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Novel Porphyrin-Cholic Acid Conjugates as Receptors for Biologically Important Anions

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A series of novel receptors showing high binding affinity in aqueous media for biologically important anions are reported. These naturally chromophoric porphyrin-based receptors contain cholic acids connected via quaternary alkyl ammonium amido linkages.

INTRODUCTION

During the past few decades, supramolecular recognition has received a great deal of attention [1]. A current trend in anion sensor design has involved appending the host with a chromophore or fluorophore either covalently or non-covalently yielding impressive optical and fluorescent anion sensors.

Recently, it has been shown that various porphyrin conjugates can be used for anion [2–4] and saccharide sensing in protic media [5]. The novelty of the present receptors arises from the attachment of the recognition elements to the porphyrin chromophore through multifunctional spacers, providing H-bonding and coulombic binding sites, which enhances the interaction of the recognition sites with the porphyrin core. We believe this approach will greatly expand the utility of the porphyrin scaffold for the construction of receptors in general. The porphyrin platform is additionally ideal for receptor design because it presents a convergent surface that can be functionalized with a variety of recognition elements thus creating a binding pocket for anionic target guests. Our receptors were designed not only to recognize important biological anions, but also express cooperativity between the four arms on the porphyrin scaffold, each containing three hydroxy groups on steroidal concave face, with the amide and quaternized nitrogen functionality spacer, ultimately providing an efficient binding pocket.

In this communication we report the synthesis as well as NMR and mass spectral characterizations of novel quaternary alkyl ammonium porphyrin-cholic acid amides **1**–**4** (Fig. 1). These compounds have been prepared by synthetic protocol based on conversion of the corresponding bile acid to amide with dimethylaminoalkylamine, which was in turn converted to the porphyrin conjugate by alkylation with tetrakis(bromomethyl)porphyrin according to previously reported procedures [6–9].

RESULTS AND DISCUSSION

In order to characterize the conformations of **1,** ^{1}H NMR measurements were performed in different solvents. In tetradeuteriomethanol the resonances of the methyls 18 and 19 were split in two lines with a 3:1 intensity ratio and both were strongly shielded from the cholic acid without porphyrin moiety (Fig. 2). We interpret this spectral data to indicate that in methanol the hydrophobic sites of the cholic acid moieties are folded above the porphyrin ring, causing a strong ring current effect on the angular methyls of the steroidal nucleus. This finding further implies that the hydrophilic site of the steroid is directed toward the solvent. Thus, this conformation may be stabilized by both hydrophobic interactions between the hydrophobic site of the steroid and porphyrin, and by hydrogen bonding between the hydroxyl groups of the steroid and the solvent. However, owing to steric hindrance only one steroidal nucleus at a time is likely capable localizing near the porphyrin ring

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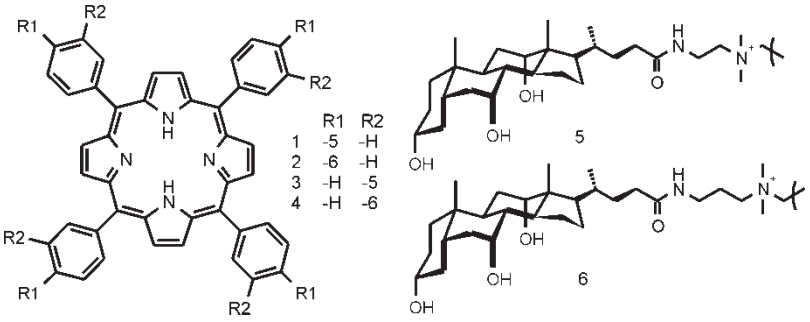

FIGURE 1 Structures of **1**–**4**.

system. In DMSO-d_6 the resonances of the angular methyls 18 and 19 are also shielded but are not split in two resolved signals. This suggests that in this latter solvent the dynamics of the steroidal moieties are faster than in methanol, resulting in time averaged chemical shifts. In order to better understand these phenomena, receptor **1** is currently being investigated with molecular modelling.

Receptors **1**–**4** were designed for complexation of biologically important substances, like oligosaccharides and nucleotides under physiological conditions. Here we present results for the efficacy and selectivity of receptors **1**–**4**, towards nucleotide phosphates as model analytes. Owing to the sensitivity limits of NMR, and mainly solubility problem of receptors in buffer for NMR concentration range, binding studies of ATP, ADP, and AMP with **1**–**4** were carried out using electronic (visible) spectroscopy.

