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Regiospecific One-point Cleavage of Capped β–Cyclodextrin by Taka-amylase A

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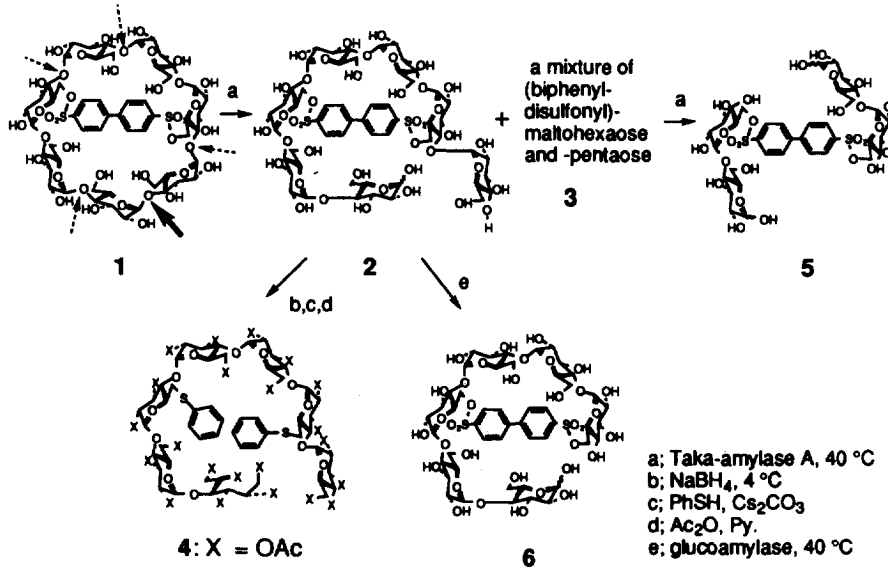
Abstract: A cyclic compound **2** composed of glucoses and biphenyl was prepared in 21% yield through regiospecific cleavage of the glucose-wall of $6^A,6^D$-di-*O*-(*p*,*p*'-biphenyldisulfonyl)-β-cyclodextrin **1** by Taka-amylase A. Copyright © 1996 Elsevier Science Ltd

The direct and practical preparation of linear maltooligosaccharides from cyclodextrins has been difficult. The hydrolysis of a parent cyclodextrin catalysed by acid or by Taka-amylase A (TAA) gives glucose or glucose and maltose as the only isolable products. Simple modification of the cyclodextrin hydroxyls can provide the enzymatic hydrolysis with some selectivity. Mono- and disubstituted cyclodextrins can selectively be transformed to the corresponding mono- or disubstituted linear maltooligosaccharides.[1-3] Nevertheless, this reaction still suffers from the fast decomposition of some glucosidic bonds in the linear maltooligosaccharides formed by direct ring-opening of the macrocycles of cyclodextrins. As the result, the



Scheme 1. Regiospecific Cleavage of the Capped β-Cyclodextrin **1** by Taka-amylase A

isolated products are no longer those of the one-point cleavage. We report here specific, enzymatic one-point cleavage of the macrocycle of capped cyclodextrin (Scheme 1).

