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Spectrophotometric determination of binding constants between some aminocyclodextrins and nitrobenzene derivatives at various pH values

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Dedicated to Professor Domenico Spinelli on the occasion of his 70th birthday, in recognition of his outstanding contributions to physical organic chemistry

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Abstract—The inclusion capacity of three modified cyclodextrins—namely mono-(6-*N*,*N*-dimethylamino-6-deoxy)- (**3**), mono-6-(2-aminoethyl)-amino-6-deoxy- (**4**) and mono-6-(2-*N*,*N*-dimethylaminoethyl)-amino-6-deoxy- (**5**) β-cyclodextrin, with six *para*-substituted nitrobenzenes (**A**–**F**) has been investigated at three different pH values. Molecular interactions in inclusion complexes have also been investigated by means of molecular mechanics (MM2/QD) models. The desolvation of the cyclodextrin is the most important factor in determining the binding ability of the various hosts. However, for a given host, electrostatic and van der Waals interactions and the formation of a hydrogen bond between the donor amino group and the oxygen atom of a secondary hydroxyl group are the most important contributions in determining the binding constant of different guests.

1. Introduction

Cyclodextrins (CDs) are very promising materials in several fields: their actual or potential uses in pharmaceuticals, foods, cosmetics or chemicals are summarized in some recent monographs.[1] Furthermore, cyclodextrins have been extensively studied as enzyme models[2] and as reagents or catalysts for regio- and stereo-selective synthesis.[3]

Cyclodextrins are widely used as hosts to form inclusion complexes with small- and medium-sized organic molecules.[4] Complexation reactions involving cyclodextrins are highly important in drug delivery systems and also for the separation and food industries.[5] These reactions also serve as excellent models for understanding general inclusion phenomena, as well as enzyme–substrate interactions.[6] Despite a large quantity of experimental and theoretical work, less attention has been paid to the problem of the ultimate factors governing the host–guest inclusion phenomena.[7]

From a thermodynamic point of view, it has been proposed that the inclusion process can be dissected into a series of ideal steps which can be summarized as: (i) desolvation of the guest; (ii) internal desolvation (partial or total) of the host cavity; (iii) inclusion of the guest; and (iv) reorganization of the solvent pool.[8] This scheme provides the general basis to discuss the role of a given effect in the process. Furthermore, the linear relationship empirically found between enthalpy (ΔH^0) and entropy ($T\Delta S^0$) variations ('enthalpy–entropy compensation' effect)[7,9] has recently been interpreted in terms of the main role being assumed by host cavity desolvation and by the conformational changes induced on the host itself by the inclusion process.[9a,e]

The relative importance of different steps of the inclusion process changes on going from α- to γ-cyclodextrins. Moreover, it can be changed by modifying one or more functional groups (hydroxyl groups). For example, weak interactions such as electrostatic forces (one of the factors determining the step (iii) have been found to be effective in the selectivity and enhancement of binding properties in amino-β-cyclodextrin.[10] At present, only a limited number of systematic thermodynamic studies using modified cyclodextrins has been reported.[9f–g,11]

Recently,[12] we have reported data describing the ability of native β-cyclodextrin (**1**) and mono-(6-amino-6-deoxy)-β-cyclodextrin (**2**) to bind aromatic compounds having a nitro or an amino group. Electrostatic and van der Waals interactions and the formation of a hydrogen bond between the donor amino group and the oxygen atom of a secondary hydroxyl group were found to be the most important contributions in determining complex stability. In order to analyze the binding ability of modified cyclodextrins more deeply,

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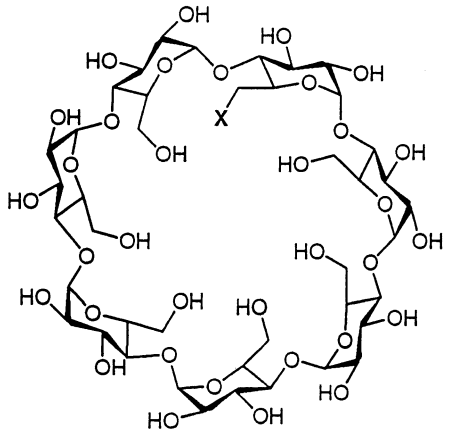

