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Synthesis and Evaluation of Microtubule Assembly Inhibition and Cytotoxicity of Prenylated Derivatives of *cyclo*-L-Trp-L-Pro

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Abstract—The synthesis of three isoprenylated derivatives of *cyclo*-L-Trp-L-Pro is described. These substances have been evaluated for cytotoxic activity in rat normal fibroblast 3Y1 cells and have also been evaluated in vitro for the inhibition of microtubule assembly.

Introduction

The tryprostatins,[1] cyclotryprostatins[2] and spirotryprostatins[3] (Fig. 1) are a class of prenylated fungal metabolites produced from *Aspergillus fumigatus*. Osada et al. have demonstrated that these substances are cell cycle inhibitors interfering with the G2/M phase progression in G2/M synchronous cultures of tsFT210 cells.[1–5] The primary target of tryprostatin A and cyclotryprostatins A and B are microtubules which induce M-phase specific inhibition and microtubule disassembly. Cell cycle inhibitors are considered to be promising candidates as anticancer drugs and have also received a considerable amount of attention recently as probes of the cell cycle.[4]

The tryprostatin family of secondary metabolites are the consequence of several modes of isoprenylation of the tryptophan moiety of the simple cyclic dipeptide progenitor *cyclo*-L-Trp-L-Pro. The structurally most interesting and complex members of this family are the spirotryprostatins which, curiously, display among the weakest biological activity of this family of cell cycle inhibitors.[5,6] On the other hand, the very simple substance tryprostatin A has been shown to inhibit tau or MAP2-dependent microtubule assembly. The presence of the methoxy group on the aromatic ring reduces the cytotoxicity while enhancing the specificity against microtubule disruptive activities. For example, the IC_{50} of tryprostatin A was determined by MTT assay to be 400 μM whereas tryprostatin B exhibited an IC_{50} value of 4 μM. The interesting profile of cell cycle inhibitory activity displayed by the tryprostatin family has prompted us to investigate the synthesis of some very simple and readily prepared analogues of tryprostatin B.

Results and Discussion

Synthesis of tryprostatin B analogues

The disposition of the dimethylallyl moiety at the 2-position of tryprostatins A and B that is essential for biological activity, suggested that the display of the isoprene group at either the indole nitrogen or the tryptophyl amide nitrogen, might closely mimic the display of this side-chain in the natural products. We therefore designed and synthesized compounds **5**, **7** and **10**, which can be viewed as analogues of tryprostatin B and evaluated their biological activities. The simple tryprostatin B analogues targeted in this study (compounds **5**, **7** and **10**) were prepared as shown in Schemes 1 and 2.

The simple indole *N*-prenylated compound **5** has been previously reported by Sammes et al. by a five-step procedure starting with *N*-CBz-L-tryptophan.[7] We have devised an alternate, four-step route from *N*-Boc-L-tryptophan (**1**) as shown in Scheme 1. Dimethylallylation of **1** with prenyl bromide in the presence of sodium hydride in DMF yielded the desired *N*-prenylated substance **2** in 68% yield. Peptide coupling of L-proline methyl ester to **2** gave the dipeptide **3** in 69% yield.

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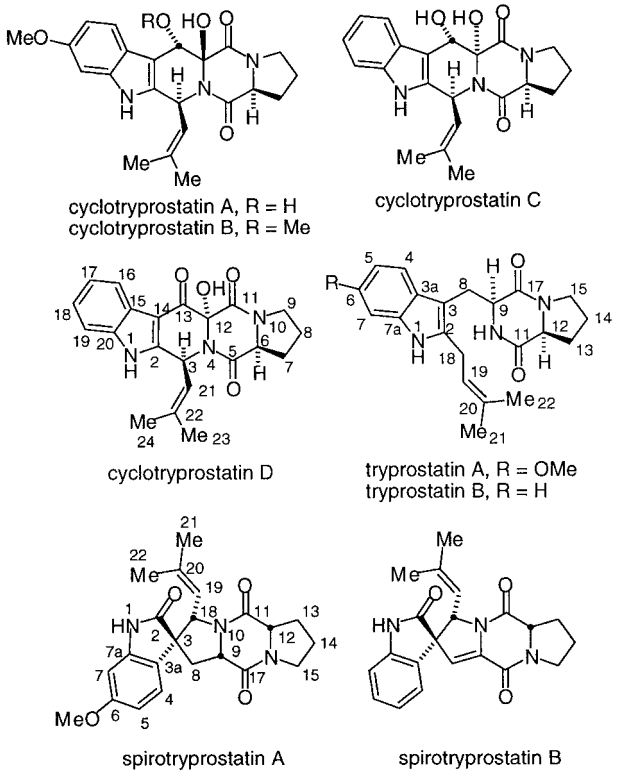
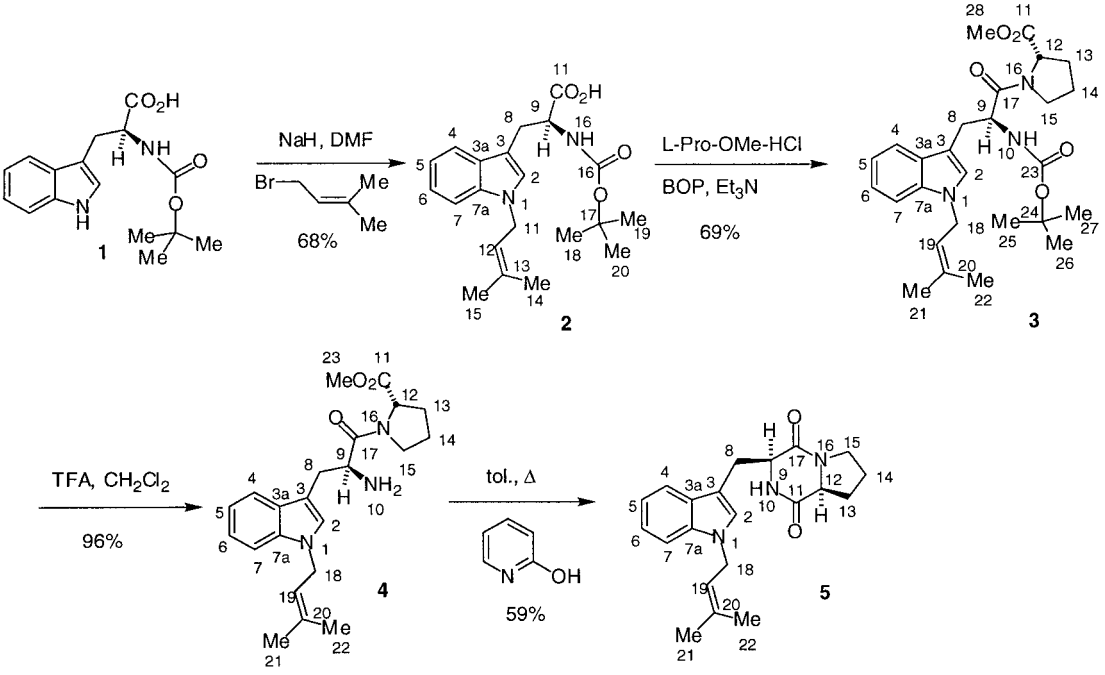


Figure 1.

