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New C_2-symmetric 2,2′-bipyridine crown macrocycles for enantioselective recognition of amino acid derivatives

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Abstract—A series of new C_2-symmetric 2,2′-bipyridine-contaning crown macrocycles **1**–**4** has been developed for enantiomeric recognition of amino acid derivatives. These new macrocycles have been showed to be strong complexing agents for primary organic ammonium salts (with K up to 4.83×10^5 M^{-1} and $-\Delta G_0$ up to 32.4 kJ mol^{-1}) and also useful chromophores for UV–vis titration studies. These macrocyclic hosts exhibited enantioselective binding towards the (*S*)-enantiomer of phenylglycine methyl ester hydrochloride (**Am1**) with $K_{(S)}/K_{(R)}$ up to 2.10 ($\Delta\Delta G_0 = -1.84$ kJ mol^{-1}) in CH_2Cl_2 with 0.25% CH_3OH. The structure–binding relationship studies showed that the aromatic subunit and the ester group of the ammonium guests are both important for good enantioselectivity. In addition, the host–guest complexes have been studied using various NMR experiments.

1. Introduction

One of the most remarkable features of many biomolecules, such as enzymes, is their abilities to distinguish enantiomers in biological reactions. Most of the amino acids and their derivatives, which are basic building blocks of many biologically important molecules, are chiral. The development of artificial receptors for these interesting compounds becomes an important research area because it can provide valuable information for a better understanding of the interactions between molecules in nature. Moreover, the studies of their recognition properties may also lead to the development of useful molecular devices and materials in biochemical[1,2] and pharmaceutical studies,[3] separation processes,[4] catalysis[5] and sensing.[6]

In the passed decade, chiral pyridine-containing macrocycles have been an attractive research area due to their ability of chiral discrimination towards organic ammonium salts and the amino acid derivatives.[7–11] The pyridine subunit of these macrocycles were reported to be important for the tripod hydrogen bonding formation with the primary ammonium salts and the π–π interaction with the aromatic moiety of the ammonium guests.[7] Since, we have been developing various types of pyridine-containing ligands for asymmetric catalysis,[12] we are interested in developing new artificial receptors for enantiomeric recognition of amino acid derivatives based on 2,2′-bipyridine (bpy).

Bpys have been used widely as metal chelating ligands due to their strong chelating ability towards various metals and ease of functionalization.[13] For instance, the bpy–ruthenium complexes have been studied extensively due to their interesting photochemical and other properties.[14] The results of these studies had led to the development of various types of bpy–ruthenium complexes-based sensors.[15] In contrast, the study of the interaction between bpy-containing macrocycles[16] and organic cationic substrates remains an unexplored area. Herein, we report the synthesis of a series of new bpy crown macrocycles (Fig. 1), and the study of their enantiomeric recognition properties towards amino acid derivatives and chiral organic ammonium salts.

2. Results and discussion

2.1. Preparation of new chiral bpy crown macrocycles

Bpy crown macrocycles **1**–**4** could be readily prepared via the two-step one-pot protocol shown in Scheme 1, which involved deprotonation of (*R*,*R*)-6,6′-bipyridinediol **5**[17] followed by cyclization with the appropriate ethyl glycol

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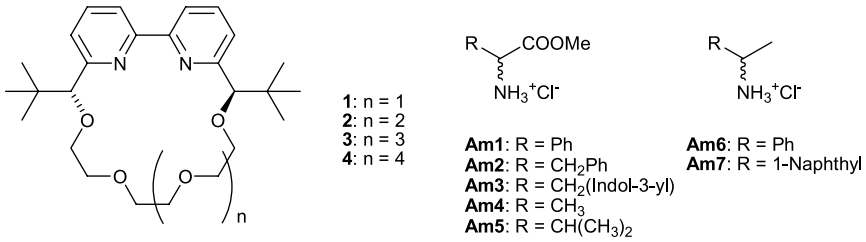
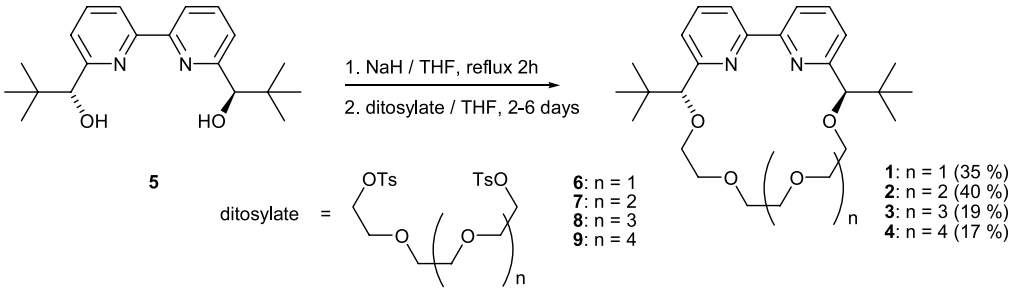


Figure 1. Chiral bpy crown macrocycles **1**–**4** (hosts) and chiral organic ammonium salts **Am1**–**Am7** (guests).



