

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

Sumaira Liaquat,^{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

Received: 25.09.2013; Accepted after revision: 11.10.2013

Abstract: A convenient synthesis of chiral *N*-, *O*-, and *S*-acyl mono- 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 prepara-

tions 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 procedures²⁵ and were reacted with tryptophan, tyrosine, 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 (Et₃N, DIPEA, K₂CO₃, 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**

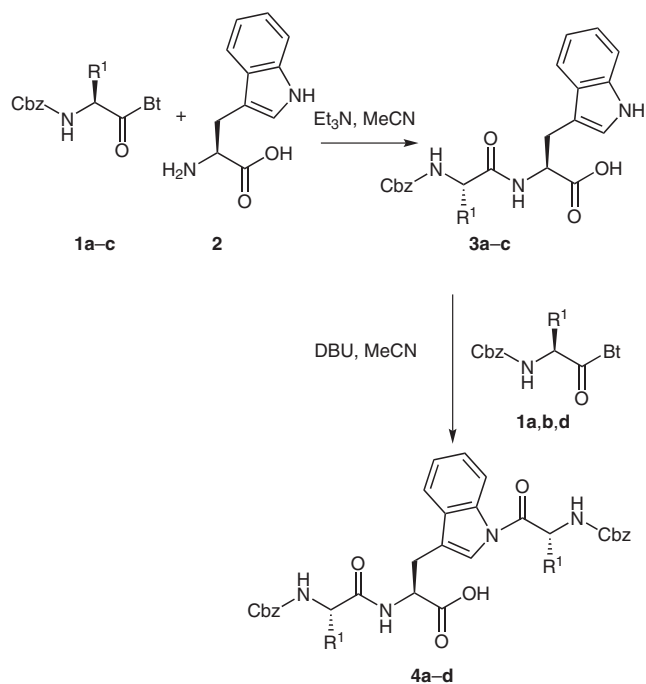
| Product 4 | Yield (%) | Mp (°C) |
|--------------------------------------|-----------|---------|
| Z-Gly-L-Trp(Z-L-Phe)-OH, 4a | 79 | 48–50 |
| Z-L-Ala-L-Trp(Z-L-Phe)-OH, 4b | 78 | 56–58 |
| Z-L-Val-L-Trp(Z-Gly)-OH, 4c | 75 | 54–56 |
| Z-L-Val-L-Trp(Z-L-Ala)-OH, 4d | 76 | 52–54 |

SYNTHESIS 2014, 46, 0067–0072

Advanced online publication: 26.11.2013

DOI: 10.1055/s-0033-1340089; Art ID: SS-2013-M0645-OP

© Georg Thieme Verlag Stuttgart · New York



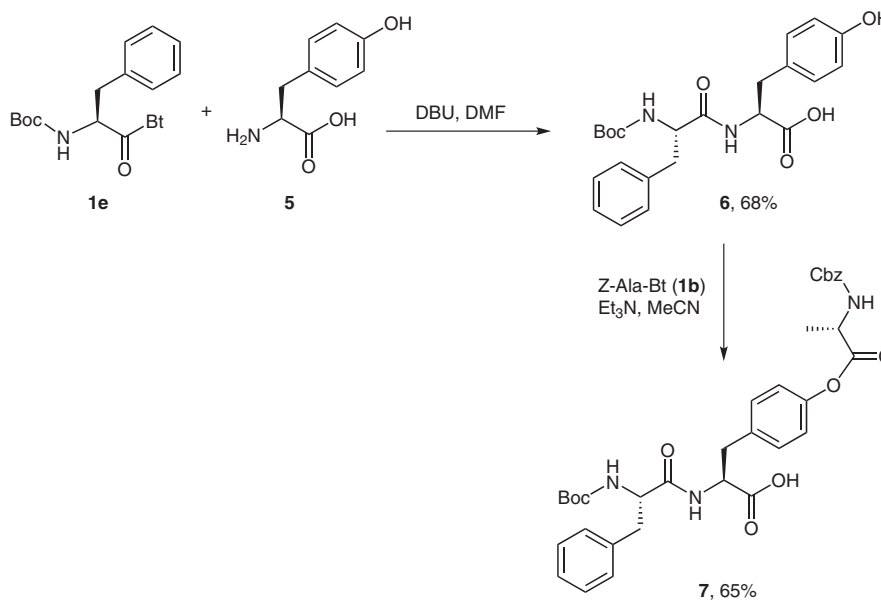
Scheme 1 Preparation of *N*-acyl monoisotriptides **4a–d**

