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Induced Circular Dichroism and Structural Assignment of the Cyclodextrin Inclusion Complexes of Bicyclic Azoalkanes

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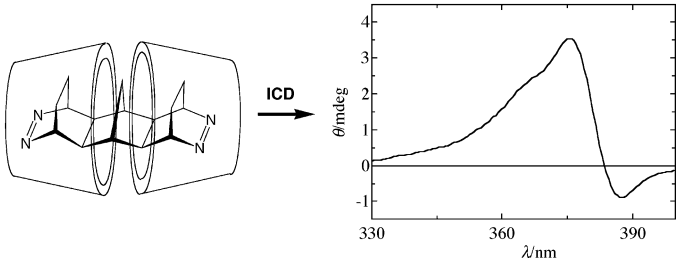
Received September 8, 2004



The stoichiometries and binding constants of the host−guest complexes between the bicyclic azoalkanes **1**−**6** and α-, *β*-, and *γ*-cyclodextrins (CDs) and the induced circular dichroism (ICD) of the complexes were analyzed. Assisted by proximity relationships obtained from 2D ROESY NMR spectra, the signs and intensities of the ICD spectra are interpreted in terms of the solution structures (co-conformations) of the CD complexes. The ICD assignments are based on the orientation-intensity ICD rules of Harata and Kodaka, which relate the ICD signs and intensities to the relative orientation of the electric dipole transition moment of the n,*π** azo chromophore to the CD axis. The influence of the size of the guest and the host is discussed and the effect of introducing an additional chromophore (either a phenyl or a second azo group) on the ICD spectra is demonstrated.

Introduction

Induced circular dichroism (ICD) is a sensitive spectroscopic tool used to study the solution structures of achiral chromophoric guests with chiral host molecules.[1−4] Spectral changes caused by the inclusion of guest molecules are frequently more exaggerated in circular dichroism compared to UV−vis spectrophotometry. The method is particularly useful for the structure analysis of natural α-, *β*-, and *γ*-cyclodextrins (CDs) as inherently chiral hosts. The structural information obtained from ICD complements the information from X-ray/neutron diffraction[5] and that from NMR techniques,[6] which are restricted in that they refer to the solid state or in that they yield only proximity relationships in solution. Ultimately, a detailed knowledge of the solution structures of host−guest complexes, as can be obtained by ICD, may improve the understanding of molecular recognition phenomena in enzyme−substrate interaction or catalysis, and it may help to advance structure−activity relationships in supramolecular chemistry.

The ICD of bicyclic azo compounds in cyclodextrins has recently been investigated.[7−12] The fascination for studying such simple *cis*-azoalkanes arises from the fact that

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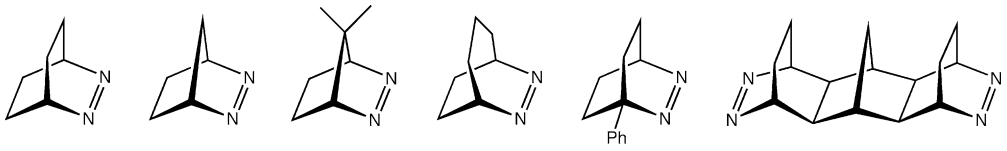
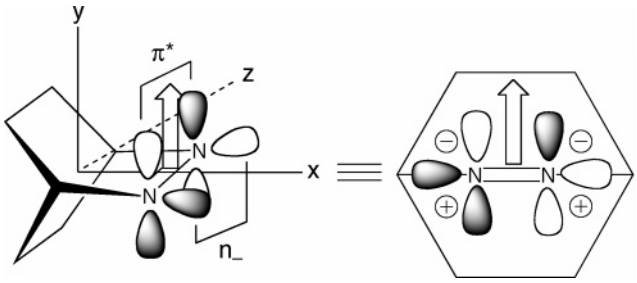
10.1021/jo048420k CCC: $30.25
Published on Web 12/03/2004

CHART 1



1 **2** **3** **4** **5** **6**

SCHEME 1



the azo group (−N=N−) is one of the smallest and simplest chromophores, but in contrast to the carbonyl group (which could compete in size) it possesses a nonvanishing electric dipole transition moment ("is allowed"), which facilitates interpretations of ICD spectra.

The diazirines[7,8,12] and derivatives of 2,3-diazabicyclo-[2.2.2]oct-2-ene (**1**),[9−11] which have been examined so far, were sufficiently small to be deeply immersed in their 1:1 host−guest complexes. Moreover, from the direction of the electric dipole transition moment of the azo near UV n_,π^* transition, which is directed along the azo π orbitals[9] (arrows along the y axis in Scheme 1), the solution structures can often be assigned by ICD,[2,3] namely the relative orientation of the guest with respect to the host. The latter has been referred to as the "co-conformation" of the host−guest complex.[13]

We have shown[9−11] that the rules of Harata[1] and Kodaka[14] (Scheme 2) can be successfully applied in these cases. This was of considerable interest since previous structural assignments based on ICD have been made for aromatic chromophores with π,π^* electronic configuration while that of aliphatic azo compounds is n,π^*. In the present work, we selected the bicyclic azoalkanes **1**−**6** as guest molecules to investigate large structural variations on the complexation by cyclodextrins. In addition, and in contrast to our previous studies, we have also varied the size of the host by using α-, β-, and γ-cyclodextrin, which are composed of 6, 7, and 8 α-D-glucose units.[3]

Experimental Section

Materials. Compounds **1**−**5** were synthesized according to literature procedures.[15−18] They were purified by sublimation (only **1**−**4**), followed by recrystallization. Compound **6** was a generous gift from Prof. S. F. Nelsen;[19] this compound was purified by sublimation before use. α-, β-, and γ-cyclodextrins were purchased from Fluka and used without purification. Deuterium oxide (>99.8%) was purchased from Glaser AG, Basel, Switzerland.