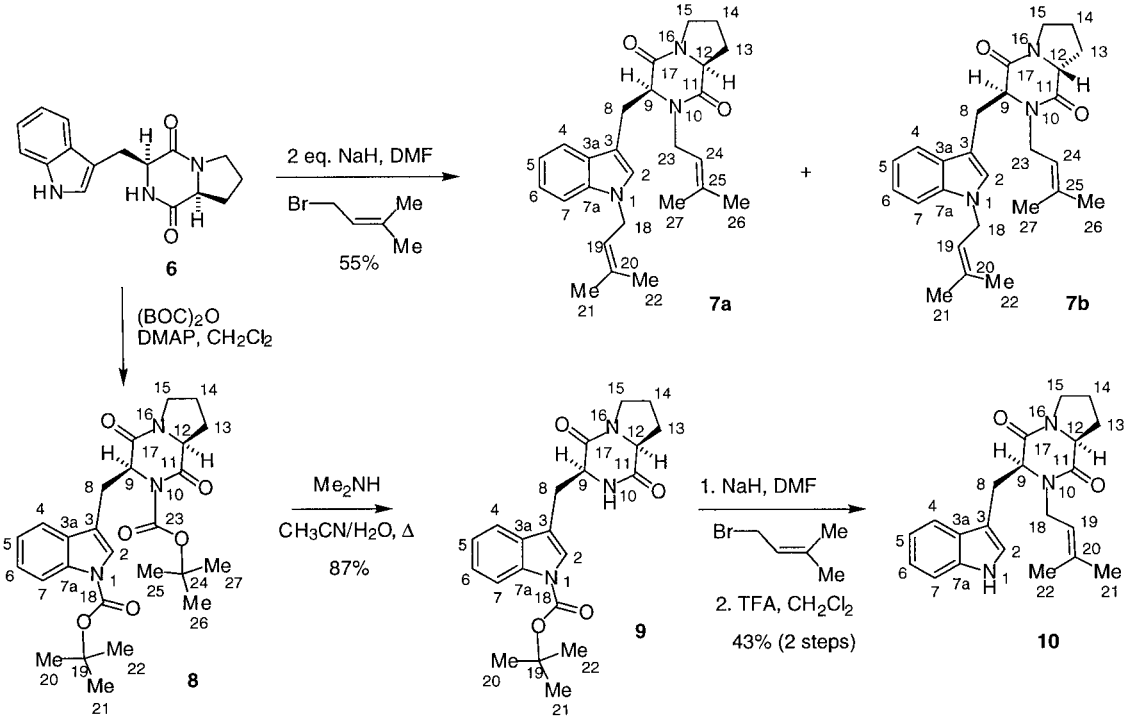
Cleavage of the *t*-Boc group with trifluoroacetic acid in methylene chloride (96%) followed by cyclization of the incipient amino methyl ester (**4**) with 2-hydroxypyridine in hot toluene provided **5** in 59% yield.

The synthesis of compounds **7** and **10** was conducted as shown in Scheme 2 using *cyclo*-L-Trp-L-Pro (**6**, also known as brevianamide F) as the key starting material. Simply treating **6** with 2 equivalents of sodium hydride in DMF in the presence of prenyl bromide furnished **7a** (*syn*-) in 55% isolated, purified yield. In addition, approximately 7% of the *anti*-epimer **7b** was detected by ^{1}H NMR and isolated.[8] The optical purity of compound **7b** was determined by acid hydrolysis (6N HCl, 110 °C, 24 h) and thin layer chromatography (TLC) recovery of proline (silica gel, eluted with EtOH). Chiral high-performance liquid chromatography (HPLC) analysis (Chiralcel WH) of the proline recovered from the hydrolysis reaction indicated a 29:71 ratio of L-Pro:D-Pro. The relative and absolute stereochemical assignment for **7b** was established through independent synthesis of *cyclo*-L-Trp-D-Pro followed by prenylation with 2 equivalents of sodium hydride in DMF in the presence of prenyl bromide that furnished an authentic specimen of **7b**. Thus, base-catalyzed epimerization of the proline residue of **7a** occurs to a more significant extent than that for the tryptophyl residue under the basic conditions of the alkylation reaction. Subjecting the *syn*-compound (**7a**) to the hydrolysis conditions (6N HCl, 110 °C, 24 h) followed by TLC isolation and chiral HPLC analysis as above, revealed a 83:17 ratio of L-Pro:D-Pro. Thus, the optical integrity (83:17 er) of **7a** was partially compromised under the basic prenylation conditions. Despite extensive effort, we were unable to resolve the individual enantiomers of either **7a** or **7b** by chiral HPLC. Both the *syn*- (**7a**) and *anti*- (**7b**) isomers of this diprenylated substance were subjected to cell cycle inhibition and cytotoxicity evaluation.

For the selective installation of the isoprene unit on the amide nitrogen, we found that conversion of **6** to the di-*N*-*t*-Boc substrate **8** (90%) followed by selective removal of the amide-derived *N*-*t*-Boc group could be accomplished by treatment of **8** with dimethylamine in water at reflux temperature which provided **9** in 87% yield.[9] Treatment of **9** with NaH in DMF in the presence of



Scheme 1.



Scheme 2.

Table 1. Biological activity of tryprostatin analogues

Compound	Concentration (μM)	Cell cycle	Cell proliferation (%)	In vitro microtubule assembly (%)[a]
5	500	Slightly toxic		
	250	No effect	176	98.5[c]
7a	50	Toxic	ND[b]	4.6[c]
	25	No effect		93.3[c]
7b	250	Toxic	ND	36.6±11.0[d]
	100	Arrest	84	
	50	No effect	143	
10	500	Slightly toxic		
	250	No effect	154	90.1[c]

[a]250 μM of tryprostatin A exhibited 71.4% assembly in this study.
[b]This compound was toxic to cells at the indicated concentration; thus, cell proliferation percent could not be obtained.
[c]Results are the mean of two independent assays.
[d]Results are the mean ±S.D. (n = three experiments).

prenyl bromide (57%) followed by cleavage of the *t*-Boc group from the indole nitrogen with TFA provided **10** in 76% yield.

Biological activity. The effects of compounds **5**, **7** and **10** on cell cycle control and microtubule assembly were examined and the results are shown in Table 1 and Figure 2.

Compounds **7a** and **7b** were the most toxic compounds of the four tryprostatin B analogues evaluated. Compound **7b** completely inhibited cell proliferation at 100 μM but this inhibition was not cell cycle dependent. Compound **7b** also inhibited microtubule assembly strongly (64% inhibition at 250 μM). Compound **7a** is the most potent compound of those tested displaying 4.6% microtubule assembly at 50 μM as compared to 36.6% for **7b** at 5-fold higher concentration (250 μM). In addition, substance **7a** was highly cytotoxic to cells down to 50 μM concentration (see Table 1 and Fig. 2). Compounds **5** and **10** slightly inhibited cell proliferation at concentrations above 500 μM but neither of these derivatives inhibited microtubule assembly in vitro.

