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Synthesis of novel chiral polyamide macrocycles containing pyridyl side-arms and their molecular recognition properties

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Abstract—Seven novel C_2-symmetrical macrocycles containing pyridyl units have been prepared by the cyclic condensation of homochiral diamide intermediates with 2,6-pyridinedicarbonyl dichloride under high dilution at room temperature. The molecular recognition of these homochiral macrocycles for amino acid derivatives has been characterized by various spectroscopic methods such as IR, FAB-MS, fluorescence and UV–vis. The macrocycle **11** exhibited significant chiral recognition towards the enantiomers of D- and L-alanine methyl ester hydrochlorides for which association constants have been determined. Molecular modeling was also used to simulate the interaction mode between the hosts and the guests.

1. Introduction

Molecular recognition has been the focus of supramolecular chemistry as one of fundamental processes in biochemical systems.[1] Recent successes in imitating the natural phenomena using synthetic artificial receptors have shown that biological behavior can be engineered into relatively simple molecules.[2] Therefore, the design and synthesis of different kinds of synthetic macrocycles and studies on their molecular recognition abilities have been one of the focuses in the fields of life science and material science. In particular, optical active macrocyclic receptors and their enantioselective recognition of chiral compounds have been attracting much attention.[3]

We report here the synthesis of seven novel chiral polyamide macrocycles and a study of their enantiomeric recognition for amino acid derivatives by spectroscopic methods. These macrocycles have C_2-symmetry axis and their chirality derives from L-amino acid derivatives.[4] Because they have amide groups similar to cyclopeptides, they can be classified as cyclophanes or cyclopseudopeptides.[5] In addition, pyridine units as building blocks are incorporated into the ring structure and side-arm, not only providing proton acceptors at the pyridyl nitrogens, but also bringing rigidity into the ring.[6] All the structural characteristics of the macrocycles make them form a certain conformation via weak forces such as hydrogen bonds and π-stacking interactions, thus offering a chiral environment.

2. Results and discussion

2.1. Synthesis

In this paper, seven new chiral macrocycles are prepared. The synthesis of macrocycles **6**, **7** and **8** as an example is shown in Scheme 1. The tosylamine **1** was transformed into its sodium salt, and then reacted with 2,6-bis(bromomethyl)pyridine to give compound **2**. Detosylation of compound **2** in concentrated H_2SO_4 gave 2,6-bis(*N*-picolylaminomethyl)-pyridine **3**. However, if the compound **3** was prepared by reacting 2-aminomethylpyridine with 2,6-bis(bromomethyl)-pyridine, the products were so complicated that the desired product was hard to separate and purify. Next, the diamine **3** was condensed with *Z*-protected valine in the presence of DCC to give compound **4**. It should be noticed that *Z*-protected valine must be mixed with DCC for 0.5 h to form the symmetric anhydride before the addition of compound **3** to the mixture, otherwise, the yield of acylated product is very low. Subsequently, compound **4** was deprotected with

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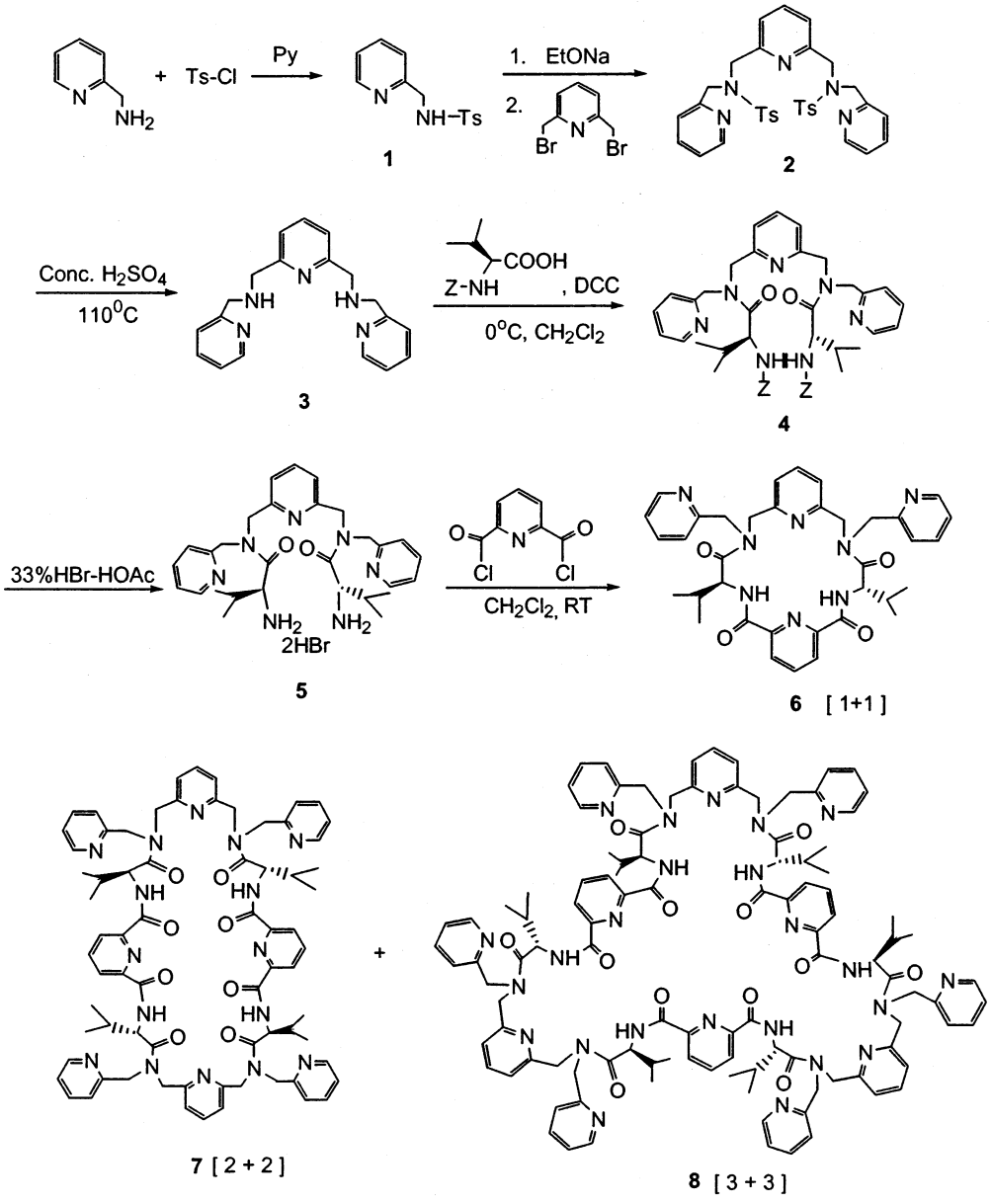


