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SYNTHESIS OF CYCLO (L-TRANS-(4-HYDROXYPROLINYL)-L-PHENYLALANINE)

by

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ABSTRACT

The diketopiperazine *Cyclo* (L-trans-(4-Hydroxyprolinyl)-L-phenylalanine)**1** has been synthesized by coupling a suitably protected form of L-trans-hydroxy proline with L-phenylalanine ethyl ester, deprotection of the amino group followed by an deacetimisation and finally desilylation. This final product has spectral data that agree with the already reported data in literature.

Keywords: diketopiperazine, hydroxyproline, phenylalanine

**For correspondence and reprints*

INTRODUCTION

Diketopiperazines are cyclic dipeptides, which are quite common in nature and a number have been isolated from higher animals and microorganisms. They are also formed from peptides by enzymatic hydrolysis. The proline residue is often encountered as a component of naturally occurring 2,5-diketopiperazines (Mann *et al.*, 1994, Schmitz *et al*. 1983). These heterocyclic compounds have diverse biological properties. Kuhkla *et al.* (1985) has identified cyclo-L-His- L-Pro in human blood. Recently Ienega *et al.* (1987) isolated two diketopiperazines cyclo (L-*trans*-OH-Pro-L-Pro) and cyclo(L-*trans*-OH-Pro-L-Leu) from rabbit skin tissue and these compounds were reported to act as plant growth regulators and promoting rice *et al*. germination.

Adamczeski *et al*. (1989) isolated cyclo(L-*trans*-(4-hydroxyprolinyl)-L-phenylalanine), a naturally occurring cyclic dipeptide from Jaspidae, a sponge found on coral reefs throughout Fiji. It was subsequently identified in the South African sponge *Jaspis digonoxea (*Rudi *et al.* 1994). We have recently isolated the same compound from a fungal culture medium.

In this paper we report a synthesis of the cyclodipeptide *cyclo*(L-*trans*-(4-hydroxyprolinyl)-L-phenylalanine), which is in principle amenable to larger scale preparations and is sufficiently flexible to provide access to synthetic analogues. Retrosynthetic analysis led to the obvious disconnection at the amide linkages to give hydroxyproline and phenylalanine as the two precursors.

MATERIALS AND METHODS

Infrared spectra (IR) were recorded on a Mattson 1000 FTIR spectrometer in the range 4000 to 500 cm^{-1}. ^{1}H NMR and ^{13}C-NMR spectra were recorded on a Bruker Spectrospin at 250 MHz with TMS as internal standard in $CDCl_3$ as solvent. Mass spectra were recorded on MS-50 Kratos for Electron Impact spectra, VG7070E for chemical ionisation and VGZABSPEC for high-resolution spectra. Chromatography refers to 'flash column' technique over silica gel (70-230 mesh). Thin layer chromatography (TLC) was carried out on plates precoated with silica. Visualisation was achieved by exposure to an iodine atmosphere.

The chemicals used were of analytical grade except methanol, which was of General Purpose Grade (GPR). Methanol was distilled and dried using molecular sieves prior to use. Ethyl acetate was also distilled prior to use in column chromatography.

N-(Benzyloxycarbonyl) L-trans-4-hydroxyproline 4

L-*Trans*-4-hydroxyproline (1.0 g, 7.63 mmol) was dissolved in 4M NaOH solution (4 ml, 16.01 mmol, 2.1 eq). The reaction mixture was cooled to 0ºC. Benzyl chloroformate (1.56g, 9.15 mmol, 1.2 eq) was added dropwise over a period of 20 minutes with continuous stirring while maintaining the temperature of the medium at 0ºC. The mixture was then stirred for a further 2 hours while the temperature was allowed to rise gradually to room temperature. The reaction was monitored by TLC (methanol:glacial acetic acid, 19:1). The reaction mixture was then extracted with diethyl ether (2x15 ml). The resulting aqueous phase was collected and acidified with HCl (1:1) until Congo red to pH paper. The liberated oil was extracted with ether (5x15 ml) and dried (Na_2SO_4). Evaporation of the solvent on the rotary evaporator gave the product (1.95 g, 95 %) as a pale yellow viscous liquid; IR: 3400(VOH), 1705, 1693(VCO); ^{1}H NMR ($CDCl_3$) 7.1-7.3 (5 H, m), 5.1 (2 H, m), 4.5 (2 H, m), 3.6 (1 H, bs, OH), 3.5 (2H, m), 2.3 (1 H, m); 2.2 ppm (1 H, m); m/z 265 (M^+, 8%), 220 (13), 176 (40), 130 (64), 91 (100).

