Chem Biol Drug Des 2012; **80:** 194–202

Research Article

doi: 10.1111/j.1747-0285.2011.01303.x

Cysteinoyl- and Cysteine-containing Dipeptidoylbenzotriazoles with Free Sulfhydryl Groups: Easy Access to N-terminal and Internal Cysteine Peptides

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N-Protected cysteines 4a–c each with a free sulfhydryl group were prepared in 70–75% yields by treatment of L-cysteine with 1-(benzyloxycarbonyl) benzotriazole (Cbz-Bt) 1a, *N*-(*tert*-butyloxy-carbonyl)benzotriazole (Boc-Bt) 1b, and 1-(9-fluorenyl-methoxy-carbonyl)benzotriazole (Fmoc-Bt) 1c, respectively. N-Protected, free sulfhydryl cysteines 4a–c were then converted into the corresponding N-protected, free sulfhydryl cysteinoylbenzotriazoles 7a–c (70–85%), which on treatment with diverse amino acids and dipeptides afforded the corresponding N-protected, free sulfhydryl N-terminal cysteine dipeptides 8a–e and tripeptides 8f–h in 73–80% yields. N-Protected, free sulfhydryl cysteine-containing dipeptides 9a,b were converted into the corresponding N-protected, free sulfhydryl dipeptidoylbenzotriazoles 10a,b (69–81%), which on treatment with amino acids, dipeptides, and a tripeptide afforded internal cysteine tripeptides 11a–c, tetrapeptides 11d,e and pentapeptide 11f, each containing a N-protected, free sulfhydryl groups in 70–90% yields under mild conditions. Treatment of N-protected, free sulfhydryl cysteinoylbenzotriazole 7a with diamines 12a,b afforded directly the cysteine-containing disulfide-bridged cyclic peptides 14a,b in 50% yields.

Key words: acylation, benzotriazole methodology, cysteine, N-protected(α-aminoacyl)benzotriazole, small peptides

Received 26 July 2011, revised 5 December 2011 and accepted for publication 5 December 2011

Cysteine peptides are diagnostically and therapeutically important in many areas of biomedical research (1–6) and thus attractive targets for drug discovery. Consequently, interest in cysteine peptide synthesis has increased significantly (7–9), including the preparation of unsymmetrical disulfides (10,11), formation of oligodeoxynucleotide (ODN)-peptide covalent linkages (12), and the synthesis of cyclic peptides (13).

N-Terminal cysteine peptides with the cysteine residues in the free thiol form are frequently needed (14,15). Syntheses of such cysteine peptides usually require initial SH-protection or regioselective intra- or intermolecular disulfide bridge formation (14,15), and such maximum protection has been the norm during the coupling steps in solid-phase peptide synthesis (16,17).

N-Cbz, *N*-Fmoc, and *N*-Boc-L-cysteine units are valuable building blocks for solid-phase peptide synthesis (18–20). They are available (i) in the form of disulfides which may subsequently be cleaved to the free SH group or (ii) with SH protected with Fmoc (18–20). Cysteine residues with SH protected by groups such as Fmoc or Boc are prone to side reactions such as (i) β-elimination leading to the formation of dehydroalanine derivatives under basic reaction conditions, followed by subsequent conjugate nucleophilic addition, for example, of piperidine (1) (ii) oxidation (18,21), (iii) alkylation (18,21), and (iv) acyl migration from sulfur to nitrogen (22).

We now report N-protected, SH-unprotected cysteinoylbenzotriazoles and cysteine-containing dipeptidoylbenzotriazoles which allow the efficient synthesis of N-terminal cysteine-containing peptides and internal cysteine-containing peptides, respectively, both with free sulfhydryl groups. Subsequent cyclization under mild conditions in the case of bis-cysteine-containing peptides forms disulfide-bridged cyclic peptides. We employ three different N-protecting groups (Cbz, Boc, and Fmoc) for the synthesis of the cysteine peptide sequences, all of which were compatible with reaction conditions convenient for peptide synthesis.

Materials and Methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR

spectra were recorded in $CDCl_3$ or DMSO-d_6 with TMS for ^1H (300 MHz) and ^{13}C (75 MHz) as an internal reference. Elemental analyses were performed on a Carlo Erba–1106 instrument (Carlo Erba Reagents, Val de Reuil, France). CH_2Cl_2 was dried and distilled over CaH_2, whereas THF was used after distillation over Na-benzophenone. Mass spectrometry was carried out on Agilent 6210 TOF-MS with electrospray ionization (ESI).

Preparation of N-Cbz, *N*-Boc-, and *N*-Fmoc-1*H*-benzotriazoles 1a–c

N-Cbz, *N*-Boc-, and *N*-Fmoc-1*H*-Benzotriazoles **1a–c** were prepared according to literature procedures (23,26).

N-Cbz-1*H*-Benzotriazole (1a) (23,24)

1a was synthesized according to literature method. (23,24) White microcrystals, yield 85%, mp 108–110 °C (lit., (23) 108.0–110.0 °C); ^1H NMR ($CDCl_3$) δ 8.12 (t, J = 9.3 Hz, 2H), 7.68–7.60 (m, 1H), 7.60–7.54 (m, 2H), 7.54–7.39 (m, 4H), 5.64 (s, 2H); ^{13}C NMR (DMSO–d_6) δ 148.2, 145.1, 134.4, 131.2, 130.5, 128.8, 128.7, 128.6, 125.9, 120.1, 113.3, 69.9.

N-Boc-1*H*-Benzotriazole (1b) (25,26)

1b was synthesized according to literature method (25,26). White microcrystals, yield 50%, mp 70.0–72.0 °C (lit., (25,26) 62.0–63.0 °C); ^1H NMR ($CDCl_3$) δ 8.22–8.02 (m, 2H), 7.64 (t, J = 7.8 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 1.77 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 146.8, 146.1, 131.8, 130.0, 125.6, 120.4, 113.7, 87.1, 28.2.

N-Fmoc-1*H*-Benzotriazole (1c)

A mixture of (9*H*-fluoren-9-yl)methyl chloroformate (1 g, 3.87 mmol) and benzotriazole (0.92 g, 7.74 mmol) in CH_2Cl_2 (20 mL) was stirred for 3 h at 10 °C. The precipitate formed was filtered off and discarded. The filtrate was evaporated under reduced pressure to give a crude product, which was washed with diethyl ether and recrystallized from CH_2Cl_2-hexanes to afford *N*-Fmoc-1*H*-benzotriazole. White microcrystals, yield 88%, mp 90–91 °C; ^1H NMR (DMSO-d_6) δ 8.09–8.18 (m, 1H), 7.91 (d, J = 7.2 Hz, 2 H), 7.81 (d, J = 7.2, 2H), 7.51–7.39 (4H, m), 7.38–7.29 (2H, m), 7.23 (br s, 1H), 5.10 (d, J = 5.1 Hz, 2H), 4.58 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 148.0, 145.0, 143.1, 140.9, 130.8, 130.1, 127.9, 127.3, 125.8, 125.0, 120.2, 119.9, 113.0, 69.5, 46.2. Anal. Calcd for $C_{21}H_{15}N_3O_2$: C, 73.89; H, 4.43; N, 12.31. Found: C, 73.84; H, 4.06; N, 12.29.

