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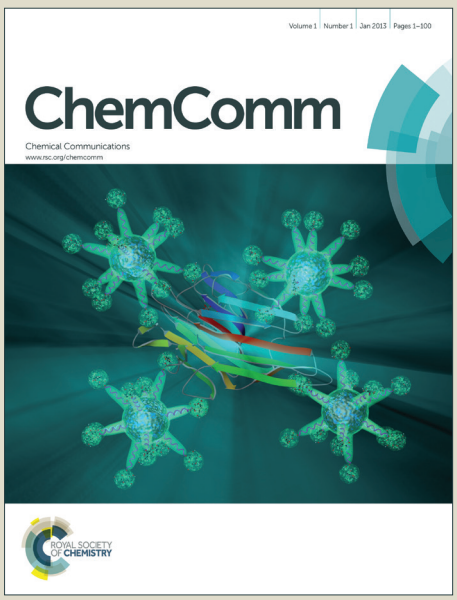

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ARTICLE TYPE

Chemoselective recognition with phosphonate cavitands: the ephedrine over pseudoephedrine case.

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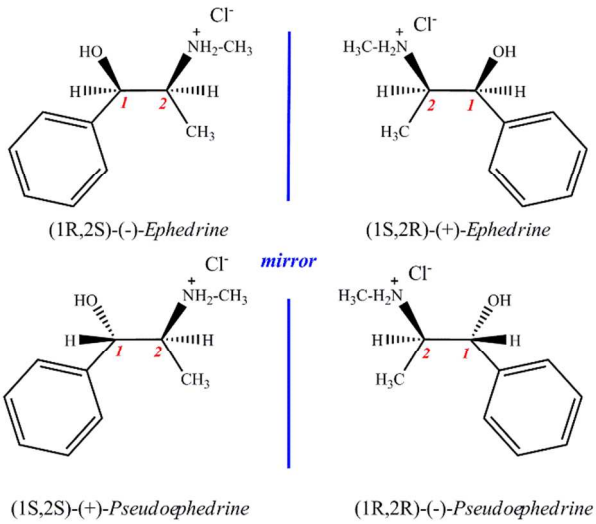
Complete discrimination of ephedrine and pseudoephedrine both in solution and in the solid state was achieved with a phosphonate cavitand receptor. The molecular origin of the epimer discrimination was revealed by the crystal structure of the respective complexes.

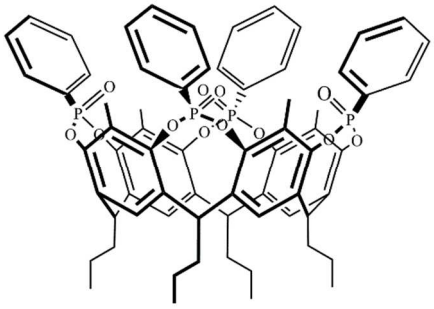
Ephedrine and pseudoephedrine, epimers differing in the configuration of the carbon bearing the hydroxyl group (Scheme 1), are stimulants that mimic adrenaline effects to the body. In Nature they can be found in *Ephedra* plants where only the (-)-ephedrine and the (+)-pseudoephedrine forms occur. Depending on their absolute configuration, different stereoisomers of chiral compounds have significantly different biological activity. In fact, (-)-ephedrine is typically used to counteract orthostatic hypotension and for weight control while (+)-pseudoephedrine is used as a nasal decongestant and cold/flu tablet. Moreover, they both have the capacity to increase the blood pressure of patients but ephedrine is more effective.