The striking similarity between the biological activity of compounds **7a** and **7b** and tryprostatin B indicate that the corresponding methoxy derivatives should display decreased toxicity and enhanced selectivity for inhibition of the cell cycle similar to that of tryprostatin A. It should also be noted that *cyclo*-L-Trp-L-Pro (brevianamide F, **6**) was completely inactive in the cell cycle inhibition and microtubule assembly assays at 250 μM concentration (data not shown). This clearly indicates the significance of the display of the isoprene moiety as an obligate functional array for the expression of biological activity in this family of alkaloids. The syntheses of the methoxy-substituted substances and related tryprostatin analogues are under investigation and their biological activities will be reported in due course.

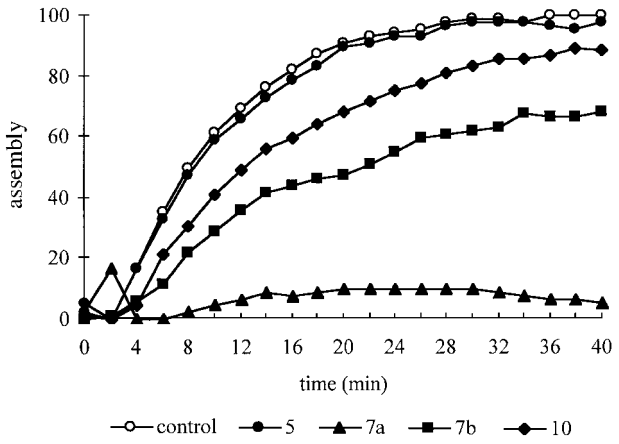


Figure 2. Microtubule assembly assay as determined by turbidity. The concentration of 7a was 50 μM; all other compounds were assayed at 250 μM.

Experimental

Cell culture and proliferation assay

Rat normal fibroblast 3Y1 cells[10] were grown in Dulbecco's modified MEM culture medium supplemented with 10% fetal calf serum under a humidified atmosphere containing 5% CO_2.

Exponentially growing 3Y1 cells were treated with compounds **5**, **7** and **10** for 24 h. The distribution of DNA content was determined by flow cytometry and relative cell numbers (cell number at 24 h per initial cell number at 0 h×100) were counted. MTT assay is a colorimetric assay using 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide. The cell viability was determined by this assay with minor modifications.[11]

Preparation of microtubule and turbidity assay (in vitro microtubule assembly assay)

Calf brain microtubule protein was prepared by two cycles of assembly–disassembly[12] and stored at −80 °C in Mes buffer (100 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), 1 mM EGTA and 0.5 mM $MgCl_2$ at pH 6.8. Protein concentrations were determined by using the Dc Protein Assay (BioRad, Hercules, CA). Microtubule assembly was monitored by the turbidity assay as described previously.[13] In brief, microtubule protein (2.0 mg/mL in Mes buffer) was incubated at 37 °C and the change in absorbance at 350 nm was monitored over time. To examine the effect of compounds **5**, **7** and **10** on polymerization, the microtubule protein was preincubated with 1% DMSO containing various concentrations of each compound at 0 °C and polymerization was initiated with the addition of 1 mM GTP and with warming to 37 °C. The distribution of DNA content was determined as previously described.[14]

Synthesis of compounds 5, 7 and 10

***N*-Prenyl-*N*′-Boc-L-tryptophan (2).** To a stirred solution of *N*-Boc-L-Trp (**1**) (2.045 g, 6.72 mmol) under an Ar atmosphere in 10 mL of dry DMF at 0 °C (ice bath), was added NaH (733 mg of a 55% oil suspension, 16.80 mmol, 2.5 equiv). The mixture was stirred at 0 °C for 10 min and prenyl bromide (1.502 g, 10.08 mmol, 1.5 equiv, 1.17 mL) was then added. The reaction mixture was stirred for 1 h at 0 °C, then for 2 h at rt. The reaction was quenched with 50 mL of water and washed with 25 mL of hexane. The hexane wash was discarded; the aqueous layer was acidified with 1 M aqueous $NaHSO_4$ until the pH = 3. The mixture was extracted with CH_2Cl_2 (3×25 mL); the organic layers were combined, washed with water (2×25 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 2.04 g of crude reaction product **2** as a yellowish oil, which was used directly in the following reaction without further purification (68% yield). Attempts to purify this substance through crystallization from several solvents were unsuccessful. Considering that this compound was difficult to purify, a small portion was transformed into its methyl ester in the following reaction for characterization.

***N*-Prenyl-*N*′-Boc-L-tryptophan methyl ester.** To a stirred solution of crude **2** (133 mg, 0.358 mmol) in 1 mL of MeOH at 0 °C, was added a solution of $TMSCHN_2$ (2 M solution in hexane) via syringe until the N_2 evolution ceased and the yellow color was persistent. Removal of the solvent under reduced pressure gave a residue that was purified by means of silica gel column chromatography, using toluene:EtOAc (16:1) as eluent to yield 73 mg of pure ester (53% yield). Optical rotation: $[\alpha]_D = +26.0$ (CH_2Cl_2, *c* 1.23). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.51 (1H, d, *J* = 8.0 Hz, H-7), 7.28 (1H, d, *J* = 8.0 Hz, H-4), 7.18 (1H, dd, *J* = 8.0, 8.0 Hz, H-6), 7.08 (1H, dd, *J* = 8.0, 8.0 Hz, H-5), 6.88 (1H, s, H-2), 5.33 (1H, m, H-19), 5.04 (1H, d, *J* = 7.6 Hz, H-10), 4.62 (3H, m, H-9, H-11, H-11′), 3.66 (3H, s, H-21), 3.25 (2H, brs, H-8, H-8′), 1.80 (3H, br. s, H-21), 1.75 (3H, brs, H-22), 1.42 (9H, s, H-18, H-19, H-20). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.76 (s, C-11), 155.21 (s, C-16), 136.31 (s, C-7a*), 136.14 (s, C-13*), 128.36 (s, C-3a), 126.00 (d, C-2), 121.52 (d, C-6**), 119.84 (d, C-12), 119.05 (d, C-5**), 118.85 (d, C-4), 109.5 (s, C-7), 108.52 (s, C-3), 79.70 (s, C-17), 54.23 (d, C-9), 52.13 (q, C-21), 43.95 (t, C-11), 28.29 (q, C-25, C-26, C-27), 27.91 (t, C-8), 25.63 (q, C-14), 17.99 (q, C-15) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3391, 3048, 2976, 2931, 1746, 1714, 1613, 1503, 1468, 1391, 1366, 1251, 1209, 1167, 1060, 1014, 856, 778, 739 cm^{-1}. HRMS (FAB+) calcd for $C_{22}H_{30}N_2O_4$: 386.2206; found 386.2202 (M+).

