NMR Studies of Four Isomers of Decahydroisoquinoline-3(*S*)-carboxylic Acid and a Potent HIV Proteinase Inhibitor incorporating the (*S,S,S*) Isomer

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The stereochemistry and conformations of two *cis* and two *trans* bridged isomers of decahydroisoquinoline-3(*S*)-carboxylic acid (DHIQ) have been elucidated by NMR spectroscopy. A potent HIV proteinase inhibitor, Ro 31-8959, incorporating the (*S,S,S*)-isomer of DHIQ has also been examined, and critical conformational features compared with those found in the X-ray structure of the enzyme-bound inhibitor.

Replication of the human immunodeficiency virus (HIV) requires the correct functioning of a virally-encoded aspartyl proteinase, essential for maturation of the *gag* and *gag-pol* polyproteins, and hence formation of the viral core proteins and enzymes.[1-3] The proteinase is a clear target for the design and synthesis of selective, tight binding, competitive inhibitors. Examination of the cleavage sites in the virus polyprotein gene products revealed an unusual propensity for Phe–Pro and Tyr–Pro amide bonds, rarely observed with mammalian enzymes, and therefore potentially a target for selective inhibition of HIV proteinase.

Inhibitors based on residues associated with these cleavage sites were synthesised, with the scissile amide bond replaced by transition state mimetics. From the early lead compound, Z.Asn.Pheψ[CH(OH)CH_2]Pro.OBu^t (**1**) (IC_{50} *vs.* HIV proteinase = 150 nmol dm^{-3}) considerable effort was concentrated on exploring the proline binding site (S_1') since modelling studies suggested that larger groups could be accommodated. Among a range of proline analogues, the decahydroisoquinoline–3-carboxylic acid (DHIQ) group was suggested, synthesised, and successfully incorporated into inhibitors.[4] One of these inhibitors containing the (*S,S,S*)-isomer of DHIQ as the *tert*-butylamide and with quinaldyl (quinoline-2-carbonyl) replacing the benzyloxycarbonyl group *i.e.* Ro 31-8959 (**2**), ($Ki \leqslant 0.1$ nmol dm^{-3}) is now undergoing clinical investigation in AIDS patients.

In this paper, we report the detailed NMR studies required to determine the stereochemistry and conformations of the four DHIQ isomers, and the further examination of the solution conformation of the inhibitor **2**. This work was carried out prior to the determination of the X-ray structure of Ro 31-8959 (**2**) bound to HIV proteinase.[5] A comparison of the solution and bound conformations was carried out.

Experimental

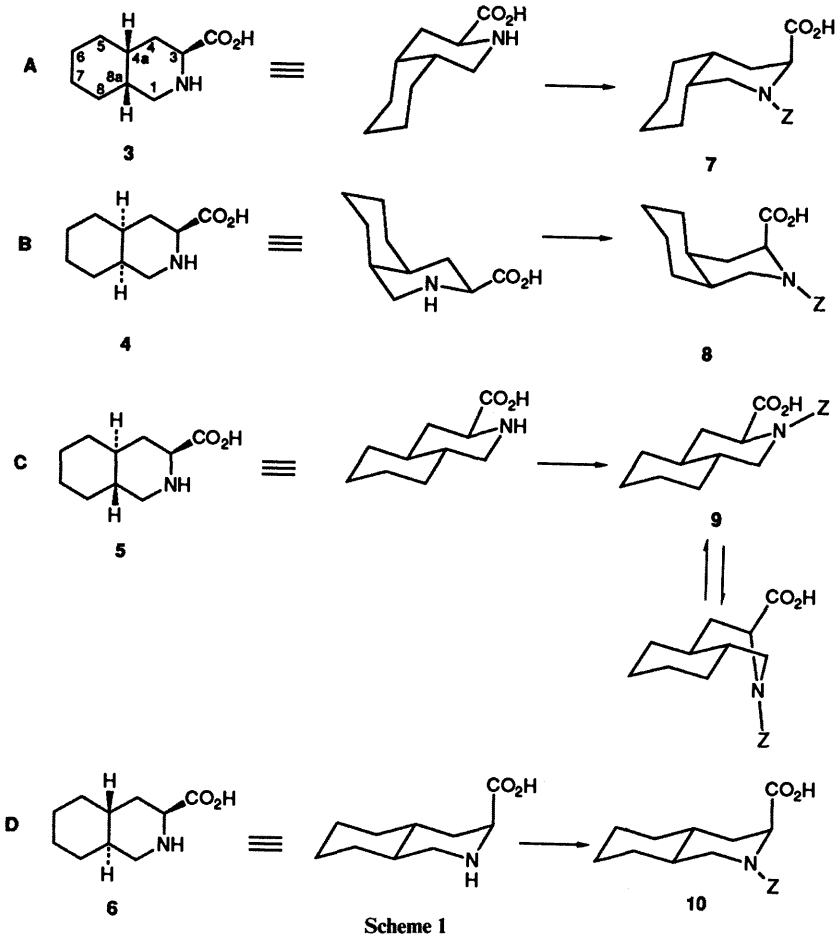
The synthesis of the compounds examined in this paper will be described elsewhere. The DHIQ samples (*ca.* 2 mg) were prepared by suspension of the solid in 99.8% D_2O (Aldrich) and then addition of sodium deuterioxide (NaOD) solution to pH > 11. 1-D spectra were acquired using presaturation where necessary to suppress the solvent peak. The parameters used were the following: SW = 3000 Hz, Time Domain = 16 K, pulse width 30°. 2-D spectra were obtained with 2 K × 1 K matrices, recycle delay 1.6 s and with sine-bell or sine-bell shifted weighting in both dimensions. The NMR spectra were obtained at 21 ± 1 °C on Bruker AC-400 and AM-400 spectrometers using TSP (sodium [2,2,3,3-$^2H_4$]-3-trimethylsilylpropionate) as internal reference. 2-D COSY spectra were obtained using the standard Bruker microprograms COSY.AU, COSY-HG.AU, and COSYPHHG.AU. In the case of Ro 31-8959, methanol was used as solvent using TMS (tetramethylsilane) as internal standard. Addition of deuteriated trifluoroacetic acid caused protonation of the tertiary nitrogen of the DHIQ ring.

