Binding of Nucleosides, Nucleotides and Anionic Planar Substrates by Bis-Intercaland Receptor Molecules

Sylvain Claude, Jean-Marie Lehn,* Frédéric Schmidt and Jean-Pierre Vigneron

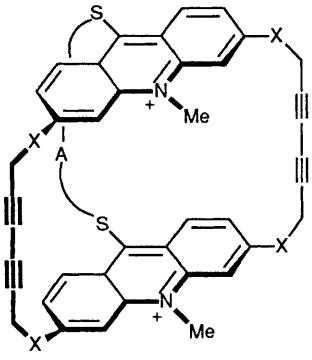
Chimie des Interactions Moléculaires, Collège de France, 11, Place Marcelin Berthelot, 75005 Paris, France†

The macrobicyclic and acyclic bis-intercaland receptor molecules **1**–**4** complex strongly, *via* stacking effects, planar anionic, as well as neutral substrates, such as nucleosides, nucleotides and aromatic carboxylates.

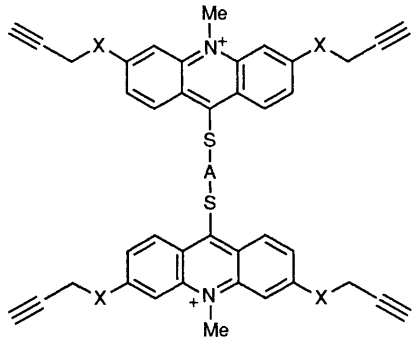
The design of receptor molecules capable of binding flat, organic substrates is of particular interest in view of the importance of compounds containing aromatic and heterocyclic groups in chemistry as well as in biology.

An approach to such binding agents rests on the development of molecules containing two flat residues of sufficient area, positioned at a distance suitable for the intercalation of planar substrates, in a manner reminiscent of the fixation of intercalators between the plateaus of base pairs in double-stranded polynucleotides and nucleic acids.[1,2] More or less rigid, acyclic and macrocyclic bis-intercalands have been

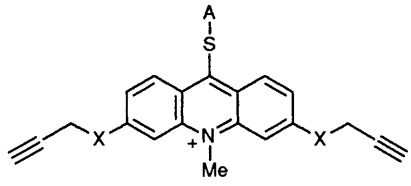
† ER 285 of the CNRS.



1a^{2+} X = O, A = — $(CH_2)_6$—
1b^{2+} X = O, A = — $CH_2CH_2OCH_2CH_2$—
1c^{2+} X = O, A = — $CH_2CH_2OCH_2CH_2OCH_2CH_2$—
2^{2+} X = NH, A = — $CH_2CH_2OCH_2CH_2$—



3a^{2+} X = O
3b^{2+} X = O
3c^{2+} X = O
4^{2+} X = NX



5^{+} X = O, A = — CH_2CH_2Me
6^{+} X = NH, A = — CH_2CH_2Me

synthesized[3–10] and the binding of planar species has been described. Thus, for instance, neutral receptors complex neutral substrates in organic solvents [3d,4,9] and positively charged receptors interact with anionic molecules.[6,8] Crystal structure determinations have shown that the complexes formed result from face-to-face association between the planar residues in the receptors and the substrates.[3–5]

We have described previously[10] the synthesis of the macrobicyclic bis-intercalands **1** and **2** and of their flexible acyclic precursors **3** and **4** containing two acridine residues that are known to form face-to-face donor–acceptor complexes.[11] We now describe the ability of the positively charged *N*-methylated derivatives $\mathbf{1}^{2+}$ and $\mathbf{2}^{2+}$ and of their acyclic dimeric and monomeric analogues $\mathbf{3}^{2+}$, $\mathbf{4}^{2+}$ and $\mathbf{5}^{+}$, $\mathbf{6}^{+}$ to strongly bind in *aqueous* solution both anionic and neutral substrates containing planar residues.‡

The addition of a series of molecules to aqueous solutions of $\mathbf{1}^{2+}$–$\mathbf{4}^{2+}$ ($\sim 10^{-5}$ mol dm^{-3}) caused pronounced changes in their electronic absorption spectrum, leading to a decrease in the intensity of the band located around 400 or 470 nm. The stability constants of the complexes have been determined by analysis of the optical density changes $\Delta D = f(S_0)$ as a function of the amount of added substrate S_0 (Tables 1 and 2).§

The stoichiometry of the complexes formed by $\mathbf{1}^{2+}$–$\mathbf{4}^{2+}$ was found to be 1:1 for all substrates, indicating that a well-defined species is generated.

