Tri-, Tetra- and Pentapeptidoylbenzotriazoles: Novel Synthetic Intermediates

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Abstract: Conveniently synthesized novel *N*-(protected α-tri-, tetra- and pentapeptidoyl)benzotriazoles couple in aqueous acetonitrile solution at 0 °C with free amino acids, dipeptides, and tripeptides to afford *N*-terminal-protected polypeptides (61–92%) including those containing unprotected OH, SH, and indole NH groups, with no detectable racemization.

Key words: acylation, peptides, coupling, amino acids, medicinal chemistry

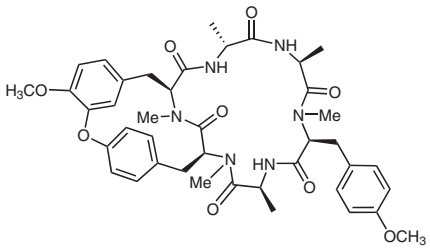

Figure 1 Cyclic hexapeptide RA-VII

Over 40 peptides are available as pharmaceuticals and more than 100 others are in clinical trials.[1] Peptides and their derivatives are used extensively in the field of medicine as anticancer drug carriers,[2] as probes for molecular imaging and disease diagnosis,[3] and they play important roles in drug design and discovery.[4] Several peptides showed antibacterial activity,[5] and naturally occurring antibiotic peptides play a role as effectors of innate immunity.[6] Many naturally occurring tetra-, penta-, hexa-, and other polypeptides with molecular weights in the range of 530–840 Da are biologically active. For example, linear tetrapeptide belamide A, from the marine cyanobacterium *Symploca* sp., exhibits classic tubulin destabilizing antimitotic characteristics;[7a] dolastatins 10 and 15 from the sea-hare *Dolabella auricularia* display extraordinary cytotoxicity towards cancer cells (IC_{50} value 2.9 nM),[7b,c] and S–S tetrapeptides show high affinity and selectivity for the μ-opioid receptor and show antioxidant properties towards mitochondria.[8] Other tetrapeptides are potent against recombinant BACE1 (β-site amyloid precursor protein cleaving enzyme 1), up to an IC_{50} value of 34.6 nM from KMI-927.[9] BACE1, which is involved in amyloid β-peptide production in Alzheimer's disease, is a major target for current drug design. Synthetic potent antimicrobial hexapeptides have great potential for basic research and drug discovery.[10]

Cyclic hexapeptide RA-VII (Figure 1), extracted from *Rubiae radix*, exhibits antitumor activity,[11a] causes a conformational change in the actin molecule and induces G2 arrest by inhibiting cytokinesis;[11b] Ceratospongamides are bioactive cyclic heptapeptides isolated from the Indonesian red alga *Ceratodictyon spongiosum* and symbiotic sponge *Sigmadocia symbiotica*;[12] rolloamides A and B, extracted from the caribbean marine sponge *eurypon laughlini*, display growth-suppressive activity against diverse cancer cell lines.[13]

Efficient syntheses of peptides and their conjugates need (i) high yields, (ii) fast reactions, and (iii) preservation of chirality. Coupling reagents used in the synthesis of biologically active peptides and their analogues include: (i) carbodiimides (DCC, DIC, EDC),[14,15] in combination with additives such as 1-hydroxybenzotriazole (HOBt),[16,17] 1-hydroxy-7-aza-benzotriazole (HOAt), and analogues;[18] (ii) phosphonium (PyBOP, HPyOPfp)[19–21] and uronium salts[22,23] of HOBt or HOAt; (iii) 1*H*-benzimidazolium type coupling reagent (CMBI);[24] (iv) mixed anhydrides,[25] or (v) carboxylic acid fluorides.[22] However, most protocols using these coupling reagents require prior protection and subsequent deprotection of various amino acid functional groups, exclusion of moisture, and usually involve column chromatographic isolation from by-products.

Tetra-, penta-, hexa- and longer peptides are usually prepared using solid-phase peptide synthesis (SPPS)[26–31] or enzymatic synthesis,[32,33] but many of these techniques suffer from low overall yields and are typically unsuitable for large-scale reactions (see conclusion section).

N-(Protected α-aminoacyl)benzotriazoles or *N*-(protected α-dipeptidoyl)benzotriazoles were previously reported to be effective reagents for the synthesis of di-, tri-, and tetrapeptides.[34,35] We now demonstrate the use of novel *N*-(protected tri-, tetra- and pentapeptidoyl)benzotriazoles as advantageous laboratory bench coupling reagents for the synthesis of tetra-, penta-, hexa-, and heptapeptides. Our *N*-(protected tri-, tetra- and pentapeptidoyl)benzotriazoles: (i) form amide bonds easily at low temperatures; (ii) can be stored in the crystalline state at room temperature for at least five months; (iii) provide products in good yields with no detectable racemization; (iv) are prepared from commercially available and inexpensive reagents, and (iv) are easily purified (no HPLC required).

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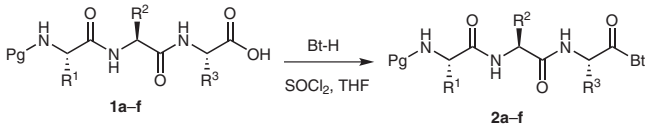
Table 1 Preparation of *N*-(Pg-α-tripeptidoyl)benzotriazoles **2a–f**



1a–f **2a–f**

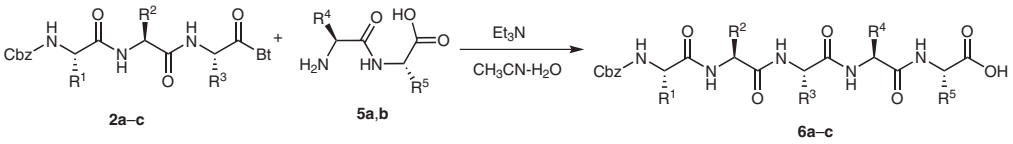
Entry	Pg	R^1	R^2	R^3	Product **2**		Yield (%)	Mp (°C)
1	Cbz	Me	Bn	H	**2a**	Cbz-L-Ala-L-Phe-Gly-Bt	80	186–187
2	Cbz	Bn	H	H	**2b**	Cbz-L-Phe-Gly-Gly-Bt	80	137–139
3	Cbz	Bn	Me	Me	**2c**	Cbz-L-Phe-L-Ala-L-Ala-Bt	73	190–192
4	Fmoc	Bn	Me	*i*-Pr	**2d**	Fmoc-L-Phe-L-Ala-L-Val-Bt	76	162–164
5	Cbz	Me	*i*-Bu	Me	**2e**	Cbz-L-Ala-L-Leu-L-Ala-Bt	86	175–176
6	Cbz	Me	$MeS(CH_2)_2$	Me	**2f**	Cbz-L-Ala-L-Met-L-Ala-Bt	81	188–189

N-(Protected α-tripeptidoyl)benzotriazoles, *N*-(protected α-tetrapeptidoyl)benzotriazoles, and *N*-(protected α-pentapeptidoyl)benzotriazoles are relatively insensitive to water, indeed, we now use these reagents in aqueous solution as efficient peptide coupling reagents for the preparation of tetra-, penta-, hexa, and heptapeptides on a relatively large scale. Preliminary experiments indicate that these reagents will be useful for the introduction of peptide fragments into diverse scaffolds by reactions with N-, O-, S- and C-nucleophiles.

Treatment of *N*-(Cbz- or Fmoc)tripeptides **1a–f**[34,36] with four equivalents of benzotriazole and one equivalent of thionyl chloride in tetrahydrofuran or dichloromethane at –45 °C for four hours, gave *N*-(protected α-tripeptidoyl)benzotriazoles **2a–f** in 73–86% yield (Table 1). The crystalline products **2a–f** were characterized by ^1H and ^{13}C NMR spectroscopy and by elemental analyses. They are stable at 20 °C for at least five months.

N-(Pg-α-Tetrapeptides) **4a–l** were prepared in yields of 70–92% by peptide coupling between *N*-(Cbz- or Fmoc-tripeptidoyl)benzotriazoles **2a–f** and amino acids **3a–i** in aqueous acetonitrile in the presence of triethylamine at 0 °C (Table 2). These amino acids include L-Cys-OH having a free SH group, L-Ser-OH with a free hydroxy group, L-Trp-OH with a free NH group, and L-Met-OH. Retention of enantiopurity of the product **4i** was confirmed by chiral HPLC analysis using a Chirobiotic T column (detection at 254 nm, flow rate 1 mL/min, and MeOH as the eluting solvent). The diastereomer **4i** showed a single retention-time peak in chiral HPLC at 2.32 min, whereas the corresponding diastereomeric mixture **(4i + 4i′)** showed two peaks at 2.32 and 2.47 min.

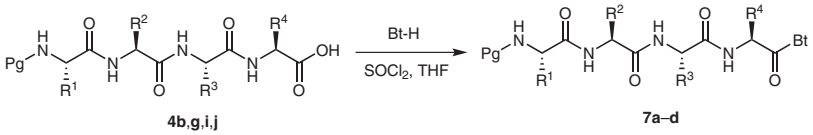
Treatment of *N*-(Cbz-α-tripeptidoyl)benzotriazoles **2a–c** with unprotected dipeptides Gly-L-Ala-OH (**5a**) and Gly-L-Leu-OH (**5b**) in aqueous acetonitrile at 0 °C gave *N*-Cbz-pentapeptides **6a–c** in 73–81% yield; the products were characterized by ^1H, ^{13}C NMR spectroscopy and by elemental analysis (Scheme 1).



