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Novel Enantioselective Receptors for N-Protected Glutamate and Aspartate

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Abstract: A series of chiral bisthiourea macrocycles **1**–**4** have been prepared and their binding properties with various dicarboxylate salts have been examined by using NMR titration and isothermal calorimetry experiments. Macrocycle **1**, in particular, favours the 1:1 binding of N-protected L-glutamate and aspartate, but favours 1:2 binding of the corresponding D-amino acids in polar solvents (dimethyl sulfoxide and acetonitrile). The macrocycles, however, do not bind carboxylates at all in the less competitive solvent chloroform. The binding properties of these macrocyles are sensitive to small structural changes as demonstrated by the altered binding properties of macrocycles **2**–**4** compared with **1**.

Keywords: enantioselectivity · receptors · solvent effects

Introduction

Molecular recognition lies at the heart of biochemistry. Biochemical processes all inherently require selective molecular recognition and complexation to be successful. Matching the degree of exquisite control and efficiency exhibited by natural systems is a challenging goal for the host-guest chemist to emulate, and has led to much interest in the design of a diverse range of synthetic receptors for important biological substrates.[1] Enantioselective recognition of biologically relevant molecules, however, remains a major challenge.[2] Although numerous receptors have been developed for dicarboxylic acids and dicarboxylates,[3] only a few enantioselective receptors for chiral dicarboxylates have been described.[4] In our efforts to develop novel receptors for dicarboxylates, we have targeted N-protected amino acid dicarboxylates (e.g. glutamate and aspartate). We recently described the chiral bisthiourea macrocycle **1** which was specifically designed to bind to glutamate through eight hydrogen bonds.[5] It incorporates thiourea moieties intended to provide the primary interaction with the carboxylates and additional amide hydrogens to act as hydrogen-bonding donors for the lone pairs of the guest carboxylate oxygens (Figure 1). Pyrido units were expected to help preorganise the macrocycle,[6] and chirality was provided by the (*S*,*S*)-1,2-diphenylethylene diamine spacer.

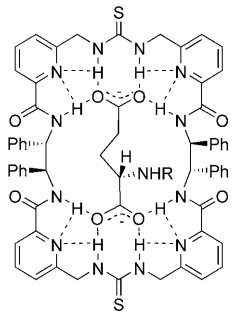

Figure 1. Schematic of proposed mode of complexation of N-Boc-L-glutamate by macrocycle **1** via eight hydrogen bonds.

Macrocycle **1** formed a strong 1:1 complex with N-Boc-L-glutamate dicarboxylate in polar solvents (DMSO and CH_3CN). However, only a weak 1:1 complex was formed with N-Boc-D-glutamate dicarboxylate, and a 1:2 (host/guest) complex was favoured instead. Surprisingly the macrocycle did not bind carboxylates at all in the less competitive solvent $CDCl_3$. Indeed ^{1}H NMR studies indicated that the macrocycle adopted a wrapped conformation with two-fold C_2 symmetry in $CDCl_3$, which was unsuitable for binding carboxylates.

In order to gain a better understanding of the unusual host–guest complexation properties of **1** we have now carried out further binding studies with macrocycle **1**, and with analogous macrocycles **2**–**4**, using ^{1}H NMR and isothermal calorimetric titrations, as well as computational studies on macrocycle **1**. Here we describe in detail the results of these studies.

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Results and Discussion

Synthesis: Macrocycle **1** was synthesised as previously described by first coupling (1*S*,2*S*)-1,2-diphenylethylene diamine with pyridyl acid **5** to give bisphthalimide **6**, which was deprotected by using hydrazine hydrate. Treatment of the resulting diamine **7** with CS_2 and DCC produced the bis(isothiocyanate) **8**. A further equivalent of diamine **7** was added to bis(isothiocyanate) **8**, in the presence of DMAP, by syringe pump, over three hours to afford macrocyclic bisthiourea **1** in 26% yield (Scheme 1).

An analogous, but more conformationally constrained, macrocycle **2** was readily prepared by an identical sequence, but starting with (*S*,*S*)-1,2-diaminocyclohexane. Pyridyl acid **5** was coupled with the diaminocyclohexane tartrate salt by using a two-phase (water/dichloromethane) solvent system, as attempted isolation of the free diamine from the tartrate salt was frustrated by low yields. The bisphthalimide **9** was converted to the bis(isothiocyanate) **11** and reaction with a further equivalent of diamine **10** gave the desired macrocycle **2** in 72% yield. The high yield (relative to the formation of macrocycle **1**) is probably due to the conformational constraint of the diamine, which favours the macrocyclisation over the formation of by-products.

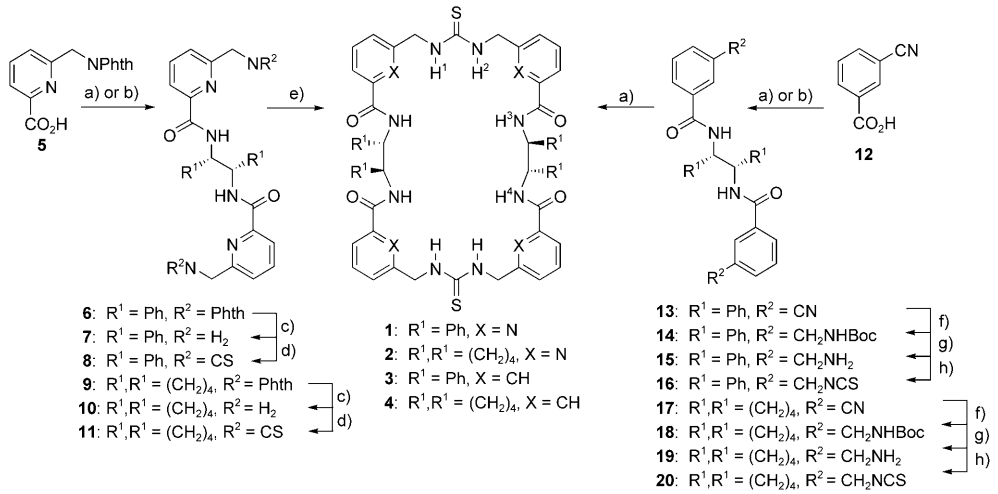
To probe the role of the pyrido unit in preorganising macrocycles **1** and **2**, the analogous macrocycles **3** and **4**, with the pyrido units replaced by benzo units, were also prepared. Commercially available 3-cyanobenzoic acid **12** was coupled with (1*S*,2*S*)-(−)-1,2-diphenylethylenediamine or (1*S*,2*S*)-(−)-1,2-diaminocyclohexane to afford bis(cyanobenzamide) **13** and **17**, respectively. The cyano groups were reduced using sodium borohydride/$NiCl_2$ and, to prevent dimerisation by-products, the diamine was trapped in situ using di-*tert*-butyl dicarbonate,[7] to provide the protected diamines **14** and **18**, respectively. Removal of the Boc protecting groups and treatment of the resulting diamines with thiophosgene produced the bis(isothiocyanates) **16** and **20**. Again, a further equivalent of diamine was added to the corresponding bis(isothiocyanate), in the presence of triethylamine, by syringe pump, over three hours to afford the macrocycles **3** and **4** in 17 and 29% yield, respectively.

Conformational studies: As described previously macrocycle **1** in [D_6]DMSO or in CD_3CN gave a simple ^{1}H NMR spectrum consistent with the expected four-fold D_2 symmetry of the macrocycle.[5] At room temperature the ^{1}H NMR spectrum of **1** in $CDCl_3$ shows very broad signals, but gives a well-resolved spectrum at −40 °C (Figure 2) indicating twofold C_2 symmetry, with, for example, two different signals for the thiourea NH protons ($\delta = 9.0$ and 8.1 ppm) and for the amide NH protons ($\delta = 10.1$ and 7.9 ppm) (Table 1).

Further ^{1}H NMR studies, by using torsion angle dynamics with NOE and scalar coupling constant constraints,[8] allowed us to determine the conformation of macrocycle **1** in $CDCl_3$ at −40 °C.

The conformation determined by using this approach (Figure 3) shows that the macrocycle adopts a twisted and wrapped conformation stabilised by two sets of three intramolecular hydrogen bonds between one of the amide carbonyls and three NH's from a thiourea and the other amide. This hydrogen-bonding motif has been observed by us previously in the dimeric crystal structure of the related acyclic monothiurea **21** (Figure 4).[6b] Further stabilisation of the wrapped conformation of **1** is achieved by weak hydrogen bonds between the pyridine nitrogen and adjacent NH groups.

The more conformationally constrained macrocycle **2** was only sparingly soluble in CD_3CN, but in [D_6]DMSO also



Scheme 1. a) $SOCl_2$; (1*S*,2*S*)-(−)-1,2- diphenylethylenediamine, DMAP, CH_2Cl_2 (68% for **6**, 48% for **13**); b) diphenylchlorophosphate; (1*S*,2*S*)-(−)-1,2-diaminocyclohexane (tartrate salt), H_2O, K_2CO_3 (52% for **9**, 67% for **17**); c) $N_2H_4{\cdot}H_2O$, EtOH (80–85%); d) CS_2, DCC, CH_2Cl_2; e) simultaneous addition of 1 equiv of **7** (or **10** or **15** or **19**) and 1 equiv of **8** (or **11** or **16** or **20**, respectively) by syringe pumps over 3 h to a solution of DMAP or Et_3N in CH_2Cl_2 (26% for **1**, 72% for **2**, 17% for **3**, 29% for **4**, over steps d and e or h and e); f) di-*tert*-butyl dicarbonate, $NiCl_2$, $NaBH_4$ (64% for **14** and **18**); g) TFA, CH_2Cl_2 (quant.); h) thiophosgene, K_2CO_3, CH_2Cl_2, H_2O. DMAP = 4-dimethylaminopyridine, DCC = *N*,*N*′-dicyclohexylcarbodiimide, TFA = trifluoroacetic acid.

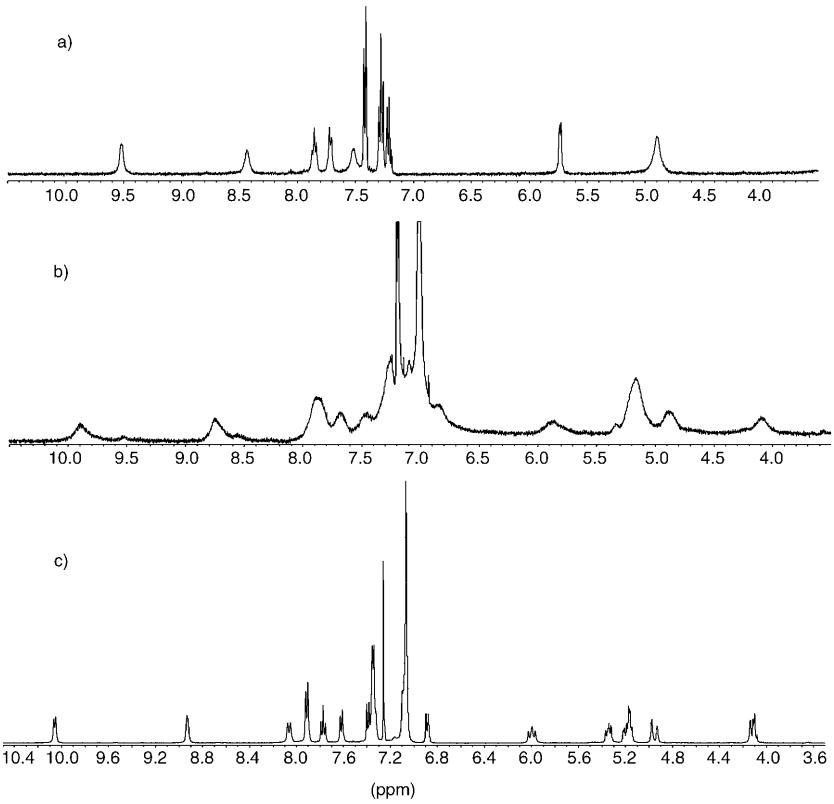


Figure 2. ^{1}H NMR spectra of macrocycle **1** in: a) [D_6]DMSO at room temperature; b) $CDCl_3$ at room temperature; c) $CDCl_3$ at −40 °C.

Table 1. ^{1}H NMR chemical shifts for NH signals of macrocycles **1**–**4** in $CDCl_3$ and [D_6]DMSO.

