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Porphyrin-Polyazacryptand Conjugates: Novel Receptors for Nucleotides

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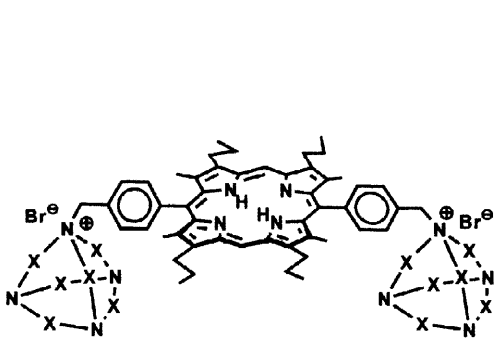
Abstract: The novel covalent conjugates **1** and **2** of porphyrins and tetraaza macrotricycles have been prepared. The compounds dissolve freely in aqueous buffer at neutral pH and bind nucleotides with good affinity ,but insignificant selectivity. Heterodimer formation with porphyrin carboxylic anions was detected in the gas phase corroborating the prediction of force-field calculations

Porphyrins constitute very popular building blocks in supramolecular chemistry due to their unique and well defined shape, their ready availability, their peculiar spectroscopic and physico-chemical properties, their versatile and predictable chemistry resting upon the extraordinary stability of the parent macrocycle and a rich variety of natural counterparts for comparison [1]. No wonder that these attractive features promoted their use as tectones in supramolecular assemblies [2], as biomimetic catalysts [3], as templates in peptide folding [4] or as ring expanded versions in the selective binding of anions [5] to mention just a few areas of recent activity. Of course, all these applications are subject to influences from the molecular environment, i.e. primarily from solvation effects. When studied in water, however, porphyrins tend to aggregate owing to their flat, plate-like structure thus aggravating and eventually obstructing meaningful quantitative investigations.



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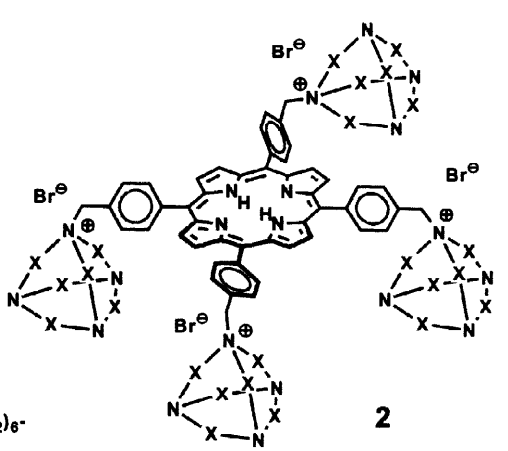
X = -(CH$_2$)$_6$-



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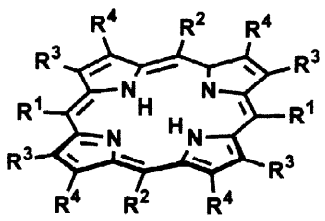
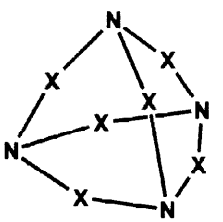
In an attempt to exploit the advantageous properties of the porphyrin nucleus in host-guest binding while circumventing its flaws we designed the conjugates **1** and **2** composed of readily accessible porphyrins to which tetraaza macrotricycles were attached at the meso positions. Under neutral conditions in water the aliphatic amino N-atoms are expected to be protonated giving the conjugate an extraordinarily high overall positive charge. Thus, the compounds are water soluble while the porphyrin core itself remains unprotonated [6] and presents a rather hydrophobic surface. In spite of the amphipatic character aggregation of this construct should be minor owing to the strong electrostatic repulsion and the symmetrical substitution pattern of the central moiety. The macrotricyclic units form cages under these conditions which have extensively been used in anion inclusion complexation [7, 8] and may in principle contribute to the observed guest anion binding selectivity on this basis. The prime reason for their incorporation into these conjugates, however, originated in their high charge density at physiological pH-values that emerges from the enforced connectivity of the mutually repulsive ammonium sites.



	R^1	R^2	R^3	R^4
3	p-$C_6H_4CH_2Br$	H	CH_3	C_3H_7
4	p-$C_6H_4CH_2Br$	H	H	H
5	p-$C_6H_4COO^{\ominus}$	H	CH_3	C_4H_9
6	p-$C_6H_4COO^{\ominus}$	H	H	H



7 X = -$(CH_2)_6$-

The synthesis started with the recently prepared porphyrin diphenyl- [9] and tetraphenyl [10] halomethyl derivatives **3** and **4** serving as alkylating agents in Menshutkin alkylations of the macrotricyclic amine **7** [11]. As already observed in earlier alkylations [12] the reaction conditions could be tuned to provide excellent yields of the monoquaternized products. Slow addition of solutions of **3** or **4** over 8 hours to a refluxing solution of the amine **7** in chloroform gave **1** and **2** in almost 90% yield.

