Approaches to Pseudopeptidic Ergopeptines. Synthesis and Molecular Structure of an α-Aza-phenylalanine-containing Oxa-cyclol

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A synthesis of the tetrahedral adduct (oxa-cyclol) **6** structurally related to the peptide portion of ergotamine and possessing an α-aza-phenylalanine residue in place of the central phenylalanine is described. The reaction sequence comprises acylation of *cyclo*-(-azaPhe-Pro-) with (*S*)-2-benzyloxypropionyl chloride, followed by chemoselective hydrogenolytic removal of the *O*-benzyl protecting group. The intermediate *N*-(α-hydroxyacyl)-*cyclo*-(-azaPhe-Pro-) undergoes spontaneous ring enlargement leading stereospecifically to the tetrahedral adduct **6** tautomeric with the 9-membered cyclodepsitripeptide *cyclo*-(-Lac-azaPhe-Pro-) **7**. The stereochemistry of **6** has been confirmed by an X-ray crystallographic analysis which provides, in addition, detailed information on the structural and conformational features of the newly formed pseudopeptide system.

The peptide portion of ergot alkaloids (ergopeptines; Fig. 1) is characterized by the presence of two unusual structural features: the residue of an α-hydroxy-α-amino acid and the free OH of a tetrahedral adduct to a carbonylic carbon atom. This unique heterocyclic-peptidic system contributes to the selective affinity

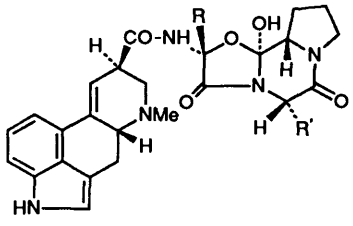


Fig. 1 Structure of ergopeptine alkaloids (oxa-cyclol type)

for a range of neurotransmitter receptors and controls the pharmacokinetic properties of ergopeptines, providing at the same time the main site for metabolic attack.[1-4] Whereas a systematic variation of the side chains of this moiety has been carried out,[1] including deletion and introduction of additional chiral centres,[3] very few backbone modifications have been performed. These are actually confined to the well known aza-[5,6] and thia-[7-9] cyclols and to the fortuitous, albeit interesting, Birch reduction of the carbonylic carbon in the six-membered ring.[10] This lack of skeletal modifications reflects the difficulty inherent in maintaining, inside a small heterocyclic system, the variety of steric and electronic factors which stabilize the tetrahedral adduct with respect to the corresponding α-hydroxyacyldioxo-piperazine and 9-membered peptide-lactone tautomeric forms.

We started recently a research program aimed at synthesizing backbone-modified analogues of the heterocyclic-peptidic system of ergopeptines and in determining the nature of the stable tautomeric forms together with their conformational properties.[11] In this paper we report results for the introduction of an α-aza-phenylalanine residue (azaPhe) in place of the central phenylalanine in a structural model related to ergotamine. It is worth noting here that the absolute configuration of the chiral centres of ergopeptines has a strong influence on the receptor binding and bioactivity. The aza-amino acids, on the other hand, are well suited to maintaining their side chains in a spatial orientation different from, but roughly intermediate to, that of the corresponding residues of L and D configuration.[12] Thus, a synthetic route to aza-analogues of ergopeptines seems a useful approach.

Acylation of the tetrahydrotriazine-dione ring of *cyclo*-(-aza-Phe-Pro-) **3** with a Lac [lactic acid; (*S*)-2-hydroxypropionic acid] residue (Schemes 1 and 2),* represents a convenient synthetic route to the desired backbone-modified analogues. The reaction gives an α-hydroxyacyl derivative as a first product; this should lead, depending upon the relative stability, to the tautomeric tetrahedral adduct (oxa-cyclol) **6** or to the 9-membered cycloaza-peptide *cyclo*-(-Lac-azaPhe-Pro-) **7**.

In order to obtain the starting *cyclo*-(-azaPhe-Pro-) **3**, Boc-azaPhe-ON*p* **1** was synthesized from *tert*-butyl 3-benzylcarbazate[13] and coupled with H-Pro-OMe to give the *N*-protected methyl ester Boc-azaPhe-Pro-OMe **2** (Scheme 1). When the latter was kept at room temperature in 2 mol dm^{-3} hydrogen chloride–ethyl acetate to remove the *tert*-butoxycarbonyl protecting group, spontaneous ring closure to give *cyclo*-(-azaPhe-Pro-) **3** took place, thus evidencing the strong tendency of the proline-containing α-aza-dipeptide intermediate azaPhe-Pro-OMe to undergo semicarbazide NH/ester CO intramolecular interaction.