2a–c **5a,b** **6a–c**

6a: R^1 = Me, R^2 = Bn, R^3 = H, R^4 = H, R^5 = Me, 73%
6b: R^1 = Bn, R^2 = H, R^3 = H, R^4 = H, R^5 = *i*-Bu, 79%
6c: R^1 = Bn, R^2 = Me, R^3 = Me, R^4 = H, R^5 = *i*-Bu, 81%

Scheme 1 Preparation of *N*-(Cbz-α-pentapeptides) **6a–c** from *N*-(Cbz-α-tripeptidoyl)benzotriazoles **2a–c** and unprotected dipeptides **5a** and **5b**

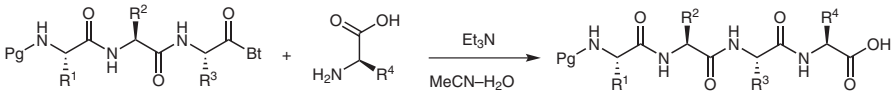


4b,g,i,j **7a–d**

7a: R^1 = Me, R^2 = Bn, R^3 = H, R^4 = Me, 75%
7b: R^1 = Bn, R^2 = Me, R^3 = *i*-Pr, R^4 = *i*-Bu, 78%
7c: R^1 = Me, R^2 = *i*-Bu, R^3 = Me, R^4 = Me, 84%
7d: R^1 = Me, R^2 = $MeS(CH_2)_2$, R^3 = Me, R^4 = Me, 86%

Scheme 2 *N*-(Pg-α-Tetrapeptidoyl)benzotriazoles **7a–d** (for details, see the experimental section)

Table 2 Preparation of *N*-(Pg-α-tetrapeptides) **4a–l** from *N*-(Pg-α-tripeptidoyl)benzotriazoles **2a–f** and Unprotected Amino Acids



2a–f **3a–i, (3b+3b')** **4a–l, (4i+4i')**

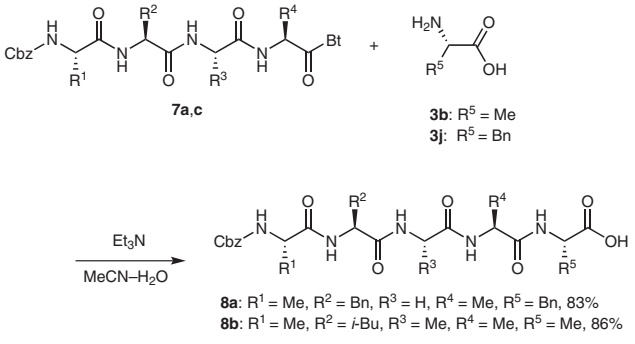
2	3		Product		Yield (%)
2a	**3a**	L-Ile-OH	**4a**	Cbz-L-Ala-L-Phe-Gly-L-Ile-OH	75
2a	**3b**	L-Ala-OH	**4b**	Cbz-L-Ala-L-Phe-Gly-L-Ala-OH	86
2b	**3c**	L-Trp-OH	**4c**	Cbz-L-Phe-Gly-Gly-L-Trp-OH	80
2b	**3d**	L-Val-OH	**4d**	Cbz-L-Phe-Gly-Gly-L-Val-OH	83
2c	**3e**	L-Met-OH	**4e**	Cbz-L-Phe-L-Ala-L-Ala-L-Met-OH	76
2c	**3f**	L-Asp-OH	**4f**	Cbz-L-Phe-L-Ala-L-Ala-L-Asp-OH	70
2d	**3g**	L-Leu-OH	**4g**	Fmoc-L-Phe-L-Ala-L-Val-L-Leu-OH	90
2d	**3e**	L-Met-OH	**4h**	Fmoc-L-Phe-L-Ala-L-Val-L-Met-OH	88
2e	**3b**	L-Ala-OH	**4i**	Cbz-L-Ala-L-Leu-L-Ala-L-Ala-OH	88
2e	(**3b+3b′**)	DL-Ala	(**4i+4i′**)	Cbz-L-Ala-L-Leu-L-Ala-DL-Ala-OH	85
2f	**3b**	L-Ala-OH	**4j**	Cbz-L-Ala-L-Met-L-Ala-L-Ala-OH	92
2a	**3h**	L-Cys-OH	**4k**	Cbz-L-Ala-L-Phe-Gly-L-Cys-OH	82
2b	**3i**	L-Ser-OH	**4l**	Cbz-L-Phe-Gly-Gly-L-Ser-OH	72

N-(Pg-α-Tetrapeptidoyl)benzotriazoles **7a–d** were prepared in 75–86% yields by treatment of *N*-(Cbz- or Fmoc)tetrapeptides **4b**, **4g**, **4i**, and **4j** with four equivalents of benzotriazole and one equivalent of thionyl chloride in either tetrahydrofuran or dichloromethane at –20 °C for four hours (Scheme 2).

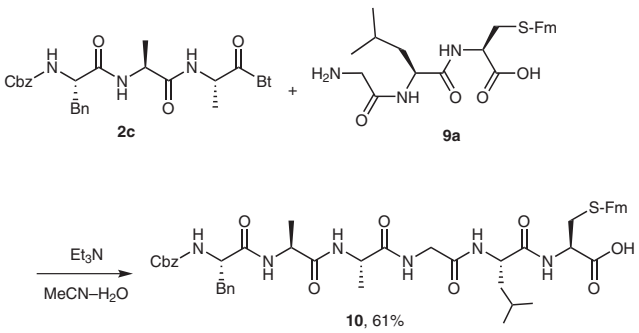
Treatment of *N*-(Cbz-α-tetrapeptidoyl)benzotriazoles **7a** and **7c** with free amino acids **3b** and **3i** in aqueous acetonitrile at 0 °C gave *N*-Cbz-pentapeptides **8a** and **8b** (83–86%), which were characterized by ^{1}H, ^{13}C NMR spectroscopy and by elemental analysis (Scheme 3).

N-Cbz-Hexapeptide **10** was synthesized in 61% yield by coupling the *N*-(Cbz-α-tripeptidoyl)benzotriazole **2c** with previously prepared tripeptide Gly-Leu-Cys(S-Fm)-OH (**9a**)[36] in aqueous acetonitrile at 0 °C; the product was characterized by ^{1}H, ^{13}C NMR spectroscopy and by HRMS analysis (Scheme 4).

To illustrate the synthetic utility of our tetrapeptidoyl benzotriazoles, we prepared N-protected hexapeptides **11a–d** by coupling *N*-(Pg-tetrapepeptidoyl)benzotriazoles **7a–d** with unprotected dipeptides **5a–c** in aqueous acetonitrile at 0 °C in 80–87% yield (Table 3). Two of these compounds (**11c** and **11d**) were previously prepared by Bordusa and co-workers using enzymatic peptide synthesis[32] consisting of three steps: (i) preparation of a peptide thioester as acyl donor; (ii) preparation of a free amino peptide segment (using solid-phase synthesis protocols) as acyl acceptor, and finally (iii) peptide coupling of the acyl donor and acyl acceptor using enzymatic catalysis.

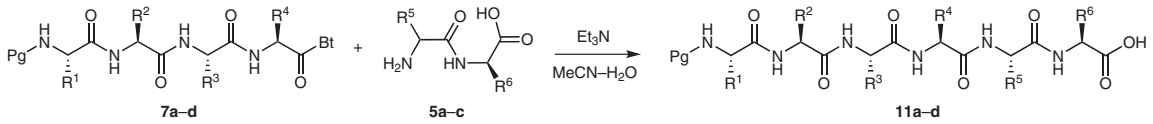


Scheme 3 Preparation of *N*-Cbz-α-pentapeptides **8a** and **8b** from *N*-(Cbz-α-tetrapeptidoyl)benzotriazoles **7a** and **7c**, and free amino acids **3b** and **3i**



Scheme 4 Preparation of *N*-(Cbz-α-hexapeptide) **10** from *N*-(Cbz-α-tripeptidoyl)benzotriazole **2c** and tripeptide **9a**

Table 3 Preparation of *N*-(Pg-α-Hexapeptides) **11a–d** from *N*-(Pg-α-Tetrapeptidoyl)benzotriazoles **7a–d** and Unprotected Dipeptides **5a–c**



7a–d **5a–c** **11a–d**

7a: R^1 = Me, R^2 = Bn, R^3 = H, R^4 = Me
7b: R^1 = Bn, R^2 = Me, R^3 = *i*-Pr, R^4 = *i*-Bu
7c: R^1 = Me, R^2 = *i*-Bu, R^3 = Me, R^4 = Me
7d: R^1 = Me, R^2 = $MeS(CH_2)_2$, R^3 = Me, R^4 = Me

11a: R^1 = Me, R^2 = Bn, R^3 = H, R^4 = Me, R^5 = H, R^6 = *i*-Bu
11b: R^1 = Bn, R^2 = Me, R^3 = *i*-Pr, R^4 = *i*-Bu, R^5 = H, R^6 = Me
11c: R^1 = Me, R^2 = *i*-Bu, R^3 = Me, R^4 = Me, R^5 = Me, R^6 = H
11d: R^1 = Me, R^2 = $MeS(CH_2)_2$, R^3 = Me, R^4 = Me, R^5 = Me, R^6 = H

7	Dipeptide 5		Product 11		Yield (%)	Lit. yield (%)
7a	**5b**	Gly-L-Leu-OH	**11a**	Cbz-L-Ala-L-Phe-Gly-L-Ala-Gly-L-Leu-OH	80	–
7b	**5a**	Gly-L-Ala-OH	**11b**	Fmoc-L-Phe-L-Ala-L-Val-L-Leu-Gly-L-Ala-OH	82	–
7c	**5c**	L-Ala-Gly-OH	**11c**	Cbz-L-Ala-L-Leu-L-Ala-L-Ala-L-Ala-Gly-OH	87	67[28]
7d	**5c**	L-Ala-Gly-OH	**11d**	Cbz-L-Ala-L-Met-L-Ala-L-Ala-L-Ala-Gly-OH	84	71[28]

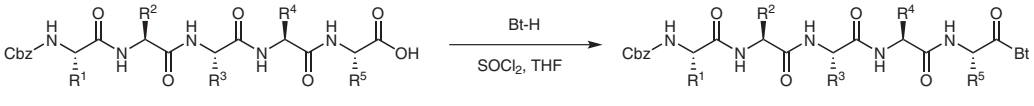
The procedure developed by Bordusa[32] includes (i) peptide coupling reactions using solutions of concentrations 10^{-8} to 10^{-7} M for acyl donors, 3×10^{-6} to 1.5×10^{-5} M for enzymes, with acyl acceptor concentration and amounts of solution unspecified; (ii) reaction times of 16 to 120 hours; (iii) crude products purified by preparative HPLC; (iv) isolation of the resulting peptides (details not specified). The compounds prepared were analyzed by HPLC, but no details of melting points, CHN analyses, or mass spectroscopic analyses of the final products were provided. The multiple reaction steps involved in the procedure, the use of enzymes, and the preparative HPLC required for purification suggests that the use of this procedure would be difficult for the preparation of such peptides in more than very small quantities. Our products **11a–d** were each characterized by ^{1}H, ^{13}C NMR spectroscopy and by elemental analyses.

