Enantioselective Complexation of Chiral Oxirane Derivatives by an Enantiopure Cryptophane in Water

Nicolas de Rycke, Marion Jean, Nicolas Vanthuyne, Thierry Buffeteau, T. Brotin

Enantioselective Complexation of Chiral Oxirane Derivatives by an Enantiopure Cryptophane in Water.

Nicolas De rycke[†], *Marion Jean*,[§] *Nicolas Vanthuyne*,[§] *Thierry Buffeteau*[*,‡] *and Thierry Brotin*,[*,†]

[†]Univ Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR 5182, Université Lyon 1, Laboratoire de Chimie, 46 allée d'Italie, F69364, LYON, France

[§] Laboratoire de Chimie Moleculaire Organique (UMR 6517 – CNRS), Boite 562—Faculté des Sciences St Jerôme, Universite d'Aix-Marseille III,Av. Escadrille Normandie-Niemen, 13397 Marseille Cedex 20, France

[‡] Institut des Science Moléculaires, Bordeaux University 351, Cours de la Libération, 33405 Talence, France

e-mail: thierry.brotin@ens-lyon.fr

RECEIVED DATE :

* to whom correspondence should be addressed.

† Laboratoire de Chimie de l'ENS-LYON

‡ Institut des Sciences Moléculaires

Abstract: In this article we show that optically active cryptophane **1** bearing phenol functions can efficiently bind the two enantiomers of epichlorohydrin, 1,2-butyloxirane, and bioxirane derivatives in aqueous solution. The binding process is characterized by the presence of high-field shifted ^{1}H NMR signals, which are specific of the encapsulated species.

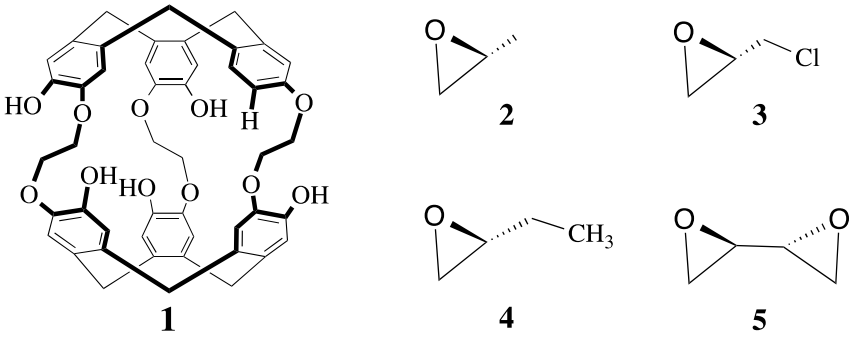
In all cases, an enantioselective effect has been measured and a relationship between the molecular volume and the affinity constants can be clearly established, even though the flexibility of the guest molecule seems also to play a role. In this series, the bioxirane guest derivative is a particularly interesting example since this compound possesses two stereogenic centers. Thus, in solution, three different couple of diastereomers can be obtained in presence of the optically active host **1**. Electronic Circular Dichroism spectroscopy (ECD) has also been used to characterize these complexes. As previously observed with the methyloxirane derivative, the ECD spectra recorded for the ^1L_b region show specific cotton bands, which are characteristic of each diastereomers formed in solution.

Introduction: cryptophane derivatives are a well-known family of organic covalently bound capsules whose binding properties have been studied for nearly 40 years.[1] In the past, these molecules have received a lot of attention because these organic systems can accommodate different atoms or molecules within their cavity. For instance, it has been shown that neutral species (methane, xenon, halogenomethanes) or charged species (alkali cations, ammonium, anions) could easily enter the cavity of the cryptophane derivatives depending on the cavity size of the host and the nature of the substituents grafted on the benzene rings.[2,3] Interestingly, the encapsulation process strongly modifies the physical properties of both guest and host molecules thus leading to important spectroscopic changes. For instance, the encapsulated guest usually possesses new original physical properties that can be used for practical applications. As an example, the Xe@cryptophane complexes have received a lot of attention over the last few years since these systems show remarkable physical properties that can be exploited for MRI biosensing applications.[4] Beside their interesting binding properties, it can also be noticed that the overwhelming majority of the cryptophane derivatives show an inherently chiral structure. This second characteristic has not

been studied as much as the binding properties of cryptophanes probably because these molecules are difficult to obtain in their enantiopure form. Collet and co-workers pioneered this work in 1987 but this is only in 2003 that new approaches have been proposed to obtain new enantiopure cryptophane derivatives.[5,6] Combined together, these two characteristics lead to original organic capsules that can be used for the complexation of optically active guest molecules.[7] However, despite the high interest for these chiral capsules the examples reported in the literature are rare. The difficulties to find small chiral guest molecules that show a good size matching with the cavity of these hosts are the main reasons that explain the scarcity of the examples reported in the literature. Collet and co-workers were the first to take advantage of the chiral structure of a cryptophane derivative to bind the enantio-enriched CHFClBr derivative. Later in 2012, we have shown that optically active methyloxirane **2** (also called propylene-oxide), could easily enter the cavity of the enantiopure cryptophane **1** in aqueous solution.[8] The association between (R)-(+) and (S)-(-)-methyloxirane and **1** gave rise to two diastereomers that could be easily distinguished by ^{1}H NMR spectroscopy, thanks to the strong shielding effect induced by the chiral cavity.[9] A moderate enantioselective effect was also observed between the two enantiomers of methyloxirane and specific ECD signals have been obtained for the two pair of diastereomers.

These interesting results prompted us to investigate other oxirane derivatives with host **1**. For instance small oxirane derivatives such as Epichlorohydrin **3** (V_{vdw} = 72.1 Å^3), 1,2-Butyloxitrane **4** (V_{vdw} = 74 Å^3) and Bioxirane **5** (V_{vdw} = 70.6 Å^3) have retained our attention because they possess small Vn der Waals volume ad they are therefore able to enter the cavity of **1** (Scheme 1). However, these molecules possess a somewhat larger molecular volume than methyloxirane (V_{vdw} = 56.9 Å^3) and their ability to enter the cavity of host **1** is questionable. In this series, compound **5** is particularly interesting since this compound possesses two stereogenic centers. Thus, three different types of diastereomers can be formed in solution in

presence of the chiral host **1**. In this article, we examine the ability of these enantiopure derivatives to enter the cavity of host **1** under different experimental conditions. For this study, ^{1}H NMR spectroscopy seems to be the method of choice to study these complexes since a slow exchange regime is usually observed between the guest molecules and the cryptophane derivatives. The ECD spectra of the different diastereomers are also reported at 278 K.

