Pergamon

Tetrahedron Letters 42 (2001) 2751–2753

TETRAHEDRON LETTERS

Aggregation behaviour and binding properties of an L-lysine appended glycoluril receptor

Beatriu Escuder,* Alan E. Rowan, Martinus C. Feiters and Roeland J. M. Nolte

Department of Organic Chemistry, University of Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, The Netherlands

Received 29 January 2001; revised 1 February 2001; accepted 20 February 2001

Abstract—A new L-lysine appended receptor based on diphenylglycoluril is described. This compound self-assembles to form well-defined tubules in chloroform and vesicles in water. The host molecule strongly binds Magneson both in chloroform and in water, and L-dopa in water mimicking an adrenergic receptor.

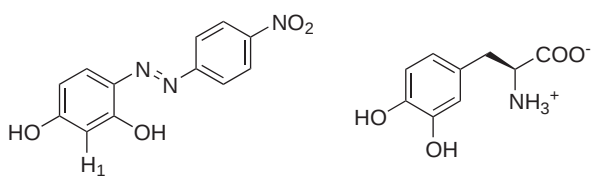
Cell-surface receptors embedded in biomembranes play an important role in the recognition and signalling of biologically relevant molecules, like hormones and neurotransmitters amongst others. Artificial receptors are capable of mimicking this function, which can lead to a better understanding of the natural processes and additionally to the development of new artificial systems for use in drug delivery, catalysis, etc.[1] Diphenylglycoluril derived receptors have been extensively studied in the past decade. These clip-shaped molecules are efficient receptors for phenolic guests (e.g. resorcinol), which are bound via H-bonding, π–π interactions and by a cavity effect. Crown-ether functionalized clip molecules are also able to bind alkaline metal ions, as well as ammonium salts,[2] in addition to complexing neutral aromatic guests.

Here, we describe the synthesis and the aggregation behaviour of a diphenylglycoluril based receptor with N^{α}-Boc-L-lysine arms (**2**) and the recognition of Magneson (**3**) and L-dopa (**4**) (Fig. 1) by this host. The attachment of amino acid residues, capable of additional H-bonding and electrostatic interactions, introduces an extra feature with potential improvement in the binding of multifunctional guests.

Compound **2** was prepared in 62% yield from the diphenylglycoluril tetrachloride derivative **1** and N^{α}-Boc-L-lysine using Finkelstein conditions, as shown in Scheme 1.[3] ^{1}H NMR studies on solutions of **2** in $CDCl_3$ showed the presence of broad peaks, in contrast to the sharp resonances observed for solutions of the previously reported *N*-functionalized hosts of this type.[2] This broadening is a result of strong aggregation of the molecules in this solvent.

This aggregation was further confirmed by transmission electron microscopy. As can be seen in Fig. 2A inset, compound **2** self-assembles to form well-defined thin tube-like assemblies with diameters of ca. 50 Å. After a few hours, these tubular structures further self-organized to give a flat array of aligned and superimposed layers of tubes (see Fig. 2A).

In contrast to the previously reported receptors,[2] compound **2** contains amino acid arms which are potential sites for additional interactions. This feature is thought to be responsible for the observed aggregation behaviour because intermolecular hydrogen bonds can now be formed between the molecules of **2** leading to well-defined structures. The amino acid arms also introduce the possibility of recognizing multifunctional guests, e.g. hydroxyaromatic amino acids and amino alcohols. For example, L-dopa (**4**) is not soluble in chloroform but in the presence of 1 equiv. of compound **2** it is fully solubilized and can be observed by

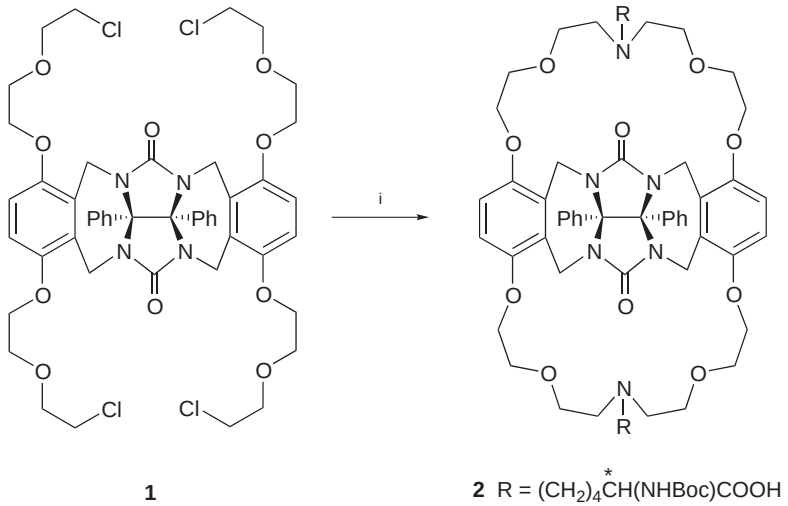


3 4

Figure 1.

