Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

New Cylindrical Peptide Assemblies Defined by Extended Parallel β-Sheets

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1. Preparation of the compounds

General Methods.

All chemicals commercially obtained were utilised as received. Thin-layer chromatography (TLC) was carried out using Merck aluminium sheets with silica gel 60 $F_{254.}$ The compounds were visualised with an Oliphant (6W – 254 nm tube) UV lamp and/or potassium permanganate stain (1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 mL 10% $NaOH_{(aq)}$ and 200 mL water). Flash column chromatography was carried out using Merck Kiselgel 60 (230-400 mesh). HPLC purification was performed on Hewlett Packard Series 1100 HPLC machine using Gemini and Discovery columns. All yields reported are isolated yields judged to be homogenous by TLC and NMR spectroscopy. ^{1}H NMR spectra were recorded on a 300 or 600 MHz spectrometer. ^{1}H NMR spectra (600 MHz) and ^{13}C NMR spectra (151 MHz) on a Varian Inova spectrometer. All spectra are reported relative to TMS (δ_H = 0.00 ppm), $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.0 ppm), $(CD_3)_2SO$ (δ_H = 2.50 ppm, δ_C = 39.5 ppm), D_2O (δ_H = 4.79 ppm) or CD_3OD (δ_H = 3.31 ppm, δ_C = 49.0 ppm). Chemical shifts (δ) are reported in ppm and all coupling constants were calculated to one decimal place. Spin multiplicities are represented by the following signals: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). IR spectra were recorded on a Perkin Elmer Spectrometer using using 20 mM solutions in DMSO or by solid deposition on surface. ESI high resolution mass spectra (FTMS) were recorded at the Adelaide Proteomics Centre, University of Adelaide, Australia on an LTQ Orbitrap XL ETD spectrometer injected via a syringe pump at a flow rate of 5 μL/min. Mass spectra analysis was performed using the XCalibur software (Version 2.0.7, Thermo Fisher Scientific). Melting points were measured on a microscope hot stage melting point apparatus and are uncorrected. Oven dried glassware was used in all reactions carried out under an inert atmosphere (either dry nitrogen or argon). All starting materials and reagents were obtained commercially unless otherwise stated. Removal of solvents “under reduced pressure” refers to the process of bulk solvent removal by rotary evaporation (low vacuum pump) followed by application of high vacuum pump (oil pump) for a minimum of 30 min. High-performance liquid chromatography (HPLC) purification of peptide products was carried out using a Phenomenex C18 column (250 x 21.2 mm), a photodiode array detector, and a Sedex evaporative light scattering detector. Both water / acetonitrile / TFA (10 / 90 / 0.001 by v / v) and water / TFA (100 / 0.001 by v / v) solutions were used for mobile phases.

A General Procedure: Peptide coupling reaction

To a stirred solution of the *N*-protected amino acid derivative (1.0 eq) in dry CH_2Cl_2 (50-75 mM) was added EDC (1.3 eq), HOBt.H_2O (1.5 eq) and DIPEA (3.0 eq). The solution was stirred at rt overnight, diluted with CH_2Cl_2 and the organic layer washed twice with 1M $HCl_{(aq)}$, saturated aqueous $NaHCO_{3(aq)}$ and brine. The organic layer was dried over $MgSO_{4(s)}$ and the solvent removed *in vacuo*. See individual experiments for details.

B. General Procedures: Hydrolysis of methyl esters

The methyl ester (1.0 eq) was dissolved in THF (100 mM). The solution was stirred at 0^{o}C for 15 min. 1M LiOH (5.0 eq) was added slowly and the mixture was stirred vigorously at rt for 16 h. The mixture was diluted with EtOAc and acidified to pH 7 with 2M $HCl_{(aq)}$. The aqueous layer was extracted with EtOAc (2×25 mL). The combined organic layer was washed with brine and dried over $MgSO_{4(s)}$. The solvent was removed *in vacuo* to give the crude product. For details, see individual experiments.

C. General Procedures: Hydrolysis of tert-butyl esters

Compound containing tert-butyl esters (0.2 mmol) was dissolved in CH_2Cl_2 (5 mL) under a nitrogen atmosphere. Trifluoroacetic acid (2 mL) was added and the solution was stirred at rt for 4 h. The solvent was removed under vacuum. For details, see individual experiments.

D. General Procedures: Conversion of amines to azides

Sodium azide (35 mmol) was suspended in anhydrous acetonitrile (35 mL) and the mixture cooled in an ice bath with stirring. Triflic anhydride (29 mmol) was added dropwise over 10 min and the cooled solution stirred for 2 h. The mixture was filtered through cotton wool to give crude triflic azide. A suspension of N-protected amino acid amine (24 mmol) was vigorously stirred in acetonitrile (100 mL) and water (40 mL), triethylamine (73 mmol) and $CuSO_{4(s)}$ (0.24 mmol) were added. The resulting solution was cooled in an ice bath and the triflic azide solution added dropwise. After 30 min, the ice bath was removed and the reaction was allowed to stir at rt overnight. The acetonitrile was removed *in vacuo* and the remaining aqueous solution washed with EtOAc (2 × 100 mL). The aqueous phase was then acidified with 2M $HCl_{(aq)}$ and extracted with EtOAc (3 × 100 mL), dried over $MgSO_{4(s)}$, filtered and the solvent removed *in vacuo* to give an oil. Chloroform was added, the solution refrigerated, and the triflyl amine by-product removed by filtration. The solvent was evaporated *in vacuo*, and the residue purified by flash column chromatography (1% CH_3OH in CH_2Cl_2) to give the amino acid azide as a pale yellow oil. This was suspended in methanol, cooled in ice, and 20% (v/v) thionyl chloride was added portion wise. The solution was stirred in ice for 1 h and then at rt for 18 h before being concentrated *in-vacuo*.

E. General Procedures: One pot esterification and N-BOC cleavage

A suspension of *N*-Boc carboxylic acid in methanol was cooled in an ice bath and 20% (v/v) thionyl chloride was added-portion wise. The solution was stirred in ice for 1 h and then at rt for 18 h before being concentrated *in-vacuo*.

F. General Procedures: Ring Closing Click Reaction (RCC)

To solution of the azide alkyne in anhydrous CH_2Cl_2 (1.6 mM) under nitrogen atmosphere was added DBU (3.0 eq) while the solution was stirred vigorously at rt. After 15 min, Cu(I)Br (1.0 eq) was added. The mixture was stirred for 7 hrs. The reaction was quenched by adding 3M $HCl_{(aq)}$. The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 500 mL). The combined organic layer was washed with water (500 mL), brine (500 mL), dried over $MgSO_{4(S)}$, filtered, and concentrated *in-vacuo*. The product was purified by flash column chromatography (1% CH_3OH in CH_2Cl_2) to give a white solid. See individual experiments for details.

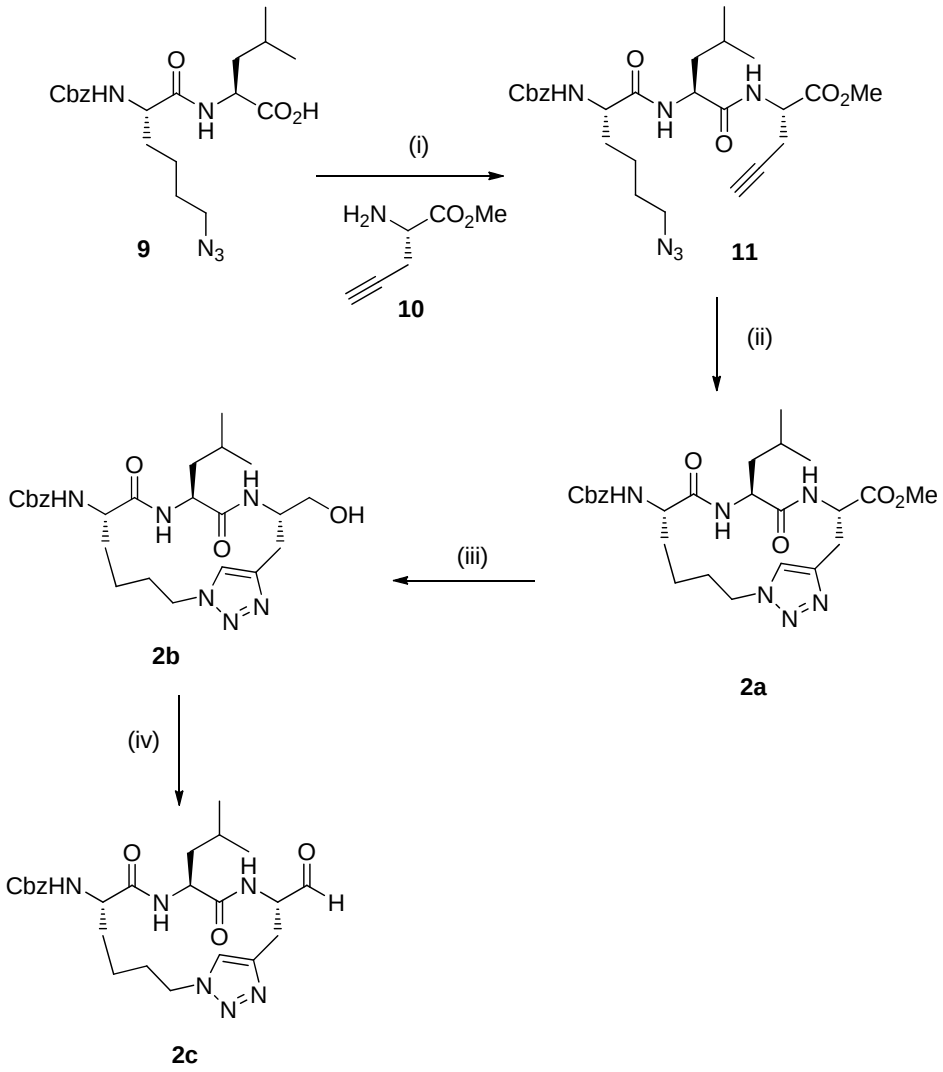
G. General Procedures: Lithium borohydride reduction of methyl ester to alcohol

A solution of the methyl ester in anhydrous THF (0.1M), under an atmosphere of nitrogen, was cooled in ice. 2M $LiBH_4$ in THF was added (3.0 eq). The mixture was stirred in ice for 1 h and then at rt for a further 17 h. Methanol was added to quench the reaction and the mixture was stirred at rt for 10 min before being concentrated *in-vacuo*. The residue was partitioned between EtOAc and 1M $KHSO_{4(aq)}$.The aqueous phase was extracted twice with chloroform and each organic extract separately was washed with brine, before being combined, dried over $MgSO_{4(s)}$, filtered and concentrated *in-vacuo*. The residue was purified by reverse phase HPLC.

H. General Procedures: Oxidation of an amino alcohol.

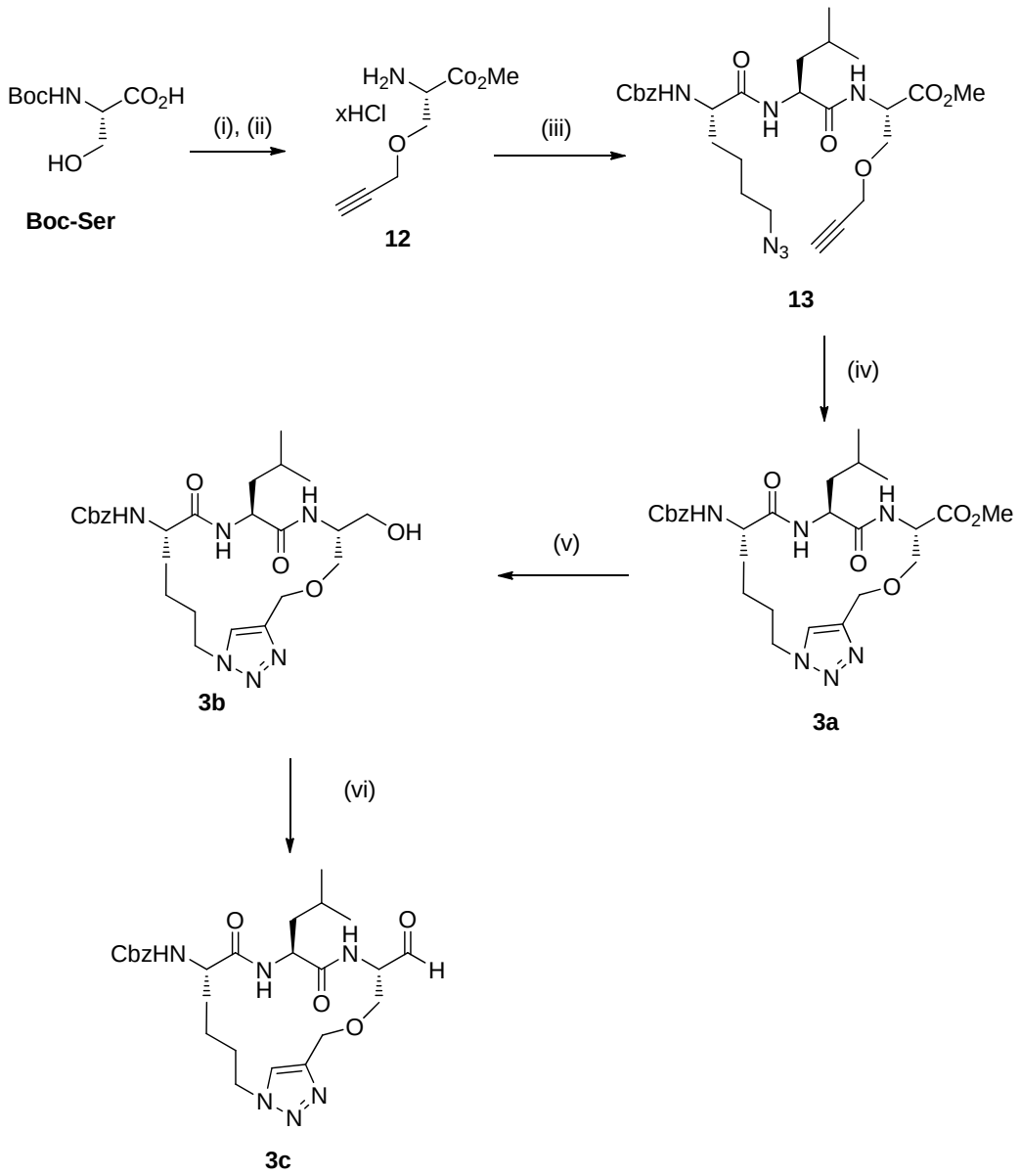
The alcohol (1.0 eq) was suspended in anhydrous CH_2Cl_2 (5 mM) under a nitrogen atmosphere. Dess-Martin periodinane reagent (3.0 eq) was added to the solution with vigorous stirring under a nitrogen atmosphere. The reaction mixture was stirred for 1.25 h at rt and then quenched by adding saturated $NaHCO_{3(aq)}$ containing 20 mM solution of $Na_2S_2O_{5(aq)}$. The aqueous layer was extracted with CH_2Cl_2 (2×10 mL) and the combined organic phase was washed with water (2×10 mL) and brine (20 mL). The organic layer was dried over $Na_2SO_{4(s)}$ and concentrated *in vacuo* to give a white solid that was purified by flash column chromatography (99:1 CH_2Cl_2/CH_3OH).

2. Scheme S1[a]



[a]Reagents and conditions: (i) **10**, EDCI, HOBt, DIPEA, (85%); (ii) CuBr, DBU, CH_2Cl_2 (75%); (iii) $LiBH_4$, THF (55%); (iv) DMP, CH_2Cl_2 (80%).

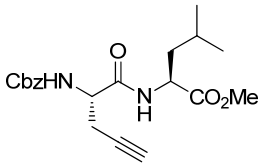
3. Scheme S2[a]



[a]Reagents and conditions: (i) NaH, *Propargyl bromide, DMF* (55%); (ii) $SOCl_2$, MeOH (85%); (iii) **9**, EDCI, HOBt, DIPEA, (75%); (iv) CuBr, DBU, CH_2Cl_2 (75%); (v) $LiBH_4$, THF (57%); (vi) DMP, CH_2Cl_2 (78%).

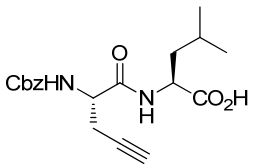
4. Synthesis and characterization

(*S*)-tert-Butyl 2-((*S*)-2-(((Benzyloxy)carbonyl)amino)pent-4-ynamido)-4-methylpentanoate 5



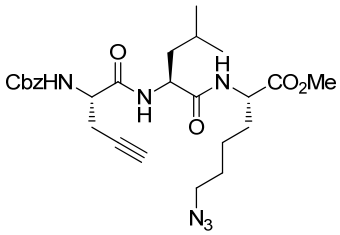
Carboxylic acid **4** (2.50 g, 10.11 mmol) was reacted with (*S*)-Leu-OMe·HCl according to General Procedure A. The crude product was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give compound **5** (3.0 g, 80%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 – 7.29 (m, 5H), 6.65 (s, 1H), 5.56 (s, 1H), 5.22 – 5.08 (m, 2H), 4.68 – 4.56 (m, 1H), 4.43 – 4.35 (m, 1H), 3.73 (s, 3H), 2.88 – 2.80 (m, 1H), 2.64 – 2.58 (m, 1H), 2.08 (t, *J* = 2.6 Hz, 1H), 1.70 – 1.60 (m, 2H), 1.59 – 1.51 (m, 1H), 0.95 – 0.89 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.8, 169.4, 156.0, 135.9, 128.5, 128.3, 128.1, 79.1, 71.8, 67.3, 53.1, 52.2, 50.9, 41.5, 24.7, 22.6, 22.4, 21.9. HRMS (ES) 397.1772 $(M+Na)^+$; $C_{20}H_{26}N_2O_5Na$ requires 397.1739.

(*S*)-2-((*S*)-2-(((Benzyloxy)carbonyl)amino)pent-4-ynamido)-4-methylpentanoic acid 6



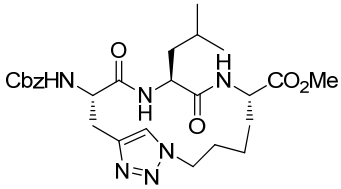
Dipeptide **5** (2.0 g, 5.5 mmol) in THF (35 mL) was hydrolysed according to General Procedure B. The residue was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **6** as yellow oil (2.4 g, 85%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 5H), 6.84 (d, *J* = 5.9 Hz, 1H), 5.72 (s, 1H), 5.13 (s, 2H), 4.65 – 4.58 (m, 1H), 4.47 – 4.39 (m, 1H), 2.83 – 2.76 (m, 1H), 2.62 (dd, *J* = 16.9, 4.5 Hz, 1H), 2.07 (t, *J* = 2.2 Hz, 1H), 1.75 – 1.64 (m, 2H), 1.63 – 1.56 (m, 1H), 0.95 – 0.91 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.9, 170.1, 156.1, 135.8, 128.6, 128.3, 128.1, 78.9, 72.0, 67.4, 53.2, 51.0, 41.0, 24.8, 22.7, 22.3, 21.8. HRMS (ES) 383.1589 $(M+Na)^+$; $C_{19}H_{24}N_2O_5Na$ requires 383.1583.

(5*S*,8*S*,11*S*)-Methyl 11-(4-azidobutyl)-8-isobutyl-3,6,9-trioxo-1-phenyl-5-(prop-2-yn-1-yl)-2-oxa-4,7,10-triazadodecan-12-oate 8



Carboxylic acid **6** (2.0 g, 5.5 mmol) was coupled with amine **7** according to General Procedure A. The crude product was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **8** as a white solid (2.4 g, 85 %). mp >230 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.30 (m, 5H), 6.68 (d, *J* = 7.7 Hz, 1H), 6.65 (d, *J* = 6.5 Hz, 1H), 5.57 (d, *J* = 6.2 Hz, 1H), 5.13 (s, 2H), 4.56 (dd, *J* = 13.2, 7.7 Hz, 1H), 4.47 (dd, *J* = 14.1, 8.6 Hz, 1H), 4.42 – 4.32 (m, 1H), 3.75 (s, 3H), 3.31 – 3.21 (m, 2H), 2.87 – 2.80 (m, 1H), 2.64 (dd, *J* = 6.8, 2.6 Hz, 1H), 2.61 (dd, *J* = 6.8, 2.6 Hz, 1H), 2.09 (t, *J* = 2.6 Hz, 1H), 1.92 – 1.81 (m, 1H), 1.75 – 1.51 (m, 5H), 1.44 – 1.31 (m, 2H), 0.97 – 0.86 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.3, 171.1, 169.8, 155.9, 135.8, 128.6, 128.4, 128.2, 78.9, 72.2, 67.5, 53.3, 52.4, 52.0, 51.9, 51.0, 40.7, 31.8, 28.3, 24.7, 22.8, 22.4, 22.0. HRMS (ES) 529.2770 $(M + H)^+$; $C_{26}H_{37}N_6O_6$ requires 529.2775.

