Synthesis of the cyclic heptapeptides Axinastatin 2 and Axinastatin 3[1]

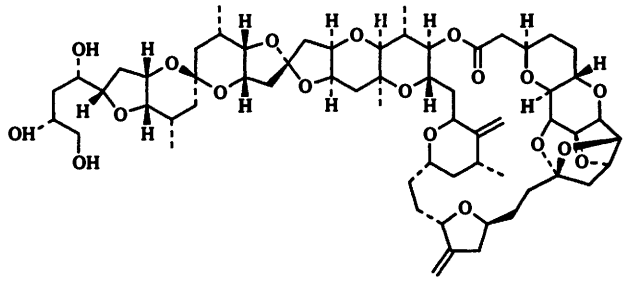
George R. Pettit,* Jeffrey W. Holman and Gerard M. Boland

Cancer Research Institute, Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604 USA

Practical total syntheses of axinastatins 2 2b and 3 2c were completed by employing Fmoc protection for the N-terminal, and *tert*-butyl ester blocking for the C-terminal, units of the amino acid and peptide intermediates. Generally, diethyl phosphorocyanidate proved effective for formation of the peptide bond, and in the one exception, asparagine, the *o*-nitrophenyl active ester proved to be useful. For the final cyclization reaction BOP-Cl was found especially effective.

Introduction

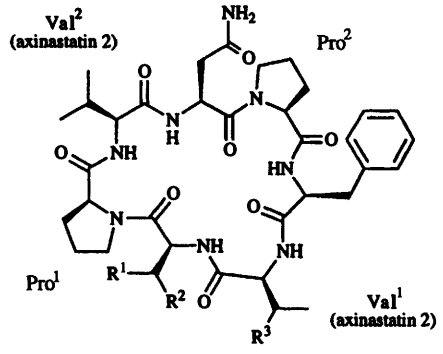
While searching for powerful antineoplastic constituents of an *Axinella* sp. (marine Porifera) from the Republic of Palau, we isolated (10^{-6} to 10^{-7}% yields) the remarkably potent (log molar GI_{50} −8 to −10) polyethers halichondrin B **1** and homo-



1 Halichondrin B

halichondrin B.[2] One of the major difficulties encountered in these polyether isolations was separation from trace (~ 10^{-6}%) cyclic peptide constituents such as the cycloheptapeptides axinastatins 1 **2a**,[2] 2 **2b**[2,3a] and 3 **2c**.[3a] When these peptides were finally obtained pure [evidenced by TLC, HPLC, 500 MHz NMR and high-resolution fast-atom bombardment mass spectrometry (HRFABMS) studies] their structures were determined and each was evaluated against a selection of animal (P388 lymphocytic leukaemia) and human cancer (*e.g.*, ovarian and colon) cell lines. All were found to be cell growth inhibitory in the range GI_{50} 0.1–0.01 μg ml^{-1}. Only about 6 mg each of axinastatins 2 and 3 was isolated. Thus, for extended biological evaluations, it became necessary to devise practical total syntheses of both peptides. Our earlier concern[3a] that axinastatins 2 and 3 might in some manner be serving as a carrier for a halichondrin present at a level too small to detect by our best physical and chemical techniques further enhanced the need for specimens **2b** and **2c** prepared by synthesis. For parallel reasons, we have also begun synthesis of axinastatin 4 obtained from an Indian Ocean *Axinella* sp. halichondrin fraction.[3b] In the present contribution, we have described the solution-phase synthesis of axinastatins 2 **2b** and 3 **2c**.

For synthesis of cyclic peptides **2b** and **2c** we used a modification[4a,b] of a relatively new solution-phase strategy which involved fluoren-9-ylmethoxycarbonyl (Fmoc) N-terminal protection, *tert*-butyl protection at the C-terminus, and formation of a peptide bond with diethyl phosphorocyanidate (DEPC), a method we have also used to obtain stylopeptide 1.[4c] Extension of the peptide chain was accomplished in a stepwise manner beginning with *tert*-butylproline. Due to the low yields



2a Axinastatin 1 ($R^1 = R^2 = R^3 = CH_3$)
2b Axinastatin 2 ($R^1 = Pr^i$, R^2 =H, $R^3 = CH_3$)
2c Axinastatin 3 ($R^1 = Pr^i$, R^2 =H, $R^3 = CH_2CH_3$)

reported[5] for the direct esterification of proline with 2-methylpropene, we prepared (62% yield) the previously unreported *N*-Fmoc-Pro-OBu^t **3** by the acid-catalysed reaction of Fmoc-proline with 2-methylpropene.[6] Cleavage of the Fmoc group was performed with diethylamine in acetonitrile. The product was used in the subsequent coupling step without further purification. The DEPC-promoted coupling between Pro-OBu^t and *N*-Fmoc-Leu was investigated using various conditions. Shiori[7] has reported DEPC-mediated couplings in dimethylformamide (DMF) using triethylamine as the base and *N*-Boc- or -Z-protection. Two subsequent studies showed[8,9] that triethylamine caused significant cleavage when *N*-Fmoc protection was employed. However, diisopropylethylamine (DIEA) was found[8,9] to have little effect on the Fmoc group. Furthermore, the stability of the Fmoc group towards DIEA has been demonstrated[10] recently using a series of solution-phase benzotriazol-1-yloxy(tripyrrolidine)phosphonium hexafluorophosphate (pyBOP) coupling reactions with *N*-Fmoc-amino acids.

When two mole equivalents of DIEA were used, the dipeptide **4** was obtained in 62% yield. However, the yield was not reproducible and this may have been due to the kinetics of the cleavage of the Fmoc group by the base. For this reason we chose to use a heterogeneous system in order to provide a small but steady concentration of base. Thus, the base was changed to anhydrous sodium carbonate and the solvent to dichloromethane. A similar procedure was reported by Veber and colleagues[11a] who performed a diphenylphosphoryl azide coupling step in the presence of sodium hydrogen carbonate. Since we assumed the use of this base would be detrimental, the reaction of Pro-OBu^t with *N*-Fmoc-Leu was accomplished

