JOC*Article*

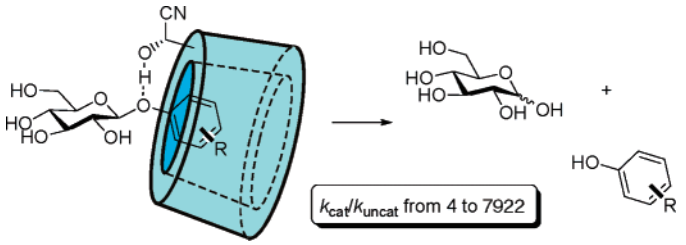
Four Orders of Magnitude Rate Increase in Artificial Enzyme-Catalyzed Aryl Glycoside Hydrolysis

Fernando Ortega-Caballero, Jeannette Bjerre, Line Skall Laustsen, and Mikael Bols*

Department of Chemistry, University of Aarhus, Langelandsgade 140, DK-8000 Aarhus C, Denmark

mb@chem.au.dk

Received April 28, 2005



(6^A*R*,6^D*R*)-6^A,6^D-Di-*C*-cyano-β-cyclodextrin (**1**) and 6^A,6^D-di-*C*-cyano-α-cyclodextrin (**2**) were synthesized and shown to catalyze hydrolysis of aryl glycosides into glucose and phenol with a reaction following Michaelis−Menten kinetics. At pH 8.0 and 59 °C hydrolysis of 4-nitrophenyl α-glucopyranoside was catalyzed by **1** with $K_M = 10.5 \pm 1.5$ mM, $k_{cat} = 1.42(\pm 0.09) \times 10^{-4}$ s^{-1}, and $k_{cat}/k_{uncat} = 7922$. Catalysis was observed with a concentration of **1** as low as 10 μM. Hydrolysis of the other aryl glycosides containing stereochemical variation in the sugar-moiety and 4-nitro-, 2-nitro-, 2-aldehydo-, and 2,4-dinitro- were also catalyzed by **1** and **2** with k_{cat}/k_{uncat} ranging from 4 to 7100. Hydrolysis of a phenyl β-D-glucoside or the thioglycoside tolylthio β-D-glucoside was also catalyzed. From a series of prepared analogues of **1** it was found that the catalysis was associated with the hydroxyl groups α to the nitril groups. The monocyanohydrin 6-*C*-cyano-β-cyclodextrin (**3**) was also found to catalyze the hydrolysis of 4-nitrophenyl β-glucopyranoside with $k_{cat}/k_{uncat} = 1356$. It was proposed that the cyclodextrin cyanohydrins **1**−**3** catalyze the hydrolysis by general acid catalysis on the bound substrate.

Introduction

A holy grail in bioorganic chemistry is the creation of artificial enzymes with the ability to mimic the rate enhancements of natural enzymes. This goal has been pursued by investigation of modified proteins such as genetically engineered enzymes or catalytic antibodies or by synthesizing small organic molecules with the ability to mimic the active site.[1] As artificial enzymes have been reported to achieve rate enhancements (k_{cat}/k_{uncat}) between 10^1 and 10^5, and natural enzymes typically have k_{cat}/k_{uncat} values of 10^6−10^{17}; this goal, though difficult, does not appear too elusive.[2]

Glycosidases are enzymes that catalyze hydrolysis of glycosides employing a two-step mechanism involving general acid catalysis and nucleophilic catalysis being performed by two carboxylates in the active site.[3] The rate enhancements caused by glycosidases are among the highest known among enzymes.[4] Incorporation of two carboxylates into a cyclodextrin creates a weak artificial aryl glycosidase, but the catalysis does not occur by protonation but rather by electrostatic stabilization of the transition state.[5]

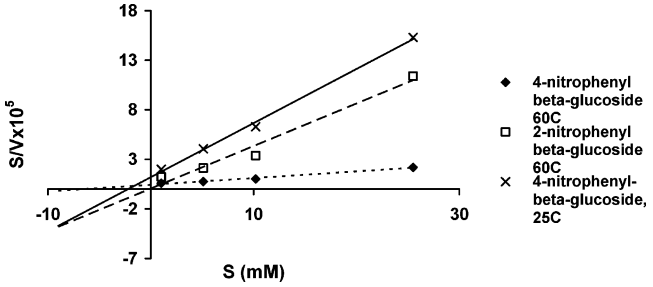


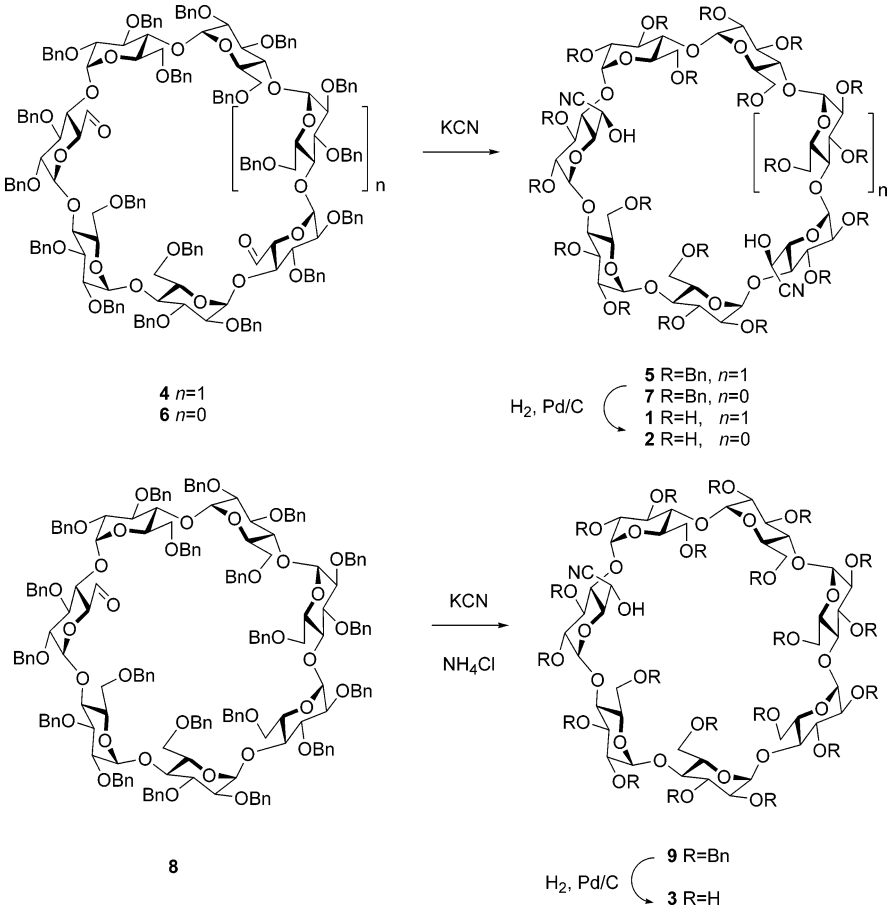
FIGURE 1. Hanes plot for **1**-catalyzed hydrolysis of different substrates at pH 7.4 and 59 °C.

We have recently reported that the cyclodextrin dicyanohydrin **1** is an artificial glycosidase with a k_{cat}/k_{uncat} of 1000 for the hydrolysis of 4-nitrophenyl-β-D-glucoside (Figure 1).[6] We have now found that **1** and its α-cyclodextrin analogue **2** both, under optimal conditions, catalyze this reaction with a k_{cat}/k_{uncat} of up to 8000. We

(1) Kirby, A. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 551−553.

10.1021/jo050861w CCC: $30.25
Published on Web 08/04/2005

SCHEME 1. Synthesis of Dicyanohydrins 1 and 2, and Monocyanohydrin 3



also report that a β-cyclodextrin with a single cyanohydrin group gives a k_{cat}/k_{uncat} of over 1000 as well as results with other substrates.

