Approaches to Pseudopeptidic Ergopeptines. Part 2.† Consequences of the Incorporation of an α-Azaproline Residue into the Oxacyclolic System

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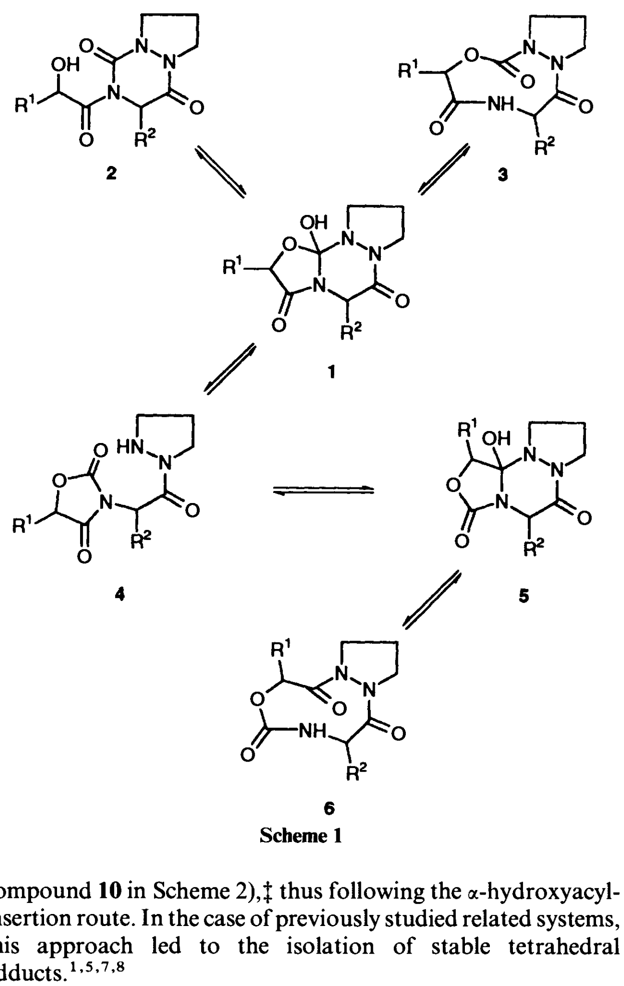
As part of a programme to synthesize pseudopeptidic ergopeptines, the introduction of an α-azaproline residue in place of native proline into an ergotamine-like oxacyclolic system has been investigated. Starting material *N*-[(*R*)-2-benzyloxypropionyl]cyclo(-Phe-azaPro-) **10** was prepared following two alternative synthetic routes and was subjected to reductive O-debenzylation. N,O-Acyl transfer on the resulting *N*-[(*R*)-2-hydroxypropionyl]cyclo(Phe-azaPro-) **14** leads, through a new type of four-heteroatom tetrahedral adduct, to (5*R*)-5-methyl-3-{(1*S*)-2-phenyl-1-[(pyrazolidin-1-yl)-carbonyl]ethyl}oxazolidine-2,4-dione **16**, as a unique isolable tautomer. Structural and conformational details of compound **14**, as revealed by X-ray analysis, are reported and compared with those of previously studied related models.

Chemical and biochemical aspects concerning the tetrahedral intermediates (oxacyclols) found in the peptide portion of ergot alkaloids (ergopeptine alkaloids) are the subject of constant interest.[1-5] It is well established that the stability of this type of tetrahedral adduct is dependent on structural and electronic factors related to the nature, configuration and sequence of the involved residues.

We recently started a research programme aimed at studying ergopeptine analogues obtained by introducing α-aza-amino acid residues into the cyclolic system. The introduction of an α-azaphenylalanine residue (azaPhe) in place of phenylalanine in a structural model related to ergotamine has been previously reported.[5] In this case a tetrahedral adduct, possessing chemical stability and stereoelectronic features analogous to those found in natural and synthetic non-pseudo derivatives, was isolated.

As a continuation of the research in this field we examine here the introduction of an α-azaproline residue (azaPro), in place of native proline, into an ergotamine-like peptidic system. As can be appreciated by examining Scheme 1, this replacement can give rise to acyl-transfer reactions which are more complex than those connected with proline-containing models[6] including the previously reported azaPhe-containing analogue.[5] The reason is due to the nature of the acyl-transfer intermediate **1** which possesses the tetrahedral cyclolic carbon atom bonded to four, instead of the usual three, heteroatoms. Thus, besides the *N*-(α-hydroxyacyl)tetrahydrotriazinediones **2** and the pseudopeptidic lactones **3**, this adduct can generate oxazolidine-2,4-dione derivatives **4**, derived from proton transfer to the azaPro nitrogen atom. This new species can in turn give rise, through the three-heteroatom adduct **5**, to 9-membered cyclic urethanes **6**. Further tautomers, derived by the attack of the exocyclic hydroxy group of compounds **2** on the alternative carbonyl carbon of the tetrahydrotriazinedione ring, have not been considered in Scheme 1 due to the unfavourable steric and conformational factors connected with the heterocyclic system that would be formed.

In order to gain information on the equilibria reported in Scheme 1 the synthesis of an analogue of type **2**, containing a DLac [D-lactic acid; (*R*)-2-hydroxypropionic acid] residue bound to the cyclo(-Phe-azaPro-) ring, was considered (see



