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Oxyazapeptides: Synthesis, Structure Determination, and Conformational Analysis

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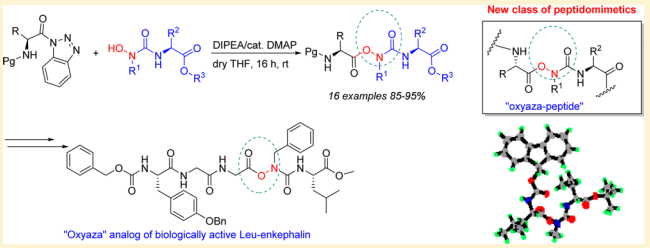
Supporting Information

ABSTRACT: Herein we report the synthesis, X-ray structure determination, and conformational analysis of a novel class of heteroatom-modified peptidomimetics, which we shall call "oxyazapeptides". Substituting the typical native N-C$^{\alpha}$ bond with an O-N$^{\alpha}$ bond creates a completely new, previously unknown family of peptidomimetics, which are hydrolytically stable and display very interesting conformational behavior. Force field calculations revealed that the barrier to rotation around the O-N$^{\alpha}$ bond in oxyazapeptides is five times lower than that around the N-N$^{\alpha}$ bond in azapeptides. Also, conformational analysis supported by X-ray suggests that the oxyaza moiety can effectively induce β-turns, which can make the newly discovered oxyazapeptide scaffold a useful tool for drug discovery and for design of biologics.



INTRODUCTION

Peptides and proteins play vital roles in biological and physiological processes. Natural peptides are widely used as drugs. However, they often need to be modified to circumvent certain problems related to drug delivery, including (i) affinity for specific receptors, (ii) metabolic stability toward endogenous proteases, (iii) appropriate biodistribution and bioavailability, and (iv) duration of action.[1,2] Such problems have been addressed by the design of peptidomimetics that may be devoid of many of the undesirable properties of natural peptides.[1] Once the structure of a natural active peptide is known, key amino acid residues necessary for receptor recognition can be identified by single amino acid modification of the peptide ligand using novel α-substituted amino acids and/or amide bond replacements. The modification of the peptide backbone (Figure 1A) with a heteroatom leading to depsipeptides (Figure 1B)[3] and azapeptides (Figure 1C)[4] has proved to be a useful strategy to design peptidomimetics.

Azapeptides are a peptidomimetics family in which substitution of the easily rotatable C$^{\alpha}$-C(O) bond in natural peptides by a more rigid urea N$^{\alpha}$-C(O) bond causes significant changes to both the chemical and the biological properties (Figure 1C). Azapeptides prefer a limited conformational space with dihedral angle values close to those of a polyproline II helix and other types of β-turns.[5−7] A systematic study involving sequential replacement of amino acid residues by their aza-counterparts of their effects on backbone conformation and activity was called "aza-amino acid scanning".[7−9]

The introduction of an α-hydroxy acid into a peptide sequence results in the formation of an ester bond, also called a depsipeptide bond (Figure 1B).[10] "Amide-to-ester" substitution is a versatile tool for investigating the effect of backbone H bonds on the 3D structure formation and stability of proteins.[11] Recently, many depsipeptides, such as enniatins[12] and cycloocta depsipeptide PF1022A,[13] have been found to be biologically active. Thus, Dyker et al. synthesized a novel class of pseudopeptides named "azadepsipeptides" (Figure 1D) and applied the method to the synthesis of a bis-aza analogue of the antiparasitic cyclooctadepsipeptide PF1022A.[14]

In the present study, we describe *de novo* design, synthesis, and characterization of oxyazapeptides in which an amino acid is replaced by an aza-hydroxy acid (Figure 1E). Oxyazapeptides can be considered as the depsipeptide analogues of azapeptides where the α-amino group of an aza-amino acid is replaced by a hydroxyl group. The effect of "amide-to-ester" substitution on the well-known limited conformational space of azapeptides[15,16] was studied computationally. Conformational analysis based on molecular mechanics calculations revealed that oxyazapeptides should adopt a β-turn secondary structure and enjoy greater conformational freedom, which could render

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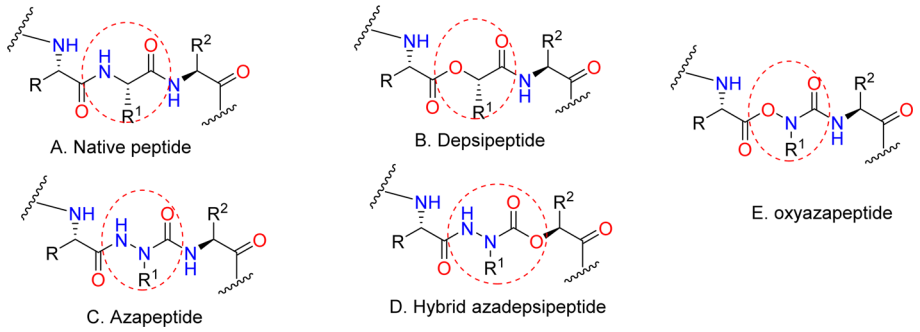


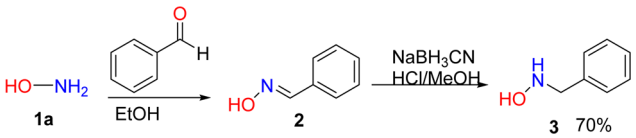
Figure 1. General structures of (A) native peptides, (B) depsipeptides, (C) azapeptides, (D) hybrid azadepsipeptides, and (E) oxyazapeptides.

them more adaptive to varying steric demand of biological interactions. Thus insertion of aza-hydroxy acid units into biologically active peptides, i.e., a type of "aza-hydroxy acid scan", would illuminate structure−activity relationships. The newly developed synthetic protocol was validated by the synthesis of an oxyaza analogue of Leu-enkephalin, an endogenous neurotransmitter.

RESULTS AND DISCUSSION

Synthesis of Oxyaza-di-, -tri-, and -tetrapeptides. The reaction of hydroxylamine **1a** with benzaldehyde afforded the corresponding oxime **2**, which was reduced with sodium cyanoborohydride ($NaCNBH_3$) to give *N*-benzylhydroxylamine **3** in 70% overall yield (Scheme 1).

Scheme 1. Synthesis of *N*-Benzylhydroxylamine



α-Amino acid ester hydrochloride salts **4a−d** were converted into active acyl imidazoles **5a−d** by reaction with carbonyldiimidazole (CDI) in the presence of 2.5 equiv of DIPEA (Hünig's base) in dry methylene chloride. Compounds **5a−d** were taken to the next step without further purification. Stirring **5a−d** with hydroxylamine **1a** or *N*-methylhydroxylamine **1b** or *N*-benzylhydroxylamine **3** at 20 °C for 16 h in dry THF containing 1 equiv of DIPEA afforded free oxyaza-dipeptides **6a−f** in 85−92% yields (Scheme 2, Table 1). No column chromatography was needed to purify the products, and a simple extractive workup using 2 N HCl gave oxyaza-dipeptides displaying satisfactory ^1H and ^{13}C NMR spectra.

