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Synthesis of Cyclodextrins with Carboxylic Acids at the Secondary Rim and an Evaluation of Their Properties as Chemzymes for Glycoside Hydrolysis

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Seven β-cyclodextrin-mono-, -di- or -tetra-*O*-acetic acids were prepared and investigated for their properties as catalysts of the hydrolysis of four nitrophenyl glycosides.

Introduction

The mild conditions, selectivity and speed of biochemical processes greatly exceed ordinary chemical reactions. This efficiency is achieved exclusively through enzyme catalysis. Enzymes use binding and proximity effects to achieve large rate enhancements (up to 10^{23})[1] for specific reactions and substrates. It would be highly desirable if chemists could mimic the enzyme's catalytic process and learn how to create catalysts suited for otherwise impossible processes. Such chemzymes[2] or enzyme mimics[3] have been reported to catalyze enzyme-like transformations from a variety of substrates.[4]

Interestingly, glycoside hydrolysis is one enzymatic transformation that is both biologically widespread and important, and has some of the highest enzymatic rate enhancements observed.[1] It is also one of the enzymatic reactions that has been subject to the most detailed study.[5] Retaining glycosidases have two carboxylic acids in the active site with one acting as an acetal protonator and the other as a nucleophile. Inverting glycosidases also have two carboxylic acids with similar functions except that the nucleophile now is a general base on a water molecule making the latter nucleophilic.[6] In the retaining enzymes the distance between the carboxylate groups is 5.0 Å, whereas it is 10.5 Å in the inverting glycosidases. The detailed information available about glycosidases,[6] and the stoichiometric simplicity of the reaction catalyzed by these enzymes, makes the glycosidase reaction ideal to mimic.

With cyclodextrin-based mimics of glycosidases progress has been made.[2,4] The role of the cyclodextrin is to bind an aromatic group of an aryl glycoside in the cavity with the sugar protruding. Catalytic groups (carboxylic acids, cyanohydrin and so on) attached to the cyclodextrin rim can then potentially catalyze the cleavage reaction. Indeed, cyclodextrin itself has been shown to be able to catalyze hydrolysis of aryl glycosides. α-Cyclodextrin can accelerate the hydrolysis of 4-nitrophenyl glycosides by up to 6-fold at pH = 12.5,[7] whereas it was shown that α- or β-cyclodextrin can accelerate the hydrolysis of 2-(deoxyglycosyl)pyridinium salts at pH = 7.[8] However, cyclodextrins where the C-6 carbon atoms have been oxidized to carboxylate groups were more efficient. At pH = 7–8 a hydrolysis rate increase (k_{cat}/k_{uncat}) of up to 10^3 was achieved with a compound that had two carboxylic acid groups at the A and D residues.[9] A compound with a single carboxylic acid group at the primary rim did, however, not catalyze hydrolysis. The catalysis by these compounds was believed to be caused by electrostatic effects.

Because phenyl glycosides can bind to cyclodextrins with the monosaccharide protruding from either face it may be anticipated that carboxylic acid groups at the secondary rim could also catalyze hydrolysis. Indeed, we recently observed[10] catalysis from a per-*O*-methylated β-cyclodextrin having a carboxymethyl or carboxyethyl group at the 2-OH position, which was surprising because (1) a single carboxylate group was sufficient for catalysis, (2) the corresponding 6-*O*-carboxymethyl derivative was inactive, and (3) previously the unmethylated 2-*O*-carboxymethyl-β-cyclodextrin was found inactive (though on different glycoside substrates).[8] To obtain more insight into these puzzling results we have, in this paper, synthesized a full series of mono-*O*-carboxymethyl-β-cyclodextrins with and without permethylation as well as a di- and a tetra-*O*-carboxymethyl compound (**7**–**9** and **19**–**22**; Figure 1). We find that the 2-*O*-carboxymethyl-substituted cyclodextrins generally have a very low or non-existing activity regardless of the position of the catalytic group and were not able to confirm the level of catalysis previously reported in 2-*O*-carboxymethyl-per-*O*-methylcyclodextrin.

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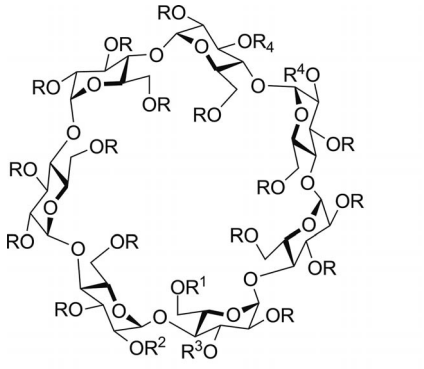
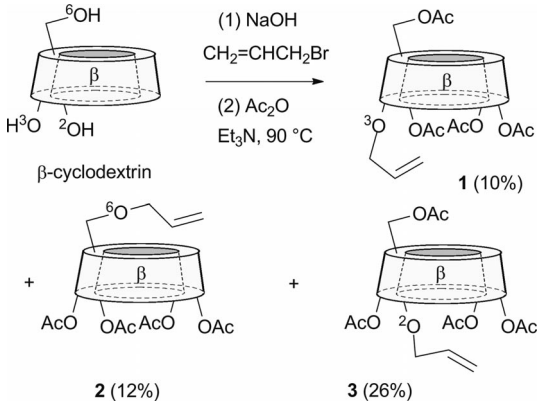


β-cyclodextrin: R = R^1 = R^2 = R^3 = R^4 = H
7: R^3 = CH_2COOH, R = R^1 = R^2 = R^4 = H
8: R^1 = CH_2COOH, R = R^2 = R^3 = R^4 = H
9: R^2 = CH_2COOH, R = R^1 = R^3 = R^4 = H
19: R^2 = R^3 = CH_2COOH, R = R^1 = R^4 = CH_3
20: R^2 = CH_2COOH, R = R^1 = R^3 = R^4 = CH_3
21: R^3 = CH_2COOH, R = R^1 = R^2 = R^4 = CH_3
22: R^2 = R^3 = R^4 = CH_2COOH, R = R^1 = CH_3

Figure 1. *O*-Carboxymethylated cyclodextrins prepared in this work.

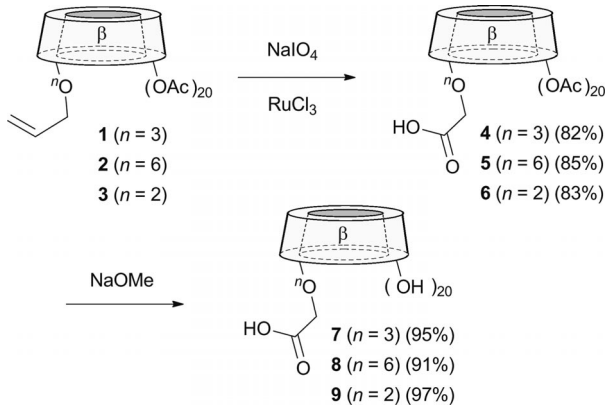
Results and Discussion

Compounds **7**–**9** and **20**–**21** (Figure 1) were all prepared by mono-allylation of β-cyclodextrin as outlined in Scheme 1. The synthesis was based on previously reported allylations.[11] β-Cyclodextrin was treated with allyl bromide in aqueous NaOH, which gave a mixture of unreacted, mono-, di- and polyallylated β-cyclodextrins. The monoallylated fraction was separated from the unreacted and overreacted fractions by chromatography on silica gel by using 2-propanol/aqueous ammonia. After peracetylation of the monoallyl fraction with Ac_2O/Et_3N at elevated temperatures, it was possible to separate the isomers by chromatography to give **1**, **2** and **3** (eluted in that order) in 10, 12 and 26% yield, respectively.

