Design, Synthesis, and Evaluation of Phe-Gly Mimetics: Heterocyclic Building Blocks for Pseudopeptides

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Enantiopure heterocyclic Boc-protected Phe-Gly dipeptidomimetics containing 1,3,4-oxadiazole, 1,2,4-oxadiazole, and 1,2,4-triazole ring systems have been synthesized as building blocks in the synthesis of pseudopeptides. Three derivatives (**1**−**3**) have the carboxylic acid function directly bound to the heterocyclic ring, and three derivatives (**4**−**6**) have an extra methylene group between the heterocyclic ring and the acid function to allow for an increased conformational flexibility. The mimetics were used as Phe-Gly replacements in the biologically active peptides dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) and substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH_2, SP). The pseudopeptide synthesis was performed using solid-phase methodology on a MBHA-resin using Boc-chemistry. The biological evaluation was performed by testing the μ- and δ-opioid receptor affinities of the dermorphin pseudopeptides and the NK_1 receptor affinities of the SP pseudopeptides. The results showed that all mimetics except **3** were excellent replacements of Phe-Gly in dermorphin since they displayed affinities for the μ-receptor (IC_{50} = 12−31 nM) in the same range as dermorphin itself (IC_{50} = 6.2 nM). The agonist activity of three pseudopeptides at human μ-receptors was also evaluated. It was shown that the tested compounds retained their agonist activity. The SP pseudopeptides showed considerably lower affinities (IC_{50} > 1 μM) for the NK_1 receptor than SP itself (IC_{50} = 1.5 nM) indicating that the Phe-Gly replacements prevent the pseudopeptides from adopting bioactive conformations.

Introduction

Although several different strategies have been used in the development of peptidomimetic compounds, it is still not fully understood how to rationally convert a peptide into a nonpeptide while maintaining the desired biological activity. Commonly used strategies for peptide mimicry involve replacements of amino acids, replacements of amide bonds or oligopeptides, or introduction of global or local conformational constraints.[2−13] We are interested in the structural and biological effects caused by replacement of important dipeptide fragments in biologically interesting peptides with nonpeptidic moieties.[14,15] Frequently, only a small part of the peptide (4−8 amino acids), the binding epitope, is responsible for the recognition and binding to the receptor. These key amino acids can be used as the starting point for the design of nonpeptidic receptor ligands. The replacements may be designed by computer modeling to display optimal similarities with the natural oligopeptide fragments in both three-dimensional and physicochemical terms.

In the present report, we describe the design, synthesis, and biological evaluation of six heterocyclic Phe-Gly mimetics (**1**−**6**) (Chart 1).[16] The compounds contain 1,3,4-oxadiazole,[17−20] 1,2,4-oxadiazole,[21−24] and 1,2,4-triazole[25−27] ring systems as amide bond replacements. The three heterocycles are known as efficient ester and/or amide isosteres,[28] but only the 1,2,4-triazole has previously been used in peptide mimicry.[29,30] Although similar in size and shape, the ring systems show variations in aromatic, electrostatic, and hydrogen-bonding properties: (i) all three heterocycles can participate in hydrogen bonding, the oxadiazoles as acceptors and the triazole as both acceptor and donor; (ii) the triazole and the 1,3,4-oxadiazole rings are aromatic, whereas the 1,2,4-oxadiazole is better described as a conjugated diene;[24] and (iii) only the 1,2,4-triazole displays any acidic or basic properties with pK_a values of 2.19 (as base) and 10.26 (as acid).[27] These variations provide an opportunity to evaluate properties of importance for amide bond mimicry and to determine the characteristics of the peptide backbone that are crucial for bioactivity.

The mimicking ability of the dipeptide mimetics was evaluated after incorporation of the compounds into substance P (SP, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2)[31,32] and dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2)[33−35] as replacements for Phe-Gly.

Relevant structure−activity relationship studies have been performed on both SP and dermorphin. The C-terminal hexapeptide amide of SP (SP_{6-11})[36] and the N-terminal tetrapeptide amide of dermorphin[37] represent minimum sequences required for receptor recognition and binding. Several SP_{6-11} pseudopeptides[38] comprising Phe^8-Gly^9 replacements have been reported: e.g. ψ[CH=CH],[39] ψ[CF=CH],[40] ψ[$COCH_2$],[41] ψ[NHCO],[42]

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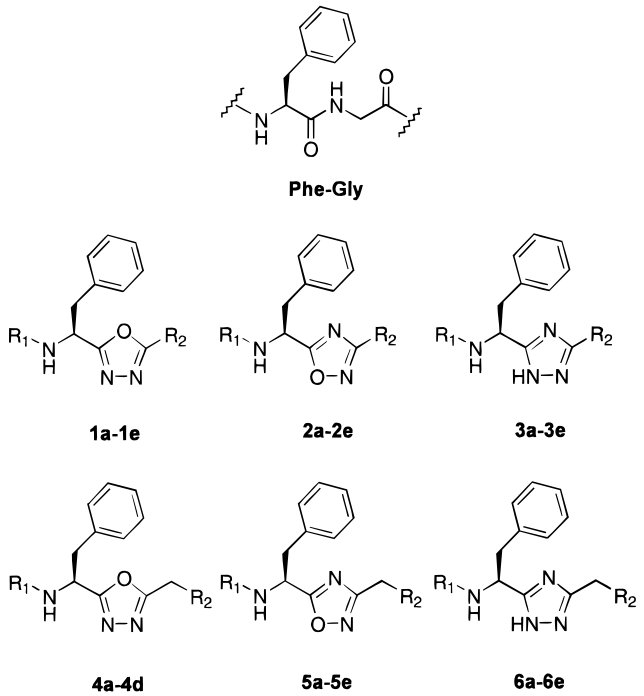
Chart 1. Structures of the Phe-Gly Mimetics



a: R_1=Ac; R_2=CONHMe
b: R_1=Boc; R_2=COOMe or COOEt
c: R_1=Boc; R_2=COOH or COO^-K^+
d: R_1=H-Tyr-D-Ala; R_2=Tyr-Pro-SerNH_2
e: R_1=H-Arg-Pro-Lys-Pro-Gln-Gln-Phe; R_2=Leu-MetNH_2

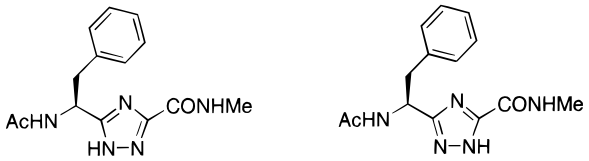
and ψ[CH_2O].[43] The biological activities were investigated in guinea pig ileum assays showing relative potencies of 24% (ψ[CH═CH]),[39] 70% (ψ[$COCH_2$]),[41] 22% (ψ[NHCO]),[42] and 25% (ψ[CH_2O])[43] of that of the natural peptide. The ψ[CF═CH][8−9]−SP pseudopeptide showed a binding affinity for the rat NK_1 receptor equal to SP (IC_{50} = 2 nM).[40] These results suggest that the Phe-Gly amide bond in SP is important for the biological activity and that it can be modified to some extent without detrimental effects on the biological activity.

Several studies on dermorphin have stressed the importance of the relative orientation of the aromatic groups of Tyr[1] and Phe[3] for receptor selectivity.[44−46] Early studies using pseudopeptides implied that the Phe[3]-Gly[4] amide bond contributes to the bioactivity of dermorphin either by intramolecular hydrogen bonding or by direct interaction with the receptor.[47,48] However, more recent studies on ψ[*E*,CH═CH][3−4]-dermorphin,[49] which retains quite high affinity for the μ-receptor, indicate that the influence of the Phe-Gly amide bond on the receptor interaction is independent of Gly[4]NH hydrogen bonding. Also other Phe[3]-Gly[4] dermorphin or N-terminal pseudopeptides with isosteric moieties such as ψ[NHCO],[47] ψ[$COCH_2$],[48] ψ[CH_2S],[50] and ψ[CH_2SO][50] show lower potencies than dermorphin in both binding assays and functional tests.

The biological evaluation of the pseudopeptides synthesized in this study was performed by testing the μ- and δ-opioid receptor affinities of the pseudopeptides **1d**−**6d** and NK_1 receptor affinities of pseudopeptides **1e**−**3e**, and **5e**, and **6e**. The agonist activity of pseudopeptides **2d**, **4d**, and **5d** at human μ-receptors was also evaluated.

Design

All derivatives were subjected to conformational analysis by molecular mechanics calculations to address the question of geometrical similarities between the designed structures and the natural fragment. Conformational searches were performed using the AMBER force field[51] as implemented in the MacroModel program (version 4.5).[52] The calculations were performed on the N-acetylated and C-terminally methyl-amidated (Ac-Phe-Gly-NHMe) derivatives to better simulate the Phe-Gly moiety within a peptide chain. Two tautomers of the 1,2,4-triazole derivatives were used for further calculations and comparisons [**3a**(1*H*), **3a**(2*H*) and **6a**(1*H*), **6a**(2*H*)].[53] Low-energy conformations ($\Delta E_s \leq 20$ kJ/mol) of all derivatives were identified and imported



3a(1*H*) **3a**(2*H*)

into the Sybyl program (version 6.1)[54] for further analysis. Only 12 unique conformers were found by the conformational search of Ac-Phe-Gly-NHMe. Among these we identified three stretched conformations (maximum distance between terminal ends), two β-turn conformations, four conformations with a γ-turn in the Gly part, and one conformation with a γ-turn in the Phe part. The latter conformation was highest in energy. The stretched conformations were among the low-energy conformations, whereas the β-turn conformations were higher in energy.

To study the geometrical similarities, all individual low-energy conformations of each mimetic were superimposed on the 12 low-energy conformations of Ac-Phe-Gly-NHMe. In total six atoms were fitted, the amide oxygen and nitrogen atoms of the dipeptide and the corresponding atoms in the mimetics. Thus, the 12 conformations of Ac-Phe-Gly-NHMe were superimposed on 48 conformations each of **1a** and **2a**, 36 conformations of **3a**(1*H*), 38 conformations of **3a**(2*H*), 116 conformations of **4a**, 74 conformations of **5a**, 72 conformations of **6a**(1*H*), and 108 conformations of **6a**(2*H*). An SPL algorithm was developed to efficiently handle the extensive amount of data collected. This algorithm allowed an evaluation of the quality of the individual fits by calculating the rms value multiplied with the excess volume of the fit. The excess volume was obtained by subtracting the van der Waals volume of the Phe-Gly conformation from the combined van der Waals volume of both fitted molecules. Comparisons of fits to one stretched and one β-turn low-energy conformation of Ac-Phe-Gly-NHMe, to which the peptidomimetics have energetically 'allowed' conformations with close resemblance (good fits), are shown in Figure 1. The geometric comparisons of **1a**−**3a** with Ac-Phe-Gly-NHMe in the stretched conformation show good fits (Figure 1A), but the same comparisons with the turn conformation are less attractive (Figure 1C). The geometric comparison using the mimetics containing a methylene spacer (**4a**−**6a**) produce considerably better fits with the turn

