Tetrahedron Vol. 48, No. 20, pp. 4115-4122, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00

Total Synthesis of the Proposed Structure of Dolastatin 15[1]

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(*Received in Belgium* 4 *March* 1992)

Key Words : Dolastatin, cytotoxic, pseudopeptide, PyCloP, N-methyl amino acid.

Abstract : Efficient synthesis of dolastatin 15 (**1**) was accomplished following a convergent strategy. The pyrrolidinone cycle of **5** was obtained by thermic cyclization of the corresponding Meldrum's adduct **4**. The methylation of the enol function was performed under Mitsunobu conditions. On the other hand, the peptide part **10** was elongated by using PyCloP as coupling reagent. The ester linkage between both segments was efficiently performed under conditions we previously described by using a mixed anhydride activation with isopropenyl chlorocarbonate. Final compound **12** was found to exhibit physical properties slightly different from those recently reported by Pettit and co-workers for the natural dolastatin 15 and their synthesis product.

INTRODUCTION

For several years we have been engaged in the study of biological action mechanisms of potent antineoplastic cyclodepsipeptides of marine origin,[2a] and we recently focused our efforts on the preparation of dolastatins 10[2b] and 15. Dolastatins constitute a new family of promising antineoplastic pseudopeptides isolated from the marine mollusc *Dolabella auricularia* by Pettit and co-workers.[3] Dolastatin 15 (**1**),[3b] for which a structure was proposed on the basis of crystallographic and NMR considerations, markedly inhibits growth of the P388 lymphocytic leukemia cell line with an ED_{50} value of 2.4 ng/mL.

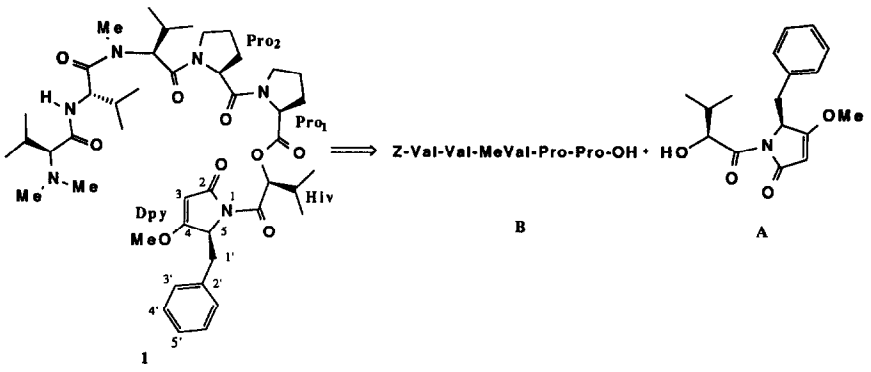
The first total synthesis of this compound was recently published by Pettit and co-workers.[4] We also undertook, simultaneously, the total synthesis of dolastatin 15; we obtained a compound with characteristics different from the recently described synthetized one and the natural one. We report herein our results.

DISCUSSION

Dolastatin 15 is a lipophilic pentapeptide esterified by an N-acyl pyrrolidone. We delineated these two subunits as retrosynthetic targets (scheme I) with the key step of our approach being the preparation of the subunit A by a methodology that we elaborated previously for the synthesis of statine.[5]

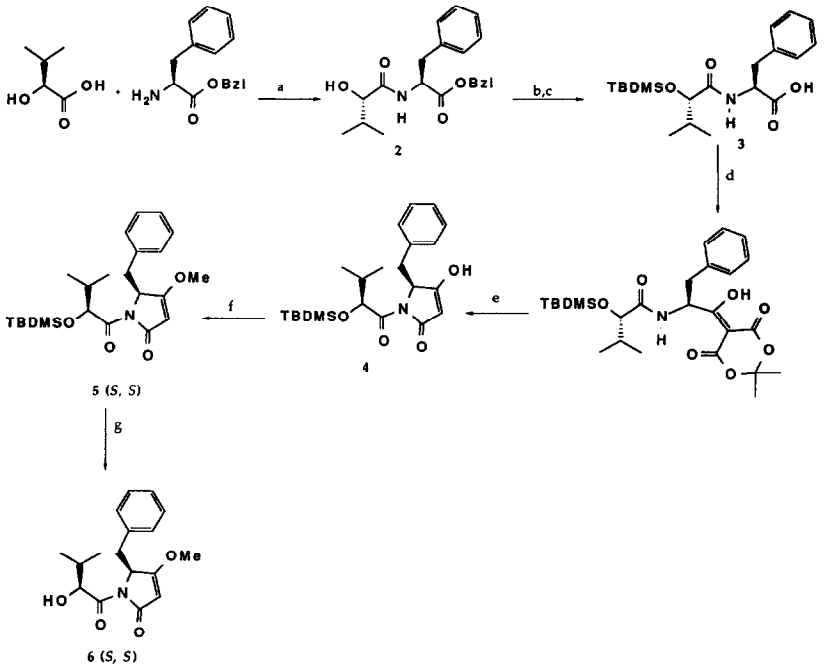
In a first set of experiments we attempted the direct introduction of the L-hydroxy isovaleric acid into the N1 position of the pyrrolidone ring under various conditions (symmetrical anhydride/DMAP, mixed anhydrides). In all cases the reaction failed, and the starting material was recovered unmodified. We then modified our strategy and prepared the cyclic part starting from the N-acylated amino acid **2**, even though epimerization of the phenylalanine residue was expected in this case (Scheme II).[6] Thus, L-hydroxy isovaleric acid was coupled to phenylalanine benzyl ester using PyBOP (trispyrrolidino-oxybenzotriazolyl phosphonium hexafluorophosphate) as reagent,[7] leading after chromatography on silica gel (ethylacetate/hexane ; 50/50) to compound **2** (98%). The hydroxyl group was then protected (TBDMSiCl, imidazole)[8] and the ester function hydrogenolyzed to furnish **3** which crystallized on trituration with ether-hexane (82% overall yield from **2**).

