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Convenient synthesis of C-terminal di- and tri-peptide amides from *N*-protected dipeptidoylbenzotriazoles

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ABSTRACT

N-Protected dipeptidoylbenzotriazoles react with aqueous ammonia to give dipeptide primary amides (77–98%) and with *N*-unprotected α-amino amides to afford tripeptide primary amides (82–86%).

1. Introduction

Naturally occurring peptides are essential for many cellular functions.[1] Peptides modified at the C-terminus are substrates or inhibitors for a variety of proteolytic enzymes.[2] The incorporation of a primary amide at the C-terminus is common modification:[3] around 50% of biologically active peptides contain such functionality.[4] Peptidyl α-carboxamides are especially widely distributed in animals and elicit vital physiological effects:[1a] examples include oxytocin,[5] lutenizing hormone releasing hormone (LHRH),[6] neurokinins,[7] and vasopressin and gastrine.[8]

Peptidyl α-carboxamides are also found within protease inhibitors. For example, the pseudo pentapeptide L-682,679 (**1**) is a highly potent and selective inhibitor of HIV-1 protease (IC_{50}=0.42 nM, Fig. 1) (**11**) and L-685,458 (**2**) is a potent inhibitor of γ-secretase (IC_{50}=17 nM) of potential therapeutic benefit in the treatment of Alzheimer's disease and other neurological disorders (Fig. 1).[9] Cathepsin L (EC 3.4.22.16) is an example of the papain-like cysteine proteases, which are ubiquitously distributed in the lysosomes of cells with prominent roles in nonspecific intracellular protein breakdown,[10] and physiologically important extra-cellular functions.[11]

Recently, an N^{ω}-nitroarginine-containing dipeptide amide [L-Arg^{NO2}-L-Lys-NH_2 (LL) (**3**)] and [D-Lys-D-Arg^{NO2}-NH_2 (DD) (**4**)] (Fig. 2)[12] showed a remarkable selectivity (hundreds- to thousands-fold) for inhibition of the neuronal isozyme of nitric oxide synthase (*n*NOS) over two other isozymes.[13] This *n*NOS selectivity has implications in the potential treatment of strokes,[14] migraine headaches,[15] and Alzheimer's disease.[16] Moreover, L-Leu-x-amides (where x is any amino acid residue) support growth of a leucine auxotroph as well as the free peptide.[17]

N-Acylbenzotriazoles are neutral efficient coupling reagents for N-acylation,[18] C-acylation,[19] and O-acylation.[20] *N*-(Aminoacyl)-benzotriazoles prepared from *N*-protected α-amino acids were successfully utilized for synthesis of di-, tri-, and tetrapeptides.[21] As part of the synthetic strategy for the modification of peptides at the C-terminus, we now report the synthesis of C-terminal protected dipeptides **10a–l**, (**10a**+**10a′**),[22] (**10e**+**10e′**) and tripeptide amides **13a**,**b** and (**13a**+**13a′**) by treating *N*-protected dipeptidoylbenzotriazoles **9a–l**, (**9a**+**9a′**), and (**9e**+**9e′**) with aqueous ammonia or with unprotected α-amino carboxamides **12a**,**b**, respectively.

2. Results

2.1. Synthesis of *N*-protected dipeptidoylbenzotriazoles

Treating Cbz-protected amino acids **5a–e** and (**5b′**) with 4 equiv of benzotriazole and 1 equiv of $SOCl_2$ in THF or CH_2Cl_2 at 20 °C for 2 h, following the published procedure,[21a,c] gave *N*-(Cbz-α-aminoacyl)benzotriazoles **6a–e** and (**6b′**) in 78–95% (Scheme 1). Peptide coupling was achieved by the reaction of **6a–e** and (**6b′**) with unprotected amino acids **7a–g** and (**7a′**) in aqueous acetonitrile in the presence of Et_3N for 10–40 min.[21b] After washing with 6 M HCl, the resulting dipeptides **8a–l**, (**8a**+**8a′**), and (**8e**+**8e′**) were obtained in 71–95% yields (Scheme 2 and Table 1). These reactions proceed without detectable racemization of the chiral center, as evidenced by their NMR and HPLC analysis.[21a,c,e]

Cbz-protected dipeptidoylbenzotriazoles **9a–l**, (**9a**+**9a′**), and (**9e**+**9e′**) were prepared in 64–91% yield from Cbz-protected dipeptides **8a–l**, (**8a**+**8a′**), and (**8e**+**8e′**) by reaction with 4 equiv of benzotriazole and 1 equiv of $SOCl_2$ at −15 °C for 4–6 h (Scheme 2 and Table 2).[21b–d]

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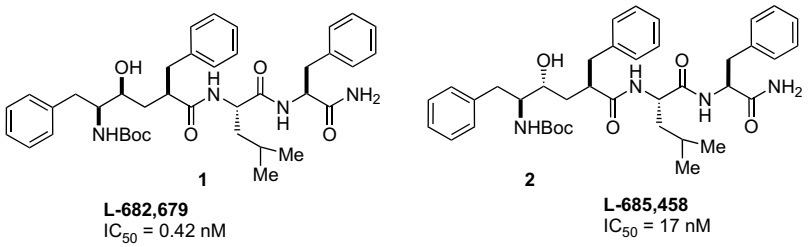


Figure 1. Chemical structures of the aspartyl protease inhibitors L-682,679 and L-685,458.

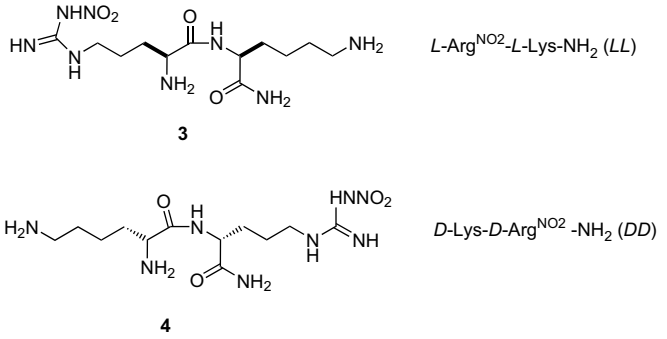


Figure 2. Chemical structures of L-Arg^{NO2}-L-Lys-NH_2 (LL) and D-Lys-D-Arg^{NO2}-NH_2 (DD).

2.2. Synthesis of *N*-protected dipeptide primary amides

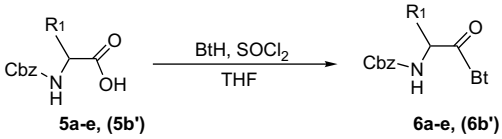
Treatment of the *N*-protected dipeptidoylbenzotriazoles **9a–l**, (**9a**+**9a′**), and (**9e**+**9e′**) with excess aqueous ammonium hydroxide in THF at 0 °C for 2 h afforded the corresponding dipeptide primary amides **10a–l**, (**10a**+**10a′**), and (**10e**+**10e′**) in 77–98% yield (Scheme 2 and Table 3). The structures of **10a–l**, (**10a**+**10a′**), and (**10e**+**10e′**) were supported by spectral data and elemental analyses.