Synthesis of N-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteines 4a–c

To a solution of L-cysteine **2** (1 mmol) and triethylamine (1 mmol) in CH_3CN-H_2O (3:1, 8 mL) was added the corresponding *N*-Cbz, *N*-Boc-, and *N*-Fmoc-1*H*-benzotriazoles **1a–c** (1 mmol). The mixture was stirred at room temperature for 2 h. Solvent was removed under reduced pressure, and ethyl acetate (10 mL) was added. The organic layer was washed with 2 N HCl and brine. Evaporation to the solvent followed by recrystalization (AcOEt: hexanes = 3:1) gave *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteine **4a–c**.

N-Cbz-L-Cys-OH (4a) (27)

White microcrystals, yield 75%, mp 67–68 °C; ^1H NMR (DMSO-d_6) δ 12.96 (s, 1H), 8.03 (s, 1H), 7.35 (s, 5H), 5.04 (s, 2H), 4.27 (s, 1H), 3.14 (d, J = 13.5 Hz, 1H), 2.91 (t, J = 15 Hz, 1H), 1.23 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 172.1, 156.3, 137.0, 128.5, 128.2, 128.0, 65.8, 56.7, 25.7. Anal. Calcd for $C_{11}H_{13}N_4O_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.65; H, 4.90; N, 5.36.

N-Boc-L-Cys-OH (4b)

Oil, yield 71%, (Lit., (28) 75–78 °C); ^1H NMR (DMSO-d_6) δ 12.81 (br s, 1H), 7.15 (d, J = 8.1 Hz, 1H), 4.18 (s, 1H), 3.08 (d, J = 12.6 Hz, 1H), 2.88 (t, J = 11.1 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 172.5, 155.4, 78.4, 52.8, 28.2. Anal. Calcd for $C_8H_{15}NO_4S$: C, 43.42; H, 6.83; N, 6.33. Found: C, 43.77; H, 6.54; N, 6.10.

N-Fmoc-L-Cys-OH (4c)

White microcrystals, yield 70%, mp 134–136 °C (lit., (29) 140–142 °C); ^1H NMR (DMSO-d_6) δ 12.80 (br s, 1H), 7.90 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 7.2 Hz, 2H), 7.79–7.60 (m, 3H), 7.43 (t, J = 7.2 Hz, 2H), 4.32 (d, J = 6 Hz, 2H), 4.26 (d, J = 6.3 Hz, 1H), 4.20–4.08 (m, 1H), 2.90 (d, J = 8.1 Hz, 1H), 2.84–2.65 (m, 1H), 1.26 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 171.4, 169.1, 156.5, 143.8, 140.7, 127.6, 127.1, 125.2, 120.1, 65.8, 54.2, 46.6, 25.7.

S-Acylation of N-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteines 6a–c

To a solution of *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteines **4a–c**. (1 mmol) in THF (5 mL) was added a solution of *N*-Cbz, *N*-Boc-, and *N*-Fmoc-L-cysteines **4a–c** (1 mmol) in THF (5 mL) in the presence of $KHCO_3$ (1 mmol). The heterogeneous mixture was then stirred at room temperature for 12 h. THF was evaporated, and the solution was acidified with HCl, and the solid formed was taken in ethyl acetate and washed with HCl (1*N*, 3×) and brine (3×) and then dried over sodium sulfate. Evaporation of ethyl acetate under reduced pressure afforded N-protected *S*-acyl cysteines **6a–c**.

N-Cbz-L-Cys(*S*-4-NO_2-Bz)-OH (6a)

White microcrystals, yield 80%, mp 155–156 °C; ^1H NMR (DMSO-d_6) δ 7.60 (d, J = 6.9 Hz, 1H), 7.37 (s, 9H), 5.06 (s, 2H), 4.14 (d, J = 4.2 Hz, 1H), 2.87 (br s, 1H), 2.74 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 197.6, 172.3, 155.6, 137.7, 137.0, 129.3, 128.4, 128.0, 127.8, 126.3, 125.2, 65.5, 53.9, 30.7. Anal. Calcd for $C_8H_{15}NO_4S.H_2O$: C, 51.18; H, 4.29; N, 6.63. Found: C, 51.26; H, 4.67; N, 6.60.

N-Boc-L-Cys(*S*-4-NO_2-Bz)-OH (6b)

White microcrystals, yield 85%, mp 175–177 °C; ^1H NMR (DMSO-d_6) δ 7.69 (s, 2H), 7.45 (s, 3H), 4.20 (br s, 1H), 3.65 (br s, 1H), 3.28 (br s, 1H), 1.22–1.58 (m, 9H); ^{13}C NMR (DMSO-d_6) δ 199.8, 171.5, 155.7, 155.5, 137.1, 129.1, 128.3, 127.8, 126.5, 78.1, 65.4, 29.6, 28.2. Anal. Calcd for $C_{15}H_{18}N_2O_7S$: C, 46.64; H, 4.90; N, 7.56. Found: C, 46.26; H, 4.51; N, 7.25.

N-Fmoc-L-Cys(*S*-4-OMe-Bz)-OH (6c)

White microcrystals, yield 78%, mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 8.30 (dd, J = 8.4, 5.4 Hz, 1H), 8.18 (d, J = 9.3 Hz, 1H), 7.86–7.94 (m, 2H), 7.86–7.78 (m, 1H), 7.76–7.62 (m, 2H), 7.49–7.38 (m, 3H), 7.34 (t, J = 6.3 Hz, 2H), 7.21 (d, J = 8.7 Hz, 1H), 4.50–4.10 (m, 3H), 3.92 (s, 3H), 3.37 (br s, 1H), 3.28–3.09 (m, 1H), 3.08–2.85 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 211.8, 181.2, 167.1, 154.6, 151.7, 147.4, 138.9, 138.3, 138.1, 137.6, 135.8, 130.4, 67.6, 62.1, 57.6, 54.3, 27.5. Anal. Calcd for $C_{26}H_{23}NO_6S$: C, 65.39; H, 4.85; N, 2.93. Found: C, 65.37; H, 4.58; N, 3.26.

Synthesis of N-(*N*′- Cbz-and *N*′-Fmoc-L-Cys)-1*H*-benzotriazoles 7a,c

Thionyl chloride (1 mmol) was added to a solution of 1*H*-benzotriazole (4 mmol) in anhydrous THF (30 mL) at 25 °C, and the reaction mixture was stirred for 20 min. *N*-Cbz- and *N*-Fmoc-L-cysteines **4a–c** (1 mmol) were added to the reaction mixture, which was stirred for 2 h at 0–5°C. The white precipitate which formed was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (15 mL), and the solution was washed with sat. Na_2CO_3 solution (3 × 5 mL), sat. NaCl solution (5 mL), and dried with ($MgSO_4$). Removal of the solvent under reduced pressure gave **7a–c**, which were recrystallized from CH_2Cl_2-hexanes.

