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A Conformational Comparison of Cyclo-[(S)-His-(S)-Leu] and Cyclo-[(S)-His-(S)-Phe], Catalysts for the Asymmetric Addition of HCN to Aldehydes

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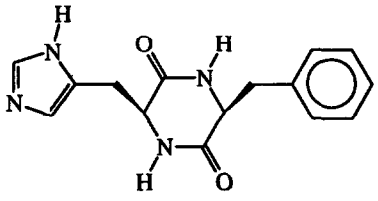
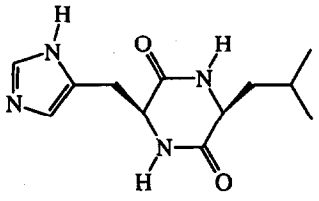
Abstract: *Cyclo-[(S)-His-(S)-Leu] adopts a conformation in solution in which the imidazole ring is folded over the diketopiperazine ring. This is the opposite conformation to that determined for cyclo-[(S)-His-(S)-Phe], and may explain the opposite sense of asymmetric induction between the two catalysts.*



(1)



(2)

Cyanohydrins are versatile, difunctional, starting materials for the preparation of a range of chemical products[1]. However, the traditional methods of preparing cyanohydrins give a racemic product[2] which needs to be resolved if it is to be used in the synthesis of homochiral products. Thus there has been much recent interest in the preparation of optically pure cyanohydrins[3], and in particular in the use of chiral catalysts for the asymmetric synthesis of cyanohydrins from carbonyl compounds and a cyanide source[4]. Numerous catalysts have been investigated for this reaction including; enzymes[5], polymers[6], organometallic reagents[7], and cyclic dipeptides[8-10] (diketopiperazines). Of these, the cyclic dipeptides initially discovered by Inoue *et al.* have been the most widely studied, with *cyclo*-[(S)-His-(S)-Phe] (1) catalysing the formation of (R)-cyanohydrins[9], and *cyclo*-[(S)-His-(S)-Leu] (2) catalysing the formation of (S)-cyanohydrins[10]. Although the synthetic utility of catalysts (1) and (2) has been extensively investigated, little is known about the mechanism of this catalytic, asymmetric, carbon-carbon bond forming reaction. Mechanistic studies on these compounds have been hindered by their amorphous nature which has prevented an X-ray analysis. The conformation of catalyst (1) in solution has been investigated[11], as has the nature of the interaction between catalyst (1) and HCN[12] and four possible transition state models have been proposed for the asymmetric induction[13,14]. In

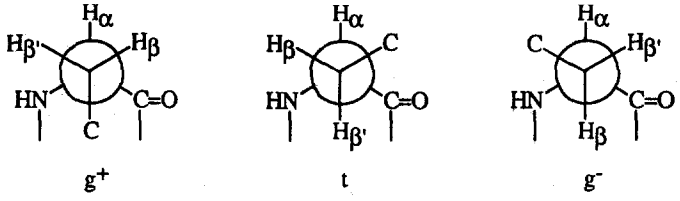
this manuscript, the conformations adopted by catalysts (1) and (2) are compared, and differences in the ground state catalyst conformations are used to suggest an explanation for the opposite senses of asymmetric induction that they exhibit during cyanohydrin synthesis.

Previous Conformational Analyses

The conformation of catalyst (1) in DMSO and CD_3OD has previously been determined by variable temperature nmr and molecular mechanics techniques[11,12]. The global minimum energy conformation was found to be the structure shown in Figure 1a in which the phenyl ring is preferentially in the g^+ rotomer (Figure 2) and so is folded over the diketopiperazine ring. Variable temperature nmr experiments showed that rotation occurs about the Phe α-β bond, but that the population of the g^+ conformer increases as the temperature is lowered, thus matching the predictions made by molecular mechanics calculations. The diketopiperazine ring was shown to be flat. Protonation of the imidazole ring of compound (1) was found not to significantly alter its conformation[12].

Figure 1: Previously Determined Conformations of Compounds (1) and (2).

Figure 2: Newman Projections of the Three Staggered Conformers about a C_α-C_β Bond.



The conformation of compound (2) has previously been investigated both by Tanihara *et al.*[15], and by Arena *et al.*[16]. Combining the results from these two papers, the conformation of catalyst (2) has been investigated at RT in both DMSO and D_2O, and the structure of protonated (2) has been studied in D_2O. Both sets of authors concluded that catalyst (2) adopts a structure of the type shown in Figure 1b in which the imidazole ring adopts a g^+ conformation and is bent over the diketopiperazine ring. Arena *et al.* further

suggested that upon protonation of the imidazole, rotation about the His β-γ bond occurs so that the imidazole ring is no longer planar above the diketopiperazine.

Both these conformational analyses of compound (2) were carried out under different conditions to our previous study of catalyst (1). Thus in order to allow a direct comparison between the structures of the two compounds, we have conducted a variable temperature nmr study of catalyst (2) in DMSO and CD_3OD as previously described for catalyst (1). In the absence of a crystal structure, we have chosen to investigate the solution conformation of these catalysts under as wide a range of conditions as possible, this being limited by the low solubility of the catalysts in non polar solvents.

Variable Temperature NMR Results for Compound (2).

