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The absolute configuration of an inherently chiral phosphonatocavitand and its use toward the enantioselective recognition of L-adrenaline

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Dedicated to Professor Henri Kagan on the occasion of his 80th birthday

ABSTRACT

An inherently chiral AB*ii* diphosphonato cavitand (±)-**4** bearing a single quinoxaline bridging moiety was synthesized and resolved by chiral HPLC. Its chiroptical properties were investigated and VCD experiments allowed the determination of its absolute configuration. Distinguishable diastereomeric complexes in solution with L-adrenaline were observed by ^{1}H and ^{31}P NMR together with a noticeable enantio-discrimination at 253 K (dr ~2:1) in favor of the dextrorotatory cavitand (+)-**4**.

1. Introduction

With the aim of mimicking biological functions in living systems, the design and the preparation of artificial molecular receptors has generated a tremendous number of molecules with different skeletons, sizes, and shapes.[1] In this particular field, enantioselective recognition using chiral host molecules has emerged, leading also to various applications in asymmetric catalysis and chiral separations.[2] Calixarenes and resorcin[4]arenes are known to be good candidates for the design of such chiral supramolecular receptors, as illustrated by the increasing number of these derivatives obtained in the last 15 years.[3] However, in most compounds, chirality is induced by the addition of stereogenic centers onto the narrow or the wide rim of the calixarene, with the aromatic cavity only being used as a rigid scaffold. In contrast to these chiral molecules, inherently chiral molecules have been also described. As defined by Böhmer et al. in this case, '*the chirality is not based on a chiral group or subunit but in the absence of symmetry plane, an inversion center or an alternating axis in the molecule as a whole*'.[3a] Several inherently chiral calix[*n*]arene (*n* = 4–8) have already been reported, to the best of our knowledge, but only a few inherently chiral resorcin[4]arene based cavitands have been described and only one has been resolved. In these examples, the desymmetrization was caused by bridging the adjacent resorcinol moieties with different groups (sites A, B, C, and D in Fig. 1) affording the cavitand structure.

Using this strategy, only two ways can lead to chiral compounds. The first one is to have at least three different sites of the four available, giving compounds of type ABCD or AABC. The only example in this category reported so far is the AABH cavitand (±)-**1** described by Cram et al., where H stands for the non-bridged site (Fig. 2).[4] The second method is to have three identical sites and the fourth one being unsymmetrical, leading to AAAB type chiral molecules. Two examples have been reported by Soncini and Dalcanale et al. (cavitand **2** in Fig. 2),[5] and Rebek Jr. et al. (cavitand **3**, Fig. 2).[6] Cavitand **2** was resolved using menthol as a chiral auxiliary, to produce the first enantiopure inherently chiral cavitands (+)-**2** and (−)-**2**. Complexation studies have been performed with host (±)-**2**, but only in the gas phase and with achiral guests. The lack of strong binding groups and the rather achiral character of the inner cavity precluded its use as an efficient enantioselective receptor in solution.[7]

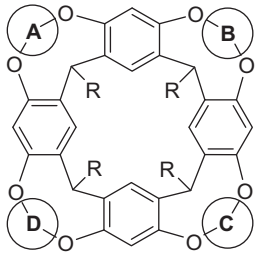

Figure 1. The resorcin[4]arene structure with its four possible bridging sites: A, B, C, and D.

The versatility of the cavitands based on the phosphorylated resorcin[4]arenes such as the tetraphosphonatocavitands,[8] which show remarkable binding properties toward alcohols,[9] metal ions,[10] and ammonium cations,[11] are good candidates for the

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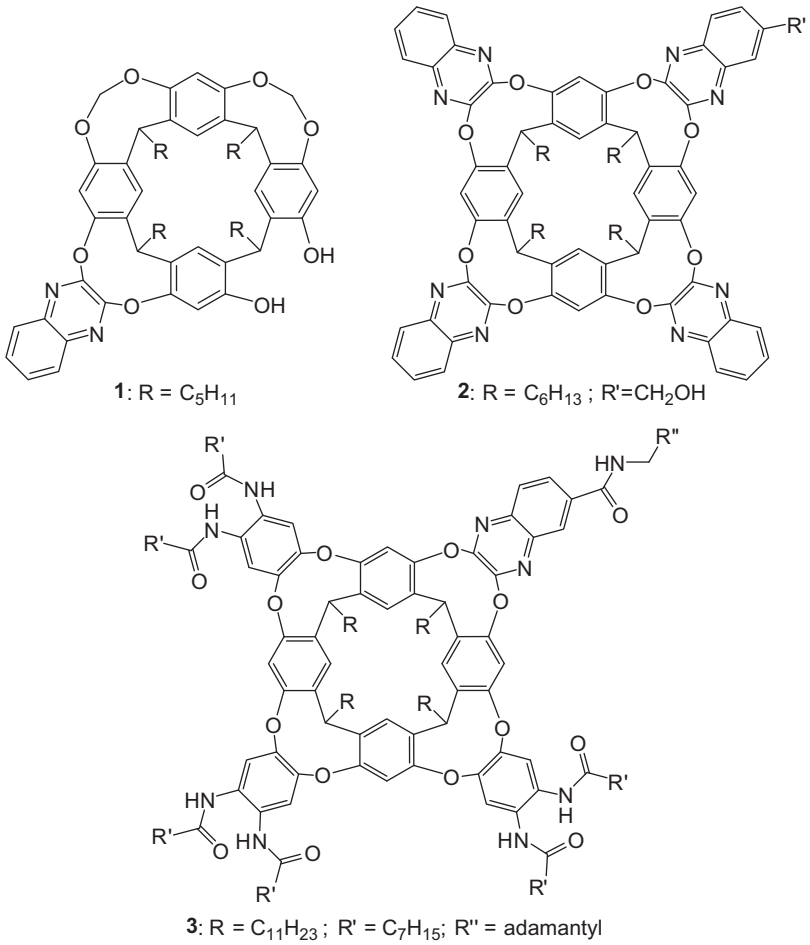


Figure 2. Examples of inherently chiral cavitands of type AABH **1** and AAAB **2** and **3**.

design of inherently chiral cavitands. For instance, the new host (±)-**4** was obtained by adding a quinoxaline bridge to an AB*ii* type phosphonatocavitand (Fig. 3). As stated above, AB (AC) defines the proximal (distal) positions of the two phosphorus groups, while *ii* corresponds to the inward orientations of the two P=O groups. Thus, the AB*ii* structure possesses; (i) two P=O bonds oriented toward the cavity (complexation sites for ammonium guests); (ii) a quinoxaline bridge that can favor π-interactions with guests bearing aromatic groups; (iii) free phenol OH groups for H-bonding; and (iv) an aromatic cavity for guest encapsulation through CH–π or cation–π interactions.

