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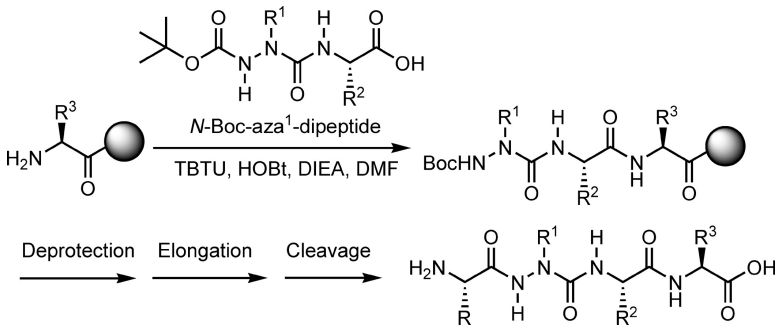

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Aza-Amino Acid Scan for Rapid Identification of Secondary Structure Based on the Application of *N*-Boc-Aza[1]-Dipeptides in Peptide Synthesis

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Abstract: Azapeptides, peptide analogues in which the α-carbon of one or more of the amino acid residues is replaced with a nitrogen atom, exhibit propensity for adopting β-turn conformations. A general protocol for the synthesis of azapeptides without racemization on solid phase has now been developed by introducing the aza-amino acid residue as an *N*-Boc-aza[1]-dipeptide. This approach has been validated by the synthesis of six *N*-Boc-aza[1]-dipeptides and their subsequent introduction into analogues of the C-terminal peptide fragment of the human calcitonin gene-related peptide (hCGRP). By performing an aza-amino acid scan of such antagonist peptides, a set of aza-hCGRP analogues was synthesized to examine the relationship between turn secondary structure and biological activity.

Introduction

Promising leads for drug discovery, natural peptides suffer from rapid metabolism, poor bioavailability, and short duration of action that compromise their application as drugs in clinic.[1−5] Peptide mimics that do not possess such shortcomings have been prepared by modification of the side chain and the backbone of native peptides as well as by the application of secondary structure surrogates.[6−15] New strategies for effectively analyzing peptide structures to identify biologically active conformations are needed to accelerate the transition from peptide to mimic.

Traditional approaches for studying peptide structure−activity relationships feature typically the sequential replacement of the amino acids in the peptide chain with alternative amino acids to assess their importance for biological activity. For example, replacement of an amino acid residue in the lead sequence by either alanine, its enantiomer, or proline may respectively reveal the significance of side chains, configuration, and conformation for activity.[16] Related scanning techniques have recently been described involving systematic introduction of *N*-alkylamino acids into peptides to ascertain the importance of amide protons in hydrogen-bond interactions.[17−20] By employing chemical approaches for systematically modifying a peptide sequence with structural constraints that favor particular geometry, new generic tools may be developed for identifying active conformers involved in peptide−receptor interactions.

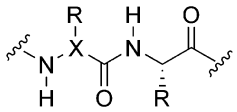

Figure 1. Schematic representation of a peptide and an azapeptide.

Azapeptides possess one or more amino acids in which the central α-carbon is replaced by nitrogen (Figure 1).[21] Electronic repulsion between the adjacent nitrogen atoms has been suggested to account for the type I and II β-turn geometry that aza-amino acid residues adopt in peptides, as predicted by computation[22−24] and observed by spectroscopic[25,26] and crystal-

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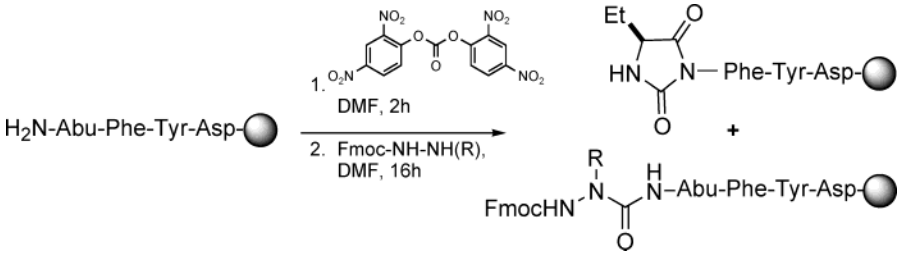
Scheme 1. Synthesis of Azapeptides on Solid Phase Is Usually Accompanied by Hydantoin Formation[33]



