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Interactions of Omeprazole and Precursors with β-Cyclodextrin Host Molecules

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Abstract

β-Cyclodextrin (β-CD) was mixed with omeprazole and some of its precursors in aqueous or water/ethanol solutions, and the resulting crystalline products have been characterized by elemental analysis, thermogravimetry, powder X-ray diffraction (XRD), FTIR and ^{13}C CP MAS NMR spectroscopy. In the case of 2-chloromethyl-4-methoxy-3,5-dimethylpyridine·HCl, it was found that the solid product always consisted of pure β-CD hydrate. On the other hand, a 2 : 1 (host-to-guest) inclusion complex was obtained between β-CD and 2-methoxy-2-mercaptobenzimidazole. The thioether intermediate 5-methoxy-2-[(3,5-dimethyl-4-methoxy-2-pyridine)methylthio]-1H-benzimidazole and its sulfoxide derivative (omeprazole) both formed 1 : 1 inclusion complexes with β-CD. Powder XRD indicates that the crystal packing of β-CD host molecules is herringbone-type for the 2 : 1 complex, and channel-type for the 1 : 1 complexes. *Ab initio* calculations were carried out to investigate the host–guest interactions. It was found that the interaction with the pyridine fragment is wholly repulsive, due to the presence of several ring substituents. On the other hand, the inclusion of the benzimidazole fragment is energetically favored, but highly dependent on the orientation of the substituent methoxy group.

Introduction

Omeprazole (OPZ) is an anti-acid drug with a widespread use in the treatment of gastric hyperacidity related diseases, such as gastric or duodenal ulcer, esophagitis and gastroesophageal reflux [1, 2]. OPZ inhibits the proton pump present in gastric mucosa cells, thus reducing acid secretion. Recently, a renewed interest for this compound has arisen as new therapeutic properties are being discovered. OPZ was evaluated for potential anti-inflammatory activity, with positive results [3]. The drug may protect gastric mucosa cells from inflammation, whether it is caused by *H. pilori* infection or by long-term administration of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) with harmful secondary effects on the stomach. In this way, omeprazole will bring a double therapeutic action in gastric hyperacidity disorders, reducing not only acid secretion but also the tissue inflammation caused by aggressive agents. Other studies investigated the mechanism of action of OPZ, finding that it forms a covalent bond with the membrane proton pump. This type of enzyme also exists in yeasts, being vital to their metabolism [4]. Omeprazole can, therefore, be used as a fungicide, featuring activity against the yeast *Candida albicans* [5], the most common pathogen in fungal infections of the mucosas.

The synthesis of omeprazole involves the formation of a thioether through the reaction of a 2-chloromethylpyridine derivative and a mercaptobenzimidazolic compound, followed by the oxidation of the corresponding sulfoxide. Omeprazole is a weak base and is stable under alkaline conditions. The main pharmaceutical drawbacks are related to the physicochemical instability to heat, light, and acidic media. Moreover, the low aqueous solubility of omeprazole, approximately 0.4% at 25 °C, is responsible for low dissolution rates and hence low bioavailability [6]. One way to overcome these problems is the complexation of OPZ with cyclodextrins such as β-CD or γ-CD. This is an established procedure to improve the biopharmaceutical properties of drugs with poor water solubility [7]. OPZ-cyclodextrin inclusion compounds have been prepared by kneading, spray-drying, coprecipitation, and freeze-drying [8, 9]. In the present work, we have carried out a comparative study of the interaction of β-CD with omeprazole and the intermediates used in the omeprazole synthesis. The adducts formed have been characterized in the solid-state by a range of techniques and *ab initio* calculations have been performed to investigate possible host–guest inclusion geometries.

