Cyclodextrin derivatives with cyanohydrin and carboxylate groups as artificial glycosidases[1]

Fernando Ortega-Caballero and Mikael Bols

Abstract: Two cyclodextrin derivatives (**1** and **2**) were prepared in an attempt to create glycosidase mimics with a general acid catalyst and a nucleophilic carboxylate group. The catalysts **1** and **2** were found to catalyse the hydrolysis of 4-nitrophenyl β-D-glucopyranoside at pH 8.0, but rapidly underwent decomposition with loss of hydrogen cyanide to convert the cyanohydrin to the corresponding aldehyde. The initial rate of the catalysis shows that the cyanohydrin group in these molecules functions as a good catalyst, but that the carboxylate has no positive effect. The decomposition product aldehydes display little or no catalysis. A mechanism for the decomposition is suggested.

Key words: biomimicry, enzyme model, kinetics, intramolecular reaction.

Résumé : On a préparé deux dérivés de la cyclodextrine (**1** et **2**) dans le but de créer des imitations de glycosidase avec un catalyseur acide général et un groupe carboxylate nucléophile. On a trouvé que les catalyseurs **1** et **2** catalysent bien l'hydrolyse β-D-glucopyranoside de 4-nitrophényle, à un pH de 8,0, mais qu'ils subissent rapidement une décomposition avec perte de cyanure d'hydrogène qui transforme en aldéhyde correspondant. La vitesse initiale de la catalyse montre que le groupe cyanohydrine des ces molécules fonctionne comme un bon catalyseur, mais que le carboxylate n'a pas d'effet positif. Les aldéhydes obtenus comme produits de décomposition ne présentent pas, ou peu, d'activité catalytique. On propose un mécanisme pour la décomposition.

Mots clés : bio-imitation, modèle d'enzyme, cinétique, réaction intramoléculaire.

[Traduit par la Rédaction]

Introduction

Supramolecular chemistry is a science that attempts to understand and use chemistry beyond the molecule (1). A fascinating research area is aimed at using supramolecular chemistry to achieve catalysis thereby creating what is *de-facto* artificial enzymes (2).

We have recently reported some of the first examples of artificial glycosidases (3). The most effective type discovered was cyclodextrin derivatives, such as **A** (Fig. 1), containing a cyanohydrin group at the primary rim (4). For these catalysts, a k_{cat}/k_{uncat} of up to 8000 was obtained for the hydrolysis of aryl glycosides (5). Based on structure–activity analysis of a series of derivatives, it was found that both the cyano group and the cyanohydrin OH were essential for catalysis, indicating that the acidity of this group was essential. It is therefore suggested that **A** works by acid catalysis as shown in Fig. 1.

A second type of cyclodextrin, such as **B**, having two carboxylate or sulphate groups at the primary rim was also found to catalyse aryl glycoside cleavage. While these catalysts were found relatively inefficient (with k_{cat}/k_{uncat} of up to 10–50), at normal conditions (6), increasing the phosphate concentration led to k_{cat}/k_{uncat} values of up to 1000 (7). Since this type of artificial enzyme has both functional groups negatively charged, the catalysis was believed to be mainly electrostatic.

The observation, in this latter case, that increasing the phosphate concentration increased the catalytic rate, was taken as evidence for the involvement of nucleophilic substitution with phosphate and suggested that the catalysis could be increased by the presence of a nucleophile. Indeed, the catalysis by the better catalyst **A** is also boosted by phosphate, but not in a linear fashion. It was therefore proposed to incorporate a nucleophilic group into a cyanohydrin-containing cyclodextrin with the goal of achieving general acid catalysis and nucleophilic catalysis in the same molecule. It was decided to attempt to use a carboxylate as the nucleophile so that the desired catalyst essentially would become a hybrid on **A** and **B**. In this paper we report the synthesis of two such molecules (**1** and **2**), each having a cyanohydrin and a carboxylate group at the primary rim, only differing in the chain length between carboxylate and cyclodextrin (Fig. 1). Both compounds were found to be catalytic, but undergo rapid decomposition of the cyanohydrin group under the reaction conditions.

Received 14 September 2005. Published on the NRC Research Press Web site at http://canjchem.nrc.ca on 12 May 2006.

This paper is dedicated to Professor Szarek with thanks for his mentorship of one of us (MB), and for his contribution to science in general.

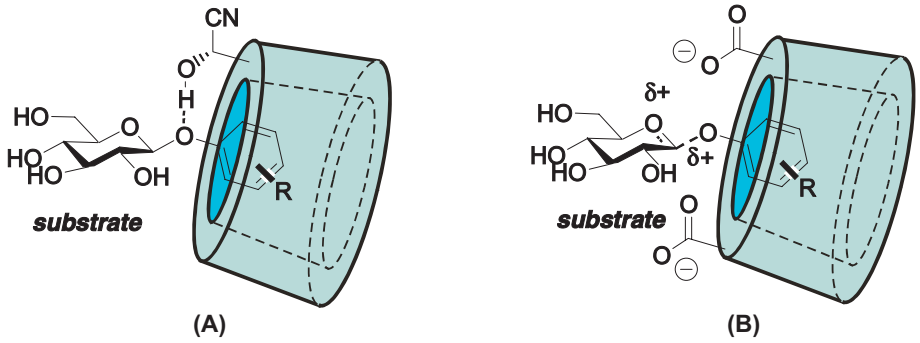
F. Ortega-Caballero and M. Bols.[2] Department of Chemistry, University of Aarhus, DK-8000 Aarhus, Denmark.

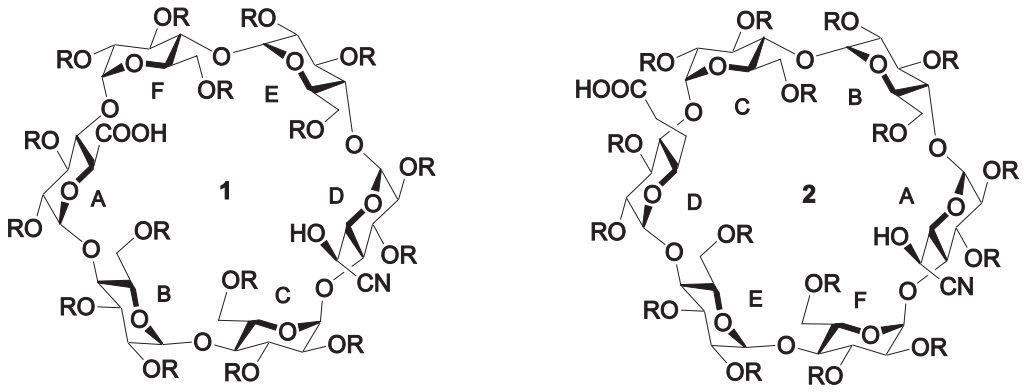
[1]This article is part of a Special Issue dedicated to Professor Walter A. Szarek.

[2]Corresponding author (e-mail: mb@chem.au.dk).