Solvent	Macrocycle	NH^1	NH^2	NH^3	NH^4
$CDCl_3$	**1**[a]	9.0	8.1	7.9	10.1
	2	8.7	8.1	7.7	9.2
[D_6]DMSO	**1**	8.3		9.4	
	2	8.6		9.0	
	3	7.9		9.0	
	4	7.9		8.2	

[a] Spectrum recorded at −40 °C. All other spectra recorded at room temperature.

gave a simple ^{1}H NMR spectrum consistent with the expected four-fold D_2 symmetry of the macrocycle. In $CDCl_3$ **2** gave a ^{1}H NMR spectrum at room temperature similar to that found for **1** and consistent with the wrapped conformation identified for **1** at −40 °C. Detailed 2D NMR studies (COSY and NOESY ^{1}H NMR experiments) allowed full assignment of all the ^{1}H NMR signals for **2**. In particular, the thiourea NH protons have two different signals at $\delta = 8.7$ and 8.1 ppm, while the amide NH protons have signals at $\delta = 9.2$ and 7.7 ppm (Table 1). However, in the case of **2** the spectrum in $CDCl_3$ was well-resolved even at room temperature, indicating that the greater conformational constraints imposed by the cyclohexyl diamine units encourages the wrapped conformation.

Macrocycles **3** and **4** in which the pyridyl units in **1** and **2** are replaced by benzo units, were soluble in DMSO and trifluoroethanol, but not in CH_3CN or in $CHCl_3$. Again in [D_6]DMSO **3** and **4** gave a simple ^{1}H NMR spectrum consistent with the expected D_2 symmetry of the macrocycle. Comparing the ^{1}H NMR spectra of **3** and **4** with the spectra of **1** and **2** in [D_6]DMSO, reveals that in the former pair the signals for the NH protons (amide and thiourea) are in each case more upfield than the corresponding signals for the latter pair of macrocycles (Table 1). This is consistent with the notion that the pyrido nitrogens are forming weak hydrogen bonds with the adjacent NH's and therefore help to preorganise the macrocycle in DMSO—even though in this solvent the macrocycles do not form the wrapped conformation identified in $CDCl_3$.

Binding studies: Binding studies were carried out using ^{1}H NMR titrations and isothermal calorimetry (Table 2). Data from the ^{1}H NMR titrations were fitted to 1:1 or 1:2 (host/guest) binding isotherms by using the NMRTit HG software.[9] Where

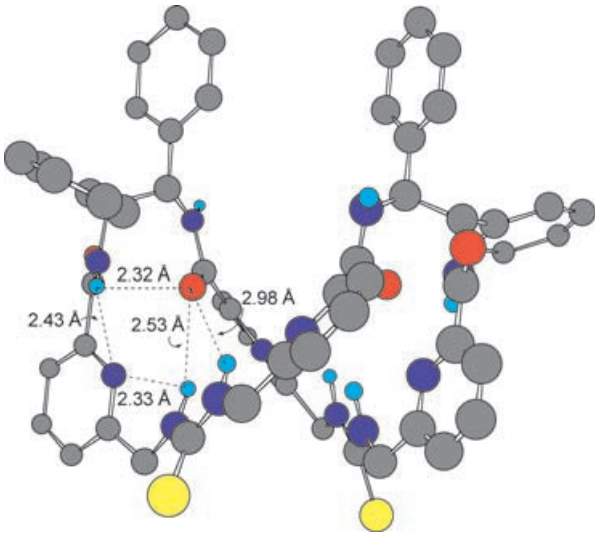


Figure 3. Conformation of macrocycle **1** in $CDCl_3$ as determined by ^{1}H NMR showing intramolecular H-bond lengths.

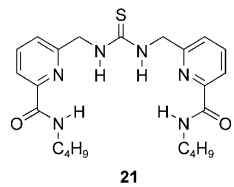


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Figure 4. Acyclic thiourea **21**.

Table 2. Binding data for macrocycles (MC) **1**–**3** with various carboxylate salts in CH_3CN and DMSO.

				NMR titration data [a]			ITC titration data[a]						
Entry	MC	Solvent[b]	Guest[c]	$K_a^{1:1}$ [M^{-1}]	$K_a^{1:2}$ [M^{-1}]	$\Delta G^{1:1}$ [kJ mol^{-1}]	$K_a^{1:1}$ [M^{-1}]	$\Delta H^{1:1}$ [kJ mol^{-1}]	$T\Delta S^{1:1}$ [kJ mol^{-1}]	$\Delta G^{1:2}$ [kJ mol^{-1}]	$K_a^{1:2}$ [M^{-1}]	$\Delta H^{1:2}$ [kJ mol^{-1}]	$T\Delta S^{1:2}$ [kJ mol^{-1}]
1	**1**	CH_3CN	N-Boc-L-Glu	$>10^4$	–	−25.3	2.7×10^4	−4.5	20.8	–	<100	–	–
2	**1**	CH_3CN	N-Boc-L-Glu	–	$>10^4$	–	<100	–	–	−26.8	4.9×10^4	−6.7	20.1
3	**1**	DMSO	N-Boc-L-Glu	3700	–	−19.2	2300	−10.7	8.5	–	<100	–	–
4	**1**	DMSO	N-Boc-D-Glu	individual binding constants could not be reliably determined by either method									
5	**1**	CH_3CN	acetate	–	$>10^4$	−13.2	200	−2.7	10.5	−20.8	5100	−5.8	15.0
6	**1**	CH_3CN	N-Ac-L-Glu	$>10^4$	5100	−22.4	1.0×10^4	2.4	24.8	−17.3	1200	2.0	19.3
7	**1**	CH_3CN	N-Ac-D-Glu	4500	$>10^4$	−19.6	3100	−5.9	13.8	−23.4	1.6×10^4	12.5	35.9
8	**1**	DMSO	N-Ac-L-Glu	$>10^4$	1000	binding constants could not be reliably determined by calorimetry							
9	**1**	DMSO	N-Ac-D-Glu	800	$>10^4$	−15.1	500	3.5	18.6	−24.7	2.5×10^4	−6.4	18.4
10	**1**	CH_3CN	N-Boc-L-Asp	$>10^4$	<100	binding constants could not be reliably determined by calorimetry							
11	**1**	CH_3CN	N-Boc-D-Asp	individual binding constants could not be reliably determined by either method									
12	**1**	DMSO	N-Boc-L-Asp	1200 at 363 K[d]									
13	**1**	DMSO	N-Boc-D-Asp	weak binding at 363 K[d]									
14	**1**	DMSO	DiBz-L-tartrate	–	211								
15	**1**	DMSO	DiBz-D-tartrate	–	103								
16	**2**	CH_3CN	N-Boc-L-Glu	–[e]	–[e]	−18.5	1800	−21.8	−3.3	–	<100	–	–
17	**2**	CH_3CN	N-Boc-D-Glu	–[e]	–[e]	–	<100	–	–	−19.0	2100	−11.7	7.2
18	**2**	DMSO	N-Boc-L-Glu	–[f]	–[f]	−15.0	430	−7.8	7.2	–	<100	–	–
19	**2**	DMSO	N-Boc-D-Glu	individual binding constants could not be reliably determined by either method									
20	**2**	CH_3CN	N-Boc-L-Asp	–[e]	–[e]	−20.5	3900	−14.7	5.8	–	<100	–	–
21	**2**	CH_3CN	N-Boc-D-Asp	–[e]	–[e]		<100			19.0	2100	−8.4	10.5
22	**2**	DMSO	N-Boc-L-Asp	individual binding constants could not be reliably determined by either method									
23	**2**	DMSO	N-Boc-D-Asp	individual binding constants could not be reliably determined by either method									
24	**3**	DMSO	N-Boc-L-Glu	–[f]	–[f]	−25.3	2.7×10^4	−5.9	19.4	−14.8	390	−8.6	6.2
25	**3**	DMSO	N-Boc-D-Glu	–[f]	–[f]	−13.8	260	−4.4	9.4	−24.5	1.9×10^4	−8.0	16.5

[a] Association constants are reported to two significant figures with estimated errors of ± 20%. The reported value of $K_a^{1:2}$ refers to the binding constant for the association of the 1:1 complex with the second equivalent of guest. The overall binding constant is therefore the product of the reported $K_a^{1:1}$ and $K_a^{1:2}$. In general the errors on thermodynamic data are estimated at ± 0.5 kJ mol^{-1}, but where 1:1 and 1:2 association constants are both significant (entries 6, 7, 9, 24, and 25) the errors may be larger. In some cases where both binding stoichiometries are competing the titration data (from one or both of the titration methods) could not be fitted at all reliably (entries 4, 8, 11, 19, 22 and 23), although strong binding was generally apparent from the data. [b] Deuterated solvents, CD_3CN and [D_6]DMSO, were used for NMR titration experiments. [c] All guests were used as the tetrabutylammonium salts. [d] Binding kinetics were slow (on the NMR timescale) at room temperature and NMR titration experiments were carried out at 90 °C, see text. Calorimetry experiments were not carried out at this temperature. [e] NMR titration experiments were not carried out in CD_3CN because of solubility problems with macrocycle **2** in this solvent. [f] Binding constants could not be reliably determined by NMR titrations.

binding was dominated by either 1:1 or 1:2 complexation, reliable association constants (K_a) could be determined and binding stoichiometry was confirmed using Job plots.[10] In cases where binding was weak it was often not possible to distinguish between 1:1 and 1:2 complexation, since the data could be fitted to both binding isotherms. In some cases it was apparent that there was strong overall binding, and both 1:1 and 1:2 complexes were formed, but neither was dominant. In these cases the individual association constants could not always be reliably determined. Job plots with these systems also gave ambiguous results as the plots showed broad maxima at stoichiometries typically of ~1:1.5. Some of the data obtained gave binding constants at the upper limit of detectability by using NMR titrations (10^4–10^5 M^{-1}) and it was concluded that in these cases $K_a > 10^4$ M^{-1}, but more precise values could not be reliably obtained from these titrations.[11]

Isothermal calorimetric binding data[12–14] was fitted using a one- or two-site binding model and, again, where binding was dominated by either 1:1 or 1:2 complexation, reliable and reproducible association constants could be determined. When overall binding was weak, or when 1:1 and 1:2 association constants were both significant then, as with the NMR titrations, the individual association constants could not always be reliably determined.

In general titration experiments were performed more than once to obtain reproducible data. Where the association constant could be determined for the same complex by using both isothermal calorimetry and ^{1}H NMR titrations, agreement between the two methods was generally good.

Isothermal calorimetry has the considerable advantage that it allows direct determination of the association constants and the enthalpy (ΔH) of binding. Hence the Gibbs free energy (ΔG) and entropic contribution (ΔS) can be calculated. In general we found that using isothermal calorimetry with these macrocycles, where binding was predominantly either 1:1 or 1:2 complexation, the association constants (and hence the Gibbs free energy) could be reproduced to a high degree of accuracy. The enthalpy–entropy balance in host–guest binding can be very sensitive to solvent composition and to the presence of small quantities of water, particularly when the bulk solvent is relatively apolar,[15] so for all

titration experiments solvents and the samples of host and guest were carefully dried prior to use.

Binding studies by using macrocycle 1: Initial investigation of the binding properties of receptor **1** with N-Boc-glutamate dicarboxylate has been described previously.[5] In summary, addition of the N-Boc-L-glutamate bis(tetrabutylammonium) salt to a solution of **1** in CD_3CN led to a strong 1:1 association ($K_a^{1:1} > 10^4\,M^{-1}$), and significant downfield shifts of the ^1H NMR NH signals, consistent with the formation of strong hydrogen bonds and the proposed mode of binding (Figure 1). The binding data obtained from the addition of N-Boc-D-glutamate could not be fitted to a 1:1 binding isotherm, but could be fitted to a 1:2 (host/guest) binding isotherm and yielded $K_a^{1:2} > 10^4\,M^{-1}$. Binding stoichiometry was confirmed by Job plots[10] and is further evident from the ^1H NMR titrations since saturation is rapidly reached after addition of ~1 equivalent of the N-Boc-L-glutamate salt, whereas saturation is reached only after addition of ~2 equivalents of N-Boc-D-glutamate salt (Figure 5). The relatively smooth titration curve obtained with N-Boc-D-glutamate, and lack of an obvious transition after addition of only one equivalent of the guest, suggests that binding of the second guest molecule is stronger than binding of the first (positive co-operativity), such that the receptor tends to saturate both binding sites simultaneously, that is, the 1:1 complex is only present in very small amounts relative to uncomplexed receptor or the 2:1 complex.