Spectroscopic Measurements. All experiments were performed at ambient temperature in D_2O. Generally, experiments were performed with 4 mM (ICD) or 2 mM (UV, NMR) solutions of the particular azoalkane. Due to limited solubility, we used only 0.5 mM of **5** and **6** for all measurements. ICD spectra were obtained with a circular dichrograph (0.2-nm resolution, 10 accumulations) by using a blank water−CD solution without guest for background correction. UV spectra were obtained with 0.1-nm resolution. ^{1}H and 2D ROESY NMR spectra were analyzed with the software package MestReC;[20] the 2D ROESY spectra were obtained for saturated solutions.

Computational Studies. All calculations were carried out with the Hyperchem package.[21] The AMBER-S parametrization for saccharides was employed.[22] The azoalkanes were kept frozen in the AM1-optimized geometries.

Results

The complexation behavior of azoalkanes **1**−**6** by α-, β-, and γ-CD complexes has been analyzed by UV spectrophotometry and by ICD and NMR spectroscopy.[11,23] We have consistently employed D_2O as solvent, also in the UV and ICD experiments, to be consistent with the NMR studies, and to exclude variances due to solvent isotope effects, which are well-known in the complexation by CDs.[24]

Formation of Inclusion Complexes. The bicyclic azoalkanes show a bathochromic shift in the UV absorption spectra with the enlargement of the cycle,[25] e.g., from 340 nm for **2**, to 365 nm for **1**, to 375 nm for **4** (all in water). Complexation by CD induced also a bathochromic shift, e.g., by ca. 6−8 nm in β-CD·**1**−**4** and by 1.5 nm in β-CD·**5**; an example is shown in Figure 1. The UV bathochromic spectral shifts of the azo absorption along with the NMR upfield shift of the guest protons, both observed upon addition of CD, signaled the formation of the respective host−guest inclusion complexes. The complexation-induced solvatochromic shift is due to the altered microenvironment, namely the increased polarizability inside the cavity,[26] while the NMR shift is characteristic for the shielding by the aliphatic host upon inclusion of the guest.[6]

2D-NMR ROESY experiments were performed to produce more detailed information on the mode of associa-

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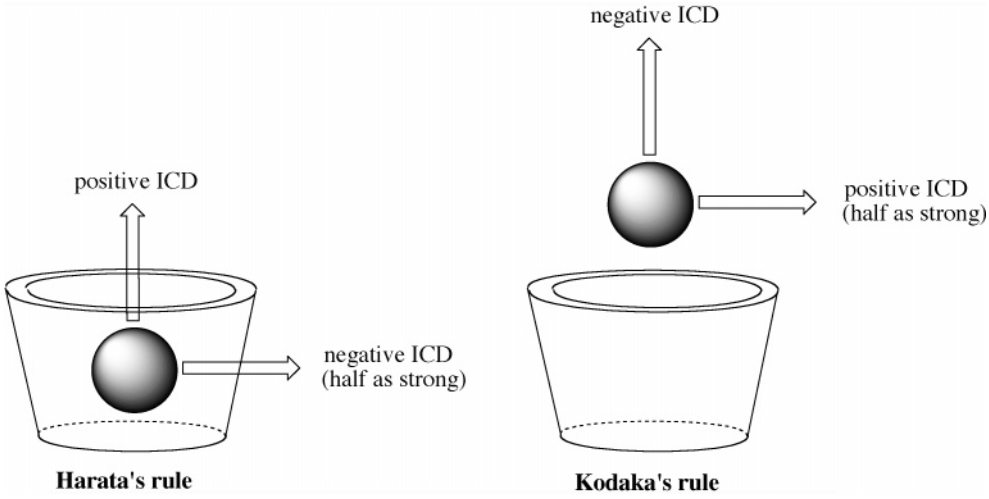
SCHEME 2[a]



[a] Expected ICD signs and relative magnitudes in dependence on the direction of the electric dipole transition moment (shown as arrows).

tion and the inclusion geometry of the complexes with the bichromophoric guests **5** and **6**. The 2D ROESY spectra of the β-CD inclusion complex of **6** (mixture of 1:1 and 2:1 complexes) revealed cross-peaks between the H-3 and H-5 protons of CD and all protons of **6**, as expected for a deeply immersed symmetrical guest. The 2D-ROESY spectra of the complex **5**·β-CD (Figure 2) are in line with a preferential complexation and deep inclusion of the phenyl group; there are strong cross-peaks of the aromatic protons with H-5 and H-6, but the cross-peak with H-3 is much stronger for the ortho than for the meta and para protons, signaling a deep inclusion where the ortho protons reside near H3, cf. Scheme 3. It should be noted that the meta and para proton resonances merge to a multiplet upon complexation, but are separate in the uncomplexed form (500 MHz), thereby allowing unambiguous peak assignments in the aromatic region. The cross-peaks between H-3 and H-5 and the anti and syn protons of the bicyclic substructure of **5** are much weaker, suggesting that the azo bicycle is positioned outside or near the upper rim of the CD.

