Synthesis of Virenamide B, a Cytotoxic Thiazole-Containing Peptide

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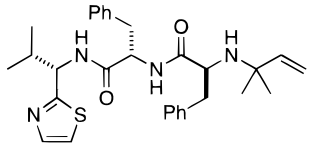
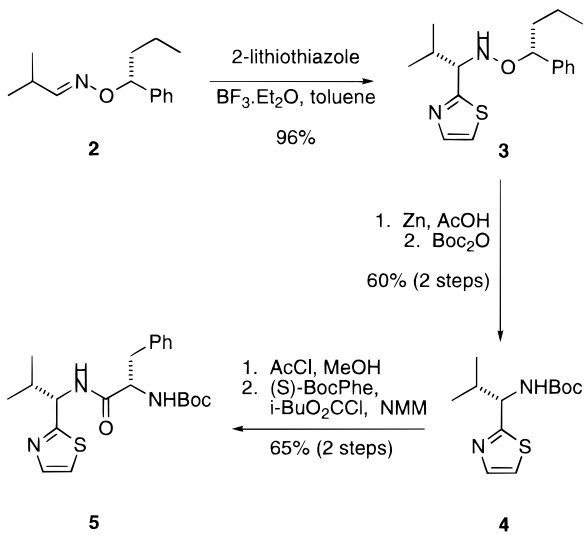
Marine organisms are a rich source of novel peptides, both cyclic and acyclic, many of which show high levels of biological activity.[1−4] For example, the linear thiazole-containing peptide dolastatin 10 is one of the most potent antineoplastic agents known,[5] and other thiazole-containing cyclic peptides also exhibit pronounced levels of cytotoxicity.[2−4] In continuation of our interest in the synthesis of biologically active thiazoles,[6−8] we now report the first synthesis of the cytotoxic linear peptide virenamide B (**1**).



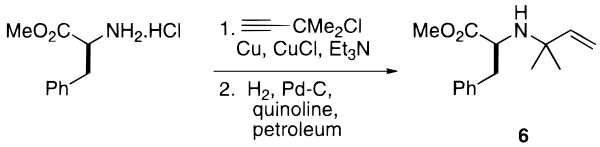
virenamide B **1**

Virenamide B is a member of a series of five cytotoxic linear peptides isolated from the Australian ascidian *Diplosoma virens*.[9,10] The structure, which was assigned on the basis of extensive NMR experiments, incorporates a 1-(2-thiazolyl)ethylamine fragment and two phenylalanine residues, one of which bears an inverted prenyl group at the *N*-terminus. We have recently reported a new asymmetric synthesis of 1-(2-thiazolyl)ethylamines based on the diastereoselective addition of 2-lithiothiazoles to (*R*)- and (*S*)-*O*-(1-phenylbutyl) oxime ethers;[11] the starting point for the synthesis of virenamide B was the (*E*)-(*R*)-oxime ether **2**.[12] Addition of 2-lithiothiazole gave the hydroxylamine **3** in excellent yield (96%) and diastereomeric excess (>95% as judged from the ^{1}H NMR spectrum of the product). Cleavage of the N−O bond using the zinc/acetic acid/ultrasound protocol[13] gave the corresponding amine, which was converted into its *N*-Boc derivative **4** for purification and characterization. Removal of the Boc group (77%) and coupling with *N*-Boc-phenylalanine gave the thiazole dipeptide **5** in good yield.



2 **3** **4** **5**

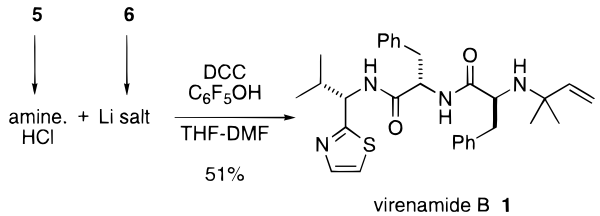
The *N*-terminal amino acid derivative was obtained from phenylalanine methyl ester hydrochloride by *N*-propargylation using 3-chloro-3-methylbutyne under copper-catalyzed conditions,[14] followed by Lindlar reduction to give the amino ester **6**. The final coupling reaction was