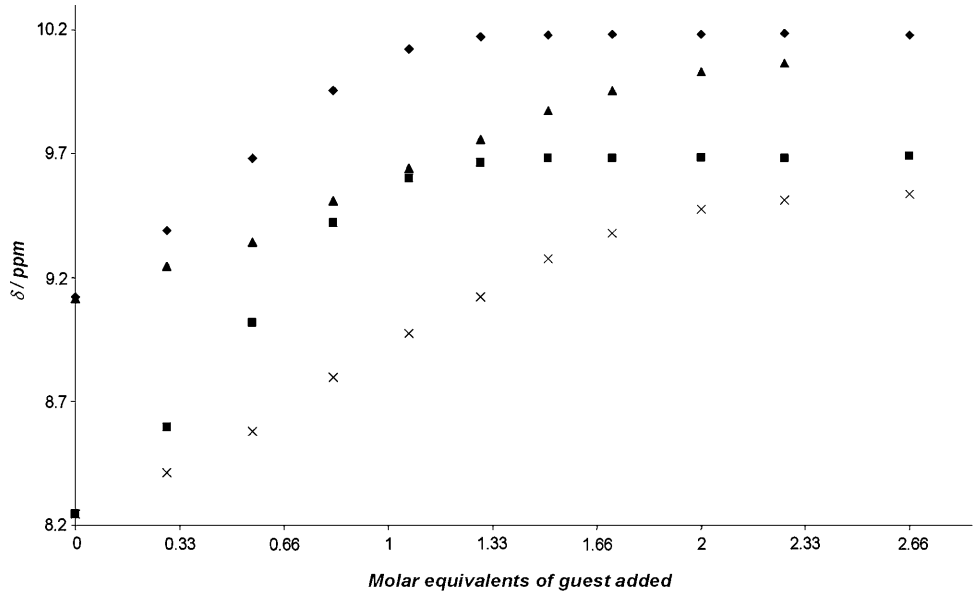

Figure 5. Binding titration curves for macrocycle **1**, showing the shift of the amide (◆: Boc-L-Glu, ▲: Boc-D-Glu) and the thiourea (■: Boc-L-Glu, ×: Boc-D-Glu) NH protons on addition of the bis(tetrabutylammonium) salts of N-Boc-L-glutamate and N-Boc-D-glutamate in CD_3CN as solvent, indicating the strong 1:1 binding with the former, and 2:1 binding with the latter.

Isothermal calorimetric binding data obtained with N-Boc-L-glutamate salt confirmed the strong 1:1 binding ($\Delta G = -25.3\,kJ\,mol^{-1}$, $K_a^{1:1} = 2.7\times10^4\,M^{-1}$), which is dominated by the entropic contribution ($T\Delta S = 20.8\,kJ\,mol^{-1}$), indicating that complexation is promoted by the release of solvent molecules from the host and guest to bulk solvent. The calorimetric data obtained with the N-Boc-D-glutamate salt indicated a small 1:1 binding constant and strong 1:2 (host/guest) binding ($\Delta G = -26.7\,kJ\,mol^{-1}$, $K_a^{1:2} = 4.9\times10^4\,M^{-1}$), again confirming the results of the NMR titrations. Conformational changes in the receptor on binding N-Boc-D-glutamate are clearly evidenced by the large shifts in many of the CH signals in the ^1H NMR spectrum of the receptor on addition of the guest.

Binding studies with macrocycle **1** and N-Boc-L-glutamate in [D_6]DMSO gave a similar picture, with a simple 1:1 binding, obtained by either NMR titration ($\Delta G = -20.4\,kJ\,mol^{-1}$, $K_a^{1:1} = 3.7\times10^3\,M^{-1}$) or isothermal calorimetry ($\Delta G = -19.2\,kJ\,mol^{-1}$, $K_a^{1:1} = 2.3\times10^3\,M^{-1}$), again with a large entropic contribution ($T\Delta S = 8.5\,kJ\,mol^{-1}$). Binding data for N-Boc-D-glutamate, however, could not be reliably fitted to give individual binding constants.

In the less competitive solvent, $CDCl_3$, addition of either enantiomer of the glutamate salt did not lead to any discernible change in the ^1H NMR spectrum of the macrocycle, which is attributed to the high energetic cost of breaking the intramolecular hydrogen bonds to open the tightly wrapped conformation adopted by the receptor in chloroform.

To confirm the above interpretation of 1:2 complexation of N-Boc-D-glutamate binding studies have now been carried out with tetrabutylammonium acetate. Significant changes in the ^1H NMR spectrum of the receptor were noted after the addition of the acetate guest in CD_3CN. Downfield shifts of the thiourea NH ($\Delta\delta_{sat} = 1.03$ ppm), the amide NH ($\Delta\delta_{sat} = 0.70$ ppm) and the benzylic CH signals ($\Delta\delta_{sat} = 0.47$ ppm) were recorded. Saturation was reached after the addition of two equivalents of acetate and the binding data could be fitted to the expected 1:2 stoichiometry ($\Delta G = -23.0\,kJ\,mol^{-1}$, $K_a^{1:2} > 10^4\,M^{-1}$). As with N-Boc-D-glutamate, the ^1H NMR titration curve obtained with acetate gave no indication of an intermediate 1:1 complex which is consistent with a small or negligible 1:1 binding constant. Isothermal calorimetric data obtained with the acetate salt also indicated a small 1:1 binding constant (ΔG ~$-13\,kJ\,mol^{-1}$, $K_a^{1:1}$ ~$200\,M^{-1}$) and strong 1:2 (host/guest) binding ($\Delta G = -20.8\,kJ\,mol^{-1}$, $K_a^{1:2} = 5100\,M^{-1}$), confirming the results of the NMR titrations.

In order to probe the binding properties of macrocycle **1** in more detail binding studies with a series of dicarboxylate substrates have also now been carried out. Firstly, to assess the influence of the bulky *tert*-butyloxycarbonyl protecting group of the glutamate substrates, binding studies were conducted with both enantiomers of N-Ac-glutamate.

Addition of N-Ac-L-glutamate salt to a solution of **1** in CD_3CN led to significant downfield shifts of the amide NH and thiourea NH ($\Delta\delta_{sat} > 1.0$ ppm) but in contrast to the titration with N-Boc-L-glutamate saturation was not reached after addition of one equivalent of the salt, and the titration curves showed a significantly sigmasoidal shape, suggesting that both 1:1 and 1:2 complexes are formed (Figure 6).

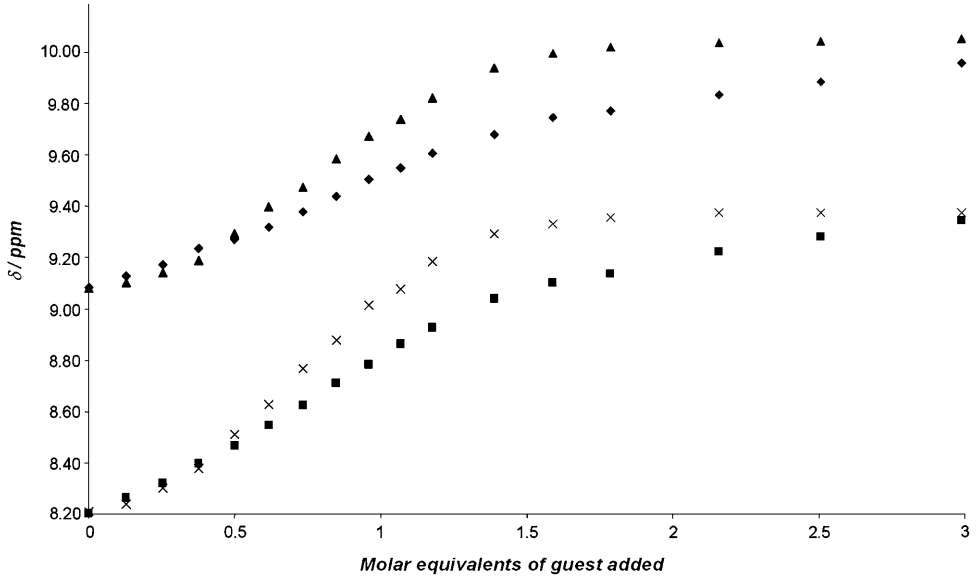

Figure 6. Binding titration curves for macrocycle **1**, showing the shift of a) the amide (◆: Ac-L-Glu, ▲: Ac-D-Glu) and b) the thiourea (■: Ac-L-Glu, ×: Ac-D-Glu) NH protons on addition of the bis(tetrabutylammonium) salts of N-Ac-L-glutamate and N-Ac-D-glutamate in CD_3CN as solvent (1:1 [host]:[guest] is reached when [guest] = 1.5 mM).

Indeed the data could be fitted to give a large $K_a^{1:1} > 10^4\,M^{-1}$, but also a significant $K_a^{1:2} = 5100\,M^{-1}$. Similar downfield shifts of the amide NH and thiourea NH were observed on addition of N-Ac-D-glutamate salt to a solution of **1** in CD_3CN. The NMR titration data could be fitted to give a large $K_a^{1:1} = 4500\,M^{-1}$ but a larger $K_a^{1:2} > 10^4\,M^{-1}$. Similar results were obtained in $[D_6]$DMSO. The NMR titration data indicates that for N-Ac-L-glutamate 1:1 complexation is dominant, whereas for N-Ac-D-glutamate 1:2 binding is the dominant, but in neither case is the selectivity as pronounced as with the two enantiomers of N-Boc-glutamate. Isothermal calorimetry data with these guests in both CH_3CN and DMSO confirmed the NMR results and binding in all cases is dominated by the entropic contribution.

To assess the ability to bind dicarboxylates with shorter chain lengths, binding studies have been conducted with both enantiomers of N-Boc-aspartate and dibenzoyl tartrate.

Addition of either enantiomer of N-Boc-aspartate tetrabutylammonium salt to a solution of **1** in CD_3CN at room temperature led to dramatic changes in the ^1H NMR spectrum of the macrocycle with considerable downfield shifts of the thiourea NH ($\Delta\delta_{sat} > 1.5$ ppm), the amide NH ($\Delta\delta_{sat} > 1.0$ ppm) and the benzylic CH signals ($\Delta\delta_{sat} > 0.5$ ppm). The titration data for N-Boc-L-aspartate showed that saturation was essentially reached after addition of one equivalent of the substrate and could be readily fitted to a 1:1 isotherm ($K_a^{1:1} > 10^4\,M^{-1}$), but the binding data for N-Boc-D-aspartate could not be fitted to a simple 1:1 or 1:2 binding stoichiometry and indicate that both 1:1 and 1:2 complexes were formed, but neither was dominant. Similarly isothermal calorimetry data with these guests could not be fitted reliably.

In $[D_6]$DMSO, addition of N-Boc-L-aspartate to macrocycle **1** at room temperature caused a broadening of the signals in the ^1H NMR spectrum of the receptor. During the addition to the host solution it was possible to observe one set of peaks for the unbound macrocycle, which decreased in intensity, and a new set of peaks (thiourea NH: $\delta = 9.7$ ppm; amide NH: $\delta = 10.3$ ppm), which increased in intensity on addition of the guest and were attributed to the bound host–guest complex. This new set of broad signals became reasonably well-resolved after the addition of four equivalents of guest. It was concluded that binding of N-Boc-L-aspartate by macrocycle **1** in $[D_6]$DMSO at room temperature was slow on the NMR time-scale. In order to overcome the slow binding kinetics, a ^1H NMR titration was carried out at 90 °C. At this temperature the ^1H NMR spectrum of macrocycle **1** in $[D_6]$DMSO was well-resolved and the signals of the amide and thiourea protons were both ~0.9 ppm upfield compared to the spectrum in $[D_6]$DMSO at room temperature. Addition of N-Boc-L-aspartate to macrocycle **1** at 90 °C in $[D_6]$DMSO led to downfield shifts of the thiourea NH ($\Delta\delta_{sat} = 0.64$ ppm) and the amide NH ($\Delta\delta_{sat} = 0.31$ ppm) signals. No significant shift was noted for the benzylic CH protons. The binding data could be fitted to a 1:1 binding isotherm ($\Delta G = -17.6$ kJ mol^{-1}, $K_a^{1:1} = 1.2 \times 10^3\,M^{-1}$). Addition of N-Boc-D-aspartate to macrocycle **1** at 90 °C in $[D_6]$DMSO produced less pronounced downfield shifts of the thiourea NH ($\Delta\delta_{sat} = 0.42$ ppm) and amide NH ($\Delta\delta_{sat} = 0.21$ ppm) protons and the slope of the titration curve indicated very weak binding and only ~25% of saturation was reached after the addition of five equivalents of guest. The data could not be

fitted unambiguously to a 1:1 or 1:2 (host/guest) binding isotherm. Isothermal calorimetry experiments were not performed at these elevated temperatures.