Scheme 1. Synthesis of chiral bpy crown macrocycles **1**–**4**.

ditosylate (**6**–**9**). To determine the optimal conditions for the cyclization process, reaction of diol **5** with triethylene glycol ditosylate (**6**) was first investigated using potassium hydride in THF under high dilution conditions. Since bpy crown macrocycle **1** contains a pseudo 18-crown-6 frame work, the presence of potassium ions was expected to give the optimal template effect on the cyclization process.[18] After 2 days stirring at room temperature, diol **5** was consumed and the MS-ESI analysis of the crude product mixture shows the formation of the bpy crown macrocycle. However, only 15% of the cyclization product (**1**) was isolated. Bpy crown macrocycle **1** was characterized unambiguously by elemental analysis, MS-ESI and NMR experiments. The ^{1}H and ^{13}C NMR spectra of **1** show only one set of signals for the two pyridine rings of the bpy subunit and a singlet for the two *t*-butyl groups, which indicate that the C_2-symmetry of bpy diol **5** is unaffected after the incorporation of the crown moiety. Moreover, the signals corresponding to the ethylene glycol units are also well resolved, which suggested that the bpy crown macrocycle is well accommodated in the pseudo 18-crown-16 frame work.

The cyclization protocol was then investigated using sodium hydride under the same reaction conditions. Surprisingly, these conditions afforded the cyclization product in a much higher isolated yield (35%). These results suggested that the template effect of alkaline metal ion may not be effective in this system, due to the rigid bpy subunit of the macrocycle. In addition, the solvent effect had also been studied. Cyclization using NaH in DMF under the high dilution condition gave a similar yield of **1** (33%) in 2 days. However, switching to DMSO resulted in only trace amounts of the cyclization product with 56% of diol **5** recovered under the same conditions.

After optimizing the cyclization conditions, diol **5** was deprotonated using sodium hydride in THF followed by treatment of the corresponding ditosylate (**7**–**9**) under the high dilution conditions to afford the desired chiral bpy crown macrocycles **2**–**4** in 17–40% isolated yields (Scheme 1). The structures of this new series of bpy crown macrocycles were characterized using the same methods as **1**. However, reaction between **5** and di(ethylene glycol)ditosylate gave only trace amounts of the expected cyclization product. This poor result may be due to the high ring strain of the bipyridino-15-crown-5 structure of the cyclization product.

2.2. Enantiomeric recognition studies using UV–vis method

With the new macrocyclic hosts prepared, we first examined the binding properties of bpy crown macrocycle **1** towards (*R*)-(−)-2-phenylglycine methyl ester hydrochloride ((*R*)-**Am1** in Fig. 1) using the UV–vis titration method. The concentration of the macrocyclic host was fixed at 2.5×10^{-5} M in CH_2Cl_2 with 0.25% of CH_3OH because of the solubility of the guest. The UV−vis signals with the guest concentration varied from 0 to 6×10^{-5} M were then observed. As shown in Figure 2, the absorption peak at

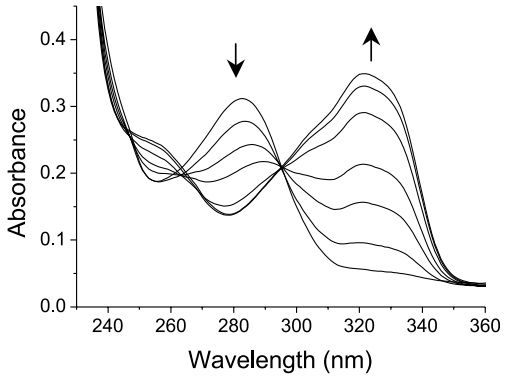


Figure 2. UV–vis titration of **1** with (*R*)-**Am1** at 298 K in CH_2Cl_2 containing 0.25% CH_3OH: [**1**]$=2.5\times10^{-5}$ M and [(*R*)-**Am1**]$=0$, 0.5, 1, 1.5, 3, 4.5, 6×10^{-5} M.

