Molecular Recognition of Nucleotides, Nucleosides, and Sugars by Aminocyclodextrins[1]

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Abstract: β-Cyclodextrins bearing two or seven aminomethyl groups in the 6-position (compounds **1a,b**) are shown to act as polytopic receptors for nucleotides. **1a** discriminates the guest compounds on the basis of nucleobase nature, sugar type (oxy- or deoxyriboses), and position of the phosphate groups (3′ or 5′). For the first time such selectivity toward different moieties of nucleotides is realized by one artificial receptor simultaneously and in water as a solvent. **1b**, although considerably less selective, exhibits very high sensitivities with binding constants of up to 3×10^6 M^{-1}. Comparison between complexes with inorganic phosphates, ribose phosphates, riboses, and nucleotides allows quantification of the different binding contributions: up to 20 kJ/mol for ionic interactions (as a function of the number of salt bridges) and 10–13 kJ/mol for the ribose unit. The nucleobases, although discriminated, are shown to contribute repulsive rather than attractive forces to complex formation. Complexation-induced NMR chemical shifts and intermolecular NOEs allow insight into the supramolecular structures. These results are in line with molecular mechanics simulations (CHARMm) which also show that the strong electrostatic interactions with **1b** draw the substrate further away from the secondary interaction sites and thus lower the selectivity. The sugar moiety is shown by NOE to rest in the cyclodextrin cavity. This complexation, in contrast to usual expectations, is stronger for the more hydrophilic ribose derivatives in comparison to the deoxy compounds. Selective broadening of some proton signals is ascribed, on the basis of T_1 measurements, to complex dissociation processes rather than slow tumbling effects.

Introduction

The selective complexation of nucleotides and nucleosides by synthetic host compounds has been the focus of an increasing number of publications.[2,3] These studies can make essential contributions toward the understanding of noncovalent interactions involving nucleic acids, as well as toward the development of new diagnostic tools. Most of the reported synthetic hosts rely on complexation through hydrogen bonding with a nucleobase. Consequently, many of these systems, with a few exceptions,[3] were studied in lipophilic solvents. In aqueous solutions, the major driving forces for the formation of supramolecular complexes can be ion pairing with the phosphate groups and base stacking or/and solvophobic forces acting on the nucleobases and/or the sugar parts. The latter part, until now, has been hardly addressed. The present paper illustrates how both salt bridges and lipophilic interactions can, on the basis of aminocyclodextrins as hosts, lead to simultaneous differentiation between the site and nature of the phosphate groups, the different nucleobases, and the presence or absence of hydroxyl groups in the furanoses.[4]

Cyclodextrins (CyD), highly water soluble cyclic oligomers of glucose, being effective artificial receptors for organic molecules[5] can serve as a potential basis for nucleotide recognition in water. Indeed, among the numerous guest molecules recognized by CyDs, special attention has recently been attracted to nucleic acid units.[6,7] Creation of CyD-based nucleotide receptors seems to be very challenging in view of regioselective ribonuclease mimics[6a,c] and applications for nucleoside prodrug delivery.[7] However, the studies made so far on the complexation of nucleobase derivatives with CyD's[6b,8,9] have revealed that the binding is very weak and restricted mainly to adenine derivatives. One reason for this is that host–guest interactions with CyD's are mainly of a hydrophobic nature, whereas nucleotides possess low hydrophobicity.

We have shown earlier that the interactions of many organic anions with ammonium centers can be quantified by a rather constant contribution to the binding free energy in water of −5

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± 1 kJ/mol for each salt bridge.[10] The same value has been found for binding of catecholate anions to amino-CyD's;[11] this general trend is also followed for interactions of charged CyD's with several organic[12] and inorganic[13] ions.

In following up this approach, we synthesized β-CyD derivatives **1** containing differently charged primary binding sites for anionic guests and investigated their binding to various nucleotides and related compounds.

Experimental Section

Materials. All guest compounds and β-CyD were purchased from Aldrich and used without further purification.

$6^A,6^D$-Bis(deoxy)-$6^A,6^D$-bis(methylamino)-β-CyD (1a) was synthesized in a two-step procedure including selective "capping" of β-CyD with 4,4'-biphenyldisulfonylchloride, as described by Tabushi, and purified as described previously.[14] ^{1}H NMR (δ, ppm): 4.93 (H1, d), 4.89 (H1*, d), 3.97 (H5*, br.t), 3.80–3.74 (H3, H3*, m), 3.72–3.62 (H5, H6, m), 3.52–3.45 (H4, H2, H2*, m), 3.36 (H4*, br.t), 3.18–3.13 (H6*, dd), 2.58 (CH_3N, s). ^{13}C NMR (δ, ppm): 103.0 (C1), 102.4 (C1*), 84.3–82.0 (C4, C4*), 74.0–72.5 (C2, C2*, C3, C3*, C5), 67.8 (C5*), 61.6 (C6), 50.9 (C6*), 34.7 (CH_3N) (Atoms marked with a star refer to amino-substituted glucose units.)

6-Heptakis(deoxy)-6-heptakis(methylamino)-β-CyD (1b) was prepared by pertosylation of the sixth (primary) hydroxy groups of β-CyD with *p*-toluenesulfonyl chloride followed by substitution of the tosylate by methylamine in methanol.[15] ^{1}H NMR (δ, ppm): 4.95 (H1, d), 3.85 (H5, br.t), 3.69 (H3, t), 3.47 (H2, dd), 3.38 (H4, t), 3.1–3.0 (H6, A, B, m), 2.55 (CH_3N, s). ^{13}C NMR (δ, ppm): 101.0 (C1), 81.9 (C4), 71.9, 71.3 (C2, C3), 67.7 (C5), 49.5 (C6), 33.8 (CH_3N).

Spectroscopy. NMR studies were performed with a Bruker AM-400 spectrometer in D_2O solutions using TMS/CCl_4 as an external standard. Proton chemical shifts were reproducible within 0.001 ppm. Complexation-induced shift (CIS) values were determined either in an equimolar mixture of host and guest compounds (normally 10^{-2} mol/L each), when high binding constants provided a >99% degree of complexation, or as the limiting value of the NMR titration curve, calculated by nonlinear regression[16] (for ribose–CyD interactions).