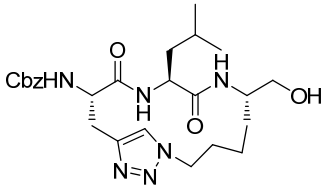
(6*S*,9*S*,12*S*)-Methyl 12-(((Benzyloxy)carbonyl)amino)-9-isobutyl-8,11-dioxo-1,7,10,15,16-pentaazabicyclo[12.2.1]heptadeca-14(17),15-diene-6-carboxylate 1a



The amino acid azide alkyne **8** (1.0 g, 1.89 mmol) was cyclised according to General Procedure F. The crude product was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **1a** as a white solid (750 mg, 75%). mp >230 ºC; IR: amide I, 1641 cm^{-1}; amide II, 1532 cm^{-1}; N-H stretch, 3292 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.31 (d, *J* = 9.1 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.70 (s, 1H), 7.43 – 7.28 (m, 5H), 7.08 (d, *J* = 7.9 Hz, 1H), 5.15 – 5.00 (m, 2H), 4.56 – 4.41 (m, 2H), 4.38 – 4.21 (m, 3H), 3.61 (s, 3H), 3.04 (dd, *J* = 14.5, 8.8 Hz, 1H), 2.95 (dd, *J* = 14.6, 4.2 Hz, 1H), 1.96 – 1.73 (m, 2H), 1.67 – 1.32 (m, 6H), 1.16 – 0.98 (m, 1H), 0.95 – 0.73 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.1, 17.3, 169.0, 155.2, 142.0, 136.8, 128.2, 127.6, 127.5, 122.2, 65.3, 52.9, 51.8, 50.7,

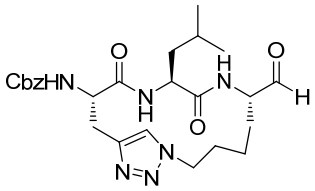
48.9, 40.9, 29.5, 28.1, 27.0, 23.9, 22.3, 22.2, 20.7. HRMS (ES) 529.2771 $(M + H)^+$; $C_{26}H_{37}N_6O_6$ requires 529.2775.

Benzyl ((6*S*,9*S*,12*S*)-6-(hydroxymethyl)-9-isobutyl-8,11-dioxo-1,7,10,15,16-pentaazabicyclo[12.2.1]heptadeca-14(17),15-dien-12-yl)carbamate 1b



Ester **1a** (600 mg, 1.1 mmol) was reduced according to General Procedure G and the crude product was purified by HPLC to give **1b** as a white solid (310 mg, 55%). mp >230 ºC; IR: amide I, 1637 cm^{-1}; amide II, 1535 cm^{-1}; N-H stretch, 3281 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.08 (d, J = 8.4 Hz, 1H), 7.80 – 7.64 (m, 2H), 7.42 – 7.29 (m, 5H), 6.94 (d, J = 7.8 Hz, 1H), 5.17 – 4.93 (m, 2H), 4.59 (t, J = 5.3 Hz, 1H), 4.54 – 4.39 (m, 1H), 4.36 – 4.24 (m, 2H), 4.18 (m, 1H), 3.88 – 3.71 (m, 1H), 3.29 – 3.14 (m, 2H), 3.11 – 2.93 (m, 2H), 2.12 – 1.91 (m, 1H), 1.67 – 1.30 (m, 6H), 1.26 – 1.10 (m, 1H), 1.03 – 0.98 (m, 1H), 0.94 – 0.72 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.6, 169.4, 155.7, 142.3, 137.5, 128.7, 128.2, 128.0, 123.01, 65.8, 64.5, 53.5, 51.6, 49.6, 49.0, 41.5, 30.3, 28.6, 28.1, 24.5, 23.0, 22.7, 21.4. HRMS (ES) 501.2819 $(M + H)^+$; $C_{25}H_{37}N_6O_5$ requires 501.2825.

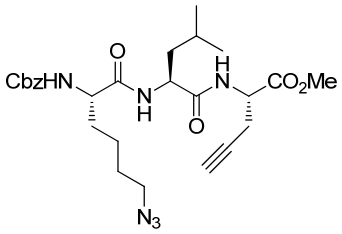
Benzyl ((6*S*,9*S*,12*S*)-6-formyl-9-isobutyl-8,11-dioxo-1,7,10,15,16 pentaazabicyclo[12.2.1]heptadeca-14(17),15-dien-12-yl)carbamate 1c



Alcohol **1b** (200 mg, 0.39 mmol) was oxidised according to General Procedure H and the crude product was purified by chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **1c** as a white solid (150 mg, 80 %) that contained a trace of the starting alcohol **1b** by ^{1}H NMR. mp 173-176 ºC; IR: amide I, 1636cm^{-1}; amide II, 1535 cm^{-1}; N-H stretch, 3280 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.42 (s, 1H), 8.26 (d, J = 8.7 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.68 (s, 1H), 7.44 – 7.28 (m, 5H), 7.11 (d, J = 8.0 Hz, 1H), 5.10 – 5.01 (m, 2H), 4.51 – 4.39 (m, 1H), 4.35 – 4.07 (m, 4H), 3.05 (dd, J = 14.4, 8.9 Hz, 1H), 2.96 (dd, J = 14.4, 3.9 Hz, 1H), 2.02 – 1.77 (m, 2H), 1.68 – 1.49 (m, 2H), 1.48 – 1.31 (m, 4H), 1.08 – 0.95 (m, 1H), 0.92

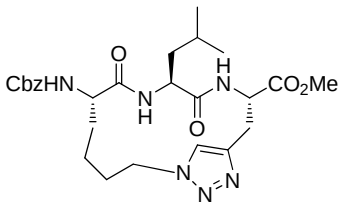
– 0.76 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 200.9, 172.0, 169.0, 155.2, 141.9, 136.8, 128.2, 127.6, 127.5, 122.3, 65.3, 55.8, 53.1, 51.0, 48.8, 40.9, 27.9, 27.2, 26.4, 24.0, 22.4, 22.1, 20.3. HRMS (ES) 499.2661 $(M + H)^+$; $C_{25}H_{35}N_6O_5$ requires 499.2669.

(5*S*,8*S*,11*S*)-Methyl 5-(4-azidobutyl)-8-isobutyl-3,6,9-trioxo-1-phenyl-11-(prop-2-yn-1-yl)-2-oxa-4,7,10-triazadodecan-12-oate 11



Carboxylic acid **9** (1.5 g, 3.55 mmol) was coupled with amine **10** according to General Procedure A. The crude product was purified by column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **11** as a white solid (1.56 g, 83 %). mp >230 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.30 (m, 5H), 6.76 (d, J = 6.6 Hz, 1H), 6.39 (d, J = 6.8 Hz, 1H), 5.28 (d, J = 6.9 Hz, 1H), 5.11 (s, 2H), 4.73 – 4.64 (m, 1H), 4.455 – 4.44 (m, 1H), 4.24 – 4.12 (m, 1H), 3.79 (s, 3H), 3.26 (t, J = 6.6 Hz, 2H), 2.84 – 2.69 (m, 2H), 2.10 – 1.99 (m, 1H), 1.96 – 1.82 (m, 1H), 1.77 – 1.50 (m, 6H), 1.50 – 1.36 (m, 2H), 0.93 (dd, J = 13.1, 6.4 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.4, 171.3, 170.4, 156.1, 135.9, 128.6, 128.3, 128.13, 78.1, 71.8, 67.2, 54.7, 52.8, 51.7, 51.1, 50.7, 41.1, 31.9, 28.4, 24.7, 22.8, 22.6, 22.2, 21.9. HRMS (ES) 529.2776 $(M + H)^+$; $C_{26}H_{37}N_6O_6$ requires 529.2775.

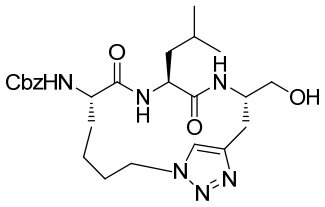
(6*S*,9*S*,12*S*)-Methyl 6-(((Benzyloxy)carbonyl)amino)-9-isobutyl-7,10-dioxo-1,8,11,15,16-pentaazabicyclo[12.2.1]heptadeca-14(17),15-diene-12-carboxylate 2a



The amino acid azide alkyne **11** (1.4 g, 2.60 mmol) was cyclised according to General Procedure F. The crude product was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **2a** as a white solid (1.0 g, 72%). mp >230 ºC; IR: amide I, 1638cm^{-1}; amide II, 1536 cm^{-1}; N-H stretch, 3277 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60 (d, J = 9.0 Hz, 1H), 8.18 (d, J = 8.9 Hz, 1H), 7.52 (s, 1H), 7.40 – 7.27 (m, 5H), 7.16 (d, J = 7.1 Hz, 1H), 4.99 (s, 2H), 4.80 – 4.74 (m, 1H), 4.40 (dd, J = 16.1, 7.9 Hz, 1H), 4.30 (t, J =

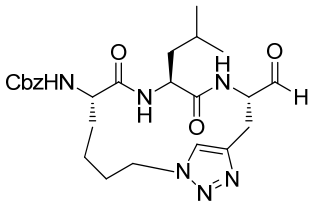
5.6 Hz, 2H), 4.03 (dd, *J* = 13.4, 6.7 Hz, 2H), 3.68 (s, 3H), 3.20 (dd, *J* = 15.1, 2.8 Hz, 1H), 3.04 – 2.88 (m, 2H), 1.82 – 1.69 (m, 3H), 1.67 – 1.38 (m, 3H), 1.36 – 1.20 (m, 1H), 0.93 – 0.81 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.4, 171.1, 170.3, 155.1, 142.4, 137.0, 128.1, 127.6, 127.5, 122.5, 65.0, 53.5, 52.1, 50.1, 48.5, 41.4, 31.2, 30.5, 28.9, 27.3, 23.8, 22.4, 22.2, 20.6. HRMS (ES) 529.2763 $(M + H)^+$; $C_{26}H_{37}N_6O_6$ requires 529.2775.

Benzyl ((6*S*,9*S*,12*S*)-12-(hydroxymethyl)-9-isobutyl-7,10-dioxo-1,8,11,15,16-pentaazabicyclo[12.2.1]heptadeca-14(17),15-dien-6-yl)carbamate 2b



Ester **2a** (900 mg, 1.71 mmol) was reduced according to General Procedure G and the crude product was purified by HPLC to give **2b** as a white solid (450 mg, 53%). mp >230 ºC; IR: amide I, 1634cm^{-1}; amide II, 1536 cm^{-1}; N-H stretch, 3279 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, *J* = 9.1 Hz, 1H), 8.06 (d, *J* = 9.3 Hz, 1H), 7.45 (s, 1H), 7.38 – 7.26 (m, 5H), 7.12 (d, *J* = 7.2 Hz, 1H), 4.98 (m, 2H), 4.39 – 4.21 (m, 4H), 4.16 – 4.02 (m, 2H), 3.35 – 3.23 (m, 3H), 2.99 (dd, *J* = 15.3, 2.4 Hz, 1H), 2.58 (dd, *J* = 15.2, 12.3 Hz, 1H), 1.80 – 1.70 (m, 1H), 1.63 – 1.45 (m, 4H), 1.43 – 1.30 (m, 2H), 0.98 – 0.73 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.1, 170.2, 155.1, 143.8, 137.0, 128.1, 127.5, 127.5, 122.1, 65.0, 63.8, 53.4, 50.4, 49.3, 48.3, 41.6, 31.29, 29.0, 27.4, 23.9, 22.5, 22.2, 20.5. HRMS (ES) 523.2651 $(M + Na)^+$; $C_{25}H_{36}N_6O_5Na$ requires 523.2645.

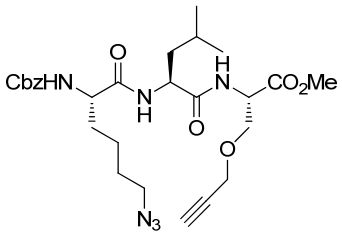
Benzyl ((6*S*,9*S*,12*S*)-12-formyl-9-isobutyl-7,10-dioxo-1,8,11,15,16-pentaazabicyclo[12.2.1]heptadeca-14(17),15-dien-6-yl)carbamate 2c



Alcohol **2b** (300 mg, 0.59 mmol) was oxidised according to General Procedure H and the crude product was purified by chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **2c** as a white solid (233 mg, 77 %). mp 168-171 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.48 (d, *J* = 8.7 Hz, 1H), 8.20 (d, *J* = 8.9 Hz, 1H), 7.53 (s, 1H), 7.38 – 7.26 (m, 5H), 7.21 (d, *J* = 7.3 Hz, 1H), 5.04 – 4.93 (m, 2H), 4.77 – 4.64 (m, 1H), 4.40 (dd, *J* = 15.6, 8.5 Hz,

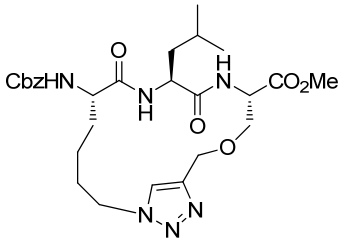
1H), 4.30 (t, *J* = 5.6 Hz, 3H), 4.14 – 3.95 (m, 2H), 3.26 (dd, *J* = 15.2, 3.0 Hz, 1H), 2.76 (dd, *J* = 15.0, 12.3 Hz, 1H), 1.83 – 1.68 (m, 1H), 1.66 – 1.54 (m, 2H), 1.54 – 1.33 (m, 4H), 0.98 – 0.72 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 199.9, 171.9, 170.3, 155.0, 142.3, 137.0, 128.1, 127.6, 127.4, 122.7, 65.0, 56.5, 53.5, 50.4, 48.4, 41.3, 31.0, 28.8, 24.5, 23.9, 22.4, 22.1, 20.6. HRMS (ES) 499.2663 $(M + H)^+$; $C_{25}H_{35}N_6O_5$ requires 499.2669.

(5*S*,8*S*,11*S*)-Methyl 5-(4-azidobutyl)-8-isobutyl-3,6,9-trioxo-1-phenyl-11-((prop-2-yn-1-yloxy)methyl)-2-oxa-4,7,10-triazadodecan-12-oate 15



Carboxylic acid **9** (1.5 g, 3.57 mmol) was coupled with amine **12** according to General Procedure A. The crude product was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **13** as a white solid (1.53 g, 77 %). mp 196-200 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 5H), 6.66 (d, *J* = 7.9 Hz, 1H), 6.42 (d, *J* = 7.6 Hz, 1H), 5.29 (d, *J* = 7.3 Hz, 1H), 5.11 (s, 2H), 4.78 – 4.68 (m, 1H), 4.56 – 4.45 (m, 1H), 4.24 – 4.11 (m, 4H), 4.00 (dd, *J* = 9.5, 3.1 Hz, 1H), 3.77 (s, 3H), 3.73 (d, *J* = 2.6 Hz, 1H), 3.26 (t, *J* = 6.7 Hz, 3H), 2.48 (t, *J* = 2.3 Hz, 1H), 1.99 – 1.79 (m, 1H), 1.77 – 1.50 (m, 4H), 1.51 – 1.35 (m, 2H), 1.02 – 0.87 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 171.4, 170.2, 156.3, 136.1, 128.7, 128.4, 128.2, 78.9, 75.4, 69.1, 67.3, 58.7, 54.7, 52.8, 52.5, 51.8, 51.2, 41.6, 32.2, 28.5, 24.8, 23.0, 22.7, 22.1. HRMS (ES) 559.2874 $(M + H)^+$; $C_{27}H_{39}N_6O_7$ requires 559.2880.

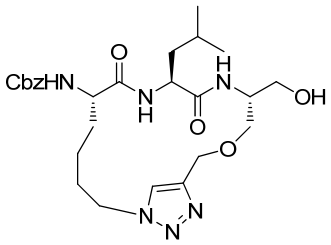
(6*S*,9*S*,12*S*)-Methyl 6-(((Benzyloxy)carbonyl)amino)-9-isobutyl-7,10-dioxo-14-oxa-1,8,11,17,18-pentaazabicyclo[14.2.1]nonadeca-16(19),17-diene-12-carboxylate 3a



The amino acid azide alkyne **15** (1.4 g, 2.50 mmol) was cyclised according to General Procedure F. The crude product was purified by flash column chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **3a** as a white solid (1.1 g, 80%). mp >230 ºC; IR: amide I,

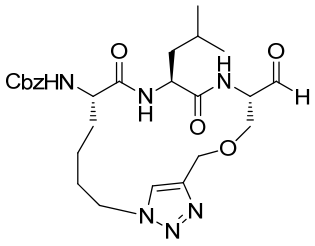
1635 cm^{-1}; amide II, 1534 cm^{-1}; N-H stretch, 3302 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.7 Hz, 1H), 7.86 (s, 1H), 7.41 – 7.27 (m, 5H), 7.18 (d, J = 7.5 Hz, 1H), 5.01 – 4.96 (m, 2H), 4.74 (dd, J = 12.1, 7.6 Hz, 1H), 4.60 (d, J = 12.3 Hz, 1H), 4.52 – 4.43 (m, 2H), 4.41 – 4.28 (m, 2H), 4.14 – 4.03 (m, 1H), 3.69 (d, J = 4.3 Hz, 1H), 3.67 (s, 3H), 3.54 (dd, J = 10.4, 7.2 Hz, 1H), 1.87 – 1.77 (m, 1H), 1.67 (dd, J = 7.2, 3.7 Hz, 1H), 1.62 – 1.42 (m, 3H), 1.38 (t, J = 7.1 Hz, 2H), 1.09 – 0.99 (m, 2H), 0.86 (d, J = 6.5 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.7, 170.3, 170.0, 155.2, 143.2, 137.0, 128.1, 127.6, 127.5, 123.9, 68.1, 65.0, 62.5, 53.54, 52.0, 50.8, 50.2, 49.0, 41.6, 31.2, 29.0, 23.8, 22.7, 21.9, 21.2. HRMS (ES) 559.2874 $(M + H)^{+}$; $C_{27}H_{39}N_6O_7$ requires 559.2880.

Benzyl ((6*S*,9*S*,12*R*)-12-(hydroxymethyl)-9-isobutyl-7,10-dioxo-14-oxa-1,8,11,17,18-pentaazabicyclo[14.2.1]nonadeca-16(19),17-dien-6-yl)carbamate 3b



Ester **3a** (1.0 g, 1.89 mmol) was reduced according to General Procedure G and the crude product was purified by HPLC to give **3b** as a white solid (499 mg, 52%). mp >230 ºC; IR: amide I, 1634 cm^{-1}; amide II, 1534 cm^{-1}; N-H stretch, 3287 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.99 (d, J = 8.6 Hz, 1H), 7.91 – 7.79 (m, 2H), 7.36 – 7.23 (m, 5H), 7.10 (d, J = 7.6 Hz, 1H), 5.04 – 4.90 (m, 2H), 4.56 – 4.45 (m, 2H), 4.43 – 4.21 (m, 3H), 4.07 (dd, J = 13.1, 7.0 Hz, 1H), 3.98 – 3.86 (m, 1H), 3.44 – 3.25 (m, 4H), 3.21 – 3.14 (m, 1H), 1.88 – 1.73 (m, 1H), 1.69 – 1.59 (m, 1H), 1.58 – 1.45 (m, 3H), 1.41 – 1.24 (m, 2H), 1.10 – 0.97 (m, 2H), 0.88 – 0.75 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.3, 170.8, 155.7, 143.8, 137.6, 128.7, 128.1, 128.0, 124.6, 67.8, 65.6, 62.5, 60.8, 54.0, 51.1, 50.0, 49.6, 42.1, 32.1, 29.7, 24.4, 23.4, 22.3, 21.8. HRMS (ES) 531.2925 $(M + H)^{+}$; $C_{26}H_{39}N_6O_6$ requires 531.2931.

Benzyl ((6*S*,9*S*,12*S*)-12-formyl-9-isobutyl-7,10-dioxo-14-oxa-1,8,11,17,18-pentaazabicyclo[14.2.1]nonadeca-16(19),17-dien-6-yl)carbamate 3c



Alcohol **3b** (300 mg, 0.56 mmol) was oxidised according to General Procedure H and the crude product was purified by chromatography on silica gel (1% CH_3OH in CH_2Cl_2) to give **3c** as a white solid (227 mg, 76 %). mp 140-144 ºC; IR: amide I, 1634 cm^{-1}; amide II, 1529 cm^{-1}; N-H stretch, 3282 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.43 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.90 (s, 1H), 7.34 (d, J = 3.7 Hz, 5H), 7.20 (d, J = 7.7 Hz, 1H), 5.14 – 4.91 (m, 2H), 4.76 – 4.58 (m, 2H), 4.57 – 4.45 (m, 2H), 4.35 (t, J = 5.4 Hz, 2H), 4.20 – 4.01 (m, 1H), 3.78 – 3.60 (m, 2H), 1.90 – 1.71 (m, 1H), 1.66 – 1.34 (m, 5H), 1.28 – 1.20 (m, 2H), 1.10 – 0.98 (m, 1H), 0.92 – 0.70 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 200.0, 172.6, 170.8, 155.7, 143.6, 137.6, 128.7, 128.1, 128.0, 124.5, 66.6, 65.6, 63.0, 57.9, 54.1, 50.8, 49.5, 42.1, 31.8, 29.6, 24.4, 23.3, 22.3, 21.9; HRMS (ES) 529.2769 $(M + H)^+$; $C_{26}H_{37}N_6O_6$ requires 529.2775.

