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Research Article

Synthesis, self-association and chiroselectivity of isotopically labeled trianglamine macrocycles in the ion trap mass spectrometer†

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Abstract: The synthesis and self-association of chiral isotopically labeled trianglamine macrocycles under electrospray mass spectrometer conditions in an ion trap are described. A moderate diastereoselectivity in the self-association process was observed providing a synthetic model system for the investigation of chiral self-association in the gas phase. The first non-covalently bound dimer exclusively bonded through aromatic–aromatic interactions was observed in the gas phase. Evidence for self-association in solution was observed by diffusion nuclear magnetic resonance spectroscopy. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: deuterium labeling; supramolecular chemistry; mass spectrometry; chiral recognition; macrocyclic chemistry

Molecular recognition is an area of chemistry aiming at the understanding of complementarity of size shape and functionality at the supramolecular level. It explores weak interaction between two or more molecules over short distances resulting in complex molecular assemblies.[1,2] At this supramolecular level chirality can be expressed through the diastereoselective formation of molecular clusters through self-assembly of homochiral building blocks.[3,4] Mass spectrometry has been shown to provide an experimental technique suitable for the study of these diastereoselective self-assembly processes in the gas phase. Early reports demonstrated the self-association of homochiral tartrate derivatives in the gas phase.[5,6] Most prominently homochiral amino acids, in particular serine, were reported to form non-covalent clusters readily generated by electrospraying or sublimation and observed and characterized through ESI mass spectrometry.[7–9]

Studies on such chiral supramolecules have been motivated to a large extent by the possibility that such self-assembled clusters of homochiral molecules play an important role in the origin of homochirality in biological systems, exemplified by the preference of amino acids for the L-configuration or sugars for the D-configuration.

Our group has studied for some time the chemistry of homochiral polyamine and polyimine macrocycles, which we named trianglimines and trianglamines.[10–15] In the course of our investigation we observed frequently that under ESI mass spectrometric conditions the homochiral macrocycles did assemble in the gas phase into larger clusters. Since trianglamines are readily available in both enantiomeric forms we decided to investigate the chiroselection of this self-assembly process in detail and report the finding of our studies in this publication.

Results and discussions

Synthesis of trianglimines

For our studies concerned with the chiroselection of homochiral trianglamine macrocycles through mass spectrometry we required macrocycles in both enantiomeric forms that were isotopically labelled in order to distinguish between diastereomeric clusters in the mass spectrometer. If one of either enantiomers of a given compound is isotopically labelled the two

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†Dedicated to the memory of Professor John Jones.

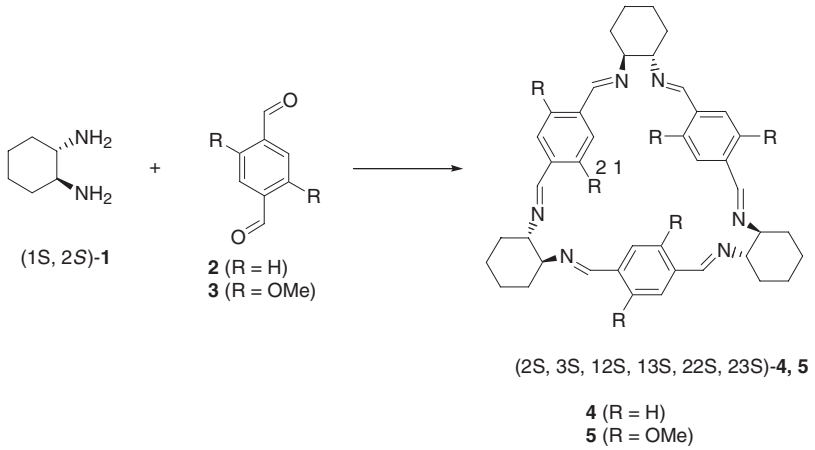

Figure 1 Synthesis of trianglimine macrocycles **4** and **5**.

enantiomers and hence any diastereomeric clusters formed through self-assembly can be readily distinguished through mass spectrometry through their characteristic *m/z* values. Compounds that are enantiomeric but differ in their isotopic substitution pattern are frequently referred to as quasi-enantiomeric compounds.[16]

Trianglimines were synthesized as reported previously using a [3 + 3] cyclocondensation between either enantiomer of *trans*-1,2-diaminocyclohexane **1** and terephthalaldehyde **2** or 2, 4-dimethoxyterephthalaldehyde **3** that gave the 30 membered ring macrocycles **4** and **5** in almost quantitative yield (see Figure 1). The *all-R* enantiomer of **4** has been previously reported by Gawronski and co-workers[17] and the *all*-R enantiomer of compound **5** by our group.[10] The spectroscopic properties of the trianglamine macrocycles were as expected and are given in the experimental section.