298 nm for **1** decreased gradually upon addition of the guest and a new absorption peak for the inclusion complex started to appear at 320 nm forming the isosbestic point at 305 nm. In contrast, binding studies using dimethylether of **5** led to only small and irregular change in the UV spectrum. This result indicated that the crown portion of the macrocycle is essential for binding. The Job's plot based on the absorbance at 320 nm supported the 1:1 stoichiometry of the host–guest complex (Fig. 3).

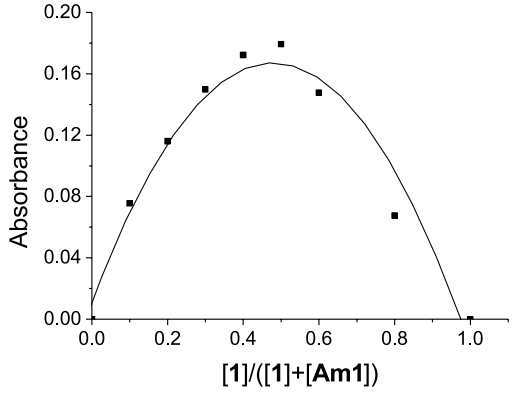


Figure 3. Job's plot of bpy crown macrocycle **1** and (*R*)-**Am1** in CH_2Cl_2 containing 0.25% CH_3OH at 298 K with [**1**]+[(*R*)-**Am1**]=5.0×10^{-5} M.

$$\frac{A_0}{A_0 - A} = \frac{\varepsilon_0}{\varepsilon_0 - \varepsilon}\left(1 + \frac{1}{K[\mathbf{Am}]_i}\right) \quad (1)$$

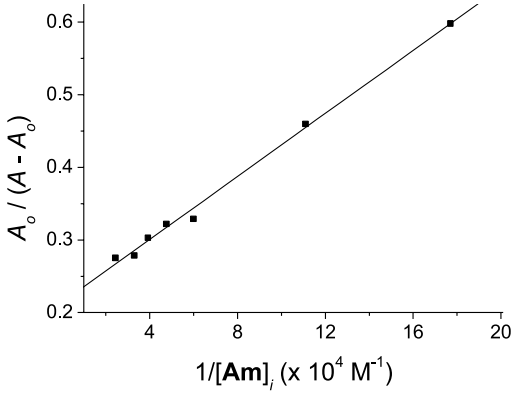


Figure 4. The plot of $A_0/(A-A_0)$ versus $1/[\mathbf{Am}]_i$ at 320 nm based on the 1:1 binding model of bpy crown macrocycle **1** with (*R*)-**Am1**.

The equilibrium binding constant (K) was estimated using Eq. 1, where A and A_0 are the absorbance of the inclusion complex at $\lambda_{max}=320$ nm with concentration of free **Am**=$[\mathbf{Am}]_i$ and 0, respectively, ε_0 and ε are the molar absorption coefficients for the free and **Am**-bound bpy macrocycle at $\lambda_{max}=320$ nm, respectively. Since the assumption of $[\mathbf{Am}]_i$ being equal to the total concentration of **Am** is not adequate in our system, the values of $[\mathbf{Am}]_i$ were calculated using the Taylor's series approximation.[19] The plot of $A_0/(A_0-A)$ against $1/[\mathbf{Am}]_i$ showed good linear relationship with $R=0.998$ for (*R*)-**Am1** (Fig. 4) and 0.992 for (*S*)-**Am1**. The binding constants were determined by the ratio of *y*-intercept to the slope,[20] which gave $K_{(R)}=9.86\pm0.77\times10^4\ M^{-1}$ and $K_{(S)}=20.7\pm1.29\times10^4\ M^{-1}$. These equilibrium binding constants are considered to be large among the analogous pyridine-containing crown macrocycles.[7,8]

To study the effect on the cavity size of the macrocyclic hosts, the binding properties of bpy crown macrocycles **2**–**4** towards **Am1** were studied using the same UV–vis titration method. As shown in Table 1, all the bpy crown macrocycles showed enantioselectivity towards (*S*)-**Am1** regardless of the cavity size of the macrocycles. Bpy crown macrocycle **1** showed the best enantiomeric recognition ability towards **Am1** with $K_{(S)}/K_{(R)}$ equals 2.1 ($\Delta\Delta G_0=-1.84$ kJ mol^{-1}). The high enantioselectivity of **1** might due to its pseudo 18-crown-6 frame work, which seems to provide a good environment for hydrogen bonding and π–π interaction with the guest molecule.[7]