Firstly, we examined dependence of UV/Vis spectra as a function of solvent, results are summarized on Fig. 3 showing aggregation for pure aqueous media. To preclude possible receptor self-aggregation of the receptor, which can be problematic with macrocyclic receptors, binding affinities were examined in solvent mixtures. UV/VIS and PLS methodologies revealed

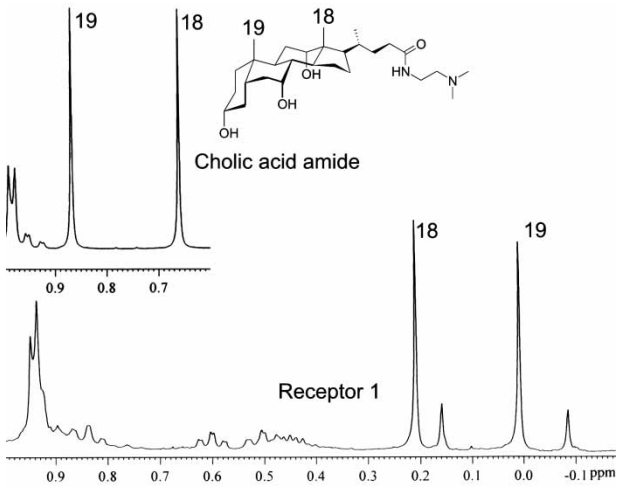

FIGURE 2 Above: ^{1}H NMR subspectrum of cholic acid dimethylaminoethyl amide. Below: ^{1}H NMR subspectrum of **1** in methanol-d_4. δ(Me-18)/ppm = 0.21 (9H) and 0.16 (3H). δ(Me-19)/ppm = 0.01 (9H) and − 0.09 (3H).

no aggregation at micromolar concentration ranges used in this study. Fig. 3 shows UV/VIS spectra of **1** in different solvents. 1 mM HEPES (*N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid)) buffer solution (aq, pH 7.2) was used to eliminate the pH effect.

UV/VIS titrations showed that all the tested substrates influenced the absorbance level of the receptors **1**–**4**. The systematic binding studies were performed by recording the absorbance at $\lambda_{max} = 420$ nm as a function of substrate/receptor, [G]/[H]. Binding constants (K_a) were calculated from the absorbance changes at the Soret maximum (ΔA) assuming a 1:1 stoichiometry for complexes (eqn. 1) [6].

$$\frac{1}{\Delta A} = \frac{1}{K_a[G](\Delta A)_\infty} + \frac{1}{\Delta A_\infty} \quad (1)$$

[G] is the nucleotide concentration, and $(\Delta A)_\infty$ is the extrapolated absorbance change where nucleotide concentration goes to infinity. Stoichiometries of the complexes were determined by Job's plot. Inorganic anions (chloride, nitrate, sulfate and bisphosphate), which have also been tested, did not show any binding with these receptors. Fig. 4 illustrates the absorbance of receptor **1** as a function of ATP:**1**-ratio.

Table I summarizes the log K_a values and stoichiometries of ATP, ADP and AMP with receptors **1**–**4**.

The lack of selectivity for selected biologically important anions is rationalized by data from Table I. Due to flexibility of receptors, not only desired 1:1 complex is formed, but also other type of complexes, like 2:1, with nearly the same efficacy. This tendency leads to the loss of selectivity. From mechanistic point of view, coulombic type of interaction is combined with simultaneous contribution from nucleobase for overall effect, probably based on π-π stacking with porphyrin nucleus leading to the observed behaviour.