lographic methods.[27−30] Although controversy remains over the role of aza-amino acids in β-turn formation, X-ray crystal structures of linear, L-proline-containing azapeptide analogues have revealed type I and II β-turns in which the aza-amino acid residue is situated, respectively, at the $i + 1$ and $i + 2$ position and the α-nitrogen assumes a pyramidal structure in all but one structure.[27,28] Aza-dipeptides and aza-tripeptides have also been studied by NMR spectroscopy in solvents of varying polarity using DMSO to disrupt hydrogen bonding.[23,25] These studies, combined with data from IR experiments and *ab initio* calculations,[22] all have led to the accepted model in which noncyclic aza-amino acids adopt either the $i + 1$ or $i + 2$ position in an intramolecularly hydrogen-bonded β-turn structure.[25] For example, the aza-tripeptide Boc-Phe-azaLeu-Ala-OMe was shown by NMR and IR spectroscopy to prefer a type II β-turn geometry in which the aza-amino acid adopted the $i + 2$ position.[23] Cyclic aza-amino acid residues, such as azaPro and azaPip, exhibit high cis-amide isomer populations N-terminal to the aza-residue in peptides and type VI β-turn geometry as observed by NMR and IR spectroscopy and X-ray analysis.[26,29−32] Energetically stable cis-peptide bonds have also been predicted to be favored in studies of azaGly containing peptides by *ab initio* and DFT methods.[24] These studies indicate that systematic replacement of the amino acids in a peptide sequence by their aza-amino acid counterpart could serve as a general means for scanning for bioactive β-turn conformations.

Azapeptide synthesis has, however, been challenging, requiring a combination of hydrazine[34,35] and peptide chemistry.[33,36] Typically, an N-protected N′-substituted hydrazine is reacted with a "carbonyl donor" such as an isocyanate or an active carbamate.[21] A significant drawback in the implementation of these protocols on solid phase has been intramolecular hydantoin formation of the resin-bound isocyanate or active carbamate intermediate prior to reaction with the substituted hydrazine (Scheme 1).[33] Although hydantoin formation may be prevented by the use of the *N*-2-hydroxy-4-methoxybenzyl group as reversible amide protection, this method requires two additional synthesis steps and employs less reactive carbamate instead of isocyanate intermediates.[37]

Application of azapeptides in the study of biologically active peptides has had significant success. For example, azapeptides have exhibited longer duration of action relative to natural peptides, presumably because of increased resistance to proteases[36,38] and because ureas are generally more chemically stable than amides.[39] The azaVal analogue of bovine angiotensin II exhibited increased duration of pressor action relative to its Val counterpart.[40] Azapeptides have also been synthesized and evaluated as inhibitors of cysteine[41−44] and serine[39,45] proteases. Azapeptide analogues of peptide hormones have been synthesized, including thyrotropin-releasing hormone (TRH),[46] oxytocin,[47] eledoisin,[48] enkephalin,[49] and luliberin (LHRH).[50] An example of the latter, Zoladex is used clinically for the treatment of prostate cancer.[51] To enhance the application of azapeptides in peptide science and medicinal chemistry, we present now a general strategy for solid-phase azapeptide synthesis, suitable for aza-amino acid scanning, based on the synthesis of *N*-Boc-aza[1]-dipeptides and their introduction into peptides.

Results and Discussion

N-Boc-aza[1]-dipeptides were synthesized by the reaction of *N*-Boc-*N*′-alkyl hydrazines with isocyanates derived from α-amino benzyl esters and subsequent hydrogenolytic ester cleavage (Scheme 2).

The synthesis of N-protected N′-substituted hydrazines has been the subject of much research, and different methodologies have proven effective for different N′-substituents.[55] *N*-Boc-protected hydrazine derivatives have been prepared for making aza-analogues of most of the proteinogenic amino acids.[52] We