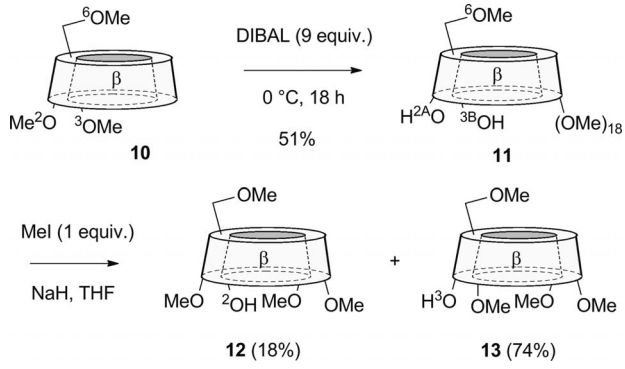


Scheme 1. Monoallylation of β-cyclodextrin.

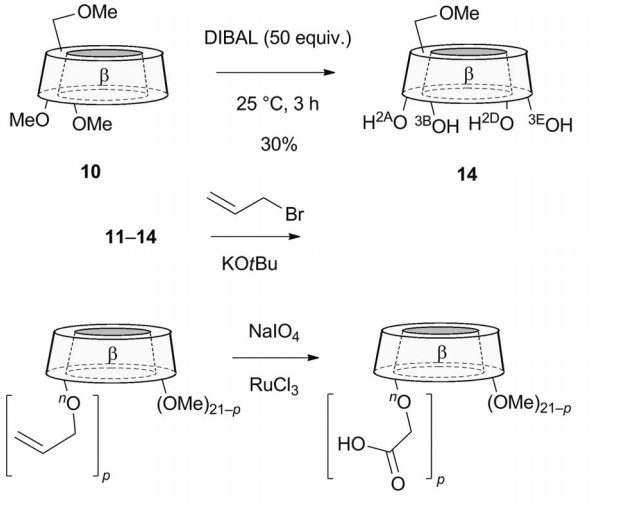
Synthesis of the three possible carboxymethyl-substituted β-cyclodextrins (**7**–**9**) then proceeded easily from **1**, **2** or **3** by the procedure outlined in Scheme 2. Oxidative cleavage with $RuCl_3/NaIO_4$ in aqueous acetonitrile gave acids **4**–**6** in 82–85% yield. Zemplen deacetylation with NaOMe in methanol gave **7**–**9** in 91–97% yield (Scheme 2).



Scheme 2. Synthesis of mono-*O*-carboxymethyl-cyclodextrins **7**–**9**.



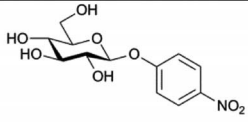
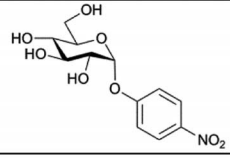
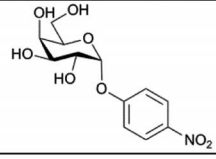
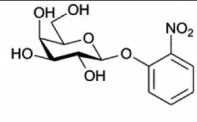
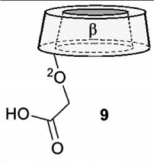
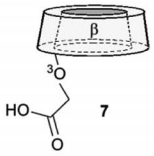
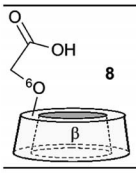
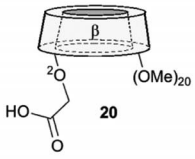
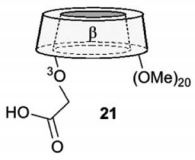
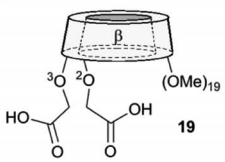
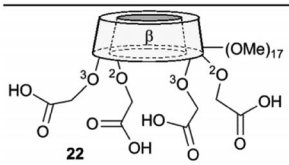
Scheme 3. Synthesis of monools **12** and **13**.



Scheme 4. Synthesis of per-*O*-methylated acids **19**–**22**.

The *O*-methylated derivatives were prepared by using the selective demethylation strategy reported by the Sollogoub group.[12] Reaction of per-*O*-methyl-β-cyclodextrin with Diisobutylaluminium hydride (DIBAL) gave 2A,3B-diol (according to the Sollogoub nomenclature) in 51% yield (Scheme 3). Other products such as monools, triols and tetraols are also formed in this reaction, and unreacted starting material is also present. Compound **11** was separated by using chromatography and then treated with 1 equiv. of MeI and NaH in THF to produce a mixture of monools **12** and **13**. Not surprisingly the 2-OH group, which is more acidic,[13] is alkylated more readily under these conditions, and chromatographic separation gave **12** and **13** in 18 and 74% yield, respectively (Scheme 3). Sollogoub et al.[12] have also described more extensive demethylation of **10**, and this chemistry was also tried. Reaction of **10** with 50 equiv. of DIBAL at room temperature gave the tetraol **14**, which was isolated in 30% yield after chromatography (Scheme 4). Many other products were also formed during this reaction. Compounds **11**, **12**, **13**, and **14** were all *O*-allylated by using

Table 1. Kinetic data for the hydrolysis of the various glycoside substrates (4–25 mM) in phosphate buffer (500 mM, pH = 8.0) by various catalysts (0.6 mM).

Catalyst	Substrate 			
 **9**	no catalysis	no catalysis	no catalysis	no catalysis
 **7**	no catalysis	$k_{cat} = 3.5\times10^{-6}$ s^{-1} $K_M = 56\pm28$ mM $k_{cat}/k_{uncat} = 80\pm39$	no Michaelis–Menten catalysis	no Michaelis–Menten catalysis
 **8**	no catalysis	no catalysis	no catalysis	no catalysis
 **20**	$k_{cat} = 4.4\times10^{-7}$ s^{-1} $K_M = 13\pm4$ mM $k_{cat}/k_{uncat} = 23.1\pm4.7$	not determined	not determined	no catalysis
 **21**	no Michaelis–Menten catalysis	$k_{cat} = 9.5\times10^{-7}$ s^{-1} $K_M = 4.8\pm2$ mM $k_{cat}/k_{uncat} = 17.5\pm3.7$	no catalysis	no catalysis
 **19**	no Michaelis–Menten catalysis	$k_{cat} = 5.4\times10^{-6}$ s^{-1} $K_M = 63.2\pm13$ mM $k_{cat}/k_{uncat} = 105\pm11$	$k_{cat} = 5.1\times10^{-7}$ s^{-1} $K_M = 25.6\pm16$ mM $k_{cat}/k_{uncat} = 19\pm10$	no Michaelis–Menten catalysis
 **22**	no Michaelis–Menten catalysis	not determined	no Michaelis–Menten catalysis	no Michaelis–Menten catalysis

excess allyl bromide and potassium *tert*-butoxide in dimethyl sulfoxide (DMSO). This gave the expected mono-, di- or tetra-*O*-allylated products **15**–**18** in 71–87% yield. Oxidation of **15**–**18** with $RuCl_3/NaIO_4$ gave the corresponding mono-, di- and tetracarboxylic acids **19**–**22** smoothly in 63–79% yield.

