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ARTICLE TYPE

Solvent control in the formation of supramolecular host-guest complexes of isoniazid with *p*-sulfonatocalix[4]arene

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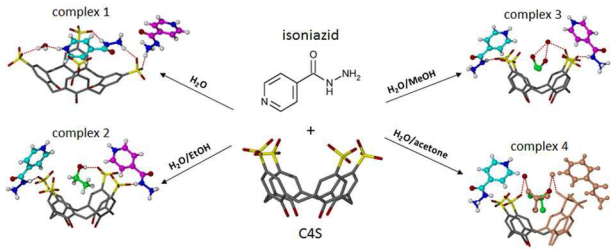
Here we demonstrate solvent role in the cocrystallization of anti-tuberculosis drug isoniazid INH with macrocyclic host *p*-sulfonatocalix[4]arene C4S. The host-guest inclusion complex of INH with C4S crystallizes from aqueous solution. Altering the crystallization medium to water-cosolvent mixtures results in the formation of C4S-INH *exo* complexes, where the host cavity becomes occupied by a solvent molecule.

Tuberculosis is an infectious disease caused by various strains of mycobacteria. It is the second cause of death for a single infectious disease after HIV. The treatment of tuberculosis is difficult and long, and usually requires a combination of several drugs. Unfortunately, there is a growing problem with drug-resistant tuberculosis as defined by resistance to the two most effective drugs: isoniazid and rifampicin Therefore, new pharmaceutical formulations of anti-tubercular antibiotics with prolonged activity and improved properties are needed. The supramolecular complexation of active pharmaceutical ingredients (APIs) with the macrocyclic host molecules offers the opportunity to manipulate physicochemical properties of pharmaceutical agents, improve bioavailability and reduce side effects.[1] A host-guest cocrystallization strategy, where macrocycle stands for a host and API for a guest, may lead to the discovery and exploitation of new multi-component forms of APIs with improved pharmaceutical properties. The macrocycles effectively used as cocrystallizing agents include cyclodextrins,[2] calix[n]arenes,[3] cucurbit[n]urils,[4] resorcin[n]arenes[5] and pyrogallo[n]arenes.[6] Water-soluble *p*-sulfonatocalix[n]arenes have been extensively studied as host systems for various bioactive molecules in the last several years.[7] However, no structural studies on the *p*-sulfonatocalix[n]arene complexes with anti-tuberculosis drugs have been reported so far. Here we wish to present our results on the cocrystallization of anti-tuberculosis drug isoniazid INH with a macrocyclic host *p*-sulfonatocalix[4]arene C4S in different solvent media.

Isoniazid has been shown to be a versatile supramolecular reagent forming neutral molecular complexes (cocrystals)[8] and molecular salts[9] with different carboxylic acid molecules, as well as with other APIs.[10] We report here new multi-component crystalline forms of INH with coformer *p*-sulfonatocalix[4]arene where the drug is complexed both *endo* and *exo* with respect to the macrocycle. Being a relatively strong acid C4S induces a proton transfer from its sulfonic acid groups to both hydrazide and pyridine functions of isoniazid. In this way the ionizable C4S acts



Scheme 1 Crystallization conditions of complexes 1-4 of *p*-sulfonatocalix[4]arene C4S with isoniazid.

simultaneously as host and counterion towards INH. The diprotonated isoniazid dication has multiple hydrogen bonding functionality that can be realized in numerous hydrogen bonding patterns. Nevertheless, the main interactions between tetraanion host C4S and dication INH as guest are salt bridges, and resulting complexes can be considered as supramolecular host-guest salts. The 1:2 host-guest stoichiometry dictated by the charge balance in the structure is preserved in all four crystalline complexes described below. Solvent was found to play a crucial role in the complexation process guiding the formation of exclusion *versus* inclusion host-guest complexes, Scheme 1. It should be mentioned that the influence of the solvent on the formation of the solid-state host-guest assemblies based on pyrogallol[4]arene macrocycles has recently been studied by Atwood and coworkers.[11]