5. Enzyme assays

1. m-Calpain Inhibition Assay[1]

m-Calpain, partially purified from sheep lung by ion-exchange chromatography, was diluted in a mixture containing 20 mM MOPS, 2 mM EGTA, 2 mM EDTA, 0.5% *β*-mercapto ethanol (pH 7.5), to give a linear response over the course of the assay. The substrate solution (0.0005% BODIPY-FL casein in 10 mM $CaCl_2$, 0.1 mM NaN_3, and 0.1% mercaptoethanol) was prepared fresh each day. Calpain inhibition assays were performed in 96-well black *Whatman*© plates at 25 °C. Calpain control assays contained 50 μL of sample buffer and 100 μL of substrate solution was added to initiate the reaction. The progress of the reaction was followed for 10 min in a (BMG) Fluorostar with excitation at 485 nm and emission at 530 nm. For inhibitor assays, the sample buffer was replaced with 50 μL of inhibitor dissolved in DMSO (2% total concentration) diluted in water. The percentage inhibition was determined as 100 times the activity with inhibitor present divided by the activity of the control assay. The reported IC_{50} values are the average of triplicate determinations.

1. A. D. Abell, M. A. Jones, A. T. Neffe, S. G. Aitken, T. P. Cain, R. J. Payne, S. B. McNabb, J. M. Coxon, B. G. Stuart, D. Pearson, H. Y. Y. Lee and J. D. Morton, *J. Med. Chem.*, 2007, **50**, 2916.

6. X-ray Crystallographic Determination of the Structure of 1a

A sample of **1a** (5 mg) was crystallized from a 1:1 mixture of methanol/chloroform, by slow evaporation over a period of 8-10 days. A colourless, thin, rod-shaped crystal was slected for single crystal X-ray crystallography. The crystal was mounted under oil onto a plastic loop and X-ray data collected at low temperatures with Mo-$K\alpha$ radiation (λ = 0.71073 Å). Data was collected on an Oxford Diffraction X-Calibur diffractometer and corrected for polarisation and Lorenztian effects, and absorption corrections applied using a multi-scan method. Structures were solved by direct methods using SHELXS-97[2] and refined by full-matrix least squares on F^2 by SHELXL-97.[3] Unless otherwise stated, all non-hydrogen atoms were refined anisotropically and hydrogen atoms were included as invariants at geometrically estimated positions. Diagrams were generated using the program X-Seed[4] as an interface to POV-Ray.[5] CCDC number 868566 contains the full crystal data for this structure. These data can be obtained from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

A view of the asymmetric unit of **1a** with the atoms shown as ellipsoids at the 50% probability level is shown in Figure SI 1.

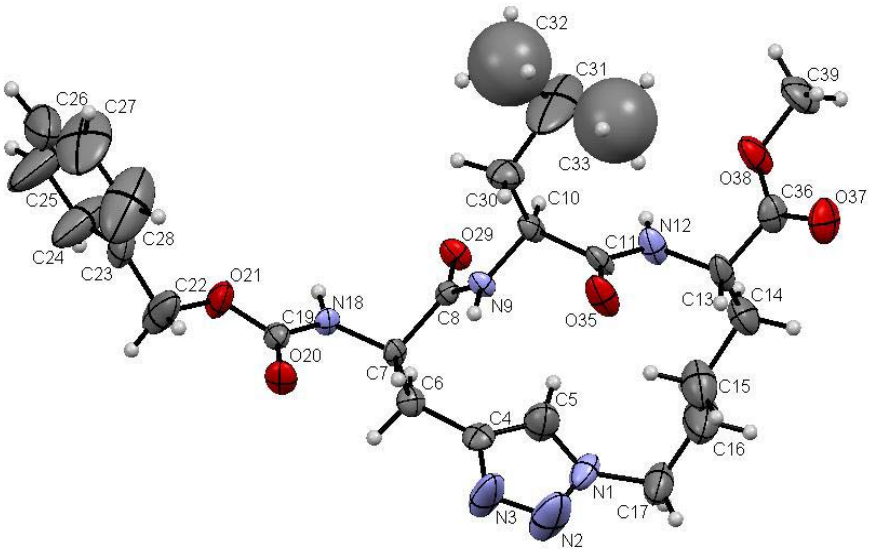


Figure S1. The asymmetric unit of the structure of **1a** with the atoms shown as ellipsoids at the 50% probability level.

The leucine side chain of **1a** is disordered and the methyl carbon atoms of this moiety were modelled with fixed U_{iso} parameters and DFIX restraints to maintain a chemically sensible structure.

2. G. M. Sheldrick, *Acta Cryst.* 1990, ***A46***, 467.
3. G. M. Sheldrick, *SHELXL-97* University of Göttingen, Göttingen, Germany, 1997.
4. L. J. Barbour, *J. Supramol. Chem.* 2001, ***1***, 189.
5. Persistence of Vision Raytracer Pty. Ltd, *POV-Ray* Williamstown, Australia, 2003-2008.

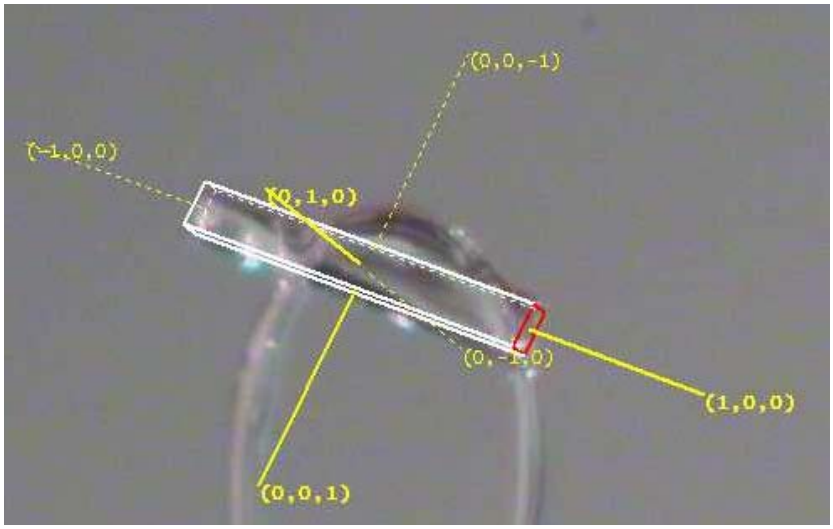
7. SEM Measurement

SEM Images of Macrocycles 1a and 2a

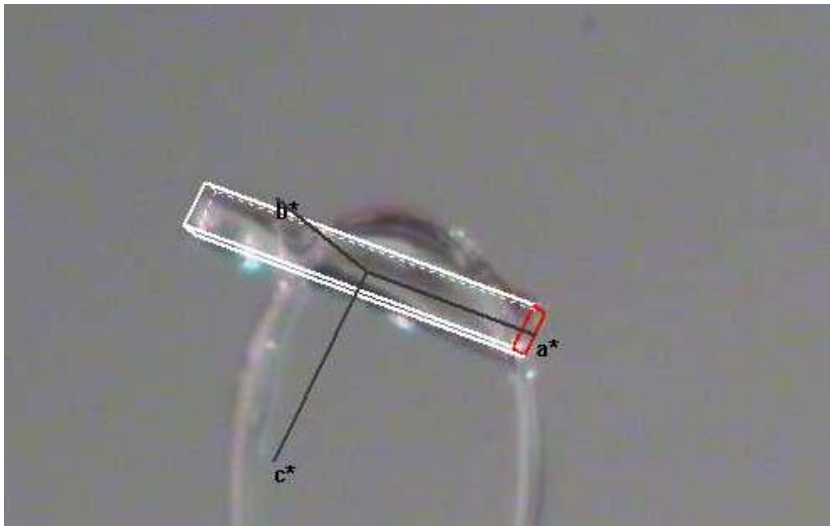
The SEM images where collected on a Philips XL20 Tungsten filament Scanning Electron Microscope. The samples were prepared by depositing solid samples of macrocycles **1a** and **2a** on 12 mm stubs. Large rod-like fibres were observed (Figure 4(a) in manuscript). The morphology of the solid samples of compounds **1a** and **2a** are consistent with bundling of the self-assembling nanotubular structures.

8. Face Indexing of 1a

CrysAlisPro was used to face index the crystal used for data collection. The indexed faces and the orientation of the reciprocal axis are shown in Figure S2. As crystals of **1a** have an orthorhombic unit cell, the *reciprocal* lattice vectors a*, b*, c* are parallel to the unit cell axes a, b, c, respectively.[6] Thus the *a*-axis is aligned along the long length of the needle and the direction of assembling of the nanotubular structure Figure S3.



(a)



(b)

Figure S2 (a) A view of the crystal of **1a** used for the structure determination and the Miller indices of the faces. (b) The orientation of the reciprocal lattice, which for an orthorhombic crystal system corresponds to the unit cell orientation.

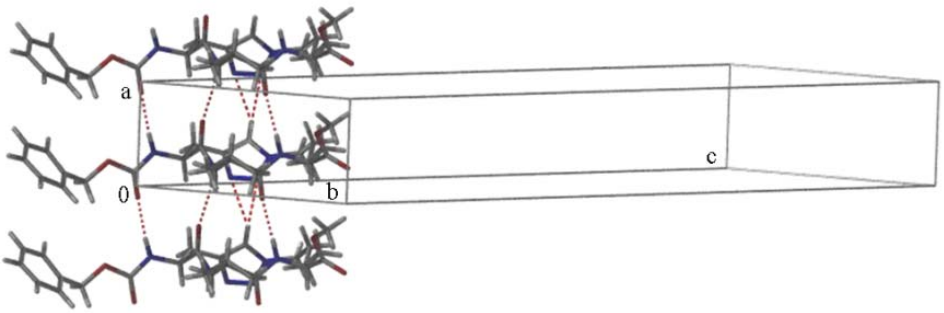


Figure S3. The packing of the nanotubular structure **1a** and the unit cell showing the alignment of the nanotubes along the *a*-axis.

6 Hammond, C. The Basics of Crystallography and Diffraction, Oxford University Press, Oxford, U.K., **2001.**

9. Infrared spectra

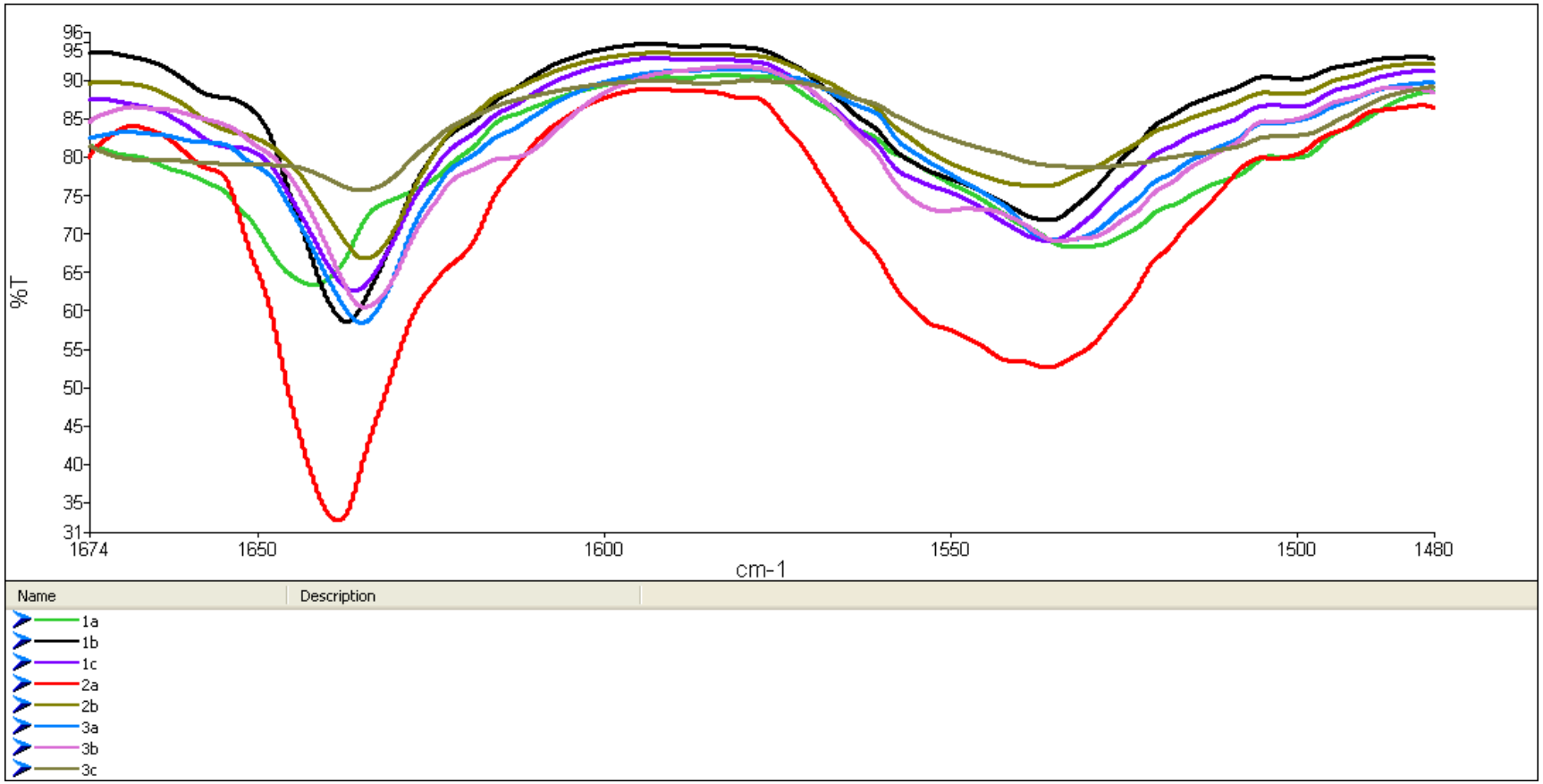


Figure S4. Overlay of infrared spectra of amide I and amide II regions (**1a**, **1b**, **1c**, **2a**, **2b**, **3a**, **3b** and **3c**) of nanotube crystals.

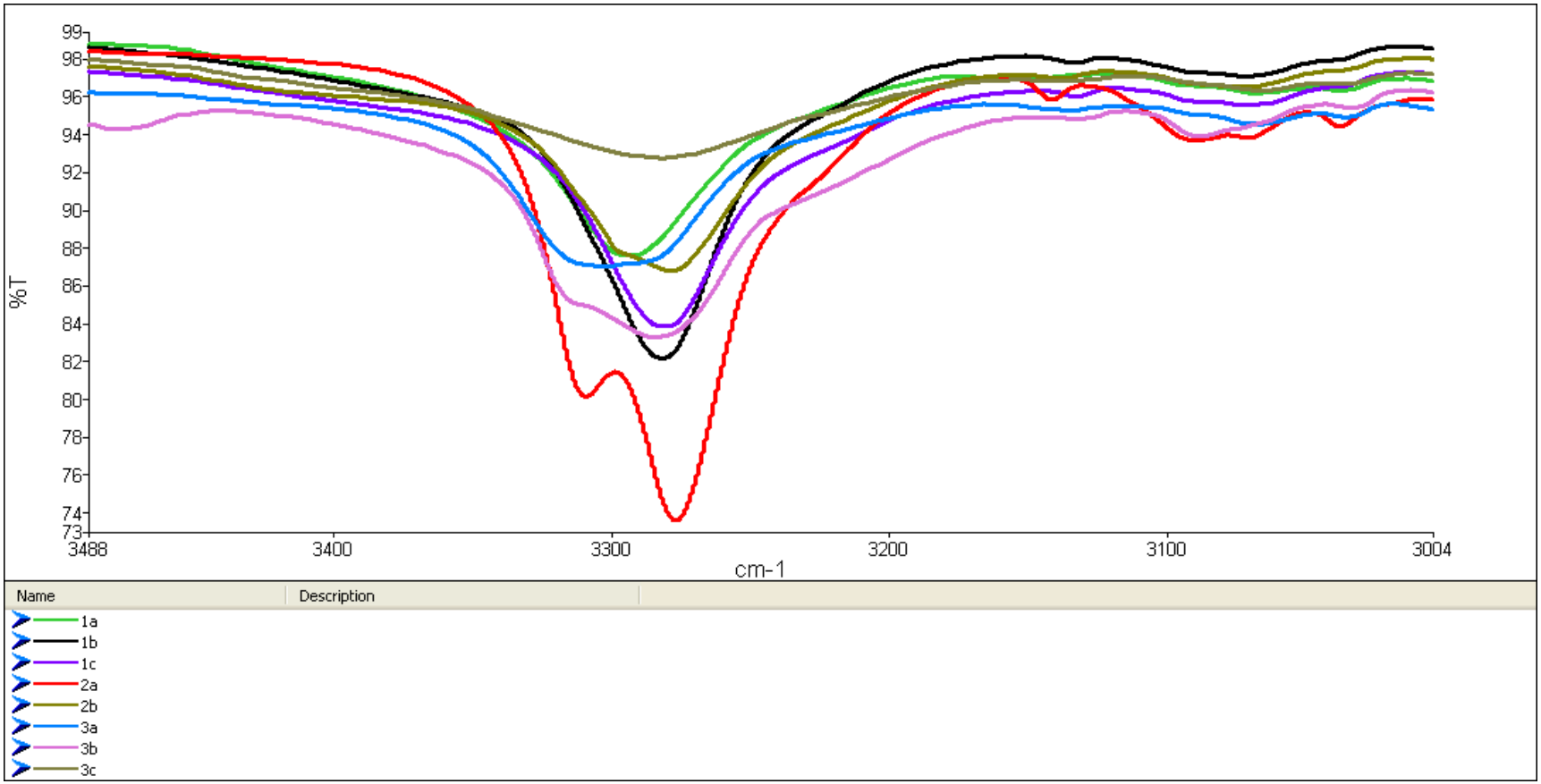


Figure S5. Overlay of infrared spectra of N-H stretch regions (**1a**, **1b**, **1c**, **2a**, **2b**, **3a**, **3b** and **3c**) of nanotube crystals.

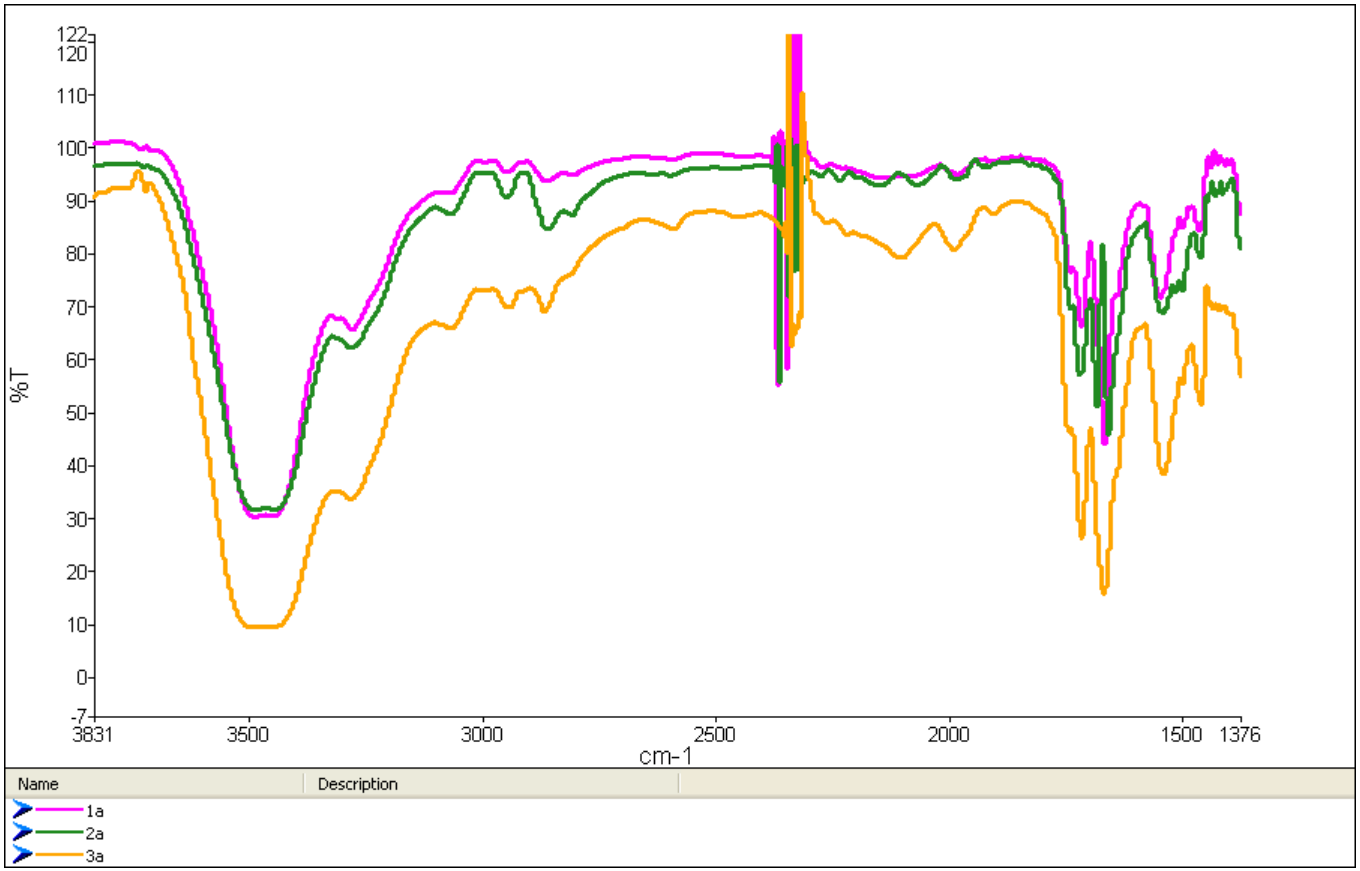


Figure S6. Overlay of infrared spectra of amide I, amide II and N-H stretch regions (**1a**, **2a** and **3a**) in solution (20 mM DMSO).

