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Synthesis of Constrained Peptidomimetics Containing 2-Oxo-1,3-oxazolidine-4-carboxylic Acids

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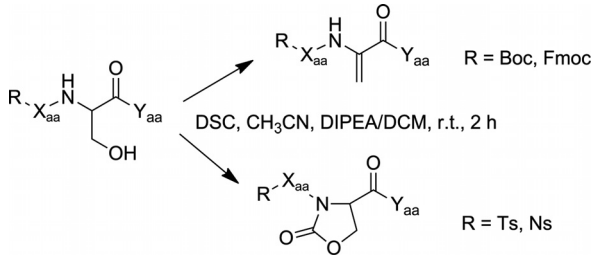
Sulfonyl peptides containing a serine or threonine residue undergo cyclization with bis(succinimidyl) carbonate (DSC) and diisopropylethylamine (DIPEA) to give peptides with a 2-oxo-1,3-oxazolidine-4-carboxylate (Oxd) group, either in solution or in the solid phase. The position of the serine or threonine residue in the sequence is relatively unimportant. Under the same conditions, the corresponding Fmoc- or Boc-peptides gave dehydration products, in agreement to previous studies. The protocol constitutes a valuable approach to the preparation of oxazolidinone-containing peptides, which are a recently emerging class of constrained peptidomimetics. As a representative example, an Oxd analogue of the endogenous opioid peptide endomorphin-1, characterized by an all-*trans* conformation, was readily prepared.

Introduction

In principle, many naturally occurring, biologically active peptides can be regarded as potential pharmaceutical agents. However, there are intrinsic weaknesses that hinder their clinical use, in particular, their rapid metabolism in vivo, which can lead to short half-life stability, and their poor permeation across membrane barriers. These inherent limitations can be bypassed by adopting the peptidomimetic strategy, which involves altering the structure of a peptide. Peptidomimetics very often contain non-amino acidic elements, but still bear identifiable similarity to the parent peptides, and either imitate or inhibit their biochemical effects.[1]

During the course of our studies on biologically active mimetics of naturally occurring opioid peptides,[2] we became interested in the preparation of dehydroalanine (DHA) containing peptides[3] as intermediates for the synthesis of sequences including substituted (*S*)- or (*R*)-tryptophans.[4] We obtained the DHA residue (80–90%) by dehydration of Boc- or Fmoc-protected serine-containing peptides with bis(succinimidyl) carbonate (DSC), according to a procedure reported in the literature (Scheme 1).[5] Interestingly, we observed that, on submitting the corresponding sulfonyl peptides to the same reaction conditions, the serine residue underwent cyclization to form the 2-oxo-1,3-oxazolidine-4-carboxylate (Oxd; Scheme 1).



Scheme 1. Different reactivities of Fmoc-, Boc-, or sulfonyl-peptides containing serine.

Peptides containing an oxazolidinone constitute a relatively uncommon class of peptidomimetics.[6] In some cases, they have been utilized as prodrugs[7] or as intermediates for the preparation of other peptidomimetics.[8] In medicinal chemistry, the oxazolidinone ring is present in some bioactive peptides or mimetics, such as the GPIIb/IIIa integrin antagonists, which are based on the (oxo-oxazolidinyl)-methyl scaffold,[9] or the HIV-1 protease inhibitors, which incorporate an oxo-*N*-phenyloxazolidine-5-carboxylic acid moiety, that are designed to improve the cellular antiviral potency.[10] Very recently, oxazolidin-2-one-containing peptides have found applications in the field of foldamers, which are short synthetic oligomers that tend to assume ordered conformations.[11] Short Oxd-peptides were shown to form well-defined 3D structures, such as the dimeric Boc-(Phe-D-Oxd)$_2$-OBn, which forms nanofibers with an antiparallel β-sheet structure.[12]

The synthesis of the oxazolidin-2-one ring from an amino alcohol and a carbonate or a dicarbonate (CDI, diethyl or diphenyl carbonate, Boc_2O, etc.) is well documented.[13] However, this work describes a new, highly efficient

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synthesis of some representative examples of Oxd-peptides directly from a peptide sequence, either in solution or in the solid phase.

Furthermore, to demonstrate the utility of the procedure in medicinal chemistry, a constrained analogue of the endogenous opioid peptide endomorphin-1 (EM1),[14] H-Tyr-Pro-Trp-PheNH$_2$, with the 2-oxo-oxazolidine-4-carboxylate in position 2 was prepared as a proline mimetic; the compound was characterized by a *trans* conformation of the preceding peptide bond.

Results and Discussion

Peptides with DHA were obtained by treating a mixture of the corresponding serine-containing *N*-Boc- or Fmoc-protected peptides and DSC in acetonitrile with a solution of DIPEA in dichloromethane at room temperature (Scheme 1). However, when the same conditions were applied to *N*-sulfonyl di-, tri-, or tetrapeptides **1** having the serine (or threonine) residue in different positions, the reaction gave the *N*-sulfonyl peptides **2**, containing a 2-oxo-oxazolidine-4-carboxylate ring, without significant formation of either DHA or aziridine[15] (Scheme 2).

