Supplementary Material (ESI) for Organic & Biomolecular Chemistry

ELECTRONIC SUPPLEMENTARY INFORMATION

The chemical ligation of selectively *S*-acylated cysteine peptides to form native peptides *via* 5-, 11- and 14-membered cyclic transition states

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Materials and methods:

Starting materials and solvents were purchased from commercial sources and used without further purification. Melting points were determined on a Fisher melting apparatus and are uncorrected. Microwave experiments were performed on a Discover® Benchmate, in 10 mL vials from CEM Corporation. HPLC analyses were performed on reverse phase gradient Phenomenex (Torrace, CA) Synergi 4u Hydro-RP 80A (2 x 150 mm; 4 um; S/N=106273-5) plus C18 guard column (2mm x 4 mm) using 0.2% acetic acid in H_2O as the eluting solvent and Agilent 1100 G1314A UV/Vis detector; wavelength = 254 nm. Mass spectrometry was done on Thermo-Finnigan (San Jose, CA) LCQ spectrophotometer with electro spray ionization (ESI). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a 300 MHz NMR spectrometer with acetone-d_6 or DMSO-d_6 as solvents. *J* values are given in Hz. Elemental analyses were performed on a Carlo Erba-1106 instrument.

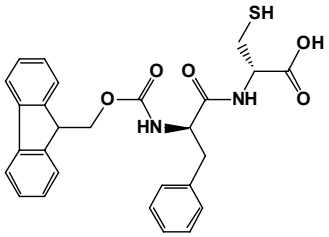
The starting *N*-(protected-α-aminoacyl)benzotriazoles **1a-h** (*N*-Fmoc-L-Phe-Bt **1a**, *N*-Fmoc-L-Met-Bt **1b**, *N*-Fmoc-L-Gly-Bt **1c**, *N*-Cbz-L-Thr-Bt **1d**, *N*-Cbz-Leu-Bt **1e**, *N*-Fmoc-Leu-Bt **1f**, 4-Me-PhCOBt **1g**, *N*-Cbz-Ala-Bt **1h**) and *N*-Fmoc-dipeptidoylbenzotriazole **6** were prepared in 75-90% yields from corresponding *N*-protected amino acids following our previously published one-step procedure.[1,2]

General procedure for preparation of peptide coupling products 2a-d, 7 and 11

N-(Protected-α-aminoacyl)benzotriazole **1** or *N*-Fmoc-dipeptidoylbenzotriazole **6** was suspended in acetonitrile and a solution of either cysteine or H-Gly-L-Cys(S-Z-L-Ala)-OH **5b** in water containing equivalent amount of triethyl amine was added. The mixture was stirred at 20 ^{o}C until the TLC revealed complete consumption of the starting materials. Acetonitrile was removed under reduced pressure and the residue formed was taken in ethyl acetate, extracted with HCl (2*N*) and brine. Ethyl acetate was concentrated under reduced pressure and hexane was

added and the turbid solution was left to crystallize overnight at -20 °C. The solid obtained was filtered, dried to give the corresponding target.

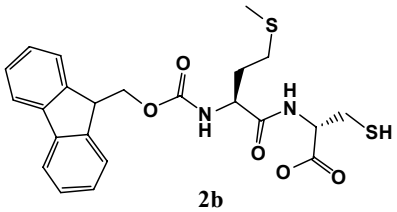
Fmoc-L-Phe-L-Cys-OH 2a



2a

White microcrystals; yield: 98%; 164.0-166.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.44 (t, J = 8.1 Hz, 1H, 2.77-2.97 (m, 3H), 3.06 (dd, J = 13.6, 3.3 Hz, 1H), 4.15-4.19 (m, 3H), 4.32-4.38 (m, 1H), 4.44-4.50 (m, 1H), 7.16-7.43 (m, 9H), 7.62-7.71 (m, 3H), 7.87 (d, J = 7.6 Hz, 2H), 8.37 (d, J = 7.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.7, 37.5, 46.7, 54.5, 60.0, 65.7, 120.2, 125.4, 125.5, 126.4, 127.2, 127.7, 128.1, 129.3 138.2, 140.8, 143.8, 143.9, 155.9, 171.6, 171.8. Calcd for $C_{27}H_{26}N_2O_5S$: C, 66.10; H, 5.34; N, 5.71. Found: C, 65.98; H, 5.41; N, 5.51.

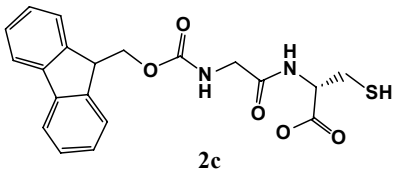
Fmoc-L-Met-L-Cys-OH 2b



2b

White microcrystals; yield: 88%; 97.0-99.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83-0.86 (m, 1H), 1.24 (br s, 1H), 1.85-1.92 (m, 2H), 1.95-2.05 (m, 3H), 2.42-2.47 (m, 1H), 2.79-2.90 (m, 2H), 4.18-4.35 (m, 4H), 4.42-4.44 (m, 1H), 7.33 (t, J = 6.6 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.65 (d, J = 8.1 Hz, 1H), 7.74 (t, J = 5.7 Hz, 2H), 7.90 (d, J = 4.5 Hz, 2H), 8.22 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.3, 22.8, 24.2, 37.4, 46.5, 50.3, 55.9, 65.6, 120.0, 125.2, 125.3, 126.2, 127.0, 127.6, 128.0, 129.2, 138.1, 140.6, 143.7, 143.8, 155.7, 171.6, 173.9. Calcd for $C_{23}H_{26}N_2O_5S_2$: C, 58.21; H, 5.52; N, 5.90. Found: C, 58.48; H, 5.53; N, 5.64.