We prepared *N*-(Cbz-α-pentapeptidoyl)benzotriazoles **12a** and **12b** in 78–85% yields by treatment of *N*-(Cbz-pentapeptides) **8a** and **8b** with four equivalents of benzotriazole and one equivalent of thionyl chloride in either tetrahydrofuran or acetonitrile at –30 °C (Scheme 5). Products **8a** and **8b** were characterized by ^{1}H and ^{13}C NMR spectroscopy and by elemental analysis.

We also prepared *N*-Cbz-hexapeptide **13** via another route by coupling *N*-(Cbz-pentapepeptidoyl)benzotriazoles (**12b**) with L-Phe-OH (**3j**) in aqueous acetonitrile at 0 °C in 85% yield (Scheme 6).

Treatment of *N*-(Cbz-α-pentapeptidoyl)benzotriazoles **12a** and **12b** with unprotected dipeptides **5c** and **5d** in aqueous acetonitrile at 0 °C gave *N*-Cbz-heptapeptides **14a** and **14b**, respectively, in 79–82% yield; the products were characterized by ^{1}H and ^{13}C NMR spectroscopic and by elemental analyses (Scheme 7).

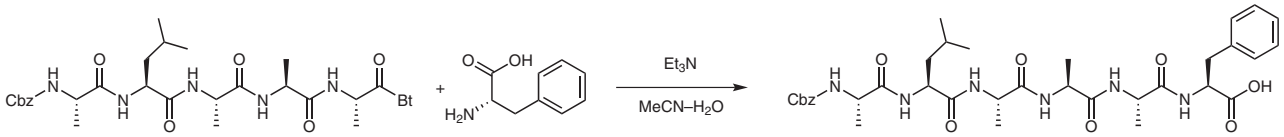
In conclusion, *N*-polypeptidoylbenzotriazoles are advantageous coupling reagents that (i) are sufficiently reactive to form amide bonds at ambient temperature; (ii) are stable enough to resist side reactions and can be stored in the crystalline state at room temperature for at least five months; (iii) 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*-



8a: R^1 = Me, R^2 = Bn, R^3 = H, R^4 = Me, R^5 = Bn
8b: R^1 = Me, R^2 = *i*-Bu, R^3 = Me, R^4 = Me, R^5 = Me

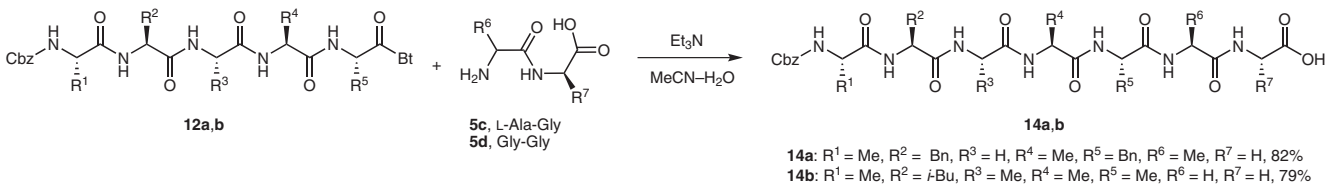
12a, 78%
12b, 85%

Scheme 5 *N*-(Cbz-α-pentapeptidoyl)benzotriazoles **12a** and **12b** (for details, see the experimental section)



12b **3j** **13**, 85%

Scheme 6 Preparation of *N*-Cbz-α-hexapeptide **13** from *N*-(Cbz-α-pentapeptidoyl)benzotriazole (**12b**) and L-Phe-OH (**3j**)



12a,b

5c, L-Ala-Gly
5d, Gly-Gly

14a,b

14a: R1 = Me, R2 = Bn, R3 = H, R4 = Me, R5 = Bn, R6 = Me, R7 = H, 82%
14b: R1 = Me, R2 = i-Bu, R3 = Me, R4 = Me, R5 = Me, R6 = H, R7 = H, 79%

Scheme 7 Preparation *N*-(Cbz-α-heptapeptides) **14a** and **14b** from *N*-(Cbz-α-pentapeptidoyl)benzotriazoles **12a** and **12b** and unprotected dipeptides **5c** and **5d**

Table 4 General Comparison of Common Coupling Procedures with Solution-Phase Coupling using Tri-, Tetra-, and Pentapeptidoyl Benzotriazoles

	Conventional SPPS[26–28]	Automated SPPS[27,28]	Microwave-assisted SPPS[29–31]	Enzymatic peptide synthesis[32,33]	Solution-phase using tri-, tetra- and pentapeptidoyl benzotriazoles
Reagents used	PyBOP, PyBroP, HOBt, DIC, DCC, HATU, HBTU	PyBOP, PyBroP, HOBt, DIC, DCC, HATU, HBTU	PyBOP, PyBroP, HOBt, DIC, DCC, HATU, HBTU	PyBOP, DIC and enzymes (trypsin, α-chymotrypsin, V8 protease)	as above
Coupling time	45–360 min	60 min	1.5–60 min	960–7200 min	30–150 min
Reagent per substrate	3–10 equiv	3–16 equiv	3–16 equiv	5 equiv	1 equiv
Moisture-sensitive	yes	yes	yes	yes	no
Large-scale feasible	no	no	no	no	yes
Protection required	for SH, OH and indole NH	for SH, OH and indole NH	for SH, OH and indole NH	for SH, OH and indole NH	not required except for NH_2
Yields	7–37	0–57	0–64	55–92	70–92
Purification	HPLC	HPLC	HPLC	RP-HPLC	recrystallization

(Pg-α-tripeptidoyl)benzotriazoles, *N*-(Pg-α-tetrapeptidoyl)benzotriazoles, and *N*-(Pg-α-pentapeptidoyl)benzotriazole reagents allow efficient peptide couplings to generate tetra-, penta-, hexa-, and heptapeptides in quantity. A comparison of reagent stoichiometries and protocols for some common SPPS coupling procedures with the solution-phase coupling procedures using tri-, tetra- and pentapeptidoyl benzotriazoles is given in Table 4.

Melting points were determined with a MelTemp apparatus and are uncorrected. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded with a Gemini 300 MHz spectrometer in DMSO-d_6, unless otherwise specified, with TMS as internal standard. The data are reported as follows: chemical shift in parts per million (ppm, δ units) and spin–spin coupling (*J*, Hz). Mass spectra were obtained with a Thermo-Finnigan LCQ spectrometer operating in the electrospray ionization (ESI) mode. CH_2Cl_2 was dried and distilled over CaH_2; THF was used after distillation over Na/benzophenone.

Preparation of *N*-(Protected tripeptidoyl)benzotriazoles 2a–f; General Procedure

Thionyl chloride (5 mmol) was added to a solution of 1*H*-benzotriazole (20 mmol) in anhydrous THF (15 mL) at 20 °C, and stirred for 20 min. The reaction mixture was cooled to –45 °C, then N-protected tripeptide **1a–f** (5 mmol), dissolved in anhydrous THF (5 mL), was added dropwise, and the mixture was stirred for 4 h. The white precipitate that formed during the reaction was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (100 mL) and the solution was washed with saturated Na_2CO_3 (3 × 50 mL), brine (3 × 50 mL), and dried over Na_2SO_4. Removal of the solvents under reduced pressure gave products **2a–f**.

Cbz-L-Ala-L-Phe-Gly-Bt (2a)

Yield: 80%; white microcrystals (CH_2Cl_2); mp 186.0–187.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.81 (t, *J* = 5.6 Hz, 1 H), 8.30 (d, *J* = 8.1 Hz, 1 H), 8.23 (d, *J* = 7.8 Hz, 1 H), 8.01 (d, *J* = 8.4 Hz, 1 H), 7.81 (t, *J* = 7.7 Hz, 1 H), 7.64 (t, *J* = 7.7 Hz, 1 H), 7.44 (d, *J* = 8.1 Hz, 1 H), 7.35–7.15 (m, 10 H), 5.10–4.95 (m, 4 H), 4.75–4.60 (m, 1 H), 4.10–4.00 (m, 1 H), 3.12 (dd, *J* = 13.8, 4.2 Hz, 1 H), 2.92 (dd, *J* = 13.8, 3.9 Hz, 1 H), 1.14 (d, *J* = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.2, 171.9, 168.4, 155.6, 145.2, 137.5, 136.9, 131.0, 130.5, 129.3, 128.3, 128.0, 127.7, 126.6, 126.2, 120.1, 113.7, 65.4, 53.5, 50.2, 42.6, 37.7, 18.1.

Anal. Calcd for $C_{28}H_{28}N_6O_5$: C, 63.63; H, 5.34; N, 15.90. Found: C, 63.57; H, 5.295; N, 15.86.

Cbz-L-Phe-Gly-Gly-Bt (2b)

Yield: 80%; white microcrystals (CH_2Cl_2); mp 137.0–139.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.59 (t, *J* = 5.3 Hz, 1 H), 8.44 (t, *J* = 5.4 Hz, 1 H), 8.29 (d, *J* = 8.4 Hz, 1 H), 8.22 (d, *J* = 8.1 Hz, 1 H), 7.81 (t, *J* = 7.7 Hz, 1 H), 7.68–7.48 (m, 2 H), 7.40–7.20 (m, 10 H), 5.10–5.00 (m, 2 H), 4.94 (s, 2 H), 4.40–4.28 (m, 1 H), 3.92 (d,

J = 6.0 Hz, 2 H), 3.08 (dd, J = 13.8, 3.6 Hz, 1 H), 2.85–2.70 (m, 1 H).

^{13}C NMR (DMSO-d_6): δ = 171.9, 169.7, 168.5, 155.9, 145.2, 138.1, 136.9, 131.0, 130.5, 129.2, 128.2, 128.0, 127.6, 127.4, 126.6, 126.2, 120.1, 113.7, 65.2, 56.2, 42.5, 41.8, 37.4.

Anal. Calcd for $C_{27}H_{26}N_6O_5$: C, 63.03; H, 5.09; N, 16.33. Found: C, 62.76; H, 5.02; N, 16.25.

