Solution-Phase Synthesis of Chiral *N*-, *O*-, and *S*-Acyl Isopeptides

Sumaira Liaqat,[a,b] Siva S. Panda,[a] Abdul Rauf,[b] Abdulrahman O. Al-Youbi,[c] Alan R. Katritzky*[a,c]

[a] Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA

[b] Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

[c] Chemistry Department, King Abdulaziz University, Jeddah 21589, Saudi Arabia
Fax +1(352)3929199; E-mail: katritzky@chem.ufl.edu

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Abstract: A convenient synthesis of chiral *N*-, *O*-, and *S*-acyl monoiso- and diisopeptides from di- and tripeptides containing tryptophan, tyrosine, and cysteine units using benzotriazole is reported in solution phase.

Key words: amino acids, peptides, chirality, benzotriazole method, solution-phase synthesis

Solid-phase peptide synthesis (SPPS) has been used routinely for the synthesis of peptides and proteins. However, the synthesis of 'difficult sequence' containing peptides is still a challenge in peptide chemistry since these peptides are often obtained in low yield and purity by SPPS.[1–3] The difficult sequences are generally hydrophobic and prone to aggregation in solvent during chain elongation and final purification. This is attributed to inter/intramolecular hydrophobic interactions and hydrogen-bond networks formed among resin-bound peptide chains, resulting in the formation of extended secondary structures such as β-sheets.

Kiso and co-workers reported that 21% D-Val was detected during the synthesis of Boc-Thr(Fmoc-Val) via solid phase, while epimerization was completely avoided in the solution phase.[4] In addition, due to the presence of an additional amino group, *N*-, *O*-, or *S*-acyl isopeptides are generally hydrophilic, which is advantageous in effective purification by HPLC. The native peptides are then generated from the corresponding *N*-, *O*, or *S*-acyl isopeptide via an N-to-N,[5] O-to-N,[6] or S-to-N[7–9] intramolecular acyl migration reaction. The strategy facilitates the synthesis of peptides with 'difficult sequences'. The *O*-acyl isopeptide method has already been used in various fields including peptide synthesis,[4a,10–14] 'click peptide' ('switch peptide') concept,[15–18] macromolecules,[19] peptide localization,[20] protein splicing,[21] and proteomics.[22]

We now report the efficient single-step preparation of chiral *N*-, *O*, or *S*-acyl isopeptides incorporating tryptophan, tyrosine, and cysteine. *N*-Acylbenzotriazoles are advantageous for N-, O-, C-, and S- acylation,[23] especially where the corresponding acid chlorides are unstable or prone to racemization. *N*-[Protected (Pg)-α-aminoacyl]- and *N*-(Pg-dipeptidoyl)benzotriazoles have enabled fast preparations of biologically relevant peptides and peptide conjugates in high yields and purity, under mild reaction conditions, with full retention of the original chirality.[23,24]

N-(Pg-α-aminoacyl)benzotriazoles **1a**–**e** and *N*-(Pg-dipeptidoyl)benzotriazoles **1f**–**h** were prepared following reported procedures[25] and were reacted with tryptophan, tryrosine, and cysteine to obtain the corresponding di- and tripeptides. These were reacted further with *N*-(Pg-α-aminoacyl)benzotriazoles and *N*-(Pg-dipeptidoyl)benzotriazoles to obtain mono- and diisotripeptides and -tetrapeptides.

Synthesis of Tryptophan Isopeptides

Benzotriazolides **1a**–**c** were coupled with free tryptophan (**2**) at 0 to 20 °C in the presence of triethylamine in acetonitrile to give Cbz-protected dipeptides **3a**–**c** (Table 1). These dipeptides **3a**–**c** were N-acylated by (Cbz-protected-α-aminoacyl)benzotriazoles **1a**,**b**,**d** in the presence of a base (Et_3N, DIPEA, K_2CO_3, or DBU) in acetonitrile to obtain protected monoisotripeptides **4a**–**d** (Table 2). DBU gave better results than the other evaluated bases (Scheme 1).

Table 1 Preparation of N-Protected Dipeptides **3a**–**c** Containing a Tryptophan Unit

Product **3**	Yield (%)	Mp (°C)	Lit. mp (°C)
Z-Gly-L-Trp-OH, **3a**	79	139–141	142–143[26]
Z-L-Ala-L-Trp-OH, **3b**	78	154–155	154–155[27]
Z-L-Val-L-Trp-OH, **3c**	77	183–185	185–187[27]

Table 2 Preparation of *N*-Acyl Monoisotripeptides **4a**–**d**

Product **4**	Yield (%)	Mp (°C)
Z-Gly-L-Trp(Z-L-Phe)-OH, **4a**	79	48–50
Z-L-Ala-L-Trp(Z-L-Phe)-OH, **4b**	78	56–58
Z-L-Val-L-Trp(Z-Gly)-OH, **4c**	75	54–56
Z-L-Val-L-Trp(Z-L-Ala)-OH, **4d**	76	52–54

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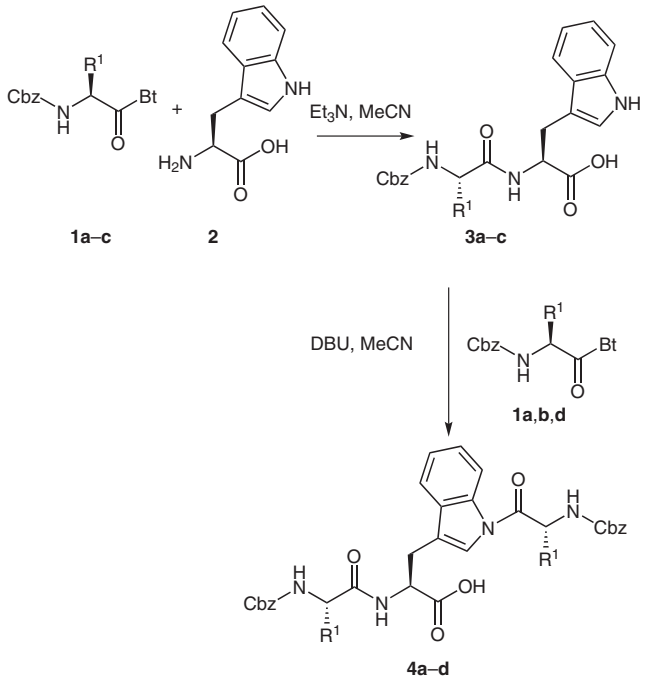