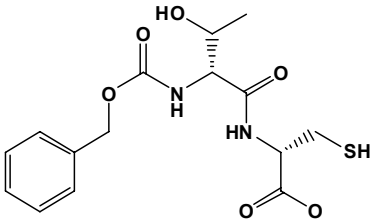
Fmoc-L-Gly-L-Cys-OH 2c



2c

White microcrystals; yield: 84%; 90.0-91.0°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42 (t, J = 8.7 Hz, 1H), 2.79-2.88 (m, 2H), 3.70-3.71 (m, 2H), 4.24-4.30 (m, 3H), 4.42-4.50 (m, 1H), 7.33 (t, J = 7.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.57-7.61 (m, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.89 (d, J = 7.5 Hz, 2H), 8.18 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.7, 43.2, 46.6, 54.2, 65.8, 120.1, 125.2, 127.0, 127.6, 140.7, 144.0, 156.5, 169.1, 171.4. Calcd for $C_{20}H_{20}N_2O_5S$: C, 59.99; H, 5.03; N, 7.00. Found: C, 59.65; H, 4.94; N, 7.01.

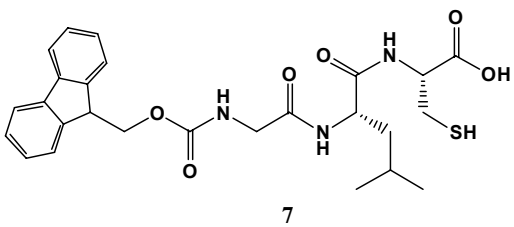
Cbz-L-Thr-L-Cys-OH 2d



2d

White microcrystals; yield: 74%; 68.0-70.0°C. ^{1}H NMR (300 MHz, acetone-d_6) δ 1.18-1.21 (m, 3H), 2.91-3.08 (m, 1H), 4.24-4.27 (m, 2H), 4.74-4.79 (m, 1H), 5.12 (br s, 3H), 6.43 (d, J = 6.9 Hz, 1H), 7.30-7.41 (m, 5H), 7.74 (d, J = 7.8 Hz, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 19.8, 27.0, 55.3, 61.1, 67.4, 68.4, 128.9, 129.0, 129.6, 128.4, 157.6, 171.5, 171.8. Calcd for $C_{15}H_{20}N_2O_6S$: C, 50.55; H, 5.66; N, 7.86. Found: C, 51.19 ; H, 5.92 ; N, 7.33 .

Fmoc-GLy-L-Leu-L-Cys-OH 7

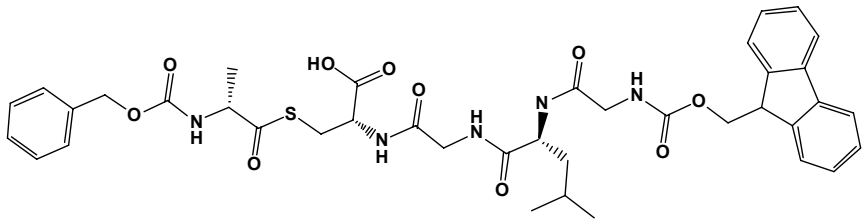


7

White microcrystals; yield: 88%; 170.0-172.0°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85-0.89 (m, 6H), 1.31-1.63 (m, 2H), 1.63-1.70 (m, 1H), 2.46-2.48 (m, 1H), 2.72-2.92 (m, 2H), 3.66 (d, J = 5.4 Hz, 2H), 4.15-4.32 (m, 3H), 4.37-4.49 (m, 2H), 7.34 (t, J = 7.2 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.57 (t, J

= 5.7 Hz, 1H), 7.73 (d, *J* = 7.2 Hz, 2H), 7.90 (d, *J* = 7.5 Hz, 2H), 8.15 (d, *J* = 8.1 Hz, 1H), 8.26 (d, *J* = 7.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.7, 23.0, 24.0, 25.3, 41.0, 43.3, 46.6, 50.8, 54.4, 65.7, 120.1, 125.2, 127.1, 127.6, 140.7, 143.8, 156.5, 168.8, 171.4, 172.1. Anal. Calcd for $C_{26}H_{31}N_3O_6S$: C, 60.80; H, 6.08; N, 8.18. Found: C, 60.56; H, 6.21; N, 8.20.

Fmoc-Gly-L-Leu-Gly-L-Cys(*S*-Z-L-Ala)-OH 11



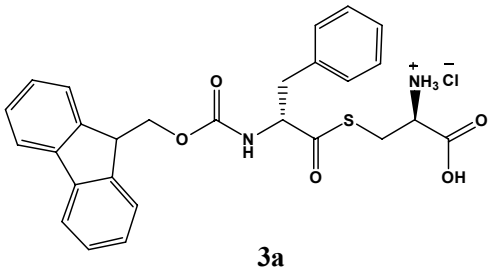
11

White microcrystals; yield: 80%; 192.0-194.0°C. ^{1}H NMR (300 MHz, acetone-d_6) δ 0.83-0.93 (m, 6H), 1.27 (br s, 1H), 1.38 (d, *J* = 7.2 Hz, 3H), 1.60-1.75 (m, 3H), 3.26-3.33 (m, 1H), 3.44-3.51 (m, 1H), 3.74-4.03 (m, 4H), 4.22-4.46 (m, 5H), 4.61-4.71 (m, 1H), 5.11 (br s, 2H), 6.84-6.86 (m, 1H), 7.14-7.15 (m, 1H), 7.30-7.43 (m, 9H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.63-7.73 (m, 3H), 7.85 (d, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) δ 18.5, 22.6, 23.9, 25.9, 41.9, 43.8, 45.5, 48.5, 51.8, 53.0, 53.5, 58.4, 67.6, 68.0, 121,3, 126.7, 128.5, 129.0, 129.2, 129.7, 138.4, 142.6, 145.5, 157.4, 158.2, 170.4, 171.4, 172.1, 173.8, 202.5. Anal. Calcd for $C_{39}H_{45}N_5O_{10}S$. H_2O : C, 60.37; H, 5.85; N, 9.03. Found: C, 60.98; H, 6.34; N, 8.08.