Results

Stereochemistry and Conformation of DHIQ Isomers.— Hydrogenation of tetrahydroisoquinoline-3(*S*)-carboxylic acid (or the corresponding *tert*-butylamide) should yield four possible DHIQ isomers **3–6** (Scheme 1). Thermodynamic considerations suggest that the three isomers which can adopt conformations with an equatorial carboxy group, *i.e.* **A**, **B** and **C**, should be favoured, at the expense of the *trans* bridged isomer, **D**, where the carboxy group is forced to occupy an axial orientation.

A ^1H NMR analysis of the crude hydrogenation product indicated that three major isomers were produced, all with H_α multiplets (for the *CH*·CO proton) indicating an axial orientation (one large and one small vicinal coupling). A similar result was obtained by hydrogenation of 3-methylisoquinoline, where two *cis* and one *trans* bridged isomers were obtained, all preferring conformations with equatorial methyl groups.[6] No significant quantity of isomer **D** was apparent, for which the α proton should appear as a narrow multiplet. Some side products appeared to be the result of hydrogenolysis of the CH_2–N bond. The ratios of **A**, **B** and **C** could be varied by altering the conditions used for hydrogenation.

In this work the three isomers **A**, **B** and **C** were isolated as the free imino acids and examined by ^1H NMR spectroscopy. For solubility reasons all the spectra of the DHIQ isomers were run in basic solution, where the carboxylic acid is fully ionised. To determine stereochemistry and conformation it was necessary to assign the multiplets for the protons on C-1, C-3, C-4, C-4a and C-8a and, if possible, obtain individual vicinal coupling constants. Problems were caused by extensive overlap in the high field region of the resonances due to the protons on C-5, C-6, C-7, C-8 together with the critical multiplets for protons on C-4a, C-8a and C-4. 2-D COSY spectra enabled most of the desired overlapping resonances to be assigned. Fortunately the multiplets for the protons in the nitrogen-containing ring, *i.e.* those on C-1, C-3, C-4, C-4a and C-8a, had chemical shifts sufficiently spread to allow coupling constants to be estimated by first-order analysis of splittings. Moreover, the breadth of the well-resolved multiplets in the COSY off-diagonal peaks enabled a clear distinction between axial and equatorial protons, particularly in phase sensitive spectra. This data, together with the readily assigned multiplets for protons on C-1