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Experimental

Materials and methods

Powder XRD data were collected on a Philips X'pert diffractometer using Cu Kα radiation filtered by Ni (λ = 1.5418 Å). Water contents in samples were determined by thermogravimetry (TGA) using a Shimadzu TGA-50 thermogravimetric analyzer at a heating rate of 1 °C min^{-1} under air, with a flow rate of 30 mL min^{-1}. Infrared spectra were recorded on a Mattson Mod 7000 FTIR spectrophotometer using KBr pellets. Solid state ^{13}C CP MAS NMR spectra were recorded at 100.62 MHz, on a 9.4 T Bruker Avance 400 spectrometer (25 °C, 4.5 μs ^{1}H 90° pulses, 2.0 ms contact time, 9 kHz spinning rate and 4 s recycle delays). Chemical shifts are quoted in parts per million from TMS.

Distilled water, analytical grade ethanol and 1,4-dioxane were used as solvents. β-CD ($C_{42}H_{70}O_{35}\cdot nH_2O$, n $\sim$ 11) was kindly donated by Laboratories Roquette (Lestrem, France) and recrystallized prior to use. The precursors 2-chloromethyl-4-methoxy-3,5-dimethylpyridine·HCl (**1**) [10], 2-methoxy-2-mercaptobenzimidazole (**2**) [11], 5-methoxy-2-[(3,5-dimethyl-4-methoxy-2-pyridine)methylthio]-1H-benzimidazole (**3**) [12, 13], and 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl-1H- benzimidazole (omeprazole) (**4**) [14–16] were prepared according to published procedures. ^{13}C CP MAS NMR of **2**: δ = 165.9, 154.9, 131.9, 124.9, 109.7, 107.3, 97.4, 56.8. ^{13}C CP MAS NMR of **3**: δ = 162.9, 155.0, 152.4, 150.9, 149.7, 147.8, 138.8, 136.7, 125.5, 122.5, 114.2, 104.5, 103.4, 98.9, 97.5, 60.0, 58.4, 53.8, 36.7, 15.3, 10.4. ^{13}C CP MAS NMR of **4**: δ = 164.1, 157.8, 149.7, 135.5, 125.7, 121.5, 112.7, 91.8, 57.5, 12.4, 8.7.

*Preparation of benzimidazole·β-CD (**2a**)*

β-CD (0.20 g, 0.17 mmol) was dissolved in a mixture of water and ethanol (10 mL, 12 : 7 vol/vol) at 80 °C, and 2-methoxy-2-mercaptobenzimidazole (**2**) (0.016 g, 0.09 mmol) added. The resulting solution was left to evaporate for about five hours, until the volume reduced by half and small crystalline nuclei began to form on the solution surface. At this point the solution was cooled slowly and after a few hours colorless prismatic crystals (0.5–1.5 mm) were obtained. The crystals were rinsed with 1,4-dioxane (2 mL) followed by two washings with ethanol. Yield: 155 mg (65%). $(C_8H_8ON_2S)\cdot 2(C_{42}H_{70}O_{35})\cdot 20H_2O$ (2810.5): calcd C 39.31, H 6.74, N 0.99, S 1.14, H_2O 12.8%; found C 39.72, H 6.39, N 0.78, S 0.82, H_2O 11.9% (TGA to 85 °C). IR (KBr): ν = 3401 s, 2924 m, 1642 m, 1620 m, 1496 (sh), 1460 m, 1455 m, 1419 m, 1383 m, 1369 m, 1336 m, 1300 m, 1258 m, 1205 m, 1156 s, 1118 (sh), 1103 (sh), 1081 s, 1028 vs, 1001 (sh), 946 m, 939 m, 889 w, 869 m, 782 w, 757 m, 707 m, 600 m, 578 m, 529 m cm^{-1}. ^{13}C CP MAS NMR: δ = 155.4, 150.5, 133.4, 125.6, 110.2, (all guest-C), 103.8, 103.0, 102.4, 101.3 (β-CD, C-1), 84.9, 84.0, 83.1, 82.0, 81.2, 80.5, 78.2 (β-CD, C-4), 76.0, 74.8, 73.6, 72.6, 72.2, 71.5 (β-CD, C-2,3,5), 63.6, 61.8, 59.5 (β-CD, C-6), 55.6 (guest-OMe).

