# Selective binding of L-glutamate derivative in aqueous solvents†

**Sandra Bartoli, Tariq Mahmood, Abdul Malik, Sally Dixon and Jeremy D. Kilburn***

***Received 22nd February 2008, Accepted 25th March 2008***
***First published as an Advance Article on the web 25th April 2008***
**DOI: 10.1039/b803089h**

A chiral bisguanidinium macrocycle binds *N*-Boc-L-glutamate in a 1 : 1 stoichiometry with significant selectivity in competitive solvent (DMSO–$H_2O$).

## Introduction

Recognition of polar molecules by synthetic receptors in aqueous solvents is a formidable challenge because of the high degree of solvation that has to be overcome. The development of synthetic receptors for enantioselective binding of L-glutamate and L-aspartate derivatives has been of particular interest because of the critical role these amino acids play in the central nervous system as excitatory transmitters. Several groups have successfully developed receptors able to bind a range of dicarboxylates[1–8] (including glutamate and aspartate) in protic solvents, typically using polyamine macrocyclic receptors (which generally exist, at physiological pH, as a mixture of several protonated forms, all of which may contribute to binding),[1,2] the metal complexes derived from such polyamines,[3] or appropriately functionalised guanidinium or amidinium derivatives.[4–6] However, examples of enantioselective receptors for such dicarboxylates in protic solvents remain rare.[7]

We recently described bisthiourea macrocycles **1** and **2** which it was anticipated could serve as enantioselective receptors for dicarboxylates, such as glutamate or aspartate, using two thiourea–carboxylate interactions with additional hydrogen bonds between the macrocyclic amides and the carboxylate groups (Fig. 1). Macrocycles **1** and **2** proved to be highly selective for the 1 : 1 binding of *N*-Boc-L-glutamate in both acetonitrile and the more competitive solvent dimethylsulfoxide (DMSO),[9] with the glutamate guest accommodated within the macrocyclic cavity [Fig. 1, i)]. Binding of *N*-Boc-D-glutamate on the other hand was predominantly 1 : 2 (host–guest) stoichiometry in DMSO [Fig. 1, ii)], with a weak 1 : 1 (host H + guest G) binding constant and a significantly stronger stepwise 1 : 2 (HG + G) binding constant. The apparent cooperativity with stronger binding of the second anionic guest than the first, a positive allosteric effect, is at first sight surprising but the two anionic guests may be a significant distance apart and therefore experience little electrostatic repulsion when both are bound. Furthermore, macrocycle **1** adopts a wrapped conformation in chloroform that is stabilised by a number of intramolecular hydrogen bonds, particularly from an amide carbonyl to the thiourea NHs and adjacent amide NH, a hydrogen bonding motif previously described by the dimeric pair crystal structure of acyclic pyridyl thiourea analogues of **2**.[10] In chloroform, macrocycle **1** does not bind carboxylates at all because the energy required to reorganise the macrocycle into a suitable binding conformation (*i.e.* to break the intramolecular hydrogen bonds) is not compensated by binding interactions that would thereby be established. In DMSO, solvation of the intramolecular hydrogen bonds weakens the preference for a tightly wrapped conformation unsuitable for binding a carboxylate guest, but binding of the first carboxylate group may still require a significant reorganisation of the molecule and consequent energetic penalty, which does not then have to be paid when a second carboxylate is bound.

In order to develop similarly selective receptors able to bind glutamate in more competitive aqueous solvent systems, we have now prepared bisguanidinium macrocycle **3** and investigated its binding properties with glutamate and aspartate derivatives. In 50 : 50 DMSO–$H_2O$ solution, macrocycle **3** forms a strong 1 : 1 complex with *N*-Boc-L-glutamate, but 1:2 complexation is preferred with *N*-Boc-D-glutamate or either enantiomer of *N*-Boc-aspartate.

## Results

### Synthesis

Direct conversion of bisthiourea macrocycle **1** to bisguanidinium macrocycle **3**, *via* treatment of an intermediate *S*-alkyl bisthiouronium macrocycle with ammonia, was not successful. The desired macrocycle was instead assembled *via* carbodiimide mediated condensation of a biscarbamoyl thiourea with a bisamine;[11] *via* an efficient synthetic strategy involving use of the bisamine as both precursor and condensation partner to the bis-carbamoyl thiourea (Scheme 1).

Efficient coupling of 3-cyanobenzoic acid with (1*S*,2*S*)-diphenylethylene diamine, followed by nitrile reduction, also in good yield, gave bisamine **7**. Conversion to a bis-carbomyl thiourea **8**, upon treatment of **7** with benzyloxycarbonyl isothiocyanate,[12] was straightforward. Coupling of bisthiourea **8** with bisamine **7** gave the protected bisguanidine macrocycle **9**, in a modest optimised yield of 29% critically dependent upon the use of 2 equivalents of DMAP in this step; the first demonstration of carbamoyl thiourea–amine condensation utility in macrocyclisation. Removal of the Cbz protecting groups was readily achieved by hydrogenolysis to give the desired macrocycle **3**, as its bis(hexafluorophosphate) salt.

