Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Influence of Host's Substitution on the Orientation of the Guest: Pseudo-rotaxanes of Charged Cyclodextrins with Methyl Orange in Solution

Nikolaos Mourtzis[a]; Kyriaki Eliadou[a]; Konstantina Yannakopoulou[a]
[a] Institute of Physical Chemistry, National Center for Scientific Research "Demokritos", Athens, Greece

Online publication date: 29 October 2010

To cite this Article Mourtzis, Nikolaos , Eliadou, Kyriaki and Yannakopoulou, Konstantina(2004) 'Influence of Host's Substitution on the Orientation of the Guest: Pseudo-rotaxanes of Charged Cyclodextrins with Methyl Orange in Solution', Supramolecular Chemistry, 16: 8, 587 — 593
To link to this Article: DOI: 10.1080/10610270412331317530
URL: http://dx.doi.org/10.1080/10610270412331317530

Supramolecular Chemistry, December 2004 Vol. 16 (8), pp. 587–593

Taylor & Francis
Taylor & Francis Group

Influence of Host's Substitution on the Orientation of the Guest: Pseudo-rotaxanes of Charged Cyclodextrins with Methyl Orange in Solution

NIKOLAOS MOURTZIS, KYRIAKI ELIADOU and KONSTANTINA YANNAKOPOULOU*

Institute of Physical Chemistry, National Center for Scientific Research "Demokritos", Aghia Paraskevi 15310, Athens, Greece

Received (in Southampton, UK) 7 June 2004; Accepted 7 September 2004

Methyl Orange (MO), an azo-dye molecule with an inherent dipole moment, has been used as a probe to explore the influence of anionic or cationic substituents of cyclodextrins (CDs) on the mode of insertion to form pseudorotaxanes, using NMR spectroscopy. MO is oriented in a single mode inside the βCD cavity, with the dimethylamino group localized at the secondary side. This orientation is completely reversed when MO enters the anionic sodium *heptakis*[6-deoxy-6-(3-thiopropionate)]-βCD (βpsp) cavity, whereas inside the cationic *heptakis*(6-deoxy-6-amino)-βCD hydrochloride, MO flips once more, to adopt the same orientation as in βCD. In the latter case the water solubility of MO is significantly lowered. The disposition of the guest in βCD and in each βCD derivative in a single mode was attributed principally to anti-parallel dipole–dipole stabilization. In the wider γCD, the availability of more cavity space leads to 1:2 and 2:2 host/guest stoichiometries and the effect of dipoles is of secondary significance. In the anionic sodium *octakis*[6-deoxy-6-(3-thiopropionate)]-γCD, MO is positioned as in βpsp, but a 1:2 adduct is also detected. Finally, MO does not dissolve in *octakis*(6-deoxy-6-amino)-γCD hydrochloride solution.

Keywords: 2D NMR; Methyl orange; Anionic–cationic cyclodextrin; Anti-parallel dipoles

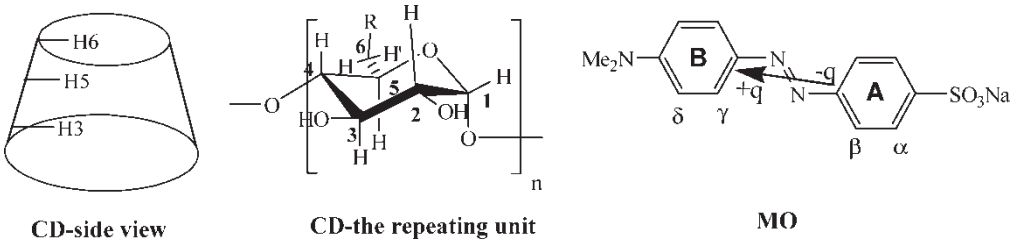
INTRODUCTION

The features that influence the structures of cyclodextrin (CD) [1] pseudorotaxanes in the solution using NMR spectroscopy and in the solid state using X-ray crystallography have been a subject of study in our laboratory [2–5]. Of particular interest has been the investigation of CD pseudorotaxanes with conjugated end-disubstituted guest molecules [6]. In the present work, a non-symmetrically end-disubstituted conjugated molecule, methyl orange (MO), is studied as the insert in natural CD cavities (Scheme 1) as well as in synthetically modified CDs bearing either anionic or cationic groups on their primary (narrow) side. The guest molecule has the additional characteristic of possessing an overall dipole moment along its extended axis [7]. Our point of interest was to investigate whether the charge on the host can dictate the orientation of MO inside the cavity.