***N*-Prenyl-*N*′-Boc-L-tryptophyl-L-proline methyl ester (3).** To a stirred solution of crude **2** (1.144 g, 3.071 mmol if 100% pure) in 10 mL of THF was added proline methyl ester hydrochloride (1.017 g, 6.142 mmol, 2 equiv). The resulting mixture was cooled to 0 °C and Et_3N (684 mg, 6.756 mmol, 2.2 equiv, 938 μL) was added dropwise via syringe over 5 min. To this mixture was added 1-hydroxybenzotriazole (415 mg, 3.071 mmol, 1 equiv) followed by the addition of DCC in small portions (665 mg, 3.22 mmol, 1.05 equiv). The ice bath was removed, and the temperature was allowed to reach 25 °C. The reaction was complete after 6 h at that temperature (TLC analysis). The reaction was worked up by first filtering

off the DCU, followed by washing with Et_2O (5×10 mL) and combining the filtrate with the washings. The solvents were removed under reduced pressure and the residue was taken up in 100 mL EtOAc. The resulting solution was washed with 5% aqueous $NaHCO_3$ (25 mL), 10% aqueous citric acid solution, again with 5% aqueous $NaHCO_3$ (25 mL), and brine (25 mL), and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 1.47 g of the crude reaction product, which was separated by means of silica gel column chromatography, using hexane:EtOAc 1:1 as eluent to give 1.018 g of pure **3** as a colorless glass (69% yield).

Optical rotation: $[\alpha]_D = -17.6$ (CH_2Cl_2, *c* 1.23). ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ 7.53 (1H, d, *J* = 8.0 Hz, H-4), 7.32 (1H, d, *J* = 8.0 Hz, H-7), 7.13 (1H, s, H-2), 7.09 (1H, dd, *J* = 8.0, 8.0 Hz, H-6*), 6.99 (1H, dd, *J* = 8.0, 8.0 Hz, H-5*), 6.48 (1H, br s, H-10), 5.31 (1H, br dd, *J* = 7.2, 7.2 Hz, H-19), 4.65 (2H, d, *J* = 6.8 Hz, H-18, H-18′), 4.45 (1H, m, H-9), 4.33 (1H, dd, *J* = 8.8, 5.2 Hz, H-12), 3.57 (1H, m, H-15), 3.32 (1H, ddd, *J* = 9.6, 6.4, 6.4 Hz, H-15′), 3.02 (1H, dd, *J* = 14.4, 5.6 Hz, H-8), 2.89 (1H, dd, *J* = 14.4, 8.0 Hz, H-8′), 2.13 (1H, m, H-13), 1.70–1.89 (3H, m, H-13′, H-14, H-14′), 1.77 (3H, br s, H-21**), 1.69 (3H, brs, H-22**), 1.28 (9H, s, H-25, H-26, H-27). ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C): δ 172.88 (s, C-11*), 171.16 (s, C-17*), 136.60 (s, C-23**), 135.77 (s, C-7a**), 128.74 (s, C-20**), 127.60 (s, C-3a), 121.60 (d, C-2), 121.10 (d, C-5), 119.16 (d, C-19), 119.16 (d, C-6), 118.99 (d, C-4), 110.30 (d, C-7), 110.13 (s, C-3), 78.83 (s, C-24), 59.32 (d, C-9), 52.20 (d, C-12), 47.11 (t, C-15), 44.11 (t, C-18), 29.19 (t, C-13), 28.78 (s, C-25, C-26, C-27), 27.83 (t, C-8), 25.85 (q, C-21), 25.21 (t, C-14), 18.36 (q, C-22) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3433, 3306, 3051, 2975, 2932, 2867, 1747, 1731, 1644, 1496, 1455, 1366, 1251, 1171, 1098, 1054, 1014, 859, 739 cm^{-1}. HRMS (FAB+) calcd for $C_{27}H_{38}N_3O_5$: 484.2811; found 484.2803 (M + H).

***N*-Prenyl-L-tryptophyl-L-proline methyl ester (4).** To a stirred solution of compound **3** (821 mg, 1.698 mmol) in 2 mL of dry CH_2Cl_2 at 0 °C was added 2 mL of TFA under an Ar atmosphere. The mixture was allowed to stir for 6 h. The solvent was removed under reduced pressure at 0 °C and the resulting residue was taken up in 100 mL of EtOAc. The solution was washed with 5% aqueous Na_2CO_3 (25 mL), brine (25 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 514 mg of crude reaction product, which, by TLC analysis was found to contain a significant amount of the diketopiperazine **4**. For this reason, together with the fact that the purification of this crude mixture proved to be very difficult, the crude product was used directly in the next step (96% crude yield).

***N*-Prenyl-*cyclo*-L-tryptophyl-L-proline (5).**[15] To a stirred solution of crude compound **4** (350 mg, 0.767 mmol) in 10 mL of dry toluene at 0 °C under an Ar atmosphere was added 2-hydroxypyridine (0.1 equiv, 0.077 mmol, 0.7 mg). The resulting solution was refluxed for 6 h. The toluene was then removed under reduced pressure and the resulting residue was purified by means of silica gel column chromatography, using the toluene:EtOAc:MeOH (12:10:1) as eluent to give 151 mg of the pure diketopiperazine **5** (59% yield).

Optical rotation: $[\alpha]_D = -107.9$ (CH_2Cl_2, *c* 0.80). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.61 (1H, d, *J* = 7.5 Hz, H-4), 7.37 (1H, d, *J* = 7.5 Hz, H-7), 7.27 (1H, dd, *J* = 7.5, 7.5 Hz, H-6*), 7.16 (1H, dd, *J* = 7.5, 7.5 Hz, H-5*), 7.05 (1H, s, H-2), 5.88 (1H, br s, H-10), 5.41 (1H, ddqq, *J* = 7.0, 7.0, 1.5, 1.5 Hz, H-19), 4.70 (2H, d, *J* = 7.0 Hz, H-18, H-18′), 4.39 (1H, dd, *J* = 10.6, 3.7 Hz, H-12), 4.09 (1H, dd, *J* = 7.5, 7.5 Hz, H-9), 3.78 (1H, ddd, *J* = 15.0, 3.7, 0.7 Hz, H-8), 3.56–3.70 (2H, m, H-15, H-15′), 2.98 (1H, dd, *J* = 15.0, 10.6 Hz, H-8′), 2.35 (1H, m, H-13), 1.97–2.10 (2H, m, H-13′, H-14), 1.88–1.95 (1H, m, H-14′), 1.88 (3H, br s, H-21), 1.82 (3H, brs, H-22). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.56 (s, C-17*), 165.81 (s, C-11*), 136.96 (s, C-7a**), 136. 84 (s, C-20**), 127.61 (s, C-3a), 126.76 (d, C-2), 122.36 (d, C-5), 119.84 (d, C-19), 119.66 (d, C-6), 118.85 (d, C-4), 110.16 (d, C-7), 108.45 (s, C-3), 59.43 (d, C-9), 54.87 (d, C-12), 45.60 (t, C-15), 44.31 (t, C-18), 28.51 (t, C-13), 27.03 (t, C-8), 25.90 (q, C-21), 22.83 (t, C-14), 18.31 (q, C-22) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3361, 3244, 3053, 2975, 2923, 2874, 1777, 1668, 1546, 1466, 1374, 1312, 1218, 1168, 1129, 1014, 919, 847, 738, 700 cm^{-1}. HRMS (FAB+) calcd for $C_{21}H_{25}N_3O_2$: 352.2025; found 252.2021 (M + H).