The cyclodextrin acids **7**–**9** and **19**–**22** were tested for catalysis of aryl glycoside hydrolysis. The hydrolysis of four nitrophenyl glycosides (Table 1) at 60 °C and pH = 8.0 was monitored with and without the presence of a cyclodextrin derivative (0.6 mM) at various concentrations. The phosphate concentration was 0.5 M, and the native cyclodextrins gave no catalysis under these conditions. Where catalysis was observed the data were analyzed for saturation kinetics. In this study the unprotected cyclodextrin derivatives **7**–**9** were found to be surprisingly poor catalysts (Table 1). Neither the 2-*O*-carboxymethyl (**9**) nor the 6-*O*-carboxymethyl (**8**) compounds showed any catalysis at pH = 8.0. The 3-*O* analogue **7** showed catalysis with 3 substrates, but only Michaelis–Menten kinetics with α-glucopyranoside. In this case the substrate binding was rather poor (K_M = 53 mM). The rate acceleration that occurs when the substrate is bound inside the cavity is k_{cat}/k_{uncat} = 80.

The *O*-methylated cyclodextrin acids all displayed some catalysis, though in many cases saturation kinetics could not be observed, and in general the catalysis was poor. In the cases where Michaelis–Menten catalysis was observed k_{cat} varied in the range $4.4–54 \times 10^{-7}$ s^{-1}, which is lower than was observed with diacids.[9] The highest rate acceleration was seen with diacid **19**, where a rate acceleration of 105 was observed for the hydrolysis of 4-nitrophenyl α-D-glucopyranoside. This obviously means that having two carboxylate groups is better than one, and that the likely cause for this is the higher probability (in **19**) that the substrate will bind close to an acid. On the other hand **22**, which has four acid groups, does not work. Catalysis was seen but not involving the cyclodextrin cavity, and it is possible that having four methylcarboxylate groups at the primary rim is too many to allow effective binding of these substrates. It is obvious from models that there is no steric hindrance in **22** as the carboxymethyl groups are small, and there is plenty of space at the secondary face when four (or even more) of these groups are attached. Therefore, it appears more likely that it is electrostatic effects that make the productive binding of the substrate unfavorable in the case of **22**.

More specifically the catalysis by **20**, which was previously reported[10] to give a k_{cat} value of 2.7×10^{-5} s^{-1}, was found in this study to give a 50 times lower value. Repeating the synthesis of **20** by using the method described in that paper, which was oxidation of the allyl group in two steps by ozone and sodium chlorate,[10] did not change the value. Thus, we could not confirm our preliminary results with **20**.

Conclusions

Our results consistently show that a β-cyclodextrin with an *O*-carboxymethyl group is a rather poor catalyst of the aryl glycoside hydrolysis reaction regardless of whether the remaining hydroxy groups are *O*-methylated or not.

Experimental Section

General: Solvents were dried by means of a solvent purification system. All reagents were used as received. Solvent evaporation was carried out by using a rotary evaporator. Glassware used for water-free reactions was dried at 130 °C for 2 h before use. Chromatography columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC plates (Merck, 60, F_{254}) were visualized by spraying with cerium sulfate (1%) and molybdic acid (1.5%) in 10% H_2SO_4 and heating until colored spots appeared. ^1H NMR, ^{13}C NMR and COSY spectra were recorded with a Bruker 500 MHz instrument.

Per-*O*-acetyl-3^A-*O*-allyl-β-cyclodextrin (1), Per-*O*-acetyl-6^A-*O*-allyl-β-cyclodextrin (2) and Per-*O*-acetyl-2^A-*O*-allyl-β-cyclodextrin (3): To a stirred solution of β-cyclodextrin hydrate (10.0 g, 8.8 mmol) and sodium hydroxide (3.87 g, 96.8 mmol) in water (44 mL), allyl bromide (381 μL, 4.4 mmol) was slowly added, and the resulting emulsion was stirred at room temp. for 12 h. The mixture was then neutralized with sulfuric acid, concentrated to dryness, and a mixture of mono-allyl-β-cyclodextrin regioisomers was separated from β-cyclodextrin and di- and polysubstituted β-cyclodextrin derivatives by chromatography on silica gel (elution mixture 1-PrOH/H_2O/concd. aq. NH_3, 10:2.5:1). The resulting mixture of regioisomers was peracetylated: acetic anhydride (20 mL) and triethylamine (20 mL) were added, and the mixture was stirred and heated to 90 °C for 3 h. The reagents were then co-evaporated with toluene, and the residue was dissolved in EtOAc (400 mL), extracted with HCl (1 M, 400 mL) and H_2O (3 × 400 mL). Then the organic phase was dried with $MgSO_4$ and concentrated under reduced pressure. Purification by using a silica gel column afforded compound **1** (10%, 800 mg) as a white foam. R_f = 0.32 (CH_2Cl_2/MeOH, 30:1). $[\alpha]_D$ = +124.1 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.96–5.88 (m, 1 H, C*H*=CH_2), 5.49 (dd, *J* = 10.1, 8.9 Hz, 1 H, 3-H), 5.36–5.25 (m, 6 H, 3-H, CH=CH_2), 5.14–5.03 (m, 8 H, 1-H, CH=CH_2), 4.81–4.67 (m, 7 H, 2-H), 4.61–4.46 (m, 8 H, 6-H, OCH_2CH=CH_2), 4.38 (dd, *J* = 12.5, 3.7 Hz, 1 H, 6-H), 4.30–4.04 (m, 13 H, 6-H, OCH_2CH=CH_2, 5-H), 3.90 (dd, *J* = 9.6, 1.6 Hz, 1 H, 5-H), 3.80 (dd, *J* = 10.0, 8.8 Hz, 1 H, 3-H), 3.77–3.65 (m, 6 H, 4-H), 3.58 (t, *J* = 9.2 Hz, 1 H, 4-H), 2.14–1.98 (m, 60 H, OAc) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.03, 170.96, 170.87, 170.77, 170.71, 170.69, 170.66, 170.61, 170.52, 170.51, 170.49, 170.40 (C=O), 169.73, 169.68, 169.66, 169.59, 169.53, 169.48 (C=O), 135.63 (*C*H=CH_2), 116.01 (CH=*C*H_2), 97.73, 97.48, 97.19, 96.73, 96.47, 96.38 (C-1), 81.09 (C-4), 77.66 (C-4), 77.34 (C-3), 76.67 (C-4), 75.81 (C-4), 75.74 (C-4), 74.87 (O*C*H_2CH=CH_2), 72.17, 72.01, 71.46, 71.12, 70.97, 70.86, 70.82, 70.67, 70.61, 70.50, 70.33, 69.84, 69.78, 69.68, 69.36 (C-2, C-3, C-5), 62.85, 62.64, 62.53 (C-6), 21.27, 21.16, 21.11, 20.96, 20.91, 20.85 (CH_3) ppm. HRMS (MALDI-TOF): calcd. for $C_{85}H_{114}O_{55}Na^+$ 2037.602; found 2037.794.