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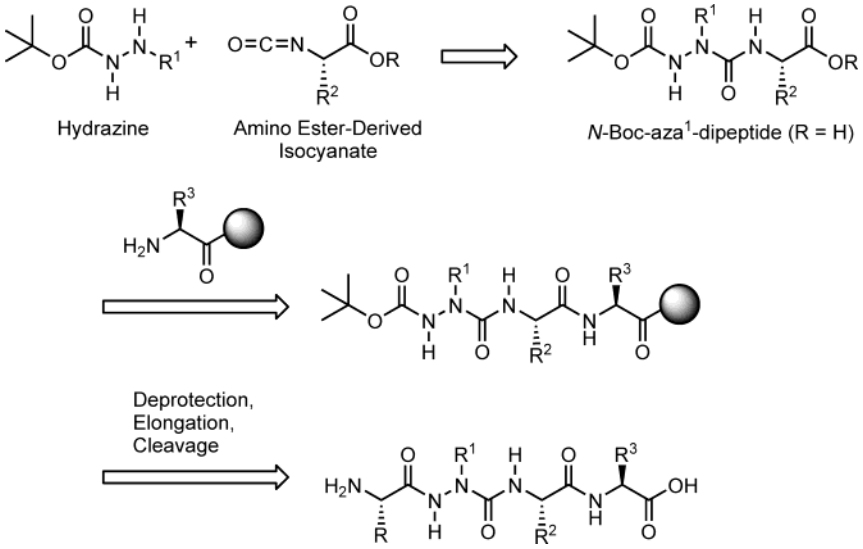
Scheme 2. *N*-Boc-Aza[1]-Dipeptide Strategy for Azapeptide Synthesis

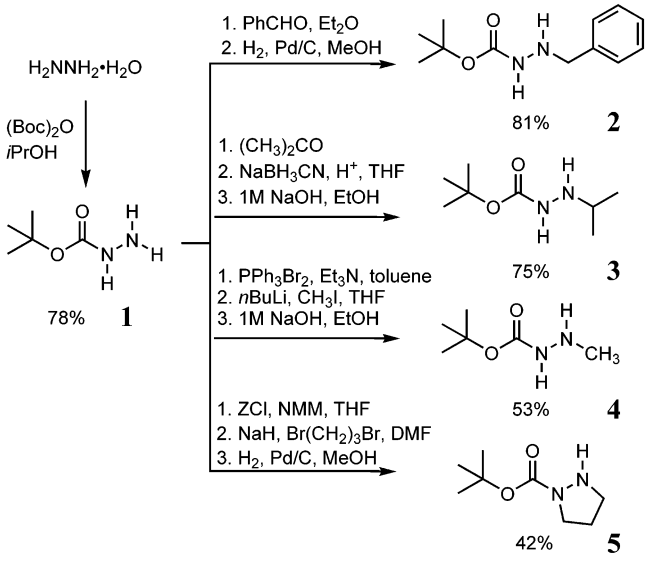


synthesized hydrazines to mimic four amino acid side-chains: Phe, Val, Ala, and Pro. Condensation of *tert*-butyl carbazate **1** with the appropriate aldehyde or ketone gave an acyl hydrazone that was reduced best by catalytic hydrogenation or hydride addition if the substituent was, respectively, aromatic or aliphatic. Benzyl- and isopropyl-substituted hydrazines **2** and **3** were isolated in 81% and 75% yields, respectively, using these procedures.[52,53] Attempts to synthesize azaAla precursor **4** by condensation of carbazate **1** with formaldehyde followed by reduction, as well as by alkylation of **1** with iodomethane, did not render the desired product in our hands. Methyl hydrazine **4** was synthesized by alkylation of the corresponding triphenyl phosphinimine with iodomethane in 53% yield.[54] Pyrazolidine **5** was synthesized by acylation of carbazate **1** with benzylchloroformate prior to alkylation with 1,3-dibromopropane and removal of the Cbz group by hydrogenolysis over palladium-on-carbon in methanol (Scheme 3).[46]

Amino ester isocyanates **6**−**9** were synthesized from the corresponding L-amino benzyl ester hydrochloride salts using a 1.93 M solution of phosgene in toluene containing 120 mol % of pyridine at 0 °C for 2h.[56] Isocyanates **6**−**9** were employed immediately in reactions with hydrazines **1**−**5**. All *N*-Boc-aza[1]-dipeptide benzyl esters were obtained in excellent yields (90−98%) after purification by silica gel chromatography, with the exception of *N*-Boc-azaVal-Gly-OBn **12**, which was isolated in 34% yield. *N*-Boc-aza[1]-dipeptides **16**−**21** were finally prepared by hydrogenolysis of benzyl esters **10**−**15** using palladium-on-carbon in ethanol (Scheme 4).

