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Papers

Dedicated to Dr. Maria Zaharescu
on the occasion of her 85th anniversary

BEZIMIDAZOLIUM BROMIDE DERIVATIVE INCLUSION COMPLEXES WITH NATIVE AND MODIFIED BETA-CYCLODEXTRINS

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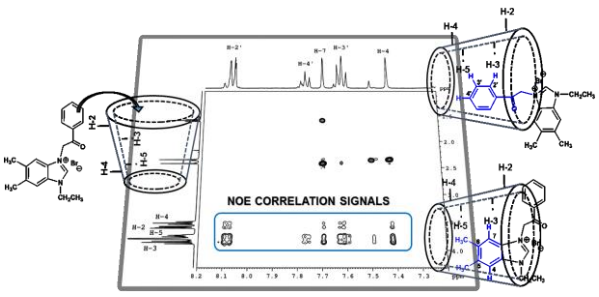
The interactions between four native and modified beta-cyclodextrins and a benzimidazolium bromide salt were analyzed through UV-Vis and NMR Spectroscopy. The new benzimidazolium salt was obtained by simple and efficient conversion of *N*-1 substituted 5,6-dimethylbenzimidazole with phenacyl bromide in acetone. In all cases, the complexes stoichiometry was 1:1, as determined from UV-Vis titrations. Based on the values for association constants, the strength of the interactions with benzimidazolium bromide was weakest with the methyl substituted beta-cyclodextrin and strongest with the sulfobutylether substituted beta-cyclodextrin. Through-space NOE experiments were used to investigate the structural aspects of inclusion process. The obtained NOE correlations indicate coexistence of two inclusion modes: one with the phenacyl group inside the cyclodextrin cavity and the second one with dimethyl-substituted benzene ring inside the cavity. The imidazole ring and the ethyl substituent have been proven to remain outside the cyclodextrin cavity in both inclusion modes.



INTRODUCTION

To date, a plethora of derivatives containing benzimidazole scaffold are described in the literature, with proven biological and pharmacological activities, such as antidepressant, anthelmintic or antiviral.[1-6] In two recent studies, Van Oosten *et al.* and Rouphael *et al.*, have shown that micromolar concentrations of omeprazole, a benzimidazole inhibitor of animal proton pumps, can improve tomato plants growth and increase their tolerance to salinity stress.[7,8] Recently, we have published several papers on synthesis and properties of small bioactive nitrogen containing molecules that can stimulate plants growth or improve the tolerance to environmental stressors.[9-13]

In a 2017 minireview, Gravel *et al.* analyzed the idea that the toxicity of compounds containing

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in their structure imidazolium and benzimidazolium salts can be tailored towards potent drugs. Driven by the never-ending fight against drug resistant bacteria, research groups are continuously searching for new antibacterial alternative compounds. The authors pointed out that a special research interest goes towards compounds able to attack the bacterial membrane.[14] In this respect, their group demonstrated in several studies the transmembrane transporters properties of novel imidazolium and benzimidazolium containing derivatives. They also showed that the transmembrane transport can be controlled by the formation of an inclusion complex with β-cyclodextrin and that a host-guest competitive assay can be used to modulate the toxicity of these salts.[15-17]

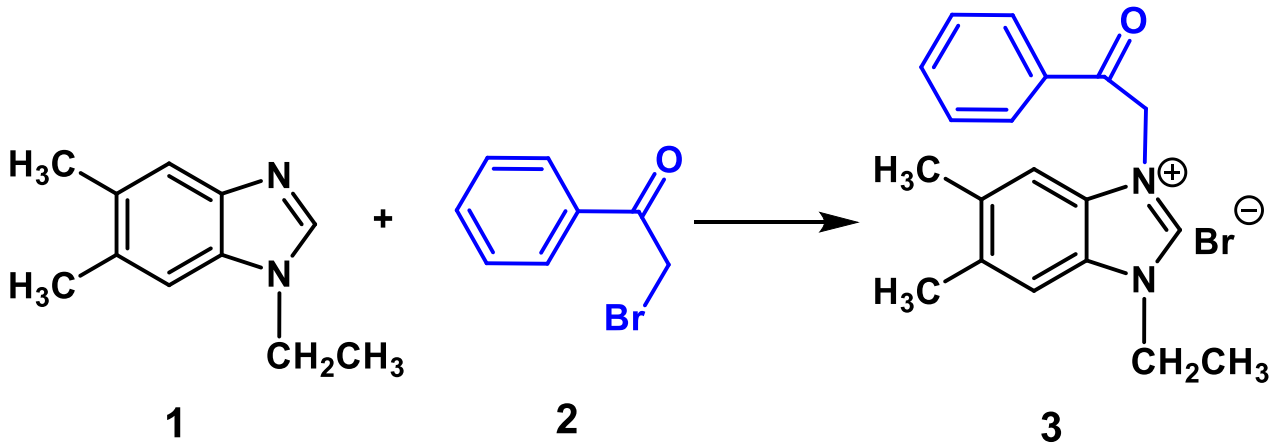
Complexation of native and modified cyclodextrins with different compounds have found numerous applications in all life sciences including medicine,[18] food industry,[19] cosmetics and personal care,[20] agriculture[21] or in analytical chemistry for chromatographic separation and purification aspects.[22] Cyclodextrins, also known as Schardinger dextrines, are stable cyclic compounds containing *α*-(1-4)-linked glucopyranose units. Both natural and chemically modified forms have unique properties owing to their truncated cone-like structures that favor the encapsulation of various molecules, generally by improving their water solubility.[23]