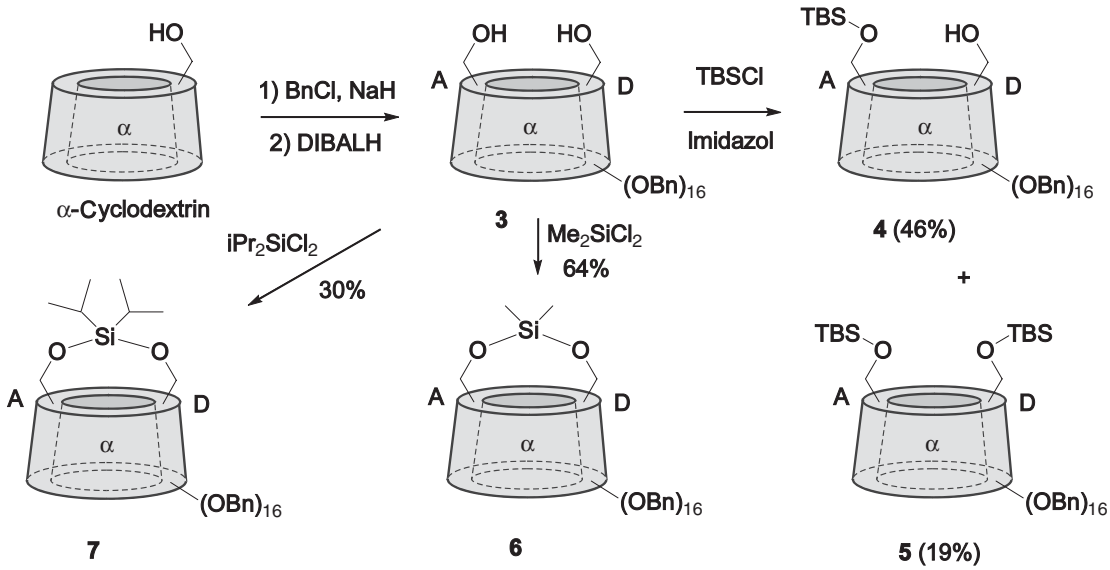
 doi:10.1139/V06-039

Fig. 1. Proposed mode of catalysis of cyclodextrin cyanohydrins (**A**) and cyclodextrin diacids (**B**).





Scheme 1. Synthesis of silylated derivatives **4**–**7**.



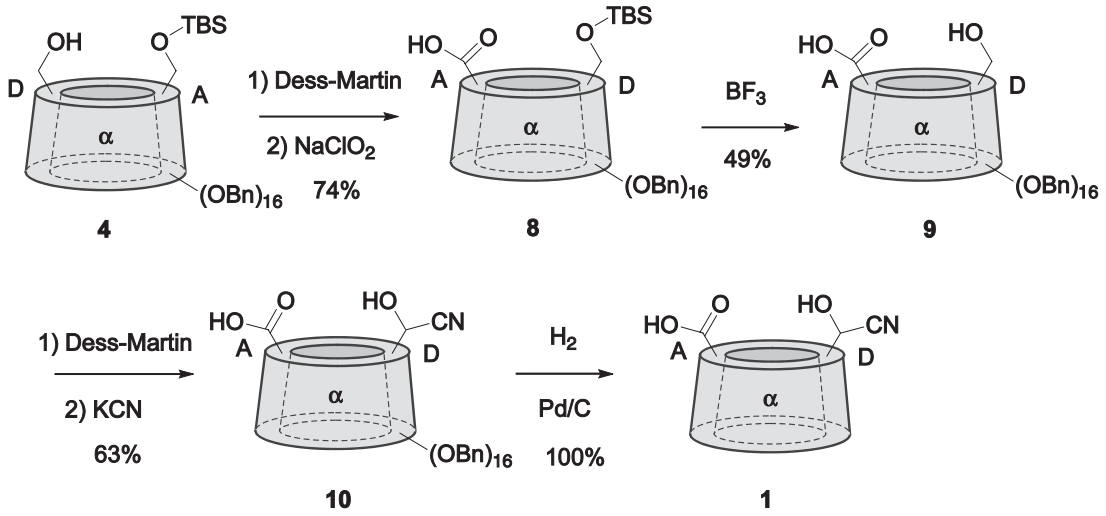
Results and discussion

Synthesis

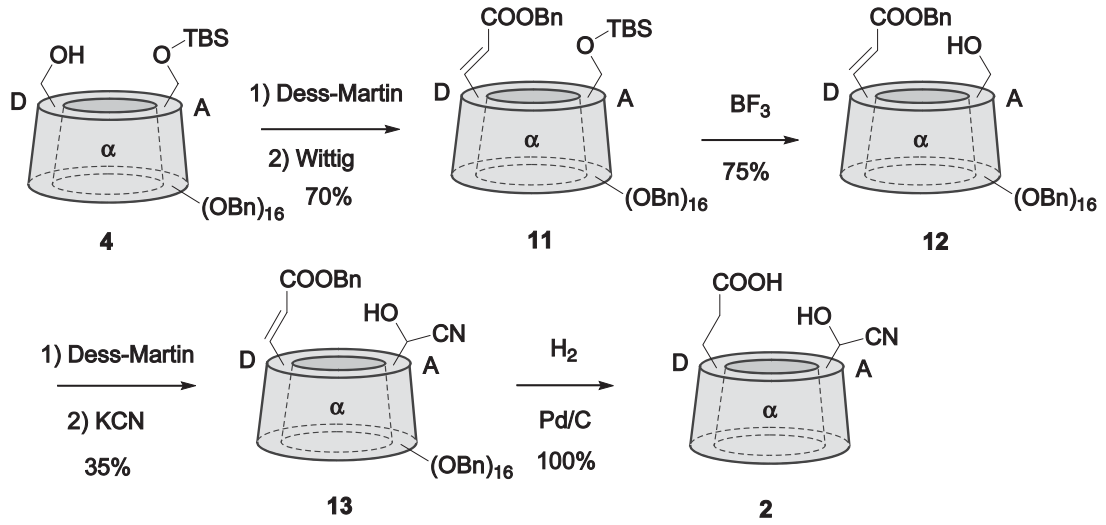
The synthesis of **1** and **2** was carried out from the hexadecabenzylated diol **3** readily available from α-cyclodextrin in two steps (Scheme 1) (8). Monosilylation of the diol **3** with TBSCl–imidazol was, however, not very selective, leading to a mixture of the mono- and di-silylated products (**4** and **5**), which could be separated by column chromatography to obtain them in 46% and 19% yield, respectively.

In an attempt to obtain **4** in a higher yield, a two-step synthesis procedure was attempted involving synthesis of silicon-bridged molecules **6** and **7** and subsequent attack on silicon with a *tert*-butyl organometalic reagent. The synthesis of the silicon diethers proceeded reasonably well; reaction of **3** with 1 equiv. of Me_2SiCl_2–pyridine gave **6** in 64% yield (Scheme 2). Similar reaction of **3** with *i*-Pr_2SiCl_2–

Scheme 2. Synthesis of **1**.



Scheme 3. Synthesis of **2**.



pyridine gave **7** in 30% yield. However, reaction of either **6** or **7** with a selection of organometalic reagents, such as methyl or *tert*-butyl magnesium chloride or butyllithium, gave neither a reaction nor loss of the protection group. Therefore, we had to satisfy ourselves with the relatively modest yield of **4**, obtained by direct silylation of **3**.

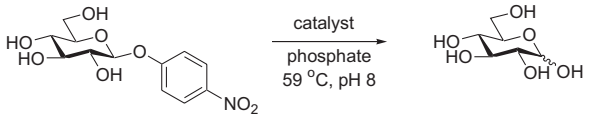
Oxidation of **4** to the aldehyde with Dess–Martin's periodinane, followed by subsequent oxidation to the carboxylic acid with $NaClO_2$, gave the acid **8** in 74% yield (Scheme 2). Removal of the TBS group with BF_3 afforded **9** in 49% yield. Oxidation of the remaining primary alcohol of **9** with Dess–Martin's periodinane and reaction of the resulting aldehyde with KCN then gave us **10** in 63% yield. Finally, hydrogenolysis of the benzyl groups gave target **1** in quantitative yield.

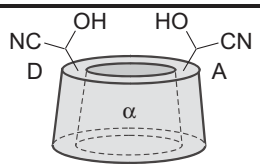
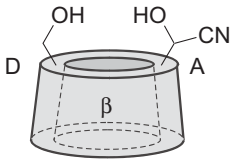
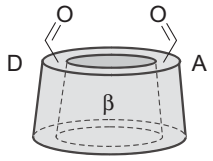
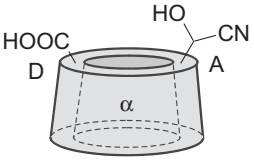
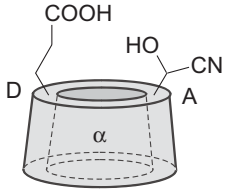
A related procedure was used to obtain **2**. Oxidation of **4** to the aldehyde was followed by Wittig reaction with benzyl 2-triphenylphosphonium acetate, which led to Wittig adduct **11** in 70% yield (Scheme 3). Deprotection of the silyl group with BF_3 gave structure **12**. As **12** was subjected to oxidation and reaction with KCN, the aldehyde was converted to the cyanohydrin **13**, albeit in a rather low yield of 35%. Hydrogenolysis eventually gave saturation of the double bond and removal of all protection groups affording a quantitative yield of target **2**.