Although carbamate N-protection has been shown to be superior to amide N-protection at preventing racemization of α-amino acids during peptide synthesis,[58] to the best of our knowledge, there have been no reports on the use of urea nor semicarbazide-protected α-amino acids in peptide chemistry.

Scheme 3. Synthesis of Substituted Hydrazines[46,52−54]



The configurational integrity of the C-terminal amino acid residue during the synthesis and application *N*-Boc-aza[1]-dipeptides was ascertained by the solution-phase synthesis of aza-tripeptides *N*-Boc-azaPhe-Ala-Phe-OMe **22**. *N*-Boc-azaPhe-(L)Ala-OH **17** was coupled to both L- and D-HCl•Phe-OMe using TBTU, HOBt, and DIEA in acetonitrile (Scheme 5). The diastereomeric purity of the resulting aza-tripeptides (*S*,*S*)- and (*S*,*R*)-**22** was measured by integration of the diastereotopic methyl ester singlets at 3.20 and 3.26 ppm, respectively, in the ^{1}H NMR spectrum taken in benzene-d_6. Spectral analysis of aza-tripeptide (*S*,*S*)-**22** during incremental additions of (*S*,*R*)-**22** established that (*S*,*S*)-**22** was of >99% purity (>99:1 d.r.), the limits of detection. Azapeptides can thus be made in high stereoisomeric purity.[57]

Solution-phase synthesis of **22** was examined because of the presence of its sequence in a peptide antagonist of the potent vasodilator, human calcitonin gene-related peptide (hCGRP, **23**),

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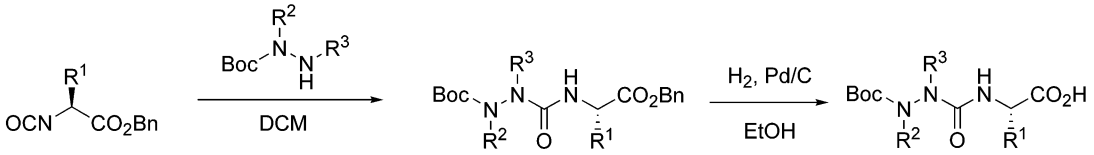
Scheme 4. Synthesis of *N*-Boc-Aza[1]-Dipeptides **16**−**21**



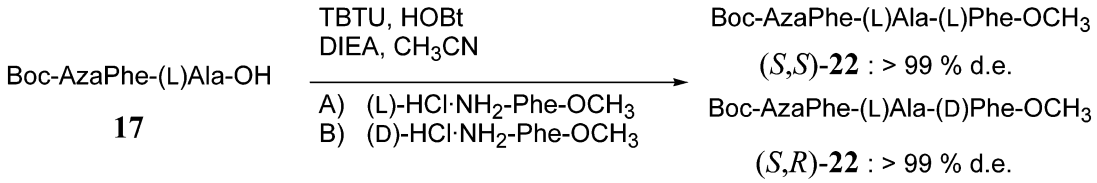
6 : R^1 = *i*Pr
7 : R^1 = Me
8 : R^1 = H
9 : R^1 = Bn

1 : R^2 = H; R^3 = H
2 : R^2 = H; R^3 = Bn
3 : R^2 = H; R^3 = *i*Pr
4 : R^2 = H; R^3 = Me
5 : $R^2 = R^3 = (CH_2)_3$

10 : R^1 = *i*Pr; R^2 = H; R^3 = Bn
11 : R^1 = Me; R^2 = H; R^3 = Bn
12 : R^1 = H; R^2 = H; R^3 = *i*Pr
13 : R^1 = Bn; R^2 = H; R^3 = Me
14 : R^1 = Bn; $R^2 = R^3$ $(CH_2)_3$
15 : R^1 = Bn; $R^2 = R^3$ = H

16 : R^1 = *i*Pr; R^2 = H; R^3 = Bn
17 : R^1 = Me; R^2 = H; R^3 = Bn
18 : R^1 = H; R^2 = H; R^3 = *i*Pr
19 : R^1 = Bn; R^2 = H; R^3 = Me
20 : R^1 = Bn; $R^2 = R^3$ $(CH_2)_3$
21 : R^1 = Bn; $R^2 = R^3$ = H

