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ORIGINAL ARTICLE

Direct synthesis of novel amphiphilic cyclodextrin

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Abstract A novel amphiphilic cyclodextrin derivative was obtained by controlled esterification of lauric acid chloride on the primary face of the native β-cyclodextrin in a one step synthesis. The characterization of the substitution degree and isomer structure was performed by mass and NMR spectroscopies. A specific purification procedure by sublimation was developed in order to eliminate the excess of lauric acid molecules in the reaction product. The sublimation efficiency was assessed by differential scanning calorimetry (DSC) in quantifying the remaining fatty acid. In this way the duration of the sublimation process could be optimized.

Keywords Amphiphilic β-cyclodextrin · Synthesis · Characterization · DSC

Introduction

The use of drugs (newly-developed or older ones) for therapeutic applications implies the design of a galenic formulation, i.e., of tools for optimizing the expected pharmaceutical effect for a given pathology. The use of carriers belonging to different classes allows solving these problems:

- Nanoparticles of high molecular mass polymers or lipid assemblies such as lipid vesicles or liposomes. These are used as reservoirs for drug delivery.
- Cage molecules such as cyclodextrins and derivatives dedicated to serve as hosts for the drug at a molecular level.

In the final aim combining both, inclusion capacity of cyclodextrin's hydrophobic cavity and the transport properties of amphiphilic structures, amphiphilic cyclodextrins can be used to prepare new supramolecular assemblies such as beads, microparticles or nanoparticles. They can also be associated with preformed phospholipidic membranes of liposomes. The literature is very abundant about amphiphilic cyclodextrins but most of the described works induced the synthesis of persubstituted cyclodextrins referred to ''medusa-like'' [1], ''skirt-shaped'' [2] or ''bouquets'' molecules [3], leading to very poor solubility and absence of self-organization in aqueous media. Grafting one single moiety on the cyclodextrin core allows increasing its amphiphilic character while preserving lyotropic properties in water. Such derivatives can then be either inserted by their hydrophobic part into lipidic systems such as liposomes or self-organize into micelles leaving the cyclodextrin cavities outside and available for including active guests [4]. However some difficulties have been encountered with mono substituted CD's, namely, the expensive synthetic cost, the tedious synthesis and purification. Based on previous results, the goal of this present work was to obtain neither

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persubtituted nor monosubtituted amphiphilic cyclodextrins but partially substituted cyclodextrins grafted specifically onto the primary face with fatty acids with degrees of substitution not exceeding seven hydrocarbon chains per molecule. To envisage further applications in pharmaceutics or in cosmetics, industrial scaling should be considered so it appeared essential to produce the cyclodextrin derivatives in only one-step synthesis with good yield and high purity.

Here, the synthesis pathway of new amphiphilic cyclodextrin derivatives resulting from the grafting of lauric acid chloride onto the primary hydroxyl groups was reported as well as their chemical structure characterization. A specific purification step was carried out by sublimation in order to eliminate the excess of fatty acid remaining in the reaction product. Quantification of lauric acid by enthalpy measurements performed by DSC allowed to monitor the efficiency of the sublimation process.

Experimental part

β-cyclodextrin was purchased from Wacker and all other reagents from Acros organics. The solvents were of HPLC grade and all reactions were carried out in oven-dried glassware. Characterization and structure determination were achieved by NMR DMX 300 spectrometer operating at 300.13 MHz. ^{13}C-NMR spectra were recorded in pyridine-D_5, the probe temperature was regulated to 338 K. The molecular structure was further confirmed by mass spectrometry. Mass spectra were recorded in methanol on a quadrupole single Waters-Micromass spectrometer (ZQ) equipped with an electrospray ionization source (Z-spray). Purification of samples was achieved by sublimation experiments. The sublimation step was performed in a Büchi Glass Oven B-580 equipped with a system for sublimation. In all experiments, the temperature was maintained at 413 K with a pressure below 1 mbar.

For each batch, β-cyclodextrin (2.5 g) and the desired volume of lauric acid chloride (e.g., 5.2 mL for 10 eq.) were added to 50 mL pyridine or water. The mixture was stirred at room temperature or 0°C for different times (from 15 min up to 65 h, depending on the degree of substitution required), the reaction was quenched by addition of a HCl 2 N aqueous solution (pH = 2), then concentrated by evaporation and filtered.