Intermolecular NOE measurements were carried out by a standard NOEDIF program with both irradiation and relaxation delays approximately three times longer than the longest proton relaxation time in the system. Experiments were made with concentrations of both host and guest compounds of (4–6) × 10^{-2} mol/L in samples without degassing. After ~200 scans for each irradiation frequency, differential spectra were obtained by subtraction of one reference spectrum.

T_1 values were measured without degassing by the inversion–recovery method and calculated by standard programs. pD Values for NMR titrations were adjusted within 0.02 pD units. Spectral assignments of the amino-CyD ^{1}H signals were made by COSY-45 experiments.

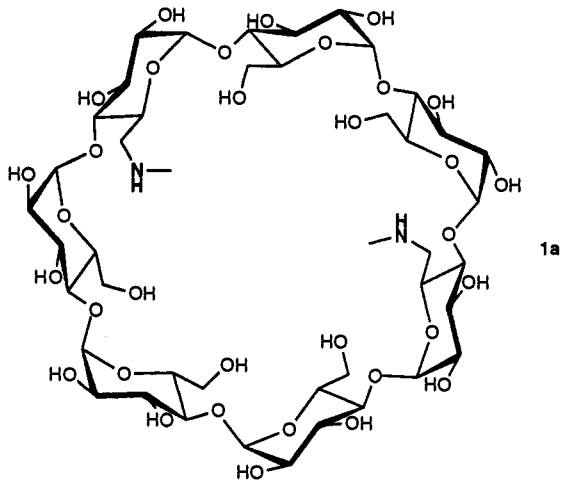
Binding Constants. As the complexation of nucleotides with aminocyclodextrins is highly pH-dependent, the binding constants were determined by potentiometric titrations, as described for other receptors.[17] In a standard run a 5 × 10^{-3} mol/L solution of a nucleotide (and an equal concentration of **1**) in 0.1 mol/L background NaCl was acidified with HCl and titrated with 0.005 mol/L NaOH. Between 50 and 100 points of each titration curve obtained with a 702 SM Titrino setup (Schott AG, Mainz) were fitted, and the equilibrium constants were calculated using the program BEST.[18] Errors in duplicate runs were below 5%. One *K* value obtained for comparison by NMR titration showed agreement within 10%. An NMR spectrum of **1a** (1 equiv) with a mixture of 5'-AMP and 5'-GMP (1 equiv each) showed stronger shift changes for 5'-AMP, thus providing independent evidence for the complexation selectivity.

Binding constants with non-ionogenic compounds (i.e. riboses) were measured by NMR titrations by monitoring the chemical shift of the CyD's H3 proton, as described in ref 16. Satisfactory fits were observed in all cases on the basis of the assumption of a 1:1 complex stoichiometry.

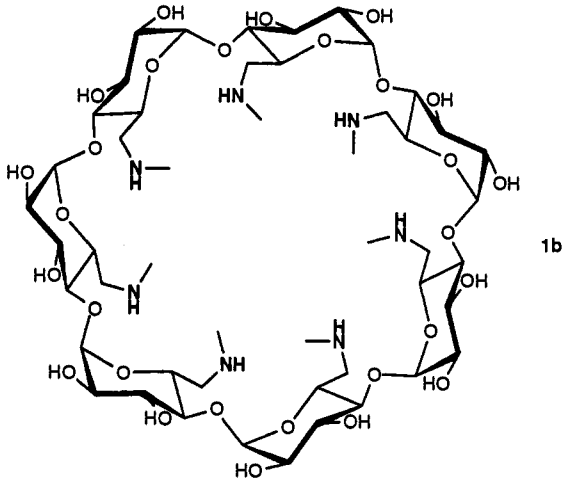
Force-Field Simulations were carried out with CHARMm/QUANTA programs[19] with the dielectric constant equal to unity and the charge distribution calculated by the Gasteiger method.

Results and Discussion

In order to optimize selectivities and to clarify the binding mechanisms between modified CyDs and nucleotides, nucleosides, *etc.*, we used the CyD derivatives **1a** and **1b** containing two or



1a



1b

seven methylamino groups in the sixth positions, respectively. A number of nucleotides and related compounds with different numbers of ionic groups and structural features (Table 1) were used as guest molecules.

Complex Formation and Geometry. Since both host and guest compounds are ionic, it is reasonable to expect the strongest binding to occur in the pH range where CyD's amino groups are protonated and positively charged[20] and where phosphate groups

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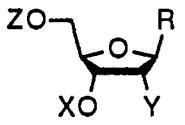
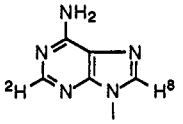
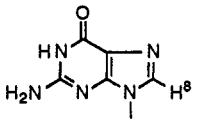
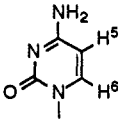
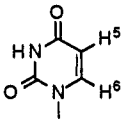
(20) The pK_a value of the pure **1a** was found to be 8.7, whereas deprotonation of **1b** proceeds in a fairly wide pH range (7.5–9.5), probably, because of the cooperativity in neighboring glucose units.

Table 1. Structures of Nucleotides and Related Compounds Recognized by **1**



	R	X	Y	Z
5'-AMP		H	OH	PO_3^{2-}
3'-AMP	-"-	PO_3^{2-}	OH	H
5'-ATP	-"-	H	OH	$P_3O_7^{4-}$
d-5'-AMP	-"-	H	H	PO_3^{2-}
5'-GMP		H	OH	PO_3^{2-}
d-5'-GMP	-"-	H	H	PO_3^{2-}
5'-CMP		H	OH	PO_3^{2-}
Cytidine	-"-	H	OH	H
d-5'-CMP	-"-	H	H	PO_3^{2-}
5'-UMP		H	OH	PO_3^{2-}
d-5'-UMP	-"-	H	H	PO_3^{2-}
RP (ribose-5-phosphate)	OH	H	OH	PO_3^{2-}
d-RP	OH	H	H	PO_3^{2-}

of the nucleotides exist in anionic form.[21] Indeed, the ^{1}H NMR spectrum (Figure 1b) of the mixture of 5'-AMP with **1b** at pH 7.0, where both above conditions are held, shows at least two pieces of evidence for complexation. These are, first, the chemical shifts of some host and guest signals compared to those of the free compounds (cf. values in Table 2) and, second, the strong line broadening of the guest proton signals, especially pronounced for the H8 proton of the nucleobase (8.3–8.4 ppm for 5'-AMP, Figure 1). The same phenomena have been detected with other nucleotides, with the line broadening being observed in H8 of purine and H6 of pyrimidine bases, i.e. those closest to the glycosidic bond.