N-(Benzyloxycarbonyl) L-trans-4-hydroxyproline ethyl ester 5

To a stirred solution of the N-protected hydroxyproline (1.95 g, 7.33 mmol) in dry DMF (10 ml) at 0ºC were added anhydrous potassium carbonate (1.12 g, 8.07 mmol, 1.1 eq) and ethyl iodide (1.2 ml, 2.28 g, 14.67 mmol, 2.0 eq). The temperature of the mixture was allowed to rise to room temperature. Stirring was continued for 15 hours and the reaction was monitored by TLC (EtOAc, R_f 0.6). The reaction mixture was then diluted with water (25 ml) and was extracted with ethyl acetate (5x25 ml). The organic layer was dried (Na_2SO_4) and evaporated to give the product (2.150 g, 100%) as a viscous yellow oil; IR: 3400 (VOH), 1743 cm^{-1} (VCO of ester), 1670 cm^{-1} (VCO amide); ^{1}H NMR ($CDCl_3$) 7.3 (5 H, m), 5.1 (2 H, m), 4.5 (2 H, m), 4.2 (1 H, q, *J* 7), 4.0 (1 H, q, *J* 7), 3.65 (2 H, m), 3.6 (1 H, s, OH), 2.3 (1 H, m), 2.1 (1 H, m), 1.1 (3 H, dt, *J* 7); m/z 293 (M^+, 2%), 220 (6), 176 (10), 91 (100).

N-(Benzyloxycarbonyl)- L-trans-4- t-butyldimethylsilyloxyproline ethyl ester 6

To a solution of the partially protected hydroxyproline derivative 5 (2.15 g, 7.33 mmol) in DMF (10 ml) were added *t*-butyldimethylsilyl chloride (1.326 g, 8.80 mmol, 1.2 eq) and imidazole (0.699 g, 10.26 mmol, 1.4 eq). The reaction mixture was stirred continuously for 14 hours and the reaction was monitored by TLC (EtOAc, 0.86). When the reaction was complete, the mixture was diluted with water (15 ml) and extracted with ethyl acetate (5x10 ml). The organic layer was dried (Na_2SO_4) and evaporated. A pale yellow viscous liquid (2.808 g, 94%) was obtained, IR: 1709(VCO of ester), 1655 (VCO of amide); ^{1}H NMR ($CDCl_3$) 7.3 (5 H, m), 5.1 (2 H, m), 4.5 (2 H, m), 4.2 (1 H, q, *J* 7), 4.0 (1 H, q, *J* 7), 3.6 (2 H, m), 2.3 (1 H, m), 2.1 (1 H, m), 1.1 (3 H, t, *J* 7), 0.8 (9 H, m), 0.05 (6 H, s); m/z 407 (M^+, 0.1) 392 (0.1), 350 (8), 306 (37), 290 (6), 165 (4), 142 (12), 91 (100).