With both enantiomers of the trianglimine macrocycles **4** and **5** in hand we obtained the unlabelled compounds by reduction with $NaBH_4$ and their quasi-enantiomeric deuterated counterparts by reduction with $NaBD_4$, respectively, in excellent yields.

The hexadeuterated compounds d^6-*all-R*-**6**, d^6-*all-S*-**6**, d^6-*all-R*-**7** and d^6-*all-S*-**7** were obtained as single diastereomers (de > 98%) as judged by their ^{1}H- and ^{2}H-NMR spectra, creating an additional six stereogenic centers (Figure 2). The ESI mass spectra of the hexadeuterated macrocycles indicate a deuteration degree of 94–95%. To account for the observed stereoselectivity we assume that attack of the nucleophile on the six imine bonds occurs in all six cases from the outside of the macrocycle, in case of the *all-R* compound from the *pro-R* face and in case of the *all-S* macrocycles from the *pro-S* face as shown in Figure 3.

This assumption allows well unambiguous assignment of the two diastereotopic benzylic protons with the 'in-plane' hydrogen appearing at 3.56 ppm and the 'out of plane' hydrogen appearing at 3.81 ppm. A ^{1}H–^{1}H-NOESY spectrum of compound **7** reveals that the 'in-plane' hydrogen appearing at 3.56 ppm shows close special proximity to the cyclohexane moiety, whereas the 'out-of-plane' hydrogen shows close spatial proximity to the OMe substituent of the aromatic ring (see Figure 3).

To the best of our knowledge this is the first example of a synthesis of all quasi-enantiomeric forms of a single isotopically labelled compound. A complete set of quasi-enantiomeric compounds has been reported by Eames and co-workers. [18,19]

The Circular Dichroism spectra of the four quasi-enantiomeric trianglamines showed an exciton-coupled doublet with a negative chirality for the *all-R* enantiomer and positive chirality for the *all-S* enantiomer. Interestingly, the $\Delta\varepsilon$ values for the deuterated compounds were lower if compared with the protonated compounds. A similar observation where introduction of deuterium labels was affecting the chirooptical properties of a pair of compounds has been reported by Eames and co-workers.[20]

Optimization of conditions and self-association of macrocycles

In any ESI mass spectroscopy experiment, a series of experimental parameters need to be carefully chosen.[21] Among the most important parameters are solvent, the ionization mode, cone temperature, cone voltage and gas flow rate. Other parameters can be optimized automatically by creation of a suitable tune file. Since all our macrocycles are characterized by six basic

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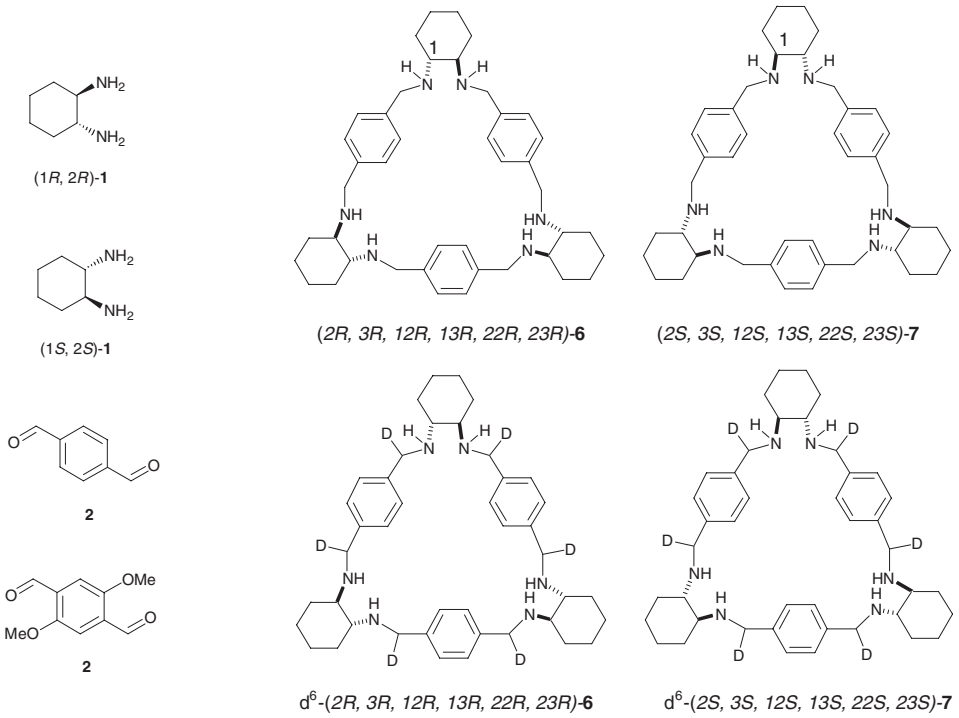

Figure 2 Synthesis of quasi-enantiomeric trianglamine macrocycles **6** and **7**.

