General Approach to the Synthesis of Persubstituted Hydrophilic and Amphiphilic *β*-Cyclodextrin Derivatives

Tomáš Kraus, Miloš Buděšínský, and Jiří Závada*

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

zavada@uochb.cas.cz

Received January 16, 2001

Heptakis(2,3,6-tri-*O*-allyl)-*β*-cyclodextrin **2** was converted to heptakis[2,3,6-tri-*O*-(2,3-dihydroxypropyl)]-*β*-cyclodextrin **3** by osmium tetroxide-catalyzed dihydroxylation. A diastereomeric mixture of **3** was treated with sodium periodate followed by sodium borohydride to give heptakis[2,3,6-tri-*O*-(2-hydroxyethyl)]-*β*-cyclodextrin **5** in 86% yield. Compound **5** could be quantitatively transformed into heptakis(2,3,6-tri-*O*-carboxymethyl)-*β*-cyclodextrin **6** by TEMPO-mediated oxidation. The same reaction sequence was also applied to heptakis(2,6-di-*O*-allyl-3-*O*-methyl)-*β*-cyclodextrin **8**, heptakis-(2,3-di-*O*-allyl-6-*O*-methyl)-*β*-cyclodextrin **12**, and heptakis(2,3-di-*O*-allyl-6-*O*-butyl)-*β*-cyclodextrin **16**; the analogous corresponding hydroxyethyl and carboxymethyl derivatives were isolated in high yields. All products were proved to be chemically uniform.

Introduction

Modified cyclodextrins with pendent hydroxyalkyl,[1] carboxyalkyl,[1] or plain carboxyl groups[2] have been a subject of intense studies in recent years. The substitution gives the parent cyclodextrins desirable properties: while the hydrophobic interior remains essentially unaffected and retains its ability to form inclusion adducts with lipophilic molecules, the hydrophilization of the exterior enhances solubility in aqueous media. This, in common with the intrinsic chirality of the macrocycle, one may find important applications particularly in analytical and medicinal chemistry (chiral selectors,[3,4] drug delivery systems,[5] viral inhibitors,[6] and other multivalent ligands[7−9]).

The hydroxyalkyl (2-hydroxyethyl, 2-hydroxypropyl) and carboxymethyl derivatives of cyclodextrins have so far been available as polydisperse mixtures of incompletely substituted regioisomeric products,[10] characterized solely by the average degree of substitution. Specific problems in applications of such mixtures have been encountered due to the varying degree of substitution and/or differences in the regioisomeric composition.[11] Obviously, chemically homogeneous cyclodextrin derivatives could be studied more precisely and exploited more effectively than the poorly defined polydisperse mixtures.

For these reasons, the development of synthetic procedures allowing a preparation of the chemically uniform persubstituted derivatives represents a challenging task[12] of a broad interest. In this paper, we report a versatile method allowing the simple preparation of a variety of *β*-cyclodextrin derivatives facially persubstituted with 2,3-dihydroxypropyl, 2-hydroxyethyl, and carboxymethyl moieties in high yields.

Results and Discussion

Previously, we and others reported[13,14] the use of ethyl diazoacetate as an efficient reagent to introduce exhaustively carboxymethyl groups into O(6) or O(3) positions of cyclodextrins. However, the applicability of this reagent is limited to the substrates which are soluble in nonpolar solvents. Accordingly, we failed to achieve carboxymethylation of the parent (very polar) cyclodextrins in this way. Also, recent attempts of exhaustive perhydroxyethylation failed.[15]

(1) The Web of Science database (ISI) has registered over 800 papers related to hydroxypropyl cyclodextrin derivatives and about 90 related to carboxymethyl derivatives since 1990. For a major review on the topic see: Jicsinsky, L.; Hashimoto, H.; Fenyvesi, É. In *Cyclodextrin Derivatives*; Supramolecular Chemistry (Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Lehn, J.-M., Eds.); Vol. 3, Cyclodextrins (Szejtli, J., Osa, T., Eds.); Pergamon: Oxford, U.K., 1996; p 57.

(2) Kraus, T.; Buděšínský, M.; Závada, J. *Eur. J. Org. Chem.* **2000**, 3133−3137 and references therein.

(3) Snopek, J.; Smolková-Keulemansová, E.; Cserháti, T., Stalcup, A. M.; Gahm, K. H. In *Cyclodextrins in Analytical Separation Methods*; Comprehensive Supramolecular Chemistry (Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Lehn, J.-M., Eds.); Vol. 3, Cyclodextrins (Szejtli, J., Osa, T., Eds.); Pergamon: Oxford, U.K., 1996; p 515.

(4) Vespalec, R.; Bocek, P. *Chem. Rev.* **2000**, *100*, 3715−3753.

(5) Nagai, T.; Ueda, H. In *Aspects of Drug Formulation with Cyclodextrins*; Comprehensive Supramolecular Chemistry (Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Lehn, J.-M., Eds.); Vol. 3, Cyclodextrins (Szejtli, J., Osa, T., Eds.); Pergamon: Oxford, U.K., 1996; p 441.

(6) Leydet, A.; Moullet, C.; Roque, J. P.; Witvrouw, M.; Pannecouque, C.; Andrei, G.; Snoeck, R.; Neyts, J.; Schols, D.; De Clercq, E. *J. Med. Chem.* **1998**, *41*, 4927−4932.

(7) Fulton, D. A.; Stoddart, J. F. *Org. Lett.* **2000**, *2*, 1113−1116.

(8) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2755−2794.

(9) Borman, S. *Chem. Eng. News* **2000**, *78*, 48−53.

(10) To the best of our knowledge, only per(6-*O*-carboxymethyl-2,3-di-*O*-methyl)-α- and *β*-cyclodextrin (ref 13) and per(3-*O*-carboxymethyl)-*β*-cyclodextrin (ref 14) have been prepared chemically uniform.