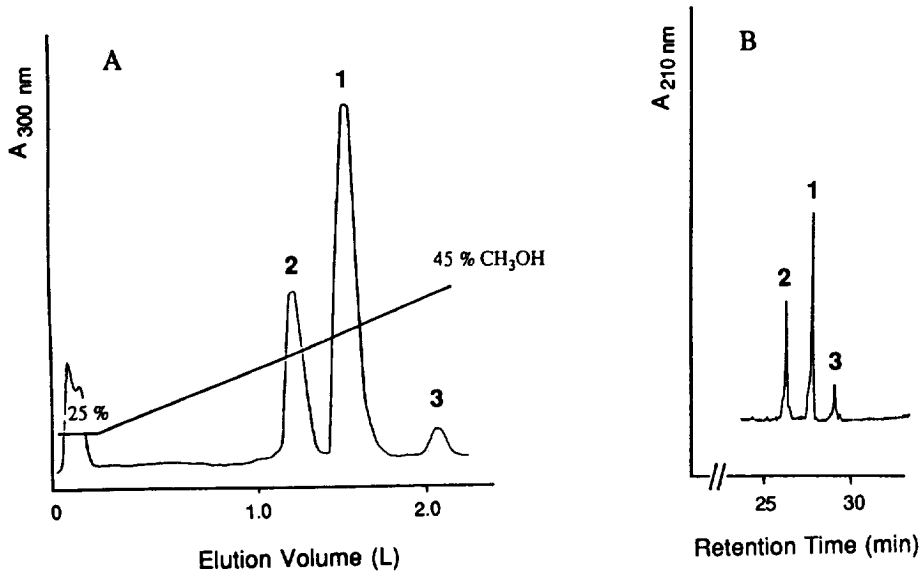


Figure 1. Reversed-phase column chromatography (Merck Lobar column Rp18, size B) (A) and HPLC (YMC-Pack AQ-313) (B) of the mixture obtained by the hydrolysis of **1** with Taka-amylase A for 4 h at 40 °C, where a linear gradient elution from 25% (1 L) to 45% (1 L) of aqueous MeOH or from 10% (30 mL) to 60% (30 mL) aqueous CH_3CN (flow rate, 1.0 mL/min) was applied, respectively.

$6^A,6^D$-Di-*O*-(*p,p'*-biphenyldisulfonyl)-β-cyclodextrin **1**[4] (200 mg) was hydrolyzed enzymatically by TAA (40 mg) in 0.2 M acetate buffer solution (pH 5.6) at 40 °C for 4 h to give **2** (42 mg, 21% and 44% based on the charged and consumed amount of **1**, respectively) and **3** (21 mg) together with the recovery of **1** (50%) (Fig. 1). The FAB mass (FABMS) spectrum of **2** showed the expected ions [M + H^+] and [M + Na^+], suggesting a structure of $6^Y,6^Z$-di-*O*-(*p,p'*-biphenyldisulfonyl)maltoheptaose. The ^{13}C-NMR spectrum (Fig. 2) shows that **2** exists as a mixture of α- and β-anomers, demonstrating the cleavage of macrocycle. The capped positions (Y and Z) were determined by the conversion of **2** to the acetate **4** and analysis of the FAB mass spectral fragmentation (Scheme 2) which suggested that the two phenylthio groups were attached to the third and sixth glucose units from the reducing end.[5] Therefore, **2** is 6",6""-di-*O*-(*p,p'*-biphenyldisulfonyl)maltoheptaose. The attachment of one phenylthio group to the position 6"" was further confirmed by the conversion of **2** by enzymatic hydrolysis with glucoamylase (*Rhyzopus niveus*)[6] at 40 °C in acetate buffer solution (pH 4.7) to the compound showing the FABMS ions [M + H^+] and [M + Na^+] corresponding to those of di-*O*-(*p,p'*-biphenyldisulfonyl)maltohexaose **6**.

Monitoring the progress of the hydrolysis of **1** with TAA demonstrates that **2** gives **3**, which was

shown to be a mixture of di-*O*-(*p*,*p*'-biphenyldisulfonyl)maltohexaose and -pentaose by the FABMS spectrum. The prolonged reaction time (2 d) led to **5** as the final major product which can be reasoned from the previous observations in the cases of 6-*O*-tosylcyclodextrins and the related compounds. The enzymatic hydrolyses of 6-*O*-tosyl-α- or -β-cyclodextrins, $6^{A},6^{D}$-di-*O*-ditosyl-β-cyclodextrin, and the related compounds with TAA gave the 6'-substituted maltoses as the final products,[2,3] whereas longer linear