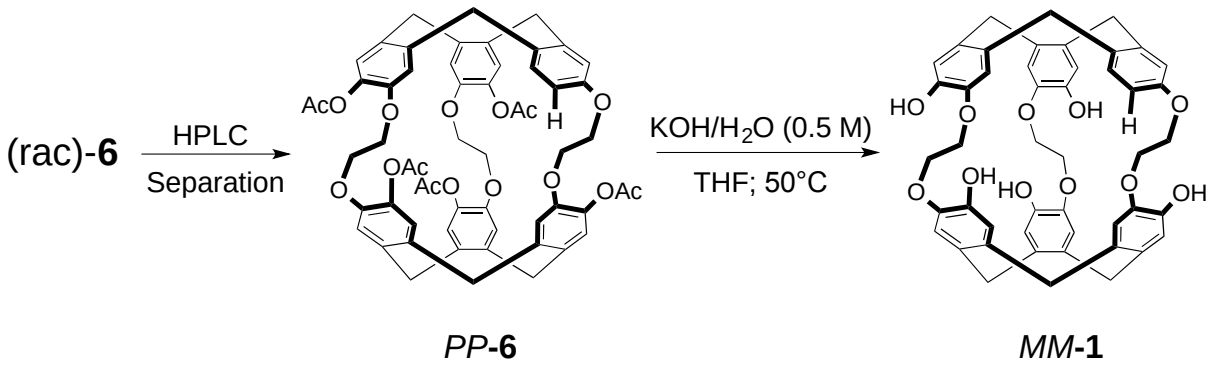


Scheme 1: structure of cryptophane **1** and chiral oxiranes (methyloxirane-**2**, Epichlorohydrin-**3**; epoxybutane-**4**; Bioxirane-**5**) used in this study. Only a single stereomer is shown for each compound.

Results and Discussion:

Optical resolution of compound 1: the preparation of compound **1** in its racemic form has been previously reported in the literature.[10] The separation of the two enantiomers of **1** has also been reported from a multi-steps synthesis using cryptophanol-A as a starting material.[11,12] This approach allowed us to obtain the two enantiomers of **1** in fair quantities and with very high enantiomeric excess (ee ~ 100%). However, this method is time consuming and in this study we have preferred to use another approach based on the separation of the two enantiomers of compound **6** by HPLC chromatography on a semi-preparative column. Compound **6** is the protected version of **1**. The separation of this compound was preferred because it shows higher solubility in organic solvents. The two

enantiomers of **1** can then be easily recovered by hydrolysis under basic solution of the two enantiomers separation of **6** (scheme 2).



Scheme 2: synthetic procedure use for the separation of the two enantiomers of **1**. The synthesis of (rac)-**6** is reported ref X.

The two enantiomers of compound **6** have been successfully separated on a semi-preparative Welk-O1 column chromatography using a chloroform/ethanol mixture (see experimental section for more details). This allowed us to obtain these two enantiomers in fair quantities and very high enantiomeric excess (ee > 99.5 % for both enantiomers; see Supp. Info S1-S2). Finally, hydrolysis of the two enantiomers of **6** under basic conditions, gives rise to the two enantiomers *MM*-**1** and *PP*-**1**.

^{1}H NMR Spectroscopy of the oxirane complexes: The previous study performed in aqueous solution with the methyloxirane derivative has shown that ^{1}H NMR spectroscopy is a valuable tool to extract information about these complexes. Indeed, in aqueous solution the host-guest association occurs under a slow exchange regime, even at room temperature. This means that the encapsulated species can be clearly identified on the ^{1}H NMR spectrum. In addition, thanks to the strong shielding effect induced by the six aromatic rings, the ^{1}H NMR signals of the encapsulated molecule appears at negative chemical shift (reference TMS). For instance,

the encapsulated methyloxirane in host **1** give rise to several sets of ^{1}H NMR signals between 0.0 and -2.0 ppm.

Compound **1** is only soluble in H_2O (D_2O) under basic conditions. Thus the ^{1}H NMR spectra of **1** with the different chiral guests have been recorded in LiOD/D_2O, NaOH/D_2O, KOH/D_2O and CsOH/D_2O at a concentration of 0.1 M. At this concentration the oxirane molecules are stable and no decomposition was observed during the time of our experiments. The spectra have been recorded at 278 K in order to decrease the exchange dynamics and to detect more easily the ^{1}H NMR signals corresponding to the encapsulated species.

At 275 K, the efficient binding of the (rac)-Epichlorohydrin by host *PP*-**1** occurs in LiOH (0.1 M). The association between guest **3** and host **1** gives rise to additional ^{1}H NMR signals. As expected, these signals appear at lower frequencies and at negative chemical shifts with respect to TMS taken as a reference. Thus, several ^{1}H NMR signals located between +0.4 ppm and -1.0 ppm can be clearly identified on the spectrum of the (rac)-**3**@*PP*-**1** complexes. The presence of numerous ^{1}H NMR signals and the large difference in intensity observed between some of these signals suggest that the two diastereomers can be distinguished and that some enantioselectivity takes place. To confirm these results, the two enantiomers (R)-(-)**3** and (S)-(+)**3** have been introduced separately using a single enantiomer of **1**. For instance, under the same conditions the (R)-(-)-**3**@*PP*-**1** diastereomer shows a simplified ^{1}H NMR spectrum compared to that of (rac)-**3**@*PP*-**1**. Thus, three sets of protons are observed for this complex at 0.15 ppm, -0.3 and -0.8 ppm, respectively. In contrast, the (S)-(+)-**3**@*PP*-**1** diastereomer shows five well-resolved signals, located at 0.2, - 0.35, - 0.55, - 0.65 and - 0.95 ppm, respectively. Assuming a 1:2.5 ratio in favour of the (S)-(+)-**3**@*PP*-**1** diastereomer, the summation of these two spectra reproduce very well the ^{1}H NMR spectrum observed with the (rac)-**3**@*PP*-**1**. It is noteworthy that the ^{1}H NMR spectrum of the (S)-(+)**3**@*PP*-**1** diastereomer is identical to that observed for the (R)-(-)-**3**@*MM*-**1** diastereomer. Similarly,

the ^{1}H NMR spectrum of the (R)-(-)-**3**@*PP*-**1** is identical to that of the (S)-(+)-**3**@*PP*-**1** diastereomer.