These phenomena are reversible with the change of temperature and with pH shifts. For example, acidification of the solution to pD 2.0, where the phosphate group is completely protonated, leads to the totally recovered line sharpness (Figure 1a) and no chemical shifts with respect to pure nucleotide are observed within the experimental error. The same holds for increasing pD to 11.5, which leads to the neutralization of the CyD's protonated amino groups (Figure 1c).

Additional evidence for the complexation can be obtained (besides from NOE and shielding differences, see below) by comparison with a *nucleoside* molecule lacking the phosphate group. 5'-CMP in a mixture with **1b** at intermediate pH values

(21) The relevant phosphate pK_a's of the nucleotides are 6.0–6.5 (Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solutions*; Butterworths: London, 1965).

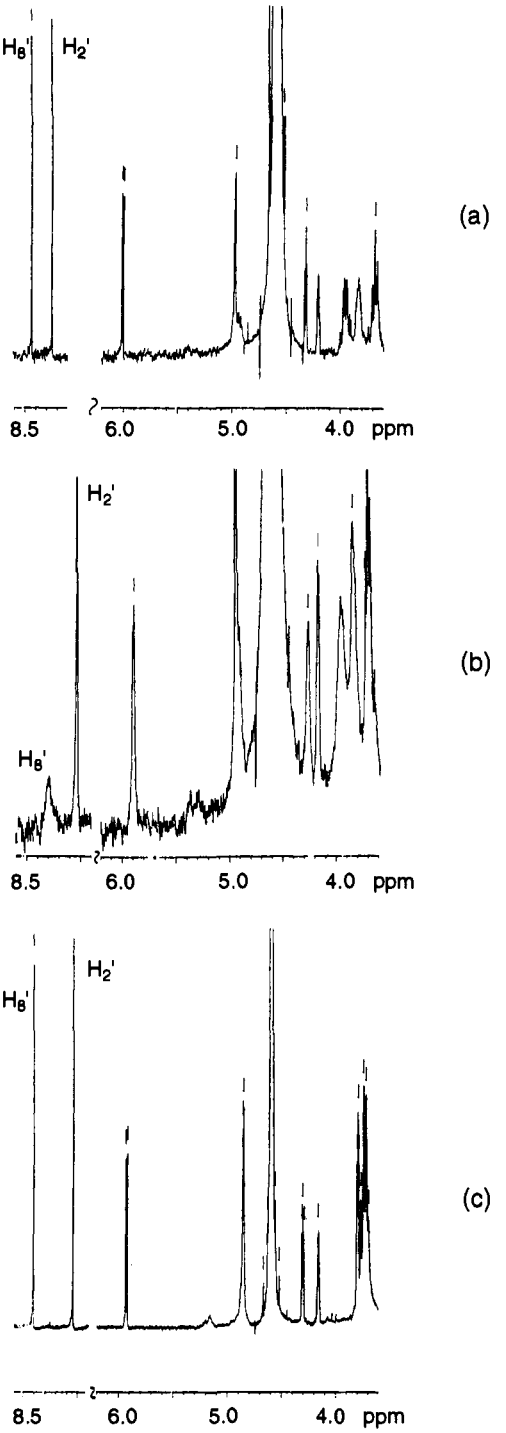


Figure 1. ^{1}H NMR spectra of the mixture of 5'-AMP and **1b** (both 10^{-2} mol/L) (a) at pD 2.0, (b) at pD 7.0, and (c) at pD 11.5.

Table 2. Complexation-Induced ^{1}H NMR Chemical Shifts (CIS) of **1b** by Different Guest Molecules at pH 6.0 and 25 °C

	CIS, ppm							
guest	H1	H5	H3	H2	H4	H6(a)	H6(b)	CH_3N
5'-AMP	0.01	–0.16	–0.12	0.00	0.02	0.01	0.08	0.01
3'-AMP	0.04	–0.12	–0.16	0.01	0.06	0.01	0.17	0.05
5'-GMP	0.03	–0.23	–0.12	0.01	0.01	0.02	0.13	0.04
5'-UMP	0.04	–0.19	–0.10	0.02	0.06	0.02	0.15	0.05
5'-CMP	0.03	–0.18	–0.09	0.01	0.06	0.02	0.13	0.05
RP	0.04	–0.11	–0.06	0.02	0.09	0.02	0.12	0.05
ribose	<0.01	<0.01	–0.05	[a]	[a]	<0.01	<0.01	<0.01

[a] Overlapped with guest signals.

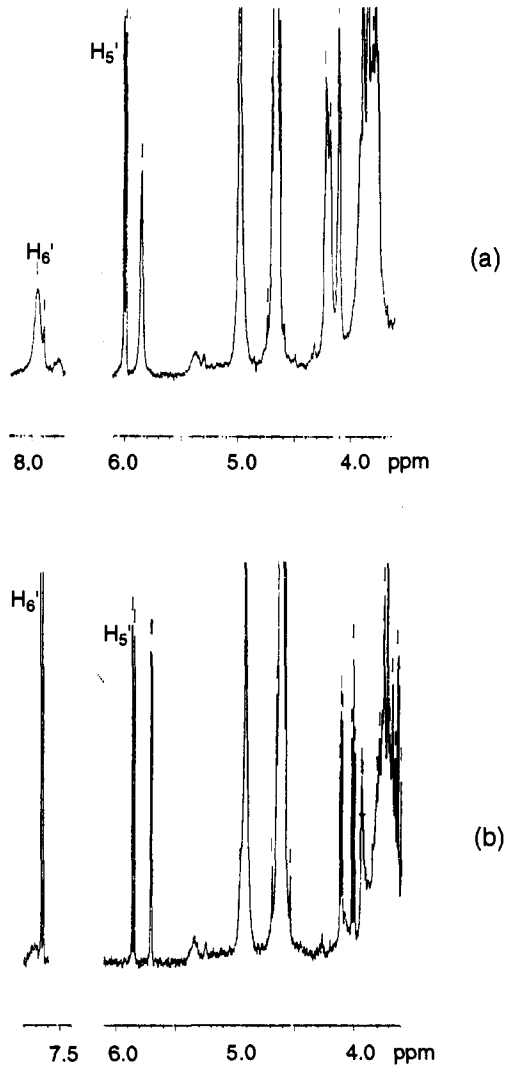


Figure 2. ^{1}H NMR spectra of the mixtures of (a) 5′-CMP and **1b** (both 10^{-2} mol/L) at pD 8.5 and (b) cytidine and **1b** (both 10^{-2} mol/L) at pD 8.5.