*School of Chemistry, University of Southampton, Southampton, UK SO17 1BJ. E-mail: jdk1@soton.ac.uk; Fax: +44 (0)2380 596805; Tel: +44 (0)2380 593596*

† Electronic supplementary information (ESI) available: NMR and ITC titration data for all binding studies. See DOI: 10.1039/b803089h

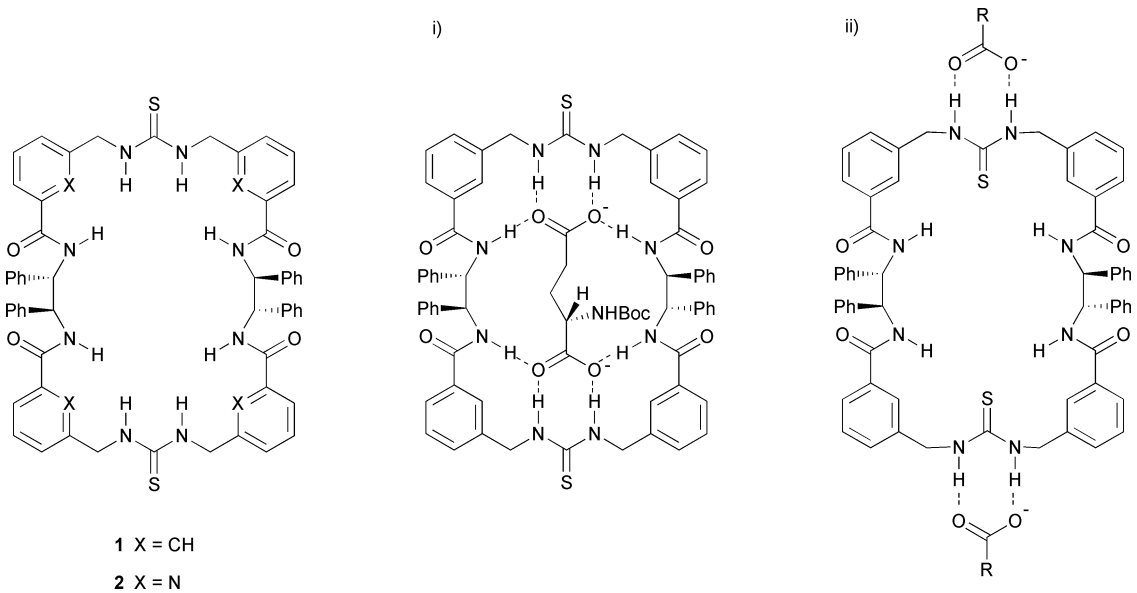
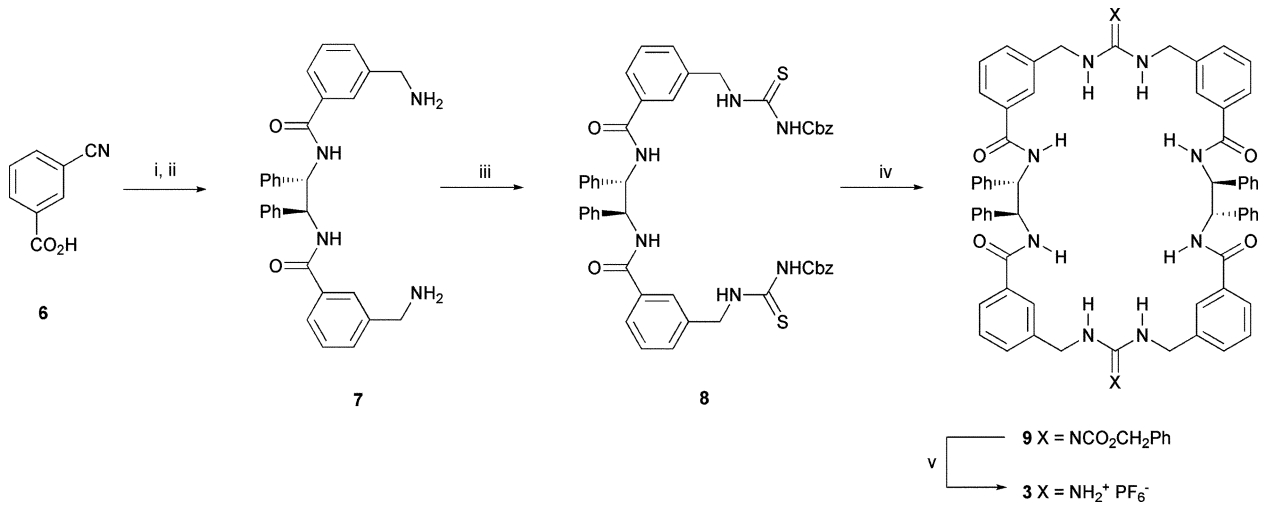



**Fig. 1** i) 1 : 1 complex of bisthiourea macrocycle **1** with *N*-Boc-L-glutamate ($K_a^{1:1} = 2.7 \times 10^4$ M$^{-1}$, $K_a^{1:2} = 3.9 \times 10^2$ M$^{-1}$ in DMSO); ii) 1 : 2 complex of bisthiourea macrocycle **1** with *N*-Boc-D-glutamate ($K_a^{1:1} = 2.6 \times 10^2$ M$^{-1}$, $K_a^{1:2} = 1.9 \times 10^4$ M$^{-1}$ in DMSO).




**Scheme 1** *Reagents and conditions*: i) $(PhO)_2P(O)Cl$, $NEt_3$, $CH_2Cl_2$, then (1*S*,2*S*)-diphenylethylene diamine, $K_2CO_3$, $H_2O$, 90%; ii) $H_2$, Pd/C, TFA–DMF–MeOH, then $NEt_3$, 83%; iii) $PhCH_2O_2CNCS$, DMF–$CH_2Cl_2$, 73%; iv) **7**, EDC, $CH_2Cl_2$–DMF, DMAP, 29%; v) $H_2$, Pd/C, MeOH–DMF, then $HPF_6$, $CH_2Cl_2$–MeOH, 77%.

