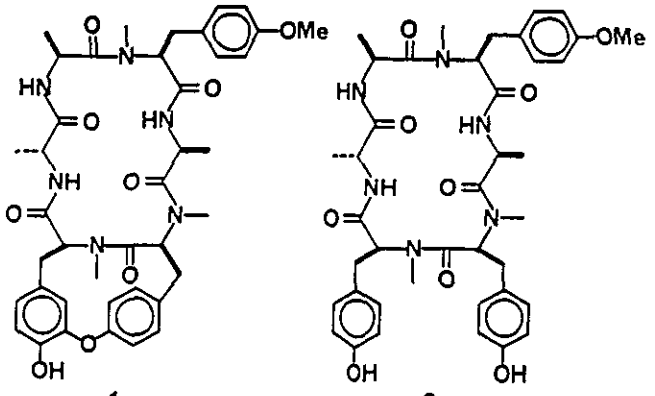
SYNTHESIS OF CYCLO-N-METHYL-L-TYR-N-METHYL-L-TYR-D-ALA-L-ALA-O,N-DIMETHYL-L-TYR-L-ALA, A CYCLIC HEXAPEPTIDE RELATED TO THE ANTITUMOR AGENT DEOXYBOUVARDIN

Robert B. Bates,* Susan L. Gin, Mark A. Hassen, Victor J. Hruby,
Kim D. Janda, George R. Kriek, Jon-Pierre Michaud, and Daniel B. Vine

Department of Chemistry
University of Arizona, Tucson, Arizona 85721, U.S.A.

Abstract—A synthesis of the title cyclic hexapeptide is described. Its lack of antitumor activity shows that the 14-membered ring of deoxybouvardin is needed for activity. Efforts to oxidatively couple its phenolic groups failed to give deoxybouvardin.

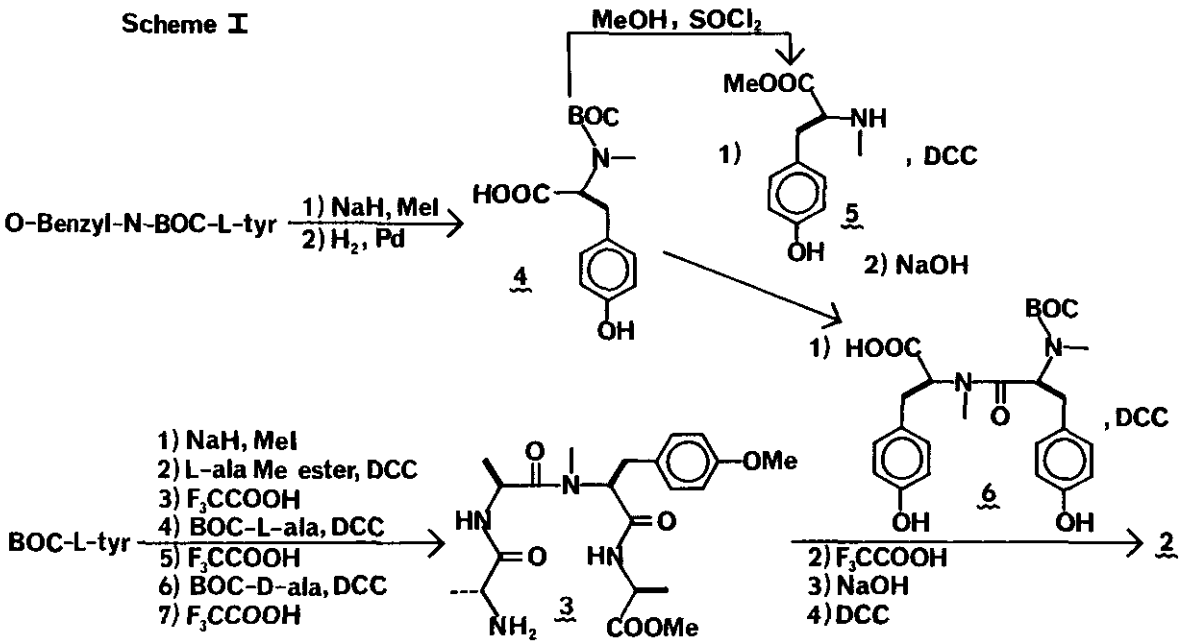
Bouvardin and deoxybouvardin (1), minor constituents of Bouvardia ternifolia, are potent antitumor agents.[1,2] We wish to report that as part of a program of synthesizing these substances and analogs, we have synthesized cyclic hexapeptide 2, and that it lacks antitumor activity though it differs from deoxybouvardin (1) by only two hydrogen atoms.