Further elution afforded compound **2** (12%, 966 mg) as a white foam. R_f = 0.27 (CH_2Cl_2/MeOH, 30:1). The analytical data for **2** are in agreement with those reported previously.[11]

Further elution afforded compound **3** (26%, 2.1 g) as a white foam. R_f = 0.21 (CH_2Cl_2/MeOH, 30:1). $[\alpha]_D$ = +111.5 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.82–5.74 (m, 1 H, C*H*=CH_2), 5.34–5.14 (m, 9 H, 3-H, CH=CH_2), 5.09–4.98 (m, 6 H, 1-H), 4.86 (d, *J* = 3.3 Hz, 1 H, 1-H), 4.83–4.71 (m, 6 H, 2-H), 4.58–4.46 (m,

7 H, 6-H), 4.30–4.15 (m, 7 H, 6-H), 4.13–4.07 (m, 6 H, 5-H), 4.02–3.92 (m, 3 H, 5-H, OCH_2CH=CH_2), 3.71–3.57 (m, 7 H, 4-H), 3.27 (dd, J = 10.0, 3.3 Hz, 1 H, 2-H), 2.09–1.97 (m, 60 H, OAc) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.05, 170.96, 170.80, 170.74, 170.60, 170.52, 170.46, 170.43, 170.39, 170.28, 169.66, 169.42, 169.40, 169.37, 169.19 (C=O), 134.45 (*C*H=CH_2), 117.81 (CH=*C*H_2), 98.45, 97.25, 97.10, 96.92, 96.67, 96.61, 96.52 (C-1), 78.42 (C-4), 77.83 (C-2), 77.75, 76.94, 76.69, 76.65, 76.33 (C-4), 72.74 (C-3), 72.21 (O*C*H_2-CH=CH_2), 71.39, 70.90, 70.82, 70.74, 70.60, 70.53, 70.47, 70.16, 69.98, 69.61, 69.54, 69.49, 69.25 (C-3, C-2, C-5), 63.01, 62.56 (C-6), 21.02, 20.93, 20.81 (CH_3) ppm. HRMS (MALDI-TOF): calcd. for $C_{85}H_{114}O_{55}Na^+$ 2037.602; found 2037.670.

Per-*O*-acetyl-3^A-*O*-carboxymethyl-β-cyclodextrin (4): Compound **1** (375 mg, 0.186 mmol) was dissolved in acetonitrile (4 mL), and a saturated aqueous solution of sodium periodate (4 mL), an aqueous ruthenium(III) chloride solution (5%, 50 μL) was added, and the reaction mixture was stirred at room temp. The reaction was monitored by using TLC ($CHCl_3$/MeOH, 10:1). After disappearance of the starting material (around 3 h), the mixture was extracted with $CHCl_3$ (3 × 15 mL). The collected $CHCl_3$ extracts were washed with $Na_2S_2O_5$ (1%, 3 × 30 mL) to remove traces of Ru salts and dried with $MgSO_4$. The solvent was evaporated and the residue purified by chromatography on a silica gel column (elution mixture $CHCl_3$/MeOH, 20:1, 10:1) to give **4** (310 mg, 82%). R_f = 0.31 ($CHCl_3$/MeOH, 10:1). The analytical data for **4** are in agreement with those reported previously.[11]

Per-*O*-acetyl-6^A-*O*-carboxymethyl-β-cyclodextrin (5): Compound **2** (402 mg, 0.199 mmol) was subjected to the procedure described for the preparation of **4** to give **5** (345 mg, 85%). R_f = 0.48 ($CHCl_3$/MeOH, 10:1). The analytical data for **5** are in agreement with those reported previously.[11]

Per-*O*-acetyl-2^A-*O*-carboxymethyl-β-cyclodextrin (6): Compound **3** (849 mg, 0.422 mmol) was subjected to the procedure described for the preparation of **4** to give **6** as a white foam (710 mg, 83%). R_f = 0.41 (H_2O/2-propanol/EtOAc, 1:3:3). $[a]_D$ = +113.7 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.36–5.22 (m, 7 H, 3-H), 5.10–5.00 (m, 7 H, 1-H), 4.79–4.69 (m, 6 H, 2-H), 4.55–4.45 (m, 7 H, 6-H), 4.30–3.94 (m,16 H, 6-H, 5-H, OCH_2COOH), 3.77–3.58 (m, 7 H, 4-H), 3.38 (dd, J = 10.1, 2.8 Hz, 1 H, 2-H), 2.10–1.98 (m, 60 H, OAc) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.83, 170.79, 170.75, 170.66, 170.58, 170.51, 170.43, 170.41, 170.40 (OAc), 170.08 (COOH), 169.69, 169.60, 169.51 (OAc), 99.85, 97.28, 97.06, 96.78, 96.53 (C-1), 78.88 (C-2), 78.49, 78.04, 77.34, 77.02, 76.55, 76.34, 76.21 (C-4), 72.62, 71.01, 70.93, 70.67, 70.59, 70.52, 70.36, 70.28, 70.01, 69.93, 69.76, 69.63, 69.52 (C-3, C-2, C-5), 68.22 (O*C*H_2COOH), 62.84, 62.76, 62.63, 62.38 (C-6), 21.17, 20.97, 20.91, 20.83, 20.77 (OAc) ppm. HRMS (MALDI-TOF): calcd. for $C_{84}H_{112}O_{57}Na^+$ 2055.576; found 2055.520.

3^A-*O*-Carboxymethyl-β-cyclodextrin (7): Compound **4** (300 mg, 0.148 mmol) was dissolved in a solution of NaOMe in MeOH (0.1 M, 6 mL), and the solution was stirred at room temp. overnight. Then water (6 mL) was added, the solution was neutralized with Dowex 120 in H^+ form, and the solvents were evaporated. The solid was dissolved in water (10 mL), the treatment with Dowex was repeated once more, and **7** was obtained by freeze-drying (165 mg, 95%). The analytical data for **7** are in agreement with those reported previously.[11]

6^A-*O*-Carboxymethyl-β-cyclodextrin (8): Compound **5** (320 mg, 0.158 mmol) was subjected to the procedure described for the preparation of **7** to give **8** (172 mg, 91%). The analytical data for **8** are in agreement with those reported previously.[11]

2^A-*O*-Carboxymethyl-β-cyclodextrin (9): Compound **6** (500 mg, 0.247 mmol) was subjected to the procedure described for the preparation of **7** to give **9** (280 mg, 97%). The analytical data for **9** are in agreement with those reported previously.[8]