The addition of the tetrabutylammonium salts of both enantiomers of dibenzoyl tartrate in [D_6]DMSO at room temperature led to small downfield shifts in the thiourea NH ($\Delta\delta_{sat}$ ~0.3 ppm), suggesting weak binding. The binding data for both enantiomers could be fitted to a 1:2 (host/guest) isotherm ($K_a^{1:2}=210\,M^{-1}$ for dibenzoyl-L-tartrate and $100\,M^{-1}$ for dibenzoyl-D-tartrate). Presumably the tartrates substrates are too bulky to allow 1:1 complexation and even 1:2 binding with the receptor is weak compared to the 1:2 binding of glutamate and aspartate carboxylates. With such weak binding constants isothermal calorimetry was not attempted with these guests.

Binding studies by using macrocycle 2: Binding studies with the chiral bisthiourea macrocycles **2**, **3** and **4** were carried out to probe the effects of the structural modifications on the receptor on the binding properties. Binding studies with macrocycle **2** and N-protected glutamate and aspartate, as their tetrabutylammonium salts, were carried out by using ^{1}H NMR titrations and isothermal calorimetry. ^{1}H NMR spectra of 1:1 mixtures of both enantiomers of N-Boc-glutamate and N-Boc-aspartate with **2** in $CDCl_3$ were obtained in chloroform. As expected, addition of either enantiomer of the guests did not lead to any discernible change in the ^{1}H NMR spectrum of the macrocycle. Thus, as with macrocycle **1**, macrocycle **2** adopts a conformation unsuitable for binding in $CDCl_3$ and the energy required to reorganise the host into a suitable binding conformation, by breaking the intramolecular hydrogen bonds, is not compensated for by the host–guest binding interactions that would be established.

^{1}H NMR titration experiments in CD_3CN were hampered by the limited solubility of macrocycle **2** in this solvent. The problem was addressed by running isothermal calorimetric titrations, which allowed the use of host solutions at much lower concentrations. With both N-Boc-glutamate and N-Boc-aspartate, macrocycle **2** has binding properties very similar to those of macrocycle **1**. Data for N-Boc-L-glutamate and N-Boc-L-aspartate indicated formation of strong 1:1 complexes with **2**, while both N-Boc-D-glutamate and N-Boc-D-aspartate form predominantly 1:2 complexes. Notably, however, the association constants with **2** are at least an order of magnitude lower than the corresponding association constants with **1**.

In DMSO, however, binding data for both enantiomers of N-Boc-glutamate and N-Boc-aspartate could not be reliably fitted to simple 1:1 or 1:2 binding isotherms using ^{1}H NMR titrations. In ^{1}H NMR titrations, shifts of the thiourea NH ($\Delta\delta_{sat} > 0.2$ ppm), the amide NH ($\Delta\delta_{sat} > 0.16$ ppm) and the benzylic CH signals ($\Delta\delta_{sat} > 0.17$ ppm) were observed but saturation was not reached even after addition of several equivalents of the guest, suggesting that binding was weak, and again probably weaker than the corresponding complexation with macrocycle **1**. Using isothermal calorimetry only with N-Boc-L-glutamate as the guest could the data be fitted, and indicated relatively weak 1:1 binding ($\Delta G = -15.0\,kJ\,mol^{-1}$, $K_a^{1:1}=530\,M^{-1}$).

Binding studies by using macrocycles 3 and 4: Complexation studies with macrocycles **3** and **4** were restricted, due to their limited solubility, to titration experiments in DMSO and used only the enantiomers of N-Boc-glutamate. Addition of N-Boc-L-glutamate to macrocycle **3** in [D_6]DMSO led to significant downfield shifts of the ^{1}H NMR signals for the thiourea NH ($\Delta\delta_{sat} > 0.5$ ppm) and the amide NH ($\Delta\delta_{sat} > 0.3$ ppm), but saturation was not reached after the addition of the first equivalent of N-Boc-L-glutamate, and the binding data could not be fitted to a simple 1:1 or 1:2 (host/guest) isotherm. The calorimetric binding data obtained with N-Boc-L-glutamate salt indicated a strong 1:1 binding ($K_a^{1:1} = 2.7\times10^4\,M^{-1}$) and a moderate 1:2 association ($K_a^{1:2}=390\,M^{-1}$).

Addition of the N-Boc-D-glutamate tetrabutylammonium salt to **3** also led to significant downfield shifts of the ^{1}H NMR signals for the thiourea NH ($\Delta\delta_{sat}$ ~1 ppm), amide NH ($\Delta\delta_{sat}$ ~0.4 ppm) protons of the macrocycle. Saturation was reached after the addition of two equivalents of guest but again the binding data could not be fitted to a simple 1:1 or 1:2 (host/guest) binding model. Isothermal calorimetry data obtained with the N-Boc-D-glutamate salt and **3**, however, indicated a moderate 1:1 binding constant ($K_a^{1:1}=260\,M^{-1}$) but a much stronger 1:2 association ($K_a^{1:2}=1.9\times10^4\,M^{-1}$).

Addition of either enantiomers of N-Boc-glutamate to a solution of receptor **4** in DMSO, on the other hand, did not lead to any significant shifts in the ^{1}H NMR spectrum of the neat host, suggesting no or very weak binding.

Computational studies: Force field computational studies were undertaken in order to shed further light on the conformational preferences of macrocycle **1** in the solvents $CHCl_3$, DMSO and CH_3CN and the origin of the binding selectivities experimentally determined for macrocycle **1** with the enantiomers of N-Boc-glutamate. The computational method used in the calculations is dependent on the solvent (see Experimental Section). With $CHCl_3$ as the solvent, the generalized Born/surface area (GB/SA) continuum solvation model was used[16] and conformational searches performed using a mixed mode Monte Carlo (MC) search algorithm. For DMSO and CH_3CN, explicit solvation models were used in molecular dynamics (MD) simulations. With the exception of the MD simulations for which Impact 2.7[17] was used, all computations were performed using Macromodel 8.0 or 8.1.[18]

The global minimum geometry for **1** obtained from the conformational search with $CHCl_3$ as solvent has a C_2 geometry stabilized by strong intramolecular hydrogen bonds (Figure 7) in agreement with the experimental ^{1}H NMR data and similar to the conformation previously predicted by torsion angle dynamics (Figure 3).

From the MD simulations in CH_3CN and DMSO, a D_2-type conformation is predominant (also in agreement with

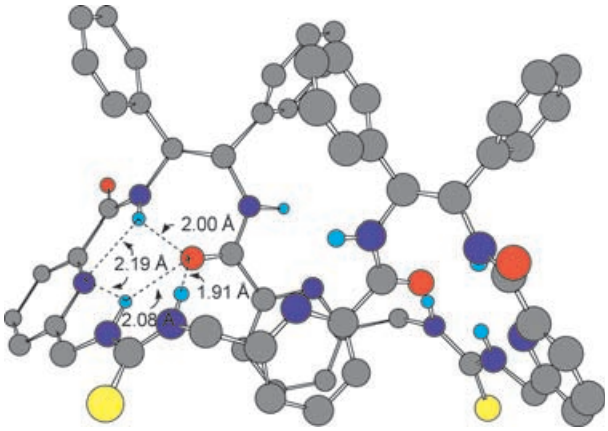

Figure 7. Conformation of macrocycle **1** in $CDCl_3$ as determined by molecular modeling showing intramolecular H-bond lengths.

^{1}H NMR data)—a conformation which is more favourable for accepting a substrate into the large open cavity (Figure 8).

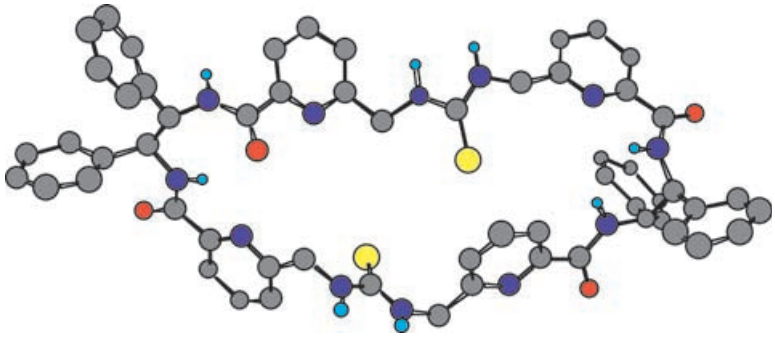

Figure 8. Conformation of macrocycle **1** in DMSO as determined by molecular modeling.

Geometries of the 1:1 binding complex of macrocycle **1** with both enantiomers of N-Boc-glutamate were determined in both $CHCl_3$ and CH_3CN. For each enantiomer the same binding modes were obtained with $CHCl_3$ or CH_3CN as solvent. According to these calculations the dicarboxylate ligand forms eight hydrogen bonds with the macrocycle, each carboxylate oxygen forming hydrogen bonds with either two amide or two thioamide hydrogens (Figures 9 and 10), which differs from the model originally proposed in which each carboxylate oxygen forms hydrogen bonds to both an amide and a urea hydrogen (Figure 1).

Although geometries for complexation of **1** with N-Boc-glutamate enantiomers in $CHCl_3$ were located computationally, no binding could be detected experimentally in this solvent. The energy required to reorganise the tightly wrapped macrocycle **1** (Figure 7) into a conformation favourable for accepting the N-Boc-glutamate substrates was calculated based on the lowest energy conformations of the macrocycle from the conformational searches in the bound (with N-Boc-glutamate) and the unbound state (Table 3). The reorganisation energy for binding either enantiomer (~300 kJ mol^{-1}) was found to be much greater than the apparent energy gain from binding (~100 kJ mol^{-1}). Of course the values of energies obtained from force field calculations will not be quantitatively accurate. However, an energy difference of ~200 kJ mol^{-1} is substantial and hence, the energetically unfavourable binding of N-Boc-glutamate enantiomers to macrocycle **1** in $CHCl_3$ is not experimentally observed.

The computational methods employed were not, however, able to reproduce the binding selectivity of N-Boc-L-glutamate over N-Boc-D-glutamate for 1:1 binding (computed $\Delta\Delta H_{L-D}$ ~+7 kJ mol^{-1}), reflecting the fact that the force field parameters used in the MD simulations were insufficient for accurate calculation of the complexation energies.

