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

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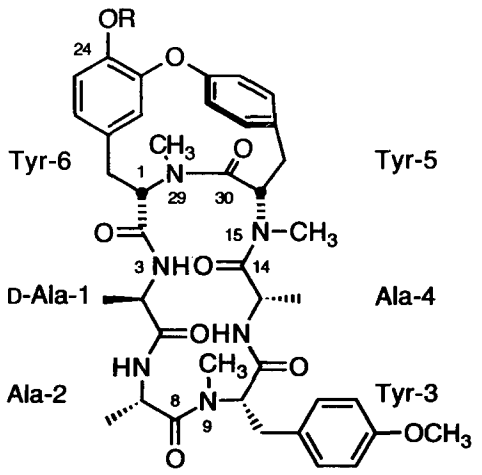
Abstract—The synthesis and evaluation of the analogues **3** and **4** of deoxybouvardin (**1**) and RA-VII (**2**), which contain modifications in the tetrapeptide subunit, are described. Unlike the natural products and similar to our prior disclosure, the agents **3** and **4**, which substitute $(Gly)_4$ and $(Gly)_3$ for the D-Ala-Ala-NMe-Tyr(OMe)-Ala tetrapeptide subunit, exist in single rigid conformations in which the central cycloisodityrosine amide adopts its preferred *trans* stereochemistry and both were found to be biologically inactive. Copyright © 1996 Elsevier Science Ltd

Introduction

Deoxybouvardin (**1**)[1] and RA-VII (**2**)[2] are representative members of a large class of naturally occurring bicyclic hexapeptides[2–16] which possess potent 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 responsible for their antitumor activity.

In recent studies we have detailed the synthesis of a range of analogues of **2** in efforts to define the structural and conformational properties of the natural products contributing to their biological properties.[21–32] Included in these efforts was the disclosure of **3** and **4** which contain fundamental modifications within the tetrapeptide subunit in which $(Gly)_4$ and $(Gly)_3$ were substituted for the D-Ala-Ala-NMe-Tyr(OMe)-Ala tetrapeptide.[32] More recently, we disclosed an improved synthesis of the authentic L,L-cycloisodityrosine derivatives,[33] which resulted in documentation of a remarkably facile epimerization and the reassignment of the stereochemistry of prior cycloisodityrosine intermediates. Herein, we report the resynthesis and evaluation of authentic **3** and **4** employing this authentic (9*S*,12*S*)-cycloisodityrosine and the reassignment of our prior derivatives[32] as **5** and **6**. Analogous to our prior observations and conclusions made with **5** and **6**, **3** and **4** were found to exist in single rigid conformations in which the central amide in the cycloisodityrosine subunit adopts its inherently preferred *trans* stereochemistry and both were found to be biologically inactive.

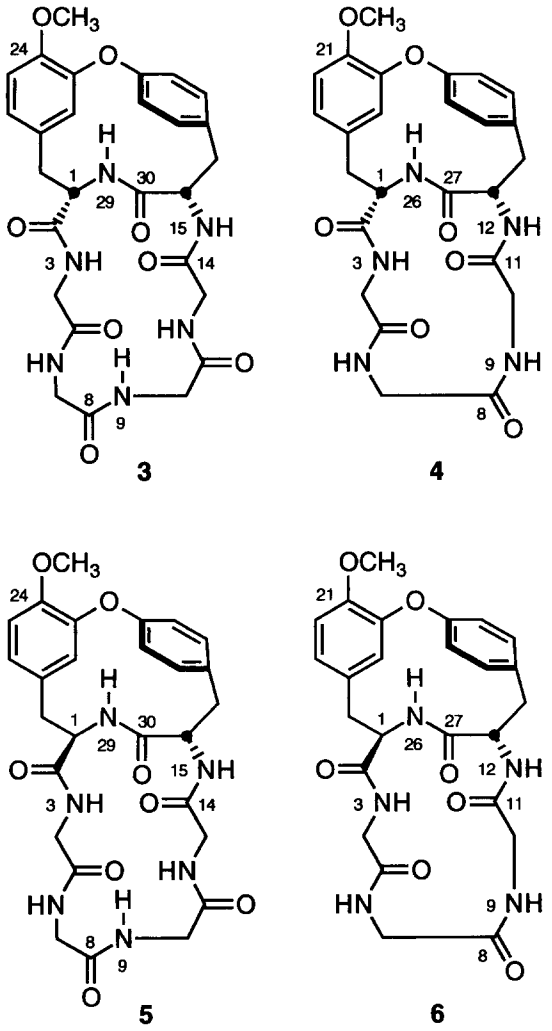


1, R = H, deoxybouvardin
2, R = CH_3, RA-VII

Results

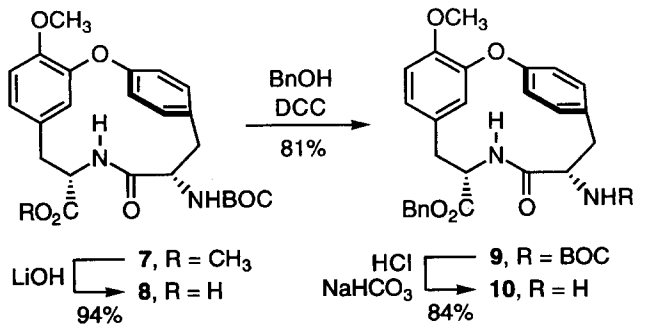
Synthesis of L,L-cycloisodityrosine benzyl ester (10). The benzyl ester of cycloisodityrosine employed in the synthesis of **3** and **4** was prepared from L,L-*N*-BOC-cycloisodityrosine methyl ester (**7**)[33] by saponification (2 equiv $LiOH-H_2O$, 3:1:1 THF:CH_3OH:H_2O, 25 °C, 6 h, 94%) followed by benzyl ester formation (1.2 equiv BnOH, 1.2 equiv DCC, CH_2Cl_2, 0–25 °C, 3 h, 81%). Subsequent acid-catalyzed *N*-BOC deprotection (2.2 N HCl:EtOAc, 25 °C, 0.5 h) and liberation of the free amine (saturated aqueous $NaHCO_3$) provided **10** (84%, Scheme 1).