So far, complexation and separation of the two epimers have been achieved using chiral receptors. Cyclodextrins (CD) have been used to discriminate between the two via high-performance liquid chromatography (HPLC),[1] capillary electrophoresis (CE)[2] and gas chromatography-mass spectrometry (GC-MS)[3] with CD embedded in the stationary phase. Chiral amido resorcinarenes complexed preferentially ephedrine in the gas phase.[4] More recently, Dutasta and coworkers reported the chemoselective complexation of ephedrine/pseudoephedrine in solution using an inherently chiral diphosphonate cavitand.[5] The levorotatory cavitand showed complete preference for ephedrine over pseudoephedrine, while its dextrorotatory enantiomer showed a less pronounced opposite trend. By comparison, more flexible achiral calix[4]arene phosphonates bind both epimers in water with similar strength.[6] Till now, the lack of crystal structures of the host-guest complexes have prevented a molecular level understanding of the observed discrimination.

In this paper, we report the chemoselective recognition of ephedrine over pseudoephedrine with an achiral cavitand, revealing the molecular origin of this selectivity via crystal structure determination of the corresponding complexes.

Phosphonate cavitands are a versatile class of synthetic receptors,[7] capable of binding inorganic and organic cations[5,8] as well as neutral molecules.[9] Their molecular recognition properties have been exploited in gas sensing,[9] supramolecular polymers,[10] surface self-assembly,[11] and product protection.[12] With their π-basic cavity, and the presence of four H-bond acceptor groups at the upper rim, these cavitands are especially suitable for interacting with $-NH_2^+CH_3$ residues. This specific recognition mode allowed to build sensors for the recognition of sarcosine in urine[13] and illicit drugs in water.[14]





Tiiii[C_3H_7, CH_3, Ph]

Scheme 1. Chemical structures of the diastereoisomers ephedrine and pseudoephedrine hydrochlorides and of the **Tiiii**[C_3H_7, CH_3, Ph] (from now on **Tiiii**) receptor.

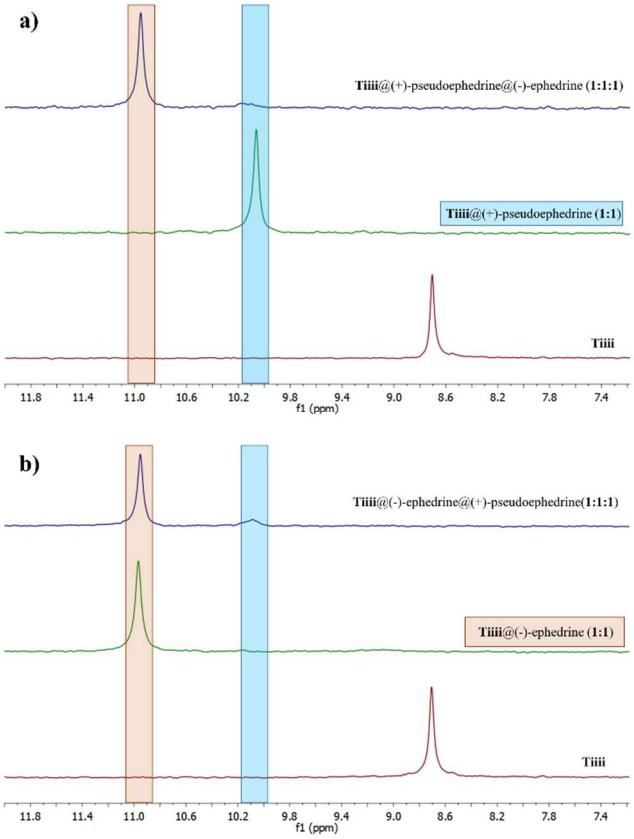


Figure 1. ^{31}P NMR (400MHz, MeOD, -20°C) of the competitive titrations. a) **Tiiii** before (red spectrum), after addition of 1.eq. of pseudoephedrine (green spectrum) and 1.eq. of ephedrine (blue spectrum) hydrochlorides; b) **Tiiii** before (red spectrum), after addition of 1.eq. of ephedrine (green spectrum) and 1.eq. of pseudoephedrine (blue spectrum) hydrochlorides.