(11) Supposedly identical commercial products sometimes exhibit variable capabilities of chiral resolution: (a) Salvador, A.; Varesio, E.; Dreux, M.; Veuthey, J. L. *Electrophoresis* **1999**, *20*, 2670−2679. (b) Szeman, J.; Ganzler, K.; Salgo, A.; Szejtli, J. *J. Chromatogr. A* **1996**, *728*, 423−431.

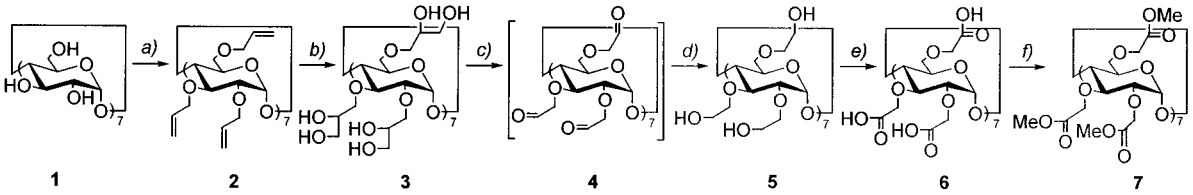
(12) The general complexity of this selectivity problem has been recently outlined (ref 2).

(13) Kraus, T.; Buděšínský, M.; Závada, J. *Carbohydr. Res.* **1997**, *304*, 81−84.

(14) Guillo, F.; Hamelin, B.; Jullien, L.; Canceill, J.; Lehn, J.-M.; De Robertis, L.; Driguez, H. *Bull. Soc. Chim. Fr.* **1995**, *132*, 857−866.

10.1021/jo010046q CCC: $20.00
Published on Web 05/25/2001

Scheme 1[a]



[a] Key: (a) allyl bromide, NaH, DMF; (b) OsO_4, 4-methylmorpholine *N*-oxide, acetone−H_2O; (c) $NaIO_4$, H_2O; (d) $NaBH_4$, H_2O; (e) 1/TEMPO, NaClO, KBr, H_2O, pH = 10; 2/Dowex Li^+ 3/separation of LiCl, LiBr; 4/Dowex H^+; (f) CH_2N_2, MeOH.

Looking for an alternative strategy, we have turned our attention to the allylic group as a convenient synthon. The selection of the allyl function appears advantageous for two important reasons. First, it is known that allyl groups can be introduced[6,16,17] effectively into various positions of the cyclodextrin skeleton. Second, a stepwise oxidation of the allylic functions opens an access to a plethora of novel hydrophilic intermediates, interesting on their own right. We have chosen heptakis(2,3,6-tri-*O*-allyl)-*β*-cyclodextrin[6] **2** as a model compound.

At the onset, we examined a direct (one-pot) transformation of the allyls into the carboxymethyl groupings, employing RuO_4 as an established[18,19] mild reagent for such purposes. Treatment of the perallylated *β*-cyclodextrin **2** with 2 mol % of the ruthenium tetroxide in the acetonitrile−water−carbon tetrachloride mixture under various conditions using $NaIO_4$ as the auxiliary oxidant lead, however, always to an inseparable mixture of products attributable to a nonselective oxidation. Similarly, we failed also with the related (milder) oxidation tandem OsO_4−$NaIO_4$ directed to the one-pot oxidation of the allyls into the aldehyde (−CH_2CHO) groups.[20]

Following these failures, we focused our attention on the individual oxidation steps outlined in Scheme 1. Aiming first at the selective dihydroxylation of the allylic groups, we employed OsO_4-catalyzed oxidation with 4-methylmorpholine *N*-oxide as the auxiliary oxidant.[21] Using 0.5 mol % of osmium tetroxide, a complete dihydroxylation of **2** has been attained within 48 h at room temperature in the water−acetone mixture. Significantly, a prolongation of the reaction time as well as increasing the amounts of both the participating oxidants did not cause overoxidation, demonstrating a remarkably high selectivity of the reagent. Analytical samples of heptakis-[2,3,6-tri-*O*-(2,3-dihydroxypropyl)]-*β*-cyclodextrin **3** were isolated by passing the reaction mixture through an ion-exchanger in H^+ cycle. FAB-MS analysis revealed only one significant peak at *m*/*z* 2712 $[M + Na]^+$ corresponding to the expected molecular mass of **3**. In accord with expectation, the obtained product **3** represents a mixture of diastereomers, as evidenced by line broadening in NMR spectra.

In the next step, the oxidative cleavage of the diol moieties in **3** was smoothly achieved with $NaIO_4$ in the aqueous solution. Isolation of the resulting (unstable) aldehyde derivative **4** was not attempted. Instead, the crude product was reduced with $NaBH_4$ and the resulting heptakis[2,3,6-tri-*O*-(2-hydroxyethyl)]-*β*-cyclodextrin **5** was isolated by reversed-phase chromatography in 86% yield.

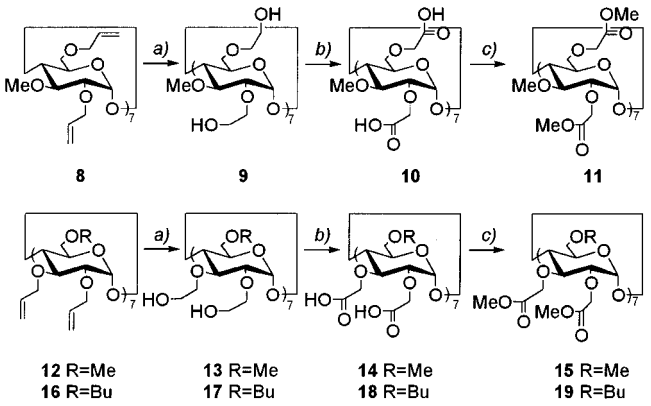
In the last step, the aqueous solution of the cyclodextrin **5** was oxidized with TEMPO−NaClO−NaBr system[23] at pH = 10, which recently proved to be remarkably efficient and selective in a similar situation.[2] After 2 h stirring at room temperature, the reaction mixture was quenched with methanol, and the reduced form of the TEMPO reagent was extracted with ether. The very polar carboxymethyl derivative **6** could not be isolated from the reaction mixture by desalination on a reversed-phase column. Instead, the aqueous layer was passed through an ion-exchanger in Li^+ cycle and the eluted lithium salts were taken up to dryness; double trituration of the solids with hot ethanol removed the soluble LiCl and LiBr salts. The ethanol-insoluble residue was converted into the free acid **6** by ion exchange (H^+ cycle) of the aqueous solution in 94% overall yield. A convenient monitoring of the oxidation was attained on TLC plates, employing samples of the corresponding methyl ester **7** obtained by treatment of the carboxylic acid **6** with diazomethane in methanol. The stepwise facial oxidative transformation of the perallylated *β*-cyclodextrin **2** into the chemically uniform hydrophilized derivatives **5** and **6** has been thus successfully accomplished.