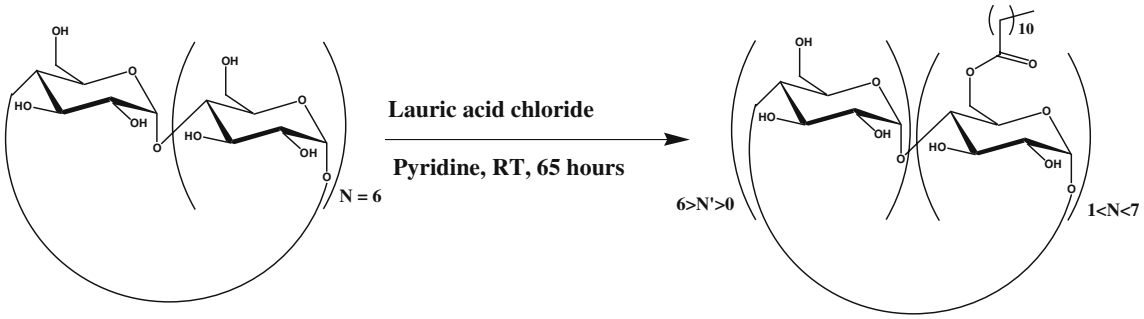
Calorimetry measurements were performed by using a DSC 7 (PERKIN ELMER) equipped with cooling device (Intracooler II) supported by Pyris Thermal Analysing Systems 3.52. Recordings were obtained by measuring the heat transfer either by cooling or heating the samples at a constant rate of 1°C/min in the 20–60°C temperature range. All samples, in the range of 1–6 mg, were introduced and sealed in 40 μL aluminium pans (PERKIN ELMER: pans B014–3021 and covers B014-3004) and stored at room temperature (≈20°C). An empty sealed pan was used as reference. For the DSC curves, the onset temperatures and peak areas were determined using standard analysing routines. Highly purified lauric acid (99.95% purity controlled by glass capillary gas–liquid chromatography of the methyl ester; impurity detected 0.05% myristic acid) was used as a standard for enthalpy determination. The enthalpy of fusion of this fatty acid is 8.53 Kcal/mole. This value was accurately determined with a Calvet calorimeter by the Centre de Recherche de Microcalorimétrie et de Thermochimie (CNRS, Marseille) [5].

Results and discussion

The self-assembly properties of the amphiphilic cyclodextrins seem to be governed by the three-dimensional structure of the substituted derivatives [6]. Based on this hypothesis, the regioselective control of the grafting hydrophobic moieties is a key-point to induce the dedicated self-organization properties in aqueous media. More specifically, for β-cyclodextrin, it should be essential that substitution is only located on the primary face of the cyclodextrin with an average number of grafting between two (to inhibit the self-inclusion process occurring with monosubstitution of fatty chain) and seven (to avoid the insolubility in water of persubtituted amphiphilic cyclodextrins).

The strategy of this synthesis was based on only one step, easy to apply, and allowing to obtain reproducible batches with a given average substitution rate per molecule that could be varied. The regioselective esterification of β-cyclodextrin was based on nucleophilic character of primary hydroxyl groups in a basic solvent such as pyridine (Sch. 1). The control of substitution of the primary face of the cyclodextrin was function of three experimental parameters such as amount and ratio of reagents, temperature and reaction time. The main results are reported by Table 1.

Depending on both reaction time and lauric acid chloride to cyclodextrin molar ratio, the grafting efficiency was varied from 0 to 7 chains onto the primary face. The reaction was optimised with 10 equivalents of lauric acid chloride for a reaction time of 65 h at room temperature. Product characterization were performed by mass spectroscopy that showed the presence of a mixture of grafted cyclodextrins with degree of substitution ranging from 1 to 7 chains per molecule



Scheme 1 Scheme of synthesis

Table 1 Degree of substitution of β-cyclodextrin primary hydroxyls by lauric acid chloride as a function of reaction conditions

Equivalents of lauric acid chloride	Temperature[a]	Time	Solvent	Number of grafted chains
1	RT	65 h	Pyridine	0–2
2	RT	65 h	Pyridine	0–3
3	253 K	15 min	Pyridine	0 and 1
3	273 K	2 h 30	Pyridine	0 and 1
3	RT	2 h 30	Pyridine	0 and 1
3	RT	65 h	Pyridine	0–4
6	253 K	65 h	Pyridine	0–5
6	RT	65 h	Pyridine	0–5
6	RT	65 h	Water	0 and 1
8	RT	65 h	Pyridine	0–6
10	RT	65 h	Pyridine	1–7

[a] RT: room temperature

(Fig. 1) and only on the primary face as attested by NMR experiments. The chemical shifts of carbons C2 and C3 are consistent with the presence of free hydroxyls and the other hand the downfield shift observed for carbons C6 are in agreement with chemical modification such as esterification. The absence of native β-cyclodextrin was noticed.