Discussion

Macrocycle **1** was previously identified as having rather unique binding properties.[5] It proved to be highly enantioselective forming a 1:1 complex with N-Boc-L-glutamate with an association constant at least two orders of magnitude larger than the corresponding association constant for the 1:1 complex with N-Boc-D-glutamate. Furthermore, it is a generally held view that binding interactions between polar functionalities (e.g. hydrogen bonds) will lead to strong complexation in a non-polar solvent (typically $CHCl_3$), but weak (or negligible) complexation in more polar (competitive) solvents or solvent mixtures, and numerous examples of this phenomenon exist. For example, *N*-tolyl-*N'*-*n*-butylurea binds tetrabutylammonium benzoate with $K_a = 1300\,M^{-1}$ in $CDCl_3$ and $K_a = 150\,M^{-1}$ in [D$_6$]DMSO,[19] and thiourea **21** binds the TBA salt of N-Ac-L-Phe with $K_a = 4800\,M^{-1}$ in $CDCl_3$ and $K_a = 680\,M^{-1}$ in 10% [D$_6$]DMSO/$CDCl_3$.[6b] The lack of binding exhibited by macrocycle **1** in $CDCl_3$, in contrast to the results observed in CH_3CN and DMSO, was thus remarkable. However, NMR studies revealed that in $CDCl_3$ the macrocycle adopts a tightly wrapped conformation that is stabilized by a number of intramolecular hydrogen bonds (Figure 3). This hydrogen-bonding motif has been previously observed in the crystal structure of **21** which forms a dimeric pair in the solid-state.[6b] We postulated that the energy required to reorganize the macrocycle into a suitable binding conformation (i.e., to break the intramolecular hydrogen bonds) was not compensated for by binding interactions that would thereby be established. The more polar solvents, CH_3CN and DMSO, can solvate the hydrogen bonding functionality of the macrocycle leading to a less rigidly constrained molecule, and this is confirmed by the ^{1}H NMR

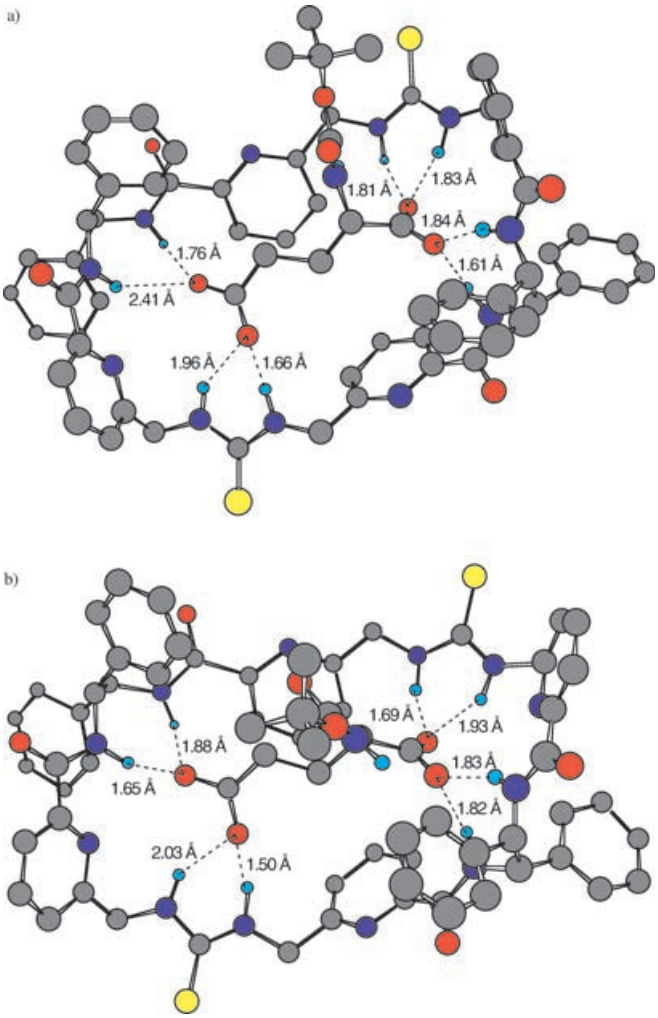

Figure 9. Geometry of the 1:1 complex of macrocycle **1** with: a) N-Boc-L-glutamate; b) N-Boc-D-glutamate, in CH_3CN as determined by molecular modeling showing intermolecular H-bond lengths.

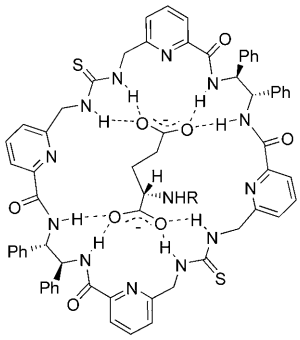

Figure 10. Schematic of mode of complexation of Boc-L-glutamate by macrocycle **1** via eight hydrogen bonds determined by molecular modeling.

spectra which reflect the four-fold D_2 symmetry of the molecule in these solvents. This hypothesis has now been verified by the computational studies which identify a similar lowest energy tightly wrapped conformation for macrocycle **1** in $CHCl_3$ as previously determined by the NMR studies, and a more flexible and open cavity in CH_3CN and DMSO. The computational studies also verify that the energy required to reorganise the wrapped conformation of the macrocycle in $CHCl_3$ into an open conformation suitable for complexation of carboxylates far exceeds the binding energy that would result from such complexation.

Table 3. Reorganisation energy of macrocycle **1** in $CHCl_3$ (Figure 7) into a conformation suitable for binding of N-Boc-D,L-glutamate (Figure 9) versus the subsequent energy gain from binding.

	ΔH [kJ mol^{-1}]	
Energy type[a]	L complex	D complex
binding energy	−100.0	−100.8
reorganisation energy	+296.9	+303.7

[a] As calculated using the MMFFs force field.[25] See text for details.

Solvation of the macrocycle by the more polar solvents allows tight binding of the N-Boc-L-glutamate salt—albeit with some conformational reorganization of the receptor (and associated energetic cost), as evidenced by the large shifts of various CH signals in the ^{1}H NMR spectrum on addition of the guest. Binding of the N-Boc-L-glutamate salt, in these solvents, is driven to a large extent by entropy. The same cavity is less willing to accommodate the enantiomeric guest (N-Boc-D-glutamate) in a simple 1:1 binding mode, and instead binds two N-Boc-D-glutamate guests with a small binding constant for the first glutamate and a significantly larger binding constant for the second (positive cooperativity). At first sight this may seem surprising since binding of the first carboxylate should introduce an electrostatic repulsion which might be expected to disfavour binding of a second carboxylate. However, the two carboxylate guests may bind on opposite faces, and at opposite ends of the macrocycle, in which case the electrostatic repulsion between the two guests should be small. Furthermore, if binding of the first N-Boc-D-glutamate requires considerable reorganization of the receptor, and consequent energetic cost, then, once the first carboxylate is bound, the receptor may bind the second carboxylate without significant additional reorganisation and associated energetic cost. Conformational changes in the receptor, on binding N-Boc-D-glutamate in acetonitrile, were, again, clearly evidenced by the large shifts in many of the CH signals of the receptor in the ^{1}H NMR spectrum, particularly the benzylic CH's ($\Delta\delta_{sat} >$ 0.5 ppm), on addition of N-Boc-D-glutamate.

The binding studies now reported with acetate show that macrocycle **1** forms a relatively weak 1:1 complex with acetate but a strong 1:2 (host/guest) complex. Complexation with acetate led to significant shifts in the NH signals and many of the CH signals of the receptor in the ^{1}H NMR spectrum, very similar to those observed on complexation with N-Boc-D-glutamate. These results are entirely consistent with the hypothesis previously suggested for the complexation of N-Boc-D-glutamate which cannot be as readily accommodated in the cavity of the macrocyle to form a stable 1:1 complex with both carboxylates bound simultaneously.

Not surprisingly macrocycle **1** can also bind N-Ac-glutamate dicarboxylate salts, and binds N-Ac-L-glutamate with a preference for 1:1 binding stoichiometry, whereas 2:1 binding of N-Ac-D-glutamate is preferred. However, the selectivities are not as pronounced as found with the sterically more demanding N-Boc-glutamate substrates. The 1:1 binding of N-Boc-L-glutamate appears in fact to be stronger than the binding of N-Ac-L-glutamate and suggests that the former substrate fits particularly well in the binding pocket. Although the computational studies were unable to calculate or differentiate between the binding energies of the two enantiomers of N-Boc-glutamate, the low energy structures found for the 1:1 binding complex of macrocycle **1** and N-Boc-L-glutamate do indicate that the *tert*-butyl group is in close contact with one of the phenyl residues of the macrocycle, and may thus benefit from a CH–π interaction providing additional stabilisation.[20] It is also noteworthy that the 1:1 binding of N-Ac-L-glutamate is slightly endothermic while the 1:1 binding of N-Boc-L-glutamate is slightly exothermic, but this is compensated to a large extent by the reduced gain in entropy on binding the latter. This would appear to be a typical example of enthalpy–entropy compensation as often found in host–guest systems,[21] and one can speculate that N-Boc-L-glutamate is more rigidly bound (in comparison with N-Ac-L-glutamate), with a stronger enthalpic contribution from hydrogen bonding and possibly CH–π interactions, but with an entropic penalty associated with the greater restriction of the motion of the guest.

Binding studies with N-Boc-L-aspartate (with a shorter separation of the two carboxylates) reveal that macrocycle **1** can also comfortably accommodate this guest to form a strong 1:1 complex in both CH_3CN and DMSO, although the binding kinetics are surprisingly slow in DMSO. However, again the macrocycle is not able to discriminate so readily the other enantiomer and titration experiments in both solvents suggest that with N-Boc-D-aspartate both 1:1 and 1:2 complexes are readily formed. Macrocycle **1** is, however, sensitive to the overall steric bulk of the guest molecule and did not form a strong 1:1 complex with either enantiomer of dibenzoyl tartrate.

We have also now studied the binding properties of several analogous macrocycles **2**–**4**. Macrocycle **2** exhibits some similarities to the binding properties of macrocycle **1**, with a preference for formation of 1:1 complexes with the L-enantiomers of N-Boc-glutamate and N-Boc-aspartate, and 1:2 complexes with the D-enantiomers. However, binding is generally weaker with this macrocycle than with **1** and competition between 1:1 and 1:2 binding stoichiometries is apparent. Furthermore, whereas binding with macrocycle **1** is generally dominated by the entropic contribution, this is less significant for the binding with macrocycle **2**. Thus the greater conformational constraints imparted by the cyclohexyl unit (in comparison to the diphenylethylene unit in **1**) disfavour binding of carboxylates, and also appear to favour the wrapped conformation observed in $CDCl_3$, as evidenced by the well-resolved spectrum obtained in this solvent at room temperature (which is only obtained at −40 °C for macrocycle **1**).

Macrocycles **3** and **4** are both insoluble in $CHCl_3$ and CH_3CN, but macrocycle **3** does form strong complexes with both enantiomers of N-Boc-glutamate in DMSO. The binding selectivities for the two enantiomers of N-Boc-glutamate are similar to those with macrocycle **1**, but binding is in fact stronger with macrocycle **3**. Macrocycle **4** on the other hand appeared not to bind N-Boc-glutamate at all. Thus replacement of the pyrido units in macrocycles **1** and **2** with benzo units in **3** and **4** significantly alters the binding properties. Analogous changes have been investigated in a number of related receptor systems and based on this previous work the pyrido unit was expected to help in preorganising the receptor for carboxylate recognition (Figure 1) with weak hydrogen bonds from adjacent amide and thiourea protons and the pyrido nitrogen.[6] Conversely, however, the presence of the pyrido nitrogen can reduce the anion complexation ability of such systems (relative to the benzo analogue) due to electrostatic repulsion.[22] The ^{1}H NMR signals for the NH protons (amide and thiourea) of **1** and **2** are in each case significantly more downfield than the corresponding signals for macrocycles **3** and **4**, confirming the anticipated interaction of the pyrido nitrogen and adjacent amide and thiourea protons in the former pair of macrocycles. The potential electrostatic repulsion between the pyrido nitrogen and carboxylate oxygens does not, however, appear to hamper the ability of **1** and **2** to complex carboxylates, and the computational studies do suggest an alternative mode of binding to that originally anticipated with each carboxylate oxygen forming hydrogen bonds with either two amide or two thiourea hydrogens (Figures 9 and 10) as opposed to each carboxylate oxygen forming hydrogen bonds to both an amide and a thiourea hydrogen adjacent to a pyridine (Figure 1). This alternative mode would appear to minimise repulsive interactions between carboxylate oxygens and pyrido lone-pairs.

Conclusion

Overall macrocycle **1** is a potent receptor for the N-protected amino acids glutamate and aspartate, with novel enantioselective and solvent-dependent binding properties. Studies on related macrocycles **2**–**4** indicate that if the binding properties of **1** are not entirely unique, they are sensitive to small structural changes, and it is largely fortuitous that **1** was the first of the series of such macrocycles that we prepared and led to the discovery of the unusual binding properties. The detailed study described herein gives some insight into the various energetic contributions to the total binding free energy of even such simple substrates. More specifically the study has also suggested an alternative mode of binding of carboxylate anion by the amidopyridyl thiourea motif to that originally conceived, which minimises the repulsive interaction between the bound anion and the pyridine lone pair (Figures 1 and 10).

Further studies in our laboratory are aimed at developing related macrocycles that can bind glutamate and aspartate derivatives in even more competitive solvents, that is water.

Experimental Section

General methods: Reactions were carried out in solvents of commercial grade and, where necessary, distilled prior to use. THF was distilled under nitrogen from benzophenone and sodium. CH_2Cl_2 was distilled from calcium hydride, as was petroleum ether where the fraction boiling between 40 and 60 °C was used. TLC was conducted on foil backed sheets coated with silica gel (0.25 mm) which contained the fluorescent indicator UV_{254}. Column chromatography was performed on Sorbsil C60, 40–60 mesh silica.