***N*,*N*′-Diprenyl-*cyclo*-L-tryptophan-L-proline (7).**[15] To a stirred solution of *cyclo*-L-Trp-L-Pro (brevianamide F, **6**) (247 mg, 0.872 mmol) in 9 mL of dry DMF under an Ar atmosphere, at 0 °C, was added NaH (77 mg of a 60% oil suspension, 1.919 mmol, 2.2 equiv). The mixture was stirred at 0 °C for 15 min and prenyl bromide (650 mg, 4.36 mmol, 5 equiv, 508 μL) was added. The reaction mixture was stirred for 1 h at 0 °C and then 2 h at rt. The reaction was then quenched with 5% aqueous $NaHCO_3$ (50 mL) and extracted with dichloromethane (2×50 mL). The organic phases were combined, washed with brine (2×50 mL), and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 340 mg of crude reaction product, which was separated by means of radial chromatography on silica gel, using hexane:EtOAc 1:1 as eluent, to give 247 mg of slightly impure product as a slightly yellowish oil (68% yield). Pure **7a** was obtained through column chromatography, using toluene:EtOAc 3:2 as eluent to give 200 mg of **7a** as a colorless oil (55% yield). $R_f = 0.21$. Approximately 7% of the *trans*-epimer, **7b**, was detected by ^{1}H NMR. This compound was purified by PTLC using toluene:EtOAc 3:2 as eluent. $R_f = 0.25$.

(7a) Optical rotation: $[\alpha]_D = -21.5$ ($CHCl_3$, *c* 0.019). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.57 (1H, d, *J* = 7.7 Hz, H-4), 7.20 (1H, d, *J* = 8.1 Hz, H-7), 7.13 (1H, ddd, *J* = 7.0, 7.0, 1.1 Hz, H-6*), 7.04 (1H, ddd, *J* = 7.7, 7.7, 1.1 Hz, H-5*), 6.77 (1H, d, *J* = 2.4 Hz, H-2), 5.26 (1H, ddqq, *J* = 7.0, 7.0, 1.5, 1.5 Hz, H-24), 5.17 (1H, ddqq, *J* = 7.0, 7.0, 1.5, 1.5 Hz, H-19), 4.81 (1H, dd, *J* = 14.7, 5.9 Hz, H-18), 4.59 (2H, d, *J* = 7.0 Hz, H-23, H-23′), 4.29 (1H, dd,

J = 4.0, 4.0 Hz, H-9), 3.53–3.68 (3H, m, H-8, H-12, H-18′), 3.36 (1H, ddd, J = 6.6, 9.2, 16.1 Hz, H-15), 3.15 (1H, dd, J = 14.7, 4.4 Hz, H-8′), 2.89 (1H, ddd, J = 4.8, 10.6, 10.6 Hz, H-15′), 1.79 (3H, br s, H-26**), 1.75 (6H, brs, H-27,** H-21), 1.71 (3H, brs, H-22**), 1.66† (1H, m, H-13), 1.27 (1H, m, H-14), 0.68 (1H, m, H-14′),-0.29 (1H, m, H-13′). (†This signal was partly obscured under the methyl group singlets.) ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.41 (s, C-17*), 164.62 (s, C-11*), 138.20 (s, C-7a**), 136. 14 (s, C-25**), 135.60 (s, C-20**), 128.03 (s, C-3a), 127.22 (d, C-2), 121.63 (d, C-5***), 119.77 (d, C-24), 119.69 (d, C-4), 119.00 (d, C-6***), 117.90 (d, C-19), 109.14 (d, C-7), 106.83 (s, C-3), 59.54 (d, C-9), 59.14 (d, C-12), 44.22 (t, C-15), 43.98 (t, C-23), 40.25 (t, C-18), 28.14 (t, C-13), 26.97 (t, C-8), 25.74 (q, C-26****), 25.52 (q, C-21****), 20.49 (t, C-14), 18.02 (q, C-27*****), 17.93 (q, C-22*****) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3286, 3051, 2969, 2931, 1654, 1464, 1458, 1375, 1363, 1357, 1313, 1265, 1194, 1106, 1032, 1014, 978, 843, 741 cm^{-1}. HRMS (FAB+) calcd for $C_{26}H_{34}N_3O_2$: 420.2651; found 420.2648 (M + H).

The optical integrity of this sample was determined by subjecting the sample to acid hydrolysis (6N HCl, 110 °C, 24 h) followed by evaporation to dryness. The sample was re-suspended in 1 mL of water and the pH was adjusted to neutrality with dilute NaOH. The sample was concentrated to approximately 200 μL and loaded onto an analytical silica gel TLC plate (20×20 cm) and eluted with ethanol. The proline band was scraped off the plate and washed from the powdered silica gel with ethanol. The ethanol was evaporated, dissolved in 1 mL of water and passed through a Dowex 50WX2-100 (H^+) ion-exchange column and eluted with water followed by 2% NH_4OH. The NH_4OH eluate was evaporated and the residue was dissolved in a small volume of water for HPLC analysis. Chiral HPLC analysis was performed on a Chiralcel WH column, eluted with the following solvent system: 28 mL of solvent A (A = *n*-butanol:acetone, 1:1) + 12 mL of solvent B (water: acetic acid, 23:7). Co-injection with authentic L-proline and D-proline revealed a ratio of 83:17, L-proline:D-proline. Authentic L-proline was subjected to the same TLC isolation and chiral HPLC analysis procedure as above; chiral HPLC analysis revealed that no racemization attended this procedure.