Our group used benzimidazolium salts mainly as intermediates in synthesis of various pyrrolo[1,2-a]benzimidazole and pyrrolo[1,2-a]quinoxaline derivatives.[24-29] Another group is showing recent activity in this research area.[30] Following our previous researches on bezimidazolium salts and their cyclodextrin inclusion complexes,[31,32] we report on the synthesis of 1-ethyl-3-[2-phenyl-2-oxoethyl]-5,6-dimethylbenzimidazolium bromide (**3**) and its complexes with native beta-cyclodextrin (**BCD**) and modified hydroxypropyl- (**HPBCD**), methyl- (**MeBCD**) and sulfobutylether-beta-cyclodextrins (**SBEBCD**).

RESULTS AND DISSCUSION

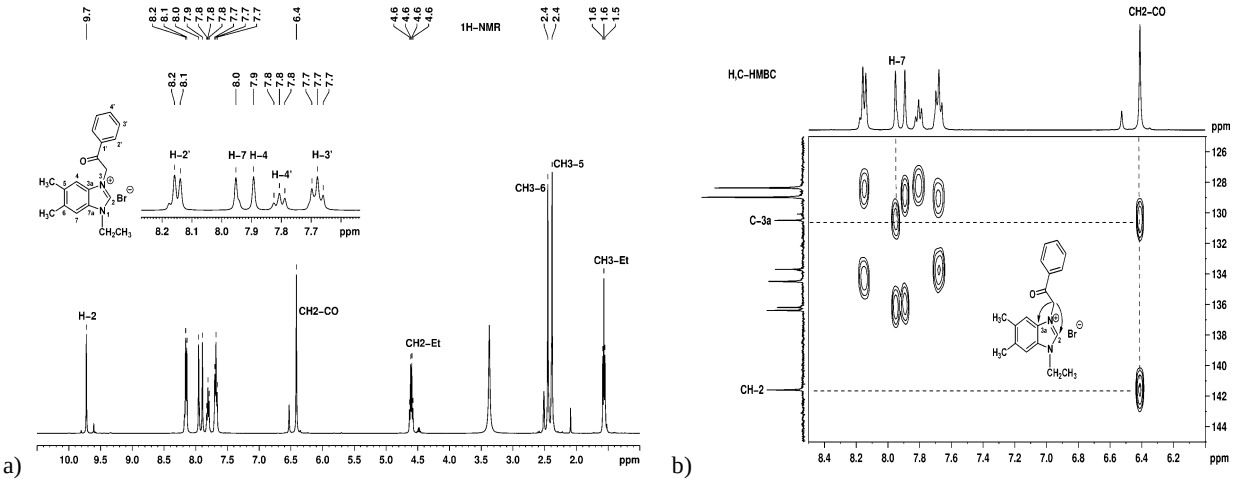
Benzimidazolium salt synthesis

The new benzimidazolium salt **3** was obtained by simple and efficient conversion of *N*-1 substituted 5,6-dimethylbenzimidazole (**1**) with phenacyl bromide (**2**) in acetone, as presented in Scheme 1.



Scheme 1. The synthetic route for benzimidazolium salt **3**.

