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Key Analogs of the Tetrapeptide Subunit of RA-VII and Deoxybouvardin

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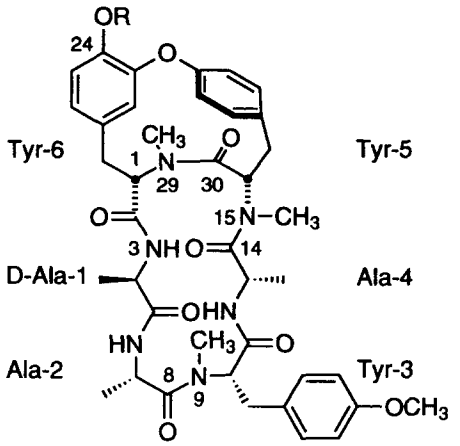
Abstract—The synthesis and evaluation of two key analogs **3** and **4** of the potent antitumor antibiotics deoxybouvardin (**1**) and RA-VII (**2**) which contain fundamental modifications in the tetrapeptide subunit are described. Unlike the natural products, these agents **3** and **4**, which substitute $(Gly)_4$ and $(Gly)_3$ for the D-Ala-Ala-NMe-Tyr(OMe)-Ala tetrapeptide subunit, adopt conformations in which the central amide in the cycloisodityrosine subunit adopts its inherently preferred *trans* stereochemistry and both were found to be biologically inactive.

Introduction

Deoxybouvardin (**1**)[1] and RA-VII (**2**)[2] constitute the initial and representative members of a large class of naturally occurring bicyclic hexapeptides[2–16] which possess potent and efficacious antitumor activity.[17,18] Both bouvardin and RA-VII have been shown to inhibit protein synthesis[17–19] through eukaryotic 80S ribosomal binding[20] with inhibition of both amino acyl *t*-RNA binding and peptidyl *t*-RNA translocation and this is presently thought to be the site of action for the antitumor activity.



1, R = H, deoxybouvardin

2, R = CH_3, RA-VII

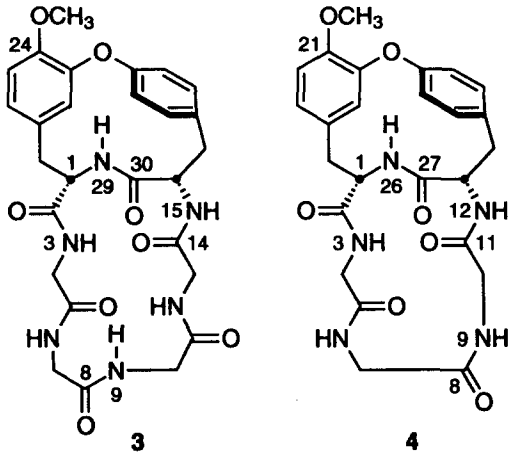
Although the initial examination of the agents led to the logical proposal that the 14-membered cycloisodityrosine-derived ring served the functional role of inducing and maintaining a rigid, normally inaccessible conformation within a biologically active tetrapeptide housed in the 18-membered cyclic hexapeptide,[1] our more recent studies have shown that it is the cycloisodityrosine subunit that constitutes the agent pharmacophore and that it is the tetrapeptide that induces and maintains the normally inaccessible 14-membered ring conformation found in the natural products.[21–36] The systematic examination of all N-desmethyl derivatives of RA-VII revealed that the key element of the tetrapeptide is the N^{15} methyl group which is essential for maintenance of the natural conformational properties of **2**.[21] The N^9 methyl group was found not to be essential and its removal led to adoption of a single, biologically active conformation and the N^{29} methyl group, once thought to be essential to the adoption of the C^{30}-N^{29} *cis* amide was found not to be essential and its removal did not alter the conformational or biological properties of **1** and **2**.[21]

Herein, we report the synthesis and evaluation of two additional key analogs of **1** and **2** which contain fundamental modifications within the tetrapeptide subunit of the agents which serve to reaffirm the conclusions drawn from our recent efforts. These analogs **3** and **4**, which substitute $(Gly)_4$ and $(Gly)_3$ for the D-Ala-Ala-NMe-Tyr(OMe)-Ala tetrapeptide subunit, adopt conformations in which the central amide in the cycloisodityrosine subunit adopts its inherently preferred *trans* stereochemistry and were found to be biologically inactive.

Synthesis of $(Gly)_3$ and $(Gly)_4$ Subunits

BOCNH-Gly-Gly-Gly-OC$_6$F$_5$ (19) and BOCNH-Gly-Gly-Gly-Gly-OC$_6$F$_5$ (21)

The preparation of the agents **3** and **4** requires the tetrapeptide $(Gly)_4$ and tripeptide $(Gly)_3$ subunits **19** and **21**, respectively. Initial efforts to prepare these two subunits focused on linear peptide couplings starting with N-BOC–glycine (**5**) and glycine methyl ester hydrochloride (**6**) and diethyl cyanophosphonate (DECP)[37] was



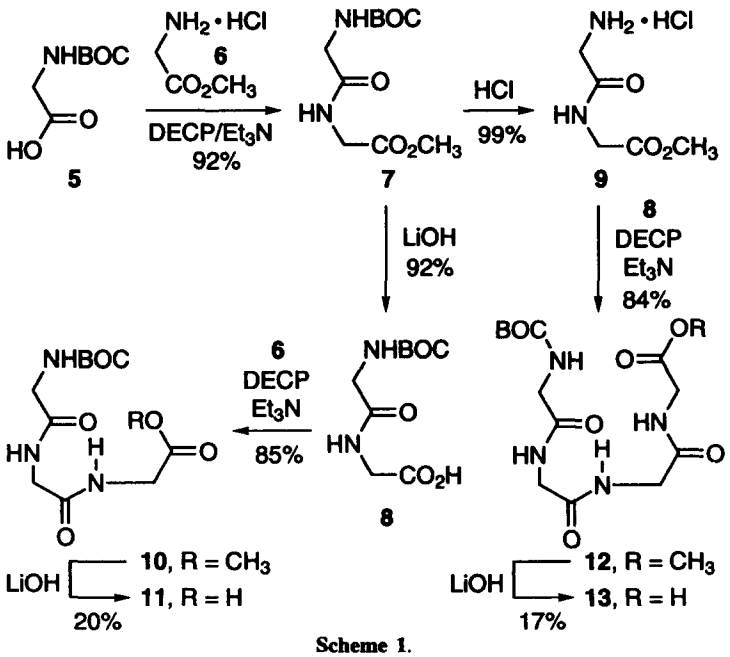
3 4

found to be the best reagent for the glycine couplings. Whereas EDCI (50–60%), DCC (40–50%), EDCI–HOBt (60–70%), and DCC–DMAP (50–60%) provided modest results, DECP gave superb coupling efficiencies (85–95%). Coupling of N-BOC–glycine (**5**) with glycine methyl ester hydrochloride (**6**, 1.5 equiv DECP, 2.5 equiv Et_3N, THF, 25 °C, 4 h, 92%) provided **7**. Methyl ester hydrolysis (4.0 equiv LiOH, THF:CH_3OH:H_2O 3:1:1, 25 °C, 2 h, 92%) of **7** or acid-catalyzed N-BOC deprotection (4.0 M HCl:EtOAc, 25 °C, 30 min, 99%) of **7** provided BOC-Gly-Gly-OH (**8**) and Gly-Gly-OMe hydrochloride (**9**), respectively. Coupling of **8** with **6** and **9** (1.5 equiv DECP, 2.5–3.0 equiv Et_3N, THF, 25 °C, 2 h) provided BOC-Gly-Gly-Gly-OMe (**10**, 85%) and BOC-Gly-Gly-Gly-Gly-OMe (**12**, 84%), respectively. Subsequent saponification of **10** and **12** (2–4 equiv LiOH, THF:CH_3OH:H_2O 3:1:1, 25 °C, 2–4 h) followed by normal work-up with acidic neutralization and organic solvent extraction provided BOC-Gly-Gly-Gly-OH (**11**, 20%) and BOC-Gly-Gly-Gly-Gly-OH (**13**, 17%) in very poor isolation yields. Efforts to improve the conversion by changing the work-up procedures to use ion-exchange resin purification of the crude lithium salt of the carboxylic acid did improve the isolation yield (40–50%) but provided **11** and **13** in a lower state of purity (Scheme 1).

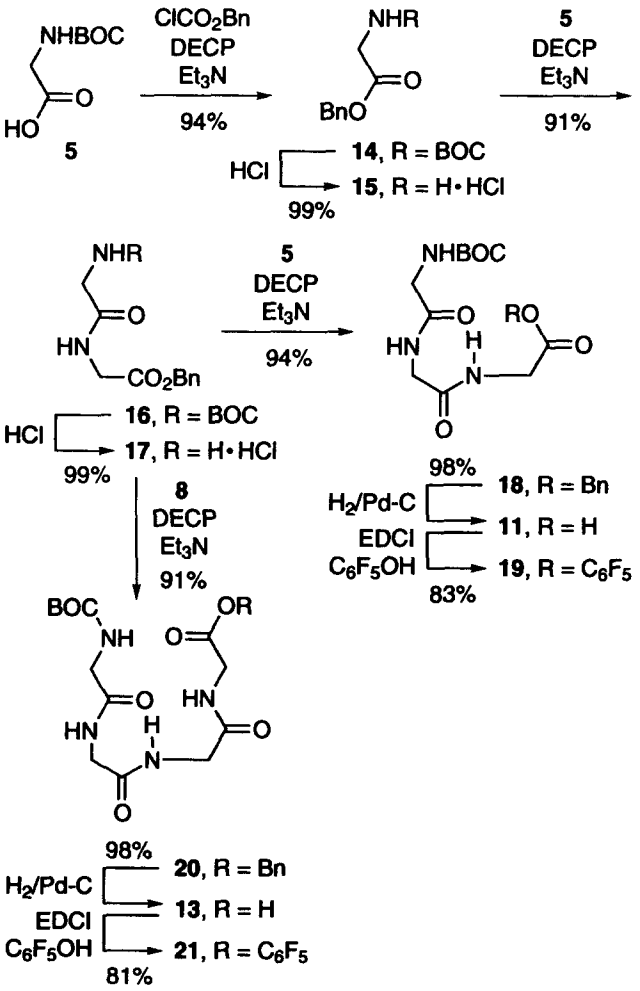
To avoid the difficulties associated with the isolation of **11** and **13** from hydrolytic de-esterifications, the corresponding benzyl esters were prepared with the intention of employing hydrogenolysis deprotection in conventional organic solvents to provide the pure acids **11** and **13** directly in good conversions (Scheme 2). Treatment of N-BOC–glycine (**5**) with benzyl chloroformate (1.0 equiv, 1.1 equiv Et_3N, 0.3 equiv DMAP, CH_2Cl_2, 0 °C, 1 h, 94%)[38] provided N-BOC–glycine benzyl ester (**14**). Acid-catalyzed N-BOC deprotection (4.0 M HCl:EtOAc, 25 °C, 30 min, 99%) followed by coupling of the crude hydrochloride salt **15** with N-BOC–glycine (**5**, 1.0 equiv, 1.5 equiv DECP, 2.5 equiv Et_3N, 25 °C, 4 h, 91%) provided BOC-Gly-Gly-OBn (**16**) in excellent conversion. N-BOC deprotection of **16** (4.0 M HCl:EtOAc, 25 °C, 30 min, 99%) followed by coupling of the resulting Gly-Gly-OBn hydrochloride (**17**) with **5** and **8** under the same reaction conditions (1.5 equiv DECP, 2.5 equiv Et_3N, 25 °C, 4–6 h), respectively, provided BOC-Gly-Gly-Gly-OBn (**18**, 94%) and BOC-Gly-Gly-Gly-Gly-OBn (**20**, 91%). Subsequent hydrogenolysis of **18** and **20** (H_2, 10% Pd-C, CH_3OH, 25 °C, 2 h) provided the corresponding acids **11** and **13** in quantitative yield and in very high purity. Esterification of **11** and **13** (1.1 equiv EDCI, 1.1 equiv C_6F_5OH, CH_2Cl_2, 25 °C, 4 h) provided the pentafluorophenyl activated esters **19** (83%) and **21** (81%), respectively.

Synthesis of 3 and 4

Direct coupling of the 14-membered cycloisodityrosine free amine **22**[36] with BOC-Gly-Gly-Gly-OC_6F_5 (**19**) and BOC-Gly-Gly-Gly-Gly-OC_6F_5 (**21**) under mild condi-



Scheme 1.

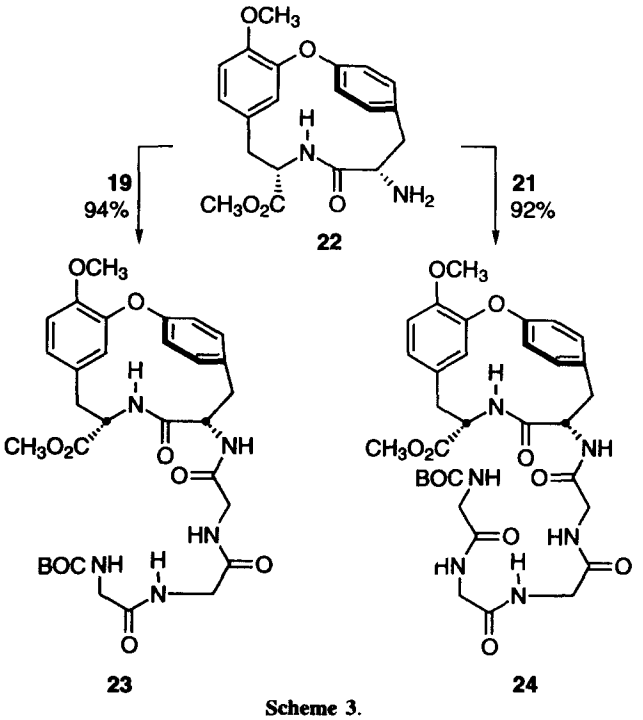


Scheme 2.