The ^1H nmr spectrum of compound (2) was recorded in DMSO-d_6 solution at temperatures of 30°C to 130°C, and the chemical shifts and coupling constants are reported in Table 1. The corresponding data recorded in CD_3OD solution at temperatures between -70°C and +40°C are reported in Table 2. Chemical shifts are accurate to better than 0.1ppm, and coupling constants to +/- 0.2Hz. Examination of the data in Table 1, shows that the most significant change in chemical shift as the temperature increases occurs for the leucine β-protons which shift to lower field. This is consistent with these protons being shielded by the imidazole ring (histidine residue in g^+ conformation) at low temperatures, but becoming less shielded at higher temperatures due to rotation about the histidine α–β bond, and hence increased population of the histidine g^- and t conformers. Thus these results confirm that the previously determined structure[15,16] is the most populated conformation at low temperatures, but indicate that other conformations are also populated at temperatures around -20°C which are commonly utilised for asymmetric cyanohydrin forming reactions. The leucine δ-protons are also shifted to lower field at high temperature, suggesting that the shielding caused by the imidazole ring extends as far as the leucine methyl groups. By contrast, no significant change in the chemical shift of the leucine γ-proton is observed, this may be due either to the fact that this peak occurs as an unresolved multiplet at all temperatures, or it could indicate that the predominant conformation about the leucine β-γ bond places this proton pointing down towards the diketopiperazine, and hence well away from the influence of the imidazole ring. The leucine α-proton shows a small shift to lower field as the temperature is raised, whilst the histidine α-proton shows no significant shift. Of the two histidine β-protons, one shows a slight shift to lower field on raising the temperature, whilst the other shows no temperature dependence of the chemical shift.

Table 1 also shows that the spectrum of compound (2) becomes better resolved as the temperature is raised, thus some peaks which appear as broad signals at room temperature, resolve into multiplets at higher temperatures. The magnitude of the $His_{\alpha-\beta1}$ coupling constant shows little or no variation with temperature, whilst the magnitude of the $His_{\alpha-\beta2}$ coupling constant increases with temperature from 6.5 to 7.5Hz. It is possible to use the magnitude of the $His_{\alpha-\beta}$ coupling constants to calculate the population of the g^+ conformer *via* Karplus type equations (Equations 1-3). The parameters for these equations were derived from a study of the related diketopiperazine *cyclo*[(S)-His-(S)-Trp][17], and the same approach has been applied to a variety of other diketopiperazines[18]. In principle, the populations of the other two rotomers can also be

Table 1: Selected Variable Temperature NMR Results for Compound (2) Dissolved in DMSO-d_6.

Temp (°C)	Leu Amide	His Amide	His α-CH	Leu α-CH	His β1-CH_2	His β2-CH_2	Leu γ-CH	Leu β1-CH_2	Leu β2-CH_2	Leu δ1-CH_3	Leu δ2-CH_3
30	8.08ppm	7.91ppm	4.04ppm	3.67ppm	2.9-3.2ppm		1.6-1.9ppm	1.2-1.4ppm	0.9-1.1ppm	0.79ppm	0.78ppm
	br s	br s	br	br	2H m		m	br m	br m	d *J* 6.5	d *J* 6.5
50	7.99ppm	7.81ppm	4.05ppm	3.69ppm	2.99ppm	2.88ppm	1.6-1.8ppm	1.2-1.4ppm	0.9-1.1ppm	0.81ppm	0.80ppm
	br s	br s	br	br	dd *J* 14.7,4.0	dd *J* 14.7,6.5	m	m	br m	d *J* 6.5	d *J* 6.5
60	7.95ppm	7.77ppm	4.05ppm	3.70ppm	3.00ppm	2.86ppm	1.7-1.8ppm	1.3-1.5ppm	1.0-1.2ppm	0.83ppm	0.82ppm
	br s	br s	br	br	dd *J* 14.9,4.0	dd *J* 14.8,6.6	m	m	m	d *J* 6.5	d *J* 6.5
70	7.86ppm	7.68ppm	4.03ppm	3.70ppm	3.01ppm	2.86ppm	1.6-1.9ppm	1.36ppm ddd	1.0-1.2ppm	0.84ppm	0.83ppm
	br s	br s	br	br	dd *J* 14.6,4.3	dd *J* 14.8,7.0	m	*J* 13.3,8.2,5.1	m	d *J* 6.6	d *J* 6.6
80	7.80ppm	7.62ppm	4.04ppm	3.71ppm	3.03ppm	2.86ppm	1.6-1.9ppm	1.42ppm ddd	1.16ppm ddd	0.85ppm	0.82ppm
	br s	br s	br	dd *J* 7.5,5.2	dd *J* 14.7,4.4	dd *J* 14.7,7.1	m	*J* 13.3,8.0,5.1	*J* 13.7,7.8,6.0	d *J* 6.6	d *J* 6.6
90	7.74ppm	7.57ppm	4.06ppm	3.73ppm	3.04ppm	2.86ppm	1.7-1.8ppm	1.44ppm ddd	1.16ppm ddd	0.84ppm	0.83ppm
	br s	br s	dd *J* 7.0,4.3	dd *J* 7.3,5.7	dd *J* 14.7,4.3	dd *J* 14.7,7.2	m	*J* 13.4,8.0,5.1	*J* 13.7,7.8,6.0	d *J* 6.6	d *J* 6.5
100	exch	exch	4.05ppm	3.73ppm	3.05ppm	2.86ppm	1.7-1.9ppm	1.46ppm ddd	1.21ppm ddd	0.85ppm	0.84ppm
			dd *J* 7.3,4.5	dd *J* 7.4, 5.3	dd *J* 14.7,4.3	dd *J* 14.8,7.3	m	*J* 13.3,7.9,5.2	*J* 13.7,7.5,6.1	d *J* 6.6	d *J* 6.5
110	exch	exch	4.06ppm	3.74ppm	3.06ppm	2.86ppm	1.7-1.9ppm	1.47ppm ddd	1.23ppm ddd	0.86ppm	0.84ppm
			dd *J* 7.5,4.4	dd *J* 7.4,5.3	dd *J* 14.7,4.3	dd *J* 14.5,7.4	m	*J* 13.2,7.8,5.3	*J* 13.7,7.6,6.0	d *J* 6.7	d *J* 6.6
120	exch	exch	4.07ppm	3.75ppm	3.07ppm	2.86ppm	1.7-1.9ppm	1.51ppm ddd	1.25ppm ddd	0.86ppm	0.85ppm
			dd *J* 7.3,4.3	dd *J* 7.4,5.4	dd *J* 14.8,4.3	dd *J* 14.8,7.5	m	*J* 13.1,7.8,5.3	*J* 13.7,7.3,6.1	d *J* 6.6	d *J* 6.6
130	exch	exch	4.07ppm	3.76ppm	3.08ppm	2.86ppm	1.7-1.9ppm	1.54ppm ddd	1.26ppm ddd	0.87ppm	0.86ppm
			dd *J* 7.6,4.3	dd *J* 7.4,5.3	dd *J* 14.8,4.3	dd *J* 14.8,7.5	m	*J* 13.0,7.7,5.3	*J* 13.7,7.4,6.2	d *J* 6.6	d *J* 6.6