6

51% (2 steps)

achieved as follows. Ester **6** was hydrolyzed using lithium hydroxide in aqueous THF. Evaporation of the solvent gave the solid lithium salt. Dipeptide **5** was deprotected in methanolic HCl (from AcCl/MeOH) to give the amine hydrochloride, which was used without purification. Coupling of the lithium carboxylate with the amine hydrochloride was carried out in THF−DMF in the presence of DCC and pentafluorophenol and gave virenamide B (**1**) in 51% yield. The ^{1}H and ^{13}C NMR spectra



virenamide B **1**

of the product closely match those described for the natural product, although the measured optical rotation {$[\alpha]_D^{25}$ −77 (*c* 0.1, $CHCl_3$)} differs by a factor of 10 from the extremely high value reported earlier {$[\alpha]_D^{25}$ −775 (*c* 0.1, $CHCl_3$)}.[9] Correspondence with the authors of the original paper has uncovered an error;[15] the correct specific rotation of the natural material is $[\alpha]_D^{25}$ −77.5 (*c* 0.1, $CHCl_3$), which closely matches that of our synthetic material.

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Experimental Section

For general experimental details, see reference 16.

(*E*)-(*R*)-(+)-*O*-(1-Phenylbutyl) Isobutyraldoxime (2). Obtained in 54% yield from the cleavage of (*R*)-*N*-(1-phenylbutoxy) phthalimide (2.13 g, 7.22 mmol) with hydrazine monohydrate (0.49 mL, 10.10 mmol) in ethanol (21 mL) and subsequent condensation of the hydroxylamine with isobutyraldehyde (2.62 mL, 28.88 mmol) as previously described;[12] a colorless oil, $[\alpha]_D^{22}$ +9.9 (*c* 0.95, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (6H, m), 5.02 (1H, t, J = 7.2 Hz), 2.44 (1H, m), 1.93 (1H, m), 1.71 (1H, m), 1.34 (2H, m), 1.05 (3H, d, J = 6.8 Hz), 1.03 (3H, d, J = 6.8 Hz), 0.92 (3H, t, J = 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.0, 142.6, 128.2, 127.2, 126.8, 84.5, 38.4, 29.3, 20.3, 20.2, 18.9, 14.1 (Me).

(*R,S*)-*N*-(1-Phenylbutoxy)-1-(2-thiazolyl)-2-methyl-1-propylamine (3). A solution of 2-lithiothiazole was prepared as follows. Thiazole (0.85 mL, 12.25 mmol) was dissolved in ether (5.5 mL) under nitrogen and cooled to −78 °C. *n*-Butyllithium (4.90 mL, 2.5 M, 12.25 mmol) was added dropwise to the solution over 15 min. The mixture turned pale yellow and was stirred for a further 30 min.

(*R*)-*O*-(1-Phenylbutyl) isobutyraldoxime (**2**) (0.897 g, 4.09 mmol) was dissolved in toluene (9 mL) under nitrogen. The solution was cooled to −78 °C and stirred for 15 min. Boron trifluoride diethyl etherate (1.55 mL, 12.25 mmol) was added, and the mixture was stirred for an additional 15 min. 2-Lithiothiazole (12.25 mL, 12 mmol) was added dropwise to the mixture, care being taken not to allow the temperature to rise above −70 °C. The mixture was stirred until complete consumption of the starting material, whereupon saturated ammonium chloride solution was added. The reaction mixture was allowed to warm to room temperature and was then extracted with ether. The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was purified by column chromatography on silica gel eluting with EtOAc/light petroleum to give the title compound as a pale yellow oil (1.187 g, 96%, de >95%): $[\alpha]_D^{20}$ +45.2 (*c* 1.0, $CHCl_3$); IR (film) 3369 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (1 H, d, J = 3.3 Hz), 7.37−7.23 (6 H, m), 5.67 (1 H, br s), 4.59 (1 H, dd, J = 5.6, 8.3 Hz), 4.10 (1 H, d, J = 7.0 Hz), 1.98 (1 H, m), 1.64 (1 H, m), 1.43 (1 H, m), 1.27−1.20 (2 H, m), 0.89 (3 H, d, J = 11.4 Hz), 0.78 (3 H, t, J = 7.2 Hz), 0.76 (3 H, d, J = 11.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 142.9, 141.8, 128.4, 127.4, 126.7, 126.5, 118.5, 85.6, 69.1, 38.6, 31.7, 19.4, 19.2, 18.9, 14.0; MS (CI) *m*/*z* (relative intensity) 304 (M^+, 2%), 172 (58), 133 (71), 91 (100), 77 (20); HRMS calcd for $C_{17}H_{24}N_2OS$ (M) 304.1609, found 304.1618.

