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The application of quasi-enantiomeric trianglamine macrocycles as chiral probes for anion recognition in ion trap ESI mass spectrometry

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Abstract—This paper reports the synthesis and application of quasi-enantiomeric trianglamine macrocycles for chiral analysis using ion trap ESI mass spectrometry. The quasi-enantiomeric macrocycles were shown to display chiroselection towards a series of enantiomerically pure carboxylic acids and therefore act as chiral probes for anions in the gas phase and in solution.

1. Introduction

Few topics in the chemical and biological sciences have attracted as much interest as chirality. Chirality represents an intrinsic property of all the building blocks of life including amino acids, carbohydrates, DNA and proteins and therefore constitutes an essential feature of life itself.[1]

Chiral analysis or the reliable determination of enantiomeric excess plays an important aspect in the development of asymmetric synthesis. There is a huge demand within the pharmaceutical and related industries for reliable and accurate analytical methods that provide rapid and efficient information on enantiomeric purity. Conventionally, chiral NMR[2] and chiral separation (chiral GC, HPLC or capillary electrophoresis)[3,4] along with chiro-optical methods play, by far, the most important role in chiral analysis. Regulatory authorities nowadays request the provision of at least two alternative and independent measures of enantiomeric purity prior to drug licensing.[5] It is obvious that if any of the two traditional methods fails, this legal requirement cannot be met. Hence, there is a strong demand for alternative techniques. These problems highlighted the call for novel methods in the determination of enantiomeric purity. Any novel method or techniques need; (i) to take into account the problems associated with traditional techniques, (ii) to be rapid and efficient; (iii) to compete with traditional methods in terms of accuracy and robustness and (iv) must use analytical equipment that is readily available within the industry. It must furthermore be cost effective and simple. Ideally it should also be applicable to high-throughput development methods. Herein, we report such a technology using chiral probes in ESI mass spectrometry.

Soft ionisation mass spectrometry techniques, in particular electrospray (ESI), are routinely used to observe host guest complexes formed via non-covalent interactions in the mass spectrometer. The principle of chiral recognition using mass spectrometry was first reported by Fales almost 30 years ago.[6] Sawada et al.[7,8] expanded on this basic observation and reported on the interaction between chiral crown ethers and quasi-enantiomeric ammonium cations using FAB mass spectrometry. The term quasi-enantiomeric signifies the use of two enantiomeric compounds, in which one enantiomeric compound is substituted with a different isotope pattern.[9] Using this technique, it is feasible to discriminate between two diastereomeric host guest complexes in the mass spectrometer using their unique masses. Lebrilla et al. extended the general principle using ion molecule reactions in the gas phase between cyclodextrins and D- and L-amino acids in ESI mass spectrometry.[10–12] The principle of chiral recognition in the mass spectrometer was clearly demonstrated. However, in Sawada's FAB, an ionisation technique was required and therefore the method was lacking general scope. In Lebrilla's case, both enantiomers of a given guest molecule are required in pure form and the method requires more extensive calibrations of each ion molecule reaction along with fairly specialised mass spectrometrical equipment. Again, the scope of the method is not general and does not satisfy

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the many criteria for an efficient and rapid technique. Other approaches of chiral mass spectrometry have been reported including kinetic resolution using chiral metal complexes or collision fragmentation experiments using chiral clusters. The state of the art of the subject was recently reviewed by Tao and Cooks.[13] Furthermore, all previously reported work on chiral recognition in the mass spectrometer was only applied to the recognition of chiral cationic species.[14–26] Herein, we report for the first time a synthetic system that is able to bind chiral anions.

2. Results and discussion

Following the pioneering work by Gawronski,[27] we have recently reported on a new class of chiral macrocyclic compounds, which we have named trianglimines. Trianglimines are formed in [3+3] cyclocondensation reaction based on the formation of six C=N imine bonds between diaminocyclohexane and terephthal-aldehyde.[28–31] The six imine bonds can be conveniently reduced using $NaBH_4$ or $NaBD_4$ to give the corresponding hexaamine trianglamines **3** and **4**. Using diamino-cyclohexane **1**, both enantiomers of any macrocycle are readily available using tartrate resolution techniques. Using this approach we have been able to synthesise enantiomeric macrocycles **3** and **4** and quasi-enantiomeric macrocycles d^6-**3** and d^6-**4** in excellent yields.[27–29] Reduction of **2** with $NaBD_4$ proceeded in high diastereoselectivity producing only one diastereoisomer of the hexadeuterated hexaamine macrocycles, as judged by their ^1H and ^2H NMR spectra. Presumably attack of the D-nucleophile occurs from outside of the macrocycle (Fig. 1).

