Molecular Recognition of Nucleosides, Nucleotides and Anionic Planar Substrates by a Water-soluble Bis-intercaland-type Receptor Molecule

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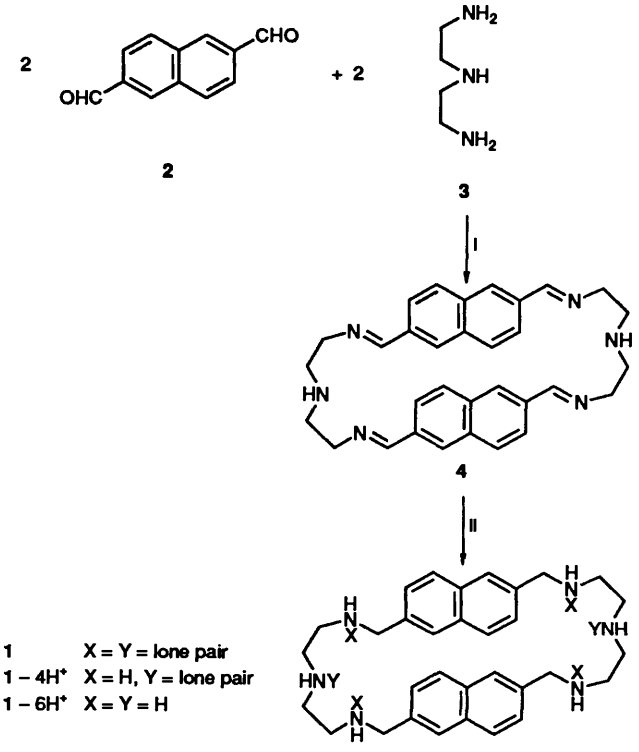
The water-soluble macrocyclic bis-intercaland receptor molecule **1** has been prepared *via* the tetraimine **4** obtained by multiple amine–aldehyde condensation. It has been found to complex strongly planar anionic substrates such as aromatic carboxylates and nucleotides. The corresponding association constants in aqueous solution have been determined by ^{1}H NMR and fluorescence measurements. The complexes present high stabilities and reveal interesting selectivities, in particular, among the nucleobases, with preferential binding of guanine-containing species.

Previous reports from our laboratory have described the synthesis and the binding properties of bis-intercaland-type macrocyclic receptor molecules.[1-3] By analogy to the fixation of intercalators between the plateaux of base pairs in double-stranded nucleic acids, these compounds contain two planar subunits of large area situated at a distance suitable for the intercalation of flat organic substrates. If the subunits possess photochemical or electrochemical properties the cyclo-intercaland receptor may be capable of performing photo- or electro-induced reactions on the bound substrate species.[4] Other types of intercaland receptor molecules have been studied recently [see for instance in ref. 2(*b*)]. We now report the preparation of the simple molecular receptor **1** and its ability to bind, in its protonated form, flat organic substrates in water.

Results and Discussion

Synthesis and Properties of Receptor **1**.—Compound **1** was isolated as its hexachlorhydrate **1**·6H$^+$ (72% yield) by $NaBH_4$ reduction of the tetraimine **4** (m.p. = 237–239 °C) (Scheme 1).



1 X = Y = lone pair
1-4H$^+$ X = H, Y = lone pair
1-6H$^+$ X = Y = H

Scheme 1 *Reagents:* i, CH_3CN, room temp.; ii, $NaBH_4$, CH_3OH, reflux, then water and HCl

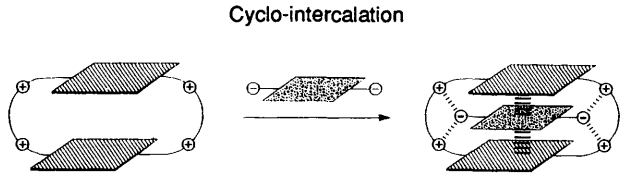


Fig. 1 Schematic representation of the cyclointercalation process for the binding of a dianionic substrate by a cyclo-bis-intercaland such as **1**·4H$^+$

The latter was readily obtained (80% yield) *via* the previously described[5,6] very efficient 2 + 2 condensation between diethylenetriamine (DIEN) and dialdehydes, here naphthalene-2,6-dicarbaldehyde[7] prepared from commercially available dimethyl naphthalene-2,6-dicarboxylate ($LiAlH_4$ reduction followed by Swern oxidation).[8] The mass spectrum indicates that the 2 + 2 compound **1** is obtained and not a 1 + 1 species, the formation of which is precluded by the relative sizes of the components and the rigidity of the naphthalene unit.

