Electronic Supplementary Information

Two consecutive aza-amino acids in peptides promote stable β-turn formation in water

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General information

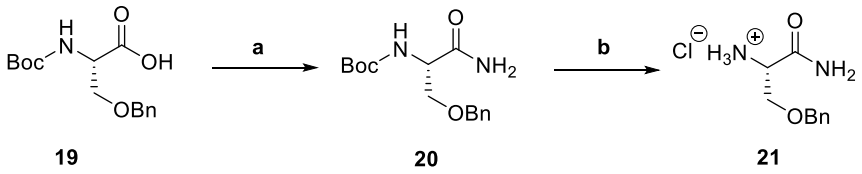
Usual solvents were purchased from commercial sources and DCM was dried and distilled over CaH_2. Thin-layer chromatography (TLC) analyses were performed on silica gel 60 F250 (0.26 mm thickness) plates. The plates were visualized with UV light (λ = 254 nm) or stained by a 4 % solution of phosphomolybdic acid or ninhydrin in EtOH. NMR spectra were recorded on an ultrafield Bruker AVANCE 300 (^{1}H, 300 MHz, ^{13}C, 75 MHz), a Bruker AVANCE 400 (^{1}H, 400 MHz, ^{13}C, 100 MHz), a Bruker AVANCE 500 (^{1}H, 500 MHz, ^{13}C, 125 MHz) or a Bruker AVANCE 600 (^{1}H, 600 MHz, ^{13}C, 150 MHz) spectrometer. Chemical shifts δ are in ppm with the solvent resonance as the internal standard (^{1}H NMR, $CDCl_3$: δ = 7.26 ppm, DMSO: δ = 2.50 ppm, CD_3OD and CD_3OH: δ = 3.31 ppm; ^{13}C NMR, $CDCl_3$: δ = 77.16 ppm, DMSO: δ = 39.52 ppm, CD_3OD and CD_3OH: δ = 49.00 ppm), and the following abbreviations are used: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quintuplet (qt), multiplet (m), broad multiplet (brm), and broad singlet (brs), broad doublet (brd). Mass

spectra were obtained using a Bruker Esquire electrospray ionization apparatus. HRMS were obtained using a TOF LCT Premier apparatus (Waters) with an electrospray ionization source. The purity of compounds was determined by HPLC-MS on Agilent 1260 Infinity. Column: ATLANTIS T3 column (C18, 2.1 x 150mm-3μm), mobile phase: ACN/H_2O + 0.1% TFA (gradient 1-30% in 15 or 20 min). Preparative HPLC were performed on Agilent 1260 Infinity II. Column: Pursuit (C18 10 x 250μm-5μm), mobile phase: ACN/H_2O + 0.1% formic acid.

Compounds **7**, **8**, **9**, **10** and **22** were prepared as reported in our previous literature[1] and **13a** was synthesized according to published method.[2]

Synthesis of compounds 21 and 13b

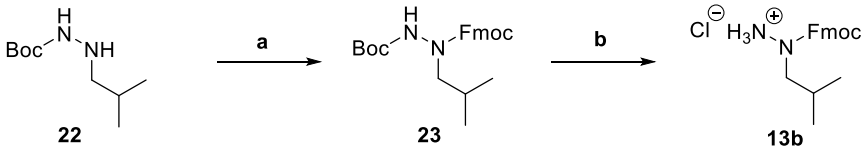
For the natural amino acid residue Ser(Bzl)-NH_2, the synthesis is described in scheme S1 and started from the coupling of Boc-Ser(Bzl)-OH **19** with ammonia using EDC and HOBt in a mixture of DCM and DMF, providing compound **20** in 78% yield. Then the cleavage of the Boc group of compound **20** afforded the hydrochloride salt of Ser(Bzl)-NH_2 (compound **21**, Yield: 85%) which can be directly used in the coupling reactions.



19 **20** **21**

Scheme S1. Synthesis of Ser(Bzl)-NH_2·HCl **21**. *Reagents and conditions:* (a) EDC, HOBt, NH_4OH, DCM/DMF, r.t, 3 h, 78%; (b) 4 M HCl in dioxane, r.t, 5 h, 85%.

For the synthesis of 1-Fmoc-1-isobutylhydrazine·HCl **13b** (described in scheme S2), compound **22** was protected by a Fmoc group using 9-fluorenylmethyl chloroformate to give compound **23** in 78% yield. The Boc group of **23** was cleaved using a solution of HCl in dioxane to give compound **13b** (Yield: 94%).



22 **23** **13b**

Scheme S2. Synthesis of 1-Fmoc-1-isobutylhydrazine·HCl **13b.** *Reagents and conditions:* (a) 9-fluorenylmethyl chloroformate, collidine, DCM, r.t, 4 h, 78%; (b) 4 M HCl in dioxane, MeOH, r.t, overnight, 94%.

Experimental section and characterization

(S)-2-(2-acetyl-1-(4-aminobutyl)hydrazine-1-carbonyl)-N-(1-amino-3-hydroxy-1-oxopropan-2-yl)hydrazine-1-carboxamide·HCOOH (4). To a solution of **12** (39 mg, 0.07 mmol) in 22 mL mixture of AcOH/H_2O (10/1) was added 10% Pd/C (8 mg). The mixture was stirred at room temperature under a hydrogen atmosphere overnight. Then the suspension was filtrated through a pad of celite and the filtrate was lyophilized to give a white solid. The crude was purified by preparative HPLC (Linear gradients of 0.5-10 % ACN in H_2O containing 0.1% formic acid) to give **4** as a white solid (17 mg, 0.04 mmol, yield: 64%).

HRMS (ESI): Calcd for $[C_{11}H_{23}N_7O_5 + H]^+$: 334.1833, found: 334.1834.

HPLC purity: ATLANTIS T3 column (C18, 2.1 x 150mm-3µm); ACN /H_2O + 0.1 % TFA, gradient 1–30 % in 15 min; R_t = 1.90 min, 100 %.

(S)-2-(2-acetyl-1-(4-aminobutyl)hydrazine-1-carbonyl)-N-(1-amino-3-hydroxy-1-oxopropan-2-yl)-1-methylhydrazine-1-carboxamide·HCOOH (5). Compound **5** was synthesized from **18a** (50 mg, 0.09 mmol) as a white solid (20 mg, 0.05 mmol, yield: 58%) by following the synthesis procedure of **4** (Preparative HPLC of **5** was performed using linear gradients of 0.5-15 % ACN in H_2O containing 0.1% formic acid).

HRMS (ESI): Calcd for $[C_{12}H_{25}N_7O_5 + Na]^+$: 370.1809, found: 370.1812.

HPLC purity: ATLANTIS T3 column (C18, 2.1 x 150mm-3µm); ACN /H_2O + 0.1 % TFA, gradient 1–30 % in 15 min; R_t = 1.96 min, 100 %.

(S)-2-(2-acetyl-1-(4-aminobutyl)hydrazine-1-carbonyl)-N-(1-amino-3-hydroxy-1-oxopropan-2-yl)-1-isobutylhydrazine-1-carboxamide·HCOOH (6). Compound **6** was synthesized from **18b** (60 mg, 0.10 mmol) as a white solid (25 mg, 0.06 mmol, yield: 59%) by following the synthesis procedure of **4** (Preparative HPLC of **6** was performed using linear gradients of 0.5-20 % ACN in H_2O containing 0.1% formic acid).

HRMS (ESI): Calcd for $[C_{15}H_{31}N_7O_5 + H]^+$: 390.2459, found: 390.2460.

HPLC purity: ATLANTIS T3 column (C18, 2.1 x 150mm-3µm); ACN /H_2O + 0.1 % TFA, gradient 1–30 % in 15 min; R_t = 9.74 and 10.24 min, 100 %.

tert-butyl (S)-3-(4-(((benzyloxy)carbonyl)amino)butyl)-9-carbamoyl-4,7-dioxo-12-phenyl-11-oxa-2,3,5,6,8-pentaazadodecanoate (11). To a solution of **10** (433 mg, 0.84 mmol) in acetonitrile (30 mL) were successively added Ser(Bzl)-NH_2·HCl **21** (213 mg, 0.92 mmol) and Et_3N (584 µL, 4.20 mmol) at room temperature. The mixture was stirred at room temperature for 24 h. After removing the volatiles under reduced pressure, the crude was purified by chromatography on silica gel (3% MeOH in EtOAc) to give **11** as a colorless oil (365 mg, 0.59 mmol, yield: 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (brs, 2H), 7.49 – 7.19 (m, 11H), 7.08 (brs, 1H), 6.54 (brs, 1H), 6.29 (brs, 1H), 5.46 (brs, 1H), 5.04 (s, 2H), 4.53 – 4.38 (m, 3H), 3.81 (m, 1H), 3.71 – 3.24 (m, 3H), 3.17 – 2.99 (m, 2H), 1.52 – 1.34 (m,

13H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.2, 159.7, 158.8, 156.8, 154.9, 137.5, 136.7, 128.5(2C), 128.4(2C), 128.1, 128.0, 127.7 (4C), 82.3, 73.3, 69.6, 66.5, 53.9, 48.4, 40.6, 28.2(3C), 26.8, 23.9; HRMS (ESI): Calcd for $[C_{29}H_{41}N_7O_8 + Na]^+$: 638.2914, found: 638.2914.

benzyl (S)-(10-acetamido-4-carbamoyl-6,9-dioxo-1-phenyl-2-oxa-5,7,8,10-tetraazatetradecan-14-yl) carbamate (12). To a solution of **11** (100 mg, 0.16 mmol) in dioxane (2 mL) was added HCl 4M in dioxane (2.44 mL, 9.75 mmol) under ice-cooling. The mixture was stirred at room temperature for 3 h. The volatiles were evaporated under reduced pressure to give a white solid. The solid was dissolved in DCM (6 mL) followed by the addition of acetic anhydride (20 µL, 0,21 mmol) and pyridine (43 µL, 0.41 mmol) at room temperature. The mixture was stirred overnight. The volatiles were evaporated under reduced pressure to give a crude, which was purified by chromatography on silica gel (5-10% MeOH in DCM) to give **12** as a white solid (68 mg, 0.12 mmol, yield: 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (brs, 1H), 8.66 (brs, 1H), 7.81 (s, 1H), 7.42 – 7.07 (m, 13H), 6.33 (d, J = 8.1 Hz, 1H), 5.00 (s, 2H), 4.49 (s, 2H), 4.26 (dt, J = 8.3, 5.2 Hz, 1H), 3.67 (dd, J = 9.7, 5.7 Hz, 1H), 3.57 (dd, J = 9.7, 4.6 Hz, 1H), 3.36 – 3.20 (m, 2H, overlap with H_2O), 2.98 (q, J = 6.3 Hz, 2H), 1.86 (s, 3H), 1.48 – 1.32 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 169.2, 158.2, 157.6, 156.0, 138.2, 137.2, 128.3(2C), 128.1(2C), 127.7(2C), 127.4(2C), 127.3(2C), 72.1, 70.3, 65.1, 53.2, 47.4, 45.5, 26.5, 24.1, 21.0; HRMS (ESI): Calcd for $[C_{26}H_{35}N_7O_7 + H]^+$: 558.2676, found: 558.2674.

1-Fmoc-1-isobutylhydrazine·HCl (13b). To a solution of **23** (550 mg, 1.34 mmol) in MeOH (10 mL) was added HCl 4M in dioxane (6.7 mL, 26.81 mmol) under ice-cooling. The mixture was stirred at room temperature overnight. Then the volatiles were evaporated under reduced pressure to give a white solid. The solid was washed with cyclohexane (10 ml), filtered and dried under reduced pressure to give compound **13b** as a white solid (437 mg, 1.26 mmol, yield: 94%). ^{1}H NMR (300 MHz, Methanol-d_4): δ 7.81 (d, J = 7.4 Hz, 2H), 7.62 (d, J = 7.4 Hz, 2H), 7.46 – 7.27 (m, 4H), 4.82 (d, J = 4.4 Hz, 2H), 4.30 (t, J = 4.4 Hz, 1H), 2.92 (d, J = 7.5 Hz, 2H), 1.55 (m, 1H), 0.60 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, Methanol-d_4): δ 156.1, 144.8(2C), 142.9(2C), 128.9(2C), 128.3(2C), 125.6(2C), 121.0(2C), 69.4, 57.2, 48.4, 27.7, 19.5(2C); HRMS (ESI): Calcd for $[C_{19}H_{22}N_2O_2 + H]^+$: 311.1760, found: 311.1759.

1-((9H-fluoren-9-yl)methyl) 2-(4-nitrophenyl) 1-methylhydrazine-1,2-dicarboxylate (14a). To a solution of 1-Fmoc-1-methylhydrazine **13a** (300 mg, 1.12 mmol) in dry DCM (20 mL) was added pyridine (0.27 ml, 3.36 mmol) at room temperature under an argon atmosphere. Then a solution of 4-nitrophenylchloroformate (338 mg, 1.68 mmol) in dry DCM (10 mL) was added dropwise under ice-cooling. The mixture was stirred at room temperature overnight. The reaction mixture was successively washed with 10 % aqueous citric acid, 10 % aqueous K_2CO_3, and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product (solid) was added to

DCM (5 mL) and stirred under ice-cooling for 5 min. The precipitate was filtered and dried under reduced pressure to give **14a** as a white solid (370 mg, 0.85 mmol, yield: 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.51 (d, *J* = 7.5 Hz, 2H), 7.38 – 6.96 (m, 7H), 4.45 (d, *J* = 6.7 Hz, 2H), 4.19 (t, *J* = 6.7 Hz, 1H), 3.21 (s, 3H); HRMS (ESI): Calcd for $[C_{23}H_{19}N_3O_6 + Na]^+$: 456.1172, found: 456.1182.

1-((9H-fluoren-9-yl)methyl) 2-(4-nitrophenyl) 1-isobutylhydrazine-1,2-dicarboxylate (14b). To a suspension of 1-Fmoc-1-isobutylhydrazine·HCl **13b** (430 mg, 1.24 mmol) in dry DCM (20 mL) was added pyridine (1 mL, 12.42 mmol) at room temperature under an argon atmosphere. Then a solution of 4-nitrophenylchloroformate (626 mg, 3.11 mmol) in dry DCM (20 mL) was added dropwise under ice-cooling. The mixture was stirred at room temperature overnight. The reaction mixture was successively washed with 10 % aqueous citric acid, 10 % aqueous K_2CO_3, and brine, dried over anhydrous Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (10-15% EtOAc in cyclohexane) to give **14b** as a white solid (350 mg, 0.74 mmol, yield: 59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.39 – 6.97 (m, 7H), 4.46 (d, *J* = 6.3 Hz, 2H), 4.14 (t, *J* = 6.3 Hz, 1H), 3.44 – 2.99 (m, 2H), 1.77 (m, 1H), 1.01 – 0.50 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.4, 155.2, 153.0, 145.2, 143.6(2C), 141.5(2C), 128.0(2C), 127.2(2C), 125.2(2C), 124.9(2C), 121.9 (2C), 120.1(2C), 68.5, 57.9, 47.2, 26.8, 19.9(2C); HRMS (ESI): Calcd for $[C_{26}H_{25}N_3O_6 + Na]^+$: 498.1636, found: 498.1637.

(9H-fluoren-9-yl)methyl 5-((tert-butoxycarbonyl)amino)-2-methyl-4,11-dioxo-13-phenyl-12-oxa-2,3 ,5,10-tetraazatridecanoate (15a). To a solution of **14a** (370 mg, 0.85 mmol) in acetonitrile (30 mL) were successively added **7** (259 mg, 0.77 mmol) and DIPEA (0.18 ml, 1.03 mmol) at room temperature. The mixture was stirred at room temperature for 5 h. After removing the volatiles under reduced pressure, the residue was dissolved in EtOAc (40 mL). The solution was successively washed with 10 % aqueous K_2CO_3, 10 % aqueous citric acid, and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (1-2% MeOH in DCM) to give **15a** as a white solid (382 mg, 0.61 mmol, yield: 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (d, *J* = 7.5 Hz, 2H), 7.51 (d, *J* = 7.5 Hz, 2H), 7.37 – 7.12 (m, 9H), 7.01 (brs, 2H), 4.99 (s, 2H), 4.80 (s, 1H), 4.49 – 4.24 (brm, 2H), 4.16 (t, *J* = 6.6 Hz, 1H), 3.63 – 3.27 (m, 2H), 3.26 – 2.97 (m, , 5H), 1.46 (s, 4H), 1.39 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.5, 157.1(from rotamers), 156.8(2C), 154.6, 143.8(2C), 141.3(2C), 136.8, 128.5(2C), 128.0 (3C), 127.8(2C), 127.2(2C), 125.2(2C), 120.0(2C), 82.1, 68.5, 66.6, 48.3, 47.1, 40.7, 38.7, 38.1(from rotamers), 28.2(3C), 27.1, 24.1; HRMS (ESI): Calcd for $[C_{34}H_{41}N_5O_7 + H]^+$: 632.3084, found: 632.3091.