NMR analysis showed no detectable racemization for compounds **10a–l**. No signals from their corresponding diastereomers were observed in the NMR spectra of **10a–l** suggesting the enantiopurity of the *N*-protected dipeptide amides. For example, the ^1H NMR spectrum of **10a** gave a clear doublet for the methyl protons of L-Ala fragment at 1.19 ppm, whereas a multiplet was observed for the corresponding diastereomeric mixture (**10a**+**10a′**) ranging from 1.08 to 1.23 ppm. Furthermore, compound **10a** and the corresponding diastereomeric mixture (**10a**+**10a′**) were subjected to HPLC analysis using Chirobiotic T column (detection at 254 nm, flow rate 1.0 mL/min, and 100% MeOH as solvent). As expected, HPLC analysis of the enantiopure LL-dipeptide amide **10a** showed a signal peak at 3.86 min. In contrast, two peaks of equal intensity at 3.67 and 3.79 min were observed for the corresponding diastereomeric mixture (**10a**+**10a′**).

2.3. Synthesis of *N*-protected tripeptide primary amides

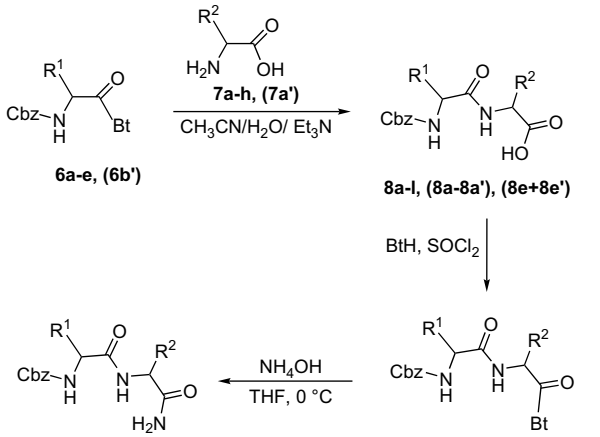
Synthesis of *N*-protected tripeptide primary amides **13a**,**b** and (**13a**+**13a′**) was achieved by reacting the *N*-protected dipeptidoylbenzotriazoles **9a**,**b** and (**9a**+**9a′**) (described above) with *N*-unprotected-α-amino amides **12a**,**b** (prepared by treating the corresponding *N*-Fmoc-α-aminoacylbenzotriazoles **11a** (Fmoc-L-Met-Bt) and **11b** (Fmoc-Gly-Bt) with aqueous ammonia followed by N-deprotection with piperidine utilizing a literature method[23] in acetonitrile at −15 °C for 6 h to afford the corresponding *N*-protected tripeptide amides **13a**,**b** and (**13a**+**13a′**) in 82–86% yields (Scheme 3 and Table 4).

NMR spectroscopic analysis of the tripeptide amide **13a** showed approximately 25% racemization. The ^1H NMR spectrum of **13a** displayed two separate doublets with different intensities for the methyl protons of L-Ala fragment at 1.13 ppm and 1.26 ppm,



Bt = Benzotriazol-1-yl
R^1: a, *L*Trp; b, *L*-Ala; b', *DL*-Ala; c, *L*-Met; d, L-Phe; e, *D*-Val

Scheme 1. Compound numbers written within brackets represent a diastereomeric mixtures or racemates; compound numbers without brackets represent enantiomers.



Scheme 2.

Table 1
Preparation of dipeptides **8**

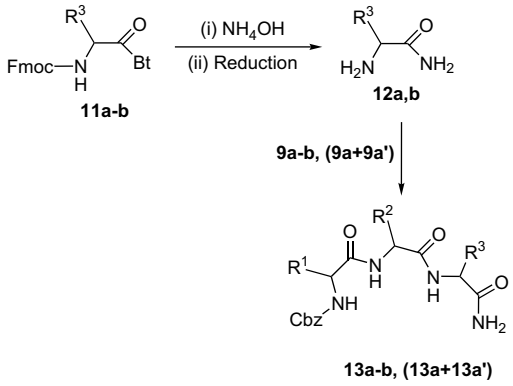
N-(Cbz-α-aminoacyl) benzotriazoles	Amino acid	Product	Yield[a] (%)
6a	L-Ala, **7a**	Cbz-L-Trp-L-Ala-OH, **8a**	95
6a	DL-Ala, (**7a′**)	Cbz-L-Trp-DL-Ala-OH, (**8a**+**8a′**)	93
6a	L-Trp, **7b**	Cbz-L-Trp-L-Trp-OH, **8b**	86
6a	Gly, **7c**	Cbz-L-Trp-Gly-OH, **8c**	93
6b	L-Trp, **7d**	Cbz-L-Ala-L-Trp-OH, **8d**	79
6b	L-Glu(OBn), **7e**	Cbz-L-Ala-L-Glu(OBn)-OH, **8e**	91
(**6b′**)	L-Glu(OBn), **7e**	Cbz-DL-Ala-L-Glu(OBn)-OH, (**8e**+**8e′**)	81
6b	L-Pro, **7f**	Cbz-L-Ala-L-Pro-OH, **8f**	78
6c	L-Trp, **7d**	Cbz-L-Met-L-Trp-OH, **8g**	86
6d	L-Met, **7f**	Cbz-L-Phe-L-Met-OH, **8h**	70
6d	L-Glu(OBn), **7e**	Cbz-L-Phe-L-Glu(OBn)-OH, **8i**	93
6d	L-Asp(OBn), **7g**	Cbz-L-Phe-L-Asp(OBn)-OH, **8j**	79
6d	L-Lys, **7h**	Cbz-L-Phe-L-Lys-OH, **8k**	71
6f	L-Asp(OBn), **7g**	Cbz-D-Val-L-Asp(OBn)-OH,[b] **8l**	83

[a] Isolated yield.
[b] Novel compound.

Table 2
Conversion of dipeptides **8** into *N*-Cbz-dipeptidoylbenzotriazoles **9**

Reactant	Product	Yield[a] (%)
8a	Cbz-L-Trp-L-Ala-Bt, **9a**	79
(**8a**+**8a**′)	Cbz-L-Trp-DL-Ala-Bt, (**9a**+**9a**′)[b]	64
8b	Cbz-L-Trp-L-Trp-Bt, **9b**	89
8c	Cbz-L-Trp-Gly-Bt, **9c**[b]	75
8d	Cbz-L-Ala-L-Trp-Bt, **9d**	78
8e	Cbz-L-Ala-L-Glu(OBn)-Bt, **9e**	91
(**8e**+**8e**′)	Cbz-DL-Ala-L-Glu(OBn)-Bt, (**9e**+**9e**′)	79
8f	Cbz-L-Ala-L-Pro-Bt, **9f**[b]	76
8g	Cbz-L-Met-L-Trp-Bt, **9g**[b]	73
8h	Cbz-L-Phe-L-Met-Bt, **9h**	79
8i	Cbz-L-Phe-L-Glu(OBn)-Bt, **9i**	80
8j	Cbz-L-Phe-L-Asp(OBn)-Bt, **9j**	81
8k	Cbz-L-Phe-L-Lys-Bt, **9k**	63
8l	Cbz-D-Val-L-Asp(OBn)-Bt, **9l**[b]	88

[a] Isolated yield.
[b] Novel compound.



Scheme 3.

respectively. However for compound **13b** no signals for any corresponding diastereomer were observed. The enantiopurity of tripeptide amide **13b** was further confirmed by HPLC analyses using Chirobiotic T column (detection at 254 nm, flow rate 1 mL/min, and MeOH as solvent): tripeptide amide **13b** gave a single retention time, whereas the diastereomeric mixture (**13a**+**13a**′) showed two retention times as expected (Table 4).