Cbz-L-Phe-L-Ala-L-Ala-Bt (2c)
Yield: 73%; white microcrystals (CH_2Cl_2); mp 190.0–192.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.83 (d, J = 5.7 Hz, 1 H), 8.29 (d, J = 8.1 Hz, 1 H), 8.23 (dd, J = 13.2, 8.4 Hz, 2 H), 7.80 (t, J = 7.7 Hz, 1 H), 7.64 (t, J = 7.7 Hz, 1 H), 7.49 (d, J = 8.7 Hz, 1 H), 7.35–7.15 (m, 10 H), 5.65–5.55 (m, 1 H), 4.92 (s, 2 H), 4.45–4.35 (m, 1 H), 4.30–4.20 (m, 1 H), 2.99 (dd, J = 10.8, 3.8 Hz, 1 H), 2.75–2.60 (m, 1 H), 1.57 (d, J = 7.2 Hz, 3 H), 1.28 (d, J = 6.9 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.5, 171.8, 171.2, 155.8, 145.3, 138.1, 137.0, 131.1, 129.2, 128.2, 128.0, 127.6, 127.3, 126.7, 126.2, 125.3, 120.2, 113.9, 65.1, 56.0, 48.6, 47.6, 37.4, 18.2, 16.6.

Anal. Calcd for $C_{29}H_{30}N_6O_5{\cdot}H_2O$: C, 62.13; H, 5.39; N, 14.99. Found: C, 62.11; H, 5.27; N, 14.56.

Fmoc-L-Phe-L-Ala-L-Val-Bt (2d)
Yield: 76%; white microcrystals (CH_2Cl_2); mp 162.0–164.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.59 (d, J = 6.3 Hz, 1 H), 8.35–8.20 (m, 2 H), 7.87 (d, J = 7.2 Hz, 1 H), 7.84–7.75 (m, 1 H), 7.70–7.54 (m, 4 H), 7.43–7.35 (m, 2 H), 7.34–7.20 (m, 6 H), 7.18 (d, J = 6.9 Hz, 1 H), 5.60 (t, J = 6.0 Hz, 1 H), 4.51–4.45 (m, 1 H), 4.30–4.20 (m, 1 H), 4.15–4.10 (m, 3 H), 3.05–2.95 (m, 1 H), 2.80–2.70 (m, 1 H), 2.45–2.30 (m, 1 H), 1.26 (d, J = 6.6 Hz, 3 H), 1.10–0.95 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.0, 171.3, 171.1, 155.8, 145.3, 143.7, 140.6, 138.2, 131.1, 130.4, 129.2, 128.0, 127.6, 127.0, 126.8, 126.2, 125.3, 120.2, 120.0, 113.9, 65.7, 57.6, 56.0, 47.8, 46.5, 37.4, 30.1, 19.3, 18.1, 17.9.

Anal. Calcd for $C_{38}H_{38}N_6O_5$: C, 69.28; H, 5.81; N, 12.76. Found: C, 69.16; H, 5.90; N, 12.75.

Cbz-L-Ala-L-Leu-L-Ala-Bt (2e)
Yield: 86%; white microcrystals (CH_2Cl_2–hexanes); mp 175.0–176.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.82 (d, J = 6.3 Hz, 1 H), 8.29 (d, J = 6.3 Hz, 1 H), 8.22 (d, J = 6.3 Hz, 1 H), 7.87 (d, J = 7.2 Hz, 1 H), 7.80 (t, J = 6.0 Hz, 1 H), 7.64 (t, J = 6.0 Hz, 1 H), 7.50–7.40 (m, 1 H), 7.38–7.25 (m, 5 H), 5.60–5.50 (m, 1 H), 5.01 (s, 2 H), 4.48–4.40 (m, 1 H), 4.10–4.00 (m, 1 H), 1.70–1.60 (m, 1 H), 1.56 (d, J = 6.6 Hz, 3 H), 1.50–1.45 (m, 2 H), 1.17 (d, J = 6.6 Hz, 3 H), 0.95–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.4, 172.2, 171.7, 155.6, 145.3, 137.0, 131.0, 130.6, 128.3, 127.6, 126.6, 120.1, 113.9, 65.3, 50.2, 50.1, 48.6, 41.0, 24.1, 23.1, 21.6, 18.1, 16.5.

Anal. Calcd for $C_{26}H_{32}N_6O_5$: C, 61.40; H, 6.34; N, 16.52. Found: C, 61.49; H, 6.51; N, 16.10.

Cbz-L-Ala-L-Met-L-Ala-Bt (2f)
Yield: 81%; white microcrystals (CH_2Cl_2); mp 188.0–189.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.86 (d, J = 5.4 Hz, 1 H), 8.30 (d, J = 8.1 Hz, 1 H), 8.23 (d, J = 8.1 Hz, 1 H), 7.99 (d, J = 7.8 Hz, 1 H), 7.81 (t, J = 7.2 Hz, 1 H), 7.64 (t, J = 7.7 Hz, 1 H), 7.48 (d, J = 7.5 Hz, 1 H), 7.38–7.25 (m, 5 H), 5.65–5.55 (m, 1 H), 5.00 (s, 2 H), 4.50–4.40 (m, 1 H), 4.10–4.00 (m, 1 H), 2.49–2.40 (m, 2 H), 2.05 (s, 3 H), 1.95–1.80 (m, 2 H), 1.57 (d, J = 7.2 Hz, 3 H), 1.17 (d, J = 6.9 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.4, 171.8, 171.4, 155.8, 145.3, 137.0, 131.1, 130.6, 128.3, 127.6, 126.7, 120.2, 113.9, 65.3, 51.2, 50.1, 48.7, 32.2, 29.3, 18.0, 16.5, 14.7.

Anal. Calcd for $C_{25}H_{30}N_6O_5S$: C, 57.02; H, 5.74; N, 15.96. Found: C, 56.63; H, 5.76; N, 15.67.

Preparation of Tetrapeptides 4a–l and Pentapeptides 6a–c; General Procedure
N-(Protected tripeptidoyl)benzotriazoles **2a–f** (0.2 mmol) were added at 0 °C to a solution of α-amino acid **3a–i** and/or dipeptide **5a** or **5b** (0.2 mmol) in a mixture of MeCN (7 mL) and H_2O (3 mL) in the presence of Et_3N (0.24 mmol). The reaction mixture was then stirred at 0 °C until the starting material was completely consumed (40–100 min for tetrapeptides and 100–150 min for pentapeptides). After 4 M HCl (1 mL) was added, the solution was concentrated under reduced pressure. The residue was extracted with EtOAc (20 mL), washed with 4 M HCl (3 × 10 mL) and brine (3 × 10 mL), and then dried over anhydrous (Na_2SO_4). Evaporation of the solvent gave the desired products **4a–l** and **6a–c** in pure forms.

Cbz-L-Ala-L-Phe-Gly-L-Ile-OH (4a)
Yield: 75%; white microcrystals; mp 90.0–92.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 8.30–8.20 (m, 1 H), 7.99–7.85 (m,2 H), 7.41 (d, J = 7.5 Hz, 1 H), 7.36–7.30 (br s, 5 H), 7.25–7.15 (m, 5 H), 5.00 (br s, 2 H), 4.52–4.45 (m, 1 H), 4.22 (dd, J = 8.4, 6.0 Hz, 1 H), 4.05–3.95 (m, 1 H), 3.80–3.75 (m, 2 H), 3.10–2.90 (m, 1 H), 2.85 (dd, J = 20.4, 13.5 Hz, 1 H), 1.85–1.75 (m, 1 H), 1.45–1.30 (m, 1 H), 1.25–1.08 (m, 4 H), 0.90–0.70 (m, 6 H).

^{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 $C_{28}H_{36}N_4O_7$: C, 62.21; H, 6.71; N, 10.36. Found: C, 62.01; H, 6.79; N, 10.46.

Cbz-L-Ala-L-Phe-Gly-L-Ala-OH (4b)
Yield: 86%; white microcrystals (CH_2Cl_2–hexanes); mp 172.0–174.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 8.26 (t, J = 5.6 Hz, 1 H), 8.08 (d, J = 6.9 Hz, 1 H), 7.98 (d, J = 7.8 Hz, 1 H), 7.41 (d, J = 7.5 Hz, 1 H), 7.35–7.30 (m, 5 H), 7.25–7.15 (m, 5 H), 5.10–4.90 (m, 2 H), 4.50–4.45 (m, 1 H), 4.30–4.15 (m, 1 H), 4.10–3.95 (m, 1 H), 3.71 (d, J = 5.7 Hz, 2 H), 3.05 (dd, J = 14.1, 4.5 Hz, 1 H), 2.81 (dd, J = 13.8, 9.3 Hz, 1 H), 1.28 (d, J = 7.2 Hz, 3 H), 1.12 (d, J = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.4, 171.1, 168.3, 155.6, 137.6, 136.9, 129.2, 128.3, 128.0, 127.8, 126.2, 65.5, 53.9, 50.2, 47.5, 41.7, 37.4, 18.1, 17.3.

Anal. Calcd for $C_{25}H_{30}N_4O_7$: C, 60.23; H, 6.07; N, 11.24. Found: C, 60.34; H, 6.20; N, 11.17.

Cbz-L-Phe-Gly-Gly-L-Trp-OH (4c)
Yield: 80%; white microcrystals (CH_2Cl_2); mp 141.0–143.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.75 (br s, 1 H), 10.86 (s, 1 H), 8.45–8.30 (m, 1 H), 8.15 (d, J = 7.2 Hz, 1 H), 8.10–8.05 (m, 1 H), 7.60–7.50 (m, 2 H), 7.40–7.10 (m, 13 H), 7.06 (t, J = 7.7 Hz, 1 H), 7.00 (t, J = 7.2 Hz, 1 H), 4.93 (br s, 2 H), 4.55–4.45 (m, 1 H), 4.35–4.20 (m, 1 H), 3.90–3.60 (m, 4 H), 3.17 (dd, J = 14.1, 5.2 Hz, 1 H), 3.10–3.00 (m, 2 H), 2.80–2.65 (m, 1 H).

^{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 $C_{32}H_{33}N_5O_7 \cdot H_2O$: C, 62.23; H, 5.71; N, 11.34. Found: C, 62.25; H, 5.96; N, 9.56.