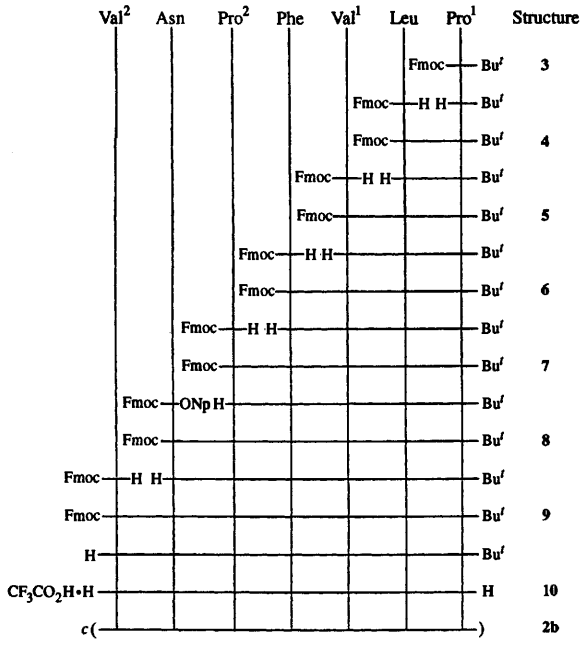


Fig. 1 Synthesis of axinastatin 2 **2b**

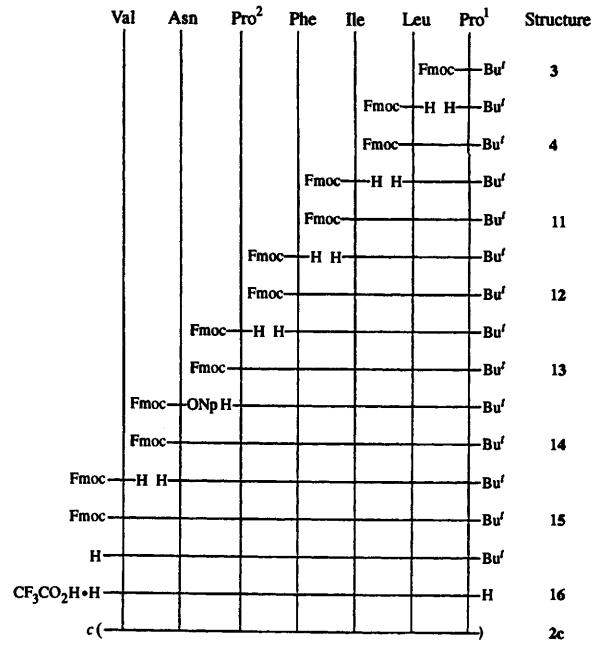


Fig. 2 Synthesis of axinastatin 3 **2c**

(89% yield) in dichloromethane using two mole equivalents of DEPC and three mole equivalents of sodium carbonate. At this point the syntheses of axinastatins 2 and 3 became divergent and will be discussed separately.

In the approach to axinastatin 2 (Fig. 1) peptides **5–7** were prepared in high yield using the new DEPC–sodium carbonate coupling procedure. In each case the product appeared diastereomerically pure by both ^{1}H and ^{13}C NMR spectroscopy, implying that either the diastereomer(s) formed by partial racemization were separated in the chromatographic step and/or the coupling reactions proceeded with an insignificant degree of racemization. The structures of the peptides were confirmed by UV, IR, ^{1}H and ^{13}C NMR spectroscopy combined with low- and high-resolution FAB mass spectral studies and elemental analyses. Except for reactions involving asparagine, the synthesis of axinastatin 2 progressed very efficiently. The coupling of N^{α}-Fmoc-Asn to peptide **7** to give hexapeptide **8** proved troublesome and gave a consistently low (28%) yield. Although Fmoc-Asn is known [12] to undergo dehydration of the unprotected amide in carbodiimide-activated peptide-bond-forming reactions, the use of active esters results in minimal side-chain dehydration.[13,14] The application of Asn *p*-nitrophenyl esters has provided useful examples.[14] Thus, Fmoc-peptide **7** was deprotected and allowed to react with N^{α}-Fmoc-Asn *o*-nitrophenyl ester under the conditions described by Bodanszky [15] to afford peptide **8** in 63% yield.

Cyclization of heptapeptide **9** to provide axinastatin 2 was accomplished as follows. The Fmoc protecting group was removed followed by cleavage of the OBu^t ester group with trifluoroacetic acid (TFA) to provide TFA salt **10**. The cyclization of compound **10** was realized using a procedure developed by Bates [16] for synthesis of similar cyclic heptapeptides. By this means, a solution of peptide **10** and DIEA in dichloromethane was added dropwise to a solution of BOP-Cl [17] in the same solvent. After one week at room temperature, the product **2b** was isolated (30% yield). The synthetic axinastatin 2 **2b** was identical by TLC, high-field (500 MHz) ^{1}H NMR and IR spectral measurements with the natural product. The optical rotation of the synthetic sample was significantly more negative, -217 *vs.* $-153° \times 10^{-1}$ cm^2 g^{-1}, than that of the natural product, which points to the possibility of the latter being associated with a chemically undetected contaminant or complex (see below).

For synthesis of axinastatin 3 (Fig. 2), peptides **11–13** and **15** were prepared by the procedure employed for preparation of axinastatin 2. Hexapeptide **14** was formed in good yield using the *o*-nitrophenyl ester of N^{α}-Fmoc-Asn. Interestingly, many of the resonances in the ^{13}C NMR spectra of peptides **11–15** were accompanied by smaller (~15%) peaks. We have previously demonstrated [18,19] that an isoleucine-derived unit can readily lead to conformational isomers observable in the ^{1}H and ^{13}C NMR spectra. Peptide **15** was deprotected using diethylamine followed by TFA (in dichloromethane) to give amino acid **16**, which was subjected to the cyclization reaction (see above) to give axinastatin 3 in 23% yield (after chromatographic purification). The synthetic peptide **2c** was found (TLC, HPLC, IR and ^{1}H NMR) to be identical with the natural product. In this case the optical rotations of the synthetic sample of axinastatin 3 and the natural product, -190 *vs.* -185×10^{-1} deg cm^2 g^{-1} respectively, were quite comparable.

Biological evaluation of the synthetic axinastatin 2 and axinastatin 3 against the P388 lymphocytic leukaemia cell line employing the same procedures as used earlier with the natural cyclic peptides showed cell growth inhibitory activity about 10- to 100-fold less (*e.g.*, GI_{50} 5.0 μg ml^{-1} for synthetic peptide **2c**) as compared with the natural products. The result of these biological experiments was further evidence that such natural cyclic peptides are able to complex with or otherwise carry trace (too small for usual NMR and chromatographic detection) amounts of exceptionally potent antineoplastic compounds such as the sponge halichondrins/halistatins.[3a,20] Additional biological studies of axinastatins 2 and 3 are under way.

Experimental

The Fmoc L-amino acids, N^{α}-Fmoc-L-asparagine-ONp ester, and diethyl phosphorocyanidate (DEPC) were obtained from Sigma-Aldrich Co. and used without further purification. The *N*,*N*-bis(2-oxooxazolidin-3-yl)phosphorodiamidic chloride (BOP-Cl) was supplied by TCI America. All solvents were redistilled prior to use. Acetonitrile and dichloromethane were dried over 4 Å-type molecular sieves. Anhydrous DMF was redistilled from ninhydrin. All reactions were carried out in dry glassware under argon. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Solvents were removed on a rotary evaporator with a bath temp < 30 °C. Unless otherwise noted, the penultimate or final isolation step

for each peptide involved flash chromatography on silica gel and the respective elution solvent is given for each new compound. Commercial TLC silica gel plates were obtained from Analtech, Inc. The TLC plates were visualized at 254 nm or by dipping in 3% cerium(IV) sulfate in 1.5 M H_2SO_4 followed by charring. Column chromatography was performed using 230–400 mesh ASTM silica from EM Science.