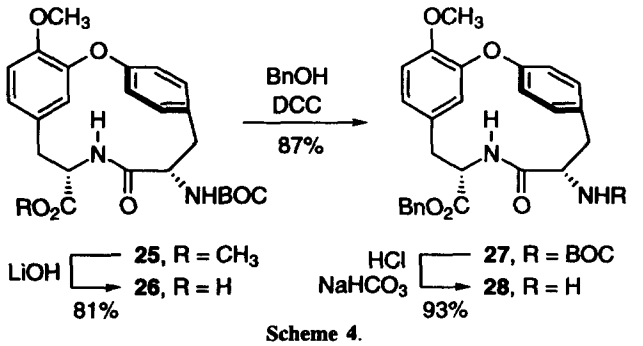
tions (THF, 25 °C, 2–4 h) provided **23** (94%) and **24** (92%), respectively, in excellent conversions (Scheme 3). Subsequent hydrolysis of the hexapeptides **23** and **24** (2.0 equiv LiOH, THF:CH_3OH:H_2O 3:1:1, 25 °C, 4–6 h) and aqueous work-up provided the corresponding acids in low isolation yields (20–30%). Similar to observations made in the synthesis of **11** and **13**, the aqueous work-up required of the saponification proved problematic.

Consequently, the benzyl ester **27** was prepared and incorporated into the synthesis. Saponification of methyl ester of **25**[36] (3.0 equiv LiOH, THF:CH_3OH:H_2O 3:1:1, 25 °C, 6 h, 81%) followed by benzyl ester formation of the resulting acid **26**[39] (1.1 equiv BuOH, 1.1 equiv DCC, 0.3 equiv DMAP, CH_2Cl_2, 0–25 °C, 2 h, 87%) provided **27** in good conversion. Subsequent acid-catalyzed N-BOC deprotection (3.8 M HCl:EtOAc, 25 °C, 30 min, 93%) provided free amine **28** (Scheme 4).

Coupling of **28** with **19** and **21** (THF, 25 °C, 2–4 h) provided **29** (88%) and 32 (93%), respectively. Sequential hydrogenolysis of the benzyl esters **29** and **32** (H_2, 10% Pd-C, CH_3OH, 25 °C 2 h, 96%), acid-catalyzed N-BOC deprotection of the resulting acids **30** and **33** (3.8 M HCl:EtOAc, 25 °C, 50 min, 100%), and subsequent macrocyclization of **31** and **34** upon treatment with diphenyl phosphorazidate[40] (DPPA, 3.0 equiv, 10.0 equiv $NaHCO_3$,[41] 0.002 M DMF, 4 °C, 72 h) provided **3** (83%) and **4** (87%) in excellent conversions (Schemes 5 and 6).



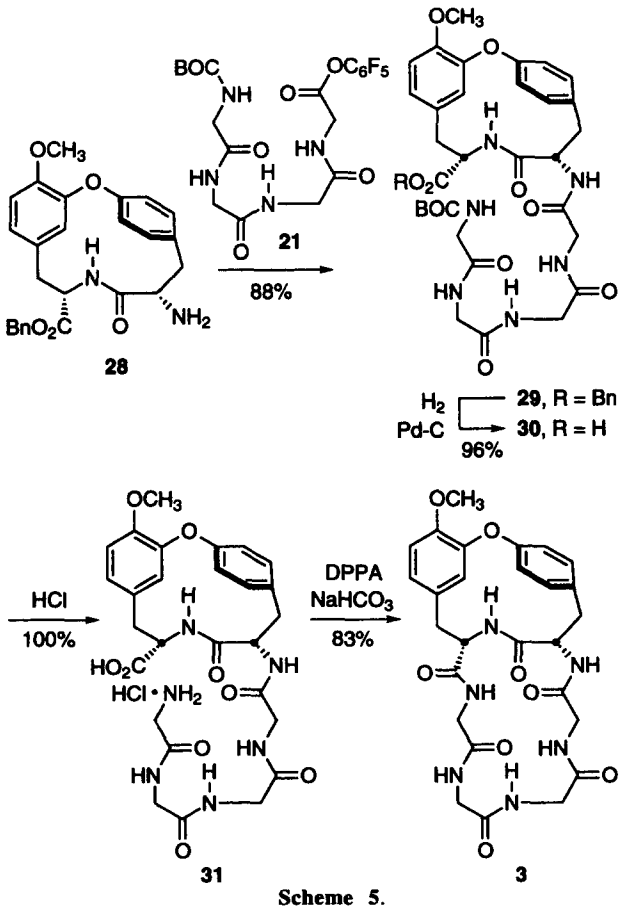
Scheme 3.



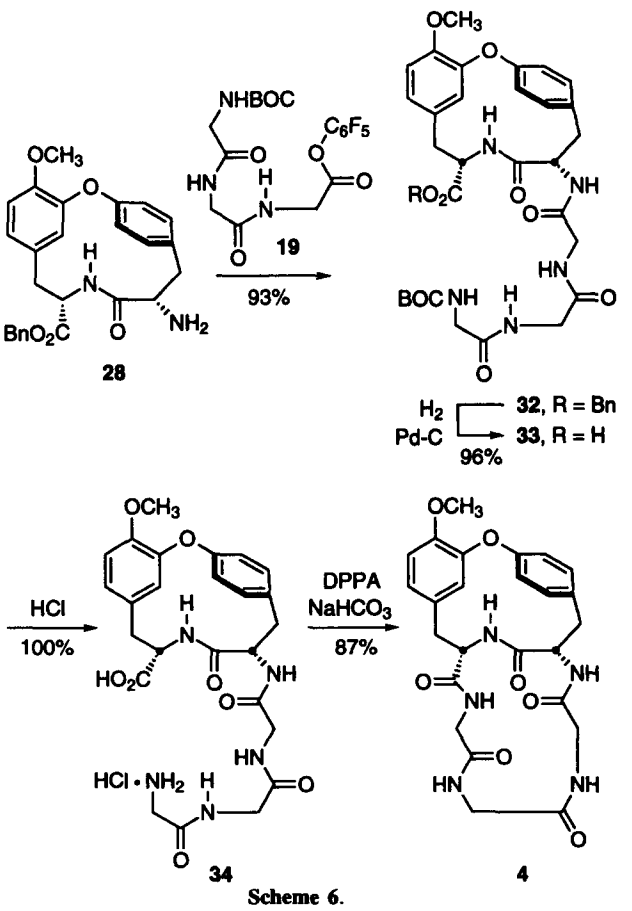
Scheme 4.

Conformational Properties of 3

Compound **3** was subjected to extensive spectroscopic studies including complete ^1H NMR, ^{13}C NMR and 2D ^1H–^1H ROESY NMR in efforts to establish its solution conformational properties. Unlike the naturally occurring bicyclic hexapeptides which adopt a major and a minor solution conformation,[21,22] the ^1H NMR spectrum of **3** revealed a single solution conformation present in all solvents. In contrast to the predominant solution conformation found in deoxybouvardin (**1**) and RA-VII (**2**) which was observed to possess a characteristic N^{29}-C^{30} *cis* amide and corresponds closely to the X-ray structure found for bouvardin,[1] the conformation of **3** was found to lack the diagnostic C^1-H/C^{16}–H NOE crosspeaks in the 2D ^1H–^1H ROESY NMR spectrum and to exhibit a set of C^1-H/N^{29}-H and C^{16}-H/N^{29}–H NOE



Scheme 5.



Scheme 6.

crosspeaks diagnostic of a *trans* C^{30}-N^{29} amide stereochemistry central to a typical type II β-turn structure capped with a Gly^1-NH--O=C-Gly^4 hydrogen bond. As disclosed in our earlier studies,[21,22] this solution conformation of **3** was similar to that of the synthetic N^9,N^{15},N^{29}-desmethyl RA-VII. This solution conformation of **3** was also found to possess a *trans* C^8-N^9 amide central to another typical type II β-turn capped with a Gly^4-NH--O=C-Gly^1 hydrogen bond and to possess C^2-N^3, C^5-N^6, C^{11}-N^{12} and C^{14}-N^{15} *trans* amides. Diagnostic of this conformation, the 2D ^1H–^1H ROESY NMR spectrum exhibited strong C^7-H/N^9-H and C^{10}-H/N^9–H NOEs but no C^7-H/C^{10}–H NOE diagnostic of a *trans* C^8-N^9 amide. Similarly characteristic of the *trans* amide stereochemistry, no crosspeaks between C^1-H/C^4-H, C^4-H/C^7-H, C^{10}-H/C^{13}-H and C^{13}-H/C^{16}-H were observed. The establishment of the two hydrogen bonds in the solution conformation of **3** was derived from the observation of amide NH exchange rates and solvent dependent chemical shift perturbations. These studies revealed that only N^3-H and N^{12}-H were engaged in H-bonding to a comparable extent (δ = 7.94 and 7.58, $t_{1/2}$ exchange = 10 h, DMSO-d_6) while N^6-H, N^9-H, N^{15}-H, and N^{29}-H were fully solvent accessible and not engaged in the intramolecular H-bonding (δ = 8.31, 8.33, 8.51 and 8.66, $t_{1/2}$ exchange ≤ 10 min, DMSO-d_6). An exhaustive conformational search of **3**[42] conducted to locate all accessible *trans* amide conformations followed by further minimization of the individual conformations with imposition of NOE distance constraints (± 15%) derived from the ^1H–^1H ROESY NMR (100 kJ $Å^{-2}$) and fixed amide torsional angles (180 ± 10°, 1000 kJ mol^{-1}) revealed that only two located conformations fit the imposed NOE distance constraints and satisfied the unrestrained hydrogen bonding constraints (Fig. 1). Both conformations were found to match not only the NOE distance constraints, but all other unrestrained experimental results surprisingly well. First, the unrestrained transannular hydrogen bond distances for the Gly^1-NH--O=C-Gly^4 and Gly^4-NH--O=C-Gly^1 are 2.34 and 2.48 Å, or 2.20 and 2.27 Å, respectively, in these conformations. In the first, they cap two typical type II β-turns. In addition, the calculated coupling constants for the six amide protons and their α-protons matched the experimental values well without imposing deliberate restraints (Table 1).

Characteristic of both conformations is the *trans* N^{29}-C^{30} amide central to the cycloisodityrosine subunit constrained to a typical type II β-turn capped by a tight Gly^1-NH--O=C-Gly^4 hydrogen bond. The first of the two conformations bears a second type II β-turn at the N^9-C^8 amide capped by a tight Gly^4-NH--O=C-Gly^1 hydrogen bond and resembles the conformation of the natural products in this region. In the second, the agent adopts a more compact conformation and the second β-turn no longer resembles that found in the natural products. Although we were not able to distinguish between the

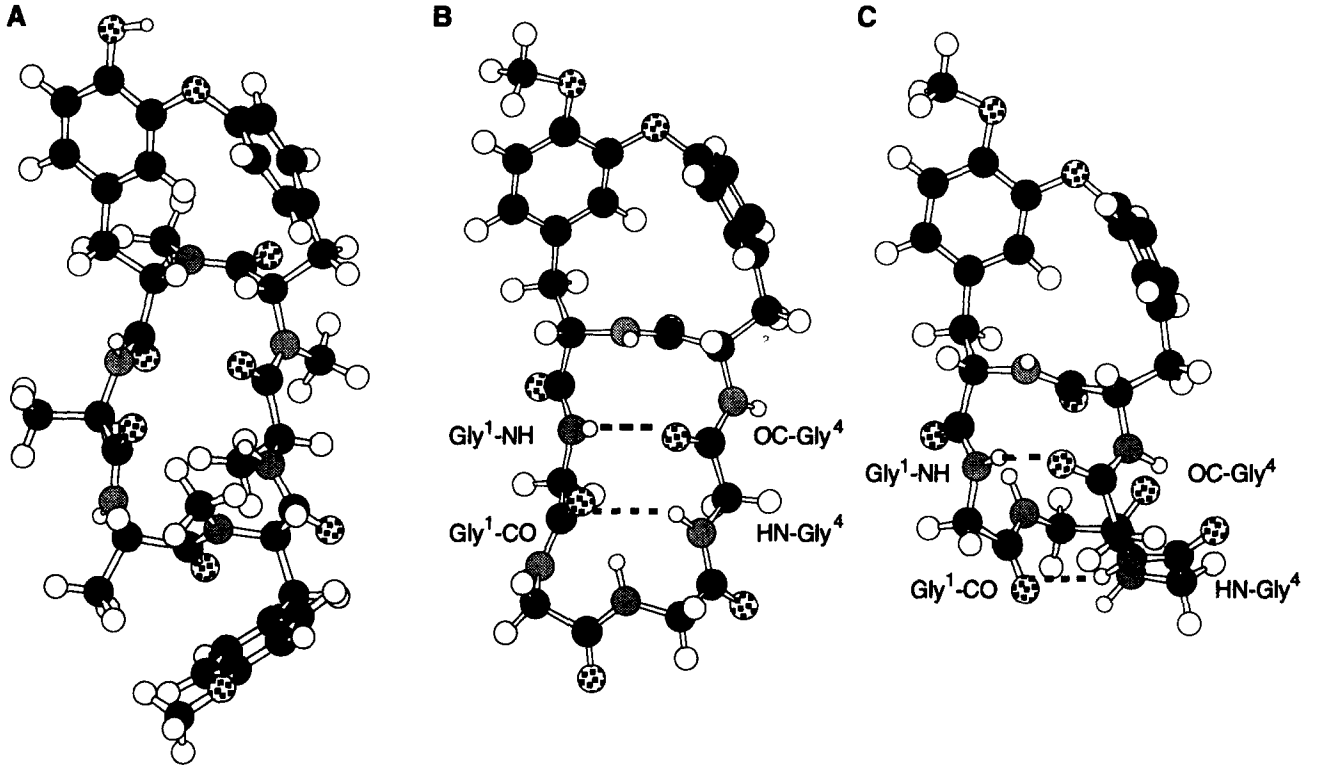


Figure 1. (A) X-Ray crystal structure of deoxybouvardin (1), (B) solution phase conformation of **3** (3a), (C) solution phase conformation of **3** (3b).