.

10. Electrospray Ionisation Mass Specta

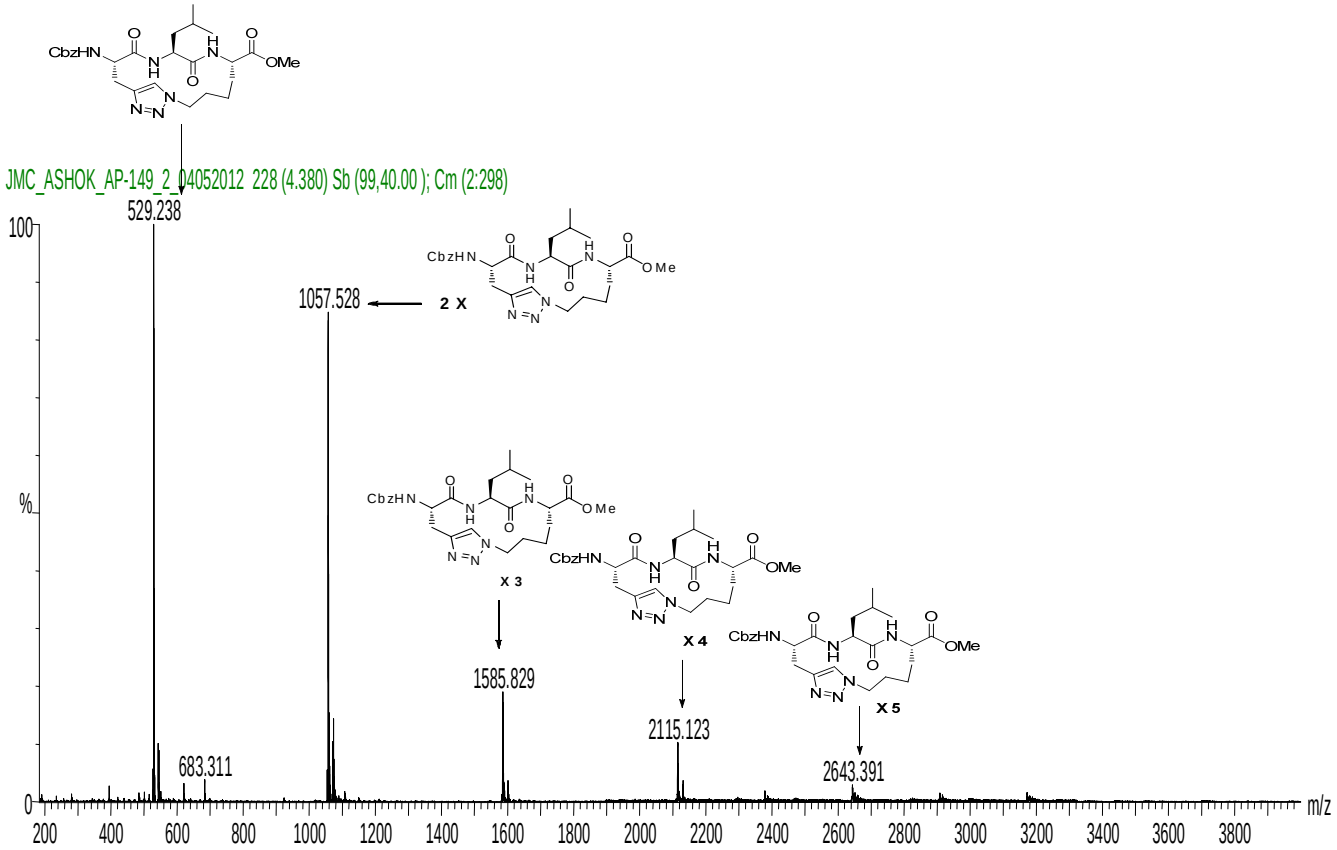


Figure S7. ESI-MS spectrum of compound **1a** $[M+ H]^+$ showing the monomer, dimer, trimer tetramer and pentamer,.

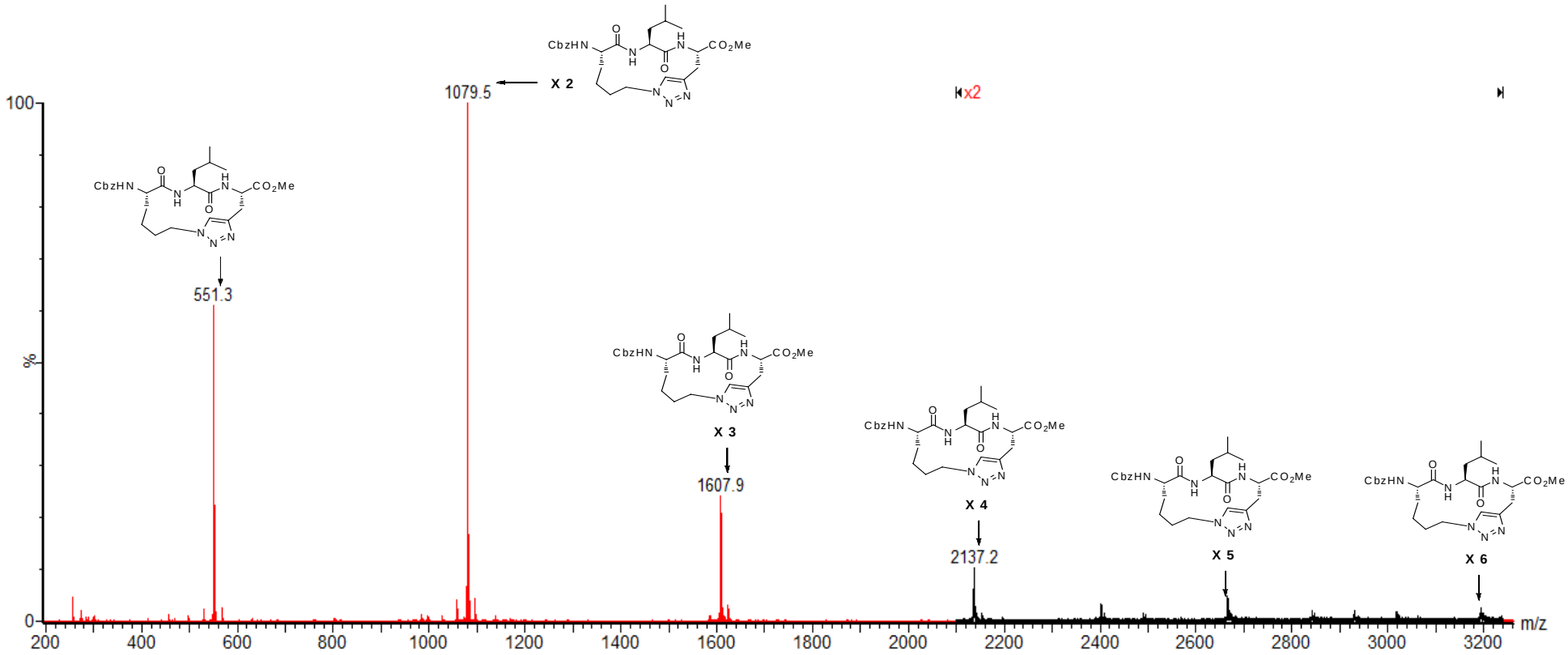


Figure 8 ESI-MS spectrum of compound **2a** $[M+ Na]^+$ showing the monomer, dimer, trimer, tetramer, pentamer, and hexamer.

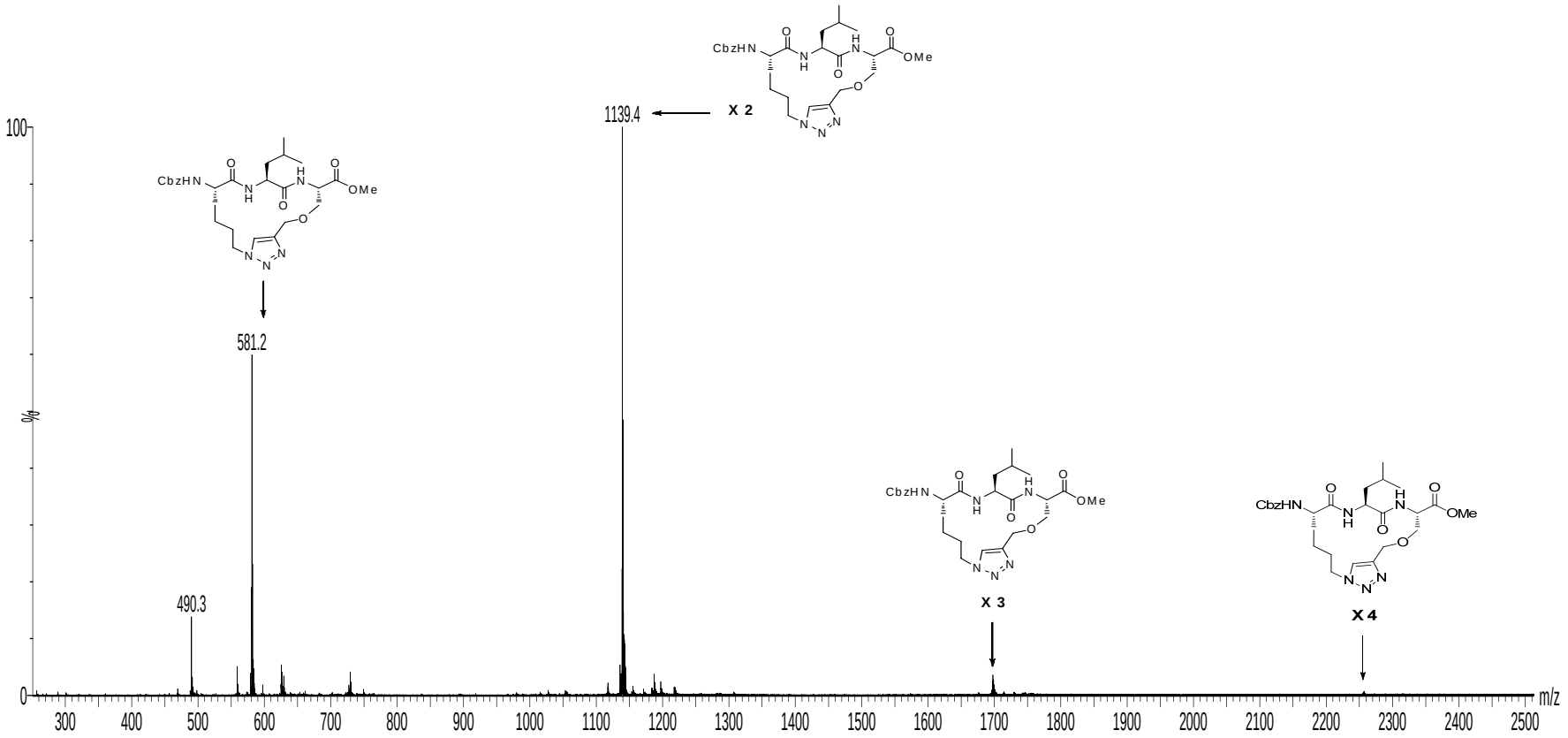


Figure S9. ESI-MS spectrum of compound **3a** $[M+ Na]^+$ showing the monomer, dimer, trimer and tetramer .

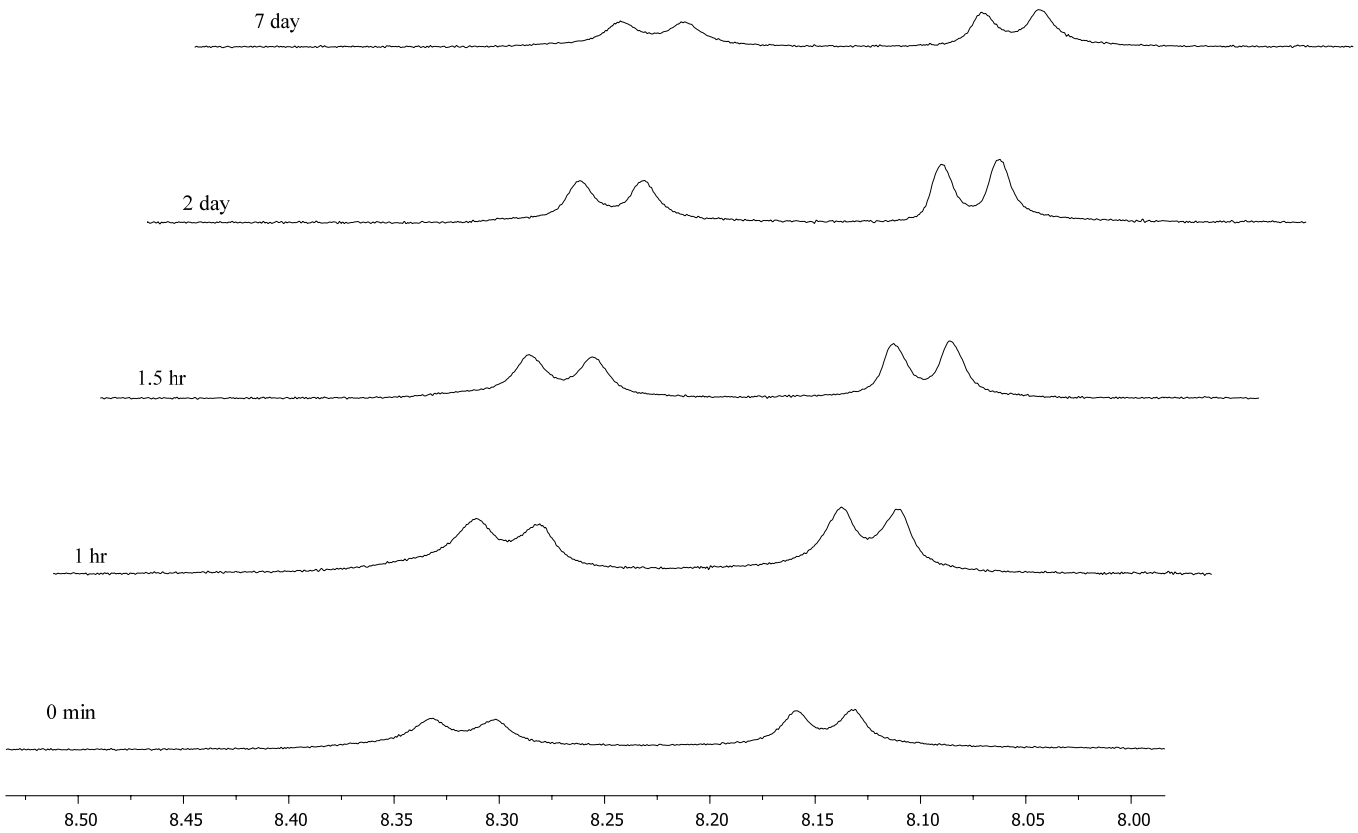
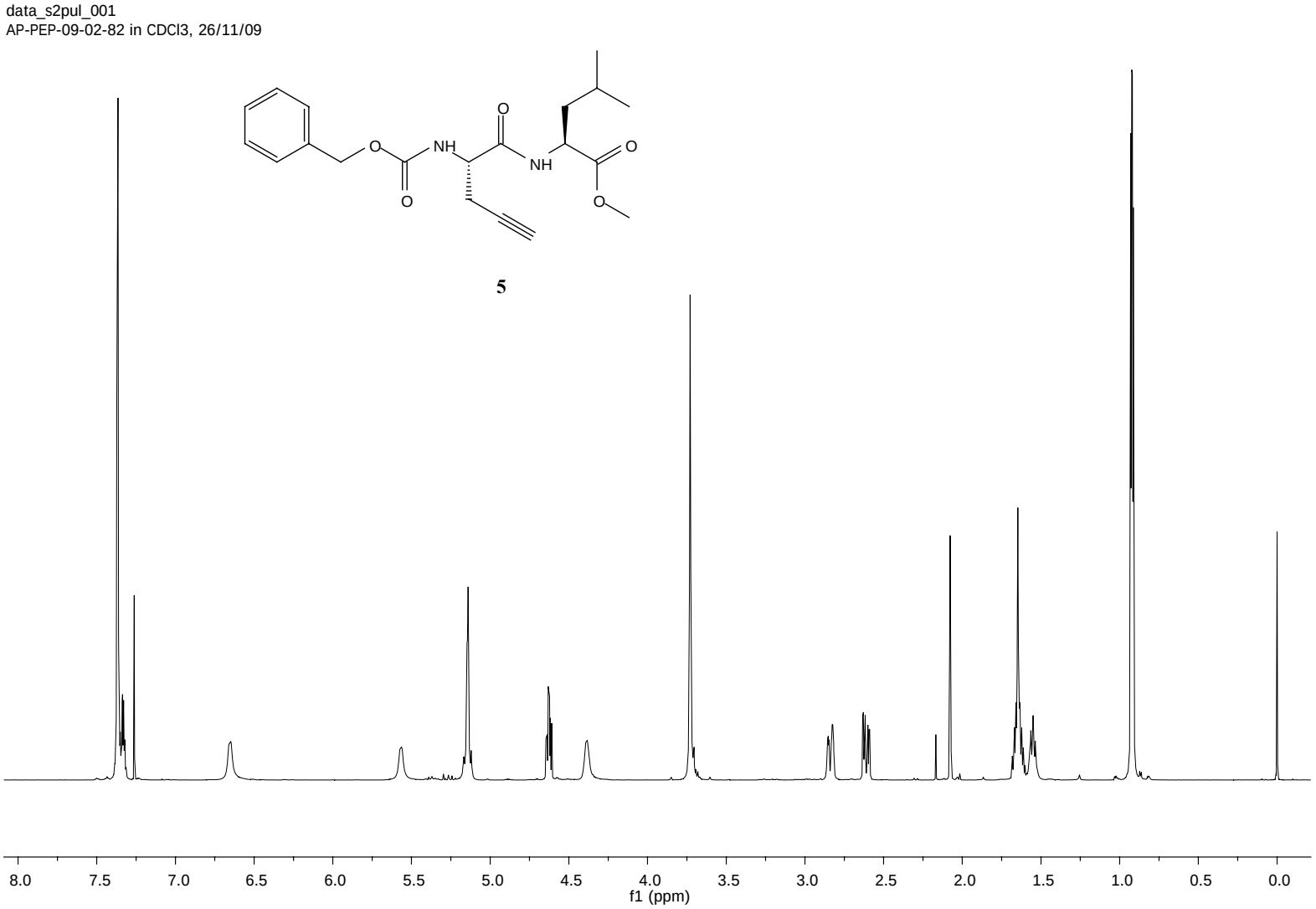


Figure S10 Relative H/D exchange rates for the amide protons of **1a** (20% DMSO/ CD_3OD), (Lys NH δ 8.33 and LeuNH, δ 8.15). A progressive upfield shift is apparent, possibly due to a lengthening of the intermolecular interaction.