In all cases a marked hypochromic effect was observed, the absorption coefficients for the receptors in the complexes being 10–90% smaller than those of the unbound species (Table 1). Such hypochromism is considered to be characteristic of the formation of stacked structures between π systems,[14] involving in particular nucleic acid bases, and may provide information about the geometry of the species.[15] It is found to be larger the more stable the complex formed by a given receptor (Table 1).

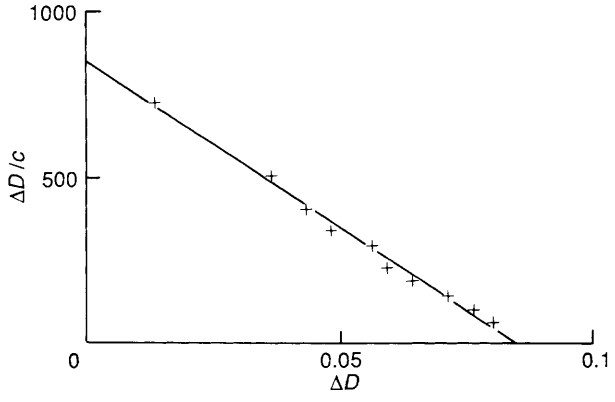


Fig. 1 Plot of $\Delta D/c$ *versus* c (ΔD: optical density change at 400 nm; c: concentration of substrate) in the case of the receptor $\mathbf{1b}^{2+}$ and the substrate terephthalate TP^{2-} for determination of the stability constant of the complex by linear regression in the Benesi–Hildebrand treatment; points are experimental, curves are calculated

Taken together, the 1:1 stoichiometry and the hypochromism suggest a sandwich type structure for the complexes, the substrates being located between the two flat acridine units of receptors $\mathbf{1}^{2+}$–$\mathbf{4}^{2+}$. They could penetrate more or less deeply into the box-like cavity of the macrobicyclic species $\mathbf{1}^{2+}$ and $\mathbf{2}^{2+}$ or be held in a chelating fashion between the two acridine residues of the acyclic bis-intercalands $\mathbf{3}^{2+}$ and $\mathbf{4}^{2+}$.

The stability of the complexes formed in aqueous solution is remarkably high for the receptors containing two intercalator units, being up to about three orders of magnitude higher than for the reference compounds $\mathbf{5}^{+}$ and $\mathbf{6}^{+}$ (Table 1). The macrobicyclic receptors $\mathbf{1}^{2+}$ and $\mathbf{2}^{2+}$ bind less strongly by factors of about 5 to 10 than the acyclic analogues $\mathbf{3}^{2+}$ and $\mathbf{4}^{2+}$. This could be due, at least in part, to a partial occupation of the cavity by the central 9,9′ bridge and/or to the rigidity of the diacetylene bridges that may keep the acridine walls somewhat too far apart. Indeed, in the crystal structure of **1a**[16] the distance between the acridine units is *ca.* 7.5 Å, which leaves a 4 Å wide cavity for planar aromatic substrates about 3–4 Å

‡ The *N*-methylated derivatives have been obtained by treating the parent compounds with methyl trifluoromethanesulphonate in refluxing 1,2-dichloroethane. Their microanalytical and spectral properties were in agreement with their structure.