N-(Benzyloxycarbonyl)- L-trans-4- t-butyldimethylsilyloxyproline 7

To a stirred ice-cold solution of the fully protected hydroxyproline derivative 6 (2.80 g, 6.87 mmol) in THF (10 ml) was added lithium hydroxide (0.29 g, 6.87 mmol, 1 eq). The reaction mixture was stirred for 2 hours and the reaction was monitored by TLC (EtOAc). When the reaction was complete, the solvent was evaporated. A pale yellow paste (2.582 g, 99 %) was obtained. The crude product was used in the next step without purification. A pale yellow viscous liquid (2.808 g, 94%) was obtained, IR: 3400 (VOH); m/z 402 ($M^+ + Na^+$, 41) 319 (60), 304 (100), 288 (41).

N-(Benzyloxycarbonyl) - L-trans-4- t-butyldimethylsilyloxy prolinyl-L-phenylalanine ethyl ester 9

To a solution of the foregoing partially protected hydroxyproline derivative **7** (2.50 g, 6.59 mmol) in DCM (10 ml) were added phenylalanine ethyl ester hydrochloride (1.514 g, 6.59 mmol, 1 eq), triethylamine (2 ml), and DCC (1.36 g, 6.59 mmol). A precipitate of N, N^1-dicyclohexylurea started to separate immediately and the amount gradually increased. After 5 hours of continuous stirring at room temperature, the urea derivative was filtered off and washed with dichloromethane. The product **9** was a pale yellow liquid, which solidified to colourless crystals on cooling (2.819 g, 77 %); IR 1658 cm^{-1} (VCO of amide), 1731 cm^{-1} (VCO of ester); ^{1}H NMR ($CDCl_3$) 7.1-7.3 (10 H, m), 5.1 (2 H, m), 5.0 (1 H, m), 4.4 (1 H, m), 4.1 (3H, m), 3.9 (1 H, m), 3.7 (2 H, m), 3.6 (1 H, m), 2.3 (1 H, m), 2.0 (1 H, m), 1.1(12 H, m), 0.05 (6 H, s); m/z 554 (M^+, 0.25) 497 (2), 453 (2), 290 (4), 224 (46), 143 (33), 91 (19), 86 (100).

L-trans-4-(t-Butyldimethylsilyloxy)- prolinyl- L-phenylalanine ethyl ester 10

To a solution of the protected peptide **9** (2.700g, 4.86 mmol) in methanol (20 ml) were added cyclohexene (10 ml) and a catalytic amount of activated palladium carbon. The mixture was refluxed for 10 hours and the reaction was monitored by TLC (EtOAc, R_f 0.3).

When the reaction was completed, the mixture was passed through a short column of silica (15 cm). Ethyl acetate was initially used for elution to remove unwanted substances, followed by methanol, which eluted the N-deprotected peptide. On evaporation of the methanol fractions, the product **10** (1.987 g, 97%) was obtained as a viscous pale yellow substance; IR 3421-3282(V_{NH}), 1743 (VCO); ^{1}H NMR ($CDCl_3$) 7.3 (5 H, m), 5.0 (1 H, m), 4.5 (1 H, m), 4.1 (4 H, m), 3.7 (2 H, m), 3.4 (1 H, m), 2.3 (1 H, m), 2.0 (1 H, m), 1.2-1.1(12 H, m), 0.05 ppm (6 H, s) ^{13}C NMR ($CDCl_3$) 175.7, 170.4, 128.5, 70, 65, 60.5, 55, 49.3, 37, 33.8 and 14.2-24.9ppm; m/z 420 (M^+, absent) 318 (2), 290 (2), 224 (18), 176 (4), 149 (94), 91 (62), 60 (100).

cyclo[L-trans-(4-(t-Butyldimethylsilyloxy)-prolinyl)-L-phenylalanine 11.