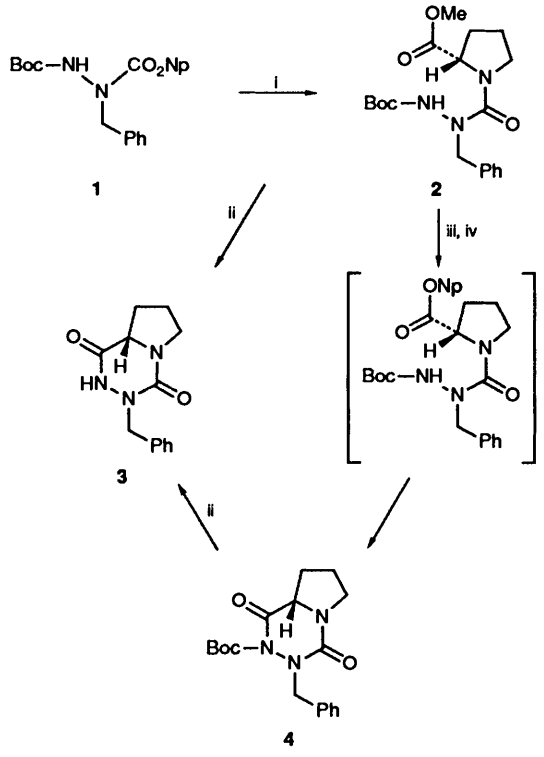
On the basis of this result and in order to gain information on the scarcely known chemistry of cycloaza-peptides, direct base-catalysed cyclization of the linear methyl ester **2**, to produce the *N*-protected cycloaza-peptide **4**, was attempted. Different bases and reaction conditions gave only unsatisfactory results. Alkaline hydrolysis of **2**, followed by carboxy activation to *p*-nitrophenyl ester (Scheme 1) led, however, to spontaneous ring closure to give the desired *N*-Boc-*cyclo*-(-azaPhe-Pro-) **4**. Conventional acidolytic removal of the Boc protecting group of **4** gives *cyclo*-(-azaPhe-Pro-) in good yield; compound **4**, on the other hand, is preferentially cleaved by methanolic hydrazine to the linear dipeptide derivative Boc-azaPhe-Pro-NH-NH_2. Thus, selective cleavage at the exocyclic or at the endocyclic CO–N bond of **4** can be performed. Spectroscopic properties of *cyclo*-(-azaPhe-Pro-) **3** are in accordance with the assigned

* Amino acid and peptide nomenclature follows the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.*, 1984, **138**, 9.

Table 1 Selected ^1H and ^{13}C NMR data[a] for compounds **3**, **4**, **5** and **6**

Residue		**3**[b]		**4**[c]		**5**[c]		**6**[c]	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Lac	C^α					4.8 q (7.0)	75.88	4.35 q (6.8)	73.74
	C^β					1.15 d	18.67	1.45 d	19.23
	CH_2O					4.4, 4.65 ABq (11.4)	72.16		
	CO						171.49[d]		166.75
azaPhe	C^β	4.25, 4.95 2AXd (16.0)	49.06	4.25, 5.15 2AXd (15.0)	52.27	4.45, 5.25 2AXd (14.7)	50.78	4.45, 5.5 2AXd (14.5)	50.12
	CO		153.80		156.06		155.76		152.03
	NH	10.45 s							
	OCON				148.04				
	CMe_3			1.6 s	27.96				
	CMe_3				85.29				
Pro	C^α	3.9 m	57.11	3.55 m	59.16	3.75 m	59.39	3.55 m	63.82
	C^β	1.85–2.1 m	26.74	1.8–2.0 m	27.05	1.9 m, 2.15 m	27.04	1.7–2.0 m	26.48
	C^γ	1.8–2.0 m	22.78	2.0–2.2 m	23.45	1.9–2.1 m	23.54	1.7–2.0 m	22.82
	C^δ	3.25–3.45 m	44.79	3.3–3.4 m	44.35	3.4 m, 3.6 m	44.26	3.45 m, 3.6 m	47.19
	CO (or COH)		165.78		170.08		170.52[d]	2.05 d (2.0)	100.20

[a] δ(ppm) from tetramethylsilane. Coupling constants (in parentheses, Hz), for mutually coupled protons are given only once, at their first occurrence in the Table. [b] Solvent $(CD_3)_2SO$. [c] Solvent $CDCl_3$. [d] Assignments may be interchanged.