In order to further explore the recognition ability of this type of cavitand, we have extended our study towards the diastereomeric methamphetamine precursors ephedrine and pseudoephedrine hydrochlorides (see Scheme 1). In solution, the complexation was observed both *via* ^{31}P NMR and ^{1}H NMR spectroscopy by following the downfield shift of the ^{31}P signals of the host (from 8.49 ppm for free **Tiiii** to 10.19 ppm and 11.01 ppm for pseudoephedrine and ephedrine complexes respectively, Figure S1) or the highfield shift of the N-CH_3 and the O-CH protons of the two guests (Figures S2, S3 and S4).

Competitive ^{31}P NMR experiments have been conducted at -20°C to determine the complexation preference of **Tiiii**. In a first experiment, one equivalent of pseudoephedrine hydrochloride was added to a methanol solution of **Tiiii** cavitand causing the diagnostic downfield shift of the host ^{31}P signal from 8.70 ppm for free **Tiiii** to 10.06 ppm. The subsequent addition of one equivalent of ephedrine hydrochloride induced a further shift to 10.95 ppm (Figure 1a). Vice versa, the addition of ephedrine first induced the diagnostic shift to 10.97 ppm, while the subsequent addition of pseudoephedrine did not cause any change in the spectrum (Figure 1b). The two competition experiments agree on demonstrating the complete preference of **Tiiii** for binding ephedrine over pseudoephedrine in solution (see Figure S5 for corresponding ^{1}H NMR titrations showing the diagnostic highfield shift of the N-CH_3 protons of the guests). Decomplexation of the guests can be easily performed by neutralizing the charge of the ammonium ion removing one of its protons with a hindered base like DBU.[15]

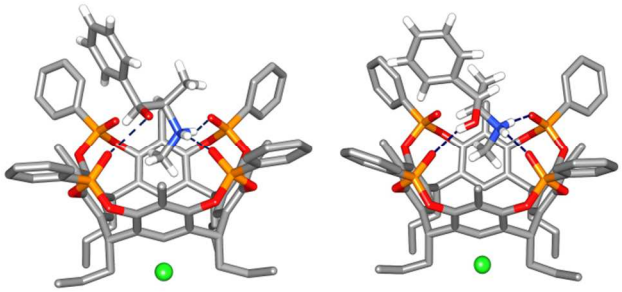


Figure 2. Molecular structure of **Tiiii@**ephedrine hydrochloride·7H_2O·CH_3OH (**1**) (left) and of **Tiiii@**pseudoephedrine hydrochloride·6CH_3OH (**2**) (right). Colour code: P, orange; O, red; C, grey; H, white; Cl, green; hydrogen bonds, blue dotted lines. Only the hydrogen atoms of the guest are shown. Lattice solvent molecules have been removed for clarity.

Given the achiral nature of **Tiiii**, we then set up a series of solid state experiments to identify the origin of this selectivity. The molecular structures of the complexes formed by **Tiiii** and the hydrochloride drugs in their racemic form have been elucidated through single-crystal X-ray diffraction analysis (see Figure 2). Crystals were obtained from slow evaporation at low temperature of a chloroform/methanol solution containing the host and the guest. At first glance, the two structures in the solid state look quite similar for the two epimers, showing the same interaction mode of the two guests with the **Tiiii** cavity. Indeed, a more thorough examination reveals that the spatial arrangement of the substituents in ephedrine and pseudoephedrine influence the interactions of the host with the guests.

In both cases the complexation of the epimers is the result of the simultaneous cooperation of a strong, not directional (and therefore not selective) electrostatic attraction with the chloride anion and weaker, directional (and thus selective) interactions of the ephedrine and pseudoephedrine cations with the **Tiiii** cavity.

The chloride anion is located at the lower rim of the cavitand stabilized by hydrogen bonds with the terminal aliphatic chains. The complexation of the cations is due to the formation of i) hydrogen bonds between the P=O groups of the cavitand and the -$^{+}NH_2$ and OH groups of the drugs and ii) C-H•••π interactions between the aromatic cavity of the host and the methyl groups directly attached to the nitrogen atom of the diastereoisomers (see ESI for the detailed geometrical parameters).

