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Chromogenic Chemosensors for *N*-Acetylaspartate Based on Chiral Ferrocene-Bearing Thiourea Derivatives

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A series of chiral ferrocene-containing thiourea derivatives have been synthesized, and their enantioselective recognition properties were investigated by UV/Vis titration. Receptors **5a** and **6a** exhibit excellent chiral recognition abilities towards *N*-acetylaspartate. The differences in solution colour can be observed directly by the naked eye. The minimum energy structures of **5a** and **6a** were also obtained by theoretical DFT calculations.

Introduction

Molecular recognition lies at the heart of biochemistry. All biomedical processes inherently require selective molecular recognition and successful complexation. Matching the degree of exquisite control and efficiency exhibited by natural systems is a challenging goal for host-guest chemists to emulate and has led to much interest in the design of a diverse range of synthetic receptors for important biological substrates.[1] Anions, especially chiral anions, play numerous fundamental roles in biological and chemical processes as exemplified by the majority of enzymes binding anions as either substrates or cofactors, and many anions act as ubiquitous nucleophiles, bases, redox agents and phase-transfer catalysts. The enantioselective recognition and sensing of biologically relevant anions by artificial host molecules has emerged recently as a key research theme within the generalized area of supramolecular chemistry.[2]

Aspartate and glutamate have been thoroughly studied as excitatory amino acid neurotransmitters.[3] D-aspartate is also present in appreciable quantities in fetal tissues and especially in fetal brain and is a potent agonist of the excitatory amino acid receptors, which play a role in brain function and development. Taking into account their critical importance, some research effort has been devoted to the synthesis of chiral receptors, which are capable of detecting and distinguishing between the enantiomers of these anions.[2c,4]

Ferrocene (Fc) systems have a well developed organic chemistry, allowing for their attachment to a wide variety of functional groups. In the past, Fc derivatives that bind to and allow the electrochemical sensing of cations[5] and anions[6] have been reported widely. The various bonding and nonbonding interactions that allow these charged species to be electrochemically recognized have been reviewed in detail.[7] Many planar chiral Fcs were used as chiral ligands in a series of metal-catalyzed processes,[8] which indicate the potential application of chiral Fc derivatives in the enantioselective recognition of chiral materials.

To the best of our knowledge, chiral anion receptors based on Fc are still rare,[9] which inspired us to use Fc as the preorganised platform and introduce strong hydrogen-bond donors such as amides and thioureas to this system. In this work, we aimed at the synthesis of a series of chiral ferrocenium/Fc derivatives bearing thiourea units **5a**–**5e**, **6a**–**6e** and the determination of their binding properties towards *N*-protected aspartate or glutamate by UV/Vis spectroscopy. The main carboxylate binding sites in these receptors are the thiourea moieties, and the two amide groups should provide additional hydrogen-bonding functionalities.[4a,b,10] Based on the good preorganised structure supplied by the Fc unit, the two chiral centres in the two arms should enable enantioselective recognition, while the process of recognition could transduce to an optical signal through the *p*-nitro phenyl chromogenic group. The results obtained from UV/Vis titration experiments indicate that receptors **5a** and **6a** have excellent enantioselective recognition abilities towards *N*-acetylaspartate (NAA); obvious colour differences could be observed by the naked eye.

Results and Discussion

Synthesis

The synthesis of compounds **5a**–**5d** and **6a**–**6d** is outlined in Scheme 1 and Scheme 2. The structures of four reference

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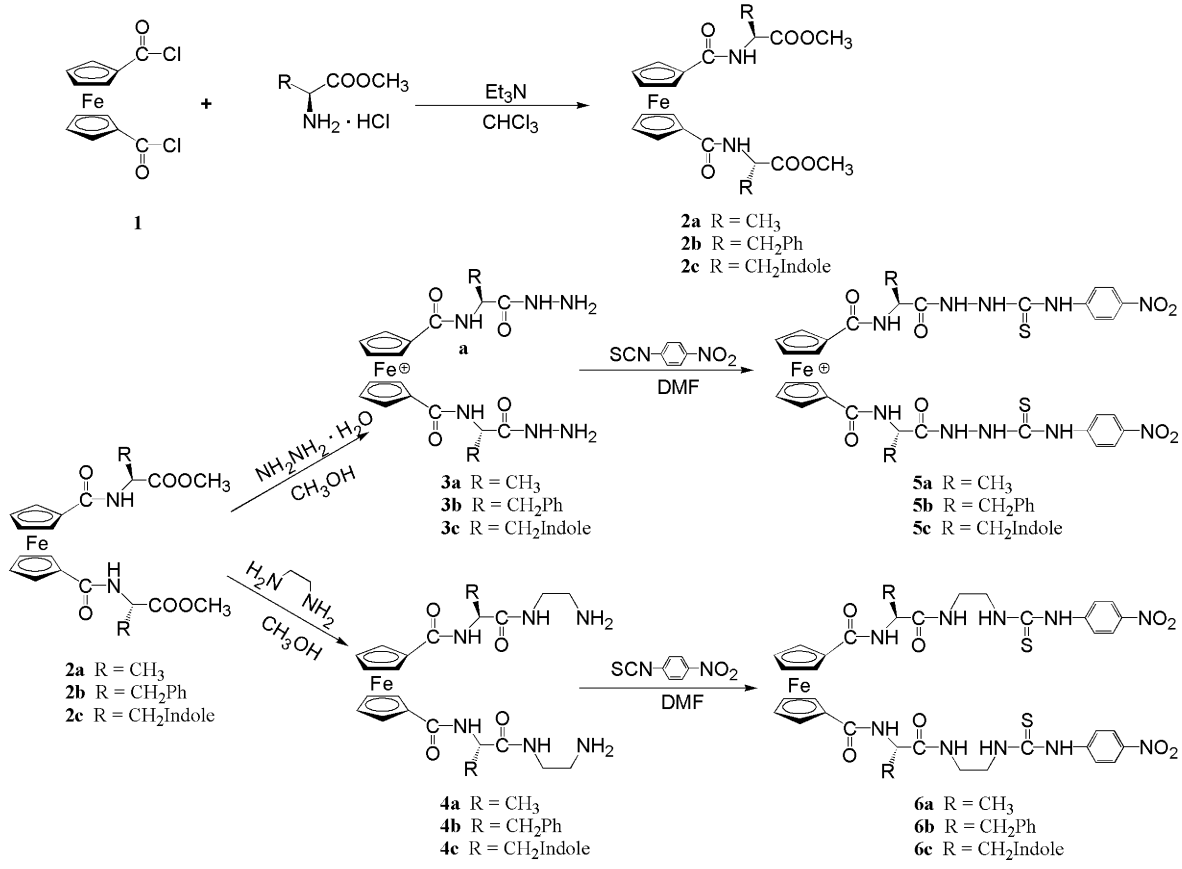
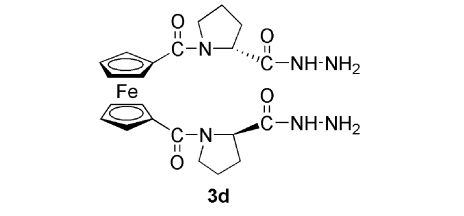
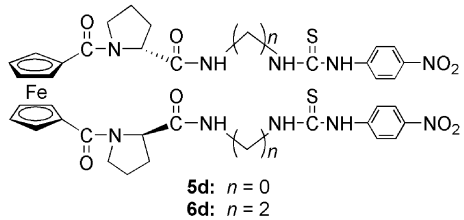
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.