N-Acylbenzotriazoles are advantageous reagents to construct peptides, peptidomimetics, and peptide conjugates.[17−20] *N*-Pg-(*α*-Aminoacyl)benzotriazoles **7a−g** were prepared following our reported procedure[17−19] and then coupled with free oxyaza-dipeptides **6a−f** in dry THF containing 1 equiv of DIPEA and a catalytic amount of DMAP (Scheme 3, Table 2) to give *N*-Pg-oxyaza-tripeptide esters **8a−j** in 85−93% yields. In an attempt to show that no racemization occurs during any stage of the reactions, we also conducted reactions between the Cbz-Ala-Bt **7b**, **7b** + **7b′** (both L and DL forms), and oxyaza-dipeptide **6a**. The absence of racemization in the oxyazapeptide (**8b** + **8b′**) was deduced from the ^1H NMR, and the retention of the chirality was further confirmed by chiral HPLC analysis using a (*S*,*S*) Welk-O1 column (MeCN/H_2O 50:50, flow rate 0.15 mL/min, detection at 210 nm). The diastereomer **8b** showed a single retention-time peak at 13.56 min, while its corresponding diastereomeric mixture (**8b** + **8b′**) showed two well separated peaks at 13.46 and 16.52 min. In our previous studies we also demonstrated that chirality of the reaction is maintained on *N*-acylation with *N*-acylbenzotriazoles for peptides, depsipeptides, and azapeptides.[17−19]

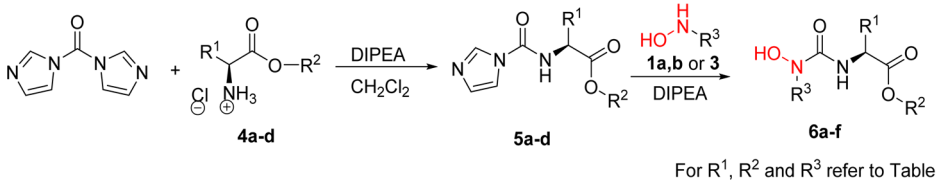
Oxyazapeptide esters **10a−f** were prepared in solution by treatment of *N*-Pg-(*α*-dipeptidoyl)benzotriazoles **9a−e** with free oxyaza-dipeptides **6b−e** in THF containing 1 equiv of DIPEA and a catalytic amount of DMAP for 16 h at 20 °C. All compounds were isolated without column chromatography (Scheme 3, Table 3). The target compounds were characterized by ^1H NMR, ^{13}C NMR, and elemental analysis. No detectable

Table 1. Construction of Oxyaza-dipeptides 6a−f

entry	R^3	R^1	R^2	**6**, yield[a] (%)
1	H	$CH(CH_3)_2$	$C(CH_3)_3$	HO-azaGly-Val-O^{t}Bu **6a**, 88
2	CH_3	$CH(CH_3)_2$	$C(CH_3)_3$	HO-azaAla-Val-O^{t}Bu **6b**, 90
3	CH_2Ph	$CH(CH_3)_2$	$C(CH_3)_3$	HO-azaPhe-Val-O^{t}Bu **6c**, 85
4	CH_3	$CH_2CH(CH_3)_2$	CH_3	HO-azaAla-Leu-OMe **6d**, 92
5	CH_2Ph	$CH_2CH(CH_3)_2$	CH_3	HO-azaPhe-Leu-OMe **6e**, 87
6	CH_3	CH_2Ph	CH_3	HO-azaAla-Phe-OMe **6f**, 90

[a]Isolated yield.

Scheme 2. Construction of Oxyaza-dipeptides 6a−f



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Scheme 3. Synthesis of Oxyaza-tri- and -tetrapeptides

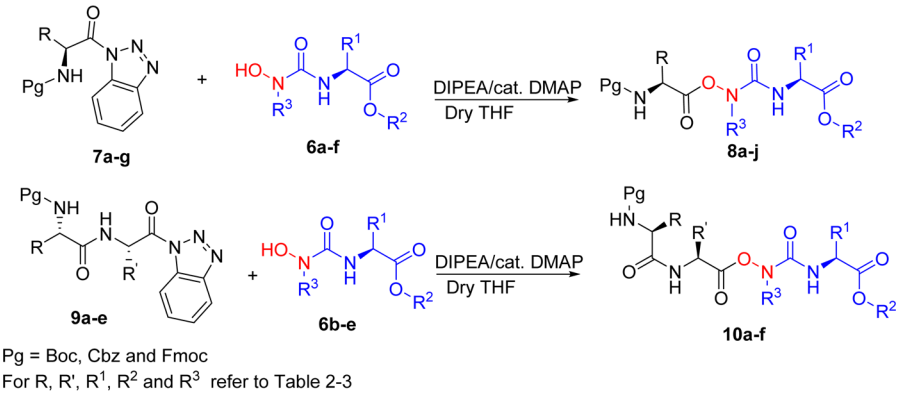

Table 2. Preparation of Oxyaza-tripeptides 8a−j

entry	RCOBt **7a−g**	**6a−f**	oxyaza-tripeptide **8a−j**, yield[a] (%)
1	Cbz-Gly-Bt **7a**	**6b**	Cbz-Gly-*O*-azaAla-Val-O^{t}Bu **8a**, 87
2	Cbz-Ala-Bt **7b**	**6a**	Cbz-Ala-*O*-azaGly-Val-O^{t}Bu **8b**, 87
3	Cbz-DL-Ala-Bt **7b** + **7b′**	**6a**	Cbz-DL-Ala-*O*-azaGly-Val-O^{t}Bu **8b** + **8b′**, 90
4	Cbz-Phe-Bt **7c**	**6d**	Cbz-Phe-*O*-azaAla-Leu-OMe **8c**, 91
5	Cbz-Phe-Bt **7c**	**6e**	Cbz-Phe-*O*-azaPhe-Leu-OMe **8d**, 90
6	Cbz-Phe-Bt **7c**	**6f**	Cbz-Phe-*O*-azaAla-Phe-OMe **8e**, 95
7	Boc-Gly-Bt **7d**	**6c**	Boc-Gly-*O*-azaPhe-Val-O^{t}Bu **8f**, 87
8	Boc-*β*Ala-Bt **7e**	**6c**	Boc-*β*Ala-*O*-azaPhe-Val-O^{t}Bu **8g**, 85
9	Fmoc-Leu-Bt **7f**	**6b**	Fmoc-Leu-*O*-azaAla-Val-O^{t}Bu **8h**, 89
10	Fmoc-Leu-Bt **7f**	**6c**	Fmoc-Leu-*O*-azaPhe-Val-O^{t}Bu **8i**, 93
11	Fmoc-Phe-Bt **7g**	**6c**	Fmoc-Phe-*O*-azaPhe-Val-O^{t}Bu **8j**, 92

[a]Isolated yield.

Table 3. Preparation of Oxyaza-tetrapeptides 10a−f

entry	**9a−e**	**6b−e**	oxyaza-tetrapeptide **10a−f**, yield[a] (%)
1	Cbz-Ala-Met-Bt **9a**	**6c**	Cbz-Ala-Met-*O*-azaPhe-Val-O^{t}Bu **10a**, 90
2	Cbz-Phe-Met-Bt **9b**	**6b**	Cbz-Phe-Met-*O*-azaAla-Val-O^{t}Bu **10b**, 86
3	Cbz-Ala-Met-Bt **9a**	**6d**	Cbz-Ala-Met-*O*-azaAla-Leu-OMe **10c**, 84
4	Boc-Gly-Gly-Bt **9c**	**6d**	Boc-Gly-Gly-*O*-azaAla-Leu-OMe **10d**, 87
5	Boc-Ala-*β*Ala-Bt **9d**	**6e**	Boc-Ala-*β*Ala-*O*-azaPhe-Leu-OMe **10e**, 84
6	Boc-Ala-Gaba-Bt **9e**	**6b**	Boc-Ala-Gaba-*O*-azaAla-Val- O^{t}Bu **10f**, 88

[a]Isolated yield.

racemization of the *N*-Pg-oxyaza-tetrapeptides esters was observed by chiral HPLC analysis.

Validation of the Synthetic Methodology. We aimed to utilize our methodology and to show general applicability and scope of these "oxyaza"-type novel peptidomimetics by synthesizing a peptidomimetic version of the biologically important pentapeptide Leu-enkephalin.[21] Leu-enkephalin is an endogenous opioid peptide neurotransmitter found naturally in the brains of many animals, including humans. Its amino acid sequence is Tyr-Gly-Gly-Phe-Leu. Protected tyrosine **11** was first activated and coupled with Gly-Gly to give tripeptide **12**. Then by using standard benzotriazole methodology the acid group was activated, and coupling of compound **13** with free oxyaza-dipeptide **6e** in dry THF in the presence of 1.0 equiv of DIPEA and 10% catalytic amount of DMAP gave the target oxyaza analogue of Leu-enkephalin **14** (Scheme 4).