Table 1. Comparison of the calculated[a] and observed[b] ^{1}H NMR coupling constants of **3**

	Coupling Constant (J, Hz)		
	Calculated		Observed
	3a	3b	
Gly^1-NH/$Gly^{1\alpha}$-H_1	2.5	5.4	4.8
Gly^1-NH/$Gly^{1\alpha}$-H_2	6.2	6.9	6.0
Gly^2-NH/$Gly^{2\alpha}$-H_1	5.8	5.5	5.8
Gly^2-NH/$Gly^{2\alpha}$-H_2	6.2	6.1	6.2
Gly^3-NH/$Gly^{3\alpha}$-H_1	2.7	5.7	6.0
Gly^3-NH/$Gly^{3\alpha}$-H_2	8.7	6.6	6.0
Gly^4-NH/$Gly^{4\alpha}$-H_1	3.3	5.1	3.8
Gly^4-NH/$Gly^{4\alpha}$-H_2	4.8	7.3	6.4
Tyr^5-NH/$Tyr^{5\alpha}$-H	7.8	6.7	6.6
$Tyr^{5\alpha}$-H/$Tyr^{5\beta}$-H_β	4.5	4.5	5.0
$Tyr^{5\alpha}$-H/$Tyr^{5\beta}$-H_α	11.6	11.6	12.0
Tyr^6-NH/$Tyr^{6\alpha}$-H	6.6	6.3	6.6
$Tyr^{6\alpha}$-H/$Tyr^{6\beta}$-H_β	2.5	2.5	–
$Tyr^{6\alpha}$-H/$Tyr^{6\beta}$-H_α	11.8	11.8	11.8

[a]Taken from the computer generated models (Fig. 1). [b]DMSO-d_6.

two possible conformations, this was readily achieved with the natural products themselves through side chain NOEs which are not possible with **3**. It is likely, though not unambiguous, that **3** behaves similarly and the first of the two conformations, illustrated in Figure 1, constitutes the single solution conformation observed. Importantly, unlike the natural products **1** and **2**, the synthetic agent **3** adopts only a single solution conformation. This solution conformation possesses the inherently preferred C^{30}-N^{29} as well as C^8-N^9 *trans* amides which are central to two typical type II β-turns capped with Gly^1-NH--O=C-Gly^4 and Gly^4-NH--O=C-Gly^1 hydrogen bonds, respectively.

Conformational Properties of 4

Like compound **3**, the agent **4** was found to adopt a single solution conformation in all solvents (e.g. DMSO-d_6, 15% CD_3OD:$CDCl_3$). The 2D ^{1}H–^{1}H ROESY NMR spectrum of **4** exhibited several valuable sets of ^{1}H–^{1}H NOE crosspeaks which helped establish its solution conformation. Similar to compound **3** and diagnostic of a C^{27}-N^{26} *trans* amide central to the 14-membered cycloisodityrosine subunit, strong C^1-H and C^{13}-H/N^{26}–H NOE crosspeaks were observed and no NOE evidence for the *cis* amide (C^1-H/C^{13}–H NOE) was detected in the 2D ^{1}H–^{1}H ROESY NMR spectrum. This *trans* C^{27}-N^{26} amide central to a typical type II β-turn was also maintained by a Gly^1-NH--O=C-Gly^3 hydrogen bond. Characteristic to this hydrogen bond, different amide NH exchange rates and solvent dependent chemical shift perturbations were observed in the studies. The high field chemical shift of N^3-H (δ = 7.83, DMSO-d_6) and longer NH exchange rate ($t_{1/2}$ exchange = 10 h, DMSO-d_6) revealed that the N^3-H was engaged in

intramolecular H-bonding while N^6-H, N^9-H, N^{12}-H, and N^{26}-H were not (δ = 8.28, 8.25, 7.68 and 8.72, $t_{1/2}$ exchange $\leq$ 30 min). In sharp contrast to **3**, which contains an 18-membered tetrapeptide ring, a characteristic *cis* C^8-N^9 amide was observed in the 15-membered tripeptide ring. The C^7-H/C^{10}–H NOE crosspeak in the 2D ^{1}H–^{1}H ROESY NMR spectrum of **4** constitutes the strongest observed NOE. Expectedly absent are NOE crosspeaks between N^9-H and C^7-H that would be present if **4** adopts a *trans* C^8-N^9 amide bond. Similarly distinct was an NOE crosspeak between N^9-H and N^{12}-H and its appearance further served to establish the existence of the *cis* C^8-N^9 amide conformation and the relative peptide backbone orientation in the triglycine subunit. The remaining three amides (C^2-N^3, C^5-N^6 and C^{11}-N^{12}) adopt the inherently favored *trans* stereochemistry since no NOEs were observed between C^1-H/C^4-H, C^4-H/C^7-H and C^{10}-H/C^{13}-H. Consistent with the *trans* C^2-N^3 amide assignment, strong NOE crosspeaks between C^1-H and C^4-H/N^3-H were observed.

The conformational search of **4**[42] with the imposition of the distance (± 15%, 100 kJ Å^{-2}) and torsional angle (100 kJ mol^{-1}) constraints derived from the 2D ^{1}H–^{1}H ROESY NMR was conducted. The amide geometries were fixed at *cis* (0° ± 10° dihedral angle) or *trans* (180° ± 10°) to match the experimentally observed stereochemistry. Consistent with expectations, only one located low energy conformation fitted the imposed NOE distance constraints and satisfied the unrestrained hydrogen bonding constraints (Fig. 2). This lower energy conformation provided calculated coupling constants for the five amide protons and their α protons that match those experimentally observed (Table 2). The unrestrained transannular hydrogen bond distance for Gly^1-NH--O=C-Gly^3 is 2.96 Å in this conformation and caps a typical type II β-turn.

This change within the tetrapeptide subunit of the natural products exerted a profound effect on the solution conformational properties. The C^{27}-N^{26} amide central to the 14-membered cycloisodityrosine subunit adopts its inherently preferred *trans* stereochemistry but the C^8-N^9 amide adopts a disfavored *cis* amide stereochemistry. This disfavored *cis* C^8-N^9 amide stereochemistry most likely is derived from the preferred maintenance of Gly^1-NH--O=C-Gly^3 hydrogen bond stabilizing the typical type II β-turn structure surrounding the central *trans* C^{27}-N^{26} amide.

Cytotoxic Activity

The comparative *in vitro* cytotoxic activity of **1**–**4** is detailed in Table 3. Consistent with prior observations in which the simple removal of the N^{15} methyl group

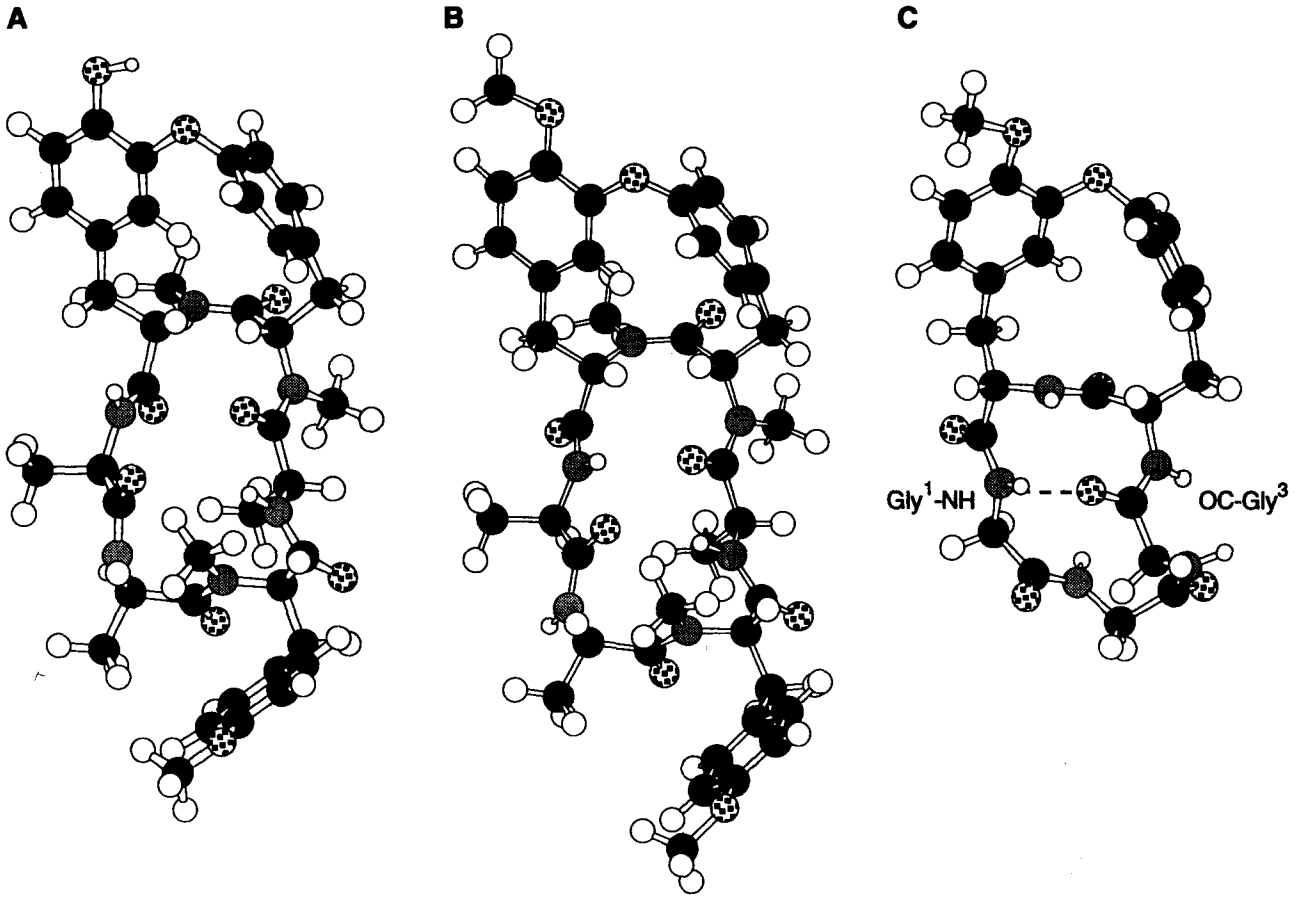


Figure 2. (A) X-Ray crystal structure of deoxybouvardin (**1**), (B) major solution phase conformation of RA-VII (**2**), (C) solution phase conformation of **4**.

Table 2. Comparison of the calculated[a] and observed[b] ^{1}H NMR coupling constants of **4**

	Coupling Constant (J, Hz)	
	Calculated	Observed
Gly^1-NH/$Gly^{1\alpha}$-H_1	5.3	5.5
Gly^1-NH/$Gly^{1\alpha}$-H_2	7.0	5.5
Gly^2-NH/$Gly^{2\alpha}$-H_1	5.0	6.1
Gly^2-NH/$Gly^{2\alpha}$-H_2	7.1	6.1
Gly^3-NH/$Gly^{3\alpha}$-H_1	4.6	5.8
Gly^3-NH/$Gly^{3\alpha}$-H_2	6.3	5.8
Tyr^4-NH/$Tyr^{4\alpha}$-H	8.3	6.8
$Tyr^{4\alpha}$-H/$Tyr^{4\beta}$-H_β	4.5	5.2
$Tyr^{4\alpha}$-H/$Tyr^{4\beta}$-H_α	11.6	12.0
Tyr^5-NH/$Tyr^{5\alpha}$-H	6.9	7.1
$Tyr^{5\alpha}$-H/$Tyr^{5\beta}$-H_β	2.5	–
$Tyr^{5\alpha}$-H/$Tyr^{5\beta}$-H_α	11.8	11.2

[a]Taken from the computer generated model (Fig. 2). [b]DMSO-d_6.

resulted in the adoption of a conformation possessing a *trans* N^{29}-C^{30} amide and in the loss of potent cytotoxic activity, the simplified agent **3** was much less potent than the natural products **1** and **2** (1000–2000 ×) and **4** was inactive (> 10,000 ×).

Table 3. *In vitro* cytotoxic activity

Agent	IC_{50} (L1210, μg mL^{-1})
Deoxybouvardin (**1**)	0.002
RA-VII (**2**)	0.001
3	2
4	>10

Experimental

BOC-Gly-Gly-OMe (7)

A suspension of N-BOC–glycine (**5**, 1.75 g, 10 mmol) and glycine methyl ester hydrochloride (**6**, 1.25 g, 10 mmol, 1.0 equiv) in anhydrous THF (20 mL) was treated with diethyl cyanophosphonate (DECP, 2.45 g, 2.28 mL, 15 mmol, 1.5 equiv) and Et_3N (2.53 g, 3.48 mL, 25 mmol, 2.5 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 4 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 5 × 6 cm, 0–5% CH_3OH:$CHCl_3$ gradient elution) afforded **7** (2.27 g, 2.46 g theoretical, 92%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.83 (*br s*, 1H, CONHCH$_2$), 5.33 (*br s*, 1H, NHBOC), 4.03 (*d*, 2H, J = 5.4 Hz, $CH_2CO_2CH_3$), 3.83 (*d*, 2H, J = 5.0 Hz, CH_2NHBOC), 3.73 (*s*, 3H, CO_2CH_3), 1.44 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.2, 169.9, 156.1, 80.3, 52.4, 44.1, 41.0, 28.3 (3C); IR (neat) ν_{max} 3327, 2978, 1750, 1676, 1529, 1440, 1369, 1251, 1213, 1171, 1050, 1031, 915, 862, 734 cm^{-1}; FABHRMS (NBA) m/z 247.1298 (M^+ + H, $C_{10}H_{18}N_2O_5$ requires 247.1294).

^{1}H NMR ($CDCl_3$, 400 MHz) with irradiation at δ 6.83 (*br s*, CONHCH$_2$) led to the collapse of the signal at δ 4.03 (*d*, $CH_2CO_2CH_3$) to a singlet; irradiation at δ 5.33 (*br s*, NHBOC) led to the collapse of the signal at δ 3.83 (*d*, CH_2NHBOC) to a singlet.