Spectra were recorded at 250MHz on Brucker AM250 spectrometer, using a concentration of 5mg of compound (2) in 0.5ml of solvent. The assignment of diastereotopic β and δ protons as 1 and 2 is arbitary, with the lower field proton being assigned as 1. Coupling constants are in Hz, and have not been corrected for second order effects.

Table 2: Selected Variable Temperature NMR Results for Compound (2) Dissolved in CD_3OD.

Temp (°C)	His α-CH	Leu α-CH	His β1-CH_2	His β2-CH_2	Leu γ-CH	Leu β1-CH_2	Leu β2-CH_2	Leu δ1-CH_3	Leu δ2-CH_3
40	4.44ppm t *J* 4.5	4.01ppm dd *J* 8.9,4.6	3.39ppm dd *J* 14.8,5.2	3.24ppm dd *J* 14.8,4.5	1.7-1.9ppm m	1.51ppm ddd *J* 13.7,9.0,4.7	0.9-1.2ppm m	1.04ppm d *J* 6.5	
30	4.44ppm t *J* 4.4	4.00ppm dd *J* 9.0,4.6	3.40ppm dd *J* 14.7,5.0	3.22ppm dd *J* 14.7,4.5	1.7-1.9ppm m	1.48ppm ddd *J* 13.8,8.9,4.6	0.8-1.1ppm m	1.04ppm d *J* 6.5	1.03ppm d *J* 6.7
20	4.45ppm t *J* 4.2	4.01ppm dd *J* 9.1,4.5	3.42ppm dd *J* 14.8,4.8	3.22ppm dd *J* 14.8, 4.3	1.7-1.9ppm m	1.46ppm ddd *J* 13.8,9.2,4.6	0.92ppm ddd *J* 13.9,9.4,4.9	1.05ppm d *J* 6.6	1.02ppm d *J* 6.5
0	4.44ppm br	3.98ppm dd *J* 9.4,3.9	3.43ppm br	3.19ppm br	1.7-1.9ppm m	1.40ppm ddd *J* 13.8,9.4,4.2	0.7-0.8ppm m	1.03ppm d *J* 6.6	1.02ppm d *J* 6.5
-30	4.45ppm br	3.9-4.0ppm m	3.42ppm br	3.15ppm br	1.7-1.9ppm m	1.33ppm br	0.51ppm br	1.00ppm br	
-40	4.45ppm br	3.95ppm br	3.43ppm br	3.15ppm br	1.6-1.8ppm br m	1.32ppm br	0.43ppm br	1.02ppm d *J* 5.8	
-50	4.46ppm br	3.94ppm br	3.43ppm br	3.14ppm br	1.6-1.8ppm br m	1.31ppm br	0.36ppm br	1.00ppm br	
-60	4.46ppm br	3.94ppm br	3.43ppm br	3.13ppm br	1.6-1.8ppm br m	1.30ppm br	0.28ppm br	1.00ppm br	
-70	4.46ppm br	3.94ppm br	3.43ppm br	3.12ppm br	1.6-1.8ppm br m	1.29ppm br	0.19ppm br	1.00ppm br	

Spectra were recorded at 250MHz on Brucker AM250 spectrometer, using a concentration of 5mg of compound (2) in 0.5ml of solvent. The assignment of diastereotopic β and δ protons as 1 and 2 is arbitary, with the lower field proton being assigned as 1. Coupling constants are in Hz, and have not been corrected for second order effects.

obtained, however this requires the diastereotopic assignment of the His_{β} protons. For free rotation about the α–β bond, *ie* equal populations of the g^+, t and g^- conformers, equations (1-3) predict coupling constants $^3J_{\alpha\beta}$ of 5.7Hz and $^3J_{\alpha\beta'}$ of 6.8Hz.

$$^3J_{\alpha\beta} = 2.6g^+ + 2.4t + 12.1g^- \quad (1)$$

$$^3J_{\alpha\beta'} = 5.2g^+ + 12.4t + 2.7g^- \quad (2)$$

$$g^+ + t + g^- = 1 \quad (3)$$

Application of the Karplus equation to the data in Table 1, shows that at 50°C, the His residue spends 60% of its time in the g^+ rotomer, but at 130°C this has fallen to 40%. These results are entirely consistent with the Leu_{β} protons becoming less shielded as the temperature increases. The $Leu_{\alpha-\beta}$ coupling constants are only resolved at temperatures above 70°C, and they show no significant variation with temperature. The observed coupling constants correspond to a population of the g^+ rotomer of 30%.

