Cyclic aromatic oligoamides as highly selective receptors for the guanidinium ion†

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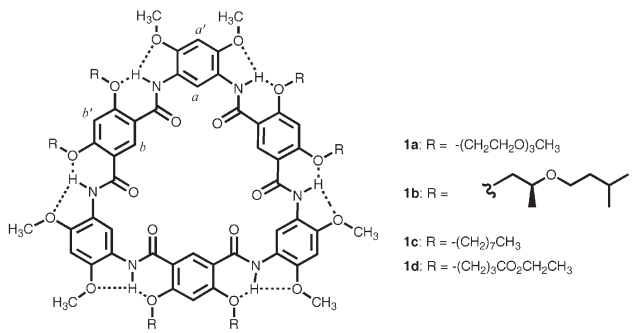
A class of six-residue, shape-persistent aromatic oligoamide macrocycles bind the guanidinium ion with very high selectivity.

Recognition of guanidinium and alkylguanidinium cations has attracted interest due to the involvement of the arginine residue in many biological recognition processes and control mechanisms.[1] Arginine is crucial to the functioning of nucleic acid-binding proteins[2–4] and enzymes.[5] It also serves as the precursor for the biosynthesis of nitric oxide.[6] Unlike most inorganic cations, the guanidinium ion is much larger and has a flat, non-spherical shape with six hydrogen bond (H-bond) donors. Therefore, recognition of this flat cation will be most effective using hosts with H-bond acceptors placed in the same plane. Elegant systems of guanidinium/arginine receptors have been developed by Bell[7] and Schrader.[8] Other examples of guanidinium/arginine recognition include those described by Dougherty,[9] Lehn,[10] Reinhoudt,[11] and Shinkai.[12] In this paper we would like to describe a class of extremely selective receptors for the guanidinium ion.

We recently reported the one-step preparation of a class of shape-persistent, six-residue aromatic oligoamide macrocycles shown by the general structure **1**.[13] These macrocycles have a well-defined internal cavity with six inward pointing amide oxygen atoms. A simple molecular modelling study† suggested that the guanidinium ion should fit snugly into the cavity of these macrocycles by forming six strong (short) H-bonds.



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Indeed, MALDI experiments revealed binding of guanidinium ion by **1a**. The MALDI spectrum of macrocycle **1a** alone revealed a strong peak at *m*/*z* 1890.6, corresponding to the complex of **1a** with Na^+ in the matrix.† Upon mixing **1a** with one equivalent of guanidinium hydrochloride, a new peak appeared at *m*/*z* 1926.1 (Fig. 1a), corresponding to the 1:1 complex (**1a**·**G**) between **1a** and the guanidinium cation (**G**). The original **1a**·Na^+ peak became minuscule in the presence of the guanidinium ion. To test the selectivity of **1a**, competition experiments were performed by mixing guanidinium hydrochloride (1 equiv.) with LiCl, NaCl, KCl, RbCl, CsCl, and NH_4Cl (1 equiv. each). Fig. 1b shows that, in the presence of these other salts, the **1a**·**G** peak was still the dominant one. It is particularly interesting that the ammonium ion did not compete with the guanidinium ion for binding with **1a** at all, which is in contrast to many other guanidinium receptors. ESI experiments on macrocycles **1** and guanidinium thiocyanate gave

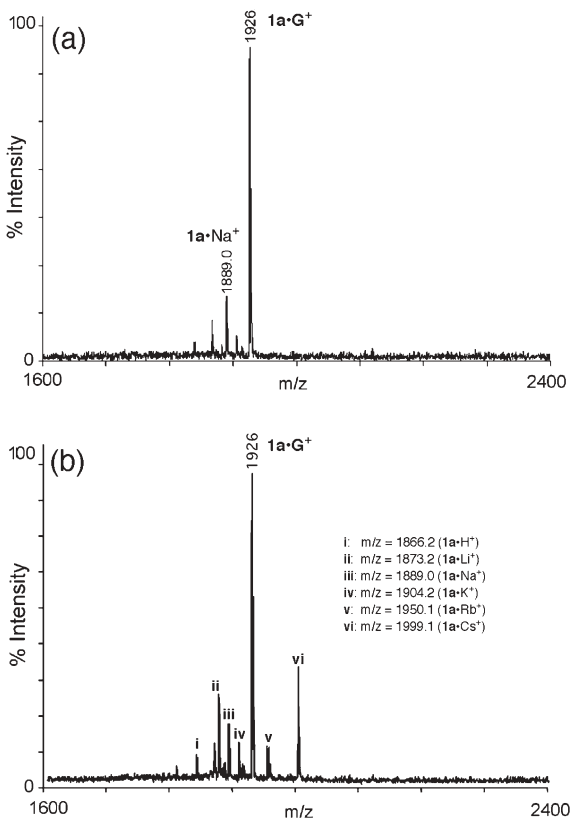

Fig. 1 MALDI-TOF spectra of (a) **1a** and guanidinium hydrochloride (**G**) (1:1), and (b) **1a** and 1 equivalent of each of guanidine hydrochloride (**G**), LiCl, NaCl, KCl, RbCl, CsCl, and NH_4Cl.