X = **1** : OH
2 : NH_2
3 : $N(CH_3)_2$
4 : $NHCH_2CH_2NH_2$
5 : $NHCH_2CH_2N(CH_3)_2$

Figure 1. Cyclodextrin hosts **1**–**5**.

we have collected data for the inclusion complexes formed between three different cyclodextrins—namely mono-(6-*N*,*N*-dimethylamino-6-deoxy)- (**3**), mono-6-(2-amino-ethyl)-amino-6-deoxy- (**4**) and mono-6-(2-*N*,*N*-dimethyl-aminoethyl)-amino-6-deoxy- (**5**) β-cyclodextrin (Fig. 1)—and six *p*-nitro benzene derivatives (**A**–**F**, Fig. 2).

The native β-cyclodextrin was modified in order to have variations in its properties, such as basicity, dipole moment, ability to act as a hydrogen bond donor and solvation. Moreover, the tail amino group in **4** and to a greater extent in **5**, could itself be included into the cyclodextrin cavity so competing with the guest. The guests were chosen in such a way to have variations in molecular properties such as, for example, dipole moment, molecular volume, ability to act as hydrogen bond donor/acceptor, hydrophobicity and solvation. Binding constants were measured spectrophotometrically at 298.1 K in aqueous phosphate buffer solution at pH 6, 8 and 11. Such values were chosen in order to avoid acid catalyzed hydrolysis of the host, and also, on the grounds of pK_a values (for **3** and **5** these have been determined in this

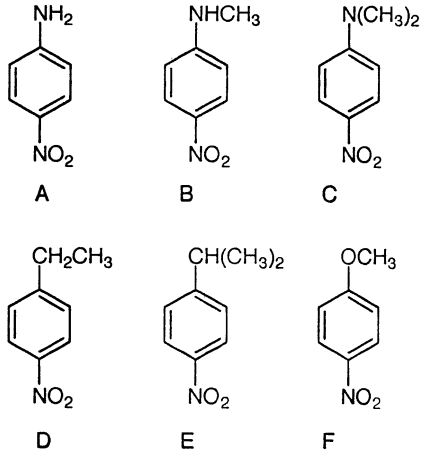

Figure 2. Nitrobenzene guests **A**–**F**.

Table 1. pK_a values and percentages of the protonated species for cyclodextrins **2**–**4**

Host	pK_{BH^+}	p$K_{BH_2^{++}}$	pH	% B	% BH^+	% BH_2^{++}
2	8.72[a]		6	–	100	
			8	16	84	
			11	100	–	
3	8.18[b]		6	–	100	
			8	40	60	
			11	100	–	
4	9.42[c]	5.33[c]	6	–	82	18
			8	4	96	–
			11	97	3	–
5	8.69[b]	4.66[b]	6	–	96	4
			8	17	83	–
			11	100	–	–

[a] From Ref. 13.
[b] This work; value reproducible within a ±5% error.
[c] From Ref. 11a.

work); **3**–**5** are allowed to pass from a medium in which they are present almost completely as free base to a medium in which they are present almost completely as conjugate acid (**3**–**5**H^+). As we had already observed, binding constants of CD change with medium acidity.[12] This has been interpreted as a consequence of variations in internal desolvation related to the charge present on host. Molecular mechanics (MM2/QD) models were also investigated to support interpretations about the trends in binding constants.

2. Results and discussion

The pK_a values for aminocyclodextrins **2**–**5** are reported in Table 1. As we can see, protonated **2**[13] and **3** and monoprotonated **4**[11a] and **5** show pK_a values ranging from 8.18 (for **3**) up to 9.42 (for **4**). It is possible to suppose that these values relate to the protonation of the primary nitrogen atom in **2** and **4** and the tertiary one in **3** and **5**, and hence methylation of amino group causes an increase in acidity of about 0.6 pK units. Diprotonated aminocyclodextrins **4**H_2^{++} and **5**H_2^{++} show pK_a values of 5.33 and 4.66, respectively. The different acidities could be a consequence of a decrease in solvation of **5**H_2^{++} due to the effect of the methyl groups present on the head nitrogen atom. On the basis of pK_a values, the actual predominant species for each pH value can be easily calculated (Table 1).