* Corresponding author. Present address: Dep. Química Inorgànica i Orgànica, Universitat Jaume I, 12080 Castelló, Spain. E-mail: escuder@qio.uji.es

0040-4039/01/$ - see front matter
PII: S0040-4039(01)00297-0



Scheme 1. (i) N^{α}-Boc-L-lysine (3 equiv.), Na_2CO_3, NaI, CH_3CN, reflux, 5 days, 62%.

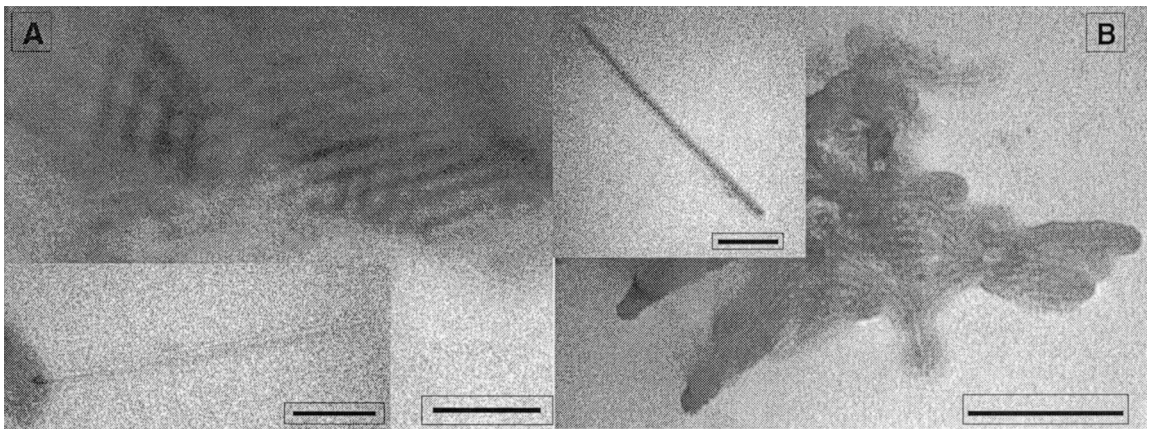


Figure 2. (A) Transmission electron micrographs of compound **2** in chloroform (Pt-shadowing, bars represent 100 nm). (B) *idem*, after addition of L-dopa (**4**) (Pt-shadowing, bars represent 200 nm).

^{1}H NMR. Transmission electron microscopy studies were carried out on a mixture of host molecule **2** and L-dopa (**4**). As can be seen in Fig. 2B inset, similar tubes were formed as described above, the difference being that the diameters of the aggregates had increased from 50 Å to ca. 120 Å. This phenomenon can be ascribed to the binding of L-dopa by the host molecules. After several hours, the rigid tube-like architectures became more flexible and self-organized to give lamellar structures (Fig. 2B).

The binding of Magneson (**3**) in host molecule **2** was studied in $CHCl_3$ by UV–vis titration and an association constant of $K_{ass}=(2.2\pm0.6)\times10^4$ M^{-1} was measured. This value is one order of magnitude higher than the values reported before for the binding of Magneson with other basket-shaped receptors.[2b] ^{1}H NMR studies on the host–guest complex in $CDCl_3$ revealed a shift in the resonances of the hydrogen atoms of Magneson and also a splitting of some of the signals of this guest due to slow exchange between bound and non-bound (H-bonded) guest molecules. NOESY experiments revealed NOE contacts between the guest H_1 proton (see Fig. 1) and the protons of the crown ether moieties and the aromatic walls of receptor **2**, confirming complexation within the cavity of the latter molecule.

In a subsequent series of experiments, the binding and aggregation behaviour of compound **2** in water was studied. A small amount of **2** was dissolved in 50 μL of MeOH and injected in water at 25°C to a final concentration of 1 mg/mL. After sonicating for 30 min at 40°C and standing for several hours at room temperature, transmission electron micrographs (not shown) were taken which showed the presence of vesicles with diameters ranging between 50 and 150 nm. The vesicular structure was confirmed by *cryo* scanning electron microscopy, as can be seen in Fig. 3.

Conductivity experiments were carried out in order to determine the critical aggregation concentration (CAC) of **2** in water at 25°C. On diluting an aqueous solution of this compound a change in the equivalent conductance was measured at ca. 10^{-6} M, indicating that at this concentration a change in the aggregation state of the molecules took place, i.e. from intermolecular H-

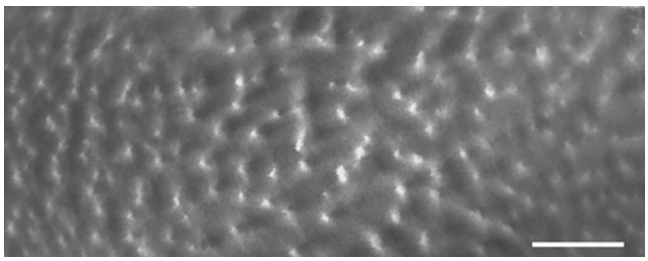


Figure 3. *Cryo* scanning electron micrograph of a dispersion of compound **2** in water (bar represents 1 μm).

