Enantioselective Recognition of Histidine and Lysine Esters by Porphyrin Chiral Clefts and Detection of Amino Acid Conformations in the Bound State

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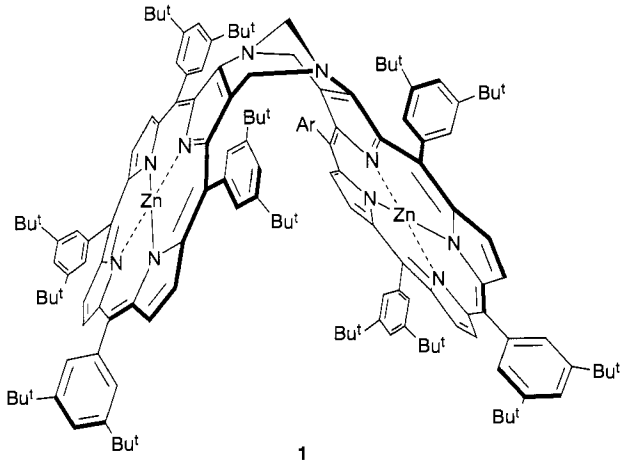
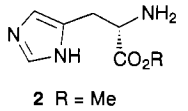
Resolution of the bisporphyrin Tröger's base analogue **1** affords homochiral clefts that tightly bind histidine esters in 80–86% e.e. and lysine benzyl ester in 48% e.e.; the histidine esters are bound in fixed conformations that can be readily detected by ^{1}H NMR spectroscopy as a result of the large dispersion of proton resonances by the ring currents of the two porphyrins.

There is considerable interest in receptors that will enantioselectively bind α-amino acids and related compounds. We have recently reported the synthesis and α,ω-diaminoalkane binding of novel bismetalloporphyrin analogues of Tröger's base, compounds of C_2 symmetry that contain a relatively rigid, well-defined, chiral molecular cleft with convergent metal ion binding sites.[1] The metal–metal separation within the helically twisted, concave cavity is 8.4–9.0 Å, by extrapolation from the structural details obtained from the X-ray crystal structure of a dipalladium(II) derivative.[1] The molecules possess the basis for at least three-point asymmetric interaction with a guest molecule and thus have the potential for enantioselective recognition. We now report the resolution of the dizinc(II) bisporphyrin Tröger's base analogue **1** and preliminary studies which establish that histidine esters **2** and **3** are bound with very good enantioselectivity, and the more conformationally flexible lysine benzyl ester **4** with moderate enantioselectivity. We have also shown that we can analyse the exact conformers of bound histidine esters which will enable an in-depth analysis of the binding interactions and the basis for the observed enantioselectivity. The chiral discrimination in the binding of histidine methyl and benzyl esters results from restrictions imposed by the chiral cavity on the conformations that the amino acid derivatives can adopt.

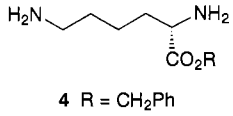
Resolution of racemic **1** was affected on a chiral HPLC column.† The (−)-enantiomer {$[\alpha]_D^{20}$ − 2000 (*c* 1.2 × 10^{-2}, $CHCl_3$)} eluted first and was obtained in 100% e.e. and the (+)-enantiomer {$[\alpha]_D^{20}$ + 2000 (*c* 1.2 × 10^{-2}, $CHCl_3$)} was purified to at least 98% e.e. The extremely high optical rotation of these enantiomers is consistent with their helicity.[2] The resolved enantiomers, (+)-**1** and (−)-**1**, show mirror-image circular dichroism spectra and a split Cotton effect in the Soret region of the electronic spectrum (Fig. 1) and very high molar ellipticities ($[\theta]_{425}$ = 2.7 × 10^6 deg cm^2 dmol^{-1}) are observed. This splitting is due to the chiral exciton coupling of the two identical chromophores and can be used to assign the absolute stereochemistry of the separated enantiomers of **1**; (−)-**1** shows a negative first Cotton effect and therefore the two porphyrin rings constitute a left-handed screwness in this enantiomer.[3]