Crystallization of INH and C4S from aqueous solution afforded block-shaped crystals of complex **1** which was solved and refined in the triclinic space group P-1. The asymmetric unit of **1** comprises one C4S, two isoniazid dications and several water molecules. One of the isoniazid molecules resides inside the C4S macrocyclic cavity, while another one is complexed outside in the crystal lattice, Fig. 1. The horizontal inclusion of isoniazid induces the pinched cone conformation of C4S, which is elucidated by S···S distances between opposite sulfonate groups of 8.0 and 13.1 Å. Such an arrangement allows maximum non-covalent interactions not only between host and included guest, but also between isoniazid dication and others C4S anions in the crystal lattice. The protonated hydrazide group of the included INH is situated in the window between two adjacent sulfonate groups at the upper C4S rim. The pyridinium ring penetrates the cavity and is slanted towards another sulfonate

group of the host due to favourable electrostatic interaction. The angle between included pyridinium ring of INH and a plane defined by four bridging methylene groups of C4S is 71º. The terminal ammonium group of INH forms one hydrogen bond with the sulfonate oxygen of the

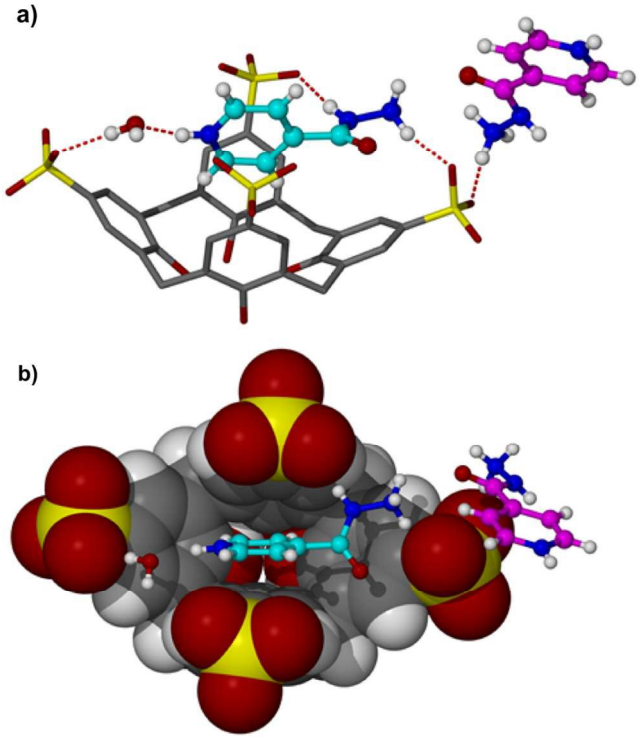


Fig. 1 The asymmetric unit of complex 1 a) highlighting some hydrogen bonds between host and guest, C4S in stick mode and INH and water in ball-and-stick mode b) C4S in the space-filled mode and INH and water in ball-and-stick mode The crystallographically independent **INH** guests are displayed in different colours (blue included into **C4S** cavity and violet complexed *exo*). Water molecules and some hydrogen atoms removed for clarity.

C4S forming the actual inclusion complex (N-H···O distance 2.75 Å), and two hydrogen bonds (N-H···O distances 2.69 and 2.80 Å) with sulfonate oxygen atoms of two adjacent C4S macrocycles. The secondary amino group of INH is hydrogen bonded to the sulfonate oxygen of the C4S host (2.70 Å). The pyridinium nitrogen is hydrogen bonded to a water molecule (2.65 Å) that sits just above the cavity and is further hydrogen bonded to sulfonate oxygen atom at the C4S rim. It should be noted that the architecture of the C4S-INH inclusion complex is quite different from the proposed earlier structures based on solution and molecular modelling studies.[12] It was suggested on the base of NMR and DFT theoretical calculations that INH is included preferentially through the hydrazide moiety inside host cavity. The authors did not consider conformational flexibility of the host and the possibility of its deformation from cone to quite 'pinched' cone. Albeit, the host-guest complexation mode in solution might be quite different from that observed by us in the solid state. The externally complexed INH interacts with the C4S anions in somewhat different way. Namely, its ammonium group forms two hydrogen bonds to the oxygen atoms of two C4S anions and one hydrogen bond to water molecule. The secondary amino group of the hydrazide fragment and pyridinium nitrogen are hydrogen bonded to sulfonate oxygen atoms of adjacent C4S anions.

C4S assembles into a typical alternate bilayer (Fig. 2) *via* O-H···O hydrogen bonding from hydroxyl groups at the lower rim to sulfonate oxygen atoms at the upper rims of adjacent C4S, as well as π-π stacking and C-H···π interactions between neighbouring C4S anions. The INH cations and water molecules are arranged between the C4S bilayers interacting generously with sulfonate functionalities of C4S forming a hydrophilic layer.