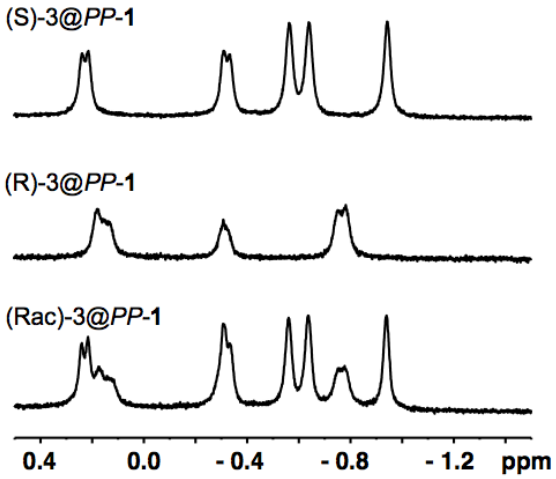


Figure 1: ^{1}H NMR spectrum of *PP*-**1** in presence of (S)-(+)-**3**, (R)-(-)-**3** and (rac)-Epichlorhydrin-**3** recorded in LiOD/D_2O (0.1 M) at 275 K. Only the spectral region corresponding to the encapsulated guest is shown.

A change of the experimental conditions does not change significantly the shape of the ^{1}H NMR spectra. For instance, in NaOD/D_2O (0.1 M) with the two enantiomers *PP*-**1** or *MM*-**1** we observe the same high-field shifted ^{1}H NMR signals (see Supp. Info. S3). However, in KOD/D_2O (0.1 M) we can notice a significant decrease in intensity of these signals but these spectra remain identical to those previously recorded in NaOD/D_2O (see Supp. Info. S4). In CsOD/D_2O no signal is detected in that region suggesting that complexation of guest **3** does not take place under these conditions. It is noteworthy that a similar solvent effect was previously observed with methyloxirane under the same experimental conditions. Indeed, It has been shown that host **1** shows a good affinity for the K^+ cation and a binding constant K = 1000 M^{-1} has been measured in LiOH/H_2O (0.1 M) by titration experiments. Other alkali cations such as Rb^+ and Cs^+ cations are also well recognized in LiOH/H_2O or NaOH/H_2O solutions and exhibit higher affinity for this host. (REF 10) Thus, herein the K^+ cation acts as

a competitor and a significant decrease in affinity of guest **3** for the host **1** is observed in KOH/H_2O solution. In $CsOH/H_2O$, it has been shown that the affinity of **1** for the Cs^+ cation is about 10^6 larger than the association constant observed with the K^+ cation. Thus, in these conditions, no complexation takes place even in presence of a large excess of guest **3**.

The association constants between optically active guest **3** and enantiopure host **1** are reported as a function of the nature of the solvents (table 1 in Supp. Info. S5). Since a slow exchange regime is observed in all cases, the determination of the binding constants is facilitated and the different concentration needed to calculate the binding constant K can be directly extracted from the ^1H NMR spectrum. A repetition of these experiments led to an uncertainty value of 20 % on the measure of the binding constant K. The calculation of the binding constants reveals that *MM*-**1** shows some enantioselectivity in presence of **3**. For instance, the binding constants for the two diastereomers (R)-(-)-**3**@*MM*-**1** and (S)-(+)-**3**@*MM*-**1** have been measured to be K = 117 M^{-1} and 45 M^{-1}, respectively at 278 K. This leads to an enantioelective ratio (R)-(-)-**3**@*MM*-**1**/(S)-(+)-**3**@*MM*-**1** = 2.6, which is similar to that measured previously from the ^1H NMR spectrum of the (rac)-**3**; (R)-(-)-**3**@*MM*-**1**/(S)-(+)-**3**@*MM*-**1** ~ 3.0. This ratio is similar to that calculated with methyloxirane. However, in the case of compound **3**, the binding constants are significantly smaller than those calculated with the two enantiomers of the methyloxirane derivative. This difference of affinity between these two guests can be explained by the large difference in their molecular volume. Indeed, methyloxirane (V_{vdw} = 57.0 $Å^3$) has a smaller volume than the eipchlorohydrin derivative (V_{vdw} = 72.1 $Å^3$) and it shows a better size matching with the cavity of host **1** (V_{vdw} ~ 95 $Å^3$). The replacement of the $LiOD/D_2O$ solution by $NaOD/D_2O$ (0.1 M) gives similar results. However, in this solvent slightly higher binding constants K = 170 M^{-1} and K = 70 M^{-1} have been calculated for the two (R)-(-)-**3**@*MM*-**1** and (S)-(+)-**3**@*MM*-**1** complexes, respectively. A similar result was also observed in the case of methyloxirane. In KOD/D_2O (0.1 M), the

association constants for these two complexes decrease significantly but some enantioselectivity is still observed in these conditions.

In NaOD/D_2O (0.1 M), the 1,2-butyloxirane-**4** derivative is also well recognized by host **1** (see figure 2). For instance, in presence of host *MM*-**1**, (rac)-**4** reveals several high-field shifted ^{1}H NMR signals between 0.0 and -2.5 ppm. The use of a single enantiomer of **4** leads to a simplification of the ^{1}H NMR spectrum. For instance, the ^{1}H NMR spectrum of the (R)-(+)**4**@*MM*-**1** complex is reduced to five well-resolved signals. In contrast, the other diastereomer complex (S)-(-)**4**@*MM*-**1** exhibits in the same region six ^{1}H NMR signals. The sum of these two spectra allows us to reproduce the ^{1}H NMR spectrum obtained for (rac)-**4**@*MM*-**1**. Interestingly, we have noticed that host **1** does not accommodate 2,3-butyloxirane, an isomer of compound **4**, whereas these two molecules have the similar molecular volume. The higher rigidity of 2,3-butyloxirane can explain the difference of behaviour between the two isomers and the difficulties for this compound to enter the cavity of host **1**.