Results and Discussion

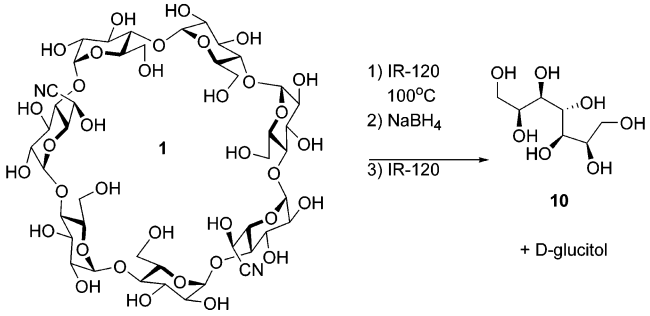
Synthesis. The cyclodextrin cyanohydrins were synthesized from the partially benzylated cyclodextrin monoand diols that can be prepared by the method of Pearce and Sinaÿ.[7] Oxidation of these alcohols with Dess–Martin's reagent affords aldehydes that were reacted with potassium cyanide giving cyanohydrins. Thus **1** and **2** were prepared by reaction of dialdehydes **4**[8] and **6**[9] with potassium cyanide giving the dicyanohydrins **5** and **7** in 77% and 80% yield, respectively (Scheme 1). The dicyanohydrin **5** is essentially one stereoisomer, while **7** could contain more than one stereoisomer. Hydrogenolysis of **5** and **7** with palladium on carbon catalysis gave **1** and **2** in quantitative yield.

Similarly the β-cyclodextrin monoaldehyde **8**[10] was reacted with KCN giving cyanohydrin **9** in 86% yield (Scheme 1). This was followed by hydrogenolysis of the benzyl protection groups of **9** giving **3** in 73% yield. The cyanohydrin synthesis appears essentially stereoselective and a single diastereomer is in any case obtained after purification. The β-dicyanohydrin **1** was one diastereomer, while **2** and **3** at least had a major stereoisomer.

(2) (a) Murakami, Y.; Kikuchi, J. I.; Hisaeda, Y.; Hayashida, O. *Chem. Rev.* **1996**, *96*, 721–758. (b) Motherwell, W. B.; Bingham, M. J.; Six, Y. *Tetrahedron* **2001**, *57*, 4663–4686. (c) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146–153. (d) Breslow, R.; Schmuck, C. *J. Am. Chem. Soc.* **1996**, *118*, 6601–6605. (e) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882–5883. (f) Akiike, T.; Nagano, Y.; Yamamoto, Y.; Nakamura, A.; Ikeda, H.; Veno, A.; Toda, F. *Chem. Lett.* **1994**, 1089–1092. (g) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1994**, *116*, 7893–7894. (h) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011. (i) Ikeda, H.; Horimoto, Y.; Nakata, M.; Ueno, A. *Tetrahedron Lett.* **2000**, *41*, 6483–6487. (j) Chou, D. T. H.; Zhu, J.; Huang, X. C.; Bennet, A. J. *J. Chem. Soc., Perkin Trans.* **2001**, *2*, 83–89. (k) Kunishima, M.; Yoshimura, K.;, Morigaki, H.; Kawamata, R.; Terao, K.; Tani, S. *J. Am. Chem. Soc.* **2001**, *123*, 10760–10761. (l) Ren, X. J.; Xue, Y.; Zhang, K.; Liu, J. Q.; Luo, G. M.; Zheng, J.; Mu, Y.; Shen, J. C. *FEBS Lett.* **2001**, *507*, 377–380. (m) Yu, J. X.; Zhao, Y. Z.; Holterman, M.; Venton, D. L. *Bioorg. Med. Chem.* **2002**, *10*, 3291–3299. (n) Ren, X. J.; Xue, Y.; Liu, J. Q.; Zhang, K.; Zheng, J.; Luo, G.; Guo, C. H.; Mu, Y.; Shen, J. C. *ChemBioChem* **2002**, *3*, 356–363. (o) Liu, Y.; Li, B.; Li, L.; Zhang, H. Y. *Helv. Chim. Acta* **2002**, *85*, 9–18. (p) Kataky, R.; Morgan, E. *Biosens. Bioelectron.* **2003**, *18*, 1407–1417. (q) Chan, W. K.; Yu, W. Y.; Che, C. M.; Wong, M. K. *J. Org. Chem.* **2003**, *68*, 6576–6582. (r) Milovic, N. M.; Badjic, J. D.; Kostic, N. M. *J. Am. Chem. Soc.* **2004**, *126*, 696–697. (s) Rousseau, C.; Christensen, B.; Pedersen, T. E.; Bols, M. *Org. Biomol. Chem.* **2004**, *2*, 3476–3482. (t) Fukudome, M.; Okabe, Y.; Sakaguchi, M.; Morikawa, H.; Fujioka, T.; Yuan, D. Q.; Fujjta, K. *Tetrahedron Lett.* **2004**, *45*, 9045–9048. (u) Dong, Z. Y.; Liu, J. Q.; Mao, S. Z.; Huang, X.; Yang, B.; Ren, X. J.; Luo, G. M.; Shen, J. C. *J. Am. Chem. Soc.* **2004**, *126*, 16395–16404. (v) Doug, T. H.; Chou, J. Z.; Huang, X.; Bennet, A. J. *J. Chem. Soc., Perkin Trans. 2* **2001**, *83–89*. (w) Ohe, T.; Kajiwara, Y.; Kida, T.; Zhang, W.; Nakatsuji, Y.; Ikeda, I. *Chem. Lett.* **1999**, 921–922.

(3) Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11–18.
(4) Wolfenden, R. *Acc. Chem. Res.* **2001**, *34*, 938–945.
(5) Rousseau, C.; Nielsen, N.; Bols, M. *Tetrahedron Lett.* **2004**, *45*, 8709–8711.
(6) Ortega-Caballero, F.; Rousseau, C.; Christensen, B.; Petersen, T. E.; Bols M. *J. Am. Chem. Soc.* **2005**, *127*, 3238–3239.
(7) (a) Pearce, A. J.; Sinaÿ, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 3610–3612. (b) Lecourt, T.; Herault, A.; Pearce, A. J.; Sollogoub, M.; Sinaÿ, P. *Chem. Eur. J.* **2004**, *10*, 2960–2971.
(8) Hardlei, T.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2880–2885.
(9) Rousseau, C.; Christensen, B.; Bols, M. *Eur. J. Org. Chem.* **2005**, *13*, 2734–2739.
(10) Rousseau, C.; Ortega-Caballero, F.; Nordstrøm, L. U.; Christensen, B.; Petersen, T. E.; Bols, M. *Chem. Eur. J.* In press.

SCHEME 2. Determination of the Stereochemistry of 1 by Degradation to D-*glycero*-D-*gluco*-Heptitol