*Preparation of thioether·β-CD (**3a**)*

β-CD (0.20 g, 0.17 mmol) was dissolved in a mixture of water and ethanol (10 mL, 3 : 1 vol/vol) at 70 °C, and 5-methoxy-2-[(3,5-dimethyl-4-methoxy-2-pyridine)methylthio]-1H-benzimidazole (**3**) (0.050 g, 0.17 mmol) added. The resulting solution was stirred overnight and cooled slowly. Small crystals formed during 1 week. Yield: 110 mg (40%). $(C_{17}H_{19}O_2N_3S)\cdot(C_{42}H_{70}O_{35})\cdot 8H_2O$ (1608.5): calcd C 44.05, H 6.58, N 2.61, S 1.99, H_2O 9.0%; found C 44.36, H 6.43, N 2.47, S 1.83, H_2O 8.8% (TGA to 75 °C). IR (KBr): ν = 3360 vs, 2924 s, 1631 m, 1591 m, 1569 m, 1450 (sh), 1423 m, 1411 m, 1383 m, 1372 m, 1334 m, 1305 (sh), 1265 m, 1251 m, 1234 m, 1202 m, 1158 s, 1100 (sh), 1080 s, 1055 (sh), 1028 vs, 1001 (sh), 944 (sh), 937 m, 862 m, 754 m, 703 m, 663 w, 607 (sh), 574 m, 529 m cm^{-1}. ^{13}C CP MAS NMR: δ = 162.1, 155.7, 154.8, 152.2, 150.7, 149.0, 138.6, 134.9, 125.5, 124.6, 122.0, 116.1, 113.6, 108.6 (all guest-C), 102.8 (β-CD, C-1), 98.2, 95.3, 92.8 (all guest-C), 82.4, 80.6, 79.1 (β-CD, C-4), 72.1 (β-CD, C-2,3,5), 59.7 (β-CD, C-6), 54.6, 53.1, 37.6, 36.8, 36.6, 14.9, 11.9, 11.4 (all guest-C).

*Preparation of omeprazole·β-CD (**4a**)*

Omeprazole (0.026 g, 0.09 mmol) was added to a solution of β-CD (0.10 g, 0.09 mmol) in aqueous NaOH (5 mL, pH 12) at room temperature. The resulting mixture was stirred for 20 h and then left to settle in a refrigerator. The product, a yellowish white precipitate, was separated by decantation and dried in a desiccator. Yield: 40 mg (27%). $(C_{17}H_{19}O_3N_3S)\cdot(C_{42}H_{70}O_{35})\cdot 8H_2O$ (1624.5): calcd C 43.62, H 6.51, N 2.59, S 1.97, H_2O 8.8%; found C 43.48, H 6.05, N 2.67, S 1.94, H_2O 7.7% (TGA to 75 °C). IR (KBr): ν = 3374 vs, 2924 m, 1628 m, 1587 m, 1566 m, 1510 m, 1461 (sh), 1428 m, 1410 m, 1384 m, 1372 (sh), 1333 m, 1311 m, 1270 w, 1252 w, 1231 w, 1205 m, 1158 s, 1101 (sh), 1080 s, 1056 vs, 1028 vs, 1004 (sh), 967 (sh), 945 m, 939 (sh), 885 w, 862 m, 837 m, 822 m, 810 m, 754 m, 703 m, 670 w, 664 w, 608 w, 576 m, 530 m, 476 w, 442 w, 431 w cm^{-1}. ^{13}C CP MAS NMR: δ = 163.0, 157.1, 149.2, 136.5, 125.2, 120.8, 111.9 (all guest-C), 102.8 (β-CD, C-1), 90.9 (guest-C), 82.6, 80.5, 79.4 (β-CD, C-4), 72.2 (β-CD, C-2,3,5), 59.7 (β-CD, C-6), 57.1, 11.7, 8.1 (all guest-C).