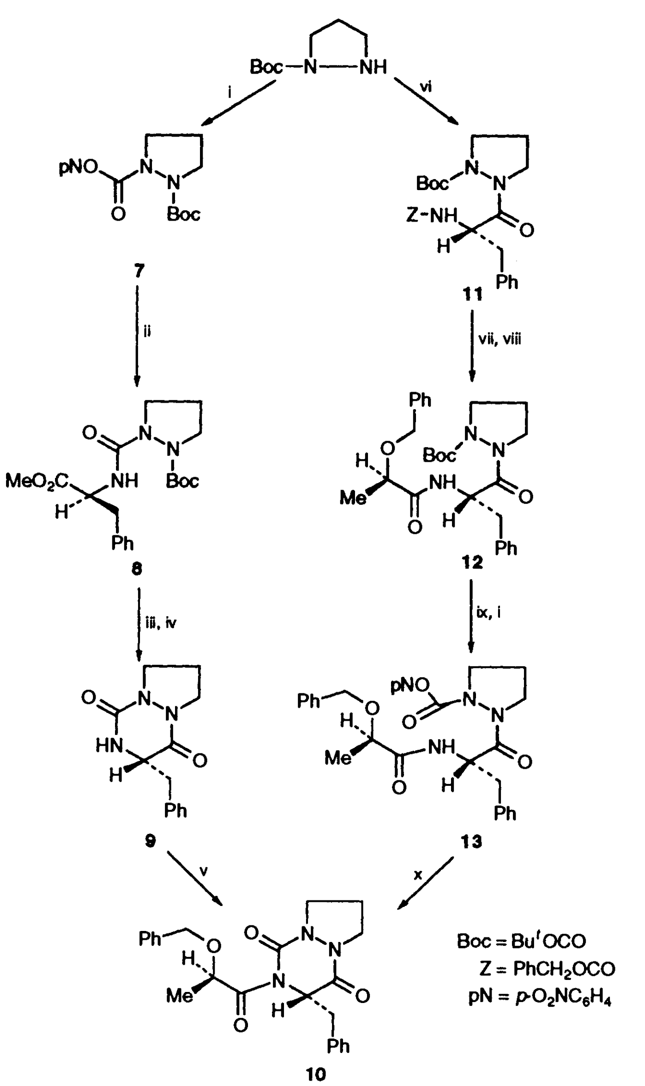
Scheme 1

compound **10** in Scheme 2),‡ thus following the α-hydroxyacyl-insertion route. In the case of previously studied related systems, this approach led to the isolation of stable tetrahedral adducts.[1,5,7,8]

The starting compound, cyclo(-Phe-azaPro-) **9**, was synthesized from *N*-(*tert*-butoxycarbonyl)pyrazolidine[9] (Scheme

† Part 1 is ref. 5.

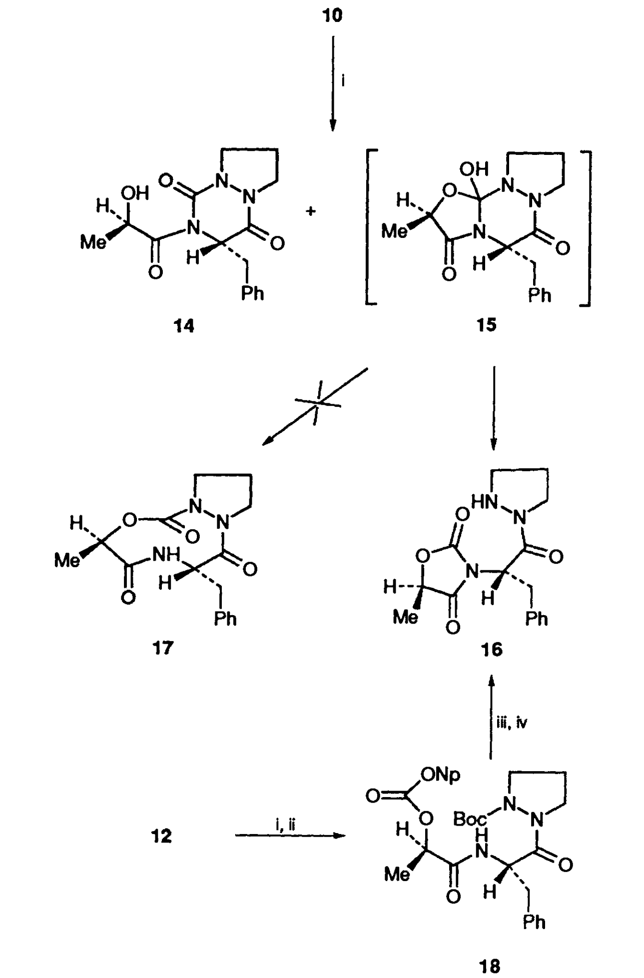
‡ Amino acid and peptide nomenclature follows the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.*, 1984, **138**, 9.



Scheme 2 *Reagents and conditions:* i, *p*-nitrophenyl chloroformate, *N*-methylmorpholine, THF, 0 °C, 3 h; ii, Phe-OMe, DMAP, DMF, room temp., 72 h; iii, HCl, 1,4-dioxane, room temp., 2 h; iv, 5% aq. acetic acid, 75 °C, 40 h; v, *O*-Bzl-DLac-Cl, pyridine-1,4-dioxane, 90 °C, 48 h; vi, Z-Phe-OH, DCC, THF, 0 °C, 4 h, then 5 °C, overnight; vii, H_2, 10% Pd/C, 80% aq. MeOH, room temp., 3 h; viii, *O*-Bzl-DLac-OH, DCC, THF–MeOH, 0 °C, 4 h; then 5 °C, overnight; ix, TFA, room temp., 1 h; x, NaH, DMF, 0 °C, 2 h

2). Acidolytic removal of the Boc-protecting group in Boc-azaPro-Phe-OMe **8** did not produce spontaneous ring closure to the tetrahydrotriazinedione ring system, as *had* been observed in the case of the previously studied model Boc-azaPhe-Pro-OMe.[5] This reflects the absence, in the deprotected intermediate azaPro-Phe-OMe as compared with azaPhe-Pro-OMe, of both the primary N-terminal NH_2 and the central CO-(aza)Pro junction; this latter, due to its tendency to adopt *cis* geometry, favours the cyclization.[10] Cyclo(-Phe-azaPro-) **9** was obtained in 33% yield by heating azaPro-Phe-OMe in aq. acetic acid, according to the procedure of Dutta and Morley.[9]

N-Acylation of cyclo(-Phe-azaPro-) **9** with (*R*)-2-benzyloxypropionyl chloride under different reaction conditions gave unsatisfactory results; by operating at 90 °C for 48 h in 1,4-dioxane–pyridine, *N*-[(*R*)-2-benzyloxypropionyl]cyclo(-Phe-azaPro-) **10** could be obtained in 15% yield. In order to overcome this limiting step, an alternative route to compound **10**, based on the synthesis of the pseudopeptide **13**, was examined (Scheme 2). The linear precursor **13**, due to the presence of the C-terminal azaPro residue, should undergo



Scheme 3 *Reagents and conditions:* i, H_2, 10% Pd/C, MeOH, room temp., 6 h; ii, *p*-nitrophenyl chloroformate, pyridine, room temp., 48 h; iii, NaH, DMF, 0 °C, 3 h; iv, TFA, room temp., 2 h

cyclization to give the desired tetrahydrotriazinedione derivative, as observed in the formation of *N*-acyldioxopiperazine systems from peptides containing a carboxy-activated C-terminal proline.[11]

Synthesis of compound **13** is reported in Scheme 2. The starting *N*-Boc-pyrazolidine[9] was coupled with Z-Phe-OH to give the protected pseudodipeptide Z-Phe-azaPro-OBu^t **11**, which was then extended at the N-terminus to provide *O*-Bzl-DLac-Phe-azaPro-OBu^t **12**. N-Deprotection, followed by treatment of the resulting hydrazide with *p*-nitrophenyl chloroformate, gave the desired active derivative **13** in good yields.