Synthesis of 3. Coupling of **10** with **11**[32] (THF, 25 °C, 4 h, 90%) followed by sequential hydrogenolysis of the benzyl ester **12** (H_2, 10% Pd–C, CH_3OH, 25 °C, 2 h, 97%), acid-catalyzed *N*-BOC deprotection of the resulting acid **13** (2.2 N HCl–EtOAc, 25 °C, 50–60 min, 100%), and subsequent macrocyclization of **14** upon treatment with DPPA[34] (5.0 equiv, 10 equiv $NaHCO_3$,[35] 0.003 M DMF, 4 °C, 72 h) provided **3** (77% from **13**) in good yield (Scheme 2). Compound **3** proved distinct from that previously disclosed,[32] and the



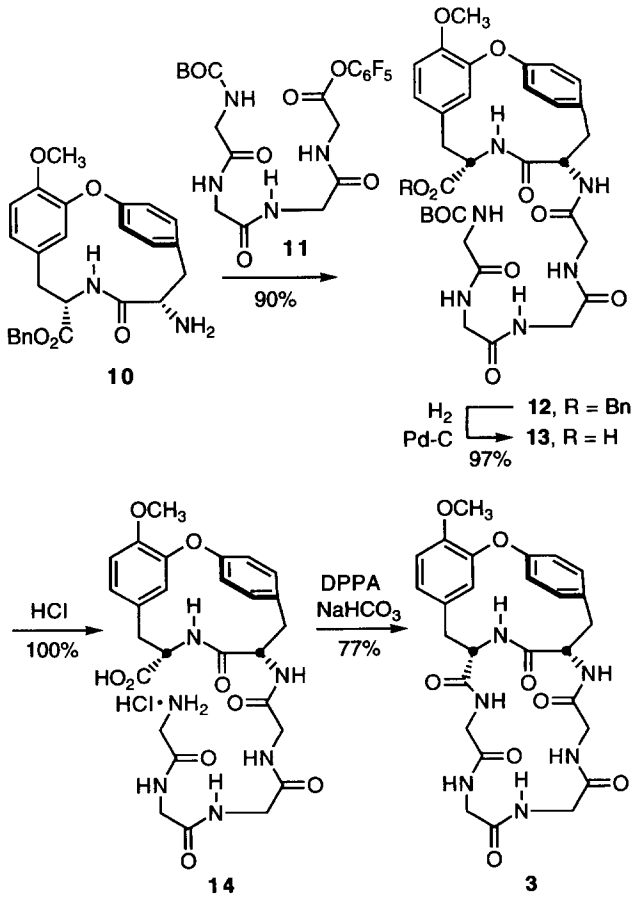
prior sample is necessarily reassigned the structure **5** possessing the (1*R*)-configuration.

Synthesis of 4. Similarly, coupling of **10** with **15**[32] (THF, 25 °C, 2 h, 91%) and sequential benzyl ester hydrogenolysis, acid-catalyzed *N*-BOC deprotection, and subsequent macrocyclization of **18** (5 equiv DPPA,[34] 10 equiv $NaHCO_3$,[35] 0.003 M DMF, 4 °C, 72 h) provided **4** (63%) in good yield (Scheme 3). Compound **4** was found to be distinct from the prior sample[32] which necessitates its reassignment to structure **6** possessing the (1*R*)-configuration.



Scheme 1.

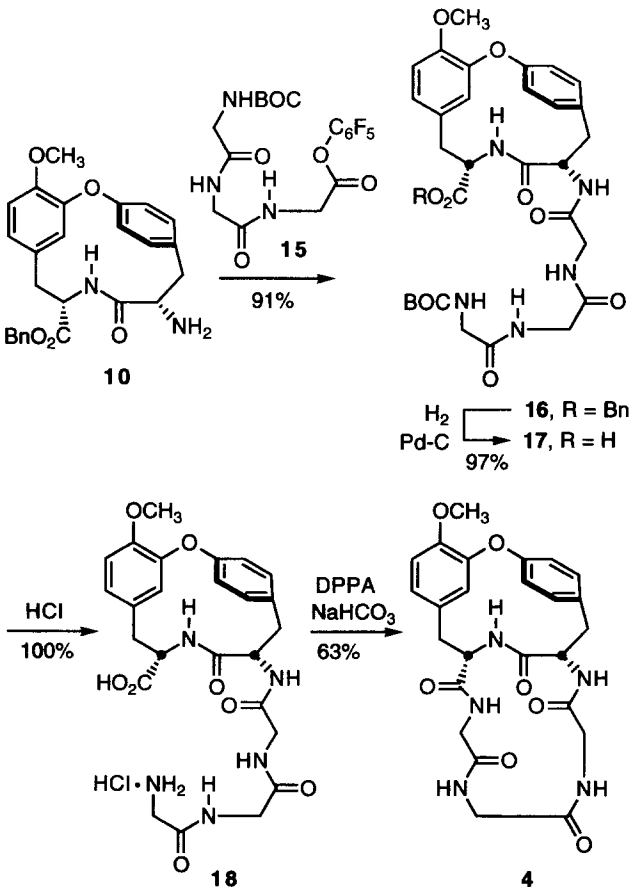
Conformational properties of 3. Compound **3** was subjected to extensive spectroscopic studies including complete ^{1}H NMR, and 2-D ^{1}H-^{1}H ROESY NMR in efforts to establish its solution conformational properties. Unlike the naturally occurring bicyclic hexapeptides which adopt a major and a minor solution conformation, the ^{1}H NMR spectrum of **3** revealed a single solution conformation present in all solvents. In contrast to the predominant solution conformation found in deoxybouvardin and RA-VII which was observed to possess a characteristic N^{29}-C^{30} *cis* amide and corresponds closely to the X-ray structure found for bouvardin, the conformation of **3** was found to lack the diagnostic C^{α}-H/C^{16}-H NOE crosspeak in the 2-D ^{1}H-^{1}H ROESY NMR spectrum and to exhibit a set of C^{α}-H/N^{29}-H and C^{16}-H/N^{29}-H NOE crosspeaks diagnostic of a *trans* C^{30}-N^{29} amide stereochemistry central to a typical type II β-turn capped with a Gly^1-NH--O=C-Gly^4 hydrogen bond. 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, in fact, to possess six *trans* amides. Diagnostic of this conformation, the 2-D ^{1}H-^{1}H 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^{α}-H/C^4-H, C^4-H/C^7-H, C^{10}-H/C^{13}-H and C^{13}-H/C^{16}-H were