11. ^{1}H and ^{13}C NMR spectra



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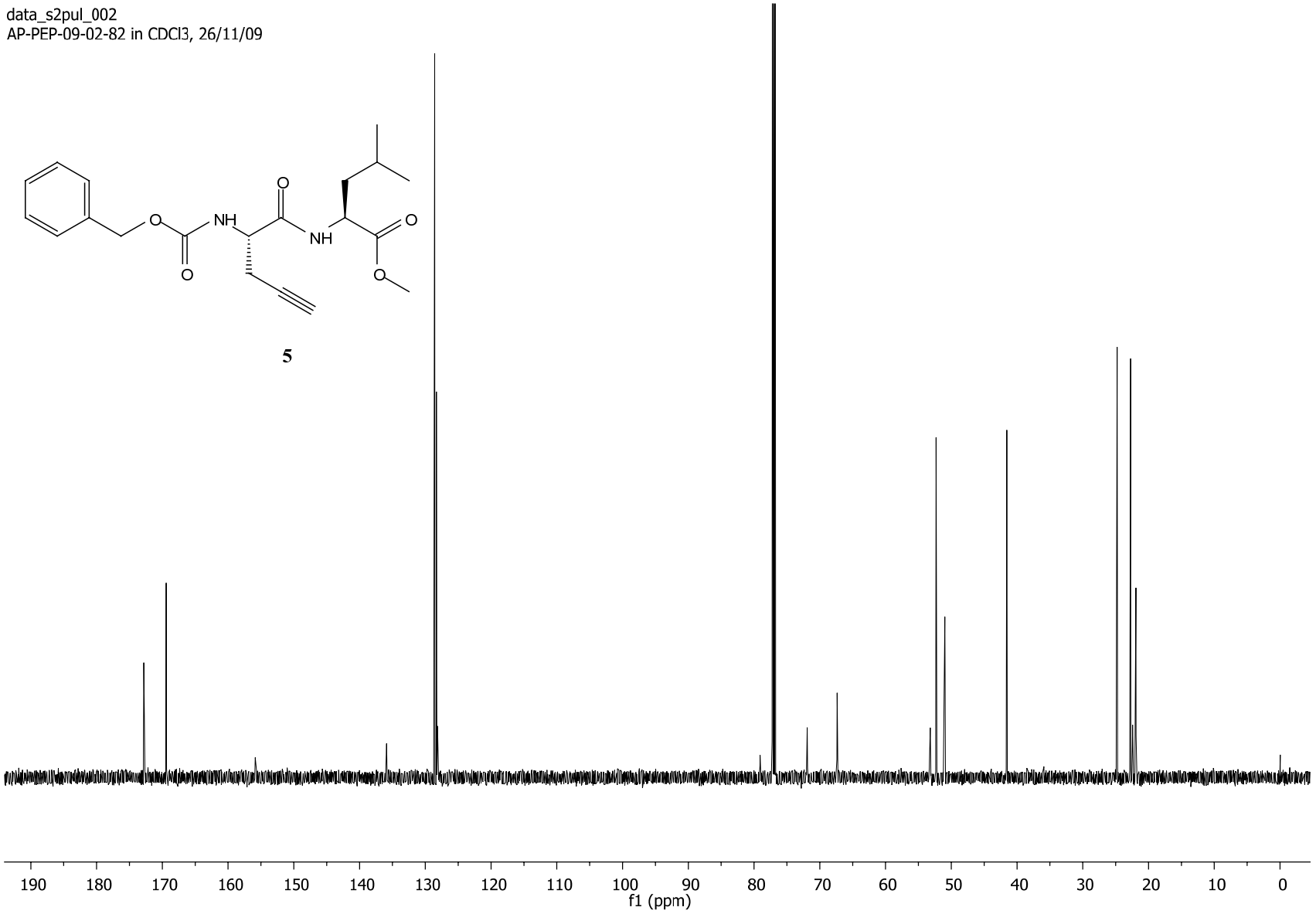

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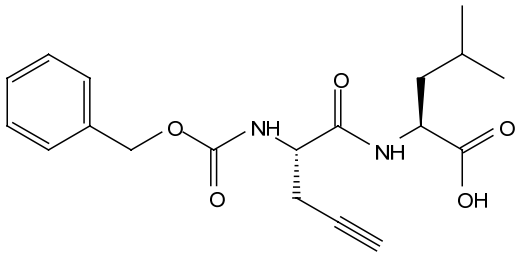
APPEP10135



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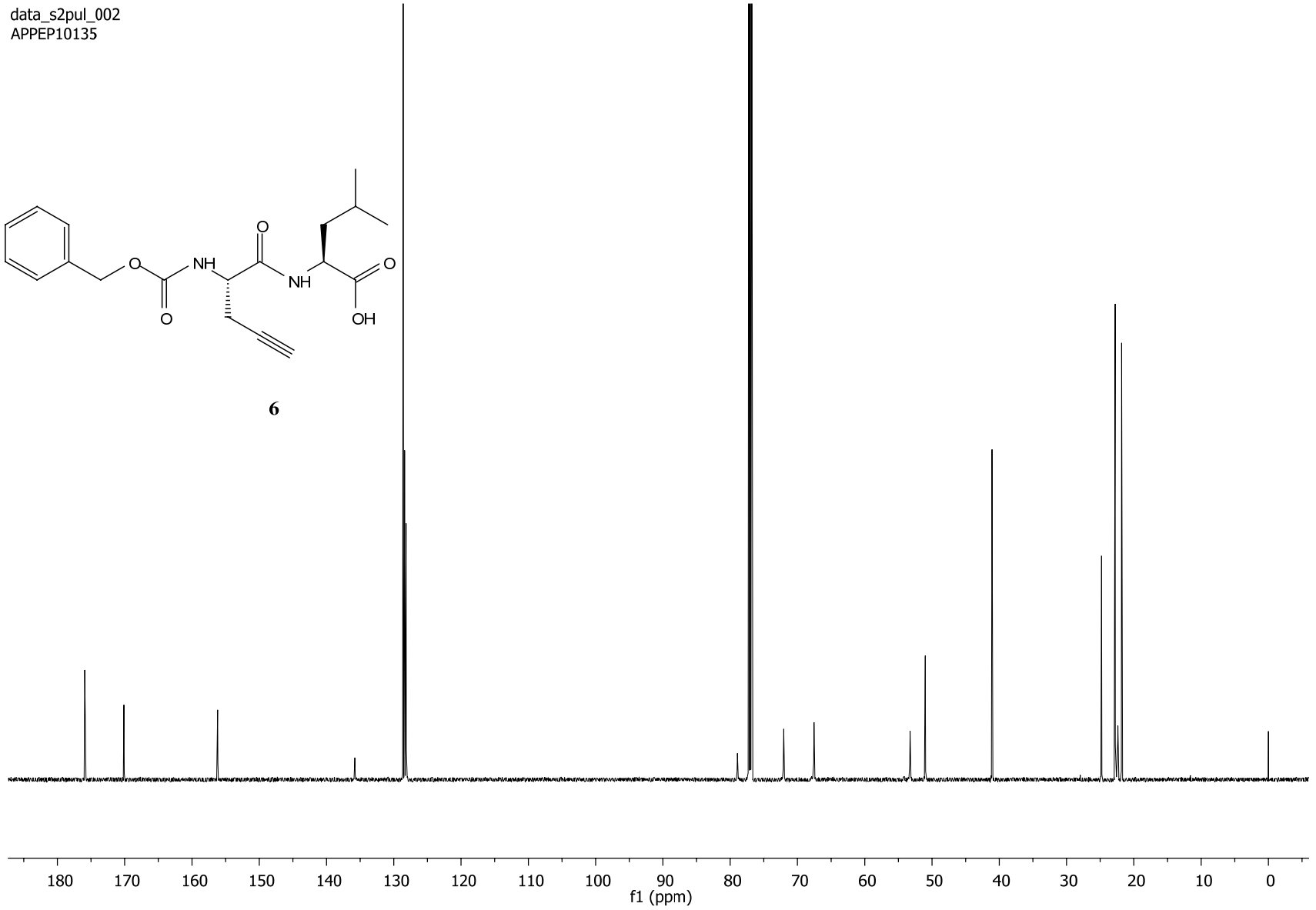
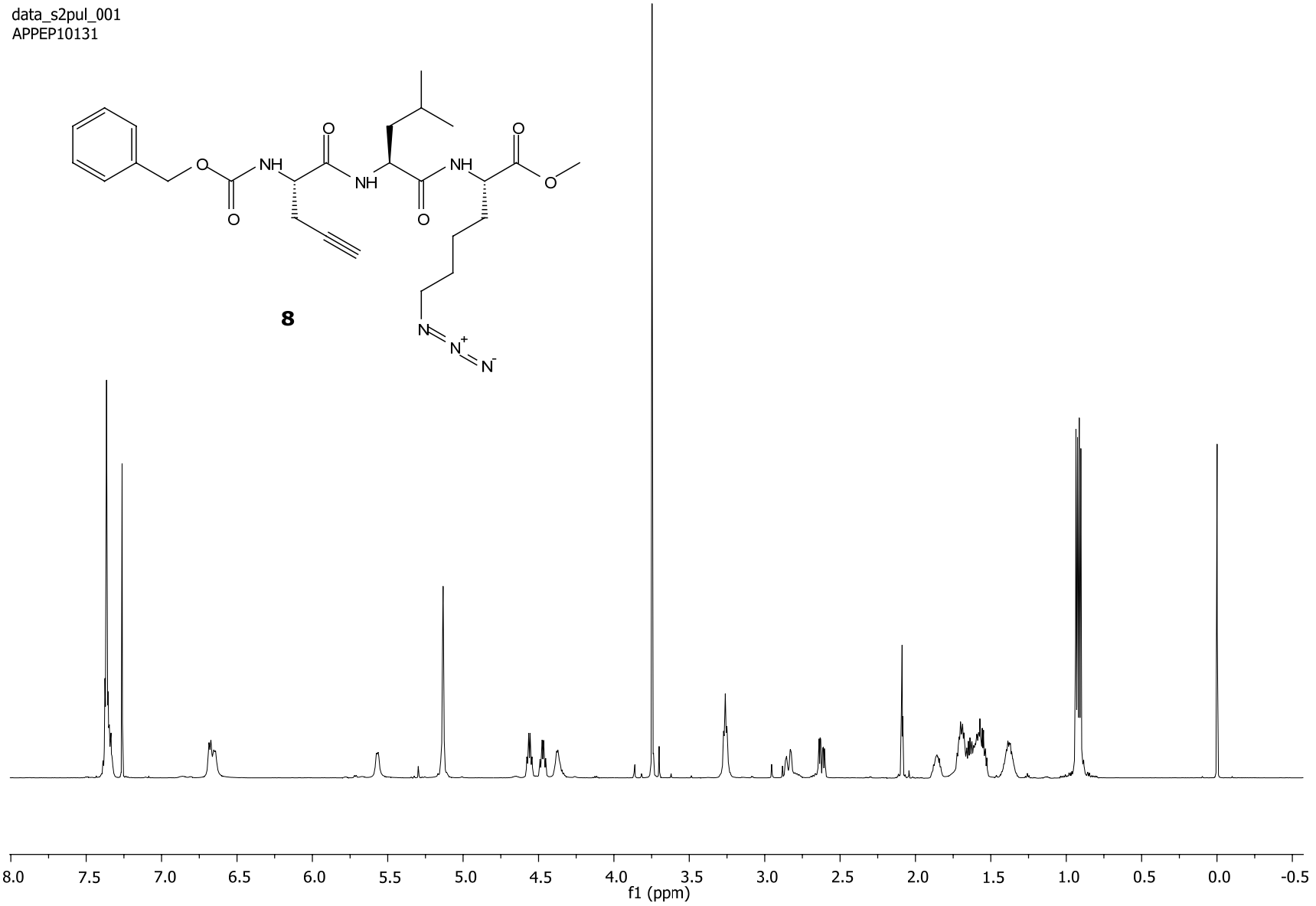
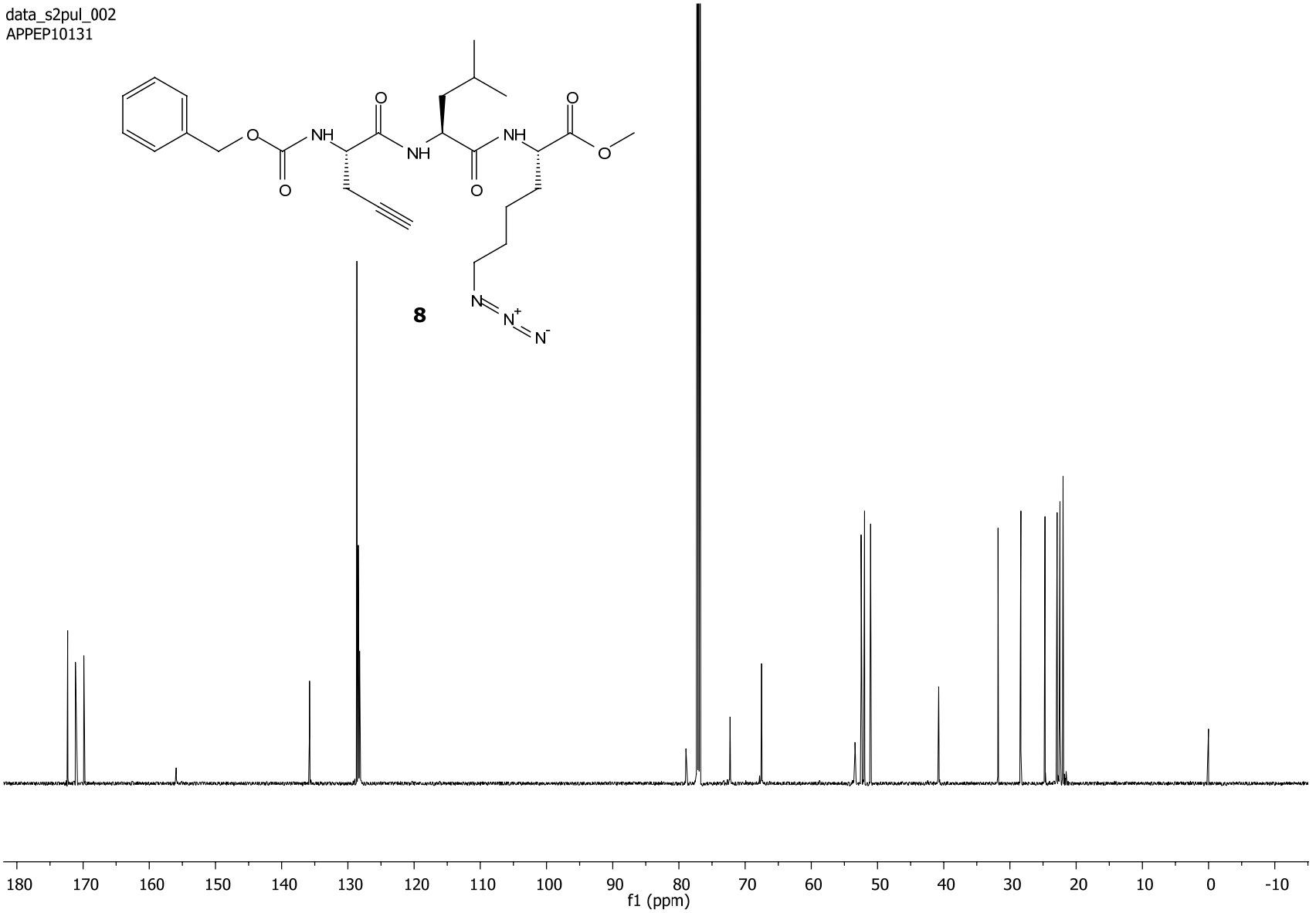


6





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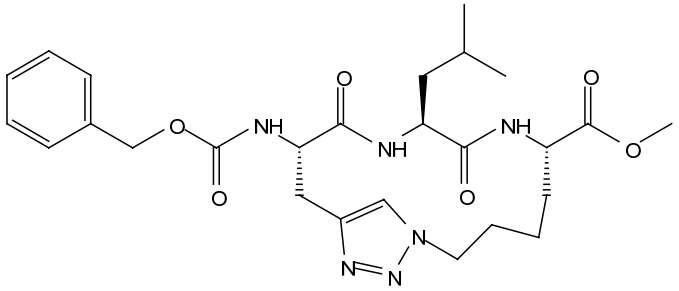
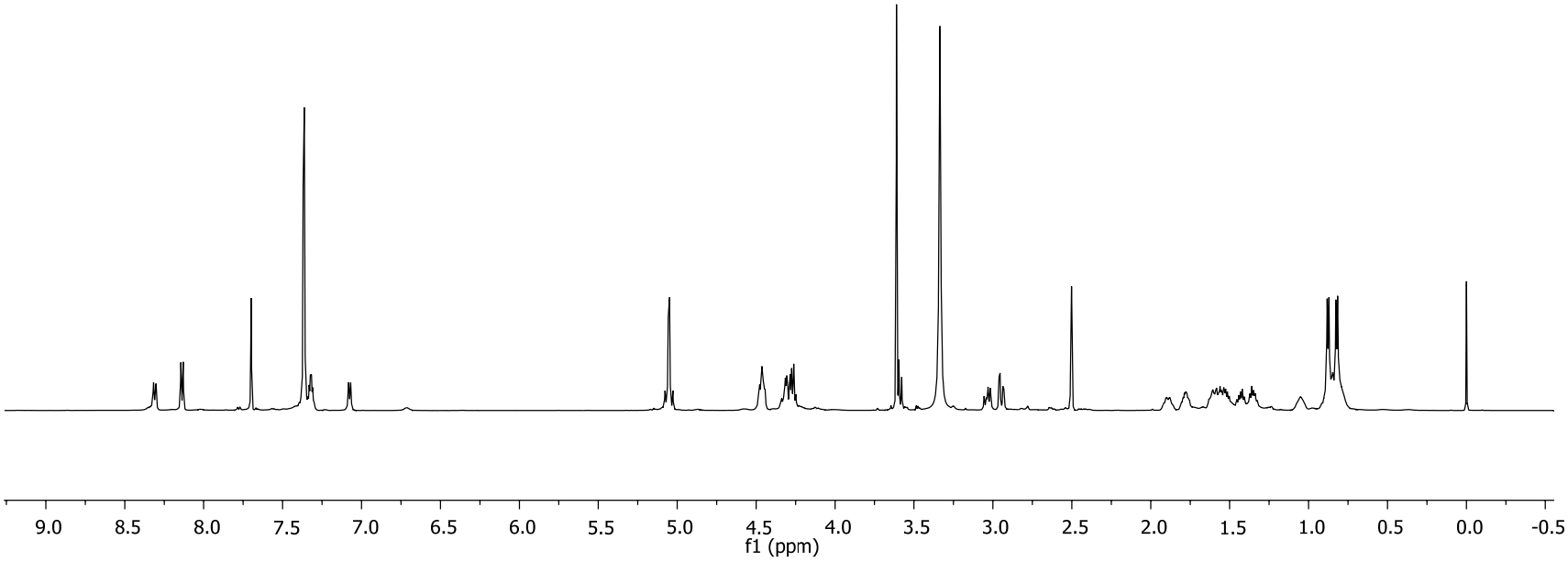


1a



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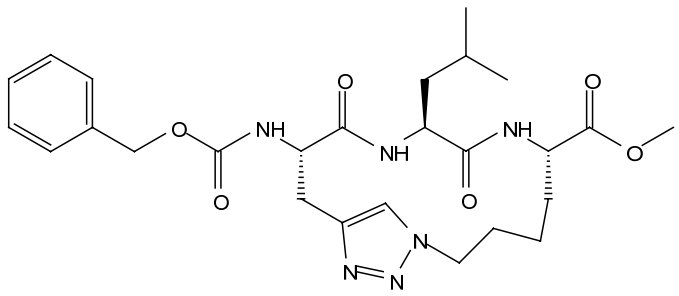