Further information for the complex formation came from MS study. An ESI-TOF MS measurement of an equimolar mixture of receptor **1** and AMP in water:methanol 1:1 solution showed a triply-charged ion with an *m/z* value of 975.93 (in addition to some other ions, such as $[\mathbf{1} + 2\mathrm{Br}]^{2+}$, $[\mathbf{1} + \mathrm{Br}]^{3+}$, and $[\mathrm{M}]^{4+}$). The observed ion may correspond to an adduct formed by **1** and $^{-}OPO_2HOCH_2C_9$

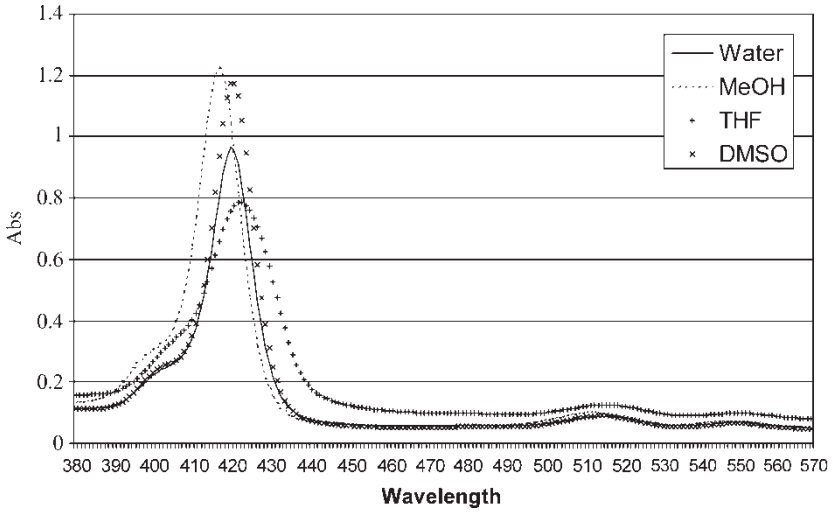

FIGURE 3 UV/VIS spectra of **1** in different solvents.

$H_{102}N_5O_3$. The partial ESI-TOF mass spectra of **1** as well as **1**:AMP are presented in Fig. 5.

In summary, we have demonstrated that novel quaternary alkyl ammonium porphyrin-cholic acid amides **1**–**4** exhibit strong binding, but poor selectivity toward ATP, ADP, and AMP. These preliminary findings inspire us to continue synthetic work toward preparing a series of more rigid and preorganized receptors exhibiting this same strong binding, and moreover selectivity for specific anion substrates. Additionally, these properties are currently being studied by molecular modelling.

EXPERIMENTAL

UV/Vis measurements were performed with Perkin Elmer Lambda 25 UV/VIS spectrometer. NMR experiments were run with Bruker Avance DRX 500 FT NMR spectrometer equipped with a z-gradient accessory and 5 mm diameter inverse detection probehead working at 500.13 MHz for proton. Mass spectrometric measurements were performed using Micromass LCT time of flight (TOF) mass spectrometer with electrospray ionization (ESI). Elemental analysis was performed using VarioEL III elemental analyzer. All reagents were purchased from Sigma-Aldrich or Fluka.

Receptors **1**–**4** were synthesized by following general procedure: Cholic acid amides were prepared by reacting cholic acid (7.3 mmol) with *N,N′*-diisopropylcarbodiimide (7.7 mmol) in $CHCl_3$ (100 mL) at room temperature with catalytic *N*-hydroxysuccinimide. After two hours, 2-dimethylaminoethylamine or 3-dimethylamino-1-propylamine (7.4 mmol) was added to the reaction mixture followed by pyridine (0.5 mL) (r.t. 2 days, yield 70–80%). The resulting cholic acid amide (120 μmol) was subsequently refluxed with the porphyrin derivative (20 μmol) (H_2TPP(*p*-BrCH_2)$_4$ or H_2TPP(*m*-BrCH_2)$_4$ prepared according to the

