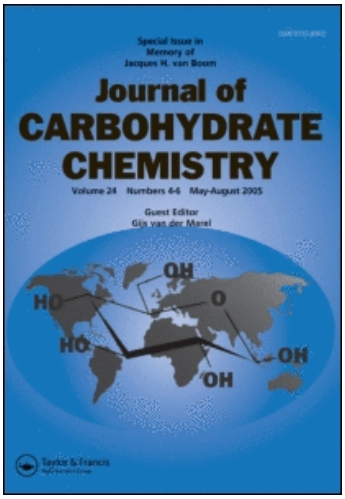

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Noriko Nakanishi[a]; Yoshiko Nagafuchi[a]; Kyoko Koizumi[a]
[a] Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan

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PREPARATION, ISOLATION AND CHARACTERIZATION OF POSITIONAL ISOMERS OF TRIGLUCOPYRANOSYL-CYCLOMALTOHEPTAOSE

Noriko Nakanishi, Yoshiko Nagafuchi, and Kyoko Koizumi*

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663, Japan

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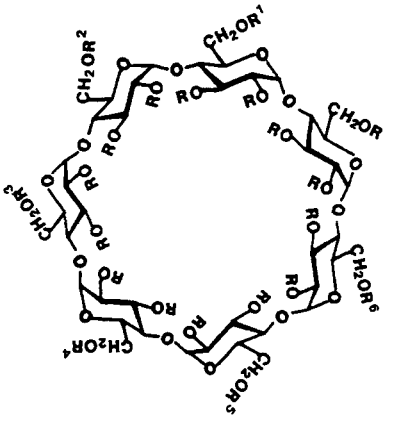
ABSTRACT

The positional isomers of triglucopyranosyl-cyclomaltoheptaose (β-cyclodextrin, βCD) were chemically synthesized by glucosylations of tris(2,3-di-*O*-acetyl)-tetrakis(2,3,6-tri-*O*-acetyl)-βCDs with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetoimidoyl-α-D-glucopyranose in the presence of trifluoromethanesulfonic acid in dichloromethane, followed by debenzylation and deacetylation. The desired $6^1,6^X,6^Y$-tri-*O*-(α-D-glucopyranosyl)-βCDs were isolated from each reaction mixture containing their configurational isomers by HPLC and characterized by ^{13}C NMR spectroscopy.

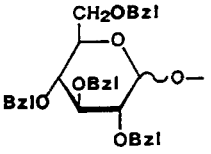
INTRODUCTION

Previously,[1] four regioisomeric tri-*O*-(α-D-glucopyranosyl)-cyclomaltoheptaoses (β-cyclodextrins, βCDs) were isolated from a mixture of glucopyranosyl-βCDs, prepared by glucoamylolysis of a mixture of maltosyl-βCDs produced from maltose and βCD by the reverse action of *Klebsiella pneumoniae* pullulanase. To confirm the structures of these regioisomers by chemical synthesis, five positional isomers of $6^1,6^X,6^Y$-tris-*O*-(*tert*-butyldimethylsilyl)-βCDs as key glucosyl intermediates were prepared, isolated and characterized.[2]

In this paper, we describe synthesis and isolation of the authentic samples of triglucopyranosyl-βCDs and furthermore, present characteristic chromatographic behavior of configurational isomers of each triglucopyranosyl-βCD.



	R	R^1	R^2	R^3	R^4	R^5	R^6
1	Ac	H	Ac	H	Ac	H	Ac
2	Ac	H	H	Ac	Ac	H	Ac
3	Ac	H	H	Ac	Ac	Ac	H
4	Ac	H	H	H	Ac	Ac	Ac
5	Ac	g	Ac	g	Ac	g	Ac
6	Ac	g	g	Ac	Ac	g	Ac
7	Ac	g	g	Ac	Ac	Ac	g
8	Ac	g	g	g	Ac	Ac	Ac
9	H	G	H	G	H	G	H
10	H	G	G	H	H	G	H
11	H	G	G	H	H	H	G
12	H	G	G	G	H	H	H

g : 

