An Improved Method of Synthesis of per-6-deoxy-6-iodo-β-cyclodextrin

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Summary: A number of per-6-substituted-6-deoxy-β-cyclodextrin derivatives have been synthesized for many years, of them, per-6-deoxy-6-iodo-β-cyclodextrin is of great importance in supramolecular chemistry. By reviewing all published papers related to synthesis of per-6-deoxy-6-iodo-β-cyclodextrin and combining with our experimental verification, this work has reported an improved method of synthesis of per-6-deoxy-6-iodo-β-cyclodextrin. The approach mainly uses adding a certain amount of methanol instead of removing DMF before adjusting pH. We think this project is simple, efficient, high repeatable and suitable for large-scale preparation, superior even to all the previously reported methods. The synthesis of bromo, chloro substituted -per-6-β-CD may also refer to this improved method. At the same time, the detailed introduction of operation process and precautions will serve as a reference and guidance to the beginner who will engage in the study on the chemical synthesis reaction of sugar and cyclodextrin.

Keywords: Cyclodextrin(CD), Per-6-substituted-6-deoxy-β-CD, Per-6-deoxy-6-iodo-β-CD(1), N,N-dimethyl formamide(DMF).

Introduction

Per-(6-deoxy-6-halogen) β-cyclodextrin (CD) plays an important role in the area of supramolecular chemistry, which includes bromo, chloro and iodo substituted-per-6-β-CD (the structure of per-(6-deoxy-6-halogen) β-CD is shown in Fig. 1). These substitutes are either the starting materials [1, 2] or intermediates for the preparation of β-CD derivatives. The functionalized β-CD, formed by grafting different groups with β-CD substitutes, has the functions of preventing and treating diseases (such as neuromuscular blocker in anaesthesia [1], inhibition alzheimer's disease formation [2], suppression anthrax lethal toxin [3]); recognizing proteins [4] and magnetic separation [5] of proteins; delivering hydrophobic drugs [6-8]; efficient gene delivery [9]; acting as artificial ion channels [10] and so on. Among these halogen substituents, per-6-deoxy-6-iodo-β-CD (hereinafter 1) is the most important. It has many names in the literature, such as per-iodo-per-6-deoxy-β-CD, per-6-iodo-β- cyclodextrin [2, 6, 9, 11]; heptaiodide [3, 16]; per-iodo-β-cyclodextrin [7]; per-(6-deoxy-6-iodo)- β-CD [10, 12, 13]; Per-6-iodo-6-deoxy-β-CD [14]; heptakis (6-deoxy-6-iodo)-cyclomaltoheptaose [4, 15]. Over the past twenty years, since A. Gadelle and J. Defaye [12] first reported the preparation of compound 1, there are many papers [3, 4, 6, 9-16] describing its synthetic technology. However, the workup procedure is proved to be unsatisfactory in our hands, they all required partial or complete removal of the DMF in the reaction mixture. As we know it is very difficult to remove DMF using conventional rotary evaporation method. In this article, we developed a new approach of manufacturing compound 1 by adding a certain amount of methanol instead of removing DMF before adjusting pH. We believe that this process is simple, efficient, and of high repeatability and large-scale preparation, superior even to all the previous methods. The product 1 was monitored by NMR spectroscopy (^{1}H, ^{13}C-NMR, NOE, COSY and HSQC). Furthermore, in order to better serve the beginners of supramolecular chemistry and increase their success opportunity of experiment, we have reviewed all relevant articles published and made a comparative analysis in our research, and then optimized the reaction conditions and workup procedures, summarized and formulated the detailed specification of the preparation.

Experimental

Raw materials

β-CD was purchased from ROQUETTE and underwent freezing dry during 48 h. DMF(Alfa Aesar) was distilled from C_aH_2 under diminished pressure, and then stored in a bottle with molecular sieve and argon. triphenylphosphine(Alfa Aesar) was desiccator-dried before use. Toluene, dichloromethane, 2-propanol were obtained from Sigma-Aldrich.Thin layer chromatography (TLC) analysis was carried out on silica coated aluminum plates from Merck using silica gel 60. All other chemicals were of reagent grade quality and used without further purification from the commercial suppliers.