N-(*N*′-Cbz-L-Cys)-1*H*-benzotriazole (7a)

White microcrystals, yield 70%, mp 155–156 °C (lit., (30) 144–147 °C); ^{1}H NMR (DMSO-d_6) δ 8.41 (d, J = 7.2 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.82 (t, J = 8.1 Hz, 1H), 7.74–7.60 (m, 1H), 7.54–7.30 (m, 5H), 7.02 (br s, 1H), 6.05–5.82 (s, 1H), 4.95–5.20 (s, 2H), 3.61–3.45 (m, 1H), 3.25–3.10 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 170.3, 156.1, 145.2, 136.5, 131.2, 130.5, 128.3, 127.9, 127.8, 126.8, 120.1, 113.9, 66.0, 53.2, 37.2. Anal. Calcd for $C_{17}H_{16}N_4O_3S.1/2H_2O$: C, 63.56; H, 4.67; N, 12.35. Found: C, 63.78; H, 4.67; N, 12.65.

N-(*N*′-Fmoc-L-Cys)-1*H*-benzotriazole (7c)

White microcrystals, yield 85%, mp 169–171°C; ^{1}H NMR (DMSO-d_6) δ 8.08–7.80 (m, 5H), 7.72 (t, J = 6.6 Hz, 2H), 7.58–7.24 (m, 6H), 4.59–4.22 (m, 3H), 3.68 (d, J = 3.3 Hz, 1H), 3.10–3.24 (m, 1H), 3.06–2.88 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.3, 171.5, 156.1, 143.8, 140.8, 128.0, 127.7, 127.1, 125.3, 120.1, 65.8, 53.1, 46.7, 31.4. Anal. Calcd for $C_{14}H_{18}N_4O_3S$: C, 64.90; H, 5.11, N, 12.60. Found: C, 64.85; H, 4.99; N, 12.97.

Synthesis of N-(*N*′-Boc-L-Cys)-1*H*-benzotriazole (7b)

To Boc-L-Cysteine (0.221 g, 1 mmol), dissolved in dichloromethane (30 mL), benzotriazole (0.119 g, 1 mmol) and dicyclohexylcarbodiimide (0.206 g, 1 mmol) were added. The reaction mixture was stirred at 0–5 °C, overnight. Dicyclohexylurea was filtered off, and the dichloromethane was evaporated. The residue was recrystallized from dichloromethane (20 mL) and hexanes (25 mL).

N-(*N*′-Boc-L-Cys)-1*H*-benzotriazole (7b)

White microcrystals, yield 80%, mp 173–175 °C; ^{1}H NMR (DMSO-d_6) δ 7.91 (s, 2H), 7.44 (s, 2H), 7.23 (d, J = 3.5 Hz, 1H), 4.19 (s, 1H,), 3.00–2.60 (m, 2H), 1.77 (d, J = 12.0 Hz, 1H), 1.37 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 170.7, 156.6, 155.5, 131.2, 128.6, 126.8, 120.2, 113.9, 79.0, 52.9, 28.1, 27.6. Anal. Calcd for $C_{14}H_{18}N_4O_3S$: C, 52.16; H, 5.63; N, 17.38. Found: C, 52.34; H, 5.75; N, 17.50.

General procedure for N-terminal cysteine-containing N-Cbz-, *N*-Boc-, and *N*-Fmoc-dipeptides 8a–e, (8a + 8a′) and tripeptides 8f–h

N-(*N*′-Cbz-, *N*′-Boc- and *N*′-Fmoc-L-cys)-1*H*-benzotriazoles **7a–c** (1 equiv.) were added at room temperature to a solution of amino acid or dipeptide (1 equiv.) in MeCN/H_2O (7:3 mL) in the presence of triethylamine (1 equiv.). The reaction mixture was stirred at room temperature until the starting material was completely consumed, as observed by TLC using hexanes-ethyl acetate (2:1) as eluent. Aq. 6 N HCl solution was then added, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (20 mL), and the organic extract was washed with 6 N HCl solution, sat. NaCl solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the desired products, which were recrystallized from chloroform- hexanes or ethyl acetate-hexanes.

N-Cbz-L-Cys-L-Phe-OH (8a) (27)

White microcrystals, yield 80%, mp 131–132°C; ^{1}H NMR (DMSO-d_6) δ 12.88 (s, 1H), 8.24–8.18 (m, 1H), 7.75–7.52 (m, 1H), 7.30–7.18 (m, 6H), 7.30–7.10 (m, 4H), 5.05 (s, 2H), 4.55–4.40 (m, 1H), 4.32 (br s, 1H), 3.24–2.90 (m, 3H), 2.88–2.72 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.5, 170.0, 155.9, 137.2, 136.8, 129.2, 128.3, 128.1, 127.8, 127.7, 126.4, 65.6, 53.6, 53.5, 36.5, 22.7. Anal. Calcd for $C_{20}H_{22}N_2O_5S$: C, 59.69; H, 5.51; N, 6.96. Found: C, 59.47; H, 5.57; N, 7.01.

N-Cbz-L-Cys-DL-Phe-OH (8a + 8a′) (27)

White microcrystals, yield 80%; ^{1}H NMR (DMSO-d_6) δ 12.90 (br s, 1H), 7.42–7.16 (m, 12H), 5.10–5.00 (m, 2H), 4.55–4.42 (m, 1H), 4.40–4.30 (m, 1H), 3.20–2.73 (m, 4H), 1.23 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 172.6, 170.4, 170.1, 156.7, 156.0, 138.4, 137.3, 136.9, 129.2, 128.9, 128.4, 128.2, 127.8, 127.7, 126.8, 126.5, 69.7, 69.1, 65.7, 65.0, 54.4, 53.6, 36.6, 35.4, 33.6. Anal. Calcd for $C_{20}H_{22}N_2O_5S$: C, 59.69; H, 5.51; N, 6.96. Found: C, 59.75; H, 5.60; N, 7.00.

N-Fmoc-L-Cys-L-Phe-OH (8b)

White microcrystals, yield 75%, mp 145–146 °C; ^{1}H NMR (DMSO-d_6) δ 13.00 (br s, 1H), 8.39 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 7.8 Hz, 2H), 7.71 (d, J = 8.7 Hz, 1H), 7.65 (dd, J = 6.9, 4.2 Hz, 2H), 7.44–7.15 (m, 9H), 4.60–4.48 (m, 1H), 4.47–4.35 (m, 1H), 4.25–4.10 (m, 2H), 4.03 (dd, J = 14.0, 6.9 Hz, 1H), 3.09 (dd, J = 13.7, 3.3 Hz, 1H), 3.00–2.77 (m, 3H), 2.44 (t, J = 8.4 Hz, 1H,); ^{13}C NMR (DMSO-d_6) δ 171.8, 171.5, 155.8, 143.8, 140.7, 138.1, 129.3, 128.1, 127.6, 127.1, 126.3, 125.3, 120.1, 65.7, 56.0, 54.4, 46.6, 37.5, 25.7. Anal. Calcd for $C_{27}H_{26}N_2O_5S$: C, 66.10; H, 5.34; N, 5.71. Found: C, 65.97; H, 5.51; N, 5.38.

