The Efficient Preparation of Di- and Tripeptides by Coupling *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles with Unprotected Amino Acids

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Abstract: *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles **2** and N-protected peptidoylbenzotriazoles **6** are coupled in aqueous acetonitrile solution with free amino acids or dipeptides to prepare: (i) 22 chirally pure dipeptides **5a**–**v** (in an average yield of 82%) from *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles **2** and unprotected amino acids, (ii) five chiral tripeptides **7a**–**e** (in an average yield of 75%) from N-protected peptidoylbenzotriazoles **6** and unprotected amino acids, (iii) one chiral tripeptide **7g** (62%) from *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazole **2a** and the free dipeptide **8**. In all, *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazole derivatives of 17 of the 20 naturally occurring amino acids were used, including those containing the following unprotected side chain functionalities: alcoholic –OH (Ser), indole –NH (Trp), imidazole –NH, phenolic –OH (Tyr), $–CONH_2$ (Gln, Asn), –SH (Cys), $–CO_2H$ (Glu, Asp), and –S-S (Cystine). Support for the complete retention of chirality was obtained by parallel experiments involving D-Ala, L-Ala, and DL-Ala for the preparation of di- and tripeptides. This and other evidence for chiral integrity was supported by NMR and HPLC analyses.

Key words: N-(protected α-aminoacyl)benzotriazole, acylating reagent, dipeptides, *N*-Cbz-peptidoylbenzotriazole, tripeptides

Many biologically active peptides function as enzymes, inhibitors, immune bodies, peptide hormones, and antibiotics. Peptides are also implicated in the onset or continuation of various diseases. Other roles include participation in mushroom toxins and snake venoms. Large peptides can be found and isolated from natural sources, but small peptides are usually prepared synthetically.

In the formation of peptide bonds by chemical synthesis, protection of the non-participating amino group is mandatory. Other functional groups, such as alcohols (–OH in Ser) or phenols (–OH in Tyr), are also frequently protected, especially in reactions with highly activated intermediates (e.g. acid halides, mixed anhydrides, or *O*-acylisoureas).[1] However, such functional groups (but not the thiol group in cysteine) can be left unprotected when selective acylating agents of moderate activity such as azides, or active esters are utilized.[1a–d]

Peptide coupling of unprotected amino acids has been achieved by acyl azides,[1d,2] *N*-hydroxysuccinimide esters,[3] and *p*-nitrophenyl esters[4] of N-protected amino acids with minimal racemization. However, there are problems associated with these protocols. Acyl azides are typically difficult to separate due to problems with washing and drying, are often unstable, and are difficult to store.[1d,5] It has been reported[6] that undesired side reactions occur during the preparation of *N*-hydroxysuccinimide esters by coupling N-protected amino acids and *N*-hydroxysuccinimide with carbodiimide. Reactions with phenyl esters can be complicated by difficulty in the complete removal of the liberated acidic phenol, especially when the peptides produced do not precipitate out.[6]

We have extensively applied *N*-acylbenzotriazoles for N-acylation[7] C-acylation[8] and O-acylation.[9] During this work, peptide coupling of *N*-(Cbz-aminoacyl)benzotriazoles with unprotected amino acids produced peptides in 85–95% yield with minimal racemization.[10] *N*-(Cbz-α-Aminoacyl)benzotriazoles prepared from N-protected α-amino acids with unfunctionalized side-chains such as Ala, Val, and Phe coupled successfully with unprotected amino acids (Ala, Val, Phe, Ser, Trp) in the presence of Et_3N in partially aqueous solution (CH_3CN/H_2O). *N*-Cbz-Peptidoylbenzotriazoles from N-protected dipeptides, *N*-Cbz-Ala-Phe-OH and *N*-Cbz-Phe-Ala-OH reacted analogously. Recently, we prepared *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles derived from Tyr, Trp, Cys, Met, and Gln containing unprotected side-chain functionality,[10b] and successfully coupled them with α-amino acids (L-Ala, L-Phe) in partially aqueous medium to form the expected peptides. NMR and HPLC analysis supported the preservation of the original chirality. These findings were further supported by NMR and HPLC comparison of the diastereomeric dipeptides prepared by coupling *N*-(Cbz-Tyr, Trp, Cys, Met, Gln and Fmoc-Trp, Met acyl)benzotriazole with H-DL-Ala-OH, H-DL-Phe-OH.[10b]

Herein we report (i) the extension of our preparation of *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles to Ser, Asn, Glu, Asp, Met, and Cystine (with unprotected alcoholic, primary amide, CO_2H, SMe, and S–S functional groups), (ii) peptide coupling of these *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles with free α-amino acids including Ala, Phe, Val, and also Trp, Ser, Gln, Met, Glu, and Cys, all with unprotected side-chain functionality, (iii) preparation of N-protected peptidoylbenzotriazoles from N-protected dipeptides, and (iv) preparation of N-protected tripeptides carrying unprotected side-chain functionalities.

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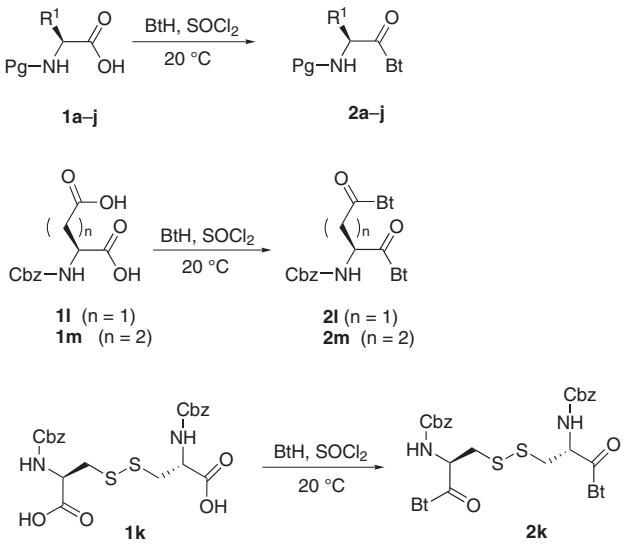
Preparation of *N*-(Cbz- and Fmoc-α-aminoacyl)benzotriazoles from *N*-Cbz- or Fmoc-α-Amino Acids

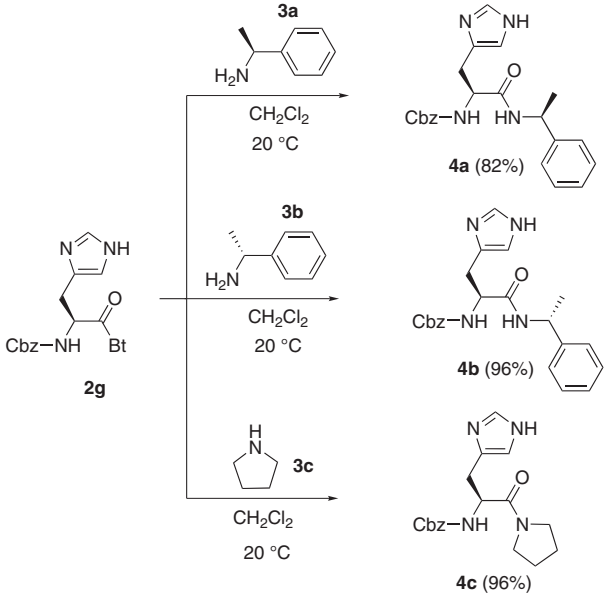
Preparations of *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles **2a–f**, **2h–j**, **2n** were carried out by the previously reported procedure:[10] the *N*-Cbz- or Fmoc-α-amino acid (**1a–f**, **1h–j**, **1n**) was treated with a mixture of four equivalents of 1*H*-benzotriazole and one equivalent of thionyl chloride in THF at room temperature for two hours to yield the desired **2a–f**, **2h–j**, **2n** in 72–95% (Scheme 1). Compounds **2k–m** were synthesized utilizing one equivalent of the dicarboxylic acid (**1k–m**) with eight equivalents of 1*H*-benzotriazole and two equivalents of thionyl chloride in THF at room temperature for two hours. Cbz-Asn-Bt (**2e**) was directly crystallized from methanol to remove unreacted 1*H*-benzotriazole. Attempted isolation of **2e** from 1*H*-benzotriazole by acid or base wash failed. Fmoc-Ser-Bt (**2j**) was isolated from a mixture by washing with 4 N HCl solution; we could not isolate **2j** by washing with saturated Na_2CO_3 solution or column chromatography using ethyl acetate–hexanes (2:1) and the prolonged reaction caused some decomposition of **2j**. Compounds **2e–f**, **2j–n** are novel and were characterized using ^1H and ^{13}C NMR spectroscopy, elemental analysis, and ORP.



Bt = Benzotriazol-1-yl
Pg = Protecting group (Z, Fmoc)
Amino acids with R^1: Trp, Tyr, Gln, Cys, Met, Asn, Ser, and His.

Scheme 1 Preparation of *N*-(Cbz- and Fmoc-aminoacyl)benzotriazoles **2a–n**

Preparation of Cbz-His-Bt (**2g**) was carried out in the same manner as described above. Although the formation of **2g** in the reaction mixture was supported by ^1H NMR spectra, **2g** could not yet be isolated in pure form. Compound **2g** underwent decomposition when washed with saturated Na_2CO_3 solution or when subjected to column chromatography. Thus, formation of **2g** was further supported by the isolation of *N*-Cbz-amino amides **4a–c** obtained from reactions of crude **2g** with amines **3a–c** (Scheme 2).



Scheme 2 Preparation of Cbz-L-His-amides **4a–c**

NMR analysis of compounds **4a–c** revealed no detectable racemization (<5%). ^1H NMR analysis of compounds **4a–c** showed an evident doublet for the amide NH proton. No signal from the corresponding diastereomer was observed in the NMR analysis of the methyl protons of **4a** and **4b**. ^1H NMR showed a clear doublet for the methyl protons present in compounds **4a** and **4b** at 1.33 and 1.28 ppm, respectively, suggesting LL-configuration is more downfield shifted as compared to LD-configuration. ^{13}C NMR gave singlets for all carbonyl and methyl carbons for **4a** and **4b**, further supporting the enantiopurity of the compounds.

Cbz-Pro-Bt (**2n**) was prepared using the same methodology, although a complicated spectrum was observed in the NMR analysis due to the formation of rotamers. The presence of an additional protection-free amino group in the side-chain functionality of L-Lys and L-Arg necessitates the preparation of doubly N-protected (L-Arg and L-Lys acyl)benzotriazoles, since we plan experiments in this direction.

Preparation of N-Protected Dipeptides with Additional Unprotected Side-Chain Functionality

Peptide-coupling reactions were carried out between *N*-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles derived from Trp, Tyr, Gln, Met, Ser, Cystine, Asp, and Glu **2a–c**,**f**,**h–m** with diverse L- and D-amino acids (Ala, Val, Phe, Trp, Ser, Tyr, Gln, Glu, and Cys) in partially aqueous solution (MeCN–H_2O) in the presence of Et_3N for one hour (Scheme 2 and Table 2). The crude products were washed with 6 N HCl solution to remove the by-product, BtH. The dipeptides **5f–h** prepared from *N*-Cbz-Gln-Bt (**2c**) were

isolated by precipitation from the reaction mixture upon acidification. Enantiopure dipeptides **5a–u** (LL-configuration) and **5v** (LD-configuration) were obtained in 70–95% yields in high purity without the use of any chromatographic techniques (Table 1 and Scheme 3).

Diastereomeric mixtures **5a + 5a′**, **5i + 5v**, **5q + 5q′** (LL- and DL-configuration) were prepared from *N*-(Cbz-L-Trp, L-Met, L-CyS-S acyl)benzotriazoles and DL-Ala-OH, using the same conditions as mentioned for the enantiopure LL-dipeptides (Table 2). Compounds **5a–v** and the diastereomeric mixtures **5a + 5a′**, **5i + 5v**, and **5q + 5q′** were obtained in 70–95% yields and were further recrystallized from $CHCl_3$–hexane for ^1H and ^{13}C NMR spectroscopy, elemental analysis, and ORP.

NMR Studies for the Chirality of 5a–v

NMR analysis showed no detectable racemization (<5%) for the enantiopure LL-dipeptides **5a–u** and the LD-dipeptide **5v**. For compounds **5a–v**, ^1H NMR analysis for each compound revealed two sets of doublets for the two –NH protons ranging from 7.5–8.6 ppm. However, for each of the diastereomeric mixtures **5a + 5a′**, **5i + 5v**, **5q + 5q′**, the two NH-proton signals appeared as four sets of doublets. The methyl protons of the L-Ala fragment showed as a clear doublet in each of the enantiopure LL-dipeptides **5a**, **5i**, and **5q**, whereas it was observed as two sets of doublets for each of the corresponding diastereomeric mixtures **5a + 5a′**, **5i + 5v**, and **5q + 5q′**. For example, the ^1H NMR analysis of **5q** indicated an evident doublet for the methyl protons at 1.29 ppm, whereas two sets of doublets were obtained at 1.27 and 1.29 ppm for the corresponding diastereomeric mixture **5q + 5q′**.

The methyl protons of **5i** (LL) were observed at 1.28 ppm, whereas the signal for the methyl protons for **5v** (LD) appeared at 1.26 ppm. These results were then carefully compared with the diastereomeric mixture **5i + 5v**, which gave a multiplet at 1.24–1.29 ppm, further highlighting the chirality of the enantiopure dipeptides **5i** and **5v**. ^{13}C NMR for each diastereomeric mixture **5a + 5a′**, **5i + 5v**, and **5q + 5q′** displayed doublets for most aliphatic and carbonyl carbons, but no significant difference was observed for the aromatic carbons.