Catalysis

The glycosidase activity of **1** and **2** was investigated by monitoring their influence on the hydrolysis of 4-nitrophenyl-β-D-glucopyranoside at pH 8 and 59 °C (Table 1). These reactions were monitored by following the increase in absorption at 400 nm. Similarly to previously investigated cyclodextrin cyanohydrins (4, 5), both **1** and **2** were found to be catalytic. However, in contrast, the catalysis was found to decline rapidly as a function of time. As shown in Figs. 2 and 3, the progress curves were initiated by a boost of a rapid reaction, which in 1 to 2 h declined to become a steady-state rate that was identical or slightly higher than the background reaction rate. Since over the time period only a small amount of substrate was converted, the phenomenon cannot be caused by substrate depletion. Furthermore, since the change in rate, depending on the condi-

Table 1. Kinetic parameters for the **1** and **2** catalysed hydrolysis of β-glucoside at pH 8.0 and 59 °C.



Structure	Catalyst	k_{cat} ($x10^{-5}$ s^{-1})	K_m	k_{cat}/k_{uncat}
	Ref. 5	6.96±0.29	6.52±1.10	2688
	Ref. 5	1.47±0.12	30.6±4.9	1067
	Ref. 5	0.13±0.02	4.50±2.47	55
	**1**[a]	2.02±0.12	7.17±1.73	1102
	1[b]	0.07±0.02	11.98±6.99	43
	**2**[a]	1.07±0.06	9.25±1.59	563
	2[b]	—[c]	—[c]	—[c]

[a]Initial catalytic rate.
[b]End catalytic rate.
[c]No catalysis.

tions, was complete before even one turnover occurred, it is extremely unlikely that product inhibition or covalent reaction between substrate and catalyst is the reason for the decline in the hydrolysis rate. As a control, the catalyst (**1** or **2**) was incubated for 2 h in buffer at 59 °C before being added to the substrate, and under these conditions the rapid reaction onset disappears and the initial rate is identical to the end rate. It is therefore safe to conclude that the catalyst undergoes a chemical reaction – decomposition reaction under the reaction conditions.

By fitting a line either to the progress curve over the first 0.5 h or after 3 h, it was possible to determine an initial rate of reaction and an end reaction rate (Table 1). For **2**, the end rate is identical to the background rate, i.e., there is no catalysis. For the other cases, a catalytic rate is obtained by subtracting the background rates, giving catalysed rates. These catalysed rates follow, both for the initial rate and the end rate, Michaelis–Menten kinetics,giving the K_M and k_{cat} values as shown in Table 1. The k_{cat} is used together with the uncatalysed rate to calculate a k_{cat}/k_{uncat} value.

As demonstrated, the values for k_{cat}/k_{uncat} for the initial reaction is 1102 and 567 for **1** and **2**, respectively. This is very similar to the rate accelerations obtained with other cyclodextrin cyanohydrins. Thus, the α-cyclodextrin dicyanohydrin gave a k_{cat}/k_{uncat} of 2688 under these conditions, while β-cyclodextrin monocyanohydrin gave k_{cat}/k_{uncat} = 1067 (Table 1) (5). It is therefore probable that **1** and **2** are able to initially function with the cyanohydrin in-

Scheme 4. Two possible mechanisms for the decomposition of **1**.

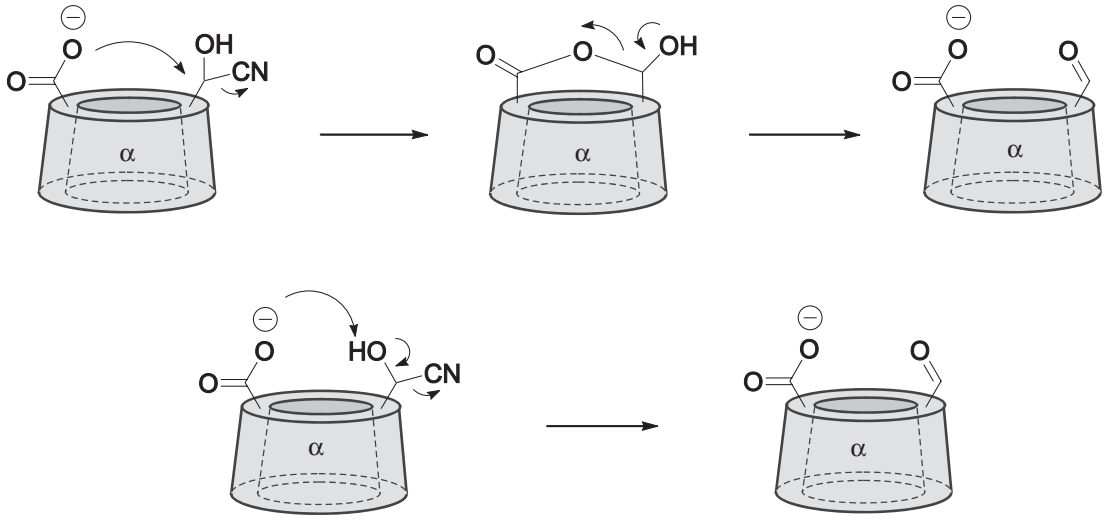


Fig. 2. Progress curve for the hydrolysis of 4-nitrophenyl β-D-glucopyranoside (pH 8, T = 59 °C, 50 mmol/L of phosphate buffer) in the presence of **1** or nothing (background).

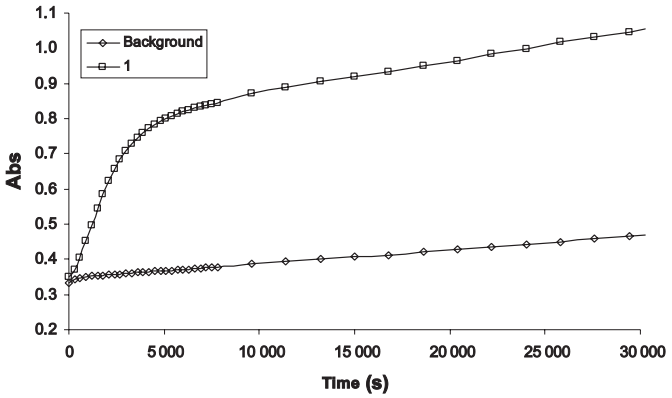
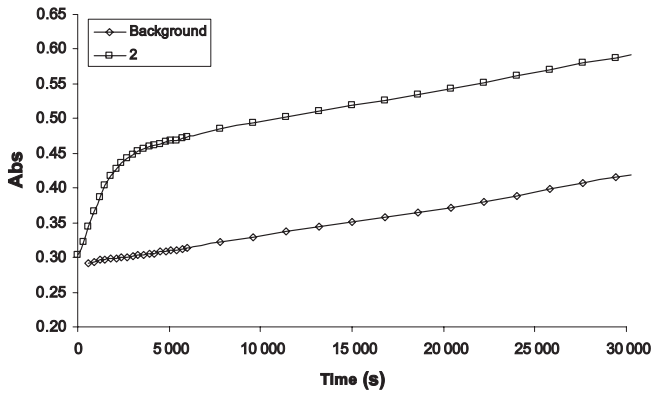


Fig. 3. Progress curve for the hydrolysis of 4-nitrophenyl β-D-glucopyranoside (pH 8, T = 59 °C, 50 mmol/L of phosphate buffer) in the presence of **2** or nothing (background).



tact and having the intended catalytic role. However, it was also observed that this initial catalysis was not higher than that of the previous catalysts, indicating that there is none of the intended cooperative catalysis between carboxylate and cyanohydrin.