§ The measurements were performed at 20 °C in aqueous buffer at constant ionic strength (sodium cacodylate 0.01 mol dm^{-3}, pH = 7.8, sodium sulphate 0.01 mol dm^{-3}) and at concentrations of ligands $\mathbf{1}^{2+}$–$\mathbf{6}^{+}$ around 10^{-5} mol dm^{-3}. In this range self-association due to the tendency of acridines to aggregate[12] may be avoided, and the large absorption coefficients make satisfactory measurements possible. Beer's law was followed below 2×10^{-5} mol dm^{-3}; the absorption coefficients ε were determined from the variation of the optical density D with concentration. Data treatment was performed by: (*i*) a Benesi–Hildebrand linear regression analysis (8–10 optical density values measured at 397 or at 475 nm for the series $\mathbf{1}^{2+}$, $\mathbf{3}^{2+}$, $\mathbf{5}^{+}$ or $\mathbf{2}^{2+}$, $\mathbf{4}^{2+}$, $\mathbf{6}^{+}$, respectively); (*ii*) the SPECFIT programme[13] using 13 optical density values measured at 7 different wavelengths at 10 nm intervals (from 380–440 nm for $\mathbf{1}^{2+}$, $\mathbf{3}^{2+}$, $\mathbf{5}^{+}$; from 490–550 nm for $\mathbf{2}^{2+}$, $\mathbf{4}^{2+}$, $\mathbf{6}^{+}$). A typical curve is shown in Fig. 1.

Table 1 Electronic absorption data λ_{max} (nm), ε, substrate binding constants, log K_s, and hypochromicity [H] for the bis-intercaland receptors $\mathbf{1}^{2+}$–$\mathbf{4}^{2+}$, for $\mathbf{5}^{+}$, $\mathbf{6}^{+}$ and for their complexes[a]

		log K_s, [H] for the substrates[b]				
Receptor	λ_{max} (ε)	TP^{2-}	2,6-NDC^{2-}	$AQDS^{2-}$	AMP^{2-}	A
$\mathbf{1a}^{2+}$	396	3.54	4.25	5.60	4.08	4.00
	(40 000)	[0.33]	[0.44]	[0.60]	[0.22]	[0.24]
$\mathbf{1b}^{2+}$	397	4.00	4.59	5.13	3.79	3.87
	(40 000)	[0.25]	[0.30]	[0.50]	[0.20]	[0.21]
$\mathbf{1c}^{2+}$	397	3.84	4.45	5.75	3.92	3.86
	(40 000)	[0.47]	[0.43]	[0.50]	[0.40]	[0.36]
$\mathbf{2}^{2+}$	469	3.72	4.43	5.95[c]	4.08	3.99
	(30 000)	[0.36]	[0.50]	[0.22][a]	[0.25]	[0.25]
$\mathbf{3a}^{2+}$	397	4.70	5.21	6.78	4.83	4.73
	(43 000)	[0.69]	[0.65]	[0.81]	[0.60]	[0.49]
$\mathbf{3b}^{2+}$	400	4.36	4.75	5.67	4.79	4.61
	(42 000)	[0.14]	[0.45]	[0.87]	[0.34]	[0.22]
$\mathbf{3c}^{2+}$	405	4.33	4.82	—	5.05	4.89
	(40 000)	[0.14]	[0.21		[0.13]	[0.19]
$\mathbf{4}^{2+}$	470	—	4.94[c]	6.36[c]	—	—
	(59 000)		[0.22][a]	[0.69][a]		
$\mathbf{5}^{+}$	398	2.25	2.93	3.28	2.34	—
	(26 900)	[0.16]	[0.30]	[0.35]	[0.18]	
$\mathbf{6}^{+}$	482	1.82	2.95	—	2.04	—
	(35 800)	[0.38][a]	[0.37][a]		[0.23][a]	

[a] Experimental conditions, see footnote §; standard deviations ≤±0.1; all receptors $\mathbf{1}^{2+}$–$\mathbf{6}^{+}$ have been used as their hydrogenosulphate salts. Hypochromicity $H = (D_0-D_{lim})/D_0$, where D_0 and D_{lim} are respectively the optical densities of the free receptor and of the complex (limiting value determined) measured at 400 nm, except for [$\mathbf{2}^{2+}$, $AQDS^{2-}$] (415 nm), [$\mathbf{4}^{2+}$, 2,6-NDC^{2-}] and [$\mathbf{4}^{2+}$, $AQDS^{2-}$] (470 nm), and for the complexes of $\mathbf{6}^{+}$ (475 nm). [b] TP^{2-}: Terephthalate; NDC^{2-}: 2,6-naphthalene dicarboxylate; $AQDS^{2-}$: 2,6-anthraquinone disulphonate; AMP^{2-}: adenosine monophosphate; A: adenosine; all anionic substrates have been used as the sodium salts. [c] Standard deviation ≤±0.2.