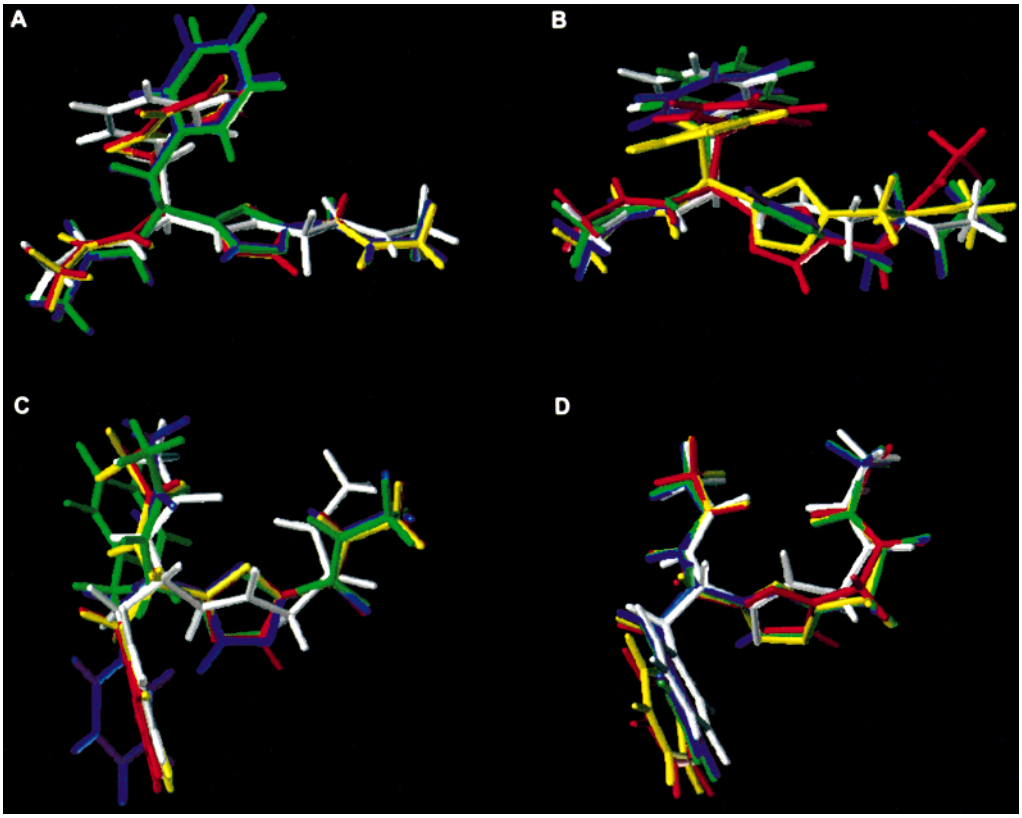


Figure 1. Geometric comparisons of Ac-Phe-Gly-NHMe and the Phe-Gly mimetics **1a**−**3a** in a stretched conformation (A) and in a β-turn conformation (C) and the corresponding comparisons of Ac-Phe-Gly-NHMe and **4a**−**6a** (B and D, respectively). Six atoms were fitted in the comparisons: the amide oxygen and nitrogen atoms of the dipeptide and the corresponding atoms of the mimetics. Ac-Phe-Gly-NHMe is shown in white, **1a** and **4a** in yellow, **2a** and **5a** in green, **3a**(1*H*) and **6a**(1*H*) in blue, and **3a**(2*H*) and **6a**(2*H*) in red.

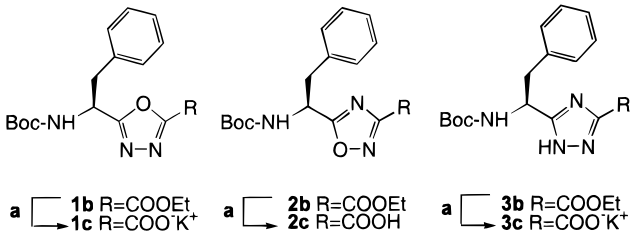
conformation of Ac-Phe-Gly-NHMe (Figure 1D) compared to the stretched conformation (Figure 1B), demonstrating that the additional conformational flexibility inherent in these mimetics can improve the similarity of certain geometric properties.

Not only geometrical and conformational similarities but also the electrostatical properties should be taken into account when considering structural comparisons. Therefore, the conformations used in the fits were imported into the Spartan program (version 4.0),[55] and electrostatic potentials were calculated from AM1 atomic charges.[56] In Figures 2 and 3 the electron density surfaces of the compounds are displayed and color-coded according to magnitude. The results obtained for Ac-Phe-Gly-NHMe and **4a** (Figure 3) were compared with ab initio calculations using the 6-31G* basis set.[57] The energy density surfaces obtained by both levels of calculations were in good agreement (data not shown) thereby providing support for the use of the less time-consuming semiempirical calculations. None of the mimetics perfectly matched the electrostatic properties of the parent dipeptide. However, the oxadiazole derivatives seem to be better mimetics of Ac-Phe-Gly-NHMe than the triazole derivatives in terms of electrostatics, the 1,3,4-oxadiazoles being the best in both series of mimetics.

Chemistry

Synthesis. The syntheses and determination of the enantiopurities of **1b**−**3b** (Scheme 1) have been reported previously.[16] Herein, we have prepared a similar series

Scheme 1[a]



[a] (a) 1 M aq KOH/EtOH.

of heterocyclic Phe-Gly mimetics with a methylene spacer between the carbonyl group and the ring system (**4b**, **5c**, and **6b**).

The 1,3,4-oxadiazoles[17−20] were obtained by dehydration of the appropriate diacylhydrazines (Scheme 2).[58−60] Diacylhydrazine **7**, derived from Boc-L-phenylalanine hydrazide[16] and methyl malonyl chloride, was treated with thionyl chloride and pyridine to afford a 1,2,3,4-oxathiadiazole *S*-oxide intermediate.[61] By heating the crude intermediate, sulfur dioxide was eliminated, and **4b** was formed (Scheme 2). The low yield of **4b** (31%) is probably due to difficulties in achieving sufficiently dry conditions in the formation of the intermediate.

The 1,2,4-oxadiazoles[21−24] were derived from activated carboxylic acids and amidoximes (Scheme 3).[62,63] The use of Boc-amino acids as starting material required mild reaction conditions during the cyclization due to the acid-sensitive protecting group and the risk for racemization.[16,64] Thus, the symmetrical anhydride of Boc-L-Phe was heated to reflux with **8** in pyridine[16] to

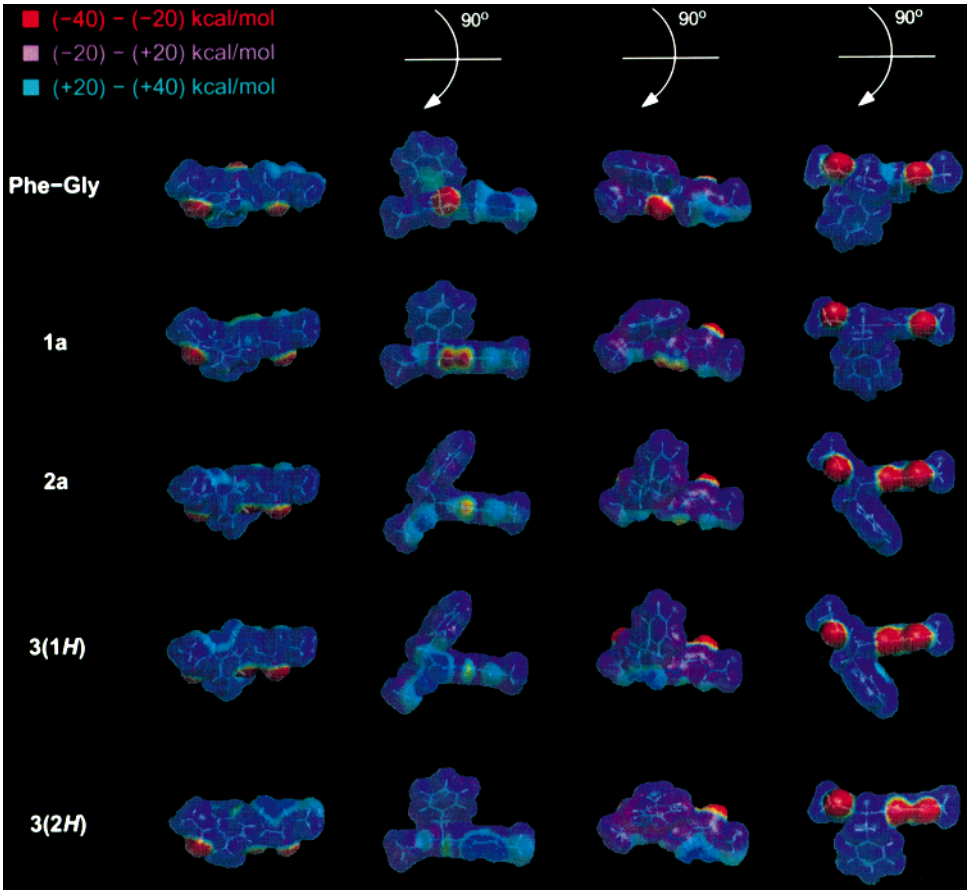
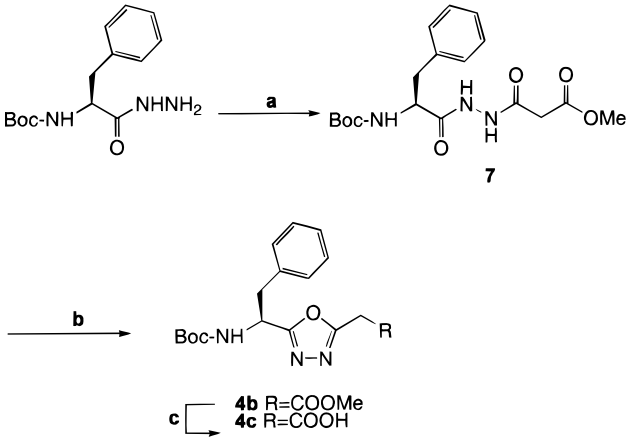


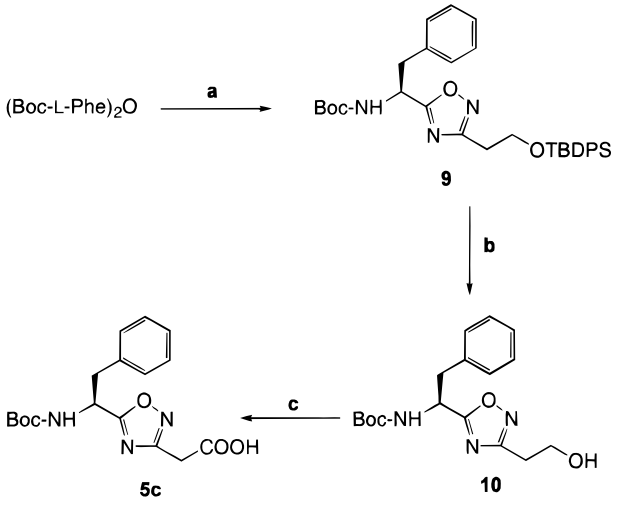
Figure 2. Comparisons of electron density surfaces of Ac-Phe-Gly-NHMe and **1a**−**3a** in stretched conformations (see Figure 1A), displayed and color-coded according to magnitude. Four different orientations are shown, to allow for accurate comparisons (rotation in 90° increments around the *x*-axis). Red areas represent potentials ranging from −40 to −20 kcal/mol, purple areas represent potentials ranging from −20 to +20 kcal/mol, and blue areas represent potentials ranging from +20 to +40 kcal/mol.

Scheme 2[a]



[a] (a) $ClCOCH_2COOMe$, Et_3N, THF, −30 °C to rt; (b) (i) $SOCl_2$/pyridine, THF, 0 °C, (ii) toluene, △; (c) (i) 1 M aq KOH/MeOH, (ii) H^+.

Scheme 3[a]



[a] (a) $TBDPSOCH_2CH_2C(NOH)NH_2$ (**8**), pyridine, △; (b) tetrabutylammonium fluoride (1 M in THF), HOAc, THF, rt; (c) Jones' reagent (0.67 M), acetone, 0 °C.

obtain **9** in 63% yield (Scheme 3). Desilylation of the protected alcohol with tetrabutylammonium fluoride (TBAF) in THF afforded racemic **10**. However, addition of glacial acetic acid prevented the racemization. Dry conditions were used in the deprotection, and the pH of the reaction mixture was carefully controlled to avoid basic conditions. The alcohol **10** was oxidized to the carboxylic acid **5c** by Jones' reagent.

