Highly Diastereoselective Peptide Chain Extensions of Unprotected Amino Acids with *N*-(Z-α-Aminoacyl)benzotriazoles

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Received 13 May 2004; revised 8 June 2004

Abstract: Coupling an unprotected amino acid or dipeptide in partially aqueous solution with a readily available *N*-(Z-α-aminoacyl)benzotriazole or *N*-(Z-α-aminopetidoyl)benzotriazole affords N-terminal-protected di-, tri-, and tetrapeptides in yields of 85–98% (average 95% for **2a–i**, 93% for **4a–f** and **4a′**, 86% for **5a–b**) with minimal epimerization.

Key words: benzotriazole methodology, *N*-(Z-α-aminoacyl)benzotriazole, peptide coupling, peptides

The many coupling reagents[1a,b] developed for the formation of amide bonds in the synthesis of biologically active peptides and their analogs[2a–c] include: (i) carbodiimides in combination with additives such as 1-hydroxybenzotriazole (HOBt),[3a,b] 1-hydroxy-7-azabenzotriazole (HOAt) and analogs[4] or *N*-hydroxysuccinimide (HOSu);[5] (ii) phosphonium[6,7] and uronium salts[8,9] of HOBt or HOAt; (iii) *N*-acylazoles such as 1,1′-carbonylbis(1*H*-imidazole) (CDI);[10] (iv) mixed anhydrides;[11] or (v) carboxylic acid fluorides.[12a,b]

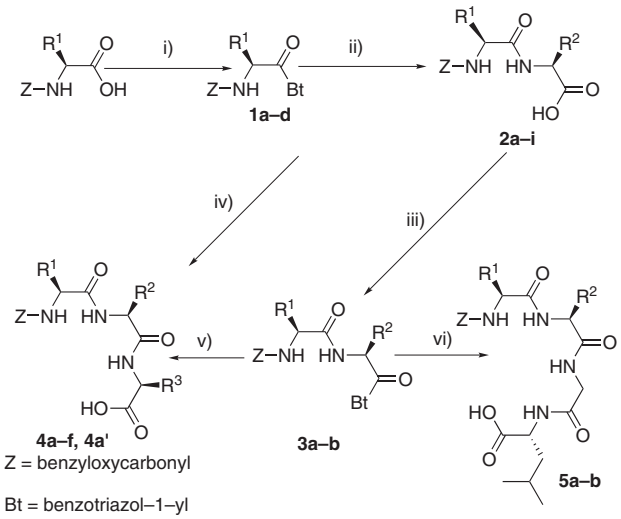
A commonly encountered problem in peptide synthesis is epimerization of the amino acid component during activation of the carboxylic acid group. Many of the coupling reagents require prior protection and subsequent deprotection of various amino acid functional groups.[13] Coupling reactions with such reagents are frequently moisture sensitive. Furthermore, isolation and purification processes often involve column chromatography due to the formation of by-products from the coupling reagents.

The literature reveals that the reactions of N-protected C-activated amino acids with unprotected amino acids have been less explored as compared to their reactions with C-protected amino acids. Hegarty in 1980 reported peptide coupling of unprotected amino acids with imidoyl halides RC(=NNR′$_2$)X (derivatives of acid hydrazides) as condensation reagents; they observed 1.0–21.0% of racemization at pH 7.2–9.3.[14] *N*-Hydroxysuccinimide esters of amino acids couple with unprotected amino acids in dioxane in the presence of sodium hydroxide.[15,16] Recent one-pot two-step preparations of di- and tripeptides couple unprotected amino acids in aqueous acetonitrile with *p*-nitrophenyl esters of N-protected amino acids in 15–98% yields, with high retention of chirality.[16]

N-Acylbenzotriazoles are neutral efficient coupling reagents for: (i) preparation of primary, secondary, and tertiary amides;[17] (ii) C-acylation of pyrroles and indoles,[18] and 2-methylfuran and thiophene;[19] and (iii) acylation of primary and secondary alkyl cyanides.[20]

N-Acylbenzotriazoles are sufficiently reactive to form amide bonds at ambient temperature, but stable enough to resist side reactions. We previously prepared aminoamides from 1-(α-Boc-aminoacyl)benzotriazoles and amines in 82–99% yields with no detectable racemization.[21] Advantageously, *N*-acylbenzotriazoles are usually crystalline and can be stored at room temperature for long periods. We report herein the preparation of N-terminal protected peptides by reactions of *N*-acylbenzotriazoles with unprotected amino acids in aqueous/organic solvents in a broadly applicable simple and efficient coupling method (Scheme 1).

The Z group is a favorite protecting group due to (i) its stability towards both acidic and basic conditions, (ii) easy purification of solid Z-protected amino acids and peptides, and (iii) its ready cleavage by hydrogenation.[1b,16]



Scheme 1 *Reagents and conditions*: i) $SOCl_2$, BtH at 25 °C; ii) unprotected amino acid, Et_3N in MeCN–H_2O; iii) $SOCl_2$, BtH at 0 °C; iv) H-Gly-Leu-OH or H-Gly-Gly-OH, Et_3N in MeCN–H_2O; v) unprotected amino acid, Et_3N in MeCN–H_2O; vi) Gly-Leu-OH, Et_3N in MeCN–H_2O.

SYNTHESIS 2004, No. 16, pp 2645–2652
Advanced online publication: 06.10.2004
DOI: 10.1055/s-2004-831255; Art ID: M03404SS

The Boc group is also a popular protecting group,[5,22] but is not preferred under strongly acidic conditions.

Chiral 1-(α-Boc-aminoacyl)benzotriazoles were previously prepared by the reaction of $BtSO_2Me$ with Boc-protected amino acids in refluxing THF in the presence of Et_3N with no detectable racemization.[21] Although Z-Ala-OH and Z-Phe-OH produced the corresponding *N*-acylbenzotriazole derivatives with 15–50% of racemization under these conditions, our recently developed mild alternative procedure for the preparation of *N*-acylbenzotriazoles proved beneficial.[23] Under this protocol, the *N*-Z-amino acid was reacted with four equivalents of benzotriazole and one equivalent of $SOCl_2$ in CH_2Cl_2 at room temperature for 2 hours to give *N*-(Z-α-aminoacyl)benzotriazoles **1a–d** in 85–95% yields; compounds **1a–c** were obtained with minimal racemization (Table 1).

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

Entry	Compound	Yield (%)	Mp (°C)	$[\alpha]_D^{25}$
1	Z-Ala-Bt (**1a**)	95	114–115	−0.8
2	Z-Val-Bt (**1b**)	91	73–74	−32.5
3	Z-Phe-Bt (**1c**)	88	151–152	+18.6
4	Z-DL-Ala-Bt (**1d**)	94	112–113	–
5	Z-Phe-Ala-Bt (**3a**)	85	180–181	−57.1
6	Z-Ala-Phe-Bt (**3b**)	90	148–149	−8.7

To test the optical purity of *N*-(Z-α-aminoacyl)benzotriazoles **1a–c** prepared by the above procedure with commercially available enantiomerically pure unprotected amino acids,[23] we have performed ^1H NMR analysis of the crude dipeptides **2**. Thus, Z-DL-Ala-L-Phe-OH, prepared by coupling Z-DL-Ala-Bt (**1d**) with L-Phe-OH, showed two separate doublets for the methyl protons at 1.25 and 1.20 ppm corresponding to the LL- and DL-diastereomers, respectively. In comparison, Z-L-Ala-L-Phe-OH (**2a**) prepared by the coupling of Z-L-Ala-Bt (**1a**) with L-Phe-OH showed a single doublet in the ^1H NMR spectrum at 1.25 ppm. Similarly partially racemized Z-L-Phe-Bt with L-Ala formed two diastereomers (Z-DL-Phe-L-Ala-OH) with signals at 1.32 and 1.23 ppm in the ^1H NMR spectrum while Z-L-Phe-L-Ala-OH (**2f**) prepared from Z-L-Phe-Bt (**1c**) and L-Ala-OH showed a single doublet for the methyl group at 1.32 ppm (see Figure 1). Compounds **1a–d** are novel compounds which were fully characterized by ^1H and ^{13}C NMR spectroscopy and elemental analysis.