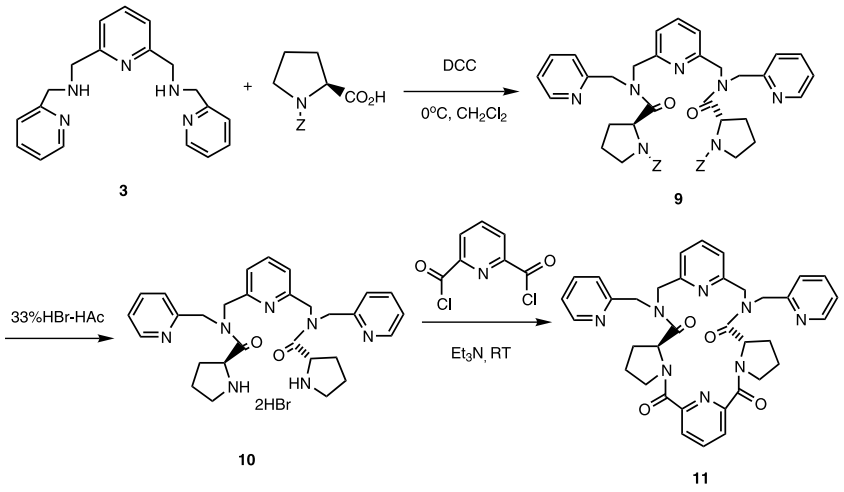
Scheme 1. Preparation of macrocycles **6**–**8**.

33% HBr–HOAc to afford compound **5**. Then three new chiral macrocycles **6**, **7** and **8** were prepared by acylation of chiral diamine dihydrobromide intermediate **5** with 2,6-pyridinedicarbonyl dichloride at high dilution (10^{-2} M) at room temperature. The macrocycles **6**, **7** and **8** are the products of [1+1], [2+2] and [3+3] cyclization and the corresponding yields are 15.6%, 5.1% and 3.7%, respectively. To our knowledge, product **8**, a 54-membered macrocycle, has seldom reported previously. With the increase of ring size, the yield reduced, which may be ascribed to the collision ratio of two terminal units decreasing with the increase in chain length in the intramolecular cyclization. The structures of the new chiral macrocycles were confirmed by ^{1}H NMR, IR, MALDI-TOF-MS spectra and elemental analyses.

Similarly, the macrocycles **11**, **15**, **16** and **19** can be synthesized according to the above-mentioned method, as shown in Schemes 2–4. It should be noticed that the intermediate **12** was prepared by 2,6-bis(bromomethyl)pyridine with a great excess of benzylamine.[7]

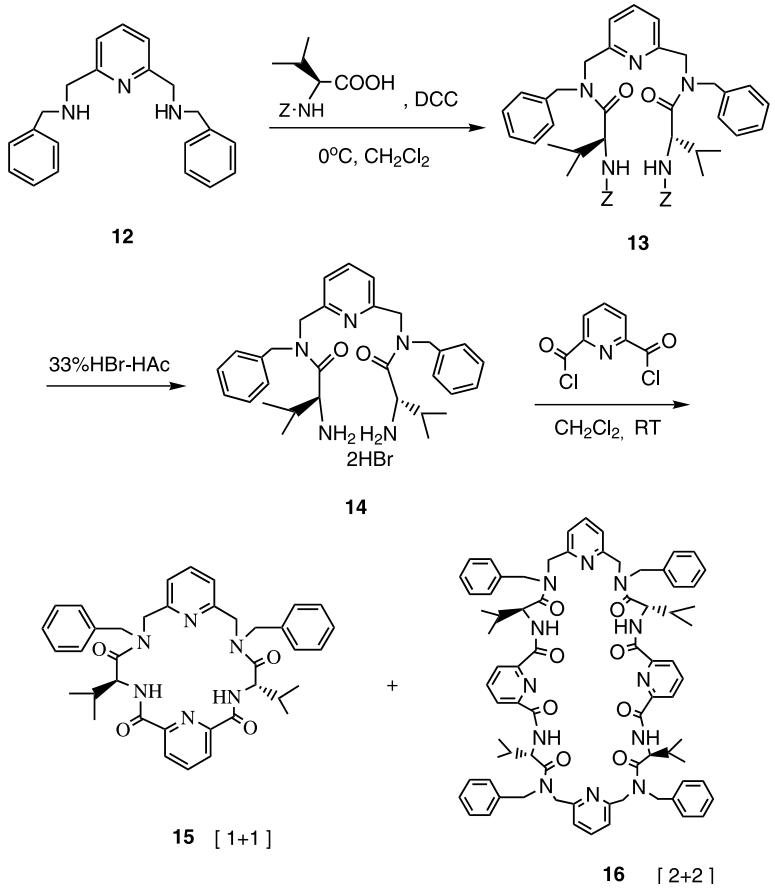
2.2. Molecular recognition

The main purpose of synthesizing these artificial receptors is to study their molecular recognition for guest molecules. Molecular recognition can be characterized by various spectroscopic methods, such as ultraviolet-visible (UV–vis), fluorescence and infrared (IR) spectroscopy, which are powerful tools used for the examination of the recognition ability of new chiral macrocycles. Different methods work together to show



3 9 10 11

Scheme 2. Preparation of macrocycle **11**.



