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ORIGINAL ARTICLE

Synthesis and inclusion properties study of some mono 6-amino β-cyclodextrin dimers bridged by N,N-succinyldiamide linkers

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Abstract Methylated and partially methylated cyclodextrin homo- and heterodimers linked by diamidosuccinic bridges were synthesised and their inclusion properties were evaluated using NMR and isothermic microcalorimetric measurements ITC. The selective binding of ligands, such as bisadamantyl derivatives, to the cavities of unprotected cyclodextrin dimers showed the equimolar formation of bidendate inclusion complexes (2:2, two ligand guest to two cavities host).

Keywords Cyclodextrins · Permethylated cyclodextrins · Selectively methylated cyclodextrins · Inclusion properties · Microcalorimetry ITC · Inclusion complex molarity · NMR study of inclusion complexes

Introduction

Because of their large hydrophobic cavities, cyclodextrins (**1**, CD) and β-cyclodextrin (**1,** n= 2) β-CD in particular, are often used to study inclusion phenomena [1]. These cavities allow the cyclodextrins to host various chemically different molecules. This guest-lodging capacity can be modified by altering the cavity size, or by selective substitution of the hydroxyls on two rims of the CDs (the primary and secondary hydroxyl sides). The CD can also form different supramolecular aggregates with substrates at different molar ratios. In this manner, it is possible to form complexes with various CDs in host–guest ratio of 1:2, 2:1 and 2:2 as well as to improve the selectivity of the complexation (e.g. studies with aromatic dyes [2, 3]) (Fig. 1).

The inclusion and complex molarity studies led to the evaluation of the storage capacity of these cavities, and are a further step in preliminary studies, particularly in the context of further practical applications [4] which include, in particular, pharmacological and environmental uses (decontamination by hosting heavy metal ions) [5, 6]. They can serve as models for vectorisation, studying drug transports, absorption-loading and release of pharmaceuticals, temporary or permanent masking or maintaining drugs in solution, as well as their potential environmental (decontamination) or biological applications is concerned [2, 7, 8].

β-CD units can also be incorporated into bridged oligomers. These molecules are usually obtained from specific synthetic routes. Their β-CD units, when linked together by covalent bonds, adopt specific conformations which enable them to host larger molecules. The dimers can interact with these guests, and develop specific affinities toward them on both monodentate and polydentate bases. The creation of such templates is an interesting step toward the nanochemical assembling of different binding capacity sites, for local, controlled and specific complexations with large host molecules [9]. Finally, the design of specific receptors often uses the CD oligomers as models for different recognition site assemblies within a single molecular

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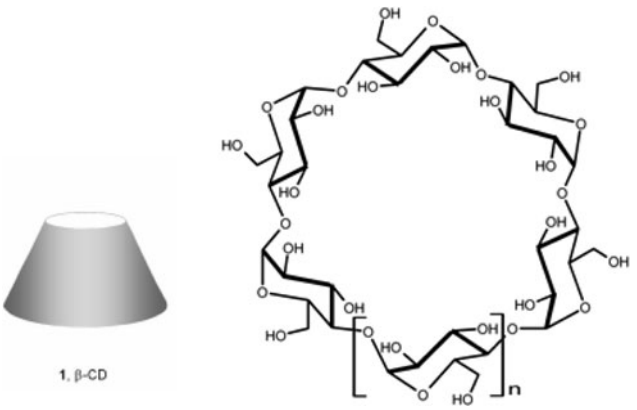


Fig. 1 : β-cylcodextrin (**1**) n = 2

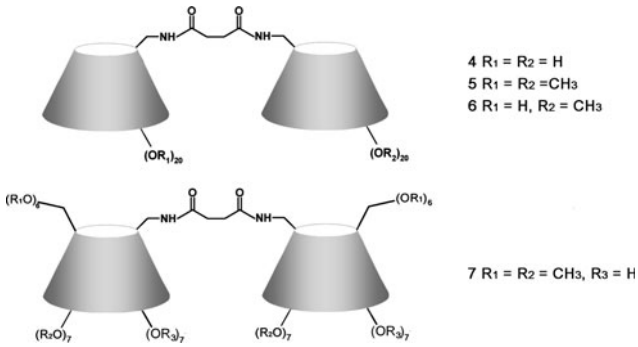


Fig. 2 Dimer structures

structure. The CD's role in these schemes, however, is usually associated with its hydrophobic interaction on the cavity level [10, 11].

In particular, CD dimers are often synthesised to obtain improved complexation and selectivity, because of the conformational preferences of such an organised assembly.

In the case of β-CD dimers, two β-CD units can each bind to one of the guest's complexation sites, or can instead incorporate it in a cooperative manner. The complexation could then take into account the different binding to the secondary or primary faces of the β-CD, for example. Polydentate guests can be retained by more than one cavity; as a result, the overall binding capacities of the β-CD dimers are greater than those of the monomer [12, 13].

Several molecules could serve as a bridge linker: the shorter derivatives of diacids (in particular, succinic acid) offer relatively simple chemistry for linking two β-CD via, for instance, a diamide bond between amino groups coming from two different β-CD substrates [14, 15] (Fig. 2).