1a

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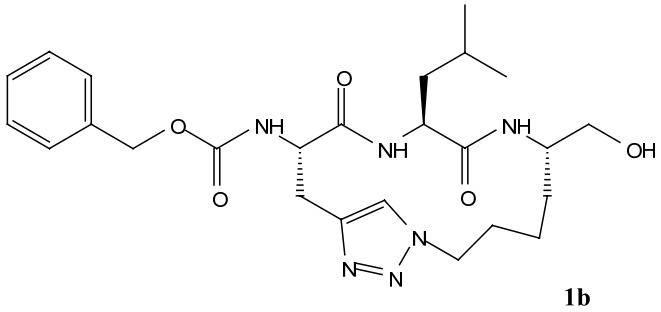
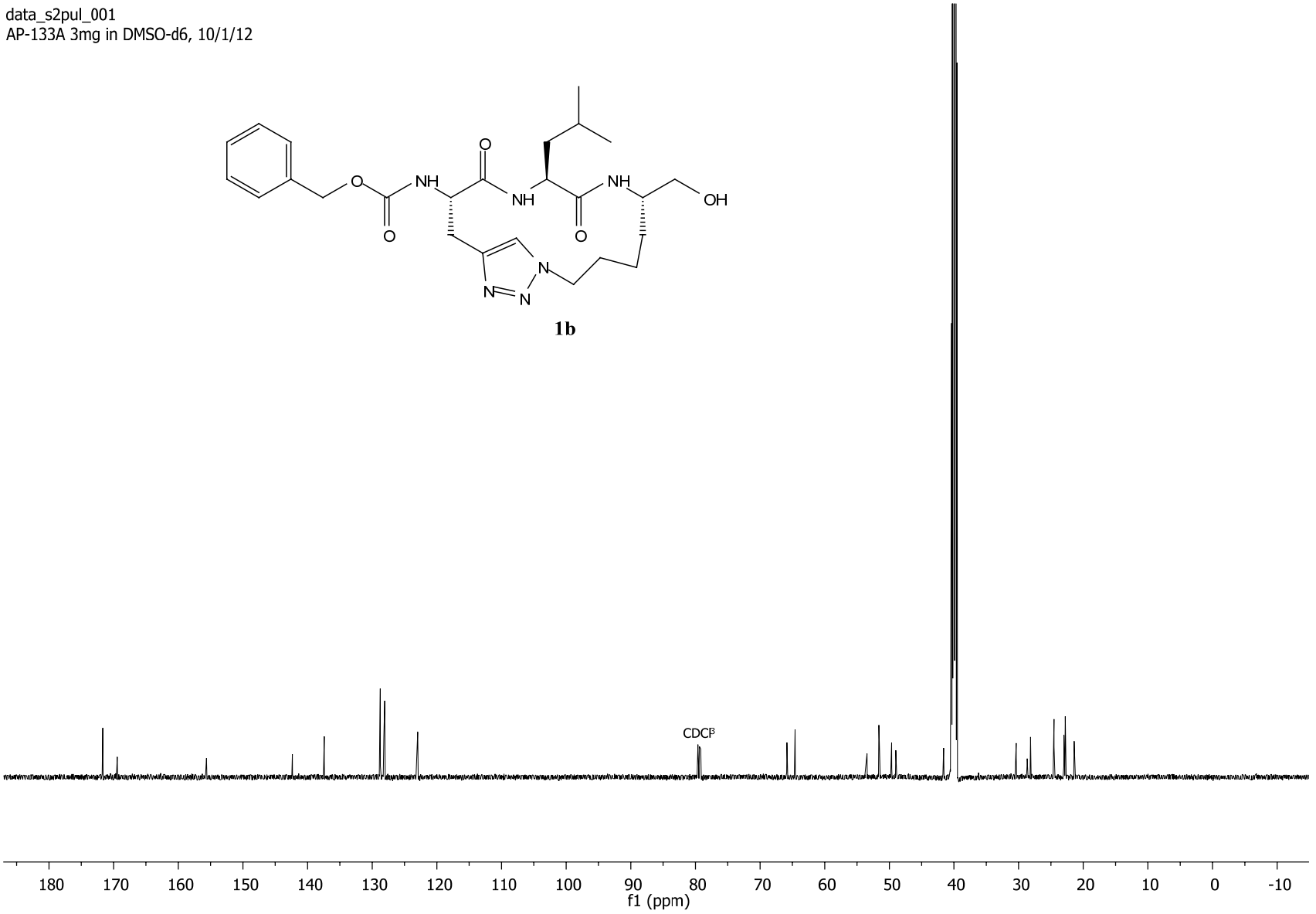
AP-133A 3mg in DMSO-d6, 10/1/12

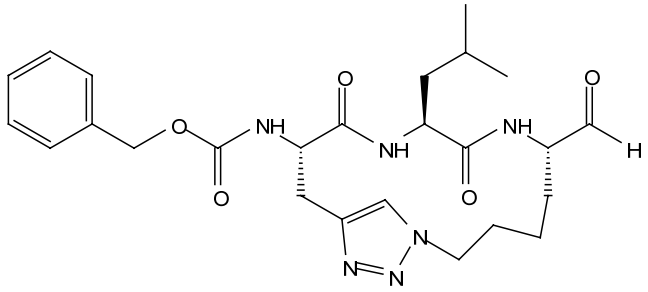


1b

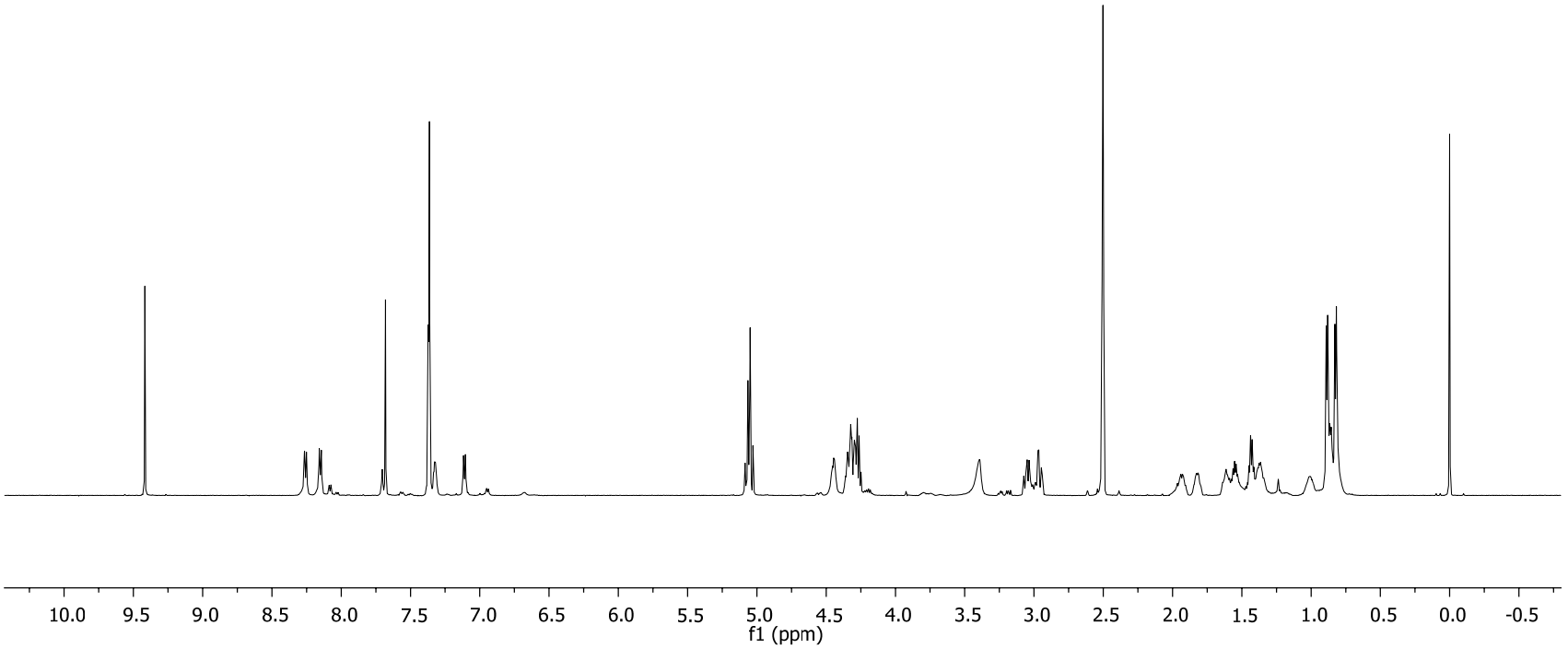


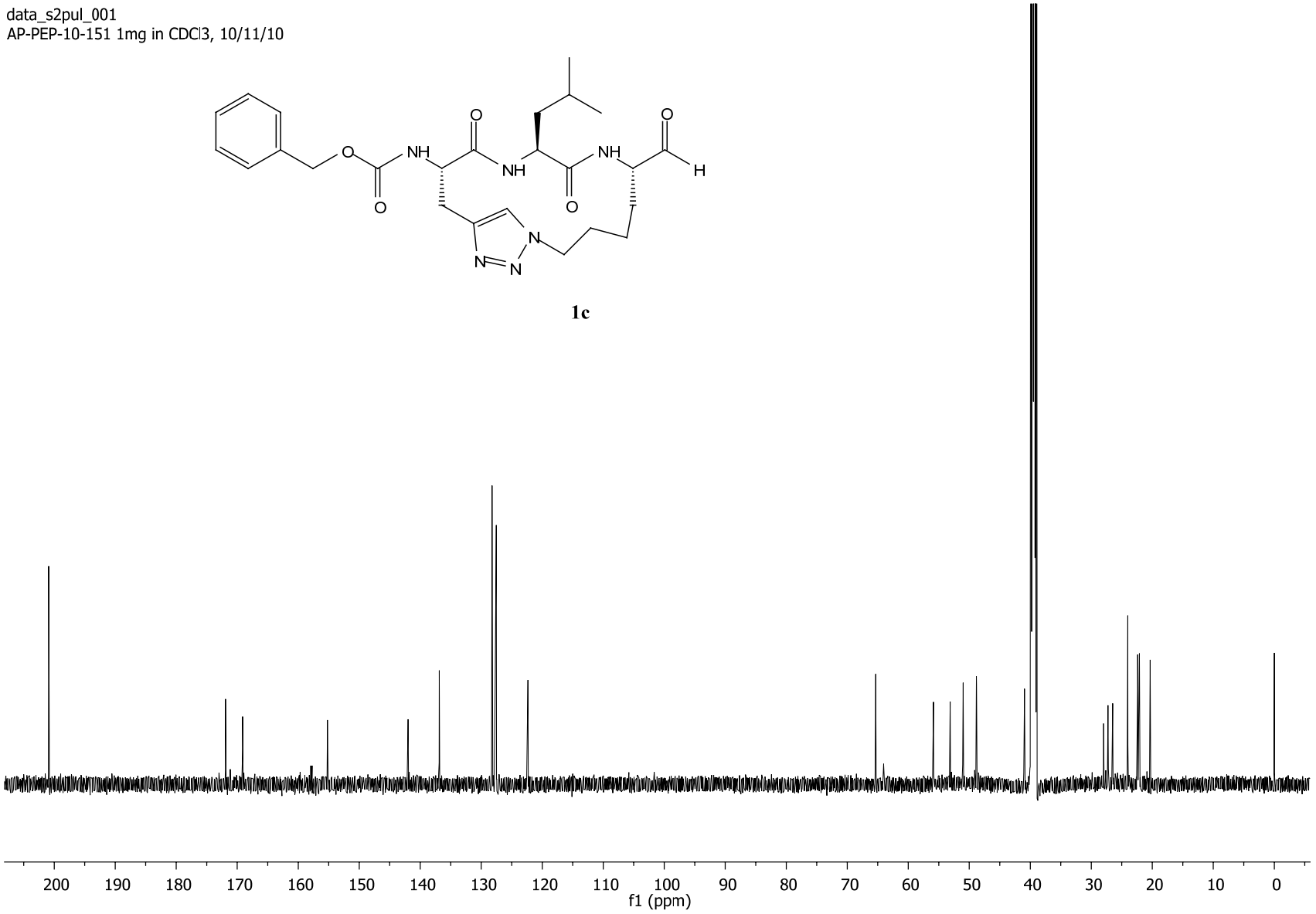
1b

data_PRESAT_001
AP-PEP-10-151 1mg in CDCl3, 10/11/10



1c





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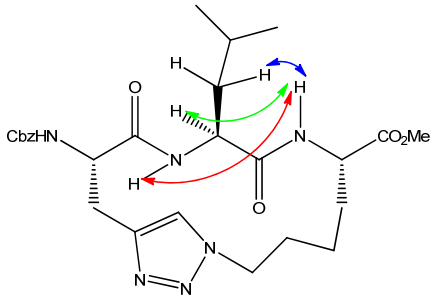
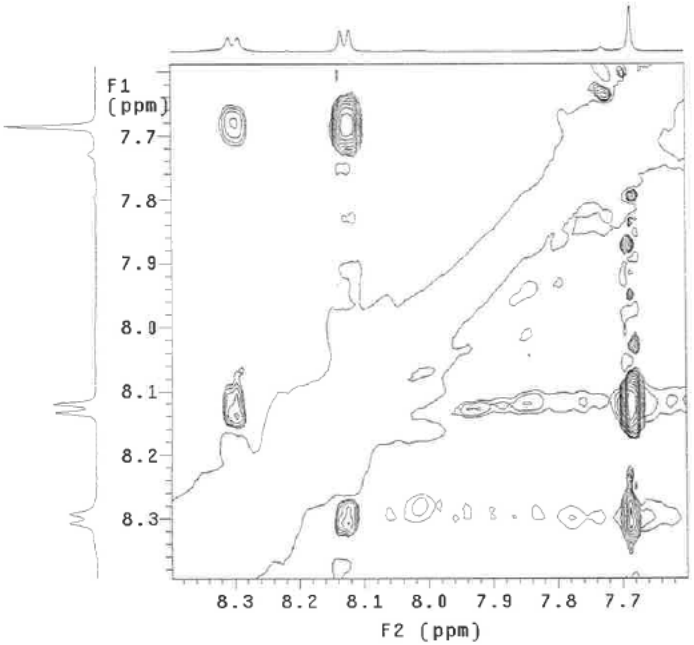
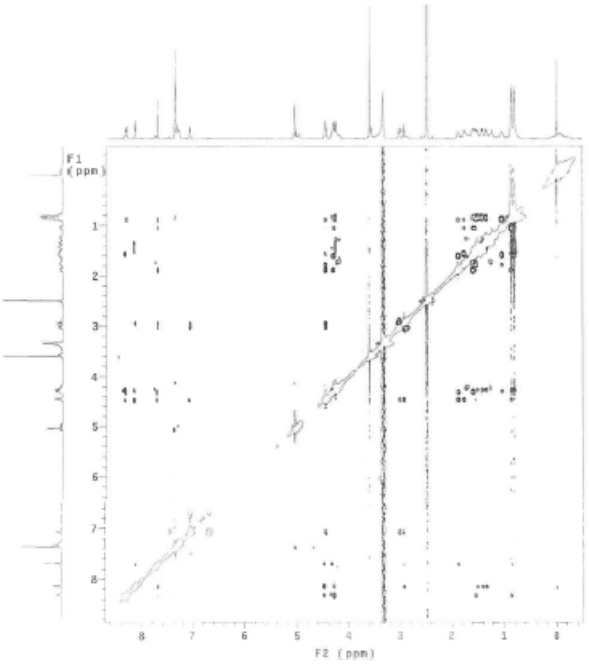
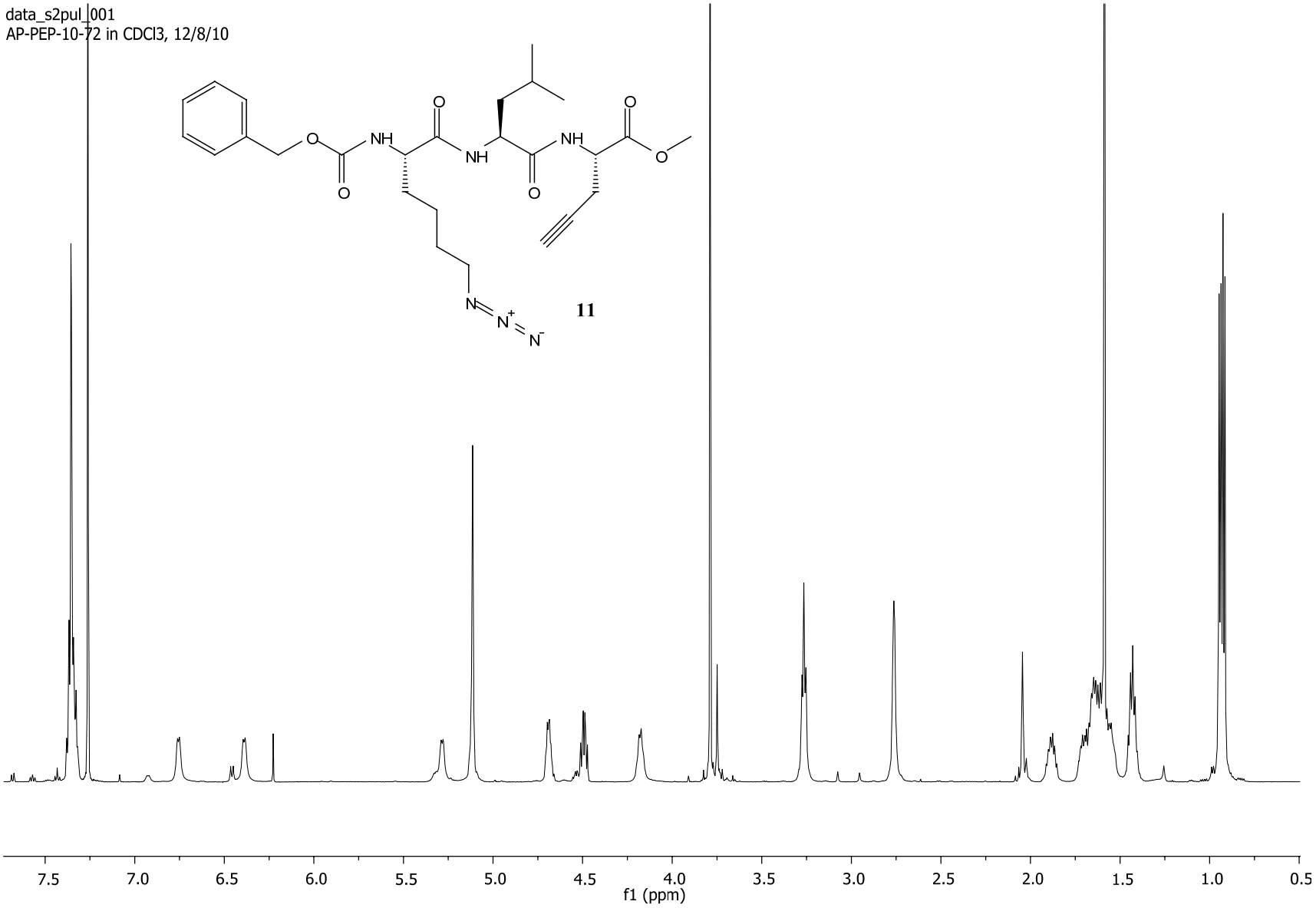