Both conjugates qualified as artificial receptors for the biologically important nucleotide anions which possess a complementary architecture consisting in a hydrophilic but negatively charged sugar phosphate moiety connected to an electron-poor heteroaromatic base [13]. In buffered aqueous solution molecular complexes of 1:1 stoichiometry were formed and could be determined by UV titration at λ = 419-422 nm. Binding events happening in the vicinity of the porphyrin chromophor were quantified using the Benesi-Hildebrand method, and the association constants obtained are shown in tab.1. Encapsulation binding of the guest anions to the tetrahedral cavity moieties known to occur with considerably smaller affinity [7] are invisible to this method.

There is surprisingly little variation in the absolute binding affinity irrespective of the host compound or the nucleotide used. Though the state of ionization differs between –2 in the monophosphates and –4 in ATP

there is no observable correlation with K_{ass} indicating the minor importance of charge interactions. Instead, stacking interactions of the heteroaromatic moieties seem to dominate the host- guest relationship showing an unusual preference for the pyrimidine nucleotides over the purine analogs with host **1** . The overall guest selectivity is small though, supporting the view that both hosts bring about guest binding by providing an unscreened hydrophobic surface at the porphyrin domain. In accord, complexes of higher guest-host stoichiometries were observed at elevated guest concentrations.

Table 1: 1:1 Association constants K_{ass} [M^{-1}] in aqueous buffer solution (HEPES; pH 7.4) of **1** and **2** with nucleotides determined by UV titration using Benesi-Hildebrandt analysis.

	host **1**		host **2**	
substrate	K_{ass} [M^{-1}] $\times 10^5$	log K_{ass}	K_{ass} [M^{-1}] $\times 10^5$	log K_{ass}
AMP^{2-}	4.3	5.63	1.9	5.27
ADP^{3-}	9.95	6.00		
ATP^{4-}	3.0	5.48	4.0	5.61
GMP^{2-}	2.3	5.35	7.0	5.84
CMP^{2-}	10.5	6.02	4.8	5.68
TMP^{2-}	10.1	6.0	4.1	5.61
UMP^{2-}	5.3	5.72	2.8	5.45

The association of **1** and **2** with guest anions in the gas phase was directly observed in Laser-desorption-mass-spectra. Whereas the anions of terephthalic acid, benzene tetracarboxylic acid and naphthalene- or anthracene disulfonic acids failed to give molecular ions of complex species mixing of **1** with **5** or **2** with **6** yielded strong signals of the respective heterodimeric molecular complexes. Figure 1 depicts the energy-minimized structure [14] of the **5** ⊂ **1** associate, which corroborates the expected and plausible mutual orientation of these planar molecules.

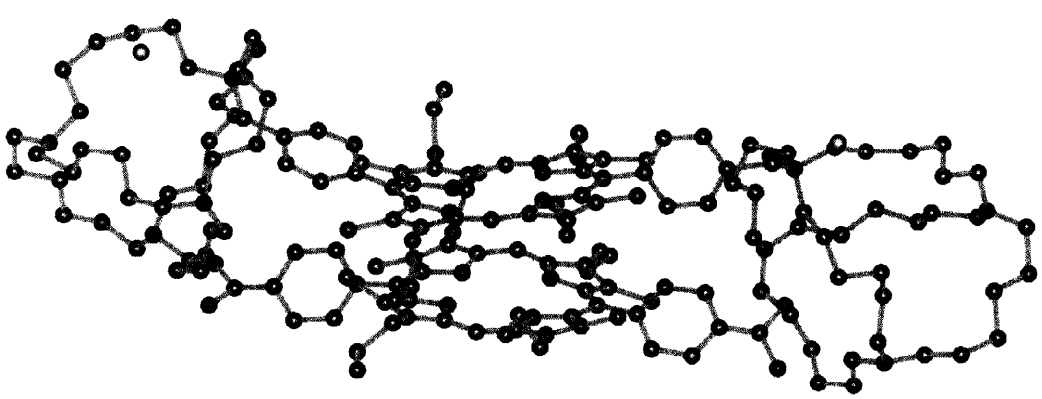


Figure 1: Energy minimized structure of the molecular complex of **1** (as the bis-hydrobromide salt) and **5** in the gas phase. The hydrogen atoms are omitted for clarity.

EXPERIMENTAL

General Methods

All solvents were purchased from commercial sources and used as received. The reactions and purities of compounds were monitored by TLC performed on precoated silica gel plates (Merck Kieselgel 60F $_{254}$ in chloroform-hexane 2:1 vol for starting bromomethylporphyrins **3** and **4** and in chloroform – methanol 10:1 up to 5:1 vol for the products **1** and **2**. ^{1}H NMR spectra were recorded on Bruker AC 250 and AM360 instruments with tetramethylsilane (TMS) as standard. Fast atom bombardment mass spectra (FAB-MS) were determined using a Varian VG70-250S double focusing magnetic sector mass spectrometer with an ionization potential of 70 eV. MALDI spectra were recorded with (1,8,9-trihydroxyanthracene – dithranol, Aldrich) and without matrix on a Biflex Bruker instrument. UV-VIS titration experiments were performed on a Cary 400 Varian Instrument.

