated by black dotted lines.



Computational modelling of compound **6ad**

Computational modelling of compound **6ad** was performed using MOLOC software.[S6] The crystal structure of the interaction of p53 peptide bound to MDM2 receptor (PDB ID 1YCR) was used for modelling.[S7]

The 2D structure of compound **6ad** was converted into a 3D structure and energy minimized in the absence of the receptor. Next the 3D structure of compound **6ad** was manually placed into the MDM2 receptor and the indol ring of compound **6ad** was aligned to the indol ring of W23 of the p53 peptide. The tert-butyl amide group of compound **6ad** was oriented into the F19 pocket of MDM2 as seen in other small molecule MDM2 cocrystal structures (PDB ID 3TU1, 3TJ2, 4MDN).[S8] Next the macrocycle was energy optimized in the MDM2 receptor using the standard settings of the force filed of MOLOC.

The structure was rendered using PYMOL (the PyMOL molecular graphics system, version 1.2r3pre, Schrödinger, LLC).

References:

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