Cbz-L-Phe-Gly-Gly-L-Val-OH (4d)
Yield: 83%; white microcrystals (CH_2Cl_2–hexanes); mp 96.0–98.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.38–8.30 (m, 1 H), 8.06–7.90 (m, 2 H), 7.55 (d, *J* = 8.4 Hz, 1 H), 7.35–7.18 (m, 10 H), 5.00–4.90 (m, 2 H), 4.35–4.30 (m, 1 H), 4.17 (dd, *J* = 8.4, 6.0 Hz, 1 H), 4.05–3.75 (m, 4 H), 3.06 (dd, *J* = 13.8, 3.9 Hz, 1 H), 2.80–2.70 (m, 1 H), 2.10–2.05 (m, 1 H), 0.88 (d, *J* = 6.9 Hz, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.9, 172.0, 169.0, 168.9, 156.0, 138.2, 137.0, 129.3, 128.3, 128.1, 127.7, 127.5, 126.3, 65.3, 57.2, 56.2, 42.1, 41.7, 37.4, 29.9, 19.2, 18.0.

Anal. Calcd for $C_{26}H_{32}N_4O_7 \cdot H_2O$: C, 58.86; H, 6.46; N, 10.56. Found: C, 58.78; H, 6.16; N, 10.14.

Cbz-L-Phe-L-Ala-L-Ala-L-Met-OH (4e)
Yield: 76%; white microcrystals (CH_2Cl_2); mp 182.0–184.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 8.19 (d, *J* = 7.2 Hz, 1 H), 8.12 (d, *J* = 7.8 Hz, 1 H), 8.00 (d, *J* = 7.5 Hz, 1 H), 7.52 (d, *J* = 8.1 Hz, 1 H), 7.35–7.20 (m, 10 H), 4.94 (s, 2 H), 4.40–4.20 (m, 4 H), 3.40–3.35 (m, 2 H), 3.05–2.95 (m, 1 H), 2.80–2.65 (m, 1 H), 2.00 (s, 3 H), 2.00–1.95 (m, 1 H), 1.89–1.80 (m, 1 H), 1.25–1.20 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.1, 172.2, 171.7, 171.3, 155.9, 138.2, 137.0, 129.2, 128.3, 128.0, 127.6, 127.4, 126.8, 126.2, 65.2, 56.1, 50.8, 48.1, 47.9, 37.4, 30.8, 29.6, 18.2, 14.6.

Anal. Calcd for $C_{28}H_{36}N_4O_7S$: C, 58.72; H, 6.34; N, 9.78. Found: C, 58.45; H, 6.71; N, 9.70.

Cbz-L-Phe-L-Ala-L-Ala-L-Asp-OH (4f)
Yield: 70%; white microcrystals (CH_2Cl_2–hexanes); mp 173.0–175.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.28–8.15 (m, 1 H), 8.13 (d, *J* = 7.8 Hz, 1 H), 8.08–8.00 (m, 1 H), 7.54–7.45 (m, 1 H), 7.38–7.15 (m, 10 H), 4.93 (s, 2 H), 4.55–4.48 (m, 1 H), 4.35–4.20 (m, 3 H), 3.10–2.90 (m, 1 H), 2.75–2.68 (m, 1 H), 2.67–2.55 (m, 2 H), 1.30–1.15 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.2, 171.8, 171.6, 171.2, 155.8, 138.1, 137.0, 129.2, 128.2, 128.0, 1 27.6, 127.3, 126.2, 65.1, 56.0, 48.5, 48.0, 47.8, 37.4, 35.9, 18.2.

Anal. Calcd for $C_{27}H_{32}N_4O \cdot H_2O$: C, 56.44; H, 5.96; N, 9.75. Found: C, 55.79; H, 5.82; N, 9.40.

Fmoc-L-Phe-L-Ala-L-Val-L-Leu-OH (4g)
Yield: 90%; white microcrystals (CH_2Cl_2); mp 223.0–225.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.50 (br s, 1 H), 8.26 (d, *J* = 6.6 Hz, 1 H), 8.15 (d, *J* = 7.8 Hz, 1 H), 7.87 (d, *J* = 7.2 Hz, 2 H), 7.75 (d, *J* = 9.0 Hz, 1 H), 7.62 (t, *J* = 7.05 Hz, 2 H), 7.40 (t, *J* = 7.4 Hz, 2 H), 7.35–7.23 (m, 5 H), 7.22–7.15 (m, 2 H), 4.45–4.30 (m, 1 H), 4.25–4.20 (m, 2 H), 4.18–4.00 (m, 3 H), 3.10–2.95 (m, 1 H), 2.80–2.70 (m, 1 H), 2.10–1.90 (m, 1 H), 1.70–1.65 (m, 1 H), 1.60–1.45 (m, 2 H), 1.30–1.20 (m, 3 H), 0.95–0.70 (m, 12 H).

^{13}C NMR (DMSO-d_6): δ = 173.8, 171.9, 171.3, 170.8, 155.8, 143.7, 140.6, 138.2, 129.2, 128.0, 127.6, 127.0, 126.2, 125.3, 120.0, 65.7, 57.3, 56.0, 50.1, 48.2, 46.6, 37.5, 30.9, 24.3, 22.9, 21.3, 19.2, 18.0.

Anal. Calcd for $C_{38}H_{46}N_4O_7$: C, 68.04; H, 6.91; N, 8.35. Found: C, 67.79; H, 7.12; N, 8.21.

Fmoc-L-Phe-L-Ala-L-Val-L-Met-OH (4h)
Yield: 88%; white microcrystals (CH_2Cl_2); mp 196.0–198.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 8.35–8.20 (m, 2 H), 7.87 (d, *J* = 7.2 Hz, 2 H), 7.79 (d, *J* = 8.4 Hz, 1 H), 7.62 (t, *J* = 7.4 Hz, 2 H), 7.40 (t, *J* = 7.1 Hz, 2 H), 7.35–7.20 (m, 7 H), 7.19–7.12 (m, 1 H), 4.45–4.16 (m, 5 H), 4.15–4.00 (m, 2 H), 3.05–2.95 (m, 1 H), 2.80–2.74 (m, 1 H), 2.48–2.40 (m, 2 H), 2.05–1.80 (m, 6 H), 1.30–1.20 (m, 3 H), 0.90–0.70 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.0, 171.9, 171.3, 170.9, 155.7, 143.7, 140.6, 138.2, 129.2, 127.9, 127.6, 127.0, 126.2, 125.2, 120.0, 65.6, 57.4, 56.0, 50.8, 48.2, 46.5, 37.4, 30.8, 30.6, 29.6, 19.1, 18.0, 14.6.

HRMS (ESI$^+$): *m*/*z* [M + H]$^+$ calcd for $C_{37}H_{44}N_4O_7S$: 689.3003; found: 689.3005; *m*/*z* [M + Na]$^+$ calcd for $C_{37}H_{44}N_4O_7SNa$: 711.2823; found: 711.2854; *m*/*z* [M – H + 2Na]$^+$ calcd for $C_{37}H_{43}N_4O_7SNa_2$: 733.2642; found: 733.2676.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-OH (4i)
Yield: 88%; white microcrystals (EtOH); mp 232.0–234.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.50 (br s, 1 H), 8.09 (d, *J* = 6.9 Hz, 1 H), 7.95–7.85 (m, 2 H), 7.46 (d, *J* = 7.5 Hz, 1 H), 7.38–7.28 (m, 5 H), 5.01 (s, 2 H), 4.35–4.22 (m, 2 H), 4.20–4.12 (m, 1 H), 4.10–4.00 (m, 1 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.26 (d, *J* = 7.2 Hz, 3 H), 1.20 (d, *J* = 7.2 Hz, 6 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.4, 171.7, 171.5, 155.6, 137.0, 128.3, 127.7, 65.4, 50.9, 50.1, 47.7, 47.4, 40.8, 24.1, 23.1, 21.6, 18.2, 17.2.

Anal. Calcd for $C_{23}H_{34}N_4O_7$: C, 57.73; H, 7.16; N, 11.71. Found: C, 57.42; H, 7.24; N, 11.44.

Cbz-L-Ala-L-Leu-L-Ala-DL-Ala-OH (4i+4i′)
Yield: 85%; white microcrystals (CH_2Cl_2); mp 226.0–228.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.07 (d, *J* = 7.2 Hz, 1 H), 7.90 (d, *J* = 7.5 Hz, 2 H), 7.45 (d, *J* = 7.2 Hz, 1 H), 7.38–7.26 (m, 5 H), 5.01 (s, 2 H), 4.30–4.21 (m, 2 H), 4.20–4.12 (m, 1 H), 4.10–4.00 (m, 1 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.25 (d, *J* = 7.2 Hz, 3 H), 1.19 (d, *J* = 7.2 Hz, 6 H), 0.90–0.50 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.4, 171.7, 171.5, 155.6, 137.0, 128.3, 127.7, 65.4, 50.9, 50.1, 47.7, 47.5, 40.8, 24.1, 23.1, 21.6, 18.2, 17.2.

Anal. Calcd for $C_{23}H_{34}N_4O_7$: C, 57.73; H, 7.16; N, 11.71. Found: C, 57.57; H, 7.36; N, 11.60.

Cbz-L-Ala-L-Met-L-Ala-L-Ala-OH (4j)
Yield: 92%; white microcrystals (MeOH); mp 242.0–244.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.53 (br s, 1 H), 8.14 (d, *J* = 6.9 Hz, 1 H), 8.05–7.93 (m, 2 H), 7.50 (d, *J* = 7.2 Hz, 1 H), 7.38–7.30 (m, 5 H), 5.01 (s, 2 H), 4.38–4.24 (m, 2 H), 4.23–4.18 (m, 1 H), 4.10–4.00 (m, 1 H), 2.48–2.40 (m, 2 H), 2.02 (s, 3 H), 1.95–1.75 (m, 2 H), 1.26 (d, *J* = 7.2 Hz, 3 H), 1.23–1.18 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 174.0, 172.5, 171.8, 170.5, 155.7, 137.0, 128.4, 127.7, 65.4, 51.7, 50.1, 47.9, 47.5, 32.1, 29.4, 18.2, 18.0, 17.2, 14.7.

Anal. Calcd for $C_{22}H_{32}N_4O_7S$: C, 53.21; H, 6.50; N, 11.28. Found: C, 53.19; H, 6.61; N, 11.08.

Cbz-L-Ala-L-Phe-Gly-L-Cys-OH (4k)
Yield: 82%; white microcrystals (CH_2Cl_2); mp 165.0–167.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.35–8.28 (m, 1 H), 8.11 (d, *J* = 7.8 Hz, 1 H), 7.98 (d, *J* = 7.8 Hz, 1 H), 7.41 (d, *J* = 7.8 Hz, 1 H), 7.36–7.30 (m, 5 H), 7.25–7.20 (m, 5 H), 5.00 (s, 2 H), 4.55–4.40 (m, 2 H), 4.05–3.95 (m, 1 H), 3.80 (d, *J* = 4.2 Hz, 2 H), 3.10–3.00 (m, 2 H), 2.90–2.75 (m, 2 H), 1.12 (d, *J* = 6.9 Hz, 3 H).

