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A convenient preparation of 6-oligo(lactic acid)cyclomaltoheptaose as kinetically degradable derivative for controlled release of amoxicillin

Jian Shen, Aiyou Hao *, Guangyan Du, Huacheng Zhang, Hongyuan Sun

School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, PR China

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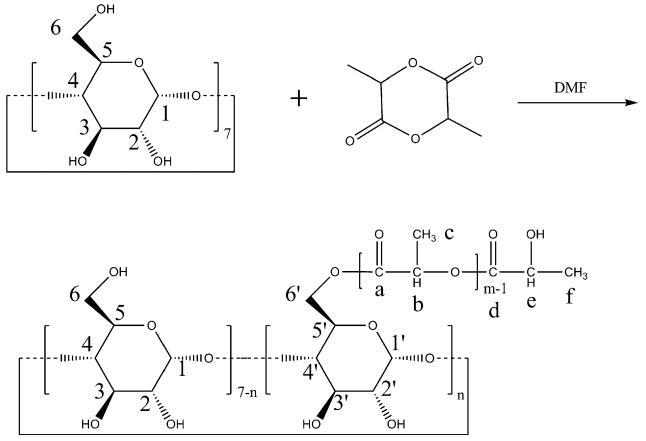
ABSTRACT

6-Oligo(lactic acid)cyclomaltoheptaose (6-OLA-βCD) with an average substitution of about 7.0 lactic acid units was prepared as a new water-soluble cyclomaltoheptaose (βCD) derivative (solubility of about 70.7-fold that of βCD), based on the ring-opening polymerization of 3,6-dimethyl-1,4-dioxane-2,5-dione (lactide). The product was characterized by ^{1}H NMR, ^{13}C NMR, IR, and MS spectroscopy. The complexation of amoxicillin with 6-OLA-βCD was found to be much stronger than that with βCD at first, and then 6-OLA-βCD was shown to decompose moderately into βCD and lactic acid. 6-OLA-βCD might be greatly valuable in a controlled release system for Amoxicillin (AMX).

1. Introduction

Cyclomaltooligosaccharides (cyclodextrins, CDs) are skirt-shaped (1→4)-linked cyclic glucooligosaccharides. The most accessible CD representatives are composed of 6, 7, and 8 glucose units named cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose (α-, β-, and γCD), respectively. The particular structure of CDs allows various guest molecules to be included to form inclusion complexes. CDs are potential candidates for drug carrier system because of their abilities to alter physical, chemical, and biological properties of guest molecules through the formation of inclusion complexes. The most common pharmaceutical application of CDs is to enhance the solubility, stability, and bioavailability of drug molecules. However, the most commercially accessible βCD has a relatively low solubility both in water and in organic solvents, which would limit its applications in pharmaceutical formulations. In the past decades, various kinds of βCD derivatives such as hydroxypropyl-βCD, hydroxybutyl-βCD, and methyl-βCD[1–8] have been prepared for improving the physicochemical properties and inclusion capacity of natural βCD as drug carriers. Here, a novel βCD derivative modified with a degradable and biocompatible oligo(lactic acid) (OLA) group,[9] 6-oligo(lactic acid)cyclomaltoheptaose (6-OLA-βCD) (Scheme 1), was prepared for the purpose of extending the pharmaceutical application of βCD. 6-OLA-βCD was demonstrated as a kinetically degradable carrier for the controlled release of the common drug Amoxicillin (AMX).



Scheme 1. Preparation method for 6-OLA-βCD.

2. Results and discussion

The synthetic approach for 6-OLA-βCD is based on the ring-opening polymerization of 3,6-dimethyl-1,4-dioxane-2,5-dione (lactide) in the presence of βCD (Scheme 1).

* Corresponding author. Tel.: +86 053188363306.
E-mail addresses: haiay@sdu.edu.cn (J. Shen), haoay@sdu.edu.cn (A. Hao).

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N,*N*′-Dimethylformamide (DMF) was found to be the appropriate medium in which the reaction of βCD with lactide could be carried out well without need of any additional catalyst. The reaction position was shown to be the C-6 hydroxyl of βCD in view of the clear shift of C-6 from 59.96 to 64.76 ppm in the ^{13}C NMR spectrum, with no clear shift for C-2 and C-3 when compared with the original ^{13}C NMR spectrum[10] of βCD. Preferential esterification at the primary hydroxyl position (OH-6) of the CD is probably due to the fact that the primary hydroxyl groups are sterically less hindered when compared with the secondary ones (OH-2 and OH-3).[11–15]