Scheme 1 Preparation of *N*-acyl monoisotripeptides **4a**–**d**

Synthesis of Tyrosine Isopeptide

The benzotriazolide **1e** was coupled with free tyrosine (**5**) at 0 to 20 °C in the presence of DBU in DMF to give Boc-protected dipeptide **6**. The dipeptide **6** was O-acylated by *N*-(Pg-α-aminoacyl)benzotriazole **1b** in the presence of triethylamine to obtain the protected monoisotripeptide **7** (Scheme 2).

Synthesis of Cysteine Isopeptides

The benzotriazolides **1b**–**h** were coupled with free cysteine (**8**) at 0 to 20 °C in the presence of triethylamine in acetonitrile to give N-protected di- and tripeptides **9a**–**f** (Table 3, Scheme 3). These di- and tripeptides **9a**–**f** were S-acylated by *N*-(Pg-α-aminoacyl)benzotriazoles and dipeptidoylbenzotriazoles in the presence of potassium bicarbonate to obtain protected monoisotri-, -tetra-, and -pentapeptides **10a**–**h** (Table 4, Scheme 3).

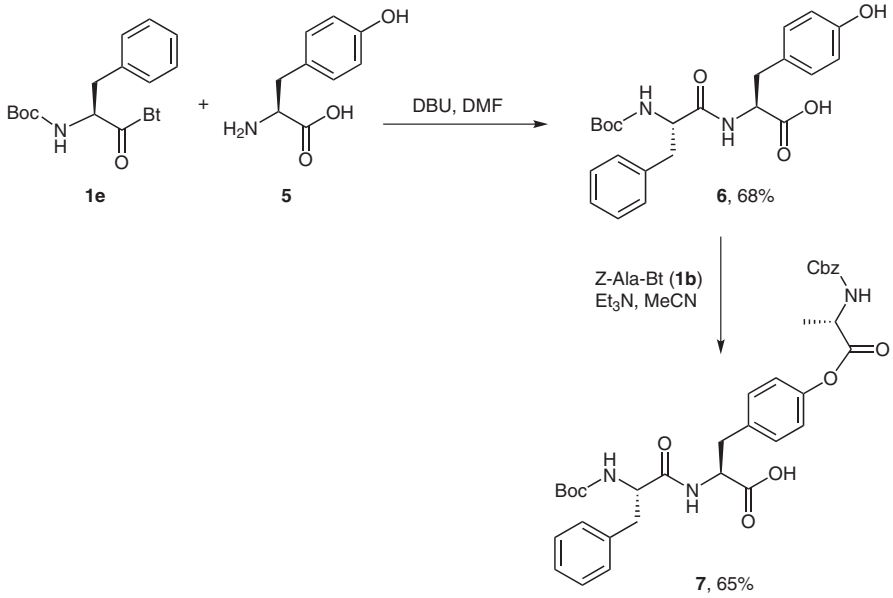
Table 3 Preparation of N-Protected Peptides **9a**–**f** Containing a Cysteine Unit

Product **9**	Yield (%)	Mp (°C)
Z-L-Ala-L-Cys-OH, **9a**	96	170–171
Z-L-Val-L-Cys-OH, **9b**	96	169–170
Z-L-Phe-L-Cys-OH, **9c**	98	125–126
Z-L-Phe-Gly-L-Cys-OH, **9d**	90	156–158
Z-L-Phe-L-Ala-L-Cys-OH, **9e**	92	177–179
Z-L-Ala-L-Phe-L-Cys-OH, **9f**	95	170–172

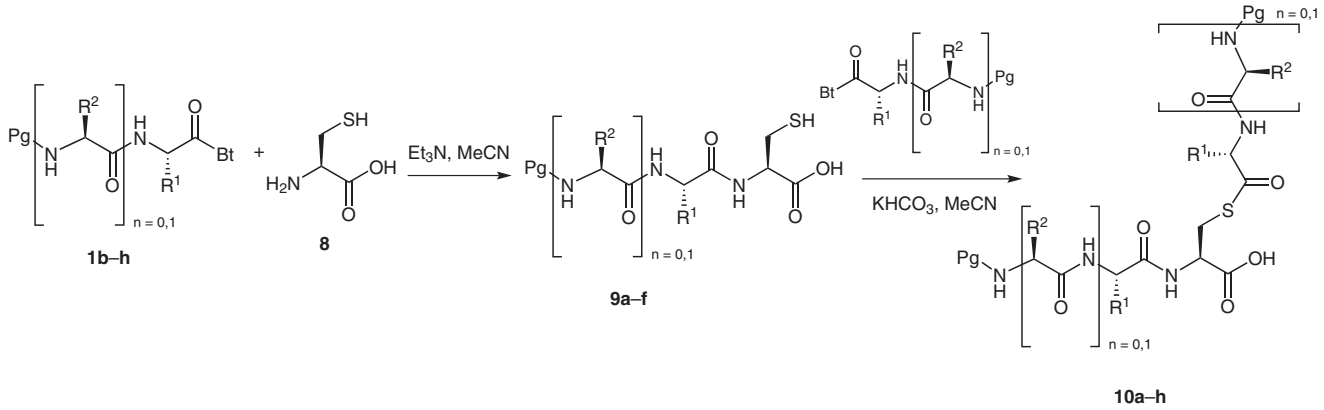
Table 4 Preparation of *S*-Acyl Peptides **10a**–**h**