It is important to note that in the tetraphosphonatocavitand hosts the phosphorus bridges are often prochiral and as a result, the phosphorus become chiral when the symmetry of the molecule is broken, especially in partially bridged phosphonatocavitands. This introduces new stereogenic centers depending on the inward or outward orientation of the phosphorus substituents (vide infra). Herein, the configuration at the phosphorus atom is important, but does not interfere with the inherent chirality of the host, which is closely related to the three-dimensional arrangement of the molecular cavity and its curvature. In this study, the *cR* or *cS* nomenclature used to define the configuration of the inherently chiral

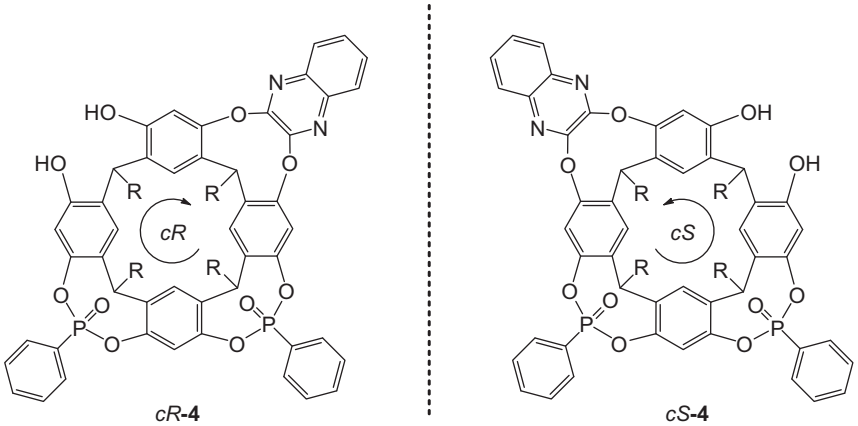


Figure 3. The two enantiomers of cavitand (±)-**4** (R = $C_{11}H_{23}$) of AB*ii* type bearing two phosphonate bridging groups and one quinoxaline bridge (wide rim up).

cavitands refers to that one proposed for the calixarene structures, which considers the curvature of the cavity and the usual sequence rules for the bridging moieties.[12]

Herein, we report on the synthesis and the resolution of the chiral diphosphonatocavitand (±)-**4**. Its absolute configuration was determined by VCD and correlated to CD measurements. The complexation properties of **4** toward an ammonium guest of biological relevance, namely L-adrenaline have been investigated.

2. Results and discussion

2.1. Synthesis

The acid-catalyzed co-condensation between resorcinol and dodecanal,[13] led predominantly to resorcin[4]arene **5** (Scheme 1).[14] The partially bridged compounds, with inward orientation of the P=O groups, were obtained by following the two-step synthesis previously described for the synthesis of triphosphonatocavitands.[15] Compound **5** was treated with two equivalents of dichlorophenylphosphine in the presence of pyridine followed by the in situ addition of sulfur.[16] This gave rise to three major compounds: the trithiophosphonate *iii*PS derivative **6** (19%) and the two dithiophosphonate cavitands AC*ii*PS **7** (8%) and AB*ii*PS **8** (24%), which were separated by column chromatography. If this method has already been reported,[17] the isolation of compounds of type AB*ii*PS is unprecedented.

Contrary to the compounds of type *iii* or AC*ii*, the AB*ii* structure can be desymmetrized by adding a third and different bridge onto the crown of the cavitand. Prior to introducing dissymmetry to this compound, AB*ii*PS **8** was first transformed into AB*ii*PO **9** using a slight excess of *m*-CPBA (3 equiv) as an oxidant; the reaction occurred with retention of configuration, and the P=O groups adopted the inward orientation.[18] In the ^{31}P NMR spectra, the signal at 78 ppm which is characteristic of the thiophosphorylated species disappears to give a single peak around 8 ppm, which is characteristic of the phosphorylated species.[19] The phosphonatocavitand **9** was isolated in 89% yield after chromatography. The quinoxaline bridges have been already used for the preparation of deep cavity cavitands.[20] In the present case, the addition of 1 equiv of 2,3-dichloroquinoxaline leads mainly to compound (±)-**4** bearing one quinoxaline bridge, and to the bis-quinoxaline derivative **10**, which were isolated by column chromatography.

2.2. Vase-kite conformations

Cavitands bearing four quinoxaline bridges are known to exist as two conformational isomers described as vase and kite forms. Interconversion between these two forms can be triggered either with temperature or pH variations.[21] The vase-kite switching is strongly solvent-dependent and was observed only in apolar and non-aromatic solvents such as $CDCl_3/CS_2$, CD_2Cl_2, and $C_2D_2Cl_4$.[22] The conformational equilibrium was followed by ^{1}H NMR by the monitoring of the chemical shift of the methine protons situated near the quinoxaline moieties. A low field resonance corresponds to the vase form whereas a high field shift corresponds to the kite form. It has been proven that in apolar and non-aromatic solvents, the vase form of cavitands bearing four quinoxaline bridges was predominant above room temperature while the kite form was predominant at low temperatures (<223 K).[23] If this behavior has been well studied for cavitands bearing four quinoxaline bridges, very little data are available for the mixed-bridged cavitands. Diederich et al. showed that the absence of one quinoxaline bridge switches off the VT-triggered conformational exchange in CD_2Cl_2, with the cavitand being fixed in the vase conformation over the entire temperature range from 203 to 313 K.[24] The pH-triggered vase-kite exchange was still observed as a result of the developing Coulombic repulsion in the vase geometry. As such behavior could occur with compounds **4** and **10**, VT-NMR experiments in CD_2Cl_2 and $CDCl_3$, and pH titration studies in $CDCl_3$, were performed for these two mixed-bridged cavitands. In both cases, no significant ^{1}H NMR chemical shift of the methine proton facing the quinoxaline bridge was observed, either by lowering the temperature (down to 213 K), or after the addition of trifluoroacetic acid (up to 10 equiv). As a consequence, for these mixed-bridged cavitands the vase-kite interconversion is absent and, according to the NMR data, the vase form predominates (Fig. 4).

2.3. Chiroptical properties

The resolution of (±)-**4** was performed by semi-preparative HPLC using a Chiralpak IC column eluted with hexane/ethanol/$CHCl_3$ 80/10/10 mixture. This method afforded enantiopure (−)-**4** {t_R = 4.8 min; $[\alpha]_D^{25} = -34$ (*c* 0.59, $CHCl_3$)} and (+)-**4** {t_R = 10.5 min; $[\alpha]_D^{25} = +34$ (*c* 0.59, $CHCl_3$)} with a productivity of 2 mg of pure enantiomer (ee >99%) per hour. The CD spectra of a CH_2Cl_2 solution of (+)-**4** ($c \sim 4 \times 10^{-5}$ M) displayed bisignated curves with a positive Cotton effect around 235 nm and two consecutive negative Cotton effects at 245 and 290 nm (Fig. 5).[25] As expected, a solution of (−)-**4** in CH_2Cl_2 showed an identical and inverted signal, confirming the enantiomeric relationship between (+)-**4** and (−)-**4**.