Nevertheless, the ^{13}C-NMR spectrum showed two carbonyl groups (ester and carboxylic acid) indicating the presence of remaining free lauric acid (Fig. 2). It is well known that purification of cyclodextrin derivatives and especially of amphiphilic ones is still a real challenge.

Indeed, usual procedures such as liquid–liquid extraction or centrifugation were not conclusive in our case. Regarding the physical behaviour of lauric acid, we envisaged to use the sublimation procedure.

To find suitable conditions, successive sublimation experiments during increasing time from 30 min to 6 h were undertaken from the same sample batch. Each sublimation run was carried out on 200 mg total product obtained with 10 equivalents lauric acid chloride. DSC measurements allowed to follow the sublimation efficiency by quantifying the lauric acid weight ratio remaining in the samples. In the temperature range explored, the cyclodextrin derivative did not show any thermal event (Fig. 3). The recorded thermograms indeed showed only one endothermic peak with onset temperature at 43.5 ± 1°C that corresponds to the fusion of free lauric acid chains [5]. The amount of lauric acid was determined from the surface areas of the endothermic peaks and that of a pure lauric acid sample used as standard. The slight shift observed between the onset temperatures of the standard (44.5 ± 0.5°C) and lauric acid in the samples can be explained by interactions between the hydrocarbon chains of the free acid molecules and those grafted onto the cyclodextrin through binary phase diagram approach [7].

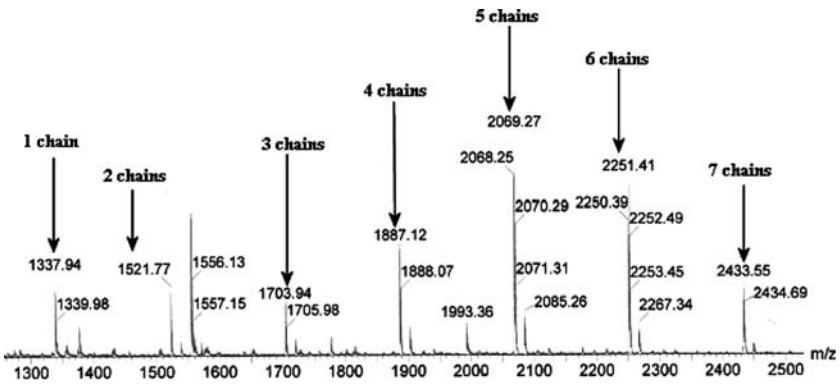


Fig. 1 Mass spectrum $[M + Na]^+$ of a sample batch obtained with 10 equivalents lauric acid chloride