Optimization of the reaction conditions led to the adoption of some minor changes with respect to the conditions described in the literature, such as the replacement of acetonitrile with *N*,*N*-dimethylformamide (DMF); the modifications resulted in excellent yields (Table 1) and reduced reaction times.

Table 1. Yields of the Oxd-peptides **2** prepared as described in Scheme 2.

1	R	X_{aa}	Y_{aa}	R^1	**2** [%][a]	**3** [%][a]
a	4-MeC_6H_4	Pro	OMe	H	trace	82
b	4-MeC_6H_4	–	PheNH$_2$	H	80	trace
c	4-MeC_6H_4	Pro	PheNH$_2$	H	85	trace
d	4-MeC_6H_4	Ala	PheNH$_2$	H	88	–
e	4-MeC_6H_4	Ala	PheNH$_2$	Me	85	–
f	4-O_2NC_6H_4	Ala	PheNH$_2$	H	90	–
g	4-MeC_6H_4	Ala-Ala	PheNH$_2$	H	95	–
h[b]	4-MeC_6H_4	Ala	PheOH[c]	H	77[d]	–
i	4-MeC_6H_4	Tyr	Trp-PheNH$_2$	H	85	–

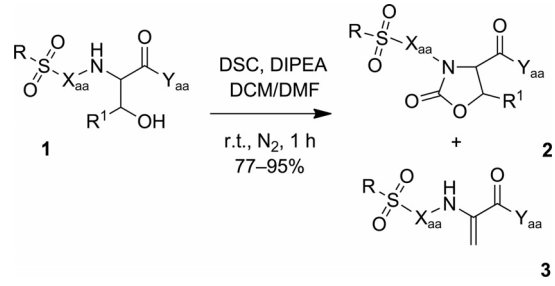
[a] Determined after isolation by flash chromatography on silica gel. [b] Ts-Ala-Ser-Phe-Wang. [c] Reaction performed on a solid phase (Wang resin), followed by peptide cleavage. [d] Determined after isolation by semipreparative RP-HPLC.

Tosyl (Ts) protected amino acids were prepared by allowing the amino acids, TsCl, and $NaHCO_3$ in H_2O/dioxane to react according to the literature;[16] the synthesis of 4-O_2N$C_6H_4SO_2$-(Nosyl or Ns)-Ala-OH was performed with Ns-Cl in H_2O at pH = 8.[17]

The dipeptide ester Ts-Pro-Ser-OMe (**1a**) was obtained in solution from Ts-Pro-OH and H-Ser-OMe·HCl under standard conditions by using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole hydrate (HOBt) as activating agents. The sulfonyl di-, tri-, and tetrapeptide-amides of general sequence RSO_2-X_{aa}-Ser/Thr-Y_{aa}-NH_2 (**1b**–**g** and **1i**) were readily obtained by standard solid-phase peptide synthesis (SPPS) on a Rink amide resin with Fmoc-protected amino acids, with *N*,*N*′-dicyclohexyl carbodiimide (DCC) and HOBt as coupling agents. After cleavage with trifluoroacetic acid (TFA) in the presence of scavengers, the peptides were isolated by flash chromatography on silica gel and analyzed by RP-HPLC and ES-MS (see Experimental Section, Table 2).

Alternatively, the Ts-peptide **1h** was prepared on Wang resin by using Fmoc-protected amino acids, DCC, and HOBt; Ts-Ala-Ser-Phe-Wang was then utilized for the following cyclization reaction on a solid phase prior to cleavage.

The cyclization reaction of sulfonyl-peptides **1a**–**g** and **1i** was performed in solution (Scheme 2). The corresponding Oxd-peptide amides **2** were isolated by flash chromatography on silica gel (purity higher than 95% by RP-HPLC analysis), whereas the Oxd-peptide acid **2h** was obtained after cleavage from the resin (see below). The Oxd-peptides were characterized by IR, NMR, RP-HPLC, ES-MS, and HRMS (FAB) analyses. ^{1}H NMR resonances were assigned by using 2D gCOSY techniques.



Scheme 2. Synthesis of Oxd-containing peptides **2**.

Only the reaction of dipeptide Ts-Pro-Ser-OMe (**1a**), containing a serine methyl ester, gave the dehydration product Ts-Pro-DHA-OMe (**3a**) as the major product (82%, Table 1). Apparently, in this case, the comparatively high acidity of the α-H atom of the serine methyl ester with respect to that of the serine amides (**1b**–**i**)[18] promoted the elimination of the intermediate Ser-*O*-succinimidyl carbonate.

In contrast, all of the other Ts or Ns oligopeptides containing Ser or Thr amides gave the corresponding Oxd-peptides, as reported in Table 1. This is a significant result, because, provided the sulfonyl group is present, these conditions allow the introduction of the Oxd group directly into oligopeptides. Indeed, when the protecting group is a carbamate, such as Boc, Fmoc, or Cbz, the reaction of peptides containing a Ser amide gives rise to the formation of DHA, as previously reported.[5a,5e] For confirmation, we submitted the corresponding Fmoc- or Boc-protected versions of **1d** (and **1f**), **1g**, and **1h** to the same conditions described above; as expected, in all cases, the reactions gave dehydration products in variable yields, without any trace of Oxd (data not shown).