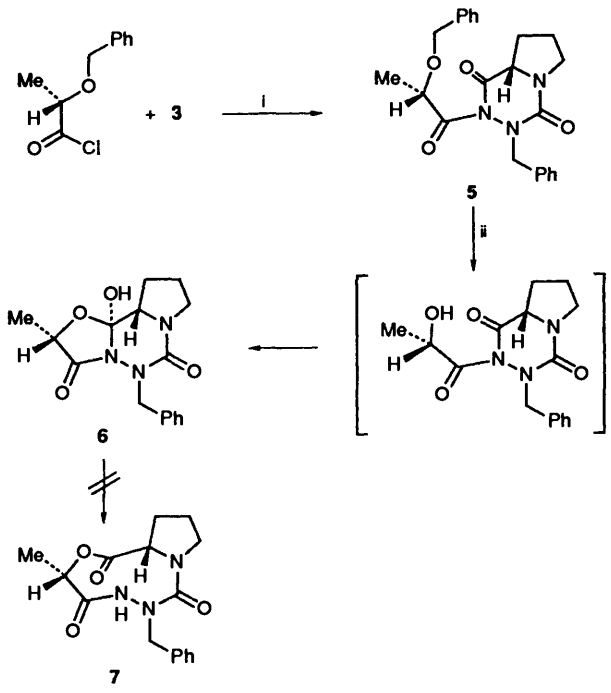
Scheme 1 *Reagents and conditions:* i, Pro-OMe, *N,N*-dimethylaminopyridine, *N,N*-dimethylformamide, room temp., 96 h; ii, HCl, ethyl acetate, room temp., 2 h; iii, 1 mol dm^{-3} NaOH, MeOH, room temp., 1 h; iv, *p*-nitrophenol, dicyclohexylcarbodiimide, CH_2Cl_2, 0 °C, 5 h, 5 °C, overnight.

structure. In the ^1H NMR spectrum (Table 1) the azaPhe β-H_2 protons of **3** appear downfield, in accordance with their benzylaminic character and exhibit, as compared with the linear precursor **2**, a large difference in the chemical-shift values ($\Delta\delta$ 0.7 ppm). The structure of compound **4** is in accordance with its chemical and spectroscopic properties; in particular, the ^{13}C NMR spectrum reveals three distinct CO signals centred at δ 170.08, 156.06 and 148.04, in accord with the different chemical environments of each carbonyl group.

Room-temperature *N*-acylation of *cyclo*-(-azaPhe-Pro-) **3** with (*S*)-2-benzyloxypropionyl chloride was found to be ineffective, probably owing to the steric interaction occurring between the *N*-benzylic side chain and the acylating residue. However, good yields of *N*-[(*S*)-2-benzyloxypropionyl]-*cyclo*-(-azaPhe-Pro-) **5** were obtained by treating *cyclo*-(-azaPhe-Pro-) **3** with 6 equiv. of (*S*)-2-benzyloxypropionyl chloride in dioxane at 90 °C for 20 h; no epimerization took place during the latter *N*-acylation. Treatment of **5** with hydrazine hydrate gave, in fact, (*S*)-2-benzyloxypropionylhydrazide and *cyclo*-(-azaPhe-Pro-) **3**, both of good optical purity. Thus, hydrazinolysis of the *N*-acyl-derivative **5**, yielding the cycloazadipeptide **3**, shows a different regioselectivity with respect to the same reaction performed on the *N*-Boc-derivative **4** and parallels that shown by related *N*-acyl-dioxopiperazines, which are preferentially cleaved by the same reagent at the exocyclic imide carbonyl.[7]

The selective hydrogenolytic *O*-debenzylation of **5**, in the presence of the *N*-benzyl group of the azaPhe residue, is a critical point of the synthetic strategy adopted here. We found, however, that treatment of **5** with Pd–H_2 (25 °C; 1 atm) in glacial acetic acid gave a single product in which only the *O*-benzyl substituent was cleaved. Further work is now in progress in order to study this selectivity and to define the *N*-debenzylation conditions which can actually transform azaPhe peptides into their azaGly counterparts.

On the basis of spectral (see Table 1) and chemical properties, confirmed by X-ray crystallographic analysis, the structure of the tetrahedral adduct **6**, tautomeric with the cyclodepsitripeptide *cyclo*-(-Lac-azaPhe-Pro-) (Scheme 2) was assigned to the *O*-debenzylated compound. Compound **6** evidences chemical stability comparable to that of natural ergot alkaloids (oxacyclols) and synthetic peptide cyclols. In particular, it can be stored unchanged at room temperature for months; chromatographic analysis and NMR spectra taken in $CDCl_3$ and $(CD_3)_2SO$ show no evidence of tautomeric equilibria. It is cleaved by methanolic hydrazine to give *cyclo*-(-azaPhe-Pro-) **3**. The ^{13}C NMR spectrum reveals only two carbonyl signals and a singlet at δ 100.2 consistent with the carbon bound to three heteroatoms. In the ^1H NMR spectrum of **6** the α-H of the oxazolidin-4-one ring is found shifted to high field (0.45 ppm) relative to the same proton in the *N*-(*O*-benzyl)-lactyl precursor **5** as it is found in related carba systems.[14] The exchangeable



Scheme 2 *Reagents and conditions:* i, pyridine, dioxane, 90 °C, 20 h; ii, H_2, 10% Pd/C, acetic acid, room temp., 7 h.