The relative molecular weight of 6-OLA-βCD could be controlled by the proportion of the two reactants. The molar ratio of βCD to lactide was tested from 1:1 to 1:8 for the purpose of providing valuable functional βCD derivatives with different molecular weights. Among the derivatives, a typical product 6-OLA-βCD shows strong complexation ability with AMX and fine water solubility of about 133 g/100 mL which is 70.7-fold that of βCD (1.88 g/100 mL) at room temperature. The average substitution degree (*n*) of OH-6 of βCD and the average chain length (*m*) of 6-oligo(lactic acid) groups attached to βCD could be calculated by the proton integration of H-f, H-c, H-1, and H-b in the ^{1}H NMR spectrum (Fig. 1) of the typical product 6-OLA-βCD, where *n* is about 1.55 and *m* is about 4.49, and thus the average molecular weight of 6-OLA-βCD is about 1637. A molecular weight 1637 of 6-OLA-βCD means an average substitution of about 7.0 lactic acid units per βCD.

The subsequent esterification which could even preferentially occur at the secondary OH group of the lactate branch is perhaps due to the enhanced steric restriction of the other OH-6 groups with the increasing of the average substitution degree of βCD. The result of *n* and *m* indicates the competition of the OH-6 of βCD to the secondary OH of the lactate branches when the average substitution degree (*n*) of the OH-6 of βCD is about 1.55, the average chain length (*m*) of 6-oligo(lactic acid) groups attached to βCD would increase to 4.48.

Some of the structural components of 6-OLA-βCD could also be shown in the mass spectrum[16] (Fig. 2). In the range of mass from 1296 to 1873, the most abundant fragment ions (*m*/*z* 1440.9, 1585.0, and 1729.8), separated with a clear interval of 144 amu which exactly corresponds to the molecular weight of one lactide, correspond to 4, 6, and 8 lactic acid repeating units being linked to βCD through the ring-opening polymerization of lactide, respectively.

The inclusion complex of AMX with 6-OLA-βCD was directly prepared by mixing AMX and 6-OLA-βCD (MW≈1637) in a 1:1 proportion and kneading for about 45 min. The formation of the inclusion complex was confirmed by the DSC-thermograms (Fig. 3) where the inclusion complex of AMX with 6-OLA-βCD (Fig. 3d) shows the absence of peaks corresponding to AMX while a simple physical mixture of AMX with 6-OLA-βCD (Fig. 3c) still shows peaks at about 83 °C, 203 °C, and 332 °C corresponding to AMX (Fig. 3b) and 6-OLA-βCD (Fig. 3a), respectively.