(9H-fluoren-9-yl)methyl 5-((tert-butoxycarbonyl)amino)-2-isobutyl-4,11-dioxo-13-phenyl-12-oxa-2,

3,5,10-tetraazatridecanoate (15b). Compound **15b** was synthesized from **14b** (350 mg, 0.74 mmol) and **7** (236 mg, 0.70 mmol) as a white solid (374 mg, 0.56 mmol, yield: 79%) by following the synthesis procedure of **15a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.44 – 7.27 (m, 10H), 7.11 (brs, 1H), 5.08 (s, 3H), 4.63 – 4.41 (m, 2H), 4.23 (t, J = 6.1 Hz, 1H), 3.76 – 3.36 (m, 2H), 3.35 – 3.05 (m, 4H), 1.78 (m, 1H), 1.63 – 1.39 (m, 13H), 0.94 – 0.71 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.3, 157.2(from rotamers), 156.7, 156.5, 154.3, 143.8(2C), 141.3(2C), 136.7, 128.5(2C), 128.0(3C), 127.7(2C), 127.1(2C), 124.9(2C), 119.9(2C), 81.9, 68.1, 67.3(from rotamer), 66.6, 58.0, 57.4(from rotamer), 48.2, 47.5(from rotamer), 47.1, 40.6, 28.1(3C), 27.1, 26.7, 24.1, 20.0(2C); HRMS (ESI): Calcd for $[C_{37}H_{47}N_5O_7 + Na]^+$: 696.3373, found: 696.3367.

tert-butyl 2-(4-(((benzyloxy)carbonyl)amino)butyl)-2-(2-methylhydrazine-1-carbonyl)hydrazine-1-carboxylate (16a). **15a** (390 mg, 0.62 mmol) was treated with 20%(v/v) piperidine/DMF (10 mL). The mixture was stirred at room temperature for 30 min. After removing the volatiles, the residue was dissolved in EtOAc (40 mL). The solution was washed with brine, dried over anhydrous Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (3-5% MeOH in DCM) to give compound **16a** as a colorless gel (230 mg, 0.56 mmol, yield: 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 – 7.27 (m, 5H), 6.82 (brs, 2H), 5.17 – 4.96 (m, 3H), 3.75 – 3.38 (m, 2H), 3.30 – 2.84 (m, 3H), 2.59 (s, 3H), 1.55 (s, 4H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.8, 156.9, 154.6, 136.8, 128.6(2C), 128.2(3C), 82.4, 66.8, 48.2, 40.8, 39.8, 28.3(3C), 27.3, 24.3; HRMS (ESI): Calcd for $[C_{19}H_{31}N_5O_5 + H]^+$: 410.2398, found: 410.2411.

tert-butyl 2-(4-(((benzyloxy)carbonyl)amino)butyl)-2-(2-isobutylhydrazine-1-carbonyl)hydrazine-1-carboxylate (16b). Compound **16b** was synthesized from **15b** (363 mg, 0.54 mmol) as a white solid (221 mg, 0.49 mmol, yield: 91%) by following the synthesis procedure of **16a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (brs, 1H), 7.29 – 7.15 (m, 5H), 6.96 (brs, 1H), 5.33 (brs, 1H), 4.99 (s, 2H), 3.43 (brs, 3H), 3.19 – 2.95 (m, 2H), 2.53 (d, *J* = 6.8 Hz, 2H), 1.64 (m, 1H), 1.44 (brs, 4H), 1.37 (s, 9H), 0.83 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0, 156.8, 154.8, 136.7, 128.5(2C), 128.0(3C), 81.9, 66.6, 60.3, 48.1, 40.7, 28.2(3C), 27.1, 26.8, 24.3, 20.6(2C); HRMS (ESI): Calcd for $[C_{22}H_{37}N_5O_5 + H]^+$: 452.2873, found: 452.2867.

tert-butyl (S)-3-(4-(((benzyloxy)carbonyl)amino)butyl)-9-carbamoyl-6-methyl-4,7-dioxo-12-phenyl-11-oxa-2,3,5,6,8-pentaazadodecanoate (17a). Triphosgene (41 mg, 0.14 mmol) was dissolved in dry DCM (2 mL) under an argon atmosphere. A solution of **16a** (143mg, 0.35 mmol) and DIPEA (60μL, 0.35 mmol) in dry DCM (4 mL) was added dropwise under ice-cooling. The mixture was stirred at room temperature for 20 min, then Ser(Bzl)-NH_2·HCl **21** (76 mg, 0.33 mmol) and DIPEA (122 uL, 0.70 mmol) were added successively. The mixture was stirred at room temperature overnight. DCM (30 mL) was added to the reaction and the solution was washed with brine, dried over anhydrous

Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (4-5% MeOH in DCM) to give compound **17a** as a white solid (100 mg, 0.16 mmol, yield: 46%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (brs, 1H), 7.57 (brs, 1H), 7.38 – 7.14 (m, 10H), 6.99 (brs, 1H), 6.33 (brs, 1H), 5.76 (brs, 1H), 5.17 (brs, 1H), 4.99 (s, 2H), 4.54 – 4.34 (m, 3H), 3.82 (m, 1H), 3.71 – 3.24 (m, 3H), 3.16 – 3.02 (m, 2H), 2.99 (s, 3H), 1.60 – 1.29 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.7, 158.8, 157.5, 157.0, 155.0, 137.7, 136.7, 128.6(2C), 128.5(2C), 128.1(3C), 127.8, 127.8(2C), 82.4, 73.3, 69.8, 66.7, 54.8, 48.5, 40.7, 36.4, 28.3(3C), 27.0, 24.0; HRMS (ESI): Calcd for $[C_{30}H_{43}N_7O_8 + Na]^+$: 652.3071, found: 652.3066.

tert-butyl (S)-3-(4-(((benzyloxy)carbonyl)amino)butyl)-9-carbamoyl-6-isobutyl-4,7-dioxo-12-phenyl-11-oxa-2,3,5,6,8-pentaazadodecanoate (17b). Compound **17b** was synthesized from **16b** (198 mg, 0.44 mmol) as a white solid (160 mg, 0.24 mmol, yield: 54%) by following the synthesis procedure of **17a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (brs, 2H), 7.34 – 7.11 (m, 10H), 6.96 (brs, 1H), 6.23 (brs, 1H), 5.61 (brs, 1H), 5.06 (brs, 1H), 5.00 (s, 2H), 4.51 – 4.36 (m, 3H), 3.97 – 3.80 (m, 1H), 3.74 – 3.35 (m, 3H), 3.29 – 2.80 (m, 4H), 1.75 (m, 1H), 1.51 – 1.34 (m, 13H), 0.91 – 0.74 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.6, 158.6, 157.3, 156.9, 154.9, 137.6, 136.6, 128.5(2C), 128.4(2C), 128.1(3C), 127.7, 127.7(2C), 82.4, 73.1, 69.7, 66.6, 56.1, 54.5, 48.4, 40.5, 28.1(3C), 27.0, 26.5, 23.8, 20.2, 20.1; HRMS (ESI): Calcd for $[C_{33}H_{49}N_7O_8 + Na]^+$: 694.3540, found: 694.3532.

benzyl (S)-(10-acetamido-4-carbamoyl-7-methyl-6,9-dioxo-1-phenyl-2-oxa-5,7,8,10-tetraazatetradecan-14-yl)carbamate (18a). To a solution of **17a** (100 mg, 0.16 mmol) in dioxane (1 mL) was added HCl 4M in dioxane (2 mL, 8.00 mmol) under ice-cooling. The mixture was stirred at room temperature for 3 h. Then the reaction was evaporated under reduced pressure to give a white solid. The solid was dissolved in DCM (8 mL) with pyridine (129 uL, 1.60 mmol). Acetic anhydride (45 uL, 0.48 mmol) was added and the mixture was stirred at room temperature overnight. DCM (10 mL) was added and the mixture was successively washed water and brine, dried over anhydrous Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (4-10% MeOH in DCM) to give compound **18a** as a white solid (69 mg, 0.12 mmol, yield: 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.58 (brs, 1H), 8.63 (brs, 1H), 7.56 – 6.88 (m, 11H), 6.62 (brs, 1H), 6.26 (brs, 1H), 5.49 (brs, 1H), 4.94 (s, 2H), 4.39 (s, 2H), 4.22 (d, J = 6.5 Hz, 1H), 3.84 – 2.52 (m, 9H), 1.84 (s, 3H), 1.50 – 1.22 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.7, 171.0, 159.1, 157.5, 157.0, 137.6, 136.6, 128.5(2C), 128.4(2C), 128.1, 127.9, 127.8(4C), 73.3, 69.6, 66.6, 55.4, 48.5, 40.6, 36.3, 26.9, 24.0, 20.9; HRMS (ESI): Calcd for $[C_{27}H_{37}N_7O_7 + H]^+$: 572.2827, found: 572.2829.

benzyl (S)-(10-acetamido-4-carbamoyl-7-isobutyl-6,9-dioxo-1-phenyl-2-oxa-5,7,8,10-tetraazatetradecan-14-yl)carbamate (18b). Compound **18b** was synthesized from **17b** (130 mg, 0.19 mmol) as a white solid (90 mg, 0.15 mmol, yield: 76%) by following the synthesis procedure of **18a**. ^{1}H NMR

(300 MHz, $CDCl_3$): δ 9.62 (brs, 1H), 8.44 (brs, 1H), 7.74 – 7.00 (m, 11H), 6.74 (brs, 1H), 5.91 (brs, 1H), 5.38 (brs, 1H), 4.97 (s, 2H), 4.43 (s, 2H), 4.25 (m, 1H), 3.89 – 2.65 (m, 8H), 1.87 (s, 3H), 1.75 (m, 1H), 1.51 – 1.12 (m, 4H), 0.88 – 0.54 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.7, 171.1, 159.1, 157.5, 157.0, 137.6, 136.6, 128.5(2C), 128.4(2C), 128.1, 127.9, 127.8(4C), 73.3, 69.6, 66.6, 56.4(2C), 48.8, 40.6, 27.1, 26.6, 23.9, 20.9, 20.2, 20.1; HRMS (ESI): Calcd for $[C_{30}H_{43}N_7O_7 + H]^+$: 614.3297, found: 614.3305.

tert-butyl (S)-(1-amino-3-(benzyloxy)-1-oxopropan-2-yl)carbamate (20). Boc-ser(Bzl)-OH **19** (2 g, 6.77 mmol) was dissolved in 12 mL DCM/DMF. EDC (2.81 g, 10.16 mmol) and HOBt were added at room temperature. The mixture was stirred for 30 min and then 7M NH_3 in MeOH (2.9 mL, 20.32 mmol) were added. The reaction was stirred at room temperature for 4 h and then evaporated under reduced pressure. The residue was dissolved in EtOAc (30 mL) and washed with 10% $NaHCO_3$ aqueous solution and brine successively, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica column chromatography using (10% MeOH in DCM) as eluent to give **20** as a white solid (1.56 g, 5.30 mmol, yield: 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.06 (m, 5H), 6.43 (brs, 1H), 6.04 (brs, 1H), 5.43 (d, *J* = 7.2 Hz, 1H), 4.55 – 4.39 (m, 2H), 4.23 (m, 1H), 3.79 (dd, *J* = 9.4, 4.2 Hz, 1H), 3.51 (dd, *J* = 9.4, 6.4 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 155.6, 137.5, 128.6(2C), 128.0, 127.9(2C), 80.3, 73.5, 70.0, 53.8, 28.4(3C); HRMS (ESI): Calcd for $[C_{15}H_{22}N_2O_4 + Na]^+$: 317.1477, found: 317.1473.

Ser(Bzl)-NH₂·HCl (21). To a solution of **20** (1.60 g, 5.44 mmol) in dioxane (20 mL) was add HCl 4M in dioxane (27 mL, 108.78 mmol) under ice-cooling. The mixture was stirred at room temperature for 5 h. Then the volatiles were evaporated under reduced pressure to give a white solid. The solid was washed with Et_2O (20 ml), filtered and dried under reduced pressure to give compound **21** as a white solid (1.06 g, 4.6 mmol, yield: 85%). ^{1}H NMR (300 MHz, Methanol-d_4): δ 7.42 – 7.24 (m, 5H), 4.70 – 4.54 (m, 2H), 4.13 (dd, *J* = 6.2, 3.9 Hz, 1H), 3.95 – 3.76 (m, 2H); ^{13}C NMR (75 MHz, Methanol-d_4): δ 169.9, 138.5, 129.5(2C), 129.1(3C), 74.6, 69.1, 54.5; HRMS (ESI): Calcd for $[C_{10}H_{14}N_2O_2 + H]^+$: 195.1128, found: 195.1126.

1-((9H-fluoren-9-yl)methyl) 2-(tert-butyl) 1-isobutylhydrazine-1,2-dicarboxylate (23). 1-Boc-2-isobutylhydrazine **22** (400 mg, 2.12 mmol) and collidine (415 µL, 3.19 mmol) were dissolved in dry DCM (20 mL) under an argon atmosphere. Then a solution of 9-fluorenylmethyl chloroformate in dry DCM (9 mL) was added dropwise under ice-cooling. The mixture was stirred at room temperature for 4 h. DCM (20 mL) was added and the mixture was successively washed with 10 % aqueous citric acid and brine, dried over anhydrous Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (10% EtOAc in cyclohexane) to give compound **23** as a white solid (680 mg, 1.66 mmol, yield: 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ

7.64 (dd, J = 7.4 Hz, 2H), 7.50 (d, J = 7.4 Hz, 2H), 7.33 – 7.12 (m, 4H), 6.45 (s, 1H), 4.35 (brd, 2H), 4.12 (t, J = 6.8 Hz, 1H), 3.18 (brd, 2H), 1.73 (brs, 1H), 1.35 (d, J = 10.4 Hz, 9H), 0.76 (brd, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.7, 155.5, 154.8(from rotamer), 143.9(2C), 141.4(2C), 127.8(2C), 127.2(2C), 125.2(2C), 120.0(2C), 81.6, 68.3, 58.0, 57.6(from rotamer), 47.3, 28.3(3C), 26.8, 20.0(2C); HRMS (ESI): Calcd for $[C_{24}H_{30}N_2O_4 + Na]^+$: 433.2103, found: 433.2090.

NMR data of compounds 4-6

NMR data for compound 4 (Ac-aLys-aGly-Ser-NH_2) in water

Table S1. ^{1}H NMR chemical shifts of diaza-tripeptide **4** in water (11 mM) at 278 K

Residue	δ NH (ppm)	δ H_α (ppm)	δ H_β (ppm)	δ Other protons (ppm)
Ac				CH_3 2.09
aLys[1]	n.d.		3.70, 3.38	γ CH_2 1.62, δ CH_2 1.67, ε CH_2 3.00
				NH_3^+ n.d.
aGly[2]	n.d.	n.d.		
Ser[3]	6.65	4.32	3.90, 3.86	OH n.d.
$NH_{2\ Z,\ E}$	n.d.			

n.d. not detected

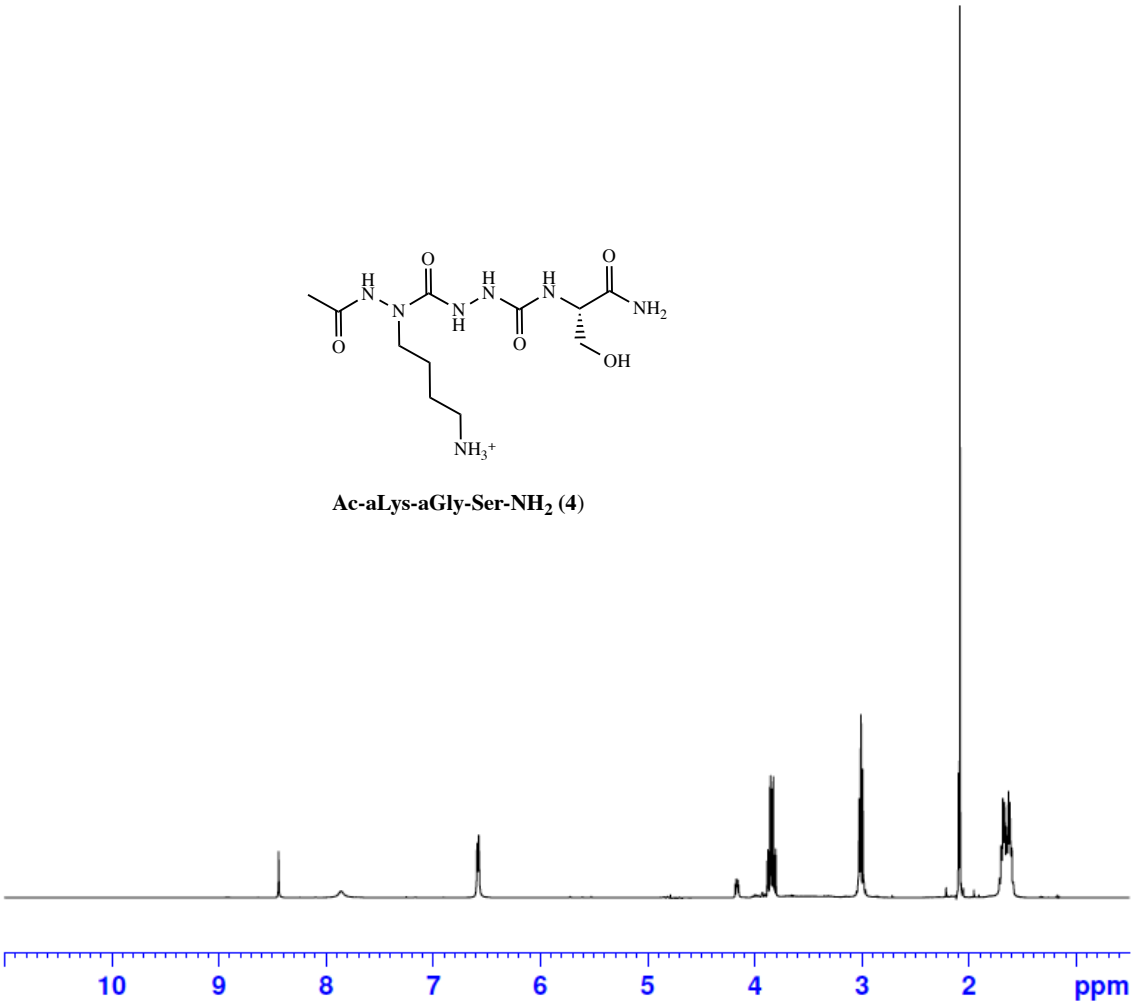
Table S2. ^{13}C NMR chemical shifts of diaza-tripeptide **4** in water (11 mM) at 308 K

Residue	δ CO (ppm)	δ C_α (ppm)	δ CH_β (ppm)	δ Other carbons (ppm)
Ac	176.5			CH_3 22.9
aLys[1]	161.8		50.5	γ CH_2 26.2, δ CH_2 26.7, ε CH_2 42.1
aGly[2]	162.7			
Ser[3]	179.5	60.1	65.5	

Table S3. NMR conformational parameters for **4** in water

Residue	Δδ NH/ΔT (ppb/K)	$^{3}J_{HN\text{-}H\alpha}$ (Hz)[a]	$^{3}J_{H\alpha\text{-}H\beta(\beta')}$ (Hz)[a]
aLys[1]	n.d.		
aGly[2]	n.d.		
Ser[3]	–3.4	7.7	5.5, 3.6
$NH_{2\ Z,\ E}$	n.d.		