Table 2 shows that essentially the same dependence of chemical shift upon temperature is observed at low temperatures in methanol as at high temperatures in DMSO. Thus the most significant temperature variation is seen in the Leu_{β} protons which move to higher field as the temperature is reduced. The effect is most noticeable for the higher field Leu_{β} proton (β2), which at -70°C occurs at 0.19ppm. These results again suggest that as the temperature is reduced, the population of the His g^+ rotomer increases. The population of the His g^+ rotomer calculated from the $His_{\alpha-\beta}$ coupling constants is also consistent with this hypothesis, thus at 40°C the g^+ rotomer has a population of 70%, whilst at 20°C this has risen to 80%. Unfortunately, below 20°C the peaks become broad and coupling information cannot be extracted. The $Leu_{\alpha-\beta}$ coupling constants can only be extracted at temperatures of 0°C and above, and show little variation with temperature within this range. The population of the Leu g^+ rotomer is constant at 20% over the temperature range of 40°C to 0°C.

Protonation of the Imidazole Ring

It has previously been suggested[15,18,19] that the reason for the formation of folded conformations of the type shown in Figure 2 in diketopiperazines containing one or more aromatic amino acids is due to π–π electron interactions between the aromatic ring, and the diketopiperazine amides. Thus it was anticipated that protonation of the imidazole ring might have an effect on the conformation adopted by compound (2). Hence variable temperature nmr spectra of compound (2) were obtained in both DMSO-d_6, and CD_3OD after protonation of the imidazole ring with TFA[20], and the results are reported in Tables 3-4.

Comparison of the data in Table 3 with that in Table 1, reveals that the temperature dependence of both chemical shifts and coupling constants is significantly reduced on protonation of compound (2). In particular, the temperature variation of the Leu_{β} protons is much reduced, this may be partially due to the reduction in the electron density of the imidazole ring causing it to exhibit a smaller shielding effect. The $His_{\alpha-\beta}$ coupling constant shows little or no variation with temperature, and corresponds to a population of the g^+ rotomer of 65-70%. The $Leu_{\alpha-\beta}$ coupling constants also shows only a small variation with temperature, which suggests that the population of the g^+ rotomer is approximately 30% over the whole temperature range. It should also

Table 3: Selected Variable Temperature NMR Results for Protonated Compound (2) Dissolved in DMSO-d_6.

Temp (°C)	Leu Amide	His Amide	His α-CH	Leu α-CH	His β-CH_2	Leu γ-CH	Leu β1-CH_2	Leu β2-CH_2	Leu δ1-CH_3	Leu δ2-CH_3
20	8.28ppm	8.09ppm	4.19ppm	3.7-3.8ppm	3.07ppm	1.5-1.8ppm	1.42ppm ddd	1.14ppm ddd	0.77ppm	
	s	s	t *J* 5.1	m	2H d *J* 5.5Hz	m	*J* 14.4,8.5,4.9	*J* 14.6,8.0,5.5	d *J* 6.6	
30	8.22ppm	8.03ppm	4.17ppm	3.7-3.8ppm	3.06ppm	1.5-1.8ppm	1.41ppm ddd	1.14ppm ddd	0.76ppm	
	s	s	t *J* 5.2	m	2H d *J* 5.5Hz	m	*J* 13.5,8.5,4.9	*J* 13.7,8.0,5.7	d *J* 6.6	
40	8.14ppm	7.97ppm	4.18ppm	3.74ppm	3.07ppm	1.5-1.8ppm	1.44ppm ddd	1.17ppm ddd	0.77ppm	
	s	s	t *J* 5.4	t *J* 7.4	2H d *J* 5.6Hz	m	*J* 13.7,8.1,5.1	*J* 13.4,7.6,5.7	d *J* 6.5	
50	8.12ppm	7.95ppm	4.20ppm	3.76ppm	3.09ppm	1.6-1.9ppm	1.46ppm ddd	1.19ppm ddd	0.79ppm	
	s	s	t *J* 5.3	t *J* 6.4	2H d *J* 5.6	m	*J* 13.4,8.1,5.1	*J* 13.6,7.6,6.0	d *J* 6.6	
60	8.06ppm	7.90ppm	4.21ppm	3.77ppm	3.10ppm	1.6-1.9ppm	1.48ppm ddd	1.22ppm ddd	0.80ppm	
	s	s	t *J* 5.5	t *J* 6.3	2H d *J* 5.6	m	*J* 13.3,8.1,5.2	*J* 13.6,7.5,6.1	d *J* 6.6	
70	8.00ppm	7.86ppm	4.21ppm	3.78ppm	3.11ppm	1.6-1.9ppm	1.50ppm ddd	1.24ppm ddd	0.82ppm	0.80ppm
	s	s	t *J* 5.5	dd *J* 7.3,5.2	2H d *J* 5.6	m	*J* 13.3,8.1,5.2	*J* 13.5,7.3,6.2	d *J* 6.6	d *J* 6.6
80	exch	exch	4.22ppm	3.78ppm	3.12ppm	1.6-1.9ppm	1.51ppm ddd	1.25ppm ddd	0.82ppm	0.81ppm
			t *J* 5.5	t *J* 8.3	2H d *J* 5.6	m	*J* 13.4,7.9,5.2	*J* 13.5,7.5,6.0	d *J* 6.6	d *J* 6.6
90	exch	exch	4.22ppm	3.7-3.8ppm	3.12ppm	1.6-1.9ppm	1.53ppm ddd	1.25ppm ddd	0.83ppm	0.82ppm
			td *J* 5.6,1.0	m	2H d *J* 5.5	m	*J* 13.4,7.8,5.2	*J* 13.6,7.4,6.1	d *J* 6.6	d *J* 6.6
100	exch	exch	4.22ppm	3.7-3.8ppm	3.14ppm	1.6-1.9ppm	1.54ppm ddd	1.29ppm ddd	0.83ppm	0.83ppm
			td *J* 5.6,1.0	m	2H d *J* 5.5	m	*J* 12.9,7.7,5.2	*J* 13.6,7.4,6.1	d *J* 6.5	d *J* 6.6

Spectra were recorded at 250MHz on Brucker AM250 spectrometer, using a concentration of 5mg of protonated compound (2) in 0.5ml of solvent. The assignment of diastereotopic β and δ protons as 1 and 2 is arbitary, with the lower field proton being assigned as 1. Coupling constants are in Hz, and have not been corrected for second order effects.