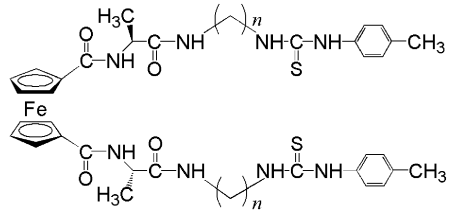
Scheme 1. The synthesis of compounds **5a**–**5c**, **6a**–**6c**.

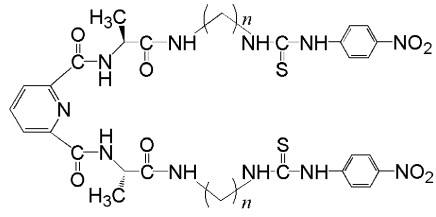
compounds **5e**, **6e**, **7a**–**7b** are outlined in Scheme 3. They are soluble in CH_3OH, DMSO and DMF. Due to the effects of paramagnetism, compounds **3a**–**3c** and **5a**–**5c** did not provide signals by liquid NMR spectroscopy, so their structures were characterized by IR, ESI-MS and elemental analysis. We also used solid-state NMR to detect the structure of **5a**, but we could not clearly identify NMR signals due to very strong coupling. We characterized the structures of other compounds by IR, ESI-MS, ^1H and ^{13}C NMR spectroscopy and elemental analysis. In order to clarify this phenomenon, we synthesized **3d** and **5d**. Interestingly, both of them provided clear NMR signals, so the paramagnetism could be attributed to the effect of the amides. Because Fc undergoes a fast one-electron oxidation to its cationic form (Fc^+), for compound **3a**–**3c**, the oxidized ferrocenium cat-





Scheme 2. The structures of compounds **3d**, **5d** and **6d**.





Scheme 3. The structures of reference compounds **5e**, **6e**, **7a** and **7b**.

ion could be stabilized by intramolecular hydrogen bonding,[7c] and the possible charge-transfer (CT) processes are shown in the Supporting Information (Scheme S1). The paramagnetic character of ferrocenium hampered the use of NMR spectroscopy in this oxidation state. If the intramolecular hydrogen bonding were obstructed by ethyl or tertiary amine, as in **4a**–**4c** or **3d**, the ferrocenium could not exist stably, and these compounds favoured their Fc forms and exhibited normal NMR signals.

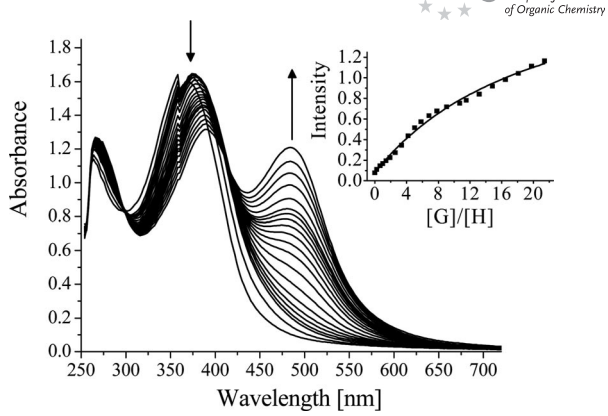


Figure 1. UV/Vis absorption spectra of receptor **5a** (5.0×10^{-5} mol dm^{-3}) upon the addition of various amounts of *N*-Ac-L-aspartate in DMSO at 25 °C. Equivalents of anion: 0, 0.25, 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.5, 11.5, 13, 15, 16.5, 18, 20 and 21.5. The curve-fitting line through the data points at 500 nm is shown in the inset, where G is the guest and H is the host. The correlation coefficient (R) of nonlinear curve fitting is 0.9947.

UV/Vis Spectroscopy

Nowadays, the development of colorimetric anion sensing is particularly challenging since visual detection can give immediate qualitative information, and it is becoming increasingly appreciated in term of quantitative analysis;[11] thus, UV/Vis spectra titration is a proper method to investigate the enantioselective recognition abilities of these receptors. It is known that NAA is present in brain tissue[12] at concentrations exceeded only by glutamate among the amino acids, and NAA is confined almost exclusively in vertebrates to nervous tissue including the retina.[13] Taking into account the critical importance of NAA, we chose NAA as the guest and recorded UV/Vis spectra for a solution of **5a** or **6a** in the absence or presence of *N*-acetyl-L- or D-aspartate, with Bu_4N^+ as the counter cation. We chose DMSO as the solvent considering the solubility of both the receptors and anions.

The UV/Vis absorption spectra of **5a** upon the addition of *N*-acetyl-L-aspartate are shown in Figure 1. In the absence of anion, **5a** had an absorption maximum at 370 nm, which can be assigned to an intramolecular charge-transfer (ICT) absorption band.[14] With the addition of L-aspartate to the solution of **5a** in DMSO (5.0×10^{-5} mol dm^{-3}), the characteristic absorption peak of the host at 370 nm decreased gradually with a redshift (of approximately 20 nm), and a new absorption peak at 484 nm was produced. The new absorption suggested the formation of the complex between **5a** and L-aspartate. Gradually increasing the concentration of anion changed the colour of the solution from yellowish to red, which could be observed by the naked eye directly.