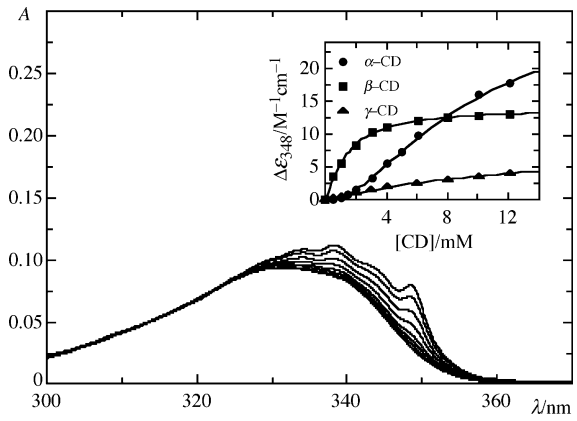


FIGURE 1. UV titration of azoalkane **3** (2.0 mM) by α-CD. The inset shows the corresponding UV titration plot and fitting at λ = 348 nm according to a 2:1 complexation model (eq 2) for α-CD and according to a 1:1 complexation model (eq 1) for β-CD and γ-CD.

Stoichiometry of Complexes. In the majority of the cases, an isosbestic point was observed for the UV titration, e.g., at 372 nm for azoalkane **5** (see inset of Figure 3), which is consistent with a 1:1 complexation mode. Exceptions were the complexation of azoalkane **3** by α-CD and **6** with β-CD, for which the competitive formation of 2:1 complexes (with a single guest and two CDs) could be confirmed through spectroscopic titration plots (see below). The stoichiometry of the complexes was further confirmed through ^{1}H NMR Job plot analysis,[27,28] namely for the **5**·β-CD and **6**·β-CD complexes. For this purpose, the complexation-induced chemical shift of the bridgehead guest protons ($\Delta\delta H_b$) was plotted against the [guest]/([guest] + [host]) concentration ratios. The resulting continuous variation plots (Figure 4) revealed maxima at 0.5 and 0.4, in line with a 1:1 complexation stoichiometry for **5** and a 2:1 complexation pattern for **6**. The maximum for **6** falls somewhat above the theoretical value of 0.33,[28] which can be rationalized in terms of the small second binding constant compared to the first one (130 versus 17 000 M^{-1}, see Table 1) and the low concentrations employed, which in turn were restricted

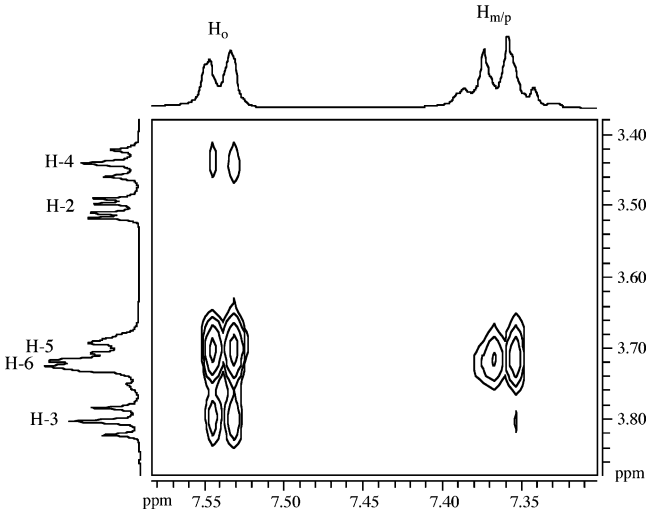


FIGURE 2. Diagnostic region of the ROESY NMR spectrum for the β-CD·**5** complex.

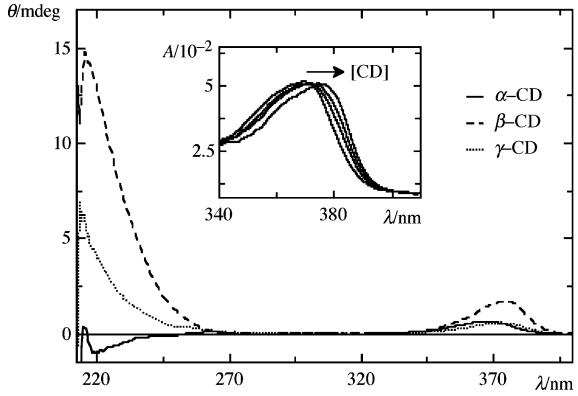


FIGURE 3. ICD spectra of azoalkane **5** (0.5 mM) in the presence of CDs (12 mM). The inset shows the corresponding UV titration plot in the presence of β-CD.

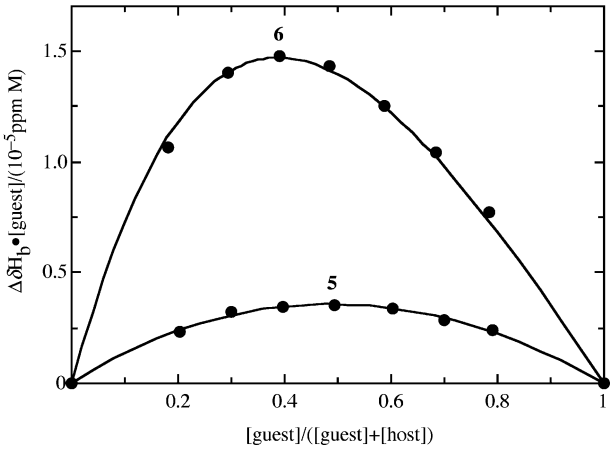
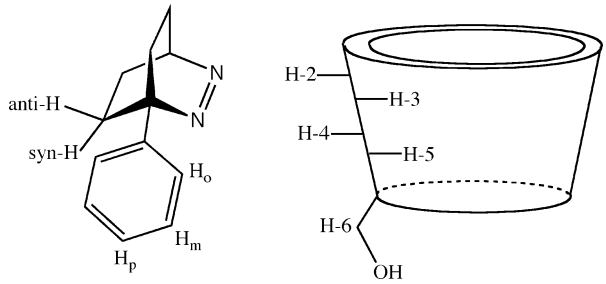