(7b) Optical rotation: $[\alpha]_D$ = +9.1 (CH_2Cl_2, *c* 0.44). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.53 (1H, d, J = 7.7 Hz, H-4), 7.24 (1H, d, J = 8.1 Hz, H-7), 7.15 (1H, ddd, J = 7.0, 7.0, 1.1 Hz, H-6), 7.06 (1H, m, H-5), 6.83 (1H, s, H-2), 5.24 (1H, m, H-24), 5.14 (1H, m, H-19), 4.62 (2H, d, J = 14.7, 5.9 Hz, H-23, H-23′), 4.52 (1H, dd, J = 5.9, 14.7 Hz, H-18), 4.19 (1H, dd, J = 4.0, 4.0 Hz, H-9), 3.56 (1H, dd, J = 8.8, 14.7 Hz, H-18′), 3.42 (2H, m, H-8, H-15), 3.18 (1H, dd, J = 4.8, 15.0 Hz, H-8′), 3.15 (1H, ddd, J = 2.2, 9.5, 11.5 Hz, H-15′), 2.24 (1H, dd, J = 6.2, 10.98 Hz, H-12), 1.90(1H, ddd, J = 5.9, 5.9, 11.8 Hz, H-13), 1.80 (3H, br s, H-26*), 1.72 (6H, br. s, H-27,* H-21*), 1.70† (1H, m, H-14), 1.65 (3H, br. s, H-22*), 1.58 (1H, m, H-13′), 1.19 (1H, m, H-14′). (†This signal was partly obscured under the methyl group singlets.) ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.22 (s, C-17*), 165.74 (s, C-11*), 137.70 (s, C-7a**), 136.43 (s, C-25**), 136.43 (s, C-20**), 128.02 (s, C-3a), 127.26 (d, C-2), 121.9 (d, C-5***), 119.9 (d, C-24), 119.3 (d, C-4), 119.07 (d, C-6***), 118.57 (d, C-19), 109.47 (d, C-7), 107.98 (s, C-3), 61.84 (d, C-9), 57.90 (d, C-12), 44.78 (t, C-15), 44.08 (t, C-23), 41.16 (t, C-18), 29.12 (t, C-13), 27.04 (t, C-8), 25.84 (q, C-26****), 25.64 (q, C-21****), 21.66 (t, C-14), 18.02 (q, C-27*****), 17.85 (q, C-22*****) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3047, 2971, 2920, 2878, 1658, 1447, 1371, 1291, 1207, 979, 743 cm^{-1}.

An independent synthesis of **7b** was conducted following exactly the same procedure as described for the synthesis of **7a** and **7b** from brevianamide F as described below. The *cyclo*-L-trp-D-pro was obtained in 66% overall yield from *N*-*t*-BOC-D-proline. The dialkylation was conducted as described above with prenyl bromide yielding **7b** in 45% yield. $[\alpha]_D$ = +33.5 (CH_2Cl_2, *c* 0.275). Subjecting this sample to the hydrolysis conditions described above followed by chiral HPLC analysis of the recovered proline, revealed a ~85:15 ratio of D-proline: L-proline. Subjecting brevianamide F (*cyclo*-L-Trp-L-Pro) to the acidic hydrolysis conditions, TLC separation of the proline produced and chiral HPLC analysis revealed exclusive formation of L-proline.

***cyclo*-D-Proline-L-tryptophan.** *N*-*t*-Boc-D-proline (73 mg, 0.34 mmol), was stirred with L-tryptophan methyl ester (97 mg, 0.34 mmol), BOP reagent (173 mg, 0.34 mmol) and Et_3N (52 μL, 1.1 equiv) in acetonitrile (5.1 mL) at rt for 3 h. A saturated aqueous solution of NaCl was added and the reaction was extracted four times with EtOAc. The combined organic layers were washed with 2N HCl, water, 10% $NaHCO_3$ (aq), water and brine successively. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The product was partially purified by flash silica gel column chromatography using 4% MeOH in CH_2Cl_2 as eluant and carried forward in the next step without further characterization. The resultant dipeptide, *N*-*t*-Boc-D-Pro-L-Trp-OMe, was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 °C. Trifluoroacetic acid (0.5 mL) was added, the ice bath was removed and the mixture was stirred for an additional 3 h. A saturated solution of $NaHCO_3$ was added until the solution became basic and the organic layer was separated from the aqueous phase. The aqueous layer was extracted three more times with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude free amine was then dissolved in toluene (1.25 mL) with 2-hydroxypyridine (4 mg) and the solution was refluxed overnight under argon. The solvent was removed under reduced pressure and the diketopiperazine was purified by flash column chromatography using 4% MeOH/CH_2Cl_2 as the eluant. Precipitation from ethyl acetate and hexanes gave an amorphous white powder. Yield: 63 mg, (66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (1H, m), 1.70 (1H, m), 1.81 (1H, m), 2.07 (1H, m), 2.83 (1H, dd, J = 6.6, 10.6 Hz), 3.15 (1H, m) 3.18 (1H, dd, J = 4.0, 14.6× Hz), 3.39 (1H, dd, J = 6.2, 14.6 Hz), 3.54 (1H,

ddd, $J=8.8$, 8.8, 12.1 Hz), 4.23 (1H, ddd, $J=4.0$, 4.0, 6.6 Hz), 5.92 (1H, bs), 7.04 (1H, d, $J=2.2$ Hz), 7.11 (1H, ddd, $J=1.1$, 8.0, 8.0 Hz), 7.18 (1H, ddd, $J=1.1$, 8.0, 8.0 Hz), 7.34 (1H, d, $J=8.0$ Hz), 7.60 (1H, d, $J=8.0$ Hz), 8.17 (1H, bs). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.6, 29.0, 30.8, 45.1, 57.9, 58.4, 109.4, 111.2, 118.8, 119.9, 122.5, 124.1, 126.9, 136.1, 165.3, 169.3. I.R. (NaCl neat) 3260, 2924, 2884, 1651, 1455, 1338, 1302, 1104, 1010, 743 cm^{-1}. $[\alpha]_{D25}=+50.9°$ (*c* 0.23, CH_2Cl_2). HRMS (FAB+) calcd for $C_{16}H_{18}N_3O_2$: 284.1399; found 284.1389.

***N,N′*-Di-Boc-*cyclo*-L-tryptophan-L-proline (8).**[15] Crystalline *cyclo*-L-Trp-L-Pro (brevianamide F, **6**) (1.5 g, 5.298 mmol) was suspended in 10 mL of dry CH_2Cl_2 under an Ar atmosphere. To this suspension, dimethylaminopyridine (DMAP) (64.7 mg, 0.53 mmol, 0.1 equiv) and triethylamine (1.072 g, 10.60 mmol, 1.48 mL, 2 equiv) were added. The mixture was cooled to −18 °C (ice-salt bath), and di-*tert*-butyldicarbonate ($(Boc)_2O$) (2.312 g, 10.60 mmol, 2 equiv) was added in one portion. The mixture was stirred at that temperature for 30 min for 2 h at 0 °C and 2 h at rt. The reaction was diluted with 100 mL of dichloromethane and washed with 1% aq $KHSO_4$ soln (2×50 mL), then with 5% $NaHCO_3$ aq soln (50 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 2.63 g of crude reaction product, which was separated by silica gel column chromatography, using toluene: EtOAc 2:1 as eluent to give 2.30 g of **8** as a viscous colorless oil (90% yield).

$[\alpha]_D=+77.21$ (CH_2Cl_2, *c* 1.18). IR (neat, NaCl): 2980, 2934, 2878, 1777, 1731, 1667, 1457, 1455, 1369, 1323, 1292, 1257, 1155, 1084, 1018, 965, 917, 852, 768, 748, 732 cm^{-1}. HRMS (FAB+) calcd for $C_{26}H_{33}N_3O_6$: 483.2369; found 483.2368 (M+).