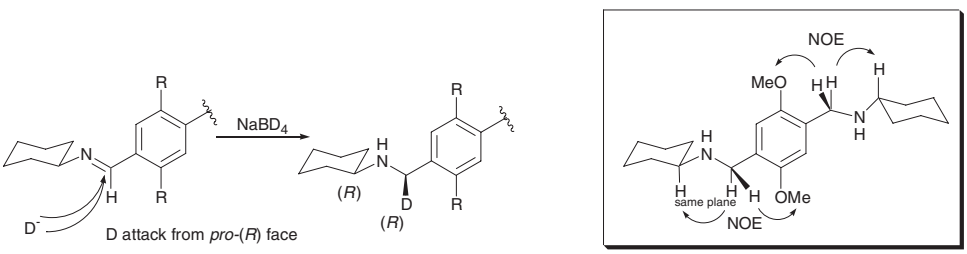

Figure 3 Stereoselective reduction of compound **7** and NOESY data of **7**.

nitrogen atoms we choose to conduct all experiments in the positive ion mode. As a solvent we choose methanol throughout, which dissolves all macrocycles of interest and which allows efficient proton transfer and therefore ionization of the macrocycles.

Conditions for the molecular ions of macrocycles **6** were optimized using the automated tune routine. When changing the cone temperature below 300°C we observed two additional signals at m/z 1297, double the original m/z value, and at m/z 1945, triple the original m/z value. Again we obtained a tune file for these cluster ions.

Upon decrease in the cone temperature the relative intensity of the dimeric and trimeric clusters was dramatically increasing. Figure 4 shows some representative spectra recorded at 250, 200 and 150°C cone temperatures. Data of the relative intensities of the dimeric and trimeric molecular complex ions are given in Table 1.

Two alternative binding modes for the observed clusters are feasible. Firstly, a dimer or trimer, respectively, formed by aromatic aromatic interactions and three N–H–N hydrogen bonds between the amine functionalities of two or three macrocycles, respectively, could be formed. Secondly, the formation of a catenane-type structure is feasible with two interlocked macrocycles.[22] We reasoned that the latter version should show some distinct signals in the NMR spectra of the macrocycles, which could not be observed. Additionally, the formations of the dimer and trimer signals are strongly concentration dependent. On increase in the macrocycle concentration the intensity ratio, monomer/dimer decreases significantly (see Table 1). Furthermore, with hexadeuterated

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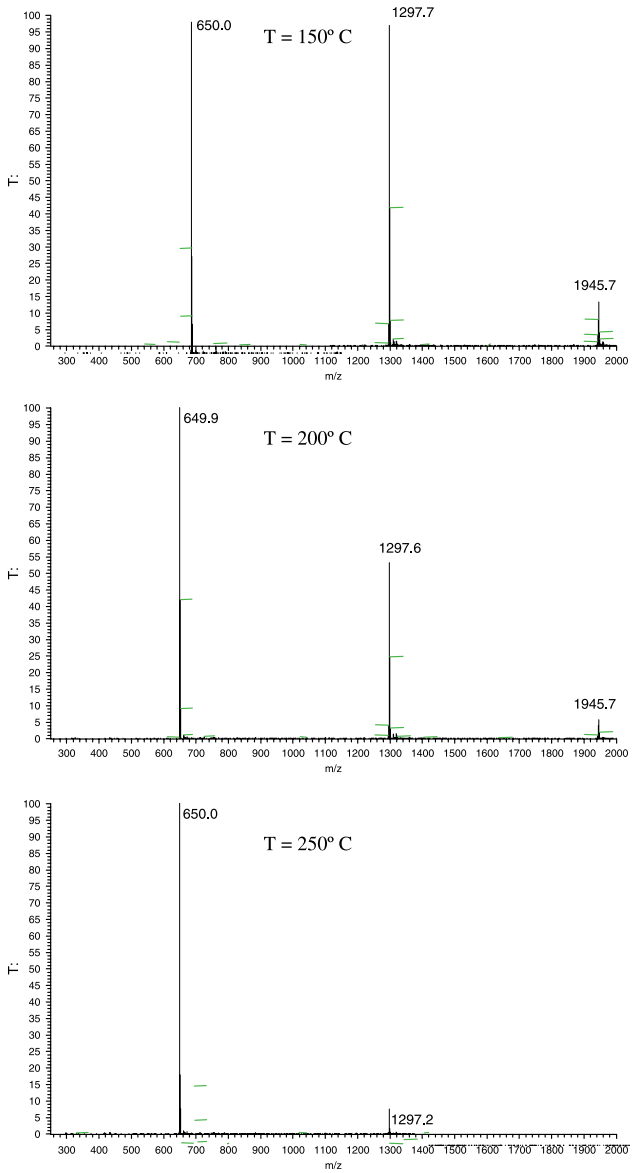