Heptakis-2,3,6-tri-*O*-methyl-β-cyclodextrin (10): β-Cyclodextrin (12 g, 10.57 mmol) was dissolved in DMSO (300 mL). NaH (60%, 20 g, 528 mmol, 50 equiv.) was added, and the mixture stirred at room temp. under nitrogen for 1 h. Iodomethane (33 mL, 528 mmol, 50 equiv.) was then added dropwise at 0 °C. The mixture was stirred overnight, slowly warming to room temp. The reaction was quenched by adding H_2O (around 500 mL) at 0 °C, and then extracted with Et_2O (5 × 300 mL). The combined organic extracts were washed with brine, dried with $MgSO_4$, filtered, and the solvent was removed in vacuo. The residue was subjected to flash chromatography ($CHCl_3$/MeOH, 60:1) to give **10** (13.5 g, 90%). The analytical data for **10** are in agreement with those reported previously.[14]

2^A,3^B-Dihydroxy-per-*O*-methyl-β-cyclodextrin (11): A solution of DIBAL-H in toluene (1.0 M, 19.44 mmol, 9 equiv., 19.44 mL) was added to a solution of compound **10** (3.09 g, 2.16 mmol) in anhydrous toluene (77.5 mL) at 0 °C under nitrogen. Then the mixture was stirred at 0 °C for 18 h under nitrogen. Aqueous HCl (1 M) was carefully added dropwise and the mixture was stirred vigorously at room temperature for 10 min. The toluene phase was separated and the aqueous layer was extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were washed with brine, dried with $MgSO_4$, filtered, and the solvent was removed in vacuo. The residue was subjected to flash chromatography (CH_2Cl_2/MeOH, 40:1) to give **11** (1.55 g, 51%). The analytical data for **11** are in agreement with those reported previously.[12]

2^A-Hydroxy-per-*O*-methyl-β-cyclodextrin (12) and 3^A-Hydroxy-per-*O*-methyl-β-cyclodextrin (13): NaH (60%, 18.16 mg, 0.454 mmol, 1.0 equiv.) was added to a solution of **11** (636 mg, 0.454 mmol) in anhydrous THF (60 mL) at 0 °C under nitrogen. After stirring at 0 °C for 1 h, CH_3I (0.454 mmol, 1.0 equiv., 0.028 mL) was added. The reaction mixture was stirred at 0 °C for 6 h and then kept at room temperature under nitrogen for a further 12 h. CH_3OH was added dropwise to quench the reaction, and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2, washed with brine, dried with $MgSO_4$, filtered and the solvent removed in vacuo. Purification on a silica column afforded compound **12** as a white foam (18%, 120 mg); R_f = 0.34 (EtOAc/MeOH, 10:1). Further elution afforded compound **13** as a white foam (74%, 477 mg); R_f = 0.22 (EtOAc/MeOH, 10:1). The analytical data for **12** and **13** are in agreement with those reported previously.[15]

2^A,3^B,2^D,3^E-Tetrahydroxy-per-*O*-methyl-β-cyclodextrin (14): DIBAL-H (160 mmol, 50 equiv., 1 M in toluene, 160 mL) was added to a stirred solution of permethylated cyclodextrin **10** (4.57 g, 3.2 mmol) in anhydrous toluene (40 mL) at room temp. under nitrogen and stirred for 3 h. The solution was cooled to 0 °C, the reaction quenched with aqueous HCl (1 M), and the mixture was stirred vigorously at room temperature for 30 min. The toluene phase was collected and the aqueous phase extracted with ethyl acetate (3 × 200 mL). The combined organic phases were washed with brine, dried with $MgSO_4$, and the solvent was removed in vacuo. The residue was subjected to flash chromatography (CH_2Cl_2/CH_3OH, 30:1) to give **14** as white foam (1.3 g, 30%). The analytical data for **14** are in agreement with those reported previously.[12]

2^A,3^B-Diallyl-per-*O*-methyl-β-cyclodextrin (15): $(CH_3)_3COK$ (176 mg, 1.57 mmol, 10 equiv.) was added to a solution of **11** (220 mg, 0.157 mmol) in anhydrous DMSO (2 mL) at room temp.

under nitrogen. After stirring for 5 min, allyl bromide (2.36 mmol, 0.2 mL, 20 equiv.) was added, and the mixture was stirred at room temp. The reaction was monitored by using TLC (CH_2Cl_2/MeOH, 15:1). The product has a larger R_f value than the starting compound. After disappearance of the starting compound and the mono-allylation compound, water was carefully added to quench the reaction, and the mixture was extracted with CH_2Cl_2 (3×10 mL). The collected CH_2Cl_2 extracts were washed with brine, dried with $MgSO_4$, and the solvent was removed in vacuo. The residue was subjected to flash chromatography (CH_2Cl_2/CH_3OH, 100:1) to give **15** as white foam (203 mg, 87%). R_f = 0.46 (EtOAc/CH_3OH, 10:1). $[\alpha]_D$ = +51.7 (c = 0.4, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.09–6.02 (m, 1 H, ^{3B}CH = CH_2), 5.98–5.90 (m, 1 H, ^{2A}CH = CH_2), 5.30–5.23 (m, 2 H, ^{3B}CH=CH_2, ^{2A}CH=CH_2), 5.16–5.08 (m, 9 H, 1-H, ^{3B}CH=CH_2, ^{2A}CH=CH_2), 4.52 (dd, J = 12.2, 5.7 Hz, 1 H, $^{3B}OCH_2CH$=CH_2), 4.27 (dd, J = 12.2, 5.7 Hz, 1 H, $^{3B}OCH_2CH$=CH_2), 4.20–4.12 (m, 2 H, $^{2A}OCH_2CH$=CH_2), 3.93 (dd, J = 10.6, 3.4 Hz, 1 H, 6-H), 3.87–3.77 (m, 13 H, 6-H, 5-H), 3.69–3.48 [m, 57 H, ^{B}3-H, OCH_3 (C3), 4-H, 6′-H, 3-H, OCH_3 (C2)], 3.38–3.37 [m, 21 H, OCH_3 (C6)], 3.33 (dd, J = 9.6, 3.5 Hz, 1 H, ^{A}2-H), 3.23–3.18 (m, 6 H, 2-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 136.49 (^{3B}CH = CH_2), 135.51 (^{2A}CH = CH_2), 117.09 (^{2A}CH = CH_2), 115.93 (^{3B}CH = CH_2), 99.11, 99.10, 99.04, 99.02 (C-1), 82.23, 82.20, 82.16, 82.12, 82.08, 81.98, 81.91, 81.88, 81.84 (C-2, C-3), 80.60, 80.59, 80.30, 80.08, 79.98, 79.89, 79.85, 79.79, 79.76 (C-4, ^{B}C-3, ^{A}C-2), 74.76 ($^{3B}OCH_2CH$=CH_2), 72.05 ($^{2A}OCH_2CH$=CH_2), 71.60, 71.45 (C-6), 71.18, 71.08, 70.95 (C-5), 61.69, 61.56, 61.51 [OCH_3 (C3)], 59.12 [OCH_3 (C6)], 58.70, 58.55 [OCH_3 (C2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{67}H_{116}O_{35}Na^+$ 1503.719; found 1503.719.