G : D-Glucopyranosyl-

RESULTS AND DISCUSSION

Synthesis. Positional isomers of $6^1,6^X,6^Y$-tris-*O*-(*tert*-butyldimethylsilyl)-βCDs, whose structures had been unambigously established, were used as the intermediates for chemical syntheses of authentic positional isomers of $6^1,6^X,6^Y$-tri-*O*-(α-D-glucopyranosyl)-βCDs. Each of four regioisomers of the intermediate, except for $6^1,6^2,6^4$-tris-*O*-(*tert*-butyldimethylsilyl)-βCD, which was very hard to isolate and purify by HPLC, was acetylated and *O*-desilylated to give tris(2,3-di-*O*-acetyl)-tetrakis(2,3,6-tri-*O*-acetyl)-βCDs (**1**, **2**, **3**, and **4**) as the required glucosyl acceptors.

Glucosylation of **1**—**4** was achieved by reaction with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetoimidoyl-α-D-glucopyranose in dichloromethane in the presence of molecular sieves using trifluoromethanesulfonic acid as promoter.[3] Each of glucosylation products (**5**—**8**) was hydrogenated in 10 % formic acid−methanol in the presence of Pd-C and then *O*-deacetylated to afford tri-*O*-(α-D-glucopyranosyl)-βCDs together with its configurational isomers.

Separation and Characterization. Figures 1, 2, 3 and 4 show the elution profiles of configurational isomers of $6^1,6^3,6^5$-, $6^1,6^2,6^5$-, $6^1,6^2,6^6$-, and $6^1,6^2,6^3$-tri-*O*-(D-glucopyranosyl)-βCDs (**9**, **10**, **11**, and **12**), respectively. In each chromatogram, the main peak corresponds to the desired compound (**13**, **14**, **15**, or **16**) having three α-(1→6) linkages, and the relationship between its elution order in respective configurational isomers and the positions of three branches on βCD-ring is characteristic. Namely, the main peak in each chromatogram elutes faster with increasing proximity of the positions of three branches. In the cases of diglucopyranosyl-βCDs[1] and also diglucopyranosyl-αCDs[4] all of configurational isomers having two α-(1→6) linkages eluted most slowly. The ratios of configurational isomers, calculated from the peak areas in each chromatogram, are summarized in Table 1. Although eight kinds of configurational isomers were theoretically considered, it seemed that isomers having three or two β-(1→6) linkages were scarcely produced. The main and some other components were isolated by chromatography on an ODS column (250 × 10 mm i.d.) with methanol−water (5:95−8:92) as the eluent.

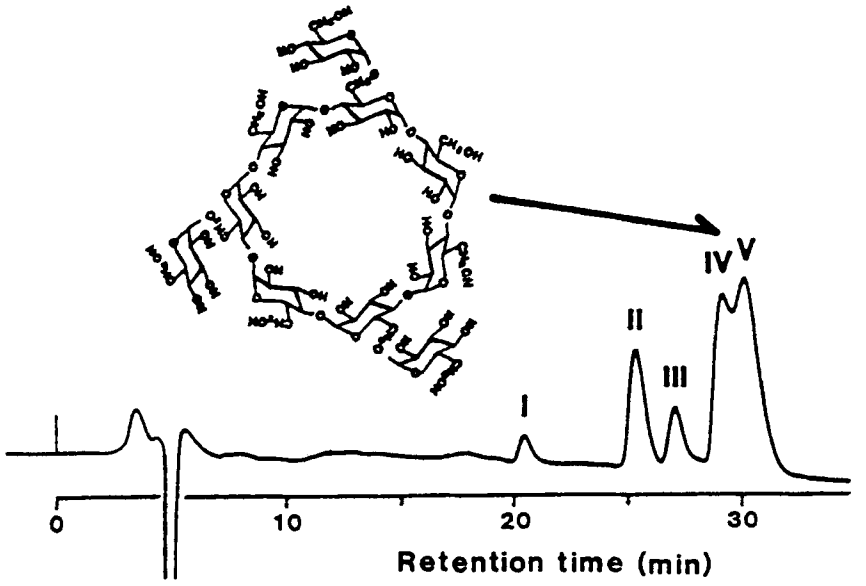


Fig. 1. Elution profiles of $6^1,6^3,6^5$-tri-*O*-(D-glucopyranosyl)-βCDs (**9**). V: $6^1,6^3,6^5$-tri-*O*-(α-D-glucopyranosyl)-βCD (**13**). Chromatographic conditions: column, YMC-Pack AQ-312-3 ODS (150 × 6 mm i.d.); eluent, methanol−water (6 : 94); flow rate, 0.7 mL/min; temperature, 30 °C.