Importantly, the outlined procedures turned out to be generally applicable for a similar transformation of other facially allyl-persubstituted *β*-cyclodextrins. Thus, e.g., heptakis(2,6-di-*O*-allyl-3-*O*-methyl)-*β*-cyclodextrin[16] **8** was transformed into heptakis[2,6-di-*O*-(2-hydroxyethyl)-3-*O*-methyl]-*β*-cyclodextrin **9** in 72% isolated yield (Scheme 2) and subsequently converted into heptakis(2,6-di-*O*-carboxymethyl-3-*O*-methyl)-*β*-cyclodextrin **10**, isolated by desalination on a reversed-phase column in 97%. For TLC monitoring, the free carboxylic acid **10** was converted into the corresponding methyl ester **11** in a quantitative yield.

Also, cyclodextrins allyl-persubstituted only at the secondary face could be hydrophilized in an analogous manner; e.g., heptakis(2,3-di-*O*-allyl-6-*O*-methyl)-*β*-cyclodextrin **12** has been converted, stepwise, into heptakis-[2,3-di-*O*-(2-hydroxyethyl)-6-*O*-methyl]-*β*-cyclodextrin **13** and heptakis(2,3-di-*O*-carboxymethyl-6-*O*-methyl)-*β*-cy-

(15) Ravoo, B. J.; Davies, J. E. D. *Angew. Chem., Int., Ed. Engl.* **2000**, *39*, 4324−4326 and references therein.
(16) Bergeron, R. J.; Meeley, M. P. *Bioorg. Chem.* **1976**, *5*, 121−126.
(17) Baer, H. H.; Shen, Y.; Gonzáles, F. S.; Berenguel, A. V.; García, J. I. *Carbohydr. Res.* **1992**, *235*, 129−139.
(18) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936−3938.
(19) Ghosh, M.; Dulina, R. G.; Kakarla, R.; Sofia, M. J. *J. Org. Chem.* **2000**, *65*, 8387−8390.
(20) Pappo, R.; Allen, D. S.; Lemieux, R. U.; Johnson, S. W. *J. Org. Chem.* **1956**, *21*, 478−479.
(21) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973−1976.
(22) Szejtli J. In *Chemistry, Physical and Biological Properties of Cyclodextrins*; Comprehensive Supramolecular Chemistry (Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Lehn, J.-M., Eds.); Vol. 3, Cyclodextrins (Szejtli, J., Osa, T., Eds.); Pergamon: Oxford, U.K., 1996; p 13.
(23) De Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Carbohydr. Res.* **1995**, *269*, 89−98.

Scheme 2[a]



[a] Key: (a) 1/OsO_4, 4-methylmorpholine *N*-oxide, acetone−H_2O, 2/$NaIO_4$, H_2O, 3/$NaBH_4$, H_2O; (b) TEMPO, NaClO, KBr, H_2O, pH = 10; (c) CH_2N_2, MeOH.

clodextrin **14** in 77% and 94% yields, respectively. Significantly, a quite analogous transformation could be accomplished successfully also with the lipophilic homologue **16** producing the amphiphilic derivatives **17** and **18** in very satisfactory yields (82% and 85%, respectively).

In contrast to the parent compound,[22] the newly prepared 2-hydroxyethylated and carboxymethylated β-cyclodextrin derivatives exhibit high solubility in water (for compounds **5**, **6**, **9**, **10**, **13**, and **14** unlimited) as well as other polar solvents (alcohols). They are strongly hygroscopic—compounds **5**, **6**, **9**, and **13** melt due to the absorption of the air-moisture in loosely closed vials within several days. Amphiphilic character of **17** allows good solubility of this compound in a range of solvents from water to chlorinated hydrocarbons.

Structural identity and homogeneity of the products were proven by ^1H and ^{13}C NMR spectra, which confirmed the preserved symmetry of the molecules. A line broadening effect was observed with hydroxyethyl and carboxymethyl derivatives; however, it was significantly reduced in DMSO-d_6 at elevated temperature (50 °C).

Conclusion

A simple versatile method for the preparation of chemically uniform per-2,3,6-, per-2,3-, and per-2,6-hydroxyethylated and carboxymethylated β-cyclodextrin derivatives has been described. In addition, the diastereomeric mixture of analogous 2,3-dihydroxypropylated derivatives, bearing up to 42 hydroxylic groups per molecule of β-cyclodextrin, can be isolated during the course of the transformation. The persubstitution was achieved via (i) introduction of allyl groups into the desired positions and alkylation of the remaining free (if any) hydroxylic groups; (ii) dihydroxylation of the allylic double bonds; (iii) diol cleavage and reduction to hydroxyethyl derivative in "one pot"; and (iv) TEMPO-mediated oxidation to carboxymethyl derivative. The individual steps of the reaction sequence proved to be highly selective, affording very good to excellent yields of the desired products. It is assumed that the described method will be generally applicable for the preparation of analogous cyclodextrin derivatives also in the α- and γ-series and for similar transformation of oligo- and polysaccharides (starch, cellulose) as well. Undoubtedly, the newly introduced groupings will allow further structure tuning more easily then the sterically congested hydroxylic groups of the native cyclodextrins.