As shown in Figure 2, we observed similar phenomena when *N*-Ac-D-aspartate was added to a solution of **5a** in DMSO (5.0×10^{-5} mol dm^{-3}). With the increasing concentration of D-aspartate, the new band at 484 nm increased gradually, 18.5 equiv. of anion led the absorbance intensity (at 484 nm) to approach 0.73 and the solution colour of solution changed from yellowish to nacarat. While the intensity approached 1.05 with the same amount of L-aspartate, the solution colour already changed from yellowish to red. Clear differences in the absorbance spectra indicated that **5a** had a good enantioselective recognition ability towards NAA. We attributed the colour change to an obvious increase of absorption in the visible region at 484 nm, which can be ascribed to a CT interaction between the electron-rich, donor nitrogen of the thiourea units and the electron-deficient *p*-nitrophenyl moieties. When the receptor bound an NAA, hydrogen bonds formed stable complexes, and electron density in the supramolecular system increased considerably. This enhanced the CT interaction between the electron-rich and -deficient moieties, resulting in a visible colour change.[15] When a protic solvent such as CH_3OH was added to the red solution of **5a** and L-aspartate or the nacarat solution of **5a** and D-aspartate in DMSO, the colour of both solutions became yellowish. These phenomena indicated that the addition of a protic solvent destroyed the complexation between **5a** and *N*-Ac-L- or D-aspartate, demonstrating that the interactions between **5a** and L- or D-aspartate depended very much on hydrogen-bonding interactions.[11,16] In many previous studies, it has been reported that similar Fc receptors bound anions or neutral molecules through hydrogen bonding.[6,17] In this system, we did not observe clear isosbestic points; this may be due to the change of interaction mode. At lower concentrations of aspartate, a 1:1 complex may be formed between one arm of the receptor and the anion, and the electron density in the supramolecular system may have increased, which may have enhanced the CT interactions between the electron-rich donor units and electron-deficient *p*-nitrophenyl moieties, resulting in the new absorption peak. With increasing amounts of anion, the other receptor arm will participate in the interaction; two arms could bind an anion in a "sandwich" mode, resulting in an extension of the supramolecular system and increased CT interactions with an enhancement of the absorption at 484 nm. The satisfactory result [the correlation coefficient (R) was > 0.99] of nonlinear curve fitting (at 500 nm) confirmed that **5a** and *N*-Ac-L- or D-aspartate formed a 1:1 complex (see the insets of Figure 1 and Figure 2);[18] in addition, the association constants (K_A) for D- and L-aspartate were different [$K_{A(L)}$ = 981.1 dm^3 mol^{-1}; $K_{A(D)}$ = 93.5 dm^3 mol^{-1}], corresponding to an L/D selectivity [$K_{A(L)}/K_{A(D)}$] of 10.49 for NAA.

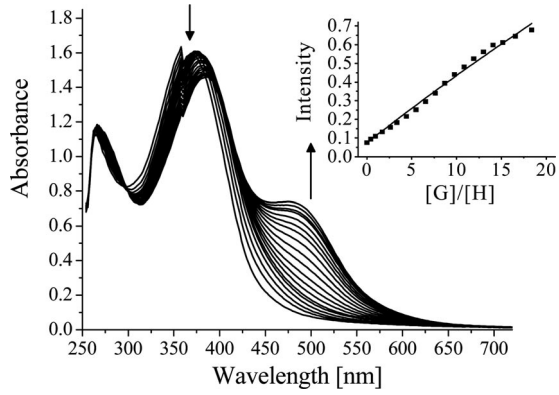


Figure 2. UV/Vis absorption spectra of receptor **5a** (5.0×10^{-5} mol dm^{-3}) upon the addition of various amounts of *N*-Ac-D-aspartate in DMSO at 25 °C. Equivalents of anion: 0, 0.4, 1.0, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, 12, 13, 14, 15, 16.5 and 18.5. The curve-fitting line through the data points at 500 nm is shown in the inset, where G is the guest and H is the host. The correlation coefficient (R) of non-linear curve fitting is 0.9926.

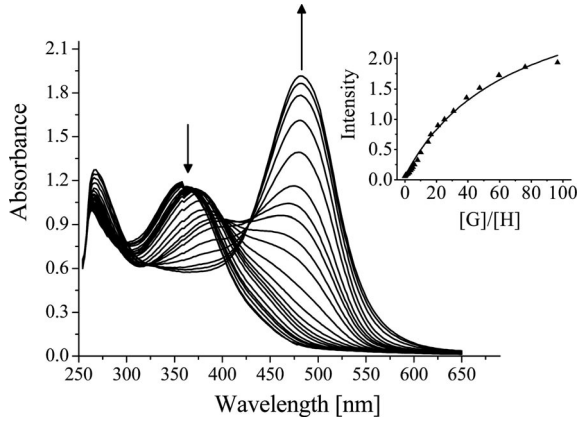


Figure 3. UV/Vis absorption spectra of receptor **6a** (5.0×10^{-5} mol dm^{-3}) upon the addition of various amounts of *N*-Ac-L-aspartate in DMSO at 25 °C. Equivalents of anion: 0, 0.35, 0.75, 1.5, 2.0, 2.75, 3.6, 4.5, 5, 6, 8, 10, 14.5, 16.5, 20, 25, 30, 40, 47.5, 60, 75 and 95. The curve-fitting line through the data points at 484 nm is shown in the inset, where G is the guest and H is the host. The correlation coefficient (R) of non-linear curve fitting is 0.9909.

Receptor **6a** showed a similar response to **5a** upon addition of *N*-Ac-L-aspartate. In the absence of the anion, the UV/Vis spectrum of **6a** in DMSO (5.0×10^{-5} mol dm^{-3}) exhibited an intramolecular CT absorption band (λ_{max} = 360 nm). With the addition of L-aspartate, the ICT band shifted to 370 nm, and we observed a new absorption band at 484 nm (see Figure 3). The solution colour changed from yellowish to red, which could also be attributed to the classical CT interaction.[15,16] But when *N*-Ac-D-aspartate was added to the solution of **6a**, as shown in Figure 4, we observed different phenomena. With the increasing concentration of anion, the characteristic absorption peak of the host at 360 nm decreased slightly with a redshift (of approximately 14 nm); only a weak new absorption peak at 484 nm was produced, and 30 equiv. of D-aspartate led the absorbance intensity (at 484 nm) to approach only 0.23, while the solution colour changed from yellowish to yellow. These results contrasted strongly with those of the **6a**/L-aspartate titration, where the absorbance at 484 nm approached 1.14 with 30 equiv. of L-aspartate, and the solution colour changed from yellowish to red. Obvious differences in the absorbance spectra and solution colour indicated that **6a** had good enantioselective recognition abilities towards the enantiomers of NAA. The satisfactory nonlinear curve fitting at 484 nm (the correlation coefficient was > 0.99) confirmed that **6a** and L- or D-aspartate formed a 1:1 complex (see the insets of Figure 3 and Figure 4). The K_A values of the two enantiomers with **6a** were very different [$K_{A(L)}$ = 297.9 dm^3 mol^{-1}; $K_{A(D)} = 3.91 \times 10^3$ dm^3 mol^{-1}], which corresponded to a D/L selectivity [$K_{A(D)}/K_{A(L)}$] of 13.1 for NAA.

