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ORIGINAL ARTICLE

Host–guest complexation of omeprazole, pantoprazole and rabeprazole sodium salts with cyclodextrins: an NMR study on solution structures and enantiodiscrimination power

Jordi Redondo · Anna Capdevila · Isabel Latorre · Jordi Bertrán

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Abstract The application of different cyclodextrins (CDs) as NMR chiral solvating agents (CSAs) for the sodium salts of the proton-pump inhibitors omeprazole, pantoprazole (sesquihydrate) and rabeprazole was investigated. It was proved that the formation of diastereomeric host–guest complexes in D_2O solution between the CDs and those substrates permitted the direct ^1H NMR discrimination of the enantiomers of the sodium salts of these compounds without the need of previous working-up. Rotating frame nuclear overhauser effect spectroscopy (ROESY) was used to ascertain the solution geometries of the host–guest complexes. The results suggested a preferential binding of the benzimidazole moiety of the guest molecules within the macrocyclic cavity of α-CD, whereas the higher dimensions of β- and γ-CD also permitted the inclusion of the highly substituted pyridine moieties. Moreover, the solution stoichiometries and the binding constants of the complexes formed with pantoprazole at room temperature were determined by ^1H and ^{19}F NMR titration. Diffusion-filtered Spectroscopy was applied to obtain clean spectra without the interference of the HOD signal.

Keywords ^1H and ^{19}F NMR · Omeprazole · Pantoprazole · Rabeprazole · Enantiorecognition · ROESY

Introduction

Omeprazole (5(6)-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl)-1*H*-benzo[d]imidazole, **1**)[1] and its analogues pantoprazole (5(6)-(difluoromethoxy)-2-((3,4-dimethoxypyridin-2-yl)methylsulfinyl)-1*H*-benzo[d]imidazole, **2**) and rabeprazole (2-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methylsulfinyl)-1*H*-benzo[d]imidazole, **3**) are currently among the most widely prescribed drugs [2]. They act as potent proton-pump inhibitors (PPIs) and exhibit, therefore, therapeutic activity against gastric disorders like peptic ulcer, reflux esophagitis or gastroesophageal reflux [3]. The basic structure of this sort of PPI compounds consists of a benzimidazole group linked to a pyridine ring through a sulfoxide group, with the sulfur atom constituting a center of chirality (see Fig. 1). Both pantoprazole and rabeprazole are currently marketed as racemic mixtures, whereas omeprazole is sold as racemate and also as the *S*-enantiomer (esomeprazole, (*S*)-**1**) [4]. Concerning stability, they rapidly undergo degradation in acidic solution, but are quite stable under alkaline conditions. This is one of the reasons why these compounds are also marketed in the form of alkaline salts (omeprazole and esomeprazole as magnesium or sodium salts; rabeprazole and pantoprazole as sodium salts, this last in the solid form of sesquihydrate).

The chiral nature of omeprazole-like drugs implies, at least in theory, the possibility of finding enantioselectivity in their pharmacokinetical behavior. In this sense, and despite some controversy concerning the balance between

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J. Redondo (✉) · A. Capdevila · I. Latorre · J. Bertrán
Department of Research and Development, Esteve Química S.A., Caracas Street 17-19, 08030 Barcelona, Spain
e-mail: jredondo@eqesteve.com

[1] Omeprazole and its analogues present tautomerism in the benzimidazole group [1]. Therefore, both tautomers must be considered in compounds **1** and **2** when using the systematic nomenclature. For simplicity, only the 5-substituted tautomers are represented in Fig. 1.

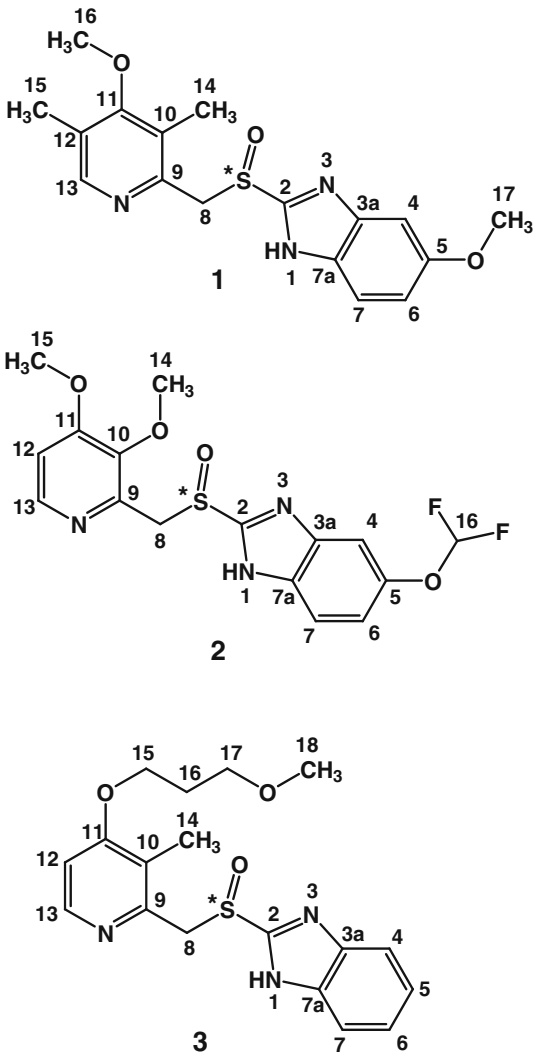

Fig. 1 Molecular structures and atom numbering of compounds **1**–**3**

commercial pressure and real efficacy emerged from clinical trials, esomeprazole is alleged by its inventor to have advantages over the racemic drug in terms of less interindividual metabolic dependence and, hence, in terms of bioavailability [5]. This perspective of finding therapeutic advantages in the single enantiomers of the drugs (the so-called "chiral switching") has triggered the need of having enantioselective synthesis or chemical resolution methods capable of producing enantiopure compounds in high yields, as well as the development of adequate analytical methods to monitor and quantify the optical purity of the samples [6, 7].