Computational details

All *ab initio* calculations were performed using the GAUSSIAN98w package [17]. Omeprazole molecular structures were fully optimized at the HF/6-31G* standard level [18], starting from several distinct geometries. Harmonic vibrational wavenumbers were calculated at the same level using analytical second derivatives. Concerning the inclusion compounds, the possible inclusion geometries were evaluated by a set of single point calculations, using the two-layer approximation of Morokuma and co-workers [19–21] (ONIOM

keyword of GAUSSIAN 98). Omeprazole was treated at upper layer (6–31G*), while β-CD was set at lower layer (Stevens/Basch/Krauss ECP minimal basis set [22, 23]).

Results and discussion

Synthesis and characterization

The precursor compounds **1–3** (Chart 1) are partially soluble in water and therefore a coprecipitation method from solution was used to prepare β-CD inclusion complexes. Specifically, the solids **1–3** were added to a solution of β-CD in water/ethanol at 70–80 °C. Concentration and/or cooling of the resulting solutions led to the precipitation of crystalline products. In the case of 2-chloromethyl-4-methoxy-3,5-dimethylpyridine·HCl (**1**), it was found that the solid product always consisted of pure β-CD hydrate. On the other hand, the other precursors 2-methoxy-2-mercaptobenzimidazole (**2**) and 5-methoxy-2-[(3,5-dimethyl-4-methoxy-2-pyridine)methylthio]-1H-benzimidazole (**3**) formed 2 : 1 and 1 : 1 (host-to-guest) inclusion complexes, respectively, with β-CD, as evidenced by elemental analysis (C,H,N,S), thermogravimetry, powder X-ray diffraction and ^{13}C MAS NMR spectroscopy. The products are designated as benzimidazole·β-CD (**2a**) and thioether·β-CD (**3a**). In the case of compound **2a**, a stoichiometric 2 : 1 complex was obtained even when cyclodextrin was treated with a two-fold molar excess of compound **2**. This can be taken as evidence for a real inclusion phenomenon [24]. A 1 : 1 inclusion complex with omeprazole (**4**) as the guest, designated as omeprazole·β-CD (**4a**), was obtained by addition of omeprazole to β-CD dissolved in aqueous sodium hydroxide solution (pH 12) at room temperature. Elemental analysis and thermogravimetry indicate that the encapsulation of the organic guest molecules to give **2a–4a** is accompanied by a slight decrease in the overall crystal water contents, relative to that of pure β-CD hydrate (which contains ca. 15.0% water by weight, i.e., ca. 11 water molecules per formula unit). Thus, the 2 : 1 adduct **2a** contains about 10 water molecules per formula unit, while the 1 : 1 adducts **3a** and **4a** contain about 8 water molecules per formula unit.

The powder XRD patterns of pure β-CD hydrate, compounds **2–4** and **2a–4a** are shown in Figure 1. The pattern of **2a** resembles that of pure β-CD hydrate, although there are substantial changes in the intensities of various peaks with some slight changes in the 2θ values. This suggests that the major phase in **2a** comprises β-CD molecules arranged in a herringbone-type pattern, as found in either pure β-CD hydrate or β-CD inclusion compounds with small alcohols [25]. In this type of arrangement, the guest and host usually form a monomeric complex, leading to a 1 : 1 stoichiometry. The stoichiometry found for **2a** (2 : 1) is therefore surprising and difficult to explain in the absence of a crystal structure determination. One possibility is that alternating β-CD molecules in the structure do not contain organic guests. This is a rare occurrence but has been reported, for example, for the 2 : 1 complex between β-CD and S-(+)-ibuprofen [26].