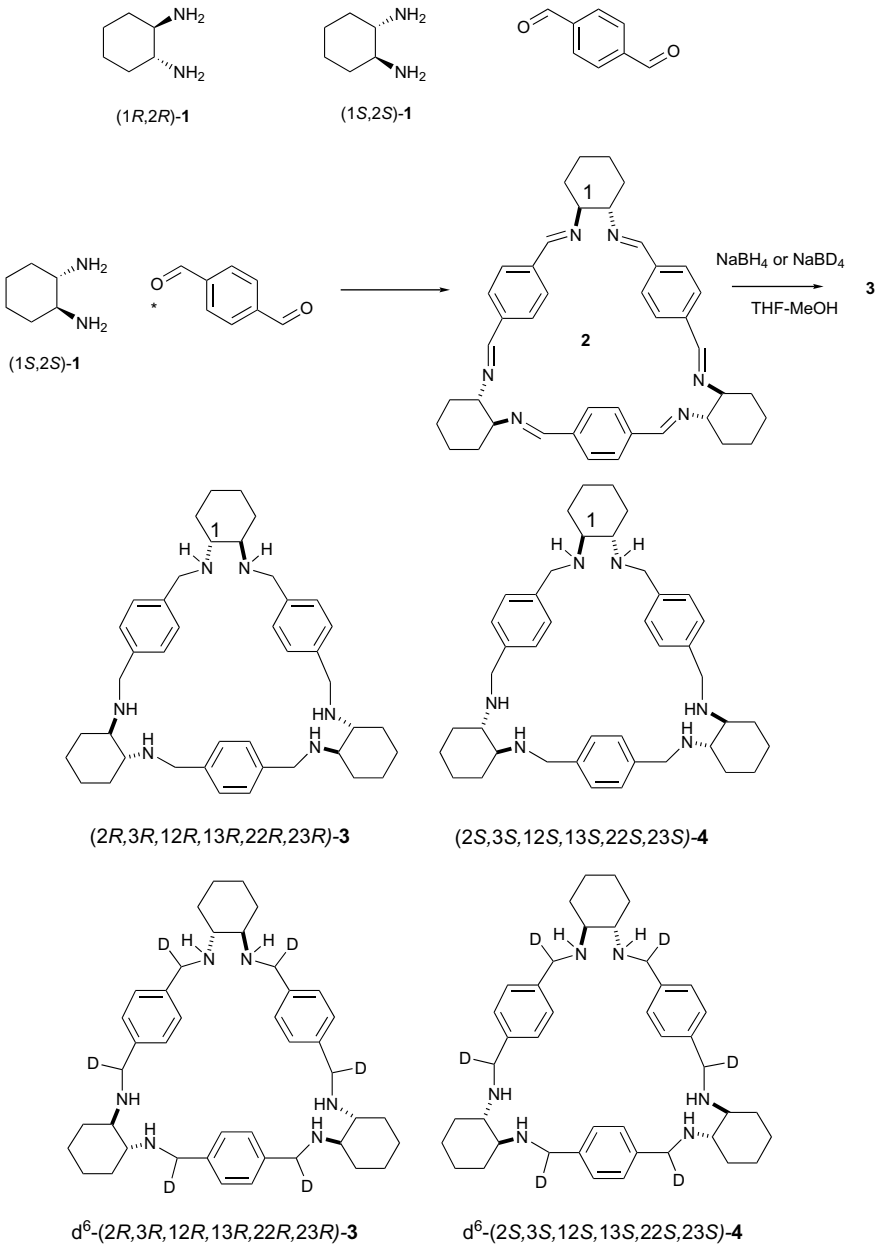

Figure 1. Trianglamine macrocycles **3** and **4**.

Both pairs of quasi-enantiomeric macrocycles can be easily distinguished in an ESI mass spectrum due to their m/z difference of six. If mixed in solution (either $CHCl_3$ or methanol) with a series of enantiomerically pure carboxylic acids **5**–**9** (see Fig. 2), the molecular ion of the parent macrocycles and of a two diastereomeric host guest complex formed between the acids and the macrocycle can be clearly observed in the ESI mass spectrum in the positive ion mode. Tandem MS/MS experiments clearly show that in all cases the host guest complex molecular ion observed, corresponds to the host guest complex postulated since fragment ions corresponding to the macrocycles are observed. Targeted fragmentations[32,33] of each of the two host guest molecular ions (at m/z 858 for **3·6**, at m/z 864 for d^6-**4·6**, ions (at m/z 854 for **3·7**, at m/z 860 for d^6-**4·7** and at m/z 783 for **3·L-8**, at m/z 789 for d^6-**4·L-8**) revealed significant differences in the stability of the two diastereomeric molecular host guest complex ions. In each case, the complex ion corresponding to the major diastereomer required increased collision energy for fragmentation as determined by the ratio of the intensities of the complex ion and the fragment ion at constant collision energy. From the relative intensities of the signals corresponding to the molecular ions of the pair of **3** and **4** and the relative intensities of the diastereomeric molecular ions of the host guest complexes observed, it becomes obvious that the molecular ions of the latter formed with considerable diastereomeric excess, for example, **3·5** and d^6-**4·5** (see Fig. 3).

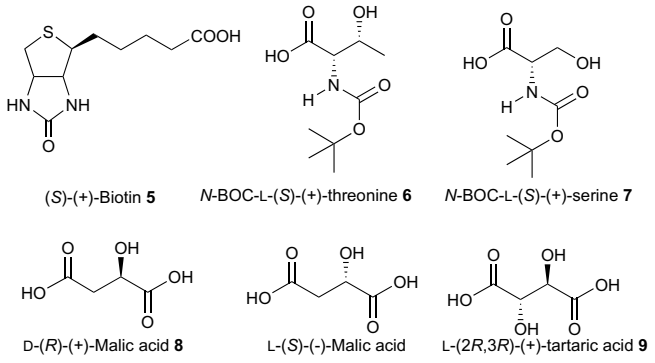

Figure 2.