Product **10**	Yield (%)	Mp (°C)
Z-L-Phe-L-Cys(Z-Gly)-OH, **10a**	45[a]	171–173
Z-L-Val-L-Cys(Z-Gly)-OH, **10b**	84	145–147
Z-L-Ala-L-Cys(Z-L-Phe)-OH, **10c**	86	142–143
Z-L-Ala-L-Phe-L-Cys(Z-L-Ala)-OH, **10d**	97	167–169
Z-L-Ala-L-Cys(Z-L-Ala-L-Phe)-OH, **10e**	95	161–163
Z-L-Phe-Gly-L-Cys(Z-L-Ala)-OH, **10f**	94	169–171
Z-L-Phe-L-Ala-L-Cys(Z-L-Ala-)-OH, **10g**	96	170–171
Z-L-Phe-Gly-L-Cys(Z-L-Ala-L-Phe)-OH, **10h**	98	146–148

[a] Compound was isolated by extraction with EtOAc.



Scheme 2 Preparation of *O*-acyl monoisotripeptide **7**



Scheme 3 Preparation of *O*-acyl monoisotripeptides **10a–h**

In summary, *N*-peptidoylbenzotriazoles are advantageous coupling reagents that (i) are sufficiently reactive to form amide bonds at ambient temperature; (ii) are stable enough to resist side reactions and can be stored in the crystalline state at room temperature; (iii) provide good yields without detectable racemization; (iv) are almost always crystalline; (v) are relatively insensitive to moisture and can be used in aqueous solution, and (vi) are inexpensive to prepare. Hence *N*-(Pg-α-aminoacyl)benzotriazole and *N*-(Pg-α-dipeptidoyl)benzotriazole reagents allow efficient peptide couplings to generate monoisopeptides via N-, O-, and S-acylation.

Commercial reagents were purchased from Sigma-Aldrich and were used without purification. Solvents were purified by distillation. Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$ or CD_3OD on Mercury or Gemini NMR spectrometers operating at 300 MHz for ^1H (with TMS as an internal standard) and 75 MHz for ^{13}C. Elemental analyses were performed on a Carlo Erba-EA1108 instrument. Analytical TLC was performed on E. Merck silica gel 60 F254 plates and visualized by UV and $KMnO_4$ staining. Flash column chromatography was performed on E. Merck silica gel 60 (40–63 mm). Yields refer to chromatographically and spectroscopically pure compounds. Mass spectrometry was done with electrospray ionization (ESI).

Cbz-Protected Dipeptides 3a–c; General Procedure

To the respective *N*-(Pg-α-aminoacyl)benzotriazole **1a–c** (0.5 mmol) in MeCN (10 mL) was added a solution of tryptophan (**2**; 102 mg, 0.5 mmol) and Et_3N (0.5 mL) in H_2O (3 mL). The reaction mixture was stirred for 8 h at 0 °C. The mixture was acidified by aq 1 M HCl and extracted with EtOAc (10 mL). The organic layer was washed with aq 1 M HCl (3 mL) and brine (5 mL), and dried ($MgSO_4$). After evaporation of solvent, the residue was triturated with Et_2O and the solid formed was filtered and dried under vacuum to give dipeptides **3a–c**, respectively (Table 1).

[(Benzyloxy)carbonyl]glycyl-L-tryptophan (3a)

Yield: 0.3 g (79%); white solid; mp 139–141 °C (Lit.[26] mp 142–143 °C).

^1H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 10.87 (d, *J* = 2.4 Hz, 1 H), 8.08 (d, *J* = 7.7 Hz, 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.48–7.22 (m, 7 H), 7.14 (s, 1 H), 7.07 (t, *J* = 7.5 Hz, 1 H), 6.99 (t, *J* = 7.4 Hz, 1 H), 5.03 (s, 2 H), 4.74–4.28 (m, 1 H), 3.77–3.52 (m, 2 H), 3.18 (dd, *J* = 14.7, 5.2 Hz, 1 H), 3.05 (dd, *J* = 14.6, 7.8 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 174.2, 169.9, 157.4, 138.0, 137.0, 129.3, 128.7, 128.2, 124.6, 121.9, 119.4, 119.1, 112.3, 110.6, 66.4, 53.9, 44.2, 28.1.

Anal. Calcd for $C_{21}H_{21}N_3O_5$: C, 63.79; H, 5.35; N, 10.63. Found: C, 63.71; H, 5.226; N, 10.37.

Dipeptides **3b**,**c** gave also physical and spectral data in conformity with the reported values.[26,27]

Protected Monoisotripeptides 4a–d; General Procedure

To a precooled solution of tryptophan containing the appropriate peptide **3a–c** (0.5 mmol) in MeCN (10 mL) and Et_3N (1.5 equiv) at 0 °C was added a solution of *N*-(Pg-α-aminoacyl)benzotriazole **1a**,**b**, or **d** (0.5 mmol) in MeCN (3 mL). After completion of the reaction (8 h), the reaction mixture was acidified with aq 1 M HCl and then extracted with EtOAc (10 mL). The organic layer was washed with H_2O (10 mL) and dried (Na_2SO_4). Evaporation of the solvent gave the desired product **4a–d**, respectively, which was recrystallized from EtOAc–hexanes (Table 2).

1-{[(Benzyloxy)carbonyl]-L-phenylalanyl}-N^{α}-{[(benzyloxy)carbonyl]glycyl}-L-tryptophan (4a)

Yield: 0.52 g (79%); off-white solid; mp 48–50 °C.