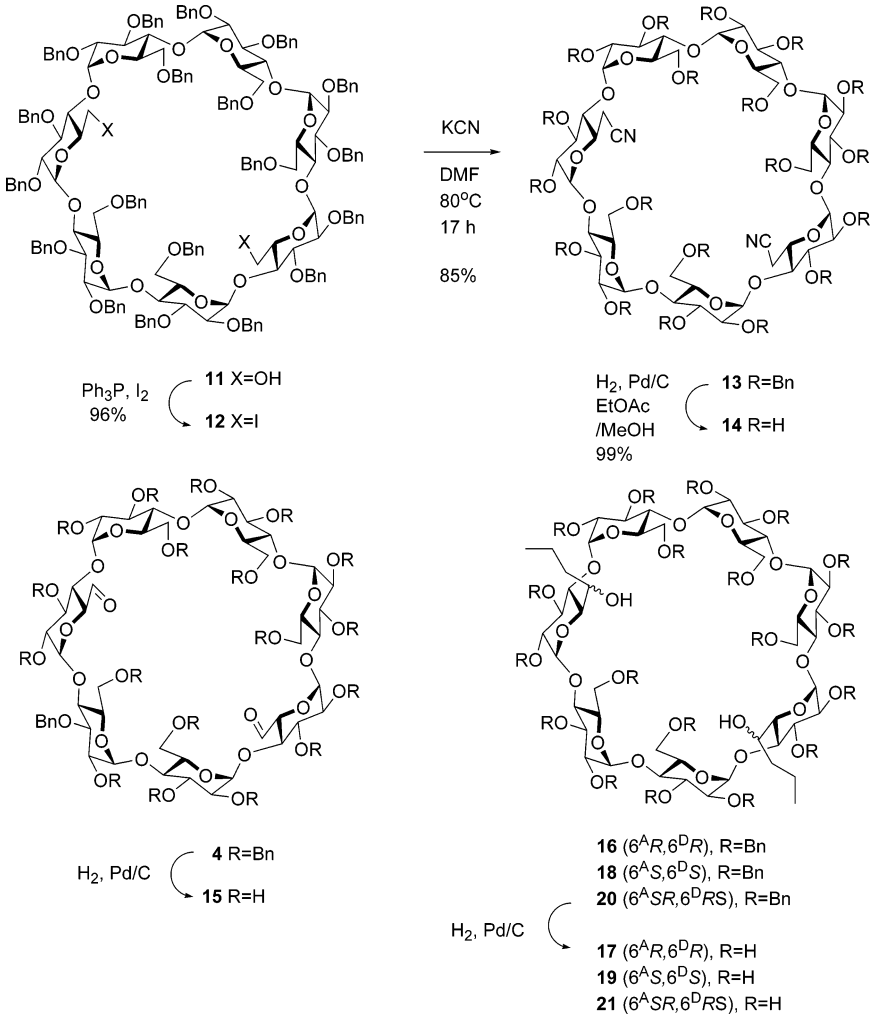


Compound **1** was shown to be the *R*,*R* isomer by the method outlined in Scheme 2. Hydrolysis of **1** with IR-120 followed by $NaBH_4$ reduction gave a 5:2 mixture of D-glucitol and a heptitol **10** that by comparison with ^{13}C NMR data[11] was shown to have the D-*glycero*-D-*gluco*-configuration. This stereochemistry is consistent with attack by cyanide from the outside of the cyclodextrin dialdehyde **4** and the cyanohydrin hydroxyl groups in **1** pointing toward the inner face.

For comparison with the cyanohydrin compounds **1**−**3**, a number of the β-cyclodextrin analogues **14**, **15**, **17**, **19**, and **21** were prepared (Schemes 3). Dinitrile **14** was obtained by conversion of the corresponding known benzylprotected A,D diol **11** into the diiodide **12** with Ph_3P, I_2, and imidazole in 96% yield as has been reported by Sinaÿ.[7] This diiodide was substituted with KCN giving dinitrile **13** in 85% yield, and deprotected by hydrogenolysis in EtOAc/MeOH giving dinitrile **14** in 99% yield (Scheme 3). The dialdehyde **15** was obtained by similar hydrogenolysis of the protected dialdehyde **4**[8] (Scheme 3). NMR of **15** shows that in aqueous solution it is exclusively on the hydrate form and it is therefore interesting to compare its catalytic properties with those of corresponding hydroxyl compounds. The di-6^A,6^D-*C*-propyl-substituted derivates **17**, **19**, and **21** were prepared by hydrogenolysis of the known stereoisomeric benzylprotected allyl derivatives **16**, **18**, and **20** (Scheme 3) that have previously been reported by us.[8] These compounds have restricted conformational freedom of the substituted C6's disallowing substituents in the *tg* conformation.[8] This means that the OH groups in **17** are in the *gt* conformation pointing in toward the inner rim, while those of **19** are in the *gg* conformation and are not. Compound **21** is a mixture of diastereoisomers each having one OH directed toward the inner rim.

Catalysis. Compound **1** was found by NMR to catalyze the conversion of 4-nitrophenyl β-D-glucopyranoside into

SCHEME 3. Synthesis of β-Cyclodextrin Derivative 14, 15, 17, 19, and 21



glucose and nitrophenol. Thus after leaving **1** for several days at 60 °C with 4-nitrophenyl β-D-glucopyranoside in phosphate buffer/D_2O, α- and β-glucose and 4-nitrophenol can be observed. The catalysis can be inhibited by addition of cyclopentanol confirming that the cyclodextrin cavity is involved in the process. No catalysis is observed with either β-cyclodextrin or mandelonitrile showing that the supramolecular positioning of binding cavity and cyanohydrin group is essential for catalysis. By following the formation of phenolate in this reaction by UV spectroscopy the process can be quantified. For one substrate, the phenyl glucoside **30**, the reaction was also followed by determining the amount of formed glucose using oxidation with 3,5-dinitrosalicylic acid, which gave essentially the same result as obtained by following phenolate formation (Table 1). The catalyzed reaction, which is obtained by subtraction of parallel uncatalyzed reactions, follows Michaelis−Menten kinetics as is seen in the Hanes plot (Figure 1). These kinetic experiments were carried out with substrate in concentrations of 1−50 mM and cyclodextrin catalyst in concentrations from 0.01 to 0.42 mM with the latter considerably smaller.

The kinetic constants for the catalysis by **1** of the hydrolysis of different nitrophenyl glycosides under different conditions are shown in Table 1. The kinetic constants were calculated by nonlinear least-squares fitting of V_{cat} vs S at four different substrate concentrations chosen in the interval $S = 0.2$ to 5 K_m (typically 1−50 mM). In 50 mM phosphate buffer at 59 °C, a K_m of 1−10 mM is obtained for reaction of the β-glucoside **22**, with an optimum k_{cat} at pH 8.0 of 5.9×10^{-5} s^{-1}, which is about 3000 times faster than the uncatalyzed reaction. Hydrolysis of the stereoisomeric substrates **23**, **24**, **25**, and **26** (an ortho isomer) was also catalyzed with efficacy, at pH 7.4, based on a k_{cat} value of **26** (β-*galacto*) > **22** (β-*gluco*) > **23** (α-*gluco*) > **25** (α-*galacto*) > **24** (α-*manno*) or based on a k_{cat}/k_{uncat} value of **22** > **23** > **26** > **25** > **24**. The variation in catalyzed rate is relatively small, about 4-fold, while the variation in k_{cat}/k_{uncat} is up to 10-fold.

By increasing the phosphate concentration the catalytic rate increases somewhat, so that a k_{cat}/k_{uncat} value of 7922 is obtained for the hydrolysis of **23** (Table 1). However, from a series of experiments of increasing phosphate buffer concentration it is seen that no simple relationship between phosphate concentration and k_{cat} exists for hydrolysis of **22** (Table 1, see also Figure S1 in the Supporting Information) so it is most plausible the increase is mainly due the change in ion strenght.

The reaction in D_2O with pD = 8.0 was about twice as fast as the reaction at pH 8.0 showing an solvent deuterium isotope effect of $k_H/k_D = 1.5$.

Adding electron withdrawing substituents in the phenyl group in the substrates **27** and **28** did not change the catalyzed hydrolysis rate very much, but because the uncatalyzed rate was increased the k_{cat}/k_{uncat} drops down to 51 and 6-fold, respectively. The K_m is essentially unchanged. Even though the substrate is still bound quite tightly the binding may be altered by the introduction of substituents. The phenyl derivative **30** is a somewhat slower substrate. For this substrate the k_{cat}/k_{uncat} becomes 1200. All together these results show that the catalyzed reaction depends little on the leaving group ability.

Remarkably the dideoxy derivative **29** is not a substrate despite it being a much more reactive glycoside than its structural parent **23**. The binding mode may be disrupted by the larger lipophilic surface this substrate has.

Interestingly the thioglycoside **32** is a substrate as well giving a k_{cat} of 1.8×10^{-4} s^{-1} and a k_{cat}/k_{uncat} of 19 (Table 1). Thiophenol has a pK_a of 6.6 and is thus between 4-nitrophenol and 2-chloro-4-nitrophenol in leaving group ability.

In Table 2 is shown the kinetic data obtained with the α-cyclodextrin dicyanohydrin **2**. This catalyst has a similar pH profile as was observed with **1**: The optimum catalysis is found at pH 8.0 in phosphate buffer. Increasing the buffer concentration increases the catalytic rate so that a k_{cat}/k_{uncat} value of 6−7000 is obtained for hydrolysis of **22** and **23** in 0.5 M phosphate buffer. A study of other buffers showed that catalysis in borate, Hepes, or glycin buffers were less efficient giving a k_{cat}/k_{uncat} value of 157−955. Compound **2** also catalyzed the hydrolysis of helicin **31**, quite effectively in terms of k_{cat}, but the background is very high as well leading to a mediocre k_{cat}/k_{uncat} value of 95.