X-ray Structure Determination. It was deemed important to confirm the structure of a representative example of this new peptidomimetics family. Thus, an X-ray crystal structure was determined of compound **8h** (Figure 2), which crystallizes in the orthorhombic space group $P2_12_12_1$. This unambiguously confirmed the structure and absolute configuration of **8h**.

Conformational Analysis. Conformational behavior of oxyazapeptides would be most interesting to study in comparison with azapeptides. This can be done by rotations around the common dihedral angles ϕ and ψ. Here, the angle ϕ denotes rotation about the N-N$^\alpha$ (or O-N$^\alpha$) bond, and the angle ψ is rotation about the bond linking the N$^\alpha$ and the carbonyl carbon (Figure 3). Rotations around these angles are expected to proceed differently for aza- and oxyazapeptides, because oxyazapeptides have a set of distinct features such as (i) the absence of hydrogen at the α-atom, (ii) shorter C-O and O-N bond lengths comparing to C-N and N-N bond lengths, and (iii) much less double bond character of the ester C-O bond comparing to the amide C-N bond. To make the calculations simpler, model aza-**15** and oxyaza-dipeptide **16** were drawn by cutting the bulky protecting groups and substituting them with methyl groups. These structures were geometry optimized using the MMX force field (as implemented in the PCModel v. 9.3 software). The optimized structures are displayed in Figure 3. One can see that the main difference between **15** and **16** is the dihedral angle ϕ, equal to 178° and 103.5° in **15** and **16**, respectively. In azapeptide **15**, angle ϕ is *trans* due to a certain double-bonded character of the hydrazine N-N bond. By contrast, oxyazapetide **16** renders a *gauche* conformation, because rotation around the O-N bond is much less hindered.

Energy barriers to rotations can also provide interesting information. To calculate rotational barriers, the optimized structures **15** and **16** were subjected to the Dihedral Driver procedure, as implemented in PCModel. The torsional energy plots are shown in Figure 4. The most striking difference between the aza- and oxyazapeptide is the barrier to rotation around the N(O)-N$^\alpha$ bond. In azapeptide **15**, the barrier is as high as 35 kcal/mol, while in oxyazapeptide **16** it is only 7 kcal/mol. The shape of the potential energy profile also exhibits a sharp difference: it is a steep double maximum in **15** and a shallow single maximum in **16**. The symmetric maxima on the ϕ torsional energy plot of **15** occur almost exactly at −90° and 90°, while the single maximum of **16** corresponds to a pure *cis* conformation. In structure **15**, these maxima can be associated

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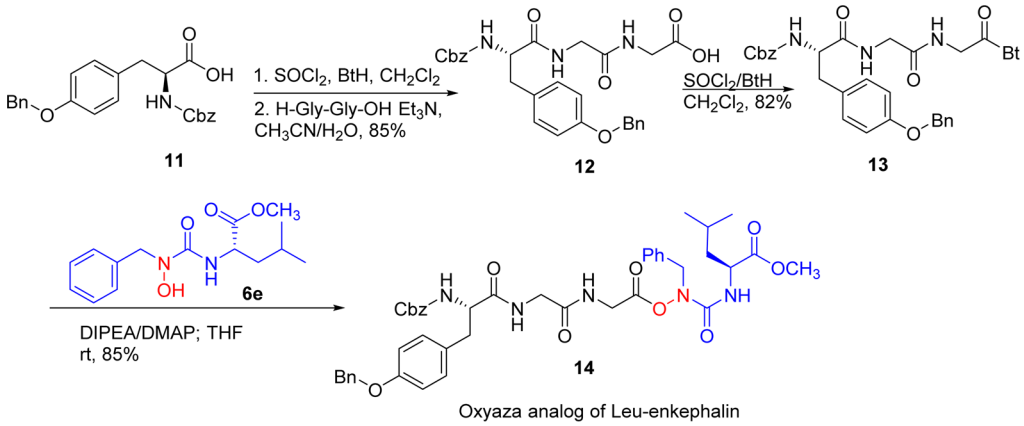
Scheme 4. Synthesis of Oxyaza Analogue of Leu-enkephalin 14



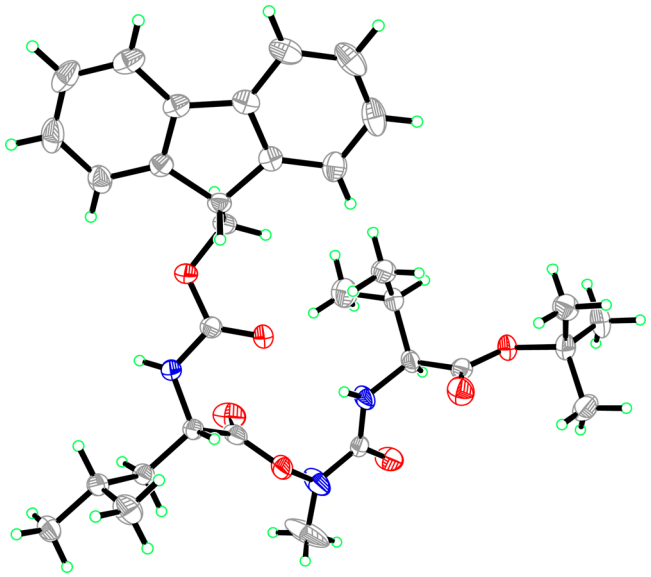


Figure 2. X-ray crystal structure of **8h**.

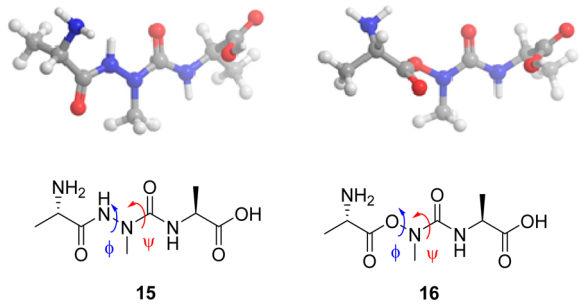


Figure 3. Optimized structures of azapeptide **15** and oxyazapeptide **16**.

with the rehybridization in the hydrazine fragment and a significant repulsion by the methyl groups, while in **16** the maximum is due to the shorter N-O bond. As the influence of the varying heteroatom drops as the distance increases, rotation around another important bond described by dihedral angle ψ, N(O)-N$^{\alpha}$-C′-N, proceeds in a very similar way in both structures. As seen in Figure 4 (right column), the barrier heights (both close to 15 kcal/mol) and the shapes of the torsional energy profiles are very similar in structures **15** and **16**. It was found that inclusion of an implicit solvation does not significantly change the conformational behavior, and the gas phase and solvation calculations were in a good qualitative agreement. To test this, we capped the free amino groups in **15** and **16** with acetyls and carried out calculations with the GB/SA model, the results of which are given in Figure 4S and 5S of the Supporting Information. Therefore the rest of the conformational analysis was done with gas-phase force field calculations.

Interestingly, the ϕ and ψ torsional angles observed in the X-ray structure (Figure 2) of **8h** are in excellent agreement with these gas phase calculations. In the solid state, the ϕ angle is 91.3(2)°, while the ψ angle is 177.9(2)°.