Alteration crystallization medium from water to 1:1 water-alcohol mixtures results in the dramatic changes in the complexation mode between host and guest. Namely, in the presence of cosolvent the INH is complexed explicitly *exo* with respect to the host cavity which becomes occupied with ethanol (complex **2**) or methanol (complex **3**) molecule, Fig. 3. The 1:2 C4S-INH stoichiometry is preserved in the ethanol and methanol complexes, but both crystallographically independent INH cations are kept outside the cavity. The preference for the inclusion of solvent molecule can be explained in terms of energetically demanding deformation of the macrocycle required to accommodate INH guest. It is known that INH is only sparingly soluble in ethanol and methanol,[13] therefore addition of these cosolvents affects also the crystallization kinetics. Namely, while crystallization from water takes from several days to several weeks, the crystals are formed within minutes or hours from 1:1 water-alcohol mixtures. The complexes crystallized in the presence of ethanol and methanol are isostructural, only differing slightly in the solvent (guest) orientation inside macrocyclic cavity.

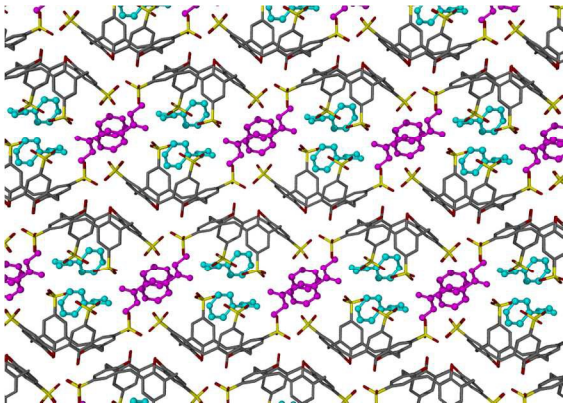


Fig. 2 Packing of the **C4S-INH** host–guest complex **1** viewed along the *a* axis. Water molecules and hydrogen atoms removed for clarity.

The macrocycle in both complexes is in conical conformation with S···S distances between opposite sulfonate groups being 10.4 and 10.0 Å for ethanol, and 10.1 and 10.1 for methanol. In the case of ethanol the model of substitutional disorder in macrocyclic cavity was adopted, namely the cavity is occupied with ethanol (SOF of 85 %) and water (SOF of 15%). The ethanol is oriented in the cavity to form hydrogen bond from its hydroxyl group to one of the sulfonate oxygen atoms of C4S host (O-H···O distance 2.71 Å,) Fig. 3a. The smaller methanol was refined as disordered into two positions inside C4S cavity (SOF of 63 and 37%). The position of methanol is not favorable for hydrogen bonding to the host sulfonate groups, instead of this

hydrogen bonding to water molecules is realized (O-H···O distances 2.74 and 2.97 Å), Fig.3b. Besides these differences in the solvent inclusion behaviour, the two structures are very similar in terms of C4S interactions with INH and packing in the crystal lattice. Therefore only the complex with ethanol will be described further in details.

The external complexation of INH cations still allows rich interactions with C4S anions. Each INH cation forms three hydrogen bonds via its pyridinium, secondary amine and ammonium functions with sulfonate groups of three C4S anions in the crystal lattice. The terminal ammonium group of INH also forms two hydrogen bonds with water molecules. There are also multiple C-H···O interactions from INH to C4S sulfonate groups (distance range 3.07-3.38 Å), as well as π-π stacking between INH pyridinium ring and outer surface of C4S (the centroid-centroid distances are 3.61 and 3.84 Å). The positioning of INH guests outside the macrocyclic cavity disrupts the traditional bilayer packing motif of C4S leading to up-down strands of C4S anions intersected with the INH cations, Fig. 4.