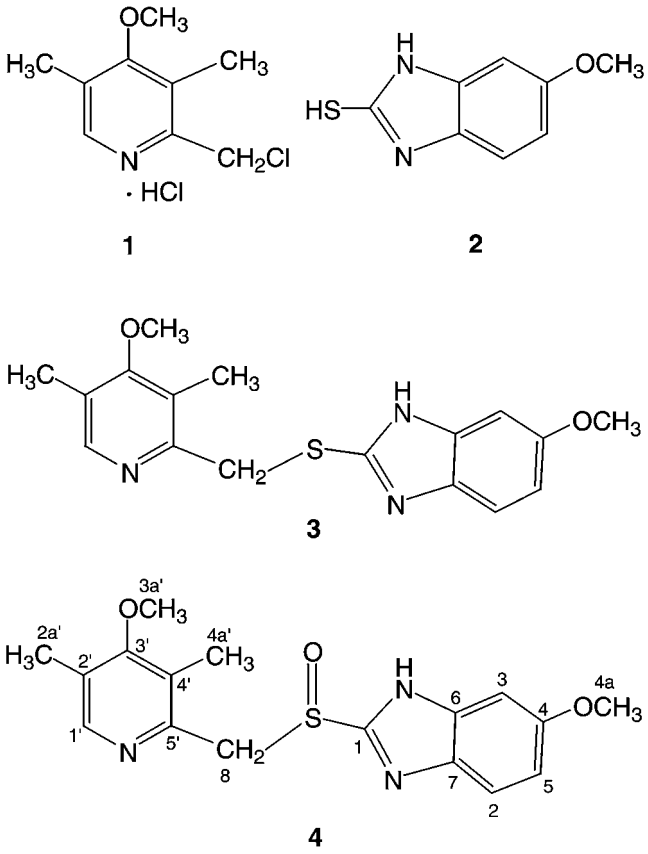

Chart 1.

Comparing the powder XRD patterns of **2** and **2a**, it is evident that **2a** does not contain measurable amounts of a phase corresponding to pure non-included **2**.

Compounds **3a** and **4a** give rise to powder XRD patterns that are very different from those of the pure components. This is a good indication for the formation of true inclusion complexes, with crystal structures different from the herringbone-type [24]. Figure 1 shows a simulated powder diffraction pattern calculated from the crystal structure data for the 1 : 1 (host : guest) β-CD inclusion compound of ethyl 4-aminobenzoate (benzocaine) [27]. This compound exhibits the typical channel-type structure consisting of head-to-head dimers of β-CD molecules stacked along the crystallographic c axis. The coincidence of the calculated pattern with the experimental patterns of **3a** and **4a**, especially at low angles (3–20 °2θ), indicates that the β-CD host molecules are arranged very similarly. The diffraction peaks for **4a** are generally weaker and broader than the corresponding peaks for **3a**, indicating a lower degree of crystallinity for **4a**. The stoichiometry found for **3a** and **4a** (1 : 1) is surprising, given the size of the guest molecules, i.e., it is difficult to envisage how β-CD : guest units (see *ab initio* calculations below) will group to form a columnar arrangement of dimers. A 2 : 1 stoichiometry, wherein one guest molecule is encapsulated within a β-CD head-to-head dimer, might seem more reasonable. However, this can be ruled out, because it has been shown both experimentally (failure to prepare a complex with **1**) and theoretically (see