1 2

Scheme I shows the synthetic route to 2. NMR analysis of the peptides containing N-methyl groups was complicated by the observance of approximately equal amounts of cis and trans forms about the N-methyl peptide bonds and about each BOC amide bond; in several such cases, the spectrum was simplified by remeasuring it at a temperature high enough to make rotation about such bonds faster than the NMR measurement.[2] Tetrapeptide 3 was prepared in 23% yield from BOC-L-tyrosine; the tripeptide obtained in the fifth step was shown by X-ray analysis[3] to crystallize in the cis form about the peptide bond bearing the N-methyl group, whereas NMR shows cis and trans forms to be present in equal amounts in solution[2]. Dipeptide 6 was prepared in 74% yield from O-benzyl-N-BOC-L-tyrosine. Tetrapeptide 3 and dipeptide 6 were combined as shown to give cyclic hexapeptide 2 in 7% yield.

Scheme I



BOC = *t*-Butoxycarbonyl
DCC = N,N'-dicyclohexylcarbodiimide

Cyclic hexapeptide 2, unlike 1, was found to be inactive in a P-388 test involving leukemia in mice. It is not surprising that this difference should occur, as the 14-membered ring in 1 no doubt greatly changes the conformation from those favored in 2.[2] Many attempts to oxidatively couple the adjacent tyrosines of 2 to give 1 (presumably mimicking the biogenesis of 1) failed.[4]

EXPERIMENTAL SECTION

General. Melting points were determined on a Kofler hot stage and are uncorrected. NMR spectra were recorded on Varian T-60 and Bruker WM-250 spectrometers. Mass spectra were run on a Varian MAT 311A spectrometer. Elemental analyses were carried out by Huffman Laboratories, Inc., Wheat Ridge, Co.

O,N-Dimethyl-N-BOC-L-tyr. BOC-tyr (30.7 g, 0.10 mol, Vega Biochemicals) in THF (500 mL, distilled from Na) was cooled to 0° C and methyl iodide (30 mL) and NaH (26 g of 50% dispersion in mineral oil) were added. After stirring 1 h at 0°C and 25°C for 16 h, 8 mL ethyl acetate was added followed by water dropwise until gas evolution ceased. Most of the solvent was evaporated, the residue was washed 3 X 200 mL pentane, diluted with 100 mL water, acidified with citric acid, and extracted 3 X 100 mL ethyl acetate. The combined ethyl acetate solutions were washed 2 X 150 mL saturated sodium thiosulfate, once with 100 mL saturated sodium chloride, dried over magnesium sulfate, and evaporated, leaving 29 g (88%) of product as a very viscous liquid; ^{1}H NMR ($CDCl_3$,δ) 10.1 (s, COO<u>H</u>), 7.1 (~d, 2H, J=9 Hz), 6.8 (~d, 2H, J=9 Hz), 3.7 (s, OC<u>H</u>$_3$), 3.1 (m, C<u>H</u>$_2$), 2.7 (s, NC<u>H</u>$_3$), 1.3 (s, C(C<u>H</u>$_3$)$_3$).