Experimental Section

General Procedure. NMR spectra were recorded at 500 MHz (^1H) and 125.7 MHz (^{13}C). 2D-COSY spectra were used for structural assignment of protons. Carbon signals were assigned using heteronuclear $^1H-^{13}C$ 2D-HMQC spectra. FAB-MS spectra in positive (pos) or negative (neg) mode were recorded using a mixture of glycerol-thioglycerol (T+G) or 2-hydroxyethyl disulfide (DS) as matrixes. Preparative reversed-phase (RP) chromatography was done with a medium-pressure column (1.8 × 28 cm, C-18 modified silica gel, 15 μm, 50 g). Other employed separation procedures were reported elsewhere.[2] All reagents and solvents were commercial products and were used as received unless otherwise noted. β-Cyclodextrin was dried at 100 °C for 8 h over P_2O_5 in vacuo. Compound **8** was prepared according to the known procedure.[16] Correct elemental analysis could not be obtained for compound **3** and carboxymethyl derivatives **6**, **10**, **14**, **18** unless variable numbers of water molecules are taken into account. Thus, calculations based on accurate weights of these compounds (molarity, optical rotation, yields) were corrected with respect to the water content as deduced from elemental analysis.

Heptakis(2,3,6-tri-*O*-allyl)-β-cyclodextrin (2) was prepared by modification of a known procedure.[6] Freshly dried β-cyclodextrin (974 mg, 0.858 mmol) was dissolved in dry DMF (50 mL) under argon atmosphere. Sodium hydride (1.442 g of 60% oil suspension, 36.0 mmol) was washed with petroleum ether (3 × 5 mL) and added to the reaction mixture, followed by allyl bromide (3.90 mL, 45.0 mmol). The reaction flask, equipped with a vertical condenser, was placed into an ultrasonic bath and sonicated[24] for 45 min at 35 °C. Then the ultrasonic bath was removed and the reaction mixture was stirred with a magnetic bar at room temperature. A slightly exothermic reaction, accompanied by evolution of gas, took place. After 12 h, the reaction was quenched with MeOH (1 mL). The volatiles were evaporated under reduced pressure at 40 °C and the residue was partitioned between H_2O (100 mL) and Et_2O (200 mL). The organic layer was washed with brine (3 × 50 mL), dried over $MgSO_4$, and evaporated. Flash chromatography of the residue using gradient elution from neat CH_2Cl_2 to CH_2Cl_2−MeOH 98:2 provided **2** (830 mg, 49%) as a colorless oil: $R_f = 0.30$ (TLC, toluene−EtOH 25:1); $[\alpha]^{25}_D = +92°$ (*c* 0.5, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 1998 for $[M + Na]^+$. Anal. Calcd for $C_{105}H_{154}O_{35}$: C, 63.80; H, 7.86. Found: C, 63.43; H, 7.93.

Heptakis[2,3,6-tri-*O*-(2-hydroxyethyl)]-β-cyclodextrin (5). Compound **2** (595 mg, 0.301 mmol) was dissolved in acetone (10 mL), and 4-methylmorpholine *N*-oxide monohydrate (1.112 g, 9.49 mmol) was added, followed by H_2O (2.5 mL). Osmium tetroxide (0.397 mL of 2.5 wt % solution in *tert*-butyl alcohol, 31.7 μmol) was added to the stirred cloudy mixture. After 1 h, another portion of H_2O (3.5 mL) was added.[25] The homogeneous reaction mixture was kept for 48 h at room temperature under argon with moderate stirring and occasional shaking. Then acetone was evaporated, and the aqueous solution was passed through a column of Dowex 50 (H^+ cycle, 15 mL) and eluted with H_2O (50 mL). The eluate was degassed and bubbled with argon and cooled to 0−5 °C under argon atmosphere, and $NaIO_4$ (2.030 g, 9.49 mmol) was added with stirring. The mixture was kept at room temperature for 2 h. Then it was cooled to 0 °C again, and $NaBH_4$ (838 mg, 22.15 mmol) was gradually added with stirring, maintaining the temperature of the reaction mixture at 0−5 °C for another 60 min, and the mixture was allowed to react at room temperature for 30 min. The excess $NaBH_4$ was decomposed, under cooling, with HCl (2 N), and the pH was adjusted to 4−5. The

(24) Sonication turned out to be essential for the reproducible course of the reaction.

(25) The successive addition of water during the reaction is crucial to ensure solubility of the intermediates in the solution.