HPLC Analysis for the Chirality of 5a, 5i, and 5q

The enantiopurity of the dipeptides **5a**, **5i**, and **5q** was further confirmed with HPLC analyses using Chirobiotic T column [detection at 254 nm, flow rate 1.0 mL/min, and MeOH–H_2O (1:1) as solvent]. For each of the LL-dipeptides **5a**, **5i**, **5q**, the HPLC results showed a single peak. By contrast, two peaks were observed for the corresponding diastereomeric mixtures **5a + 5a′**, **5i + 5v**, **5q + 5q′**, confirming the enantiopurity of the LL-dipeptides **5a**, **5i**,

Table 1 Conversion of N-Protected α-Amino Acids into N-Protected (α-Aminoacyl)benzotriazoles

Entry	Reagent	Product	Yield (%)[a]	Mp (°C)	$[\alpha]_D^{23}$
1	*N*-Cbz-L-Trp-OH (**1a**)[b]	*N*-Cbz-L-Trp-Bt (**2a**)[b]	95	100–101	+48.8
2	*N*-Cbz-L-Tyr-Bt (**1b**)[b]	*N*-Cbz-L-Tyr-Bt (**2b**)[b]	86	165–166	+64.3
3	*N*-Cbz-L-Gln-OH (**1c**)[b]	*N*-Cbz-L-Gln-Bt (**2c**)[b]	72	161–162	–37.5
4	*N*-Cbz-L-Cys-OH (**1d**)[b]	*N*-Cbz-L-Cys-Bt (**2d**)[b]	76	144–147	–169.0
5	*N*-Cbz-L-Asn-OH (**1e**)	*N*-Cbz-L-Asn-Bt (**2e**)	72	142–143	–30.1
6	*N*-Cbz-L-Met-OH (**1f**)	*N*-Cbz-L-Met-Bt (**2f**)	95	105–107	–49.3
7	*N*-Cbz-L-His-OH (**1g**)	*N*-Cbz-L-His-Bt (**2g**)	90[c]	–[c]	–[d]
8	*N*-Fmoc-L-Trp-OH (**1h**)[b]	*N*-Fmoc-L-Trp-Bt (**2h**)[b]	90	88–90	+17.6
9	*N*-Fmoc-L-Met-OH (**1i**)[b]	*N*-Fmoc-L-Met-Bt (**2i**)[b]	87	98–100	–103.9
10	*N*-Fmoc-L-Ser-OH (**1j**)	*N*-Fmoc-L-Ser-Bt (**2j**)	68	100–101	–44.8
11	*N*-Cbz-L-CyS-S-diOH (**1k**)	*N*-Cbz-L-CyS-S-diBt (**2k**)	88	180–182	–151.7
12	*N*-Cbz-L-Asp-diOH (**1l**)	*N*-Cbz-L-Asp-diBt (**2l**)	86	140–143	+29.3
13	*N*-Cbz-L-Glu-diOH (**1m**)	*N*-Cbz-L-Glu-diBt (**2m**)	92	150–152	–6.0
14	*N*-Cbz-L-Pro-OH (**1n**)[e]	*N*-Cbz-L-Pro-Bt (**2n**)[e]	74	oil	–139.6

[a] Isolated yield.
[b] Previously prepared.[10b]
[c] Crude yield, no melting point available.
[d] Characterized by the preparation of amino amides, see experimental section.
[e] Rotamers.

Table 2 Preparation of *N*-Cbz-Dipeptides **5a–v** from *N*-Protected (α-Aminoacyl)benzotriazoles **2a–c,f,h–m**, and Free Amino Acids

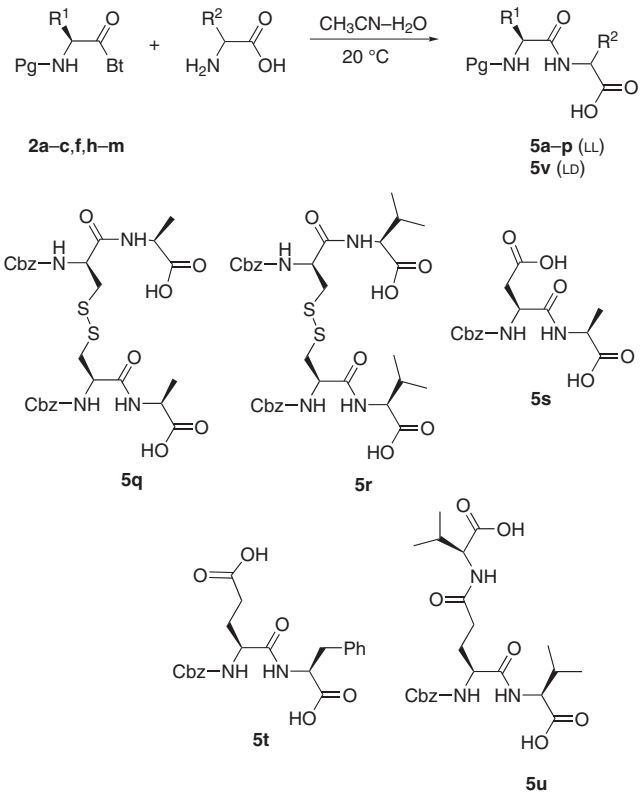
Entry	Reactant	Amino acid	Product	Yield (%)[a]	Mp (°C)	$[\alpha]_D^{23}$
1	**2a**	L-Ala	Cbz-L-Trp-L-Ala-OH (**5a**)	90	140–144	–23.9
2	**2a**	L-Cys	Cbz-L-Trp-L-Cys-OH (**5b**)	86	140–144	–28.5
3	**2a**	L-Ser	Cbz-L-Trp-L-Ser-OH (**5c**)	86	133–138	–11.4
4	**2a**	L-Trp	Cbz-L-Trp-L-Trp-OH (**5d**)	85	188–190	–15.6
5	**2b**	L-Trp	Cbz-L-Tyr-L-Trp-OH (**5e**)	90	114–119	+2.4
6	**2c**	L- Ala	Cbz-L-Gln-L-Ala-OH (**5f**)	72	211–213	–2.1
7	**2c**	L-Gln	Cbz-L-Gln-L-Gln-OH (**5g**)	47	132–134	+26.7
8	**2c**	L-Val	Cbz-L-Gln-L-Val-OH (**5h**)	66	186–188	+5.5
9	**2f**	L-Ala	Cbz-L-Met-L-Ala-OH (**5i**)	95	137–138	–9.0
10	**2f**	L-Met	Cbz-L-Met-L-Met-OH (**5j**)	95	120–122	–8.4
11	**2f**	L-Trp	Cbz-L-Met-L-Trp-OH (**5k**)	82	172–173	+8.2
12	**2f**	L-Glu	Cbz-L-Met-L-Glu-OH (**5l**)	94	119–120	–8.2
13	**2h**	L-Ser	Fmoc-L-Trp-L-Ser-OH (**5m**)	87	114–115	–13.7
14	**2i**	L-Ser	Fmoc-L-Met-L-Ser-OH (**5n**)	88	157–159	–2.3
15	**2i**	L-Glu	Fmoc-L-Met-L-Glu-OH (**5o**)	93	148–150	–18.1
16	**2j**	L-Ala	Fmoc-L-Ser-L-Ala-OH (**5p**)	72	131–132	–2.3
17	**2k**	L-Ala	$(Cbz\text{-L-CyS-S-L-Ala-OH})_2$ (**5q**)	96	158–160	–102.2
18	**2k**	L-Val	$(Cbz\text{-L-CyS-S-L-Val-OH})_2$ (**5r**)	91	98–100	–80.4
19	**2l**	L-Ala	Cbz-L-Asp-L-Ala-OH (**5s**)[b]	75	112–113	–57.8
20	**2m**	L-Phe	Cbz-L-Glu-L-Phe-OH (**5t**)[b]	88	168–170	+15.2
21	**2m**	L-Val	Cbz-L-Glu-L-di-Val-OH (**5u**)	71	87–88	–11.1
22	**2f**	D-Ala	Cbz-L-Met-D-Ala-OH (**5v**)	95	157–158	–33.4
23	**2a**	DL-Ala	Cbz-L-Trp-DL-Ala-OH (**5a** + **5a′**)[c,d]	94	93–95	–18.4
24	**2f**	DL-Ala	Cbz-L-Met-DL-Ala-OH (**5i** + **5v**)[d]	92	141–142	–5.4
25	**2k**	DL-Ala	$(Cbz\text{-L-CyS-S-DL-Ala-OH})_2$ (**5q** + **5q′**)[c,d]	91	153–154	–118.4

[a] Isolated yields.
[b] Mono-substituted product.
[c] **a′** and **q′** are LD-configuration.
[d] Diastereomeric mixture.

5q. These results also indicated shorter retention times for the LL-configuration as compared to the LD-configuration (Table 5).

Preparation of N-Protected Peptidoylbenzotriazoles from N-Protected Dipeptides

N-Cbz-Dipeptides **5a**,**e**,**i**,**v**, and the diastereomeric mixture **5i** + **5v** were successfully converted into the corresponding benzotriazole derivatives **6a–d**, and the diastereomeric mixture **6c** + **6d** respectively (Scheme 4). The reactions were carried out at –10 °C by following the same simple procedure as used for **2**, until the starting materials **5** were completely consumed. The products **6** were obtained with no detectable racemization, as evidenced by their ^1H and ^{13}C NMR spectra. Initially, a reaction of **5i** was carried out at 20 °C following the procedure used for **2a–n**, but the product **6c** was then obtained with partial racemization. Compounds **6a**,**c**,**d**, and the mixture **6c** + **6d** were obtained by an aqueous workup, but **6b** was purified by column chromatography due to the loss of **6b** when subjected to base wash. Compound **6a–d** and the mixture **6c** + **6d** were obtained in 76–87% yields without the use

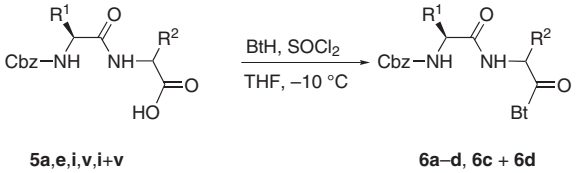


Scheme 3 Preparation of LL- and LD-dipeptides **5a–v;** for designation of R^1 and R^2, refer to Table 2

of any chromatographic techniques with the exception of **6b** (Table 3). Compounds **6a–d** and the mixture **6c + 6d** were recrystallized using $CHCl_3$–hexane and characterized using ^1H and ^{13}C NMR spectroscopy, elemental analysis, and ORP.

NMR Evidence for the Chirality of 6a–d

NMR analysis demonstrated the absence of racemization for compounds **6a–d**. ^1H NMR showed two clear doublets for the two –NH protons of each of compounds **6a–d**. The observed chemical shift for the methyl protons of L-Ala in **6c** was 1.57 ppm, whereas the methyl protons in **6d** were seen at 1.55 ppm. In addition, no signals from the other corresponding diastereomer were observed in the NMR spectrum of **6c** and **6d**, suggesting the enantiopurity of the N-protected peptidoyl benzotriazoles. The NMR analysis also gave a singlet for the methyl protons (SMe) from *N*-Cbz-L-Met fragment. ^{13}C NMR displayed a singlet for each carbonyl carbon for compounds **6a–d**. The methyl carbons of compounds **6a**,**c**,**d** also appeared as singlets in ^{13}C NMR.



Scheme 4 Preparation of *N*-Cbz-dipeptidoylbenzotriazoles **6a–d** and diastereomeric mixture **6c + 6d**; for detailed structures, see Table 3

On the other hand, the mixture **6c + 6d** displayed a complicated NMR spectrum. In ^1H NMR, two sets of doublets were obtained for all –NH protons. In addition, an unresolved triplet for the methyl protons from L-Ala and two singlets for the methyl protons (SMe) of the *N*-Cbz-L-Met fragment were also observed in the ^1H NMR. All aliphatic carbons with the exception of the benzyl CH_2 carbon and two carbonyl carbons appeared as sets of two singlets in ^{13}C NMR.

Preparation of N-Protected Tripeptides Carrying Unprotected Side-Chain Functionality

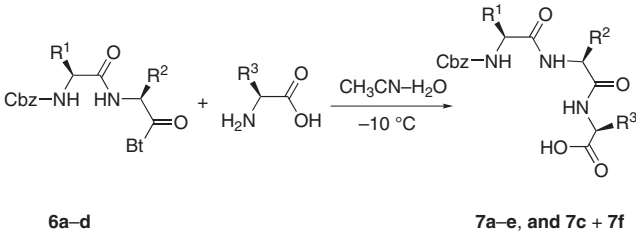
Tripeptides **7a–e**, **7g** and the diastereomeric mixture **7c + 7f** were prepared by two different methods: (i) peptide-coupling reactions between compounds **6a–c**, and the mixture **6c + 6d** with an unprotected amino acid afforded **7a–e** and the diastereomeric mixture **7c + 7f** (Scheme 5 and Table 4) and (ii) peptide-coupling reaction between **2a** and the unprotected dipeptide **8** gave **7g** (Scheme 6).