Table 4: Selected Variable Temperature NMR Results for Protonated Compound (2) Dissolved in CD_3OD.

Temp (°C)	His α-CH	Leu α-CH	His β1-CH_2	His β2-CH_2	Leu γ-CH	Leu β1-CH_2	Leu β2-CH_2	Leu δ1-CH_3	Leu δ2-CH_3
0	4.56ppm	4.1-4.2ppm	3.54ppm	3.38ppm	1.9-2.1ppm	1.7-1.9ppm	1.6-1.7ppm	1.05ppm	1.04ppm
	t *J* 4.8	m	dd *J* 15.1,5.4	dd *J* 15.1,4.5	m	m	m	d *J* 6.4	d *J* 6.4
-10	4.56ppm	4.12ppm	3.54ppm	3.42ppm	1.9-2.1ppm	1.7-1.9ppm	1.6-1.7ppm	1.05ppm	1.03ppm
	t *J* 4.9	dd *J* 8.6,5.3	dd *J* 15.1,5.4	dd *J* 15.1,4.6	m	m	m	d *J* 6.4	d *J* 6.4
-20	4.56ppm	4.12ppm	3.54ppm	3.39ppm	1.9-2.1ppm	1.8-1.9ppm	1.6-1.8ppm	1.05ppm	1.03ppm
	t *J* 4.7	dd *J* 8.7,5.3	dd *J* 15.1,5.4	dd *J* 15.1,4.6	m	m	m	d *J* 6.2	d *J* 5.1
-30	4.55ppm	4.1-4.2ppm	3.54ppm	3.39ppm	1.8-2.0ppm	1.7-1.9ppm	1.6-1.8ppm	1.05ppm	1.03ppm
	t *J* 5.0	br	dd *J* 15.1,5.4	dd *J* 15.0,4.5	br	br	m	d *J* 5.8	d *J* 5.8
-40	4.56ppm	4.1-4.2ppm	3.54ppm	3.39ppm	1.8-2.1ppm	1.7-1.9ppm	1.6-1.8ppm	1.05ppm	1.03ppm
	br t	br	hidden	dd *J* 15.1,4.6	br	br	m	d *J* 6.0	d *J* 6.0
-50	4.56ppm	4.1-4.2ppm	3.53ppm	3.3-3.4ppm	1.9-2.1ppm	1.8-2.0ppm	1.6-1.8ppm	1.05ppm	1.03ppm
	br t	br	hidden	br	br	br	br	d *J* 6.0	d *J* 5.9
-60	4.56ppm	4.1-4.2ppm	3.53ppm	3.3-3.4ppm	1.9-2.1ppm	1.7-1.9ppm	1.6-1.8ppm	1.0-1.1ppm	
	br t	br	hidden	br	br	br	br	br	

Spectra were recorded at 250MHz on Brucker AM250 spectrometer, using a concentration of 5mg of protonated compound (2) in 0.5ml of solvent. The assignment of diastereotopic β and δ protons as 1 and 2 is arbitary, with the lower field proton being assigned as 1. Coupling constants are in Hz, and have not been corrected for second order effects. Hidden indicates that the peak was partially obscured by the solvent resonance.

be noted that over the entire temperature range, the His_{β} protons occur as a single resonance, in contrast to the situation observed in Tables 1, 2, and 4. The same lack of temperature dependence of the chemical shifts and coupling constants of protonated catalyst (2) is seen by comparing the data in Table 2 with that in Table 4. Thus the chemical shifts of the Leu_{β} protons show no variation with temperature, nor do the $His_{\alpha-\beta}$ coupling constants vary, and they suggest a population of the His g^+ rotomer of 70%.

Molecular Modelling Results

In order to explain the variable temperature nmr results obtained for protonated and unprotonated forms of catalyst (2), a molecular mechanics study of the catalyst was carried out using the methods reported previously for catalyst (1)[11,12]. Thus a randomly drawn structure corresponding to compound (2) was first energy minimised until the energy gradient was <0.1KJmol^{-1} using the TNCG minimiser and the AMBER forcefield as implemented within the Macromodel-3D[21] program. The resulting minimum energy structure was then used as the starting structure for a accessible conformational space search. Thus each of the rotatable bonds was rotated in 60° increments, and the conformation of the diketopiperazine ring was varied by assigning one of the bonds as a ring closure bond, and rotating the remaining bonds in 60° increments. The Macromodel program automatically discards those structures where rotation of the bonds within the diketopiperazine would result in an unreasonable bond length for the ring closure bond (<100pm or >200pm). Each of the remaining structures was energy minimised as described above, duplicate structures being automatically discarded to produce a set of minimum energy conformations. All structures within 50KJmol^{-1} of the global minimum energy conformation were retained for further examination.

For the unprotonated catalyst, the calculations were carried out *in vacuo*, and in simulated chloroform and water solvents (the only environments supported by the Macromodel program). However, examination of the 50 lowest energy conformers in each case showed that only the calculations carried out in water were able to reproduce the populations determined by nmr. In particular, the calculations carried out *in vacuo*, or in chloroform severely underestimated the population of the His g^+ rotomer. Details of each conformer of compound (2) with a predicted population of >2% at 20°C in water are given in Table 5. For the His residue, the populations of the g^+, t, and g^- rotomers (calculated at 20°C in water) were predicted to be 78, 16, and 6% respectively (with the five lowest energy conformers being in the g^+ rotomer), whilst for the Leu residue, the same populations were calculated as 14, 14, and 72% (with the two lowest energy conformers containing the g^- rotomer). The conformer populations given above differ slightly from those in Table 5, as they are based on an analysis of all conformers within 50KJmol^{-1} of the global minimum. The same calculations were carried out on the protonated catalyst, and again the results in a simulated water solvent most closely match the experimentally observed results. These results are shown in Table 6 for each conformer with a predicted population of >2% at 20°C. Thus, for the His residue the populations of the g^+, t, and g^- rotomers (calculated at 20°C) were predicted to be 62, 30, and 8% respectively (with the global minimum being in the g^+ rotomer), whilst for the Leu residue, the same populations were calculated as 12, 21, and 67% (with the global minimum containing the g^- rotomer). For both unprotonated and protonated forms of compound (2), the molecular mechanics calculations predict a predominantly flat diketopiperazine ring.