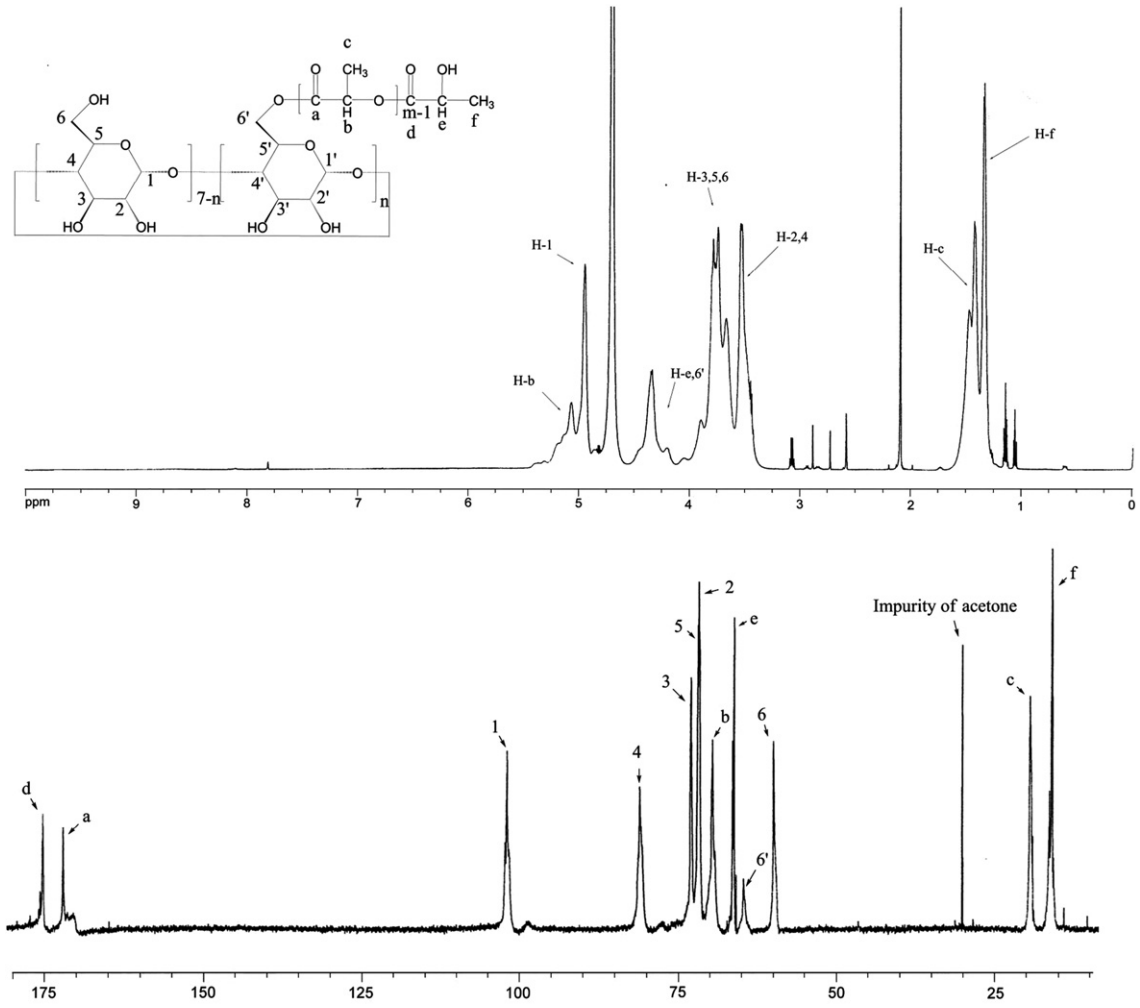


Figure 1. ^{1}H (upper) and ^{13}C NMR spectra of 6-OLA-βCD (MW ≈ 1637) in D_2O.

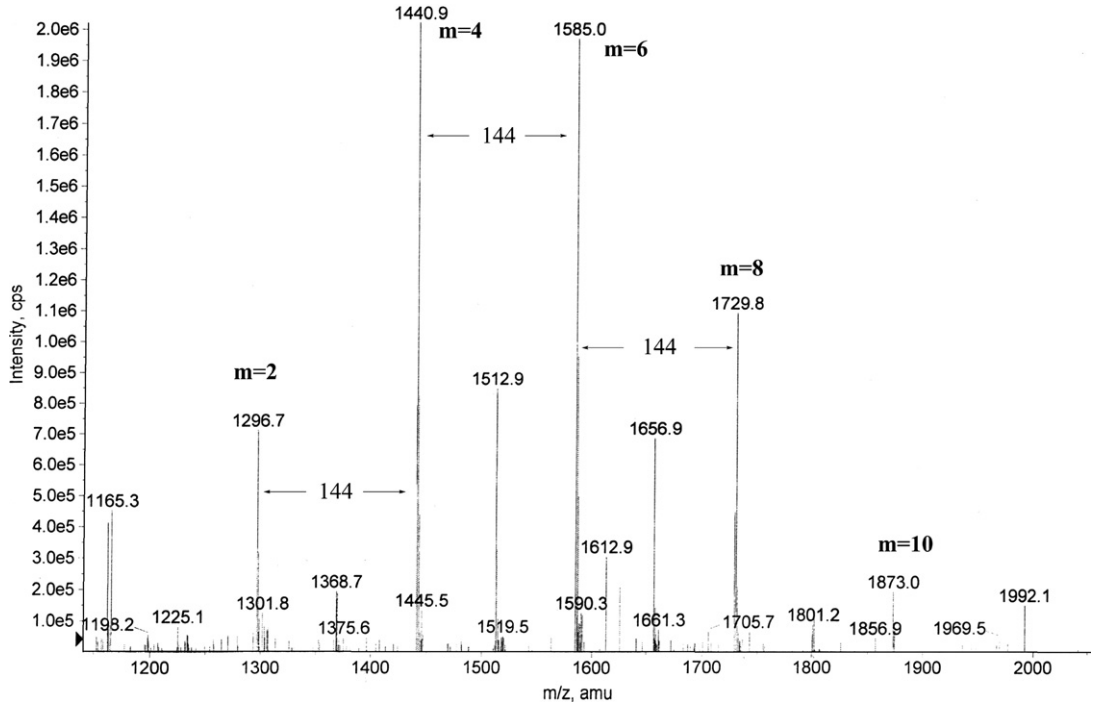


Figure 2. Positive ESI mass spectrum of 6-OLA-βCD (MW ≈ 1637).