Scheme 1

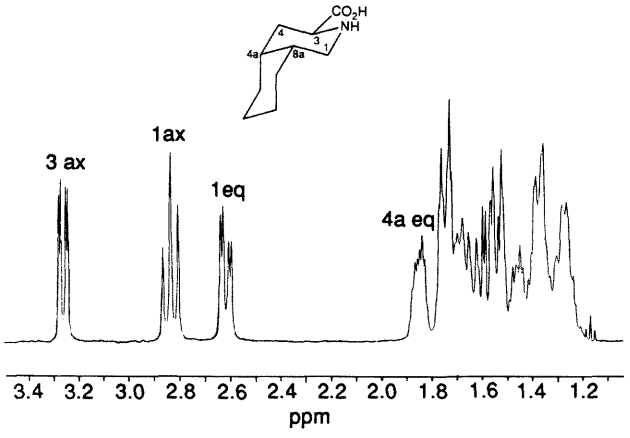


Fig. 1 The 1D 400 MHz proton magnetic resonance spectrum of the amino acid **A** (**3**) in aqueous solution at pH *ca.* 11

and C-3 provided all the necessary information. For example in isomers **A**, **B** and **C** the C-3 proton is clearly axial with a large and a small coupling to the C-4 protons. In isomers **B** and **C** the C-4 axial proton has a large coupling to the bridge C-4a proton which is therefore axial (in the piperidine ring). In isomer **A** on the other hand both the C-4 protons have *small* couplings to the bridge proton which is therefore equatorial (in the piperidine ring). The orientation of the proton on C-8a is easily assigned by mere inspection of the C-1 proton multiplets. A typical 1D spectrum is shown in Fig. 1.

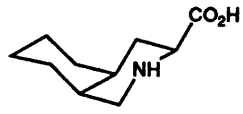
The elusive isomer **D**, with the C-3 carboxyl group axial, was synthesised separately by an unequivocal route.[7] The stereochemistry and conformation were confirmed by the observation of small couplings to the C-3 proton (2.2 and 5.5 Hz) and large couplings from the C-4 axial proton to the bridge C-4a proton (11.9 Hz) and from the C-1 axial to the C-8a proton (11.2 Hz). All the chemical shifts and coupling constants for the piperidine rings of **A**, **B**, **C** and **D** are recorded in Table 1.

The availability of three of the DHIQ isomers as the *N*-benzyloxycarbonyl derivatives **7**–**9** led to some interesting observations concerning the conformations of these compounds. It is well established that *N*-acylation of piperidines leads to a strong preference for the axial orientation for substituents on C-2 or C-6, due to the destabilizing $A^{1,3}$ effect.[8] Thus, for example, the preferred conformation of *N*-acetylpipecolic acid has the carboxy group axially orientated, both in solution[9] and in the crystal.[10] If, as expected, the benzyloxycarbonyl group has the same effect, the derivatives of isomers **A**–**D** may adopt different conformations as shown (Scheme 1). An axial position for the carboxy group can readily be achieved in isomers **A** and **B** by ring inversion in each case, conserving the chair–chair conformation (**7** and **8**). Evidence for this transformation is seen in the ^{1}H NMR spectra of the two compounds where in both cases the proton alpha to the carboxy group appears as a narrow multiplet typical of an equatorially oriented proton. Although not confirmed experimentally, it would be expected that isomer **D** would not change conformation following *N*-acylation (**10**). In isomer **C** the rigid *trans* decalin backbone will not allow an axial carboxy group, unless the piperidine ring were to flip to a boat conformation. The NMR evidence indicates that the *N*-benzyloxycarbonyl derivative of isomer **C** exists largely as the chair–chair conformation **9** despite the large steric penalty. The coupling constants around the piperidine ring suggest that the ratio is *ca.* 90:10: chair–chair:chair–boat. This is based on analysis of the first-order multiplets for the protons on C-1, C-3 and C-4 [δ(CD_3OD): H-1ax, 2.71; H-1eq, 3.80; H-3, 4.03; H-4eq, 2.02; H-4ax, 1.44. Coupling constants (J): 1ax8a, 10.8; 1eq8a, 4.9; 3ax4ax, 9.7; 3ax4eq, 5.8; 4ax4a, 10.5;

Table 1 NMR data for decahydroisoquinoline isomers **A**, **B**, **C** and **D**[a]

A (3) 

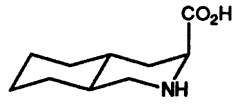
Proton[b]	δ[c]	Protons	J
1eq	2.62	3ax4eq	3.0
1ax	2.84	3ax4ax	11.6
3ax	3.26	4eq4ax	13.0
4eq	1.75	4eq4a	3.1
4ax	1.57	4ax4a	4.4
4a(eq)	1.86	4a8a	small[d]
8a(ax)	1.75	8a1eq	4.4
		8a1ax	11.2
		1eq1ax	13.0

B (4) 