Mps were recorded on a Kofler hot stage or Electrothermal 9100 apparatus and were uncorrected. The ^1H NMR spectra were recorded in the stated solvent, employing either a Varian Gemini-300 or Varian VXR-500S instrument at 300 and 500 MHz, respectively. In addition, the ^1H NMR spectral assignments were determined with the aid of ^1H–^1H homonuclear chemical-shift correlation (COSY) spectra which were obtained with the VXR-500S instrument. *J* Values are given in Hz. All the ^{13}C NMR spectra were recorded in the solvent noted with the Gemini-300 instrument at 75.5 MHz. The ^{13}C NMR spectral assignments were decided with the aid of either attached proton test (APT) or distortionless enhancement by polarization transfer (DEPT) spectra which were obtained with either the VXR-500S or the Gemini-300 instrument. UV spectra were recorded in chloroform with a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer. Optical rotations were obtained with a Perkin-Elmer 241 polarimeter at ambient temperature, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Mattson Instruments 2020 Galaxy FTIR instrument. EI mass spectra were recorded using a FINNIGAN MAT312 (70 eV). FABMS measurements were made at the Midwest Center for Mass Spectrometry, Lincoln, NE. Elemental analyses were determined at Galbraith Laboratories, Inc., Knoxville, TN.

tert-Butyl *N*-Fmoc-L-prolinate 3

A solution composed of *N*-Fmoc-L-proline (10.0 g, 30 mmol), conc. sulfuric acid (0.3 ml) and dry dichloromethane (60 ml) in a pressure bottle was cooled to −72 °C and liquified 2-methylpropene (50 ml, −0.4 mol) was added dropwise (*via* cannula). After the addition was complete, the bottle was securely sealed with a septum and the solution was stirred at room temperature for 17 h. Upon cooling of the mixture to −72 °C, the septum was carefully removed and the mixture was vigorously stirred at room temperature for 1 h to remove the excess of 2-methylpropene. The solution was carefully poured into saturated aq. sodium hydrogen carbonate (30 ml) and the organic layer was washed successively with saturated aq. sodium hydrogen carbonate (2 × 20 ml), water (until neutral, 3 × 20 ml) and brine. Removal of the solvent *in vacuo* gave a solid, which was separated by flash chromatography on silica gel (2:2:1 hexane–CH_2Cl_2–EtOAc) to afford a solid, which was recrystallized from methanol to give ester **3** (7.3 g, 62%) as needles, mp 109–110 °C; R_f 0.60 (1:1 EtOAc–hexane), R_f 0.68 (98:2 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ −90 (*c* 0.25, $CHCl_3$); λ_{max}(CH-Cl_3)/nm (log ε) 234 (3.85), 266 (4.30), 289 (3.78) and 300 (3.84); ν_{max}(Nujol)/cm^{-1} 1728, 1709, 1159, 1123 and 743; δ_C(75.5 MHz; $CDCl_3$) (two conformers) 23.0/23.9 (Pro γ), 27.7 (^{t}Bu CH_3), 29.6/30.8 (Pro β), 46.2/46.8 (Pro δ), 47.0/47.1 (Fmoc CH), 59.4/59.86 (Pro α), 67.2/67.5 (Fmoc CH_2), 81.1/81.3 (^{t}Bu C), 120.0 (Fmoc ArCH), 125.2 (Fmoc ArCH), 125.3 (Fmoc ArCH), 125.5 (Fmoc ArCH), 127.1 (Fmoc ArCH), 127.2 (Fmoc ArCH), 127.7 (Fmoc ArCH), 141.3/141.4 (Fmoc ArC), 143.8/144.1 (Fmoc ArC), 144.3/144.5 (Fmoc ArC), 154.7/154.9 (carbamate CO) and 172.0/172.2 (ester CO); δ_H(300 MHz; $CDCl_3$) 1.44/1.47 [9 H, 2 s (two conformers), ^{t}Bu], 1.85–2.35 (5 H, complex), 3.45–3.80 (2 H, complex), 4.15–4.50 (3 H, complex), 7.32 (2 H, t, *J* 7.3), 7.40 (2 H, t, *J* 7.3), 7.61 (2 H, t, *J* 7.3 and 7.77 (2 H, d, *J* 7.3); *m/z* (EI) 393 (1%, M^+), 320 (1, M^+ − ^{t}BuO), 292 (12, M^+ − ^{t}BuOCO), 178 (100, dibenzofulvene), 165 (40), 152 (24), 114 (35), 89 (9), 70 (75) and 57 (75) (Found: C, 73.7; H, 7.0; N, 3.55. Calc. for $C_{24}H_{27}NO_4$: C, 73.26; H, 6.92; N, 3.56%).

Generally, ester **3** did not require chromatographic purification and the crude product was recrystallized from methanol.

General procedure for peptide-bond-forming reactions

A solution of the *N*-Fmoc amino acid or peptide *tert*-butyl ester (25.4 mmol) in dry acetonitrile (200 ml) was stirred under argon at room temperature and diethylamine (200 ml) was added. After 1 h, the excess of diethylamine and acetonitrile were removed, a fresh aliquot of acetonitrile (100 ml) was added, and the mixture was evaporated to dryness. To another flask were added the appropriate Fmoc amino acid (25.4 mmol) and dry dichloromethane (250 ml). The solution was cooled to −5 °C and stirred while DEPC (4.25 ml, 28.0 mmol) was added. After the mixture had been stirred for 10 min at 0 °C, a dry dichloromethane (150 ml) solution of the unit from *N*-Fmoc deprotection was added *via* cannula. Anhydrous sodium carbonate (8.1 g, 76.2 mmol) was added and the reaction mixture was stirred under argon and allowed to warm to room temperature over a period of 3 h. A further aliquot of DEPC (4.25 ml, 28.0 mmol) was added and the solution was stirred at room temperature for another 2 h. The solution was filtered and the filtrate was evaporated to dryness to give a viscous oil (generally) which was purified as described in the following experiment.