FIGURE 4. Job plot showing the normalized variation of the complexation-induced shift of the bridgehead protons of azoalkanes **5** and **6** in the presence of β-CD versus molar ratio.

SCHEME 3



by the solubility of azoalkane **6** (0.5 mM). Both nonideal conditions can result in a shift of the Job plot maximum away from the theoretical value.[28]

Spectroscopic Titrations and Binding Constants. The complexation-induced UV or NMR shifts were employed to determine the binding constants with α-, β-, and γ-CD by spectroscopic titration methods (see inset of Figure 1). In the case of 1:1 complexation, eq 1 was employed in a nonlinear data fitting procedure.[29] In detail, the change in the absorption maximum ($\Delta\epsilon_{obs}$, eq 1a, for **1**, **2**, **3**, and **4**) or the chemical shift of the particular protons ($\Delta\delta_{obs}$, eq 1b, for **5**) at a constant guest concentration, $[G]_0$, were plotted against the total CD concentration, $[CD]_0$, to afford the binding constants (Table 1).

TABLE 1. Binding Constants of the CD·1−6 Complexes from UV, ^{1}H NMR, and ICD Titrations[a]

	K (M^{-1})		
azoalkane	α-CD	β-CD	γ-CD
1[b]	50	1100	6
2[c]	30	190	∼0
3[c]	80 [20][f]	1000	70
4[c]	10	800	30
5[d]	230	2800	60
6	∼0[d]	17000 [130][e,f]	240[d]

[a] The error in the data is 10%. [b] From refs 11 and 23. [c] UV titration. [d] ^{1}H NMR titration. [e] ICD titration. [f] Binding constant for formation of the 2:1 complex given in square brackets.

$$\Delta\epsilon_{obs} = \frac{(\epsilon_{CD\cdot G} - \epsilon_G)[G]}{[G]_0} \quad (1a)$$

or

$$\Delta\delta_{obs} = \frac{(\delta_G - \delta_{CD\cdot G})[G]}{[G]_0} \quad (1b)$$

with

$$[G] = \frac{K[G]_0 - K[CD]_0 - 1 + \sqrt{(K[G]_0 + K[CD]_0 + 1)^2 - 4K^2[G]_0[CD]_0}}{2K}$$

Complexation of azoalkane **3** by α-CD was accompanied by a number of peculiarities, which included the absence of an isosbestic point, the development of vibrational fine structure in the absorption band (Figure 1), and unusually large molar ellipticities (Table 2). The formation of a 2: 1 complex needed to be invoked in this case, as well as for complexation of **6** by β-CD, since the titration plots could not be satisfactorily fitted by eq 1 (Figure 5). A nonlinear fitting procedure according to a 1:1 and 2:1 complexation pattern was used to determine the binding constants K_1 and K_2 in these cases (Table 1).

Note that the 2:1 fitting process requires the nontrivial numerical solution of the system of coupled equations in eq 2, since an analytical solution[30] is not available. Several programs have been applied for specific examples[31−34] and a few general programs to solve the problem have also been described.[35,36] In the present work, we have used both the coupled-equation module implemented in the program ProFit[29] as well the cubic-equation method[7,33] to obtain reproducible and self-consistent data. Experimentally, UV-spectrophotometric titration plots (eq 2a) were advantageous for the complexation of azoalkane **3** by α-CD due to the large changes in the emerging fine-structured bands (Figure 1), while for the azoalkane **6**·

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TABLE 2. ICD Effects[a] and Molar Ellipticities[b] of CD·1−6 Complexes

	α-CD		β-CD		γ-CD	
azoalkane	θ (mdeg)	Δε (mdeg M^{-1} cm^{-1})	θ (mdeg)	Δε (mdeg M^{-1} cm^{-1})	θ (mdeg)	Δε (mdeg M^{-1} cm^{-1})
1	14	0.31	20	0.17	1.5	0.17
2	5.0	0.15	16	0.19	0.0	
3	44	0.79 [2.06][c]	−11	−0.09	2.6	0.07
4	5.0	0.04	3.5	0.03	6.5	0.19
5[d]	0.7	0.06	1.7	0.11	0.6	0.09
6[d]	0.0		3.5 [−0.6][c,e]	0.65 [−0.07][c,e]	2.5	0.24

[a] ICD effect obtained in D_2O with 12 mM host and 4.0 mM guest unless stated differently. [b] Molar ellipticity calculated as $\Delta\epsilon = \theta/(32982\ l\ c)$, cf. ref 37 with l = path length and c = actual concentration of complex in solution calculated by using the binding constants in Table 1. [c] The molar ellipticity of the 2:1 complex, obtained by fitting of the titration data according to a 2:1 complexation model (eq 2), is given in square brackets. [d] 0.5 mM guest due to limited solubility. [e] Two signals with opposite signs, cf. Figure 5.