Table 2 Stability constants log K_s for the binding of various substrates[a] by the bicyclic bis-intercaland receptor $\mathbf{1b}^{2+}$ [b]

B^-	OP^{2-}	MP^{2-}	TP^{2-}	2,6-NDC^{2-}	1,8-NDC^{2-}
3.45	3.28	3.75	4.00	4.59	3.80
1,5-NDS^{2-}	1,3,5-BTC^{3-}	1,2,4,5-BTC^{4-}	1,4,5,8-NTC^{4-}		
3.91	3.50	3.88	4.00		
T	C	U	G	dU	
3.86	3.94	4.05	4.30[c]	4.06	
TMP^{2-}	CMP^{2-}	UMP^{2-}	GMP^{2-}	ADP^{3-}	ATP^{4-}
4.05	3.94	33.86	4.09	3.84	3.91

[a] B^-: Benzoate; OP^{2-}: orthophthalate; MP^{2-}: metaphthalate; 1,8-NDC^{2-}: 1,8-naphthalene dicarboxylate; 1,5-NDS^{2-}: 1,5-naphthalene disulphonate; 1,3,5-BTC^{3-}: 1,3,5-benzenetricarboxylate; 1,2,4,5-BTC^{4-}: 1,2,4,5-benzene tetracarboxylate; T: thymidine; C: cytidine; U: uridine; G: guanosine; dU: 2′-deoxyuridine; TMP^{2-}, CMP^{2-}, UMP^{2-}, GMP^{2-}: monophosphates of T, C, U, G, respectively; ADP^{3-}: adenosine diphosphate; ATP^{4-}: adenosine triphosphate; all anionic substrates have been used as the sodium salt; see also footnote [b] of Table 1. [b] Experimental conditions see footnote §; standard deviations ≤±0.1. [c] Standard deviation ±0.15.

thick. In contrast, with the flexible acyclic compounds $\mathbf{3}^{2+}$ and $\mathbf{4}^{2+}$ close contact is possible between the two acridine units and the substrate sandwiched between them. The differences in binding constants between the receptors containing different bridges (**a**, **b**, **c**) or nitrogens ($\mathbf{2}^{2+}$, $\mathbf{4}^{2+}$) in place of oxygens ($\mathbf{1}^{2+}$, $\mathbf{3}^{2+}$) are not large enough for a detailed structural interpretation of the values determined. One may, however, note that $\mathbf{3a}^{2+}$ appears to bind more strongly than $\mathbf{3b}^{2+}$ and $\mathbf{3c}^{2+}$.

The selectivity of complexation of different substrates presents two interesting features. The binding constants increase markedly with the size of the flat substrates, by factors of 10–100 as one goes from one to two or three rings (compare TP^{2-}, 2,6-NDC^{2-} and $AQSD^{2-}$, Table 1). This corresponds to an increase in contact area between the substrates and the acridine groups. In contrast, for substrates of similar size, there is no clear trend of increasing stability with the number of negative charges, as is seen for the binding of benzene derivatives bearing one to four carboxylate functions or of AMP^{2-}, ADP^{3-} and ATP^{4-} by receptor $\mathbf{1b}^{2+}$ (Tables 1 and 2). Thus, electrostatic effects appear to play only a minor role, perhaps due to a compensation between charge–charge attraction and increasing solvation with the number of negative charges. The appreciably stronger binding of 2,6-NDC^{2-} with respect to its 1,8-NDC^{2-} isomer may be due to a structural selectivity involving a better receptor–substrate fit.