When *N*-Ac-L- or D-aspartate was added to a solution of **5a** or **6a** in DMSO, colour changes of the solution are shown in the Figure 5. In each case, we added 10 equiv. of anion. When the receptors bound the anions effectively, hydrogen bonds were constructed to form stable complexes, and the electron density in the supramolecular system increased considerably to enhance the CT interaction between the thiourea units and the electron-deficient *p*-nitrophenyl moieties, which resulted in a visible colour change. The re-

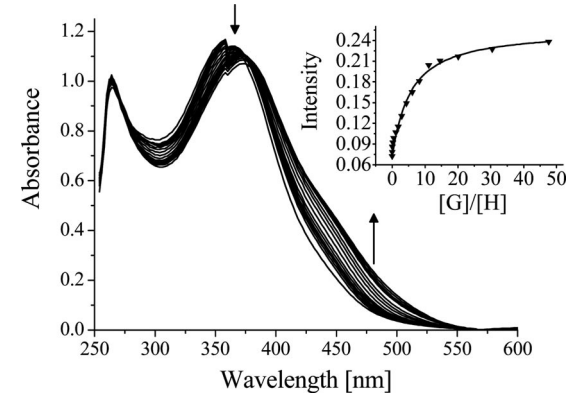


Figure 4. UV/Vis absorption spectra of receptor **6a** (5.0×10^{-5} mol dm^{-3}) upon the addition of various amounts of *N*-Ac-D-aspartate in DMSO at 25 °C. Equivalents of anion: 0, 0.05, 0.1, 0.2, 0.5, 1.2, 1.8, 3.0, 4.5, 6.0, 8.5, 11.0, 14.5, 20, 30 and 47.5. The curve-fitting line through the data points at 484 nm is shown in the inset, where G is the guest and H is the host. The correlation coefficient (R) of non-linear curve fitting is 0.9928.

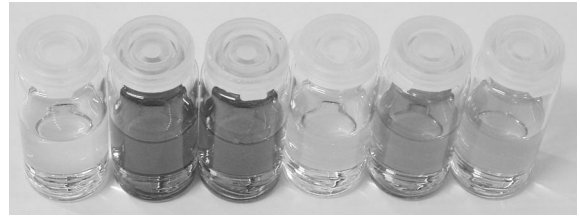


Figure 5. Effects of *N*-Ac-L- or D-aspartate (10 equiv., as Bu_4N^+ salts) on solution colour changes of **5a** or **6a** (1×10^{-4} mol dm^{-3}) in DMSO. From left to right: (a) **5a**; (b) **5a** + L-aspartate; (c) **5a** + D-aspartate; (d) **6a**; (e) **6a** + L-aspartate; (f) **6a** + D-aspartate.

markable colour changes from yellowish to nacarat or yellow of **6a** with *N*-Ac-L- or D-aspartate, respectively, revealed that **6a** can be used as a colorimetric chemosensor for the enantiomers of NAA.

Receptors **5a** and **6a** have similar structures; both of them could form stable 1:1 complexes with NAA, but their enantioselective recognition abilities towards NAA are nearly opposite. Compound **6a** had stronger interactions with D enantiomers (the D/L selectivity was 13.1), while **5a** was inclined to bind L enantiomers (the L/D selectivity was 10.49). In addition, the K_A values were also different. $K_{A(\mathbf{6a}+\text{D-aspartate})}$ was much larger than $K_{A(\mathbf{5a}+\text{L-aspartate})}$, while $K_{A(\mathbf{6a}+\text{L-aspartate})}$ was also larger than $K_{A(\mathbf{5a}+\text{D-aspartate})}$, suggesting that **6a** could bind the guest through stronger hydrogen-bonding interactions with NAA, possibly due to the more complementary structure of **6a** for NAA than that of **5a**. We presume that these receptors may have different preorganised structures and could interact with the enantiomers through different hydrogen-bonding sites. We carried out quantum chemical calculations to explain these phenomena further.

We used continuous variation methods to determine the stoichiometric ratios of the complexes formed between the receptors **5a** and **6a** and the anion guests.[19] We maintained the total concentration of the host and NAA constant (1.0×10^{-4} mol dm^{-3}) in DMSO, and varied the mole fraction of the guest {[G]/([H]+[G])} continuously. Figure 6 shows the Job plots for **5a** (at 500 nm) and **6a** (at 484 nm) with L- or D-aspartate in DMSO. When the mole fraction of the guest was 0.50, the absorption reached a maximum, demonstrating that **5a** and **6a** both formed a 1:1 complex with L- or D-aspartate.

We also investigated the interactions between receptors **5a** or **6a** and the enantiomers of *N*-acetyl-, *N*-Boc- or *N*-benzoyl-protected aspartate or glutamate by UV/Vis titration experiments. The results are listed in Table 1 and show that **5a** and **6a** have lower chiral recognition abilities towards other aspartate and glutamate derivatives. For the 1:1 complex, a K_A can be calculated using the following Equation (1) in Origin 7.0.[20] A represents the absorption intensity, and C_H and C_G are the respective concentrations