β-CD complex ICD titrations produced the best results (eq 2b, Figure 5), because the 1:1 and 2:1 complexes have opposite and therefore very characteristic ICD effects (see below). In both cases, the concentration of guest was kept constant to allow plots of the spectral shifts against the total cyclodextrin concentration, $[CD]_0$.

$$\epsilon_{obs} = \frac{\epsilon_G[G] + \epsilon_{CD\cdot G}[CD\cdot G] + \epsilon_{CD_2\cdot G}[CD_2\cdot G]}{[G]_0} \quad (2a)$$

or

$$\theta_{obs} = \frac{\theta_{CD\cdot G}[CD\cdot G] + \theta_{CD_2\cdot G}[CD_2\cdot G]}{[G]_0} \quad (2b)$$

with

$$[G] = [G]_0 - [CD\cdot G] - [CD_2\cdot G]$$

$$[CD\cdot G] = \frac{1 + Y + Z - \sqrt{(1 + Y + Z)^2 - 4YZ}}{2K_1}$$

$$[CD_2\cdot G] = \frac{K_2[CD\cdot G][CD]_0 - K_2[CD\cdot G]^2}{1 + K_2[CD\cdot G]}$$

where

$$Y = K_1([CD]_0 - [CD_2\cdot G]) \text{ and } Z = K_1([G]_0 - [CD_2\cdot G])$$

Induced Circular Dichroism. The evolution of the ICD spectra of azoalkanes **1**−**6** upon addition of α-, β-, or γ-CD was also analyzed to obtain information on the complex geometries. The signs and intensities of the ICD signals as well as molar ellipticities are compiled in Table 2. The absolute intensity of the ICD effects can be directly compared in terms of the molar ellipticity values which extrapolate the observed ICD effects to quantitative complexation. The ICD effects were very different among the various guest molecules (e.g., as shown for β-CD in Figure 6) and the type of CD (e.g., as shown for azoalkane **3** in Figure 7). In the case of azoalkane **3** with α-CD and **6** with β-CD, ICD titrations were performed to obtain independent values for the binding constants and to corroborate the 2:1 stoichiometry and, thus, to complement the results from NMR and UV titrations.

Force-Field Calculations. Further insights into the specific modes of inclusion of azoalkanes **1**−**6** in cyclodextrins were obtained by structure optimizations with the AMBER force field according to our previously described protocol.[9,10] Force-field calculated structures have been demonstrated, for azoalkane **1** and one bridgehead-alkylated derivative, to be qualitatively consistent with experimental ICD effects,[9] while the quantitative analysis required the consideration of the entire ensemble of energetically accessible conformations.[10] Since we were well aware from the previous studies that the careful evaluation of complex stabilities required more detailed work than the simple energy minimizations available within the scope of the present experimental study, we employed the present calculations merely to

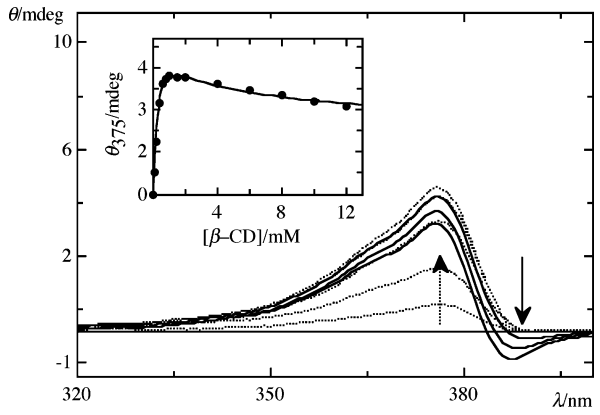


FIGURE 5. ICD titration of the bis-azoalkane **6** (0.5 mM) with β-CD. The inset shows the corresponding titration plot and fitting at λ = 375 nm according to a 2:1 complexation model (eq 2).

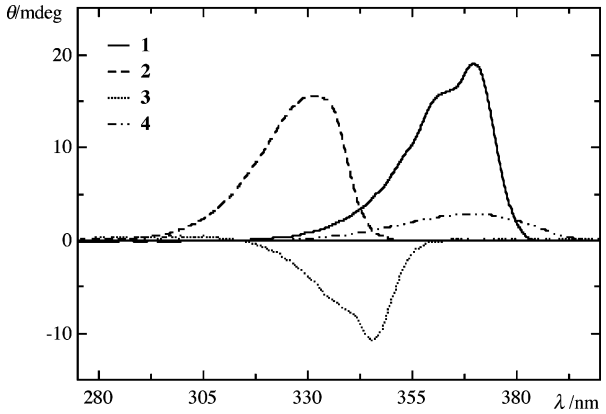


FIGURE 6. ICD spectra of azoalkanes **1**−**4** (4.0 mM) in the presence of β-CD (12 mM).

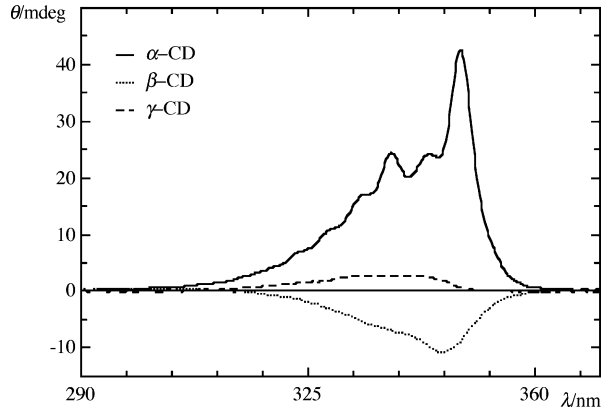


FIGURE 7. ICD spectra of azoalkane **3** (4.0 mM) in the presence of different CDs (12 mM).