Cyclization of compound **13** under mild reaction conditions, such as treatment at room temperature with aq. alkaline buffer[12] or with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in benzene,[11,13] gave unsatisfactory results. Good yields of the *N*-acyltetrahydrotriazinedione **10** were obtained when compound **13** was treated at 0 °C with NaH in dry *N*,*N*-dimethylformamide (DMF).

Hydrogenolytic O-debenzylation of compound **10** (Scheme 3) afforded two isomeric products which could be separated and characterized as the *N*-(α-hydroxyacyl)tetrahydrotriazinedione **14** and the oxazolidine-2,4-dione derivative **16**. Neither the oxacyclol **15**, intermediate in the formation of compound **16**, nor the tautomeric 9-membered pseudopeptidic lactone **17** (Scheme 3) was isolated or detected. Structures assigned to compounds **14** and **16** are based on chemical and spectroscopic evidence, and for compound **14** the structure is supported by

X-ray crystallographic analysis. In the adopted reaction and isolation conditions (see Experimental section) compounds **14** and **16** are isolated in 60 and 28% yield, respectively.

Significant spectral data of **14** are: the ^{13}C NMR spectrum (Table 1) shows three CO signals centred at δ_C 177.15, 161.74 and 147.22. A δ_C-value significantly lower than 147 is expected for the sp^3 cyclolic carbon of compound **15**. In fact, the sp^3 carbon of a three-heteroatom cyclolic ring in ergopeptine alkaloids appears at $\delta_C \sim 103$ [14] and that of a four-heteroatom adduct, previously studied by A. P. K. Orrell and J. D. Wallis, at δ_C 117.1.[15] The ^1H NMR spectrum in [$^2H_6$]dimethyl sulfoxide (see Experimental section) shows the exchangeable proton as a doublet coupled to the DLac C$^\alpha$H. It is interesting to note that the downfield shift, typical of the C$^\alpha$H protons involved in the $(CO)_2N$ imide system of *N*-acyldioxopiperazines,[16] is maintained in the aza-analogue **14** as well as in the O-protected precursor **10**, whose DLac and Phe C$^\alpha$H protons appear at δ_H 4.9; 5.5 and 4.85; 5.25, respectively; the Phe C$^\alpha$H proton of the non-N-acylated cyclopseudodipeptide **9** resonates, on the other hand, at δ 4.1 (Table 1). Although, in the solid state, compound **14** is stable enough to be stored at room temperature for weeks, in methanol solution a slow conversion into compound **16** is observed, presumably through formation and collapse of the tetrahedral adduct **15** (Scheme 3). This intramolecular *N,O*-carbonyl transfer, which involves a 5-membered ring closure and a 6-membered ring opening, is practically complete in a week at room temperature, as deduced by TLC and ^1H NMR monitoring.

The IR spectrum of compound **16** shows three bands in the carbonyl region, centred at 1660, 1740 and 1815 cm^{-1}; the last absorbance is unusual and characteristic of oxazolidine-2,4-diones.[17] In the ^1H NMR spectrum ($CDCl_3$) the exchangeable proton appears at δ 4.0 as a triplet, coupled to the pyrazolidine CH_2N; the ^{13}C NMR spectrum ($CDCl_3$) reveals three CO signals, resonating at δ_C 173.69, 167.64 and 155.04 (Table 1). Unlike *N*-(α-hydroxyacyl)tetrahydrotriazinedione **14**, compound **16** is stable on storage and does not show a tendency toward tautomeric transformations (Scheme 1). The structure assigned to compound **16** has been confirmed by comparison with a sample obtained by following a different synthetic approach (Scheme 3). Starting from the N,O-protected pseudopeptide **12**, the *p*-nitrophenyl carbonate **18** was synthesized and subjected to ring closure, by using NaH under reaction conditions analogous to those adopted for the cyclization of the active ester **13**. Subsequent removal of the Boc protecting group from the intermediate N-protected oxazolidine-2,4-dione derivative gave a mixture of two products possessing almost identical chromatographic behaviour and spectral properties. Accurate analysis of the data revealed that the desired compound **16** was formed together with a diastereoisomeric form. This result seems to be due to partial epimerization involving the DLac C$^\alpha$H which, in the intermediate **18**, is adjacent to the electron-attracting *p*-nitrophenyl carbonate group.

In order to confirm the structural assignment and to relate the results to previously studied models, an X-ray crystallographic analysis of compound **14** has been undertaken. It is worth observing that although several pseudopeptides containing α-aza-amino acid residues have been studied, only a few reports give detailed information on the structural and conformational features of the new analogues.[5,18,19]

Fig. 1 shows the molecular structure and the numbering scheme of compound **14**; Table 2 reports a selection of bond lengths and angles together with relevant torsion angles. Full lists of bond lengths and angles, fractional coordinates and thermal parameters have been deposited with the CCDC.*

* See Instructions for Authors, in the January issue.

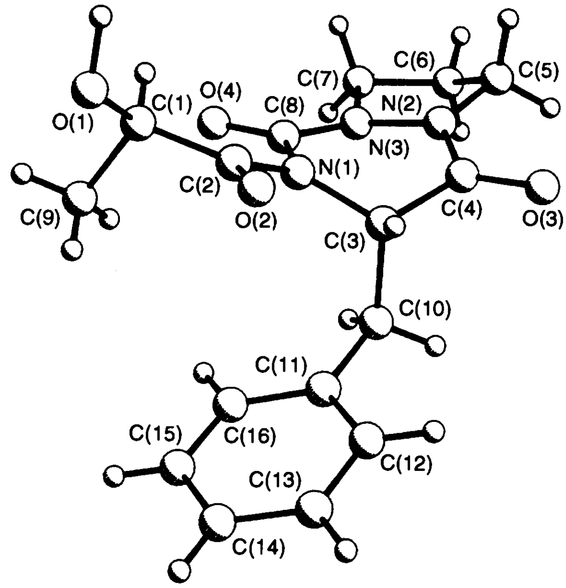


Fig. 1 Molecular structure and atomic numbering scheme of compound **14**

As reported in Table 2, the lengths of the three endocyclic CO–N bonds of the tetrahydrotriazinedione ring are 1.327, 1.328 and 1.418 Å for N(2)–C(4), N(3)–C(8) and N(1)–C(8), respectively. The lengthening of the latter bond is related to the presence of the acylating group at N(1), in accordance with previous observations in related systems.[20] In the azaPro pyrazolidine ring the N–N distance (1.403 Å) is significantly smaller than the average value observed for N–C$^\alpha$H (1.48 Å) in the pyrrolidine ring of proline residues[19] and very similar to the N–N distance observed in the azaPhe residue (1.393 Å) of the previously studied pseudopeptidic oxacyclol.[5]