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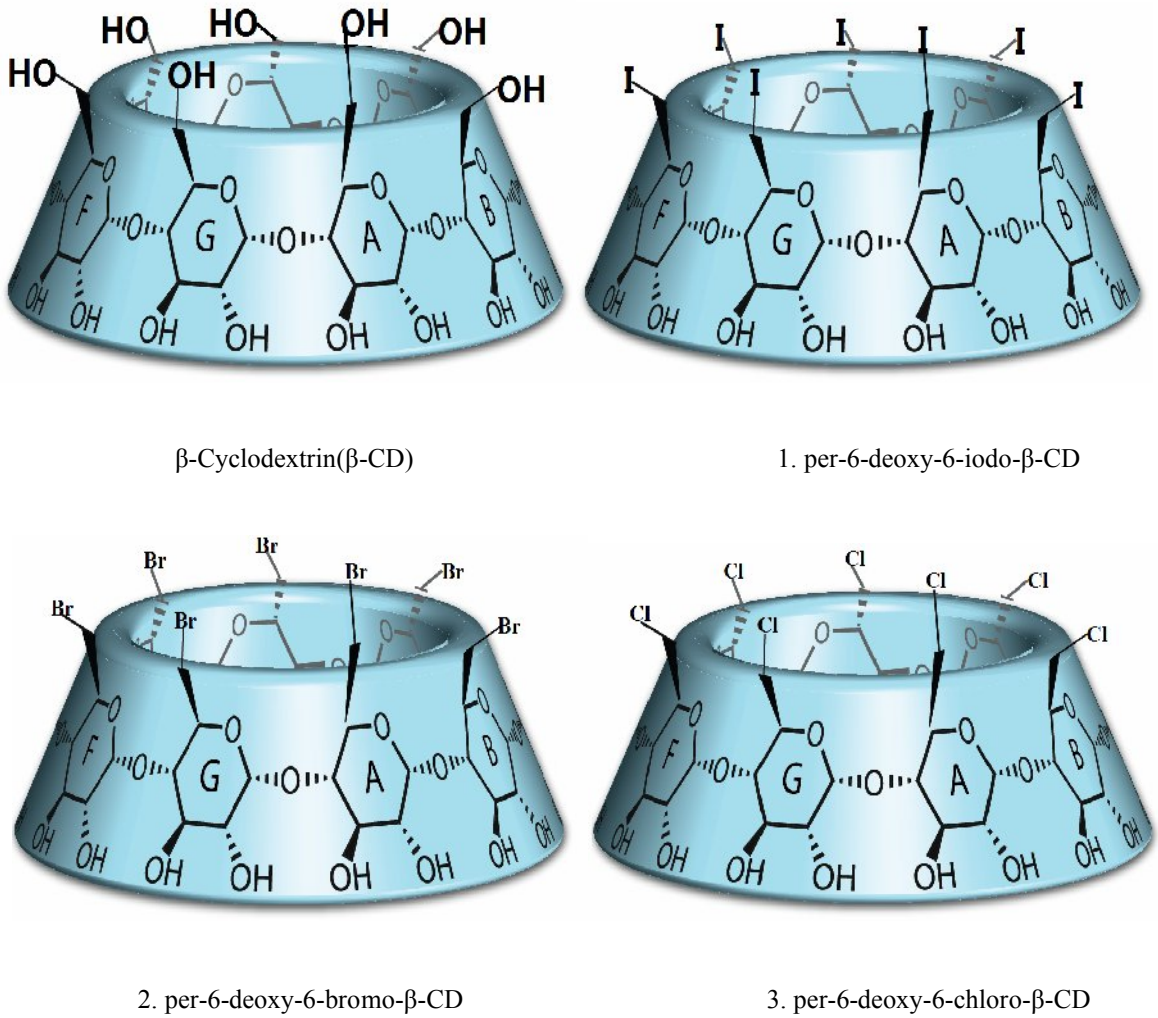


Fig. 1: structure of per (6-deoxy-6-halogen) β-CD.