Coupling reactions of **1a–d** were carried out with diverse unprotected amino acids in partially aqueous solution ($MeCN–H_2O$) in the presence of Et_3N for 10 to 40 minutes. After washing with 6 N HCl, the resulting peptides **2a–i** were obtained in 85–98% yields (Table 2). The crude products were estimated to be >95% pure and their absence of epimerization was established by ^1H NMR experiments. Thus, Z-L-Phe-Bt (**1c**) was reacted with racemic DL-Ala-OH. While enantiopure Z-L-Phe-L-Ala-OH (**2f**) showed the methyl group on the alanine fragment at 1.32 ppm as a doublet, the methyl groups in diastereomers Z-L-Phe-D-Ala-OH and Z-L-Phe-L-Ala-OH resonated at 1.23 and 1.32 ppm, respectively. Furthermore, the dipeptides were analyzed by HPLC (detection at 254 nm, flow rate 1.0 mL/min, and solvents 50:50 MeOH–H_2O contained 0.1% TFA); while Z-L-Phe-DL-Ala-OH gave two peaks at 15.1 and 19.8 min, Z-L-Phe-L-Ala-OH (**2f**) showed only a single peak at 15.1 min. This result confirmed minimal epimerization in the reaction.

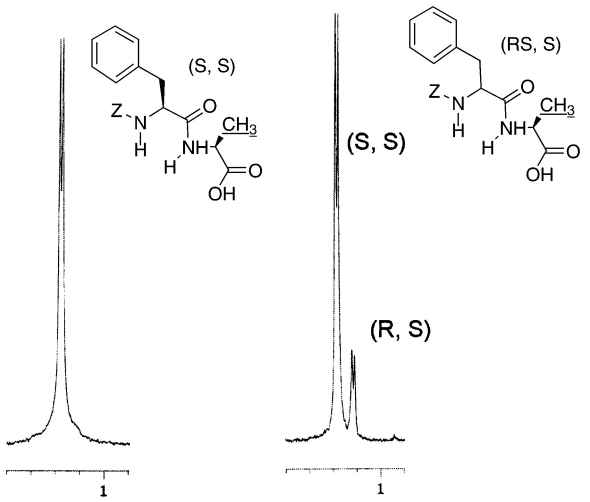


Figure 1 ^1H NMR spectra of compound **2f** (left) and partially racemized **2f** (right) in $CDCl_3$ (CH_3 signal in L-Ala)

Z-Phe-Ala-Bt (**3a**) and Z-Ala-Phe-Bt (**3b**) were prepared from *N*-Z-protected dipeptides by reaction with benzotriazole and $SOCl_2$ at 0 °C for 2 h. This reaction proceeded at 0 °C without visible racemization in NMR (i.e. <5.0% as indicated by ^1H NMR of the crude products), and gave **3a** and **3b** in 85% and 90% yields, respectively (Table 1). However, at 25 °C, 5–15% racemization was observed: the methyl group in Z-L-Phe-DL-Ala-Bt showed peaks at 1.58 ppm (LL) and 1.47 ppm (LD). Compounds **3a** and **3b** are novel compounds and were fully characterized by ^1H and ^{13}C NMR spectroscopy and elemental analysis.

Tripeptides **4a–f** were prepared according to two different protocols: (i) reactions of *N*-(Z-α-aminoacyl)benzotriazoles **1a**, **1b**, and **1c** with free dipeptides, H-Gly-L-Leu-OH and H-Gly-Gly-OH; (ii) reactions of *N*-(Z-α-aminopeptidoyl)benzotriazoles **3a** and **3b** with free amino acids (see Scheme 1 and Table 3). The reaction conditions were similar to those described above for the preparation of the dipeptides **2a–i**, but longer reaction times (around 30 to 120 min) were required. After workup as described above for the preparation of the dipeptides **2a–i**, the enantiopure tripeptides **4a–f** were obtained in 85–98% yields (Table 4). In order to check the enantiopurity, a racemic mixture of Z-L-Ala-Gly-L-Leu-OH (**4a**) and Z-D-Ala-Gly-L-Leu-OH (**4a′**) was prepared from racemic com-

Table 2 Preparation of *N*-Z-Dipeptides from *N*-(Z-α-Aminoacyl)benzotriazoles and Unprotected Amino Acids

Entry	RCOBt Reactant	Amino Acid	Product	Yield (%)	Previous Work		
					Reagent	Yield (%)	Lit.
1	**1a**	Phe	Z-Ala-Phe-OH (**2a**)	90	Ketone oxime[a]	79[b]	–[24a]
2	**1a**	Ser	Z-Ala-Ser-OH (**2b**)	85	1,3-Thiazoline-2-thione[c]	54[d]	–[25]
3	**1a**	Try	Z-Ala-Trp-OH (**2c**)	97	DCC	79	–[26]
4	**1b**	Phe	Z-Val-Phe-OH (**2d**)	98	Rhodium chloride[e]	77	–[27]
5	**1b**	Try	Z-Val-Trp-OH (**2e**)	96	DCC/HOBt	–[f]	–[28]
6	**1c**	Ala	Z-Phe-Ala-OH (**2f**)	98	(±)-Benzoin[g]	64	–[29]
7	**1c**	Val	Z-Phe-Val-OH (**2g**)	95	HOSu[h]/DCC	54	–[30]
8	**1c**	Phe	Z-Phe-Phe-OH (**2h**)	98	DBP[i]	91[j]	–[31]
9	**1c**	Ser	Z-Phe-Ser-OH (**2i**)	96	*p*-Nitrophenol	67	–[16]

[a] (i) Di-2-pyridyl ketone or acetone oxime, EDC and DMAP; (ii) Phe-OH with phosphazene base.
[b] Overall yield of the two-step reaction.
[c] (i) DCC is required; (ii) Ser-OH, Et_3N in THF–H_2O (1:1).
[d] Overall yield of the two-step reaction.
[e] Tris(triphenyl phosphane)rhodium(I) chloride for cleavage of allyl-C-terminal protection.
[f] No data.
[g] (i) Addition of DCC and DMAP, followed by NH_4OAC and HOAc; (ii) O_2, hν, then Ala-OH.
[h] 1-Hydroxysuccinimide.
[i] 1-(α-Diazobenzyl)pyrene for activation of the amino acid.
[j] Yield by photolysis of the ester in the three-step reaction.

