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COMMUNICATION

A polyoxapolyaza macrobicyclic receptor for the recognition of zwitterions†

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A polyoxapolyaza heteroditopic macrobicyclic compound ($btpN_4O_3$) was synthesized. The acid–base behaviour of the compound as well as its binding ability for zwitterionic amino acids were studied by potentiometry at 298.2 ± 0.1 K in H_2O–MeOH (50 : 50 v/v) and at I = 0.10 ± 0.01 M in NMe_4TsO. The $H_nbtpN_4O_3^{n+}$ showed preference for amino acids containing tetrahedral anionic groups.

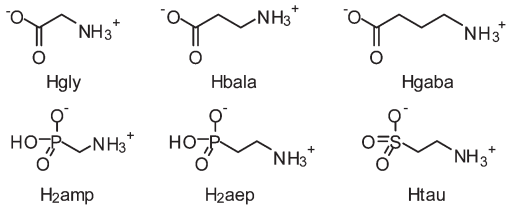
Amino acids are very important anion containing substrates, not only for their use as building blocks of proteins but also for their role in the metabolism, neurotransmission and biosynthesis.[1] Thus, the design of artificial receptors for the selective recognition and sensing of amino acids in aqueous solutions may have important applications. Consequently, over the last thirty years supramolecular chemists have strived to design receptors selective for amino acids.[2] However, this task has proven extremely challenging due to the intrinsic characteristics of amino acids. The zwitterionic nature of the amino acids at physiological pH requires both the existence of a cation and an anion binding site in the receptor, which from a synthetic point of view is not a trivial task. With this in mind, much work in the area has focused on the recognition of derivatized amino acids in which either the carboxylate or the ammonium group is protected.[3] Moreover, strong binding of zwitterions in aqueous solutions is hampered by the high energetic cost of desolvation of the double ion. Indeed, few receptors were conceived to work in aqueous solutions.[4]

Very recently two heteroditopic hemicryptophanes were shown to be able to encapsulate zwitterionic amino acids in up to 20% water in MeCN medium through a combination of hydrogen bonding, cation–π and anion–π interactions.[5]

Another possible approach for binding zwitterionic species in more competitive aqueous solutions consists in developing heteroditopic macrobicyclic compounds in which the anion binding sites are positively charged ammonium groups and the cation binding sites are ether groups. Macrobicyclic compounds offer the possibility to encapsulate substrates within their well defined cavities and allow the placement of several binding units in strategic points of the molecule for multiple and cooperative binding.[6] It was shown that tris(2-aminoethyl)amine (tren) capped macrobicyclic polyammonium receptors strongly bind anions in aqueous media by combining electrostatic and hydrogen bonding interactions,[6c,d] and they were particularly useful in the binding of carboxylates.[4e] In addition, it was recently demonstrated that a new family of benzene capped polyammonium cryptands are also useful for the binding of anions in aqueous solutions.[7] On the other hand, triethanolamine capped and benzene capped macrobicyclic receptors have been shown to bind ammonium,[8] amino alcohols and amino acids.[9] Thus it was intended to join these two types of compounds to produce "hybrid" polyoxapolyaza cryptands which were believed to be suitable for the recognition of the amino acids depicted in Scheme 1.

Mixed polyoxapolyaza macrobicyclic receptors have been first prepared by Bharadwaj *et al.*[10] and only recently their ability to bind anions has been evaluated.[11] However the mixed anion–cation encapsulation has never been attempted in this class of molecules, perhaps due to their relatively small cavities. Herein a new mixed polyoxapolyaza macrobicyclic compound is described, with a larger cavity than the ones previously reported by Bharadwaj *et al.*, and its ability to bind zwitterionic amino acids in a highly competitive aqueous solution is evaluated.

The synthesis of heteroditopic macrobicyclic compounds requires two critical steps: formation of a tripodal intermediate and macrobicyclization through a [1 + 1] "tripod–tripod



Scheme 1 Target zwitterionic substrates.