N-Cbz-L-Cys-L-Ser-OH (8c)

Oil. Yield 73%; ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1H), 7.50–7.20 (m, 6H), 5.04 (s, 2H), 4.50 (s, 1H), 4.13 (s, 1H), 3.62 (br s, 1H), 3.59–3.40 (m, 1H), 3.18 (br s, 1H), 3.08 (d, J = 6.9 Hz, 1H), 2.97 (br s, 1H), 1.18 (t, J = 7.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 181.0, 180.8, 166.8, 147.7, 138.9, 138.3, 138.2, 77.7, 76.6, 70.3, 64.5, 29.0. Anal. Calcd for $C_{14}H_{18}N_2O_6S$: C, 49.11; H, 5.30; N, 8.18. Found: C, 49.52; H, 4.89; N, 7.77.

N-Fmoc-L-Cys-L-Trp-OH (8d)

White microcrystals, yield 77%, mp 136–137 °C; ^{1}H NMR (DMSO-d_6) δ 12.88 (br s, 1H), 10.85 (s, 1H), 8.35 (d, J = 7.8 Hz, 1H), 7.90–7.80 (m, 2H), 7.74–7.50 (m, 4H), 7.50–7.14 (m, 6H), 7.08 (t, J = 7.2 Hz, 1H), 6.99 (t, J = 6.9 Hz, 1H), 4.49 (dd, J = 7.2, 2.1 Hz, 1H), 4.44–4.32 (m, 1H), 4.22–4.02 (m, 3H), 3.16 (dd, J = 14.7, 3.6 Hz, 1H,), 3.06–2.75 (m, 3H), 2.42 (t, J = 8.1 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 172.1, 171.4, 155.8, 143.7, 140.6, 136.1, 127.6, 127.3, 127.0, 125.3, 123.9, 120.8, 120.0, 118.5, 118.2, 111.3, 110.1, 65.7, 55.4, 54.4, 46.6, 27.8, 25.5. Anal. Calcd for $C_{29}H_{27}N_3O_5S$: C, 65.77; H, 5.14; N, 7.93. Found: C, 65.37; H, 5.58; N, 7.46.

N-Boc-L-Cys-L-Ala-OH (8e) (31)

Yellow microcrystals, yield 83%, mp 144–145 °C; ^{1}H NMR (DMSO-d_6) δ 12.62 (s, 1H), 8.40–8.00 (m, 1H), 7.07 (d, J = 8.7 Hz, 1H), 4.42–4.10 (m, 2H), 3.24–3.00 (m, 1H), 2.99–2.64 (m, 1H), 1.80–0.80 (m, 13H); ^{13}C NMR (DMSO-d_6) δ 174.1, 170.4, 155.6, 78.8, 53.6, 47.9, 28.4, 24.7, 17.4. Anal. Calcd for $C_{11}H_{20}N_2O_5S$: C, 45.19; H, 6.90; N, 9.58. Found: C, 45.32; H, 6.48; N, 10.00.

N-Cbz-L-Cys-Gly-Gly-OH (8f)

White microcrystals, yield 75%, mp 159–161 °C; ^{1}H NMR (DMSO-d_6) δ 8.83 (d, J = 8.7 Hz, 1H), 8.68 (d, J = 7.5 Hz, 1H), 8.13 (br s, 1H), 7.42–7.28 (m, 5H), 5.10–4.95 (m, 2H), 4.66–4.53 (m, 1H), 3.64–3.52 (m, 4H), 3.15–3.05 (m, 1H), 2.98–2.82 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.8, 172.3, 171.1, 155.6, 142.9, 129.3, 128.3, 128.0, 127.8, 126.2, 65.5, 56.2, 41.8, 24.7. Calcd for $C_{15}H_{19}N_3O_6S.H_2O$: C, 46.50; H, 5.46; N, 10.85. Found: C, 46.85; H, 5.60; N, 11.12.

N-Cbz-L-Cys-L-Leu-Gly-OH (8g)

White microcrystals, yield 80%, mp 162–164 °C; ^{1}H NMR (DMSO-d_6) δ 8.74 (s, 1H), 8.01(d, J = 7.5 Hz, 1H), 7.76 (s, 1H), 7.37 (s, 5H), 5.18–4.90 (m, 2H), 4.25–4.10 (m, 2H), 3.98 (br s, 1H), 3.63–3.46 (m, 2H), 3.12–2.92 (m, 1H), 1.61 (s, 1H), 1.48 (s, 2H), 0.85 (br s,); ^{13}C NMR (DMSO-d_6) δ 174.2, 172.9, 170.9, 156.1, 140.7, 127.6, 127.1, 125.3, 65.7, 58.3, 53.3, 46.7, 40.6, 24.9, 24.2, 23.1, 21.5. Anal. Calcd for $C_{19}H_{27}N_3O_6S$: C, 53.63; H, 6.40; N, 9.88. Found: C, 53.86; H, 6.22; N, 9.49.

N-Cbz-L-Cys-Gly-L-Ala-OH (8h)

White microcrystals, yield 80%, mp 143–145 °C; ^{1}H NMR (DMSO-d_6) δ 12.80 (s, 1H), 8.34 (br s, 1H), 8.06 (d, J = 7.2 Hz, 1H), 7.72 (dd, J = 11.4, 9.3 Hz, 1H), 7.40–7.28 (m, 5H), 5.04 (s, 3H), 4.40–4.14 (m, 2H), 3.73 (d, J = 5.7 Hz, 1H), 3.22–3.08 (m, 1H), 2.96–2.80 (m, 1H), 1.26 (d, J = 7.2 Hz, 3H), 0.85 (d, J = 7.5 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 173.9, 172.2, 168.2, 156.1, 136.9, 128.3, 127.8, 127.7, 65.6, 53.9, 53.1, 47.5, 41.9, 17.3. Calcd for $C_{16}H_{21}N_3O_6S$: C, 50.12; H, 5.52; N, 10.96. Found: C, 49.81; H, 5.65; N,11.01.

Synthesis of N-(*N'*-Cbz- and *N'*-Fmoc-α-dipeptidoyl)-1*H*-benzotriazoles 10a,b

Thionyl chloride (1 mmol) was added to a solution of 1*H*-benzotriazole (4 mmol) in anhydrous THF (30 mL) at 20 °C, and the reaction mixture was stirred for 20 min. *N*-Cbz- and *N*-Fmoc-L-cysteine dipeptides **9a**,**b** (1 mmol) were added to the reaction mixture at −30°C, which was stirred for 4 h at −30 °C. The white precipitate which formed was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (15 mL), and the solution was washed with sat. Na_2CO_3 solution (3 × 5 mL), sat. NaCl solution (5 mL), and dried with ($MgSO_4$). Removal of the solvent under reduced pressure gave **10a**,**b** which were recrystallized from CH_2Cl_2-hexanes.