pound **1d** with H-Gly-L-Leu-OH for comparison with the enantiopure tripeptide **4a**. The ^1H NMR of the mixture (**4a** + **4a′**) showed broadened peaks for protons of two methyl groups in the *iso*-butyl group and complicated multiplets for three NH protons (7.55, 7.91 and 8.17 ppm) while **4a** gave two sharp doublets for the methyl groups and two doublets and a broad singlet for the NH protons. In the ^{13}C NMR spectrum, the **4a**/**4a′** mixture of diastereomers gave separate signals at 50.0 [from Z-L-Ala-Gly-L-Leu-OH (**4a**)] and 50.2 ppm [from Z-D-Ala-Gly-L-Leu-OH (**4a′**)], but many other signals from **4a** and **4a′** overlapped. Moreover, HPLC was utilized to confirm the negligible racemization; **4a** showed a single peak at 11.7 min when a mixture of **4a** and **4b** showed two peaks at 11.7 and 14.1 min (detection at 230 and 254 nm, flow rate 1.0 mL/min, and solvents 50:50 MeOH–H_2O contained 0.1% TFA). Tripeptides **4b**, **4e**, and **4f** are novel compounds, and were

Table 3 Preparation of *N*-Z-Tripeptides (i) from *N*-(Z-α-Aminoacyl)benzotriazoles and Unprotected Dipeptides (Entries 1–4) (ii) from *N*-(Z-α-Aminopeptidoyl)benzotriazoles and Unprotected Amino Acids (Entries 5–7)

Entry	RCOBt Reactant	Amino Acid or Dipeptide	Product	Yield (%)	Previous Work		
					Reagent	Yield (%)	Lit.
1	**1a**	Gly-Leu	Z-Ala-Gly-Leu-OH (**4a**)	93	–	–	–
2	**1d**	Gly-Leu	Z-DL-Ala-Gly-Leu-OH (**4a + 4a′**)	94	–	–	–
3	**1b**	Gly-Leu	Z-Val-Gly-Leu-OH (**4b**)	85	–	–	–
4	**1c**	Gly-Gyl	Z-Phe-Gly-Gly-OH (**4c**)	98	*p*-Nitrophenol	84	–[32]
5	**3a**	Ala	Z-Phe-Ala-Ala-OH (**4d**)	92	Ethyl chloroformate[a]	71[b]	–[33]
6	**3a**	Ser	Z-Phe-Ala-Ser-OH (**4e**)	94	–	–	–
7	**3b**	Try	Z-Ala-Phe-Trp-OH (**4f**)	95	–	–	–

[a] Coupling of Z-Phe-OH with Aal-Ala-OMe.
[b] Overall yield (The coupling reaction 89% and the C-terminal deprotection 80%).

characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis, and optical rotation.

Reactions of **3a** and **3b** with Gly-L-Leu-OH for 2–4 hours gave tetrapeptides **5a** and **5b** in 86% and 85% yields, respectively (Table 4). These novel compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis, and optical rotation.

Table 4 Preparation of *N*-Z-Tetrapeptides from *N*-(Z-α-Aminopeptidoyl)benzotriazoles and an Unprotected Dipeptide

Entry	RCOBt Reactant	Dipeptide	Product	Yield (%)
1	**3a**	Gly-Leu	Z-Phe-Ala-Gly-Leu-OH (**5a**)	86
2	**3b**	Gly-Leu	Z-Ala-Phe-Gly-Leu-OH (**5b**)	85

In summary, *N*-acylbenzotriazoles derived from N-protected amino acids or peptides have been introduced as new coupling reagents. The peptide coupling reaction utilizing the *N*-acylbenzotriazole derivatives and unprotected amino acids proceeds with minimal epimerization in partially aqueous solution under mild conditions.

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 ^{1}H (300 MHz) and ^{13}C (75 MHz) as the internal reference unless specified otherwise. The HPLC was performed with Chirobiotic T column 4.6 × 250 mm, detection at 254 nm, flow rate 1.0 mL/min, and solvents (MeOH–H_2O contained 0.1% TFA). THF was distilled from sodium metal in the presence of benzophenone under N_2 immediately prior to use. Amino acids and peptides are L-configuration unless specified otherwise.

Preparation of 1a–d and 3a–b; General Procedure

Thionyl chloride (595 mg, 5 mmol) was added to a solution of 1*H*-benzotriazole (2.38 g, 20 mmol) in anhyd THF (15 mL) at 25 °C, and the reaction mixture was stirred for 20 min. To the mixture, was added N-protected amino acid (5 mmol) dissolved in anhyd THF (5 mL) dropwise, and stirred for 1 h at 25 °C. For compounds **3a** and **3b**, the mixture was cooled to 0 °C, and *N*-Z-dipeptide (5 mmol) dissolved in anhyd THF (5 mL) was added dropwise, and stirred at 0 °C for 2 h. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (EtOAc–hexanes, 1:1 for **1a–d**, $CHCl_3$–hexanes, 1:1 for **3a** and **3b**) to give the desired product. Further purification was performed by recrystallization from $CHCl_3$–hexanes for the purpose of elemental analysis.

Benzyl *N*-[(1*S*)-2-(1*H*-1,2,3-Benzotriazol-1-yl)-1-methyl-2-oxoethyl]carbamate (Z-Ala-Bt, 1a)

Colorless fine needles (95%); mp 114–115 °C; $[\alpha]_D^{25}$ –0.8 (*c* = 1.8, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.69 (d, *J* = 7.0 Hz, 3 H, CH_3), 5.11 (d, *J* = 12.2 Hz, 1 H, O OCH_2Ph), 5.17 (d, *J* = 12.2 Hz, 1 H, OCH_2Ph), 5.69 (d, *J* = 7.6 Hz, 1 H, NH), 5.78–5.84 (m, 1 H, NCHCO), 7.14 (br s, 1 H, ArH), 7.36–7.42 (m, 4 H), 7.50–7.55 (m, 1 H, ArH in Bt), 7.67 (td, *J* = 8.1, 0.8 Hz, 1 H, ArH in Bt), 8.13 (d, *J* = 8.2 Hz, 1 H, ArH in Bt), 8.26 (d, *J* = 8.1 Hz, 1 H, ArH in Bt).

^{13}C NMR ($CDCl_3$): δ = 19.0, 50.5, 67.2, 114.3, 120.3, 126.5, 128.1, 128.2, 128.5, 130.7, 131.1, 136.0, 146.0, 155.6, 172.2.

Anal. Calcd for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.97; N, 17.27. Found: C, 63.21; H, 4.88; N, 17.40.

Benzyl *N*-[(1*S*)-1-(1*H*-1,2,3-Benzotriazol-1-ylcarbonyl)-2-methylpropyl]carbamate (Z-Val-Bt, 1b)

Colorless needles (91%); mp 73–74 °C; $[\alpha]_D^{25}$ –32.5 (*c* = 2.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 0.97 (d, *J* = 6.8 Hz, 3 H, CHCH_3), 1.13 (d, *J* = 6.8 Hz, 3 H, CHCH_3), 2.48–2.54 [m, 1 H, CHC*H*(CH_3)$_2$], 5.13 (d, *J* = 12.4 Hz, 1 H, OCH_2Ph), 5.16 (d, *J* = 12.4 Hz, 1 H, OCH_2Ph), 5.68 (d, *J* = 9.0 Hz, 1 H, NH), 5.77 (dd, *J* = 9.0, 4.5 Hz, 1 H, NCHCO), 7.15 (br s, 1 H, ArH), 7.36 (br s, 4 H, ArH), 7.50–7.55 (m, 1 H, ArH in Bt), 7.64–7.69 (m, 1 H, ArH in Bt), 8.13 (d, *J* = 8.3 Hz, 1 H, ArH in Bt), 8.27 (d, *J* = 8.2 Hz, 1 H, ArH in Bt).