The proposed chemical structure was verified through NMR spectroscopy analysis. Due to very poor water solubility, the initial NMR analysis was performed in DMSO-d_6 as solvent. The assignment of the proton and carbon signals was done using standard 2D NMR homo- and heteronuclear correlations such as: H,H-COSY, H,C-HSQC and H,C-HMBC. Both proton and carbon chemical shifts values and signals multiplicities correspond with the proposed structure. In the proton spectrum (Fig. 1a), phenacyl protons resonate at the following chemical shifts: 6.4 (singlet, CH_2CO), 7.7 (triplet, H-3'), 7.8 (triplet, H-4') and 8.2 ppm (doublet, H-2'), whereas the benzimidazole protons were assigned at 1.5 (triplet, CH_3), 2.4 (two singlets for CH_3 groups), 4.6 (quartet, CH_2), 7.9 (singlet, H-4), 8.0 (singlet, H-7) and 9.7 ppm (singlet, H-2). The success of *N*-acylation is confirmed by the three-bond correlation signals between phenacyl's CH_2 group and benzimidazole CH-2 and quaternary C-3a, as exemplified in the long-range H,C-HMBC spectrum from Fig. 1b.



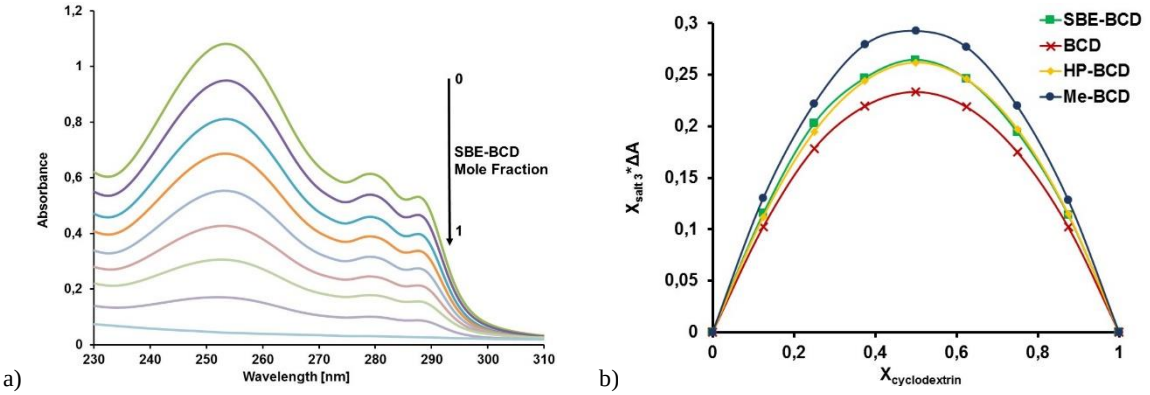
a) b)

Fig. 1 – a) The ^{1}H-NMR spectrum corresponding to benzimidazolium salt **3**, recorded in DMSO-d_6, with annotated assignments; **b**) detailed low-field region from the long-range H,C-HMBC spectrum showing the correlation signals that prove the success of *N*-acylation.

Stoichiometry of the complexes

The first step in characterization of cyclodextrin's inclusion complexes is the stoichiometry determination, which depends on both the nature and size of inner cavity and guest size. It is well known that cyclodextrin inner cavity is slightly hydrophobic while the exterior is hydrophilic. Beta-CDs inner cavity has a diameter of 6.0-6.5 Å and can accommodate non-polar specific sized molecules, forming inclusion complexes stabilized through hydrogen bonds or Van der Waals forces.

In this study, the stoichiometry of the four inclusion complexes was determined through UV-Vis spectroscopy, using the continuous variation method (Job's method). For this analysis, four sets of solutions were prepared by mixing aqueous compound **3** solutions with aqueous beta-cyclodextrins solutions, so that the total concentrations were kept constant and mole fractions (X) were varied from 0 to 1. Absorption spectra (Fig. 2a) show a maximum absorbance at λ_{max}= 254 nm and Job plots were obtained from the graphical representation of the $X\Delta A$ *versus* X, where ΔA is the difference in absorbance between free benzimidazolium salt and the one measured in cyclodextrins' presence. All plots show inflexion points at $X_{cyclodextrin}$= 0.5 indicating 1:1 stoichiometry, as presented in Fig. 2b.



a) b)

Fig. 2 – a) UV-Vis spectra of nine **SBEBCD-3** solutions, with mole fractions varied for 0 to 1; b) Job plots for the four cyclodextrins-**3** mixtures, obtained from UV-Vis titrations, showing 1:1 stoichiometry.