## Binding characterisation

Macrocycle **3** gave a well-resolved $^1$H NMR spectrum in $d_6$-DMSO and in 5% $CD_3OD$–$CDCl_3$, consistent with the expected $D_2$ symmetry. Binding studies with macrocycle **3** were first conducted by $^1$H NMR titration in $d_6$-DMSO (Fig. 2).†[13,14] Addition of *N*-Boc-L-glutamate (as the bis(tetrabutylammonium) dicarboxylate salt) led to downfield shifts of the guanidinium NH resonance ($\Delta\delta_{max} > 1.3$ ppm) and amide NH resonance ($\Delta\delta_{max} > 1.0$ ppm), consistent with the formation of hydrogen bonds. The titration curve did not reach saturation (6.4 equiv. added guest) and data could not be fitted to a simple 1 : 1 (host–guest) binding isotherm, but to a 1 : 2 (host–guest) binding isotherm in which binding is dominated by a large 1 : 1 association (H + G) ($K_a^{1:1} > 10^4$ M$^{-1}$) with a small contribution from sequential 1 : 2 binding (HG + G) ($K_a^{1:2} \sim 2 \times 10^3$ M$^{-1}$). A similar downfield shift of the amide NH resonance was observed on addition of 5.6 equiv. *N*-Boc-D-glutamate, (also as the bis(tetrabutylammonium) dicarboxylate salt, $\Delta\delta_{max} \sim 1.2$ ppm), to macrocycle **3** and, once again, a sigmoidal data plot was obtained which could not be fitted assuming 1 : 1 binding and indicates two binding stoichiometries.[15] Fitting using a 1 : 2 (host–guest) binding isotherm indicated that binding of *N*-Boc-D-glutamate is dominated by 1 : 2 binding ($K_a^{1:1} \sim 10^3$ M$^{-1}$, $K_a^{1:2} > 10^4$ M$^{-1}$). Hence macrocycle **3** shows similar binding selectivity, and preference for 1 : 1 binding with *N*-Boc-L-glutamate, in DMSO, as the thiourea analogue **1**.

Investigation of the binding properties of macrocycle **3** in a more competitive solvent system, 50% $H_2O$–DMSO in which the macrocycle gives a poorly resolved $^1$H NMR spectrum,[16] was made using isothermal calorimetry (ITC).[17–19] Titration of **3**

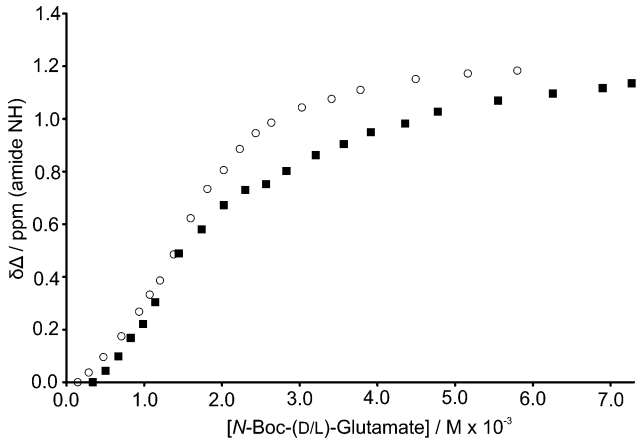



**Fig. 2** NMR binding titration of macrocycle **3** with *N*-Boc-L-glutamate (■), [**3**]/mM = 1.75, and *N*-Boc-D-glutamate (○), [**3**]/mM = 1.30, in $d_6$-DMSO.

with the bis(tetrabutylammonium) dicarboxylate salt of *N*-Boc-L-glutamate gave a titration curve (Fig. 3)† which could be fitted using a two-site binding model, giving a large 1 : 1 binding constant, $K_a^{1:1}$ $3.8 \times 10^4$ $M^{-1}$ ($\Delta G^{1:1} = -26.1$ kJmol$^{-1}$), and a smaller sequential 1 : 2 (host–guest) binding constant, $K_a^{1:2} = 5.3 \times 10^3$ $M^{-1}$. The data indicate that the predominant 1 : 1 binding is both enthalpically and, to a lesser extent, entropically favourable (Table 1, entry 1), whereas the subsequent binding of the second equivalent is largely driven by favourable entropy.

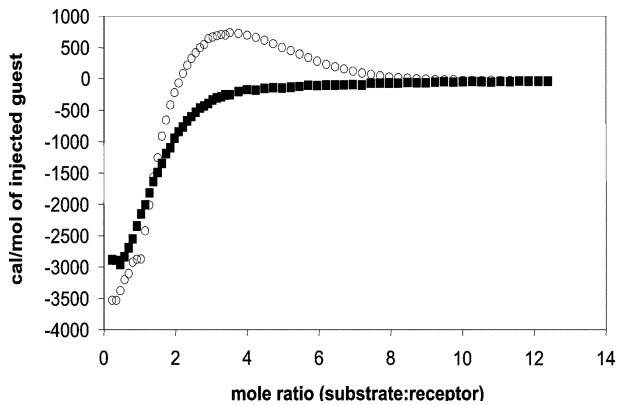



**Fig. 3** ITC data for macrocycle **3** with *N*-Boc-L-glutamate (■) and *N*-Boc-D-glutamate (○) in 50 : 50 DMSO–$H_2O$.

Titration of **3** with the bis(tetrabutylammonium) dicarboxylate salt of *N*-Boc-D-glutamate indicated a large 1 : 2 (host–guest) binding constant, $K_a^{1:2}$ $1.4 \times 10^4$ $M^{-1}$ ($\Delta G^{1:1} = -24.0$ kJ mol$^{-1}$), and a smaller 1 : 1 binding component, $K_a^{1:1}$ $2.9 \times 10^3$ $M^{-1}$ (Table 1, entry 2). ITC titration of macrocycle **3** with the two enantiomers of *N*-Boc-aspartate was also carried out, and in both cases gave predominantly 1 : 2 binding ($K_a^{1:2} > 10^4$ $M^{-1}$) (Table 1, entries 3 and 4), and a titration curve very similar to that observed for *N*-Boc-D-glutamate. In each of these three cases the dominant 1 : 2 binding was largely driven by the enthalpic contribution, whereas the smaller 1 : 1 binding was enthalpically very unfavourable, and was driven by a large entropic contribution.