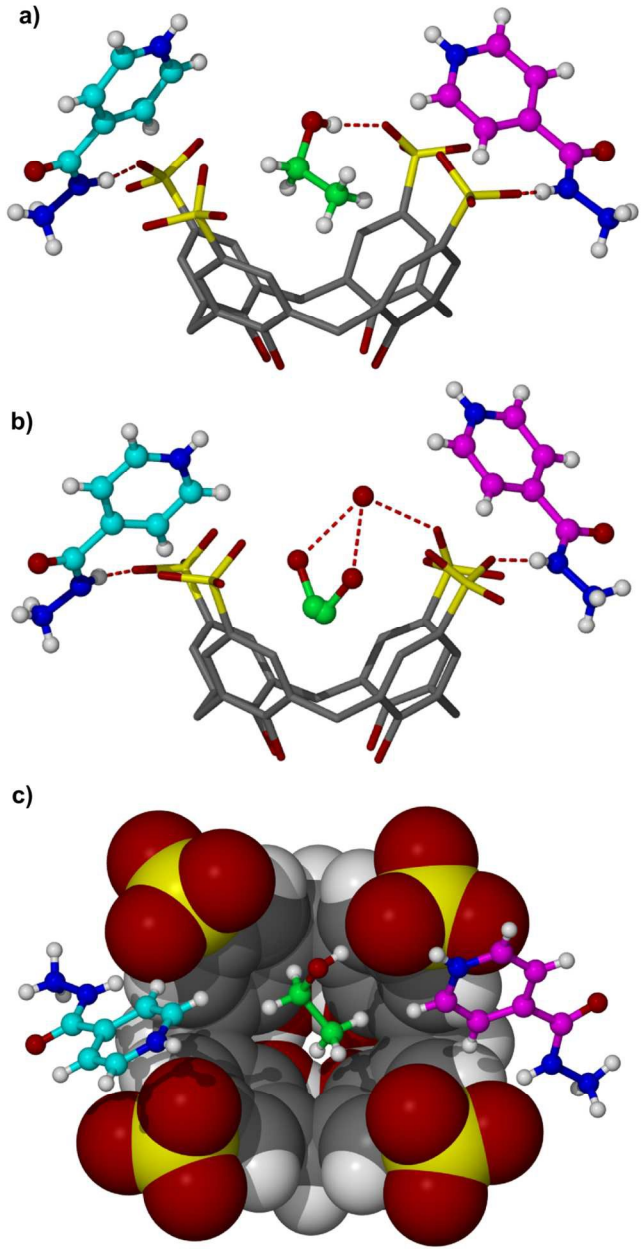


Fig. 3 The asymmetric unit of complex **2** (a) and complex **3** (b), **C4S** in stick mode and **INH** and solvent molecules in ball-and-stick mode. The crystallographically independent **INH** guests complexed outside macrocyclic cavity are displayed in different colours; (c) the asymmetric unit of complex **2, C4S** in the space-filled mode and INH and ethanol in ball-and-stick mode. Water molecules and some hydrogen atoms removed for clarity.

When water-acetone is used instead of water-alcohol mixture the crystallization of higher symmetry complex **4** in the space group *C*2/*c* is observed. The asymmetric unit contains half of the C4S anions, half of the acetone molecule residing close to 2-fold axis inside host cavity, one INH dication and four water molecules, Fig. 5. The C4S is in a slightly pinched cone conformation with S···S distances between opposite sulfonate groups being 9.7 and 10.8 Å. The acetone occupies two positions inside the C4S cavity due its closeness to a special position. One of the methyl groups of acetone points down to the bottom of the cavity with C-H···π short contact to one of the C4S phenyl rings (distance C-

H···centroid 3.41 Å), while another methyl group and carbonyl function are in the plane of sulfonate groups. The C4S-INH stoichiometry is 1:2 as in all three previous complexes. Despite higher symmetry the interaction mode between C4S and *exo* kept INH in the complex with acetone is very similar to the corresponding complexes with ethanol and methanol. The sole crystallographically independent INH dication forms three hydrogen bonds via its pyridinium, secondary amine and ammonium functions with sulfonate groups of three C4S anions in the crystal lattice. The terminal ammonium group of INH also forms two hydrogen bonds with water molecules. The overall packing of the acetone complex is also very close to packing mode of the complexes with ethanol and methanol except that here all INH cations are symmetry equivalent, Fig. 6.

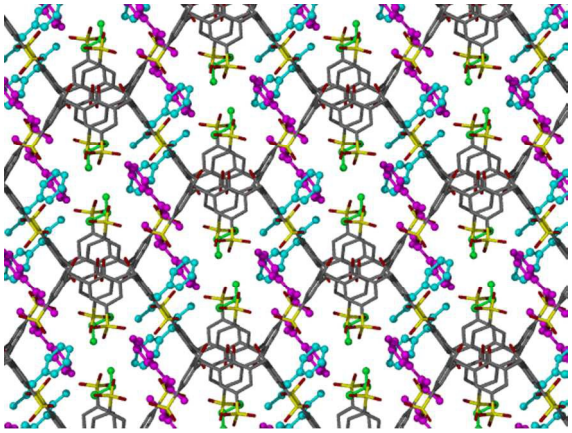


Fig. 4 Packing of the **C4S-INH** host–guest complex **2** viewed along the *c* axis. Water molecules and hydrogen atoms removed for clarity.