Figure 4 Expanded ESI mass spectra of compound **6** (0.01 mM in MeoH) at different cone temperature.

macrocycle **d^6-6** in hand some cross-over experiments were carried out. For a 1:1 ratio of *all-R*-**6** and d^6-*all-R*-**6**, the molecular ions of the dimer appeared as a three-line pattern indicating a statistical mixture of *all-R*-**6** ● *all-R*-**6**, d^6-*all-R*-**6** ● *all-R*-**6** and d^6-*all-R*-**6** ● d^6-*all-R*-**6** that formed very rapidly. Increase in concentration of *all-R*-**6** resulted in an immediate change of the pattern again to a statistical mixture. Since formation of a catenane requires slow ring opening we interpret this observation of an indication that a non-catenane structure was formed. As additional evidence for the formation of a non-catenane dimer we carried out tandem MS experiments. The molecular complex ion at *m/z* 1297 was stored in the ion trap of the mass spectrometer. By choosing a collision energy of 10%, the tandem MS spectra showed only one fragment ion at *m/z* 649. This experiment shows that the dimeric structure can be fragmented at very low fragmentation energies and points towards a non-catenane-type dimer. Fragmentation of a catenane dimer would involve breaking of a covalent bond requiring a significantly higher fragmentation energy.

For the hexa-amine dimer we needed to distinguish between the individual contributions of aromatic–aromatic interactions and N–H–N hydrogen bonding. Should the methanol solution be saturated with NaF or TBAF the fluoride ions should act as strong competitors in hydrogen bonding and therefore disrupt the dimeric

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Table 1 Dependence of intensities of dimeric and trimeric molecular clusters on change of cone temperature and concentration of compound *all-R*-**6** in MeOH solution

Parameters at 0.01 mM Cone temperature (°C)	Intensity *m/z* 649 *all-R*-**6** (%)	Intensity *m/z* 1297 *all-R*-**6** • *all-R*-**6** (%)	Intensity *m/z* 1945 (*all-R*-**6**)$_3$ (%)
125	92	100	16
150	100	97	12
175	100	65	8
200	100	53	7
225	100	18	1
250	100	7	0
300	100	0	0
Parameters at 150°C cone temperature Concentration in mM			
1	88	100	18
0.1	94	100	14
0.05	100	100	12
0.01	100	97	12
0.005	100	91	9
0.001	100	81	8

structure if hydrogen bonding is the main contributing factor in the dimerization process. Indeed addition of fluoride dramatically reduces the intensity of the molecular ion of the dimeric species. Similarily, addition of protic acids should strengthen the interactions in the dimeric species. However, addition of mineral acids leads to very different results, namely the formation of trianglamine anion complexes in the gas phase, which will not be discussed here.

Interestingly we also observed formation of a dimeric structure at low cone temperatures for the hexa-imine macrocycles *all-R*-**4** and *all-R*-**5**, which clearly lack the NH functionalities capable of forming hydrogen bonds. We propose that such dimeric structures are formed exclusively through a series of aromatic–aromatic interactions of the face-to-face type. The formation of non-covalent clusters in the gas phase exclusively formed through aromatic aromatic interaction has been reported in ruthenium-based system by Gourdon and co-workers.[23] Although it needs to be mentioned that in this example additional cation–π-interactions of the polycationic ruthenium complexes might have contributed to the overall stability of the observed cluster.

Chiroselection of quasi-enantiomeric macrocycles in the gas phase

With the quasi-enantiomeric macrocycles and optimized mass spectrometrical conditions in hand we started investigating the chiroselection properties of the macrocycles **6** and **7** in a self-association process. As standard mass spectrometric conditions we choose a cone temperature of 150°C at a total concentration of 0.01 mM of both quasi-enantiomeric macrocycles using a standard tune file obtained for the dimeric molecular complex ion. Experiments were carried out at three stoichiometries using 2:1, 1:1 and 1:2 ratios of the two quasi-enantiomeric macrocycles.