In DMSO-d_6 at 80 °C, this compound appears as a mixture of two predominant rotamers in a ∼2:1 proportion that interconvert slowly on the NMR time scale. For that reason, both ^{1}H and ^{13}C spectra include the signals for both major and minor rotamers.[16] ^{1}H NMR ($CDCl_3$, 25 °C, 400 MHz): major rotamer: δ 8.08 (1H, d, $J=7.9$ Hz, H-7), 7.47 (1H, d, $J=7.9$ Hz, H-4), 7.27 (1H, t, $J=7.0$ Hz, H-6) 7.25 (1H, bs, H-2), 7.19 (1H, t, $J=7.3$ Hz, H-5), 4.93 (1H, dd, $J=2.6$, 4.7 Hz, H-9), 3.84 (1H, dd, $J=6.2$, 11.1 Hz, H-12), 3.59 (1H, m, H-15), 3.56 (1H, dd, $J=2.9$, 14.7 Hz, H-8), 3.46 (1H, dd, $J=5.0$, 14.7 Hz, H-8′) 3.09 (1H, ddd, $J=4.4$, 1,.3, 10.3 Hz, H-15′), 1.77 (1H, m, H-13), 1.63 (9H, s, H-20, H-21, H-22), 1.56 (9H, s, H-25, H-26, H-27), 1.48 (1H, m, H-14), 1.25 (1H, m, H-14′). 0.11 (1H, m, H-13′); minor rotamer: δ 8.10 (1H, d, $J=7.9$ Hz, H-7), 7.52 (1H, d, $J=7.6$ Hz, H-4), 7.57 (1H, bs, H-2), 7.29 (1H, ddd, $J=1.2$, 7.3, 7.3 Hz, H-6), 7.22 (1H, ddd, $J=0.9$, 7.3, 7.3 Hz, H-5), 5.03 (1H, dd, $J=5.6$, 5.6 Hz, H-9), 3.48 (1H, m, H-15), 3.31 (3H, m, H-8, H-8′, H-15′), 3.13 (1H, dd, $J=6.5$, 9.4 Hz, H-12), 2.11 (1H, ddd, $J=5.9$, 5.9, 10.9 Hz, H-13), 1.84 (2H, m, H-13′, H-14), 1.64 (9H, s, H-20, H-21, H-22), 1.45(1H, m, H-14′), 1.40 (9H, s, H-25, H-26, H-27). ^{13}C NMR ($CDCl_3$, 25 °C, 100 MHz): major rotamer: δ 165.85 (s, C-17*), 164.02 (s, C-11*), 150.98 (s, C-18**), 149.30 (s, C-23**), 134.14 (s, C-7a*), 130.16 (s, C-3a*), 126.16 (d, C-2), 124.73 (d, C-6), 122.58 (d, C-5), 119.64 (d, C-4), 115.01 (d, C-7), 113.71, (s, C-3), 84.07 (s, C-19), 83.73 (s, C-24), 60.53 (d, C-12), 60.21 (d, C-9), 44.73 (t, C-15), 28.89 (t, C-8), 28.89 (t, C-13, q, C-20, C-21, C-22***), 27.59 (q, C-25, C-26, C-27***), 20.57 (t, C-14) (*assignments for signals with the same superscript may be interchanged) minor rotamer: δ 167.25 (s, C-17*), 164.95 (s, C-11*), 150.11 (s, C-18**), 149.35 (s, C-23**), 135.38 (s, C-7a*), 129.90 (s, C-3a*), 125.25 (d, C-2), 124.81 (d, C-6), 122.73 (d, C-5), 118.94 (d, C-4), 115.27 (d, C-7), 114.29, (s, C-3), 84.34 (s, C-19), 83.96 (s, C-24), 61.13 (d, C-9), 59.20 (d, C-12), 45.18 (t, C-15), 29.16 (t, C-13), 28.09 (q, C-20, C-21, C-22***), 27.95 (t, C-8), 27.65 (q, C-25, C-26, C-27***), 21.95 (t, C-14) (*assignments for signals with the same superscript may be interchanged).

***N*-Boc-*cyclo*-L-tryptophan-L-proline (9).**[15] To a stirred suspension of *N,N′*-di-Boc-*cyclo*-L-Trp-L-Pro (**8**) (2.00 g, 4.135 mmol) in 100 mL of MeCN under an Ar atmosphere was added dimethylamine (40% aq soln, 3.0 mL). The solution was heated to reflux for 90 min. The solvent was removed under reduced pressure and the residue was separated by means of silica gel column chromatography, using hexane:EtOAc:MeOH (4:5:1) as eluent to give 1.48 g of **9** as a colorless glass (93% yield). This fraction was further purified using column chromatography, with CH_2Cl_2:MeOH 25:1 as eluent to give 1.39 g of pure **9** as a colorless glass (87% yield).

Optical rotation: $[\alpha]_D=+4.45$ (CH_2Cl_2, *c* 0.88). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05 (1H, br d, $J=8.0$ Hz, H-7), 7.51 (1H, d, $J=7.6$ Hz, H-4), 7.44 (1H, s, H-2), 7.25 (1H, ddd, $J=7.6$, 7.6, 1.2 Hz H-6), 7.17 (1H, ddd, $J=7.6$, 7.6, 1.2 Hz, H-5), 7.11 (1H, brs, H-10 (N-H)), 4.19 (1H, ddd, $J=6.4$, 4.4, 4.4 Hz, H-9), 3.51 (1H, ddd, $J=8.8$, 8.8, 12.0 Hz, H-15), 3.15–3.25 (3H, m, H-8, H-12, H-15′), 3.13 (1H, dd, $J=14.8$, 4.8 Hz, H-8′), 2.09 (1H, ddd, $J=6.4$, 6.4, 11.8 Hz, H-13), 1.83 (1H, m, H-14), 1.71 (1H, m, H-13′), 1.58 (9H, s, H-20, H-21, H-22), 1.51 (1H, m, H-14′). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.44 (s, C-17*), 165.56 (s, C-11*), 149.68 (s, C-18), 135.57 (s, C-7a*), 130.05 (s, C-3a*), 125.48 (d, C-2), 124.90 (d, C-6), 122.89 (d, C-5), 119.30 (d, C-4), 115.39 (d, C-7), 114.71, (s, C-3), 84.16 (s, C-19), 58.16 (d, C-9), 57.97 (d, C-12), 45.45 (t, C-15), 30.49 (t, C-13), 29.10 (q, C-20, C-21, C-22), 28.34 (t, C-8), 21.86 (t, C-14) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3236, 2979, 2927, 2876, 1732, 1664, 1452, 1370, 1332, 1308, 1256, 1228, 1159, 1109, 1085, 1017, 865, 765, 747, 729, 699 cm^{-1}. HRMS (FAB+) calcd for $C_{21}H_{25}N_3O_4$: 383.1845; found 383.1842 (M+).

***N*-Boc-*N′*-prenyl-*cyclo*-L-tryptophan-L-proline.**[15] To a stirred solution of compound **9** (925 mg, 2.412 mmol) in 4 mL of dry DMF under an Ar atmosphere at 0 °C, NaH was added (58 mg of a 60% oil suspension, 2.412 mmol, 1 equiv). The mixture was stirred at 0 °C for 30 min and prenyl bromide (719 mg, 4.83 mmol, 2 equiv, 502 μL) was added. The reaction mixture was stirred for 1 h at 0 °C and then 2 h at rt. The reaction was then quenched with 5% aqueous $NaHCO_3$ (50 mL)

and extracted with CH_2Cl_2 (2×50 mL). The organic phases were combined, washed with brine (2×50 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 1.34 gm of crude reaction product, which was separated by means of radial silica gel chromatography, using toluene:EtOAc 1:1 to 2:3 as eluent to give 640 mg of *N*-Boc-*N*′-prenyl-*cyclo*-L-tryptophan-L-proline as a colorless oil (57% yield).