NMR spectra were recorded on a Bruker DRX 400 (^{1}H: 400 MHz, ^{13}C: 100.6 MHz), at 25 °C and Chemical shifts are reported in ppm (d) using tetramethylsilane (TMS) as the internal reference given in ppm according to calibration to the corresponding solvents $CDCl_3$ (^{1}H:7.26 ppm, ^{13}C:77.00 ppm) and CD_3OD (^{1}H: 3.31 ppm, ^{13}C: 49.05ppm). Mass spectra were performed on a MicroTof Bruker. IR spectra were recorded on a Tensor 27 Bruker.

Improved procedure for the synthesis of compound **1**

Desiccator-dried triphenylphosphine (10.5 g, 40 mmol) was dissolved in 40 ml dry anhydrous DMF in a 250 mL flask equipped with a condenser and iodine (10.1 g, 39.79 mmol) in small portions was added to this solution carefully with vigorous stirring, followed after 30 min, the freeze dried β-CD (2.16 g, 1.9 mmol) was added to this dark brown solution and stirred more than 24 h at 70 °C under N_2 atmosphere protection. Through the TLC (developing agent, ethyl acetate: isopropanol: water = 2: 1: 1, charring with 10% H_2SO_4 in ethanol), if the result showed completed disappearance of the starting material β-CD, then finished the reaction and placed until room temperature, methanol was added with the half volume of the reaction solution and stirring was continued for 30 min. The reaction mixture was then cooled to about 15 ℃, and the pH was adjusted to 9-10 by the addition of 3 M sodium methoxide in methanol, with simultaneous cooling. The solution was kept at room temperature for 30 min to destroy the formate esters formed in the reaction, after which the reaction mixture was poured into 200-300 ml methanol to form a precipitate, which was collected by filtration

(fritted glass No. 3), and washed with methanol and water until colourless. The product was dried in a vacuum oven at 60 ℃ under high vacuum, and then a white powder in 80-90% yield was obtained.

^{13}C-NMR (101 MHz, DMSO-*d6*) δ: 102.12(C_1), 85.94 (C_4), 72.16 (C_5), 71.91(C_3), 70.94(C_2), 9.46(C_6).

^{1}H-NMR (400 MHz, DMSO-*d6*) δ,ppm: 6.01 (d,J=6.8 Hz,7H,C_2-OH), 5.91 (d, J=1.8 Hz,7H,C_3-OH), 4.98 (d,J = 3.2 Hz,7H,C_1-H), 3.79 (d,J=9.3 Hz,7H,C_6-Ha), 3.68–3.53 (m,14H,C_3-H, C_5-H), 3.52–3.20 (m,21H,C_2-H,C_4-H,C_6-H_b).

ESI-MS: $[M+Na]^+$ calculated for $C_{42}H_{63}O_{28}I_7$,1926.6711; found 1926.7493.

IR (KBr): ν (cm^{-1}) 681, 749, 937, 1034, 1099, 1150, 1366, 1410, 1655, 1730, 2911,3026, 3336.