Despite its relatively low sensitivity, NMR spectroscopy is probably an unsurpassed analytical technique regarding to speed and easiness of sample preparation. The main requirement for achieving enantiodiscrimination by NMR is the use of an enantiomerically pure agent capable of reacting or complexing with the studied substrate, thus generating the corresponding diastereomeric species that

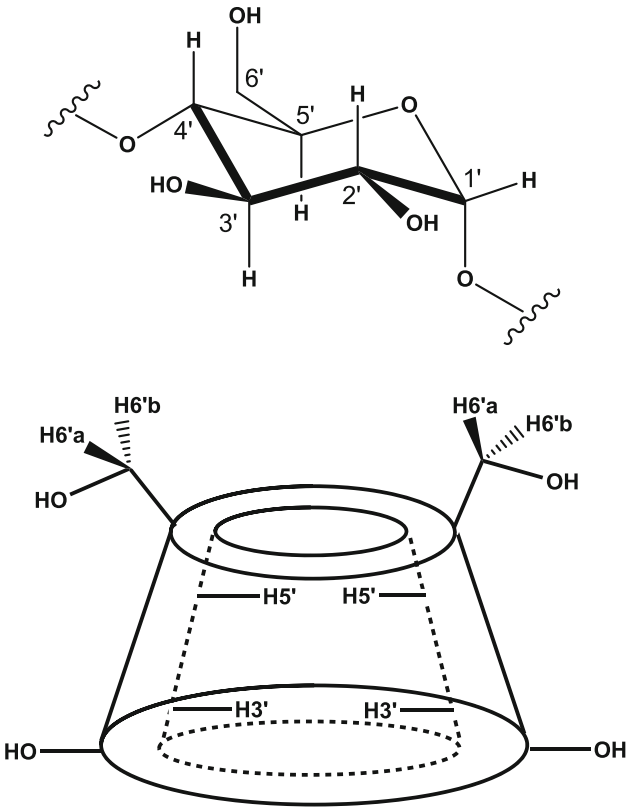

Fig. 2 Numbering of the glucose unit and scheme of the cyclodextrin geometry

can lead, in principle, to observe different signals in the spectra. Chiral solvating agents (CSAs) [8, 9], chiral derivatizing agents (CDAs) [10, 11] and, more recently, chiral liquid crystal solvents [12, 13] compose the current standard equipment for the detection and quantification of enantiomers by NMR. And among all those analytical tools, natural cyclodextrins (CDs) stand out not only by their easy availability but also by their application to a broad range of medium-size organic molecules as are most of the pharmaceutical compounds [14, 15].

Native cyclodextrins are macrocyclic oligosaccharides formed by 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucose units bonded through $\alpha(1 \rightarrow 4)$-type linkages. The molecular structure of a CD resembles to a truncated cone with all secondary hydroxyls (2-OH and 3-OH) placed on the wider rim of the torus-shaped molecule whilst the primary hydroxyls (6-OH) crown the narrower rim (see Fig. 2). Such geometry defines a hydrophobic cavity (average diameters: α-CD, 5.7 Å; β-CD, 7.8 Å; γ-CD, 9.5 Å) [16] where a guest molecule or a part of it can fit to establish a supramolecular association (host–guest complex) in water solution. It is convenient to classify the methyne hydrogen atoms of these macrocycles in two categories: the "inner" protons (H-3′ and H-5′), which point to the center of the torus, and the "outer" protons (H-2′ and H-4′), which are

directly in contact with the surrounding solvent. On the other way, the methylene groups (H-$6'_{ab}$) are the only mobile enough to change significantly its orientation depending on the interaction of the primary OHs with the solvent molecules and/or the complexed guests.

Cyclodextrins and their derivatives are widely used chiral auxiliaries for different separation techniques like high performance liquid chromatography [17], gas chromatography [18], capillary electrophoresis [19] and, of course, NMR [20]. The resolving power of these molecules relies on its ability to form a pair of diastereomeric host–guest complexes between the natural enantiomer of the macrocycle ((+)-R) and the two enantiomers of the studied substrate.

Although it can be found in the literature a good number of chromatographic methods for the chiral analysis of omeprazole and other analogue compounds [21–23], there is no by far the same amount of published reports concerning NMR chiral techniques for this family of drugs. A certain NMR enantiodiscrimination using cyclodextrins on omeprazole in aqueous basic solutions was mentioned by Figueiras et al. in a work of 2006 [24]. On the other hand, the use of (S)-BINOL as efficient NMR CSA for ome-, lanso-, panto- and rabeprazole was also recently reported [25]. The lack of application of other chiral NMR reagents to that sort of important compounds probably lies in their high instability in aqueous solutions at pHs below 7, what makes very difficult the use of typical water-soluble CSAs like chiral organic acids, as well as in the absence of functional groups capable of reacting easily with derivatizing agents, like Mosher acids.

As a part of the development studies carried out in our laboratory for the production of the PPI compounds, it was important to achieve a fast and reliable NMR method for the determination of the optical purity directly in the final products of the synthesis (the sodium salts of ome-, panto- and rabeprazole). Taking into account the limited solubility of the alkaline salts in solvents other than water or alcohols, it was decided to explore the use of water-soluble chiral auxiliaries like the three natural cyclodextrins (α-, β-, and γ-CD) which, given their easy availability, appeared to be the first choice as appropriate CSAs for those kind of compounds. In addition, an NMR study on the most probable solution geometries of the host–guest complexes formed with the CDs was completed.

Materials and methods

The sodium salts of compounds **1**, (*S*)-**1**, **2** and **3** were obtained from our laboratory using proprietary synthetic processes [26–28]. All the cyclodextrins were purchased from Sigma-Aldrich. The absolute configuration of (*S*)-**1** and its optical purity was determined by using the chiral methods described in the European Pharmacopoeia [29]. NMR measurements were performed on a Varian Mercury spectrometer operating at 400.1 MHz for ^{1}H and 376.4 MHz for ^{19}F. All ^{1}H NMR spectra were externally referenced (coaxial tube) to the methyl signal of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) dissolved in D_2O. The ^{19}F NMR spectra were recorded without reference. The host–guest complexes were prepared by dissolving weighted quantities of the CD and the PPI compounds in D_2O (the concentrations were around 40 mM), and the assignment of their ^{1}H NMR spectra was accomplished by means of standard NMR techniques (mainly COSY and 1D NOESY). The 2D ROESY spectra were recorded at 25 °C, using a mixing time of 300 ms, 16 scans per FID and 128 repetitions. Some ROESY experiments were repeated at lower temperature (10 °C) in order to observe more intense intermolecular ROEs. To obtain the diffusion-filtered spectra, the standard Varian sequence BPPLED was applied, using a gradient pulse of 4 ms and a diffusion delay of 150 ms.

Results and discussion

Firstly, as we decided to form the host–guest complexes using the commercial cyclodextrins without the previous D_2O lyophilization usually employed in NMR to deuterate their hydroxyl groups, it was found convenient to suppress the intense ^{1}H peak from HDO ($\delta \sim 4.8$) in order to gain more signal-to-noise for the resonances of interest. Unfortunately, the attenuation of such huge peak by using conventional signal suppression techniques based on selective irradiation (like PRESAT or WET) unavoidably affected the signals from the diastereotopic protons H-8_A and H-8_B of the PPI compound (two doublets with a large geminal coupling constant of about 12 Hz), which resonate approximately at the same frequency of the HDO protons. To solve this inconvenience, it was employed a method based on Diffusion Ordered Spectroscopy (DOSY) described by Esturau et al. in 2006 [30], consisting of the application of a Bipolar Gradient Pulse Pair Longitudinal Eddy Current (BPPLED) sequence. With this methodology, it was possible to obtain diffusion-filtered ^{1}H NMR spectra in which the water signal resulted substantially attenuated while the signals from the complex remained essentially unaltered thus permitting the inspection of the proton resonances from the CH_2–SO group of the substrate.