Table 1. ^{1}H NMR Data

compd	H-1 J(1,2)	H-2 J(2,3)	H-3 J(3,4)	H-4 J(4,5)	H-5 J(5,6)	H-6 J(5,6')	H-6′ J(6,6')	OMe, OBu	other protons
									$OCH_2CH{=}CH_2$
2	5.17	3.35	~3.73	~3.77	~3.77	3.91	3.58		4.49, 4.26 (2H); 6.01 (1H); 5.22, 5.08 (2H)
	3.7	9.4	*a*	*a*	3.4	1.0	10.7		4.17, 4.14 (2H); 5.91 (1H); 5.26, 5.12 (2H)
									4.01, 3.97 (2H); 5.88 (1H); 5.25, 5.15 (2H)
12	5.16	3.36	~3.75	~3.72	~3.76	3.90	3.52	3.35	4.18, 4.14 (2H); 5.91 (1H); 5.26, 5.12 (2H)
	3.7	9.5	*a*	*a*	3.2	1.3	10.5		4.48, 4.27 (2H); 6.01 (1H); 5.22, 5.08 (2H)
16	5.16	3.33	3.70	3.77	3.75	3.94	3.51	3.46 (2H), 1.56 (2H),	4.48, 4.26 (2H); 6.01 (1H); 5.22, 5.08 (2H)
	3.6	9.5	*a*	*a*	~3.0	<1.5	10.7	1.36 (2H), 0.91 (3H)	4.17, 4.14 (2H); 5.92 (1H); 5.26, 5.11 (2H)
									OCH_2CH_2OH
5	5.22	3.22	~3.55	~3.66	3.72	3.93	3.54		3.86, 3.75 (2H), 3.55 (2H), 4.43 (OH)
	3.5	9.8	8.8	9.8	*a*	3.5	11.3		3.66, 3.60 (2H), 3.53 (2H), 4.34 (OH)
									3.47, 3.40 (2H), 3.52 (2H), 4.41 (OH)
9	5.11	3.20	3.41	3.57	3.72	3.85	3.56	3.54	3.66, 3.58 (2H), 3.55 (2H), (OH)[a]
	3.6	9.8	*a*	9.9	4.0	1.7	11.2		3.46, 3.43 (2H), 3.51 (2H), (OH)[a]
13	5.15	3.24	~3.56	~3.57	3.71	3.82	3.47	3.19	3.85, 3.74 (2H), 3.54 (2H), 4.42 (OH)
	3.6	9.5	~9.5	~10.5	4.2	1.5	11.0		3.65, 3.61 (2H), 3.53 (2H), 4.32 (OH)
17	5.16	3.22	~3.53	~3.58	3.71	3.86	3.49	3.38 (2H), 1.49 (2H),	3.85, 3.74 (2H), 3.53 (2H), 4.43 (OH)
	3.6	9.6	*a*	*a*	3.6	1.8	10.8	1.34 (2H), 0.89 (3H)	3.63, 3.59 (2H), 3.54 (2H). 4.33 (OH)
									OCH_2COOH
6	5.29	3.36	3.60	3.69	3.77	3.94	3.68		4.57, 4.24 (2H), 12.20 (COOH)
	3.7	9.6	8.4	8.4	*a*	3.8	11.8		4.27, 4.15 (2H), 12.20 (COOH)
									4.02, 3.99 (2H), 12.20 (COOH)
10	5.19	3.31	3.43	3.59	3.76	3.89	3.67	3.54	4.26, 4.14 (2H), 12.20 (COOH)
	3.6	9.8	8.5	9.8	4.0	~1.5	11.2		4.02, 3.98 (2H), 12.20 (COOH)
14	5.24	3.35	~3.60	~3.60	3.73	3.79	3.50	3.26	4.57, 4.23 (2H), 12.20 (COOH)
	3.7	~9.6	*a*	*a*	4.4	<1.5	10.7		4.27, 4.16 (2H), 12.20 (COOH)
18	5.24	3.35	3.58	3.63	3.71	3.84	3.50	3.39 (2H), 1.50 (2H),	4.56, 4.21 (2H), 12.20 (COOH)
	3.8	9.3	8.7	9.2	4.0	<2	11.0	1.34 (2H), 0.89 (3H)	4.28, 4.14 (2H), 12.20 (COOH)
									OCH_2COOMe
7	5.32	3.46	~3.71	~3.82	~3.85	3.94	~3.77		4.76, 4.39 (2H), 3.71 (3H)
	3.7	9.8	*a*	*a*	*a*	4.2	11.0		4.46, 4.19 (2H), 3.71 (3H)
									4.17, 4.11 (2H), 3.67 (3H)
11	5.24	3.37	3.57	3.66	3.86	3.94	3.87	3.63	4.45, 4.23 (2H), 3.72 (3H)
	3.8	9.7	8.7	9.7	4.5	1.6	10.8		4.19, 4.13 (2H), 3.71 (3H)
15	5.29	3.45	3.71	~3.78	3.84	~3.78	3.58	3.37	4.76, 4.38 (2H), 3.67 (3H)
	3.8	9.5	*a*	*a*	*a*	<1	10.4		4.44, 4.20 (2H), 3.71 (3H)
19	5.30	3.45	3.70	3.82	3.75	3.86	3.55	3.43 (2H), 1.56 (2H),	4.76, 4.40 (2H), 3.67 (3H)
	3.8	9.8	8.6	9.8	3.8	1.3	10.8	1.36 (2H), 0.91 (3H)	4.45, 4.18 (2H), 3.71 (3H)

[a] Value of parameter could not be determined.

Table 2. ^{13}C NMR Data

compd	C-1	C-2	C-3	C-4	C-5	C-6	OMe, OBu	other carbons
								$OCH_2CH{=}CH_2$
2	98.73	79.22	80.04	79.07	71.00	69.07		74.48, 136.23, 116.80
								72.19, 135.40, 116.73
								72.14, 134.90, 115.62
12	98.67	79.21	78.81	80.00	70.90	71.43	58.95	74.49, 136.16, 116.84
								72.24. 135.60, 115.70
16	98.66	79.29	80.13	78.97	71.16	69.51	71.19, 31.80, 19.36, 13.92	74.49, 136.31, 116.64
								72.14, 135.48, 115.60
								OCH_2CH_2OH
5	97.16	80.26	79.87	77.85	70.80	69.39		74.38, 60.60; 72.51, 60.56; 72.21, 60.21
9	98.02	80.31	81.82	78.73	70.78	69.40	60.74	72.47, 60.70; 72.12, 60.22
13	97.20	80.10	79.85	78.02	70.68	71.17	58.33	74.42, 60.56; 72.26, 60.56
17	97.18	80.24	79.88	78.08	70.79	69.34	70.22, 31.31, 18.82, 13.57	74.47, 60.55; 72.25, 60.55
								OCH_2COOH
6	98.38	79.02	80.86	78.97	70.79	69.80		70.15, 171.47; 68.25, 171.31; 67.60, 171.02
10	98.36	79.68	81.88	79.13	70.78	69.85	60.65	68.15, 171.31; 67.36, 171.23
14	98.57	78.98	80.92	79.22	70.60	71.07	58.28	70.08, 171.62; 67.79, 170.97
18	98.72	78.88	80.89	79.29	70.74	69.08	70.27, 31.28, 18.77, 13.58	70.11, 171.61; 67.73, 170.95
								OCH_2COOMe
7	99.88	80.10	81.18	80.39	71.16	70.33		70.79, 170.65, 51.57
								68.78, 170.57, 51.55
								68.60, 170.57, 51.41
11	99.93	80.61	82.19	80.52	71.08	70.46	61.45	68.53. 170.92, 51.64
								68.33, 170.67, 51.55
15	99.78	80.17	81.35	80.23	71.10	71.29	59.00	70.80, 170.63, 51.56
								68.87, 170.56, 51.44
19	99.71	80.20	81.36	80.20	71.29	69.34	71.26, 31.74, 19.29, 13.89	70.76, 170.63, 51.50
								68.76, 170.61, 51.39

