Cyclol Formation from Tripeptides containing β-Alanine

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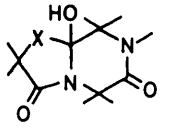
Attempts to synthesize stable tetrahedral intermediates (cyclols) from β-alanine containing precursors are described. Cyclization of *N*-(*N*-benzyloxycarbonyl-β-alanyl)-Phe-Pro-ONp gave *N*-(*N*-benzyloxycarbonyl-β-alanyl)-*cyclo*-(Phe-D-Pro). Cyclization of *N*-(*N*-benzyloxycarbonyl-Ala)-βAla-Pro-ONp and of *N* [*N*-(*R*)-(α-hydroxyisovaleryl)-βAla]-Pro-ONp afforded the corresponding anhydrocyclols. The first example of an oxa-cyclol related to a ten-membered cyclodepsitripeptide was synthesized by acylating *cyclo*-(βAla-Pro) with α-benzyloxypropionyl chloride followed by hydrogenolytic removal of the *O*-benzyl protecting group.

CHEMICAL and biochemical aspects of stable tetrahedral intermediates are receiving increasing attention.[1] In the peptide field, several tetrahedral intermediates (cyclols) have been isolated.[2] All these compounds possess the general structure (1) and can be obtained by cyclizing linear precursors containing activated *C*-terminal α-imino-acids or by acylating diketopiperazines with α-substituted acids. Peptides containing a stable cyclolic structure, known at the present time, are tautomeric with nine-membered cyclic tripeptides containing one secondary amidic bond (CONHR) in the ring and, as shown in structure (1), they have the cyclolic carbon atom



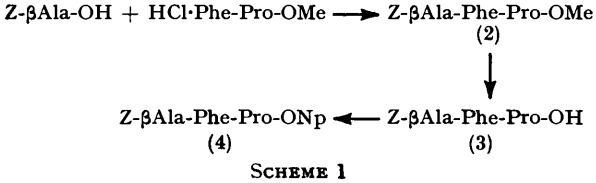
(1)

X = O
X = S
X = NCO_2R

(bonded to three heteroatoms) located at the juncture of a five- with a six-membered ring.

In continuation of our work in this field, and in order to examine the possibility of obtaining cyclols related to ten-membered cyclotripeptides, we report here results concerning the cyclization of linear tripeptides containing a β-amino-acid residue. Three models have been considered, namely Z-βAla-Phe-Pro, Z-Ala-βAla-Pro, and HyIv-βAla-Pro.

Synthesis of Z-βAla-Phe-Pro (3) and its activation to the corresponding *p*-nitrophenyl ester (4) are shown in Scheme 1. Owing to the presence of *C*-terminal proline, and that of the urethane protecting group on the *N*-terminal residue,[3] compound (4) should represent a suitable model to verify the possibility of isolating an

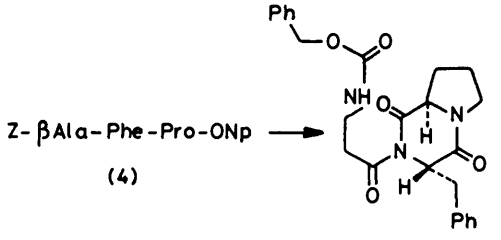


SCHEME 1

aza-cyclol with the cyclolic carbon atom at the juncture of two six-membered rings.

Cyclization of the active ester (4) was performed by following two different procedures: (i) treatment of compound (4) for 2 h at room temperature with alkaline aqueous buffer; and (ii) treatment of compound (4) with sodium hydride (1.2 equiv.) in dry *N*,*N*-dimethylformamide (DMF). Both procedures have already been successfully employed to synthesize type (1) aza-[3] and oxa-cyclols [4] starting from the corresponding linear peptide *p*-nitrophenyl esters.

By following procedure (i), 60% of the active ester (4) was hydrolysed to the corresponding acid; the neutral fraction consisted of one component containing starting material and *cyclo*-(Phe-D-Pro) as impurities. Purification by column chromatography afforded a cyclic compound (25—30% yield) to which structure (5) was assigned. No difference in the results occurred when



sodium hydride–DMF was used to cyclize the active ester (4); the acyldiketopiperazine (5) was in fact the only cyclic compound which could be isolated (in 15% yield). Chemical and spectroscopic properties of compound (5) are in accordance with the assigned structure and with the expected [2,5] epimerization at the chiral centre of the proline residue. Treatment with methanolic hydrazine at room temperature gives Z-βAla-$NHNH_2$ and *cyclo*-(Phe-D-Pro) in high yield; [3b] in the ^1H n.m.r. spectrum the α-H protons of the proline and phenylalanine residues are found at characteristically high [2,6] and low [2,7] field, respectively (δ 2.7 and 5.35), and the NH proton appears as triplet coupled to the β-H_2 of the β-alanine residue. In the ^{13}C n.m.r. spectrum (see the Table) four singlets from the carbonyl carbons are present and there are no signals in the range 90—110 p.p.m.[8] (cyclolic carbon atoms).

^{13}C N.m.r. data [a] from compounds (5), (10), (22), and (23) [b]

Residue		δ/p.p.m. (5)	(10)	(22)	(23)
Z-β-Alanine or β-Alanine	C-α	36.53t	36.03t	35.83t	34.49t
	C-β	40.35t	39.64t	37.51t	37.60t
	CON	173.66s *	169.42s *	170.49s [c]	171.07s [c]
	COO	156.27s			
	CH_2O	66.70t			
Pyrrolidine	C-α	59.00d [c]	113.18s	58.70d	65.04d
	C-β	29.24t	31.54t	28.89t	27.50t
	C-γ	22.07t	21.58t	22.26t	22.65t
	C-δ	44.84t	48.43t	48.44t	47.76t
	CO (or corresponding carbon atom)	169.65s [c]	120.61s	167.56s [c]	107.51s
Remaining residue	C-α	59.98d [c]	57.80d	76.75d	73.39d
	C-β	37.91t	17.39q	18.13q	17.73q
	CON	164.38s [c]	168.84s [c]	176.50s [c]	171.46s [c]
	COO		152.82s		
	CH_2O		68.35t	72.26t	

[a] Chemical shifts relative to $SiMe_4$, for $CDCl_3$ solutions. [b] Recorded on a Bruker WH-90 (22.63 MHz) instrument. [c] Values may be interchanged.