Scheme I



Treatment of **3** by isopropenyl chlorocarbonate (IPCC) in the presence of Meldrum's acid and DMAP, followed by heating the resulting adduct in refluxed acetonitrile, yielded **4** as an off-white solid after elimination of the solvent (86% crude yield).[5b] Methylation of the enolic function was conducted under Mitsunobu conditions as described recently for tetronic acids,[9] to provide **5**. Alternatively, alkylation by diazomethane afforded a complex mixture containing in particular the product arising from the O-

Scheme II



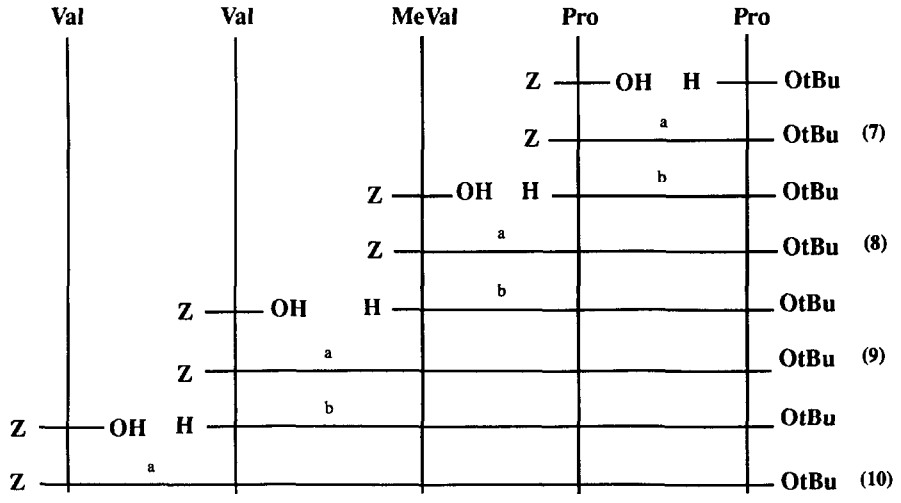
(a) PyBOP (1 equiv), iPr_2EtN (1 equiv), CH_2Cl_2, rt, 2 h, 98%; (b) tBuMe2SiCl (1.1 equiv), imidazole (3.5 equiv), DMF, rt, 16h, 82%; (c) H_2, Pd-C 10%, MeOH, rt, 1 h, 100%; (d) Meldrum's acid (1.1 equiv), dimethylaminopyridine (DMAP)(2.5 equiv), isopropenyl chlorocarbonate (IPCC) (1.1 equiv), CH_2Cl_2, -10 °C, 15 min; (e) D , CH3CN, 30 min, 86% for two steps; (f) Ph_3P (1.2 equiv), MeOH (1.2 equiv), DEAD (1.2 equiv), THF, rt, 16 h, 60%; (g) CF_3CO_2H, rt, 5 min, 86%.

methylation of the amide oxy group.[10]

As expected,[6] the reaction proceeded with partial epimerisation; HPLC analysis of crude **5** showed that it was a mixture of both epimers in a 9:1 ratio. The activation of an N-acyl amino acid can undergo a complete inversion of its chiral center.[11] Thus, in order to unambigueously identify the major component, the same reaction was carried out starting from the **3** (*S,R*) diastereoisomer. In this latter case, a mixture of both isomers of **5** was also obtained, but in the inverted 1:3 ratio.[12] Therefore, we could conclude that the major compound was the **5** (*S,S*) epimer in the first experiment, and the **5** (*S,R*) epimer in the second one.

Both epimers were easily separated by column chromatography to provide **5** (*S,S*) in a 60% yield. Treatment of **5** (*S,S*) by trifluoroacetic acid afforded the free hydroxyl compound **6** (*S,S*) (86%, $[\alpha]^{20}_D$= +313° (*c* 1, either MeOH or $CHCl_3$), mp = 59-60°C; lit.[4] $[\alpha]^{20}_D$= +285° (*c* 0.002, solvent not given, oil).

Scheme III



(a) PyCloP (1.5 equiv), iPr_2EtN (4 equiv), CH_2Cl_2, 5 min at 0 °C and then 3 h, at rt; (b) H_2, Pd-C 10%, MeOH, rt, 2 h.