BOC-Gly-Gly-OH (8)

A solution of **7** (1.48 g, 6.0 mmol) in THF:CH_3OH:H_2O (3:1:1, 15 mL) was treated with LiOH·H_2O (1.0 g, 24 mmol, 4.0 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred under Ar at 25 °C for 2 h. The organic solvents were removed under a stream of N_2 and the residue was treated with H_2O (5 mL), EtOAc (10 mL) and 15% aqueous citric acid (pH 3.0). The two layers were separated and the aqueous phase was extracted with EtOAc (4 × 10 mL). The combined EtOAc extracts were washed with H_2O (5 mL) and saturated aqueous NaCl (5 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude acid (1.28 g, 1.39 g theoretical, 92%) was recrystallized from 80% EtOAc:hexane to afford **8** (1.16 g, 1.39 g theoretical, 83%) as a white powder: mp 128–130 °C dec. (white powder, EtOAc:hexane); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.60 (*br s*, 1H, CO_2H), 8.07 (*t*, 1H, J = 5.8 Hz, CONHCH$_2$), 7.00 (*t*, 1H, J = 6.2 Hz, NHBOC), 3.77 (*d*, 2H, J = 5.8 Hz, CH_2CO_2H), 3.57 (*d*, 2H, J = 6.2 Hz, CH_2NHBOC), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3, 169.8, 155.8, 78.1, 43.1, 40.6, 28.3 (3C); IR (KBr) ν_{max} 3363, 2984, 2936, 2544, 1738, 1691, 1623, 1530, 1441, 1413, 1369, 1289, 1224, 1166, 1058, 989, 948, 860, 783, 661 cm^{-1}; FABHRMS (NBA) m/z 233.1140 (M^+ + H, $C_9H_{16}N_2O_5$ requires 233.1137).

Gly-Gly-OMe Hydrochloride (9)

A solution of **7** (200 mg, 0.813 mmol) in 4.0 M HCl:EtOAc (2 mL) was stirred at 0 °C for 10 min and 25 °C for 25 min. The volatiles were removed *in vacuo* and the residue was dried thoroughly under vacuum to afford **9** (147 mg, 148 mg theoretical, 99%) as a white solid, which was used directly in the following reaction without further purification. For **9**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.90 (*t*, 1H, J = 5.8 Hz, CONH), 8.17 (*br s*, 3H, $^+NH_3$), 3.98 (*d*, 2H, J = 5.8 Hz, $CH_2CO_2CH_3$), 3.67 (*s*, 3H, CO_2CH_3), 3.63 (*br s*, 2H, $CH_2{}^+NH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.0, 166.6, 51.9, 40.9, 40.0; IR (KBr) ν_{max} 3346, 2964, 2883, 1743, 1666, 1550, 1464, 1412, 1375, 1230, 1100, 957, 883 cm^{-1}.

BOC-Gly-Gly-Gly-OMe (10)

A suspension of **8** (116 mg, 0.5 mmol) and glycine methyl ester hydrochloride (**6**, 63 mg, 0.5 mmol, 1.0 equiv) in anhydrous THF (4 mL) was treated with DECP (122 mg, 114 μL, 0.75 mmol, 1.5 equiv) and Et_3N (126.3 mg, 174 μL, 1.25 mmol, 2.5 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at

25 °C for 2 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 6 cm, 0–10% CH_3OH:$CHCl_3$ gradient elution) afforded **10** (257 mg, 303 mg theoretical, 85%) as a colorless oil, which solidified upon standing: ^1H NMR (DMSO-d_6, 400 MHz) δ 8.29 (*t*, 1H, *J* = 5.8 Hz, Gly^3-NH), 8.11 (*t*, 1H, *J* = 5.6 Hz, Gly^2-NH), 7.03 (*t*, 1H, *J* = 5.9 Hz, Gly^1-NH), 3.86 (*d*, 2H, *J* = 5.8 Hz, $Gly^{3\alpha}$-H), 3.75 (*d*, 2H, *J* = 5.6 Hz, $Gly^{2\alpha}$-H), 3.64 (*s*, 3H, CO_2CH_3), 3.59 (*d*, 2H, *J* = 5.9 Hz, $Gly^{1\alpha}$-H), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.2, 169.8, 169.5, 155.9, 78.2, 51.8, 43.3, 41.7, 40.6, 28.3 (3C); IR (KBr) ν_{max} 3311, 2979, 2930, 1698, 1650, 1539, 1516, 1454, 1395, 1368, 1211, 1158, 1058, 1031, 978, 944, 873, 760 cm^{-1}; FABHRMS (NBA) m/z 304.1500 (M^+ + H, $C_{12}H_{21}N_3O_6$ requires 304.1509).

^1H NMR (DMSO-d_6, 400 MHz) with irradiation at δ 8.29 (*t*, Gly^3-NH) led to the collapse of the signal at δ 3.86 (*d*, $Gly^{3\alpha}$-H) to a singlet; irradiation at δ 8.11 (*t*, Gly^2-NH) led to the collapse of the signal at δ 3.75 (*d*, $Gly^{2\alpha}$-H) to a singlet; irradiation at δ 7.03 (*t*, Gly^1-NH) led to the collapse of the signal at δ 3.59 (*d*, $Gly^{1\alpha}$-H) to a singlet.

BOC-Gly-Gly-Gly-Gly-OMe (12)

A suspension of **8** (140 mg, 0.6 mmol) and **9** (110 mg, 0.6 mmol, 1.0 equiv) in anhydrous THF (5 mL) was treated with DECP (147 mg, 136 μL, 0.9 mmol, 1.5 equiv) and Et_3N (182 mg, 250 μL, 1.8 mmol, 3.0 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 2 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 8 cm, 0–10% CH_3OH:$CHCl_3$ gradient elution) afforded **12** (181 mg, 216 mg theoretical, 84%) as a white solid: mp 153–155 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.28 (*t*, 1H, *J* = 6.0 Hz, Gly^4-NH), 8.20 (*t*, 1H, *J* = 6.0 Hz, Gly^3-H), 8.06 (*t*, 1H, *J* = 5.7 Hz, Gly^2-NH), 7.03 (*t*, 1H, *J* = 6.0 Hz, Gly^1-NH), 3.86 (*d*, 2H, *J* = 6.0 Hz, $Gly^{4\alpha}$-H), 3.76 (*d*, 2H, *J* = 5.7 Hz, $Gly^{2\alpha}$-H), 3.75 (*d*, 2H, *J* = 6.0 Hz, $Gly^{3\alpha}$-H), 3.64 (*s*, 3H, CO_2CH_3), 3.59 (*d*, 2H, *J* = 6.0 Hz, $Gly^{1\alpha}$-H), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.3, 169.8, 169.4, 169.2, 155.9, 78.2, 51.8, 43.3, 42.1, 41.7, 40.6, 28.3 (3C); IR (KBr) ν_{max} 3296, 3086, 2978, 1654, 1554, 1418, 1373, 1282, 1248, 1178, 1031, 987, 949, 871 cm^{-1}; FAB-HRMS (NBA) m/z 361.1723 (M^+ + H, $C_{14}H_{24}N_4O_7$ requires 361.1723).

^1H NMR (DMSO-d_6, 400 MHz) with irradiation at δ 8.28 (*t*, Gly^4-NH) led to the collapse of the signal at δ 3.86 (*d*, $Gly^{4\alpha}$-H) to a singlet; irradiation at δ 8.20 (*t*, Gly^3-NH) led to the collapse of the signal at δ 3.75 (*d*, $Gly^{3\alpha}$-H) to a singlet; irradiation at δ 8.06 (*t*, Gly^2-NH) led to the collapse of the signal at δ 3.76 (*d*, $Gly^{2\alpha}$-H) to a singlet; irradiation at δ 7.03 (*t*, Gly^1-NH) led to the collapse of the signal at δ 3.59 (*d*, $Gly^{1\alpha}$-H) to a singlet.

N-*[(tert-Butyloxy)carbonyl]-glycine benzyl ester (14)*

A solution of N-BOC–glycine (**5**, 3.50 g, 20 mmol) in anhydrous CH_2Cl_2 (40 mL) was treated with Et_3N (2.23 g, 3.1 mL, 22 mmol, 1.1 equiv) and benzyl chloroformate (3.41 g, 2.86 mL, 20 mmol, 1.0 equiv) at 0 °C under Ar. The resulting reaction mixture was stirred at 0 °C for 5 min before DMAP (733 mg, 6.0 mmol, 0.3 equiv) was slowly added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h before being treated with CH_2Cl_2 (40 mL) and saturated aqueous $NaHCO_3$ (40 mL). The organic phase was washed successively with H_2O (20 mL), 1 N aqueous HCl (2 × 20 mL), H_2O (2 × 20 mL), and saturated aqueous NaCl (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (SiO_2, 5 × 8 cm, 10–30% EtOAc:hexane gradient elution) afforded **14** (4.99 g, 5.30 g theoretical, 94%) as a white solid: mp 66–67 °C (white powder, EtOAc:hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.38 (*m*, 5H, ArH), 5.17 (*s*, 2H, CO_2CH_2Ph), 5.02 (*br s*, 1H, NHBOC), 3.94 (*d*, 2H, *J* = 5.6 Hz, Gly^{α}-H), 1.43 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 155.8, 135.2, 128.6 (2C), 128.5, 128.4 (2C), 80.0, 67.1, 42.5, 28.3 (3C); IR (KBr) ν_{max} 3328, 2981, 2938, 1755, 1685, 1544, 1455, 1407, 1369, 1296, 1250, 1185, 1052, 964, 916, 865, 754,702, 634 cm^{-1}.

Glycine benzyl ester hydrochloride (15)

A solution of **14** (2.65 g, 10.0 mmol) in 4.0 M HCl:EtOAc (10 mL) was stirred at 0 °C for 10 min and 25 °C for 30 min. The volatiles were removed *in vacuo* and the residue was dried thoroughly under vacuum to afford **15** (2.0 g, 2.02 g theoretical, 99%) as a white solid, which was used directly in the following reaction without further purification. For **15**: ^1H NMR (DMSO-d_6, 400 MHz) δ 8.61 (*br s*, 3H, $^+NH_3$), 7.32–7.46 (*m*, 5H, ArH), 5.25 (*s*, 2H, CO_2CH_2Ph), 3.87 (*br s*, 2H, Gly^{α}-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.6, 135.3, 128.5 (2C), 128.4, 128.3 (2C), 66.8, 39.6; IR (KBr) ν_{max} 3446, 2963, 2861, 2675, 2629, 1759, 1586, 1554, 1501, 1405, 1367, 1248, 1141, 1059, 985, 912, 736, 697 cm^{-1}.

BOC-Gly-Gly-OBn (16)

A suspension of N-BOC–glycine (**5**, 700 mg, 4.0 mmol) and glycine benzyl ester hydrochloride (**15**, 800 mg, 4.0 mmol, 1.0 equiv) in anhydrous THF (20 mL) was treated wtih DECP (980 mg, 0.91 mL, 6.0 mmol, 1.5 equiv) and Et_3N (1.01 g, 1.4 mL, 10.0 mmol, 2.5 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 4 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 3 × 8 cm, 0–5% CH_3OH:$CHCl_3$ gradient elution) afforded **16** (1.18 g, 1.29 g theoretical, 91%) as a colorless oil: ^1H NMR ($CDCl_3$, 400 MHz) 7.28–7.33 (*m*, 5H, ArH), 6.89 (*br s*, 1H, $CONHCH_2$), 5.36 (*br s*, 1H, NHBOC), 5.13 (*s*, 2H, CO_2CH_2Ph), 4.04 (*d*, 2H, *J* = 5.4 Hz, $CH_2CO_2CH_2Ph$), 3.81 (*d*, 2H, *J* = 5.3 Hz, CH_2NHBOC), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 169.9, 169.6, 156.0, 135.1, 128.6, 128.5, 128.3, 80.2, 67.2, 44.1, 41.2, 28.3 (3C); IR (neat) ν_{max} 3321, 2982, 2935, 1752, 1716, 1682, 1526, 1456, 1392, 1368, 1283, 1170, 1034, 981, 945, 863, 803, 754, 699 cm^{-1}; FABMS (NBA–NaI) m/z 323 (M^+ + H, $C_{16}H_{22}N_2O_5$).

Gly-Gly-OBn Hydrochloride (17)

A solution of **16** (966 mg, 0.3 mmol) in 4.0 M HCl:EtOAc (5 mL) was stirred at 0 °C for 10 min and 25 °C for 30 min. The volatiles were removed *in vacuo* and the residue was dried thoroughly under vacuum to afford **17** (653 mg, 666 mg theoretical, 98%) as a white solid, which was used directly in the following reaction without further purification. For **17**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.03 (*t*, 1H, *J* = 5.8 Hz, CONH), 8.30 (*br s*, 3H, $^+NH_3$), 7.32–7.40 (*m*, 5H, ArH), 5.17 (*s*, 2H, $COCH_2Ph$), 4.03 (*d*, 2H, *J* = 5.8 Hz, $CH_2CO_2CH_2Ph$), 3.63 (*br s*, 2H, $CH_2^+NH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) 169.4, 166.7, 135.9, 128.5 (2C), 128.2 (2C), 128.1, 66.1, 40.7, 40.0; IR (KBr) ν_{max} 3218, 3075, 2926, 2862, 1734, 1689, 1565, 1522, 1408, 1389, 1362, 1245, 1220, 1098, 1016, 963, 918, 748, 702 cm^{-1}.

