Hydrogen-bond recognition of cyclic dipeptides in water

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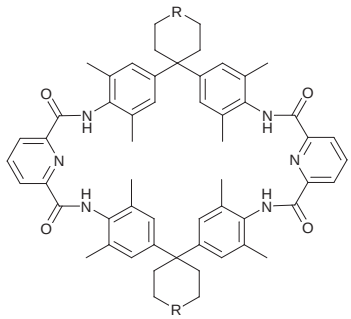
An amide macrocycle with a highly preorganised cavity containing both polar and non-polar recognition sites forms stable complexes with cyclic dipeptides in water *via* amide–amide hydrogen-bonds, NH–π hydrogen-bonds and hydrophobic contacts.

Host–guest systems have been extensively studied in organic solvents and the requirements for designing efficient selective receptors are well-understood.[1] In contrast, the development of comparable systems which function in water has proved much more challenging, because the compounds are not only more difficult to handle, but also more difficult to understand due to the complex behaviour of the solvent. Hydrophobic cavities have been the focus of synthetic recognition systems in water,[2] but there are a limited number of examples where hydrogen-bonding sites have been used in conjunction with hydrophobic binding to provide selective binding in water.[3] It is this arrangement that is the characteristic feature of protein binding pockets which usually have complicated arrays of polar and non-polar sites, and the interplay of their recognition and desolvation properties is one of the factors that makes it difficult to disentangle the complexities of biological recognition.[4] Here we describe a simple synthetic host–guest system which allows us to study this interplay of polar and non-polar binding interactions in water.

The synthesis and recognition properties of **1** have been reported.[5] The water soluble analogue **2** was prepared in the



1 R = CH_2
2 R = N^+Me_2 Cl^-

same way. The two quaternary ammonium centres on the receptor periphery were sufficient to confer good water solubility on the macrocyle, and ^{1}H NMR dilution experiments showed no evidence of any aggregation or micelle formation at millimolar concentrations.

Single crystals of **2** suitable for X-ray crystallography of were grown from a water–MeCN mixture.‡ The macrocycle cavity is filled by a cluster of water molecules in the crystal [Fig. 1(*a*)]. Although the waters are within H-bonding distance of each other and sites on the macrocycle (2.8–3.1 Å), partial occupancy and the poor quality of the X-ray data preclude a detailed assignment of the H-bond network. We have previously obtained an X-ray crystal structure of the organic soluble

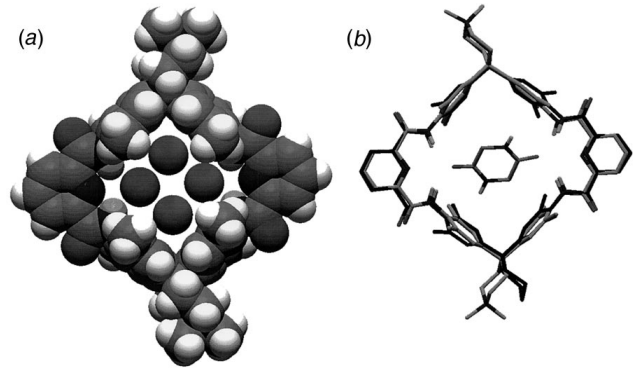
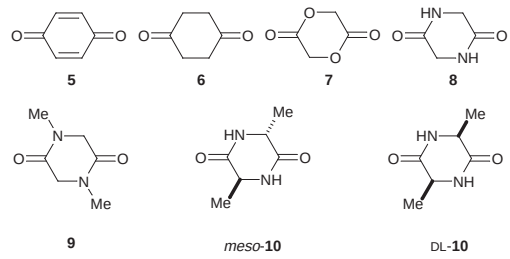


Fig. 1 (*a*) The X-ray crystal structure of **2** showing the positions of the water molecules which solvate the cavity. (*b*) The X-ray crystal structure of **2** superimposed on the X-ray crystal structure of the **1**·**8** complex.

analogue **1** complexed with glycine anhydride **8**,[5] and Fig. 1(*b*) shows this structure superimposed on the structure of **2**. The only difference between the chemical structures of the two macrocycles is the replacement of cyclohexane by quaternised piperidine on the periphery of the macrocycle. The only difference between the conformations of the macrocycles in the two X-ray structures is the orientation of one of these peripheral groups: the geometry of the cavity and arrangement of functional groups is identical, which reflects the high degree of preorganisation conferred on this system by the intramoelcular pyridine–amide hydrogen-bonds.