The 1,2,4-triazoles[25−27] were formed by thermal dehydration of suitable acylamidrazones (Scheme 4). The acylamidrazone **11** was obtained by reacting Boc-L-phenylalanine hydrazide with ethyl *β*-amino-*β*-ethoxyacrylate.[65] Heating of the crude **11** at 155 °C afforded the 1,2,4-triazole derivative **6b** in 57% yield (over two steps) (Scheme 4).

Determination of the Enantiomeric Excess. The enantiopurities of **6b** and **10** were determined by analyses on chiral stationary phases using a straight

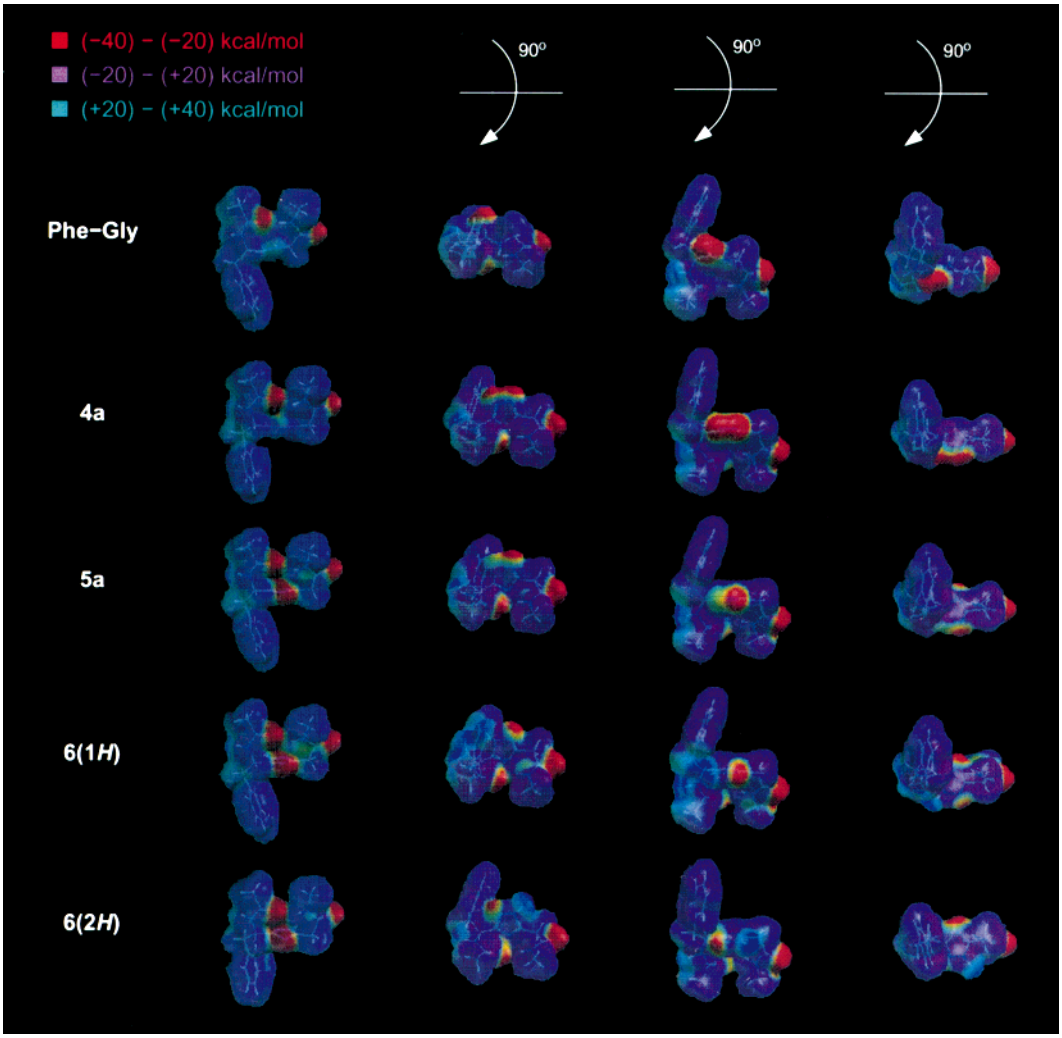
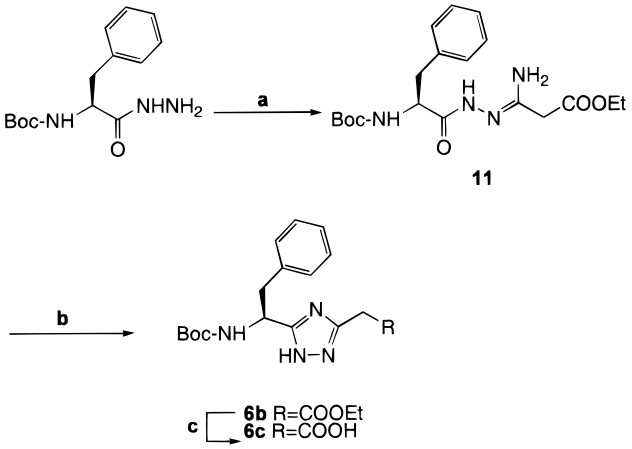


Figure 3. Comparisons of electron density surfaces of Ac-Phe-Gly-NHMe and **4a**−**6a** in β-turn conformations as shown in Figure 1D, displayed and color-coded according to magnitude (see legend to Figure 2 for color coding).

Scheme 4[a]



[a] (a) EtOC(NH)CH_2COOEt·HCl, Et_3N, THF, rt; (b) xylenes, △; (c) (i) 1 M aq KOH/EtOH, (ii) H^+.

phase HPLC system (Table 1).[16] It was not possible to analyze the enantiomeric purity of the free carboxylic acid **5c** on the stationary phases used (Chiralcel OD-H and Chiralpak AD). Instead, we determined the enantiopurity of the precursor **10** (>95% ee).[66] Racemic **10** was available (see above), thus allowing a strict determination of the % ee. Racemic **6b** was obtained by using Boc-D,L-phenylalanine hydrazide as starting material. Hence, the retention times for both enantiomers could be determined. The % ee of **6b** was >95%.[66] The % ee of **4b** could not be established since no reproducible chromatograms could be obtained.

Synthesis of Pseudopeptides. The six Phe-Gly mimetics were used as building blocks in the solid-phase peptide syntheses (SPPS)[67] of the SP and dermorphin pseudopeptides. The syntheses were performed manually in plastic syringes in ca. 50-μmol scale on *p*-methylbenzhydrylamine (MBHA) resins.

Derivatives **1b**−**4b** and **6b** were hydrolyzed before being coupled to the resin-bound peptide. The crude acids, **2c**, **4c**, and **6c**, were used in the coupling reactions, but the carboxylic acids of **1b** and **3b** were not possible to isolate as they decarboxylated during the workup of the reaction mixture. However, the carboxylates **1c** and **3c** were stable and could be successfully coupled to the peptide. The acid **2c** decarboxylated somewhat slower than the acids derived from **1b** and **3b** but could not be stored and had to be used in the coupling reaction immediately after isolation. It is well-known that 1,2,4-oxadiazole 3-acids,[68] 1,3,4-oxadiazole acids,[69−71] and 1,2,4-triazole 3-acids,[72,73] as well as other heteroaromatic acids,[74,75] are readily decarboxylated.

To synthesize pseudopeptides **1d**−**6d**, **1e**−**3e**, **5e**, and

Table 1. Physical Data of Compounds **4b**, **5c**, **6b**, and **10**

compd	yield (%)	mp (°C)	$[\alpha]_D$ (deg)	t_R (min)[a]	% ee	formula[b]
4b	31	59.5−61[c]	−31.5[d]	ND[e]	ND[e]	$C_{18}H_{23}N_3O_5 \cdot 1/4H_2O$
5c	64	163.5−164.5[f]	−23.5[g]	ND[e]	ND[e]	$C_{17}H_{21}N_3O_5$[h]
6b	57[i]	106.5−107[c]	−24.2[d]	22.1/13.8[j]	>95	$C_{19}H_{26}N_4O_4 \cdot 1/4H_2O$
10	75	84−84.5[c]	−20.8[k]	30.9/26.4[l]	>95	$C_{17}H_{23}N_3O_4$

[a] Retention times for both enantiomers given as *L*/*D*. [b] Except when noted, the compounds gave elemental analyses for C, H, and N within 0.4% of the theoretical values. [c] Recrystallized in Et_2O/pentane. [d] *c* 1.0, $CHCl_3$. [e] Not determined. [f] Recrystallized in $CHCl_3$. [g] *c* 1.1, $CHCl_3$. [h] C: calcd, 58.78; found, 58.30. [i] Yield calculated from Boc-L-Phe. [j] Analysis run on a Chiralpak AD column at 30 °C: mobile phase, hexane/2-propanol/Et_2NH (80:20:0.1); flow rate, 0.5 mL/min; detection wavelength, 225 nm. [k] *c* 0.9, $CHCl_3$. [l] Analysis run on a Chiralcel OD-H column at ambient temperature: mobile phase, hexane/2-propanol/Et_2NH (90:10:0.1); flow rate, 0.5 mL/min; detection wavelength: 211 nm.

Table 2. Characterization of the Synthesized Pseudopeptides

compd	MS (MW) [$M^+ + H$]	t_R (min)	amino acid analysis
dermorphin			Tyr 1.92 (2); Ala 0.98 (1); Phe 0.96 (1); Gly 1.00 (1); Pro 0.93 (1); Ser 0.99 (1)[a]
1d	814.8 (813.8)	22.0[b,c]	Tyr 1.98; Ala 0.99; Phe 1.00; Gly (NA);[d] Pro 1.02; Ser 1.00
2d	815.5 (813.8)	22.7[b,c]	Tyr 1.98; Ala 1.00; Phe 0.67; Gly (NA); Pro 1.00; Ser 1.00
3d	815.3 (812.8)	20.6[b,c]	Tyr 2.02; Ala 1.00; Phe (NA); Gly (NA); Pro 0.98; Ser 1.00
4d	828.4 (827.8)	34.0[e,f]	Tyr 2.00; Ala 1.01; Phe 0.96; Gly (NA); Pro 1.00; Ser 1.02
5d	828.8 (827.8)	21.0[b,c]	Tyr 2.04; Ala 1.04; Phe 1.00; Gly (NA); Pro 1.00; Ser 0.99
6d	828.8 (826.9)	20.2[b,c]	Tyr 1.97; Ala 0.97; Phe 0.07; Gly (NA); Pro 1.00; Ser 1.00
SP			Arg 0.98 (1); Pro 1.83 (2); Lys 1.01 (1); Gln 1.95 (2); Phe 1.92 (2); Gly 1.00 (1); Leu 0.98 (1); Met 0.79 (1)[a]
1e	1359.8 (1358.6)	31.4[c,g]	Arg 0.97; Pro 2.02; Lys 1.01; Gln 2.02; Phe 1.99; Gly (NA); Leu 1.00; Met 0.91
2e	1361.3 (1358.6)	32.6[c,g]	Arg 0.98; Pro 2.01; Lys 1.02; Gln 2.01; Phe 1.86; Gly (NA); Leu 1.00; Met 0.94
3e	1359.6 (1357.6)	26.9[c,h]	Arg 1.00; Pro 1.94; Lys 1.05; Gln 2.07; Phe 1.04; Gly (NA); Leu 1.06; Met 1.01
5eα	1373.6 (1372.7)	32.0[c,i]	Arg 0.99; Pro 2.00; Lys 1.00; Gln 2.01; Phe 1.92; Gly (NA); Leu 1.01; Met 0.97
5eβ	1373.2 (1372.7)	33.0[c,i]	Arg 0.98; Pro 1.97; Lys 0.99; Gln 2.04; Phe 1.91; Gly (NA); Leu 1.00; Met 0.99
6e	1373.8 (1371.1)	30.2[f,j]	Arg 0.98; Pro 1.96; Lys 1.00; Gln 2.01; Phe 1.01; Gly (NA); Leu 1.00; Met 0.94

[a] Theoretical equivalents of each amino acid in the natural peptide are given in parentheses. [b] 0−60% CH_3CN/H_2O/0.1%TFA, 1 mL/min, 30 min. [c] Analyses run on a μBondapak C-18 column (Waters), 8 × 100 mm. [d] NA, not analyzed. [e] 15−60% CH_3CN/H_2O/0.1%TFA, 5 mL/min, 90 min. [f] Preparative HPLC run on a μBondapak C-18 column (Waters), 25 × 100 mm. [g] 5−65% CH_3CN/H_2O/0.1%TFA, 0.5 mL/min, 30 min. [h] 20−60% CH_3CN/H_2O/0.1%TFA, 0.5 mL/min, 30 min. [i] 0−60% CH_3CN/H_2O/0.1%TFA, 0.5 mL/min, 30 min. [j] 15−60% CH_3CN/H_2O/0.1%TFA, 5 mL/min, 60 min.