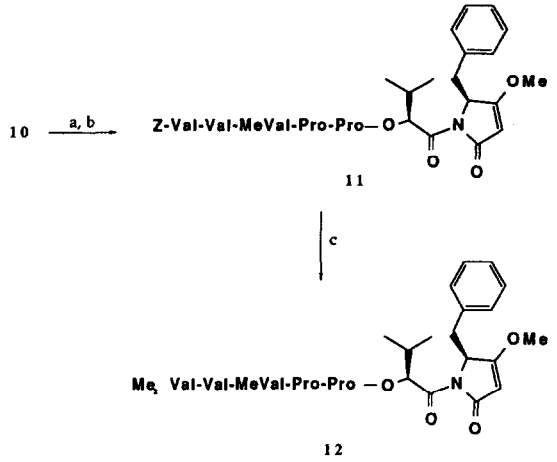
In the same manner, **5** (*S,R*) led to **6** (*S,R*) ($[\alpha]^{20}_D$= -210° (*c* 1, MeOH) or -212° (*c* 1, $CHCl_3$), oil).[12]

The peptide part B (scheme I) was elongated following a stepwise procedure starting from ProOtBu as depicted in scheme III. PyCloP, whose efficiency was recently demonstrated for coupling N-alkyl aminoacids, was used as reagent.[13] Thus, pentapeptide **10** was obtained in a 32% overall yield from ProOtBu.

The tert-butyl protecting group of **10** was removed in trifluoroacetic acid and the resulting acid was linked to **6** using our previous described conditions (IPCC, DMAP),[6] to lead after chromatography (ethyl acetate /hexane ; 85 : 15) to **11** (70%) (Scheme IV). Final N-deprotection of **11** and bismethylation of the terminal amino group were achieved directly by hydrogenolyzis of the benzyloxycarbamate function in the presence of a large excess of aqueous formaldehyde to provide **12** in 80% yield after HPLC purification on RP8 silica gel and precipitation by trituration in methanol.

The IR and mass spectra of **12** exhibited the same characteristics as the natural dolastatin 15 (**1**) described by Pettit *et al.*.[3b] In the case of the NMR spectrum, however, some minor differences were observed which can probably be explained by differences in either temperature, concentration or the higher field used for **1** (400 instead of 360 MHz). However, compound **12** differed somewhat from the natural compound and the synthetized product described by Pettit *et al.*[4] on the basis of analytical data. The optical rotation measured for **12** was -87° (*c* 0.4, MeOH) compared to -26° (*c* 0.01, MeOH) for **1**,[3b] and -48.2° (*c*

Scheme IV



(a) CF_3CO_2H, rt, 2 h, 82%; (b) 6 (1 equiv), Et_3N (1.6 equiv), dimethylaminopyridine (DMAP) (0.4 equiv), isopropenyl chlorocarbonate (IPCC) (1.1 equiv), CH_2Cl_2, 5 min at 0 °C then 2 h at rt, 76% (c) H_2, Pd-C 10%, 37 % aqueous HCHO (32 equiv), MeOH, rt, 48 h, 80%.

0.11, MeOH) for the product synthetized by Pettit's group.[4] There was also a difference in the melting point: 167-170 °C for **12**, 143-148 °C for **1** and 112-114 °C for their synthetized product.

The differences between **1** and both synthetized compounds can probably be explained by the lack of purity of **1** (only 6.2 mg isolated from 1600 kg of wet sea hare). On the other hand, the disagreements between **12** and Pettit's product are more disconcerting and may be related to the differences in the physical characteristics of the intermediate compound **6**. Epimerisation was expected during the cyclisation to N-acyl tetramic acid. Thus, we effectively isolated both epimers **5** (***S,R***) and **5** (***S,S***), and unequivocally established their configuration and their diastereomeric purity after separation. As a consequence, compound **6** (***S,S***) showed higher optical rotation than the product synthetized by Pettit.

Compound **12** was active against the U.S. National Cancer Institute's P388 lymphocytic leukemia cell line with an ED_{50} of 0.48 ± 0.08 ng / mL at 24 h and 0.11 ± 0.03 ng / mL at 48 h. These values indicate that compound **12** has slightly better activity than **1** (ED_{50} = 2.4 ng / mL).[3b]

In conclusion, we unambigously synthesized compound **12** which exhibited qualitative spectroscopic data (NMR, MS, IR) quite similar to those reported by Pettit and co-workers for dolastatin 15.[3b] On the basis of quantitative criteria (melting point, optical rotation), both synthetized products showed higher purity than the natural one. However, the differences in the physical properties of the two synthetized compounds are more difficult to explain. This problem is now under investigation in our laboratory.

Acknowledgments. We are indebted to Dr. S. Cros (CNRS Toulouse) for antineoplastic assays, to Dr A. Cavé (CNRS Montpellier) for NMR measurements, and to Dr. J. C.Promé (CNRS Toulouse) for MS determinations. We are grateful to Dr S. Salhi for her help in the revision of this manuscript.