Scheme 2.

observed. The establishment of the two hydrogen bonds which cap the two type II β-turns was derived from the observation of amide NH exchange rates and solvent dependent chemical shift perturbations. These studies revealed that the chemical shifts of only N^3-H and N^{12}-H were not perturbed upon changes in solvent and to exhibit the smallest δ in DMSO-d_6 (δ = 7.55 and 7.31), while N^6-H, N^9-H, and N^{15}-H were fully solvent accessible, exhibited the greatest solvent dependent chemical shift perturbations, and were not engaged in the intramolecular hydrogen bonding (DMSO-d_6, δ = 8.48, 8.46, and 8.66, respectively). The N^{29}-H also exhibited an intermediate degree of solvent shielding (DMSO-d_6, δ = 7.76) and may also be engaged in a weak hydrogen bond or simply sterically less accessible to the solvent. Solvent exchange rates for the six amides were conducted in 25% CD_3OD_3–$CHCl_3$ and confirmed these observations. The N^3-H and N^{12}-H exhibited very slow exchange rates ($t_{1/2} \geq 36$ h) consistent with their participation in tight intramolecular hydrogen bonding. In contrast, the N^6-H, N^9-H, and N^{15}-H exchanged rapidly ($t_{1/2} \leq 1$ h) and the N^{29}-H only slightly slower ($t_{1/2} = 2$ h).

An exhaustive conformational search of **3**[35–37] 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 ^{1}H-^{1}H ROESY



Scheme 3.

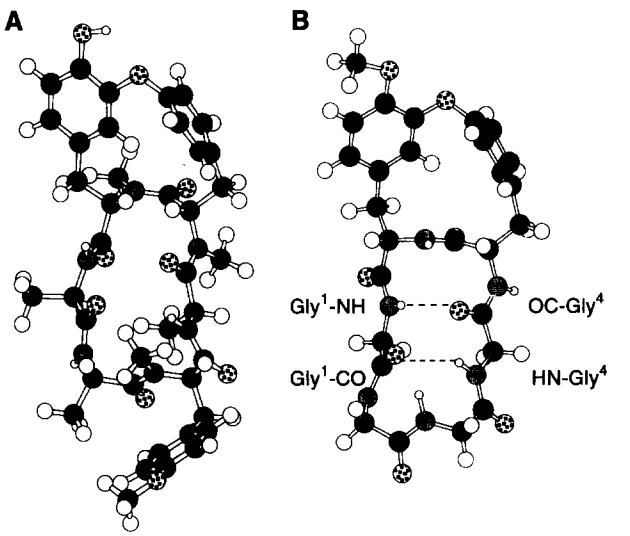


Figure 1. (A) X-ray crystal structure of deoxybouvardin (**1**).[13] (B) Solution phase conformation of **3**.

NMR (100 kJ Å^{-2}) and fixed amide torsional angles (180 ± 10°, 1000 kJ mol^{-1}) revealed that only one located conformation fit the imposed NOE distance constraints and satisfied the unrestricted hydrogen-bonding constraints (Fig. 1). The hydrogen-bond distances for Gly^1-NH--O=C-Gly^4 and Gly^4-NH--O=C-Gly^1 were 2.34 and 2.48 Å, respectively. 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 this conformation 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. It also 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

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

	Coupling constant (*J*, Hz)	
	Calculated	Observed
Gly^1-NH/Gly$^{1\alpha}$-H$_1$	2.5	1.6
Gly^1-NH/Gly$^{1\alpha}$-H$_2$	6.2	8.5
Gly^2-NH/Gly$^{2\alpha}$-H$_1$	5.8	5.6
Gly^2-NH/Gly$^{2\alpha}$-H$_2$	6.2	6.4
Gly^3-NH/Gly$^{3\alpha}$-H$_1$	2.7	5.0
Gly^3-NH/Gly$^{3\alpha}$-H$_2$	8.7	6.8
Gly^4-NH/Gly$^{4\alpha}$-H$_1$	3.3	2.6
Gly^4-NH/Gly$^{4\alpha}$-H$_2$	4.8	6.4
Tyr^5-NH/Tyr$^{5\alpha}$-H	7.8	4.2
Tyr$^{5\alpha}$-H/Tyr$^{5\beta}$-H$_\beta$	4.5	4.2
Tyr$^{5\alpha}$-H/Tyr$^{5\beta}$-H$_\gamma$	11.6	13.9
Tyr^6-NH/Tyr$^{6\alpha}$-H	6.6	7.4
Tyr$^{6\alpha}$-H/Tyr$^{6\beta}$-H$_\beta$	2.5	—
Tyr$^{6\alpha}$-H/Tyr$^{6\beta}$-H$_\gamma$	11.8	11.0

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

Table 2. Cytotoxic activity

Agent	IC_{50} (L1210, μg/mL
1, deoxybouvardin	0.002
2, RA-VII	0.001
3	>10
4	>10
5	2
6	>10

resembles the predominant conformation of the natural products in this region. Unlike the natural products, **3** adopts only a single solution conformation which 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 hydrogen bonds.

Cytotoxic activity. The in vitro cytotoxic activity of **1–6** is summarized in Table 2. Consistent with prior observations in which agents that adopt a *trans* N^{29}-C^{30} amide were found to be biologically inactive, the agents **3** and **4**, like **5** and **6**, exhibited no cytotoxic activity and were >10,000× less potent than **1** and **2**.