MO ($pK_a = 3.4$) is a common azo-dye indicator molecule known to form inclusion complexes with CDs, as studied previously mainly by spectrophotometric methods. In the crystalline state [8] the 2:1 host–guest complex formed with αCD, was characterized by high disorder of the guest. In solution, using UV–Vis spectroscopy, the αCD/MO complex has been regarded as a 1:1 species alone [9,10] or in coexistence with the 2:1 adduct [11]. Based on kinetic measurements, other researchers [12] have suggested that MO is included in αCD with its $-SO_3^-$ group exiting the primary side. No 2D NMR data exist to describe the structure in solution. With βCD, there seems to be an agreement that only the 1:1 complex is present, [9–11,13] however, no NMR data exist to unequivocally describe the orientation of MO, either. Finally, γCD is generally accepted to form a strong 1:2 host–guest complex, [9,11,14] and detailed kinetics support a mechanism [14] where a weak 1:1 complex slowly draws up a second MO molecule to become a 1:2 adduct, which eventually develops into a 2:2 species by threading one more γCD molecule. Again, structural evidence from NMR data is not available.

*Corresponding author. E-mail: dyanna@chem.demokritos.gr

ISSN 1061-0278 print/ISSN 1029-0478 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10610270412331317530



SCHEME 1 General structure of the cyclodextrin hosts (βCD:R = OH, n = 7; γCD:R = OH, n = 8; βpsp:R = SCH_2CH_2COONa, n = 7; γpsp:R = SCH_2CH_2COONa, n = 8, βpNH_2:R = NH_2, n = 7; γpNH_2:R = NH_2, n = 8) and methyl orange (MO).

We focused on using NMR spectroscopy in aqueous solution to determine the structures of the complexes between MO and β- and γCD, as well as the anionic CDs, sodium *heptakis*[6-deoxy-6-(3-thiopropionate)]-βCD (βpsp), and sodium *octakis*-[6-deoxy-6-(3-thiopropionate)]-γCD (γpsp) and cationic, *heptakis*(6-amino-6-deoxy)-βCD hydrochloride (βpNH_2) and *octakis*(6-amino-6-deoxy)-γCD hydrochloride (γpNH_2). We anticipated that the different charges on the host and the direction of their associated dipole would influence the orientation of the guest.

RESULTS AND DISCUSSION

The NMR experiments were conducted at pH 7.3, where the natural CDs are not charged (pKa of αCD is 12.3) [15] and MO and βpsp, γpsp exist as the corresponding sodium salts. The experiments with per-amino cyclodextrins, βpNH_2 and γpNH_2, were carried out at pH 6.0 where they are mostly protonated [16]. Preliminary investigation of the ^{1}H NMR spectra of αCD/MO revealed very broad signals of both guest and host protons at room temperature, not much influenced by changing the temperature from 278 K to 313 K. We chose, therefore, not to pursue αCD/MO further since clear and detailed data could be collected from complexes of β- and γ-CD and their derivatives, which all fell within the fast exchange condition.

βCD/MO COMPLEX

2D ROESY NMR experiments of βCD/MO, gave a clear picture of the non-covalent interactions between host and guest molecules. The N-methyl protons of MO show (Fig. 1a) very strong correlation with the cavity protons H3 only, immediately showing that this group is positioned at the secondary side of the host.

Similarly, protons H_δ of ring B (Fig. 1b) display very strong interaction with H3 and very weak with H5, supporting the above orientation. In line with these, protons H_γ show a weaker cross-peak with H3 than with H5, but nothing with H6,6′, indicating that these protons approach the primary side but are located below the primary rim. Protons H_β and H_α of ring A of MO give a very strong and a very weak correlation, respectively, with H6,6′ only. The above data is consistent with an unambiguous orientation of MO inside the βCD cavity: the NMe_2 group is at the secondary face, ring B is completely inside, the azo-bridge is also inside but toward the primary face, and ring A is completely outside the primary side in the aqueous medium (Scheme 2a).