6e, couplings of the Phe-Gly mimetics to the resin-bound peptides were performed by using various coupling strategies. Compounds **3c**, **4c**, and **6c** were coupled to the dermorphin peptide-resin using HBTU/HOBt/DIPEA as coupling reagents, whereas **1c**, **2c**, and **5c** were activated as mixed anhydrides by treatment with ethyl chloroformate/Et_3N. Compounds **1c**−**3c** and **6c** were coupled to the SP peptide-resin using the same conditions as above. We failed to couple enantiopure L-**5c** using these conditions or by using HATU/HOAt/DIPEA or PyBrOP/NMM as coupling reagents. However, incorporation of racemic **5c** into SP by automated SPPS using HBTU/HOBt/DIPEA was successful. The epimers of the resulting **5e** (**5e**α and **5e**β) were separated by preparative reversed-phase HPLC (Table 2). We failed to synthesize the pseudopeptide **4e**. Introduction of **4e** in the peptide chain using either HBTU/HOBt/DIPEA or PyBrOP/NMM as coupling reagents was unsuccessful.

Boc-protecting groups are generally removed from N^{α} of the amino acid residues by treatment with TFA. However, since we had found that deprotection of Boc-protected 1,2,4-oxadiazole derivatives using TFA results in racemization,[76] deprotection with iodotrimethylsilane (TMSI)[77,78] was used for the resin-bound 1,2,4-oxadiazole-based Phe-Gly mimetics. TMSI was also used to deprotect the 1,3,4-oxadiazole- and 1,2,4-triazole-based mimetics.

The pseudopeptides were cleaved from the resins and purified by preparative reversed-phase HPLC on a C-18 column. An exception was **3d** which was purified on an IMAC column[79] in order to facilitate the separation of the pseudopeptide from shorter peptide fragments. The pseudopeptides were characterized by HPLC analysis, plasma desorption mass spectrometry, and amino acid analysis (Table 2).

Biological Results and Discussion

NK_1 Receptor Binding. The affinities of the SP pseudopeptides (**1e**−**3e**, **5e**, and **6e**) for the rat NK_1 receptor were determined in displacement screening experiments using the radioligand 3,4-[^{3}H]-(L-Pro^2)SP. All pseudopeptides displayed considerably lower affinities to the rat NK_1 receptor than SP itself ($IC_{50SP} = 1.5 \pm 0.2$ nM). IC_{50} values were >1000 nM for all pseudopeptides except **5e**α. Compound **5e**α was subjected to more

Table 3. μ-Opioid Receptor Affinities for the Dermorphin Pseudopeptides

ligand	[^{3}H]DAMGO, IC_{50} (nM)[a]
DAMGO	3.5 ± 0.6
dermorphin	6.2 ± 1.5
1d	20 ± 9
2d	17 ± 5
3d	490 ± 67
4d	31 ± 4
5d	12 ± 1
6d	21 ± 2

[a] The IC_{50} values are given as the mean ± SEM.

careful measurements, and its IC_{50} value was determined to be 307 ± 47 nM. This difference in affinity observed between the epimeric pseudopeptides containing the 1,2,4-oxadiazole derivative **5** (**5e**α and **5e**β) agrees with receptor binding data obtained with (D-Phe^8)SP, which is significantly less potent than SP.[80] The incorporation of the heterocyclic mimetics in the peptide sequence may affect the ability of the pseudopeptides to adopt a bioactive conformation. However, the reduced affinity may also be related to the differences in electrostatic properties between the mimetics and natural peptides.

***μ*- and *δ*-Opioid Receptor Binding.** Affinities of the pseudopeptides **1d**−**6d** for the rat μ-opioid receptors were determined by displacement of the potent and highly selective enkephalin-related radioligand [^{3}H]-DAMGO[81,82] (Table 3). All dipeptide mimetics except **3** are excellent Phe-Gly replacements in dermorphin, since the corresponding pseudopeptides displayed high affinities for the μ-receptor (IC_{50} = 12−31 nM). Pseudopeptide **3d** showed lower affinity (IC_{50} = 490 ± 67 nM). Preliminary screening experiments of δ-opioid receptor affinities using the radioligand [^{3}H]DPDPE[83] indicated that none of the pseudopeptides had any appreciable affinity for the δ-receptor (IC_{50} > 500 nM). Hence, the pseudopeptides appear to be μ-selective.

In preliminary experiments, three of the pseudopeptides (**2d**, **4d**, and **5d**) were evaluated as agonists at human μ-receptors expressed in 293S-hMOR cells[84] by studying their ability to stimulate the binding of [^{35}S]-GTPγS to G-proteins coupled to μ-receptors.[85,86] All three peptides produced dose-dependent increases in [^{35}S]GTPγS binding. The pseudopeptides also produced higher E_{max} values than morphine (E_{max} = 53%) or fentanyl (E_{max} = 59%; DAMGO = 100%)[87] and can therefore be considered as agonists. The potency ranking was the same as the affinity ranking obtained from the binding analysis. The EC_{50} value for **5d** was 869 nM (n = 2; 833, 906) as compared with 170 ± 42 nM for DAMGO.

No strict affinity difference was observed between the pseudopeptides containing the more conformationally restricted Phe-Gly mimetics (**1**−**3**) and the more flexible derivatives (**4**−**6**). Thus, the novel dermorphin pseudopeptides appear to be able to adopt an energetically accessible bioactive conformation. In addition, the spatial arrangement of the Tyr^1 and Phe^3 side chains does not appear to have been negatively affected by the introduction of heterocyclic moieties. The retained affinity of the pseudopeptides is noteworthy as the Phe^3-Gly^4 structural element has been suggested to be of critical importance for dermorphin activity (see above). The results obtained also support the hypothesis that Gly^{4}NH is not required for μ-receptor activity.[49]

Concluding Remarks

The present study demonstrates that 1,2,4-oxadiazole, 1,3,4-oxadiazole, and 1,2,4-triazole derivatives with high enantiopurities can be obtained from Boc-L-Phe and that dipeptide mimetics based on these ring systems can be used as building blocks in solid-phase syntheses of dermorphin and SP pseudopeptides.

Insertion of the novel dipeptide mimetics into SP led to a dramatic loss in affinity for the rat NK_1 receptor, the most potent pseudopeptide being 150-fold less potent than SP in terms of NK_1 receptor affinity. Hence, the heterocyclic mimetics used herein are not useful as mimics of Phe-Gly in SP. The reduction in affinity of the pseudopeptides might be due to undesirable steric, electrostatic, or other properties such as an inability to adopt energetically favorable bioactive conformations. However, during the design process, we used force-field calculations to establish that the conformational preferences of the synthesized Phe-Gly mimetics were quite similar to those of Phe-Gly. Therefore, it is more likely that other (yet unidentified) properties of the Phe-Gly mimetics are detrimental to the activity of the SP pseudopeptides.

In contrast to the above, insertion of the dipeptide mimetics into dermorphin led to potent and selective pseudopeptides—they had high affinities for μ-receptors and low affinities for δ-receptors. Furthermore, preliminary experiments indicate that these μ-receptor ligands are agonists. It has been demonstrated that the Phe-Gly fragment is important for the activity of dermorphin. Hence, the successful replacement of Phe-Gly in dermorphin is a demonstration of molecular mimicry.

It is apparent from the present study that the mimicking ability of a dipeptide mimetic will vary from peptide to peptide. This is a consequence of the inability of a dipeptide replacement to mimic all inherent properties of the natural fragment.

Experimental Section

Biochemistry. 1. NK_1 Receptor Binding. Membranes were prepared from whole rat brain minus cerebellum. The tissue was homogenized in 10 vol of 0.32 M sucrose in the cold. The homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was saved and centrifuged at 18000*g* for 20 min at 4 °C. The pellet was saved, thoroughly mixed with 4 vol of distilled water, and incubated for 8 min at 25 °C to cause lysis. The lysate was centrifuged at 10000*g* for 20 min at 4 °C, and the supernatant with membranes was retained. The membrane suspension was diluted with membrane buffer, 50 mM Tris-HCl buffer, pH 7.4, at 25 °C. About 10 000 cpm of 3,4-[^{3}H]-(L-Pro^2)SP in 75 μL of tracer buffer, 0.75 μg of membrane protein in 100 μL of membrane buffer, and peptide competitor in 25 μL of H_2O were incubated at 25 °C for 30 min. The tracer buffer was 70 mM Tris-HCl, 8.36 mM $MnCl_2$, pH 7.4, 10.67 mg/mL bacitracin, 1.067 mg/mL phosphoramidon, 5.33 mg/mL BSA. Incubations were terminated by filtration through Whatman GF-C membranes presoaked in 0.3% poly(ethylenimine) solution for at least 3 h at 4 °C. Each assay included triplicates of all samples with competitor and SP standard and triplicates of two controls. The experiment was repeated three times, and mean values ± SEM are given.

2. *μ*- and *δ*-Opioid Receptor Binding. Membranes were prepared according to methods described above. The radioligand [^{3}H]DAMGO[81,82] was used in the μ-receptor binding assay and [^{3}H]DPDPE[83] in the δ-receptor assay. In the

μ-receptor binding assay, unlabeled DAMGO was used as standard, in the δ-receptor binding assay, Met-enkephalin. Other conditions were the same as for the NK_1 receptor binding.

3. Agonist Binding. Membranes were prepared from HEK-293S cells that had been subjected to stable transfection of the cloned human μ-opioid receptor (293S-hMOR cells).[84] The [^{35}S]GTPγS binding assay[87] was modified from Lorenzen et al.[85] and Traynor and Nahorski.[86] The data were fitted to a sigmoidal curve, and best-fit values were calculated for the E_{max} (maximal effect) and EC_{50} (concentration producing half-maximal response). DAMGO was used as standard, and its maximal effect was used to define a reference of 100%.

Molecular Modeling. The molecular modeling and molecular mechanics calculations were performed on a Silicon Graphics Indy workstation. The N-acetylated and C-terminally amidated derivatives (Ac-Phe-Gly-NHMe, **1a**−**6a**) were constructed in the program MacroModel (version 4.5)[52] and energy-minimized using the implemented AMBER force field.[51] Low-energy conformations were identified by a random conformational search about all rotable bonds (1000 steps), since this method can rapidly explore a large part of the conformational space.[88] The lowest-energy conformation resulting from that search was thereafter used as the starting conformation of a more systematic search based on the SUMM method (5000 steps).[89] Energy minimizations were performed using the AMBER force field with a maximum of 50 iterations. Low-energy conformations (ΔE_s < 20 kJ/mol) obtained by both searches were combined and energy-minimized to an rms gradient < 0.001 kJ/Å mol. Conformations were defined as duplicates when the residuals from the least-squares atom-by-atom superimposition were <0.25 Å for all non-hydrogen atoms. The average number of duplicates was at least 50 for each derivative.