The coupling reactions were performed using the method (i) at –10 °C, and NMR analysis showed minimal racemization. However, extensive racemization was observed

Table 3 Conversion of *N*-Cbz-Dipeptides **5a**,**e**,**i**,**v**, and the Mixture **5i + 5v** into *N*-Cbz-Dipeptidoylbenzotriazoles **6a–d**, **6c + 6d**

Reactant	Product	Yield (%)[a]	Mp (°C)	$[\alpha]_D^{23}$
Cbz-L-Trp-L-Ala-OH (**5a**)	Cbz-L-Trp-L-Ala-Bt (**6a**)	78	176–177	–27.7
Cbz-L-Trp-L-Trp-OH (**5d**)	Cbz-L-Trp-L-Trp-Bt (**6b**)	76	152–154	+10.1
Cbz-L-Met-L-Ala-OH (**5i**)	Cbz-L-Met-L-Ala-Bt (**6c**)	85	104–105	–19.3
Cbz-L-Met-D-Ala-OH (**5v**)	Cbz-L-Met-D-Ala-Bt (**6d**)	87	135–137	–7.6
Cbz-L-Met-DL-Ala-OH (**5x**)	Cbz-L-Met-DL-Ala-Bt (**6c + 6d**)	84	96–97	–10.6

[a] Isolated yields.



Scheme 5 Preparation of tripeptides **7a–e** and **7c + 7f** from *N*-Cbz-dipeptidoylbenzotriazoles **6a–d**, **6c + 6d** with unprotected amino acids

Table 4 Preparation of Tripeptides **7a–e** and **7c + 7f** from *N*-Cbz-Dipeptidoylbenzotriazoles **6a–d** and **6c + 6d** with Unprotected Amino Acids

Reactant	Amino Acid	Product	Yield (%)[a]
6a	L-Cys	Cbz-L-Trp-L-Ala-L-Cys-OH (**7a**)	86
6b	L-Trp	Cbz-L-Trp-L-Trp-L-Trp-OH (**7b**)	87[b]
6c	L-Ala	Cbz-L-Met-L-Ala-L-Ala-OH (**7c**)	86
6c	L-Ser	Cbz-L-Met-L-Ala-L-Ser-OH (**7d**)	83[c]
6c	L-Trp	Cbz-L-Met-L-Ala-L-Trp-OH (**7e**)	92[d]
6c + 6d	L-Ala	Cbz-L-Met-DL-Ala-L-Ala-OH (**7c + 7f**)[e]	88

[a] Isolated yields.
[b] Diastereomeric ratio 3:1.
[c] Diastereomeric ratio 4:1.
[d] Diastereomeric ratio 4:1.
[e] Diastereomeric mixture.

when the coupling was carried out under similar conditions at room temperature.

NMR and HPLC Evidence for the Chirality of 7a–e

NMR analysis demonstrated no detectable racemization for the preparation of **7a** and **7c**, and **7b**, **7d**, **7e** with some degree of racemization. ^{1}H NMR of **7a** and **7c** showed clear doublets for all –NH and the methyl protons from L-Ala. For compounds **7b**,**d**,**e**, –NH protons appeared as two sets of doublets, indicating diastereomeric mixtures; the ratios of the two sets of doublets for **7b**,**d**,**e** were summarized in Table 4. The different diastereomeric ratios for compounds **7b**,**d**,**e** suggest differences in the extent of racemization; however, no precise reason could be attributed to the observation.

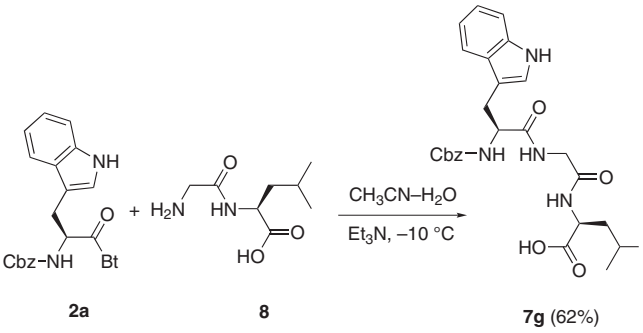
To confirm the extent of racemization, Cbz-L-Met-L-Ala-L-Ala-OH (**7c**) and Cbz-L-Met-DL-Ala-L-Ala-OH (**7c + 7f**) were subjected to HPLC analysis. A single peak was observed for **7c** at 12.4 min, whereas the diastereomeric mixture **7c + 7f** noticeably separated into two peaks at 12.4 and 15.6 min (Table 5).

Table 5 HPLC Analyses

Entry	Compound	t_R (min)[a]
5a	Cbz-L-Trp-L-Ala-OH	11.0
5a + 5a′	Cbz-L-Trp-DL-Ala-OH	11.0, 12.9
5i	Cbz-L-Met-L-Ala-OH	11.7
5i + 5v	Cbz-L-Met-DL-Ala-OH	11.7, 12.3
5q	Cbz-L-CyS-S-L-Ala-OH	11.6
5q + 5q′	Cbz-L-CyS-S-DL-Ala-OH	11.6, 12.2
7c	Cbz-L-Met-L-Ala-L-Ala-OH	12.4
7c + 7f	Cbz-L-Met-DL-Ala-L-Ala-OH	12.4, 15.6

[a] For conditions, see the experimental section.

Previously, preparation of tripeptides was also achieved by the fragment-coupling method using diverse modes of C-terminal activation of the dipeptide-like acyl azides,[11a] carbodiimides,[11b] *p*-nitrophenyl esters,[11c] and alkyl chloroformates.[11d] These methods employed amino acid esters bearing alkyl side-chains, but no such reaction utilizing unprotected amino acids in aqueous solution was reported. Although these known methods produced tripeptides in two steps, involving the peptide coupling and hydrolysis of the esters, the efficiency and the overall yield were moderate in the range of 51–62%. Thus, these methodologies are not particularly attractive due to prolonged reaction times, harsh reaction conditions, and low yields, especially with functionalized amino acids. These associated drawbacks with previous methods strongly suggest that our method offers an added advantage for the synthesis of tripeptides in shorter reaction times, utilizing mild reaction conditions.



Scheme 6 Preparation of tripeptide **7g** from *N*-Cbz-(α-aminoacyl)benzotriazole **2a** and unprotected dipeptide **8**

The second methodology utilizes a coupling reaction between N-protected (α-aminoacyl)benzotriazoles **2a** and an unprotected dipeptide **8**. The desired coupling reaction was achieved using dipeptides having less sterically hindered amino acid fragments like Gly, L-Leu (Scheme 5). However, analogous reactions failed to give the desired tripeptides, probably due to the hydrolysis of *N*-(Cbz- or Fmoc-aminoacyl)benzotriazoles when bulky amino acid

fragments such as L-Trp and L-Met were present at the N-terminus of dipeptides. This hydrolysis could be due to the steric hindrance at the N-terminus of dipeptides, leading to competition between the hydrolysis of *N*-(Cbz- or Fmoc-aminoacyl)benzotriazole and the coupling reaction.

In conclusion, we have prepared *N*-(Cbz- or Fmoc- Trp, Tyr, Gln, Asn, Cys, Met, Ser, Glu, Asp, Pro, His, and Cystine acyl)benzotriazoles **2a–n** and N-protected peptidoylbenzotriazoles **6a–d**, and the diastereomeric mixture **6c + 6d**. These C-activated amino acid derivatives with unprotected side-chain functionalities undergo peptide couplings with unprotected amino acids or amino acid esters to give numerous di- and tripeptides in an average yield of 82% and 75%, respectively. The compounds were obtained without using any chromatography for purification. The original chirality of the resulting products was preserved in >97% ee, evidenced by NMR and HPLC. Both *N*-(Cbz- or Fmoc-aminoacyl)benzotriazoles and N-protected peptidoylbenzotriazoles are easy to handle due to their solidity and stability under ambient atmosphere, and their coupling reactions can be carried out either in a purely organic solvent or in a partly aqueous solution.

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with TMS for ^1H (300 MHz) and ^{13}C (75 MHz) as the internal reference. *N*-Cbz- and Fmoc-amino acids and amino acids, purchased from Fluka and Acros, were used without further purification. HPLC analyses were performed on Beckman system gold programmable solvent module 126, using Chirobiotic T column (4.6 × 250 mm), detection at 254 nm, flow rate of 1.0 mL/min and MeOH–H_2O (1:1) as eluting solvent.

_N_-(Cbz- and Fmoc-aminoacyl)benzotriazoles 2a–f, 2h–j, 2n; General Procedure

$SOCl_2$ (0.37 mL, 5 mmol) was added to a soln of 1*H*-benzotriazole (2.380 g, 20 mmol) in anhyd THF (15 mL) at 20 °C, and the reaction mixture was stirred for 20 min at 40–50 °C. To the reaction mixture at 0 °C, the N-protected amino acid **1** (5 mmol), dissolved in dry THF (5 mL), was added dropwise, and stirred for 2 h at 20 °C. The white precipitate 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 6N HCl soln (3 × 50 mL) or sat. Na_2CO_3 soln (3 × 50 mL), sat. NaCl soln (50 mL), and dried over $MgSO_4$. Removing solvents under reduced pressure gave products **2a–f**,**2h–j**,**2n**, which were recrystallized from $CHCl_3$–hexanes for elemental analysis. In case of **2e**, the product was obtained by washing with Et_2O, followed by recrystallization using MeOH. Compounds **2a–d**,**2h–i** are previously reported by us.[10b] Compounds **2e–f**,**2j**,**2n** are novel and fully characterized by NMR and elemental analysis.

Benzyl _N_-[(1S)-2-(1*H*-1,2,3-benzotriazol-1-yl)-1-(1*H*-imidazol-4-ylmethyl)-2-oxoethyl]carbamate (2g)

$SOCl_2$ (0.37 mL, 5 mmol) was added to a soln of 1*H*-benzotriazole (2.380 g, 20 mmol) in anhyd THF (15 mL) at 20 °C, and the reaction mixture was stirred for 20 min at 20 °C. To the reaction mixture, *N*-Cbz-L-His-OH (**1g**) (1.455 g, 5 mmol) was added, and stirred for 3 h at 20 °C. The white precipitate formed during the reaction was filtered off, and the solvent was completely removed under reduced pressure. The residue obtained was dissolved in min amount of CH_2Cl_2 (10 mL) and was subsequently used for the next reaction.

_N_-(Cbz- and Fmoc-diaminoacyl)benzotriazoles 2k–m; General Procedure

$SOCl_2$ (0.73 mL, 10 mmol) was added to a soln of 1*H*-benzotriazole (4.760 g, 40 mmol) in anhyd THF (30 mL) at 20 °C, and the reaction mixture was stirred for 20 min at 40–50 °C. To the reaction mixture at 0 °C, the N-protected amino acid **1** (5 mmol), dissolved in anhyd THF (5 mL) was added dropwise, and stirred for 2 h at 20 °C. The white precipitate 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 soln was washed with sat. Na_2CO_3 soln (3 × 50 mL), sat. NaCl soln (50 mL), and dried over $MgSO_4$. Removing solvents under reduced pressure gave products **2k–m**, which were recrystallized from $CHCl_3$–hexanes for elemental analysis.

_N_-[(1*S*)-2-Benzotriazolyl-1-(carbamoylmethyl)-2-oxoethyl](phenylmethoxy)carboxamide (*N*-Cbz-Asn-Bt, 2e)

White microcrystals; yield: 1.323 g (72%); mp 142–143 °C; $[\alpha]_D^{23}$ –30.1 (*c* 1.66, DMF).

^1H NMR (DMSO-d_6): δ = 2.77 (dd, *J* = 15.4, 7.6 Hz, 1 H), 2.91 (dd, *J* = 15.4, 5.7 Hz, 1 H), 5.03 (s, 2 H), 5.80–5.82 (m, 1 H), 7.00–7.05 (m, 1 H), 7.33 (s, 5 H), 7.53 (s, 1 H), 7.60–7.66 (m, 1 H), 7.77–7.82 (m, 1 H), 8.20–8.28 (m, 3 H).

^{13}C NMR (DMSO-d_6): δ = 36.8, 51.3, 66.1, 114.1, 120.4, 126.9, 128.0, 128.2, 128.6, 130.9, 131.3, 136.8, 145.5, 156.1, 170.6, 171.4.

Anal. Calcd for $C_{18}H_{17}N_5O_4$: C, 58.85; H, 4.66; N, 19.06. Found: C, 58.60; H, 4.52; N, 18.23.

Benzyl _N_-[(1*S*)-1-(1*H*-1,2,3-benzotriazol-1-ylcarbonyl)-3-(methylsulfanyl)propyl]carbamate (*N*-Cbz-Met-Bt, 2f)

White microcrystals; yield: 1.461 g (76%); mp 105–107 °C; $[\alpha]_D^{23}$ –35.6 (*c* 1.66, DMF).

^1H NMR (DMSO-d_6): δ = 2.05 (s, 3 H), 2.01–2.15 (m, 1 H), 2.22–2.28 (m, 1 H), 2.60–2.72 (m, 2 H), 5.06 (s, 2 H), 5.62–5.69 (m, 1 H), 7.36 (s, 5 H), 7.62–7.67 (m, 1 H), 7.79–7.84 (m, 1 H), 8.22–8.31 (m, 3 H).

^{13}C NMR (DMSO-d_6): δ = 14.2, 29.5, 29.7, 53.4, 65.9, 114.0, 120.2, 126.7, 127.8, 127.9, 128.4, 130.7, 131.1, 136.6, 145.3, 156.3, 171.9.