As with β-cyclodextrin dicyanohydrin **1** the catalysis performed by **2** of hydrolysis of 2-chloro-4-nitrophenyl glucoside **27** and 2,4-dinitrophenyl glucoside **28** is not enhanced compared to the 4-nitrophenyl glycosides despite the much higher leaving group ability of these groups. As the background hydrolysis rate is much higher for these substrates k_{cat}/k_{uncat} is comparatively low particularly for **28**. These substrates are bound with a similar strength at **22**, however, suggesting that poor fit is not the reason for the drop in k_{cat}/k_{uncat}. For the dideoxy sugar **29** no catalysis was observed, and it is possible that any catalysis is overshadowed by the much higher background hydrolysis of this substrate.

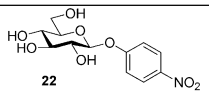
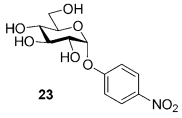
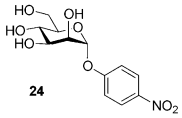
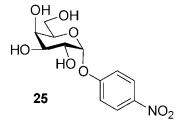
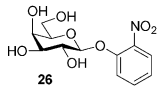
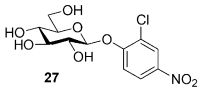
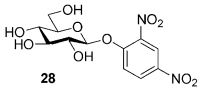
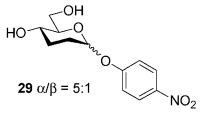
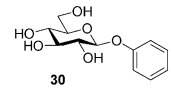
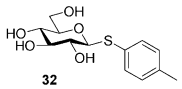
The β-cyclodextrin monocyanohydrin **3** was also found catalytic on substrates **22**−**23** and **25**−**26** (Table 3). Depending on the substrate the catalysis is 2−20 times slower than that by **1**. Nevertheless it is clear that one cyanohydrin group is sufficient for catalysis and that two merely increase catalysis by increasing the chance for productive binding of substrate.

The catalytic power of other CD derivatives toward 4-nitrophenyl β-D-glucopyranoside (**22**) hydrolysis is shown in Table 4. The propyl analogues **17**, **19**, and **21** afford no catalysis, similarly to β-cyclodextrin and regardless of whether the two OH groups point toward or away from the cavity. This shows that the cyano groups are essential. Dinitrile **14** is catalytic, but with a 250 times lower catalytic power, showing that the cyanohydrin OH groups are very important for the catalysis. These experiments show that the role of the cyano group must be in its electron withdrawing capacity increasing the acidity of the hydroxyl group. The dihydrate **15** is a comparatively good catalyst with a k_{cat}/k_{uncat} value about 60 times lower than that of **1**.

The above results can be summated to the following main points: (1) A single cyanohydrin at the primary rim is sufficient to promote catalysis. (2) The cyano group itself promotes little catalysis. (3) The acidity of the 6-OH group appears to be important, since unmodified cyclo-

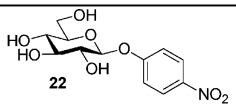
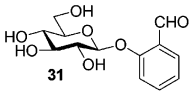
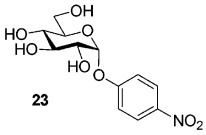
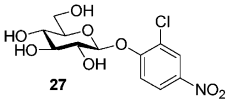
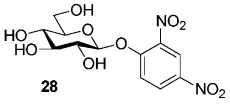
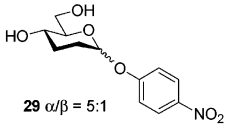
(11) Angyal, S. J.; Le Fur, R. *Carbohydr. Res.* **1984**, *126*, 15−26.

TABLE 1. Kinetic Parameters for the Dicyanohydrin-β-cyclodextrin (1)-Catalyzed Hydrolysis of Various Glycosides at Different pH and 59 °C[a]

Substrate	pH	Phosphate (mM)	k_{cat} ($x10^{-5}$ s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
 **22**	6.2	50	1.03±0.21	4.14±3.64	421
	6.6	50	1.83±0.02	4.97±1.21	989
	7.0	50	3.13±0.05	5.90±0.43	2577
	7.4	50	5.02±0.34	4.60±1.48	3141
	7.7	50	5.55±0.26	4.54±1.04	2247
	8.0	50	5.87±0.73	10.32±4.15	3116
	8.0	25	6.66±0.34	3.61±0.97	2487
	8.0	100	6.32±0.47	3.34±1.33	2055
	8.0	175	9.02±0.45	5.26±1.23	3212
	8.0	250	12.3±0.43	7.75±1.02	5340
	8.0	350	14.7±0.41	8.74±0.87	5759
	8.0	500	14.2±0.68	6.25±1.26	6396
	7.4*	50	0.40±0.03	6.67±1.17	920
	8.0[@]	50	4.04±0.16	3.10±0.68	2888
 **23**	7.4	50	3.05±0.41	12.7±3.7	2234
	8.0	500	14.2±0.9	10.5±1.5	7922
 **24**	7.4	50	1.84±0.11	2.65±0.55	279
 **25**	7.4	50	2.40±0.12	1.46±0.36	513
 **26**	7.4	50	4.52±0.01	1.62±1.55	512
 **27**	8.0	50	5.26±0.41	7.17±1.30	51
 **28**	8.0*	50	0.93±0.19	5.44±2.82	6
 **29** α/β = 5:1	8.0*	50	No catalysis		-
 **30**	8.0[†]	50	3.30±0.21[#]	3.03±0.88[#]	Not dtmnd
			3.27±0.15[§]	0.63±0.19[§]	1200
 **32**	8.0	50	18.2±0.8	0.06±0.03	19

[a] The reactions were followed by measuring absorption at 400 nm. *: at 25 °C. †: at 90 °C; #: followed by measuring absorption at 290 nm. §: followed by measuring glucose formation. @: in D_2O. Concentration of **1** was 0.42 mM.

TABLE 2. Kinetic Parameters for the Dicyanohydrin-α-cyclodextrin 2-Catalyzed Hydrolysis of Various Glycosides at Different pH and 59 °C[a]

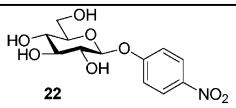
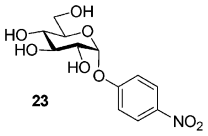
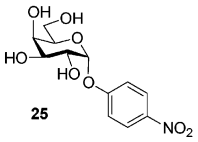
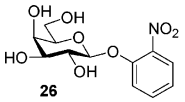
Substrate	pH	Phosphate (mM)	k_{cat} (x 10^{-5} s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
 **22**	6.2	50	0.93±0.05	6.34±1.04	227
	6.6	50	1.44±0.07	9.30±1.53	849
	7.0	50	2.16±0.05	4.50±0.47	1013
	7.4	50	4.59±0.10	4.69±0.33	1660
	7.7	50	5.34±0.46	7.56±2.50	2629
	8.0	50	6.96±0.29	6.52±1.10	2688
	7.4	100	4.82±0.33	4.85±1.05	2279
	7.4	250	3.01±0.08	3.34±0.34	812
	8.0	500	20.3±2.9	6.63±3.86	7101
	8.0	Borate-HCl	1.31±0.00	8.09±0.18	955
	8.9	Borate-HCl	0.64±0.10	-	227
	7.4	HEPES	0.30±0.03	7.78±1.35	157
	8.5	Glycin-NaOH	0.89±0.17	12.32±5.21	277
 **31**	7.4	50	5.31±1.21[#]	4.51±2.70[#]	95
 **23**	8.0	500	16.5±2.0	14.8±3.6	6138
 **27**	8.0	50	3.70±0.68	7.12±3.04	58
 **28**	8.0*	50	0.54±0.03	7.77±0.77	4
 **29** α/β = 5:1	8.0*	50	No catalysis		-

[a] The reactions were followed by measuring absorption at 400 nm. *: at 25 °C. #: followed by measuring absorption at 376 nm. Concentration of (**2**) was 0.49 mM.

dextrins or propyl cyclodextrins **17**, **19**, and **21** not are catalytic, while **15** is. (4) The catalytic rate does not follow leaving group ability. Thus a good leaving group such as 2,4-dinitrophenol ($pK_a = 4.0$), 2-chloro-4-nitrophenol ($pK_a = 5.45$), and tolylthiophenol ($pK_a = 6.6$) in the substrate does not give a faster hydrolysis than 4-nitrophenol ($pK_a = 7.15$). Nor were the 4-nitrophenol glycosides significantly better substrates than **30** even though phenol ($pK_a = 9.9$) is an almost 1000-fold worse leaving group. (5) A solvent deuterium isotope effect of 1.5 is observed. (6) No clear pH dependency is observed, but in phosphate buffer the catalytic rate increases with increasing pH. (7) There is no clear phosphate dependence though the catalytic rate in general increases with increasing the phosphate concentration.

