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ELECTRONIC SUPPORTING INFORMATION

Designing hybrid foldamers: The effect on the peptide conformational bias of β- versus α- and γ-linear residues in alternation with (1*R*,2*S*)-2-aminocyclobutane-1-carboxylic acid

Sergio Celis,[a] Esther Gorrea,[a] Pau Nolis,[b] Ona Illa,[a] Rosa M. Ortuño*[a]

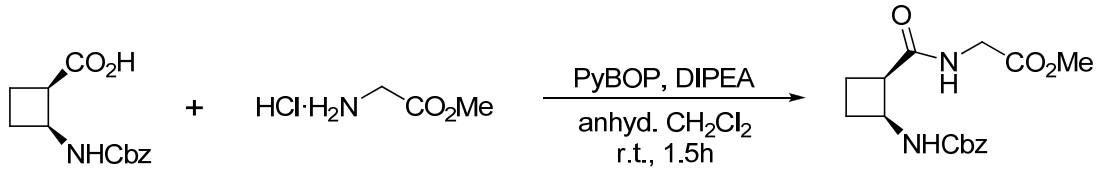
a: Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. E-mail: rosa.ortuno@uab.es; fax: (34) 935811265

b: Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

SUMMARY

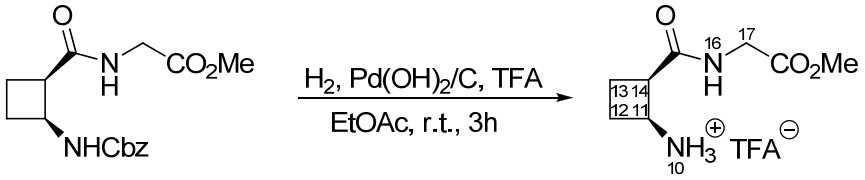
Experimental procedures

Dipeptide 5:



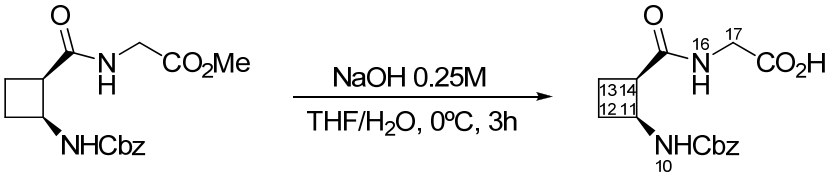
DIPEA (3.6 mL, 21 mmol) and PyBOP (2.95 g, 5.7 mmol) were added to a solution of acid **1** (1.28 g, 5.14 mmol) in anhydrous dichloromethane (20 mL). After five minutes stirring, methyl glycine hydrochloride (0.71 g, 5.7 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed *in vacuo* and the resulting solid purified by silica gel chromatography using hexane-ethyl acetate (1:5) as eluent to afford **5** (1.28 g, 78%) as a white solid. δ_H (250 MHz, $CDCl_3$): 1.94 (m, 1H), 2.08 (m, 1H), 2.19 - 2.41 (complex signal, 2H), 3.28 (m, 1H), 3.73 (s, 3H), 3.84- 4.05 (complex signal, 2H), 4.51 (quint, J = 8.5 Hz, 1H), 5.07 (broad s, 2H), 5.78 (broad s, 1H), 6.00 (broad s, 1H), 7.27 - 7.38 (broad s, 5H). Spectroscopic data are consistent with those reported in the literature.[1]

Dipeptide 8:



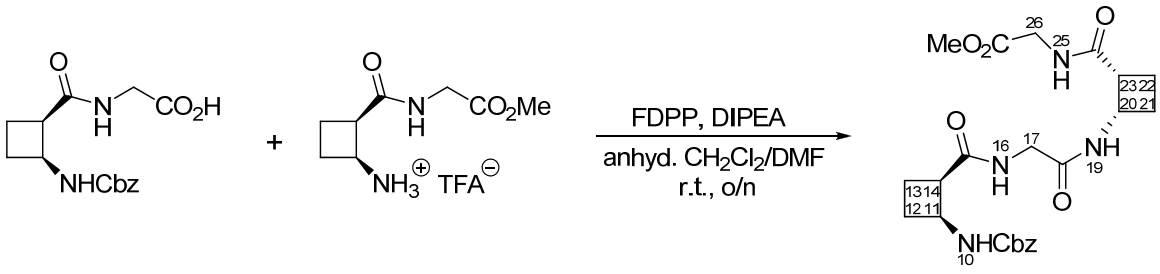
TFA (0.07 mL, 0.93 mmol) was added to a solution of dipeptide **5** (230 mg, 0.72 mmol) in EtOAc (20 mL). The mixture was hydrogenated over 10% $Pd(OH)_2/C$ (84 mg) at room temperature at 6-7 atm for 3 h. The catalyst was removed by filtration through Celite® and washed successively with ethyl acetate and methanol. The filtrate was evaporated *in vacuo* to provide **8** (216 mg, quantitative yield) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 2.15 - 2.48 (complex signal, 4H), 3.43 (m, 1H), 3.71 (s, 3H), 3.96 (broad s, 2H), 4.00 (m, 1H); δ_C (62.5 MHz, MeOH-d_4) 22.5, 27.5, 42.5, 48.2, 53.6, 173.0, 176.0.

Dipeptide 11:



To an ice-cooled solution of dipeptide **5** (120 mg, 0.37 mmol) in a 1:2 mixture of THF - water (21 mL), a 0.25 M NaOH (3.5 mL) was added. The mixture was stirred at 0 ºC for 3 h. The mixture was washed with CH_2Cl_2 (1 x 20 mL) before being acidified to pH 2 with 2M HCl. The aqueous layer was extracted with EtOAc (3 x 20 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding carboxylic acid **11** (110 mg, 96%) as a white solid. This compound was used directly in next step without further purification. Crystals, mp 124-128 ºC (from EtOAc). $[\alpha]_D$ = - 44 (*c* 0.40, EtOAc). IR (ATR): ν 3316, 2951, 1695, 1650, 1537 cm^{-1}. δ_H (250 MHz, $CDCl_3$) 1.90 (m, 1H), 2.10 (m, 1H), 2.15 - 2.35 (complex signal, 2H), 3.32 (m, 1H), 3.61 - 4.30 (complex signal, 2H), 4.49 (quint, J = 8.5 Hz, 1H), 5.02 (d, J = 12.2 Hz, 1H), 5.08 (d, J = 12.2 Hz, 1H), 5.89 (d, J = 8.5 Hz, 1H), 6.35 (broad s, 1H), 7.27 - 7.41 (broad s, 5H); δ_C (62.5 MHz, $CDCl_3$) 18.2, 29.1, 41.4, 46.3, 46.7, 66.9, 128.2, 128.3, 128.6, 136.6, 157.9, 171.4, 172.4; *m/ z* (ESI): Found, 329.1115 $[M + Na]^+$. Calcd. for $C_{15}H_{18}N_2O_5Na$: 329.1108.

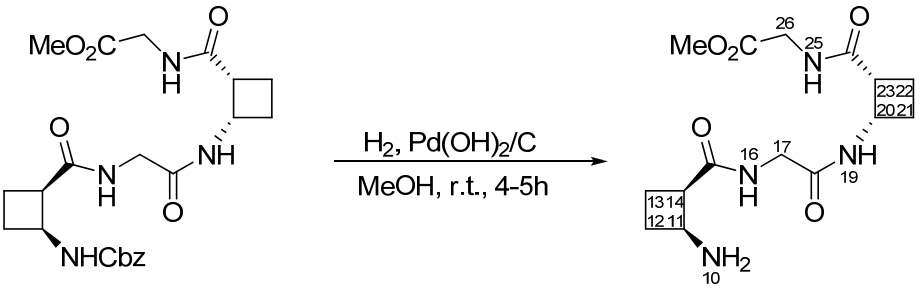
Tetrapeptide 14:



DIPEA (0.4 mL, 2.3 mmol) and FDPP (0.21 g, 0.55 mmol) were added to a solution of acid **11** (150 mg, 0.49 mmol) in a 20:1 mixture of anhydrous CH_2Cl_2 - DMF (21 mL). After five minutes stirring dipeptide **8** (147 mg, 0.49 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and DMF lyophilized. The crude was dissolved in EtOAc (20 mL) and the resulting solution was washed once with saturated aqueous $NaHCO_3$ (15 mL) and H_2O (15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum. The resulting residue

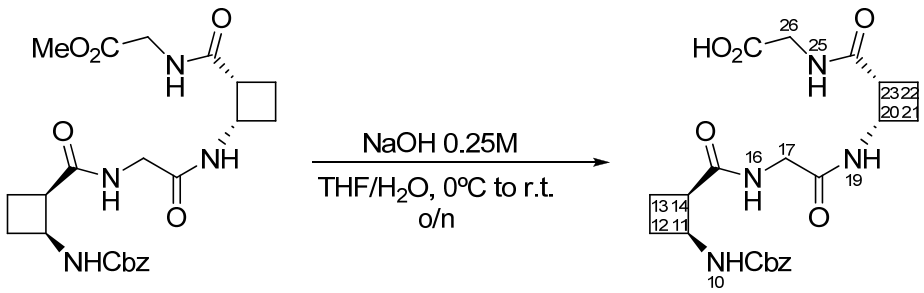
was then purified by Et_2O washes, stirring and disaggregating the solid to provide **14** (120 mg, 52%) as a white solid. Crystals, mp 175-179 ºC (from Et_2O). $[\alpha]_D$ = - 124 (*c* 0.29, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3307, 3067, 2950, 1703, 1650, 1536; δ_H (600 MHz, $CDCl_3$) 1.83 - 1.93 (complex signal, 2H), 2.03 (m, 1H), 2.09 (m, 1H), 2.12 - 2.20 (complex signal, 3H), 2.31 (m, 1H), 3.19 (m, 1H), 3.28 (m, 1H), 3.67 (dd, *J* = 17 Hz, *J* = 4.8 Hz, 1H), 3.74 (s, 3H), 3.76 (dd, *J* = 18 Hz, *J* = 5.0 Hz, 1H), 4.01 (dd, *J* = 17 Hz, *J* = 6.0 Hz, 1H), 4.20 (dd, *J* = 18 Hz, *J* = 6.2 Hz, 1H), 4.49 (quint, *J* = 8.5 Hz, 1H), 4.67 (quint, *J* = 8.5 Hz, 1H), 5.01 (d, *J* = 12 Hz, 1H), 5.06 (d, *J* = 12 Hz, 1H), 6.28 - 6.35 (complex signal, 2H), 6.50 (broad s, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 7.28 - 7.39 (complex signal, 5H); δ_C (150 MHz, $CDCl_3$) 18.1, 18.4, 28.9, 29.6, 41.4, 43.1, 44.9, 45.6, 46.0, 46.7, 52.7, 66.7, 128.1, 128.2, 128.6, 136.5, 156.0, 169.3, 171.4, 173.2, 173.5; *m/ z* (ESI): Found, 497.2007 $[M + Na]^+$. Calcd. for $C_{23}H_{30}N_4O_7Na$: 497.2007.

Tetrapeptide 17:



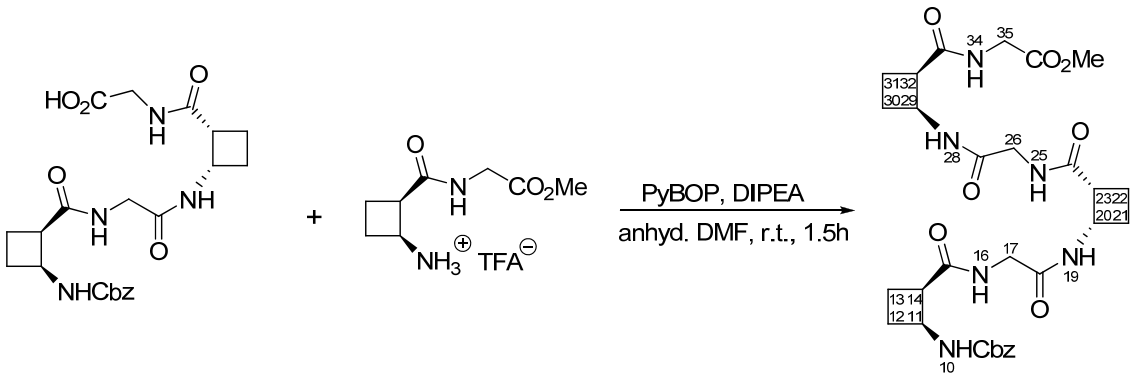
A solution of tetrapeptide **14** (130 mg, 0.27 mmol) in MeOH (20 mL) was hydrogenated over 10% $Pd(OH)_2/C$ (40 mg) at room temperature at 7-8 atm for 5 h. The catalyst was removed by filtration through Celite® and washed successively with dichloromethane and methanol. The filtrate was evaporated *in vacuo* to provide **17** (94 mg, quantitative yield) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 1.91 - 2.44 (complex signal, 8H), 3.34 - 3.53 (complex signal, 2H), 3.72 - 4.04 (complex signal, 5H), 3.75 (s, 3H), 4.47 (q, *J* = 8.5 Hz, 1H).

Tetrapeptide 20:



To an ice-cooled solution of tetrapeptide **14** (130 mg, 0.27 mmol) in a 1:1 mixture of THF - water (100 mL), 0.25 M NaOH (3 mL, 0.75 mmol) was added. The mixture was stirred from 0 ºC to room temperature overnight. The mixture was washed with CH_2Cl_2 (1 x 40 mL) before being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding carboxylic acid **20** (100 mg, 79%) as a white solid. This compound was used directly in next step without further purification. Crystals, mp 143-146 ºC (from EtOAc). $[\alpha]_D$ = - 53 (*c* 0.63, DMSO). ν_{max} (ATR)/cm^{-1} 3328, 2950, 1750, 1687, 1650, 1626, 1561, 1535. δ_H (250 MHz, DMSO-d_6) 1.75 - 2.02 (complex signal, 4H), 2.03 - 2.28 (complex signal, 4H), 3.21 - 3.34 (complex signal, 2H), 3.56 - 3.85 (complex signal, 4H), 4.35 (quint, J = 8.2 Hz, 1H), 4.57 (quint, J = 8.2 Hz, 1H), 4.99 (broad s, 2H), 7.28 (m, 1H), 7.30 - 7.37 (complex signal, 5H), 7.86 - 7.96 (complex signal, 2H), 8.00 (m, 1H). δ_C (150 MHz, DMSO-d_6) 17.8 (2C), 28.7, 28.8, 40.9, 41.9, 44.4, 44.7, 44.9, 46.6, 65.2, 127.5, 127.7, 128.4, 137.2, 155.2, 168.4, 171.9, 172.1, 172.3; *m/ z* (ESI): Found, 483.1852 $[M + Na]^+$. Calcd. for $C_{22}H_{28}N_4O_7Na$: 483.1850.

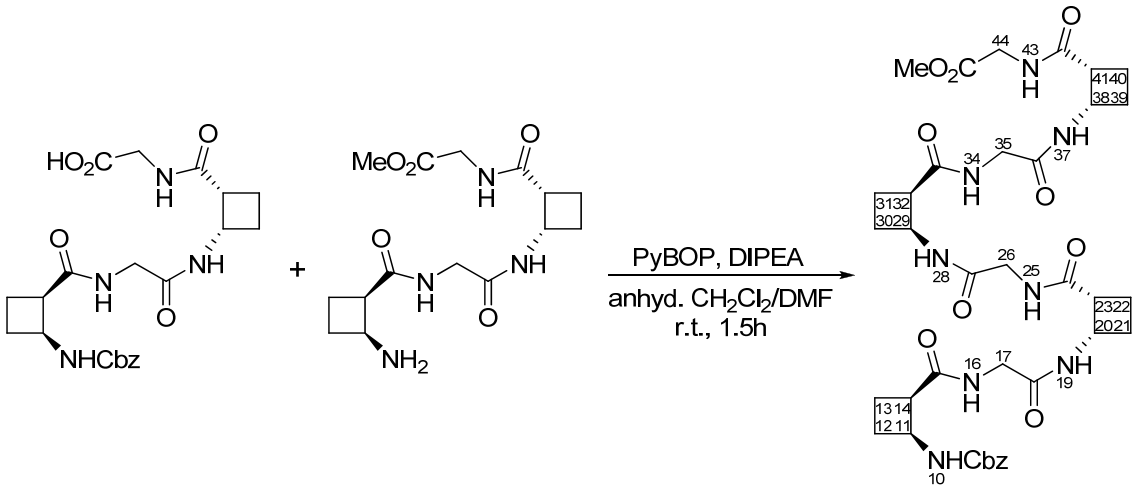
Hexapeptide 23:



DIPEA (0.36 mL, 2.1 mmol) and PyBOP (0.15 g, 0.29 mmol) were added to a solution of acid **20** (100 mg, 0.22 mmol) in anhydrous DMF (3mL). After five minutes stirring dipeptide **8** (66 mg, 0.22 mmol) was added and the mixture was stirred at room temperature for 1.5 h. DMF was

lyophilized and the resulting crude was dissolved in EtOAc (20 mL).The solution was washed once with H_2O (15 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum. The resulting residue was then purified by Et_2O washes, stirring and disaggregating the solid and by silica gel chromatography using CH_2Cl_2 - MeOH (20:1) as eluent to provide **23** (40 mg, 30%) as a pale yellow solid. Crystals, mp 163-166 ºC (from CH_2Cl_2); $[\alpha]_D$ = - 60 (*c* 0.59, DMSO). ν_{max} (ATR)/cm^{-1} 3294, 3068, 2947, 1731, 1687, 1687, 1633, 1529. δ_H (250 MHz, DMSO-d_6) 1.70 - 2.00 (complex signal, 6H), 2.03 - 2.26 (complex signal, 6H), 3.12 - 3.27 (complex signal, 3H), 3.54 - 4.06 (complex signal, 6H), 3.64 (s, 3H), 4.30 (quint, J = 8.5 Hz, 1H), 4.43 - 4.64 (complex signal, 2H), 5.00 (broad s, 2H), 7.23 (m, 1H), 7.29 - 7.41 (complex signal, 5H), 7.81 - 7.95 (complex signal, 3H), 8.04 - 8.10 (complex signal, 2H). δ_C (250 MHz, DMSO-d_6) 17.7, 17.9, 28.3, 28.6, 28.9, 40.4, 41.9, 44.3, 44.4, 44.6, 44.7, 44.8, 46.5, 51.7, 65.1, 127.4, 127.6, 128.3, 137.1, 152.4, 168.3, 168.5, 170.6, 172.0, 172.4. *m/ z* (ESI): Found, 651.2755 $[M + Na]^+$. Calcd. for $C_{30}H_{40}N_6O_9Na$: 651.2749.

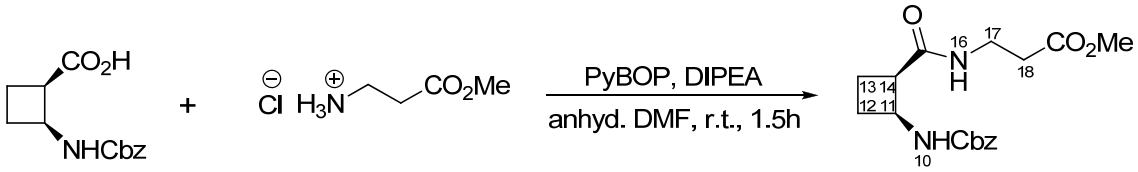
Octapeptide 25:



DIPEA (0.14 mL, 0.82 mmol) and PyBOP (0.18 g, 0.35 mmol) were added to a solution of acid **20** (130 mg, 0.29 mmol) in a 12:1 mixture of anhydrous CH_2Cl_2 - DMF (19.5 mL). After five minutes stirring amine **17** (99 mg, 0.29 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed *in vacuo* and DMF was lyophilized. The resulting crude was purified by Et_2O washes, stirring and disaggregating the to provide **25** (70 mg, 32%) as a pale yellow solid. Crystals, mp 182-185 ºC (from Et_2O). $[\alpha]_D$ = - 226 (*c* 0.25, DMSO). ν_{max} (ATR)/cm^{-1} 3294, 3066, 2947, 1726, 1688, 1635, 1533. δ_H (360 MHz, DMSO-d_6) 1.73 - 2.00 (complex signal, 8H), 2.04 - 2.22 (complex signal, 8H), 3.18 - 3.30 (complex signal, 4H), 3.56 - 4.05 (complex signal, 8H), 3.64 (s, 3H), 4.30 (m, 1H), 4.45 - 4.62 (complex signal, 3H), 4.99 (broad s, 2H), 7.24 - 7.39 (complex signal, 6H), 7.83 - 7.98 (complex signal,

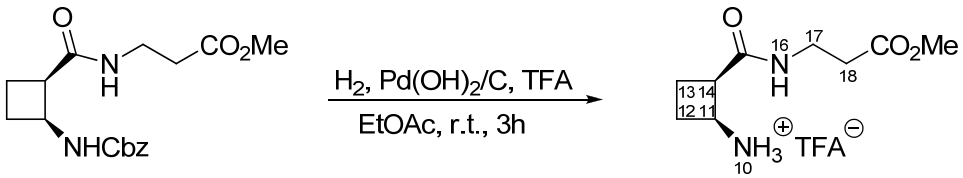
4H), 8.04 - 8.10 (complex signal, 3H). δ_C (90 MHz, DMSO-d_6) 17.7, 17.9, 28.3, 28.6, 28.8, 29.0, 40.7, 41.8, 41.9, 44.3, 44.5, 44.7, 44.7, 44.9, 46.5, 51.7, 65.1, 127.4, 127.6, 128.3, 137.2, 155.1, 168.3, 168.5, 168.6, 170.6, 172.0, 172.5, 174.4; *m/ z* (ESI): Found, 805.3480 $[M + Na]^+$. Calcd. for $C_{38}H_{51}N_7O_{11}Na$: 805.3491.

Dipeptide 6:



DIPEA (0.4 mL, 2.4 mmol) and PyBOP (0.35 g, 0.67 mmol) were added to a solution of acid **1** (140 mg, 0.42 mmol) in anhydrous DMF (5 mL). After five minutes stirring, methyl β-alanine hydrochloride **3** (80 mg, 0.55 mmol) was added and the mixture was stirred at room temperature for 1.5 h. EtOAc (50 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (4 x 50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated and the remaining DMF was lyophilized. The residue was purified by silica gel chromatography using EtOAc as eluent to afford **6** (170 mg, 90%) as a white solid. δ_H (250 MHz, $CDCl_3$) 1.83 – 2.09 (complex signal, 2H), 2.20 – 2.41 (complex signal, 2H), 2.42 – 2.58 (complex signal, 2H), 3.15 (m, 1H), 3.46 (q, J = 6.2 Hz, 2H), 3.68 (s, 3H), 4.47 (quint, J = 8.5 Hz, 1H), 5.06 (broad s, 2H), 5.78 (broad d, J = 8.5 Hz, 1H), 6.02 (broad s, 1H), 7.28 – 7.37 (broad s, 5H). Spectroscopic data are consistent with those reported in the literature.[2]

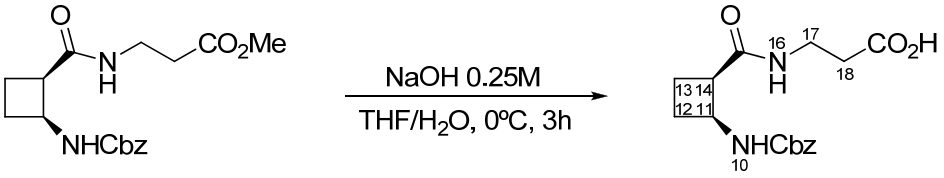
Dipeptide 9:



TFA (0.12 mL, 1.6 mmol) was added to a solution of dipeptide **6** (530 mg, 1.6 mmol) in EtOAc (20 mL). The mixture was hydrogenated over 10% $Pd(OH)_2/C$ (160 mg) at room temperature at 6-7 atm for 3 h. The catalyst was removed by filtration through Celite® and washed successively with ethyl acetate and methanol. The filtrate was evaporated *in vacuo* to provide **9** (498 mg, quantitative yield) as a yellow oil. This compound was used in next step without

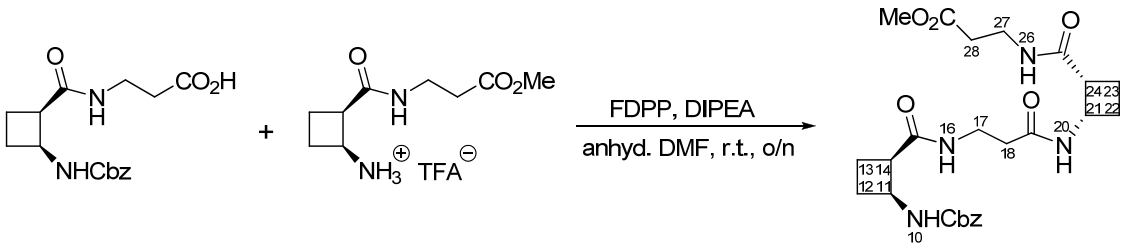
further purification. δ_H (250 MHz, MeOH-d_4) 2.12 – 2.43 (complex signal, 4H), 2.20 – 2.41 (complex signal, 2H), 2.56 (t, J = 6.6 Hz, 2H), signal for H_{14} under methanol residual peak, 3.46 (t, J = 6.6 Hz, 1H), 3.47 (t, J = 6.7 Hz, 1H), 3.69 (s, 3H), 3.96 (q, J = 8.0 Hz, 1H).

Dipeptide 12:



To an ice-cooled solution of dipeptide **6** (710 mg, 2.10 mmol) in a 1:2 mixture of THF - water (120 mL), 0.25 M NaOH (19 mL) was added. The mixture was stirred at 0 ºC for 3 h. The mixture was washed with CH_2Cl_2 (1 x 50 mL) before being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding dipeptide **12** (630 mg, 93%) as a white solid. This compound was used directly in next step without further purification. δ_H (250 MHz, $CDCl_3$) 1.86 (m, 1H), 2.09 (m, 1H), 2.19 – 2.38 (complex signal, 2H), 2.41 – 2.54 (complex signal, 2H), 3.19 – 3.42 (complex signal, 2H), 3.59 (m, 1H), 4.43 (quint, J = 8.5 Hz, 1H), 5.01 (d, J = 12.3 Hz, 1H), 5.10 (d, J = 12.3 Hz, 1H), 6.05 (broad d, J = 8.5 Hz, 1H), 6.83 (broad s, 1H), 7.24 – 7.38 (broad s, 5H). Spectroscopic data are consistent with those reported in the literature.[3]

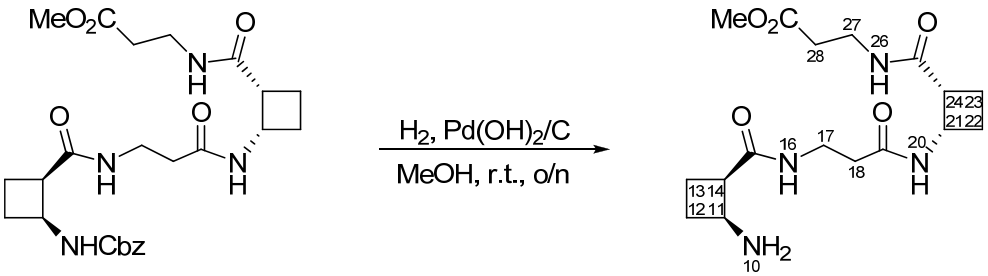
Tetrapeptide 15:



DIPEA (1.3 mL, 7.6 mmol) and FDPP (2.2 g, 5.7 mmol) were added to a solution of acid **12** (460 mg, 1.44 mmol) in anhydrous DMF (5 mL). After five minutes stirring dipeptide **9** (453 mg, 1.44 mmol) was added and the mixture was stirred at room temperature overnight. EtOAc (30 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (4 x 30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated and the remaining DMF was lyophilized. The organic layer was dried over anhydrous $MgSO_4$,

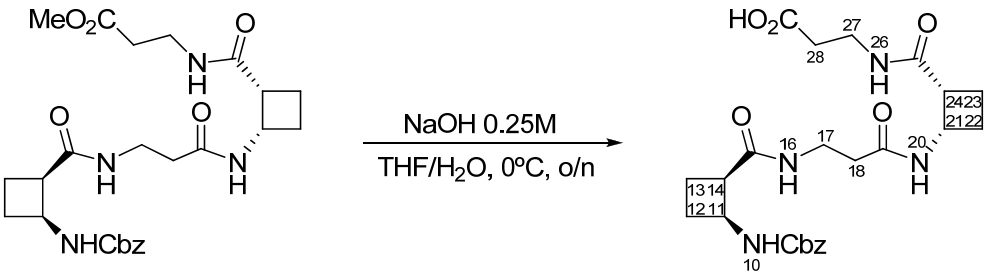
filtered and the solvent removed under vacuum. The resulting residue was purified by silica gel chromatography using CH_2Cl_2 – MeOH (15:1) as eluent to afford **15** (430 mg, 60%) as a white solid. δ_H (250 MHz, $CDCl_3$) 1.80 – 2.41 (complex signal, 10H), 2.45 – 2.54 (complex signal, 2H), 3.17 (m, 1H), 2.21 – 2.36 (complex signal, 2H), 3.40 – 3.55 (complex signal, 2H), 3.61 (m, 1H), 3.69 (s, 3H), 4.47 (m, 1H), 4.61 (m, 1H), 4.97 – 5.15 (complex signal, 2H), 6.02 (broad s, 1H,) 6.18 (broad s, 1H), 6.67 (broad s, 1H), 6.99 (broad s, 1H), 7.27 – 7.40 (broad s, 5H). Spectroscopic data are consistent with those reported in the literature.[3]

Tetrapeptide 18:



A solution of tetrapeptide **15** (200 mg, 0.40 mmol) in MeOH (40 mL) was hydrogenated over 10% $Pd(OH)_2/C$ (60 mg) at room temperature at 7-8 atm for 5 h. The catalyst was removed by filtration through Celite® and washed successively with dichloromethane and methanol. The filtrate was evaporated *in vacuo* to provide **18** (147 mg, quantitative yield) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 2.15 – 2.48 (complex signal, 4H), 3.43 (m, 1H), 3.71 (s, 3H), 3.96 (broad s, 2H), 4.00 (m, 1H); δ_C (62.5 MHz, MeOH-d_4) 22.5, 27.5, 42.5, 48.2, 53.6, 173.0, 176.0.

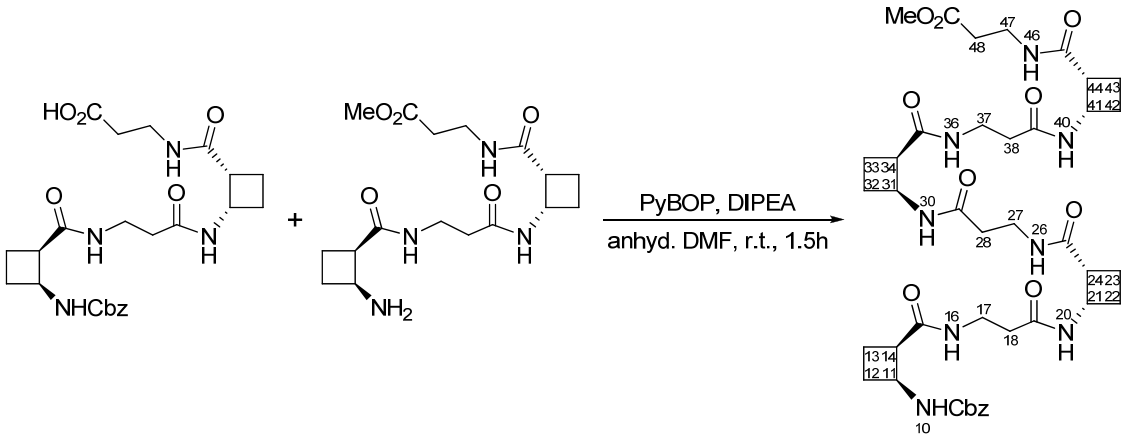
Tetrapeptide 21:



To an ice-cooled solution of tetrapeptide **15** (200 mg, 0.40 mmol) in a 1:1 mixture of THF - water (100 mL), 0.25 M NaOH (4.0 mL, 1.0 mmol) was added. The mixture was stirred from 0 ºC to room temperature overnight. The mixture was washed with CH_2Cl_2 (1 x 40 mL) before

being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding tetrapeptide **21** (180 mg, 94%) as a white solid. This compound was used directly in next step without further purification. Crystals, mp 167-170 ºC (from EtOAc). $[\alpha]_D$ = – 68 (*c* 0.29, DMSO). ν_{max} (ATR)/cm^{-1} 3310, 3030, 2950, 1693, 1648, 1522. δ_H (250 MHz, DMSO-d_6) 1.67 – 1.84 (complex signal, 2H), 1.85 – 2.01 (complex signal, 2H), 2.06 – 2.24 (complex signal, 6H), 2.28 – 2.43 (complex signal, 2H), 3.07 – 3.31 (complex signal, 6H), 4.25 (quint, *J* = 8.25 Hz, 1H), 4.46 (quint, *J* = 8.25 Hz, 1H), 5.00 (broad s, 1H), 7.18 (broad d, *J* = 8.25 Hz, 1H), 7.24 – 7.40 (complex signal, 5H), 7.54 – 7.67 (complex signal, 2H), 7.91 (broad d, *J* = 8.15 Hz, 1H). δ_C (62.5 MHz, DMSO-d_6) 17.8, 17.9, 27.3, 27.7, 34.1, 34.7, 35.3, 44.8, 44.9, 45.1, 46.6, 65.2, 127.6, 127.7, 128.3, 137.1, 155.1, 169.7, 171.6, 172.9. *m/ z* (ESI): Found, 511.2167 $[M + Na]^+$. Calcd. for $C_{24}H_{32}N_4O_7Na$: 511.2163.