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 monoisotriptide **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**



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

(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).

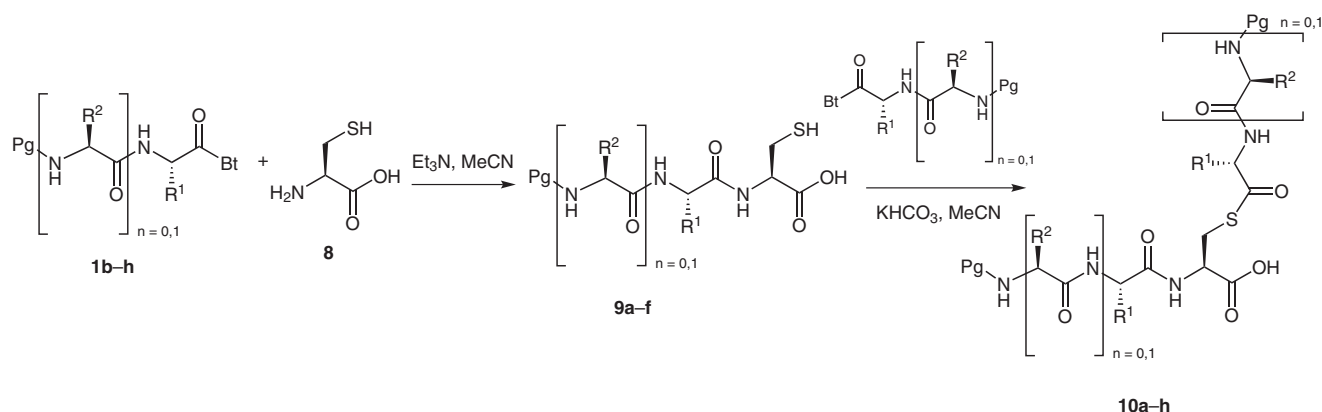
Table 3 Preparation of *N*-Protected Peptides **9a–f** Containing a Cysteine Unit

| 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 3 Preparation of *O*-acyl monoisotriptides **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 CDCl₃ or CD₃OD on Mercury or Gemini NMR spectrometers operating at 300 MHz for ¹H (with TMS as an internal standard) and 75 MHz for ¹³C. 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 KMnO₄ 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 Et₃N (0.5 mL) in H₂O (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 (MgSO₄). After evaporation of solvent, the residue was triturated with Et₂O 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).

¹³C 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 C₂₁H₂₁N₃O₅: 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 Monoisotriptides 4a–d; General Procedure

To a precooled solution of tryptophan containing the appropriate peptide **3a–c** (0.5 mmol) in MeCN (10 mL) and Et₃N (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 H₂O (10 mL) and dried (Na₂SO₄). 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).

¹³C 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 C₃₈H₃₆N₄O₈: 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).

¹³C 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 C₃₉H₃₈N₄O₈: 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.

¹H 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).

¹³C 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 C₃₄H₃₆N₄O₈: 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.

¹H 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).

¹³C 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 C₃₅H₃₈N₄O₈: 641.2617; found: 641.2626.