Optical rotation: $[\alpha]_D = +29.7$ (CH_2Cl_2, *c* 1.33). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05 (1H, br d, *J* = 7.6, H-7), 7.47 (1H, d, *J* = 7.6 Hz, H-4), 7.31 (1H, s, H-2), 7.24 (1H, m, H-6), 7.18 (1H, m, H-5), 5.08 (1H, br dd, *J* = 7.2, 7.2 Hz, H-19), 4.38 (1H, dd, *J* = 14.8, 6.0 Hz, H-18), 4.19 (1H, dd, *J* = 4.8, 4.8 Hz, H-9), 3.55 (1H, dd, *J* = 14.8, 8.8 Hz, H-18′), 3.44 (1H, ddd, *J* = 8.8, 8.8, 11.6 Hz, H-15), 3.32 (1H, dd, *J* = 14.8, 4.4 Hz, H-8), 3.12 (1H, dd, *J* = 14.8, 5.2 Hz, H-8′), 3.09 (1H, m, H-15′), 2.76 (1H, dd, *J* = 11.2, 6.8 Hz, H-12), 1.99 (1H, ddd, *J* = 6.4, 6.4, 12.4 Hz, H-13), 1.73 (1H, m, H-14), ∼1.64† (2H, m, H-13′, H-14), 1.68 (3H, brs, H-21), 1.61 (9H, s, H-25, H-26, H-27), 1.57 (3H, brs, H-22), 1.27 (1H, m, H-14′). †Signal party buried under the H-21 and H-25,26,27 singlets. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.82 (s, C-17*), 165.30 (s, C-11*), 149.24 (s, C-23), 137.59 (s, C-7a**), 135.23 (s, C-20**), 129.75 (s, C-3a), 125.15 (d, C-2), 124.66 (d, C-6), 122.49 (d, C-5), 118.96 (d, C-4), 118.37 (d, C-19), 115.06 (d, C-7), 114.20 (s, C-3), 83.88 (s, C-24), 61.41 (d, C-9), 57.92 (d, C-12), 44.93 (t, C-15), 41.64 (t, C-18), 28.99 (t, C-13), 28.01 (q, C-25, C-26, C-27), 27.12 (t, C-8), 25.68 (q, C-21), 21.61 (t, C-14), 17.67 (q, C-22) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3448, 3309, 3114, 3052, 2977, 2932, 2886, 1732, 1667, 1453, 1371, 1335, 1256, 1157, 1085, 1018, 854, 768, 748, 701 cm^{-1}. HRMS (FAB+) calcd for $C_{26}H_{34}N_3O_4$: 452.2549. Found 452.2532 (M + H).

***N*′-Prenyl-*cyclo*-L-tryptophan-L-proline (10).**[15] To a reaction vessel containing *N*-Boc-*N*′-prenyl-*cyclo*-L-tryptophan-L-proline obtained as described above (355 mg, 0.762 mmol) was added TFA (2 mL) under an Ar atmosphere at 0 °C for 2 h with magnetic stirring. The TFA was then removed under reduced pressure at 0 °C and the resulting residue was dissolved in 50 mL of CH_2Cl_2. The solution was washed with 5% aqueous Na_2CO_3 (25 mL) and brine (25 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave 250 mg of the crude reaction product, which was separated by means of radial silica gel chromatography, using CH_2Cl_2:MeOH 50:1 as eluent to give 213 mg of **10** as a colorless oil (76% yield).

Optical rotation: $[\alpha]_D = +70.1$ (CH_2Cl_2, *c* 0.83). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.41 (1H, br s, H-1), 7.55 (1H, d, *J* = 7.5 Hz, H-4), 7.30 (1H, d, *J* = 7.5 Hz, H-7), 7.14 (1H, dd, *J* = 7.5, 7.5 Hz, H-6*), 7.08 (1H, dd, *J* = 7.5, 7.5 Hz, H-5*), 6.92 (1H, d, *J* = 2.4 Hz, H-2), 5.13 (1H, br dd, *J* = 7.2, 7.2 Hz, H-19), 4.55 (1H, dd, *J* = 14.4, 5.6 Hz, H-18), 4.22 (1H, dd, *J* = 4.0, 4.0 Hz, H-9), 3.60 (1H, dd, *J* = 14.4, 8.8 Hz, H-18′), 3.49 (1H, dd, *J* = 14.8, 3.2 Hz, H-8), 3.42 (1H, ddd, *J* = 8.4, 8.4, 12.0 Hz, H-15), 3.19 (1H, dd, *J* = 14.8, 4.8 Hz, H-8′), 2.97 (1H, ddd, *J* = 2.0, 9.2, 12.0 Hz, H-15′), 2.18 (1H, dd, *J* = 10.8, 6.4 Hz, H-12), 1.89 (1H, br ddd, *J* = 6.4, 6.4, 12.8 Hz, H-13), 1.73 (3H, brs, H-21), 1.70 (1H, m†, H-14), 1.67 (3H, br. s, H-22), 1.56 (1H, m, H-13′), 1.14 (1H, m, H-14′). †This signal was buried between the methyl group singlets. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.54 (s, C-17*), 165.62 (s, C-11*), 137.93 (s, C-7a****), 136. 01 (s, C-20****), 127.23 (s, C-3a), 123.96 (d, C-2), 122.42 (d, C-5***), 119.74 (d, C-6***), 118.90 (d, C-4**), 118.39 (d, C-19), 111.11 (d, C-7**), 109.31 (s, C-3), 61.68 (d, C-9), 57.88 (d, C-12), 44.76 (t, C-15), 41.15 (t, C-18), 29.18 (t, C-13), 27.06 (t, C-8), 25.82 (q, C-21), 21.57 (t, C-14), 17.87 (q, C-22) (assignments for signals with the same superscript may be interchanged). IR (neat, NaCl): 3281, 3069, 2979, 2027, 2879, 1667, 1659, 1651, 1637, 1455, 1338, 1256, 1252, 1215, 1154, 1105, 1010, 971, 921, and 741 cm^{-1}. HRMS (FAB+) calcd for $C_{21}H_{26}N_3O_2$: 352.2025; found 352.2020 (M + H).

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15. The relative stereochemistry of this compound was determined by extensive ^{1}H NMR NOE studies.
16. The two rotamers can actually be separated by successive elution of 1:1 hexanes:ethyl acetate by silica gel PTLC. The two compounds were found to be rotamers and not diastereomers since each rotamer was converted to compound **9** by treatment with dimethylamine. The product (**9**) from each rotamer displayed identical TLC mobility, ^{1}H NMR and ^{13}C NMR spectra.