The end k_{cat}/k_{uncat}, on the other hand, is 43 and no catalysis occurred for **1** and **2**, respectively (Table 1). This in likelihood means that the cyanohydrin has been blocked or destroyed. There are two reasonable possibilities of how this might occur. Either the free carboxylic acid might form an ester, a lactone, with the cyanohydrin hydroxyl group or another alcohol, which either blocks the catalytic group or the cavity to an extent that the substrate cannot enter. Alternatively, the carboxylate may function as an internal nucleophile and perform nucleophilic catalysis, not as intended on the substrate, but on the cyanohydrin, making it undergo hydrolysis to the aldehyde and cyanide. This latter explanation makes more chemical sense: lactonization of hydroxy acids is normally not thermodynamically favorable at pH 8, so it is not very plausible that a lactone would be the major form of **1** or **2** at pH 8. Further, the relatively weak catalysis obtained from decomposed **1** does indeed suggest that the compound has been converted to an aldehyde, since we have previously found that β-cyclodextrin dialdehyde is a weak catalyst (Table 1).

To support either hypothesis, the decomposition of **1** in buffer was followed by MS spectroscopy at different intervals, and the initial peak corresponding to M + Na of **1** was over time converted to new peak corresponding to M + Na of the corresponding cyclodextrin acidaldehyde. It is therefore clear that the rapidly declining catalytic activity of **1** and **2** is due to rapid decomposition of the cyanohydrin into the aldehyde, and we propose the intramolecular mechanisms outlined in Scheme 4 for this conversion. Thus, either attack of carboxylate ion on the cyanohydrin leads to substitution of this group and formation of a lactone hemiacetal that subsequently cleaves readily. Alternatively, the carboxylate acts as a general base catalyst, assisting the spontaneous decomposition of the cyanohydrin. At present these mechanisms are indistinguishable. It is remarkable that both **1** and **2** undergo this cleavage with ease despite the fact that the carboxylate is placed at rather different positions, though it is also clear that **2** undergoes decomposition faster. Nevertheless, the observation that **1** undergoes this reaction at all suggests that a considerable flexibility is present in the cyclodextrin.

In summary, we have prepared two new cyclodextrin derivatives that contain a cyanohydrin and a carboxylate group on the primary face of the cyclodextrin. Both compounds are able to catalyse the hydrolysis of glycosides with a rate that is equivalent to the rate previously seen with other cyclodextrin cyanohydrins, but rapidly lose activity. This decomposition was found to occur as the cyanohydrin cleaved into the corresponding aldehyde and cyanide, and the carboxylate group is believed to catalyse this process.

Experimental

All reagents were used as purchased without further purification. TLC was performed on Merck silica gel 60 F_{254} plates with detection by charring with cerium sulphate and ammonium heptamolybdat, and by UV light when applicable. Flash column chromatography was performed on Fluka silica gel (230–400 mesh) as the stationary phase. Optical rotations were recorded on a PerkinElmer 241 polarimeter at room temperature. IR spectra were recorded on a PerkinElmer FT-IR Paragon 1000. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (δ_H, δ_C 0.00). *J* values are given in Hz. MALDI-TOF mass spectra were recorded on a Bruker Daltonics mass spectrometer (Bruker) using an α-cyano-4-hydroxy-cinnamic acid (HCCA) matrix.

6^A-*O*-*tert*-Butyldimethylsilyl-2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin (4) and 6^A,6^D-di-*O*-*tert*-butyldimethylsilyl-2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin (5)

A solution of **3** (2.00 g, 0.83 mmol), imidazole (113 mg, 1.66 mmol), and TBDMSCl (150 mg, 0.99 mmol) in anhydr. DMF (20 mL) was stirred under a N_2 atmosphere at room temperature for 48 h. The reaction mixture was diluted with EtOAc and the organic phase was washed several times with water, dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 1:4 → 2:7), to first afford **5** (19%) and then **4** (955 mg, 46%) as white foams. **4**: $[\alpha]_D$ +32.2° (*c* 1.0, CH_3Cl). IR (KBr, cm^{-1}): 3482, 3063, 3029, 2926, 2857, 1951, 1733, 1605, 1496, 1453, 1359, 1094, 1027. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33–7.08 (m, 80H, aromatic-H), 5.57 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.47 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.36 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.32 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.21 (t, 2H, ^2J = 10.8 Hz, CH_2Ph), 5.04 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 4.95–4.80 (m, 13H), 4.70 (t, 2H, ^2J = 12.4 Hz), 4.59–4.34 (m, 21H), 4.25–4.08 (m, 15H), 4.00–3.81 (m, 14H), 3.73–3.60 (m, 7H), 3.56 (dd, 1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.50–3.43 (m, 3H), 3.41 (dd, 1H, $^3J_{1,2}$ = 3.0 Hz, $^3J_{2,3}$ = 9.8 Hz, H-2), 3.36 (dd, 1H, $^3J_{1,2}$ = 3.0 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 2.56 (bs, 1H, OH), 0.87 (s, 9H, SiC(CH_3)$_3$), 0.00 (s, 6H, $SiCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 139.4–137.9 (C_{ipso}), 128.3–126.8 (CH aromatic), 98.7 (C-1), 98.1 (C-1), 98.0 (C-1), 97.9 (C-1), 97.8 (C-1), 97.7 (C-1), 81.4, 81.3, 81.0, 80.8, 80.5, 80.0, 79.8, 79.6, 79.4, 79.2, 79.1, 79.0, 78.6, 78.2, 76.1, 76.0, 75.9, 75.8, 75.7, 75.6, 74.8, 74.6, 73.3, 73.1, 73.0, 72.9, 72.6, 72.4, 72.2, 71.9, 71.8, 71.6, 71.4, 71.3, 69.3, 69.2, 68.8 (CH_2, CH), 62.5 (C-6), 61.4 (C-6), 60.3 (C-6), 26.0 (SiC(CH_3)$_3$), 18.3 (SiC), –4.7 ($SiCH_3$), –4.9 ($SiCH_3$). MALDI-TOF-MS *m*/*z* calcd. for $C_{154}H_{170}O_{30}SiNa$: 2550.144; found: 2550.526 [M]$^+$. **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.30–7.12 (m, 80H, aromatic-H), 5.31–5.20 (m, 8H), 5.14–5.11 (m, 4H), 4.91–4.86 (m, 6H), 4.62–4.41 (m, 19H), 4.33 (t, 4H, ^2J = 12.4 Hz), 4.23–3.94 (m, 21H), 3.80 (d, 1H, ^3J = 8.4 Hz), 3.66–3.50 (m, 10H), 3.42 (dd, 2H, $^3J_{1,2}$ = 3.4 Hz, $^3J_{2,3}$ = 9.4 Hz, H-2), 0.91 (s, 18H, SiC(CH_3)$_3$), 0.01 (s, 6H, $SiCH_3$), 0.00 (s, 6H, $SiCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 139.3–138.1 (C_{ipso}), 128.3–126.8 (CH aromatic), 98.3 (C-1), 98.1 (C-1), 98.0 (C-1), 81.3, 81.1, 80.7, 79.2, 79.1, 79.0, 78.1, 78.0, 75.8, 75.4, 75.3, 73.5, 73.4, 73.0, 72.8, 72.6, 72.4, 71.6, 71.4, 69.1, 69.0 (CH_2, CH), 62.3 (C-6), 26.0 (SiC(CH_3)$_3$), 18.2 (SiC), –4.9 ($SiCH_3$), –5.2 ($SiCH_3$).