Experimental

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 (8). A solution of (9*S*,12*S*)-7[33] (24 mg, 0.05 mmol) in THF:CH_3OH:H_2O (3:1:1, 2 mL) was treated with LiOH–H_2O (4.3 mg, 0.10 mmol, 2.0 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 steady stream of N_2 and the residue was treated with H_2O (2 mL), EtOAc (5 mL) and 10% aq HCl (pH 3.0). The two layers were separated, and the aq phase was extracted with EtOAc (4×5 mL). The combined EtOAc extracts were washed with H_2O (2 mL) and satd aqueous NaCl (2 mL), dried ($MgSO_4$), and concentrated in vacuo to afford **8** (21.9 mg, 23.3 mg theoretical, 94%) as a white solid, which was directly used in the following reaction without further purification: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25–7.30 (m, 2H, C15- and C18-H), 7.14 (dd, 1H, *J*=2.0, 8.2 Hz, C16- or C17-H), 7.04 (dd, 1H, *J*=2.0, 8.2 Hz, C17- or C16-H), 6.73 (d, 1H, *J*=8.2 Hz, C5-H), 6.56 (dd, 1H, *J*=2.0, 8.2 Hz, C6-H), 6.01 (br s, 1H, N10-H), 5.10 (br s, 1H, NHBOC), 4.90 (d, 1H, *J*=2.0 Hz, C19-H), 4.56 (m, 1H, C12-H), 4.10 (m, 1H, C9-H), 3.93 (s, 3H, $ArOCH_3$), 3.48 (dd, 1H, *J*=4.6, 13.6 Hz, C13-Hβ), 2.88–2.96 (m, 2H, C13-Hα and C8-Hβ), 2.74 (dd, 1H, *J*=7.3, 16.2 Hz, C8-Hα), 1.47 (s, 9H, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3313, 2954, 2862, 1759, 1707, 1662, 1585, 1497, 1441, 1364, 1226, 1200, 1164, 1092, 1046, 908, 867, 774, 728 cm^{-1}; FABHRMS (NBA–CsI): *m/e* 589.0965 (M^+ + Cs, $C_{24}H_{28}N_2O_7$ requires 589.0951).

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 (9). A solution of **8** (8.0 mg, 0.018 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was treated with benzyl alcohol (2.3 mg, 2.2 μL, 0.021 mmol, 1.2 equiv), DCC (4.4 mg, 0.021 mmol, 1.2 equiv), and DMAP (1.1 mg, 0.0088 mmol, 0.5 equiv) at 0 °C under Ar. The resulting reaction mixture was stirred at 0 °C for 1 h and 25 °C for 2 h before the solvent was removed in vacuo. Chromatography (SiO_2, 1×5 cm, 10–30% EtOAc–hexane gradient elution) afforded **9** (7.7 mg, 9.6 mg theoretical, 81%) as a colorless oil, which solidified upon standing: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25–7.36 (m, 7H, C15- and C18-H and ArH), 7.05 (m, 2H, C16- and C17-H), 6.75 (d, 1H, *J*=8.2 Hz, C5-H), 6.56 (dd, 1H, *J*=2.0, 8.2 Hz, C6-H), 5.80 (br s, 1H, N10-H), 5.21 (d, 1H, *J*=12.3 Hz, CO_2CHH), 5.13 (d, 1H, *J*=12.3 Hz, CO_2CHH), 5.08 (br s, 1H, NHBOC), 5.00 (d, 1H, *J*=2.0 Hz, C19-H), 4.57 (m, 1H, C12-H), 4.26 (m, 1H, C9-H), 3.93 (s, 3H, $ArOCH_3$), 3.50 (dd, 1H, *J*=4.8, 13.4 Hz, C13-Hβ), 2.75–2.95 (m, 3H, C8-H_2 and C13-Hα), 1.48 (s, 9H, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 2926, 2851, 1717, 1672, 1518, 1261, 1163, 1130, 1026, 912, 867, 803, 728 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 679.1435 (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 (10). A solution of **9** (15.2 mg, 0.028 mmol) in 2.2 N HCl–EtOAc (2.0 mL) was stirred at 25 °C for 30 min. The volatiles were removed in vacuo and the residue was treated with satd aq $NaHCO_3$ (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 satd aq NaCl (2 mL), dried ($MgSO_4$), and concentranted in vacuo. Flash chromatography (SiO_2, 1×4 cm, 0–8% CH_3OH–$CHCl_3$ gradient elution) afforded **10** (10.5 mg, 12.5 mg theoretical, 84%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.34 (m, 6H, C15- or C18-H and ArH), 7.23 (br s, 1H, N10-H), 7.12 (dd, 1H, *J*=2.0, 8.2 Hz, C18- or C15-H), 7.10 (dd, 1H, *J*=2.0, 8.2 Hz, C16- or C17-H), 7.00 (dd, 1H, *J*=2.0, 8.2 Hz, C17- or C16-H), 6.73 (d, 1H, *J*=8.2 Hz, C5-H), 6.56 (dd, 1H, *J*=2.0, 8.2 Hz, C6-H), 5.12 (d, 1H, *J*=12.3 Hz, CO_2CHH), 5.10 (d, 1H, *J*=12.3 Hz, CO_2CHH), 5.03 (d, 1H, *J*=2.0 Hz, C19-H), 4.19 (m, 1H, C9-H), 3.93 (s, 3H, $ArOCH_3$), 3.90 (m, 1H, C12-H), 3.49 (dd, 1H, *J*=3.0, 11.4 Hz, C13-Hβ), 2.83 (d, 1H, *J*=16.0 Hz, C8-Hβ), 2.81 (t, 1H, *J*=11.4 Hz, C13-Hα), 2.70 (dd, 1H, *J*=10.5, 16.0 Hz, C8-Hα), 1.71 (br s, 2H, NH_2); IR (neat) ν_{max} 3345, 2926, 2841, 1744, 1662, 1588, 1502, 1441, 1263, 1206, 1174, 1128, 1024, 974, 903, 867, 789, 728 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 579.0890 (M^+ + Cs, $C_{26}H_{26}N_2O_5$ requires 579.0896).

BOC-Glycyl-glycyl-glycyl-glycyl-L-tyrosyl-*O*-methyl-L-tyrosine cyclic 5^4→6^3 ether, benzyl ester (12). A solution of **10** (2.8 mg, 0.0062 mmol) in anhyd THF (0.5 mL) was treated with **11**[32] (3.8 mg, 0.0075 mmol, 1.2 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. Chromatography