Table 5: Molecular Mechanics Results on Compound (2) Calculated in Water.

Conformer Energy (KJ/Mol)	Population (%)[1]	Histidine Conformation	Leucine Conformation	Diketopiperazine Conformation
-126.7	23.3	g^+	g^-	Flat
-126.3	19.8	g^+	g^-	Flat
-123.7	6.9	g^+	t	Flat
-123.5	6.4	g^+	g^-	Boat
-123.3	5.9	g^+	t	Flat
-123.3	5.9	t	g^-	Flat
-123.2	5.7	t	g^-	Flat
-122.9	5.0	g^+	g^+	Boat
-122.8	4.8	t	g^+	Flat
-122.4	4.1	g^+	g^+	Boat
-121.6	3.0	g^-	g^-	Flat
-121.2	2.5	g^-	g^-	Flat
-121.0	2.3	g^+	g^-	Flat
-120.9	2.2	g^+	g^-	Flat
-120.6	2.0	$g^\pm$	g^-	Flat

1) Populations are calculated at 298.15K

Conformational Conclusions

In DMSO and methanol solutions, molecular mechanics calculations and variable temperature nmr studies suggest that the global minimum energy conformation of compound (2) is represented by the structure shown in Figure 2, in which the imidazole ring is folded over the diketopiperazine and is shielding the Leu β-protons. However, both the His and Leu residues are rotating, and hence other conformers have appreciable populations. As would be expected for this model, the observed coupling constants indicate that the population of the g^+ rotomer increases as the temperature decreases due to the other higher energy conformations becoming less populated. Both the nmr and molecular mechanics calculations are consistent with this conclusion, however the nmr data are also consistent with an alternative situation in which the global minimum energy conformer would be any structure other than that shown in Figure 2, and a number of conformers of the type shown in Figure 2 are only slightly higher in energy. This would result in the His g^+ conformers being the most highly populated at all but the very lowest energies, despite the global minimum energy conformation being of a different form. However, no evidence for this was found from the molecular mechanics calculations[22].

Table 6: Molecular Mechanics Results on Protonated Compound (2) Calculated in Water.

Conformer Energy (KJ/Mol)	Population (%)[1]	Histidine Conformation	Leucine Conformation	Diketopiperazine Conformation
-247.9	28.8	g^+	g^-	Flat
-245.9	12.9	t	t	Flat
-245.4	10.5	t	g^-	Flat
-245.0	8.9	g^+	g^+	Boat
-244.7	7.9	g^+	g^-	Flat
-244.5	7.3	g^+	g^-	Boat
-242.5	3.3	g^-	t	Flat
-242.4	3.1	g^+	g^+	Boat
-242.4	3.1	g^-	g^-	Flat
-242.0	2.7	g^+	g^-	Boat
-241.9	2.6	g^+	t	Flat
-241.8	2.5	t	g^-	Flat
-241.7	2.4	t	t	Flat
-241.5	2.2	g^-	g^-	Flat
-241.2	1.9	t	g^-	Flat

1) Populations are calculated at 298.15K

The molecular modelling results do not explain why protonation of the imidazole ring results in less shielding of the Leu β-protons, since the population of the His g^+ rotomer is predicted not to undergo a major change on protonation (78 to 65%), nor do they explain why the ^1H nmr spectrum of protonated catalyst (2) is far less temperature dependent than the unprotonated spectrum. A change in the conformation of the diketopiperazine ring would account for both the decrease in the shielding of the Leu β-protons, and the lack of temperature dependence of the nmr spectrum, as in a boat shaped conformation, even when the His residue occupies the g^+ rotomer, the imidazole ring does not appreciably shield the Leu β-protons. However, neither the molecular mechanics calculations, nor the nmr data indicate this to be the case. The two calculated $His_{\alpha-\beta}$ coupling constants for the flat and boat shaped conformations of catalyst (2) are 3.0 and 3.5Hz in both cases, so these cannot be used to determine the conformation of the diketopiperazine ring. However, at high temperatures in DMSO the protonated catalyst shows a ^5J coupling of 1.0Hz between the two α-protons, this coupling is not seen at lower temperatures due to the decrease in resolution as the temperature decreases. It has previously been shown[23], that the magnitude of this ^5J coupling is dependent upon the conformation of the diketopiperazine ring. In DMSO, a 1.0Hz coupling constant corresponds to a flat diketopiperazine ring. Conformations with boat shaped diketopiperazine rings would have ^5J values of between 1.0 and 1.8Hz depending upon the amount by which the ring is bent away from planarity, and assuming that the boat was

formed such that the side chains were in the least hindered positions. As a number of rapidly interconverting conformations are present in solution, the observed coupling constant will be a weighted average of the value for each of these. However, the observed value of 1.0Hz is clearly inconsistent with a major population of boat shaped conformers. Thus it appears that the changes in the nmr spectra observed upon protonating compound (2) are due to rotation about the His β-γ bond as previously suggested to occur in D_2O[16]. This rotation places the imidazole ring away from the Leu β- and δ-protons, and thus accounts for the lack of shielding of the Leu protons.

