



Synthetic assembly of two β -cyclodextrins through a trehalose moiety as a linker¹



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ABSTRACT

Synthetic assembly of two β -cyclodextrins (CDs) was efficiently accomplished using a 6,6'-di-iodotrehalose derivative as a unique linker by means of a one-pot reaction in liquid ammonia for Birch reduction and subsequent S_N2 replacement. The unique sulfide-linked CD dimer displayed a wider hydrophobic region due to the cavity of the CDs and a part of similar structure of a trehalose linker. An inclusion property of the CD dimer was tentatively examined using TNS (2-p-toluidinylnaphthalene-6-sulfonate) as a model guest compound.

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Cyclodextrins (CDs) are known as cyclic α 1-4 linked D-glucosyl oligomers [1,2], and β -CD is composed of seven glucosyl residues and shows unique characteristics including extremely poor solubility in water and a high inclusion property of hydrophobic guest molecules due to the tight fitting to the appropriate cavity size [3]. In order to effectively include a larger guest compound than the cavity capacity of the corresponding CD, considerable effort has been directed toward syntheses of a variety of CD dimers [4–6]. For example, Breslow's group reported the synthesis of a dimeric CD via a sulfide linkage, and the dimeric CD showed excellent inclusion properties against cholesterol and 6-(4-*tert*-butylanilino) naphthalene-2-sulphonic acid (BNS) sodium salt by means of a cooperative binding system [7]. Phenyl and pyridyl type linkers between CDs were also used for assembly of two CDs [8,9] and selective disruption of protein aggregation by the CD dimers was observed [10]. In addition to the aromatic linkers, amidoalkyl type linkers were also used for construction of CD dimers, and the CD dimers showed enzymatic properties [11]. Simple alkyl linkers

were used for the preparation of CD dimers by means of a simple acid-catalyzed condensation between alkyl diols and one of the primary alcohols [12,13]. We have developed an efficient one-pot reaction involving Birch reduction and subsequent S_N2 replacement in order to construct various multivalent substrates [14,15]. This method includes generation of thiolate anion from thiol or benzylthioether by Birch reduction and Williamson-type sulfide synthesis using the corresponding thiolate anion as a nucleophile. Thus, a thiobenzylated CD was treated with Na in liquid NH_3 followed by addition of tri- or tetravalent alkyl halides to afford the corresponding cluster-type derivatives [14].

Trehalose is composed by two glucopyranosyl units linked by an α 1–1 glycosidic bond. We have reported chemical modification of the trehalose as a starting material for preparation of L-iduronic acid [16]. Trehalose-capped CDs were developed by Cucinotta et al. [17,18] and Fujita et al. [19]. β -CD was capped by a trehalose moiety to give the corresponding capped CD derivative having trehalose: CD ratio of 1:1 [17,18], and γ -CD was capped by trehalose to yield a derivative having a trehalose: CD ratio of 1:1 [19]. No CD dimer has been prepared using trehalose as a bridge between two CDs. In this paper, we describe an efficient synthesis of a new class of CD dimer **1** in which CDs were linked through trehalose. Fig. 1 shows a trehalose-linked CD dimer that can recognize large hydrophobic compounds such as cholesterol.

¹ This work is dedicated to the memory of Professor Hiroyoshi Kuzuhara.

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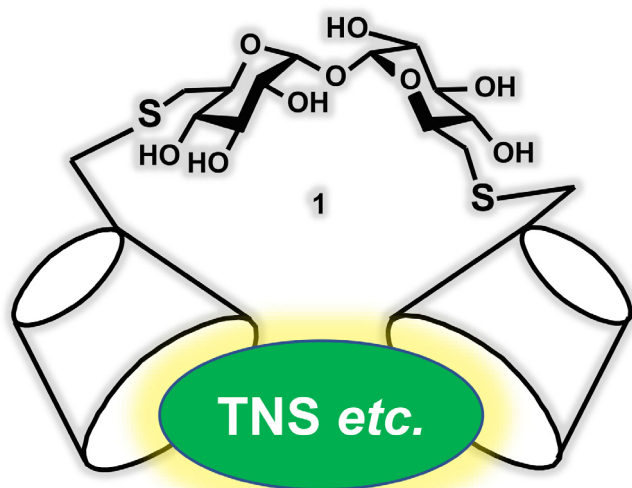


Fig. 1. CD dimer linked by a trehalose bridge.

Primary alcohols at C-6 positions on trehalose **2** were directly substituted by iodine atoms by modified Vilsmeier-Haack reaction [20,21] to afford iodide **3**, which was not able to be purified at this stage. Thus, all of the hydroxy groups in **3** were temporarily acetylated, and the crude acetate was crystallized from EtOH to give pure **4** in 83.3% yield (2 steps) having mp. at 191–193°C. Transesterification of acetate **4** was carried out in MeOH by means of Zemplén's manner [22] to yield the corresponding halogenoalcohol **5**, which was crystallized from EtOH to give a white crystalline mass (82.3% yield). On the other hand, β -CD was converted into the thiobenzyl derivative **7** and thiol derivative **8** by the method previously reported [14] Figure Scheme 1.