Overall the data suggest that there is a precise and tight fit within the macrocyclic cavity for the preferred guest, *N*-Boc-L-glutamate, with strong hydrogen bonding interactions to both carboxylate groups of the glutamate (Fig. 4) and enthalpically favourable 1 : 1 binding. Sequential binding, to form the 1 : 2 complex, presumably involves displacement of one carboxylate functionality of the internally bound glutamate and binding to the carboxylate of a second glutamate, with no net increase in hydrogen bonding, but leading to a much more flexible complex. Formation of the 1 : 2 complex from the 1 : 1 complex might therefore be expected to be enthalpically neutral but entropically favourable, as observed experimentally. Tight binding of *N*-Boc-D-glutamate (and similarly *N*-Boc-L- and D-aspartate) is presumably not possible within the cavity of macrocycle **3** and instead initial 1 : 1 binding may only involve a single carboxylate–thiourea interaction on the outside of the macrocycle. The data indicate that this process is both highly endothermic and entropically favourable, presumably because this first binding event involves substantial reorganisation of macrocycle **3**, breaking of a number of intramolecular hydrogen bonds and desolvation of tightly bound solvent molecules (in contrast binding of *N*-Boc-L-glutamate described above leads to a much more rigid 1 : 1 complex with significantly greater hydrogen bonding leading to an enthalpically much more favourable process). Sequential binding of a second molecule of *N*-Boc-D-glutamate (and similarly *N*-Boc-L- and D-aspartate) then involves an enthalpically favourable formation of a second carboxylate–thiourea interaction on the outside of the macrocycle and without any substantial further reorganisation of the macrocycle. While it is important not to over-interpret the thermodynamic data obtained from the ITC experiments or rule out other binding stoichiometries, this explanation satisfactorily rationalises the experimental data and is entirely consistent with the binding properties observed with the original thiourea macrocycle when binding the two enantiomers of *N*-Boc-glutamate in DMSO. Unfortunately we were unable to obtain any detailed structural information about the complexation from NMR studies due to the poor solubility and

**Table 1** ITC binding data for macrocycle **3** with biscarboxylate salts in 50 : 50 DMSO–$H_2O$[a]

| Entry | Guest | $K_a^{1:1}$/$M^{-1}$ | $\Delta G^{1:1}$/kJ mol$^{-1}$ | $\Delta H^{1:1}$/kJ mol$^{-1}$ | $T\Delta S^{1:1}$/kJ mol$^{-1}$ | $K_a^{1:2}$/$M^{-1}$ | $\Delta G^{1:2}$/kJ mol$^{-1}$ | $\Delta H^{1:2}$/kJ mol$^{-1}$ | $T\Delta S^{1:2}$/kJ mol$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *N*-Boc-L-Glu | $3.8 \times 10^4$ | −26.1 | −18.1 | 8.0 | $5.3 \times 10^3$ | −21.6 | −2.2 | 19.4 |
| 2 | *N*-Boc-D-Glu | $2.9 \times 10^3$ | −19.8 | 39.3 | 59.1 | $1.4 \times 10^4$ | −24.0 | −22.5 | 1.5 |
| 3 | *N*-Boc-L-Asp | $1.1 \times 10^3$ | −17.4 | 61.7 | 79.1 | $1.1 \times 10^4$ | −23.3 | −19.2 | 4.1 |
| 4 | *N*-Boc-D-Asp | $2.5 \times 10^3$ | −19.4 | 46.9 | 66.3 | $1.4 \times 10^4$ | −23.9 | −19.5 | 4.4 |

[a] Association constants are reported to two significant figures with estimated errors of ±20%. $K_a^{1:2}$ refers to the binding constant for stepwise association of the 1 : 1 complex with a second equivalent of guest, the overall binding constant is therefore the product of reported $K_a^{1:1}$ and $K_a^{1:2}$.

