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2-*O*-Substituted-3,6-per-anhydro-α-Cyclodextrin as Potential Biocompatible Agents for the Selective Complexation of Heavy Metal Ions with Special Attention to Lead ⋆

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Abstract. We report on the synthesis, characterization and ionic complexation properties of hexakis (2-*O*-acetyl-3,6-anhydro) cyclomaltohexaose and hexakis (2-*O*-methyl-3,6-anhydro) cyclomaltohexaose using thin-layer chromatography and Nuclear Magnetic Resonance spectroscopy. The selectivity towards cations depends on chemical modification of the hydroxyl groups and a very high specificity can be obtained in the case of lead for methylated derivatives.

Key words: (3,6-anhydro) cyclodextrin, hexakis (2-*O*-acetyl-3,6-anhydro) cyclomaltohexaose, hexakis (2-*O*-methyl-3,6-anhydro) cyclomaltohexaose, lead, ionic complexation, Nuclear Magnetic Resonance, thin-layer chromatography.

1. Introduction

Cyclodextrins (CDs) are known for their ability to form inclusion complexes with a large variety of organic compounds [1]. In order to increase and extend their binding properties, many chemical modifications have been performed on the CD ring. Most modifications only slightly affect the structure of the parent derivatives although they can induce very important structural modifications. Anhydration between the OH-3 and OH-6 hydroxyl groups of D-glucopyranose residues represent a typical case. It indeed transforms the $^4C_1$ chair conformation of the glucose unit into the $^1C_4$ form and leads to per-3,6-anhydro-CDs. This modification of the conformation results in a significant change in the properties of CDs since per-3,6-anhydro-CDs are unable to complex organic molecules but can bind cations due to their reversed (inside-out) structure [3].

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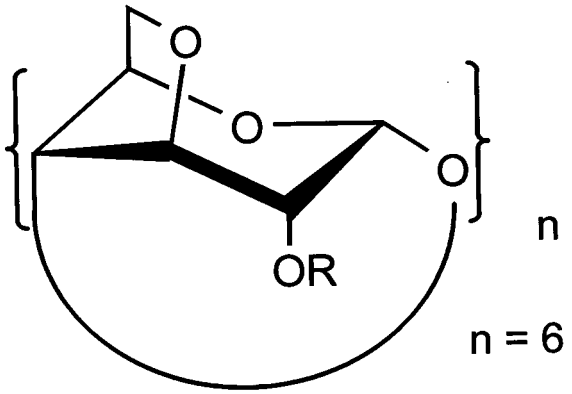

Figure 1. Structure of **1** (R = Ac) and **2** (R = Me).

It is well documented [3, 4, 7] that in the case of the per-3,6-anhydro-α-CD (6 anhydro glucose units) the selectivity towards cations is in the following order: $Pb^{2+} > Sr^{2+} > K^{+} > NH_4^{+} > Na^{+}$. As these results cannot be rationalized in terms of ion size and/or charge only, it was suspected that the hydroxyl function could play a key role in the observed selectivity.

In order to check this assumption, the chemical modification of the OH-2 hydroxyl groups of per-3,6-anhydro-CDs was also considered. This involves one [5], several or all [6] secondary hydroxyl groups of the 3,6-anhydro units and allows the design of custom-made systems.

Yamamura *et al*. [5] synthesized heptakis (mono-2-*O*-*p*-phenylazobenzoate-3,6-anhydro)-β-CD and attempted to demonstrate that this compound exhibits a better selectivity towards alkali ions compared to dibenzo-18-crown-6. The first example of a persubstituted 3,6-anhydro derivative was reported by Ashton *et al*. [6]. These authors prepared heptakis (2-*O*-methyl-3,6-anhydro) cyclomaltoheptaose from natural β-CD and not from the per-3,6-anhydro-β-CD but did not investigate the ionic complexation properties of this new derivative.

To date, attempts to prepare persubstituted derivatives and to investigate their properties in terms of ionic complexation were never achieved. For this reason, particular attention was paid to (3,6-anhydro) persubstituted compounds. As 3,6-anhydro-α-CD exhibits a selectivity towards heavy metals (lead being one of the best candidates) while the β and γ derivatives do not, it appeared more attractive to investigate the effects of chemical modification of 3,6-anhydro-α-CD only.