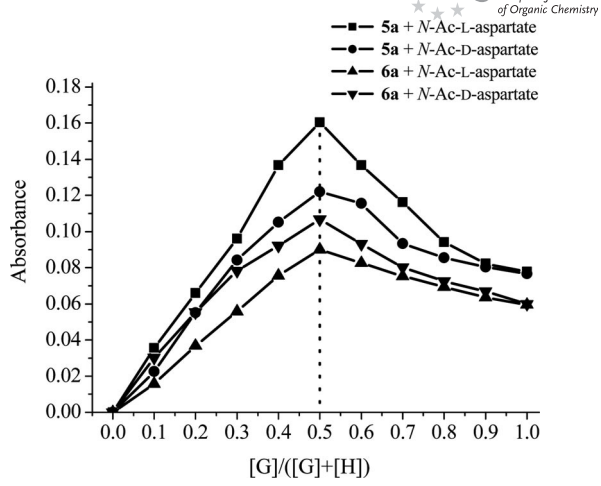


Figure 6. Job plots of **5a** (at 500 nm) and **6a** (at 484 nm) interacting with *N*-Ac-L- or D-aspartate. The total concentration of the host (H) and guest (G) was 1.0×10^{-4} mol dm^{-3} in DMSO.

of host and anion guest. The K_A values and correlation coefficients (R) obtained by a nonlinear least-squares analysis of A versus C_H and C_G are listed in Table 1.

$$A = A_0 + \frac{A_{lim} - A_0}{2C_0}\{C_H + C_G + 1/K_A - [(C_H + C_G + 1/K_A)^2 - 4\,C_H\,C_G\,]^{1/2}\} \quad (1)$$

From the data listed in Table 1, we found that receptor **5a** showed a good enantioselective recognition ability towards *N*-acetyl-glutamate with a D/L selectivity [$K_{A(D)}/K_{A(L)}$] of 2.64, while **6a** also exhibited a weak chiral recognition for this anion. When *N*-Boc-aspartate or *N*-benzoyl-aspartate was used, these receptors showed no enantioselectivities. On the other hand, control binding experiments with **5a** or **6a** and malate under the same conditions revealed no chiral recognition effect. These results demonstrated that the acetyl group of NAA played an important role in the chiral recognition process and could largely influence the complexation.

Another interesting phenomenon we found was that the K_A values between receptors and various *N*-protected glutamates were generally larger than those with the correspond-

Table 1. K_A values, correlation coefficients (R) and enantioselectivities (K_L/K_D) of receptors **5a** or **6a** with L- or D-aspartate or glutamate in DMSO at 25 °C.

Entry	Guest[a]	**5a**			**6a**		
		K_A [dm^3 mol^{-1}][b]	R	K_D/K_L	K_A [dm^3 mol^{-1}][b]	R	K_D/K_L
1	*N*-Ac-L-Asp	981.1 ± 45.2	0.9937	–	297.9 ± 24.2	0.9909	–
2	*N*-Ac-D-Asp	93.5 ± 8.6	0.9926	0.095	(3.91 ± 0.34) × 10^3	0.9912	13.1
3	*N*-Boc-L-Asp	(4.30 ± 0.39) × 10^3	0.9915	–	(1.75 ± 0.11) × 10^3	0.9938	–
4	*N*-Boc-D-Asp	(5.30 ± 0.42) × 10^3	0.9918	1.23	(1.88 ± 0.09) × 10^3	0.9968	1.07
5	*N*-Bz-L-Asp	(4.97 ± 0.35) × 10^3	0.9915	–	(5.22 ± 0.19) × 10^3	0.9977	–
6	*N*-Bz-D-Asp	(4.07 ± 0.28) × 10^3	0.9936	0.82	(4.68 ± 0.30) × 10^3	0.9928	0.90
7	*N*-Ac-L-Glu	(1.48 ± 0.12) × 10^3	0.9928	–	(2.05 ± 0.19) × 10^3	0.9906	–
8	*N*-Ac-D-Glu	(3.91 ± 0.21) × 10^3	0.9962	2.64	(1.41 ± 0.12) × 10^3	0.9915	0.69
9	*N*-Boc-L-Glu	(9.27 ± 0.66) × 10^3	0.9910	–	(2.96 ± 0.20) × 10^3	0.9947	–
10	*N*-Boc-D-Glu	(1.17 ± 0.72) × 10^4	0.9902	1.26	(4.38 ± 0.24) × 10^3	0.9964	1.48
11	*N*-Bz-L-Glu	(1.23 ± 0.12) × 10^4	0.9935	–	(8.18 ± 0.79) × 10^3	0.9905	–
12	*N*-Bz-D-Glu	(1.30 ± 0.14) × 10^4	0.9922	1.06	(8.36 ± 0.81) × 10^3	0.9910	1.02

[a] The anions were used as their Bu_4N^+ salts. [b] All error values were obtained by the results of nonlinear curve fitting.

ing *N*-protected aspartate. For example, $K_{A(\mathbf{5a}+N\text{-Ac-L-Glu})}$ was 1480 $dm^3 mol^{-1}$, while $K_{A(\mathbf{5a}+N\text{-Ac-L-Asp})}$ was 981.1 $dm^3 mol^{-1}$, indicating that both receptors have stronger hydrogen-bonding interactions with glutamate than with aspartate. We presume the carboxylate group of glutamate could approach the hydrogen-bonding groups of the receptors closer due to its longer alkyl chain, promoting the complexation of host and guest.[16]

In order to investigate possible interaction mode, we synthesized a series of reference compounds **5b**–**5e**, **6b**–**6e**, **7a** and **7b** and studied their bonding properties towards NAA by UV/Vis titration. The data listed in Table 2 demonstrate that these receptors formed 1:1 complexes with NAA, but most of them did not have chiral recognition abilities towards this anion, except **5d**, which exhibited good selectivity for the aspartate (the D/L selectivity was 2.92). We designed receptors **5b**–**5d** and **6b**–**6d** to determine the influence of the different amino acid chiral centres, and the results indicated that only the alanine unit could supply the proper steric conformation and enable enantioselective recognition. Increasing the steric hindrance by introducing the benzyl or indole group to the chiral centre did not enhance the chiral discrimination process.

Table 2. K_A values and enantioselectivities (K_D/K_L) of receptors **5b**–**7b** with *N*-acetyl-L- or -D-aspartate in DMSO at 25 °C.