The syntheses of Z-Ala-βAla-Pro-ONp (9) and HyIv-βAla-Pro-ONp (19) are shown in Schemes 2 and 3. The tripeptides (9) and (19) contain the β-amino-acid residue in the central position and have been designed in order to give information about aza- and oxa-cyclols in which the cyclolic carbon atom is located at the juncture of a five- with a seven-membered ring.

Z-Ala-OH + HCl·βAla-OMe ⟶ Z-Ala-βAla-OMe (6)

Z-Ala-βAla-Pro-OH (8) ⟶ Z-Ala-βAla-Pro-ONp (9)

SCHEME 2

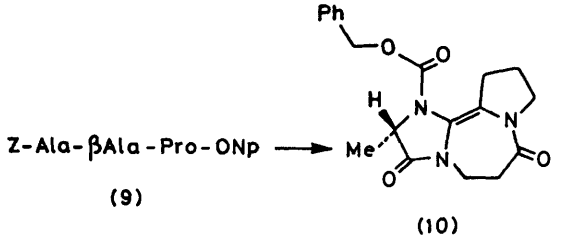
Attempted cyclization of compound (9) in aqueous medium by procedure (i) was unsuccessful; under these conditions no cyclization products could be detected and the prevailing reaction was the hydrolysis of the active ester. By using DMF with sodium hydride the cyclization product (10) was isolated in low yield together with different amounts of hydantoin derivatives. The side-reaction leading to the elimination of benzyl alcohol could be almost completely suppressed, and the yield of the cyclization product enhanced, through an accurate regulation of the reaction conditions (slow addition of 2.2 equiv. of NaH to the solution of the active ester cooled to −10 °C). The ^{13}C n.m.r. spectrum of compound (10) (Table) shows three carbonyl singlets and two additional singlets at δ 113.18 and 120.61. Only one doublet attributable to an α-H is present (at δ 57.80); in the ^1H n.m.r. spectrum on the other hand the Ala α-H quartet appears at δ 4.5. The mass spectrum shows intense signals at *m/e* 355 (*M*) and 220 (*M* − $PhCH_2OCO$); the peak at *m/e* 70 (pyrrolinium ion), characteristic of proline-containing peptides, is not significant.

The anhydrocyclol (10) was also obtained by refluxing the active ester (9) in dry benzene with diazabicycloundecene (DBU). Treatment of Z-Ala-βAla-Pro (8) with acetic anhydride–sodium acetate at 100 °C under the conditions usually adopted to prepare *N*-acyldiketopiperazines from *N*-acyl-X-Pro-OH dipeptides [2] did not afford the anhydrocyclol (10); this compound was, however, obtained in good yield when treatment with acetic anhydride–sodium acetate was carried out at 140 °C for 2 h.

HyIv-OH + HCl·βAla-OMe ⟶ HyIv-βAla-OMe (15) ⟶ HyIv-βAla-OH (16)

HyIv-βAla-Pro-OBzl (17) ⟶ HyIv-βAla-Pro-OH (18) ⟶ HyIv-βAla-Pro-ONp (19)

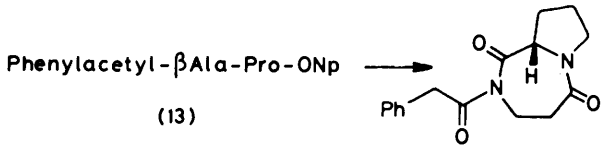
SCHEME 3



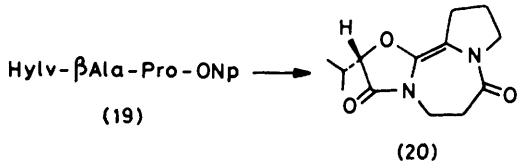
(10)

As is well known, seven-membered cyclodipeptides are highly strained and, unlike diketopiperazines, are not easily formed. Owing to the presence of a rigid N–C(α) bond in *C*-terminal proline,[9] however, formation of the anhydrocyclol (10) from the corresponding cyclol should involve an intermediate *N*-(*N*-benzyloxycarbonylalanyl)-*cyclo*-(βAla-Pro), rather than an intermediate ten-membered cyclotripeptide. In order to substantiate this hypothesis, *N*-phenylacetyl-βAla-Pro-ONp (13) was synthesized and subjected to cyclization conditions. In accordance with the results obtained in the cyclization of

the tripeptide (9), cyclization of compound (13) to *N*-phenylacetyl-*cyclo*-(βAla-Pro) (14) required non-aqueous conditions and an excess of sodium hydride. Good yields of (14) were also obtained by refluxing compound (13) with DBU in dry benzene, and by using acetic anhydride–sodium acetate at 140 °C.



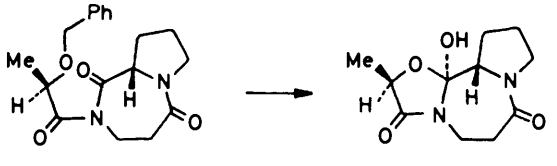
Cyclization of HyIv-βAla-Pro-ONp (19) gave essentially the same results as those obtained in the cyclization of Z-Ala-βAla-Pro-ONp (9). Isolation and purification of the anhydrocyclol (20) was, however, found to be difficult because of the instability of the product.