Compound **1** is soluble in acidic aqueous solution. Its pK_as were found to be 2.50, 2.85, 7.39, 8.12, 8.85, 9.47.[9] As in macrocycles studied earlier,[10] the pK_as of the central NH functions are very low and, under the conditions used for the binding studies (pH = 6, pyridine–CF_3CO_2D buffer), **1** is mainly in its tetraprotonated form **1**·4H$^+$.

The protonated receptor molecule **1** brings together two flat naphthalene subunits with two positively charged diethylenetriamine binding sites. It may thus be expected to complex molecular substrates in aqueous solution by a combination of hydrophobic (stacking) and electrostatic effects (Fig. 1). It delineates a flat-walled molecular cavity suitable for inclusion of planar organic species. The repulsive interactions between the positive charges of the protonated nitrogen atoms may be expected to prevent or at least to hinder the collapse of the cavity. On the other hand, the diethylenetriamine units are flexible enough to allow the cavity to adjust to substrate thickness for optimal stacking.

Substrate Binding by the Tetraprotonated Receptor **1**·4H$^+$.—In the presence of increasing amounts of protonated **1**, the ^{1}H NMR signals of various anionic substrates were found to undergo marked $\Delta\delta$ upfield shifts, indicating that complexation had occurred (Table 1). The stability constants of the complexes were determined by analysis of the $\Delta\delta = f[C]/[S]$ curves ([C] and [S] being the ligand and substrate concentrations,

† UPR 285 of the CNRS.

Table 1 Stability constants (log K_s) and limiting ^1H NMR upfield shifts ($\Delta\delta_{lim}$) calculated for the complexes of macrocyclic receptor molecule **1** with anionic and neutral molecular substrates

Substrate[a]	log K_s[b] (NMR)	$\Delta\delta_{lim}$[c]	log K_s[e] (fluorim.)
M^{2-}	3.5	245.1	
F^{2-}	4.4	488.9	
OP^{2-}	3.6	82.5	
MP^{2-}	5	387.7	
TP^{2-}	5.2	330.0	
A	2.2	230.3	
G	3.3	33.2	
C	1.6	233	
U	1.9	175.8	
AMP^{2-}	4.3	234.4	4.1
ADP^{3-}	5.1[d]	*d*	5.0
ATP^{4-}	5.2[d]	*d*	5.1
GMP^{2-}	4.9	49.8	4.6
CMP^{2-}	3.7	207.3	3.6
UMP^{2-}	4.1	124.8	3.8

[a] M^{2-}, maleate; F^{2-}, fumarate; OP^{2-}, orthophthalate; MP^{2-}, metaphthalate; TP^{2-}, terephthalate; A, adenosine; G, guanosine; C, cytidine; U, uridine; AMP^{2-}, GMP^{2-}, CMP^{2-}, UMP^{2-}: monophosphates of A, G, C, U respectively; ADP^{3-}, adenosine diphosphate; ATP^{4-}, adenosine triphosphate. [b] K_s and $\Delta\delta_{lim}$ are calculated from the plots of substrate chemical shifts as a function of the macrocycle: substrate ratio obtained by diluting, at *ca.* 23 °C, an aqueous mixture of **1** (2.5 mmol dm^{-3}) and the substrate (0.25 mmol dm^{-3}) with an aqueous solution of the substrate at the same concentration (0.25 mmol dm^{-3}). In this manner, the concentration of the substrate was kept constant (0.25 mmol dm^{-3}), while the concentration of **1** varied from 2.5 to 0.075 mmol dm^{-3}. The solutions were adjusted to pH 6 {[$^2H_5$]pyridine (0.01 mmol dm^{-3})–CF_3CO_2D buffer}. In order to cross-check the results, competition experiments were performed between different substrates; thus competition experiments (1) between F^{2-} taken as reference substrate and M^{2-}, TP^{2-}, AMP^{2-}, GMP^{2-}, UMP^{2-}, (2) between MP^{2-} as reference and TP^{2-}, (3) between AMP^{2-} as reference and GMP^{2-}, gave K_s values fully consistent with the direct method. All anionic substrates were used as sodium salts. [c] Calculated maximum upfield chemical shifts (in Hz at 200 MHz) induced by binding on the vinylic or aromatic or anomeric protons of the substrate. [d] These stability constants were too high to be accurately determined owing to limitations of the NMR method at high K_s values. [e] The fluorescence spectra (λ_{exc} = 300 nm) were obtained at *ca.* 23 °C by adding to an aqueous solution (A) of **1**, an aqueous mixture (B) of substrate and **1** in which **1** is at the same concentration as in (A). In these experiments the concentration of **1** remained constant (c = 0.01 mmol dm^{-3}) while the concentration of the substrate changed from 0.05 to 100 mmol dm^{-3}; the solutions were adjusted to pH 6 {[$^2H_5$] pyridine (0.01 mmol dm^{-3})–CF_3CO_2D buffer}.