Association constants of the complexes

The binding ability into the host cyclodextrins and the stability of the formed complexes were evaluated from the association constants. In the case of NMR spectroscopy, the host-guest interactions are followed in proton spectra by observing chemical shift variation upon complexation of either host or guest signals. The randomly substituted beta-CDs used in this study have very broad signals for glucopyranose unit that overlap in the region 3.4–4.0 ppm and cannot be used to follow the

host-guest interactions. By contrast, the benzimidazole salt aromatic signals are well resolved and differentiated, being good candidates for observing the interactions with host cyclodextrins.

Due to the low water solubility of the salt **3**, sets of solutions were prepared so that salt concentration was kept constant at 10^{-3} M and cyclodextrins concentration was increased from 0 to 15×10^{-2} M, depending on the cyclodextrins' water solubility at room temperature. The NMR version of Benesi-Hildebrand equation was used to determine the association constants from the chemical shifts' variation of benzimidazolium salt signals.[33]

$$1/\Delta\delta = 1/(K_a\Delta\delta_{max}[H]_0) + 1/\Delta\delta_{max},$$

where $\Delta\delta$ is the chemical shift variation upon addition of cyclodextrin, $[H]_0$ is cyclodextrin concentration and K_a is the association constant.

The plot of $1/\Delta\delta$ as a function of cyclodextrins concentration reciprocal is linear, as exemplified in Fig. 3 for benzimidazolium H-2' proton, with the slope $1/K_a\Delta\delta_{max}$ and intercept $1/\Delta\delta_{max}$. From these plots, the following values for the association constants were obtained: 60 M^{-1} for **BCD**, 55 M^{-1} for **HPBCD**, 482 M^{-1} for **SBEBCD** and 44 M^{-1} for **MeBCD** complexes.

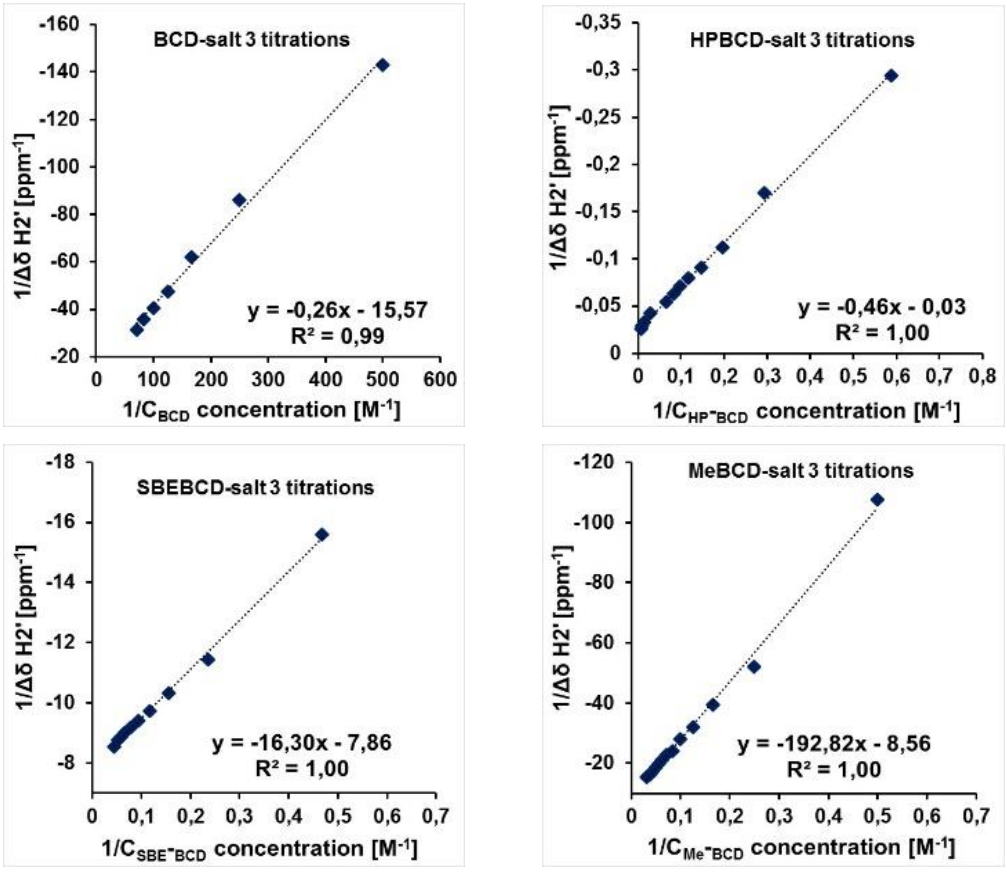


Fig. 3 – Plots of the Benesi-Hildebrand data treatment for the benzimidazolium salt H-2' chemical shift displacement in the presence of the four cyclodextrines.