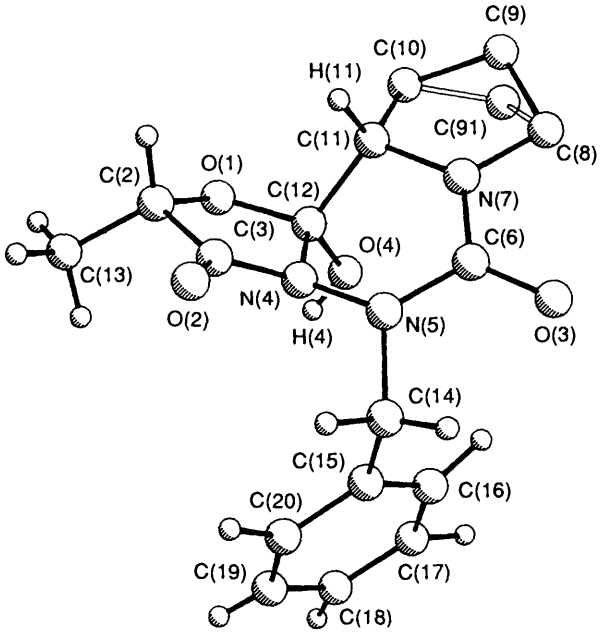


Fig. 2 Molecular structure and atomic numbering scheme of the tetrahedral adduct **6**

OH proton appears at δ 2.0 (in $CDCl_3$) as a sharp doublet (J 2.0 Hz), long-range coupled to the Pro α-H, a finding indicating the involvement of these two protons in a W conformation.[15] This structural feature, which is characteristic of ergot peptides and has been confirmed for the aza analogue **6** by X-ray crystallographic analysis, implicates an *anti*-orientation of the hydroxy group with respect to the Pro α-H and defines at the same time the absolute configuration of the new formed chiral centre. Notwithstanding the replacement of a nitrogen atom for the Phe α-CH, the α-hydroxyacyl incorporation reaction leading to the aza analogue **6**, then

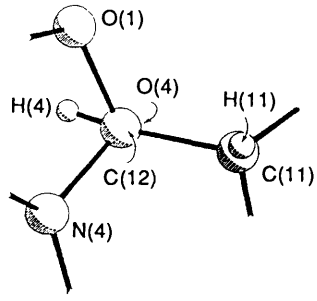


Fig. 3 A partial plot of **6** looking down the C(12)–O(4) bond

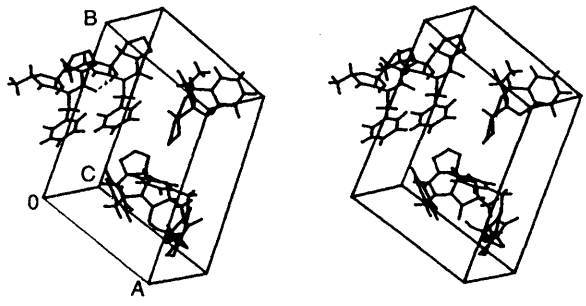


Fig. 4 The crystal packing of the oxa-cyclol **6**

follows a stereospecific course in which *syn* arrangement of the OH group with respect to the Pro α-H does not take place.

A variety of peptide bolynd surrogates and backbone modifications have been recently reported in the attempt to modulate the action of bioactive peptides.[12,16,17] However, the attention payed to the evaluation of the conformational consequences connected with the pseudopeptide approach is comparatively low[18] and pertinent data are just emerging in the literature.[19,20] In this context and in order to confirm the stereochemistry at the newly formed chiral centre, an X-ray analysis of **6** was undertaken.

Fig. 2 shows the molecular structure together with the numbering scheme of **6**. Table 2 reports a selection of the relevant bond lengths and angles for non-hydrogen atoms. The mean value of bond distances involving hydrogen atoms is 0.96(6) Å and the mean values of angles are 109(3)° and 120(3)° for C(sp^3) and C(sp^2) carrier atoms, respectively. In Table 3 a selection of the most significant torsion angles is reported. Full lists of bond lengths and angles, fractional coordinates and thermal parameters have been deposited with the CCDC.*

The oxazolidine-4-one ring adopts an envelope C_s-C(12) conformation with C(12) displaced 0.298 Å from the plane of the other four ring atoms towards C(11). The central oxo-tetrahydrotriazine ring presents a distorted boat conformation with approximate C_s symmetry through C(6) and C(12), these atoms being displaced by 0.132 and 0.664 Å respectively out of the mean plane of the other four ring atoms. As is frequently found in crystal structures of proline-containing peptides,[21,22] the C_γ of the proline residue is disordered and the corresponding 5-membered pyrrolidine ring can be described by two half-chair (C_2) conformations, C_2-N(7) or C_2-C(11), depending on which disordered position of C(9) is considered.