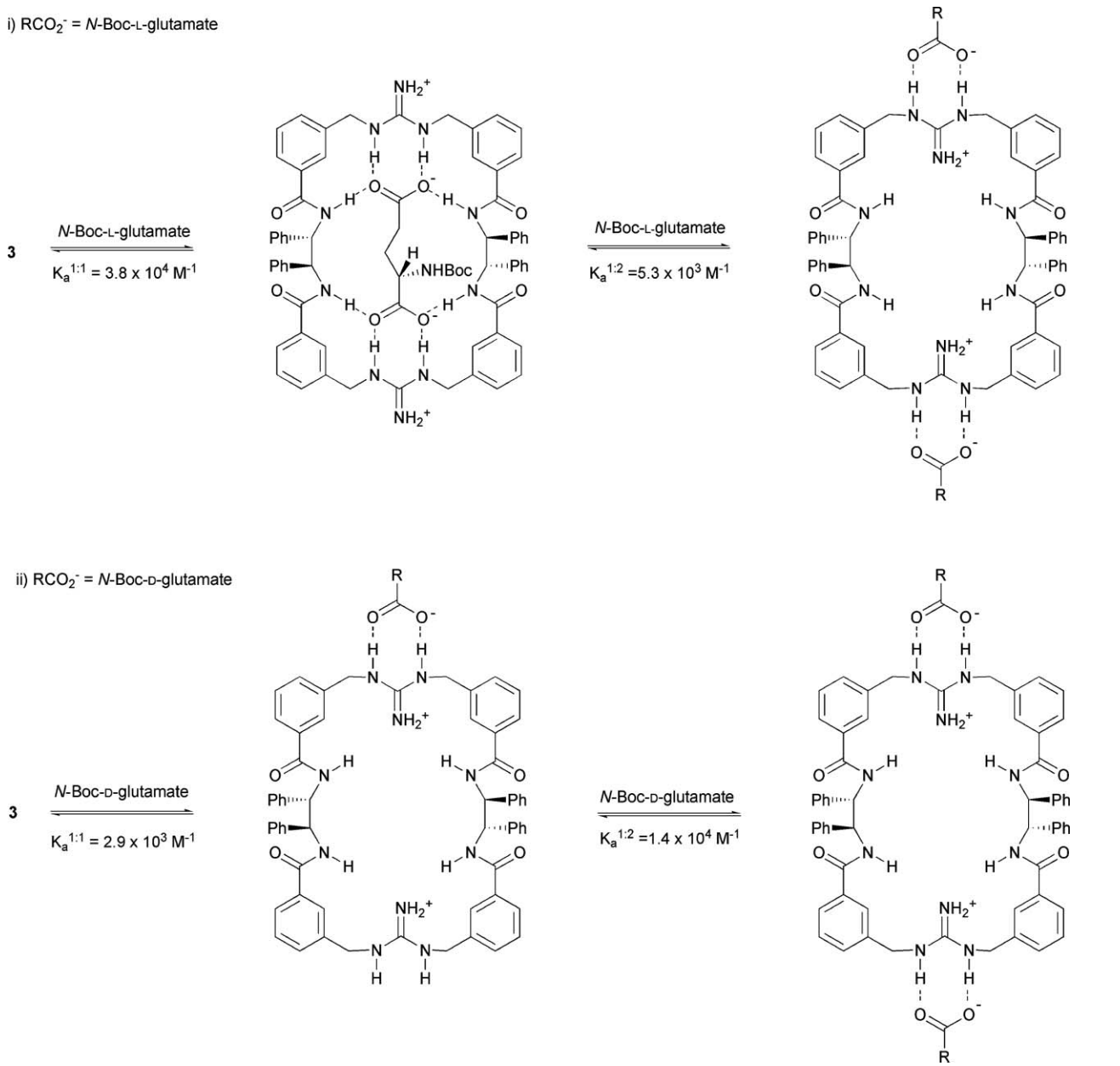



**Fig. 4** Proposed mode of sequential binding of i) *N*-Boc-L-glutamate and ii) *N*-Boc-D-glutamate by macrocycle **3**.

poorly resolved spectra of the complexes obtained in 50% DMSO–$H_2O$.

## Conclusions

Bisguanidinium macrocycle **3** is able to bind strongly with biscarboxylates in a highly competitive aqueous solvent system, demonstrating an important advance over the class of bisthiourea receptors we have reported previously. Macrocycle **3** is also able to discriminate for the 1 : 1 binding of *N*-Boc-L-glutamate against both its enantiomer *N*-Boc-D-glutamate and the smaller aspartate derivatives. ITC binding studies were also carried out with macrocycle **3** and using unprotected, zwitterionic L-glutamate as guest in 50% $H_2O$–DMSO (using a tris buffer) but in this case indicated very weak binding ($K_a^{1:1} < 10^2$ $M^{-1}$). Studies are under way to synthesise macrocycles which are both more water soluble and incorporate additional functionality to stabilise interactions with the ammonium group of unprotected glutamate.

## Experimental

### General techniques

Reactions were carried out in solvents of commercial grade and, where necessary, distilled prior to use. Reactions requiring a dry atmosphere were conducted in oven dried glassware under nitrogen. $CH_2Cl_2$ was distilled from calcium hydride, as was

petroleum ether where the fraction boiling between 40 and 60 °C was used. Where degassed solvents were used, a stream of nitrogen was passed through them immediately prior to use, unless otherwise stated. TLC was conducted on foil backed sheets coated with silica gel (0.25 mm) and containing fluorescent indicator $UV_{254}$. Column chromatography was performed on Sorbsil C60, 40–60 mesh silica.

$^1$H and $^{13}$C NMR spectra were recorded on Bruker AV300, AM300 or DPX400 spectrometers. $^1$H chemical shifts are reported as values in ppm referenced to residual solvent. The following abbreviations are used to denote multiplicity and may be compounded: s = singlet, d = doublet, t = triplet, q = quartet, fs = fine splitting. Coupling constants, *J*, are measured in Hertz (Hz). $^{13}$C spectra were proton decoupled and referenced to solvent. The number of adjacent protons was determined by DEPT experiments. Low resolution mass spectra were recorded on a Waters ZMD mass spectrometer, single quadrupole, 2700 autosampler in methanol or acetonitrile. Accurate mass spectra were recorded on a VG analytical 70–250-SE double focussing mass spectrometer. Melting points were determined in open capillary tubes using a Gallenkamp Electrothermal melting point apparatus and are uncorrected. Microanalyses were performed by MEDAC Ltd., Surrey.

### $^1$H NMR titration experiments[13]

$^1$H NMR titration experiments were conducted on a Brüker AM 300 spectrometer at 298 K. A sample of host was dissolved in the deuterated solvent. A portion of this solution was used as the host NMR sample and the remainder used to dissolve a sample of the guest, so that the concentration of the host remained constant throughout the titration. Guest stock solutions were typically prepared such that 10 μL of that solution contained 0.1 equivalents of guest with respect to host, unless otherwise stated. Successive aliquots of the guest solution were added to the host NMR sample and the $^1$H NMR spectrum recorded after each addition. Changes in chemical shift of guanidinium and amide NH host signals, as a function of guest concentration, were analysed using NMRTit HG software, assuming a 1 : 1 or 1 : 2 binding stoichiometry.