Through space NOE interactions

From the NMR titration experiments performed for determination of association constants, we obtained for the benzimidazolium salt signals small chemical shifts variations, between ± 0.01 and ± 0.05 ppm. In these cases, additional information about the host-guest interactions are obtained from two dimensional nuclear Overhauser effect experiments (NOE), such as H,H-ROESY (rotating frame NOE), where cross signals are expected due to spatial proximity (below 5 Å) between host and guest protons. For recording the ROESY experiments, 1:1 (molar ratio) mixtures in D_2O of benzimidazolium salt **3** and **BCD**, **MeBCD**, **HPBCD** and **SBEBCD** respectively were prepared. As we previously reported,[27] in D_2O these benzimidazolium salts undergo easy deuteration of CH_2CO (6.4 ppm) and imidazolic CH-2 (9.7 ppm) protons. Consequently, their corresponding signals are no longer present in the proton spectra and no information about NOE interactions could be obtained for these sites.

The ROESY spectra for the four analyzed mixtures are presented in Fig. 4. In all cases, NOE correlation peaks were observed between cyclodextrin's glucopyranose protons and aromatic protons from phenacyl and benzimidazol groups. Taking into account the dynamic nature of the host-guest interactions, these NOE correlations indicate two possible inclusion modes: one with the phenacyl group inside the cyclodextrin cavity and the second one with dimethyl-substituted benzene ring inside (Fig. 4e). These two inclusion modes are clearly seen in ROESY spectra of **SBEBCD-3** and **BCD-3** mixtures, presented in Figs. 4a and 4c. Aside from the correlation peaks with cyclodextrins inner cavity protons (H-3 and H-5), NOE signals were also observed between the salt and the cyclodextrins outer protons (H-2 and H-4). This is better seen in the case of **BCD**, where it is a clear separation between inner and outer cyclodextrin protons signals. Additional, for **SBEBCD-3** mixture we obtained NOE correlations between salt **3** and sulfobutylether residue, supporting the dynamic equilibrium between complexed and free forms.