N-(*N'*-Cbz-L-Phe-L-Cys)-1*H*-benzotriazole (10a)

White microcrystals, yield 81%, mp 154–156 °C; ^{1}H NMR ($CDCl_3$) δ 8.19 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.56–7.48 (m, 1H), 7.36–7.22 (m, 12H), 5.33 (d, J = 8.1 Hz, 1H), 5.09 (s, 2H), 4.54 (t, J = 7.2 Hz, 1H), 3.66–3.12 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 172.6, 167.9, 155.7, 146.0, 136.3, 135.1, 131.0, 129.5, 128.8, 128.7, 128.3, 128.2, 127.6, 126.8, 120.6, 114.4, 67.2, 49.6, 37.7, 18.8. Anal. Calcd for $C_{26}H_{25}N_5O_4S$: C, 62.01; H, 5.00; N, 13.91. Found: C, 62.32; H, 5.22; N, 14.18.

N-(*N'*-Fmoc-Gly-L-Cys)-1*H*-benzotriazole (10b)

White microcrystals, yield 69%, mp 140–142 °C; ^{1}H NMR (DMSO-d_6) δ 8.41–8.14 (m, 1H), 7.96–7.81 (m, 3H), 7.78–7.58 (m, 4H), 7.50–7.28 (m, 6H), 4.28 (br s, 3H), 3.74 (dd, J = 17.4, 5.1 Hz, 2H), 3.42–3.25 (m, 2H), 1.23 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 171.8, 169.8, 156.4, 145.7, 143.8, 140.7, 131.2, 130.5, 127.6, 127.4, 1125.2, 124.9, 120.1, 113.9, 65.8, 53.9, 46.6, 46.0, 31.0. Anal. Calcd for $C_{26}H_{23}N_5O_4S$; C, 62.26; H, 4.62; N, 13.96. Found: C, 62.56; H, 4.67; N, 14.34.

Synthesis of internal cysteine containing free sulfhydryl N-protected tripeptides 11a–c, tetrapeptides 11d,e, and pentapeptide 11f

N-(*N'*-Cbz- and *N'*-Fmoc-α-dipeptidoyl)-1*H*-benzotriazoles **10a**,**b** (1 equiv.) were added at room temperature to a solution of amino acid or dipeptide or tripeptide (1 equiv.) in MeCN: H_2O (7:3 mL) in the presence of triethylamine (1 equiv.). The reaction mixture was stirred at 0–5°C for 2 h. Aq. 6 N HCl solution was then added, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, and the organic layer was washed with 6 N HCl solution, sat. NaCl solution, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the desired products, which

were recrystallized from chloroform-hexanes or ethyl acetate-hexanes.

N-Cbz-L-Phe-L-Cys-L-Phe-OH (11a)

White microcrystals, yield 90%, mp 179–180 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 7.5 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.40–7.14 (m, 1H), 6.98 (m, 16H), 5.05–4.82 (m, 3H), 4.68–4.50 (m, 1H), 4.44–4.20 (m, 1H), 3.32–2.90 (m, 4H), 2.75 (dd, J = 13.8, 10.8 Hz, 2H), 1.25 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 171.7, 167.1, 156.6, 138.1, 137.5, 129.8, 129.0, 128.9, 128.4, 128.0, 127.1, 66.1, 54.0, 53.3, 38.2, 37.2, 26.7. Anal. Calcd for $C_{29}H_{31}N_3O_6S$: C, 63.37; H, 5.68; N, 7.64. Found: C, 63.77; H, 5.81; N, 8.01.

N-Cbz-L-Phe-L-Cys-L-Trp-OH (11b)

White microcrystals, yield 79%, mp 196–198 °C; ^{1}H NMR (DMSO-d_6) δ 10.86 (s, 1H), 8.34 (s, 1H), 8.15 (d, J = 7.2 Hz, 1H), 8.03 (s, 1H), 7.44–7.24 (m, 14H), 6.98 (t, J = 7.2 Hz, 1H), 4.93 (s, 2H), 4.58–4.42 (m, 1H), 4.30 (br s, 1H), 3.24–3.00 (m, 4H), 2.75 (t, J = 12.3 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 173.1, 171.9, 168.8, 168.5, 155.9, 138.1, 136.9, 136.0, 129.2, 128.2, 128.0, 127.6, 127.4, 127.2, 126.2, 123.7, 120.9, 118.3, 118.1, 111.3, 109.6, 65.2, 56.1, 53.0, 42.1, 41.7, 37.4, 27.1. Anal. Calcd for $C_{31}H_{32}N_4O_6S$: C, 63.25; H, 5.48; N, 9.22. Found: C, 63.55; H, 5.78; N, 9.52.

N-Fmoc-Gly-L-Cys-L-Leu-OH (11c)

White microcrystals, yield 70%, mp 162–164 °C. ^{1}H NMR (DMSO-d_6) δ 8.74 (d, J = 7.2 Hz, 1H), 7.95 (d, J = 6.0 Hz, 1H), 7.84 (d, J = 7.5 Hz, 2H), 7.74 (dd, J = 7.2, 3.6 Hz, 2H), 7.37 (t, J = 7.2 Hz, 2H), 7.29 (t, J = 7.2 Hz, 2H), 4.35–4.20 (m, 1H), 4.11 (s, 2H), 3.65–3.40 (m, 2H), 3.15–2.85 (m, 4H), 1.69–1.55 (m, 1H), 1.55–1.38 (m, 2H), 7.37 (t, J = 6.9 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 172.4, 171.2, 167.5, 146.1, 140.4, 127.3, 126.9, 126.8, 125.0, 124.9, 119.9, 53.5, 51.8, 46.5, 41.0, 36.0, 34.7, 24.2, 23.1, 21.4. Anal. Calcd for $C_{26}H_{31}N_3O_6S$: C, 60.80; H, 6.08; N, 8.18. Found: C, 61.21; H, 6.12; N, 8.43.

N-Cbz-L-Phe-L-Cys-Gly-Gly-OH (11d)

White microcrystals, yield 84%, mp 186–187 °C; ^{1}H NMR ($CDCl_3$) 7.65 (s, 1H), 7.64–7.24 (m, 13H), 5.05–4.90 (m, 2H), 5.90–4.80 (m, 1H), 4.50 (br s, 1H), 4.04–3.72 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 173.2, 170.9, 156.9, 136.7, 136.2, 129.4, 128.6, 128.2, 127.9, 127.0, 67.2, 56.6, 42.9, 41.5, 38.2, 29.9. Anal. Calcd for $C_{24}H_{28}N_4O_7S$: C, 55.80; H, 5.46; N, 10.85. Found: C, 56.11; H, 5.48; N, 11.21.