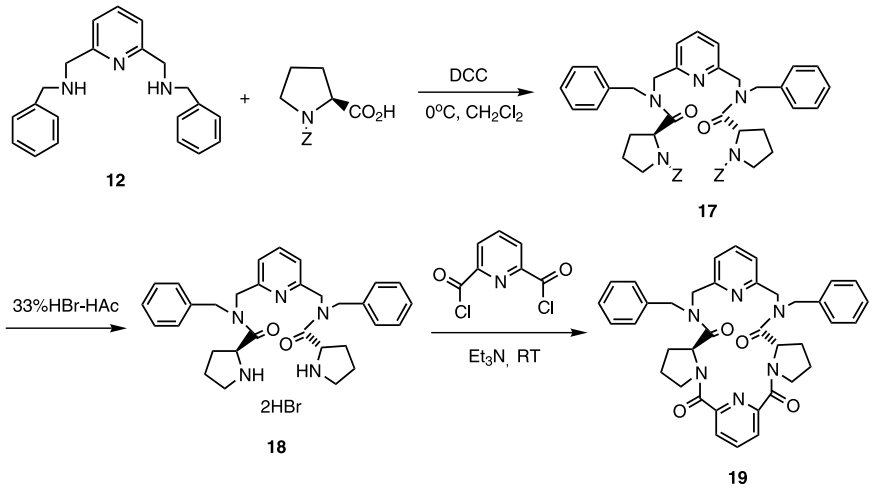
12 13 14 15 [1+1] 16 [2+2]

Scheme 3. Preparation of macrocycles **15** and **16**.

that homochiral macrocycles can possess chiral molecular recognition ability for organic molecules. Amino acids and derivatives, as the fundamental building blocks of biological systems, are the most attractive objectives. Herein, amino acid methyl ester hydrochlorides and dipeptides were selected as the guest molecules. We only use [1+1] macrocycles as the hosts, because the functional groups of macrocycles as building blocks instead of the size of the macrocycle plays the main role in molecular recognition based on our previous research.[3]

2.3. IR spectroscopy

Infrared spectroscopy is a convenient and widely used method for the study of molecular recognition.[8] For the polyamide macrocycles, the C=O double bond has an obvious characteristic peak at 1650 cm^{-1}. Hydrogen



Scheme 4. Preparation of macrocycle **19**.

bond formation makes the peak position shift to lower frequency, which can indicate an interaction between the host and the guest.

From the IR data in Table 1, it can be seen that when the macrocycles and the guests were mixed, the stretching vibrations of C=O and N–H all shift to lower frequency, which shows that there are hydrogen bonds formed between the hosts and the guests. Among the items, the macrocycle **6** has an significant recognition for D-phe-OMe·HCl. Compared with the macrocycle **19**, macrocycle **6** demonstrates better chiral recognition for D- or L-phenylalanine methyl ester hydrochloride. Generally, the shift value of D-amino acid methyl ester hydrochloride is greater than that of L-isomer, demonstrating that the macrocycle has the enantiomeric discrimination ability between D- and L-isomers of amino acid methyl ester hydrochlorides.

2.4. FAB-MS

Fast atom bombardment mass spectrometry (FAB-MS) as a soft ionization technique is effective for a variety of supramolecular complexes.[9] FAB mass spectra in Table 2 demonstrate that the host-guest 1+1 molecular ion peaks appear in some host–guest systems. We can see that both macrocycle **6** and macrocycle **19** can bind amino acid methyl ester hydrochlorides. The hydrochlorides containing a benzene ring bind well with the macrocycles. Because the benzene ring in the guest can have π–π stacking with the pyridine in the macrocycle, the size of benzene ring also matches very well to the cavity size of macrocycle. There is no molecular ion peak when the guest is alanine methyl ester hydrochloride. There is also no molecular ion peak of complexes between macrocycle **6** and CPH, possibly because of its bulky size compared to the macrocycle. Furthermore, there is no evidence that one macrocycle binds two guests, so the binding stoichiometry can be inferred as 1:1.

Table 1. IR Data of macrocycles with the guest molecules (ν/cm^{-1})

Host + Guest	$\nu_{C=O}$	$\Delta\nu_{C=O}$	ν_{N-H}	$\Delta\nu_{N-H}$
6	1669		3426	
6 + L-Ala-OMe·HCl	1658	11	3422	4
6 + D-Ala-OMe·HCl	1651	18	3420	6
6 + L-Phe-OMe·HCl	1659	10	3420	6
6 + D-Phe-OMe·HCl	1650	19	3390	36
6 + L-Val-Obz·HCl	1655	14	3420	6
6 + BPHME	1659	10	3392	34
6 + CPH	1657	12	3443	−17
19	1648		–	
19 + L-Phe-OMe·HCl	1644	4	–	–
19 + D-Phe-OMe·HCl	1642	6	–	–

*Measured in 1:1 mole ratio of the host–guest mixture in the solid state. Ala-OMe: alanine methyl ester; Phe-OMe: phenylalanine methyl ester; Val-OBz: valine benzyl ester; BPHME: linear dipeptide *Z*-Phe-His-OMe. CPH: cyclodipeptide Phe-His.

Table 2. FAB-MS data of the macrocycles with amino acid derivatives[a]

Host + Guest	M + 1 Peak	Relative intensity[b]
6 + L-Ala-OMe·HCl	649 (H)	S
6 + D-Ala-OMe·HCl	649 (H)	S
6 + L-Phe-OMe·HCl	828 (H + G)	W
6 + D-Phe-OMe·HCl	828 (H + G)	W
6 + L-Val-OBz·HCl	856 (H + G)	W
6 + BPHME	1099 (H + G)	W
6 + CPH	649 (H)	S
19 + L-Phe-OMe·HCl	822 (H + G)	W
19 + D-Phe-OMe·HCl	822 (H + G)	W

[a] Measured in a 1:1 mole ratio of the host-guest mixture.
[b] S = Strong; W = Weak.

2.5. Fluorescence spectroscopy

Fluorescence spectroscopy is known to have high sensitivity compared to other spectroscopic methods.[10]

Therefore, the enantiomeric recognition for D- and L-amino acid derivatives by these homochiral macrocycle receptors has been characterized by fluorescence spectra. It is evident from the differences in these fluorescence intensity and fluorescence maxima that these chiral ligands exhibit chiral recognition. As shown in Table 3, both fluorescence intensity and fluorescence maximum exhibit some differences. The intensities of fluorescence increase (or decrease) according to the environment of fluorescence molecules. When mixed with the guests, the fluorescence intensities of the macrocycles **6** and **11** increase, while the fluorescence intensities of the macrocycles **15** and **19** decrease. The reason for this phenomenon may be that the nitrogen in the pyridine ring as a binding site interacts with proton by static force, but there is no such effect for a benzene ring.

2.6. UV–vis

UV–vis spectroscopy is a convenient and widely used method for the study of binding phenomena.[11] When the receptor (or substrate) absorbs light at different wavelengths in the free and complexed states, the differences in ultraviolet spectrophotometry may suffice for estimation of molecular recognition.