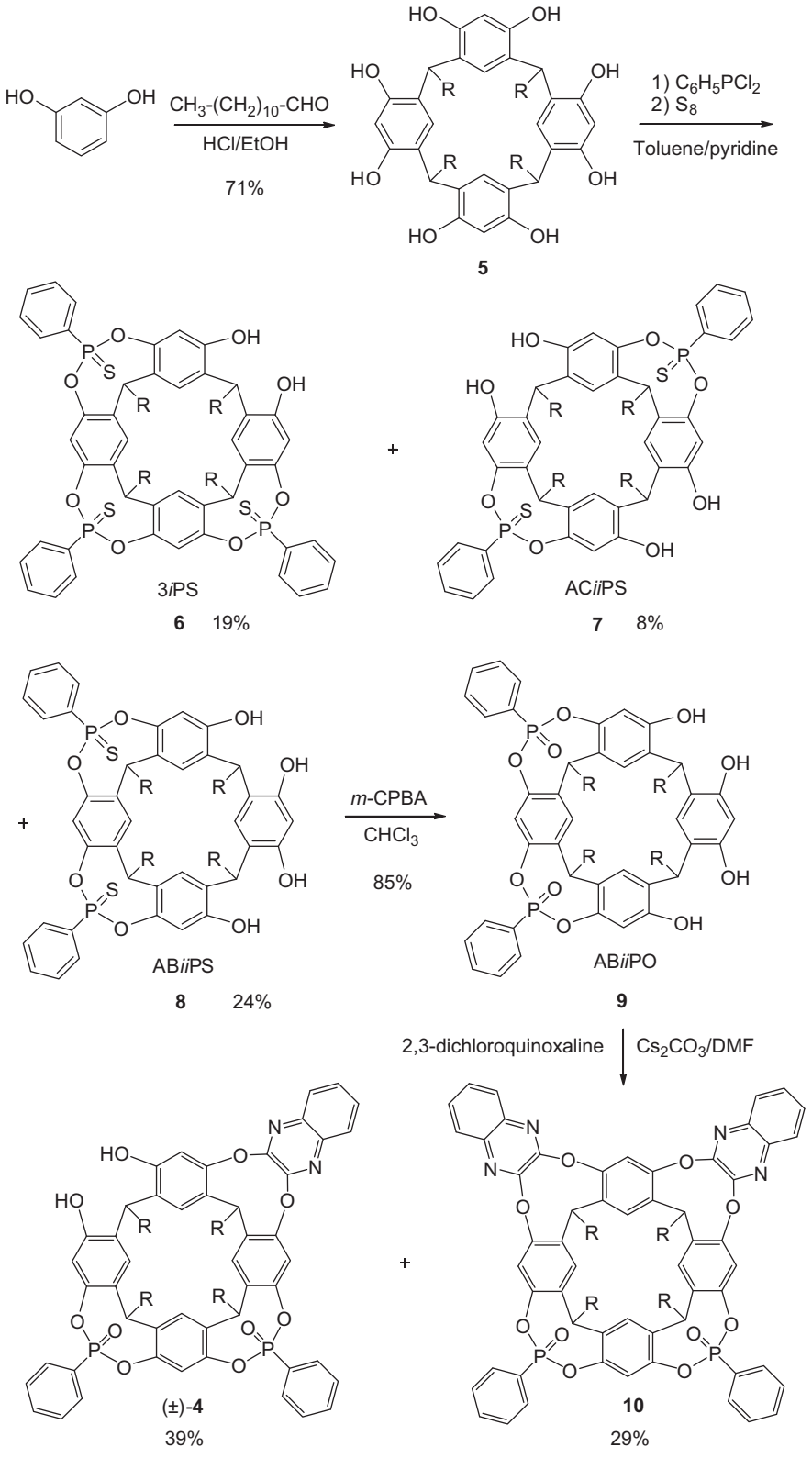
2.4. Determination of the absolute configuration by VCD

Recently, vibrational circular dichroism (VCD) spectroscopy has become a valuable complementary or alternative method for predicting the absolute configuration of non-racemic compounds.[26] Although a rather large variety of structurally different chemical substances have been analyzed, to the best of our knowledge, no experimental and theoretical investigations on inherently chiral calixarene type molecules have been reported. The absolute configuration of the enantiomers of cavitand **4** was assigned from the comparison between experimental and calculated VCD spectra. Due to the size of the molecule, two approximations were considered for the calculations of the IR and VCD spectra, performed at the DFT level using B3PW91 functional and 6-311G(d) basis set: (i) the $C_{11}H_{23}$ groups at the narrow rim were substituted successively by CH_3 and CH_2CH_3 groups (*m***4**-CH_3 and *m***4**-CH_2CH_3, respectively, in Fig. 6); (ii) only two vase conformations (*I* and *II*) for each simplified cavitand have been used for the calculations of IR (Fig. 7) and VCD (Fig. 8) spectra. These two vase conformations only differ by the relative orientation of the hydroxyl groups. For both models, the energy for conformation (*I*) is about 1.5 kJ mol^{-1} lower than that for conformation (*II*).

Despite these two approximations, the agreement between experimental and calculated IR and VCD spectra is quite good. The calculated spectra were very similar using *m***4**-CH_3(*I*) or *m***4**-CH_2CH_3(*I*) on the one hand, and *m***4**-CH_3(*II*) or *m***4**-CH_2CH_3(*II*) on the other hand (Figs. 7 and 8). Particularly, the intensities, the signs and the shapes of the bands 1–7 are well reproduced mainly in the calculated spectra of the conformations *m***4**-CH_3(*I*) or *m***4**-CH_2CH_3(*I*), whereas a better agreement is obtained for the bands 8–11 with the conformation *m***4**-CH_3(*II*) or *m***4**-CH_2CH_3(*II*). The vibrational modes that are associated to these bands concern essentially the core of the cavitand and do not seem to be strongly affected by the four R chains. Thus, we can reasonably use these bands in order to safely assign the absolute configuration c*R* to (−)-**4**.

2.5. Recognition of L-adrenaline guest

Taking advantage of the functionalities, the size and the geometry of cavitand (±)-**4**, we examined its recognition properties to-



Scheme 1. Synthesis of cavitand (±)-**4** (R = $C_{11}H_{23}$).

ward selected enantiopure ammonium guests of biological relevance bearing aromatic moieties. We turned our attention to L-adrenaline L-**11**, which matches such criteria. This compound is known to take part in complex biological processes such as signaling pathways, storing, and retrieving information in the neuronal system. Only few examples of adrenergic synthetic hosts have been described.[27]

Upon the addition of a solution of 1 equiv of (±)-**4** in $CDCl_3$ to L-adrenaline picrate salt [L-**11**][pic] (insoluble in $CDCl_3$), the complete solubilization of the guest was obtained and a highfield

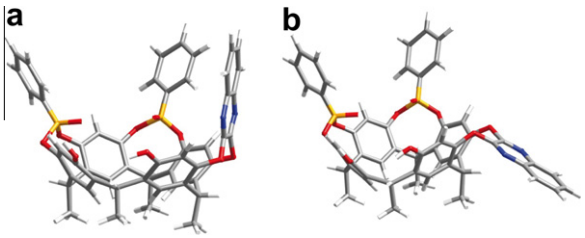


Figure 4. Model structures of the vase (a) and kite (b) conformations of the quinoxaline-bridged AB*ii* cavitand **4** (long alkyl chains have been replaced by CH_3 groups).

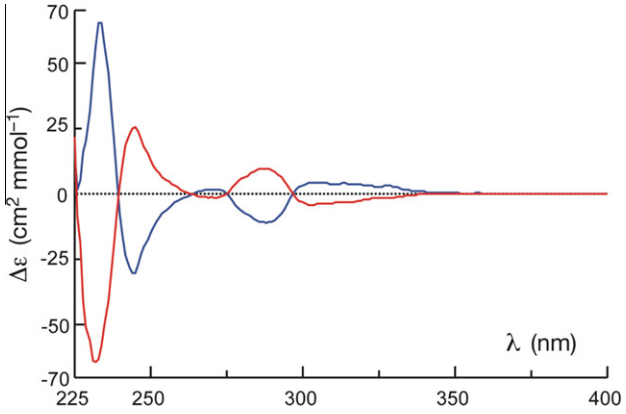


Figure 5. CD spectra of (+)-**4** (blue) and (−)-**4** (red) in CH_2Cl_2 ($c \sim 4 \times 10^{-5}$ M).