Scheme 5. Coupling of *N*-Boc-Aza[1]-Dipeptides to Amino Acids Using Standard Peptide Conditions Is Achieved in Higher than 99 % d.e.[57]



Scheme 6. Synthesis of Peptides **24**−**29** on Solid Phase Using Oxime Resin

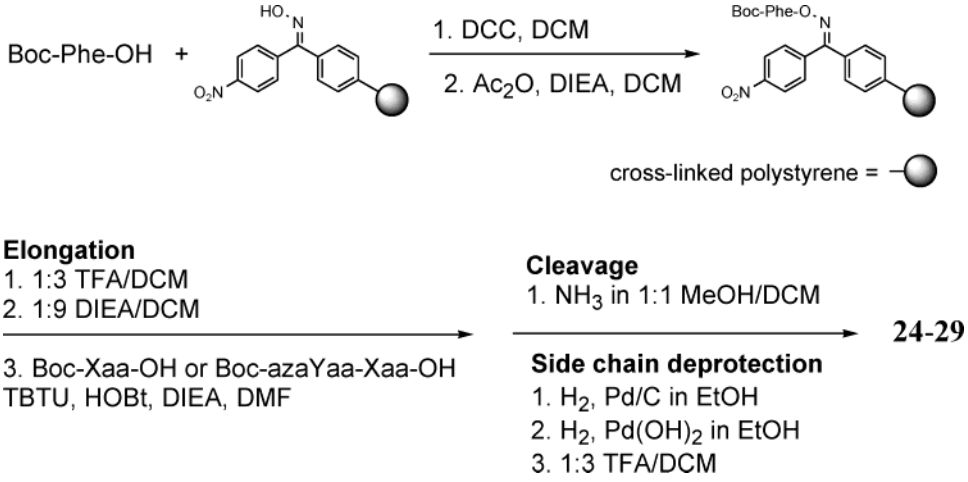


Table 1. Sequence of α-hCGRP (**23**), Peptide Antagonists **24** and **25**, and Azapeptides **26**−**29**

(**23**) α-hCGRP	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF–NH_2				
compound	sequence	t_R (min)[a]	formula	MS (calcd for $[M + H]^+$)	HPLC purity
(**24**) [D^{31}, P^{34}, F^{35}]$CGRP_{29-37}$	PTDVGPFAF−NH_2	13.0	$C_{46}H_{64}N_{10}O_{12}$	949.4 (949.4)	92
(**25**) [D^{31}, P^{34}, F^{35}]$CGRP_{27-37}$	FVPTDVGPFAF−NH_2	15.1	$C_{60}H_{82}N_{12}O_{14}$	1195.9 (1195.9)	>99
(**26**) [D^{31}, P^{34}, $azaF^{35}$]$CGRP_{29-37}$	PTDVGP***F***AF−NH_2	13.2	$C_{45}H_{63}N_{11}O_{12}$	950.5 (950.5)	91
(**27**) [D^{31}, $azaP^{34}$, F^{35}]$CGRP_{29-37}$	PTDVG***P***FAF−NH_2	12.7	$C_{45}H_{63}N_{11}O_{12}$	950.5 (950.5)	88
(**28**) [$azaF^{27}$, D^{31}, P^{34}, F^{35}]$CGRP_{27-37}$	***F***VPTDVGPFAF−NH_2	17.0	$C_{59}H_{81}N_{13}O_{14}$	1196.3 (1196.6)	>99
(**29**) [D^{31}, P^{34}, $azaF^{35}$]$CGRP_{27-37}$	FVPTDVGP***F***AF−NH_2	14.3	$C_{59}H_{81}N_{13}O_{14}$	1196.6 (1196.6)	92

[a] 20−80% acetonitrile in H_2O containing 0.01% TFA over 20 min, in vol: 10 μL.

a 37-amino acid neuropeptide that is characterized by a disulfide loop between residues 2−7, an α-helix between residues 8−18, and an aminated C-terminus (Table 1).[59] Structure activity studies have found that C-terminus analogues of hCGRP, such as peptides **24** and **25**, exhibit antagonist activity and have been suggested to adopt bioactive turn conformations.[60] To explore the power of aza-amino acid scanning, our synthetic approach was used to make aza-analogues of the 9 and 11 residue peptide antagonists **24** and **25**. Because aza-amino acids have been shown to adopt $i + 1$ and $i + 2$ positions of β-turns, azapeptides **26**−**29** were synthesized to study the importance of turn regions about the aromatic residues for antagonist activity. Employing aza[1]-dipeptide fragments, hydantoin formation was avoided during azapeptide synthesis on solid phase. Azapeptides analogues **26**−**29** were prepared for evaluation as potential hCGRP antagonists (Table 1). Scheme 6 shows the synthesis of peptides **24**−**29** on solid phase using oxime resin.