respectively). The stoichiometry of the complexes formed was found to be 1 : 1 for all substrates, indicating that a well-defined species had been generated. The very large upfield shifts observed for the bound substrates agree with the formation of inclusion complexes, molecular cryptates, in which the bound species is contained in the molecular cavity and more or less inserted between the flat walls (see Fig. 1).

Both structural and electrostatic factors are expected to contribute to the stability and the selectivity of complexation. The stronger binding of fumarate as compared with maleate indicates that structural effects dominate purely electrostatic interactions which should favour maleate, the substrate of higher charge density (see also ref. 11). There is a remarkable structural selectivity in the benzenedicarboxylate series. The receptor **1** displays linear recognition among the three isomers and the very strong binding of the terephthalate anion TP^{2-} resulting from both electrostatic and hydrophobic effects, reveals significant complementarity between the receptor and the substrate. Compared with a related receptor[11] of ellipsoidal shape, the stability constant K_s is increased by a factor of six indicating better complementarity between the planar dianionic compound and the flat-faced receptor **1**.

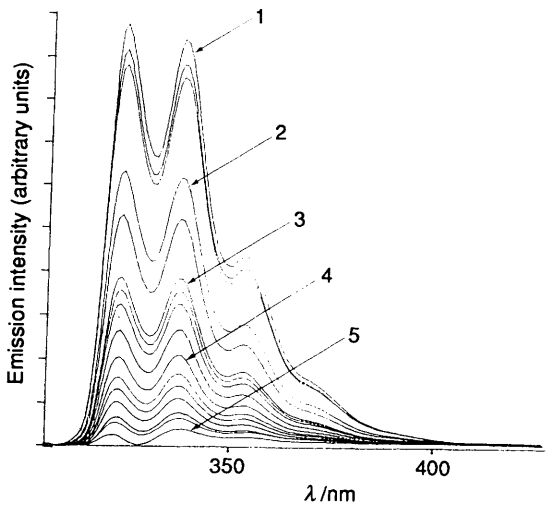


Fig. 2 Fluorimetric titration of **1** (10^{-5} mmol dm^{-3}) with UMP (see Table 1, footnote *e*): [UMP]/[**1**]: 1, 0; 2, 7.8; 3, 20.4; 4, 47.2; 5, 464

The complexation of nucleotides presents some interesting features. The NMR method gave an initial set of K_s values obtained at [S] = 0.25 mmol dm^{-3} (see Table 1, footnote *b*). But this method is not reliable for high K_s values and, besides, owing to overlapping of NMR signals, determining the NMR shifts of the substrate upon addition of **1** proved to be difficult and sometimes inaccurate. A second set of K_s values was obtained by fluorescent measurements at [C] = 0.01 mmol dm^{-3} (see Table 1, footnote *e*). Upon addition of the substrate the fluorescence intensity of **1** decreased steadily and reached a limiting value at high proportion of substrate (Fig. 2). Benesi–Hildebrand treatment of the data gave the stability constants K_s (Table 1). The results are in good agreement with those obtained by the NMR method. Owing to the influence of the lower concentration of substrate with respect to the buffer, these calculated values are slightly inferior to those obtained by the NMR method but the ratios of stability constants obtained by the two methods are fully consistent. The stability of the complexes increases with the number of negative charges in the substrate as seen for the strong binding of AMP^{2-}, ADP^{3-} and ATP^{4-} and for the complexation of the neutral substrates adenosine A, guanosine G, cytidine C and uridine U compared with their charged counterpart AMP^{2-}, GMP^{2-}, CMP^{2-} and UMP^{2-}. Thus, in contrast with the results obtained with a class of cyclointercalands studied earlier,[2b] electrostatic effects appear to play a major role in the present case. The selectivity of complexation of the doubly charged monophosphate derivatives AMP^{2-}, GMP^{2-}, CMP^{2-} and UMP^{2-} should be noted. The binding constants increase markedly with the size of the flat substrate, purine derivatives being more strongly complexed that pyrimidine ones. A significant selectivity is also observed within these two series of compounds: GMP^{2-} and UMP^{2-} being, respectively, more strongly complexed that AMP^{2-} and CMP^{2-}. Similarly, G is bound more strongly than A by an order of magnitude and U more strongly than C. These selectivities may be of interest for the design of reagents for the site-specific binding and modification of simple stranded nucleic acids.