To conclude, various supramolecular host-guest compounds of isoniazid with *p*-sulfonatocalix[4]arene crystallize in different modes depending on the solvent system applied. The arrangement of isoniazid *exo* or *endo* to the host macrocyclic cavity can be regulated by the proper choice of the solvent. The host-guest inclusion complex characterized by pinched cone conformation of C4S crystallizes from aqueous solution. Changing the crystallization medium to water-cosolvent (ethanol, methanol or acetone) binary mixtures results in the crystallization of host-guest complexes where INH is complexed explicitly *exo* and the macrocyclic cavity becomes occupied by a solvent molecule. Such solvent guided cocrystallization is likely to represent an important strategy for obtaining multiple crystalline forms of the host-guest complexes with active pharmaceutical ingredients.

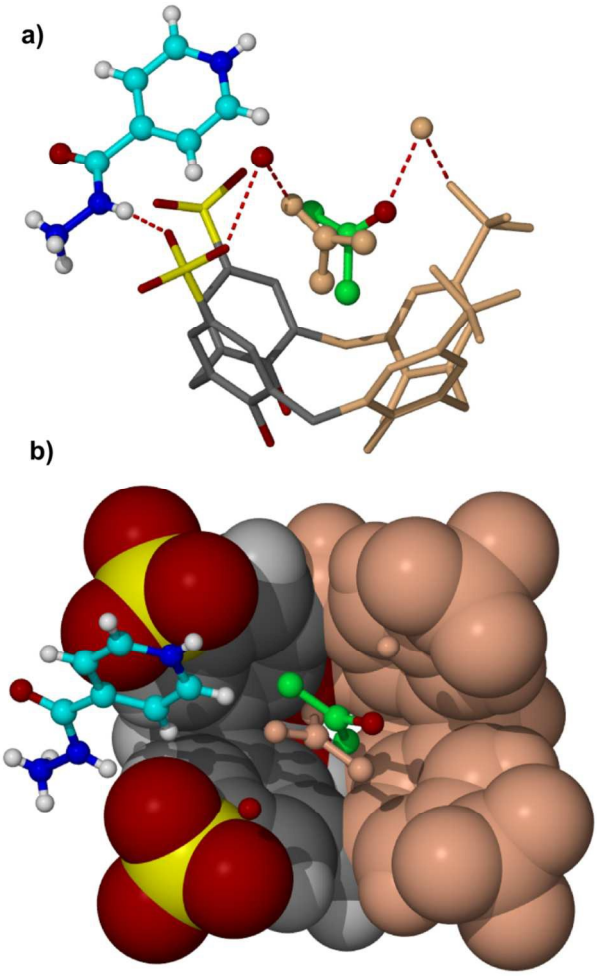


Fig. 5 The asymmetric unit of complex **4;** the symmetry generated part of the structure is coloured in beige**;** a) **C4S** in stick mode and **INH** and solvent molecules in ball-and-stick mode; b) **C4S** in the space-filled mode and **INH** and solvent in ball-and-stick mode. Water molecules and some hydrogen atoms removed for clarity.

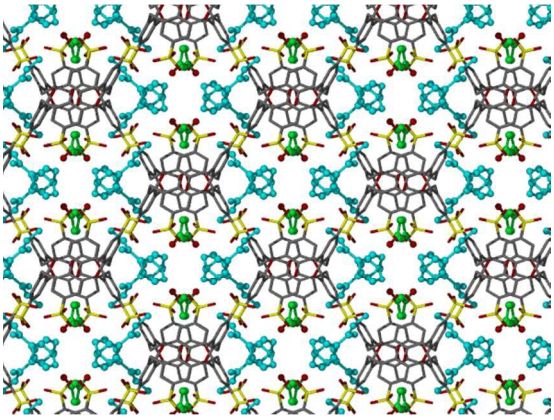


Fig. 6 Packing of the **C4S-INH** host–guest complex **4** viewed along the *c* axis. Water molecules and hydrogen atoms removed for clarity.

