High Field NMR Techniques and Molecular Modelling Study of the Inclusion Complexes of the Nootropic Drug Tenilsetam (CAS-997) in Cyclodextrins

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The inclusion complexes of the chiral anti-amnesic drug 3-(2-thienyl)piperazin-2-one (Tenilsetam, CAS-997) with α-, β- and γ-cyclodextrins (CDs) were studied by high resolution ^{1}H NMR spectroscopy and molecular modelling (MACROMODEL interactive computer program). The partial inclusion of the guest from the secondary hydroxyl side of α-CD was observed in aqueous solution, as well as a deeper insertion into β-CD from the same side; no interactions were evidenced with γ-CD. Diastereoisomeric pairs were observed for both α- and β-CD inclusion complexes. ROESY experiments allowed us to detect the dipolar contacts between protons of the thiophene ring and those of the internal cavity of β-CD. The possible geometry of the complex edifice derived from experimental results was consistent with those obtained by molecular modelling. The formation of hydrogen bonds provides specific interaction sites to differentiate the diastereoisomeric intermolecular inclusion complexes in terms of conformational host–guest adaptation according to available experimental NMR data.

The relatively hydrophobic cavities of the doughnut-shaped cyclodextrins (CDS) are known for their ability to bind organic molecules in aqueous solution and in the crystalline state by non-covalent interactions.[1] The internal diameter of the cavity and the size of the guest molecules are of primary importance for the formation of inclusion complexes. This enables the CDs to behave as molecular receptors towards guest species. The complexation driving forces have been attributed to hydrophobic interactions, van der Waals–London dispersion forces and hydrogen bonding. Pharmaceutical applications of CDs as carriers are encouraged owing to the useful modification observed of the physico–chemical properties of the complexed guest relative to those of the free molecule.[1,2] Analytical applications of the inclusion phenomenon in the separation of structural, geometrical and optical isomers are also growing rapidly. The use of CDs for resolution of antipods can be highly advantageous since it does not require derivatization of the racemic substance. Chiral analysis can be performed in aqueous solutions by ^{1}H NMR spectroscopy using high frequency spectrometers.[3] The pseudo C_n symmetric cavity of CDs, however, sometimes includes *R* and *S* guests equally, annulling their potential value as effective reagents. Host–guest specific interaction sites have to exist for CDs to recognize the chirality of optically active guests. Compared to other techniques, NMR spectroscopy provides a powerful method to study inclusion phenomena because both host and guest molecules are simultaneously observed.[4] Application of high magnetic field and specific techniques can provide a large amount of information useful to determine the disposition of the guest relative to the host molecule in solution. Guest protons which are 3–4 Å apart from internal-cavity protons are expected to experience dipolar contacts. NOESY experiments, or the more sensitive ROESY variant, can be used to highlight this type of interactions involving through-space information exchange.[4] Detailed inspection of the energy-minimized structures obtained by theoretical calculations are complementary to NMR results to gain insights into the three-dimensional complex structures. The value of the molecular modelling method in the analysis of the geometry of the inclusion compounds as well as of the topology of the host–guest interactions and in the rationalization of the NMR information was recently outlined.[4] Recently, studies on the structural and conformational properties of the chiral nootropic drug† 3-(2-thienyl)piperazin-2-one (Tenilsetam, CAS-997) have been reported.[5] In the present paper, detailed investigations on the interactions of Tenilsetam (CAS) with α-, β- and γ-CD were carried out. High resolution ^{1}H NMR spectroscopy was used to get information on the reality of the inclusion and on the three-dimensional structure of the supermolecules in terms of chiral recognition. From dipolar correlation maps (ROESY), the intermolecular spatial proximities of protons of guest and β-CD were investigated. The geometries of the inclusion edifice derived from these experimental data were compared to structures obtained by molecular modelling and force field calculations in order to explore the possible chiral selectivity sites.