Chemistry: General.[16] Toluene was dried over sodium, and dimethylformamide (DMF) was bubbled with N_2 (g) and then kept over molecular sieves (3 Å). The amino acid analyses[90] were performed at the Department of Biochemistry, Biomedical Centre, Uppsala University, Uppsala, Sweden. Ethyl 5-[(*S*)-1-(*tert*-butyloxycarbonylamino)-2-phenylethyl]-1,3,4-oxadiazole-2-carboxylate (**1b**),[16] ethyl 5-[(*S*)-1-(*tert*-butyloxycarbonylamino)-2-phenylethyl]-1,2,4-oxadiazole-3-carboxylate (**2b**),[16] ethyl 5-[(*S*)-1-(*tert*-butyloxycarbonylamino)-2-phenylethyl]-1,2,4-triazole-3-carboxylate (**3b**),[16] and ethyl β-amino-β-ethoxyacrylate hydrochloride[65] were prepared according to known procedures. Data on elemental analyses, optical rotations, and melting points of **4b**, **5c**, **6b**, and **10** are listed in Table 1.

Methyl 2-{5-[(*S*)-1-(*tert*-Butyloxycarbonylamino)-2-phenylethyl]-1,3,4-oxadiazol-2-yl}acetate (4b). The reaction was carried out in dry THF under nitrogen atmosphere. $SOCl_2$ (63 μL, 0.87 mmol) was added to a chilled (0 °C) solution of **7** (253 mg, 0.67 mmol) and pyridine (140 μL, 1.7 mmol) in THF. The mixture was stirred at 0 °C for 2 h. The resulting salt was filtered off and the filtrate was concentrated in vacuo. The residue was redissolved in toluene and heated to reflux for 1.5 h. The reaction was monitored by TLC (Et_2O/pentane, 9:1). The solvent was evaporated and the residue was purified by column chromatography (Et_2O/pentane, 2:1) affording 74 mg (31%) of pure **4b**: ^{1}H NMR ($CDCl_3$) δ 7.27−7.08 (m, 5H), 5.27 (m, 1H), 5.18 (m, 1H), 3.93 (s, 2H), 3.75 (s, 3H), 3.32−3.15 (AB-system, 2H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 167.10, 166.45, 160.56, 154.72, 135.09, 129.29, 128.59, 127.13, 80.40, 52.83, 48.28, 39.66, 31.54, 28.14.

2-{5-[(*S*)-1-(*tert*-Butyloxycarbonylamino)-2-phenylethyl]-1,2,4-oxadiazol-3-yl}acetic Acid (5c). Jones' reagent (457 μL, 0.31 mmol, 0.67 M) was added to a chilled solution (0 °C) of **10** (51 mg, 0.15 mmol) in acetone. The reaction was monitored by TLC (Et_2O). After 12 h the mixture was filtered and the solvent evaporated. The residue was dissolved in Et_2O and extracted with saturated aqueous $NaHCO_3$. The aqueous phase was acidified with 1 M aqueous HCl and then extracted with Et_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated affording 34 mg (64%) of pure **5c**: ^{1}H NMR (acetone-d_6) δ 7.25−7.15 (m, 5H), 6.70 (br d, 1H), 5.22−5.10 (m, 1H), 3.78 (s, 2H), 3.29 (dd, J = −13.7 Hz, J = 6 Hz, 1H), 3.16 (dd, J = 9.2 Hz, 1H), 1.28 (s, 9H); ^{13}C NMR (acetone-d_6) δ 181.05, 169.40, 166.26, 156.23, 137.81, 130.48, 129.4, 127.89, 80.02, 50.82, 39.83, 32.64, 28.69.

Ethyl 2-{5-[(*S*)-1-(*tert*-Butyloxycarbonylamino)-2-phenylethyl]-1,2,4-triazol-3-yl}acetate (6b). Crude **11** was heated in xylenes in a silicon oil bath at 155 °C. The reaction was monitored by TLC (EtOAc/pentane, 3:2). After 30 min the solvent was evaporated. Purification of the residue by column chromatography [(i) EtOAc/pentane, 2:3, (ii) EtOAc/pentane, 1:1] afforded 110 mg (57%, over two steps) of pure **6b**: ^{1}H NMR ($CDCl_3$) δ 12.6 (br, 1H), 7.95−7.00 (m, 5H), 5.78 (br s, 1H), 5.13 (m, 1H), 4.20 (q, J = 7.2 Hz, 2H), 3.85 (s, 2H), 3.28−3.07 (m, 2H), 1.34 (s, 9H), 1.27 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 169.24, C-3 and C-5 not visible, 155.51, 136.73, 129.36, 128.28, 126.60, 79.91, 61.55, 49.54, 40.45, 33.48, 28.23, 14.04.

1-(Methoxycarbonylacetyl)-2-[(*S*)-2-(*tert*-butyloxycarbonylamino)-3-phenylpropanoyl]hydrazine (7). The reaction was carried out in dry THF under nitrogen atmosphere. Methyl malonyl chloride (248 μL, 2.3 mmol) was added to a chilled solution (−30 °C) of Boc-L-phenylalanine hydrazide[16] (433 mg, 1.6 mmol) and *N*-methylmorpholine (NMM) (255 μL, 2.3 mmol) in THF. The mixture was stirred for 4 h while the temperature was gradually increased to room temperature and then kept at ambient temperature. The reaction was monitored by TLC (EtOAc). The salt formed was filtered off and the filtrate was concentrated. The residue was partitioned between EtOAc and water. The organic layer was extracted with saturated aqueous $NaHCO_3$, 10% aqueous citric acid, and brine, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (EtOAc/pentane, 2:1) affording 340 mg (58%) of pure **7**: mp 158−159 °C; $[\alpha]_D$ −10.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.21 (br s, 1H), 10.05 (br s, 1H), 7.27−7.15 (m, 5H), 5.78 (d, 1H), 4.79 (m, 1H), 3.74 (s, 3H), 3.44 (s, 2H), 3.18−2.90 (AB-system, 2H), 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.24, 168.01, 161.49, 155.58, 136.46, 129.41, 128.39, 126.75, 80.05, 53.82, 52.60, 39.93, 38.56, 28.28. Anal. Calcd for $C_{18}H_{25}N_3O_6$: C, 57.0; H, 6.6; N, 11.1. Found: C, 56.8; H, 6.6; N, 10.9.

3-(*tert*-Butyldiphenylsilyloxy)propanamide Oxime (8). (a) Preparation of 3-(*tert*-Butyldiphenylsilyloxy)propionitrile.[91] The reaction was carried out in dry THF under nitrogen atmosphere. *tert*-Butyldiphenylchlorosilane (18.5 mL, 73 mmol) was added to a solution of 3-hydroxypropionitrile (2.46 mL, 36 mmol), Et_3N (12.7 mL, 91 mmol), and (dimethylamino)pyridine (DMAP) (178 mg, 1.5 mmol) in THF. The reaction mixture was stirred at room temperature for 3.5 h. The solvent was evaporated. The residue was dissolved in CH_2Cl_2 and extracted with saturated aqueous $NaHCO_3$, saturated aqueous citric acid, and brine. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2/pentane, 2:3) affording 8.6 g (76%) of pure 3-(*tert*-butyldiphenylsilyloxy)-propionitrile: mp 50−51 °C (CH_2Cl_2/pentane); ^{1}H NMR ($CDCl_3$) δ 7.70−7.35 (m, 10H), 3.84 (t, J = 6.3 Hz, 2H), 2.53 (t, 2H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 135.49, 132.67, 129.86, 127.82, 117.89, 59.02, 26.66, 21.41, 19.12. Anal. Calcd for $C_{19}H_{23}NOSi$: C, 73.7; H, 7.5; N, 4.5. Found: C, 73.6; H, 7.6; N, 4.4.

(b) A solution of hydroxylamine hydrochloride (3.8 g, 55 mmol) and K_2CO_3 (3.8 g, 27 mmol) in water was added to 3-(*tert*-butyldiphenylsilyloxy)propionitrile (8.5 g, 27 mmol) dissolved in EtOH. The mixture was stirred at room temperature for 23 h. The solvent was evaporated and the residue was redissolved in Et_2O. The mixture was filtered and the filtrate was dried with Na_2SO_4. After filtration the solvent was evaporated. Crystallization of the residue (Et_2O/pentane) afforded 5.4 g (57%) of pure **8**: mp 83−84 °C; ^{1}H NMR ($CDCl_3$) δ 7.70−7.25 (m, 10H), 5.04 (br s, 2H), 3.88 (t, J = 5.6 Hz, 2H), 2.37 (t, 2H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 153.88, 135.51, 132.90, 129.98, 127.86, 62.42, 33.61, 26.82, 19.06. Anal. Calcd for $C_{19}H_{26}N_2O_2Si$: C, 66.6; H, 7.6; N, 8.2. Found: C, 66.8; H, 7.8; N, 8.0.

5-[(*S*)-1-(*tert*-Butyloxycarbonylamino)-2-phenylethyl]-

3-[2-(*tert*-butyldiphenylsilyloxy)ethyl]-1,2,4-oxadiazole (9). The symmetrical anhydride was generated by addition of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (558 mg, 2.7 mmol) to a solution of Boc-L-phenylalanine (1.43 g, 5.4 mmol) in dry CH_2Cl_2. The mixture was stirred at 0 °C for 1 h. The resulting *N*,*N*′-dicyclohexylurea (DCU) was filtered off and the filtrate was concentrated. The residue was redissolved in pyridine and a solution of **8** (926 mg, 2.7 mmol) in pyridine was added dropwise. The mixture was heated to reflux for 3 h and the reaction was monitored by TLC (EtOAc). The solvent was evaporated, and the residue was dissolved in Et_2O and extracted with water, 10% aqueous citric acid, and saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2/pentane, 4:1) affording 940 mg (63%) of pure **9** as an oil: $[\alpha]_D$ −9.4° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.65−7.00 (m, 15H), 5.29 (m, 1H), 5.06 (m, 1H), 4.00 (t, J = 5.4 Hz, 2H), 3.29−3.08 (m, 2H), 2.96 (t, 2H), 1.40 (s, 9H), 1.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 178.29, 168.16, 154.68, 135.52, 135.04, 133.39, 129.68, 129.25, 128.62, 127.69, 127.22, 80.43, 60.81, 49.26, 39.98, 29.51, 28.20, 26.70, 19.14. Anal. Calcd for $C_{33}H_{41}N_3O_4Si$: C, 69.3; H, 7.2; N, 7.3. Found: C, 69.2; H, 7.4; N, 7.4.

5-[(*S*)-1-(*tert*-Butyloxycarbonylamino)-2-phenylethyl]-3-(2-hydroxyethyl)-1,2,4-oxadiazole (10). The reaction was carried out in dry THF under nitrogen atmosphere. A mixture of tetrabutylammonium fluoride (800 μL, 0.80 mmol, 1 M solution in THF) and 30 μL of acetic acid in THF was added to a solution of **9** (281 mg, 0.49 mmol) in THF. The reaction was monitored by TLC (Et_2O). After stirring at room temperature for 2 h the solvent was evaporated. The residue was redissolved in Et_2O and extracted with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography [(i) Et_2O/pentane, 1:2, (ii) Et_2O] affording 123 mg (75%) of pure **10**: ^{1}H NMR ($CDCl_3$) δ 7.30−7.00 (m, 5H), 5.3 (br, 1H), 5.1 (br, 1H), 3.95 (t, J = 5.9 Hz, 2H), 3.31−3.14 (m, 2H), 2.96 (t, 2H), 2.30 (t, 1H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 178.78, 168.48, 154.89, 135.04, 129.23, 128.71, 127.35, 80.61, 59.42, 49.36, 39.80, 29.20, 28.21.