BOC-Gly-Gly-Gly-OBn (18)

A suspension of **17** (260 mg, 1.0 mmol) and N-BOC–glycine (**5**, 175 mg, 1.0 mmol, 1.0 equiv) in anhydrous THF (4 mL) was treated with DECP (245 mg, 228 μL, 1.5 mmol, 1.5 equiv) and Et_3N (253 mg, 348 μL, 2.5 mmol, 2.5 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 4 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 8 cm, 0–10% CH_3OH:$CHCl_3$ gradient elution) afforded **18** (354 mg, 379 mg theoretical, 94%) as a colorless oil, which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.53 (*t*, 2H, *J* = 5.8 Hz, Gly^2-NH and Gly^3-NH), 7.25–7.33 (*m*, 5H, ArH), 5.81 (*t*, 1H, *J* = 5.3 Hz, Gly^1-NH), 5.08 (*s*, 2H, CO_2CH_2Ph), 3.98 (*d*, 2H, *J* = 5.8 Hz, Gly$^{3\alpha}$-H), 3.97 (*d*, 2H, *J* = 5.8 Hz, Gly$^{2\alpha}$-H), 3.76 (*d*, 2H, *J* = 5.3 Hz, Gly$^{1\alpha}$-H), 1.36 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 169.9, 169.8, 156.5, 135.2, 128.6 (2C), 128.4, 128.2 (2C), 80.1, 67.0, 44.1, 42.7, 41.1, 28.2 (3C); IR (KBr) ν_{max} 3321, 2979, 2933, 1749, 1666, 1536, 1454, 1368, 1246, 1171, 1031, 947, 861, 750 cm^{-1}; FABHRMS (NBA) m/z 380.1822 (M^+ + H, $C_{18}H_{25}N_3O_6$ requires 380.1822).

BOC-Gly-Gly-Gly-OH (11)

A solution of **18** (160 mg, 0.422 mmol) in anhydrous CH_3OH (5 mL) was treated with 10% Pd-C (24 mg, 15% wt equiv) and stirred under an atmosphere of H_2 (1 atm) at 25 °C for 2 h. The reaction mixture was filtered through Celite (CH_3OH wash), concentrated *in vacuo*, and dried thoroughly under vacuum to afford **11** (120 mg, 122 mg theoretical, 98%) as a white solid: mp 88–90 °C (dec); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.78 (*t*, 1H, *J* = 5.8 Hz, Gly^3-NH), 7.73 (*t*, 1H, *J* = 5.8 Hz, Gly^2-NH), 6.45 (*t*, 1H, *J* = 5.8 Hz, Gly^1-NH), 3.94 (*d*, 4H, *J* = 5.8 Hz, Gly$^{3\alpha}$ and Gly$^{2\alpha}$-H), 3.77 (*d*, 2H, *J* = 5.8 Hz, Gly$^{1\alpha}$-H), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (acetone-d_6, 100 MHz) δ 171.4, 171.2, 170.5, 157.1, 79.6, 44.7, 43.0, 41.4, 28.5 (3C); IR (KBr) ν_{max} 3322, 2981, 2937, 1667, 1540, 1407, 1369, 1252, 1168, 1032, 947, 861, 754, 656 cm^{-1}; FABHRMS (NBA) m/z 290.1364 (M^+ + H, $C_{11}H_{19}N_3O_6$ requires 290.1352).

BOC-Gly-Gly-Gly-OC$_6$F$_5$ (19)

A suspension of **11** (87 mg, 0.3 mmol) in anhydrous CH_2Cl_2 (3 mL) was treated with EDCI (63.5 mg, 0.33 mmol, 1.1 equiv) and C_6F_5OH (61 mg, 0.33 mmol, 1.1 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 4 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 2 × 10 cm, 40–80% EtOAc:hexane gradient elution) afforded **19** (114 mg, 137 mg theoretical, 83%) as a colorless oil, which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61 (*t*, 1H, *J* = 5.8 Hz, Gly^3-NH), 7.30 (*t*, 1H, *J* = 5.8 Hz, Gly^2-NH), 5.52 (*t*, 1H, *J* = 5.6 Hz, Gly^1-NH), 4.36 (*d*, 2H, *J* = 5.8 Hz, Gly$^{3\alpha}$-H), 4.04 (*d*, 2H, *J* = 5.8 Hz, Gly$^{2\alpha}$-H), 3.80 (*d*, 2H, *J* = 5.6 Hz, Gly$^{1\alpha}$-H), 1.41 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 169.9, 166.0, 156.7, 140.9 (2C, *J* = 4.0, 8.0, 21.0 and 253.0 Hz, C2' and C6'), 139.7 (*J* = 8.0, 8.0, 21.0, 21.0 and 253.0 Hz, C4'), 137.9 (2C, *J* = 4.0, 8.0, 21.0, 21.0 and 252.0 Hz, C3' and C5'), 124.5 (*J* = 4.0, 8.0, 8.0 and 21.0 Hz, C1'), 81.0, 44.5, 42.8, 40.5, 28.2 (3C); IR (KBr) ν_{max} 3304, 2999, 2923, 1800, 1720, 1672, 1651, 1524, 1394, 1370, 1252, 1167, 1118, 1051, 1002, 891, 864, 787, 687, 649 cm^{-1}; FABHRMS (NBA) m/z 456.1211 (M^+ + H, $C_{17}H_{18}F_5N_3O_6$ requires 456.1194).

BOC-Gly-Gly-Gly-Gly-OBn (20)

A suspension of **8** (232 mg, 1.0 mmol) and **17** (259 mg, 1.0 mmol, 1.0 equiv) in anhydrous THF (6 mL) was treated with DECP (245 mg, 228 μL, 1.5 mmol, 1.5 equiv) and Et_3N (253 mg, 348 μL, 2.5 mmol, 2.5 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 6 h before the solvent was removed *in vacuo*. Flash chromatography (SiO_2, 3 × 10 cm, 0–10% CH_3OH:$CHCl_3$ gradient elution) afforded **20** (396 mg, 436 mg theoretical, 91%) as a white solid, which was recrystallized from 10% CH_3OH:iPr_2O to afford **20** (341 mg, 436 mg theoretical, 78%) as a white powder. For **20**: mp 174–175 °C (white powder, 10% CH_3OH:iPr_2O); ^{1}H NMR (DMSO-d_6, 400 MHz) 8.33 (*t*, 1H, *J* = 5.9 Hz, Gly^4-NH), 8.21 (*t*, 1H, *J* = 5.7 Hz, Gly^3-NH), 8.06 (*t*, 1H, *J* = 5.7 Hz, Gly^2-NH), 7.33–7.42 (*m*, 5H, ArH), 7.03 (*t*, 1H, *J* = 5.9 Hz, Gly^1-NH), 5.19 (*s*, 2H, CO_2CH_2Ph), 3.92 (*d*, 2H, *J* = 5.9 Hz, Gly$^{4\alpha}$-H), 3.76 (*d*, 4H, *J* = 5.7 Hz, Gly$^{3\alpha}$- and Gly$^{2\alpha}$-H), 3.60 (*d*, 2H, *J* = 5.9 Hz, Gly$^{1\alpha}$-H), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) 169.8, 169.7, 169.4, 169.2, 155.9, 136.0, 128.5 (2C), 128.1, 128.0 (2C), 78.2, 65.9, 43.3, 42.1, 41.8, 40.7, 28.2 (3C); IR (KBr) ν_{max} 3360, 3306, 2980, 2934, 1740, 1690, 1650, 1533, 1426, 1403, 1363, 1247, 1208, 1167, 1028, 981, 867, 726, 696 cm^{-1}; FABHRMS (NBA) m/z 437.2044 (M^+ + H, $C_{20}H_{28}N_4O_7$ requires 437.2036).

BOC-Gly-Gly-Gly-Gly-OH (13)

A solution of **20** (218 mg, 0.5 mmol) in anhydrous CH_3OH (10 mL) was treated with 10% Pd-C (33 mg, 15% wt equiv) and stirred under an atmosphere of H_2 (1 atm) at 25 °C for 2 h. The reaction mixture was filtered through Celite (CH_3OH wash), concentrated *in vacuo*,

and dried thoroughly under vacuum to afford **13** (170 mg, 173 mg theoretical, 98%) as a white solid, which was recrystallized from 10% CH_3OH:iPr_2O to afford **13** (146 mg, 173 mg theoretical, 84%) as a white powder. For **13**: mp 126–128 °C dec. (white powder, 10% CH_3OH:iPr_2O); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.19 (*t*, 1H, *J* = 5.8 Hz, Gly^4-NH), 8.12 (*t*, 1H, *J* = 5.7 Hz, Gly^3-NH), 8.06 (*t*, 1H, *J* = 5.5 Hz, Gly^2-NH), 7.03 (*t*, 1H, *J* = 6.0 Hz, Gly^1-NH), 3.73–3.85 (*m*, 6H, $Gly^{4\alpha}$-, $Gly^{3\alpha}$- and $Gly^{2\alpha}$-H), 3.59 (*d*, 2H, *J* = 6.0 Hz, $Gly^{1\alpha}$-H), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.2, 169.8, 169.2, 169.1, 155.9, 78.2, 43.3, 42.1, 41.8, 40.8, 28.3 (3C); IR (KBr) ν_{max} 3308, 3089, 2982, 2934, 1650, 1552, 1418, 1369, 1281, 1251, 1170, 1030, 950, 892, 866, 789, 689 cm^{-1}; FABHRMS (NBA) m/z 347.1580 (M^+ + H, $C_{13}H_{22}N_4O_7$ requires 347.1567).

BOC-Gly-Gly-Gly-Gly-OC_6F_5 (21)

A suspension of **13** (173 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (5 mL) was treated with EDCI (106 mg, 0.55 mmol, 1.1 equiv) and C_6F_5OH (101 mg, 0.55 mmol, 1.1 equiv) at 25 °C under Ar. The resulting clear reaction solution was stirred at 25 °C for 4 h. The precipitate was collected, washed with anhydrous CH_2Cl_2 (3 × 5 mL), and dried thoroughly under vacuum to afford **21** (208 mg, 256 mg theoretical, 81%) as a white powder, which was sufficiently pure enough to use directly in the following reaction without further purification. For **21**: ^1H NMR (DMSO-d_6, 400 MHz) δ 8.59 (*t*, 1H, *J* = 5.8 Hz, Gly^4-NH), 8.26 (*t*, 1H, *J* = 6.0 Hz, Gly^3-NH), 8.06 (*t*, 1H, *J* = 5.6 Hz, Gly^2-NH), 7.04 (*t*, 1H, *J* = 5.9 Hz, Gly^1-NH), 4.34 (*d*, 2H, *J* = 5.8 Hz, $Gly^{4\alpha}$-H), 3.80 (*d*, 2H, *J* = 6.0 Hz, $Gly^{3\alpha}$-H), 3.77 (*d*, 2H, *J* = 5.6 Hz, $Gly^{2\alpha}$-H), 3.59 (*d*, 2H, *J* = 5.9 Hz, $Gly^{1\alpha}$-H), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.89, 169.85, 169.3, 166.7, 155.9, 140.6 (2C, *J* = 4.0, 8.0, 21.0 and 253.0 Hz, C2' and C6'), 139.3 (*J* = 8.0, 8.0, 21.0, 21.0 and 253.0 Hz, C4'), 137.7 (2C, *J* = 4.0, 8.0, 21.0, 21.0 and 252.0 Hz, C3' and C5'), 125.2 (*J* = 4.0, 8.0, 8.0 and 21.0 Hz, C1'), 78.2, 43.3, 42.1, 41.7, 40.3, 28.2 (3C); IR (KBr) ν_{max} 3307, 3079, 2975, 2931, 1794, 1654, 1522, 1416, 1370, 1286, 1249, 1166, 1123, 995, 944, 903, 867, 702 cm^{-1}; FABHRMS (NBA–NaI) m/z 535.1228 (M^+ + Na, $C_{19}H_{21}F_5N_4O_7$ requires 535.1228).

BOC-Glycyl-glycyl-glycyl-L-tyrosyl-O-methyl-L-tyrosine cyclic $4^4 \rightarrow 5^3$ ether, methyl ester (23)

A solution of **22** (1.8 mg, 0.0049 mmol) in anhydrous THF (0.5 mL) was treated with **19** (2.4 mg, 0.00535 mmol, 1.1 equiv) at 25 °C under Ar before the resulting reaction mixture was stirred at 25 °C for 2 h under Ar. The solvent was removed under a steady stream of N_2. Flash chromatography (SiO_2, 1 × 3 cm, 0–7% CH_3OH:$CHCl_3$ gradient elution) afforded **23** (2.9 mg, 3.1 mg theoretical, 94%) as a white solid: mp > 250 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.46 (*d*, 1H, *J* = 6.4 Hz, Tyr-NH), 8.03 (*d*, 1H, *J* = 8.5 Hz, Tyr-NH), 7.99 (*t*, 1H, *J* = 5.8 Hz, Gly-NH), 7.96 (*t*, 1H, *J* = 5.6 Hz, Gly-NH), 7.69 (*br s*, 1H, Gly-NH), 7.43 (*dd*, 1H, *J* = 2.0, 8.3 Hz, $Tyr^{4\beta a}$-H), 7.13 (*dd*, 1H, *J* = 2.0, 8.3 Hz, $Tyr^{4\beta b}$-H), 7.11 (*dd*, 1H, *J* = 2.0, 8.3 Hz, $Tyr^{4\epsilon a}$-H), 6.90 (*dd*, 1H, *J* = 2.0, 8.3 Hz, $Tyr^{4\epsilon b}$-H), 6.87 (*d*, 1H, *J* = 8.2 Hz, $Tyr^{5\epsilon a}$-H), 6.64 (*dd*, 1H, *J* = 1.9, 8.2 Hz, $Tyr^{5\delta a}$-H), 5.06 (*d*, 1H, *J* = 1.9 Hz, $Tyr^{5\delta b}$-H), 4.49 (*m*, 1H, $Tyr^{4\alpha}$-H), 4.22 (*m*, 1H, $Tyr^{5\alpha}$-H), 3.85 (*s*, 3H, Tyr^5-OCH_3), 3.78–3.81 (*m*, 4H, four Gly^{α}-H), 3.62 (*d*, 2H, *J* = 5.8 Hz, Gly^{α}-H), 3.60 (*s*, 3H, CO_2CH_3), 3.07 (*dd*, 1H, *J* = 5.0, 12.0 Hz, $Tyr^{4\beta}$-H_β), 2.79 (*dd*, 1H, *J* = 8.4, 15.8 Hz, $Tyr^{5\beta}$-H_α), 2.73 (*t*, 1H, *J* = 12.0 Hz, $Tyr^{4\beta}$-H_α), 2.61 (*d*, 1H, *J* = 15.8 Hz, $Tyr^{5\beta}$-H_β), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 3405, 2958, 2930, 1727, 1655, 1518, 1438, 1364, 1263, 1201, 1164, 1130, 1024, 970, 887, 833, 800 cm^{-1}; FABHRMS (NBA) m/z 642.2745 (M^+ + H, $C_{31}H_{39}N_5O_{10}$ requires 642.2775).