Moreover, one can also consider the solubility of the ensemble created. For instance, the β-CDs, which have high water solubility, could be modified to be amphiphilic, by the alkylation of the hydroxyl groups of the "native" β-CD precursor. Alternatively, an amino group could be introduced via specific monoamination on the primary face of sugar, of 6-hydroxy via its monotosylate **2**, to mono 6-amino β-CDs (**3**), which would offer a direct way to use this bond as a substrate to these dimers by succinic acid linker via its double amidation [16]. It is also expected that such a system will show an improved selectivity toward some guest substrates. These types of molecules were reported by Easton [17, 18], Liu [19], and Reinhoudt [20], which were studied further for their supramolecular capacity by dyes inclusion [21– 24].[1]

In our study, we report on the synthesis of three new β-CD dimers, all linked by the *N*,*N*-disuccinylamido linker. First, three homodimers were built with, respectively, permethylated β-CD (**5**, β-CD met or β-CD trimet), selectively 2,6-dimethylated β-CD (**7**, β-CD dimet) and, as a reference for this series, the "native" β-CD dimer (**4**) already synthesised by Easton [17, 18]. Finally, one mixed heterodimer (**6**) was synthesised using one methylated β-CD (**5**) and one "native" β-CD (**1**). Some difficulties are reported with the synthesis of pure dimers when these have free hydroxyls (of compounds **4** and **6**).

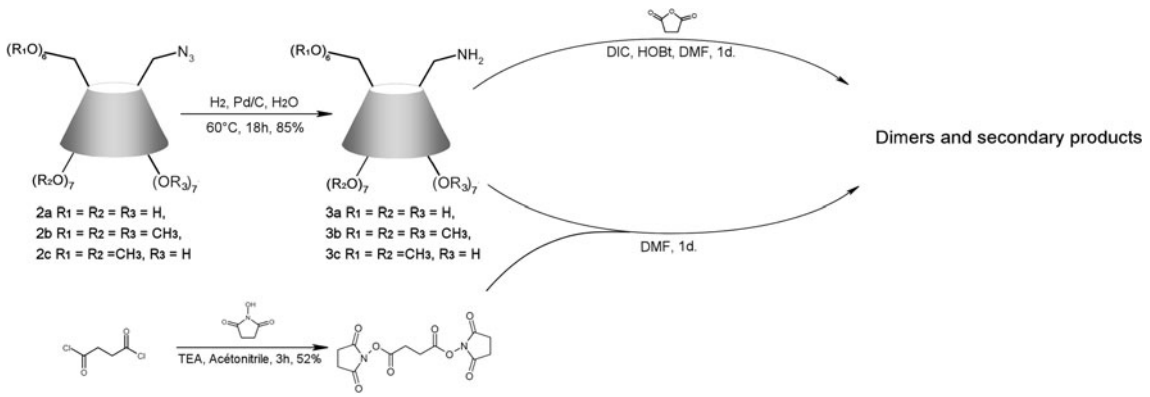
The synthesis and physicochemical properties of these four dimers are discussed in this study. In particular, NMR and different mass spectroscopies (e.g., MALDI) were used to identify the complex structures obtained and the presence of oligomers thought to be formed during the synthesis. Molecular modelling and microcalorimetry were used to study more specifically the inclusion properties of the modified and reference cavities, using mainly a bisadamantyl-EDTA disodium salt (**8**) derivative, as a guest compound [25, 26].[2]

In this way, we can evaluate the influence of the methylation level of the individual β-CD on the accessibility of

[1] The primary and secondary hydroxyl groups of CD are often subjected to etherification and esterification in order to improve their solubility in organic medium. The most popular ether derivatives of CDs remain their permethylated (**3b**) and (2-hydroxypropyl) analogs.

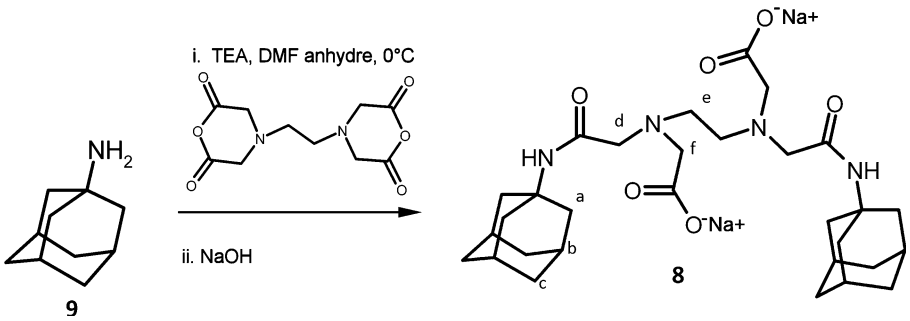
Footnote 1 continued
The commercially available mixture of the last compounds is a random mixture of different proportion of partially-hydroxyalkylated isomers which retain their interesting solubility and inclusion properties. Interestingly, such modified CDs can also be used in sensitive drug delivery; the lack of precise information on the degree and positions of alkylation turns out to be relatively unimportant, when compared to the beneficial role of the 2-hydroxypropyl CD derivatives, as well as to the low cost of such usually impossible-to-separate mixtures [21, 22].

[2] The similar study with a common dye, methyl orange (MO), was also performed but most of our seven MO suppliers failed to offer the product pure enough to perform the quantitative studies involving microcalorimetry. Under the mass spectroscopic studies the presence of inclusion complexes with this dye was however confirmed. After several verifications and numerous attempts of purifications using many different analytical techniques this ligand study was abandoned because of the impossibility of having the pure compound sample. It is amazing that this problem did not invalidate many previously published works in ITC



Scheme 1 Synthetic pathways for dimers **4–7**

Scheme 2 Synthesis of **8** from amine **9**



the dimer's cavities. The flexibility of the conformation adopted by these dimers, as tested with the bisadamantyl **8**, should define the molarity of the complex formed when this ligand is interacting with the host cavities. It is expected that the ligand binds to at least one of two β-CD cavities according to its conformations and the availability of access modified by methylation. The second binding of the ligand is accessibility-dependent, as well, but could also be of inclusion or capping nature only. Microcalorimetry seems to be much simpler and faster than NMR spectroscopy for such studies, because of the large number of protons involved and hidden within the cavities, e.g. H-5, H-3 overlapping chemical shift signals. These two techniques, which can be considered complementary, were used in our study where we attempted, using NMR, to deduce the relative position of the guest in the CD by observing the chemical shift of protons involved in complexation [27, 28].