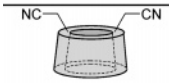
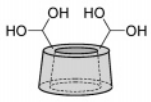
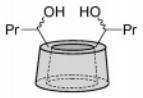
Points 1−3 indicate that the hydroxyl group and its acidity is important for the catalysis. Two roles of the OH group can be imagined: protonation of the exocyclic glycoside oxygen by general acid catalysis or nucleophilic catalysis by the alcoholate substituting the aryloxy group. The lack of importance of leaving group ability (point 4) and the solvent isotope effect (point 2) suggest that this role

TABLE 3. Kinetic Parameters for the Cyanohydrin-β-cyclodextrin (3)-Catalyzed Hydrolysis of Various Glycosides at Different Phosphate Concentrations and 59 °C[a]

Substrate	pH	Phosphate (mM)	k_{cat} (x 10^{-5} s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
 **22**	8.0	50	1.47±0.12	30.6±4.9	1067
	8.0	500	2.88±0.48	4.69±3.09	1356
 **23**	8.0	50	0.69±0.12	2.10±1.46	311
	8.0	500	7.56±2.32	12.7±7.8	1299
 **25**	8.0	50	0.39±0.08	0.00±0.96	100
 **26**	8.0	50	0.17±0.03	10.0±3.4	14

[a] The reactions were followed by measuring absorption at 400 nm. Concentration of **3** was 0.27 mM.

TABLE 4. Kinetic Parameters for the Catalysis by Different Cyclodextrin Derivatives of the Hydrolysis of 4-Nitrophenyl-β-D-glucoside at pH 7.4 and 59 °C in 50 mM Phosphate Buffer[a]

Structure	Catalyst	k_{cat} (x 10^{-5} s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
	**14**	0.009±0.003	4.90±4.24	4
	**15**	0.13±0.02	4.50±2.47	55
	**17** ($6^A R,6^D R$)	No catalysis		-
	19 ($6^A S,6^D S$)	No catalysis		-
	21 ($6^A S,6^D R/6^A R,6^D S$)	No catalysis		-

[a] The reactions were followed by measuring absorption at 400 nm. The catalyst concentration was 2.2 mM.

is general acid catalysis as shown in Figure 2, since this will have a rate determining step that involves deuterium transfer and is independent of leaving group ability.

The pH dependency (point 6) and the phosphate dependency (point 7) suggest that the mechanism may be more complicated than can be elucidated from the present data. The pH profile appear to be closely associated to the ionization of phosphate since no rate increase as a function of pH is seen in other buffers. A small amount of phosphate clearly has a positive effect, since the rate is much higher in phosphate buffer, and the most obvious reason for that could be nucleophilic catalysis by secondary phosphate. However, the k_{cat} increase obtained by increasing the phosphate concentration 10−20 times is only 2−3. Perhaps this behavior can be explained by a change in rate determining step, so that when phosphate is not present the cleavage of the C−OAryl bond becomes rate determining.

The stereochemistry of the cyanohydrin is likely to be important though this has not been demonstrated. Previ-

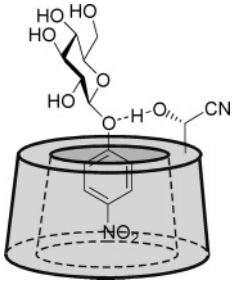


FIGURE 2. Proposed mechanism for the catalysis.

ous work on 6-*C*-substituted cyclodextrins showed that these derivatives have very restricted conformational freedom along the C5−C6 bond as both OH and alkyl substituents shun the *tg* conformation.[4] Therefore an important feature in **1**, **2**, and **3** could be that the *R* isomers having the cyanohydrin 6-OH groups are fixed in the *gt* conformation pointing toward the binding site.

In summary cyclodextrin cyanohydrins have been shown to be remarkably efficient artificial enzymes catalyzing hydrolysis of aryl glycosides with rate increases close to 10^4. A single cyanohydrin is required for the catalysis. The catalysis is believed to be general acid catalysis with the cyanohydrin OH delivering a proton to the exocyclic oxygen of the glycoside. Phosphate appears to have an influence and may assist in displacing the aryloxy group in the second step. Thus cyclodextrin cyanohydrins mimic part of mechanistic apparatus of natural glycosidases as the cyanohydrin mimics a protonating carboxylate. Including other parts of this apparatus such as nucleophilic catalysis may improve the catalysis further.

Experimental Section

$6^A,6^D$-Di-*C*-cyano-$2^{A-G},3^{A-G},6^{B,C,E-G}$-nonadecakis-*O*-benzyl-β-cyclodextrin (5). A mixture of potassium cyanide (649 mg, 9.96 mmol) and ammonium chloride (802 mg, 15 mmol) in water (10 mL) was added at 0 °C to a solution of **4**[8] (200 mg, 0.07 mmol) in ether/MeOH (1:1) (10 mL). The reaction mixture was stirred overnight at room temperature. The organic solvent was removed in a vacuum and the water phase was extracted with CH_2Cl_2. The organic layer was washed, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc/pentane 1:4 → 1:3) to afford **5** (156 mg, 77%) as a white foam: $[\alpha]_D$ +41.8 (*c* 1.0, $CHCl_3$); IR (KBr) 3410, 3029, 2924, 2867, 2242 (CN), 1496, 1453, 1358, 1208, 1095, 1040 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.50−7.01 (m, 95H, aromatic-H), 5.78 (d, 1H, $^3J_{1,2}$ = 4.0 Hz, H-1), 5.75 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.40−5.31 (m, 4H), 5.20−5.15 (m, 2H), 5.11−4.97 (m, 6H), 4.90−4.21 (m, 44H), 4.18−3.97 (m, 14H), 3.95−3.84 (m, 5H), 3.79−3.47 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.9−138.0 (C_{ipso}), 128.9−126.8 (CH aromatic), 120.3 (CN), 119.5 (CN), 100.8 (C-1), 100.2 (C-1), 99.5 (C-1), 98.3 (C-1), 98.0 (C-1), 97.5 (C-1), 97.3 (C-1), 82.3, 82.1, 81.9, 81.7, 81.4, 81.2, 81.1, 80.9, 80.6, 80.2, 79.9, 79.8, 78.9, 78.4, 76.7, 76.3, 76.2, 75.6, 75.4, 74.9, 74.6, 74.5, 74.0, 73.9, 73.7, 73.6, 73.5, 73.4, 73.2, 73.1, 73.0, 72.9, 72.7, 72.5, 72.4, 72.2, 72.1, 72.0, 71.8, 71.6, 71.1, 70.0, 69.8, 69.1, 66.1 (CH_2, CH), 60.4 (*C*H(OH)CN), 59.9 (*C*H(OH)CN); MALDI-TOF-MS *m/z* calcd for $C_{177}H_{182}O_{35}N_2Na$ 2918.2420, found 2918.1184.

($6^AR,6^DR$)-$6^A,6^D$-Di-*C*-cyano-β-cyclodextrin (1). Compound **5** (415 mg, 0.14 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (12 mL). Then Pd/C (42 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **1** (169 mg, 100%) as a white solid: $[\alpha]_D$ +89.4 (*c* 1.0, H_2O); IR (KBr) 3363, 2932, 2258 (CN), 1678, 1425, 1203, 1156, 1030 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ (ppm) 5.05 (br s, 3H, H-1), 4.95 (br s, 4H, H-1), 4.00 d, 1H, ^3J = 8.8 Hz), 3.91−3.42 (m, 30H); ^{13}C NMR (100 MHz, D_2O) δ (ppm) 119.1 (CN), 102.1 (C-1), 102.0 (C-1), 81.6, 81.5, 81.4, 81.3, 81.2, 80.0, 73.2, 73.1, 72.5, 72.3, 72.1, 72.0, 71.9 (CH), 60.7, 60.5, 59.6 (CH_2, *C*H(OH)CN); MALDI-TOF-MS *m/z* calcd for $C_{44}H_{68}O_{35}N_2Na$ 1207.3500, found 1207.3739.