BOC-Glycyl-glycyl-glycyl-glycyl-L-tyrosyl-O-methyl-L-tyrosine cyclic $5^4 \rightarrow 6^3$- ether, methyl ester (24)

A solution of **22** (3.7 mg, 0.01 mmol) in anhydrous THF (0.5 mL) was treated with **21** (5.6 mg, 0.01 mmol, 1.1 equiv) at 25 °C under Ar, and the resulting reaction mixture was stirred at 25 °C for 4 h under Ar before the solvent was removed under a steady stream of N_2. Flash chromatography (SiO_2, 1 × 3 cm, 0–10% CH_3OH:$CHCl_3$ gradient elution) afforded **24** (6.4 mg, 7.0 mg theoretical, 92%) as a white solid: mp > 250 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.60 (*d*, 1H, *J* = 6.2 Hz, Tyr-NH), 8.22 (*d*, 1H, *J* = 6.3 Hz, Tyr-NH), 8.20 (*t*, 1H, *J* = 5.8 Hz, Gly-NH), 8.09 (*t*, 1H, *J* = 5.9 Hz, Gly-NH), 8.06 (*t*, 1H, *J* = 5.5 Hz, Gly-NH), 7.42 (*dd*, 1H, *J* = 2.1, 8.3 Hz, $Tyr^{5\delta a}$-H), 7.14 (*dd*, 1H, *J* = 2.1, 8.3 Hz, $Tyr^{5\delta b}$-H), 7.09 (*dd*, 1H, *J* = 2.1, 8.3 Hz, $Tyr^{5\epsilon a}$-H), 7.03 (*t*, 1H, *J* = 5.7 Hz, Gly-NH), 6.92 (*dd*, 1H, *J* = 2.1, 8.3 Hz, $Tyr^{5\epsilon b}$-H), 6.87 (*d*, 1H, *J* = 8.3 Hz, $Tyr^{6\epsilon a}$-H), 6.64 (*dd*, 1H, *J* = 1.8, 8.3 Hz, $Tyr^{6\delta a}$-H), 4.99 (*d*, 1H, *J* = 1.8 Hz, $Tyr^{6\delta b}$-H), 4.48 (*m*, 1H, $Tyr^{5\alpha}$-H), 3.83 (*s*, 3H, Tyr^6-OCH_3), 3.76–3.79 (*m*, 6H, three Gly^{α}-H), 3.65 (*dd*, 1H, *J* = 6.5, 10.3 Hz, $Tyr^{6\alpha}$-H), 3.60 (*d*, 2H, *J* = 5.8 Hz, Gly^{α}-H), 3.59 (*s*, 3H, CO_2CH_3), 3.08 (*dd*, 1H, *J* = 5.3, 12.3 Hz, $Tyr^{5\beta}$-H_β), 2.80 (*dd*, 1H, *J* = 10.7, 16.1 Hz, $Tyr^{6\beta}$-H_α), 2.73 (*t*, 1H, *J* = 12.3 Hz, $Tyr^{5\beta}$-H_α), 2.61 (*d*, 1H, *J* = 16.1 Hz, $Tyr^{6\beta}$-H_β), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 3412, 2927, 2850, 1727, 1656, 1519, 1439, 1368, 1263, 1130, 1024, 975, 887, 800 cm^{-1}; FABHRMS (NBA–CsI) m/z 831.1988 (M^+ + Cs, $C_{33}H_{42}N_6O_{11}$ requires 831.1966).

12(S)-[[N-(tert-Butyloxy)carbonyl]amino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.$1^{3,7}$]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylic acid (26)

A solution of **25**[25] (9.0 mg, 0.019 mmol) in THF: CH_3OH:H_2O (3:1:1, 0.5 mL) was treated with LiOH:H_2O (2.4 mg, 0.057 mmol, 3.0 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 6 h. The organic solvents were removed under a stream of N_2 and the residue was treated with H_2O (1.0 mL), EtOAc (3.0 mL), and 15% aqueous citric acid (pH 3.0). The two layers were separated and the aqueous phase was extracted with EtOAc (3 × 3 mL). The combined EtOAc extracts were washed with H_2O (2.0 mL) and saturated aqueous NaCl (2.0 mL), dried ($MgSO_4$), and

concentrated *in vacuo* to afford **26** (7.0 mg, 8.7 mg theoretical, 81%) as a colorless oil, which solidified as a white solid upon standing: $[\alpha]_D^{25}$ –24 (*c* 0.09, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C18-H), 7.17 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C15-H), 7.07 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C17-H), 6.96 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C16-H), 6.73 (*d*, 1H, *J* = 8.2 Hz, C5-H), 6.55 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C6-H), 6.50 (*br s*, 1H, N10-H), 5.39 (*d*, 1H, *J* = 8.8 Hz, NHBOC), 4.96 (*d*, 1H, *J* = 2.2 Hz, C19-H), 4.13 (*m*, 1H, C9-H), 4.08 (*dd*, 1H, *J* = 7.6, 10.2 Hz, C13-H), 3.92 (*s*, 3H, C4-OCH_3), 3.24 (*dd*, 1H, *J* = 5.0, 12.2 Hz, C13-H_β), 2.93 (*d*, 1H, *J* = 16.6 Hz, C8-H_β), 2.86 (*t*, 1H, *J* = 12.2 Hz, C13-H_α), 2.73 (*dd*, 1H, *J* = 10.8, 16.6 Hz, C8-H_α), 1.42 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8, 172.3, 157.4, 155.6, 152.3, 146.7, 134.1, 132.4, 130.9, 129.9, 125.1, 124.8, 121.4, 114.7, 111.6, 80.9, 58.3, 56.0, 54.5, 38.5, 34.1, 28.3 (3C); IR (KBr) ν_{max} 3422, 2923, 2856, 1718, 1661, 1585, 1517, 1441, 1364, 1284, 1162, 1130, 1029, 977, 890, 832, 803 cm^{-1}; FABHRMS (NBA) m/z 457.1970 (M^+ + H, $C_{24}H_{28}N_2O_7$ requires 457.1975).

Benzyl 12(S)-[[N-(tert-Butyloxy)carbonyl]amino]-4-methoxy-11-oxo-10-aza-2-oxatricyclo[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (27)

A solution of **26** (5.0 mg, 0.011 mmol) in anhydrous CH_2Cl_2 (0.5 mL) was treated with benzyl alcohol (1.3 mg, 1.3 μL, 0.012 mmol, 1.1 equiv), 1,3-dicyclohexylcarbodiimide (DCC, 2.5 mg, 0.012 mmol, 1.1 equiv), and 4-dimethylaminopyridine (DMAP, 0.4 mg, 0.0033 mmol, 0.3 equiv) at 0 °C under Ar. The resulting reaction mixture was stirred at 0 °C for 1 h before being warmed to 25 °C for 2 h. The solvent was removed *in vacuo* and the residue was directly purified by flash chromatography (SiO_2, 1 × 5 cm, 10–30% EtOAc:hexane gradient elution) to afford **27** (5.2 mg, 6.0 mg theoretical, 87%) as a colorless oil, which solidified upon standing: $[\alpha]_D^{25}$ +48 (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C18-H), 7.27–7.37 (*m*, 5H, ArH), 7.22 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C15-H), 7.09 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C17-H), 6.99 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C16-H), 6.74 (*d*, 1H, *J* = 8.3 Hz, C5-H), 6.55 (*dd*, 1H, *J* = 2.2, 8.3 Hz, C6-H), 5.69 (*d*, 1H, *J* = 7.4 Hz, N10-H), 5.15 (*d*, 1H, *J* = 12.4 Hz, CO_2CHH), 5.13 (*d*, 1H, *J* = 7.6 Hz, NHBOC), 5.08 (*d*, 1H, *J* = 12.4 Hz, CO_2CHH), 5.03 (*d*, 1H, *J* = 2.2 Hz, C19-H), 4.10–4.13 (*m*, 2H, C9- and C12-H), 3.93 (*s*, 3H, C4-OCH_3), 3.27 (*dd*, 1H, *J* = 5.1, 12.2 Hz, C13-H_β), 2.87 (*t*, 1H, *J* = 12.2 Hz, C13-H_α), 2.85 (*d*, 1H, *J* = 16.8 Hz, C8-H_β), 2.67 (*dd*, 1H, *J* = 11.0, 16.8 Hz, C8-H_α), 1.44 (*s*, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4, 170.9, 157.2, 156.7, 152.3, 146.7, 135.3, 134.6, 132.5, 130.5, 129.7, 128.5, 128.3 (3C), 128.0 (2C), 125.1, 124.7, 121.3, 114.6, 111.6, 80.3, 67.0, 58.4, 56.1, 54.1, 39.1, 34.3, 28.3 (3C); IR (KBr) ν_{max} 3328, 2928, 2851, 1627, 1577, 1518, 1364, 1311, 1245, 1161, 1130, 1088, 971, 892, 837 cm^{-1}; FABHRMS (NBA–CsI) m/z 679.1449 (M^+ + Cs, $C_{31}H_{34}N_2O_7$ requires 679.1420).

Benzyl 12(S)-amino-4-methoxy-11-oxo-10-aza-2-oxatricyclo-[12.2.2.1^{3,7}]nonadeca-3,5,7(19),14,16,17-hexaen-9(S)-carboxylate (28)

A solution of **27** (7.0 mg, 0.013 mmol) in 3.8 M HCl:EtOAc (0.5 mL) was stirred at 25 °C for 30 min. The volatiles were removed *in vacuo* and the residue was treated with saturated aqueous $NaHCO_3$ (1.5 mL). The resulting aqueous solution was extracted with EtOAc (4 × 4 mL). The combined EtOAc extracts were washed with H_2O (2 mL) and saturated aqueous NaCl (2 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (SiO_2, 1 × 4 cm, 0–8% CH_3OH:$CHCl_3$ gradient elution) afforded **28** (5.3 mg, 5.7 mg theoretical, 93%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.37 (*m*, 6H, ArH and C18-H), 7.19 (*dd*, 1H, *J* = 2.2, 8.3 Hz, C15-H), 7.07 (*dd*, 1H, *J* = 2.2, 8.3 Hz, C17-H), 7.00 (*dd*, 1H, *J* = 2.2, 8.3 Hz, C16-H), 6.74 (*d*, 1H, *J* = 8.2 Hz, C5-H), 6.56 (*dd*, 1H, *J* = 2.2, 8.2 Hz, C6-H), 5.69 (*br s*, 1H, N10-H), 5.18 (*d*, 1H, *J* = 12.2 Hz, CO_2CHH), 5.08 (*d*, 1H, *J* = 12.2 Hz, CO_2CHH), 5.07 (*d*, 1H, *J* = 2.2 Hz, C19-H), 4.20 (*dd*, 1H, *J* = 7.1, 9.8 Hz, C9-H), 3.92 (*s*, 3H, C4-OCH_3), 3.32 (*m*, 1H, C12-H), 3.19 (*d*, 1H, *J* = 12.2 Hz, C13-H_β), 2.88 (*d*, 1H, *J* = 16.6 Hz, C8-H_β), 2.79 (*t*, 1H, *J* = 12.2 Hz, C13-H_α), 2.70 (*dd*, 1H, *J* = 11.0, 16.6 Hz, C8-H_α), 1.81 (*br s*, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.7, 157.1, 152.3, 146.7, 135.2, 132.2, 130.9, 130.3, 129.6, 128.8, 128.6, 128.5, 128.2, 125.1, 124.6, 121.2, 114.8, 111.6, 67.3, 60.2, 56.1, 54.0, 34.2, 29.7; IR (KBr) ν_{max} 3436, 3046, 2954, 1718, 1667, 1590, 1513, 1436, 1415, 1267, 1225, 1205, 1128, 1021, 980, 882, 836, 805, 764 cm^{-1}; FABHRMS (NBA) m/z 447.1920 (M^+ + H, $C_{26}H_{26}N_2O_5$ requires 447.1920).

^1H NMR ($CDCl_3$, 400 MHz) with irradiation at δ 5.69 (*br s*, N10-H) led to the collapse of the signal at δ 4.20 (*dd*, C9-H) to a doublet; irradiation at δ 3.32 (*m*, C12-H) led to the collapse of the signal at δ 2.79 (*t*, C13-H_α) to a doublet; irradiation at δ 3.19 (*d*, C13-H_β) led to the collapse of the signal at δ 2.79 (*t*, C13-H_α) to a doublet; irradiation at δ 2.88 (*d*, C8-H_β) led to the collapse of the signal at δ 2.70 (*dd*, C8-H_α) to a doublet.