^1H NMR (DMSO-d_6): δ = 12.70 (br s, 1 H), 10.87 (s, 1 H), 8.09 (d, *J* = 7.8 Hz, 1 H), 7.66 (d, *J* = 8.6 Hz, 1 H), 7.54 (d, *J* = 7.8 Hz, 1 H), 7.49–7.24 (m, 16 H), 7.12–6.94 (m, 3 H), 5.04–4.80 (m, 4 H), 4.58–4.46 (m, 1 H), 4.28–4.14 (m, 1 H), 3.75–3.55 (m, 2 H), 3.27–2.98 (m, 4 H).

^{13}C NMR (DMSO-d_6): δ = 173.3, 173.2, 169.0, 156.5, 156.0, 137.9, 137.1, 137.0, 136.1, 129.1, 128.3, 128.3, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.2, 123.7, 120.9, 118.4, 118.2, 111.4, 109.6, 65.5, 65.3, 55.5, 52.9, 43.3, 36.5, 27.2.

HRMS (–ESI-TOF): *m*/*z* $[M - H]^-$ calcd for $C_{38}H_{36}N_4O_8$: 675.2460; found: 675.2477.

N^{α}-{[(Benzyloxy)carbonyl]-L-alanyl}-1-{[(benzyloxy)carbonyl]-L-phenylalanyl}-L-tryptophan (4b)

Yield: 0.53 g (78%); off-white solid; mp 56–58 °C.

^1H NMR (DMSO-d_6): δ = 12.69 (s, 1 H), 10.86 (s, 1 H), 8.04 (d, *J* = 7.7 Hz, 1 H), 7.65 (d, *J* = 8.4 Hz, 1 H), 7.53 (d, *J* = 7.9 Hz, 1 H), 7.39–7.19 (m, 18 H), 7.06 (t, *J* = 7.5 Hz, 1 H), 6.98 (t, *J* = 7.4 Hz, 1 H), 5.08–4.90 (m, 4 H), 4.49 (q, *J* = 7.0 Hz, 1 H), 4.28–4.04 (m, 2 H), 3.23–3.02 (m, 3 H), 2.91–2.71 (m, 1 H), 1.21 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 173.3, 173.2, 172.5, 156.0, 155.6, 137.9, 137.0, 136.0, 129.1, 128.3, 128.3, 128.2, 128.2, 127.8, 127.7, 127.6, 127.5, 127.2, 126.4, 123.7, 120.9, 118.4, 118.2, 111.3, 109.6, 65.4, 65.3, 55.5, 52.9, 49.9, 39.5, 36.5, 27.0, 18.2.

HRMS (–ESI-TOF): *m/z* [M – H]$^-$ calcd for $C_{39}H_{38}N_4O_8$: 689.2617; found: 689.2637.

N^{α}-{[(Benzyloxy)carbonyl]-L-valyl}-1-{[(benzyloxy)carbonyl]glycyl}-L-tryptophan (4c)
Yield: 0.46 g (75%); off-white solid; mp 54–56 °C.

^{1}H NMR (DMSO-d_6): δ = 12.59 (br s, 1 H), 10.85 (s, 1 H), 8.15 (d, *J* = 7.5 Hz, 1 H), 7.53 (d, *J* = 7.8 Hz, 1 H), 7.38–7.22 (m, 12 H), 7.20–7.16 (m, 1 H), 7.06 (t, *J* = 7.5 Hz, 1 H), 6.97 (t, *J* = 7.4 Hz, 1 H), 5.12–4.95 (m, 4 H), 4.49 (dd, *J* = 13.8, 7.3 Hz, 2 H), 4.09–3.54 (m, 2 H), 3.22–2.80 (m, 2 H), 1.95 (dd, *J* = 14.1, 7.4 Hz, 1 H), 0.82 (t, *J* = 7.2 Hz, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.2, 171.2, 159.4, 156.1, 137.1, 136.1, 128.4, 128.3, 127.8, 127.7, 123.6, 120.9, 118.4, 111.3, 109.6, 65.4, 59.9, 52.9, 30.5, 27.1, 19.2, 18.1.

HRMS (–ESI-TOF): *m/z* [M – H]$^-$ calcd for $C_{34}H_{36}N_4O_8$: 627.2460; found: 627.2488.

1-{[(Benzyloxy)carbonyl]-L-alanyl}-N^{α}-{[(benzyloxy)carbonyl]-L-valyl}-L-tryptophan (4d)
Yield: 0.48 g (76%); off-white solid; mp 52–54 °C.

^{1}H NMR (DMSO-d_6): δ = 8.15 (s, 1 H), 7.85–7.72 (m, 2 H), 7.57 (dd, *J* = 10.7, 7.3 Hz, 1 H), 7.45–7.19 (m, 12 H), 7.09–6.92 (m, 2 H), 5.10–4.90 (m, 4 H), 4.78 (t, *J* = 6.4 Hz, 1 H), 4.27–4.12 (m, 1 H), 4.04 (dd, *J* = 7.2, 3.7 Hz, 1 H), 3.47–3.09 (m, 2 H), 2.10–1.90 (m, 1 H), 1.36 (t, *J* = 7.1 Hz, 3 H), 0.88 (d, *J* = 7.4 Hz, 3 H), 0.84 (d, *J* = 6.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 176.5, 174.9, 174.8, 173.9, 158.2, 138.1, 138.0, 137.8, 129.6, 129.5, 129.0, 128.8, 128.8, 127.1, 124.8, 122.5, 120.0, 119.4, 115.7, 112.5, 67.5, 61.9, 54.5, 50.8, 32.1, 28.6, 19.8, 18.8, 18.0.