^{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, 54.3, 53.9, 50.2, 45.5, 41.9, 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, 56.48; H, 5.89; N, 10.46.

Cbz-L-Phe-L-Gly-L-Gly-L-Ser-OH (4l)
Yield: 72%; white microcrystals (CH_2Cl_2–hexanes); mp 65.0–67.0 °C.

^{1}H NMR (DMSO-d_6): δ = 7.70–7.60 (m,1 H), 7.40–7.00 (m, 12 H), 6.00–5.80 (m, 1 H), 5.00–4.90 (m, 2 H), 4.88–4.80 (m, 1 H), 4.52–4.40 (m, 1 H), 4.05–3.70 (m, 6 H), 3.15–3.00 (m, 1 H), 2.90–2.80 (m, 1 H).

^{13}C NMR (DMSO-d_6): 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 $C_{24}H_{28}N_4O_8$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.26; H, 5.75; N, 11.34.

Cbz-L-Ala-L-Phe-Gly-Gly-L-Ala-OH (6a)
Yield: 73%; white microcrystals (CH_2Cl_2); mp 154.0–156.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.59 (br s, 1 H), 8.38–8.35 (m, 1 H), 8.2 (d, *J* = 6.9 Hz, 1 H), 8.08–8.00 (m, 1 H), 8.00–7.90 (m, 1 H), 7.38–7.25 (m, 6 H), 7.24–7.20 (m, 5 H), 5.00 (br s, 2 H), 4.60–4.50 (m, 1 H), 4.05–3.95 (m, 1 H), 3.85–3.65 (m, 4 H), 3.10–3.00 (m, 1 H), 2.90–2.75 (m, 1 H), 1.27 (d, *J* = 7.2 Hz, 2 H), 1.11 (d, *J* = 6.9 Hz, 5 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.3, 171.3, 168.8, 168.3, 155.6, 137.6, 136.9, 129.2, 128.3, 127.9, 127.7, 126.2, 65.4, 53.7, 50.2, 47.4, 42.1, 41.6, 37.6, 18.1, 17.2.

Anal. Calcd for $C_{27}H_{33}N_5O_8{\cdot}H_2O$: C, 56.54; H, 5.80; N, 12.21. Found: C, 56.46; H, 5.67; N, 10.71.

Cbz-L-Phe-Gly-Gly-Gly-L-Leu-OH (6b)
Yield: 79%; white microcrystals (CH_2Cl_2); mp 202.0–204.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.59 (s, 1 H), 8.36–8.10 (m, 1 H), 8.14–8.00 (m, 3 H), 7.58 (d, *J* = 8.1 Hz, 1 H), 7.40–7.20 (m, 10 H), 4.94 (s, 1 H), 4.40–4.15 (m, 2 H), 3.85–3.65 (m, 6 H), 3.10–3.00 (m, 1 H), 2.80–2.70 (m, 1 H), 1.70–1.58 (m, 1 H), 1.55–1.45 (m, 2 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 171.9, 169.1, 168.9, 168.6, 155.9, 138.2, 137.0, 129.2, 128.3, 128.0, 127.7, 127.4, 126.2, 65.3, 56.2, 50.2, 42.2, 41.6, 37.4, 24.3, 22.9, 21.4.

Anal. Calcd for $C_{29}H_{37}N_5O_8{\cdot}H_2O$: C, 57.89; H, 6.53; N, 11.64. Found: C, 57.59; H, 6.37; N, 11.30.

Cbz-L-Phe-L-Ala-L-Ala-Gly-L-Leu-OH (6c)
Yield: 81%; white microcrystals (CH_2Cl_2); mp 198.0–200.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 8.25–7.85 (m, 4 H), 7.55–7.45 (m, 1 H), 7.30–7.10 (m, 10 H), 4.94 (br s, 2 H), 4.40–4.15 (m, 4 H), 3.80–3.70 (m, 2 H), 3.10–2.95 (m, 1 H), 2.80–2.70 (m, 1 H), 1.65–1.58 (m, 1 H), 1.55–1.45 (m, 2 H), 1.25–1.20 (m, 6 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.3, 171.8, 171.3, 168.6, 155.9, 138.2, 137.0, 129.2, 128.3, 128.0, 127.7, 127.4, 126.2, 65.2, 56.1, 50.2, 48.3, 41.7, 37.4, 24.2, 22.9, 21.4, 18.1, 15.2.

Anal. Calcd for $C_{31}H_{41}N_5O_8$: C, 60.87; H, 6.76; N, 11.45. Found: C, 60.78; H, 6.76; N, 11.09.

Preparation of *N*-(Protected tetrapeptidoyl)benzotriazoles 7a–d; General Procedure
Thionyl chloride (5 mmol) was added to a solution of 1*H*-benzotriazole (20 mmol) in anhydrous THF (15 mL) at 20 °C, and the mixture was stirred for 30 min. The reaction mixture was cooled to –20 °C, then N-protected tetrapeptides **2b**, **2g**, **2i**, or **2j** (5 mmol), dissolved in anhydrous THF (5 mL), were added dropwise, and the mixture was stirred for 4 h. The white precipitate that formed during the reaction was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (100 mL) and the solution was washed with saturated Na_2CO_3 (3 × 30 mL), and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave the desired products **7a–d**.

Cbz-L-Ala-L-Phe-Gly-L-Ala-Bt (7a)
Yield: 75%; white microcrystals (CH_2Cl_2–hexanes); mp 212.0–214.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.71 (d, *J* = 5.4 Hz, 1 H), 8.35–8.25 (m, 2 H), 8.22 (d, *J* = 6.9 Hz, 1 H), 8.00 (d, *J* = 7.5 Hz, 1 H), 7.81 (t, *J* = 7.7 Hz, 1 H), 7.65 (t, *J* = 7.7 Hz, 1 H), 7.43 (d, *J* = 6.6 Hz, 1 H), 7.35–7.30 (m, 5 H), 7.25–7.18 (m, 5 H), 5.70–5.60 (m, 1 H), 5.10–4.95 (m, 2 H), 4.55–4.40 (m, 1 H), 4.05–3.95 (m, 1 H), 3.82 (d, *J* = 5.1 Hz, 2 H), 3.05–2.95 (m, 1 H), 2.90–2.75 (m, 1 H), 1.57 (d, *J* = 6.0 Hz, 3 H), 1.11 (d, *J* = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.5, 171.8, 171.2, 169.1, 155.6, 145.3, 137.6, 136.9, 131.1, 130.6, 129.2, 128.3, 128.0, 127.7, 126.7, 126.2, 120.2, 113.9, 65.5, 54.0, 50.2, 48.5, 41.5, 37.3, 18.0, 16.8.

Anal. Calcd for $C_{31}H_{33}N_7O_6$: C, 62.09; H, 5.55; N, 16.35. Found: C, 62.06; H, 5.71; N, 15.32.

Fmoc-L-Phe-L-Ala-L-Val-L-Leu-Bt (7b)
Yield: 78%; white microcrystals (CH_2Cl_2–hexanes); mp 206.0–208.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.85–8.75 (m, 1 H), 8.35–8.18 (m, 3 H), 7.87 (d, *J* = 6.9 Hz, 2 H), 7.85–7.74 (m, 2 H), 7.70–7.55 (m, 4 H), 7.45–7.35 (m, 2 H), 7.33–7.15 (m, 7 H), 5.70–5.60 (m, 1 H), 4.45–4.38 (m, 1 H), 4.35–4.30 (m, 1 H), 4.20–4.00 (m, 3 H), 3.10–2.95 (m, 1 H), 2.73 (dd, *J* = 18.9, 11.4 Hz, 1 H), 2.10–1.90 (m, 1 H), 1.85–1.65 (m, 2 H), 1.25–1.15 (m, 3 H), 1.12–1.05 (m, 2 H), 0.98–0.79 (m, 12 H).

^{13}C NMR (DMSO-d_6): δ = 172.0, 171.8, 171.5, 171.3, 155.8, 145.3, 143.7, 140.6, 138.2, 131.0, 130.5, 129.2, 128.0, 127.6, 127.0, 126.7, 126.2, 125.3, 120.0, 114.0, 65.6, 64.9, 57.1, 56.0, 51.2, 48.2, 46.5, 37.4, 30.8, 24.7, 23.0, 21.0, 19.1, 18.0, 15.2.

Anal. Calcd for $C_{44}H_{49}N_7O_6$: C, 68.46; H, 6.40; N, 12.70. Found: C, 68.07; H, 6.77; N, 12.59.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-Bt (7c)
Yield: 84%; white microcrystals ($CHCl_3$–hexanes); mp 205.0–206.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.74 (d, *J* = 5.7 Hz, 1 H), 8.29 (d, *J* = 8.1 Hz, 1 H), 8.22 (d, *J* = 7.8 Hz, 1 H), 7.95 (d, *J* = 7.5 Hz, 1 H), 7.87 (d, *J* = 8.1 Hz, 1 H), 7.80 (t, *J* = 7.1 Hz, 1 H), 7.64 (t, *J* = 7.2 Hz, 1 H), 7.45 (d, *J* = 7.2 Hz, 1 H), 7.38–7.30 (m, 5 H), 5.60–5.50 (m, 1 H), 5.01 (s, 2 H), 4.38–4.22 (m, 2 H), 4.10–4.00 (m, 1 H), 1.55 (d, *J* = 7.2 Hz, 4 H), 1.45–1.35 (m, 2 H), 1.25 (d, *J* = 6.9 Hz, 3 H), 1.18 (d, *J* = 7.2 Hz, 3 H), 0.95–0.75 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.4, 172.3, 171.7, 171.5, 155.6, 145.3, 137.0, 131.0, 130.6, 128.3, 127.7, 126.7, 120.2, 113.9, 65.3, 50.7, 50.1, 48.5, 47.5, 40.8, 24.1, 23.1, 21.5, 18.1, 16.6.

Anal. Calcd for $C_{29}H_{37}N_7O_6$: C, 60.09; H, 6.43; N, 16.91. Found: C, 60.07; H, 6.62; N, 16.46.