The above results suggest that intermediate *N*-(α-amidoacyl)- or *N*-(α-hydroxyacyl)-*cyclo*-(βAla-Pro) can equilibrate with the corresponding aza- or oxa-cyclols. In the conditions adopted for the formation of the seven-membered cyclic intermediates, however, cyclolic forms are not stable and only the corresponding anhydro-derivatives (10) and (20) can be isolated.

By following these indications the synthesis of a cyclol related to a ten-membered cyclodepsitripeptide was attempted following a different approach.[5]

Cyclo-(βAla-Pro) (21) was synthesized and acylated with (*R*)-α-benzyloxypropionyl chloride to give *N*-[(*R*)-α-benzyloxypropionyl]-*cyclo*-(βAla-Pro) (22). Deprotection with Pd–H_2 in glacial acetic acid gave a crystalline compound to which the oxa-cyclol structure (23) was assigned. Treatment of compound (23) at room



temperature with methanolic hydrazine gave *cyclo*-(βAla-Pro); in the ^{1}H n.m.r. spectrum the α-H of the lactyl residue is found shifted to high field (0.50 p.p.m.) relative to the imidic precursor (22);[2,3b] the exchangeable proton appears at δ 7.3 [in $(CD_3)_2SO$] as a sharp doublet (long range coupled to α-H Pro); signals from the βAla β-H_2 and lactyl α-H protons are not affected by exchange with D_2O. The i.r. spectrum ($CHCl_3$) shows a large band centred at 3 300 cm^{-1} and no absorptions in the regions of lactone carbonyls and the *trans*-amide II band (1 480—1 575 cm^{-1}). The ^{13}C n.m.r. spectrum reveals only two carbonyl signals and a singlet at δ 107.5 p.p.m.; this resonance is in accordance with the presence, in compound (23), of a non-protonated carbon bonded to three heteroatoms.[10] The mass spectrum does not show ions heavier than $M + 1$. No evidence of isomerization into the ten-membered cyclodepsitripeptide or into the α-hydroxyacyl derivative could be deduced from the spectral data.

Failure to obtain an aza-cyclol from the *N*-(β-benzyloxycarbonylamidoacyl)diketopiperazine intermediate, as in the case of the tripeptide (4), confirms that cyclols containing two condensed six-membered rings are not stable and cannot be isolated despite the mild cyclization conditions and the favourable system examined. It is worth noting in this context that *N*-(β-aminoacyl)-diketopiperazines,[9] in which a more nucleophilic group is contained, easily isomerize into the corresponding ten-membered cyclotripeptides which represent the stable tautomers.

The isolation of the oxa-cyclol (23) indicates that trans-annular interaction can also occur in ten-membered cyclotripeptides and that the resulting cyclols, built up from a five-membered ring condensed with a seven-membered ring, are stable enough to be isolated. This finding parallels the behaviour of *N*-(α-hydroxyacyl)-caprolactams.[11] These compounds do not show a tendency to be converted into the corresponding ten-membered cyclic tautomers and in solution exist as cyclols [11a] or in equilibrium with the cyclolic forms.[11b]

EXPERIMENTAL

M.p.s are uncorrected. I.r. spectra were recorded with a Perkin-Elmer 521 spectrophotometer. ^{1}H N.m.r. spectra were recorded with a Varian EM 390 spectrometer ($SiMe_4$ as internal standard). ^{13}C N.m.r. spectra were recorded with a Varian CFT 20 (20 MHz) instrument unless otherwise indicated. Mass spectra were determined with a Hewlett-Packard 5980 A spectrometer operating at 70 eV. Optical rotations were taken at 20 °C with a Schmidt-Haensch 16065 polarimeter.

N-(N-*Benzyloxycarbonyl*-β-*alanyl*)-L-*phenylalanyl*-L-*proline Methyl Ester* (2).—To a solution of L-phenylalanyl-L-proline methyl ester hydrochloride (6.42 g) and *N*-benzyloxycarbonyl-β-alanine (4.6 g) containing dicyclohexylcarbodi-imide (4.24 g) in tetrahydrofuran (THF) (80 ml) and methanol (10 ml), *N*-methylmorpholine (2.08 g) was added with stirring. After 4 h at 0 °C and 12 h at 5 °C the mixture was filtered and the resulting solution evaporated under reduced pressure. The residue, dissolved in ethyl acetate, was washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water, dried and evaporated to give an oily residue (8.7 g). Column chromatography (silica, ethyl acetate as eluant) gave *compound* (2) (5.2 g) as an oil, $[\alpha]_D$ −40° (c 1.00 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1 740, 1 710, and 1 660—1 635 cm^{-1}; δ($CDCl_3$) 2.3 (2 H, m, α-H_2 βAla), 3.7 (3 H, s, OMe), 4.5 (1 H, m, α-H Pro), 5.0 (1 H, m, α-H Phe), 5.4 (1 H, t, *J* 3.0 Hz, NH βAla), and 7.1 (1 H, d, *J* 8.5

Hz, NH Phe) (Found: C, 64.6; H, 6.5; N, 8.65. $C_{26}H_{31}N_3O_6$ requires C, 64.85; H, 6.5; N, 8.7%).

N-(N-*Benzyloxycarbonyl*-β-*alanyl*)-L-*phenylalanyl*-L-*proline* p-*Nitrophenyl Ester* (4).—To a solution of the methyl ester (2) (4.6 g), 2N-sodium hydroxide (9.6 ml) in methanol (25 ml) was added. After 6 h at room temperature the solution was evaporated under reduced pressure and the residue taken up in water. The aqueous alkaline solution was washed with ethyl acetate, acidified, and extracted with chloroform. The organic layer was washed with water, dried, and evaporated to give Z-βAla-Phe-Pro-OH (3) (4.3 g) which was used without further purification.