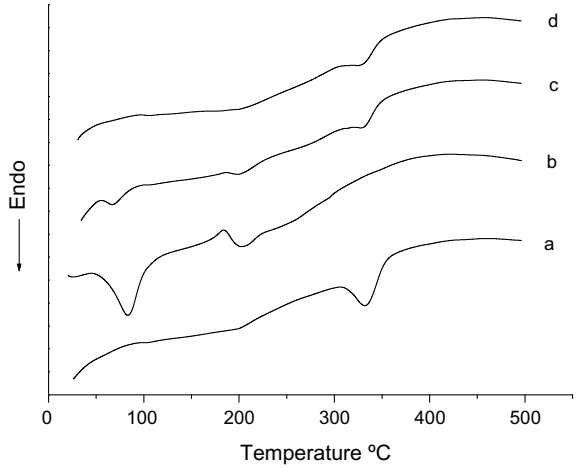


Figure 3. DSC-thermograms of 6-OLA-βCD (MW ≈ 1637, a) AMX (b), and 1:1 AMX/6-OLA-βCD (MW ≈ 1637) systems: physical mixture (c), and inclusion samples (d).

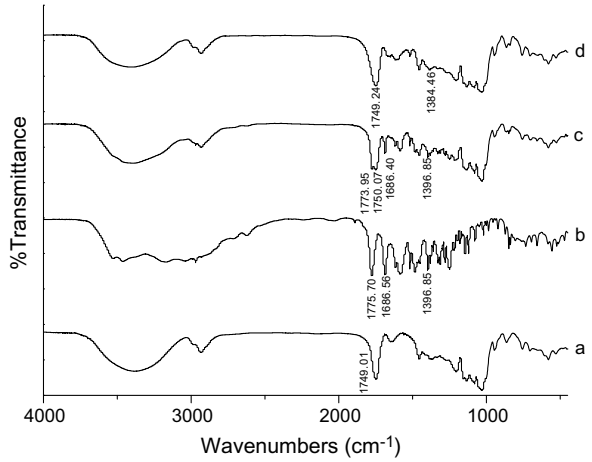


Figure 4. IR spectra of 6-OLA-βCD (MW ≈ 1637, a), AMX (b), and 1:1 AMX/6-OLA-βCD (MW ≈ 1637) systems: physical mixture (c), and inclusion samples (d).

The complex formation of AMX with 6-OLA-βCD was also confirmed by IR spectroscopy (Fig. 4). In comparison with the simple physical mixture of AMX with 6-OLA-βCD (Fig. 4c), the sharp amide stretching absorption at 1686 cm^{-1} of AMX as the guest included in 6-OLA-βCD becomes flat and weak, and a new absorption band at 1384 cm^{-1} appears, while the absorption band of AMX (Fig. 4b) at 1396 cm^{-1} disappears.

The effect of 6-OLA-βCD on the stability and solubility of AMX was investigated compared with βCD (Fig. 5). It is obvious that the hydrolysis of AMX could be decelerated by 6-OLA-βCD (MW ≈ 1637) while it is slightly accelerated by βCD. For instance, the hydrolysis of AMX when complexed with 6-OLA-βCD (MW ≈ 1637) in water is about 18% after 8 days, while it would be 68% when AMX is alone and 89% when complexed with βCD.

As shown in the phase-solubility diagram of AMX in the presence of 6-OLA-βCD and βCD, respectively (Fig. 6), AMX becomes more soluble in a solution of 6-OLA-βCD (MW ≈ 1637, 0.1 mol L^{-1}) with about 29.3-fold increase compared to pure water. However, the increase is only about 10.3-fold in the saturated solution of βCD as compared to pure water.

The time-dependent decomposing process of 6-OLA-βCD (MW ≈ 1637) in water was detected by TLC and ^{1}H NMR. As shown in Figure 7, we could find that the decomposing rate of 6-OLA-βCD is fast at the beginning and then becomes slow gradually, and finally 6-OLA-βCD decomposes to βCD (R_f = 0.36, in this solvent system) and lactic acid, which was confirmed by ^{1}H NMR (Fig. 8, Sample III).