^{1}H NMR spectra were obtained at 300 MHz on Bruker AC300 and Bruker AM 300 spectrometers and at 400 MHz on a Bruker DPX400 spectrometer. ^{13}C NMR spectra were obtained at 75 MHz on Bruker AC300 and Bruker AM 300 spectrometers and at 100 MHz on a Bruker DPX400 spectrometer. Spectra were referenced with respect to the residual solvent peak for the deuterated solvent. Infrared spectra were obtained on BIORAD Golden Gate FTS 135. All melting points were measured in open capillary tubes using a Gallenkamp Electrothermal Melting Point Apparatus and are uncorrected. Optical rotations were measured on a PolAr2001 polarimeter using the solvent stated, the concentration given is in g per 100 mL. Electrospray mass spectra were obtained on a Micromass platform with a quadrupole mass analyser. FAB spectra were obtained on a VG Analytical 70–250-SE normal geometry double focusing mass spectrometer. High-resolution accurate mass measurements were carried out at 10,000 resolution using mixtures of polyethylene glycols and/or polyethylene glycol methyl ethers as mass calibrants for FAB. Semi-preparative reverse-phase HPLC was carried out using a Hewlett Packard HP1100 Chemstation with an automated fractions collector and a Phenomenex C18 prodigy 5 μm (250 mm × 10 mm) column. Water (0.1% TFA) and acetonitrile (0.04% TFA) gradient was employed using elution conditions: time 0 min: 10% CH_3CN/H_2O; 25 min: 90% CH_3CN/H_2O; 40 min: 10% CH_3CN/H_2O. Calorimetric experiments were performed on an Isothermal Titration Calorimeter from Microcal Inc., Northampton, Massachusetts, USA.

Synthetic details

Diamide 6: Pyridyl acid **5** (1.84 g, 6.5 mmol) was suspended in CH_2Cl_2 (15 mL) and cooled to 0 °C. Triethylamine (1.0 mL, 7.15 mmol) was slowly added and a clear solution obtained. Diphenylchlorophosphate (1.35 mL, 6.5 mmol) was added and the solution stirred for 1 h at 0 °C. (1*S*,2*S*)-1,2-Diphenyl-1,2-ethylenediamine-L-tartrate salt (1.18 mg, 3.25 mmol) was suspended in water (7 mL) and K_2CO_3 (2.0 g, 10.7 mmol) added. After 30 min, the solution of diamine was added to the mixed anhydride solution at 0 °C. The resulting mixture was stirred for 2 h at 0 °C, and then at room temperature for 14 h. The organic phase was extracted, washed with 2 M HCl (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic layer was dried over magnesium sulphate and the solvent evaporated in vacuo. The crude material was purified by column chromatography (30% ethyl acetate/petrol ether up to 50% ethyl acetate/petrol ether) to give diamide **6** as a white solid (1.1 g, 46%). R_f = 0.3 (ethyl acetate); m.p. 106–107 °C; $[\alpha]_D^{21}$ = 15.0 (c = 1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (br s, 2H, CHN*H*Ph), 7.97–7.77 (m, 10H, ArH), 7.73 (t, 2H, J = 8 Hz, PyrH), 7.32 (d, 2H, J = 8 Hz, PyrH), 7.22–7.02 (m, 10H, ArH), 5.36 (br s, 2H, C*H*NHPh), 5.07 (d, 2H, J = 16 Hz, PhthNCH_AH$_B$), 5.01 (d, 2H, J = 16 Hz, PhthNCH$_A$$H_B$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.1 (0), 164.1 (0), 154.2 (0), 149.5 (0), 145.8 (0), 138.7 (0), 138.2 (1), 134.2 (1), 132.4 (1), 128.5 (1), 127.7 (1), 123.7 (1), 123.6 (1), 121.2 (1), 59.2 (1), 42.6 (2); IR (neat): ν_{max} = 3230 (m), 1770 (w), 1710 (s), 1670 (m), 1510 (m), 1385 (s), 945 cm^{-1} (s); MS ES$^+$: m/z: 741 $[M+H]^+$, 763 $[M+Na]^+$; HRMS (FAB): m/z: calcd for $C_{44}H_{32}N_6O_6Na$: 763.2281, found 763.2281 $[M+Na]^+$.

Diamine 7: Hydrazine monohydrate (80 μL, 1.64 mmol) was added to a solution of diamide **6** (600 mg, 0.82 mmol) in ethanol (5 mL) and the mixture heated at reflux for 8 h. The solvent was removed in vacuo to afford a white solid which was redissolved in 2 M HCl (5 mL). The solution was refluxed for 30 minutes, after which the insoluble material was removed by filtration. The aqueous solution was basified to pH 9 with 1 M NaOH and the precipitated diamine was extracted with CH_2Cl_2 (5 × 10 mL). The organic layer was dried over $MgSO_4$ and the solvent evaporated in vacuo to afford diamine **7** as a white solid (330 mg, 84%). M.p. 73–75 °C; $[\alpha]_D^{21}$ = 29.4 (c = 1, 10% methanol/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.13 (br s, 2H, N*H*CO), 7.91 (d, 2H, J = 7 Hz, PyrH), 7.64 (t, 2H, J = 7 Hz, PyrH), 7.24 (d, 2H, J = 7 Hz, PyrH), 7.19–7.12 (m, 10H, ArH), 5.63 (br s, 2H, C*H*NH), 3.98 (br s, 4H, CH_2NH$_2$), 1.78 (s, 4H, NH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.0 (0), 149.1 (0), 138.9 (0), 137.9 (0), 128.9 (1), 128.7 (1), 127.9 (1), 127.8 (1), 124.1 (1), 120.4 (1), 59.7 (1), 47.5 (2); IR (neat): ν_{max} = 3362 (w), 1659 (m), 1590 cm^{-1} (w); MS ES$^+$: m/z: 481 $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{28}H_{29}N_6O_2$: 481.2346, found 481.2359 $[M+H]^+$.

Macrocycle 1: Carbon disulfide (414 μL, 6.86 mmol) was added to a mixture of diamine **7** (165 mg, 0.50 mmol) in dry CH_2Cl_2 (10 mL) at −10 °C and the mixture stirred for 1 hour. *N*,*N*′-Dicyclohexylcarbodiimide (200 mg, 0.49 mmol) was added and the mixture stirred for 45 min at −10 °C, then 30 min at room temperature. The excess carbon disulfide and solvent were removed in vacuo yielding the crude bis(isothiocyanate) **8** as a pale yellow solid. Bis(isothiocyanate) **8** in dry CH_2Cl_2 (10 mL) and a further one equivalent of diamine **7** (165 mg, 0.20 mmol) in dry CH_2Cl_2 (10 mL) were simultaneously added over 3 h via syringe pump addition to a solution of DMAP (20 mg, 10% by weight) in CH_2Cl_2 (50 mL) under a slow stream of nitrogen. After stirring at room temperature for 16 h, the excess solvent was removed in vacuo. The resultant yellow residue was purified by column chromatography (30% ethyl acetate/petrol ether up to neat ethyl acetate) yielding macrocycle **1** as a white solid (20.4 mg, 20%) R_f = 0.48 (ethyl acetate); $[\alpha]_D^{21}$ = 146.0 (c = 1, CH_2Cl_2); m.p. 153–154 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 90 °C): δ = 9.38 (d, 4H, J = 6 Hz, NHCO), 8.27 (t, 4H, J = 5 Hz, NHCS), 7.93 (t, 4H, J = 7 Hz, PyrH), 7.84 (d, 4H, J = 7 Hz, PyrH), 7.59 (d, 4H, J = 7 Hz, PyrH), 7.48–7.26 (m, 20H, Ph), 5.82–5.77 (m, 2H, C*H*NHCO), 5.03 (dd, 4H, J = 16, 5 Hz, CH_AH$_B$(Ar)NHCS), 4.95 (dd, 4H, J = 16, 5 Hz, CH$_A$$H_B$(Ar)-NHCS); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.1 (0), 157.3 (0), 149.4 (0), 139.8 (0), 138.7 (0), 128.5 (1), 128.0 (1), 127.6 (1), 127.4 (1), 124.8 (1), 120.8 (1), 58.1 (1), 49.2 (2); IR (neat): ν_{max} = 3310 (br), 1670 (s), 1525 (m), 1455 cm^{-1} (w); MS ES$^+$: m/z: 1045 $[M+H]^+$, 1067 $[M+Na]^+$; HRMS (ES$^+$): m/z: calcd for $C_{58}H_{52}S_2N_{12}O_4$: 1045.3754, found 1045.3748 $[M+H]^+$.

Diamide 9: Pyridyl acid **5** (1.23 g, 4.3 mmol) was suspended in CH_2Cl_2 (10 mL) and cooled to 0 °C. Triethylamine (0.66 mL, 4.73 mmol) was slowly added and a clear solution obtained. Diphenylchlorophosphate (0.89 mL, 4.3 mmol) was added and the solution stirred for 1 hour at 0 °C. (1*S*,2*S*)-1,2-Diaminocyclohexane-L-tartrate salt (564 mg, 2.15 mmol) was suspended in water (3.3 mL) and K_2CO_3 (980 mg) added. After 30 min, the solution of diamine was added to the mixed anhydride solution at 0 °C and the resulting mixture stirred at 0 °C for 2 h, then at room temperature for 14 h. The organic phase was extracted, washed with 2 M HCl (5 mL) and saturated aqueous $NaHCO_3$ (5 mL). The organic layer was dried over magnesium sulphate and the solvent evaporated in vacuo. The crude material was purified by column chromatography (80% ethyl acetate/petrol ether) to afford diamide **9** as a white solid (706 mg, 52%). R_f = 0.49 (ethyl acetate); m.p. 122–124 °C; $[\alpha]_D^{21}$ = 150.7 (c = 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, 2H, J = 8 Hz, NH), 7.95–7.89 (m, 10H, ArH), 7.76 (dd, 4H, J = 7, 3 Hz, PhthH), 7.29 (d, 2H, J = 8 Hz, PyrH), 5.04 (s, 4H, NCH$_2$), 3.81 (m, 2H, NHC*H*), 2.06 (m, 2H), 1.75 (m, 2H), 1.50 (m, 2H), 1.39 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.0 (0), 163.9 (0), 157.2 (0), 149.5 (0), 138.0 (0), 134.1 (1), 132.2 (1), 123.6 (1), 123.3 (1), 121.0 (1), 53.1 (1), 42.6 (2), 31.9 (2), 24.5 (2); IR (neat): ν_{max} = 3260 (m), 1730 (s), 1653 (m), 1535 cm^{-1} (s); MS ES$^+$: m/z: 643 $[M+H]^+$, 665 $[M+Na]^+$; HRMS (ES$^+$): m/z: calcd for $C_{36}H_{30}N_6NaO_6$: 665.2125, found 665.2122 $[M+Na]^+$.

Diamine 10: Hydrazine monohydrate (59.2 μL, 1.22 mmol) was added to a solution of diamide **9** (392 mg, 0.61 mmol) in ethanol (3 mL) and the mixture heated at reflux for 8 h. The solvent was removed in vacuo to

afford a white solid which was redissolved in 2 M HCl (3 mL). The solution was refluxed for 30 min, after which the insoluble material was removed by filtration. The filtrate was basified to pH 9 with 1 M NaOH and washed with CH_2Cl_2 (5×10 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent evaporated in vacuo to afford diamine **10** as a white foam (193 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.54 (brs, 2H, NHCO), 7.96 (d, 2H, J=8 Hz, PyrH), 7.70 (t, 2H, J=8 Hz, PyrH), 7.29 (d, 2H, J=8 Hz, PyrH), 4.10–3.90 (m, 6H, CH_2NH$_2$ and C*H*NH), 2.27 (m, 2H), 2.13 (m, 6H, CH_2 and NH_2), 1.85 (m, 2H), 1.48 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.8 (0), 148.2 (0), 136.8 (0), 126.5 (1), 122.8 (1), 119.3 (1), 52.9 (1), 45.9 (2), 31.3 (2), 23.8 (2); IR (neat): ν_{max}=3430 (m), 1703 (s), 1547 cm^{-1} (m); MS ES$^+$: m/z: 383 $[M+H]^+$; HRMS (ES$^+$): m/z: calcd for $C_{20}H_{27}N_6O_2$: 383.2195, found 383.2188 $[M+H]^+$.