For this purpose, we prepared hexakis (2-*O*-acetyl-3,6-anhydro) cyclomaltohexaose **1** and hexakis (2-*O*-methyl-3,6-anhydro) cyclomaltohexaose **2**. In the first derivative, the acetyl group appeared to be convenient in terms of ionic complexation because of its enhanced complexing properties and potential selectivity.

The permethylated derivative was selected since the oxygen atom of the additional ether group exhibits a less acidic character compared to the parent derivative. This difference could be highly informative to explain the role of the hydroxyl

groups regarding the complexation of ionic species. We report here on the synthesis and the characterization of **1** and **2** to demonstrate the role of the chemical modification of 3,6-anhydro-α-CD on the ionic complexation properties using both thin-layer chromatography and Nuclear Magnetic Resonance (NMR) spectroscopy.

2. Experimental

2.1. MATERIALS AND METHODS

Thin-layer chromatography experiments were performed on Polygram Ionex 25-SA-Na plates. The commercial plates covered with cation exchange resin were supplied in the sodium form and were further exchanged for the relevant cation by soaking in a 5% salt solution [7]. After drying, the CD was applied and 4 immersions were performed with water followed by charring with 10% H_2SO_4. In the case of **2**, acetonitrile-water (50–50 v/v) was used as eluant.

Mass spectrometry experiments were performed on a SCIEX spectrometer using the electrospray infusion mode. Preparative HPLC was carried out with a Delta Pack apparatus (Waters Associates) equipped with an Evaporative Light Scattering Detector (ELSD).

^{1}H NMR experiments were performed using Bruker AC200 and DRX500 spectrometers operating at 200 MHz and 500 MHz, respectively. One-dimensional (1D) NMR spectra were collected using 16K data points. Chemical shifts were measured in ppm downfield from external tetramethylsilane (TMS). D_2O was obtained from Euriso-Top (France).

2.2. SYNTHESIS OF HEXAKIS (2-*O*-ACETYL-3,6-ANHYDRO) CYCLOMALTOHEXAOSE (**1**)

200 mg (0.23 mmole) of dry 3,6-per-anhydro-α-CD was stirred in pyridine (2 mL) and acetic anhydride (2 mL) at 70 °C for 10 hours. The reaction was followed by NMR spectroscopy. After completion, the solvent was removed under reduced pressure and the crude product was dissolved in water, filtered and taken to dryness. Preparative HPLC using methanol-water (50 : 50, v : v) as eluant on a μBondapak C_{18} column afforded chromatographically pure **1** (206 mg, 80%). m/z: 1139.3 [M + Na]$^+$; ^{1}H NMR (500 MHz, 298K, D_2O): δ_H: 5.45 (H-1, d, $J_{1,2}$ 2.9), 4.97 (H-2, t, $J_{2,3}$ 4.7), 4.73 (H-5, t, $J_{5,4}$ 2.4, $J_{5,6}$ 0.0), 4.58 (H-3, t, $J_{3,4}$ 5.0), 4.34 (H-6, d, $J_{6,6''}$ −10.8), 4.26 (H-4, dd), 4.08 (H-6′, dd, $J_{6',5}$ 2.7), 2.25 (CH_3, s). Solubilities at 20 °C: Water: 38 mmol L^{-1}; chloroform: 51 mmol L^{-1}.

2.3. SYNTHESIS OF HEXAKIS 2-*O* METHYL (3,6 ANHYDRO) CYCLOMALTOHEXAOSE (**1**)

Dry 3,6-per-anhydro-α-CD (50 mg, 0.057 mmole) and NaH (50 mg, 2 mmole) were stirred in dry DMF (10 mL) for 35 minutes under dry nitrogen. Methyl iodide

(200 μl, 2.2 mmole) was added slowly and the mixture was further stirred for 15 minutes. The solvent was removed under reduced pressure. The crude product was lyophilized twice. Conventional chromatography on a silica column [6] or preparative HPLC using gradient elution from water to aqueous 30% CH_3CN on a Y.M.C. PBMN polyamine column afforded chromatographically pure **2** (45 mg, 83%). m/z: 971.1 $[M+Na]^+$; ^{1}H NMR (500 MHz, 298K, D_2O): δ_H: 5.33 (H-1, d, $J_{1,2}$ 3.3), 4.63 (H-3, t, $J_{3,4}$ 5.1, $J_{2,3}$ 4.8), 4.65 (H-5, t, $J_{5,4}$ 2.5, $J_{5,6}$ 0.0), 4.33 (H-6, d, $J_{6,6'}$ −11.0), 4.28 (H-4, dd), 4.08 (H-6′, dd, $J_{6',5}$ 2.8), 3.74 (H-2, t), 3.56 (CH_3, s). Solubilities at 20 °C: Water: 100 mmol L^{-1}; chloroform: 40 mmol L^{-1}.