The influence of the decomposition of 6-OLA-βCD on the stability of inclusion complex was studied using the formula[17] $1/\Delta A = 1/(a \cdot K \cdot [H]^n) + 1/a$, where K is the stability constant of the complex, ΔA is the absorption deviations from a drug standard solution, [H]

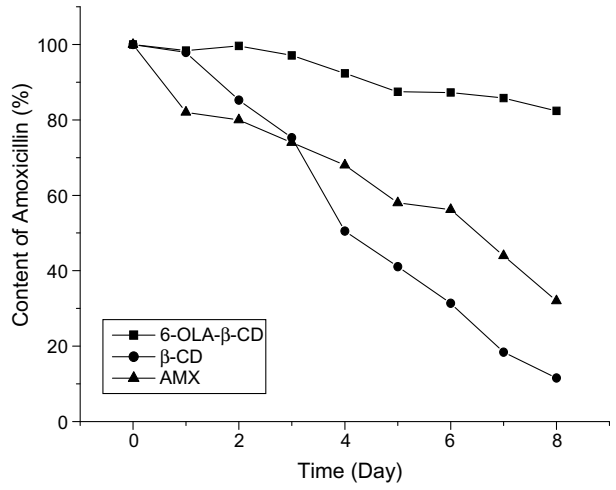


Figure 5. Hydrolysis of AMX in free form (▲), complexed with 6-OLA-βCD (MW ≈ 1637, ■) and βCD (●) in water.

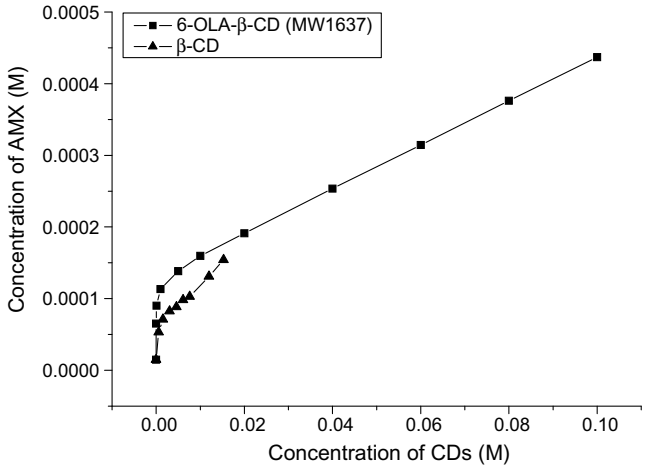


Figure 6. Phase-solubility diagram of the AMX complex with 6-OLA-βCD (MW ≈ 1637) and βCD, respectively.

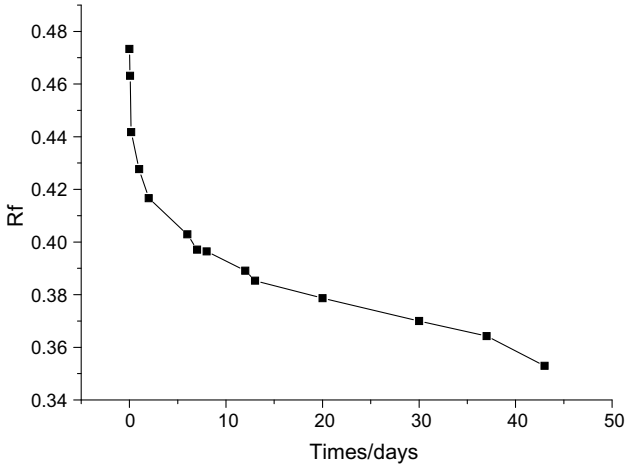


Figure 7. Decomposition of 6-OLA-βCD (MW ≈ 1637) by TLC in aqueous solution (pH 6.0) at room temperature.

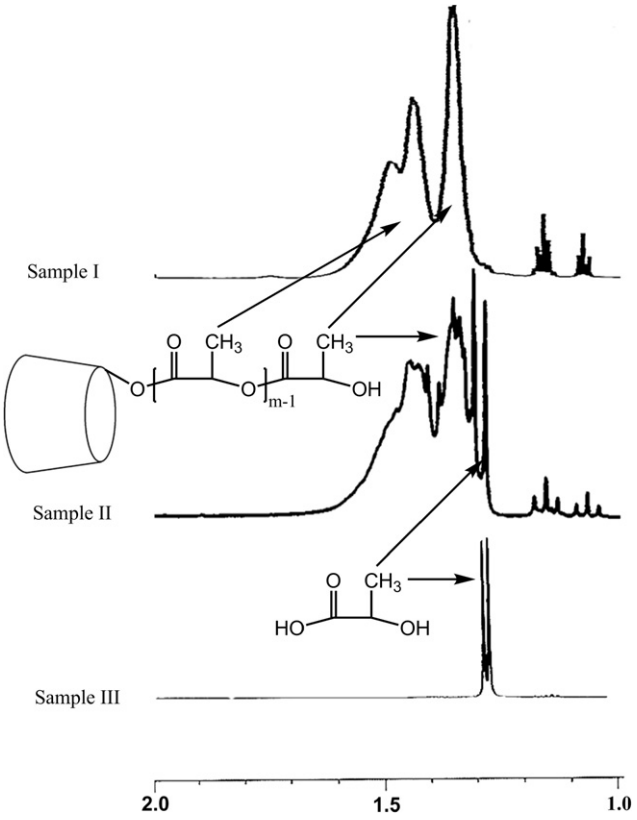


Figure 8. Selected regions of the ^{1}H NMR spectra in D_2O of Sample I, II, and III (Sample I is an extemporaneously prepared aqueous solution of 6-OLA-βCD, Sample II is an aqueous solution of 6-OLA-βCD stored for 12 days, and Sample III is an aqueous solution of 6-OLA-βCD which has decomposed to βCD).