Cbz-L-Ala-L-Met-L-Ala-L-Ala-Bt (7d)
Yield: 86%; white microcrystals (CH_2Cl_2–hexanes); mp 183.0–185.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.78 (d, J = 5.4 Hz, 1 H), 8.30 (d, J = 8.4 Hz, 1 H), 8.22 (d, J = 8.1 Hz, 1 H), 8.05–7.93 (m, 2 H), 7.80 (t, J = 7.4 Hz, 1 H), 7.64 (t, J = 7.7 Hz, 1 H), 7.49 (d, J = 6.9 Hz, 1 H), 7.40–7.30 (m, 5 H), 5.60–5.20 (m, 1 H), 5.01 (s, 2 H), 4.39–4.28 (m, 2 H), 4.10–4.02 (m, 1 H), 2.45–2.40 (m, 2 H), 1.98 (s, 3 H), 1.94–1.70 (m, 2 H), 1.56 (d, J = 7.2 Hz, 3 H), 1.30–1.22 (m, 3 H), 1.19 (d, J = 6.9 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.5, 171.7, 170.5, 155.7, 145.3, 136.9, 131.1, 130.6, 128.3, 127.7, 126.7, 120.2, 113.9, 65.3, 51.6, 50.1, 48.5, 47.6, 32.1, 29.4, 18.0, 16.9, 16.6, 14.6.

Anal. Calcd for $C_{28}H_{35}N_7O_6S$: C, 56.27; H, 5.90; N, 16.40. Found: C, 55.88; H, 6.08; N, 15.20.

Preparation of Pentapeptides 8a, 8b, hexapeptides 10, and 11a–d; General Procedure

N-(Protected tripeptidoyl)benzotriazole **2c** (0.2 mmol) or *N*-(protected tetrapeptidoyl)benzotriazole **7a–d** (0.2 mmol) were added at 0 °C to a solution of tripeptide **9a** and/or amino acids **3b**, **3j** or dipeptide **5a–c** (0.2 mmol) in a mixture of MeCN (7 mL) and H_2O (3 mL) in the presence of Et_3N (0.24 mmol). The reaction mixture was then stirred at 0 °C until the starting material was completely consumed as observed by TLC (hexanes–EtOAc, 2:1). HCl (4 N, 1 mL) was added and the solvent was removed under reduced pressure. The residue obtained was dissolved in EtOAc (20 mL), and the organic extract was washed with 4 M HCl (3 × 5 mL), brine (3 × 5 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the desired products **8a**, **8b**, **10**, and **11a–d**.

Cbz-L-Ala-L-Phe-L-Gly-L-Ala-L-Phe-OH (8a)

Yield: 83%; white microcrystals (CH_2Cl_2); mp 193.0–195.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.28–8.16 (m, 2 H), 8.04–7.90 (m, 2 H), 7.42 (d, J = 7.5 Hz, 1 H), 7.36–7.10 (m, 15 H), 5.10–4.90 (m, 2 H), 4.55–4.45 (m, 1 H), 4.48–4.25 (m, 2 H), 4.05–3.95 (m, 1 H), 3.71 (d, J = 5.1 Hz, 2 H), 3.10–3.00 (m, 2 H), 2.95–2.75 (m, 2 H), 1.20–1.10 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.7, 172.3, 172.0, 171.2, 168.0, 155.6, 137.6, 137.5, 136.9, 129.2, 129.1, 128.3, 128.2, 128.0, 127.7, 126.4, 126.2, 65.5, 53.8, 53.5, 50.2, 47.8, 41.9, 37.5, 36.6, 18.4, 18.1.

Anal. Calcd for $C_{34}H_{39}N_5O_8$: C, 63.24; H, 6.09; N, 10.85. Found: C, 62.95; H, 6.13; N, 10.67.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-L-Ala-OH (8b)

Yield: 86%; white microcrystals (MeOH); mp 236.0–238.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.60 (br s, 1 H), 8.10 (d, J = 7.5 Hz, 1 H), 7.95–7.85 (m, 3 H), 7.45 (d, J = 7.2 Hz, 1 H), 7.36–7.28 (m, 5 H), 5.01 (s, 2 H), 4.30–4.16 (m, 4 H), 4.10–4.00 (m, 1 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.26 (d, J = 7.2 Hz, 3 H), 1.19 (d, J = 6.6 Hz, 9 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.4, 171.8, 171.7, 171.6, 155.7, 137.0, 128.3, 127.7, 65.4, 50.9, 50.1, 48.0, 47.8, 47.4, 40.8, 24.1, 23.1, 21.6, 18.2, 18.0, 17.2.

Anal. Calcd for $C_{26}H_{39}N_5O_8{\cdot}H_2O$: C, 55.01; H, 7.28; N, 12.34. Found: C, 55.53; H, 7.23; N, 12.14.

Cbz-L-Phe-L-Ala-L-Ala-Gly-L-Leu-L-Cys(S-Fm)-OH (10)

Yield: 61%; white microcrystals (MeOH); mp 194.0–196.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.65–8.55 (m, 1 H), 8.35–8.28 (m, 1 H), 8.25–8.12 (m, 1 H), 8.10–8.05 (m, 1 H), 8.00–7.95 (m, 1 H), 7.88–7.80 (m, 2 H), 7.78–7.70 (m, 2 H), 7.68–7.55 (m, 1 H), 7.45–7.10 (m, 10 H), 4.93 (br s, 1 H), 4.45–4.25 (m, 4 H), 4.20–4.10 (m, 1 H), 3.85–3.65 (m, 3 H), 3.18–3.05 (m, 3 H), 3.02–2.98 (m, 2 H), 2.80–2.70 (m, 2 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.28–1.10 (m, 3 H), 0.90–0.70 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.4, 171.8, 171.4, 168.4, 166.2, 155.9, 146.0, 140.5, 138.2, 137.0, 129.3, 129.0, 128.3, 128.0, 127.7, 127.4, 127.0, 126.2, 125.0, 119.9, 65.2, 56.2, 53.1, 51.4, 48.4, 46.5, 42.0, 41.2, 37.4, 35.9, 34.4, 24.1, 23.2, 21.6, 18.2.

HRMS (ESI$^+$): m/z [M + Na]$^+$ calcd for $C_{48}H_{56}N_6O_9SNa$: 915.3722; found: 915.3764; m/z [M – H + 2Na]$^+$ calcd for $C_{48}H_{55}N_6O_9SNa_2$: 937.3541; found: 937.3579.

Cbz-L-Ala-L-Phe-Gly-L-Ala-Gly-L-Leu-OH (11a)

Yield: 80%; white microcrystals (MeOH); mp 152.0–154.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.60 (br s, 1 H), 8.30–8.22 (m, 1 H), 8.20–8.16 (m, 1 H), 8.15–8.10 (m, 1 H), 8.08–7.95 (m, 2 H), 7.45–7.40 (m, 1 H), 7.38–7.30 (m, 5 H), 7.25–7.18 (m, 5 H), 5.10–4.95 (m, 2 H), 4.52–4.45 (m, 1 H), 4.30–4.15 (m, 1 H), 4.10–3.95 (m, 1 H), 3.80–3.65 (m, 4 H), 3.05–3.00 (m, 1 H), 2.88–2.75 (m, 1 H), 1.65–1.60 (m, 1 H), 1.58–1.42 (m, 2 H), 1.30–1.25 (m, 1 H), 1.23 (d, J = 6.9 Hz, 3 H), 1.12 (d, J = 7.2 Hz, 3 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 174.0, 172.4, 171.2, 168.6, 168.4, 155.6, 137.6, 136.9, 129.3, 128.4, 128.0, 127.8, 126.2, 65.5, 53.9, 50.2, 48.4, 42.1, 41.7, 37.4, 24.3, 22.9, 21.4, 18.1.

Anal. Calcd for $C_{33}H_{44}N_6O_9$: C, 59.27; H, 6.63; N, 12.57. Found: C, 59.35; H, 6.67; N, 12.06.

Fmoc-L-Phe-L-Ala-L-Val-L-Leu-Gly-L-Ala-OH (11b)

Yield: 82%; white microcrystals (CH_2Cl_2–hexanes); mp 143.0–145.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.53 (br s, 1 H), 8.30–8.22 (m, 1 H), 8.25–8.14 (m, 1 H), 8.10–8.00 (m, 1 H), 7.87 (d, J = 7.5 Hz, 2 H), 7.65–7.50 (m, 3 H), 7.40 (t, J = 7.4 Hz, 2 H), 7.35–7.20 (m, 7 H), 7.20–7.12 (m, 1 H), 4.40–4.35 (m, 1 H), 4.30–4.22 (m, 2 H), 4.21–4.16 (m, 1 H), 4.15–4.05 (m, 3 H), 3.75–3.65 (m, 1 H), 3.05–2.95 (m, 1 H), 2.80–2.70 (m, 1 H), 2.52–2.50 (m, 1 H), 2.50–2.47 (m, 1 H), 2.04–1.90 (m, 1 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.30–1.15 (m, 6 H), 0.90–0.60 (m, 12 H).

^{13}C NMR (DMSO-d_6): δ = 174.0, 172.3, 172.2, 171.4, 171.0, 168.4, 155.8, 143.7, 140.6, 138.2, 129.3, 128.0, 127.6, 127.0, 126.2, 125.3, 120.1, 65.7, 56.0, 48.2, 47.5, 46.6, 41.8, 40.7, 37.5, 24.3, 24.2, 23.2, 23.0, 21.7, 21.3, 19.2, 18.1, 17.9, 17.3.

Anal. Calcd for $C_{43}H_{54}N_6O_9$: C, 64.64; H, 6.81; N, 10.52. Found: C, 64.87; H, 7.06; N, 9.26.

HRMS (ESI$^+$): m/z [M + Na]$^+$ calcd for $C_{43}H_{54}N_6O_9Na$: 821.3844; found: 821.3863; m/z [M – H + 2Na]$^+$ calcd for $C_{43}H_{54}N_6O_9Na_2$: 843.3664; found: 843.3664.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-L-Ala-Gly-OH (11c)[32]

Yield: 87%; white microcrystals (MeOH); mp 240.0–242.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.15–8.05 (m, 1 H), 8.00–7.90 (m, 4 H), 7.48–7.42 (m, 1 H), 7.38–7.30 (m, 5 H), 5.01 (s, 2 H), 4.30–4.20 (m, 4 H), 4.10–4.00 (m, 1 H), 3.75–3.65 (m, 2 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.35–1.15 (m, 12 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.4, 172.3, 171.7, 171.6, 171.1, 155.6, 137.0, 128.3, 127.7, 65.4, 50.9, 50.1, 48.1, 47.9, 40.8, 24.1, 23.1, 21.6, 18.4, 18.1, 18.0.