shows effects similar to those with 5′-AMP (Figure 2a). Under the same conditions the neutral cytidine molecule does not display any spectral changes (Figure 2b).

As nucleotide and amino-CyD molecules change their protonation states, chemical shifts of the protons close to the ionogenic groups should evidently be affected by ionization processes. Indeed, the signal of the methyl groups attached to the nitrogen atom in **1b** moves considerably upfield with increasing pD (Figure 3a), yielding the same ionization pH range as given by potentiometric titrations. Similar behavior is observed for the H2 proton of adenine responsible for deprotonation of the nucleobase amino group (Figure 3a). However, the signals of some guest protons, for instance, H1′ and H2′ in the ribose moiety, despite their being far from ionogenic groups, also exhibit a pH-dependence. Moreover, the respective curves (Figure 3b) have a bell-like shape with the right side corresponding to ionization of the CyD's CH_3N group. The fact that these protons are sensitive to deprotonation of the CyD may indicate that the ribose unit is involved in the complexation. The left sides of the curves obviously correspond to ionization of the guest phosphate groups. It is seen that the respective pK_a values are shifted to the acidic field. These effects have been described for other guests[22] and are important for binding constant determinations (see the Experimental Section).

The chemical shifts of **1b** induced by complexation with different compounds are very informative with respect to the

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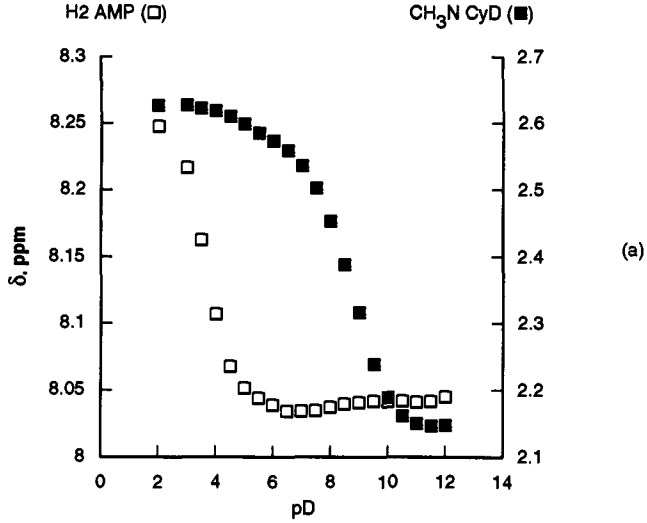


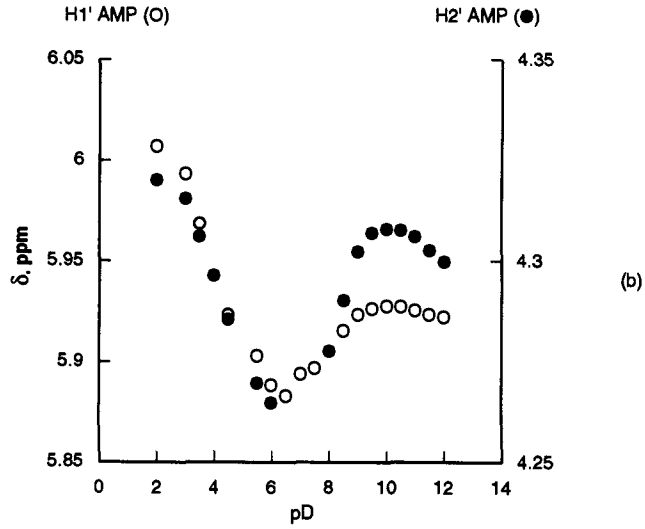


Figure 3. ^{1}H NMR chemical shifts of the protons in the mixture of 5′-AMP and **1b**, plotted *vs* pD.

complex geometry (Table 2). All the guest compounds studied exert considerable shielding effects on H3 and H5 CyD protons, which are located inside the CyD cavity. This means that the guest molecule is included inside the cavity.[23] Another signal that is shifted strongly upfield is that of the H6 proton, which is close to the interacting ion pair. There is a remarkable increase in the H6–H6 magnetic nonequivalence upon complexation. As far as other protons are concerned, their complexation shifts are much less and often within experimental error.

The CIS values of the nucleotides are indicative with respect to complexation (see examples in Figure 4) but are less conclusive with respect to the inclusion modes. The signals of both ribose and nucleobase moieties are mainly shifted slightly upfield, which confirms the interaction of the whole nucleotide molecule with the host compound.

The most reliable evidence of the complex structure can be obtained from studies of the *intermolecular* NOE. As shown in Figure 5, irradiation of the H3 and H5 protons of **1b** gives rise to considerable enhancements of the ribose signals. Such effects have been shown to provide semiquantitative information about

(23) The directions of these shifts is similar to those reported by Komiyama (ref 6b), but their absolute values are higher. These values are comparable for all the nucleotides and ribose phosphate but are much smaller for ribose itself. Therefore, the shielding effects are caused very likely by the charged phosphate group rather than nucleobase or ribose units.