The reaction of the dipeptide Ts-Ser-Phe-NH_2 (**1b**) gave Ts-Oxd-Phe-NH_2 (**2b**) in good yield and only traces of the

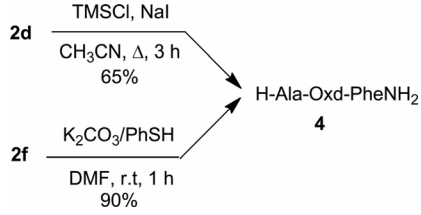
dehydration product (**3b**). The tripeptides Ts-Pro-Ser-Phe-NH_2 (**1c**) and Ts-Ala-Ser-Phe-NH_2 (**1d**), containing a tosyl group that was not directly appended to the Ser residue, gave the corresponding Oxd-peptides **2c** and **2d**, respectively, in high yields.

In a similar way, Ts-Ala-Thr-Phe-NH_2 (**1e**), which contains a Thr residue, gave the corresponding tripeptide with a *trans*-5-methyl-2-oxo-oxazolidine-4-carboxylate (**2e**). In this case, the (2*S*,3*R*) stereochemistry of the threonine residue was retained, as determined from the ^{1}H NMR coupling constant of the 4-H–5-H doublet (J = 4.8 Hz).

The reaction could also be performed with a different sulfonyl group; thus, the Nosyl-peptide Ns-Ala-Ser-Phe-NH_2 (**1f**), gave the expected product Ns-Ala-Oxd-Phe-NH_2 (**2f**) with comparable yield. Furthermore, the tetrapeptide Ts-Ala-Ala-Ser-Phe-NH_2 (**1g**), having the Ser residue in position 3 of the sequence, underwent cyclization to the corresponding Oxd-tetrapeptides Ts-Ala-Ala-Oxd-Phe-NH_2 (**2g**) in almost quantitative yield.

To expand the synthetic scope of the reaction, the Oxd-tripeptide acid Ts-Ala-Oxd-Phe-OH (**2h**) was synthesized entirely on a solid phase (Table 1). The resin-bound precursor Ts-Ala-Ser-Phe-Wang (**1h**) was prepared under standard conditions, and then subjected to cyclization by treatment with a moderate excess of DSC and DIPEA in dichloromethane/DMF (4:1) for 2 h. The cleavage of the Oxd-peptide was performed by treatment with two consecutive portions of 10% TFA in dichloromethane and scavengers. Analysis of the crude reaction mixture by RP-HPLC and ES-MS analysis revealed the presence of the peptide Ts-Ala-Ser-Phe-OH (ca. 5%). The peptide **2h** was isolated by semipreparative RP-HPLC with satisfactory yield (77%, based on an average resin loading of 0.6 mmol/g).

The feasibility of sulfonyl group removal under reasonably mild conditions was then investigated (Scheme 3). The tripeptide Ts-Ala-Oxd-PheNH_2 (**2d**) was treated with iodotrimethylsilane[19] to give the deprotected product H-Ala-Oxd-PheNH_2 (**4**) in moderate yield, together with a mixture of by-products. As an alternative, treatment of **2d** with SmI_2/pyrrolidine/water,[20] was attempted, but this method gave **4** in only low yield.



Scheme 3. Removal of Ts from **2d** and Ns from **2f** affording the deprotected Oxd-containing peptide **4**.

In contrast, cleavage of the Ns group was much easier (Scheme 3), and treatment of Ns-Ala-Oxd-PheNH_2 (**2f**) with K_2CO_3/PhSH[21] gave **4** in good yield. The superior performance of Ns compared with Ts is not unexpected.[22] Based on this result, the Ns group can be regarded as the best choice for the preparation of deprotected Oxd-peptides.

Finally, to demonstrate the usefulness of Oxd-peptides as conformationally defined peptidomimetics containing a pseudo-proline,[6] an analogue of the endogenous opioid peptide endomophin-1 (EM1), H-Tyr-Pro-Trp-PheNH_2 was prepared. Among the different natural or synthetic opioid peptides,[23] EM1 is unique for high receptor affinity and selectivity towards the μ-opioid receptor (MOR). However, despite many structural investigations performed on EM1 and its analogues, the identification of the bioactive conformation remains elusive.[24] Extensive NMR studies revealed that, in solution, EM1 exists as an approximate 1:3 *cis*/*trans* mixture of conformers with respect to the Tyr^1–Pro^2 peptide bond.

The tetrapeptide Ts-Tyr-Ser-Trp-Phe-NH_2 (**1i**) gave Ts-Tyr-Oxd-Trp-Phe-NH_2 (**2i**) in satisfactory yield. In solution, the Oxd-tetrapeptide **2i** adopts an all-*trans* conformation of the peptide bonds, as revealed by the NMR spectrum of the compound, which shows a single set of sharp resonances (Figure 1).

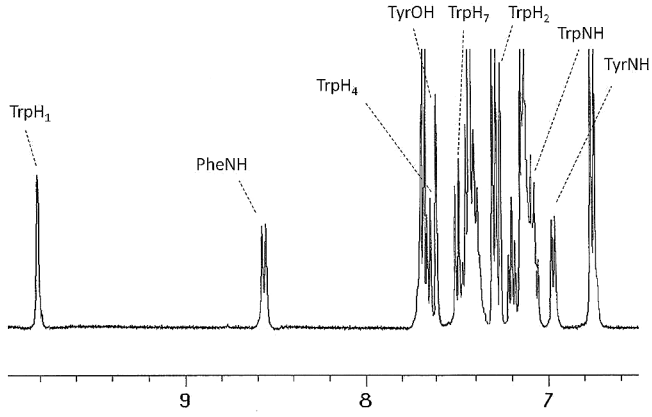


Figure 1. ^{1}H NMR spectrum of **2i**, showing the amide and aromatic region.

