

Supporting Information

The Thioacid–Azide Reaction with Alkyl Azides: Prospects and Limitations in the Field of Amide and Thioamide Synthesis

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Materials & Methods

Reagents and solvents, unless stated otherwise, were purchased from commercial suppliers and used without further purification. The resins as well as Fmoc-protected natural L-amino acids were purchased from Novabiochem. Thin-layer chromatography (TLC) was performed with precoated silica gel plates and visualized by UV light ($\lambda = 254$ nm) or KMnO_4 solution. The reaction mixtures were purified by column chromatography over silica gel (60–240 mesh). Azido glycine,¹ γ -azido butanoic acid² and Boc-Nle(N_3)-OH³ were synthesised following known protocols.

The NMR spectra were recorded on 600 or 400 MHz spectrometers at room temperature. Chemical shifts (δ) are reported in ppm relative to residual solvent peak (CDCl_3 ; 7.26 ppm (^1H) and 70 ppm (^{13}C), D_2O : 4.79 ppm (^1H), $(\text{CD}_3)_2\text{SO}$: 2.50 ppm (^1H) and 39.52 ppm (^{13}C)). The analytical HPLC-MS was applied from WatersTM with a 717plus autosampler, a 600S controller, 2 pumps 616 and a 2489 UV/Visible detector connected to a 3100 mass detector (low resolution, all Waters Corporation, Milford, Massachusetts, USA). The RP-HPLC-column was a Kromasil C18 (5 μm , 250x4.6mm with a flow rate of 0.8 mL/min, MeCN/ H_2O (0.1% TFA)). For fluorescence LC-UV a Jasco FP-2020Plus fluorescence detector (JASCO Inc., Easton, Maryland, USA) was attached used with the analytical HPLC. Data was processed by Empower Pro software (Waters Corporation).

HPLC purification of the peptides was performed on a JASCO LC-2000 Plus system using a reversed phase C18 column (5 μm , 25 _ 250 mm, constant flow of 16.0 ml/min: 5 min at 7% MeCN (with 0.1% TFA), gradient 7–95% MeCN (with 0.1% TFA) over 30 min), consisting of a Smartline Manager 5000 with interface module, two Smartline Pump 1000 HPLC pumps, a 6-port-3-channel injection valve with 2.5 mL loop, a UV detector (UV-2077) and a high pressure gradient mixer.

The analytical HPLC-MS was applied from WatersTM with a 717plus autosampler, a 600S controller, 2 pumps 616 and a 2489 UV/Visible detector connected to a 3100 mass detector (low resolution, all Waters Corporation, Milford, Massachusetts, USA). The RP-HPLC-column was a Kromasil C18 (5 μm , 250x4.6mm with a flow rate of 0.8 mL/min, MeCN/ H_2O (0.1% TFA)). For fluorescence LC-UV a Jasco FP-2020Plus fluorescence detector (JASCO Inc., Easton, Maryland, USA) was attached used with the analytical HPLC. Data was processed by Empower Pro software (Waters Corporation).

High-resolution mass spectra (HRMS) were collected with an Agilent 6210 ToF LC/MS system (Agilent Technologies, Santa Clara, California, USA) using as an eluent water and acetonitrile in a 1:1 mixture (with 0.1 % TFA) at a flow rate of 0.2 mL/min.

Experimental Procedures

N- α -Fluorenylmethoxycarbonyl-(ϵ -*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-ylamino))-L-Lysin (Fmoc-Lys(NBD)-OH)⁴

N- α -Fluorenylmethoxycarbonyl-L-lysine (500 mg, 1.04 mmol) and NBD-Cl (310 mg, 1.55 mmol) were dissolved in a mixture of water (15 mL) and methanol (10 mL). NaHCO₃ (262 mg, 3.12 mmol) was added. The solution was stirred for 2.5 h at 55 °C. The reaction was cooled down to room temperature and 1 N HCl was added until pH 2. The mixture was extracted with diethylether. The organic layers were combined, washed with brine and dried over MgSO₄. Concentration and purification by column chromatography (CH₂Cl₂/MeOH, 10/1 (v/v)) yielded 1.06 g (2.00 mmol, 96 %) of product as orange green metallic crystals: $R_f = 0.23$, ¹H-NMR ((CD₃)₂SO, 400 MHz): $\delta = 12.61$ (s, 1H), 9.50 (s, 1H), 8.42 (d, $J = 8.9$ Hz, 1H), 7.84 (d, $J = 7.6$ Hz, 2H), 7.70 (dd, $J = 7.6, 4.9$ Hz, 2H), 7.65 (d, $J = 8.1$ Hz, 1H), 7.38 (t, $J = 7.5$ Hz, 2H), 7.29 (t, $J = 7.5$ Hz, 2H), 6.31 (d, $J = 9.1$ Hz, 1H), 4.30-4.25 (m, 2H), 4.19 (t, $J = 7.0$ Hz, 1H), 4.03-3.92 (m, 1H), 3.48-3.37 (m, 2H), 1.87-1.58 (m, 4H), 1.52-1.40 (m, 2H). ¹³C-NMR (CD₃CN, 100 MHz): $\delta = 174.0$ (C), 156.2 (C), 145.1 (d, $J = 3.4$ Hz, C), 144.4 (C), 144.1 (C), 143.8 (C), 140.7 (d, $J = 2.5$ Hz, C), 137.8 (CH₂), 127.6 (CH₂), 127.1 (CH₂), 125.3 (CH₂), 120.6 (C), 120.1 (CH₂), 99.0 (CH), 65.6 (CH₂), 53.8 (CH), 46.7 (CH), 43.2 (CH₂), 30.5 (CH₂), 27.1 (CH₂), 23.2 (CH₂). HRMS for C₂₇H₂₆N₅O₇⁺, m/z: [M+H⁺] calcd.: 532.1827, found: 532.1829.

Peptide Synthesis

Peptides were synthesised with ABI 433A Peptide Synthesizer (Applied Biosystems, Inc., Foster City, California, USA) via standard Fmoc-based conditions (Fast-moc protocol with HOBt/HBTU conditions) on a preloaded Fmoc-Gly-Wang resin (0.79 mmol/g). Non-canonical amino acids (2 eq.) were coupled manually with HATU (2 eq.), HOBt (2 eq.) and DIPEA (2 eq.) overnight. All peptides were purified by HPLC (MeCN/H₂O (0.1 % TFA)) and lyophilised to yield pure peptides:

1: H₂N-Nle(N₃)RHRKIK(NBD)RDNG-OH (TFA salt): 15.62 mg (7.26 μ mol, 7 %)

HRMS: m/z: [M+H⁺] calcd.: 798.9200, found: 798.9233.

9: N₃-GGESGEH-OH: 29.7 mg (48.1 μmol, 48 %)

HRMS: m/z: [M+H⁺] calcd.: 618.2114, found: 618.2080.

10: N₃-(CH₂)₃C(O)-GESGEG-OH: 26.1 mg (40.4 μmol, 40 %)

HRMS: m/z: [M+H⁺] calcd.: 646.2427, found: 646.2386.

Thioacid–azide reaction with azido peptide 1

Azido peptide **1** was dissolved (see Table 1 for solvent/concentration). After addition of thioacetic acid (**2**) (20 eq.) the reaction was shaken for 2 d (see Table 1 for temperature). Conversion was checked by LC-fluorescence (ex: 460 nm, em: 540 nm, Figure S1–S7) and peaks were assigned by LC-UV-MS: LRMS: m/z (calculated): [M+2H]²⁺ 799.4 (azido peptide **1**), 806.9 (amide **3**), 820.4 (acetylated peptide **4**), 786.4 (amine peptide **5**), 814.9 (thioamide **6**); m/z (experimental): [M+2H]²⁺ (ESI-ToF) 799.0 (**1**), 806.9 (**3**), 820.5 (**4**), 786.5 (**5**), 815.0 (**6**).

Gradient A (Table 1, entry 1–2 and 5–6): 5 min at 5% MeCN (with 0.1% TFA), gradient 5–60% MeCN (with 0.1% TFA) over 45 min, gradient 60–100% MeCN (with 0.1% TFA) over 4 min.

Gradient B (Table 1, entry 3 and 4): 5 min at 0% MeCN (with 0.1% TFA), gradient 0–100% MeCN (with 0.1% TFA) over 30 min.

Thioacid–azide reaction with azido peptides 9 and 10

Azido peptide **9** or **10** was dissolved (see Table 2 for solvent/concentration). After addition of thioacetic acid (**2**) (20 eq.) the reaction was shaken at 40 °C for 2 d. The reaction mixture was diluted in water, lyophilised and redissolved in D₂O. Conversion and product ratios of **11** and **12** were determined by ¹H-NMR, HMBC and HMQC.

N-(2-(2-(2-(2-azidoacetamido)ethoxy)ethoxy)ethyl)-2,2,2-trifluoroacetamide (13)

Azido glycine (41 mg, 0.41 mmol, 1 eq.) and Boc-DOOA (98 mg, 0.39 mmol, 0.95 eq.) were dissolved in dry dichloromethane and DMAP (25 mg, 0.20 mmol, 0.5 eq.) was added. The reaction was cooled to 0 °C and EDC·HCl (86 mg, 0.45 mmol, 1.1 eq.) was added in portions. The reaction mixture was stirred overnight at rt. It was then diluted with dichloromethane (30 mL) and the organic phase was washed with conc. Na₂CO₃ solution (2 x 20 mL), citric acid (0.1 M, pH 4.5, 2 x 20 mL), water (1 x 20 mL) and brine (1 x 20 mL). The organic phase was dried over MgSO₄, filtered and the solvent was removed *in vacuo*. This yielded *tert*-butyl

(2-(2-(2-(2-azidoacetamido)ethoxy)ethoxy)ethyl)carbamate in 96 % (0.124 g, 0.37 mmol) as a transparent oil with slight impurities: $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): $\delta = 7.27$ (bs, 1H), 6.75 (bs, 1H), 3.99 (s, 2H), 3.64-3.48 (m, 10H), 3.38-3.32 (m, 2H), 1.46 ppm (s, 9H); $^{13}\text{C-NMR}$ (CDCl_3 , 150 MHz): $\delta = 166.18$, 155.49, 78.88, 69.83, 69.68, 69.03, 57.97, 52.25, 39.88, 38.69, 27.92 ppm; HRMS for $\text{C}_{13}\text{H}_{26}\text{N}_5\text{O}_5^+$, m/z: $[\text{M}+\text{H}^+]$ calcd.: 332.1934, found: 331.1937. *tert*-Butyl (2-(2-(2-(2-azidoacetamido)ethoxy)ethoxy)ethyl)carbamate (0.124 g, 0.37 mmol) was treated with a solution of TFA (20 % in dichloromethane, 2 mL) for 30 min. Afterwards, the solvent was removed *in vacuo* and remaining TFA was removed by co-evaporation with dichloromethane and by high vacuum. The flask was then flooded with argon and the residue was dissolved in dry methanol (1 mL). Triethylamine (0.16 mL, 1.17 mmol, 3 eq.) and ethyl fluoroacetate (70 mg, 0.49 mmol, 1.25 eq.) were added under vigorous stirring and the reaction was kept at rt for 2 h. The solvent was removed *in vacuo*. The resulting oil was dissolved in a mixture of $\text{H}_2\text{O}:\text{MeCN}$ and purified by preparative HPLC ($t_r = 25.9$ min, 6 % MeCN for 5 min, 6 % to 80 % MeCN in 50 min). This yielded compound **13** in 52 % (64 mg, 0.16 mmol) as a transparent oil: $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): $\delta = 7.07$ (s, 1H), 6.70 (s, 1H), 4.00 (s, 2H), 3.70-3.44 ppm (m, 12H); $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): $\delta = 157.45$, 117.70, 166.72, 70.27, 70.12, 69.46, 68.64, 52.60, 39.58, 38.99 ppm; IR (liquid, cm^{-1}): 2113 (w, N_3); HRMS for $\text{C}_{10}\text{H}_{17}\text{F}_3\text{N}_5\text{O}_4^+$, m/z: $[\text{M}+\text{H}^+]$ calcd.: 328.1233, found: 328.1234.

1-Azido-11-hydroxy-3,6,9-trioxaundecane (14)

Tetraethylene glycol (21.31 g, 109 mmol, 7.8 eq.) was dissolved in dry dichloromethane (90 mL) under argon atmosphere and triethylamine (2.12 g, 21 mmol, 1.5 eq.) was added. The mixture was cooled to 0 °C and tosyl chloride (2.66 g, 14 mmol, 1 eq.) was added in portions. After stirring for 30 min at 0 °C, the reaction mixture was warmed to rt and stirred overnight. The solution was further diluted with dichloromethane (90 mL) and the organic phase was extracted with citric acid (0.1 M, pH 4.5, 3 x 100 mL), water (2 x 100 mL) and brine (1 x 100 mL). The organic phases were collected, dried over Na_2SO_4 , filtered and the solvent was removed *in vacuo*. The oily residue was used without further purification (4.8 g). The oil (2.00 g, 5.74 mmol) was dissolved in abs. ethanol (30 mL), sodium azide (0.41 g, 6.31 mmol, 1.1 eq.) was added and the solution was reacted under reflux overnight. The reaction mixture was diluted with dichloromethane (50 mL) and water (50 mL). The organic phase was extracted with water (3 x 50 mL) and the combined aqueous phases were re-extracted with dichloromethane (3 x 50 mL). The organic phase was dried over Na_2SO_4 , filtered and the solvent was removed *in vacuo*. This yielded compound **13** as a slightly yellow oil (1.02 g,

4.65 mmol, 81 %): $^1\text{H-NMR}$ (CDCl_3 , 300 MHz): $\delta = 3.74\text{-}3.64$ (m, 10H), 3.63-3.60 (m, 2H), 3.40 (t, $J = 4.86$ Hz), 2.57 ppm (bs); $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz): $\delta = 72.38, 70.62, 70.58, 70.51, 70.26, 69.97, 61.66, 50.67$ ppm; IR (liquid, cm^{-1}): 2100 (w, N_3). The analytical data is in accordance with the literature.⁵

***N*-(2,5-dioxo-9,12-dioxa-3,6-diazatetradecan-14-yl)-2,2,2-trifluoroacetamide (15)**

Compound **13** (28 mg, 0.086 mmol) was dissolved in a solution of NH_4OH (0.1 M, pH 4)/DMF (7:3, 4.3 mL, 20 mM) and thioacetic acid (65 mg, 0.86 mmol, 10 eq.) was added. The reaction mixture was stirred for 2 d at 40 °C. The solvent was then removed *in vacuo* and the resulting residue was dissolved in a H_2O :MeCN mixture and lyophilised. The crude reaction mixture was checked by $^1\text{H-NMR}$ (CDCl_3 , 600 MHz). Purification by semi-preparative HPLC ($t_r = 22.3$ min, 10 % MeCN for 10 min, 10 % to 90 % MeCN in 60 min) yielded compound **15** in 50 % (13 mg, 0.043 mmol): $^1\text{H-NMR}$ (CDCl_3 , 600 MHz): $\delta = 7.57$ (bs, 1H), 6.73 (bs, 1H), 6.60 (bs, 1H), 3.91 (d, $J = 5.3$ Hz, 2H) 3.67-3.60 (m, 6H), 3.56 (dd, $J = 10.2, 5.1$ Hz, 4H), 3.46 (dd, $J = 10.4, 5.2$ Hz, 2H), 2.05 ppm (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz): $\delta = 171.47, 169.35, 157.67$ (q, $J = 31.25$ Hz, $\text{C}(\text{O})\text{CF}_3$), 116.08 (q, $J = 238.75$ Hz, CF_3), 70.44, 70.41, 69.56, 68.92, 43.57, 39.92, 39.47, 22.93 ppm; HRMS for $\text{C}_{12}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_5^+$, m/z : $[\text{M}+\text{H}^+]$ calcd.: 360.1205, found: 360.1205.

2,2,2-trifluoro-*N*-(5-oxo-2-thioxo-9,12-dioxa-3,6-diazatetradecan-14-yl)acetamide (16)

Compound **13** (21 mg, 0.064 mmol) was dissolved in a solution of KCl/HCl (0.1 M, pH 2, 3.2 mL, 20 mM) and thioacetic acid (48 mg, 0.64 mmol, 10 eq.) was added. The reaction mixture was stirred for 2 d at 40 °C. The solvent was then removed *in vacuo* and the resulting residue was dissolved in a H_2O :MeCN mixture and lyophilised. The crude reaction mixture was checked by $^1\text{H-NMR}$ (CDCl_3 , 300 MHz). Purification by semi-preparative HPLC ($t_r = 22.3$ min, 10 % MeCN for 10 min, 10 % to 90 % MeCN in 60 min) yielded compound **16** in 30 % (7 mg, 0.019 mmol): $^1\text{H-NMR}$ (CDCl_3 , 600 MHz): $\delta = 8.17$ (bs, 1H), 6.99 (bs, 1H), 6.45 (bs, 1H), 4.28 (d, $J = 4.5$ Hz, 2H), 3.68-3.49 (m, 12H), 2.61 ppm (s, 3H); $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz): $\delta = 201.40, 167.48, 157.51$ (q, $J = 31.25$ Hz, $\text{C}(\text{O})\text{CF}_3$), 116.01 (q, $J = 238.75$ Hz, CF_3), 70.55, 70.46, 69.63, 68.85, 49.15, 39.81, 39.57, 33.76 ppm; HRMS for $\text{C}_{12}\text{H}_{21}\text{F}_3\text{N}_3\text{O}_4\text{S}^+$, m/z : $[\text{M}+\text{H}^+]$ calcd.: 344.1433, found: 344.1430.

***N*-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)acetamide (17)**

1-Azido-11-hydroxy-3,6,9-trioxaundecane (72 mg, 0.33 mmol) (**14**) was dissolved in a solution of NH₄OAc (0.1 M, pH 4)/DMF (7:3, 17.6 mL, 20 mM) and thioacetic acid (0.25 g, 3.30 mmol, 10 eq.) was added. The reaction mixture was stirred for 2 d at 40 °C. The solvent was then removed *in vacuo* and the resulting residue was dissolved in a H₂O:MeCN mixture and lyophilised. The crude reaction mixture was checked by ¹H-NMR (CDCl₃, 300 MHz). Purification by preparative HPLC (t_r = 5.4 min, 6 % for 5 min, 6 % to 80 % MeCN in 50 min) yielded compound **17** in 18 % (14 mg, 0.06 mmol): ¹H-NMR (CDCl₃, 300 MHz): δ = 7.33 (bs, 1H), 3.72-3.71 (m, 4H), 3.68-3.55 (m, 8H), 3.53-3.50 (m, 2H), 3.43-3.39 (m, 2H), 3.13 (bs), 1.97 ppm (s, 3H); ¹³C-NMR (CDCl₃, 75 MHz): δ = 170.60, 72.49, 70.59, 70.32, 70.08, 69.88, 69.86, 61.44, 39.20, 22.81 ppm; HRMS for C₁₀H₂₂NO₅⁺, m/z: [M+H⁺] calcd.: 236.1492, found: 236.1554.

***N*-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)thioacetamide (18)**

1-Azido-11-hydroxy-3,6,9-trioxaundecane (**14**) (78 mg, 0.36 mmol) was dissolved in a solution of KCl/HCl (0.1 M, pH 2, 17.8 mL, 20 mM) and thioacetic acid (0.31 g, 3.36 mmol, 10 eq.) was added. The reaction mixture was stirred for 2 d at 40 °C. The solvent was then removed *in vacuo* and the resulting residue was dissolved in a H₂O:MeCN mixture and lyophilised. The crude reaction mixture was checked by ¹H-NMR (CDCl₃, 300 MHz). Purification by preparative HPLC (t_r = 19.8 min, 6 % for 5 min, 6 % to 80 % MeCN in 50 min) yielded the desired PEG-Thioacetamide **18** in 32 % (28.9 mg, 0.114 mmol): ¹H-NMR (CDCl₃, 300 MHz): δ = 9.41 (s), 3.89-3.84 (m, 2H), 3.75-3.70 (m, 4H), 3.70-3.61 (m, 10H), 3.00 (bs), 2.55 ppm (s, 3H); ¹³C-NMR (CDCl₃, 75 MHz): δ = 72.46, 70.67, 70.22, 69.86, 69.71, 69.64, 61.24, 46.13, 33.30 ppm; HRMS for C₁₀H₂₂NO₄S⁺, m/z: [M+H⁺] calcd.: 252.1270, found: 252.1270.

Analytical Data

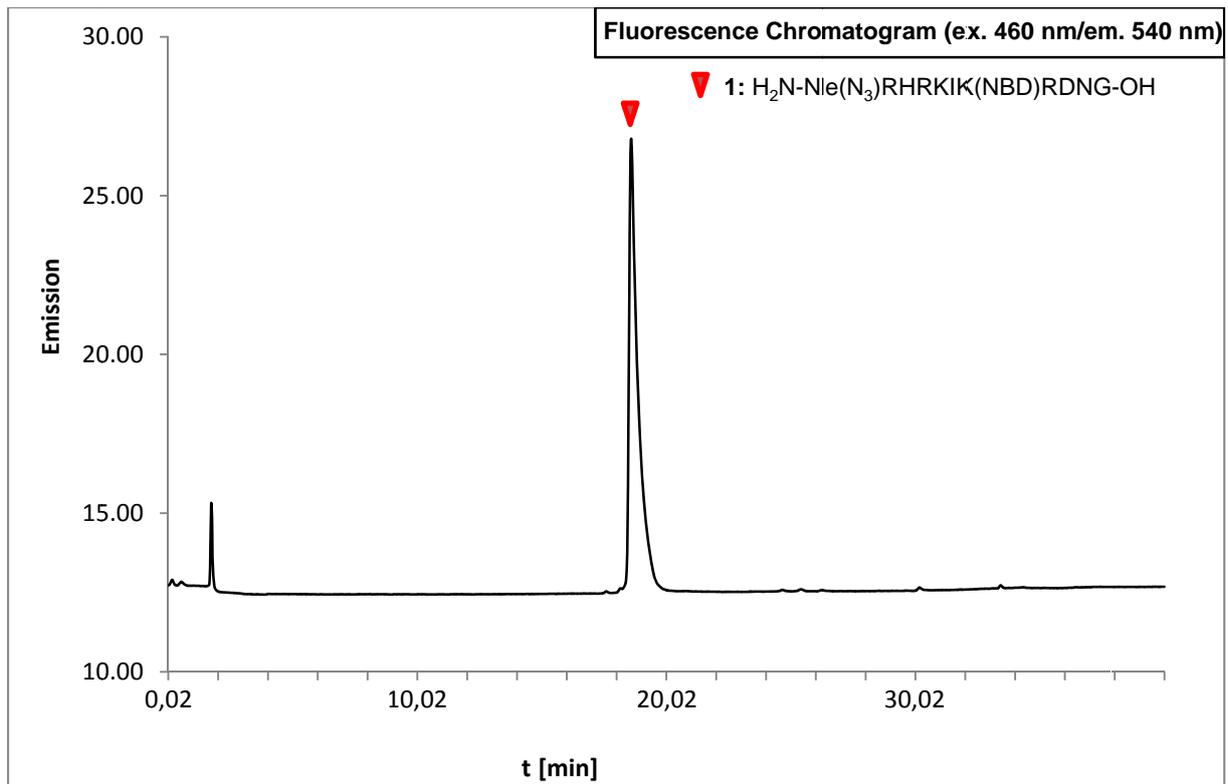


Figure S1: LC-fluorescence chromatogram of peptide 1.