Five atoms of the six-membered tetrahydrotriazinedione ring are nearly coplanar; the C(3) atom, corresponding to the Phe C$^\alpha$ atom, is displaced 0.548 Å out of the mean plane of the other ring atoms; the conformation of the six-membered ring can be thus described as an approximate sofa;[21] however, the presence of a pseudo-binary axis ($\Delta C_2 = 7°$), passing through the middle point of the N(1)–C(3) and N(2)–N(3) bonds, shows that the shape of the ring is strongly distorted towards a half-chair conformation. The pyrazolidine ring adopts an envelope C_S–C(6) conformation with the C(6) atom, corresponding to the azaPro C$^\gamma$ atom, displaced 0.463 Å out from the plane of the other four ring atoms.

An interesting stereochemical feature of compound **14** concerns the chirality of N(3) which replaces the C$^\alpha$ atom of the proline residue. The deviation of N(3) from the plane of its substituents is larger than that of the other two nitrogen atoms of the molecule. The sum of the bond angles around N(1), N(2) and N(3) is, in fact, 360.0, 358.6 and 357.6°, respectively, and the distance from the plane of their substituents 0.003, 0.094 and 0.123 Å, respectively. As can be deduced from Fig. 1, the pyramidality of the azaPro N(3) is such as to direct the incipient sp^3 lone pair in the opposite direction to that of the C$^\alpha$–H bond of the L-phenylalanine residue. Thus, at least in the solid state, the azaPro mimics a proline residue possessing *R*(D) absolute configuration. This stereochemical preference can be rationalized by considering the formation of a pseudo-dioxopiperazine system of *trans* type; in accord with literature findings on proline-containing dioxopiperazines, the more stable diastereoisomers[22,23] are, in fact, the *trans*-forms.

The benzylic side chain of the Phe residue adopts a *quasi*-axial orientation and a rotameric state which can be described as extended towards the nitrogen. This arrangement is not

Table 1 Selected ^1H and ^{13}C NMR data[a] for compounds **9**, **10**, **14** and **16**

	9		**10**		**14**		**16**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
DLac (or 5-Methyloxazolidine-2,4-dione)								
C^{α}			4.85q (6.5)	75.45	4.9q (6.5)	68.86	4.65q (7.0)	75.19
C^{β}			1.5d	17.65	1.45d	21.81	1.35d	16.49
OH					3.3br			
CH_2O			4.4, 4.5 ABq (11.2)	72.24				
CO				174.24		177.15		173.69
OCON								155.04
Phe								
C^{α}	4.1m	56.84	5.25m	56.99	5.5m	56.94	5.4dd	56.25
C^{β}	2.9dd (9.2, 13.8) 3.2dd (3.7, 13.8)	37.69	3.05dd (4.4, 14.0) 3.15dd (5.4, 14.0)	37.30	3.05dd (4.5, 14.0) 3.15dd (6.0, 14.0)	37.04	3.45dd 3.6dd	32.81
CO		160.22		162.65		161.74		167.64
NH	5.5app. s							
azaPro (or pyrazolidine)								
C^{β}	3.55m, 3.7m[b]	44.68[c]	3.45m, 3.9m[d]	44.32[e]	3.6m, 4.0m[f]	44.51[g]	3.0m (CH_2NHN)	44.69[h]
C^{γ}	2.0–2.2m	22.91	1.7–2.0m	22.54	1.9m, 2.1m	22.59	2.05m	26.99
C^{δ}	3.75m[b]	45.47[c]	2.8m, 3.4m[d]	44.53[e]	3.0m, 3.65m[f]	44.92[g]	3.5m (CH_2NNH)	48.39[h]
CO		154.49		147.19		147.22		
NH							4.0t (6.0)	

[a] Chemical shifts from $SiMe_4$ in $CDCl_3$ solution; J/Hz in parentheses. The assignments for proton-bearing carbons were confirmed by APT experiments. [b–h] Assignments may be interchanged.

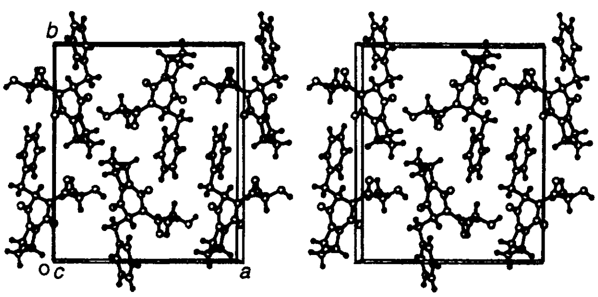


Fig. 2 The crystal packing of compound **14** along the *c* axis

characteristic of cyclodipeptides containing an aromatic side chain; these prefer a folded conformation in which the aromatic ring is oriented face-to-face over the heterocyclic ring.[24] The extended conformation found in the crystal is not preferred in $CDCl_3$ solution; the Phe $C^{\alpha}H–C^{\beta}H_2$ vicinal coupling constants (4.5 and 6.0 Hz; Table 1) indicate a significant contribution by the folded rotamer. An extended conformation, however, is preferred in the case of the non-N-acylated cyclopseudodipeptide **9** as indicated by the value (3.7 and 9.2 Hz; Table 1) of the corresponding vicinal coupling constants.

Fig. 2 shows the stereoview of the crystal packing of compound **14**. The molecules are held together by van der Waals forces and by hydrogen bonds. Each molecule is involved in two hydrogen bonds with the molecules related by the two-fold screw axis parallel to *a*, through the hydroxy group and the O(3) atom, forming infinite chains. The geometrical parameters of this interaction are: O(1)···O(3) = 2.772(4) Å; C(4)–O(3)···O(1) = 169.9°; H–O(1)···O(3) = 1.772 Å.

Conclusions.—The present results suggest that, in contrast with related azaPhe-containing systems,[5] the ergot-like four heteroatom-ring system tetrahedral adducts **1**, deriving from the azaPro *versus* Pro replacement, do not represent stable prototropic tautomers. Isolable forms are in fact the *N*-(α-hydroxyacyl)tetrahydrotriazinediones **2** (see compound **14**) and the new oxazolidine-2,4-dione derivatives **4** (see compound **16**), in which the proton is located on the alcoholic oxygen and the acylpyrazolidine nitrogen, respectively. In the case under study a tendency of the *N*-α-hydroxyacyl derivative of type **2** to rearrange into the oxazolidinedione form of type **4** has also been observed. This latter species, despite the contemporary presence of the nucleophilic acylhydrazine NH and the oxazolidinedione carbonyl groups, has been found to be stable under the reaction conditions adopted.