Anal. Calcd for $C_{19}H_{20}N_4O_3S$: C, 59.36; H, 5.24; N, 14.57. Found: C, 59.48; H, 5.19; N, 14.62.

Benzyl _N_-[(1*S*)-2-(1*H*-1,2,3-benzotriazol-1-yl)-1-(1*H*-imidazol-4-ylmethyl)-2-oxoethyl]carbamate (Cbz-His-Bt, 2g)

Crude yield: 95%; no mp and optical rotation are available. Characterization of 2g was carried out by the preparation of amino amides **4a–c**.

Crude ^1H NMR (DMSO-d_6): δ = 3.35–3.43 (m, 1 H), 3.46–3.50 (m, 1 H), 5.06 (s, 2 H), 5.88 (q, *J* = 8.3 Hz, 1 H), 7.30–7.37 (m, 5 H), 7.54 (s, 1 H), 7.65–7.70 (m, 1 H), 7.82–7.87 (m, 1 H), 8.30 (q, *J* = 8.3 Hz, 2 H), 8.54 (d, *J* = 7.2 Hz, 1 H), 9.13 (s, 1 H).

9*H*-Fluoren-9-ylmethyl-_N_-[(1*S*)-2-(1*H*-1,2,3-benzotriazol-1-yl)-1-(hydroxymethyl)-2-oxoethyl]carbamate (*N*-Fmoc-Ser-Bt, 2j)

White microcrystals; yield: 1.457 g (68%); mp 100–101 °C; $[\alpha]_D^{23}$ –44.8 (*c* 1.66, DMF).

^1H NMR (DMSO-d_6): δ = 4.18–4.23 (m, 1 H), 4.30–4.60 (m, 4 H), 5.73–5.75 (m, 1 H), 7.26–7.30 (m, 2 H), 7.38 (t, *J* = 7.4 Hz, 2 H), 7.60–7.69 (m, 3 H), 7.79 (t, *J* = 7.6 Hz, 1 H), 7.87 (d, *J* = 7.7 Hz, 2 H), 8.06–8.22 (m, 1 H), 8.25–8.29 (m, 1 H), 8.52–8.58 (m, 1 H).

^{13}C NMR (DMSO-d_6): δ = 46.5, 54.2, 60.6, 60.8, 66.0, 113.8, 120.0, 120.2, 125.1, 126.8, 127.0, 127.6, 130.5, 131.2, 140.7, 143.5, 145.3, 155.9, 168.1.

ESI-FTICR-MS: *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{20}N_4O_4$: 451.1377; found: 451.1417.

Benzyl *N*-[(1*S*)-1-({[(2*S*)-2-(benzylcarbonyl)amino-3-(1*H*-1,2,3-benzotriazol-1-yl)-3-oxopropyl]disulfanyl}methyl)-2-(1*H*-1,2,3-benzotriazol-1-yl)-2-oxoethyl]carbamate (*N*-Cbz-Cystine-diBt, 2k)

White microcrystals; yield: 3.056 g (88%); mp 180–182 °C; $[\alpha]_D^{23}$ –109.6 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 3.18 (dd, *J* = 13.8, 9.9 Hz, 2 H), 3.55 (dd, *J* = 13.8, 10.4 Hz, 2 H), 5.06 (s, 2 H), 5.90–5.93 (m, 2 H), 7.28–7.38 (m, 10 H), 7.59–7.64 (m, 2 H), 7.80 (t, *J* = 7.6 Hz, 2 H), 8.12 (d, *J* = 8.2 Hz, 2 H), 8.21 (d, *J* = 8.3 Hz, 2 H), 8.39 (d, *J* = 7.4 Hz, 2 H).

^{13}C NMR (DMSO-d_6): δ = 22.0, 30.8, 36.6, 53.5, 58.7, 66.8, 126.4, 127.4, 128.0, 128.2, 128.3, 129.1, 135.6, 137.3, 150.4, 171.0, 172.7, 173.6.

Anal. Calcd for $C_{34}H_{30}N_8O_6S_2$: C, 57.45; H, 4.25; N, 15.76. Found: C, 57.67; H, 4.26; N, 15.70.

Benzyl *N*-[(1*S*)-3-(1*H*-1,2,3-benzotriazol-1-yl)-1-(1*H*-1,2,3-benzotriazol-1-ylcarbonyl)-3-oxopropyl]carbamate (*N*-Cbz-Asp-diBt, 2l)

White microcrystals; yield: 2.019 g (86%); mp 140–143 °C; $[\alpha]_D^{23}$ +21.3 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 4.19 (dd, *J* = 17.8, 8.3 Hz, 1 H), 4.30 (dd, *J* = 17.8, 4.8 Hz, 1 H), 5.06 (s, 2 H), 6.15 (q, *J* = 7.1 Hz, 1 H), 7.33–7.36 (m, 5 H), 7.60–7.78 (m, 2 H), 7.79 (t, *J* = 7.2 Hz, 1 H), 7.83 (t, *J* = 7.7 Hz, 1 H), 8.21–8.32 (m, 4 H), 8.54 (d, *J* = 6.4 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 36.8, 50.4, 66.0, 113.9, 114.0, 120.1, 120.2, 126.6, 126.8, 127.7, 127.9, 128.3, 130.5, 130.7, 131.0, 131.2, 136.5, 145.4, 145.5, 155.9, 168.9, 170.0.

Anal. Calcd for $C_{24}H_{19}N_7O_4$: C, 61.40; H, 4.08; N, 20.89. Found: C, 61.22; H, 3.94; N, 20.78.

Benzyl *N*-[(1*S*)-1-acetyl-4-(1*H*-1,2,3-benzotriazol-1-yl)-4-oxobutyl]carbamate (*N*-Cbz-l-Glu-diBt, 2m)

White microcrystals; yield: 2.224 g (92%); mp 150–152 °C; $[\alpha]_D^{23}$ –4.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.40–2.47 (m, 1 H), 2.55–2.64 (m, 1 H), 3.72 (t, *J* = 6.8 Hz, 2 H), 5.06 (s, 2 H), 5.74 (q, *J* = 7.6 Hz, 1 H), 7.32–7.36 (s, 5 H), 7.57–7.61 (m, 2 H), 7.75 (t, *J* = 7.8 Hz, 1 H), 7.81 (t, *J* = 7.8 Hz, 1 H), 8.15 (d, *J* = 8.2 Hz, 1 H), 8.23–8.26 (m, 3 H), 8.44 (d, *J* = 6.8 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 24.9, 31.3, 53.5, 65.9, 113.9, 114.0, 120.0, 126.3, 126.7, 127.8, 127.9, 128.3, 130.5, 130.7, 130.8, 131.1, 136.6, 145.3, 145.4, 156.3, 171.4.

Anal. Calcd for $C_{25}H_{21}N_7O_4$: C, 62.11; H, 4.38; N, 20.28. Found: C, 62.23; H, 4.29; N, 20.28.

Benzyl (2*S*)-2-(1*H*-1,2,3-benzotriazol-1-ylcarbonyl)tetrahydro-1*H*-pyrrole-1-carboxylate (*N*-Cbz-Pro-Bt, 2n)

Colorless oil; yield: 1.664 g (95%); $[\alpha]_D^{23}$ –139.6 (*c* 1.83, DMF).

^{1}H NMR (DMSO-d_6) (two rotameric forms): δ = 1.90–2.10 (m, 2 H), 2.20–2.84 (m, 1 H), 2.50–2.54 (m, 1 H), 3.52–3.67 (m, 2 H), 4.89–5.04 (m, 1 H), 5.14 (s, 1 H), 5.70–5.82 (m, 1 H), 6.95–6.98 (m, 2 H), 7.41–7.42 (m, 3 H), 7.67 (t, *J* = 7.7 Hz, 1 H), 7.84 (t, *J* = 7.6 Hz, 1 H), 8.21–8.33 (m, 2 H).

^{13}C NMR (DMSO-d_6): δ = 23.3, 24.1, 29.7, 30.7, 46.6, 47.2, 58.9, 59.6, 66.3, 134.0, 120.2, 126.8, 126.9, 127.5, 127.6, 127.8, 127.9, 128.4, 130.6, 131.1, 136.1, 136.7, 145.3, 153.2, 154.2, 171.0.

Anal. Calcd for $C_{19}H_{18}N_4O_3$: C, 65.13; H, 5.18; N, 15.99. Found: C, 64.94; H, 5.23; N, 15.79.

Amino Amides 4a–c; General Procedure

Amine **3** was added to an ice-cold soln of crude **2g** (1 mmol) in CH_2Cl_2 (10 mL) dropwise, until the reaction mixture turned alkaline (pH 8–9). The reaction mixture was then stirred at 20 °C until the starting material was completely consumed as observed on ^{1}H NMR. It was washed with sat. Na_2CO_3 soln (3 × 20 mL) and sat. NaCl soln (20 mL), and then dried over $MgSO_4$. Evaporation of the solvent gave the desired products **4a**–**c**, which were further recrystallized from $CHCl_3$–hexane, unless specified otherwise.

Benzyl *N*-((1*S*)-1-(1*H*-imidazol-4-ylmethyl)-2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl)carbamate (4a)

White microcrystals; yield: 0.322 g (82%); mp 183–185 °C; $[\alpha]_D^{23}$ –26.4 (*c* 1.75, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.33 (d, *J* = 6.9 Hz, 3 H), 2.69–2.86 (m, 2 H), 4.31 (dd, *J* = 13.4, 7.7 Hz, 1 H), 4.83–4.92 (m, 1 H), 5.00 (s, 2 H), 6.74 (s, 1 H), 7.17–7.21 (m, 4 H), 7.25–7.37 (m, 8 H), 7.54 (s, 1 H), 8.35 (d, *J* = 7.7 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 22.4, 30.8, 47.7, 54.7, 65.3, 113.2, 125.7, 126.4, 127.6, 127.7, 128.1, 128.3, 134.6, 136.5, 137.1, 144.6, 155.7, 170.5.

Anal. Calcd for $C_{22}H_{24}N_4O_3$: C, 67.33; H, 6.16; N, 14.28. Found: C, 67.64; H, 6.38; N, 14.33.

Benzyl *N*-((1*S*)-1-(1*H*-imidazol-4-ylmethyl)-2-oxo-2-{[(1*R*)-1-phenylethyl]amino}ethyl)carbamate (4b)

White microcrystals; yield: 0.377 g (96%); mp 139–140 °C; $[\alpha]_D^{23}$ +16.2 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.28 (d, *J* = 7.1 Hz, 3 H), 2.80–2.83 (m, 2 H), 4.25–4.32 (m, 1 H), 4.84–4.92 (m, 1 H), 4.99 (s, 2 H), 6.83 (s, 1 H), 7.17–7.43 (m, 11 H), 7.56 (s, 1 H), 8.27 (d, *J* = 8.2 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 22.3, 30.8, 47.7, 54.6, 65.3, 113.3, 125.9, 126.5, 127.6, 127.7, 128.1, 128.3, 134.7, 136.5, 137.6, 144.4, 155.7, 170.5.

Anal. Calcd for $C_{22}H_{24}N_4O_3$: C, 67.33; H, 6.16; N, 14.28. Found: C, 67.69; H, 6.45; N, 14.32.

Benzyl *N*-[(1*S*)-1-(1*H*-imidazol-4-ylmethyl)-2-oxo-2-(1-pyrrolidinyl)ethyl]carbamate (4c)

White microcrystals; yield: 0.329 g (96%); mp 133–134 °C; $[\alpha]_D^{23}$ +24.8 (*c* 1.83, DMF).

^{1}H NMR ($CDCl_3$): δ = 1.80–1.87 (m, 4 H), 2.93–3.08 (m, 2 H), 3.23–3.61 (m, 4 H), 4.73 (q, *J* = 6.9 Hz, 1 H), 5.03 (d, *J* = 12.4 Hz, 1 H, A part of AB system), 5.07 (d, *J* = 12.4 Hz, 1 H, B part of AB system), 6.09 (d, *J* = 8.2 Hz, 1 H), 6.80 (s, 1 H), 7.30–7.34 (s, 5 H), 7.49 (s, 1 H).

^{13}C NMR ($CDCl_3$): δ = 24.2, 26.1, 30.1, 46.3, 46.8, 52.9, 67.1, 77.4, 128.2, 128.3, 128.7, 135.3 (2C), 136.5, 156.2, 170.3.

Anal. Calcd for $C_{18}H_{22}N_4O_3$: C, 63.14; H, 6.48; N, 16.36. Found: C, 63.10; H, 6.54; N, 16.32.