[a] measured at 309 K; n.d. not detected



Ac-aLys-aGly-Ser-NH_2 (4)

Figure S1: 1D ^{1}H NMR spectrum (500.3 MHz) of diaza-tripeptide **4** in water (11 mM) at 308 K

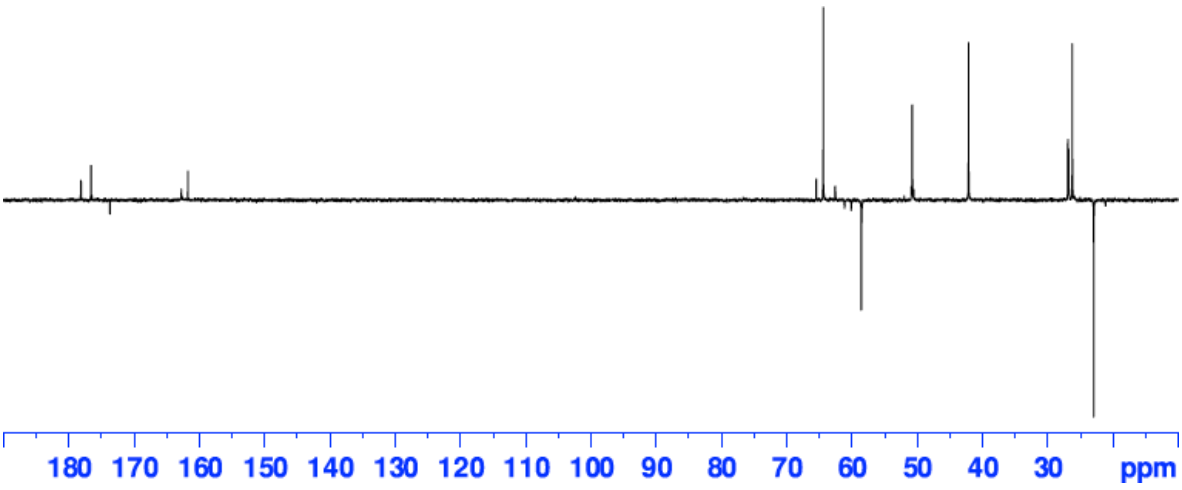
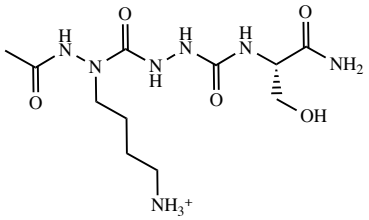


Figure S2: 1D ^{13}C DEPTQ NMR spectrum (125.8 MHz) of diaza-tripeptide **4** in water (11 mM) at 308 K



Ac-aLys-aGly-Ser-NH$_2$ (4)

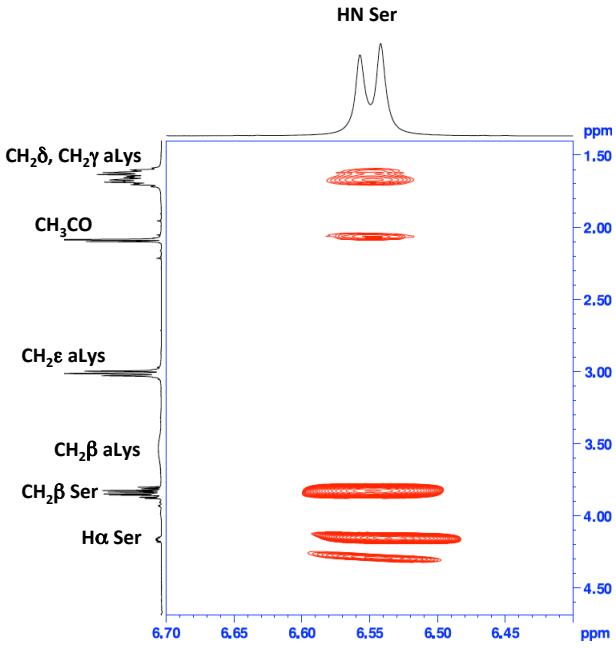


Figure S3: Zoom of 2D ^{1}H-^{1}H ROESY spectrum (500 MHz, mixing time of 250 ms) of **4** in water (11 mM) at 308 K, showing the correlations of Ser HN proton.

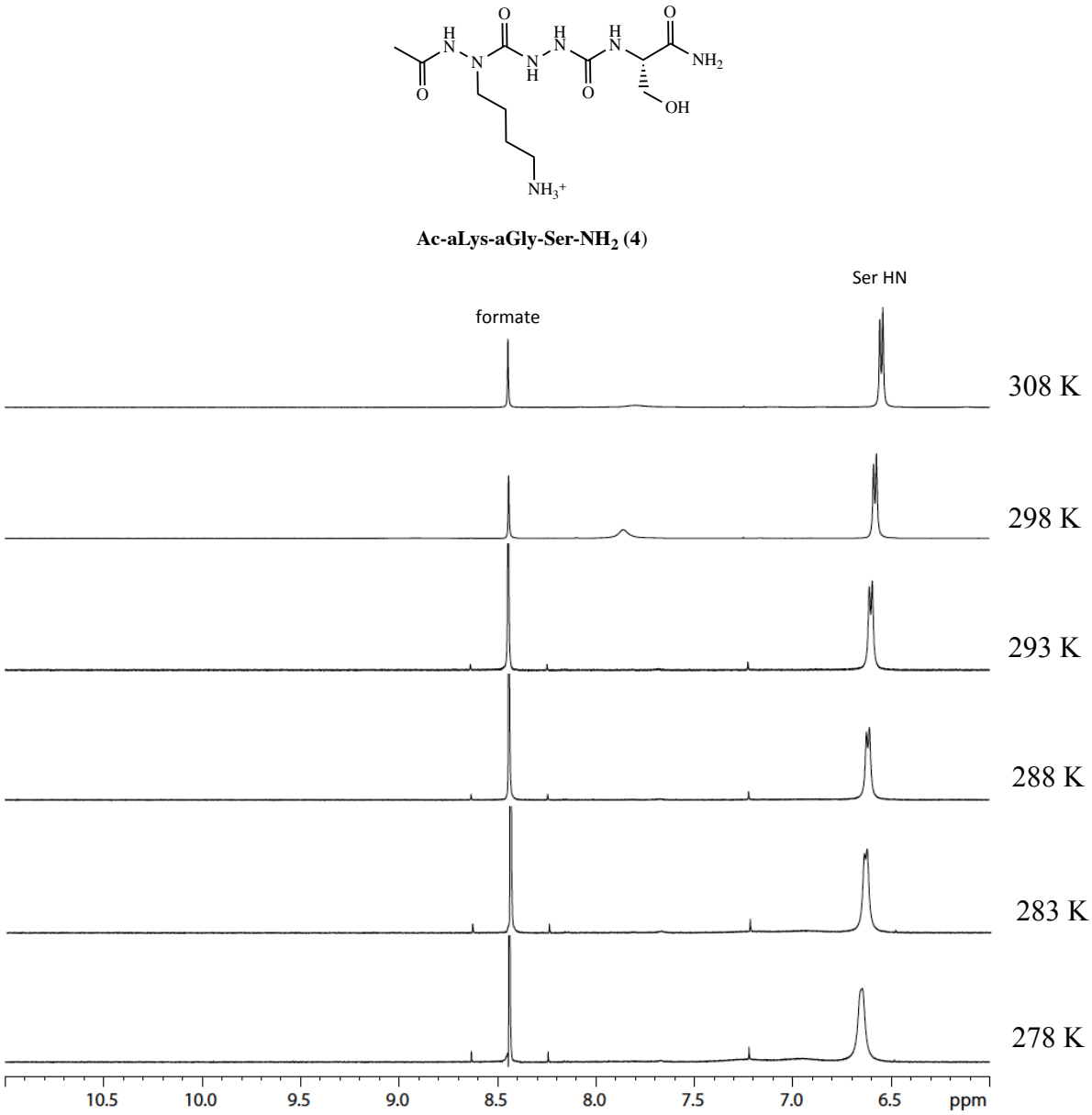


Figure S4: Variation of the Ser NH proton chemical shift of **4** in water (11 mM) in the temperature range 278 K–308 K (500.3 MHz)

NMR data for compound 4 (Ac-aLys-aGly-Ser-NH_2) in methanol

Table S4. ^{1}H NMR chemical shifts of diaza-tripeptide **4** in methanol (19.4 mM) at 298 K

Residue	δ NH (ppm)	δ H_α (ppm)	δ H_β (ppm)	δ Other protons (ppm)
Ac				CH_3 2.04
aLys[1]	n.d.		3.59, 3.53	γ CH_2 1.57, δ CH_2 1.72, ε CH_2 2.93, 2.85
				NH_3^+ n.d.
aGly[2]	9.06	7.73		
Ser[3]	6.51	4.10	3.84	OH n.d.
$NH_{2\ Z,\ E}$	n.d.			

n.d. not detected

Table S5. ^{13}C NMR chemical shifts of diaza-tripeptide **4** in methanol (19.4 mM) at 298 K

Residue	δ CO (ppm)	δ C_α (ppm)	δ CH_β (ppm)	δ Other carbons (ppm)
Ac	172.8			CH_3 21.1
aLys[1]	159.7		49.1	γ CH_2 25.0, δ CH_2 25.5, ε CH_2 40.7
aGly[2]	161.2			
Ser[3]	177.0	58.4	64.8	

Table S6. NMR conformational parameters for **4** in methanol

Residue	δ NH [a]	Δδ NH/ΔT (ppb/K)	$^3J_{HN\text{-}H\alpha}$ (Hz) [b]	$^3J_{H\alpha\text{-}H\beta(\beta')}$ (Hz) [b]
aLys[1]	10.71	–5.8		
aGly[2]	9.50, 8.28 (α)	–4.6, –7.9 (α)		
Ser[3]	6.60	–1.4	7.0	5.3, 4.5

[a] signal at 228 K

[b] average value, measured at 298 K

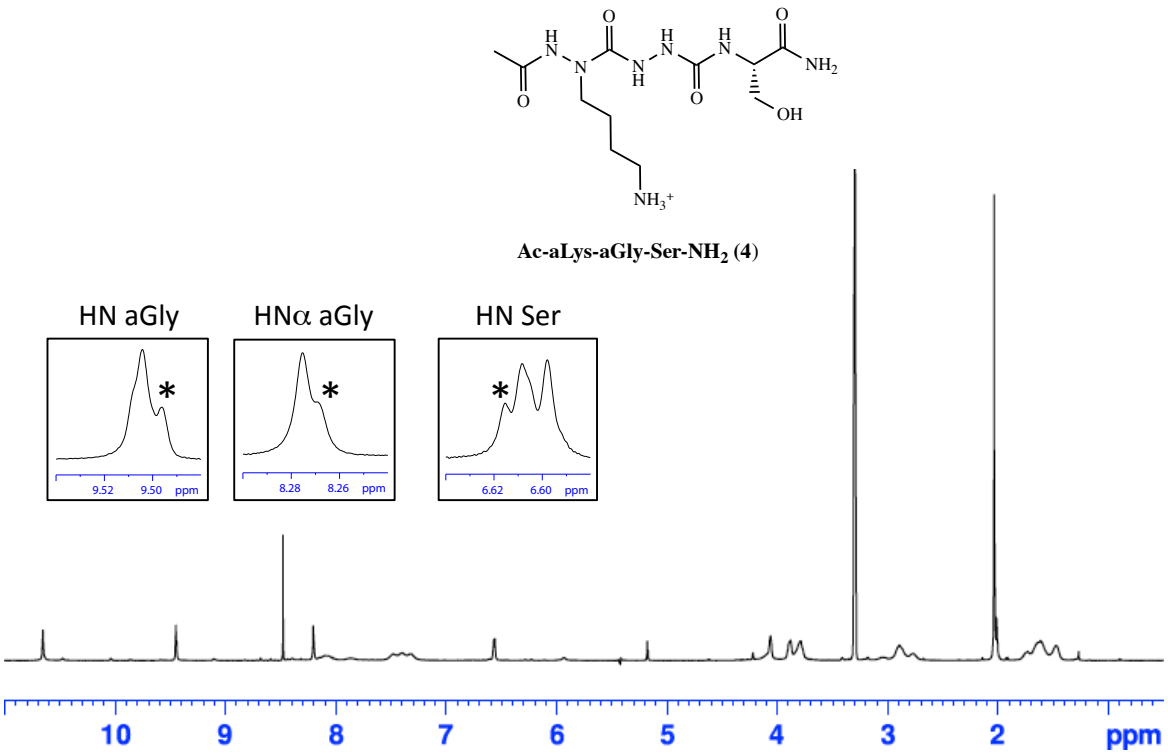


Figure S5: 1D ^{1}H NMR spectrum (600.4 MHz) of diaza-tripeptide **4** in methanol (19.4 mM) at 228 K. The insets show the presence of minor forms (indicated by asterisks) for the HN protons of aGly and Ser.

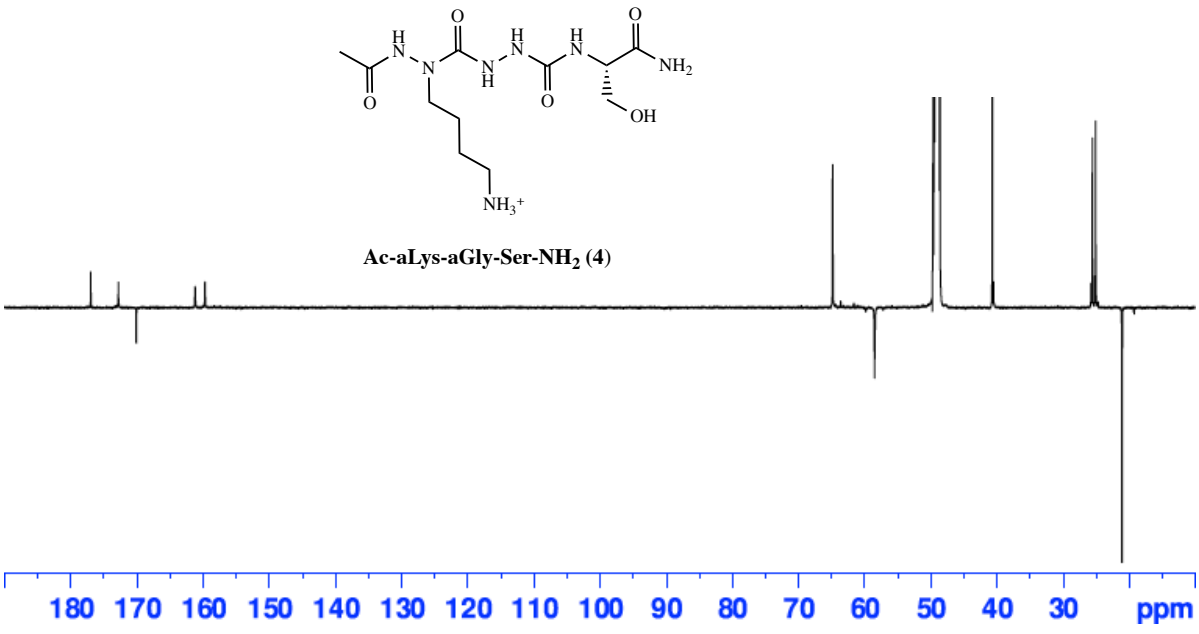
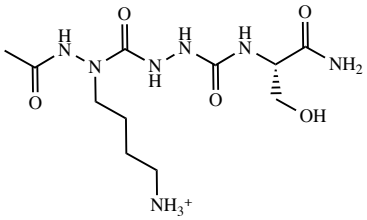


Figure S6: 1D ^{13}C DEPTQ NMR spectrum (125.8 MHz) of diaza-tripeptide **4** in methanol (19.4 mM) at 298 K



Ac-aLys-aGly-Ser-NH$_2$ (4)