General procedure for the preparation of the porphyrin-tetraamine conjugates 1 and 2.

The reactions were carried out by alkylation of the macrotricyclic amine 7 with porphyrins **3** and **4** . Slow addition of the *p*-bromomethyl derivatives **3** and **4** to a chloroform solution of amine **7** (1.1.molar equivalent of **7** for each bromomethyl group of porphyrin) was employed in order to prevent higher alkylation of the cage amine **7**.

Preparation of conjugate 1

5,15-Bis(1-azonia-8,15,22-triazatricyclo[13.13.6.6.^{8,22}]tetracontylmethylphen-4-yl)-3,7,13,17-tetramethyl-2,8,12,18-tetra-*n*-propylporphyrin bis-bromide (**1**)

The solution of 87.4 mg (0.1 mmol) of bis(4-bromomethylphenyl)porphyrin derivative 3 in 5 ml of chloroform was added under argon over a period of 3 hours to the solution of 123 mg (0.22 mmol) of amine **7** in 50 ml of chlorofom. After addition the reaction mixture was refluxed for another 24 hours. The reaction mixture was then evaporated to a volume of 10 ml and 5 ml of diethyl ether was added. The clean product **1** precipitated and was isolated by filtration in 95.5 % yield. : **^{1}H NMR** (360 MHz, $CDCl_3$): 10.20 (2H, s); 8.18 (4H, d); 7.95 (4H, d); 5.43 (4H, s); 3.92 (8H, bs); 3.53 (6H, bs); 2.42 (12H, s); 2.32 (18H, bs); 2.18 (8H, kv); 1.99 (6H, bs); 1.49 and 1.39 (42H, 2 bs); 1.24 (12H, t); -2.46 (2H, s).**^{13}C NMR** (90 MHz, $CDCl_3$): 14.37, 14.41, 26.34, 28.43, 33.53, 48.09, 48.16, -49.97m, , 105.09, 125.60, 136.18, 141.01, 143.96, 144.33, 159.22.

MALDI-TOF MS: $C_{122}H_{202}N_{12}Br_2$ *m/z* = 1914 $[M+H+Br]^+$, 1994 $[M+H+2Br]^+$, **electrospray MS**: *m/z* = 917.9 $[M+2H]^{2+}/2$).

Preparation of conjugate 2:

5,10,15,20-Tetrakis(1-azonia-8,15,22-triazatricyclo[13.13.6.6.^{8,22}]tetracontylmethylphen-4-yl)porphyrin tetrakis-bromide **(2)**

The solution of 98.6 mg (0.1 mmol) of tetrakis(*p*-bromomethyl)porphyrin derivative **4** in 5 ml of chloroform was slowly added under argon to the solution of 246 mg of amine **7** (4.4 molar equivalent) in 50 ml of chloroform under reflux. After addition the reaction mixture was refluxed for another 24 hours, the course of reaction was followed by TLC using dichloromethane-methanol 5:1vol for development. The reaction mixture was evaporated to dryness and the residue was redissolved in 10 ml of chlorofom-methanol 10:1 vol. A small amount of an insoluble byproduct was filtered off and 5 ml of diethyl ether was added slowly to the filtrate to precipitate the product which was isolated by filtration after 1 hour obtaining **2** in 88 % yield . **^{1}H NMR** (360 MHz, $CDCl_3$, 10 % CD_3OD) δ = 8.77 (8H, m,); 8.25 (8H, m,); 7.89 (8H, m,), 5.18 (8H, s,); 3.58 (12H, bs,); 2.25 (36H, s,); 1.96 (12H, bs,); 1.49 and 1.3 (84H, bs,); -2.20 (2H, s,). **MALDI-TOF MS** $C_{192}H_{322}N_{20}Br_4$ *m/z* = 3228 $[M+H+4Br]^+$, 1534 $[M+2Br]^{2+}/2$; 808 $[M+4H+4Br]^{4+}/4$; **electrospray MS**: *m/z* = 645 $[M+H]^{5+}/5$; 808 $(M+4H, 4Br)^{4+}/4$.

Complex formation of receptor 1 (M_1 = 1830) with **5** (M_5 = 775): **MALDI-TOF** *m/z* = 2605.95 $[M_1+M_2+H]^+$ 1506.79 $[(M_1+2Br) + M_2, TBA)]^{2+}/2$. Complexation of receptor **2** with porphyrin tetraacid **6**: **MALDI-TOF** *m/z* = 3696 $[M_2+ M_6 + H]^+$.

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14. MM+ forcefield calculation as implemented in the Hyperchem 3.0 molecular modelling package was used.