All three nitrogen atoms of **6** show pyramidal character, the sum of the bond angles around N(4), N(5) and N(7) being 352.6, 354.6 and 357.4° and the distance from the plane of their substituents 0.222, 0.189 and 0.134 Å, respectively. Relevant is

* For full details of the Cambridge Crystallographic Data Centre deposition scheme see 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 1*, 1993, issue 1.

the deviation from planarity shown by the azaPhe N(4), the corresponding value found in dihydroergotamine being 0.116 Å.[23]

The pyramidalization of the contiguous nitrogen atoms N(4)–N(5) of the azaPhe is such as to direct the two incipient sp^3 lone pairs in opposite directions. The benzylic side chain at N(5) takes a pseudo-equatorial orientation leaving the nitrogen lone-pair *cisoidal* with the Pro Hα; thus, the azaPhe residue mimics a phenylalanine residue with *S* (L) absolute configuration, as it is found in natural ergot peptides.

The OH group at C(12) adopts a rotameric state in which the system of four bonds H–O(4)–C(12)–C(11)–H is in a nearly planar W geometry corresponding to that inferred by ^{1}H NMR data in $CDCl_3$ solution (see pertinent torsion angles in Table 3). This rotameric preference indicates an *exo* anomeric $n\rightarrow\sigma^*_{C\,X}$ stereoelectronic effect[24,25] which places both the oxygen O(4) lone-pairs antiperiplanar to the bonds which C(12) makes with the two heteroatoms O(1) and N(4) (see Fig. 3). In this conformation the two O(4) lone pairs and the corresponding C(12)–O(1) and C(12)–N(4) antibonding σ* orbitals can overlap. The high shortening of the C(12)–O(4) bond distance observed in **6** (1.357 Å as compared with 1.432 Å standard value)[26] clearly indicates that this stabilizing $n\rightarrow\sigma^*$ electronic interaction is operating. Fig. 4 shows the crystal packing, indicating an intermolecular hydrogen bond of 2.674(4) Å between the carbonyl O(3) atom and the hydroxy O(4) atom of another molecule translated by a *z* unit. The distance between O(3) and the hydrogen at O(4) is 1.93(5) Å and the angle between the C(6)–O(3) bond and the O(4) atom engaged in the hydrogen bond is 119.6(2)°. It is interesting to note that in this intermolecular hydrogen bond scheme the above discussed W planar arrangement, with the characteristic rotameric state of the free hydroxy group, is maintained.

Table 2

(a) Selected bond lengths (Å) with esds in parentheses

O(1)–C(2)	1.447(4)	C(6)–O(3)	1.246(4)
O(1)–C(12)	1.411(4)	N(7)–C(8)	1.478(5)
C(2)–C(3)	1.510(5)	N(7)–C(11)	1.469(4)
C(2)–C(13)	1.501(7)	C(8)–C(9)	1.54(1)
C(3)–N(4)	1.362(4)	C(8)–C(9′)	1.51(1)
C(3)–O(2)	1.217(5)	C(9)–C(10)	1.49(1)
N(4)–N(5)	1.393(4)	C(9′)–C(10)	1.45(1)
N(4)–C(12)	1.466(4)	C(10)–C(11)	1.533(5)
N(5)–C(6)	1.377(4)	C(11)–C(12)	1.521(5)
N(5)–C(14)	1.474(4)	C(12)–O(4)	1.357(4)
C(6)–N(7)	1.347(4)	O(4)–H(4)	0.78(5)

(b) Selected bond angles (°) with esds in parentheses

C(12)–O(1)–C(2)	110.5(3)	C(11)–N(7)–C(8)	111.5(3)
C(3)–C(2)–O(1)	104.7(3)	C(9)–C(8)–N(7)	103.5(4)
C(13)–C(2)–O(1)	110.4(3)	C(9′)–C(8)–N(7)	101.0(5)
C(13)–C(2)–C(3)	114.2(4)	C(10)–C(9)–C(8)	105.6(5)
N(4)–C(3)–C(2)	105.7(3)	C(10)–C(9′)–C(8)	108.5(7)
O(2)–C(3)–C(2)	127.9(3)	C(11)–C(10)–C(9)	104.7(5)
O(2)–C(3)–N(4)	126.3(4)	C(11)–C(10)–C(9′)	106.2(5)
N(5)–N(4)–C(3)	122.5(3)	C(10)–C(11)–N(7)	104.2(3)
C(12)–N(4)–C(3)	111.8(3)	C(12)–C(11)–N(7)	108.1(3)
C(12)–N(4)–N(5)	118.3(2)	C(12)–C(11)–C(10)	117.2(3)
C(6)–N(5)–N(4)	118.7(3)	N(4)–C(12)–O(1)	102.8(2)
C(14)–N(5)–N(4)	115.0(3)	C(11)–C(12)–O(1)	112.9(3)
C(14)–N(5)–C(6)	120.9(3)	C(11)–C(12)–N(4)	105.9(2)
N(7)–C(6)–N(5)	117.3(3)	O(4)–C(12)–O(1)	111.7(3)
O(3)–C(6)–N(5)	119.9(3)	O(4)–C(12)–N(4)	113.9(3)
O(3)–C(6)–N(7)	122.8(3)	O(4)–C(12)–C(11)	109.4(3)
C(8)–N(7)–C(6)	121.1(3)	C(15)–C(14)–N(5)	112.3(3)
C(11)–N(7)–C(6)	124.8(3)	H(4)–O(4)–C(12)	110.0(3)