(*S*)-(−)-*N*-(*tert*-Butoxycarbonyl)-1-(2-thiazolyl)-2-methylpropylamine (4). Zinc dust (8.37 g, 128 mmol) was added to a mixture of (*R,S*)-*N*-(1-phenylbutoxy)-1-(2-thiazolyl)-2-methyl-1-propylamine (**3**) (0.967 g, 3.2 mmol) in acetic acid/water (20 mL, 1:1). The mixture was placed in a sonic bath at 40 °C, and the was reaction followed by TLC until completion. The zinc was filtered and washed with ether. The filtrate was basified with sodium hydroxide solution (3 M), and the aqueous layer was exhaustively extracted with dichloromethane. The extracts were combined, dried (Na_2SO_4), filtered, and evaporated. The residue was dissolved in dichloromethane (7 mL), and di-*tert*-butyl dicarbonate (4 mmol) and DMAP (cat.) were added. The mixture was stirred at room temperature for 12 h. Saturated aqueous sodium bicarbonate (10 mL) was added, and the mixture was stirred for 10 min. The mixture was extracted with dichloromethane, and the organic extracts were combined, dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash chromatography on silica gel, eluting with EtOAc/light petroleum to give the title compound as a colorless oil (60%, ee 92% by HPLC): $[\alpha]_D^{20}$ −33.6 (*c* 1.1, $CHCl_3$); IR (film) 3299, 1704 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (1 H, d, J = 3.3 Hz), 7.19 (1 H, d, J = 3.3 Hz), 5.37 (1 H, br d, J = 8.9 Hz), 4.86 (1 H, dd, J = 5.5, 8.9 Hz), 2.34−2.23 (1 H, m), 1.40 (9 H, br s), 0.91 (3 H, d, J = 6.8 Hz), 0.86 (3 H, d, J = 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 155.5, 142.5, 118.3, 79.7, 57.7, 33.6, 28.3, 19.2, 17.5; MS (EI) *m*/*z* (relative intensity) 257 (MH^+, 76%), 183 (100); HRMS calcd for $C_{12}H_{21}N_2O_2S$ (MH) 257.1323, found 257.1322.

(*S*)-(−)-1-(2-Thiazolyl)-2-methyl-1-propylamine Hydrochloride. Acetyl chloride was added dropwise to a stirred solution of (*S*)-(−)-*N*-(*tert*-butoxycarbonyl)-1-(2-thiazolyl)-2-methylpropylamine (200 mg, 0.78 mmol) in dry MeOH (9 mL). After 3 h the solvent was removed in vacuo to give the title compound as a colorless solid (154 mg, 77%): mp 185−186 °C (from *i*-propanol/ether); $[\alpha]_D^{25}$ −16.9 (*c* 1.18, MeOH); IR (KBr) 3438 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (1 H, d, J = 3.3 Hz), 7.70 (1 H, d, J = 3.3 Hz), 4.60 (1 H, d, J = 6.9 Hz), 2.36 (1 H, m), 1.11 (3 H, d, J = 6.9 Hz), 0.97 (3 H, d, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 142.5, 120.8, 57.3, 32.5, 17.3, 17.0; MS (EI) *m*/*z* (relative intensity) 157 ($[M - Cl]^+$, 3%), 113 (100); HRMS calcd for $C_7H_{13}N_2S$ (M − Cl) 157.0799, found 157.0802. Anal. calcd for $C_7H_{13}ClN_2S\cdot0.5H_2O$: C, 41.7; H, 7.0; N, 13.9. Found: C, 41.95; H, 6.7; N, 13.9.