N-Boc-aza[1]-dipeptides **16**, **17**, and **20** were incorporated into azapeptides **26**−**29** using a Boc protection strategy on oxime

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resin.[61] Removal of the Boc group was carried out using 25% TFA in DCM (30 min) followed by neutralization in 10% DIEA in DMF (10 min). Of particular note, the Boc removal from the aza-amino acid required longer reaction times and was carried out during peptide synthesis for 2 h instead of the 30 min period necessary for performing the analogous deprotection with natural amino acid residues. Peptide couplings were carried out with 3 equiv each of the appropriate Boc-protected amino acid or *N*-Boc-aza[1]-dipeptides, TBTU, HOBt, and DIEA, in DMF for 2 h. When coupling to aza-amino acid residues, extended reaction times (16 h) were employed between washing of the resin and addition of fresh reagents, because acylation of aza-amino acid residues had been reported to be slower than similar couplings to ordinary amino acid residues.[36] Final cleavage of the peptide from the resin was performed using a 1:1 mixture of saturated NH_3 in MeOH/DCM. Removal of the side-chain-protecting groups [Thr(OBn) and Asp(OBn)] was carried out by hydrogenolysis first over $Pd(OH)_2$, followed by hydrogenolysis over Pd/C in ethanol. The Boc group of the final peptide was removed using 25% TFA in DCM, and the peptides **24**−**29** were purified by HPLC to furnish 10−20% yields based on initial loading of 0.3 mmol/g. Characterization of the peptides was carried out by LC−MS, and purity was assessed by UV peak integration.

Conclusion

N-Boc-azadipeptides containing different aza-amino acids (azaGly, azaVal, azaPhe, azaAla, and azaPro) have been synthesized in solution and demonstrated to be configurationally stable building blocks for solution and solid-phase peptide synthesis. The utility of this methodology was illustrated by the solid-phase synthesis of azapeptides **26**−**29** possessing 9 and 11 residues using standard peptide coupling conditions on oxime resin. We are currently working with collaborators to evaluate the biological activity of azapeptides **26**−**29** as potential hCGRP antagonists.

Experimental Section

General Data. Solvents and reagents were purified as specified in the Supporting Information. Mass spectral data and HRMS was obtained by the Université de Montréal Mass Spectrometry facility.

General Protocol for the Synthesis of *N*-Boc-Aza[1]-Dipeptide Benzyl Esters (10−15). L-Amino acid benzyl ester hydrochloride (1 equiv) and pyridine (4 equiv) were dissolved in DCM, cooled to 0 °C, treated with a 1.93 M solution of phosgene in toluene (1.2 equiv), and stirred at 0 °C for 2 h. The reaction mixture was extracted twice with the appropriate aliquots of chilled 0.5M HCl, water, and brine. The DCM layer was dried over Na_2SO_4 and treated with a solution of the appropriate Boc-hydrazine (**1**−**5**, 2 equiv) and DIEA (2 equiv) in DCM. The reaction was stirred at RT for 16 h. The solvent was evaporated, and the products were purified by silica gel chromatography.

Boc-AzaPhe-L-Ala-OBn (11, 0.75 g, 95% yield): mp = 108−109 °C; R_f = 0.84 (10% MeOH in DCM); $[\alpha]^{20}_D$ 2.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR 300 MHz ($CDCl_3$): δ 7.40−7.23 (m, 10H), 5.98 and 5.95 (2 s, N−H's, 2H), 5.21 (d, 1H, J = 12 Hz), 5.15 (d, 1H, J = 12 Hz), 4.59 (q, 1H, J = 7 Hz), 1.45 and 1.42 (s and d, 12H);^{13}C NMR 75 MHz ($CDCl_3$): δ 173.6, 157.1, 154.4, 136.2, 135.6, 129.1, 128.9, 128.7, 128.5, 128.2, 127.9, 82.4, 67.1, 50.7, 49.4, 28.4, 19.2; HRMS (FAB) *m*/*e*: 428.2185 (calcd for $C_{23}H_{30}N_3O_5$ (M + H$^+$), 428.2179).