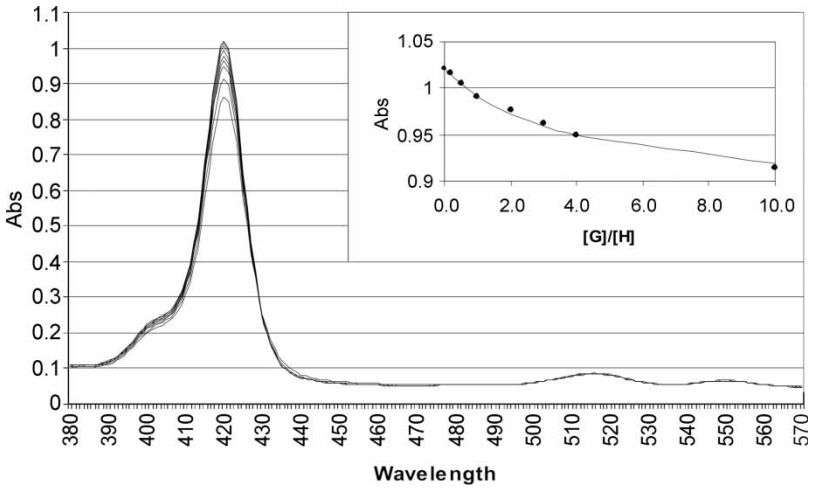

FIGURE 4 UV/VIS titration of **1** (2.3 μM) with ATP in 1 mM HEPES:MeOH (2:1). Insert: Dots denote measured data points and solid line is calculated curve. [G] is concentration of ATP and [H] is concentration of **1**.

TABLE I Log K_a values and stoichiometries of **1**–**4** with ATP, ADP, and AMP

Receptor	Nucleotide	Log K_a	Stoichiometry nucleotide:receptor
1	AMP	5.7	1:1
1	ADP	5.4	2:1
1	ATP	5.1	2:1
2	AMP	5.9	1:1
2	ADP	5.4	1:1
2	ATP	5.8	1:1
3	AMP	5.8	1:1
3	ADP	5.5	1:1
3	ATP	5.4	1:1
4	AMP	5.6	1:1
4	ADP	5.6	1:1
4	ATP	5.2	2:1

reference [8]) in $CHCl_3$ (30 mL) for 1 day giving desired products (yield 80–90%).

5,10,15,20-Tetrakis[*N*,*N*-dimethyl-*N*-(3α,7α,12α-trihydroxy-5β-cholan-24-oyl amidoethyl) ammonium-*p*-tolyl] porphyrin tetrabromide (1)

^{1}H NMR: δ(500 MHz, CD_3OD): −0.09 (3H, s), 0.01 (9H, s), 0.16 (3H, s), 0.21 (9H, s), 0.40–2.40 (118H), 2.85 (1H, s), 2.95 (3H, s), 3.16 (4H, m), 3.44 (24H, d), 3.65 (8H, m), 3.83 (4H, m), 3.92 (4H, m), 4.80 (12H, s), 5.01 (8H, s), 8.11 (8H, d), 8.39 (8 H, d), 8.95 (8H, br). MS (ESI-TOF): $m/z = 645$ $[M]^{4+}$, 887 $[M + Br]^{3+}$, 1371 $[M + 2Br]^{2+}$. M.W.($C_{160}H_{234}N_{12}O_{16}$) = 2581.72. Anal calcd. for $C_{160}H_{234}N_{12}O_{16}$: C, 66.24; H, 8.13; N, 5.79. Found C, 63.35; H, 7.76; N, 5.36.

5,10,15,20-Tetrakis[*N*,*N*-dimethyl-*N*-(3α,7α,12α-trihydroxy-5β-cholan-24-oyl amidopropyl) ammonium-*p*-tolyl] porphyrin tetrabromide (2)

^{1}H NMR: δ(500 MHz, CD_3OD): −1.15 (3H, d), −0.82 (9H, s), 0.02–0.27 (20H), 0.59–2.24 (106 H), 2.58 (2H, s), 3.22 (8H, m), 3.34 (4H, s), 3.40 (24H, d), 3.52 (12H, m), 4.52 (2H, s), 4.80 (12H, s), 4.98 (8H, m), 8.06 (8H, d), 8.38 (8H, d), 8.96 (8H, br). MS (ESI-TOF): $m/z = 659$ $[M]^{4+}$, 906 $[M + Br]^{3+}$, 1399 $[M + 2Br]^{2+}$. M.W.($C_{164}H_{242}N_{12}O_{16}$) = 2637.83. Anal calcd. for $C_{164}H_{242}N_{12}O_{16}$: C, 66.60; H, 8.24; N, 5.68. Found C, 64.45; H, 8.07; N, 5.65.