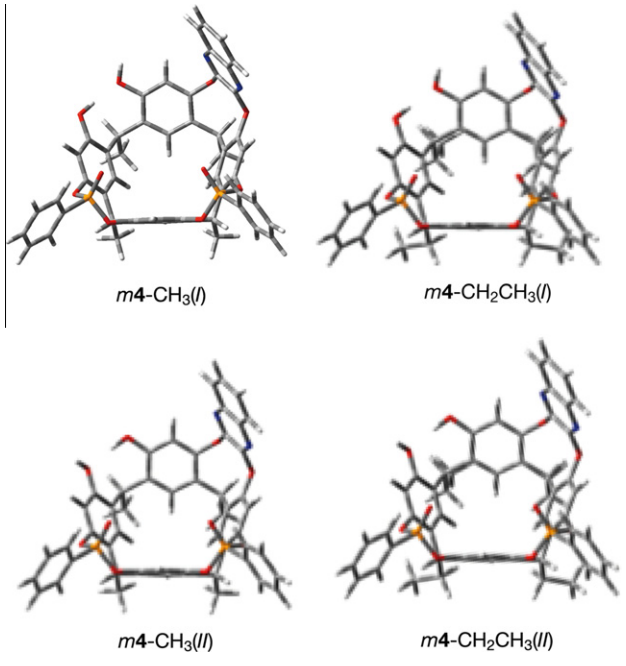


Figure 6. Optimized vase conformations of the simplified models of compound **4**: *m*4-CH_3(*I*), *m*4-CH_3(*II*), *m*4-CH_2CH_3(*I*) and *m*4-CH_2CH_3(*II*) at the B3PW91/6-311G(d) level.

chemical shift of the NCH_3 protons was noticed in the ^{1}H NMR spectrum ($\delta = -1.3$ ppm) indicating the complexation of this guest into the cavity of (±)-**4** (Fig. 9b). The two expected diastereomeric complexes were not observed by NMR at room temperature due to the fast host–guest exchange rate on the NMR timescale. At 253 K

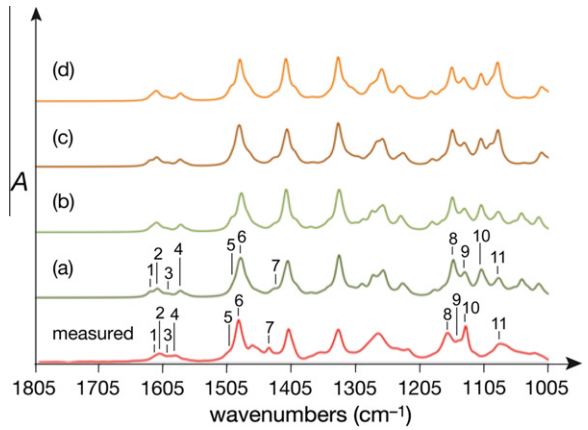


Figure 7. Comparison of the experimental IR spectrum of (±)-**4** with predicted IR spectra of the stable conformation of *m*4-CH_2CH_3(*I*) (a), *m*4-CH_2CH_3(*II*) (b), *m*4-CH_3(*I*) (c) and *m*4-CH_3(*II*) (d), at the B3PW91/6-311G(d) level. For predicted spectra, wave numbers are scaled by the factor 0.9676.

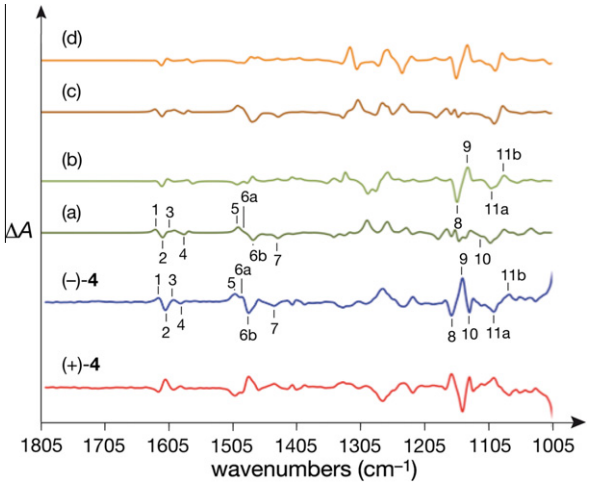


Figure 8. Comparison of experimental VCD spectra of (+)-**4** and (−)-**4** with predicted VCD spectra of the stable conformation of *m*4-CH_2CH_3(*I*) (a), *m*4-CH_2CH_3(*II*) (b), *m*4-CH_3(*I*) (c) and *m*4-CH_3(*II*) (d), at the B3PW91/6-311G(d) level. For predicted spectra, wave numbers are scaled by the factor 0.9676.

the host–guest exchange becomes slow on the ^{1}H NMR timescale and the methyl resonance of the guest split into two signals corresponding to the two diastereomers, namely [(+)-**4**][L-**11**][pic] and [(−)-**4**][L-**11**][pic] (Fig. 9c). The same occurs in the ^{31}P NMR spectrum as two sets of doublets, which overlap as a triplet-like signal, were obtained. The association constants $K_{(+)} \sim 435\ M^{-1}$ and $K_{(-)} \sim 140\ M^{-1}$ for the complexes [(+)-**4**][L-**11**][pic] and [(−)-**4**][L-**11**][pic], respectively, were determined by ^{1}H NMR at 253 K. This gives a rough estimation of the $K_{(+)}/K_{(-)}$ ratio of 3:1, indicating that (+)-**4** better accommodates L-adrenaline. In the presence of a 1:0.4 host–guest ratio, a 2:1 diastereomeric ratio was observed on the ^{1}H and ^{31}P NMR spectra at 253 K in favor of [(+)-**4**][L-**11**][pic] (Fig. 9d). The use of enantiopure (+)-**4** and (−)-**4** allowed us to assign each diastereomeric pair (Fig. 9e and f). Under similar conditions, more free cavitand is observed in the ^{31}P NMR spectrum when using (−)-**4** (Fig. 9f) compared to (+)-**4** (Fig. 9e). As [L-**11**][pic] is insoluble in $CDCl_3$, cavitand **4**, which plays the role of a solid–liquid extractant, in its dextrorotatory form better extracts this guest to the liquid phase compared to the laevorotatory enantiomer. This clearly indicates the preference of L-adrenaline guest toward the (+)-**4** enantiomer.