To a solution of compound (3) (2.8 g) and *p*-nitrophenol (1.7 g) in ethyl acetate (50 ml), dicyclohexylcarbodi-imide (1.3 g) was added at 0 °C with stirring. After 2 h at 0 °C and 12 h at 5 °C, the mixture was filtered and the solution repeatedly washed with saturated aqueous sodium carbonate and water. After drying and evaporation, the residue (3.1 g) was chromatographed (silica, chloroform–diethyl ether, 1 : 1 as eluant) to give the *active ester* (4) (2.5 g) as an oil, $[\alpha]_D$ −45° (*c*, 1.00 in $CHCl_3$), $\nu_{max.}$ 1 765, 1 710, and 1 660—1 630 cm^{-1}; δ($CDCl_3$) 2.4 (2 H, t, α-H_2 βAla), 4.6 (1 H, m, α-H Pro), 5.0 (1 H, m, α-H Phe), 5.5 (1 H, t, *J* 5.5 Hz, NH βAla), and 7.0 (1 H, d, *J* 8.5 Hz, NH Phe); *m/e* M^+ absent, 449 (*M* − 139, 1.0%), 358 (*M* − 139 −91, 3.5), 139 (*p*-nitrophenol, 25), and 91 (CH_2Ph, 100).

N-(N-*Benzyloxycarbonyl*-β-*alanyl*)-*cyclo*-(L-*phenylalanyl*-D-*prolyl*) (5).—*Procedure* (i). To a solution of the active ester (4) (0.98 g) in dioxan (50 ml), aqueous 0.1M-sodium hydrogen carbonate (25 ml) and aqueous 0.1M-sodium carbonate (25 ml) were added. After 2 h at room temperature the reaction mixture was evaporated under reduced pressure. The residue was partitioned between water and chloroform and the organic layer washed with saturated sodium carbonate solution and water. After drying and removal of the chloroform, the residue (0.38 g) was purified by preparative t.l.c. ethyl acetate–hexane, (9 : 1 as eluant) to afford the N-*acyldiketopiperazine* (5) (180 mg) as a foam, $[\alpha]_D$ −94° (*c*, 1.00 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 725 and 1 670 cm^{-1}; δ($CDCl_3$) 1.5—2.2 (4 H, m, β- and α-H_2 Pro), 2.7 (1 H, m, α-H Pro), 3.1—3.2 (4 H, m, α-H_2 βAla and β-H_2 Phe), 3.4—3.7 (4 H, m, β-H_2 βAla and -H_2 Pro), 5.1 (2 H, s, CH_2O), 5.35 (1H, X-part of ABX system, α-H Phe), 5.40 (1 H, t, *J* 6.0 Hz, NH), and 7.1—7.5 (10 H, m, Ph); *m/e* 449(M^+, 7.5%), 358(*M* − CH_2Ph, 22), 342(*M* − OCH_2Ph, 23), 314(358 − CO_2, 22), 244(diketopiperazine, 26), and 91(CH_2Ph, 100) (Found: C, 66.6; H, 6.05; N, 9.05. $C_{25}H_{27}N_3O_5$ requires C, 66.8; H, 6.05; N, 9.35%).

Procedure (ii). To a solution of the active ester (4) (0.50 g) in dry *N*,*N*-dimethylformamide (DMF) (12 ml), sodium hydride (80% in white oil; 32 mg) was added at 0 °C with stirring. After 6 h at 0 °C and 12 h at room temperature, ice-cold aqueous sodium hydrogen carbonate and then methylene chloride were added. The organic layer was washed with 1N-sodium carbonate and water. Drying and evaporation gave a residue which, after preparative t.l.c., afforded *compound* (5) in 15% yield.

N-(N-*Benzyloxycarbonyl*-L-*alanyl*)-β-*alanine Methyl Ester* (6).—To a solution of β-alanine methyl ester hydrochloride (5.0 g) and *N*-benzyloxycarbonyl-L-alanine (8.0 g) containing dicyclohexylcarbodi-imide (7.35 g) in ethyl acetate (100 ml), *N*-methylmorpholine (3.6 g) was added with stirring. After 4 h at 0 °C and 12 h at 5 °C, the mixture was filtered, diluted with ethyl acetate and washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water. The residue obtained after drying and evaporation was crystallised (ethyl acetate–hexane) to give *compound* (6) (7.0 g), m.p. 96—97 °C, $[\alpha]_D$ −13° (*c* 1.00 in $CHCl_3$), $\nu_{max.}$ (KBr) 1 730, 1 690, and 1 655 cm^{-1}; δ($CDCl_3$) 1.3 (3 H, d, *J* 6.5 Hz, Me Ala), 2.5 (2 H, m, α-H_2 βAla), 3.45 (2 H, m, β-H_2 βAla), 3.65 (3 H, s, OMe), 4.25 (1 H, m, α-H Ala), 5.1 (2 H, s, CH_2O), 5.8 (1 H, d, *J* 7.5 Hz, NH Ala), 6.95 (1 H, br, t, NH βAla), and 7.3 (5 H, m, Ph) (Found: C, 58.4; H, 6.5; N, 8.9. $C_{15}H_{20}N_2O_5$ requires C, 58.4; H, 6.5; N, 9.1%).

N-(N-*Benzyloxycarbonyl*-L-*alanyl*)-β-*alanine Hydrazide* (7).—To a solution of methyl ester (6) (3.0 g) in methanol (13 ml), hydrazine hydrate (4.3 ml) was added and the mixture was set aside for 20 h at room temperature. The precipitate was collected and recrystallised from methanol to afford *compound* (7) (2.1 g), m.p. 187—188 °C, $[\alpha]_D$ −10.5° (*c*, 1.00 CH_3CO_2H), $\nu_{max.}$ (KBr) 1 675, 1 645, and 1 620 cm^{-1}; δ-[$(CD_3)_2SO$] 1.2 (3 H, d, *J* 7.5 Hz, Me Ala), 2.2 (2 H, m, α-H_2 βAla), 3.25 (2 H, m, β-H_2 βAla), 4.05 (1 H, m, α-H Ala), 4.2 (2 H, br s, NH_2), 5.05 (2 H, s, CH_2O), 7.4 (6 H, m, NH Ala and Ph), 7.9 (1 H, br t, NH βAla), and 9.1 (1 H, br s, N*H*-NH_2) (Found: C, 54.45; H, 6.4; N, 18.1. $C_{14}H_{20}N_4O_4$ requires C, 54.5; H, 6.5; N, 18.2%).