Hence it appears that the conformation adopted by catalyst (2) is independent of solvent, as the same conformation is observed in DMSO, CD_3OD, and D_2O. Only protonation of the imidazole ring appears to change the conformation. Thus it is likely that the conformation determined here will not be affected by the presence of HCN (We have previously shown that the interaction of HCN with catalyst (1) is not ionic[12]) or an aldehyde during an asymmetric cyanohydrin forming reaction. The conformation of catalyst (2) shown in Figure 1b may therefore be the catalytically active conformation, although it is clear from both the molecular modelling and nmr results, that other low energy conformers exist, and have significant populations.

Comparison with Catalyst (1) and Possible Relevance to Asymmetric Cyanohydrin Formation

Catalyst (2) catalyses the the addition of HCN to aldehydes, giving the (S)-enantiomer of the cyanohydrin. By contrast, catalyst (1) catalyses the same reaction but gives the (R)-enantiomer of the cyanohydrin. The conformation of catalyst (1) has previously been determined[11,12], and found to adopt a conformation in which the phenyl ring folds over the diketopiperazine[24] as shown in Figure 1a. This conformation of catalyst (1) results in the shielding of the top face of the imidazole ring by the phenyl ring, whilst in catalyst (2), it is the bottom face of the imidazole ring that is shielded by the diketopiperazine ring. As it would seem likely that the imidazole ring is involved in the catalysis (by reacting either with the aldehyde[13], or with the HCN[14]), this opposite accessability of the two faces may be relevent to the opposite enantiomer of the cyanohydrin produced by the two catalysts, although the mechanism of this reaction has not yet been determined. It is however possible that catalysis occurs through a conformation other than the ground state structures, as both catalysts (1) and (2) have been shown to interconvert between a range of low energy conformations. Further work on the asymmetric synthesis of cyanohydrins catalysed by synthetic peptides, and on the mechanisms of these reactions is underway and will be reported in due course.

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References

1) Jackson, W.R., Jacobs, H.A., Jayatilake, G.S., Matthews, B.R., Watson, K.G.; *Aust. J. Chem.*, **1990**, 43, 2043; Becker, W., Freund, H., Pfeil, E., *Angew. Chem., Int. Ed. Engl.*, **1965**, 4, 1079; Brussee, J., Roos, E.C., v.d.Gen, A.; *Tetrahedron Lett.*, **1988**, 29, 4485; Ziegler, T., Horsch, B., Effenberger, F.; *Synthesis*, **1990**, 575; Matthews, B.R., Gountzos, H., Jackson, W.R., Watson, K.G.; *Tetrahedron Lett.*, **1989**, 30, 5157; Jackson, W.R., Jacobs, H.A., Matthews, B.R., Jayatilake, G.S., Watson, K.G.; *Tetrahedron Lett.*,**1990**, 31, 1447.

2) March, J., 'Advanced Organic Chemistry: Reactions, Mechanisms, and Structure 2nd Ed.', McGraw Hill, London, **1977**, p873.

3) Choi, V.M.F., Elliott, J.D., Johnson, W.S.; *J. Org. Chem.*, **1983**, 48, 2294; Choi, V.M.F., Elliott, J.D., Johnson, W.S.; *Tetrahedron Lett.*, **1984**, 25, 591; Reetz, M.T., Kessler, K., Jung, A.; *Angew. Chem., Int. Ed. Engl.*, **1985**, 24, 989; Wang, Y-F., Chen, S-T., Liu, K. K-C., Wong, C-H.; *Tetrahedron Lett.*, **1989**, 30, 1917; Effenberger, F., Gutterer, B., Ziegler, T., Eckhardt, E., Aichholz, R.; *Liebigs Ann. Chem.*, **1991**, 47; Inagaki, M., Hatanaka, A., Mimura, M., Hiratake, J., Nishioka, T., Oda, J.; *Bull. Chem. Soc. Jpn.*, **1992**, 65, 111; Inagaki, M., Hiratake, J., Nishioka, T., Oda, J.; *J. Org. Chem.*, **1992**, 57, 5643; Garcia-Ruano, J.L., Martin-Castro, A.M., Rodriguez, J.H.; *J. Org. Chem.*, **1992**, 57, 7235.

4) For a recent review of this area see North, M.; *Synlett*, **1993**, 807.

5) Ziegler, T., Horsch, B., Effenberger, F.; *Synthesis*, **1990**, 575; Becker, W., Freund, H., Pfeil, E., *Angew. Chem., Int. Ed. Engl.*, **1965**, 4, 1079; Effenberger, F., Ziegler, T., Forster, S.; *Angew. Chem., Int. Ed. Engl.*, **1987**, 26, 458; Effenberger, F., Horsch, B., Weingart, F., Ziegler, T., Kuhner, S.; *Tetrahedron Lett.*, **1991**, 32, 2605; Zandbergen, P., Van der Linden, J., Brussee, J., Van der Gen, A.; *Synthetic Commun.*, **1991**, 21, 1387; Ognyanov, V.I., Datcheva, V.K., Kyler, K.S.; *J. Am. Chem. Soc.*, **1991**, 113, 6992; Huuhtanen, T.T., Kanerva, L.T.; *Tetrahedron Asymm.*, **1992**, 3, 1223.

6) Tsuboyama, S.; *Bull. Chem. Soc. Jpn.*, **1962**, 35, 1004; Tsuboyama, S.; *Bull. Chem. Soc. Jpn.*, **1965**, 38, 354; Tsuboyama, S.; *Bull. Chem. Soc. Jpn.*, **1966**, 39, 698; Danda, H., Chino, K., Wake, S.; *Chem. Lett.*, **1991**, 731.