The binding constants for the different possible complexes between hosts **3**–**5** and guests **A**–**F** are reported in Table 2 and illustrated graphically in Fig. 3. Furthermore, data previously collected for hosts **1** and **2** are also reported, for a useful comparison.

The collected binding constants are not particularly high, compared to literature reports for similar guests.[7] The inclusion constant values show that aminocyclodextrins are on the whole worse hosts than native cyclodextrin **1**. Among aminocyclodextrins **2**–**5**, **3** is generally the best host, **2** is generally the worst host at pH=6 and 8, whereas at pH=11 it has binding ability comparable to **4** and **5**. The binding ability of **4** and **5** is similar to or better than that of **2**. This means that the pendant group on the primary rim is not a hindrance to the inclusion of substrate. The fact that

Table 2. Measured binding constants K (M^{-1})

pH	Guest	Host				
		1	**2**	**3**	**4**	**5**
6	**A**	380±40[a]	160±25[a]	330±30	385±25	285±45
	B	1080±80[a]	190±80[a]	605±60	590±60	610±70
	C	710±50[a]	180±50[a]	890±50	640±80	700±45
	D	400±160[a]	170±35[a]	280±60	120±35	205±70
	E	1450±65[a]	600±120[a]	695±100	400±50	520±70
	F	175±30	85±20	105±25	280±30	80±15
8	**A**	350±30[a]	250±30[a]	470±40	425±65	350±50
	B	800±80[a]	210±80[a]	685±90	630±50	640±50
	C	660±70[a]	240±50[a]	1180±100	610±25	690±80
	D	240±40[a]	170±35[a]	320±120	210±80	185±60
	E	1100±250[a]	660±50[a]	890±100	615±90	750±100
	F	205±60	195±60	215±25	335±35	225±65
11	**A**	340±30[a]	330±30[a]	730±80	260±40	300±60
	B	560±60[a]	510±60[a]	1350±110	550±70	715±90
	C	580±40[a]	550±40[a]	1140±40	650±20	645±65
	D	400±160[a]	300±160[a]	400±100	175±25	210±80
	E	1100±130[a]	780±90[a]	1250±110	475±150	615±100
	F	170±20	240±25	520±60	200±25	145±45

[a] From Ref. 12.

measured constants for **3** appear systematically higher than those found for **2** may be attributed to easier internal desolvation of the host due to the dimethylated pendant group. The binding ability of each CD changes with the acidity of the medium. For native cyclodextrin the host–guest interactions are better at pH=6 than at pH=11, in fact at this value the secondary hydroxyl groups of **1** are partially dissociated.[1a] For aminocyclodextrins, a different behavior on decreasing the pH value is shown by **2** and **3** and by **4** and **5**. For the former two hosts, the progressive protonation causes an increasing difficulty in desolvating the cavity so that a corresponding decrease is observed in binding abilities. No decrease is observed in binding abilities for **4** and **5** going from pH=11 to pH=8. Keeping in mind that these hosts should be already protonated at pH=8 (Table 1) this finding seems to confirm that the first protonation step involves the tail nitrogen atom of the pendant group. Thus, it does not affect the internal desolvation of the host cavity but may reinforce its polar character. Trends in binding constants are opposite, for **1** compared to **2–5**, as a consequence of the different charge present on hosts, confirming the already noticed modified binding ability of electrically charged cyclodextrins.[12] This behavior has been attributed to a lack of desolvation of the host.

In order to evaluate how the guest–host interactions depend on guest properties, we report in Table 3 the calculated free energies of solvation[14] for **A–F**, as well as hydrophobic substituent constants π.[15]

There is no correlation between guest solvation energies and binding constants; despite its higher solvation energy *p*-nitro-*N*,*N*-dimethylaniline (**C**, R=–$N(CH_3)_2$) is more strongly bound than both *p*-nitroanisole (**F**, R=–OCH_3) and *p*-nitroethylbenzene (**D**, R=–CH_2CH_3). It is also possible to observe a lack in correlation between hydrophobic parameters and binding constants; despite its higher hydrophobicity **D** (R=–CH_2CH_3) is included to a lesser extent than **C** (R=–$N(CH_3)_2$). Binding constant values seem to