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†Electronic supplementary information (ESI) available: Experimental details; NMR (^1H, ^{13}C, COSY, NOESY, and HMQC), ESI-mass spectra of $btpN_4O_3$; table with the protonation constants of $btpN_4O_3$ and of the amino acids; table with overall and stepwise association constants (log $\beta_{H_nL_rA_a}$). Crystal data of $btpN_4O_3$ together with the refinement details are also given. CCDC 877715. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25725d

coupling" strategy.[12] The synthetic strategy requires a bifunctional building block in which one of the functionalities is protected while the other is available to react and produce tripodal compounds that are the precursors of the desired heteroditopic macrobicyclic receptors. We have previously reported the use of this strategy and 4-(diethoxymethyl)benzaldehyde as the bifunctional building block to prepare a polyaza macrobicyclic compound analogous to $btpN_4O_3$ in 79% yield.[13] $btpN_4O_3$, being a mixed polyoxapolyaza macrobicyclic compound, required the use of (4-diethoxymethylphenyl)methanol as the bifunctional building block and a slightly different synthetic procedure, as outlined in Scheme 2.

The (4-diethoxymethylphenyl)methanol **(2)** has an acetal-protected aldehyde group and an OH group available to react with 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene **(1)** in a Williamson ether synthesis procedure. After deprotection of the aldehyde groups the trialdehyde tripodal intermediate **(3)** was obtained and reacted with tren in a Schiff-base condensation cyclization step.

Surprisingly, with the same reaction conditions used to prepare the polyaza analogue (reagents at 5.6 mM in MeCN at r.t.) only polymeric materials were obtained in the attempted synthesis of $btpN_4O_3$. Since the only difference in the synthetic strategy is the use of a 2,4,6-triethylbenzene derived tripodal intermediate in the synthesis of $btpN_4O_3$ instead of a tren derived one in the synthesis of the polyaza analogue, it seems that the cyclization reaction requires a certain degree of flexibility of the tripodal intermediate. Indeed it has been pointed out that the rigidity of the 2,4,6-triethylbenzene scaffold requires the use of suitably preorganized spacers, namely meta-substituted aromatic units, in the synthesis of macrobicyclic compounds.[7b,14] This is why there are fewer 2,4,6-triethylbenzene derived macrobicyclic compounds reported in the literature when compared with the tren derived ones. In order to circumvent this problem the cyclization was tried in refluxing MeCN and in ten times more diluted conditions. With this method $btpN_4O_3$ was obtained after sodium borohydride reduction in 40% yield.

The single crystal X-ray diffraction determination of $btpN_4O_3$ revealed that one acetonitrile molecule is hosted in the macrobicyclic cavity as shown in Fig. 1. The three independent N–H···N hydrogen bonds between the acetonitrile and secondary amine binding groups of $btpN_4O_3$ are established with N···N distances of 3.27(2), 3.30(2) and 3.40(2) Å and the N–H···N corresponding angles of 167.4(14)°, 162.8(15)° and 160.1(14)°, respectively (for crystal data and refinement details, see Table S1 of ESI†).

The protonation constants of $btpN_4O_3$ were determined by potentiometry in H_2O–MeOH (50 : 50 v/v) solution at 298.2 K and ionic strength 0.10 M in NMe_4TsO. The results are collected in Table S2 in the ESI† and the corresponding species distribution diagram represented in Fig. 2.

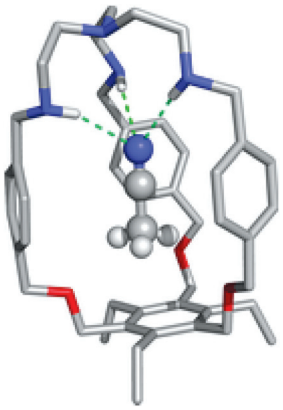


Fig. 1 X-ray single crystal structure of $btpN_4O_3$ showing an MeCN solvent molecule encapsulated in the macrobicyclic binding pocket. Hydrogen bonds are shown as dashed lines and the C–H hydrogen atoms of the receptor, apart from the N–H binding sites, have been omitted for clarity.