EXPERIMENTAL

General Methods. Column chromatographic separations were performed on silica gel (63-200 μm) and TLC analyses on aluminum sheets precoated with silica gel. Melting points were measured on a Totolli apparatus. Optical rotations were evaluated on a Schmidt et Haensch polarimeter and were at ± 1°. ^1H NMR spectra were recorded on a WM-360 (360MHz) Bruker spectrometer. Mass spectra were run at the

Service d'Analyse de l'USTL. Elemental analyses were measured at the Service de Microanalyse de l'ENSCM. HPLC analyses were performed on a Gold Beckman apparatus using either a C8 Ultrabase, 5 µm 150 x 4.6 mm from S.F.C.C.-Shandon (column 1) or a Protein C4, 5 µm, 150 x 4.6 mm from Vydac (column 2); water with 0.1% TFA was solvent A and acetonitrile with 0.1% TFA, solvent B.

Benzyl N-[(2*S*)-2-hydroxy-isovaleryl]-L-phenylalaninate (2). To a solution of phenylalanine benzyl ester (tosylate salt) (8.24 g, 19.3 mmol), and L-hydroxy-isovaleric acid (2.50 g, 21.2 mmol) in methylene chloride (20 mL), were added N-methylmorpholine (7,0 mL, 63.6 mmol), and PyBOP (11.02 g, 21.2 mmol), and the mixture was stirred at room temperature for 2 h. After addition of ethyl acetate (200 mL), the reaction was washed with 5% $KHSO_4$ (2 x 30 mL), 5% $NaHCO_3$ (2 x 30 mL), and brine (30 mL). The mixture was dried (Na_2SO_4), and the solvent removed under reduced pressure. The residue was chromatographed (200 g silica gel, hexane/ethyl acetate, 50:50) to yield **2** which crystallized in Et_2O/ hexane (7.4 g, 98%): mp 71-72 °C; R_f 0.57 (ethyl acetate/hexane, 50:50); $[\alpha]_D^{20}$ = -36° (*c*, 1, MeOH); ^1H NMR ($CDCl_3$) δ 0.73 (d, *J* = 6.8 Hz, 3 H), 0.95 (d, *J* = 6.9 Hz, 3 H), 2.02-2.08 (m, 1 H), 2.52 (b, 1 H), 3.07 and 3.13 (ABX, J_1 = 14.0 Hz, J_2 = 6.6 Hz, J_3 = 6.2 Hz, 2 H), 3.93 (d, *J* =3.3 Hz, 1 H), 4.92-4.98 (m , 1 H), 5.10 (d, *J* = 12.2 Hz, 1 H), 5.16 (d, *J* = 12.2 Hz, 1 H), 6.82 (d *J* = 7.7 Hz, 1 H), 7.02-7.04 (m, 2 H), 7.19-7.35 (m, 8 H). Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.70; H, 6.76; N, 4.31.

N-[(2*S*)-O-*tert*-Butyldimethylsilyl-2-hydroxy-isovaleryl]-L-phenylalanine (3). To a solution of **2** (7.12 g, 20.3 mmol) and imidazole (3.45 g, 50.8 mmol) in DMF (30 mL) was added *tert*-butyldimethylsilyl chloride (4.0 g, 26.4 mmol). The reaction was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue dissolved in ethyl acetate (200ml). the mixture was washed with water (3 x 30 mL), dried (Na_2SO_4), and concentrated in vacuum. After column chromatography on silica gel (300 g, hexane/ethyl acetate, 80:20), the corresponding silyl ether was obtained as an oil (7.8 g, 80%). This compound was immediately solubilized in methanol (100 mL) and hydrogenolyzed (atm. pressure) on 10% palladium over charcoal (2 g) for 1 h at room temperature. The reaction was filtered and the solvent removed under reduced pressure to yield **3** which crystallized after trituration in hexane (6.3 g, 100%): mp 61-62 °C; R_f 0.39 (ethyl acetate/hexane/acetic acid, 50:50:1); $[\alpha]_D^{20}$ = -20° (*c* 1, MeOH); ^1H NMR ($CDCl_3$) δ -0.03 (s, 3 H), 0.02 (s, 3 H), 0.68 (d, *J* = 6.8 Hz, 3 H), 0.83 (s, 9 H), 0.83 (d, *J* = 6.9 Hz, 3 H), 1.95-2.02 (m, 1H), 3.08-3.20 (m, 2 H), 3.94 (d, *J* =3.0 Hz, 1 H), 4.88-4.93 (m, 1 H), 6.90 (d, *J* = 8.3 Hz, 1 H), 7.15-7.29 (m, 5 H). Anal. Calcd for $C_{20}H_{33}NO_4Si$: C, 63.29; H, 8.76; N, 3.69. Found: C, 63.48; H, 8.49; N, 4.07.

(5*S*)-1-[(2*S*)-O-*tert*-Butyldimethylsilyl-2-hydroxy-isovaleryl]-2-oxo-4-methoxy-5-benzyl-3-pyrroline (5). To a cooled (-10 °C) solution of **3** (4.0 g, 10.6 mmol), Meldrum's acid (1.67 g, 11.6 mmol), and DMAP (3.22 g, 26.4 mmol) in ethanol free methylene chloride (20 mL), was added dropwise (0.5 h) a solution of isopropenyl chloroformate (1.40 mL, 11.6 mmol) in methylene chloride (5 mL). The reaction was stirred 2 h at -10 °C. A precipitate appeared. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (150 mL) and washed with 5% $KHSO_4$ (2 x 30 mL), dried (Na_2SO_4), and concentrated in vacuum.