Conclusions.—The present study indicates that an α-aza-amino acid residue can be introduced into a tripeptidic model analogous to that of ergopeptines without destabilizing the tetrahedral adduct structure in favour of the corresponding *N*-α-hydroxyacyclic and cyclotripeptidic tautomeric forms. Furthermore, it has been shown that nucleophilic attack by the α-hydroxyacyl group on the cyclic pseudopeptide *cyclo*-(-azaPhe-Pro-) follows a stereospecific course leading to a single tetrahedral adduct, possessing conformational and stereoelectronic features analogous to those found in natural ergopeptines. Studies are now in progress in our laboratories aimed at synthesizing backbone modified ergopeptines as well as new models containing different α-aza-amino acids, including azaPro residues.

Experimental

M.p.s are uncorrected. TLC was performed on pre-coated silica gel Merck 60F 254 plates developed with $CHCl_3$–hexane (99:1) (R_{FA}), $CHCl_3$–MeOH (95:5) (R_{FB}), $CHCl_3$–MeOH (97:3) (R_{FC}). Optical rotations were taken at 20 °C with a Schmidt-Haensch Polartronic D polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^{1}H (300 MHz) and ^{13}C (75.43 MHz) NMR spectra were determined with tetramethylsilane as internal standard using a Varian XL-300 instrument.

N-tert-*Butoxycarbonylazaphenylalanine* p-*Nitrophenyl Ester* **1**.—To a stirred solution of *tert*-butyl 3-benzylcarbazate (25.7 g, 115.8 mmol) in tetrahydrofuran (150 cm^3), *p*-nitrophenyl chloroformate (23.3 g, 115.8 mmol) in tetrahydrofuran (50 cm^3) and *N*-methylmorpholine (11.7 g, 115.8 mmol) in tetrahydrofuran (50 cm^3) were added dropwise at 0 °C during 30 min. After 3 h at 0 °C the precipitate was filtered off and the resulting

Table 3 Selected torsion angles (°) with esds in parentheses

O(1)–C(2)–C(3)–N(4)	−2.8(4)	N(7)–C(11)–C(12)–O(1)	164.3(3)
C(2)–C(3)–N(4)–N(5)	164.5(3)	N(7)–C(11)–C(12)–O(4)	−70.6(3)
C(2)–C(3)–N(4)–C(12)	15.1(4)	N(7)–C(11)–C(12)–N(4)	52.6(3)
C(3)–N(4)–C(12)–O(1)	−21.4(3)	C(8)–C(9)–C(10)–C(11)	32.9(7)
C(3)–N(4)–C(12)–C(11)	97.2(3)	C(8)–N(7)–C(11)–C(10)	11.3(4)
C(3)–N(4)–N(5)–C(6)	−128.0(3)	C(8)–N(7)–C(11)–C(12)	136.6(3)
C(3)–N(4)–N(5)–C(14)	77.5(4)	C(9)–C(8)–N(7)–C(11)	8.5(5)
N(4)–N(5)–C(6)–N(7)	14.7(4)	C(9)–C(10)–C(11)–N(7)	−27.2(5)
N(4)–N(5)–C(14)–C(15)	57.5(4)	C(11)–C(12)–O(1)–C(2)	−94.7(3)
N(4)–C(12)–O(1)–C(2)	18.9(3)	C(11)–C(12)–N(4)–N(5)	−53.6(3)
N(5)–C(14)–C(15)–C(16)	66.9(5)	C(11)–C(12)–O(4)–H(4)	−174(4)
N(5)–C(6)–N(7)–C(11)	−10.6(5)	H(11)–C(11)–C(12)–O(4)	175(3)
C(6)–N(7)–C(11)–C(10)	−150.4(3)	C(12)–O(1)–C(2)–C(3)	−10.8(4)
C(6)–N(7)–C(11)–C(12)	−25.0(4)	C(12)–N(4)–N(5)–C(6)	19.6(4)
N(7)–C(8)–C(9)–C(10)	−25.7(7)	C(14)–N(5)–C(6)–O(3)	−14.2(5)