The rigidity, chirality and asymmetric magnetic field generated by the very large ring current effects of the porphyrin rings of **1** provide a binding site with very large dispersion of the resonances of protons within the cavity. As a consequence,





2 R = Me
3 R = CH_2Ph



4 R = CH_2Ph

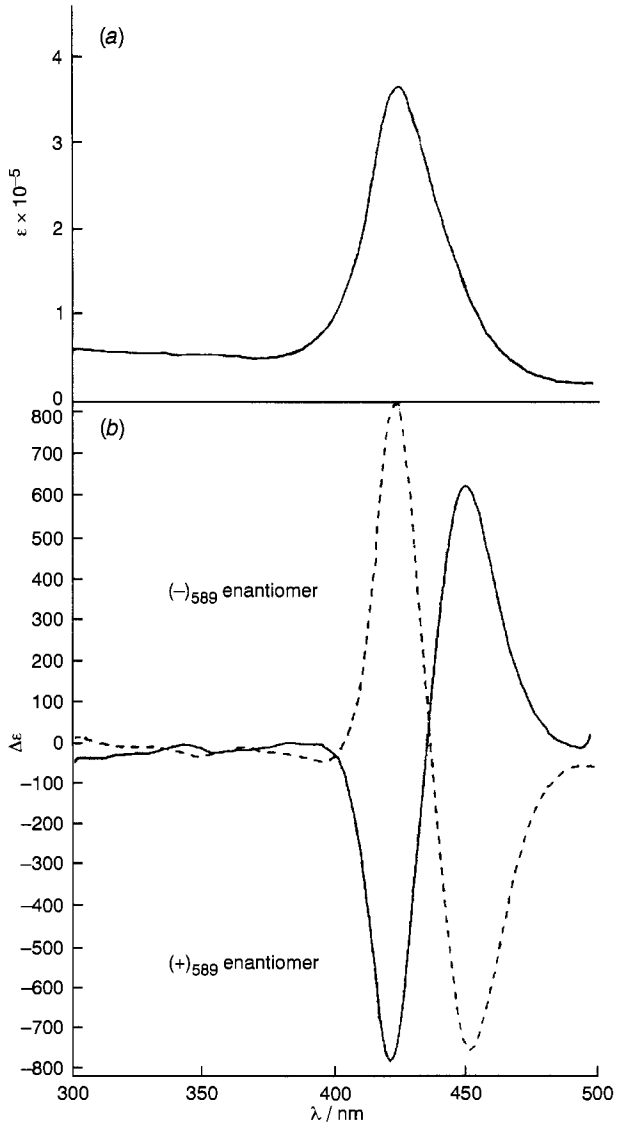


Fig. 1 (*a*) The Soret band of (+)$_{589}$-bisporphyrin **1** in the UV spectrum (1.9 × 10^{-6} mol dm^{-3} in chloroform at room temp.). (*b*) CD spectra of the enantiomers of bisporphyrin **1** (1.9 × 10^{-6} mol dm^{-3} in chloroform at room temp.).

resonances of diastereotopic geminally coupled protons are so well dispersed that ^{1}H NMR spectra of tightly bound ligands can be analysed by first-order methods. This has facilitated the study of the enantioselective recognition properties of the dizinc(II) host **1** with amino acid derivatives which can exploit the two metal binding sites in the cavity.