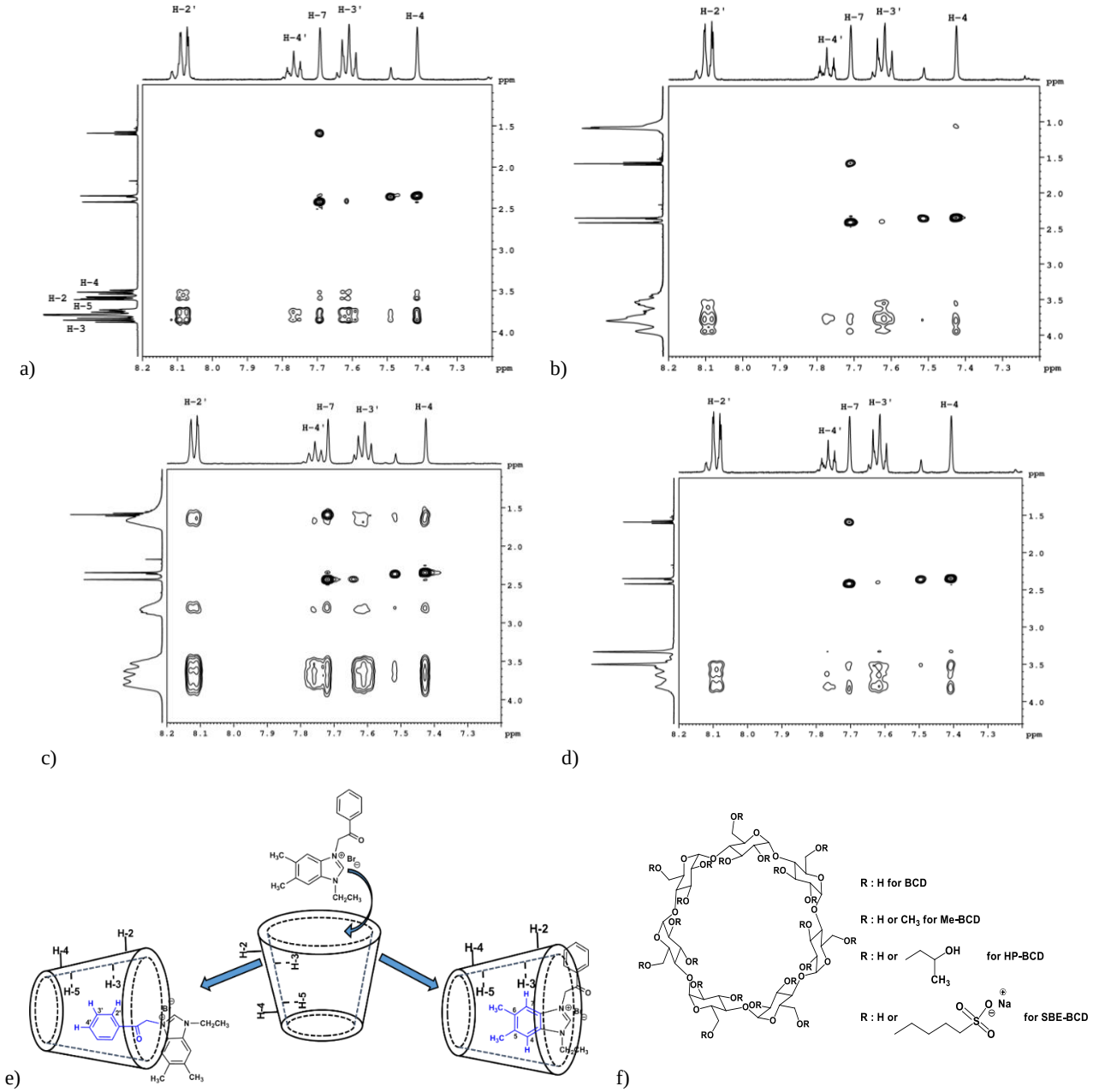


Fig. 4 – H,H-ROESY spectra, recorded with water suppression, for the 1:1 mixtures: a) **BCD-3**; b) **HPBCD-3**; c) **SBEBCD-3**; d) **MeBCD-3**; e) graphical representation of the two possible inclusion modes of salt **3** inside native BCD cavity and f) chemical structures of the four cyclodextrins.

EXPERIMENTAL

Materials and Methods

The NMR spectra were recorded on Bruker Avance III 400 MHz spectrometer equipped with a 5 mm inverse-detection z-gradient multinuclear probe. For all the experiments performed in this study, we used Bruker pulse programs included in TopSpin 3.1, acquisition and processing software. Unambiguous signals assignments in proton and carbon spectra of newly synthesized benzimidazolium bromide were based on information obtained from homo- and heteronuclear bidimensional correlation experiments like COSY, HSQC and HMBC. ROESY experiments were recorded with water signal suppression, with a mixing time of 200 miliseconds. ^{1}H NMR spectra were recorder at constant temperature of 27°C and were referenced on the solvent residual peak (4.8 ppm for D_2O and 2.51 ppm for DMSO).

UV-Vis absorption spectra were obtained using a SPECORD210Plus spectrometer (Analityk Jena, Germany), in rectangular 10 mm path length quartz cuvettes, at room temperature.

Commercially available β-cyclodextrin hydrate (99%, Aldrich), 2-hydroxypropyl-β-cyclodextrin (Alfa Aesar), sulfobutylether-β-cyclodextrin (Ligand Pharmaceuticals Inc.) and Methyl-β-cyclodextrin (Aldrich) were used without further purification.

Synthesis of 1-ethyl-3-[2-phenyl-2-oxoethyl]-5,6-dimethylbenzimidazolium bromide (3)

To a solution of 5 mmole of 1-ethyl-5,6-dimethylbenzimidazoline (**1**) in 30 ml acetone, 5 mmole of phenacyl bromide (**2**) was added. The reaction mixture was heated at reflux temperature for 3 h and left overnight at room temperature. The solid was filtered off, washed on the filter with 10 ml mixture of acetone-diethyl ether 1:1 and recrystallized from methanol/diethyl ether.