We use a UV spectral titration method to obtain the association constant. When the concentration of the macrocycle is much smaller than that of the guest ($[H]_o \ll [G]_o$), the modified Hilderbrand–Benesi equation can be employed under these conditions.[12]

Table 3. Fluorescence data of the macrocycles with enantiomers of amino acid methyl ester hydrochlorides

Host[a]+Guest[a]	λ_{max}(nm)[b]	$\Delta\lambda$ (nm)	I/I_o (relative intensity)
6	361.2		
6+L-Ala-OMe·HCl	362.5	1.3	1.07
6+D-Ala-OMe·HCl	362.0	0.8	1.24
6+L-Phe-OMe·HCl	361.6	0.4	1.12
6+D-Phe-OMe·HCl	363.0	1.8	1.23
11	382.1		
11+L-Ala-OMe·HCl	374.0	−8.1	1.18
11+D-Ala-OMe·HCl	380.1	−2.0	1.26
11+L-Phe-OMe·HCl	368.4	−13.7	1.89
11+D-Phe-OMe·HCl	372.9	−9.2	1.52
15	371.1		
15+L-Ala-OMe·HCl	376.4	5.3	0.56
15+D-Ala-OMe·HCl	378.7	7.6	1.11
15+L-Phe-OMe·HCl	362.4	−8.7	0.77
15+D-Phe-OMe·HCl	372.0	0.9	0.71
19	377.4		
19+L-Ala-OMe·HCl	379.8	2.4	0.49
19+D-Ala-OMe·HCl	380.4	3.0	0.49
19+L-Phe-OMe·HCl	381.2	3.8	0.65
19+D-Phe-OMe·HCl	378.2	0.8	0.52

[a] Equimolar amounts of the macrocycles and salts were dissolved in the solvent (CH_3OH/CH_3CN=1:30) with the concentration of 2×10^{-5} M.

[b] λ_{ex}=300 nm.

$$5[G]_o[H]_o/\Delta A = 1/K\Delta\varepsilon + [G]_o$$

$[H]_o$: the concentration of macrocycle; $[G]_o$: the concentration of the guest; ΔA: the change of UV–vis intensity of the macrocycle upon addition of amino acid methyl ester hydrochloride; K: association constant; $\Delta\varepsilon$: the molar extinction coefficient.

Fig. 1 shows the absorption maximum of the macrocycle **11** gradually increases upon the addition of D-alanine methyl ester hydrochloride with varying concentrations. The plots of calculated $[G]_o[H]_o/\Delta A$ values as a function of $[G]_o$ give good straight lines, the result is shown in Fig. 2. From the linear plot, we can obtain the association constant for the interaction of the macrocycle **11** with D-Ala-OMe·HCl as $K_D=8.12\times10^2$ M^{-1} (r=0.992) and that of the macrocycle **11** with L-Ala-OMe·HCl as $K_L=2.03\times10^3$ M^{-1} (r=0.982). Therefore, the selectivity ratio is 2.5.

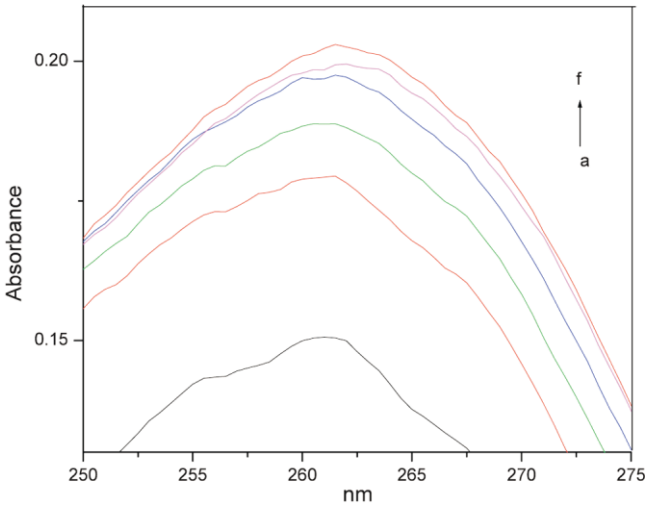


Figure 1. UV–vis spectra of **11** (1×10^{-5} mol dm^{-3}) in the presence of D-Ala-OMe·HCl. a: 0; b: 6×10^{-4}; c: 8×10^{-4}; d: 10×10^{-4}; e: 14×10^{-4}; f: 16×10^{-4} mol dm^{-3}.

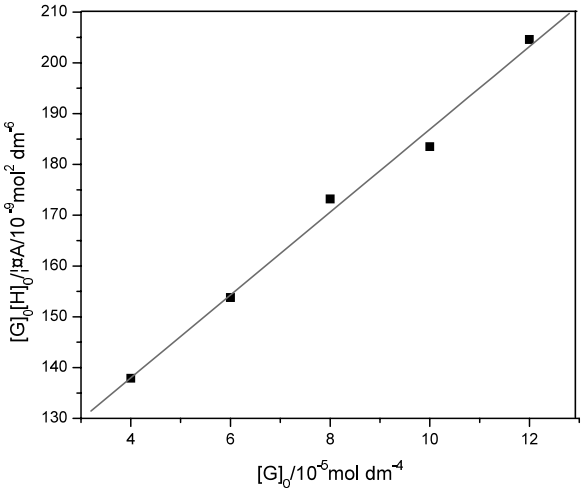


Figure 2. Typical plot of $[G]_o[H]_o/\Delta A$ versus $[G]_o$ for the inclusion complexation of **11** with D-Ala-OMe·HCl in the solvent (CH_3OH:CH_3CN=1:30).

2.7. Molecular modeling

In order to study further the structure and interaction mode between the host and the guest, we also use computer modeling to simulate the binding. Just from the IR data, we can see that the hosts and the guests interact with each other by hydrogen bonding between amide groups and protons. Based on our previous research,[3] we think there are tripod hydrogen bonds formed between the macrocycles and the guests. Here, the macrocycles **6** and **11** were chosen as candidates for the conformational search using Sybyl6.4 performed on Silicon Graphics O2 (R5000) workstations. Fig. 3 represents the predicted conformation of the macrocycle **6** combining with L-phenylalanine methyl ester hydrochloride and Fig. 4 represents the macrocycle **11** with D-alanine methyl ester hydrochloride. From the energy data in Table 4, we can see that when the macrocycles combine with the guests, the total energy values are lower. It can also be inferred that both of the macrocycles **6** and **11** have a better enantiomeric discrimination towards the D-isomer than to L-isomer, because the energy of the complex of the D-isomer with macrocycle is lower than that of L-isomer. The molecular modeling is in agreement with the experimental conclusions.