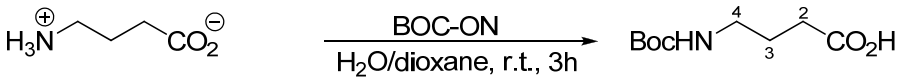
Octapeptide 26:



DIPEA (0.90 mL, 5.3 mmol) and PyBOP (0.40 g, 0.77 mmol) were added to a solution of acid **21** (180 mg, 0.37 mmol) in anhydrous DMF (5 mL). After five minutes stirring amine **18** (167 mg, 0.37 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed *in vacuo* and DMF was lyophilized. The resulting crude was purified by Et_2O washes, stirring and disaggregating the solid and by silica gel chromatography using CH_2Cl_2 – MeOH (15:1) as eluent to afford octapeptide **26** (190 mg, 27%) as a white solid. Crystals, mp 242-247 ºC (from CH_2Cl_2); $[\alpha]_D$ = – 73 (*c* 0.22, DMSO). ν_{max} (ATR)/cm^{-1} 3298, 3067, 2949, 1647, 1533. δ_H (360 MHz, DMSO-d_6) 1.67 – 2.25 (complex signal, 22H), 2.35 – 2.46 (complex signal, 2H), 3.07 – 3.26 (complex signal, 12H), 3.58 (s, 3H), 4.26 (quint, *J* = 8.5 Hz, 1H), 4.38 – 4.54 (complex signal, 3H), 5.00 (complex signal, 2H), 7.21 (m, 1H), 7.26 – 7.37

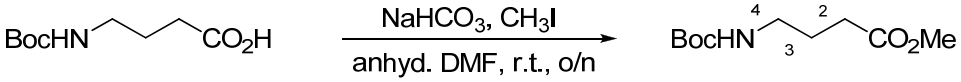
(complex signal, 5H), 7.53 – 7.77 (complex signal, 4H), 7.87 – 8.01 (complex signal, 3H). δ_C (90 MHz, DMSO-d_6) 17.7, 17.9, 27.3, 27.8, 28.6, 29.0, 33.8, 34.6, 35.2, 35.4, 44.8, 45.3, 46.6, 51.4, 65.2, 127.6, 127.7, 128.3, 137.1, 155.1, 169.7, 171.5, 171.6, 171.8. *m/ z* (ESI): Found, 861.4123 $[M + Na]^+$. Calcd. for $C_{42}H_{59}N_7O_{11}Na$: 861.4117.

4-*tert*-butoxycarbonylaminobutyric acid:



Et_3N (4.04 mL, 29.1 mmol) and BOC-ON (5.24 g, 21.3 mmol) were added to a solution of GABA (2.00 g, 19.4 mmol) in a 1:1 mixture of dioxane – H_2O (80 mL). The mixture was stirred at room temperature for 3 hours. H_2O (100 mL) was added and the resulting solution was washed with EtOAc (2 x 60 mL) before being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x 60 mL) and the organic layer was washed with 5% citric acid solution (2 x 50 mL), saturated aqueous NaCl (2 x 50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding carboxylic acid (3.39 g, 86%) as a white solid. This compound was used directly in next step without further purification. δ_H (250 MHz, $CDCl_3$) 1.44 (s, 9H), 1.82 (quint., J = 7.0 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 3.18 (complex signal, 2H), 4.70 (broad s, 1H). Spectroscopic data are consistent with those reported in the literature.[4]

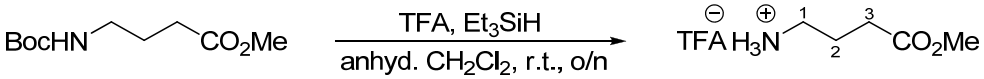
Methyl 4-(*tert*-butoxycarbonylamino)butanoate:



$NaHCO_3$ (2.80 g, 33.4 mmol) and CH_3I (5.20 mL, 83.5 mmol) were added to a solution of 4-*tert*-butoxycarbonylaminobutyric acid (3.39 g, 16.8 mmol) in anhydrous DMF (30 mL). The mixture was stirred at room temperature overnight. CH_3I excess was removed with a nitrogen flow. H_2O (10 mL) was added and the resulting solution extracted with EtOAc (2 x 50 mL), was washed with saturated aqueous NaCl (1 x 40 mL) and with H_2O (1 x 40 mL). The resulting organic layer was then dried over anhydrous $MgSO_4$, filtered and solvents removed under vacuum. The solid was purified by silica gel chromatography using EtOAc – hexane (1:3) to afford Methyl 4-(*tert*-butoxycarbonylamino)butanoate (2.58 g, 71%) as a white solid. δ_H (250 MHz, $CDCl_3$) 1.43 (s, 9H), 1.81 (quint., J = 7.0 Hz, 2H), 2.36 (t, J = 7.3 Hz, 2H), 3.16 (q, J =

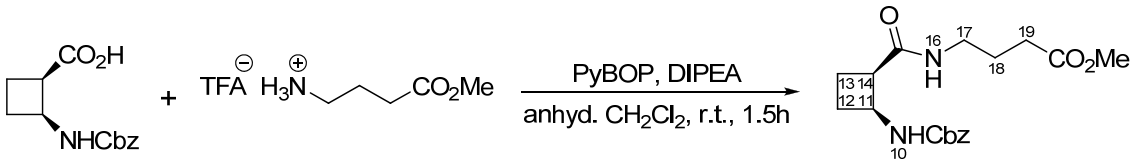
6.7 Hz, 2H), 3.67 (s, 3H), 4.61 (broad s, 1H). Spectroscopic data are consistent with those reported in the literature.[4]

4-Methyl-4-oxobutan-1-aminium trifluoroacetate, 4:



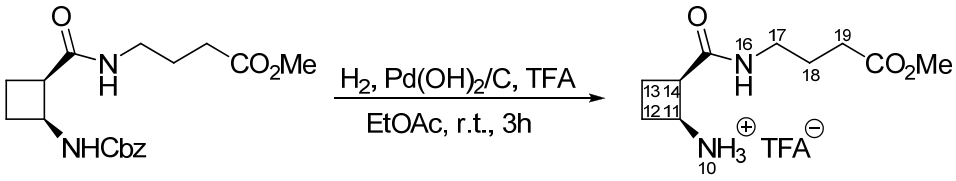
Et_3SiH (2.1 mL, 13.1 mmol) and TFA (5.1 mL, 66.2 mmol) were added to a solution of methyl 4-(*tert*-butoxycarbonylamino)butanoate (1.1 g, 5.1 mmol) in anhydrous CH_2Cl_2 (20 mL). The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* to provide salt **4** (1.17 g, quantitative yield) as a colourless oil. This compound was used in next step without further purification. δ_H (250 MHz, $CDCl_3$) 1.93 – 2.08 (complex signal, 2H), 2.59 (t, J = 6.3 Hz, 2H), 3.11 – 3.26 (complex signal, 2H), 3.73 (s, 3H). Spectroscopic data are consistent with those reported in the literature.[4]

Dipeptide 7:



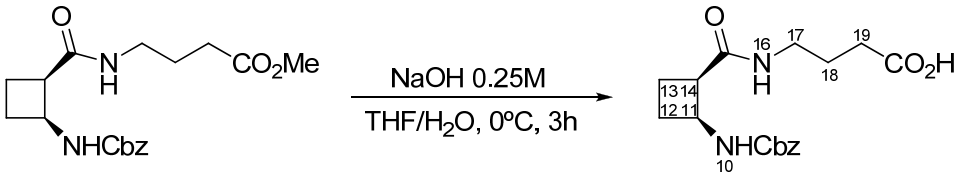
DIPEA (7.0 mL, 41 mmol) and PyBOP (3.5 g, 6.8 mmol) were added to a solution of acid **1** (1.61 g, 6.5 mmol) in anhydrous CH_2Cl_2 (20 mL). After five minutes stirring, **4** (1.50 g, 6.5 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed *in vacuo*, EtOAc (50 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (1 x 20 mL), saturated aqueous NaCl solution (1 x 20 mL) and H_2O (1 x 20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated. The residue was purified by silica gel chromatography using hexane – EtOAc (1:5) as eluent to afford **7** (1.80 g, 80%) as a white solid. Crystals, mp 82 – 86 ºC (from EtOAc). $[\alpha]_D$ = –174 (*c* 0.38, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3325, 2951, 1733, 1689, 1645, 1526. δ_H (400 MHz, $CDCl_3$) 1.70 – 1.8 (complex signal, 2H), 1.91 (m, 1H), 2.05 (m, 1H), 2.22 – 2.38 (complex signal, 4H), 3.08 – 3.29 (complex signal, 3H), 3.65 (s, 3H), 4.46 (quint, J = 8.5 Hz, 1H), 4.98 – 5.12 (complex signal, 2H), 5.64 – 5.79 (broad s, 2H), 7.27 – 7.41 (broad s, 5H). δ_C (100 MHz, $CDCl_3$) 18.7, 24.9, 29.6, 31.6, 39.0, 46.6, 46.7, 51.9, 66.8, 128.2, 128.3, 128.7, 136.7, 155.9, 173.2, 173.9. *m/ z* (ESI): Found, 371.1579 $[M + Na]^+$. Calcd. for $C_{18}H_{24}N_2O_5Na$: 371.1577.

Dipeptide 10:



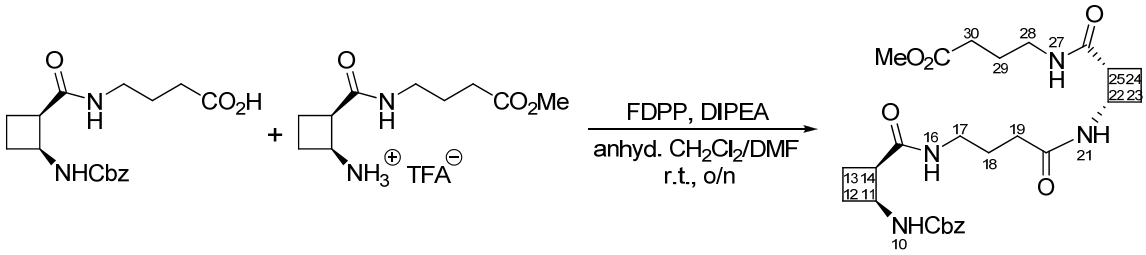
TFA (0.18 mL, 2.3 mmol) was added to a solution of dipeptide **7** (640 mg, 1.8 mmol) in EtOAc (20 mL). The mixture was hydrogenated over 10% $Pd(OH)_2/C$ (200 mg) at room temperature at 6-7 atm for 3 h. The catalyst was removed by filtration through Celite® and washed successively with ethyl acetate and methanol. The filtrate was evaporated *in vacuo* to provide **10** (603 mg, quantitative yield) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 1.81 (quint., J = 7.3 Hz, 2H), 2.13 (m, 1H), 2.22 – 2.47 (complex signal, 2H), 3.23 (t, J = 6.8 Hz, 1H), 3.24 (t, J = 6.9 Hz, 1H), H_{14} under methanol residual peak, 3.67 (s, 3H), 3.98 (m, 1H).

Dipeptide 13:



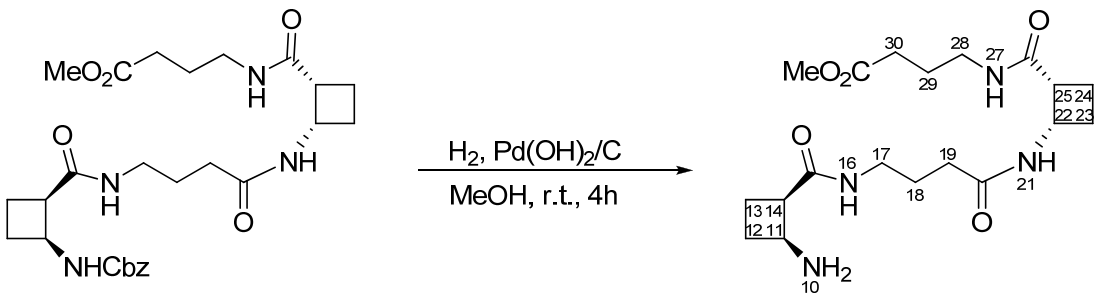
To an ice-cooled solution of dipeptide **7** (640 mg, 1.9 mmol) in a 1:2 mixture of THF – water (105 mL), 0.25 M NaOH (20 mL) was added. The mixture was stirred at 0 ºC for 3 h. The mixture was washed with CH_2Cl_2 (1 x 40 mL) before being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x 60 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding carboxylic acid **13** (560 mg, 91%) as a white solid. This compound was used directly in next step without further purification. Crystals, mp 138-141 ºC (from EtOAc). $[\alpha]_D$ = –31 (*c* 0.31, MeOH). ν_{max} (ATR)/cm^{-1} 3306, 2947, 1684, 1639, 1526. δ_H (360 MHz, $CDCl_3$) 1.61 – 1.79 (complex signal, 2H), 1.87 (m, 1H), 2.07 (m, 1H), 2.23 – 2.35 (complex signal, 4H), 3.12 – 3.28 (complex signal, 3H), 4.43 (quint, J = 8.5 Hz, 1H), 5.01 (d, J = 12.3 Hz, 1H), 5.06 (d, J = 12.3 Hz, 1H), 6.08 (broad d, J = 8.5 Hz, 1H), 6.36 (broad s, 1H), 7.24 7.36 (broad s, 5H). δ_C (90 MHz, $CDCl_3$) 18.2, 24.8, 28.9, 31.4, 38.9, 46.6, 47.0, 66.9, 128.2, 128.3, 128.7, 136.5, 156.3, 173.6, 177.9. *m/ z* (ESI): Found, 357.1419 $[M + Na]^+$. Calcd. for $C_{17}H_{22}N_2O_5Na$: 357.1421.

Tetrapeptide 16:



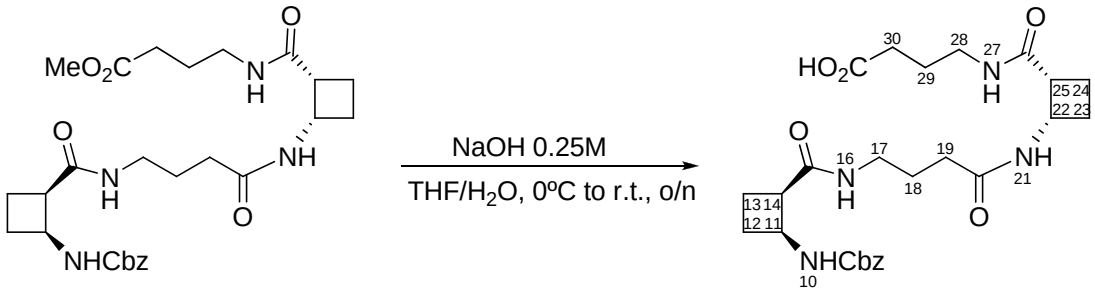
DIPEA (1.85 mL, 10.8 mmol) and FDPP (1.0 g, 2.6 mmol) were added to a solution of acid **13** (710 mg, 2.1 mmol) in anhydrous CH_2Cl_2 (15 mL). After five minutes stirring dipeptide **10** (494 mg, 2.1 mmol) was added and the mixture was stirred at room temperature overnight. EtOAc (20 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (1 x 20 mL), saturated aqueous NaCl (1 x 20 mL) and H_2O (1 x 20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated. The resulting residue was purified by Et_2O and pentane washes, stirring and disaggregating the solid to afford **16** (680 mg) as a white solid. Crystals, mp 146 – 150 ºC (from Et_2O). $[\alpha]_D$ = –84 (*c* 1.35, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3308, 3067, 2948, 1735, 1688, 1639, 1527. δ_H (600 MHz, $CDCl_3$) 1.62 – 1.81 (complex absoprtion, 4H), 1.85 – 1.96 (complex absoprtion, 2H), 2.01 (m, 1H), 2.05 – 2.16 (complex absoprtion, 3H), 2.23 – 2.39 (complex absoprtion, 6H), 3.04 – 3.29 (complex signal, 6H), 3.64 (s, 3H), 4.44 (quint, *J* = 8.4 Hz, 1H), 4.62 (quint, *J* = 8.4 Hz, 1H), 4.98 (d, *J* = 12.2 Hz, 1H), 5.05 (d, *J* = 12.2 Hz, 1H), 6.14 (broad s, 1H), 6.23 (d, *J* = 8.4 Hz, 1H), 6.53 (broad s, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.26 – 7.36 (complex absoprtion, 5H). δ_C (150 MHz, $CDCl_3$) 18.5, 19.1, 24.7, 25.2, 28.6, 29.1, 31.3, 33.5, 38.6, 38.8, 44.9, 45.9, 46.3, 46.6, 52.0, 66.6, 128.1, 128.2, 128.6, 136.4, 155.9, 172.5, 173.4, 174. *m/ z* (ESI): Found, 553.2629 $[M + Na]^+$. Calcd. for $C_{27}H_{38}N_4O_7Na$: 553.2633.

Tetrapeptide 19:



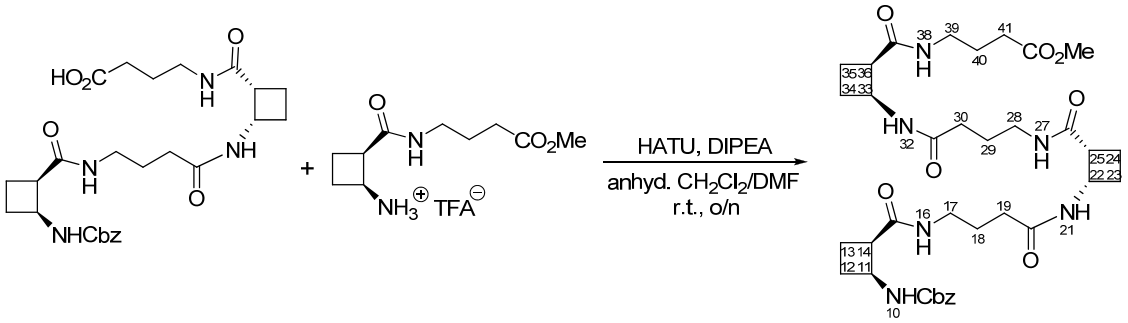
A solution of tetrapeptide **16** (260 mg, 0.49 mmol) in MeOH (25 mL) was hydrogenated over 10% $Pd(OH)_2/C$ (80 mg) at room temperature at 7-8 atm for 4 h. The catalyst was removed by filtration through Celite® and washed successively with dichloromethane and methanol. The filtrate was evaporated *in vacuo* to provide **19** (190 mg, 98%) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 1.67 – 2.39 (complex signal, 16H), 3.10 – 3.27 (complex signal, 6H), 3.66 (s, 3H), 3.71 (m, 1H), 4.57 (q, J = 8.5 Hz, 1H).

Tetrapeptide 22:



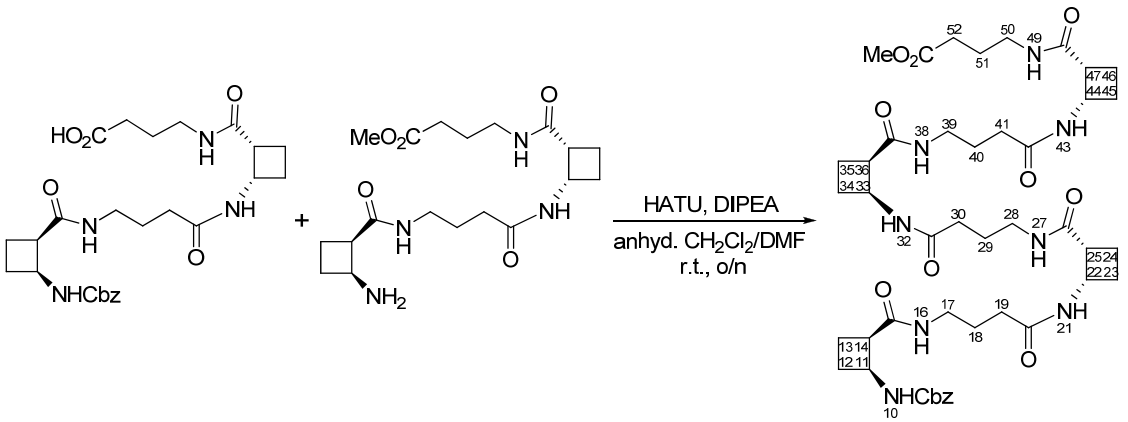
To an ice-cooled solution of tetrapeptide **16** (450 mg, 0.85 mmol) in a 1:1 mixture of THF - water (200mL), 0.25 M NaOH (11.0 mL, 2.75 mmol) was added. The mixture was stirred from 0º C to room temperature overnight. The mixture was washed with CH_2Cl_2 (1 x 100 mL) before being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 x 30 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding carboxylic acid **22** (450 mg, quantitative yield) as a white solid. This compound was used directly in next step without further purification. Crystals, mp 133-137 ºC (EtOAc). $[\alpha]_D$ = –45 (*c* 0.31, DMSO). ν_{max} (ATR)/cm^{-1} 3307, 3065, 2946, 1688, 1639, 1544, 1527. δ_H (250 MHz, DMSO-d_6) 1.47 – 1.64 (complex signal, 4H), 1.71 – 2.05 (complex signal, 6H), 2.08 – 2.24 (complex signal, 6H), 2.92 – 3.08 (complex signal, 4H), 3.10 – 3.19 (complex signal, 2H), 4.25 (quint, J = 8.5 Hz, 1H), 4.47 (quint, J = 8.5 Hz, 1H), 4.97 (broad s, 1H), 7.16 (broad d, J = 8.5 Hz, 1H), 7.26 – 7.38 (complex signal, 5H), 7.57 – 7.66 (complex signal, 2H), 7.82 (broad d, J = 8.5 Hz, 1H). δ_C (62.5 MHz, DMSO-d_6) 17.8, 17.9, 24.7, 25.5, 27.5, 27.7, 31.1, 32.6, 44.7, 45.1, 45.4, 46.6, 65.2, 127.6, 127.7, 128.3, 137.2, 155.1, 171.2, 171.6, 171.7, 174.3. *m/ z* (ESI): Found, 539.2479 $[M + Na]^+$. Calcd. for $C_{26}H_{16}N_4O_7Na$: 539.2476.

Hexapeptide 24:



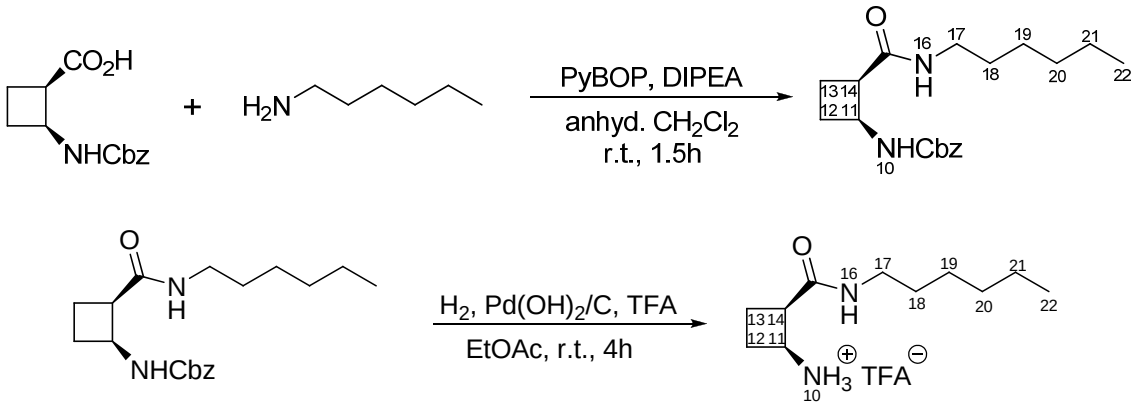
DIPEA (0.40 mL, 2.3 mmol) and HATU (135 mg, 0.36 mmol) were added to a solution of acid **22** (152 mg, 0.29 mmol) in a 25:1 mixture of anhydrous CH_2Cl_2 –DMF (26 mL). After five minutes stirring dipeptide **10** (95 mg, 0.29 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the DMF was lyophilized. The resulting residue was then purified by H_2O, Et_2O and pentane washes, stirring and disaggregating the solid to provide **24** (75 mg, 36%) as a pale yellow solid. Crystals, mp 172-175 ºC (from Et_2O). $[\alpha]_D$ = –106 (*c* 0.42, DMSO). ν_{max} (ATR)/cm^{-1} 3304, 3068, 2946, 1735, 1688, 1641, 1530. δ_H (250 MHz, DMSO-d_6) 1.45 – 1.66 (complex signal, 6H), 1.72 – 2.08 (complex signal, 10H), 2.08 – 2.32 (complex signal, 8H), 2.91 – 3.07 (complex signal, 6H), 3.07 – 3.21 (complex signal, 3H), 3.57 (s, 3H), 4.26 (quint, *J* = 8.4 Hz, 1H), 4.37 – 4.55 (complex signal, 2H), 4.98 (broad s, 2H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.25 – 7.39 (complex signal, 5H), 7.53 – 7.65 (complex signal, 3H), 7.74 – 7.86 (complex signal, 2H). δ_C (250 MHz, DMSO-d_6) 17.8, 17.9, 24.6, 25.5, 27.4, 27.5, 27.7, 30.7, 32.6, 37.7, 38.1, 44.6, 45.0, 45.3, 46.6, 51.2, 65.1, 127.5, 127.7, 128.3, 137.1, 155.0, 171.1, 171.2, 171.5, 173.1. *m/ z* (ESI): Found, 735.3689 $[M + Na]^+$. Calcd. for $C_{36}H_{52}N_6O_9Na$: 735.3688.

Octapeptide 27:



DIPEA (0.26 mL, 1.5 mmol) and HATU (0.28 g, 0.75 mmol) were added to a solution of acid **22** (260 mg, 0.50 mmol) in a 20:1 mixture of anhydrous CH_2Cl_2 –DMF (21 mL). After fifteen minutes stirring amine **19** (198 mg, 0.50 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the DMF was lyophilized. The resulting residue was then purified by H_2O, Et_2O, pentane and CH_2Cl_2 washes, stirring and disaggregating the solid to provide **27** (280 mg, 62%) as a pale yellow solid. Crystals, mp 230-234 ºC (from Et_2O). $[\alpha]_D$ = –154 (*c* 0.34, DMSO). ν_{max} (ATR)/cm^{-1} 3230, 3063, 2945, 1735, 1688, 1639, 1528. δ_H (250 MHz, DMSO-d_6) 1.46 – 1.66 (complex signal, 8H), 1.71 – 2.23 (complex signal, 22H), 2.23 – 2.32 (complex signal, 2H), 2.89 – 3.07 (complex signal, 8H), 3.08 – 3.21 (complex signal, 4H), 3.57 (s, 3H), 4.20 (quint, *J* = 8.3 Hz, 1H), 4.40 – 4.52 (complex signal, 3H), 4.98 (s, 2H), 7.16 (broad d, *J* = 8.2 Hz, 1H), 7.26 – 7.38 (complex signal, 5H), 7.52 – 7.67 (complex signal, 4H), 7.77 – 7.89 (complex signal, 3H). δ_C (62.5 MHz, DMSO-d_6) 17.9 (4C), 24.6, 25.5, 25.6, 25.7, 27.4, 27.5, 27.7, 30.7, 32.6 (3C), 37.7, 38.1, 44.6, 44.7, 45.1, 45.3, 45.4, 46.6, 51.2, 65.1, 127.5, 127.7, 128.3, 137.2, 155.0, 171.2, 171.5, 171.6, 173.1. *m/ z* (ESI): Found, 917.4753 $[M + Na]^+$. Calcd. for $C_{46}H_{67}N_7O_{11}Na$: 917.4743.

Compound 28:

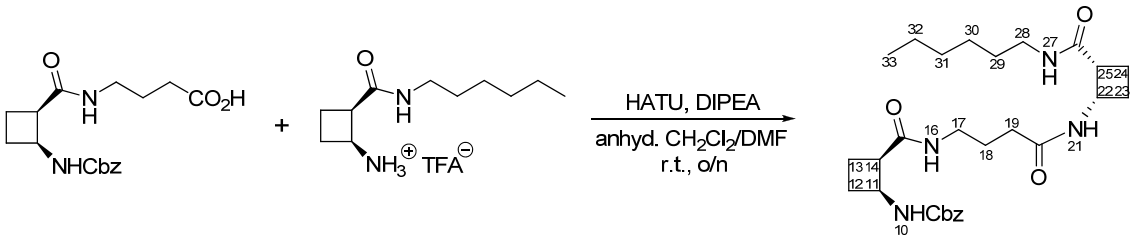


DIPEA (0.45 mL, 2.6 mmol) and PyBOP (0.55 g, 1.1 mmol) were added to a solution of acid **1** (0.22 g, 0.89 mmol) in anhydrous CH_2Cl_2 (20 mL). After five minutes stirring, hexylamine (0.13 mL, 0.98 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed *in vacuo*, EtOAc (50 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (1 x 20 mL), saturated aqueous NaCl solution (2 x 20 mL) and H_2O (1 x 20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated. The residue was purified by silica gel chromatography using hexane – EtOAc (1:1) as eluent to afford the Cbz protected compound (220 mg, 76%) as a white solid. Crystals, mp 83-86 ºC (from hexane – EtOAc). $[\alpha]_D$ = –43 (*c* 0.51, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3316, 2927,

2856, 1688, 1642, 1545, 1524. δ_H (250 MHz, $CDCl_3$) 1.01 (broad s, 3H), 1.17 (complex signal, 6H), 1.90 – 2.05 (complex signal, 2H), 2.23 – 2.38 (complex signal, 2H), 3.08 – 3.22 (complex signal, 3H), 4.45 (complex signal, 1H), 4.89 – 5.12 (complex signal, 2H), 5.55 (broad s, 1H), 5.75 (broad s, 1H). δ_C (62.5 MHz, $CDCl_3$). 13.9, 18.3, 22.5, 26.5, 29.0, 29.6, 31.4, 39.4, 46.1, 46.5, 66.4, 76.6, 77.2, 77.7, 127.8, 127.9, 128.3, 136.4, 155.7, 172.7. *m/ z* (ESI): Found, 355.1989 $[M + Na]^+$. Calcd. for $C_{19}H_{28}N_2O_5Na$: 355.1992.

TFA (0.05 mL, 0.6 mmol) was added to a solution of Cbz protected compound (190 mg, 0.57 mmol) in EtOAc (20 mL). The mixture was hydrogenated over 10% $Pd(OH)_2/C$ (60 mg) at room temperature at 6-7 atm for 4 h. The catalyst was removed by filtration through Celite® and washed successively with ethyl acetate and methanol. The filtrate was evaporated *in vacuo* to provide **28** (178 mg, quantitative yield) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 0.93 (broad s, 3H), 1.22–1.43 (complex signal, 8H), 1.43–1.62 (complex signal, 2H), 2.15 (m, 1H), 2.23–2.51 (complex signal, 3H), 3.20 (m, 1H), 3.97 (m, 1H).

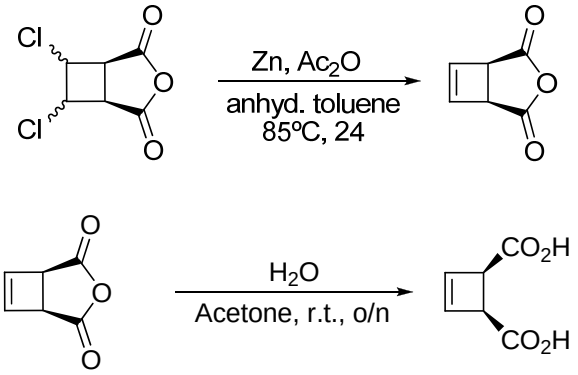
Compound 29



DIPEA (0.43 mL, 2.5 mmol) and HATU (200 mg, 0.53 mmol) were added to a solution of acid **13** (140 mg, 0.41 mmol) in a 20:1 mixture of anhydrous CH_2Cl_2 –DMF (10.5 mL). After ten minutes stirring compound **28** (178 mg, 0.57 mmol) was added with 10 mL of anhydrous CH_2Cl_2 and 1 mL of anhydrous DMF and the mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the DMF was lyophilized. The resulting crude was purified by H_2O and Et_2O washes, stirring and disaggregating the solid. The residue was lately purified by silica gel chromatography using CH_2Cl_2 – MeOH (10:1) as eluent to afford **29** (85.2 mg, 40%) as a white solid. Crystals, mp 148-152 ºC (from Et_2O). $[\alpha]_D$ = 166.7 (*c* 0.58, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3305, 2934, 2858, 1688, 1639, 1530, 1454. δ_H (250 MHz, $CDCl_3$) 0.89 (broad s, 3H), 1.28 (complex signal, 6H), 1.44 – 1.48 (complex signal, 4H), 1.73 – 2.17 (complex signal, 6H), 2.31 – 2.36 (complex signal, 4H), 3.18 – 3.23 (complex signal, 6 H), 4.45 – 4.51 (complex signal, 1H), 4.63 – 4.66 (complex signal, 1H), 5.07 (broad s, 2H), 5.66 (broad s, 1H), 6.02 (broad s, 1H), 6.31 (broad s, 1H), 7.06 (broad s, 1H), 7.34 (complex signal,

5H). δ_C (62.5 MHz, $CDCl_3$). 14.1, 18.7, 19.5, 22.7, 25.1, 26.7, 28.8, 29.5, 29.8, 31.6, 33.8, 38.9, 39.7, 45.2, 46.0, 46.4, 46.9, 54.7, 66.8, 128.3, 128.6, 136.7, 155.9, 172.4, 173.4. *m/ z* (ESI): Found, 537.3059 $[M + Na]^+$. Calcd. for $C_{28}H_{42}N_4O_5Na$: 537.3047.