2^A-*O*-Allyl-per-*O*-methyl-β-cyclodextrin (16): Compound **12** (120 mg, 0.085 mmol) was subjected to the procedure for the preparation of **15** to give **16** (87 mg, 71%). R_f = 0.33 (EtOAc/2-propanol, 10:1). $[\alpha]_D$ = +140.9 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.99–5.91 (m, 1 H, CH=CH_2), 5.33 (dd, J = 17.3, 1.7 Hz, 1 H, CH=CH_2), 5.15–5.12 (m, 7 H, CH=CH_2, 1-H), 5.07–5.05 (m, 1 H, 1-H), 4.23 (dd, J = 12.9, 5.8 Hz, 1 H, OCH_2CH=CH_2), 4.12 (dd, J = 12.9, 5.3 Hz, 1 H, OCH_2CH=CH_2), 3.89–3.79 (m, 14 H, 6-H, 5-H), 3.66–3.56 [m, 35 H, OCH_3 (C3), 4-H, 6′-H], 3.54–3.49 [m, 25 H, 3-H, OCH_3 (C2)], 3.38–3.37 [m, 21 H, OCH_3 (C6)], 3.33 (dd, J = 9.6, 3.5 Hz, 1 H, 2-H), 3.20–3.18 (m, 6 H, 2-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 135.46 (CH=CH_2), 116.85 (CH=CH_2), 99.51 (^{A}C-1), 99.13 (C-1), 82.19, 82.11, 82.01, 81.96, 81.91, 81.84 (C-2, C-3), 80.55, 80.40, 80.32, 80.23, 79.91 (C-4, ^{A}C-2), 71.87 (OCH_2CH=CH_2), 71.60, 71.52 (C-6), 71.15, 71.10, 71.04, 70.97 (C-5), 61.76, 61.73, 61.65, 61.59 [OCH_3 (C3)], 59.12 [OCH_3 (C6)], 58.77, 58.74, 58.63, 58.61 [OCH_3 (C2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{65}H_{114}O_{35}Na^+$ 1477.703; found 1477.385.

3^A-Allyl-per-*O*-methyl-β-cyclodextrin (17): Compound **13** (369 mg, 0.261 mmol) was subjected to the procedure for the preparation of **15** to give **17** (297 mg, 78%). R_f = 0.39 (EtOAc/CH_3OH, 10:1). $[\alpha]_D$ = +155.5 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.09–6.01 (m, 1 H, CH=CH_2), 5.26 (dd, J = 17.3, 1.8 Hz, 1 H, CH=CH_2), 5.15–5.10 (m, 8 H, CH=CH_2, 1-H), 4.49 (dd, J = 11.9, 5.7 Hz, 1 H, OCH_2CH=CH_2), 4.26 (dd, J = 11.9, 5.7 Hz, 1 H, OCH_2CH=CH_2), 3.92 (dd, J = 10.6, 3.8 Hz, 1 H, 6-H), 3.87–3.77 (m, 13 H, 6-H, 5-H), 3.70–3.55 [m, 33 H, ^{A}3-H, OCH_3 (C3), 4-H, 6′-H], 3.53–3.47 [m, 27 H, 3-H, OCH_3 (C2)], 3.37–3.36 [m, 21 H, OCH_3 (C6)], 3.22–3.17 (m, 7 H, 2-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 136.28 (CH=CH_2), 116.02 (CH=CH_2), 99.21, 99.09, 98.82 (C-1), 82.20, 82.15, 81.96, 81.89 (C-2, C-3), 80.54, 80.51, 80.34, 80.26, 79.94 (C-4, ^{A}C-3), 74.85 (OCH_2CH=CH_2), 71.59, 71.49 (C-6), 71.16, 71.05, 71.01 (C-5), 61.66, 61.63, 61.59, 61.56 [OCH_3 (C3)], 59.10, 59.09 [OCH_3 (C6)], 58.87, 58.74, 58.65, 58.63, 58.59 [OCH_3 (C2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{65}H_{114}O_{35}Na^+$ 1477.703; found 1477.721.

2^A,3^B,2^D,3^E-Tertraallyl-per-*O*-methyl-β-cyclodextrin (18): $(CH_3)_3$COK (1.077 g, 9.6 mmol, 30 equiv.) was added to a solution of **14** (439 mg, 0.32 mmol) in anhydrous DMSO (10 mL) at room temp. under nitrogen. After stirring for 5 min, allyl bromide (9.6 mmol, 1.108 mL, 40 equiv.) was added, and the mixture was stirred at room temp. The reaction was monitored by using TLC (CH_2Cl_2/MeOH, 15:1) and HRMS (MALDI-TOF). The product has a larger R_f value than the starting compound. After 3 h, water was carefully added to quench the reaction, and the mixture was extracted with CH_2Cl_2 (3×20 mL). The collected CH_2Cl_2 extracts were washed with brine, dried with $MgSO_4$, and the solvent was removed in vacuo. The residue was subjected to flash chromatography (CH_2Cl_2/CH_3OH, 200:1) to give **18** as white foam (410 mg, 84%). R_f = 0.62 (EtOAc/CH_3OH, 10:1). $[\alpha]_D$ = +123.4 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.10–6.02 (m, 2 H, $^{B,E}CH$=CH_2), 5.97–5.90 (m, 2 H, $^{A,D}CH$=CH_2), 5.29–5.23 (m, 4 H, $^{A,D}CH$=CH_2, $^{B,E}CH$=CH_2), 5.17–5.06 (m, 11 H, 7× 1-H, $^{A,D}CH$=CH_2, $^{B,E}CH$=CH_2), 4.53 (m, 2 H, $^{B,E}OCH_2CH$=CH_2), 4.26 (dd, J = 12.3, 5.7 Hz, 2 H, $^{B,E}OCH_2CH$=CH_2), 4.19–4.11 (m, 4 H, $^{A,D}OCH_2CH$=CH_2), 3.94 (dd, J = 10.5, 3.2 Hz, 2 H, 6-H), 3.85–3.68 (m, 14 H, 5× 6-H, 7× 5-H, 2× 3-H), 3.67–3.48 [m, 49 H, 7× 4-H, 5× OCH_3 (C-3), 7× 6-H, 5× 3-H, 5× OCH_3 (C-2)], 3.38–3.30 [m, 23 H, 7× OCH_3 (C-6), 2× 2-H], 3.23–3.17 (m, 5 H, 5× 2-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 136.52, 136.49 ($^{B,E}CH$=CH_2), 135.52, 135.50 ($^{A,D}CH$=CH_2), 117.06, 117.04 ($^{A,D}CH$=CH_2), 115.97, 115.94 ($^{B,E}CH$=CH_2) 99.17, 99.16, 99.05, 99.03, 98.99 (C-1), 82.19, 82.13, 82.06, 81.95, 81.88, 81.80 (C-2, C-3), 80.30, 80.03, 79.97, 79.92, 79.81, 79.79, 79.76, 79.62 (C-2′, C-4, C-3′), 74.82 ($^{B,E}OCH_2CH$=CH_2), 72.00, 71.95, 71.64, 71.52, 71.39 ($^{A,D}OCH_2CH$=CH_2, C-6, C-6′) 71.22, 71.20, 70.98, 70.96, 70.92, 70.89, 70.84 (C-5), 61.73, 61.71, 61.66, 61.63, 61.61, 61.44, 61.42 [OCH_3 (C-3)], 59.24, 59.22, 59.10, 59.09, 58.99, 58.96, 58.84, 58.81, 58.71, 58.69, 58.59, 58.57 [OCH_3 (C-6), OCH_3 (C-2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{71}H_{120}O_{35}Na^+$ 1555.750; found 1555.706.