Experimental

Materials. α-, β- and γ-cyclodextrin (Fluka AG, Switzerland) were used as received and freeze-dried from deuterium oxide (CEA, France). CAS-997 (gift from Cassella AG, Germany) was recrystallized from ethyl acetate (m.p. 118 °C) and freeze-dried from D_2O.

NMR spectroscopy. ^{1}H NMR spectra of mixtures of the drug and CDs in D_2O were recorded at 298 K using a Bruker AMX500 spectrometer operating at 500.13 MHz. Chemical shifts are given relative to external tetramethylsilane (TMS). The COSY-45 and ROESY experiments were performed using pulse sequences already described.[7,8]

Computational methodology. Calculations were performed using the MACROMODEL program[9] running on a high resolution Tektronic 4111 graphics systems controlled by a Digital VAX-VMS 3500 computer. The molecular structure of the guest was generated by the manual drawing routine in MACROMODEL, while the starting structures of α-, β- and γ-CD were created using the Grow subroutine of the carbohydrate mode. Frames of high radial symmetry were attained for CDs as previously reported.[10] Energy minimization of geometries and conformations of the isolated host and *R,S* guest molecules were performed by using the MM2 force field

† The term 'nootropic' ('cognition-activator') indicates a new class of drugs for treatment of the main age-related brain syndromes.[6]

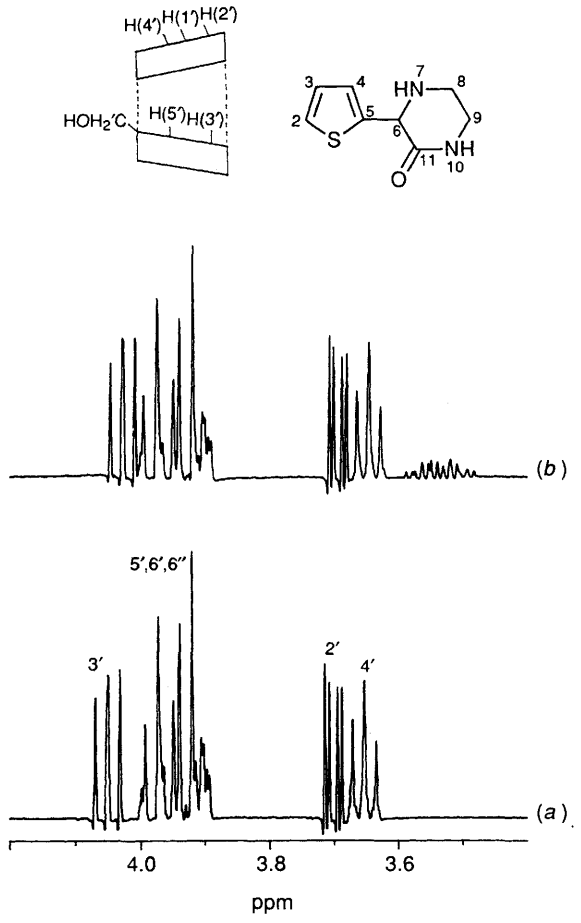

Fig. 1 Partial 500 MHz ^{1}H NMR spectra of (*a*) free α-CD (10 mmol dm^{-3}) and (*b*) α-CD (5 mmol dm^{-3})/CAS (5 mmol dm^{-3}) solutions in D_2O at 298 K with assignment of the signals from the CD moiety

method (the root mean squares of the gradient vectors were less than 0.01 kJ $Å^{-1}$). The optimized CDs structures were in good agreement with the experimental data derived from X-ray analysis.[11–13] Rigid body docking experiments were carried out to position the guest in the cavity of the CDs and repeated to check its safe location inside the torus cavity in order to obtain the absolute energy minimum. Energy minimization of the structures was performed until a gradient of about 3 kJ $Å^{-1}$ was reached. Successive PRCG routines accomplished fully relaxed optimization of the obtained minimum energy docked structure, until 0.01 kJ $Å^{-1}$ gradient.