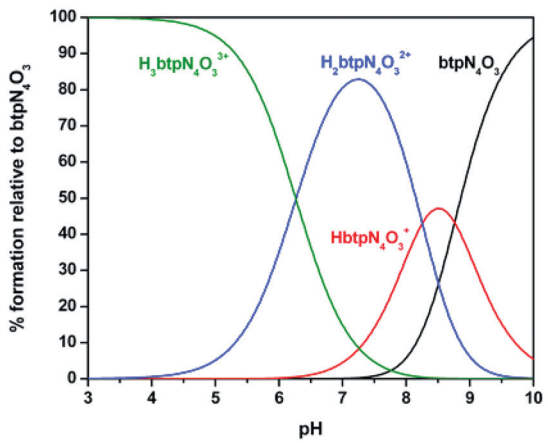
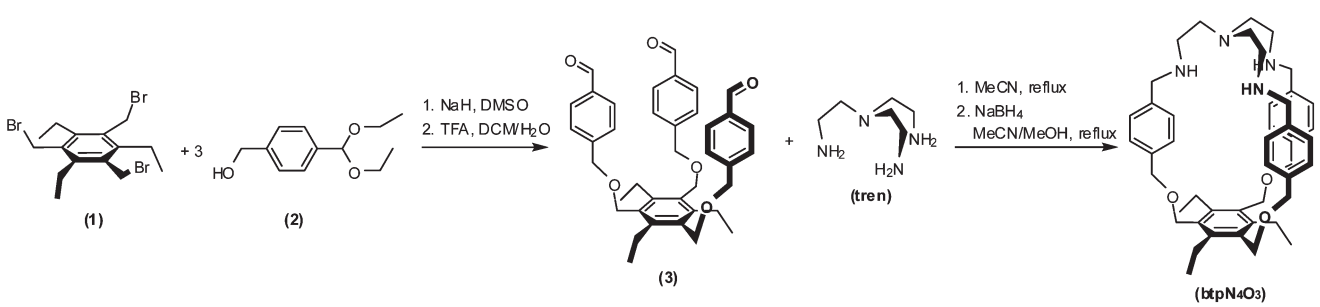


Fig. 2 Species distribution diagram of the protonation of $btpN_4O_3$ in H_2O–MeOH (50 : 50 v/v) solution at 298.2 K and 0.10 M NMe_4TsO. $C_{btpN_4O_3} = 1.0 \times 10^{-3}$ M.



Scheme 2 Synthetic procedure of $bptN_4O_3$.

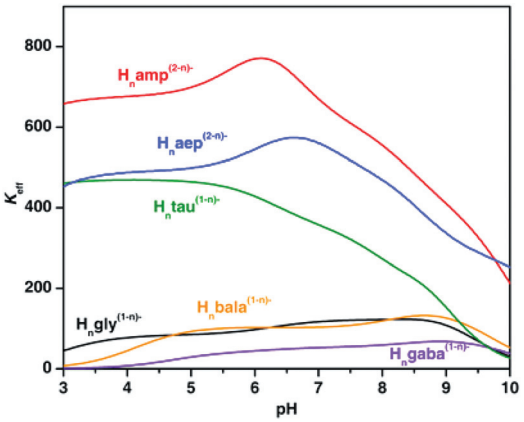


Fig. 3 Plot of K_{eff} *versus* pH for the associations formed between the indicated amino acids and $H_nbtpN_4O_3^{n+}$, in H_2O–MeOH (50 : 50 v/v) solution at 298.2 K and 0.10 M NMe_4TsO.

The acid–base behaviour of $btpN_4O_3$ is straightforward: three protonation constants were found in the working pH region (3.0–10.0), corresponding to the successive protonations of the secondary amines. The tertiary amine is very acidic due to the nearby presence of three positive charges, hence its protonation constant cannot be obtained in the working pH region. As shown in Fig. 2, the fully protonated form of the receptor, $H_3btpN_4O_3^{3+}$, exists as the main species below pH ≈ 6.2. This receptor species exhibits a compartment with three protonated amines to interact with the anionic group of the amino acids through electrostatic interactions and hydrogen bonding. The other compartment has three ether oxygen atoms available to accept hydrogen bonds from the ammonium group of the substrates and an aromatic unit which may contribute to the establishment of cation–π interactions.