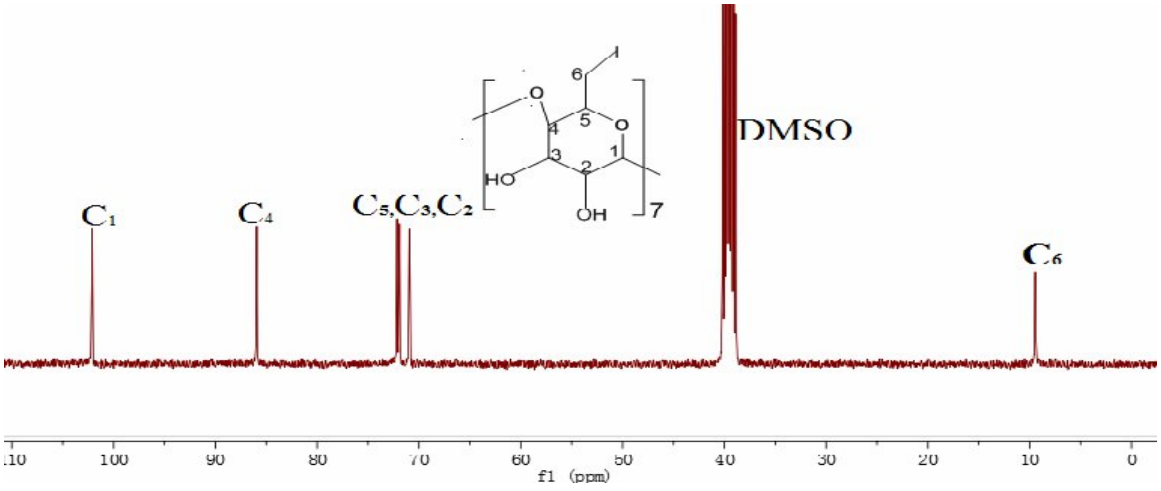


Fig. 2: ^{13}C-NMR of per-(6-deoxy-6-iodo)-β-CD(DMSO-d6, 25 °C).

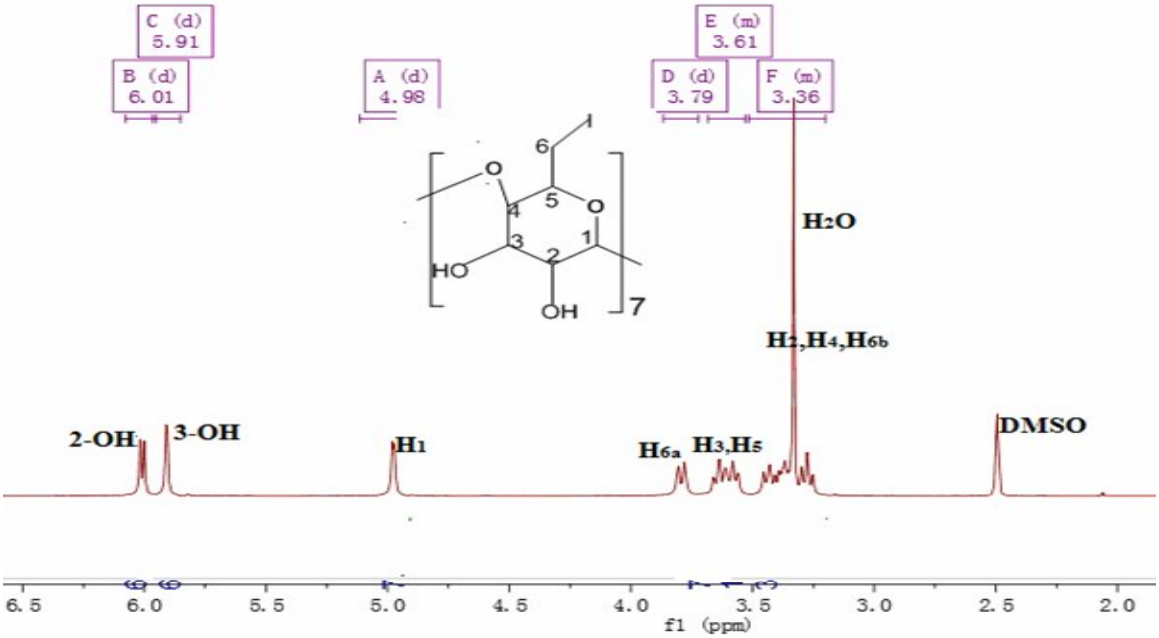


Fig. 3: ^{1}H-NMR of per-(6-deoxy-6-iodo)-β-CD (DMSO-d6, 25 °C).