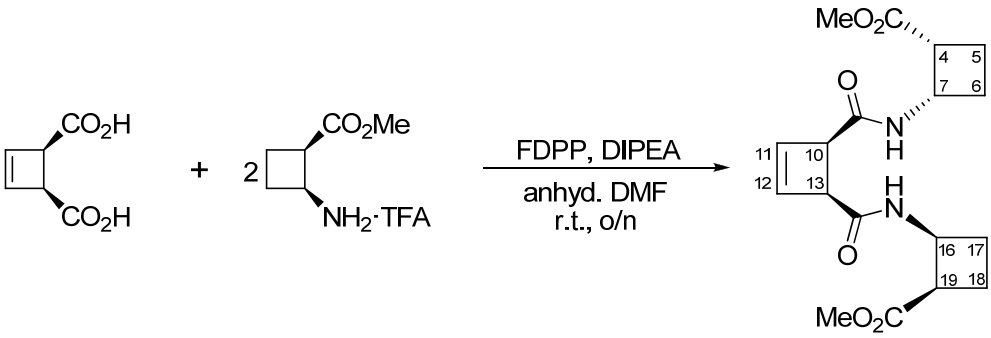
Compound 31



In a two-necked round bottom flask distilled acetic anhydride (10 mL, 0.11 mol) was added to a solution of dicloroadducts **30** (2.0 g, 10 mmol) in anhydrous toluene (10 mL). The resulting solution was stirred and heated until it reached 45º C. Previously activated dried dust Zn (15 g, 0.23 mol) was then added and the mixture was vigorously stirred at 85º C for 24 h. The catalyst and salts were removed by filtration through Celite® and washed successively with toluene and CH_2Cl_2. The filtrate was evaporated *in vacuo* and a dense dark oil was obtained. The crude was then purified by distillation (50-100º C) under reduced pressure (1 mbar) to provide the desired intermediate (649 mg, 51%) as a crystalline white solid. δ_H (250 MHz, $CDCl_3$) 4.05 (s, 2H), 6.49 (s, 2H). δ_C (62.5 MHz, D_2O) 47.8, 139.5, 167.9. Spectroscopic data are consistent with those reported in the literature.[5]

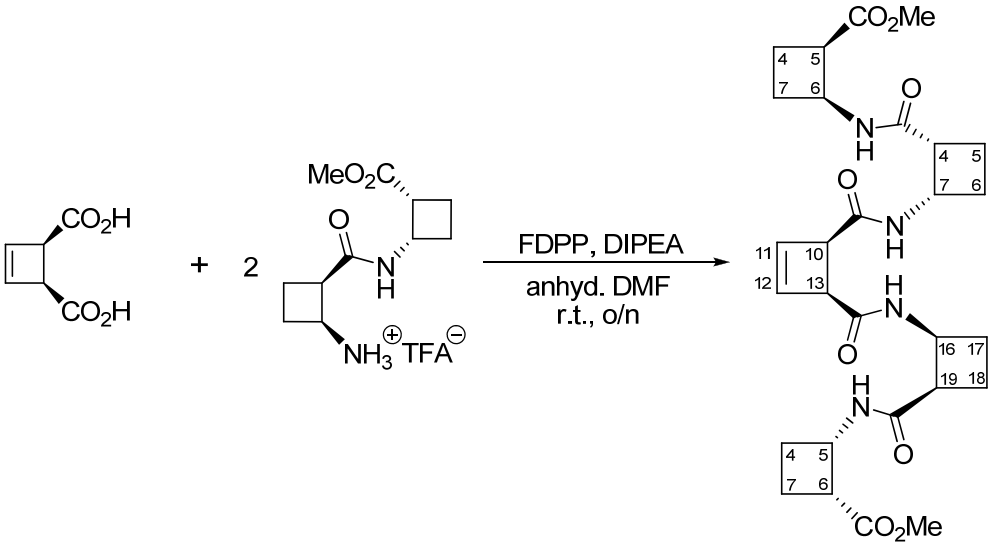
H_2O (1.2 mL, 67 mmol) was added to a solution of the intermediate (0.83 g, 6.7 mmol) in acetone (21 mL) and the mixture was stirred overnight at room temperature. Solvent was removed under reduced pressure and H_2O lyophilized to provide diacid **31** (0.95 g, quantitative yield) as a white solid. No further purification was needed. δ_H (250 MHz, D_2O) 4.11 (s, 2H), 6.35 (s, 2H). δ_C (62.5 MHz, D_2O) 51.9, 139.8, 178.4. Spectroscopic data are consistent with those reported in the literature.[6]

Pseudodipeptide 33



31 (640 mg, 4.5 mmol), FDPP (3.5 g, 9.1 mmol) and DIPEA (5.3 mL, 13.5 mmol) were dissolved in the minimum amount of anhydrous DMF under a nitrogen atmosphere. In another flask, monomer **32** (2.17 g, 8.9 mmol) was dissolved in the minimum amount of anhydrous DMF. DIPEA (4.6 mL, 27 mmol) was added to the later flask. Then the solution was transferred to the first flask with the use of a cannula. The mixture was stirred overnight at room temperature. The solvent was then removed *in vacuo* and the resulting residue was purified by silica gel chromatography using CH_2Cl_2 – MeOH (15:1) as eluent to afford **33** (260 mg, 20%) as a pale white solid. Crystals, mp 107-109 ºC (from CH_2Cl_2). $[\alpha]_D$ = –65 (*c* 0.40, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3293, 2942, 1718, 1648, 1523, 1433. δ_H (360 MHz, $CDCl_3$) 1.92 – 2.04 (complex signal, 4H), 2.14 – 2.33 (complex signal, 4H), 3.29 – 3.37 (complex signal, 2H), 3.71 (s, 6H), 3.76 – 3.80 (complex signal, 2H), 4.67 (q, *J* = 8.6 Hz, 2H), 6.26 (d, J = 2.9 Hz, 1H), 6.38 (d, J = 2.9 Hz, 1H), 6.77 (broad d, J = 8.9 Hz, 1H), 6.85 (broad d, J = 8.9 Hz, 1H). δ_C (90 MHz, $CDCl_3$) 19.3, 19.4, 29.3, 29.4, 44.0, 44.2, 44.3, 51.0, 51.5, 51.8, 136.8, 138.8, 169.1, 169.5, 174.6. *m/ z* (ESI): Found, 387.1529 $[M + Na]^+$. Calcd. for $C_{18}H_{24}N_2O_6Na$: 387.3798.

Pseudotetrapeptide 35



31 (72.2 mg, 0.51 mmol), FDPP (0.43 g, 1.1 mmol) and DIPEA (0.65 mL, 1.7 mmol) were dissolved in anhydrous DMF (5 mL) under a nitrogen atmosphere. After ten minutes stirring dipeptide **34** (334 mg, 0.98 mmol) was added with the minimum amount of anhydrous DMF and the mixture was stirred at room temperature overnight. DMF was lyophilized and the resulting residue was dissolved in EtOAc (50 mL). The solution was washed with saturated aqueous $NaHCO_3$ (4 x 20 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated. The resulting residue was purified by silica gel chromatography using EtOAc and then MeOH as eluent to afford **35** (40 mg, 14%) as a white solid. Crystals, mp 65-69 ºC (from CH_2Cl_2). $[\alpha]_D$ = –158 (*c* 0.96, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3282, 2949, 1724, 1642, 1512, 1436. δ_H (360 MHz, $CDCl_3$) 1.83 – 2.40 (complex signal, 16H), 3.07 – 3.23 (complex signal, 2H), 3.33 – 3.47 (complex signal, 2H), 3.67 – 3.76 (complex signal, 2H), 3.69 (s, 6H), 4.49 – 4.81 (complex signal, 4H), 6.20 (d, *J* = 2.8 Hz, 1H), 6.36 (d, J = 2.8 Hz, 1H), 6.67 (broad d, J = 8.6 Hz, 1H), 6.76 (broad d, J = 8.5 Hz, 1H), 7.04 (broad d, J = 8.7 Hz, 1H), 7.10 (broad d, J = 8.5 Hz, 1H). δ_C (90 MHz, $CDCl_3$) 19.0, 19.1, 19.2, 28.7, 28.9, 29.1, 29.3, 44.6, 44.7, 44.8, 45.6, 51.0, 51.8, 51.9, 137.0, 138.4, 169.7, 170.2, 172.3, 172.4, 174.6, 174.7. *m/ z* (ESI): Found, 559.2755 $[M + H]^+$. Calcd. for $C_{28}H_{39}N_4O_8$: 559.4431.

NMR studies for tetrapeptide 14

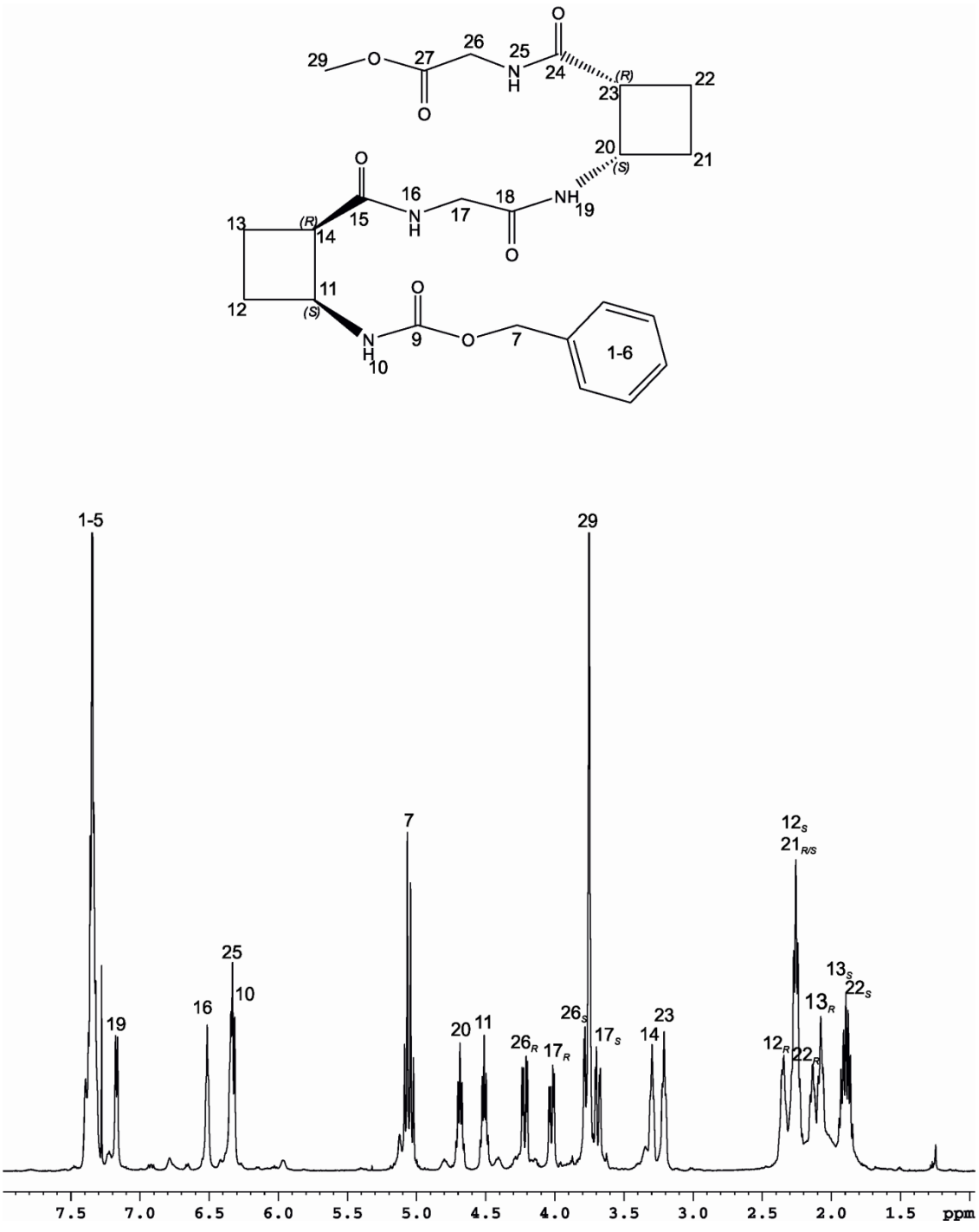


Figure S1. ^{1}H-NMR assignment of tetrapeptide **14** in $CDCl_3$ recorded at 270 K (600 MHz)

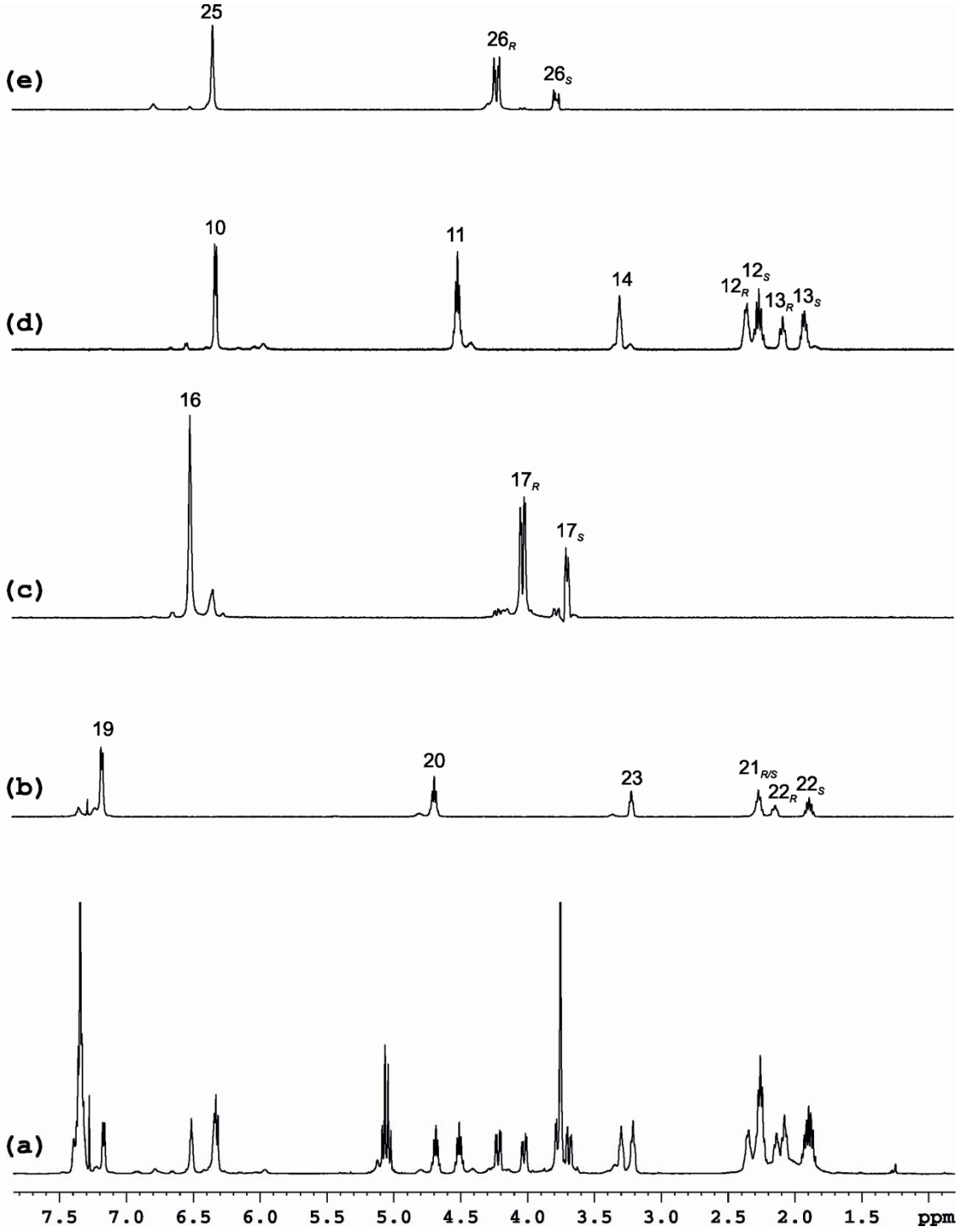


Figure S2. 1D selective TOCSY NMR experiments used for product characterization of tetrapeptide **14**. TOCSY mixing time was set to 60 ms in all the experiments. Experiments were performed at 270 K in $CDCl_3$ (600 MHz). (a) ^{1}H-NMR for visual comparison purposes. (b) NH_{19} selective TOCSY. (c) NH_{16} selective TOCSY. (d) NH_{10} selective TOCSY. (e) NH_{16} selective TOCSY.

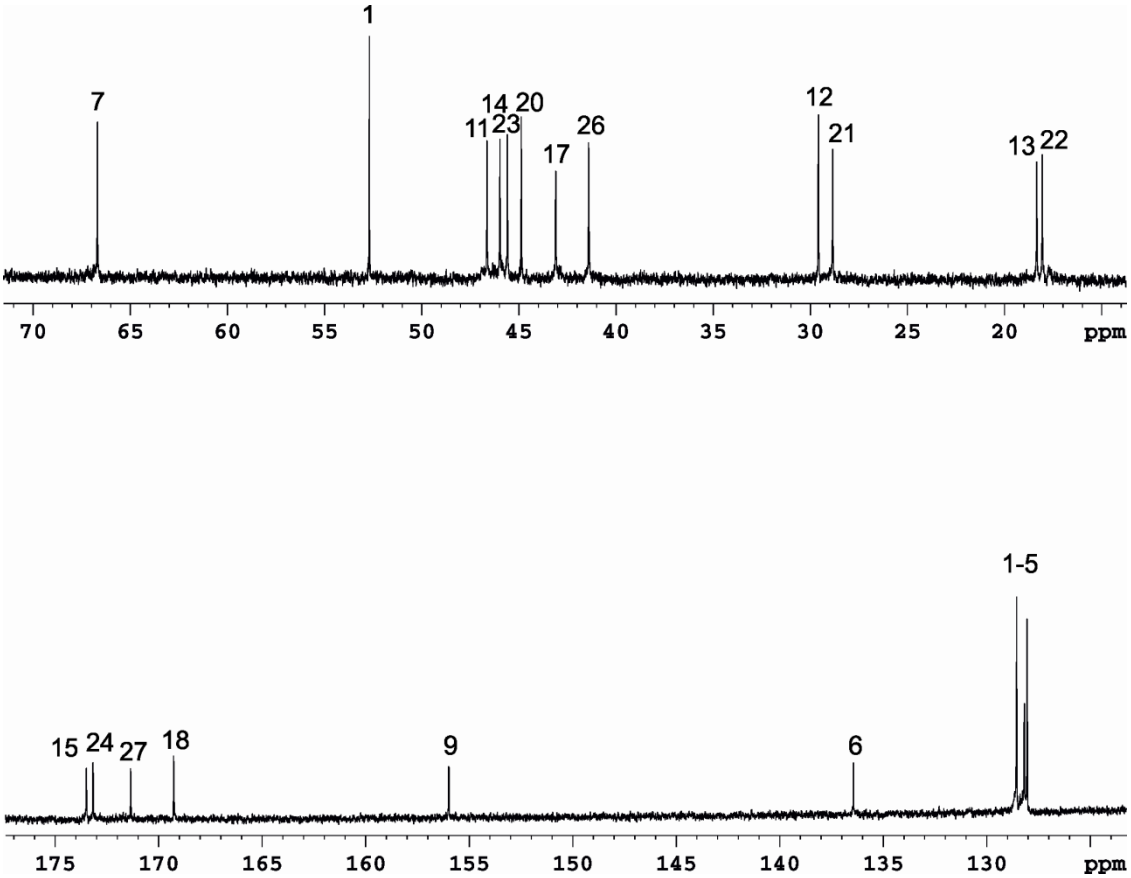


Figure S3. ^{13}C-NMR assignment of tetrapeptide **14** in $CDCl_3$ recorded at 270 K (600 MHz)

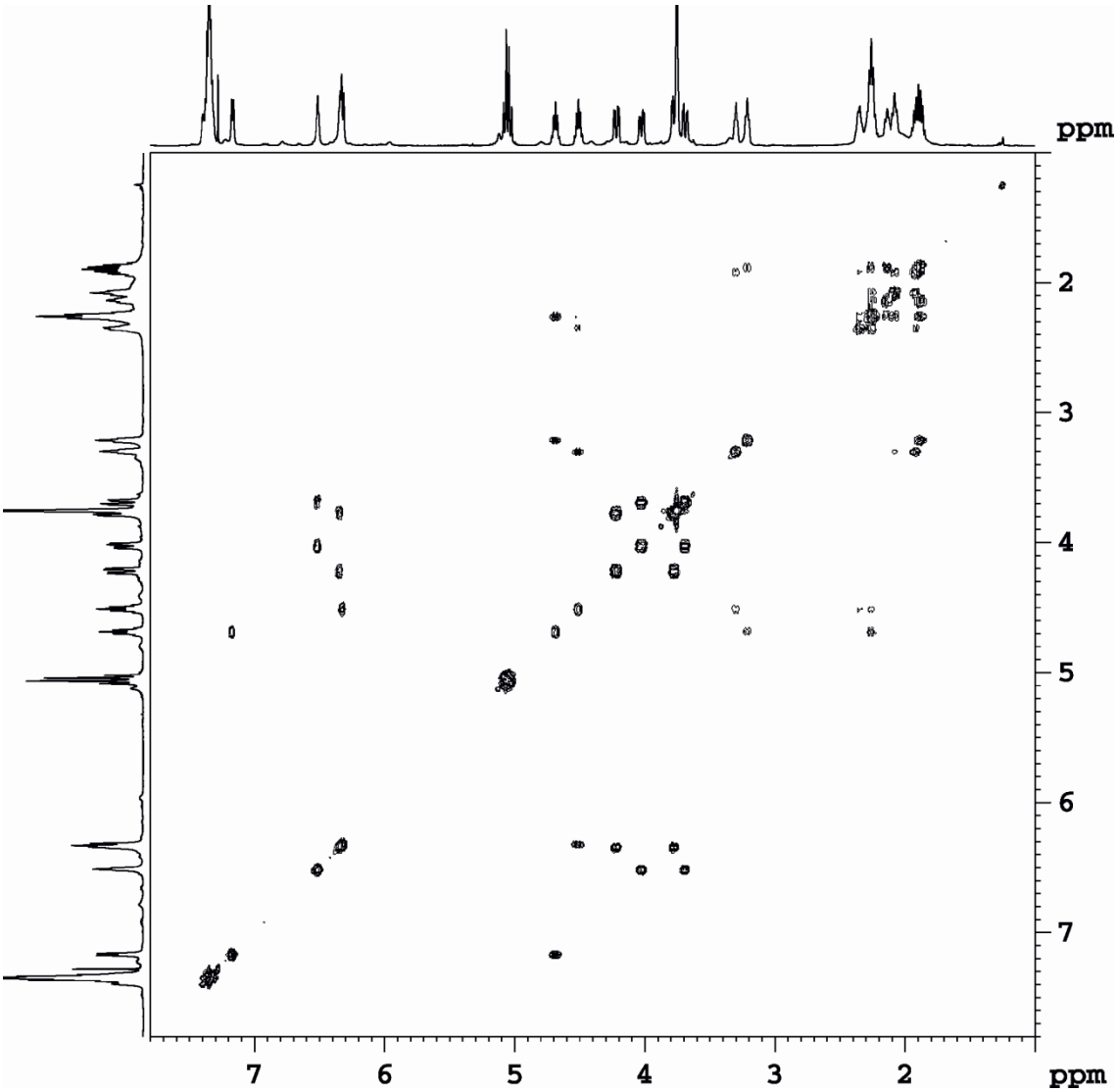


Figure S4. COSY NMR spectrum of tetrapeptide **14** in $CDCl_3$ recorded at 270 K (600 MHz)

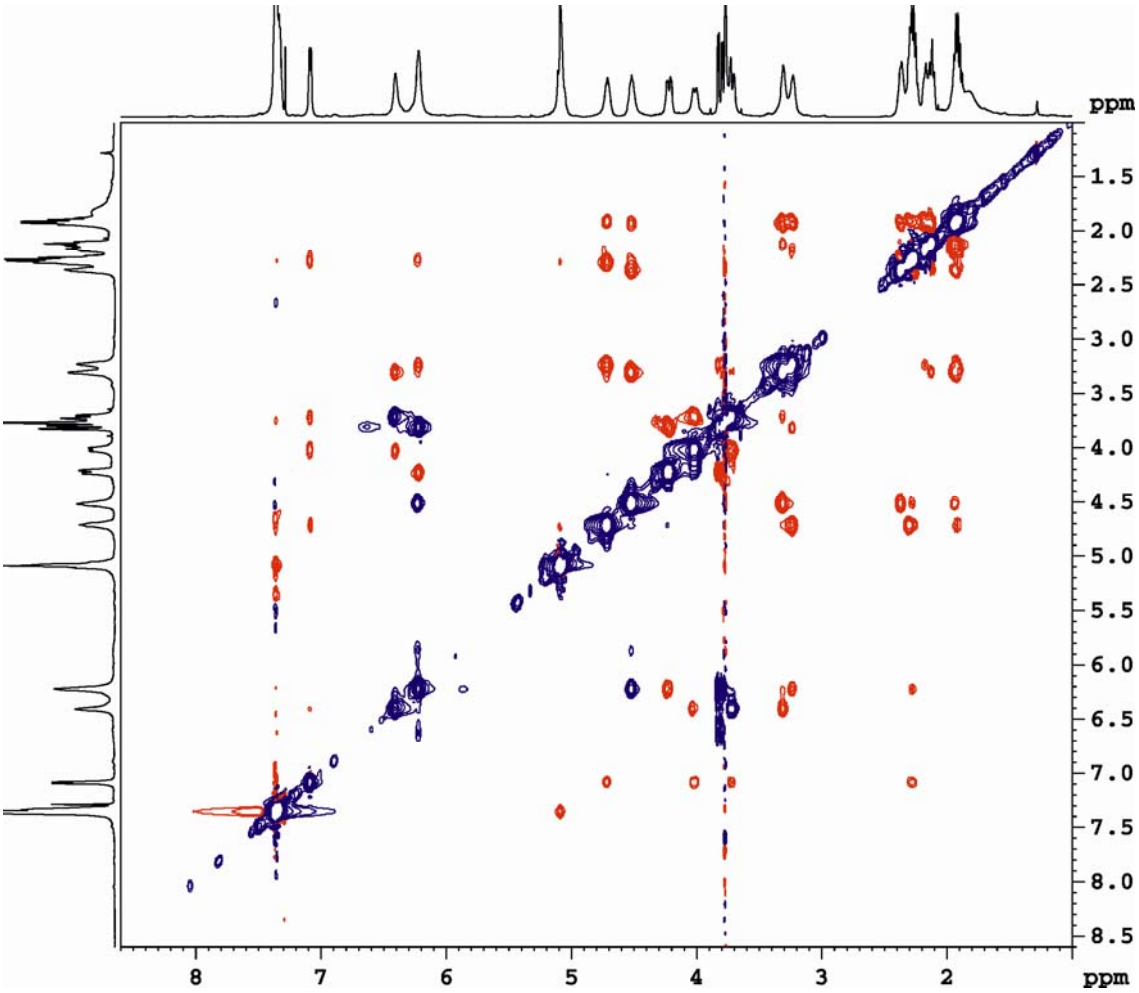


Figure S5. ROESY NMR spectrum of tetrapeptide **14** in $CDCl_3$ recorded at 298 K (600 MHz)

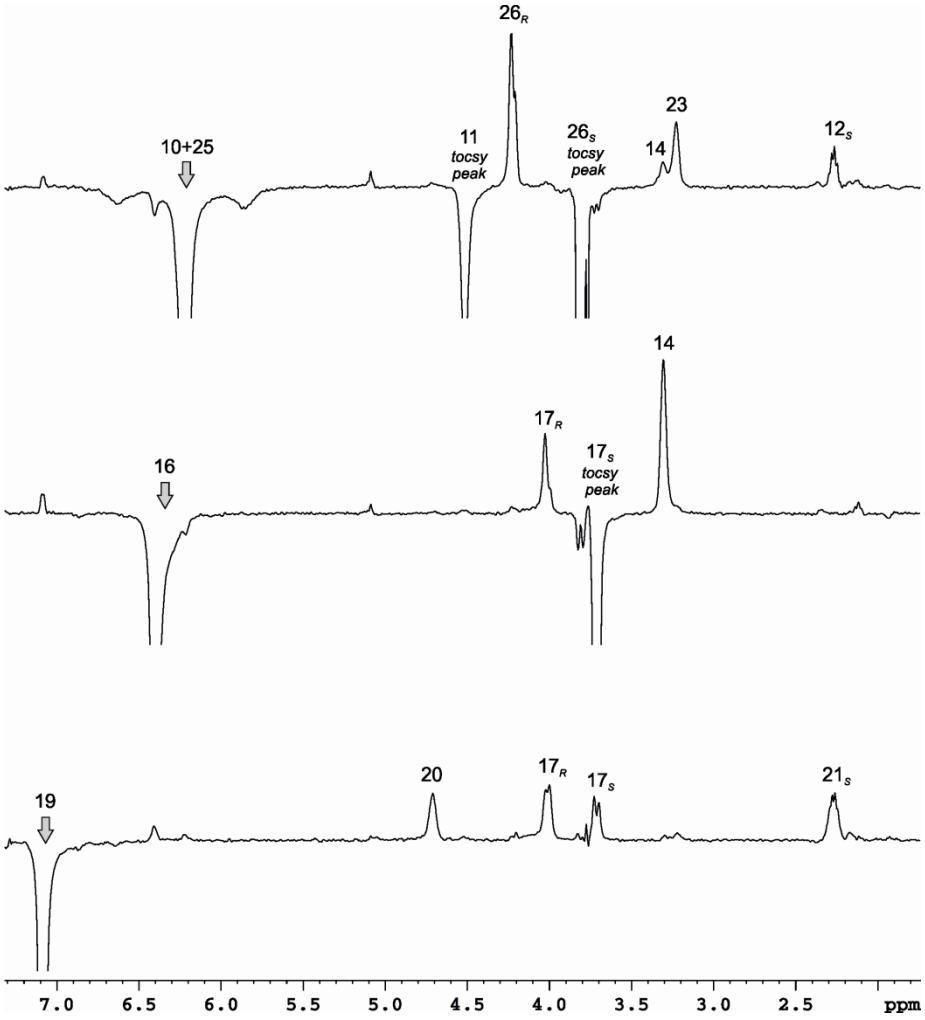


Figure S6. 1D ROESY slices extracted from 2D spectrum for tetrapeptide **14** in $CDCl_3$ recorded at 298 K (600 MHz).