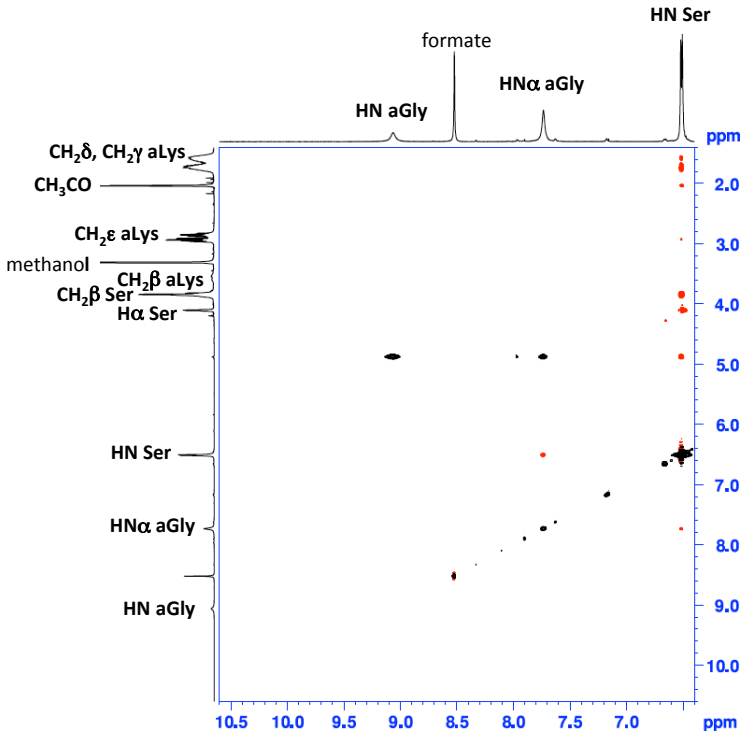


Figure S7: 2D ^{1}H-^{1}H ROESY spectrum (500.3 MHz, mixing time 250 ms) of diaza-tripeptide **4** in methanol (19.4 mM) at 298 K, showing the correlation peaks of NH protons

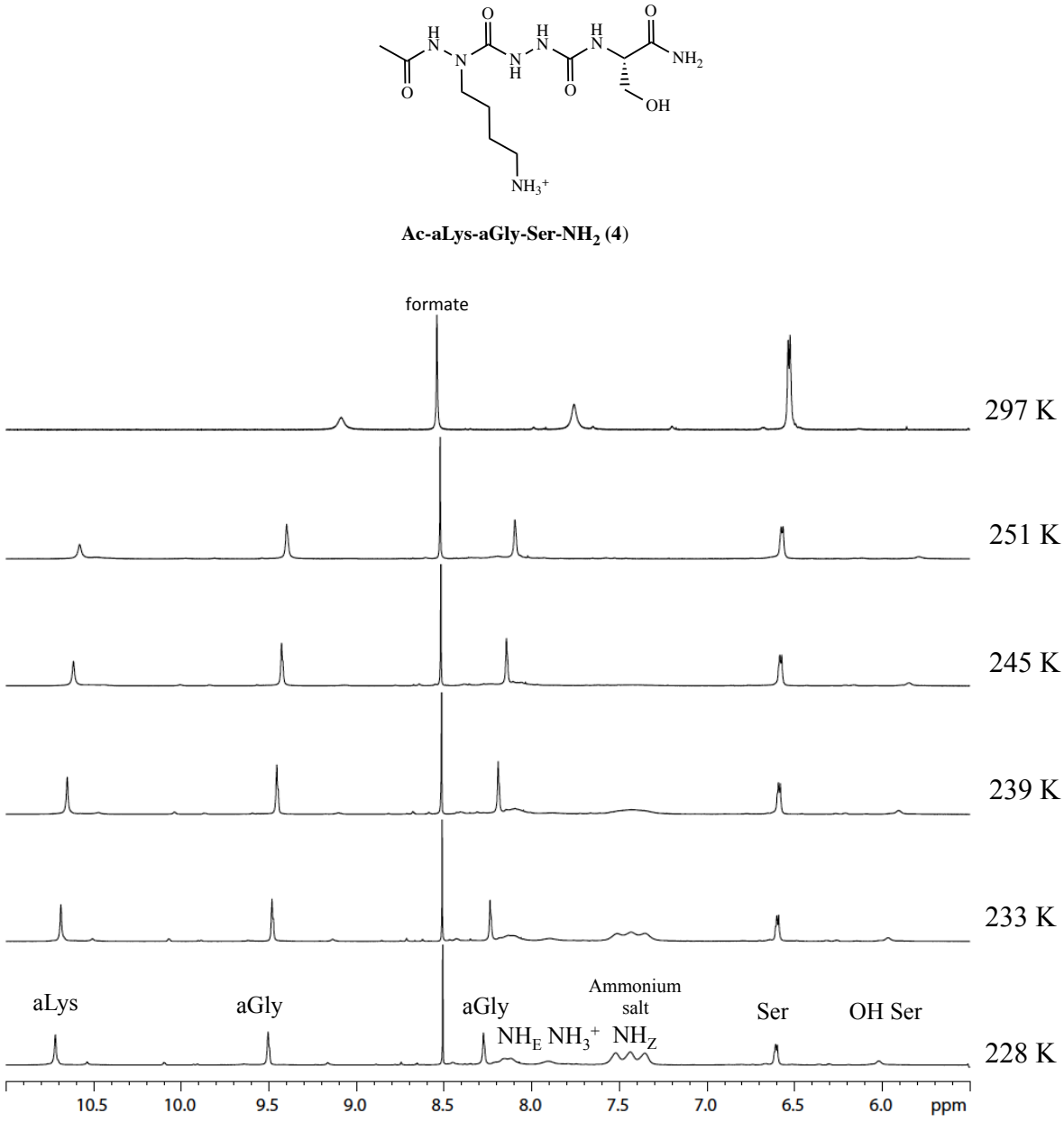


Figure S8: Variation of the amide NH proton chemical shifts of **4** in methanol (19.4 mM) in the temperature range 228 K–297 K (600.4 MHz)

NMR data for compound 5 (Ac-aLys-aAla-Ser-NH_2) in water

Table S7. ^{1}H NMR chemical shifts of diaza-tripeptide **5** in water (11 mM) at 278 K

Residue	δ NH (ppm)	δ H_α (ppm)	δ H_β (ppm)	δ Other protons (ppm)
Ac				CH_3 2.10
aLys[1]	10.55, 10.48		3.70, 3.38	γ CH_2 1.63, δ CH_2 1.68, ε CH_2 3.00
				NH_3^+ 7.62
aAla[2]	9.51, 9.45, 9.04		3.06	
Ser[3]	6.97, 6.89, 6.75	4.27	3.86	OH n.d.
$NH_{2\ Z,\ E}$	7.30, 7.24			
	7.86,7.78,7.75,7.66			

Table S8. ^{13}C NMR chemical shifts of diaza-tripeptide **5** in water (11 mM) at 308 K

Residue	δ CO (ppm)	δ C_α (ppm)	δ CH_β (ppm)	δ Other carbons (ppm)
Ac	176.6			CH_3 22.9
aLys[1]	160.8		50.9	γ CH_2 26.2, δ CH_2 26.8, ε CH_2 42.1
aAla[2]	162.5		38.8	
Ser[3]	178.4	59.2	64.4	

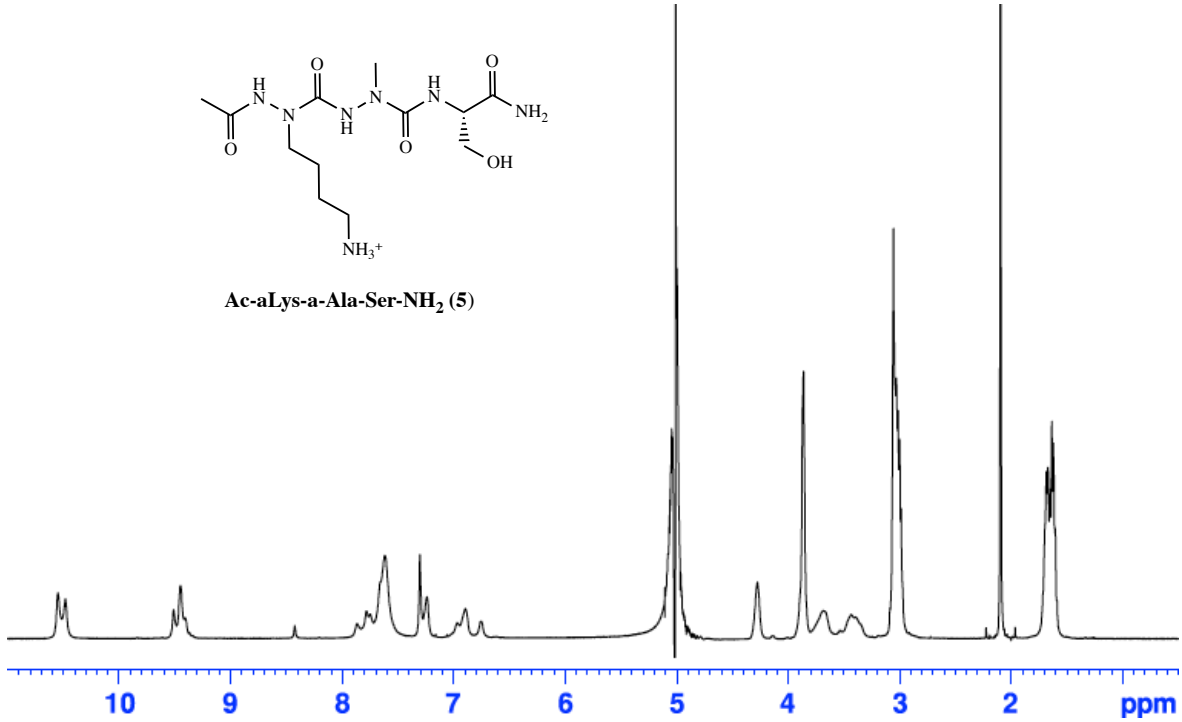


Figure S9: 1D ^{1}H NMR spectrum (500.3 MHz) of diaza-tripeptide **5** in water (11 mM) at 278 K

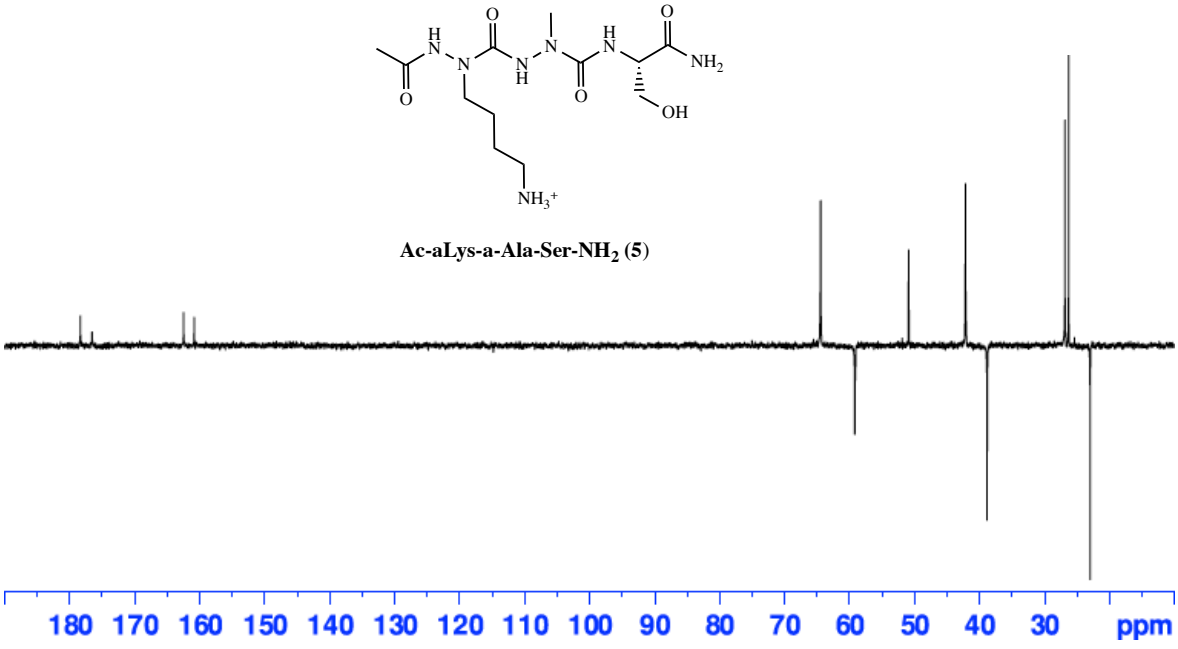
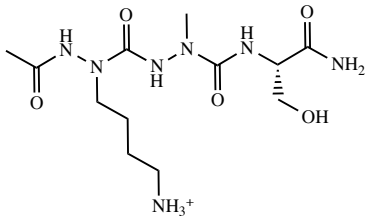


Figure S10: 1D ^{13}C DEPTQ NMR spectrum (125.8 MHz) of diaza-tripeptide **5** in water (11 mM) at 308 K



Ac-aLys-a-Ala-Ser-NH$_2$ (5)

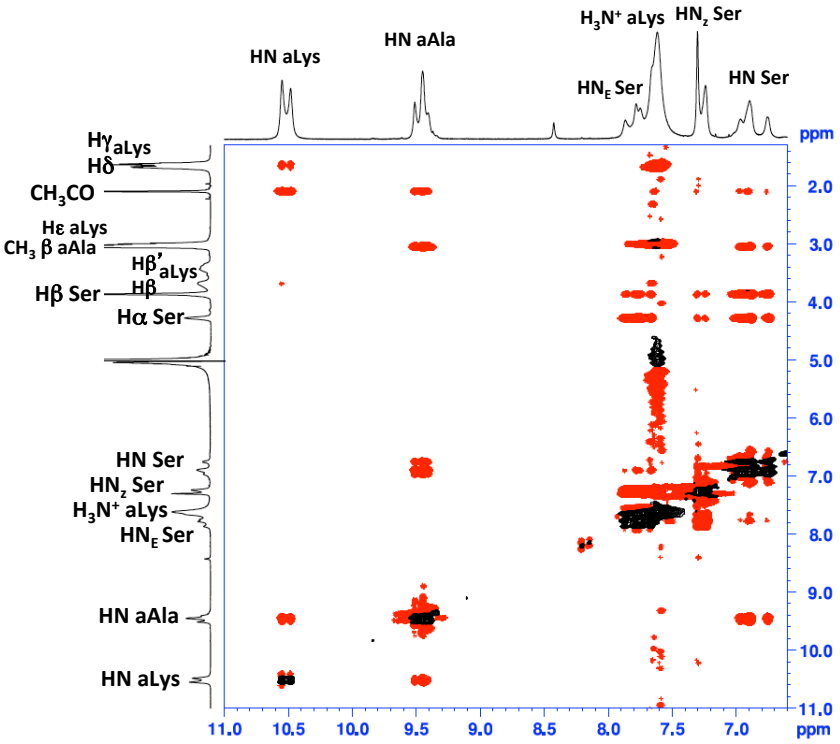


Figure S11: 2D ^{1}H-^{1}H ROESY spectrum (500.3 MHz, mixing time of 250 ms) of **5** in water (11 mM) at 278 K, showing the correlation peaks of NH protons for the different conformers. Diagonal peaks and exchange peaks are in black while ROE cross-peaks of opposite sign are in red.