^{13}C NMR spectroscopy data (D_2O) revealed that **A**, **B1** and **B3** as described in a previous paper[1] coincided with **13**, **15** and **14**, respectively. Compound **16** had not been found in a mixture of glucopyranosyl-βCDs prepared previously by an enzymatic method.[1] Consequently, **B2** prepared previously,[1] was assigned as $6^1,6^2,6^4$-tri-*O*-(α-D-glucopyranosyl)-βCD.

Figure 5 shows ^{13}C NMR spectra of $6^1,6^2,6^3$-tri-*O*-(α-D-glucopyranosyl)-βCD (**16**) and a configurational isomer. The signals in the spectra were assigned by reference to the previously reported data[1] and the assign-

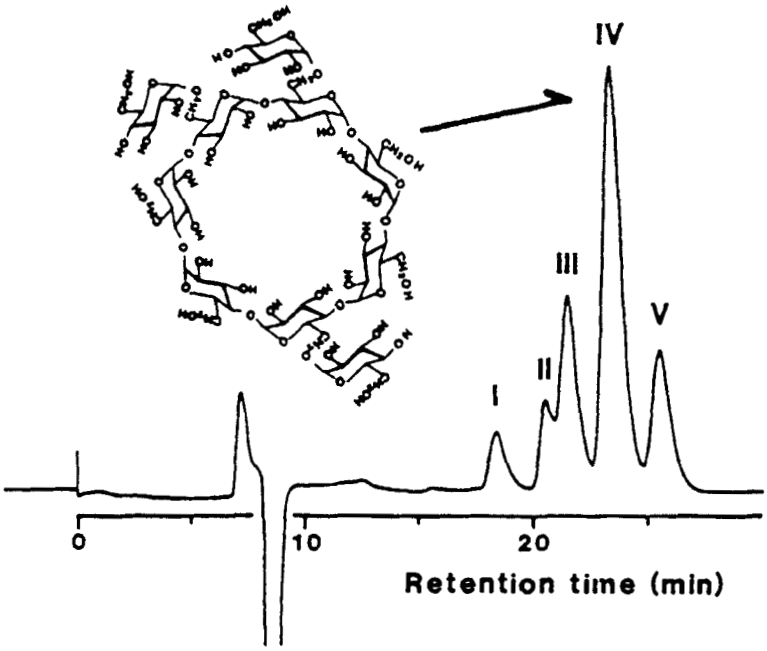


Fig. 2. Elution profiles of $6^1,6^2,6^5$-tri-*O*-(D-glucopyranosyl)-βCDs (**10**). IV: $6^1,6^2,6^5$-tri-*O*-(α-D-glucopyranosyl)-βCD (**14**). Chromatographic conditions: column, YMC-Pack A-312-3 ODS (150 × 6 mm i.d.); eluent, methanol −water (7 : 93); flow rate, 0.4 mL/min; temperature, 25 ℃.

ment of three kinds of C-6 signals were confirmed by the distortionless enhancement by polarization transfer (DEPT) method.[5] The C-1 resonances of the glucose residues of the CD ring, side-chain residues α-(1→6)-linked and β-(1→6)-linked to the CD ring appeared at δ 102.5−102.7, 99.9−100.3, and 104.0 ppm, respectively. In the ^{13}C NMR spectrum of the main product, corresponding to the peak I in **Fig. 4**, three α-(1→6)-linked