***N*-Fmoc-Leu-Pro-Bu^t 4.** Final isolation as noted above (General section) was by flash chromatography on silica gel (2:1 hexane–EtOAc as eluent) to give dipeptide **4** (11.5 g, 89%) as a foamy solid, mp 50–52 °C; R_f 0.44 (hexane–EtOAc 1:1); $[\alpha]_D^{20}$ −59 (*c* 1.04, $CHCl_3$); λ_{max}(log ε)/nm 232 (3.93), 266 (4.32), 288 (3.78) and 300 (3.84); ν_{max}(Nujol)/cm^{-1} 3200, 1736, 1720, 1641, 1252, 1223, 1154 and 739; δ_C(75.5 MHz; $CDCl_3$) 21.9 (Leu γ), 23.7 (Pro γ), 24.8 (Leu δ), 25.1 (Leu δ), 28.2 (^{t}Bu CH_3), 29.3 (Pro β), 42.4 (Leu β), 47.1 (Pro δ), 47.5 (Fmoc CH), 51.2 (Leu α), 60.0 (Pro α), 67.4 (Fmoc CH_2), 81.7 (^{t}Bu C), 120.5 (Fmoc ArCH), 125.8 (Fmoc ArCH), 127.6 (Fmoc ArCH), 128.2 (Fmoc ArCH), 141.9 (Fmoc ArC), 144.5 (Fmoc ArC), 144.6 (Fmoc ArC), 157.0 (urethane CO), 171.8 (amide or ester CO) and 172.0 (ester or amide CO); δ_H(300 MHz; $CDCl_3$) 0.97 (3 H, d, *J* 6.7, Leu δ), 1.035 (3 H, d, *J* 6.7, Leu δ), 1.46 (9 H, s, ^{t}Bu), 1.56 (1 H, septet, *J* 6.7, Leu γ), 1.73–2.24 (6 H, complex, Pro β, Pro γ and Leu β), 3.53–3.63 (1 H, complex, Pro δ), 3.69–3.78 (1 H, complex, Pro δ), 4.21 (1 H, t, *J* 6.8, Fmoc CH), 4.34 (2 H, d, *J* 6.8, Fmoc CH_2), 4.41 (1 H, dd, *J* 8.7 and 4.6, Pro α), 4.57 (1 H, dt, *J* 9.0 and 4.5, Leu α), 5.53 (1 H, d, *J* 9.0, Leu NH), 7.31 (2 H, tt, *J* 7.5 and 1.5, Fmoc ArH), 7.40 (2 H, t, *J* 7.5, Fmoc ArH), 7.60 (2 H, d, *J* 7.5, Fmoc ArH) and 7.760 (2 H, d, *J* 7.5, Fmoc ArH); *m/z* (EI) 506 (2%, M^+), 433 (3, M^+ − ^{t}BuO), 394 (1), 336 (1), 308 (6), 264 (4), 209 (4) and 178 (100, dibenzofulvene) (Found: C, 70.95; H, 7.7; N, 5.7. Calc. for $C_{30}H_{38}N_2O_5$: C, 71.12; H, 7.56; N, 5.53%).

***N*-Fmoc-Val-Leu-Pro-OBu^t 5.** Chromatographic separation (95:5 hexane–Pr^{i}OH) gave peptide **5** (12 g, 96%) as a foamy solid, mp 92–96 °C; R_f 0.50 (1:1 hexane–EtOAc); $[\alpha]_D^{20}$ −60 (*c* 1.02, $CHCl_3$); λ_{max}(log ε)/nm 229 (3.91), 267 (4.22), 289 (3.68) and 300 (3.74); ν_{max}(Nujol)/cm^{-1} 3292, 1736, 1728, 1638, 1534, 1152, 1031 and 739; *m/z* (FAB) 606 (37%, $[M + H]^+$), 384 (73) and 277 (100) {HRMS(FAB) Found: $[M + H]^+$, 606.3547. Calc. for $C_{35}H_{48}N_3O_6$: [M + H], 606.3543} (Found: C, 69.2; H, 8.0; N, 6.8. Calc. for $C_{35}H_{47}N_3O_6$: C, 69.40; H, 7.82; N, 6.94%).

***N*-Fmoc-Phe-Val-Leu-Pro-OBu^t 6.** Chromatography and elution with 2:3 hexane–ethyl acetate gave tetrapeptide **6** (5.8 g, 92%) as a foamy solid, mp 129–131 °C; R_f 0.29 (2:3 hexane–EtOAc), R_f 0.24 (9:1 hexane–Pr^{i}OH); $[\alpha]_D^{20}$ −57 (*c* 1.18, $CHCl_3$); λ_{max}(log ε)/nm 232 (3.99), 257 (4.24), 267 (4.27), 289 (3.74) and 300 (3.79); ν_{max}(Nujol)/cm^{-1} 3273, 1738, 1703, 1636, 1537, 1152 and 739; *m/z* (FAB) 753 (47%, $[M + H]^+$), 582 (25), 469 (48) and 179 (100) {HRMS(FAB) Found: $[M + H]^+$, 753.4241. Calc. for $C_{44}H_{57}N_4O_7$: [M + H], 753.4227}

(Found: C, 69.8; H, 7.7; N, 7.6. Calc. for $C_{44}H_{56}N_4O_7$: C, 70.19; H, 7.50; N, 7.44%).

***N*-Fmoc-Pro-Phe-Val-Leu-Pro-OBu^t 7.** Elution with 1:7 hexane–ethyl acetate followed by crystallization from methanol–water led to peptide **7** (3.5 g, 77%) as a solid, mp 144–146 °C; R_f 0.23 (1:7 hexane–EtOAc); $[\alpha]_D^{23}$ −93 (*c* 1.09, $CHCl_3$); λ_{max}(log ε)/nm 229 (3.95), 267 (4.26), 289 (3.70) and 300 (3.76); ν_{max}(neat)/cm^{-1} 3295, 1736, 1707, 1638, 1545, 1233, 1154, 1123, 758 and 741; *m/z* (FAB) 850.5 (5%, $[M + H]^+$), 794 (1), 679 (4), 628 (7), 467 (9), 229 (12), 179 (100) and 116 (34) {HRMS(FAB) Found: $[M + H]^+$, 850.4758 [Δ 0.3 ppm]. Calc. for $C_{49}H_{64}N_5O_8$: $[M + H]$, 850.4754} (Found: C, 69.3; H, 7.5; N, 8.2. Calc. for $C_{49}H_{63}N_5O_8$: C, 69.24; H, 7.47; N, 8.24%).