bonded aggregates to monomers or smaller oligomers.[4] The binding of Magneson in host **2** was studied in water above this CAC. The binding constant was estimated by UV titration to be $(4.4\pm1.2)\times10^4$ M^{-1}. From the plot of the absorbance change versus the host concentration it was derived that the host–guest binding ratio was 1:0.5. This suggests that in the vesicles of **2** only half of the binding sites, viz. those at the outer surface, are accessible for the guest molecules and strongly supports the presence of well-defined vesicular aggregates.[2b]

The binding of adrenaline and related compounds by artificial receptors in water and in organic solvents has been studied in the literature with varying results.[5] We investigated the binding of L-dopa (**4**) in receptor molecule **2** in a buffered aqueous solution of pH 8 above the CAC by competition experiments using Magneson as a UV probe.[6] The binding constant was estimated to be $(4.3\pm1.5)\times10^3$ M^{-1}, which is one of the highest values reported for binding of this guest in water. The high affinity for L-dopa is a result of a combination of several molecular recognition interactions between the cavity and the lysine-functionalized tails with the substrate. This combination of multiple interactions is similar to the binding of these kind of substrates to the natural receptors.[7]

In conclusion, we have shown that molecular basket **2** displays a well-defined aggregation behaviour in water and in organic solvents. Moreover, it is demonstrated that this compound is a good receptor for a multifunctional phenolic guest, such as Magneson, and the pharmaceutically important guest L-dopa in water. These studies are currently being extended to other guests in water with the objective being to develop an adrenergic receptor mimic.

Acknowledgements

B.E. thanks the EC for a post-doctoral Marie Curie TMR grant.

References

1. (a) *Comprehensive Supramolecular Chemistry*. *Supramolecular Reactivity and Transport: Bioorganic Systems*; Murakami, Y., Ed.; Elsevier: Amsterdam, 1996; (b) Kikuchi, J.-I.; Murakami, Y. *J. Incl. Phen. Mol. Rec. Chem.* **1998**, *32*, 209–221; (c) Kikuchi, J.-I.; Ariga, K.; Ikeda, K. *Chem. Commun.* **1999**, 547–548.
2. (a) Rowan, A. E.; Elemans, J. A. A. W.; Nolte, R. J. M. *Acc. Chem. Res.* **1999**, *32*, 995–1006 and references cited therein; (b) Schenning, A. P. H. J.; Escuder, B.; van Nunen, J. L. M.; de Bruin, B.; Rowan, A. E.; van der Gaast, S. J.; Feiters, M. C.; Nolte, R. J. M. *J. Org. Chem.* **2001**, *66*, 1538–1547.
3. A mixture of compound **1** (4 g, 4.05 mmol), NaI (40 g, 0.27 mol) and Na_2CO_3 (13.3 g, 0.13 mols) in 500 mL of acetonitrile was refluxed under nitrogen for 4 h. Then, N^{α}-Boc-L-lysine (3 g, 12.2 mmol) was added in small portions over a period of 2 days and the mixture was refluxed for 1 week. After filtration and evaporation of the solvent, the crude white solid was suspended in $CHCl_3$ and washed with 10% citric acid and water. The organic layer was dried (Na_2SO_4) and concentrated under vacuum. After column chromatography (neutral alumina, eluent 0.1–0.5% MeOH/$CHCl_3$, v/v) pure **2** was obtained as a yellowish powder (3.35 g, 62%). Mp 128°C. $[\alpha]_D^{20}=+5.85$ ($c=0.65$, $CHCl_3$). ^1H NMR ($CDCl_3$): 1.2–2.0 (m+s, broad, 32H); 2.3–3.0 (m, 12H); 3.5–4.6 (m, 30H); 5.5 (m, 4H); 6.71 (m, 4H); 7.10 (s, broad, 10H). FAB-MS $m/z=1335.8$ $[M+H]^+$. Anal. calcd for $C_{70}H_{94}N_8O_{18}$: C, 62.95; H, 7.09; N, 8.39. Found: C, 62.71; H, 6.78; N, 8.17.
4. Evans, D. F.; Wennerström, H. *The Colloidal Domain. Where Physics, Chemistry, Biology and Technology Meet*; VCH: New York, 1994; p. 144 and references cited therein.
5. (a) Schrader, T. *J. Org. Chem.* **1998**, *63*, 264–272; (b) Herm, M.; Schrader, T. *Chem. Eur. J.* **2000**, *6*, 47–53 and references cited therein; (c) Lamarque, L.; Miranda, C.; Navarro, P.; Escartí, F.; García-España, E.; Latorre, J.; Ramírez, J. A. *Chem. Commun.* **2000**, 1337–1338.
6. Connors, K. A. *Binding Constants*; Wiley: New York, 1987. In a typical competition procedure, Magneson was added to the vesicles dispersed in an aqueous phosphate buffered solution (pH 8) and the resulting mixture was titrated with L-dopa following the change in the UV–vis absorption at 450 nm.
7. Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hilbert, M. *J. Med. Chem.* **1992**, *35*, 3448–3462.