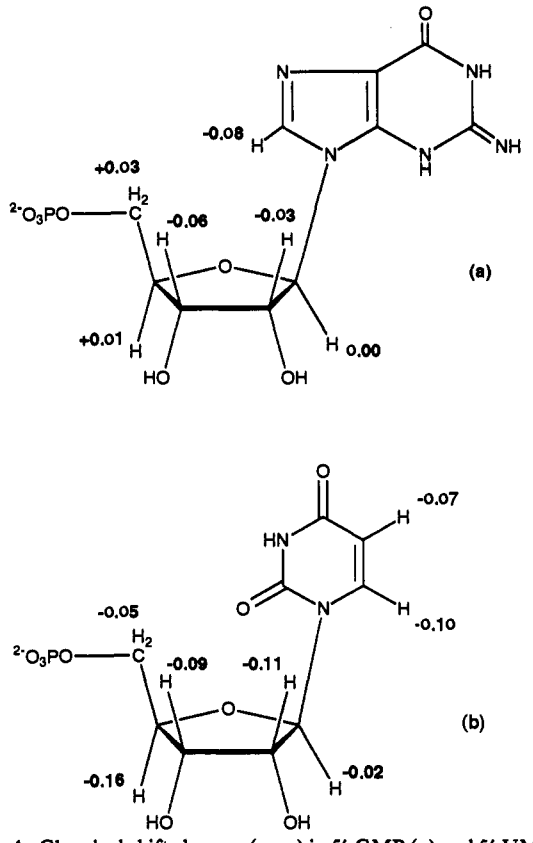


Figure 4. Chemical shift changes (ppm) in 5′-GMP (a) and 5′-UMP (b) induced by complexation with **1b**.

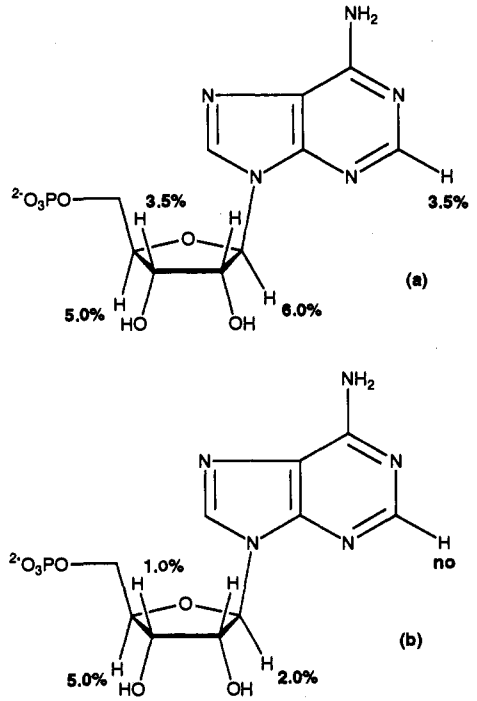


Figure 5. Nuclear Overhauser enhancements of the 5′-AMP signals on irradiation of H3 (a) and H5 (b) of **1b** in the mixture of 5′-AMP and **1b** (both 5×10^{-2} mol/L) at pD 6.0.

the inclusion complex geometry,[24] and these results undoubtedly mean that the ribose unit is located inside the CyD cavity.

*Intra*molecular NOEs in CyD's were also observed, and it should be in principle possible to use these for NOE–distance calibrations. However, since the orientation of the host and guest compounds is time-averaged and spin relaxation might be on time scales similar to that of the chemical exchange between the conformers, we only discuss the relative proximity of the particular protons. For instance, the *inter*molecular enhancements given in Figure 5 (5–6%) are comparable with the NOEs *within* the CyD molecule obtained upon irradiation of the H1 proton of **1b** at H2 (9%) and H4 (10%) signals. Since H2–H1 and H4–H1 distances taken from the CHARMm simulated structure (see Figure 7) are 2.4 and 2.3 Å, respectively, the distance between nucleotide ribose protons and H3 and H5 of the CyD should be within *ca.* 3 Å, which corresponds to the intracavity position of the ribose ring. It should be noted that irradiation of the CyD protons provides better information about intermolecular NOEs than irradiation of the nucleotide protons because in the second case we can expect the enhancement only in one out of seven glucose units which is closest to the guest.

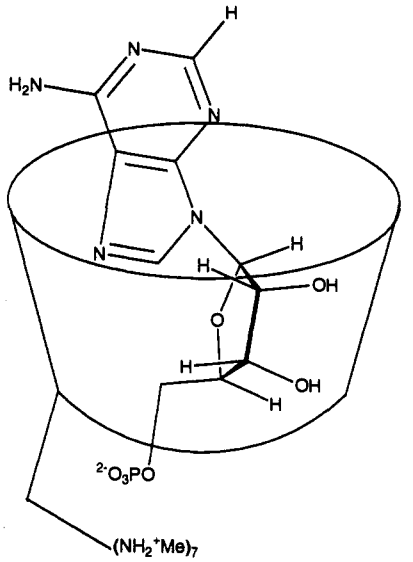


Figure 6. Schematic presentation of the proposed inclusion complex structure of 5′-AMP dianion and **1b** heptacation.

No intermolecular NOEs exceeding experimental error (~0.5%) have been observed on irradiation of the H1, H2, and H4 protons of **1b** located at the outer side of the host molecule, which eliminates conformations with binding outside the cavity.

The above results are consistent with the inclusion complex structure as shown in Figure 6. Here the ion-pair proximity provides the strong electrostatic interaction. The ribose unit and part of the nucleobase are accommodated within the cavity. The fact that the H2 signal of the nucleobase also shows the NOE with H3 of **1b** (Figure 5a) is in accordance with force-field simulations of this structure, demonstrating that the respective protons are within 3 Å. One also cannot exclude here the possibility of the complexation of adenine with a second CyD molecule at the high concentrations used for NOE measurements. However, the respective binding constants do not normally exceed 100 L/mol[6b,7] (Table 3). NMR titrations of 5′-CMP and 5′-AMP (2×10^{-3} M) with **1b** (up to 7×10^{-3} M) showed formation of only 1:1 complexes, which we considered in CIS and binding constant analyses.