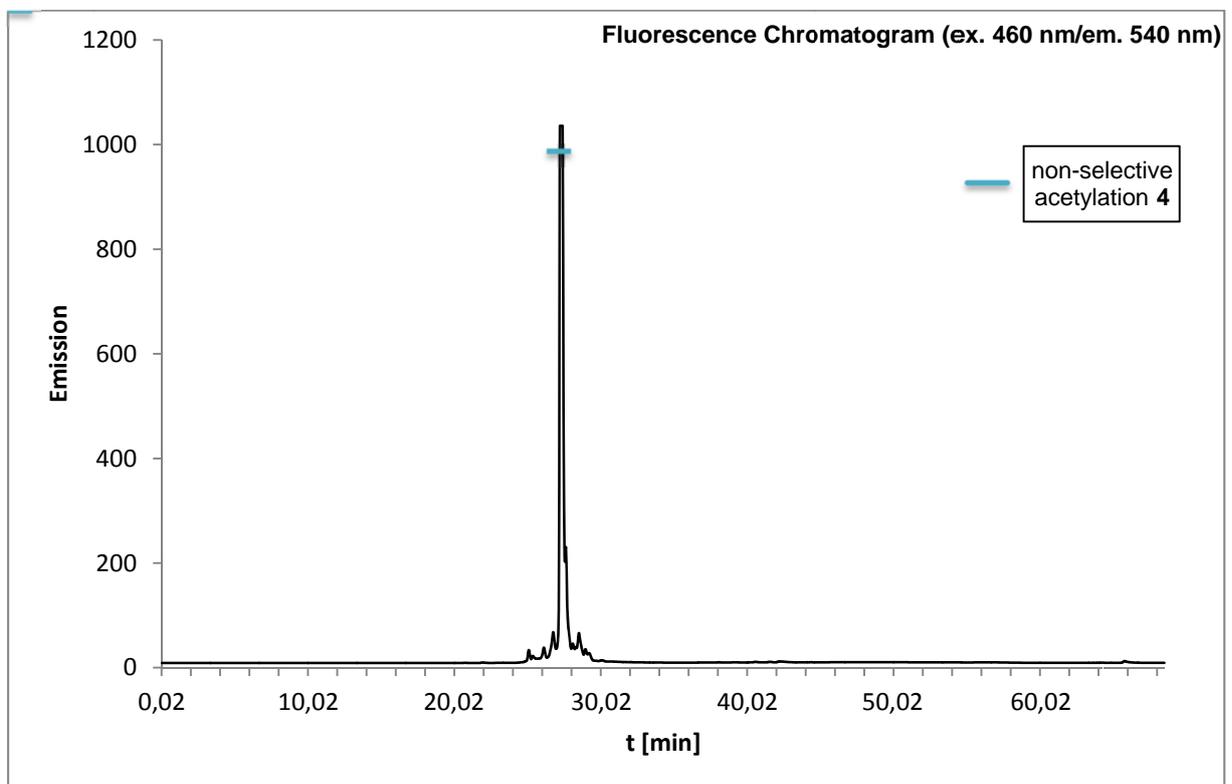


Figure S2: LC-fluorescence chromatogram (Table 1, entry 1, Gradient A).

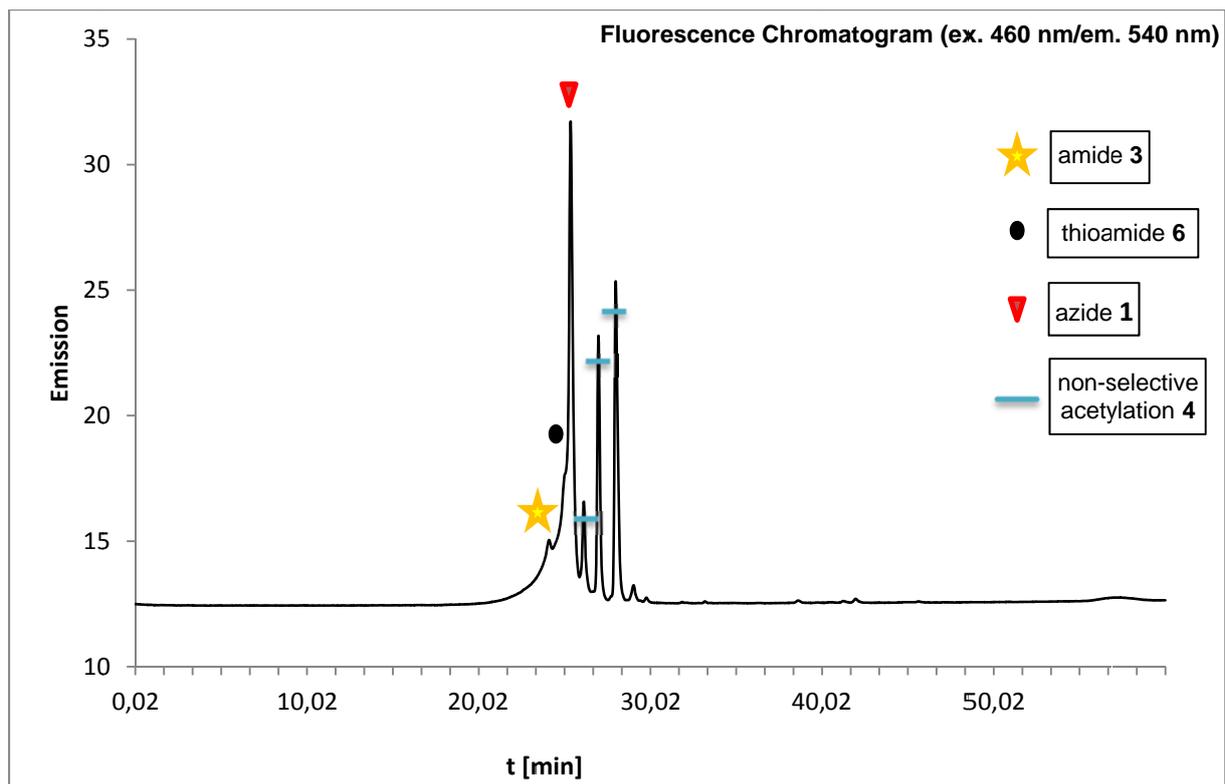


Figure S3: LC-fluorescence chromatogram (Table 1, entry 2, Gradient A).

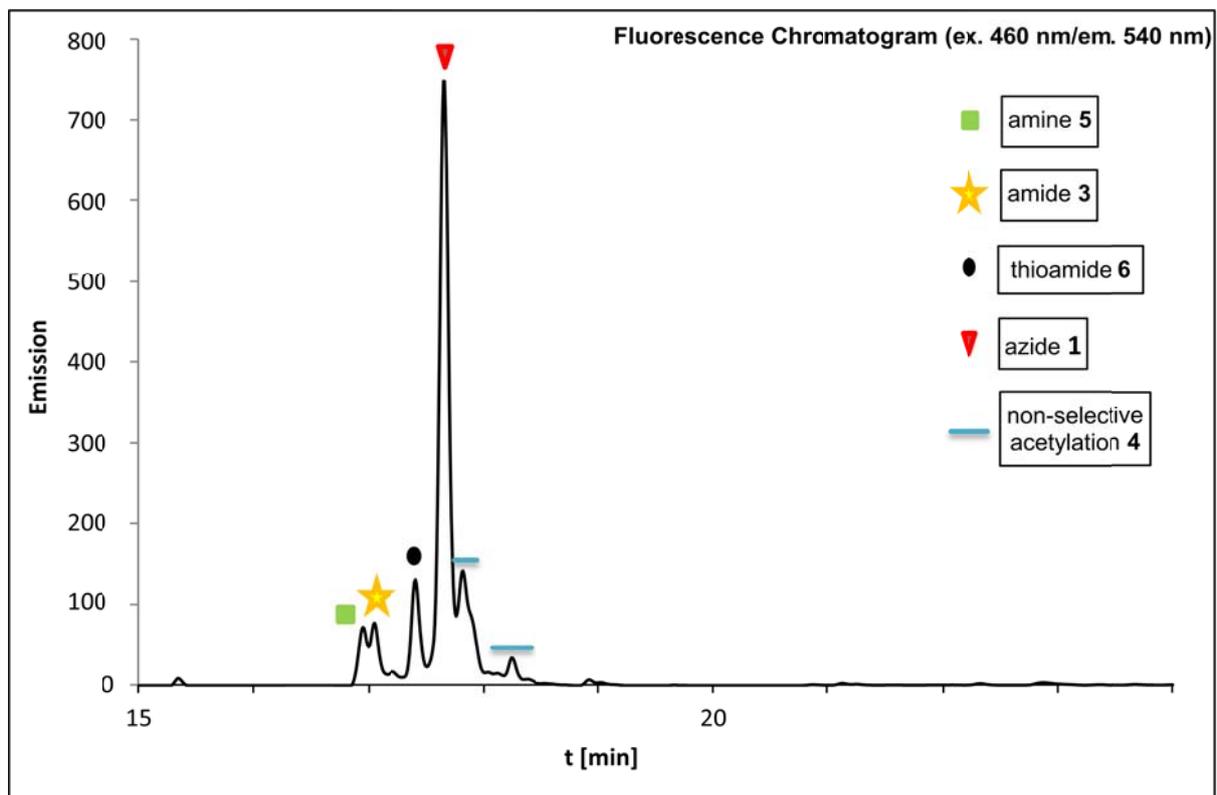


Figure S4: LC-fluorescence chromatogram (Table 1, entry 3, Gradient B).

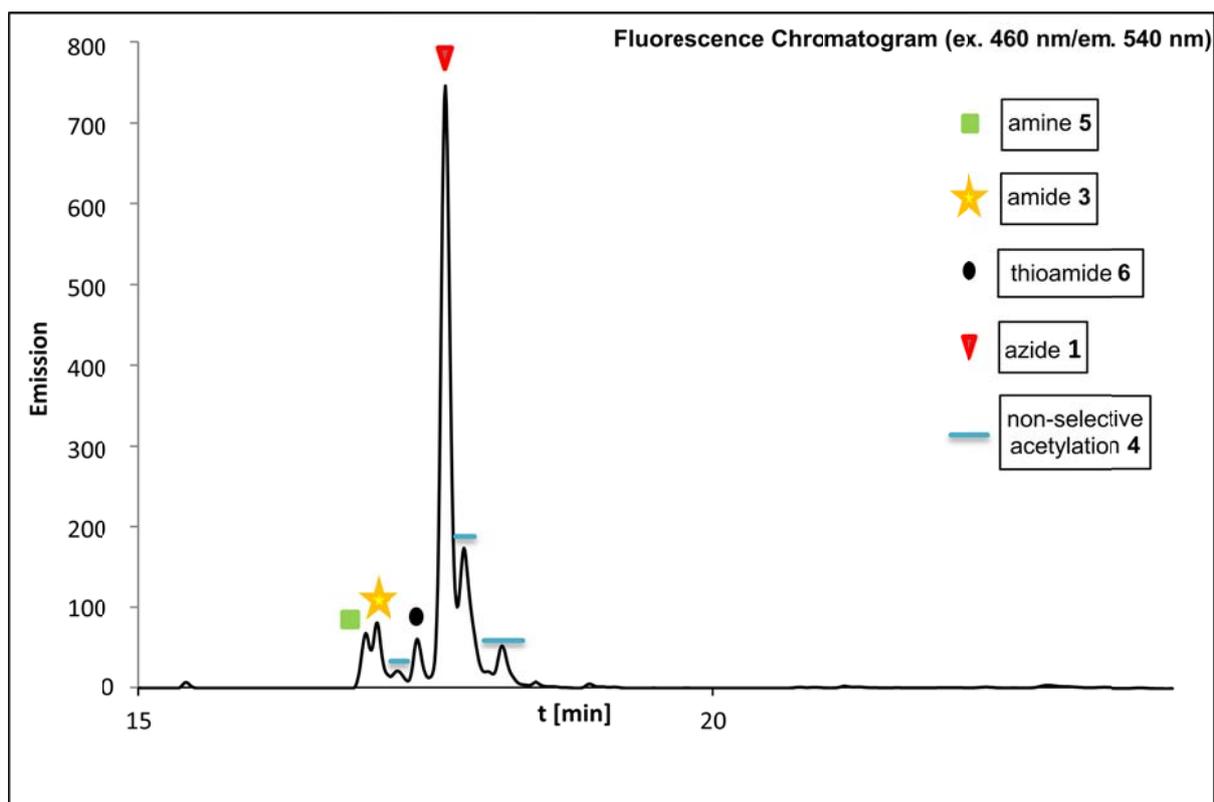


Figure S5: LC-fluorescence chromatogram (Table 1, entry 4, Gradient B).

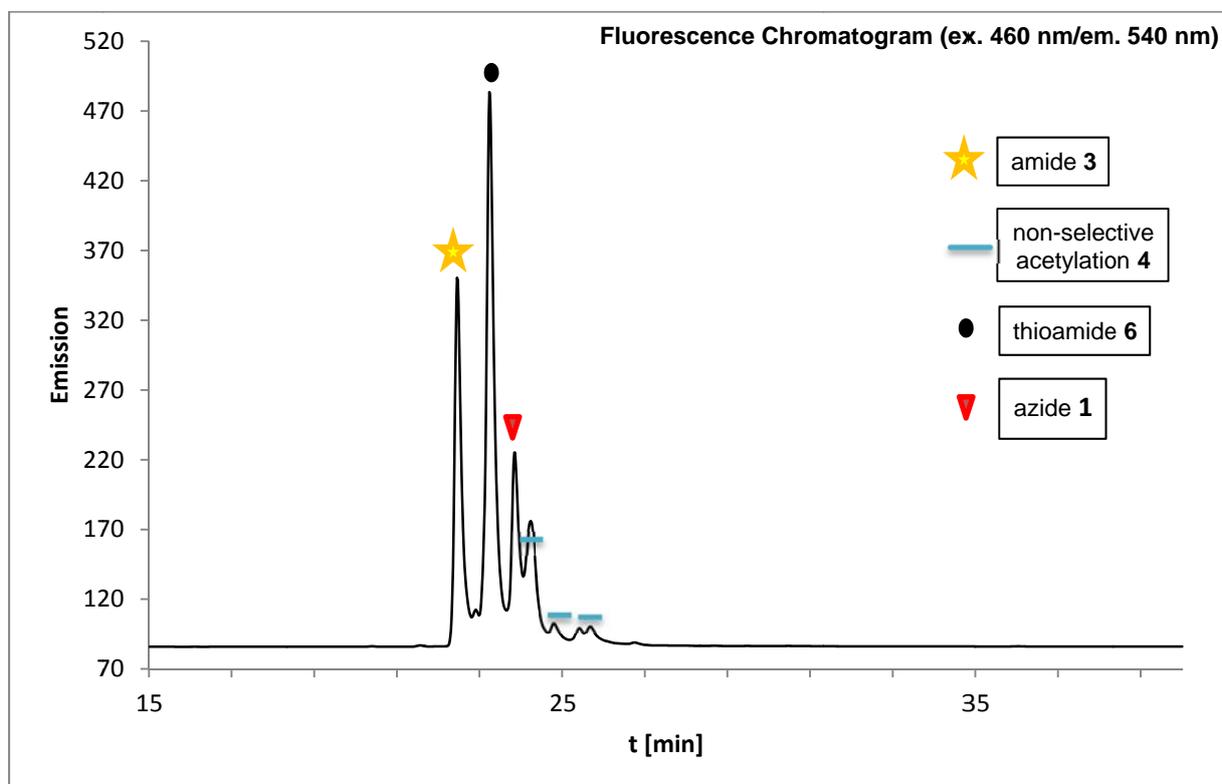


Figure S6: LC-fluorescence chromatogram (Table 1, entry 5, Gradient A).

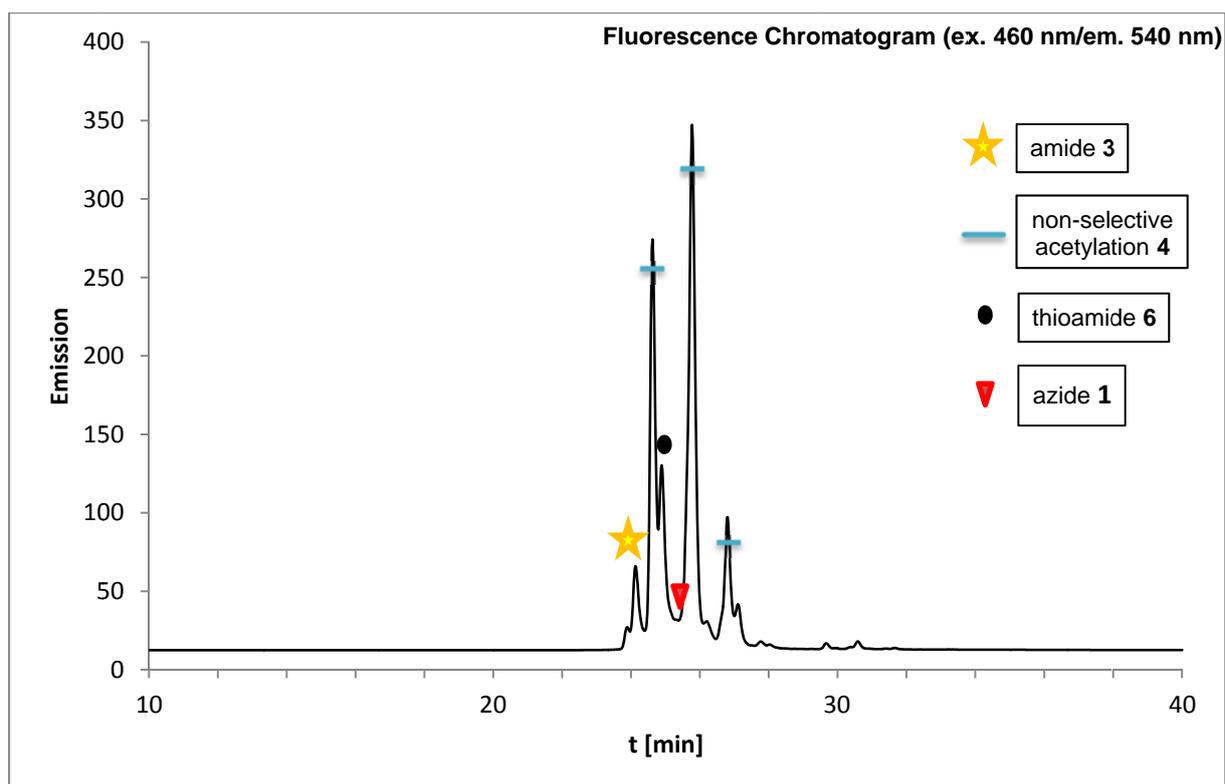


Figure S7: LC-fluorescence chromatogram (Table 1, entry 6, Gradient A).

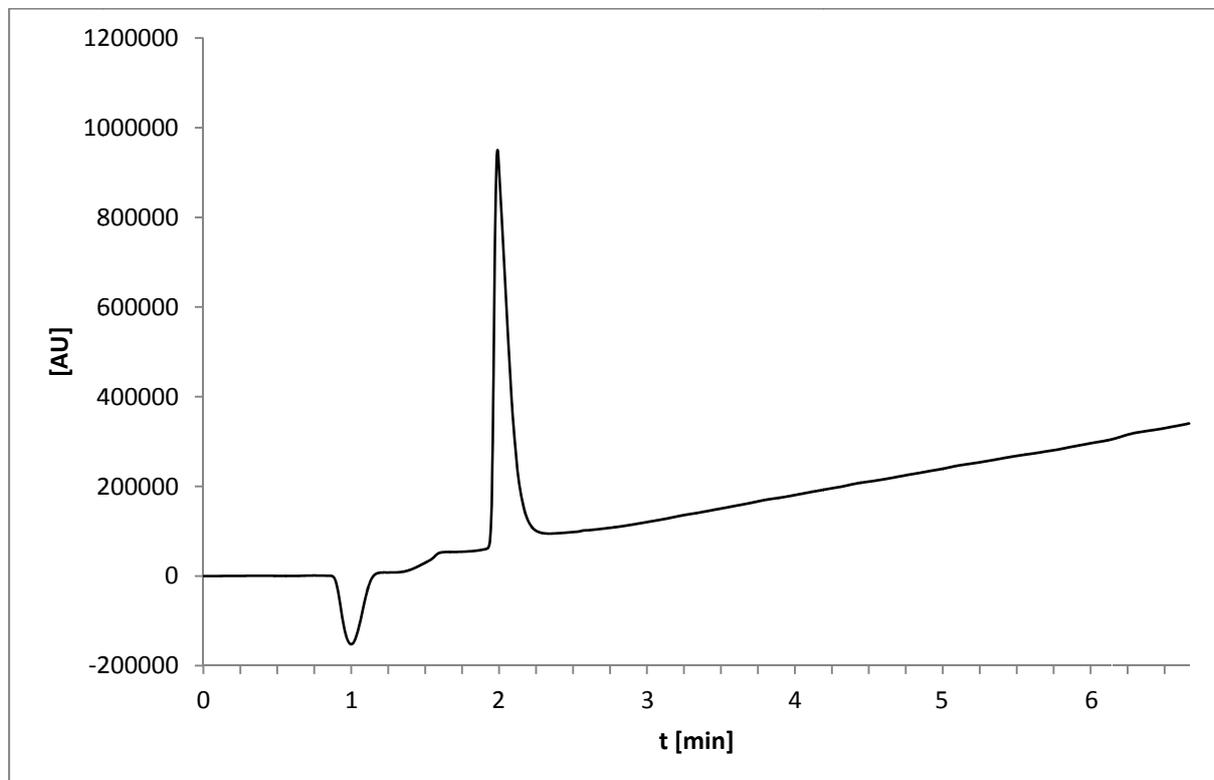


Figure S8: HPLC-UV trace of azido glycine peptide **9** ($\lambda = 220$ nm).