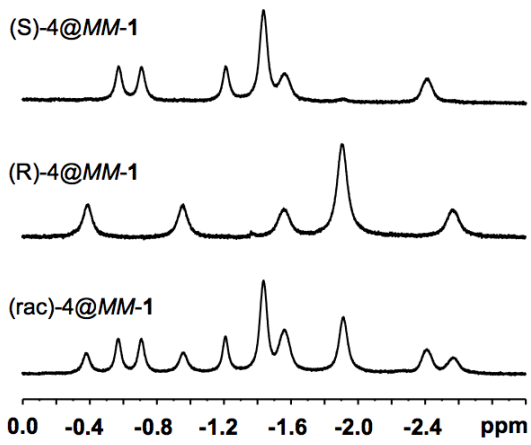


Figure 2: ^{1}H (500 MHz) NMR spectra of the (S)-**4**@*MM*-**1**, (R)-**4**@*MM*-**1**, (rac)-**4**@*MM*-**1** complexes recorded in NaOD/D_2O (0.1 M) at 275 K. Only the high-field region (0.0 to - 2.5 ppm) is shown.

A measure of the binding constants shows a weak enantioselectivity in favour of the (S)-(-)**4**@*MM*-**1** diastereomer (see Table 2 in Supp. Info. S4). Indeed, a weak binding constant K = 16 M^{-1} has been measured for this complex whereas the other diastereomer (R)-

(+)**4**@*MM*-**1** shows a lower association constant K = 9 M^{-1}. The difference of affinity between guests **3** and **4** is large is probably a consequence of the larger molecular volume of guest **4** (V_{vdw} = 74 $Å^3$) that does fit well with the cavity of the host.

Compound **5** represents the most interesting compound for these complexation studies. Indeed, 2,2'-bioxirane (**5**) (**CAUTION; very toxic**) possesses two stereogenic centers. Thus, three different stereomers can be obtained: the (2R,2'R)-(-)-2,2'-bisoxirane, the (2S,2'S)-(+)-2,2'-bisoxirane and the meso-derivative, which is achiral. In presence of the chiral host *PP*-**1**, three different diastereomers can thus be formed in solution. To our knowledge, this is the first time that a chiral guest with two stereogenic centers is used for complexation studies with an enantiopure cryptophane. This compound has been chosen because it possesses a van der Waals volume (V_{vdw} =70.6 $Å^3$), which is very close to that of guest **3**.[13] Thus, this compound is expected to enter the cavity of **1** under the same conditions. It is noteworthy that, even though the two enantiomers of **5** are commercially available, we have encountered a lot of difficulties to purchase these compounds. Thus, we decided to prepare the two enantiomers (2R, 2'R)-(-)-2,2'-bisoxirane and (2S, 2'S)-(+)-2,2'-bisoxirane from a known procedure.[14] The two enantiomers (ee ~ 100%) have been purified by distillation and obtained as crystals with low melting points (mp = 23°C). Buffeteau and co-workers have recently reported the chiroptical properties of the two enantiomers of compound **5**.[15] On the other hand, the meso stereoisomer has been purchased from a chemical company.

The ^{1}H NMR spectra (high-field region) of the three diastereomers recorded in NaOD/D_2O at 275 K are reported in figure 3. These spectra show large difference between them that depends on the stereochemistry of guest **5**. For instance, in this solvent the meso-stereomer shows six well-resolved ^{1}H NMR signals with the same intensity and the encapsulation of (meso)-**5** by host *PP*-**1** represents an interesting textbook case. Indeed, the (meso)-**5** derivative possesses a plane of symmetry and it is therefore achiral. Consequently, each pair

of protons (H_a, $H_{a'}$; H_b, $H_{b'}$ and H_c, $H_{c'}$) of this stereoisomer is enantiotopic and the ^{1}H NMR spectrum of the free guest is characterized by three sets of protons signals (spectrum not shown). However, a different situation occurs when this molecule is introduced into a chiral environment such as the cavity of *PP*-**1**. Thus, under these conditions, a splitting of each pair of proton takes place thus leading to six well-resolved ^{1}H NMR signals for the encapsulated (meso)-stereomer, as observed in figure 3. A change of the nature of the solvent gives rise to similar results and in LiOD/D_2O similar spectra are obtained. A different situation occurs with the two optically active (2R,2'R)-(-)-**5** and (2S,2'S)-(+)-**5** enantiomers. Indeed, each pair of protons (H_a, $H_{a'}$; H_b, $H_{b'}$ and H_c, $H_{c'}$) present on these two derivatives is homotopic. Thus, the two (2R,2'R)-(-)-**5**@*PP*-**1** and (2S,2'S)-(+)-**5**@*PP*-**1** diastereomers show a simplified ^{1}H NMR spectra with respect to the spectrum observed with the (meso)-**5**@*PP*-**1**. Indeed, when placed in a chiral environment, each pair of protons is still characterized by a single ^{1}H NMR signal. However, it can be easily seen that the two diastereomers (2R,2'R)-(-)-**5**@*PP*-**1** and (2S,2'S)-(+)-**5**@*PP*-**1** can be easily distinguished since they present large chemical shift differences.

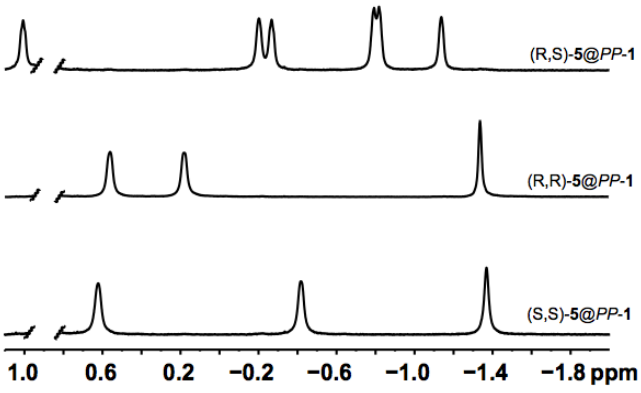


Figure 3: ^{1}H (400 MHz) NMR spectra of the (2R,2'R)-**5**@*PP*-**1**, (2S,2'S)-**5**@*PP*-**1** and (meso)-**5**@*PP*-**1** complexes recorded in NaOD/D_2O (0.1 M) at 275 K. Only the high-field region (1.0 to - 2.0 ppm) is shown. A signal (methyl group) corresponding to diethyl ether has been removed for clarity.