N-(N-*Benzyloxycarbonyl*-L-*alanyl*)-β-*alanyl*-L-*proline* (8).—To a solution of the hydrazide (7) (8.55 g) in glacial acetic acid (11 ml) and 1N hydrochloric acid (200 ml), sodium nitrite (1.9 g), dissolved in the minimum of cold water, was added with stirring at −5 °C. After 10 min the solid was filtered, dissolved at −5 °C in THF (110 ml), and added dropwise to a stirred solution cooled to 0 °C of L-proline (3.3 g) in aqueous 1N-sodium hydroxide. Stirring was continued at 0 °C for an additional hour during which time 1N-sodium hydroxide was added to keep the reaction mixture alkaline. THF was evaporated under reduced pressure and the resulting aqueous solution was washed with ethyl acetate. Acidification followed by extraction with chloroform gave the *tripeptide acid* (8) (7.75 g) which was purified by column chromatography (silica gel, ethyl acetate–acetic acid, 97 : 3 as eluant) to give an oily product, pure by n.m.r. and t.l.c., $[\alpha]_D$ −54.0° (*c* 1.00 in MeOH), δ[$(CD_3)_2SO$] 1.2 (3 H, d, *J* 7.5 Hz, Me Ala), 1.6—2.2 (4 H, m, β-H_2 and γ-H_2 Pro), 2.2—2.5 (2 H, m, α-H_2 βAla), 3.1—3.6 (4 H, m, δ-H_2 Pro and β-H_2 βAla), 4.0 (1 H, m, α-H Ala), 4.2 (1 H, m, α-H Pro) 5.0 (2 H, s, CH_2O), 7.3 (6 H, m, NH Ala and Ph), and 7.8 (1 H, br t, NH βAla).

N-(N-*Benzyloxycarbonyl*-L-*alanyl*)-β-*alanyl*-L-*proline* p-*Nitrophenyl Ester* (9).—This compound was prepared by following the procedure reported for the active ester (4). Starting from the tripeptide (8) (2.9 g) the crude *p*-nitrophenyl ester (9) (3.0 g) was obtained. Purification by column chromatography (silica gel, ethyl acetate as eluant), afforded compound (9) as an oil, pure by n.m.r. and t.l.c.; this could be used without further purification, δ($CDCl_3$) 1.3 (3 H, d, *J* 7.5 Hz, Me Ala), 4.2 (1 H, m, α-H Ala), 4.6 (1 H, m, α-H Pro), 5.05 (2 H, s, CH_2O), 5.7 (1 H, d, *J* 7.5 Hz, NH Ala), 7.1 (1 H, br t, NH βAla), 7.3 (7 H, m, arom.), and 8.25 (2 H, two lines, arom.).

Anhydroaza-cyclol (10).—To a solution, cooled to −10 °C, of the ester (9) (2.15 g) in dry DMF (140 ml), sodium hydride (80% in white oil; 277 mg) was added with stirring. After 6 h at 0 °C and 12 h at room temperature, the reaction mixture was worked up as described for compound (5) to give the cyclol (10) as a foam (1.15 g). Purification (column chromatography; silica gel, ethyl acetate as eluant) and crystallisation from ethyl acetate–diethyl ether afforded the *anhydro-cyclol* (10) (0.55 g), m.p. 110—111 °C, $[\alpha]_D$ −28° (*c*

1.00 in $CHCl_3$), $\nu_{max.}$ (KBr) 1 690, 1 615, and 1 595 cm^{-1}; δ($CDCl_3$) 1.5 (3 H, d, *J* 6.8 Hz, Ala Me), 1.7—2.8 (4 H, m, β- and γ-H_2 Pro), 2.6—2.9 (2 H, m, α-H_2 βAla), 3.5—3.8 (2 H, m, δ-H_2 Pro), 3.70 and 4.20 (2 H, m, β-H_2 βAla), 4.5 (1 H, q, *J* 6.8 Hz, α-H Ala), 5.25 (2 H, s, CH_2O), and 7.40 (5 H, m, Ph); *m/e* 355(M^+, 30%), 220(M − $OCOCH_2Ph$, 25), 166(46), and 91(100) (Found: C, 61.45; H, 5.95; N, 11.4. $C_{19}H_{21}N_3O_4 \cdot H_2O$ requires C, 61.1; H, 6.2; N, 11.25%).

N-(N-*Phenylacetyl*-β-*alanyl*)-L-*proline Methyl Ester* (11).—To a solution of L-proline methyl ester hydrochloride (4.15 g) and *N*-phenylacetyl-β-alanine [12] (5.2 g) containing dicyclohexylcarbodi-imide (5.15 g) in ethyl acetate (70 ml) and methanol (20 ml), *N*-methylmorpholine (2.5 g) was added with stirring. After 2 h at 0 °C and 12 h at 5 °C, the mixture was filtered and the resulting solution evaporated under reduced pressure. Work-up gave 7.0 g of residue. Column chromatography (silica gel, ethyl acetate–methanol, 95 : 5 as eluant) afforded the *methyl ester* (11) (6.0 g) as an oil, δ($CDCl_3$) 1.8—2.2 (4 H, m, β- and γ-H_2 Pro), 2.5 (2 H, m, α-H_2 βAla), 3.3—3.6 (4 H, m, δ-H_2 Pro and β-H_2 βAla), 3.5 (2 H, s, CH_2Ph), 3.7 (3 H, s, OMe), 4.4 (1 H, m, α-H Pro), 6.7 (1 H, br t, NH), and 7.3 (5 H, m, arom.); $\nu_{max.}$ ($CHCl_3$) 1 740 and 1 640 cm^{-1} (Found: C, 63.9; H, 6.85; N, 8.7. $C_{17}H_{22}N_2O_4$ requires C, 64.1; H, 7.0; N, 8.8%).