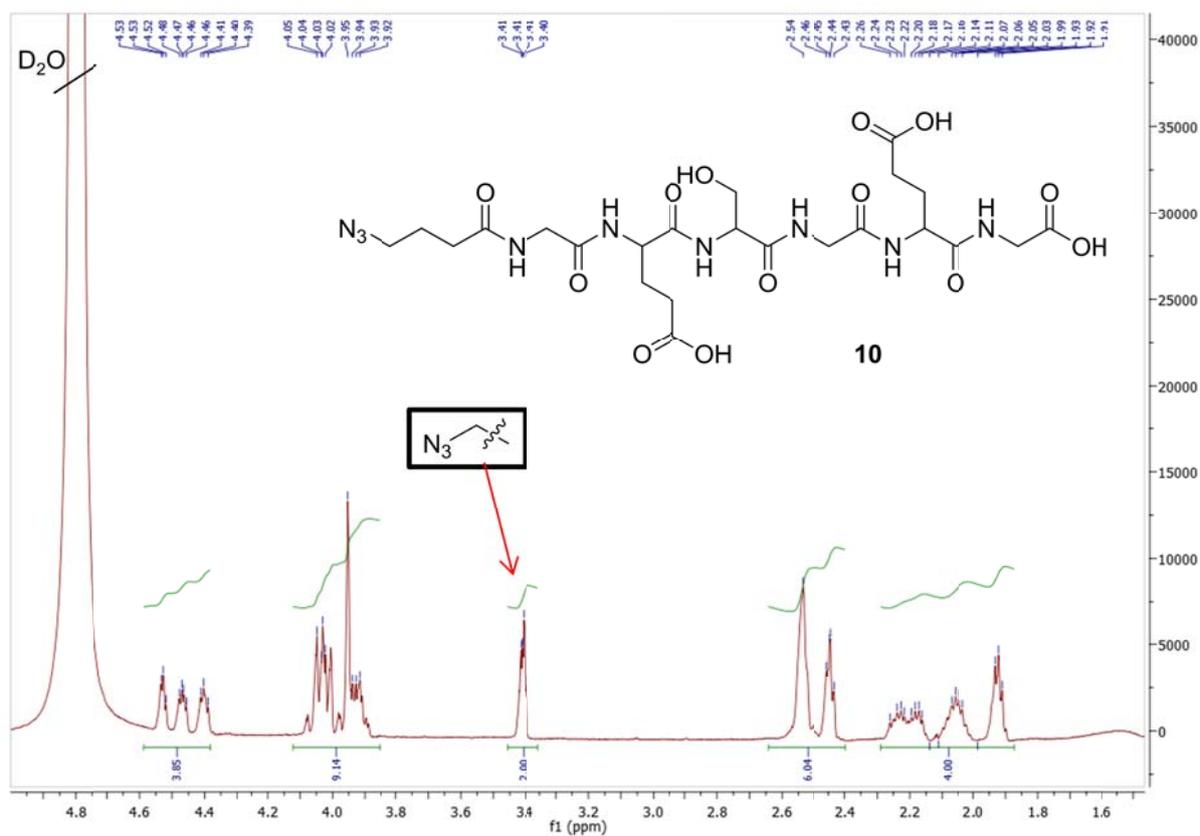


Figure S11: $^1\text{H-NMR}$ of azido peptide **10**.

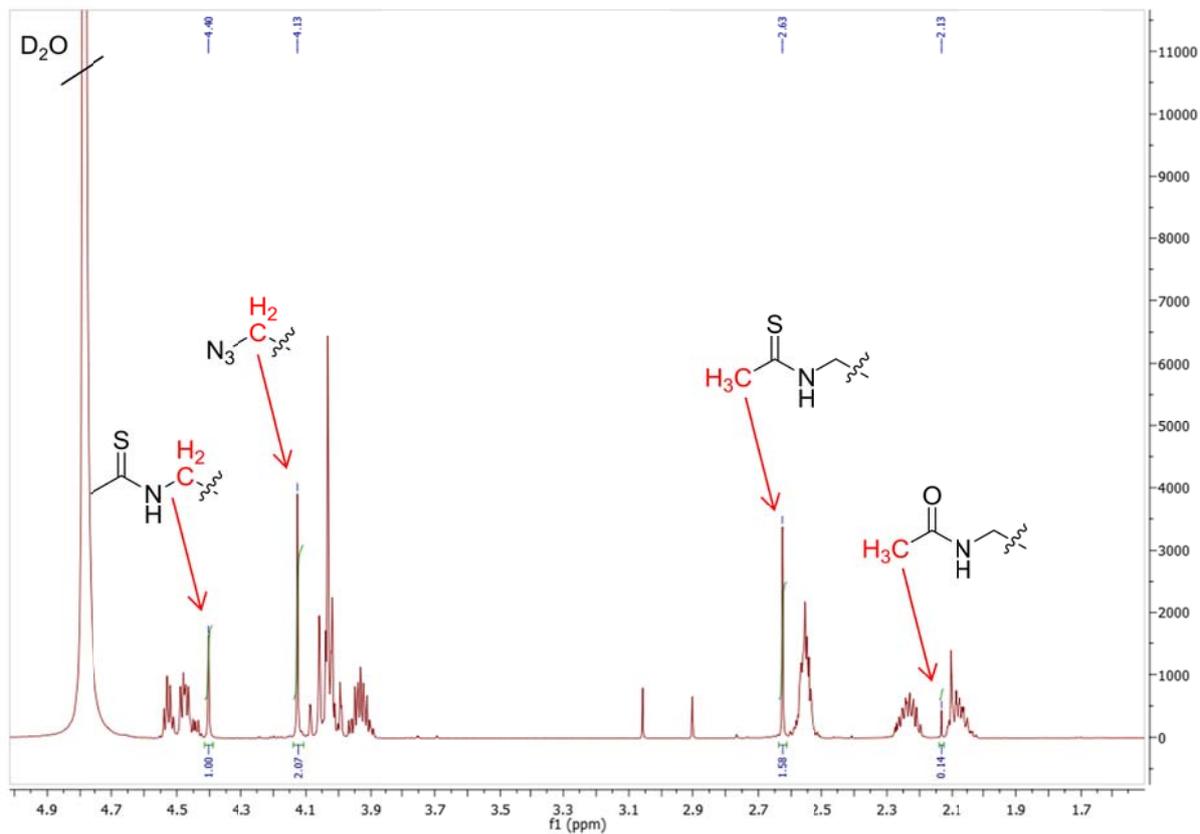


Figure S12: $^1\text{H-NMR}$: reaction of azido glycine peptide **9** & thioacetic acid (**2**) (Table 2, entry 1).

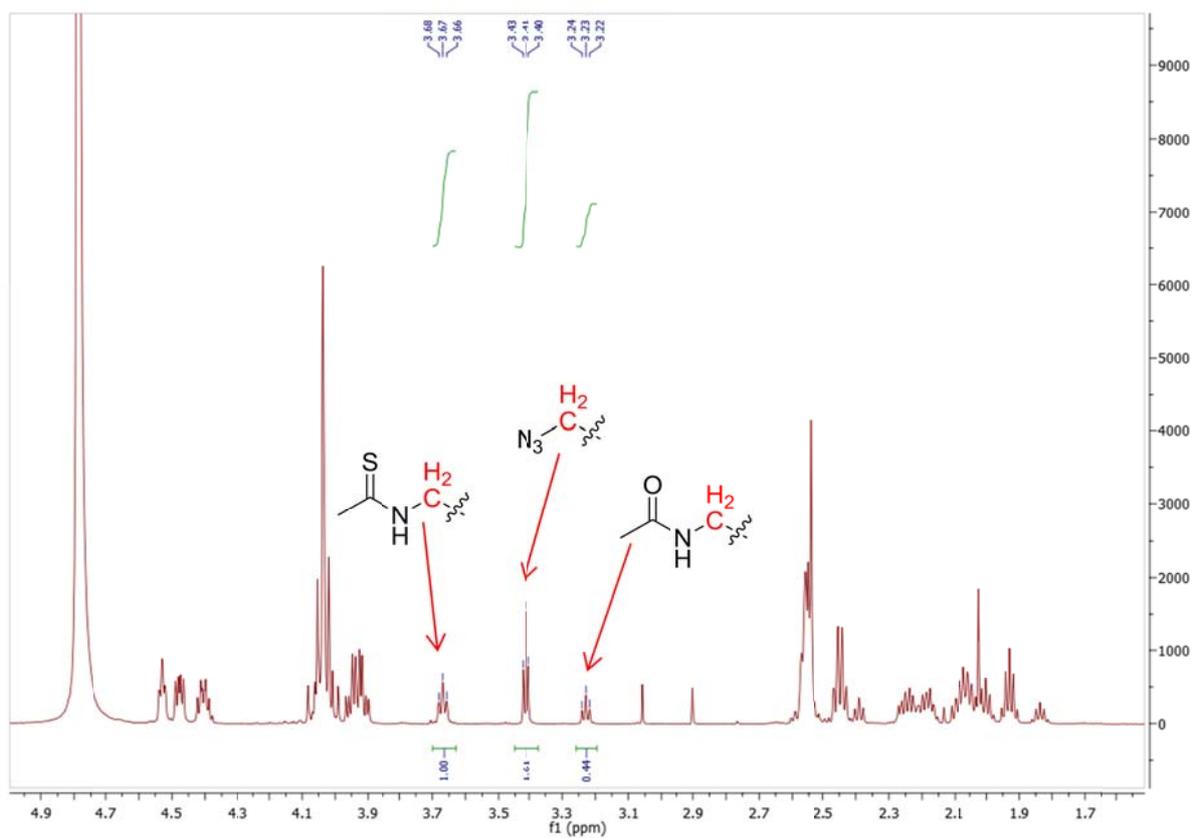


Figure S13: ¹H-NMR: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 2).

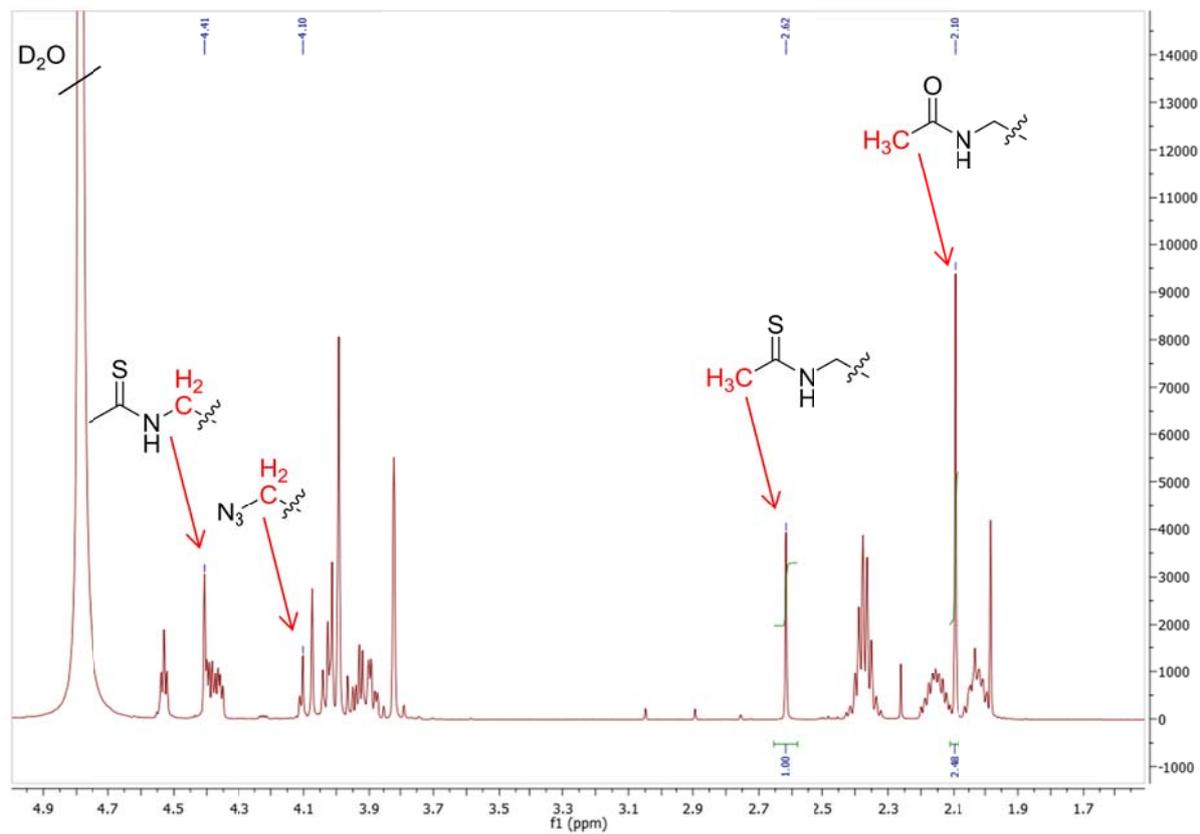


Figure S14: ¹H-NMR: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 3).

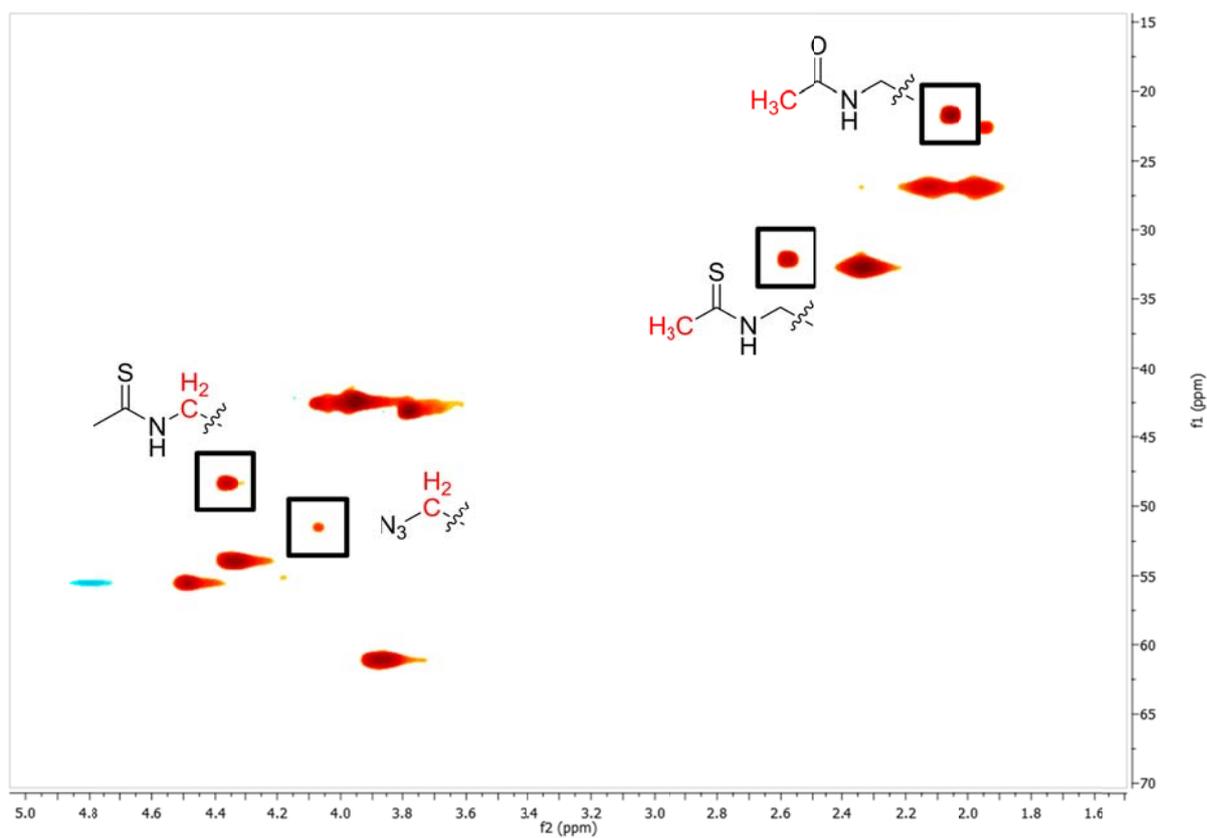


Figure S15: HMQC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 3).

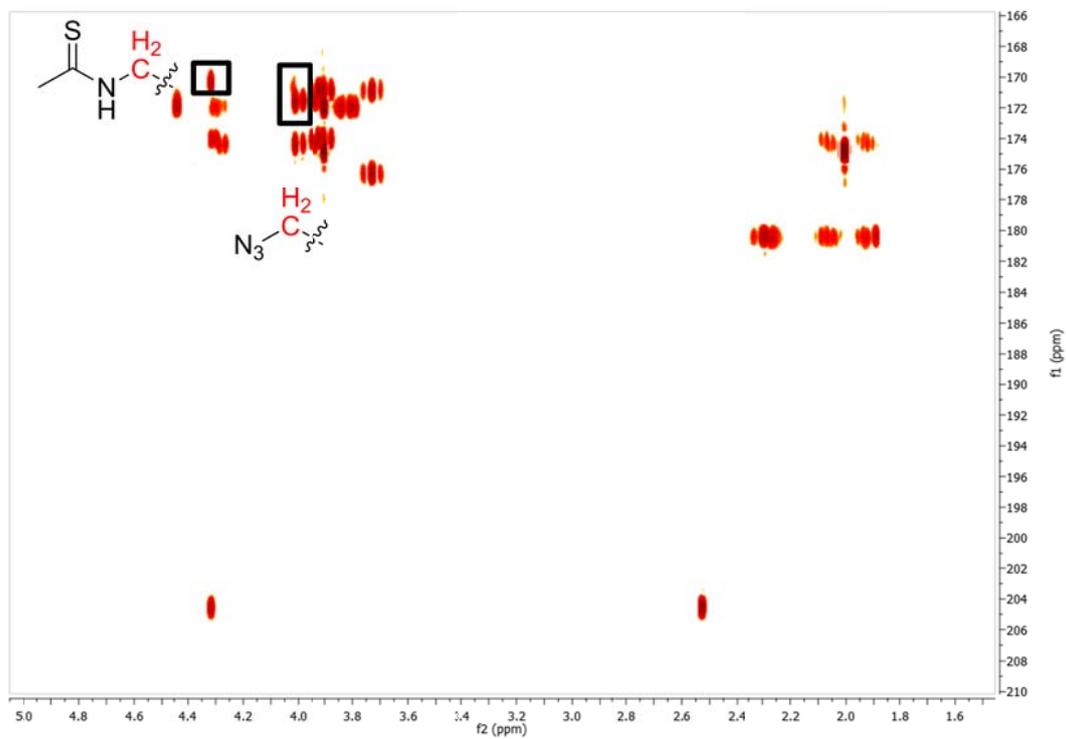


Figure S16: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 3).

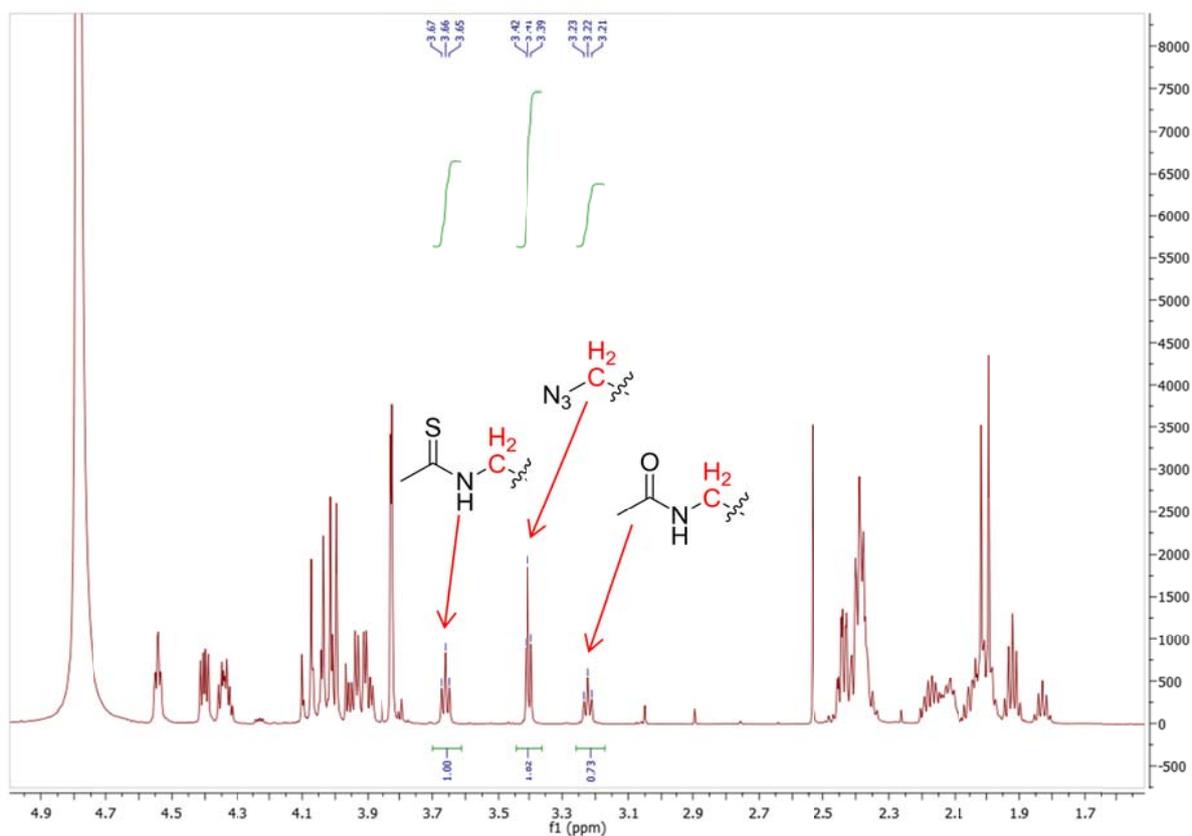


Figure S17: ¹H-NMR: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 4).