Macrocycle 2: Carbon disulfide (207 μL, 3.7 mmol) was added to a mixture of diamine **10** (100 mg, 0.26 mmol) in dry CH_2Cl_2 (5 mL) at −10 °C and the mixture stirred for 1 h. *N*,*N*′-Dicyclohexylcarbodiimide (107 mg, 0.50 mmol) was added and the mixture stirred for a further 45 min at −10 °C then 30 min at room temperature. The excess carbon disulfide and solvent were removed in vacuo yielding the crude bis(isothiocyanate) **11** as a white solid. Bis(isothiocyanate) **11** in dry CH_2Cl_2 (5 mL) and a further one equivalent of diamine **10** (100 mg, 0.26 mmol) in dry CH_2Cl_2 (5 mL) were simultaneously added over 3 h via syringe pump addition to a solution of DMAP (10 mg, 10% by weight) in CH_2Cl_2 (25 mL) under a slow stream of nitrogen. After stirring at room temperature for 16 h, the excess solvent was removed in vacuo and the resultant yellow residue purified by column chromatography (ethyl acetate) to yield macrocycle **2** as a white solid (156 mg, 72%). R_f=0.54 (ethyl acetate); m.p. 240 °C (decomp); $[\alpha]_D^{21}$ =165.5 (c=1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.24 (brs, 2H, NHCO), 8.73 (brs, 2H, NHCS), 8.15 (d, 2H, J=8 Hz, NHCO), 8.00 (d, 2H, J=7 Hz, PyrH), 7.79 (t, 2H, J=7 Hz, PyrH), 7.72 (brd, 2H, J=7 Hz, NHCS), 7.36 (d, 2H, J=7 Hz, PyrH), 7.23 (brs, 4H, PyrH), 6.85 (brs, 2H, PyrH), 5.42 (m, 2H, C*H*NH), 5.14 (brd, 2H, J=17 Hz, CH_AH$_B$NH), 4.88 (brd, 2H, J=17 Hz, CH$_A$$H_B$NH), 4.34 (d, 2H, J=12 Hz, CH$_C$$H_D$NH), 4.12 (m, 2H, CH$_C$$H_D$NH), 3.69 (m, 2H, C*H*NH), 2.57 (m, 2H), 2.12 (m, 2H), 1.82 (m, 4H), 1.56 (m, 2H), 1.53 (m, 2H), 1.31 (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 182.0 (0), 164.1 (0), 154.2 (0), 148.6 (0), 138.5 (1), 120.0 (1), 117.9 (1), 54.5 (2), 49.0 (1), 30.8 (2), 24.0 (2); MS ES$^+$: m/z: 850 $[M+H]^+$; IR (neat): ν_{max}=3310 (br), 2360 (w), 2330 (w), 1670 (m), 1525 (m), 1450 (w), 1360 (w), 1255 cm^{-1} (w); HRMS (ES$^+$): m/z: calcd for $C_{42}H_{49}N_{12}O_4S_2$: 849.3444, found 849.3448 $[M+H]^+$.

Bisnitrile 13: 3-Cyanobenzoic acid (867 mg, 5.9 mmol) was refluxed in neat thionyl chloride (12 mL) for 4 h. The solvent was removed in vacuo and the residue dissolved in dry CH_2Cl_2 (5 mL). (1*S*,2*S*)-(−)-1,2-Diphenylethylenediamine (400 mg, 1.8 mmol) and DMAP (1.15 g, 9.40 mmol) were added and the mixture stirred at room temperature for 2 d. The solvent was removed in vacuo and the residue purified by column chromatography (50% ethyl acetate/petrol ether) to give bisnitrile **13** as a white solid (420 mg, 48%). R_f=0.33 (50% ethyl acetate/petrol ether); m.p. 243–244 °C; $[\alpha]_D^{23}$ = −102.7 (c=0.8, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.06 (s, 2H, ArH), 7.98 (d, 2H, J=8 Hz, NH), 7.81 (d, 2H, J=8 Hz, ArH), 7.56 (t, 2H, J=8 Hz, ArH), 7.32 (d, 2H, J=8 Hz, ArH), 7.20–7.10 (m, 10H, Ph), 5.58 (d, 2H, J=8 Hz, NHC*H*); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 171.6 (0), 166.5 (0), 140.3 (0), 136.4 (0), 135.9 (1), 132.6 (1), 132.2 (1), 130.6 (1), 129.3 (1), 128.9 (1), 128.6 (1), 119.0 (0), 58.4 (1); IR (neat): ν_{max}=3287 (b), 2981 (w), 2927 (w), 1696 (s), 1631 cm^{-1} (s); MS ES$^+$: m/z: 471 $[M+H]^+$, 963 $[2M+Na]^+$; HRMS (ES$^+$): m/z: calcd for $C_{60}H_{44}N_8NaO_4$: 963.3377, found 963.3369 $[2M+Na]^+$.

Dicarbamate 14: Di-*tert*-butyl dicarbonate (1.25 g, 5.72 mmol) and $NiCl_2$ (371 mg, 2.86 mmol) were added to a solution of **13** (673 mg, 1.43 mmol) in THF (15 mL) and MeOH (10 mL). Sodium borohydride (757 mg, 20.0 mmol) was carefully added to the mixture at 0 °C and the mixture stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (20 mL) and saturated aqueous $NaHCO_3$ (10 mL). The insoluble precipitate was removed by filtration and washed with ethyl acetate (2×15 mL). The combined organic layers were dried over $MgSO_4$ and evaporated in vacuo and triturated with $CHCl_3$ to afford dicarbamate **14** as a white solid (618 mg, 64%). R_f=0.83 (5% MeOH in CH_2Cl_2); m.p. 187–188 °C; $[\alpha]_D^{23}$=−40.5 (c=1.0, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.97 (d, 2H, J=8 Hz, N*H*CH), 7.59–7.54 (m, 4H, ArH), 7.46–7.32 (m, 10H, ArH), 7.31–7.25 (m, 4H, ArH), 7.15 (t, 2H, J=7 Hz, NHCO), 5.67 (d, 2H, J=8 Hz, C*H*NH), 4.14 (d, 4H, J=8 Hz, CH_2NH), 1.37 (s, 18H, CH_3); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 166.5 (0), 155.7 (0), 140.6 (0), 140.4 (0), 134.8 (0), 129.6 (1), 128.1 (1), 127.8 (1), 127.2 (1), 126.7 (1), 125.9 (1), 125.3 (1), 77.8 (0), 57.2 (1), 43.1 (2), 28.2 (3); IR (neat): ν_{max}=3295 (m), 2973 (w), 1689 (s), 1630 (s), 1528 (s), 1361 (m), 1243 cm^{-1} (m); MS ES$^+$: m/z (%): 679 $[M+H]^+$, 701 $[M+Na]^+$ (100), 1379 (15) $[2M+Na]^+$; HRMS (ES$^+$): m/z: calcd for $C_{40}H_{46}N_4NaO_6$: 701.3315, found 701.3320 $[M+Na]^+$.

Diamine 15: Dicarbamate **11** (312 mg, 0.46 mmol) was dissolved in TFA/CH_2Cl_2 1:1 (10 mL) and the solution stirred at room temperature for 2 h. Toluene (10 mL) was added and the solvent evaporated in vacuo to give the bis-TFA salt as a clear oil (294 mg, quantitative). The salt was dissolved in CH_2Cl_2 (10 mL), washed with 10% aqueous K_2CO_3 (10 mL), dried with $MgSO_4$ and evaporated in vacuo to give the diamine **15** as a white foam (206 mg, 70%). M.p. 283 °C; $[\alpha]_D^{23}$ = −89.1 (c=0.7, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.29 (brs, 2H, N*H*CH), 7.77–7.53 (m, 4H, ArH), 7.43 (d, 2H, J=8 Hz, ArH), 7.35–7.10 (m, 12H, ArH), 5.69 (d, 2H, J=8 Hz, C*H*NH), 4.02 (brs, 4H, CH_2NH$_2$), 1.77 (s, 4H, NH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 166.7 (0), 141.4 (0), 140.6 (0), 134.7 (0), 127.7 (1), 127.5 (1), 127.4 (1), 126.7 (1), 126.5 (1), 126.0 (1), 125.2 (1), 57.4 (1), 45.6 (2); IR (neat): ν_{max}=3298 (w), 1633 cm^{-1} (s); MS ES$^+$: m/z: 479 $[M+H]^+$, 501 $[M+Na]^+$, 979 $[2M+Na]^+$; HRMS (ES$^+$): m/z: calcd for $C_{30}H_{31}N_4O_2$: 479.2448, found 479.2442 $[M+H]^+$.

Macrocycle 3: Diamine **15** (200 mg, 0.42 mmol) was dissolved in CH_2Cl_2 (5 mL) and 0.5 M aqueous K_2CO_3 added (5 mL). After stirring for 20 min, thiophosgene (127 μL, 1.67 mmol) was added directly to the organic layer and the solution stirred for 16 h. The organic layer was washed with 2 M aqueous HCl (10 mL), dried over $MgSO_4$ and the solvent evaporated in vacuo to give crude bis(isothiocyanate) **16** as a pale yellow foam. The bisisocyanate in dry CH_2Cl_2 (5 mL) and a further one equivalent of diamine **15** (200 mg, 0.42 mL) in dry CH_2Cl_2 (5 mL) were simultaneously added over 3 h via syringe pump addition to a solution of triethylamine (116 μL, 0.84 mmol) in dry CH_2Cl_2 (50 mL) under a slow stream of nitrogen. The mixture was stirred at room temperature for 16 h. Macrocycle **3** precipitated out of solution as a yellow solid. The compound was removed by filtration and recrystallised (DMF/diethyl ether) to give macrocycle **3** as a pale yellow solid (68 mg, 17%). M.p. 240 °C (decomp); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.00 (brs, 4H, NHCO), 7.95 (brs, 4H, NHCS), 7.61–7.57 (m, 8H, ArH), 7.36 (m, 16H, ArH), 7.22–7.20 (m, 8H, ArH), 7.14–7.12 (m, 4H, ArH), 5.66 (4d, H, J=5 Hz, C*H*NH), 4.68 (brs, 8H, CH_2NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 168.5 (0), 164.4 (0), 142.7 (0), 136.8 (0), 131.9 (1), 130.2 (1), 129.6 (1), 129.4 (1), 128.9 (1), 128.4 (1), 127.9 (1), 59.4 (1), 50.6 (2); IR (neat): ν_{max}=3271 (b), 1637 (s), 1529 cm^{-1} (s); MALDI MS: m/z: 1041 $[M+H]^+$, 1063 $[M+Na]^+$; elemental analysis calcd (%) for $C_{62}H_{56}N_8O_4S_2$: C 71.54, H 5.42; N 10.76, S 6.16; found C 71.11, H 5.28, N 10.36, S 5.66.

Bisnitrile 17: Diphenylchlorophosphate (4.15 mL, 0.02 mol) was added to a solution of 3-cyanobenzoic acid (3 g, 0.02 mol) and triethylamine (3.1 mL, 0.022 mol) in CH_2Cl_2 (15 mL) and the solution stirred for 1 h at 0 °C. (1*S*,2*S*)-1,2-Diaminocyclohexane-L-tartrate salt (2.62 g, 0.01 mol) was suspended in water (15 mL) and K_2CO_3 (4.5 g, 0.03 mol) added. After 30 min, the diamine solution was added to the mixed anhydride solution at 0 °C. The resulting mixture was stirred at 0 °C for 2 h and then at room temperature for 14 h. The organic phase was washed with 2 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and then dried over $MgSO_4$. The solvent was evaporated in vacuo and the residue purified by flash column chromatography (ethyl acetate) to give bisnitrile **17** as a white solid (3.61 g, 97%). R_f=0.60 (ethyl acetate); m.p. 236–237 °C; $[\alpha]_D^{22}$ = 191 (c=1.0, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.50 (d, 2H, J=8 Hz, NH), 8.05 (s, 2H, ArH), 7.95 (d, 2H, J=8 Hz, ArH), 7.88 (d, 2H, J=8 Hz, ArH), 7.57 (t, 2H, J=8 Hz, ArH), 3.91 (m, 2H, C*H*NH), 1.72 (m, 2H), 1.50 (m, 4H), 1.26 (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 164.5 (0), 135.9 (0), 134.4 (0), 131.9 (1), 130.7 (1), 129.6

(1), 118.3 (0), 111.3 (1), 53.1 (1), 31.4 (2), 24.7 (2); IR (neat): $\nu_{max}=3273$ (m), 1630 (s), 1541 cm^{-1} (s); MS ES$^+$: *m/z*: 373 $[M+H]^+$, 410 $[M+Na]^+$; HRMS (ES$^+$): *m/z*: calcd for $C_{44}H_{40}N_8NaO_4$: 767.3065, found 767.3065; elemental analysis calcd (%) for $C_{22}H_{20}N_4O_2$: C 70.95, H 5.41, N 15.04; found C 70.77, H 5.41, N 14.93.