The residue was dissolved in acetonitrile (100 mL) and refluxed for 0.5 h. The solvent was removed under reduced pressure. The resulting pyrrolidinone (**4**) was then methylated under the conditions described by Bajwa and Anderson.[6] Thus, to a stirred solution of the preceeding product (2.6 g, 11.4 mmol), methanol (0.55 mL, 13.7 mmol), and triphenylphosphine (3.59 g, 13.7 mmol) in THF (100 mL) was added dropwise a solution of diethylazodicarboxylate (2.15 mL, 13.7 mmol) in THF (50 mL). The reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue chromatographed on silica gel (200 g, hexane/ethyl acetate, 70:30) to furnish **5** which crystallized by trituration in pentane (2.65 g, 60%): mp 98-99 °C; R_f 0.56 (hexane/ethyl acetate, 30:70); t_R 5.98 min (column 2; gradient 50 to 70% B in 10 min and 1.5 mL/min; t_R 6.20 min for the (*S*,*R*) epimer); $[\alpha]_D^{20}$ = +195° (*c* 1, MeOH); ^1H NMR (DMSO *d6*) δ 0.05 (s, 3H), 0.08 (s, 3 H), 0.77 (d, *J* = 6.7 Hz, 3 H), 0.90 (d, *J* = 6.7 Hz, 3 H), 0.93 (s, 9 H), 1.83-1.92 (m, 1 H), 3.08 (dd, J_1 = 13.8 Hz, J_2 = 2.5 Hz, 1 H), 3.48 (dd, J_1 =

13.8 Hz, J_2 = 5.2 Hz, 1 H), 3.82 (s, 3 H), 4.86 (dd, J_1 = 2.8 Hz, J_2 = 4.9 Hz, 1 H), 5.12 (s, 1 H), 5.20 (d, J = 3.0 Hz, 1 H), 6.96-6.98 (m, 2 H), 7.21-7.23 (m, 3 H). Anal. Calcd for $C_{23}H_{35}NO_4Si$: C, 66.15; H, 8.45; N, 3.35. Found: C, 66.35; H, 8.38; N, 3.67.

(5*S*)-1-[(2*S*)-2-hydroxy-isovaleryl]-2-oxo-4-methoxy-5-benzyl-3-pyrroline (6). Compound **5** (1.60 g, 3.84 mmol) was dissolved in TFA (10 mL) and allowed to stand at room temperature for 5 min. The solvent was removed under reduced pressure and the residue chromatographed on silica gel (100 g, hexane/ethyl acetate, 70:30) to yield **6** which crystallized by triturating in pentane (1.00 g, 86%): mp 59-60 °C; R_f 0.45 (hexane/ethyl acetate, 30:70); t_R 6.06min (column 1; gradient 40 to 70% B in 10 min; t_R 5.75min for the (*S,R*) epimer); $[\alpha]_D^{20}$ = +313° (*c* 1, MeOH); ^{1}H NMR (DMSO *d6*) δ 0.81 (d, J = 6.8 Hz, 3 H), 0.88 (d, J = 6.8 Hz, 3H), 1.87-1.96 (m, 1 H), 3.01 (dd, J_1 = 13.7 Hz, J_2 = 2.7 Hz, 1 H), 3.49 (dd, J_1 = 13.7 Hz, J_2 = 5.0 Hz, 1 H), 3.82 (s, 3H), 4.82-4.84 (m, 1 H), 4.89-4.92 (m, 2 H), 6.94-6.96 (m, 2 H), 7.19-7.25 (m, 3H). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 66.97; H, 7.11; N, 4.66.

Peptidic synthesis, typical procedure. To a cooled solution (0 °C) of the amino ester hydrochloride (1 equiv), the N-protected amino acid (1.5 equiv), and PyCloP (1.5 equiv) in ethanol free methylene chloride (1 mL/mmol), was added diisopropylethylamine (4 equiv). The reaction was stirred for 5 min at 0 °C and then for 3 h at room temperature. The mixture was diluted with ethyl acetate, washed with 5% $KHSO_4$, 5% $NaHCO_3$, and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuum and the residue chromatographed on silica gel (hexane/ethyl acetate).

Hydrogenolysis, typical procedure. The N-protected amino acids were solubilized in a mixture of methanol (10 mL/mmol) and 12N HCl (1 equiv), and hydrogenolysed over 10% Pd on charcoal overnight.

***tert*-Butyl [*N*-(benzyloxycarbonyl)-L-prolyl]-L-prolinate (7).** Yield 3.41 g (85%): mp 88-90 °C (methylene chloride/hexane); R_f 0.17 (methylene chloride/methanol, 97:3); $[\alpha]_D^{20}$ = -119° (*c* 1, MeOH; lit.: -118°); ^{1}H NMR (DMSO *d6*), two conformers (1:1) δ 1.36 (s, 9 H), 1.66-2.01 (m, 6 H), 2.08-2.27 (m, 2 H), 3.31-3.52 (m, 3.5 H), 3.62-3.68 (m, 0.5 H), 4.05 (dd, J_1 = 3.0 Hz, J_2 = 8.0 Hz, 0.5 H), 4.16 (dd, J_1 = 4.5 Hz, J_2 = 7.5 Hz, 0.5 H), 4.50 (dd, J_1 = 3.0 Hz, J_2 = 8.0 Hz, 0.5 H), 4.52 (dd, J_1 = 3.0 Hz, J_2 = 8.0 Hz, 0.5 H), 4.91 and 5.02 (AB, J = 13.0 Hz, 1 H), 5.04 and 5.08 (AB, J = 11.2 Hz, 1 H), 7.25-7.41 (m, 5H).