In LiOD/D_2O (0.1 M) at 275 K, the calculated binding constants shows a higher affinity (K = 70 M^{-1}) of the *PP*-**1** enantiomer for the (2R,2'R)-(-)-**5**. A similar value K = 80 M^{-1} has been calculated with the (2S,2'S)-(+)-**5**@*MM*-**1** complex (see Supp. Info. S6). In contrast, the the diastereomers (2S,2'S)-(+)-**5**@*PP*-**1** and (2R,2'R)-(-)-**5**@*MM*-**1** show lower association constants that have been estimated to be K = 20 M^{-1} and K = 30 M^{-1}, respectively. On another hand, the determination of the binding constants for (meso)-**5**@*PP*-**1** and (meso)-**5**@*MM*-**1**, gave similar K = 70 M^{-1} values. As previously observed with methyloxirane and guest **3**, slightly higher binding constants have been calculated in NaOD/D_2O (see Supp. Info. X).

All combined together, the ^1H NMR spectroscopy shows that guests **3**-**5** are well recognized by the enaniopure hosts *PP*-**1** and *MM*-**1** in LiOD/D_2O, NaOD/D_2O. In KOD/D_2O, the K^+ cation is a competitor and a decrease of the association constants is observed with guest **3** and **5**. For guests **3**-**5**, an enantioselectivity effect is measured in these solvents. The binding constants measured in all cases are moderate but the differences observed between the different guest molecules show that a clear correlation could be established between the association constants K and their molecular volume. For instance, for a given solvent, epichlorhydrin (**3**, V_{vdw} = 72.1 $Å^3$) shows higher binding constants than 1,2-butyloxirane (**4**, V_{vdw} = 74 $Å^3$). It is noteworthy that these values are significantly smaller than those previously calculated for the two enantiomers of methyloxirane (V_{vdw} = 57 $Å^3$). (ref 9) The two enantiomers of **5** (V_{vdw} = 70.6 $Å^3$) have a slightly smaller volume than guest **3** and show also lower affinity for host **1**. However, it can be noticed that guest **5** is made of two oxirane cycles and is therefore more rigid than guest **3** that possesses a single oxirane cycle. Consequently, guest **5** cannot change its conformation as easily as guest **3** to match with the cavity of the host **1**. A similar effect has also been observed with the two isomers of butyloxirane. Indeed, the 2,3-butyloxirane appears to be more rigid than 1,2-butyloxirane (**4)**

and it cannot enter the cavity of the host, even though the molecular volumes of these two molecules are expected to be identical.

ECD Spectroscopy of the oxirane complexes 3, 4 and 5: as previously demonstrated with the complexation of the two enantiomers of methyloxirane with host **1**, Electronic Circular Dichroism (ECD) spectroscopy is a valuable tool to characterize these supramolecular complexes in solution. Indeed, with this technique it is possible to visualize the spectral modifications of the host upon encapsulation. In the case of host **1**, the ECD signals originating from the solvent or the oxirane guest is not a problem since both species only absorb light below 220 nm. Thus, there is no overlap between these signals and the ECD signals of the host, at least for the two ^1L_a and ^1L_b transitions located between 240 and 300 nm. Previously, we have reported that at 293 K, the complexation of the two enantiomers of methyl-oxirane (**2**) with the chiral host **1** gives rise to specific ECD spectra in the ^1L_b region of the UV-visible spectrum. The differences observed between these ECD signals are small but they appear to be characteristic of each diastereomers and are not the consequence of artefacts. Interestingly, a comparison of these spectra with the ECD spectrum of the racemic mixture allowed us to confirm the higher affinity of the (R)-(+)-**2** enantiomer for *PP*-**1**. At 278 K the difference between the two spectra is even more pronounced (see Supp. Info. S7). Based on these interesting results, we decided to record at 278 K the ECD spectra of optically active guests **3**, **4** and **5** molecules with the two enantiomers of **1**.

The ECD spectra of *PP*-**1** and *MM*-**1** enantiomers recorded in NaOD (0.1 M) at 278 K in presence of (rac)-**3**, (R)-(-)-**3**, (S)-(+)-**3** guests are reported in figure 4. Another series of ECD spectra recorded at 278 K in LiOD (0.1 M) are also shown in Supp. Info. S8. As observed for the methyloxirane guest, the ECD spectra of guest **3** recorded in NaOH (0.1 M) show clear difference between the different diastereomers, at least for the ^1L_b region of the

UV-vis spectrum. For instance, between 270 and 340 nm, we observe that the (S)-**3**@*PP*-**1** diastereomer exhibits two ECD bands in this region: a weak ECD signal could be clearly detected with a maximum at 320 nm and a bigger ECD band is also present at 295 nm. As expected for enantiopure materials, the same ECD signals with the same intensities are obtained for the (R)-**3**@*MM*-**1** diastereomer. Interestingly, it can be notice that different spectra could be obtained with the two other (R)-(-)-**3**@*PP*-**1** and (S)-(+)-**3**@*MM*-**1** diastereomers. With these two complexes, the ECD spectra show a single broad ECD band with a shoulder at 310 ppm. A superposition of these spectra shows clear differences and these differences cannot be attributed to artefacts and are characteristic of these complexes. Interestingly, we noticed that the ECD of the rac-**3**@*PP*-**1** and rac-**3**@*MM*-**1** diastereomers, showed a lot of similarities with the ECD spectra recorded for the (S)-(+)-**3**@*PP*-**1** and (R)-(-)-**3**@*MM*-**1** diastereomers. Thus, it can be deduced that host *PP*-**1** shows a clear preference for the (S)-(+)-**3** enantiomer. These experiments demonstrate that enantioselective complexation of guest **3** can also be detected by ECD spectroscopy. These experiments nicely confirm our results previously obtained by ^{1}H NMR spectroscopy (see table 1). In LiOH/H_2O (0.1 M), similar ECD spectra have been obtained (see Supporting Information S8).

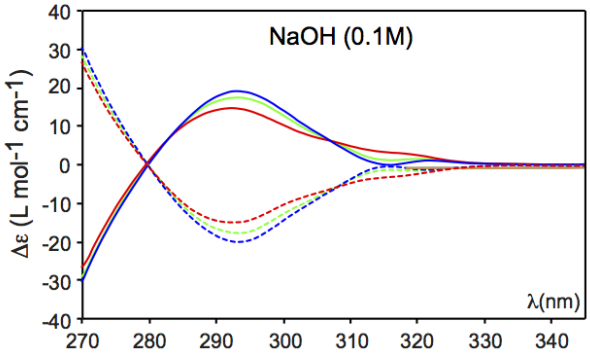


Figure 4: ECD spectra of (R)-(-)-**3**@*PP*-**1** (red curve); (S)-(+)-**3**@*PP*-**1** (blue curve); (rac)-**3**@*PP*-**1** (green curve) recorded in NaOH/H_2O (0.08 M) at 278 K. The ECD spectra of (S)-

(+)-**3**@*MM*-**1** (red dashed curve); (R)-(-)-**3**@*MM*-**1** (blue dashed curve); (rac)-**3**@*PP*-**1** (green dashed curve) are also shown.