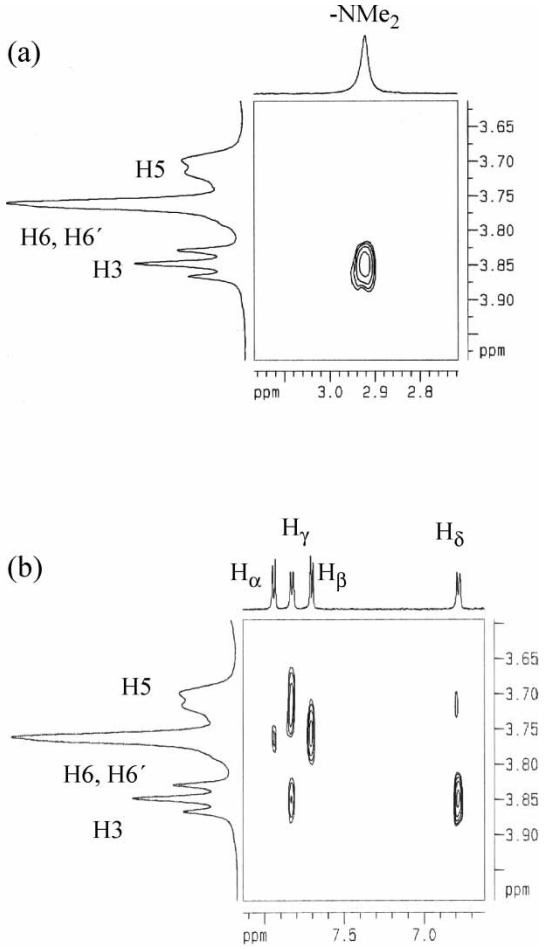
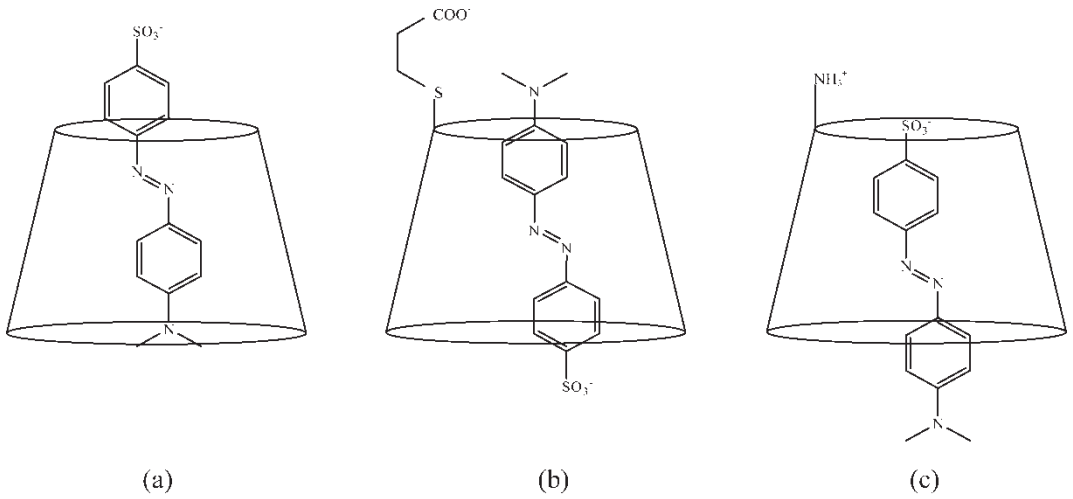


FIGURE 1 2D ROESY spectrum of the βCD/MO complex (pH = 7.3, 298 K), (a) aliphatic and (b) aromatic region.



SCHEME 2 Proposed structures of (a) βCD/MO, (b) βpsp/MO, (c) βpNH$_2$/MO.

When excess MO was used, there was no evidence for dimerisation of the guest, unlike Congo Red [4] or other azo-dyes, [11] which strongly aggregate in solution. Besides, no interaction between the NMe_2 group and protons H_α–H_γ was found, no dependence of the ^{1}H NMR chemical shifts of MO on its concentration (0.3 mM to 3.0 mM), and no evidence for 2:1 host/guest complex, even at concentration ratio 12 mM/3 mM. The observed structure in solution is due to a single complex, that is, unlike previous examples of aliphatic end-disubstituted guests, [2] MO has a unique direction inside βCD and no orientational isomerism is observed. A possible stabilization of the $-SO_3^-$ group of the guest, through extensive hydration from the aqueous medium explains only why one MO ring is included, resulting in a complex rather than a pseudorotaxane. What seems to be of definite importance is that the guest orients itself inside the cavity with its dipole moment anti-parallel to that of βCD. We have previously demonstrated [17] by semi-empirical MO calculations that βCD has a dipole moment directed from the secondary towards the primary side, the value of which varies from 2.9 Debye to 14.9 Debye, depending upon the orientation of the primary hydroxyl groups relative to the cavity axis. Earlier computational work has shown [18] that aromatic guests should enter the CD cavity with their dipole moment anti-parallel to that of cyclodextrins. Screening of several of the published crystal structures of 1:1 βCD complexes confirms that host-guest anti-parallel dipoles are found in many of them, as in βCD/*n*-nonanoic acid, [19] βCD/*trans*-cinnamic acid, [20] βCD/t-butyl benzoic acid, [21] βCD/m-nitroaniline, [22] although the strong tendency of βCD to form dimers and crystal packing forces are very important factors in the solid that do not exist in solution and may dominate over dipole considerations. Such an example is the crystal structure of 4-amino-4′-nitrobiphenyl/βCD, [23] where the nitro-group is located at the secondary side, but host *and* guest molecules exist as dimers, stable entities that make up the crystal. Therefore the consequence of the dipole–dipole interactions could be amplified and better assessed, if a charged host is used, such as the anionic βCD derivative, considered next.

βpsp/MO COMPLEX

Sodium *heptakis*[6-deoxy-6-(3-thiopropionate)]-βCD (βpsp), negatively charged at neutral pH, also forms a complex with MO. It was found that protons H_γ and H_δ of ring B show cross-peaks with H5 (Fig. 2a) and weaker with H6, H6′ as well as with the $-SCH_2-$ and $-CH_2COO^-$ protons of the thiopropionate substituent (Fig. 2b). No correlation is found between βpsp and protons of ring A.