6^A,6^D-Di-*O*-methylsilyl-2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin (6)

Dimethylsilyl chloride (60 μL, 0.50 mmol) was added to a solution of **3** (1.00 g, 0.41 mmol) in anhydrous pyridine (22 mL) and the reaction mixture was stirred at room temperature under a N_2 atmosphere for 48 h. The reaction mixture was concentrated under reduced pressure and toluene was added and pyridine was azeotroped. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 2:7) to afford **6** (650 mg, 64%) as white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35–7.07 (m, 80H, aromatic-H), 5.85 (d, 2H, $^3J_{1,2}$ = 4.0 Hz, H-1), 5.64 (d, 2H, ^2J = 10.4 Hz, C*H*Ph), 5.25 (d, 2H, ^2J = 10.4 Hz, C*H*Ph), 5.01 (d, 2H, ^2J = 10.8 Hz, C*H*Ph), 5.93 (t, 4H, ^2J = 12.0 Hz, CH_2Ph), 4.83–4.75 (m, 8H), 4.64 (d, 2H, ^2J = 10.0 Hz), 4.57–4.41 (m, 19H), 4.30–4.09 (m, 16H), 4.02–3.98 (m, 4H), 3.85 (bd, 2H, *J* = 7.6 Hz), 3.75 (d, 2H, *J* = 10.4 Hz), 3.70 (dd, 2H, $^3J_{1,2}$ = 3.6 Hz, $^3J_{2,3}$ = 10.0 Hz, H-2), 3.59 (t, 2H, *J* = 9.2 Hz), 3.53–3.44 (m, 8H), 0.00 (s, 6H, $SiCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 139.5–137.8 (C_{ipso}), 128.3–126.1 (CH aromatic), 99.2 (C-1), 98.5 (C-1), 96.4 (C-1), 81.6, 81.5, 81.1, 80.8, 80.4, 80.3, 78.9, 77.8, 76.8, 76.5, 76.1, 73.6, 73.5, 73.3, 73.1, 72.8, 72.1, 71.9, 71.8, 69.8, 69.2, 68.9 (CH_2, CH), 63.1 (C-6), –4.1 ($SiCH_3$). MALDI-TOF-MS *m*/*z* calcd. for $C_{150}H_{160}O_{30}SiNa$: 2492.0661; found: 2492.306 [M]$^+$.

6^A,6^D-Di-*O*-isopropylsilyl-2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin (7)

Diisopropylsilyl chloride (27 μL, 0.15 mmol) was added to a solution of **3** (300 mg, 0.12 mmol) in anhydrous pyridine (7 mL) and the reaction mixture was stirred at room temperature under a N_2 atmosphere for 19 h. The reaction mixture was concentrated under reduced pressure and toluene was added and pyridine was azeotroped. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 2:7), to afford **7** (95 mg, 30%) as white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26–6.86 (m, 80H, aromatic-H), 5.73 (d, 1H, $^3J_{1,2}$ = 4.4 Hz, H-1), 5.45 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.13–5.00 (m, 3H), 4.86–4.72 (m, 6H), 4.66–4.58 (m, 4H), 4.49–4.24 (m, 18H), 4.20–3.90 (m, 16H), 3.85–3.82 (m, 3H), 3.77–3.67 (m, 3H), 3.56 (dd, 1H, $^3J_{1,2}$ = 4.4 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.52–3.40 (m, 6H), 3.36–3.30 (m, 5H), 1.17 (t, 2H, ^3J = 7.2 Hz, SiCH), 0.95 (d, 3H, ^3J = 7.6 Hz, SiCH(CH_3)$_2$), 0.90 (d, 3H, ^3J = 7.2 Hz,

SiCH(CH_3)$_2$), 0.88 (d, 3H, ^3J = 8.0 Hz, SiCH(CH_3)$_2$), 0.86 (d, 3H, ^3J = 7.2 Hz, SiCH(CH_3)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 139.5–137.8 (C_{ipso}), 129.0–126.0 (CH aromatic), 99.6 (C-1), 98.6 (C-1), 96.1 (C-1), 81.4, 81.2, 81.1, 80.9, 80.6, 79.9, 78.9, 77.9, 76.5, 75.9, 73.6, 73.4, 73.0, 72.8, 72.6, 72.0, 71.7, 70.2, 69.4, 68.3 (CH_2, CH), 63.3 (C-6), 18.1 (SiCH), 17.3–17.2 (SiCH(CH_3)$_2$). MALDI-TOF-MS *m/z* calcd. for $C_{154}H_{168}O_{30}SiNa$: 2548.1287; found: 2549.343 $[M]^+$.

6^D-*O*-*tert*-Butyldimethylsilyl-2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin-6^A-carboxylic acid (8)

To a solution of **4** (336 mg, 0.13 mmol) in CH_2Cl_2 (13 mL) was added Dess–Martin periodinane reagent (141 mg, 0.33 mmol) and the reaction mixture was stirred for 2 h and then quenched by addition of Et_2O (13 mL) and satd. aq. $NaHCO_3$ containing 3.0 g of $Na_2S_2O_3$ (13 mL). After stirring for an additional hour, the solution was diluted with Et_2O (50 mL) and washed successively with satd. aq. $NaHCO_3$ (30 mL) and water (30 mL). The organic phase was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was dissolved in a mixture of *t*-BuOH (9.5 mL), THF (4 mL), and 2-methyl-2-butene (4 mL), and $NaClO_2$ (240 mg, 2.66 mmol) and NaH_2PO_4 (266 mg) in water (4 mL) were added. The reaction mixture was stirred overnight and then quenched with 1 mol/L aq. HCl (10 mL) and extracted with EtOAc (3 × 50 mL). The organic phase was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent, EtOAc–pentane, 2:7 → 2:5 and 2:5, containing 1% HCOOH) to afford **8** (251 mg, 74%) as white foam. $[\alpha]_D$ +28.6° (*c* 1.0, CH_3Cl). IR (KBr, cm^{-1}): 3442, 3063, 3030, 2927, 1724 (COOH), 1497, 1453, 1360, 1094, 1027. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38–7.10 (m, 80H, aromatic-H), 5.79 (d, 1H, ^3J = 3.6 Hz, H-1), 5.74 (d, 1H, ^3J = 3.6 Hz, H-1), 5.55 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.51 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.22 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 5.18 (d, 1H, ^2J = 11.2 Hz, C*H*Ph), 5.02–4.74 (m, 16H), 4.71–3.93 (m, 50H), 3.84–3.31 (m, 19H), 3.63 (dd, 1H, $^3J_{1,2}$ = 4.0 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 0.88 (s, 9H, SiC(CH_3)$_3$), 0.00 (s, 6H, SiCH$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.9 (CO), 139.3–136.4 (C_{ipso}), 128.7–126.3 (CH aromatic), 99.3 (C-1), 98.4 (C-1), 97.9 (C-1), 97.5 (C-1), 97.1 (C-1), 96.3 (C-1), 82.8, 81.3, 81.2, 80.8, 80.6, 80.5, 80.0, 79.9, 79.8, 79.2, 79.1, 78.9, 78.1, 77.9, 76.4, 76.2, 76.1, 75.8, 75.4, 74.1, 73.7, 73.5, 73.3, 73.2, 72.9, 72.7, 72.6, 72.2, 71.8, 71.6, 71.5, 71.2, 70.8, 70.3, 70.1, 69.5, 68.9, 68.8, 62.4 (CH_2, CH), 26.0 (SiC(*C*H$_3$)$_3$), 18.3 (SiC), –4.5 (SiCH$_3$), –4.6 (SiCH$_3$). MALDI-TOF-MS *m/z* calcd. for $C_{154}H_{168}O_{31}SiNa$: 2564.1237; found: 2565.203 $[M]^+$.