N-(N-*Phenylacetyl*-β-*alanyl*)-L-*proline* (12).—To a solution of the dipeptide methyl ester (11) (3.6 g) in methanol (30 ml), 2N-sodium hydroxide (11 ml) was added. After 5 h at room temperature, the solution was evaporated under reduced pressure and the residue taken up in water. The aqueous solution was washed with ethyl acetate, acidified, and extracted with chloroform. The organic layer was washed with water, dried, and evaporated to give 3.0 g of residue. Purification by column chromatography (silica gel, ethyl acetate–acetic acid, 97 : 3 as eluant) afforded *compound* (12) (2.5 g) as a foam, pure by n.m.r. and t.l.c., $\nu_{max.}$ ($CHCl_3$) 1 725 and 1 650 cm^{-1}; δ[$(CD_3)_2SO$] 1.7—2.2 (4 H, m, β- and γ-H_2 Pro), 2.3—2.6 (2 H, m, α-H_2 βAla), 3.2—3.6 (4 H, m, β-H_2 βAla and δ-H_2 Pro), 4.4 (1 H, m, α-H Pro), 7.25 (5 H, m, arom.), and 8.1 (1 H, br t, NH).

N-*Phenylacetyl*-cyclo-(β-*alanylprolyl*) (14).—By following the procedure adopted for the activation of compounds (3) and (8), *N*-(*N*-phenylacetyl-β-alanyl)-L-proline *p*-nitrophenyl ester (13) was prepared and used without further purification. Treatment of the ester (13) (0.84 g) under the same conditions as those adopted for the synthesis of the anhydro-cyclol (10) afforded, after preparative t.l.c. (silica gel, ethyl acetate–methanol 95 : 5 as eluant), the *title compound* (14) (90 mg) as an oil, $\nu_{max.}$ ($CHCl_3$) 1 720—1 705 and 1 640—1 615 cm^{-1}; δ($CDCl_3$) 1.5—2.3 (4 H, m, β- and γ-H_2 Pro), 2.75 (2 H, m, α-H_2 βAla), 3.5 (2 H, m, δ-H_2 Pro), 4.3 (2 H, s, CH_2Ph), 4.65 and 3.5 (2 H, m, β-H_2 βAla), 4.75 (1 H, m, α-H Pro), and 7.3 (5 H, m, Ph); *m/e* 286 (M^+, 37.5%), 216(M − 70, 45), 167(M − $PhCH_2CO$, 12), and 70(pyrrolinium, 100) (Found: C, 67.0; H, 6.3; N, 9.6. $C_{16}H_{18}N_2O_3$ requires C, 67.1; H, 6.3; N, 9.8%).

N-(L-α-*Hydroxyisovaleryl*)-β-*alanine Methyl Ester* (15).—To a stirred ice-cold solution of L-α-hydroxyisovaleric acid (25.4 g) in THF (250 ml), β-alanine methyl ester hydrochloride (30.0 g) and *N*-methylmorpholine (21.7 g) in methanol (50 ml) were added. After addition of 1-hydroxybenzotriazole (29.0 g) and dicyclohexylcarbodimide (44.3 g), the mixture was stirred for 1 h at 0 °C and 3 h at room temperature. The solid was removed by filtration and the solution evaporated under reduced pressure. The residue taken up in chloroform was washed with 2N-citric acid, saturated aqueous sodium hydrogen carbonate, and water. Drying and evaporation afforded a residue (24 g). Crystallisation from diethyl ether–hexane gave *compound* (15) (18 g), m.p. 41—42 °C, $[\alpha]_D$ −37.0° (*c* 1.00 in $CHCl_3$), δ($CDCl_3$) 0.78 (3 H, d, *J* 7.5 Hz, Me), 0.96 (3 H, d, *J* 7.5 Hz, Me), 2.1 (1 H, m, β-H HyIv), 2.55 (2 H, m, α-H_2 βAla), 3.55 (2 H, m, β-H_2 βAla), 3.68 (3 H, s, OMe), 3.90 (1 H, m, α-H HyIv), 4.3 (1 H, br d, OH), and 7.3 (1 H, br t, NH) (Found: C, 53.05; H, 8.4; N, 6.7. $C_9H_{17}NO_4$ requires C, 53.2; H, 8.4; N, 6.9%).

N-(L-α-*Hydroxyisovaleryl*)-β-*alanine* (16).—To a solution of the methyl ester (15) (13.1 g) in methanol (170 ml), 2N-sodium hydroxide (35 ml) was added. After 1 h at room temperature the solution was evaporated and the residue taken up in water. The aqueous alkaline solution was washed with diethyl ether, acidified, and extracted for 20 h in a continuous extractor with chloroform. After drying and evaporation, the residue (10 g) was crystallised from diethyl ether–hexane to give *compound* (16) (7.5 g), m.p. 76—78 °C, $[\alpha]_D$ +51.0° (*c* 1.00 in $CHCl_3$), δ[$(CD_3)_2SO$] 0.75 (3 H, d, *J* 7.0 Hz, Me), 0.90 (3 H, d, *J* 7.0 Hz, Me), 2.0 (1 H, m, β-H HyIv), 2.4 (2 H, m, α-H_2 βAla), 3.4 (2 H, m, β-H_2 βAla), 3.7 (1 H, d, *J* 4.5 Hz, α-H HyIv), and 7.8 (1 H, br t, NH) (Found: C, 50.6; H, 7.85; N, 7.35. $C_8H_{15}NO_4$ requires C, 50.8; H, 8.0; N, 7.4%).