***N*-Fmoc-Asn-Pro-Phe-Val-Leu-Pro-OBu^t 8.** A solution of compound **7** (703 mg, 0.83 mmol) and diethylamine (30 ml) in dry acetonitrile (30 ml) was stirred at room temperature under argon. After 1 h, the excess of diethylamine and acetonitrile were removed (bath temp. < 30 °C), a fresh aliquot of acetonitrile (100 ml) was added and the solution was evaporated to dryness (1.5 h at 0.05 mmHg). Anhydrous DMF (8 ml) was added to the residue and the mixture was stirred under argon until all of the solid had dissolved (*ca.* 15 min). Next, *N*-hydroxybenzotriazole (HOBt) (115 mg, 0.85 mmol) followed by N^{α}-Fmoc-Asn-ONp (523 mg, 1.10 mmol) were added and the solution was stirred under argon at room temperature for 2.5 h. The solvent was removed and separation of the residue by flash chromatography on silica gel (EtOAc) was followed by trituration with diethyl ether (20 ml). The precipitate thus formed was collected, and dried under vacuum (0.05 mmHg for 3 h) to give hexapeptide **8** as a solid (0.50 g, 63%), mp 170–173 °C; R_f 0.12 (ethyl acetate); $[\alpha]_D^{20}$ −93 (*c* 1.0, $CHCl_3$); λ_{max}(log ε)/nm 228 (3.99), 267 (4.27), 289 (3.71) and 300 (3.76); ν_{max}(Nujol)/cm^{-1} 3383, 3341, 3287, 3190, 1723, 1661, 1636, 1534, 1242, 1155, 1030 and 743; *m/z* (FAB) 986.5 (100%, $[M + Na]^+$), 884 (4), 793 (11), 680 (19), 581 (14), 434 (12) and 133 (14) {HRMS(FAB) Found: $[M + H]^+$, 986.5001. Calc. for $C_{53}H_{69}N_7O_{10}Na$: $[M + Na]$, 986.5003} (Found: C, 65.7; H, 7.25; N, 10.0. Calc. for $C_{53}H_{69}N_7O_{10}$: C, 66.02; H, 7.21; N, 10.17%).

***N*-Fmoc-Val-Asn-Pro-Phe-Val-Leu-Pro-OBu^t 9.** The fraction eluted with 99:1 ethyl acetate–methanol was triturated with diethyl ether to give peptide **9** (234 mg, 69%) as a solid, mp 211–213 °C; R_f 0.19 (EtOAc–MeOH, 99:1); $[\alpha]_D^{20}$ −86 (*c* 1.0, $CHCl_3$); λ_{max}(log ε)/nm 230 (4.02), 267 (4.29), 289 (3.73) and 300 (3.79); ν_{max}(neat)/cm^{-1} 3304, 1724, 1643, 1530, 1242, 1155 and 756; δ_C(75.5 MHz; CD_3OD) 19.0 (Val^2 γ), 19.3 (Val^1 γ), 19.9 (Val^2 γ), 20.0 (Val^1 γ), 22.4 (Leu δ), 23.8 (Leu δ), 25.1 (Pro^2 γ), 25.9 (Leu γ), 26.0 (Pro^1 γ), 28.4 (^{t}Bu CH_3), 30.2 (Pro^1 β), 30.6 (Pro^2 β), 32.1 (Val^2 β), 32.3 (Val^1 β), 37.4 (Phe β), 38.4 (Asn β), 41.6 (Leu β), 48.4 (Pro^2 β), 48.6 (Fmoc CH), 49.0 (Pro^1 δ), 49.1 (Val^2 α), 51.0 (Asn α), 57.3 (Phe α), 60.5 (Pro^2 α), 61.6 (Val^1 α), 62.3 (Leu α), 62.5 (Pro^1 α), 68.3 (Fmoc CH_2), 82.9 (^{t}Bu C), 121.5 (Fmoc ArCH), 126.8 (Fmoc ArCH), 128.2 (Ph *p*-CH), 128.8 (two overlapping resonances, Ph *m*-CH), 129.4 (Fmoc ArCH), 130.0 (Fmoc ArCH), 130.9 (Ph *o*-CH), 140.2 (Ph C), 143.2 (Fmoc ArC), 145.7 (Fmoc ArC), 145.9 (Fmoc ArC), 159.2 (carbamate CO), 173.1 (ester or amide CO), 173.4 (ester or amide CO), 173.6 (ester or amide CO), 173.7 (ester or amide CO), 174.4 (ester or amide CO), 174.5 (ester or amide CO), 174.8 (ester or amide CO) and 175.5 (ester or amide CO); δ_H(300 MHz; $CDCl_3$) 0.90–0.956 (18 H, overlapping d, Val^1 γ, Val^2 γ and Leu δ), 1.40 (9 H, s, ^{t}Bu), 1.57 (2 H, t, *J* 6.8, Leu β), 1.65–1.79 (3 H, complex, Pro^1 β, Pro^2 β and Leu γ), 1.86–2.19 (7 H, complex, Val^2 β, Pro^2 γ, Pro^1 γ, Pro^2 β, and Pro^1 β), 2.42 (1 H, distorted septet, *J* 6.8, Val^1 β), 2.63 (1 H, dd, A part of ABX, *J* 14.8 and 4.1, Asn β), 2.94 (1 H, dd, B part of ABX, *J* 8.1 and 14.8, Asn β), 3.04 (1 H, dd, A part of ABX, *J* 13.9 and 12.2, Phe β), 3.37 (1 H, dd, B part of ABX, *J* 13.9 and 4.0, Phe β), 3.52–3.79 (4 H, complex, Pro^1 δ and Pro^2 δ), 3.95 (1 H, t, *J* 6.8, Val^2 α), 4.01 (1 H, distorted dt, *J* 7.4 and 1.1, Val^1 α), 4.22 (1 H, t, *J* 6.9, Fmoc CH), 4.31–4.45 (4 H, overlapping m, Fmoc CH_2, Pro^1 α and Pro^2 α), 4.62–4.72 (2 H, overlapping m, Leu α and Phe α), 5.02 (1 H, dt, *J* 7.5 and 4.0, Asn α), 5.46 (1 H, br d, *J* 7.5, Val^2 NH), 6.27 (1 H, br s, Asn NH), 6.65 (1 H, br s, Asn NH), 7.08 (1 H, br d, *J* 4.4, Asn NH), 7.18–7.30 (7 H, complex, PhH, Leu NH and Val^1 NH), 7.32 (2 H, partly obscured t, *J* 6.8, Fmoc ArH), 7.41 (2 H, t, *J* 7.2, Fmoc ArH), 7.52 (1 H, br d, *J* 8.4, Phe NH), 7.60 (2 H, d, *J* 6.7, Fmoc ArH) and 7.77 (2 H, d, *J* 7.2, Fmoc ArH); *m/z* (FAB) 1063.6 (32%, $[M + H]^+$), 892.5 (12), 779 (15), 680 (7), 572 (4), 533 (7), 436 (7), 344 (7) and 179 (100) {HRMS(FAB) Found: $[M + H]^+$, 1063.5882. Calc. for $C_{58}H_{79}N_8O_{11}$: $[M + H]$, 1063.5867} (Found: C, 65.4; H, 7.5; N, 10.45. Calc. for $C_{58}H_{78}N_8O_{11}$: C, 65.52; H, 7.39; N, 10.54%).