Entry	Host	*N*-Ac-L-Asp[a] K_A [$dm^3 mol^{-1}$][b]	*N*-Ac-D-Asp[a] K_A [$dm^3 mol^{-1}$][b]	K_D/K_L
1	**5b**	$(1.12 \pm 0.09) \times 10^3$	$(1.44 \pm 0.12) \times 10^3$	1.29
2	**6b**	555.1 ± 42.8	423.5 ± 36.2	0.76
3	**5c**	$(2.02 \pm 0.17) \times 10^3$	$(2.52 \pm 0.20) \times 10^3$	1.25
4	**6c**	$(3.19 \pm 0.20) \times 10^3$	$(4.57 \pm 0.43) \times 10^3$	1.43
5	**5d**	779.5 ± 43.7	$(2.28 \pm 0.14) \times 10^3$	2.92
6	**6d**	700.6 ± 38.1	879.9 ± 64.3	1.25
7	**5e**	102.3 ± 9.2	145.6 ± 13.5	1.42
8	**6e**	155.8 ± 12.6	187.9 ± 16.3	1.20
9	**7a**	$(8.99 \pm 0.90) \times 10^3$	$(8.35 \pm 0.72) \times 10^3$	0.93
10	**7b**	$(1.19 \pm 0.20) \times 10^4$	$(1.65 \pm 0.14) \times 10^4$	1.38

[a] The anions were used as their Bu_4N^+ salts. [b] All error values were obtained by the results of nonlinear curve fitting.

Receptors **5e** and **6e** have similar structures to those of **5a** and **6a**, respectively, except that the electron-repulsive *p*-tolyl units substitute for *p*-nitrophenyl units. Thiourea is regarded as a hydrogen-bond donor; introducing an electron-repulsive unit will significantly reduce the acidities of the thiourea protons, leading to its hydrogen-bond-donating ability becoming much weaker. The data obtained from UV/Vis titration supported this reasoning, as the K_A between **5e** and *N*-Ac-L-aspartate was only 102.3 $dm^3 mol^{-1}$, which is much smaller than that of **5a** with L-aspartate (K_A = 981.1 $dm^3 mol^{-1}$). In the same way, the K_A of **6e** with D-aspartate was 187.9 $dm^3 mol^{-1}$, much smaller than that of **6a** with D-aspartate (K_A = 3910 $dm^3 mol^{-1}$). Unlike the excellent enantioselective recognition abilities of **5a** and **6a**, we found receptors **5e** and **6e** did not have chiral recognition abilities towards NAA. These results indicate that the thiourea groups linked to the electron-withdrawing *p*-nitrophenyl group played a critical role in the complexation; the enantiomeric selectivity largely depended on different hydrogen-bonding abilities.

In the design of chemosensors, the selection of a supramolecular platform is critically important because it supplies the proper preorganised structure for the recognition. In this work, we introduced Fc as a supramolecular platform and prepared a series of Fc derivatives bearing thiourea groups, which exhibited excellent chiral recognition abilities. In order to compare this platform with possible alternatives, we synthesized two reference compounds (**7a** and **7b**) based on 2,6-pyridine dicarboxylic acid, and their bonding properties towards NAA are listed in Table 2 (Entries 9 and 10). The K_A between **7a** and L-aspartate was 8.99×10^3 $dm^3 mol^{-1}$, while that of **7b** with L-aspartate was 1.19×10^4 $dm^3 mol^{-1}$, and both of them were significantly larger than the values of the corresponding Fc derivatives (**5a** and **6a**), demonstrating that **7a** and **7b** had substantially stronger interactions with aspartate than did the Fc derivatives. We presume that 2,6-pyridine dicarboxylic acid has a constrictive structure, as the two functional arms of **7a** or **7b** could only stretch in specific directions, and thus, are inclined to bind the aspartate in a "sandwich" mode, while intense hydrogen-bonding interactions could be formed because the carboxylate units of the anion approach the thiourea binding units closely. Although **7a** and **7b** bound NAA intensely, neither of them showed chiral selectivity for NAA, which further demonstrates the advantages of Fc as the supramolecular platform for this system. The preorganised structure and favourable conformation of Fc derivatives led to the excellent chiral recognition abilities.

On the basis of the good enantioselective recognition abilities of receptors **5a** and **6a** towards NAA, we employed them as probes to detect the enantiomeric composition of this anion. We studied the UV/Vis absorbance changes of **5a** and **6a** versus the enantiomeric composition of NAA. As shown in the Figure 7, the absorbance intensity of **5a** decreased linearly with increasing D isomer content of the NAA, and we also observed a similar linear decrease when **6a** was used as the probe. Thus, these receptors can be used as colorimetric sensors to readily determine the enantiomeric composition of NAA.[21]

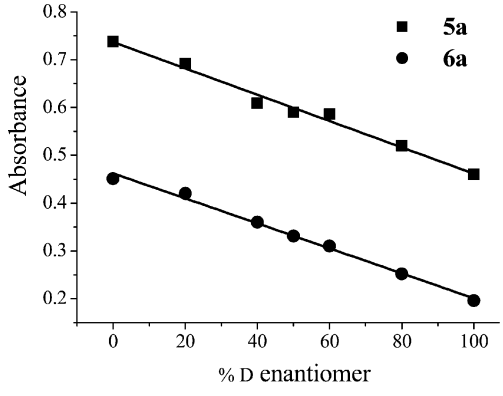


Figure 7. The UV/Vis absorbance intensities of receptor **5a** (at 500 nm) or **6a** (at 484 nm) at 5.0×10^{-5} mol dm^{-3} versus the enantiomeric composition of NAA at 5.0×10^{-4} mol dm^{-3}.

Theoretical Calculations

In the UV/Vis titrations described above, we found receptors **5a** and **6a** had excellent enantioselective recognition abilities towards NAA, but the D/L selectivity was nearly opposite. Receptor **5a** was inclined to bind L enantiomers, while **6a** had stronger affinity to D enantiomers. We presumed these two receptors had different preorganised structures; their two functional arms containing multiple hydrogen-bonding sites stretched in different directions.

In order to obtain information about the possible structures of **5a** and **6a**, we carried out quantum chemical calculations at the DFT level of theory. The minimum energy structures are shown in Figure 8. We found that **5a** adopted a stretched conformation, with the two functional arms of this molecule occupying different sides of the Fc; the minimum distance between the two phenyl groups was 18.24 Å, while the distance between the two sulfurs was 13.70 Å. Receptor **6a** adopted a completely different conformation, with the two functional arms on the same side of the Fc; the minimum distance between the two phenyl groups was 13.54 Å, while the distance between the two sulfurs was 14.08 Å. These structures can be described as "foldout" structures; receptor **5a** looks likes an "open foldout", while **6a** looks like a "closed foldout", with the Fc unit controlling these open/closed conformations like an axis of foldout. Based on these models, we presume NAA may adopt a stretched conformation when it interacts with **5a**, while it may contract when it interacts with **6a** due to the limited interarm space. Different interaction modes could induce the different chiral recognition abilities towards the enantiomers.