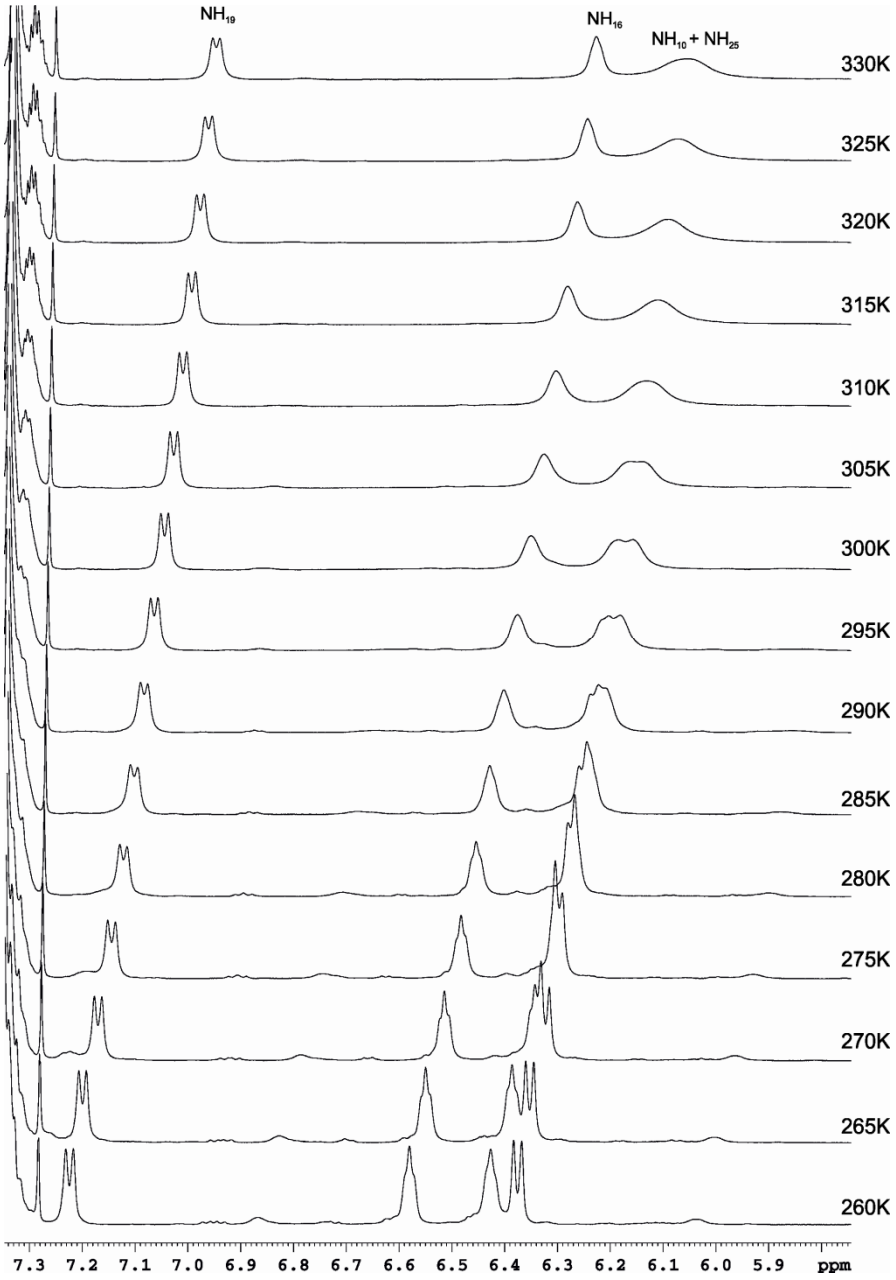


Figure S7. variable temperature ^{1}H-NMR spectra of tetrapeptide **14** in $CDCl_3$ (600 MHz).

Temperature coefficients extracted:

NH_{19} shifts -3.9 ppb/K, NH_{16} shifts -5.1 ppb/K, NH_{25} shifts -5.3 ppb/K, NH_{10} shifts -4.6 ppb/K

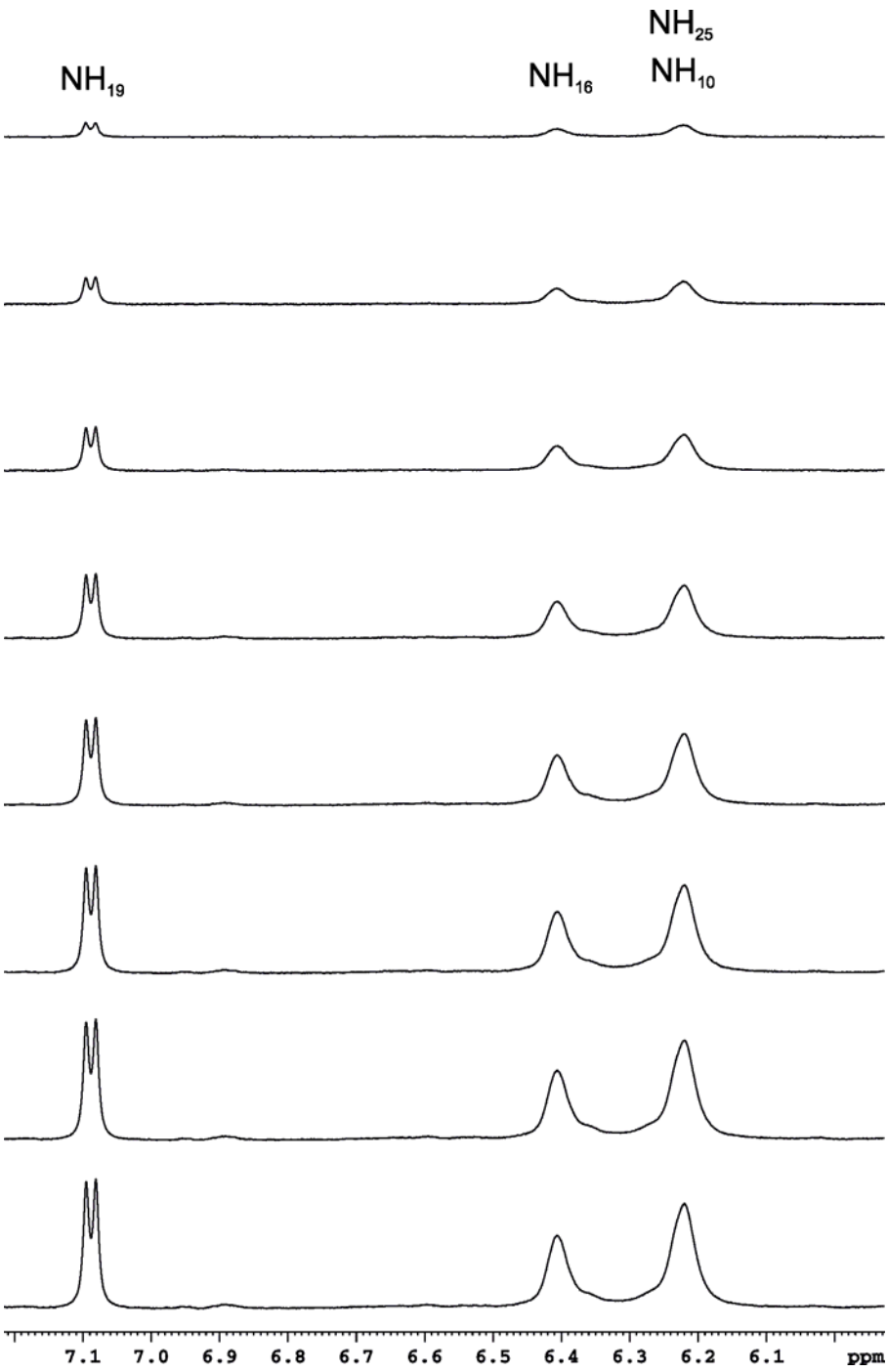


Figure S8. Self-diffusion NMR studies in $CDCl_3$ of tetrapeptide **14** at 600 MHz at 298 K. BPLED spectra are recorded with a diffusion time of 100 ms, with a gradient duration of 0.5 ms and with a gradient strength from 2% to 95% in eight lineal steps from (a) to (h). All proton signals decay approximately at the same rate.

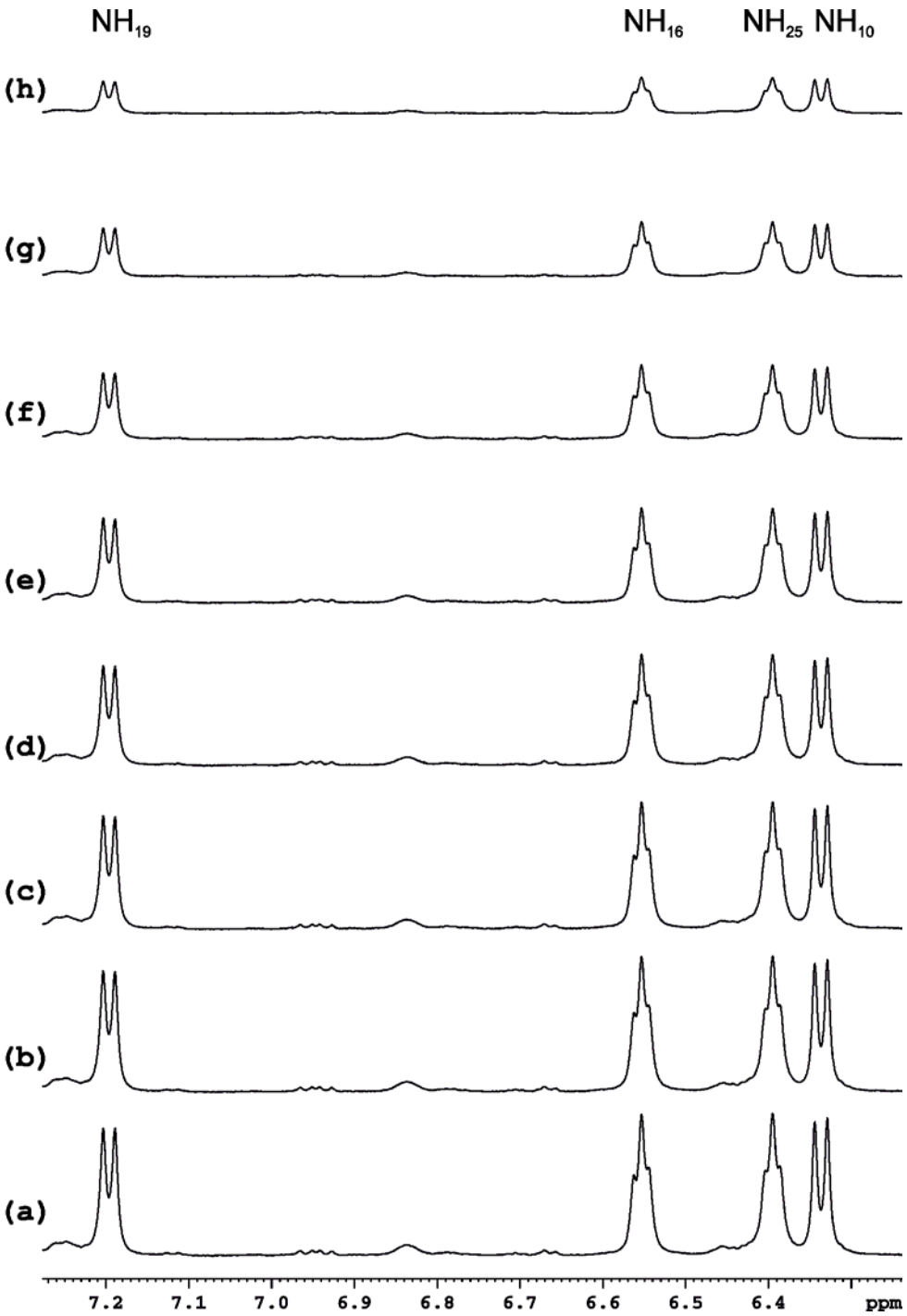


Figure S9. Self-diffusion NMR studies in $CDCl_3$ of tetrapeptide **14** at 600 MHz at 270 K. BPLED spectra are recorded with a diffusion time of 20 ms, with a gradient duration of 0.5 ms and with a gradient strength from 2% to 95% in eight lineal steps from (a) to (h). All proton signals decay approximately at the same rate.

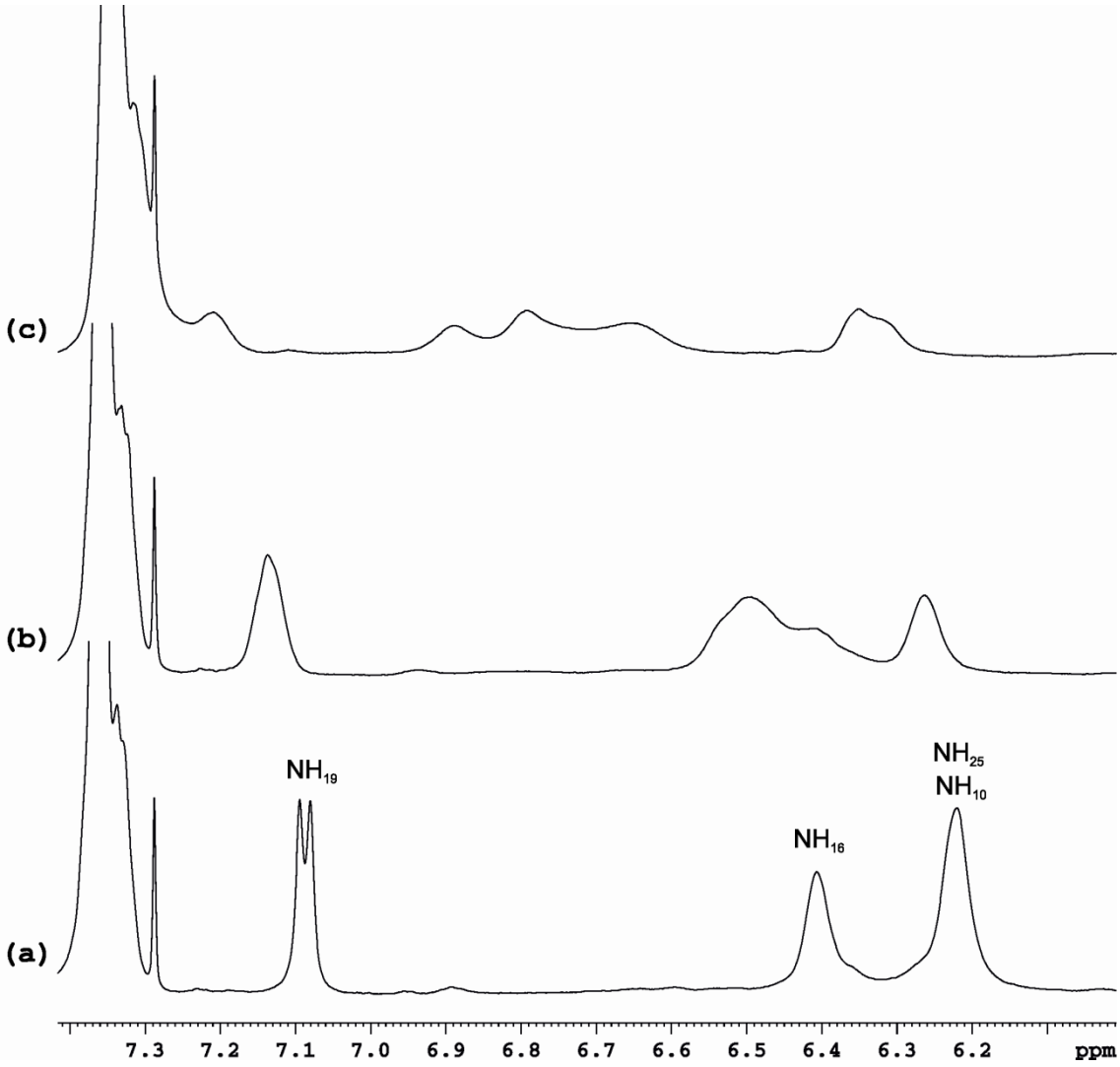


Figure S10. MeOD exchange experiments.

NMR studies for tetrapeptide 16

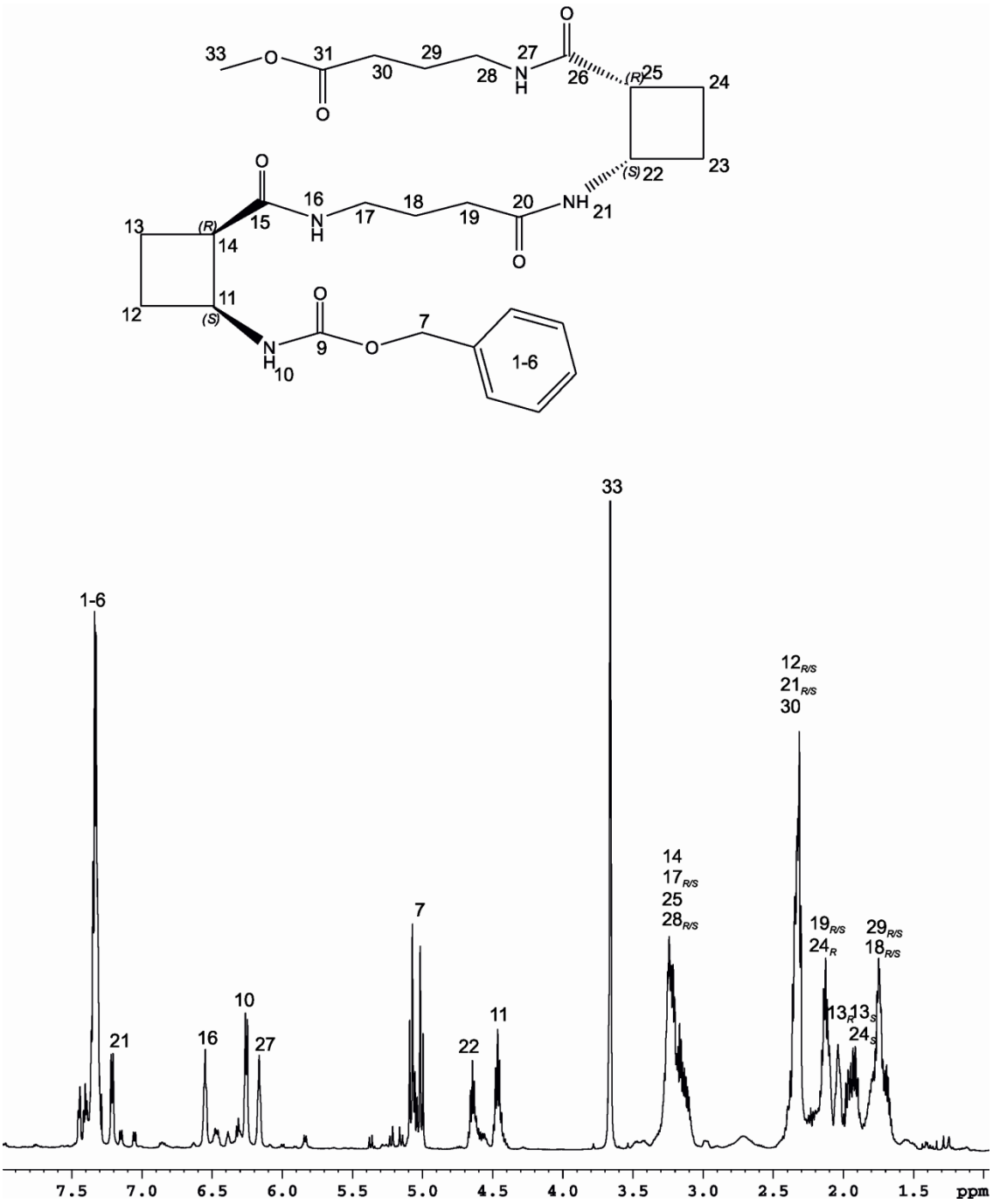


Figure S11. ^{1}H-NMR assignment of tetrapeptide **16** in $CDCl_3$ recorded at 270 K (600 MHz)

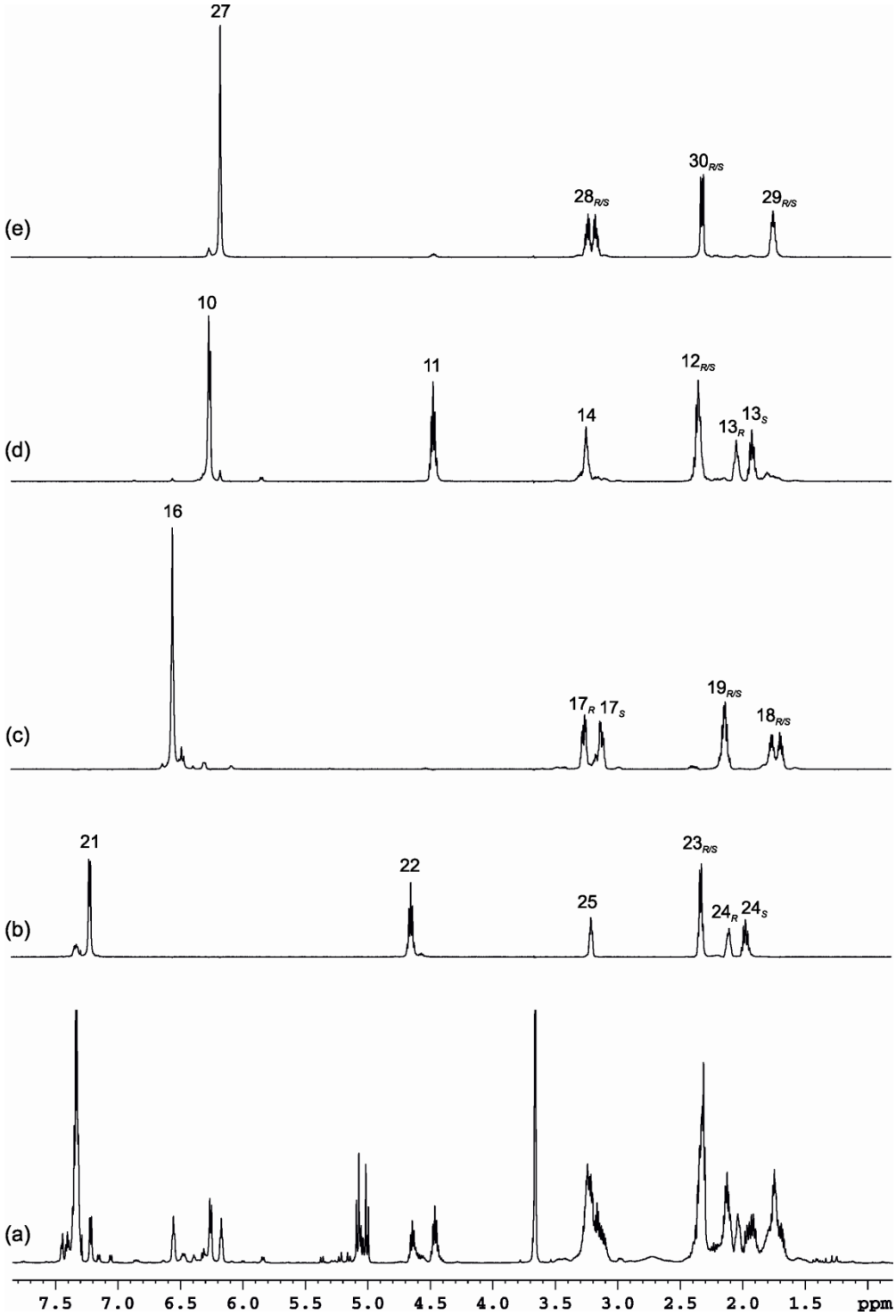


Figure S12. 1D selective TOCSY NMR experiments used for product characterization of tetrapeptide **16**. TOCSY mixing time was set to 60 ms in all the experiments. Experiments were performed at 270 K in $CDCl_3$ (600 MHz). (a) ^{1}H-NMR for visual comparison purposes. (b) NH_{21} selective TOCSY. (c) NH_{16} selective TOCSY. (d) NH_{10} selective TOCSY. (e) NH_{27} selective TOCSY.

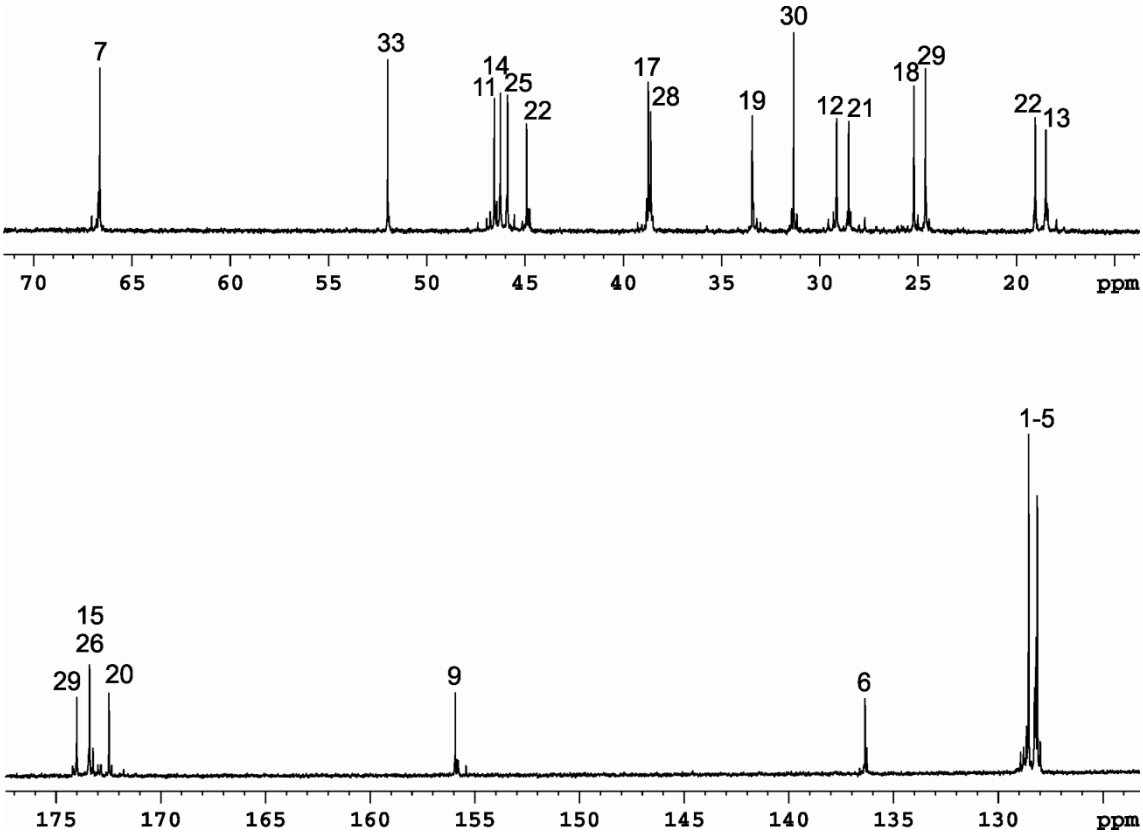


Figure S13. ^{13}C-NMR assignment of tetrapeptide **16** in $CDCl_3$ recorded at 270 K (600 MHz)

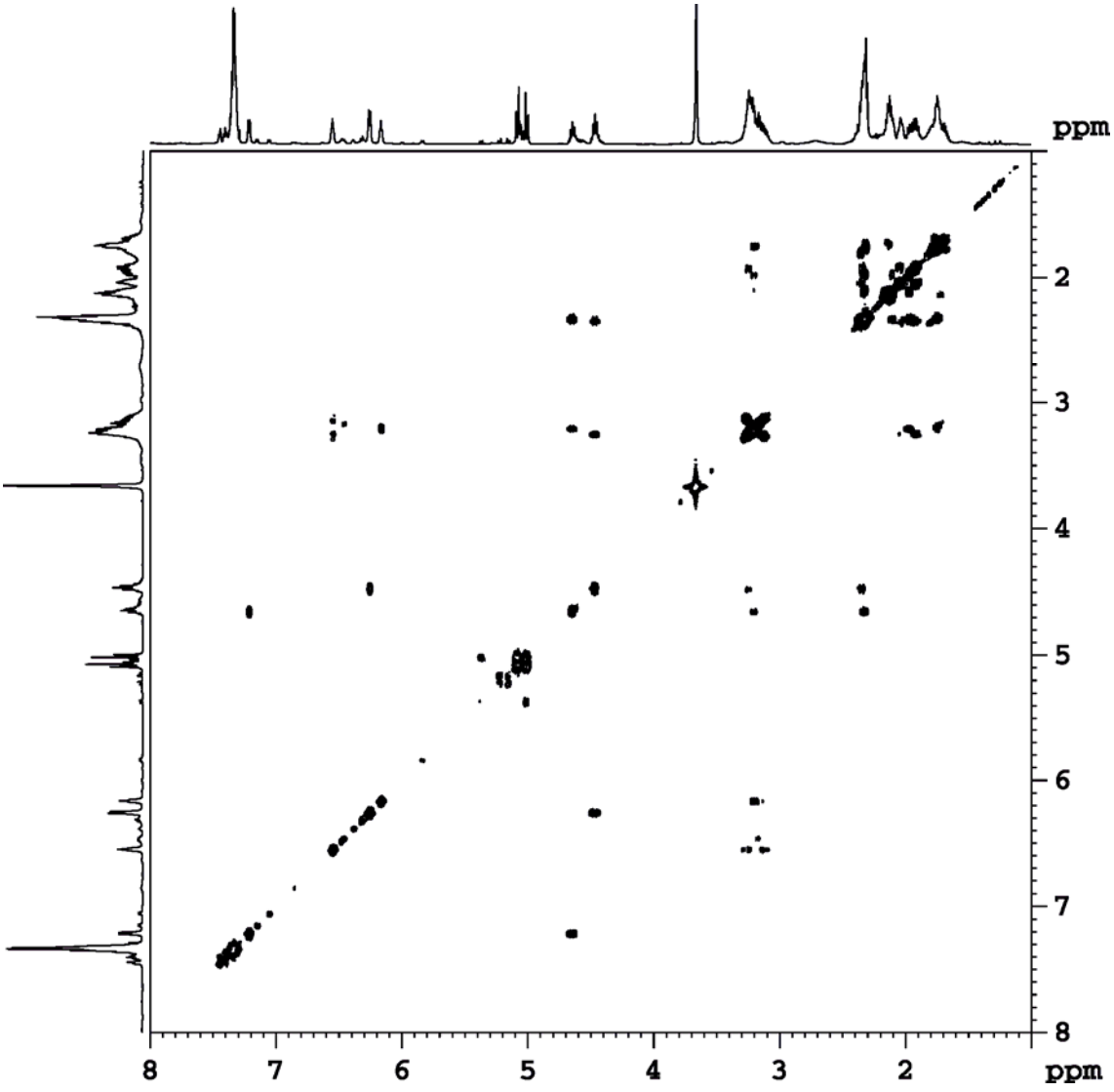


Figure S14. COSY NMR spectrum of tetrapeptide **16** in $CDCl_3$ recorded at 270 K (600 MHz)

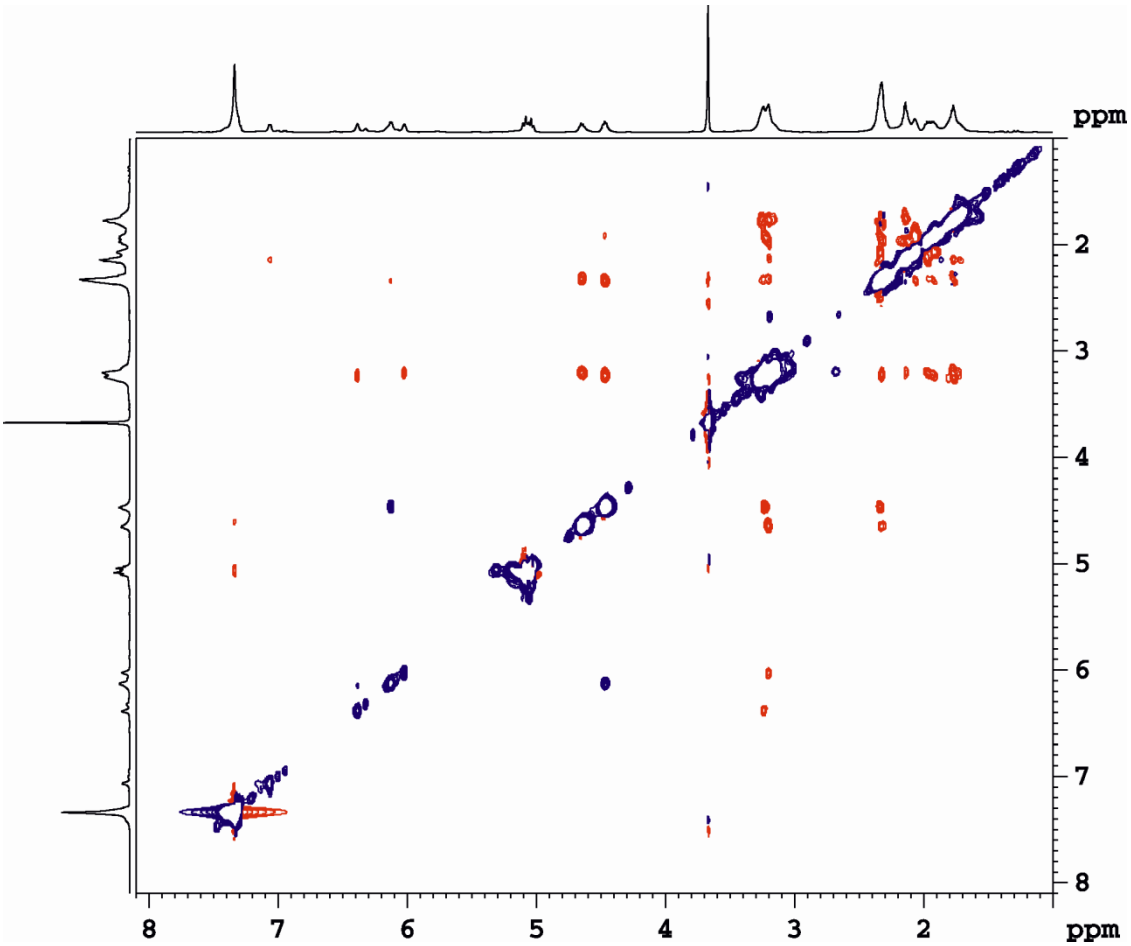


Figure S15. ROESY NMR spectrum of tetrapeptide **16** in $CDCl_3$ recorded at 298 K (600 MHz)

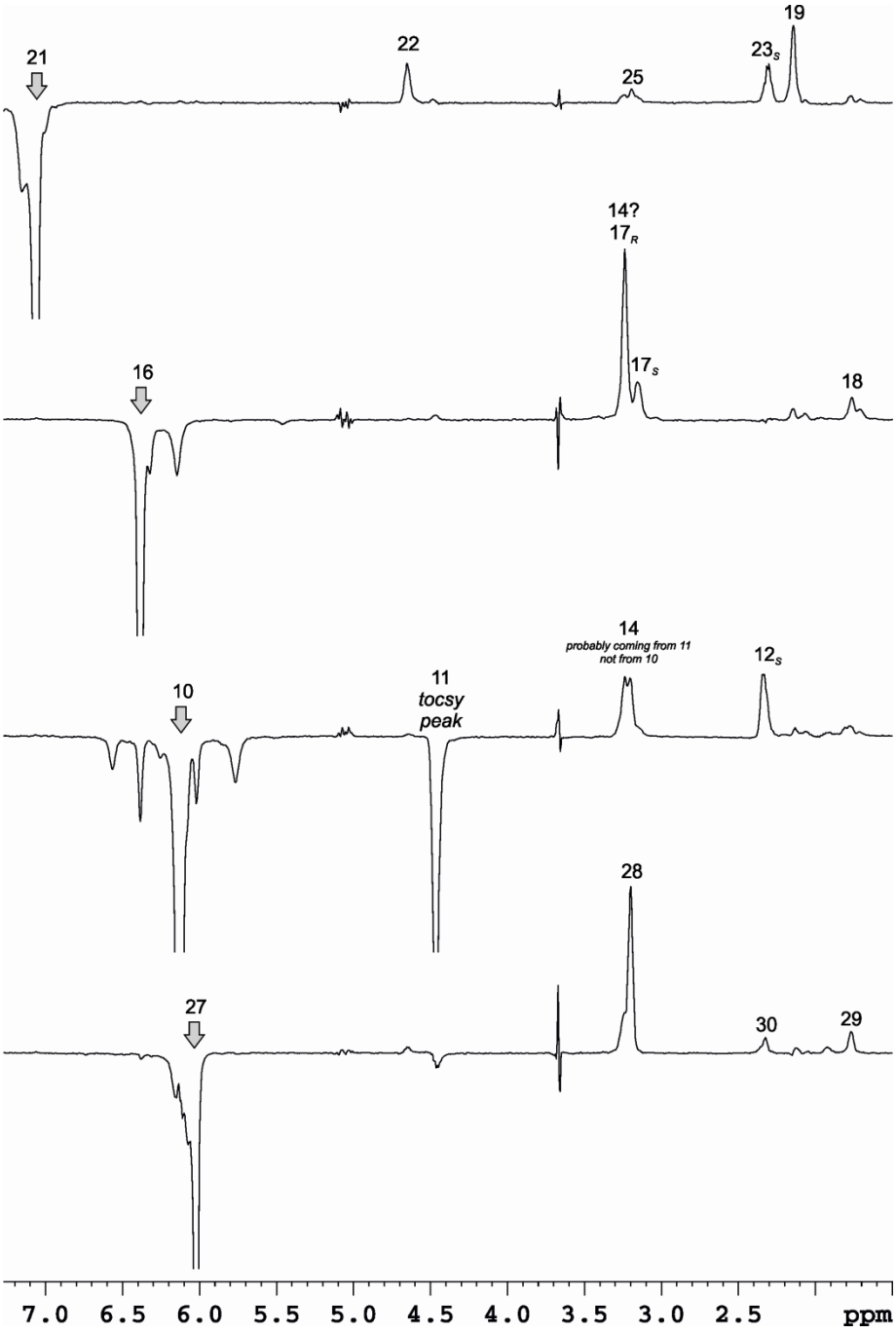


Figure S16. 1D ROESY slices extracted from 2D spectrum for tetrapeptide **16** in $CDCl_3$ recorded at 298 K (600 MHz).