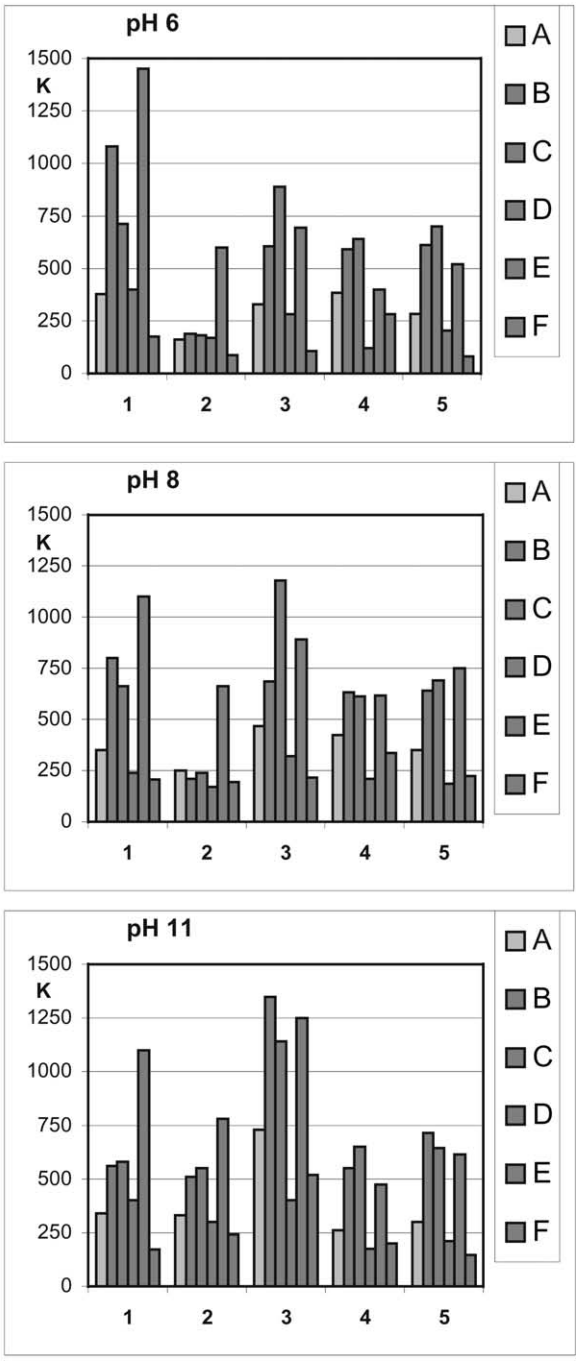

Figure 3. Histograms showing the binding constants K.

Table 3. Calculated (HF/3-21G/COSMO) free energies of desolvation and π hydrophobic substituents constants for guests **A–F**

Guest	ΔG_w^0 (kJ mol^{-1})[a]	π[b]
A	37.6	−1.51
B	36.5	−0.75
C	28.7	−0.10
D	15.9	0.74
E	15.3	1.25
F	26.2	−0.30

[a] See Ref. 14.
[b] See Ref. 15.

indicate that at least a combination of three substrate properties, i.e. hydrophobicity, ability to form hydrogen bonds as well as dipolar character, contribute to determine the entity of host–guest interaction. Data relative to alkylbenzenes **D** (R=$-CH_2CH_3$) and **E** (R=$-CH(CH_3)_2$) show that the more hydrophobic isopropyl derivative (**E**) is included more strongly than the less hydrophobic ethyl derivative (**D**) irrespective of both pH value and host. Data relative to isosteric **B** (R=$-NHCH_3$), **D** (R=$-CH_2CH_3$) and **F** (R=$-OCH_3$) compounds show a more favorable inclusion for **B** with respect to **D** and **F**. These two have a similar tendency towards inclusion, in some cases we found binding constants higher for **D** than for **F** in other cases opposite. Therefore, it seems possible to conclude that hydrophobicity has about the same effect on inclusion as the dipolar character and the ability to act as hydrogen bond acceptor. The same result can be obtained by comparing the binding constants of **C** (R=$-N(CH_3)_2$) and **E** (R=$-CH(CH_3)_2$) with hosts **3**–**5**. It is interesting to notice that for **1** and **2** we had found that, according to hydrophobic properties, **E** is included better than **C**. The different behavior of hosts **3**–**5** with respect to **1** and **2** could be attributed to the partial (**4** and **5**) or total (**3**) lack of acidic hydrogen atoms on the amino group bonded to cyclodextrin. The comparison between the binding constants of **B** (R=$-NHCH_3$) and **D** (R=$-CH_2CH_3$) points out the more relevant role played, in the inclusion process, by hydrogen bond donation than hydrophobicity.