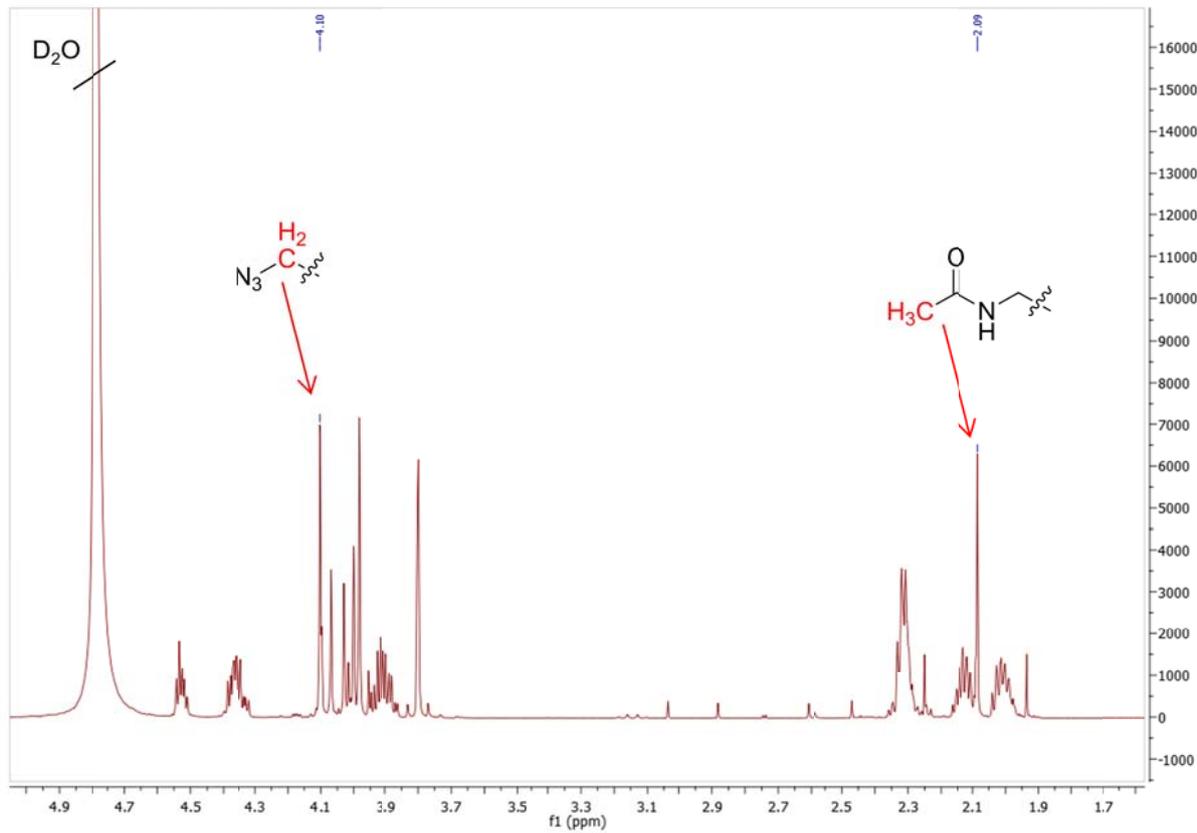


Figure S18: ¹H-NMR: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 5).

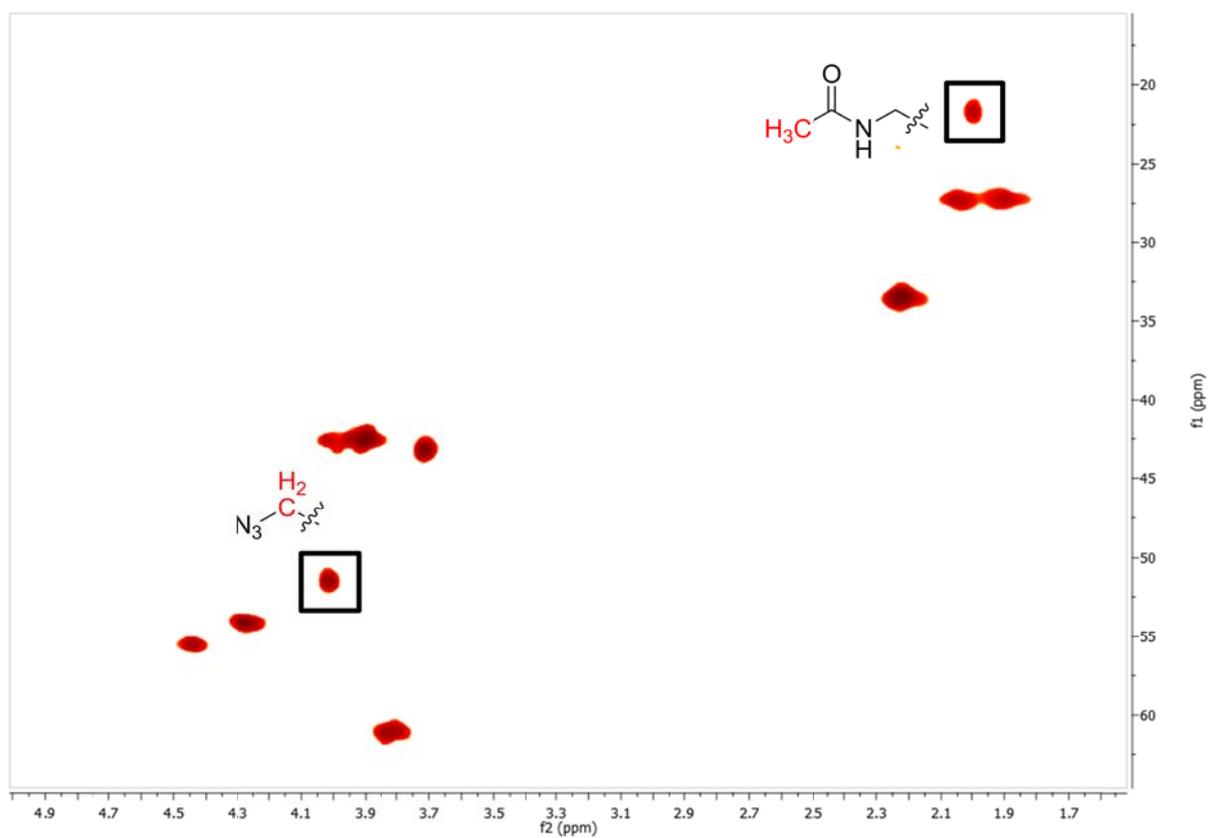


Figure S19: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 5).

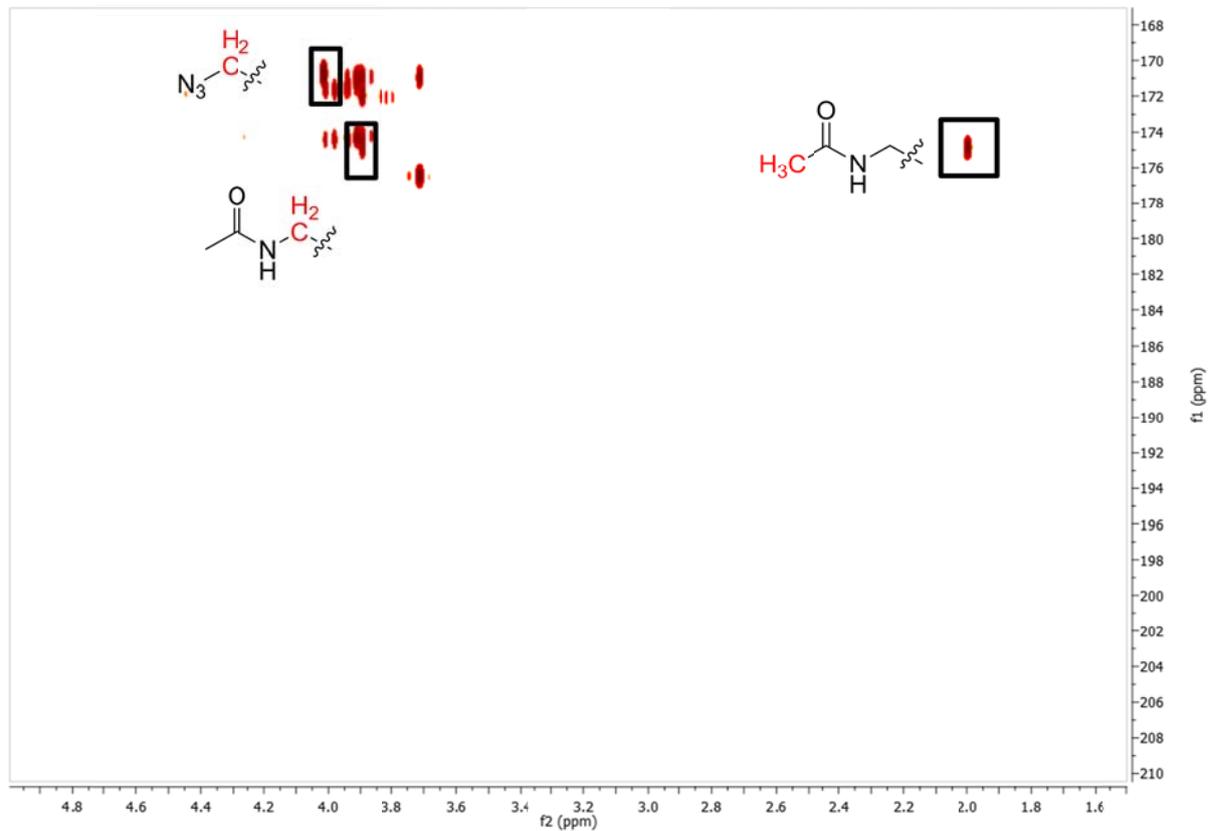


Figure S20: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 5).

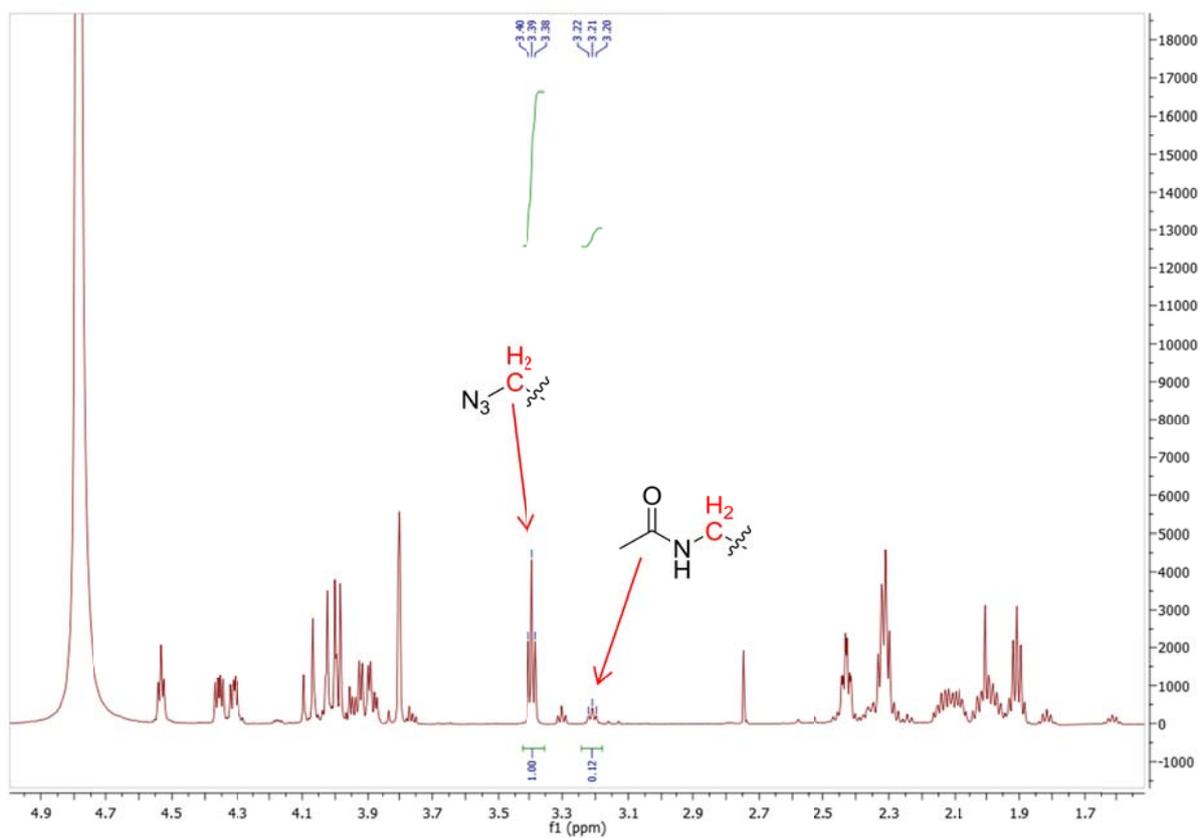


Figure S21: $^1\text{H-NMR}$: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 6).

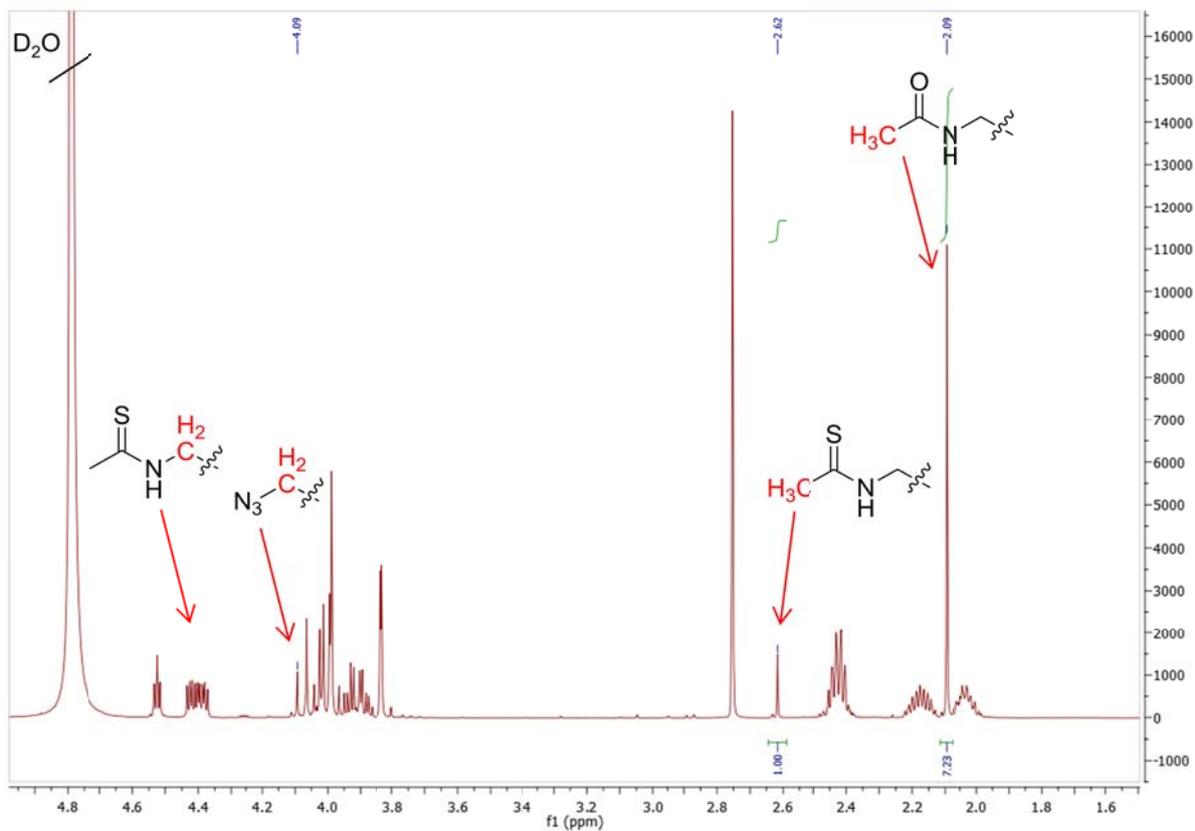


Figure S22: $^1\text{H-NMR}$: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 7).

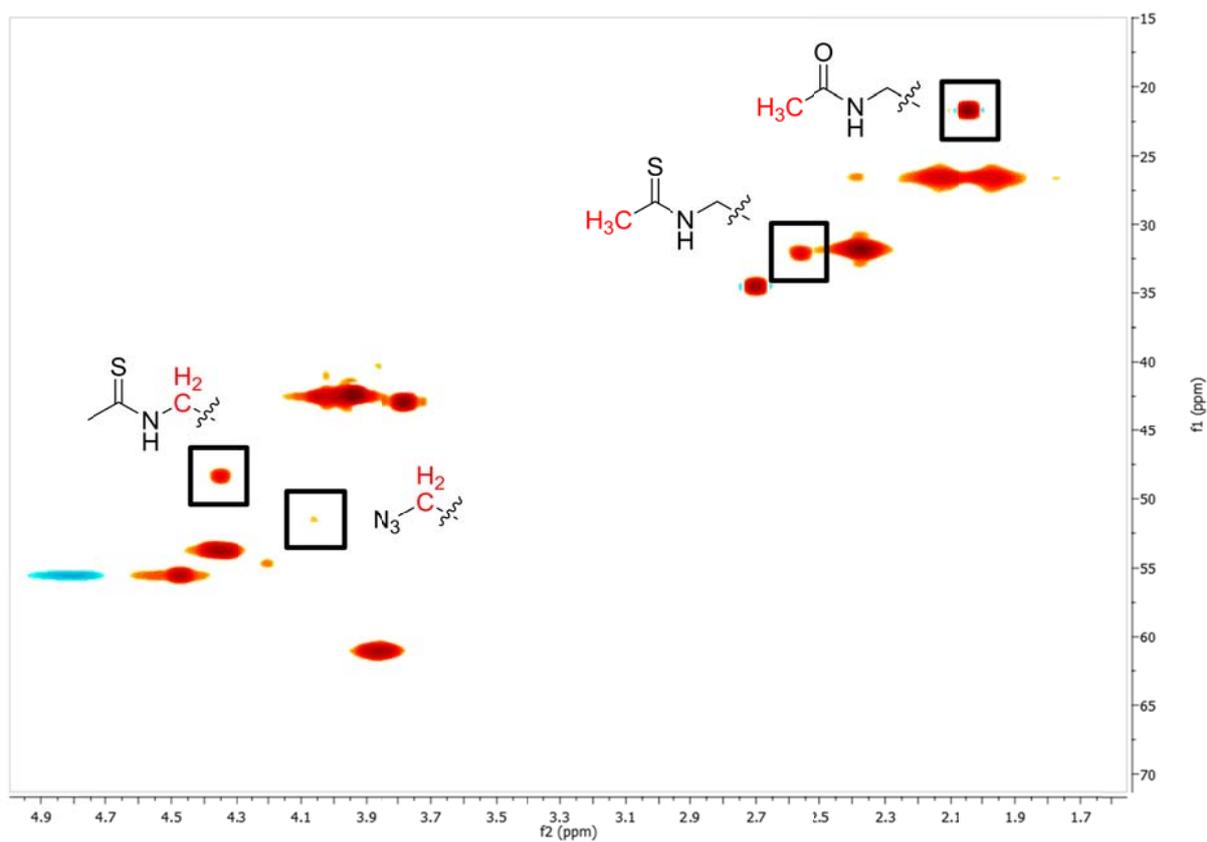


Figure S23: HMQC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 7).

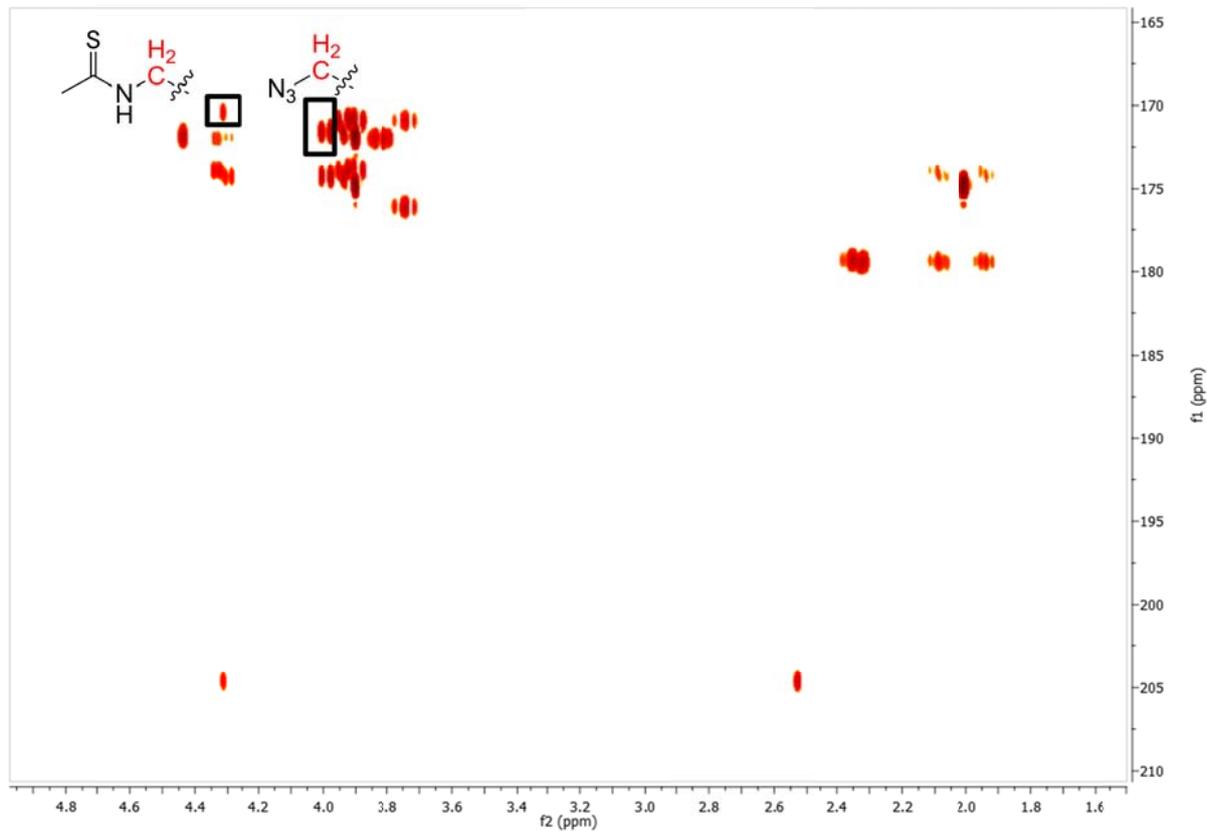


Figure S24: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 7).