The marked changes in fluorescence observed on binding also indicate that receptors such as **1** represent potential probes for the detection of nucleotides and their derivatives. Even larger and/or more selective effects might be obtained with cyclo-bis-intercalands containing other heterocyclic units such as acridine derivatives, *etc.*[12]

Conclusions

The tetraprotonated receptor molecule $\mathbf{1}\cdot 4H^+$ displays re-

markable binding stabilities and selectivities towards negatively charged and neutral organic substrates. The increase of binding strength with the size as well as with the charge of the substrates indicates that both stacking and electrostatic effects contribute to the stability of the complexes, *i.e.*, to molecular recognition by receptor **1**. Analogues of **1** with flat walls of larger area should give more stable and selective complexes. Furthermore the results obtained also point to the ability of receptor **1** to discriminate significantly between the nucleobases, a feature of interest for the exploration of site-selective binding of **1** to oligonucleotides and nucleic acids.

Experimental

General.—All commercially available chemicals employed were reagent grade and used without further purification. Melting points were determined on an Electrothermal digital melting-point apparatus. Proton NMR spectra were recorded on a Bruker AC 200 spectrometer. The microanalyses and the mass spectra were performed at the *Service Central de Microanalyse du CNRS*, Lyon.

2,5,8,19,22,25-*Hexaaza*[9,9](2,6)*naphthalenophane*-1,8,18,-25-*tetraene* **4**.—A solution of diethylenetriamine (DIEN) (645 mg, 6.2 mmol) in CH_3CN (250 cm^3) was added dropwise, under N_2, to a well-stirred solution of naphthalene-2,6-dicarbaldehyde (1.15 g, 6.2 mmol) in CH_3CN (250 cm^3). The reaction was stirred at room temp. for 36 h after which the deposited solid was filtered off and dried. This crude product was recrystallised from CH_2Cl_2 to give the title compound **4** (1.29 g, 82%), m.p. 237–239 °C; δ(200 MHz; $CDCl_3$; TMS) 3.09 (8 H, t), 7.32 (4 H, dd, J = 8.6 Hz), 7.61 (4 H, d, J = 8 Hz), 7.81 (4 H, s) and 8.44 (4 H, s); m/z (FAB^+) 503 (MH^+, 97%) (Found: C, 72.6; H, 7.0; N, 15.9; O, 4.5. $C_{32}H_{34}N_6 \cdot 1.5\,H_2O$ requires C, 73.2; H, 6.85; N, 15.7; O, 4.23%).

2,5,8,19,22,25-*Hexaaza*[9,9](2,6)*naphthalenophane* **1**·6H^+.—A solution of sodium borohydride (570 mg, 15 mmol) in absolute CH_3OH (10 cm^3) was added dropwise, at room temp. under N_2, to a solution of the tetraimine **4** (458 mg, 0.9 mmol) in absolute CH_3OH (20 cm^3). After 45 min of refluxing and cooling at room temp., water (3 cm^3) and aqueous NaOH solution (6 mol dm^{-3}; 0.5 cm^3) were added. The solvents were evaporated off under vacuum at 40 °C and the residue was dissolved in hot THF. After cooling a saturated solution of HCl in THF was added and the precipitate was filtered off and dried. It was dissolved in a minimum volume of THF–water mixture and diffusion of THF gave crystals of **1**·6HCl (334 mg; 72%). **1** δ(200 MHz; D_2O; pH = 6; Bu^{t}OH) 1.81 (8 H, m), 2.09 (8 H, m), 3.12 (8 H, s), 6.18 (4 H, d, J = 9 Hz), 6.42 (4 H, d, J = 9 Hz) and 6.63 (4 H, s); m/z (FAB^+) 511.6 (MH^+, 42%) (Found: C, 50.9; H, 6.6; N, 10.9; Cl, 27.6; O, 4.2. $C_{32}H_{42}N_6$·6HCl·2H_2O requires C, 50.19; H, 6.80; N, 10.98; Cl, 27.84; O, 4.01).

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