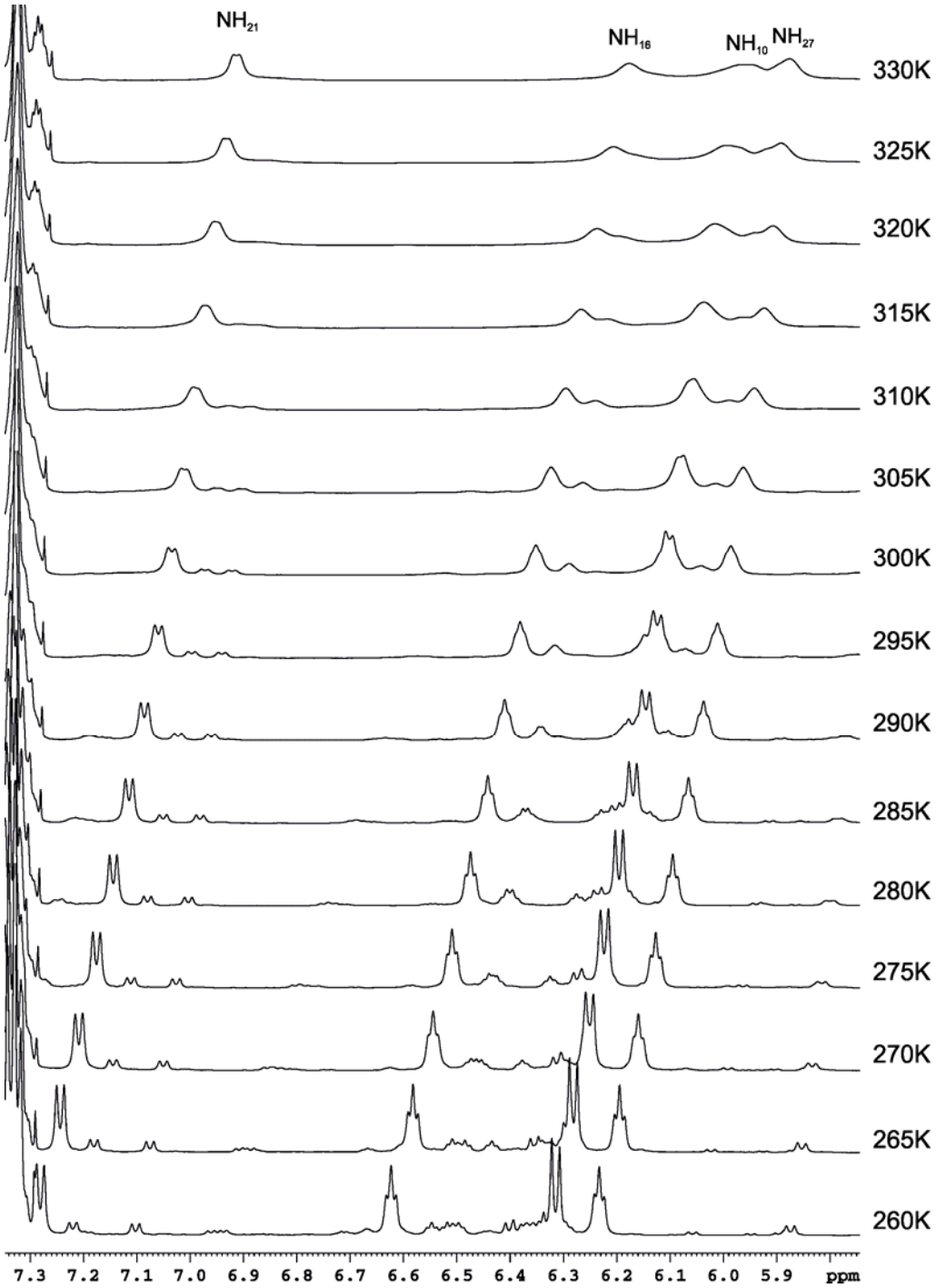


Figure S17. Variable temperature ^{1}H-NMR spectra of tetrapeptide **16** in $CDCl_3$ (600 MHz).

Temperature coefficients:

NH_{21} shifts -5.3 ppb/K, NH_{16} shifts -6.4 ppb/K, NH_{27} shifts -5.1 ppb/K , NH_{10} shifts -5.1 ppb/K

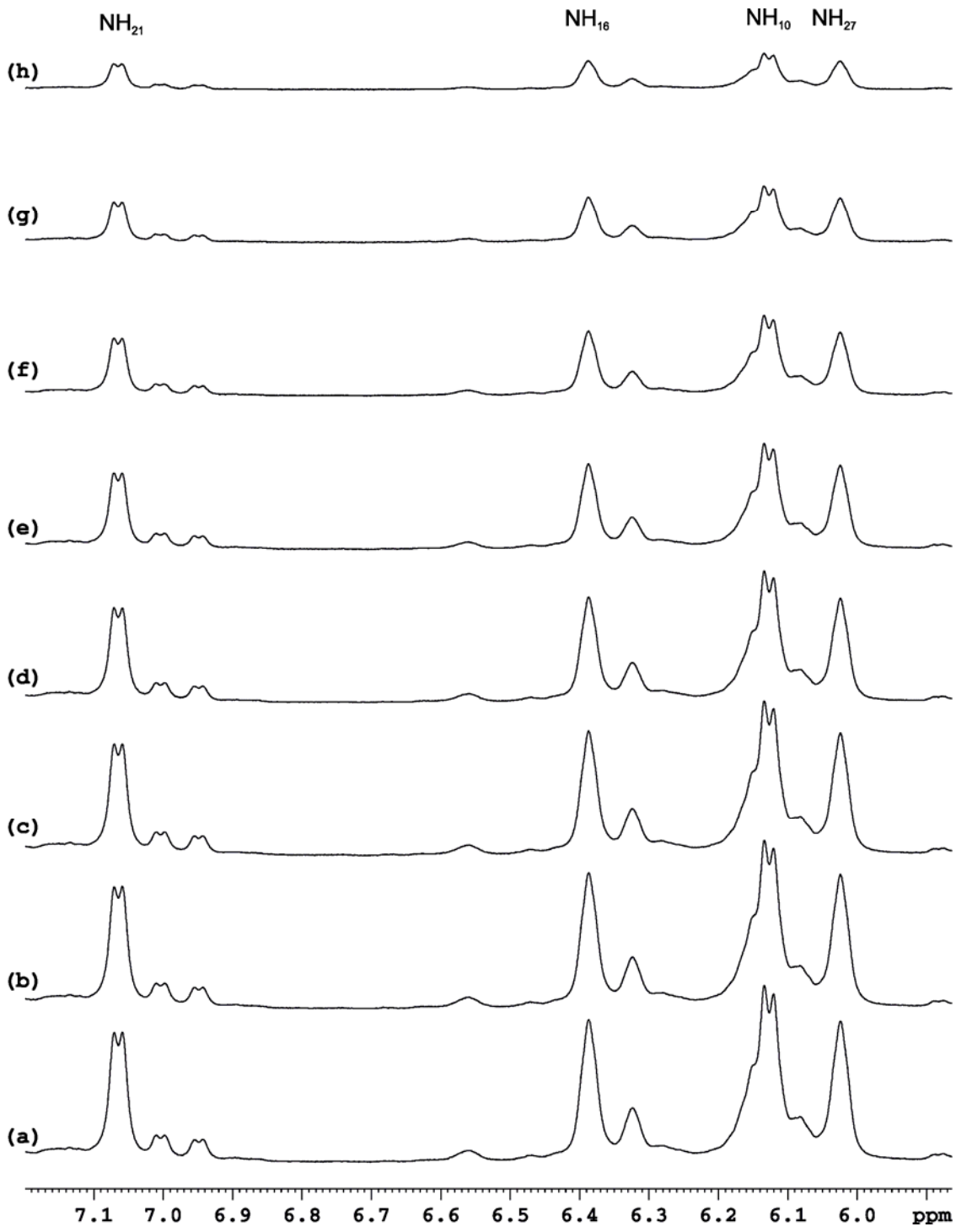


Figure S18. Self-diffusion NMR studies in $CDCl_3$ of tetrapeptide **16** at 600 MHz at 298 K. BPLED spectra are recorded with a diffusion time of 50 ms, with a gradient duration of 0.5 ms and with a gradient strength from 2% to 95% in eight lineal steps from (a) to (h). All proton signals decay approximately at the same rate.

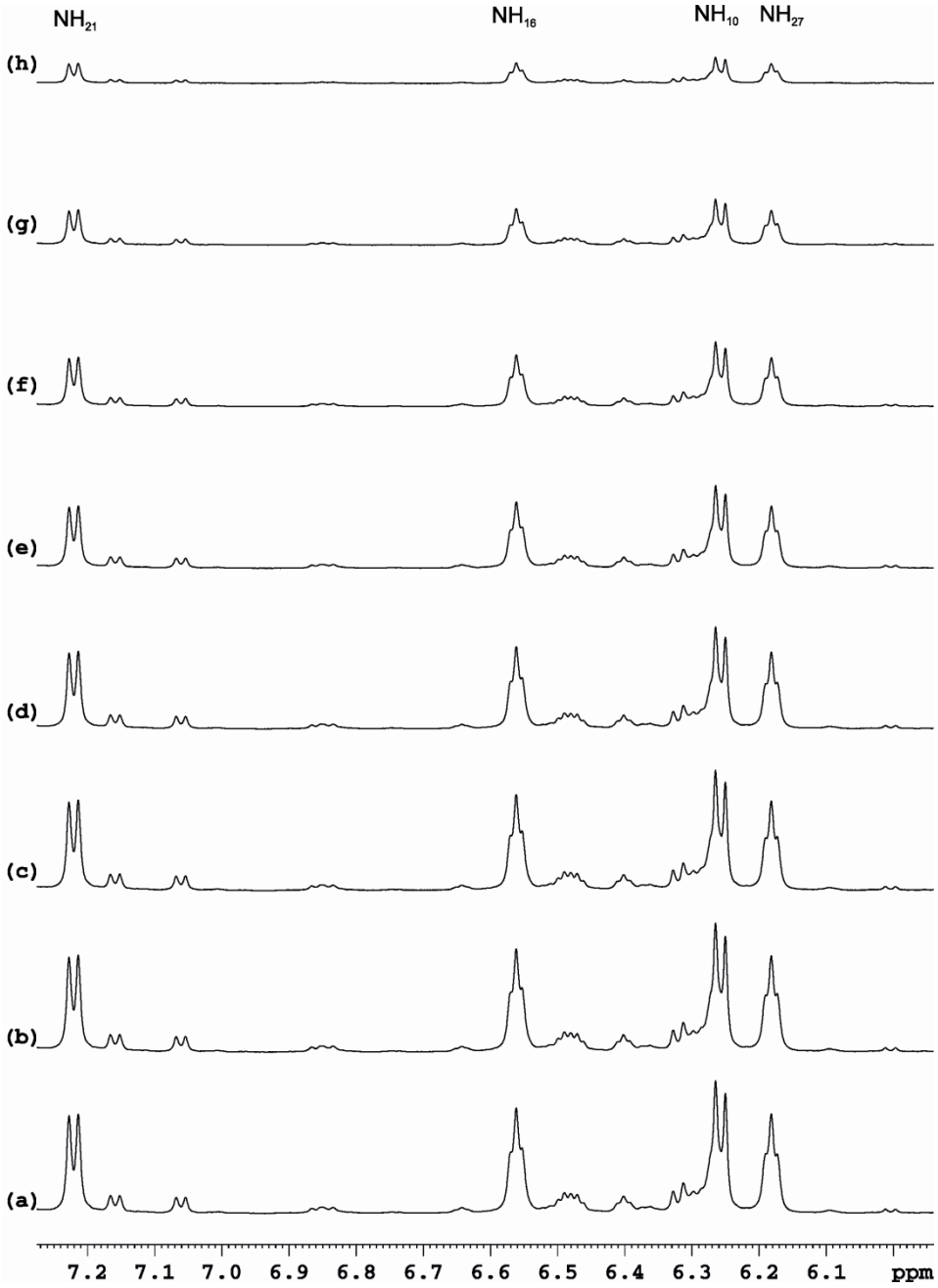


Figure S19. Self-diffusion NMR studies in $CDCl_3$ of tetrapeptide **16** at 600 MHz at 270 K. BPLED spectra are recorded with a diffusion time of 35 ms, with a gradient duration of 0.5 ms and with a gradient strength from 2% to 95% in eight lineal steps from (a) to (h). All proton signals decay approximately at the same rate.

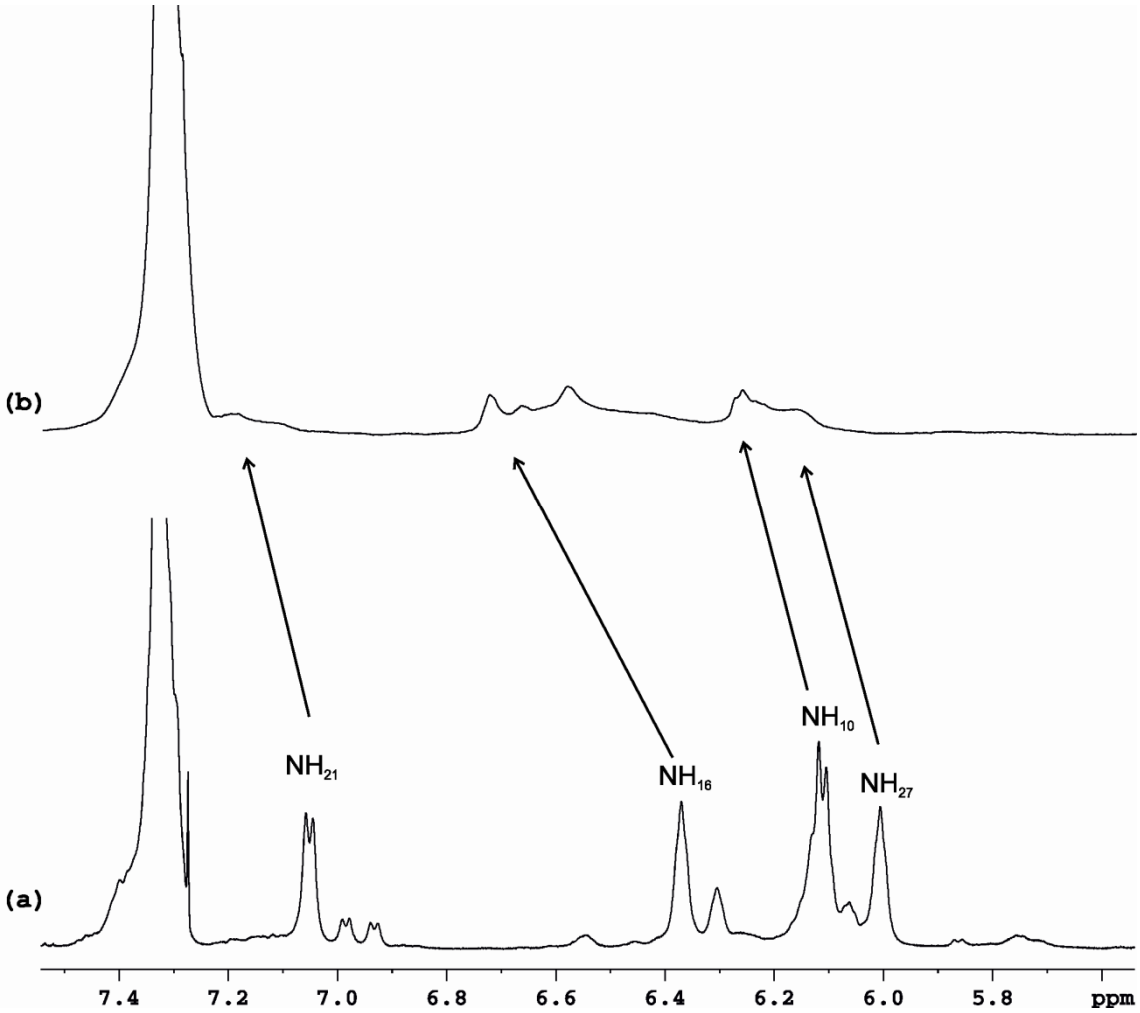


Figure S20. MeOD exchange experiments.

NMR experiments for pseudodipeptide 33

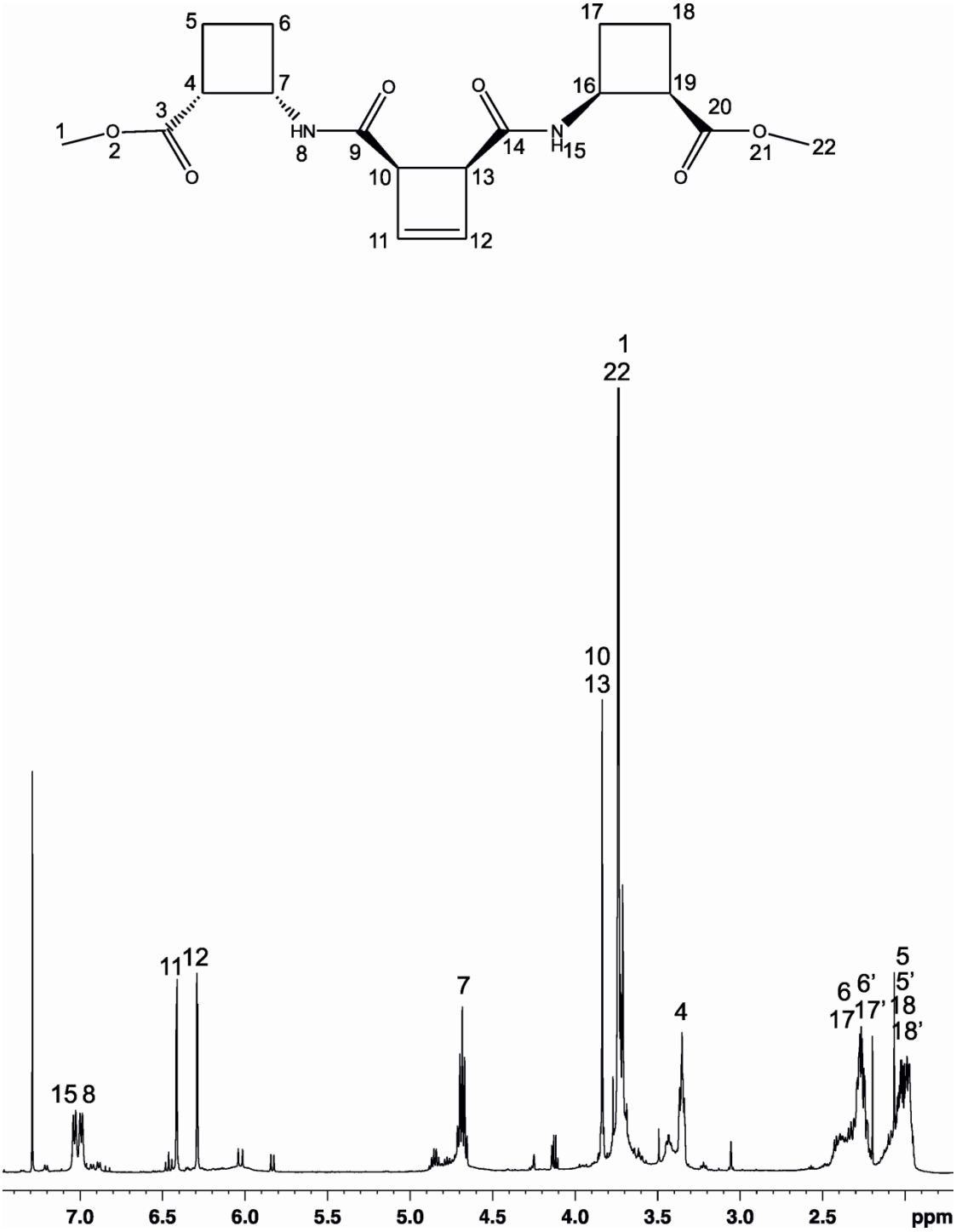


Figure S21. ^{1}H-NMR assignment of pseudodipeptide **33** in $CDCl_3$ recorded at 273 K (600 MHz)

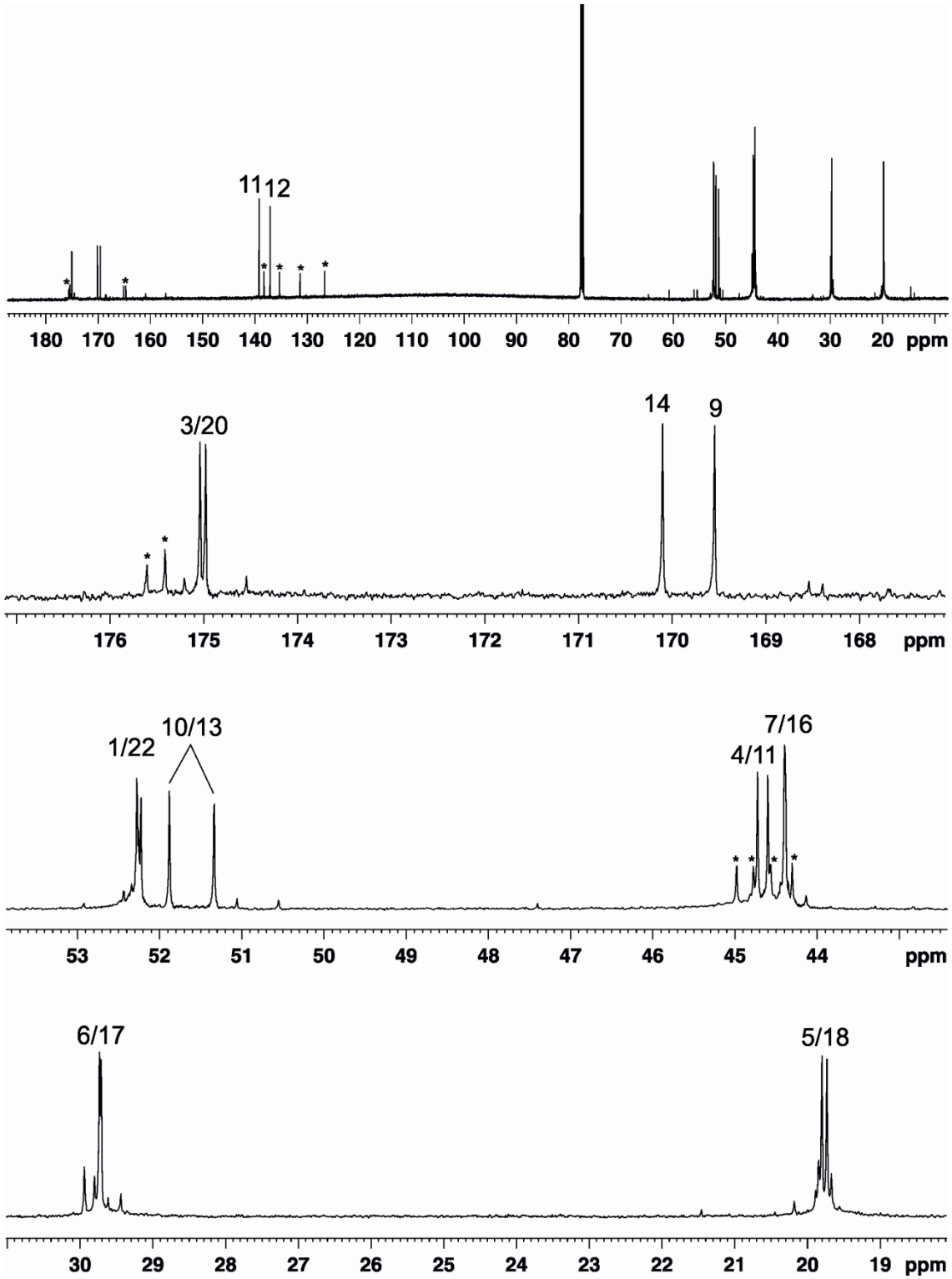


Figure S22. ^{13}C-NMR assignment of pseudodipeptide **33** in $CDCl_3$ recorded at 298K (600 MHz)

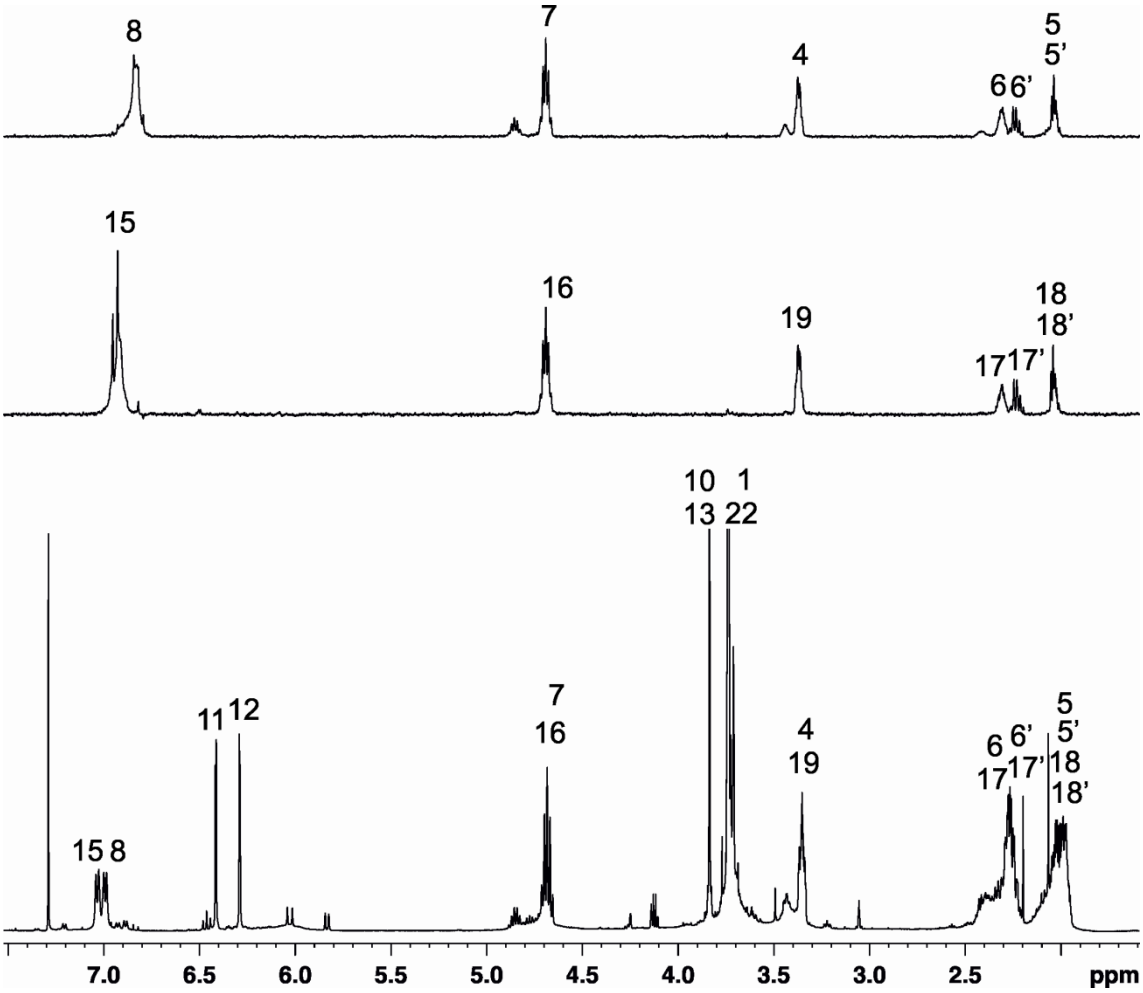


Figure S23. 1D selective TOCSY NMR experiments used for product characterization of pseudodipeptide **33**. TOCSY mixing time was set to 60 ms in all the experiments. Experiments were performed at 298 K in $CDCl_3$ (600 MHz). (a) ^{1}H-NMR for visual comparison purposes. (b) NH_{15} selective TOCSY. (c) NH_8 selective TOCSY.