7) Narasaka, K., Yamada, T., Minanikawa, H.; *Chem. Lett.*, **1987**, 2073; Minanikawa, H., Hayakawa, S., Yamada, T., Iwasawa, N., Narasaka, K.; *Bull. Chem. Soc. Jpn.*, **1988**, 61, 4379; Hayashi, M., Matsuda, T., Oguni, N.; *J. Chem. Soc., Chem. Commun.*, **1990**, 1364; Hayashi, M., Matsuda, T., Oguni, N.; *J. Chem. Soc., Perkin Trans. 1.*, **1992**, 3135; Hayashi, M., Miyamoto, Y., Inoue, T., Oguni, N.; *J. Chem. Soc., Chem. Commun.*, **1991**, 1752; Mori, A., Nitta, H., Kudo, M., Inoue, S.; *Tetrahedron Letts.*, **1991**, 32, 4333; Abe, H., Nitta, H., Mori, A., Inoue, S.; *Chem. Lett.*, **1992**, 2443; Nitta, H., Yu, D., Kudo, M., Mori, A., Inoue, S.; *J. Am. Chem. Soc.*, **1992**, 114, 7969; Mori, A., Ohno, H., Nitta, H., Tanaka, K., Inoue, S.; *Synlett*, **1991**, 563; Ohno, H., Nitta, H., Tanaka, K., Mori, A., Inoue, S.; *J. Org. Chem.*, **1992**, 57, 6778; Kobayashi, S., Tsuchiya, Y., Mukaiyama, T.; *Chem. Lett.*, **1991**, 541.

8) Oku, J., Ito, N., Inoue, S.; *Makromol. Chem.*, **1979**, 180, 1089.

9) Oku, J., Inoue, S.; *J. Chem. Soc., Chem. Commun.*, **1981**, 229; Oku, J., Ito, N., Inoue, S.; *Makromol. Chem.*, **1982**, 183, 579; Matthews, B.R., Jackson, W.R., Jayatilake, G.S., Wilshire, C., Jacobs, H.A.;

Aust. J. Chem., **1988**, 41, 1697; Kobayashi, Y., Asada, S., Watanabe, I., Hayashi, H., Motoo, Y., Inoue, S.; *Bull. Chem. Soc. Jpn.*, **1986**, 59, 893; Asada, S., Kobayashi, Y., Inoue, S.; *Makromol. Chem.*, **1985**, 186, 1755; Kobayashi, Y., Hayashi, H., Kitaui, K., Inoue, S.; *Chem. Lett.*, **1986**, 931.

10) Mori, A., Ikeda, Y., Kinoshita, K., Inoue, S.; *Chem. Lett.*, **1989**, 2119.

11) North, M.; *Tetrahedron*, **1992**, 48, 5509.

12) Hogg, D.J.P., North, M.; *Tetrahedron*, **1993**, 49, 1079.

13) Hogg, D.J.P., North, M., Stokoe, R.B., Teasdale, W.G.; *Tetrahedron Asymm.*, **1993**, 4, 1553.

14) Callant, D.; Coussens, B., Van der Maten, T., deVries, J.G., deVries, N.K.; *Tetrahedron Asymm.*, **1992**, 3, 401; Tanaka, K., Mori, A., Inoue, S.; *J. Org. Chem.*, **1990**, 55, 181; Jackson, W.R., Jayatilake, G.S., Matthews, B.R., Wilshire, C.; *Aust. J. Chem.*, **1988**, 41, 203.

15) Tanihara, M., Imanishi, Y., Higashimura, T.; *Biopolymers*, **1977**, 16, 2217.

16) Arena, G., Impellizzeri, G., Maccarrone, G., Pappalardo, G., Sciotto, D., Rizzarelli, E., *J. Chem. Soc., Perkin Trans. 2.*, **1992**, 371.

17) Sheinblatt, M., Andorn M., Rudi, A.; *Int. J. Pept. Prot. Res.*, **1988**, 31, 373.

18) Sheinblatt, M.; *Int. J. Pept. Protein Res.*, **1991**, 38, 8; Kojima, Y., Yamashita, T., Nishide, S., Hirotsu, K., Higuchi, T.; *Bull. Chem. Soc. Jpn.*, **1985**, 58, 409; Sheinblatt, M.; *J. Chem. Soc., Perkin Trans. II*, **1990**, 127.

19) Kopple, K.D., Marr, D.H.; *J. Am. Chem. Soc.*, **1967**, 89, 6193; Kopple, K.D., Ohnishi, M.; *J. Am. Chem. Soc.*, **1969**, 91, 962; Ziauddin, Kopple, K.D.; *J. Org. Chem.*, **1970**, 35, 253; Lin, C.F., Webb, L.E.; *J. Am. Chem. Soc.*, **1973**, 95, 6803; Arena, G., Impellizzeri, G., Maccarrone, G., Pappalardo, G., Sciotto, D., Rizzarelli, E.; *Thermochimica Acta*, **1989**, 154, 97.

20) The protonated catalyst was obtained by dissolving compound (2) in excess trifluoroacetic acid at room temperature. After ten minutes, the excess trifluoroacetic acid was evaporated *in vacuo* at room temperature, and the residual protonated compound (2) was thoroughly washed with ether to remove the last traces of trifluoroacetic acid. The product was further dried *in vacuo* at room temperature, before being dissolved in the appropriate deuterated solvent for the nmr work.

21) Still, W.C., Mohmadi, F., Richards, N.G.J., Guida, W.C., Lipton, M., Liskamp, R., Chang, G., Hendrickson, T., DeGunst, F., Hasel, W.; Macromodel 3D., Version 3.1, Columbia Univesrsity, New York, NY, 1991.

22) The authors thank the referee of this manuscript for bringing this possibility to our attention.

23) Davies, D.B., Khaled, Md.A.; *J. Chem. Soc., Perkin Trans. II*, **1976**, 1238.

24) Our previous report[11] of the detection of a minor conformation of compound (1) would appear to be incorrect, as we have now obtained nmr spectra of this catalyst which do not show peaks corresponding to this conformer.

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