solution was filtered through C-18 modified silica gel (2 g) on a glass frit, and the sorbent was washed with H_2O (10 mL), followed by H_2O−MeOH 50:50 (60 mL). The MeOH-containing portion was collected, then concentrated to approximately 20 mL and charged onto an RP column. Subsequent chromatography (gradient elution from H_2O to H_2O−MeOH 60:40)

afforded **5** (533 mg, 86%) as a white amorphous solid after drying over P_2O_5 at 80 °C in vacuo for 10 h: R_f = 0.67 (RP TLC, H_2O−MeOH 50:50); $[\alpha]^{25}_D$ = +113° (*c* 0.5, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C): see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2082 for $[M + Na]^+$. Anal. Calcd for $C_{84}H_{154}O_{56}$: C, 48.97; H, 7.53. Found: C, 48.50; H, 7.75.

Isolation and Characterization of the Intermediate Diastereomeric Mixture of Heptakis[2,3,6-tri-*O*-(2,3-dihydroxypropyl)]-β-cyclodextrin (3). Compound **2** (71.0 mg, 35.9 μmol) was treated with osmium tetroxide and 4-methylmorpholine *N*-oxide for 48 h as described above. After evaporation of acetone, the aqueous solution was passed through a column of Dowex 50 (2 mL) in H^+ cycle and purified by filtration through a Pasteur pipet (4 × 20 mm, C-18 modified silica gel); gradient elution from H_2O−MeOH 98:2 to 90:10 afforded **3** (96.0 mg, 98% calcd for dihydrate) as a colorless oil after drying at 50 °C in vacuo over P_2O_5 for 10 h: ^1H NMR (DMSO-d_6, 25 °C): δ 3.10−3.82 (m), 3.94 (bs, 7H), 4.42−4.70 (m, 49H), 5.26 (bs, 7H); FAB-MS (T+G, pos) *m*/*z* 2712 for $[M + Na]^+$. Anal. Calcd for $C_{105}H_{196}O_{77}\cdot 2H_2O$: C, 46.25; H, 7.39. Found: C, 46.30; H, 7.45.

Heptakis(2,3,6-tri-*O*-carboxymethyl)-β-cyclodextrin (6). Hydroxyethyl derivative **5** (0.206 g, 0.100 mmol) was dissolved in H_2O (5 mL) and potassium bromide (1 M, 2.1 mL, 2.1 mmol), and then TEMPO (0.328 g, 2.1 mmol) and sodium hydrogen carbonate (0.5 M, 5 mL) were added successively. Sodium hypochlorite (0.61 M, 13.8 mL, 8.4 mmol), precooled to 0 °C, was added to the stirred reaction mixture. The pH of the solution was adjusted to 10.0 by addition of NaOH (0.5 M) and maintained for 2 h at room temperature. Then MeOH (1 mL) was added, and the color of the solution turned from red to yellow. After 1 min, TEMPO was extracted with ether (5 × 30 mL) and the pH of the solution was brought to ∼5 by addition of HCl (3 M). The colorless reaction mixture was charged onto a column of Dowex 50 (40 mL) prepared in Li^+ cycle and eluted with H_2O (200 mL). The eluate was evaporated to a thick syrup, and ethanol (100 mL) was added with stirring. The resulting suspension was warmed to about 60 °C, sonicated, and left to cool. The fine white precipitate was collected, and the trituration procedure was repeated once more. The resulting solid was dissolved in H_2O (2 mL) and passed through a column of Dowex 50 (15 mL) in H^+ cycle. The eluate was concentrated to a volume of about 2 mL and lyophilized to give **6** (0.226 g, 94%, calcd for trihydrate) as a white powder after drying at 50 °C in vacuo over P_2O_5 for 10 h: $[\alpha]^{25}_D$ = +76° (*c* 0.5, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C): see Tables 1 and 2; FAB-MS (DS, neg) *m*/*z* 2352 for $[M - H]^-$. Anal. Calcd for $C_{84}H_{112}O_{77}\cdot 3H_2O$: C, 41.90; H 4.94. Found: C, 41.95; H, 4.86.

Heptakis(2,3,6-tri-*O*-methoxycarbonylmethyl)-β-cyclodextrin (7). Carboxylic acid **6** (50 mg, 20.8 μmol, calcd for trihydrate) was dissolved in MeOH (4 mL) and placed into an ice−water cooling bath. An ethereal solution of diazomethane was added dropwise with stirring until the yellow color of the reaction mixture persisted. After 10 min, MeOH was evaporated and the residue (55 mg) was dissolved in CH_2Cl_2. The solution was filtered through a Pasteur pipet filled with silica gel (4 × 10 mm) and eluted with CH_2Cl_2−MeOH 9:1. Evaporation of the eluate gave **7** (54 mg, 98%) as a white amorphous solid: R_f = 0.5 ($CHCl_3$−MeOH 9:1); $[\alpha]^{25}_D$ = +54 (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2672 for $[M + Na]^+$. Anal. Calcd for $C_{105}H_{154}O_{77}$: C, 47.60; H, 5.86. Found: C, 47.75; H, 5.93.