Computational Study of β-Turn Induction. It is known[6,22] that azaamino acid residues are instrumental in inducing β-turns and other helical structures in peptides. It is believed that this ability stems from a restricted rotation around the N$^{\alpha}$-C′ bond, which is due to a partial double-bonded character of the latter. Conformational analysis of a simple dipeptide with one azaamino unit (For-Ala-azaAla-NH$_2$) revealed that a global minimum structure was one having a set of dihedral angles consistent with a β-turn structural motif.[6] As the oxyaza unit enjoys at least one additional degree of freedom, which is the rotation around the C-O ester bond, one would expect oxyazapeptides to attain a β-turn structure with even greater ease. To explore the ability of the oxyaza unit to induce a β-turn, we ran a full conformational search (MMX force field) of a model For-Ala-Ala-NH$_2$ dipeptide in which one of the C$^{\alpha}$ atoms was replaced by nitrogen and the adjacent amide N was replaced by oxygen. For consistency, this model oxyaza-dipeptide was made isosteric to For-Ala-azaAla-NH$_2$ previously published by Lee et al.[6] The conformational search found that a β-turn conformer was the global minimum on the potential energy surface.

This oxyaza-dipeptide **17** conformer is displayed in Figure 5, with the isosteric azadipeptide **18** also shown for the sake of comparison. The dihedral angles ϕ_1, ψ_1 and ϕ_2, ψ_2 characterizing rotations in oxyaza-dipeptide **17** are found to be −67°, 109°, 109° and 2°, which is very close to the type II β-turn structure.[15,16] As seen in Figure 5, the isosteric azadipeptide **18** also makes a β-turn conformation, but according to the set of the dihedral angles −77°, 76°, 179°, and −3.7°, the hydrazine moiety prefers a *trans* conformation, which can be explained by the steric preference of the adjacent urea group.

Comparative analysis of hydrogen bond contacts in structures **17** and **18** also adds to an understanding of the steric consequences of the "amide-to-ester" replacement. As

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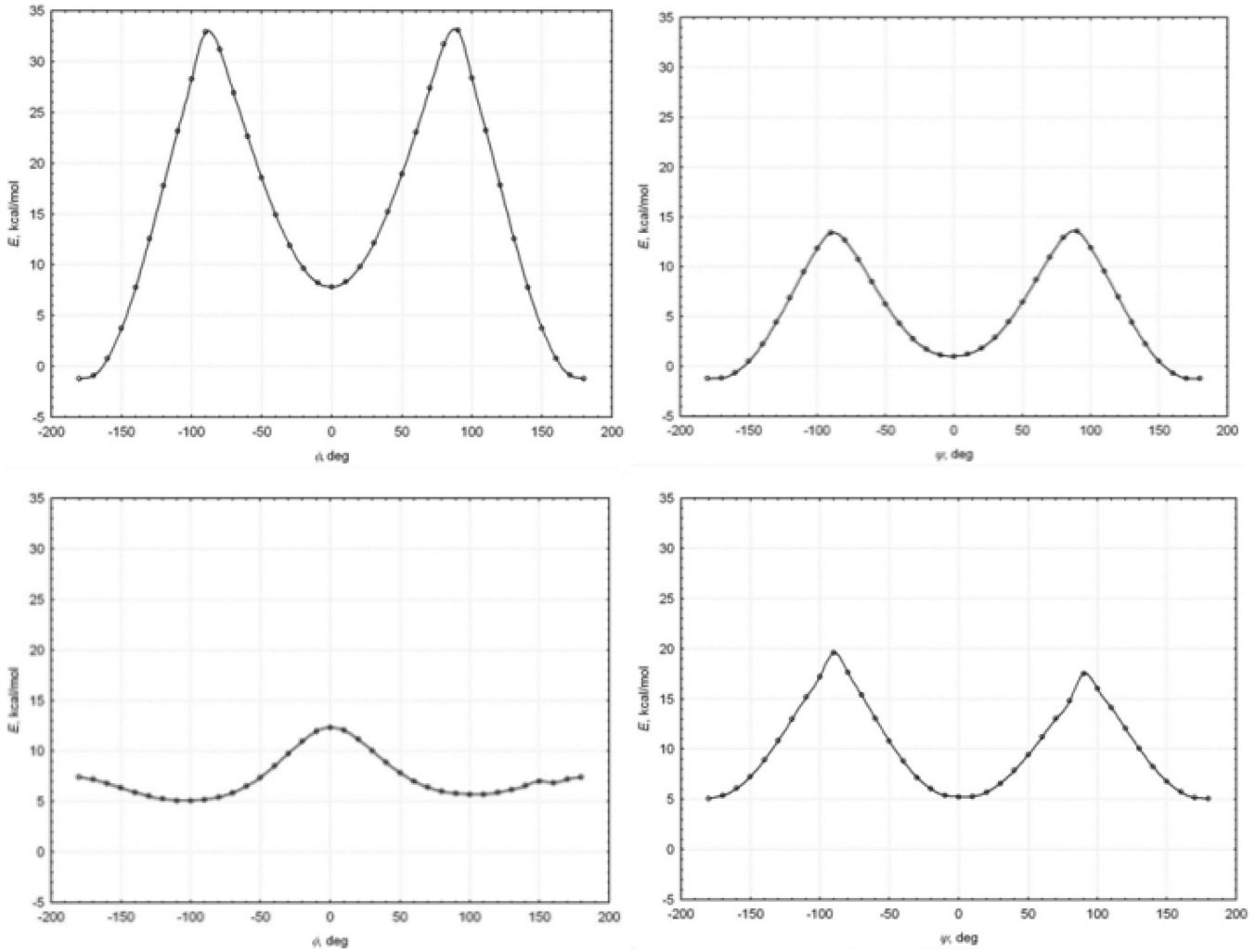

Figure 4. Torsional energy plots for dihedral angles ϕ (left) and ψ (right) in azapeptide **15** (upper row) and oxyazapeptide **16** (lower row).

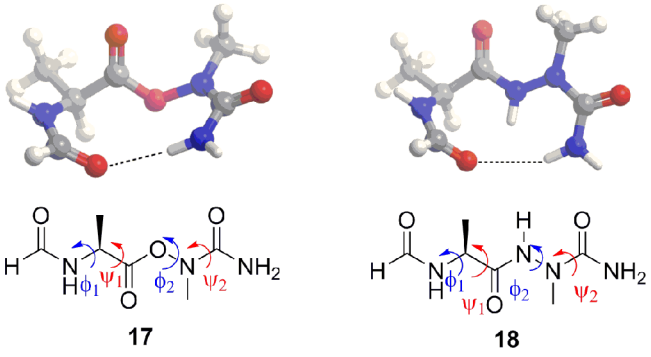

Figure 5. Molecular structures of β-turn structures of oxyazapeptide **17** (left) and azapeptide **18** (right); the C10 hydrogen bonds are indicated by the dotted lines.

seen in Figure 5, an unobstructed C10 (10-membered intramolecular cycle) hydrogen bond is formed in the oxyazadipeptide **17**, which is characterized by the O1-H(N4) separation of 2.25 Å and the O1-H(N4)-N4 angle of 167°. In the X-ray structure of oxyaza tripeptide **8h** (Figure 2), the respective contact length is slightly longer (2.66 Å) and the O-H-N angle is somewhat smaller (155°), which is most likely due to the steric influence of the valine's bulky isopropyl group. In azadipeptide structure **18**, the respective separation and angle are 2.61 Å and 154.2°, which render the respective hydrogen bond a weaker one compared to that in **17**. Another structural feature of azapeptides, which oxyazapeptides are free of, is the O-H(N2) contact. In structure **18**, this contact (2.10 Å and 135.7°) making a C7 hydrogen bond makes the structure a γ-turn rather than a β-turn. On the other hand, O-H(N2) should be a weaker hydrogen bond than O1-H(N4) (because of the strong angular dependence of hydrogen bond strength) and therefore can be considered as one exerting assistance to the primary O1-H(N4) in keeping the β-turn conformation. As distinguished from **18**, the β-turn conformation of oxyaza structure **17** is supported by a single although stronger hydrogen bond O1-H(N4). As a result, the increased backbone flexibility and a stronger C10 hydrogen bond in oxyazapeptides can put them on a par with azapeptides or even make better β-turn inducers that can be used in the synthesis of artificial peptidomimetics.