After optimizing the cavity size of the macrocyclic host, the effect on the structure of the ammonium guest was then investigated. A number of amino acid derivatives and chiral organic ammonium salts were submitted for the enantiomeric recognition studies with bpy crown macrocycle **1** and the results were summarized in Table 2. Generally, the ammonium guests containing an aromatic side-chain exhibited higher enantioselectivity than those containing an alkyl side-chain. The ammonium guest bearing a phenyl side-chain (**Am1**) showed the strongest binding between the host and the guest, and it also gave the highest $K_{(S)}$ to $K_{(R)}$ ratio (Table 2, entries 1–4). However, the benzyl derivative, phenylalanine methyl ester hydrochloride (**Am2**), showed a significantly lower enantioselectivity. These results suggested that the extra methylene group of the benzyl moiety in **Am2** may orientate the phenyl ring of **Am2** poorly for π–π interaction with the bpy subunit of the host. In addition, tryptophan methyl ester hydrochloride (**Am3**) was

Table 1. Enantiomeric recognition studies of bpy crown macrocycles **1**–**4** towards **Am1** in CH_2Cl_2 with 0.25% CH_3OH at 298 K using UV–vis titration method[a]

Entry	Host	Guest[b]	K ($\times10^4$ M^{-1})	$K_{(S)}/K_{(R)}$	$-\Delta G_0$ (kJ mol^{-1})	$\Delta\Delta G_0$[c] (kJ mol^{-1})	R
1	**1**	(*R*)-**Am1**	9.9±0.8	2.1	29	−1.9	0.998
2	**1**	(*S*)-**Am1**	21±1.3		30		0.992
3	**2**	(*R*)-**Am1**	7.5±0.5	1.6	28	−1.2	0.996
4	**2**	(*S*)-**Am1**	12±0.6		29		0.998
5	**3**	(*R*)-**Am1**	7.6±0.6	1.3	28	−0.6	0.997
6	**3**	(*S*)-**Am1**	9.7±1.6		28		0.986
7	**4**	(*R*)-**Am1**	7.8±0.8	1.8	28	−1.4	0.991
8	**4**	(*S*)-**Am1**	14±1.1		29		0.992

[a] The concentration of the hosts: 2.5×10^{-5} mol dm^{-3}.
[b] **Am1**: 2-Phenylglycine methyl ester hydrochloride.
[c] $\Delta\Delta G_0=-nRT\ln(K_{(S)}/K_{(R)})$.

Table 2. Enantiomeric recognition studies of bpy crown macrocycle **1** towards amino acid derivatives and chiral organic ammonium salts at 298 K using UV–vis and NMR titration methods

Entry	Method	Guest[a]	K ($\times 10^2$ M^{-1})	$K_{(S)}/K_{(R)}$	$-\Delta G_0$ (kJ mol^{-1})	$\Delta\Delta G_0$[b] (kJ mol^{-1})	R
1	UV–vis[c]	(*R*)-**Am1**	990±77	2.1	29	−1.9	0.998
2	UV–vis[c]	(*S*)-**Am1**	2100±130		30		0.992
3	UV–vis[c]	(*R*)-**Am2**	300±42	1.6	26	−1.2	0.996
4	UV–vis[c]	(*S*)-**Am2**	480±41		27		0.998
5	UV–vis[c]	(*R*)-**Am4**	470±37	1.0	27	−0.1	0.998
6	UV–vis[c]	(*S*)-**Am4**	490±66		27		0.995
7	UV–vis[c]	(*R*)-**Am5**	81±9	1.3	22	−0.8	0.993
8	UV–vis[c]	(*S*)-**Am5**	110±9		23		0.988
9	NMR[d]	(*R*)-**Am1**	2.7	3.7	14	−3.2	—
10	NMR[d]	(*S*)-**Am1**	10		17		—
11	NMR[d]	(*R*)-**Am6**	6.0	1.1	16	0.1	—
12	NMR[d]	(*S*)-**Am6**	5.7		16		—

[a] **Am1**: 2-Phenylglycine methyl ester hydrochloride; **Am2**: Phenylalanine methyl ester hydrochloride; **Am4**: Alanine methyl ester hydrochloride; **Am5**: Valine methyl ester hydrochloride; **Am6**: (α-Phenylethyl)ammonium chloride.
[b] $\Delta\Delta G_0 = -nRT \ln (K_{(S)}/K_{(R)})$.
[c] The concentration of **1** is 2.5×10^{-5} mol dm^{-3} in CH_2Cl_2 with 0.25% CH_3OH.
[d] The initial concentration of **1** is 5.1×10^{-3} M in CD_2Cl_2 with 10% CD_3OD.

also submitted for the recognition study. However, the equilibrium binding constant could not be determined because of overlap between the UV signals corresponding to the host and the guest.