HRMS (–ESI-TOF): *m/z* [M – H]$^-$ calcd for $C_{35}H_{38}N_4O_8$: 641.2617; found: 641.2626.

(*S*)-3-(4-{[((Benzyloxy)carbonyl)-L-alanyl]oxy}phenyl)-2-{(*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanamido}propanoic Acid (7)
To Boc-Phe-Bt (**1e**; 183 mg, 0.5 mmol) in MeCN (10 mL) was added a solution of tyrosine (**5**; 91 mg, 0.5 mmol) and DBU (1.0 mmol) in DMF (5 mL). The reaction mixture was stirred for 6 h at 20 °C. The mixture was acidified with aq 2 M HCl and extracted with EtOAc (10 mL). The organic layer was washed with aq 2 M HCl (3 mL) and brine (5 mL), and dried ($MgSO_4$). The crude product **6** was treated with Z-Ala-Bt (**1b**; 109 mg, 0.34 mmol) in the presence of Et_3N (1.5 equiv) in MeCN–H_2O (7 mL:3 mL) at 0 °C. After completion of the reaction (6 h), the mixture was acidified with aq 4 M HCl. The solution was then extracted with EtOAc (10 mL), the EtOAc layer was washed with H_2O (10 mL), and dried (Na_2SO_4). Evaporation of the solvent gave the desired product **7**; yield: 0.41 g (65%); white solid; mp 171–173 °C.

^{1}H NMR (DMSO-d_6): δ = 8.12 (d, *J* = 8.0 Hz, 1 H), 7.95 (d, *J* = 6.9 Hz, 1 H), 7.40–7.15 (m, 13 H), 6.98 (d, *J* = 8.3 Hz, 2 H), 6.88 (d, *J* = 8.6 Hz, 1 H), 5.07 (s, 2 H), 4.48 (s, 1 H), 4.32 (s, 1 H), 4.18 (s, 1 H), 3.13–2.89 (m, 3 H), 2.69 (t, *J* = 12.3 Hz, 1 H), 1.42 (d, *J* = 7.0 Hz, 3 H), 1.28 (s, 9 H).

^{13}C NMR (DMSO-d_6): δ = 172.7, 171.8, 171.7, 156.0, 155.2, 149.1, 138.2, 136.9, 135.1, 130.4, 129.2, 128.4, 128.0, 127.9, 127.8, 126.2, 121.2, 78.1, 65.6, 55.8, 53.3, 49.6, 37.4, 36.0, 28.1, 16.8.

HRMS (–ESI-TOF): *m/z* [M – H]$^-$ for $C_{34}H_{39}N_3O_9$: 632.2614; found: 632.2599.

N-Protected Di- and Tripeptides 9a–f; General Procedure
To the corresponding N-protected aminoacyl- and dipeptidoylbenzotriazole **1b**–**h** (0.5 mmol) in MeCN (10 mL) was added a solution of cysteine (**8**; 61 mg, 0.5 mmol) and Et_3N (0.5 mL) in H_2O (3 mL). The reaction mixture was stirred for 4 h at 0 °C. The mixture was acidified with aq 4 M HCl and extracted with EtOAc (10 mL). The organic layer was washed with aq 4 M HCl (3 mL) and brine (5 mL), and dried (Na_2SO_4). After evaporation of the solvent, the residue was triturated with Et_2O–hexanes (1:1) and the solid formed was filtered and dried under vacuum to give the respective dipeptides **9a–c** and tripeptides **9d–f** (Table 3).

[(Benzyloxy)carbonyl]-L-alanyl-L-cysteine (9a)
Yield: 0.31 g (97%); white solid; mp 170–171 °C.

^{1}H NMR (DMSO-d_6): δ = 8.67 (d, *J* = 7.9 Hz, 1 H), 7.92–7.81 (m, 1 H), 7.81–7.65 (m, 5 H), 5.52–5.35 (m, 2 H), 4.99–4.86 (m, 1 H), 4.61–4.45 (m, 1 H), 3.65–3.33 (m, 2 H), 1.65 (d, *J* = 7.1 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 173.1, 172.2, 156.1, 137.4, 128.8, 128.2, 128.2, 65.9, 51.9, 50.4, 31.1, 18.7.

Anal. Calcd for $C_{14}H_{18}N_2O_5S$: C, 51.52; H, 5.56; N, 8.58. Found: C, 51.83; H, 5.55; N,9.10.

[(Benzyloxy)carbonyl]-L-valyl-L-cysteine (9b)
Yield: 0.17 g (96%); white solid; mp 169–170 °C.

^{1}H NMR (DMSO-d_6): δ =12.84 (s, 1 H), 8.16 (d, *J* = 7.7 Hz, 1 H), 7.45–7.20 (m, 6 H), 5.04 (s, 2 H), 4.57–4.27 (m, 1 H), 3.94 (dd, *J* = 8.9, 6.8 Hz, 1 H), 2.92–2.71 (m, 2 H), 2.43 (t, *J* = 8.5 Hz, 1 H), 2.09–1.86 (m, 1 H), 0.89 (d, *J* = 6.7 Hz, 3 H), 0.85 (d, *J* = 6.6 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 171.4, 156.1, 137.1, 128.3, 127.8, 127.6, 65.4, 60.0, 54.3, 30.3, 25.5, 19.2, 18.1.

Anal. Calcd for $C_{16}H_{22}N_2O_5S$: C, 54.22; H, 6.26; N, 7.90. Found: C, 54.26; H, 6.37; N, 7.82.

[(Benzyloxy)carbonyl]-L-phenylalanyl-L-cysteine (9c)
Yield: 0.39 g (98%); white solid; mp 125–126 °C.