In summary, the following conclusions can be drawn from the aforementioned facts: (1) The chiral macrocycles show obvious interactions with amino acid methyl ester hydrochlorides, and some of them recognize D- and L-enantiomers of amino acid methyl ester hydrochlorides significantly; (2) It is effective to use various spectroscopic methods together to determine the enantiomeric recognition ability for D- and L-enantiomers of amino acid methyl ester by these artificial receptors.

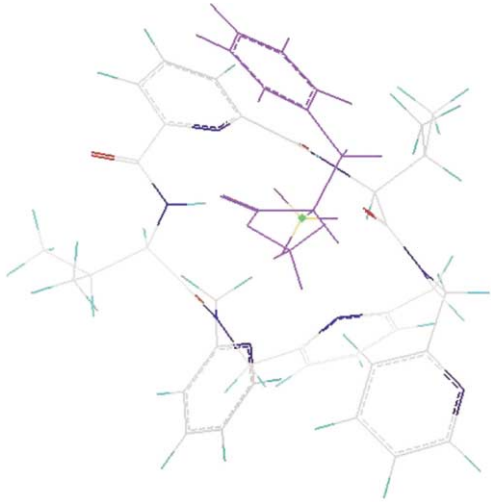


Figure 3. Complex conformation formed by the macrocycle **6** with L-Phe-OMe·HCl.

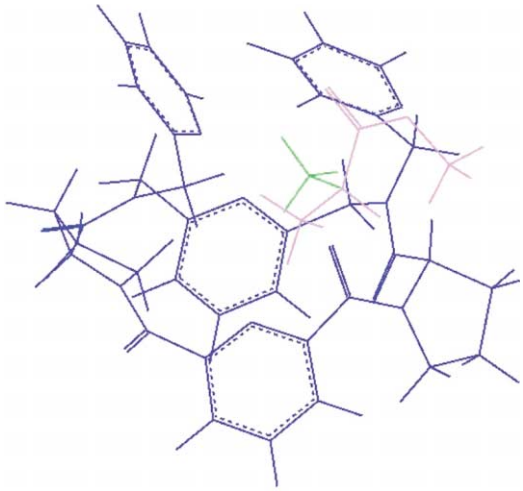


Figure 4. Complex conformation formed by the macrocycle **11** with D-Ala-OMe·HCl.

Table 4. Energy data of optimized conformation of macrocycles with enantiomers of amino acid methyl ester hydrochlorides (kcal/mol)

Host + Guest	Energy	ΔE
6	38.535	
6 + L-Phe-OMe·HCl	10.785	−27.750
6 + D-Phe-OMe·HCl	3.279	−34.256
11	18.130	
11 + L-Ala-OMe·HCl	−6.305	−24.435
11 + D-Ala-OMe·HCl	−14.070	−32.200

Firstly, the initial structures of the molecules were input. These structures were then successively refined using minimization options of Sybyl with Tripos force field. The entire complex was then minimized using 5000 steps of steepest descent, followed by conjugate gradient algorithm leading to a maximum derivative of less than a 0.010 kcal/(mol Å^2) (root-mean-square (rms) energy gradient). Gasterger–Huckel charges were employed throughout.

3. Experimental

3.1. General comments

Infrared spectra were measured on a Bruker Vector 22 spectrometer. ^{1}H NMR spectra were recorded on a Bruker ARX 400 spectrometer. Chemical shifts are indicated in δ values (ppm) downfield from internal TMS. FAB-mass spectra were measured on VG-ZAB-HS mass spectrometer. Elemental analyses were carried out on Elementar Vario EL instrument. Melting points were taken on an XT-4 melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin–Elmer 241 MC spectrometer. Commercial grade solvents were used without further purification unless specified. CH_2Cl_2 was distilled from calcium hydride. Starting materials were purchased from the Acros chemical company unless otherwise noted. Compound **1** was prepared according to the literature.[13] as was compound **12**.[7]

3.2. Spectra measurement

The fluorescence spectra were measured at 25°C with 970-CRT fluorescence spectrometer. The excitation wavelength of the fluorescence spectra was 300 nm and the excitation and emission slits were 10 nm. A solution of nitrile–methanol (30:1 v/v) was used as the solvent. The UV–vis spectra were measured at 25°C with TU-1901 UV spectrometer. The maximum wavelength is 262 nm. A solution of nitrile-methanol (30:1 v/v) was used as the solvent. The concentration of the host is 1×10^{-5} M with the increasing concentration of the added guest.

3.3. 2,6-Bis(*N*-tosyl-picolylaminomethyl)pyridine 2

To a stirred suspension of the sulfonamide **1** (2.62 g, 10 mmol) in absolute EtOH (30 mL), NaOEt prepared by dissolving sodium (0.247 g, 10.7 mmol) in EtOH (10 mL) was added dropwise at rt. The reaction mixture was refluxed for 5 h and then cooled. After removing ethanol, the precipitate was collected by rapid filtration and dried to give white solid (2.16 g, 76.5%).

A mixture of 2,6-bis(bromomethyl)pyridine (1.31g, 4.95 mmol) and tosylate sodium salt (2.8 g, 9.9 mmol) was stirred in absolute ethanol (40 mL) at ambient temperature for 8 h. After removing EtOH, the residue was purified by column chromatography on silica gel using ethyl acetate–petroleum ether (1:1) as eluent to give sticky liquids, which was recrystallized from hexane to give white solid (1.66 g, 53.5% yield). Mp 72–74°C, MS (FAB): m/z 626 $(M+H)^+$; IR (KBr): ν 3455, 2361, 1646, 1595, 1435, 1336, 1153 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.35–8.33 (t, 1H), 7.67–7.06 (m, 18H), 4.48 (s, 4H), 4.36 (s, 4H), 2.39 (s, 6H). Anal. calcd for $C_{33}H_{33}N_5O_4S_2$: C, 63.14%; H, 5.30%; N, 11.16%. Found: C, 63.03%; H, 5.36%; N, 11.13%.