Among the ammonium guests bearing alkyl side-chains, alanine methyl ester hydrochloride (**Am4**) showed almost no enantioselectivity and the more bully valine methyl ester hydrochloride (**Am5**) showed slightly higher enantioselectivity than **Am4**. These results suggested that the steric effect of the ammonium guest is important for good enantioselectivity when no aromatic group is available for π–π interaction. In addition, (α-phenylethyl)ammonium chloride (**Am6**) and (α-(1-naphthyl)ethyl)ammonium chloride (**Am7**) were used to study effect of the binding properties without the ester subunit. Unfortunately, no new UV signal corresponding to the host–guest complex was observed upon addition of **Am6**, and the UV signals between **Am7** and the macrocyclic host **1** was found to be overlapped.

2.3. Enantiomeric recognition studies using NMR titration method

Since the binding studies with **Am6** and **Am7** could not be carried out using UV–vis spectroscopic method, their binding studies with bpy crown macrocycle **1** were then carried out using the NMR titration method.[21–23] The binding study with **Am1** using the NMR titration method was also carried out for comparison. A 10% of CD_3OD in CD_2Cl_2 solution was used due to the solubility of the ammonium guests. The initial concentration of **1** was set to be 5.1×10^{-3} M in order to obtain reasonable ^1H NMR signals and this solution was titrated with 40.5×10^{-3} M of the ammonium guest in the same solvent system with tetramethylsilane (TMS) as the internal standard. By using the non-linear least squares treatment, the equilibrium binding constants and the chemical shift of the host–guest complex signal of interested (δ_c) can be calculated through the minimization of the error function F (Eq. 2),[24]

$$F = \Sigma(\delta_{obsd} - \delta_{ave})^2$$

$$= \Sigma[\delta_{obsd,i} - X_{f,i}\delta_f - (1 - X_{f,i})\delta_c]^2 \quad (2)$$

where δ_{obsd} is the chemical shift of the bpy crown macrocycle signal of interest and δ_{ave} is the weighted average of the same signal or the free and complexed bpy crown macrocycle. δ_f is the chemical shift of the same signal of the free crown macrocycle and X_f is the mole fraction of the free bpy crown macrocycle.

As shown in Table 2 entries 9–12, the equilibrium binding constants for **Am1** and **Am6** observed under the NMR conditions (10% CD_3OD in CD_2Cl_2) are much smaller (about 200-fold for (*S*)-**Am1** and more than 300-fold for (*R*)-**Am1**) than those observed under the UV–vis conditions (0.25% CH_3OH in CH_2Cl_2). To investigate this dramatic effect on different methanol content, the recognition study between **1** and **Am1** was carried out in CH_2Cl_2 with 10% CH_3OH using the UV–vis method. These conditions also gave small K values ($K_{(S)}=3500$ M^{-1} and $K_{(R)}=1800$ M^{-1}), which is roughly in the same order of magnitude as the results obtained by the NMR titration method. Though the NMR study showed almost no enantioselectivity for **Am6**, it showed the good binding ability of **Am6** towards **1**, which was not observed by the UV–vis method. These results suggested that the ester subunit of the ammonium guest is important for the formation of the new UV signal for the host–guest complex, and it is also important for the high enantiomeric recognition ability. The equilibrium binding constant between **Am7** and **1** could not be determined due to overlap between signals corresponding to the naphthalane ring and the bpy moiety.

2.4. Characterization of the host–guest complex

The host–guest complexes were characterized using various NMR experiments. 2D NOESY and ROESY spectra of a roughly 1:1 mixture of **1** and (*S*)-**Am1** in CD_2Cl_2 with 5%

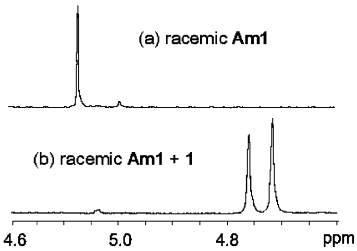


Figure 5. The signals correspond to the α-protons of (±)-**Am1** (5.0×10^{-3} M) in CD_2Cl_2 with 5% CD_3OD with (a) no bpy crown macrocycle, and (b) bpy crown macrocycle **1** (5.0×10^{-3} M).

CD_3OD did not show any correlation between the phenyl protons of the ammonium guest and the bpy protons of the macrocyclic host. These results could be due to the fast exchange rate between the host and the guest molecules.[7,23] The ^{1}H NMR spectrum of (±)-**Am1** (with the (*S*)-enantiomer in slightly excess) showed a singlet signal for the α-protons (Fig. 5a), which was spilt into two peaks in the presence of **1** (Fig. 5b). Moreover, the ^{1}H NMR spectrum of **1**·(*S*)-**Am1** complex showed a distinct 2:3 pattern for the phenyl proton signals (Fig. 6), indicating the π-systems of **1** and (*S*)-**Am1** are in close vicinity.