2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-Hexadecakis-*O*-benzyl-α-cyclodextrin-6^A-carboxylic acid (9)

To a solution of **8** (463 mg, 0.18 mmol) in CH_2Cl_2 (5.5 mL) was added $BF_3{\cdot}Et_2O$ (0.2 mL). The reaction mixture was stirred for 1.5 h at room temperature, diluted with CH_2Cl_2, and poured in ice water. The mixture was basified with aq. NaOH (1 mol/L) and the phases were separated. The organic layer was acidified with aq. HCl (1 mol/L), dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent, EtOAc–pentane, 1:4 containing 1% HCOOH) to afford **9** (218 mg, 49%) as white foam. $[\alpha]_D$ +35.0° (*c* 0.5, CH_3Cl). IR (KBr, cm^{-1}): 3442 (OH), 3031, 2924, 1746 (COOH), 1496, 1454, 1354, 1095, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26–6.97 (m, 80H, aromatic-H), 5.66 (d, 1H, ^3J = 4.0 Hz, H-1), 5.64 (d, 1H, ^3J = 4.0 Hz, H-1), 5.42 (d, 1H, ^2J = 10.0 Hz, C*H*Ph), 5.36 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.07 (d, 2H, ^2J = 10.4 Hz, CH_2Ph), 4.85–4.76 (m, 5H), 4.73–3.89 (m, 60H), 3.79 (t, 3H, ^3J = 8.6 Hz), 3.74–3.46 (m, 14H), 3.42 (dd, 2H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.37 (d, 2H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.22–3.16 (m, 2H), 3.11 (t, 1H, ^3J = 9.0 Hz, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1 (CO), 139.5–136.2 (C_{ipso}), 128.8–126.3 (CH aromatic), 99.3 (C-1), 99.0 (C-1), 98.1 (C-1), 97.9 (C-1), 97.8 (C-1), 96.5 (C-1), 83.4, 81.9, 81.6, 81.5, 81.3, 81.2, 80.9, 80.6, 80.5, 79.9, 79.5, 79.4, 79.2, 78.0, 76.6, 76.4, 76.2, 75.4, 74.1, 74.0, 73.8, 73.6, 73.5, 73.4, 73.3, 73.2, 72.8, 72.5, 72.2, 71.9, 71.8, 71.4, 71.0, 70.8, 70.2, 70.0, 69.3, 68.9, 68.1, 61.0 (CH_2, CH). MALDI-TOF-MS *m/z* calcd. for $C_{148}H_{154}O_{31}Na$: 2450.0372; found: 2450.447 $[M]^+$.

6^D-*C*-Cyano-2$^{A–F}$,3$^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin-6^A-carboxylic acid (10)

To a solution of **9** (217 mg, 0.09 mmol) in CH_2Cl_2 (9 mL) was added Dess–Martin periodinane reagent (95 mg, 0.22 mmol) and the reaction mixture was stirred 2 h and then quenched by addition of Et_2O (9 mL) and satd. aq. $NaHCO_3$ containing 0.36 g of $Na_2S_2O_3$ (9 mL). After stirring for an additional hour, the solution was diluted with Et_2O (30 mL) and washed successively with satd. aq. $NaHCO_3$ (25 mL) and water (25 mL). The organic phase was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. To a solution of the residue in Et_2O (3 mL) and MeOH (5 mL) were added KCN (164 mg, 2.52 mmol) and NH_4Cl (225 mg, 4.20 mmol) in water (10 mL). The reaction mixture was stirred for 20 h at room temperature. After that, the organic solvent was removed and the aqueous phase was extracted with CH_2Cl_2. The organic layer was washed with water, dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 1:4 → 1:4 containing 1% HCOOH) to afford **10** (130 mg, 63%) as a white foam. $[\alpha]_D$ +38.6° (*c* 1.0, CH_3Cl). IR (KBr, cm^{-1}): 3031, 2927, 2247 (CN), 1741 (COOH), 1497, 1454, 1355, 1096, 1045. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25–6.96 (m, 80H, aromatic-H), 5.71 (d, 1H, ^3J = 3.2 Hz, H-1), 5.62 (d, 1H, ^3J = 4.0 Hz, H-1), 5.39 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.34 (d, 1H, ^3J = 3.6 Hz, H-1), 5.32–5.21 (m, 7H), 5.13 (d, 1H, ^2J = 15.6 Hz, C*H*Ph), 5.05 (m, 2H), 4.95–4.84 (m, 3H), 4.77–4.48 (m, 40H), 4.46–4.13 (m, 42H), 4.12–3.70 (m, 54H), 3.68–3.13 (m, 35H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.9 (CO), 169.9 (CO), 139.6–136.2 (C_{ipso}), 128.9–126.2 (CH aromatic), 118.4 (CN), 117.2 (CN), 100.4 (C-1), 99.6 (C-1), 99.4 (C-1), 98.5 (C-1), 98.1 (C-1), 97.9 (C-1), 97.6 (C-1), 97.1 (C-1), 97.0 (C-1), 96.3 (C-1), 84.4, 83.6, 81.5, 81.2, 81.1, 80.9, 80.5, 80.4, 80.3, 80.1, 80.0, 79.7, 79.6, 79.5, 79.4, 79.2, 79.1, 79.0, 78.6, 78.1, 76.5, 76.4, 76.2, 76.0, 75.7, 75.6, 75.4, 74.6, 74.3, 74.2, 74.0, 73.9, 73.7, 73.6, 73.5, 73.4, 73.3, 73.2, 73.1, 73.0, 72.9, 72.8, 72.7,

72.6, 72.5, 72.4, 72.3, 72.1, 72.0, 71.7, 71.6, 71.4, 71.3, 71.2, 71.0, 70.8, 70.6, 70.4, 70.2, 70.1, 69.9, 69.5, 69.2, 68.9, 68.8, 63.0 (CH_2, CH), 60.4 (*C*H(OH)CN). MALDI-TOF-MS *m/z* calcd. for $C_{149}H_{152}O_{31}NNa$: 2474.0246; found: 2474.316 $[M]^+$.

6^D-*C*-Cyano-α-cyclodextrin-6^A-carboxylic acid (1)

Compound **10** (130 mg, 0.05 mmol) was dissolved in a mixture of MeOH–EtOAc (1:1, 5 mL). Then Pd/C (13 mg) and TFA (cat) were added and the mixture was stirred overnight under a hydrogen atmosphere. Filtration over Celite and evaporation of the solvent gave **1** (54 mg, 100%) as a white solid. $[\alpha]_D$ +67.2° (*c* 0.5, H_2O). IR (KBr, cm^{-1}): 3423, 2942, 2246 (CN), 1679 (COOH), 1437, 1205, 1151, 1034. ^1H NMR (400 MHz, D_2O) δ: 5.14–5.07 (m, 6H, H-1), 4.02–3.63 (m, 40H), 3.32 (m, 1H). ^{13}C NMR (100 MHz, D_2O) δ: 163.3 (CO), 163.0 (CO), 119.0 (CN), 101.8 (C-1), 101.7 (C-1), 101.6 (C-1), 101.5 (C-1), 101.4 (C-1), 101.3 (C-1), 100.7 (C-1), 82.0, 81.7, 81.6, 81.5, 81.4, 81.3, 81.2, 80.9, 74.8, 73.1, 73.0, 72.9, 72.7, 72.4, 72.2, 72.1, 71.9, 71.7, 71.6, 71.5, 71.3, 65.4, 60.7, 60.3, 60.0, 59.7, 68.8, 63.0 (CH, CH_2, *C*H(OH)CN). MALDI-TOF-MS *m/z* calcd. for $C_{37}H_{57}O_{31}NNa$: 1034.2812; found: 1034.127 $[M]^+$.