(SiO_2, 1×5 cm, 0–10% CH_3OH–$CHCl_3$ gradient elution) afforded **12** (4.3 mg, 4.8 mg theoretical, 90%) as a white solid mp >250 °C (dec); ^1H NMR (CD_3OD, 400 MHz) δ 7.39 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\delta a}$-H), 7.28–7.30 (m, 5H, ArH), 7.19 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\delta b}$-H), 7.00 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\varepsilon a}$-H), 6.91 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\varepsilon b}$-H), 6.79 (d, 1H, J=8.2 Hz, $Tyr^{6\varepsilon a}$-H), 6.54 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{6\delta a}$-H), 5.16 (d, 1H, J=2.0 Hz, $Tyr^{6\delta b}$-H), 5.03 (d, 1H, J=12.8 Hz, CO_2CHH), 4.99 (d, 1H, J=12.8 Hz, CO_2CHH), 4.64 (m, 1H, $Tyr^{5\alpha}$-H), 4.06 (m, 1H, $Tyr^{6\alpha}$-H), 3.95 (s, 3H, $ArOCH_3$), 3.71–3.91 (m, 8H, Gly^{α}-H), 3.30–3.34 (m, 1H, $Tyr^{5\beta}$-Hβ, partially obscured by solvent), 2.99 (dd, 1H, J=8.3, 16.2 Hz, $Tyr^{6\beta}$-Hα), 2.95 (dd, 1H, J=6.9, 12.0 Hz, $Tyr^{5\beta}$-Hα), 2.83 (d, 1H, J=16.2 Hz, $Tyr^{6\beta}$-Hβ), 1.44 (s, 9H, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3299, 3086, 2924, 2852, 1647, 1554, 1519, 1417, 1368, 1250, 1174, 1028, 864, 668 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 907.2245 (M^++Cs, $C_{39}H_{46}N_6O_{11}$ requires 907.2279).

Cyclo (glycyl-glycyl-glycyl-glycyl-L-tyrosyl-*O*-methyl-L-tyrosyl) cyclic $5^4 \rightarrow 6^3$ ether (3). A solution of **12** (4.3 mg, 0.0055 mmol) in anhydrous CH_3OH (2 mL) was treated with 10% Pd–C (1.0 mg, 25% 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** (3.7 mg, 3.8 mg theoretical, 97%) as a white solid, which was directly used in the following reaction without further purification.

A solution of **13** (3.7 mg, 0.0053 mmol) in 2.2 M HCl–EtOAc (1.0 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 **14** (3.3 mg, 3.3 mg theoretical, 100%) as a white solid, which was used directly in the next reaction.

A solution of **14** (3.3 mg, 0.0053 mmol) in anhydrous DMF (2.0 mL) was cooled to 0 °C and treated with $NaHCO_3$ (4.6 mg, 0.0053 mmol, 10.0 equiv) and diphenylphosphoryl azide (DPPA, 7.5 mg, 6.0 μL, 0.027 mmol, 5.0 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 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–$CHCl_3$ (4×4 mL). The combined organic extracts were washed with H_2O (2 mL) and satd aq NaCl (2 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 1×5 cm, 0–15% *i*PrOH–$CHCl_3$ gradient elution) afforded **3** (2.3 mg, 3.0 mg theoretical, 77%) as a white solid: mp >250 °C; $[\alpha]_D^{25}$ +15 (*c* 0.1, 50% CH_3OH–$CHCl_3$); ^1H NMR (25% CD_3OD–$CDCl_3$, 400 MHz) δ 7.65 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\delta b}$-H), 7.16 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\delta a}$-H), 7.13 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\varepsilon a}$-H), 6.90 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{5\varepsilon b}$-H), 6.76 (d, 1H, J=8.2 Hz, $Tyr^{6\varepsilon a}$-H), 6.60 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{6\delta a}$-H), 4.96 (d, 1H, J=2.0 Hz, $Tyr^{6\delta b}$-H), 3.58–4.61 (m, 10H, $Tyr^{5\alpha}$-, $Tyr^{6\alpha}$- and eight Gly^{α}-H), 3.90 (s, 3H, $ArOCH_3$), 3.45 (dd, 1H, J=6.8, 14.2 Hz, $Tyr^{5\beta}$-Hβ), 2.81–3.07 (m, 3H, $Tyr^{5\beta}$-Hα and $Tyr^{6\beta}$-H_2); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.66 (d, 1H, J=4.2 Hz, Tyr^5-NH), 8.48 (dd, 1H, J=5.6, 6.4 Hz, Gly^2-NH), 8.46 (dd, 1H, J=5.0, 6.8 Hz, Gly^3-NH), 7.89 (dd, 1H, J=2.3, 8.2 Hz, $Tyr^{5\delta b}$-H), 7.76 (d, 1H, J=7.4 Hz, Tyr^6-NH), 7.55 (d, 1H, J=7.8 Hz, Gly^1-NH), 7.31 (d, 1H, J=6.4 Hz, Gly^4-NH), 7.13 (dd, 1H, J=2.3, 8.2 Hz, $Tyr^{5\delta a}$-H), 7.12 (dd, 1H, J=2.3, 8.2 Hz, $Tyr^{5\varepsilon b}$-H), 6.94 (dd, 1H, J=2.3, 8.2 Hz, $Tyr^{5\varepsilon a}$-H), 6.88 (d, 1H, J=8.3 Hz, $Tyr^{6\varepsilon a}$-H), 6.64 (dd, 1H, J=2.0, 8.2 Hz, $Tyr^{6\delta a}$-H), 4.91 (d, 1H, J=2.0 Hz, $Tyr^{6\delta b}$-H), 4.39 (m, 1H, $Tyr^{5\alpha}$-H), 4.29 (dd, 1H, J=7.7, 17.2 Hz, $Gly^{4\alpha}$-Ha), 4.10 (dd, 1H, J=8.5, 16.8 Hz, $Gly^{1\alpha}$-Ha), 3.89 (dd, 1H, J=2.6, 17.2 Hz, $Gly^{4\alpha}$-Hb), 3.82 (s, 3H, $ArOCH_3$), 3.81 (dd, 1H, J=6.8, 15.8 Hz, $Gly^{3\alpha}$-Ha), 3.76 (dd, 1H, J=5.2, 11.0 Hz, $Tyr^{6\alpha}$-H), 3.69 (dd, 1H, J=6.6, 14.8 Hz, $Gly^{2\alpha}$-Ha), 3.62 (d, 1H, J=5.6, 14.8 Hz, $Gly^{2\alpha}$-Hb), 3.59 (dd, 1H, J=4.9, 15.8 Hz, $Gly^{3\alpha}$-Hb), 3.46 (dd, 1H, J=1.6, 16.8 Hz, $Gly^{1\alpha}$-Hb), 3.31 (dd, 1H, J=4.2, 13.9 Hz, $Tyr^{5\beta}$-Hβ), 3.03 (d, 1H, J=13.9 Hz, $Tyr^{5\beta}$-Hα), 2.89 (dd, 1H, J=11.0, 16.2 Hz, $Tyr^{6\beta}$-Hα), 2.73 (d, 1H, J=16.2 Hz, $Tyr^{6\beta}$-Hβ); IR (neat) ν_{max} 3491, 3341, 2919, 1628, 1531, 1461, 1426, 1327, 1260, 1210, 1129, 1033, 981, 863, 789 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 699.1158 (M^++Cs, $C_{27}H_{30}N_6O_8$ requires 699.1179).