1-(Ethoxycarbonylacetimidyl-2-[(*S*)-2-(*tert*-butyloxycarbonylamino)-3-phenylpropanoyl]hydrazine (11). Boc-L-Phenylalanine hydrazide[16] (143 mg, 0.51 mmol) dissolved in EtOH was added to a solution of ethyl β-amino-β-ethoxyacrylate hydrochloride (100 mg, 0.51 mmol) and Et_3N (71 μL, 0.51 mmol) in EtOH.[92] The mixture was stirred at room temperature for 12 h. The product precipitated after addition of water and cooling. The solids were filtered off and the crude product was used in the ring closure reaction without further purification.

General Peptide Synthesis. The pseudopeptides were prepared using N^{α}-*tert*-butyloxycarbonyl (Boc)-protected amino acid derivatives on an MBHA (*p*-methylbenzhydrylamine) resin. If needed, the functional groups in the side chains were protected with appropriate groups: the N^{γ} in Arg with a *p*-toluenesulfonyl group (Tos), Lys with a *p*-chlorobenzyloxycarbonyl (Cl-Z) group, and the Tyr and Ser with a benzyl group (Bn). The coupling reactions were carried out at room temperature in a 5-mL disposable syringe with a porous polyethylene disk as a filter (ca. 50-μm pore size). A stainless steel needle was attached to the syringe. The syringe was rotated in a carousel during the deprotection and coupling reactions.

1. Coupling and Deprotection Procedures for the Amino Acids. The Met-Leu-MBHA and Tyr(Bn)-Pro-Ser(Bn)-MBHA resins were prepared in advance to be used in the syntheses of the SP and dermorphin pseudopeptides, respectively. The reaction cycle for coupling of amino acids to the resin-bound peptide chain and deprotection of the N-terminal amino acid was as follows: The Boc-protecting group of the N-terminal of the resin-bound peptide sequence was removed with concd TFA (8 min). The peptide-resin was washed with DMF (4 × 1 min + 2 × 3 min) and then neutralized with 3 equiv of *N*,*N*-diisopropylethylamine (DIPEA) in DMF for 10 min. A solution of 3 equiv of the Boc-amino acid, 3 equiv of 1-hydroxybenzotriazole hydrate (HOBt), 3 equiv of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), or *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), and 6 equiv of DIPEA in DMF was added to the neutralized peptide-resin. The coupling reaction was run for 30 min while rotating the syringe. The peptide-resin was washed with DMF (4 × 1 min + 2 × 3 min).

2. Deprotection Procedures for the Phe-Gly Analogues. Deprotection of N^{α} of the Phe-Gly mimetics was accomplished by addition of a solution of 2 equiv of TMSI in CH_2Cl_2 and rotating the syringe for 20 min. After removal of the soluble material, 50 μL of MeOH in CH_2Cl_2 was added and the syringe was rotated for an additional 10 min.

3. Cleavage of the Peptide from the Resin. Thioanisole (100 μL) and 1,2-ethanediol (50 μL) were added to the resin. The mixture was stirred at room temperature for 10 min, before TFA (1 mL) was added. After 10 min, trifluoromethanesulfonic acid (TFMSA) (100 μL) was added and the mixture was stirred for an additional 2 h. Cold ether was added to the filtrate, and the precipitated peptide and the resin were filtered off. The peptide was dissolved in TFA and filtered, leaving the resin on the frit. Cold ether was added to the filtrate, and the precipitated peptide was filtered off. The crude pseudopeptides (10−40 mg) were purified using preparative reversed-phase HPLC. The fractions containing the pseudopeptide were pooled and freeze-dried providing 1−8 mg of pure product. The pseudopeptides were analyzed by reversed-phase HPLC, plasma desorption mass spectrometry, and amino acid analysis[90] (Table 2). Amino acid analyses of the pseudopeptides containing the oxadiazole-based mimetics resulted in the formation of a fragment analyzed as phenylalanine. However, the 1,2,4-triazole ring system did not seem to be affected by the conditions used during the hydrolysis.

H-Tyr-D-Ala-{5-[(*S*)-1-amino-2-phenylethyl]-1,3,4-oxadiazol-2-ylcarbonyl}-Tyr-Pro-Ser-NH$_2$ (1d). A Tyr(Bn)-Pro-Ser(Bn)-MBHA resin (50 μmol) was used. Compound **1b** (52 mg, 144 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (158 μL) for 30 min at room temperature. The mixture was concentrated and the residue was carefully dried. The crude carboxylate (**1c**) was dissolved in dry CH_2Cl_2/DMF (4:1). Half of the volume was chilled to −10 °C and treated with NMM (9.5 μL, 86 μmol) and ethyl chloroformate (8.3 μL, 86 μmol). The mixture was stirred at −10 °C for 30 min and then drawn up into the syringe. The coupling was run at room temperature for 2 h. The procedure was repeated with the remaining **1c**. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 653 μg/mg.

H-Tyr-D-Ala-{5-[(*S*)-1-amino-2-phenylethyl]-1,2,4-oxadiazol-3-ylcarbonyl}-Tyr-Pro-Ser-NH$_2$ (2d). A Tyr(Bn)-Pro-Ser(Bn)-MBHA resin (50 μmol) was used. Compound **2b** (54.0 mg, 150 μmol) was hydrolyzed in EtOH with aqueous 1 M KOH (165 μL) for 20 min at room temperature. The mixture was concentrated and redissolved in MeOH. Half of the volume was treated with H^+ ion-exchange resin (Dowex 50 W × 8), filtered, and concentrated. The residue was redissolved in Et_2O, dried ($MgSO_4$), filtered, and concentrated. The crude acid (**2c**) was dissolved in CH_2Cl_2/DMF (4:1). The solution was chilled to −10 °C and treated with NMM (12.4 μL, 112 μmol) and ethyl chloroformate (11.0 μL, 112 μmol). The mixture was stirred at −10 °C for 30 min. The reaction mixture was drawn up into the syringe and the coupling reaction was run at room temperature for 2 h. The procedure was repeated with the remaining volume of the MeOH solution from the hydrolysis. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 729 μg/mg.

H-Tyr-D-Ala-{5-[(*S*)-1-amino-2-phenylethyl]-1,2,4-triazol-3-ylcarbonyl}-Tyr-Pro-Ser-NH$_2$ (3d). A Tyr(Bn)-Pro-Ser(Bn)-MBHA resin (50 μmol) was used. Compound **3b** (35.7 mg, 99 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (218 μL) for 14 h at 65 °C. The mixture was concentrated and

the residue was carefully dried. HBTU (22.8 mg, 60 μmol), HOBt (8.1 mg, 60 μmol), and DIPEA (60 μL, 345 μmol) were added to a solution of the crude **3c** in DMF and the mixture was drawn up into the syringe. The coupling was run for 3 h. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 220 μg/mg.

H-Tyr-D-Ala-{2-[5-((*S*)-1-amino-2-phenylethyl)-1,3,4-oxadiazol-2-yl]acetyl}-Tyr-Pro-Ser-NH_2 (4d). A Tyr(Bn)-Pro-Ser(Bn)-MBHA resin (50 μmol) was used. Compound **4b** (72 mg, 199 μmol) was hydrolyzed in MeOH with 1 M aqueous KOH (258 μL) for 3 h at room temperature. The solvent was evaporated and the residue was partitioned between Et_2O and water. The Et_2O layer was extracted with saturated aqueous $NaHCO_3$. The $NaHCO_3$ phase was acidified with 1 M aqueous HCl and extracted with Et_2O. The Et_2O layer was dried ($MgSO_4$), filtered, and concentrated to afford the crude acid **4c**. HBTU (21.8 mg, 57.6 μmol), HOBt (7.8 mg, 57.6 μmol), and DIPEA (60 μL, 345 μmol) were added to 20 mg of **4c** in DMF. The mixture was drawn up into the syringe and the coupling was run for 4 h. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 1260 μg/mL.

H-Tyr-D-Ala-{2-[5-((*S*)-1-amino-2-phenylethyl)-1,2,4-oxadiazol-3-yl]acetyl}-Tyr-Pro-Ser-NH_2 (5d). A Tyr(Bn)-Pro-Ser(Bn)-MBHA resin (60 μmol) was used. Ethyl chloroformate (8.6 μL, 90 μmol) was added to a chilled mixture (−10 °C) of **5c** (21 mg, 60 μmol) and NMM (9.9 μL, 90 μmol) in CH_2Cl_2/DMF (4:1). The mixture was stirred at −10 °C for 30 min and was drawn up into the syringe. The coupling was run at room temperature for 2 h. The procedure was repeated with another 22 mg of **5c**. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 514 μg/mL.

H-Tyr-D-Ala-{2-[5-((*S*)-1-amino-2-phenylethyl)-1,2,4-triazol-3-yl]acetyl}-Tyr-Pro-Ser-NH_2 (6d). A Tyr(Bn)-Pro-Ser-(Bn)-MBHA resin (50 μmol) was used. Compound **6b** (66 mg, 176 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (388 μL) for 5 h at room temperature. The mixture was concentrated and the residue was carefully dried and redissolved in EtOH. The solution was treated with H^+ ion-exchange resin (Dowex 50 W × 8), filtered, and concentrated affording crude **6c**. HBTU (19 mg, 50 μmol), HOBt (6.8 mg, 50 μmol), and DIPEA (52 μL, 300 μmol) were added to 17.3 mg of the crude **6c** in DMF. The mixture was drawn up into the syringe and the coupling was run for 2 h. The procedure was repeated with an additional 17.3 mg of **6c**. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 567 μg/mg.

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-{5-[(*S*)-1-amino-2-phenylethyl]-1,3,4-oxadiazol-2-ylcarbonyl}-Leu-Met-NH_2 (1e). A Leu-Met-MBHA resin (41.5 μmol) was used. Compound **1b** (60 mg, 166 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (198 μL) for 30 min at room temperature. The mixture was concentrated and the residue carefully dried by repeated evaporation with benzene. The crude carboxylate (**1c**) was dissolved in dry CH_2Cl_2/DMF (1:1). Half of the volume was chilled to −10 °C and treated with NMM (10 μL, 91.3 μmol) and ethyl chloroformate (8.7 μL, 91.3 μmol). The mixture was stirred at −10 °C for 25 min and then drawn up into the syringe. The coupling reaction was run at room temperature for 2 h. The procedure was repeated with the remaining **1c**. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 691 μg/mg.

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-{5-[(*S*)-1-amino-2-phenylethyl]-1,2,4-oxadiazol-3-ylcarbonyl}-Leu-Met-NH_2 (2e). A Leu-Met-MBHA resin (41.5 μmol) was used. Compound **2b** (60 mg, 166 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (198 μL) for 20 min at room temperature. The mixture was concentrated and redissolved in MeOH. Half of the volume was treated with H^+ ion-exchange resin (Dowex 50 W × 8), filtered, and concentrated. The crude acid (**2c**) was dissolved in dry THF. The solution was chilled to −5 °C and treated with Et_3N (16 μL, 166 μmol) and ethyl chloroformate (23 μL, 166 μmol). The mixture was stirred at −5 °C for 30 min. The resulting triethylammonium chloride was filtered off and the filtrate was concentrated. The residue was redissolved in DMF and drawn up into the syringe. The coupling reaction was run at room temperature for 2.5 h. The procedure was repeated with the remaining volume of MeOH from the hydrolysis. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 472 μg/mL.