Results and discussion

Synthesis

The synthesis of the specific β-CD dimers is quite difficult, especially for the so-called "native" dimer with its free hydroxyls (**4**). Despite the presence of many synthetic by-products already reported in our previous work [29, 30] as well as by Easton [17, 18], the yield and purity of dimers is quite high.

β-CD was initially monotosylated according to a modified Bittman procedure [31]. This allowed the incorporation of an azido group on the CD (**2 a,b,c**), which was reduced to a mono-amino compound (**3a,b,c**) as described in Scheme 1.

Then, two different pathways were followed to synthesise these dimers: both used the activated ester methodology,[3] but with reagents used in the reverse order. The first pathway used succinic anhydride (0.5 eq.) and amino-CD in DMF. The second pathway used an activated ester created from succinyl dichloride with N-hydroxysuccinimide, which was then reacted with mono-6-amino β-CD [31–34]. There was no important difference observed between these two pathways for either permethylated (**5**) or 2,6-dimethylated β-CD dimers (**7**) (Scheme 2).

However, for compounds **4** and **6**, these pathways displayed important differences according to the conditions used. These conditions were optimised by varying the

[3] Active ester methodology, details see Hocquelet, C.: Étude de dérivés de molécules cages, PhD thesis, Université d'Orleans, 2007. Some of the results reported in this paper were also presented as a poster at 13th International Cyclodextrin Symposium, May, 2006 (Abstract of papers) Hocquelet, C., Jankowski, C.K., Alves, S., Boutellier, L., Mauclaire, L. Effect of methylation on the inclusion capacity of β-cyclodextrin dimmers.

temperature of the reaction and the concentration of mono-6-amino β-CD (**3a**). This finally led to the production of dimers only, or to dimers mixed with trimers. The trimers, as observed by MALDI spectroscopy in particular, are formed at lower ratio of amine-activated esters. This indicates that trimers can only be formed if the intermolecular reaction is favoured. The mass determination of this trimer leads to the conclusion that it originated from the esterification of the free 6-hydroxyl group of mono-6-amino β-CD (**3a**). When concentrated media were chosen, the reactivity of the free primary hydroxyls increased to nearly maximum levels for a primary amine with an activated ester. As there were six primary free hydroxyls for one amine, a high rate of trimerisation was observed.

Both pathways yield only the dimer at very low concentration of **3**, but as the concentration of the amine increased, so did the concentration of the trimer. This phenomenon was also observed when the temperature was increased from 25 to 40 °C which was an indication of the intermolecular pathway of trimer formation. However, the exact structure of trimer obtained, MALDI apart, was not further elucidated mainly because of the difficulty of its separation.

The synthesis of CD homodimers **5** and **7** reached high yields of 74 and 97%, respectively, while two other dimers with free hydroxyls **4** and **6** were obtained with 74 and 77% yields, respectively, referred here to the azide reduction step only, and their purification by column chromatography gave very pure products as asserted by mass spectrometry. The intense protonated and cationised molecular ions were observed using ESI–MS. NMR high resolution spectra enabled unambiguous identification of all proton and carbon signals, such as the succinic linker methylenes, anomeric nuclei, etc. (Experimental part).

Methylation level, as estimated from integration of these signals, showed that the number of methyls associated to the dimers built with di- and trimethylated CD units corresponded to the expected values for these compounds. As far as the reciprocal orientation of two CD units in solvents used for recording NMR spectra in DMSO for all dimers, this corresponds to the *trans* (anti) conformation (without the through- space interactions between two CD units).

However, molecular modelling calculations show that the dimers have good propensity for the conformation where the two CD units are bound by H-bonds for native–native dimer **4**, and that this stabilising interaction is much weaker or absent for the homodimer built with dimethylated glucose units (dimet–dimet) **6** and similar but build with tri or permethylated glucose units (trimet–trimet) **5** dimers. The hetero dimer **7** showed by far a preference for the anti conformation.

In these gas phase calculations we considered three conformations, allowing the entire β-CD unit to rotate around the succinyl diamide bond of the CD-dimer, until two calculated faces of the CD are arranged in sec–sec, sec–prim, and prim–prim bonding interaction orientation of two β-CD residues especially pertinent for the free hydroxyl CD unit containing dimers.

Ligand complexation studies via Isothermal Microcalorimetry (ITC)

Further studies of these dimers were carried out to establish the molarity of ligand to host ratio as well as to observe the behaviour of two β-CD residues in the presence of guests having bonding capacity toward one or two β-CD residues.

In particular, because of the availability of the mixed dimer offering discriminatory access to the cavity according to the methylation of the one of two β-CDs, the entire series of β-CD dimers should be tested. These two studies should be done in order to compare and complete each of the observations from these studies**.**

The method used was isothermal microcalorimetry ITC, which was considered as a proven technique for such studies and enabled the determination of the association constant for any given complex formed between the β-CD host and the ligand. In particular, it was expected to quantify the molarity of the ligand–host complex, as well as indicate how many β-CD units participate in the complexation of said ligand, and in which roles (inclusion or capping). These results were consequently challenged by two more techniques—NMR and the molecular modelling evaluation of structural energies as described in following sections.

Microcalorimetry (ITC) was used in accordance to the methodology developed by Gibb [2, 35].[4] Using this method one can calculate from the titration curves the thermodynamic functions ΔH and ΔS, then calculate ΔG and deduce the association constant for the complexes formed.