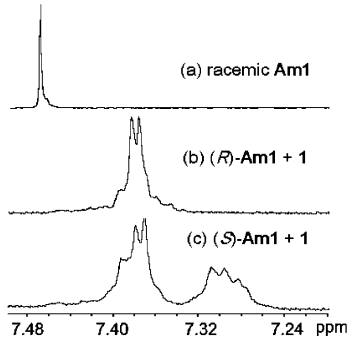


Figure 6. The signals correspond to the benzene rings of (a) (±)-**Am1** alone (5.0×10^{-3} M); (b) (*R*)-**Am1** (5.0×10^{-3} M) and (c) (*S*)-**Am1** (5.0×10^{-3} M) with bpy crown macrocycle **1** (5.0×10^{-3} M) in CD_2Cl_2 with 5% CD_3OD.

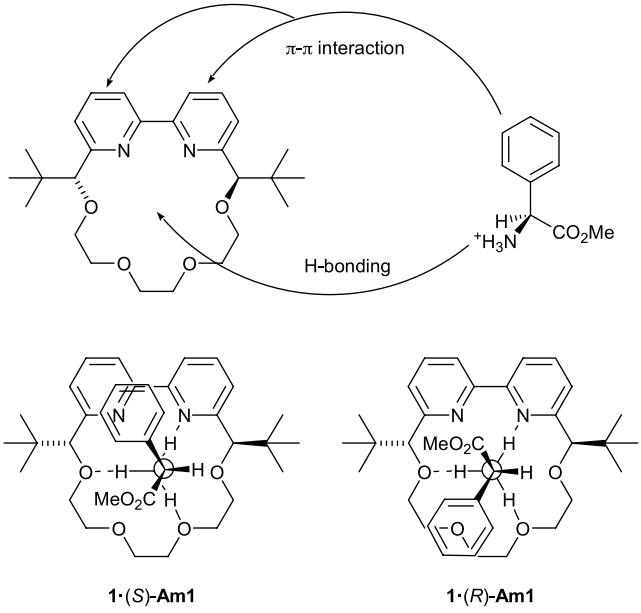


Figure 7. Interactions between bpy crown macrocycle **1** with **Am1**.

Although, the 2D NOESY and ROESY experiments showed no correlation between **1** and (*S*)-**Am1**, the structure–binding relationship studies and the ^{1}H NMR spectra strongly suggested that the presence of π–π interaction between bpy crown macrocycle **1** and **Am1**. Figure 7 showed the conformations of the **1**·(*S*)-**Am1** and **1**·(*R*)-**Am1** complexation models with the ester group and the side-chain at the α-position pointing away from the *t*-butyl group of the macrocyclic host. The **1**·(*S*)-**Am1** complexation model is considered to be more stable due to the extra stabilization energy from the π–π interaction between the bpy subunit of the macrocyclic host and the phenyl ring of the ammonium guest. These transition state models seem to be able to account for the high enantioselectivity of (*S*)-**Am1**.

3. Conclusion

In summary, we have developed a series of new C_2-symmetric bpy crown macrocycles (**1**–**4**) and studied their enantiomeric recognition properties towards a number of amino acid derivatives and chiral organic ammonium salts using UV–vis and NMR methods. The macrocycles were found to be strong chelating agents for organic ammonium salts (with K up to 4.8×10^5 M^{-1}) and useful chromophore for UV–vis titration studies. Bpy crown macrocycle **1**, bearing the pseudo 18-crown-6 type structure, exhibited the highest enantioselectivity towards (*S*)-**Am1** with $K_{(S)}/K_{(R)}$ equals 2.1. The structure–binding relationship studies showed that the π–π interaction between the phenyl group of the ammonium guest and the bpy subunit of the macrocycle host is important for high enantioselectivity. This observation was also supported by the NMR studies of the **1**·(*S*)-**Am1** complex. Moreover, the recognition study of **1** with **Am6** suggested that the ester group of the ammonium guests is important for both high enantioselectivity and the new UV signal formation for the host–guest complex.

4. Experimental

4.1. Materials and apparatus

2,6-Bipyridinediol (**5**) was prepared according to the literature procedures.[15] AR-grade CH_2Cl_2 and CH_3OH were used for the UV–vis titration experiments. The ^{1}H and ^{13}C NMR spectra were obtained by a 300 MHz instrument. The 2D NOESY and ROESY spectra were obtained by a 500 MHz instrument.