It is worth mentioning that the diastereoselectivities measured carry an error of ±10% between two experiments under identical conditions, presumably due to the inhomogeneity of the infused ESI spray solution.[33] In two control experiments using, for example, L-malic acid and racemic malic acid and L-*N*-Boc serine and racemic *N*-Boc-serine as the guest molecules, we could clearly show that the observed intensities were not artefacts of the measurement, since in case of the racemic guest mixtures equal intensities of the two diastereomeric host guest molecular ions could be observed (Table 1).

To obtain more insight on the origins of the diastereoselections, we performed a series of additional ESI experiments[33] focusing on L-malic acid as a guest. Reduction of the cone temperature resulted in an increased intensity of the host guest molecular complex ion, however, in a reduced diastereoselectivity. An increase in the cone temperature resulted in a decrease of the intensity of the host guest complex ion and an increase of the diastereoselectivity. This finding suggests that the diastereoselectivity observed is presumably operating under thermodynamic control. Reduction of the ion trap scan time results in no change of the observed diastereoselectivities. This finding suggests that the origin of the diastereoselectivity results from the solution chemistry rather than the actual gas phase chemistry taking place in the ion trap itself. Variation in the concentration of host and guest allows the determination of the two gas phase equilibrium constants K_R and K_S where (*R*)- and (*S*)-denote the absolute configuration of the macrocycles. Correlation of the MS-diastereoselectivities with solution data using NMR shift titrations failed in the case of L-malic acid **8** and *N*-Boc L-serine **7** due to unfavourable solubilities and signal overlap. In case of L-*N*-Boc threonine **6**, two NMR shift titrations for a 1:1 stoichiometry as shown by a JOB analysis, were carried out using *all*-(*R*)-**3** and *all*-(*S*)-**4** showing that in line with the MS stabilities the formation of *all*-(*R*)-**3·6** ($K_S = 860\ M^{-1}$) is favoured over the formation of *all*-(*S*)-**4·6** ($K_R = 780\ M^{-1}$). For all other host guest pairs, the ratio of K_S/K_R solution equilibrium constants[34] was determined using a novel circular dichroism (CD) assay described below. In all cases investigated, small differences in the equilibrium constants of the diastereomeric complexes in solution could be observed.

The CD assay is based on a competitive binding experiment using binding of a single enantiomer of a guest molecule to a racemic mixture of macrocycles *all*-(*R*)-**3** and *all*-(*S*)-**4**. Figure 4 shows the CD spectra obtained illustrating the principle. Enantiomerically pure macrocycle *all*-(*R*)-**3** displays a typical bisignate CD spectrum as shown in magenta. A mixture of D-**8** and *all*-(*R*)-**3** shows a composition curve corresponding to a mixture of free guest and host and host guest complex. It can be clearly seen that in the CD spectrum of this mixture, an increased molar elipticity in the near UV region between 220 and 230 nm and a decreased molar elipticity around 250 nm if compared to *all*-(*R*)-**3** was observed. This change of the CD curve could be attributed to the presence of the host guest complex of D-**8**·*all*-(*R*)-**3**. The CD spectrum of the racemic mixture of *all*-(*R*)-**3** and *all*-(*S*)-**4** shows no CD spectrum as expected. The CD spectrum of the racemic mixture of *all*-(*R*)-**3** and *all*-(*S*)-in the presence of D-**8** corresponds to a composite curve of a mixture of both enantiomeric free macrocyclic hosts, free guest and the two diastereomeric host guest complexes. In solution the two enantiomeric macrocycles compete for binding with the guest. From the shape of the CD spectrum it can be deduced that free *all*-(*S*)-**4** and *all*-(*R*)-**3**·D-**8** are present in excess in solution. Hence *all*-*R*-**3**·D-**8** complex is formed preferentially over *all*-(*S*)-**4**·D-**8**. Carrying out the experiment at at least two different concentrations of the guest allowed us to estimate the ratio of the binding constants K_S/K_R (Table 2).[34]

In the cases of *N*-BOC-L-threonine **6** and *all*-*R*-**3** and *N*-Boc-L-threonine **6** and *all*-(*S*)-**4** ^1H–^1H-NOESY spectra in combination with ASIS considerations[35] allowed struc-