Experimental curves of these titrations, done in both directions—dimer by ligand solutions or vica versa-ligand by dimer solutions, are compared (fitted) to the theoretical curves and the molarity of complex was evaluated from this last step (Table 1). In all cases the dimer studies were compared to the studies of the same ligand with the model β-CD (**1**).

Titration of CDs by **8**

Bisadamantyl EDTA tetracetate disodium salt (**8**) used in the experiments was of very high purity (see Experimental). Using the ITC microcalorimetry techniques, the following results were obtained for model β-CD (**1**), two

[4] See footnote 3.

Table 1 Thermodynamical data obtained via ITC with solutions of CD-compounds **1**, **4** and **7**.

	Host (mM)	Guest (mM)	$K_{ax}10^{-4}$ (M^{-1})	ΔH (kJ $mole^{-1}$)	ΔH	TΔS	ΔG	n ligand/receptor
1	0.852	4.002	3.9 ± 0.1	−52.0 ± 0.1	−26.0 ± 0.1	0.2 ± 0.1	−26.2 ± 0.03	0.509 (1:2)
4	0.402	4.002	32.9 ± 0.8	−53.1 ± 0.1	−26.5 ± 0.1	5.0 ± 0.1	−31.5 ± 0.1	0.995 (1:1)
7	1.635	16.067	0.3 ± 0.03	−41.7 ± 0.1	−20.8 ± 0.1	−0.9 ± 01	−19.9 ± 0.03	1.17

All thermodynalmical values of this table are expressed in kJ/mole

dimers **4** and **7** (Table 1) and the adamantyl ligand. Two selected dimers had different CD-cavity access and the aim of this experiment was to determine the molarity of association of the bidentate ligand in these dimers.

The results were interpreted as follows:

-with the model *β*-CD (**1**), the ligand formed a 2:1 complex, while for dimer **4** with the same ligand showed a 1:1 ratio, which corresponds to the complexation of one adamantly radical by one *β*-CD cavity.

-for the more hindered dimer **7**, the same ligand shows complexation constants 12 times weaker than for dimer **4** and experimental stoichiometry of 1.17. This could lead to the conclusion that there is much weaker interaction between the *β*-CD cavity and the adamantyl head in formation of the complex.

Looking for the most plausible explanation of these results, rephrase the higher degree of methylation of the cavity should be advanced as a possibility. It is also possible that distortion of the CD cavity takes place. In order to further study this distortion NMR and molecular modelling experiments were performed. When the opposite titration was performed (**8** by solution of **7**) the ratio of ligand to receptor was at 0.98.

NMR studies of the binding of bis-adamantyl **8** *by CD-dimers*

Having in hand binding results for dimers **7** and **4** with bis-adamantyl ligand **8**, as described in previous sections, two sets of NMR experiments were performed in order to confirm these findings (Figs. 3, 4, 5).

First, small but significant alterations of the chemical shifts of this series of protons witnessed the ligand–dimer binding for both compounds.

In the absence of a guest, all the lines for compound **4** appeared broad on the ^{1}H spectrum, certainly due to polymerisation. This lack of spectral resolution impeded a further structural assignment.

This was not the case for compound **7**. In the absence of a guest, the signals of the anomeric protons of compound **7** were spread over a range of 0.35 ppm and almost all separated (Fig. 3), this indicates that a part of the linker enters the CD cavities, as already observed [36]. After addition of bis-adamantyl **8**, these signals moved closer. They were

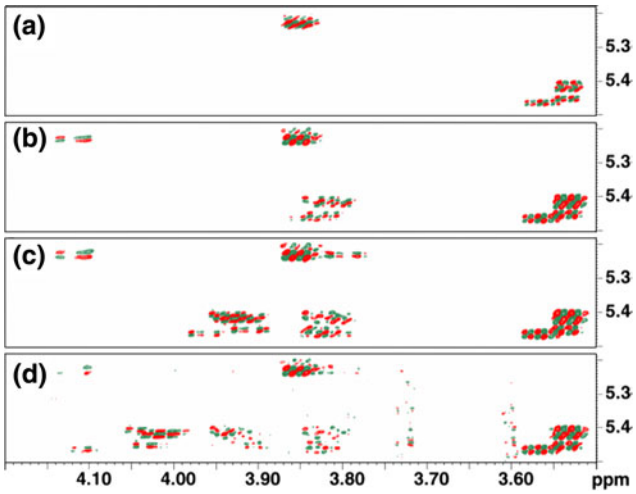


Fig. 3 Semi-soft experiments performed after selective excitation of the anomeric region of dimer **7**. **a** COSY showing the H1–H2 cross-peaks, **b** one-step RELAY adding the H1–H3 cross-peaks, **c** two-step RELAY adding the H1–H4 cross-peaks, **d** three-step RELAY where the H1–H5 cross-peaks appear

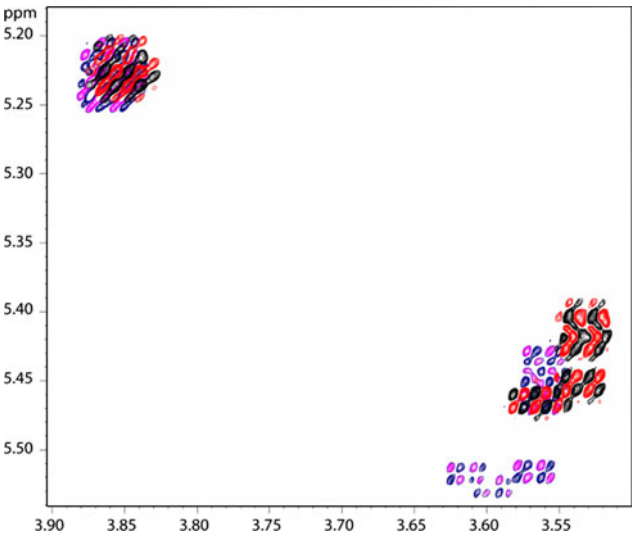


Fig. 4 Superimposition of two Semi-soft COSY experiments performed on dimer **7** in the absence (*magenta, blue*) and in the presence (*red, black*) of bis-adamantyl derivative **8**. The selective excitation covered the whole H-1 region and the phase-sensitive mode was used. Displayed region: H1 → H2 cross-peaks. Temperature: 300°K; Magnetic field 14 T