Traditional syntheses of primary carboxamides (i) treat acids or derived acid halides, anhydrides or esters with ammonia.[24] However, the exothermic reactions of ammonia with acyl halides must be controlled; acid anhydrides and ammonia can also form imides.[25] Additionally, these methods are often not suitable for selective amidation.[26] Other reported methods include: (ii) ammonolysis of *N*-protected amino acids with diaminomethane dihydrochloride,[27] (iii) treatment of acids with ammonia-releasing agents (NH_4OH, NH_4Cl or $(NH4)_2CO_3$) in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluoro-phosphate (PyBOP) and *N*-hydroxybenzotriazole (HOBt),[28] 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxy-7-aza-benzotriazole (HOAt) or HOBt (**48**), *N*,*N*′-dicylcohexyl-carbodiimide (DCC) and HOAt or HOBt,[29] *p*-tolylsulfonyl chloride (TsCl),[30] or *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ).[31] Difficulties can arise from the insolubility of starting materials, competitive hydrolysis or partial racemization in approaches (ii) and (iii).

Peptidyl α-carboxamides have also been prepared previously by several solid phase and biosynthetic methods. Solid phase syntheses include (iv) acidolytic cleavage of peptide from support-bound benzylamine- or benzhydrylamine-based handles,[32] (but side-chain deprotection takes place in concert with cleavage); (v) ammonolysis of support-bound esters of side-chain deprotected peptide with liquid ammonia or ammonia/MeOH solution,[33] or ammonia/THF,[34] (limited to side-chain deprotected peptides and requiring well-sealed containers, as trace amounts of moisture can result in the production of acid as a contaminant);[35] and (vi) deprotection and cleavage of solid-supported *N*-tetrachloro-phthaloyl peptides.[36]

Biosynthetic techniques include: (vii) oxidative cleavage of polypeptides by peptidylglycine alpha-amidating monooxygenase (PAM);[37] (viii) enzymatic couplings of an acyl donor component (esters or thioesters) with an acyl acceptor component (amino acid amides).[38] Enzymatic approaches have attracted interest for years because they take place in an aqueous medium and occur with high stereo- and regio-selectivity in mild conditions of pH and temperature with no need for side-chain protection. However, they may be restricted to specific substrates, require long reaction times, and/or give the products in low yield.[38]

We have shown that our methodology offers convenient preparative and work-up procedures, and uses inexpensive reagents to prepare *N*-protected di- and tripeptide primary amides from readily available *N*-protected dipeptidoylbenzotriazoles in good yields (77–98%) as compared with literature methods.

3. Summary

In summary, we have developed a convenient and efficient approach for a broad range of chirally pure dipeptide primary amides (average yield 88%), by treating the corresponding *N*-protected dipeptidoylbenzotriazoles with aqueous ammonia. The approach was extended to the preparation of tripeptide primary amides (average yield 83%) by using some of the same substrates with *N*-unprotected α-amino amides. These results, coupled with the simplicity of isolation and purification procedures, make this approach competitive with literature methods.

Table 3
Conversion of **9** into dipeptide amides **10**

Reactant	Product	Yield[a] (%)	Mp (°C)	$[\alpha]_D^{23}$
9a	Cbz-L-Trp-L-Ala-NH_2, **10a**[b]	79	168–170	−9.5
(**9a**+**9a**′)	Cbz-L-Trp-DL-Ala-NH_2,[c] (**10a**+**10a**′)	95	157–160	−20.7
9b	Cbz-L-Trp-L-Trp-NH_2, **10b**	96	192–194	−36.6
9c	Cbz-L-Trp-Gly-NH_2, **10c**	86	151–155	−26.4
9d	Cbz-L-Ala-L-Trp-NH_2, **10d**	77	201–202	−7.7
9e	Cbz-L-Ala-L-Glu(OBn)-NH_2, **10e**	98	162–163	−3.0
(**9e**+**9e**′)	Cbz-DL-Ala-L-Glu(OBn)-NH_2, (**10e**+**10e**′)	90	138–139	−4.7
9f	Cbz-L-Ala-L-Pro-NH_2, **10f**	95	161–162	−37.6
9g	Cbz-L-Met-L-Trp-NH_2, **10g**	91	218–220	−12.7
9h	Cbz-L-Phe-L-Met-NH_2, **10h**	94	208–210	−14.8
9i	Cbz-L-Phe-L-Glu(OBn)-NH_2, **10i**	96	205–207	−10.6
9j	Cbz-L-Phe-L-Asp(OBn)-NH_2, **10j**	98	162–163	−3.0
9k	Cbz-L-Phe-L-Lys-NH_2, **10k**	92	193–195	−8.5
9l	Cbz-D-Val-L-Asp(OBn)-NH_2, **10l**	88	195–196	−32.9

[a] Isolated yield.
[b] HPLC for **10a**: 3.86 min.
[c] HPLC for (**10a**+**10a**′): 3.67 and 3.79 min.

Table 4
Conversion of **9a**,**b** and (**9a**+**9a**′) into tripeptide amides **13**

Reactant	Amino amide	Product	Yield[a] (%)	Mp (°C)	$[\alpha]_D^{23}$
9a	Met-NH_2, **12a**	Cbz-L-Trp-L-Ala-Met-NH_2, **13a**	82	222–224	−33.30
(**9a**+**9a**′)	Gly-NH_2, **12b**	Cbz-L-Trp-DL-Ala-Gly-NH_2,[b] (**13a**+**13a**′)	82	164–166	−17.92
9b	Gly-NH_2, **12b**	Cbz-L-Trp-L-Trp-Gly-NH_2,[c] **13b**	86	220–221	−15.36

[a] Isolated yield.
[b] HPLC for (**13a**+**13a**′): 9.90 and 11.82 min.
[c] HPLC for **13b**: 8.17 min.

4. Experimental

4.1. General

All mps are uncorrected. NMR spectra were recorded on a Varian Gemini (300 MHz) spectrometer in DMSO-d_6 with TMS for ^{1}H (300 MHz) and ^{13}C (75 MHz) as an internal reference. IR spectra were recorded on a Unicam SP-1200 infrared spectrophotometer using KBr Wafer technique. *N*-Cbz-amino acids and amino acids were purchased from Fluka (Buchs, Switzerland) and Acros (Suwanee, GA, USA), were used without further purification. Elemental analyses were performed on a Carlo Erba-1106 instrument. Optical rotation values were measured with the use of the sodium D line. 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 1.0 mL/min, and with methanol as solvent.