Dipeptide 5. (*tert*-Butoxycarbonyl)-phenylalanine (281 mg, 1.06 mmol) was dissolved in dry THF (12 mL) under nitrogen and cooled to 0 °C. *N*-Methylmorpholine (0.222 mL, 2.12 mmol) and *iso*-butyl chloroformate (0.137 mL, 1.06 mmol) were added sequentially to the solution and stirred for 30 min. (*S*)-(−)-1-(2-Thiazolyl)-2-methyl-1-propylamine hydrochloride (170 mg, 0.88 mmol) in dry DMF (3 mL) was added in one portion, and the mixture was stirred for 1 h. Water, brine, and EtOAc were added, and the layers were separated. The aqueous phase was further extracted with EtOAc. The combined EtOAc extracts were washed with water, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by column chromatography on silica gel eluting with EtOAc/light petroleum (1:4 to 1:2) to give the title compound as a colorless solid (363 mg, 85%): mp 164−165 °C (from EtOAc/light petroleum); $[\alpha]_D^{20}$ −49.50 (*c* 1.01, MeOH); IR (KBr) 3405, 3309, 1680, 1652 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (1 H, d, J = 3.3 Hz), 7.26−7.12 (6 H, m), 6.80 (1 H, br d, J = 8.6 Hz), 5.16 (1 H, d, J = 6.2 Hz), 5.13 (1 H, d, J = 6.2 Hz), 4.38 (1 H, m), 3.06 (2 H, d, J = 6.9 Hz), 2.31−2.26 (1 H, m), 1.41 (9 H, br s), 0.88 (3 H, d, J = 6.9 Hz), 0.86 (3 H, d, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.8, 155.4, 142.5, 136.5, 136.5, 129.2, 128.6, 126.8, 118.3, 80.2, 56.1, 55.9, 38.0, 33.4, 28.2, 19.0, 17.8; MS (CI) *m*/*z* (relative intensity) 404 (MH^+, 49%), 142 (100); HRMS calcd for $C_{21}H_{30}N_3O_3S$ (MH) 404.2008, found 404.2005. Anal. calcd for $C_{21}H_{29}N_3O_3S$: C, 62.5; H, 7.2; N, 10.4. Found: C, 62.45; H, 7.3; N, 10.3.

(*S*)-(+)-Methyl 2-[(2-Methylbut-3-en-2-yl)amino]3-phenylpropanoate (6). (*S*)-Phenylalanine methyl ester hydrochloride (2 g, 9.27 mmol), copper metal (58.9 mg, 0.927 mmol), and copper(I) chloride (92 mg, 0.927 mmol) were suspended in THF (14 mL) and cooled to 0 °C. Triethylamine (2.84 mL, 20.4 mmol) followed by 3-chloro-3-methylbut-1-yne (1.20 mL, 10.7 mmol) in THF (6 mL) were added to the solution, and the mixture was stirred for 3 h. The mixture was diluted with ether and filtered through a pad of Celite. The filtrate was evaporated, and the residue was partly purified by column chromatography on silica gel eluting with EtOAc/light petroleum (1:10) to give a pale yellow oil, which was used without further purification.

Quinoline (0.95 mL) and 10% palladium on charcoal (100 mg) were added to the yellow oil dissolved in light petroleum. A balloon filled with hydrogen gas was fitted, and the mixture was stirred for 30 min. The mixture was filtered, the solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel eluting with ether/light petroleum (1:20) to give the title compound as a colorless oil (1.168 g, 51%): $[\alpha]_D^{22}$ +24.73 (*c* 1.86, $CHCl_3$); IR (film) 3328, 2971, 1737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (5 H, m), 5.51 (1 H, dd, J = 10.4, 17.6 Hz), 4.96−4.89 (2 H, m), 3.58 (3 H, s), 3.43 (1 H, dd, J = 6.3, 7.7 Hz), 2.87 (1 H, dd, J = 6.6, 13.2 Hz), 2.79 (1 H, J = 7.7, 13.2 Hz), 1.07 (3 H, s), 1.04 (3 H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 145.6, 137.5, 129.4, 128.2, 126.6, 112.3, 57.7, 54.6, 51.5, 41.6, 27.1, 26.7; MS (EI) *m*/*z* (relative intensity) 248 (MH^+, 4%), 188 (87), 156 (97), 120 (99), 91 (100); HRMS calcd for $C_{15}H_{21}NO_2$ (MH) 248.1651, found 248.1650.