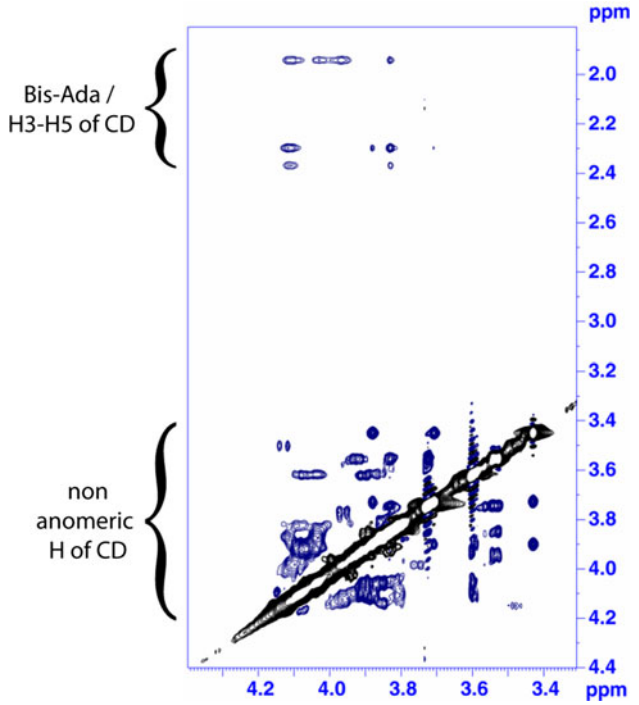


Fig. 5 Dimer **7** or **4** ROESY partial contour plot of an off-resonance ROESY experiment (mixing time of 200 ms) showing dipolar interactions involving the H-2 to H-6 protons of the cyclodextrin rim Bottom part: intra-CD interactions; top part: host–guest interactions

separated into two groups, with respective integrals (for instance measured on the H1-H2 cross peaks in the COSY map) had a ratio of 3:4. Therefore, these two groups could not correspond to the two CD rims of the dimer, but rather to respectively, 3 and 4 proton pairs from low field to high field. This seems to indicate that the two rims participate to the guest binding. The absence of significant chemical shift variation of some H-3 on binding (as observed on semi-soft one-step relay experiments (see Fig. 4) suggests that the $COO^{-}Na^{+}$ moieties of the adamantly ligand do not enter in the CD cavities.

Apart from the modification of these particular chemical shifts of CD moieties observed when complexed by the ligand in both dimeric structures with bis-adamantyl, the contact interactions were studied as well (semi-soft NOESY and 2D ROESY) to show the interactions between protons from the interior cavity of the sugar dimer and ligand.

The presence of host–guest through-space interactions, for example between protons from the interior cavity of the dimer sugar rings and guest ligand, were also investigated for both compounds. In particular, off-resonance ROESY experiments [37–41] were performed.

For dimer **4**, the ROESY map unambiguously revealed some dipolar interactions between the CD protons and the guest protons, but as no detailed spectral assignment had been possible on such a spectrum, it was impossible to check wether they concerned internal protons of the CD cavities. This experiment (2D ROESY) clearly showed the inclusion of the ligand and possible dipolar interactions involving the H-2 and H-6 protons of the cyclodextrin rims and the guest.

For the second complex bis-adamantyl bound to dimer **7**, cross-peaks between cyclodextrin H-3 and H-5 protons and guest protons on the 2D ROESY map clearly proved the inclusion of the ligand. However, due to reasons of symmetry of both the host and guest molecules it was impossible at this stage of the study to go further and to determine for instance the direction of the insertion into the cavity (for example, via the adamantly or the carboxylic moieties). Access of the ligand to the primary or secondary hydroxyl sides of the CD is also impossible to determine.

Similar NMR studies for two remaining dimers, **5** and **6**, although attempted using the same set of experiments, were however inconclusive, mainly due to the highly overlapping areas of their proton spectra (even at 500 MHz).

Conclusion

In this study, four dimers of cyclodextrin bound by the succinyldiamide linker were successfully synthesised and fully characterised with the help of mass and NMR polynucleic spectroscopies. Analysis of these high molecular weight compounds, having for example, different methylation levels as an additional analytical difficulty, confirmed the proposed structures as well as enabled identification of some secondary minor products from the succinic coupling reaction, such as, for example, the trimer.

The presence of the two CD cavities of these dimers was an interesting phenomenon the inclusion properties of these molecules. Using microcalorimetry, first the molarity of such complexes was established using β-CD (**1**) as a model compound. The inclusion of bisadamantyl derivative **8** corresponded to an equimolar 1:1 ratio for dimer **7** and for the more hindered slightly smaller, **4**, a 1:1.17 ratio, while β-CD (**1**) displayed a 2:1 ratio. This observation alone confirmed that access to one of the two CD cavities is more difficult for such a ligand and the discrimination of two methylated (to different levels) CD was observed.