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† Electronic supplementary information (ESI) available: Experimental procedures; NMR, MALDI, and ESI spectra. Modelled structure of macrocycle **1**. See http://dx.doi.org/10.1039/b504411a

nearly identical results to those from the MALDI experiments,† suggesting that selective complexation of the guanidinium ion is indeed an inherent property of **1**. In fact, the selective formation of a 1:1 complex of the guanidinium ion with any one of macrocycles **1a–d** was a general phenomenon that was independent of the side chains of **1**. This is consistent with the binding of the guanidinium ion in the internal cavity of these macrocycles.

MALDI experiments indicated that the complexation of guanidinium ion was a slow process. It was found that the mixture of macrocycle **1a** and one equivalent of guanidinium tetraphenylborate in chloroform containing 10% methanol, if measured immediately after sample preparation, gave spectra showing complexation to both the guanidinium and sodium ions. However, if the measurement was delayed for one hour after sample preparation, the **1a**·**G** peak became the dominant one.†

The slow binding process hampered the determination of the association constant of complex **1a**·**G**. ^{1}H NMR titration experiments led to data points that were not interpretable. Attempts to determine the binding strength using isothermal titration calorimetry (ITC) did not succeed either. Nevertheless, the high binding strength of **1a**·**G** was clearly demonstrated by measuring ^{1}H NMR spectra in solvents of increasing polarity. For example, in acetone-d_6, the ^{1}H NMR spectrum of the 1:1 mixture of **1a** and guanidinium thiocyanate showed significant shifts for the signals of guanidinium NH_2 (>0.5 ppm), and aromatic protons *a* (~−0.2 ppm) and *b* (~0.9 ppm) when compared to the spectra of the free guanidinium salt and **1a** in the same solvent.† The solvent-dependent behavior of guanidinium and aromatic protons were then systematically examined in $CDCl_3$ containing CH_3OH. When both **1a** and **G** were present, the ^{1}H NMR signals of the guanidinium NH_2 and aromatic protons *a* and *b* mostly appeared at downfield positions as compared to the corresponding signals of free guanidinium ion and **1a** (Fig. 2). The shifts of guanidinium signals in the presence and absence of **1a** showed two opposite trends: with an increasing proportion of methanol, the NH_2 signal of free guanidinium ion showed large downfield shifts, while that of the guanidinium ion in the presence of **1a** (1 equiv.) showed small upfield shifts (Fig. 2a). The guanidinium ion also affected the behavior of the aromatic protons *a* and *b* (Fig. 2b and 2c). As the polarity of the mixed solvent increased, both protons *a* and *b* shifted downfield in the presence of guanidinium salt (~1 equiv.). In the absence of the guanidinium ion, these two aromatic protons showed small upfield shifts with increasing solvent polarity. These results suggest that the guanidinium and aromatic protons of the free and bound forms of **1a** and **G** were placed under two very different environments. That such differences between the free and bound forms of **1a** and **G** were still observed in up to 50% methanol[14] in $CDCl_3$ is consistent with binding of the guanidinium cation to **1a** in very polar environment.

To pinpoint the binding site, a two-dimensional (NOESY) NMR spectrum of **1b**·**G** was recorded in acetone-d_6. As shown in Fig. 3, the observed NOE between the guanidinium NH_2 signal and the interior aromatic protons *a* has provided convincing evidence for the binding of the guanidinium ion in the internal cavity of **1b**. Surprisingly, no NOE was detected between the guanidinium protons and the interior aromatic protons *b*. This suggests the possibility of a non-planar conformation for macrocycle **1b** when binding guanidinium ion. In such a conformation, protons *b* are not in the same plane shared by the