The *trans* conformation of **2i** was confirmed by comparison of the NMR spectroscopic data with that of Oxd-peptides described in the literature.[11,12] In Oxd-peptides, the carbonyl group of the cycle introduces a constraint that forces the pseudo-peptide bond to always have the *trans* conformation. This feature should be of help to investigate the bioactive conformation of EM1, which is an aspect that is under investigation.

Conclusions

In this paper we have illustrated the ready synthesis of some representative peptides incorporating a 2-oxo-oxazolidine-4-carboxylate starting from sulfonyl peptides containing Ser or Thr. Interestingly, the outcome of the reaction was practically independent of the distance between the Ser or Thr residues and the sulfonyl group. Tosyl- or Nosyl-protected peptides gave equally excellent results; these sulfonyl groups can be removed under a variety of mild conditions, in particular, the removal of Nosyl gave the deprotected peptide in good yields. The cyclization reaction was

performed either in solution or on a solid phase, making the entire process a promising approach to the preparation of this class of constrained peptidomimetics and foldamers. As a representative example, an all-*trans* Oxd analogue of EM1 was prepared.

Experimental Section

General Methods: Unless stated otherwise, standard chemicals were obtained from commercial sources and used without further purification. Flash chromatography of **1** was performed on silica gel (230–400 mesh) by using mixtures of distilled solvents. Compounds **1** were analyzed by RP-HPLC and ES-MS. RP-HPLC was performed on an ODS column [4.6 μm particle size, 100 Å pore diameter, 250 μm, DAD 210 nm, from H_2O/CH_3CN (9:1) to H_2O/CH_3CN (2:8) in 20 min] at a flow rate of 1.0 mL/min, followed by 10 min at the same composition. Semipreparative RP-HPLC purification of compounds **2** were performed on a C18 column [7 μm particle size, 21.2 mm × 150 mm, from H_2O/CH_3CN (8:2) to 100% CH_3CN in 10 min] at a flow rate of 10 mL/min. The purity of **2** (more than 95%) was assessed by analytical RP-HPLC under the above conditions. Semipreparative and analytical RP-HPLC of compound **4** were performed under the same conditions as reported for **2**, but with the addition of 0.1% TFA in the mobile phase. Compounds **2** and **4** were analyzed by RP-HPLC, ES-MS, and HRMS (FAB). ^{1}H NMR spectra were recorded at 400 MHz at room temperature in $CDCl_3$/[D_6]DMSO (2:1) by using 5 mm tubes; ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts are reported as δ values relative to the $CDCl_3$ signal.

Synthesis of 1: A measure of Fmoc-Rink amide resin (0.5 g, 1.1 mmol/g, resin particle size: 100–200 mesh) was introduced into a reactor of an automated synthesizer apparatus. Fmoc was removed with DMF/piperidine (4:1) (5 mL) under mechanical shaking. After 15 min, the suspension was filtered, the resin was washed with CH_2Cl_2 (5 mL) and treated, while shaking, with a second portion of DMF/piperidine (4:1). After 40 min, the suspension was filtered, and the resin was washed three times in sequence with CH_2Cl_2 (5 mL) and CH_3OH (5 mL). The resin was swollen in CH_2Cl_2 (5 mL), and a solution of the *N*-protected amino acid (1.1 mmol) and HOBt (1.1 mmol) in DMF (4 mL) was added, followed by DCC (1.1 mmol). The mixture was mechanically shaken, and, after 3 h, the resin was filtered and washed three times with the sequence CH_2Cl_2 (5 mL) and CH_3OH (5 mL). Coupling efficacy was determined by means of the Kaiser test. The resin-bound peptide was suspended in a solution of TFA (4.8 mL), H_2O (0.20 mL), and PhOH (0.050 g), in CH_2Cl_2 (5 mL), and mechanically shaken at room temp. After 2 h, the mixture was filtered, the resin was washed with 10% TFA in Et_2O (2 × 5 mL) and Et_2O (2 × 5 mL). Filtrate and washes were collected, and solvent and volatiles were removed under N_2 flow at room temp. The resulting residue was suspended in Et_2O, and the crude solid that precipitated was triturated and collected by using a centrifuge. The crude solid was purified by flash chromatography on silica gel (eluent: EtOAc; column size: 10 cm × 1.5 cm), giving **1** (70–80%). The products were analyzed by RP-HPLC and by MS, and the results are summarized in Table 2.

Table 2. RP-HPLC and ES-MS analyses of **1**.

1	ES-MS [M + 1] found/calcd.	Purity [%][a]	1	ES-MS [M + 1] found/calcd.	Purity [%][a]
a	371.1/371.1	85	**b**	406.1/406.2	87
c	503.2/503.1	91	**d**	477.2/477.1	89
e	491.2/491.1	93	**f**	508.2/508.2	86
g	548.2/548.1	84	**i**	755.3/755.2	90

[a] Determined by analytical RP-HPLC; for conditions, see General Methods.