Dicarbamate 18: Di-*tert*-butyl dicarbonate (2.34 g, 10.7 mmol) and $NiCl_2$ (695 mg, 5.36 mmol) were added to a solution of bisnitrile **17** (1.0 g, 2.68 mmol) in THF (10 mL) and MeOH (15 mL). Sodium borohydride (1.42 g, 38.0 mmol) was carefully added at 0 °C and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the precipitate dissolved in ethyl acetate (20 mL) and saturated aqueous $NaHCO_3$ (10 mL). The insoluble precipitate was filtered off and washed with ethyl acetate (2×10 mL). The combined organic layers were dried over $MgSO_4$ and evaporated in vacuo to afford a white solid. The crude material was purified by column chromatography (2% MeOH/$CHCl_3$) to yield dicarbamate **18** as a white solid (925 mg, 91%). $R_f=0.58$ (ethyl acetate); m.p. 101–102 °C; $[\alpha]_D^{22}=58.1$ (c=0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (s, 2H, ArH), 7.58 (d, 2H, *J*=8 Hz, ArH), 7.41–7.30 (m, 4H, ArH), 6.75 (brs, 2H, NH), 5.00 (brs, 2H, NH), 4.28 (d, 4H, *J*= 6 Hz, CH_2N), 3.99 (m, 2H, C*H*NH), 2.21 (m, 2H), 1.83 (m, 2H), 1.43 (m, 22H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.1 (0), 167.9 (0), 139.7 (0), 134.6 (0), 130.6 (1), 128.9 (1), 126.1 (1), 125.8 (1), 79.8 (0), 54.7 (1), 44.5 (2), 32.5 (2), 28.5 (2), 24.9 (3); IR (neat): $\nu_{max}=3303$ (b), 2981 (w), 2927 (w), 2852 (w), 1696 (s), 1631 cm^{-1} (s); MS ES$^+$: *m/z*: 581 $[M+H]^+$, 603 $[M+Na]^+$; HRMS (ES$^+$): *m/z*: calcd for $C_{32}H_4N_4NaO_6$: 603.3153, found 603.3148 $[M+Na]^+$.

Diamine 19: Dicarbamate **18** (500 mg, 0.86 mmol) was dissolved in 1:1 solution of TFA and CH_2Cl_2 (10 mL). The solution was stirred at room temperature for 2 h. Toluene (10 mL) was added and the solvent evaporated in vacuo to give a clear oil. Trituration of the oil with diethyl ether provided the trifluoroacetate salt of diamine **19** as a white solid (450 mg, 86%). M.p. 170 °C (decomp); $[\alpha]_D^{22}$ = 61 (*c*=1.0, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.26 (brs, 2H, NHCO), 7.86 (s, 2H, ArH), 7.71 (d, 2H, *J*=8 Hz, ArH), 7.54 (d, 2H, *J*=8 Hz, ArH), 7.45 (m, 2H, ArH), 4.47 (brs, 6H, NH_3^+), 4.03 (brs, 4H, CH_2NH$_3^+$), 3.95 (m, 2H, C*H*NH), 1.90 (m, 2H), 1.70 (m, 2H), 1.52 (m, 2H), 1.30 (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.6 (0), 134.8 (0), 133.7 (0), 130.9 (1), 128.1 (1), 127.7 (1), 126.4 (1), 52.6 (1), 41.7 (2), 31.2 (2), 24.2 (2); IR (neat): $\nu_{max}=3290$ (m), 2935 (b), 1667 (m), 1635 (s), 1533 (s), 1179 (s), 1120 cm^{-1} (s).

Macrocycle 4: Diamine **19** (200 mg, 0.53 mmol) was dissolved in CH_2Cl_2 (10 mL) and 0.5 M aqueous K_2CO_3 added (5 mL). Thiophosgene (160 μL, 2.1 mmol) was added directly to the organic layer and the solution stirred for 16 h. The organic phase was extracted and washed with 2 M HCl (10 mL), dried over $MgSO_4$ and the solvent evaporated in vacuo to give bis(isothiocyanate) **20** as a pale yellow foam. The bisisocyanate in dry CH_2Cl_2 (5 mL) and a further one equivalent of diamine **15** (200 mg, 0.53 mmol) in dry CH_2Cl_2 (5 mL) were simultaneously added over 3 h via syringe pump addition to a solution of triethylamine (183 μL, 1.3 mmol) in dry CH_2Cl_2 (50 mL) under a slow stream of nitrogen. The mixture was stirred at room temperature for 16 h. Macrocycle **4** precipitated out of solution as a pale yellow solid and purified by semi-preparative RP-HPLC (127 mg, 29%). M.p. 228–229 °C; $[\alpha]_D^{22}$ = 104 (*c*=0.5, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.20 (d, 4H, *J*=7 Hz NHCO), 7.92 (brs, 4H, NHCS), 7.66 (m, 4H, ArH), 7.33 (brs, 8H, ArH), 4.63 (s, 8H, CH_2NH), 3.94 (m, 4H), 1.91 (m, 4H), 1.75 (m, 4H), 1.53 (m, 4H), 1.31 (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 167.0 (0), 162.8 (0), 137.5 (0), 134.8 (0), 128.0 (1), 126.5 (1), 125.4 (1), 120.8 (1), 52.9 (1), 35.7 (2), 30.7 (2), 24.6 (2); IR (neat): $\nu_{max}=3276$ (b), 3056 (w), 2983 (w), 2862 (w), 1636 (s), 1534 cm^{-1} (s); MS MALDI in DMSO: *m/z*: 867 $[M+Na]^+$; elemental analysis calcd (%) for $C_{46}H_{52}N_8O_4S_2$: C 65.38, H 6.20, N 13.25, S 7.59; found C 65.07, H 5.87, N 13.02, S 7.15.

Computational details

Macrocycle solvent dependent conformational preferences: For macrocycle **1** in $CHCl_3$, to locate the lowest energy conformations, 5000 steps of the mixed mode MCMM/LMCS conformational search algorithm were performed. Within the MCMM/LMCS algorithm, a mixture of Monte Carlo Multiple Mimima (MCMM) steps[23] or low mode conformational steps (LMCS) is used.[24] For MCMM steps, 2 to 23 ($N-1$) of the defined macrocyclic torsions (where N is the number of defined torsions) were randomly adjusted; for LMCS steps, the low frequency eigenvectors ("soft" vibrational modes) in the system were used to direct the search. The definition of ring opening/closures allowed for the torsional variations within the macrocycle. A 1:1 ratio of Monte Carlo torsional moves to low mode moves was used. The Monte Carlo Structure Selection (MCSS) option used was a "random walk" search where the most recent structure within a 75 kJ mol^{-1} energy window of the current "global" minimum was used as the starting structure for the subsequent MC step. Finally, the MMFFs force field[25] was used in combination with the GB/SA solvation model for $CHCl_3$.[16] Computations were performed using MacroModel 8.0.[18]

Since there is no GB/SA parametrization available within MacroModel for the solvents DMSO and CH_3CN, for studies of macrocycle **1** in these solvents explicit solvation models were used. The macrocycle was therefore imbedded in previously equilibrated cubic boxes containing either 512 DMSO or CH_3CN solvent molecules with experimental densities. For CH_3CN, fully flexible six-site all-atom OPLS-AA(2001)[26] CH_3CN models were equilibrated in a $35.42\times35.42\times35.42$ Å^3 dimensional box over a series of simulations and temperatures first in the isothermal–isochoric (NVT) ensemble (T=50, 150, 225 and 298.15 K) and then the isothermal-isobaric (NPT) ensemble (T=298.15 K) so that the experimental density[27] of acetonitrile at 298.15 K and 1 atm pressure (0.7857 g cm^{-3}) was close to reproduced. In the case of DMSO, a $39.28\times39.28\times39.28$ Å^3 box was used corresponding to an experimental density of 1.096 g cm^{-3}.[27] Final boxes for each solvent were within 2% of the experimental densities. Two starting conformations for macrocycle **1** were used for the simulations: the C_2 conformation found as the most stable comformation in $CHCl_3$; and a more planar open type conformation where the intramolecular hydrogen bonds for the C_2 conformation did not have to be broken. Overlapping solvent molecules and those giving rise to repulsive interactions with macrocycle were removed and short minimizations (1000 steps, steepest descent algorithm) performed to remove bad contacts. MD simulations by using periodic boundary conditions (PBC) and the Verlet algorithm in the isothermal-isobaric ensemble (NPT) were subsequently performed (298.15 K, 1 atm). A 30 ps equilibration period was followed by a 60 ps data collection phase using a timestep of 1 fs. The OPLS-AA-(2001) force field[26] was used due to the unavailability of MMFFs for use with Impact 2.7.[17] Conformational preferences of the macrocycle were analyzed from the resulting MD trajectories for the data collection phase. For both solvents a D_2 type conformation located starting from the open "unwrapped" conformation was found to be energetically more stable. The C_2 conformation remained close to its wrapped conformation over the time period of the simulations—which in DMSO and CH_3CN is unfavorable compared to having D_2 symmetry. Finally, as stated MD simulations were performed using Impact 2.7.[17]

Solvent dependent binding preferences: For macrocycle **1** and 1:1 binding of N-Boc-D,L-glutamate, binding in the two solvents $CHCl_3$ and CH_3CN was computed as follows. Again for $CHCl_3$, the GB/SA model was used in a mixed mode MCMM/LMCS search. There were a number of different conditions used to those for macrocycle **1** alone. Importantly, global ligand translations and rotations were also included and were seen as crucial to success of the conformational search. In total, 2 to 5 torsions were adjusted each MCMM step, with a 1:1 ratio of MC torsional moves and/or ligand translation to the low mode moves. A MCSS usage-directed structure selection option was used so that the least investigated structure was used as the starting point for the next MC step. 10000 MCMM/LMCS steps by using the MMFFs force field were performed with an energy window of 25 kJ mol^{-1} above the global minimum used for saving conformations. Computations were performed using MacroModel 8.1.

For binding in CH_3CN, MD simulations using the Verlet algorithm were performed in explicit solvent. Macrocycle **1** with bound enantiomer (L or D N-Boc-glutamate) were introduced into the previously equilibrated (as described above) cubic box containing 512 CH_3CN molecules. Overlapping solvent molecules and those giving rise to repulsive van der Waals (vdW) interactions with the complex were then removed and short minimizations (1000 steps, steepest descent algorithm) performed to remove

any bad contacts. Minimizations were followed by NPT simulations (temperature 298.15 K; pressure 1 atm) using PBC: 200 ps equilibration preceded 400 ps data collection phases using a timestep of 1 fs for both L and D bound complexes. The OPLS-AA(2001) force field was used, with a non-bond cutoff of 10 Å for van der Waals interactions and an Ewald treatment for electrostatics.[28] For the binding enthalpies in CH_3CN, ΔH_L can be calculated as follows:

$$\Delta H_L = H_{R-L} - H_R - H_L \quad (1)$$

where H_{R-L} corresponds to the average enthalpy of the complex of the macrocyclic receptor with the L enantiomer from the MD simulations, and H_R and H_L correspond to the enthalpies for the isolated macrocycle and L enantiomer conformations, respectively. An analogous expression exists for ΔH_D. However, as the isolated enantiomeric energies are the same ($H_L = H_D$), MD simulations of the receptor bound L and D complexes in CH_3CN were sufficient to compute $\Delta\Delta H_{L-D}$ (298.15 K):

$$\Delta\Delta H_{L-D} = H_{R-L} - H_{R-D} \quad (2)$$

Individual H_{R-L} (and H_{R-D}) values were summed from the following enthalpic components:

$$H_{R-L} = [H_{intra} + H_{non-b} + H_{solv}]_{L-complex} \quad (3)$$

where H_{intra} and H_{non-b} are the intramolecular and non-bond interaction energies within the L (or D) complexes and H_{solv} is the solvation energy of the complex. Simulations were performed by using Impact 2.7.

Finally, to extract the most representative and predominant type of conformations from the MD trajectories, conformations were superimposed and clustered into groups of similar conformations according to root mean square (RMS) distances between heavy atoms. A representative conformation was obtained for each cluster. The representative conformation from the largest cluster is the most important, and is reported and discussed in the results. Clustering calculations were performed using NMRclust 1.2.[29]

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