^{1}H NMR (400.1 MHz, DMSO-d_6), δ (ppm): 9.72 (s, 1H, H2), 8.15 (t, *J*=8 Hz, 2H, H2',6'), 7.95 (s, 1H, H7), 7.95 (s, 1H, H4), 7.80, (t, *J*=8 Hz, 1H, H4'), 7.68 (t, *J*=8 Hz, 2H, H3',5'), 6.41 (s, 2H, CH_2-3), 4.60 (q, *J*=7 Hz, 2H, CH_2-1), 2.45 (s, 3H, CH_3-5), 2.38 (s, 3H, CH_3-6), 1.56 (t, *J*=7 Hz, 3H, CH_3-1); ^{13}C NMR (100.6 MHz, DMSO-d_6), δ (ppm): 191.2 (C=O), 141.6 (C2), 136.4 (C6), 136.2 (C5), 134.5 (C4'), 133.7 (C1'), 130.5 (C3a), 129.0 (C3',5'), 128.4 (C2',6',7a), 113.4 (C4), 113.1 (C7), 53.1 (CH_2-3), 42.1 (CH_2-1), 19.9 (CH_3-5,6), 14.3 (CH_3-1).

Beta-cyclodextrin (BCD): ^{1}H NMR (400.1 MHz, DMSO-d_6), δ (ppm): 5.08, 5.08 (d, *J*=4 Hz, 7H, H1), 3.98 (t, *J*=9 Hz, 7H, H3), 3.90 (s, 7H, H6), 3.88 (d, *J*=10 Hz, 7H, H5), 3.67 (dd, *J*=4 Hz, *J*=10 Hz, 7H, H2), 3.60 (t, *J*=9 Hz, 7H, H4).

Sulfobutylether-beta-cyclodextrin (SBEBCD): ^{1}H NMR (400.1 MHz, DMSO-d_6), δ (ppm): 5.29-5.15 (H1), 4.09-3.54 (H2, H3, H4, H5, H6, CH_2 bound to C6), 3.02-3.00 (CH_2), 1.85 (2×CH_2).

Methyl-beta-cyclodextrin (MeBCD): ^{1}H NMR (400.1 MHz, DMSO-d_6), δ (ppm): 5.31-5.10 (H1), 4.02-3.62 (H2, H3, H4, H5, H6), 3.60 (CH_3).

2-hydroxypropyl-beta-cyclodextrin (HPBCD): ^{1}H NMR (400.1 MHz, DMSO-d_6), δ (ppm): 5.28-5.11 (H1), 4.05-3.53 (H2, H3, H4, H5, H6), 1.19-1.17 (CH_3).

Preparation of the benzimidazolium bromides-cyclodextrins solutions

For stoichiometry determination through UV-VIS spectroscopy, a series of solutions in distilled water were prepared by keeping constant the concentration (10^{-5} M) of the two components while varying their molar ratio between 0 and 1.

For determination of the association constants, other series of solutions in deuterated water (D_2O) were prepared by keeping constant the concentration (10^{-3} M) for benzimidazolium salt **3** and varying the cyclodextrins concentration as it follows: 0 to 1.4×10^{-2} M (for **BCD**), 0 and 1.53 M^{-1} (for **HPBD**), 0 and 3.15×10^{-2} M (for **MeBCD**) and 0 and 2×10^{-3} M (for **SBEBCD**).

CONCLUSIONS

The interactions between native and three modified beta-cyclodextrins with a newly synthesized benzimidazolium bromide salt were analyzed through UV-Vis and NMR spectroscopy. From UV-Vis titrations we obtained a 1:1 stoichiometry for all four analyzed cyclodextrine-benzimidazolium salt mixtures. The binding ability into the host cyclodextrins and the stability of the formed complexes were evaluated from ^{1}H-NMR titrations. The NMR version of Benesi-Hildebrand equation was used to determine the association constants from the chemical shifts' variation of benzimidazolium salt signals. Based on the values for association constants, the strength of the interactions with benzimidazolium bromide increases in the order **MeBCD** < **HPBCD** < **BCD** < **SBEBCD**. In the ROESY spectra of the four analyzed mixtures we observed NOE correlation peaks between cyclodextrin's glucopyranose protons and aromatic protons from phenacyl and benzimidazol groups. Taking into account the dynamic nature of the host-guest interactions, these NOE correlations indicated the coexistence of two inclusion modes: one with the phenacyl group inside the cyclodextrin cavity and the second one with dimethyl-substituted benzene ring inside the cavity.

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