TFA–Val-Asn-Pro-Phe-Val-Leu-Pro 10. A solution of peptide **9** (37 mg, 0.035 mmol) in dry acetonitrile (10 ml) was stirred and diethylamine (10 ml) was added. The solution was stirred at room temperature for 1 h. The solvent was removed and TFA (2 ml) was added to the residue. The solution was kept under argon at room temperature for 1 h, excess of TFA was removed, diethyl ether (15 ml) was added and the solvent was removed. A further amount of diethyl ether (15 ml) was added and the precipitated solid was collected to yield TFA salt **10** (31 mg, 98%) as an off-white solid, which was used directly in the cyclization step.

Axinastatin 2 2b. A three-necked flask equipped with a 200 ml pressure-equalizing dropping funnel was cooled and dry dichloromethane (700 ml) and BOP-Cl (0.62 g, 2.45 mmol) were added under argon. The solution was cooled to 0 °C and part of a solution of peptide **10** (0.15 g, 0.167 mmol) and DIEA (4.7 ml) in dry dichloromethane (700 ml) was added to the dropping funnel *via* cannula. The peptide solution was added dropwise over a period of 1 h (the dropping funnel was filled periodically *via* cannula with the remaining solution). After complete addition, the solution was stirred at room temperature under argon for 7 days. The dichloromethane was removed and the residue was dissolved in ethyl acetate (200 ml)–water (100 ml). The ethyl acetate phase was washed successively with water (100 ml), saturated aq. sodium hydrogen carbonate (2 × 100 ml) and brine (50 ml) and dried over anhydrous sodium sulfate. The solvent was removed and the residue was separated by flash chromatography (twice) on silica gel and elution with 1:1 hexane–propan-2-ol followed by recrystallization from methanol–water to afford axinastatin **2** (39 mg, 30%) as a solid, mp 335–337 °C (decomp. 280 °C) compared with the natural sample, mp 280–282 °C, R_f 0.36 (1:1 hexane–Pr^{i}OH); $[\alpha]_D^{24}$ −217 (*c* 0.16, MeOH), {lit.,[3a] $[\alpha]_D^{25}$ −153 (*c* 0.17, MeOH)}; ν_{max}(NaCl)/cm^{-1} 3271, 2959, 2872, 1630, 1520, 1447 and 1414; δ_H(500 MHz; $CDCl_3$) 0.89 (3 H, d, *J* 6.5), 0.95 (9 H, m), 1.02 (6 H, dd, *J* 7.0 and 2.0), 1.14–1.32 (complex), 1.65–2.03 (complex), 2.23 (1 H, ddt, *J* 12.5, 6.5 and 2.0), 2.37 (1 H, hex, *J* 6.5), 2.59 (1 H, dd, *J* 11.5 and 6.5), 2.91 (1 H, dd, *J* 14.5 and 12.0), 3.11 (1 H, dd, *J* 13.5 and 5.5), 3.28 (1 H, dd, *J* 13.5 and 4.5), 3.44–3.52 (2 H, complex), 3.59 (1 H, br t, *J* 8.3), 3.67 (1 H, dt, *J* 12.5 and 4.0), 4.03 (1 H, dd, *J* 9.5 and 7.0), 4.16 (1 H, overlapping t, *J* 7.0), 4.18 (1 H, overlapping t, *J* 9.5), 4.34 (1 H, dt, *J* 12.5 and 3.0), 4.38 (1 H, d, *J* 7.0), 4.68 (1 H, poorly resolved dd, *J* 3.5), 4.77 (1 H, ddd, *J* 11.5, 9.5 and 4.5), 5.41 (1 H, br s), 6.24 (1 H, br s), 6.59 (1 H, br d, *J* 4.0), 7.17–7.24 (5 H, m, Ph), 7.51 (1 H, d, *J* 9.5), 7.96 (1 H, d, *J* 8.0) and 8.03 (1 H, d, *J* 4.5).

***N*-Fmoc-Ile-Leu-Pro-OBu^t 11.** Separation by flash chromatography on silica gel (5:4 hexane–EtOAc) followed by gravity chromatography on silica gel (same solvent mixture) gave tripeptide **11** as a foamy solid (6.5 g, 86%), mp 97–100 °C; R_f 0.33 (5:4 hexane–EtOAc); $[\alpha]_D^{25}$ −59 (*c* 1.05, $CHCl_3$); λ_{max}(log ε)/nm 230 (3.92), 266 (4.23), 289 (3.68) and 300 (3.74); ν_{max}(NaCl)/cm^{-1} 3293, 1736, 1703, 1640, 1537, 1244, 1154, 1036, 758 and 741 {HRMS(FAB) Found: $[M + H]^+$, 620.3698. Calc. for $C_{36}H_{50}N_3O_6$: $[M + H]$, 620.3699} (Found: C, 69.5; H, 8.2; N, 6.8. Calc. for $C_{36}H_{49}N_3O_6$: C, 69.76; H, 7.97; N, 6.78%).

***N*-Fmoc-Phe-Ile-Leu-Pro-OBu^t 12.** Tetrapeptide **12** was isolated by gradient column chromatography using 6:5→1:1 hexane–EtOAc to yield the pure peptide as a foamy solid (4.75 g, 64%). A 0.52 g sample was precipitated from methanol–water (*ca.* 1:3) to give a solid (0.50 g), mp 133–136 °C (softens at 119 °C); R_f 0.32 (hexane–EtOAc, 1:1), 0.34 (hexane–Pr^{i}OH, 93:7); $[\alpha]_D^{25}$ −59 (*c* 1.0, $CHCl_3$); λ_{max}(log ε)/nm 230 (3.96), 267 (4.26), 289 (3.69) and 300 (3.75); ν_{max}(Nujol)/cm^{-1} 3273, 1736, 1703, 1638, 1543, 1258, 1231 and 1154; *m/z* (FAB) 767 (73%, $[M + H]^+$), 711 (7), 596 (27), 483 (42) and 178 (100) {HRMS(FAB) Found: $[M + H]^+$, 767.4380. Calc. for $C_{45}H_{59}N_4O_7$: [M + H], 767.4383} (Found: C, 70.25; H, 7.9; N, 7.5. Calc. for $C_{45}H_{58}N_4O_7$: C, 70.47; H, 7.62; N, 7.30%).

***N*-Fmoc-Pro-Phe-Ile-Leu-Pro-OBu^t 13.** Chromatography (twice) using 1:4 hexane–ethyl acetate as eluent provided a total of 3.5 g (74%) of peptide **13** as a foamy solid. A sample was precipitated from methanol–water (*ca.* 1:3) to give pure peptide **13** as a solid, mp 148–151 °C; R_f 0.35 (1:7 hexane–EtOAc); $[\alpha]_D^{25}$ −96 (*c* 1.0, $CHCl_3$); λ_{max}(log ε)/nm 230 (3.97), 267 (4.28), 289 (3.69) and 300 (3.76); ν_{max}(NaCl)/cm^{-1} 3293, 1738, 1709, 1638, 1547, 1221, 1154, 758 and 743 {HRMS(FAB) Found: $[M + H]^+$, 864.4927. Calc. for $C_{50}H_{66}N_5O_8$: [M + H], 864.4911} (Found: C, 69.4; H, 7.6; N, 8.1. Calc. for $C_{50}H_{65}N_5O_8$: C, 69.50; H, 7.58; N, 8.11%).