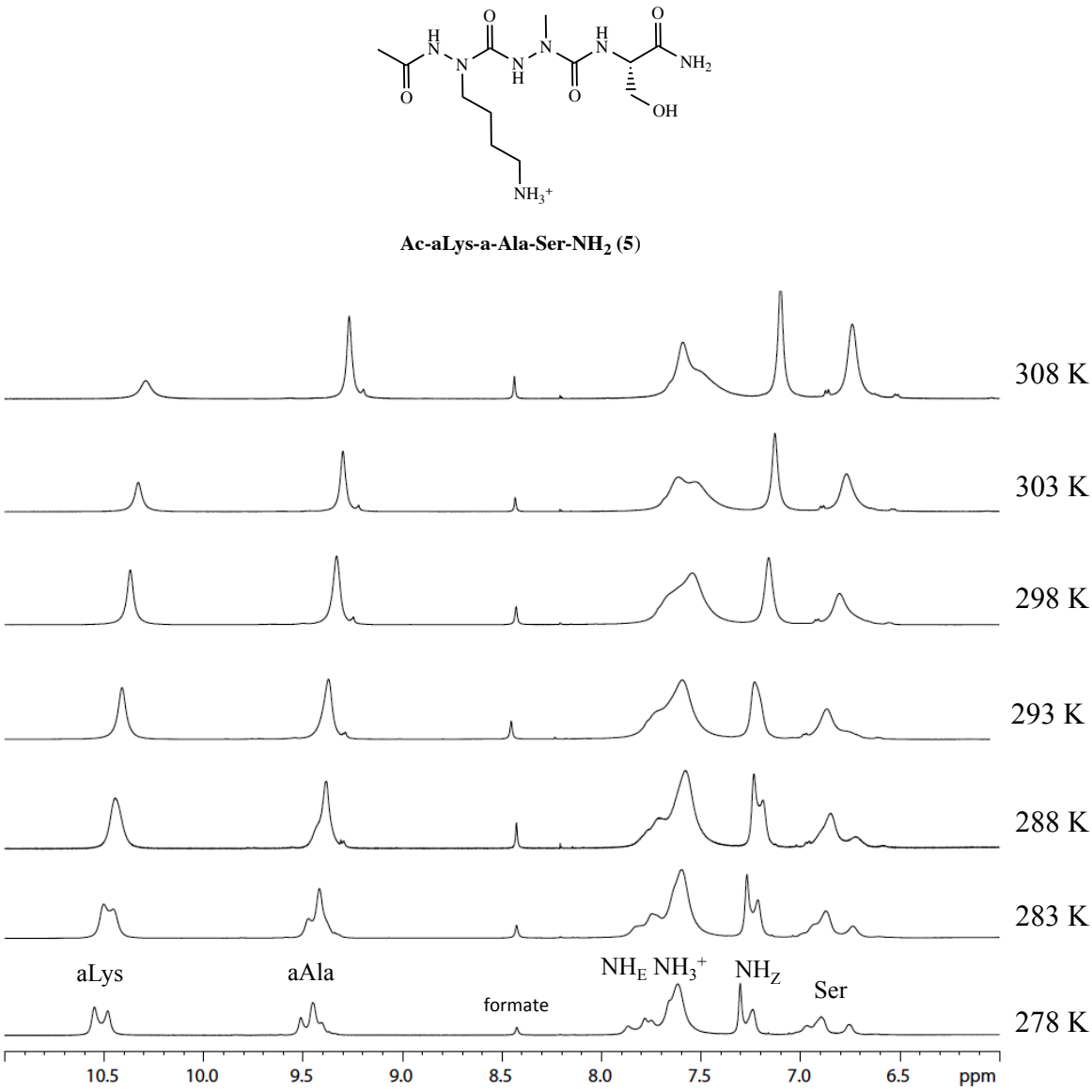


Figure S12: Variation of the amide NH proton chemical shifts of **5** in water (11 mM) in the temperature range 278 K–308 K (500.3 MHz)

NMR data for compound 5 (Ac-aLys-aAla-Ser-NH_2) in methanol

Table S9. ^{1}H NMR chemical shifts of diaza-tripeptide **5** in methanol (27.9 mM) at 298 K

Residue	δ NH (ppm)	δ H_α (ppm)	δ H_β (ppm)	δ Other protons (ppm)
Ac				CH_3 2.06
aLys[1]	n.d.		3.74, 3.39	γ CH_2 1.59 δ CH_2 1.73, ε CH_2 2.94
				NH_3^+ n.d.
aAla[2]	9.37		3.07	
Ser[3]	6.67	4.24	3.86	OH n.d.
$NH_{2\ Z,\ E}$	7.17, 7.57			

Table S10. ^{13}C NMR chemical shifts of diaza-tripeptide **5** in methanol (27.9 mM) at 298 K

Residue	δ CO (ppm)	δ C_α (ppm)	δ CH_β (ppm)	δ Other carbons (ppm)
Ac	173.6			CH_3 21.0
aLys[1]	158.8		49.0	γ CH_2 25.1, δ CH_2 25.8, ε CH_2 40.6
aAla[2]	160.4		36.6	
Ser[3]	176.1	57.8	63.6	

Table S11. Amide proton chemical shifts and NMR conformational parameters for compound **5** in methanol. A, B, C and D letters correspond to the four observed conformers, with respective populations of 42%, 27%, 19%, 12%.

Residue	δ NH (ppm) [a]	Δδ NH/ΔT (ppb/K)	$^3J_{HN\text{-}H\alpha}$ (Hz) [a]	$^3J_{H\alpha\text{-}H\beta\beta'}$ (Hz) [b]
aLys[1]	10.71 (A), 10.69 (B)	–4.8 (A), –5.4 (B)	-	
	10.76 (C), 10.67 (D)	–5.0 (C), –5.5 (D)		
aAla[2]	9.82 (A), 9.71 (B)	–4.4 (A), –4.8 (B)	-	
	9.68 (C), 9.58 (D)	–4.0 (C), –4.2 (D)		
Ser[3]	6.98 (A), 6.83 (B)	–3.3 (A), –2.6 (B)	8.2 (A), 8.0 (B)	5.0, 4.8
	6.81 (C), 6.69 (D)	–3.0 (C), –2.2 (D)	7.6 (C), br (D)	
$NH_{2\ Z}$	7.70 (A), 7.78 (B)	–7.1 (A), –7.8 (B)		
	7.64 (C), 7.70 (D)	–6.8 (C), –7.1 (D)		
$NH_{2\ E}$	7.83 (A), 7.94 (B)	–4.9 (A), –5.6 (B)		
	8.07 (C), 8.11 (D)	–5.8 (C), –5.4 (D)		
OH	5.95 (A), 6.19 (B)	–8.1 (A), –7.0 (B)		
	5.93 (C), 6.09 (D)	–12.2 (C), –7.3 (D)		

[a] measured at 228 K; br, broad peak
[b] average value, measured at 298 K

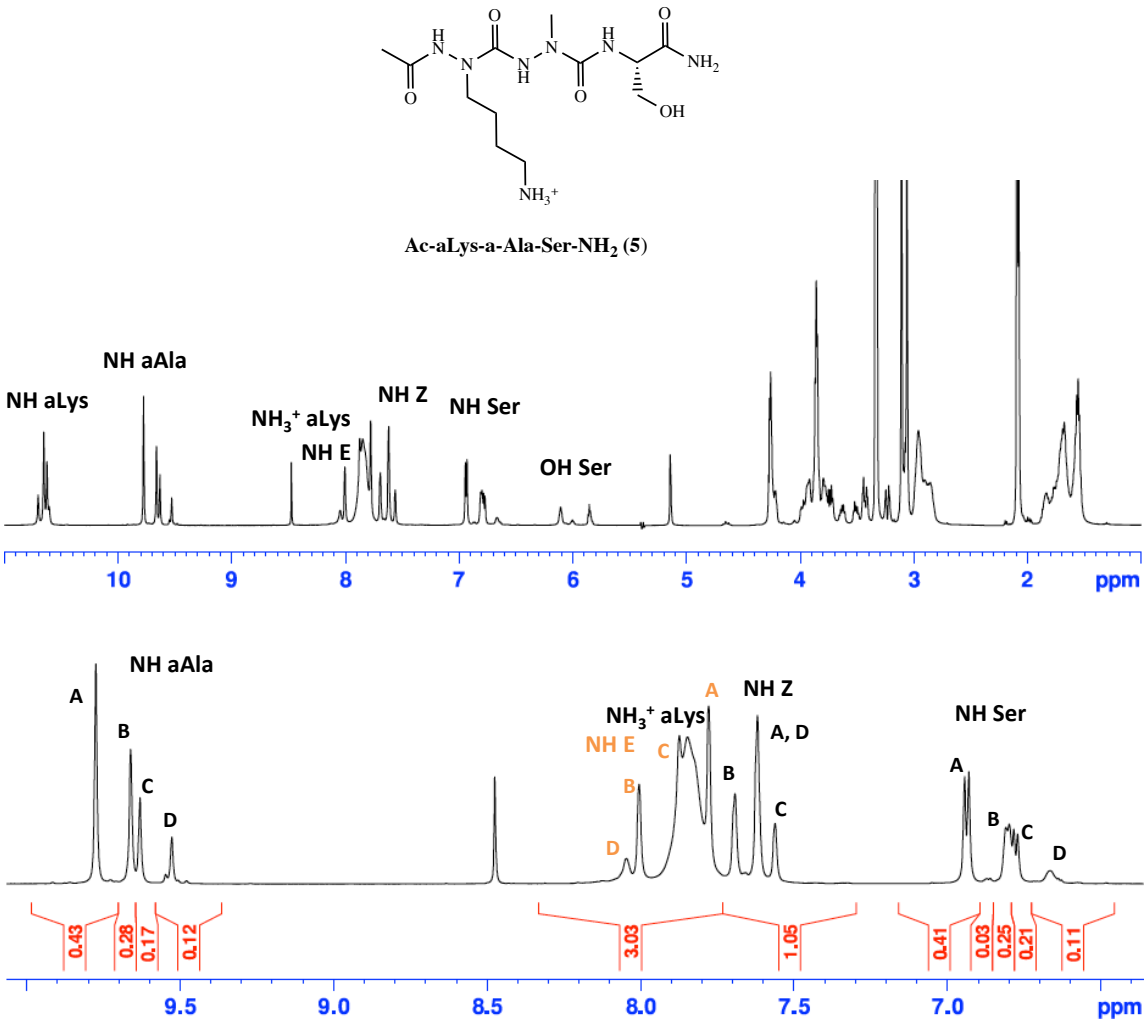


Figure S13: 1D ^{1}H NMR spectrum (600.4 MHz) of diaza-tripeptide **5** in methanol (27.9 mM) at 228 K with integration of amide proton signals for quantifying the population of the four conformers A, B, C and D

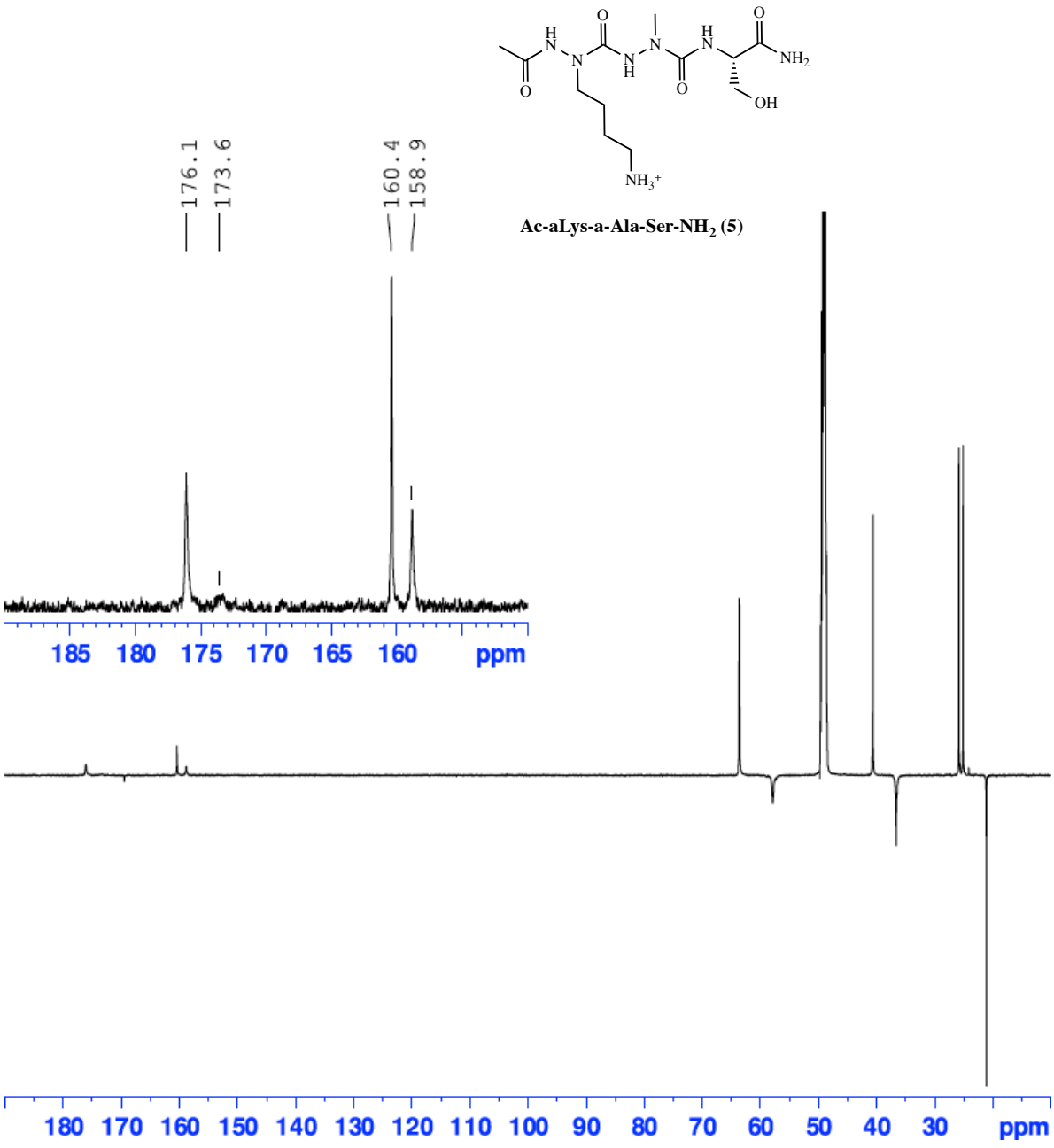
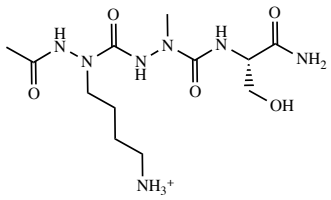


Figure S14: 1D ^{13}C DEPTQ NMR spectrum (125.8 MHz) of diaza-tripeptide **5** in methanol (27.9 mM) at 298 K



Ac-aLys-a-Ala-Ser-NH$_2$ (5)

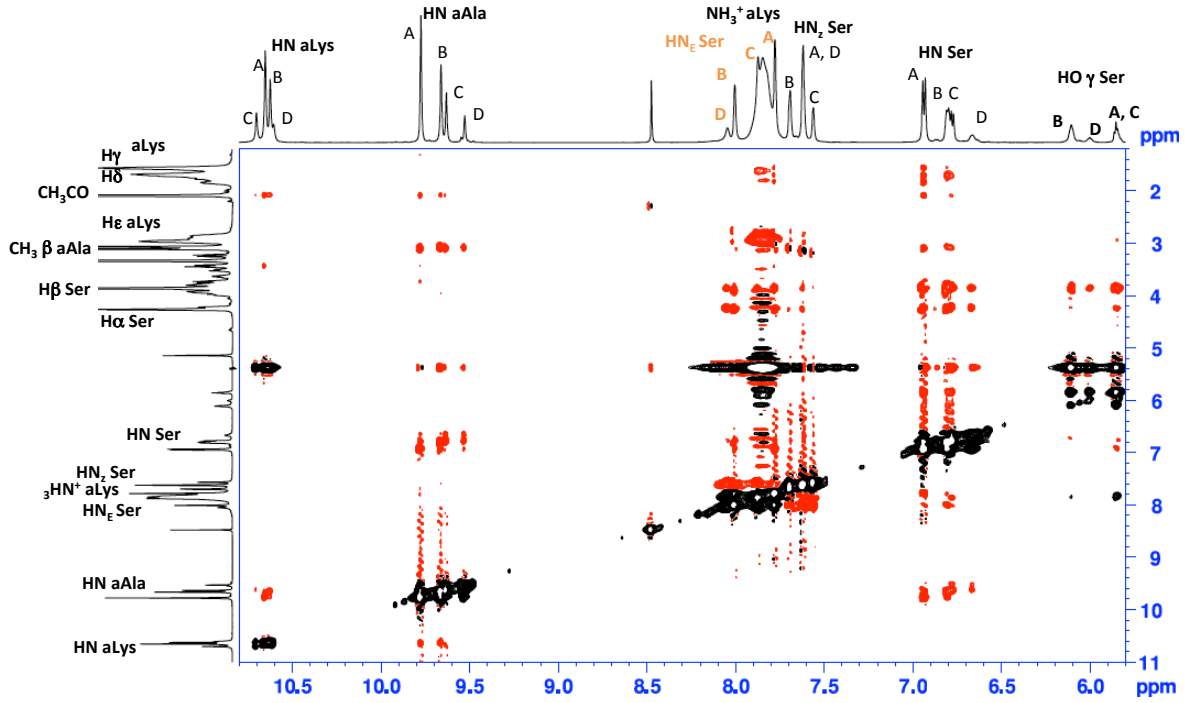


Figure S15: Expansion of the 2D ^{1}H-^{1}H ROESY spectrum (600.4 MHz, mixing time of 250 ms) of **5** in methanol (27.9 mM) at 239 K, showing the correlation peaks of NH protons for the four conformers A, B, and C and D. Diagonal peaks and exchange peaks are in black while ROE cross-peaks of opposite sign are in red.