BOC-Glycyl-glycyl-glycyl-glycyl-L-tyrosyl-O-methyl-L-tyrosine cyclic 5^4→6^3 ether, benzyl ester (29)

A solution of **28** (4.5 mg, 0.010 mmol) in anhydrous THF (1 mL) was treated with **21** (5.7 mg, 0.011 mmol, 1.1 equiv) at 25 °C under Ar. The resulting reaction mixture was stirred at 25 °C for 4 h under Ar before the solvent was removed under a steady stream of N_2. Flash chromatography (SiO_2, 1 × 3 cm, 0–10% CH_3OH:$CHCl_3$ gradient elution) afforded **29** (6.8 mg, 7.7 mg theoretical, 88%) as a white solid: mp > 250 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.65 (*d*, 1H, *J* = 6.2 Hz, Tyr-NH), 8.28 (*d*, 1H, *J* = 8.6 Hz, Tyr-NH), 8.19 (*t*, 1H, *J* = 5.6 Hz, Gly-NH), 8.06 (*t*, 2H, *J* = 5.6 Hz, two Gly-NH), 7.42 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.32–

7.39 (*m*, 5H, ArH), 7.14 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.10 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 7.03 (*t*, 1H, *J* = 5.8 Hz, Gly-NH), 6.92 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.87 (*d*, 1H, *J* = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.63 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{6\delta a}$-H), 5.09 (*d*, 1H, *J* = 12.8 Hz, CO_2CHH), 5.05 (*d*, 1H, *J* = 12.8 Hz, CO_2CHH), 4.98 (*d*, 1H, *J* = 2.2 Hz, Tyr$^{6\delta b}$-H), 4.49 (*m*, 1H, Tyr$^{5\alpha}$-H), 3.83 (*s*, 3H, $ArOCH_3$), 3.60–3.81 (*m*, 9H, Tyr$^{6\alpha}$ and eight Gly$^{\alpha}$-H), 3.08 (*dd*, 1H, *J* = 4.7, 11.8 Hz, Tyr$^{5\beta}$-H_β), 2.81 (*dd*, 1H, *J* = 2.5, 16.6 Hz, Tyr$^{6\beta}$-H_β), 2.76 (*dd*, 1H, *J* = 10.6, 11.8 Hz, Tyr$^{5\beta}$-H_α), 2.63 (*dd*, 1H, *J* = 12.4, 16.6 Hz, Tyr$^{6\beta}$-H_α), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 3421, 2964, 2933, 1655, 1518, 1441, 1364, 1263, 1226, 1164, 1026, 887, 862, 836, 805 cm^{-1}; FABHRMS (NBA–CsI) m/z 907.2322 (M^+ + Cs, $C_{39}H_{46}N_6O_{11}$ requires 907.2279).

Cyclo(glycyl-glycyl-glycyl-glycyl-L-tyrosyl-O-methyl-L-tyrosyl) cyclic 5^4→6^3 ether (3)

A solution of **29** (6.0 mg, 0.0078 mmol) in anhydrous CH_3OH (2 mL) was treated wtih 10% Pd-C (1.0 mg, 17% wt equiv) and stirred under an atmosphere of H_2 (1 atm) at 25 °C for 2 h. The reaction mixture was filtered through Celite (CH_3OH wash), concentrated *in vacuo*, and dried thoroughly under vacuum to afford **30** (5.1 mg, 5.3 mg theoretical, 96%) as a white solid: mp > 250 °C (dec); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.16 (*br s*, 1H, Tyr-NH), 8.06 (*br s*, 1H, Tyr-NH), 7.79 (*m*, 2H, two Gly-NH), 7.54 (*m*, 2H, two Gly-NH), 7.38 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.11 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\delta b}$-H), 7.08 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 6.89 (*dd*, 1H, *J* = 2.2, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.83 (*d*, 1H, *J* = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.59 (*dd*, 1H, *J* = 2.0, 8.3 Hz, Tyr$^{6\delta a}$-H), 5.15 (*d*, 1H, *J* = 2.0 Hz, Tyr$^{6\delta b}$-H), 4.32 (*m*, 1H, Tyr$^{5\alpha}$-H), 4.12 (*d*, 2H, *J* = 5.0 Hz, Gly$^{\alpha}$-H), 3.82 (*s*, 3H, $ArOCH_3$), 3.75 (*d*, 2H, *J* = 6.4 Hz, Gly$^{\alpha}$-H), 3.72 (*d*, 2H, *J* = 5.6 Hz, Gly$^{\alpha}$-H), 3.70 (*m*, 1H, Tyr$^{6\alpha}$-H), 3.60 (*d*, 2H, *J* = 5.9 Hz, Gly$^{\alpha}$-H), 3.12 (*dd*, 1H, *J* = 5.0, 11.6 Hz, Tyr$^{5\beta}$-H_β), 2.56–2.71 (*m*, 3H, Tyr$^{5\beta}$-H_α and two Tyr$^{6\beta}$-H), 1.38 (*s*, 9H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 3397, 2923, 2851, 1733, 1654, 1538, 1512, 1441, 1262, 1220, 1128, 1026, 887, 805 cm^{-1}; FABHRMS (NBA–CsI) m/z 817.1844 (M^+ + Cs, $C_{32}H_{40}N_6O_{11}$ requires 817.1809).

A solution of **30** (5.0 mg, 0.0073 mmol) in 3.8 M HCl:EtOAc (0.5 mL) was stirred at 0 °C for 10 min and 25 °C for 50 min. The volatiles were removed *in vacuo* and the residue was dried thoroughly under vacuum to afford **31** (4.5 mg, 4.5 mg theoretical, 100%) as a white solid, FABMS (NBA–CsI) m/z 585 (M^+ + H, $C_{27}H_{32}N_6O_9$), which was used directly in the next reaction.

A solution of **31**·HCl (4.5 mg, 0.0073 mmol) in anhydrous DMF (4.0 mL) was cooled to 0 °C and treated with $NaHCO_3$ (6.0 mg, 0.073 mmol, 10.0 equiv) and diphenylphosphoryl azide (DPPA, 6.0 mg, 4.7 μL, 0.022 mmol, 3.0 equiv) under Ar. The resulting reaction mixture was stirred at 4 °C for 72 h before the solvent was removed *in vacuo*. The residue was then treated with H_2O (2.0 mL) and 15% *i*PrOH:$CHCl_3$ (4 mL), and the aqueous layer was extracted with 15% *i*PrOH:$CHCl_3$ (3 × 4 mL). The combined organic extracts were washed with H_2O (2 mL) and saturated aqueous NaCl (2 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography (SiO_2, 0.5 × 8 cm, 0–15% *i*PrOH:$CHCl_3$ gradient elution) afforded **3** (3.4 mg, 4.1 mg theoretical, 83%) as a white solid: mp > 250 °C; $[\alpha]_D^{25}$ +24 (*c* 0.05, 50% CH_3OH:$CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.66 (*d*, 1H, *J* = 6.6 Hz, Tyr^6-NH), 8.51 (*t*, 1H, *J* = 6.0 Hz, Gly^3-NH), 8.33 (*d*, 1H, *J* = 6.6 Hz, Tyr^5-NH), 8.31 (*dd*, 1H, *J* = 5.8, 6.2 Hz, Gly^2-NH), 7.94 (*dd*, 1H, *J* = 4.8, 6.0 Hz, Gly^1-NH), 7.58 (*dd*, 1H, *J* = 3.8, 6.4 Hz, Gly^4-NH), 7.49 (*dd*, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\delta a}$-H), 7.14 (*dd*, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\epsilon a}$-H), 7.12 (*dd*, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\delta b}$-H), 6.91 (*dd*, 1H, *J* = 2.1, 8.3 Hz, Tyr$^{5\epsilon b}$-H), 6.86 (*d*, 1H, *J* = 8.3 Hz, Tyr$^{6\epsilon a}$-H), 6.62 (*dd*, 1H, *J* = 2.0, 8.3 Hz, Tyr$^{6\delta a}$-H), 4.94 (*d*, 1H, *J* = 2.0 Hz, Tyr$^{6\delta b}$-H), 4.24 (*p*, 1H, *J* = 5.8 Hz, Tyr$^{5\alpha}$-H), 3.83 (*s*, 3H, $ArOCH_3$), 3.52–3.94 (*m*, 9H, Tyr$^{6\alpha}$-H and eight Gly$^{\alpha}$-H), 3.19 (*dd*, 1H, *J* = 5.0, 12.0 Hz, Tyr$^{5\beta}$-H_β), 2.80 (*t*, 1H, *J* = 12.0 Hz, Tyr$^{5\beta}$-H_α), 2.76 (*dd*, 1H, *J* = 11.8, 15.2 Hz, Tyr$^{6\beta}$-H_α), 2.64 (*d*, 1H, *J* = 15.2 Hz, Tyr$^{6\beta}$-H_β); ^{1}H NMR (15% CD_3OD:$CDCl_3$, 400 MHz) δ 7.42 (*dd*, 1H, *J* = 2.2, 8.2 Hz, Tyr$^{5\delta a}$-H), 7.18 (*dd*, 1H, *J* = 2.2, 8.2 Hz, Tyr$^{5\delta b}$-H), 7.12 (*dd*, 1H, *J* = 2.2, 8.2 Hz, Tyr$^{5\epsilon a}$-H), 6.93 (*dd*, 1H, *J* = 2.2, 8.2 Hz, Tyr$^{5\epsilon b}$-H), 6.75 (*d*, 1H, *J* = 8.2 Hz, Tyr$^{6\epsilon a}$-H), 6.58 (*dd*, 1H, *J* = 1.8, 8.2 Hz, Tyr$^{6\delta a}$-H), 4.91 (*d*, 1H, *J* = 1.8 Hz, Tyr$^{6\delta b}$-H), 4.27 (*dd*, 1H, *J* = 5.0, 11.6 Hz, Tyr$^{5\alpha}$-H), 3.66–4.06 (*m*, 12H, Tyr$^{6\alpha}$-, Tyr^6-OCH_3 and eight Gly$^{\alpha}$-H), 3.18 (*dd*, 1H, *J* = 5.0, 12.0 Hz, Tyr$^{5\beta}$-H_β), 2.91 (*t*, 1H, *J* = 12.0 Hz, Tyr$^{5\beta}$-H_α), 2.75–2.82 (*m*, 2H, Tyr$^{6\beta}$-H_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.0, 170.4, 170.2, 169.5, 169.0, 168.9, 156.8, 152.1, 146.1, 134.4, 132.6, 131.9, 130.1, 124.6, 124.1, 121.1, 115.0, 112.0, 57.1, 55.8, 43.5, 42.6, 41.8, 36.9, 34.5; IR (KBr) ν_{max} 3313, 2923, 2851, 1613, 1529, 1492, 1467, 1435, 1372, 1328, 1306, 1255, 1199, 1154, 1108, 1048, 874, 802, 770, 747 cm^{-1}; FABHRMS (NBA–CsI) m/z 699.1206 (M^+ + Cs, $C_{27}H_{30}N_6O_8$ requires 699.1179).

^{1}H NMR (15% CD_3OD:$CDCl_3$, 400 MHz) with irradiation at δ 7.42 (*dd*, Tyr$^{5\delta a}$-H) led to the collapse of the signal at δ 7.18 (*dd*, Tyr$^{5\delta b}$-H) to a doublet and to the collapse of the signal at δ 7.12 (*dd*, Tyr$^{5\epsilon a}$-H) to a doublet; irradiation at δ 6.93 (*dd*, Tyr$^{5\epsilon b}$-H) led to the collapse of the signal at δ 7.18 (*dd*, Tyr$^{5\delta b}$-H) to a doublet and to the collapse of the signal at δ 7.12 (*dd*, Tyr$^{5\epsilon a}$-H) to a doublet; irradiation at δ 6.58 (*dd*, Tyr$^{6\delta a}$-H) led to the collapse of the signal at δ 6.75 (*d*, Tyr$^{6\epsilon a}$-H) to a doublet and to the collapse of the signal at δ 4.91 (*d*, Tyr$^{6\delta b}$-H) to a singlet; irradiation at δ 3.18 (*dd*, Tyr$^{5\beta}$-H_β) led to the collapse of the signal at δ 4.27 (*dd*, Tyr$^{5\alpha}$-H) to a doublet and to a change of the signal at δ 2.91 (*t*, Tyr$^{5\beta}$-H_α).

The ^{1}H–^{1}H ROESY NMR spectrum of **3** (DMSO-d_6) displayed diagnostic NOE crosspeaks for Tyr^6-NH/Tyr$^{6\delta b}$-H, Tyr^6-NH/Tyr$^{5\alpha}$-H, Tyr^6-NH/Tyr$^{6\alpha}$-H, Gly^3-NH/Gly^4-NH, Gly^3-NH/Gly$^{2\alpha}$-H, Gly^3-NH/Gly$^{3\alpha}$-H, Gly^2-NH/Gly$^{2\alpha}$-H, Gly^2-NH/Gly$^{1\alpha}$-H, Gly^1-NH/Tyr$^{6\alpha}$-H, Gly^1-NH/

$Gly^{1\alpha}$-H, Gly^{4}-NH/$Gly^{4\alpha}$-H, $Tyr^{5\delta a}$-H/$Tyr^{5\alpha}$-H, $Tyr^{5\delta a}$-H/$Tyr^{5\epsilon a}$-H, $Tyr^{5\delta a}$-H/$Tyr^{5\beta}$-H_β, $Tyr^{5\epsilon a}$-H/$Tyr^{6\delta b}$-H, $Tyr^{5\delta b}$-H/$Tyr^{5\epsilon b}$-H, $Tyr^{5\delta b}$-H/$Tyr^{5\beta}$-H_α, $Tyr^{5\epsilon b}$-H/$Tyr^{6\delta b}$-H, $Tyr^{6\epsilon a}$-H/Tyr^{6}-OCH_3, $Tyr^{6\epsilon a}$-H/$Tyr^{6\delta a}$-H, $Tyr^{6\delta a}$-H/$Tyr^{6\beta}$-H_β, $Tyr^{6\delta b}$-H/$Tyr^{6\alpha}$-H, $Tyr^{5\alpha}$-H/$Tyr^{5\beta}$-H_β, $Tyr^{6\alpha}$-H/$Tyr^{6\beta}$-H_β, $Tyr^{5\beta}$-H_β/$Tyr^{5\beta}$-H_α, $Tyr^{6\beta}$-H_α/$Tyr^{6\beta}$-H_β.