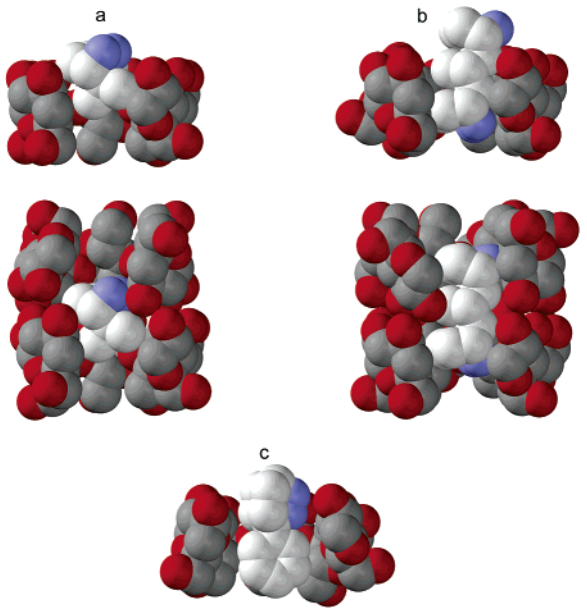
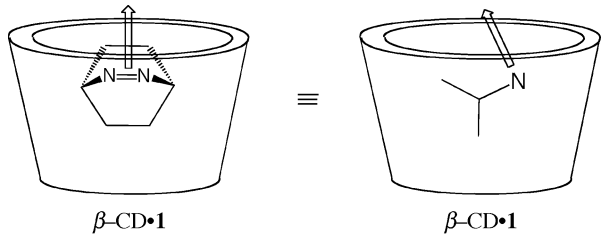


FIGURE 8. Representative AMBER-optimized CD inclusion complex structures shown in cross section; hydrogen atoms are omitted for clarity: (a) 1:1 and 2:1 complexes of azoalkane **3** with α-CD, (b) 1:1 and 2:1 complexes of azoalkane **6** with β-CD, and (c) 1:1 complex of azoalkane **5** with β-CD.

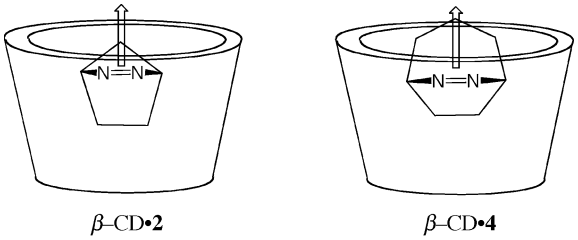
corroborate experimental findings and interpretations, but refrained from making predictions or assigning co-conformations solely on the basis of force-field calculations.

Some key structures relevant for the present interpretations are shown in Figure 8. Most importantly, the calculations confirmed the high propensity of the *gem*-dimethyl group in azoalkane **3** to protrude into the α-CD cavity, which is retained in its 2:1 complex (structure a, Figure 8). For azoalkane **6**, it is noteworthy that the 1:1 complexation results in a somewhat tilted inclusion geometry, while the formation of the 2:1 complex induces an orientation more parallel to the imaginary axes of the host molecules (structure b, Figure 8). Finally, the phenyl group of azoalkane **5** was shown to have a higher affinity toward complexation than the bicyclic azo group (structure c, Figure 8), and in this case the guest protruded also deepest into the host cavity.

SCHEME 4



SCHEME 5



Discussion

We have previously studied the β-CD complexes of azoalkane **1** and its bridgehead-substituted derivatives.[9,11] This study has now been extended to the smaller α-CD and the larger γ-CD as host molecules. In addition, we varied the guest by selecting the smaller 2,3-diazabicyclo[2.2.1]hept-2-ene **2** and its 7,7-dimethyl derivative **3**, the larger 2,3-diazabicyclo[2.2.3]non-2-ene **4**, the 1-phenyl derivative **5**, and the bis-azoalkane **6**. The azoalkanes **5** and **6** are of special interest since they possess two chromophores, which could respond differently to complexation by the host. Generally speaking, two different modes of complexation appeared viable: a 1:1 complexation with a deep immersion of the entire guest or one chromophoric residue of the guest and a 2:1 complexation in which the guest is "shared" by two CDs.

Monochromophoric Azoalkanes. The monochromophoric derivatives **1**−**4** and their β-CD complexes will be discussed first. We have previously demonstrated, based on ICD and NMR experiments as well as molecular dynamics calculations, that azoalkane **1** prefers a lateral co-conformation in its β-CD complex (Scheme 4), unless the steric demand of bridgehead substituents induces a frontal co-conformation.[9−11] The driving force for formation of the lateral conformation derives from the better hydrophobic interaction for immersing the nonpolar ethano bridge into the cavity as opposed to inclusion of the azo group, which retains interactions with the water molecules near the upper rim region. Note that the electric dipole transition moment in the lateral co-conformation is oriented approximately along the CD axis (Scheme 4). A positive ICD effect is expected in this case (Scheme 2), as experimentally observed (Table 2). The positive ICD effects observed for the β-CD complexes of azoalkanes **2** and **4** (Table 2) can be understood in terms of a lateral co-conformation (Scheme 5) as well, although in these cases it cannot be readily differentiated whether the smaller or larger bridge protrudes into the β-CD cavity. Force-field calculations indicate a higher stability for inclusion of the ethano bridge for both derivatives with β-CD, suggesting that the methano bridge of **2** or the propano bridge of **4** is too small or too large, respectively, to promote a good fit. Note also the

lower binding constants of **2** and **4** relative to **1**, which support the improved goodness-of-fit for azoalkane **1**.