These results were supported challenged by high resolution NMR study and further confirmed by molecular modelling. From the most interesting NMR results one can confirm that the bis-adamantyl ligand is entering the dimer cavity, however, it is impossible at this stage to determine (because of the symmetry of the ligand which

averages this interaction), the direction of insertion into the cavity (e.g., via the adamantly head or the carboxylic moieties). Access of the ligand from the primary or secondary hydroxyl sides of CD, although predicted by molecular modelling, was however impossible to demonstrate by NMR.

All observations resulting from the use of several different techniques confirmed binding of the ligand by the dimeric cavities of CD. One can conclude that access to the methylated cavity of CD was reduced but the selectivity of ligand binding was slightly improved.

In this access study, however, the organic solvent solubility was limited because of well documented limitations of this parameter for some CDs.

Experimental part

Materials and methods

Starting CDs were purchased from Roquettes Frères (France). Most of the reagents and solvents used in this study came from Sigma–Aldrich and used without further purification. TLC was performed on Silica Gel 60 F254 plates (E. Merck) followed by charring with 10% (v/v) H_2SO_4 or UV revelation. Routine NMR experiments used to monitor synthesis were performed using a Bruker DRX500 spectrometer operating at 500 and ca125 MHz for ^1H and ^{13}C, respectively. In all cases, samples were prepared in deuterium oxide or in d_6-DMSO (Euriso-Top, Saclay, France) or as indicated, and measurements were performed at 25 °C. Chemical shifts (δ) are given relative to external $(CH_3)_4Si$ (0 ppm) and calibration was performed using the signal from the residual protons of the solvent as a secondary reference. Coupling constants are expressed in Hz, multiplicity of proton resonance signals: s singlet, d doublet, t triplet, l large (broad), m multiplet. Selected 2D experiments were run on these compounds in order to unambiguously assign signals. The mass spectra were recorded on triple quadrupole Quattro II Micromass ESI–MS system as solutions of ca 0.1 mg mL^{-1} in methanol/water (1:1) introduced with Harward Apparatus syringe-pump. The working range of the instrument was from *m/z* 100–2000. For the details of ESI analytical conditions see refs [21, 29, 30]. MALDI-TOF spectra were recorded for superior than 1500 mass compounds only on MALDI-TOF Voyager DE, Applied Biosystem of Université de Lille using DHB matrix and usual protein calibration standards within 120–5700 mass range. The acceleration voltage was fixed at 20 kV and number of laser shots at 100. Some of these spectra were recorded using the Université de Paris VI facilities.

2D High resolution NMR experiments

These NMR experiments were run on a BRUKER Avance II 600 spectrometer equipped with a TCI cryoprobe.

The semi-soft experiments used an excitation sculpting scheme [36] made of a hard 90° pulse followed by a 180° soft pulse bracketed by identical pulsed field gradients. This soft pulse was constituted by a Q3 gaussian cascade [37] of 13 ms centred on the H1 region. Such an approach gave quasi-uniform excitation of the whole H1 region with in-phase signals, and has already proven to be very useful for the assignment of protons in CD dimers [38, 39].

The off-resonance ROESY experiment [40] used an rf field strength of 10 kHz shifted by 7700 Hz (effective angle = 54° 7). The adiabatic version of the sequence using a trapezoidal spin-lock pulse during the mixing time of a NOESY experiment was employed [41].

NMR sample preparation

1.4 mg of compound **4** (or **7**) was dissolved in 500 µL D_2O in a medium-wall 5 mm OD NMR tube. After the ^1H spectrum and the semi-soft 2D experiments, 0.89 mg of **8** was added to the solution. The final solution was perfectly limpid.

Microcalorimetry

TIC experiments were modelled on Gibb and Tato [35] procedures on VP-ITC isothermal titration apparatus at 25 °C in phosphate buffer (pH 7.2, 8.0 or as indicated). Concentration of CD hosts and eight guests were adjusted according to formation constants and cavity characteristics. Typical concentrations for these experiments were within a 0.5–1.0 and 10 mM range and prepared in stock water solution of equal volume of NaH_2PO_4 (0.1413 g L^{-1}) and Na2HPO4 (3.7455 g L^{-1}).

Titrations were carried out via 150 injections of 3 µL over a period of 6 min, of guest into the cell containing CD host and then were repeated in the opposite way of addition. Fit of theoretical values to the experimental ones, using enthalpy, formation constants and stoichiometric calorimetric titration thermograms obtained for all three CD's and compound **8** are available on request from the corresponding author (CKJ, Canada).

Synthesis

Glucose units of CD numbering (for NMR nuclei, carbons or protons, identification) follows the rule A, B, C….G (e.g., β-CD), where A is the first glucose, B the second etc. H-$6'^{B}$ means one of the number 6 protons of the second glucose (also named II in the alternative nomenclature).

Compounds **3a, 3b, 3c** were synthesised according to method described by Carofliglio [42] by reduction of compound **2a, 2b, 2c**, respectively, and purified using ionic exchange resin BioRad AG 50 W-X4 (50–100 mesh). The residue was acidified at a pH of 4–5. The aqueous solution was put into the resin column, eluted with 900 mL of water and then with 400 mL of aqueous ammonia (10%). The pure compound appeared in ammonia fractions. These fractions were evaporated almost to dryness and lyophilized, yields were 80%, 85%, 82% for compounds **3a, 3b, 3c,** respectively.

Compound **3a**

Rf = 0.35 ($BuOH/MeOH/H_2O/NH_3$, 3:3:3:1); NMR H-1 (D_2O, 500.13 MHz) δ (ppm): 5.09–5.13 (7H, H-1); 3.86–4.01 (H-3/H-6^{B-G}/H-5/H-$6'^{B-G}$); 3.68 (dd large, H-4); 3.62 (tl, H-2); 3.51 (t, 1H); 3.19 (d, 1H, H-6^{A}); 2.95 (dd, 1H, H-$6'^{A}$).