Since the preparation of an alkyl halide-type trehalose derivative **5** and β -CD derivatives **7** and **8** as precursors of thiolate was accomplished, our attention was turned toward the condensation

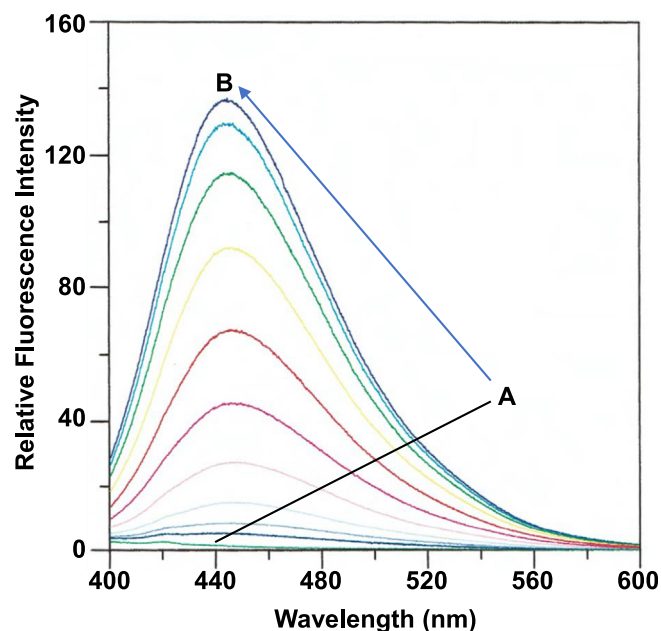
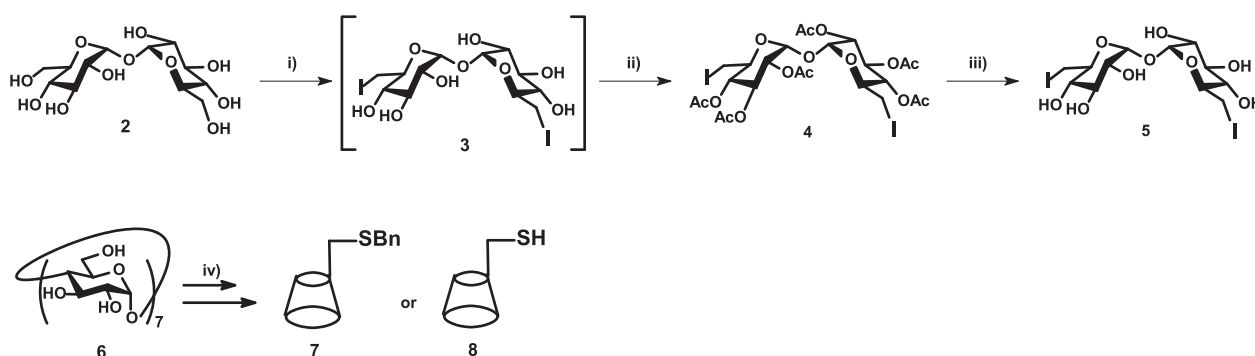
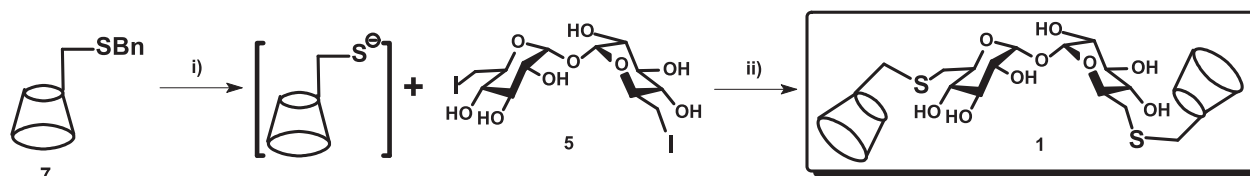


Fig. 2. Changes in emission spectra of TNS at λ_{ex} 366 nm (in 0.1 M phosphate buffer, pH 7.0, at ambient temperature). The CD dimer **1** concentrations (M) are 0, 1.95×10^{-6} , 3.91×10^{-6} , 7.81×10^{-6} , 1.57×10^{-5} , 3.13×10^{-5} , 6.25×10^{-5} , 1.25×10^{-4} , 2.5×10^{-4} , 5.0×10^{-4} , and 1.0×10^{-3} , read from A to B. The concentration of TNS is 1.0×10^{-5} M.

of each derivative. A coupling reaction between a halide **5** and a thiolate of **8** in liquid NH_3 at -33°C in the presence of Na was initially examined, and the reaction mixture was purified by chromatography on Sephadex G-25 with 5% aq AcOH as the eluent to give β -CD dimer **1** in 16% yield after lyophilization. Structural elucidation of **1** was performed by a combination of ^1H NMR, Fab MS, and elemental analysis, and the results are shown in the [supporting information](#). Because thiol **8** includes a corresponding air oxidation product such as an S–S dimer as an impurity, the reaction



Scheme 1. Reagents and conditions: i) I_2 , PPh_3 , DMF, 80°C , 4 h, then 3 M NaOMe in MeOH (pH 9), rt, 30 min; ii) Ac_2O , Pyr, rt, on; iii) NaOMe, MeOH, $0^\circ\text{C} \rightarrow \text{rt}$, 2 h; iv). Ref. 14.