All energy calculations were carried out with MACROMODEL's default values for the relative permittivities and non-bonded cut off distances.* In order to obtain reliable theoretical results it is important that the force field is appropriate for the molecules under study. In the case of enantiomers and their interactions with the molecular cavity of CDs, eventual, if any, deficiencies in the force field can reasonably be assumed to be identical for both the isomers and thus very similar for the diastereoisomeric pair. The lack of a suitable solvent effect model forced us to exclude water from all calculations.† The neglect of solvation effects is a commonly

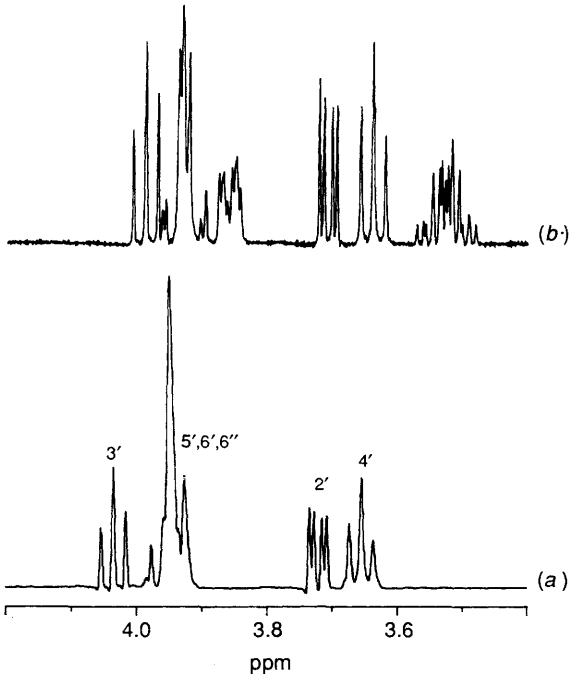

Fig. 2 Partial 500 MHz ^{1}H NMR spectra of (*a*) free β-CD (10 mmol dm^{-3}) and (*b*) β-CD (5 mmol dm^{-3})/CAS (20 mmol dm^{-3}) solutions in D_2O at 298 K with assignment of the signals from the CD moiety

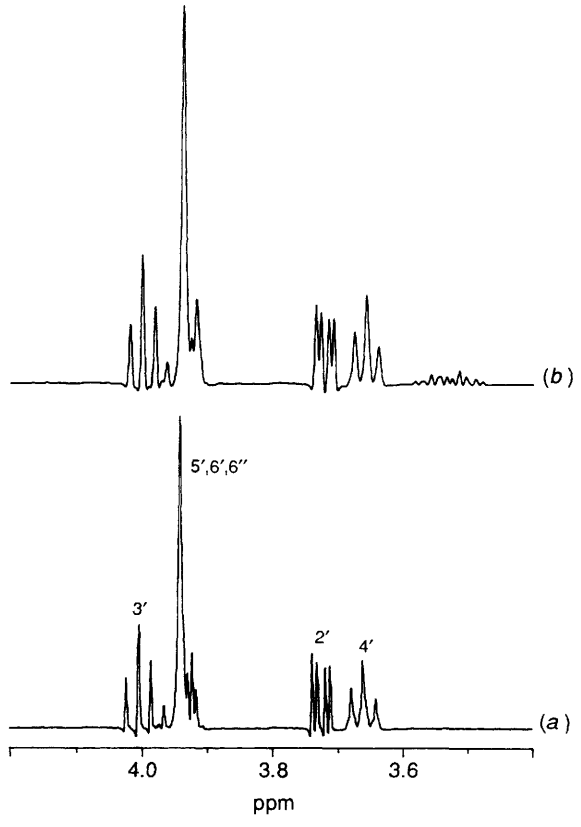

Fig. 3 Partial 500 MHz ^{1}H NMR spectra of (*a*) free γ-CD (10 mmol dm^{-3}) and (*b*) γ-CD (5 mmol)/CAS (5 mmol dm^{-3}) solutions in D_2O at 298 K with assignment of the signals from the CD moiety

adopted computational procedure in simulation of complexed structures. In spite of the serious limitations inherent in this approach (hydrophobic interactions provide the major force for complexation phenomena) the agreement of these theoretical results with physical reality supports the validity of the