In order to have information about the interplay between the different factors examined above and of their influence on the energetic of binding, we generated computational models of our complexes, as 'naked' isolated species in the gas phase, using the molecular mechanics MM2[16] force field. It should be remembered that the desolvation energy should control the ultimate stability of complex and determine the relative ability of any given host in inclusion process. However, for a specific host, when the desolvation energy is nearly constant, the differences in binding constants can be to attribute to specific interactions related to the structure and electronic properties of the guest. It should be also noticed that no entropic effect is taken into account by calculations. However, this could be an unimportant simplification if enthalpy–entropy compensation effect is operative. Indeed, in this case, the entropic term is linearly correlated to binding energy. The entropic term has previously been found to be unimportant for several classes of compounds when very similar averaged unit variations in $\Delta G^0$and ΔH^0 have been calculated by the effect of adding a methylene to guest. For example,[7] in the case of β-cyclodextrin, the averaged unit increments in ΔG^0 and ΔH^0 are -2.8 and -3.3 kJ mol^{-1}, respectively. This clearly indicates that although the entropic term can control the ultimate stability of the complex it can be nearly constant for similar compounds. When it is not possible to neglect the entropic term, only the Gibbs energy values can give the correct indication of relative stability of adducts. Therefore, data collected in Table 4 (see later) are, in our opinion, useful to assess the factors governing the inclusion process. We took into account nine possible hosts, namely **1**–**5** and their conjugate acid forms $\mathbf{2}H^+$–$\mathbf{5}H^+$. Consistent with the discussion reported above, we consider $\mathbf{4}H^+$ and $\mathbf{5}H^+$ as protonated exclusively on the terminal N atom of the ancillary chain. From a structural point of view, dynamic simulations (Section 4) generally predict that the guest is accommodated in the host cavity bearing its nitro group towards the primary rim, in agreement with literature reports.[17] This arrangement is consistent with the polarization of the cavity, because all the guests have a dipolar momentum with the negative side oriented towards the nitro group.

The predicted energies (ΔE_{cplx}) associated with the naked binding interaction, dissected in the contributions due to variation in steric strain (ΔE_{strain}), van der Waals (ΔE_{vdW}) and electrostatic interactions (ΔE_{elst}), are reported in Table 4.

Data confirm our previous report that, at least in the gas phase, van der Waals and dipolar–electrostatic interactions afford the main sources of energy stabilization, while the contribution from release of steric strain is much less important. Further inspection of the data reported in Table 4 reveals other interesting trends. Smaller average stabilizing contributions due to van der Waals interactions are found for complexes with neutral host **5** than with neutral hosts **1**–**4**, in agreement with a loss of interaction between the cavity and the bulky pendant group upon complex formation. Average electrostatic contributions increase in the series **2**∼**3**<**1**<**4**, according to the increasing dipolar character of the host cavity as a function of the pendant group. The average ΔE_{elst} contribution for complexes with host **5** appears to be higher. This may also be explained with the partial inclusion of the pendant group in the cavity of the isolated host, interfering with the mutual interaction of the –OH groups on the primary rim: so guest inclusion, causing the expulsion of the pendant group, allows a better organization of the hydroxyl network. Strain contributions ΔE_{strain} for hosts **4** and **5** are slightly destabilizing (different to **1**, **2** and **3**) probably because of negative effects on the conformation of the longer pendant group. Passing to the complexes with the protonated hosts $\mathbf{2}H^+$, $\mathbf{3}H^+$ and $\mathbf{4}H^+$ we notice a strong average increase in the complex stabilization energy ΔE_{cplx} that appears to be mainly due to electrostatic contributions (about 50–60 kJ mol^{-1}), while minor variations are shown in the ΔE_{vdW} values. At the same time, strain interactions become (on average) more destabilizing by an amount of about 20 kJ mol^{-1} as a consequence of the accommodation of the guests to maximize electrostatic interactions. Host $\mathbf{5}H^+$ has apparently a different trend, because comparison with **5** shows that ΔE_{elst} increases by a smaller amount, while the strain contribution becomes now more stabilizing. Probably also this finding can be attributed to the partial inclusion of the charged pendant in the isolated host, associated with an amount of steric strain that is released upon complex formation. On the other hand, contributions to electrostatic interactions are actually comparable to those for the other protonated hosts.