Finally, the NMe_2 group shows medium interactions with H5 and $-SCH_2-$ and weak with $-CH_2COO^-$, suggesting that extension of the cavity beyond H6,6′ has become useful in surrounding MO. The resulting complex is expected to have a 1:1 stoichiometry due to size restriction. The ROESY data show that the $-NMe_2$ group is outside the "normal" cavity, among the methylene arms of the primary side, ring B as well as the azo-bridge are inside the cavity but toward the secondary side, and ring A practically out in the aqueous medium. (Scheme 2b).

This positioning of MO can be easily explained if the repulsive electrostatic forces between the $-SO_3^-$ group (MO) and $-COO^-$ groups (βpsp) are taken into consideration, together with the overall electrostatic stabilization through favorable anti-parallel orientation of dipoles, in agreement with the argument invoked previously. In fact, the dipole

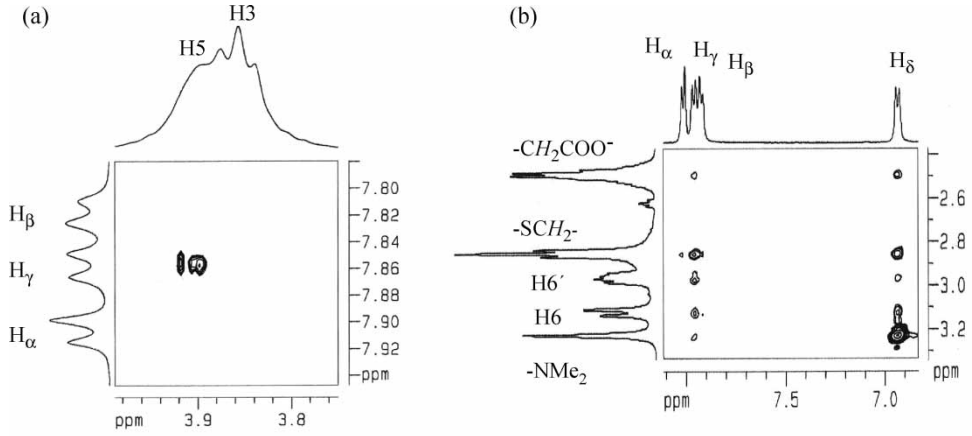


FIGURE 2 2D ROESY spectrum of the βpsp/MO complex (pH = 7, 298 K) at (a) 5 mM/5 mM and (b) 10 mM/1 mM ratios.

moment of βpsp was calculated (MOPAC, AM1) to be approximately −8 Debye, i.e. quite strong and opposite in direction of that of βCD. For the calculation, the thiopropionate substituents were clustered along the cavity axis, similarly to the reported X-ray structure of γpsp in a very stable drug complex [24]. The observed orientation of a charged guest, directed by the introduction of charged groups on the host's structure can be a useful tool in the design of supramolecular systems.

βpNH$_2$/MO

In the presence of the cationic βpNH$_2$ hydrochloride at pH 6 and 7, the solubility of MO was dramatically reduced to a value much lower than that of the free MO (∼4 mM). As a consequence, the guest peaks' integrals were about 3–8% of those of the host and the corresponding 2D ROESY spectra showed weak cross-peaks. Correlation was observed (Fig. 3) between H_α and both cavity protons H3 and H5, whereas H_β and H_γ nearly overlapped and interacted with H3 only. No cross-peaks were observed between H_δ, NMe_2 and protons of βpNH$_2$. This weak but definite data show that the small amount of MO that is retained in solution has its ring A inside the βpNH$_2$ cavity with the sulphonate group at the periphery of the amino-groups, which leaves the aromatic ring B completely outside the secondary face. Therefore, the orientation of MO is reversed compared to βpsp, and is similar to that in βCD but here ring A is included as opposed to ring B. The very small solubility of MO in the aqueous solution of βpNH$_2$ observed in the present case is reminiscent of the precipitation of proteins by the addition of amine salts in their solution.

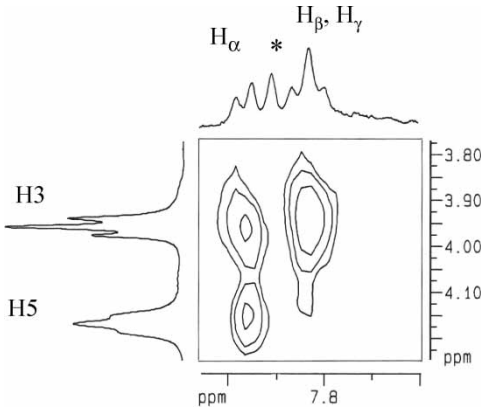


FIGURE 3 2D ROESY spectrum of the βpNH$_2$/MO complex (pH = 6, 298 K), *unremovable Ph_3PO impurity.