^{13}C NMR ($CDCl_3$): δ = 16.9, 19.6, 31.6, 59.4, 67.3, 114.3, 120.3, 126.4, 128.1, 128.5, 130.6, 131.0, 136.0, 146.0, 156.2, 171.5.

Anal. Calcd for $C_{19}H_{20}N_4O_3$: C, 64.76; H, 5.72; N, 15.90. Found: C, 64.82; H, 5.77; N, 15.80.

Benzyl *N*-[(1*S*)-2-(1*H*-1,2,3-Benzotriazol-1-yl)-1-benzyl-2-oxoethyl]carbamate (Z-Phe-Bt, 1c)

Colorless plates (89%); mp 151–152 °C; $[\alpha]_D^{25}$ +18.6 (*c* = 2.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 3.23 (dd, *J* = 13.9, 7.7 Hz, 1 H, CHCH_2Ph), 3.49 (dd, *J* = 13.9, 4.9 Hz, 1 H, CHCH_2Ph), 5.09 (s, 2 H, OCH_2Ph), 5.51 (d, *J* = 8.2 Hz, 1 H, NH), 6.05–6.10 (m, 1 H, NCHCO), 7.12–7.14 (m, 2 H), 7.23–7.33 (m, 8 H), 7.53–7.58 (m, 1 H, ArH in Bt), 7.66–7.72 (m, 1 H, ArH in Bt), 8.16 (d, *J* = 8.1 Hz, 1 H, ArH in Bt), 8.24 (d, *J* = 8.1 Hz, 1 H, ArH in Bt).

^{13}C NMR ($CDCl_3$): δ = 38.8, 55.6, 67.2, 114.3, 120.4, 126.6, 127.4, 128.1, 128.5, 128.7, 129.2, 130.8, 134.9, 146.0, 155.7, 170.8.

Anal. Calcd for $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99. Found: C, 69.19; H, 5.11; N, 14.05.

Benzyl *N*-[2-(1*H*-1,2,3-Benzotriazol-1-yl)-1-methyl-2-oxoethyl]carbamate (Z-DL-Ala-Bt, 1d)

Colorless crystals (94%); mp 112–113 °C.

^{1}H NMR ($CDCl_3$): δ = 1.69 (d, *J* = 7.0 Hz, 3 H, CH_3), 5.11 (d, *J* = 12.2 Hz, 1 H, OCH_2Ph), 5.17 (d, *J* = 12.2 Hz, 1 H, OCH_2Ph), 5.69 (d, *J* = 7.6 Hz, 1 H, NH), 5.78–5.84 (m, 1 H, NCHCO), 7.14 (br s, 1 H), 7.36 (s, 4 H), 7.50–7.55 (m, 1 H, ArH in Bt), 7.64–7.70 (m, 1 H, ArH in Bt), 8.13 (d, *J* = 8.2 Hz, 1 H, ArH in Bt), 8.26 (d, *J* = 8.1 Hz, 1 H, ArH in Bt).

^{13}C NMR ($CDCl_3$): δ = 38.8, 55.6, 67.2, 114.3, 120.4, 126.6, 127.4, 128.1, 128.5, 128.7, 129.2, 130.8, 130.9, 134.9, 135.9, 146.0, 155.7, 170.8.

Anal. Calcd for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.97; N, 17.27. Found: C, 63.24; H, 4.96; N, 17.26.

Benzyl *N*-((1*S*)-2-{[(1*S*)-2-(1*H*-1,2,3-Benzotriazol-1-yl)-1-methyl-2-oxoethyl]amino}-1-benzyl-2-oxoethyl)carbamate (Z-Phe-Ala-Bt, 3a)

Colorless needles (85%); mp 180–181 °C; $[\alpha]_D^{25}$ –57.1 (*c* = 0.83, $CHCl_3$).

^{1}H NMR (DMSO-d_6): δ = 1.61 (d, *J* = 7.1 Hz, 3 H, CHCH_3), 2.70–2.78 (m, 1 H, CHCH_2Ph), 3.07 (dd, *J* = 13.6, 3.0 Hz, 1 H, CHCH_2Ph), 4.34–4.41 (m, 1 H, NC*H*CO), 4.93 (s, 2 H, OCH_2Ph), 5.63 (apparent q, *J* = ca. 6.5 Hz, 1 H, NCHCO), 7.21–7.35 (m, 10 H), 7.55 (d, *J* = 8.8 Hz, 1 H, NH), 7.65 (t, *J* = 7.6 Hz, 1 H, ArH in Bt), 7.82 (t, *J* = 7.7 Hz, 1 H, ArH in Bt), 8.25 (d, *J* = 8.3 Hz, 1 H,

ArH in Bt), 8.31 (d, *J* = 8.4 Hz, 1 H, ArH in Bt), 9.02 (d, *J* = 5.5 Hz, 1 H, NH).

^{13}C NMR (DMSO-d_6): δ = 16.6, 37.3, 48.6, 55.6, 65.1, 113.9, 120.1, 126.2, 126.6, 127.4, 127.6, 127.9, 128.2, 129.1, 130.6, 131.0, 136.9, 137.9, 145.3, 155.8, 171.7, 172.0.

Anal. Calcd for $C_{26}H_{25}N_5O_4$: C, 66.23; H, 5.34; N, 14.85. Found: C, 65.80; H, 5.48; N, 14.52.

Benzyl *N*-((1*S*)-2-{[(1*S*)-2-(1*H*-1,2,3-Benzotriazol-1-yl)-1-benzyl-2-oxoethyl]amino}-1-methyl-2-oxoethyl)carbamate (Z-Ala-Phe-Bt, 3b)

Colorless microcrystals (90%); mp 148–149 °C; $[\alpha]_D^{25}$ –8.7 (*c* = 2.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 1.34 (d, *J* = 7.0 Hz, 3 H, CHCH_3), 3.22 (dd, *J* = 14.0, 7.8 Hz, 1 H, CHCH_2Ph), 3.47 (dd, *J* = 14.0, 4.8 Hz, 1 H, CHCH_2Ph), 4.30–4.33 (m, 1 H, NCHCO), 5.07 (d, *J* = 12.2 Hz, 1 H, OCH_2Ph), 5.13 (d, *J* = 12.2 Hz, 1 H, OCH_2Ph), 5.34 (d, *J* = 6.2 Hz, 1 H, NH), 6.20–6.23 (m, 1 H, NCHCO), 7.04–7.35 (m, 11 H), 7.51–7.57 (m, 1 H, ArH in Bt), 7.65–7.70 (m, 1 H, ArH in Bt), 8.15 (d, *J* = 8.2 Hz, 1 H, ArH in Bt), 8.22 (d, *J* = 8.0 Hz, 1 H, ArH in Bt).