An additional problem related to the detection of H-8_{AB} signals lied in the progressive exchange of these protons with deuterons in D_2O under alkaline conditions. As the methylene group is slightly acidic due to the stabilization of the resulting carbanion by the vicinal sulphoxide function, the basicity of the PPI sodium salts solutions is high enough

Table 1 Assignment of ^{1}H NMR signals from the sodium salts of compounds **1–3** (400 MHz, D_2O, 25 °C; reference: 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS))

Omeprazole sodium salt			Pantoprazole sodium salt			Rabeprazole sodium salt		
δ (ppm)	multiplicity, J(Hz)	Assignment	δ (ppm)	Multiplicity, J(Hz)	Assignment	δ (ppm)	Multiplicity, J(Hz)	Assignment
7.95	s	H-13	8.08	d, 5.7	H-13	7.98	d, 5.8	H-13
7.33	d, 8.8	H-7	7.62	d, 8.8	H-7	7.51	m	H4/H-7
6.98	d, 2.5	H-4	7.44	d, 2.3	H-4	7.06	m	H-5/H-6
6.66	dd, 8.8; 2.5	H-6	7.01	dd, 8.8; 2.3	H-6	6.61	d, 5.8	H-12
4.57	d, 12.7	H-8A	6.95	d, 5.7	H-12	4.64	d, 12.7	H-8A
4.29	d, 12.7	H-8B	6.78	t, 75.0 ($J_{H\text{-}F}$)	H-16	4.49	d, 12.7	H-8B
3.67	s	H-17	4.79	d, 12.7	H-8A	3.83	m	H-15
3.35	s	H-16	4.60	d, 12.7	H-8B	3.34	t, 6.4	H-17
2.00	s	H-15	3.86	s	H-15	3.13	s	H-18
1.71	s	H-14	3.68	s	H-14	1.79	quint., 6.2	H-16
–	–	–	–	–	–	1.73	s	H-14

to trigger H–D exchange at ambient temperature.[2] Complete deuteration on this position (i.e. disappearing of methylene ^{1}H resonances) was observed for several cases in a few hours after the preparation of the samples. It was mandatory, therefore, to acquire the diffusion-filtered spectra of the complexes in a short period of time.

Finally, it should be noted that different ^{1}H NMR signals for the complexed and the free forms of the substrates were not observed in any case, thus indicating that, as expected in cyclodextrin complexes at room temperature, the exchange between free and bound forms is fast on the NMR time scale.

NMR enantiodiscrimination of the substrates

^{1}H NMR spectroscopy

The enantiodiscrimination power of the CSAs is directly related to the stability of the formed complexes in solution. With cyclodextrins, the main factors involved in the stabilization of their host–guest complexes are hydrogen bonds and van der Waals forces. The other key factor is the role played by the solvent. In the case of an organic molecule in water solution in presence of a CD, the hydrophobic effect drives the more apolar moieties of the guest to shelter into the macrocyclic cavity. Concomitant with this process, the release of high-enthalpy water molecules from the CD cavity to the bulk solvent has been invoked as another important factor in the stabilization of the complexes. In consequence, compounds like the studied sodium salts, which present good water solubility at ambient temperature, will not show, in principle, a great tendency towards complexation with a CD and, therefore, it was expected not to find a remarkable induced nonequivalence ($\Delta\Delta\delta = |\delta_R - \delta_S|$) of the guest protons (Table 1).

Despite this outlook, and as can be observed in Table 2, the magnetic nonequivalence induced on the guest protons at ambient temperature by one equivalent of any of the three CDs resulted to be important in most cases and, therefore, the signal splitting was large enough to accomplish an accurate integration of the respective resonances from each enantiomer, allowing the quantitation of optical purity for the three PPI compounds using the CDs as chiral agents.

More in detail, it was found that complexation of compound **1** with α-CD produced a remarkable splitting in the pyridine proton signal (H-13, $\Delta\Delta\delta$: 0.043 ppm), as well as in 14-Me and 16-OMe signals ($\Delta\Delta\delta$: 0.027 and 0.033 ppm, respectively). On the other hand, complexation with β-CD induced important non-equivalence also on 14-Me and 16-OMe (see Table 2 and Fig. 3), and a smaller splitting in signals from the aromatic protons H-4, H-6, H-7 and H-13 (see Table 2 and Fig. 4).

On the contrary, γ-CD induced less enantiodifferentiation on this substrate (the maximum nonequivalence occurred on MeO-16 ($\Delta\Delta\delta$: 0.012 ppm)), probably due to loose binding as a consequence of the greater dimensions of this macrocyclic cavity. Regarding compound **2**, both α- and β-CD resulted to be convenient CSAs, since they induced appreciable nonequivalence on H-13 and MeO-14 protons (see Fig. 5). Finally, compound **3** was the one which presented the less enantiodifferentiation; only the signal from one of the methylenic protons H-8 appeared to be sufficiently split in the complex with β-CD ($\Delta\Delta\delta$: 0.013 ppm), as can be observed in Fig. 6. As expected, the increasing of the amount of CD to displace the host–guest equilibrium towards the associated forms permitted a certain improvement of the signal separation.

[2] The measured pDs of freshly prepared solutions were in all cases around 10.

Table 2 Chemical shift non-equivalence ($\Delta\delta = |\delta_R - \delta_S|$, ppm) for protons of compounds **1**–**3** (sodium salts) in the presence of one equivalent of different cyclodextrins (D_2O, 22 °C)

Comp	CD	H6	H4	H5	H7	H8A	H8B	H12	H13	H15	H16	H14	H17	H18
1	*α*	0.000	0.000	–	0.000	0.000	0.022	–	0.043	0.000	0.033	0.027	nd	–
	β	0.011	0.007	–	0.014	nd	nd	–	0.015	0.000	0.046	0.032	0.006	–
	γ	0.010	0.000 (bd)	–	0.010	nd	0.006	–	0.000	0.000 (bd)	0.012	0.003	nd	–
2	*α*	0.000	0.012	–	0.007	nd	0.009	0.007	0.027	0.000	0.000	0.016	–	–
	β	0.000	0.006	–	0.007	0.000	0.024	0.005	0.017	0.000	0.000	0.028	–	–
	γ	0.006	0.005	–	0.003	nd	0.037	0.009	0.007	0.00	0.003	0.015	–	–
3	*α*	0.000	0.000	0.000	0.000	nd	0.005	0.005	0.007	0.000	0.000	0.005	0.000	0.000
	β	0.000	0.000	0.000	0.000	0.008	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	γ	bd	bd	bd	bd	nd	nd	0.008	0.000	0.000	0.000	0.006	0.000	0.010