General procedure for preparation of *S*-acyl-isodipeptides 3a-b

To a suspension of *N*-(protected-α-aminoacyl)benzotriazoles **1a,b** (1 mmol) in acetonitrile (10 mL) was added a solution of cysteine HCl (157 mg, 1 mmol) in water (1 mL) and triethylamine (0.14 mL, 1 mmol). The hetrogenous reaction mixture was stirred at room temperature for 2 h. Then concentrated HCl (0.1 mL) was added and acetonitrile was removed. The residue was filtered and washed with ethyl acetate, dried to give the corresponding *S*-acyl-isodipeptides **3a-b**.

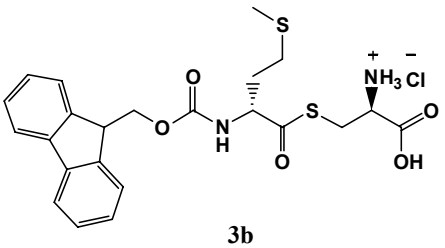
Fmoc-L-Phe-*S*-Cys(NH_3Cl)-OH 3a



3a

White microcrystals; yield: 63%; 158.0-160.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85 (t, J = 11.7 Hz, 1H), 3.17 (dd, J = 13.5, 3.0 Hz, 1H), 3.39 (d, J = 5.7 Hz, 2H), 4.16 (d, J = 6.9 Hz, 2H), 4.25 (d, J = 6.3 Hz, 2H), 4.38- 4.46 (m, 1H), 7.18-7.36 (m, 7 H), 7.42 (t, J = 7.05 Hz, 2H), 7.64 (t, J = 6.0 Hz, 2H),7.89 (d, J = 7.5 Hz, 2H), 8.30 (d, J = 8.7 Hz, 1H), 8.66 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.0, 36.1, 46.6, 51.6, 62.6, 65.9, 120.1, 125.3, 126.5, 127.1, 127.7, 128.3, 129.1, 137.4, 140.7, 143.6, 155.9, 169.0, 200.0 Anal. Calcd for $C_{27}H_{27}ClN_2O_5S$: C, 61.53; H, 4.97; N, 5.32. Found: C, 61.86; H, 5.10; N, 5.30.

Fmoc-L-Met-*S*-Cys(NH_3Cl)-OH 3b



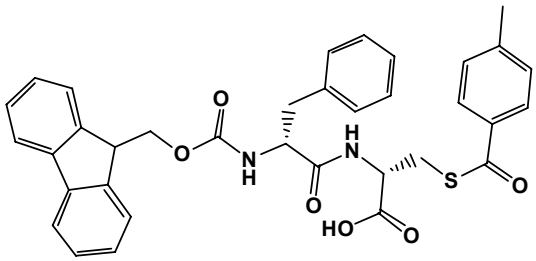
3b

White microcrystals; yield: 35%; 150.0-152.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80-1.94 (m, 2H), 1.94-1.99 (m, 1H), 2.04 (s, 3H), 2.41-2.45 (m, 1H), 3.32-3.34 (m, 2H), 4.10-4.14 (m, 1H), 4.26, (t, J = 6.9 Hz, 1H), 4.38-4.39 (m, 3H), 7.34 (t, J = 6.9 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.73 (d, J = 6.3 Hz, 2H), 7.91 (d, J = 7.5 Hz, 2H), 8.18 (d, J = 7.5 Hz, 1H), 8.53 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5, 27.8, 29.5, 30.3, 46.7, 51.5, 59.9, 65.8, 120.1, 125.2, 127.1, 127.7, 140.7, 143.6, 143.7, 156.1, 169.0, 200.5. Anal. Calcd for $C_{23}H_{27}ClN_2O_5S_2$: C, 54.05; H, 5.33; N, 5.48. Found: C, 54.05; H, 5.36; N, 5.44.

General procedure for *S*-acylation of cysteine-peptides

Dipeptide **2a** or tripeptide **7** together with equimolar amount of *N*-acylbenzotriazole was suspended in acetonitrile at 0-5 ^{o}C. Then a solution of $KHCO_3$ in water was added dropwise over 15 minutes. The solution was stirred at the same temperature and monitored by TLC for starting material consumption. After completion of the reaction, the solution was acidified with 2*N* HCl, then aceonitrile was removed under reduced pressure and the residue formed was taken in ethyl acetate, extracted with 2*N* HCl, brine. Ethyl acetate was concentrated under reduced pressure and hexanes was added until the solution is turbid and the solution was left to crystallize in the freezer. The solid obtained was filtered, dried to give the corresponding targets **4a-d, 8**.

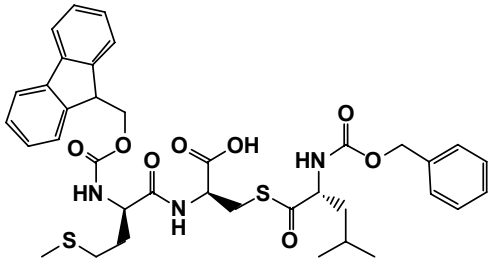
Fmoc-L-Phe-L-Cys(*S*-P-toluoyl)-OH 4a



4a

White microcrystals; yield: 78%; 152.0-154.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.34 (s, 3H), 2.79 (t, 12.6 Hz, 1H), 3.05 (dd, *J* = 13.2, 3 Hz Hz, 1H), 3.29-3.37 (m, 1H), 3.62 (dd, *J* = 4.8, 13.5 Hz, 1H), 4.07-4.14 (m, 3H), 4.28-4.38 (m, 1H), 4.48-4.53 (m, 1H), 7.19 (d, *J* = 6.9 Hz, 1H), 7.23-7.32 (m, 9H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 6.0 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 2H), 7.88 (d, *J* = 7.2 Hz, 2H), 8.54 (d, *J* = 8.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.2, 29.3, 37.4, 38.8, 46.6, 51.7, 56.1, 65.8, 120.1, 125.3, 125.4, 126.3, 126.7, 127.0, 127.1, 127.7, 128.1, 129.3, 129.6, 133.7, 138.2, 140.7, 143.7, 143.8, 144.6, 155.8, 171.5, 171.8, 189.0 Anal. Calcd for $C_{35}H_{32}N_2O_6S$: C, 69.06; H, 5.30; N, 4.60. Found: C, 68.70; H, 4.92; N, 4.75. 128.8, 129.8133.8, 134.9, 145.0, 168.4, 171.1, 190.0. Anal. Calcd for $C_{20}H_{22}N_2O_4S$: C, 59.05; H, 5.74; N, 7.25. Found: C, 58.70; H, 5.72; N, 6.94.