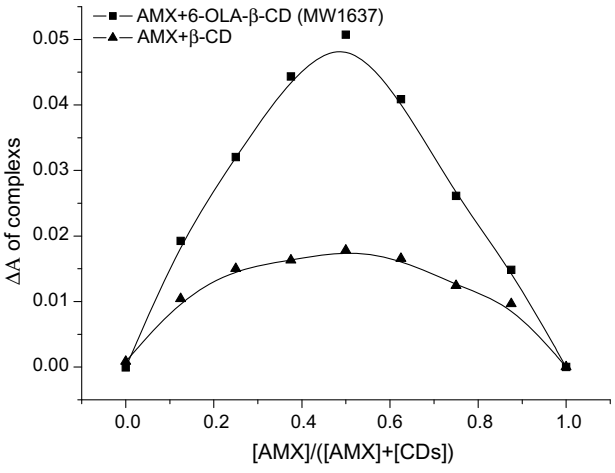


Figure 9. Job's plots of the absorbance changes at 272 nm upon complexation of AMX with 6-OLA-βCD (MW ≈ 1637, ■) and βCD (▲) at 25 °C with a concentration sum ([AMX] + [CDs]) at 8.0×10^{-4} M.

is the concentration of 6-OLA-βCD or βCD, and n is the stoichiometry of the complex.

The stoichiometry of the complex was determined by the Job's plot method[18–21] based on varying the respective molar ratio of CD and AMX at a constant total concentration. As shown in Figure 9, a maximum value at 0.5 M fraction indicates a complex composition of one AMX molecule per one CD, and that means $n = 1$ and a formula of $1/\Delta A = 1/(a \cdot K \cdot [H]) + 1/a$.

The complex stability constant K of AMX with 6-OLA-βCD (Table 1) could be calculated by the formula $1/\Delta A = 1/(a \cdot K \cdot [H]) + 1/a$ based on Figure 10, where Sample I is an extemporaneously prepared aqueous solution of 6-OLA-βCD (MW ≈ 1637), Sample II is an aqueous solution of 6-OLA-βCD (MW ≈ 1637) stored for 12 days before complexing with AMX, and Sample III is an aqueous solution of 6-OLA-βCD (MW ≈ 1637) which had decomposed to βCD completely (Fig. 8). It is obvious that the decomposition of 6-OLA-βCD causes a descending trend of the inclusion complex stabilities (represented in Scheme 2).

Table 1
The stability constant of different samples of 6-OLA-βCD (MW ≈ 1637) with AMX