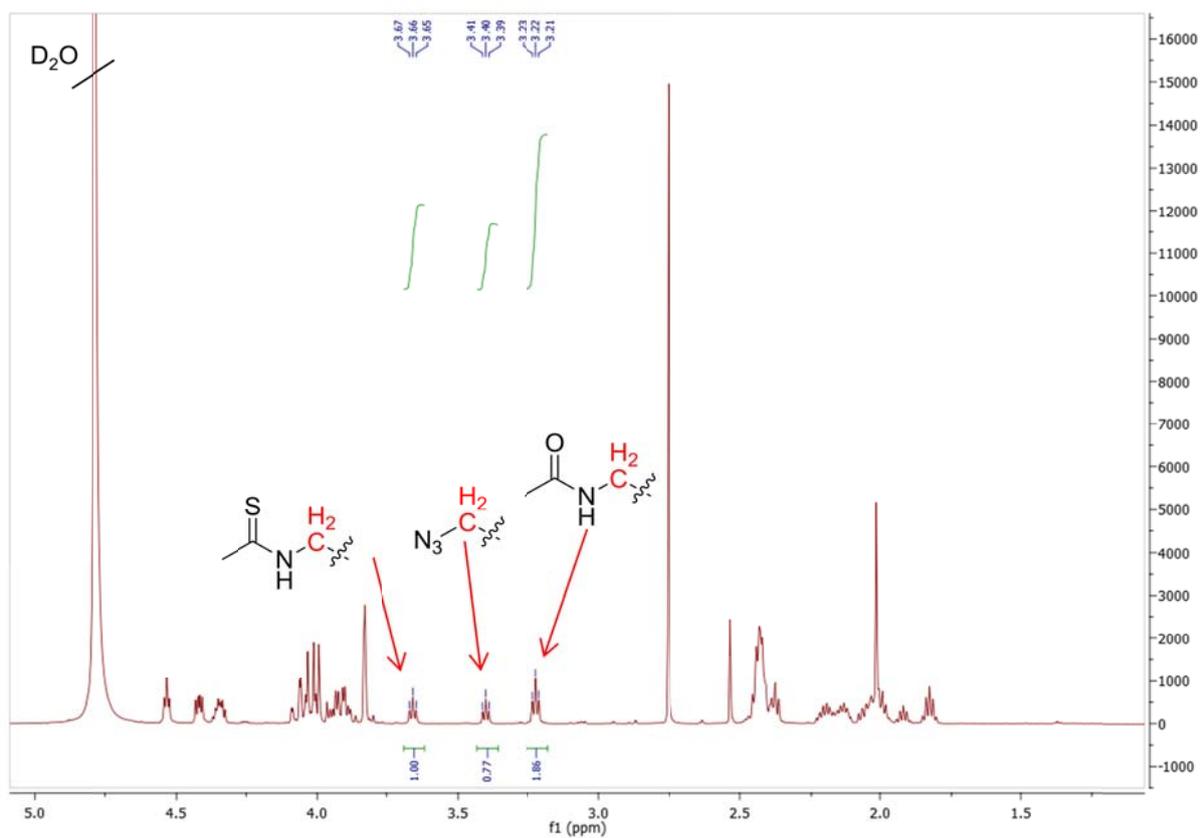


Figure S25: $^1\text{H-NMR}$: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 8).

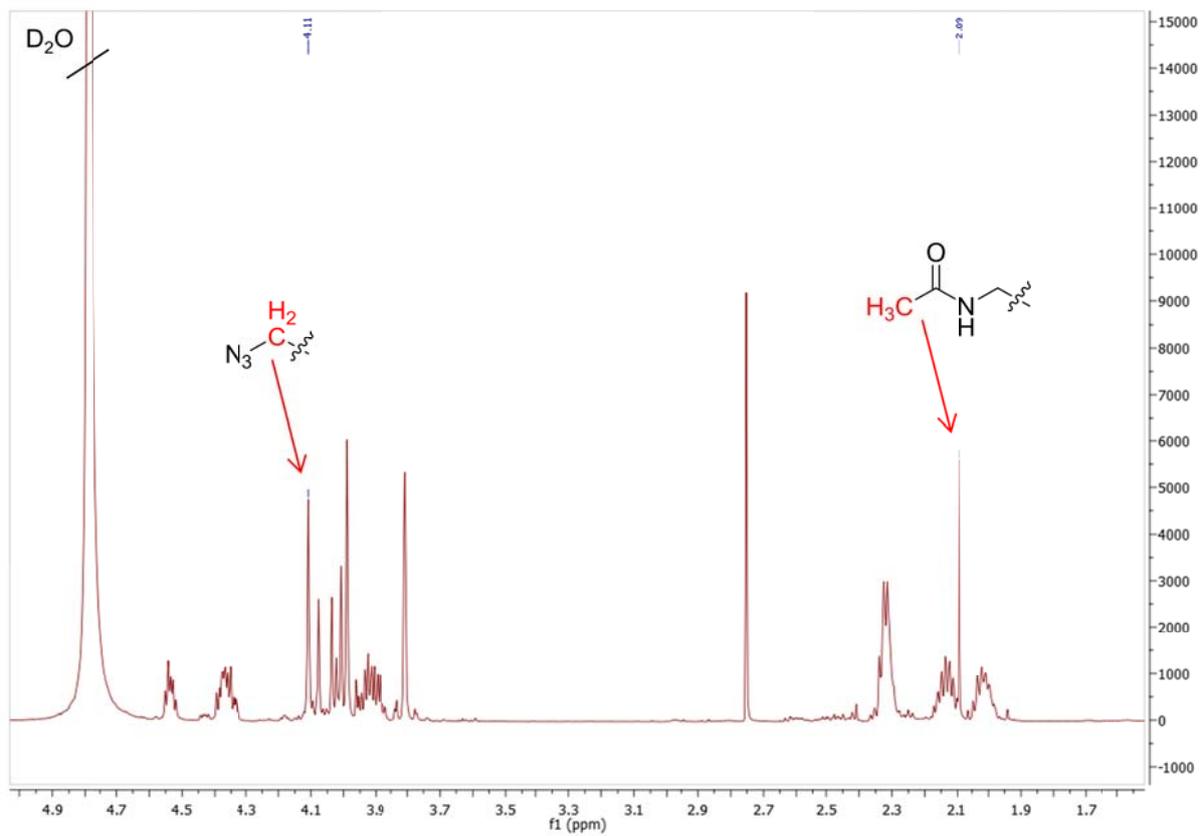


Figure S26: $^1\text{H-NMR}$: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 9).

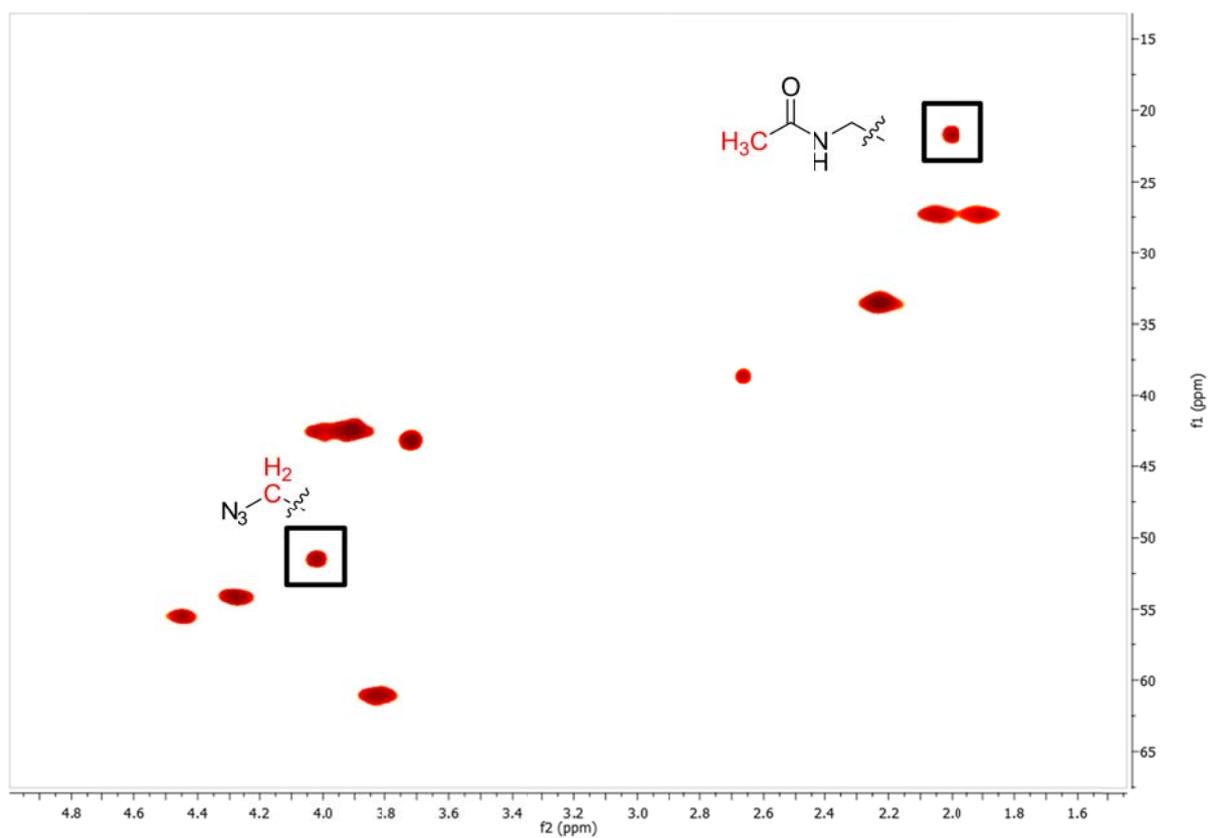


Figure S27: HMQC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 9).

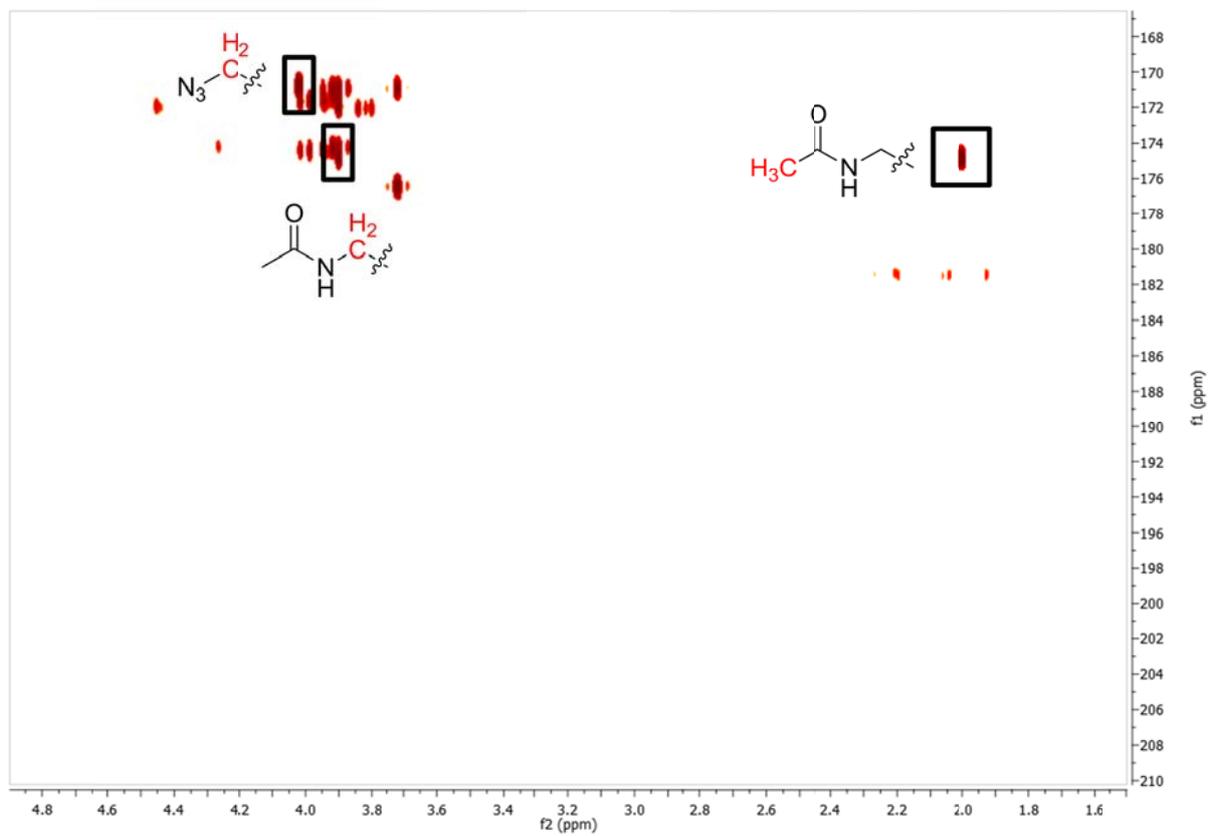


Figure S28: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 9).

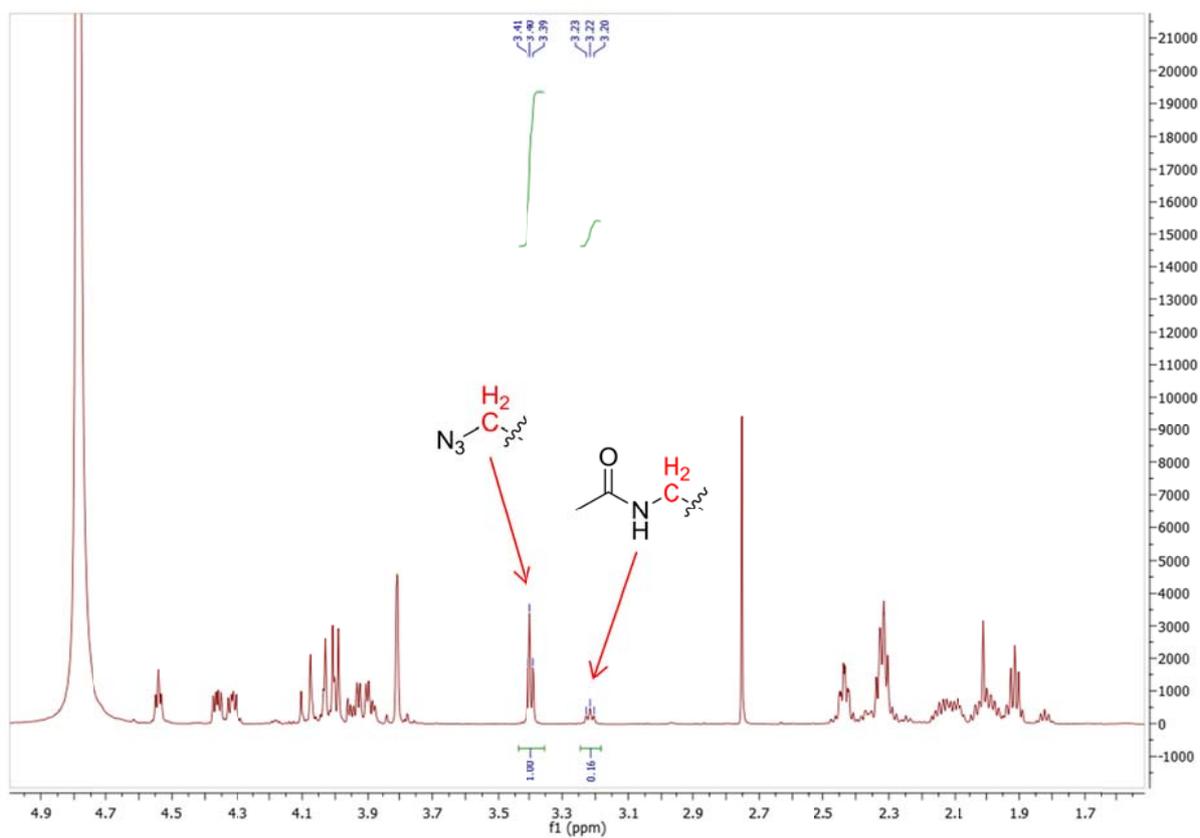


Figure S29: $^1\text{H-NMR}$: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 10).

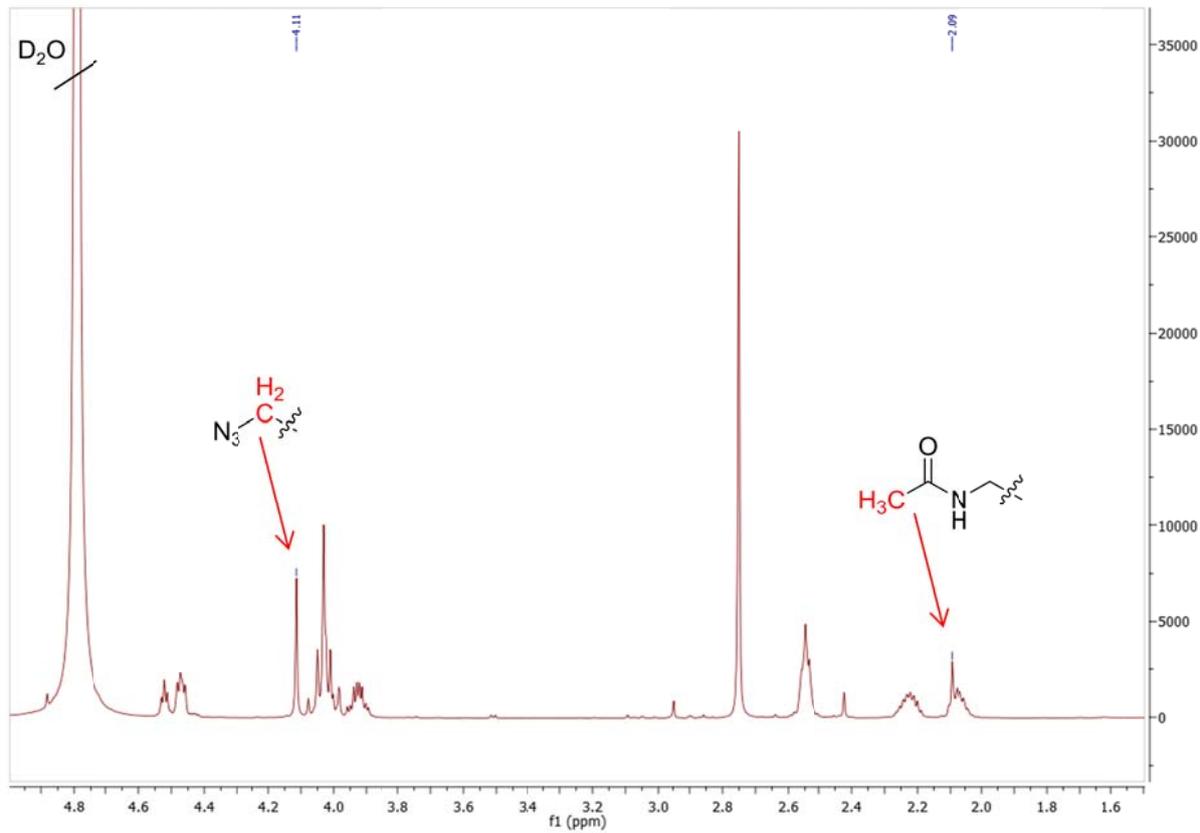


Figure S30: $^1\text{H-NMR}$: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 11).

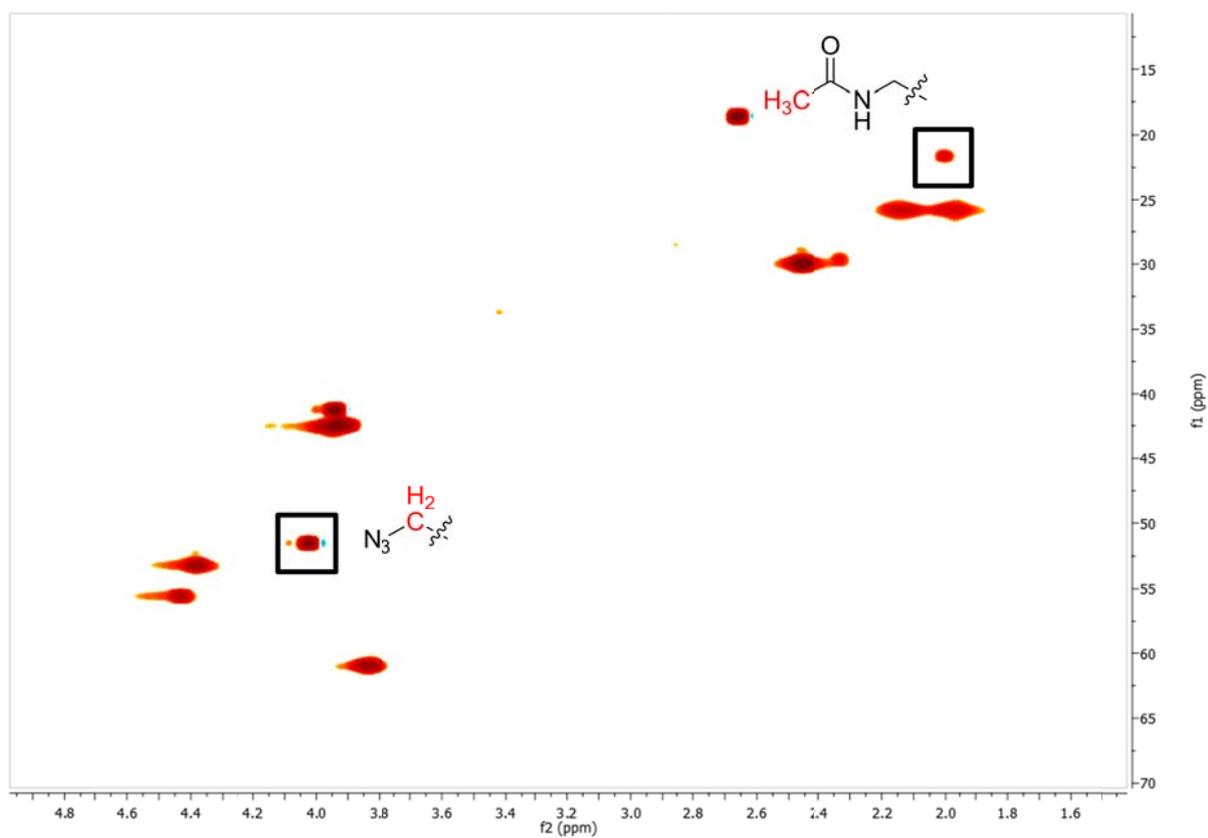


Figure S31: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 11).