3. Results and Discussion

3.1. SYNTHESIS AND CHARACTERIZATION OF **1** AND **2**

Among the classical reactions of carbohydrates, acetylation leads to protection of hydroxyl groups. With natural CDs this reaction is carried out in pyridine and acetic anhydride mixture (50 : 50, v : v) at 70 °C for 4–6 h [8]. In the case of 3,6-anhydro-α-CD similar conditions are efficient but a 10-h reaction time is required for a complete conversion.

The permethylation reaction of 3,6-anhydro-α-CD requires the same conditions as the permethylation of natural CDs [9]. Therefore **2** was prepared by reaction of the corresponding alkoxide with methyl iodide in anhydrous DMF. **2** was purified either by preparative HPLC or by conventional chromatography on a silica column. After purification the chemical structures of **1** and **2** were assessed by mass spectrometry and NMR. 1D NMR experiments were performed on 3 mM solutions of **1** and **2** and the complete assignment of both derivatives was achieved using 2D NMR experiments (COSY, DQS ...) as described elsewhere [10]. Figure 2 displays the comparison of the ^{1}H NMR spectra of 3,6-anhydro-α-CD, **1** and **2**, respectively.

The ^{1}H NMR spectra of **1** (Figure 2(b)) and **2** (Figure 2(c)) show that H-1, H-2 and H-4 experience the largest modifications of chemical shifts compared to the parent derivative (Figure 2(a)) upon chemical modification of the hydroxyl groups.

In comparison with the hydroxyl groups, the acetyl and methyl groups induce larger steric effects which may lead to structural distorsions and therefore to variations of the chemical shifts of the signals of the H-1 and H-4 protons. Concerning H-2, it was expected that the acetyl and methyl groups located on C-2 would induce an important shift of this proton. Conversely, H-6 and H'-6 are weakly modified owing to their positions on the rigid ether bridge.

3.2. CHROMATOGRAPHIC INVESTIGATIONS OF THE COMPLEXATION PROPERTIES OF PERSUBSTITUTED DERIVATIVES **1** AND **2**

In order to evidence the scavenging capacities of **1** and **2** towards cations a preliminary study was performed using thin-layer chromatography on ion-loaded plates.

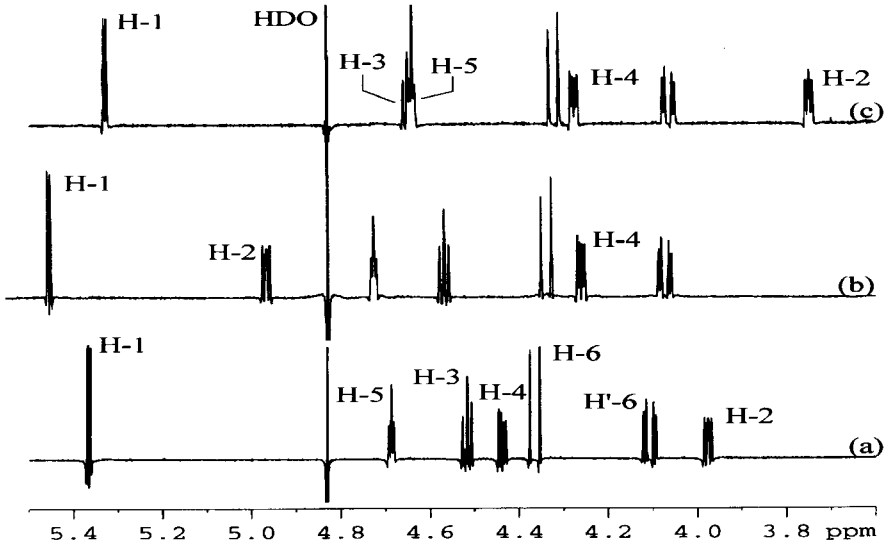

Figure 2. Partial ^{1}H NMR spectra (500 MHz, 298 K, D_2O) of 3,6-anhydro-α-CD (a), **1** (b) and **2** (c).