The increase of binding strength with the size and its insensitivity to the charge of the substrate indicate that substrate binding by receptors $\mathbf{1}^{2+}$–$\mathbf{4}^{2+}$ is dominated by stacking features that involve both van der Waals interactions and hydrophobic effects.

This is also in line with the remarkably strong complexation of neutral substrates, the nucleosides A, T, C, U, G and dU (Tables 1 and 2). The stability constants are comparable to those of the corresponding doubly charged monophosphate derivatives, the nucleotides AMP^{2-}, TMP^{2-}, CMP^{2-}, UMP^{2-} and GMP^{2-}.

Finally, preliminary studies also indicate that the bis-intercalands $\mathbf{1}^{2+}$–$\mathbf{4}^{2+}$ interact strongly with single-stranded and double-stranded nucleic acids (for related work see ref. 7*b*).

Received, 22nd April 1991; Com. 1/01886H

References

1 W. Saenger, *Principles of Nucleic Acid Structure*, Springer, Heidelberg, 1984; *Nucleic Acids in Chemistry and Biology*, eds. G. M. Blackburn and M. J. Gait, IRL Press, Oxford, 1990.

2 H. M. Berman and P. R. Young, *Ann. Rev. Biophys. Bioeng.*, 1981, **10**, 87; E. Westhof and M. Sundaralingam, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 1852; L. P. G. Wakelin, *Med. Res. Rev.*, 1986, **6**, 275.

3 (*a*) C. W. Chen and H. W. Whitlock, Jr., *J. Am. Chem. Soc.*, 1978, **100**, 4921; (*b*) E. J. Jarvi and H. W. Whitlock, *J. Am. Chem. Soc.*, 1982, **104**, 7196; (*c*) R. E. Sheridan and H. W. Whitlock, Jr., *J. Am. Chem. Soc.*, 1988, **110**, 407; (*d*) K. M. Neder and H. W. Whitlock, Jr., *J. Am. Chem. Soc.*, 1990, **112**, 9412.

4 J. Jazwinski, A. J. Blacker, J.-M. Lehn, M. Cesario, J. Guilhem and C. Pascard, *Tetrahedron Lett.*, 1987, **28**, 6057.

5 P. R. Ashton, B. Odell, M. V. Reddington, A. M. Z. Shawin, J. F. Stoddart and D. J. Williams, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1550.

6 A. J. Blacker, J. Jazwinski and J.-M. Lehn, *Helv. Chim. Acta*, 1987, **70**, 1.

7 (*a*) A. Hamilton, J. L. Sessler and J.-M. Lehn, *J. Am. Chem. Soc.*, 1986, **108**, 5158; (*b*) A. Slama-Schwok and J.-M. Lehn, *Biochemistry*, 1990, **29**, 7895.

8 J.-M. Lehn, F. Schmidt and J.-P. Vigneron, *Tetrahedron Lett.*, 1988, **29**, 5255.

9 S. C. Zimmerman, C. M. VanZyl and G. S. Hamilton, *J. Am. Chem. Soc.*, 1989, **111**, 1373; *J. Am. Chem. Soc.*, 1991, **113**, 183, 196; and references cited therein.

10 S. Claude, J.-M. Lehn and J.-P. Vigneron, *Tetrahedron Lett.*, 1989, **30**, 941.

11 L. Toupet, A. Miniewicz and C. Ecolivet, *Acta Crystallogr., Sect. C*, 1989, **45**, 1044.

12 See for instance: R. Larsson and B. Norden, *Acta Chem. Scand.*, 1970, **24**, 2583.

13 H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **32**, 95; 257; 1133.

14 See for instance: J. Bolte, C. Demuynck and J. Lhomme, *J. Am. Chem. Soc.*, 1976, **98**, 613, and references cited therein.

15 F. Seyama, K. Akahori, Y. Sakata, S. Misumi, M. Aida and C. Nagata, *J. Am. Chem. Soc.*, 1988, **110**, 2192, and references cited therein.

16 J. Guilhem and C. Pascard, unpublished results.