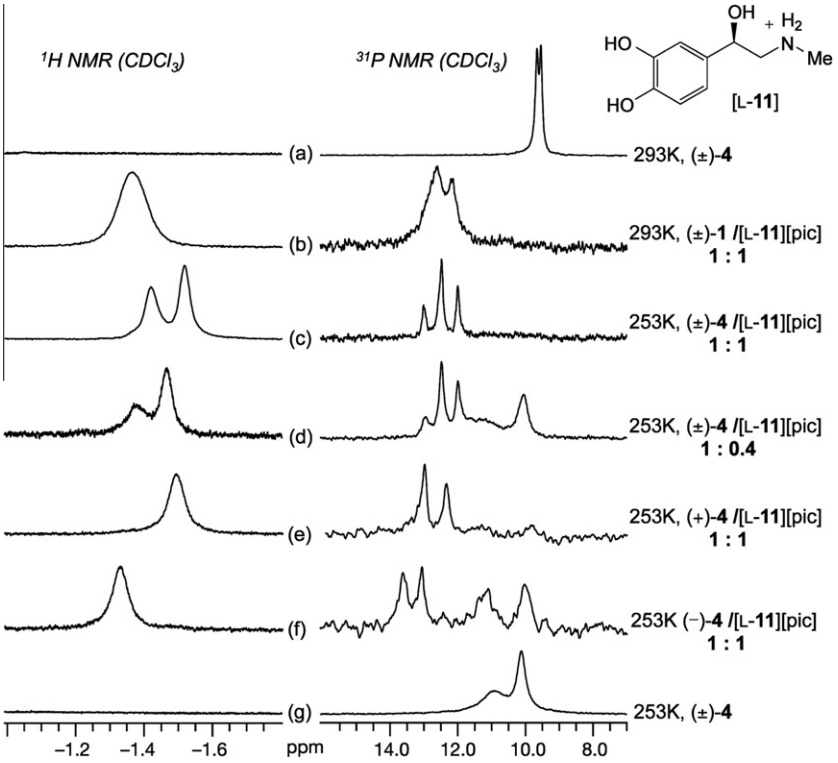


Figure 9. ^{1}H and ^{31}P NMR spectra ($CDCl_3$) of (a) (±)-**4** (293 K); (b) (±)-**4**/[L-**11**][pic] 1:1 (293 K); (c) (±)-**4**/[L-**11**][pic] 1:1 (253 K); (d) (±)-**4**/[L-**11**][pic] 1:0.4 (253 K); (e) (+)-**4**/[L-**11**][pic] 1:1 (253 K); (f) (−)-**4**/[L-**11**][pic] 1:1 (253 K); (g) (±)-**4** (253 K).

3. Conclusion

In conclusion, we have demonstrated that the new chiral AB*ii* phosphonatocavitand (±)-**4**, obtained by desymmetrization of the cavity, has been successfully resolved by semi-preparative chiral HPLC. The calculations of the IR and VCD spectra performed at the DFT level (B3PW91 functional) and the comparison with the experimental data allows us to predict the absolute configuration of each enantiomer. Compound (±)-**4** was then used as a discriminating host toward L-adrenaline. Cavitand (+)-**4** showed a preference toward L-adrenaline, with regard to the laevorotatory enantiomer. Ongoing efforts are made to better understand the non-covalent interactions between receptors and neurotransmitters. Other types of guest molecules are being investigated to broaden the field of application of this new chiral phosphonatocavitand, as well as the development of other chiral and enantiopure host molecules.

4. Experimental

4.1. General

Reactions were carried out using commercial available reagents in oven-dried apparatus. Toluene, Et_2O, and THF were dried and distilled from sodium benzophenone under nitrogen just before use. CH_2Cl_2 was dried over powdered CaH_2 and distilled under nitrogen just before use. NMR experiments were recorded on 200 MHz (4.7 T) or 500 MHz (11.7 T) NMR spectrometers. *J* Coupling constants are in hertz; chemical shifts are in δ values relative to Me_4Si (^{1}H and ^{13}C) or H_3PO_4 85% (^{31}P). ^{13}C and ^{31}P NMR spectra are proton decoupled. Spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), integration and assignment. HRMS was recorded on a commercial apparatus (ESI Source, TOF). Mass spectra were acquired on an ion trap instrument, detecting positive (+) ions in the ESI mode. Electronic Circular Dichroism (ECD) spectra were recorded on a JASCO J-815 CD spectrometer in a 1.0 cm quartz cell; λ are given in nanometer and molar circular dichroic absorptions $\Delta\varepsilon$ in $cm^2\ mmol^{-1}$.

4.2. Enantioseparation

Resolution of enantiomers was performed using a Lachrom Elite HPLC driven by an EZChrom software, with a L-2130 pump, a L-2200 sampler, a L-2350 oven, a L-2455 diode array detector, and a Jasco CD-2455 circular dichroism detector. The separation was obtained with successive additions (500 μL each, every 15 min) on a semi-preparative chiral column Chiralpak IC (250 × 10 mm, 5 μm) thermostated at 30 °C with UV detector at 254 nm and CD detector at 277 nm. Enantiomeric excesses (ees) were determined by HPLC using commercial analytical chiral column Chiralpak IC (250 × 4.6 mm, 5 μm) using same conditions. Optical rotations were reported as follows: $[\alpha]_D^{25}$ (*c* g/100 mL, in solvent) and were performed on a Perkin–Elmer 241 MC, equipped with a sodium lamp (589 nm) and a 10 cm double envelop cell thermostated at 25 °C.

4.2.1. Cavitand (−)-4

The resolution was performed on the semi-preparative HPLC starting from 40 mg of (±)-**4**; 18 mg of (−)-**4** were recovered by collecting the fractions eluted between 3.5 and 6 min: t_R = 4.8 min (hexane/ethanol/chloroform 8/1/1, 1 ml/min); $[\alpha]_D^{25} = -34$ (*c* 0.59, $CHCl_3$).

4.2.2. Cavitand (+)-4

The resolution was performed on the semi-preparative HPLC starting from 40 mg of (±)-**4**; 18 mg of (+)-**4** were recovered by collecting the fractions eluted between 8 and 13 min: t_R = 10.5 min (hexane/ethanol/chloroform 8/1/1, 1 ml/min); $[\alpha]_D^{25} = +34$ (*c* 0.59, $CHCl_3$).

4.3. IR and VCD measurements

Infrared (IR) and vibrational circular dichroism (VCD) spectra were recorded on a Bruker PMA 50 accessory coupled to a Vertex70 Fourier transform infrared spectrometer. A photoelastic modulator (Hinds PEM 90) set at l/4 retardation was used to modulate the handedness of the circular polarized light at 50 kHz. Demodulation was performed by a lock-in amplifier (SR830 DSP). An optical low-pass filter (<1800 cm^{-1}) before the photoelastic modulator was used to enhance the signal/noise ratio. A transmission cell equipped with CaF_2 windows and a 210 μm spacer was used. All solutions were prepared from 7.4 mg sample in 220 μL CD_2Cl_2. The VCD spectra of each pure enantiomer were measured and subtracted with each other in order to eliminate artifacts. For the individual spectra of the enantiomers, about 12,000 scans were averaged at 4 cm^{-1} resolution (corresponding to 3 h measurement time). For the infrared spectrum the cell filled with CD_2Cl_2 served as a reference. The spectra are presented without smoothing and further data processing.

4.4. DFT calculations

The geometry optimizations, vibrational frequencies, IR absorption, and VCD intensities were calculated with Density Functional Theory (DFT) using B3PW91 functional and a 6-311G(d) basis set. Frequencies were scaled by a factor of 0.9676. The IR absorption and VCD spectra were constructed from calculated dipole and rotational strengths assuming Lorentzian band shape with a half-width at half maximum of 6 cm^{-1}. All calculations were performed using GAUSSIAN03.[28]

4.5. Synthesis of cavitands 6–8

The azeotropic distillation, by means of a Dean and Stark apparatus, of a suspension of **5** (2.12 g, 1.92 mmol) in toluene (190 mL) was performed overnight under dry argon to remove traces of water from the starting material. Afterwards, pyridine (1 mL, 12.4 mmol) and dichlorophenylphosphine (0.52 mL, 3.83 mmol) were added dropwise at 0 °C. The resultant mixture was stirred at 0 °C for 45 min and then allowed to reach room temperature. Sulfur (0.184 g, 5.75 mmol) was then added and the solution was heated at reflux temperature for 6 h. The resulting mixture was filtered at room temperature and the filtrate was concentrated under vacuum to give a beige residue (3.40 g). Silica gel column chromatography (eluent CH_2Cl_2/ethyl acetate: from 98:2 to 90:10) of the residue afforded the *iii* compound **6** (0.555 mg, 0.37 mmol, 19%) as a white solid.[15] Further elution with CH_2Cl_2/ethyl acetate: from 90:10 to 80:20 afforded the *ACii* compound **7** (0.222 mg, 0.16 mmol, 8%) as a white solid. Further elution with CH_2Cl_2/ethyl acetate: from 80:20 to 70:30 afforded the *ABii* compound **8** (0.679 mg, 0.46 mmol, 24%) as a white solid.