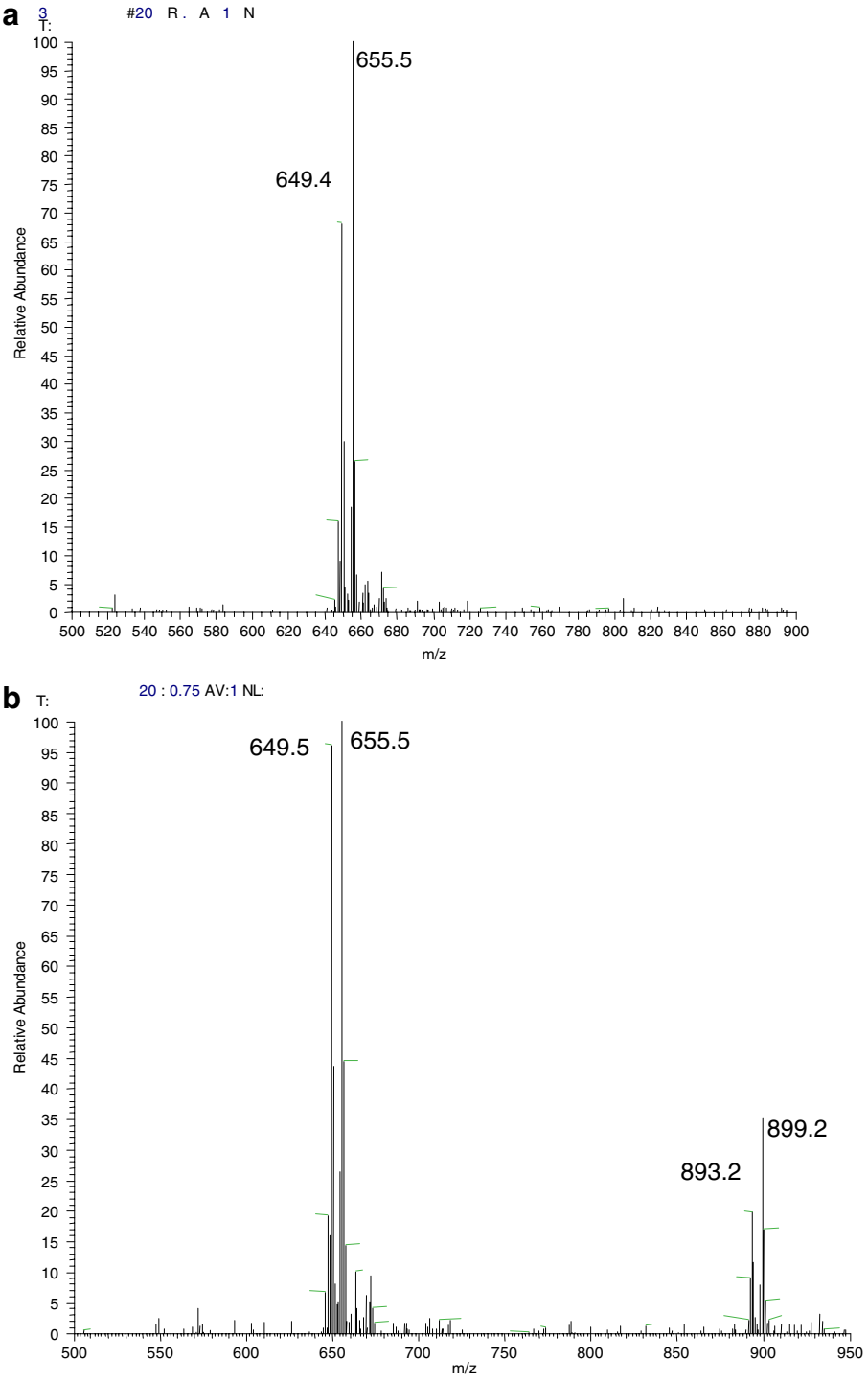

Figure 3. ESI spectrum of 10 μM MeOH solution of (a) macrocycle **3**, d^6-**4** and (b) macrocycle **3**, d^6-**4** in the presence of (*S*)-biotin **5** at 250 °C cone temperature.

ture determination of the host guest complexes in solution. Figure 5 shows the minimised structure of threonine complex *all*-(*R*)-**3**·**6** as a representative example.

The CH_3 group of the threonine points inside the central hole of the macrocyclic cavity, as indicated by a significant highfield shift of the CH_3 protons in the host guest complex when compared to the free threonine. Both the NH and anomeric proton display a downfield shift indicating their spatial proximity to the edges of the aromatic deshielding cone. The carboxylate group forms a salt bridge to a protonated ammonium nitrogen side. The threonine OH forms a hydrogen bond to a neighbouring NH functionality. The differences in stability between the two diastereomeric complexes *all*-(*R*)-**3**·**6** and *all*-(*S*)-**4**·**6** arise most likely due to repulsive interactions between the aromatic side walls of

Table 1. Observed diastereoselectivity for acids **5**–**9** binding 10 μM solutions

Macrocycle	Guest	Solvent	de (%)
3 and d^6-**4**	**5**	$CHCl_3$	28[a]
3 and d^6-**4**	**5**	MeOH	35[a]
4 and d^6-**3**	**5**	MeOH	12[b]
3 and d^6-**4**	**6**	MeOH	22[a]
3 and d^6-**4**	**6**	MeOH	9[b]
3 and d^6-**4**	D-**7**	MeOH	17[a]
3 and d^6-**4**	L-**7**	MeOH	17[a]
3 and d^6-**4**	L-**8**	MeOH	14[a]
3 and d^6-**4**	L-**8**	MeOH	7[b]
3 and d^6-**4**	D-**8**	MeOH	14[a]
3 and d^6-**4**	D-**8**	MeOH	7[b]
3 and d^6-**4**	**9**	MeOH	8[a]
3 and d^6-**4**	*rac*-**8**	MeOH	0[a]

[a] Using 300 °C cone temperature.
[b] Using 250 °C cone temperature.