2^A,3^B-*O*-Dicaboxymethyl-per-*O*-methyl-β-cyclodextrin (19): Compound **15** (500 mg, 0.338 mmol) was dissolved in acetonitrile (9 mL), then saturated aqueous sodium periodate (9 mL) and 5% aqueous ruthenium(III) chloride (100 μL) were added, and the reaction mixture was stirred at room temp. The reaction was monitored by using TLC ($CHCl_3$/MeOH, 10:1). After disappearance of the starting compound, the mixture was extracted with $CHCl_3$ (3×15 mL). The collected $CHCl_3$ extracts were washed with 1% $Na_2S_2O_5$ (3×30 mL) to remove traces of Ru salts and dried with $MgSO_4$. The solvent was evaporated, and the product was purified by chromatography on silica gel [CH_2Cl_2/MeOH, 30:1 (1% formic acid)] to give **19** (405 mg, 79%). R_f = 0.10 (H_2O/2-propanol/EtOAc, 1:3:3). $[\alpha]_D$ = +114.7 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.22 (d, J = 3.3 Hz, 1 H, 1-H), 5.19 (d, J = 3.6 Hz, 1 H, 1-H), 5.14 (d, J = 3.2 Hz, 2 H, 1-H), 5.12–5.10 (m, 2 H, 1-H), 5.08 (d, J = 3.4 Hz, 1 H, 1-H), 4.63 (d, J = 17.6 Hz, 1 H, OCH_2-COOH), 4.45 (d, J = 17.7 Hz, 1 H, OCH_2COOH), 4.38 (d, J = 17.1 Hz, 1 H, OCH_2COOH), 4.31 (d, J = 17.0 Hz, 1 H, OCH_2-COOH), 4.04 (dd, J = 10.5, 2.1 Hz, 1 H, 6-H), 3.95 (dd, J = 10.6, 3.7 Hz, 1 H, 6-H), 3.90–3.70 [m, 17 H, 5× 6-H, 7× 5-H, 1× ^{A}3-H, 1× ^{B}3-H, 1× OCH_3 (C-3)], 3.69–3.46 [m, 52 H, 7× 4-H, 7× 6′-H, 5× OCH_3 (C-3), 5× 3-H, 6× OCH_3 (C-2)], 3.42–3.36 [m, 22 H, 7× OCH_3 (C-6), 1× 2-H], 3.22–3.18 (m, 6 H, 6× 2-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 172.33 (COOH), 171.51

(COOH), 99.64, 99.48, 99.46, 99.43, 99.27, 99.26, 99.12, 99.10, 98.89, 98.87, 98.79 (C-1), 82.72, 82.49, 82.33, 82.05, 81.99, 81.94, 81.87, 81.68, 81.62, 81.31, 81.19, 80.78, 80.67, 80.55, 80.24, 79.79 (C-2, C-3, C-4), 71.88, 71.64, 71.50, 71.39, 71.34, 71.28, 71.22, 71.15, 71.01, 70.80, 70.42 (C-6, C-5, O*C*H$_2$COOH), 68.69 (O*C*H$_2$-COOH), 62.44, 61.80, 61.71, 61.68, 61.60, 61.57, 61.41, 61.25, 61.22 [OCH$_3$(C-3)], 59.22, 59.14, 59.11, 59.05, 58.68, 58.66, 58.52, 58.49, 58.47, 58.42, 58.40 [OCH$_3$(C-6), OCH$_3$(C-2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{65}H_{112}O_{39}Na^+$ 1539.667; found 1539.729.

2^A-*O*-Carboxymethyl-per-*O*-methyl-β-cyclodextrin (20): Compound **16** (42.2 mg, 0.029 mmol) was subjected to the procedure for the preparation of **19** to give **20** as a white foam (31 mg, 73%). R_f = 0.29 (H$_2$O/2-propanol/EtOAc, 1:3:3). $[a]_D$ = +69.2 (*c* = 0.7, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 5.18 (d, *J* = 3.6 Hz, 1 H, 1-H), 5.14 (dd, *J* = 8.8, 3.7 Hz, 2 H, 1-H), 5.10 (dd, *J* = 4.6, 3.8 Hz, 2 H, 1-H), 5.07 (d, *J* = 3.5 Hz, 1 H, 1-H), 4.97 (d, *J* = 3.4 Hz, 1 H, 1-H), 4.45 (d, *J* = 16.5 Hz, 1 H, OC*H*$_2$COOH), 4.20 (d, *J* = 16.4 Hz, 1 H, OC*H*$_2$COOH), 3.98 (dd, *J* = 10.2, 3.1 Hz, 1 H, 6-H), 3.90–3.75 (m, 12 H, 6-H, 5-H), 3.72–3.45 [m, 61 H, 5-H, 4-H, OC*H*$_3$ (C-3), 6′-H, OC*H*$_3$ (C-2), 3-H], 3.39–3.34 [m, 22 H, OC*H*$_3$ (C-6), 2-H], 3.21–3.17 (m, 6 H, 2-H) ppm. ^{13}C NMR (126 MHz, CDCl$_3$): δ = 170.83 (COOH), 99.63, 99.54, 99.52, 99.20, 99.15, 98.94, 98.91 (C-1), 82.62, 82.36, 82.14, 82.04, 81.95, 81.90, 81.77, 81.61, 81.33, 80.79, 80.63, 80.38, 79.93, 79.55 (C-2, C-3, C-4), 72.09, 71.98 (C-6), 71.88 (^{A}C-5), 71.66, 71.58 (C-6), 71.25, 71.04, 70.97, 70.88 (C-5), 70.61 (^{A}C-6), 69.78 (O*C*H$_2$COOH), 61.71, 61.64, 61.58, 61.55, 61.40, 61.20 [OCH$_3$ (C-3)], 59.39, 59.36, 59.30, 59.26, 59.16, 59.11, 59.01, 58.92, 58.48, 58.45, 58.34, 58.33 [OCH$_3$ (C-6), OCH$_3$ (C-2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{64}H_{112}O_{37}Na^+$ 1495.677; found 1495.597.