The binding of L-histidine methyl ester **2** to (±)-**1** was initially monitored by ^{1}H NMR. When 0.4 equiv. of amino acid ester were employed [Fig. 2(*a*)] the NH_2, α-CH and β-CH_2 proton resonances of the guest now lie between δ −0.1 and −5.3; such large upfield shifts of the resonances of all these protons is only consistent with the amino acid ester being bound within the bisporphyrin cavity.[4] The system is clearly in slow exchange, indicating strong binding within the cavity. In Fig. 2(*a*) both diastereoisomeric complexes are observed although there is a strong preference for binding to (+)-**1**. The signals from the minor diastereoisomer are marked with an asterisk and when further ligand is added they increase in intensity. This interpretation was confirmed by repeating the ^{1}H NMR experiments with 0.4 equiv. of guest **2** and either 1 equiv. of resolved (+)-**1** [Fig. 2(*b*)], or 1 equiv. of (−)-**1** [Fig. 2(*c*)] which allowed full assignment of the spectra. The ratio of the two sets of signals in Fig. 2(*a*) corresponds to the enantioselectivity observed with this ligand; the 93:7 ratio of the two diastereoisomeric complexes corresponds to an enantiomeric excess of 86%. This is an impressive enantioselectivity corresponding to a difference in binding free energies for the two diastereoisomeric complexes of $\Delta\Delta G = -6.4$ kJ mol^{-1}, which is higher than any previously reported for porphyrin-based hosts binding neutral amino acid derivatives.[5]

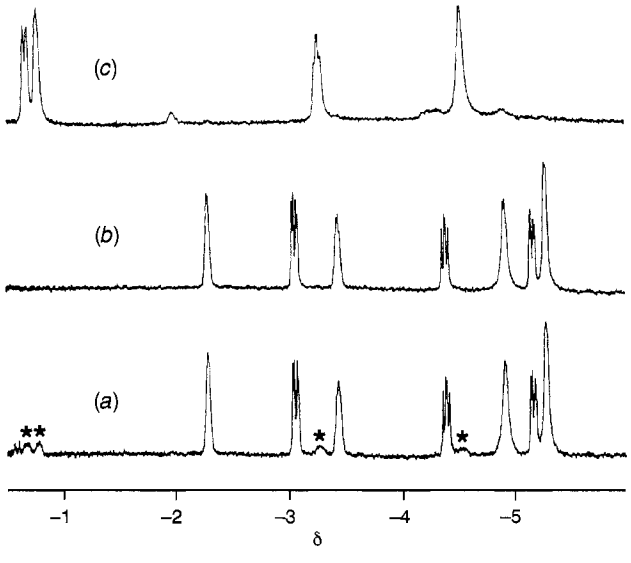


Fig. 2 ^{1}H NMR (400 MHz, $CDCl_3$) spectra showing the upfield bound ligand resonances from 0.4 equiv. of **2** and: (*a*) 1 equiv. of (±)-**1**; (*b*) 1 equiv. of resolved (+)-**1**; and (*c*) 1 equiv. of resolved (−)-**1**

The ^{1}H NMR spectrum of the (+)-**1**·**2** complex [Fig. 2(*b*)] reveals that L-histidine methyl ester binds to this host in two distinct conformations in the ratio 55:45 (corresponding to $\Delta\Delta G = -0.50$ kJ mol^{-1}). There are two distinct sets of bound signals for the ligand and the magnitude of the two $^3J_{\alpha\beta}$ coupling constants for each of these sets suggests that the two conformations are rotamers about the side chain angle χ_1 (the torsion angle around the α-CH and β-CH_2); (Table 1). The $^3J_{\alpha\beta}$ values of 2 and 5 Hz [for the pro-(*S*) ($H_{\beta S}$) and pro-(*R*) ($H_{\beta R}$) protons] for the major conformer suggest a *gauche–gauche* (*gg*) arrangement of the α-CH and β-CH_2 protons whereas the values of 10 and 5 Hz for the other set of signals implies either a *gauche–trans* (*gt*) or a *trans–gauche* (*tg*) conformation. Although we cannot distinguish between the *gt* and *tg* conformers on the basis of coupling constant evidence alone, consideration of the N···N distances between the NH_2 and Im(N_ε) suggests that the *tg* conformer would be bound preferentially. The N···N distance is 4.9 Å in this conformer (*cf.* 6.1 Å for the *gt* conformer) which is virtually identical to the *gg* conformer distance of 4.8 Å. These values are appropriate for the 8.4–9.0 Å separation of metal centres, allowing for a Zn–N distance of 2.0 Å.[6] This host can thus distinguish between the different conformers of the guest and the two of most suitable size are 'frozen out' in the bound complexes.