***N*-Fmoc-Asn-Pro-Phe-Ile-Leu-Pro-OBu^t 14.** A solution composed of pentapeptide **13** (3.1 g, 3.59 mmol), diethylamine (60 ml) and dry acetonitrile (60 ml) was stirred under argon at room temperature for 1 h. The solvent and excess of diethylamine were removed *in vacuo*. Acetonitrile (20 ml) was added and the solvent was again evaporated off (*in vacuo* at 0.1 mmHg for 2 h). The residue was dissolved in dry DMF (44 ml) and HOBt (494 mg, 3.66 mmol) and N^{α}-Fmoc-Asn-ONp (2.22 g, 4.66 mmol) were added. The solution was stirred under argon at room temperature for 2 h. The DMF was removed at room temperature and the residue was separated by flash chromatography on silica (ethyl acetate as eluent) to give hexapeptide **14** (2.48 g, 71%) as a solid, mp 157–159 °C; R_f 0.31 (7:3 hexane–Pr^{i}OH); $[\alpha]_D^{25}$ −89 (*c* 1.0 $CHCl_3$); λ_{max}(log ε)/nm 229 (3.91), 267 (4.23), 289 (3.59) and 300 (3.67); ν_{max}(NaCl)/cm^{-1} 3306, 3212, 1723, 1645, 1530, 1246, 1154, 1034, 914 and 737 {HRMS(FAB) Found: $[M + H]^+$, 978.5336. Calc. for $C_{54}H_{72}N_7O_{10}$: [M + H], 978.5341} (Found: C, 65.6; H, 7.75; N, 9.8. Calc. for $C_{54}H_{71}N_7O_{10}$·0.5 H_2O: C, 65.70; H, 7.35; N, 9.93%).

***N*-Fmoc-Val-Asn-Pro-Phe-Ile-Leu-Pro-Obu^t 15.** Employing 95:5 CH_2Cl_2–MeOH as eluent followed by precipitation from dichloromethane–ethyl acetate gave the product **15** (1.2 g, 52%) as a solid, mp 210–211 °C; R_f 0.22 (EtOAc), 0.34 (97:3 EtOAc–MeOH); $[\alpha]_D^{25}$ −82 (*c* 1.0, $CHCl_3$); λ_{max}(log ε)/nm 229 (3.97), 267 (4.26), 289 (3.67) and 300 (3.74); ν_{max}(NaCl)/cm^{-1} 3304, 3065, 1723, 1659, 1530, 1242, 1155 and 736; δ_C(75.5 MHz; $CDCl_3$) 11.1 (Ile δ), 15.4 (Ile γ), 17.7 (Val γ), 18.9 (Val γ), 21.6 (Leu δ), 23.0 (Leu δ), 23.7 (Pro^2 γ), 24.4 (Leu γ), 24.6 (Pro^1 γ and Ile γ), 27.7 (^{t}Bu CH_3), 28.8 (Pro^1 β), 29.1 (Pro^2 β), 31.0 (Val β), 35.8 (Phe β and Ile β), 37.3 (Asn β), 40.5 (Leu β), 46.7 (Pro^1 δ), 46.9 (Fmoc CH), 47.3 (Pro^2 δ), 47.7 (Val α), 49.2 (Asn α), 55.6 (Phe α), 58.9 (Pro^2 α), 59.9 (Ile α), 60.1 (Leu α), 60.9 (Pro^1 α), 67.0 (Fmoc CH_2), 81.2 (^{t}Bu C), 120.1 (Fmoc ArCH), 125.2/125.3 (Fmoc ArCH), 126.6 (Ph *p*-CH), 127.2 (Ph *m*-CH), 127.9 (Fmoc ArCH), 128.4 (Fmoc ArCH), 129.3 (Ph *o*-CH), 138.4 (Ph C), 141.4 (Fmoc ArC), 144.0 (Fmoc ArC), 156.7 (carbamate CO), 171.1 (ester or amide CO), 171.5 (ester or amide CO), 171.7 (two signals, ester or amide CO), 172.3 (two signals, ester or amide CO) and 173.1 (ester or amide CO); δ_H(300 MHz; $CDCl_3$) 0.83–0.94 (18 H, complex, Val γ, Leu δ, Ile δ and Ile γ CH_3), 1.18–1.33 (2 H, complex, Ile γ CH_2), 1.40 (9 H, s, ^{t}Bu), 1.52–1.72 (4 H, complex, Pro β and Leu β), 1.86–2.18 (7 H, complex, Val β, Pro β and Pro γ), 2.67 (1 H, d, A part of ABX, *J* 13.1, Asn β), 2.95 (1 H, dd, B part of ABX, *J* 15.4 and 14.7, Asn β), 3.05 (1 H, t, A part of ABX, *J* 12.8, Phe β), 3.31 (1 H, d, B part of ABX, *J* 11.3, Phe β), 3.47–3.82 (4 H, complex, Pro δ), 4.11–4.47 (5 H, complex, Pro α, Fmoc CH_2 and Fmoc CH), 4.54–4.70 (3 H, complex, Asn α, Ile α, and Phe α), 4.95 (1 H, br m, Val α), 5.75 (1 H, br t, Asn NH), 6.28 (1 H, br s, Asn NH), 7.13–7.32 (11 H, complex Fmoc ArH, PhH, Asn NH, Phe NH, Ile NH, Leu NH), 7.39 (2 H, t, *J* 7.2, Fmoc ArH), 7.59 (2 H, d, *J* 7.2, Fmoc ArH) and 7.75 (2 H, *J* 7.2, Fmoc ArH) {HRMS(FAB) Found: $[M + H]^+$, 1077.6014. Calc. for $C_{59}H_{81}N_8O_{11}$: [M + H], 1077.6024} (Found: C, 64.9; H, 7.7; N, 10.2. Calc. for $C_{59}H_{80}N_8O_{11}$·H_2O: C, 64.68; H, 7.55; N, 10.23%).

TFA–Val-Asn-Pro-Phe-Ile-Leu-Pro 16. A solution of peptide **15** (512 mg, 0.53 mmol) in dry acetonitrile (100 ml) was stirred under argon and diethylamine (100 ml) was added. The solution was stirred at room temperature for 1 h and solvent was removed *in vacuo*. Dry dichloromethane (20 ml) and TFA (20 ml) were added and the remaining isolation of the TFA salt was conducted as summarized for obtaining TFA salt **10**.