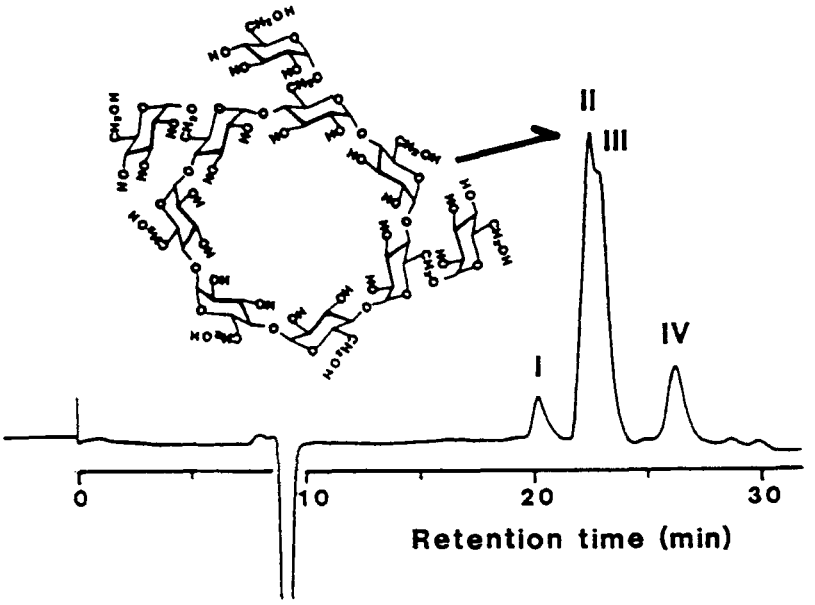


Fig. 3. Elution profiles of $6^1,6^2,6^6$-tri-*O*-(D-glucopyranosyl)-βCDs (**11**). II: $6^1,6^2,6^6$-tri-*O*-(α-D-glucopyranosyl)-βCD (**15**). Chromatographic conditions: eluent, methanol −water (8 : 92); flow rate, 0.3 mL/min ; others as in **Fig. 2.**

C-1 signals and no β-(1→6)-linked C-1 signal were observed; hence the main product was tri-*O*-(α-D-glucosylated) product. The compound corresponding to the last peak IV showed two α-(1→6)-linked and one β-(1→6)-linked C-1 signals, confirming that it was a configurational isomer having two α-(1→6)- and one β-(1→6)-linkages. The minor components corresponding to the peak IV in **Fig. 1**, and the peaks III and V in **Fig. 2** had two α-(1→6)- and one β-(1→6)-linkages. The spectral data relating to C-6 signals were consistent with the structures of these configurational isomers. The large downfield shift of three C-6 signals