* Distance-dependent relative permittivity $\varepsilon = 1.0$; VDW cut off distance = 8 Å; electrostatic cut off distance = 13 Å; H bonding cut off distance = 4 Å.

† Previously, we reported calculations carried out incorporating explicit solvent molecules (about one hundred water molecules) to β-CD structure demonstrating the hydrophobic character of the cavity of the calculated β-CD.[10]

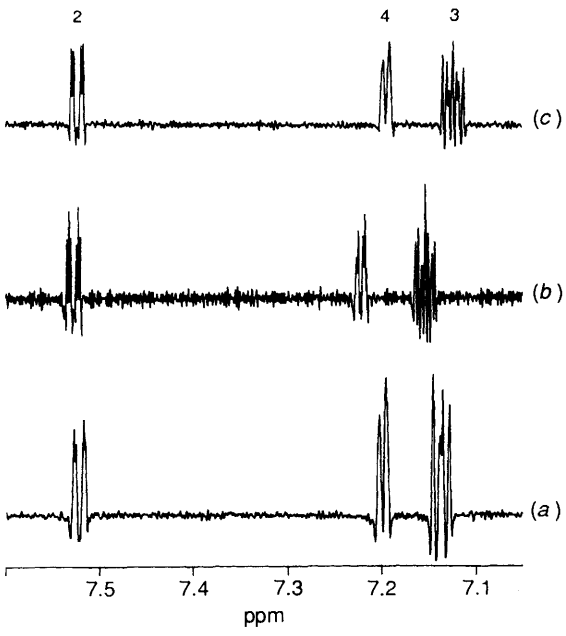

Fig. 4 Partial 500 MHz ^{1}H NMR spectra in D_2O at 298 K of aromatic region of (*a*) free CAS (5 mmol dm^{-3}), (*b*) α-CD (5 mmol dm^{-3})/CAS (5 mmol dm^{-3}) and β-CD (2 mmol dm^{-3})/CAS (8 mmol dm^{-3}) solutions

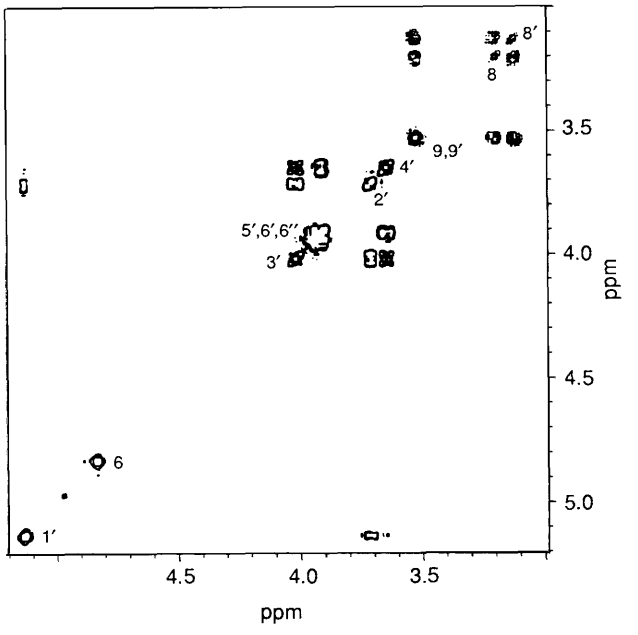

Fig. 5 Partial 500 MHz homonuclear ^{1}H–^{1}H chemical shift correlation spectrum in D_2O at 298 K of the β-CD (5 mmol dm^{-3})/CAS (5 mmol dm^{-3}) solution.

modelling method.[4,14] Calculated overall gas-phase binding energies included van der Waals and charge interactions as attractive forces producing a complex.