4.2. *N*-(Cbz-α-aminoacyl)benzotriazoles 6: general procedure

N-(Cbz-α-aminoacyl)benzotriazoles **6a–e** and (**6b′**) were prepared according to the literature procedure.[18,20,21a]

4.3. Dipeptides 8: general procedure

N-Cbz-protected dipeptides **8a–l**, (**8a**+**8a′**), and (**8e**+**8e′**) were prepared according to the literature procedure.[38,20,21a]

*4.3.1. (3S)-4-(Benzyloxy)-3-[((2R)-2-[(benzyloxy)carbonyl]amino-3-methylbutanoyl)amino]-4-oxobutanoic acid (Cbz-D-Val-L-Asp(3-OBn)-OH, **8l**)*

Yield: 83%; white microcrystals; mp 114–115 °C; $[\alpha]_D^{23}$ −19.3 (*c* 1.65, DMF). ^{1}H NMR: δ=8.42 (d, *J*=7.8 Hz, 1H), 7.39–7.30 (m, 11H), 5.12 (s, 2H), 5.07 (s, 2H), 4.65 (dd, *J*=13.5, 7.0 Hz, 1H), 3.96 (t, *J*=7.8 Hz, 1H), 2.91 (dd, *J*=16.3, 5.6 Hz, 1H), 2.75 (dd, *J*=16.4, 7.2 Hz, 1H), 1.98–1.92 (m, 1H), 0.84 (t, *J*=6.5 Hz, 6H). ^{13}C NMR: δ=172.1, 171.2, 170.2, 156.3, 137.3, 136.1, 128.6, 128.5, 128.2, 128.0, 127.9, 127.8, 65.9, 65.5, 60.0, 48.7, 36.1, 30.7, 19.2, 18.0. IR: ν_{max} 3430–2500 (br), 1705, 1645, 1620, 1590, 1425 cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_2O_7$: C, 63.15; H, 6.18; N, 6.14. Found: C, 62.82; H, 6.34; N, 6.29.

4.4. *N*-Cbz-protected dipeptidoylbenzotriazoles 9: general procedure

N-Cbz-protected dipeptidoylbenzotriazoles **9a–l**, (**9a**+**9a′**), and (**9e**+**9e′**) were prepared according to the literature procedure.[18,21]

*4.4.1. Benzyl N-((1S)-2-[2-(1H-1,2,3-benzotriazol-1-yl)-1-methyl-2-oxoethyl]amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl)carbamate (Cbz-L-Trp-DL-Ala-Bt, (**9a**+**9a′**))*

Yield: 64%; white microcrystals; mp 142–144 °C; $[\alpha]_D^{23}$ −8.4 (*c* 1.81, DMF). ^{1}H NMR: δ=10.84 (s, 1H), 9.04–8.97 (m, 1H), 8.32–8.24 (m, 2H), 7.82 (t, *J*=7.7 Hz, 1H), 7.73–7.63 (m, 2H), 7.44 (d, *J*=8.7 Hz, 1H), 7.35–7.19 (m, 7H), 7.06–6.97 (m, 2H), 5.67–5.63 (m, 1H), 4.93 (d, *J*=3.7 Hz, 2H), 4.45–4.41 (m, 1H), 3.16–3.12 (m, 1H), 2.94–2.91 (m, 1H), 1.61–1.49 (m, 3H). ^{13}C NMR: δ=172.6, 172.3, 171.9, 155.9, 155.7, 145.4, 137.0, 136.1, 131.1, 130.7, 128.3, 127.7, 127.5, 127.4, 127.2, 126.9, 126.7, 124.1, 124.0, 120.8, 120.2, 118.6, 118.2, 114.0, 111.3, 110.0, 109.9, 65.3, 65.0, 55.1, 54.9, 48.7, 48.6, 31.0, 27.8, 16.7, 16.6. IR: ν_{max} 3100, 3060, 2915, 1715, 1650, 1620, 1410 cm^{-1}. Anal. Calcd for $C_{28}H_{26}N_6O_4$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.61; H, 5.27; N, 16.43.

*4.4.2. Benzyl N-((1S)-2-[2-(1H-1,2,3-benzotriazol-1-yl)-2-oxoethyl]amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl)carbamate (Z-L-Trp-L-Gly-Bt, **9c**)*

Yield: 75%; white microcrystals; mp 186–187 °C; $[\alpha]_D^{23}$ −27.3 (*c* 1.73, DMF). ^{1}H NMR: δ=10.87 (s, 1H), 8.89 (t, *J*=5.1 Hz, 1H), 8.30 (d, *J*=8.2 Hz, 1H), 8.25 (d, *J*=8.2 Hz, 1H), 7.82 (t, *J*=7.6 Hz, 1H), 7.72 (d, *J*=7.7 Hz, 1H), 7.64 (t, *J*=7.7 Hz, 1H), 7.56 (d, *J*=8.5 Hz, 1H), 7.37–7.23 (m, 7H), 7.08 (t, *J*=7.3 Hz, 1H), 7.00 (t, *J*=7.3 Hz, 1H), 5.02 (t, *J*=5.9 Hz, 2H), 4.97 (s, 2H), 4.49–4.44 (m, 1H), 3.25 (dd, *J*=14.4, 3.4 Hz, 1H), 3.00 (dd, *J*=14.4, 10.5 Hz, 1H). ^{13}C NMR: δ=173.0, 169.6, 155.9, 145.3, 137.0, 131.0, 130.6, 128.3, 127.7, 127.5, 127.2, 126.6, 124.0, 120.9, 120.2, 118.5, 118.2, 113.8, 111.3, 110.2, 65.3, 55.4, 42.6, 27.9. Anal. Calcd for $C_{27}H_{24}N_6O_4$: C, 65.31; H, 4.87; N, 16.93. Found: C, 65.63; H, 4.99; N, 16.55.

*4.4.3. Benzyl N-(1S)-2-[(2S)-2-(1H-1,2,3-benzotriazol-1-ylcarbonyl)tetrahydro-1H-pyrrol-1-yl]-1-methyl-2-oxoethylcarbamate (Cbz-L-Ala-L-Pro-Bt, **9f**)*

Yield: 76%; white microcrystals; mp 52–54 °C; $[\alpha]_D^{23}$ −29.8 (*c* 1.66, DMF). ^{1}H NMR: δ=8.34 (d, *J*=8.2 Hz, 1H), 8.26 (d, *J*=8.1 Hz, 1H), 7.84 (t, *J*=7.7 Hz, 1H), 7.71–7.61 (m, 2H), 7.42–7.33 (m, 5H), 5.76 (dd, *J*=8.8, 5.1 Hz, 1H), 5.04 (AB, J_{AB}=16.2, 12.6 Hz, 2H), 4.46 (t, *J*=7.2 Hz, 1H), 3.88–3.76 (m, 2H), 2.50–2.46 (m, 1H), 2.25–2.10 (m, 3H), 1.27 (d, *J*=7.0 Hz, 3H). ^{13}C NMR: δ=171.4, 170.7, 155.8, 145.5, 137.2, 131.3, 130.8, 128.5, 128.0, 127.9, 127.0, 120.4, 114.1, 65.6, 59.3, 48.1, 47.0, 29.0, 25.3, 16.7. Anal. Calcd for $C_{22}H_{23}N_5O_4$: C, 62.70; H, 5.50; N, 16.62. Found: C, 62.49; H, 5.57; N, 16.91.