γCD/MO COMPLEX

When MO was added to an aqueous solution of γCD significant shifts of the cavity protons were observed, a clear indication of inclusion, as expected. Surprisingly, H6 and H6′ appeared at different chemical shifts with gradual increase of MO concentration, indicating that there is a clear preference regarding their direction relative to the cavity. The 2D ROESY NMR spectra (Fig. 4) show that protons H_α interact strongly with H3 and moderately with H5, showing that they are inside the cavity and close to the secondary side. Both protons H_β and H_γ show strong correlation with H3 and H5 and medium with H6 and H6′, which is unexpected for a 1:1 complex. Moreover, protons H_δ have weak correlation with H3 and stronger with H5. Lastly, the $-NMe_2$ protons interact with both H3, H5 of γCD (Fig. 4b) but also with H_α and H_β of ring A (Fig. 4a), clearly a result of dimerisation of MO inside the cavity.

If a head-to-tail π−π-stacking arrangement occured we would observe cross-peaks among aromatic protons of rings A and B, [4] at concentrations as high as 30 mM or above but there are no such signals. This obvious host–guest 1:2 stoichiometry is not the only one present. In the continuous

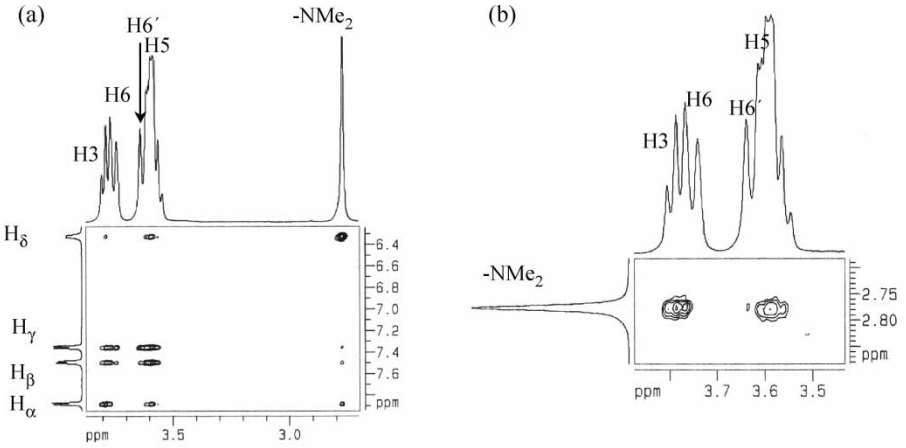


FIGURE 4 2D ROESY spectrum of the γCD/MO complex (pH = 7, 298 K).

variation plots (Fig. 5a) of the three relevant γCD protons, the chemical shift changes of H3 give a maximum at a ratio [γCD]/[MO] = 1:2 and those of H5, H6′ at 1:1. The corresponding plot for the MO protons (Fig. 5b) has a maximum at 1:1 ratio for H_α, whereas for H_β–H_δ and —NMe_2 it shows a mixed stoichiometry between 1:1 and 1:2.

Similarly the plateau in the titration plot appears at [γCD]/[MO] ratio greater than 1:1 for H3 and exactly at 1:1 for H6′ (Fig. 6). Based on all available data we propose that the first MO molecule is placed with the —NMe_2 group completely outside the primary side and the azo-linkage and ring A directed to the secondary side. The second MO molecule adopts a head-to-tail orientation with respect to the first one, with its —NMe_2 end inside the cavity, allowing the methyl protons to interact with H_α and H_β of the first MO, thus the rest of the second MO molecule remains outside (Scheme 3a).

Considering the size of the host and the length of the guest, in combination with the predominant 1:2 structure of Scheme 3a, additional complexation of a second γCD ring to afford a 2:2 structure is visualized in Scheme 3b. This would nicely correspond to the Job plot data regarding the 1:1 ratio and would account for the non-uniform behavior of the protons in the titration and the observed NOE correlations. Besides, kinetic measurements have shown previously [14] the 1:2 and 2:2 stoichiometry for this system.

γpsp/MO COMPLEX

Similar experiments were carried out with the *octakis*[6-deoxy-6-(3-thiopropionate)]-γCD (γpsp). As with βpsp, here also chemical shift changes of the thiopropionate chain protons were observed, in addition to those of cavity protons H3, H5, and H6′. A line broadening effect disturbed both the host and the guest signals at room temperature resulting in poor spectral resolution. Cooling at 283 K increased the broadening of peaks. Therefore all experiments were conducted at 313 K, where the signals of H_γ and H_δ were broad singlets and those of H_α and H_β relatively sharp doublets. The corresponding continuous variation plots showed a nearly 1:1 stoichiometry, when observing the γpsp shifts (Fig. 7a) and protons of ring A, whereas protons of ring B showed a host:guest ratio nearly 1:2 (Fig. 7b).