As opposed to other guests which can be disordered inside the cavity[16] due to a very small barrier of rotation,[17] in the present case no such fluxionality has been noted in the solid state.

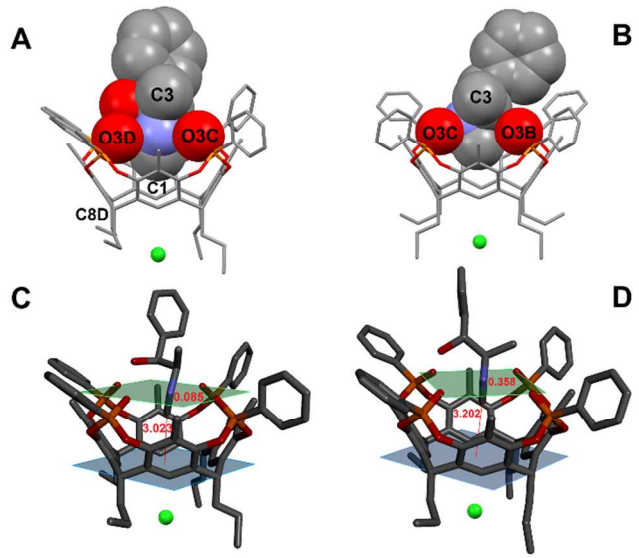


Figure 3. Top: perspective view of **1** (A) and **2** (B) with the guest and two oxygen atoms of the cavitand shown in space filling mode. Bottom: least-squares planes passing through the oxygen atoms of the P=O groups (green) and through the C8 atoms of the lower rim (blue) for **1** (C) and **2** (D). Hydrogen atoms, lower rim chains and lattice solvent molecules have been omitted for clarity.

It must be emphasized that this supramolecular architecture is not the result of the self-assembly in the crystal lattice of the **Tiiii** cavity with the drug hydrochloride ionic pair, but it exists as a whole isolated entity prior to the growth of the lattice. This has been proved by solution experiments and corroborated by molecular modelling calculations showing that the molecular geometries of the two complexes in the gas phase mirror those in the solid state (see ESI).

The ion-pair separations of ephedrine or pseudoephedrine is influenced by the different steric hindrance of the two epimers which prevents pseudoephedrine, rather than ephedrine, to enter more deeply within the **Tiiii** cavity. Figure 3A shows a lateral view of **1** with the nitrogen atom of the guest and its two hydrogen-bonded oxygens from the P=O groups in the foreground. Looking at the space filling mode, it is easy to notice that the methyl group C3 does not interfere with the hydrogen

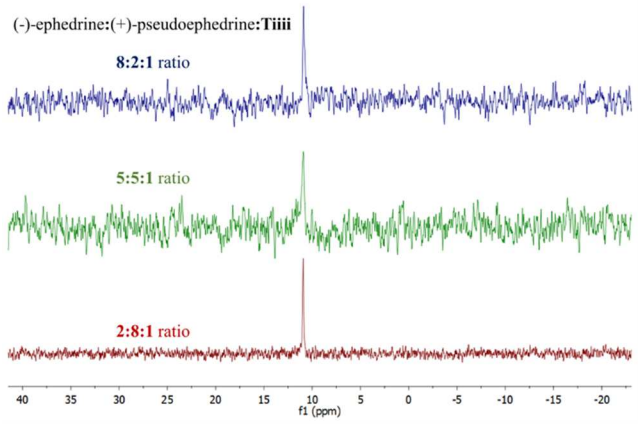


Figure 4. ^{31}P NMR (400MHz, MeOD, 25°C) of the filtered crystals obtained from different guests:**Tiiii** ratio. The guests are hydrochlorides.