A control experiment showed that using a 1:1 mixture of *all-R*-**6** and d^6-*all-R*-**6** three signals for the dimeric species are observed in a statistical ratio of 1:2:1 or at 2:1 stoichiometry of 4:4:1.

If two quasi-enantiomeric trianglamine macrocycles, e.g. of *all-R*-**6** and d^6-*all-S*-**6** were mixed at a 1:1 stoichiometry, the relative intensities of the three resulting molecular complex ions of *all-R*-**6** • *all-R*-**6**, *all-R*-**6** • d^6-*all-S*-**6** and d^6-*all-S*-**6** • d^6-*all-S*-**6** were significantly different if compared with the statistical mixture observed for two labeled compounds showing the identical absolute configuration. This change of signal intensities is also pointing towards a diastereoselective self-association process in the gas phase. Figure 5 shows a representative example of a mass spectrum of a mixture of two quasi-enantiomeric macrocycles at a 2:1 stoichiometry. In addition to the expected signals representing the dimeric species, additional signals arising due to dimeric structures that have been incompletely deuterated or that have suffered from H–D exchange can be identified.

For the determination of the diastereoselectivities from the experimental mass spectrometrical data, we firstly assume that both quasi-enantiomeric compounds *all-R*-**6** and d^6-*all-S*-**6** are ionized with the same efficiency. Looking at the experimental data it

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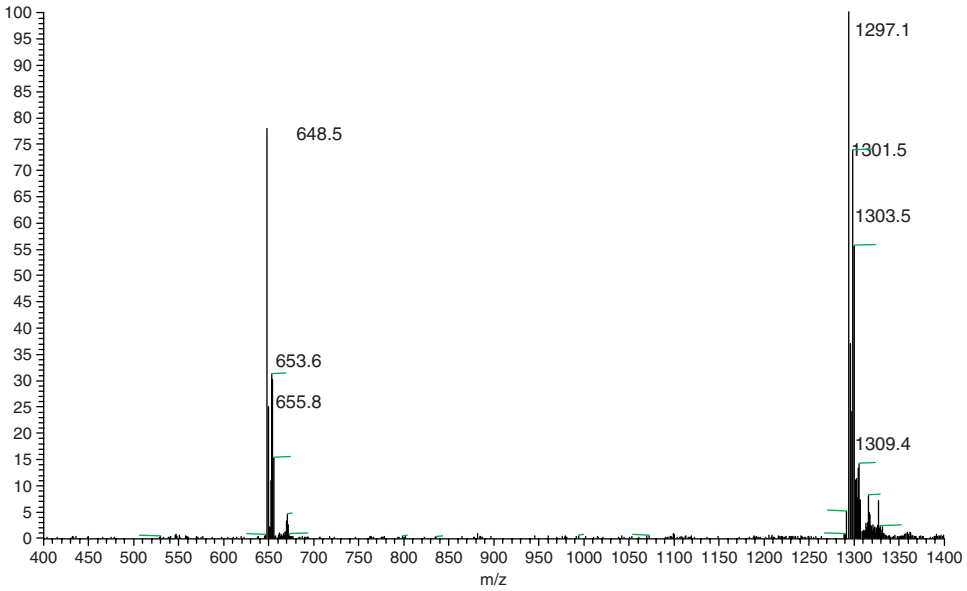

Figure 5 ESI mass spectrum of a 2:1 mixture of *all-R*-**6** and d^6-*all-S*-**6** in MeOH at 0.01 mM concentration and 150°C cone temperature.

becomes obvious that the diastereomeric molecular complex ions of *all-R*-**6** ● *all-R*-**6**, *all-R*-6 ● d^6-*all-S*-**6** and d^6-*all-S*-**6** ● d^6-*all-S*-**6** are despite their structural resemblance not ionized with the same efficiency. In particular, the molecular ion of d^6-*all-S*-**6** ● d^6-*all-S*-**6** is showing reduced ionization efficiency in all experiments. We have currently no explanation of why this duodecadeuterated structure is ionized less efficiently if compared with its protonated enantiomer. As a consequence of this observation the diastereoselectivity was determined experimentally using two values firstly the ratio of *all-R*-**6** ● *all-R*-**6**: *all-R*-6 ● d^6-*all-S*-**6** was termed de (H) and secondly the ratio of *all-R*-**6** ● d^6-*all-S*-**6**: d^6-*all-S*-**6** ● d^6-*all-S*-**6** was termed de (D). The diastereoselectivities were determined at three different stoichiometries as stated above and the average value is given in Table 2. In all cases the homo dimeric structure, e.g. *all-R*-**6** ● *all-R*-**6** was found to be the major diastereomer formed as opposed to the heterodimeric structure *all-R*-**6** ● *all-S*-**6**.