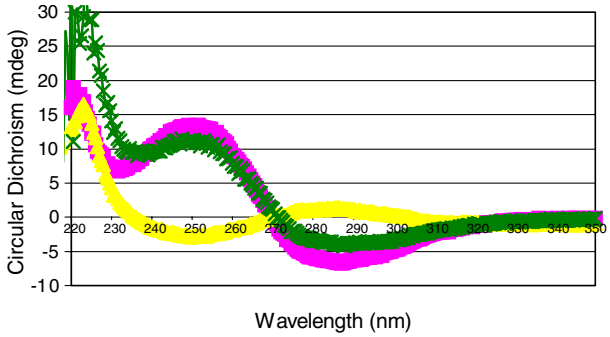

Figure 4. CD spectra in methanol for chiral recognition assay: magenta curve green curve *all*-(*R*)-**3** (0.1 mM); green curve *all*-(*R*)-**3** + D-**8** (0.1 mM each) and yellow curve *all*-(*R*)-**3**, *all*-(*S*)-**4** (0.05 mM each) and D-**8** (0.1 mM).

Table 2. Solution equilibrium constants determined

Host	Guest	de (%) by MS[a]	K_S/K_R by CD[b]	K_S/K_R by NMR[c]
3	**6**	22	1.2	1.1
3	**7**	17	1.3	—
3	**8**	14	1.4	—

[a] Determined in $CHCl_3$ at 250 °C cone temperature.
[b] In MeOH at 500 MHz.
[c] In $CDCl_3$ at 500 MHz.

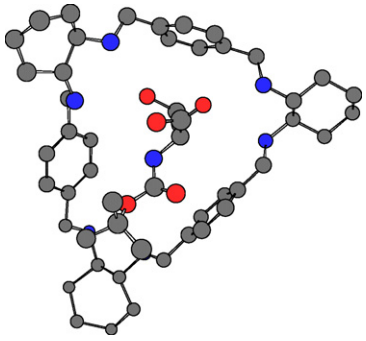

Figure 5. Structure of host guest complex between *N*-BOC-L-threonines **7** and **3** from NOE and ASIS NMR data.[35]

the macrocycle and the sterically demanding BOC-group as judged by the difference of intensities of the NOESY cross peak between the signals of the BOC protons and the aromatic protons of the macrocycle.

3. Conclusion

In conclusion we have achieved the first synthesis of both quasi-enantiomeric pairs of a macrocycle that can act as a receptor for chiral anions in the gas phase. Supported by a series of works on the synthesis of triangalamine macrocycles, we are able to judiciously tune the functionalities, size and electronic effects within the chiral host molecule to allow adjustment and complementarity to the guest molecule thus offering promising scope for the method in the future.[28–31] We have thoroughly investigated mass spectral parameters that influence the chiral selection in the gas phase. We have shown that chiral selection occurs in both gas phase under thermodynamic control and chiral selection occurs in solution phase shown through the introduction of a novel CD based assay that allows screening for chiral recognition in solution.

4. Experimental

4.1. General procedures

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-500 MHz spectrometer. δ values are quoted relative to teramethylsilane ($\delta = 0.00$ ppm) or chloroform ($\delta = 7.23$ ppm) for ^1H NMR and relative to chloroform ($\delta_C = 77.0$ ppm) for ^{13}C NMR. Coupling constants J are in hertz. Microanalysis was carried out using a Leeman CE 440 automatic elemental analyser. Infrared spectra were determined on a Perkin Elmer 200 Spectrometer. Optical rotations were determined on a Bellingham + Stanley ADO 220 polarimeter. Optical rotations were determined at at least two concentrations. The highest concentration is stated in the Experimental. Circular Dichroism spectra were obtained using an Advanced Photophysics Chirascan spectrometer using 10 mm rectanglar cuvettes. The mass spectra were recorded using a Thermo Finnigan DECA XP Plus ion trap mass spectrometer fitted with an electrospray ion source by direct infusion. All chemicals/reagents were purchased from the Aldrich Chemical Company. Solvents were dried using the usual procedures and reagents used without further purification unless stated otherwise. *all*-(*R*)-**2**, *all*-(*R*)-**3** and[27] (1*R*,2*R*)-**1** were obtained by the published procedure.[36]

4.1.1. (2*S*,3*S*,12*S*,13*S*,22*S*,23*S*)-4,11,14,21,24-Hexa-aza-(2,3:12,13:22,23)-tributano-(6,9:16,19:26,29)-trietheno-(2*H*,3*H*,12*H*,13*H*,22*H*,23*H*)-hexahydro-(30)-annulene 2. Terephthaldehyde (1.34 g, 10 mmol) in dichloromethane (8.3 ml) was added to a solution of (1*S*,2*S*)-diaminocyclohexane **1** (1.14 g, 10 mmol) in dichloromethane (5 ml). The mixture was stirred at room temperature for 3 h, after which the solvent was evaporated in vacuum and the crude compound recrystallised from ethyl acetate to give the title product **2** as white needles (5.7 g, 90%); mp >360 °C; IR

ν_{max} (Nujol)/cm^{-1} 1642 C=N; $[\alpha]_D^{25} = +354$ (*c* 0.5, CH_2Cl_2, 1-dm); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.14 (6H, s, N=C*H*), 7.52 (12H, s, Ar-H), 3.37 (6H, m, C*H*-N), 1.80 (24H, m, –CH_2–), 1.48 (6H, m, –CH_2–); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 160.3, 137.9, 128.1, 74.0, 32.6, 24.3; *m*/*z* (ESI) 637.8 (M^++H, 100%).