Solution-Phase Synthesis of Oxd-peptides 2b–g and 2i: DSC (0.45 mmol) was added to a stirred solution of **1** (0.3 mmol) in CH_2Cl_2/DMF (4:1) (3 mL) followed by DIPEA (0.45 mmol) at room temp., under an inert gas. After 1 h, the solvent was removed under reduced pressure, the residue was diluted with 0.1 M HCl (5 mL), and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/EtOAc, 1:1; column size: 15 cm × 1.0 cm) to give **2** (80–95%; purity higher than 95% as determined by analytical RP-HPLC). Under the same reaction conditions and workup protocol, Ts-Pro-Ser-OMe (**1a**) gave Ts-Pro-DHA-OMe (**3a**) (82%; 95% pure by analytical RP-HPLC).

Solid-Phase Synthesis of 2h: Wang resin pre-loaded with Fmoc-Phe (0.5 g, 0.4–0.8 mmol/g, resin particle size: 100–200 mesh) was introduced into a reactor of an automated synthesizer apparatus. Fmoc removal and couplings with the following *N*-protected amino acids were performed as described above; the quantities of the reagents were calculated based on an average resin loading of 0.6 mmol/g. The resin-bound peptide was suspended in CH_2Cl_2/DMF (4:1) (5 mL), and DSC (0.75 mmol) and DIPEA (0.75 mmol) were added at room temp. under an inert gas. After 2 h, the mixture was filtered, and the resin-bound peptide was washed three times in sequence with CH_2Cl_2 (5 mL) and CH_3OH (5 mL). The resin-bound peptide was suspended in a mixture of TFA (1 mL), H_2O (0.33 mL), ethanedithiol (0.33 mL), and PhOH (0.33 mL), in CH_2Cl_2 (8 mL), and mechanically shaken at room temp. After 2 h, the mixture was filtered, the resin was washed with 5% TFA in Et_2O (2 × 5 mL) and Et_2O (2 × 5 mL). The cleavage procedure was repeated, and all of the filtrates and washes were collected; solvent and volatiles were removed under an N_2 flow at room temp. The resulting residue was suspended in Et_2O, and the crude solid that precipitated was triturated and collected by using a centrifuge. The Oxd-peptide acid **2i** was isolated by semipreparative RP-HPLC (see General Methods) (77% based on the average resin loading of 0.6 mmol/g; 96% pure by analytical RP-HPLC).

Removal of the Ts Group: To a suspension of sodium iodide (0.045 g, 0.3 mmol) in CH_3CN (3 mL), TMSCl (0.038 mL, 0.3 mmol) was added, and the mixture was stirred at 0 °C under an inert gas for 10 min. To this stirred suspension, a solution of **2d** (0.10 g, 0.2 mmol) in CH_3CN (3 mL) was added, and the reaction mixture was heated to reflux for 3 h. The solvent was removed under reduced pressure, and the residue was diluted with satd. aqueous Na_2CO_3 (5 mL) and extracted with EtOAc (4 × 5 mL). The collected organic layers were dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by semipreparative RP-HPLC (see General Methods) to afford **4** (0.045 g, 65%; 95% pure by analytical RP-HPLC).

Removal of the Ns Group: To a stirred solution of **2f** (0.11 g, 0.2 mmol) in DMF (4 mL), PhSH (0.026 mL, 0.24 mmol) and K_2CO_3 (0.83 g, 0.6 mmol) were added under an inert gas, and the mixture was stirred at room temp. for 1 h. The solvent was removed under reduced pressure, and compound **4** was isolated as described above (0.063 g, 90%; 96% pure by analytical RP-HPLC).

Ts-Pro-DHA-OMe (3a): IR (nujol): $\tilde{\nu}$ = 3050, 1720, 1690 cm^{-1}. ^{1}H NMR: δ = 1.58–1.80 (m, 3 H, ProHβ, ProHγ), 2.15 (m, 1 H, ProHβ), 2.42 (s, 3 H, $TsCH_3$), 3.20 (m, 1 H, ProHδ), 3.46 (m, 1 H,

ProHδ), 3.85 (s, 3 H, $COOCH_3$), 4.15 (dd, J = 3.2, 8.6 Hz, 1 H, ProHα), 5.91 (s, 1 H, =CH), 6.57 (s, 1 H, =CH), 7.33 (d, J = 8.0 Hz, 2 H, ArH), 7.74 (d, J = 8.0 Hz, 2 H, ArH), 9.15 (br. s, 1 H, NH) ppm. ^{13}C NMR: δ = 19.1, 22.0, 22.4, 40.2, 50.5, 56.8, 105, 125.3, 129.5, 136.3, 138.4, 141.5, 165.3, 171.5 ppm. ES-MS: calcd. for [M + 1] 371.1; found 371.2.