CONCLUSIONS

We have designed, synthesized, and structurally studied a new family of peptidomimetics termed "oxyazapeptides" in which the normal N-C^{α} bond is replaced by an O-N^{α} bond. This class of compounds are conformationally more labile, due to a lower barrier to rotation around the O-N and C-O bonds, compared to those around the N-C and C-N bonds in native peptides. As

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a result, oxyazapeptides enjoy a higher degree of conformational freedom that might make them potentially more adaptable to varying steric demand in receptor binding. The conformational analysis suggests that the oxyaza moiety can effectively induce β-turns in peptidomimetics and thus serve a useful synthetic auxiliary in the design of small peptide based drugs. More importantly, these newly discovered oxyazapeptides can be a useful tool for drug discovery and for the targeted design of biologics.

EXPERIMENTAL SECTION

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded with TMS for ^{1}H (300 MHz) and ^{13}C (75 MHz) as an internal reference. Reaction progress was monitored by thin-layer chromatography (TLC) and visualized by UV light. DCM was dried and distilled over CaH_2, whereas tetrahydrofuran (THF) was used after distillation over Na-benzophenone. Carbonyldiimidazole (CDI), hydroxylamine **1a**, *N*-methylhydroxylamine **1b**, sodium cyanoborohydride, benzaldehyde, and L-amino methyl/*tert*-butyl ester hydrochloride **4a**–**d** were purchased from chemical supply companies and used without further purification. *N*-Pg-(α-Aminoacyl)-benzotriazoles **7a**–**g**, *N*-Pg-(α-dipeptidoyl)benzotriazoles **9a**–**d**, and Cbz-Tyr-(OBn)-Gly-Gly-Bt **13** were prepared according to the literature methods.

Computational Details. All calculations were done with the PCModel software ver. 9.3, Serena Software. The MMX force field was used. The ground state structures were identified through full conformational searches using the GMMX routine of PCModel. The implicit solvent model used was GB/SA (Generalized Born/Surface Area) with the analytical method of Still. The solvent dielectric constant was taken as 78.30 (water) and the internal dielectric constant was taken as 1, which are default settings in PCModel. Full conformational search was also done with the GB/SA solvent method. The torsional potentials were calculated with the Dihedral Driver procedure as implemented in PCModel; start and final angles were −180° and 180°, respectively, with the step equal to 10°.

General Methods for the Preparation of Oxyaza-dipeptide 6a–f. To a suspension of L-amino methyl/*tert*-butyl ester hydrochloride **4a**–**d** (1.0 mmol, 1.0 equiv) in DCM (20 mL) at 20 °C were added 2.5 equiv of DIPEA and CDI (carbonyldiimidazole, 1.1 equiv). The reaction mixture was stirred for 3 h at rt, and the organic layer was washed with water (2 × 20 mL), $NaHCO_3$ (3 × 20 mL), and brine solution (2 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum to give oily monosubstituted imidazole derivative **5a**–**d**. The residue **5a**–**d** (1.0 equiv) was dissolved in dry THF (20 mL) and reacted with *N*–alkylhydroxylamine **1a,b** or **3** (1.0 equiv) in the presence of DIPEA (1.0 equiv) at 20 °C overnight. The reaction mixture was poured into a separatory funnel, washed with water (2 × 20 mL), 2 N HCl (3 × 20 mL), and brine solution (2 × 20 mL), and dried over $MgSO_4$, and the solvent evaporated under vacuum to give oxyazadipeptide **6a**–**f**.

*HO-azaGly-Val-O^{t}Bu (**6a**).* Compound **6a** was prepared according to the given general procedure for **6a**–**f**. White microcrystals (0.408 g, 88%); mp 125.0–127.0 °C; $[\alpha]^{20}_D$ −10.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (br s, 1H), 7.30 (s,1H), 6.42 (d, *J* = 9.0 Hz, 1H), 4.28 (dd, *J* = 9.0, 4.5 Hz, 1H), 2.24–2.06 (m, 1H), 1.45 (s, 9H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 162.0, 82.3, 58.1, 31.6, 28.2, 19.1, 17.8; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{10}H_{20}N_2O_4Na$ 255.1315, found 255.1326.

*HO-azaAla-Val-O^{t}Bu (**6b**).* Compound **6b** was prepared according to the given procedure for **6a**. White microcrystals (0.443 g, 90%); mp 88.0–91.0 °C; $[\alpha]^{20}_D$ −4.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1H), 6.37 (d, *J* = 8.7 Hz, 1H), 4.22 (dd, *J* = 8.9, 4.7 Hz, 1H), 3.13 (s, 3H), 2.18–2.11 (m, 1H), 1.46 (s, 9H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 161.3, 82.2, 58.7, 38.8, 31.4, 28.2, 19.2, 17.9. Anal. Calcd for $C_{11}H_{22}N_2O_4$: C, 53.64; H, 9.00; N, 11.37. Found: C, 53.52; H, 9.27; N, 11.04.

*HO-azaPhe-Val-O^{t}Bu (**6c**).* Compound **6c** was prepared according to the given procedure for **6a**. White microcrystals (0.548 g, 85%); mp 90.0–92.0 °C; $[\alpha]^{20}_D$ −20.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.12 (m, 5H), 6.41 (d, *J* = 9.0 Hz, 1H), 4.64–4.45 (m, 2H), 4.19 (dd, *J* = 8.7, 4.8 Hz, 1H), 2.16–1.97 (m, 1H), 1.39 (s, 9H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 160.7, 137.1, 128.9, 128.5, 127.5, 82.1, 77.7, 77.3, 76.9, 58.7, 54.8, 31.6, 28.2, 19.1, 17.9. Anal. Calcd for $C_{17}H_{26}N_2O_4$: C, 63.33; H, 8.13; N, 8.69. Found: C, 63.38; H, 8.40; N, 8.65.

*HO-azaAla-Leu-OMe (**6d**).* Compound **6d** was prepared according to the given procedure for **6a**. Low melting solid (0.402 g, 92%); $[\alpha]^{20}_D$ −26.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.23 (d, *J* = 8.1 Hz, 1H), 4.38–4.29 (m, 1H), 3.67 (s, 3H), 3.07 (s, 3H), 1.70–1.46 (m, 3H), 0.87 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 161.0, 52.5, 51.9, 41.3, 38.3, 25.0, 23.1, 21.9. Anal. Calcd for $C_9H_{18}N_2O_4$: C, 49.53; H, 8.31; N, 12.84. Found: C, 49.34; H, 8.60; N, 12.46.

*HO-azaPhe-Leu-OMe (**6e**).* Compound **6e** was prepared according to the given procedure for **6a**. White microcrystals (0.256 g, 87%); mp 114.0–116.0 °C; $[\alpha]^{20}_D$ −12.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.13 (m, 5H), 6.30 (d, *J* = 8.1 Hz, 1H), 4.60 (d, *J* = 15.0 Hz, 1H), 4.52 (d, *J* = 15.0 Hz, 1H), 4.42–4.34 (m, 1H), 3.65 (s, 3H), 1.70–1.48 (m, 3H), 0.88 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 161.0, 52.5, 51.9, 41.3, 38.3, 25.0, 23.1, 21.9. Anal. Calcd for $C_{15}H_{22}N_2O_4$: C, 61.21; H, 7.53; N, 9.52. Found: C, 61.30; H, 7.92; N, 9.49.

*HO-azaAla-Phe-OMe (**6f**).* Compound **6f** was prepared according to the given procedure for **6a**. Low melting solid (0.454 g, 90%); $[\alpha]^{20}_D$ −20.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.38–7.18 (m, 3H), 7.16–7.08 (m, 2H), 6.34 (d, *J* = 7.8 Hz, 1H), 4.76–4.60 (m, 1H), 3.68 (s, 3H), 3.10–3.02 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 160.7, 136.1, 129.3, 128.8, 127.3, 54.4, 52.5, 38.5, 38.4. Anal. Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.52; H, 6.69; N, 10.73.