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-{5-[(*S*)-1-amino-2-phenylethyl]-1,2,4-triazol-3-ylcarbonyl}-Leu-Met-NH_2 (3e). A Leu-Met-MBHA resin (40.4 μmol) was used. Compound **3b** (50 mg, 139 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (319 μL) for 14 h at 65 °C. The mixture was concentrated and the residue carefully dried. HBTU (30.6 mg, 81 μmol), HOBt (10.9 mg, 81 μmol), and DIPEA (42 μL, 242 μmol) were added to **3c** dissolved in DMF and the mixture was drawn up into the syringe. The coupling reaction was run for 2 h. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 638 μg/mg.

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-{2-[5-((*S*)-1-amino-2-phenylethyl)-1,2,4-oxadiazol-3-yl]acetyl}-Leu-Met-NH_2 (5e). The couplings of **5c** to the Leu-Met-NH_2 resin did not succeed. However, in one successful experiment racemic **5c** was incorporated into SP by automated solid-phase synthesis using HBTU/HOBt/DIPEA as coupling reagents. The epimers were separated by preparative reversed-phase HPLC (Table 2). Peptide content: **5e**α, 638 μg/mg; **5e**β, 645 μg/mg.

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-{2-[5-((*S*)-1-amino-2-phenylethyl)-1,2,4-triazol-3-yl]acetyl}-Leu-Met-NH_2 (6e). A Leu-Met-MBHA resin (40 μmol) was used. Compound **6b** (130 mg, 353 μmol) was hydrolyzed in EtOH with 1 M aqueous KOH (778 μL) for 1.5 h at reflux. The mixture was concentrated and the residue was carefully dried and redissolved in EtOH. The solution was treated with H^+ ion-exchange resin (Dowex 50 W × 8), filtered, and concentrated. HBTU (22.8 mg, 60 μmol), HOBt (8.1 mg, 60 μmol), and DIPEA (63 μL, 360 μmol) were added to 21 mg of **6c** in DMF. The mixture was drawn up into the syringe and the coupling reaction was run for 2 h. The deprotection of N^{α} of the Phe-Gly residue with TMSI and the remaining coupling and deprotection steps were performed as described above. Peptide content: 666 μg/mg.

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Supporting Information Available: ^1H and ^{13}C NMR spectral assignments for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Abbreviations: Ac, acetyl; Bn, benzyl; Boc, *tert*-butoxycarbonyl; Cl-Z, *p*-chlorobenzyloxycarbonyl; DAMGO, Tyr-D-Ala-Gly-Me-Phe-$NHCH_2CH_2OH$; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCU, *N*,*N*′-dicyclohexylurea; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, (dimethylamino)pyridine; DPDPE, Tyr-cyclo(D-Pen-Gly-Phe-D-Pen)-OH; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; IMAC, immobilized metal affinity chromatography; MBHA,

p-methylbenzhydrylamine; NMM, *N*-methylmorpholine; Pen, penicillamine; PyBrOP, bromotrispyrrolidinophosphonium hexafluorophosphate; SP, substance P; SPL, Sybyl programming language; SPPS, solid-phase peptide synthesis; TBAF, tetrabutylammonium fluoride; TBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFMSA, trifluoromethanesulfonic acid; TMSI, iodotrimethylsilane; Tos, *p*-toluenesulfonyl.

(2) Spatola, A. F.; Darlak, K. Amide bond surrogates: Pseudopeptides and macrocycles. *Tetrahedron* **1988**, *44*, 821−833.

(3) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. Emerging approaches in the molecular design of receptor-selective peptide ligands: Conformational, topographical and dynamic considerations. *Biochem. J.* **1990**, *268*, 249−262.

(4) Hölzemann, G. Peptide conformation mimetics. *Kontakte (Darmstadt)* **1991**, 3−12 (Part 1) and 55−63 (Part 2).

(5) Chorev, M.; Goodman, M. A dozen years of retro-inverso peptidomimetics. *Acc. Chem. Res.* **1993**, *26*, 266−273.

(6) Freidinger, R. M. Toward peptide receptor ligand drugs: Progress on nonpeptides. *Prog. Drug. Res.* **1993**, *40*, 33−98.

(7) Giannis, A.; Kolter, T. Peptidomimetics for receptor ligands − discovery, development, and medicinal perspectives. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267.

(8) Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. Concepts and Progress in the Development of Peptide Mimetics. *J. Med. Chem.* **1993**, *36*, 3039−3049.

(9) Adang, A. E. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; Van Staveren, C. J. Case histories of peptidomimetics: progression from peptides to drugs. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 63−78.

(10) Gante, J. Peptidomimetics − tailored enzyme inhibitors. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720.

(11) Liskamp, R. M. J. Conformationally restricted amino acids and dipeptides, (non)peptidomimetics and secondary structure mimetics. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1−19.

(12) Nowick, J. S.; Smith, E. S.; Pairish, M. Artificial β-sheets. *Chem. Soc. Rev.* **1996**, *25*, 401−415.

(13) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. Design and synthesis of conformationally constrained amino acids as versatile scaffolds and peptide mimetics. *Tetrahedron* **1997**, *53*, 12789−12854.

(14) Jenmalm, A.; Luthman, K.; Lindeberg, G.; Nyberg, F.; Terenius, L.; Hacksell, U. Novel peptidomimetics: Inhibitors of substance P endopeptidase. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1693−1698.

(15) Li, Y.-L.; Luthman, K.; Hacksell, U. Novel L-Phe-Gly mimetics. *Tetrahedron Lett.* **1992**, *33*, 4487−4490.

(16) Borg, S.; Estenne-Bouhtou, G.; Luthman, K.; Csöregh, I.; Hesselink, W.; Hacksell, U. Synthesis of 1,2,4-oxadiazole-, 1,3,4-oxadiazole-, and 1,2,4-triazole-derived dipeptidomimetics. *J. Org. Chem.* **1995**, *60*, 3112−3120.

(17) Behr, L. C. 1,3,4-Oxadiazoles. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Ed.; Wiley: New York, 1962; pp 263−282.

(18) Nesynov, E. P.; Grekov, A. P. The chemistry of 1,3,4-oxadiazole derivatives. *Russ. Chem. Rev.* **1964**, *33*, 508−515.

(19) Hetzheim, A.; Möckel, K. Recent advances in 1,3,4-oxadiazole chemistry. In *Advances in Heterocyclic Chemistry*; Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1966; pp 183−224.

(20) Hill, J. 1,3,4-Oxadiazoles. In *Comprehensive Heterocyclic Chemistry*; Potts, K. T., Ed.; Pergamon Press: Oxford, 1984; pp 427−446.

(21) Behr, L. C. 1,2,4-Oxadiazoles. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Ed.; Wiley: New York, 1962; pp 245−262.

(22) Eloy, F. A review of the chemistry of 1,2,4-oxadiazoles. *Fortschr. Chem. Forsch.* **1965**, *4*, pp 807−876.

(23) Clapp, L. B. 1,2,4-Oxadiazoles. *Adv. Heterocycl. Chem.* **1976**, *20*, 65−116.

(24) Clapp, L. B. 1,2,3- and 1,2,4-Oxadiazoles. In *Comprehensive Heterocyclic Chemistry*; Potts, K. T., Ed.; Pergamon Press: Oxford, 1984; pp 365−392.

(25) Potts, K. T. The chemistry of 1,2,4-triazoles. *Chem. Rev.* **1961**, *61*, 87−127.

(26) Temple, C., Jr. *1,2,4-Triazoles*; John Wiley & Sons: New York, 1981; Vol 37.

(27) Polya, J. B. 1,2,4-Triazoles. In *Comprehensive Heterocyclic Chemistry*; Potts, K. T., Ed.; Pergamon Press: Oxford, 1984; pp 733−790.

(28) See ref 16 and refs 6−8 therein.

(29) Burrell, G.; Evans, J. M.; Hadley, M. S.; Hicks, F.; Stemp, G. Benzopyran potassium channel activators related to cromakalim − Heterocyclic amide replacement at position 4. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1285−1290.

(30) Thompson, S. K.; Eppley, A. M.; Frazee, J. S.; Darcy, M. G.; Lum, R. T.; Tomaszek, T. A., Jr.; Ivanoff, L. A.; Morris, J. F.; Sternberg, E. J.; Lambert, D. M.; Fernandez, A. V.; Petteway, S. R., Jr.; Meek, T. D.; Metcalf, B. W.; Gleason, J. G. Synthesis and antiviral activity of a novel class of HIV-1 protease inhibitors containing a heterocyclic P1′−P2′ amide bond isostere. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2441−2446.

(31) Pernow, B. Substance P. *Pharmacol. Rev.* **1983**, *35*, 85−141.

(32) Regoli, D.; Boudon, A.; Fauchère, J.-L. Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.* **1994**, *46*, 551−599.

(33) De Castiglione, R. Structure−activity relationships in dermorphin-like peptides. In *Highlights in Receptor Chemistry*; Melchiorri, C., Giannella, M., Eds.; Elsevier Sci: Amsterdam, 1984; pp 149−168.

(34) De Castiglione, R.; Rossi, A. C. Structure−activity relationships of dermorphin synthetic analogues. *Peptides* **1985**, *6* (Suppl. 3), 117−125.

(35) Melchiorri, P.; Negri, L. The Dermorphin Peptide Family. *Gen. Pharmacol.* **1996**, *27*, 1099−1107.

(36) Cascieri, M. A.; Huang, R.-R. C.; Fong, T. M.; Cheung, A. H.; Sadowski, S.; Ber, E.; Strader, C. D. Determination of the amino acid residues in substance P conferring selectivity and specificity for the rat neurokinin receptors. *Mol. Pharmacol.* **1992**, *41*, 1096−1099.

(37) Salvadori, S.; Sarto, G.; Tomatis, R. Synthesis and pharmacological activity of dermorphin and its *N*-terminal sequences. *Int. J. Pept. Protein Res.* **1982**, *19*, 536−542.

(38) Pseudopeptides are defined as modified peptides containing sequences that are not peptidergic. Pseudopeptides can be obtained by, e.g., isosteric replacement of peptide bonds or replacement of larger fragments in the peptide with nonpeptidic structures.

(39) Cox, M. T.; Gormley, J. J.; Hayward, C. F.; Petter, N. N. Incorporation of *trans*-olefinic dipeptide isosteres into enkephalin and substance P analogues. *J. Chem. Soc., Chem. Commun.* **1980**, 800−802.

(40) Allmendinger, T.; Felder, E.; Hungerbühler, E. Fluoroolefin dipeptide isosteres − II. Enantioselective synthesis of both antipodes of the Phe-Gly dipeptide mimic. *Tetrahedron Lett.* **1990**, *31*, 7301−7304.

(41) Ewenson, A.; Laufer, R.; Chorev, M.; Selinger, Z.; Gilon, C. Ketomethylene pseudopeptide analogues of substance P: Synthesis and biological activity. *J. Med. Chem.* **1986**, *29*, 295−299.

(42) Chorev, M.; Rubini, E.; Gilon, C.; Wormser, U.; Selinger, Z. Synthesis of partially modified retro-inverso substance P analogues and their biological activity. *J. Med. Chem.* **1983**, *26*, 129−135.

(43) Roubini, E.; Laufer, R.; Gilon, C.; Selinger, Z.; Roques, B. P.; Chorev, M. Pseudopeptide analogues of substance P and leucine enkephalinamide containing the ψ[CH_2O] modification: Synthesis and biological activity. *J. Med. Chem.* **1991**, *34*, 2430−2438.

(44) Wilkes, B. C.; Schiller, P. W. Conformation-activity relationship of cyclic dermorphin analogues. *Biopolymers* **1990**, *29*, 89−95.