*4.4.4. Benzyl N-[(1S)-1-([(1S)-2-(1H-1,2,3-benzotriazol-1-yl)-1-(1H-indol-2-ylmethyl)-2-oxoethyl]-aminocarbonyl)-3-(methylsulfanyl)propyl]carbamate (Cbz-L-Met-L-Trp-Bt, **9g**)*

Yield: 73%; white microcrystals; mp 171–173 °C; $[\alpha]_D^{23}$ +18.0 (*c* 1.58, DMF). ^{1}H NMR: δ=10.94 (s, 1H), 8.91 (d, *J*=5.8 Hz, 1H), 8.29 (d, *J*=8.1 Hz, 1H), 8.22 (d, *J*=8.2 Hz, 1H), 7.82 (t, *J*=7.6 Hz, 1H), 7.66 (t, *J*=7.6 Hz, 1H), 7.59–7.55 (m, 2H), 7.38–7.28 (m, 7H), 7.06 (t, *J*=7.5 Hz, 1H), 6.96 (t, *J*=7.3 Hz, 1H), 5.96–5.90 (m 1H), 5.04 (AB, J_{AB}=17.3, 12.4 Hz, 2H), 4.28–4.26 (m, 1H), 3.53 (dd, *J*=14.7, 4.8 Hz, 1H), 3.37–3.31 (m, 1H), 2.51 (t, *J*=7.8 Hz, 2H), 2.07 (s, 3H), 1.94–1.85 (m, 2H). ^{13}C NMR: δ=172.2, 171.4, 155.9, 145.3, 136.9, 136.0, 131.0, 130.5, 128.3, 127.8, 128.6, 126.7, 124.2, 121.0, 120.2, 118.4, 118.0, 114.0, 111.4, 108.7, 65.7, 53.8, 53.4, 31.7, 29.5, 26.9, 14.6. Anal. Calcd for $C_{30}H_{30}N_6O_4S$: C, 63.14; H, 5.30; N, 14.73. Found: C, 62.98; H, 5.21; N, 15.04.

*4.4.5. Benzyl (3S)-4-(1H-1,2,3-benzotriazol-1-yl)-3-[((2R)-2-[(benzyloxy)carbonyl]amino-3-methylbutanoyl)amino]-4-oxobutanoate (Cbz-D-Val-Asp(OBn)-Bt, **9l**)*

Yield: 86%; white microcrystals; mp 109–110 °C; $[\alpha]_D^{23}$ −10.8 (*c* 1.80, DMF). ^{1}H NMR: δ=9.05 (d, *J*=6.7 Hz, 1H), 8.27 (d, *J*=8.4 Hz, 1H), 8.21 (d, *J*=8.2 Hz, 1H), 7.81 (t, *J*=7.6 Hz, 1H), 7.64 (t, *J*=7.7 Hz, 1H), 7.36–7.29 (m, 11H), 5.98 (dd, *J*=13.7, 6.7 Hz, 1H), 5.17–4.98 (m, 4H), 3.97 (dd, *J*=15.3, 6.7 Hz, 1H), 3.30 (dd, *J*=16.8, 5.4 Hz, 1H), 3.01 (dd, *J*=16.8, 8.4 Hz, 1H), 1.96–1.89 (m, 1H), 0.81 (d, *J*=6.9 Hz, 3H), 0.75 (d, *J*=6.6 Hz, 3H). ^{13}C NMR: δ=171.8, 169.9, 169.6, 156.3, 145.5, 137.2, 135.8, 131.2, 130.8, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 126.9, 120.4, 114.1, 66.3, 65.6, 59.9, 49.7, 35.5, 30.6, 19.2, 18.0. Anal. Calcd for $C_{30}H_{31}N_5O_6$: C, 64.62; H, 5.60; N, 12.56. Found: C, 64.55; H, 5.59; N, 12.28.

4.5. Dipeptide primary amides 10: general procedure

To a solution of *N*-protected dipeptidoylbenzotriazoles **9a–l**, (**9a**+**9a′**), and (**9e**+**9e′**) (0.2 mmol) in THF (10 mL) was added NH_4OH (2 mmol) in THF (10 mL) at 0 °C and the reaction mixture was stirred for 2 h at 0 °C. THF was removed in vacuo, and the solid formed was dissolved in EtOAc (50 mL), washed with 5% Na_2CO_3 (3×15 mL), dried over $MgSO_4$. Removing solvents under reduced pressure gave an oil, which was recrystallized from EtOAc/hexanes to give **10a–l**, (**10a**+**10a′**), and (**10e**+**10e′**).

*4.5.1. Benzyl N-[(1S)-2-[(1S)-2-amino-1-methyl-2-oxoethyl]-amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl]carbamate (Cbz-L-Trp-L-Ala-NH$_2$, **10a**)*

Yield: 79%; white microcrystals; mp 168–170 °C; $[\alpha]_D^{23}$ −9.5 (*c* 1.42, DMF). ^{1}H NMR: δ=10.86 (s, 1H), 8.09 (d, *J*=7.4 Hz, 1H), 7.68 (d, *J*=7.7 Hz, 1H), 7.47 (d, *J*=8.1 Hz, 1H), 7.39–7.27 (m, 7H), 7.20 (s, 1H), 7.13–7.08 (m, 2H), 7.03–6.99 (m, 1H), 4.99 (s, 2H), 4.36–4.25 (m, 2H), 3.18 (dd, *J*=14.6, 4.0 Hz, 1H), 2.77 (dd, *J*=14.3, 9.9 Hz, 1H), 1.26 (d, *J*=7.0 Hz, 3H). ^{13}C NMR: δ=174.2, 171.4, 155.9, 137.0, 136.1, 128.3, 127.7, 127.4, 127.3, 123.9, 120.9, 118.6, 118.2, 111.3, 110.2, 65.3, 55.6, 48.0, 27.7, 18.5. IR: ν_{max} 3250, 3090, 3045, 2925, 1705, 1640, 1530, 1515, 1490, 1440 cm^{-1}. Anal. Calcd for $C_{22}H_{24}N_4O_4$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.95; H, 6.00; N, 13.32.

*4.5.2. Benzyl N-((1S)-2-[(1S)-2-amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl]amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl)carbamate (Cbz-L-Trp-L-Trp-NH$_2$, **10b**)*

Yield: 96%; white microcrystals; mp 192–194 °C; $[\alpha]_D^{23}$ −36.6 (*c* 1.68, DMF). ^{1}H NMR: δ=10.84 (s, 1H), 10.81 (s, 1H), 8.03 (d, *J*=7.7 Hz, 1H), 7.61 (d, *J*=7.7 Hz, 2H), 7.44–7.44 (m, 2H), 7.34–7.27 (m, 5H), 7.24–7.22 (m, 2H), 7.16 (s, 1H), 7.11–7.08 (m, 2H), 7.06–7.03 (m, 2H), 6.99–6.94 (m, 2H), 4.94 (s, 2H), 4.56–4.52 (m, 1H), 4.33–4.28 (m, 1H), 3.18–2.99 (m, 3H), 2.88 (d, *J*=14.2, 10.2 Hz, 1H). ^{13}C NMR: δ=173.4, 171.7, 156.0, 137.1, 136.2, 136.2, 128.5, 127.8, 127.6, 127.6, 127.4, 123.9, 123.7, 121.0, 118.7, 118.4, 111.5, 111.4, 110.4, 110.1, 65.5, 55.9, 53.3, 28.0, 27.8. Anal. Calcd for $C_{30}H_{29}N_5O_4$: C, 68.82; H, 5.58; N, 13.38. Found: C, 68.65; H, 5.62; N, 13.40.