To a solution of **10** (1.98g, 4.70 mmol) in toluene (15 ml) were added a few crystals of DMAP. The mixture was refluxed for 2 hours and the reaction was monitored by TLC (EtOAc, R_f 0.65). When the reaction was complete, the mixture was filtered and the filtrate was evaporated to give **11** as a viscous yellow liquid (1.728 g, 98%); ^{1}H NMR ($CDCl_3$) 7.3 (5 H, m), 5.0 (1 H, m), 4.6 (1 H, m), 4.1 (1 H, m), 3.7 (1 H, m), 3.5 (1 H, m), 3.2 (2 H, m), 2.3 (1 H, m), 2.1 (1 H, m), 1.1(9 H, m), 0.05 ppm (6 H, s); ^{13}C NMR ($CDCl_3$) 172.7, 170.5, 128.5, 70, 65, 61.5, 55, 52, 37.5, 33.9, 25 and 14.6 ppm ; m/z 374 (M^+, absent) 306 (52), 224 (7), 176(4), 91(100) .

cyclo(L-trans-(4-hydroxyprolinyl)-L-phenylalanine) 1.

To a solution of dipeptide **11** (1.728g, 4.60 mmol) in THF (10 ml) was added TBAF (2.7 ml, 2.4 g, 9.21 mmol, 2 eq). The reaction mixture was stirred continuously for 2 hours and the reaction was monitored by TLC (EtOAc, R_f 0.2). On completion of the reaction, the solvent was evaporated. The crude product was then purified by column chromatography (3:1 EtOAc:MeOH) to give the cyclic dipeptide **1** as a pale yellow viscous oil (0.934 g, 78%); IR 3200-3400 (VOH), 1700 and 1670 cm^{-1} (VCO of amide); ^{1}H NMR ($CDCl_3$) 7.2 (5 H, m), 6.3 (1 H, s, OH), 5.0 (1 H, m), 4.7 (1 H, m), 4.4 (1 H, m), 4.0 (1 H, m), 3.6 (1 H, m), 3.0(2 H ,m), 2.1(1 H, m), 1.8(1 H, s); ^{13}C NMR ($CDCl_3$) 171.8, 170.1, 136.0, 129.1, 128.5, 69.2, 61.5, 55.6, 53.3, 37.9 and 37.0 ppm.

RESULTS AND DISCUSSION

The synthesis of the cyclodipeptide involves two key steps: coupling of two amino acids, L-*trans*-4-hydroxyproline **2** and L-phenylalanine **3,** in suitably protected forms and the intramolecular cyclisation of the resulting dipeptide (Scheme 1).

For our synthetic purposes we required a suitably protected derivative of L-*trans*-4-hydroxyproline. The plan was to sequentially protect the secondary amine as its benzyloxycarbamate and the alcohol as its silyl ether. This was executed as follows:

The NH group of the hydroxyproline was protected as the oxycarbamate by the treatement with benzylchloroformate in the presence of sodium hydroxide, which furnished the *N*-protected hydroxyproline **4** as a pale yellow viscous liquid in 95 % yield. The carboxylic acid group was esterified using ethyl iodide and anhydrous potassium carbonate in DMF, and the protected hydroxyproline **5** was obtained as a yellow viscous liquid. In addition to other peaks the ^{1}H NMR spectrum of **5** showed two sets of triplets at d 0.8 ppm corresponding to 3 protons (CH_3) and two sets of quartets at 4.2 and 4.4 ppm corresponding to CH_2 of the ethyl ester group. This may be due to restricted rotation about the amide bond. Treating compound **5** with t-butyl dimethyl silyl chloride and imidazole in DMF (Klebe, 1972, Pierce, 1968) at room temperature finally protected the hydroxyl group. The fully protected pro-

line derivative **6** was thus obtained as a pale yellow viscous liquid in 94 % yield. The ^{1}H NMR spectrum of **6** showed peaks at d 1.1 ppm (t, 3 H, CH_3) and d 0.8 ppm (m, 9 H, t-butyl) and d 0.05 ppm (s, 6 H, 2 x CH_3 of silyl group). Deprotection of the carboxylic acid group of **6** was performed using lithium hydroxide in tetrahydrofuran, and the expected compound **7** was obtained in 99% yield.

The other precursor for the synthesis of the diketopiperazine was phenylalanine ethyl ester **8**. Phenylalanine ethyl ester hydrochloride is commercially available, but was also prepared in quantitative yield by refluxing phenylalanine in absolute ethanol in the presence of thionyl chloride.