### Isothermal calorimetry titration experiments[19]

All experiments were performed using an isothermal titration calorimeter from Microcal Inc. (Northampton, MA). In a typical experiment, a 0.6 mM receptor solution is added to the calorimetric cell. A 40 mM solution of guest is introduced in 65 injections: 30 injections of 2.5 μL followed by 35 injections of 5.0 μL to a total of 250 μL added guest. The solution is continuously stirred to ensure rapid mixing and kept at 30 °C, through the combination of an external cooling bath and an internal heater. Dilution effects are determined by performing a blank experiment in which the same guest solution is added to pure solvent and the heat signature subtracted from the raw titration to produce the final binding curve. Binding parameters are determined by applying either one-site or two-sites models, using the Origin software provided. These methods rely on standard nonlinear least-squares regression (Levenberg–Marquard method) to fit the curves, taking into account the change in volume that occurs during the calorimetric titration.

### Synthetic procedures

(1*S*,2*S*)-(−)-1,2-Diphenyl-1,2-ethylenediamine-L-tartrate was prepared according to the method of Corey and co-workers.[20] Benzyloxycarbonyl isothiocyanate was prepared according to the method of Groziak and Townsend.[12] Preparation of *N*,*N*′-(1*S*,2*S*)-diphenyl-ethyl-1,2-diylbis-3-aminomethylbenzamide (**7**) has been reported previously.[9b]

### *N*,*N*′-(1*S*,2*S*)-Diphenyl-ethyl-1,2-diylbis-[3-(*N*′-benzyl-*N*″-carbobenzyloxy-thioureido)-benzamide 8

*N*,*N*′-(1*S*,2*S*)-Diphenyl-ethyl-1,2-diylbis-3-aminomethylbenzamide (**7**) (250 mg, 0.52 mmol) was taken into a mixture of $CH_2Cl_2$ (10 mL) and dimethylformamide (DMF) (1 mL). Benzyloxycarbonyl isothiocyanate (252 mg, 1.3 mmol) was added and the mixture stirred for 8 h, during which time formation of a precipitate was observed, before concentration *in vacuo*. The residue was suspended in $CH_2Cl_2$ (5 mL) and the solid collected by filtration and washed with $CH_2Cl_2$ (2 × 5 mL) and $Et_2O$ (2 × 5 mL). Drying *in vacuo* gave the title compound as a white solid (330 mg, 73%): $R_f = 0.25$ (3% MeOH in DCM); mp = 155–156 °C; $^1$H NMR (400 MHz, $d_6$-DMSO): $\delta$ = 4.30 (4H, brs, C*H*$_2$NH), 5.17 (4H, s, $CH_2O$), 5.65 (2H, d, *J* = 6.5 Hz, CH), 7.10–7.50 (26H, m, ArH), 7.63 (2H, s, ArH), 9.04 (2H, d, *J* = 7.0 Hz, N*H*CH), 10.24 (2H, br s, N*H*CH$_2$), 11.23 (2H, s, NHCbz) ppm; $^{13}$C NMR (100 MHz, $d_6$-DMSO): $\delta$ = 47.8 ($CH_2$), 57.2 (CH), 67.0 ($CH_2$), 125.9 (CH), 126.7 (CH), 127.0 (CH), 127.4 (CH), 128.0 (CH), 128.4 (CH), 128.5 (CH), 128.6 (CH), 130.4 (CH), 135.2 (C), 135.7 (C), 138.2 (C), 140.8 (C), 153.4 (C), 166.6 (C), 180.1 (C) ppm; MS ($ES^+$): *m*/*z*: 865 ($[M + H]^+$, 90%) 887 ($[M + Na]^+$, 85%).

### Macrocycle 9

*N*,*N*′-(1*S*,2*S*)-Diphenyl-ethyl-1,2-diylbis-[3-(*N*′-benzyl-*N*″-carbobenzyloxy-thioureido)-benzamide **8** (60 mg, 0.069 mmol) was taken into $CH_2Cl_2$ (10 mL) and DMF (10 mL). 1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC) (53 mg, 0.276 mmol) and 4-dimethylaminopyridine (DMAP) (15 mg, 0.14 mmol) were then added. To this vigorously stirred solution, a solution of *N*,*N*′-(1*S*,2*S*)-diphenyl-ethyl-1,2-diylbis-3-aminomethylbenzamide (**7**) (34 mg, 0.071 mmol) in $CH_2Cl_2$–DMF (15 mL of a 2 : 1 mixture) was added and the resulting mixture stirred for 36 h before concentration *in vacuo*. Purification by column chromatography. ($SiO_2$ eluted with $CH_2Cl_2$ → EtOAc–$CH_2Cl_2$ (5 : 1) then $SiO_2$ eluted with 2% MeOH in $CH_2Cl_2$) gave the title compound as a white solid (28 mg, 29%); mp 171–172 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 4.24 (8H, brs, C*H*$_2$NH), 5.09 (4H, s, $CH_2O$), 5.64 (4H, br s, CHPh), 7.04 (4H, d, *J* = 7.0 Hz, NHCO), 7.12 (4H, br s, NHCN), 7.20–7.40 (46H, m, ArH) ppm; $^{13}$C NMR (100 MHz, 5% $CD_3OD$–$CDCl_3$): $\delta$ = 44.5 ($CH_2$), 59.3 (CH), 66.8 ($CH_2$), 126.0 (CH), 127.6 (CH), 127.7 (CH), 127.8 (CH), 127.9 (CH), 128.3 (CH), 128.5 (CH), 128.7 (CH), 130.4 (C), 134.8 (C), 137.1 (C), 138.7 (C), 159.9 (C), 163.9 (C), 168.5 (C) ppm; MS ($ES^+$): m/z: 639 ($[M + 2H]^{2+}$, 100%) 1276 ($[M + H]^+$, 30%); Found: C, 71.70; H, 5.54; N, 10.57. Calc. for $C_{78}H_{70}N_{10}O_8 \cdot 2H_2O$: C, 71.43; H, 5.69; N, 10.68%.