*4.5.3. Benzyl N-[(1S)-2-[(2-amino-2-oxoethyl)amino]-1-(1H-indol-2-ylmethyl)-2-oxoethyl]carbamate (Cbz-L-Trp-Gly-NH$_2$, **10c**)*

Yield: 86%; white microcrystals; mp 151–155 °C; $[\alpha]_D^{23}$ −26.4 (*c* 1.82, DMF). ^{1}H NMR: δ=10.86 (s, 1H), 8.32 (t, *J*=5.6 Hz, 1H), 7.66 (d, *J*=7.7 Hz, 1H), 7.54 (d, *J*=7.8 Hz, 1H), 7.39–7.35 (m, 3H), 7.32–7.28 (m, 3H), 7.23–7.21 (m, 2H), 7.16–7.08 (m, 2H), 7.01 (t, *J*=7.4 Hz, 1H), 4.99 (s, 2H), 4.36–4.29 (m, 1H), 3.70 (dd, *J*=12.4, 5.7 Hz, 2H), 3.19 (dd, *J*=14.6, 4.4 Hz, 1H), 2.97 (dd, *J*=14.6, 9.7 Hz, 1H). ^{13}C NMR: δ=172.2, 171.0, 156.2, 137.1, 136.2, 128.5, 127.9, 127.7, 127.4, 124.0, 121.0, 118.6, 118.4, 111.5, 110.3, 65.5, 55.8, 42.2, 27.7. Anal. Calcd for $C_{21}H_{22}N_4O_4$: C, 63.95; H, 5.62; N, 14.20. Found: C, 64.10; H, 5.68; N, 13.82.

*4.5.4. Benzyl N-((1S)-2-[(1S)-2-amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl]amino-1-methyl-2-oxoethyl)carbamate (Cbz-L-Ala-L-Trp-NH$_2$, **10d**)*

Yield: 77%; white microcrystals; mp 201–202 °C; $[\alpha]_D^{23}$ −7.6 (*c* 1.62, DMF). ^{1}H NMR: δ=10.86 (s, 1H), 7.88 (d, *J*=8.0 Hz, 1H), 7.62 (d, *J*=7.7 Hz, 1H), 7.53 (d, *J*=7.3 Hz, 1H), 7.43–7.34 (m, 7H), 7.16–7.11 (m, 2H), 7.09–7.02 (m, 1H), 7.00 (t, *J*=7.3 Hz, 1H), 5.05 (AB, J_{AB}=19.4, 12.6 Hz, 2H), 4.50 (dd, *J*=13.1, 7.4 Hz, 1H), 4.09–4.06 (m, 1H), 3.16 (dd, *J*=14.7, 5.1 Hz, 1H), 3.03 (dd, *J*=14.6, 8.0 Hz, 1H), 1.19 (d, *J*=7.1 Hz, 3H). ^{13}C NMR: δ=173.2, 172.2, 155.8, 137.0, 136.0, 128.4, 127.8, 127.4, 123.5, 120.8, 118.5, 118.2, 111.2, 110.0, 65.5, 53.0, 50.3, 27.6, 18.0. Anal. Calcd for $C_{22}H_{24}N_4O_4$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.78; H, 5.99; N, 13.51.

*4.5.5. Benzyl (4S)-5-amino-4-[((2S)-2-[(benzyloxy)carbonyl]-aminopropanoyl)amino]-5-oxopentanoate (Cbz-L-Ala-L-Glu(4-OBn)-NH$_2$, **10e**)*

Yield: 98%; white microcrystals; mp 162–163 °C; $[\alpha]_D^{23}$ −3.0 (*c* 1.32, DMF). ^{1}H NMR: δ=7.90 (d, *J*=8.0 Hz, 1H), 7.53 (d, *J*=7.1 Hz, 1H), 7.36–7.34 (m, 11H), 7.13 (s, 1H), 5.09 (s, 2H), 5.01 (d, *J*=7.7 Hz, 2H), 4.23 (dd, *J*=13.2, 8.1 Hz, 1H), 4.05 (t, *J*=7.1 Hz, 1H), 2.37 (t, *J*=7.8 Hz, 2H), 2.09–2.00 (m, 1H), 1.84–1.77 (m, 1H), 2.40 (d, *J*=7.0 Hz, 3H). ^{13}C NMR: δ=172.9, 172.4, 155.8, 137.0, 136.2, 128.4, 128.3, 128.0, 127.9, 127.7, 65.5, 65.4, 51.5, 50.2, 30.0, 27.3, 17.9. Anal. Calcd for $C_{23}H_{27}N_3O_6$: C, 62.57; H, 6.16; N, 9.52. Found: C, 62.57; H, 6.19; N, 9.45.

*4.5.6. Benzyl (4S)-5-amino-4-[(2-[(benzyloxy)carbonyl]-aminopropanoyl)amino]-5-oxopentanoate (Cbz-DL-Ala-L-Glu(4-OBn)-NH$_2$, (**10e**+**10e′**))*

Yield: 90%; white microcrystals; mp 138–139 °C; $[\alpha]_D^{23}$ −4.7 (*c* 1.33, DMF). ^{1}H NMR: δ=8.12–7.93 (m, 1H), 7.58 (t, *J*=6.2 Hz, 1H), 7.44–7.34 (m, 11H), 7.19 (d, *J*=15.5 Hz, 1H), 5.12 (s, 2H), 5.05 (s, 2H), 4.29–4.23 (m, 1H), 4.13–4.04 (m, 1H), 2.44–2.36 (m, 2H), 2.10–1.98 (m, 1H), 1.88–1.81 (m, 1H), 1.23 (dd, *J*=7.0, 1.2 Hz, 3H). ^{13}C NMR: δ=172.9, 172.8, 172.6, 172.4, 172.3, 155.9, 155.8, 137.0, 136.9, 136.2, 128.4, 128.3, 128.0, 127.9, 127.8, 65.5, 51.4, 50.2, 30.0, 27.3, 27.0, 18.0. Anal. Calcd for $C_{23}H_{27}N_3O_6$: C, 62.57; H, 6.16; N, 9.52. Found: C, 62.42; H, 6.16; N, 9.65.

*4.5.7. Benzyl N-(1S)-2-[(2S)-2-(aminocarbonyl)tetrahydro-1H-pyrrol-1-yl]-1-methyl-2-oxoethylcarbamate (Cbz-L-Ala-L-Pro-NH$_2$, **10f**)*

Yield: 95%; white microcrystals; mp 161–162 °C; $[\alpha]_D^{23}$ −37.6 (*c* 1.88, DMF). ^{1}H NMR: δ=7.52 (d, *J*=7.4 Hz, 1H), 7.39–7.25 (m, 5H), 7.21 (br s, 1H), 6.89 (br s, 1H), 5.00 (s, 2H), 4.31 (t, *J*=7.0 Hz, 1H), 4.21 (dd, *J*=8.20, 3.5 Hz, 1H), 3.59–3.55 (m, 2H), 2.05–1.76 (m, 4H), 1.20 (d, *J*=6.7 Hz, 3H). ^{13}C NMR: δ=173.6, 170.8, 155.7, 137.1, 128.4, 127.8, 127.7, 65.3, 59.5, 48.0, 46.5, 29.2, 24.5, 16.8. Anal. Calcd for $C_{16}H_{21}N_3O_4$: C, 60.18; H, 6.63; N, 13.16. Found: C, 60.00; H, 6.73; N, 12.96.