3^A-*O*-Carboxymethyl-per-*O*-methyl-β-cyclodextrin (21): Compound **17** (305 mg, 0.21 mmol) was subjected to the procedure for the preparation of **19** to give **21** as a white foam (221 mg, 1%). R_f = 0.31 (H$_2$O/2-propanol/EtOAc, 1:3:3). $[a]_D$ = +122.3 (*c* = 1.0, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 5.71 (d, *J* = 3.4 Hz, 1 H, 1-H), 5.14–5.13 (m, 2 H, 1-H), 5.10–5.08 (m, 3 H, 1-H), 5.05 (d, *J* = 3.7 Hz, 1 H, 1-H), 4.58 (d, *J* = 17.6 Hz, 1 H, OC*H*$_2$COOH), 4.45 (d, *J* = 17.6 Hz, 1 H, OC*H*$_2$COOH), 4.11 (dd, *J* = 10.7, 2.8 Hz, 1 H, 6-H), 3.87–3.67 (m, 15 H, 6-H, 5-H, ^{A}4-H, ^{A}3-H), 3.66–3.44 [m, 58 H, 4-H, OC*H*$_3$ (C-3), 6′-H, OC*H*$_3$ (C-2), 3-H], 3.40–3.33 [m, 21 H, OC*H*$_3$ (C-6)], 3.29 (dd, *J* = 9.8, 3.4 Hz, 1 H, 2-H), 3.23 (dd, *J* = 9.4, 3.5 Hz, 1 H, 2-H), 3.19–3.14 (m, 5 H, 2-H) ppm. ^{13}C NMR (126 MHz, CDCl$_3$): δ = 172.45 (COOH), 99.80, 99.50, 99.41, 99.31, 99.00, 98.95, 98.91 (C-1), 82.69, 82.66, 82.54, 82.29, 82.21, 82.13, 82.05, 81.94, 81.89, 81.76, 81.43, 81.29, 80.88, 80.85, 80.72, 80.48, 80.40, 80.30, 80.25 (C-2, C-3, C-4, ^{A}C-3, ^{A}C-4), 71.82 (O*C*H$_2$COOH), 71.72, 71.67 (C-6), 71.54 (^{A}C-5), 71.48 (C-6), 71.37, 71.18, 71.10, 71.05, 71.01, 70.89 (C-5), 70.55 (^{A}C-6), 61.96, 61.73, 61.70, 61.57, 61.54, 61.52 [OCH$_3$ (C-3)], 59.17, 59.11, 59.09, 59.03, 58.94, 58.73, 58.66, 58.55, 58.51, 58.13 [OCH$_3$ (C-6), OCH$_3$ (C-2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{64}H_{112}O_{37}Na^+$ 1495.677; found 1495.691.

2^A,3^B,2^D,3^E-*O*-Tetracarboxymethyl-per-*O*-methyl-β-cyclodextrin (22): Compound **18** (180 mg, 0.117 mmol) was subjected to the procedure for the preparation of **19** to give **22** as a white foam (118 mg, 63%). R_f = 0.44 (H$_2$O/2-propanol/EtOAc, 1:2:2, 0.5% formic acid). $[a]_D$ = +112.1 (*c* = 1.0, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ = 5.17 (d, *J* = 3.4 Hz, 1 H, 1-H), 5.09 (t, *J* = 3.5 Hz, 2 H, 1-H), 5.04 (t, *J* = 3.3 Hz, 2 H, 1-H), 5.00 (d, *J* = 3.3 Hz, 1 H, 1-H), 4.98 (d, *J* = 3.4 Hz, 1 H, 1-H), 4.53 (dd, *J* = 17.5, 2.0 Hz, 2 H, OC*H*$_2$-COOH), 4.37–4.34 (m, 2 H, OC*H*$_2$COOH), 4.31–4.27 (m, 2 H, OC*H*$_2$COOH), 4.21 (dd, *J* = 17.1, 3.9 Hz, 2 H, OC*H*$_2$COOH), 3.96–3.92 (m, 2 H, 6-H), 3.89 (dd, *J* = 10.8, 3.9 Hz, 1 H, 6-H), 3.69 [s, 3 H, 1× OCH$_3$ (C-3)], 3.67 [s, 3 H, 1× OCH$_3$ (C-3)], 3.54 [s, 3 H, 1× OCH$_3$ (C-3)], 3.52 [m, 6 H, 2× OCH$_3$ (C-3)], 3.50 [s, 3 H, 1× OCH$_3$ (C-2)], 3.49 [s, 3 H, 1× OCH$_3$ (C-2)], 3.45 [s, 3 H, 1× OCH$_3$ (C-2)], 3.42 [m, 6 H, 1× OCH$_3$ (C-2)], 3.81–3.36 (m, 32 H, 4× 6-H, 7× 5-H, 7× 4-H, 7× 6′-H, 7× 3-H), 3.33–3.22 [m, 25 H, 7× OCH$_3$ (C-6), 4× 2-H], 3.14–3.08 (m, 3 H, 2-H) ppm. ^{13}C NMR (126 MHz, CDCl$_3$): δ = 172.12, 172.10, 171.44, 171.41 (COOH), 99.82, 99.66, 99.64, 99.50, 99.47, 99.16, 98.88, 98.87, 98.76 (C-1), 82.71, 82.57, 81.93, 81.82, 81.59, 81.49, 81.44, 81.36, 81.08, 80.90, 80.47, 80.44, 80.14, 80.03, 79.87 (C-2, C-3, C-4), 72.12, 72.02, 71.94, 71.54, 71.45, 71.38, 71.33, 71.10, 71.04, 70.94, 70.88, 70.52, 70.45 (C-6, C-5, O*C*H$_2$COOH), 68.71 (O*C*H$_2$COOH), 62.53, 62.48, 61.66, 61.64, 61.43, 61.18 [OCH$_3$ (C-3)], 59.23, 59.11, 59.05, 58.83, 58.80, 58.43, 58.39, 58.20 [OCH$_3$ (C-6), OCH$_3$ (C-2)] ppm. HRMS (MALDI-TOF): calcd. for $C_{67}H_{112}O_{43}Na^+$ 1627.647; found 1627.891.

Procedure for Determining the Rate of Hydrolysis: Each assay was performed on samples (1 mL) prepared from aqueous solutions of the appropriate nitrophenyl glycoside (0.5 mL) at different concentrations mixed with phosphate buffer (0.5 M, 0.5 mL) containing either **7**–**9** or **19**–**22** (0.58 mM) or nothing as control. The reactions were monitored at 59 °C by using UV absorption at 400 nm and typically monitored for 12 h. Rates were calculated from the slope of the progress curve of each reaction. Uncatalysed rates were obtained directly from the control samples. Catalyzed rates were calculated by subtracting the uncatalysed rate from the rate obtained from the appropriate cyclodextrin-containing sample. The catalyzed rates were used to construct a Hanes plot ([S]/V vs. [S]) from which K_m and V_{max} were determined. k_{cat} was calculated as V_{max}/[cyclodextrin]; k_{uncat} was determined as the slope from a plot of V_{uncat} vs. [S]. The following extinction coefficients were determined and used in the calculations: ε = 17.04 mM^{-1} cm^{-1} (pH = 8.0, 59 °C, 4-nitrophenolate), ε = 2.28 mM^{-1} cm^{-1} (pH = 8.0, 59 °C, 2-nitrophenolate).

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of compounds.

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