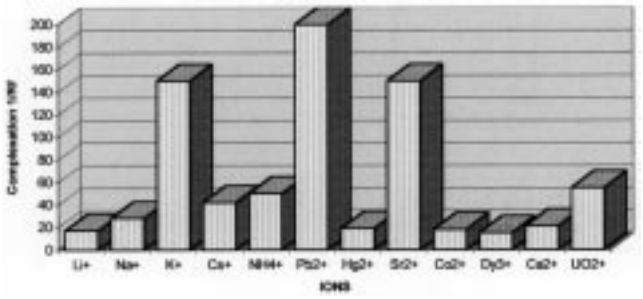

Figure 3. Ionic complexation indexes ($1/R_f$) of 3,6-anhydro-α-CD.

This method requires the usual chromatographic conditions and allows one to obtain the R_f values after migration with the pertinent CD in the eluting medium. By this qualitative approach, it is possible to estimate the strength of the complexes which is represented by $1/R_f$. Strong complexes are retained on the plate and yield high $1/R_f$ values.

Figures 3–5 display the complexation values ($1/R_f$ values) determined for 3,6-anhydro-α-CD, **1** and **2**, respectively. The histogram of Figure 3 established for 3,6-anhydro-α-CD shows that the strength of complexes is in the order $Pb^{2+} > Sr^{2+} \sim K^+ > NH_4^+ > Na^+$.

The results displayed in Figure 4 show that the affinity of **1** for Pb^{2+} and Sr^{2+} drops dramatically after acetylation while K^+ is strongly scavenged by the acetylated derivative. On the other hand, the affinities for ammonium and sodium cations remain unaffected by the chemical modification.

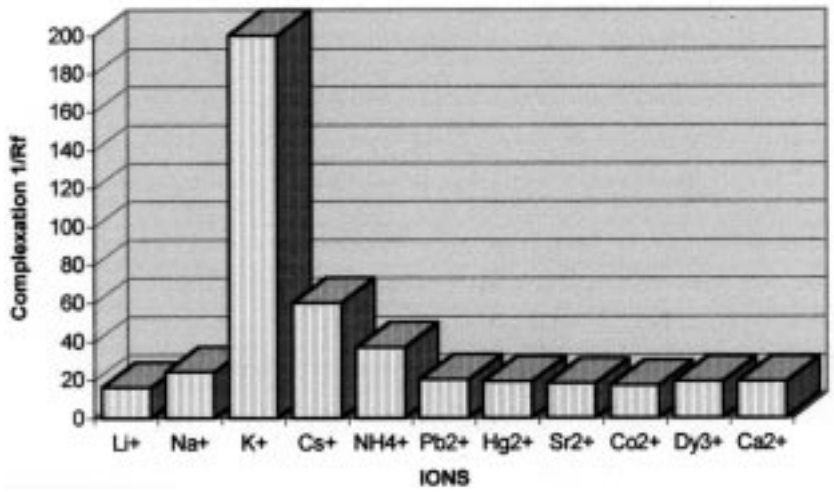

Figure 4. Ionic complexation indexes $(1/R_f)$ of **1**.

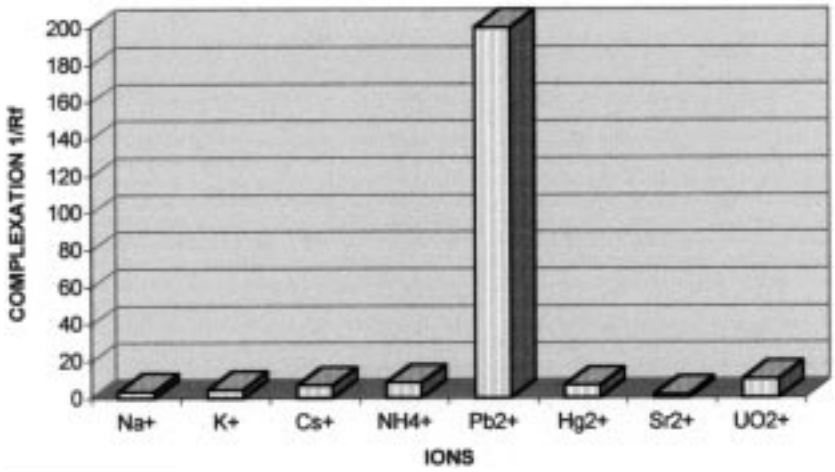

Figure 5. Ionic complexation indexes $(1/R_f)$ of **2**.