ESI–MS +: *m/z* calculated for $[M + H]^+$ 1134.15, found: 1134.5

Compound **3b**

Rf = 0.7 ($CHCl_3$/MeOH, 8:2 (v/v)); NMR H-1 (D2O, 500.13 MHz) δ (ppm) 5.36 (d, 1H, H-1^{A}, $^3J_{1,2} = 3.6$ Hz); 5.30–5.35 (m, 6H, H-$1^{B-G)}$; 3.86–3.96 (m, H-$5^{B-G)}$; 3.87–3.92 (m, H-$6^{B-G)}$; 3.83 (H-5^{A}); 3.75–3.83 (m, H-4^{B-G}); 3.76 (H-3^{A}); 3.69–3.79 (m, H-3^{B-G}); 3.71 (H-4^{A}); 3.65–3.73 (m, H-$6'^{B-G}$); 3.64–3.66 (m, OCH_3-6); 3.55–3.57 (m, OCH_3-3); 3.43 (H-2^{A}); 3.42–3.43 (m, OCH_3-2); 3.36–3.44 (m, H-2^{B-G}); 3.05 (dd, 1H, H-6^{A}, $^3J_{5,6} = 5.5$ Hz, $^3J_{6,6'} = -14.2$ Hz); 2.96 (dd, 1H, H-6′, $^3J_{5,6'} = 3.0$ Hz, $^3J_{6',6} = 14.2$ Hz);

C-13 (D_2O, 125.7 MHz) δ (ppm) 97.1–97.8 (C-1^{A-G}); 80.9–81.6 (C-3^{A-G}); 80.2–80.6 (C-2^{A-G}); 76.7–78.6 (C-4^{A-G}); 71.0–71.4 (C-6^{B-G}); 70.7–71.7 (C-5^{A-G}); 59.8–60.4 (OCH_3-6); 58.3–59.0 (OCH_3-3 CD/OCH_3-2); 41.6 (C-6^{A});

ESI–MS +: *m/z* calculated for $[M + H]^+$ 1414.59, found: 1414.8

Compound **3c**

Rf = 0.4 ($CHCl_3$/MeOH, 9:1 (v/v)); NMR H-1 (D_2O, 500.13 MHz) δ (ppm) 5.24–5.30 (7H, H-$^{A-G}$); 3.92–3.98 (H-3^{A-G}); 3.80–3.90 (H-5^{B-G}); 3.72–3.80 (H-6^{B-G}, H-$6'^{B-G}$); 3.71 (H-5^{A}); 3.61 (OCH_3-6); 3.58–3.65 (H-4^{B-G}); 3.55 (H-4^{A}); 3.43 (OCH_3-2); 3.38–3.44 (H-2^{A-G}); 3.06 (dd, 1H, H-6^{A}); 2.92 (dd, 1H, H-$6'^{A}$);

C-13 (D_2O, 125.7 MHz) δ (ppm) 99.3–99.9 (C-1^{A-G}); 81.5–82.2 (C-4^{A-G}/C-2^{A-G}); 72.4–72.7 (C-3^{A-G}); 70.4–72.7 (C-5^{A-G}/C-6^{A-G}); 59.6-59.8 (OCH_3-6); 58.8–59.0 (OCH_3-2); 41.5 (C-6^{A});

ESI–MS +: *m/z* calculated for $[M + H]^+$ 1316.40, found: 1316.8

Synthesis of dimers **4** *and* **6**

General procedure:

500 mg (213 μmol) of the corresponding amine was dissolved in 10 mL DMF. 66 mg (212 μmol) of activated ester was added and stirred at room temperature overnight. The mixture was then evaporated. The residue dissolved in water was poured into 300 mL of acetone. After filtration the precipitate was dried 3 h at 80 °C.

Yields of dimers **4** and **6** were of 95 and 74%, respectively.

Compound **4**

The coupling of two units into dimer **4** was also performed using the Reinhoudt procedure [43–46] from N-hydroxysuccinylimidyl succinate prepared in our laboratory from succinic acid dichloride with 53% yield. The purity of this reagent was checked via proton NMR (DMSO, 500 MHz) δ, ppm: 3.07 (s, 4H, CH_2 succ. chain), 2.81 (s, 8H, CH_2 succinylimidyl ring methylenes).

N,N'-Bis(6-deoxy-β-cyclodextrin-6^{A}-yl)succinylamide (**4**)

Starting amine: ***3a****, yield: 95%*

NMR H-1 (DMSO,500 MHz) **δ**, ppm: 7.61 (t, 2H, NH); 5.66–5.81 (m, 28H,OH); 4.79–4.88 (m, 14H, H-1); 4.43–4.53 (m, 14H, OH); 3.51–3.75 (m, H-3/H-4/H-5/H-6^{B-G}/H-$6'^{A}$); 3.27–3.39 (m, H-2/H-4/H-$6'^{A}$); 2.27–2.42 (m, 4H, CH_2 succ.)

C–13 (DMSO, 125.7 MHz) **δ** ppm: 171.8 (C=O), 102.1–102.2 (C-1); 83.3 (C-4^{A}); 81.4–81.9 (C-4^{B-G}); 69.9–73.03 (C-2/C-3/C-5); 59.8–59.9 (C6^{B-G}); 38.7 (C-6^{A}); 30.6 (CH_2 succ.)

MS MALDI-TOF: *m/z* calculated for $[M + Na]^+$ 2373.09, found 2371.98.