At the first sight, our data show no significant correlation between the binding constant values and the calculated ΔE_{cplx} binding energies. However, a detailed analysis reveals interesting aspects. If we consider only data referring to complexes with neutral hosts (i.e. complexes of **1** at all pH values and of **2**–**5** at pH 11), we notice that the calculated binding energies for guests **A** (R=NH_2) and **B** (R=$NHCH_3$) appear systematically too low. Keeping in

Table 4. Calculated (MM2) binding energies (kJ mol^{-1})

Guest	ΔE_{cplx}[a]	ΔE_{strain}[b]	ΔE_{vdW}[c]	ΔE_{elst}[d]	ΔE_{cplx}[a]	ΔE_{strain}[b]	ΔE_{vdW}[c]	ΔE_{elst}[d]
	Host **1**							
A	−121.5	−2.1	−50.0	−69.3				
B	−129.7	−1.4	−59.9	−68.2				
C	−137.9	−2.4	−70.0	−65.3				
D	−135.2	−2.6	−67.1	−65.3				
E	−143.9	−2.7	−75.0	−65.8				
F	−127.8	3.2	−66.3	−67.0				
	Host **2**				Host **2**H$^+$			
A	−120.3	−15.6	−52.4	−48.4	−177.3	7.6	−69.4	−115.4
B	−125.7	−16.3	−47.6	−61.9	−189.3	12.7	−73.0	−116.3
C	−135.9	−15.9	−72.3	−47.7	−183.8	7.2	−74.8	−116.1
D	−129.7	−10.6	−73.5	−45.2	−185.2	9.6	−74.3	−120.5
E	−137.3	−4.9	−82.0	−50.3	−190.6	−4.4	−98.1	−87.9
F	−126.5	−19.6	−61.0	−48.4	−186.8	−9.4	−93.3	−86.6
	Host **3**				Host **3**H$^+$			
A	−88.7	3.2	−42.2	−49.7	−156.7	8.7	−57.6	−108.0
B	−94.2	−10.8	−38.6	−45.0	−163.8	9.0	−64.5	−108.5
C	−102.3	−6.2	−50.9	−45.2	−164.9	9.0	−65.0	−109.0
D	−101.6	−9.2	−47.5	−45.0	−161.5	8.3	−60.9	−109.0
E	−108.0	−9.5	−53.3	−45.2	−166.3	1.4	−68.0	−98.9
F	−95.7	−4.6	−48.5	−45.0	−159.9	11.3	−63.3	−110.6
	Host **4**				Host **4**H$^+$			
A	−125.0	8.1	−59.2	−73.8	−160.5	37.5	−61.4	−136.5
B	−134.5	8.2	−70.5	−72.1	−164.3	24.7	−72.0	−116.9
C	−144.4	7.0	−81.8	−69.5	−171.0	24.8	−81.2	−114.7
D	−137.7	16.0	−79.6	−74.1	−174.9	17.2	−76.2	−115.8
E	−148.9	6.5	−88.5	−66.9	−179.9	17.8	−81.8	−115.8
F	−135.3	5.4	−71.3	−71.9	−165.7	20.5	−76.1	−112.7
	Host **5**				Host **5**H$^+$			
A	−129.6	3.6	−37.0	−96.2	−167.4	−6.1	−50.4	−111.0
B	−137.2	4.2	−46.2	−95.2	−174.2	−4.4	−59.3	−110.6
C	−144.4	2.3	−54.9	−91.8	−179.1	−2.3	−62.1	−114.8
D	−143.3	3.3	−53.9	−92.6	−181.6	−5.1	−63.0	−113.6
E	−148.0	2.5	−58.3	−92.1	−186.7	4.1	−69.5	−121.2
F	−138.0	0.7	−46.3	−94.9	−175.3	−10.2	−59.2	−106.9