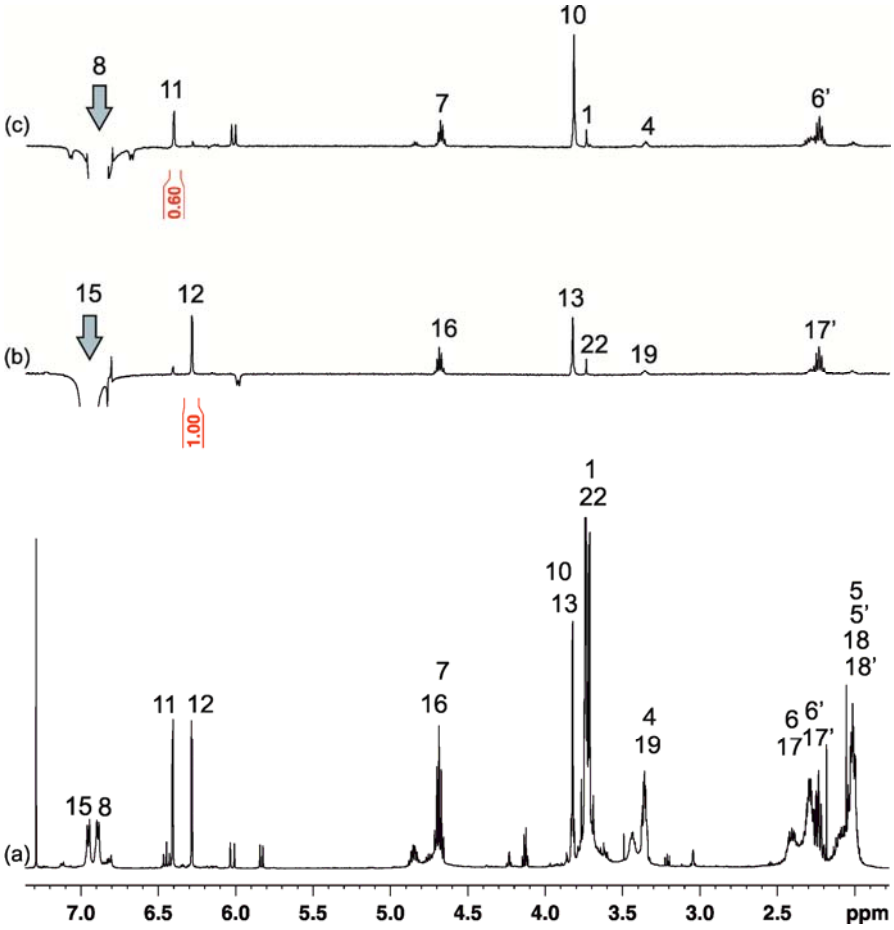


Figure S24. (a) ^{1}H-NMR of pseudodipeptide **33** in $CDCl_3$ recorded at 298 K (600 MHz) . (b) 1D-selective NOE experiment when irradiating H_{15} proton. NOE are integral for H_{12} is arbitrarily considered 1.0 (red marked). (c) 1D-selective NOE experiment when irradiating H_8 proton. NOE area integral for H_{11} is 0.6 relative to H_{15}-H_{12} NOE (red marked). Ratio of NOE area integrals H_{15}-H_{12}/H_8-H_{11} (0.67) is used for structure determination. All NOE experiments were performed in a Bruker 600 MHz spectrometer in $CDCl_3$ at 298K and using a NOE mixing time of 500ms.

Relative distances between atoms can be extracted qualitatively from NOE data integration (Figure S24). Thus, when the ratio between H_{15}-H_{12} NOE area and H_{11}-H_8 NOE area was calculated we obtained a value of 1.67 Å (1.0 a.u./0.6 a.u.). This value was then compared with the ratio of the distance among the same protons in the two possible structures theoretically calculated. When two 6-membered rings were taken into account the ratio encountered was 1.64 Å. Instead, if one 6-membered ring and one 7-membered ring are taken into account the same ratio was 1.25 Å. Therefore, the former structure considering two six-membered rings is in a much better agreement with the experimental results.

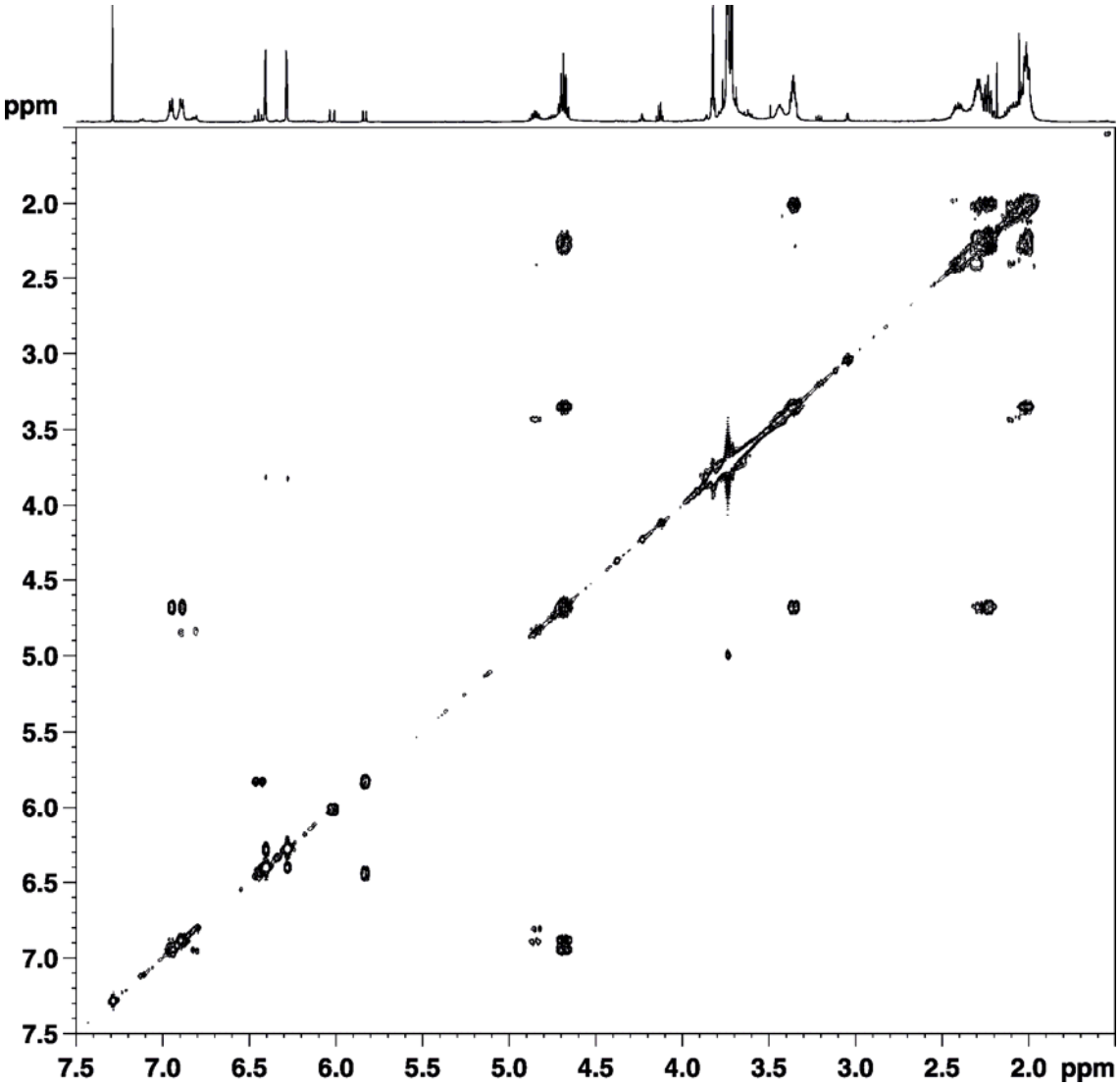


Figure S25. COSY NMR spectrum of pseudodipeptide **33** in $CDCl_3$ recorded at 298 K (600 MHz)

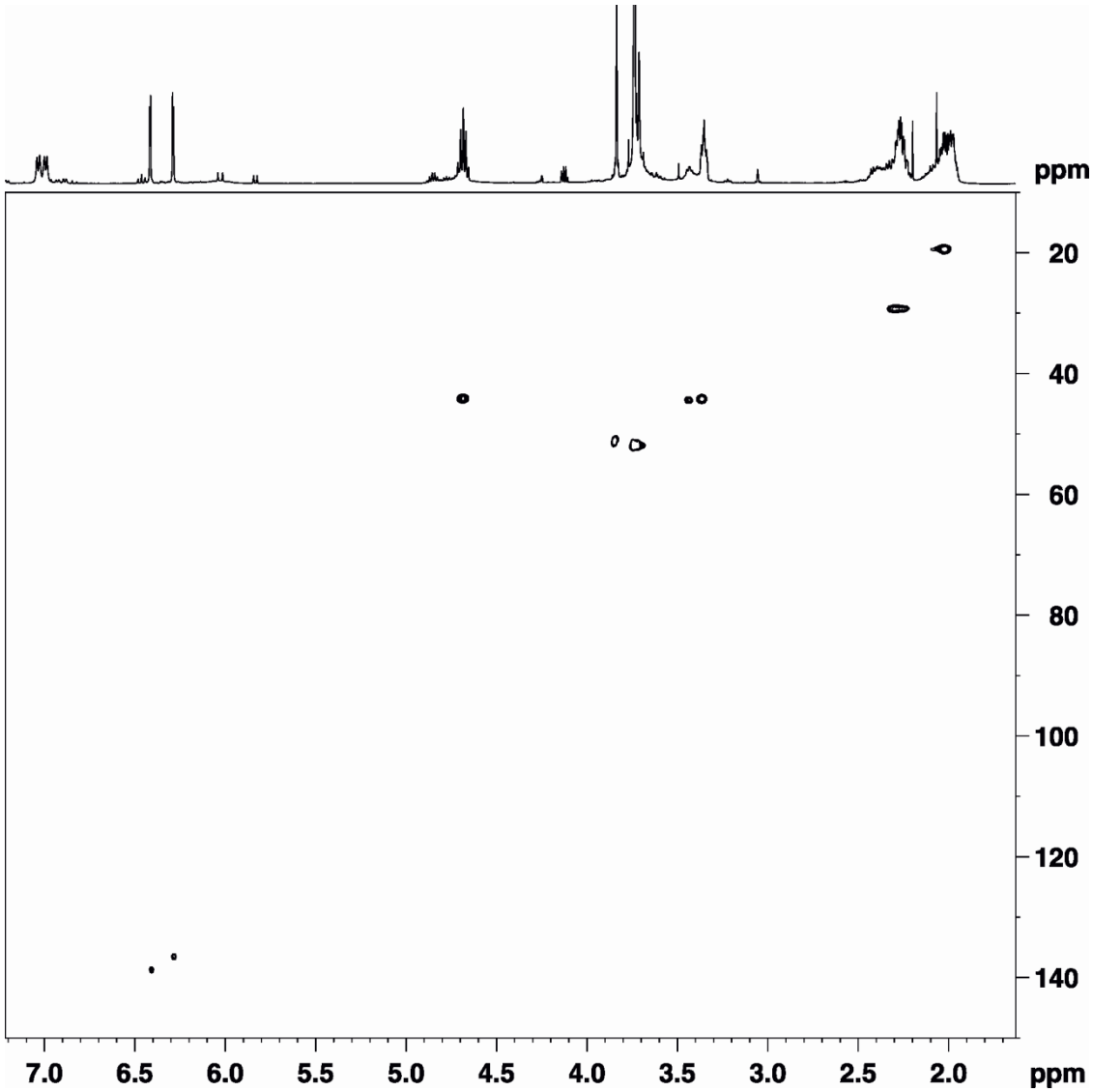


Figure S26. HSQC NMR spectrum of pseudodipeptide **33** in $CDCl_3$ recorded at 298 K (600 MHz)

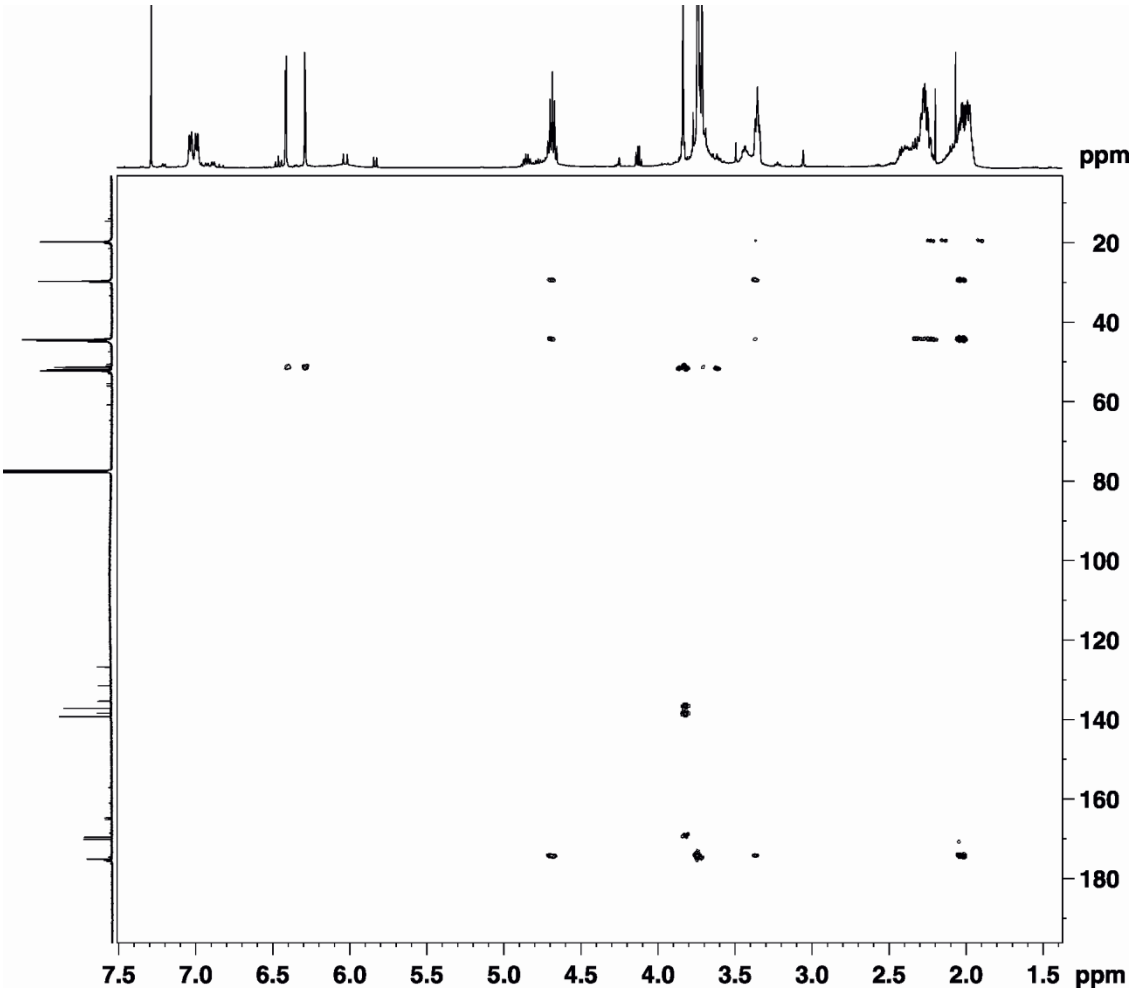


Figure S27. HMBC NMR spectrum of pseudodipeptide **33** in $CDCl_3$ recorded at 298 K (600 MHz)

NMR studies for pseudotetrapeptide 35

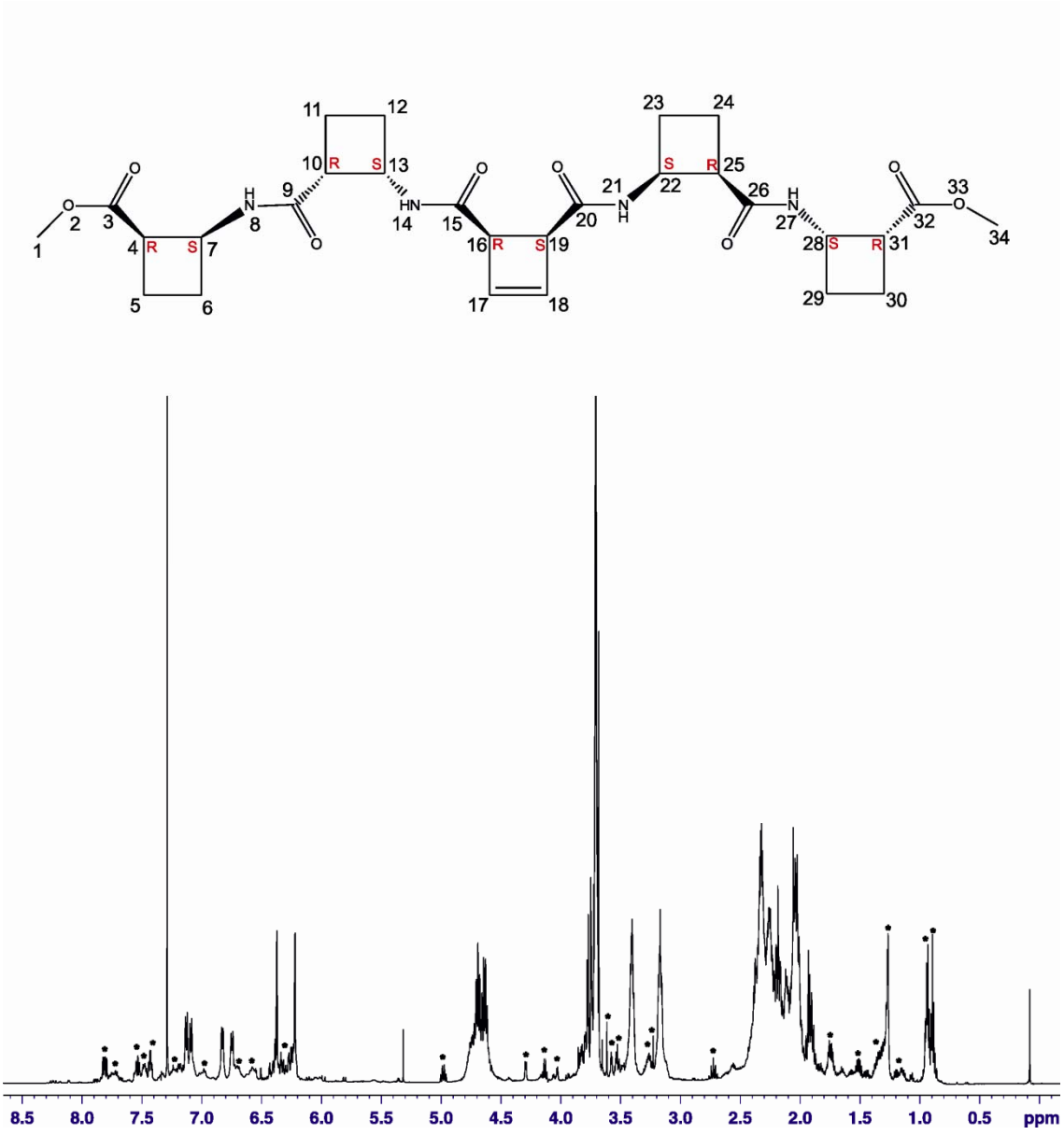


Figure S28. ^{1}H-NMR assignment of pseudotetrapeptide **35** in $CDCl_3$ recorded at 298 K (600 MHz)

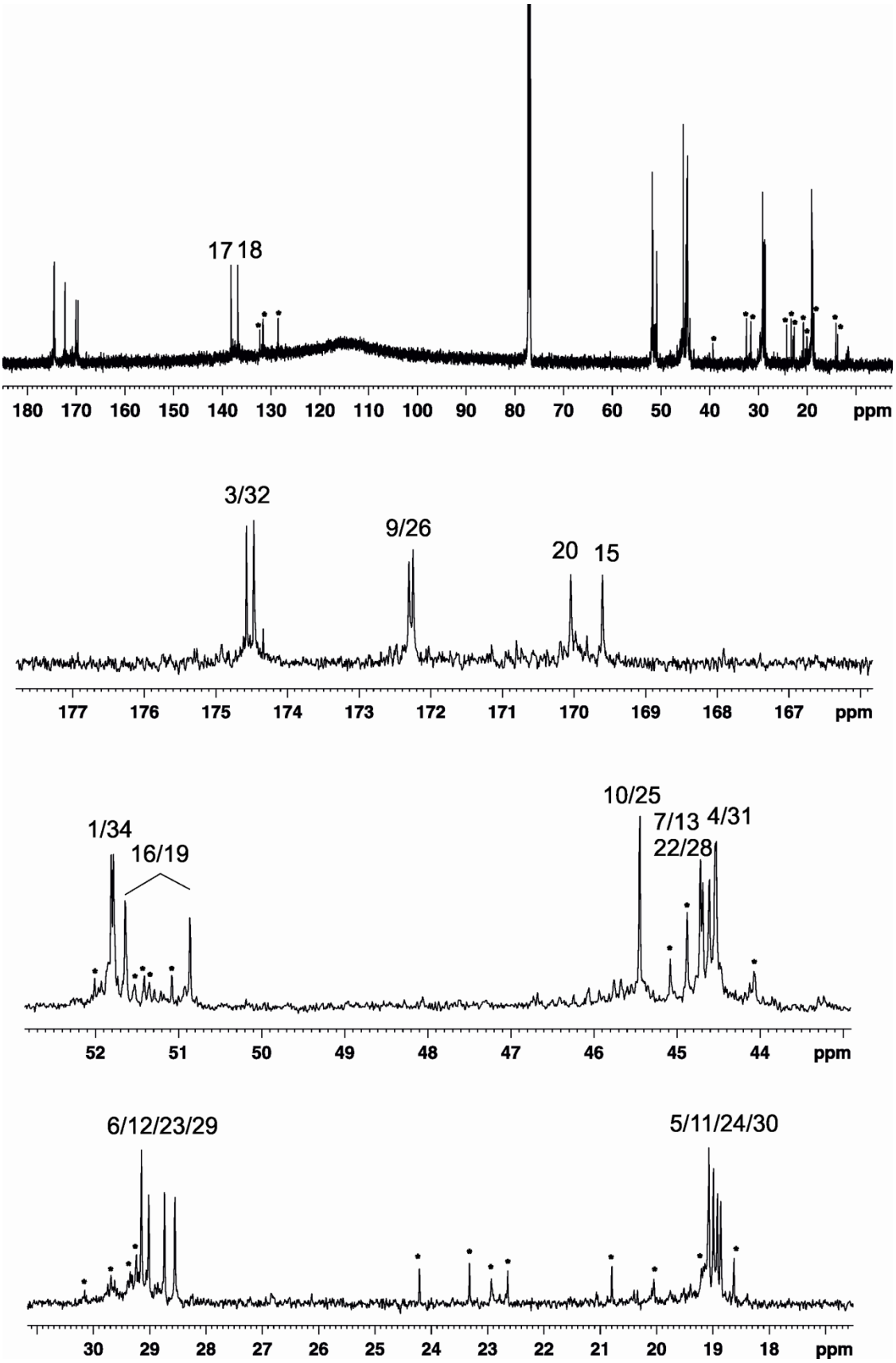


Figure S29. ^{13}C-NMR assignment of pseudotetrapeptide **35** in $CDCl_3$ recorded at 298 K (600 MHz)

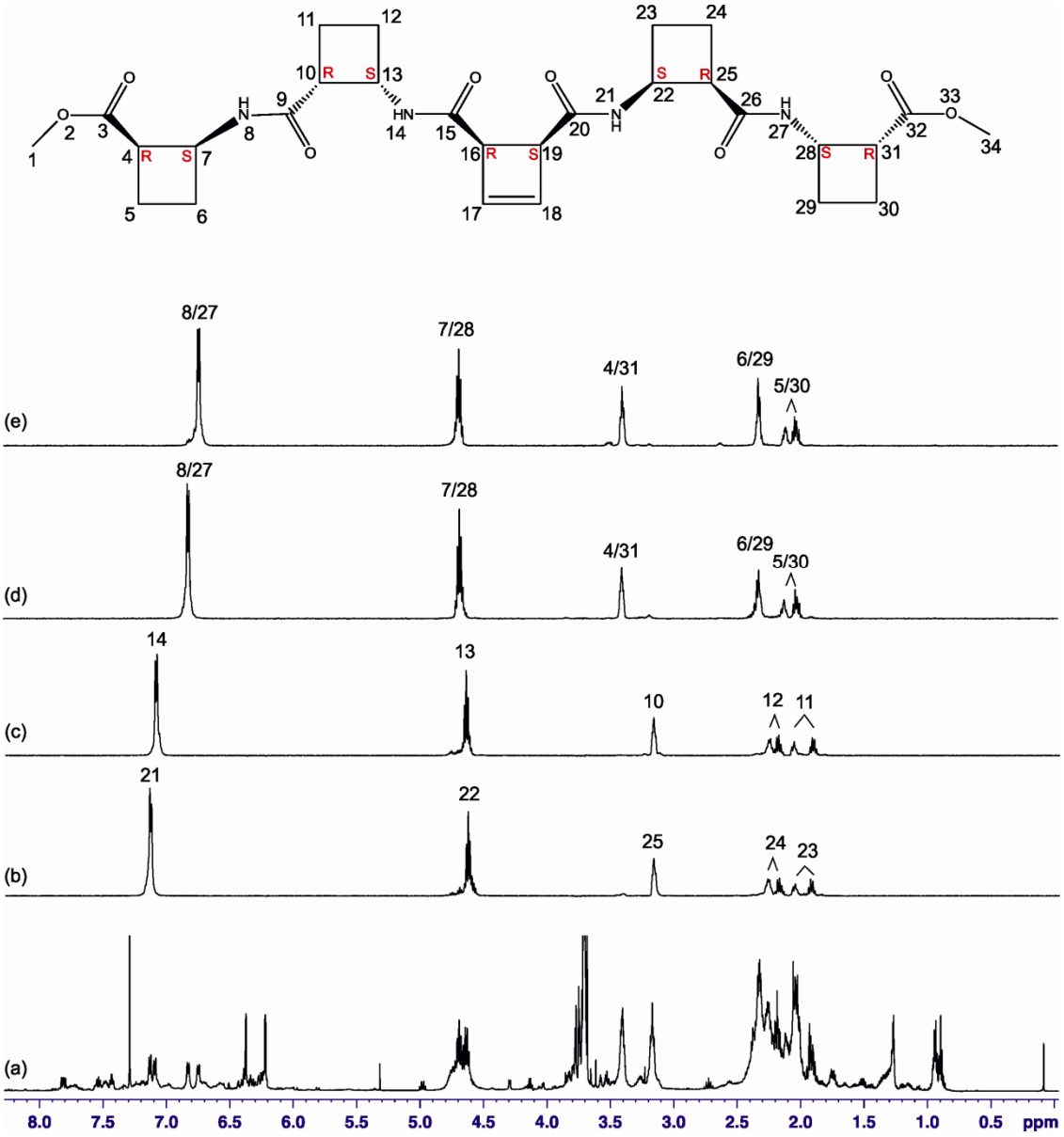


Figure S30. 1D selective TOCSY NMR experiments used for product characterization of pseudotetrapeptide **35**. TOCSY mixing time was set to 60 ms in all the experiments. Experiments were performed at 298 K in $CDCl_3$ (600 MHz). (a) ^{1}H-NMR for visual comparison purposes. (b) NH_{21} selective TOCSY. (c) NH_{14} selective TOCSY. (d) and (e) $NH_{8/27}$ selective TOCSY.

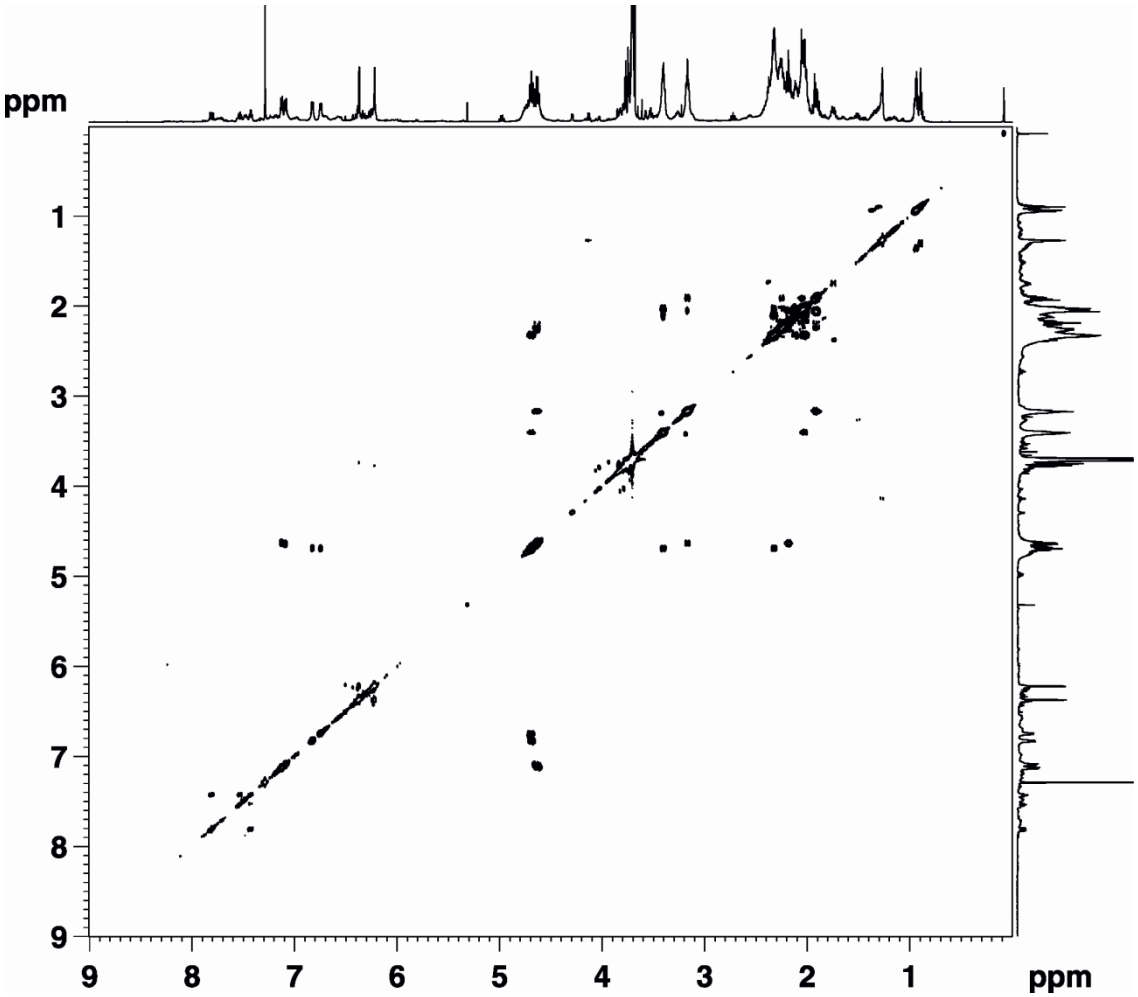


Figure S31. COSY NMR spectrum of pseudotetrapeptide **35** in $CDCl_3$ recorded at 298 K (600 MHz)

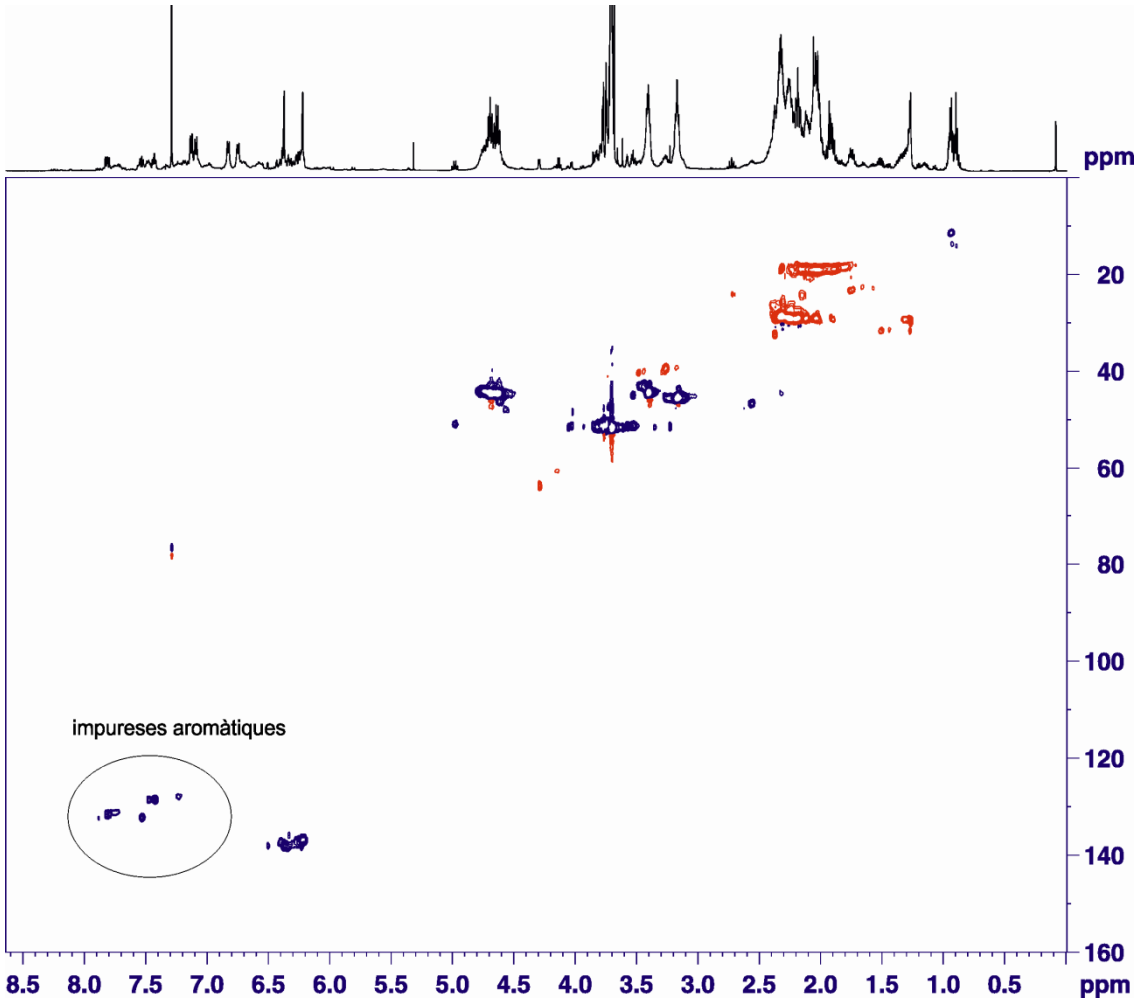


Figure S32. Edited HSQC spectrum for tetrapeptide **35** recorded at 298 K (600 MHz)