4.5.1. Cavitand 6[15]

LSIMS *m*/*z* 1541.7339 [*M*+Na]$^+$ (calcd 1541.7334). ^{31}P NMR (81.02 MHz, 300 K, $CDCl_3$): δ 78.17 (2P); 79.15 (1P). ^{1}H NMR ($CDCl_3$, 300 K, 200.13 MHz) δ 0.91 (m, 12H, $-CH_2-(CH_2)_9-CH_3$), 1.30 (m, 72, $-CH_2-(CH_2)_9-CH_3$), 2.35 (m, 8H, $-CH_2-(CH_2)_9-CH_3$), 4.44 (t, 1H, J = 8,0 Hz), 4.75 (m, 3H), 6.54 (s, 2H), 6.72 (s, 2H), 7.23 (s, 2H), 7.37 (s, 2H), 7.53 (m, 9H), 8.22 (m, 6H). ^{13}C NMR ($CDCl_3$, 300 K, 50.32 MHz) δ 14.10 ($CH_2-(CH_2)_9-CH_3$), 22.68 ($CH_2-(CH_2)_8-CH_2-CH_3$), 28.05 ($CH_2-(CH_2)_8-CH_2-CH_2-CH_3$), 29.39 ($CH_2-(CH_2)_9-CH_3$), 29.72 ($CH_2-(CH_2)_9-CH_3$), 30.96 ($CH_2-CH_2-(CH_2)_8-CH_3$), 31.94 ($CH_2-CH_2-(CH_2)_8-CH_3$), 33.85 ($CH_2-CH_2-(CH_2)_8-CH_3$), 35.74, 35.97, 111.95, 119.46, 121.72, 122.93, 128.26 (d, ^3J = 15.6 Hz), 128.37 (d, ^3J = 15.6 Hz), 129.83, 130.00, 131.03 (d, ^2J = 11.8 Hz), 131.84 (d, ^1J = 168.4 Hz), 132.73, 135.05, 135.96, 146.24, 151.57.

4.5.2. Cavitand 7

LSIMS *m*/*z* 1403.7633 [M+Na]$^+$ (calcd 1403.7633). ^{31}P NMR ($CDCl_3$, 300 K, 80.02 MHz) : δ 77.32 (2P). ^{1}H NMR ($CDCl_3$, 300 K, 200.13 MHz) δ 0.87 (m, 12H, $-CH_2-(CH_2)_9-CH_3$), 1.25 (m, 72H, $-CH_2-(CH_2)_9-CH_3$), 2.12 (m, 4H, $(-CH_2-(CH_2)_9-CH_3)_b$), 2.30 (m, 4H, $(-CH_2-(CH_2)_9-CH_3)_a$), 4.28 (t, 2H, ^3J = 7.20 Hz), 4.70 (t, 2H, ^3J = 7.20 Hz), 6.50 (s, 4H), 7.18 (s, 4H), 7.54 (m, 6H), 8.17 (dd, 4H, $^3J_{H-H}$ = 7.47 Hz, $^3J_{P-H}$ = 14.62 Hz). ^{13}C NMR ($CDCl_3$, 300 K, 50.32 MHz) δ 14.11 ($-CH_2-(CH_2)_9-CH_3$), 22.69 ($-CH_2-(CH_2)_8-CH_2-CH_3$), 27.93 ($-CH_2-(CH_2)_7-CH_2-CH_2-CH_3$), 28.06 ($-CH_2-(CH_2)_7-CH_2-CH_2-CH_3$), 29.41 ($-CH_2-(CH_2)_9-CH_3$), 29.70 ($-CH_2-(CH_2)_9-CH_3$), 31.49 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 31.94 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 33.71, 34.04 ($CH_2-CH_2-(CH_2)_8-CH_3$), 35.44, 112.61, 122.90, 128.50 (d, ^3J = 15.7 Hz), 129.07, 130.77, 130.99 (d, ^2J = 11.7 Hz), 131.66 (d, ^1J = 164.8 Hz), 133.30, 145.12 (d, ^2J = 10.9 Hz), 151.29.

4.5.3. Cavitand 8

LSIMS *m*/*z* 1381.7834 [M+H]$^+$ (calcd 1381.7822). ^{31}P NMR ($CDCl_3$, 300 K, 80.02 MHz): δ 80.69 (2P). ^{1}H NMR ($CDCl_3$, 300 K, 200.13 MHz) δ 0.85 (m, 12H, $-CH_2-(CH_2)_9-CH_3$), 1.25 (m, 72H, $-CH_2-(CH_2)_9-CH_3$), 2.12 (m, 4H, $(-CH_2-(CH_2)_9-CH_3)_b$), 2.31 (m, 4H, $(-CH_2-(CH_2)_9-CH_3)_a$), 4.24 (t, 2H, ^3J = 6.84 Hz), 4.67 (t, 2H, ^3J = 6.84 Hz), 5.54 (s, 1H), 6.54 (s, 2H), 6.67 (s, 1H), 7.03 (s, 1H), 7.25 (s, 2H), 7.32 (s, 1H), 7.56 (m, 6H), 8.15 (dd, 4H, $^3J_{H-H}$ = 7.47 Hz, $^3J_{P-H}$ = 14.94 Hz), 8.53 (s, 2H), 8.72 (s, 2H). ^{13}C NMR ($CDCl_3$, 300 K, 50.32 MHz) δ 14.11 ($-CH_2-(CH_2)_9-CH_3$), 22.69 ($-CH_2-(CH_2)_7-CH_2-CH_3$), 28.06 ($-CH_2-(CH_2)_7-CH_2-CH_2-CH_3$), 29.41 ($-CH_2-(CH_2)_9-CH_3$), 29.74 ($-CH_2-(CH_2)_9-CH_3$), 31.10 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 31.94 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 33.41 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 33.60, 35.83, 102.96, 112.23, 119.95, 122.05, 123.56, 123.96, 128.40 (d, ^3J = 15.7 Hz), 129.16, 130.34, 131.04 (d, ^2J = 11.8 Hz), 131.48 (d, ^1J = 171.5 Hz), 132.89, 136.18, 146.11, 150.16, 152.56.