In all cases $H8_A$ refers to the downfield H8 proton and $H8_B$ to the upfield H8 proton

bd broad signal, *nd* not determined due to overlapping with CD signals

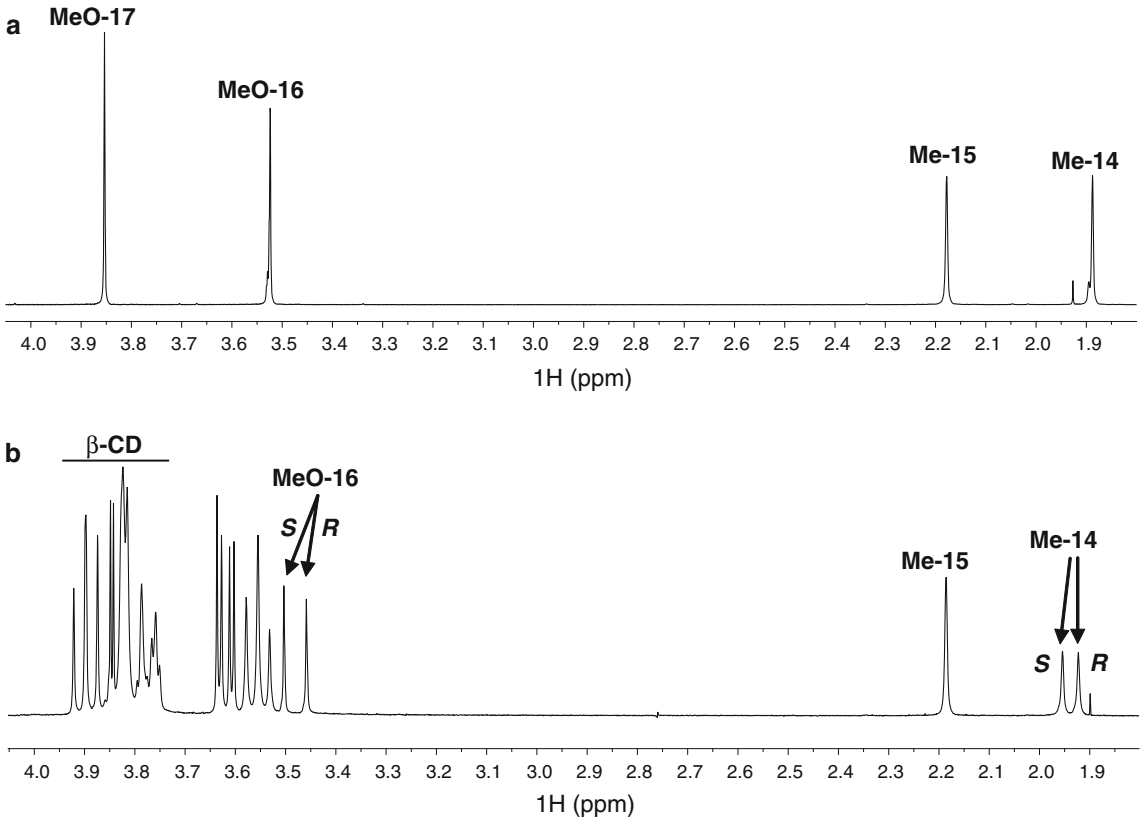

Fig. 3 ^{1}H NMR aliphatic protons signals of the sodium salt of compound **1** in absence (**a**) and presence (**b**) of one equivalent of *β*-CD

In addition to the signal splitting due to enantiodiscrimination, it has to be mentioned the occasional observation of an extra split in the ^{1}H signals of the methyl and methoxy groups of the substrates. Such effect, manifested as a more or less intense hump in the referred peaks, was previously described by Figueiras et al. [31] for omeprazole complexed with *β*- and methyl-*β*-CD in alkaline solutions, and was hypothetically attributed by these authors to different pH-dependent omeprazole conformations which affected the stereoelectronic status of the molecule. Anyway the reason, what seems to be clear is that the effect must not be related to the recognition phenomenon since it was also observed in the pure sodium salts dissolved in D_2O. On the other hand, our observations concerning the temperature dependence of those extra peaks were essentially in agreement with those reported in the above mentioned publication, although certain dispersion found in the intensity and position of the "hump" (upfield or downfield to the main peak) suggests that the effect must have a multi-factor dependence (concentration,

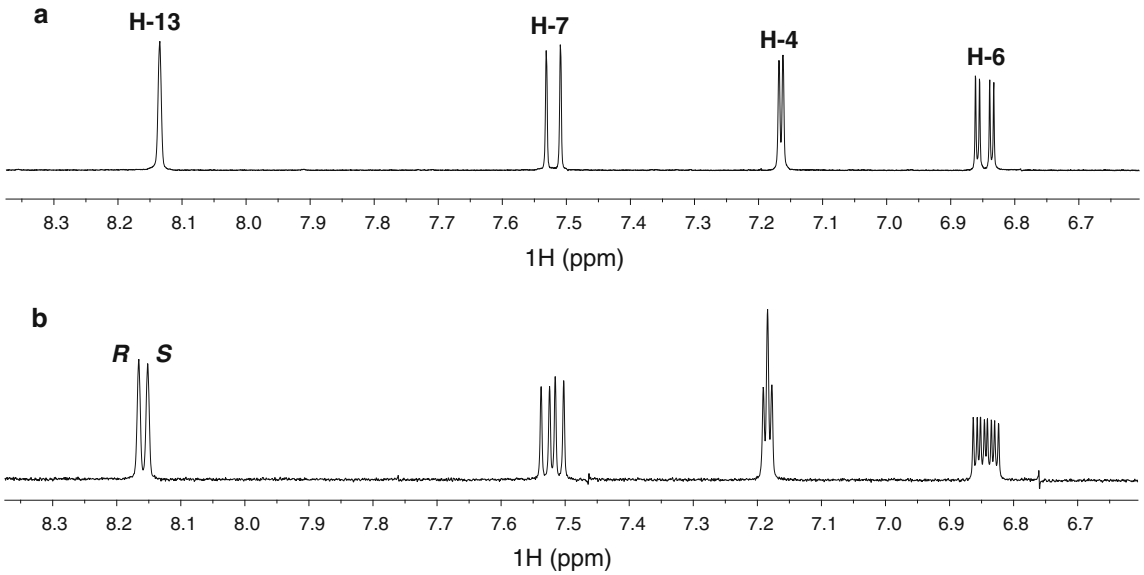

Fig. 4 ^{1}H NMR aromatic protons signals of the sodium salt of compound **1** in absence (**a**) and presence (**b**) of one equivalent of β-CD