Ts-Oxd-PheNH$_2$ (2b): IR (nujol): $\tilde{\nu}$ = 1777, 1710, 1651 cm^{-1}. ^{1}H NMR: δ = 2.43 (s, 3 H, $TsCH_3$), 3.15–3.28 (m, 2 H, PheHβ), 4.16 (dd, J = 4.2, 8.6 Hz, 1 H, 4-H), 4.53 (t, J = 8.8 Hz, 1 H, 5-H), 4.65 (q, J = 6.8 Hz, 1 H, PheHα), 5.12 (dd, J = 4.2, 8.4 Hz, 1 H, 5-H), 6.82 (br. s, 1 H, $CONH_2$), 7.23 (br. s, 1 H, $CONH_2$), 7.23–7.42 (m, 7 H, ArH), 7.76 (d, J = 8.0 Hz, 2 H, ArH), 8.60 (d, J = 8.0 Hz, 1 H, PheNH) ppm. ^{13}C NMR: δ = 19.9, 36.0, 52.7, 55.9, 65.2, 124.1, 125.7, 127.2, 127.9, 128.2, 128.3, 132.5, 133.0, 135.9, 150.2, 166.4, 171.8 ppm. HRMS (FAB): calcd. for $C_{20}H_{22}N_3O_6S$ [M + H]$^+$ 432.1229; found 432.1232.

Ts-Pro-Oxd-PheNH$_2$ (2c): IR (nujol): $\tilde{\nu}$ = 1773, 1715, 1660 cm^{-1}. ^{1}H NMR: δ = 1.52 (m, 1 H, ProHγ), 1.72 (m, 1 H, ProHγ), 1.82 (m, 1 H, ProHβ), 1.97 (m, 1 H, ProHβ), 2.35 (s, 3 H, $TsCH_3$), 3.00–3.09 (m, 3 H, ProHδ, PheHβ), 3.42 (m, 1 H, ProHδ), 4.22 (dd, J = 3.6, 9.4 Hz, 1 H, 4-H), 4.51 (t, J = 9.2 Hz, 1 H, 5-H), 4.62 (q, J = 7.2 Hz, 1 H, PheHα), 5.02 (dd, J = 3.6, 9.2 Hz, 1 H, 5-H), 5.42 (dd, J = 3.8, 9.0 Hz, 1 H, ProHα), 6.50 (br. s, 1 H, $CONH_2$), 6.73 (br. s, 1 H, $CONH_2$), 7.08–7.22 (m, 5 H, ArH), 7.24 (d, J = 8.4 Hz, 2 H, ArH), 7.62 (d, J = 8.4 Hz, 2 H, ArH), 7.78 (d, J = 8.0 Hz, 1 H, PheNH) ppm. ^{13}C NMR: δ = 17.9, 20.7, 23.1, 38.5, 40.7, 49.0, 51.1, 54.8, 56.4, 126.1, 127.8, 128.5, 129.4, 136.9, 139.1, 143.5, 152.6, 170.2, 173.1, 175.0 ppm. HRMS (FAB): calcd. for $C_{25}H_{29}N_4O_7S$ [M + H]$^+$ 529.1757; found 529.1760.

Ts-Ala-Oxd-PheNH$_2$ (2d): IR (nujol): $\tilde{\nu}$ = 1766, 1722, 1649, 1530 cm^{-1}. ^{1}H NMR: δ = 1.32 (d, J = 7.3 Hz, 3 H, $AlaCH_3$), 2.40 (s, 3 H, $TsCH_3$), 3.00–3.17 (m, 2 H, PheHβ), 4.27 (dd, J = 4.2, 9.3 Hz, 1 H, 4-H), 4.45 (t, J = 9.3 Hz, 1 H, 5-H), 4.64 (q, J = 7.4 Hz, 1 H, PheHα), 4.91 (dd, J = 4.2, 8.6 Hz, 1 H, 5-H), 5.15 (dq, J = 7.4, 9.3 Hz, 1 H, AlaHα), 6.07 (br. s, 1 H, $CONH_2$), 6.39 (br. s, 1 H, $CONH_2$), 6.41 (d, J = 9.3 Hz, 1 H, AlaNH), 7.10–7.27 (m, 7 H, ArH), 7.75 (d, J = 8.4 Hz, 2 H, ArH), 7.98 (d, J = 8.0 Hz, 1 H, PheNH) ppm. ^{13}C NMR: δ = 18.5, 20.8, 39.1, 47.8, 50.7, 54.9, 56.0, 127.1, 127.3, 128.7, 129.4, 129.8, 136.4, 139.5, 143.9, 152.4, 167.9, 173.5, 174.5 ppm. HRMS (FAB): calcd. for $C_{23}H_{27}N_4O_7S$ [M + H]$^+$ 503.1600; found 503.1598.