^{1}H NMR (DMSO-d_6): δ = 12.95 (br s, 1 H), 8.48 (d, *J* = 7.7 Hz, 1 H), 7.48 (d, *J* = 8.8 Hz, 1 H), 7.38–7.10 (m, 10 H), 5.00–4.82 (m, 2 H), 4.61–4.47 (m, 1 H), 4.39–4.24 (m, 1 H), 3.21 (dd, *J* = 13.7, 4.7 Hz, 1 H), 3.12–2.95 (m, 2 H), 2.74 (dd, *J* = 13.8, 10.9 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 171.8, 171.8, 155.8, 138.1, 137.0, 129.2, 128.3, 128.0, 127.7, 127.4, 126.3, 65.2, 56.0, 51.6, 37.5.

Anal. Calcd for $C_{20}H_{22}N_2O_5S$: C, 59.69, H, 5.51; N, 6.96. Found: C,60.10; H,5.50; N,6.83.

[(Benzyloxy)carbonyl]-L-phenylalanylglycyl-L-cysteine (9d)
Yield: 0.4 g (90%); white solid; mp 156–158 °C.

^{1}H NMR (DMSO-d_6): δ = 12.90 (s, 1 H), 8.39 (t, *J* = 5.8 Hz, 1 H), 8.07 (d, *J* = 7.9 Hz, 1 H), 7.58 (d, *J* = 8.5 Hz, 1 H), 7.40–7.10 (m, 10 H), 4.97 (d, *J* = 12.9 Hz, 1 H), 4.92 (d, *J* = 11.9 Hz, 1 H) 4.53–4.40 (m, 1 H), 4.36–4.21 (m, 1 H), 3.90–3.71 (m, 2 H), 3.04 (dd, *J* = 14.0, 4.0 Hz, 1 H), 2.95–2.72 (m, 2 H), 2.43 (t, *J* = 8.5 Hz, 1 H), 1.36 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 172.0, 171.7, 168.8, 156.0, 138.2, 137.0, 129.2, 128.3, 128.1, 127.7, 127.5, 126.3, 65.3, 56.2, 54.3, 42.0 37.3, 25.7.

Anal. Calcd for $C_{22}H_{25}N_3O_6S$: C, 57.50; H, 5.48; N, 9.14. Found: C, 57.28; H, 5.48; N, 9.00.

[(Benzyloxy)carbonyl]-L-phenylalanyl-L-alanyl-L-cysteine (9e)
Yield: 0.43 g (92%); white solid; mp 177–179 °C.

^{1}H NMR (DMSO-d_6): δ = 12.93 (br s, 1 H), 8.21 (d, *J* = 7.9 Hz, 1 H), 7.97 (d, *J* = 8.9 Hz, 1 H), 7.43 (d, *J* = 7.5 Hz, 1 H), 7.39–7.16 (m, 10 H), 5.04 (d, *J* = 12.6 Hz, 1 H), 4.98 (d, *J* = 12.4 Hz, 1 H), 4.56 (dd, *J* = 9.1, 4.6 Hz, 1 H), 4.43 (dd, *J* = 7.0, 4.4 Hz, 1 H), 4.06–3.94 (m, 1 H), 3.07 (dd, *J* = 13.6, 4.1 Hz, 1 H), 2.94–2.72 (m, 3 H), 1.19 (dd, *J* = 15.6, 8.3 Hz, 1 H), 1.12 (d, *J* = 7.1 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.3, 171.3, 170.9, 155.7, 137.6, 136.9, 129.3, 128.3, 128.0, 127.8, 127.7, 126.2, 65.4, 54.4, 53.5, 50.2, 37.2, 25.5, 18.0.

Anal. Calcd for $C_{23}H_{27}N_3O_6S$: C, 58.34; H, 5.75; N, 8.87. Found: C, 57.96; H, 5.81; N, 8.90.

[(Benzyloxy)carbonyl]-L-alanyl-L-phenylalanyl-L-cysteine (9f)
Yield: 0.44 g (95%); white solid; mp 170–172 °C.

^{1}H NMR (DMSO-d_6): δ = 12.93 (s, 1 H), 8.22 (d, *J* = 7.9 Hz, 1 H), 7.97 (d, *J* = 8.9 Hz, 1 H), 7.43 (d, *J* = 7.4 Hz, 1 H), 7.40–7.15 (m, 10 H), 5.04 (d, *J* = 12.6 Hz, 1 H), 4.98 (d, *J* = 12.4 Hz, 1 H), 4.56 (dd, *J* = 9.2, 4.6 Hz, 1 H), 4.43 (dd, *J* = 7.1, 4.4 Hz, 1 H), 4.06–3.95 (m, 1 H), 3.07 (dd, *J* = 13.8, 4.2 Hz, 1 H), 2.92–2.73 (m, 2 H), 2.44 (d, *J* = 8.7 Hz, 1 H), 1.12 (d, *J* = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.3, 171.3, 170.9, 155.7, 137.6, 136.9, 129.3, 128.3, 128.0, 127.8, 127.7, 126.2, 65.4, 54.4, 53.5, 50.2, 37.2, 25.5, 18.0.

Anal. Calcd for $C_{23}H_{27}N_3O_6S$: C, 58.34, H, 5.75; N, 8.87. Found: C, 57.96; H, 5.81; N, 8.90.

***S*-Acyl Peptides 10a–h; General Procedure**
To a precooled solution of cysteine containing the appropriate peptide **9a**–**f** (0.5 mmol) in MeCN–H_2O (7 mL:3 mL) at 0 °C was added a solution of *N*-acylbenzotriazole or *N*-(Pg-α-aminoacyl)benzotriazole **1b**–**h** (0.5 mmol) in MeCN (3 mL) with stirring followed by addition of $KHCO_3$ (0.14 g) for 10 min in four installments. After additional stirring for 2–3 h at 0 to 10 °C, the reaction mixture was acidified with aq 4 M HCl. The solution was then extracted with EtOAc (10 mL), the EtOAc layer was washed with H_2O (10 mL), and dried (Na_2SO_4). Evaporation of the solvent gave the respective desired product **10a**–**h**, which was recrystallized from EtOAc–hexanes (Table 4).