(S)-3-(4-{[(Benzyloxy)carbonyl]-L-alanyl}oxy}phenyl)-2-[(S)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropanamido}propionic 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 (MgSO₄). The crude product **6** was treated with Z-Ala-Bt (**1b**; 109 mg, 0.34 mmol) in the presence of Et₃N (1.5 equiv) in MeCN–H₂O (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 H₂O (10 mL), and dried (Na₂SO₄). Evaporation of the solvent gave the desired product **7**; yield: 0.41 g (65%); white solid; mp 171–173 °C.

¹H 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).

¹³C 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 C₃₄H₃₉N₃O₉: 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 Et₃N (0.5 mL) in H₂O (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 (Na₂SO₄). After evaporation of the solvent, the residue was triturated with Et₂O–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.

¹H 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).

¹³C 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 C₁₄H₁₈N₂O₅S: 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.

¹H 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).

¹³C 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 C₁₆H₂₂N₂O₅S: 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.

¹H 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).

¹³C 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 C₂₀H₂₂N₂O₅S: 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.

¹H 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).

¹³C 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 C₂₂H₂₅N₃O₆S: 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.

¹H 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).

¹³C 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.

1H 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–H₂O (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 KHCO₃ (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 H₂O (10 mL), and dried (Na₂SO₄). 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.

1H 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.

1H 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.

1H 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.

1H 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.

1H 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-phenylalanyl]glycyl]-L-cysteine (10f)

Yield: 0.61 g (94%); white solid; mp 169–171 °C.

1H 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.

1H 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-phenylalanyl]glycyl]-L-cysteine (10h)

Yield: 0.79 g (98%); white solid; mp 146–148 °C.

^1H 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 $[\text{M} - \text{H}]^-$ calcd for $\text{C}_{42}\text{H}_{45}\text{N}_5\text{O}_{10}\text{S}$: 810.2814; found: 810.2822.

Acknowledgment

We thank the University of Florida and the Kenan Foundation for financial support. This paper was also funded in part by generous support from King Abdulaziz University, under grant No. D-006/431. The authors, therefore, acknowledge the technical and financial support of KAU. We also thank Dr. C. D. Hall for useful suggestions and English checking.

Supporting Information for this article is available online at <http://www.thieme-connect.com/ejournals/toc/synthesis>.