Compound **6**

*N-mono(6^{A}-deoxy-β-cyclodextrin-6^{A}-yl)-N'mono[6^{A}-deoxy-2^{A},3^{A}-di-O-methyl-hexakis(2^{B-G},3^{B-G},6^{B-G}-tri-O-methyl)-β-cyclodextrin-6^{A}-yl]-succinamide (**6**)*

Starting amine: **3c** followed by addition of the second amine **3a** after 2 h, yield: 74%.

NMR H-1 (DMSO, 500 MHz) **δ**, ppm: 7.63 (m, NHC=O); 7.60 (m, NHC=O), 5.67–5.81 (OH), 5.20 (m, 1H, H-1 CD methylated), 5.11 (m, 1H, H-1 CD methylated; 5.01–5.06, 5.20 (m, 5H, H-1 CD methylated); 4.81–4.86 (m, 7H, H-1, CD native); 4.46 (m, OH); 3.20–3.76 (m, H-3/

H-4/H-5/H-6/OCH3); 3.03–3.12 (m, 7H, H-2 CD methylated); 2.35 (m, CH_2 succ.)

C-13 (125.7 MHz) **δ**, ppm: 171.67 (C=O), 101.77–102.10 (C-1 CD native); 96.86–97.77 (C-1, CD methylated); 79.09–81.68 (C-2/C-4 CD); 69.20–72.95 (CH CD); 57.68–60.64 (OCH_3/CH CD); 30.51 (CH_2 succ.)

MS-MALDI-TOF: *m/z* calculated for $[M + Na]^+$ 2652.62; found 2652.84.

Synthesis of dimers **5** *and* **7**

Corresponding amine **3** was dissolved in DMF and succinic anhydride was added. The reaction was stirred overnight. DIC and HOBt were added successively. After 1 day, the reaction mixture was evaporated to dryness. The residue was diluted in dichloromethane and extracted with water. The organic phase was purified by column chromatography with CH_2Cl_2/MeOH as eluant.

The yields of compounds **5** and **7** were respectively 77 and 74%.

Compound **5**

*N,N'-Bis[6^A-deoxy-2^A,3^A-di-O-methyl-hexakis (2^{B-G},3^{B-G},6^{B-G}-tri-O-methyl)-β-cyclodextrin-6^A-yl} succinamide (**5**)*

*Starting amine: **3b**, yield 77%* NMR H-1 (DMSO, 500 MHz), δ(ppm): 7.58 (t, 2H, NH, ^{3}JNH, 6, 4.3 Hz); 5.19 (d, 2H, H-1); 5.08 (d, 2H, H-1); 5.03–5.06 (m, 10H, 10x H-1); 3.62–3.78 (m, H-5/H-6); 3.41–3.54 (m, H-4/H-5/H-6/OCH_3); 3.34–3.41 (m, 42H, 14xOCH_3); 3.28–3.33 (m, 14H, H-3); 3.19–3.25 (m, 42H, OCH_3); 3.02–3.11 (m, 14H, 14xH-2); 2.22–2.41 (m, 4H, CH_2 succ.)

C-13 (DMSO, 125.7 MHz), δ ppm: 171.6 (C=O); 97.6–98.4 (C-1); 81.4–82.3 (C-3); 79.0–81.3 (C-2/C-3); 70.1–71.6 (C-6/C-5); 60.8–61.1 (OCH_3); 58.0–58.0 (OCH_3), 30.7 (CH_2 succ.)

MS MALDI-TOF: *m/z* calculated for $[M + Na]^+$ 2934.17; found 2934.30.

Compound **7**

*N,N'-Bis[6^A-deoxy-2^A-O-methyl-hexakis(2^{B-G},6^{B-G}-di-O-methyl)β-cyclodextrin-6^A-yl] succinamide (**7**)*

*Starting amine **3c**, yield:74%* NMR H-1 (DMSO, 500 MHz), **δ** (ppm): 7.60 (m, 2H,NH); 5.75 (d, 14H, OH-3); 5.11 (m, 2H, H-1'); 4.91–5.00 (m, H-1^{B-G}); 3.50–3.80 (m, H-3/H-5/H-3/H-4); 3.49 (s, OCH_3); 3.24 (s, OCH_3); 3.00–3.26 (m, H-2); 2.25–2.40 (m, 4H, CH_2 succ.)

C-13 (DMSO, 125.7 MHz), **δ** (ppm): 171.3 (C=O); 100.0–100.2 (C-1); 81.6–82.9 (C-4/C-2); 72.6–72.8 (C-3); 69.9–71.0 (C-5/C-6); 59.6–59.8 (OCH_3); 58.0–58.3 (OCH_3); 30.9 (CH_2 succ.)

MS MALDI-TOF; *m/z* calculated for $[M + Na]^+$ 2737.7924, found 2737.24.

*sym-Bis Adamantyldiamide(adamantyl EDTA or ethylenediamine tetra acetate disodium salt) (**8**)*

A more correct name for this compound could be disodium salt of sym-Bis-1-adamantanyldiamide EDTA (**8**) and was synthesised in our laboratory by slightly modifying the method of Tellini [44, 47], dried over magnesium sulphate for 18 h at 70 °C and recristallised from the methanol several times until the correct ESI control, where the spectrum indicated the complete removal of substrates of this synthesis. Yield: 65%.

NMR H-1 (DMSO, 500 MHz), δ, ppm: 7.41 (s, 2H, N**H**C=O); 3.35 (s, 4H, Hf); 3.10 (s, 4H, Hd); 2.68 (s, 4H, He); 2.00 (s, Hb); 1.02 (s, Ha) and 1.62 (s, 12H, Hc).

MS ESI: *m/z* calculated for $[M + Na]^+$ 581.40, found 581.71.

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