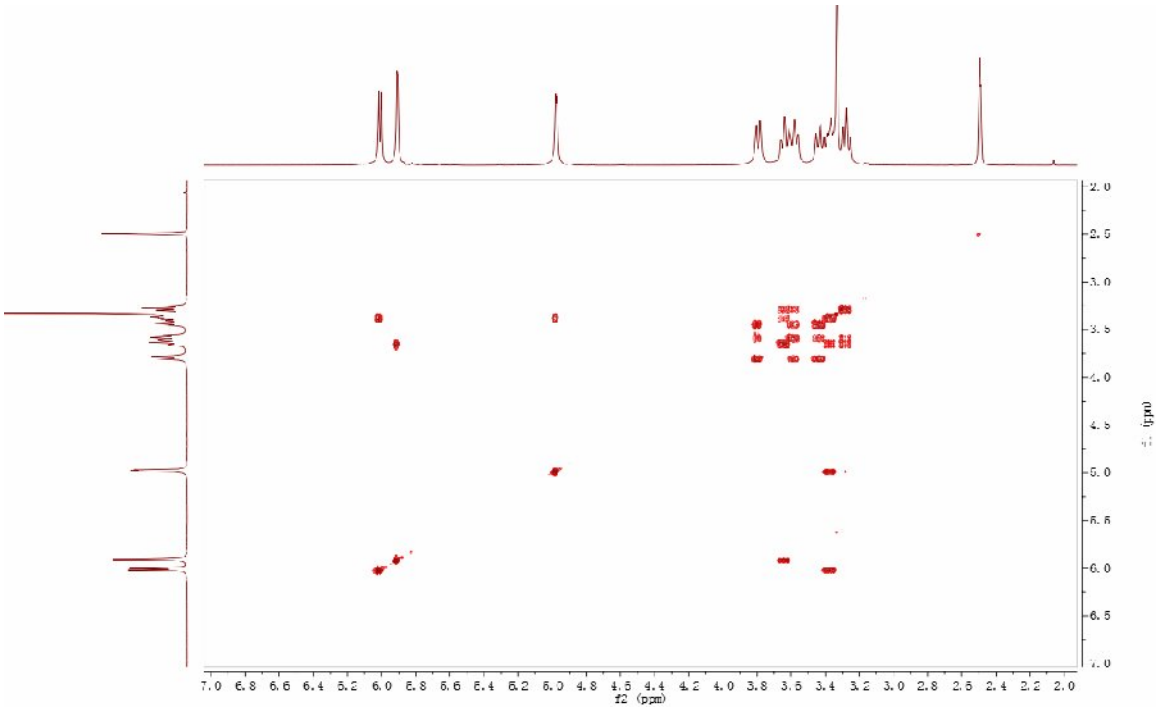


Fig. 4: ^{1}H-^{1}H COSY of per-(6-deoxy-6-iodo)-β-CD (DMSO-d6, 25 °C).