^1H NMR (DMSO-d_6, 400 MHz) with irradiation at 8.66 ppm (d, Tyr^5-NH) led to the change of the signal at 4.39 ppm (m, $Tyr^{5\alpha}$-H) to a doublet; irradiation at 8.48 ppm (dd, Gly^2-H) led to the collapse of the signal at 3.69 ppm (dd, Gly^2-Ha) to a doublet and to the collapse of the signal at 3.62 ppm (dd, $Gly^{2\alpha}$-Hb) to a doublet; irradiation at 8.46 ppm (dd, Gly^3-NH) led to the collapse of the signal at 3.81 ppm (dd, $Gly^{3\alpha}$-Ha) to a doublet and to the collapse of the signal at 3.59 ppm (dd, $Gly^{3\alpha}$-Hb) to a doublet; irradiation at 7.89 ppm (dd, $Tyr^{5\delta b}$-H) led to the collapse of the signal at 7.13 ppm (dd, $Tyr^{5\delta a}$-H) to a doublet and to the collapse of the signal at 7.12 ppm (dd, $Tyr^{5\varepsilon b}$-H) to a doublet; irradiation at 7.76 ppm (d, Tyr^6-NH) led to the collapse of the signal at 3.76 ppm (dd, $Tyr^{6\alpha}$-H) to a doublet; irradiation at 7.55 ppm (d, Gly^1-NH) led to the collapse of the signal at 4.10 ppm (dd, $Gly^{1\alpha}$-Ha) to a doublet and the signal at 3.46 ppm (dd, $Gly^{1\alpha}$-Hb) to a doublet; irradiation at 7.31 ppm (d, Gly^4-NH) led to the collapse of the signal at 4.29 ppm (dd, $Gly^{4\alpha}$-Ha) to a doublet and to the collapse of the signal at 3.89 ppm (dd, $Gly^{4\alpha}$-Hb) to a doublet; irradiation at 6.64 ppm (dd, $Tyr^{6\delta a}$-H) led to the collapse of the signal at 6.88 ppm (d, $Tyr^{6\varepsilon a}$-H) to a singlet and to the collapse of the signal at 4.91 ppm (d, $Tyr^{6\delta b}$-H) to a singlet; irradiation at 3.03 ppm (d, $Tyr^{5\beta}$-Hα) led to the collapse of the signal at 3.31 ppm (dd, $Tyr^{5\beta}$-Hβ) to a doublet; irradiation at 2.73 ppm (d, $Tyr^{6\beta}$-Hβ) led to the collapse of the signal at 2.89 ppm (dd, $Tyr^{6\beta}$-Hα) to a doublet.

The ^1H-^1H ROESY NMR spectrum of **3** (DMSO-d_6, 400 MHz) displayed diagnostic NOE crosspeaks for Tyr^5-NH/$Tyr^{5\delta b}$-H, Tyr^5-NH/$Tyr^{5\alpha}$-H, Tyr^5-NH/$Gly^{4\alpha}$-Hb, Tyr^5-NH/$Tyr^{5\beta}$-Hα, Gly^2-NH/$Gly^{1\alpha}$-Hb, Gly^2-NH/$Gly^{2\alpha}$-Ha, Gly^3-NH/Gly^4-NH, Gly^3-NH/$Gly^{3\alpha}$-Ha, Gly^3-NH/$Gly^{3\alpha}$-Hb, Gly^3-NH/$Gly^{2\alpha}$-Hb, $Tyr^{5\delta b}$-H/$Tyr^{5\varepsilon b}$-H, $Tyr^{5\delta b}$-H/$Tyr^{5\beta}$-

Hα, Tyr^{6}-NH/Gly^{1}-NH, Tyr^{6}-NH/Tyr$^{6\delta b}$-H, Tyr^{6}-NH/Tyr$^{5\alpha}$-H, Tyr^{6}-NH/Tyr$^{6\alpha}$-H, Gly^{1}-NH/Gly$^{1\alpha}$-Hb, Tyr$^{5\delta a}$-H/Tyr$^{6\delta b}$-H, Tyr$^{5\delta a}$-H/Tyr$^{5\beta}$-Hβ, Tyr$^{5\varepsilon a}$-H/Tyr$^{6\beta b}$-H, Tyr$^{6\varepsilon a}$-H/Tyr^{6}-OCH$_3$, Tyr$^{6\varepsilon a}$-H/Tyr$^{6\delta a}$-H, Tyr$^{6\delta a}$-H/Tyr$^{6\beta}$-Hβ, Tyr$^{6\delta b}$-H/Tyr$^{6\alpha}$-H, Tyr$^{6\delta b}$-H/Tyr$^{6\beta}$-Hα, Tyr$^{5\alpha}$-H/Tyr$^{5\beta}$-Hα, Tyr$^{5\alpha}$-H/Tyr$^{5\beta}$-Hβ, Gly$^{4\alpha}$-Ha/Gly$^{4\alpha}$-Hb, Gly$^{1\alpha}$-Ha,/Gl$^{1\alpha}$-Hb, Gly$^{3\alpha}$-Ha/Gly$^{3\alpha}$-Hb, Tyr$^{6\alpha}$-H/Tyr$^{6\beta}$-Hβ, Gly$^{2\alpha}$-Ha/Gly$^{2\alpha}$-Hb, Tyr$^{5\beta}$-Hβ/Tyr$^{5\beta}$-Hα, Tyr$^{6\beta}$-Hα/Tyr$^{6\beta}$-Hβ.