LL- and LD-Dipeptides 5a–v and Diastereomeric Mixtures 5a + 5a′, 5i + 5v, 5q + 5q′; General Procedure

N-Protected (aminoacyl)benzotriazoles **2a**–**c**,**f**,**h**–**m** (0.5 mmol) were added at 20 °C to a soln of α-amino acid (0.5 mmol) in MeCN–H_2O (7 mL : 3 mL) in the presence of Et_3N (0.6 mmol). The reaction mixture was then stirred at 20 °C until the starting material was completely consumed, as observed on TLC using hexane–EtOAc (2:1) as the eluent. Aq 6 N HCl soln (1 mL) was then 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 6 N HCl soln (3 × 5 mL) and sat. NaCl soln (10 mL) and dried over $MgSO_4$. Evaporation of the solvent gave the de-

sired product, which was further recrystallized from $CHCl_3$–hexane, unless specified otherwise.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-3-yl)propanoyl]amino}propanoic Acid (*N*-Cbz-L-Trp-L-Ala-OH, 5a)

White microcrystals; yield: 0.174 g (85%); mp 140–144 °C; $[\alpha]_D^{23}$ –23.9 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.32 (d, *J* = 7.2 Hz, 3 H), 2.90 (dd, *J* = 14.6, 10.4 Hz, 1 H), 3.15 (dd, *J* = 14.6, 3.2 Hz, 1 H), 4.22–4.38 (m, 2 H), 4.88–4.98 (m, 2 H), 6.96–7.04 (m, 1 H), 7.06–7.09 (m, 1 H), 7.18 (s, 1 H), 7.22–7.38 (m, 7 H), 7.70 (d, *J* = 7.7 Hz, 1 H), 8.38 (d, *J* = 7.1 Hz, 1 H), 10.82 (s, 1 H), 12.60 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.2, 27.8, 47.6, 55.2, 65.2, 110.2, 111.3, 118.2, 119.6, 120.8, 124.0, 127.3, 127.5, 127.7, 128.3, 136.1, 137.0, 155.8, 171.8, 174.1.

Anal. Calcd for $C_{22}H_{23}N_3O_5$: C, 64.54; H, 5.66; N, 10.26. Found: C, 64.44; H, 5.66; N, 10.03.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-3-yl)propanoyl]amino}-3-sulfanylpropanoic Acid (*N*-Cbz-L-Trp-L-Cys-OH, 5b)

White microcrystals; yield: 0.190 g (86%); mp 140–144 °C; $[\alpha]_D^{23}$ –28.5 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.41 (t, *J* = 8.2 Hz, 1 H), 2.76–2.88 (m, 2 H), 2.93 (dd, *J* = 14.0, 10.2 Hz, 1 H), 3.14 (dd, *J* = 14.0, 2.7 Hz, 1 H), 4.36–4.40 (m, 1 H), 4.47 (q, *J* = 6.6 Hz, 1 H), 4.94 (s, 2 H), 6.98 (t, *J* = 7.2 Hz, 1 H), 7.07 (t, *J* = 7.4 Hz, 1 H), 7.17–7.35 (m, 7 H), 7.45 (d, *J* = 8.2 Hz, 1 H), 7.66 (d, *J* = 7.4 Hz, 1 H), 8.33 (d, *J* = 7.7 Hz, 1 H), 10.82 (s, 1 H), 12.96 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 25.5, 27.7, 54.4, 55.4, 65.3, 110.1, 111.3, 118.2, 118.5, 120.9, 124.0, 127.3, 127.5, 127.7, 128.3, 136.1, 134.0, 155.9, 171.5, 172.1.

Anal. Calcd for $C_{22}H_{23}N_3O_5S$: C, 59.85; H, 5.25; N, 9.52. Found: C, 60.03; H, 5.28; N, 9.37.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-3-yl)propanoyl]amino}-3-hydroxypropanoic Acid (*N*-Cbz-L-Trp-L-Ser-OH, 5c)

White microcrystals; yield: 0.183 g (86%); mp 133–138 °C; $[\alpha]_D^{23}$ –11.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.93 (dd, *J* = 14.3, 10.3 Hz, 1 H), 3.16 (dd, *J* = 14.3, 3.3 Hz, 1 H), 3.68 (dd, *J* = 10.9, 3.8 Hz, 1 H), 3.77 (dd, *J* = 10.9, 4.9 Hz, 1 H), 4.32–4.41 (m, 2 H), 4.93 (s, 2 H), 6.96 (t, *J* = 7.4 Hz, 1 H), 7.06 (t, *J* = 7.4 Hz, 1 H), 7.17–7.40 (m, 8 H), 7.67 (d, *J* = 8.0 Hz, 1 H), 8.22 (d, *J* = 7.7 Hz, 1 H), 10.81 (s, 1 H), 12.60 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 27.8, 54.8, 55.4, 61.4, 65.2, 110.2, 112.3, 118.2, 118.6, 120.8, 123.9, 127.4, 127.7, 128.3, 136.1, 137.0, 135.8, 155.8, 172.0, 172.1.

Anal. Calcd for $C_{22}H_{23}N_3O_6$: C, 62.11; H, 5.45; N, 9.88. Found: C, 62.01; H, 5.43; N, 9.71.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-3-yl)propanoyl]amino}-3-(1*H*-indol-3-yl)propanoic Acid (*N*-Cbz-L-Trp-L-Trp-OH, 5d)

White microcrystals; yield: 0.225 g (85%); mp 188–190 °C; $[\alpha]_D^{23}$ –15.6 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.89 (dd, *J* = 14.2, 10.6 Hz, 1 H), 3.07–3.15 (m, 2 H), 3.22 (dd, *J* = 14.2, 4.8 Hz, 1 H), 4.33–4.35 (m, 1 H), 4.51–4.55 (m, 1 H), 4.93 (s, 2 H), 6.94–7.00 (m, 2 H), 7.01–7.08 (m, 2 H), 7.13 (s, 2 H), 7.19–7.38 (m, 8 H), 7.56 (d, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 7.7 Hz, 1 H), 8.28 (d, *J* = 7.4 Hz, 1 H), 10.80 (s, 1 H), 10.88 (s, 1 H), 12.7 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 27.1, 27.8, 53.0, 55.4, 65.3, 109.7, 110.2, 111.3, 11.4, 118.2, 118.4, 118.6, 120.9, 121.0, 123.7, 123.8, 127.3, 127.5, 127.7, 128.3, 136.1, 137.0, 155.8, 172.0, 173.3.

Anal. Calcd for $C_{30}H_{28}N_4O_5$: C, 68.69; H, 5.38; N, 10.68. Found: C, 67.90; H, 5.35; N, 10.65.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(4-hydroxyphenyl)propanoyl]amino}-3-(1*H*-indol-3-yl)propanoic Acid (*N*-L-Cbz-Tyr-L-Trp-OH, 5e)

White microcrystals; yield: 0.226 g (90%); mp 114–119 °C; $[\alpha]_D^{23}$ +2.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.59 (dd, *J* = 13.4, 11.0 Hz, 1 H), 2.88 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.09 (dd, *J* = 14.6, 8.1 Hz, 1 H), 3.21 (dd, *J* = 14.6, 5.2 Hz, 1 H), 4.18–4.25 (m, 1 H), 4.51 (q, *J* = 7.4 Hz, 1 H), 4.86 (d, *J* = 12.9 Hz, 1 H, A part of AB system), 4.96 (d, *J* = 12.9 Hz, 1 H, B part of AB system), 6.63 (d, *J* = 8.2 Hz, 2 H), 6.98 (t, *J* = 7.3 Hz, 2 H), 7.04–7.11 (m, 3 H), 7.17–7.18 (m, 3 H), 7.20–7.40 (m, 5 H), 7.55 (d, *J* = 7.7 Hz, 1 H), 8.24 (d, *J* = 7.4 Hz, 1 H), 9.18 (s, 1 H), 10.89 (br s, 1 H), 12.61 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 27.0, 36.7, 52.9, 56.3, 65.1, 109.6, 111.3, 114.8, 118.2, 118.4, 121.0, 123.7, 127.2, 127.4, 127.6, 128.1, 128.3, 130.1, 136.1, 137.0, 155.8, 171.7, 173.2.

ESI-FTICR-MS: *m*/*z* [M + Na]$^+$ calcd for $C_{28}H_{27}N_3O_6$: 524.1792; found: 524.1801.

(2*S*)-2-{[(2*S*)-5-Amino-2{[(benzyloxy)carbonyl]amino}-5-oxopentanoyl]amino}propanoic Acid (*N*-Cbz-L-Gln-L-Ala-OH, 5f)

White microcrystals; yield: 0.126 g (72%); mp 211–213 °C; $[\alpha]_D^{23}$ –2.1 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.28 (d, *J* = 7.1 Hz, 3 H), 1.62–1.75 (m, 1 H), 1.89–1.99 (m, 1 H), 2.05–2.20 (m, 2 H), 4.00 (q, *J* = 8.5 Hz, 1 H), 4.19 (quin, *J* = 6.8 Hz, 1 H), 5.01 (s, 2 H), 6.77 (s, 1 H), 7.25–7.42 (m, 7 H), 8.20 (d, *J* = 7.2 Hz, 1 H), 12.60 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.2, 27.9, 31.5, 47.4, 54.1, 65.4, 127.7, 127.8, 128.4, 137.0, 155.9, 171.4, 173.8, 174.1.

Anal. Calcd for $C_{16}H_{21}N_3O_6$: C, 54.69; H, 6.02; N, 11.96. Found: C, 54.44; H, 6.21; N, 11.84.

(2*S*)-5-Amino-2-{[(2*S*)-5-amino-2-{[(benzyloxy)carbonyl]amino}-5-oxopentanoyl]amino}-5-oxopentanoic Acid (*N*-Cbz-L-Gln-L-Gln-OH, 5g)

White microcrystals; yield: 0.094 g (47%); mp 132–134 °C; $[\alpha]_D^{23}$ +26.7 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.72–2.04 (m, 4 H), 2.30–2.34 (m, 4 H), 3.37 (br s, 2 H), 4.07 (q, *J* = 8.3 Hz, 1 H), 4.19–4.27 (m, 1 H), 5.05 (s, 2 H), 7.36–7.39 (m, 5 H), 7.48 (d, *J* = 8.0 Hz, 1 H), 8.20 (d, *J* = 7.4 Hz, 1 H), 12.32 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 26.2, 27.3, 30.0, 30.1, 51.1, 53.7, 65.4, 127.7, 127.8, 128.3, 137.0, 155.9, 171.6, 173.1, 173.8, 174.0.

Anal. Calcd for $C_{18}H_{24}N_4O_7$: C, 52.94; H, 5.92. Found: C, 52.70; H, 5.65.

(2*S*)-2-{[(2*S*)-5-Amino-2-{[(benzyloxy)carbonyl]amino}-5-oxopentanoyl]amino}-3-methylbutanoic Acid (*N*-Cbz-L-Gln-L-Val-OH, 5h)

White microcrystals (from H_2O); yield: 0.125 g (66%); mp 186–188 °C; $[\alpha]_D^{23}$ +5.5 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.89 (d, *J* = 6.9 Hz, 6 H), 1.66–1.76 (m, 1 H), 1.85–2.00 (m, 1 H), 2.01–2.23 (m, 3 H), 4.09 (q, *J* = 8.7 Hz, 1 H), 4.17 (dd, *J* = 8.2, 5.8 Hz, 1 H), 5.02 (s, 2 H), 6.79 (s, 1 H), 7.28–

7.39 (m, 6 H), 7.46 (d, *J* = 8.2 Hz, 1 H), 7.94 (d, *J* = 8.5 Hz, 1 H), 12.66 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.8, 19.1, 27.9, 30.0, 31.6, 54.2, 57.0, 65.4, 127.7, 127.8, 128.4, 137.0, 155.9, 172.0, 173.0, 173.9.

Anal. Calcd. for $C_{18}H_{25}N_3O_6$: C, 56.98; H, 6.64; N, 11.07. Found: C, 57.24; H, 6.80; N, 10.96.

(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]propanoic Acid (Cbz-L-Met-L-Ala-OH, 5i)

White microcrystals; yield: 0.168 g (95%); mp 137–138 °C; $[\alpha]_D^{23}$ –9.0 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.28 (d, *J* = 7.1 Hz, 3 H), 1.76–1.90 (m, 2 H), 2.03 (s, 3 H), 2.46–2.50 (m, 2 H), 4.11 (d, *J* = 5.2 Hz, 1 H), 4.19 (t, *J* = 7.2 Hz, 1 H), 5.02 (s, 2 H), 7.35 (s, 5 H), 7.48 (d, *J* = 8.2 Hz, 1 H), 8.24 (d, *J* = 6.9 Hz, 1 H), 12.55 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 17.0, 29.5, 31.8, 47.5, 53.6, 65.4, 127.7, 127.8, 128.3, 137.0, 155.9, 171.3, 174.0.

Anal. Calcd for $C_{16}H_{22}N_2O_5S$: C, 54.22; H, 6.26; N, 7.90. Found: C, 54.53; H, 6.34; N, 8.30.

(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]-3-methylsulfanylbutanoic Acid (Cbz-L-Met-L-Met-OH, 5j)

White microcrystals; yield: 0.197 g (95%); mp 120–122 °C; $[\alpha]_D^{23}$ –8.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.76–1.86 (m, 2 H), 1.89–2.00 (m, 2 H), 2.03 (s, 6 H), 2.47–2.51 (m, 4 H), 4.07–4.14 (m, 1 H), 4.28–4.35 (m, 1 H), 5.01 (s, 2 H) 7.33–7.35 (m, 5 H), 7.80 (d, *J* = 8.8 Hz, 1 H), 8.21 (d, *J* = 7.8 Hz, 1 H), 12.68 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 29.5, 29.6, 30.6, 31.7, 50.9, 53.7, 65.4, 127.7, 127.8, 128.3, 137.0, 155.9, 171.6, 173.2.