Electrostatic Interactions and Selectivity between 3′- and 5′-Phosphate Binding. The binding constant values presented in Table 3 allow a quantitative analysis of the roles of the different interactions between nucleotides and amino-CyD's. The first point to be considered is the strength of the ion-pair interactions. One can see that the binding constants of all the nucleoside monophosphates with **1b** (Table 3, items 1–9) differ to some degree, but are, in general, of the same order. The average complexation free energy is around 27.5 kJ/mol. By subtracting

(24) For recent examples of steady-state intermolecular NOEs with CyD's, see: (a) Lipkowitz, K. B.; Raghothama, S.; Yang, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554. (b) Fornasier, R.; Lucchini, V.; Scrimin, P.; Tonellato, U. *J. Inclusion Phenom.* **1986**, *4*, 291.

Table 3. Binding Constants K (M^{-1}) and Complexation Free Energies ΔG (kJ/mol) of Nucleotides and Related Compounds to Cyclodextrins at 25 °C

$$RPO_3^{2-} + CyD^{n+} = (RPO_3 \cdot CyD)^{(n-2)+}$$

		1a		**1b**		β-CyD
Nr	guest	$10^{-3}K$	$-\Delta G$	$10^{-5}K$	$-\Delta G$	K
1	5′-AMP	14.1	23.7	1.26	29.1	90[a]
2	3′-AMP	1.51	18.1	0.92	28.2	250[b]
3	d-5′-AMP	0.48	15.3	1.17	28.9	
4	5′-GMP	6.16	21.6	0.40	26.2	
5	d-5′-GMP		<15	5.89	32.9	
6	5′-CMP	0.83	16.6	0.20	24.5	
7	d-5′-CMP		<15	0.44	26.5	
8	5′-UMP	0.83	16.6	0.87	28.1	
9	d-5′-UMP		<15	0.38	26.1	
10	5′-ATP[c]	97.7	28.4	32.4	37.1	
11	RP	11.2	23.1	8.51	33.8	
12	d-RP	2.4	19.3	8.13	33.7	
13	PO_4^{3-}	0.20	13.1	0.037	20.3	
14	ribose			0.00026	8.1	1.0

[a] The value from ref 7a. The same constant as well as related ones measured elsewhere[34] by chromatography was found to be much higher, perhaps due to additional interactions with a stationary phase. [b] The value from ref 6b. [c] Tetraanion.

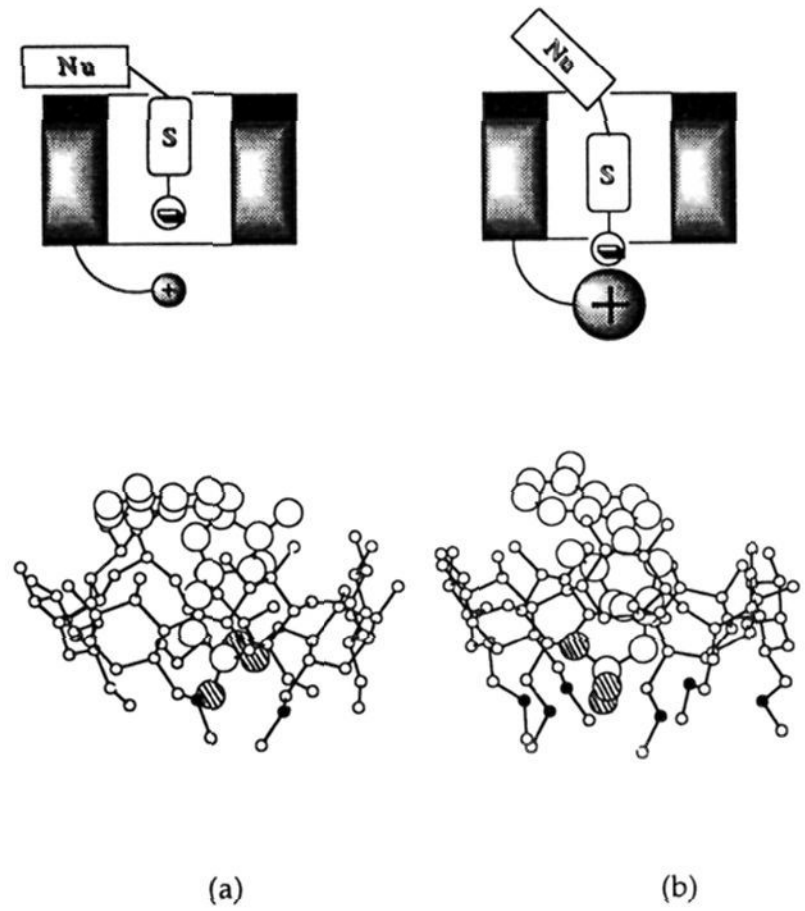
the separately measured contribution of the ribose (8.1 kJ/mol, Table 3, item 14) from this value, we obtain the increment of the ion-pair interaction between phosphate and ammonium groups of 19–20 kJ/mol. Remarkably, the inorganic phosphate ion has the same complexation energy (Table 3, item 13).

As has been mentioned in the Introduction, each salt bridge *in water is worth* 5 ± 1 kJ/mol *for electrostatic host–guest binding* if the conformations allow sufficient contact between the anion and cation. By applying this value to the complexation of nucleoside monophosphates with **1b** we can conclude that only four salt bridges (out of 21 possible in a formal sense!) materialize in the complex. In order to estimate how this number corresponds to the arrangement of the charged groups we have performed *force-field simulations of the inclusion complex structure between* 5′-AMP and **1b** (Figure 7b).

It is reasonable to accept that two oppositely charged ions form a "salt bridge" if the distance between them is close to the sum of ionic radii of atoms possessing the highest charge density (which is in our case approximately 3 Å for $NH_2R_2^+$ and phosphate oxygens[25]). If we count the salt bridges according to this rule, *the number of bridges amounts to six or seven for the nucleotide* included into **1b** (Figure 7b). The reason why the experimental data indicate only ~4 salt bridges can be seen, in analogy to earlier observations,[26,28] in the inability to simultaneously materialize optimal electrostatic and lipophilic (van der Waals) contacts.

Similar analyses for complexation of nucleoside monophosphates *with* **1a** (Table 3) *give the value for the electrostatic* contribution of about 10 kJ/mol, which corresponds to two salt bridges, according to the number of ammonium groups. Noticeably, the same value was obtained for this receptor in complexation with catecholate anions.[11] Likewise, the simulated structure of the complex between 5′-AMP and **1a** (Figure 7a) shows evidence of two salt bridges. Evidently, the host with only two cationic *groups accommodates the nucleotides optimally for both possible* electrostatic contacts.