5,10,15,20-Tetrakis[*N*,*N*-dimethyl-*N*-(3α,7α,12α-trihydroxy-5β-cholan-24-oyl amidoethyl) ammonium-*m*-tolyl] porphyrin tetrabromide (3)

^{1}H NMR: δ(500 MHz, CD_3OD): −0.86 (3H, s), −0.30 (2H, s), −0.10 (2H, s), 0.02 (3H, d), 0.16 (2H, s), 0.28 (2H, d), 0.30–2.50 (133H), 3.37 (24H, s), 3.67 (8H, m), 3.82 (8H, m), 4.52 (3H, s), 4.81 (12H, s), 4.95 (8H, m), 8.02 (4H, m), 8.12 (4H, d), 8.45 (8H, m), 8.91 (8H, br). MS (ESI-TOF): $m/z = 645$ $[M]^{4+}$, 887 $[M + Br]^{3+}$, 1371 $[M + 2Br]^{2+}$. M.W.($C_{160}H_{234}N_{12}O_{16}$) = 2581.72.

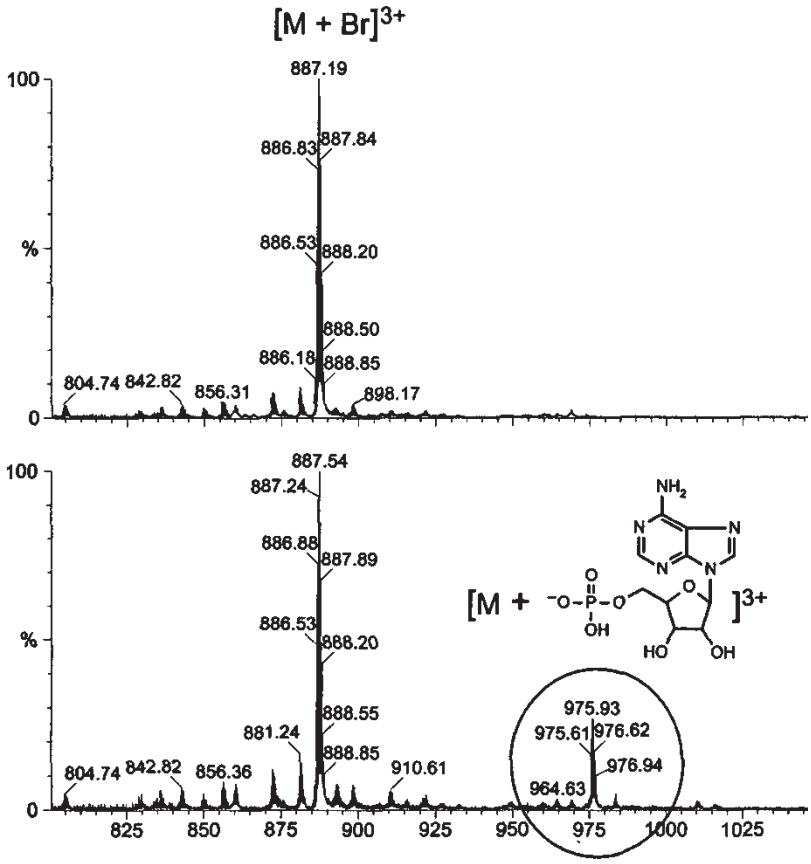

FIGURE 5 ESI-TOF MS subspectra of **1** (above) and **1** + AMP (below).

Anal calcd. for $C_{160}H_{234}N_{12}O_{16}$: C, 66.24; H, 8.13; N, 5.79. Found C, 65.82; H, 8.27; N, 5.24.

5,10,15,20-Tetrakis[*N,N*-dimethyl-*N*-(3α,7α,12α-trihydroxy-5β-cholan-24-oyl amidopropyl) ammonium-*m*-tolyl] porphyrin tetrabromide (4)

^{1}H NMR: δ(500 MHz, CD_3OD): −0.27 (2H, s), 0.00 (4H, d), 0.14 (2H, s), 0.15–3.20 (146H), 3.34 (24H, s), 3.60 (18H, m), 4.53 (2H, s), 4.81 (12H, s), 4.91 (8H, m), 8.00 (4H, m), 8.09 (4H, d), 8.42 (8H, m), 8.92 (8H, br). MS (ESI-TOF): $m/z = 659$ $[M]^{4+}$, 906 $[M + Br]^{3+}$, 1399 $[M + 2Br]^{2+}$. M.W.($C_{164}H_{242}N_{12}O_{16}$) = 2637.83. Anal calcd. for $C_{164}H_{242}N_{12}O_{16}$: C, 66.60; H, 8.24; N, 5.68. Found C, 64.72; H, 8.22; N, 5.52.

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