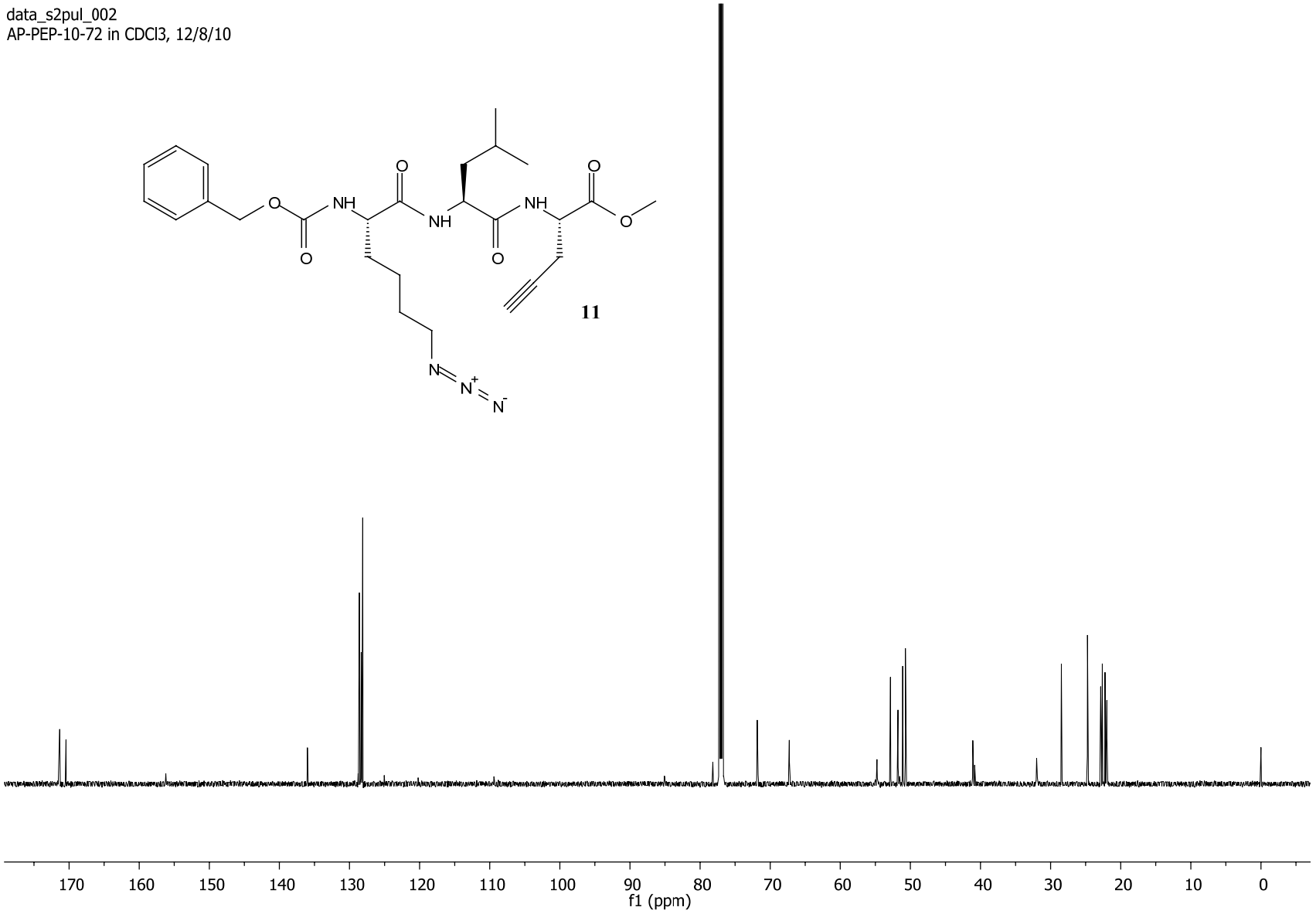
1a

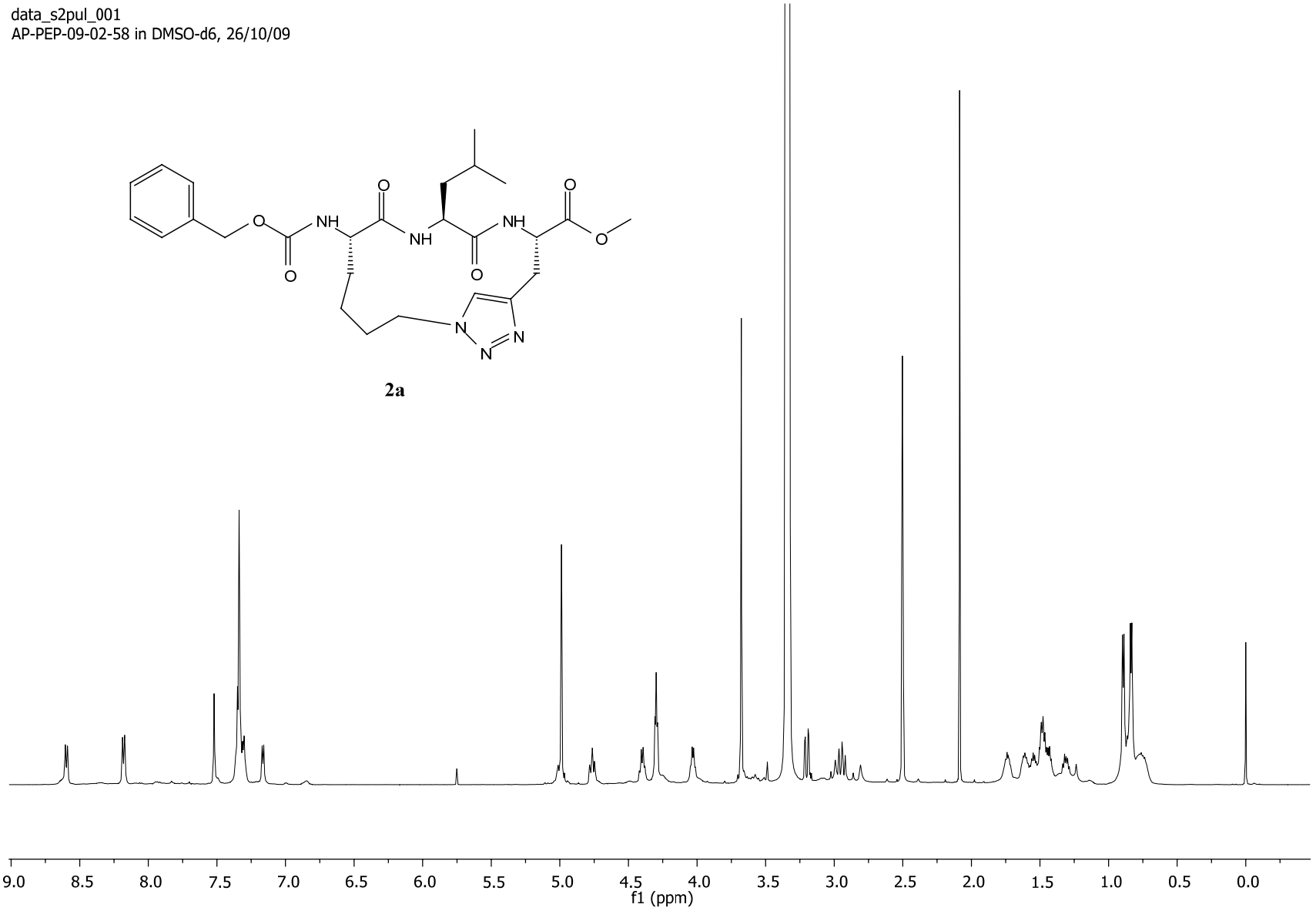
Figure S11 Intramolecular NOE cross-peaks observed for **1a** in d_6 DMSO at 20°C.



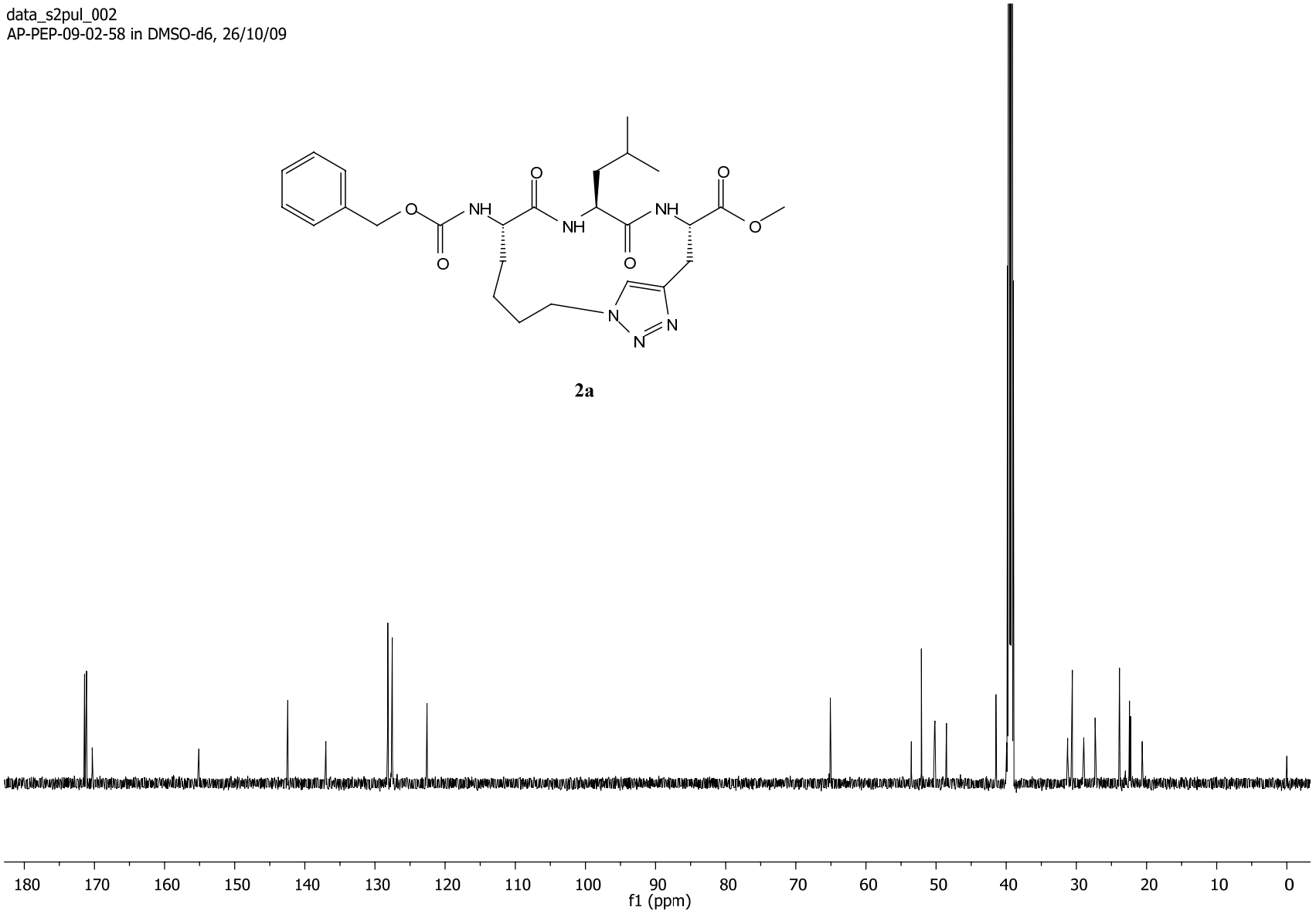








2a



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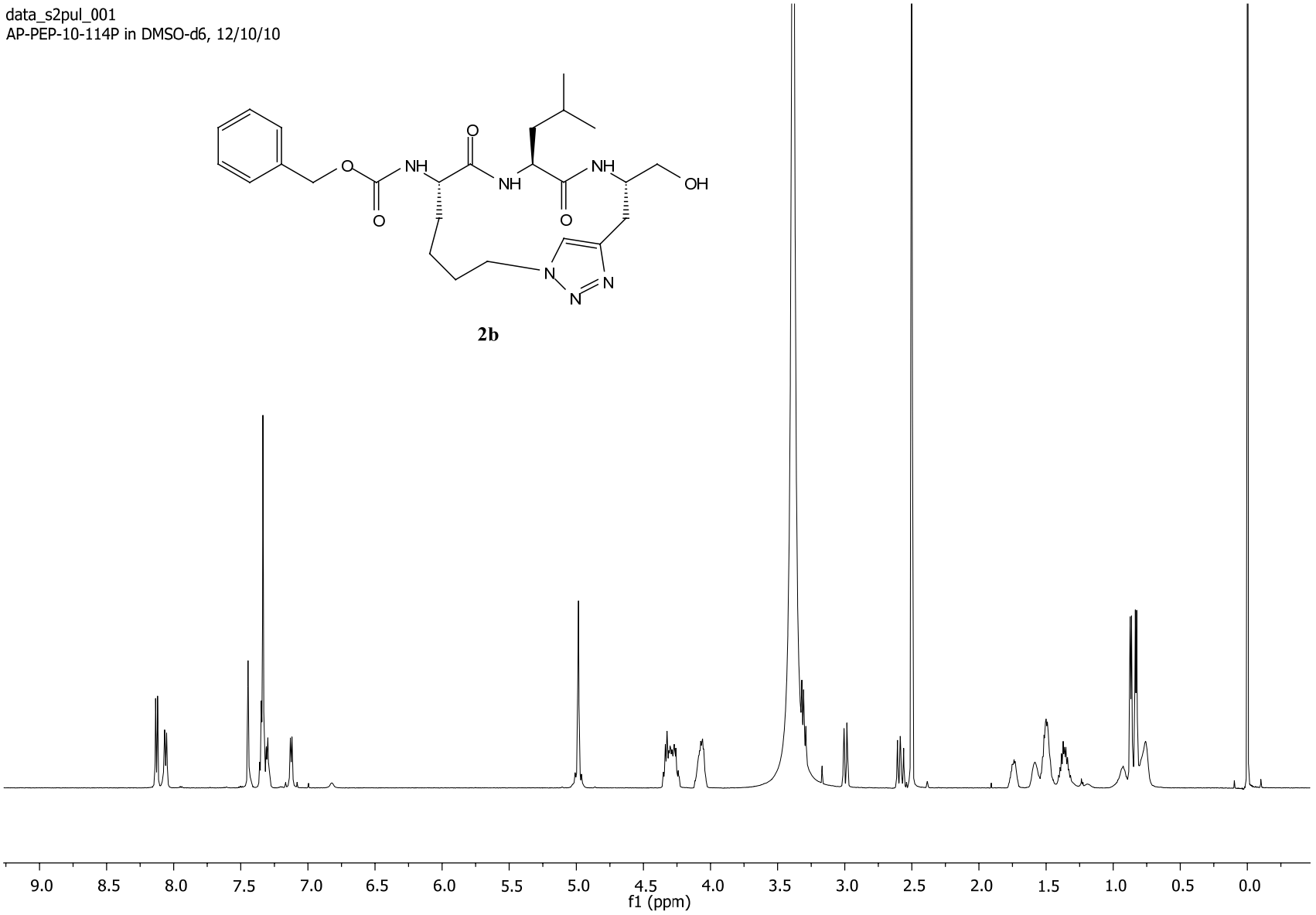
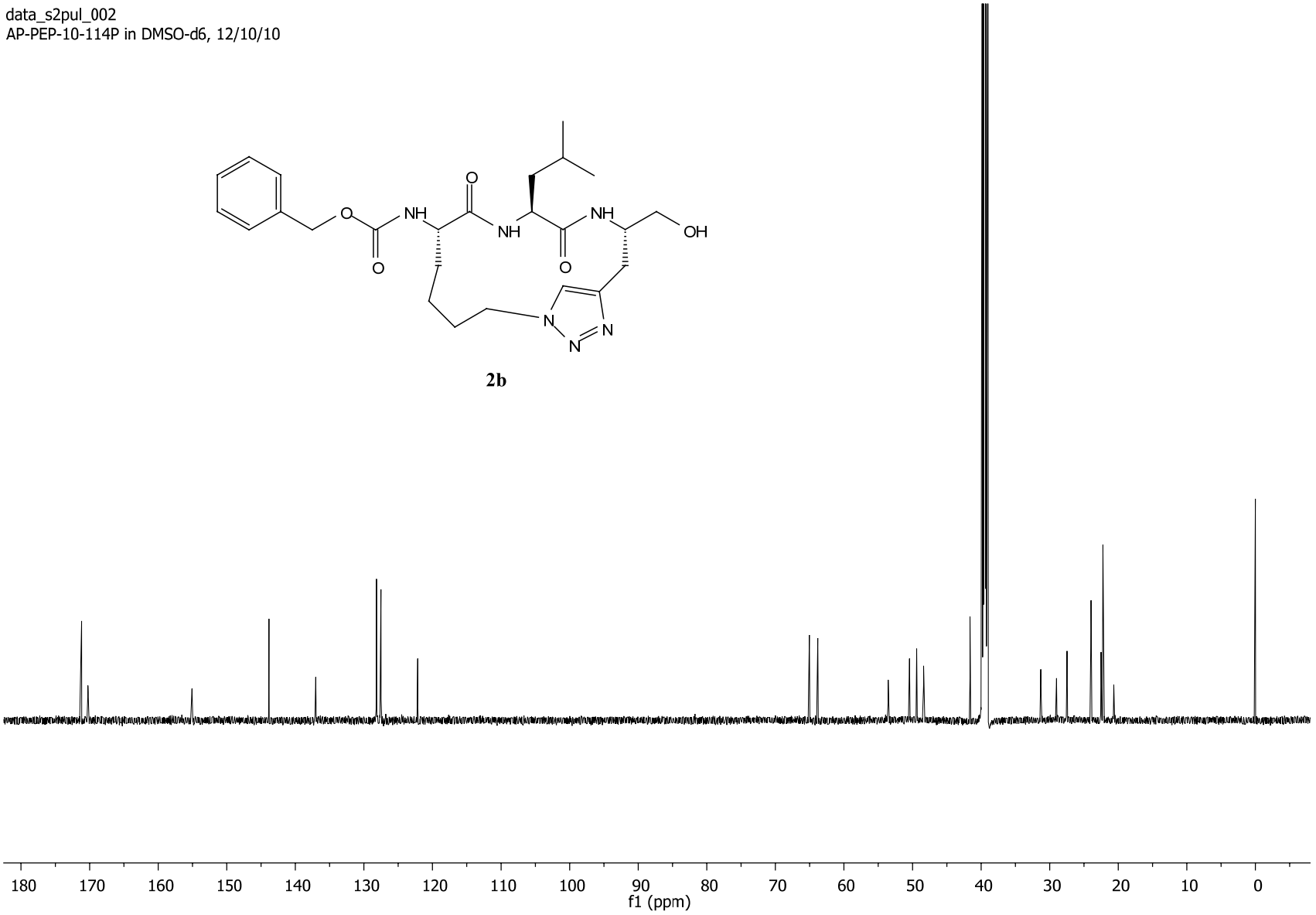