bond formation (C3-O3C 3.318(8), C3-O3D 3.866(5) Å; the distance between the methyl group C1 of the guest and the least-squares plane C8A, C8B, C8C, C8D defining the lower rim of the cavitand is 3.023 Å, Figure 3C). This is not the case for complex **2** (Figure 3B): when pseudoephedrine interacts with the cavity, the methyl group C3 is sterically hindered by two oxygen atoms of the upper rim (one of which forming a hydrogen bond with the ammonium group), preventing the guest from further entering the cavitand (C3-O3B 3.242(6), C3-O3C 3.275(6) Å and C1···C8A, C8B, C8C, C8D 3.202 Å, Figure 3D). The distance of the nitrogen atom from the chloride counteranion moves from 7.218(3) Å for **1** to 7.547(4) Å for **2** and the distances of N from the least-squares plane passing through the oxygens of the phosphonate groups are 0.085(4) and 0.358(5) Å for **1** and **2**, respectively (see Figure 3C and 3D). The steric hindrance of the methyl C3 with the oxygen atoms O3C and O3D of the P=O groups prevents pseudohephedrine to fully accommodate the $^{+}NH_2$-CH_3 moiety into the cavity, thus weakening the binding.

To further demonstrate this point, a series of competitive crystallization experiments[18] have been carried out using the pure enantiomeric forms of the two hydrochlorides [(1R,2S) and (1S,2S) for ephedrine and pseudoephedrine, respectively]. Chloroform/methanol solutions containing the host and different mole fractions of the two guests were prepared. First, the crystals of **Tiiii**@ephedrine hydrochloride·3H_2O·3CH_3OH (**3**, enantiopure guest) and **Tiiii**@pseudoephedrine hydrochloride·4H_2O (**4**, enantiopure guest) were obtained and their crystal structure were solved with X-ray diffraction analysis (Figures S9 and Figure S10, respectively). It is worthwhile to underline that the interaction mode is not influenced by the use of the guests in their racemic form or as pure enantiomer. Three vials containing ephedrine, pseudoephedrine and **Tiiii** in ratios 2:8:1, 8:2:1 and 5:5:1 (with a total guests:host ratio of 10:1) were put in the fridge and the content allowed to crystallize. X-ray diffraction analysis on the crystals formed always evidenced the sole presence of ephedrine hydrochloride inside the cavitand [compounds **Tiiii**@ephedrine hydrochloride·7H_2O·CH_3OH (**5**), **Tiiii**@ephedrine hydrochloride·2H_2O·2CH_3OH (**6**) and **Tiiii**@ephedrine hydrochloride·4H_2O·CH_3OH (**7**); see Figure S11, S12 and S13 in the ESI. The crystals were also filtered and carefully washed with cold methanol to remove all the non-complexed guests. After that, they were dissolved in deuterated chloroform to perform ^{31}P and ^{1}H NMR analysis. The spectra reported in Figures 4 and S6 confirm the exclusive presence of the ephedrine complex and the selectivity of the cavitand uptake.

In conclusion, both epimers, irrespective of their optical purity, are bound by **Tiiii** with the same set of synergistic interactions which dictates the geometry of the resulting complexes. The spatial arrangement of the methyl group C3 destabilizes the **Tiiii**@pseudoephedrine complex over the **Tiiii**@ephedrine one by partially pulling out the $^{+}NH_2$-CH_3 moiety from the cavity, leading to the selective uptake of the latter both in solution and in the solid state. This finding represents a significant example of the potential of synthetic receptors in the specific recognition of biologically relevant targets, with possible outcomes in drug delivery and biomedical applications.[19]

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Notes and references

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† Electronic Supplementary Information (ESI) available: Synthesis and characterization, materials, methodology of all experiments and additional spectral data, crystallization conditions, crystallographic information tables, ORTEP views of the reported structures, CCDC-1034901 (**1**), -1034902 (**2**), -1034903 (**3**), -1034904 (**4**), -1034905 (**5**), -1034906 (**6**) and -1034907 (**7**). For CIF files see DOI: 10.1039/b000000x/

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The molecular origin of the selective recognition of ephedrine over pseudoephedrine by an achiral phosphonate cavitand receptor was revealed by the crystal structure of the respective complexes.