Results and Discussion

The 500 MHz ^{1}H NMR spectra of the mixtures of α-, β- and γ-CD with CAS were analysed and compared to the spectra of the pure CDs to investigate the formation of inclusion complexes (Figs. 1–3). Addition of CAS to the three CDs solutions produces different effects. The inclusion of CAS in the α- and β-CD cavity in aqueous solution was supported by the modification of the ^{1}H NMR spectra of both partners. Significant upfield shift was experienced by the H(3′) protons of α-CD as an effect of thiophene ring proximity. The chemical shifts of H(5′) and H(6′) are virtually unchanged as are those of H(1′), H(2′) and H(4′) protons located on the external surface of α-CD (Fig. 1). Small downfield shifts were observed for the aromatic ring protons of CAS as the effects of changes in environment polarity on passing from the solution to the hydrophobic cavity. All these proton signals appear duplicated [Fig. 4(*b*)]. Two peaks, separated by 1.8 Hz, arose from the chiral proton H(6) of the piperazinone ring, which gave rise to a singlet in the pure compound. The effect of a cavity larger by one glucose moiety, as for β-CD, is shown in Fig. 2. The H(3′), H(5′) and H(6′) protons of β-CD experienced anisotropic shielding attributable to the included aromatic ring and resonated at higher field whereas the H(2′) and H(4′) resonances were practically unaffected. The upfield shift of H(5′) was larger than those of the H(3′) and H(6′) protons. Inclusion does not alter the chemical shifts of thiophene protons but duplication of the H(3) signal was observed [Fig. 4(*c*)]. In the aliphatic region of the spectrum, one of the H(9) methylene protons and the chiral H(6) proton of the piperazinone ring were duplicated. The separation of the two peaks arising from H(6) showed a dependence on the ratio of the components in solution, increasing with the enhancement of the β-CD concentration (being zero for a β-CD:CAS ratio of 2:8, 2.5 Hz for a β-CD:CAS ratio of 5:5 and 3.5 Hz for a β-CD:CAS ratio of 8:2).* The COSY experiment supports the assignments and excludes scalar inclusion-related additional couplings which may cause the duplication of signals (Fig. 5). The inclusion of the guest in the γ-CD cavity can be excluded because neither of the spectra of the partners in the mixture were altered (Fig. 3). This latter evidence can be easily rationalized in terms of the dimensions of the guest thiophene ring which is too small to fit into the large γ-CD cavity. Also the lack of entrapment of the guest along its long axis into the cavity has to be attributed both to geometrical considerations and to the hydrophilic character of the piperazinone ring moiety.

In the case of α- and β-CD, the experimental evidences provide unequivocal proof of inclusion. Assumptions on the orientation of the guest relative to the host can be derived from NMR data. In the most probable geometry of the complex, the α-CD molecule binds the guest species from its secondary hydroxyl side determining a shallow inclusion of the thiophene ring. However, specific interaction sites have to exist both in the cavity and at the edge of the CD for diastereoisomeric discrimination to occur. The occurrence of binding from the secondary hydroxyl side to the aromatic ring first is consistent with NMR information on the complex involving the β-CD. The thiophene ring is deeply inserted in the cavity while the piperazinone ring should be excluded from the cavity and protrudes outside towards the bulk of the solvent, interacting with the rim of the CD host. In order to get more detailed information on the real geometry of the β-CD–CAS complex, investigation of the proximities between protons of the thiophene ring and of the internal cavity have to be attained. Compared with NOESY experiments, the more sensitive ROESY variant was preferred. The ability to evidence very weak dipolar interactions makes this technique extremely powerful in investigating inclusion complexes of organic molecules in CDs. The ROESY contour plot of the β-CD–CAS complex is shown in Fig. 6. The experiment was performed at 280 K to enhance dipolar interactions. Specific dipolar contacts between aromatic protons and protons of the β-CD produce the observed cross-peaks, indicating that the H(4) thiophene proton is close to the H(3′) of the β-CD and that H(3) is deeper