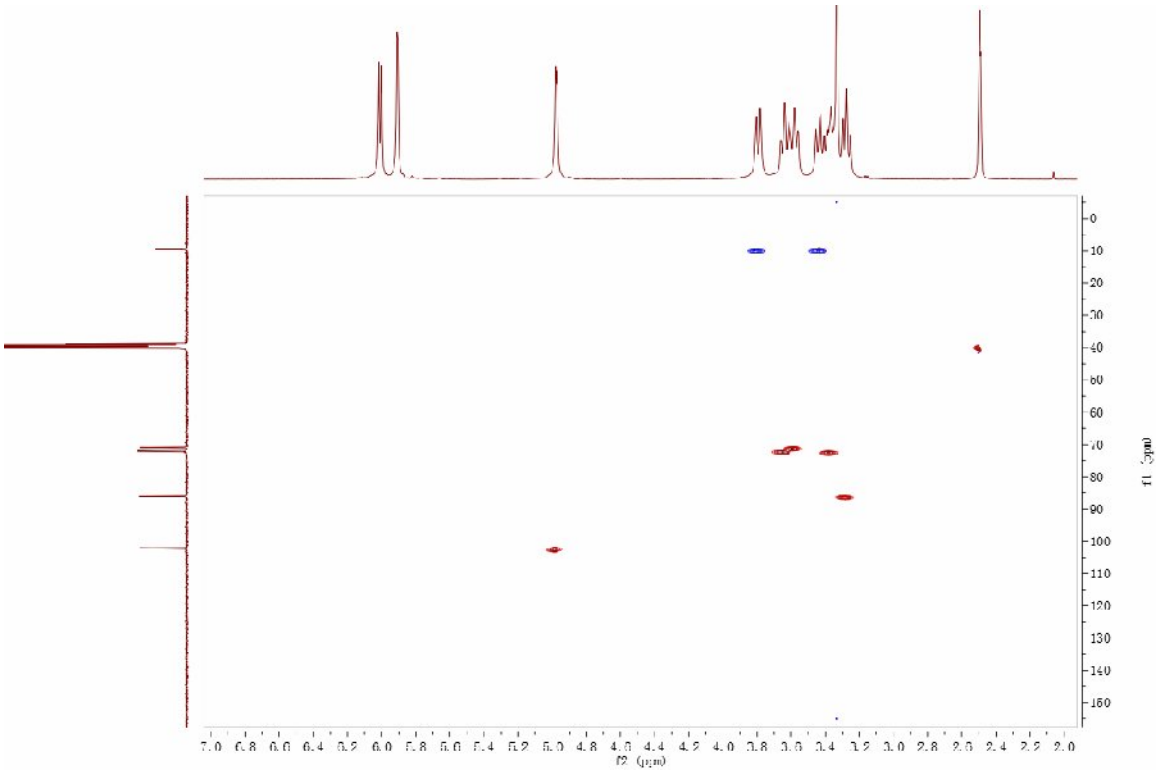


Fig. 5: HSQC of per-(6-deoxy-6-iodo)-β-CD **(1).**

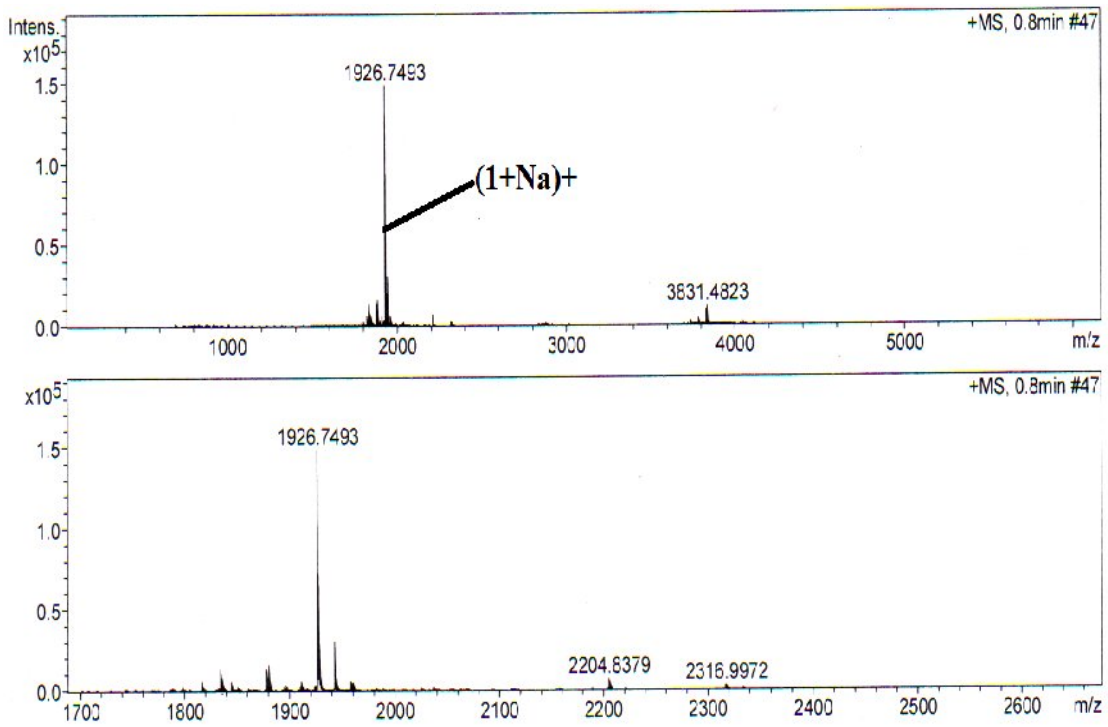


Fig. 6: Mass spectra of per-(6-deoxy-6-iodo)-β-CD**(1).**

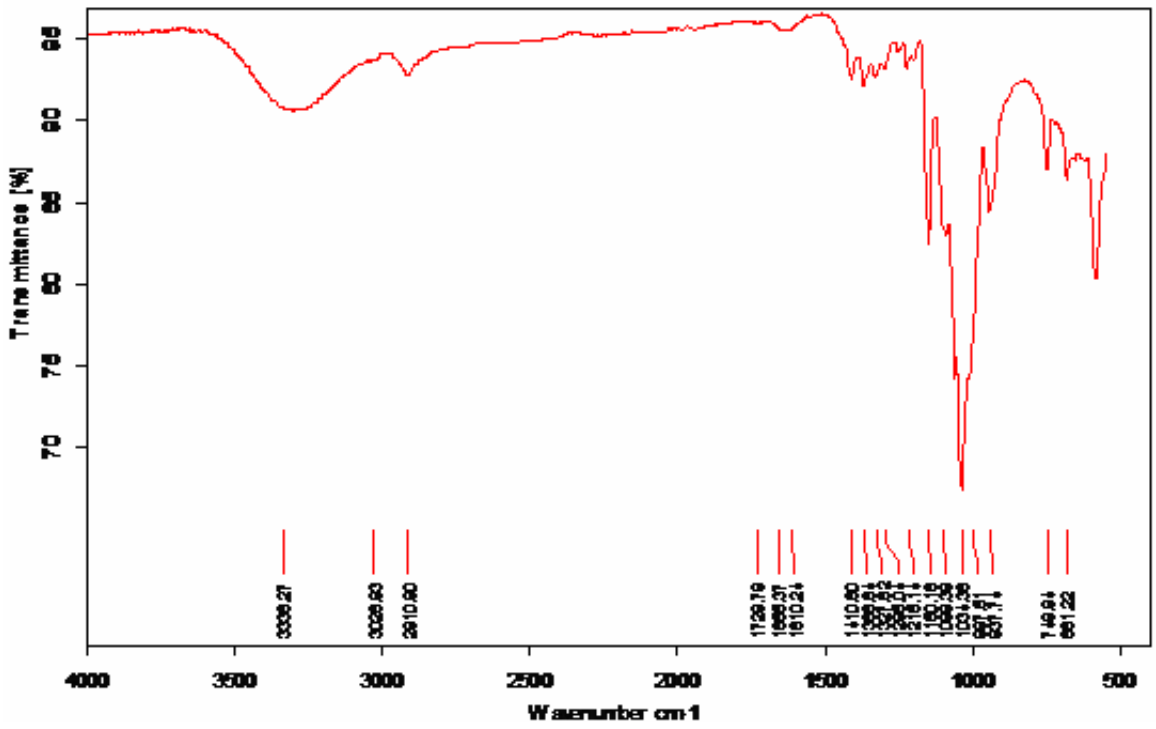