N-Cbz-L-Phe-L-Cys-Gly-L-Ala-OH (11e)

White microcrystals, yield 80%, mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 13.00 (s, 1H), 7.89 (d, J = 7.2 Hz, 2H), 7.71 (d, J = 7.5 Hz, 2H), 7.75–7.30 (m, 5H), 7.23 (br s, 5H), 5.06–4.94 (m, 2H), 4.46–4.34 (m, 1H), 4.29–4.19 (m, 2H), 3.66 (br s, 2H), 3.32–3.26 (m, 1H), 3.32–3.26 (m, 1H), 3.16–3.05 (m, 2H), 2.88 (dd, J = 14.1, 9.9 Hz, 1H), 1.20 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 172.4, 171.4, 168.7, 155.6, 137.6, 136.9, 129.2, 128.3, 128.0, 127.8, 126.2, 65.5, 54.4, 53.9, 50.2, 41.9, 40.3, 37.4, 25.7, 18.1. Anal. Calcd for $C_{25}H_{30}N_4O_7S$: C, 56.59; H, 5.70; N, 10.56. Found: C, 56.91; H, 5.75; N, 10.83.

N-Cbz-L-Phe-L-Cys-Gly-L-Ala-L-Leu-OH (11f)

White microcrystals, yield 74%, mp 188–189 °C; ^{1}H NMR (DMSO-d_6) δ 8.23 (t, J = 5.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 2H), 7.42 (s, 1H), 7.35 (br s, 5H), 7.23 (br s, 5H), 5.00 (s, 2H), 4.52 (br s, 1H), 4.22 (t, J = 6.3 Hz, 1H), 4.01 (t, J = 7.2 Hz, 1H), 3.78 (d, J = 5.7 Hz, 2H), 3.18–2.96 (m, 2H), 2.95–2.75 (m, 2H), 1.78 (br s, 1H), 1.45–1.25 (m, 1H), 1.22–1.02 (m, 5H), 0.86 (d, J = 6.9 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 172.8, 172.3, 171.1, 168.6, 155.6, 142.9, 137.7, 136.9, 129.3, 128.3, 128.0, 127.8, 126.2, 65.5, 56.2, 53.8, 50.2, 41.8, 37.5, 36.5, 24.7, 18.1, 15.6, 11.3. Anal. Calcd for $C_{31}H_{41}N_5O_8S$: C, 57.84; H, 6.42; N, 10.88. Found: C, 57.98; H, 6.44; N, 11.15.

General procedure for disulfide-bridged cysteine peptides 14a,b

N-(*N*′-Cbz-L-Cys)-1*H*-benzotriazole **7a** (0.712 g, 2 mmol) was added at room temperature to a solution of [1,4-butanediamine (0.088 g, 1 mmol) or 1,6-hexanediamine (0.116 g, 1 mmol)] in a solution of THF in the presence of triethylamine (0.2 mL). The reaction mixture was then stirred at room temperature until the starting material was completely consumed, as observed by TLC using hexanes-ethyl acetate (2:1) as development solvent. THF was removed under reduced pressure, and the residue was washed with 2 N HCl solution, diethyl ether (three times), and recrystallized from methanol-diethyl ether.

Dibenzyl((4*R*,13*R*)-5,12-dioxo-1,2-dithio-6,11-diazacyclopentadecane-4,13-diyl)dicarbamate monohydrate (14a)

White microcrystals, yield 50%, mp 156–158 °C; ^{1}H NMR (DMSO-d_6) δ 8.60–7.80 (m, 4H), 7.60–7.25 (m, 10H), 5.03 (s, 4H), 4.50–4.22 (m, 1H), 4.15–3.95 (m, 1H), 3.60–3.98 (m, 8H), 1.82 (s, 1H), 1.60–1.39 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 173.1, 173.0, 170.2, 155.9, 155.5, 155.4, 139.5, 137.2, 128.3, 127.7, 124.6, 115.1, 65.4, 55.5, 53.1, 43.1, 38.2, 25.2, 24.6. HRMS (ESI). Calcd for $C_{26}H_{32}N_4O_6S$: $[M+Na]^+$ 583.689. Found: 583.200. Anal. Calcd for $C_{26}H_{32}N_4O_6.H_2O$:C, 52.33; H, 6.08; N, 9.39. Found: C, 52.44; H, 6.34; N, 9.52.

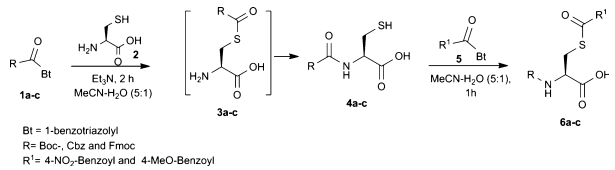
Dibenzyl((4*R*,15*R*)-5,14-dioxo-1,2-dithio-6,13-diazacyclopentadecane-4,15-diyl)dicarbamate (14b)

White microcrystals, yield 50%, mp 167–169 °C; ^{1}H NMR (DMSO-d_6) δ 8.05 (br s, 4H), 7.96–7.84 (m, 2H), 7.56–7.41 (m, 3H), 7.36 (br s, 5H), 5.95–5.52 (m, 4H), 5.12–4.98 (m, 2H), 4.26 (t, J = 7.2 Hz, 1H), 3.12–3.00 (m, 3H), 2.92–2.68 (m, 4H), 1.33 (br s, 4H), 1.20 (t, J = 7.2 Hz, 4H); ^{13}C NMR (DMSO-d_6) δ 171.2, 170.6, 156.0, 136.7, 128.2, 127.7, 127.5, 65.6, 59.5, 52.5, 52.3, 32.6, 30.7, 24.1, 22.8. Calcd for $C_{28}H_{36}N_4O_6S_2$.HCl: C, 53.79; H, 5.97; N, 8.96. Found: C, 53.64; H, 5.89; N, 9.00.

Results and Discussion

Preparation of N-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteines 4a–c

The amino group of L-cysteine **2** has been selectively acylated using *N*-Cbz-1*H*-benzotriazole **1a** (23,24), *N*-Boc-1*H*-benzotriazole **1b** (25,26), and *N*-Fmoc-1*H*-benzotriazole **1c**. According to our previously reported method (32), reactions of *N*-Cbz, *N*-Boc-, and *N*-Fmoc-1*H*-benzotriazoles **1a–c** with L-cysteine **2** at 20 °C in the presence of 2 equivalents of triethylamine in CH_3CN-H_2O (3:1) each gave selectively the corresponding N-protected, free sulfhydryl L-cysteines **2a–c** (Scheme 1, Table 1) in yields of 70–75%. As previously discussed (32), such N-acylations probably involve initial attack by the nucleophilic sulfur anion of L-cysteine at **1a–c**; intermediates **3a–c** then undergo fast *S*- to *N*-acyl transfer to give *N*-acylcysteines **4a–c** (Scheme 1).



Scheme 1: Synthesis and S-acylation of *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteine.