Anal. Calcd for $C_{28}H_{42}N_6O_9$: C, 55.43; H, 6.98; N, 13.85. Found: C, 56.07; H, 7.36; N, 12.00.

HRMS (ESI$^+$): m/z [M + Na]$^+$ calcd for $C_{28}H_{42}N_6O_9Na$: 629.2905; found: 629.2912; m/z [M – H + 2Na]$^+$ calcd for $C_{28}H_{42}N_6O_9Na_2$: 651.2725; found: 651.2729.

Cbz-L-Ala-L-Met-L-Ala-L-Ala-L-Ala-Gly-OH (11d)[32]

Yield: 84%; white microcrystals (CH_2Cl_2–hexanes); mp 254.0–256.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.60 (br s, 1 H), 8.18–8.10 (m, 1 H), 8.06–7.95 (m, 3 H), 7.92 (d, *J* = 7.8 Hz, 1 H), 7.50 (d, *J* = 7.5 Hz, 1 H), 7.40–7.30 (m, 5 H), 5.01 (s, 2 H), 4.38–4.20 (m, 4 H), 4.10–4.00 (m, 1 H), 3.80–3.70 (m, 2 H), 2.45–2.40 (m, 2 H), 2.03 (s, 3 H), 1.95–1.75 (m, 2 H), 1.25–1.15 (m, 12 H).

^{13}C NMR (DMSO-d_6): δ = 172.5, 172.3, 171.8, 171.6, 171.1, 170.6, 155.7, 137.0, 128.3, 127.7, 65.4, 51.7, 50.1, 48.0, 47.9, 40.7, 32.1, 29.4, 18.4, 18.0, 14.6.

Anal. Calcd for $C_{27}H_{40}N_6O_9S·H_2O$: C, 50.46; H, 6.27; N, 13.08. Found: C, 50.32; H, 6.44; N, 12.63.

Preparation of *N*-(Protected pentapeptidoyl)benzotriazoles 12a and 12b; General Procedure

$SOCl_2$ (1 mmol) was added to a solution of 1*H*-benzotriazole (4 mmol) in anhydrous THF or MeCN (15 mL) at 20 °C, and stirred for 30 min. The reaction mixture was cooled to –30 °C, then *N*-protected pentapeptides **8a** or **8b** (1 mmol), dissolved in anhydrous THF or MeCN (5 mL), were added dropwise and the mixture was stirred for 4 h. The white precipitate that formed during the reaction was filtered off, and the filtrate was concentrated under reduced pressure. The residue obtained was washed with Et_2O to remove excess BtH, then dried to give the desired products **12a** and **12b.**

Cbz-L-Ala-L-Phe-L-Gly-L-Ala-L-Phe-Bt (12a)

Yield: 78%; white microcrystals (MeOH); mp 188.0–190.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.95–8.85 (m, 1 H), 8.30–8.16 (m, 2 H), 8.04–7.95 (m, 2 H), 7.84–7.74 (m, 1 H), 7.65–7.58 (m, 1 H), 7.45–7.10 (m, 17 H), 5.90–5.80 (m, 1 H), 5.10–4.95 (m, 2 H), 4.55–4.35 (m, 2 H), 4.10–3.95 (m, 1 H), 3.75–3.65 (m, 2 H), 3.35–3.30 (m, 1 H), 3.25–3.15 (m, 1 H), 3.08–3.00 (m, 1 H), 2.90–2.75 (m, 1 H), 1.25–1.05 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.8, 172.3, 171.2, 171.0, 168.2, 155.6, 145.2, 137.6, 136.9, 136.5, 131.0, 130.4, 129.2, 129.0, 128.3, 128.0, 127.7, 126.7, 126.2, 120.2, 113.9, 65.4, 54.4, 53.8, 50.2, 47.6, 41.9, 37.4, 36.4, 18.2, 18.1.

Anal. Calcd for $C_{40}H_{42}N_8O_7$: C, 64.33; H, 5.67; N, 15.00. Found: C, 63.69; H, 5.60; N, 14.54.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-L-Ala-Bt (12b)

Yield: 85%; white microcrystals (CH_2Cl_2); mp 198.0–200.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.51 (d, *J* = 6.9 Hz, 1 H), 8.29 (d, *J* = 8.1 Hz, 1 H), 8.23 (d, *J* = 8.1 Hz, 1 H), 7.98–7.85 (m, 3 H), 7.81 (t, *J* = 7.2 Hz, 1 H), 7.64 (t, *J* = 8.1 Hz, 1 H), 7.45 (d, *J* = 7.5 Hz, 1 H), 7.38–7.28 (m, 5 H), 5.56 (t, *J* = 6.6 Hz, 1 H), 5.01 (s, 2 H), 4.45–4.40 (m, 1 H), 4.30–4.20 (m, 2 H), 4.10–4.00 (m, 1 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.25–1.10 (m, 9 H), 1.05–0.95 (m, 3 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 172.5, 171.8, 155.7, 145.3, 137.0, 131.1, 130.6, 128.3, 127.7, 126.7, 120.2, 114.0, 65.4, 50.9, 50.1, 48.6, 48.0, 47.8, 47.6, 40.8, 24.1, 23.1, 21.6, 18.1, 17.2, 16.6.

Anal. Calcd for $C_{32}H_{42}N_8O_7·H_2O$: C, 57.47; H, 6.63. Found: C, 57.52; H, 6.47.

Preparation of Hexapeptide 13 and Heptapeptides 14a and 14b; General Procedure

N-(Protected pentapeptidoyl)benzotriazole **12a** or **12b** (0.2 mmol) was added at 0 °C to a solution of amino acid **3j** or dipeptide **5c** or **5d** (0.2 mmol) in a mixture of MeCN (7 mL) and H_2O (3 mL) in the presence of Et_3N (0.24 mmol). The reaction mixture was stirred at 0 °C until TLC (hexanes–EtOAc, 1:1) showed complete consumption of the starting materials **12a** or **12b**, then 4 M HCl (1 mL) was added and the solvent was removed under reduced pressure. The residue obtained was dissolved in EtOAc (15 mL), and the organic extract was washed with 4 M HCl (2 × 5 mL), brine (2 × 5 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the desired products **13**, **14a**, and **14b**.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-L-Ala-L-Phe-OH (13)

Yield: 85%; white microcrystals (EtOH); mp 240.0–242.0 °C.

^{1}H NMR (DMSO-d_6): δ = 8.12– 8.04 (m, 1 H), 7.98–7.82 (m, 4 H), 7.46 (d, *J* = 7.2 Hz, 1 H), 7.36–7.30 (m, 5 H), 7.26–7.16 (m, 5 H), 5.01 (s, 2 H), 4.45–4.35 (m, 1 H), 4.30–4.15 (m, 4 H), 4.10–4.00 (m, 1 H), 3.10–3.00 (m, 1 H), 2.95–2.85 (m, 1 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.25–1.10 (m, 12 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 174.0, 172.8, 172.7, 172.0, 171.7, 171.5, 155.7, 137.4, 137.0, 129.2, 128.3, 128.2, 127.7, 126.4, 65.4, 53.5, 50.9, 50.1, 48.0, 47.8, 47.4, 40.8, 36.6, 24.1, 23.1, 21.6, 18.2, 18.1, 17.2.

Anal. Calcd for $C_{35}H_{48}N_6O_9.2\ H_2O$: C, 57.36; H, 7.15; N, 11.47. Found: C, 57.46; H, 6.90; N, 11.64.

Cbz-L-Ala-L-Phe-L-Gly-L-Ala-L-Phe-L-Ala-Gly-OH (14a)

Yield: 82%; white microcrystals (CH_2Cl_2); mp 168.0–170.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.65 (br s, 1 H), 8.28–8.16 (m, 2 H), 8.06–7.88 (m, 3 H), 7.44–7.32 (m, 7 H), 7.25–7.15 (m, 10 H), 5.08–4.90 (m, 2 H), 4.55–4.45 (m, 2 H), 4.35–4.25 (m, 2 H), 4.05–3.95 (m, 1 H), 3.78–3.65 (m, 4 H), 3.05–2.95 (m, 2 H), 2.88–2.78 (m, 2 H), 1.26–1.10 (m, 9 H).

^{13}C NMR (DMSO-d_6): δ = 172.7, 172.3, 172.0, 171.2, 170.5, 168.3, 168.0, 156.0, 137.5, 136.9, 129.2, 128.3, 128.2, 128.0, 127.7, 126.4, 126.2, 65.4, 53.8, 53.5, 50.1, 48.2, 47.8, 41.9, 37.5, 37.1, 18.7, 18.3, 18.1.

Anal. Calcd for $C_{39}H_{47}N_7O_{10}$: C, 60.53; H, 6.12. Found: C, 60.78; H, 5.87.

Cbz-L-Ala-L-Leu-L-Ala-L-Ala-L-Ala-Gly-Gly-OH (14b)

Yield: 79%; white microcrystals; mp 263.0–265.0 °C.

^{1}H NMR (DMSO-d_6): δ = 12.55 (br s, 1 H), 8.15–8.08 (m, 1 H), 8.00–7.86 (m, 4 H), 7.46 (d, *J* = 7.5 Hz, 1 H), 7.35 (br s, 6 H), 5.01 (s, 2 H), 4.30–4.20 (m, 3 H), 4.10–4.00 (m, 1 H), 3.76–3.70 (m, 1 H), 3.34 (s, 4 H), 1.65–1.55 (m, 1 H), 1.50–1.40 (m, 2 H), 1.19 (d, *J* = 6.3 Hz, 12 H), 0.90–0.80 (m, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.9, 172.4, 171.7, 171.6, 171.5, 169.0, 155.6, 137.0, 128.3, 127.7, 65.3, 50.9, 50.1, 48.0, 47.8, 47.4, 40.7, 24.1, 23.1, 21.6, 18.1, 18.0, 17.1.

Anal. Calcd for $C_{30}H_{45}N_7O_{11.}H_2O$: C, 52.85; H, 6.95. Found: C, 52.54; H, 6.96.

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

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