^{13}C NMR ($CDCl_3$): δ = 18.1, 38.5, 50.3, 54.1, 67.1, 114.3, 120.4, 126.6, 127.4, 128.1, 128.2, 128.5, 128.6, 129.2, 130.8, 131.0, 135.0, 136.0, 146.0, 156.0, 170.2, 172.1.

Anal. Calcd for $C_{26}H_{25}N_5O_4$: C, 66.23; H, 5.34; N, 14.85. Found: C, 66.25; H, 5.37; N, 14.29.

Preparation of 2a–i, 4a–f, 4a′, and 5a–b; General Procedure

N-Acylbenzotriazoles **1a–d**, **3a–b** (0.5 mmol) were added at r.t. to a solution of α-amino acid (0.5 mmol) in a solution of MeCN (7 mL) and H_2O (3 mL) in the presence of Et_3N (0.6 mmol). The reaction mixture was then stirred at r.t. until the starting material was completely consumed (10–40 min for dipeptides, 30–120 min for tripeptides, 120–240 min for tetrapeptides). After 6 N HCl (1 mL) was added, the solution was concentrated under reduced pressure. The residue was extracted with EtOAc (20 mL), washed with 6 N HCl (5 mL) and brine (10 mL), and then dried ($MgSO_4$). Evaporation of the solvent gave the desired product in pure form, which was recrystallized further from $CHCl_3$–hexanes.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}propanoyl)amino]-3-phenylpropanoic Acid (Z-Ala-Phe-OH, 2a)[24a,b]

Colorless microcrystals (90%); mp 122–124 °C (Lit.[23] mp 126–127 °C); $[\alpha]_D^{25}$ +4.1 (*c* = 1.3, CH_2Cl_2) {Lit.[24a] $[\alpha]_D^{25}$ +4.2 (*c* = 1.3, CH_2Cl_2)}.

^{1}H NMR (DMSO-d_6): δ = 1.16 (d, *J* = 7.0 Hz, 3 H, CHCH_3), 2.92 (dd, *J* = 13.6, 8.5 Hz, 1 H, CHCH_2Ph), 3.05 (dd, *J* = 13.6, 4.9 Hz, 1 H, CHCH_2Ph), 4.04–4.10 (m, 1 H, NCHCO), 4.38–4.45 (m, 1 H, NCHCO), 5.00 (s, 2 H, OCH_2Ph), 7.23–7.45 (m, 11 H, ArH and NH), 8.05 (d, *J* = 7.4 Hz, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.1, 36.5, 49.8, 53.2, 65.3, 126.3, 127.6, 127.7, 128.0, 128.2, 129.1, 136.9, 137.3, 155.4, 172.3, 172.6.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}propanoyl)amino]-3-hydroxypropanoic Acid (Z-Ala-Ser-OH, 2b)[25]

Colorless microcrystals (85%); mp 192–194 °C (Lit.[25] mp 194–196 °C); $[\alpha]_D^{25}$ +21.1 (*c* = 0.4, DMF) {(Lit.[25] $[\alpha]_D^{25}$ +21.1 (*c* = 0.4, DMF)}.

^{1}H NMR (DMSO-d_6): δ = 1.22 (d, *J* = 7.1 Hz, 3 H, CHCH_3), 3.60–3.75 (m, 2 H, CHCH_2OH), 4.13–4.18 (m, 1 H, NCHCO), 4.25–4.28 (m, 1 H, NCHCO) 5.02 (s, 2 H, OCH_2Ph), 7.35 (s, 5 H), 7.36–7.48 (m, 1 H, OH), 7.91–8.00 (m, 2 H, 2 NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.2, 49.8, 54.4, 61.2, 65.3, 127.6, 128.2, 128.2, 136.9, 155.5, 171.8, 172.5.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino} propanoyl)amino]-3-(1*H*-indol-3-yl)propanoic Acid (Z-Ala-Try-OH, 2c)[26]

Colorless microcrystals (97%); mp 154–155 °C; $[\alpha]_D^{25}$ +8.1 (*c* = 1.6, MeOH).

^{1}H NMR (DMSO-d_6): δ = 1.19 (d, *J* = 7.0 Hz, 3 H, CHCH_3), 3.07 (dd, *J* = 15.0, 8.7 Hz, 1 H, CHCH_2), 3.18 (dd, *J* = 15.0, 5.0 Hz, 1 H, CHCH_2), 4.11 (apparent q, *J* = ca. 7.1 Hz, 1 H, NCHCO), 4.44–4.51 (m, 1 H, NCHCO), 4.98 (d, *J* = 12.6 Hz, 1 H, OCH_2Ph), 5.04 (d, *J* = 12.6 Hz, 1 H, OCH_2Ph), 6.98 (t, *J* = 7.2 Hz, 1 H), 7.07 (t, *J* = 7.2 Hz, 1 H), 7.26–7.46 (m, 8 H), 7.53 (d, *J* = 7.7 Hz, 1 H), 8.06 (d, *J* = 7.7 Hz, 1 H, NH), 10.9 (s, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.1, 26.9, 49.8, 52.7, 65.3, 109.5, 111.2, 118.1, 118.3, 120.8, 123.6, 127.2, 127.6, 128.2, 136.0, 136.9, 155.5, 172.4, 173.1.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methylbutanoyl)amino]-3-phenylpropanoic Acid (Z-Val-Phe-OH, 2d)[27]

Colorless microcrystals (98%); mp 166–167 °C (Lit.[27] mp 167–168 °C); $[\alpha]_D^{25}$ –13.0 (*c* = 1.0, MeOH) {(Lit.[27] $[\alpha]_D^{25}$ –13.3 (*c* = 1.0, MeOH)}.

^{1}H NMR (DMSO-d_6): δ = 0.78–0.82 (m, 6 H, 2 CH_3), 1.87–1.99 [m, 1 H, CHCH(CH_3)$_2$], 2.89 (dd, *J* = 12.6, 9.0 Hz, 1 H, CHCH_2Ph), 3.05 (dd, *J* = 12.6, 5.2 Hz, 1 H, CHCH_2Ph), 3.85–3.91 (m, 1 H, NCHCO), 4.41–4.48 (m, 1 H, NCHCO), 5.03 (s, 2 H, OCH_2Ph), 7.18–7.35 (m, 11 H, ArH and NH), 8.17 (d, *J* = 7.7 Hz, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.0, 19.0, 30.4, 36.7, 53.2, 59.9, 65.3, 126.3, 127.5, 127.7, 128.0, 128.2, 129.0, 137.0, 137.4, 155.9, 171.0, 172.7.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-methylbutanoyl)amino]-3-(1*H*-indol-3-yl)propanoic Acid (Z-Val-Try-OH, 2e)[28,29]

Colorless microcrystals (96%); mp 187–188 °C (Lit.[28] mp 135–137 °C); $[\alpha]_D^{25}$ –6.4 (*c* = 1.5, MeOH) {(Lit.[29] $[\alpha]_D^{25}$ –6.0 (*c* = 2.63, MeOH)}.