Moderate diastereoselectivities were observed around 10–20% de. Interestingly, the diastereoselectivity depended on the cone temperature used in the experiment. With increasing cone temperature the experimentally determined diastereoselectivity was increasing. This indicates that the diastereoselection process operates under thermodynamic control.

Studies of self-association in solution

Like in most studies of molecular recognition events using mass spectrometry, the question arises whether the phenomenon observed appears only in the gas phase or is as well observable in solution. If such a phenomenon is as well experimentally verified in solution the confidence in the technique of mass spectrometry for studying molecular recognition phenomena grows dramatically. For this reason we decided to investigate the self-association process of trianglamine macrocycles in solution.

We have previously demonstrated that diffusion NMR is a powerful technique that can be used to investigate molecular recognition processes in solution.[25–26] Hence, we acquired a series of diffusion NMR spectra of trianglamines in $CDCl_3$ and D_3COD at various concentrations. The observed diffusion coefficient of the trianglamine macrocycles was indeed changing upon variation of concentration within the experimental error. With increasing concentration the experimentally

Table 2 Observed diastereoselectivity for formation of dimeric molecular complex ions at 150°C cone temperature and 0.01 mM concentration of macrocycles in MeOH solution

Compound 1	Compound 2	de (D) (%)	de (H) (%)
all-R-**6**	d^6-*all-S*-**6**	14	16
d^6-*all-R*-**6**	*all-S*-**6**	14	16
all-R-**7**	d^6-*all-S*-**7**	9	10
all-R-**6**[a]	d^6-*all-S*-**6**	15	16
all-R-**6**[b]	d^6-*all-S*-**6**	17	19
all-R-**6**[c]	d^6-*all-S*-**6**	19	21
all-R-**6**	d^6-*all-R*-**6**	0	0

[a] At 200°C cone temperature.
[b] At 225°C cone temperature.
[c] At 250°C cone temperature.

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Table 3 Diffusion coefficient, measured by ^{1}H-diffusion NMR of macrocycle **6** at various concentrations

Concentration of **6** (mM)	Diffusion coefficient D ($m^2 s^{-1}$) in $CDCl_3$	Diffusion coefficient D ($m^2 s^{-1}$) D_3COD
3	1.7×10^9	1.6×10^9
6	1.6×10^9	1.5×10^9
9	1.4×10^9	1.4×10^9
12	1.2×10^9	1.4×10^9

observed diffusion coefficient (Table 3) was decreasing pointing to an increase in the hydrodynamic radius of the species in solution. Such an observation can only be rationalized by assuming self-aggregation of the trianglamine macrocycles in solution hence clearly showing that the self-association phenomena observed in the gas phase also occur in solution.

Conclusion

In conclusion we have shown that both enantiomers of trianglimine and trianglamine macrocycles are readily obtained as well as their isotopically labeled quasi-enantiomeric counterparts. The compounds show self-association in the gas phase under ESI conditions with both dimeric and trimeric molecular clusters formed through non-covalent interactions. The mass spectrometric conditions for the observation of these clusters have been optimized. For hexa-imine macrocycles, the first non-covalent complex observed in the gas phase formed exclusively by aromatic–aromatic interactions was observed.

In case quasi-enenatiomeric macrocycles are used, diastereomeric molecular clusters can be observed in the gas phase. The non-covalent binding process exhibits a moderate diastereoselectivity between 9 and 20%. This self-association process has been shown to operate also in solution.

General experimental

^{1}H, ^{2}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer. Chemical shifts are reported as δ values in ppm relative to the solvent $CDCl_3$ as an internal standard (δ 7.26), or TMS (δ 0.00) when d_6-DMSO is used. Coupling constants (*J* and *N*) are reported in Hertz. Peak assignments in the proton NMR are abbreviated as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet and m = multiplet.

Elemental analysis was made on a Leeman Labs CE440 Elemental Analyser. The optical rotation was measured in a Bellingham-Stanley ADP220 automatic polarimeter (L.E.D. with interference filter, 589 nm). Circular dichroism spectra were recorded on a Applied Photophysics Chirascan instrument using 1 mM solutions in methanol. Infrared spectra were determined on a Perkin Elmer 2000 Spectrometer. The EI, CI, LSMS and FAB data were collected on a ThermoFinnigan Mat 95 X. All The ESI-MS was recorded on a ThermoFinnigan DECA LCQ XP Plus ion trap mass spectrometer by direct infusions.