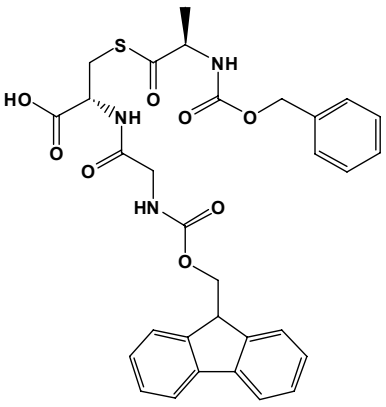
Fmoc-L-Met-L-Cys(*S*-Z-L-Leu)-OH 4b



4b

White microcrystals; yield: 83%; 84.0-86.0°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81-0.86 (m, 6H), 1.24 (brs, 1H), 1.47-1.62 (m, 2H), 1.83-1.93(m, 2H), 2.04 (s, 3H), 2.45-2.48 (m, 1H), 3.05-3.13 (m, 3H), 3.26-3.42 (m, 1H), 4.15-4.31 (m, 5H), 5.07 (s, 2H), 7.30-7.35 (m, 5H), 7.42 (t, J = 7.5 Hz, 2H), 7.59 (d, J = 8.1 Hz, 1H), 7.72 (t, J = 6.9 Hz, 2H), 7.89 (d, J = 7.2 Hz, 2H), 8.07 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6, 20.9, 22.8, 24.2, 29.3, 29.6, 31.9, 46.7, 51.5, 53.7, 59.6, 65.7, 120.1, 125.3, 127.0, 127.6, 127.8, 128.3, 136.8, 140.7, 143.7, 143.9, 155.8, 156.1, 171.4, 171.5, 201.6. Anal. Calcd for $C_{37}H_{43}N_3O_8S_2$: C, 61.56; H, 6.00; N, 5.82. Found: C, 61.80; H, 6.26; N, 5.45.

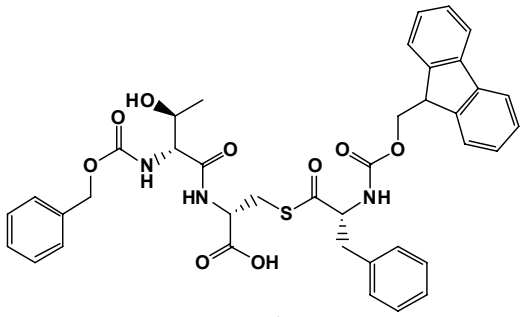
Fmoc-Gly-L-Cys(*S*-Z-L-Ala)-OH 4c



4c

White microcrystals; yield: 87%; 159.0-161.0°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (d, J = 6.6 Hz, 3H), 3.08 (dd, J = 13.2, 9 Hz, 1H), 3.25-3.39 (m, 2H), 3.65 (d, J = 5.1 Hz, 2H), 4.19-4.29 (m, 4H), 4.34-4.38 (m, 1H), 5.03-5.07 (m, 2H), 7.31-7.45 (m, 9H), 7.57 (t, J = 6 Hz, 1H), 7.72 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 7.5 Hz, 2H), 8.09 (d, J = 7.2 Hz, 1H), 8.29 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 18.3, 31.0, 45.0, 48.3, 52.9, 58.4, 67.6, 67.9, 121.2, 126.6, 128.3, 128.9, 129.1, 129.6, 138.2, 142.4, 145.4, 157.4, 157.9, 170.5, 171.9, 202.5. Anal. Calcd for $C_{31}H_{31}N_3O_8S$: C, 61.48; H, 5.16; N, 6.94. Found: C, 61.12; H, 5.22; N, 6.77.

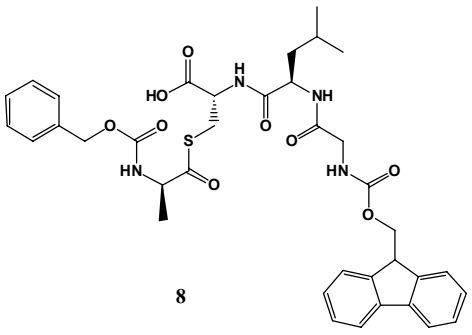
Z-L-Thr-L-Cys(*S*-Fmoc-L-Phe)-OH 4d



4d

White microcrystals; yield: 72%; 175.0-177.0°C. ^{1}H NMR (300 MHz, acetone-d_6) δ 1.18 (d, J = 5.7 Hz, 3H), 2.92-3.00 (m, 1H), 3.27-3.38 (m, 2H), 3.55 (dd, J = 13.5, 4.5 Hz, 1H), 4.13-4.16 (m, 1H), 4.25-4.31 (m, 4H), 4.53-4.60 (m, 1H), 4.72-4.80 (m, 1H), 5.02-5.14 (m, 1H), 6.33 (d, J = 7.5 Hz, 1H), 7.20-7.42 (m, 16 H), 7.61 (t, J = 8.7 Hz, 2H), 7.68 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, acetone-d_6) δ 19.7, 38.4, 48.4, 53.0, 61.0, 64.1, 64.2, 67.5, 67.9, 68.4, 121.2, 126.6, 128.0, 128.4, 129.0, 129.1, 129.7, 130.6, 138.4, 138.6, 142.5, 145.3, 145.4, 157.4, 157.7, 171.6, 172.0, 202.0. Anal. Calcd for $C_{39}H_{41}N_3O_{10}S$. H_2O: C, 62.97; H, 5.56; N, 5.65. Found: C, 62.83; H, 5.29; N, 5.76.