The recognition properties of the new receptor **2** were investigated by ^{1}H NMR titration experiments in H_2O–D_2O (9 : 1). No detectable changes were observed upon addition of benzoquinone **5** or the diester **7**. The binding constants for the



5 **6** **7** **8**

9 *meso*-**10** DL-**10**

other guests investigated are listed in Table 1. The structures of the complexes were determined from the limiting complexation-induced changes in chemical shift and a ROESY experiment for the most stable complex, which is formed with alanine anhydride **10**. The downfield shift for the signal due to the **2** amide protons shows that they form H-bonds with the carbonyl groups of the guests (Table 1). Characteristic upfield shifts are observed for the signals due to CH protons (δ −0.9 to −1.1), the amide protons (δ −0.5) and the methyl protons (δ −0.6 to −0.8) of all the guests which shows these protons are shielded by the aromatic side-walls of the macrocycle on complexation.

Table 1 ^{1}H NMR titration data: association constants and limiting changes in chemical shift for formation of 1 : 1 complexes with macrocycle **2** in water. Data for complexation with macrocycle **1** in $CHCl_3$ are shown for comparison

Guest	K_a/M^{-1} host **2** in water	$\Delta\delta$ (ppm) of **2** amide NH	K_a/M^{-1} host **1** in $CDCl_3$
5	< 5	—	230
6	94 ± 9	+0.1	850
7	< 5	—	340
8	71 ± 8	+0.3	1.0×10^6
9	100 ± 10	+0.1	—
meso-**10**[a]	100 ± 10	+0.7	—
DL-**10**	760 ± 80	+0.5	—

[a] The values for *meso*-**10** were determined by titrating a mixture of DL-**10** and *meso*-**10** into **2**. Using the data obtained previously for DL-**10**, the mixed titration could be analysed in a straightforward manner, because complexation with **2** caused the signals due to DL-**10** and *meso*-**10**, which were initially coincident, to split (these compounds are clearly bound in slightly different geometries inside the macrocycle).

These changes in chemical shift are very similar to those observed for complexation with **1** in $CHCl_3$ and suggest that the structures of all of the complexes are similar to that shown in Fig. 1(*b*).

Cyclohexane-1,4-dione **6**, glycine anhydride **8** and the bis(*N*-methyl) derivative **9** all bind with comparable affinity. We have previously measured the association constants for **1** with guests **5–8** in $CHCl_3$, and the stabilities are all substantially reduced in water, which reflects the increase in solvent competition for the hydrogen-bonding sites (Table 1). However, the selectivity in $CHCl_3$ is quite different from that in water: the association constant for **6** is reduced by an order of magnitude in water; for **5** and **7**, it is at least two orders of magnitude lower, and for **8**, it is four orders of magnitude lower. This trend reflects the relative polarity of the guests and the strength of their interaction with water: more polar guests are more difficult to desolvate in water and are therefore bound weakly. Clearly, decreasing the polarity of the guest should increase binding in water, and we therefore examined three dimethyl derivatives of glycine anhydride. For **9** and *meso*-**10**, there is no increase in affinity, and CPK models suggest that these guests do not fit properly into the cavity. However, the association constant for the other isomer DL-**10** is significantly larger, indicating good shape complementarity which allows additional hydrophobic interactions with the methyl groups to be realised. Intermolecular NOEs observed in a ROESY experiment on the **2**·**10** complex confirm that the **10** methyl groups are close to the aromatic side-walls of the receptor in the complex.

Evidence that NH–π hydrogen-bonds are involved in recognition in this system comes from the rates of exchange of the amide protons with water. H_2O–D_2O (9:1) was used as the solvent, so that we could monitor the amide signals during the NMR titrations. However, this necessitated the use of a solvent suppression sequence which removed the signals due to the amides of **8** and **10**. These protons are in fast exchange with the solvent, but the signals due to the amides of receptor **2** were unaffected by solvent suppression, because they are intramolecularly hydrogen-bonded and exchange slowly with solvent. However during the course of the titration, signals due to the **8** and **10** amide protons appeared and increased in intensity until they reached a similar intensity to the signals due to the host. This implies that complexation of these guests protects the amides from exchange with solvent in the same way as conventional hydrogen-bonds and provides direct evidence for NH–π hydrogen-bonding in these complexes.[5,6]