*4.5.8. Benzyl N-[(1S)-1-([(1S)-2-amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl]aminocarbonyl)-3-(methylsulfanyl)propyl]carbamate (Cbz-L-Met-L-Trp-NH$_2$, **10g**)*

Yield: 91%; white microcrystals; mp 218–220 °C; $[\alpha]_D^{23}$ −12.7 (*c* 1.50, DMF). ^{1}H NMR: δ=10.8 (s, 1H), 7.90 (d, *J*=7.8 Hz, 1H), 7.59 (d, *J*=7.8 Hz, 1H), 7.54 (d, *J*=7.8 Hz, 1H), 7.43 (s, 1H), 7.35–7.27 (m, 6H), 7.13–7.03 (m, 3H), 6.96 (t, *J*=7.3 Hz, 1H), 5.02 (d, *J*=4.4 Hz, 2H), 4.52–4.45 (m, 1H), 4.08–4.04 (m, 1H), 3.13 (dd, *J*=15.0, 5.1 Hz, 1H), 3.00 (dd, *J*=14.9, 8.0 Hz, 1H), 2.39 (t, *J*=7.6 Hz, 2H), 1.99 (s, 3H), 1.84–1.73 (m, 2H). ^{13}C NMR: δ=173.4, 171.3, 156.2, 137.1, 136.1, 128.5, 128.0, 127.9, 127.6, 123.6, 121.0, 118.7, 118.4, 111.4, 110.1, 65.7, 54.2, 53.2, 31.8, 29.7, 27.8, 14.7. Anal. Calcd for $C_{24}H_{28}N_4O_4S$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.69; H, 6.16; N, 11.68.

*4.5.9. Benzyl N-((1S)-2-[(1S)-1-(aminocarbonyl)-3-(methylsulfanyl)propyl]amino-1-benzyl-2-oxoethyl)carbamate (Cbz-L-Phe-L-Met-NH$_2$, **10h**)*

Yield: 94%; white microcrystals; mp 208–210 °C; $[\alpha]_D^{23}$ −14.8 (*c* 0.92, DMF). ^{1}H NMR: δ=8.13 (d, *J*=8.1 Hz, 1H), 7.61 (d, *J*=8.4 Hz, 1H), 7.40–7.19 (m, 11H), 7.15 (s, 1H), 4.98 (s, 2H), 4.36–4.27 (m, 2H), 3.06 (dd, *J*=13.8, 4.1 Hz, 1H), 2.78 (dd, *J*=13.6, 10.6 Hz, 1H), 2.50–2.46 (m, 2H), 2.07 (s, 3H), 2.00–1.95 (m, 1H), 1.85–1.81 (m, 1H). ^{13}C NMR: δ=173.1, 171.6, 156.1, 138.2, 137.1, 129.4, 128.5, 128.2, 127.9, 127.6, 126.4, 65.4, 56.4, 51.9, 38.0, 32.1, 29.7, 14.8. Anal. Calcd for $C_{22}H_{27}N_3O_4S$: C, 61.52; H, 6.34; N, 9.78. Found: C, 61.14; H, 6.39; N, 9.50.

*4.5.10. Benzyl (4S)-5-amino-4-[((2S)-2-[(benzyloxy)carbonyl]-amino-3-phenylpropanoyl)amino]-5-oxopentanoate (Cbz-L-Phe-L-Glu(4-OBn)-NH$_2$, **10i**)*

Yield: 96%; white microcrystals; mp 205–207 °C; $[\alpha]_D^{23}$ −10.6 (*c* 1.1, DMF). ^{1}H NMR: δ=8.10 (d, *J*=8.0 Hz, 1H), 7.60 (d, *J*=8.4 Hz, 1H), 7.41–7.38 (m, 5H), 7.37–7.22 (m, 11H), 7.16 (s, 1H), 5.13 (s, 2H), 4.96 (s, 2H), 4.34–4.24 (m, 2H), 3.05 (dd, *J*=13.9, 4.0 Hz, 1H), 2.78 (dd, *J*=13.6, 10.7 Hz, 1H), 2.41 (t, *J*=8.0 Hz, 2H), 2.06–1.79 (m, 2H). ^{13}C NMR: δ=172.9, 172.4, 171.6, 156.0, 138.2, 137.1, 136.4, 129.3, 128.6, 128.5, 128.2, 128.2, 128.1, 127.9, 127.6, 126.4, 65.7, 65.4, 56.4, 51.8, 37.3, 30.2, 27.5. Anal. Calcd for $C_{29}H_{31}N_3O_6$: C, 66.99; H, 6.04; N, 8.12. Found: C, 67.00; H, 6.10; N, 7.92.

*4.5.11. Benzyl (3S)-4-amino-3-[((2S)-2-[(benzyloxy)carbonyl]-amino-3-phenylpropanoyl)amino]-4-oxobutanoate (Cbz-L-Phe-L-Asp(3-OBn)-NH_2, **10j**)*

Yield: 94%; white microcrystals; mp 163–165 °C; $[\alpha]_D^{23}$ −28.6 (*c* 1.61, DMF). ^{1}H NMR: δ=8.39 (d, *J*=8.0 Hz, 1H), 7.61 (d, *J*=8.1 Hz, 1H), 7.40–7.25 (m, 17H), 5.13 (s, 2H), 4.97 (s, 2H), 4.61 (q, *J*=7.7 Hz, 1H), 4.34–4.30 (m, 1H), 3.04 (d, *J*=9.9 Hz, 1H), 2.91–2.64 (m, 3H). ^{13}C NMR: δ=172.1, 171.6, 170.3, 156.1, 138.2, 137.1, 136.2, 129.4, 128.6, 128.5, 128.2, 128.1, 128.1, 127.9, 127.6, 126.4, 65.9, 65.4, 56.4, 49.5, 37.4, 36.4. Anal. Calcd for $C_{28}H_{29}N_3O_6$: C, 66.79; H, 5.80; N, 8.34. Found: C, 66.55; H, 5.82; N, 8.20.

*4.5.12. Benzyl N-(5S)-6-amino-5-[((2S)-2-[(benzyloxy)carbonyl]-amino-3-phenylpropanoyl)amino]-6-oxohexylcarbamate (Cbz-L-Phe-L-N^6-Lys-NH_2, **10k**)*

Yield: 92%; white microcrystals; mp 193–195 °C; $[\alpha]_D^{23}$ −8.5 (*c* 1.68, DMF). ^{1}H NMR: δ=8.05 (d, *J*=8.0 Hz, 1H), 7.59 (d, *J*=8.5 Hz, 1H), 7.38–7.26 (m, 17H), 7.09 (s, 1H), 5.04 (s, 2H), 4.98 (s, 2H), 4.34–4.20 (m, 2H), 3.09–2.98 (m, 3H), 2.78 (t, *J*=12.6 Hz, 1H), 1.74–1.52 (m, 2H), 1.46–1.40 (m 2H), 1.34–1.27 (m, 2H). ^{13}C NMR: δ=173.6, 171.5, 156.2, 156.0, 138.3, 137.4, 137.2, 129.4, 128.5, 128.4, 128.2, 127.9, 127.8, 127.6, 127.5, 126.4, 65.4, 65.3, 56.4, 52.5, 40.5, 37.5, 32.7, 29.3, 22.7. Anal. Calcd for $C_{31}H_{36}N_4O_6$: C, 66.41; H, 6.47; N, 9.99. Found: C, 66.20; H, 6.37; N, 9.70.