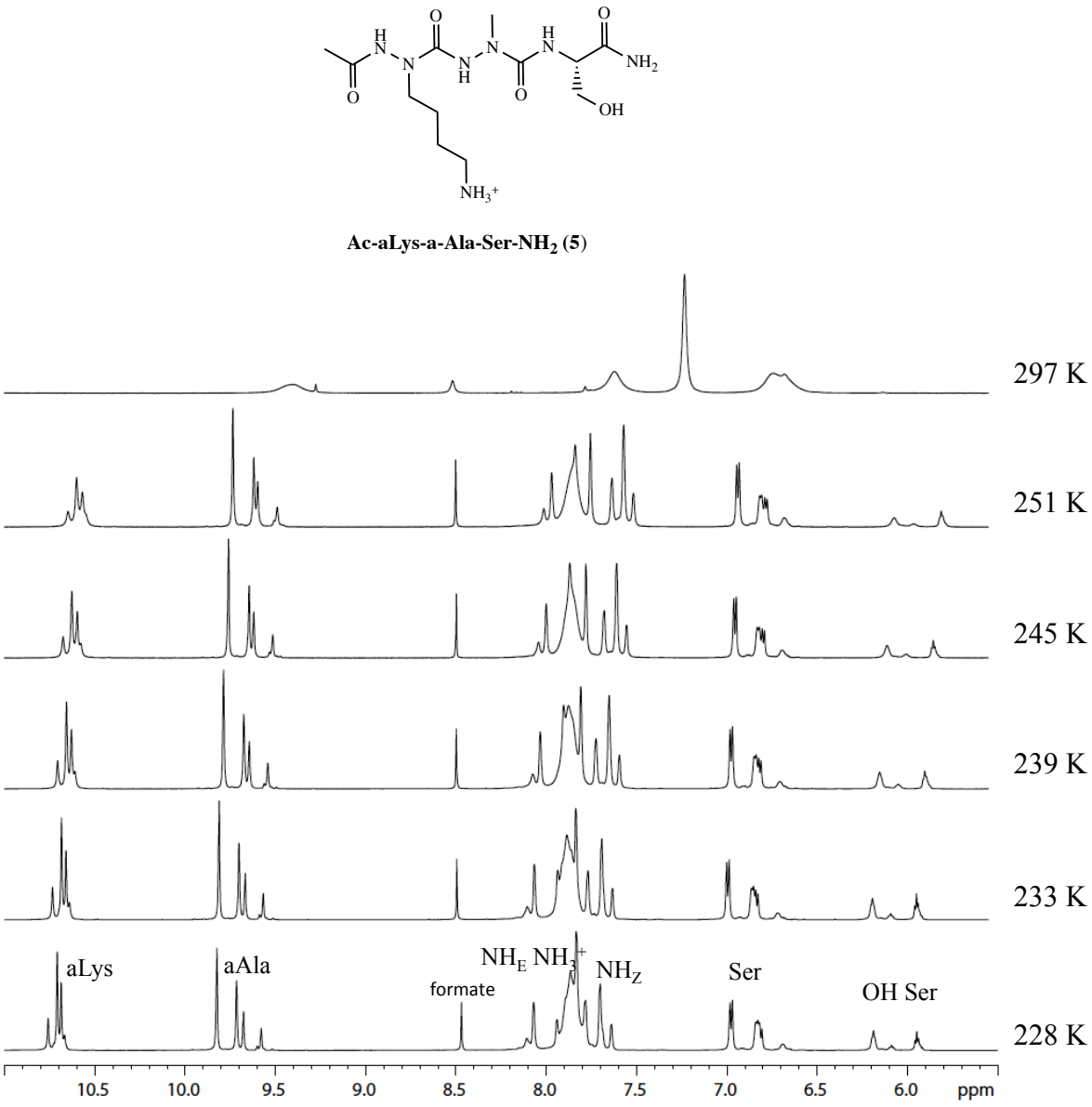


Figure S16: Variation of the amide NH proton chemical shifts of **5** in methanol (27.9 mM) in the temperature range 228 K–297 K (600.4 MHz)

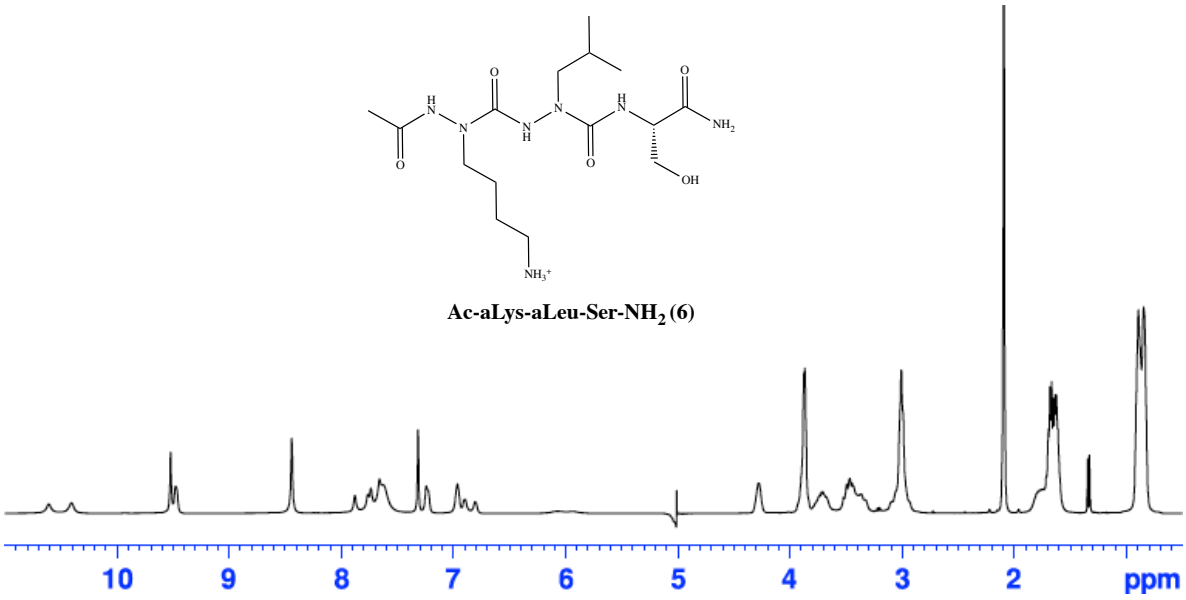
NMR data for compound 6 (Ac-aLys-aLeu-Ser-NH_2) in water

Table S12. ^{1}H NMR chemical shifts of diaza-tripeptide **6** in water (10 mM) at 278 K. A, B, C and D letters correspond to the four observed conformers.

Residue	δ NH (ppm)	δ H_α (ppm)	δ H_β (ppm)	δ Other protons (ppm)
Ac				CH_3 2.09
aLys[1]	10.61, 10.41		3.72, 3.36	γ CH_2 1.62, δ CH_2 1.66, ε CH_2 3.00
				NH_3^+ 7.62
aLeu[2]	9.52 (A, B)		3.47, 2.97	γ CH 1.76, δδ' CH_3 0.89
	9.48 (C, D)			
Ser[3]	6.96 (A, B)	4.28	3.87	OH 6.06, 5.93
	(6.89, 6.80) (C, D)			
$NH_{2\ Z}$	7.31 (A, B)			
	(7.24, 7.23) (C, D)			
$NH_{2\ E}$	(7.88, 7.76) (C, D)			
	7.74 (B), 7.66 (A)			

Table S13. ^{13}C NMR chemical shifts of diaza-tripeptide **6** in water (10 mM) at 308 K

Residue	δ CO (ppm)	δ C_α (ppm)	δ CH_β (ppm)	δ Other carbons (ppm)
Ac	176.3			CH_3 23.0
aLys[1]	160.5	-	50.9	γ CH_2 26.3, δ CH_2 26.8, ε CH_2 42.1
aLeu[2]	162.5	-	58.6	γ CH 28.7, δ,δ' CH_3 22.1
Ser[3]	178.4	59.2	64.4	



Ac-aLys-aLeu-Ser-NH$_2$ (6)

Figure S17: 1D ^{1}H NMR spectrum (500.3 MHz) of diaza-tripeptide **6** in water (10 mM) at 278 K

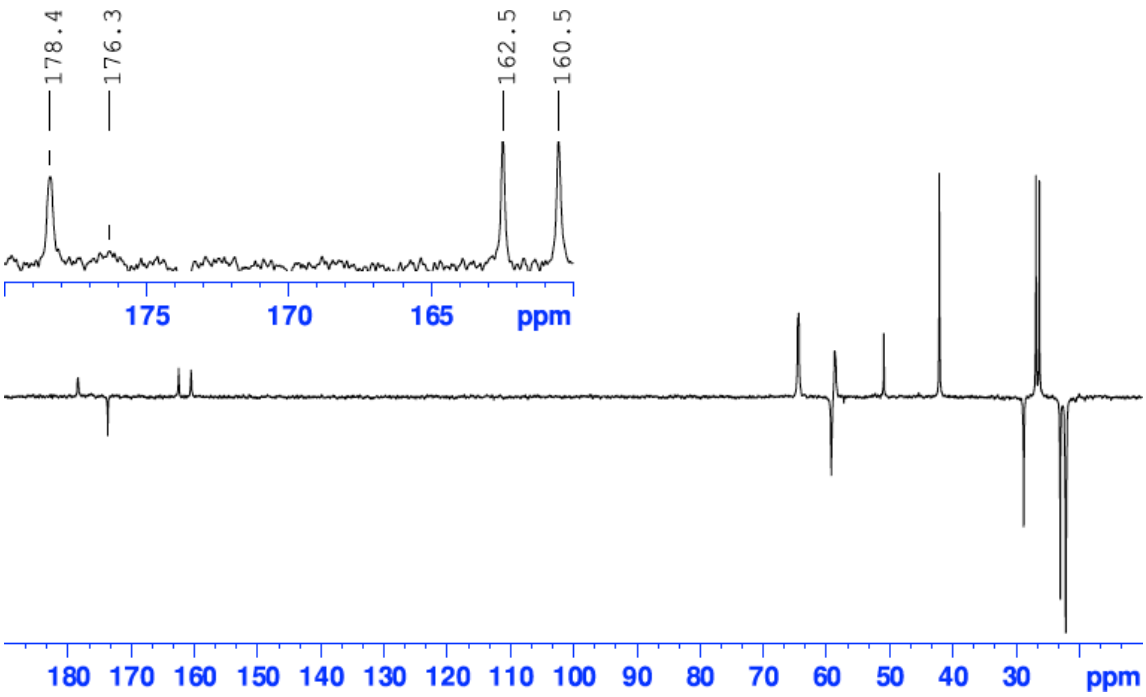
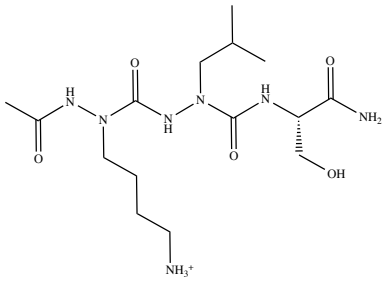


Figure S18: 1D ^{13}C DEPTQ NMR spectrum (125.8 MHz) of diaza-tripeptide **6** in water (10 mM) at 308 K



Ac-aLys-aLeu-Ser-NH$_2$ (6)

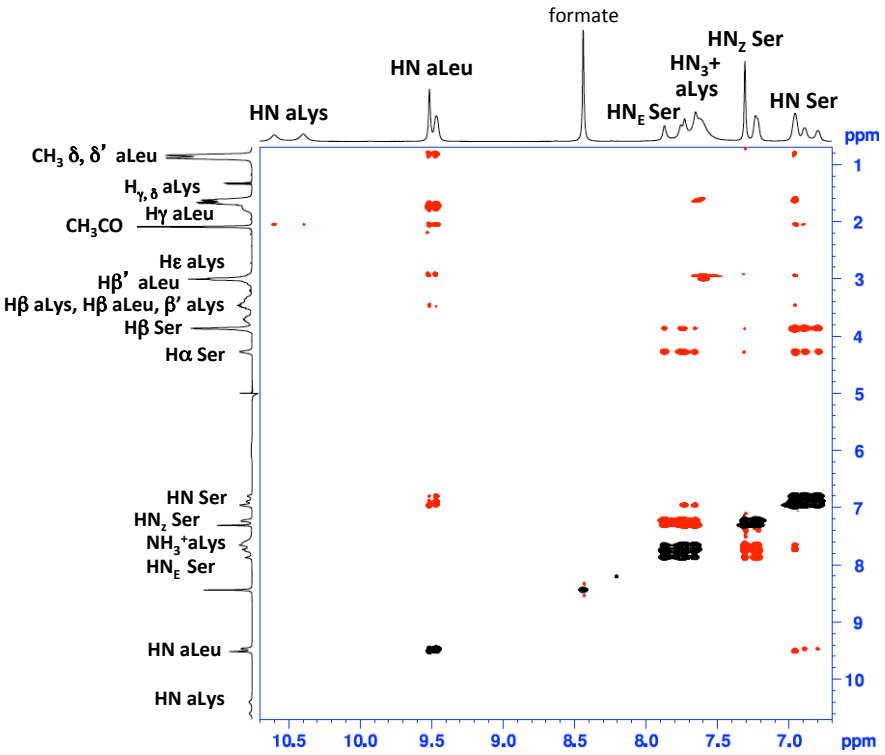
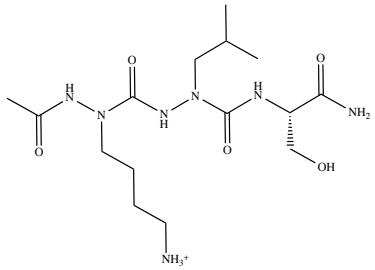


Figure S19: Zoom of the 2D ^{1}H-^{1}H ROESY spectrum (500.3 MHz, mixing time of 250 ms) of **6** in water (10 mM) at 278 K, showing the correlation peaks of NH protons for the different conformers. Diagonal peaks and exchange peaks are in black while ROE cross-peaks of opposite sign are in red.



Ac-aLys-aLeu-Ser-NH$_2$ (6)

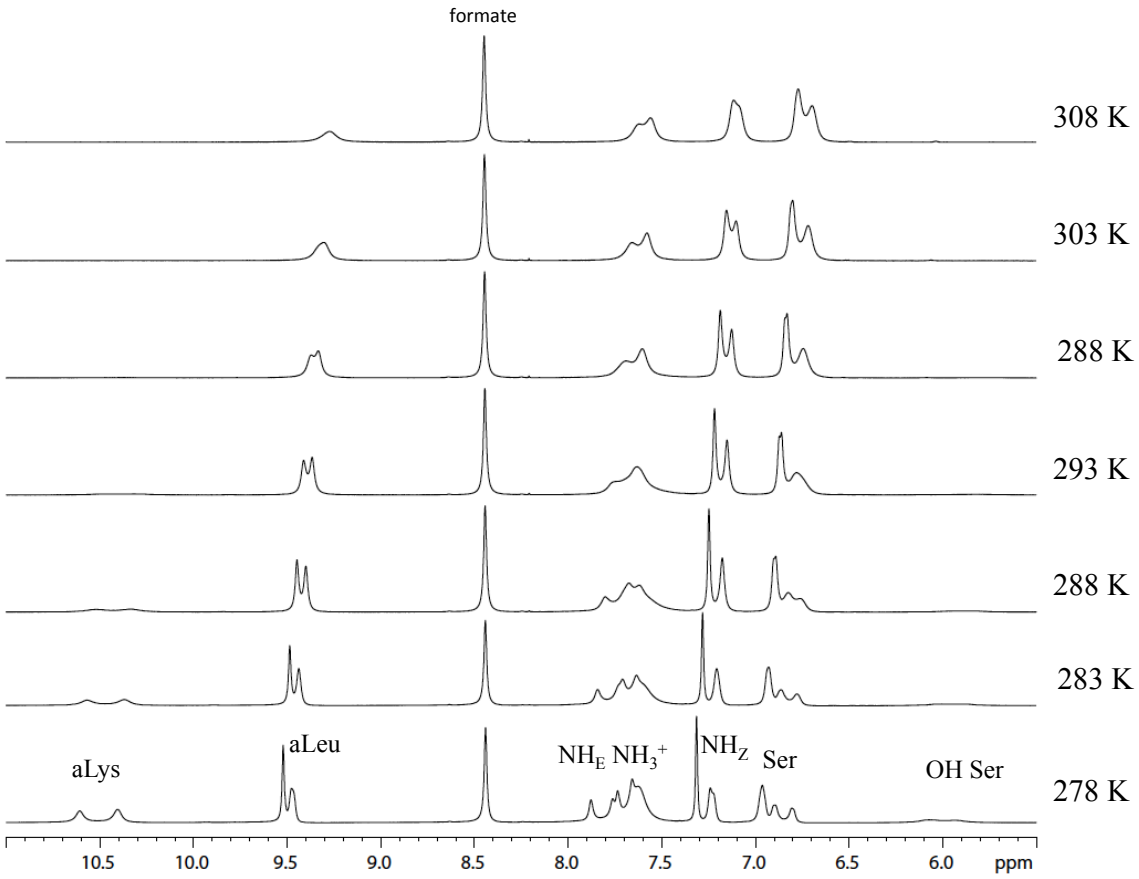


Figure S20: Variation of the amide NH proton chemical shifts of **6** in water (10 mM) in the range temperature 278 K–308 K (500.3 MHz)

NMR data for compound 6 (Ac-aLys-aLeu-Ser-NH_2) in methanol

Table S14. ^{1}H NMR chemical shifts of diaza-tripeptide **6** in methanol (25.6 mM) at 298 K

Residue	δ NH (ppm)	δ H_α (ppm)	δ H_β (ppm)	δ Other protons (ppm)
Ac				CH_3 2.06
aLys[1]	n.d.		3.73, 3.37	γ CH_2 1.58, δ CH_2 1.71, ε CH_2 2.93, 2.88
			3.62, 3.43	$NH3^+$ n.d.
aLeu[2]	n.d.		3.57, 3.00	γ CH 1.84, δ,δ' CH_3 0.90
Ser[3]	6.70, 6.52	4.24	3.87, 3.83	OH n.d.
$NH_{2\ Z,\ E}$	7.17, 7.55			

Table S15. ^{13}C NMR chemical shifts of diaza-tripeptide **6** in methanol (25.6 mM) at 298 K

Residue	δ CO (ppm)	δ C_α (ppm)	δ CH_β (ppm)	δ Other carbons (ppm)
Ac	173.9[a]			CH_3 21.1
aLys[1]	158.8	-	49.2	γ CH_2 25.1, δ CH_2 26.2, ε CH_2 40.8
aLeu[2]	160.5	-	57.3	γ CH 27.9, δ,δ' CH_3 20.6
Ser[3]	176.5	58.1	63.6	

[a] Not observed on 1D ^{13}C spectrum, assigned via $^{2}J_{CH3\text{-}CO}$ correlation peak on HMBC

Table S16. Amide proton chemical shifts and NMR conformational parameters for compound **6** in methanol. A, B, C and D letters correspond to the four observed conformers, with respective populations of 55%, 22%, 17%, 6%.