The histogram of Figure 5 shows that **2** exhibits a very high specificity for Pb^{2+} in comparison with other cations which are scavenged very weakly or not at all. From these results it appears that the chemical modification of 3,6-anhydro-α-CD modifies the specificity and particularly the capture of lead ions. Esterification inhibits the scavenging of lead while methylation strongly enhances the binding properties with a unique specificity towards lead. These chromatographic investigations have to be supported by NMR to derive more detailed information at the molecular level.

3.3. NMR INVESTIGATIONS OF THE COMPLEXATION PROPERTIES OF 3,6-ANHYDRO-α-CD, **1** AND **2**

The complexation modifies the electronic environments of the nuclei and can therefore induce shifts of their resonance frequencies. For a given nucleus, two limiting cases can be readily distinguished.

If the exchange lifetime between the free and bound species is shorter than the NMR timescale, a fast exchange regime results whereas if the lifetime is longer than the NMR timescale, the system is in the slow exchange regime. In the latter case, two signals related to the chemical shifts of the free (δ_f) and bound (δ_b) species are observed. In the fast exchange regime, a single weighted out signal (δ) is observed. This implies that slow, fast and intermediate regimes can be found in one example according to the frequency differences between the sites for a given signal.

3.3.1. *Observation of complexation in fast exchange regimes (cases of 3,6-anhydro-α-CD and* **1***)*

This situation is encountered here for the 3,6-anhydro-α-CD as exemplified by **1**. In the present cases, mixing of the CD and cations will (if complexation occurs) induce modifications of the NMR spectra of the CD.

The association constants of 1 : 1 complexes of 3,6-anhydro-α-CD were calculated by Fauvelle *et al*. [7] and confirm the following order in terms of selectivity: $Pb^{2+} > Sr^{2+} > K^+ > NH_4^+$. These values fully support the chromatographic data. ^{1}H spectra (Figure 6) of a 3 mM solution of **1** in the presence of 10 equivalents of potassium, ammonium or sodium chloride showed that H-1 and H-4 experience the largest shifts upon addition of potassium while low shift variations for the same protons are observed upon addition of ammonium and no detectable shift variations are induced by sodium. These results show that **1** exhibits selectivities towards cations in the following order: $K^+ > NH_4^+ > Na^+$, fully supporting the chromatographic data.

As described earlier, the largest shift variations are observed for H-1 and H-4 and can be explained by ring distorsions induced by complexation of ions.

3.3.2. *Observation of complexation in the slow exchange regime (case of* **2***)*

The ^{1}H NMR spectra (Figure 7a–c) illustrating the complexation of **2** with lead provide a typical situation of a slow exchange regime. Separated NMR signals for the free and bound CD are identified showing that a two-site exchange situation is encountered (Figure 7(a)).

Furthermore, the strength of the complex obtained with **2** and lead is demonstrated owing to the modifications of the ^{1}H spectrum of a 3 mM solution of **2** after addition of 1 mM or 3 mM lead nitrate solution. Indeed, in the presence of 1 mM lead nitrate, the signals of **2** broaden (Figure 7(b)). The free and bound species are clearly observed for H-1. The respective integrals corresponding to free and