Experimental

M.p.s were measured on a Büchi oil bath apparatus and are uncorrected. TLC was performed on pre-coated silica gel Merck 60F 254 plates developed with $CHCl_3$–MeOH (99:1) (R_{fA}), $CHCl_3$–MeOH (97:3) (R_{fB}) or $CHCl_3$–MeOH (98:2) (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 ($CHCl_3$) were recorded on a Perkin-Elmer 983 spectrophotometer. ^1H (300 MHz) and ^{13}C (75.43 MHz) NMR spectra were determined on a Varian XL-300 instrument for solutions in $CDCl_3$ containing tetramethylsilane as an internal standard, unless noted otherwise; *J*-values are given in Hz.

N-(tert-*Butoxycarbonyl*)*azaproline* p-*Nitrophenyl Ester* **7**.—To a stirred solution of *tert*-butoxycarbonylpyrazolidine (3.8 g, 22.0 mmol) in tetrahydrofuran (THF) (40 cm^3), solutions of *p*-nitrophenyl chloroformate (4.4 g, 22.0 mmol) in THF (10 cm^3) and *N*-methylmorpholine (2.2 g, 22.0 mmol) in THF (10 cm^3) were added dropwise at 0 °C over a period of 30 min. After 3 h at 0 °C the precipitate was filtered off and the resulting solution was evaporated to dryness. The residue was taken up in $CHCl_3$ and the solution was washed successively with 1 mol dm^{-3} $KHSO_4$, saturated aq. Na_2CO_3, and water, dried and evaporated. The residue was eluted with $CHCl_3$–MeOH (99:1) from a silica gel column to give the *active ester* **7** as an oil (6.5 g, 88%), R_{fA} 0.8 (Found: C, 53.3; H, 5.8; N, 12.4. $C_{15}H_{19}N_3O_6$ requires C, 53.4; H, 5.7; N, 12.5%); ν_{max}/cm^{-1} 1710–1730 (CO);

Table 2 Selected bond distances (a) in Å, bond angles (b) and torsion angles (c) in degrees. Estimated standard deviations are given in parentheses

(a) Intramolecular distances			
O(1)–C(1)	1.412(5)	C(1)–C(2)	1.527(6)
O(2)–C(2)	1.205(5)	C(1)–C(9)	1.516(6)
O(3)–C(4)	1.236(6)	C(3)–C(4)	1.512(6)
O(4)–C(8)	1.210(5)	C(3)–C(10)	1.546(6)
N(1)–C(2)	1.405(4)	C(5)–C(6)	1.510(6)
N(1)–C(3)	1.480(4)	C(6)–C(7)	1.504(6)
N(1)–C(8)	1.418(5)	C(10)–C(11)	1.502(6)
N(2)–N(3)	1.403(5)	C(11)–C(12)	1.377(6)
N(2)–C(4)	1.327(5)	C(11)–C(16)	1.392(7)
N(2)–C(5)	1.457(5)	C(12)–C(13)	1.398(6)
N(3)–C(7)	1.477(5)	C(13)–C(14)	1.358(8)
N(3)–C(8)	1.328(5)	C(14)–C(15)	1.371(7)
		C(15)–C(16)	1.384(7)
(b) Intramolecular bond angles			
C(2)–N(1)–C(3)	117.3(3)	O(3)–C(4)–N(2)	123.4(4)
C(2)–N(1)–C(8)	124.1(3)	O(3)–C(4)–C(3)	121.4(4)
C(3)–N(1)–C(8)	118.6(3)	N(2)–C(4)–C(3)	115.2(4)
N(3)–N(2)–C(4)	120.7(3)	N(2)–C(5)–C(6)	103.2(4)
N(3)–N(2)–C(5)	110.0(3)	C(5)–C(6)–C(7)	104.7(3)
C(4)–N(2)–C(5)	127.9(4)	N(3)–C(7)–C(6)	103.4(3)
N(2)–N(3)–C(7)	109.2(3)	O(4)–C(8)–N(1)	124.9(3)
N(2)–N(3)–C(8)	124.3(3)	O(4)–C(8)–N(3)	122.2(4)
C(7)–N(3)–C(8)	124.1(3)	N(1)–C(8)–N(3)	112.9(3)
O(1)–C(1)–C(2)	109.9(3)	C(3)–C(10)–C(11)	113.5(3)
O(1)–C(1)–C(9)	106.9(3)	C(10)–C(11)–C(12)	120.0(4)
C(2)–C(1)–C(9)	110.0(3)	C(10)–C(11)–C(16)	121.6(4)
O(2)–C(2)–N(1)	119.2(3)	C(12)–C(11)–C(16)	118.4(4)
O(2)–C(2)–C(1)	121.1(3)	C(11)–C(12)–C(13)	119.9(4)
N(1)–C(2)–C(1)	119.6(3)	C(12)–C(13)–C(14)	121.2(5)
N(1)–C(3)–C(4)	111.9(3)	C(13)–C(14)–C(15)	119.4(4)
N(1)–C(3)–C(10)	112.1(3)	C(14)–C(15)–C(16)	120.3(4)
C(4)–C(3)–C(10)	110.3(3)	C(11)–C(16)–C(15)	120.8(4)
(c) Torsion angles			
N(1)–C(2)–C(1)–C(9)	80.2(4)	N(3)–C(8)–N(1)–C(2)	154.9(3)
N(1)–C(3)–C(4)–N(2)	−24.5(5)	N(3)–C(8)–N(1)–C(3)	−24.8(4)
N(1)–C(3)–C(10)–C(11)	−76.5(4)	C(1)–C(2)–N(1)–C(8)	9.5(5)
N(1)–C(8)–N(3)–N(2)	−12.6(4)	C(3)–C(4)–N(2)–C(5)	−174.2(4)
N(2)–N(3)–C(7)–C(6)	16.9(4)	C(3)–C(10)–C(11)–C(12)	−86.1(4)
N(2)–C(5)–C(6)–C(7)	30.0(4)	C(4)–N(2)–N(3)–C(7)	−165.5(4)
N(3)–N(2)–C(4)–C(3)	−9.0(5)	C(4)–N(2)–N(3)–C(8)	31.4(5)
N(3)–N(2)–C(5)–C(6)	−20.2(4)	C(4)–C(3)–N(1)–C(8)	43.1(4)
N(3)–C(7)–C(6)–C(5)	−28.7(4)	C(5)–N(2)–N(3)–C(7)	2.1(4)

δ_H 1.45 (9 H, s, 3 × Me), 1.8–2.3 (2 H, m, γ-H_2 azaPro), 3.2–4.2 (4 H, m, β- and δ-H_2 azaPro), 7.3–7.5 (2 H, m, ArH) and 8.3 (2 H, two lines, ArH).