Fmoc-Gly-L-Leu-L-Cys(*S*-Z-L-Ala)-OH 8



8

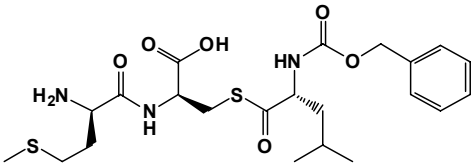
White microcrystals; yield: 75%; 174.0-176.0°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, J = 6.0Hz, 6H), 1.26 (d, J = 6.6 Hz, 3H), 1.40-1.46 (m, 2H), 1.55-1.64 (m, 1H), 3.07 (dd, J = 13.5, 8.4 Hz, 1H), 3.24-3.23 (m, 1H), 3.66 (d, J = 6.0 Hz, 3H), 4.12-4.28 (m, 5H), 4.35-4.42 (m, 1H), 5.01-5.11 (m, 2H), 7.31-7.45 (m, 9H), 7.54 (t, J = 6.3 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.90 (d, J = 7.2 Hz, 2H), 7.94 (d, J = 9.3 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 8.39 (d, J = 7.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.2, 21.6, 23.0, 24.0, 29.2, 41.2, 43.3, 46.6, 50.6, 51.5, 56.6, 65.7, 120.1, 125.2, 127.1, 127.6, 127.7, 127.9 128.4, 136.7, 140.7, 143.8, 155.8,

156.5, 168.7, 171.3, 172.0, 201.7. Anal. Calcd for $C_{37}H_{42}N_4O_9S$: C, 61.82; H, 5.89; N, 7.79. Found: C, 61.47; H, 6.08; N, 7.68.

General procedure for Fmoc deprotection of *N*-Fmoc-cysteine peptides 4b,c, 8, 11

N-Fmoc-Cysteine peptide **4b**, **c**, **8** or **11** was dissolved in dry THF at 0 ^oC under argon. Then, DBU (two equivalents) was added dropwise. The solution was stirred for 15 minutes, THF was decanted and the sticky solid was dissolved in *2N* HCl, then, the pH of the solution was adjusted to 5 using Na_2HPO_4. The solid formed was filtered off, washed with water, methanol, diethyl ether and dried to give corresponding unprotected *S*-acyl-isopeptide **5a,b, 9,** or **12**.

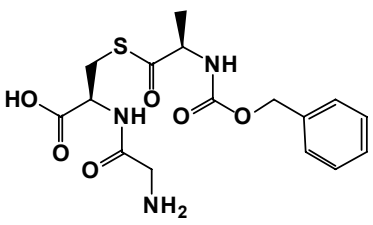
H-L-Met-L-Cys-(*S*-Z-L-Leu)-OH 5a



5a

White microcrystals; yield: 90%; 148.0-150.0 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.83-089 (m, 6H), 1.53 (dd, J = 1.6, 8.7 Hz, 2H), 1.59-1.64 (m, 1H), 1.81-1.92 (m, 3H), 2.04 (s, 3H), 2.51-2.58 (m, 1H), 3.11 (dd, J = 12.6, 6.6 Hz, 1H), 3.31 (dd, J = 12.6, 7.5 Hz, 1H), 3.59 (br s, 1H), 4.14-4.18 (m, 2H), 4.50 (br s, 2H), 5.04-5.13 (m, 2H), 7.33-7.38 (m, 5H), 8.04 (d, J = 8.1 Hz, 1H), 8.37 (brs, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5, 20.9, 22.9, 24.2, 28.8, 30.8, 32.7, 52.4, 52.6, 59.6, 65.7, 127.6 127.8, 128.3, 136.8, 156.1, 170.7, 171.3, 201.9. Anal. Calcd for $C_{22}H_{33}N_3O_6S_2.H_2O$: C, 51.04; H, 6.81; N, 8.12. Found: C, 50.72; H, 6.68; N, 7.75.

H-Gly-L-Cys-(*S*-Z-L-Ala)-OH 5b

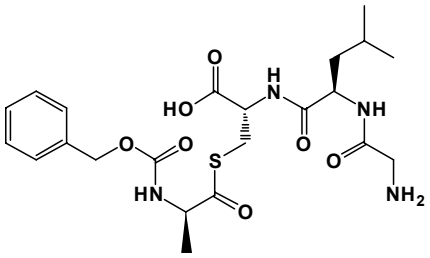


5b

White microcrystals; yield: 88%; 188.0-190.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25 (d, J = 7.2 Hz, 3H), 3.07 (dd, J = 12.3, 6.6 Hz, 1H), 3.29 (dd, J = 13.5, 3.9 Hz, 1H), 3.47 (d, J = 16.5

Hz, 1H), 3.54 (d, *J* = 13.8 Hz, 1H), 4.15-4.19 (m, 2H), 5.06 (dd, *J* = 18.6, 12.3 Hz, 2H), 7.30-7.37 (m, 5H), 8.04 (d, *J* = 7.8 Hz, 1H), 8.29 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.5, 31.4, 40.9, 52.9, 56.6, 65.7, 127.8, 128.4, 136.8, 155.8, 166.8, 171.4, 202.1. Anal. Calcd for $C_{16}H_{21}N_3O_6S$: C, 50.12; H, 5.52; N, 10.96. Found: C, 50.43; H, 5.67; N, 10.52.