N-[N-(L-α-*Hydroxyisovaleryl*)-β-*alanyl*]-L-*proline Benzyl Ester* (17).—A mixture of L-proline benzyl ester hydrochloride (1.5 g) and triethylamine (0.64 g) in ethyl acetate (10 ml) was stirred for 0.5 h at 0 °C. A solution of the acid (16) (1.2 g) in THF (15 ml) and a solution of dicyclohexylcarbodi-imide (1.3 g) in ethyl acetate (15) ml) were then added. The reaction mixture was stirred for an additional 2 h at 0 °C and for 20 h at 5 °C. After the usual work-up procedure, crude benzyl ester (17) (1.65 g) was obtained. Chromatography through silica (ethyl acetate–methanol, 95 : 5 as eluant) gave *compound* (17) (1.2 g) which was crystallised from diethyl ether, m.p. 78—80 °C, $[\alpha]_D$ −88° (*c* 1.00 in $CHCl_3$), δ($CDCl_3$) 0.80 (3 H, d, *J* 7.0 Hz, Me), 1.0 (3 H, d, *J* 7.0 Hz, Me), 1.8—2.3 (5 H, m, β- and γ-H_2 Pro and β-H HyIv), 2.5 (2 H, m, α-H_2 βAla), 3.4—3.7 (5 H, m, β-H_2 βAla, δ-H_2 Pro and OH), 3.9 (1 H, m, α-H HyIv), 4.6 (1 H, m, α-H Pro), 5.2 (2 H, AB q, CH_2Ph), 7.3 (1 H, br t, NH), and 7.4 (5 H, m, Ph) (Found: C, 63.85; H, 7.4; N, 7.35. $C_{20}H_{28}N_2O_5$ requires C, 63.8; H, 7.5; N, 7.4%).

N-[N-(L-α-*Hydroxyisovaleryl*)-β-*alanyl*]-L-*proline* p-*Nitrophenyl Ester* (19).—A solution of the benzyl ester (17) (2.0 g) in methanol (40 ml) was hydrogenated in the presence of Pd (black; 0.40 g) during 5 h. The catalyst was filtered off and the solution evaporated to give *N*-[*N*-(L-α-hydroxyisovaleryl)-β-alanyl]-L-proline (18) (1.40 g), m.p. 131—132 °C (ethyl acetate–hexane). Activation of compound (18) (1.0 g) was performed as described for compounds (4) and (9). Crude active ester (19) (1.15 g) was purified by column chromatography (silica gel, ethyl acetate–methanol 97 : 3) and by preparative t.l.c., affording the *active ester* (19) (0.70 g), δ($CDCl_3$) 0.80 (3 H, d, *J* 7.0 Hz, Me), 0.95 (3 H, d, *J* 7.0 Hz, Me), 1.9—2.5 (5 H, m, β- and γ-H_2 Pro, β-H HyIv), 2.6 (2 H, m, α-H_2 βAla), 3.45—3.80 (5 H, m, β-H_2 βAla, δ-H_2 Pro and OH), 3.90 (1 H, m, α-H HyIv), 4.65 (1 H, m, α-H Pro), 7.3 (1 H, br t, NH), 7.3 (2 H, m, arom.), and 8.25 (2 H, m, arom.).

Cyclization of the Active Ester (19).—By following the procedure reported for anhydroaza-cyclol (10), treatment of the ester (19) (0.50 g) with sodium hydride in dry DMF afforded a complex mixture from which the *anhydro-oxa-cyclol*

(20) (12 mg) was isolated by preparative t.l.c. as an unstable oily compound (silica gel, ethyl acetate as eluant), *m/e* 250(M^+, 47%), 168(12), and 139(100); δ($CDCl_3$) 0.95 (3 H, d, *J* 7.0 Hz, Me), 1.10 (3 H, d, *J* 7.0 Hz, Me), 1.6—2.3 (3 H, m, $CH_2CH_2CH_2$ and β-H HyIv), 2.70 (4 H, m, α-H_2 βAla and $CH_2C{=}C$), 3.8 [4 H, m, β-H_2 βAla and $(CH_2)_2CH_2N$], and 4.45 (1 H, d, *J* 3.5 Hz, α-H HyIv).

Cyclo-(β-*Alanylprolyl*) (21).—A solution of *N*-(β-alanyl)-L-proline *p*-nitrophenyl ester hydrochloride (2.2 g) in dry pyridine (640 ml) and dry DMF (90 ml) was set aside for 20 h at 55 °C. Evaporation of the solvents afforded a residue which, after column chromatography (silica gel, chloroform–methanol 9 : 1 as eluant), gave *compound* (21) (0.90 g), m.p. 166—167 °C (ethanol), $[\alpha]_D$ −30° (*c* 1.00 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 685, 1 650, and 1 615 cm^{-1}; δ($CDCl_3$) 1.65—2.30 (3 H, m, β-H_2 Pro and γ-H Pro), 2.45 (1 H, m, γ-H Pro), 2.75 (2 H, m, α-H_2 βAla), 3.2—3.8 (4 H, m, β-H_2 βAla and δ-H_2 Pro), 4.55 (1 H, m, α-H Pro), and 7.4 (1 H, br t, NH); *m/e* 168(M^+, 48%), 140(17), and 70(100) (Found: C, 57.0; H, 7.2; N, 16.5. $C_8H_{12}N_2O_2$ requires C, 57.1; H, 7.2; N, 16.7%).