Axinastatin 3 2c. The cyclization of heptapeptide **16** was effected using dry dichloromethane (230 ml), BOP-Cl (1.3 g, 5.10 mmol), peptide **16** (308 mg, 0.34 mmol) and DIEA (9.7 ml) as described above for producing axinastatin 2 **2b** to yield axinastatin 3 **2c** (61 mg, 23%), mp 337–340 °C (decomp. 307 °C); R_f 0.26 (9:1 EtOAc–MeOH); $[\alpha]_D^{25}$ −190 (*c* 0.22, MeOH) {lit.,[3a] $[\alpha]_D^{25}$ −185 (*c* 0.21, MeOH)}; ν_{max}(NaCl)/cm^{-1} 3312, 2963, 2876, 1636, 1524 and 1435; δ_H(300 MHz; $CDCl_3$) 0.86–0.95 (12 H, m), 1.02 (6 H, *J* 6.8), 1.39–1.49 (4 H, complex), 1.46–1.56 (1 H, m), 1.68–2.02 (13 H, complex), 2.24 (1 H, dddd, *J* 13.0, 7.5, 5.9 and 2.5), 2.37 (1 H, hex, *J* 6.8), 2.59 (1 H, dd, *J* 11.2 and 6.5), 2.92 (2 H, distorted t, *J* 11.8), 3.15 (1 H, dd, *J* 14.8 and 4.4), 3.25 (1 H, dd, *J* 13.9 and 4.8), 3.43–3.72 (4 H, complex), 4.05 (1 H, dd, *J* 9.8 and 7.7), 4.15 (1 H, dd, *J* 8.2 and 6.6), 4.28 (1 H, t, *J* 9.3), 4.33–4.40 (2 H, m), 4.67 (1 H, dd, *J* 9.7 and 5.9), 4.78 (1 H, ddd, *J* 11.4 and 4.7), 5.39 (1 H, br s), 6.45 (1 H, br s), 6.70 (1 H, br s), 7.16–7.28 (5 H, m), 7.55 (1 H, d, *J* 9.5), 7.96 (1 H, d, *J* 7.8) and 8.06 (1 H, d, *J* 4.5).

Acknowledgements

The research reported here was supported by Outstanding Investigator Grant CA-443440-1A1-07 awarded by the Division of Cancer Treatment, the U.S. National Cancer Institute, DHHS, the Robert B. Dalton Endowment Fund, Eleanor W. Libby, the Donald Ware Waddell Foundation, and the Arizona Disease Control Research Commission. We also wish to thank Drs Michael R. Boyd, Robin K. Pettit and Dr R. A. Nieman for other assistance.

References

1 Contribution 343 of the series Antineoplastic Agents. For part 342: Y. Kamano, H.-P. Zhang, A. Hino, M. Yoshida, G. R. Pettit, C. L. Herald and H. Itokawa, *J. Nat. Prod.*, 1995, **58**, 1868.

2 G. R. Pettit, C. L. Herald, M. R. Boyd, J. E. Leet, C. Dufrense, D. L. Doubek, J. M. Schmidt, R. L. Cerny, J. N. A. Hooper and K. C. Rützler, *J. Med. Chem.*, 1991, **34**, 3339.

3 (*a*) G. R. Pettit, F. Gao, R. L. Cerny, D. L. Doubek, L. P. Tackett, J. M. Schmidt and J.-C. Chapuis, *J. Med. Chem.*, 1994, **37**, 1165; (*b*) G. R. Pettit, F. Gao and R. L. Cerny, *Heterocycles*, 1993, **35**, 711.

4 (*a*) A. Trzeciak and W. Bannwarth, *Tetrahedron Lett.*, 1992, **33**, 4557; (*b*) J. Jiang, W.-R. Li and M. Joullié, *Synth. Commun.*, 1994, **24**, 187; (*c*) G. R. Pettit and S. R. Taylor, *J. Org. Chem.*, 1996, **61**, 2322.

5 R. Roeske, *J. Org. Chem.*, 1963, **28**, 1251.

6 G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, 1960, **82**, 3359.

7 Y. Hamada, S. Rishi, T. Shiori and S. Yamada, *Chem. Pharm. Bull.*, 1977, **25**, 224; T. Shiori and Y. Hamada, *Yakugaku Zasshi*, 1988, **180**, 1115 (*Chem. Abstr.*, 1989, **110**, 193336k).

8 M. Bodanszky, S. S. Deshmone and J. Martinez, *J. Org. Chem.*, 1979, **44**, 1622.

9 C.-D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E. O. Lundell and J. D. Haug, *Int. J. Pept. Protein Res.*, 1980, **15**, 59.

10 T. Høeg-Jensen, M. H. Jakobsen and A. Holm, *Tetrahedron Lett.*, 1991, **32**, 6387.
11 (*a*) S. F. Brady, R. M. Freidinger, W. J. Paleveda, C. D. Colton, C. F. Homnick, W. L. Whitter, P. Curley, R. F. Nutt and D. F. Veber, *J. Org. Chem.*, 1987, **52**, 764; (*b*) W.-R. Li, W. R. Ewing, B. D. Harris and M. M. Joullié, *J. Am. Chem. Soc.*, 1990, **112**, 7659; (*c*) S. Zimmer, E. Hoffman, G. Jung and H. Kessler, *Liebigs Ann. Chem.*, 1993, 497.
12 C. Ressler and H. Ratzkin, *J. Org. Chem.*, 1961, **26**, 3356.
13 S. Mojsov, A. R. Mitchel and R. B. Merrifield, *J. Org. Chem.*, 1980, **45**, 555.
14 C. H. Li and D. Yamashiro, *J. Am. Chem. Soc.*, 1973, **95**, 1310.
15 A. Bodanszky, M. Bodanszky, N. Chandramouli, J. Z. Kwei, J. Martinez and J. C. Tolle, *J. Org. Chem.*, 1980, **45**, 72.
16 R. B. Bates and S. Caldera, personal communication.
17 M. H. Kim and D. V. Patel, *Tetrahedron Lett.*, 1994, **35**, 5603.
18 G. R. Pettit, J. K. Srirangam, S. B. Singh, M. D. Williams, D. L. Herald, J. Barkóczy, D. Kantoci and F. Hogan, *J. Chem. Soc., Perkin Trans. 1*, 1996, 859.
19 G. R. Pettit, D. D. Burkett and M. D. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1996, 853.
20 G. R. Pettit, J. K. Srirangam, D. L. Herald, J.-P. Xu, M. R. Boyd, Z. Cichacz, Y. Kamano, J. M. Schmidt and K. L. Erickson, *J. Org. Chem.*, 1995, **60**, 8257.

Paper 6/00500D
Received 22*nd January* 1996
Accepted 28*th May* 1996