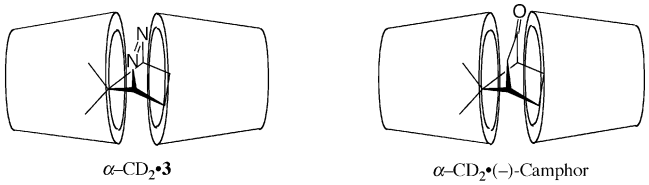
The negative ICD for the 7,7-dimethyl derivative **3** with β-CD (Table 2) stands out and suggests a significant change of the co-conformation in this complex. The dimethylmethylene group is the most hydrophobic part of the molecule and most likely to protrude into the β-CD cavity, a notion that is confirmed by force-field calculations (Figure 8). There are two alternative interpretations of the observed negative ICD effect of azoalkane **3** with β-CD. The first one would be that a deep immersion may no longer apply for **3** as a consequence of steric repulsion between the dimethylmethylene group and the walls of β-CD, which become narrower when the guest protrudes deeper into the cone-shaped cavity. This could cause a position of the azo group above the upper rim, i.e., outside the inner cavity; Kodaka's rule (Scheme 2) would then apply, which predicts the opposite ICD sign and could therefore account for the experimental result. This interpretation is disfavored by the fact that the 1:1 inclusion complex of **3** with α-CD shows a strong positive ICD (see below) even though the α-CD cavity is smaller and thus should cause the steric effect to become more pronounced, which should force the azo group outside the inner cavity of α-CD as well. The second interpretation takes into account that the tilt angle of the electric dipole transition moment relative to the CD axis in the lateral co-conformation is small but quite significant (30°, cf. Scheme 4), such that small alterations of the size and substituents on the bridge, which could affect this angle, could well increase this tilt angle and invert the sign of the ICD even for a deep inclusion complex.

The ICD effects for the γ-CD complexes of **1**−**4** are all weak and positive. Since γ-CD is sufficiently large to form inclusion complexes with all azoalkanes, and since the lateral co-conformation was found to be preferred in β-CD, we propose the same situation for γ-CD as well. Also of interest are the binding constants, which in contrast to the smaller β-CD, follow the order **4** > **1** > **2** as expected from the size and the hydrophobicity of these guests. Note that the smallest and most hydrophilic guest **2** does not show a significant binding with γ-CD at all (Table 1), and therefore produces no ICD signal.

Exclusively positive ICD effects are observed for α-CD as well, except for azoalkane **6**, which is apparently too bulky to bind significantly to this smallest host. The very strong ICD for the 7,7-dimethyl derivative **3** (Figure 7) is particularly noteworthy. In fact, UV and ICD titrations suggest the formation of a 2:1 complex for **3**, which did not need to be invoked in any other α-CD case. For example, an isosbestic point was not observed upon addition of α-CD to **3** (Figure 1). The complexation of **3** by α-CD also gave rise to the observation of a fine structure (Figures 1 and 7), which was not observed in the other cases. Hydrogen bonding is known to cause a band broadening of azo absorption bands,[26] such that the emerging fine structure can be interpreted in terms of an efficient removal of water molecules, e.g., through formation of a tight 2:1 complex.[26]

The complexation behavior of azoalkane **3** is reminiscent of the situation for the structurally related molecule

SCHEME 6



camphor,[24,38,39] which forms a 2:1 complex with α-CD as well. The *gem*-dimethyl group appears to have a high propensity for complexation with α-CD on its own, while leaving the remainder of the guest molecule sufficiently exposed to be complexed by a second α-CD. The prediction of ICD signs for 2:1 complexes cannot be achieved by means of the classical ICD intensity rules for 1:1 complexes (Scheme 2), such that no structural assignments can be reliably based on ICD. Molecular mechanics simulations (Figure 8) do confirm the favorable inclusion of the *gem*-dimethyl group in both the 1:1 and 2:1 complexes to give rise to the structures depicted in Scheme 6. In the case of camphor, a similar complex structure has been suggested on the basis of NMR longitudinal and transverse relaxation rates for the methyl protons of the corresponding 2:1 complex.[39]

Bichromophoric Azoalkanes. Azoalkanes **5** and **6** are the largest and most hydrophobic guests, as indicated by their restricted water solubility (ca. 0.5 mM), which accounts for the largest binding constants obtained for the 1:1 complexes of **5** and in particular **6** (Table 1); an exception is α-CD, which is apparently too small to complex azoalkane **6**.

The azo n,π^* absorption band of the phenyl-substituted azoalkane **5** shows a less pronounced bathochromic shift upon β-CD complexation than that of azoalkanes **1**−**4**. In contrast, **5** has a significantly larger binding constant with β-CD (2800 M^{-1}) than the previously studied derivatives of **1** (ca. 1000 M^{-1}).[11] This enhancement cannot be explained in terms of a change in the co-conformation of the azo chromophore within the cavity since different co-conformers of **1** display similar binding constants.[11] Finally, bulky bridgehead substituents are known to promote a frontal alignment and should give rise to a negative ICD effect,[9−11] which is not the case for azoalkane **5** (Table 2).

