Synthesis and Stereochemistry of Axinastatin 4

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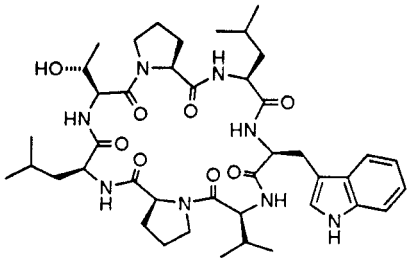
Axinastatin 4 from a marine sponge was synthesized by high-dilution BOP-Cl cyclization of Trp-Val-Pro-Leu-Thr-Pro-Leu in 94% yield (only 2.5% at normal dilution), showing the configurations of the last three amino acids to be *S*. Synthetic axinastatin 4 was devoid of cytostatic activity.

The cyclic heptapeptide axinastatin 4 (**1**, 6.1 mg, 10^{-6}% yield) was isolated from 600 kg of the marine sponge *Axinella* cf. *carteri* by Pettit et al. and characterized as *cyclo*-(Pro-Leu-Thr-Pro-Leu-Trp-Val) from MS and NMR data.[1] The configurations of the Leu and Pro units were determined to be *S*, but the configurations in the Trp, Val, and Thr units remained unknown. We now report the synthesis of axinastatin 4 (**1**).



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Using *S* amino acids, six of the seven linear heptapeptides (all except Leu-Thr-Pro-Leu-Trp-Val-Pro) that could cyclize to axinastatin 4 (**1**) were prepared by solid-phase peptide synthesis in 7−26% yields after HPLC. Initial attempts to cyclize these with *bis*(2-oxo-3-oxazoladinyl)phosphinic chloride (BOP-Cl),[2] (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP),[3] 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), and 1,3-diisopropylcarbodiimide[4] gave 0−6% yields of **1**. A procedure using high-dilution techniques was then devised that gave **1** in 94% yield from Trp-Val-Pro-Leu-Thr-Pro-Leu with BOP-Cl; the yield was only 40% when half as much solvent was used. The synthetic material was identical to the natural material by ^{1}H NMR, TLC, and HPLC. The most efficient procedure was to omit HPLC purification of the intermediate linear heptapeptide; thus, crude linear peptide (obtained in 89% yield) was cyclized in 65% yield (after HPLC) to axinastatin 4 (**1**).

This synthesis confirms the proposed structure **1** for axinastatin 4 and establishes for the first time the configurations in the Trp, Val, and Thr units as *S*. The synthetic material was not cytotoxic, suggesting that the activity observed for the natural material was due to a highly cytotoxic trace impurity, possibly synergistic with axinastatin 4 (**1**). Among related cyclic heptapeptides, synthetic stylopeptide 1[5] was similarly inactive, and synthetic axinastatins 2 and 3 possessed only 1/10−1/100 of the activity found for the natural products.[6]

Experimental Section

Linear Heptapeptides. Solid-phase peptide methods[7] employing Wang resin[8] and Fmoc amino acids were used. Thr was protected as its *tert*-butyl ether. Couplings with diisopropylcarbodiimide were monitored using bromophenol blue added directly to the beads.[9] The peptide was cleaved from the resin for 2 h at 25 °C with a 95:2.5:2.5 mixture of CF_3COOH, ethanedithiol, and anisole,[7] and purified using reversed-phase HPLC with a gradient from H_2O to 50% MeCN−H_2O.

Axinastatin 4 (1). Using oven-dried glassware under dry argon in a 250-mL round-bottom flask, linear heptapeptide Trp-Val-Pro-Leu-Thr-Pro-Leu TFA salt (4.2 mg, 0.0051 mmol) and 0.1 mL iPr_2EtN in 25 mL of CH_2Cl_2 (dried over mol sieves) were added dropwise over 3 h to a magnetically stirred solution of BOP-Cl (20 mg, 0.078 mmol, TCI America) in 25 mL of CH_2Cl_2 at 0 °C. After two additional days at 25 °C, the solvent was evaporated, the residue was taken up in EtOAc (5 mL), and the solution was washed 2 × 2 mL H_2O and 2 × 2 mL saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated. Analytical HPLC showed the residue to contain 4.0 mg (94%) of axinastatin 4 (**1**). The residue was purified by preparative HPLC (H_2O → CH_3CN over 30 min, t_R 26 min) to give material identical with natural **1** by ^{1}H NMR, TLC (R_f 0.44 in 10% MeOH−CH_2Cl_2), and HPLC.

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References and Notes

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