[a] Binding stabilisation energy (Section 4).
[b] Contribution to ΔE_{cplx} due to steric (bond length, bond angle and dihedral angle) strain.
[c] Contribution to ΔE_{cplx} due to van der Waals interactions.
[d] Contribution to ΔE_{cplx} due to electrostatic (dipole and charge) interactions.

mind that these guests are characterized by the most unfavorable desolvation energy and the least hydrophobic character, but also by the fact that they are the only hydrogen bond donors, we deduce that probably the lack in the calculation method of any explicit term describing hydrogen bond interactions may be the source of the observed fault. Indeed if the fault were due to effects related to solvation or hydrophobicity, we should expect to find the calculated energies systematically too high. With cationic guests, however, the ΔE_{cplx} values appear erratic and show absolutely no statistically significant correlation with ΔG^0 data. Nonetheless, it is interesting to notice that the calculated ΔE_{cplx} values appear systematically more favorable by an amount of about 30–60 kJ mol^{-1} with respect to those for the corresponding 'neutral' complexes, despite the fact that in comparison 'cationic' complexes show a lower (hosts **2** and **3**) or a similar (hosts **4** and **5**) stability. This is in agreement with the idea of more difficult desolvation of the cationic host. However, it is clear that the calculation methodology used is not apt to describe adequately cationic systems. In our opinion, a possible reason for this fault probably lies in the omission of an explicit solvent environment. It should also be remembered that the anionic phosphate buffer probably plays an important role. Clearly, a molecular simulation including a counterion and an explicit solvent environment may be expected to give more correct results, but it would also make computation unreasonably time-consuming.

3. Conclusions

Data reported in this work show that the inclusion process depends strongly on medium pH as well as the host structure. In particular, dimethylation of nitrogen atom of **2** causes an increase in the binding ability of cyclodextrin cavity whereas an analogous variation is not observed when methylation of primary nitrogen atom of **4** is carried out. The data collected herein seems to indicate that cyclodextrin desolvation is the most important factor in determining the binding ability of host. For a given host, however, the selectivity of substrate inclusion depends on van der Waals and electrostatic interactions as well as, where possible, on hydrogen bond between substrate and cyclodextrin. The

binding constant values, like the calculated (MM2/QD) binding energies, indicate that there is no obvious hierarchy among the factors (except for the not very important steric strain) that govern the inclusion process.

4. Experimental

4.1. Materials

Commercial **A**,**D**,**E** and **F** (Fluka, Aldrich) were purified by crystallization or distillation before use; **B** and **C** were prepared according to literature reports.[18] β-CD **1** (Fluka) was dried in a desiccator in vacuo over phosphorus pentoxide at 90°C for at least 24 h, and then used as such. Mono(6-amino-6-deoxy)-β-CD **2** was prepared according to literature reports;[19] cyclodextrins **3**,**4**[20] and **5** were prepared as follows: mono(6-iodo-6-deoxy)-β-CD[21] (4.98 g, 4 mmol) was allowed to react with a large excess (about 20 mmol) of the proper amine in dry pyridine (30 ml) at 60°C for 18 h. The solvent was then removed in vacuo and the residue was chromatographed on silica gel using a butanol–methanol–water–30% aq. ammonia (6:2:1:1) mixture as eluent. Fractions containing the desired product were collected and concentrated, and the residue was dissolved in water (20 ml) and filtered; the filtrate was concentrated in vacuo and crystallized from water–methanol–diethyl ether to afford the pure product (yield 60–80%).

4.1.1. Mono(6-*N*,*N*-dimethylamino-6-deoxy)-β-cyclodextrin (3). White powder; mp 200–205°C (decomp.). ^{1}H NMR (250 MHz, D_2O): δ 2.31 (s, 6H), 2.67–2.78 (m, 1H), 2.90 (br d, 1H, J=10.8 Hz), 3.43 (br t, 1H, J=9.4 Hz), 3.43–3.63 (m, 14H), 3.70–4.03 (m, 25H), 4.99 (br s, 7H). Anal. calcd for $C_{44}H_{75}NO_{34}$: C 45.48, H 6.51, N 1.22. Found: C 45.35, H 6.59, N 1.20.