N-(tert-*Butoxycarbonyl)azaprolyl*-L-*phenylalanine Methyl Ester* **8**.—To a stirred solution containing L-phenylalanine methyl ester (2.1 g, 12.0 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.2 g, 2.0 mmol) in DMF (6 cm^3) was added a solution of the active ester **7** (2.7 g, 8.0 mmol) in DMF (4 cm^3) in portions at room temperature. After 72 h, the reaction mixture was evaporated and the residue was taken up in ethyl acetate. The solution was repeatedly washed successively with 1 mol dm^{-3} $KHSO_4$, saturated aq. Na_2CO_3, and water, dried and evaporated. The resulting oil was chromatographed on silica gel with $CHCl_3$–MeOH (99:1) as the eluent to give *methyl ester* **8** as an oil (2.7 g, 90%), R_{fA} 0.7; $[\alpha]_D$ +18.0 (*c* 1.00, $CHCl_3$) (Found: C, 60.4; H, 7.05; N, 11.2. $C_{19}H_{27}N_3O_5$ requires C, 60.5; H, 7.2; N, 11.1%); ν_{max}/cm^{-1} 3430 (NH) and 1735 and 1670 (CO); δ_H 1.45 (9 H, s, 3 × Me), 1.8–2.2 (2 H, m, γ-H_2 azaPro), 3.0–3.2 (2 H, m, β-H_2 Phe), 3.3–3.8 (4 H, m, β- and δ-H_2 azaPro), 3.7 (3 H, s, OMe), 4.75 (1 H, m, α-H Phe), 6.05 (1 H, d, *J* 8.5, NH) and 7.1–7.4 (5 H, m, ArH).

cyclo(-L-*Phenylalanylazaprolyl*-) **9**.—An ice-cooled solution of the above described methyl ester **8** (5.5 g, 14.6 mmol) in 1,4-dioxane (30 cm^3) was treated with dry HCl gas and was then stirred for 2 h at room temperature. The solution was evaporated to dryness and the resulting oil was repeatedly taken up in dry diethyl ether. The residue was taken up in ethyl acetate and the organic layer was washed successively with saturated aq. $NaHCO_3$ and water, dried and evaporated to give azaprolyl-L-phenylalanine methyl ester (3.7 g), which was used without further purification.

According to ref. 9, a solution of the above reported methyl ester (3.5 g, 12.6 mmol) in aq. 5% acetic acid (120 cm^3) was heated on a water-bath at 75 °C for 40 h. After drying and evaporation of the mixture, the resulting oil was chromatographed on silica gel using $CHCl_3$–MeOH (98:2) as the eluent to give *title compound* **9** as an oil (1.0 g, 33%), R_{fB} 0.5; $[\alpha]_D$ −52.0 (*c* 1.00, $CHCl_3$) (Found: C, 63.6; H, 6.05; N, 17.2. $C_{13}H_{15}N_3O_2$ requires C, 63.7; H, 6.2; N, 17.1%); ν_{max}/cm^{-1} 3370 (NH) and 1660 (CO).

N-*Benzyloxycarbonyl*-L-*phenylalanylazaproline* tert-*Butyl Ester* **11**.—To a stirred solution of *N*-benzyloxycarbonyl-L-phenylalanine (5.2 g, 17.4 mmol) in THF (60 cm^3) were added solutions of dicyclohexylcarbodiimide (DCC) (3.6 g, 17.4 mmol) in THF (20 cm^3) and *tert*-butoxycarbonylpyrazolidine (3.0 g, 17.4 mmol) in THF (20 cm^3) at 0 °C. After 4 h at 0 °C and 16 h at 5 °C, the precipitate was filtered off and the resulting solution was evaporated to dryness. The residue was taken up in ethyl acetate and the solution was washed successively with 1 mol dm^{-3} $KHSO_4$, saturated aq. $NaHCO_3$, and water, dried and evaporated. The residue was eluted with $CHCl_3$–MeOH (98:2) from a silica gel column to give *title compound* **11** as an oil (6.9 g, 87%), R_{fC} 0.7; $[\alpha]_D$ +13.0 (*c* 1.00, $CHCl_3$) (Found: C, 66.05; H, 7.1; N, 9.35. $C_{25}H_{31}N_3O_5$ requires C, 66.2; H, 6.9; N, 9.3%); ν_{max}/cm^{-1} 3425 (NH) and 1720 and 1660 (CO); δ_H 1.35 (9 H, s, 3 × Me), 1.6–2.1 (2 H, m, γ-H_2 azaPro), 2.9 (2 H, m, β-H_2 Phe), 3.0–3.8 (4 H, m, β- and δ-H_2 azaPro), 4.95–5.05 (3 H, m, CH_2O and α-H Phe), 5.55 (1 H, d, *J* 8.5, NH) and 7.1–7.4 (10 H, m, ArH).

N-[(R)-2-*Benzyloxypropionyl*]-L-*phenylalanylazaproline* tert-*Butyl Ester* **12**.—The *N*-benzyloxycarbonyl derivative **11** (4.6 g, 10.1 mmol) was hydrogenated in 80% aq. MeOH (150 cm^3) in the presence of 10% Pd on activated charcoal (0.9 g). After 3 h the catalyst was filtered off and the filtrate was evaporated under reduced pressure to afford phenylalanylazaproline *tert*-butyl ester (3.2 g), which was used without further purification.

A solution of the above described N-deprotected derivative (2.3 g, 7.2 mmol) in THF (15 cm^3)–MeOH (5 cm^3) was added at 0 °C to a stirred solution containing (+)-(*R*)-2-benzyloxypropionic acid (1.95 g, 10.8 mmol) and DCC (1.5 g, 7.2 mmol) in THF (25 cm^3). After 4 h at 0 °C and 16 h at 5 °C the reaction mixture was filtered, the resulting solution was evaporated under reduced pressure and the residue was taken up in ethyl acetate. The solution was washed successively with 1 mol dm^{-3} $KHSO_4$, saturated aq. $NaHCO_3$, and water, dried and evaporated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (99:1) as the eluent to give *title compound* **12** as an oil (3.0 g, 86%), R_{fC} 0.75; $[\alpha]_D$ +16.0 (*c* 1.00, $CHCl_3$) (Found: C, 67.45; H, 7.2; N, 8.8. $C_{27}H_{35}N_3O_5$ requires C, 67.3; H, 7.3; N, 8.7%); ν_{max}/cm^{-1} 3405 (NH) and 1720 and 1660 (CO); δ_H 1.35 (3 H, d, *J* 6.8, Me), 1.4 (9 H, s, 3 × Me), 1.65–2.1 (2 H, m, γ-H_2 azaPro), 2.9 (2 H, m, β-H_2 Phe), 3.0–3.9 (4 H, m, β- and δ-H_2 azaPro), 3.9 (1 H, q, *J* 6.8, α-H Lac), 4.4 (2 H, app. s, CH_2O), 5.25 (1 H, m, α-H Phe), 7.05 (1 H, d, *J* 5.0, NH) and 7.1–7.4 (10 H, m, ArH).