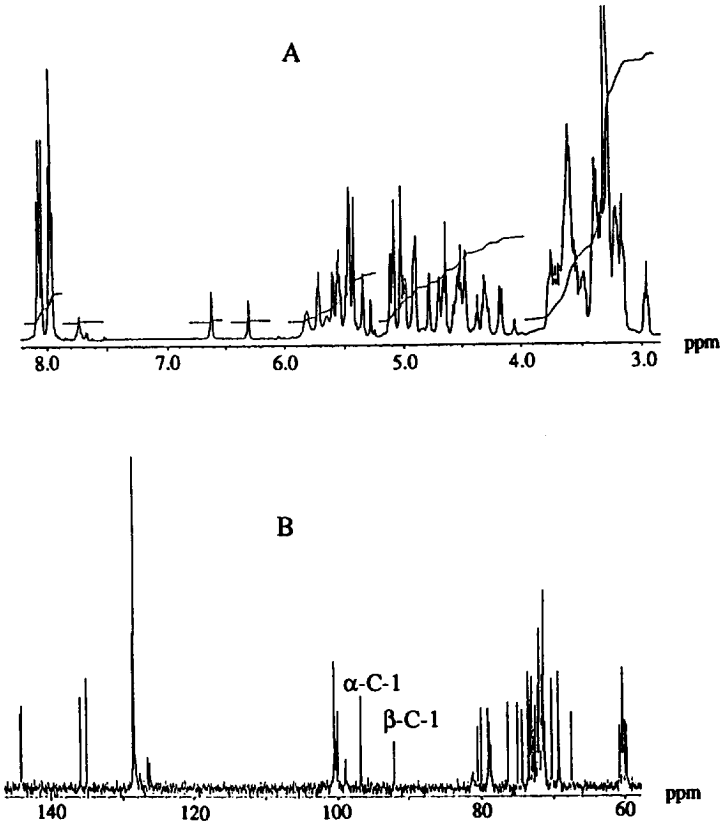
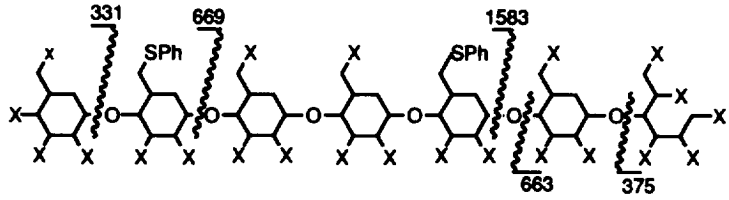


Figure 2. ^{1}H (A) and ^{13}C NMR (B) Spectra of **2** in DMSO-d_6

oligosaccharides were too small in amount to be isolated,[2] because their cleavages were much faster than those of the macrocyclic rings.[3] The present success in isolation of the intermediate **2** in moderate yield is resulted from the cyclic structure of **2** which reasonably resists the succeeding cleavages.



4: X = OAc

Scheme 2. FABMS Spectral Fragmentation Pattern of the Peracetate **4**.

It is interesting to note that in compound **1**, only one given point (shown by a bold arrow in Scheme 1) is mainly cleaved by TAA although other four points (dotted arrows) were potentially possible. This demonstrates that there is an obvious order of acceptability for enzymatic hydrolysis in the glucose-wall of capped cyclodextrin. Therefore, variation of the capped position, the capping group and the kind of cyclodextrin can give various analogues of **2**. The specifically activated oligosaccharide **2** can be easily converted to the corresponding specifically substituted linear oligosaccharides which are difficult to be prepared by any stepwise chemical method. These syntheses are now in progress in our group.

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References and Notes

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3. Fujita, K.; Tahara, T.; Koga, T.; Imoto, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3150-3154.
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5. This procedure for regiochemical determination is already established. For example, see Ref. 2.
6. Glucoamylase is an exo-splitting enzyme that consecutively remove the glucose units from the nonreducing end of starch and glycogen. Ueda, S. *Handbook of Amylases and Related Enzymes. Their Sources, Isolation Methods, Properties and Applications*; Pergamon Press: Oxford. 1988; pp.116-117.

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