* The small anisochronicity for diastereoisomeric inclusion complexes is a well documented feature.[3,10,15,16]

in the cavity. Very weak interactions with the cavity were experienced by the H(2) proton. Additional dipolar contacts with other protons of the β-CD were absent.

The modelling procedure, initiated by docking calculations, was carried out for both enantiomers of CAS in order to study the specific interactions in the edifice of the complex with the β-CD. The energy-minimized structures (Fig. 8), which were obtained after full relaxation showed the thiophene ring of the guest to be accommodated in the cavity, nearly perpendicular to the oxygen glycosidic plane, the C(2)–H(2) bond parallel to the C_n axis of the β-CD. The thiophene moiety of the *S* isomer was found to be more deeply inserted into the cavity with respect to that of the *R*-CAS. Intermolecular distances between internal cavity protons and thiophene ring protons calculated from the models were in excellent agreement with the ROESY NMR data. The piperazinone ring protrudes from the side of the secondary hydroxyl groups, but in the *S*-CAS complex it was closer to the rim of the CD than in the *R* isomer. The carbonyl oxygen was at hydrogen bond distance from a secondary hydroxyl group of the β-CD in both cases. Modelling of *R,S*-CAS–α-CD complexes produced structures showing the partial insertion of the thiophene ring into the smaller cavity from the secondary hydroxyl side, in agreement with the NMR data (Fig. 7). The carbonyl group of the piperazinone ring forms a hydrogen bond with one of the hydroxyl groups of the α-CD edge and additional specific interaction sites may be constituted by the amino group. The 'three point' interaction required for enantiomeric recognition may be verified by these close contacts allowing the chiral selectivity of CDs towards asymmetric guests.

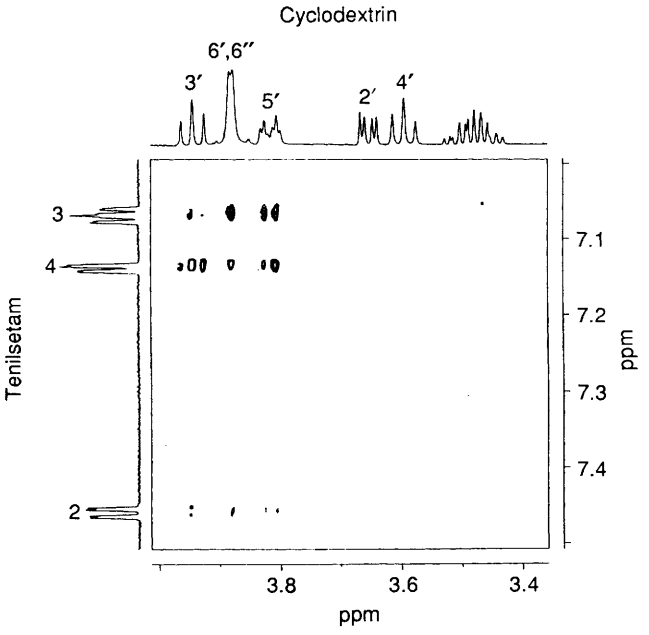

Fig. 6 Partial contour plot of a ROESY experiment performed on the β-CD (5 mmol dm^{-3})/CAS (20 mmol dm^{-3}) solution in D_2O (500 MHz, 298 K)

Conclusions

The occurrence of molecular interactions in CDs inclusion complexes can be deeply investigated using powerful NMR techniques. The modern ROESY experiment provides unequivocal proof of host–guest dipolar interactions allowing the determination of the three-dimensional structure of the inclusion complex in solution. In this technique, the molecular mechanics calculations appear as a useful complementary method in rationalizing the experimental information. The energy-minimized structures obtained by theoretical calculations in agreement with NMR data evidence the host–guest interaction sites and the presence of hydrogen bonds which stabilize the complexes revealing chiral selectivity of CDs towards asymmetric guests.