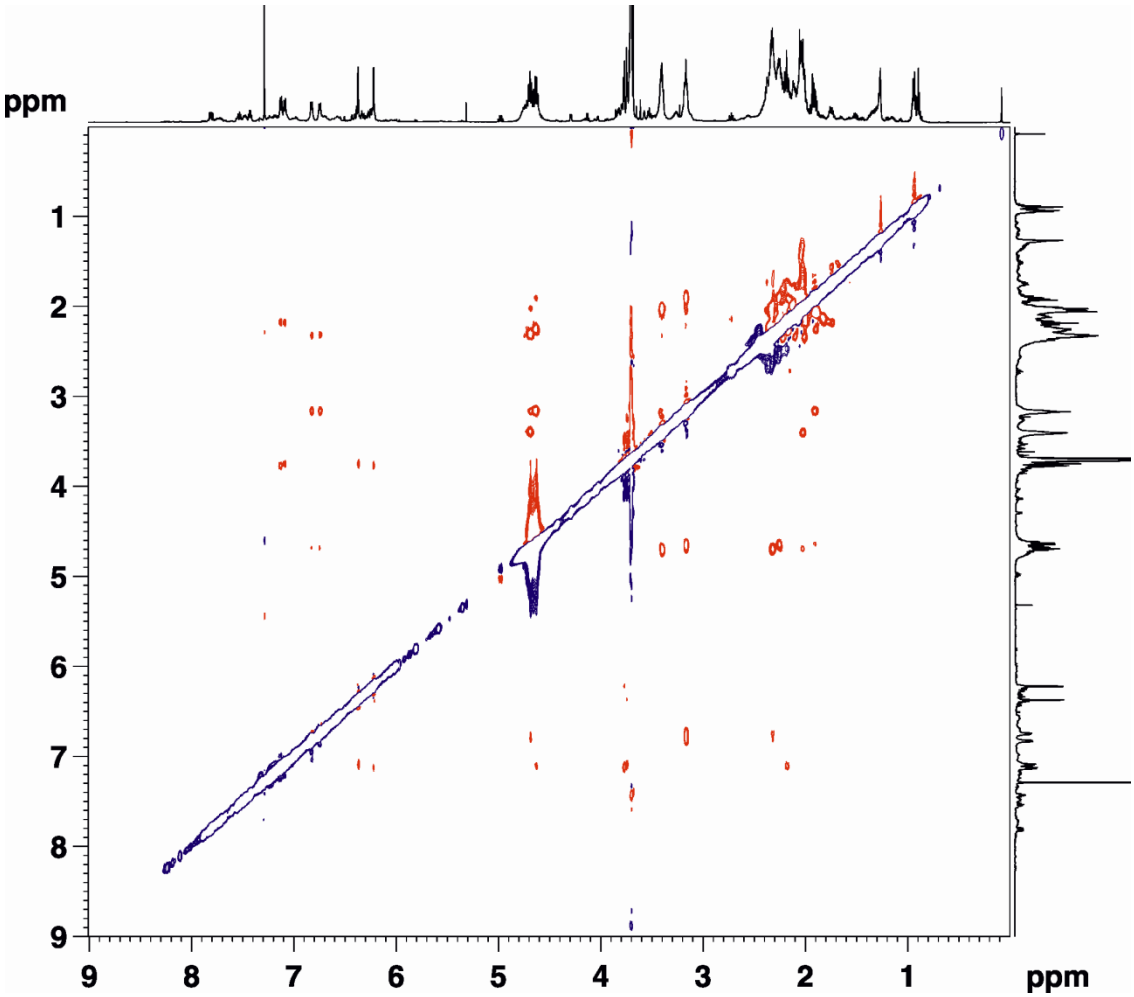


Figure S33. ROESY NMR spectrum of pseudotetrapeptide **35** in $CDCl_3$ recorded at 298 K (600 MHz)

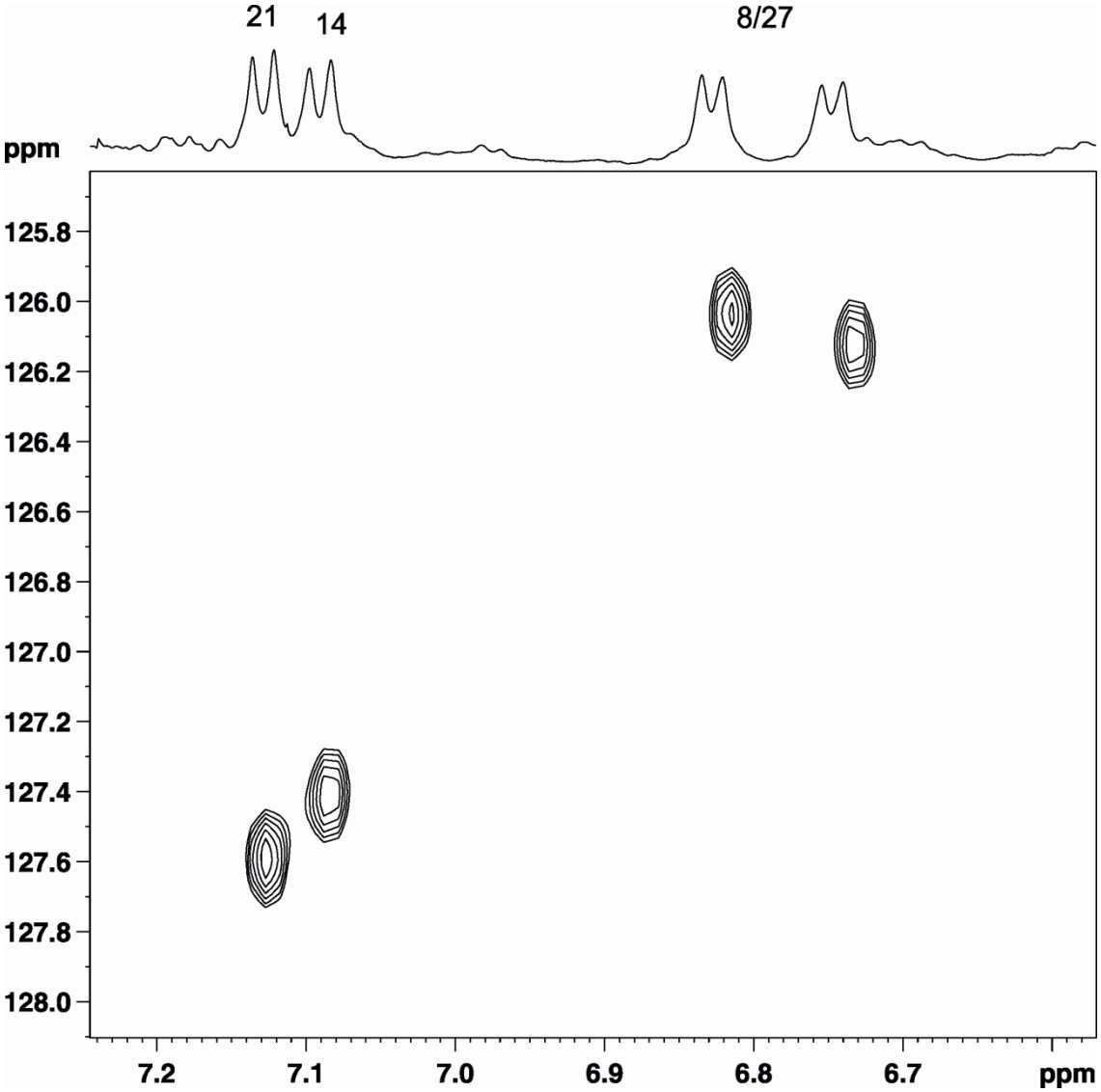
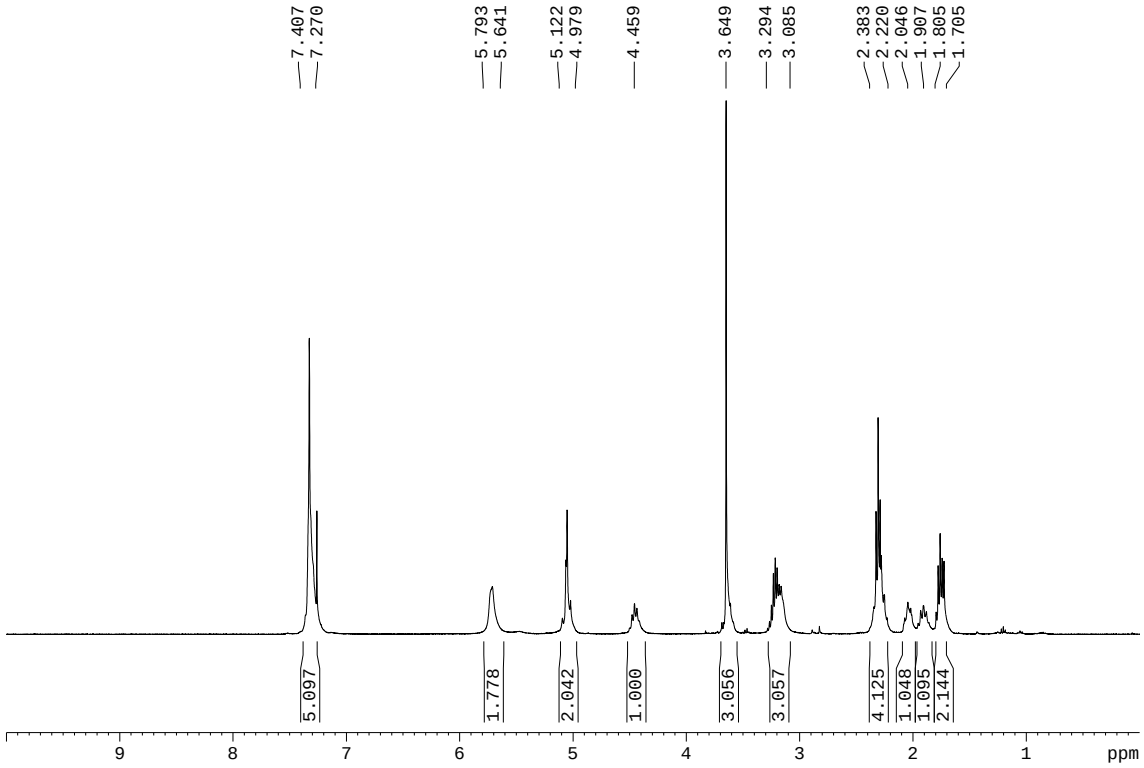
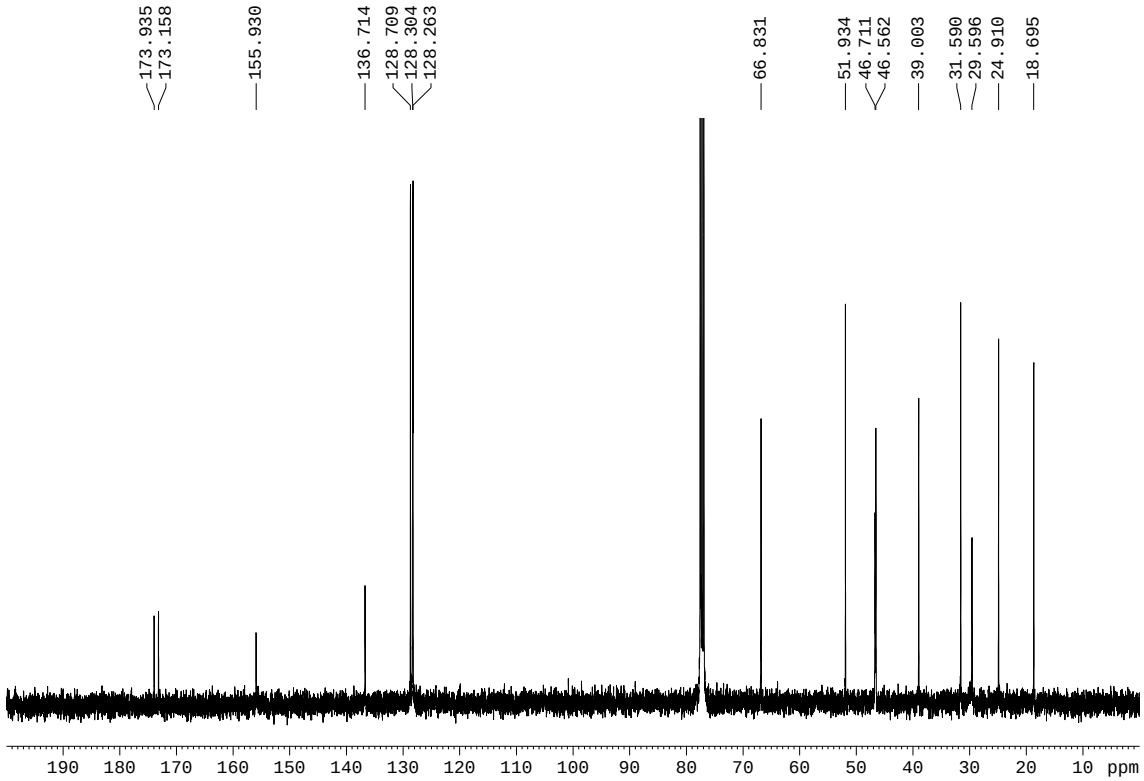


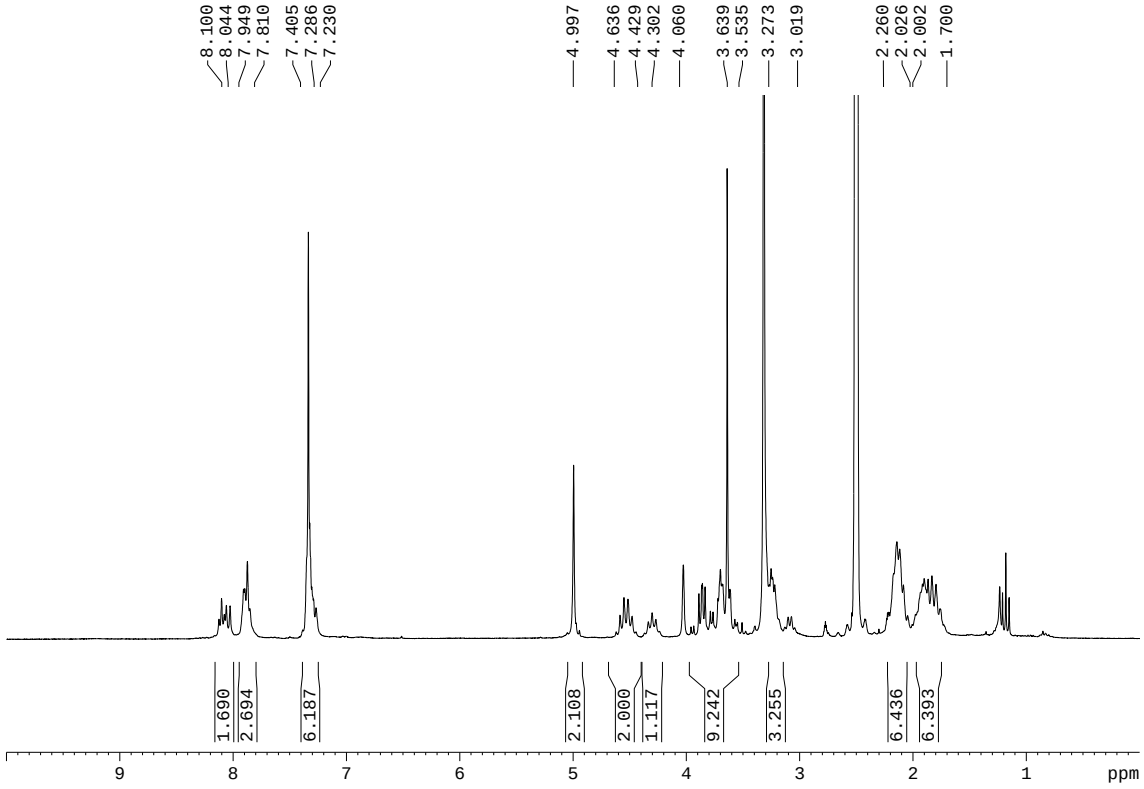
Figure S34. $^{1}H/^{15}N$-HSQC experiment of pseudotetrapeptide **35** recorded at 298 K (600 MHz)

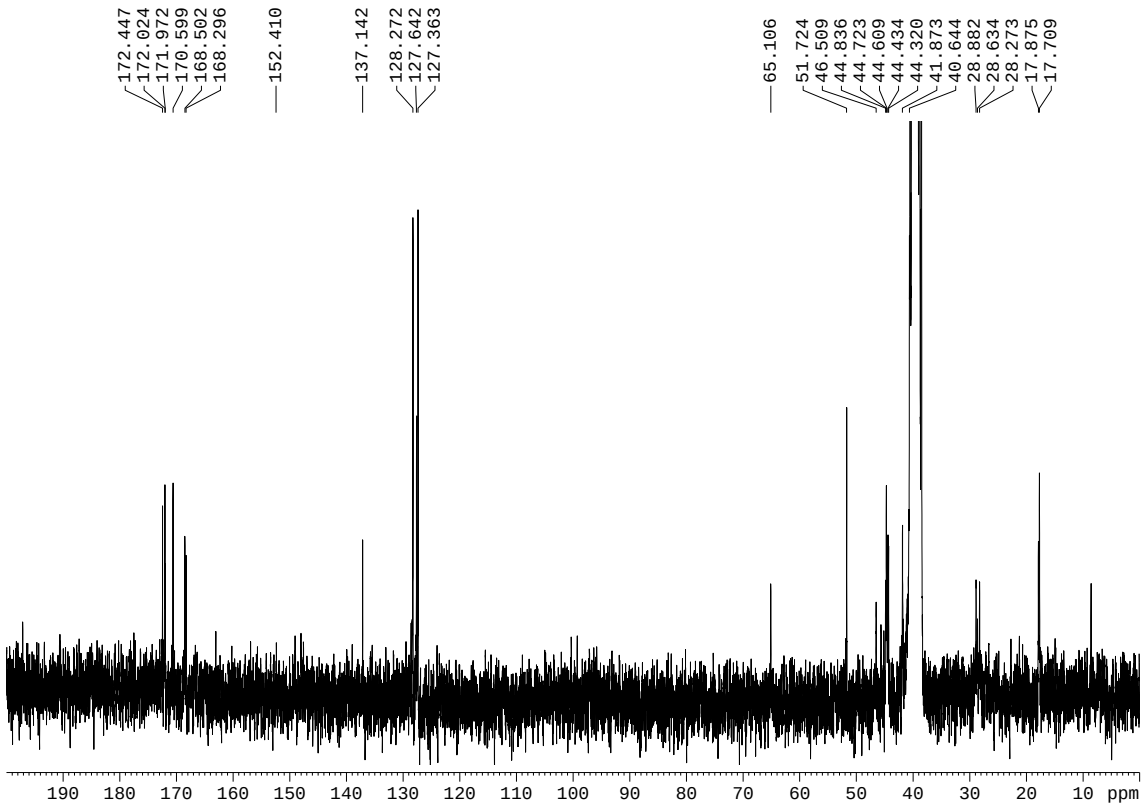
^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) spectra for compound 7



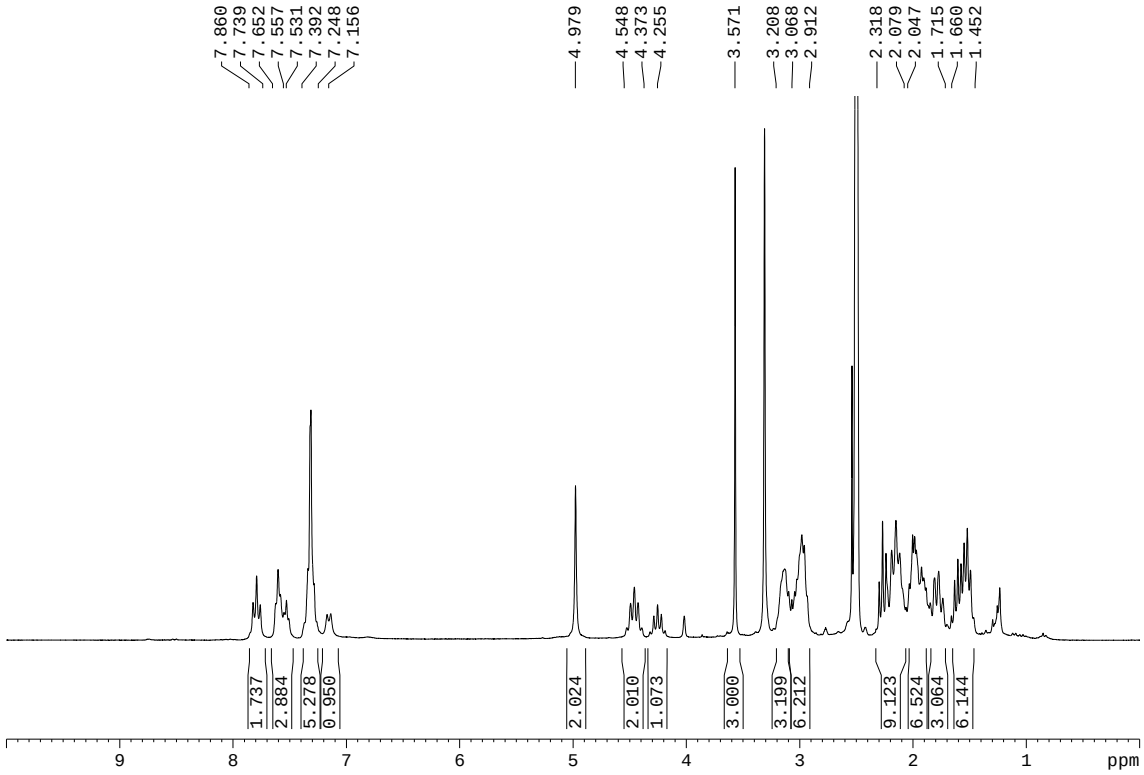


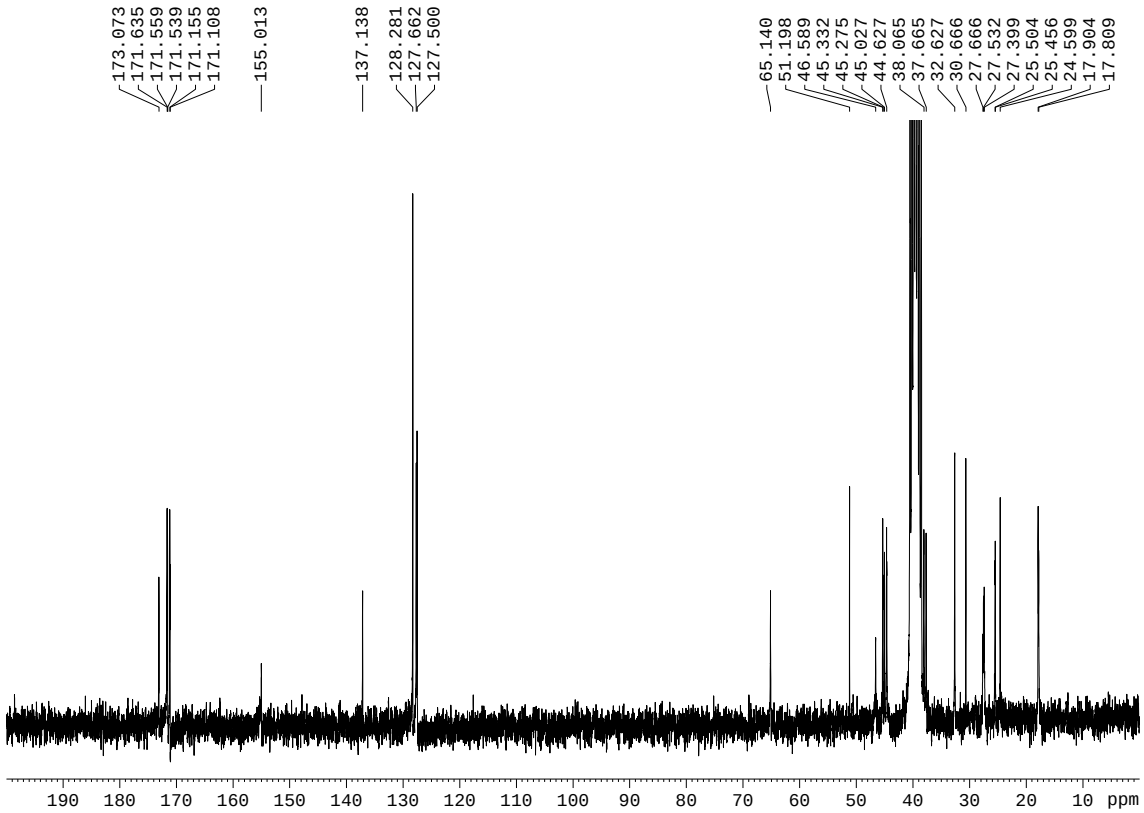
^{1}H-NMR (DMSO-d_6, 250 MHz) and ^{13}C-NMR (DMSO-d_6, 62.5 MHz) spectra for compound 23



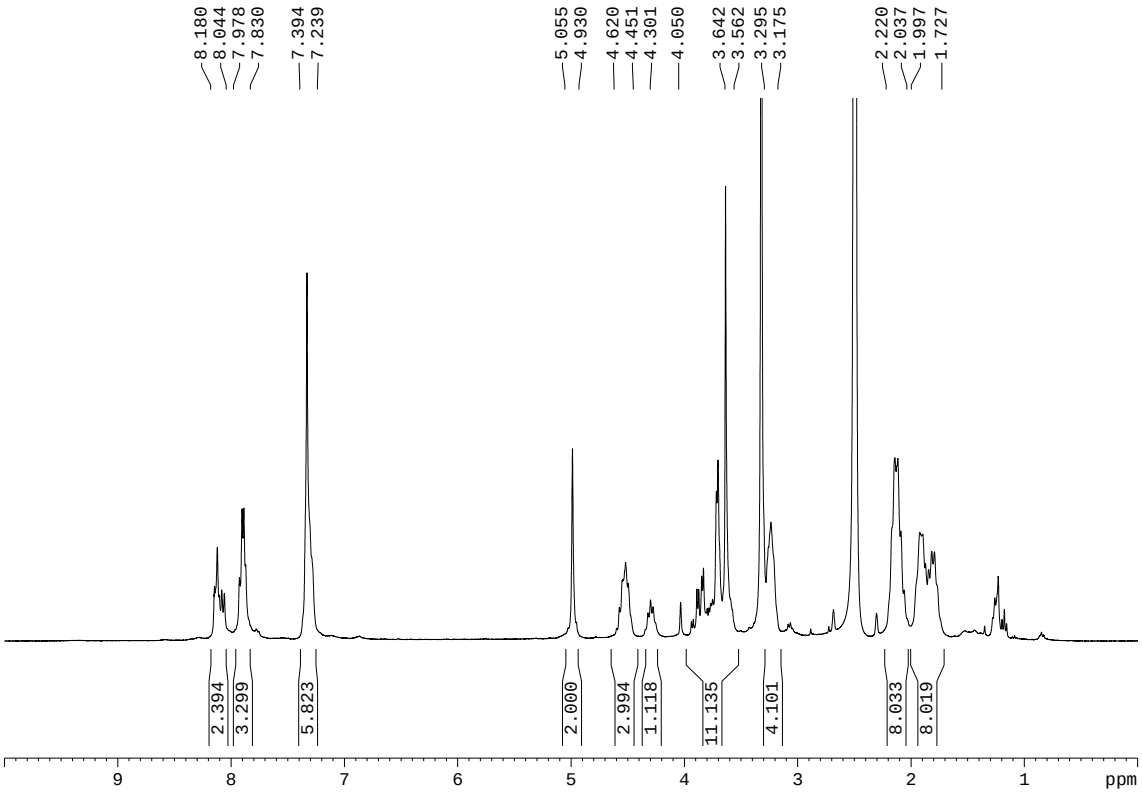


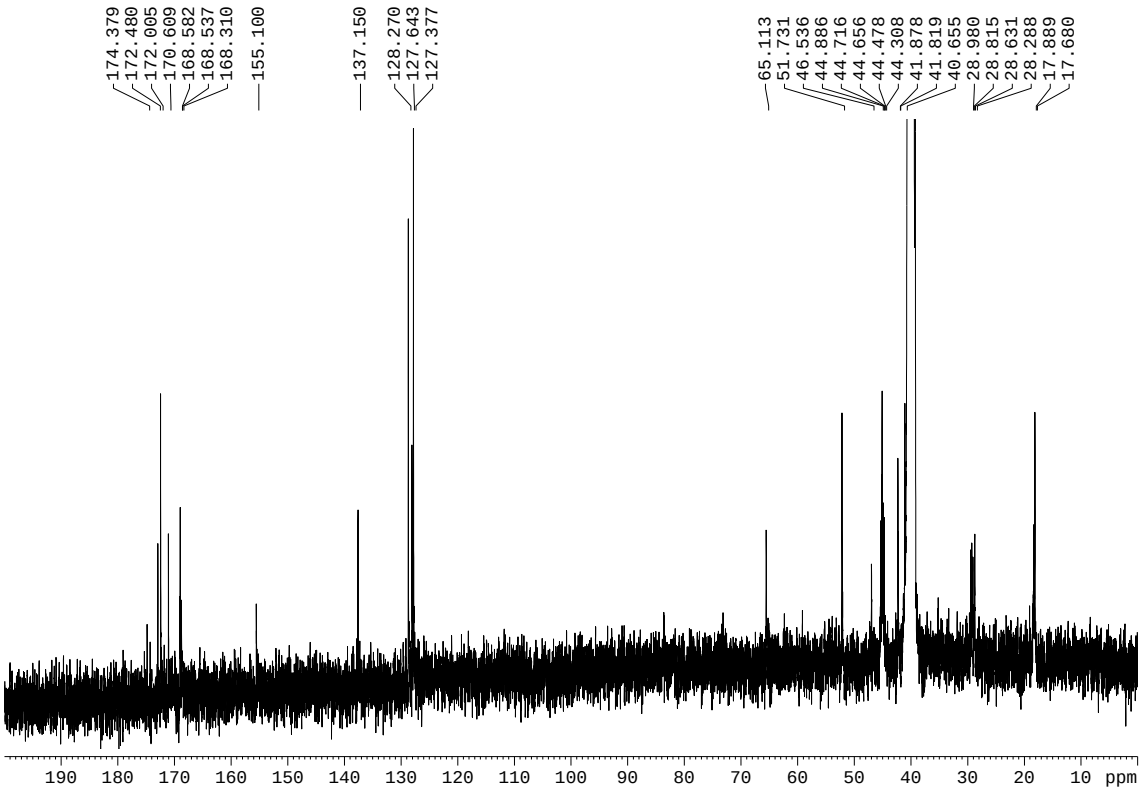
^{1}H-NMR (DMSO-d_6, 250 MHz) and ^{13}C-NMR (DMSO-d_6, 62.5 MHz) spectra for compound 24