solution evaporated to dryness. The residue was taken up in $CHCl_3$ and the solution washed with 1 mol dm^{-3} $KHSO_4$, 5% aqueous Na_2CO_3, and water, dried and evaporated. The residue was eluted with $CHCl_3$–hexane (97:3) from a silica gel column to give the *active ester* **1** as a foam (40.8 g, 91%), R_{FA} 0.6 (Found: C, 58.8; H, 5.5; N, 10.8. $C_{19}H_{21}N_3O_6$ requires C, 58.9; H, 5.4; N, 10.85%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3410 (NH) and 1760–1720 (CO); $\delta_H(CDCl_3)$ 1.45 (9 H, s, 3 × Me), 4.8 (2 H, br m, NCH_2), 6.6 (1 H, s, NH), 7.2–7.4 (7 H, m, ArH) and 8.25 (2 H, d, ArH).

N-tert-*Butoxycarbonylazaphenylalanyl*-L-*proline Methyl Ester* **2**.—To a stirred solution containing L-proline methyl ester (13.4 g, 104.0 mmol) and *N*,*N*-dimethylaminopyridine (3.2 g, 26.0 mmol) in dimethylformamide (150 cm^3), the active ester **1** (40.2 g, 104.0 mmol) in dimethylformamide (150 cm^3) was added in portions at room temperature. After 96 h, the reaction mixture was evaporated under reduced pressure and the residue taken up in ethyl acetate. The solution was repeatedly washed with 1 mol dm^{-3} $KHSO_4$, saturated aqueous Na_2CO_3, and water, dried and evaporated. The resulting oil was chromatographed on silica gel using $CHCl_3$–hexane (99:1) as the eluent to give *methyl ester* **2** as an oil (34.9 g, 89%), R_{FB} 0.8; $[\alpha]_D$ −20.0 (*c* 1.00 in $CHCl_3$) (Found: C, 60.5; H, 7.1; N, 11.2. $C_{19}H_{27}N_3O_5$ requires C, 60.45; H, 7.2; N, 11.1%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3400 (NH) and 1740 and 1645 (CO); $\delta_H(CDCl_3)$ 1.35 (9 H, s, 3 × Me), 1.7–2.2 (4 H, m, β- and γ-H_2 Pro), 3.45–3.6 (2 H, m, δ-H_2 Pro), 3.7 (3 H, s, OMe), 4.3–4.7 (3 H, m, NCH_2 and α-H Pro), 6.25 (1 H, s, NH) and 7.2–7.45 (5 H, m, ArH).

cyclo-(-*Azaphenylalanyl*-L-*prolyl*-) **3**.—A solution of the above described methyl ester **2** (33.0 g, 87.5 mmol) was stirred with 2 mol dm^{-3} hydrogen chloride in ethyl acetate (130 cm^3) for 2 h at room temperature. The resulting solid was filtered off, washed with dry ether and dried over P_2O_5 to give crude *cyclopeptide* **3** (15.4 g, 72%), m.p. 217–218 °C (from MeOH); R_{FB} 0.55; $[\alpha]_D$ −4.0 (*c* 0.50 in MeOH) (Found: C, 63.6; H, 6.2; N, 17.2. $C_{13}H_{15}N_3O_2$ requires C, 63.65; H, 6.15; N, 17.1%); ν_{max}(KBr)/cm^{-1} 3090 (NH) and 1690 and 1630 (CO).

N-tert-*Butoxycarbonyl*-cyclo-(-*azaphenylalanyl*-L-*prolyl*-) **4**.—To a solution of the methyl ester **2** (5.0 g, 13.3 mmol) in MeOH (30 cm^3) 1 mol dm^{-3} NaOH (16 cm^3) was added. After 1 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 ether, acidified to pH 3.5 with $KHSO_4$ and extracted with $CHCl_3$. The extract was dried and evaporated to afford *N-tert*-butoxycarbonyl-azaphenylalanyl-L-proline (4.6 g) which was used without further purification.

To a stirred solution of the above described peptide acid (4.3 g, 11.8 mmol) and *p*-nitrophenol (3.3 g, 23.6 mmol) in CH_2Cl_2 (70 cm^3), dicyclohexylcarbodiimide (2.4 g, 11.8 mmol) was added at 0 °C. After 5 h at 0 °C and 16 h at 5 °C, the reaction mixture was filtered and the organic layer washed with saturated aqueous Na_2CO_3 and water. It was then dried and evaporated and the residue was chromatographed on silica gel using $CHCl_3$–MeOH (99:1) as the eluent to give *title compound* **4** (3.9 g, 95%) as an oil, R_{FC} 0.6; $[\alpha]_D$ −20.0 (*c* 1.00 in $CHCl_3$) (Found: C, 62.6; H, 6.65; N, 12.1. $C_{18}H_{23}N_3O_4$ requires C, 62.6; H, 6.7; N, 12.2%); $\nu_{max}(CHCl_3)/cm^{-1}$ 1785, 1745 and 1680 (CO).