**Macrocycle 3**

Macrocycle **9** (17 mg, 0.013 mmol) was taken into MeOH–DMF (2 mL of a 1 : 1 mixture). 10% Pd/C (3 mg) was added and the mixture stirred under $H_2$ (1 atm) for 14 h. The mixture was filtered through Celite™ and the filtrate concentrated *in vacuo*. The residue obtained was then taken into $CH_2Cl_2$–MeOH (6 mL of a 5 : 1 mixture) and $HPF_6$ (10 μL of a 60% v/v aqueous solution, 0.04 mmol) was added. The solution was then concentrated *in vacuo* and the residue suspended in $H_2O$, filtration and drying *in vacuo* gave the title compound as a white solid (13 mg, 77%); mp 183–185 °C; $^1$H NMR (300 MHz, $d_6$-DMSO): $\delta = 4.43$ (8H, d, $J =$ 4.0 Hz, C$H_2$NH), 5.66 (4H, br s, CHPh), 7.00–7.80 (40H, m, ArH + $NH_2^+$), 8.03 (4H, br s, N$H$CH$_2$), 9.16 (4H, br s, NHCO) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$–$CD_3OD$): $\delta = 47.5$ ($CH_2$), 61.8 (CH), 126.2 (CH), 128.9 (CH), 129.9 (CH), 130.4 (CH), 130.6 (CH), 131.4 (CH), 131.9 (CH), 133.3 (C), 137.5 (C), 138.9 (C), 141.5 (C), 171.5 (C) ppm; MS (ES$^+$): $m/z$: 504 ([M + 2H]$^{2+}$, 93%) 526 ([M + 2Na]$^{2+}$, 100%) 1008 ([M + H]$^+$, 30%); Found: C, 55.55; H, 4.90; N, 10.77. Calc. for $C_{62}H_{60}F_{12}N_{10}O_4P_2 \cdot 2H_2O$: C, 55.77; H, 4.83; N, 10.89%.

## References and notes


1 Review: E. García-Espana, P. Díaz, J. M. Llinares and A. Bianchi, *Coord. Chem. Rev.*, 2006, **250**, 2952–2986.

2 (*a*) Original work on polyamine macrocyclic receptors for carboxylates: B. Dietrich, M. W. Hosseini, J.-M. Lehn and R. B. Sessions, *J. Am. Chem. Soc.*, 1981, **103**, 1282–1283; (*b*) E. Kimura, A. Sakonaka, T. Yatsunami and M. Kodama, *J. Am. Chem. Soc.*, 1981, **103**, 3041–3045; (*c*) E. Kimura, M. Kodama and T. Yatsunami, *J. Am. Chem. Soc.*, 1982, **104**, 3182–3187; (*d*) M. W. Hosseini and J.-M. Lehn, *J. Am. Chem. Soc.*, 1982, **104**, 3525–3527; (*e*) M. W. Hosseini and J.-M. Lehn, *Helv. Chim. Acta*, 1986, **69**, 587–603.

3 (*a*) Macrocyclic metal complexes: A. E. Martell and R. J. Motekaitis, *J. Am. Chem. Soc.*, 1988, **110**, 8059–8064; (*b*) C. Bazzicalupi, A. Bencini, A. Bianchi, V. Fusi, E. García-Espana, C. Giorgi, J. M. Llinares, J. A. Ramirez and B. Valtancoli, *Inorg. Chem.*, 1999, **38**, 620–621; (*c*) M. Boiocchi, M. Bonizzoni, L. Fabbrizzi, G. Piovani and A. Taglietti, *Angew. Chem., Int. Ed.*, 2004, **43**, 3847–3852; (*d*) B. Verdejo, J. Aguilar, A. Doménech, C. Miranda, P. Navarro, H. R. Jiménez, C. Soriano and E. García-Espana, *Chem. Commun.*, 2005, 3086–3088; (*e*) F. Li, R. Delgado and V. Félix, *Eur. J. Inorg. Chem.*, 2005, 4550–4561.

4 (*a*) Reviews: P. Blondeau, M. Segura, R. Pérez-Fernández and J. de Mendoza, *Chem. Soc. Rev.*, 2007, **36**, 198–210; (*b*) K. A. Schug and W. Lindner, *Chem. Rev.*, 2005, **105**, 67–113; (*c*) M. D. Best, S. L. Tobey and E. V. Anslyn, *Coord. Chem. Rev.*, 2003, **240**, 3–15.

5 (*a*) B. Dietrich, T. M. Fyles, J.-M. Lehn, L. G. Pease and D. L. Fyles, *J. Chem. Soc., Chem. Commun.*, 1978, 934–936; (*b*) B. Dietrich, D. L. Fyles, T. M. Fyles and J.-M. Lehn, *Helv. Chim. Acta*, 1979, **62**, 2763–2787; (*c*) P. Schiebl and F. P. Schmitdchen, *Tetrahedron Lett.*, 1993, **34**, 2449–2452; (*d*) B. R. Linton, M. S. Goodman, E. Fan, S. A. van Arman and A. D. Hamilton, *J. Org. Chem.*, 2001, **66**, 7313–7319; (*e*) J. Raker and T. E. Glass, *J. Org. Chem.*, 2002, **67**, 6113–6116; (*f*) H. Ait-Haddou, S. L. Wiskur, V. M. Lynch and E. V. Anslyn, *J. Am. Chem. Soc.*, 2001, **123**, 11296–11297; (*g*) for guanidinium derived receptors able to bind to aspartate or glutamate rich peptides, see, for example: X. Salvatella, M. W. Peczuh, M. Gairí, R. K. Jain, J. Sanchez-Quesada, J. de Mendoza, A. D. Hamilton and E. Giralt, *Chem. Commun.*, 2000, 1399–1400.