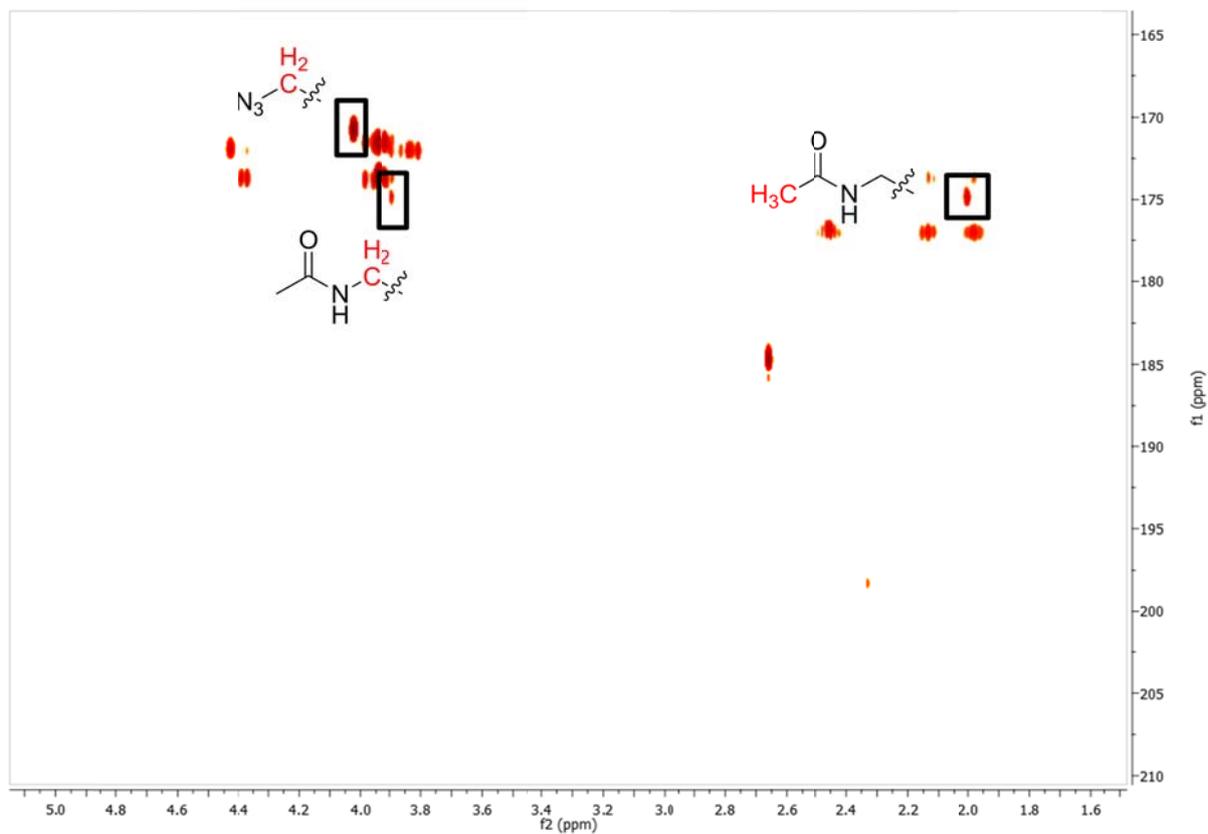


Figure S32: HMBC: reaction of azido glycine peptide **9** and thioacetic acid (**2**) (Table 2, entry 11).

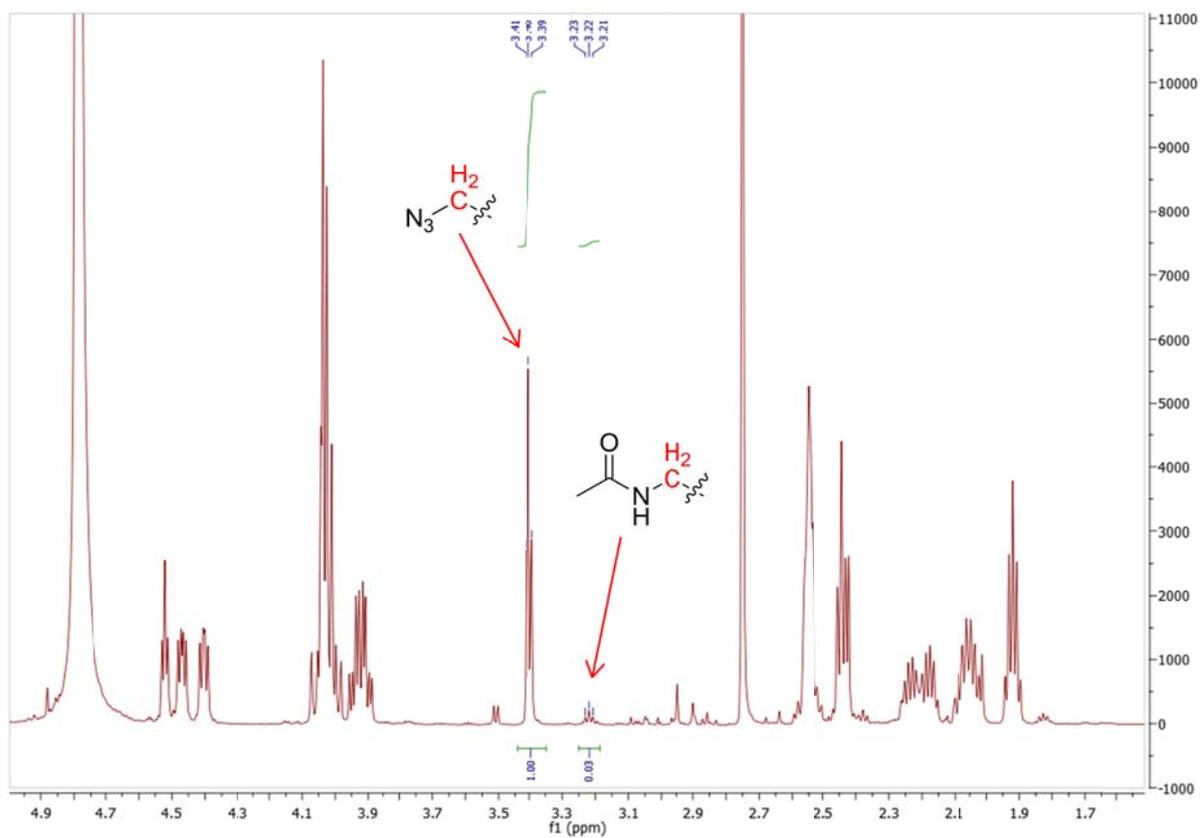


Figure S33: $^1\text{H-NMR}$: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 12).

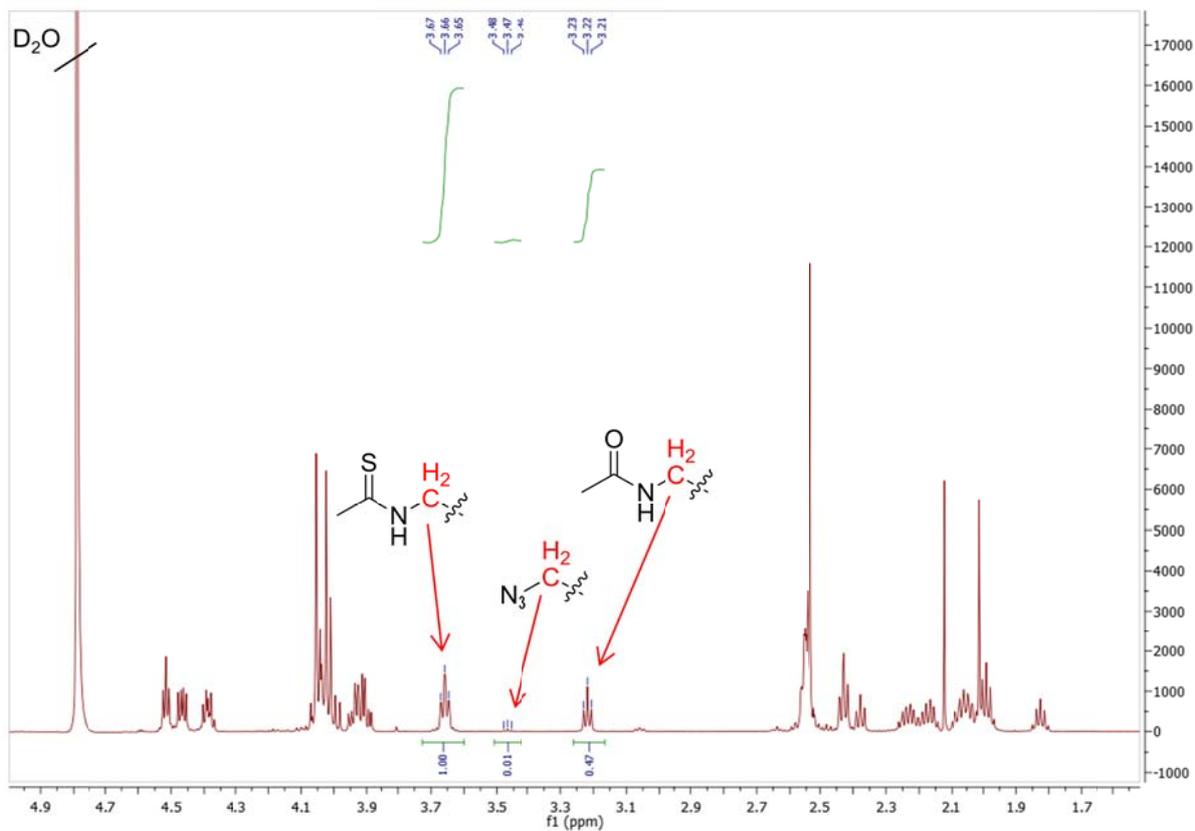


Figure S34: $^{13}\text{C-NMR}$: reaction of 4-azido butanoic acid peptide **10** and thioacetic acid (**2**) (Table 2, entry 13).

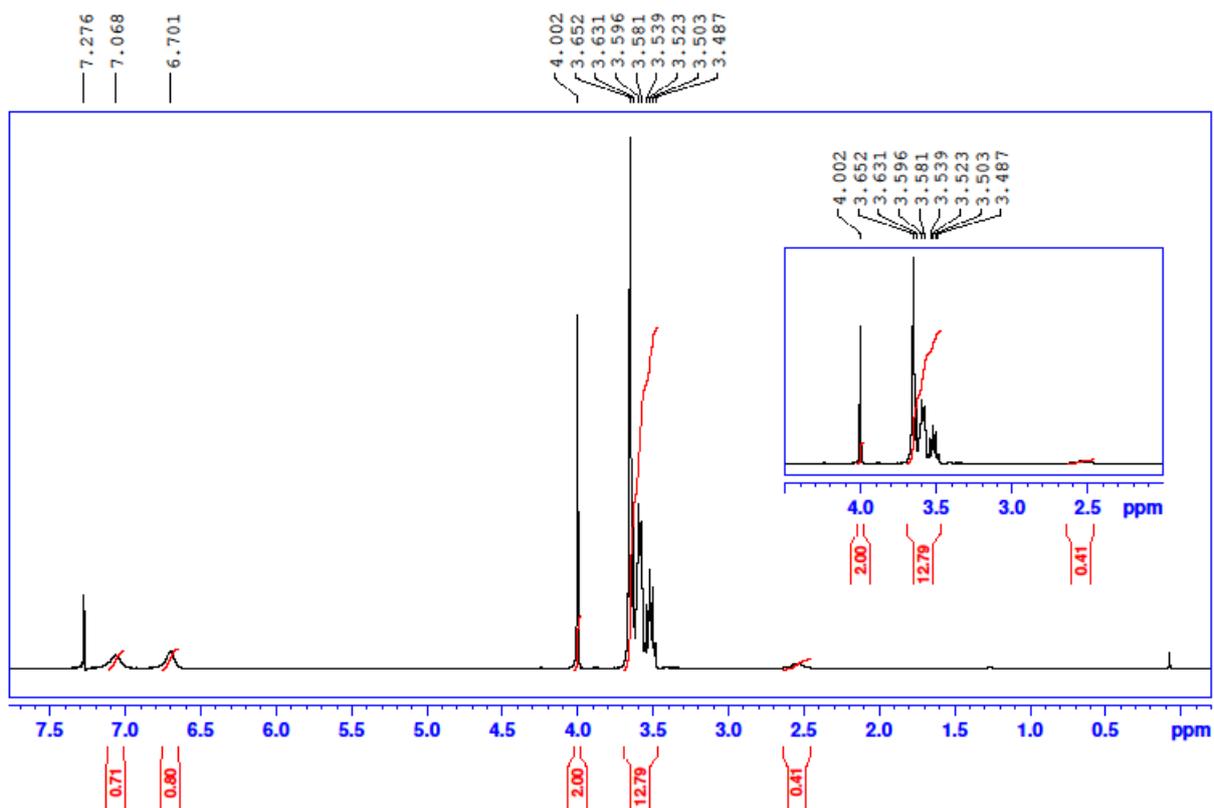


Figure S35: $^1\text{H-NMR}$ of azido compound 13.

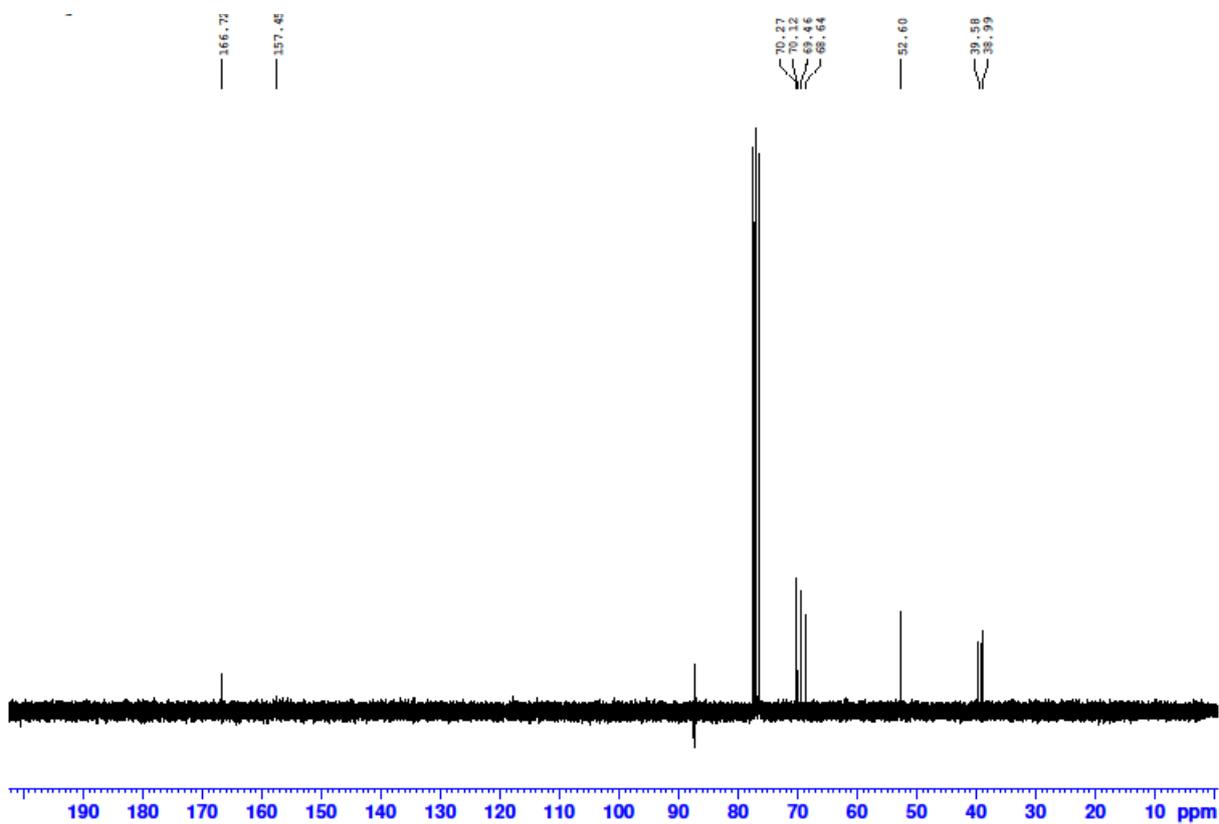


Figure S36: $^{13}\text{C-NMR}$ of azido compound 13.

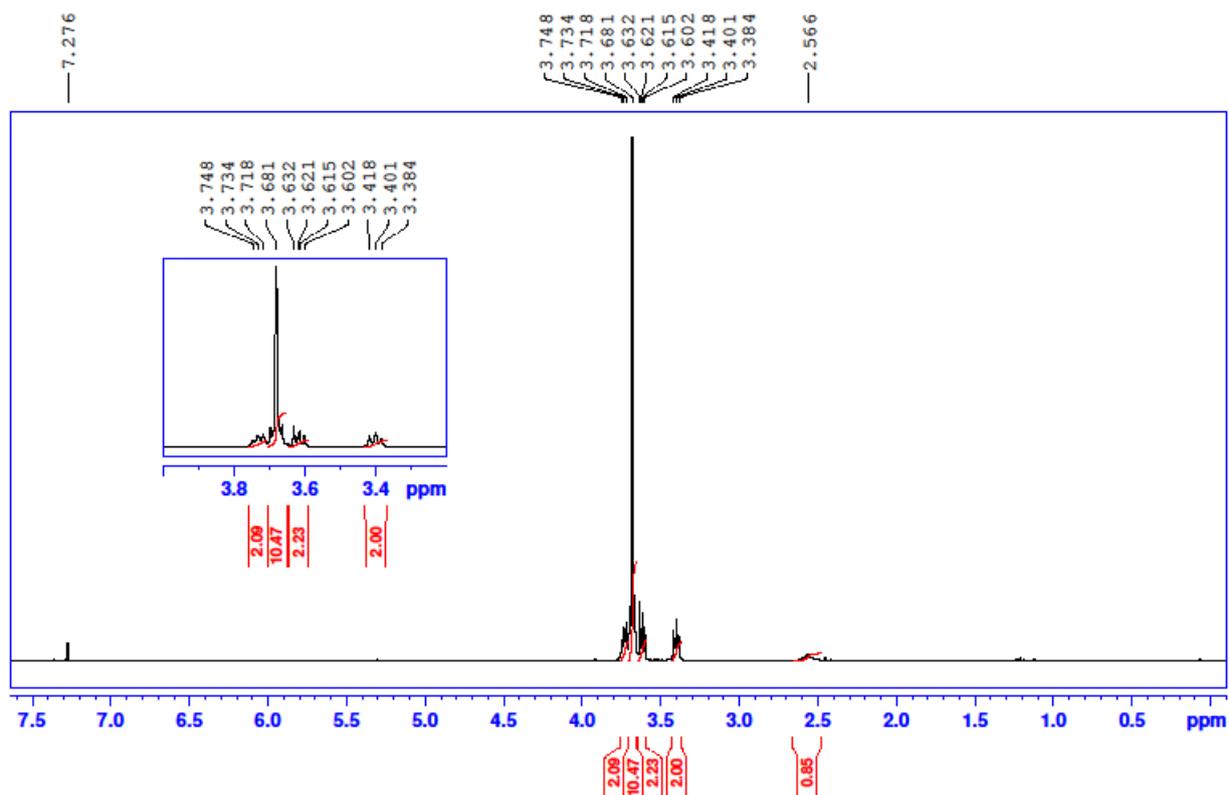


Figure S37: $^1\text{H-NMR}$ of azido compound 14.

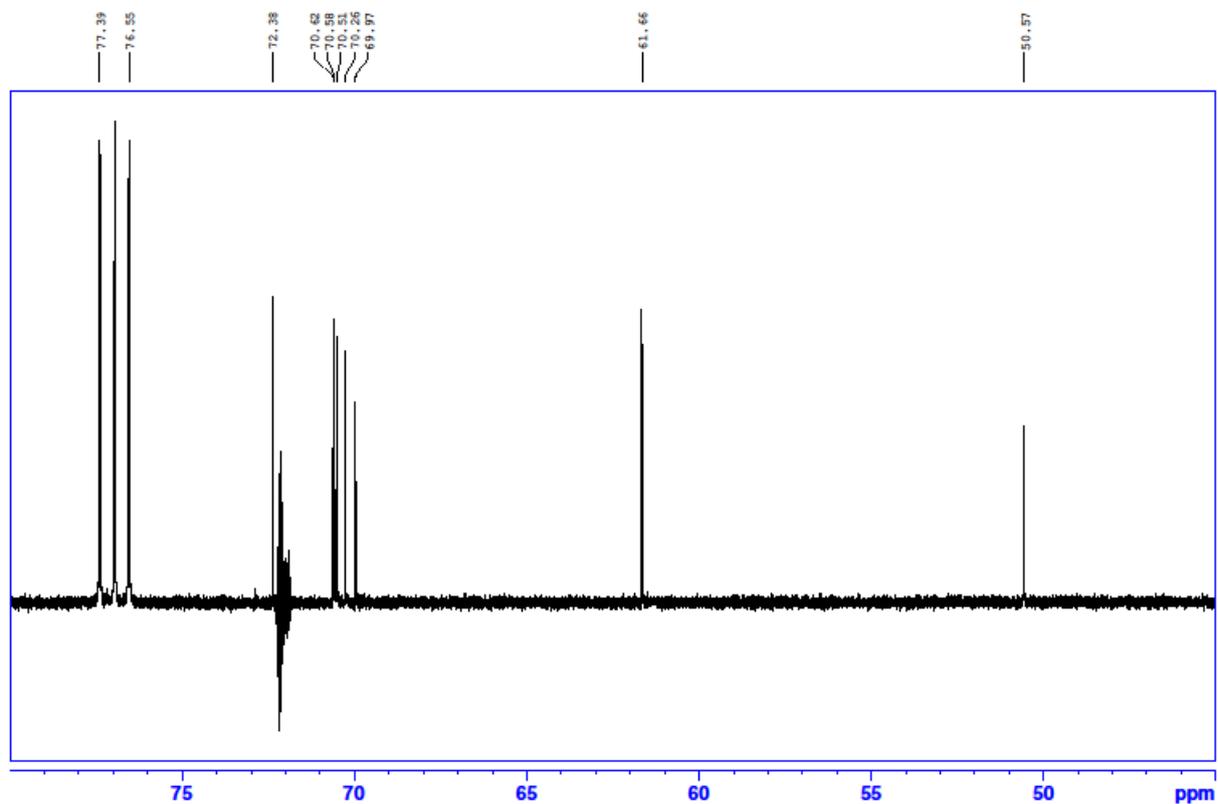


Figure S38: $^{13}\text{C-NMR}$ of azido compound 14.