Residue	δ NH (ppm) [a]	ΔδNH/ΔT (ppb/K)	$^{3}J_{HN\text{-}H\alpha}$ (Hz) [a]	$^{3}J_{H\alpha\text{-}H\beta}$ (Hz) [b]
aLys[1]	n.d.	-		
aLeu[2]	9.82 (A), 9.74 (B)	–4.3 (A), –5.0 (B)		
	9.70 (C), 9.62 (D)	–4.6 (C), –5.0 (D)		
Ser[3]	6.94 (A), 6.79 (B)	–2.7 (A), –2.9 (B)	8.2 (A), 7.7 (B)	5.0
	6.71 (C), 6.58 (D)	–2.4 (C), –2.2 (D)	7.2 (C), 6.8 (D)	
$NH_{2\ Z}$	7.69 (A), 7.76 (B)	–6.9 (A), –7.4 (B)		
	7.59 (C), 7.69 (D)	–6.4 (C), –6.9 (D)		
$NH_{2\ E}$	7.76 (A), 8.03 (B)	–4.8 (A), –5.3 (B)		
	7.97 (C), 8.13 (D)	–6.1 (C), –5.3 (D)		

[a] measured at 228 K; n.d. not detected
[b] average value, measured at 298 K.

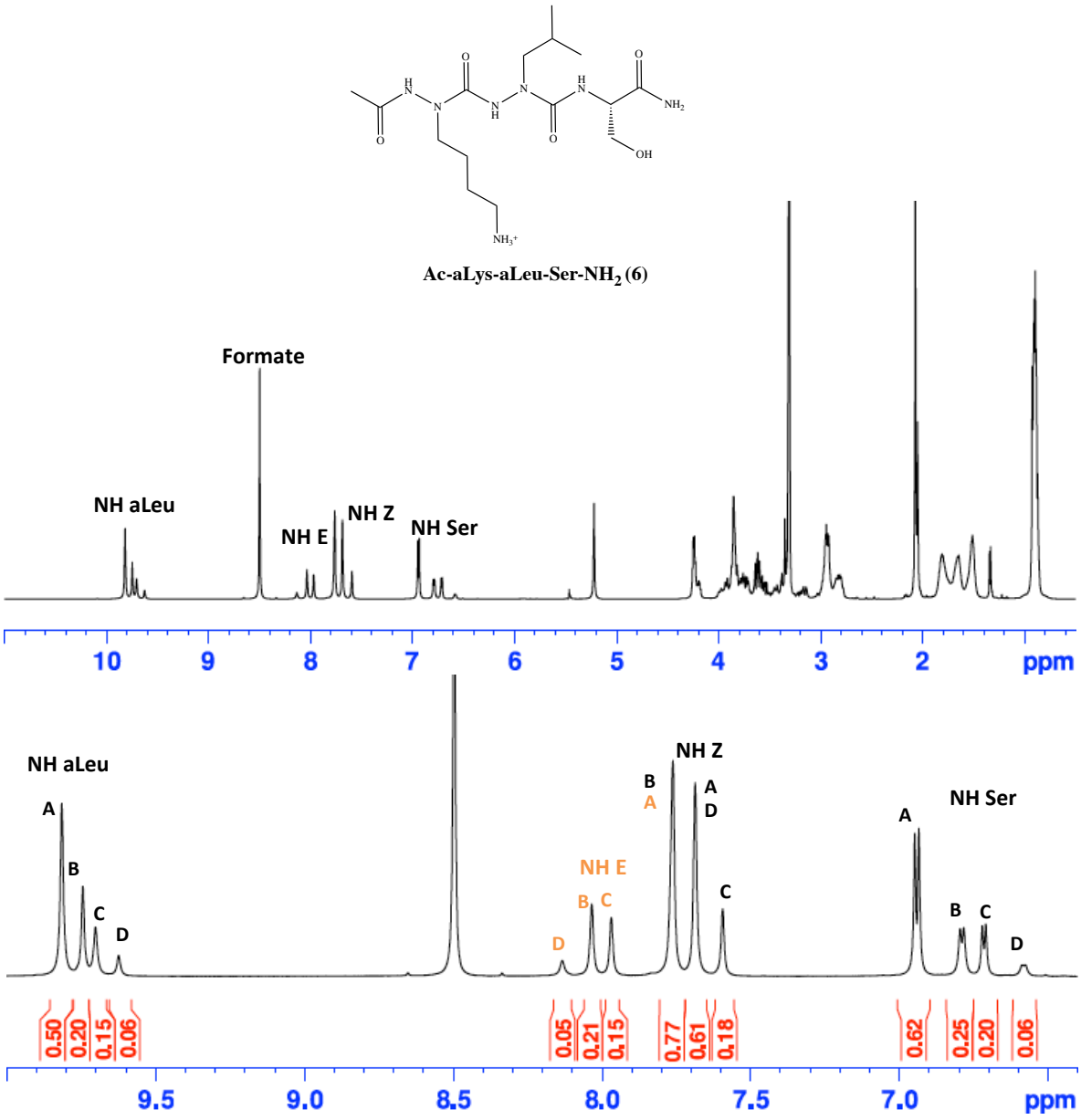
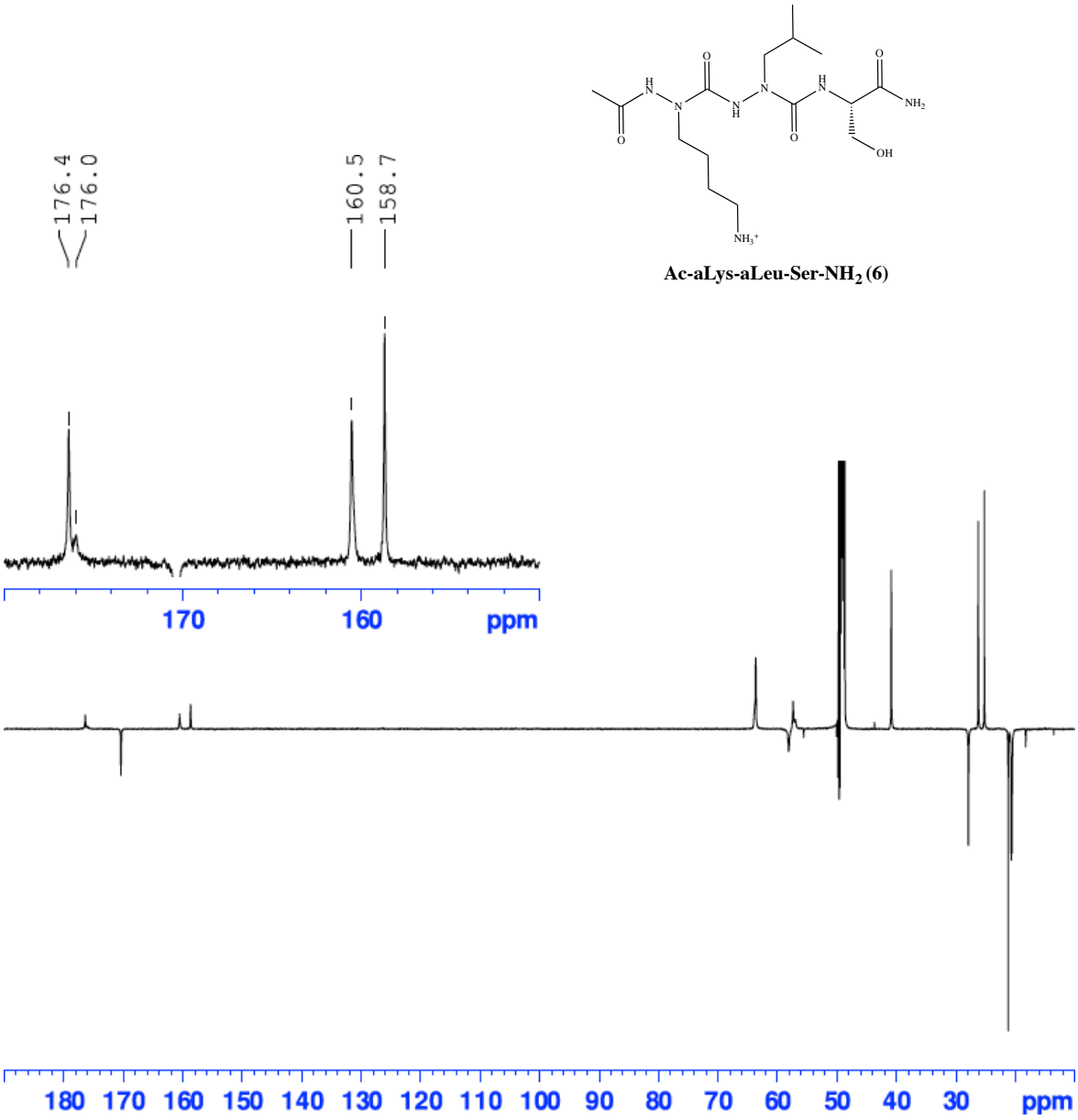


Figure S21: 1D ^{1}H NMR spectrum (600.4 MHz) of diaza-tripeptide **6** in methanol (25.6 mM) at 228 K with integration of amide proton signals for quantifying the populations of the four conformers A, B, C and D.



Ac-aLys-aLeu-Ser-NH$_2$ (6)

Figure S22: 1D ^{13}C DEPTQ NMR spectrum (125.8 MHz) of diaza-tripeptide **6** in methanol (25.6 mM) at 298 K

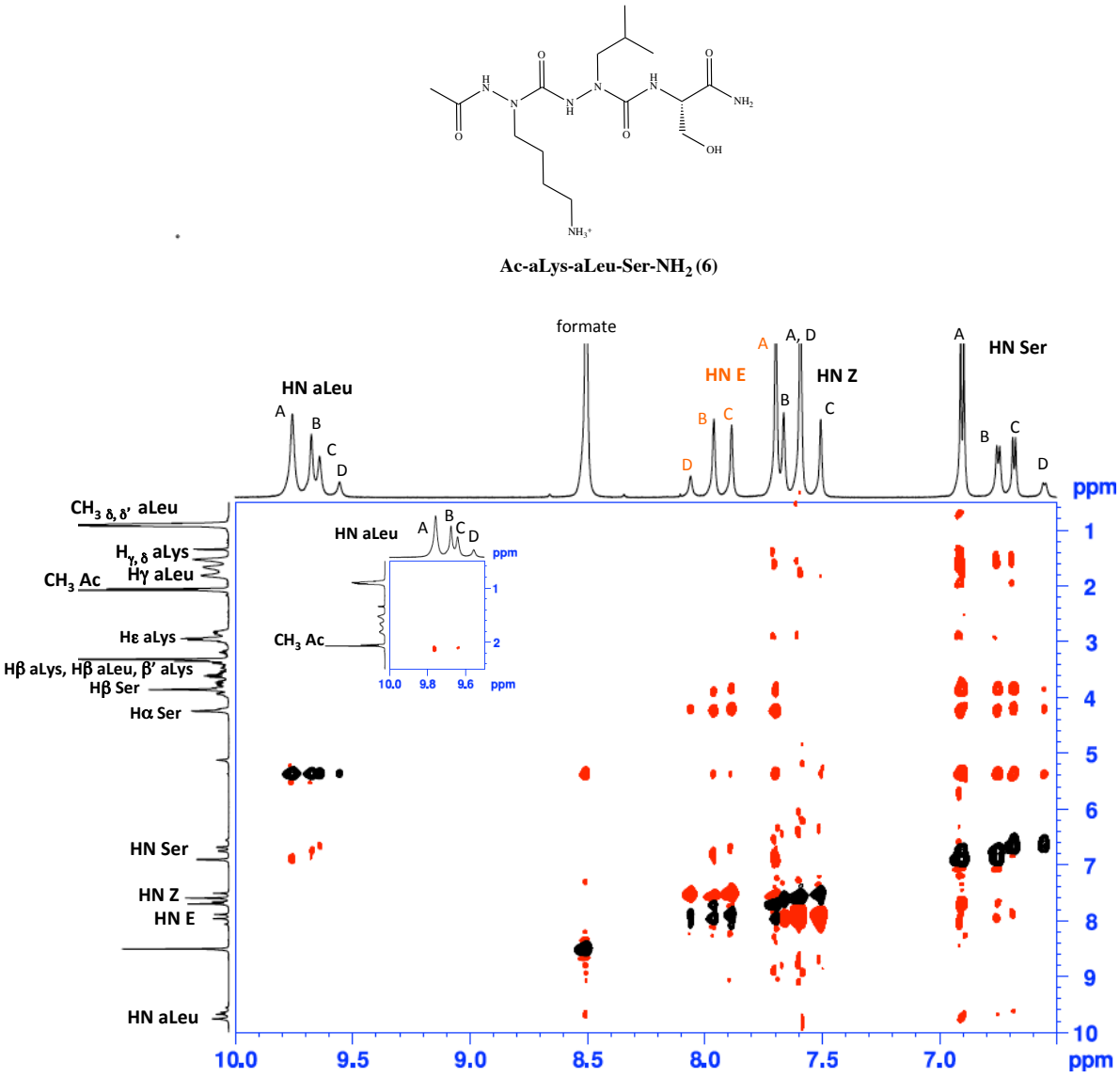
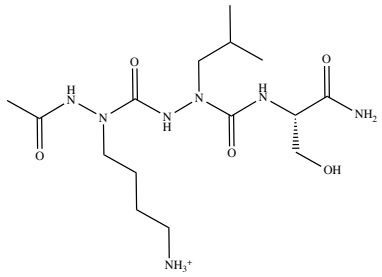


Figure S23: Expansion of the 2D ^{1}H-^{1}H ROESY spectrum (600.4 MHz, mixing time of 250 ms) of **6** in methanol (25.6 mM) at 239 K, showing the correlation peaks of NH protons. The insert shows the correlation peaks between the amide proton of aLeu and the methyl protons of the N-terminal acetyl group, observed at lower contour levels. Diagonal peaks and exchange peaks are in black while ROE cross-peaks of opposite sign are in red.



Ac-aLys-aLeu-Ser-NH$_2$ (6)