N-[(R)-2-*Benzyloxypropionyl*]-L-*phenylalanylazaproline* p-*Nitrophenyl Ester* **13**.—The preceding oil (2.8 g, 5.8 mmol) was treated with trifluoroacetic acid (TFA) (6 cm^3) at room temperature. After 1 h the solution was evaporated to dryness and the residue was repeatedly taken up in dry diethyl ether. The solvent was replaced by $CHCl_3$ and the solution was washed successively with saturated aq. $NaHCO_3$ and water, dried and evaporated to give [(*R*)-2-benzyloxypropionyl-L-phenylalanyl]pyrazolidine (2.2 g) which was used without further purification.

To a stirred solution of the above described N-deprotected derivative (1.9 g, 5.0 mmol) in THF (20 cm^3) were added solutions of *p*-nitrophenyl chloroformate (1.0 g, 5.0 mmol) in THF (5 cm^3) and *N*-methylmorpholine (0.5 g, 5.0 mmol) in THF (5 cm^3) dropwise at 0 °C over a period of 20 min. After 3 h at 0 °C the precipitate was filtered off and the resulting solution was evaporated to dryness. The residue was taken up in $CHCl_3$ and the solution was washed successively with 1 mol dm^{-3} HCl, saturated aq. Na_2CO_3, and water, dried and evaporated. The residue was eluted with $CHCl_3$–MeOH (99:1) from a silica gel column to give the *active ester* **13** as an oil (2.6 g, 96%), R_{fC} 0.75; $[\alpha]_D$ +40.0 (*c* 1.00, $CHCl_3$) (Found: C, 63.9; H, 5.4; N, 10.3. $C_{29}H_{30}N_4O_7$ requires C, 63.7; H, 5.5; N, 10.25%); ν_{max}/cm^{-1} 3405 (NH) and 1745 and 1660 (CO); δ_H 1.35 (3 H, d, *J* 6.0, Me), 1.7–2.0 (2 H, m, γ-H_2 azaPro), 2.8–3.4 (4 H, m, β-H_2 Phe and δ-H_2 azaPro), 3.7–4.0 (3 H, m, β-H_2 azaPro and α-H Lac), 4.5 (2 H, app. s, CH_2O), 5.5 (1 H, m, α-H Phe), 7.0 (1 H, br, NH), 7.1–7.5 (12 H, m, ArH) and 8.25 (2 H, m, ArH).

N-[(R)-2-*Benzyloxypropionyl*]*cyclo*(-L-*phenylalanylazaprolyl*-) **10**.—(*From compound* **13**). To a stirred solution of the active ester **13** (2.5 g, 4.6 mmol) in dry DMF (30 cm^3) was added sodium hydride (80% in white oil; 5 mmol) at 0 °C. After 3 h at 0 °C the mixture was treated with ice-cold aq. $NaHCO_3$ and ethyl acetate, and the organic layer was washed successively with saturated aq. Na_2CO_3 and water. It was then dried and evaporated and the residue was chromatographed on silica gel using $CHCl_3$–MeOH (995:5) as the eluent to give *title compound* **10** (1.3 g, 67%) as an oil, R_{fC} 0.75; $[\alpha]_D$ +130.0 (*c* 1.00, $CHCl_3$) (Found: C, 67.7; H, 6.3; N, 10.15. $C_{23}H_{25}N_3O_4$ requires C, 67.8; H, 6.2; N, 10.3%); ν_{max}/cm^{-1} 1700 and 1665 (CO).

(*From compound* **9**). A mixture of (+)-(*R*)-2-benzyloxypropionyl chloride (6.3 g, 31.8 mmol) and compound **9** (1.3 g, 5.3 mmol) in 1,4-dioxane (100 cm^3) containing dry pyridine (3.1 g, 39.8 mmol) was heated for 48 h at 90 °C. After cooling of the reaction mixture, the precipitate was filtered off and the resulting solution was evaporated to dryness. The residue was taken up in $CHCl_3$, and the solution was washed successively with 0.5 mol dm^{-3} HCl, saturated aq. $NaHCO_3$ and water, dried and evaporated. The resulting residue was chromatographed on silica gel using $CHCl_3$–MeOH (99:1) as the eluent to give title compound **10** (0.3 g, 15%).

N-[(R)-2-*Hydroxypropionyl*]*cyclo*(L-*phenylalanylazaprolyl*-) **14** *and* (5R)-5-*Methyl*-3-{(1S)-2-*phenyl*-1-[(*pyrazolidin*-1-*yl*)-*carbonyl*]*ethyl*}*oxazolidine*-2,4-*dione* **16**.—A solution of the above described *O*-benzyl derivative **10** (1.1 g, 2.7 mmol) in MeOH (60 cm^3) was kept under a stream of H_2 in the presence of 10% Pd on activated charcoal (0.3 g). After 6 h the catalyst was filtered off, the filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel using $CHCl_3$–MeOH (99:1) as the eluent.

The main component isolated by chromatography was further purified by crystallization from ethyl acetate to give *title compound* **14** (0.5 g, 60%), m.p. 160–162 °C; R_{fA} 0.4; $[\alpha]_D$ +104.0 (*c* 1.00, $CHCl_3$) (Found: C, 60.5; H, 6.1; N, 13.3. $C_{16}H_{19}N_3O_4$ requires C, 60.6; H, 6.0; N, 13.2%); ν_{max}/cm^{-1} 3520 (OH) and 1665 (CO); δ_H([$^2H_6$]dimethyl sulfoxide) 1.1 (3 H, d, *J* 6.5, Me), 1.9–2.2 (2 H, m, γ-H_2 azaPro), 3.0 (2 H, m, β-H_2 Phe), 3.2–3.9 (4 H, m, β-H_2 and δ-H_2 azaPro), 4.7 (1 H, m, α-H Lac), 5.1 (1 H, d, *J* 8.0, OH), 5.2 (1 H, m, α-H Phe) and 7.05–7.3 (5 H, m, ArH).