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

(2S 3S 12S 13S 22S 23S)-4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(7, 8′, 17, 18′, 27, 28′)-hexamethoxy-(6, 9: 16, 19: 26, 29)-trietheno-(2H, 3H, 12H, 13H, 22H, 23H)-hexahydro-(30)-annulene-5. 2,5-Diformyl-1,4-dimethoxybenzene **3** (0.2 g, 1 mmol) in dichloromethane (1.5 ml) was added to a solution of (1*S*, 2*S*)-diaminocyclohexane **1** (0.11 g, 1 mmol) in dichloromethane (1 ml). The mixture was stirred for 3 h at room temperature, the solvent was evaporated in vacuum, and the crude compound recrystallized from ethyl acetate to give the title compound **5** as white needles (0.75 g, 90%); m.p. > 200°C; $[\alpha]_D^{25} - 442.4°$ (*c* 0.2, CH_2Cl_2, 1-dm); IR ν_{max}(Nujol)/cm^{-1} 1630 (C═N); ^{1}H-NMR (270 MHz; $CDCl_3$) δ_H 8.40 (6H, s, N═C*H*), 7.23 (6H, s, Ar–*H*), 3.64 (12H, s, –OCH_3), 3.35 (6H, s, C*H*–N), 1.22–1.91 (24H, m, –CH_2); ^{13}C-NMR (68.7 MHz; $CDCl_3$) δ_C 155.6 (H*C*═N), 132.1 (C_{ar}–OCH_3), 123.8 (C_{ar}–CH═N), 100.5 (C_{ar}–H), 82.3 (R_2*C*H-N═CH); 46.8 (O–*C*H_3), 29.1 (s, N–CH–*C*H_2), 13.4 (s, C–*C*H_2–C); MS (LSIMS) *m/z* 817.4 (M^++H, 100%); CHN requires for $C_{48}H_{60}N_6O_6$: C 70.5%, H 7.4%, N 10.3%. Found: C 63.7%, H 7.7%, N 8.3% (**5**+EtOAc + 3H_2O).

(2S, 3S, 12S, 13S, 22S, 23S)-1, 4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(6, 9: 16,19: 26, 29)-trietheno-(1H, 2H, 3H, 4H, 5H, 10H, 11H, 12H, 13H, 14H, 15H, 20H, 21H, 22H, 23H, 24H, 25H, 30H)-octadecahydro-(30)-annulene

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all-S-6. Solid $NaBH_4$ (0.1 g, 2.4 mM) was added slowly to a solution of compound **4** (190 mg, 2.3 mmol) in 10 ml of 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 recrystallized from toluene giving the title product *all-S-***6** as white needles (164 mg, 85%); IR υ_{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.

(2R, 3R, 12R, 13R, 22R, 23R)-1, 4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(7, 8′, 17, 18′, 27, 28′)-trietheno-(1H, 2H, 3H, 4H, 11H, 12H, 13H, 14H, 21H, 22H, 23H, 24H)-duodecahydro-(5D, 10D, 15D, 20D, 25D, 30D)-hexadeutero (30)-annulene *d*^{6}-all-R-6. Using the above procedure 190 mg of *all-R-***4** and 0.1 g of $NaBD_4$ gave the title compound **d**^{6}-*all-R-***6** (79%) as white needles; IR υ_{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%).

(2S, 3S, 12S, 13S, 22S, 23S)-1, 4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(7, 8′, 17, 18′, 27, 28′)-trietheno-(1H, 2H, 3H, 4H, 11H, 12H, 13H, 14H, 21H, 22H, 23H, 24H)-duodecahydro-(5D, 10D, 15D, 20D, 25D, 30D)-hexadeutero (30)-annulene *d*^{6}-all-S-6. Using the above procedure 190 mg of *all-R-***4** and 0.1 g of $NaBD_4$ gave the title compound **d**^{6}-*all-*S-**6** as white needles; IR υ_{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%).