Proton	δ	Protons	J
1eq	2.70	3ax4eq	3.3
1ax	2.77	3ax4ax	11.6
3ax	3.07	4eq4ax	12.3
4eq	1.89	4eq4a	3.5
4ax	1.67	4ax4a	11.6
4a(ax)	1.88	4a8a	small[d]
8a(eq)	1.55	8a1eq	~1.0
		8a1ax	3.1
		1eq1ax	13.3

C (5) 

Proton	δ	Protons	J
1eq	2.87	3ax4eq	3.2
1ax	2.21	3ax4ax	11.8
3ax	3.13	4eq4ax	~12
4eq	1.85	4eq4a	small
4ax	~1.05	4ax4a	large[d]
4a(ax)	1.11	4a8a	large[d]
8a(ax)	~1.05	8a1eq	3.7
		8a1ax	11.4
		1eq1ax	12.2

D (6) 

Proton	δ	Protons	J
1eq	2.66	3eq4eq	2.2
1ax	2.39	3eq4ax	5.5
3eq	3.45	4eq4ax	13.3
4eq	1.95	4eq4a	3.9
4ax	1.37	4ax4a	11.9
4a(ax)	0.88	4a8a	~11
8a(ax)	1.09	8a1eq	3.9
		8a1ax	11.2
		1eq1ax	11.5

[a] Solvent NaOD/D_2O pH 11–12; compounds measured as the carboxylate anions. [b] Only piperidine ring protons assigned. [c] Referenced to internal TSP. [d] Inferred from breadth of phase sensitive COSY multiplets.

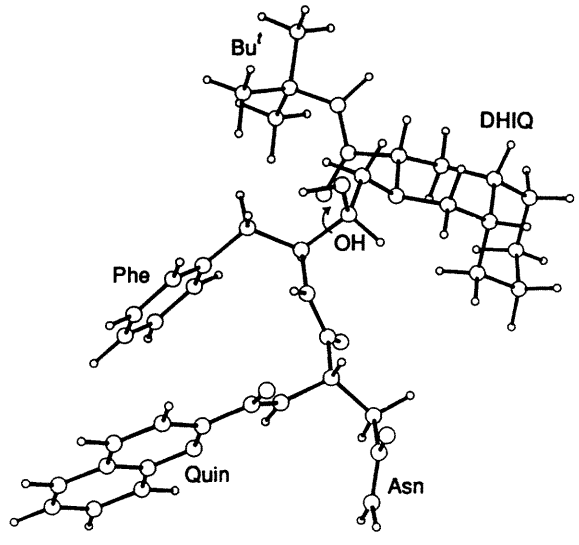


Fig. 2 The enzyme-bound conformation of Ro 31-8959 (**2**) from the X-ray structure of the HIV proteinase–inhibitor complex[5] ('Quin' = quinoline-2-carbonyl, 'DHIQ' = decahydroisoquinoline-3-carbonyl, 'Phe' = pseudo phenylalanine side chain)

4eq4a, 4.3]. The value for J_{3ax4ax} of 9.7 is reduced by *ca.* 1 Hz from that expected for the chair–chair conformation indicating either some deviation from a true chair form or the presence of *ca.* 10% of another conformation, probably the boat form.

NMR Studies on the HIV Proteinaise Inhibitor Ro 31-8959 (**2**).—Incorporation of the four DHIQ isomers **3**–**6**, as the *tert*-butylamides, into the proline (S'_1) binding site led to a new series of HIV proteinase inhibitors (**2**, **11**–**13**). Separately, the asparagine protecting group was changed from benzyloxycarbonyl to quinaldyl to further enhance binding. Of the four inhibitors **2**, **11**–**13** only one showed exceptional potency against the enzyme; that incorporating the DHIQ isomer **B** (**4**) to give inhibitor **2** Ro 31-8959 (IC_{50} *vs.* HIV proteinase = 1.9×10^{-9} mol dm^{-3} $Ki \leqslant 0.1$ nmol dm^{-3}). The other inhibitors (**11**, **12** and **13**) incorporating DHIQ isomers **3**, **5** and **6** have IC_{50} values of 6.5×10^{-8}, 3.5×10^{-7} and 8.5×10^{-6} mol dm^{-3} respectively.[11]