N-[(R)-α-*Benzyloxypropionyl*]-cyclo-(β-*alanylprolyl*) (22).—A mixture of (+)-(*R*)-α-benzyloxypropionyl chloride [13] (4.5 g) and compound (21) (2.7 g) in dry dioxan (60 ml) containing dry pyridine (1.8 ml) was heated for 15 h at 80 °C. Evaporation of the solvent gave a viscous oil which was shaken at room temperature with a mixture of diethyl ether and water for 0.5 h to hydrolyse the excess of acid chloride. The solid (22) (2.0 g) was filtered off and the organic layer was washed with 1N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water. Drying and evaporation afforded a residue which, after column chromatography (silica gel, ethyl acetate–methanol 97 : 3 as eluant), gave additional compound (22) (0.70 g). Crystallisation from ethyl acetate of the collected fractions gave pure *compound* (22) (2.0 g), m.p. 148—150 °C, $[\alpha]_D$ +66° (*c* 0.50 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 1 720 and 1 635 cm^{-1}; δ($CDCl_3$) 1.36 (3 H, d, *J* 6.0 Hz, Me), 1.5—2.3 (3 H, m, β-H_2 Pro and γ-H Pro), 2.4—2.8 (3 H, m, α-H_2 βAla and γ-H Pro), 3.4—3.8 (3 H, m, δ-H_2 Pro and β-H βAla), 4.52 (2 H, AB q, CH_2O), 4.6 (1 H, m, β-H βAla), 4.75 (1 H, m, α-H Pro), 5.08 (1 H, q, *J* 6.0 Hz, *CH*Me), and 7.33 (5 H, m, Ph); *m/e* 331(M^+ + 1, 56%), 224(100), 91(55), and 70(61) (Found: C, 65.4; H, 6.7; N, 8.5. $C_{18}H_{22}N_2O_4$ requires C, 65.4; H, 6.7; N, 8.5%).

Mild acid hydrolysis of compound (22) (190 mg) in methanol (3.0 ml) and 5N-hydrochloric acid (2.0 ml) for 5 h at room temperature gave, after work-up (+)-(*R*)-α-benzyloxypropionic acid, $[\alpha]_D$ +80° (*c* 1.00 in MeOH).[14]

Oxa-cyclol (23).—The *O*-benzyl derivative (22) (2.0 g) was hydrogenated in glacial acetic acid (23 ml) in the presence of 5% Pd on alumina (2.2 g). The catalyst was filtered off and the solution was evaporated under reduced pressure to give a crystalline residue (1.5 g). Crystallisation from ethyl acetate gave the *oxa-cyclol* (23) (1.2 g), m.p. 178—180 °C, $[\alpha]_D$ −62.5° (*c* 0.4 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3 300br, 1 720, and 1 640 cm^{-1}; δ($CDCl_3$) 1.35 (3 H, d, *J* 7.0 Hz, Me), 1.5—2.4 (4 H, m β-H_2 and γ-H_2 Pro), 2.4—2.7 (2 H, m, α-H_2 βAla), 2.9—3.9 (4 H, complex m, δ-H_2 Pro, α-H Pro and β-H βAla), 4.1 (1 H, m, β-H βAla), 4.55 (1 H, q, *J* 7.0 Hz, *CH*Me), and 6.7 (1 H, br s, OH); δ[$(CD_3)_2SO$] 1.25 (3 H, d, *J* 7.0 Hz, Me), 4.45 (1 H, q, *J* 7.0 Hz, *CH*Me), and 7.4 (1 H, d, *J* 1.5 Hz, OH); *m/e* 240 (M^+, 6%), 223(2), 170(13), and 70(100) (Found: C, 51.1; H, 7.0; N, 10.65. $C_{11}H_{16}N_2O_4{\cdot}H_2O$ requires C, 51.2; H, 7.0; N, 10.85%). Treatment with methanolic hydrazine [14] gave *cyclo*-(β-alanylprolyl), $[\alpha]_D$ −41° (*c* 0.70 in $CHCl_3$).

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REFERENCES

1 M. K. Kaloustian, M. I. Aguilar-Laurents de Gutierrez, and R. B. Nader, *J. Org. Chem.*, 1979, **44**, 666 and references therein; M. Baudy and A. Robert, *Tetrahedron Lett.*, 1980, 2517.

2 G. Lucente, F. Pinnen, G. Zanotti, S. Cerrini, W. Fedeli, and F. Mazza, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1499 and references therein.

3 (*a*) G. Lucente and A. Romeo, *Chem. Commun.*, 1971, 1605; (*b*) G. Lucente, F. Pinnen, and G. Zanotti, *Tetrahedron Lett.*, 1978, 1009.

4 P. Stutz and P. A. Stadler, *Monatsh. Chem.*, 1976, **107**, 763.

5 H. Ott, A. J. Frey, and A. Hofmann, *Tetrahedron*, 1963, **19**, 1675.

6 G. Gawne, G. W. Kenner, N. H. Rogers, R. C. Sheppard, and K. Titlestad in 'Peptides 1968,' ed. Bricas, North Holland Publishing Company, Amsterdam, 1968, p. 28.

7 P. Stutz, R. Brunner, and P. A. Stadler, *Experientia*, 1973, **29**, 936.

8 F. Conti, G. Lucente, A. Romeo, and G. Zanotti, *Int. J. Pept. Protein Res.*, 1973, **5**, 353.

9 M. Rothe, R. Theysohn, D. Muhlhausen, F. Eisenbeiss, and W. Schindler in 'Chemistry and Biology of Peptides,' ed. J. Meienhofer, Ann Arbor Science Publishers, 1972, p. 51.

10 N. J. Bach, H. E. Boaz, E. C. Kornfeld, C. Chang, H. G. Floss, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, 1974, **39**, 1372.

11 (*a*) R. G. Griot and A. J. Frey, *Tetrahedron*, 1963, **19**, 1661; (*b*) M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and Z. E. Agadzhanyan, *Tetrahedron*, 1965, **21**, 3537.

12 H. H. Freedman, *J. Am. Chem. Soc.*, 1955, **77**, 6003.

13 P. A. Levene, *J. Biol. Chem.*, 1936, **113**, 153.

14 H. Ott, R. Griot, P. A. Stadler, and A. J. Frey, *Helv. Chim. Acta*, 1963, **46**, 2306.