4.6. Synthesis of cavitand 9

m-Chloroperoxybenzoic acid (140 mg, 0.8 mmol) was added to a solution of cavitand **8** (377 mg, 0.26 mmol) in $CHCl_3$ (38 mL). The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the crude compound was recrystallized from MeOH to give **9** as a white solid (300 mg, 85%). LSIMS *m*/*z* 1421.85 [M+THF+H]$^+$ (calcd 1421.88). ^{31}P NMR ($CDCl_3$, 300 K, 202.45 MHz) : δ 8.30 (2P). ^{1}H NMR ($CDCl_3$, 300 K, 500.10 MHz) δ 0.85 (m, 12H, $-CH_2-(CH_2)_9-CH_3$), 1.25 (m, 72H, $-CH_2-(CH_2)_9-CH_3$), 2.12 (m, 4H, $(-CH_2-(CH_2)_9-CH_3)_b$), 2.27 (m, 4H, $(-CH_2-(CH_2)_9-CH_3)_a$), 4.29 (t, 2H, ^3J = 7.62 Hz), 4.60 (t, 2 H, ^3J = 7.62 Hz), 6.10 (s, 1H), 6.59 (s, 2H), 6.86 (s, 1H), 6.89 (s, 1H), 7.11 (s, 2H), 7.30 (s, 1H), 7.56 (m, 4H), 7.65 (t, 2H, ^3J = 7.31 Hz), 8.04 (dd, 4H, $^3J_{H-H}$ = 7.43 Hz, $^3J_{P-H}$ = 14.19 Hz), 8.65 (s, 2H), 10.11 (s, 2H). ^{13}C NMR ($CDCl_3$, 300 K, 50.32 MHz) δ 14.26 ($-CH_2-(CH_2)_9-CH_3$), 23.09 ($-CH_2-(CH_2)_7-CH_2-CH_3$), 28.45 ($-CH_2-(CH_2)_7-CH_2-CH_2-CH_3$), 29.79 ($-CH_2-(CH_2)_9-CH_3$), 30.13 ($-CH_2-(CH_2)_9-CH_3$), 31.23 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 32.35 ($-CH_2-CH_2-(CH_2)_8-CH_3$), 33.94, 36.40, 103.30, 111.52, 118.23, 122.96, 123.80, 124.43, 125.23, 127.91, 130.01, 132.27, 132.60, 134.87, 137.30, 146.11, 146.70, 151.81, 156.09.

4.7. Synthesis of cavitands 10 and (±)-4

Cesium carbonate (77 mg, 0.24 mmol) and 2,3-dichloro-quinoxaline (16 mg, 0.08 mmol) were added to a solution of cavitand **9** (100 mg, 0.075 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 48 h and then poured into ethyl acetate (30 mL). The solution was extracted with water (10 × 5 mL), dried over Na_2SO_4, and evaporated to dryness under vacuum to give a brown residue. The crude compound was purified by silica gel column chromatography (eluent CH_2Cl_2/THF from 99:1 to 9:1) to give

compound **10** as a white solid (35 mg, 29%). Further elution with CH_2Cl_2/THF from 9:1 to 7:3 gave compound (±)-**4** as a white solid (42 mg, 39%).

4.7.1. Cavitand 10

LSIMS *m/z* 1601.8706 $[M+H]^+$ (calcd 1601.8709). ^{31}P NMR ($CDCl_3$, 300 K, 202.45 MHz) : δ 8.14 (2P). ^1H NMR ($CDCl_3$, 300 K, 500.10 MHz) δ 0.86 (m, 12H, $-CH_2-(CH_2)_9-CH_3$), 1.26 (m, 64H, $-CH_2-CH_2-(CH_2)_8-CH_3$), 1.44 (m, 8H, $-CH_2-CH_2-(CH_2)_8-CH_3$), 2.30 (m, 8H, $-CH_2-(CH_2)_9-CH_3$), 4.67 (t, 1H, ^3J = 7.40 Hz), 5.78 (t, 1H, ^3J = 8.17 Hz), 6.81 (s, 1H), 7.19 (s, 1H), 7.21 (s, 1H), 7.45 (s, 2H), 7.45 (s, 1H), 7.45 (m, 6H), 7.50 (m, 4H), 7.83 (d, 2H, ^3J = 8.05 Hz), 7.97 (m, 6H), 8.46 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 K , 50.32 MHz) δ 14.14 ($-CH_2-(CH_2)_9-CH_3$), 22.72 ($-CH_2-(CH_2)_9-CH_3$), 27.95 ($-CH_2-(CH_2)_9-CH_3$), 28.09 ($-CH_2-(CH_2)_9-CH_3$), 29.45 ($-CH_2-(CH_2)_9-CH_3$), 29.74 ($-CH_2-(CH_2)_9-CH_3$), 31.06 ($-CH_2-(CH_2)_9-CH_3$), 31.96 ($-CH_2-(CH_2)_9-CH_3$), 32.14 ($-CH_2-(CH_2)_9-CH_3$), 34.21, 36.07, 117.26, 117.75, 118.73, 122.02, 122.51, 123.20, 127.82, 128.52, 129.28, 131.59 (d, ^3J = 16.1 Hz), 133.27 (d, ^3J = 16.1 Hz), 134.39, 134.81, 135.78, 136.41, 140.12, 145.92, 146.10, 152.28, 152.84.

4.7.2. Cavitand (±)-4

LSIMS *m/z* 1475.8478 $[M+H]^+$ (calcd 1475.8491). ^{31}P NMR ($CDCl_3$, 300 K, 202.45 MHz) : δ 9.01 (1P), 9.21 (1P). ^1H NMR ($CDCl_3$, 300 K, 500.10 MHz) δ 0.86 (m, 12H, $-CH_2-(CH_2)_9-CH_3$), 1.25 (m, 72H, $-CH_2-(CH_2)_9-CH_3$), 2.12 (m, 2H, $(-CH_2-(CH_2)_9-CH_3$), 2.28 (m, 4H, $-CH_2-(CH_2)_9-CH_3$), 4.24 (m, 1H), 4.58 (m, 1H), 4.70 (m, 1H), 5.68 (m, 1H), 6.40 (s, 1H), 6.93 (s, 1H), 7.05 (s, 1H), 7.11 (s, 1H), 7.23 (m, 3H), 7.26 (s, 1H), 7.32 (s, 1H), 7.38 (m, 1H), 7.47 (m, 2H), 7.55 (m, 3H), 7.60 (s, 1H), 7.74 (m, 2H), 7.90 (m, 2H), 8.01 (m, 2H). ^{13}C NMR ($CDCl_3$, 300 K, 50.32 MHz) δ 14.21 ($-CH_2-(CH_2)_9-CH_3$), 22.78 ($-CH_2-(CH_2)_9-CH_3$), 28.10 (m, $-CH_2-(CH_2)_9-CH_3$), 29.51 (m, $-CH_2-(CH_2)_9-CH_3$), 29.80 (m, $-CH_2-(CH_2)_9-CH_3$), 30.84 ($-CH_2-(CH_2)_9-CH_3$), 31.23 ($-CH_2-(CH_2)_9-CH_3$), 32.02 (m, $-CH_2-(CH_2)_9-CH_3$), 32.12 ($-CH_2-(CH_2)_9-CH_3$), 33.65 ($-CH_2-(CH_2)_9-CH_3$), 33.92, 34.04, 35.92, 36.25, 110.29, 111.35, 117.25, 117.35, 121.98, 122.34, 122.44, 123.58, 125.15 (d, ^1J = 206.2 Hz), 125.60 (d, ^1J = 206.2 Hz), 128.07, 128.20, 128.37, 128.52 (d, ^3J = 16.1 Hz), 128.56 (d, ^3J = 16.1 Hz), 129.27, 129.45, 129.53, 129.62, 131.15 (d, ^2J = 10.1 Hz), 131.44 (d, ^2J = 10.1 Hz), 133.23, 133.32, 134.30, 134.85, 135.94, 137.52, 145.39, 145.75, 145.97, 152.29, 152.44, 152.61, 152.85, 156.09.