Interestingly, the ECD spectra recorded at T = 293 K also show clear difference between the different diastareomers (spectra not shown). At this temperature ^{1}H NMR spectroscopy is not useful to extract useful information since these spectra only reveals broads signals with small intensities due to a faster in-out exchange dynamics (spectra not shown).

Interestingly, the ECD spectra recorded in KOH/H_2O (0.1 M) for the same complexes show complete different spectra at 293 K and 278 K (see supporting information S9). For instance, at 278K an ECD band is first observed at 310 nm ($\Delta\varepsilon$ ~ 28 L mol^{-1} cm^{-1}) and a second ECD band with a lower intensity is also visible at 290 nm ($\Delta\varepsilon$ ~ 10 L mol^{-1} cm^{-1}). Compared to the ECD spectra recorded in LiOH/H_2O and NaOH/H_2O, The difference observed in this solvent can be explained by the ability of host **1** to accommodate a K^+ cation within its cavity. The affinity of host **1** for this small cation forces the cage to modify the conformation of the three linkers in order to maximize interactions with the cation. Consequently, the ECD spectrum of **1** is strongly affected by this conformational change. It is noteworthy that, in contrast to what is observed at 293 K (see supporting information X), the two spectra recorded for the two pair of diastereomers are not exactly identical at 278 K and very small differences can be observed at this temperature. This result supports the idea that even in presence of this competitor the two enantiomers of **3** do not behave similarly with host **1**. The measures of small but different K values support this assumption.

The ECD spectra of (R)-(+) and (S)-(-)-1,2-butyloxirane (**4**) also show some differences in presence of host **1**. For instance, in NaOH (0.1 M), these ECD spectra are very similar to those previously observed for guest **3**. However, for guest 4 a comparison of these spectra

with those obtained for the (rac)-**4**@*PP*-**1** and the (rac)-**4**@*MM*-**1** did not allow us to detect with confidence an enantioselective effect (see Supp. Info. S10).

As previously mentioned compound **5** that contains two stereogenic centers is the most interesting guest molecule to be studied in this series. Indeed, in presence of host **1**, three different pairs of diastereomers can be obtained in solution. It is important to note that, in contrast to guest **2**, **3** and **4**, different experimental conditions have been used to record the ECD spectra with guest **5**. Indeed, in the case of **5** we are limited by the amount of enantiopure material and we do not have the possibility to use this material in large excess as this was achieved with guest **2**, **3** and **4**. The experimental procedure used for recording ECD spectra with guest **5** is described in detail in the experimental section. The ECD spectra recorded in $NaOH/H_2O$ (0.08 M) for the different diastereomers are shown in figure 5. The ECD spectra recorded in $LiOH/H_2O$ (0.08 M) are also reported in supporting information (Supp. Info S11). Thanks to the high enantiomeric purity of host *PP*-**1** and guests **5**, the ECD spectra of the different diastereomers reveal clear differences between them even though these differences are small. These spectra also differ from that of the ECD spectra obtained with the meso-**5** derivative that show an intermediate behaviour. The ECD spectra recorded with the other enantiomer *MM*-**1** also reveal perfect mirror images thus excluding the possibility that these spectral differences could be due to artefacts. It can be noticed that these three pair of diastereomers differ greatly from the guest free host *PP*-**1** and *MM*-**1** recorded in the same condition. Thus, this suggests that the encapsulation of the different stereomers of guest **5** have a strong impact on the conformation of the linkers of the host. In $LiOH/H_2O$ (0.08 M) identical spectra have been obtained even though in this solvent it is more difficult to distinguish the meso-**5**@*PP*-**1** (meso-**5**@*MM*-**1**) and the (2S,2'S)-(+)-**5**@*PP*-**1** ((2R,2'R)-(-)-**5**@*MM*-**1**) diastereomers.

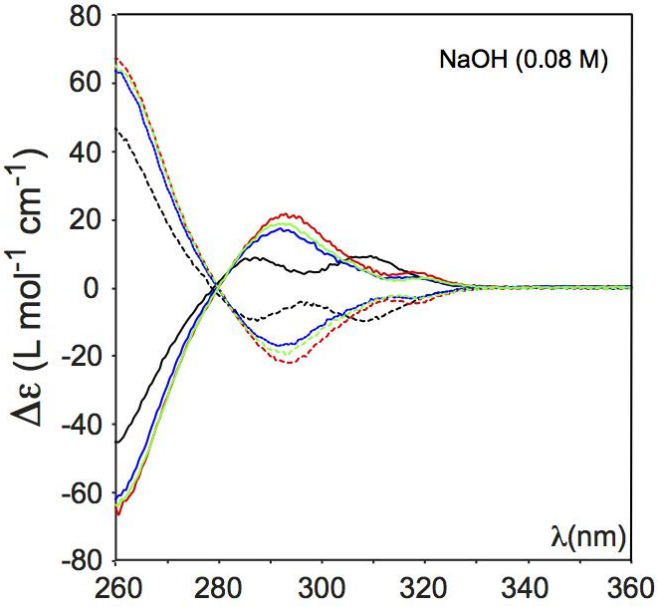


Figure 5: ECD spectra of (2R,2'R)-(-)-**5**@*PP*-**1** (red curve); (2S,2'S)-(+)-**5**@*PP*-**1** (blue curve); (meso)-**5**@*PP*-**1** (green curve) and guest free *PP*-**1** (black curves). ECD spectra of (2S,2'S)-(+)-**5**@*MM*-**1** (red dashed curve); (2R,2'R)-(-)-**5**@*MM*-**1** (blue dashed curve); (meso)-**5**@*MM*-**1** (green dashed curve) and guest free *MMP*-**1** (black curves). Spectra recorded in NaOH/H_2O (0.08 M) at 278 K.

All combined together, our results reveal that ECD spectroscopy is a valuable and extremely sensitive chiroptical tool to distinguish the different diastereomers present in solution. Interestingly, even when these experiments are performed at room temperature, ECD spectroscopy still reveals large differences between the different species. In contrast, at the same temperature, ^{1}H NMR spectroscopy does provide much informations due to a fast in-out exchange kinetics. Thus in this case only very broad signals are observed at this temperature.