Anal. Calcd for $C_{18}H_{26}N_2O_5S_2$: C, 52.19; H, 6.32; N, 6.76. Found: C, 52.09; H, 6.38; N, 6.72.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-4-(methylsulfanyl)butanoyl]amino}-3-(1*H*-indol-3-yl)propanoic Acid (Cbz-L-Met-L-Trp-OH, 5k)

White microcrystals; yield: 0.193 g (82%); mp 172–173 °C; $[\alpha]_D^{23}$ +8.2 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.78–1.88 (m, 2 H), 2.00 (s, 3 H), 2.41–2.47 (m, 2 H), 3.07 (dd, *J* = 14.5, 8.0 Hz, 1 H), 3.18 (dd, *J* = 14.5, 5.1 Hz, 1 H), 4.13–4.14 (m, 1 H), 4.46–4.48 (m, 1 H), 5.01 (d, *J* = 12.5 Hz, 1 H, A part of AB system), 5.03 (d, *J* = 12.5 Hz, 1 H, B part of AB system) 6.97 (t, *J* = 6.9 Hz, 1 H), 7.06 (t, *J* = 7.7 Hz, 1 H), 7.31–7.34 (m, 6 H), 7.46 (br s, 1 H), 7.51 (t, *J* = 7.7 Hz, 1 H), 8.13 (d, *J* = 7.4 Hz, 1 H), 10.87 (s, 1 H), 12.67 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 26.9, 29.5, 31.8, 52.9, 53.7, 65.4, 109.6, 111.3, 118.1, 118.4, 120.9, 123.6, 127.2, 127.7, 127.8, 128.3, 136.0, 137.0, 155.9, 171.4, 173.2.

Anal. Calcd for $C_{24}H_{27}N_3O_5S$: C, 61.39; H, 5.80; N, 8.95. Found: C, 61.42; H, 5.96; N, 8.78.

(2*S*)-2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-4-(methylsulfanyl)butanoyl]amino}pentanedioic Acid (Cbz-L-Met-L-Glu-OH, 5l)

White microcrystals; yield: 0.192 g (93%); mp 119–120 °C; $[\alpha]_D^{23}$ –8.2 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.74–1.87 (m, 4 H), 2.03 (s, 3 H), 2.27–2.32 (m, 2 H), 2.45–2.48 (m, 2 H), 4.08–4.16 (m, 1 H), 4.18–4.25 (m, 1 H), 5.02 (s, 2 H), 7.33–7.37 (m, 5 H), 7.49 (d, *J* = 8.0 Hz, 1 H), 8.19 (d, *J* = 7.7 Hz, 1 H), 12.44 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 14.8, 26.3, 29.7, 30.1, 31.9, 51.3, 53.8, 65.6, 127.9, 128.0, 128.5, 137.1, 156.1, 171.8, 173.3, 173.9.

Anal. Calcd for $C_{18}H_{24}N_2O_7S$: C, 52.42; H, 5.86; N, 6.79. Found: C, 52.45; H, 5.84; N, 6.70.

(*S*)-2-[(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-(1*H*-indol-3-yl)propionylamino]-3-hydroxypropionic Acid (Fmoc-L-Trp-L-Ser-OH, 5m)

White microcrystals; yield: 0.223 g (87%); mp 114–115 °C (decomposed); $[\alpha]_D^{23}$ –13.7 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.95 (dd, *J* = 14.6, 10.2 Hz, 1 H), 3.15 (dd, *J* = 14.6, 3.4 Hz, 1 H), 3.66 (dd, *J* = 10.7, 4.1 Hz, 1 H), 3.76 (dd, *J* = 10.7, 5.0 Hz, 1 H), 4.13 (s, 3 H), 4.30–4.41 (m, 2 H), 5.01 (br s, 1 H), 6.97 (t, *J* = 7.5 Hz, 1 H), 7.05 (t, *J* = 6.9 Hz, 1 H), 7.19–7.42 (m, 6 H), 7.54 (d, *J* = 8.8 Hz, 1 H), 7.62 (t, *J* = 8.2 Hz, 2 H), 7.70 (d, *J* = 7.7 Hz, 1 H), 7.87 (d, *J* = 7.4 Hz, 2 H), 8.24 (d, *J* = 7.7 Hz, 1 H), 10.81 (s, 1 H), 12.66 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 46.5, 54.7, 55.4, 61.3, 65.6, 110.3, 111.2, 118.1, 118.6, 120.0, 120.8, 123.8, 125.3, 127.1, 127.3, 127.6, 136.1, 140.6, 143.8, 155.7, 171.9, 172.0.

Anal. Calcd for $C_{29}H_{27}N_3O_6$: C, 67.82; H, 5.30; N, 8.18. Found: C, 67.42; H, 5.51; N, 8.39.

(2*S*)-2-{[(2*S*)-2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-(methylsulfanyl)butanoyl]amino}-3-hydroxypropanoic Acid (Fmoc-L-Met-L-Ser-OH, 5n)

White microcrystals; yield: 0.202 g (88%); mp 157–159 °C; $[\alpha]_D^{23}$ –2.3 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.78–1.99 (m, 2 H), 2.04 (s, 3 H), 2.49–2.51 (m, 2 H), 3.66 (dd, *J* = 11.0, 4.3 Hz, 1 H), 3.77 (dd, *J* = 11.0, 5.1 Hz, 1 H), 4.10–4.29 (m, 5 H), 5.01 (s, 1 H, OH), 7.30–7.35 (m, 2 H), 7.39–7.44 (m, 2 H), 7.59 (d, *J* = 8.3 Hz, 1 H), 7.70–7.75 (m, 2 H), 7.89 (d, *J* = 7.5 Hz, 2 H), 8.08 (d, *J* = 7.7 Hz, 1 H), 12.64 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 29.6, 31.9, 46.6, 53.7, 54.6, 61.2, 65.6, 120.1, 125.3, 127.1, 127.7, 140.7, 143.7, 143.9, 155.9, 171.6, 171.9.

Anal. Calcd for $C_{23}H_{26}N_2O_6S$: C, 60.25; H, 5.72; N, 6.11. Found: C, 60.67; H, 5.67; N, 5.75.

(2*S*)-2-[(2*S*)-2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-(methylsulfanyl)butanoyl]aminopentanedioic Acid (Fmoc-L-Met-L-Glu-OH, 5o)

White microcrystals; yield: 0.235 g (94%); mp 148–150 °C; $[\alpha]_D^{23}$ –18.1 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.75–2.01 (m, 4 H), 2.04 (s, 3 H), 2.27–2.29 (m, 2 H), 2.45–2.48 (m, 2 H), 4.09–4.29 (m, 5 H), 7.32 (t, *J* = 7.5 Hz, 2 H), 7.42 (t, *J* = 7.3 Hz, 2 H), 7.58 (d, *J* = 8.2 Hz, 1 H), 7.71–7.75 (m, 2 H), 7.89 (d, *J* = 7.7 Hz, 2 H), 8.19 (d, *J* = 7.7 Hz, 1 H), 12.42 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 26.2, 29.5, 30.0, 31.8, 46.7, 51.2, 53.6, 65.6, 120.1, 125.3, 127.1, 127.6, 140.7, 143.7, 144.0, 155.9, 171.6, 173.2, 173.8.

Anal. Calcd for $C_{25}H_{28}N_2O_7S$: C, 60.10; H, 5.64; N, 5.60. Found: C, 60.07; H, 5.64; N, 6.06.

(2S)-2-[((2S)-2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-3-hydroxypropanoyl)amino]propanoic Acid (Fmoc-L-Ser-L-Ala-OH, 5p)

White microcrystals; yield: 0.143 g (72%); mp 131–132 °C; $[\alpha]_D^{23}$ –2.3 (*c* 1.5, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.31 (d, *J* = 7.2 Hz, 3 H), 3.55 (dd, *J* = 10.5, 7.1 Hz, 1 H), 3.65 (dd, *J* = 10.5, 5.4 Hz, 1 H), 4.06–4.33

(m, 5 H), 4.44 (br s, 1 H), 7.32–7.40 (m, 2 H), 7.43–7.48 (m, 2 H), 7.45–7.90 (m, 2 H), 7.93 (d, *J* = 7.4 Hz, 2 H), 8.20 (d, *J* = 7.1 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.3, 46.6, 47.6, 57.1, 61.8, 65.7, 120.1, 125.4, 127.1, 127.7, 140.7, 143.8, 143.9, 160.0, 170.0, 174.1.

(*S*)-2-{(*S*)-2-Benzyloxycarbonylamino-3-[(*S*)-2-benzyloxycarbonylamino-2-((*S*)-1-carboxyethylcarbamoyl)ethyldisulfanyl]propionylamino}propionic Acid (Cbz-L-Cystine-L-di-Ala-OH, 5q)

White microcrystals; yield: 0.312 g (96%); mp 158–160 °C; $[\alpha]_D^{23}$ –102.2 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.29 (d, *J* = 7.4 Hz, 6 H), 2.86 (dd, *J* = 13.0, 10.9 Hz, 2 H), 3.13 (dd, *J* = 13.0, 3.3 Hz, 2 H), 4.21 (quin, *J* = 7.2 Hz, 2 H), 4.31–4.37 (m, 2 H), 5.04 (s, 4 H), 7.33–7.35 (m, 10 H), 7.59 (d, *J* = 8.6 Hz, 2 H), 8.27 (d, *J* = 7.1 Hz, 2 H), 12.58 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 17.0, 40.6, 47.7, 53.7, 65.6, 127.7, 127.8, 128.3, 136.9, 156.0, 169.9, 173.8.

Anal. Calcd for $C_{28}H_{34}N_4O_{10}S_2$: C, 51.68; H, 5.27; N, 8.61. Found: C, 51.87; H, 5.29; N, 8.26.

(*S*)-2-{(*S*)-2-Benzyloxycarbonylamino-3-[(*S*)-2-benzyloxycarbonylamino-2-((*S*)-1-carboxy-2-methylpropylcarbamoyl)ethyldisulfanyl]propionylamino}-3-methylbutyric Acid (Cbz-L-Cystine-L-di-Val-OH, 5r)

White microcrystals; yield: 0.322 g (91%); mp 98–100 °C; $[\alpha]_D^{23}$ –80.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.86 (d, *J* = 6.9 Hz, 12 H), 2.03–2.09 (m, 2 H), 2.85–2.93 (m, 2 H), 3.11–3.16 (m, 2 H), 4.16 (dd, *J* = 8.0, 5.8 Hz, 2 H), 4.34–4.41 (m, 2 H), 5.01 (d, *J* = 12.5 Hz, 2 H, A part of AB system), 5.04 (d, *J* = 12.5 Hz, 2 H, A part of AB system), 7.28–7.38 (m, 10 H), 7.66 (d, *J* = 8.8 Hz, 2 H), 7.97 (d, *J* = 8.5 Hz, 2 H), 12.71 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 17.9, 19.0, 30.0, 40.6, 53.9, 57.2, 65.8, 127.7, 127.8, 128.3, 136.9, 156.0, 170.2, 172.7.

Anal. Calcd for $C_{32}H_{42}N_4O_{10}S_2$: C, 54.38; H, 5.99; N, 7.93. Found: C, 54.45; H, 6.23; N, 7.63.

(3*S*)-3-{[(Benzyloxy)carbonyl]amino}-4-{[(1*S*)-1-carboxyethyl]amino}-4-oxobutanoic Acid (Cbz-L-Asp-L-Ala-OH, 5s)

White microcrystals; yield: 0.127 g (75%); mp 112–113 °C; $[\alpha]_D^{23}$ –57.8 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.37 (d, *J* = 7.2 Hz, 3 H), 2.60 (dd, *J* = 17.0, 5.8 Hz, 1 H), 3.05 (dd, *J* = 17.0, 9.4 Hz, 1 H), 4.45–4.50 (m, 1 H), 4.68 (q, *J* = 6.9 Hz, 1 H), 5.04 (s, 2 H), 7.30–7.38 (m, 5 H), 8.00 (d, *J* = 8.2 Hz, 1 H), 12.83 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.1, 34.7, 47.3, 49.3, 65.8, 127.8, 127.9, 128.3, 136.6, 155.7, 170.3, 173.7, 175.4.

ESI-FTICR-MS: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{18}N_2O_7$: 339.1187; found: 339.1192.

(4*S*)-4-{[(Benzyloxy)carbonyl]amino}-5-{[(1*S*)-1-carboxy-2-phenylethyl]amino}-5-oxopentanoic Acid (Cbz-L-Glu-L-Phe-OH, 5t)

White microcrystals; yield: 0.186 g (88%); mp 168–170 °C; $[\alpha]_D^{23}$ +15.2 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.79–1.84 (m, 1 H), 2.17–2.31 (m, 1 H), 2.36–2.45 (m, 2 H), 2.90 (dd, *J* = 13.7, 9.8 Hz, 1 H), 3.03 (dd, *J* = 13.7, 5.2 Hz, 1 H), 4.42–4.50 (m, 1 H), 4.66 (dd, *J* = 11.0, 2.1 Hz, 1 H), 5.02 (d, *J* = 12.9 Hz, 1 H, A part of AB system), 5.12 (d, *J* = 12.9 Hz, 1 H, B part of AB system), 7.22–7.33 (m, 10 H), 7.32 (s, 1 H), 8.59 (d, *J* = 7.6 Hz, 1 H), 12.78 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 22.0, 30.8, 36.6, 53.5, 58.7, 66.8, 126.4, 127.4, 127.9, 128.2, 128.3, 129.1, 135.6, 137.3, 150.4, 170.9, 172.7, 173.6.