Sample	I	II	III
Stability constant K (M^{-1})	2.4×10^5	1.9×10^4	137.3

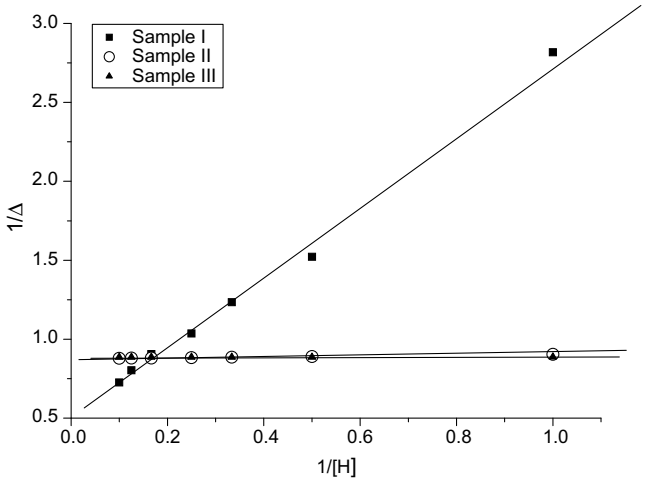
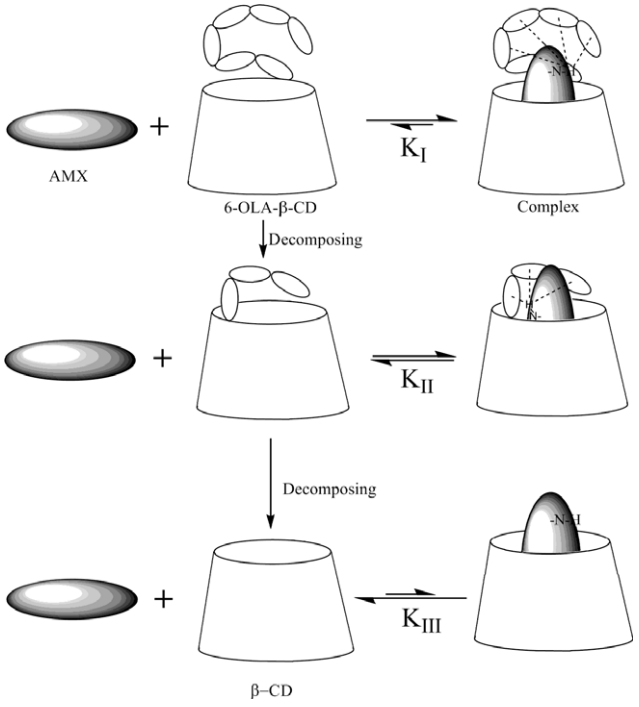
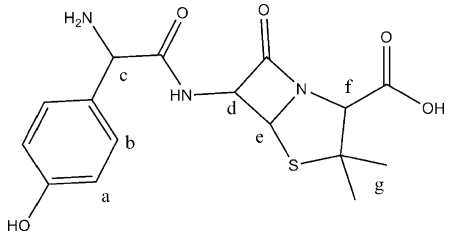


Figure 10. The plot of $1/\Delta A \sim 1/[H]$ for 6-OLA-βCD (MW ≈ 1637) and AMX at 229 nm.



Scheme 2. Possible complexation mode of 6-OLA-βCD with AMX[26,27] in water.

Table 2
^{1}H NMR Chemical shifts, δ (ppm), of C–H protons[c] in AMX and its complex with 6-OLA-βCD (MW ≈ 1637) in D_2O



Proton	AMX	6-OLA-βCD complex		βCD complex	
		AMX	Δδ[a]	AMX	Δδ[a]
H_a	7.225	7.262	0.037	7.258	0.033
H_b	6.834	6.871	0.037	6.865	0.031
H_d	5.358	5.477	0.119	5.441	0.088
H_e	5.008	5.021	0.013	4.964	−0.044[b]
H_f	4.027	4.078	0.051	4.031	0.004
H_g	1.279	1.303	0.024	1.304	0.025

[a] Chemical shift changes of AMX when complexed with 6-OLA-βCD or βCD, respectively.
[b] The negative value indicates a high field shift.
[c] The chemical shift of H_c in amoxicillin is covered by the HDO peak.

A selection of chemical shift data for AMX in its free form or complexed with 6-OLA-βCD or βCD is presented in Table 2. The chemical shift variations of all the aromatic protons of AMX when complexed with CDs show a clear evidence for the formation of the complexes.[22,23] Moreover, the chemical shift variations for H_d, H_e, H_f of AMX complexed with 6-OLA-βCD are larger in comparison to the shifts observed for AMX complexed with βCD, which indicates an additional interaction of AMX with 6-OLA-βCD as compared to βCD. The chemical shift of H_d of AMX complexed with 6-OLA-βCD is the largest, which might be related to an additional effect[24,25] of the 6-OLA branches of 6-OLA-βCD on amide group of AMX.

In conclusion, 6-oligo(lactic acid)cyclomaltoheptaose as a fine water soluble cyclomaltoheptaose derivative modified by degradable and biocompatible group is capable of enhancing the solubility and stability of Amoxicillin. The time-dependent stability of inclusion complex of 6-oligo(lactic acid)cyclomaltoheptaose with Amoxicillin caused by the kinetic decomposition of 6-oligo(lactic acid)cyclomaltoheptaose might provide a potential material in the controlled drug release carrier system.

3. Experimental

3.1. General methods

3,5-Dimethyl-1,4-dioxane-2,5-dione (lactide) was prepared according to the literature.[28] βCD was purchased from Guangdong Yunan Chemical Reagent Co. Ltd, China, recrystallized twice from distilled water and dried in vacuum for 12 h. Reagent-grade DMF (Country Medicine Reagent Co. Ltd, China) was dried over $MgSO_4$. DL-Lactic acid and acetone were all commercially available from Country Medicine Reagent Co. Lt., China and were used as received. AMX was supplied by Ji En Medicament Research Company, China.