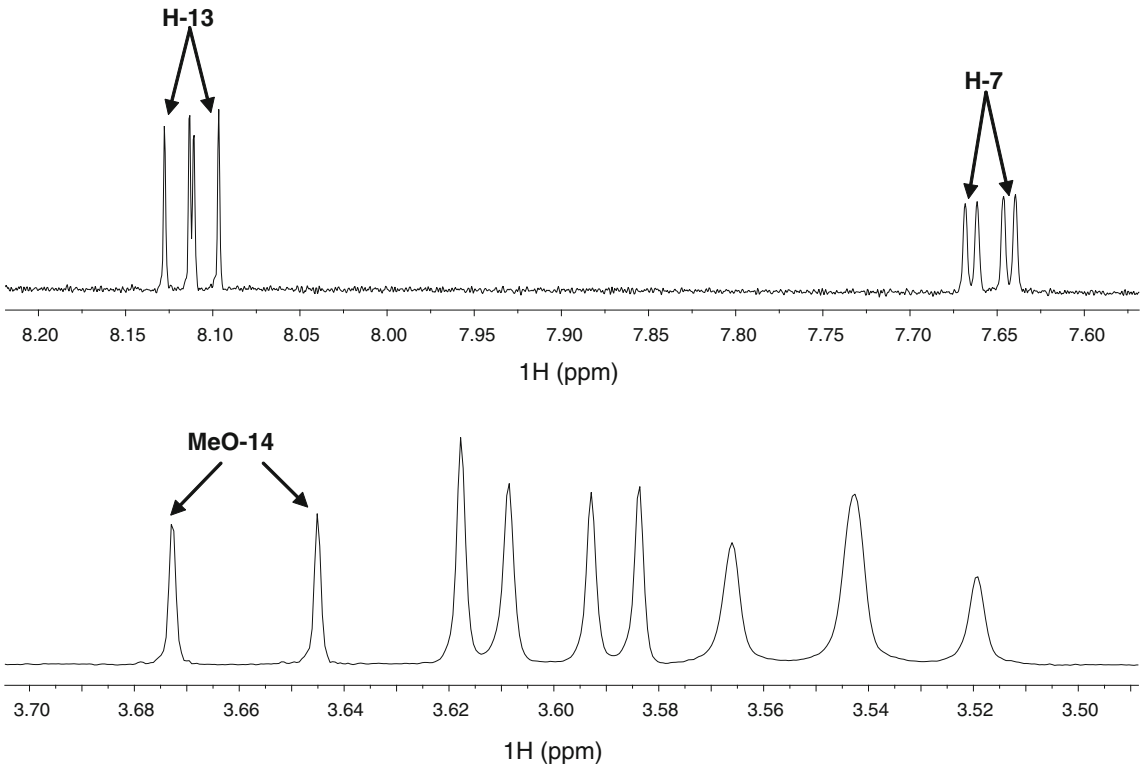

Fig. 5 Partial ^{1}H NMR spectra of compound **2** sodium salt/β-CD showing the splitting of MeO-14 and H-7, H-13 signals

host–guest equilibrium). From the experimental point of view, however, the presence of this extra splitting can complicate the quantitation of enantiomerically enriched samples by integration of the corresponding nonequivalent Me (or MeO) signals. So, for this purpose it is recommended to use any sufficiently split signal from the aromatic protons of the complexed substrate.

^{19}F NMR spectroscopy

Interestingly, the ^{19}F NMR spectra of the complexes formed with the fluorine-containing compound **2** revealed a considerable splitting of the doublet from the difluoromethyl group ($^{2}J_{FH} = 75$ Hz), in particular with α- and β-CD ($\Delta\Delta\delta = 0.085$ and 0.042 ppm, respectively).

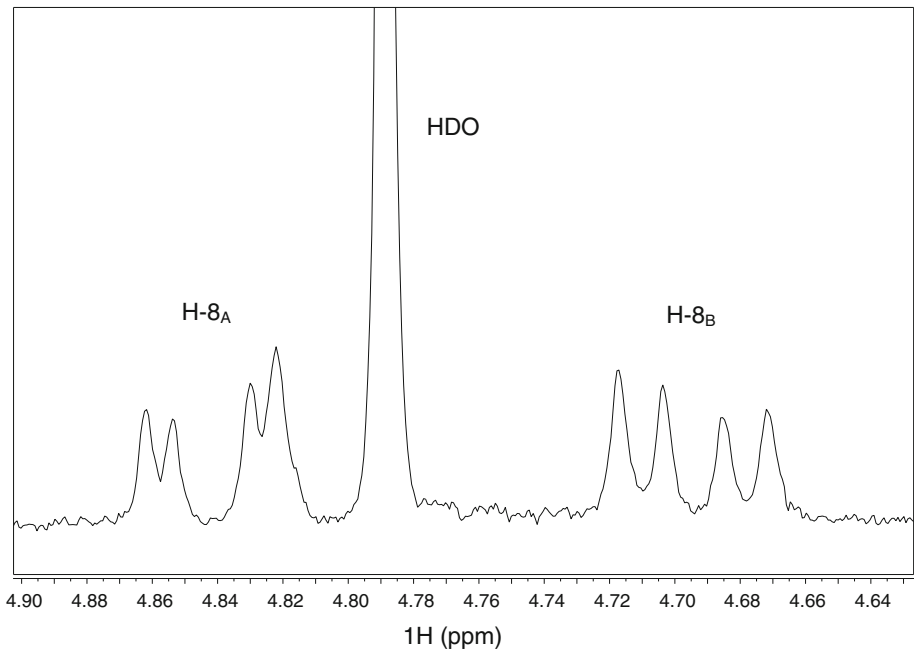

Fig. 6 Splitting of H-8 signals of compound **3** by effect of complexation with β-CD

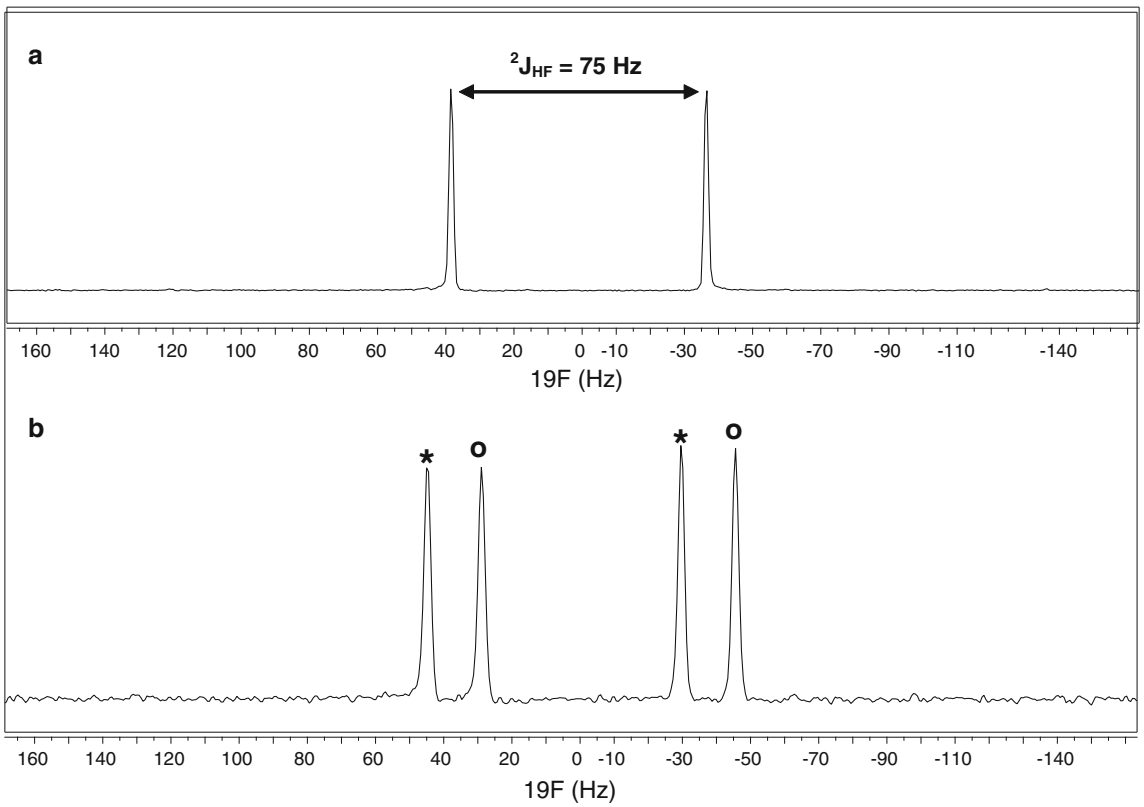

Fig. 7 ^{19}F NMR spectrum of compound **2** sodium salt in absence (**a**) and in presence (**b**) of one equivalent of β-CD