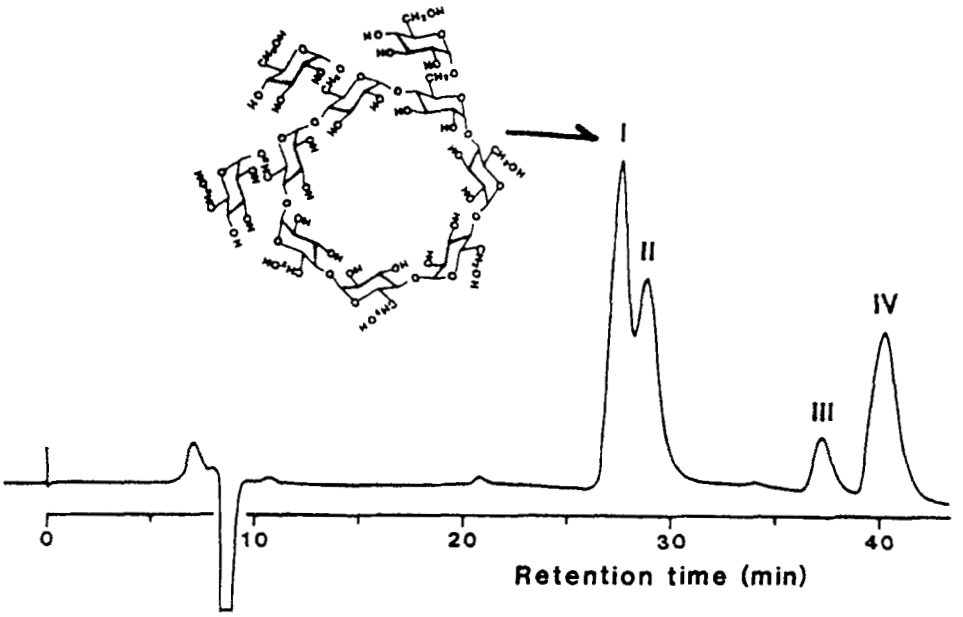


Fig. 4. Elution profiles of $6^1,6^2,6^3$-tri-*O*-(D-glucopyranosyl)-βCDs (**12**). I : $6^1,6^2,6^3$-tri-*O*-(α-D-glucopyranosyl)-βCD (**16**). Chromatographic conditions: temperature, 27 ℃; others as in **Fig. 2**.

TABLE 1. Ratios of Configurational Isomers in the Glucosylation Products

	Peak No.				
Products	I	II	III	IV	V
$6^1,6^3,6^5$-triglucosylated	1.0	5.8	3.5	8.4	<u>14.9</u>
$6^1,6^2,6^5$-triglucosylated	1.0	1.1	3.8	<u>8.6</u>	2.9
$6^1,6^2,6^6$-triglucosylated	1.0	<u>5.8</u>	3.7	1.5	
$6^1,6^2,6^3$-triglucosylated	<u>5.2</u>	4.0	1.0	3.0	

Underlined ones are the tri-*O*-(α-D-glucosylated) product in each mixture of isomers.

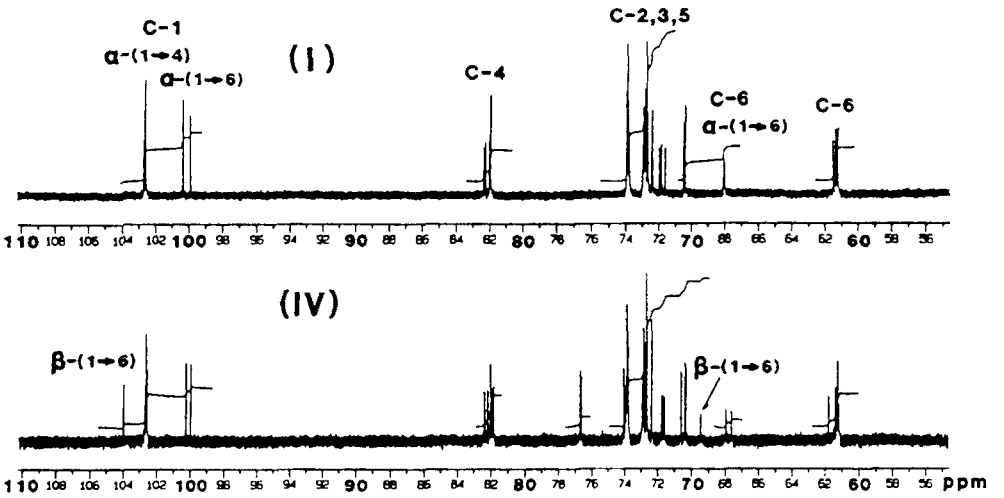


Fig. 5. ^{13}C NMR spectra of $6^1,6^2,6^3$-tri-*O*-(D-glucopyranosyl)-βCDs (**12**) in D_2O at 125.65 MHz. I : α,α,α-substituted product (**16**), IV : α,α,β-substituted isomer (**17**).

indicates that the side-chain D-glucopyranosyl residues were attached to oxygens on these carbon atoms. The signals of C-6 involved in α-(1→6)-linkage and β-(1→6)-linkage appear at δ ~68 and 69.5 ppm, respectively.

EXPERIMENTAL

General Procedures. Optical rotations were measured with a JASCO digital polarimeter, model DIP 360. Centrifugal chromatography was performed with a Harrison Centrifugal Thin Layer Chromatotron, model 7924. HPLC was conducted using a JASCO 880-PU pump, a Waters U6K universal injector, a KNAUER refractive index monitor, and a column oven SSC-3510C (Senshu). The columns used were YMC-Pack AQ-312-3 ODS (150 × 6 mm i.d.), YMC-Pack A-312-3 ODS (150 × 6 mm i.d.), YMC-Pack A-323 ODS (250 × 10 mm i.d.). A Shimadzu Chromatopac C-R3A digital integrator was used for quantitative analyses. ^{13}C NMR spectra (125.65 MHz) were recorded with a JEOL GSX-500 spectrometer for solutions in D_2O (internal Me_4Si).