4.1.2. (2*S*,3*S*,12*S*,13*S*,22*S*,23*S*)-1,4,11,14,21,24-Hexa-aza-(2,3:12,13:22,23)-tributano-(6,9:16,19:26,29)-trietheno-(1*H*,2*H*,3*H*,4*H*,5*H*,10*H*,11*H*,12*H*,13*H*,14*H*,15*H*,20*H*,21*H*,22*H*,23*H*,24*H*,25*H*,30*H*)-octadecahydro-(30)-annulene 4. Solid $NaBH_4$ (0.1 g, 2.4 mM) was added slowly to a solution of compound **2** (190 mg, 2.3 mmol) in 10 ml 1:1 mixture of THF (tetrahydrofuran) and methanol. The mixture was stirred for 2 h. The solvent was removed under vacuum. The residue was dissolved in 20 ml dicholoromethane and extracted with water (3 × 15 ml). The combined organic extracts were dried over $MgSO_4$, filtered and the solvent removed in vacuum. The residue was recrystallised from toluene to give title product **4** as white needles (164 mg, 85%); IR ν_{max} (Nujol)/cm^{-1} 3298 cm^{-1} (N*H* stretch); $[\alpha]_D^{25} = +82$ (*c* 0.5, CH_2Cl_2, 1-dm); ^{1}H NMR (500 MHz; $CDCl_3$), δ_H 7.29 (12H, s, C*H* aromatic), 3.91 (6H, d, *J* 13.1, HN–C*H*), 3.62 (6H, d, *J* 13.1, *H*N–CH), 2.24 (12H, m, CH_2), 1.74 (12H, m, CH_2), 1.25 (6H, m, CH_2), 1.04 (m, 6H, CH_2); MS (ESI) *m*/*z* 649.4 (M+H, 100%) CHN calcd for $C_{42}H_{60}N_6$ C 77.7, H 9.32, N 12.95, found C 77.6, H 9.31, N 12.9.

4.1.3. (2*R*,3*R*,12*R*,13*R*,22*R*,23*R*)-1,4,11,14,21,24-Hexa-aza-(2,3:12,13:22,23)-tributano-(7,8′,17,18′,27,28′)-trietheno-(1*H*,2*H*,3*H*,4*H*,11*H*,12*H*,13*H*,14*H*,21*H*,22*H*,23*H*,24*H*)-duodecahydro-(5D,10D,15D,20D,25D,30D)-hexadeutero-(30)-annulene d^6-3. Using the procedure given in Section 4.1.2, 190 mg of *all*-*R*-**2** and 0.1 g of $NaBD_4$ gave the title compound **d^6-3** as white needles; IR ν_{max} (Nujol)/cm^{-1} 3298 cm^{-1} (N*H* stretch); $[\alpha]_D^{25} = -79$ (*c* 0.5, CH_2Cl_2, 1-dm); ^{1}H NMR (500 MHz; $CDCl_3$), δ_H 7.29 (12H, s, C*H* aromatic), 3.62 (6H, s, *H*N–CHD), 2.24 (12H, m, CH_2), 1.74 (12H, m, CH_2), 1.25 (6H, m, CH_2), 1.04 (m, 6H, CH_2); ^{2}H NMR (76.7 MHz; $CDCl_3$) 3.9 (s, br); MS (ESI) *m*/*z* 655 (M+H, 100%).

4.1.4. (2*S*,3*S*,12*S*,13*S*,22*S*,23*S*)-1,4,11,14,21,24-Hexa-aza-(2,3:12,13:22,23)-tributano-(7,8′,17,18′,27,28′)-trietheno-(1*H*,2*H*,3*H*,4*H*,11*H*,12*H*,13*H*,14*H*,21*H*,22*H*,23*H*,24*H*)-duodecahydro-(5D,10D,15D,20D,25D,30D)-hexadeutero-(30)-annulene d^6-4. Using the procedure given in Section 4.1.2, 190 mg of *all*-(*R*)-**2** and 0.1 g of $NaBD_4$ gave the title compound d^6-**4** as white needles; IR ν_{max} (Nujol)/cm^{-1} 3298 cm^{-1} (N*H* stretch); $[\alpha]_D^{25} = +80$ (*c* 0.5, CH_2Cl_2, 1-dm); ^{1}H NMR (500 MHz; $CDCl_3$), δ_H 7.29 (12H, s, C*H* aromatic), 3.62 (6H, s, , *H*N–CHD), 2.24 (12H, m, CH_2), 1.74 (12H, m, CH_2), 1.25 (6H, m, CH_2), 1.04 (m, 6H, CH_2); ^{2}H NMR (76.7 MHz; $CDCl_3$) 3.9 (s, br); MS (ESI) *m*/*z* 655 (M+H, 100%).