*4.5.13. Benzyl (3S)-4-amino-3-[((2R)-2-[(benzyloxy)carbonyl]-amino-3-methylbutanoyl)amino]-4-oxobutanoate (Cbz-D-Val-L-Asp(3-OBn)-NH_2, **10l**)*

Yield: 88%; white microcrystals; mp 195–195 °C; $[\alpha]_D^{23}$ −33.0 (*c* 1.70, DMF). ^{1}H NMR: δ=8.46 (d, *J*=8.4 Hz, 1H), 7.49 (d, *J*=7.4 Hz, 1H), 7.40–7.23 (m, 12H), 5.06 (d, *J*=2.1 Hz, 2H), 5.02 (s, 2H), 4.68–4.61 (m, 1H), 3.75 (t, *J*=7.4 Hz, 1H), 2.89 (dd, *J*=16.1, 4.8 Hz, 1H), 2.60 (dd, *J*=16.2, 9.1 Hz, 1H), 1.91–1.85 (m, 1H), 0.84–0.78 (m, 6H). ^{13}C NMR: δ=172.2, 171.4, 170.3, 156.5, 136.9, 136.0, 128.4, 128.0, 127.9, 127.9, 65.7, 65.5, 60.8, 49.2, 36.1, 29.6, 19.0, 18.6. Anal. Calcd for $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.22. Found: C, 63.18; H, 6.48; N, 9.03.

4.6. Tripeptide primary amides 13: general procedure

A mixture of *N*-unprotected amino amide **12a**,**b** (0.2 mmol) and *N*-protected dipeptidoylbenzotriazoles **9a**–**l**, (**9a**+**9a**′), and (**9e**+**9e**′) (0.2 mmol) in acetonitrile (10 mL) was stirred for 6 h at −15 °C. Acetonitrile was removed in vacuo, and the solid formed was dissolved in EtOAc (50 mL), washed with 5% Na_2CO_3 (3×15 mL), and dried over $MgSO_4$. Removing solvents under reduced pressure gave oil, which was recrystallized from EtOAc/hexanes to give **13a**,**b** and (**13a**+**13a**′).

*4.6.1. Benzyl N-((1S)-1-[((1S)-2-[(1S)-1-(aminocarbonyl)-3-methylsulfanyl)propyl]amino-1-methyl-2-oxoethylamino)-1-(1H-indol-2-ylmethyl)-2-oxoethyl]carbamate (Cbz-L-Trp-L-Ala-L-Met-NH_2, **13a**)*

Yield: 82%; white microcrystals; mp 222–224 °C. ^{1}H NMR: δ=10.86 (s, 1H), 8.33–8.28 (m, 1H), 7.92–7.90 (m, 1H), 7.71 (d, *J*=8.0 Hz, 1H), 7.51 (d, *J*=8.1 Hz, 1H), 7.38–7.27 (m, 7H), 7.21–7.17 (m, 2H), 7.10 (t, *J*=7.4 Hz, 1H), 7.01 (t, *J*=7.3 Hz, 1H), 5.00–4.97 (m, 2H), 4.37–4.24 (m, 3H), 3.18–3.14 (m, 1H), 2.98–2.90 (m, 1H), 2.51–2.39 (m, 2H), 2.06 (s, 3H), 2.00–1.94 (m, 1H), 1.88–1.76 (m, 1H), 1.28–1.12 (m, 3H). ^{13}C NMR: δ=173.1, 172.2, 172.1, 156.1, 137.1, 136.2, 128.5, 127.8, 127.7, 127.6, 127.4, 124.1, 121.0, 118.8, 118.3, 111.4, 110.3, 65.4, 55.6, 51.8, 48.7, 32.1, 29.7, 17.9, 14.8. Anal. Calcd for $C_{27}H_{33}N_5O_5S$: C, 60.09; H, 6.16; N, 12.98. Found: C, 59.78; H, 6.25; N, 12.68.

*4.6.2. Benzyl N-[(1S)-2-(2-[(2-amino-2-oxoethyl)amino]-1-methyl-2-oxoethylamino)-1-(1H-indol-2-ylmethyl)-2-oxoethyl]-carbamate (Cbz-L-Trp-DL-Ala-Gly-NH_2, (**13a**+**13a**′))*

Yield: 86%; white microcrystals; mp 164–166 °C. ^{1}H NMR: δ=10.84 (s, 1H), 8.36–8.28 (m, 1H), 8.06–8.00 (m, 1H), 7.68–7.60 (m, 1H), 7.53–7.46 (m, 1H), 7.35–7.24 (m, 7H), 7.17–7.13 (m, 2H), 7.06 (t, *J*=7.4 Hz, 1H), 6.97 (t, *J*=7.2 Hz, 1H), 4.99 (s, 2H), 4.29–4.23 (m, 2H), 3.70–3.60 (m, 2H), 3.16–3.06 (m, 1H), 2.98–2.87 (m, 1H), 1.24–1.12 (m, 3H). ^{13}C NMR: δ=172.3, 172.2, 172.0, 170.9, 156.1, 156.0, 136.9, 137.0, 136.9, 136.1, 128.3, 127.8, 127.7, 127.5, 127.3, 124.0, 120.8, 118.6, 118.5, 118.2, 111.3, 110.1, 109.8, 65.5, 65.3, 55.7, 55.5, 48.6, 41.9, 27.6, 17.8, 17.7. HRMS calcd for $C_{24}H_{27}N_5O_5$ $[M+H]^+$ 466.2085, found 466.2063.

*4.6.3. Benzyl N-((1S)-2-[(1S)-2-[(2-amino-2-oxoethyl)amino]-1-(1H-indol-2-ylmethyl)-2-oxoethyl]amino-1-(1H-indol-2-ylmethyl)-2-oxoethyl)carbamate (Cbz-L-Trp-L-Trp-Gly-NH_2, **13b**)*

Yield: 86%; white microcrystals; mp 220–221 °C. ^{1}H NMR: δ=10.90 (s, 1H), 10.85 (s, 1H), 8.30–8.23 (m, 2H), 7.68–7.61 (m, 2H), 7.50 (d, *J*=8.2 Hz, 1H), 7.38–7.32 (m, 5H), 7.29–7.23 (m, 4H), 7.18–7.15 (m, 2H), 7.10 (t, *J*=7.4 Hz, 2H), 7.04–6.98 (m, 2H), 4.98 (s, 2H), 4.59 (q, *J*=12.9 Hz, 1H), 4.38–4.30 (m, 1H), 3.74 (dd, *J*=16.8, 6.0 Hz, 1H), 3.60 (dd, *J*=16.8, 5.3 Hz, 1H), 3.24 (dd, *J*=16.7, 5.0 Hz, 1H), 3.14–3.04 (m, 2H), 2.91 (dd, *J*=14.6, 10.2 Hz, 1H). ^{13}C NMR: δ=172.1, 171.5, 170.9, 155.9, 136.9, 136.1, 128.3, 127.7, 127.5, 127.4, 127.3, 123.8, 123.7, 120.9, 118.6, 118.4, 118.2, 111.3, 110.2, 109.9, 65.4, 55.6, 53.8, 42.1, 27.6, 27.5. Anal. Calcd for $C_{32}H_{32}N_6O_5$: C, 66.19; H, 5.55; N, 14.47. Found: C, 65.84; H, 5.61; N, 14.28.

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