2,3,4,6-Tetra-*O*-benzyl-1-*O*-trichloroacetoimidoyl-α-D-glucopyranose. To a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucose (1.0 g) in

absolute dichloromethane (10 mL) at -5℃ was added trichloroacetonitrile[6] (1.9 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[7] (55 μL). The mixture was stirred for 1 h at room temperature and diluted with chloroform. The organic layer was thoroughly washed with ice-water, dried, and concentrated (syrup 1.8 g).

Glucosylation of 1－4. To a stirred mixture of **1** (413 mg) and powdered 4 Å molecular sieves (1.0 g) in dry dichloromethane (15 mL) at -20 ℃ was added a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetoimidoyl-α-D-glucopyranose (3.3 g) in dichloromethane under nitrogen. After stirring for 30 minutes, a solution of trifluoromethanesulfonic acid (70 μL) in dichloromethane (1 mL) was added dropwise. The mixture was stirred for 1 h at -20 ℃, made neutral with triethylamine (3 mL), and filtered through Celite. The combined filtrate and washing were washed successively with M sulfuric acid, aq sodium hydrogen carbonate, and water, dried, and concentrated. Centrifugal chromatography with hexane－acetone (3 : 1) of the residue gave chromatographicaly pure $6^1,6^3,6^5$-tri-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (**5**, 629mg, 83.3%). In the same manner as described above, $6^1,6^2,6^5$-tri-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (**6**, 399 mg, 77.8%), $6^1,6^2,6^6$-tri-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (**7**, 66 mg, 54.0%) and $6^1,6^2,6^3$-tri-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-βCD peracetate (**8**, 625 mg, 43.4%) were obtained from **2** (280 mg), **3** (168 mg) and **4** (787 mg), respectively.

$6^1,6^X,6^Y$-Tri-*O*-(D-glucopyranosyl)-βCDs (9－12). A solution of **5** (332 mg), **6** (353 mg), **7** (78 mg), or **8** (451 mg) in 10% formic acid －methanol (20－40 mL) was hydrogenated in the presence of 10% Pd-C (1.3－1.5 g) in a nitrogen atmosphere for 2 h at room temperature. The catalyst was filtered off, washed with methanol, and the combined filtrate and washings were concentrated. The residue was treated with 0.05 N sodium methoxide in methanol (10－20 mL) for 1 h at room temperature, made neutral with Amberlite IR-120B [H^+] resin, filtered, and concentrated. The syrup was shown by HPLC to be comprised of a mixture of configurational isomers, **9** (133 mg, 85.5%), **10** (145 mg, 87.7%), **11** (30 mg, 82.1%), or **12** (129 mg, 61.0%). $6^1,6^3,6^5$-Tri-*O*-(α-D-glucopyranosyl)-βCD, **13** and one of its isomers were isolated from the mixture, **9** by HPLC on a column of YMC-Pack A-323 ODS (250 × 10 mm i.d.) with methanol－water (8 : 92). Similarly, 6^1, 6^2, 6^5-tri-*O*-(α-D-gluco-

pyranosyl)-βCD, **14** and its two isomers, $6^1,6^2$, 6^6-tri-*O*-(α-D-glucopyranosyl)-βCD, **15**, and $6^1,6^2,6^3$-tri-*O*-(α-D-glucopyranosyl)-βCD, **16** and its one isomer, **17** were isolated from the mixtures, **10**, **11**, and **12**, respectively. Specific rotations of $6^1,6^2,6^3$-tri-*O*-(D-glucopyranosyl)-βCDs in H_2O: **16**, $[\alpha]_D^{23}$ +149.0° ; **17**, $[\alpha]_D^{23}$ +145.7° .

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