BOC-Glycyl-glycyl-glycyl-L-tyrosyl-*O*-methyl-L-tyrosine cyclic 4^{4}→5^{3} ether, benzyl ester (15). A solution of **10** (2.8 mg, 0.006 mmol) in anhydrous THF (0.5 mL) was treated with **15**[32] (3.4 mg, 0.0075 mmol, 1.2 equiv) at 25 °C under Ar and 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. Chromatography (SiO_2, 1×5 cm, 0–8% CH_3OH–$CHCl_3$ gradient elution) afforded **16** (4.0 mg, 4.4 mg theoretical, 91%) as a white solid: mp >250 °C (dec); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.39 (dd, 1H, J=2.0, 8.2 Hz, Tyr$^{4\delta a}$-H), 7.28–7.30 (m, 5H, ArH), 7.20 (dd, 1H, J=2.0, 8.2 Hz, Tyr$^{4\delta b}$-H), 7.01 (dd, 1H, J=2.0, 8.2 Hz, Tyr$^{4\varepsilon a}$-H), 6.89 (dd, 1H, J=2.0, 8.2 Hz, Tyr$^{4\varepsilon b}$-H), 6.80 (d, 1H, J=8.2 Hz, Tyr$^{5\varepsilon a}$-H), 6.54 (dd, 1H, J=2.0, 8.2 Hz, Tyr$^{5\delta a}$-H), 5.16 (d, 1H, J=2.0 Hz, Tyr$^{5\delta b}$-H), 5.03 (d, 1H, J=12.4 Hz, CO_2CHH), 5.00 (d, 1H, J=12.4 Hz, CO_2CHH), 4.64 (m, 1H, Tyr$^{4\alpha}$-H), 4.05 (m, 1H, Tyr$^{5\alpha}$-H), 3.95 (s, 3H, $ArOCH_3$), 3.66–3.91 (m, 6H, Gly$^{\alpha}$-H), 3.30–3.34 (m, 1H, Tyr$^{4\beta}$-Hβ, partially obscured by solvent peak), 2.99 (dd, 1H, J=4.3, 16.0 Hz, Tyr$^{5\beta}$-Hα), 2.95 (dd, 1H, J=6.7, 12.7 Hz, Tyr$^{4\beta}$-Hα), 2.83 (d, 1H, J=16.0 Hz, Tyr$^{5\beta}$-Hβ), 1.44 (s, 9H, $CO_2C(CH_3)_3$); IR (neat) ν_{max} 3320, 2926, 2851, 1653, 1521, 1350, 1261, 1164, 1129, 1027, 864 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 850.2048 (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}→5^{3} ether (4). A solution of **15** (4.0 mg, 0.0055 mmol) in anhyd CH_3OH (2 mL) was treated with 10% Pd–C (1.0 mg, 25% 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 **16** (3.4 mg, 3.5 mg theoretical, 97%) as a white solid, which was used directly in the following reaction without further purification.

A solution of **16** (3.4 mg, 0.0053 mmol) in 2.2 M HCl–EtOAc (1.0 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 **17** (3.1 mg, 3.1 mg theoretical, 100%) as a white solid, which was used directly in the next reaction.

A solution of **17** (3.1 mg, 0.0053 mmol) in anhydrous DMF (2.0 mL) was cooled to 0 °C and treated with $NaHCO_3$ (4.6 mg, 0.053 mmol, 10.0 equiv) and DPPA (7.5 mg, 6.0 μL, 0.027 mmol, 5.0 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 (1.0 mL) and 15% *i*PrOH–$CHCl_3$ (4.0 mL), and the aqueous layer was extracted with 15% *i*PrOH–$CHCl_3$ (4×5 mL). The combined organic extracts were washed with H_2O (2 mL) and satd aq NaCl (2 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 1×5 cm, 0–10% *i*PrOH–$CHCl_3$ gradient elution) afforded **4** (1.8 mg, 2.9 mg theoretical, 63%) as a white solid: mp >250 °C; $[\alpha]_D^{25}$ −68 (*c* 0.1, 50% CH_3OH–$CHCl_3$); ^{1}H NMR (25% CD_3OD–$CDCl_3$, 400 MHz) δ 7.54 (dd, 1H, J=2.4, 8.4 Hz, Tyr$^{4\delta b}$-H), 7.28 (dd, 1H, J=2.4, 8.4 Hz, Tyr$^{4\delta a}$-H), 7.26 (dd, 1H, J=2.4, 8.4 Hz, Tyr$^{4\varepsilon a}$-H), 7.07 (dd, 1H, J=2.4, 8.4 Hz, Tyr$^{4\varepsilon b}$-H), 6.71 (d, 1H, J=8.2 Hz, Tyr$^{5\varepsilon a}$-H), 6.50 (dd, 1H, J=2.0, 8.2 Hz, Tyr$^{5\delta a}$-H), 4.97 (d, 1H, J=2.0 Hz, Tyr$^{5\delta b}$-H), 4.41 (t, 1H, J=5.2 Hz, Tyr$^{4\alpha}$-H), 3.58–4.14 (m, 7H, Tyr$^{5\alpha}$-H and six Gly^{2}-H), 3.87 (s, 3H, $ArOCH_3$), 3.35 (dd, 1H, J=6.8, 12.7 Hz, Tyr$^{4\beta}$-Hβ), 3.04 (dd, 1H, J=4.6, 14.6 Hz, Tyr$^{5\beta}$-Hα), 2.87–2.90 (m, 2H, Tyr$^{5\beta}$-Hβ and Tyr$^{4\beta}$-Hα); IR (neat) ν_{max} 3355, 2945, 2851, 1663, 1641, 1516, 1447, 1415, 1260, 1205, 1126, 1025, 877, 836 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 642.0986 (M^+ + Cs, $C_{25}H_{27}N_5O_7$ requires 642.0965).

^{1}H NMR (25% CD_3OD–$CDCl_3$, 400 MHz) with irradiation at 7.54 ppm (dd, Tyr$^{4\delta b}$-H) led to the collapse of the signal at 7.28 ppm (dd, Tyr$^{4\delta a}$-H) to a doublet and to the collapse of the signal at 7.07 ppm (dd, Tyr$^{4\varepsilon b}$-H) to a doublet; irradiation at 6.50 ppm (dd, Tyr$^{5\delta a}$-H) led to the collapse of the signal at 6.71 ppm (d, Tyr$^{5\varepsilon a}$-H) to a singlet and to the collapse of the signal at 4.97 ppm (d, Tyr$^{5\delta b}$-H) to a singlet.