In the case of the chiral oxirane **3**, **4** and **5**, we observe that ECD spectroscopy gives rise to specific ECD signals in the ^1L_b region of the UV-vis spectrum (270 - 300 nm). In aqueous solution, this spectral region is known to be very sensitive to the encapsulation process since it has a strong impact on the conformation of the three linkers. Herein, even tereomers that

show identical molecular volume but different spatial arrangements show different ECD spectra.

Conclusion: In this article we report a complexation study of small chiral oxirane derivatives (1,2-epichlorhydrin - **3**, 1,2-epxybutane - **4**, and bis-oxirane - **5**) with the water-soluble cryptophane **1**. The two enantiomers of compound **1** have been isolated by HPLC on chiral stationary phase and have been obtained with very high enantiomeric excess (ee ~ 100 %). ^{1}H NMR spectroscopy reveals that efficient complexation of guests **3**, **4** and **5** takes place in aqueous solution in LiOD/D_2O, NaOD/D_2O and KOD/D_2O. Compound **5** is a particularly interesting guest for this study since this molecule contains two stereogenic centers. Thus, three different pair of diastereomer can be obtained in solution in presence of the chiral host **1**. For this study, the two enantiomers (2R,2'R)-**5** and (2S,2'S)-**5** have been prepared according to a known procedure.

A slow in-out exchange dynamics is observed by ^{1}H NMR spectroscopy in all cases. In addition, a small enantioselective effect is observed for guests **3**, **4** and **5**. A correlation between the affinity of the host for these oxirane molecules and their molecular volume can be established. The flexibility of the guest seems also to play a role since 2,3-epoxybutane is not recognized whereas 1,2-epoxybutane (**4**) shows some affinity for the cavity of **1**. For this study, ECD spectroscopy appears to be a valuable tool for characterizing the different species formed in solution since specific ECD signals are obtained in the $^{1}L_b$ region of the spectrum. These results nicely confirm the previous data obtained with the chiral methyloxirane (**2**) in solution.

Experimental section:

Synthesis of optically active cryptophan-ol **1:** the two enantiomers *MM*-**1** and *PP*-**1** have been prepared by hydrolysis under basic conditions of the two enantiopure *PP*-**2** and *MM*-**2** derivatives, respectively. Compounds *MM*-**2** and *PP*-**2** have been separated by HPLC on a semi-preparative chiral chromatography and they have been obtained with an enantiomeric purity (ee > 99.5 %). Details on the separation of compounds *MM*-**2** and *PP*-**2** by HPLC are reported in the Supporting Information section. The synthesis of optically active cryptophanol-**1** has been previously reported from a multi-steps synthesis. The ^{1}H NMR spectra of *MM*-**1** and *PP*-**1** were found identical for these two approaches.

^{1}H NMR spectra of guest **3** and **4** have been recorded on a Varian 500 MHz at 278 K. ^{1}H NMR spectra of guest **5** have been recorded on a Bruker 400 MHz at 278 K. The measurement of the binding constants was achieved as follow: compound **1** was weighed in an NMR tube and a known amount of solvent (400 μL) was introduced in the tube. Then a very small amount of oxirane derivative was introduced in the NMR tube in order to have guest signals of the same intensity than the molecular host. By integration, the knowledge of the host concentration allows us to determine the concentration of both the free guest and the complex. A repetition of these experiments gives an uncertainty of 20% on the calculated binding constants.

For the two enantiomers of compounds **3** and **4** in presence of hosts *PP*-**1** and *MM*-**1**, the ECD spectra were recorded in aqueous solution (LiOH/H_2O, NaOH/H_2O and KOH/H_2O) at a concentration of 0.1 M. All the spectra were recorded at 278 K. A path length quartz cell of 1 cm (concentrations were in the range 5×10^{-5} - 1×10^{-4} M) has been used for these experiments. Spectra were recorded in the wavelength ranges of 270 – 400 nm with a 0.5 nm increment and a 1s integration time. Spectra were processed with standard spectrometer software, baseline corrected and sometimes slightly smoothed by using a third order least square polynomial fit.

For the two enantiomers of compound **5** the ECD spectra were recorded in $LiOHH_2O$ and $NaOH/H_2O$ at a concentration of 0.08 M according to the following procedure**: to be described.** A path length quartz cell of 1 cm (concentrations were in the range 5×10^{-5} - 1×10^{-4} M) has been used for these experiments. Spectra were recorded in the wavelength ranges of 270 – 400 nm with a 0.5 nm increment and a 1s integration time.

Acknowledgments: The authors are indebted to the CNRS (Chemistry Department) and to Région Aquitaine for financial support in VCD equipments. The GDR 3712 Chirafun is acknowledged for allowing a collaborative network between some partners of this project.

Keywords: Binding properties, Cryptophane, Enantioselective complexation, Electronic Circular Dichroism, Supramolecular Chemistry,

[1] T. Brotin, J.-P. Dutasta, *Chem. Rev.* **2009**, 88-130.

[2] Encapsulation of neutral species, see for instance: a) P. Satha, G. T. Illa, S. Hazra, C. S. Purohit, ChemistrySelect 2017, 2, 10699-10703. b) A. I. Joseph, S. H. Lapidus, M. C. Kane, K. T. Holman, *Angew. Chem. Int. Ed.* **2015**, *54*, 1471-1475. c) E. Dubost, N. Kotera, S. Garcia-Argote, Y ; Boulard, E. léonce, C. Boutin, P. Berthault, C. Dugave, B. Rousseau, Org. Lett. 2013, 15, 2866-2868. d) M.- J. Li, C.-H. Huang, C.-C. Lai, S.-H. Chiu. *Org. Lett.* **2012**, *14*, 6146-6149. e) M. A. Little, J. Donkin, J. fisher, M. A. Halcrow, J. Loder, M. J. Hardie, *Angew. Chem. Int. Ed.* **2012**, *51*, 764-766. f) C. givelet, J. Sun, D. Xu, T. J. Emge, A. Dhokte, R. Warmuth, *Chem. Commun.* **2011**, *47*, 4511-4513. g) A. Bouchet, T. Brotin, D. Cavagnat, T. Buffeteau, *Chem. -Eur. J.* **2010**, *16*, 4507-4518. h) K. T. Holman, S. D. Drake, J. W. Steed, G. W. Orr, J. L. Atwood, *Supramolecular Chemistry*, **2010**, *22*, 870-890. i) K. E. chaffee, H. A. Fogarty, T. Brotin, B. M. Goodson, J.-P. Dutasta, *J. Phys. Chem. A* **2009**, *113*,

13675-13684. j) G. Huber, T. Brotin, L. Dubois, H. Desvaux, J.-P. Dutasta, P. Berthault, *J. Am. Chem. Soc.* **2006**, *128*, 6239. k) K. Bartik, M. Luhmer, J.-P. Dutasta, A. Collet, J. Reisse, *J. Am. Chem. Soc.* **1998**, 120, 784-791. l) L. Garel, L, J.-P. Dutasta, A. Collet, *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1169-1171.