^{1}H NMR (DMSO-d_6): δ = 0.80–0.85 (m, 6 H, 2 CH_3), 1.91–1.98 [m, 1 H, CHCH(CH_3)$_2$], 2.99 (dd, *J* = 13.8, 9.0 Hz, 1 H, CHCH_2-3-indolyl), 3.05 (dd, *J* = 13.8, 5.2 Hz, 1 H, CHCH_2-3-indolyl), 3.90–3.95 (m, 1 H, NCHCO), 4.44–4.60 (m, 1 H, NCHCO), 5.00 (d, *J* = 12.5 Hz, 1 H, OCH_2Ph), 5.06 (d, *J* = 12.5 Hz, 1 H, OCH_2Ph), 6.97 (t, *J* = 7.3 Hz, 1 H), 7.06 (t, *J* = 7.3 Hz, 1 H), 7.18–7.37 (m, 8 H), 7.53 (d, *J* = 7.7 Hz, 1 H), 8.16 (d, *J* = 7.4 Hz, 1 H, NH), 10.86 (br s, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.0, 19.1, 27.1, 30.5, 52.8, 59.9, 65.4, 109.7, 111.3, 118.1, 118.3, 120.9, 123.6, 127.2, 127.6, 127.7, 128.3, 136.1, 137.1, 156.0, 171.2, 173.2.

Anal. Calcd for $C_{24}H_{27}N_3O_5$: C, 65.89; H, 6.22; N, 9.60. Found: C, 65.92; H, 6.33; N, 9.58.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanoyl)amino]propanoic Acid (Z-Phe-Ala-OH, 2f)[30]

Colorless microcrystals (96%); mp 157–158 °C (Lit.[30] mp 153–154 °C); $[\alpha]_D^{25}$ –9.5 (*c* = 1.0, EtOH) {(Lit.[30] $[\alpha]_D^{25}$ –10.0 (*c* = 1.90, EtOH)}.

^{1}H NMR: δ = 1.36 (d, *J* = 7.0 Hz, 3 H, CHCH_3), 3.05 (d, *J* = 6.2 Hz, 2 H, CHCH_2Ph), 4.47–4.52 (m, 2H, 2 NCHCO), 5.13 (d, *J* = 12.5 Hz, 1 H, OCH_2Ph), 5.18 (d, *J* = 12.5 Hz, 1 H, OCH_2Ph), 5.63 (d, *J* = 5.6 Hz, 1 H, NH), 6.65 (br s, 1 H, NH), 7.15–7.36 (m, 10 H), 8.80 (br s, 1 H, CO_2H).

^{13}C NMR: δ = 17.1, 37.4, 47.5, 55.9, 65.1, 126.2, 127.4, 127.6, 128.0, 128.3, 129.2, 137.0, 138.2, 155.8, 171.4, 174.0.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanoyl)amino]-3-methylbutanoic Acid (Z-Phe-Val-OH, 2g)[31]
Colorless microcrystals (95%); mp 140–142 °C (Lit.[31] mp 149–151 °C); $[\alpha]_D^{25}$ –6.2 (*c* = 2.0, MeOH) {(Lit.[31] $[\alpha]_D^{22}$ –6.3 (*c* = 2.0, MeOH)}.

^{1}H NMR (DMSO-d_6): δ = 0.90 (d, *J* = 5.2 Hz, 6 H, 2 CH_3), 2.05–2.11 [m, 1 H, CHC*H*$(CH_3)_2$], 2.69–2.77 (m, 1 H, CHCH_2Ph), 2.98–3.02 (m, 1 H, CHCH_2Ph), 4.17–4.22 (m, 1 H, NCHCO), 4.36–4.41 (m, 1 H, NCHCO), 4.94 (s, 2 H, OCH_2Ph), 7.19–7.53 (m, 11 H, ArH and NH), 8.07–8.09 (m, 2 H, NH and CO_2H).

^{13}C NMR (DMSO-d_6): δ = 17.9, 19.0, 29.9, 37.3, 55.8, 57.1, 65.1, 126.1, 127.3, 127.6, 127.9, 128.2, 129.1, 136.9, 138.0, 155.7, 171.8, 172.8.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanoyl)amino]-3-phenylpropanoic Acid (Z-Phe-Phe-OH, 2h)[32,33]
Colorless microcrystals (98%); mp 141–142 °C (Lit.[33] mp 138–139 °C); $[\alpha]_D^{25}$ –6.7 (*c* = 1.3, MeOH).

^{1}H NMR (DMSO-d_6): δ = 2.67–2.75 (m, 1 H, CHCH_2Ph), 2.93–3.14 (m, 3 H, CHCH_2Ph), 4.31–4.34 (m, 1 H, NCHCO), 4.49–4.5.1 (m, 1 H, NCHCO), 4.94 (s, 2 H, OCH_2Ph), 7.12–7.51 (m, 16 H), 8.10 (br s, 1 H, CO_2H), 8.32 (d, *J* = 7.6 Hz, 1 H, NH).

^{13}C NMR (DMSO-d_6): δ = 36.6, 37.3, 53.4, 55.9, 65.1, 126.1, 126.4, 127.3, 127.6, 127.9, 128.1, 128.2, 129.1, 136.9, 137.3, 138.0, 155.7, 171.5, 172.7.

(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-phenylpropanoyl)amino]-3-hydroxypropanoic Acid (Z-Phe-Ser-OH, 2i)[34]
Colorless microcrystals (96%); mp 140–141 °C (Lit.[34] mp 137 °C); $[\alpha]_D^{25}$ +0.6 (*c* = 1.0, MeOH) {Lit.[34] $[\alpha]_D^{22}$ +0.6 (*c* = 1.0, MeOH)}.

^{1}H NMR (DMSO-d_6): δ = 2.73 (t, *J* = 12.6 Hz, 1 H, CHCH_2Ph), 3.06–3.10 (m, 1 H, CHCH_2Ph), 3.67 (dd, *J* = 10.3, 3.3 Hz, 1 H, CHCH_2OH), 3.78 (dd, *J* = 10.3, 4.5 Hz, 1 H, CHCH_2OH), 4.32–4.42 (m, 2 H, 2 NCHCO), 4.93 (s, 2 H, OCH_2Ph), 7.24–7.46 (m, 10 H), 7.52 (d, *J* = 8.8 Hz, 1 H, NH), 8.32 (d, *J* = 7.7 Hz, 1 H, NH); two exchangeable protons (OH and CO_2H) are missing.

^{13}C NMR (DMSO-d_6): δ = 37.4, 54.6, 55.9, 61.2, 65.1, 126.1, 127.3, 127.6, 127.9, 128.2, 129.2, 136.9, 138.1, 155.7, 171.7, 171.8.

(5*S*,11*S*)-11-Isobutyl-5-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triaza-dodecan-12-oic Acid (Z-Ala-Gly-Leu-OH, 4a)
Colorless microcrystals (93%); mp 150.5–151.5 °C; $[\alpha]_D^{25}$ –13.8 (*c* = 1.3, MeOH).

^{1}H NMR (DMSO-d_6): δ = 0.83 (d, *J* = 6.2 Hz, 3 H, CH_3), 0.87 (d, *J* = 6.3 Hz, 3 H, CH_3), 1.20 (d, *J* = 7.2 Hz, 3 H, CH_3), 1.52–1.64 [m, 3 H, CH_2C*H*$(CH_3)_2$], 3.72 (d, *J* = 5.4 Hz, 2 H, NCH_2CO), 4.02–4.07 (m, 1 H, NCHCO), 4.21–4.29 (m, 1 H, NCHCO), 4.99 (d, *J* = 12.6 Hz, 1 H, OCH_2Ph), 5.06 (d, *J* = 12.6 Hz, 1 H, OCH_2Ph), 7.36 (s, 5 H), 7.55 (d, *J* = 7.0 Hz, 1 H, NH), 7.91 (d, *J* = 7.8 Hz, 1 H, NH), 8.17 (br s, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 17.8, 21.3, 22.7, 24.1, 41.7, 50.0, 65.3, 127.7, 127.7, 128.2, 136.8, 155.7, 168.5, 172.6, 173.8.

Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 58.21; H, 7.01; N, 10.59.

(11*S*)-11-Isobutyl-5-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic Acid (Z-DL-Ala-Gly-Leu-OH, 4a + 4a′)
Colorless microcrystals (94%); mp 101–105 °C.

^{1}H NMR (DMSO-d_6): δ = 0.83 (d, *J* = 6.2 Hz, 3 H), 0.88 (d, *J* = 6.3 Hz, 3 H), 1.21 (d, *J* = 7.2 Hz, 3 H), 1.52–1.62 (m, 3 H), 3.72 (d, *J* = 5.1 Hz, 2 H), 4.02–4.07 (m, 1 H), 4.24–4.26 (m, 1 H), 4.99 (d, *J* = 12.6 Hz, 1 H), 5.05 (d, *J* = 12.6 Hz, 1 H), 7.28–7.46 (m, 5 H), 7.55 (d, *J* = 7.0 Hz, 1 H), 7.90–7.97 (m, 1 H), 81.4–8.17 (m, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.8, 21.3, 22.7, 24.1, 41.7, 50.1, 50.2, 65.4, 127.7, 127.7, 128.3, 136.8, 155.8, 168.6, 172.7, 173.8.

Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 58.43; H, 6.99; N, 10.66.

(5*S*,11*S*)-11-Isobutyl-5-isopropyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic Acid (Z-Val-Gly-Leu-OH, 4b)
Colorless microcrystals (85%); mp 131.5–132.5 °C; $[\alpha]_D^{25}$ –17.1 (*c* = 1.4, MeOH).

^{1}H NMR (DMSO-d_6): δ = 0.82–0.88 (m, 12 H, 4 CH_3), 1.49–1.66 [m, 3 H, CH_2C*H*$(CH_3)_2$], 1.93–2.02 [m, 1 H, CHC*H*$(CH_3)_2$], 3.73 (d, *J* = 5.4 Hz, 2 H, NCH_2CO), 3.85 (apparent t, *J* = ca. 7.7 Hz, 1 H, NCHCO), 4.25 (apparent q, *J* = ca. 7.7 Hz, 1 H, NCHCO), 5.01 (d, *J* = 12.6 Hz, 1 H, OCH_2Ph), 5.07 (d, *J* = 12.6 Hz, 1 H, OCH_2Ph), 7.30–7.40 (m, 6 H, ArH and NH), 7.95 (d, *J* = 8.0 Hz, 1 H, NH), 8.21 (t, *J* = 5.4 Hz, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.1, 19.1, 21.3, 22.7, 24.1, 29.9, 41.6, 50.0, 60.3, 65.3, 127.6, 127.7, 128.2, 136.9, 156.2, 168.5, 171.4, 173.8.

Anal. Calcd for $C_{21}H_{31}N_3O_6$: C, 59.84; H, 7.41; N, 9.97. Found: C, 60.13; H, 7.64; N, 9.94.

(5*S*)-5-Benzyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic Acid (Z-Phe-Gly-Gly-OH, 4c)[35]
Colorless microcrystals (98%); mp 120–122 °C (Lit.[35] mp 122–125 °C); $[\alpha]_D^{25}$ –21.4 (*c* = 1.4, DMF) {Lit.[35] $[\alpha]_D^{15}$ –11.8 (*c* = 1.0, DMF)}.

^{1}H NMR (DMSO-d_6): δ = 2.76 (t, *J* = 12.3 Hz, 1 H, CHCH_2Ph), 3.03–3.08 (m, 1 H, CHCH_2Ph), 3.78 (s, 4 H, 2 NCH_2CO), 4.31 (br s, 1 H, NCHCO), 4.94 (s, 2 H, OCH_2Ph), 7.25–7.40 (m, 11 H, ArH and NH), 7.56 (d, *J* = 7.8 Hz, 1 H, NH), 8.11 (br s, 1 H, NH), 8.35 (br s, 1 H, CO_2H).

^{13}C NMR (DMSO-d_6): δ = 37.4, 40.6, 41.9, 56.2, 65.3, 126.2, 127.4, 127.6, 128.0, 128.3, 129.2, 137.0, 138.1, 155.9, 169.0, 171.1, 171.8.

(5*S*,8*S*,11*S*)-5-Benzyl-8,11-dimethyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic Acid (Z-Phe-Ala-Ala-OH, 4d)[36]
Colorless microcrystals (92%); mp 180–181 °C (Lit.[36] mp 187.5–188.5 °C); $[\alpha]_D^{25}$ –15.0 (*c* = 1.0, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.23–1.29 (m, 6 H, 2 CH_3), 2.66–2.75 (m, 1 H, CHCH_2Ph), 3.00–3.05 (m, 1 H, CHCH_2Ph), 4.17–4.35 (m, 3 H, 3 NCHCO), 4.93 (s, 2 H, OCH_2Ph), 7.19–7.33 (m, 10 H), 7.51 (d, *J* = 8.7 Hz, 1 H, NH), 8.14 (br s, 1 H, NH), 8.17 (br s, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 17.1, 18.2, 37.3, 47.4, 47.8, 55.9, 65.1, 126.1, 127.3, 127.5, 127.9, 128.2, 129.1, 136.9, 138.1, 155.8, 171.1, 171.7, 173.9.

(5*S*,8*S*,11*S*)-5-Benzyl-11-(hydroxymethyl)-8-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic Acid (Z-Phe-Ala-Ser-OH, 4e)
Colorless microcrystals (94%); mp 185.5–186.5 °C; $[\alpha]_D^{25}$ –1.4 (*c* = 1.1, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.26 (d, *J* = 7.0 Hz, 3 H, CHCH_3), 2.68–2.76 (m, 1 H, CHCH_2Ph), 3.01–3.10 (m, 1 H, CHCH_2Ph), 3.62–3.76 (m, 2 H, CHCH_2OH), 4.24–4.34 (m, 2 H, 2 NCHCO), 4.26–4.48 (m, 1 H, NCHCO), 4.92–4.94 (m, 2 H, OCH_2Ph), 7.20–7.34 (m, 10 H),

7.53 (d, *J* = 8.8 Hz, 1 H, NH), 8.04 (d, *J* = 7.7 Hz, 1 H, NH), 8.19 (d, *J* = 7.4 Hz, 1 H, NH); two exchangeable protons (OH and CO_2H) are missing.

^{13}C NMR (DMSO-d_6): δ = 18.3, 37.3, 47.9, 54.5, 56.0, 61.2, 65.1, 126.1, 127.3, 127.6, 127.9, 128.2, 129.1, 136.9, 138.1, 155.8, 171.1, 171.7, 172.1.

Anal. Calcd for $C_{23}H_{27}N_3O_7$: C, 60.38; H, 5.95; N, 9.18. Found: C, 59.84; H, 6.06; N, 9.10.