***N*-{[(Benzyloxy)carbonyl]-L-phenylalanyl}-*S*-{[(benzyloxy)carbonyl]glycyl}-L-cysteine (10a)**
Yield: 0.3 g (45%); white solid; mp 171–173 °C.

^{1}H NMR (DMSO-d_6): δ = 13.00 (s, 1 H), 8.48 (d, *J* = 6.6 Hz, 1 H), 8.03 (t, *J* = 5.6 Hz, 1 H), 7.49 (d, *J* = 8.9 Hz, 1 H), 7.41–7.13 (m, 15 H), 5.14–4.82 (m, 4 H), 4.38–4.29 (m, 2 H), 3.95 (d, *J* = 6.1 Hz, 2 H), 3.27–2.91 (m, 3 H), 2.77–2.68 (m, 1 H).

^{13}C NMR (DMSO-d_6): δ = 198.4, 171.7, 171.4, 156.5, 155.8, 138.1, 137.0, 136.7, 129.2, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.4, 65.8, 65.2, 56.0, 51.7, 50.4, 37.5, 29.1.

HRMS (–ESI-TOF): *m*/*z* [M – H]$^-$ calcd for $C_{30}H_{31}N_3O_8S$: 592.1759; found: 592.1746.

***N*-{[(Benzyloxy)carbonyl]-L-valyl}-*S*-{[(benzyloxy)carbonyl]glycyl}-L-cysteine (10b)**
Yield: 0.45 g (84%); white solid; mp 145–147 °C.

^{1}H NMR (DMSO-d_6): δ = 8.32 (d, *J* = 7.8 Hz, 1 H), 7.99 (t, *J* = 6.1 Hz, 1 H), 7.46–7.19 (m, 11 H), 5.14–4.96 (m, 4 H), 4.40–4.22 (m, 1 H), 3.94 (d, *J* = 6.4 Hz, 3 H), 3.34 (dd, *J* = 13.5, 5.3 Hz, 2 H), 3.10 (dd, *J* = 13.5, 8.4 Hz, 1 H), 2.02–1.95 (m, 1 H), 0.87(d, *J* = 6 Hz, 3 H), 0.83 (d, *J* = 6 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 198.4, 171.5, 171.3, 156.5, 156.1, 137.1, 136.8, 128.4, 128.4, 127.9, 127.8, 127.6, 65.9, 65.5, 59.9, 51.7, 50.4, 30.6, 29.1, 19.2, 17.9.

Anal. Calcd for $C_{26}H_{31}N_3O_8S$: C, 57.24; H, 5.73; N, 7.70. Found: C, 57.0; H, 5.78; N, 7.68.

***N*-{[(Benzyloxy)carbonyl)]-L-alanyl}-*S*-{[(benzyloxy)carbonyl]-L-phenylalanyl}-L-cysteine (10c)**
Yield: 0.51 g (86%); white solid; mp 142–143 °C.

^{1}H NMR (DMSO-d_6): δ = 8.24 (d, *J* = 8.1 Hz, 1 H), 8.15 (d, *J* = 7.1 Hz, 1 H), 7.44 (d, *J* = 8.3 Hz, 1 H), 7.37–7.20 (m, 15 H), 5.08–4.94 (m, 4 H), 4.43–4.34 (m, 2 H), 4.17–4.06 (m, 1 H), 3.36 (dd, *J* = 13.6, 5.5 Hz, 1 H), 3.18–3.05 (m, 2 H), 2.81 (dd, *J* = 13.9, 11.1 Hz, 1 H), 1.24 (d, *J* = 7.1 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 200.6, 172.6, 171.6, 156.0, 155.6, 137.4, 137.0, 136.8, 129.2, 128.4, 128.3, 128.2, 127.8, 127.8, 127.4, 126.5, 65.6, 65.5, 62.7, 51.5, 50.0, 36.5, 29.7, 18.3.

Anal. Calcd for $C_{31}H_{33}N_3O_8S$: C, 61.27; H, 5.47; N, 6.91. Found: C, 60.88; H, 5.35; N, 7.20.

***S*-{[(Benzyloxy)carbonyl]-L-alanyl}-*N*-{[(benzyloxy)carbonyl]-L-alanyl-L-phenylalanyl}-L-cysteine (10d)**
Yield: 0.65 g (97%); white solid; mp 173–175 °C.

^{1}H NMR (DMSO-d_6): δ = 12.92 (s, 1 H), 8.60 (d, *J* = 7.9 Hz, 1 H), 8.20 (d, *J* = 8.0 Hz, 1 H), 7.42–7.15 (m, 17 H), 5.14–4.88 (m, 4 H), 4.58 (s, 1 H), 4.42–4.24 (m, 1 H), 4.20–3.95 (m, 2 H), 3.29 (s, 1 H), 3.19–3.00 (m, 2 H), 2.88 (dd, *J* = 14.1, 10.1 Hz, 1 H), 1.21 (d, *J* = 6.9 Hz, 3 H), 1.19 (d, *J* = 6.6 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 199.8, 172.8, 172.5, 171.5, 155.6, 155.5, 137.1, 137.0, 136.9, 129.1, 128.3, 128.2, 128.1, 127.8, 127.7, 126.5, 65.4, 65.4, 60.4, 51.5, 49.9, 36.5, 29.4, 18.3, 17.9.

Anal. Calcd for $C_{34}H_{38}N_4O_9S$: C, 60.16; H, 5.64; N, 8.25. Found: C, 59.88; H, 5.66; N, 8.21.