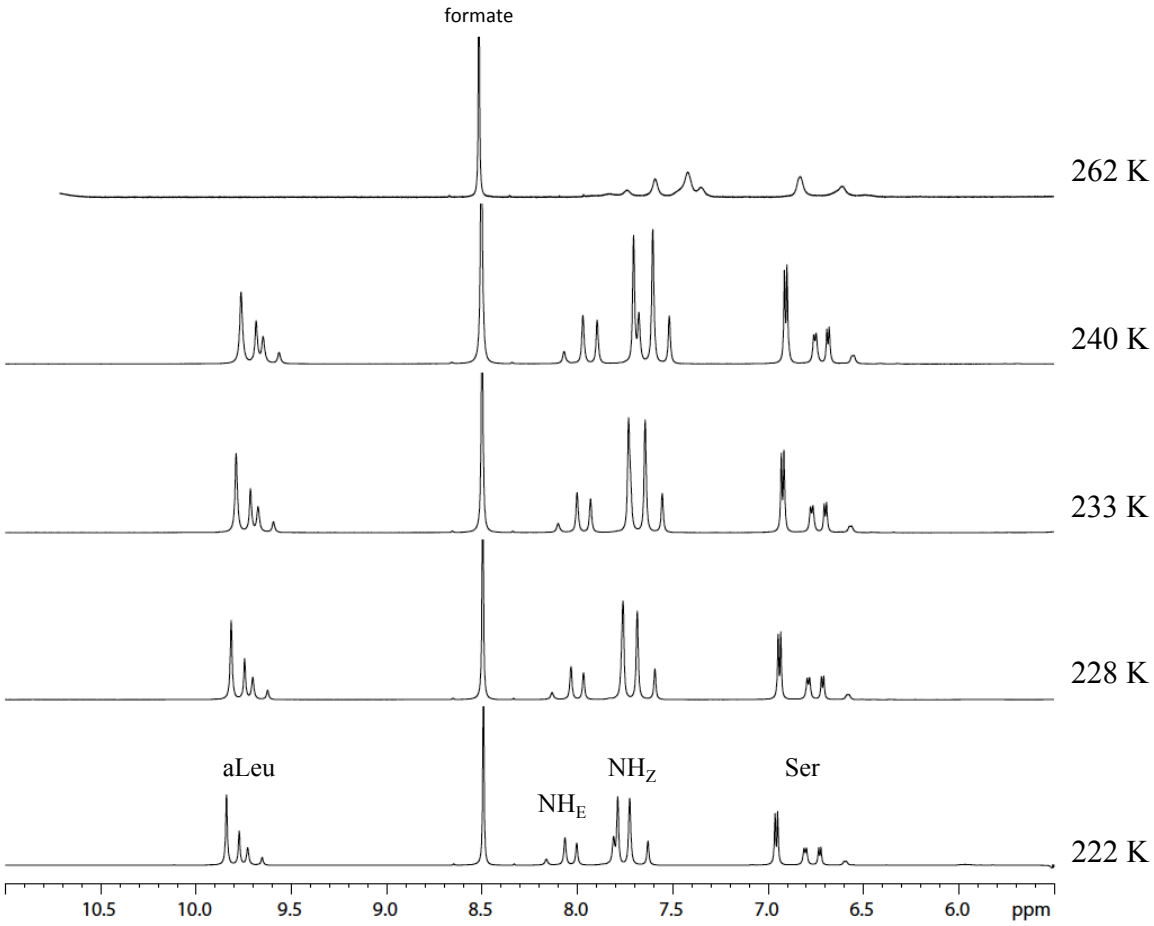
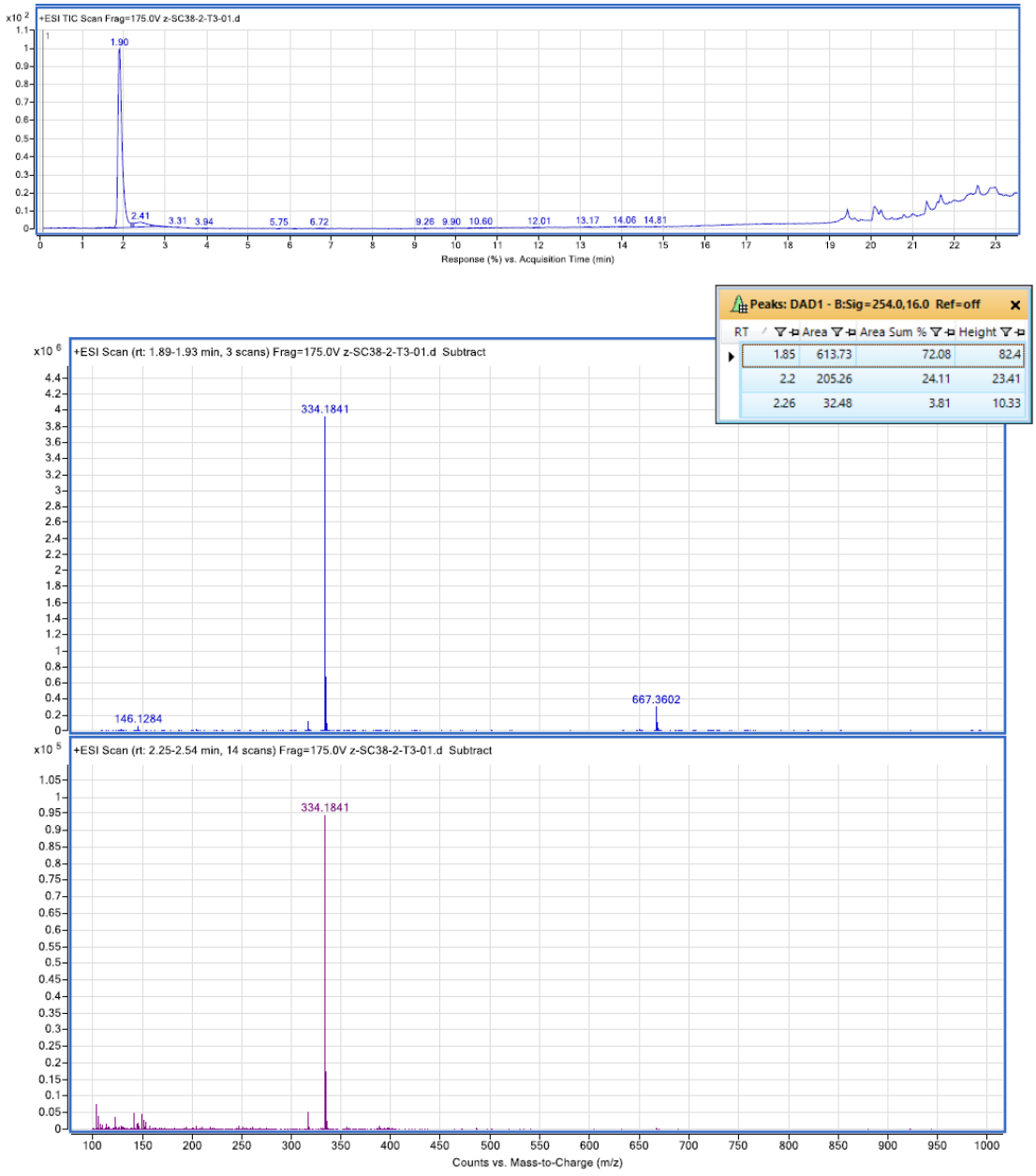


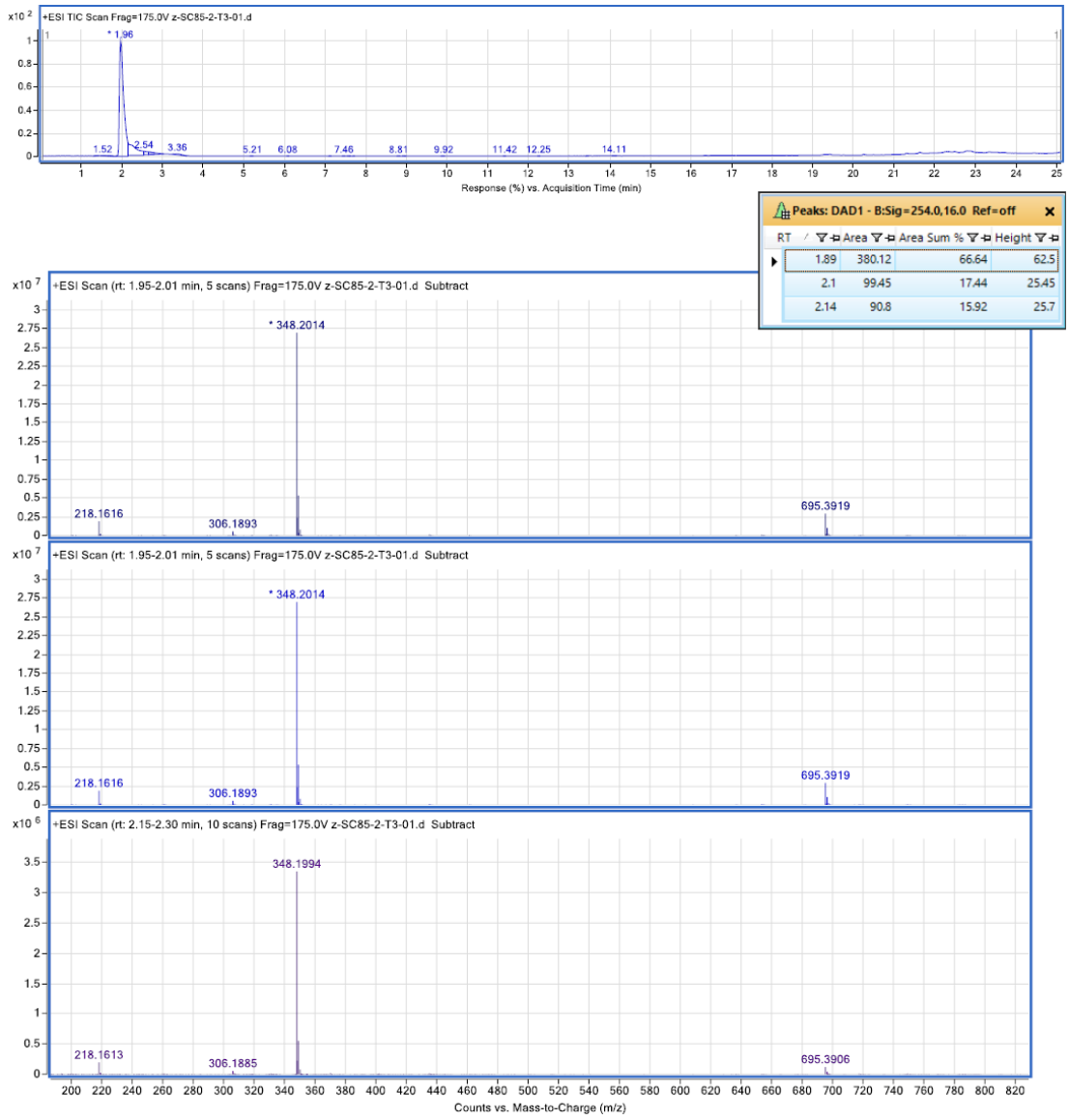
Figure S24: Variation of the amide NH proton chemical shifts of **6** in methanol (25.6 mM) in the temperature range 222 K–262 K (600.4 MHz)

HPLC-MS spectra of compounds 4-6

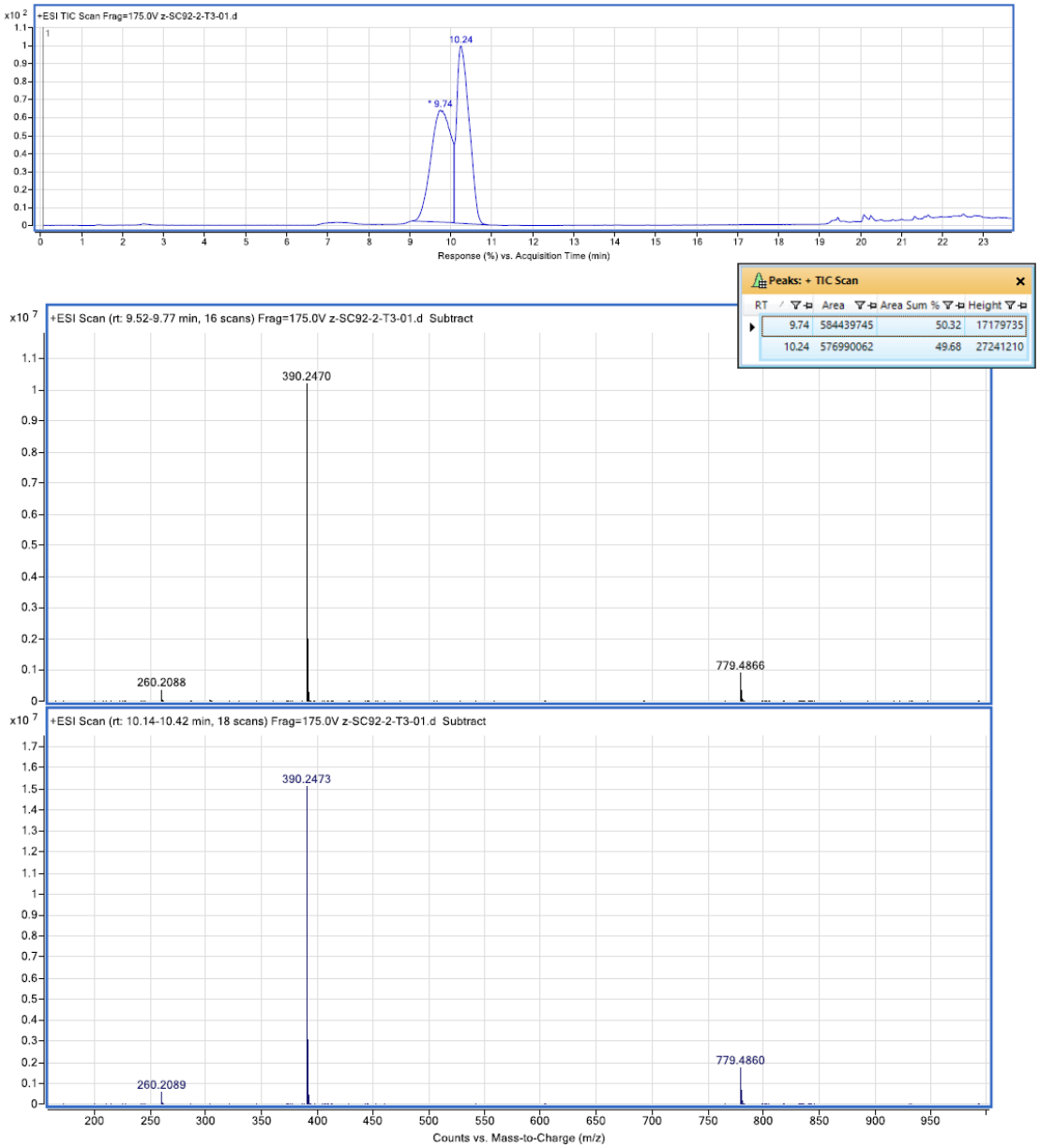
HPLC-MS spectrum of compound 4



HPLC-MS spectrum of compound 5

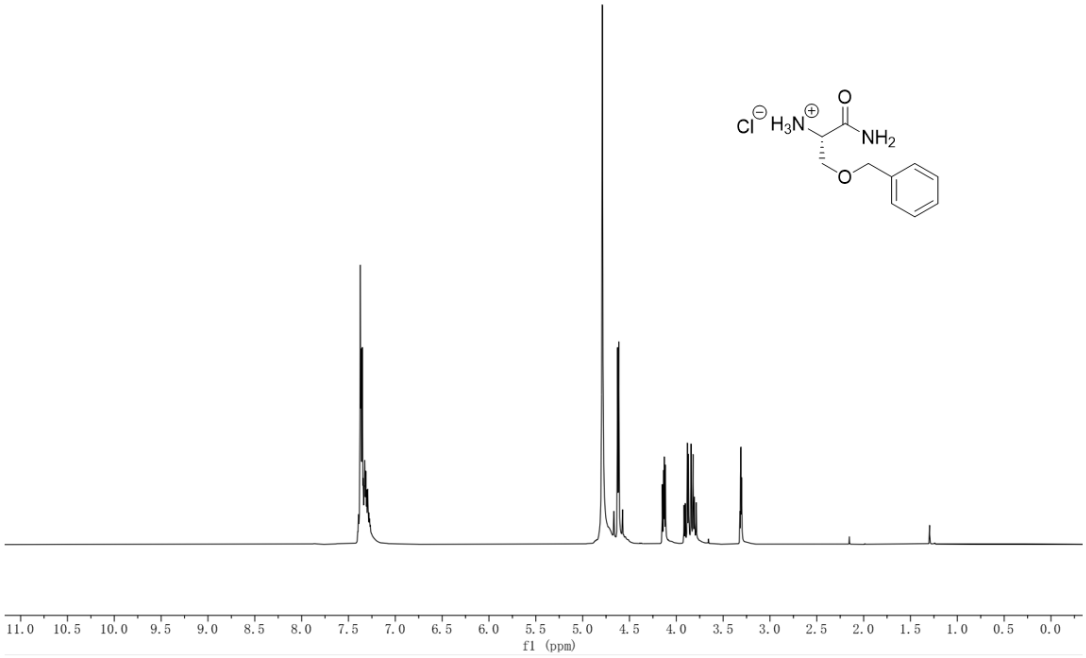


HPLC-MS spectrum of compound 6

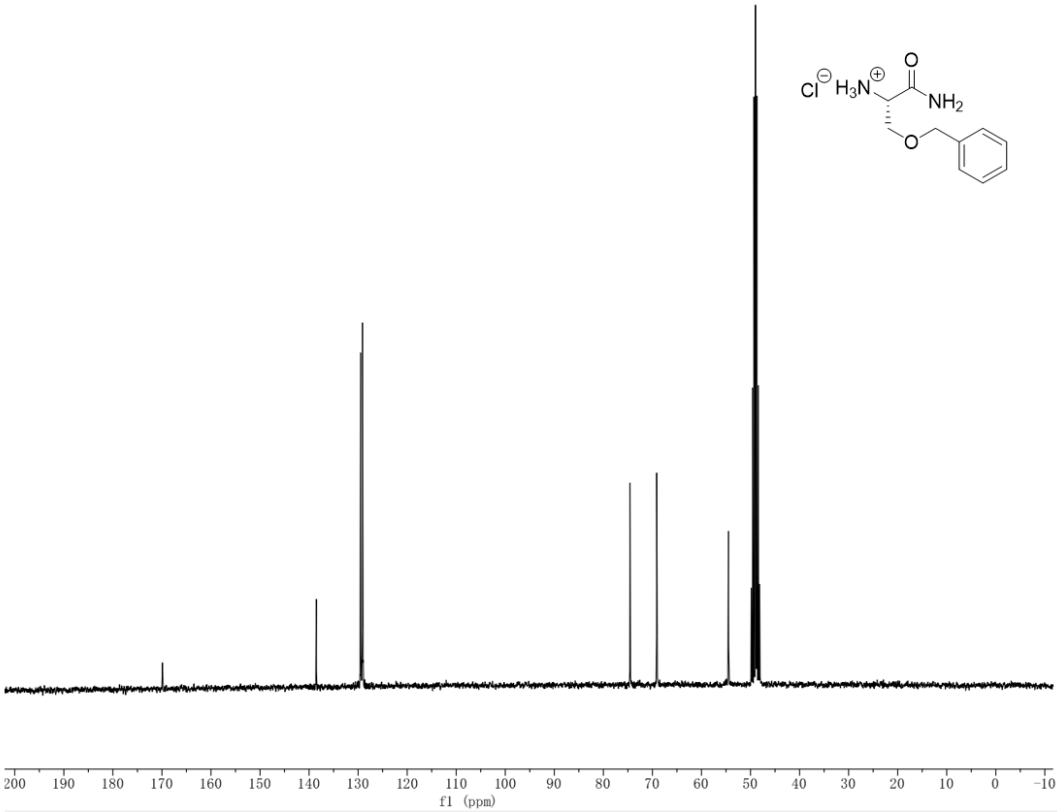


^{1}H-NMR and ^{13}C-NMR spectra of compound 21

^{1}H NMR (300 MHz, Methanol-d_4): compound 21



^{13}C NMR (75 MHz, Methanol-d_4): compound 21



Reference

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2 C. Gibson, S. L. Goodman, D. Hahn, G. Hölzemann and H. Kessler, *J. Org. Chem.*, 1999, **64**, 7388–7394.