Therefore, ^{19}F NMR can be considered an excellent way of measuring the optical purity of enantiomerically enriched samples of pantoprazole sodium salt (see Fig. 7).

Enantiomeric excess determination

The use of NMR as a quantitative technique for the assessment of the enantiomeric purity was checked in our

particular case by adding 1 equivalent of β-CD to samples of sodium esomeprazole ((*S*)-**1**) containing different amounts of the *R*-enantiomer. The corresponding ^{1}H NMR spectra were recording at 25 C in D_2O with sufficient number of scans (typically 64) in order to achieve good signal-to-noise levels. Under such conditions, it was possible to observe and integrate (after application of an adequate apodization function to the FID) several signals corresponding to the minor enantiomer in a sample of esomeprazole 98.0% ee (enrichment determined by UV-detected chiral chromatography). This method permitted a fast, routine in-process control of optically enriched samples of esomeprazole sodium salt (see Fig. 8).

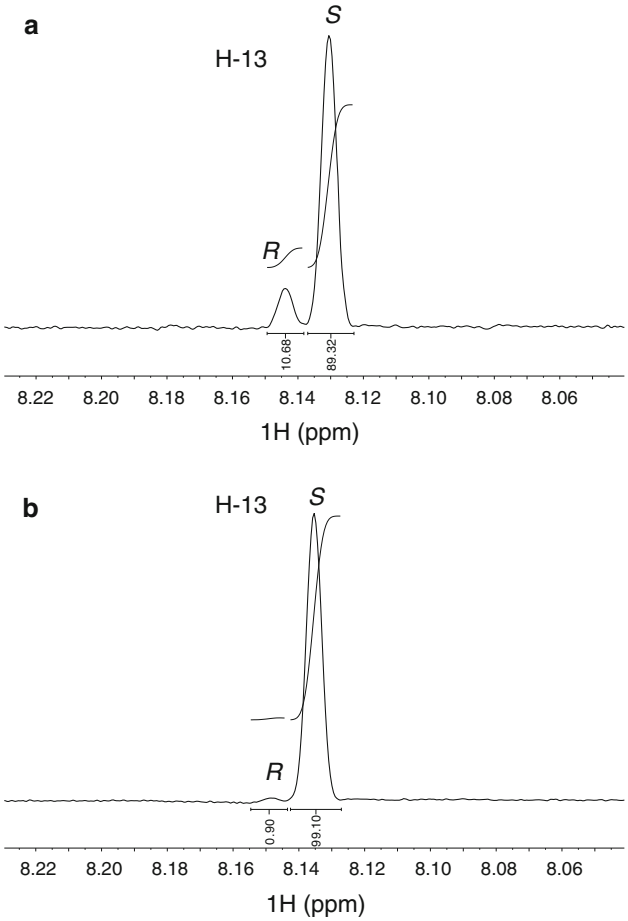

Fig. 8 Example of quantitation of residual *R*-omeprazole in esomeprazole samples using β-CD as CSA. The singlets from H-13 proton in samples 78% ee (**a**) and 98% ee (**b**) are shown

Solution geometries of the host–guest complexes

Chemical shift changes and ROESY data

It is well known that the upfield changes ($\Delta\delta = \delta_{complexed} - \delta_{free}$) displayed by the resonances of the CD inner protons (H-3′ and H-5′) in the presence of a substrate is a sign of the penetration of the guest into the macrocyclic cavity. This induced shielding can be clearly attributed to the magnetic anisotropy effect from the ring current of an inserted aromatic or heteroaromatic group. In our case, given the alkaline nature of the substrates, it was considered convenient to compare the chemical shifts of the complexed CDs to the corresponding values of the pure CDs in D_2O solution adjusted at pH 10.0 with NaOD. As can be observed in Table 3, all CD resonances manifested a certain degree of upfield shifts in the presence of any of the three substrates, but a general tendency to more important δ changes for H-3′ and H-5′ was found, especially in the case of β-CD complexes, where H-5′ signals manifested the more intense shifts (around −0.125 ppm). As expected, smaller δ increments were displayed by the outer β-CD protons resonances, although quite important changes (between −0.069 and −0.075 ppm) were also observed for the secondary methylenes (H-6′$_{ab}$). On the other hand, in the case of γ-CD, chemical shift changes of about −0.065 ppm were observed for H-3′ and/or H-5′ protons. Concerning the smallest cyclodextrin, α-CD, although H-3′ resonances displayed the greatest changes (at least in the complexes with the sodium salts of **2** and **3**), in general the shifts for the rest of protons were not very important, probably as a result of the low affinity of this small macrocycle towards the substrates.

In order to confirm the fitting of the aromatic moieties inside the CD cavities and to ascertain the average geometries of the host–guest complexes in D_2O solution, the 2D ROESY spectra of each one of the nine complexes were obtained. The results summarized in Table 4 confirmed that we were dealing with real inclusion complexes in solution, since all intermolecular dipolar couplings, although quiet weak in overall, involved exclusively the inner and/or the H-6′$_{ab}$ protons of the CDs. Clearly, the ROESY results revealed a certain dependence of the complexation mode on the size of the macrocycle. So, the smaller cyclodextrin, α-CD, showed a preferential affinity for the benzimidazole ring of the guest molecules, as all the intermolecular ROEs arose between protons of this chemical group and H-3′/H-5′/H6′$_{ab}$ CD protons, while no ROEs were detected involving neither the pyridine protons nor any of the pyridine substituents (see Fig. 9). This, so to speak, host regioselectivity of α-CD towards the benzimidazole could be related to the steric hindrance caused by the highly substituted pyridine ring of the PPI compounds. On the contrary, β- and γ-CD appeared to be large enough to admit also the pyridinic moiety, since some cross peaks were observed between the inner protons of the macrocycles and the pyridine proton H-12 and/or H-13 (see Figs. 10, 11, 12). The interaction of the benzimidazole group in the particular case of the complexation of

Table 3 ^{1}H NMR chemical shift changes ($\Delta\delta = \delta$complexed $- \delta$free, ppm) for CD protons in the presence of one equivalent of the sodium salts of compounds **1–3** (D_2O, 25 °C)