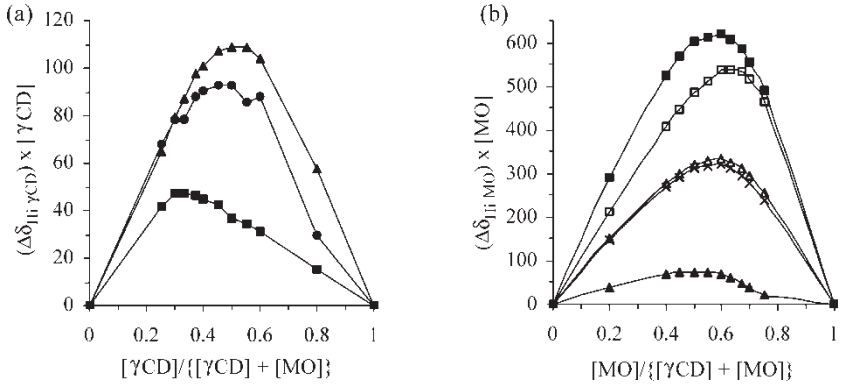


FIGURE 5 Continuous variation plots for the γCD/MO complex [(a) ■: H3, ▲: H5, ●: H6′, (b) ▲: H_α, Δ: H_β, □: H_γ, ■: H_δ, x: —NMe_2].

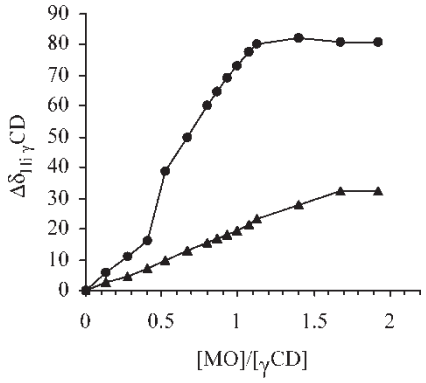


FIGURE 6 Molar ratio plot for the γCD/MO complex (▲: H3, ●: H6′).

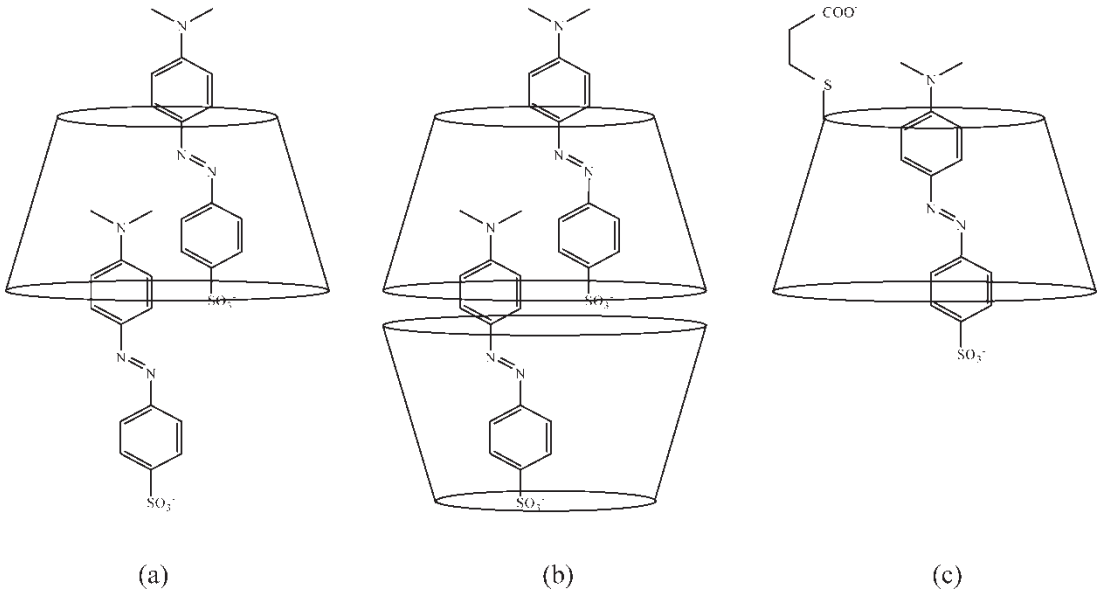
The above data indicate that the main complexation event leading to a 1:1 adduct, is followed by a second process to a 1:2 adduct mostly involving ring B of MO. The 2D ROESY interactions are generally weaker than those observed in the previous complexes, expected under the unfavorable temperature conditions (313 K) and the marginally fast rate of exchange that governs the various processes. Thus H_α protons correlate weakly only with H3. Protons H_β give a stronger cross-peak with H3 than with H5 (Fig. 8), indicating that ring A is placed at the secondary side. Protons H_γ correlate with H5, H6, H6′ and —SCH_2— and only weakly with H3, whereas H_δ interact with H5, H6′ and —SCH_2—. Ring B is thus located at the primary side and beyond. Increased flexibility of the substituent arms of the larger γpsp apparently prevents the development of an observable noe effect with the —NMe_2 group.