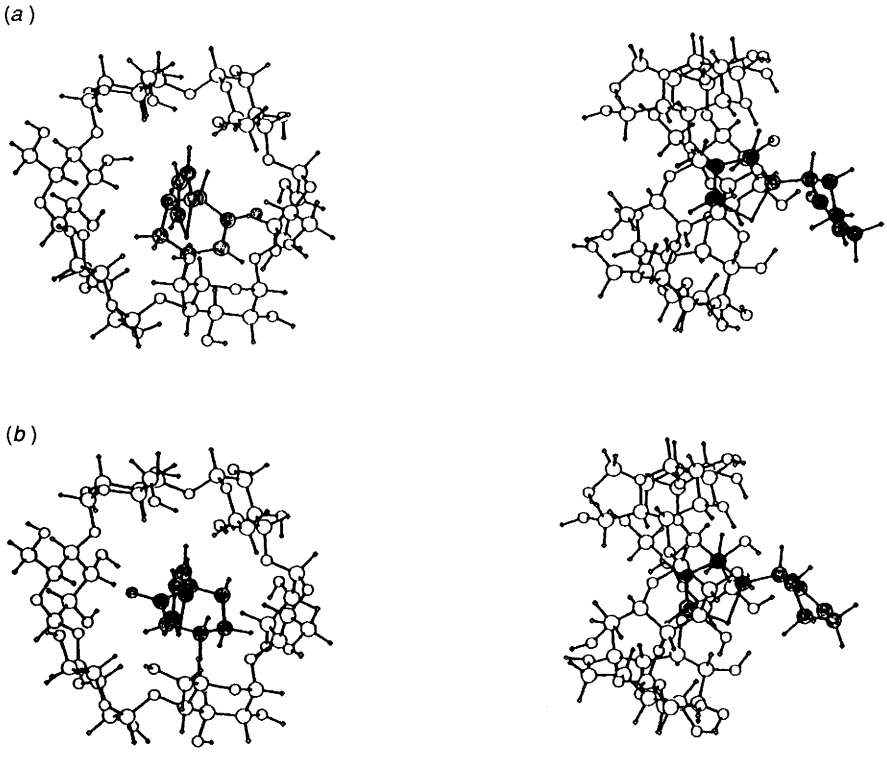

Fig. 7 Top-view (left) and side-view (right) of the computer generated structures of the inclusion complexes of enantiomers *R* (*a*) and *S* (*b*) of CAS with α-CD