H-Gly-L-Leu-L-Cys(*S*-Z-L-Ala)-OH 9



9

White microcrystals; yield: 90%; 200.0-202.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83-0.89 (m, 6H), 1.24 (d, *J* = 7.2 Hz, 3H), 1.41-1.65 (m, 3H), 3.06 (dd, *J* = 12.6, 6 Hz, 1H), 3.30 (dd, *J* = 12.9, 4.8 Hz, 1H), 3.98-4.01 (m, 2H), 4.14-4.24 (m, 3H), 5.06 (dd, *J* = 21.3, 11.7 Hz, 2H), 7.30-7.37 (m, 5H), 7.71 (d, *J* = 6.3 Hz, 1H), 8.01 (d, *J* = 7.5 Hz, 1H), 8.69 (d, *J* = 8.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.5, 21.4, 23.0, 24.2, 31.1, 40.9, 51.9, 52.5, 56.6, 65.7, 127.7, 128.3, 136.7, 155.7, 167.3, 1701.1, 171.8, 201.8. Anal. Calcd for $C_{22}H_{32}N_4O_8S$. H_2O : C, 51.35; H, 6.66; N, 10.89. Found: C, 51.73; H, 6.48; N, 10.51.

General procedure for chemical ligation of *S*-acyl-isodipeptides 3a-b to form native peptides 2a-b

The isodipeptide hydrochloride **3a, b** (0.25 mmol) was dissolved in a deoxygenated mixture of water (2 mL), acetonitrile (6 mL) and triethylamine (0.035 mL, 0.25 mmol). The mixture was stirred at room temperature for one hour under argon. The reaction was acidified to pH 1 using *2N* HCl and extracted with ethyl acetate (10 mL). The ethyl acetate layer was washed with *2N* HCl (2x2 mL), brine (1x2 mL), dried over sodium sulfate. Hexanes (5mL) was added to ethyl

acetate and the turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and dried in desiccators to give the corresponding native dipeptide **2a-d**.

General procedure for chemical ligation of *S*-acyl-isotripeptide 5c, *S*-acyl-isotetrapeptide 9 and *S*-acyl-isopentapeptide 12.

S-Acyl-isotri-, -isotetra- or -isopentapeptide (**5c**, **9** or **12**) (0.10 mmol) was suspended in deoxygenated phosphate buffer (NaH_2PO_4/ Na_2HPO_4) (pH 7.8, 2 mL) containing acetonitrile (0.5 mL) as a co-solvent. The mixture was irradiated with microwave (50 ^oC, 50 W, 1 hour) in a microwave tube under argon. The reaction was cooled to room temperature, quenched with aqueous solution of TCEP (1M, 0.1 mL) and stirred for 20 minute before acidification with 2N.HCl to pH 1. After extraction with ethyl acetate and the usual workup, the solid obtained was weighed as crude yield. Then a solution in methanol (2 mL) was made and analyzed by HPLC-MS.

HPLC chromatograms and ESI-MS Spectra of the chemical ligation of 5c, 9 and 12

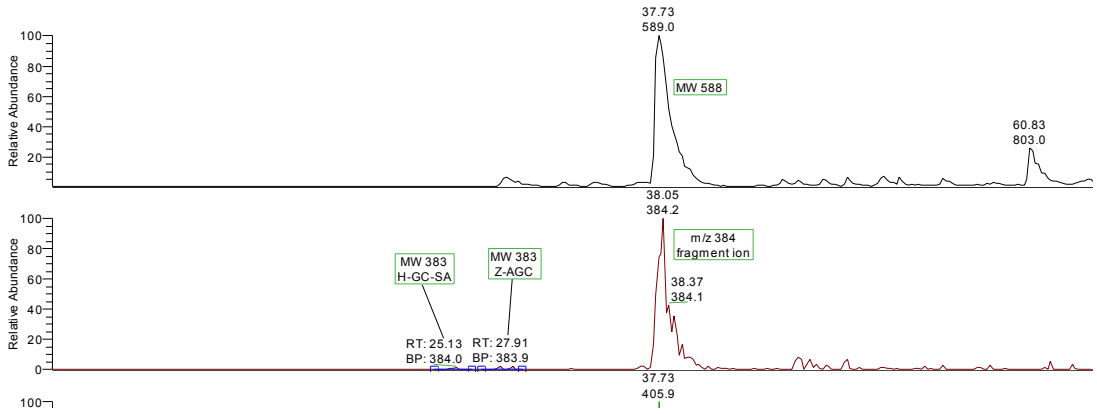


Fig. 1: HPLC-MS chromatogram of the chemical ligation experiment of *S*-acyl-isotripeptide **5c**. The expected ligation product **13** (**Z-AGC**, molecular weight 383) was detected (shaded peaks) but it was very minor component of the sample bottom trace. The most abundant peak was due to a MW 588 compound, which is due to the disproportionation product (RT 37.7 min, top trace). It produced an m/z 384 fragment ion (bottom trace, RT 38.05 min).

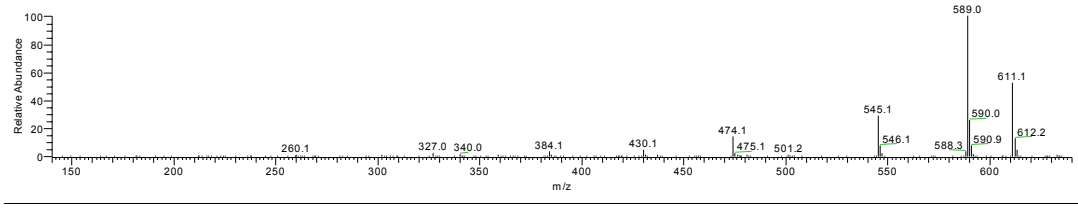


Fig.2: Mass spectrum of the disproportionation product **14** by (+)ESI-MS was due to a MW 588 compound, which produced an m/z 589 [M+H]+ ion and m/z 384 fragment ion along with other ions.