Ts-Ala-(5-Me-Oxd)-PheNH$_2$ (2e): IR (nujol): $\tilde{\nu}$ = 1776, 1716, 1633 cm^{-1}. ^{1}H NMR: δ = 1.24 (d, J = 7.2 Hz, 3 H, $AlaCH_3$), 1.33 (d, J = 6.0 Hz, 3 H, 5-CH_3), 2.36 (s, 3 H, $TsCH_3$), 3.03 (d, J = 6.4 Hz, 2 H, PheHβ), 4.22 (d, J = 4.8 Hz, 1 H, 4-H), 4.42 (t, J = 5.2 Hz, 1 H, 5-H), 4.55 (q, J = 7.3 Hz, 1 H, PheHα), 4.98 (m, 1 H, AlaHα), 5.70 (br. s, 1 H, $CONH_2$), 5.81 (br. s, 1 H, $CONH_2$), 6.17 (d, J = 7.9 Hz, 1 H, AlaNH), 7.09–7.20 (m, 5 H, ArH), 7.22 (d, J = 7.2 Hz, 2 H, ArH), 7.38 (d, J = 8.0 Hz, 1 H, PheNH), 7.65 (d, J = 7.2 Hz, 2 H, ArH) ppm. ^{13}C NMR: δ = 18.5, 20.3, 21.5, 37.8, 51.0, 54.9, 75.0, 75.9, 127.1, 127.3, 128.7, 129.2, 129.8, 136.3, 136.7, 147.5, 151.8, 167.0, 173.0, 174.7 ppm. HRMS (FAB): calcd. for $C_{24}H_{29}N_4O_7S$ [M + H]$^+$ 517.1757; found 517.1761.

Ns-Ala-Oxd-PheNH$_2$ (2f): IR (nujol): $\tilde{\nu}$ = 1769, 1724, 1648, 1532 cm^{-1}. ^{1}H NMR: δ = 1.32 (d, J = 7.0 Hz, 3 H, $AlaCH_3$), 3.00–3.15 (m, 2 H, PheHβ), 4.20 (dd, J = 4.1, 9.1 Hz, 1 H, 4-H), 4.40 (t, J = 9.1 Hz, 1 H, 5-H), 4.59 (q, J = 7.3 Hz, 1 H, PheHα), 4.81 (dd, J = 4.1, 8.5 Hz, 1 H, 5-H), 5.15 (dq, J = 7.0, 8.8 Hz, 1 H, AlaHα), 6.21 (br. s, 1 H, $CONH_2$), 6.80 (br. s, 1 H, $CONH_2$), 7.10–7.30 (m, 5 H, ArH), 7.37 (d, J = 8.8 Hz, 1 H, AlaNH), 7.79 (d, J = 8.0 Hz, 1 H, PheNH), 7.96 (d, J = 9.0 Hz, 2 H, ArH), 8.22 (d, J = 9.0 Hz, 2 H, ArH) ppm. ^{13}C NMR: δ = 19.6, 37.1, 50.2, 53.9, 55.3, 65.8, 123.7, 126.3, 127.8, 127.9, 128.9, 136.2, 146.4, 149.3, 152.3, 167.1, 172.1, 172.4 ppm. HRMS (FAB): calcd. for $C_{22}H_{24}N_5O_9S$ [M + H]$^+$ 534.1295; found 534.1300.

Ts-Ala-Ala-Oxd-PheNH$_2$ (2g): IR (nujol): $\tilde{\nu}$ = 1783, 1710, 1705, 1653, 1525 cm^{-1}. ^{1}H NMR: δ = 1.20 (d, J = 6.8 Hz, 3 H, $AlaCH_3$), 1.35 (d, J = 7.2 Hz, 3 H, $AlaCH_3$), 2.40 (s, 3 H, $TsCH_3$), 3.03 (dd, J = 6.4, 13.8 Hz, 1 H, PheHβ), 3.08 (dd, J = 6.9, 13.8 Hz, 1 H, PheHβ), 3.78 (quint, J = 7.3 Hz, 1 H, AlaHα), 4.22 (dd, J = 4.3, 9.0 Hz, 1 H, 4-H), 4.43 (t, J = 9.2 Hz, 1 H, 5-H), 4.63 (q, J = 7.1 Hz, 1 H, PheHα), 4.91 (dd, J = 4.3, 8.8 Hz, 1 H, 5-H), 5.28 (quint, J = 6.8 Hz, 1 H, AlaHα), 5.80 (br. s, 1 H, $CONH_2$), 6.50 (br. s, 1 H, $CONH_2$), 6.60 (d, J = 8.4 Hz, 1 H, AlaNH), 7.10–7.27 (m, 8 H, ArH, AlaNH), 7.72 (d, J = 8.0 Hz, 2 H, ArH), 7.81 (d, J = 7.2 Hz, 1 H, PheNH) ppm. ^{13}C NMR: δ = 16.3, 18.0, 20.5, 36.7, 47.1, 51.3, 53.6, 55.2, 64.8, 125.9, 126.1, 127.4, 128.4, 128.7, 135.6, 136.0, 142.6, 151.4, 166.8, 170.6, 171.7, 171.9 ppm. HRMS (FAB): calcd. for $C_{26}H_{32}N_5O_8S$ [M + H]$^+$ 574.1972; found 574.1977.