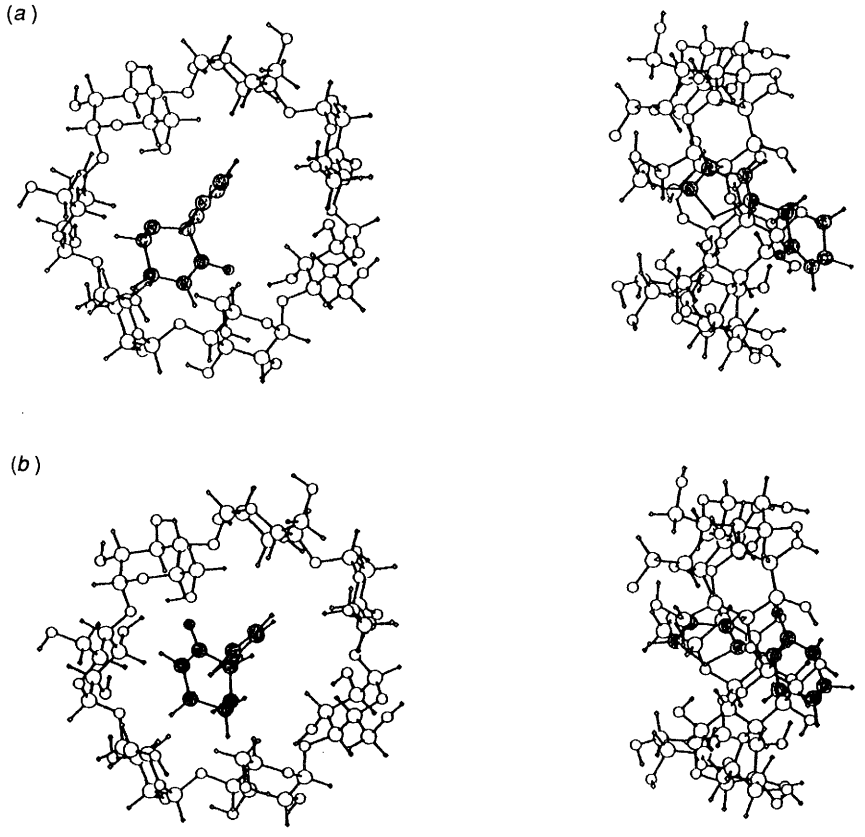

Fig. 8 Top-view (left) and side-view (right) of the computer generated structures of the inclusion complexes of enantiomers *R* (*a*) and *S* (*b*) of CAS with β-CD

Acknowledgements

Work supported by M.P.I., Italy (40%, 1990).

References

1 J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic publishers, Dordrecht, 1988.
2 D. Duchêne, *Proc. Fourth Int. Symp. on Cyclodextrins*, Kluwer Academic publishers, Dordrecht, 1988, pp. 265–275.
3 A. F. Casy and A. D. Mercer, *Magn. Reson. Chem.*, 1988, **26**, 765 and references therein.
4 (*a*) B. Perly, F. Djedaïni and P. Berthault, *New Trends in Cyclodextrins and Derivatives*, Editions de Santé, Paris, 1991, chapter 5; (*b*) B. Perly and F. Djedaïni, *New Trends in Cyclodextrins and Derivatives*, Editions de Santé, Paris, 1991, chapter 6.
5 M. E. Amato, G. Bandoli, A. Grassi, A. Marletta and B. Perly, *Eur. J. Med. Chem.*, 1991, **26**, 443.
6 (*a*) P. Tallal, *J. Clin. Psychopharmacol.*, 1985, **5**, 272; (*b*) J. Kabes, *J. Int. Med. Res.*, 1985, **13**, 185.
7 W. P. Aue, E. Bartholdi and R. R. Ernst, *J. Chem. Phys.*, 1976, **64**, 2229.
8 A. Bax and D. G. Davis, *J. Magn. Reson.*, 1985, **63**, 207.
9 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440.
10 M. E. Amato, F. Djedaïni, G. C. Pappalardo, B. Perly and G. Scarlata, *J. Pharm. Sci.*, in the press, 1992, paper No. 91-081.
11 K. K. Chacko and W. Saenger, *J. Am. Chem. Soc.*, 1981, **103**, 1708.
12 K. Lindner and W. Saenger, *Carbohydr. Res.*, 1982, **99**, 103.
13 K. Harata, *Chem. Lett.*, 1984, 641.
14 H. J. Thiem, M. Brandl and R. Breslow, *J. Am. Chem. Soc.*, 1988, **110**, 8612.
15 D. Greatbanks and R. Pickford, *Magn. Reson. Chem.*, 1987, **25**, 208.
16 K. B. Lipowitz, S. Raghothama and J. Yang, *J. Am Chem. Soc.*, 1992, **114**, 1554.

Paper 2/02116A
Received 24*th April* 1992
Accepted 16*th July* 1992