Thus the functional group interactions responsible for recognition are amide–amide hydrogen-bonds, NH–π hydrogen-bonds and hydrophobic CH–π interactions. Although it is difficult to interpret simplistic binding experiments of this type in terms of individual interaction energies,[6,7] there are some interesting observations to be made in these systems. The association constants for **6** and **8** are very similar: desolvation of **8** is much more difficult than desolvation of **6**, which suggests that either the magnitude of the NH–π interaction in water is comparable to a hydrophobic CH_2–π interaction or that the amide–amide hydrogen-bonds are stronger than the ketone–amide hydrogen-bonds despite the competition with water. Compound **8** is a very polar substrate with very few useful recognition sites for binding in water, and yet **2** is able to complex it with reasonable affinity. The water cluster which solvates **2** presents a polar recognition surface which has a lot of similarities with that of glycine anhydride [Fig. 1(*b*)]. However, the release of these water molecules to bulk solvent on guest complexation is entropically favourable and may be enthalpically favourable for the water which solvates the non-polar part of the receptor.[2a] The most stable complex is formed with DL-alanine anhydride DL-**10**, where hydrophobic interactions with the two methyl groups are responsible for the ten-fold increase the association constant relative to glycine anhydride **8**.

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Notes and references

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‡ *Crystal data* for $C_{60}H_{98}Cl_2N_8O_{18}$; M = 1290.36, crystallises from MeCN–water as long colourless needles; crystal dimensions 0.76 × 0.32 × 0.32 mm, tetragonal, a = 33.6111(15), b = 33.6111(15), c = 13.2959(6) Å, U = 15020.5(12) Å^3, Z = 8, D_c = 1.141 Mg m^{-3}, space group $P4_2/ncm$ (λ = 0.71073 Å), μ(Mo-Kα) = 0.152 mm^{-1}, F(000) = 5536, 59975 reflections, 3879 independent reflections, final R = 0.1611. The crystals were long and fibrous on attempted cleavage. The spots and resolution were very poor, hence the high final R. The complex has C_S symmetry. CCDC 182/1012.

1 *Comprehensive Supramolecular Chemistry*, ed. F. Vogtle, Pergamon, Oxford, vol. 2, 1996.
2 (*a*) S. B. Ferguson, E. M. Sanford, E. M. Seward and F. Diederich, *J. Am. Chem. Soc*., 1991, **113**, 5410; (*b*) S. Mecozzi, A. P. West and D. A. Dougherty, *J. Am. Chem. Soc*., 1996, **118**, 2307.
3 F. Diederich and D. R. Carcanague, *Helv. Chim. Acta*, 1994, **77**, 800; B. Hinzen, P. Seiler and F. Diederich, *Helv. Chim. Acta*, 1996, **79**, 942; (c) V. M. Rotello, E. A. Viani, G. Deslongchamps, B. A. Murray and J. Rebek, *J. Am. Chem. Soc*., 1993, **115**, 797; M. M. Conn, G. Deslongchamps, J. de Mendoza and J. Rebek, *J. Am. Chem. Soc*., 1993, **115**, 3548; Y. Kato, M. M. Conn and J. Rebek, *J. Am. Chem. Soc*., 1994, **116**, 3279; M. Torneiro and W. C. Still, *J. Am. Chem. Soc*., 1995, **117**, 5887.
4 A. R. Fersht and L. Serrano, *Curr. Opin. Struct. Biol*., 1993, **3**, 75.
5 F. J. Carver, C. A. Hunter and R. J. Shannon, *J. Chem. Soc*., *Chem. Commun*., 1994, 1277; H. Adams, F. J. Carver, C. A. Hunter and N. J. Osborne, *Chem. Commun*., 1996, 2529.
6 T. M. Fong, M. A. Cascieri, H. Yu, A. Bansal, C. Swain and C. D. Strader, *Nature*, 1993, **362**, 350; H. Adams, K. D. M. Harris, G. A. Hembury, C. A. Hunter, D. Livingstone and J. F. McCabe, *Chem. Commun*., 1996, 2531.
7 D. H. Williams, B. Bardsley, W. Tsuzuki and A. J. Maguire, *Chem. Biol*., 1997, **4**, 507; H. Adams, F. J. Carver, C. A. Hunter, J. C. Morales and E. M. Seward, *Angew. Chem*., *Int. Ed. Engl*., 1996, **35**, 1542.

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