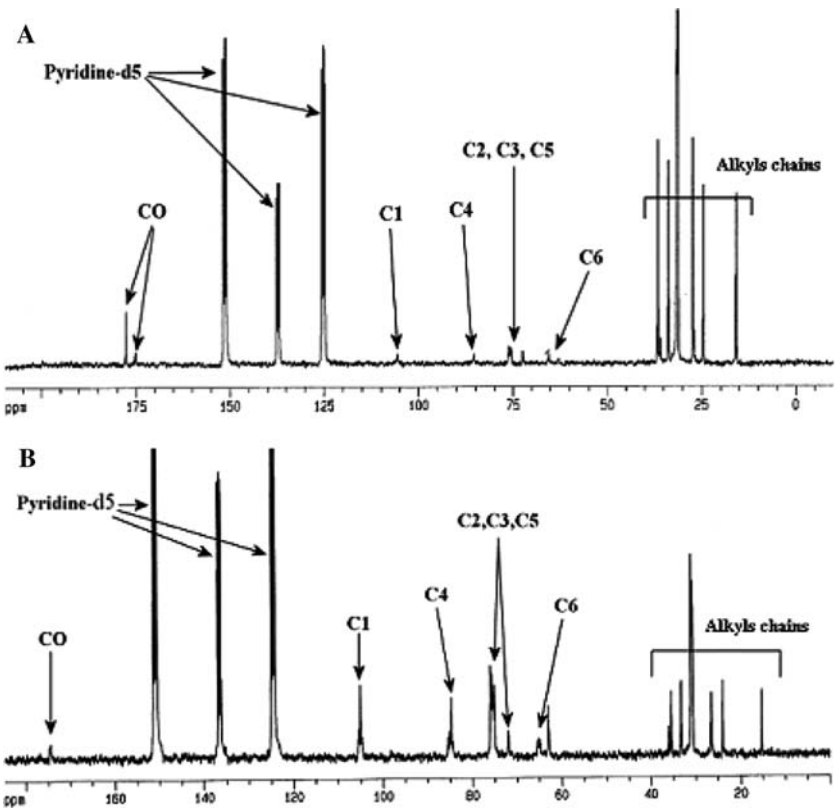


Fig. 2 ^{13}C NMR spectrum of a sample batch obtained with 10 equivalents lauric acid chloride in pyridine-D_5. (**a**) before sublimation, (**b**) after sublimation

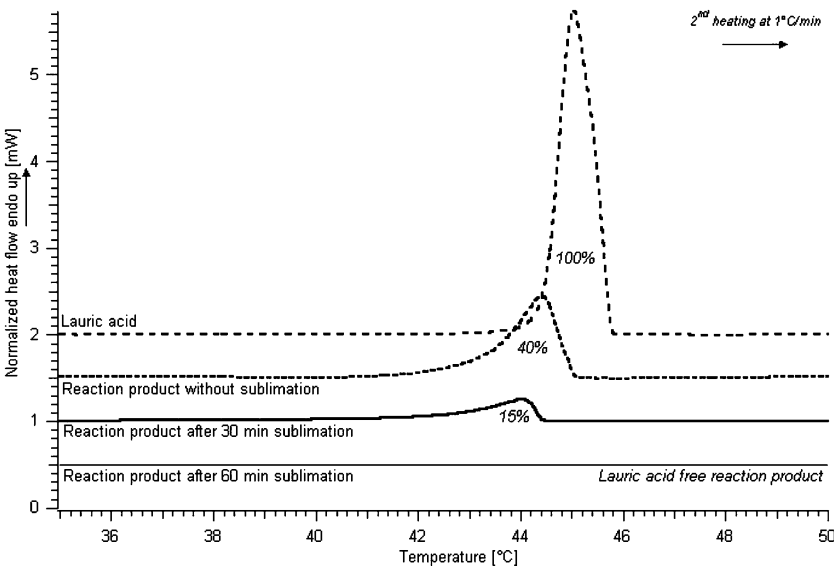


Fig. 3 Characteristic DSC heating-curves of 10 equivalent-reaction samples at different sublimation times and standard lauric acid. Recording were subsequent to a first heating from 20 to 60°C followed by a cooling from 60 to 20°C. Weight ratios (%) of lauric acid contained in the samples were calculated from the corresponding surface peak areas and that of the standard

Results demonstrated that one-hour sublimation led to complete removal of lauric acid from a 200 mg sample which initially contained 40 wt% of excess acid. This was confirmed by mass spectrometry.

^{13}C-NMR spectrum performed after sublimation also showed the disappearing of the carbonyl chemical shift corresponding to free lauric acid. The average substitution degree was then determined on the purified samples by using a decoupled carbon NMR sequence allowing integration of resonance signals of carbon atoms. In the case of the reaction at 10 acid chloride equivalents, the ratio between carbonyl group and C-1 of β-cyclodextrin gave 4.55 chains per molecule that indicated an average substitution rate between 4 and 5 grafted hydrocarbon chains.

Conclusion

This work proposed a new route of amphiphilic cyclodextrin synthesis based on a simple and easy one-step process. Suitable conditions was found to provide a final derivative consisted on a mixture of cyclodextrins grafted onto the primary face by different numbers of dodecanoyl chains and statistically ranging from 1 to 7 with an average at 4.5 chains per molecule. This product was totally devoid of free lauric acid by sublimation as attested by both DSC and mass spectrometry analyses. These conditions are particularly suitable for transposition at an industrial scale.

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