Benzyl 6^A-*tert*-butyldimethylsilyl-$2^{A–F}$,$3^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin-6^D-propenoate (11)

To a solution of BnO_2CCH_2PPhBr (496 mg, 1.01 mmol) in anhydr. THF (13 mL) was added dropwise *n*-BuLi (0.6 mL, 0.96 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was cooled to –40 °C and a solution of **4** (850 mg, 0.34 mmol) in anhydr. THF (13 mL) was added. The mixture was stirred at –40 °C for 30 min and was then allowed to reach room temperature. Ether (200 mL) was added and the mixture was washed with aq. NH_4Cl (150 mL), water (2 × 100 mL), and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 2:11) to afford **11** (625 mg, 70%) as white foam. $[\alpha]_D$ +42.6° (*c* 1.0, CH_3Cl). IR (KBr, cm^{-1}): 3030 (C=C), 2926, 1720 (CO_2Bn), 1496, 1453, 1360, 1094, 1027. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.50 (dd, 1H, $^3J_{4,5}$ = 4.0 Hz, $^3J_{trans}$ = 15.6 Hz, CH=), 7.44–7.16 (m, 85H, aromatic-H), 6.07 (d, 1H, $^3J_{trans}$ = 15.6 Hz, =$CHCO_2Bn$), 5.42 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.34 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.29–5.10 (m, 10H), 5.24 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.02 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 4.98–4.87 (m, 8H), 4.65–4.40 (m, 23H), 4.28–4.11 (m, 18H), 4.08–3.95 (m, 6H), 3.87 (bd, 2H,^3J = 8.0 Hz), 3.71–3.59 (m, 7H), 3.57–3.47 (m, 4H), 3.45–3.37 (m, 3H), 0.91 (s, 9H, $SiC(CH_3)_3$), 0.00 (s, 6H, $SiCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.9 (CO), 145.8 (CH=), 139.5–137.9 (C_{ipso}), 136.0 (C_{ipso}-CO_2Bn), 128.7–127.1 (CH aromatic), 122.0 (=*C*HCO_2Bn), 99.2 (C-1), 99.0 (C-1), 98.5 (C-1), 98.2 (2 × C-1), 98.1 (C-1), 84.5, 81.4, 81.3, 81.1, 80.9, 80.8, 80.1, 79.7, 79.6, 79.2, 78.7, 78.6, 78.4, 78.0, 76.0, 75.9, 75.5, 75.3, 73.7, 73.4, 73.2, 72.9, 72.8, 72.7, 72.6, 72.5, 72.1, 71.9, 71.5, 71.3, 69.5, 69.4, 68.9, 68.5, 66.3, 62.3, 60.5 (CH_2, CH). MALDI-TOF-MS *m/z* calcd. for $C_{163}H_{176}O_{31}SiNa$: 2680.1863; found: 2680.434 $[M]^+$.

Benzyl $2^{A–F}$,$3^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin-6^D-propenoate (12)

TBAF (0.12 mL, 0.12 mmol) was added to a solution of **11** (102 mg, 0.04 mmol) in anhydrous THF (1.2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 6 h. Aqueous NH_4Cl was added and the mixture was diluted with ether (50 mL) and washed successively with water (2 × 25 mL) and brine (25mL). The organic layer was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 2:7) to afford **12** (73 mg, 75%) as white foam. $[\alpha]_D$ +36.6° (*c* 1.0, CH_3Cl). IR (KBr, cm^{-1}): 3488 (OH), 3030 (C=C), 2924, 1722 (CO_2Bn), 1496, 1453, 1354, 1094, 1039. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.25–6.98 (m, 86H, aromatic-H and CH=), 6.00 (d, 1H, $^3J_{trans}$ = 15.6 Hz, =$CHCO_2Bn$), 5.40 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.29 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.28 (d, 1H, $^3J_{1,2}$ = 4.0 Hz, H-1), 5.20–5.13 (m, 3H), 5.06 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 4.92 (s, 2H), 4.90 (d, 1H, $^3J_{1,2}$ = 3.0 Hz, H-1), 4.84–4.69 (m, 11H), 4.64 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 4.58 (t, 2H, ^2J = 11.2 Hz), 4.46 (d, 1H, $^3J_{1,2}$ = 4.0 Hz, H-1), 4.43 (d, 1H, $^3J_{1,2}$ = 4.0 Hz, H-1), 4.39–4.20 (m, 17H), 4.15–3.95 (m, 13H), 3.89–3.66 (m, 13H), 3.61–3.57 (m, 2H), 3.50–3.33 (m, 10H), 3.31 (dd, 1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.25 (dd, 1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 2.39 (bs, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.8 (CO), 145.8 (CH=), 139.6–137.9 (C_{ipso}), 135.9 (C_{ipso}-CO_2Bn), 128.7–126.5 (CH aromatic), 122.2 (=*C*HCO_2Bn), 99.3 (C-1), 99.0 (C-1), 98.7 (C-1), 98.3 (C-1), 98.2 (C-1), 97.5 (C-1), 82.5, 81.6, 81.4, 81.3, 81.2, 80.6, 80.1, 79.9, 79.6, 79.5, 79.0, 78.7, 78.3, 76.4, 76.2, 76.1, 75.9, 75.8, 74.8, 74.5, 73.5, 73.4, 73.3, 73.2, 73.1, 73.0, 72.9, 72.5, 72.4, 72.0, 71.9, 71.8, 71.6, 69.4, 69.0, 68.9, 66.3, 61.4 (CH_2, CH). MALDI-TOF-MS *m/z* calcd. for $C_{157}H_{162}O_{31}Na$: 2566.0998; found: 2566.760 $[M]^+$.

Benzyl 6^A-*C*-cyano-$2^{A–F}$,$3^{A–F}$,6^B,6^C,6^E,6^F-hexadecakis-*O*-benzyl-α-cyclodextrin-6^D-propenoate (13)

To a solution of **12** (246 mg, 0.10 mmol) in Et_2O (3.5 mL) and MeOH (6 mL) were added KCN (189 mg, 2.91 mmol) and NH_4Cl (259 mg, 4.85 mmol) in water (11.5 mL). The reaction mixture was stirred overnight at room temperature. After that, the organic solvent was removed and the aqueous phase was extracted with CH_2Cl_2. The organic layer was washed with water, dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc–pentane, 1:4 → 2:7) to afford **13** (87 mg, 35%) as white foam. $[\alpha]_D$ +47.3° (*c* 0.8, CH_3Cl). IR (KBr, cm^{-1}): 3030 (C=C), 2924, 2246 (CN), 1721 (CO_2Bn), 1496, 1454, 1355, 1096, 1040. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.38 (dd, 1H, $^3J_{4,5}$ = 4.8 Hz, $^3J_{trans}$ = 15.6 Hz, CH=), 7.33–7.09 (m, 85H, aromatic-H), 6.17 (d, 1H, $^3J_{trans}$ = 15.6 Hz, =$CHCO_2Bn$), 5.30 (bd, 1H, ^3J = 7.6 Hz), 5.24 (d, 1H, ^2J = 10.4 Hz, CH_2Ph), 5.22 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.18 (d, 1H, ^2J = 11.2 Hz, C*H*Ph), 5.16 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.11 (d, 1H, ^2J= 10.8 Hz, CHPh), 5.08–4.98 (m, 7H), 4.91–4.76 (m, 8H), 4.67–4.57 (m, 5H), 4.51–4.39 (m, 15H), 4.35 (d, 1H, ^2J = 12.0 Hz), 4.30 (d, 1H, ^2J = 12.4 Hz), 4.18–3.77 (m, 23H), 3.71 (t, 1H, ^3J = 9.0 Hz), 3.65 (d, 1H, ^2J = 10.8 Hz), 3.58–3.47 (m, 8H), 3.45 (dd, 1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.41 (dd,

1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.3 (CO), 146.2 (CH=), 139.5–137.8 (C_{ipso}), 135.7 (C_{ipso}-CO_2Bn), 128.7–126.8 (CH aromatic), 122.5 (=*C*HCO_2Bn), 117.5 (CN), 99.5 (C-1), 99.4 (C-1), 99.3 (C-1), 98.5 (C-1), 98.4 (C-1), 98.2 (C-1), 83.6, 80.9, 80.8, 80.4, 80.2, 80.1, 80.0, 79.6, 79.3, 79.2, 78.9, 78.7, 78.3, 77.9, 76.1, 75.5, 75.4, 74.9, 73.6, 73.5, 73.4, 73.3, 73.2, 73.1, 72.9, 72.8, 72.7, 72.0, 71.7, 71.6, 69.6, 69.5, 69.0, 68.8, 68.7, 66.4, 62.9 (CH_2, CH). MALDI-TOF-MS *m*/*z* calcd. for $C_{158}H_{161}O_{31}NNa$: 2591.0950; found: 2590.501 $[M]^+$.