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References

1. (a) Lehn, J.-M. Ed., Atwood, J. L.; Davies, J. E. D.; Macnicol, D. D.; Vögtle, F. Executive Eds., *Supramolecular Reactivity and Transport: Bioorganic Systems. Comprehensive Supramolecular Chemistry*; Murakami, Y. Ed., Elsevier: Amsterdam, 1996; Vol. 4.; (b) Shirakawa, S.; Tanaka, Y.; Kobari, T.; Shimizu, S. *New J. Chem.* **2008**, *32*, 1835; (c) Hembury, G. A.; Borovkov, V. V.; Inoue, Y. *Chem. Rev.* **2008**, *108*, 1; (d) Pinkhassik, E.; Stibor, I.; Casnati, A.; Ungaro, R. *J. Org. Chem.* **1997**, *62*, 8654; (e) Scarso, A.; Rebek, J., Jr. *Supramol. Chirality* **2006**, *265*, 1.
2. (a) Moberg, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 4721; (b) Fernandes, S. A.; Nachtigal, F. F.; Lazzarotto, M.; Fujiwara, F. Y.; Marsaioli, A. J. *Magn. Reson. Chem.* **2005**, *43*, 398; (c) Yakovenko, A. V.; Boyko, V. I.; Kalchenko, V. I.; Baldini, L.; Casnati, A.; Sansone, F.; Ungaro, R. *J. Org. Chem.* **2007**, *72*, 3223.
3. (a) Visotsky, C. S. M.; Böhmer, V. *Adv. Supramol. Chem.* **2000**, *7*, 139; (b) Cherenok, S.; Dutasta, J.-P.; Kalchenko, V. *Curr. Org. Chem.* **2006**, *10*, 2307; (c) Simulescu, V.; Ilia, G. *J. Inclusion Phenom. Macrocycl. Chem.* **2010**, *66*, 3.
4. Cram, D. J.; Tunstad, L. M.; Knobler, C. B. *J. Org. Chem.* **1992**, *57*, 528.
5. Soncini, P.; Bonsignore, S.; Dalcanale, E.; Ugozzoli, F. *J. Org. Chem.* **1992**, *57*, 4608.
6. Renslo, A. R.; Tucci, F. C.; Rudkevich, D. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2000**, *122*, 4573; Renslo, A. R.; Rudkevich, D. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 7459.
7. Vincenti, M.; Dalcanale, E.; Soncini, P.; Guglielmetti, G. *J. Am. Chem. Soc.* **1990**, *112*, 445.
8. Dutasta, J.-P. *Top. Curr. Chem.* **2004**, *232*, 55.
9. Melegari, M.; Suman, M.; Pirondini, L.; Moiani, D.; Massera, C.; Ugozzoli, F.; Kalenius, E.; Vainiotalo, P.; Mulatier, J.-C.; Dutasta, J.-P.; Dalcanale, E. *Chem. Eur. J.* **2008**, *14*, 5772.
10. Bibal, B.; Tinant, B.; Declercq, J.-P.; Dutasta, J.-P. *Supramol. Chem.* **2003**, *15*, 25.
11. (a) Delangle, P.; Mulatier, J.-C.; Tinant, B.; Declercq, J.-P.; Dutasta, J.-P. *Eur. J. Org. Chem.* **2001**, 3695; (b) Yebeutchou, R. M.; Dalcanale, E. *J. Am. Chem. Soc.* **2009**, *131*, 2452.
12. Dalla Cort, A.; Mandolini, L.; Pasquini, C.; Schiaffino, L. *New J. Chem.* **2004**, *28*, 1198.
13. Timmerman, P.; Verboom, W.; Reinhoudt, D. N. *Tetrahedron* **1996**, *52*, 2663.
14. Weinelt, F.; Schneider, H. J. *J. Org. Chem.* **1991**, *56*, 5527.
15. Dubessy, B.; Harthong, S.; Aronica, C.; Bouchu, D.; Busi, M.; Dalcanale, E.; Dutasta, J.-P. *J. Org. Chem.* **2009**, *74*, 3923.
16. Bibal, B.; Tinant, B.; Declercq, J.-P.; Dutasta, J.-P. *Chem. Commun.* **2002**, 432.
17. Cantadori, B.; Betti, P.; Boccini, F.; Massera, C.; Dalcanale, E. *Supramol. Chem.* **2008**, *20*, 29.
18. Herriott, A. W. *J. Am. Chem. Soc.* **1971**, *93*, 3304.
19. Tebby, J. C. *Handbook of phosphorous-31 Nuclear Magnetic Resonance Data*; CRC Press: Boca Raton, 1991.
20. (a) Moran, J. R.; Karbach, S.; Cram, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 5826; (b) Cram, D. J. *Science* **1983**, *219*, 1177.
21. (a) Cram, D. J.; Choi, H.-J.; Bryant, J. A.; Knobler, C. B. *J. Am. Chem. Soc.* **1992**, *20*, 7748; (b) Azov, V. A.; Schlegel, A.; Diederich, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 4635; (c) Pagliusi, P.; Lagugné-Labarthet, F.; Shenoy, D. K.; Dalcanale, E.; Shen, Y. R. *J. Am. Chem. Soc.* **2006**, *128*, 12610.
22. Moran, J. R.; Ericson, J. L.; Dalcanale, E.; Bryant, J. A.; Knobler, C. B.; Cram, D. J. *J. Am. Chem. Soc.* **1991**, *113*, 5707.
23. Azov, V. A.; Diederich, F.; Lill, Y.; Hecht, B. *Helv. Chim. Acta* **2003**, *86*, 2149.
24. Roncucci, P.; Pirondini, L.; Paderni, G.; Massera, C.; Dalcanale, E.; Azov, V. A.; Diederich, F. *Chem. Eur. J.* **2006**, *12*, 4775.
25. First example of CD spectra of a chiral resorcinarene: Mann, E.; Rebek, J., Jr. *Tetrahedron* **2008**, *64*, 8484.
26. (a) Nafie, L. A.; Keiderling, T. A.; Stephens, P. J. *J. Am. Chem. Soc.* **1976**, *98*, 2715; (b) Nafie, L. A. *Annu. Rev. Phys. Chem.* **1997**, *48*, 357; (c) Stephens, P. J.; Devlin, F. J. *Chirality* **2000**, *117*, 172; (d) Freedman, T. B.; Cao, X.; Dukor, R. K.; Nafie, L. A. *Chirality* **2003**, *15*, 743; (e) Gatineau, D.; Moraleda, D.; Naubron, J.-V.; Bürgi, T.; Giordano, L.; Buono, G. *Tetrahedron: Asymmetry* **2009**, *20*, 1912; (f) Piron, F.; Vanthuyne, N.; Joulin, B.; Naubron, J.-V.; Cisma, C.; Terec, A.; Varga, R. A.; Roussel, C.; Roncali, J.; Grosu, I. *J. Org. Chem.* **2009**, *74*, 9062; (g) Brotin, T.; Cavagnat, D.; Dutasta, J.-P.; Buffeteau, T. *J. Am. Chem. Soc.* **2006**, *128*, 5533; (h) Brotin, T.; Cavagnat, D.; Buffeteau, T. *J. Phys. Chem. A* **2008**, *112*, 8464; (i) Cavagnat, D.; Buffeteau, T.; Brotin, T. *J. Org. Chem.* **2008**, *73*, 66.
27. (a) Givelet, C.; Buffeteau, T.; Arnaud-Neu, F.; Hubscher-Bruder, V.; Bibal, B. *J. Org. Chem.* **2009**, *74*, 5059; (b) Escuder, B.; Rowan, A. E.; Feiters, M. C.; Nolte, R. J. M. *Tetrahedron* **2004**, *60*, 291; (c) Molt, O.; Schrader, T. *Angew. Chem., Int. Ed.* **2003**, *40*, 3148; (d) Molt, O.; Rübeling, D.; Schäfer, G.; Schrader, T. *Chem. Eur. J.* **2004**, *10*, 4225.
28. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Rob, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 03 Rev. E01* Gaussian, Inc.: Wallingford, CT, 2003.