Methyl O,N-Dimethyl-N-BOC-L-tyr-L-alaninate. O,N-Dimethyl-N-BOC-L-tyr (20.3 g, 66 mmol) and methyl L-alaninate (6.8 g, 66 mmol, freshly prepared from the hydrochloride by bubbling ammonia through a $CHCl_3$ solution of the hydrochloride, filtering, and evaporating) in CH_2Cl_2 (200 mL) was protected with a drying tube, cooled to 0°C, and DCC (15 g) was added. After stirring 1 h at 0°C and 16 h at 25°C, urea was filtered off and the solution was washed with 5% HOAc, water, and saturated $KHCO_3$ solution, dried over $MgSO_4$, and evaporated, leaving 24.6 g (95%) of product as a viscous oil containing some urea crystals; ^{1}H NMR ($CDCl_3$, δ) 7.1 (~d, 2H, J=9 Hz), 6.8 (~d, 2H, J=9 Hz), 6.6 (m, N<u>H</u>), 4.7 (m, Tyr C<u>H</u>), 4.6 (quintet, J=7 Hz, Ala C<u>H</u>), 3.8 (s, OC<u>H</u>$_3$), 3.8 (s, OC<u>H</u>$_3$), 3.2 (m, C<u>H</u>$_2$), 2.8 (s, NC<u>H</u>$_3$), 1.4 (s, C(C<u>H</u>$_3$)$_3$), 1.4 (d, J=7 Hz, Ala CC<u>H</u>$_3$).

Methyl O,N-Dimethyl-L-tyr-L-alaninate. Methyl O,N-dimethyl-N-BOC-L-tyr-L-alaninate (15.9 g, 40 mmol) was allowed to stand for 15 min at 25°C with 160 mL of trifluoroacetic acid and then most of the solvent was removed under vacuum at 40°C. CH_2Cl_2 (20 mL) was added and the amine was extracted with 500 mL and then 100 mL of 0.1 N HCl. The aqueous solutions were washed 2 X 20 mL CH_2Cl_2, made basic with solid $KHCO_3$, saturated with solid NaCl, and extracted 3 X 100 mL CH_2Cl_2. Drying over $MgSO_4$ and evaporating left 8.4 g (76%) of amine as an oil; ^{1}H NMR ($CDCl_3$, δ) 7.6 (broad d, Ala N<u>H</u>), 7.1 (~d, 2H, J=9 Hz), 6.8 (~d, 2H, J=9 Hz), 4.6 (quintet, J=7 Hz, Ala C<u>H</u>), 3.7 (s, OC<u>H</u>$_3$), 3.7 (s, OC<u>H</u>$_3$), 2.3-3.3 (m, C<u>H</u>$_2$ and Tyr C<u>H</u>), 2.3 (s, NC<u>H</u>$_3$), 1.5 (s, Tyr N<u>H</u>), 1.4 (d, J=7 Hz, CC<u>H</u>$_3$).

On standing for several months at 25°C, this substance is converted into the diketopiperazine

cyclo-O,N-dimethyl-L-tyr-L-ala hydrate, mp 78-80°C; ^{1}H NMR ($CDCl_3$, δ) 7.0 (~d, 2H, J=9 Hz), 6.8 (~d, 2H, J=9 Hz), 6.5 (broad s, NH), 4.2 (~t, J=4 Hz, Tyr CH), 4.0 (m, Ala CH), 3.8 (s, OCH_3), 3.2 (m, CH_2), 3.1 (s, NCH_3), 2.0 (s, H_2O), 0.7 (s, CCH_3).[5]

Anal. Calcd for $C_{14}H_{18}N_2O_3 \cdot H_2O$: C, 59.99; H, 7.19; N, 9.99. Found: C, 60.22; H, 7.22; N, 10.30.

Methyl N-BOC-L-ala-O,N-dimethyl-L-tyr-L-alaninate. Methyl O,N-dimethyl-L-tyr-L-alaninate (6.18 g, 21 mmol) was coupled with excess BOC-L-ala (Vega Biochemicals) as described above except the washings with HOAc solution and water were omitted to minimize diketopiperazine formation. The crude viscous oil containing urea crystals (10.6 g, 109 %) was not further purified; ^{1}H NMR showed coupling to be complete (NCH_3 due to cis and trans peptide bonds at δ 2.9 and δ 3.0; no NCH_3 at δ 2.3).

