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B R I E F C O M M U N I C A T I O N S

CHEMO-ENZYMATIC SYNTHESIS OF HEXAKIS(6-*O*-ALLYL)CYCLOMALTOHEXAOSE

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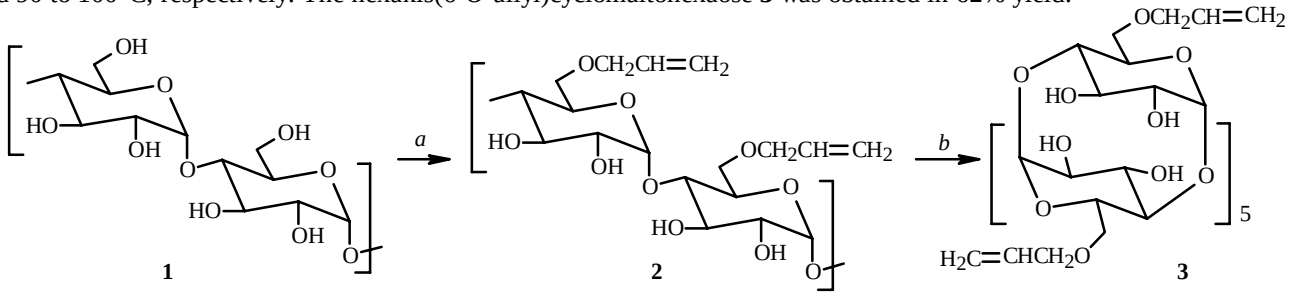
Cyclodextrins [1] (CDs) and their synthetic derivatives [2] attract much attention due to their complexation abilities. CDs consist of six, seven, or eight units of α-1,4-linked D-glucopyranose (named α-, β-, and γ-CD, respectively) and have the shape of truncated cones with secondary hydroxy groups at the 2 and 3 positions of glucose units arranged at the wider rim and primary hydroxy groups at the 6 position at the narrower rim. Hydroxy groups provide the resulting complex with hydrophilic character and also allow further synthetic transformations. In such studies, regioselectively monosubstituted CDs play an important role, but their utility is limited by complicated preparation methods.

Cyclomaltodextrin glucanotransferases (CGTase; EC 2.4.1.19) are able to convert starch into CDs, closed-ring structures in which six or more glucose units are joined by means of α-1,4 glucosidic bonds [3]. Depending on the type of CD (with six, seven, or eight glucopyranose residues: α-, β-, or γ-CD, respectively) initially formed, the CD-forming enzymes are classified as α-, β-, or γ-CGTases [4].

In this study, we present a simple method for the synthesis of hexakis(6-*O*-allyl)cyclomaltohexaose. It will indicate that CD derivatives also can be directly synthesized by the thermostable CGTase catalyzing starch derivatives.

We took advantage of the fact that in excess base the preferred outcome of the reaction of amylose with allyl bromide will be substitution in the O-6 position (Scheme 1). The crude product was isolated via precipitation with EtOH and subsequent freeze-drying. The per-6-*O*-allyl amylose **2** was obtained in 78% yield.

The extremely thermophilic anaerobic archaeon strain B1001 producing CGTase was isolated from a hot-spring environment [5]. The temperatures for per-6-*O*-allyl amylose-degrading activity and cyclodextrin synthesis activity were 110 and 90 to 100°C, respectively. The hexakis(6-*O*-allyl)cyclomaltohexaose **3** was obtained in 62% yield.



1 **2** **3**

a. CH_2=$CHCH_2Br$, NaOH; *b.* α-CGTase

Synthesis of Per-6-*O*-allyl Amylose (2). To a stirred solution of amylose (3.72 mmol, 600 mg) and sodium hydroxide (2.00 g, 50 mmol) in water (20 mL) allyl bromide (323 μL, 3.72 mmol) was added and the resulting emulsion was stirred at room temperature for 12 h. The colorless suspension was diluted with 180 mL of distilled water and neutralization was performed with HCl. The solution was evaporated to dryness. The crude product was isolated via precipitation with EtOH and subsequent freeze-drying. $[\alpha]_D$ +85.7 (*c* 1.1, H_2O). ^{13}C NMR (75MHz, D_2O): δ 134.6 ($CH_2\underline{C}H=CH_2$), 116.9 ($CH_2CH=\underline{C}H_2$), 105.1–96.0 (C-1), 78.5–74.0 (C-4), 70.3–68.2 (C-2, C-3, C-5), 68.1 ($\underline{C}H_2CH=CH_2$), 63.0 (C-6).

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Enzymatic Synthesis of Hexakis(6-*O*-allyl)cyclomaltohexaose (3). The CGTase was obtained by the previously reported way [5]. Reaction mixtures (100 mL) containing 2.5% (wt/vol) soluble per-6-*O*-allyl amylose **2** and 50 mmol/L sodium acetate buffer (pH 5.0) were incubated with the CGTase at 90°C for 24 h. The reaction was terminated at 0°C. Purification by column chromatography with $CHCl_3$–MeOH (10:1 [vol/vol]) gave 7.86 mg **3** in 62% yield. $C_{54}H_{84}O_{30}$. $[\alpha]_D$ +75.3 (*c* 1.1, H_2O). ^1H NMR (300 MHz, D_2O): δ 6.07–5.82 (m, 6H, $CH_2C\underline{H}=CH_2$), 5.39–4.81 (12H, m, $CH_2CH=C\underline{H}_2$), 5.13 (6H, d, H-1), 3.70 (6H, m, H-2), 3.95 (6H, m, H-3), 3.55 (6H, m, H-4), 3.72–3.90 (6H, m, H-5), 3.73–3.94 (12H, m, H-6), 3.61 (s, 12H, $C\underline{H}_2CH=CH_2$), 4.72 (12H, s, OH). ESIMS: *m*/*z* 1235.5 $[M+Na]^+$.

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