4.2. General mass spectrometry experiments

All MS experiments were carried out by direct infusion at a rate of 8 μl/min of solutions with a concentration of 10 μM in MeOH of all components.

For mass spectra of the macrocycles, a tune file was created using the Excalibur autotune function using a solution of 10 μM **3** in MeOH. For the mass spectra of the host guest complex ions, a tune file was created in the same way using a solution of L-malic acid and 10 μM **3**. The tune files were used throughout. Cone temperatures and scan times were changed manually leaving all other tune parameters constant.

Tandem MS experiments were carried out with an isolation width of 2 amu and a collision energy of 30%. For all diastereomeric host guest complexes the collision energy was furthermore varied using values of 15%, 20%, 25% and 30%.

Diastereoselectivities were determined by taking the arithmetic mean value for $\{[I(all\text{-}(R)\text{-}\mathbf{3}) - I(d^6\text{-}all\text{-}(S)\text{-}\mathbf{4})] + [I(all\text{-}(R)\text{-}\mathbf{3}\cdot\text{guest}) - I(d^6\text{-}all\text{-}(S)\text{-}\mathbf{4}\cdot\text{guest})]\}/2$ where *I* denotes the intensity of the mass spectral signals.

Taking into account the intensities of both quasi-enantiomeric macrocycles and diastereomeric host guest complex ions, the potential effect of ion suppression should be reduced.

4.3. NMR titrations

All ^{1}H NMR titrations were carried out in $CDCl_3$ using TMS as the internal standard on a Bruker Avance 500 MHz instrument using standard Bruker software.

A 1:1 stoichiometry was established using the method of Job using total concentrations of **7** and *all*-*R*-**3** of 0.01 M measuring at data points of $x = 0.1$, 0.3, 0.5, 0.7 and 0.9.

NMR shift titrations were carried out in $CDCl_3$ at constant concentration of *all*-*R*-**3** or *all*-*S*-**3** of 0.01 M (6.5 mg/ml). The concentration of **6** was varied and measurements were taken at a concentration of **6** of 0.12 M, 0.08 M, 0.04 M, 0.02 M, 0.01 M, 0.005 M and 0.001 M. The changes in the chemical shift of the NH proton and of the CHOH protons were monitored and used for the evaluation of the binding constant. The binding constant was obtained by standard curve fitting procedures using a 1:1 stoichiometry. The error in both curves obtained is estimated to be 5%.

4.4. ^{1}H–^{1}H-NOESY experiments

^{1}H–^{1}H-NOESY experiments were carried out using standard Bruker software of solutions of *all*-(*R*)-**3** or *all*-(*S*)-**3** of 0.03 M (19.5 mg/ml) and 0.03 M solution of **6** at 293 K in degassed $CDCl_3$ using a mixing time of 500 ms. Positive NOE effects observed from $(CH_3)_3$ of **6** to Ar-H, CH_2N and C*H*NH of **3**, NH of **6** to Ar-H of **3**, CHCH_3OH of **6** to Ar-H and CH_2N of **3** were quantified and used for structure estimation. A total of 10 molecular models with different starting geometries were minimised at the MM2 level using Chem3D and the structure showing the best agreement with the experimental NOE and ASIS data was chosen.[35] The ^{1}H NMR data for the 1:1 complex at

0.03 M are as follows: ^{1}H NMR (500 MHz; $CDCl_3$), δ_H 7.30 (12H, s, ArH), 5.27 (1H, s, C=ONH), 4.00 (6H, d, J 13.0, CH_AH_BN), 3.68 (6H, d, J 13.0, CH_AH_BN), 3.47 (1H, q, J 6.5, C*H*OH), 2.55 (6H, m, C*H*N), 2.17 (6H, m, NH), 1.43 (9H, s, $C(CH_3)_3$), 1.25–1.11 (24H, m, CH_2), 1.08 (3H, d, J 6.5, $CHCH_3$).