Methyl L-Ala-O,N-dimethyl-L-tyr-L-alaninate. The entire crude product from the reaction above was cleaved with trifluoroacetic acid as described above, yielding 4.6 g (60% over two reactions) of amine, mp 136-137°C; ^{1}H NMR ($CDCl_3$, δ) shows cis and trans forms about the N-methyl-bearing peptide bond, present in about equal amounts.[2] An X-ray study showed the crystals to be of cis peptide.[3]

Anal. Calcd for $C_{18}H_{27}N_3O_5$: C, 59.16; H, 7.45; N, 11.50. Found: C, 59.28; H, 7.67; N, 11.80.

Methyl N-BOC-D-ala-L-ala-O,N-dimethyl-L-tyr-L-alaninate. Methyl L-ala-O,N-dimethyl-L-tyr-L-alaninate (4.5 g, 15 mmol) was coupled with excess BOC-D-ala (Vega Biochemicals) as described above. The crude foam obtained (6.6 g, 106%) was used directly in the next reaction.

Methyl D-Ala-L-ala-O,N-dimethyl-L-tyr-L-alaninate (3). Cleavage of the BOC grouping from 4.0 g (9 mmol) of the above tetrapeptide with trifluoroacetic acid gave 1.8 g (61% over two reactions) of crystalline 3, mp 138-142°C; ^{1}H NMR ($CDCl_3$) shows cis and trans forms about the N-methyl-bearing peptide bond in about equal amounts, δ 2.9 and 3.0 (s, NCH_3).

O-Benzyl-N-methyl-N-BOC-L-tyr. O-Benzyl-N-BOC-L-tyr (55.6 g, 0.15 mol, mp 112-113°C, lit. 108-110°C[6]) in dry THF (700 mL) was cooled to 0°C and methyl iodide (42 mL) and NaH (25.5 g of 50% dispersion in mineral oil) were added. Reaction and workup as above for O,N-dimethyl-N-BOC-L-tyr gave 57.4 g (99%) of viscous oil; ^{1}H NMR ($CDCl_3$, δ) 9.6 (broad s, COOH), 7.3 (broad s, C_6H_5), 7.1 (~d, 2H, J=9 Hz), 6.8 (~d, 2H, J=9 Hz), 5.0 (s, CH_2), 4.6 (m, CH), 3.1 (m, CH_2), 2.7 (s, NCH_3), 1.3 (s, $C(CH_3)_3$).

N-Methyl-N-BOC-L-tyr (4). O-Benzyl-N-methyl-N-BOC-L-tyr (30 g, 78 mmol) and acetic acid (3 mL) in dioxane (150 mL) were stirred with 5% Pd on charcoal (5 g) at 25°C under a hydrogen atmosphere. After 60 h, the theoretical amount of hydrogen (1.9 L) had been taken up. Filtering

and evaporation of the solvent left a viscous oil which on trituration with hexane crystallized to give 22.5 g (98%) of product, mp 141-144°C (dec); ^{1}H NMR ($CDCl_3$, δ) 8.8 (broad s, COOH and ArOH), 7.0 (~d, 2H, J=9 Hz), 6.7 (~d, 2H, J=9 Hz), 4.1 (m, CH), 3.1 (m, CH_2), 2.7 (s, NCH_3), 1.3 (s, $C(CH_3)_3$).

Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.52; H, 7.08; N, 4.84.

Methyl N-Methyl-L-tyrosinate (5). 4 (4.0 g, 14 mmol) in 130 mL of methanol was stirred and cooled to 0°C and 10 mL of $SOCl_2$ was added dropwise. After refluxing overnight, the volatiles were blown off with nitrogen. The residual oil was washed with ether, taken up in 400 mL of chloroform, and ammonia was bubbled through this solution for 10 min. Filtration and evaporation left 2.4 g (100%) of 5, mp 109-111°C (lit.[7] 111-112°C).