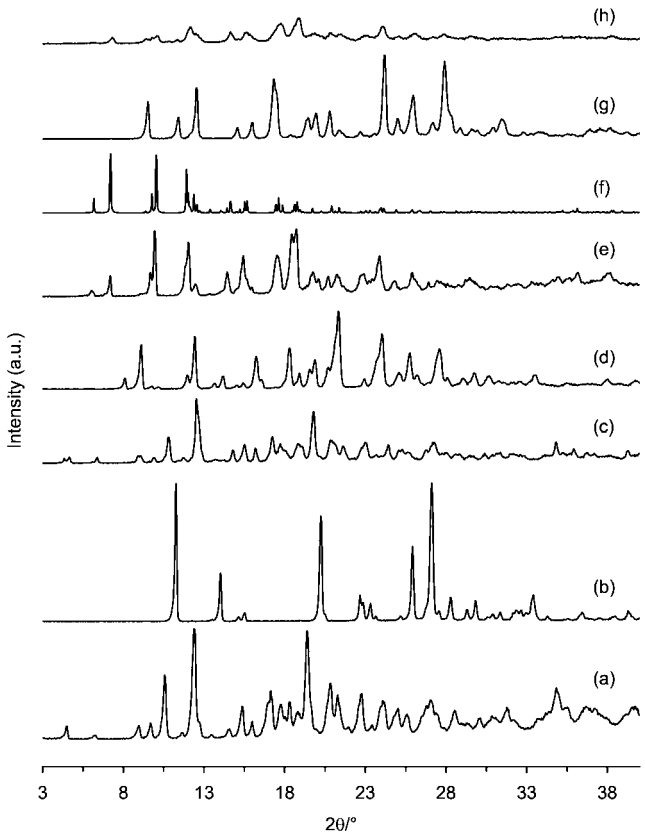

Figure 1. Powder XRD patterns of (a) plain β-CD hydrate, (b) compound **2**, (c) the adduct benzimidazole·β-CD (**2a**), (d) compound **3**, (e) the adduct thioether·β-CD (**3a**), (f) the 1 : 1 complex of β-CD with benzocaine (calculated), (g) omeprazole (**4**), and (h) the adduct omeprazole·β-CD (**4a**). The program PowderCell [28] was used to produce the calculated pattern (f) using the crystal structure data reported in the literature (guest molecules were omitted from the calculations for simplicity).

ab initio calculations below) that inclusion of the pyridine fragment of **3** and **4** is highly unfavorable.

Figure 2 shows the solid-state ^{13}C CP MAS NMR spectra of β-CD hydrate, compounds **2–4** and **2a–4a**. The spectrum of β-CD hydrate is similar to that reported previously and exhibits multiple resonances (spread over a relatively large chemical shift range) for each type of carbon atom [29-31]. This has been mainly correlated with different torsion angles about the (1 $\rightarrow$ 4) linkages for C-1 and C-4 [29, 30], and with torsion angles describing the orientation of the hydroxyl groups [31]. The different carbon resonances are assigned to C-1 (101–104 ppm), C-4 (78–84 ppm), C-2,3,5 (71–76 ppm) and C-6 (57–65 ppm). The spectrum of **2a** is similar to that of β-CD hydrate except that the resonances for the β-CD carbons are slightly broader. More significant changes are observed for **3a** and **4a**, possibly due to conformational changes arising from inclusion of guest molecules in the β-CD cavities. Thus, the β-CD carbons C-1, C-2,3,5 and C-6 are observed as single broad peaks at 102.8, 72.1 and 59.7 ppm, respectively. The inclusion of guest molecules in the β-CD cavities may force the host molecule to adopt a more symmetrical conformation, with each glucose unit in a similar environment [32, 33]. In addition to the resonances for the β-CD carbons, the spectra of **2a–4a** exhibit several relatively weak lines that can be assigned to the car-