4.2. General procedures for the synthesis of ethylene glycol ditosylate 6–9

The corresponding ethylene glycol (3.0 mmol) in dry THF (20 mL) was added dropwise to a vigorously stirred suspension of NaH (0.23 g, 9.6 mmol) in dry THF (5 mL) at 0 °C. The reaction mixture was slowly warmed and refluxed for 4 h and then cooled to 0 °C. After addition of tosyl chloride (1.43 g, 7.5 mmol) in dry THF (10 mL) at 0 °C, the mixture was warmed to room temperature and

stirred for 48 h. The reaction mixture was concentrated under reduced pressure and the residue was treated cautiously with water and extracted with diethyl ether (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by silca gel column chromatography (petroleum ether/ethyl acetate) to provide the corresponding ditosylate compound.

4.2.1. Tri(ethyl glycol)ditosylate (6) and penta(ethylene glycol)ditosylate (7). Both compounds are commercially available.

4.2.2. Tetra(ethylene glycol)ditosylate (8). The above procedures were followed with tetra(ethylene glycol) as the starting material. The isolated yield for **8** is 1.39 g (92%): ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 6H), 3.55–3.58 (m, 8H), 3.64–3.70 (m, 4H), 4.11–4.17 (m, 4H), 7.34 (d, J=8.1 Hz, 4H), 7.79 (d, J=8.7 Hz, 4H).

4.2.3. Hexa(ethylene glycol)ditosylate (9). The above procedures were followed with hexa(ethylene glycol) as the starting material. The isolated yield for **9** is 1.68 g (95%): ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 6H), 3.58–3.70 (m, 20H), 4.14–4.17 (m, 4H), 7.35 (d, J=7.8 Hz, 4H), 7.80 (d, J=8.4 Hz, 4H).

4.3. General procedures for the synthesis of bpy crown macrocycle 1–4

To a vigorously stirred suspension of NaH (0.080 g, 3.2 mmol) in dry THF (3 mL) at 0 °C was added dropwise a solution of di-*tert*-butyl-2,6-bipyridinedimethanol **6** (0.33 g, 1.0 mmol) in dry THF (10 mL). The resulting mixture was stirred at 0 °C for 30 min and heated under reflux for 2 h. The mixture was then cooled to room temperature and treated with a solution of the corresponding ethylene glycol di-*p*-tosylate (1.2 mmol) in dry THF (25 mL) dropwise. The resulting mixture was stirred at room temperature for 2–6 days. After removal of the solvent under aspirator vacuum, the residue was treated with water and extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by silca gel column chromatography (petroleum ether/ethyl acetate) to yield bpy crown macrocycle **1**–**4**.

4.3.1. (*R*,*R*)-7,18-Di-*tert*-butyl-8,11,14,17-tetraoxa-23,24-diaza-tricyclo[17.3.1.1^{2,6}]tetracosa-1(23),2(24),3,5,19,21-hexaene (1). The above procedures were followed with tri(ethylene glycol)di-*p*-tosylate **6**. Bpy crown macrocycle **1** was isolated as an off-white solid (0.15 g, 35%): mp 142–146 °C $[\alpha]_D^{25}$ −107.6 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2968, 1577, 1109; ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 18H), 2.59–2.68 (m, 2H), 2.76–2.84 (m, 2H), 3.15–3.21 (m, 2H), 3.22–3.29 (m, 2H), 3.40–3.47 (m, 2H), 3.76–3.83 (m, 2H), 4.05 (s, 2H), 7.23 (d, J=7.8 Hz, 2H), 7.72 (t, J=7.8 Hz, 2H), 7.94 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.4, 35.7, 68.5, 70.2, 71.0, 91.2, 120.4, 123.0, 135.8, 157.3, 160.4. Anal. Calcd for $C_{26}H_{38}N_2O_4$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.46; H, 8.55; N, 6.49; Positive ion MS-ESI *m*/*z*: 443 (MH^+), 465 ($M+Na^+$), 481 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{26}H_{38}N_2O_4$: 442.2832; found 442.2834.