(2*S*)-2-({(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-5-[(1-carboxy-2-methylpropyl)amino]-5-oxopentanoyl}amino)-3-methylbutanoic Acid (Cbz-L-Glu-L-di-Val-OH, 5u)

White microcrystals; yield: 0.170 g (71%); mp 87–88 °C; $[\alpha]_D^{23}$ –11.1 (*c* 2.08, DMF).

^{1}H NMR ($CDCl_3$): δ = 0.86 (d, *J* = 6.2 Hz, 6 H), 0.88 (d, *J* = 6.2 Hz, 6 H), 1.27 (s, 1 H), 2.17–2.23 (m, 4 H), 2.44 (dd, *J* = 17.5, 7.9 Hz, 1 H), 2.74 (dd, *J* = 17.4, 7.4 Hz, 1 H), 4.53 (dd, *J* = 8.3, 4.5 Hz, 1 H), 4.65 (d, *J* = 7.4 Hz, 1 H), 5.22 (d, *J* = 12.6 Hz, 1 H, A part of AB system), 5.26 (d, *J* = 12.6 Hz, 1 H, A part of AB system), 6.80 (d, *J* = 8.6 Hz, 2 H), 7.30–7.35 (m, 7 H).

^{13}C NMR ($CDCl_3$): δ = 14.3, 17.7, 19.1, 22.5, 22.9, 31.1, 31.7, 31.8, 57.5, 60.1, 68.8, 128.3, 128.7, 128.8, 135.0, 151.7, 171.2, 174.3, 174.8 (2).

ESI-FTICR-MS: *m*/*z* [M + H]$^+$ calcd for $C_{23}H_{33}N_3O_8$: 480.2340; found: 480.2360.

(*R*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]propanoic Acid (Cbz-L-Met-D-Ala-OH, 5v)

White microcrystals; yield: 0.168 g (95%); mp 157–158 °C; $[\alpha]_D^{23}$ –33.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.26 (d, *J* = 7.4 Hz, 3 H), 1.71–1.96 (m, 2 H), 2.02 (s, 3 H), 2.41–2.51 (m, 2 H), 4.09–4.15 (m, 1 H), 4.20 (quin, *J* = 7.3 Hz, 1 H), 5.07 (s, 2 H), 7.06–7.36 (m, 5 H), 7.45 (d, *J* = 8.5 Hz, 1 H), 8.17 (d, *J* = 7.4 Hz, 1 H), 12.57 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 17.4, 29.6, 31.2, 47.5, 53.7, 65.4, 127.7, 127.8, 128.3, 137.0, 155.9, 171.1, 173.9.

Anal. Calcd for $C_{16}H_{22}N_2O_5S$: C, 54.22; H, 6.26; N, 7.90. Found: C, 54.31; H, 6.42; N, 7.79.

2-{[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-(1*H*-indol-3-yl)propanoyl]amino}propanoic Acid (Cbz-L-Trp-DL-Ala-OH, 5a + 5a′)

White microcrystals; yield: 0.192 g (94%); mp 93–95 °C; $[\alpha]_D^{23}$ –18.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.23 (d, *J* = 8.0 Hz, 3 H), 1.31 (d, *J* = 7.5 Hz, 3 H), 2.85–2.95 (m, 2 H), 3.05–3.15 (m, 2 H), 4.16–4.37 (m, 4 H), 4.92–4.94 (m, 4 H), 6.94–7.00 (m, 2 H), 7.03–7.08 (m, 2 H), 7.16 (br s, 2 H), 7.23–7.37 (m, 14 H), 7.63–7.70 (m, 2 H), 8.30 (d, *J* = 7.4 Hz, 1 H), 8.36 (d, *J* = 7.4 Hz, 1 H), 10.81 (s, 2 H), 12.59 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 14.0, 17.2, 17.3, 22.1, 27.8, 28.2, 31.0, 47.5, 55.2, 55.3, 65.2, 110.0, 110.1, 111.3, 118.1, 118.6, 120.8, 123.9, 124.0, 127.3, 127.4, 127.5, 127.7, 128.3, 136.0, 136.1, 137.0, 137.1, 155.7, 155.8, 171.5, 171.8, 174.0, 174.1.

Anal. Calcd for $C_{22}H_{23}N_3O_5$: C, 64.54; H, 5.66; N, 10.26. Found: C, 64.70; H, 6.06; N, 9.69.

2-((*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino)propanoic Acid (Cbz-L-Met-DL-Ala-OH, 5i + 5v)

White microcrystals; yield: 0.63 g (92%); mp 141–142 °C; $[\alpha]_D^{23}$ –5.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.24 (d, *J* = 6.8 Hz, 3 H), 1.27 (d, *J* = 6.8 Hz, 3 H), 1.79–1.84 (m, 4 H), 2.03 (s, 6 H), 2.49–2.51 (m, 4 H), 4.09–4.22 (m, 4 H), 5.01 (s, 1 H), 5.02 (s, 1 H), 7.33–7.39 (m, 10 H), 7.43–7.49 (m, 2 H), 8.17 (d, *J* = 7.4 Hz, 1 H), 8.23 (d, *J* = 7.1 Hz, 1 H), 12.56 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 17.0, 17.3, 29.5, 29.6, 31.9, 47.5, 53.5, 53.7, 65.4, 127.7, 127.8, 128.3, 137.0, 155.9, 171.1, 171.2, 173.9, 174.0.

Anal. Calcd for $C_{16}H_{22}N_2O_5S$: C, 54.22; H, 6.26; N, 7.90. Found: C, 54.22; H, 6.20; N, 8.09.

2-{(*S*)-2-Benzyloxycarbonylamino-3-[(*S*)-2-benzyloxycarbonylamino-2-(1-carboxyethylcarbamoyl)ethyldisulfanyl]propionylamino}propionic Acid (Cbz-L-Cystine-DL-di-Ala-OH, 5q + 5q′)

White microcrystals; yield: 0.296 g (91%); mp 153–154 °C; $[\alpha]_D^{23}$ –118.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.27 (d, *J* = 6.9 Hz, 6 H), 1.29 (d, *J* = 6.9 Hz, 6 H), 2.81–2.89 (m, 4 H), 3.10–313 (m, 4 H), 4.18–4.21 (m, 4 H), 4.22–4.34 (m, 4 H), 5.04 (s, 8 H), 7.34–7.36 (m, 20 H), 7.57 (t, *J* = 9.2 Hz, 4 H), 8.26–8.31 (m, 4 H), 12.62 (s, 4 H).

^{13}C NMR (DMSO-d_6): δ = 17.0, 17.3, 40.6, 47.6, 53.6, 65.5, 127.6, 127.8, 128.3, 136.9, 156.0, 169.6, 169.9, 173.7, 173.8.

Anal. Calcd for $C_{28}H_{34}N_4O_{10}S_2$: C, 51.68; H, 5.27; N, 8.61. Found: C, 51.80; H, 5.14; N, 8.81.

***N*-Cbz-Dipeptidoylbenzotriazoles 6a–d and Diastereomeric Mixture 6a + 6d; General Procedure**

The preparation of **6a–d** and **6a + 6d** was performed at –10 °C under similar conditions as those described for **2a–n**. In the case of **6b**, the desired product was isolated by column chromatography, using EtOAc–hexanes (2:1) as eluent.

Benzyl *N*-{(*S*)-1-[(*S*)-2-benzotriazol-1-yl-1-methyl-2-oxoethylcarbamoyl]-2-(1*H*-indol-3-yl)-ethyl}carbamate (Cbz-L-Trp-L-Ala-Bt, 6a)

White microcrystals; yield: 1.991 g (78%); mp 176–177 °C; $[\alpha]_D^{23}$ –27.7 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.60 (d, *J* = 7.4 Hz, 3 H), 2.86–2.92 (m, 1 H), 3.11–3.16 (m, 1 H), 4.39–4.43 (m, 1 H), 4.92 (s, 2 H), 5.62–5.66 (m, 1 H), 6.94–6.99 (m, 1 H), 7.02–7.07 (m, 1 H), 7.22–7.34 (m, 6 H), 7.42 (d, *J* = 8.5 Hz, 2 H), 7.62–7.72 (m, 2 H), 7.79–7.84 (m, 1 H), 8.25 (d, *J* = 8.3 Hz, 1 H), 8.31 (d, *J* = 8.2 Hz, 1 H), 9.02 (d, *J* = 5.8 Hz, 1 H), 10.83 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 16.1, 27.3, 48.2, 54.4, 64.7, 109.5, 110.8, 113.5, 117.6, 118.1, 119.7, 120.3, 123.5, 126.2, 126.7, 127.0, 127.1, 127.8, 130.2, 130.6, 135.6, 136.4, 144.8, 155.3, 171.3, 171.9.

Anal. Calcd for $C_{28}H_{26}N_6O_4$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.83; H, 5.01; N, 16.19.

Benzyl *N*-{(*S*)-1-[(*S*)-2-benzotriazol-1-yl-1-(1*H*-indol-3-ylmethyl)-2-oxoethylcarbamoyl]-2-(1*H*-indol-3-yl)ethyl}carbamate (Cbz-L-Trp-L-Trp-Bt, 6b)

Pale yellow microcrystals; yield: 2.378 g (76%); mp 152–154 °C (decomposed); $[\alpha]_D^{23}$ +10.1 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 2.89 (dd, *J* = 14.6, 10.3 Hz, 1 H), 3.14 (dd, *J* = 14.6, 3.8 Hz, 1 H), 3.31–3.39 (m, 1 H), 3.53 (dd, *J* = 14.5, 5.2 Hz, 1 H), 4.40–4.48 (m, 1 H), 4.93 (s, 2 H), 5.97 (q, *J* = 5.7 Hz, 1 H), 6.90–6.97 (m, 2 H), 7.00–7.06 (m, 3 H), 7.22–7.34 (m, 8 H), 7.42 (d, *J* = 8.5 Hz, 1 H), 7.54 (d, *J* = 7.7 Hz, 1 H), 7.60–7.68 (m, 2 H), 7.79 (t, *J* = 7.6 Hz, 1 H), 8.21 (d, *J* = 8.2 Hz, 1 H), 8.26 (d, *J* = 8.5 Hz, 1 H), 8.98 (d, *J* = 6.3 Hz, 1 H), 10.82 (s, 1 H), 10.89 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 27.1, 27.7, 53.8, 55.0, 65.3, 108.7, 110.0, 111.3, 111.4, 114.0, 118.0, 118.1, 118.4, 118.5, 120.1, 120.8, 121.0, 123.9, 124.1, 126.6, 126.9, 127.2, 127.5, 127.7, 128.3, 130.5, 131.0, 136.0, 136.1, 136.9, 145.3, 155.8, 171.4, 172.6.

Anal. Calcd for $C_{36}H_{31}N_7O_4$: C, 69.11; H, 4.99; N, 15.67. Found: C, 68.97; H, 5.05; N, 15.55.

Benzyl *N*-{(*S*)-1-[(*S*)-2-benzotriazol-1-yl-1-methyl-2-oxoethylcarbamoyl]-3-methylsulfanylpropyl}carbamate (Cbz-L-Met-L-Ala-Bt, 6c)

White microcrystals; yield: 1.936 g (85%); mp 104–105 °C; $[\alpha]_D^{23}$ –19.3 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.57 (d, *J* = 7.2 Hz, 3 H), 1.75–1.99 (m, 2 H), 2.05 (s, 3 H), 2.47–2.50 (m, 2 H), 4.17–4.24 (m, 1 H), 5.01 (s, 2 H), 5.59 (quin, *J* = 6.7 Hz, 1 H), 7.33–7.35 (m, 5 H), 7.53 (d, *J* = 8.0 Hz, 1 H), 7.64 (t, *J* = 7.7 Hz, 1 H), 7.80 (t, *J* = 7.7 Hz, 1 H), 8.22 (d, *J* = 8.3 Hz, 1 H), 8.29 (d, *J* = 8.3 Hz, 1 H), 8.90 (d, *J* = 5.5 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 16.5, 29.5, 31.7, 48.6, 53.3, 65.4, 113.9, 120.2, 126.7, 127.7, 127.8, 128.3, 130.6, 131.1, 136.9, 145.3, 155.9, 171.9, 172.0.

Anal. Calcd for $C_{22}H_{25}N_5O_4S$: C, 58.01; H, 5.53; N, 15.37. Found: C, 58.07; H, 5.58; N, 15.00.

Benzyl *N*-[(*S*)-1-((*R*)-2-benzotriazol-1-yl-1-methyl-2-oxoethylcarbamoyl)-3-methylsulfanylpropyl]carbamate (Cbz-L-Met-D-Ala-Bt, 6d)

White microcrystals; yield: 1.982 g (87%); mp 135–137 °C; $[\alpha]_D^{23}$ –7.6 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.55 (d, *J* = 7.2 Hz, 3 H), 1.77–1.91 (m, 2 H), 2.03 (s, 3 H), 2.44–2.50 (m, 2 H), 4.12–4.22 (m, 1 H), 4.97–5.07 (m, 2 H), 5.58 (quin, *J* = 6.8 Hz, 1 H), 7.34–7.35 (m, 5 H), 7.47 (d, *J* = 8.2 Hz, 1 H), 7.64 (t, *J* = 7.6 Hz, 1 H), 7.81 (t, *J* = 7.7 Hz, 1 H), 8.23 (d, *J* = 8.6 Hz, 1 H), 8.29 (d, *J* = 8.3 Hz, 1 H), 8.85 (d, *J* = 5.8 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 16.7, 29.5, 31.8, 48.6, 53.5, 65.4, 114.0, 120.2, 126.7, 127.7, 127.8, 128.3, 130.6, 131.0, 136.9, 145.3, 155.9, 171.7, 171.8.