[3] Encapsulation of charged species, see for instance: a) F. Bregier, O. Hudecek, F. Chaux, M. J. Penouilh, J. C. Chambron, P. Lhotak, E. Aubert, E. Espinosa, *Eur. J. Org. chem.* **2017**, 3795-3811. b) T. Brotin, S. Goncalves, P. Berthault, D. Cavagnat, T. Buffeteau, *J. Phys. Chem. B* **2013**, *117*, 12593; c) T. Brotin, R. Montserret, A. Bouchet, D. Cavagnat, M. Linares, T. Buffeteau, *J. Org. Chem.* **2012**, *77*, 1198; d) R. M. Fairchild, K. T. Holman, *J. Am. Chem. Soc.* **2005**, *127*, 16364-16365. e) M. Miura, S. Yuzawa, M. Takeda, M. Takeda, Y. Habata, T. Tanase, S. Akabori, *Supramolacular chemistry*, **1996**, *8*, 53-66 ; f) S. Akabori, M. Miura, M. Takeda, S. Yuzawa, Y. Habata, T. Ishii, *Supramolacular chemistry*, **1996**, *7*, 187-196 ; g) L. Garel, J.-P. Dutasta, A. Collet, *J. Am. Chem. Soc.* **1993**, *115*, 11652-11653.

[4] See for instance : a) G. Milanole, B. Gao, A. Paoletti, G. Pieters, C. Dugave, E. Deutsch, S. Rivera, F. Law, J.-L. Perfettini, E. Mari, E. Léonce, C. Boutin, P. Berthault, H. Volland, F. Fenaille, T. Brotin, B. Rousseau, *Bioorg. Med.chem.* **2017**, *25*, 6653-6660; b) B. A. Riggle, Y. Wang, I. J. Dmochowski, *J. Am. Chem. Soc.* **2015**, *137*, 5542-5548; c) N. Kotera, E. Dubost, G. Milanole, E. Doris, E. Gravel, N. Arhel, T. Brotin, J.-P. Dutasta, J. Cochrane, E. Mari, C. Boutin, E. Léonce, P. Berthault B. Rousseau, *Chem. Commun.* **2015**, *51*, 11482-11484; d) N. Tassali, N. Kotera, C. Boutin, E. Léonce, Y. Boulard, B. Rousseau, E. Dubost, F. Taran, T. Brotin, J.-P. Dutasta, P. Berthault, *Anal. Chem.* **2014**, *86*, 1783-1788; e) K. K. Palaniappan, R. M. Ramirez, V. S. Bajaj, D. E. Wemmer, A. Pines, M. B. Francis, *Angew. Chem. Int. Ed.*, **2013**, *52*, 4849; f) T. K. Stevens, K. K. Palaniappan, R. M. Ramirez, M. B. Francis, D. E. Wemmer, A. Pines, A. *Magn. Reson. Med.* **2013**, *69*, 1245-1252; g) G. K. Seward, Y. Bai, N. S. Khan, I. J. Dmochowski, *Chem. Sci*. **2011**, *2*, 1103-1110; h) G. K. Seward, Y. Bai, N. S. Khan, I. J. Dmochowski, *Chem. Sci*. **2011**, *2*, 1103-1110; i) C. Hilty, T. Lowery, D. Wemmer, A. Pines, *Angew. Chem. Int. Ed.* **2006**, *45*, 70-73. j) L. Schröder, T. J. Lowery, C. Hilty, D. E. Wemmer, A. Pines, *Science*, **2006**, *314*, 446-449.

[5] J. Canceill, A. Collet, G. Gotarelli, P. palmieri, *J. Am. Chem. Soc.* **1987**, *109*, 6454-6464.

[6] T. Brotin, R. barbe, M. Darzac, J.-P. Dutasta, *Chem. Eur. J.* **2003**, *9*, 5784-5792.

[7] a) P. soulard, P. Asselin, A. Cuisset, J. R. A. Moreno, T. R. Huet, D. Petitprez, J. Demaison, T. B. Freedman, X. Cao, L. A. Nafie, J. Crassous, *PhysChemChemPhys*, **2006**, *8*, 79-92. b) J. Canceill, L. Lacombe, A. Collet, *J. Am. Chem. Soc.* **1985**, *107*, 6993-6996.

[8] A. Bouchet, T. Brotin, M. Linares, H. Ågren, D. Cavagnat, T. Buffeteau, *J. Org. Chem.* **2011**, *76*, 4178-4181.

[9] Herein, the sign of the polarimetric measurement R-(+) and R-(-) of methyloxirane refers to the specific optical rotation (SOR) of the neat compound. For this compound, it has been shown that the sign of the SOR values is solvent dependent. See for instance: Y. Kumata, J. Furukawa, T. Fueno, *Bull. Chem. Soc. Jpn.* **1970**, *43*, 3920-3921.

[10] A. Bouchet, T. Brotin, M. Linares, H. Ågren, D. Cavagnat, T. Buffeteau, *J. Org. Chem.* **2011**, *76*, 1372-1383

[11] M. darzac, T. Brotin, D. Bouchu, J.-P. Dutasta, *Chem. Commun.* **2002**, 48-49.

[12] T. Brotin, D. Cavagnat, P. Berthault, R. Montserret, T. Buffeteau, *J. Phys. Chem. B* **2012**, *116*, 10905

[13] Y. H. Zhao, M. H. Abraham, A. M. Zissimos, *J. Org. Chem*. **2003**, *68*, 7368-7373.

[14] M. A. Robbins, P. N. Devine, T. Oh. *Org. Synth*. **1999**, *76*, 101-109.

[15] N. Daugey, N. de Rycke, T. Brotin, T. Buffeteau, *Chirality*, **2018**, (ASAP)