(2S, 3S, 12S, 13S, 22S, 23S)-1, 4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(7, 8′, 17, 18′, 27, 28′)-hexamethoxy-(6, 9: 16, 19: 26, 29)-trietheno-(1H, 2H, 3H, 4H, 5H, 10H, 11H, 12H, 13H, 14H, 15H, 21H, 22H, 23H, 24H, 25H, 30H)-octadecahydro-(30)-annulene-all-S-7. To a stirred solution of compound **5** (0.127 g, 0.15 mmol) in THF:MeOH (1:1, 3 ml) gradually was added solid $NaBH_4$ (0.05 g, 1.2 mmol), and the solution was stirred for 2 h at room temperature. After removal of solvents under vacuum, the residue was extracted with CH_2Cl_2 (3×15 ml) and 10 ml water, the organic extracts were combined, dried over $MgSO_4$, filtered and the solvent removed under vacuum. Recrystallization from toluene gave the title compound *all-S-***7** as a white powder (0.1 g, 80%); m.p. 165°C; $[\alpha]_D^{25} + 217.1°$ (*c* 0.2, CH_2Cl_2, 1-dm); IR ν_{max}(Nujol)/cm^{-1}:1460, 1378; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 6.74, (6H, s, Ar–*H*), 3.81 (12H, d, *J* 12.6 Hz, CH_AH_BN), 3.59 (18H, s, OCH_3), 3.56 (12H, AB doublet, *J* 12.6, CH_AH_BN), 2.12–1.81 (36H, m, C*H*–N, CH_2 and N*H*); ^{13}C NMR (67.5 MHz; $CDCl_3$) δ_C 151.6, 128.0, 112.8, 60.8, 55.0, 45.8, 31.2, 25.1; MS (LSIMS) *m/z* 829.3 (M^+, 100%); CHN requires for $C_{48}H_{72}N_6O_6$: C 69.5%, H 8.7%, N 10.0%. Found: C 69.4%, H 8.7%, N 9.5%.

(2S, 3S, 12S, 13S, 22S, 23S)-1, 4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(7, 8′, 17, 18′, 27, 28′)-hexamethoxy-(6, 9: 16, 19: 26, 29)-trietheno-(1H, 2H, 3H, 4H, 11H, 12H, 13H, 14H, 21H, 22H, 23H, 24H)-duodecahydro-(5D, 10D, 15D, 20D, 25D, 30D)-hexadeutero-(30)-annulene-*d*^{6}-all-S-7. Using the above procedure 85 mg (0.1 mmol) of **5** and 4 mg of $NaBD_4$ gave the title compound d^6-*all-*S-**7** as a white powder (68 mg, 80%); m.p. 166°C; $[\alpha]_D^{25} + 218.2°$ (*c* 0.2, CH_2Cl_2, 1-dm); IR ν_{max}(Nujol)/cm^{-1}:1460, 1378; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 6.74, (6H, s, Ar–*H*), 3.59 (18H, s, OCH_3), 3.56 (6H, s, CH_ADN), 2.12–1.81 (36H, m, C*H*–N, CH_2 and N*H*); ^{2}H NMR (76.7 MHz; $CDCl_3$) δ_D 3.8 (s, br); ^{13}C NMR (67.5 MHz; $CDCl_3$) δ_C 151.6, 128.0, 112.8, 60.8, 55.0, 45.8, 31.2, 25.1; MS (LSIMS) *m/z* 835.4 (M^+, 100%).

(2R, 3R, 12R, 13R, 22R, 23R)-1, 4, 11, 14, 21, 24-Hexa-aza-(2, 3: 12, 13: 22, 23)-tributano-(7, 8′, 17, 18′, 27, 28′)-hexamethoxy-(6, 9: 16, 19: 26, 29)-trietheno-(1H, 2H, 3H, 4H, 11H, 12H, 13H, 14H, 21H, 22H, 23H, 24H)-duodecahydro-(5D, 10D, 15D, 20D, 25D, 30D)-hexadeutero-(30)-annulene-*d*^{6}-all-R-7. Using the above procedure 85 mg (0.1 mmol) of **5** and 4 mg of $NaBD_4$ gave the title compound **d**^{6}-*all-R-***7** as a white powder (68 mg g, 80%); m.p. 163°C; $[\alpha]_D^{25} - 219.8°$ (*c* 0.2, CH_2Cl_2, 1-dm); IR ν_{max}(Nujol)/cm^{-1}:1460, 1378; ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 6.74, (6H, s, Ar–*H*), 3.59 (18H, s, OCH_3), 3.56 (6H, s, CH_ADN), 2.12–1.81 (36H, m, C*H*–N, CH_2 and N*H*); ^{2}H NMR (76.7 MHz; $CDCl_3$) δ_D 3.8 (s, br); ^{13}C NMR (67.5 MHz; $CDCl_3$) δ_C 151.6, 128.0, 112.8, 60.8, 55.0, 45.8, 31.2, 25.1; MS (LSIMS) *m/z* 835.4 (M^+, 100%).

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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 0.01 mM in MeOH of all components.

For mass spectra of the macrocycles and dimeric cluster ions, a tune file was created using the Excalibur autotune function using a solution of 10 μM of **3** in MeOH. The tune files were used throughout. Cone temperatures were changed manually leaving all other tune parameters constant.

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