The minor fraction isolated by chromatography gave isomeric *title compound* **16** as an oil (0.2 g, 28%), R_{fA} 0.65; $[\alpha]_D$ +38.0 (*c* 1.00, $CHCl_3$) (Found: C, 60.6; H, 6.1; N, 13.25%); ν_{max}/cm^{-1} 3535 (NH) and 1815, 1740 and 1660 (CO).

N-{(R)-2-[(p-*Nitrophenoxy*)*carbonyloxy*]*propionyl*}-L-*phenylalanylazaproline* tert-*Butyl Ester* **18**.—A solution of the *O*-benzyl derivative **12** (5.0 g, 10.4 mmol) in MeOH (60 cm^3) was kept under a stream of H_2 in the presence of 10% Pd on activated charcoal (1.0 g). After 6 h the catalyst was filtered off, the solution was evaporated to dryness and the residue was eluted with $CHCl_3$–MeOH (98:2) from a silica gel column to give *N*-[(*R*)-2-hydroxypropionyl]-L-phenylalanylazaproline *tert*-butyl ester (3.2 g) as an oil.

To a stirred solution of the preceding O-deprotected derivative (3.1 g, 8.0 mmol) in pyridine (15 cm^3) was added *p*-nitrophenyl chloroformate (1.6 g, 8.0 mmol) in portions. After 48 h at room temperature the reaction mixture was evaporated under reduced pressure and the residue was taken up in ethyl acetate. The solution was repeatedly and successively washed with ice-cold 1 mol dm^{-3} HCl, saturated aq. Na_2CO_3, and water, dried and evaporated. The residue was eluted with $CHCl_3$–MeOH (98:2) from a silica gel column to give the *carbonate* **18** as a foam (3.5 g, 78%), R_{fC} 0.7; $[\alpha]_D$ + 18.0 (*c* 1.00, $CHCl_3$) (Found: C, 58.35; H, 5.7; N, 10.2. $C_{27}H_{32}N_4O_9$ requires C, 58.3; H, 5.8; N, 10.1%); ν_{max}/cm^{-1} 3415 (NH), 1770, 1720 and 1660 (CO); δ_H 1.4 (9 H, s, 3 × Me), 1.45 (3 H, d, *J* 7.0, Me), 1.8–2.1 (2 H, m, γ-H_2 azaPro), 2.95 (2 H, m, β-H_2 Phe), 3.15–4.1 (4 H, m, β- and δ-H_2 azaPro), 5.15 (1 H, q, *J* 7.0, α-H Lac), 5.3 (1 H, m, α-H Phe), 6.9 (1 H, d, *J* 7.5, NH), 7.1–7.3 (7 H, m, ArH) and 8.25 (2 H, m, ArH).

5-Methyl-3-{(1S)-2-phenyl-1-[(pyrazolidin-1-yl)carbonyl]-ethyl}oxazolidine-2,4-dione **16**.—(*From compound* **18**). To a stirred solution of compound **18** (1.8 g, 3.3 mmol) in dry DMF (30 cm^3) was added sodium hydride (80% in white oil; 6.6 mmol) at 0 °C. After 3 h at 0 °C, ice-cold aq. $NaHCO_3$ and ethyl acetate were added and the organic layer was washed successively with saturated aq. Na_2CO_3 and water. It was then dried and evaporated and the resulting residue was chromatographed on silica gel using $CHCl_3$–MeOH (97:3) as the eluent to give N-{(2'S)-2'-[(5''R)-5''-methyl-2'',4''-dioxooxazolidin-3''-yl]-3'-phenylpropionyl}azaproline *tert*-butyl ester (0.6 g).

The above described N-protected derivative (0.5 g, 1.2 mmol) was treated with TFA (2.5 cm^3) at room temperature. After 2 h the solution was evaporated to dryness and the residue was repeatedly taken up in dry diethyl ether. The solvent was replaced by $CHCl_3$ and the solution was washed successively with saturated aq. $NaHCO_3$ and water, dried and evaporated. Chromatography on silica gel using $CHCl_3$–MeOH (99:1) as the eluent afforded title compound **16** (0.2 g, 47%).

X-Ray Structure Determination of Compound **14**.—A prismatic crystal of compound **14** ($C_{16}H_{19}N_3O_4$) was obtained by slow evaporation from a solution in ethyl acetate. All measurements were made on a Rigaku AFC5R diffractometer with graphite-monochromated Cu-Kα radiation and a 12 kW rotating anode generator. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centred reflections in the range $78 < 2\theta < 80°$, corresponded to an orthorhombic cell with dimensions $a = 14.358(2)$, $b = 16.694(2)$, $c = 6.2375(8)$ Å, $V = 1495.1(3)$ Å^3. For $Z = 4$ and M = 317.3, the calculated density is 1.41 g cm^{-3}. Based on the systematic absences and the successful solution and refinement of the structure, the space group was determined to be $P2_12_12_1$. The data were collected at room temperature using the θ–2θ scan technique to a maximum 2θ-value of 124.2°. The ratio of peak counting time to background counting time was 2:1. A total of 1409 reflections was collected. The intensities of three representative reflections which were measured after every 150 reflections remained constant throughout data collection, indicating crystal and electronic stability (no decay correction was applied). The linear absorption coefficient for Cu-Kα is 8.64 cm^{-1}. An empirical absorption correction, based on azimuthal scans of several reflections, was applied to intensities. The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement.—The structure was solved by direct methods with the SIR 92 program.[25] The non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were geometrically generated except HO(1) which was detected from a Fourier difference synthesis; their positions and thermal parameters were refined by assuming the riding model approximation. The final cycle of full-matrix least-squares refinement was based on 1221 observed reflections [$I > 3.00\sigma(I)$] and 208 variable parameters and converged (largest parameter shift was 0.03 times its esd) with unweighted and weighted agreement factors of: $R = 0.048$ and $R_w = 0.077$. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ with $w = 4F_o^2/\sigma^2(F_o^2)$ and σ^2 based on counting statistics. Plots of $\Sigma w(|F_o| - |F_c|)^2$ *versus* $|F_o|$, reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.30 and −0.45 e Å^{-3} respectively. Neutral atom scattering factors and the values for $\Delta f'$ and $\Delta f''$ were taken from the International Tables for Crystallography.[26] Anomalous dispersion effects were included in F_{calc}.[27] All calculations were performed using the TEXSAN crystallographic software package (Molecular Structure Corporation, 1985).

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