(*S*)-Lithium 2-[(2-Methylbut-3-en-2-yl)amino]3-phenylpropanoate. Lithium hydroxide monohydrate (34 mg, 0.81 mmol) was added to a solution of the above ester **6** (200 mg, 0.81 mmol) in THF/water (15 mL:2 mL), and the mixture was heated under reflux for 24 h. The solution was allowed to cool

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to room temperature, and the solvent was removed in vacuo to give a colorless solid, which was used without further purification.

Deprotection of Dipeptide 5. Acetyl chloride (0.21 mL, 2.98 mmol) was added over 3 h to a solution of dipeptide **5** dissolved in dry MeOH (5 mL) under nitrogen. The solvent was removed in vacuo to give a colorless oil, which was used without further purification.

Virenamide B (1). (*S*)-Lithium 2-phenyl-*N*-2-(2-methylbut-3-ene)ethylamino carboxylate (71.2 mg, 0.298 mmol), DCC (61.4 mg, 0.298 mmol), and pentafluorophenol (54.8 mg, 0.298 mmol) were dissolved in THF under nitrogen. The above hydrochloride, dissolved in DMF (2 mL), was added dropwise to the solution. The mixture was stirred overnight, whereupon a white precipitate had formed. Ethyl acetate was added, and the solution was filtered. Saturated sodium bicarbonate solution was added to the filtrate, and the layers were separated. A further portion of sodium bicarbonate was added, and the layers were separated. The combined aqueous layers were extracted with EtOAc. The combined organic phases were washed with water, dried (Na_2SO_4), filtered, and evaporated to give a green residue. The residue was purified by column chromatography on silica gel eluting with EtOAc/light petroleum (1:2 to 2:3) to give the title compound as a colorless oil (65.5 mg, 51%): $[\alpha]_D^{25}$ −77 (*c* 0.1, $CHCl_3$) (lit.[9] $[\alpha]_D^{25}$ −775 (*c* 0.10, $CHCl_3$); IR ($CHCl_3$) 3419, 3330, 3087, 2969, 1675, 1660 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (1 H, d, J = 8.0 Hz), 7.69 (1 H, d, J = 3.4 Hz), 7.29−7.09 (11 H, m), 6.95 (1 H, d, J = 8.9 Hz), 5.21 (1 H, m), 5.16 (1 H, dd, J = 6.1, 8.9 Hz), 4.86−4.81 (2 H, m), 4.64 (1 H, dd, J = 7.2, 8.2 Hz), 3.27 (1 H, dd, J = 4.1, 9.1 Hz), 3.10 (1 H, dd, J = 7.2, 14.0 Hz), 3.05 (1 H, dd, J = 7.2, 14.0 Hz), 3.99 (1 H, dd, J = 4.1, 13.6 Hz), 2.41 (1 H, dd, J = 9.1, 13.6 Hz), 2.28 (1 H, m), 0.92 (3 H, s), 0.90 (3 H, s), 0.88 (3 H, d, J = 2.6 Hz), 0.86 (3 H, d, J = 2.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 170.6, 170.5, 144.7, 142.6, 137.1, 136.6, 129.5, 129.4, 128.6, 128.5, 127.0, 126.8, 118.3, 112.7, 58.0, 56.2, 54.8, 53.9, 39.9, 37.9, 33.2, 28.0, 25.0, 19.2, 17.8; MS (CI) *m*/*z* (relative intensity) 519 (MH^+, 11%), 188 (33), 142 (100), 120 (32); HRMS calcd for $C_{30}H_{39}N_4O_2S$ (MH) 519.2793, found 519.2798.

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Supporting Information Available: ^{13}C NMR spectra of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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