***tert*-Butyl [*N*-(benzyloxycarbonyl)-N-methyl-L-valyl]-L-prolyl-L-prolinate (8).** Compound **7** (3 g, 7.45 mmol) was hydrogenolyzed to furnish the corresponding hydrochloride salt (1.89 g, 83%).

Coupling of this salt (1.40 g, 4.59 mmol) with Z-MeVal prepared according to Benoiton *et al.*,[14] yielded compound **8** (2.36 g, 100%) as a foam: R_f 0.33 (hexane/ethyl acetate, 50:50); $[\alpha]_D^{20}$ = -142° (*c* 1, MeOH); MS (FAB$^+$) *m/e* (relative intensity) 516 (M + H$^+$, 9), 382 (8), 91 (100), 70 (80); ^{1}H NMR (DMSO *d6*), three main conformers (50:30:20) δ 0.58 (d, J = 7.0 Hz, 0.1 H), 0.69 (d, J = 6.8Hz, 0.3 H), 0.79 (d, J = 6.6 Hz, 2.7 H), 0.84 (d, J = 6.5 Hz, 1 H), 0.87 (d, J = 6.6 Hz, 0.5 H), 0.89 (d, J = 6.6Hz, 1.4 H), 1.36 (s, 9 H), 1.52-2.25 (m, 9 H), 2.61 (s, 0.6 H), 2.80 (s, 0.9 H), 2.83 (s, 1.5 H), 3.34-3.72 (m, 4 H), 3.95 (dd, J_1 = 4.8 Hz, J_2 = 8.4 Hz, 0.4 H), 4.13-4.18 (m, 0.6 H), 4.33 (d, J = 10.9 Hz, 0.3 H), 4.41 (d, J = 10.5 Hz, 0.2 H) H), 4.50 (d, J = 11.0 Hz, 0.5 H), 4.53-4.59 (m, 1 H), 4.92 and 5.12 (AB, J = 12.9 Hz, 0.4 H), 5.04 and 5.19 (AB, J = 12.2 Hz, 0.6 H), 5.08 and 5.14 (AB, J = 13.0 Hz, 1 H), 7.34-7.39 (m, 5H).

***tert*-Butyl [*N*-(benzyloxycarbonyl)-L-valyl]-(N-methyl-L-valyl)-L-prolyl-L-prolinate (9).** Compound **8** (2.20 g, 4.26 mmol) was hydrogenolysed to furnish the corresponding hydrochloride salt (1.83 g, 95%).

Coupling of this salt (1.78 g, 4.59 mmol) with Z-Val yielded compound **9** (2.35 g, 89%) as a foam; R_f 0.40 (hexane/ethyl acetate, 40:60); $[\alpha]_D^{20}$ = -170° (*c* 1, MeOH); MS (FAB$^+$) *m/e* (relative intensity) 615 (M + H$^+$, 3), 482 (1), 347 (57), 269 (7), 91 (100), 70 (51); ^{1}H NMR (DMSO *d6*), two conformers (96:4) δ 0.70 (d, J = 6.3 Hz, 3 H), 0.82 (d, J = 6.7 Hz, 3 H); 0.85 (d, J = 6.4 Hz, 3 H); 0.89 (d, J = 6.6 Hz, 3 H); 1.37 (s, 9 H), 1.73-1.83 (m, 3 H), 1.85-2.00 (m, 4 H), 2.05-2.20 (m, 3 H), 3.01 (s, 3 H), 3.47-3.55 (m, 2 H), 3.61-3.75 (m, 2 H), 4.17 (dd, J_1 = 3.9 Hz, J_2 = 8.6 Hz, 1 H), 4.22 (t, J = 8.6 Hz, 1 H), 4.50-4.54 (m, 1 H), 4.95 (d,

J = 10.2 Hz, 1 H), 4.98 and 5.07 (AB, J = 12.7 Hz, 2 H), 7.28-7.37 (m, 5H), 7.50 (d, J = 8.6 Hz, 1 H); minor conformer, distinguishable signals δ 1.42 (s), 3.05 (s).

***tert*-Butyl [*N*-(benzyloxycarbonyl)-L-valyl]-L-valyl-(N-methyl-L-valyl)-L-prolyl-L-prolinate (10).** Compound **9** (1.55 g, 2.52 mmol) was hydrogenolysed to furnish the corresponding hydrochloride salt (1.31g, 100%).