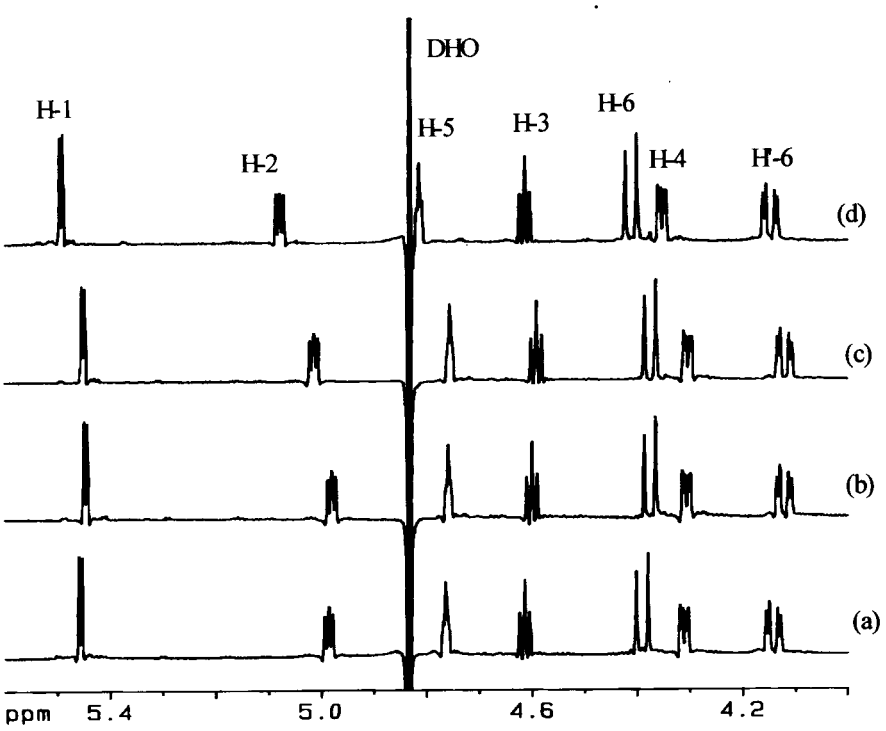

Figure 6. Partial ^{1}H NMR spectra (500 MHz, 298 K, D_2O) of **1** (1 mM) in the absence (a) and in the presence of 10 mM of Na^+ (b), NH_4^+ (c) and K^+ (d).

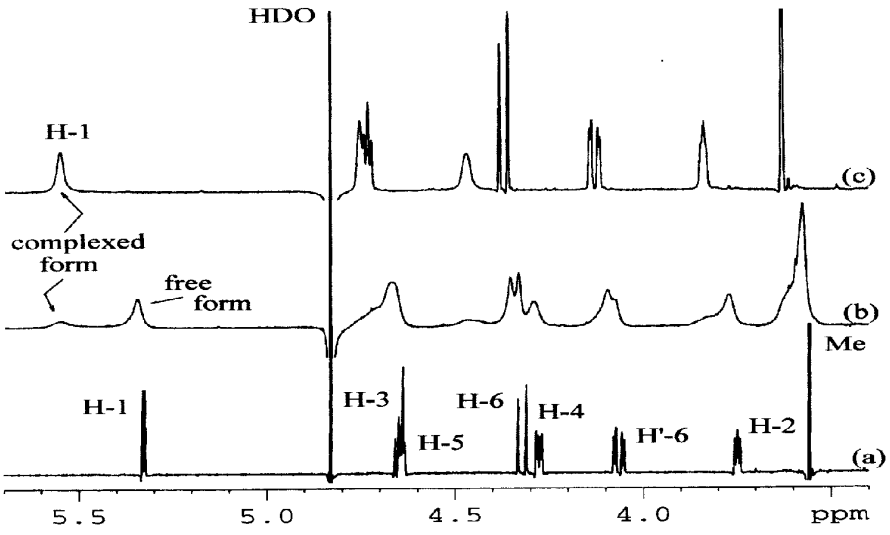

Figure 7. ^{1}H NMR spectra (500 MHz, 298 K, D_2O) of **2** (3 mM) in the absence (a) and in the presence of 1 mM (b) and 3 mM (c) of lead nitrate.

bound H-1 signals show that the lead is completely scavenged since the area corresponding to free CD corresponds to twice the area of the bound CD and that after addition of equimolar quantities of lead, the signal of the free CD has disappeared. Such a situation is encountered when the affinity constant is extremely high and therefore much higher than found for the parent compound (K_a = 2500 M^{-1}) [7]. On the other hand if one equivalent of sodium nitrate is added to the above solution the NMR signals remain unaffected. This observation confirms the lack of affinity of **2** for sodium as observed by chromatography. As far as biological applications are concerned, this is of primary importance since competition by ions such as sodium, potassium or calcium should be avoided to ensure the efficiency of the present compounds for the elimination of heavy metals in living systems.

4. Conclusion

The present data show that the chemical modification of per-3,6-anhydro-CD can orient or reverse the selectivity of these cyclodextrin derivatives towards cations with a special attention towards lead owing to its implication in human sanitary applications. A more detailed analysis of the structure of the lead-**2** complex can be expected by combining NMR data obtained from ^{13}C labelled complexes, crystallographic investigations and molecular modelling. These approaches and further chemical modifications are in progress and are expected to provide an *a priori* selection of the optimal host derivative for a given ion. Concerning **2**, the observed affinity and specificity for lead complexation as well as its solubility in aqueous medium make it a good candidate for the elimination of biological contamination by this metal. In this respect, the use of this biocompatible agent as a potential drug in the case of lead intoxication (saturnism) can be anticipated [11].

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