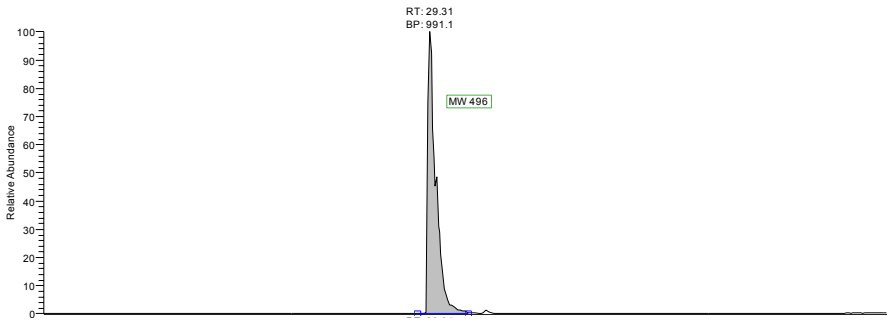


Fig. 3. HPLC-MS chromatogram of *S*-acyl-isotetrapeptide **9** used in the ligation experiment

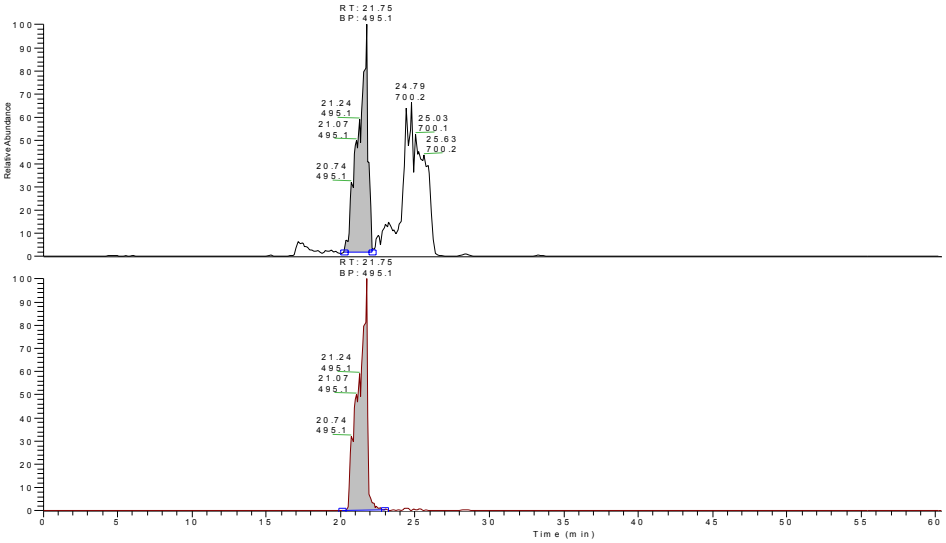


Fig. 4. HPLC-MS chromatogram of the chemical ligation experiment of *S*-acyl-isotetrapeptide **9**. The desired ligation product **15** molecular weight 496 (bottom trace, RT 21.75 min) (shaded peaks) was the most abundant peak in the sample via reverse phase gradient C18 HPLC/(-)ESI MS

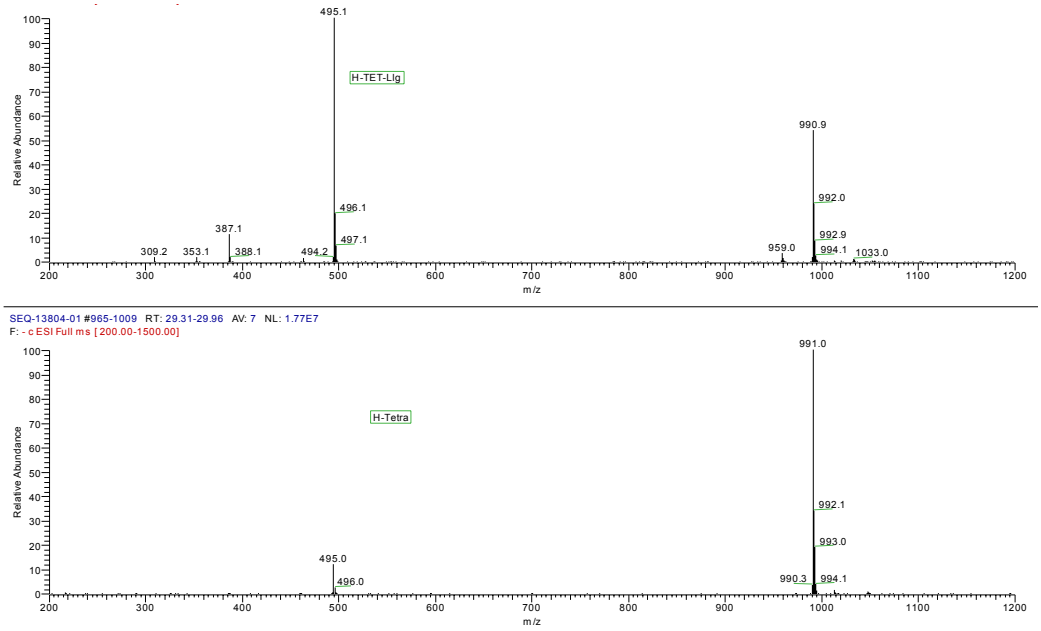


Fig. 5. The molecular weight of the desired ligation product **15** (top) and *S*-acyl-isotetrapeptide **9** (bottom); both produced m/z 495 [M-H]- ions, m/z 991 [(M-H)+M]- self-adduct ions. They clearly gave different fragmentation patterns.