BOC-Glycyl-glycyl-glycyl-L-tyrosyl-O-methyl-L-tyrosine cyclic $4^4 \rightarrow 5^3$ ether, benzyl ester (32)

A solution of **28** (3.8 mg, 0.0085 mmol) in anhydrous THF (0.5 mL) was treated with **19** (4.3 mg, 0.0094 mmol, 1.1 equiv) at 25 °C under Ar before the resulting reaction mixture was stirred at 25 °C for 2 h under Ar. The solvent was removed under a steady stream of N_2. Flash chromatography (SiO_2, 1 × 3 cm, 0–8% CH_3OH:$CHCl_3$ gradient elution) afforded **32** (5.7 mg, 6.1 mg theoretical, 93%) as a white solid: mp > 250 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.66 (*d*, 1H, *J* = 6.2 Hz, Tyr-NH), 8.28 (*d*, 1H, *J* = 8.6 Hz, Tyr-NH), 8.09 (*t*, 1H, *J* = 5.7 Hz, Gly-NH), 8.08 (*t*, 1H, *J* = 5.8 Hz, Gly-NH), 7.42 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta a}$-H), 7.32–7.39 (*m*, 5H, ArH), 7.14 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta b}$-H), 7.10 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon a}$-H), 7.04 (*t*, 1H, *J* = 5.8 Hz, Gly-NH), 6.92 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon b}$-H), 6.87 (*d*, 1H, *J* = 8.3 Hz, $Tyr^{5\epsilon a}$-H), 6.63 (*dd*, 1H, *J* = 1.9, 8.3 Hz, $Tyr^{5\delta a}$-H), 5.09 (*d*, 1H, *J* = 12.6 Hz, CO_2CHH), 5.06 (*d*, 1H, *J* = 12.6 Hz, CO_2CHH), 4.98 (*d*, 1H, *J* = 1.9 Hz, $Tyr^{5\delta b}$-H), 4.50 (*ddd*, 1H, *J* = 5.2, 9.6, 12.8 Hz, $Tyr^{4\alpha}$-H), 3.83 (*s*, 3H, $ArOCH_3$), 3.40–3.87 (*m*, 7H, $Tyr^{5\alpha}$- and six Gly^{α}-H), 3.07 (*dd*, 1H, *J* = 5.0, 12.0 Hz, $Tyr^{4\beta}$-H_β), 2.81 (*dd*, 1H, *J* = 10.8, 16.4 Hz, $Tyr^{5\beta}$-H_α), 2.75 (*t*, 1H, *J* = 12.0 Hz, $Tyr^{4\beta}$-H_α), 2.63 (*d*, 1H, *J* = 16.4 Hz, $Tyr^{5\beta}$-H_β), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$); IR (KBr) ν_{max} 3446, 2918, 2848, 1738, 1650, 1554, 1533, 1458, 1266, 1212, 1161, 1125, 1069, 1023, 875, 839, 808 cm^{-1}; FABHRMS (NBA–CsI) m/z 850.2097 (M^+ + Cs, $C_{37}H_{43}N_5O_{10}$ requires 850.2064).

Cyclo(glycyl-glycyl-glycyl-L-tyrosyl-O-methyl-L-tyrosyl) cyclic $4^4 \rightarrow 5^3$ ether (4)

A solution of **32** (5.0 mg, 0.007 mmol) in anhydrous CH_3OH (2 mL) was treated with 10% Pd-C (1.0 mg, 20% wt equiv) and stirred under an atmosphere of H_2 (1 atm) at 25 °C for 2 h. The reaction mixture was filtered through Celite (CH_3OH wash), concentrated *in vacuo*, and dried thoroughly under vacuum to afford **33** (4.2 mg, 4.4 mg theoretical, 96%) as a white solid, which was used directly in the next reaction. For **33**: mp > 250 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.41 (*br s*, 1H, Tyr-NH), 8.31 (*t*, 1H, *J* = 5.8 Hz, Gly-NH), 8.14 (*m*, 2H, Tyr-NH and Gly-NH), 8.02 (*br s*, 1H, Gly-NH), 7.39 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta a}$-H), 7.12 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta b}$-H), 7.09 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon a}$-H), 6.89 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon b}$-H), 6.83 (*d*, 1H, *J* = 8.3 Hz, $Tyr^{5\epsilon a}$-H), 6.59 (*dd*, 1H, *J* = 2.0, 8.3 Hz, $Tyr^{5\delta a}$-H), 5.06 (*d*, 1H, *J* = 2.0 Hz, $Tyr^{5\delta b}$-H), 4.40 (*m*, 1H, $Tyr^{4\alpha}$-H), 3.82 (*s*, 3H, Tyr^{5}-OCH_3), 3.45–3.87 (*m*, 7H, $Tyr^{5\alpha}$-H and three Gly^{α}-H), 3.08 (*dd*, 1H, *J* = 5.0, 11.8 Hz, $Tyr^{4\beta}$-H_β), 2.65–2.75 (*m*, 3H, $Tyr^{5\beta}$-H_2 and $Tyr^{4\beta}$-H_α), 1.40 (*s*, 9H, $CO_2C(CH_3)_3$.

A solution of **33** (3.8 mg, 0.006 mmol) in 3.8 M HCl:EtOAc (0.5 mL) was stirred at 0 °C for 10 min and 25 °C for 50 min. The volatiles were removed *in vacuo* and the residue was dried thoroughly under vacuum to afford **34** (3.4 mg, 3.4 mg theoretical, 100%) as a white solid, FABHRMS (NBA) m/z 528.2099 (M^+ + H, $C_{25}H_{29}N_5O_8$ requires 528.2094), which was used directly in the next reaction.

A solution of **34** (3.4 mg, 0.006 mmol) in anhydrous DMF (4.0 mL) was cooled to 0 °C and treated with $NaHCO_3$ (5.0 mg, 0.06 mmol, 10.0 equiv) and DPPA (5.0 mg, 4 μL, 0.018 mmol, 3.0 equiv) under Ar. The resulting reaction mixture was stirred at 4 °C for 72 h before the solvent was removed *in vacuo*. The residue was then treated with H_2O (2.0 mL) and 15% *i*PrOH:$CHCl_3$ (4 mL), and the aqueous layer was extracted with 15% *i*PrOH:$CHCl_3$ (3 × 3 mL). The combined organic extracts were washed with H_2O (2.0 mL) and saturated aqueous NaCl (2 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (SiO_2, 0.5 × 8 cm, 0–10% *i*PrOH:$CHCl_3$ gradient elution) afforded **4** (2.6 mg, 3.0 mg theoretical, 87%) as a white solid: mp > 250 °C; $[\alpha]_D^{25}$ –35 (*c* 0.05, 50% CH_3OH:$CHCl_3$); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.72 (*d*, 1H, *J* = 7.1 Hz, Tyr^5-NH), 8.28 (*t*, 1H, *J* = 6.1 Hz, Gly^2-NH), 8.25 (*t*, 1H, *J* = 5.8 Hz, Gly^3-NH), 7.83 (*t*, 1H, *J* = 5.5 Hz, Gly^1-NH), 7.68 (*d*, 1H, *J* = 6.8 Hz, Tyr^4-NH), 7.43 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta a}$-H), 7.12 (*dd*, 2H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta b}$- and $Tyr^{4\epsilon a}$-H), 6.90 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon b}$-H), 6.87 (*d*, 1H, *J* = 8.3 Hz, $Tyr^{5\epsilon a}$-H), 6.64 (*dd*, 1H, *J* = 2.0, 8.3 Hz, $Tyr^{5\delta a}$-H), 5.03 (*d*, 1H, *J* = 2.0 Hz, $Tyr^{5\delta b}$-H), 4.22 (*p*, 1H, *J* = 5.7 Hz, $Tyr^{4\alpha}$-H), 3.83 (*s*, 3H, Tyr^5-OCH_3), 3.54–3.91 (*m*, 7H, $Tyr^{5\alpha}$-H and three Gly^{α}-H), 3.27 (*dd*, 1H, *J* = 5.2, 12.0 Hz, $Tyr^{4\beta}$-H_β), 2.74 (*dd*, 1H, *J* = 11.2, 16.8 Hz, $Tyr^{5\beta}$-H_α), 2.71 (*t*, 1H, *J* = 12.0 Hz, $Tyr^{4\beta}$-H_α), 2.62 (*d*, 1H, *J* = 16.8 Hz, $Tyr^{5\beta}$-H_β); ^1H NMR (15% CD_3OD:$CDCl_3$, 400 MHz) δ 7.44 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta a}$-H), 7.16 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\delta b}$-H), 7.11 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon a}$-H), 6.91 (*dd*, 1H, *J* = 2.2, 8.3 Hz, $Tyr^{4\epsilon b}$-H), 6.76 (*d*, 1H, *J* = 8.2 Hz, $Tyr^{5\epsilon a}$-H), 6.58 (*dd*, 1H, *J* = 1.8, 8.2 Hz, $Tyr^{5\delta a}$-H), 5.05 (*d*, 1H, *J* = $Tyr^{5\delta b}$-H), 4.27 (*dd*, 1H, *J* = 5.2, 11.6 Hz, $Tyr^{4\alpha}$-H), 3.53–4.13 (*m*, 10H, $Tyr^{5\alpha}$-, Tyr^5-OCH_3 and six Gly^{α}-H), 3.28 (*dd*, 1H, *J* = 5.2, 11.6 Hz, $Tyr^{4\beta}$-H_β partially overlapped with the solvent peak), 2.81 (*t*, 1H, *J* = 11.6 Hz, $Tyr^{4\beta}$-H_α), 2.79 (*dd*, 1H, *J* = 10.8, 16.6 Hz, $Tyr^{5\beta}$-H_α), 2.67 (*d*, 1H, *J* = 16.6 Hz, $Tyr^{5\beta}$-H_β); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.7, 170.4, 169.8, 169.7, 169.3, 156.7, 151.9, 146.2, 134.6, 133.0, 131.5, 130.7, 124.5, 124.0, 121.4, 114.9, 112.1, 57.1, 55.8, 54.8, 43.8, 43.0, 42.3, 37.5, 32.8; IR (KBr) ν_{max} 3313, 2923, 2852, 1626, 1610, 1523, 1487, 1467, 1436, 1369, 1328, 1303, 1251, 1200, 1102, 1046 cm^{-1}; FABHRMS (NBA–CsI) m/z 642.0933 (M^+ + Cs, $C_{25}H_{27}N_5O_7$ requires 642.0965).

^1H NMR (15% CD_3OD:$CDCl_3$, 400 MHz) with irradiation at δ 7.44 (*dd*, $Tyr^{4\delta a}$-H) led to the collapse of the signal at δ 7.16 (*dd*, $Tyr^{4\delta b}$-H) to a doublet and to the collapse of the signal at δ 7.11 (*dd*, $Tyr^{4\epsilon a}$-H) to a doublet; irradiation at δ 6.91 (*dd*, $Tyr^{4\epsilon b}$-H) led to the collapse of the signal at δ 7.16 (*dd*, $Tyr^{4\delta b}$-H) to a

doublet and to the collapse of the signal at δ 7.11 (*dd*, $Tyr^{4\alpha a}$-H) to a doublet; irradiation at δ 6.58 (*dd*, $Tyr^{5\delta a}$-H) led to the collapse of the signal at δ 6.76 (*d*, Tyr^{5ea}-H) to a singlet and to the collapse of the signal at δ 5.05 (*d*, $Tyr^{5\delta b}$-H) to a singlet; irradiation at δ 3.28 (*dd*, $Tyr^{4\beta}$-H_β) led to the collapse of the signal at δ 4.27 (*dd*, $Tyr^{4\alpha}$-H) to a doublet and to the collapse of the signal at 2.81 (*t*, $Tyr^{4\beta}$-H_α) to a doublet; irradiation at δ 2.67 (*d*, $Tyr^{5\beta}$-H_β) led to the collapse of the signal at δ 2.79 (*dd*, $Tyr^{5\beta}$-H_α) to a doublet and a change of the signal at δ 3.94 (*m*, $Tyr^{5\alpha}$-H overlapped with Gly^{α}-H).

The ^1H–^1H ROESY NMR spectrum of **4** (DMSO-d_6) displayed diagnostic NOE crosspeaks for Tyr^5-NH/$Tyr^{5\delta b}$-H, Tyr^5-NH/$Tyr^{4\alpha}$-H, Tyr^5-NH/$Tyr^{5\alpha}$-H, Gly^2-NH/$Gly^{2\alpha}$-H, Gly^3-NH/$Gly^{3\alpha}$-H, Gly^3-NH/Tyr^4-NH, Gly^1-NH/$Tyr^{5\alpha}$-H, Gly^1-NH/$Gly^{1\alpha}$-H, Tyr^4-NH/$Tyr^{4\alpha}$-H, $Tyr^{4\delta a}$-H/Tyr^{4ea}-H, $Tyr^{4\delta a}$-H/$Tyr^{5\delta b}$-H, $Tyr^{4\delta a}$-H/$Tyr^{4\alpha}$-H, $Tyr^{4\delta a}$-H/$Tyr^{4\beta}$-H_β, $Tyr^{4\delta b}$-H/Tyr^{4eb}-H, $Tyr^{4\delta b}$-H/$Tyr^{4\beta}$-H_α, Tyr^{4ea}-H/$Tyr^{5\delta b}$-H, Tyr^{4eb}-H/$Tyr^{5\delta b}$-H, Tyr^{5ea}-H/Tyr^5-OCH_3, Tyr^{5ea}-H/$Tyr^{5\delta a}$-H, $Tyr^{5\delta a}$-H/$Tyr^{5\alpha}$-H, $Tyr^{5\delta a}$-H/$Tyr^{5\beta}$-H_β, $Tyr^{5\delta b}$-H/$Tyr^{5\alpha}$-H, $Tyr^{5\delta b}$-H/$Tyr^{5\beta}$-H_α, $Tyr^{4\alpha}$-H/$Tyr^{4\beta}$-H_β, $Gly^{2\alpha}$-H/$Gly^{3\alpha}$-H, $Tyr^{5\alpha}$-H/$Tyr^{5\beta}$-H_β, $Tyr^{4\beta}$-H_β/$Tyr^{4\beta}$-H_α, $Tyr^{5\beta}$-H_α/$Tyr^{5\beta}$-H_β.

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42. Global and close low-lying minima ($\leq$ 5 kcal mol^{-1} for **3** and **4**) were located in conformational searches with use directed Monte Carlo sampling and subsequent minimization of conformations generated by random variations (0–180°) in the available torsional angles[43] excluding those originating in the phenyl rings (Macromodel,[44] version 4.5, OPLSA force field, MCMM = 5000, MCSS = 2, 12 or 5 kcal mol^{-1} window). The global minima for **3** and **4** were located 41 and 16 times, respectively.

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