Methyl N-BOC-N-methyl-L-tyr-N-methyl-L-tyrosinate. 5 (2.1 g, 10 mmol) was coupled with 4 (3.0 g, 10 mmol) as described above, yielding 4.06 g (84%) dipeptide as a foam. A sample was chromatographed on silica gel (1:10 MeOH-CH_2Cl_2) to remove urea, but the product still did not crystallize. The ^{1}H NMR spectrum ($CDCl_3$) showed broad absorptions in the expected regions which sharpened on warming to 120°C.[2]

N-BOC-N-methyl-L-tyr-N-methyl-L-tyr (6). Methyl N-BOC-N-methyl-L-tyr-N-methyl-L-tyrosinate (3.76 g, 7.7 mmol) was stirred under N_2 with 20 mL of 2 N NaOH for 2.5 h, evaporated slightly to remove MeOH, diluted to 150 mL with water, washed 3 X 100 mL CH_2Cl_2, acidified to pH 2 with 1N HCl, and quickly saturated with NaCl and extracted 3 X 100 mL of EtOAc. After drying over $MgSO_4$, evaporation left 3.32 g (91%), mp 190-198°C (dec). Recrystallization from $CHCl_3$ gave 6, mp 208-210°C (dec); ^{1}H NMR ($CDCl_3$, low solubility) showed the absence of OCH_3.

Methyl N-BOC-N-methyl-L-tyr-N-methyl-L-tyr-D-ala-L-ala-O,N-dimethyl-L-tyr-L-alaninate. 6 (1.28 g, 2.7 mmol) was coupled with 3 (1.18 g, 2.7 mmol) as described above, giving 2.59 g (108%) of crude hexapeptide as a foam which was reacted without further purification.

Methyl N-Methyl-L-tyr-N-methyl-L-tyr-D-ala-L-ala-O,N-dimethyl-L-tyr-L-alaninate. Removal of the BOC grouping from the above hexapeptide with trifluoroacetic acid gave 1.2 g (56% over two reactions) of amine, mp 105-112°C; ^{1}H NMR ($CDCl_3$, δ) shows the BOC grouping to be removed, 6.6-7.1 (m, aromatic), 3.8 (s, OCH_3), 2.6-3.4 (m, NCH_3 and CH_2), 0.9-1.4 (m, CCH_3).

Cyclo-N-methyl-L-tyr-N-methyl-L-tyr-D-ala-L-ala-O,N-dimethyl-L-tyr-L-ala (2). The total product from the previous reaction was stirred for 2 h under N_2 with 40 mL of 2 N NaOH, vacuum was applied for a short time to remove MeOH, the pH was adjusted to 6.5 with 1N HCl, and the solvent was evaporated. DMF (300 mL), N-hydroxybenzotriazole (511 mg), and DCC (344 mg) were added. After stirring 48 h under N_2, the mixture was concentrated to 70 mL, filtered to remove NaCl and urea, washed with $CHCl_3$, and evaporated to give 2.3 g of solid. Chromatography on silica gel (20:25:2

pentane-CH_2Cl_2-MeOH) gave a fraction of 200 mg, mp 250-265°C, which on washing with $CHCl_3$ gave 140 mg (12% over two reactions) of 2 as small white crystals, mp 280-290°C. TLC indicated about 150 mg additional 2 to be present in the adjacent chromatography fractions. Fourier transform ^1H and ^{13}C NMR spectra were obtained and showed broad absorptions in the expected places, but the signals were weak due to very low solubility in $CDCl_3$. The formation of the last amide bond was supported by the insolubility of this substance in hot 1N HCl or saturated $KHCO_3$; it was soluble in 2N NaOH as expected. MS: no molecular ion peak observed with EI and CI, but FAB using glycerol gave a very strong peak for MH^+ at 759, and with NaCl added, a very strong peak for MNa^+ at 781.[8] The ^1H NMR spectrum, unlike that of 1,[2] showed very broad absorptions at room temperature due to the presence of 8 forms differing in configurations about the 3 N-methyl peptide bonds. Amino acid analysis indicated the peptide to contain equimolar amounts of ala and N-methyl-tyr.

Anal: Calcd for $C_{40}H_{50}N_6O_9$ + 6.4% non-CHN impurity: C, 59.28; H, 6.22; N, 10.37. Found: C, 59.62; H, 6.30; N, 10.08.

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REFERENCES AND NOTES

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