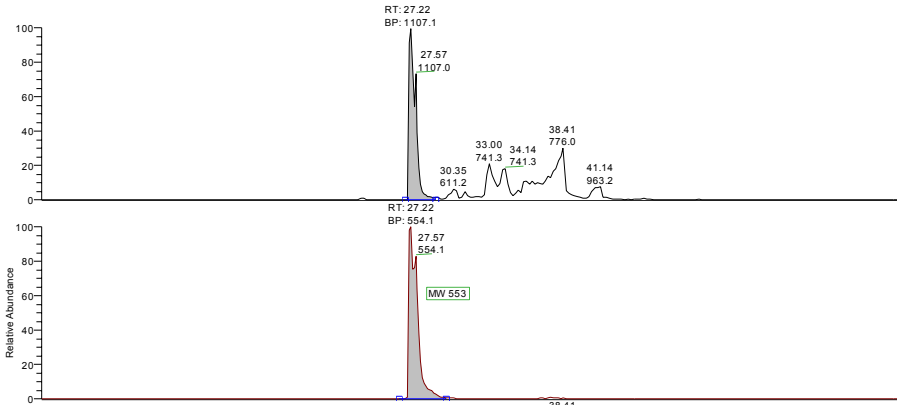


Fig 6. Compound **12** HPLC/(+)ESI-MS. The most abundant peak (shaded, top trace) was due to the MW 553 compound, m/z 554 $[M+H]^+$ ion-peak shaded in middle trace.

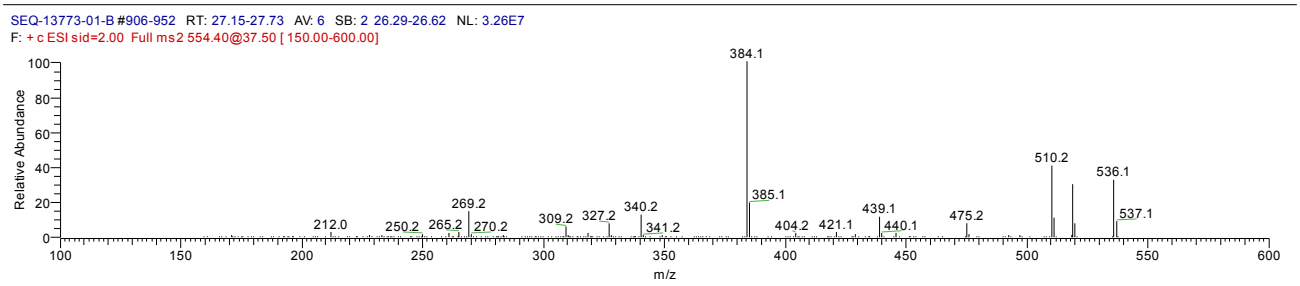


Fig. 7. Mass spectrum of *S*-acyl-isopentapeptide **12** molecular weight 553: (+)ESI-MS/MS of m/z 554 .

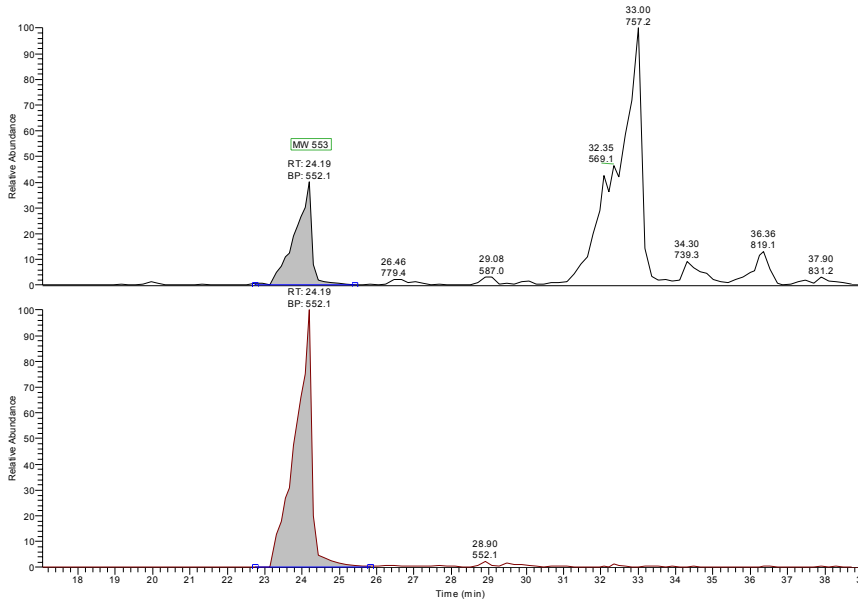


Fig 8. HPLC-MS chromatogram of the chemical ligation experiment of *S*-acyl-isopentapeptide **12.** The desired ligation product **16** molecular weight 553 was a major compound in the sample (shaded peaks)

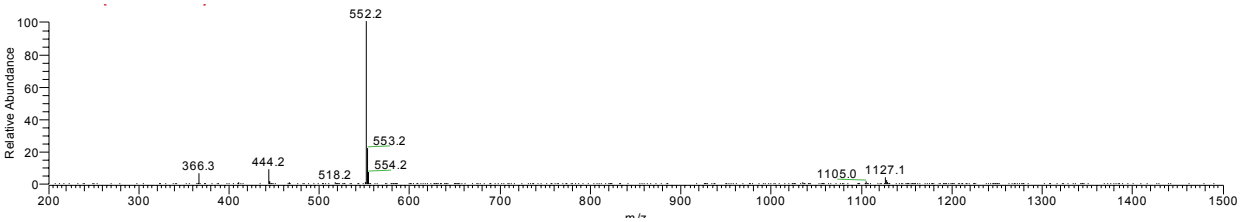


Fig.9: The desired ligation product **16** (MW 553) produced an m/z 552 [M-H]- ion, which underwent dissociation to produce m/z 444, 366.

References

[1] A. R. Katritzky, P. Angrish, K. Suzuki, *Synthesis* 2006, 411.

[2] A. R. Katritzky, N. E. Abo-Dya, S. R. Tala, K. Gyanda, Z. K. Abdel-Samii, *Org. Biomol. Chem.* 2009, **7**, 4444.