The above information sums up a picture where MO is positioned in the γpsp cavity with the dimethylamino-end at the primary side (Scheme 3c), as in βpsp. A second, practically not-included, MO molecule participates to form a 1:2 host:guest adduct, as we proposed for the γCD 1:2 complex above. Finally, the solubility of MO in the presence of γpNH2 was very low and no data were collected.

EXPERIMENTAL

βCD, γCD and Methyl Orange (MO, 96% dye content) were commercially available. Buffered aqueous solutions were prepared using Na_2HPO_4 and NaH_2PO_4 in D_2O to maintain pH 6 (βpNH_2·HCl), 7 (βpsp, γCD, γpsp and γpNH_2·HCl) or 7.3 (βCD). The NMR spectra were recorded on 500 MHz instrument at 298 K (βCD, βpsp, βpNH_2, γCD and γpNH_2) or 313 K (γpsp). For the 2D ROESY NMR spectra 300–350 ms mixing times were used at transmitter attenuation of ~30 dB using βCD, γCD, βpsp, γpsp, βpNH_2 and γpNH_2 solutions of [host]/[guest] 2:4, 30:26, 5:5 (and 10:1), 10:10, 5:5 and 2:2 mM, respectively, were used. Titrations were carried out by adding solid MO to a 30 mM solution of γCD in buffered D_2O. For the continuous variation plots solutions of 4 mM in each γCD and MO were mixed to create ten solutions with proportions from 80% to 25% for γCD and 20% to 75% MO, respectively.

Heptakis(6-deoxy-6-amino)-βCD hydrochloride (βpNH_2), and *octakis*(6-deoxy-6-amino)-γCD hydrochloride (γpNH_2), were prepared according to the literature [25,26]. Thus the per-bromo derivatives were prepared from the parent βCD and γCD in 96% and 80% yield, respectively. Then the corresponding per-azido derivatives were prepared in 98% and 64% yield, respectively which were reduced to βpNH_2 (78%)



SCHEME 3 Proposed structures of (a) γCD/MO 1:2, (b) γpsp/MO 2:2, (c) γpsp/MO.

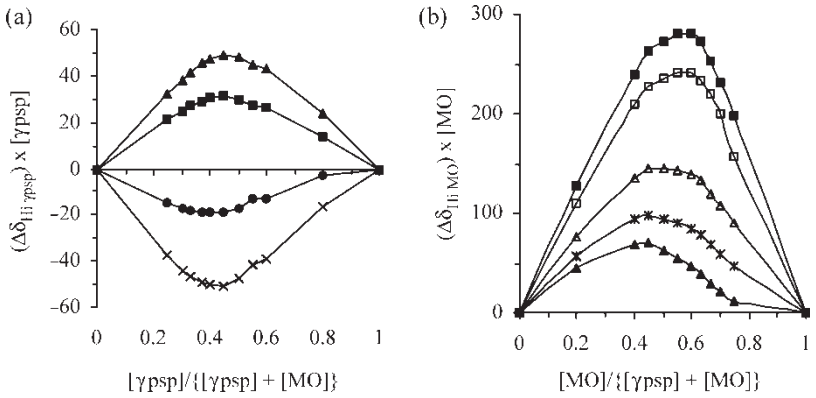


FIGURE 7 Continuous variation plots for the γpsp/MO complex [(a) ■: H3, ▲: H5, ●: H6′, x: —CH_2S—, (b) ▲: H_α, Δ: H_β, □: H_γ, ■: H_δ, x: —NMe_2].

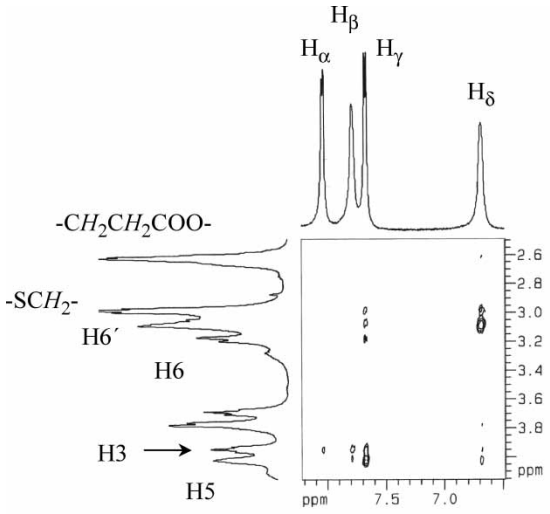


FIGURE 8 2D ROESY spectrum of the γpsp/MO complex (pH = 7, 313 K).

and γpNH_2 (80%). Sodium h*eptakis*[6-deoxy-6-(3-thiopropionate)]-βCD (βpsp) and sodium *octakis*[6-deoxy-6-(3-thiopropionate)]-γCD (γpsp) were synthesized as reported [27] and dialyzed to afford pure βpsp (57%) and γpsp (73%).Their ^{1}H- and ^{13}C-NMR and ESI-MS data agreed with literature data.