(45) Shenderovich, M. D.; Nikiforovich, G. V.; Golbraikh, A. A. Conformational features responsible for the binding of cyclic analogues of enkephalin to opioid receptors. III. Probable binding conformations of μ-agonists with phenylalanine in position 3. *Int. J. Pept. Protein Res.* **1991**, *37*, 241−251.

(46) Tourwé, D.; Verschueren, K.; van Binst, G.; Davis, P.; Porreca, D.; Hruby, V. J. Demorphin sequence with high δ-affinity by fixing the Phe side chain to trans at χ_1 *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1305−1308.

(47) Salvadori, S.; Marastoni, M.; Balboni, G.; Sarto, G.; Tomatis, R. Synthesis and opioid activity of partial retro-inverso analogues of dermorphin. *Int. J. Pept. Protein Res.* **1985**, *25*, 526−533.

(48) Marastoni, M.; Balboni, G.; Salvadori, S.; Sarto, G. P.; Tomatis, R. Structure−activity relationships of dermorphin tetrapeptides carrying a ketomethylene linkage. *Arzneim.-Forsch./Drug Res.* **1985**, *35* (II), 1630−1632.

(49) Jaspers, H.; Tourwé, D.; van Binst, G.; Pepermans, H.; Borea, P.; Ucelli, L.; Salvadori, S. Dermorphin tetra- and heptapeptide analogues containing a [3,4] amide bond replacement by a carbon−carbon double and single bond. *Int. J. Pept. Protein Res.* **1992**, *39*, 315−321.

(50) Darlak, K.; Grzonka, Z.; Spatola, A. F.; Benovitz, D. E.; Burks, T. F.; Wire, W. S. Synthesis, biological activities, and enzymatic stabilities of dermorphin pseudopeptides. *Peptides 1988* **1989**, 634−636.

(51) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. An all atom force field for simulations of proteins and nucleic acids. *J. Comput. Chem.* **1986**, *7*, 230−245.

(52) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel − An integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.

(53) The 1*H*-tautomer was observed in an X-ray crystallographic study of a serine-based triazole derivative (ref 16).
(54) SYBYL 6.1; Tripos Associates, 1699 S. Hanley Rd., Suite 303, St. Louis, MO 63144.
(55) SPARTAN 4.0; Wavefunction, Inc., 18401 Von Karman Ave., Suite 370, Irvine, CA 92715.
(56) Dewar, W. J.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: A new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.
(57) Hariharan, P. C.; Pople, J. A. Effect of *d*-functions on molecular orbital energies for hydrocarbons. *Chem. Phys. Lett.* **1972**, *16*, 217−219.
(58) Müller, E. Herstellung von Di-, Tri- und Tetraacylhydrazinen. In *Methoden der Organischen Chemie*; Müller, E., Ed.; Georg Thieme Verlag: Stuttgart, 1971; pp 127−168.
(59) Košmrlj, J.; Kocevar, M.; Polanc, S. A mild approach to 1,3,4-oxadiazoles and fused 1,2,4-triazoles. Diazenes as intermediates? *Synlett* **1996**, 652−654.
(60) Mazurkiewicz, M. R.; Grymel, M. An efficient synthesis of 1,3,4-oxadiazoles from N,N′-diacylhydrazines using $Ph_3P{\cdot}Br_2$, $Ph_3P{\cdot}CCl_4$ or $Ph_3P{\cdot}CBr_4$ adducts as condensing agents. *Polish J. Chem.* **1997**, *71*, 77−82.
(61) Golfier, M.; Guillerez, M.-G. Cyclisations dipolaires I. Mecanisme de la cyclisation des dihydrazides par le reactif $SOCl_2$/pyridine. *Tetrahedron Lett.* **1976**, 267−270.
(62) Eloy, F. R. L. The chemistry of the amidoximes and related compounds. *Chem. Rev.* **1962**, *62*, 155−183.
(63) Liang, G.-B.; Feng, D. D. An improved oxadiazole synthesis using peptide coupling reagents. *Tetrahedron Lett.* **1996**, *37*, 6627−6630.
(64) Borg, S.; Luthman, K.; Nyberg, F.; Terenius, L.; Hacksell, U. 1,2,4-Oxadiazole derivatives of phenylalanine: Potential inhibitors of substance P endopeptidase. *Eur. J. Med. Chem.* **1993**, *28*, 801−810.
(65) Glickman, S. A.; Cope, A. C. Structure of β-amino derivatives of α,β-unsaturated lactones and esters. *J. Am. Chem. Soc.* **1945**, *67*, 1017−1020.
(66) The analysis of **10** was performed on a Chiralcel OD-H column obtained from J. T. Baker, Holland. Hexane/2-propanol/Et_2NH (90:10:0.1) at a flow rate of 0.5 mL/min was used as mobile phase. The sample was dissolved in the mobile phase. The analysis of **6b** was run on a Chiralpak AD column at J. T. Baker (Deventer, Holland). Hexane/2-propanol/Et_2NH (80:20:0.1) at a flow rate of 0.5 mL/min was used as mobile phase. The sample was dissolved in EtOH.
(67) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. Solid-phase peptide synthesis: a silver anniversary report. *Int. J. Pept. Protein Res.* **1987**, *30*, 705−739.
(68) Brachwitz, H. Hydroximsäurederivate; Versuch zur Darstellung der 5-Phenyl-1,2,4-oxadiazol-3-carbonsäure. *Z. Chem.* **1972**, *12*, 130−132.
(69) Werber, G.; Buccheri, F.; Noto, R.; Gentile, M. 1,5-Dipolar cycloaddition reactions. Semicarbazone bromides, 5-alkyl (or aryl)amino-1,3,4-oxadiazole-2-carboxylic acids and their esters. *J. Heterocycl. Chem.* **1977**, *14*, 1385−1388.
(70) Dost, J.; Heschel, M.; Stein, J. Zur Herstellung von 1,3,4-Oxadiazol-2-carbonsäure-derivaten. *J. Prakt. Chem.* **1985**, *327*, 109−116.
(71) Krämer, I.; Schunack, W. Synthese und H_2-antagonistische Wirkung von *N*-[3-(3-Piperidino-methyl-phenoxy)propyl]-1,3,4-oxadiazol-2-aminen. *Arch. Pharm. (Weinheim)* **1986**, *319*, 1091−1098.
(72) Dost, J.; Stein, J.; Heschel, M. Zur Herstellung von 5-substituierten 1,2,4-Triazol-3-carbonsäurederivaten aus Oxalsäureethylester-N^1-acylamidrazonen. *Z. Chem.* **1986**, *26*, 203−204.
(73) Heschel, H.; Stein, J.; Dost, J. Über Cycliserungsversuche von Oxalsäureethylester-N^1-acyl-amidrazonen zu 5-*R*-1,4-Triazol-3-carbonsäurederivaten. *Wiss. Z. Paedagog. Hochsch. "Karl Liebknecht" Potsdam* **1987**, *31*, 45−52.
(74) Dunn, G. E.; Lee, G. K. J. Kinetics and mechanism of the decarboxylation of pyrrole-2-carboxylic acid in aqueous solution. *Can. J. Chem.* **1971**, *49*, 1032−1035.
(75) Dunn, G. E.; Lee, G. K. J.; Thimm, H. Kinetics and mechanism of decarboxylation of some pyridinecarboxylic acids in aqueous solution. *Can. J. Chem.* **1972**, *50*, 3017−3027.
(76) To determine the enantiopurity of **2b**, an amide of (−)-Mosher acid was prepared by first removing the Boc protecting group with TFA (30% in CH_2Cl_2) and then treating the free amine with (−)-Mosher acid chloride. Analysis of the amide by straight phase HPLC indicated that racemization had occurred during the deprotection with TFA. This finding was also verified by the lack of optical rotation exhibited by the free amine.
(77) Jung, M. E.; Lyster, M. A. Conversion of alkyl carbamates into amines *via* treatment with trimethylsilyl iodide. *J. Chem. Soc., Chem. Commun.* **1978**, 315−316.
(78) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. Trimethylsilyl iodide as a peptide deblocking agent. *J. Chem. Soc., Chem. Commun.* **1979**, 495−496.
(79) Hansen, P.; Lindeberg, G. Importance of the α-amino group in the selective purification of the synthetic histidine peptides by immobilised metal ion affinity chromatography. *J. Chromatogr. A* **1995**, *690*, 155−159.
(80) Wang, J.-X.; DiPasquale, A. J.; Bray, A. M.; Maeji, N. J.; Geysen, H. M. Study of stereo-requirements of substance P binding to NK1 receptors using analogues with systematic D-amino acid replacements. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 451−456.
(81) Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. Analogues of β-LPH_{61-64} possessing selective agonist activity at μ-opiate receptors. *Eur. J. Pharmacol.* **1981**, *70*, 531−540.
(82) Kosterlitz, H. W.; Paterson, S. J. Tyr-D-Ala-Gly-MePhe-NH-$(CH_2)_2$OH is a selective ligand for the μ-opiate binding site. *Br. J. Pharmacol.* **1981**, *73*, 299P.
(83) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Bis-penicillamine enkephalins possess highly improved specificity toward δ opioid receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871−5874.
(84) Valiquette, M.; Vu, H. K.; Yue, S. Y.; Wahlestedt, C.; Walker, P. Involvement of Trp-284, Val-296, and Val-297 of the human delta-opioid receptor in binding of delta-selective ligands. *J. Biol. Chem.* **1996**, *271*, 18789−18796.
(85) Lorenzen, A.; Fuss, M.; Vogt, H.; Schwabe, U. Measurement of guanine nucleotide-binding protein activation by A1 adenosine receptor agonists in bovine brain membranes: stimulation of guanosine-5′-O-(3-[^{35}S]thio)triphosphate binding. *Mol. Pharmacol.* **1993**, *44*, 115−123.
(86) Traynor, J. R.; Nahorski, S. R. Modulation of μ-opioid agonists of guanosine-5′-O-(3-[^{35}S]thio)triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. *Mol. Pharmacol.* **1995**, *47*, 848−854.
(87) Payza, K.; St-Onge, S.; LaBarre, M.; Roberts, E.; Fraser, G.; Valiquette, M.; Vu, H. K.; Calderon, S. N.; Rice, K. C.; Walker, P.; Wahlestedt, C. Potency, selectivity, and agonism of opioid ligands at cloned human δ, μ, and κ receptors. International Association for the Study of Pain: 8th World Congress on Pain Abstracts, Vancouver, Canada, 1996; p 460.
(88) Chang, G.; Guida, W. C.; Still, W. C. An internal coordinate Monte Carlo method for searching conformational space. *J. Am. Chem. Soc.* **1989**, *111*, 4379−4386.
(89) Goodman, J. M.; Still, W. C. An unbounded systematic search of conformational space. *J. Comput. Chem.* **1991**, *12*, 1110−1117.
(90) For the determination of amino acid content, samples were hydrolyzed at 110 °C for 24 h with 6 N HCl (Merck Suprapur) containing norleucine and 2 mg/mL reagent-grade phenol in thoroughly evacuated and sealed Pyrex tubes. Methonine was determined as methionine sulfone, with a sample hydrolyzed following oxidation with performic acid according to Moore (*J. Biol. Chem.* **1963**, *238*, 235). The hydrolysates were analyzed with an LKB model 4151 Alpha Plus amino acid analyzer using the ninhydrin reagent and hydrolysate analysis program essentially as described in the apparatus manual. The serine values were corrected for 10% destruction during hydrolysis.
(91) Loubinoux, B.; O'Sullivan, A. C.; Sinnes, J.-L.; Winkler, T. A new method for the synthesis of 2-carboxymethyl-2-hydroxytetrahydropyrans. *Tetrahedron* **1994**, *50*, 2047−2054.
(92) Glover, E. E.; Rowbottom, K. T.; Bishop, D. C. Synthesis and oxidation of quaternary salts of 1-aminoimidazoles. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2927−2929.

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