In the ^{1}H NMR spectrum of the (−)-**1**·**2** complex, one conformer predominates as shown in Fig. 2(*c*), but because of the broadness of the signals it is difficult to assign the exact conformation of this bound form, although it appears to be a *gt* or *tg* form.‡ The assignments for the bound upfield ligand resonances with each of the two enantiomers of **1** were determined from decoupling experiments and are given in Table 2.

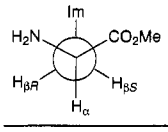
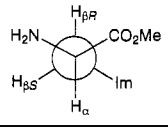
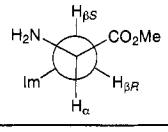
In order to quantify the thermodynamics involved in the enantioselection, binding constants were measured for com-

Table 2 Assignments for the upfield bound ligand resonances for **2** binding to **1** (the furthest downfield β-CH_2 signal is not shown in Fig. 2 as it is next to the Me_4Si signal)

	Ligand resonance in **1**·**2** (+)-**1**		(−)-**1**
	gg	*tg*	Major conformer
NH_2	−5.29	−4.92	−4.54
H_α	−2.28	−3.44	−0.68
β-CH_RH_S	−3.06[a]	−5.15[c]	−3.27
β-CH_RH_S	−0.14[b]	−4.39[d]	−0.78

[a] The assignments of the pro-(*R*) and pro-(*S*) β-protons were made on the basis of examination of molecular models of the host–guest complex and from coupling constants, they may be reversed in the case of the *gg* conformer. The observed couplings were: dd, *J* 14, *J* 5 Hz. [b] dd, *J* 14, *J* 2 Hz. [c] dd, *J* 14, *J* 5 Hz. [d] dd, *J* 14, *J* 10 Hz.

Table 1 Expected conformations of **2** around the torsion angle between the α-CH to β-CH_2 groups

	L-Histidine methyl ester		
			
Conformation	*gg*	*gt*	*tg*
χ_1/°	60	180	−60
Expected $^3J_{\alpha S}$/Hz	<5	<5	>10
Expected $^3J_{\alpha R}$/Hz	<5	>10	<5
Observed $^3J_{\alpha\beta}$/Hz	2, 5	not observed	10, 5
NH_2–Im(N_ε) distance/Å	4.8	6.1	4.9

plexation of L-histidine benzyl ester **3** and L-lysine benzyl ester **4** with each of the enantiomers of **1**. The binding constants obtained from spectrophotometric titrations§ of low concentrations of host **1** in toluene¶ with these two ligands are given in Table 3.

For **3**, both enantiomers of the host have high affinities but the (+)-enantiomer binds more tightly by a factor of 9.2-fold (corresponding to an 80% e.e.; $\Delta\Delta G = -5.4$ kJ mol^{-1}). The magnitude of the binding constant for complexation of **3** to (+)-**1** is very similar to that for complexation of 1,4-diaminobutane to **1** (1.6×10^8 dm^3 mol^{-1}).[1] This tight binding suggests a ditopic interaction of the two basic nitrogen sites on the guest with the two zinc centres; further steric interactions with the walls of the helical cavity leads to the enantioselective recognition.