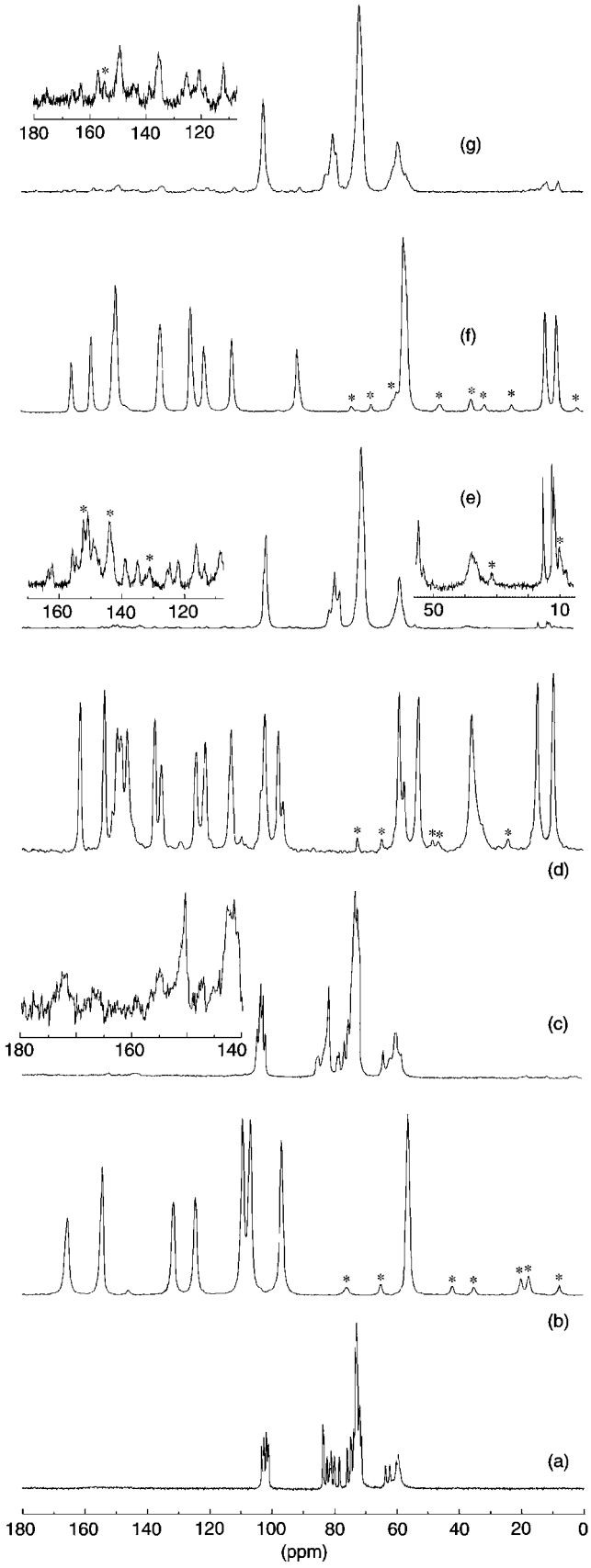

Figure 2. Solid-state ^{13}C CP MAS NMR spectra of (a) plain β-CD hydrate, (b) compound **2**, (c) the adduct benzimidazole·β-CD (**2a**), (d) compound **3**, (e) the adduct thioether·β-CD (**3a**), (f) omeprazole (**4**), and (g) the adduct omeprazole·β-CD (**4a**). Spinning sidebands are denoted by *.

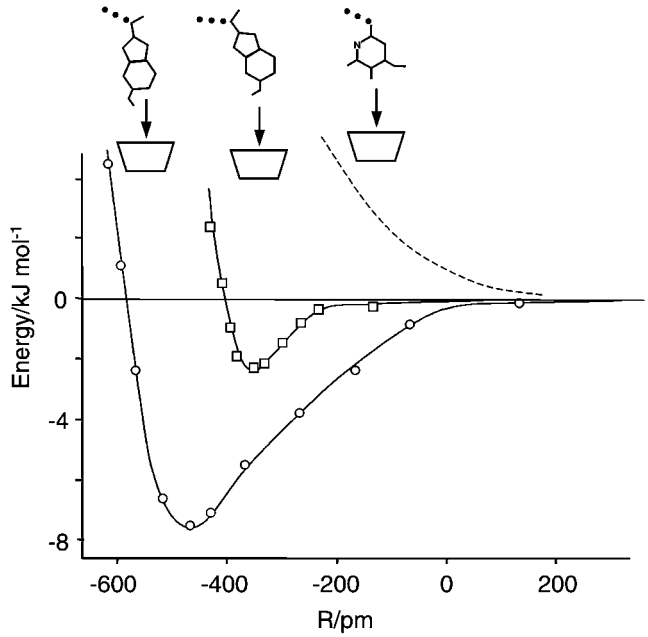

Figure 3. Energy vs host–guest distance plot for the omeprazole·β-CD complex. R is defined as the distance between the plane of the outer hydrogen atoms in the secondary hydroxyl groups of β-CD and the nearest hydrogen atom of the approaching omeprazole molecule. Negative R values refer to inclusion.

bon atoms of the guest molecules. These are weakly shifted compared with the corresponding lines for the non-included compounds **2–4**, confirming that the structural integrity of the guest molecules is retained in the included state (and that the interaction with the host molecule is weak). Similar conclusions are drawn upon comparison of the FTIR spectra for free and included compounds.