The coupling reaction of the protected hydroxyproline **7** with phenylalanine ethyl ester hydrochloride **8** was accomplished using dicyclohexylcarbodiimide and triethylamine at room temperature to furnish the dipeptide **9** in 77% yield.

The IR spectrum of **9** showed peaks at 1658 (C=O of amide) and 1731 cm^{-1} (C=O of ester). The ^{1}H NMR spectrum of the dipeptide **9** showed signals at d 7.2-7.3 ppm (m, 10 H, aromatic protons), 5.1 ppm (d, 2 H, benzylic proton of the Cbz group), 5.0 ppm (m, 1 H, proton attached to the same carbon as the silyl ether), 4.1 ppm (m, 3 H, CH_2 of ethyl ester merging with the C-H of phenylalanine moiety), 3.7 ppm (m, 2 H, benzylic protons of phenylalanine), 1.1 ppm (m, 12 H, methyl group (3 H) of ester and *t*-butyl component (9 H) of silyl group) and 0.05 ppm (s, 6 H, 2 methyl groups of silyl derivative). The other signals at 4.4 ppm (m, 1 H), 3.9 ppm (m, 1 H), 2.3 ppm (m, 1 H), 2.0 ppm (m, 1 H) corresponded to the proline protons.

The ^{13}C NMR spectrum was also consistent with the structure assigned to compound **9**.

The Z group was then removed by refluxing dipeptide **9** with cyclohexene in methanol over Pd-C to give the deprotected product **10** in 97 % yield after chromatography.

Cyclisation was carried out by refluxing the dipeptide **10** in toluene, using a catalytic amount of DMAP. The protected diketopiperazine **11** was obtained as a pale yellow paste in 98 % yield. There were no signals corresponding to the methylene (4.1 ppm) and methyl (1.1 ppm) protons of the ester group in the ^{1}H NMR spectrum of the reaction product, thus indicating that cyclisation had indeed taken place.

The last step of the reaction sequence involved deprotection of the hydroxyl group using tetrabutylammonium fluoride in tetrahydrofuran (Corey &Venkateswarlu, 1972) at room temperature. Final purification by column chromatography afforded a pure sample of the diketopiperazine, which exhibited characterization data consistent with the proposed structure and the ^{13}C NMR and ^{1}H NMR spectra were consistent with the data reported for this compound by Adamczeski *et al* (1989). The biological activity of this compound is presently under investigation and will be reported in due course.

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REFERENCES

MANN, R., DAVIDSON, R.S., HOBBS, J.B., BANTHORPE, D.V. AND HARBORNE, J.B. (1994), *Natural Products*, pp 186-187, Longmann.

SCHMITZ, F.J., VANDERAH, D.J., HOLLENBEAK, K.H., ENWALL, C.E., GOPICHAND, Y., SENGUPTA P.K., HOSSAIN, M.B., VAN DER HELM, D. (1983). Metabolites from the marine sponge *Tedania ignis*. A new atisanediol and several known diketopiperazines. *Journal of Organic Chem*istry, **48**, 3941.

KUHKLA, M.J., BRESLIN, H.J. & BOWDEN, C.R. (1985). Synthesis, Characterisation, and Anorectic Testing of the Four Stereoisomers of Cyclo(histidylproline), *Journal of Medicinal Chem*istry, **28**, 1745-1747.