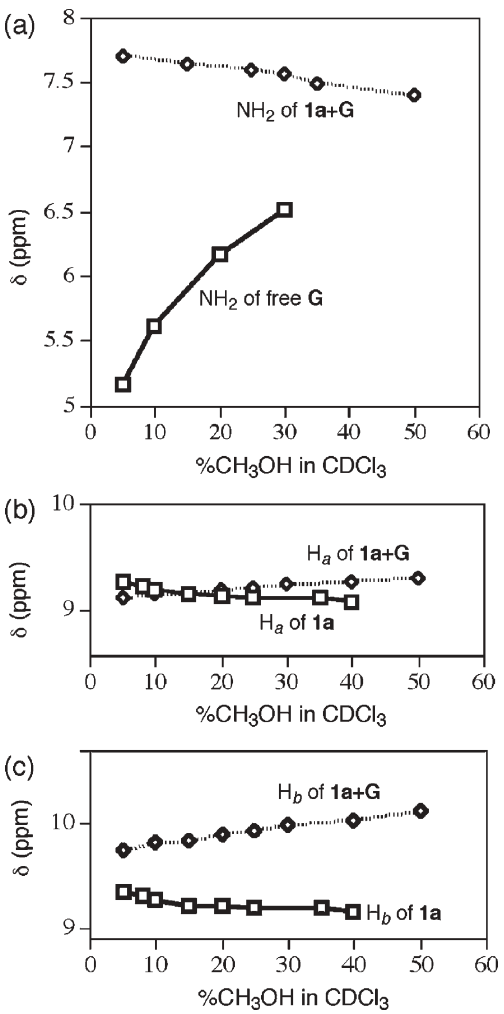

Fig. 2 Effects of varying proportions of methanol in chloroform-d_6 on the chemical shifts of (a) the protons of the free (□) and bound (◇) guanidinium ions, (b) the aromatic protons *a* of **1a** in the presence (◇) and absence (□) of the guanidinium ion, (c) the aromatic protons *b* of **1a** in the presence (◇) and absence (□) of the guanidinium ion.

guanidinium protons, protons *a* and the amide oxygens, and are probably exposed to solvent molecules. Such a conformation is consistent with the above-observed solvent-dependent shifts of protons *b*. Confirming this hypothetical conformation has to wait for the determination of the crystal structures of the complexes between macrocycles **1** and the guanidinium ion.

Initial results from MALDI experiments showed that macrocycle **1a** also selectively bound the octylguanidinium ion.† However, results from MALDI measurement in the presence of both guanidinium and octylguanidinium ions showed that **1a** slightly favored the guanidinium ion.† This is reasonable because the guanidinium ion can be fully accommodated by the cavity of **1a** while the octylguanidinium ion, with its hydrocarbon tail, is not completely compatible with the cavity. One interesting possibility is that the rigid cavities of macrocycles **1**, along with the six well-positioned amide oxygen atoms, could effectively compete with solvent molecules for hydrogen bonding, leading to stable **1**·**G** complexes in highly polar solvents such as methanol and water. This possibility will be probed with macrocycles that are fully soluble in these polar solvents.

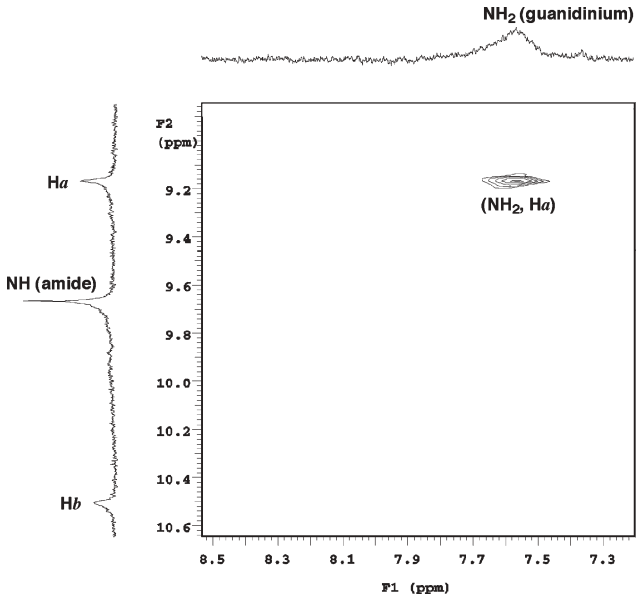

Fig. 3 The NOE between the guanidinium (NH_2) protons and the aromatic protons *a* of **1b** as revealed by the NOESY spectrum of the 1:1 mixture of **1b** and guanidinium thiocyanate (500 MHz; 2 mM in acetone-d_6; 283 K; mixing time: 0.4 s).

In summary, macrocycles **1** showed very high selectivity toward the guanidinium ion. This high selectivity is not surprising giving the persistent and rigid shape of the macrocycles. The cavity, with its well-positioned amide oxygens, fits the guanidinium ion by nearly perfect matching of H-bond donors and acceptors, which easily distinguish other cations from the guanidinium ion. In contrast, no other cations can fit well into the shape-persistent cavity to result in effective binding. With their ready availability and more importantly, their highly selective binding of guanidinium and octylguanidinium ions, these macrocycles should provide a convenient platform for designing specific receptors of other alkylguanidinium ions, arginine, and arginine-containing peptides and proteins.

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14 The ^{1}H NMR signals became too broad to allow meaningful assignment with greater than 50% methanol.