3.4. 2,6-Bis(*N*-picolylaminomethyl)pyridine 3[14]

2,6-Bis(*N*-tosyl-picolylaminomethyl)pyridine (1.66 g, 2.6 mmol) was dissolved in concentrated H_2SO_4 (10 mL) and then stirred at 110°C under nitrogen for 2 h. After cooling, the solution was diluted with water and then neutralized by aqueous 4N NaOH with cooling in an ice bath. The free amine was extracted with $CHCl_3$, dried over anhydrous Na_2SO_4 and then concentrated to dryness to give **3** as yellow liquid (0.736 g, 87.1% yield). MS (EI): m/z 319.

3.5. 2,6-Bis(*N*-picolyl-*Z*-valinylaminomethyl)pyridine 4

N-Carbobenzyloxyalanine (14.1 g, 5.71 mmol) and DCC (1.18 g, 5.71 mmol) were dissolved in dry CH_2Cl_2 (10 mL). After stirring for 0.5 h at 0°C, 2,6-bis(*N*-picolylaminomethyl)pyridine **3** (0.434 g, 1.36 mmol) in CH_2Cl_2 (10 mL) was added dropwise to the mixture at the same temperature. After stirring at rt for additional 2 h, the resulting white suspension was filtered. The filtrate was washed with 5% aqueous $NaHCO_3$ (2×25 mL) and then concentrated to dryness. The residue was dissolved in ethyl acetate (40 mL) and washed with 1N HCl (2×30 mL). The separated aqueous phase was neutralized with 1N NaOH and then extracted with ethyl acetate (2×50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. Flash chromatography on silica gel using ethyl acetate afforded colorless oil (0.91 g, 85% yield). $[\alpha]_D^{20}=-18.7$ (c 1.80, $CHCl_3$); MS (EI): m/z 785 (M^+); IR (KBr): ν 3439, 1645, 1539, 1456, 1237, 1164, 1024 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.50 (m, 2H), 7.57 (m, 3H), 7.32–7.06 (m, 14H), 5.87 (m, 1H), 5.75 (m, 1H), 5.10–4.37 (m, 14H), 2.08 (m, 2H), 0.91 (m, 12H). Anal. calcd for $C_{45}H_{51}N_7O_6$: C, 68.74%; H, 6.54%; N, 12.48%. Found: C, 68.63%; H, 6.59%; N, 12.38%.

3.6. 2,6-Bis(*N*-picolyl-valinylaminomethyl)pyridine hydrobromide 5

Compound **4** (0.8 g, 1 mmol) was dissolved in 33% HBr–HOAc (10 mL). The mixture was stirred at rt for 2 h and the solution was concentrated to dryness. Anhydrous diethyl ether (10 mL) was added to the residue, and the mixture was stirred for additional 1 h and then filtered to give a light yellow powder (ca. 100% yield). Mp 232–233°C, MS (FAB): m/z 518 $(M+H)^+$; IR (KBr): ν 3437, 2363, 1664, 1464, 1165, 1049 cm^{-1}; ^1H NMR (CD_3OD): δ 8.53 (m, 1H), 8.44 (m, 1H), 7.80–7.66 (m, 3H), 7.36–7.16 (m, 6H), 5.00–4.89 (m, 4H), 4.75 (m, 1H), 4.67 (m, 2H), 4.40 (m, 1H), 3,75 (m, 2H), 1.98 (m, 2H), 0.93 (m, 12H).

3.7. Pyridine-valine macrocycles 6, 7 and 8

A solution of freshly prepared 2,6-pyridinedicarbonyl dichloride (0.26 g, 1.27 mmol) in dry dichloromethane (20 mL) was added dropwise to well-stirred solution of diester diamine dihydrobromide **5** (0.79 g, 1.17 mmol) and triethylamine (0.98 mL, 7 mmol) in dry CH_2Cl_2 (120 mL) at 0°C over 0.5 h. The reaction mixture was stirred for additional 12 h at rt. The resulting solution was concentrated to 60 mL and was washed with water and 5% $NaHCO_3$, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel using chloroform–methanol (30:1) as eluent to give white solid **6** (95 mg, 15.6% yield), **7** (46 mg, 5.1% yield) and **8** (27 mg, 3.7% yield).

6: mp 150–151°C, $[\alpha]_D^{20}=-104.3$ (c 1.11, $CHCl_3$); MS (FAB): $m/z=649$ $(M+H)^+$; IR (KBr) ν 3452, 2926, 1669, 1593, 1437, 1323, 1220 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.16 (d, 2H, $J=4.4$ Hz), 8.05 (d, 2H, $J=7.6$ Hz), 7.90 (t, 1H, $J=7.6$ Hz), 7.67 (t, 2H, $J=7.6$ Hz), 7.36 (m, 2H), 7.19 (m, 2H), 6.56 (m, 3H), 5.71 (d, 2H, $J=15.2$ Hz), 4.92–4.09 (m, 8H), 2.57 (m, 2H), 0.98 (d, 12H, $J=6.8$ Hz). Anal. calcd: $C_{36}H_{40}N_8O_4$ C, 66.65%; H, 6.21%; N, 17.27%. Found: C, 66.47%; H, 6.23%; N, 17.13%.

7: mp 136–138°C, $[\alpha]_D^{20}=+7.9$ (c 2.77, $CHCl_3$), FABMS: m/z 1297 $(M+H)^+$; IR (KBr): ν 3455, 2966, 2352, 1645, 1523, 1434, 1330, 1173 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.53–7.00 (m, 28H), 5.29–4.03 (m, 20H), 2.37 (m, 4H), 1.01 (m, 24H). Anal. calcd: $C_{72}H_{80}N_{16}O_8\cdot H_2O$ C, 65.73%; H, 6.28%; N, 17.04%. Found: C, 65.59%; H, 6.31%; N, 17.02%.

8: mp 134–136°C, $[\alpha]_D^{20}=+23.9$ (*c* 1.12, $CHCl_3$), MS (MALDI-TOF): *m*/*z* 1945 $(M+H)^+$; IR (KBr): *v* 3470, 2966, 1641, 1525, 1432, 1330, 1212, 1170 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.52–7.09 (m, 42H), 5.13–4.10 (m, 30H), 2.27 (m, 6H), 0.95 (m, 36H). Anal. calcd: $C_{72}H_{80}N_{16}O_8 \cdot H_2O$ C, 65.74%; H, 6.28%; N, 17.04%. Found: C, 65.67%; H, 6.26%; N, 16.98%.