Coupling of this salt (0.55 g, 1.14 mmol) with Z-Val yielded compound **10** (0.55 g, 68%): mp 104-106 °C (ethyl acetate/pentane);R_f 0.20 (hexane/ethyl acetate, 30:70); $[\alpha]_D^{20}$ = -192° (*c* 0.5, MeOH); MS (FAB^+) *m/e* (relative intensity) 714 (M + H^+, 3), 446 (38), 269 (8), 91 (100), 70 (55); ^1H NMR (CD_2Cl_2), two conformers (90:10) δ 0.65 (d, J = 6.5 Hz, 3 H), 0.81 (d, J = 6.1 Hz, 6 H); 0.84 (d, J = 6.2 Hz, 6 H); 0.88 (d, J = 6.5 Hz, 3 H); 1.37 (s, 9 H), 1.71-1.85 (m, 3 H), 1.85-2.01 (m, 5 H), 2.05-2.20 (m, 3 H), 3.01 (s, 3 H), 3.47-3.55 (m, 2 H), 3.61-3.73 (m, 2 H), 3.94 (dd, J_1 = 7.0 Hz, J_2 = 8.8 Hz, 1 H), 4.17 (dd, J_1 = 4.0 Hz, J_2 = 8.7 Hz, 1 H), 4.49 (t, J = 8.6 Hz, 1 H), 4.53 (dd, J_1 = 4.6 Hz, J_2 = 8.5 Hz, 1 H), 4.95 (d, J = 10.9 Hz, 1 H), 5.02 (s, 2 H), 7.20 (d, J = 9.0 Hz, 1 H), 7.30-7.38 (m, 5H), 7.99 (d, J = 8.1 Hz, 1 H). Anal. Calcd for $C_{38}H_{59}N_5O_8$, H_2O C, 62.30; H, 8.33; N, 9.56. Found: C, 61.94; H, 8.30; N, 9.41.

(5*S*)-1-[(2*S*)-*O*-[[*N*-(benzyloxycarbonyl)-L-valyl]-L-valyl-(N-methyl-L-valyl)-L-prolyl-L-prolyl]-2-hydroxy-isovaleryl)]-2-oxo-4-methoxy-5-benzyl-3-pyrroline (11). The *tert*-butyl ester **10** (0.45 g, 0.63 mmol) was treated by a 1:1 mixture of methylene chloride and trifluoroacetic acid (0.64 mL) for 2 h at room temperature. The solvent was evaporated under reduced pressure to yield the corresponding acid which crystallized by triturating in diethyl ether (0.34 g, 82%).

To a cooled (0 °C) solution of this compound (100 mg, 152 μmol), alcohol **6** (46 mg, 152 μmol), DMAP (4 mg), and triethylamine (42 μΛ, 304 μmol) in ethanol free methylene chloride (3 mL), was added a solution of isopropenyl chlorocarbonate (40 μL, 304 μmol) in ethanol free methylene chloride (1 mL). The reaction was stirred for 15 min at this temperature and then for 4 h at room temperature. The mixture was diluted with ethyl acetate (20 mL), washed with 5% $KHSO_4$, 5% $NaHCO_3$, and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue chromatographed on silica gel (10 g, hexane/ethyl acetate, 15:85) to yield **11** as a white solid (100 mg, 70%): mp 115-117 °C; R_f 0.42 (methylene chloride/methanol/acetic acid, 95:3:2); $[\alpha]_D^{20}$ = -82° (*c* 1, MeOH); MS (FAB^+) *m/e* (relative intensity) 943 (M + H^+, 2), 498 (23), 446 (62), 154 (94), 91 (100), 70 (57); ^1H NMR (DMSO *d6*) δ 0.67 (d, J = 6.6 Hz, 3 H), 0.80-0.89 (mixture of d, 18 H), 0.98 (d, J = 6.8 Hz, 3 H), 1.71-1.81 (m, 1 H), 1.83-2.06 (m, 6 H), 2.07-2.17 (m, 3 H), 2.18-2.28 (m, 2 H), 2.95 (dd, J_1 = 13.9 Hz, J_2 = 2.9 Hz, 1H), 3.01 (s, 3H), 3.41 (dd, J_1 = 13.6 Hz, J_2 = 3.6 Hz, 1H), 3.50-3.77 (m, 4 H), 3.80 (s, 3 H), 3.94 (dd, J_1 = 7.0 Hz, J_2 = 8.9 Hz, 1H), 4.43-4.59 (m, 3 H), 4.91 (t, J = 3.8 Hz, 1H), 4.95 (d, J = 10.9 Hz, 1H), 5.02 (s, 2 H) 5.10 (s, 1 H), 5.79 (d, J = 3.1 Hz, 1H), 7.00-7.05 (m, 2 H), 7.14-7.24 (m, 4 H), 7.30-7.37 (m, 5 H), 7.99 (d, J = 8.3 Hz, 1H). Anal. Calcd for $C_{51}H_{70}N_6O_{11}$, H_2O C, 63.68; H, 7.49; N, 8.74. Found: C, 63.68; H, 7.58; N, 8.71.