Notes and references

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† Electronic Supplementary Information (ESI) available: X-ray crystallographic files in CIF format. See DOI: 10.1039/b000000x/

All reagents and solvents employed were commercially available and used as received without further purification. The *p*-sulfonatocalix[4]arene was purchased from TCI EUROPE and isoniazid from Sigma Aldrich.

‡ **Crystal data for 1**: $C_{40}H_{43.6}O_{21.2}N_6S_4$, *Mr* = 1075.5, colourless, triclinic, space group *P*-1, *a* =10.6026(9), *b* =14.4115(9), *c* = 15.2947(15) Å, α = 72.165(7), β = 85.457(7), γ = 86.934(6)º, *V* = 2216.7(3) Å^3, *Z* = 2, ρ_{calc} = 1.611 g·cm^{-3}, $\mu(Cu_{K\alpha})$ = 2.795 mm^{-1}, θ_{max} = 70.1º, 14651 reflections measured, 8342 unique, 711 parameters, *R* = 0.044, *wR* = 0.111 (*R* = 0.057, *wR* = 0.120 for all data), *GooF* = 0.82. CCDC 1029529.

Crystal data for 2: $C_{41.7}H_{54.8}O_{25.2}N_6S_4$, *Mr* = 1170.8, colourless, triclinic, space group *P*-1, *a* =12.5233(3), *b* =12.7554(4), *c* = 17.6293(5) Å, α =77.734(2), β =75.621(2), γ = 66.431(3)º, *V* = 2479.9(1) Å^3, *Z* = 2, ρ_{calc} = 1.568 g·cm^{-3}, $\mu(Mo_{K\alpha})$ = 0.288 mm^{-1}, θ_{max} = 28.2º, 30126 reflections measured, 12272 unique, 757 parameters, *R* = 0.037, *wR* = 0.098 (*R* = 0.045, *wR* = 0.103 for all data), *GooF* = 0.93. CCDC 1029530.

Crystal data for 3: $C_{41}H_{51}O_{26}N_6S_4$, *Mr* = 1172.1, colourless, triclinic, space group *P*-1, *a* =12.4798(5), *b* =12.6682(4), *c* = 17.6904(5) Å, α =77.545(3), β =75.299(3), γ = 67.843(3)º, *V* = 2482.9 (2) Å^3, *Z* = 2, ρ_{calc} = 1.568 g·cm^{-3}, $\mu(Mo_{K\alpha})$ = 0.289 mm^{-1}, θ_{max} = 28.2º, 23957 reflections measured, 12289 unique, 799 parameters, *R* = 0.045, *wR* = 0.111 (*R* = 0.056, *wR* = 0.117 for all data), *GooF* = 0.86. CCDC 1029531.

Crystal data for 4: $C_{43}H_{59}O_{27}N_6S_4$, *Mr* = 1220.2, colourless, monoclinic, space group *C*2/*c*, *a* =20.2344(4), *b* =15.2501(3), *c* = 17.8339(3) Å, β =107.069(2)º, *V* = 5260.7(2) Å^3, *Z* = 4, ρ_{calc} = 1.541 g·cm^{-3}, $\mu(Cu_{K\alpha})$ = 2.511 mm^{-1}, θ_{max} = 68.2º, 9929 reflections measured, 4802 unique, 420 parameters, *R* = 0.078, *wR* = 0.201 (*R* = 0.084, *wR* = 0.207 for all data), *GooF* = 0.89. CCDC 1029532.

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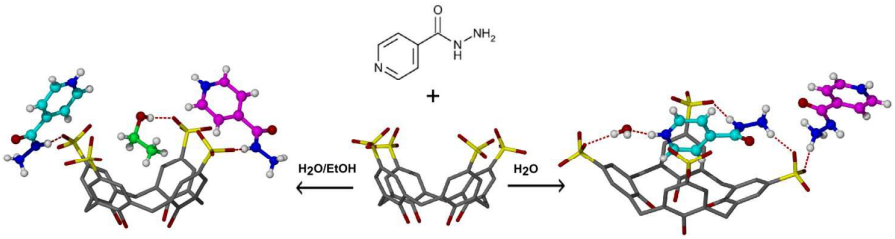

The position of the anti-tuberculosis drug isoniazid inside (*endo)* or outside (*exo)* to the macrocyclic cavity of *p*-sulfonatocalix[4]arene can be regulated by the proper choice of the solvent system.

Solvent control in the formation of supramolecular host-guest salts of isoniazid with *p*-sulfonatocalix[4]arene

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