^{13}C NMR and ^{1}H NMR spectra were performed at 400 MHz on a Bruker Avance 400M NMR. Positive ESI-MS was performed on API 4000 MS equipment with methanol as solvent. UV–vis spectra were recorded on a TU-1800pc UV–vis Spectrophotometer. DSC

was analyzed by Mettler-Toledo TGA/SDTA 851, using a scanning rate of 10 °C/min from 25 °C to 500 °C. IR spectra were performed on Avatar370 FT-IR Spectrometer. TLC analysis was performed on glass plates precoated with Silica Gel F_{254} obtained from Qingdao Haiyang Chem, China.

3.2. Preparation of 6-OLA-βCD

A solution of βCD (5.0 g, 4.4 mmol) in 100 mL DMF was heated to 85 °C under nitrogen atmosphere for 1 h. The solution was cooled to 30 °C and lactide (5 g, 34 mmol) was added successively. The mixture was then heated to 80–85 °C and the temperature was maintained for 6 h. The solvent was evaporated under diminished pressure to give an oily pale yellow residue. Dry ether was added to the residue; the resulting white precipitate was filtered and washed with acetone. The purification was repeated twice, followed by vacuum desiccation to give 6-OLA-βCD as a white powder (5.6 g, 58.5%, MW ≈ 1637). IR: ν = 3382.36 (vs, O–H), 2934.61 (s, C–H), 1749.01 (vs, C=O), 1652.22 (w, C=O), 1456.22(w, $-CH_3$), 1204.99 (s, CO–OR), 1133.95 (w, CO–OR), 1030.09 (s, C–O–C) cm^{-1}; ^1H NMR (D_2O): δ 5.06379, 4.94166 (m, 12.41H, –COC*H*-(CH_3)O– and C-1H), 4.33538 (m, 4.65H, –COC*H*(CH_3)OH and C′-6H) 3.78124–3.52218 (m, 40.45H, C-2H, C-3H, C-4H, C-5H, C-6H), 1.47668–1.26958 (m, 20.8785H, $-CH_3$); ^{13}C NMR (D_2O): δ 175.684, 175.229, 172.053 (C=O), 102.290, 102.012, 101,652 (C-1), 81.297, 81.133, 80.876 (C-4), 73.055 (C-3), 71.966, 71.838, 71.678 (C-5), 71.678 (C-2), 69.601, 69.264, 66.493, 66.411, 66.240, 65.919 (–COCH(CH_3)O–), 64.767 (C-6′), 59.964 (C-6), 19.489, 19.449, 19.386, 19.345, 19.113, 19.039 (–COCH(CH_3)O–), 16.538, 16.463, 16.081, 16.021 (–COCH(CH_3)OH. Anal. Calcd for $C_{62.88}H_{97.84}O_{13.92}$: C, 46.06; H, 5.99. Found: C, 45.83; H, 6.225.

3.3. Stability of 6-OLA-βCD

The aqueous solution of 2% 6-OLA-βCD was prepared in a NaH_2PO_4–Na_2HPO_4 buffer solution of pH 6.0 adjusted at room temperature. The decomposition of 6-OLA-βCD was monitored by TLC (5:2:1 *i*-PrOH–EtOAc–water).

3.4. Preparation of the inclusion complex of AMX with 6-OLA-βCD

AMX and 6-OLA-βCD were mixed in a 1:1 proportion and kneaded for 45 min. During this process, methanol was added to the mixture to maintain a suitable consistency. The product was dried at 40 °C for 4 h under vacuum. The dried residue was gently ground into a fine powder.

3.5. Hydrolysis of AMX

UV absorbance was used to monitor the hydrolysis of AMX[29] at 350 nm. Solutions of AMX, 1:1 AMX-βCD and 1:1 AMX-6-OLA-βCD were stored at room temperature. The absorbances were recorded with an interval of 1 day.

3.6. Solubility of AMX

An excess of AMX was mixed with aqueous solutions of increasing CDs concentrations. The samples were stirred at room temperature. After 6 h the suspensions were centrifuged at 5000 rpm for 15 min, the amount of soluble AMX in the upper liquid phase was determined by UV-spectrophotometry at 227 nm.

3.7. Inclusion ability of CDs with AMX

UV analyses were performed on aqueous solutions with a fixed AMX concentration (1.0×10^{-5} M) and varied CDs concentrations (from 2.0×10^{-6} to 2.0×10^{-7} M) at room temperature.

An aqueous solution Sample I, II, and III of 6-OLA-βCD was used. Sample I is an extemporaneously prepared aqueous solution of 6-OLA-βCD, Sample II is an aqueous solution of 6-OLA-βCD stored for 12 days before complexing with AMX, and Sample III is an aqueous solution of 6-OLA-βCD which has decomposed to βCD (TLC control).

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