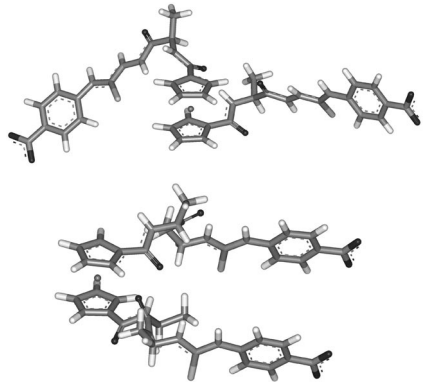


Figure 8. Calculated (B3LY/6-31G*) structures for receptors **5a** (upper) and **6a** (lower).

We tried to calculate the possible interaction mode of receptors with NAA, but we obtained no reliable data due to the complicated hydrogen-bonding system. From the minimum energy structures of these receptors, the opposite enantioselectivities can be explained to some extent, which could contribute to the further design of Fc derivatives.

Conclusion

In summary, we synthesized a series of chiral Fc derivatives containing thiourea groups and investigated their enantioselective recognition abilities towards NAA by UV/Vis titration experiments. Clear differences in the solution colour and K_A values demonstrated that receptors **5a** and **6a** had excellent chiral recognition abilities towards NAA, which could be discriminated by the naked eye. In order to investigate the possible interaction mechanism, we designed some Fc derivatives with different chiral centres or electron-repulsive groups. Comparison experiments indicated that the preorganised conformations of **5a** and **6a** and the multiple hydrogen-bonding interactions between the thiourea groups and the aspartate can contribute to the chiral recognition abilities. We also obtained minimum energy structures of **5a** and **5b** through quantum-chemical calculations at the DFT level of theory, which initially explain why these receptors show different chiral bonding properties towards the same guest. In previous studies, such chromogenic chiral Fc derivatives have been seldom reported. Based on their good optical properties and special hydrogen-bonding system, these receptors could be used as chiral chromogenic sensors for the enantiomers of NAA, while the development of these kinds of Fcs will have potential application in the research of enantioselective recognition and asymmetric catalysis.

Experimental Section

General: Ethylenediamine was distilled before use. $CHCl_3$ was washed with water and dried from $CaCl_2$, and Et_3N was dried and distilled from CaH_2 and KOH. All other commercially available reagents were used without further purification. Melting points were determined with a Reichert 7905 melting-point apparatus (uncorrected). Optical rotations were recorded with a Perkin–Elmer Model 341 polarimeter. IR spectra were obtained with a Nicolet 670 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded with a Varian Inova unity-600 MHz spectrometer. ^{13}C NMR spectra were recorded with a Varian Inova unity-600 MHz spectrometer. Mass spectra were recorded with a Finnigan LCQ advantage mass spectrometer. Elemental analysis was determined with a Carlo–Erba 1106 instrument. The UV/Vis spectra were recorded with a TU-1901 spectrophotomer. The anions were used as their Bu_4N^+ salts. 1,1′-ferrocenediacetyl chloride (**1**) was synthesized according to the methods reported in the literature.[22]

Synthesis

General Procedure for the Synthesis of the Fc Derivatives 2: To a solution of L-amino acid methyl ester hydrochloride (3 mmol) and Et_3N (4 equiv. for each amino acid methyl ester hydrochloride) in dry $CHCl_3$ (30 mL), 1,1′-ferrocenediacetyl chloride (**1**, 1.5 mmol) in dry $CHCl_3$ (20 mL) was added dropwise in an iced brine bath under a N_2 atmosphere. The reaction mixture was stirred at 0 °C for 1 h, the ice brine bath was removed, and the mixture was stirred overnight at room temperature. The reaction solution was then washed with water three times. The organic layer was collected and dried with anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel.

***N*,*N*′-[(Ferrocene-1,1′-diyl)biscarbonyl]bis(L-alanine methyl ester) (2a):** Pure product was obtained by column chromatography on silica gel [eluent: $CHCl_3/CH_3CH_2OH$ = 100:1 (v/v)] as a brown-red powder (0.50 g) in 75% yield; m.p. 162–163 °C. $[\alpha]_D^{20}$ = +346.7 (*c* =

0.015, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3349, 1716, 1659, 1522, 1439, 1384, 1373, 1311, 1231, 1179, 1054, 835, 776 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 1.39 (s, 3 H, CCH_3), 1.41 (s, 3 H, CCH_3), 3.82 (s, 6 H, OCH_3), 4.35 (s, 2 H, Cp-H), 4.55 (s, 2 H, Cp-H), 4.76 (s, 2 H, Cp-H), 4.81–4.89 (m, 2 H, C*H), 4.91 (s, 2 H, Cp-H), 7.75 (d, J = 8.4 Hz, 2 H, CONH) ppm. $C_{20}H_{24}FeN_2O_6$ (444.27): calcd. C 54.07, H 5.45, N 6.31; found C 53.82, H 5.57, N 6.20.

The characterization data of compounds **2b**–**2d** are listed in part 2.1 of the Supporting Information.

General Procedure for the Synthesis of the Fc Derivatives 3: A solution (20 mL) of **2a**–**2d** (0.5 mmol) in CH_3OH was added dropwise to a stirred solution of hydrazine hydrate (6 equiv. for **2a**–**2d**) in CH_3OH (10 mL). The mixture was stirred for 48 h under N_2 protection at room temperature. The solvent was removed under reduced pressure, and water (50 mL) was poured into the residue. The solid obtained was filtered and washed with cold CH_3CH_2OH several times and dried in vacuo to obtain the pure product.

Compound 3a: 0.12 g was obtained as a brown powder (54% yield); the compound decomposed at 196–198 °C. $[a]_D^{20}$ = +87.2 (c = 0.005, DMSO). IR (KBr): $\tilde{\nu}$ = 3442, 3262, 1661, 1569, 1541, 1458, 1377, 1315, 1276, 1202, 970 cm^{-1}. ESI-MS: m/z (%) = 445.3 (100) [M + H]$^+$. $C_{18}H_{24}FeN_6O_4$ (444.27): calcd. C 48.66, H 5.44, N 18.92; found C 48.35, H 5.60, N 18.74.