General Methods for the Preparation of Oxyaza-tri- and -tetrapeptides 8a–j, 10a–f. *N*-Pg-(α-Aminoacyl)benzotriazoles **7a**–**g** or *N*-Pg-(α-dipeptidoyl)benzotriazoles **9a**–**d** (1.0 mmol, 1.0 equiv) was dissolved in dry THF (20 mL) and reacted with oxyaza-dipeptides **6a**–**f** (1.0 equiv) in the presence of DIPEA (1.0 equiv) and a 10% cat. amount of DMAP at 20 °C overnight. The reaction mixture was poured into a separatory funnel, washed with water (2 × 20 mL), 2 N HCl (3 × 20 mL), and brine solution (2 × 20 mL), dried over $MgSO_4$, and evaporated under vacuum to give oxyaza-tripeptide **8a**–**j** or oxyaza-tetrapeptide **10a**–**f**, which were characterized by ^{1}H and ^{13}C NMR and elemental analysis.

*Cbz-Gly-O-azaAla-Val-O^{t}Bu (**8a**).* Compound **8a** was prepared according to the given general procedure for **8a**–**j**. White microcrystals (0.381 g, 87%); mp 80.0–82.0 °C; $[\alpha]^{20}_D$ −13.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.17 (m, 5H), 6.01 (d, *J* = 8.4 Hz, 1H), 5.52 (t, *J* = 5.6 Hz, 1H), 5.07 (s, 2H), 4.22 (dd, *J* = 8.4, 4.8 Hz, 1H), 3.99 (d, *J* = 5.7 Hz, 2H), 3.15 (s, 3H), 2.15–2.04 (m, 1H), 1.38 (s, 9H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 168.0, 158.8, 156.8, 136.1, 128.7, 128.5, 128.3, 82.1, 67.7, 58.9, 42.2, 38.6, 31.6, 28.2, 19.1, 17.9. Anal. Calcd for $C_{21}H_{31}N_3O_7$: C, 57.65; H, 7.14; N, 9.60. Found: C, 57.75; H, 7.52; N, 9.40.

*Cbz-Ala-O-azaGly-Val-O^{t}Bu (**8b**).* Compound **8b** was prepared according to the given general procedure for **8a**–**j**. Low melting solid (0.381 g, 87%); $[\alpha]^{20}_D$ −17.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.30–7.20 (m, 5H), 6.28 (d, *J* = 8.7 Hz, 1H), 5.69 (d, *J* = 7.2 Hz, 1H), 5.08 (d, *J* = 12.0 Hz, 1H), 5.01 (d, *J* = 12.6 Hz, 1H), 4.45–4.35 (m, 1H), 4.26 (dd, *J* = 8.7, 4.8 Hz, 1H), 2.25–2.00 (m, 1H), 1.48–1.33 (m, 12H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 171.2, 158.6, 156.2, 136.1, 128.7, 128.4, 128.2, 82.3, 67.4, 58.6, 48.9, 31.6, 28.2, 19.0, 17.9, 15.4. Anal. Calcd for $C_{21}H_{31}N_3O_7$: C, 57.65; H, 7.14; N, 9.60. Found: C, 57.50; H, 7.40; N, 9.31.

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*Cbz-(DL)Ala-O-azaGly-Val-O^{t}Bu (**8b + 8b′**).* Compound **8b** + **8b′** was prepared according to the given general procedure for **8a−j**. Low melting solid (0.394 g, 90%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.35−7.15 (m, 5H), 6.24 (br s, 1H), 5.63−5.52 (m, 1H), 5.16−4.98 (m, 2H), 4.50−4.37 (m, 1H), 4.32−4.20 (m, 1H), 2.20−2.04 (m, 1H), 1.47−1.36 (m, 12H), 0.92−0.82 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 171.2, 158.6, 156.2, 136.1, 128.7, 128.4, 128.3, 82.3, 67.6, 67.5, 58.5, 49.1, 48.9, 31.6, 28.2, 19.2, 19.1, 17.8, 15.5. Anal. Calcd for $C_{21}H_{31}N_3O_7$: C, 57.65; H, 7.14; N, 9.60. Found: C, 57.55; H, 7.42; N, 9.22.

*Cbz-Phe-O-azaAla-Leu-OMe (**8c**).* Compound **8c** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.455 g, 91%); mp 123.0−125.0 °C; $[\alpha]^{20}_D$ −17.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.00 (m, 10H), 6.46 (d, *J* = 8.1 Hz, 1H), 5.31−5.24 (m, 1H), 5.08 (d, *J* = 12.0 Hz, 1H), 4.99 (d, *J* = 12.0 Hz, 1H), 4.50−4.32 (m, 2H), 3.68 (s, 3H), 3.10−3.03 (m, 2H), 2.85 (s, 3H), 1.70−1.55 (m, 3H), 0.87 (d, *J* = 5.4 Hz, 3H), 0.84 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 170.5, 159.0, 156.4, 135.7, 134.6, 129.4, 129.2, 128.8, 128.7, 128.3, 127.9, 67.8, 54.9, 52.4, 52.1, 41.1, 38.0, 37.3, 24.8, 23.2, 21.9. Anal. Calcd for $C_{26}H_{33}N_3O_7$: C, 62.51; H, 6.66; N, 8.41. Found: C, 62.76; H, 6.80; N, 8.56.

*Cbz-Phe-O-azaPhe-Leu-OMe (**8d**).* Compound **8d** was prepared according to the given general procedure for **8a−j**. Low melting solid (0.518 g, 90%); $[\alpha]^{20}_D$ −41.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−6.95 (m, 15H), 6.67 (d, *J* = 7.8 Hz, 1H), 5.62 (d, *J* = 4.5 Hz, 1H), 5.08−4.93 (m, 2H), 4.64 (d, *J* = 15.0 Hz, 1H), 4.50 (d, *J* = 15.0 Hz, 1H), 4.29−4.18 (m, 1H), 3.64 (s, 3H), 2.77 (d, *J* = 7.2 Hz, 2H), 1.70−1.52 (m, 3H), 0.87 (d, *J* = 5.4 Hz, 3H), 0.86 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 170.4, 158.7, 156.7, 135.8, 135.4, 135.1, 129.3, 129.2, 129.2, 128.8, 128.6, 128.4, 128.3, 127.9, 127.8, 67.6, 54.9, 54.8, 52.4, 52.3, 40.9, 36.7, 24.8, 23.2, 21.9; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{32}H_{37}N_3O_7Na$ 598.2524, found 598.2550.

*Cbz-Phe-O-azaAla-Phe-OMe (**8e**).* Compound **8e** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.507 g, 95%); mp 132.0−134.0 °C; $[\alpha]^{20}_D$ −24.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33−6.98 (m, 15H), 6.57 (d, *J* = 7.8 Hz, 1H), 5.57 (d, *J* = 5.4 Hz, 1H), 5.02 (d, *J* = 12.3 Hz, 1H), 4.93 (d, *J* = 12.3 Hz, 1H), 4.63 (q, *J* = 7.2 Hz, 1H), 4.40 (q, *J* = 6.8 Hz, 1H), 3.60 (s, 3H), 3.11−2.98 (m, 4H), 2.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 170.3, 158.6, 156.5, 136.7, 135.9, 134.9, 129.5, 129.4, 129.1, 128.8, 128.6, 128.6, 128.3, 127.8, 127.1, 67.7, 55.0, 54.9, 52.4, 38.1, 38.0, 37.3. Anal. Calcd for $C_{29}H_{31}N_3O_7$: C, 0.65.28; H, 5.86; N, 7.88. Found: C, 65.40; H, 6.11; N, 7.92.