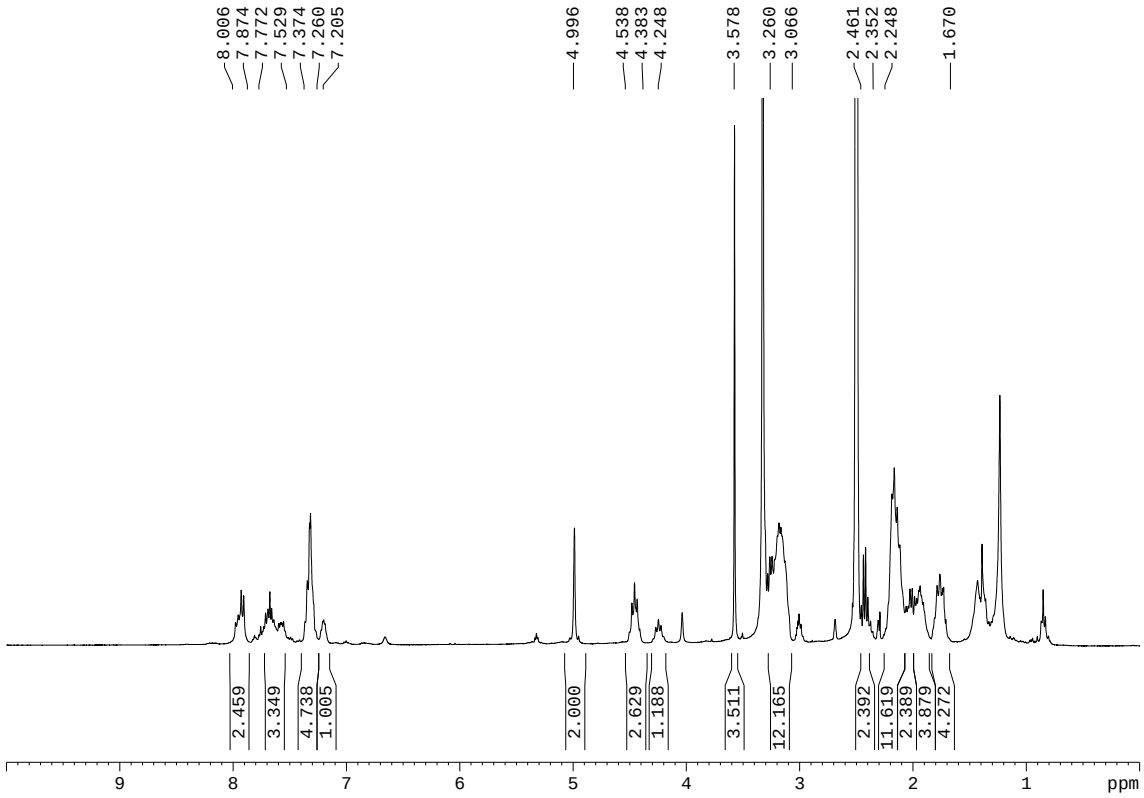


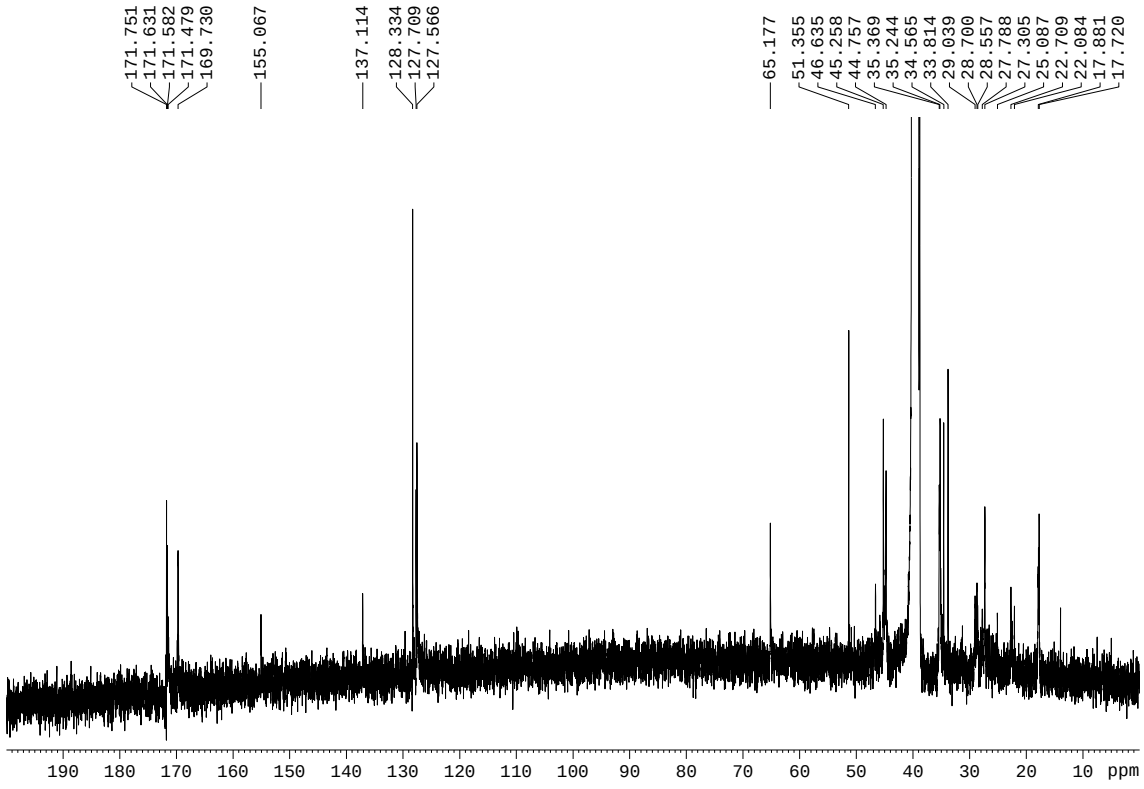
^{1}H-NMR (DMSO-d_6, 360 MHz) and ^{13}C-NMR (DMSO-d_6, 90 MHz) spectra for compound 25



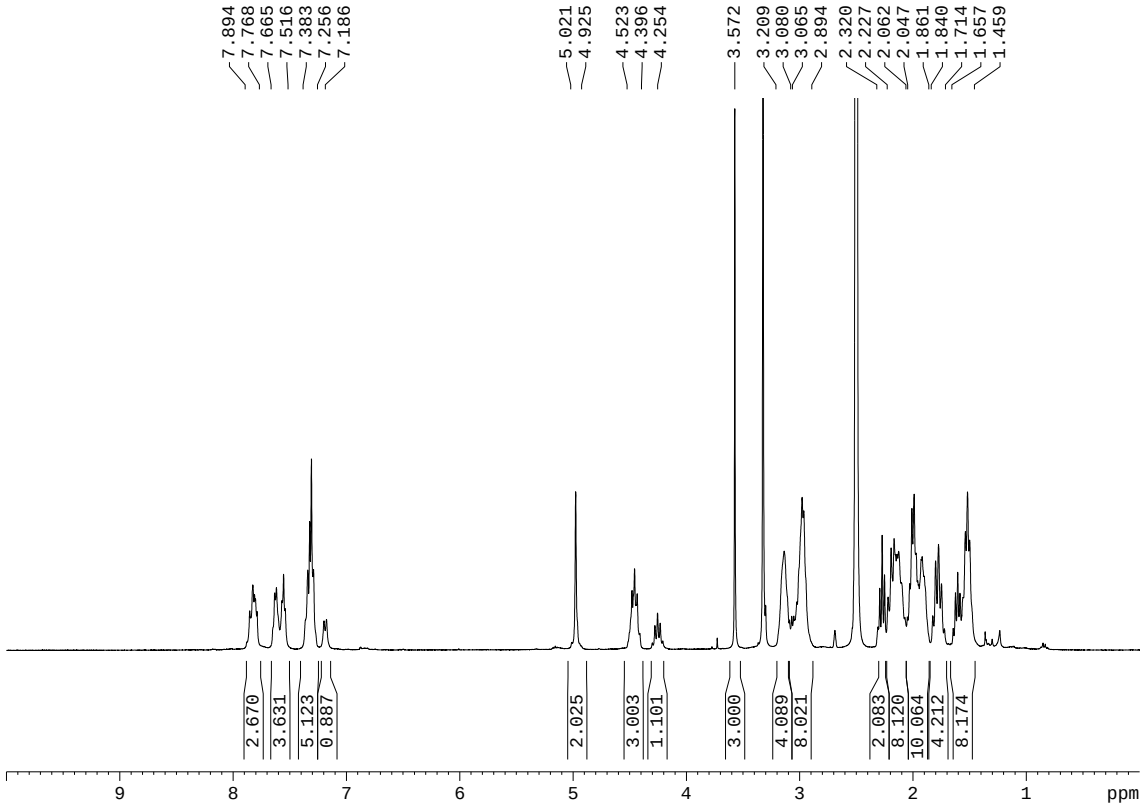


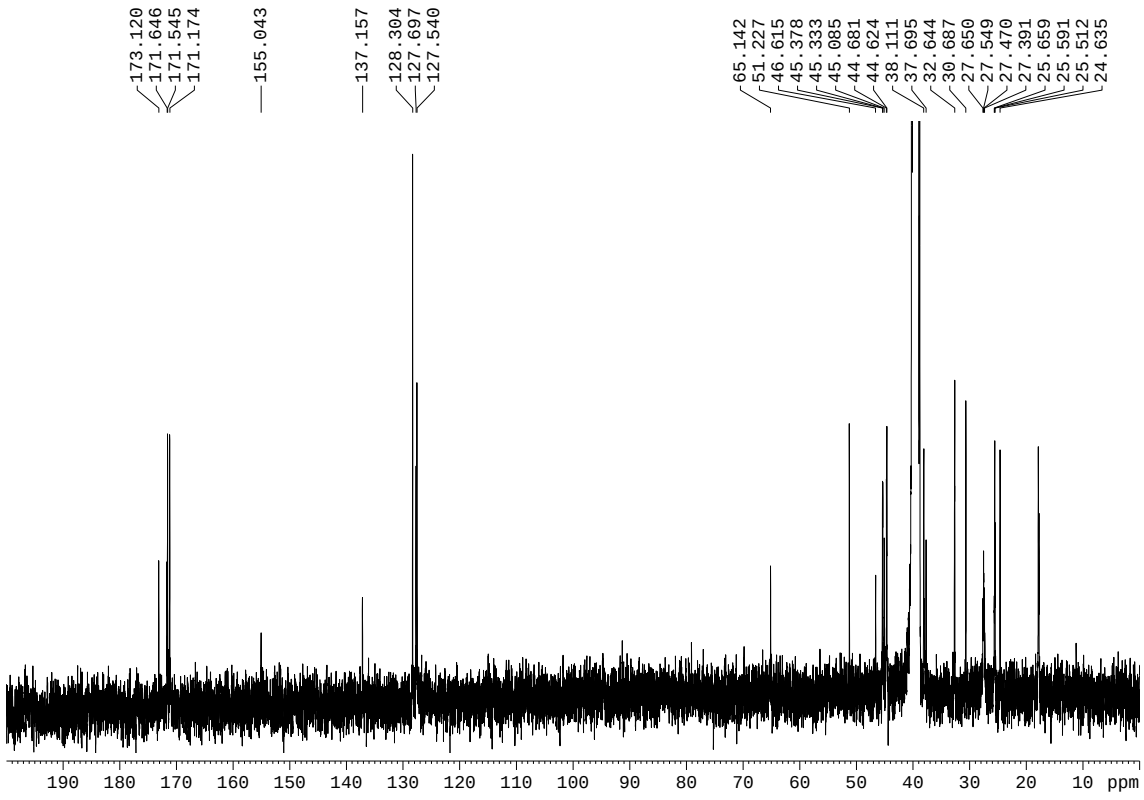
^{1}H-NMR (DMSO-d_6, 360 MHz) and ^{13}C-NMR (DMSO-d_6, 90 MHz) spectra for compound 26





^{1}H-NMR (DMSO-d_6, 360 MHz) and ^{13}C-NMR (DMSO-d_6, 90MHz) spectra for compound 27





Computational calculations

In order to understand the different conformations for the 3 tetrapeptides, theoretical calculations were carried out. β-alanine tetrapeptide **15** had been studied previously, so the study was performed with the glycine tetrapeptide **14** and the GABA one **16**.

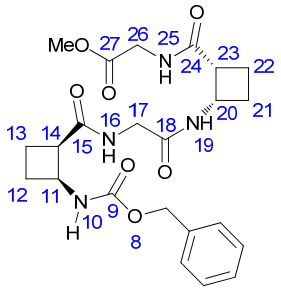


Figure S35. Numbering for tetrapeptide **14**

The calculation procedure started with restrictions extracted from NMR data. ROE values were used to define three groups of distances: 3, 4 and 5 Å, respectively, for strong, medium and weak signals (see Table 1). A margin of ± 0,5 Å was allowed for all distance restrictions.

Table 1. Distances extracted from NMR data of tetrapeptide **14**.

ROE restrictions	**Strong**	**Medium**	**Weak**
	NH_{16}-H_{14}	NH_{25}-H_{23}	NH_{10}-H_{14}
	NH_{25}-H_{26R}	NH_{16}-H_{17R}	NH_{10}-H_{12S}
		NH_{19}-H_{20}	
		NH_{19}-H_{17R}	
		NH_{19}-H_{17S}	
		NH_{19}-H_{21S}	
Distances (Å)	3 ± 0,5 Å	4 ± 0,5 Å	5 ± 0,5 Å

Apart from the ROE data, *J* coupling values were used to extract dihedral angles. $^{3}J_{NH10\text{-}H11}$ is 9.5 Hz, so applying Poulsen's equation,[7] the dihedral angle for NH_{10}-N-C_{11}-H_{11} is 180°. $^{3}J_{NH19\text{-}H20}$ is 8.5 Hz so the dihedral angle (H_{19}-N_{19}-C_{20}-H_{20}) associated is ±

158°. $^{3}J_{NH16\text{-}H17S}$ is 6.0 Hz, that is consistent with four dihedral angles (H_{16}-N_{16}-C_{17}-H_{17}): ± 23° and ± 137° whereas $^{3}J_{NH16\text{-}H17R}$ is 4.7 Hz, which represents dihedral angles of ± 37° and ± 127°. Another dihedral restriction due to glycine CH_2 protons diastereotopicity was considered for the calculations: N_{16}-C_{17}-C_{18}-N_{19}: ± 120 °. In all cases a margin of ± 20° was allowed. The possibilities arisen from these values which have a reasonable geometric sense are summarized in Table 2.

Table 2. Possible combinations of dihedral angles and resulting structure possibilities for tetrapeptide **14**.

	Structure possibilities							
Dihedral angles	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
H_{10}-N_{10}-C_{11}-H_{11}	180	180	180	180	180	180	180	180
H_{19}-N_{19}-C_{20}-H_{20}	158	-158	158	-158	158	-158	158	-158
H_{16}-N_{16}-C_{17}-H_{17R}	23	23	23	23	-23	-23	-23	-23
H_{16}-N_{16}-C_{17}-H_{17S}	127	127	127	127	127	127	127	127
N_{16}-C_{17}-C_{18}-N_{19}	120	120	-120	-120	120	120	-120	-120

	Structure possibilities							
Dihedral angles	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
H_{10}-N_{10}-C_{11}-H_{11}	180	180	180	180	180	180	180	180
H_{19}-N_{19}-C_{20}-H_{20}	158	-158	158	-158	158	-158	158	-158
H_{16}-N_{16}-C_{17}-H_{17R}	-137	-137	-137	-137	137	137	137	137
H_{16}-N_{16}-C_{17}-H_{17S}	-37	-37	-37	-37	-127	-127	-127	-127
N_{16}-C_{17}-C_{18}-N_{19}	120	120	-120	-120	120	120	-120	-120

These 16 possibilities, each of them with the distance restrictions shown in Table 1, were submitted to a conformational search. A mixed Monte-Carlo[8] /Low-Mode[9] Conformational Search was done using the MMFF (Merck Molecular Force Field)[10] force field implemented in the Macromodel 9.8 program.[11] The solvent effect was included using the GB/SA[12] method implemented in Macromodel with chloroform as

solvent. For each of the starting geometries, if the number of structures computed within a 1 kcal/mol range was too big a second conformational search was carried out limiting the margin of the angles to ± 10°. If the number of structures obtained within 1 kcal/mol was too small, the conformers within 2 kcal/mol were also considered. After the conformational search was carried out, the selected structures within the defined energy range were grouped into different families if necessary, with the criteria of similar NH-CO interactions. The most stable structure (lowest energy) of each of these families was optimized at B3LYP[13] /6-31G(d) level of theory in gas phase with Gaussian09 package.[14] To ensure that the calculated structure was a minimum, a frequency calculation was also run. Table 3 summarizes these results.

Table 3. Number of conformers within 1 kcal/mol for each structure possibility and number of optimized structure in each group for tetrapeptide **14**.

Structure possibilities	**1**	**2**	**3**[a]	**4**[a]	**5**	**6**	**7**[a]	**8**[a]
Conformers within 1 kcal/mol	14	23	38	9	8	7	7	1(4)[b]
Optimized conformers at B3LYP/6-31G(d)	1	1	0[c]	2	2	0[c]	1	1

Structure possibilities	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Conformers within 1 kcal/mol	10	4	17	1(4)[b]	5	2(3)[b]	13	22
Optimized conformers at B3LYP/6-31G(d)	1	1	3	1	1	1	2	2

[a] Conformational search with angle variation of ± 10°

[b] Number of conformers within 1 kcal/mol too small, so conformers within 2 kcal/mol are taken.

[c] Not optimized at the B3LYP/6-31G(d) level of theory because the conformers found are very similar to others of different groups.

The energies of all the computed structures were compared in terms of ΔG and finally structure A (resulting from starting structure 8) was the most stable one. Structures and relative energies of the 3 following most stable conformers are shown in Figure S36.

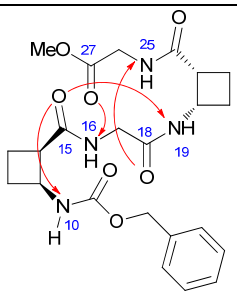
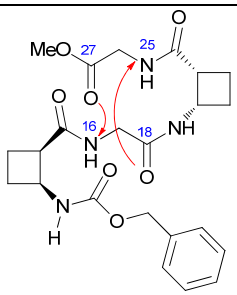
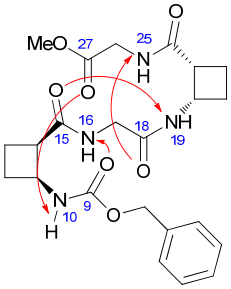
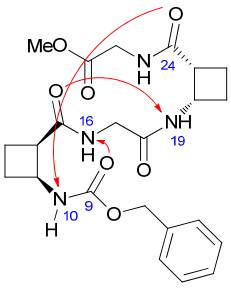
A (from 8) ΔG^0_{rel} = 0.0 kcal/mol	**B (from 12) ΔG^0_{rel} = 1.4 kcal/mol**
	
NH_{10}-CO_{15} : 2.53 Å NH_{16}-CO_{27}: 2.14 Å NH_{19}-CO_{15}: 2.08 Å NH_{25}-CO_{18}: 1.95 Å	NH_{16}-CO_{27}: 1.99 Å NH_{25}-CO_{18}: 2.04 Å
C (from 8) ΔG^0_{rel} = 2.2 kcal/mol	**D (from 7) ΔG^0_{rel} = 2.4 kcal/mol**
	
NH_{10}-CO_{27} : 1.82 Å NH_{16}-CO_{9}: 2.04 Å NH_{19}-CO_{15}: 1.94 Å NH_{25}-CO_{18}: 1.99 Å	NH_{10}-CO_{24} : 2.03 Å NH_{16}-CO_{9}: 2.04 Å NH_{19}-CO_{15}: 1.94 Å

Figure S36. Structure of the 4 most stable conformers computed for tetrapeptide **14**.

The most stable calculated conformer presents 4 possible hydrogen bonds between NH_{16}-CO_{27}, CO_{18}-NH_{25} and a bifurcated one between CO_{15} and NH_{10} and NH_{19},

respectively (Figure S37). This structure is consistent with the NMR data and represents a β-sheet type folding.

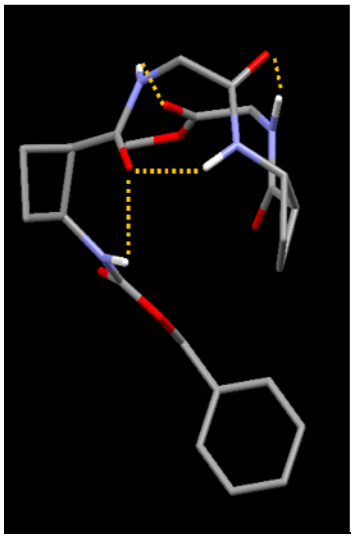


Figure S37. Most stable conformer for tetrapeptide **14**.

Results for tetrapeptide **16** were obtained in a similar manner and are shown below.

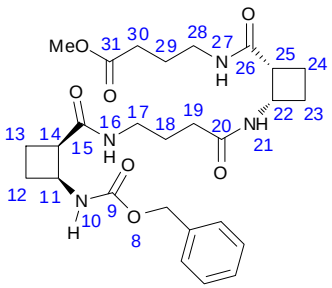


Figure S38. Numbering for tetrapeptide **16**

The calculation procedure started with restrictions extracted from NMR data. ROE values were used as explained for glycine tetrapeptide **14**. (see Table 4).

Table 4. Distances extracted from NMR data of tetrapeptide **16**.

ROE restrictions	**Strong**	**Medium**	**Weak**
	NH_{16}-H_{17R}	NH_{21}-H_{22}	NH_{16}-H_{18}
	NH_{27}-H_{28}	NH_{21}-H_{19}	NH_{16}-H_{17S}
		NH_{21}-H_{23S}	NH_{27}-H_{30}
		NH_{10}-H_{12}	NH_{27}-H_{29}
			NH_{21}-H_{25}
Distances (Å)	3 ± 0,5 Å	4 ± 0,5 Å	5 ± 0,5 Å

Apart from the ROE data, *J* coupling values were used to extract dihedral angles. $^{3}J_{NH10\text{-}H11}$ is 8.3 Hz, so applying the equation from Poulsen,[7] the dihedral angle for NH_{10}-N-C_{11}-H_{11} is ± 156°. $^{3}J_{NH19\text{-}H20}$ is 8.5 Hz so the dihedral angle (H_{19}-N_{19}-C_{20}-H_{20}) associated is ± 160°. $^{3}J_{NH16\text{-}H17S}$ is 4.7 Hz, that is consistent with four dihedral angles (H_{16}-N_{16}-C_{17}-H_{17}): ± 29° and ± 133° and $^{3}J_{NH16\text{-}H17R}$ is 6.0 Hz, which represents dihedral angles of approximately ± 29° and ± 133°, also. These last two values represent 9 possible combinations which together with the other 4 dihedral angles make a total of 36 possible combinations. In view of this situation another approximation was used to obtain starting dihedral angles for H_{16}-N_{16}-C_{17}-H_{17}. Based on a work done with a glycine amide[15] the difference of chemical shift in ppm of two diastereotopic protons was correlated with a dihedral angle associated. The difference in ppms for protons H_{17} is 0.15 ppm and for protons H_{28} is 0.06 ppm. These values correlate with dihedral angles of 75° (N_{16}-C_{17}-C_{18}-C_{19}) and 85° (N_{27}-C_{28}-C_{29}-C_{30}), respectively (see Figure S39).

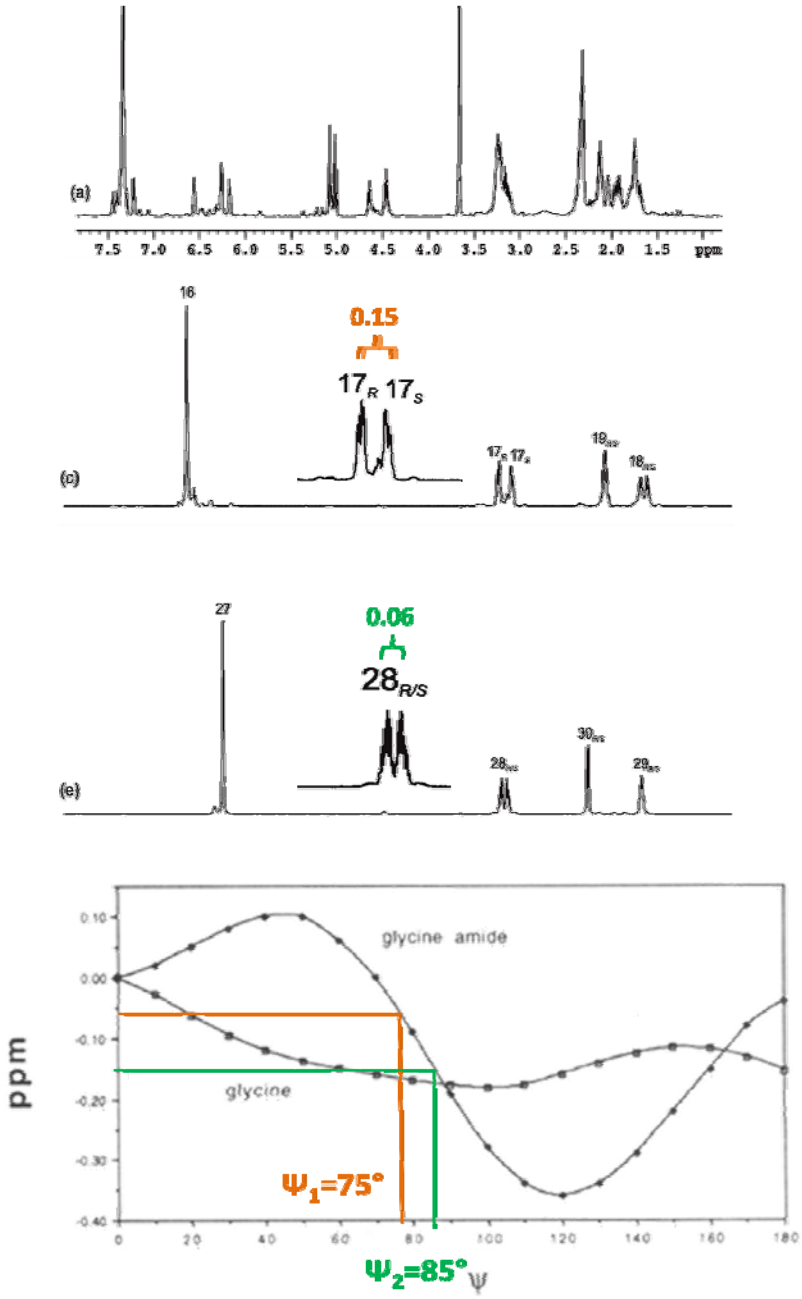


Figure S39. Differences in chemical shifts for protons H_{17} and H_{28} of tetrapeptide **16**. Correlation with dihedral angles (figure extracted from reference 15).

The possibilities arisen from these values which have a reasonable sense are summarized in Table 5. In all cases a margin of ± 20° was allowed.

Table 5. Possible combinations of dihedral angles and resulting structure possibilities for tetrapeptide **16**.

	Structure possibilities			
Dihedral angles	**1**	**2**	**3**	**4**
H_{10}-N_{10}-C_{11}-H_{11}	156	-156	156	-156
H_{16}-N_{16}-C_{17}-$H_{17R/S}$	85	85	85	85
H_{27}-N_{27}-C_{28}-$H_{28R/S}$	75	75	75	75
H_{21}-N_{21}-C_{22}-N_{22}	160	160	-160	-160

These 4 possibilities, each of them with the distance restrictions shown in Table 4, were submitted to a conformational search. For each of the starting geometries, if the number of structures computed within a 1 kcal/mol range was too big a second conformational search was carried out limiting the margin of the angles to ± 10^{o}. If the number of structures obtained within 1 kcal/mol was too small, the conformers within 2 kcal/mol were also considered.

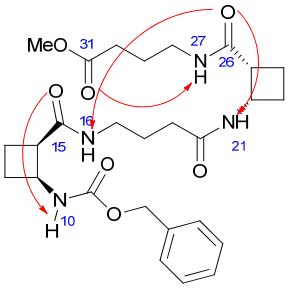
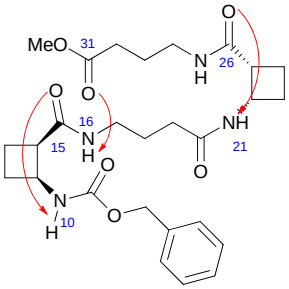
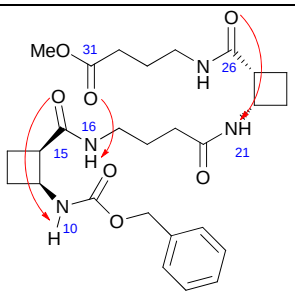
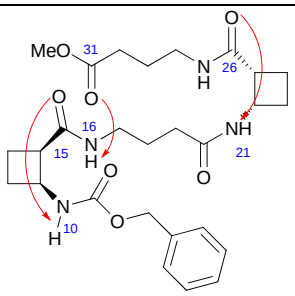
After the conformational search was carried out, the selected structures within the defined energy range were grouped into different families if necessary, with the criteria of similar NH-CO interactions, and the most stable structure (lowest energy) of each of these families was optimized at the B3LYP/6-31G(d) level of calculation in the gas phase as before. To ensure that the calculated structure was a minimum, a frequency calculation was also run. Results are summarized in Table 6.

Table 6. Number of conformers within 1 kcal/mol for each structure possibility and number of optimized structure in each group for tetrapeptide **16**.

Structure possibilities	**1**	**2**	**3**	**4**
Conformers within 1 kcal/mol	48	46	39 (104)[a]	68
Optimized conformers at B3LYP/6-31G(d)	2	4	3	4

[a] Number of conformers within 1 kcal/mol too small, so conformers within 2 kcal/mol are taken.

The energies of all the computed structures were compared in terms of ΔG and finally structure E (resulting from starting structure 4) was the most stable one. Structures and relative energies of the 3 following most stable conformers are shown too in Figure S40.

E (from 4) ΔG^0_{rel} = 0.0 kcal/mol	**F (from 2) ΔG^0_{rel} = 1.9 kcal/mol**
	
NH_{10}-CO_{15} : 2.41 Å NH_{16}-CO_{26}: 2.06 Å NH_{21}-CO_{26}: 2.15 Å NH_{27}-CO_{31}: 1.93 Å	NH_{10}-CO_{15}: 2.67 Å NH_{16}-CO_{31}: 2.06 Å NH_{21}-CO_{26}: 2.03 Å
G (from 1) ΔG^0_{rel} = 2.5 kcal/mol	**D (from 3) ΔG^0_{rel} = 2.7 kcal/mol**
	

NH_{10}-CO_{15}: 2.00 Å NH_{16}-CO_{31}: 2.04 Å NH_{21}-CO_{26}: 2.07 Å	NH_{10}-CO_{15}: 2.00 Å NH_{16}-CO_{31}: 2.04 Å NH_{21}-CO_{26}: 2.07 Å

Figure S40. Structure of the 4 most stable conformers computed for tetrapeptide **16**.

The most stable calculated conformer presents 4 possible hydrogen bonds between NH_{10}-CO_{15}, NH_{27}-CO_{31} and a bifurcated one between CO_{26} and NH_{16} and NH_{21}, respectively. This structure is consistent with the NMR data and represents a β-sheet type folding (Figure S41).

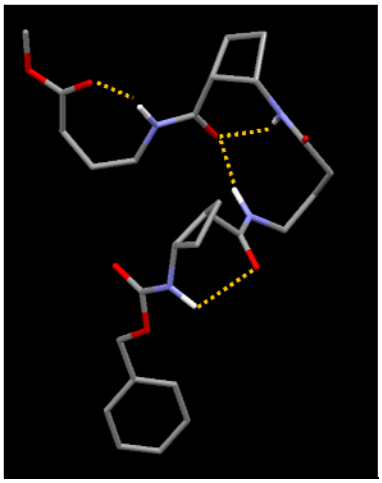


Figure S41. Most stable conformer for tetrapeptide **16**.

References:

[1] D. Fernández, E. Torres, F. X. Avilés, R. M. Ortuño and J. Vendrell, *Bioorg. Med. Chem.*, 2009, **17**, 3824.

[2] M. Martín-Vilà, E. Muray, G. P. Aguado, Á. Álvarez-Larena, V. Branchadell, C. Minguillón, E. Giralt and R. M. Ortuño, *Tetrahedron: Asymmetry*, 2000, **11**, 3569.

[3] S.Izquierdo, M. J. Kogan, T. Parella, A. G. Moglioni, V. Branchadell, E. Giralt and R. M. Ortuño, *J. Org. Chem.*, 2004, **69**, 5093.

[4] L.-X. Zhao, J. G. Park, Y.-S. Moon, A. Basnet, J. Choi, E.-K. Kim, T. C. Jeong, Y. Jahng, E.-S. Lee, *Il Farmaco,* 2004, **59**, 381.

[5] N. Gauvry, C. Comoy, C. Lescop, F. Huet, *Synthesis*, 1999, **4**, 574.

[6] M. Perrott, B. Novak, *Macromolecules*, 1996, **29**, 1817.

[7] S. Ludvigsen, K. V. Andersen and F. M. Poulsen, *J. Mol. Biol.,* 1991, **217**, 731.

[8] (a) G. Chang, W. C. Guida and W. C. Still, *J. Am. Chem. Soc*., 1989, **111**, 4379.; (b) G. Chang, W. C. Guida and W. C. Still, *J. Am. Chem. Soc*., 1990, **111**, 1419.

[9] (a) I. Kolossváry and W. C. Guida, *J. Am. Chem. Soc*., 1996, **118**, 5011.; (b) I. Kolossváry and W. C. Guida, *J. Comput. Chem.*, 1999, **20**, 1671.

[10] T. A. Halgren, *J. Comput. Chem*., 1996, **17,** 490.

[11] (a) F. Mohamadi, N. G. H. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, D. Qiu, P. S. Shenkin, F. P. Hollinger and W. C. Still, *J. Phys. Chem. A*, 1997, **101**, 3005.; (b) MacroModel 9.8 (2010) Schrodinger, LLC, New York, NY.

[12] D. Qiu, P. S. Shenkin, F. P. Hollinger and W. C. Still, *J. Phys. Chem. A*., 1997, **101**, 3005.

[13] (a) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.; (b) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785..

[14] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone , B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li , H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J .L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, Jr. J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B.

Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, (2009) Gaussian 09, Revision B.01. Gaussian, Inc., Wallingford CT.

[15] H. M. Sulzbach, P. v. R. Schleyer and H. F. Schaefer III, *J. Am. Chem. Soc.*, 1994, **116**, 3967.