The combined results suggest that the mode of complexation varies for **5** since the more hydrophobic phenyl group preferably protrudes into the cavity. This conclusion is supported by the ICD of the β-CD complex (Figure 3). The ICD spectrum shows a positive signal at 375 nm and below 270 nm, which correspond to the n,π^* band of the azo chromophore and the ^1L_a band of the phenyl group, respectively.[40] Preferential binding of the phenyl group results in a complex in which the azo chromophore is located outside the cavity, since the molecular length of **5** (ca. 10 Å) exceeds the depth of CD (8 Å), cf. Scheme 7. This conformational assignment is experimentally confirmed by 2D ROESY NMR experiments, which show

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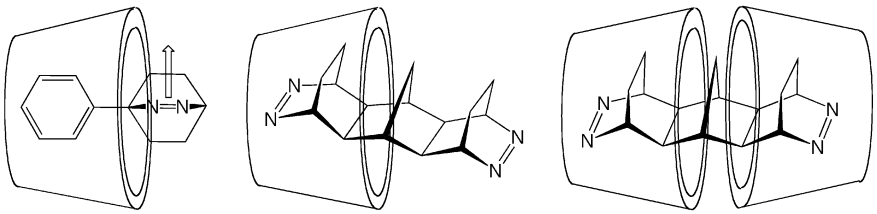
SCHEME 7



very strong cross-peaks for the aromatic protons and the inner CD protons. The small bathochromic shift of the azo group in comparison to the other azoalkanes also suggests a position outside the inner cavity. Kodaka's rule (Scheme 2) therefore applies to the azo chromophore, which predicts a positive ICD effect for the azo group, since the electric dipole transition moment lies outside and is oriented perpendicular to the cavity axis (arrow in Scheme 7). On the other hand, Harata's rule now holds for the transitions of the deeply immersed aromatic chromophore. The transition moment of the ^1L_a transition points along the CD axis, in agreement with the strong positive ICD band in the far-UV region. The azo-group-derived ICD effects for **5** in α-CD and γ-CD are also positive (Table 2) and, therefore, can be explained by assuming similar co-conformations (Scheme 7), but the negative ICD effect of the α-CD complex in the far-UV region (ca. 220 nm) remains unaccounted for (Figure 3).

The ICD spectrum of the bis-azoalkane **6** with β-CD gives rise to a distinct pattern with a very strong positive peak and a weaker negative signal at longer wavelengths (Figure 5). Again, the guest is too large (ca. 10 Å) to be completely immersed such that one or both azo groups may reside partially outside the β-CD cavity. Motivated by the interpretation of the ICD spectrum of a related bischromophoric azo compound (bis-diazirine),[8] which shows two bands with different signs as well, we considered the possibility that the bands with opposite signs originate from one azo group lying inside the cavity and the other one outside, which could readily explain this pattern due to the expected inversion of ICD signs (Scheme 2); in this case, the bathochromically shifted negative ICD band should correspond to the immersed chromophore due to the characteritic solvatochromic shift of the azo absorption band.[10,11]

The detailed ICD analysis revealed, however, that this tempting explanation cannot apply for azoalkane **6**, because the stronger positive ICD band at 375 nm first increases up to a β-CD concentration of ca. 1.5 mM, but *decreases* again at higher concentrations (Figure 5). This decrease of the positive band is accompanied by a bathochromic shift and, more importantly, an emerging weak negative band at longer wavelength. This peculiar titration behavior clearly indicates the presence of a second species, assigned to the 2:1 complex, which emerges at higher β-CD concentration and gives rise to the negative ICD band. The bathochromic shift of the negative band is in line with this interpretation because the 2:1 complex offers a better protection from the solvent. The strong positive band is consequently assigned to the ICD signal of the 1:1 complex, which decreases with increasing β-CD concentration. It should be noted that the titration behavior also excludes an interpretation of the observed band splitting in terms of exciton coupling between the two chromophores, which is also not indicated in the UV spectra of the free guest.[41,42]

The ICD effects for azoalkane **6** cannot be convincingly interpreted in terms of the orientation-intensity rules (Scheme 2), since the complexation geometry of this elongated guest molecule (Scheme 7) places the electric dipole transition moment between a perpendicular and parallel orientation, where definitive assignments cannot be made; these are further complicated by the presence of a second chromophore.

Conclusions

In summary, the complexation of bicyclic azoalkanes by cyclodextrins in aqueous solution occurs with medium to weak binding constants, and in two cases the competitive formation of 2:1 complexes could be quantitatively analyzed. The signs and intensities of the ICD effects of the azo n,π^* transition vary strongly with ring size and substitution pattern of the azoalkane as well as with the type of cyclodextrin employed. In limiting cases, the co-conformations of the complexes can be assigned on the basis of Harata's and Kodaka's rules, in combination with NMR data. In the majority of cases, the azo compounds appear to adapt a deeply immersed lateral conformation. For bichromophoric systems, competitive complexation of one chromophore and steric effects determine the solution structures of the complexes.

Acknowledgment. The authors thank Prof. S. F. Nelsen for providing a sample of compound **6**. This work is part of the Swiss National Research Program "Supramolecular Functional Materials" (NRP 47).

JO048420K

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