*Boc-Gly-O-azaPhe-Val-O^{t}Bu (**8f**).* Compound **8f** was prepared according to the given general procedure for **8a−j**. Low melting solid (0.417 g, 87%); $[\alpha]^{20}_D$ −11.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.20 (m, 5H), 6.03 (d, *J* = 8.4 Hz, 1H), 5.24 (t, *J* = 5.4 Hz, 1H), 4.81 (d, *J* = 15.3 Hz, 1H), 4.72 (d, *J* = 15.3 Hz, 1H), 4.27 (dd, *J* = 8.4, 3.3 Hz, 1H), 3.90−3.66 (m, 2H), 2.20−2.04 (m, 1H), 1.43 (s, 9H), 1.39 (s, 9H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 8.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 168.4, 158.1, 155.8, 135.2, 128.9, 128.4, 127.8, 81.8, 80.4, 59.0, 54.5, 41.4, 31.2, 28.2, 28.0, 18.8, 17.8; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{37}N_3O_7Na$ 502.2543, found 502.2524.

*Boc-βAla-O-azaPhe-Val-O^{t}Bu (**8g**).* Compound **8g** was prepared according to the given general procedure for **8a−j**. Low melting solid (0.420 g, 85%); $[\alpha]^{20}_D$ −20.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30−7.20 (m, 5H), 5.76 (d, *J* = 8.4 Hz, 1H), 5.16 (t, *J* = 5.7 Hz, 1H), 4.82 (d, *J* = 15.3 Hz, 1H), 4.69 (d, *J* = 15.3 Hz, 1H), 4.28 (dd, *J* = 8.4, 4.5 Hz, 1H), 3.30 (q, *J* = 6.0 Hz, 2H), 2.50 (dd, *J* = 6.8, 5.3 Hz, 2H), 2.15−2.03 (m, 1H), 1.41 (s, 9H), 1.37 (s, 9H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.82 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 169.9, 158.0, 155.9, 135.3, 128.9, 128.5, 128.0, 82.3, 79.6, 58.5, 54.3, 33.1, 31.6, 31.6, 28.4 28.0, 18.8, 17.7; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{25}H_{39}N_3O_7Na$ 516.2687, found 516.2680.

*Fmoc-Leu-O-azaAla-Val-O^{t}Bu (**8h**).* Compound **8h** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.518 g, 89%); mp 113.0−115.0 °C; $[\alpha]^{20}_D$ +22.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.8 Hz, 2H), 7.52 (d, *J* = 7.5 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.26 (t, *J* = 7.5 Hz, 2H), 6.29 (d, *J* = 8.7 Hz, 1H), 5.37 (d, *J* = 6.6 Hz, 1H), 4.40−4.22 (m, 4H), 4.16 (t, *J* = 7.2 Hz, 1H), 3.19 (s, 3H), 2.20−2.05 (m, 1H), 1.72−1.55 (m, 3H), 1.41 (s, 9H), 1.00−0.80 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.4, 159.0, 156.5, 143.8, 141.5, 128.0, 127.3, 125.2, 120.2, 81.9, 67.6, 59.1, 52.0, 47.3, 40.5, 38.4, 31.6, 28.2, 25.1, 22.7, 22.2, 19.1, 18.0. Anal. Calcd for $C_{32}H_{43}N_3O_7$: C, 66.07; H, 7.45; N, 7.22. Found: C, 65.7; H, 7.77; N, 7.58.

*Fmoc-Leu-O-azaPhe-Val-O^{t}Bu (**8i**).* Compound **8i** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.612 g, 93%); mp 116.0−119.0 °C; $[\alpha]^{20}_D$ −13.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.54 (d, *J* = 7.2 Hz, 2H), 7.44−7.20 (m, 9H), 6.41 (d, *J* = 8.4 Hz, 1H), 5.31 (d, *J* = 6.6 Hz, 1H), 4.95 (d, *J* = 15.3 Hz, 1H), 4.75 (d, *J* = 15.3 Hz, 1H), 4.40−4.30 (m, 3H), 4.22−4.08 (m, 2H), 2.21−2.10 (m, 1H), 1.47 (s, 9H), 1.42−1.17 (m, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.81 (d, *J* = 6.3, Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 171.1, 158.6, 156.3, 143.6, 141.3, 135.3, 128.9, 128.3, 127.8, 127.1, 127.1, 125.0, 120.0, 81.6, 67.4, 59.2, 54.7, 51.7, 47.0, 39.8, 31.3, 28.0, 24.5, 22.4, 21.8, 18.9, 17.9. Anal. Calcd for $C_{38}H_{47}N_3O_7$: C, 69.38; H, 7.20; N, 6.39. Found: C, 69.18; H, 7.40; N, 6.60.

*Fmoc-Phe-O-azaPhe-Val-O^{t}Bu (**8j**).* Compound **8j** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.636 g, 92%); mp 107.0−109.0 °C; $[\alpha]^{20}_D$ −21.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.8 Hz, 2H), 7.55−7.44 (m, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.35−7.22 (m, 10H), 7.11 (d, *J* = 7.8 Hz, 2H), 6.46 (d, *J* = 8.7 Hz, 1H), 5.33 (d, *J* = 5.7 Hz, 1H), 4.70 (s, 2H), 4.40−4.30 (m, 3H), 4.20−4.11 (m, 2H), 2.92−2.75 (m, 2H), 2.20−2.10 (m, 1H), 1.46 (s, 9H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 170.2, 158.4, 156.2, 143.6, 141.2, 135.3, 135.0, 129.1, 129.0, 128.3, 127.8, 127.6, 127.1, 127.1, 125.0, 120.0, 81.6, 67.5, 59.2, 54.8, 54.3, 47.0, 36.7, 31.3, 28.0, 19.0, 18.0; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{41}H_{45}N_3O_7Na$ 714.3150, found 714.3186.

*Cbz-Ala-Met-O-azaPhe-Val-O^{t}Bu (**10a**).* Compound **10a** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.593 g, 90%); mp 83.0−85.0 °C; $[\alpha]^{20}_D$ −31.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.14 (m, 11H), 6.46 (d, *J* = 8.7 Hz, 1H), 5.89 (d, *J* = 7.8 Hz, 1H), 5.16−4.99 (m, 2H), 4.93 (d, *J* = 15.3 Hz, 1H), 4.55 (d, *J* = 14.7 Hz, 1H), 4.40−4.05 (m, 3H), 2.37−2.10 (m, 3H), 2.09−1.71 (m, 5H), 1.43 (s, 9H), 1.28 (d, *J* = 7.2 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 171.7, 169.6, 158.6, 156.5, 136.2, 135.4, 129.0, 128.6, 128.5, 128.3, 128.0, 127.9, 81.8, 67.1, 65.9, 59.3, 54.8, 51.4, 31.4, 29.8, 29.2, 28.1, 19.2, 19.1, 18.1, 15.2. Anal. Calcd for $C_{33}H_{46}N_4O_8S$: C, 60.16; H, 7.04; N, 8.50. Found: C, 60.10; H, 7.14; N, 8.81.

*Cbz-Phe-Met-O-azaAla-Val-O^{t}Bu (**10b**).* Compound **10b** was prepared according to the given general procedure for **8a−j**. White microcrystals (0.567 g, 86%); mp 58.0−60.0 °C; $[\alpha]^{20}_D$ −23.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, *J* = 6.3 Hz, 1H), 7.30−6.96 (m, 11H), 6.61−6.52 (m, 1H), 4.98 (d, *J* = 12.3 Hz, 1H), 4.89 (d, *J* = 12.3 Hz, 1H), 4.57−4.35 (m, 1H), 4.33−4.14 (m, 2H), 3.11−2.85 (m, 5H), 2.41−2.21 (m, 1H), 2.19−1.86 (m, 7H), 1.34 (s, 9H), 0.89 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 171.0, 169.1, 158.5, 156.4, 136.3, 136.1, 129.3, 129.2, 128.3, 127.8, 127.6, 126.7, 81.6, 66.7, 60.2, 58.8, 51.3, 37.8, 37.7, 31.3, 29.7, 29.2, 27.8, 18.8, 17.7, 15.1. Anal. Calcd for $C_{33}H_{46}N_4O_8S$: C, 60.16; H, 7.40; N, 8.50. Found: C, 59.80; H, 7.14; N, 8.53.