6 (*a*) L. Sebo, B. Schweizer and F. Diederich, *Helv. Chim. Acta*, 2000, **83**, 80–92; (*b*) L. Sebo, F. Diederich and V. Gramlich, *Helv. Chim. Acta*, 2000, **83**, 93–113.

7 (*a*) I. Alfonso, F. Rebolledo and V. Gotor, *Chem.–Eur. J.*, 2000, **6**, 3331–3338; (*b*) I. Alfonso, B. Dietrich, F. Rebolledo, V. Gotor and J.-M. Lehn, *Helv. Chim. Acta*, 2001, **84**, 280–295; (*c*) A. González-Álvarez, I. Alfonso, P. Díaz, E. García-Espana and V. Gotor, *Chem. Commun.*, 2006, 1227–1229.

8 (*a*) Recent examples of related receptors for tricarboxylates in water: S. L. Tobey and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 10963–10970; (*b*) S. L. Wiskur, J. L. Lavigne, A. Metzger, S. L. Tobey, V. Lynch and E. V. Anslyn, *Chem.–Eur. J.*, 2004, **10**, 3792–3804; (*c*) C. Schmuck and M. Schwegmann, *J. Am. Chem. Soc.*, 2005, **127**, 3373–3379; (*d*) A. Frontera, J. Morey, A. Oliver, M. N. Pina, D. Quinonero, A. Costa, P. Ballester, P. M. Deya and E. V. Anslyn, *J. Org. Chem.*, 2006, **71**, 7185–7195.

9 (*a*) S. Rossi, G. M. Kyne, D. L. Turner, N. J. Wells and J. D. Kilburn, *Angew. Chem., Int. Ed.*, 2002, **41**, 4233–4236 [*NB*: macrocycle **2** is incorrectly represented as the (1*R*,2*R*)-(+)-1,2-diphenyl enantiomer of the 1*S*,2*S*-macrocycle for which binding studies are reported, in both Schemes 1 and 2 of reference 9*a*]; (*b*) A. Ragusa, S. Rossi, J. M. Hayes, M. Stein and J. D. Kilburn, *Chem.–Eur. J.*, 2005, **11**, 5674–5688.

10 G. M. Kyne, M. E. Light, M. B. Hursthouse, J. de Mendoza and J. D. Kilburn, *J. Chem. Soc., Perkin Trans. 1*, 2001, 1258–1263.

11 (*a*) B. R. Linton, A. J. Carr, B. P. Orner and A. D. Hamilton, *J. Org. Chem.*, 2000, **65**, 1566–1568; (*b*) R. J. Fitzmaurice, F. Gaggini, N. Srinivasan and J. D. Kilburn, *Org. Biomol. Chem.*, 2007, **5**, 1706–1714.

12 Preparation of CbzNCS was adapted from: M. P. Groziak and L. B. Townsend, *J. Org. Chem.*, 1986, **51**, 1277–1282.

13 NMR titration data was analysed using NMRTit HG software: A. P. Bisson, C. A. Hunter, J. C. Morales and K. Young, *Chem.–Eur. J.*, 1998, **4**, 845–851. All binding data from NMR titration experiments are provided in the ESI†.

14 The binding constants obtained for *N*-Boc-L/D-glutamate using NMR titrations could not be determined with great accuracy. The dominant binding constant in each case was at the upper limit of detectability using this method (L. Fielding, *Tetrahedron*, 2000, **56**, 6151–6170) and the value obtained for the smaller binding constant in each case was sensitive to the values used at the start of the iterative calculation and could therefore not be determined to better than 1 significant figure. Job plots give ambiguous results with broad maxima at stoichiometries of ~1–1.5.

15 C. S. Wilcox, J. C. Adrian Jr., T. H. Webb and F. J. Zawacki, *J. Am. Chem. Soc.*, 1992, **114**, 10189–10197.

16 Poor resolution of the $^1$H NMR spectrum of **3** in 50% $H_2O$–DMSO results from sparing solubility of the macrocycle. In fact, neither the amide nor guanidinium NH resonance were visible in the spectrum—presumably because of fast exchange with the water.

17 A. Cooper, *Curr. Opin. Chem. Biol.*, 1999, **3**, 557–563 and references therein.

18 (*a*) For the specific application of isothermal calorimetry to carboxylate recognition, see: ref. 5*d* and 6*a*; (*b*) B. Linton and A. D. Hamilton, *Tetrahedron*, 1999, **55**, 6027–6038; (*c*) M. Berger and F. P. Schmidtchen, *J. Am. Chem. Soc.*, 1999, **121**, 9986–9993.

19 The ITC data were obtained using a MicroCal VP-ITC isothermal calorimeter at apparent pH 6.8 and are corrected for the heat generated from dilution of the host. Data were analysed by non-linear least square fitting using the Origin software supplied by MicroCal: T. Wiseman, S. Williston, J. F. Brandts and L.-N. Lin, *Anal. Biochem.*, 1989, **179**, 131–137. All binding data from ITC experiments are provided in the ESI†.

20 (*a*) S. Pikul and E. J. Corey, *Org. Synth.*, 1993, **71**, 22; (*b*) E. J. Corey, D-H. Lee and S. Sarshar, *Tetrahedron: Asymmetry*, 1995, **6**, 3–6.