((5*S*)-1-[(2*S*)-*O*-[(*N*,*N*-dimethyl-L-valyl)-L-valyl-(N-methyl-L-valyl)-L-prolyl-L-prolyl]-2-hydroxy-isovaleryl)]-2-oxo-4-methoxy-5-benzyl-3-pyrroline (12). Compound **11** (35 mg, 37μmol), solubilized in methanol (1ml) was hydrogenolyzed over palladium 10% on charcoal, in the presence of aqueous formaldehyde (104 μL, 32 equiv). Additional formaldehyde (52 μL) was added after 16 h. The reaction was complete within 48 h. The reaction was filtered and the solvent evaporated under reduced pressure to yield a white solid (32 mg) which was purified by HPLC (column C18 Hypersil 10 μm 25 x 500 mm from Whatman; gradient from 30 to 60% acetonitrile). During evaporation of acetonitrile under reduced pressure, compound **12** precipitated. The aqueous phase was extracted with methylene chloride, and the joined organic layers were dried and concentrated to furnish **12** as an amorphous white solid (26.5 mg, 80%): mp = 167-170 °C; $[\alpha]^{20}_D$= -87° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (logε) 220 (4.48), 239 (4.46) nm. IR (KBr plate) : ν_{max} 2960, 2920, 2870, 1730, 1690, 1660, 1630, 1445, 1305, 1255, and 1185 cm^{-1}; MS (FAB^+) *m/e* (relative intensity) 837 (M + H^+, 14), 611 (11), 498 (32), 437 (3), 401 (1), 340 (100), 286 (14), 227 (21), 204 (53); ^1H NMR (CD_2Cl_2) δ 0.76 (d, J = 6.7 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.93 (d, J = 6.4 Hz, 3 H), 0.93 (d, J = 6.9 Hz, 3 H), 0.94 ((d, J = 7.0 Hz, 3 H), 0.96 (d, J = 7.1 Hz, 3 H), 0.97 (d, J = 6.5 Hz, 3 H),

1.06 (d, J = 6.9 Hz, 3 H), 1.82-1.89 (m, 1 H), 1.98 (oct, J = 6.7 Hz, 1 H), 2.03-2.10 (m, 4 H), 2.11-2.30 (m, 6 H), 2.23 (s, 6 H), 2.40 (d, J = 6.5 Hz, 1 H), 3.06 (dd, J_1 = 3.2 Hz, J_2 = 13.9 Hz, 1 H), 3.11 (s, 3 H), 3.53 (dd, J_1 = 4.6 Hz, J_2 = 13.9 Hz, 1 H), 3.58-3.64 (m, 1H), 3.68-3.74 (m, 1 H), 3.74-3.79 (m, 1 H), 3.78 (s, 3 H), 3.81-3.87 (m, 1 H), 4.61 (dd, J_1 = 4.7 Hz, J_2 = 8.3 Hz, 1H), 4.72 (dd, J_1 = 3.7 Hz, J_2 = 8.4 Hz, 1H), 4.75 (s, 1 H), 4.77 (dd, J_1 = 4.9 Hz, J_2 = 3.0 Hz, 1 H), 4.77 (dd, J_1 = 6.3 Hz, J_2 = 9.3 Hz, 1 H), 5.09 (d, J = 11.0 Hz, 1 H), 5.86 (d, J = 2.9 Hz, 1 H), 6.79 (d, J = 9.0 Hz, 1 H), 7.10-7.13 (m, 2 H), 7.20-7.23 (m, 3 H); ^{13}C NMR (CD_2Cl_2) δ 178.8 (C2 Dpy), 173.2 (CO Val), 172,3 (CO Pro1), 171.6 (CO Me_2Val), 170.6 (CO Pro2), 169.8 (C4 Dpy), 169.5 (CO Hyv), 169.1 (CO MeVal), 134.7 (C2' Dpy), 130.4 (C3' Dpy), 128.4 (C4' Dpy), 127.3 (C5' Dpy), 95.0 (C2 Dpy), 78.1 (Cα Hiv), 77.0 (Cα Me_2Val), 60.4 (C4 Dpy), 59.5 (Cα MeVal), 58.9 (Cα Pro1), 58.8 (O$\underline{C}H_3$), 58.4 (Cα Pro2), 54.0 (Cα MeVal), 48.0 (Cδ Pro1), 47.0 (Cδ Pro2), 43.1 (N($\underline{C}H_3$)$_2$ Me_2Val), 35.2 (C1' Dpy), 31.5 (Cβ Val), 30.8 (N$\underline{C}H_3$ MeVal), 29.4 (Cβ Hiv), 29.0 (Cβ Pro1), 28.8 (Cβ Pro2), 28.0 (Cβ Me_2Val), 27.6 (Cβ MeVal), 25.2 (Cγ Pro1 and Cγ Pro2), 20.3 (Cγ Me_2Val), 19.9 (Cγ Val and Cγ Hiv), 19.2 (Cγ MeVal), 18.6 (Cγ MeVal), 18.1 (Cγ Me_2Val), 18.0 (Cγ Val), 16.1 (Cγ Hiv).

REFERENCES AND NOTES

1. Abbrevations and symbols follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Eur. J. Biochem. **1984**, *138*, 9). In addition the following abbrevations are used: PyBOP, (1*H*-1,2,3-benzotriazol-1-yl-oxy)trispyrrolidinophosphonium hexafluorophosphate; PyClоP, trispyrrolidinochlorophosphonium hexafluorophosphate; IPCC, isopropenyl chlorocarbonate.
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