$6^A,6^D$-Di-*C*-cyano-$2^{A-F},3^{A-F},6^{B,C,E,F}$-hexadecakis-*O*-benzyl-α-cyclodextrin (7). A mixture of potassium cyanide (1.54 g, 23.57 mmol) and ammonium chloride (2.15 g, 40.17 mmol) in water (24 mL) was added at 0° C to a solution of dialdehyde **6**[9] (404 mg, 0.17 mmol) in ether/MeOH (12 mL/9 mL). The reaction mixture was stirred overnight at room temperature. The organic solvent was removed in a vacuum and the water phase was extracted with CH_2Cl_2. The organic layer was washed, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc/pentane 1:3 → 1:2) to afford dicyanohydrin **7** (327 mg, 80%) as a white foam: $[\alpha]_D$ +34.4 (*c* 0.25, $CHCl_3$); IR (KBr) 3335, 3031, 2928, 2865, 2247 (CN), 1496, 1454, 1355, 1208, 1165 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.42−7.14 (m, 90H, aromatic-H), 5.48 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.42−5.37 (m, 2H), 5.31−5.26 (m, 3H), 5.22 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.17 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.13−5.09 (m, 2H), 5.03−4.64 (m, 10H), 4.60−4.36 (m, 20H), 4.32−3.93 (m, 10H), 3.87−3.47 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.8−137.5 (C_{ipso}), 129.0−126.7 (CH aromatic), 119.8 (CN), 119.7 (CN), 117.8 (CN), 101.1 (C-1), 101.0 (C-1), 100.2 (C-1), 99.8 (C-1), 99.3 (C-1), 99.1 (C-1), 98.4 (C-1), 97.8 (C-1), 82.5, 82.4, 81.9, 81.7, 81.3, 81.2, 81.1, 81.0, 80.9, 80.8, 80.5, 80.3, 79.6, 79.5, 79.3, 78.7, 76.7, 76.6, 76.2, 76.1, 75.9, 75.7, 75.3, 75.2, 73.9, 73.8, 73.7, 73.5, 73.4, 73.3, 73.2, 73.1, 73.0, 72.9, 72.8, 72.7, 72.6, 72.2, 72.0, 71.8, 71.7, 71.6, 70.4, 70.0, 69.8, 69.5, 69.4, 62.9 (CH_2, CH), 60.4 (*C*H(OH)CN), 60.3 (*C*H(OH)CN); MALDI-TOF-MS *m/z* calcd for $C_{150}H_{154}O_{30}N_2Na$ 2486.0484, found 2486.973.

$6^A,6^D$-Di-*C*-cyano-α-cyclodextrin (2). Compound **7** (327 mg, 0.13 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (12 mL). Then Pd/C (33 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **2** (133 mg, 100%) as a white solid: $[\alpha]_D$ +84.6 (*c* 0.5, $CHCl_3$); IR (KBr) 3401, 2936, 2252 (CN), 1681, 1436, 1203, 1151, 1033 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ (ppm) 5.15 (br s, 1H, H-1), 5.03 (br s, 1H, H-1), 4.93 (br s, 4H, H-1), 3.95−3.39 (m, 30H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 118.2 (CN), 115.4 (CN), 101.7 (C-1), 101.6 (C-1), 81.8, 81.4, 80.1, 73.2, 72.5, 72.3, 71.9, 71.6, 60.8, 60.6, 59.6 (CH_2, CH); MALDI-TOF-MS *m/z* calcd for $C_{38}H_{58}O_{30}N_2Na$ 1045.2972, found 1045.2972.

6^A-*C*-Cyano-$2^{A-G},3^{A-G},6^{B-G}$-eiocosakis-*O*-benzyl-β-cyclodextrin (9). A mixture of potassium cyanide (4.08 g, 63 mmol) and ammonium chloride (5.05 g, 94 mmol) in water (63 mL) was added to a solution of **8**[10] (1.22 g, 0.42 mmol) in Et_2O/MeOH (1:1) (63 mL). The reaction mixture was stirred overnight at room temperature. The organic solvent was removed in a vacuum and the water phase was extracted with CH_2Cl_2. The organic layer was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography (eluent gradient, EtOAc/pentane 1:4 → 1:3) to afford **9** (1.06 g, 86%) as an oil: $[\alpha]_D$ +32.6 (*c* 1.0, $CDCl_3$); IR (film) 3364, 3030, 2925, 2868, 2230 (CN), 1496, 1453, 1356, 1207, 1094, 1040, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.32−6.86 (m, 100H, aromatic-H), 5.59 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.30−5.16 (m, 3H), 5.15−4.97 (m, 6H), 4.96−4.92 (t, 2H, *J* = 3.2 Hz), 4.91−4.88 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 4.85−4.82 (d, 1H, $^3J_{1,2}$ = 4.0 Hz, H-1), 4.76−4.24 (m, 36H), 4.08−3.77 (m, 22H), 3.76−3.38 (m, 15H), 3.40 (dd, 1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.7−138.0 (C_{ipso}), 129.3−125.5 (CH aromatic), 118.8 (CN), 100.0 (C1), 99.1 (C1), 98.6 (C1), 98.2 (C1), 98.0 (C1), 97.5 (C1), 81.8, 81.5, 81.4, 81.3, 81.2, 80.4, 80.0, 79.9, 79.8, 79.3, 79.2, 79.1, 78.9, 78.7, 77.8, 76.6, 76.2, 76.0, 75.6, 75.5, 74.8, 74.6, 73.9, 73.7, 73.6, 73.5, 73.5, 73.3, 73.1, 73.0, 72.9, 72.8, 72.7, 72.4, 72.3, 72.1, 72.0, 71.8, 71.6, 71.4, 71.3, 70.0, 69.9, 69.5, 69.4, 69.3, 69.2; MALDI-TOF-MS *m/z* calcd for $C_{183}H_{189}O_{35}NNa$ 2983.2937, found 2983.4310.

6-*C*-Cyano-β-cyclodextrin (3). Compound **9** (1.06 g, 0.36 mmol) was dissolved in 2-methoxyethanol (25 mL). Then Pd/C (107 mg) and TFA (cat) were added and the mixture was stirred at room temperature under hydrogen atmosphere until completion. Filtration through Celite and evaporation of the solvent gave **3** (303 mg, 73%) as a white solid: $[\alpha]_D$ +49.5 (*c* 0.1, H_2O); IR (film) 3364, 2938, 2079, 1684, 1203, 1141, 1054,

1033 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ (ppm) 5.07−5.03 (m, 1H, H-1), 5.0−4.92 (m, 6H, H-1), 4.00−3.36 (m, 41 H); MALDI-TOF-MS *m/z* calcd for $C_{43}H_{69}O_{35}NNa$ 1182.3548, found 1183.0570.