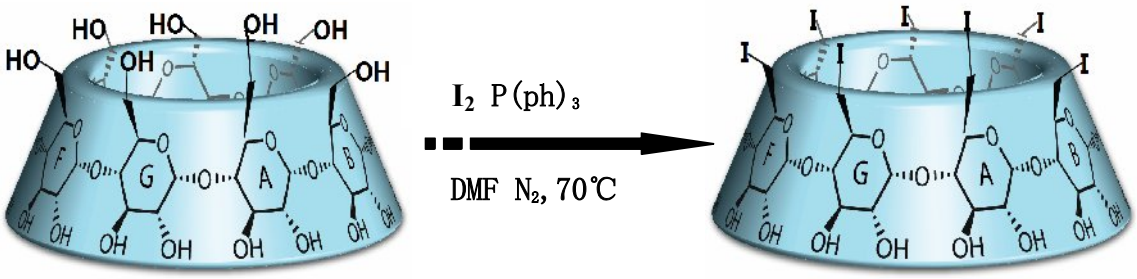


Fig. 7: IR of per-(6-deoxy-6-iodo)-β-CD **(1).**



Scheme 1: pathway of the formation of per-(6-deoxy-6-iodo)-β-CD

Results and Discussion

The synthesis of compound 1 is showed as Scheme 1.