6^A-*C*-Cyano-α-cyclodextrin-6^D-propanoic acid (2)

Compound **13** (113 mg, 0.04 mmol) was dissolved in a mixture of MeOH–EtOAc (1:1, 5 mL). Then Pd/C (12 mg) and TFA (cat) were added and the mixture was stirred overnight under a hydrogen atmosphere. Filtration over Celite and evaporation of the solvent gave **2** (46 mg, 100%) as a white solid. $[\alpha]_D$ +56.8° (*c* 1.0, H_2O). IR (KBr, cm^{-1}): 3431, 2247 (CN), 1685 (COOH), 1437, 1207, 1144, 1050. ^{1}H NMR (400 MHz, D_2O) δ: 4.94 (m, 6H, H-1), 3.89–3.77 (m, 20H), 3.49 (bs, 10H), 3.24–3.17 (m, 2H), 2.42 (bs, 1H), 2.29 (bs, 1H), 1.64 (bs, 2H). ^{13}C NMR (100 MHz, D_2O) δ: 163.3 (CO), 163.0 (CO), 118.0 (CN), 101.7 (C-1), 101.3 (C-1), 85.9, 81.6, 81.2, 73.3, 72.0, 71.8, 71.7, 70.6, 60.4, 39.5, 26.6 (CH, CH_2, *C*H(OH)CN). MALDI-TOF-MS *m*/*z* calcd. for $C_{39}H_{61}O_{31}NNa$: 1062.3125; found: 1062.284 $[M]^+$.

Procedure for determining the rate of hydrolysis

Each assay was performed on 2 mL samples prepared from 1 mL aqueous solutions of the appropriate aryl glycoside at different concentrations mixed with 1 mL of phosphate or other buffer containing either cyclodextrin derivative (0.5 mg/mL) or nothing as the control. The reactions were followed continuously at 59 °C using UV absorption at 400 nm. The reactions were monitored for 3–18 h. Velocities were determined as the slope of the progress curve of each reaction. Uncatalysed velocities were obtained directly from the control samples. Catalyzed velocities were calculated by subtracting the uncatalysed velocity from the velocity of the appropriate cyclodextrin-containing sample. The catalysed velocities 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].

Acknowledgements

We thank the Lundbeck Foundation and Forskningsrådet for Natur og Univers (FNU) for generous financial support.

References

1. J.W. Steed and J. Atwood. L. Supramolecular chemistry. John Wiley and Sons, Ltd., Chicester. 2001.
2. (*a*) A.J. Kirby. Angew. Chem. Int. Ed. Engl. **33**, 551 (1994); (*b*) Y. Murakami, J.I. Kikuchi, Y. Hisaeda, and O. Hayashida. Chem. Rev. **96**, 721 (1996); (*c*) W.B. Motherwell, M.J. Bingham, and Y. Six. Tetrahedron, **57**, 4663 (2001); (*d*) R. Breslow. Acc. Chem. Res. **28**, 146 (1995); (*e*) R. Breslow and C. Schmuck. J. Am. Chem. Soc. **118**, 6601 (1996); (*f*) R. Breslow and B. Zhang. J. Am. Chem. Soc. **114**, 5882 (1992); (*g*) T. Akiike, Y. Nagano, Y. Yamamoto, A. Nakamura, H. Ikeda, A. Veno, and F. Toda. Chem. Lett. 1089 (1994); (*h*) R. Breslow and B. Zhang. J. Am. Chem. Soc. **116**, 7893 (1994); (*i*) R. Breslow and S.D. Dong. Chem. Rev. **98**, 1997 (1998); (*j*) H. Ikeda, Y. Horimoto, M. Nakata, and A. Ueno. Tetrahedron Lett. **41**, 6483 (2000); (*k*) D.T.H. Chou, J. Zhu, X.C. Huang, and A.J. Bennet. J. Chem. Soc. Perkin Trans. 2, 83 (2001); (*l*) M. Kunishima, K. Yoshimura, H. Morigaki, R. Kawamata, K. Terao, and S. Tani. J. Am. Chem. Soc. **123**, 10760 (2001); (*m*) X.J. Ren, Y. Xue, K. Zhang, J.Q. Liu, G.M. Luo, J. Zheng, Y. Mu, and J.C. Shen. FEBS Lett. **507**, 377 (2001); (*n*) J.X. Yu, Y.Z. Zhao, M. Holterman, and D.L. Venton. Bioorg. Med. Chem. **10**, 3291 (2002); (*o*) X.J. Ren, Y. Xue, J.Q. Liu, K. Zhang, J. Zheng, G. Luo, C.H. Guo, Y. Mu, and J.C. Shen. ChemBioChem. **3**, 356 (2002); (*p*) Y. Liu, B. Li, L. Li, and H.Y. Zhang. Helv. Chim. Acta, **85**, 9 (2002); (*q*) J. Yang, B. Gabriele, S. Belvedere, Y. Huang, and R. Breslow. J. Org. Chem. **67**, 5057 (2002); (*r*) R. Kataky and E. Morgan. Biosens. Bioelectron. **18**, 1407 (2003); (*s*) W.K. Chan, W.Y. Yu, C.M. Che, and M.K. Wong. J. Org. Chem. **68**, 6576 (2003); (*t*) N.M. Milovic, J.D. Badjic, and N.M. Kostic. J. Am. Chem. Soc. **126**, 696 (2004); (*u*) C. Rousseau, B. Christensen, T.E. Pedersen, and M. Bols. Org. Biomol. Chem. **2**, 3476 (2004); (*v*) M. Fukudome, Y. Okabe, M. Sakaguchi, H. Morikawa, T. Fujioka, D.Q. Yuan, and K. Fujjta. Tetrahedron Lett. **45**, 9045 (2004); (*w*) Z.Y. Dong, J.Q. Liu, S.Z. Mao, X. Huang, B. Yang, X.J. Ren, G.M. Luo, and J.C. Shen. J. Am. Chem. Soc. **126**, 16395 (2004); (*x*) M. McNaughton, L. Engman, A. Birmingham, G. Powis, and I.A. Cotgreave. J. Med. Chem. **47**, 233 (2004); (*y*) C. Rousseau, B. Christensen, and M. Bols. Eur. J. Org. Chem. 2734 (2005); (*z*) S.H. Yoo, B.J. Lee, H. Kim, and J. Suh. J. Am. Chem. Soc. **127**, 9593 (2005).
3. For first research on artificial glycosidases, see: (*a*) T.H. Doug, J.Z. Chou, X. Huang, and A.J. Bennet. J. Chem. Soc. Perkin Trans. 2, 83 (2001); (*b*) T. Ohe, Y. Kajiwara, T. Kida, W. Zhang, Y. Nakatsuji, and I. Ikeda. Chem. Lett. 921 (1999).
4. F. Ortega-Caballero, C. Rousseau, B. Christensen, T.E. Petersen, and M. Bols. J. Am. Chem. Soc. **127**, 3238 (2005).
5. F. Ortega-Caballero, J. Bjerre, L.S. Laustsen, and M. Bols. J. Org. Chem. **70**, 7217 (2005).
6. C. Rousseau, N. Nielsen, and M. Bols. Tetrahedron Lett. **45**, 8709 (2004).
7. C. Rousseau, F. Ortega-Caballero, L.U. Nordstrøm, B. Christensen, T.E. Petersen, and M. Bols. Chem. Eur. J. **11**, 5094 (2005).
8. (*a*) A.J. Pearce and P. Sinaÿ. Angew. Chem. Int. Ed. **39**, 3610 (2000); (*b*) T. Lecourt, A. Herault, A.J. Pearce, M. Sollogoub, and P. Sinaÿ. Chem. Eur. J. **10**, 2960 (2004).