3.8. 2,6-Bis(*N*-picolyl-*Z*-prolinylaminomethyl)pyridine 9

N-Carbobenzyloxyalanine (0.47 g, 1.87 mmol) and DCC (0.39 g, 1.87 mmol) were dissolved in dry CH_2Cl_2 (5 mL). After stirring for 0.5 h at 0°C, 2,6-bis(*N*-picolylaminomethyl)-pyridine **3** (0.27 g, 0.85 mmol) in CH_2Cl_2 (5 mL) was added dropwise to the mixture at the same temperature. After stirring at rt for additional 2 h, the resulting white suspension was filtered. The filtrate was washed with 5% aqueous $NaHCO_3$ (2×25 mL) and then concentrated to dryness. The residue was dissolved in ethyl acetate (30 mL) and washed with 1N HCl (2×20 mL). The separated aqueous phase was neutralized with 1N NaOH and then extracted with ethyl acetate (2×40 mL). The organic layer was dried over Na_2SO_4 and then evaporated. Flash chromatography on silica gel using ethyl acetate–petroleum ether (1:1) as eluent afforded colorless oil (0.58 g, 87.2% yield). $[\alpha]_D^{20}=-2.0$ (*c* 3.0, $CHCl_3$); MS (FAB): *m*/*z* 782 $(M+H)^+$; IR (KBr): *v* 3391, 2954, 2362, 1655, 1591, 1416, 1352, 1202, 1121 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.52–8.42 (m, 2H), 7.70–6.74 (m, 19H), 5.15–4.45 (m, 14H), 3.67 (m, 2H), 3.52 (m, 2H), 2.06–1.80 (m, 8H). Anal. calcd for $C_{45}H_{47}N_7O_6$: C, 69.12%; H, 6.06%; N, 12.54%. Found: C, 69.07%; H, 6.10%; N, 12.49%.

3.9. 2,6-Bis(*N*-picolyl-prolinylaminomethyl)pyridine hydrobromide 10

Compound **9** (0.6 g, 0.77 mmol) was dissolved in 33% HBr–HOAc (10 mL). The mixture was stirred at rt for 2 h, and then the solution was concentrated to dryness. Anhydrous ethyl ether (25 mL) was added to the residue, and the mixture was stirred for additional 1 h. The mixture was filtered to afford light yellow powder (0.648 g, 92% yield). Mp 193–195°C; MS (FAB): *m*/*z* 514 $(M+H)^+$; IR (Kr): *v* 3418, 2941, 1668, 1541, 1462, 1368, 1239, 1168 cm^{-1}; ^1H NMR (DMSO): δ 8.73–7.18 (m, 11H), 4.95–4.66 (m, 10H), 3.22 (m, 4H), 2.50 (m, 2H), 1.89 (m, 8H).

3.10. Pyridine–proline macrocycle 11

A solution of freshly prepared 2,6-pyridinedicarbonyl chloride (0.14 g, 0.71 mmol) in dry dichloromethane (20 mL) was added dropwise to well-stirred solution of compound **10** (0.65 g, 0.71 mmol) and triethylamine (0.7 mL, 5 mmol) in dry CH_2Cl_2 (100 mL) at 0°C over 0.5 h. The reaction mixture was stirred for additional 12 h at rt. The resulting solution was concentrated to 60 mL and was washed with water and 5% $NaHCO_3$, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel using chloroform–methanol (20:1) as eluent to give white solids (47 mg, 10.3% yield). Mp 141–143°C; $[\alpha]_D^{20}=-134.6$ (*c* 1.7, $CHCl_3$); MS (FAB): *m*/*z* 648 $(M+H)^+$; IR (KBr): *v* 3447, 2966, 2361, 1646, 1438, 1338, 1211 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.61–6.51 (m, 14H), 5.64–3.44 (m, 14H), 2.31–1.67 (m, 8H). Anal. calcd for $C_{36}H_{36}N_8O_4 \cdot H_2O$: C, 65.24%; H, 5.78%; N, 16.91%. Found: C, 65.19%; H, 5.81%; N, 16.87%.

3.11. 2,6-Bis(*N*-benzyl-*Z*-valinylaminomethyl)pyridine 13

The mixture of carbobenzyloxyvaline (1.25 g, 4.96 mmol) and DCC (1.02 g, 4.96 mmol) in CH_2Cl_2 (10 mL) was stirred with cooling in an ice bath for 0.5 h. A solution of compound **17** (0.78 g, 2.48 mmol) in CH_2Cl_2 was added dropwise to the reaction mixture at the same temperature. After stirring for additional 2 h at ambient temperature, the reaction mixture was filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography on silica gel using ethyl acetate–petroleum ether (1:2) as eluent to afford viscous oil (1.45 g, 74.8% yield). $[\alpha]_D^{20}=-17.0$ (*c* 0.50, $CHCl_3$); IR (KBr): *v* 3439, 3030, 1715, 1642, 1525, 1448, 1317, 1225 cm^{-1}; MS (FAB): *m*/*z* 784 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 7.56 (m, 1H), 7.36–7.11 (m, 20H), 5.70–5.52 (m, 2H), 5.13–4.58 (m, 11H), 4.51–4.40 (m, 2H), 4.24–4.09 (m, 1H), 2.05 (m, 2H), 0.91 (m, 12H). Anal. calcd for $C_{47}H_{53}N_5O_6$: C, 72.01%; H, 6.81%; N, 8.93%. Found: C, 71.97%; H, 6.76%; N, 8.89%.

3.12. 2,6-Bis(*N*-benzyl-valinylaminomethyl)pyridine hydrobromide 14

Compound **13** (1.28 g, 1.6 mmol) was dissolved in 33% HBr–HOAc (12 mL). The mixture was stirred at rt for 2 h and then was concentrated to dryness. Anhydrous ethyl ether (25 mL) was added to the residue, and the mixture was stirred for additional 1 h. The mixture was then filtered to give a light yellow powder (1.05 g, 95% yield). Mp 231–233°C; MS (FAB): *m*/*z* 516 $(M+H)^+$; IR (KBr): *v* 3415, 2934, 2360, 1655, 1452, 1363, 1228, 1172 cm^{-1}; ^1H NMR (DMSO): δ 8.25–8.18 (m, 4H), 7.83–7.74 (m, 1H), 7.40–7.14 (m, 10H), 4.96–3.87 (m, 10H), 2.15–2.07 (m, 2H), 0.96–0.82 (m, 12H).