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References

1. Jolad, S. D.; Hoffmann, J. J.; Torrance, S. J.; Wiedhopf, R. M.; Cole, J. R.; Arora, S. K.; Bates, R. B.; Gargiulo, R. L.; Kriek, G. R. *J. Am. Chem. Soc.* **1977**, *99*, 8040; Bates, R. B.; Cole, J. R.; Hoffmann, J. J.; Kriek, G. R.; Linz, G. S.; Torrance, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1343.

2. Review: Itokawa, H.; Takeya, K. *Heterocycles* **1993**, *35*, 1467.

3. Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Mihashi, S.; Hamanaka, T. *Chem. Pharm. Bull.* **1986**, *34*, 3762.

4. Itokawa, H.; Takeya, K.; Mihara, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1424.

5. Itokawa, H.; Takeya, K.; Mori, N.; Kidokoro, S.; Yamamoto, H. *Planta Med.* **1984**, *51*, 313.

6. Itokawa, H.; Takeya, K.; Mori, N.; Sonobe, T.; Serisawa, N.; Hamanaka, T.; Mihashi, S. *Chem. Pharm. Bull.* **1984**, *32*, 3216.

7. Itokawa, H.; Takeya, K.; Mori, N.; Takanashi, M.; Yamamoto, H.; Sonobe, T.; Kidokoro, S. *Gann* **1984**, *75*, 929.

8. Itokawa, H.; Takeya, K.; Mori, N.; Hamanaka, T.; Sonobe, T.; Mihara, K. *Chem. Pharm. Bull.* **1984**, *32*, 284.

9. Itokawa, H.; Yamamiya, T.; Morita, H.; Takeya, K. *J. Chem. Soc., Perkin Trans. 1* **1992**, 455.

10. Itokawa, H.; Morita, H.; Takeya, K.; Tomioka, N.; Itai, A. *Chem. Lett.* **1991**, 2217.

11. Itokawa, H.; Morita, H.; Takeya, K.; Tomioka, N.; Itai, A.; Iitaka, Y. *Tetrahedron* **1991**, *47*, 7007.

12. Morita, H.; Yamamiya, T.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull.* **1992**, *40*, 1352.

13. Morita, H.; Kondo, K.; Hitotsuyanagi, Y.; Takeya, K.; Itokawa, H.; Tomioka, N.; Itai, A.; Iitaka, Y. *Tetrahedron* **1991**, *47*, 2757.

14. Itokawa, H.; Saitou, K.; Morita, H.; Takeya, K. *Chem. Pharm. Bull.* **1991**, *39*, 2161.

15. Itokawa, H.; Morita, H.; Takeya, K. *Chem. Pharm. Bull.* **1991**, *40*, 1050.

16. Itokawa, H.; Saitou, K.; Morita, H.; Takeya, K.; Yamada, K. *Chem. Pharm. Bull.* **1992**, *40*, 2984.

17. Hamanaka, T.; Ohgoshi, M.; Kawahara, K.; Yamakawa, K.; Tsuruo, T.; Tsukagoshi, S. *J. Pharmacobio-Dyn.* **1987**, *10*, 616; Kato, T.; Suzumura, Y.; Takamoto, S.; Ota, K. *Anticancer Res.* **1987**, *7*, 329.

18. Tobey, R. A.; Orlicky, D. J.; Deaven, L. L.; Rall, L. B.; Kissane, R. J. *Cancer Res.* **1978**, *38*, 4415.

19. Johnson, R. K.; Chitnis, M. P. *Proc. Am. Assoc. Cancer Res.* **1978**, *19*, 218; Chitnis, M. P.; Alate, A. D.; Menon, R. S. *Chemotherapy (Basel)* **1981**, *27*, 126; Chitnis, M.; Menon, R.; Adwankar, M.; Satyamoorthy, K. *Tumori* **1985**, *71*, 261.

20. Zalacain, M.; Zaera, E.; Vazquez, D.; Jiminez, A. *FEBS Lett.* **1982**, *148*, 95; Morita, H.; Yamamiya, T.; Takeya, K.; Itokawa, H.; Sakuma, C.; Yamada, J. Suga, T. *Chem. Pharm. Bull.* **1993**, *41*, 781.

21. Boger, D. L.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7364.

22. Boger, D. L.; Patane, M. A.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7357.

23. Boger, D. L.; Patane, M. A.; Zhou, J. *J. Am. Chem. Soc.* **1994**, *116*, 8544.

24. Boger, D. L.; Patane, M. A.; Jin, Q.; Kitos, P. A. *Bioorg. Med. Chem.* **1994**, *2*, 85.

25. Boger, D. L.; Zhou, J. *J. Am. Chem. Soc.* **1993**, *115*, 11426.

26. Boger, D. L.; Yohannes, D.; Zhou, J.; Patane, M. A. *J. Am. Chem. Soc.* **1993**, *115*, 3420; Boger, D. L.; Yohannes, D. *J. Am. Chem. Soc.* **1991**, *113*, 1427.

27. Boger, D. L.; Yohannes, D.; Myers, J. B. *J. Org. Chem.* **1992**, *57*, 1319.

28. Boger, D. L.; Myers, J. B.; Yohannes, D.; Kitos, P. A.; Suntornwat, O.; Kitos, J. C. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 313.

29. Boger, D. L.; Myers, J. B. *J. Org. Chem.* **1991**, *56*, 5385.

30. Boger, D. L.; Yohannes, D. *Synlett* **1990**, *1*, 33.

31. Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1988**, *53*, 487; Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1987**, *52*, 5283.

32. Boger, D. L.; Zhou, J.; Winter, B.; Kitos, P. A. *Bioorg. Med. Chem.* **1995**, *3*, 1579.

33. Boger, D. L.; Zhou, J. *J. Org. Chem.* **1996**, *61*, 3938. Boger, D. L.; Zhou, J.; Borzilleri, R. M.; Nukui, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1089.

34. Shioiri, T.; Ninomiga, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.

35. Global and close low-lying minima (5 kcal/mol 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[36] excluding those originating in the phenyl rings (Macromodel,[37] version 4.5, OPLSA force field, MCMM = 5000, MCSS = 2, 12 or 5 kcal/mol window). The global minima for **3** and **4** were located 41 and 16 times, respectively.

36. Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

37. Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. *MACROMODEL*, Columbia University, New York, 1990.

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