(5*S*,8*S*,11*S*)-8-Benzyl-11-(1*H*-indol-3-ylmethyl)-5-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triaza dodecan-12-oic Acid (Z-Ala-Phe-Try-OH, 4f)

Colorless microcrystals (95%); mp 203–204 °C; $[\alpha]_D^{25}$ –6.9 (*c* = 0.6, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.11 (d, *J* = 7.0 Hz, 3 H, CHC*H*$_3$), 2.77–2.85 (m, 1 H, CHC*H*$_2$Ar), 2.98–3.23 (m, 3 H, CHC*H*$_2$Ar), 4.00 (t, *J* = 7.2 Hz, 1 H, NCHCO), 4.45–4.60 (m, 2 H, 2 NCHCO), 4.97 (d, *J* = 12.5 Hz, 1 H, OC*H*$_2$Ph), 5.03 (d, *J* = 12.5 Hz, 1 H, OC*H*$_2$Ph), 6.98 (t, *J* = 7.1 Hz, 1 H), 7.06 (t, *J* = 7.5 Hz, 1 H), 7.18–7.21 (m, 6 H), 7.33–7.41 (m, 7 H), 7.53 (d, *J* = 7.7 Hz, 1 H, NH), 8.01 (d, *J* = 8.0 Hz, 1 H, NH), 8.32 (d, *J* = 5.6 Hz, 1 H, NH), 11.0 (br s, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.1, 27.0, 37.5, 50.1, 53.0, 53.6, 65.4, 109.5, 111.4, 118.1, 118.3, 120.8, 123.8, 126.1, 127.2, 127.7, 127.9, 128.3, 129.3, 136.0, 136.9, 137.6, 155.5, 170.9, 172.1, 173.0.

HRMS: *m*/*z* calcd for $C_{19}H_{27}N_3O_6$: 557.2400 (M); found: 557.2400.

(5*S*,8*S*,14*S*)-14-Isobutyl-5-benzyl-8-methyl-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oic Acid (Z-Phe-Ala-Gly-Leu-OH, 5a)

Colorless microcrystals (86%); mp 207.5–208.5 °C; $[\alpha]_D^{25}$ –11.2 (*c* = 1.2, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.83 [d, *J* = 6.3 Hz, 3 H, CH(C*H*$_3$)$_2$], 0.88 (d, *J* = 6.3 Hz, 3 H, CH(C*H*$_3$)$_2$], 1.14–1.24 [m, 1 H, CH$_2$C*H*(CH$_3$)$_2$], 1.24 [d, *J* = 6.8 Hz, 2 H, C*H*$_2$CH(C*H*$_3$)$_2$], 1.48–1.66 (m, 3 H, CHC*H*$_3$), 2.68–2.77 (m, 1 H, CHC*H*$_2$Ph), 3.00–3.08 (m, 1 H, CHC*H*$_2$Ph), 3.74 (d, *J* = 5.4 Hz, 2 H, NCH$_2$CO), 4.20–4.36 (m, 3 H, 3 NCHCO), 4.94 (s, 2 H, OC*H*$_2$Ph), 7.19–7.36 (m, 10 H), 7.51 (d, *J* = 8.5 Hz, 1 H, NH), 8.02 (d, *J* = 8.0 Hz, 1 H, N*H*), 8.07 (t, *J* = 5.4 Hz, 1 H, NH), 8.22 (d, *J* = 6.9 Hz, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 18.1, 21.3, 22.7, 24.1, 37.3, 41.6, 48.3, 50.0, 55.9, 65.1, 126.1, 127.3, 127.6, 127.9, 128.2, 129.1, 136.9, 138.0, 155.8, 168.4, 171.2, 172.2, 173.8.

Anal. Calcd for $C_{28}H_{36}N_4O_7$: C, 62.21; H, 6.71; N, 10.36. Found: C, 62.01; H, 6.78; N, 10.36.

(5*S*,8*S*,14*S*)-14-Isobutyl-8-benzyl-5-methyl-3,6,9,12-tetraoxo-1-phenyl-2-oxa-4,7,10,13-tetraazapentadecan-15-oic Acid (Z-Ala-Phe-Gly-Leu-OH, 5b)

Colorless microcrystals (85%); mp 149–150 °C; [α]D^{25} –26.6 (*c* = 1.1, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.84 [d, *J* = 6.3 Hz, 3 H, CH(C*H*$_3$)$_2$], 0.89 [d, *J* = 6.3 Hz, 3 H, CH(C*H*$_3$)$_2$], 1.12 (d, *J* = 7.0 Hz, 3 H, CHC*H*$_3$), 1.49–1.65 [m, 3 H, C*H*$_2$C*H*(CH$_3$)$_2$], 2.86 (dd, *J* = 13.1, 6.3 Hz, 1 H, CHC*H*$_2$Ph), 3.04 (dd, *J* = 13.1, 4.0 Hz, 1 H, CHC*H*$_2$Ph), 3.74 (d, *J* = 5.4 Hz, 2 H, NCH$_2$CO), 3.96–4.06 (m, 1 H, NCHCO), 4.25 (apparent q, *J* = ca. 7.4 Hz, 1 H, NCHCO), 4.50 (br s, 1 H, NCHCO), 4.98 (d, *J* = 12.4 Hz, 1 H, OC*H*$_2$Ph), 5.04 (d, *J* = 12.4 Hz, 1 H, OC*H*$_2$Ph), 7.14–7.28 (m, 5 H), 7.30–7.42 (m, 6 H, ArH and NH), 7.96 (d, *J* = 7.9 Hz, 1 H, NH), 8.01 (d, *J* = 8.0 Hz, 1 H, NH), 8.23 (t, *J* = 5.4 Hz, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 17.9, 21.3, 22.7, 24.1, 37.3, 41.6, 50.1, 53.7, 65.4, 126.1, 127.7, 127.9, 128.2, 129.1, 136.8, 137.6, 155.6, 168.4, 171.0, 172.2, 173.9. HRMS m/z calcd for $C_{28}H_{36}N_4O_7$ 541.2662 (M), found 541.2662.

Boc-Protected Dipeptide from Boc-Phe-Bt

Boc-Phe-Ala-OH was prepared by the procedure used for preparation of **2a–i**. This experiment showed that Boc-protected peptides can also be prepared by this method.

(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)propanoic Acid (Boc-Phe-Ala-OH)[23]

White microcrystals (98%); mp 90–93 °C (Lit.[23] mp 96 °C); $[\alpha]_D^{25}$ +9.8 (*c* = 2.0, MeOH) {Lit.[23] $[\alpha]_D^{25}$ +11.62 (*c* = 2.00, MeOH)}.

^{1}H NMR (DMSO-d_6): δ = 1.22 (d, *J* = 7.2 Hz, 3 H, CHC*H*$_3$), 1.30 [s, 9 H, C(CH$_3$)$_3$], 2.69–2.78 (m, 1 H, CHC*H*$_2$Ph), 2.90–2.97 (m, 1 H, CHC*H*$_2$Ph), 4.20–4.27 (m, 2 H, 2 NCHCO), 6.84–6.90 (m, 1 H, NH), 7.19–7.29 (m, 5 H), 8.17–8.25 (m, 1 H, NH); one exchangeable proton is missing.

^{13}C NMR (DMSO-d_6): δ = 17.5, 28.2, 37.9, 47.5, 55.4, 78.0, 126.2, 128.0, 129.3, 138.0, 155.1, 171.2, 174.0.

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