Complex	$\Delta\delta$					
	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′
α-CD/1	−0.025	−0.032	−0.024	−0.023	nd	nd
β-CD/1	−0.038	−0.038	−0.087	−0.036	−0.125	−0.075
γ-CD/1	−0.026	−0.023	−0.049	−0.025	ca. −0.070	−0.022
α-CD/2	−0.031	−0.046	−0.052	−0.025	−0.020	ca. −0.040
β-CD/2	−0.048	−0.057	−0.118	−0.046	−0.143	−0.078
γ-CD/2	−0.016	−0.017	−0.037	−0.016	−0.039	−0.021
α-CD/3	−0.036	−0.040	−0.065	−0.032	−0.025	−0.027
β-CD/3	−0.024	−0.024	−0.082	−0.017	−0.127	−0.069
γ-CD/3	−0.012	−0.011	−0.064	−0.009	−0.064	−0.002

nd not determined due to signal overlapping

Table 4 Intermolecular ROEs between inner CD protons and protons of compounds **1–3**

Compound	CD	Guest protons showing ROE with H-3′ CD protons	Guest protons showing ROE with H-5′ CD protons
1	α	H-4	(H-4; H-6)[a]
	β	H-7; H-13	H-4; H-6; H-7
	γ	H-4; H-7; H-13	H-4; H-13
2	α	H-4; H-6	H-4; H-16[b]
	β	H-4; H-7	H-4; H-7
	γ	H-6	–
3	α	H-5 + H-6; H-4 + H-7	H-5 + H-6
	β	H-5 + H-6; H-4 + H-7; H-12; H-13	H-5 + H-6; H-4 + H-7; H-12
	γ	H-5 + H-6	H-4 + H-7

[a] Both H-4 and H-6 also showed an intra-molecular ROE with MeO-17

[b] Some interaction between H6′ab and H-4 or H-16 cannot be discarded as H6′ab resonances overlap with H-5′ signals

omeprazole with β-CD and di-O-methyl-β-CD has been previously established by Braga et al. using ROESY and ab initio calculations [32].

Unfortunately, the ROESY data did not permit to establish unequivocally the preferred insertion side (via "primary" or via "secondary" rim). The fact that almost all host–guest ROE contacts involved both crowns of inner macrocyclic protons may be interpreted as the result of the coexistence of different supramolecular structures in fast exchange or, alternatively, as the consequence of a sufficiently deep insertion of the guest exclusively through the broader rim of the CD, thus permitting the interaction of the included aromatic group of the drug with H-3′, H-5′ and even H-6′ab protons.

Finally, a further evidence of the interaction between substrate molecules and macrocycles was the systematic narrowing of the line widths observed for the complexed CDs in comparison with those of their free forms, particularly in the case of H-2′ and H-4′ resonances, which shown a clear improvement both in line width and shape. This effect reveals that the conformation of the free macrocycle is more disordered than that of the complexed form, where the presence of a guest molecule must impose some dynamic and conformational restrictions.

Host–guest stoichiometry and binding constants

In connection with the structural aspects of the complexes, it is also important to know the stoichiometry of the host–guest species in solution. A very simple way to ascertain the order of the supramolecular associations is the NMR titration method, in which the chemical shift increments ($\Delta\delta$) shown by the guest are plotted against the corresponding host/guest molar ratios (or vice versa when monitoring the host $\Delta\delta$s). The intersection of the two secant lines of the experimental curve indicates the average stoichiometry of the complex in solution. The sodium salt of pantoprazole was chosen as a convenient model to study this subject, since the presence of the difluoromethyl group in this molecule offered the opportunity of monitoring also ^{19}F, a nucleus more sensitive than ^{1}H to structural and microenvironmental changes. The experimental curves obtained for the complexes of compound **2** with the three