2b



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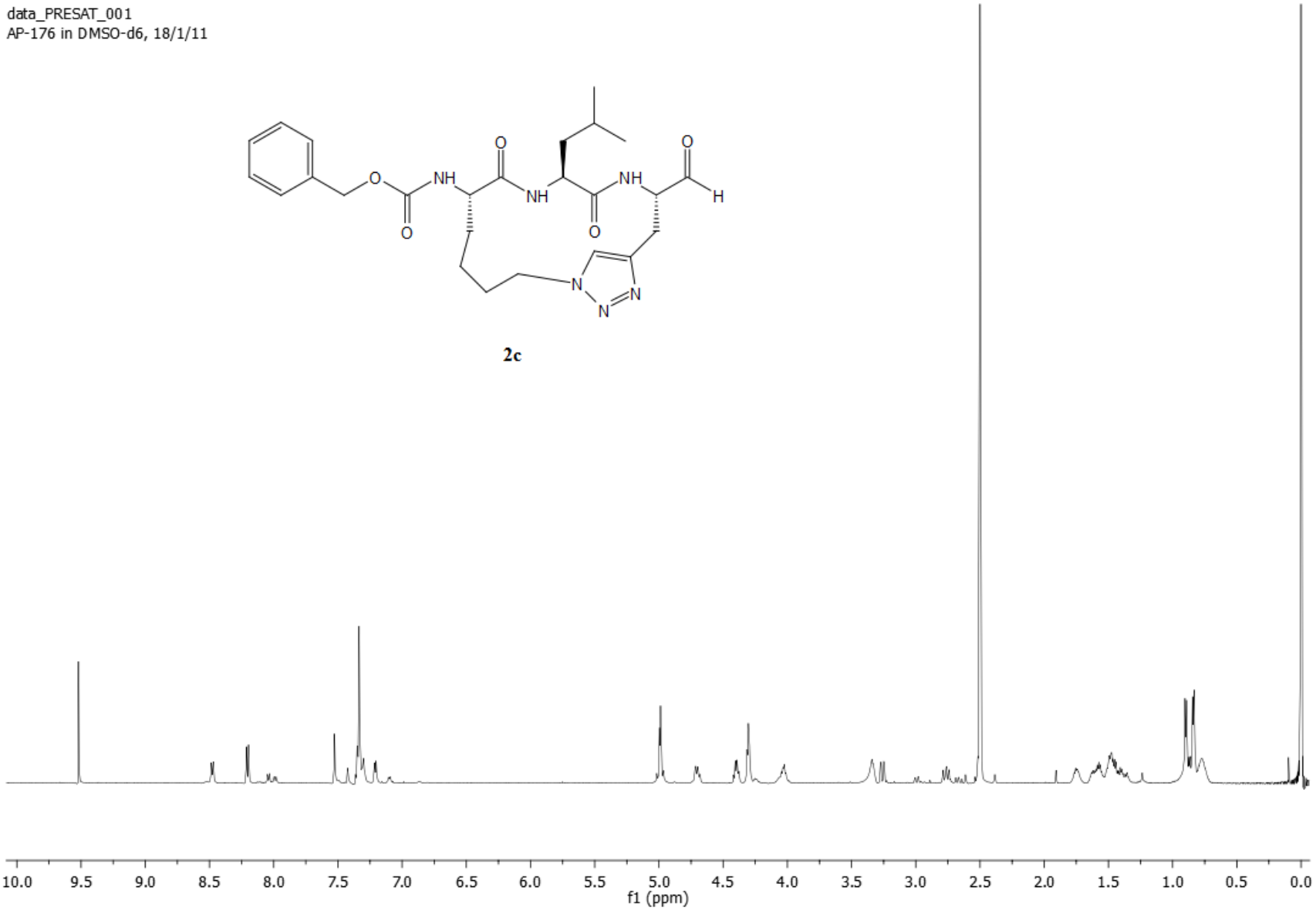
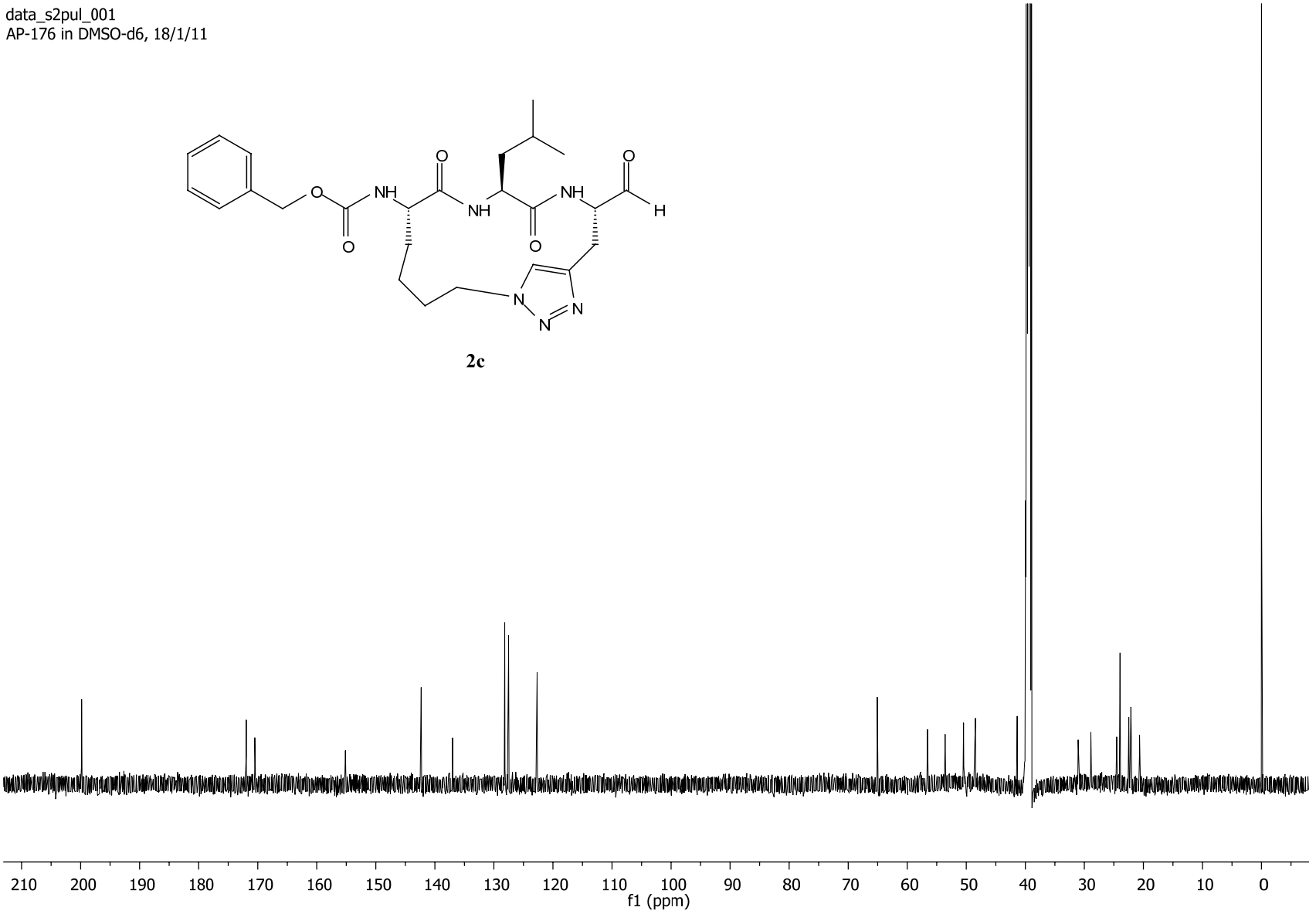
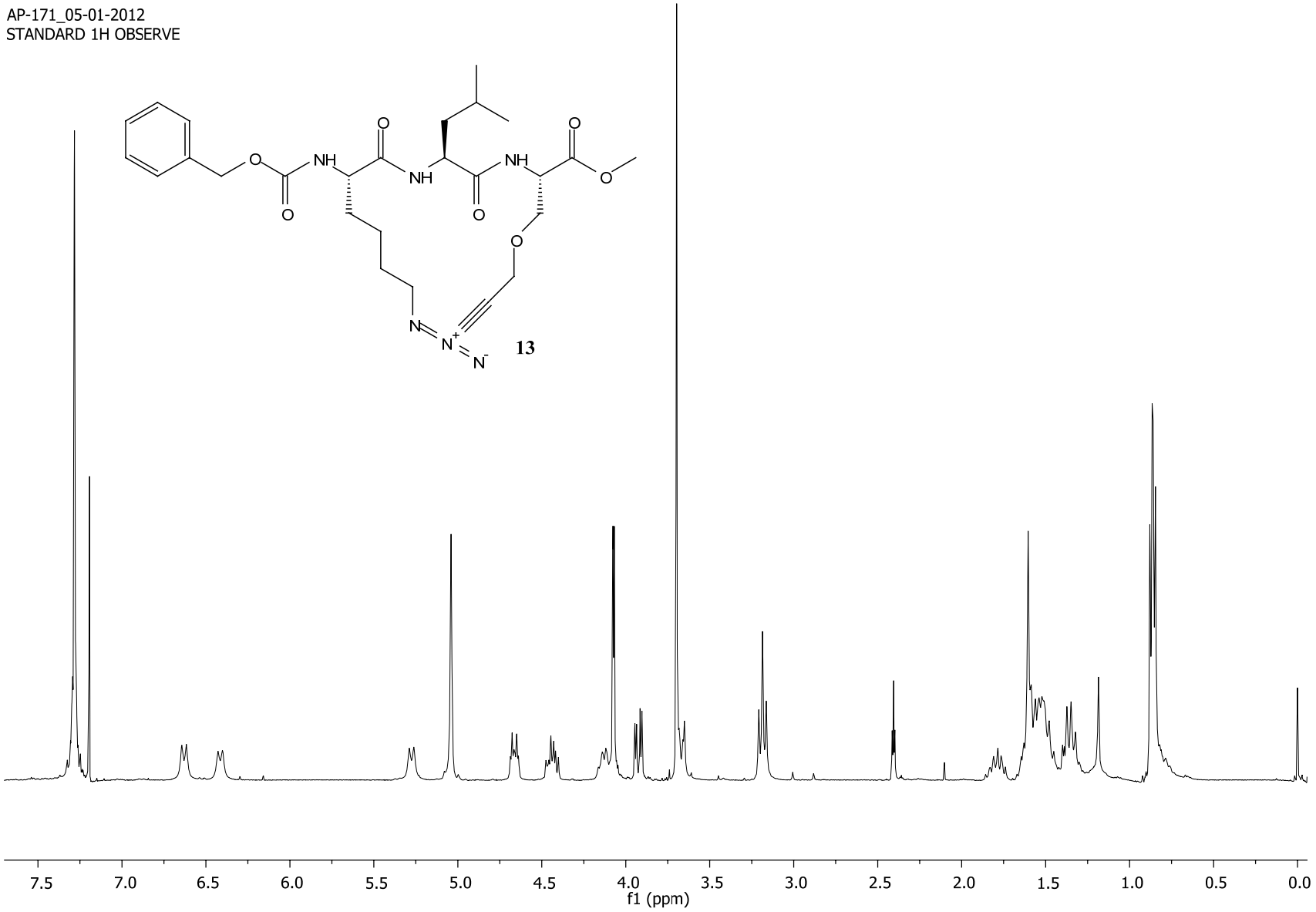
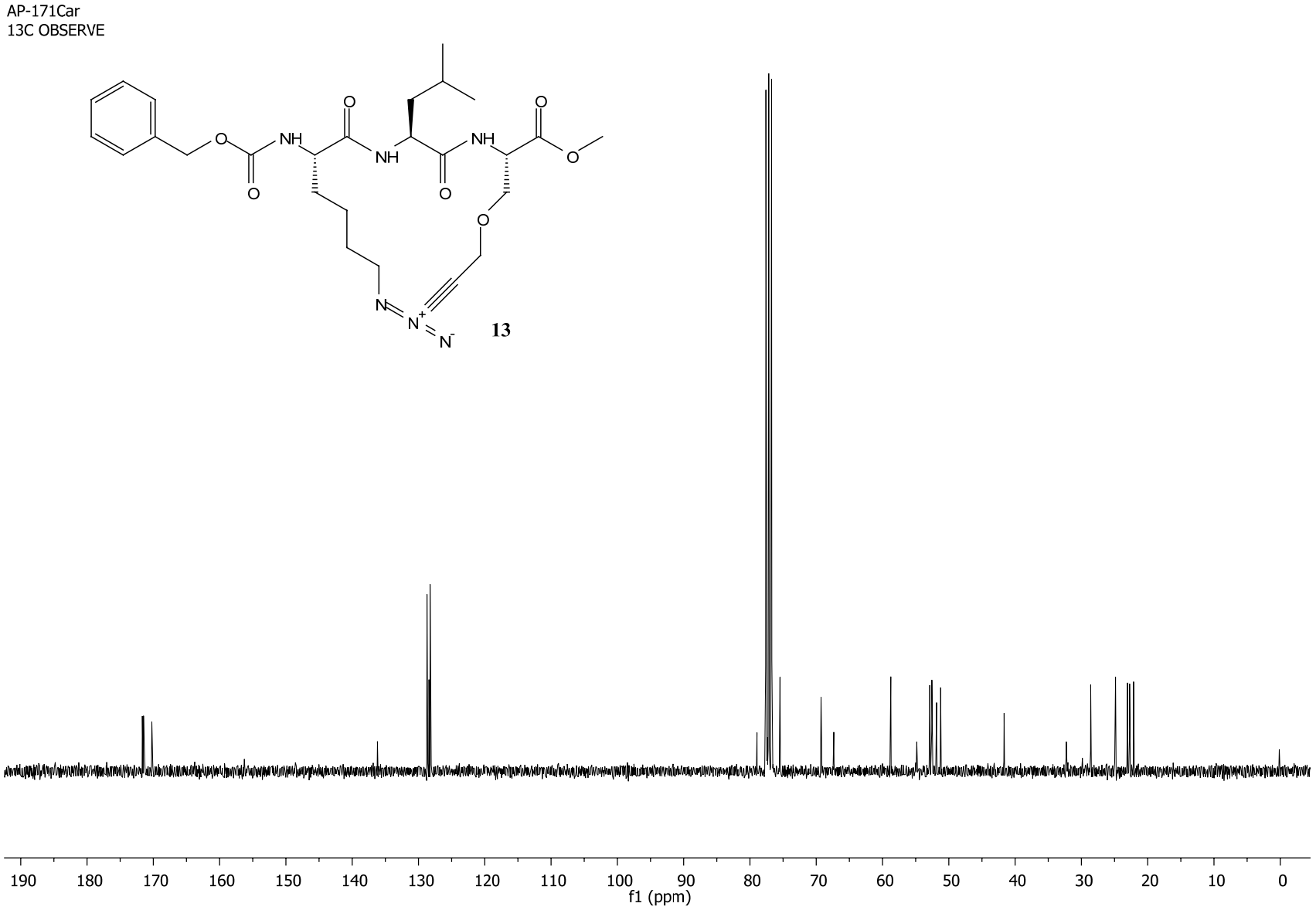


2c







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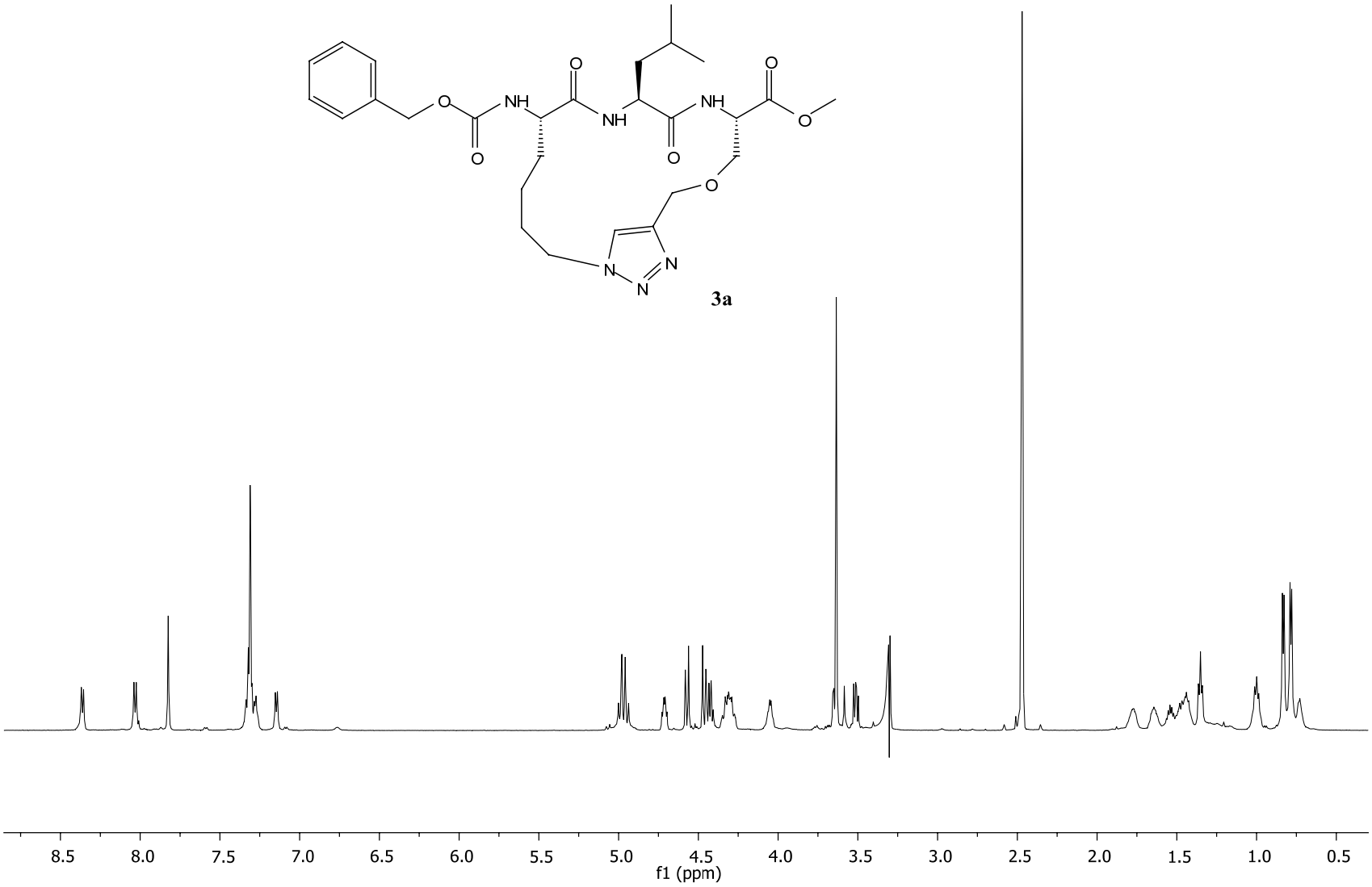
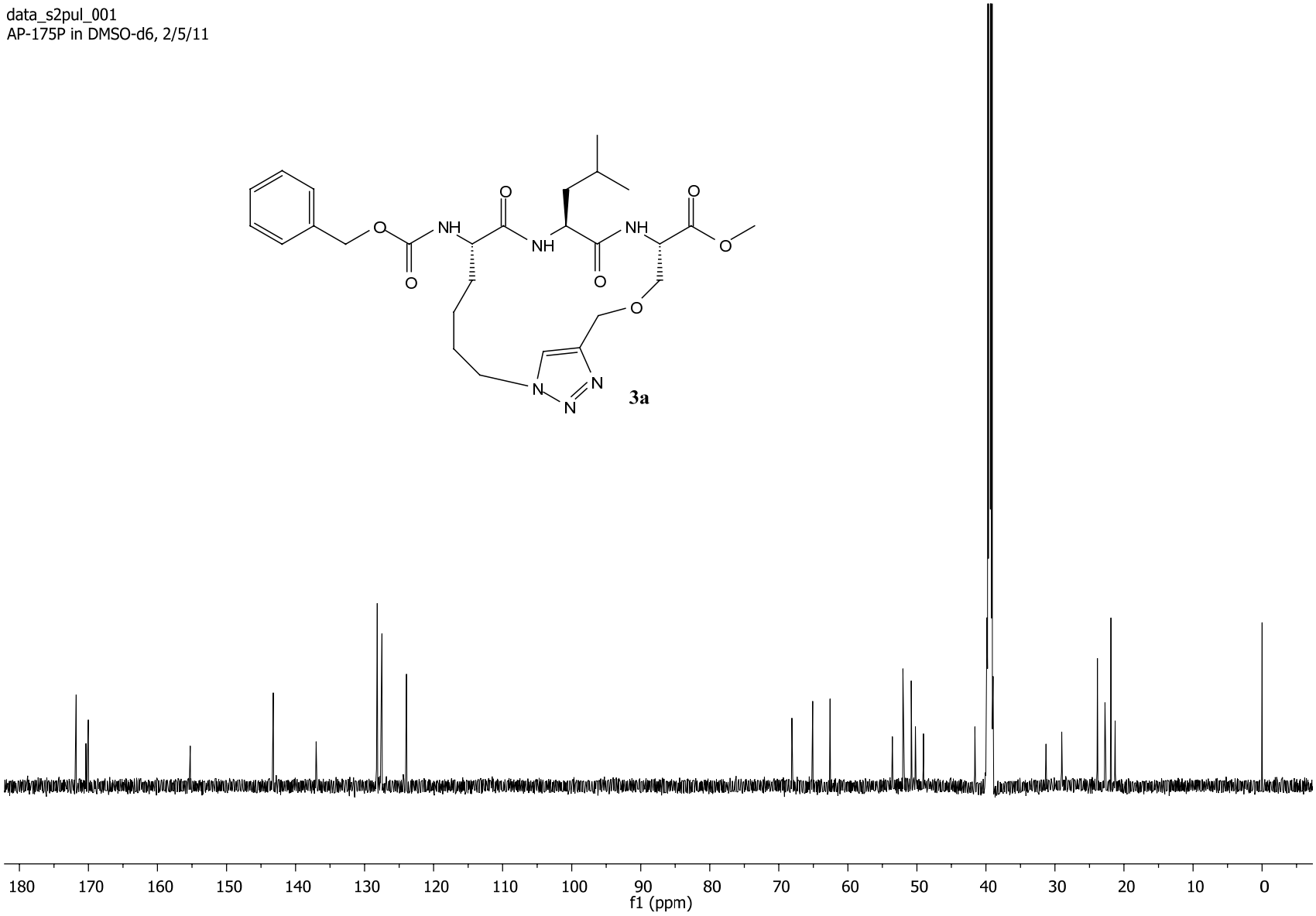
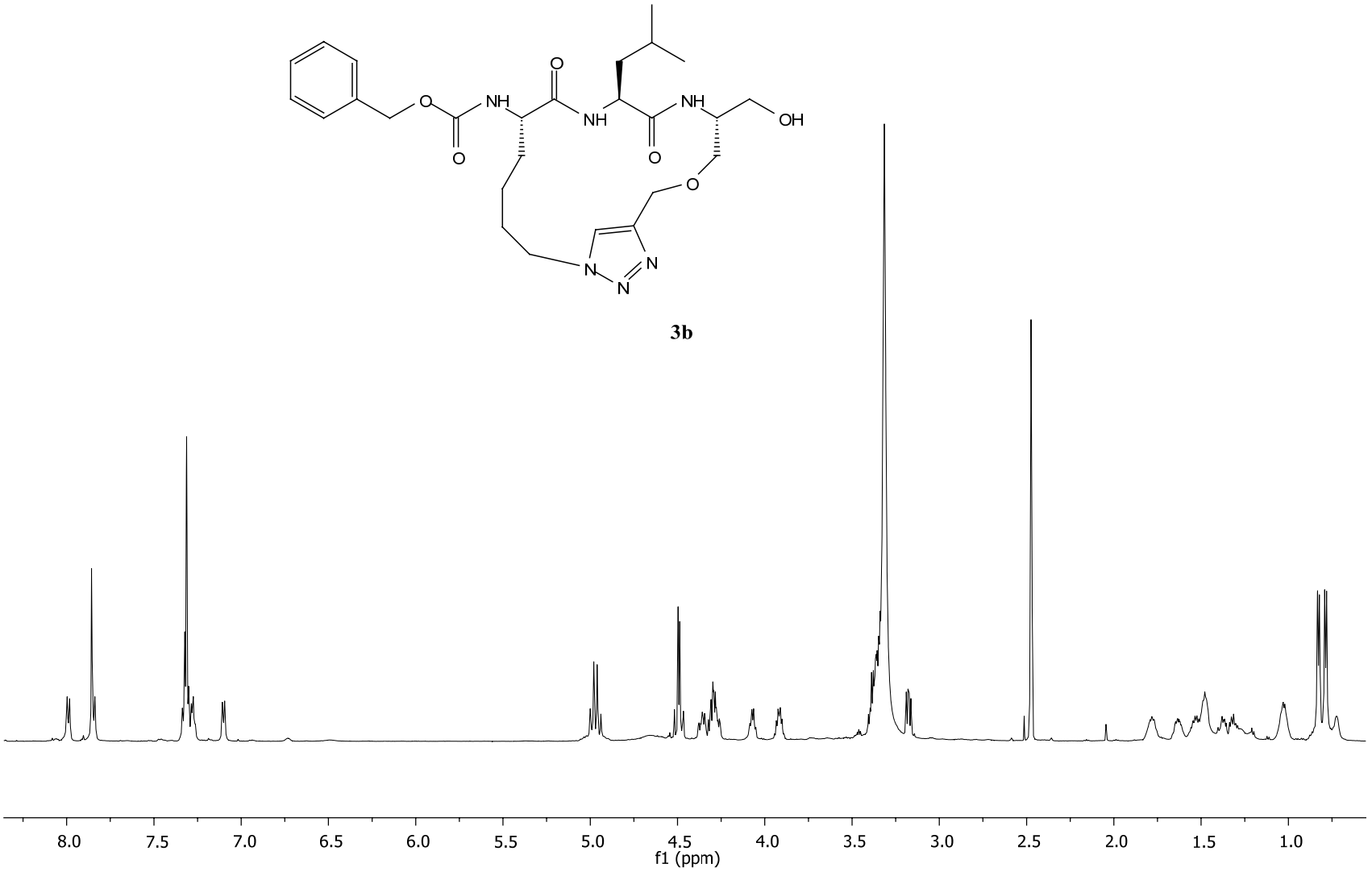
AP-175P in DMSO-d6, 2/5/11



3a



data_s2pul_001
AP-307C 3mg in DMSO-d6, 30/4/12



3b