The association constants of the protonated forms of $btpN_4O_3$ with several amino acids were determined by potentiometry in H_2O–MeOH (50 : 50 v/v) solution at 298.2 K and 0.10 M NMe_4TsO. The values obtained are collected in Table S3 of the ESI.†

Only species of 1 : 1 receptor to substrate stoichiometry were found for the different protonation states of the receptor. The protonated forms of $btpN_4O_3$ show relatively modest association constants for the binding of the studied amino acids, probably a consequence of the high energetic cost of desolvation of the substrates and of the close proximity of the anionic and cationic functionalities, which mutually attenuates their respective charges, thus lowering the effectiveness of charged receptors.[15] It is also possible that the ammonium binding sites of the receptor cause some repulsion on the ammonium group of the substrates, disfavouring the movement of the amino acids towards the receptor. In fact in the few examples of zwitterionic binding by synthetic receptors in water[4b] or mixed MeOH–H_2O,[4a–d] the reported association constants (K) are in the range 17–360. These values are lower than the ones obtained in this work.

The plot of K_{eff} *versus* pH[16] for the association of $H_nbtpN_4O_3^{n+}$ with the amino acid substrates is shown in Fig. 3. For pH values below 6.2, where the receptor is mainly in the triprotonated form and the substrates are all in their zwitterionic form, the selectivity trend for the studied amino acids is $H_2amp > H_2aep \approx Htau > Hbala \approx Hgly > Hgaba$ (Fig. 3). Interestingly, the receptor has lower affinity for amino acids containing a carboxylate group than for amino acids with a tetrahedral anionic group. This suggests that the latter are preferred due to the 3-fold symmetry of their anionic group which should be complementary to the tren subunit of the cryptand[17] and possibly due to the formation of a higher number of hydrogen bonds.

In conclusion, a polyoxapolyaza heteroditopic macrobicyclic compound was synthesized through a [1 + 1] "tripod–tripod coupling" strategy to be used as a receptor for the recognition of amino acids. The binding studies showed that the protonated receptor binds the zwitterionic amino acid substrates through a combination of hydrogen bonding, electrostatic interactions and possibly cation–π interactions with association constants in the range of 1.63–3.21 log units, the highest values in comparison with reported ones determined under identical experimental conditions (H_2O–MeOH solutions). The receptor showed a preference for amino acids containing a tetrahedral anionic group due to the 3-fold symmetry of their anionic moiety, complementary to the tren subunit of the cryptand.

Acknowledgements

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Notes and references

1 J. M. Berg, J. L. Tymoczko and L. Stryer, *Biochemistry*, W. H. Freeman & Co Ltd, New York, New York, 5th edn, 2002.
2 (*a*) J. W. Steed and J. L. Atwood, *Supramolecular Chemistry*, John Wiley and Sons, Ltd, Hoboken, New Jersey, 2nd edn, 2009; (*b*) L. Mutihac, J. H. Lee, J. S. Kim and J. Vicens, *Chem. Soc. Rev.*, 2011, **40**, 2777–2796; (*c*) Y. Zhouab and J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 52–67.
3 J. L. Sessler and A. Andrievsky, *Chem.–Eur. J.*, 1998, **4**, 159–167, and references therein.
4 (*a*) F. P. Schmidtchen, *J. Org. Chem.*, 1986, **51**, 5161–5168; (*b*) E. Kimura, H. Fujioka and M. Kodama, *J. Chem. Soc., Chem. Commun.*, 1986, 1158–1159; (*c*) A. P. de Silva, H. Q. N. Gunaratne, C. McVeigh, G. E. M. Maguire, P. R. S. Maxwell and E. O'Hanlon, *Chem. Commun.*, 1996, 2191–2192; (*d*) S.-i. Sasaki, A. Hashizume, D. Citterio, E. Fujii and K. Suzuki, *Tetrahedron Lett.*, 2002, **43**, 7243–7245; (*e*) R. J. Fitzmaurice, G. M. Kyne, D. Douheret and J. D. Kilburn, *J. Chem. Soc., Perkin Trans. 1*, 2002, 841–864.
5 (*a*) O. Perraud, V. Robert, A. Martinez and J.-P. Dutasta, *Chem.–Eur. J.*, 2011, **17**, 13405–13408; (*b*) O. Perraud, V. Robert, H. Gornitzka, A. Martinez and J.-P. Dutasta, *Angew. Chem., Int. Ed.*, 2012, **51**, 504–508.
6 (*a*) S. O. Kang, J. M. Llinares, V. W. Day and K. Bowman-James, *Chem. Soc. Rev.*, 2010, **39**, 3980–4003; (*b*) P. Mateus, N. Bernier and R. Delgado, *Coord. Chem. Rev.*, 2010, **254**, 1726–1747; (*c*) S. O. Kang, M. A. Hossain and K. Bowman-James, *Coord. Chem. Rev.*, 2006, **250**, 3038–3052; (*d*) V. McKee, J. Nelson and R. M. Town, *Chem. Soc. Rev.*, 2003, **32**, 309–325; (*e*) C. Seel and F. Vögtle, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 528–549.
7 (*a*) P. Mateus, R. Delgado, P. Brandão and V. Félix, *J. Org. Chem.*, 2009, **74**, 8638–8646; (*b*) P. Mateus, R. Delgado, P. Brandão, S. Carvalho and