Ts-Ala-Oxd-Phe-OH (2h): IR (nujol): $\tilde{\nu}$ = 2800–3450, 1766, 1722, 1716, 1649 cm^{-1}. ^{1}H NMR: δ = 1.20 (d, J = 7.2 Hz, 3 H, $AlaCH_3$), 2.41 (s, 3 H, $TsCH_3$), 3.05–3.17 (m, 2 H, PheHβ), 4.27 (dd, J = 4.1, 8.5 Hz, 1 H, 4-H), 4.36 (t, J = 9.2 Hz, 1 H, 5-H), 4.63 (q, J = 7.6 Hz, 1 H, PheHα), 4.92 (dd, J = 4.0, 9.2 Hz, 1 H, 5-H), 5.20 (m, 1 H, AlaHα), 6.30 (d, J = 9.0 Hz, 1 H, AlaNH), 7.10–7.25 (m, 5 H, ArH), 7.30 (d, J = 8.0 Hz, 2 H, ArH), 7.52 (d, J = 7.6 Hz, 1 H, PheNH), 7.73 (d, J = 8.0 Hz, 2 H, ArH), 9.32 (br. s, 1 H, COOH) ppm. ^{13}C NMR: δ = 17.9, 20.5, 38.4, 47.9, 50.1, 54.2, 56.4, 126.8, 127.7, 128.2, 129.1, 130.2, 136.3, 139.2, 144.1, 152.4, 168.0, 173.2, 176.0 ppm. HRMS (FAB): calcd. for $C_{23}H_{26}N_3O_8S$ [M + H]$^+$ 504.1441; found 504.1436.

Ts-Tyr-Oxd-Trp-PheNH$_2$ (2i): IR (nujol): $\tilde{\nu}$ = 1776, 1718, 1675, 1526 cm^{-1}. ^{1}H NMR: δ = 2.38 (s, 3 H, $TsCH_3$), 2.42 (dd, J = 8.0, 14.0 Hz, 1 H, TyrHβ), 2.82 (dd, J = 7.6, 14.2 Hz, 1 H, TrpHβ), 2.99 (dd, J = 6.4, 14.2 Hz, 1 H, TrpHβ), 3.15 (dd, J = 3.0, 14.0 Hz, 1 H, TyrHβ), 3.22 (d, J = 7.6 Hz, 2 H, PheHβ), 4.22 (dd, J = 4.1, 9.2 Hz, 1 H, 4-H), 4.50 (t, J = 9.2 Hz, 1 H, 5-H), 4.57 (q, J = 6.8 Hz, 1 H, TrpHα), 4.92 (q, J = 7.0 Hz, 1 H, PheHα), 5.02 (dd, J = 4.1, 8.8 Hz, 1 H, 5-H), 5.30 (quint, J = 6.8 Hz, 1 H, TyrHα), 5.72 (br. s, 1 H, $CONH_2$), 6.05 (br. s, 1 H, $CONH_2$), 6.75 (d, J = 8.4 Hz, 2 H, TyrArH), 6.95 (d, J = 7.8 Hz, 1 H, TyrNH), 7.02–7.15 (m, 4 H, TrpNH, TrpH-5, TyrArH), 7.19 (t, J = 7.6 Hz, 1 H, TrpH-6), 7.25 (s, 1 H, TrpH-2), 7.30 (d, J = 8.0 Hz, 2 H, ArH), 7.32–7.45 (m, 5 H, PheArH), 7.50 (d, J = 8.0 Hz, 1 H, TrpH-7), 7.61 (s, 1 H, TyrOH), 7.66 (d, J = 7.9 Hz, 1 H, TrpH-4), 7.68 (d, J = 8.0 Hz, 2 H, ArH), 8.55 (d, J = 8.8 Hz, 1 H, PheNH), 9.81 (br. s, 1 H, $TrpH_1$) ppm. ^{13}C NMR: δ = 21.4, 31.9, 36.6, 37.3, 49.3, 52.7, 59.2, 60.1, 71.3, 111.8, 112.3, 116.6, 120.2, 121.0, 123.5, 125.0, 126.2, 127.3, 129.5, 132.1, 132.3, 136.2, 137.6, 140.1, 141.3, 155.7, 156.2, 173.2, 174.0, 174.2, 176.0 ppm. HRMS (FAB): calcd. for $C_{40}H_{41}N_6O_9S$ [M + H]$^+$ 781.2656; found 781.2662.

H-Ala-Oxd-PheNH$_2$ (4): IR (nujol): $\tilde{\nu}$ = 3352, 3445, 1764, 1722, 1645 cm^{-1}. ^{1}H NMR: δ = 1.22 (d, J = 6.8 Hz, 3 H, $AlaCH_3$), 2.96 (dd, J = 9.8, 13.8 Hz, 1 H, PheHβ), 3.12 (dd, J = 4.6, 13.8 Hz, 1 H, PheHβ), 3.30 (q, J = 6.8 Hz, 1 H, AlaHα), 4.08 (dd, J = 4.6, 8.4 Hz, 1 H, 4-H), 4.36 (dd, J = 4.6, 9.2 Hz, 1 H, 5-H), 4.43 (t, J = 8.4 Hz, 1 H, 5-H), 4.50 (m, 1 H, PheHα), 7.10 (br. s, 1 H, $CONH_2$), 7.15 (br. s, 1 H, $CONH_2$), 7.16–7.30 (m, 5 H, ArH), 8.05–8.25 (m, 3 H, PheNH, AlaNH) ppm. ^{13}C NMR: δ = 17.3, 37.9, 47.7, 49.0, 56.1, 63.2, 126.0, 128.7, 129.2, 137.1, 153.4, 172.4, 173.8, 174.0 ppm. HRMS (FAB): calcd. for $C_{16}H_{21}N_4O_5$ [M + H]$^+$ 349.1512; found 349.1509.

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