4.3.2. (*R*,*R*)-7,21-Di-*tert*-butyl-8,11,14,17,20-pentaoxa-26,27-diaza-tricyclo[20.3.1.1^{2,6}]heptacosa-1(26),2(27),3,5,22,24-hexaene (2). The above procedures were followed with tetra(ethylene glycol)di-*p*-tosylate **7**. Bpy crown macrocycle **2** was isolated as an amorphous solid (0.19 g, 40%): $[\alpha]_D^{25}$ +15.6 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2953, 2868, 1571, 1438, 1107; ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 18H), 2.66–2.81 (m, 2H), 2.84–3.02 (m, 4H), 3.04–3.14 (m, 2H), 3.22–3.34 (m, 2H), 3.40–3.50 (m, 2H), 3.62–3.73 (m, 2H), 3.87–3.99 (m, 2H), 4.32 (s, 2H), 7.41 (d, J=7.5 Hz, 2H), 7.77 (t, J=7.8 Hz, 2H), 8.26 (d, J=7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.2, 35.9, 69.0, 69.8, 71.5, 72.0, 92.5, 119.1, 122.3, 135.9, 154.7, 161.3; Positive ion MS-ESI *m*/*z*: 487 (MH^+), 509 ($M+Na^+$), 525 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{28}H_{42}N_2O_5$: 486.3094; found 486.3090.

4.3.3. (*R*,*R*)-7,24-Di-*tert*-butyl-8,11,14,17,20,23-hexaoxa-29,30-diaza-tricyclo[23.3.1.1^{2,6}]triaconta-1(29),2(30),3,5,25,27-hexaene (3). The above procedures were followed with penta(ethylene glycol)di-*p*-tosylate **8**. Bpy crown macrocycle **3** was isolated as a pale yellow oil (0.10 g, 19%): $[\alpha]_D^{25}$ +19.3 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2953, 2868, 1571, 1438, 1107; ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 18H), 3.13–3.82 (m, 20H), 4.34 (s, 2H), 7.44 (d, J=7.8 Hz, 2H), 7.78 (t, J=7.8 Hz, 2H), 8.23 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.4, 36.0, 70.3, 70.5, 70.7, 70.9, 71.8, 92.1, 119.4, 122.4, 136.3, 154.9, 160.2; Positive ion MS-ESI *m*/*z*: 531 (MH^+), 553 ($M+Na^+$), 569 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{30}H_{46}N_2O_6$: 530.3356; found 530.3354.

4.3.4. (*R*,*R*)-7,27-Di-*tert*-butyl-8,11,14,17,20,23,26-hepta-oxa-32,33-diaza-tricyclo[26.3.1.1^{2,6}]tritriaconta-1(32),2(33),3,5,28,30-hexaene (4). The above procedures were followed with hexa(ethylene glycol)di-*p*-tosylate **9**. Bpy crown macrocycle **5** was isolated as a pale yellow oil (0.10 g, 17%): $[\alpha]_D^{25}$ −46.4 (*c* 0.5, CH_2Cl_2); IR (neat, cm^{-1}) 2953, 1572, 1441, 1107; ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 18H), 3.37–3.65 (m, 24H), 4.29 (s, 2H), 7.44 (d, J=7.8 Hz, 2H), 7.80 (d, J=7.8 Hz, 2H), 8.26 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 26.5, 35.8, 69.7, 70.6, 70.8, 70.9, 71.2, 91.8, 119.5, 122.4, 136.6, 155.0, 160.8; positive ion MS-ESI *m*/*z*: 575 (M^+), 597 ($M+Na^+$), 613 ($M+K^+$). HRMS *m*/*z*: Calcd for $C_{32}H_{50}N_2O_7$: 574.3618; found 574.3616.

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24. The association constant (K) for 1:1 complexation can be expressed by Eq. 3

$$K = \frac{[C]}{[H][G]} = \frac{[C]}{([H]_0 - [C])([G]_0 - [C])} \tag{3}$$

where $[H]_0$ and $[G]_0$ are the initial concentrations of the host and guest, respectively, and [H], [G] and [C] are the concentrations of the host, guest and complex at equilibrium, respectively. By substitution of $[C]=[H]_0(1-X_f)$ into Eq. 3, X_f became the unknown of the quadratic equation (Eq. 4):

$$[H]_0 X_f^2 + \left([G]_0 - [H]_0 + \frac{1}{K}\right)X_f - \frac{1}{K} = 0 \tag{4}$$

Therefore,

$$X_f = \frac{-([G]_0 - [H]_0 + 1/K) \pm \sqrt{([G]_0 - [H]_0 + 1/K)^2 - 4[H]_0(-1/K)}}{2[H]_0} \tag{5}$$

Since

$$F = \Sigma(\delta_{obsd} - \delta_{ave})^2$$

$$= \Sigma[\delta_{obsd,i} - X_{f,i}\delta_f - (1 - X_{f,i})\delta_c]^2 \tag{2}$$

K and δ_c were calculated by minimization of the error function F using a computer program developed by us.