V. Félix, *Org. Biomol. Chem.*, 2009, **7**, 4661–4637; (*c*) M. Arunachalam, I. Ravikumar and P. Ghosh, *J. Org. Chem.*, 2008, **73**, 9144–9147.
8 (*a*) R. Méric, J.-P. Vigneron and J.-M. Lehn, *J. Chem. Soc., Chem. Commun.*, 1993, 129–131; (*b*) B. Dietrich, J.-P. Kintzinger, J.-M. Lehn, B. Metz and A. Zahidi, *J. Phys. Chem.*, 1987, **91**, 6600–6606; (*c*) S. Y. Jon, J. Kim, M. Kim, S.-H. Park, W. S. Jeon, J. Heo and K. Kim, *Angew. Chem., Int. Ed.*, 2001, **40**, 2116–2119.
9 H.-J. Buschmann, E. Schollmeyer and L. Mutihac, *Thermochim. Acta*, 1998, **316**, 189–192.
10 (*a*) D. K. Chand and P. K. Bharadwaj, *Inorg. Chem.*, 1996, **35**, 3380–3387; (*b*) D. K. Chand and P. K. Bharadwaj, *Tetrahedron*, 1997, **53**, 10517–10522; (*c*) P. Ghosh, S. S. Gupta and P. K. Bharadwaj, *J. Chem. Soc., Dalton Trans.*, 1997, 935–938.
11 (*a*) M. C. Das and P. K. Bharadwaj, *Eur. J. Inorg. Chem.*, 2007, 1229–1232; (*b*) M. C. Das, S. K. Ghosh and P. K. Bharadwaj, *Dalton Trans.*, 2009, 6496–6506; (*c*) M. C. Das, S. K. Ghosh and P. K. Bharadwaj, *CrystEngComm*, 2010, **12**, 413–419; (*d*) M. C. Das, S. K. Ghosh, S. Sen and P. K. Bharadwaj, *CrystEngComm*, 2010, **12**, 2967–2974.
12 B. Dietrich, M. W. Hosseini, J.-M. Lehn and R. B. Sessions, *Helv. Chim. Acta*, 1985, **68**, 289–299.
13 P. Mateus, R. Delgado, P. Brandão and V. Félix, *Chem.–Eur. J.*, 2011, **1**, 7020–7031.
14 N. De Rycke, J. Marrot, F. Couty and O. R. P. David, *Tetrahedron Lett.*, 2010, **51**, 6521–6525.
15 J. Rebek, B. Askew, D. Nemeth and K. Parris, *J. Am. Chem. Soc.*, 1987, **109**, 2432–2434.
16 M. T. Albelda, M. A. Bernardo, E. García-España, M. L. Godino-Salido, S. V. Luis, M. J. Melo, F. Pina and C. Soriano, *J. Chem. Soc., Perkin Trans. 2*, 1999, 2545–2549.
17 C. Bazzicalupi, A. Bencini, A. Bianchi, A. Danesi, C. Giorgi and B. Valtancoli, *Inorg. Chem.*, 2009, **48**, 2391–2398.