Scheme 2. Reagents and conditions: i) Na, liquid NH_3 , -33°C ; ii) -33°C , on.

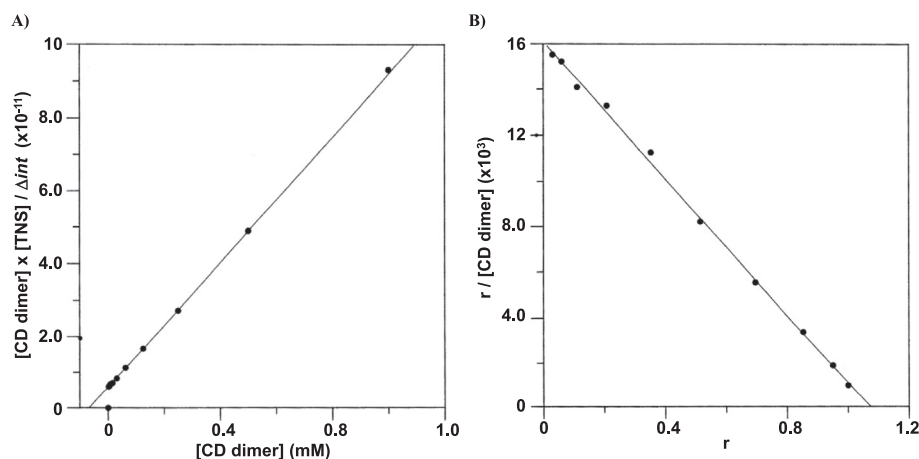


Fig. 3. Determinations of the equilibrium constants of the TNS-CD dimer complex: A) Hildebrand-Benesi plot, B) Scatchard plot.

yield was low. In contrast, a sulfide derivative **7** does not have impurities. Thiobenzyl **7** was therefore used as a better candidate for the coupling reaction between β -CD and trehalose, and the condensation reaction proceeded smoothly to afford the corresponding sulfide derivative **1** having two β -CD moieties in 60.7% yield after lyophilization. This method has valuable merits; 1) purity of the starting material as benzyl sulfide, 2) easy generation of a thiolate as a nucleophile in situ by means of Birch reduction, and 3) simple coupling reaction by means of Williamson ether synthesis (Figure Scheme 2).

Given the success of the preparation of a β -CD dimer bridged by a trehalose moiety, our next interest was in evaluation of the inclusion properties of the β -CD dimer **1** for TNS (2-p-toluidinylnaphthalene-6-sulfonate) [23] as a model guest compound. Because of its availability and easiness in handling, TNS was selected as a guest compound for a CD dimer [24–26]. Various concentrations of CD dimer **1** were measured in 0.01 mM TNS in phosphate buffer at pH 7.0, and the profiles of the emission spectra are shown in Fig. 2. No fluorescence emission of TNS was observed when CD dimer **1** was absent in the TNS solution (A). As the CD dimer concentration increased from A to B, fluorescence intensities of TNS gradually increased.

The host–guest interaction was analyzed by means of a Hildebrand-Benesi plot [27,28] and a Scatchard plot [29,30], and the results are shown in Fig. 3. For the Hildebrand-Benesi plot in Fig. 3 A), the X axis indicates the concentration of CD dimer **1** as $[CD\ dimer]$ in mM and the Y axis means $[CD\ dimer] \times [TNS] / \Delta int$, where Δint was calculated from the difference in intensity of the CD dimer-TNS complex and that of TNS alone. This relation gave a dissociation constant (K_D) of 6.837×10^{-5} M. In addition, r of the Scatchard plot in Fig. 3 used as the X axis means the ratio of Δint and Δint_{max} , where Δint_{max} was calculated from the difference in the highest intensity of the CD dimer-TNS complex and that of TNS alone. The results from the Scatchard plot gave a K_D value of 6.670×10^{-5} M and formation of the host–guest complex of 1:1.07. Kondo et al. reported the dissociation constants of β -CD–TNS complex were 0.65 mM in the 1:1 complex and 71.4 mM in the 1:2 complex [24]. Our results indicated that the host–guest complex is composed of one CD dimer and one TNS molecule, with much lower dissociation constant, and the CD dimer holds one TNS molecule by means of the cavity of each CD in the dimer as shown in Fig. 1.

In summary, a CD dimer linked by a trehalose moiety was efficiently synthesized from thiobenzyl CD and 6,6'-di-iodotrehalose by means of a one-pot reaction in liquid NH_3 involving Birch reduction of the benzyl group followed by S_N2 replacement of

the thiolate to iodide on trehalose to afford the corresponding CD dimer bridged by a trehalose moiety in good yield. The CD dimer showed unique inclusion properties for TNS, and the host–guest complex showed a 1:1 stoichiometric ratio. Results of further evaluations of the CD dimer will be presented elsewhere.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tetlet.2021.153287>.

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