Ab initio calculations

Omeprazole is known to present several conformations, arising from both the internal rotations around the C1-S-C8-C5′ single bonds and from the orientations of the substituent methoxy groups. The lowest energy structure found at the HF/6-31G* level presents an extended geometry, with a C1-S-C8-C5′ dihedral angle of ca. 177°. This structure is similar to the one reported for the crystal (179°, [34]), but with a different orientation of the methoxy group at C4. Both structures have been used to evaluate the omeprazole-β-CD inclusion process.

Figure 3 shows the most relevant results for the inclusion of omeprazole in β-CD, considering the approach by either the pyridine or the benzimidazole fragments along the major β-CD axis. The interaction with the pyridine fragment was found to be purely repulsive, as expected from the size of the fragment (arising from the presence of several substituents on the pyridine ring) and the above-mentioned difficulties in preparing an inclusion complex with precursor **1**. On the other hand, the inclusion of the benzimidazole fragment is energetically favored, but highly dependent on the orientation of the substituent methoxy group. For the C5-C4-O-C4a = 180° orientation, the shape of the fragment does not allow

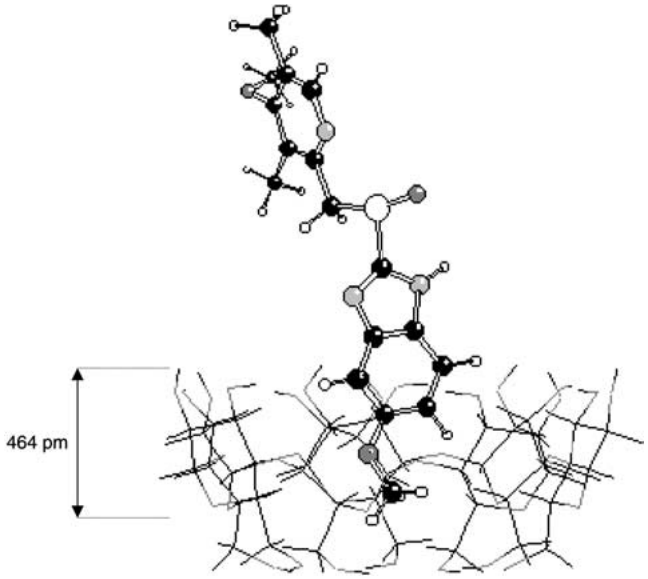

Figure 4. Schematic view of the lowest energy structure for the omeprazole·β-CD inclusion compound.

a deep inclusion, leading to a weak omeprazole-β-CD interaction (Figure 3). The C5-C4-O-C4a = 0° orientation – which is found in the crystal structure – results in a more favorable shape for inclusion and yields the best host–guest fit. The penetration of the benzimidazole fragment, measured from the H-nuclei positions (large rim OH of β-CD to methoxy group) is ca. 464 pm (Figure 4) and the calculated inclusion energy is ca. 8 kJ mol^{-1}. Despite the simplicity of the model, this energy value is a good estimate of the efficiency of the inclusion process. The minima found for the inclusion of the benzimidazole fragment can be assumed also for the inclusion of the thioether precursor **3**, as they are mainly the result of intracavity benzimidazole-β-CD interactions.

Concluding remarks

β-Cyclodextrin inclusion complexes have successfully been prepared with omeprazole and two of the intermediates involved in the synthesis of omeprazole. Both the experimental results and *ab initio* calculations indicate that the inclusion process involves encapsulation of the benzimidazole fragment in the β-CD cavity.

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