References

1. *Stereochemistry of Organic Compounds*, 1st ed.; Eliel, E. L., Wilen, S. H., Eds.; J. Wiley: New York, 1994.
2. Parker, D. *Chem. Rev.* **1991**, *91*, 1441.
3. Gubitz, G. *Chromatograph* **1990**, *30*, 555–564.
4. Bluhm, L.; Huang, J.-M.; Li, T. Y. *Anal. Bioanal. Chem.* **2005**, *382*, 592–598.
5. Collins, A. N.; Sheldrake, G. N.; Crosby, J. *Chirality in Industry*; Wiley: New York, 1997.
6. Fales, H. M.; Wright, G. J. *J. Am. Chem. Soc.* **1977**, *99*, 2339–2340.
7. Sawada, M.; Takai, Y.; Yamada, H.; Kaneda, T.; Kamada, K.; Mizooku, T.; Hirose, K.; Tobe, Y.; Naemura, K. *Chem. Commun.* **1994**, 2497–2498.
8. Sawada, M.; Yamaoka, H.; Takai, Y.; Yamada, H.; Azuma, T.; Fujioka, T.; Tanaka, T. *Chem. Commun.* **1998**, 1569–1570.
9. Eames, J.; Suggate, M. J. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 705–717.
10. Grogorean, G.; Ramirez, J.; Ahn, S.-H.; Lebrilla, C. B. *Anal. Chem.* **2000**, *72*, 4275–4281.
11. Ahn, S.; Ramirez, S. J.; Grigorean, G.; Lebrilla, C. B. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 278–287.
12. Grigorean, G.; Ramirez, J.; Ahn, S.; Lebrilla, C. B. *Anal. Chem.* **2000**, *47*, 705–717.
13. Tao, A.; Cooks, R. G. *Anal. Chem.* **2003**, 25A.
14. Guo, J.; Wu, J.; Siuzdak, G.; Finn, M. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1755–1758.
15. Liang, Y.; Bradshaw, J. S.; Izzat, R. M.; Poe, R. M.; Dearden, D. V. *Int. J. Mass Spectrom.* **1999**, *185/186/187*, 977–988.
16. Sawada, M. *Mass Spectrom. Rev.* **1997**, *16*, 73–90.
17. Shizuma, M.; Ohta, M.; Yamada, H.; Takai, Y.; Nakaoki, T.; Takeda, T.; Sawada, M. *Tetrahedron* **2002**, *58*, 4319–4330.
18. Sawada, M.; Takai, Y.; Imamura, H.; Yamada, H.; Takahashi, S.; Yamaoka, H.; Hirose, K.; Tobe, Y.; Tanaka, J. *Eur. J. Mass Spectrom.* **2001**, *7*, 447–449.
19. Shizuma, M.; Takai, Y.; Kawamura, M.; Takeda, T.; Sawada, M. *J. Mass Spectrom.* **2001**, *210/211*, 585–590.
20. Shizuma, M.; Adachi, H.; Amemura, A.; Takai, Y.; Takeda, T.; Sawada, M. *Tetrahedron* **2001**, *57*, 4567–4578.
21. Shizuma, M.; Adachi, H.; Kawamura, M.; Takai, Y.; Takeda, T.; Sawada, M. *J. Chem. Soc., Perkin Trans. 2* **2001**, 592–601.
22. Shizuma, M.; Imamura, H.; Takai, Y.; Yamada, H.; Takeda, T.; Takahashi, S.; Sawada, M. *Chem. Lett.* **2001**, *2*, 592–601.
23. Sawada, M.; Yamaoka, H.; Takai, Y.; Kawai, Y.; Yamada, H.; Azuma, T.; Fujioka, T.; Tanaka, T. *J. Mass Spectrom.* **1999**, *193*, 123–130.
24. Sawada, M.; Yamaoka, H.; Takai, Y.; Kawai, Y.; Yamada, H.; Azuma, T.; Fujioka, T.; Tanaka, T. *Chem. Commun.* **1998**, 1569–1570.
25. Sawada, M.; Shizuma, M.; Takai, Y.; Takai, Y.; Adachi, H.; Uchiyama, T. *Chem. Commun.* **1998**, 1453–1454.
26. Sawada, M.; Takai, Y.; Yamada, H.; Nishida, J.; Kaneda, T.; Arakawa, R.; Okamoto, M.; Hirose, K.; Tanaka, T.; Naemura, K. *J. Chem. Soc., Perkin Trans. 2* **1998**, 701–710.
27. Gawronski, J.; Kolbon, H.; Kwit, M.; Katrusiak, A. *J. Org. Chem.* **2000**, *65*, 5768–5773.
28. Kuhnert, N.; Straßnig, C.; Lopez-Periago, A. *Tetrahedron: Asymmetry* **2002**, *13*, 123–128.
29. Kuhnert, N.; Lopez-Periago, A.; Rossignolo, G. M. *Org. Biomol. Chem.* **2003**, *1*, 1157.
30. Kuhnert, N.; Lopez-Periago, A.; Rossignolo, G. M. *Org. Biomol. Chem.* **2005**, *3*, 525–537.
31. Kuhnert, N.; Burzlaff, N.; Patel, C.; Lopez-Periago, A. *Org. Biomol. Chem.* **2005**, *3*, 1911–1920.
32. Clifford, M. N.; Knight, S.; Kuhnert, N. *J. Agric. Food Chem.* **2005**, *53*, 3821–3832.
33. *ESI Mass Spectrometry: Fundamentals, Application and Instrumentation*; Cole, R. B., Ed.; Wiley: Chichester, 1997.
34. Rodgers, A.; Latham, H. C.; Wormell, P.; Parkinson, A.; Ismail, M.; Sanders, J. K. *Enantiomer* **1998**, *3*, 395–408.
35. Haigh, C. W.; Mallion, R. B. *Progr. NMR Spectrom.* **1980**, *33*, 303.
36. Larrow, J. F.; Jacobsen, E. N. *J. Org. Chem.* **1994**, *59*, 1939.