A further interesting fact is that isomeric adenosine monophosphates (Table 3, items 1 and 2) differ in the binding constants



(a) (b)

Figure 7. Schematic representation of electrostatically enforced configuration (high) and CHARMm-simulated structures (low; hydrogen atoms omitted) of 5′-AMP inclusion complexes with **1a** (a) and **1b** (b). Filled circles correspond to atoms bearing maximum charge, according to a Gasteiger distribution.

to **1a** by a factor of 10. This relates to the generally larger sensitivity of the weaker receptor to guest structural features which will be discussed below.

Binding of both CyD derivatives with the ATP tetraanion is, as expected, much stronger. The estimated number of salt bridges for **1b** is about six, and the respective binding constant (K = 3.2 × 10^6 M^{-1}) seems to be one of the highest known for cyclodextrin complexes.

Role of the Nucleobases. Nucleobase selectivity is one of the most important issues in synthetic receptors. The hepta-charged **1b** has similar binding constants with the nucleotides of different structure. Evidently, the strong electrostatic interaction enforces an orientation with diminished contact between the nucleobase part and the host molecule.

In contrast, in the case of **1a**, where binding is not so strong, much higher sensitivity to the nucleobase variation is observed. In a series of structurally similar nucleoside 5′-monophosphates (Table 3, items 1, 4, 6, and 8), a preference is seen for purine-based nucleotides, namely AMP and GMP, with an average difference in binding free energies of about 6 kJ/mol. Two possible explanations for this can be proposed.

The hydrophophobicities of adenine (log P(A) = 0.0) and guanine (log P(G) = –1.0), as estimated by Hansch's scale,[27] are higher than those of pyrimidine bases (log P(C) = –1.1 and log P(U) = –1.5). This could be the reason for better binding of adenine derivatives to both β-CyD and azoniacyclophane[28] but does not quite account for the binding difference between GMP and pyrimidine nucleotides. A more likely explanation is based on comparison of simulated structures of the inclusion complexes of 5′-AMP with **1a**,**1b** (Figure 7). When the nucleotide is bound to **1a**, relatively weak salt bridges allow the nucleobase to form hydrogen bonds with the secondary CyD hydroxyl groups (Figure 7a). The differentiation of purine and pyrimidine nucleotides then would be the result of the larger π-surface of the purines, with the adenine moiety providing the largest polarizability and, therefore, interactions. The very strong electrostatic interactions with **1b** pull the guest molecules closer to the CyD amino groups (Figure 7b) with subsequent lessening of the discriminating nucleobase–CyD contacts.

(25) According to CHARMm parametrization and crystal ionic radii (*Handbook of chemistry and physics;* Weast, R. C., Ed.; Chemical Rubber Co.: Cleveland, OH, 1972–73).

(26) Schneider, H.-J.; Schiestel, T.; Zimmerman, P. *J. Am. Chem. Soc.* **1992**, *114*, 7698.

(27) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525.

(28) Schneider, H.-J.; Blatter, T.; Palm, B.; Pfingstag, U.; Rüdiger V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704.

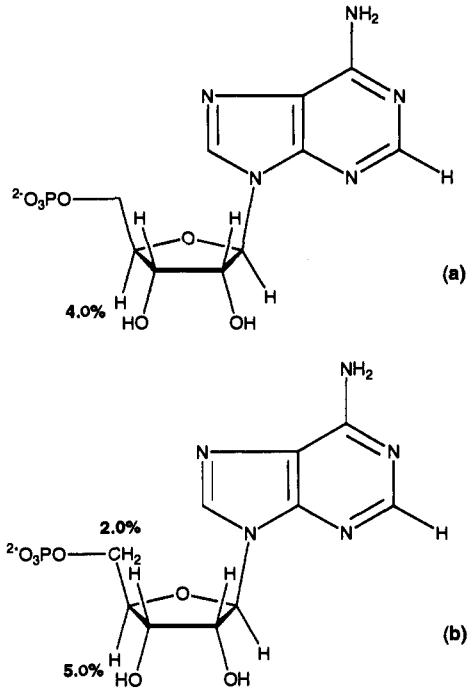


Figure 8. Nuclear Overhauser enhancements of the 5′-AMP signals on irradiation of H3 (a) and H4 (b) of **1b** (mixture of 5′-AMP and **1b** (both 5×10^{-2} mol/L) at pD 3.0).

Sugar–CyD Interactions and Selectivity between Oxy- and Deoxynucleotides. There is clear evidence that the sugar unit (ribose) plays a considerable role in the complexation, although the strong electrostatic binding by phosphate alone could also have led to complex formation without intracavity inclusion of the hydrophilic ribose:

(I) Both CIS and NOE data (Figure 5) show that the sugar resides inside the hydrophobic CyD cavity in accordance with independent measurements of the ribose binding (Table 3), with the binding free energy being higher for **1b** and lower for β-CyD. (II) Ribose 5-phosphate and deoxyribose 5-phosphate bind better to both **1a** and **1b** than nucleoside and deoxynucleotide monophosphates, respectively (Table 3). This means that the unsubstituted sugar molecule exhibits specific and attractive forces with the host. (III) As mentioned above, no CISs have been observed upon addition of **1b** to nucleotides under acidic conditions, where the phosphate group is protonated. However, in the same system, we detected intermolecular NOEs in the ribose moiety upon irradiation of H3 and H4 of **1b** (Figure 8). This indicates that the nucleotide ribose and, perhaps, the neutral phosphate group form a complex with the CyD even in the absence of ion-pair interactions. The guest is most probably located near CyD's secondary side rather than in the cavity.

What could be the nature of these sugar–CyD interactions? In recent studies of cyclodextrin–sugar interactions,[29] hydrophobicity of the sugar molecules was discussed as a partial driving force for the complexation although there was no direct correlation of binding constants with hydrophobicities.[30] In our case a more likely explanation of sugar binding to CyD is the formation of intermolecular hydrogen bonds similar to those in recently discussed complexes of bilirubin with CyD and even with linear dextrins.[31] Molecular modeling of the inclusion complex between RP and **1a** (Figure 9) indeed shows several hydrogen bonds between the host and the guest. Noticeably, some original hydrogen bonds in the CyD appear to be disrupted, with the respective hydroxyl groups being released for complexation with bulk water. The amino-CyD also provides much better binding to ribose itself in comparison to the electroneutral CyD (Table 3), again pointing out the importance of hydrogen bonds for which the protonated amino group provides an exceptionally good donor.