It was important to confirm that the *N*-alkyl group on DHIQ *tert*-butylamide was equatorially oriented, and to investigate the solution conformation of **2** as far as possible, particularly since a crystal structure was not at that time available. With *ca.* 40 non-equivalent proton resonances in this structure, assignment of chemical shifts and coupling constants required several COSY experiments on the free base and protonated forms of the inhibitor (protonated on the DHIQ ring nitrogen but not on the quinoline). Phase-sensitive COSY experiments enabled active coupling constants between multiplets to be estimated by close inspection of the off-diagonal cross peaks of connected resonances, and identifying them, where possible with peaks in the 1-D spectrum. 'Long range' COSY experiments enhanced cross peaks from multiplets having only small mutual coupling constants. In such experiments a delay of 0.08 s is introduced before and after the 90° pulse.[12] Apart from the obvious downfield shift of proton resonances for C*H*–N groups, the 'lone pair' effect[13,14] was used to identify the three CH–N protons in a *trans* diaxial relationship with the nitrogen lone pair *i.e.* the C-1 axial and C-3 axial protons in the DHIQ ring, and one of the N–CH_2 protons. These protons are high field shifted in the free base; *e.g.* 1_{ax} δ 2.18, 3_{ax} δ 2.63, whereas in

Table 2 Chemical shifts and coupling constants for Ro 31-8959 (**2**)[a]

Group	Proton	Chemical shifts[b] Free base	Protonated	Group	Protons	Coupling constants[c] Free base	Protonated
DHIQ	1eq	3.06	3.36	DHIQ	1eq1ax	*ca.* 12.0	*ca.* 13
	1ax	2.18	3.55		3ax4eq	2.2	3.4
	3ax	2.63	4.01		3ax4ax	11.0	11.0
	4eq	1.37	1.80		4eq4ax	11.0	*ca.* 12
	4ax	2.06	2.12		4eq4a	*ca.* 4	*ca.* 3
	4a	1.78	*ca.* 2.0		4ax4a	11.0	*ca.* 11
	8a	1.65	*ca.* 2.0		4a8a	*ca.* 4	small
					8a1eq	3.0	2.1
NCH_2–$CHOH$	9	*ca.* 2.68	3.37		8a1ax	2.2	2.1
	10	2.22	3.17				
	11	3.89	4.07	$NCH_2CH(OH)$	9,10	*ca.* 12.0	14.0
					9,11	*ca.* 7.2	2.1
'Phe'	α	4.27	3.98		10,11	6.4	9.2
	β_1	3.02	3.12				
	β_2	2.68	2.67	'Phe'	α,β_1	3.8	3.8
					α,β_2	11.0	10.2
Asn	α	4.93	4.83		β_1,β_2	14.0	14.0
	β_1	2.77	2.84				
	β_2	2.68	2.70	Asn	α,β_1	6.6	8.0
					α,β_2	6.8	5.6
Phenyl ring	*ortho*	7.18	7.13		β_1,β_2	15.6	16.2
	meta	6.90	6.89				
	para	6.72	6.72				
Quinoline	3′	8.52	8.50				
	4′	8.18	8.14				
	5′	8.02	8.03				
	6′	7.69	7.72				
	7′	7.84	7.86				
	8′	8.14	8.15				
	Bu^t	1.30	1.38				

[a] Solvents: free base, [$^2H_4$]methanol; protonated, [$^2H_4$]methanol + CF_3CO_2D. [b] References to internal TMS. Resonances for DHIQ protons on carbons 5,6,7 and 8 not determined. [c] Coupling constants for aryl protons as expected.

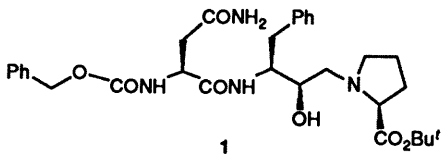
the *N*-protonated form the corresponding shifts are 3.55 and 4.01 ppm. The chemical shifts and coupling constants for all protons in **2** except the C-5, C-6, C-7 and C-8 protons of the DHIQ are recorded in Table 2.

The results confirm that the DHIQ ring adopts the same conformation as that shown in Scheme 1 (**4**) with the CONH–*tert*-butyl group equatorial and the N–CH_2 group, as expected, also equatorial in the unprotonated form. The vicinal coupling constants between the asparagine α and β protons and those for the $NCH_2CH(OH)CH$ fragment vary considerably for the free base and protonated forms of the compound. However, without stereospecific assignments of the Asn β protons, detailed conclusions concerning side chain conformational changes are open to two interpretations. Suffice it to say that no single rotameric form appears to be dominating in either free base or protonated form. Similarly for the $NCH_2CH(OH)CH$ fragment, the assignment of the diastereotopic methylene protons cannot be made uniquely without stereoselective deuteriation experiments which are not possible with the current chemical synthesis.