6^A,6^D-Dideoxy-6^A,6^D-diiodononadecakis-*O*-benzyl-β-cyclodextrin (12). A mixture of 6^A, 6^D-diol-nonadecakis-*O*-benzyl-β-cyclodextrin (**11**)[7] (1.40 g, 0.49 mmol), iodine (749 mg, 2.95 mmol), triphenylphosphine (774 mg, 2.95 mmol), and imidazole (402 mg, 5.90 mmol) in toluene (70 mL) was vigorously stirred at 75 °C for 16 h. To reaction mixture was added an equal volume of sat. $NaHCO_3$ and the mixture was stirred 5 min. An excess of iodine was removed by the addition of aqueous sat. $Na_2S_2O_3$. The organic layer was diluted with EtOAc (200 mL) and washed with water (80 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography (eluent, EtOAc/pentane 1:4 → 1:3) to afford **12** (1.45 g, 96%) as a white foam: $[\alpha]_D$ +34.4 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.22−6.96 (m, 95H, aromatic-H), 5.24 (d, 1H, $^3J_{1,2}$ = 4.0 Hz, H-1), 5.18 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.10 (d, 1H, ^2J = 12.8 Hz, C*H*Ph), 5.08 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.06 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.04 (d, 1H, ^2J = 11.2 Hz, C*H*Ph), 5.03 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.10 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 4.91 (d, 1H, ^2J = 11.2 Hz, C*H*Ph), 4.89 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.86 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.82 (d, 1H, ^2J = 11.2 Hz, C*H*Ph), 4.77 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 4.66−4.60 (m, 8H), 4.52−4.26 (m, 25H), 4.15 (t, 2H, ^3J = 8.2 Hz), 3.93−3.82 (m, 20H), 3.70−3.60 (m, 7H), 3.50−3.26 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.5−139.2 (C_{ipso}), 138.7−138.2 (C_{ipso}), 128.6−127.1 (CH aromatic), 99.3 (C-1), 98.9 (C-1), 98.6 (C-1), 98.5 (C-1), 98.1 (C-1), 83.7, 81.7, 81.1, 81.0, 80.8, 80.5, 80.3, 80.1, 79.7, 79.6, 79.5, 79.0, 78.7, 78.5, 78.0, 76.2, 75.8, 75.3, 75.1, 74.9, 73.8, 73.7, 73.6, 73.2, 73.0, 72.9, 72.8, 72.7, 72.0, 71.7, 71.6, 71.2, 70.5, 69.9, 69.5, 69.4 (CH_2, CH), 9.9 (CH_2I), 9.3 (CH_2I); MALDI-TOF-MS *m/z* calcd for $C_{175}H_{182}O_{33}I_2Na$ 3088.0550, found 3088.0424.

6^A,6^D-Di-*C*-cyano-6^A,6^D-dideoxynonadecakis-*O*-benzyl-β-cyclodextrin (13). Potassium cyanide (457 mg, 7.01 mmol) was added to a solution of **12** (1.02 g, 0.33 mmol) in DMF (25 mL). The reaction mixture was stirred at 80 °C for 17 h. The mixture was cooled and water (30 mL) and EtOAc (60 mL) were added. The aqueous layer was washed with EtOAc (30 mL) and the combined organic layer was washed with water (40 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography (eluent, EtOAc/pentane 1:3 → 1:2) to afford **13** (817 mg, 85%) as a white foam: $[\alpha]_D$ +35.7 (*c* 1.0, $CHCl_3$); IR (KBr) 3482, 2924, 2867, 2252 (CN), 1496, 1453, 1356, 1208, 1094, 1040 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.29−6.89 (m, 95H, aromatic-H), 5.27 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.09−4.97 (m, 7H), 5.00 (d, 1H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.84 (d, 1H, $^3J_{1,2}$ = 3.0 Hz, H-1), 4.80 (d, 1H, $^3J_{1,2}$ = 3.2 Hz, H-1), 4.71−4.12 (m, 36H), 4.23−3.74 (m, 26H), 3.61 (t, 2H, ^3J = 11 Hz), 3.51−3.31 (m, 10H), 3.27 (dd, 1H, $^3J_{2,3}$ = 9.8 Hz, $^3J_{1,2}$ = 3.0 Hz, H-2), 2.81(dd, 2H, $^2J_{6,6'}$ = 14.4 Hz, $^3J_{5,6}$ = 7.6 Hz, H-6_A or 6_D), 2.53 (dd, 1H, $^2J_{6,6'}$ = 17.2 Hz, $^3J_{5,6}$= 7.2 Hz, H-6_D or 6_A), 2.47 (dd, 1H, $^2J_{6,6'}$ = 17.2 Hz, $^3J_{5,6}$ = 7.8 Hz, H-$6'_D$ or $6'_A$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 139.5−138.1 (C_{ipso}), 128.8−127.0 (CH aromatic), 118.1 (CN), 117.8 (CN), 99.2 (3 × C-1), 98.8 (C-1), 98.5 (C-1), 98.3 (C-1), 98.0 (C-1), 82.1, 81.0, 80.8, 80.4, 80.2, 80.0, 79.6, 79.4, 79.0, 76.2, 75.8, 75.1, 74.5, 73.7, 73.5, 73.2, 73.0, 72.9, 72.8, 72.0, 71.8, 69.8, 69.5, 69.0, 67.9, 67.6 (CH_2, CH), 22.3 (CH_2CN), 21.8 (CH_2CN); MALDI-TOF-MS *m/z* calcd for $C_{177}H_{182}O_{33}N_2Na$ 2886.2522, found 2886.2307.

6^A,6^D-Di-*C*-cyano-6^A,6^D-deoxy-β-cyclodextrin (14). Compound **13** (1.12 g, 0.39 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (30 mL). Then Pd/C (112 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **14** (445 mg, 99%) as a white solid: $[\alpha]_D$ +86.3 (*c* 1.0, H_2O); IR (KBr) 3405, 2929, 2258 (CN), 1676, 1420, 1156, 1079, 1033 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ (ppm) 4.99 (d, 5H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.96 (s, 2H, H-1), 4.02 (br t, 1H, ^3J = 7.4 Hz, H-5), 3.78−3.68 (m, 22H), 3.61−3.47 (m, 18H), 3.44 (t, 2H, ^3J = 9.2 Hz), 3.37 (t, 2H, ^3J = 9.4 Hz), 3.08 (br d, 2H, H-6_A or 6_D), 2.83 (dd, 1H, $^2J_{6,6'}$ = 17.4 Hz, H-6_D or 6_A), 2.82 (dd, 1H, $^2J_{6,6'}$ = 16.8 Hz, H-$6'_D$ or $6'_A$); ^{13}C NMR (100 MHz, D_2O) δ (ppm) 118.8 (CN), 102.2 (C-1), 102.1 (C-1), 101.9 (C-1), 84.6, 81.5, 81.4, 73.2, 72.7, 72.2, 72.0, 67.5, 60.5 (CH), 20.7 (CH_2CN); MALDI-TOF-MS *m/z* calcd for $C_{44}H_{68}O_{33}N_2Na$ 1175.3602, found 1175.3444.

6^A,6^D-Dialdehydo-β-cyclodextrin (15). Compound **4**[8] (196 mg, 0.07 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (5 mL). Then Pd/C (20 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **15** (80 mg, 100%) as a white solid: $[\alpha]_D$ +83.6 (*c* 1.0, H_2O); IR (KBr) 3343, 2937, 1681, 1438, 1206, 1153, 1031 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ (ppm) 5.29 (br s, 2H, OH), 5.01 (br s, 2H, OH), 4.94 (br s, 7H, H-1), 3.84−3.75 (m, 31H), 3.54−3.47 (m, 20H); ^{13}C NMR (100 MHz, D_2O) δ (ppm) 102.0 (C-1), 87.4, 82.3, 81.2, 73.2, 72.9, 72.2, 71.9 (CH), 60.4 (C-6); MALDI-TOF-MS *m/z* calcd for $C_{42}H_{70}O_{37}Na$ 1189.3494, found 1189.3035 (dihydrate); for $C_{42}H_{68}O_{36}Na$ 1171.3388, found 1171.2986 (monohydrate); for $C_{42}H_{66}O_{35}Na$ 1153.3282, found 1153.2978 (dialdehyde).

(6^A*S*,6^D*S*)-6^A,6^D-Di-*C*-propyl-β-cyclodextrin (17). Compound **16**[8] (500 mg, 0.37 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (50 mL). Then Pd/C (100 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **17** (203 mg, 100%) as a white foam. $[\alpha]_D$ +94.4 (*c* 1.0, H_2O); ^1H NMR (400 MHz, D_2O, 80 °C) δ (ppm) 5.65−5.45 (m, 7H, H-1), 4.50−4.21 (m, 23H), 4.20−3.98 (m, 18H), 2.16−2.04 (m, 2H), 2.02−1.91 (m, 2H), 1.88−1.70 (m, 4H), 1.34 (br s, 6H, CH_3); ^{13}C NMR (100 MHz, D_2O) δ (ppm) 102.0 (C-1), 81.2, 81.1, 80.9, 79.9, 73.7, 73.6, 73.4, 73.2, 72.9, 72.6, 72.2, 72.1, 71.9, 67.6, 67.5, 60.3 (CH), 60.1, 59.9, 59.8 (C-6), 35.2, 18.9, 18.7, 18.6 (CH_2), 13.3 (CH_3); MALDI-TOF-MS *m/z* calcd for $C_{48}H_{82}O_{35}Na$ 1241.4534, found 1241.4782.