L-Lysine benzyl ester **4** is bound less tightly to (+)-**1** and (−)-**1** than the histidine benzyl ester (Table 3). The difference in binding free energy is also smaller (−2.5 kJ mol^{-1}) and corresponds to 48% e.e. The two nitrogen binding sites present in this ligand are linked by a more flexible chain than is the case in the histidine derivatives, and thus the enantioselective recognition of a ligand of this type is more difficult. The magnitude of the binding of **4** to both (+)-**1** and (−)-**1** is weaker than the binding of 1,5-diaminopentane to **1** (6.1×10^7 dm^3 mol^{-1}),[1] indicating that the ester group of **4** interacts sterically with the cavity walls.

The binding of **3** in [$^2H_8$]toluene was also examined by ^{1}H NMR to allow a direct comparison with the spectrophotometric results. This larger ligand behaved similarly to the methyl ester **2**; however, the ratio of bound *gg* and *tg* forms was significantly different (Fig. 3). The *tg* form was now clearly favoured by 81 : 19 (*cf.* 45 : 55 for **2** in $CDCl_3$). Nevertheless, the ratio of the diastereoisomeric complexes [*i.e.* (+)-**1**·**3** and (−)-**1**·**3**; the minor diastereoisomer is marked with an asterisk] is *ca* 9 : 1 (80% e.e.), in agreement with the spectrophotometric binding constant determinations, and only slightly changed from that seen with the methyl ester. The influence of the ester group and solvent on the conformations adopted by ligands is under further investigation.

Table 3 Binding constants for the resolved enantiomers of bisporphyrin **1**[a]

	K/dm^3 mol^{-1}		K[(+)-**1**]	
Ligand	(−)-**1**	(+)-**1**	K[(−)-**1**]	$\Delta\Delta G$/kJ mol^{-1}
3	1.2×10^7	1.1×10^8	9.2	−5.4
4	8.5×10^6	2.4×10^7	2.8	−2.5

[a] Measured in toluene at 20 °C, errors estimated at ±10%.

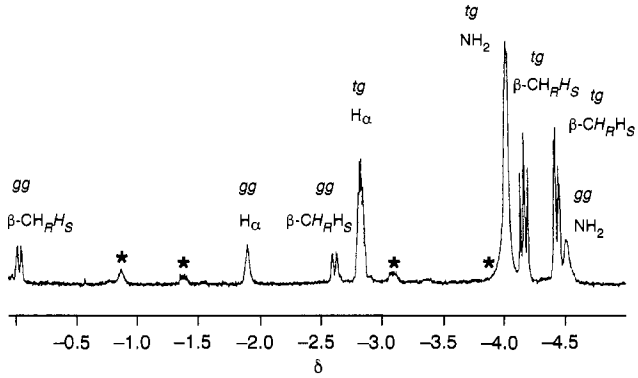


Fig. 3 ^{1}H NMR (400 MHz, [$^2H_8$]toluene) spectrum showing the upfield bound ligand resonances from 0.4 equiv. of **3** and 1 equiv. of (±)-**1**

These enantioselective recognition studies are at present being extended to examine amino acids with different functionalities and this is being combined with the preparation of mixed metal host systems where the different sites in the bisporphyrin will selectively recognise different functionalities in the guests. Synthesis of bisporphyrin Tröger's base analogues with additional peripheral functionality at the β-pyrrolic positions to further define the binding cavity is also underway. Use of these clefts in asymmetric catalysis of organic reactions should also be possible.

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Footnotes

† An HPLC column packed with a chiral stationary phase of silica gel covalently bound to (*R*)-*N*-3,5-dinitrobenzoylphenylglycine (Analytical Jones APEX Chiral AP Column) was used with 0.5% propan-2-ol in light petroleum as the eluent.

‡ Planned low-temperature ^{1}H NMR spectroscopy experiments should be informative.

§ Spectrophotometric titrations were carried out in toluene at 20.0 ± 0.2 °C under an argon atmosphere and were analysed using a Simplex least-squares curve-fitting procedure.[7]

¶ Toluene was chosen as the solvent as chloroform suffers from significant acid build-up over time which would interfere with our very low concentration host and ligand solutions.

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