*Cbz-Ala-Met-O-azaAla-Leu-OMe (**10c**).* Compound **10c** was prepared according to the given general procedure for **8a−j**. Low melting solid (0.466 g, 84%); $[\alpha]^{20}_D$ −36.0 (*c* 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, *J* = 5.4 Hz, 1H), 7.39−7.08 (m, 5H), 6.57 (d, *J* = 8.1 Hz, 1H), 5.93 (d, *J* = 7.5 Hz, 1H), 5.01 (s, 2H), 4.48−4.14 (m, 3H), 3.59 (s, 3H), 3.08 (s, 3H), 2.58−2.31 (m, 2H), 2.22−1.87 (m, 5H), 1.68−1.42 (m, 3H), 1.27 (d, *J* = 7.2 Hz, 3H), 0.85−0.80 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 173.9, 169.7, 158.9, 156.5, 136.2, 128.7, 128.4, 128.0, 67.2, 52.4, 52.1, 51.7, 50.2, 40.9, 38.1, 30.2, 29.5, 24.8, 23.1, 21.8, 17.9, 15.4; HRMS (ESI-

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TOF) m/z $[M + Na]^+$ calcd for $C_{25}H_{38}N_4O_8SNa$ 577.2303, found 577.2331.

*Boc-Gly-Gly-O-azaAla-Leu-OMe (**10d**).* Compound **10d** was prepared according to the given general procedure for **8a**–**j**. White microcrystals (0.376 g, 87%); mp 57.0–59.0 °C; $[\alpha]^{20}_D$ −6.0 (c 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (t, J = 4.8 Hz, 1H), 6.58 (d, J = 8.1 Hz, 1H), 5.70 (t, J = 5.7 Hz, 1H), 4.54–4.30 (m, 1H), 4.06 (t, J = 5.3 Hz, 2H), 3.95–3.79 (m, 2H), 3.72 (s, 3H), 3.20 (s, 3H), 1.79–1.54 (m, 3H), 1.44 (s, 9H), 0.94 (d, J = 6.0 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 171.9, 167.7, 159.0, 156.5, 80.4, 52.5, 52.1, 44.0, 41.2, 40.9, 38.4, 28.5, 24.9, 23.1, 21.7. Anal. Calcd for $C_{18}H_{32}N_4O_8$: C, 49.99; H, 7.46; N, 12.95. Found: C, 50.41; H, 7.84; N, 12.58.

*Boc-Ala-βAla-O-azaPhe-Leu-OMe (**10e**).* Compound **10e** was prepared according to the given general procedure for **8a**–**j**. White microcrystals (0.451 g, 84%); mp 61.0–63.0 °C; $[\alpha]^{20}_D$ −23.0 (c 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25–7.08 (m, 6H), 6.47 (d, J = 8.4 Hz, 1H), 5.44 (d, J = 7.8 Hz, 1H), 4.80 (d, J = 15.3 Hz, 1H), 4.63–4.42 (m, 2H), 4.11–3.95 (m, 1H), 3.62 (s, 3H), 3.52–3.20 (m, 2H), 2.45–2.28 (m, 2H), 1.60–1.47 (m, 3H), 1.32 (s, 9H), 1.19 (d, J = 7.2 Hz, 3H), 0.84 (d, J = 5.4 Hz, 3H), 0.81 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 173.8, 170.1, 158.4, 155.7, 135.5, 129.0, 128.6, 128.0, 80.0, 54.3, 52.5, 52.0, 50.1, 41.2, 35.3, 33.0, 28.5, 24.9, 23.1, 21.9, 18.6. Anal. Calcd for $C_{26}H_{40}N_4O_8$: C, 58.19; H, 7.51; N, 10.44. Found: C, 58.18; H, 7.77; N, 10.60.

*Boc-Ala-Gaba-O-azaAla-Val-O^{t}Bu (**10f**).* Compound **10f** was prepared according to the given general procedure for **8a**–**j**. White microcrystals (0.432 g, 86%); mp 124.0–126.0 °C; $[\alpha]^{20}_D$ −6.0 (c 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (br s, 1H), 6.44 (br s, 1H), 5.64 (br s, 1H), 4.38–4.22 (m, 1H), 4.20–4.06 (m, 1H), 3.54–3.35 (m, 1H), 3.17 (s, 3H), 2.54–2.33 (m, 2H), 2.15 (d, J = 4.2 Hz, 2H), 2.01–1.71 (m, 2H), 1.45 (s, 9H), 1.41 (s, 9H), 1.29 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 7.5 Hz, 3H), 0.88 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 172.0, 170.7, 159.0, 157.4, 82.1, 80.0, 58.9, 50.4, 38.1, 37.9, 31.8, 28.9, 28.5, 28.2, 24.9, 19.0, 18.5, 18.1. Anal. Calcd for $C_{23}H_{42}N_4O_8$: C, 54.96; H, 8.42; N, 11.15. Found: C, 54.96; H, 8.80; N, 11.08.

Preparation of Oxyaza Analogue of Leu-enkephalin14. The oxyaza-dipeptide **6e** (1.0 mmol, 1.0 equiv), DIPEA (1.0 mmol, 1.0 equiv), and 10% cat. amount of DMAP were dissolved in dry THF. A solution of Cbz-Tyr-(OBn)-Gly-Gly-Bt[19] **13** (1.0 mmol, 1.0 equiv) in THF (5.0 mL) was added, and the mixture was stirred for 16 h at 20 °C. The reaction mixture was monitored by TLC [EtOAc–hexanes (1:2)]. After completion of the reaction, the solvent was evaporated. The residue was dissolved in EtOAc (30 mL) and washed with 2 N HCl solution (4 × 10 mL), water (10 mL), and brine (10 mL). The solvent was dried over $MgSO_4$ and evaporated to give oxyaza analogue of Leu-enkephalin **14**. White microcrystals (0.676 g, 85%); mp 88.0–90.0 °C; $[\alpha]^{20}_D$ −7.0 (c 1.0, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45–8.32 (m, 2H), 7.56–7.17 (m, 19H), 6.91 (d, J = 8.3 Hz, 2H), 5.05 (s, 2H), 4.95 (s, 2H), 4.65 (d, J = 5.7 Hz, 1H), 4.24 (d, J = 12.3 Hz, 2H), 4.08–3.96 (m, 1H), 3.83–3.75 (m, 3H), 3.62 (s, 3H), 3.37 (s, 1H), 3.00 (dd, J = 15.3, 4.8 Hz, 1H), 2.72 (dd, J = 14.1, 3.3 Hz, 1H), 1.73–1.42 (m, 3H), 0.86 (d, J = 4.8 Hz, 3H), 0.82 (d, J = 5.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.7, 171.6, 169.4, 167.7, 157.4, 156.5, 155.6, 136.8, 136.6, 135.5, 129.9, 128.3, 128.0, 127.9, 127.7, 127.4, 127.3, 127.2, 127.1, 114.0, 68.8, 64.9, 56.1, 53.1, 51.5, 51.2, 41.4, 40.0, 36.2, 33.8, 23.8, 22.5, 20.7. Anal. Calcd for $C_{43}H_{49}N_5O_{10}$: C, 64.89; H, 6.21; N, 8.80. Found: C, 65.15; H, 6.35; N, 8.75.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H/^{13}C NMR spectra, HPLC chromatography, crystallographic data, and additional computational data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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