(6^A*R*,6^D*R*)-6^A,6^D-Di-*C*-propyl-β-cyclodextrin (19). Compound **18**[8] (109 mg, 0.37 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (20 mL). Then Pd/C (40 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **19** (43 mg, 100%) as a white foam. $[\alpha]_D$ +55.2 (*c* 1.0, H2O); ^1H NMR (400 MHz, D_2O, 80 °C) δ (ppm) 5.65−5.45 (m, 7H, H-1), 4.50−4.21 (m, 23H), 4.20−3.98 (m, 18H), 2.16−2.04 (m, 2H), 2.02−1.91 (m, 2H), 1.88−1.70 (m, 4H), 1.34 (br s, 6H, CH_3); ^{13}C NMR (100 MHz, D_2O) δ (ppm) 102.0 (C-1), 81.2, 81.1, 80.9, 79.9, 73.7, 73.6, 73.4, 73.2, 72.9, 72.6, 72.2, 72.1, 71.9, 67.6, 67.5, 60.3 (CH), 60.1, 59.9, 59.8 (C-6), 35.2, 18.9, 18.7, 18.6 (CH_2), 13.3 (CH_3); MALDI-TOF-MS *m/z* calcd for $C_{48}H_{82}O_{35}Na$ 1241.4534, found 1241.4789.

(6^A*RS*,6^D*SR*)-6^A,6^D-Di-*C*-propyl-β-cyclodextrin (21). Compound **20**[8] (350 mg, 0.37 mmol) was dissolved in a mixture of MeOH/EtOAc (1:1) (20 mL). Then Pd/C (50 mg) and TFA (cat) were added and the mixture was stirred overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave **21** (141 mg, 100%) as a white foam. $[\alpha]_D$ +91.9 (*c* 1.0, H_2O); ^1H NMR (400 MHz, D_2O, 80 °C) δ (ppm) 5.65−5.45 (m, 7H, H-1), 4.50−4.21 (m, 23H), 4.20−3.98 (m, 18H), 2.16−2.04 (m, 2H), 2.02−1.91 (m, 2H), 1.88−1.70 (m, 4H), 1.34 (br s, 6H, CH_3); ^{13}C NMR (100 MHz, D_2O) δ (ppm) 102.0 (C-1), 81.2, 81.1, 80.9, 79.9, 73.7, 73.6, 73.4, 73.2, 72.9, 72.6, 72.2, 72.1, 71.9, 67.6, 67.5, 60.3 (CH), 60.1, 59.9, 59.8 (C-6), 35.2, 18.9, 18.7, 18.6 (CH_2), 13.3 (CH_3); MALDI-TOF-MS *m/z* calcd for $C_{48}H_{82}O_{35}Na$ 1241.4534, found 1241.4660.

Hydrolysis of Compound 1. To a solution of **1** (82 mg, 0.07 mmol) in water (6 mL) was added 20 mL of Amberlite IR-120 (H^+), and the mixture was stirred at 100 °C for 48 h. The resin was removed by filtration and $NaBH_4$ (132 mg, 3.5 mmol) was added to the filtrate. Then the reaction mixture was stirred for 30 min at room temperature and Amberlite

IR-120 was added until pH was acid. The resin was removed by filtration and the solvent was removed. The residue was coevaporated with MeOH several times to remove boronic acid, to give a residue containing **10** and D-glucitol. **10**: ^{13}C NMR (100 MHz, D_2O) δ (ppm) 72.7, 72.5, 71.6, 71.5, 69.4, 62.7, 62.3. This is in agreement with the D-*glyco*-D-*gluco*-heptitol.[11]

4-Nitrophenyl 2,3-Dideoxy-α-D-glucopyranoside (29). This compound was made from 1,4,6-tri-*O*-acetyl-2,3-dideoxy-α,β-D-glucopyranose[12] and 4-nitrophenol, using a general procedure previosuly described for other arylglycosides.[13] The product nitrophenyl 2,3-dideoxy-4,6-di-*O*-acetyl-α-D-glucopyranoside was purified by Flash chromatography in pentane−ether (1:1) and was obtained in 25% yield in an unseparable mixture containing 10−20% of the β-anomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.21 (d, 2H, ^3J = 9.2 Hz, aromatic-H), 7.18 (d, 2H, ^3J = 9.2 Hz, aromatic-H), 5.66 (s, 1H, H-1), 4.83 (dt, 1H, ^3J = 5.2 Hz, H-4), 4.24 (dd, 1H, $^3J_{5,6}$ = 5.2 Hz, $^2J_{6,6'}$ = 12.0 Hz, H-6), 4.03 (dd, 1H, $^3J_{5,6'}$ = 2.0 Hz, $^3J_{6,6'}$ = 12.0 Hz, H-6′), 3.90 (ddd, 1H, $^3J_{5,6'}$ = 2.0 Hz, $^3J_{5,6}$ = 5.2 Hz, $^3J_{4,5}$ = 5.6 Hz, H-5), 2.19−2.00 (m, 4H, H-2,2′,3,3′), 2.06 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO).

The acetates were removed by conventional Zemplen deacetylation[14] giving an essentially quantitative yield of the product **29** in an unseparable mixture containing 10−20% of the β-anomer. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.21 (d, 2H, ^3J = 9.2 Hz, aromatic-H), 7.28 (d, 2H, ^3J = 9.2 Hz, aromatic-H), 5.72 (s, 1H, H-1), 3.71 (dd, 1H, $^3J_{5,6}$ = 2.8 Hz, $^2J_{6,6'}$ = 12.0 Hz, H-6), 3.65 (dd, 1H, $^3J_{5,6'}$ = 5.2 Hz, $^3J_{6,6'}$ = 12.0 Hz, H-6′), 3.60−3,63 (m, 1H, H-4), 3.47 (ddd, 1H, $^3J_{5,6'}$ = 2.8 Hz, $^3J_{5,6}$ = 5.2 Hz, H-5), 2.09−1.93 (m, 4H, H-2,2′,3,3′); HRMS *m/z* calcd for $C_{12}H_{15}O_6NNa$ 292.0797, found 292.0801.

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 (0.025−5 mg) or nothing as control. The reactions were followed continuously at 25 or 59 °C, using UV absorption at 400 nm for the nitrophenyl substrates or 376 nm for **31**. The reaction of **30** was monitored by taking out samples of the reaction at 90 °C, diluting 4 times with pH 10 buffer before measuring absorption at 290 nm. Alternatively glucose formation was determined by heating samples with 3,5-dinitrosalicylic acid at 100 °C and measuring absorption at 540 nm.[15] The reactions were monitored for 3−18 h. Velocities were determined as the slope of the progress curve of each reaction. Uncatalyzed velocities were obtained directly from the control samples. Catalyzed velocities were calculated by subtracting the uncatalyzed velocity from the velocity of the appropriate cyclodextrin containing sample. The catalyzed 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} versus [S]. The inhibition experiments were made by adding cyclopentanol (15 μL) to an catalyzed sample.

Acknowledgment. This work has been supported by the Lundbeck foundation, The Danish National Science Research Council, and the Ramón Areces Foundation (F.O.C.). We also thank Annette Nordestgaard for Technical Assistance.

Supporting Information Available: Copies of ^{13}C or ^{1}H NMR spectra of new compounds and a plot of k_{cat} vs phosphate concentration for the hydrolysis of **22** catalyzed by **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO050861W

(12) Jiang, Y.; Isobe, M. *Tetrahedron* **1996**, *52*, 2877−92.
(13) Smits, E.; Engberts, J. B. F. N.; Kellogg, R. M.; van Doren, H. A. *J. Chem. Soc., Perkin Trans. I* **1996**, 2873−2877.
(14) Shafizadeh, F.; Stacey, M. *J. Chem. Soc.* **1957**, 4612−4615.
(15) Miller, G. L. *Anal. Chem.* **1959**, *31*, 426.