Substance 1 CAS No. is 30754-23-5, chemical formula: $C_{42}H_{63}I_7O_{28}$, molecular weight: 1904.26. It's insoluble in methanol as well as in water, only soluble in DMF and DMSO. There are two kinds of methods for synthesis 1. One is to report an important reaction by Defaye and Gadelle [12]. It used the selective replacement of all the primary hydroxyl groups of β-cyclodextrin by the reaction of iodine and triphenylphosphane in DMF. Another method was introduced by Chmurski and Defaye [13], It was to a DMF solution of dried β-cyclodextrin added N-iodsuccinimide and triphenylphosphane. But the two projects all need precipitate the reaction solution in ice-water. This may result in a lot of impurities in the final product, and followed by complex purifuction [15]. Subsequently, a modified method was introduced in several literatures [6, 7, 9, 10, 11, 14, 16], which all involved the use of methanol instead of ice-water as a precipitating solvent, and the methanol method continue to be used to now. However, this procedure all required partial or complete removal of the DMF in the reaction mixture. It is not easy to remove completely DMF under reduced pressure. So we developed an improved approach of preparing 1 through adding a certain amount of methanol instead of removing DMF before adjusting pH. The addition of methanol can destroy the excess of materials and subsequent alkalinsation to pH 9-10 of the reaction mixture. This operation makes the product 1 more pure. Meanwhile, after our experiments, the reaction condition we optimized is: the molar ratio of iodine and triphenylphosphine to β-CD is 2-3:1, respectively, at 70℃, more than 24 h reaction time and all the process under N_2 protection. This reaction is sensitive to air and moisture,and strict with anhydrous, so all reagents and instruments used must be dry, and the β-CD is preferably freeze dried. The whole reaction system should be under the protection of nitrogen or argon. We verified this condition 10 times with yield 80-90%. About purification 1, thesis [6, 9, 15] introduced Soxhlet extraction the raw precipitation with methanol for 20 h. We confirmed there was no significant purification effect using this method. If one wants to get compound 1 with the highest purity, silica gel column chromatography should be employed.

Finally, there are a few mistakes we found in the literature [7, 10, 12, 15]. On page S6 of Electronic Supplementary Information (ESI) of paper [7], "β-cyclodextrin (4.32 g, 26.6 mmol equiv)" should be described in detail"β-cyclodextrin (4.32 g, 3.81 mmol, equivalent to 6-hydroxyl group 26.6 mmol)"; the article [10] wrote by Yassine El Ghoul, Ruddy Renia, *et al.,* on page 4 of ESI, the structure of 1 between C_1 and C_5 must be oxygen atom (O), rather than C (carbon atom); in the document of A. Gadelle and J. Defaye [12], on page 79 of Experiment Procedure, "added either 1 or 2 (4.32 g. 26.6 mole equiv. " ought to correct"added either 1 or 2 (3.81 mmol, equivalent to 6-hydroxyl group 26.6 mmol "; in the essay of Hans H. Baer, Antonio Vargas Berengue, *et al.,*. on page 310, under the"Heptakis (6-deoxy-6-iodo) Cyclomaltoheptose (9) ", in the 9th line "NaH_2PO_3" should be changed as "NaH_2PO_4".

Conclusion

We have developed an improved synthesis method of per-6-deoxy-6-iodo-β-CD. Addition of methanol was used instead of evaporating the DMF before adjusting pH. The optimized approach is simple, suitable for large-scale preparation, with stable yield at 80-90%. Moreover, detailed description of the experimental process and precautions are useful for the novice who will

undertake the study on the chemical synthesis reaction of sugar and cyclodextrin.

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