References

- (1) Tam, J. P.; Lu, Y.-A. *J. Am. Chem. Soc.* **1995**, *117*, 12058.
- (2) Guichou, J.-F.; Patiny, L.; Mutter, M. *Tetrahedron Lett.* **2002**, *43*, 4389.
- (3) Alexander, I.; Raimo, F.; Antje, R.; Tatjana, A.; Lothar, J. *Eur. Pat. Appl EP 2487183 A1 20120815*, **2012**; *Chem. Abstr.* **2012**, *157*, 349369.
- (4) (a) Yoshiya, T.; Taniguchi, A.; Sohma, Y.; Fukao, F.; Nakamura, S.; Abe, N.; Ito, N.; Skwarczynski, M.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Org. Biomol. Chem.* **2007**, *5*, 1720. (b) Sohma, Y.; Taniguchi, A.; Skwarczynski, M.; Yoshiya, T.; Fukao, F.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Tetrahedron Lett.* **2006**, *47*, 3013.
- (5) Popov, V.; Panda, S. S.; Katritzky, A. R. *Org. Biomol. Chem.* **2013**, *11*, 1594.
- (6) Popov, V.; Panda, S. S.; Katritzky, A. R. *J. Org. Chem.* **2013**, *78*, 7455.
- (7) Panda, S. S.; El-Nachef, C.; Bajaj, K.; Youbi, A. O.; Oliferenko, A. A.; Katritzky, A. R. *Chem. Biol. Drug. Des.* **2012**, *80*, 821.
- (8) Ha, K.; Chahar, M.; Monbaliu, J.-C. M.; Todadze, E.; Hansen, F. K.; Oliferenko, A. A.; Ocampo, C. E.; Leino, D.; Lillicotch, A.; Stevens, C. V.; Katritzky, A. R. *J. Org. Chem.* **2012**, *77*, 2637.
- (9) Katritzky, A. R.; Tala, S. R.; Dya, N. E. A.; Ibrahim, T. S.; E-Feky, S. A.; Gyanda, K.; Pandya, K. M. *J. Org. Chem.* **2011**, *76*, 85.
- (10) Coin, I.; Dolling, R.; Krause, E.; Bienert, M.; Beyermann, M.; Sferdean, C. D.; Carpino, L. A. *J. Org. Chem.* **2006**, *71*, 6171.
- (11) Taniguchi, A.; Yoshiya, T.; Abe, N.; Fukao, F.; Sohma, Y.; Kimura, T.; Hayashi, Y.; Kiso, Y. *J. Pept. Sci.* **2007**, *13*, 868.
- (12) Lecaillon, J.; Gilles, P.; Subra, G.; Martinez, J.; Amblard, M. *Tetrahedron Lett.* **2008**, *49*, 4674.
- (13) Yoshiya, T.; Kawashima, H.; Sohma, Y.; Kimura, T.; Kiso, Y. *Org. Biomol. Chem.* **2009**, *7*, 2894.
- (14) Tailhades, J.; Gidel, M.-A.; Grossi, B.; Lecaillon, J.; Brunel, L.; Subra, G.; Martinez, J.; Amblard, M. *Angew. Chem. Int. Ed.* **2010**, *49*, 117.
- (15) Taniguchi, A.; Sohma, Y.; Kimura, M.; Okada, T.; Ikeda, K.; Hayashi, Y.; Kimura, T.; Hirota, S.; Matsuzaki, K.; Kiso, Y. *J. Am. Chem. Soc.* **2006**, *128*, 696.
- (16) Taniguchi, A.; Sohma, Y.; Hirayama, Y.; Mukai, H.; Kimura, T.; Hayashi, Y.; Matsuzaki, K.; Kiso, Y. *ChemBioChem* **2009**, *10*, 710.
- (17) Mutter, M.; Chandravarkar, A.; Boyat, C.; Lopez, J.; Santos, S. D.; Mandal, B.; Mimna, R.; Murat, K.; Patiny, L.; Saucedo, L.; Tuchscherer, G. *Angew. Chem. Int. Ed.* **2004**, *43*, 4172.
- (18) Kiewitz, S. D.; Kakizawa, T.; Kiso, Y.; Cabrele, C. *J. Pept. Sci.* **2008**, *14*, 1209.
- (19) Hentschel, J.; Krause, E.; Borner, H. G. *J. Am. Chem. Soc.* **2006**, *128*, 7722.
- (20) Akira, S.; Daisuke, T.; Naomi, N.; Shugo, T.; Kohji, I.; Akira, O. *ChemBioChem* **2007**, *8*, 1929.
- (21) Perello, M. V.; Hori, Y.; Ribo, M.; Muir, T. W. *Angew. Chem. Int. Ed.* **2008**, *47*, 7764.
- (22) Boussett, S.; Perez, I. D.; Kogan, M. J.; Oliveira, E.; Giralt, E. *ACS Nano* **2009**, *3*, 3091.
- (23) Bajaj, K.; Panda, S. S.; Nacheff, C. E.; Katritzky, A. R. *Chem. Biol. Drug. Des.* **2012**, *80*, 17.
- (24) Panda, S. S.; Bajaj, K.; Meyers, M. J.; Sverdrup, F. M.; Katritzky, A. R. *Org. Biomol. Chem.* **2012**, *10*, 8985.
- (25) Katritzky, A. R.; Angrish, P.; Todadze, E. *Synlett* **2009**, 2392.
- (26) Anderson, G. W. French patent FR 1406785 A, **1965**; *Chem. Abstr.* **1965**, *63*, 72439.
- (27) Katritzky, A. R.; Suzuki, K.; Singh, S. K. *Synthesis* **2004**, 2645.