Heptakis[2,6-di-*O*-(2-hydroxyethyl)-3-*O*-methyl]-β-cyclodextrin (9) was prepared from heptakis(2,6-di-*O*-allyl-3-*O*-methyl)-β-cyclodextrin **8** (0.530 g, 0.296 mmol) analogously to **5**, with the exception of the final purification procedure: the crude reaction mixture was filtered through C-18 modified silica gel (2 g) on a glass frit, and the sorbent was washed with H_2O (10 mL) and then H_2O−MeOH 10:90 (60 mL). The MeOH-containing eluate was concentrated to approximately 10 mL and charged on an RP chromatography column (gradient elution from H_2O−MeOH 95:5 to 40:60) to give **9** (0.393 g, 72%) as a white amorphous solid after drying over P_2O_5 at 80 °C in vacuo for 10 h: R_f = 0.30 (RP TLC, H_2O−MeOH 4:6); $[\alpha]^{25}_D$ = +122° (*c* 0.5, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 1872 for $[M + Na]^+$. Anal. Calcd for $C_{77}H_{140}O_{49}$: C, 49.99; H, 7.63. Found: C, 49.55; H, 7.89.

Heptakis(2,6-di-*O*-carboxymethyl-3-*O*-methyl)-β-cyclodextrin (10) was prepared from **9** (100 mg, 54.1 μmol) analogously to **6**, with the exception of the final purification procedure: TEMPO was extracted with ether (5 × 10 mL) and the pH was brought to 1.5 by addition of 3 M HCl. The colorless reaction mixture was charged onto an RP column. Gradient elution using CH_3CN−H_2O−TFA from 4.0:95.9:0.1 to 30.0:69.9:0.1 followed by concentration and lyophilization of the eluate gave **10** (0.113 g, 97%, calcd for heptahydrate) as a white powder after drying at 50 °C in vacuo over P_2O_5 for 16 h: R_f = 0.15 (RP TLC, CH_3CN−H_2O−TFA 35.0:69.9:0.1); $[\alpha]^{25}_D$ = +87° (*c* 0.5, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C) see Tables 1 and 2; FAB-MS (DS, neg) *m*/*z* 2043 for $[M - H]^-$. Anal. Calcd for $C_{77}H_{112}O_{63}\cdot 7H_2O$: C, 42.58; H, 5.85. Found: C, 42.49; H, 5.53.

Heptakis(2,6-di-*O*-methoxycarbonylmethyl-3-*O*-methyl)-β-cyclodextrin (11) was prepared from **10** (62.0 mg, 28.6 μmol) analogously to **7**. Compound **11** (61 mg, 95%) was isolated as a colorless amorphous solid: R_f = 0.38 ($CHCl_3$−MeOH 95:5); $[\alpha]^{25}_D$ = +71° (*c* 0.7, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2264 for $[M + Na]^+$. Anal. Calcd for $C_{91}H_{140}O_{63}$: C, 45.21; H, 6.29. Found: C, 45.15; H, 6.18.

Heptakis(2,3-di-*O*-allyl-6-*O*-methyl)-β-cyclodextrin (12). Heptakis(2,3-di-*O*-allyl)-β-cyclodextrin[17] (0.303 g, 0.179 mmol) was dissolved in dry DMF (10 mL). Sodium hydride (0.168 g of 60% dispersion, 4.2 mmol) was washed with petroleum ether and added to the stirred solution under argon. The reaction mixture was placed on an ice−water bath, and methyl iodide (0.261 mL, 4.2 mmol) was added dropwise. After 30 min, the cooling bath was removed and the reaction mixture was kept under stirring at room temperature for additional 6 h. The excess of sodium hydride was decomposed by dropwise addition of MeOH (2 mL) with cooling. Solvents were evaporated in vacuo at 40 °C, and the solids were partitioned between H_2O (50 mL) and petroleum ether (100 mL). The organic layer was washed with 10% aqueous solution of sodium thiosulfate (30 mL), H_2O (2 × 30 mL) and dried over magnesium sulfate giving a yellow oily crude product (0.340 g), which was subjected to column chromatography (gradient elution from neat $CHCl_3$ to $CHCl_3$−MeOH 97:3) affording **12** (0.276 g, 86%) as a colorless oil: R_f = 0.4 (toluene−ethanol 9:1); $[\alpha]^{25}_D$ = +113° (*c* 1.1, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 1816 for $[M + Na]^+$. Anal. Calcd for $C_{91}H_{140}O_{35}$: C, 60.91; H, 7.87. Found: C, 60.55; H, 7.84.

Heptakis[2,3-di-*O*-(2-hydroxyethyl)-6-*O*-methyl]-β-cyclodextrin (13) was prepared from **12** (426 mg, 0.237 mmol) analogously to **5**, with the exception of the final purification procedure: the crude reaction mixture was filtered through C-18 modified silica gel (2 g) on a glass frit, and the sorbent was washed with H_2O (20 mL) and then with H_2O−MeOH 30:70 (60 mL). The MeOH-containing portion of the eluate was concentrated to approximately 10 mL and loaded onto an RP column. Subsequent reversed phase chromatography (gradient elution from H_2O to H_2O-MeOH 40:60) afforded **13** (0.340 g, 77%) as a white amorphous solid after drying over P_2O_5 at 80 °C in vacuo for 10 h: R_f = 0.34 (RP TLC, H_2O−MeOH 4:6); $[\alpha]^{25}_D$ = +131° (*c* 0.5, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 1872 for $[M + Na]^+$. Anal. Calcd for $C_{77}H_{140}O_{49}$: C, 49.99; H, 7.63. Found: C, 49.64; H, 7.86.

Heptakis(2,3-di-*O*-carboxymethyl-6-*O*-methyl)-β-cyclodextrin (14) was prepared from **13** (163 mg, 88.1 μmol) analogously to **6**, with the exception of the final purification procedure: TEMPO was extracted with ether (5 × 10 mL), and pH was brought to 1.5 by addition of 3 M HCl. The colorless reaction mixture was charged onto an RP column. Gradient elution using CH_3CN−H_2O−TFA from 4.0:95.9:0.1 to 30.0:69.9:0.1 (v/v) followed by concentration and lyophilization of the eluate gave **14** (0.180 g, 94%, calcd for heptahydrate)

as a white powder after repeated drying at 60 °C in vacuo over P_2O_5 for 16 h: R_f = 0.41 (RP TLC, CH_3CN-H_2O-TFA 30.0:69.9:0.1); $[\alpha]^{25}_D = +58°$ (*c* 0.5, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C) see Tables 1 and 2; FAB-MS (DS, neg) *m*/*z* 2044 for $[M - H]^-$. Anal. Calcd for $C_{77}H_{112}O_{63}\cdot 7H_2O$: C, 42.58; H 5.85. Found: C, 42.40; H, 5.65.