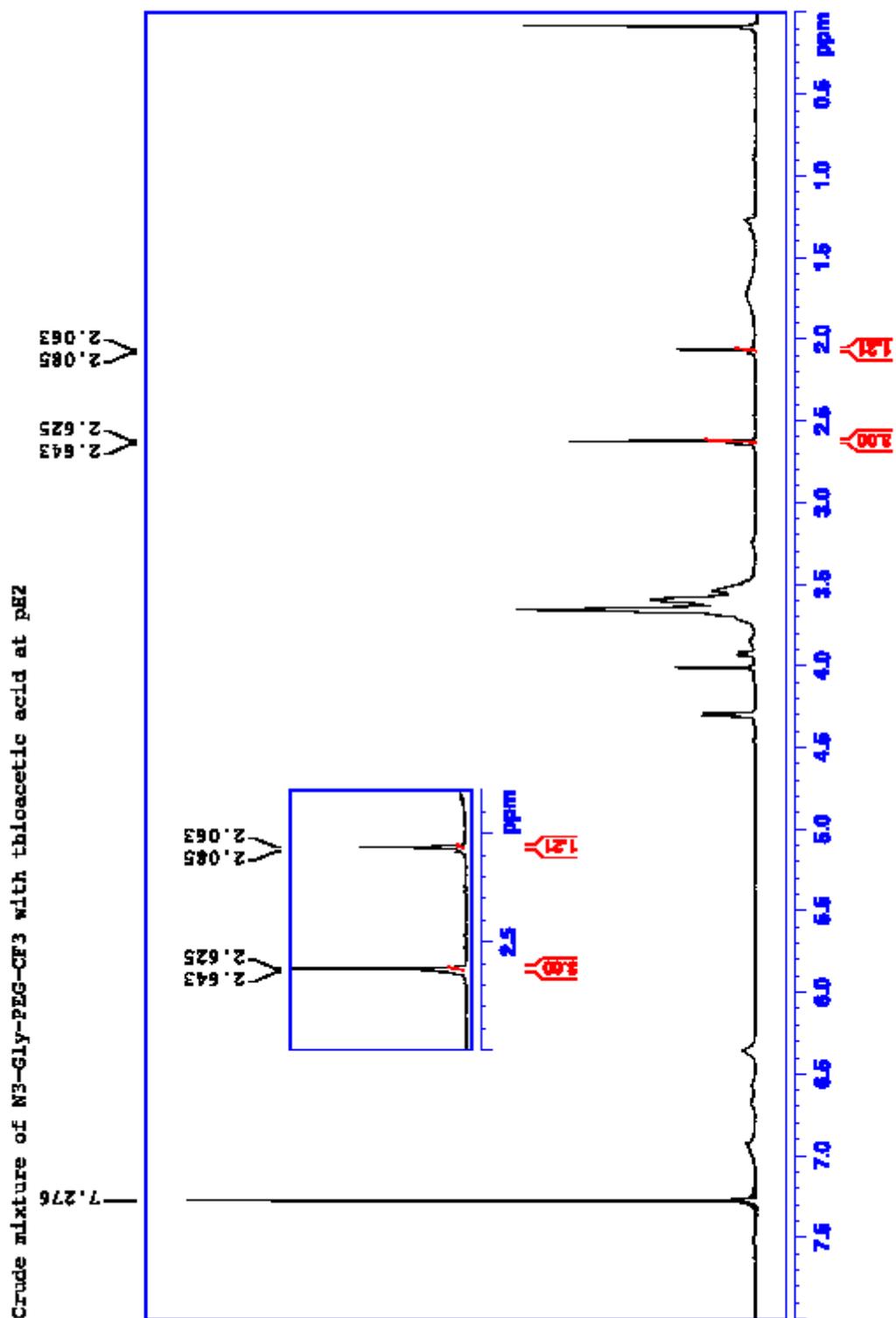


Figure S39: $^1\text{H-NMR}$ of crude reaction mixture of **13** and **2** at pH 2 (Table 3, entry 1).

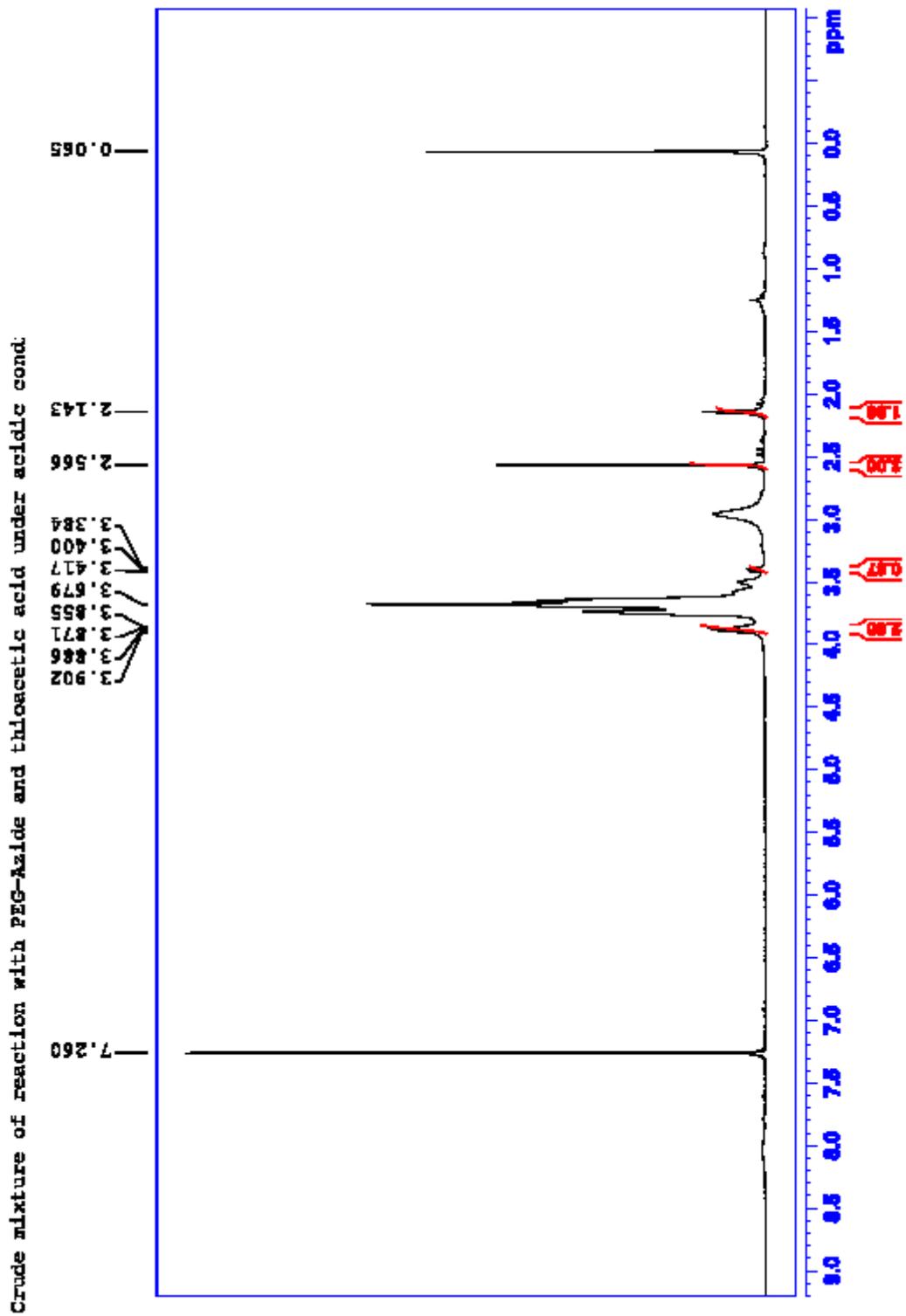


Figure S40: $^1\text{H-NMR}$ of crude reaction mixture of **14** and **2** at pH 2 (Table 3, entry 2).

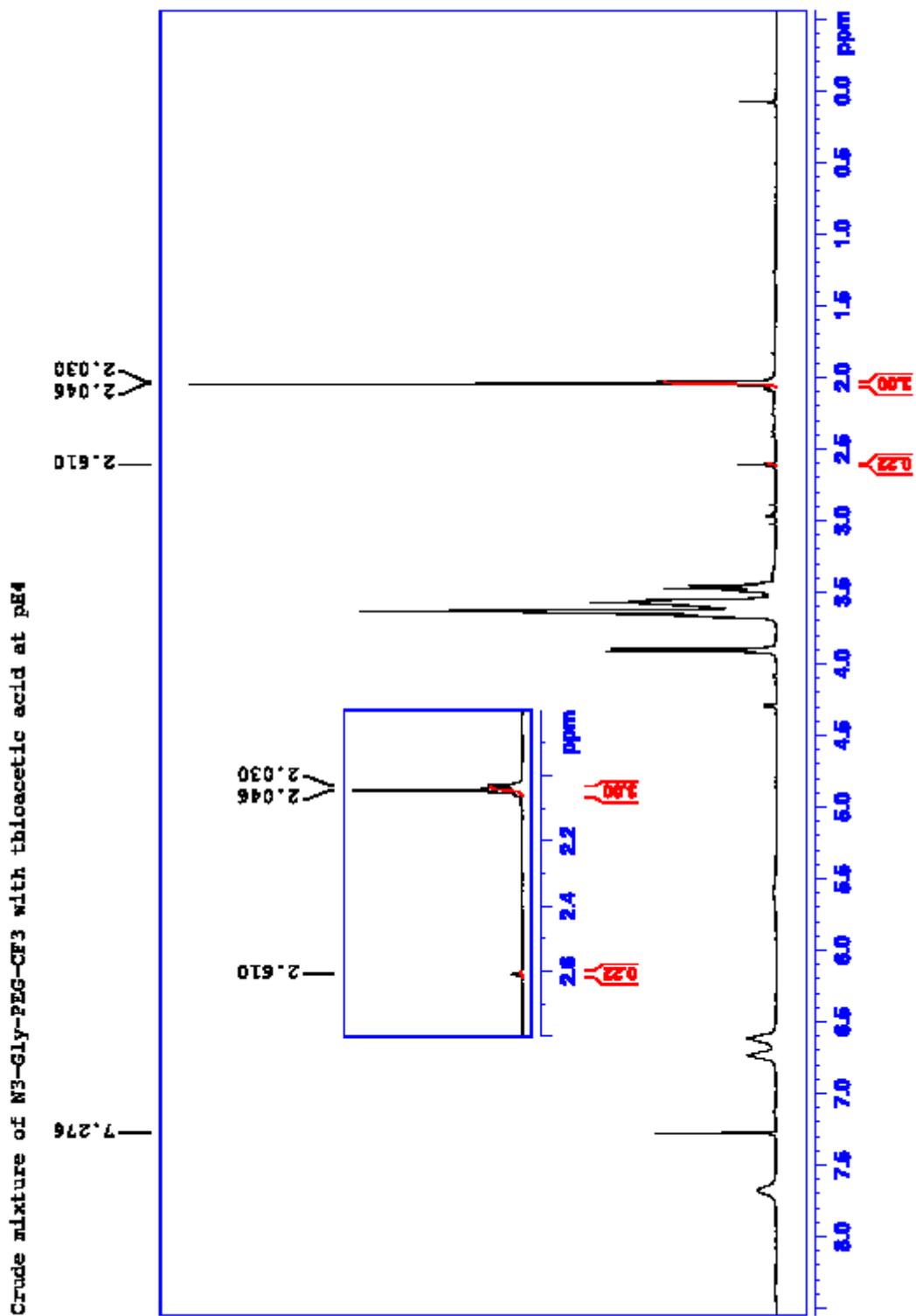


Figure S41: $^1\text{H-NMR}$ of crude reaction mixture of **13** and **2** at pH 4 (Table 3, entry 3).

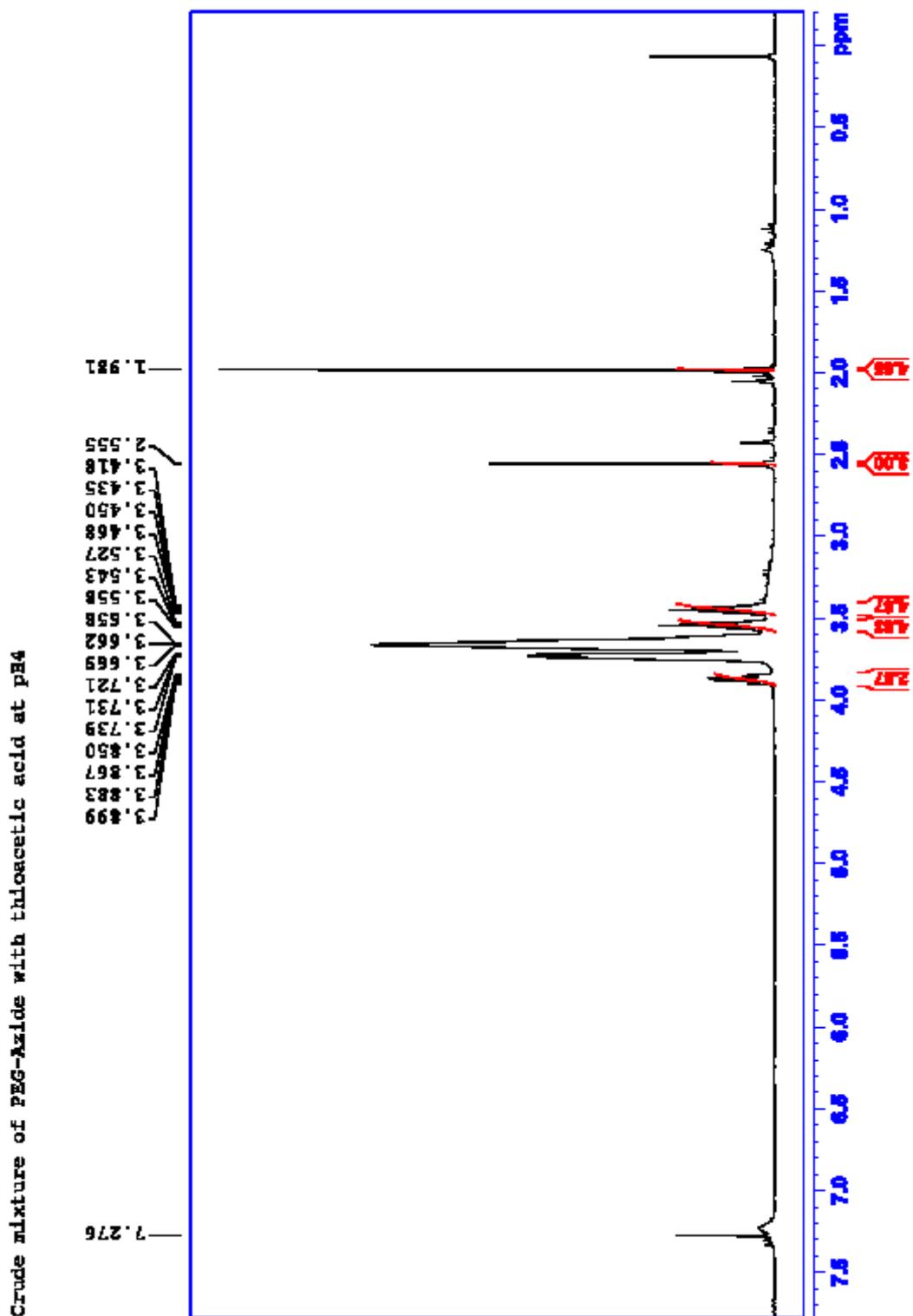


Figure S42: $^1\text{H-NMR}$ of crude reaction mixture of **14** and **2** at pH 4 (Table 3, entry 4).

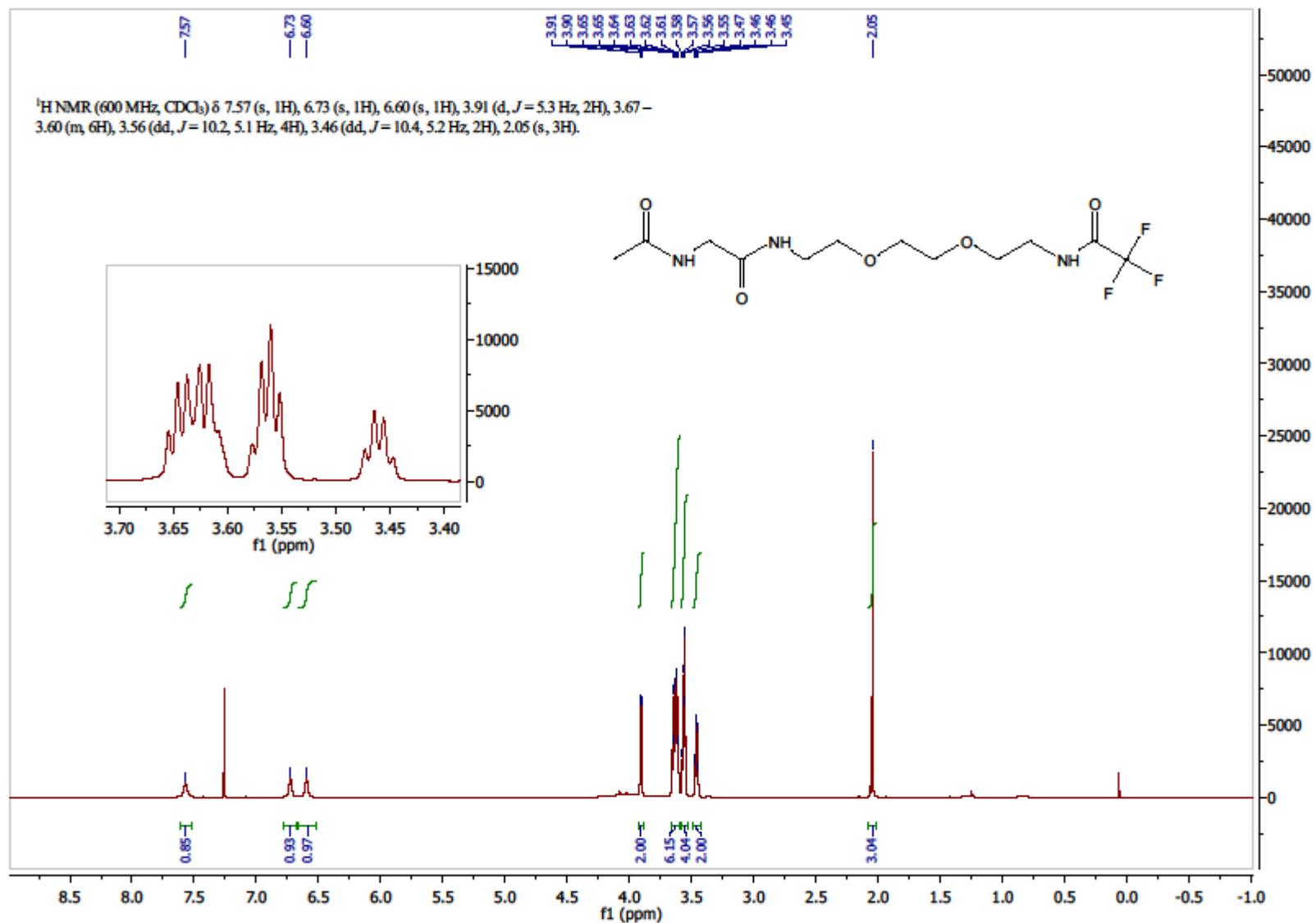


Figure S43: ¹H-NMR of acetamide 15.

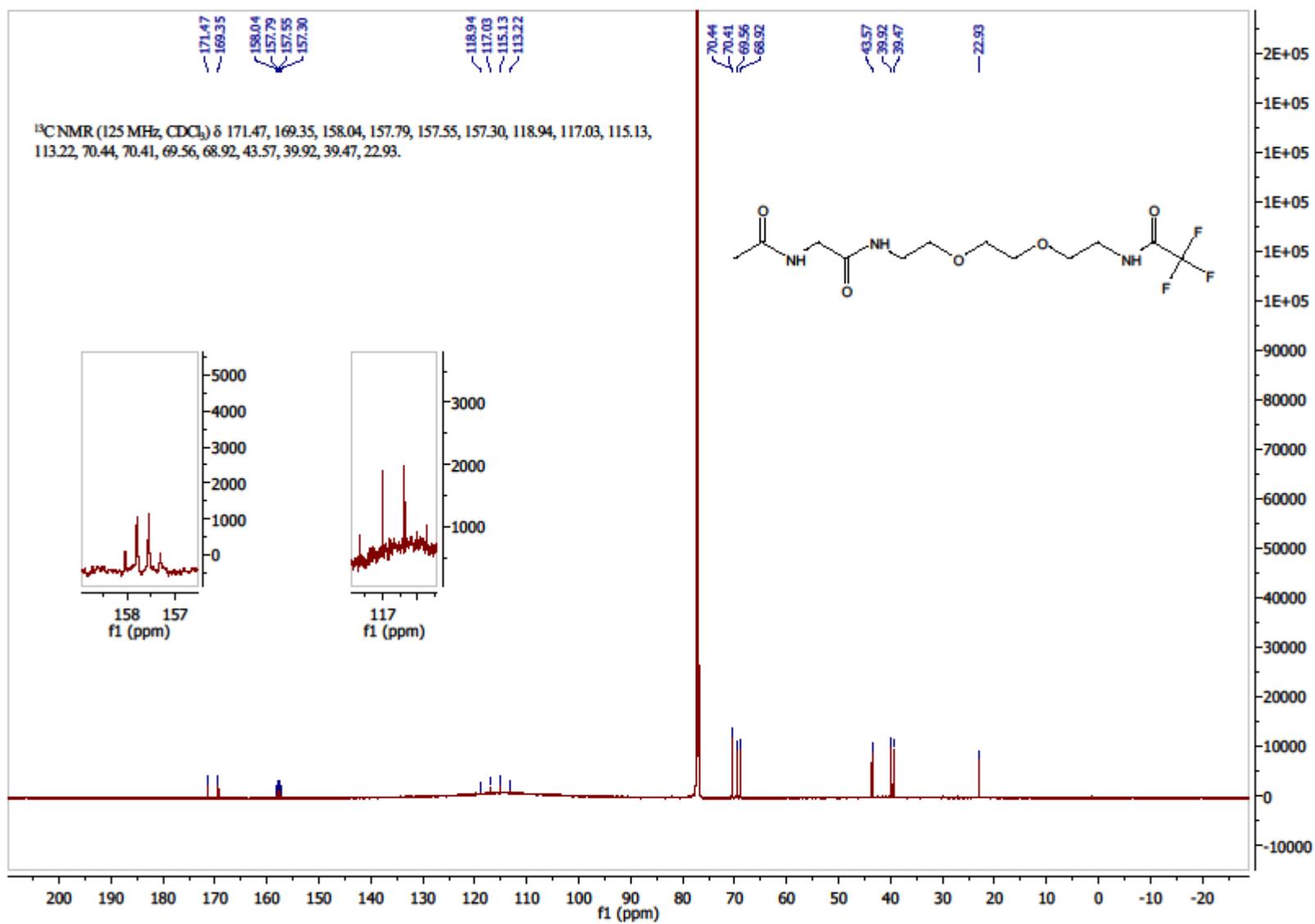


Figure S44: ¹³C-NMR of acetamide 15.