3.13. Pyridine–valine macrocycles 15 and 16

A solution of freshly prepared 2,6-pyridinedicarbonyl dichloride (0.27 g, 1.3 mmol) in dry dichloromethane (20 mL) was added dropwise to well-stirred solution of compound **14** (0.87 g, 1.3 mmol) and triethylamine (0.91 mL, 6.5 mmol) in dry CH_2Cl_2 (120 mL) at 0°C over 0.5 h. The reaction mixture was stirred for additional 12 h at rt. The resulting solution was concentrated to 60 mL and was washed with water and 5% $NaHCO_3$, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel using chloroform–methanol (100:1) as eluent to give white solid **15** (63 mg, 7.5% yield) and **16** (37 mg, 2.2% yield).

15: mp 134–136°C $[\alpha]_D^{20}=-50.2$ (*c* 2.97, $CHCl_3$); MS (FAB): *m*/*z* 647 $(M+H)^+$; IR (KBr): *v* 3482, 2961, 2366, 1661, 1524, 1426, 1317, 1223, 1172 cm^{-1}; ^1H NMR

($CDCl_3$): δ 8.16 (d, 2H, $J=7.6$ Hz), 7.90 (t, 1H, $J=7.6$ Hz), 7.35–7.21 (m, 10H), 6.55 (m, 3H), 5.81 (d, 2H, $J=13.6$ Hz), 5.17–4.26 (m, 8H), 2.84 (s, 1H), 2.05 (m, 1H), 1.13–0.97 (m, 12H). Anal. calcd for $C_{38}H_{42}N_6O_4 \cdot 0.5H_2O$: C, 69.59%; H, 6.61%; N, 12.82%. Found: C, 69.63%; H, 6.64%; N, 12.79%.

16: mp 140–142°C; $[\alpha]_D^{20}=-20.4$ (c 0.26, $CHCl_3$); MS (FAB): m/z 1293$(M+H)^+$; IR (KBr): ν 3414, 2964, 2359, 1643, 1522, 1440, 1368, 1215, 1167 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.10–6.86 (m, 32H), 5.34–4.02 (m, 20H), 2.31–2.25 (m, 4H), 1.03–0.96 (m, 24H). Anal. calcd for $C_{76}H_{84}N_{12}O_8.H_2O$: C, 69.60%; H, 6.61%; N, 12.81%. Found: C, 69.57%; H, 6.62%; N, 12.79%.

3.14. 2,6-Bis(*N*-benzyl-*Z*-prolinylaminomethyl)-pyridine 17

The mixture of carbobenzyloxyproline (3.36 g, 13.48 mmol) and DCC (2.78 g, 13.48 mmol) in CH_2Cl_2 (10 mL) was stirred with cooling in an ice bath for 0.5 h. At the same temperature, a solution of **12** (2.14 g, 6.74 mmol) in CH_2Cl_2 was added dropwise to the reaction mixture. After stirring for additional 2 h at ambient temperature, the reaction mixture was filtered. The filtrate was concentrated to dryness and the residue was purified by column chromatography on silica gel using ethyl acetate–petroleum ether (3:1) as eluent to afford viscous oil (2.83 g, 65.4% yield). $[\alpha]_D^{20}=-3.2$ (c 2.37, $CHCl_3$); MS (FAB): m/z 780 $(M+H)^+$; IR (KBr): ν 3381, 3030, 1660, 1593, 1537, 1416, 1355, 1206, 1120 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.38–7.22 (m, 21H), 5.18–5.08 (m, 4H), 4.88–4.28 (m, 10H), 3.72–3.51 (m, 4H), 2.08–1.94 (m, 8H). Anal. calcd for $C_{47}H_{49}N_5O_6$: C, 72.38%; H, 6.33%; N, 8.98%. Found: C, 72.41%; H, 6.29%; N, 8.95%.

3.15. 2,6-Bis(*N*-benzyl-*Z*-prolinylaminomethyl)pyridine hydrobromide 18

Compound **17** (2 g, 2.56 mmol) was dissolved in 33% HBr–HOAc (12 mL). The mixture was stirred at rt for 2 h and then was concentrated to dryness. Anhydrous ethyl ether (25 mL) was added to the residue, and the mixture was stirred for additional 1 h. The mixture was then filtered to give a light yellow powder (1.71 g, ca. 100% yield). Mp 166–168°C; MS (FAB): m/z 512 $(M+H)^+$; IR (KBr): ν 3415, 2928, 1653, 1451, 1366, 1233, 1169 cm^{-1}; ^1H NMR (DMSO): δ 9.45 (m, 1H), 8.64 (m, 1H), 7.80 (m, 1H), 7.38–7.16 (m, 12H), 4.71–4.40 (m, 10H), 3.29–3.20 (m, 4H), 2.34 (m, 2H), 1.92–1.82 (m, 6H).

3.16. Pyridine–proline macrocycle 19

A solution of freshly prepared 2,6-pyridinedicarbonyl dichloride (0.42 g, 2 mmol) in dry dichloromethane (20 mL) was added dropwise to well-stirred solution of compound **18** (1.34 g, 2 mmol) and triethylamine (1.41 mL, 10 mmol) in dry CH_2Cl_2 (150 mL) at 0°C over 0.5 h. The reaction mixture was stirred for additional 12 h at rt. The resulting solution was concentrated to 60 mL and was washed with water and 5% $NaHCO_3$, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel using chloroform–methanol (100:1) as eluent to give white solid **19** (85 mg, 6.6% yield). Mp 132–134°C; $[\alpha]_D^{20}=-172.4$ (c 0.35, $CHCl_3$); MS (FAB): m/z 643 $(M+H)^+$; IR (KBr): ν 3485, 2924, 1648, 1453, 1349, 1213, 1165 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–6.77 (m, 16H), 6.27–3.78 (m, 14H), 2.34–1.65 (m, 8H). Anal. calcd for $C_{38}H_{38}N_6O_4$: C, 71.01%; H, 5.96%; N, 13.07%. Found: C, 70.98%; H, 5.99%; N, 13.01%.

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