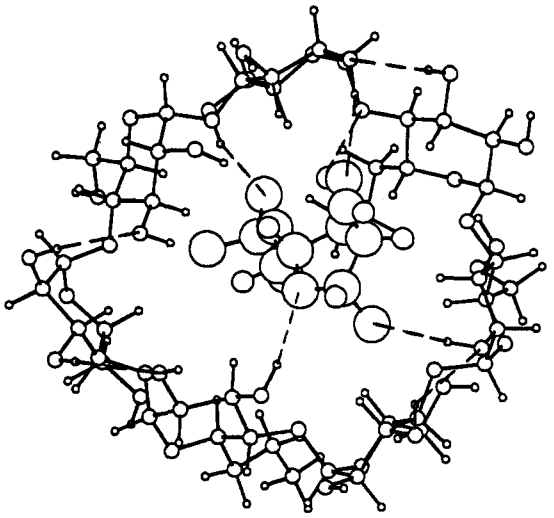


Figure 9. CHARMm-simulated structure of the RP inclusion complex with **1a**. Dashed lines correspond to calculated hydrogen bonds.

Table 4. T_1 Values of Free and Bound 5′-AMP and **1b** (pH 7.0)

proton (AMP)	proton (CyD)	T_1 free, s	T_1 bound, s
H8		0.72	0.88
H2		4.15	3.60
H1′		2.05	1.15
H3′		0.93	1.22
H4′		1.11	1.02
H5′		0.34	0.58
	H1	0.68	0.83
	H5	0.78	0.81
	H3	1.06	1.14
	H2	1.09	1.28
	H4	0.65	0.96
	H6	0.33	0.44
	CH_3N	0.79	0.91

Finally, the most useful and, in view of the hydrophobic nature of the CyD cavity, surprising result of a distinctively better complexation with the more hydrophylic ribose in comparison to deoxyribose derivatives again indicates hydrogen bonds as the dominating factor, in this case involving the 2′-OH of the sugar.

Complexation Dynamics. The signal broadening on complexation prompted some additional studies. One of the reasons for the broadening could be binding to a host molecule which might have longer correlation times, provided the system would behave like a typical biopolymer.[32] However, measurements of the host and guest longitudinal relaxation time changes in the 5′-AMP–**1b** (Table 4) system demonstrate that the aminocyclodextrin has, as expected,[24a] T_1 values even smaller than those of the guest and is still in the extreme narrowing limit. Alternatively, the broadening can be caused by some chemical exchange slow enough on the NMR time scale. Since the binding constants of the nucleotide anions to **1b** are in the range 10^5–10^7 L/mol and the association rate constants are likely to be close to the diffusion limit (10^8 L/(mol·s) or less,[33] the dissociation rate constants should be on the order of 10^3–10^1 s^{-1}. This means that any exchange process involving the complex dissociation can be slow on the NMR time scale and could provide the broadening. The latter has been found to increase with increasing the binding constant, being mostly pronounced for ATP. However, the broadening

(29) Aoyama, Y.; Nagai, Y.; Otsuke, J.; Kobayashi, K.; Toi, H. *Angew. Chem.* **1992**, *104*, 785; *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 745.
(30) . Aoyama, Y. *Trends Anal. Chem.* **1993**, *12*, 23.
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(34) Seno, M.; Lin, M.; Iwamoto, K. *J. Chromatogr.* **1990**, *523*, 293.

hardly reflects only shift differences or exchange between bound and unbound substrates because, first, the free/bound guest ratio was up to 10^5 and, second, the broadening was observed normally only for the signal of a nucleobase proton close to a glycosidic bond. This means there was no correlation between broadening and CIS values. In conclusion, the dynamic behavior could be due to exchange between syn- and anti-conformations of the nucleotide which is hampered in the complex and requires dissociation.

Conclusions

Aminocyclodextrins represent ditopic receptors in which the strength of the ionic binding contributions can be effectively tuned by the number of amino groups. In line with the geometrically possible number of salt bridges with sufficient contact between the charges, the electrostatic binding contributions are 13 kJ/mol for the doubly charged CyD **1a** and 20 kJ/mol for CyD **1b** with seven charges. The measurements, furthermore, allow factorization of the binding contributions by the sugar (ribose) part as 10 kJ/mol with **1a** and 13 kJ/mol with **1b**, which allows a larger number of hydrogen bonds from the protonated amine to the sugar. This, and the lower binding constants with the deoxyribose derivatives in spite of their higher hydrophobicity, indicates hydrogen bonds as the dominant driving force for the sugar complexation.

The nucleobases attached to the sugar units lead to the complexation free energies having been lowered by up to 9 kJ/mol in comparison to those of the ribosephosphate itself. The, nevertheless, observed selectivity with respect to the bases underlines that discrimination sites in such receptors may well be repulsive as long as the primary binding site (in our case the salt bridges) enforces sufficient contact at the discrimination sites. The lower selectivity of **1b** with respect to all three factors (positions of phosphate, oxy/deoxy sugar, and nucleobase) is, of course, in line with the general rule of most analytical systems in which high sensitivity goes at the expense of selectivity. Our results provide a consistent mechanistic scheme for such a behavior. In particular, the observed NMR shifts and the intermolecular NOEs show that the nucleotides are oriented in the host compound in such a way that allows simultaneous contact of the different substrate parts with complementary receptor parts. This inclusion mode is in line with force-field simulated models which also indicate that the stronger electrostatic interaction in **1b** pulls the substrates further into the cavity, with the consequence of weaker contact at the secondary interaction sites and therefore lower selectivity. In the rather unexplored field of artificial receptors for carbohydrates, this is the first direct experimental evidence by NMR of intracavity inclusion in a cyclodextrin and for the binding contribution of a ribose being stronger than that of a nucleobase.

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