Heptakis(2,3-di-*O*-methoxycarbonylmethyl-6-*O*-methyl)-β-cyclodextrin (15) was prepared from **14** (0.050 g, 23.0 μmol) analogously to **7**. Compound **14** (0.047 g, 92%) was isolated as a colorless amorphous foam: R_f = 0.60 ($CHCl_3$−MeOH 9:1); $[\alpha]^{25}_D = +56°$ (*c* 0.5, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2265 for $[M + Na]^+$. Anal. Calcd for $C_{91}H_{140}O_{63}$: C, 45.21; H, 6.29. Found: C, 45.15; H, 6.18.

Heptakis(2,3-di-*O*-allyl-6-*O*-butyl)-β-cyclodextrin (16). Heptakis(2,3-di-*O*-allyl)-β-cyclodextrin[17] (0.522 g, 0.308 mmol) was dissolved in dry DMF (20 mL). Sodium hydride (0.258 g of 60% dispersion, 6.46 mmol) was extracted with petroleum ether and added to the stirred solution under argon. The reaction mixture was placed on an ice−water bath, and butyl iodide (0.735 mL, 6.46 mmol) was added dropwise. After 30 min, the cooling bath was removed, and the stirred reaction mixture was protected from light and kept at room temperature for 12 h. The excess of sodium hydride was then decomposed by dropwise addition of MeOH (2 mL) with cooling. Solvents were evaporated in vacuo at 40 °C, and the solids were partitioned between water (50 mL) and petroleum ether (200 mL). The organic layer was washed with 10% aqueous solution of sodium thiosulfate (30 mL) and H_2O (2 × 30 mL) and dried over magnesium sulfate to give a yellow oily crude product (0.630 g) that was subjected to column chromatography (silica gel, 20 g, gradient elution from neat petroleum ether to petroleum ether−acetone 9:1), affording **16** (0.450 g, 70%) as a colorless oil: R_f = 0.35 (petroleum ether−acetone 8:2); $[\alpha]^{25}_D = +92$ (*c* 0.9, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2110 for $[M + Na]^+$. Anal. Calcd for $C_{112}H_{182}O_{35}$: C, 64.39; H 8.79. Found: C, 64.57; H, 8.86.

Heptakis[2,3-di-*O*-(2-hydroxyethyl]-6-*O*-butyl]-β-cyclodextrin (17) was prepared from **16** (323 mg, 0.138 mmol) analogously to **5**, with the exception of the final purification procedure: the crude reaction mixture was filtered through C-18 modified silica gel (2 g) on a glass frit, and the sorbent was washed with H_2O (10 mL) and then with neat MeOH (60 mL). The methanolic fraction was concentrated to dryness, redissolved in H_2O, and loaded onto an RP column. Subsequent RP chromatography (gradient elution in H_2O−MeOH from 50:50 to 5:95) afforded **17** (0.242 g, 82%) as a white amorphous solid after drying over P_2O_5 at 70 °C in vacuo for 20 h: R_f = 0.15 (RP TLC, H_2O−MeOH 1:9); $[\alpha]^{25}_D = +108°$ (*c* 0.8, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2166 for $[M + Na]^+$. Anal. Calcd for $C_{98}H_{182}O_{49}$: C, 54.87; H, 8.56. Found: C, 54.43; H, 8.86.

Heptakis(2,3-di-*O*-carboxymethyl-6-*O*-butyl)-β-cyclodextrin (18) was prepared from **17** (136 mg, 63.4 μmol) analogously to **6**, with the exception of the final purification procedure: TEMPO was extracted with ether (5 × 10 mL), and the pH was brought to 1.5 by addition of 3 M HCl. The colorless reaction mixture was charged onto an RP column. Gradient elution using CH_3CN-H_2O-TFA from 4.0:95.9:0.1 to 70.0:29.9:0.1 (v/v) followed by concentration and lyophilization of the eluate gave **18** (0.133 g, 85%, calcd for heptahydrate) as a white powder after repeated drying at 60 °C in vacuo over P_2O_5 for 16 h: R_f = 0.10 (RP TLC, CH_3CN-H_2O-TFA 50.0:49.9:0.1); $[\alpha]^{25}_D = +73°$ (*c* 0.6, H_2O); ^1H and ^{13}C NMR (DMSO-d_6, 50 °C) see Tables 1 and 2; FAB-MS (DS, neg) *m*/*z* 2339 for $[M - H]^-$. Anal. Calcd for $C_{98}H_{154}O_{63}\cdot 7H_2O$: C, 47.73; H, 6.87. Found: C, 47.48; H, 6.91.

Heptakis(2,3-di-*O*-methoxycarbonylmethyl-6-*O*-butyl)-β-cyclodextrin (19) was prepared from **18** (0.050 g, 20.3 μmol) analogously to **7**. Compound **19** (0.049 g, 96%) was isolated as a colorless amorphous foam: R_f = 0.50 ($CHCl_3$−MeOH 95:5); $[\alpha]^{25}_D = +41°$ (*c* 0.4, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$, 25 °C) see Tables 1 and 2; FAB-MS (DS, pos) *m*/*z* 2560 for $[M + Na]^+$. Anal. Calcd for $C_{112}H_{182}O_{63}$: C, 53.03; H, 7.23. Found: C, 53.38; H, 7.42.

Acknowledgment. This work was supported by the Grant Agency of the Czech Republic (Grant No. 203/00/0138).

Supporting Information Available: ^1H and ^{13}C spectra of compounds **2**, **5**−**7**, and **9**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010046Q