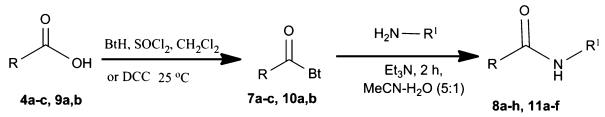
S-acylation of N-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteines 6a–c

The availability of the free SH group was shown by reacting *N*-Cbz, *N*-Boc-, and *N*-Fmoc L-cysteines **4a–c** with *N*-acylbenzotriazoles **5** at 20 °C in MeCN-H_2O (5:1) for 1 h in the presence of one equivalent $KHCO_3$ to give *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-*S*-acyl cysteines **6a–c** in 78–85% isolated yields (Scheme 1, Table 1).

Preparation of N-(*N′*-Cbz-, *N′*-Boc-, and *N′*-Fmoc-L-Cys)-1*H*-benzotriazoles 7a–c

N-Cbz- *N*-Boc-, and *N*-Fmoc free sulfhydryl cysteinoylbenzotriazoles **7a–c** (Scheme 2, Table 2) were prepared in 20–85% yield by the reactions of N-protected-L-cysteines **4a–c** each with 4 equivalents of 1*H*-benzotriazole and 1 equivalent of $SOCl_2$ in THF at 0–10 °C for 2 h. The very low-yield *N*-Boc free sulfhydryl cysteinoyl-1*H*-benzotriazoles **7b** (20%) is probably because of the partial deprotection of *N*-Boc group in the acidic medium generated by reaction of 1*H*-benzotriazole with $SOCl_2$. To improve the yield of **7b**, *N*-Boc-cysteine was treated with 1*H*-benzotriazole in the presence of DCC to afford *N*-Boc free sulfhydryl cysteinoylbenzotriazoles **7b** in 80% yield. Although the DCC method gave *N*-Boc free sulfhydryl cysteinoylbenzotriazoles **7b** in a better yield, the $SOCl_2$ method is on balance more convenient in case of *N*-Cbz and *N*-Fmoc free sulfhydryl cysteinoyl-1*H*-benzotriazoles **7a**,**c** because of the short reaction time and the easy workup. Novel coupling reagents **7a–c** are crystalline (mp's 155–175 °C), stable at 20 °C in air, and sufficiently resistant to hydrolysis to be used in partially aqueous solution.

Preparation of N-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteine-containing di- and tripeptides 8a–h, (8a + 8a′)



BtH= 1H-Benzotriazole
For complete list of R and R^1 refer to Table 2

Scheme 2: Synthesis and peptide coupling reactions of *N*-(*N′*-Cbz-, *N′*-Boc- and *N′*-Fmoc-L-Cys)-1*H*-benzotriazoles **7a–c** and *N*-(*N′*-Cbz- and *N′*-Fmoc-α-dipeptidoyl)-1*H*-benzotriazoles **10a**,**b**.

Reactions of *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-cysteinoyl-1*H*-benzotriazoles **7a–c** (each having free sulfhydryl groups) with various amino acids and dipeptides in acetonitrile-water (3:1) in the presence of triethylamine at 20 °C gave the corresponding *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteine dipeptides **8a–e** in 73–80% yields and tripeptides **8f–h** in 75–80% yields having free sulfhydryl groups (Scheme 2). This procedure allowed the synthesis of dipeptides **8d** (yield 77%) and **8c** (yield 73%) containing tryptophan and serine residues and possessing intact free indole N-H and hydroxyl groups, respectively (Scheme 2, Table 2). Novel *N*-Cbz, *N*-Boc-, and *N*-Fmoc-L-cysteine dipeptides **8a–e** and tripeptides **8f–h** were characterized by ^1H, ^{13}C NMR spectroscopy and elemental analysis. Dipeptides **8a** (**8a** + **8a′**) retained diastereomeric purity, which was evidenced by Chiral HPLC analysis using a Chirobiotic T column (detection at 254 nm, flow rate 0.5 mL/min, and MeOH-H_2O (98:2) as eluent. Thus, diastereomeric mixture (**8a** + **8a′**) showed two peaks at 3.99 and 4.77, whereas the single diastereomer **8a** showed one single peak at 4.00, which confirms that there is no detectable racemization during the reaction.

Preparation of cysteine-containing N-(*N′*-Cbz- and *N′*-Fmoc-α-dipeptidoyl)-1*H*-benzotriazoles 10a,b

N-(*N′*-Cbz- and *N′*-Fmoc-a-dipeptidoyl)-1H-benzotriazoles **10a**,**b** (Scheme 2, Table 2) were prepared in 69–81% yield by the reactions

Table 1: Synthesis and S-acylation of *N*-Cbz-, *N*-Boc-, and *N*-Fmoc-L-cysteine

Entry	RCOBt **1a–c**	RCOOH **4a–c**, Yield (%)[a]	R^1COBt **5**	N-protected S-acyl cysteines **6a–c**, Yield (%)[a]
1	Cbz-Bt **1a**	*N*-Cbz-L-Cys-OH **4a**, 75	4-NO_2-Benzoyl-Bt **5a**	*N*-Cbz-L-Cys(S-4-NO_2-Bz)-OH **6a**, 80
2	Boc-Bt **1b**	*N*-Boc-L-Cys-OH **4b**, 71	4-NO_2-Benzoyl-Bt **5a**	*N*-Boc-L-Cys(S-4-NO_2-Bz)-OH **6b**, 85
3	Fmoc-Bt **1c**	*N*-Fmoc-L-Cys-OH **4c**, 70	4-MeO-Benzoyl-Bt **5b**	*N*-Fmoc-L-Cys(S-4-MeO-Bz)-OH **6c**,78

[a]Isolated yield.

Table 2: Synthesis and peptide coupling reactions of *N*-(*N*′-Cbz-, *N*′-Boc-, and *N*′-Fmoc-L-Cys)-1*H*-benzotriazoles **7a**,**c** and *N*-(*N*′-Cbz- and *N*′-Fmoc-α-dipeptidoyl)-1*H*-benzotriazoles **10a**,**b**