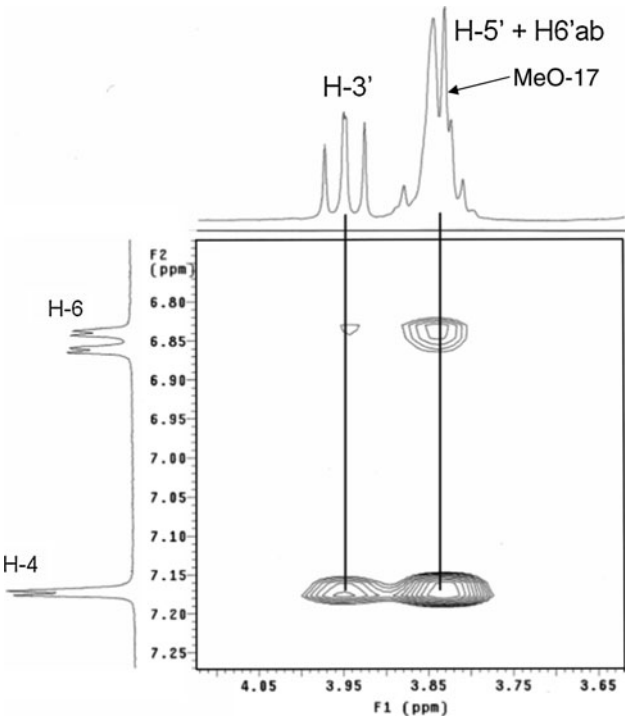

Fig. 9 Partial 2D ROESY spectrum of α-CD/**1**

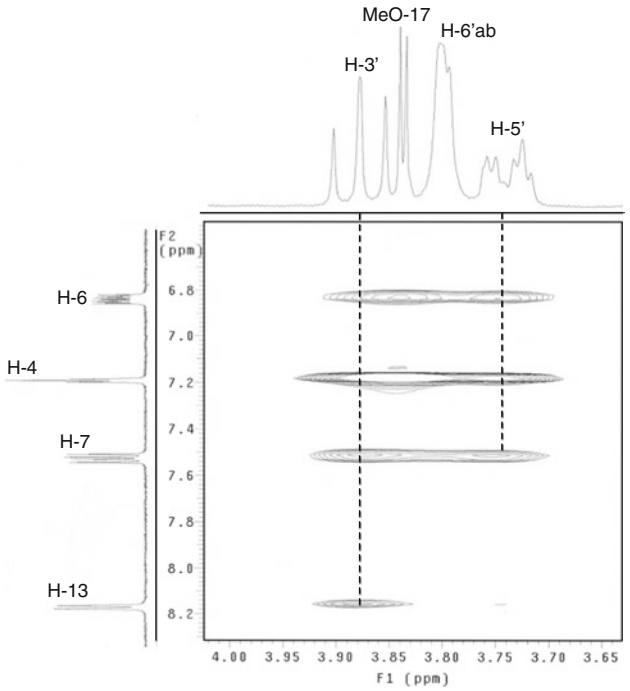

Fig. 10 Partial 2D ROESY spectrum of β-CD/**1**

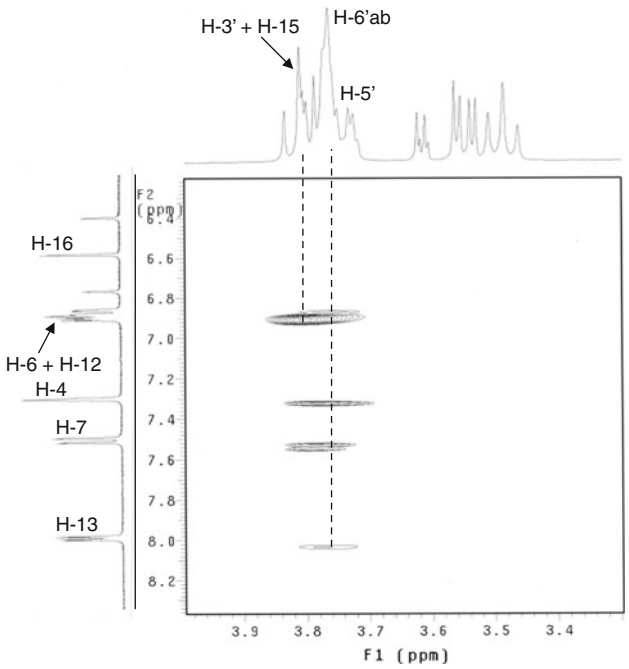

Fig. 11 Partial 2D ROESY spectrum of γ-CD/**2**

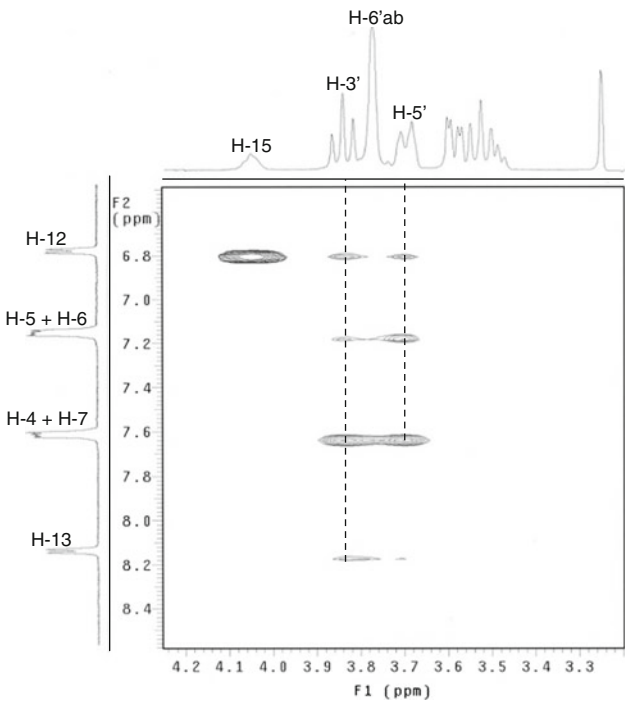

Fig. 12 Partial 2D ROESY spectrum of β-CD/**3**

CDs revealed than in all cases the average host–guest stoichiometry resulted to be 1:1.

The corresponding binding constants (K_a) were determined by using the following equation, which is the Scott's adaptation of the more general and well-known Benesi–Hildebrand method [33]:

$$[CD]/\Delta\delta = [CD]/\Delta\delta_{max} + 1/K_a\Delta\delta_{max}$$

In this equation, [CD] represents the molar concentration of the host, $\Delta\delta$ is the difference between

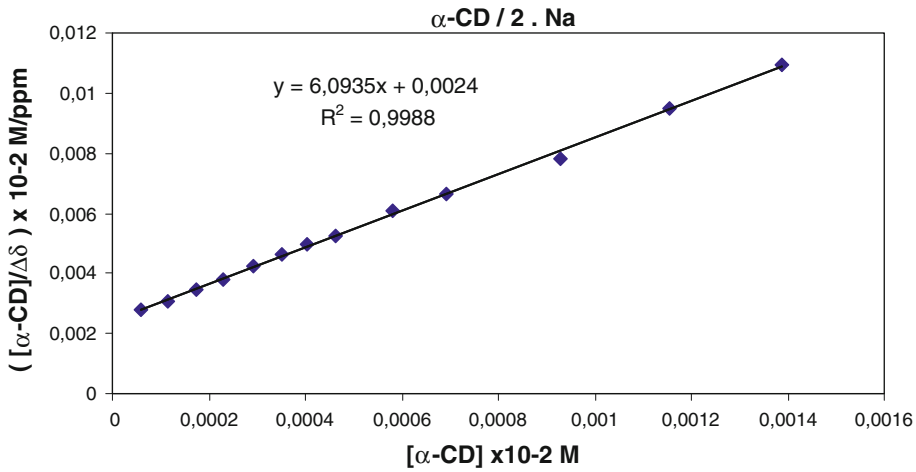

Fig. 13 Linear fit of [α-CD]/Δδ of H-16 (compound **2**) versus [α-CD]

the chemical shifts of the guest in presence of a given CD concentration and in absence of CD, and $\Delta\delta_{max}$ represents the absolute difference between the chemical shifts of the guest in absence of CD and in presence of an infinite amount of it. The value of $\Delta\delta_{max}$ equals to the inverse of the slope and the intercept of the plot with the ordinate axis to $1/K_a\Delta\delta_{max}$. The plot of [CD]/Δδ for the fluorine nucleus and several protons of the guest against [CD] yielded good linear fits for the three CD complexes of pantoprazole sodium salt (as an example, see Fig. 13),[3] a result that confirmed the 1:1 stoichiometry found before. According to this methodology, the estimated K_a's for the α-, β- and γ-CD complexes resulted to be 25, 183 and 77 M^{-1}, respectively. This result is consistent with the cyclodextrin dimensions, provided that the major stabilization factor is the steric fitting of the guest into the host cavities, since, among the three macrocycles, β-CD inner diameter is the one that better fits the dimensions of the guest benzimidazole group.

Conclusion

Despite their considerable solubility in water, the sodium salts of the PPIs ome-, panto- and rabeprazole form stable host–guest complexes with the three native cyclodextrins in aqueous solution. The existence of the corresponding diastereomeric complexes with the PPI enantiomers produced sufficient splitting of some substrate resonances to permit the monitoring of the optical purity of the sodium salt samples without the need of previous work-up, so defining a very fast an useful analytical method to apply in the chemical development of these kind of drugs. Concerning the geometry of the complexes, it has been proved via 2D ROESY the existence of a general preference for the interaction of the guest's benzimidazole group with the cavities of the cyclodextrins, although some intermolecular ROEs involving also the pyridine protons were detected in β- and γ-CD complexes, thus suggesting the coexistence of different host–guest geometries in solution. The results obtained for the complexes formed between the three CDs and pantoprazole indicated a 1:1 host/guest stoichiometry in solution.

[3] H-16 signal (for the α- and β-CD complexes) and H-4 signal (for the γ-CD complex) were found to give the best linear fits (the squares of correlation coefficients, R^2, were equal to 0.9988, 0.9892 and 0.9980 respectively).

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