References

[1] *Chem. Rev.* **1998**, *98*, issue dedicated to cyclodextrins.
[2] Eliadou, K.; Yannakopoulou, K.; Rontoyianni, A.; Mavridis, I. M. *J. Org. Chem.* **1999**, *64*, 6217.
[3] Yannakopoulou, K.; Mavridis, I. M. *Curr. Org. Chem.* **2004**, *8*(1), 25.
[4] Mourtzis, N.; Cordoyiannis, G.; Nounesis, G.; Yannakopoulou, K. *Supramol. Chem.* **2003**, *15*(7–8), 639.
[5] Giastas, P.; Mourtzis, N.; Yannakopoulou, K.; Mavridis, I. M. *J. Inclus. Phenom. Macrocyclic Chem.* **2002**, *44*, 351.
[6] Giastas, P.; Yannakopoulou, K.; Mavridis, I. M. *Acta Crystallogr.* **2003**, *B59*, 287.
[7] Rinuy, J.; Piron, A.; Brevet, P. F.; Blanchard-Dasce, M.; Girault, H. H. *Chem. Eur. J.* **2000**, *6*(18), 3434.
[8] Harata, K. *Bull. Chem. Soc. Jpn.* **1976**, *49*(6), 1493.
[9] Miyajima, K.; Komatsu, H.; Inoue, K.; Handa, T.; Nakagaki, M. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 6.
[10] Suzuki, M.; Takai, H.; Tanaka, K.; Narita, K.; Fujiwara, H.; Ohmori, H. *Carbohydr. Res.* **1996**, *288*, 75.
[11] Hirai, H.; Toshima, N.; Uenoyama, S. *Bull. Chem. Soc. Jpn.* **1985**, *58*(4), 1156.
[12] Hersey, A.; Robinson, B. H. *J. Chem. Soc. Faraday Trans. 1*, **1984**, 2039.
[13] Sanchez, A. M.; de Rossi, R. H. *J. Org. Chem.* **1996**, *61*, 3446.
[14] Clarke, R. J.; Coates, J. H.; Lincoln, S. F. *Carbohydr. Res.* **1984**, *127*(2), 181.
[15] Szejtli, J. *Chem. Rev.* **1998**, 1943.
[16] McCracken, P. G.; Ferguson, C. G.; Vizitiu, D.; Walkinshaw, C. S.; Wang, Y.; Thatcher, G. R. J. *J. Chem. Soc. Perkin Trans. 2*, **1999**, 911.
[17] Botsi, A.; Yannakopoulou, K.; Hadjoudis, E.; Waite, J. *Carbohydr. Res.* **1996**, *283*, 1.
[18] Kitagawa, M.; Hajime, H.; Sakurai, M.; Inoue, Y.; Chujo, R. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4225.
[19] Rontoyianni, A.; Mavridis, I. M. *Acta Crystallogr. C* **1996**, 2277.
[20] Kokkinou, A.; Makedonopoulou, S.; Mentzafos, D. *Carbohydr. Res.* **2000**, *328*, 135.
[21] Rontoyianni, A.; Mavridis, I. M.; Hadjoudis, E.; Duisenberg, A. J. M. *Carbohydr. Res.* **1994**, *252*, 19.
[22] Harata, K. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2782.
[23] Brett, T. J.; Liu, S.; Coppens, P.; Stezowski, J. J. *Chem. Commun.* **1999**, 551.
[24] Bom, A.; Bradley, M.; Cameron, K.; Clark, J. K.; van Egmond, J.; Feilden, H.; MacLean, E. J.; Muir, A. W.; Palin, R.; Rees, D. C.; Zhang, M.-Q. *Angew. Chem. Int. Ed.* **2002**, *41*, 266.
[25] Guillo, F.; Hamelin, B.; Jullien, L.; Canceill, J.; Lehn, J.-M.; De Robertis, L.; Driguez, H. *Bull. Soc. Chim. Fr.* **1995**, *132*, 857.
[26] Vizitiu, D.; Walkinshaw, C. S.; Gorin, B. I.; Thatcher, G. R. J. *J. Org. Chem.* **1997**, *62*, 8760.
[27] Adam, J. M.; Bennett, D. J.; Bom, A.; Clark, J. K.; Feilden, H.; Hutchinson, E. J.; Palin, R.; Prosser, A.; Rees, D. C.; Rosair, G. M.; Stevenson, D.; Tarver, G. J.; Zhang, M. Q. *J. Med. Chem.* **2002**, *45*, 1806.