***N*-{[(Benzyloxy)carbonyl]-L-alanyl}-*S*-[(benzyloxy)carbonyl]-L-alanyl-L-phenylalanyl-L-cysteine (10e)**
Yield: 0.64 g (95%); white solid; mp 161–163 °C.

^{1}H NMR (DMSO-d_6): δ = 12.92 (s, 1 H), 8.60 (d, *J* = 7.9 Hz, 1 H), 8.20 (d, *J* = 8.0 Hz, 1 H), 7.44–7.15 (m, 17 H), 5.10–4.91 (m, 4 H), 4.61–4.58 (m, 1 H), 4.38–4.26 (m, 1 H), 4.16–3.99 (m, 2 H), 3.32–3.29 (m, 1 H), 3.16–3.02 (m, 2 H), 2.88 (dd, *J* = 14.1, 10.1 Hz, 1 H), 1.21 (d, *J* = 6.9 Hz, 3 H), 1.19 (d, *J* = 6.3 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 199.8, 172.8, 172.5, 171.5, 155.6, 155.5, 137.0, 129.1, 128.3, 128.2, 128.2, 127.8, 127.7, 126.5, 65.4, 60.4, 51.5, 49.9, 36.5, 29.4, 18.3, 17.9.

Anal. Calcd for $C_{34}H_{38}N_4O_9S$: C, 60.16; H, 5.64; N, 8.25. Found: C, 60.23; H, 5.82; N, 8.31.

***S*-{[(Benzyloxy)carbonyl]-L-alanyl}-*N*-[(benzyloxy)carbonyl]-L-phenylalanylglycyl-L-cysteine (10f)**
Yield: 0.61 g (94%); white solid; mp 169–171 °C.

^{1}H NMR (DMSO-d_6): δ = 12.95 (s, 1 H), 8.34–8.19 (m, 2 H), 8.05 (d, *J* = 7.4 Hz, 1 H), 7.52 (d, *J* = 8.5 Hz, 1 H), 7.40–7.14 (m, 15 H), 5.11–4.84 (m, 4 H), 4.45–4.12 (m, 3 H), 3.83–3.63 (m, 2 H), 3.30–3.23 (m, 1 H), 3.12–2.96 (m, 2 H), 2.73 (dd, *J* = 13.8, 10.7 Hz, 1 H), 1.24 (d, *J* = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 201.6, 171.8, 171.4, 168.7, 155.9, 155.8, 138.2, 137.0, 129.2, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.4, 126.2, 65.8, 65.2, 56.7, 56.2, 51.5, 41.7, 37.4, 29.5, 17.3.

Anal. Calcd for $C_{33}H_{36}N_4O_9S$: C, 59.63; H, 5.46; N, 8.43. Found: C, 59.24; H, 5.55; N, 8.42.

***S*-{[(Benzyloxy)carbonyl]-L-alanyl}-*N*-[(benzyloxy)carbonyl]-L-phenylalanyl-L-alanyl-L-cysteine (10g)**
Yield: 0.66 g (96%); white solid; mp 170–171 °C.

^{1}H NMR (DMSO-d_6): δ = 12.92 (br s, 1 H), 8.60 (d, *J* = 7.9 Hz, 1 H), 8.20 (d, *J* = 8.1 Hz, 1 H), 7.48–7.16 (m, 17 H), 5.09–4.92 (m, 4 H), 4.65–4.52 (m, 1 H), 4.38–4.26 (m, 1 H), 4.16–4.00 (m, 2 H), 3.31–3.29 (m, 1 H), 3.16–3.02 (m, 2 H), 2.88 (dd, *J* = 14.1, 10.1 Hz, 1 H) 1.21 (d, *J* = 6.8 Hz, 3 H), 1.19 (d, *J* = 6.6 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 199.8, 172.8, 172.5, 171.5, 155.6, 155.5, 137.0, 129.1, 128.3, 128.2, 128.2, 127.8, 127.7, 126.5, 65.4, 65.4, 60.4, 49.9, 49.8, 36.5, 29.4, 18.3, 17.9.

Anal. Calcd for $C_{34}H_{38}N_4O_9S$: C, 60.16; H, 5.64; N, 8.25. Found: C, 60.35; H, 5.67; N, 8.19.

***S*-[(Benzyloxy)carbonyl]-L-alanyl-L-phenylalanyl-*N*-[(benzyloxy)carbonyl]-L-phenylalanylglycyl-L-cysteine (10h)**
Yield: 0.79 g (98%); white solid; mp 146–148 °C.

^{1}H NMR (DMSO-d_6): δ = 8.64 (d, J = 8.0 Hz, 1 H), 8.32 (t, J = 5.7 Hz, 1 H), 8.22 (d, J = 8.0 Hz, 1 H), 7.56 (d, J = 8.7 Hz, 1 H), 7.42–7.15 (m, 21 H), 5.07–4.88 (m, 4 H), 4.65–4.55 (m, 1 H), 4.42–4.26 (m, 2 H), 4.16–4.04 (m, 1 H), 3.85–3.72 (m, 2 H), 3.40–3.29 (m, 1 H), 3.16–3.03 (m, 3 H), 2.96–2.84 (m, 1 H), 2.77 (dd, J = 13.1, 10.0 Hz, 1 H), 1.21 (d, J = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 199.9, 172.8, 171.4, 168.6, 155.9, 137.1, 137.0, 129.2, 129.1, 128.3, 128.3, 128.2, 128.0, 127.8, 127.7, 127.4, 126.2, 65.2, 60.4, 56.2, 51.7, 49.9, 41.7, 36.5, 29.8, 17.9.

HRMS (–ESI-TOF): m/z [M – H]$^-$ calcd for $C_{42}H_{45}N_5O_{10}S$: 810.2814; found: 810.2822.

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