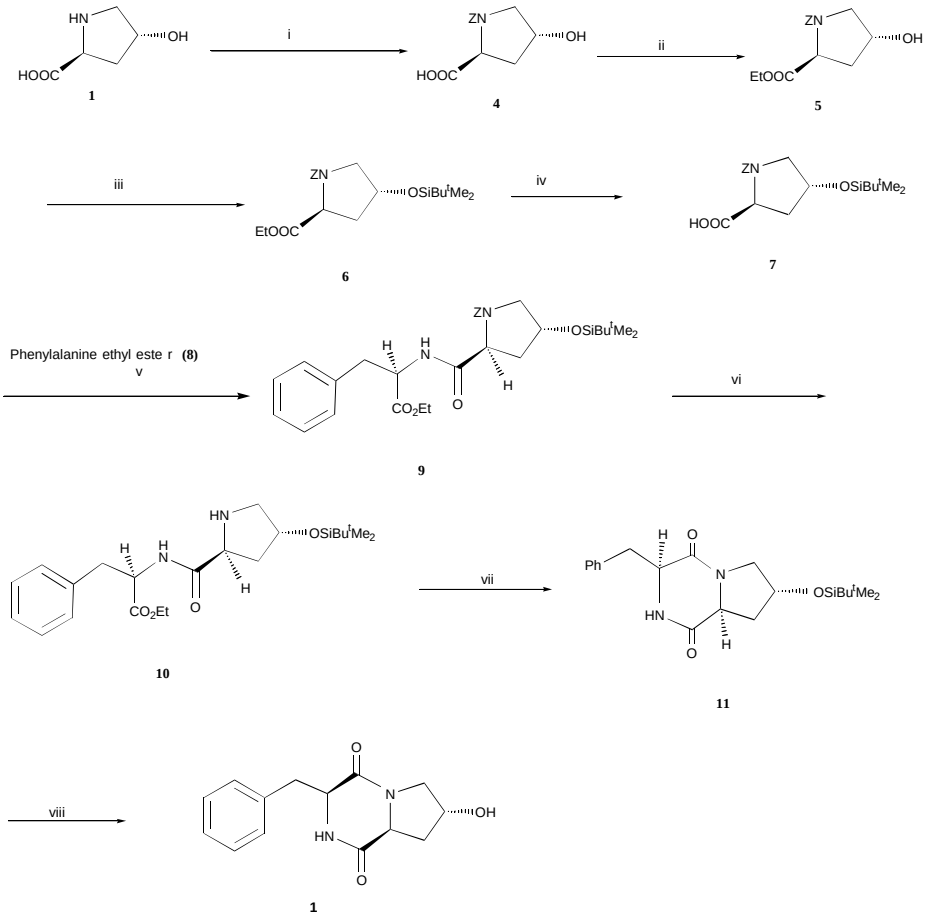
IENEGA, K., NAKAMURA, K. & GOTO, T. (1987). Bioactive compounds produced in animal tissues (I) ; Two diketopiperazine plant growth regulators containing hydroxyproline isolated from rabbit skin tissue extract. *Tetrahedron Lett*ers, **28**, 1285-1286.

ADAMCZESKI, M., QUINOA, E. & CREWS, P. (1989). Novel sponge-Derived Amino Acids Structures, Stereochemistry, and Synthesis of several New Heterocycles. *Journal of American Chemical Society*, **111**, 647-654.

RUDI, A., KASHMAN, Y., BENAYAHU, Y. & SCHLEYER, M. (1994.). Amino Acid derivatives from the Marine Sponge *Jaspidae Digonoxea*. *Journal of. Natural Products*, **57**, 829-832.

KLEBE, J.F. (1972), In *Advances in Organic Chemistry, Methods and Results*, Vol. 8 , pp. 97-178,(Ed E.C. Taylor) Wiley-Interscience, New York. Pierce, A.E. (1968), *Silylation of Organic Compounds*, Pierce Chemical Company, Rockford, Illinois,

COREY, E.J & VENKATESWARLU, A. (1972). *Journal of American Chemical Society*, **94**, 6190.



Scheme 1 *Reagents and conditions:* i,$PhCH_2OCOCl$; ii, Anhydrous K_2CO_3, C_2H_5I; iii, Bu^tMe_2SiCl, Imidazole; iv, LiOH; v, DCC; vi, Cyclohexene, Pd-C; vii, DMAP ; viii, TBAF