4.1.2. Mono-6-(2-*N*,*N*-dimethylamino-ethyl)-amino-6-deoxy-β-cyclodextrin (5). White crystals; mp 203–206°C (decomp.). ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.19 (s, 6H), 2.38 (t, 2H, J=5.7 Hz), 2.64 (t, 2H, J=5.7 Hz), 2.77 (dd, 1H, J=5.7, 12.3 Hz), 2.97 (br d, 1H, J=12.3 Hz), 3.29–3.55 (m, 15H), 3.55–3.80 (m, 25H), 4.52 (br s, 6H), 4.68 (d, 7H, J=2.7 Hz), 5.77 (br s, 14H). ^{1}H NMR (250 MHz, D_2O): δ 2.27 (s, 6H), 2.50–2.60 (m, 2H), 2.71–2.95 (m, 3H), 3.06 (br d, 1H, J=11.5 Hz), 3.43 (br t, 1H, J=9.4 Hz), 3.51–3.72 (m, 14H), 3.80–4.00 (m, 25H), 5.06 (br s, 7H). Anal. calcd for $C_{46}H_{80}N_2O_{34}$: C 45.85, H 6.69, N 2.32. Found: C 45.71, H 6.81, N 2.27.

Samples of the hosts for binding constant measurements were dried before use, keeping them for three days in a desiccator in vacuo over phosphorus pentoxide at 55°C, and were then stored in the same apparatus at 40°C.

All other commercial reagents and materials needed were used as such without further purification. Stock phosphate buffer solutions were prepared according to literature reports and used within a few days, after checking the actual pH value with a PHM82 Radiometer equipped with a GK2401C combinated electrode. Freshly double-distilled water was used for the preparation of the buffers, which were used as solvents for the preparation of the measurement solutions.

All fitting analyses were performed by means of the KALEIDAGRAPH™ 3.0.1 software delivered by Abelbeck Software.

4.2. Measurements of pK_a

(i) A precisely weighed amount (about 0.0464 g, 40 μmol) of **3** were introduced in a water-jacketted vessel thermostated at 298.1±0.3 K and were dissolved in a 0.0025 M standardized HCl solution (20 ml) under magnetic stirring. A stream of fine argon bubbles was passed for 15 min through the solution, which was then titrated with a 0.1 M standardized NaOH solution introduced into the vessel by a microsyringe. The titration was performed following the pH value with the apparatus described above. Data were finally processed fitting the pH vs. added base curve by means of the proper equation obtained analytically. (ii) A weighed amount (about 0.0482 g, 40 μmol) of **5** were dissolved with a 0.005 M standardized HCl solution (20 ml) and titrated with a 0.2 M standardized NaOH solution as just described.

4.3. Measurement of binding constants

Solutions for measurements were prepared at a fixed concentration of guest (usually about 20 μM) and at a concentration of host ranging from 0 to about 6 mM, and were filtered through a Gelman ACRODISC CR PTFE (0.45 μm) microfilter before use. UV–Vis spectra were recorded at 298.1±0.3 K on a Beckmann DU-7 spectrophotometer, and presented good isosbestic points. A suitable wavelength was chosen after recording a 'difference spectrum' by comparison of the samples without cyclodextrin and with the highest cyclodextrin concentration. The absorbances of the different solutions at the work wavelength were processed by direct non-linear regression analysis.[22]

4.4. Calculations

HF/3-21G/COSMO calculations were performed by means of the Gaussian 98[23] software from the Gaussian Inc.; MM2 calculations were performed by means of the CS Chem3D Pro™ 5.0 software package from the CambridgeSoft Corporation. Models of the hosts and of their complexes were elaborated with the aid of the 'Quenched Dynamics' (QD) method outlined by Lipkowitz.[24] The behavior of a suitable starting model of the complex at 300 K is simulated by molecular dynamics for a period of 1000 ps, in order to get a significant picture of the conformational space. Structures are sampled from the obtained 'simulation pool' and allowed to undergo full geometry optimization by simulated annealing. In this way, only a limited number of energy minima are found. Data in Table 4 refer to the absolute minimum found for each complex.

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