In contrast, the $-CH_2Ph$ side chain appears to be much more ordered with small and large α–β coupling constants (3.8 and 11.0 Hz in the free base, 3.8 and 10.2 in the protonated form). Moreover, the chemical shifts of the phenyl group ring protons appear at unusually high field suggesting that they are affected by the anisotropy of the quinoline ring, *i.e.* the phenyl ring is predominantly in the face of the quinoline ring, with the *para* (4) proton most affected. This is probably an edge-to-face interaction rather than a face-to-face orientation, since the quinoline chemical shifts are less affected. The phenyl ring high field shifts are not observed in derivatives where the quinoline-2-carbonyl group is replaced by other groups *e.g.* carbobenzoxy. Also, when the pseudo Phe alpha stereochemistry is inverted, high field shifts of the phenyl protons are not observed.

Comparisons with Enzyme-bound Conformation.—Following the completion of this work the crystal structure of Ro 31-8959 (**2**) complexed to HIV-1 proteinase was determined.[5] The bound conformation is shown in Fig. 2. It is interesting to compare the bound and solution conformations to see which features are common.

In the crystal the rigid DHIQ system shows a *cis* bridge with equatorial N–CH_2 and $CONHBu^t$ groups, as found in solution. The aromatic ring of the pseudo phenylalanine side chain is on the same side and turned towards the quinoline ring in an edge-to-face relationship, as also found in solution. The flexibility noted for other parts of the molecule in solution is obviously removed in the crystal, where the asparagine side chain and the hydroxyethylene backbone are conformationally fixed. In the bound conformation, the approximate torsion angles for the CH·CH(OH)CH_2·N fragment are as follows: C*H*–C*H*(OH) = −81°, C*H*(OH)–CH_2 = −95° and 147°. It should be noted that the actual proton coordinates are not obtained in the X-ray structure of the enzyme–inhibitor complex, so that these values are obtained by placing protons in their expected geometry for the backbone heavy atoms. However the torsion angles, constrained by the demands of the proteinase active site, clearly deviate from ideal staggered geometry and would be expected to be quite different in solution. For such torsion angles, the expected coupling constants would be 0, 0 and *ca.* 7 Hz respectively, very different from those actually observed in solution for the free base or protonated forms. The question arises as to whether the inhibitor is bound in free base or protonated form. The pK_a of the DHIQ nitrogen is *ca.* 7.1 and



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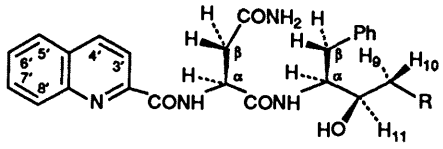

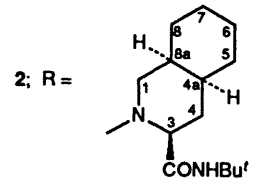

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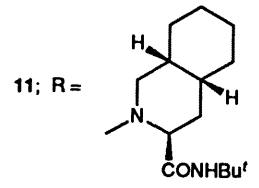

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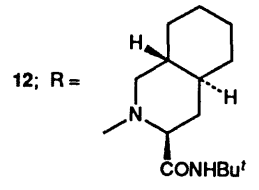

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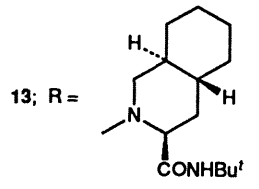

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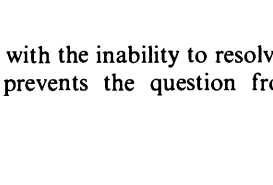

this information, together with the inability to resolve protons in the X-ray structure, prevents the question from being answered.

In conclusion, use of the DHIQ ring as a proline replacement in inhibitors of HIV proteinase provides a tight-fitting, rigid side chain binding in the S_1' pocket of the enzyme. Apart from an understandable flexibility of part of the backbone including the hydroxyethylene group, other conformational features observed in solution seem to be conserved on binding to the enzyme.

Acknowledgements

We gratefully acknowledge the assistance provided by Dr. A. Kröhn in modelling the inhibitors studied in this work, and for providing the plotted views of the bound conformation of the optimised compound **2**. We also thank Mr. B. D. Scott and Mr. M. I. Cooper for the pK_a measurements, and Drs. B. J. Graves and M. H. Hatada for the X-ray data.

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Paper 2/05392F
Received 7*th October* 1992
Accepted 18*th November* 1992