Anal. Calcd for $C_{22}H_{25}N_5O_4S$: C, 58.01; H, 5.53; N, 15.37. Found: C, 57.74; H, 5.42; N, 15.09.

Benzyl *N*-[(*S*)-1-(2-benzotriazol-1-yl-1-methyl-2-oxoethylcarbamoyl)-3-methylsulfanylpropyl]carbamate (Cbz-L-Met-DL-Ala-Bt, 6c + 6d)

White microcrystals; yield: 1.913 g (84%); mp 96–97 °C; $[\alpha]_D^{23}$ –10.6 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.55 (d, *J* = 6.5 Hz, 3 H), 1.57 (d, *J* = 6.5 Hz, 3 H), 1.78–1.94 (m, 4 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.43–2.48 (m, 4 H), 4.20–4.25 (m, 2 H), 4.98–5.06 (m, 4 H), 5.59 (quin, *J* = 6.8 Hz, 2 H), 7.33–7.37 (m, 10 H), 7.47 (d, *J* = 8.2 Hz, 1 H), 7.54 (d, *J* = 8.0 Hz, 1 H), 7.64 (t, *J* = 7.7 Hz, 2 H), 7.81 (t, *J* = 7.6 Hz, 2 H), 8.22 (d, *J* = 8.3 Hz, 2 H), 8.29 (d, *J* = 8.3 Hz, 2 H), 8.85 (d, *J* = 5.8 Hz, 1 H), 8.90 (d, *J* = 5.5 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 14.7 16.5, 16.7, 29.5, 29.6, 31.7, 31.8, 48.6, 48.7, 53.3, 53.5, 65.5, 114.0, 120.2, 126.7, 126.8, 127.7, 127.8, 128.3, 130.6, 131.1, 131.1, 137.0, 145.3, 155.9, 156.0, 171.8, 171.9, 172.0. Anal. Calcd for $C_{22}H_{25}N_5O_4S$: C, 58.01; H, 5.53; N, 15.37. Found: C, 58.15; H, 5.61; N, 15.00.

Tripeptides 7a–f; General Procedure

The preparation of tripeptides **7a–f** was carried out using the procedure described for the preparation of **5** at –10 °C instead of 20 °C.

(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(1*H*-indol-3-yl)-propionylamino]propionylamino}-3-mercaptopropionic Acid (Cbz-L-Trp-L-Ala-L-Cys-OH, 7a)

White microcrystals; yield: 0.220 g (86%); mp 114–115 °C (decomposed); $[\alpha]_D^{23}$ –23.0 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.25 (d, *J* = 6.9 Hz, 3 H), 2.44–2.46 (m, 1 H), 2.74–2.99 (m, 3 H), 3.10–3.16 (m, 1 H), 4.29–4.47 (m, 3 H), 4.93 (s, 2 H), 6.94–6.99 (m, 1 H), 7.03–7.08 (m, 1 H), 7.16 (app s, 1 H), 7.23–7.34 (m, 6 H), 7.42 (d, *J* = 8.0 Hz, 1 H), 7.66 (d, *J* = 7.7 Hz, 1 H), 8.12 (d, *J* = 7.7 Hz, 1 H), 8.25 (d, *J* = 7.5 Hz, 1 H), 10.80 (s, 1 H), 12.90 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 18.0, 25.5, 27.7, 48.1, 54.2, 55.4, 65.2, 110.1, 111.2, 118.1, 118.6, 120.8, 123.9, 127.3, 127.4, 127.6, 128.3, 136.0, 137.0, 155.9, 171.4, 171.7, 172.2.

Anal. Calcd for $C_{25}H_{28}N_4O_6S$: C, 58.58; H, 5.51; N, 10.93. Found: C, 58.86; H, 5.58; N, 11.13.

(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-3-(1*H*-indol-3-yl)-propionylamino]-3-(1*H*-indol-3-yl)propionylamino}-3-(1*H*-indol-3-yl)propanoic Acid (Cbz-L-Trp-L-Trp-L-Trp-OH, 7b)

White microcrystals; yield: 0.309 g (87%); mp 127–129 °C (decomposed); $[\alpha]_D^{23}$ –13.6 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): dr = 3:1; δ = 2.75–2.85 (m, 2 H), 2.95–3.23 (m, 4 H), 4.24–4.30 (m, 1 H), 4.45–4.56 (m, 1 H), 4.62–4.74 (m, 1 H), 4.91 (s, 2 H), 6.82–7.08 (m, 8 H), 7.17–7.36 (m, 10 H), 7.52–7.65 (m, 3 H), 8.09 (d, *J* = 7.9 Hz, 0.75 H), 8.30 (d, *J* = 8.8 Hz, 1 H), 8.39 (d, *J* = 8.0 Hz, 0.25 H), 10.72–10.84 (m, 3 H), 12.67 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 27.1, 27.7, 53.1, 55.5, 65.2, 109.5, 109.8, 109.9, 110.0, 110.2, 110.3, 111.2, 111.3, 118.1, 118.3, 118.5, 120.8, 120.9, 123.6, 123.8, 127.3, 127.4, 127.6, 128.2, 136.0, 136.8, 136.9, 155.7, 171.4, 171.6, 173.1, 173.3.

Anal. Calcd for $C_{41}H_{38}N_6O_6$: C, 69.28; H, 5.39; N, 11.82. Found: C, 68.93; H, 5.47; N, 11.83.

(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]propionylamino}propanoic Acid (Cbz-L-Met-L-Ala-L-Ala-OH, 7c)

White microcrystals; yield: 0.183 g (86%); mp 213–214 °C; $[\alpha]_D^{23}$ –13.0 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.22 (d, *J* = 7.6 Hz, 3 H), 1.27 (d, *J* = 7.4 Hz, 3 H), 1.73–1.91 (m, 2 H), 2.02 (s, 3 H), 2.43–2.51 (m, 2 H), 4.05–4.13 (m, 1 H), 4.18 (quin, *J* = 7.2 Hz, 1 H), 4.29 (quin, *J* = 7.2 Hz, 1 H), 5.02 (s, 2 H), 7.33–7.39 (m, 5 H), 7.51 (d, *J* = 7.9 Hz, 1 H), 8.01 (d, *J* = 7.5 Hz, 1 H), 8.14 (d, *J* = 7.2 Hz 1 H), 12.54 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 17.1, 18.2, 29.6, 31.7, 47.4, 47.8, 53.8, 65.4, 127.7, 127.8, 128.3, 137.0, 156.0, 171.0, 171.9, 174.0.

Anal. Calcd for $C_{19}H_{27}N_3O_6S$: C, 53.63; H, 6.40; N, 9.88. Found: C, 53.62; H, 6.44; N, 9.50.

(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]propionylamino}-3-hydroxypropanoic Acid (Cbz-L-Met-L-Ala-L-Ser-OH, 7d)

White microcrystals; yield: 0.183 g (83%); mp 177–178 °C; $[\alpha]_D^{23}$ –4.4 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): dr = 4:1; δ = 1.23 (d, *J* = 6.9 Hz, 3 H), 1.76–1.96 (m, 2 H), 2.02 (s, 3 H), 2.44–2.50 (m, 2 H), 3.60–3.74 (m, 2 H), 4.06–4.18 (m, 1 H), 4.25–4.32 (m, 1 H), 4.36 (t, *J* = 7.2 Hz, 1 H), 5.02 (s, 2 H), 7.33–7.36 (m, 5 H), 7.52 (d, *J* = 7.7 Hz, 0.8 H), 8.02 (d, *J* = 7.7 Hz, 1 H), 8.09 (d, *J* = 8.0 Hz, 0.2 H), 12.61 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 18.3, 29.6, 31.7, 47.9, 53.8, 54.6, 61.3, 65.4, 127.7, 127.8, 127.9, 128.3, 137.0, 156.0, 171.0, 171.8, 172.2.

Anal. Calcd for $C_{19}H_{27}N_3O_7S$: C, 51.69; H, 6.16; N, 9.52. Found: C, 51.57; H, 6.38; N, 9.19.

(*S*)-2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]propionylamino}-3-(1*H*-indol-3-yl)propionic Acid (Cbz-L-Met-L-Ala-L-Trp-OH, 7e)

White microcrystals; yield: 0.249 g (92%); mp 171–172 °C (decomposed); $[\alpha]_D^{23}$ +5.2 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): dr = 4:1; δ = 1.77 (d, *J* = 7.4 Hz, 3 H), 1.76–11.96 (m, 2 H), 2.00 (s, 3 H), 2.42–2.50 (m, 2 H), 3.01–3.08 (m, 1 H), 3.13–3.17 (m, 1 H), 4.08–4.09 (m, 1 H), 4.32–4.34 (m, 1 H), 4.44–4.46 (m, 1 H), 5.01 (s, 2 H), 6.94–6.99 (m, 1 H), 7.02–7.07 (m, 1 H), 7.12–7.16 (m, 1 H), 7.31–7.33 (m, 5 H), 7.48–7.56 (m, 2 H), 8.00 (d, *J* = 7.1 Hz, 0.8 H), 8.11 (d, *J* = 7.1 Hz, 1 H), 8.17 (d, *J* = 8.0 Hz, 0.2 H), 10.83 (s, 1 H), 12.65 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.6, 18.2, 27.0, 29.6, 31.7, 47.9, 52.9, 53.7, 65.4, 109.5, 111.3, 118.1, 118.3, 120.9, 123.7, 127.7, 127.8, 128.3, 136.0, 137.0, 155.9, 171.0, 171.7, 172.1, 173.1, 173.2.

Anal. Calcd for $C_{27}H_{32}N_4O_6S$: C, 59.98; H, 5.97; N, 10.36. Found: C, 59.84; H, 6.24; N, 10.19.

2-{(*S*)-2-[(*S*)-2-Benzyloxycarbonylamino-4-methylsulfanylbutyrylamino]propionylamino}propanoic Acid (Cbz-L-Met-DL-Ala-L-Ala-OH, 7f)

White microcrystals; yield: 0.187 g (88%); mp 176–177 °C (decomposed); $[\alpha]_D^{23}$ –8.6 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.24 (d, *J* = 6.7 Hz, 1.5 H), 1.26 (d, *J* = 6.7 Hz, 1.5 H), 1.29 (d, *J* = 6.7 Hz, 1.5 H), 1.32 (d, *J* = 6.7 Hz, 1.5 H), 1.82–1.97 (m, 2 H), 2.06 (s, 3 H), 2.44–2.54 (m, 1 H), 4.11–4.16 (m, 1 H), 4.20–4.28 (m, 1 H), 4.33 (quin, *J* = 7.1 Hz, 2 H), 5.11 (s, 2 H), 7.37–7.39 (m, 5 H), 7.55 (d, *J* = 7.9 Hz, 0.5 H), 7.65 (d, *J* = 7.5 Hz, 0.5 H), 8.04–8.06 (m, 1 H), 8.16–8.22 (m, 1 H), 12.57 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.5, 17.0, 17.1, 18.1, 29.5, 31.7, 47.3, 47.4, 47.3, 47.9, 53.7, 54.0, 65.3, 65.4, 127.6, 127.7, 128.2, 136.8, 137.0, 171.0, 171.5, 171.7, 173.7, 173.8.

Anal. Calcd for $C_{19}H_{27}N_3O_6S$: C, 53.63; H, 6.40; N, 9.88. Found: C, 53.45; H, 6.49; N, 9.68.

(*S*)-2-{2-[(*S*)-2-Benzyloxycarbonylamino-3-(1*H*-indol-3-yl)propionylamino]acetylamino}-4-methylpentanoic Acid (Cbz-L-Trp-L-Gly-L-Leu-OH, 7g)

White microcrystals; yield: 0.163 g (62%); mp 130–131 °C; $[\alpha]_D^{23}$ –36.5 (*c* 1.66, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.84 (d, *J* = 7.0 Hz, 6 H), 1.45–1.65 (m, 3 H), 2.94 (dd, *J* = 14.5, 10.5 Hz, 1 H), 3.13 (dd, *J* = 14.5, 3.9 Hz, 1 H), 3.70–3.74 (m, 2 H), 4.12–4.13 (m, 1 H), 4.23–4.27 (m, 1 H), 4.93 (s, 2 H), 6.96 (t, *J* = 7.4 Hz, 1 H), 7.02–7.07 (m, 1 H), 7.18 (br s, 1 H), 7.23–7.34 (m, 6 H), 7.62 (d, *J* = 7.7 Hz, 1 H), 7.74–7.77 (m, 2 H), 8.55 (s, 1 H), 10.82 (br s, 1 H), 12.60 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 21.9, 22.0, 24.3, 27.6, 41.5, 42.3, 51.5, 55.8, 65.2, 110.3, 111.2, 118.1, 118.4, 120.7, 123.8, 127.2, 127.4, 127.6, 128.2, 136.0, 137.0, 155.9, 167.9, 172.2, 174.2.

Anal. Calcd for $C_{27}H_{32}N_4O_6 \cdot H_2O$: C, 61.58; H, 6.51; N, 10.64. Found: C, 61.19; H, 6.18; N, 10.57.

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