The characterization data of compounds **3b**–**3d** were listed in part 2.2 of the Supporting Information.

General Procedure for the Synthesis of the Fc Derivatives 4: A solution (20 mL) of **2a**–**2d** (0.5 mmol) in CH_3OH was added dropwise to a stirred solution of ethylenediamine (6 equiv. for **2a**–**2d**) in CH_3OH (10 mL). The mixture was stirred for 48 h under N_2 protection at room temperature. The solvent and excess ethylenediamine were removed under reduced pressure, and the residue was washed with cold CH_3CH_2OH and dried in vacuo to give pure product.

Compound 4a: 0.18 g was obtained as a filemot powder (72% yield); the compound decomposed at 220 °C. $[a]_D^{20}$ = +97.0 (c = 0.005, DMSO). IR (KBr): $\tilde{\nu}$ = 3289, 3083, 2936, 1665, 1622, 1556, 1458, 1380, 1316, 1199, 1036, 814 cm^{-1}. ^1H NMR (600 MHz, [D_6]-DMSO): δ = 1.26–1.31 (m, 6 H, CCH_3), 1.75 (s, 4 H, D_2O-exchangable, NH_2), 2.98–3.16 (m, 8 H, NCH_2CH_2N), 4.31 (s, 2 H, Cp-H), 4.38 (s, 2 H, Cp-H), 4.46 (s, 2 H, Cp-H), 4.71–4.79 (m, 2 H, C*H), 4.91 (s, 2 H, Cp-H), 7.35 (d, J = 8.4 Hz, 2 H, CONH), 8.66 (s, 2 H, CONHC) ppm. ESI-MS: m/z (%) = 501.5 (100) [M + H]$^+$. $C_{22}H_{32}FeN_6O_4$ (500.38): calcd. C 52.81, H 6.45, N 16.80; found C 52.57, H 6.58, N 16.72.

The characterization data of compounds **4b**–**4d** were listed in part 2.3 of the Supporting Information.

General Procedure for the Synthesis of the Fc Derivatives 5 and 6: A solution of *p*-nitrophenyl isothiocyanate (0.144 g, 0.8 mmol) in dry DMF (15 mL) was added dropwise to a solution of **3** or **4** (0.4 mmol) in dry DMF (15 mL) at room temperature. The reaction mixture was then stirred at room temperature for 48 h. A large amount of water was poured into the solution. The collected precipitate was washed with $CHCl_3$ and acetone, and the solid was dried under vacuum to obtain the pure product.

Compound 5a: 0.19 g was obtained as a bottle-green powder (59% yield); m.p. 168–170 °C. $[a]_D^{20}$ = +116.1 (c = 0.005, DMSO). IR (KBr): $\tilde{\nu}$ = 3331, 1630, 1595, 1547, 1506, 1384, 1332, 1301, 1247, 1181, 1113, 851, 748 cm^{-1}. ESI-MS: m/z (%) = 804.3 (100) [M$^+$]. $C_{32}H_{32}FeN_{10}O_8S_2$ (804.64): calcd. C 47.75, H 4.01, N 17.41; found C 47.50, H 4.28, N 17.26.

Compound 6a: 0.28 g was obtained as a filemot powder (81% yield); m.p. 172–174 °C. $[a]_D^{20}$ = +30.0 (c = 0.005, DMSO). IR (KBr): $\tilde{\nu}$ = 3322, 1634, 1594, 1540, 1510, 1382, 1334, 1303, 1263, 1111, 853 cm^{-1}. ^1H NMR (600 MHz, [D_6]DMSO): δ = 1.25–1.29 (m, 6 H, CH_3), 2.70–2.81 (m, 2 H, NCH_2C), 2.85–2.92 (m, 2 H, NCH_2C), 3.57–3.62 (m, 4 H, NCH_2C), 4.25 (s, 2 H, Cp-H), 4.30 (s, 2 H, Cp-H), 4.38 (s, 2 H, Cp-H), 4.70–4.78 (m, 2 H, C*H), 4.84 (s, 2 H, Cp-H), 7.31 (d, J = 7.2 Hz, 2 H, CONH), 7.75 (s, 4 H, NO_2ArH), 7.97 (s, 2 H, CNHCS), 8.17 (s, 4 H, NO_2ArH), 8.44 (s, 2 H, CSNHAr), 10.20 (s, 2 H, CONHC) ppm. ^{13}C NMR (150 MHz, [D_6]DMSO): δ = 18.1, 38.7, 44.1, 48.9, 70.4, 71.6, 72.1, 77.1, 121.3, 123.0, 124.2, 125.2, 125.7, 142.6, 144.1, 145.7, 146.7, 169.3, 175.1, 181.0 ppm. ESI-MS: m/z (%) = 861.9 (100) [M + H]$^+$. $C_{36}H_{40}FeN_{10}O_8S_2$ (860.74): calcd. C 50.24, H 4.68, N 16.27; found C 50.01, H 4.90, N 16.40.

The characterization data of compounds **5b**–**5d**, **6b**–**6d** and **7a**–**7b** were listed in parts 2.4 and 2.5 of the Supporting Information, respectively.

Bu_4N^+ Salts: Bu_4N^+ salts of the amino acids were prepared by adding 2 equiv. of Bu_4NOH in CH_3OH to a solution of corresponding *N*-protected aspartic acid or glutamic acid (1 equiv.) in CH_3OH. The mixture was stirred at room temperature for 2 h, and the solvents were evaporated to dryness under reduced pressure. The resulting syrup was dried under high vacuum at 50 °C for 24 h, checked by NMR and stored in a desiccator.

Binding Studies: The host compounds were prepared as stock solutions in DMSO at 5×10^{-4} mol dm^{-3}. The anion was dissolved to approximately 0.015 mol dm^{-3} and 0.15 mol dm^{-3} of stock solution in DMSO. The working solutions were prepared by adding different volumes of *N*-protected aspartate or glutamate solution to a series of test tubes followed by the addition of the same amount of stock solution of host compound followed by dilution to 3.0 mL by DMSO. After being shaken for several minutes, the work solution was measured immediately. K_A values were calculated by means of a nonlinear, least-squares, curve-fitting method with ORIGIN 7.0 (Origin-Lab Corporation).

Supporting Information (see also the footnote on the first page of this article): Scheme of the possible charge transfer process of compounds **3a**–**3c**, ^1H NMR spectra of **3d** and the characterization data of reference compounds.

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