N-[(S)-2-*Benzyloxypropionyl*]-cyclo-(-*azaphenylalanyl*-L-*prolyl*-) **5**.—A mixture of (−)-(*S*)-2-benzyloxypropionyl chloride (18.0 g, 90.6 mmol) and compound **3** (3.7 g, 15.1 mmol) in dioxane (350 cm^3) containing dry pyridine (8.9 g, 113.3 mmol) was heated for 20 h at 90 °C. After cooling of the reaction mixture, the precipitate was filtered off and the resulting solution evaporated to dryness under reduced pressure. The residue was taken up in $CHCl_3$, and the solution washed with 0.5 mol dm^{-3} HCl, saturated aqueous $NaHCO_3$ and water, dried and evaporated. The resulting residue was chromatographed on silica gel using $CHCl_3$–MeOH (99:1) as the eluent to give the *title compound* **5** (4.3 g, 70%) as an oil: R_{FC} 0.7; $[\alpha]_D$ −183.0 (*c* 1.00 in $CHCl_3$) (Found: C, 67.9; H, 6.1; N, 10.2. $C_{23}H_{25}N_3O_4$ requires C, 67.8; H, 6.2; N, 10.3%); $\nu_{max}(CHCl_3)/cm^{-1}$ 1730 and 1680 (CO).

Oxa-cyclol **6**.—The *O*-benzyl derivative **5** (3.8 g, 9.3 mmol) was hydrogenated in glacial acetic acid (150 cm^3) in the presence of 10% Pd on activated charcoal (0.75 g). After 7 h the catalyst was filtered off and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (96:4) as the eluent to yield the *oxa-cyclol* **6** (1.2 g, 41%) as a crystalline residue, m.p. 188–190 °C (from ethyl acetate); R_{FC} 0.6; $[\alpha]_D$ +110.0 (*c* 1.00 in $CHCl_3$) (Found: C, 60.6; H, 6.1; N, 13.2. $C_{16}H_{19}N_3O_4$ requires C, 60.55; H, 6.0; N, 13.25%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3180br (OH) and 1740 and 1660 (CO).

X-Ray Structure Determination.—Suitable single crystals of compound **6** were obtained by slow evaporation from ethyl acetate. Approximate unit cell parameters and the space group were determined from oscillation and Weissenberg photographs. Intensity data were collected at room temperature on a Siemens P3 automatic four circle diffractometer equipped with graphite monochromator and Cu-Kα radiation, in θ–2θ scanning mode to a maximum 2θ of 139°. The refined unit cell parameters were determined by least-squares refinement of the angular setting of 15 selected reflections.

Crystal Data. $C_{16}H_{19}N_3O_4$, M = 317.3, orthorhombic, a = 13.782(10), b = 18.320(12), c = 6.376(4) Å, V = 1610(2) Å^3, Z = 4, D_c = 1.31 g cm^{-3}, Cu-Kα radiation, λ = 1.5418 Å, μ(Cu-Kα) = 8.025 cm^{-1}. Space group $P2_12_12$ from systematic absences. Background counts were taken for a time equal to that of the scan. Out of a total of 1835 independent recorded reflections, the intensities of 1575 were considered observed for $I > 3\sigma(I)$. The intensities of three standard reflections were monitored every 100 collected reflections and remained essentially constant throughout data collection. Lorentz and polarization factors were applied, but intensities were not corrected for absorption or extinction.

Structure Solution and Refinement.—The structure was solved by direct methods with the MULTAN 80 program.[27] An *E* map computed with phases of the set with the highest figures of merit revealed all the non-hydrogen atoms, which were refined isotropically. At this stage a Fourier difference synthesis showed all the hydrogen atoms in stereochemically feasible positions, but not those bound to the disordered atom C(9) and those bound to C(8) and C(10) and they were excluded in the following calculations. The non-hydrogen atoms were then refined anisotropically while the hydrogen atom positional parameters together with the individual isotropic thermal values were included in the full-matrix refinement. The four reflections 020, 021, 110 and 111 were excluded from the last cycles of refinement because they were judged to be severely influenced by the extinction effect. The function minimized was $\Sigma w\ (|F_o| - |F_c|)^2$ where $w = (\sin\theta/\lambda)^2$. A last difference synthesis showed no residual significant peaks. When the refinement was stopped the sum of the square of the ratios between the parameter shifts and the esds was 0.07. The final R,R_w and S values were 0.050, 0.053 and 1.6 respectively. All the

calculations were carried out on a Data General Eclipse MV 8000 II computer, with the crystallographic software of ref. 28.

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