General Protocol for the Synthesis of *N*-Boc-Aza[1]-Dipeptides (16−21). *N*-Boc-aza[1]-dipeptide benzyl esters (**10**−**15**) were dissolved in absolute ethanol, treated with a suspension of 10 mol % of Pd/C (10 wt %) in ethanol, and stirred under H_2 at 1 atm for 30 min. The reaction was filtered over Celite and the solvent was removed by rotary evaporation to obtain *N*-Boc-aza[1]-dipeptide (**16**−**21**).

Boc-AzaPhe-L-Ala-OH (**17**, 51 mg, 70% yield) was sublimed at 50−55 °C; R_f = 0.16 (10% MeOH in DCM); $[\alpha]^{20}_D$ 3.9° (*c* 1.0, MeOH); ^{1}H NMR 300 MHz (CD_3OD): δ 7.34−7.23 (m, 5H), 4.22 (q, 1H, J = 7 Hz), 1.48 (s, 9H), 1.39 (d, 3H, J = 7 Hz); ^{13}C NMR 75 MHz (CD_3OD): δ 176.0, 158.8, 155.6, 136.9, 129.0, 128.5, 127.6, 81.5, 50.9, 49.7, 27.5, 17.8; HRMS (FAB) *m*/*e*: 338.1716 (calcd for $C_{16}H_{24}N_3O_5$ (M + H$^+$), 338.1710).

Boc-AzaPhe-L-Ala-L-Phe-OCH$_3$ [(*S*,*S*)-22]. Aza-dipeptide **17** (78 mg, 0.23 mmol) was dissolved in 1 mL of acetonitrile, cooled to 0 °C, treated with TBTU (74 mg, 0.23 mmol) and HOBt (31 mg, 0.23 mmol), stirred at 0 °C for 10 min, and treated with a solution of L-phenylalanine methyl ester hydrochloride (99 mg, 0.45 mmol) and DIEA (89 mg, 0.69 mmol) in 0.2 mL of acetonitrile. After being stirred for 12 h, the solvent was evaporated, and the crude product was dissolved in DCM and extracted with small aliquots of concentrated $NaHCO_3$ solution, 1 M NaH_2PO_4, and brine. The solvent was evaporated to collect pure (*S*,*S*)-**22** (93 mg, 81% yield): mp = 120−122 °C; $[\alpha]^{20}_D$ 37.8° (*c* 1.0, $CHCl_3$); R_f = 0.75 (10% MeOH in DCM); ^{1}H NMR (400 MHz, C_6D_6): δ 7.26−7.04 (m, Ar, 10H) 6.12 (d, J = 7 Hz, NH, 1H), 6.02 (br s, NH, 1H), 5.01 (dd, J = 6 and 14 Hz, 1H), 4.59 (m, 1H), 3.20 (s, 3H), 3.16 (dd, J = 6 and 14 Hz, 1H), 3.05 (dd, J = 6 and 14 Hz, 1H), 3.32 (d, J = 7 Hz, 3H), 1.25 (s, 9H); ^{13}C NMR 75 MHz ($CDCl_3$): δ 172.7, 171.9, 157.4, 154.4, 136.1, 136.0, 129.5, 129.1, 129.0, 128.8, 128.2, 127.3, 82.7, 53.6, 52.6, 50.8, 50.1, 38.0, 28.3, 18.8; ESI/MS *m*/*z*: 521.2, 100% (calcd. for $C_{26}H_{34}N_4O_6Na$ (M + Na$^+$) 521.2). **Boc-AzaPhe-L-Ala-D-Phe-OCH$_3$** [(*S*,*R*)-**22**] was synthesized from **17** and D-phenylalanine methyl ester hydrochloride according to the procedure described above (83% yield); $[\alpha]^{20}_D$ −5.8° (*c* 2.0, $CHCl_3$); R_f = 0.66 (10% MeOH in DCM); ^{1}H NMR (300 MHz C_6D_6): δ 7.22−7.00 (m, Ar, 10H), 5.87 (d, J = 6 Hz, NH, 1H), 5.72 (s, NH, 1H), 5.02 (dd, J = 8 and 14 Hz, 1H), 4.52 (m, 1H), 3.26 (s, 3H), 3.12 (dd, J = 6 and 14 Hz, 1H), 2.95 (dd, J = 8 and 14 Hz, 1H), 1.28 (s, 9H), 1.18 (d, J = 7 Hz, 3H); ^{13}C NMR 75 MHz ($CDCl_3$): δ 172.7, 172.0, 157.3, 154.5, 136.2, 136.1, 129.4, 129.0, 128.9, 128.7, 128.6, 128.0, 127.2, 82.6, 53.5, 52.4, 49.9, 38.7, 37.9, 28.2; ESI/MS *m*/*z*: 521.2, 100%, (calcd. for $C_{26}H_{34}N_4O_6Na$ (M + Na$^+$) 521.2).