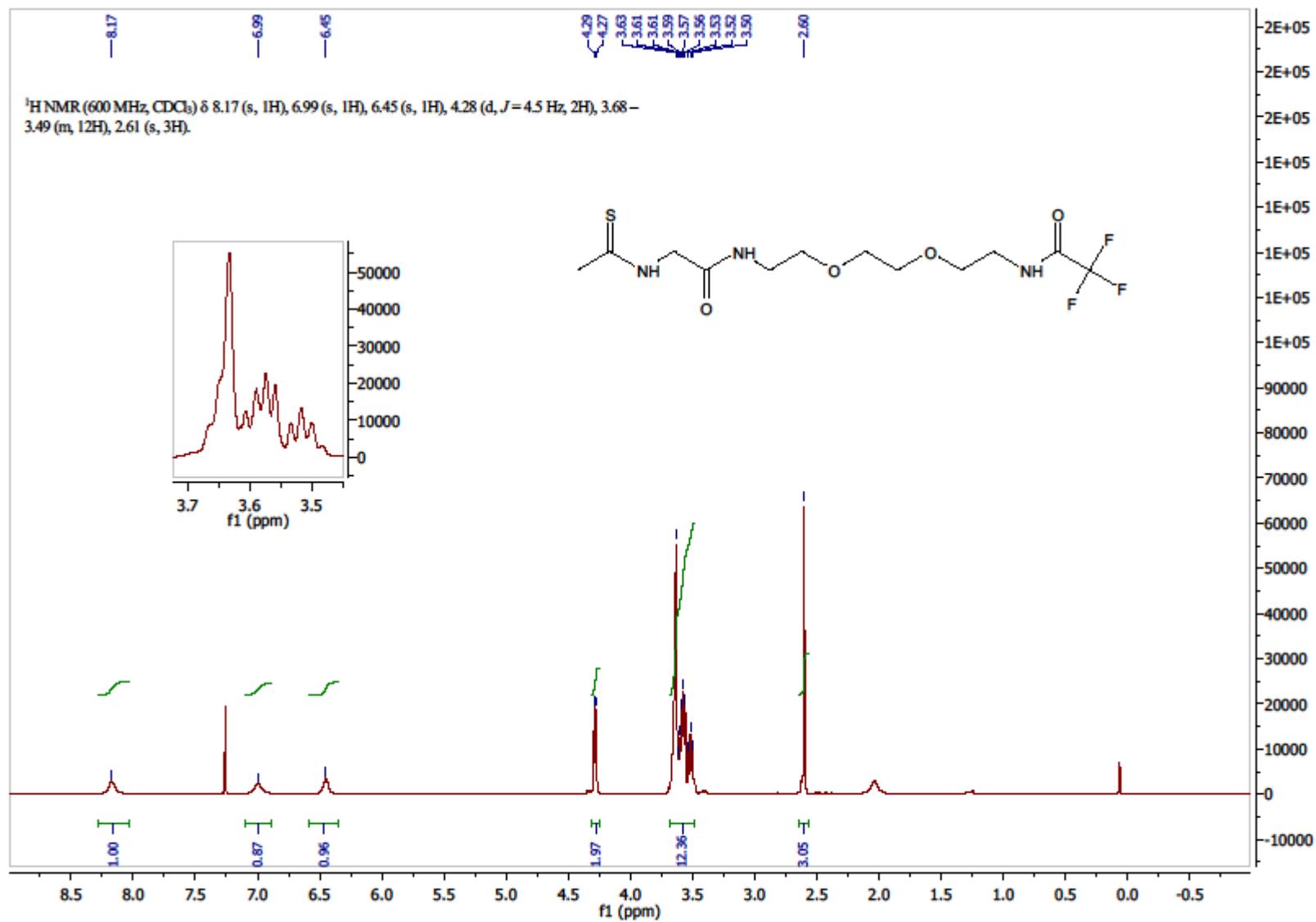


Figure S45: ¹H-NMR of thioacetamide 16.

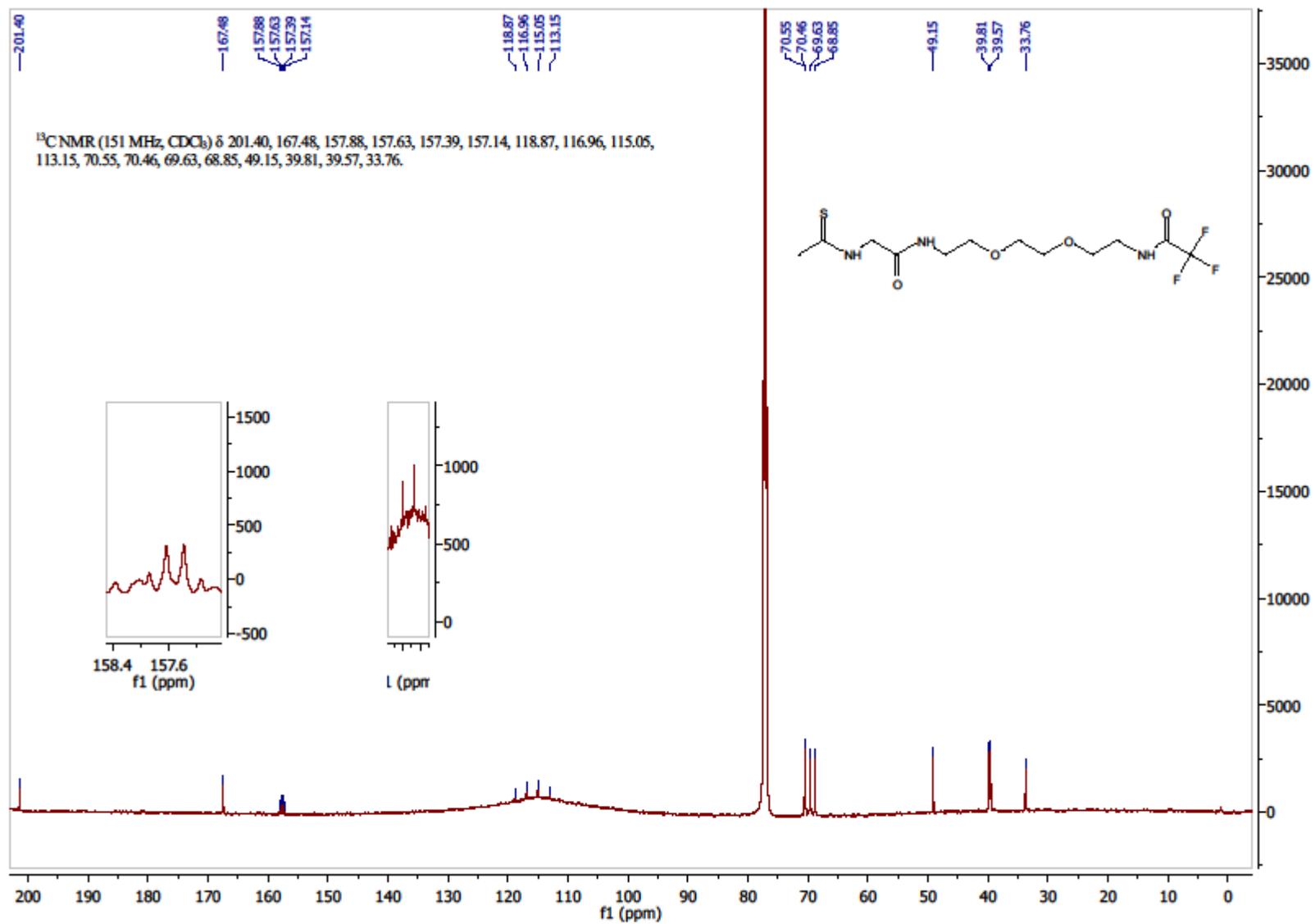


Figure S46: ¹³C-NMR of thioacetamide 16.

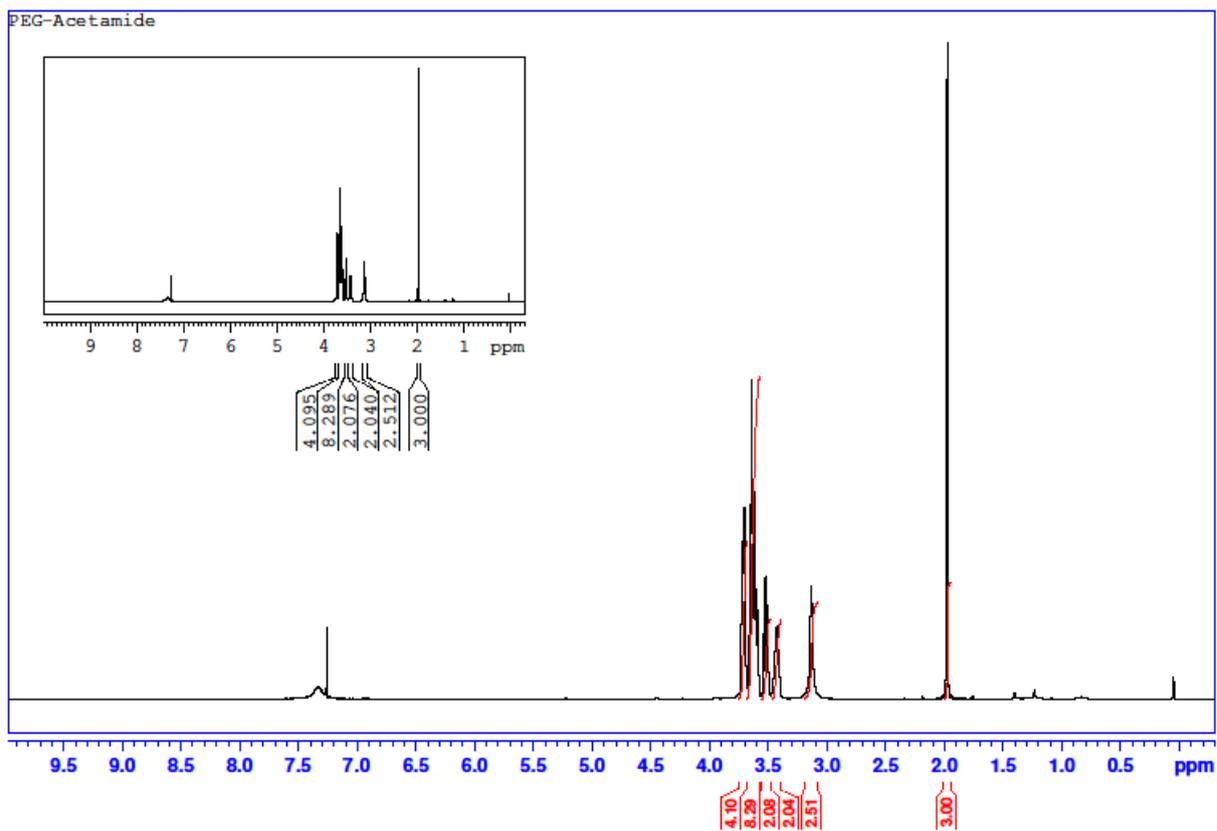


Figure S47: ^1H -NMR of acetamide 17.

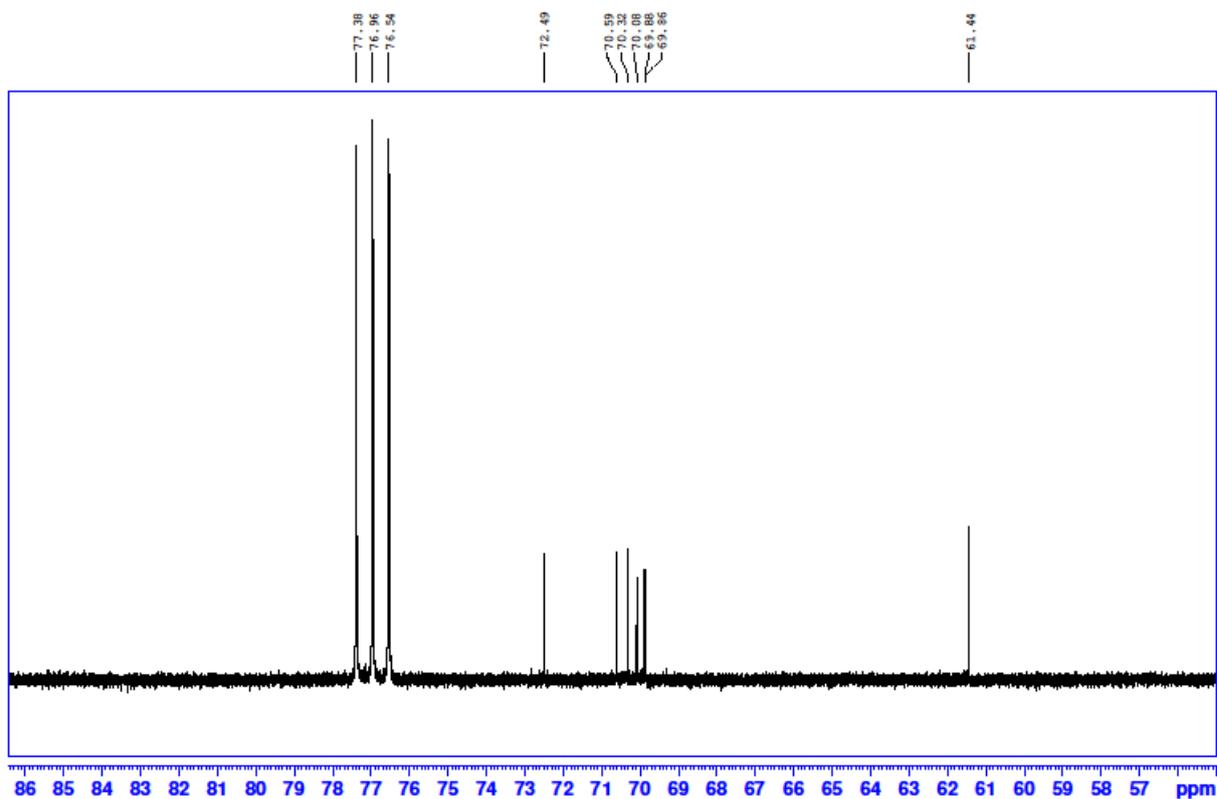


Figure S48: ^{13}C -NMR of acetamide 17.

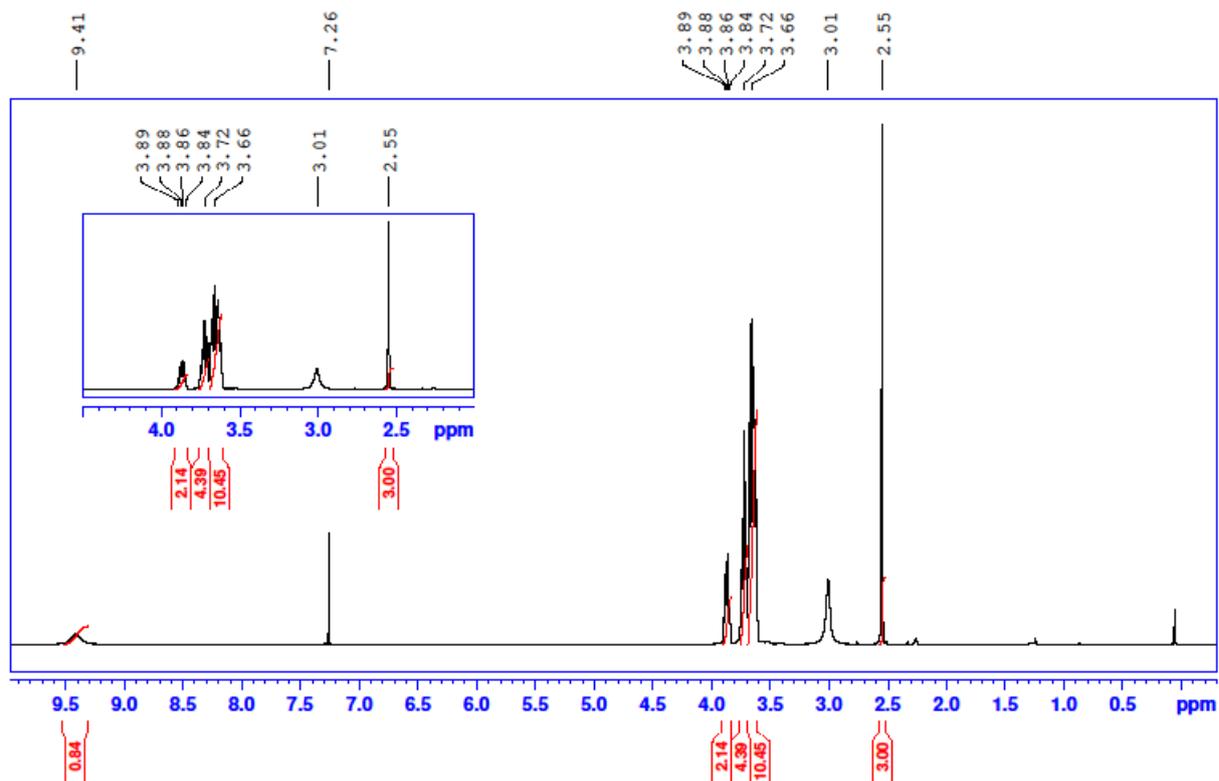


Figure S49: $^1\text{H-NMR}$ of thioacetamide **18**.

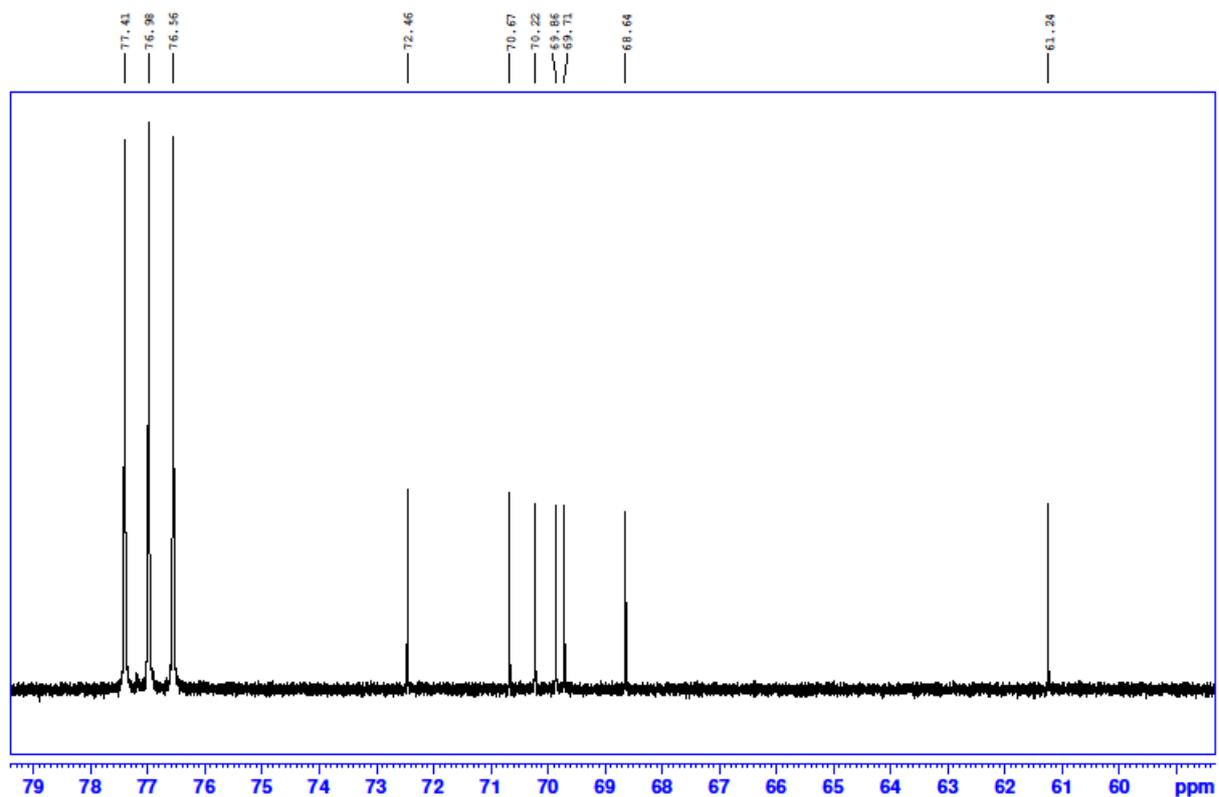


Figure S50: $^{13}\text{C-NMR}$ of thioacetamide **18**.

References

- 1 J. T. Lundquist, J.C. Pelletier, *Org. Lett.* 2001, **3**, 781.
- 2 N. Khoukhi, M. Vaultier, R. Carrié, *Tetrahedron* 1987, **43**, 1811.
- 3 Le Chevalier Isaad, F. Barbetti, P. Rovero, A.M. D'Ursi, M. Chelli, M. Chorev, A.M. Papini, *Eur. J. Org. Chem.* 2008, 5308.
- 4 G. Kragol, M. Lumbierres, J.M. Palomo, H. Waldmann, *Angew. Chem. Int. Ed.* 2004, **43**, 5839.
- 5 M. Gonçalves, K. Estieu-Gionnet, T. Berthelot, G. Lăin, M. Bayle, X. Canron, N. Betz, A. Bikfalvi, G. Déléris, *Pharm. Res.* 2005, **22**, 1411.