Entry	RCOOBt **7a–c** or **10a**,**b**, Yield (%)[a]	R^1-NH_2	Peptides **8a–h** or **11a–f**, Yield (%)[a]
1	*N*-Cbz-L-Cys-Bt **7a** (30), 70	L-Phe-OH	*N*-Cbz-L-Cys-L-Phe-OH **8a** (27), 80
2	*N*-Cbz-L-Cys-Bt **7a** (30), 70	DL-Phe-OH	*N*-Cbz-L-Cys-DL-Phe-OH **8a** + **8a′** (27), 80
3	*N*-Fmoc-L-Cys-Bt **7c**, 85	L-Phe-OH	*N*-Fmoc-L-Cys-L-Phe-OH **8b**, 75
4	*N*-Cbz-L-Cys-Bt **7a** (30), 70	L-Ser-OH	*N*-Cbz-L-Cys-L-Ser-OH **8c**, 73
5	*N*- Fmoc-L-Cys-Bt **7c**, 85	L-Trp-OH	*N*-Fmoc-L-Cys-L-Trp-OH **8d**, 77
6	*N*-Boc-L-Cys-Bt **7b**, 80	L-Ala-OH	*N*-Boc-L-Cys-L-Ala-OH **8e** (31), 83
7	*N*-Cbz-L-Cys-Bt **7a** (30), 70	H-Gly-Gly-OH	*N*-Cbz-L-Cys-Gly-Gly-OH **8f**, 75
8	*N*-Cbz-L-Cys-Bt **7a** (30), 70	H-Leu-Gly-OH	*N*-Cbz-L-Cys-L-Leu-Gly-OH **8g**, 80
9	*N*-Cbz-L-Cys-Bt **7a** (30), 70	H-Gly-L-Ala-OH	*N*-Cbz-L-Cys-Gly-L-Ala-OH **8h**, 80
10	*N*-Cbz-L-Phe-L-Cys-Bt **10a**, 81	L-Phe-OH	*N*-Cbz-L-Phe-L-Cys-L-Phe-OH **11a**, 90
11	*N*-Cbz-L-Phe-L-Cys-Bt **10a**, 81	L-Trp-OH	*N*-Cbz-L-Phe-L-Cys-L-Trp-OH **11b**, 79
12	*N*-Fmoc-Gly-L-Cys-Bt **10b**, 69	L-Leu-OH	*N*-Fmoc-L-Phe-L-Cys-L-Leu-OH **11c**, 70
13	*N*-Cbz-L-Phe-L-Cys-Bt **10a**, 81	H-Gly-Gly-OH	*N*-Cbz-L-Phe-L-Cys-Gly-Gly-OH **11d**, 84
14	*N*-Cbz-L-Phe-L-Cys-Bt **10a**, 81	H-Gly-L-Ala-OH	*N*-Cbz-L-Phe-L-Cys-Gly-L-Ala-OH **11e**, 80
15	*N*-Cbz-L-Phe-L-Cys-Bt **10a**, 81	H-Gly-L-Ala-L-Leu-OH	*N*-Cbz-L-Phe-L-Cys-Gly-L-Ala-L-Leu-OH **11f**, 74

[a]Isolated yield.

of *N*-Cbz- and *N*-Fmoc-L-cysteine-containing dipeptides **9a**,**b** prepared from previously reported procedure[31] with 4 equivalents of 1*H*-benzotriazole and 1 equivalent of $SOCl_2$ in THF at −30 °C for 4 h.

Novel coupling reagents **10a**,**b** are crystalline (mp's 140–156 °C), stable at 20 °C in air, and sufficiently resistant to hydrolysis to be used in partially aqueous solution.

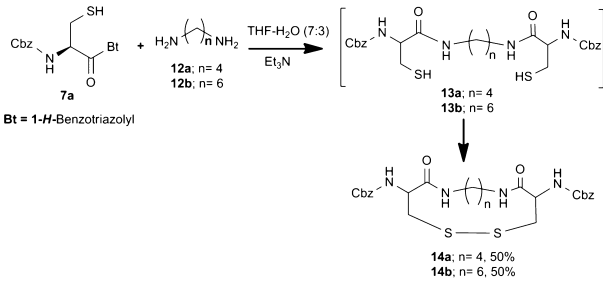
Preparation of N-Cbz- and *N*-Fmoc- internal L-cysteine-containing tri-, tetra-, and pentapeptides 11a–f

Reactions of *N*-Cbz- and *N*-Fmoc-dipeptidoyl-1*H*-benzotriazoles **10a**,**b** (each having free sulfhydryl groups) with amino acids, dipeptides, and tripeptide in acetonitrile-water (5:1) in the presence of triethylamine at 0–5°C gave the corresponding *N*-Cbz- and *N*-Fmoc free sulfhydryl internal L-cysteine-containing tripeptides **11a–c**, in 70–90% yields, tetrapeptides **11d**,**e** in 80–84% yields and pentapeptide **11f** in 74% yield having free sulfhydryl groups (Scheme 2, Table 2). This procedure allowed the synthesis of internal cysteine-containing tri-, tetra-, and pentapeptides **11a–f** having free sulfhydryl groups under mild conditions and in good yields. Novel *N*-Cbz- and *N*-Fmoc-L-cysteine tripeptides **11a–c**, tetrapeptides **11d**,**e** and pentapeptide **11f** were characterized by ^1H, ^{13}C NMR spectroscopy, and elemental analysis.

Synthesis of disulfide-bridged cysteine peptides

Disulfide bridges possess improved stability against enzymatic degradation and play critical roles in stabilizing the tertiary structures of peptides and proteins (33). Many previous efforts toward the controlled construction of disulfide bridges have required complex protection strategies for chain assembly and for selective cleavage of the sulfhydryl protecting groups (34–37).

We have now prepared cysteine-containing cyclic disulfide-bridged cyclic peptide **14a** with a 14-membered ring and **14b** with a 16-membered ring. Treatment of diamines **12a**,**b** with 2 equivalents *N*-(*N*′-Cbz-Cys)-1*H*-benzotriazole **7a** in THF-H_2O for 3 h, afforded cyclic disulfide-bridged 14- and 16-membered cyclic peptides via non-isolable intermediates **13a**,**b** which were oxidized *in situ* to give **14a**,**b** in 50% yield. We think that the reasons behind the low yields of **14a**,**b** are not only the competing thioesterification reaction, but also the partial solubility of **14a**,**b** in the extraction solvent (2N HCl) used to remove benzotriazole at the end of the reaction. (Scheme 3).



Scheme 3: Synthesis of cyclic disulfide-bridged (14–16 membered ring) peptides **14a**,**b**.

Conclusion

In conclusion, we have prepared stable crystalline N-protected, free sulfhydryl cysteinoylbenzotriazoles **7a–c** from N-protected, free sulfhydryl cysteines; and N-protected, free sulfhydryl cysteine-containing dipeptidoylbenzotriazoles from N-protected, free sulfhydryl cysteine-containing dipeptides. N-Protected, free sulfhydryl cysteinoylbenzotriazoles **7a–c** formed N-terminal cysteine-containing N-protected, free sulfhydryl dipeptides and tripeptides selectively under mild reaction conditions with no detectable racemization in good yields. Novel N-protected, free sulfhydryl, cysteine-containing dipeptidoylbenzotriazoles **10a**,**b** formed internal cysteine-containing N-

protected, free sulfhydryl tripeptides, tetrapeptides, and pentapeptide under mild reaction conditions in good yields. N-protected, free sulfhydryl cysteinoylbenzotriazole was used to form 14- and 16-membered disulfide-bridged cyclic peptides, making them efficient reagents for peptide synthesis.

Acknowledgments

We thank the University of Florida, The Kenan Foundation and King Abdulaziz University, Jeddah, Saudi Arabia for financial support. Authors thank Dr Nader E. Abo-Dya and Dr C. D. Hall for their helpful discussions. Mr T. S. Ibrahim is grateful to the ministry of higher education in Egypt for a scholarship.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Chiral HPLC diagram of **8a** and **8a + 8a′**.

Appendix S1. ^{1}H, ^{13}C NMR spectra S3.

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