Peptide Synthesis (24−29). Peptide synthesis was performed in an automated shaker using oxime resin.[61] Aspartic acid and threonine were introduced as Boc-L-Asp(Bn)-OH and Boc-L-Thr(Bn)-OH. Couplings were performed with Boc-protected amino acids (300 mol %) and *N*-Boc-aza[1]-dipeptides (**16**, **17**, and **20**, 300 mol %), respectively, with TBTU (300 mol %), HOBt (300 mol %), and DIEA (300 mol %) in DMF for 2 h. The resin was agitated with N_2 bubbles during the coupling, rinsing, and deprotection sequences. Coupling reactions were monitored by the Kaiser ninhydrin test.[62] In cases of incomplete couplings, the resin was resubmitted to the same coupling conditions. Deprotections were performed with 25% TFA in DCM (30 min), and the resin was neutralized with 10% DIEA in DMF (10 min). The peptides were obtained by cleavage from the resin with a 1:1 solution of saturated NH_3 in MeOH/DCM (30 min). The crude product was purified by LC−MS and then submitted to hydrogenolysis over 10 mol % $Pd(OH)_2$ in EtOH for 16 h at 7 atm and then 10 mol % Pd/C in EtOH at 1 atm for 30 min, followed by Boc deprotection using 1:4 TFA/DCM for 30 min. The final peptides (**24**−**29**) were purified with semipreparative LC−MS (Previal C18 column, 22 × 250 mm^2, particle size 5 μm) with solvent A, H_2O (0.01% TFA), and solvent B, acetonitrile (0.01% TFA), using a gradient of 20−80% over 20 min at a flow rate of 15 mL/min. Retention times (t_R) are reported in minutes.

PTDVGPFAF−NH_2 (**24**). Purity 92% by LC−MS (t_R = 13.1); LRMS calcd for $C_{46}H_{65}N_{10}O_{12}$ (M + H$^+$), 949.5; found, 949.4.

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FVPTDVGPFAF−NH_2 (**25**). Purity >99% by LCMS (t_R = 15.1); LRMS calcd for $C_{60}H_{83}N_{12}O_{14}$ ($M + H^+$), 1195.6; found, 1195.6. PTDVGPazaFAF−NH_2 (**26**). Purity 91% by LCMS (t_R = 13.2); LRMS calcd for $C_{45}H_{64}N_{11}O_{12}$ ($M + H^+$), 950.5; found, 950.5. PTDVGaza-PFAF−NH_2 (**27**). Purity 88% by LCMS (t_R = 12.7); LRMS calcd for $C_{45}H_{64}N_{11}O_{12}$ ($M + H^+$), 950.5; found, 950.5. azaFVPTDVGPFAF−NH_2 (**28**). Purity >99% by LC−MS (t_R = 17.0); LRMS calcd for $C_{59}H_{82}N_{13}O_{14}$ ($M + H^+$), 1196.6; found, 1196.3. FVPTDVGPazaFAF−NH_2 (**29**). Purity 92% by LC−MS (t_R = 14.3); LRMS calcd for $C_{59}H_{82}N_{13}O_{14}$ ($M + H^+$), 1196.6; found, 1196.6.

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Supporting Information Available: Experimental procedures and spectral data for compounds **1**−**22** and LC−MS spectra for **24**−**29** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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