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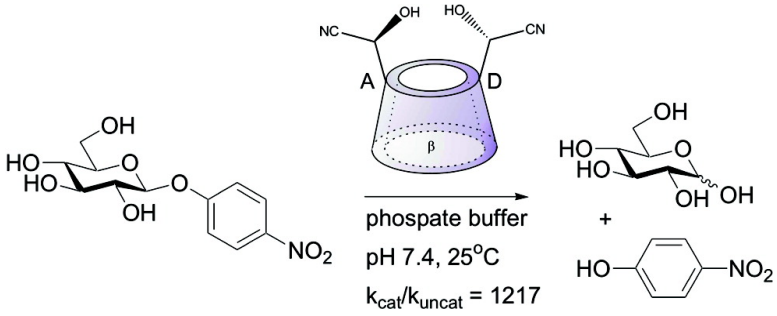

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Remarkable Supramolecular Catalysis of Glycoside Hydrolysis by a Cyclodextrin Cyanohydrin

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The mildness, selectivity, and rate of enzymatic catalysis greatly exceed ordinary chemical catalysis. Enzymes use binding and proximity effects to achieve astounding rate enhancements for specific reactions and substrates.[1] As many enzymes achieve their catalysis by surprisingly simple means, the mimicking of enzyme action appears possible.[2]

We have recently reported that the cyclodextrin diacid **1** is an artificial glycosidase with a k_{cat}/k_{uncat} of 35 for the hydrolysis of 4-nitrophenyl-β-D-glucoside (**2**) (Figure 1).[3] We report here the remarkable observation that the dicyanohydrin **3** is a significantly better artificial enzyme, achieving k_{cat}/k_{uncat} of 200−2000 for hydrolysis of aryl glycosides.

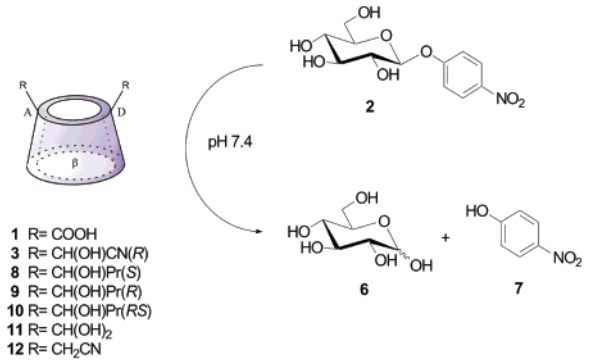

Figure 1. Glycoside hydrolysis catalyzed by β-cyclodextrin derivatives. Pr = *n*-propyl.

Compound **3** is obtained by reaction of known β-cyclodextrin dialdehyde **4**[4] with KCN in 77% yield, followed by hydrogenolysis of the benzyl protection groups (Figure 2). This gives **3** in a

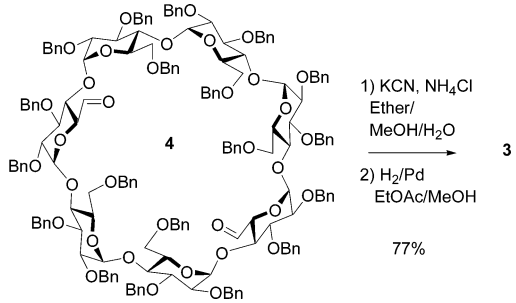

Figure 2. Synthesis of cyanohydrin **3** from known aldehyde **4**.[4]

quantitative yield. The cyanohydrin synthesis appears essentially stereoselective, and a single diastereomer is in any case obtained after purification. This isomer was shown to be the *R*,*R* isomer as outlined in Figure 3. Hydrolysis of **3** with IR-120 followed by

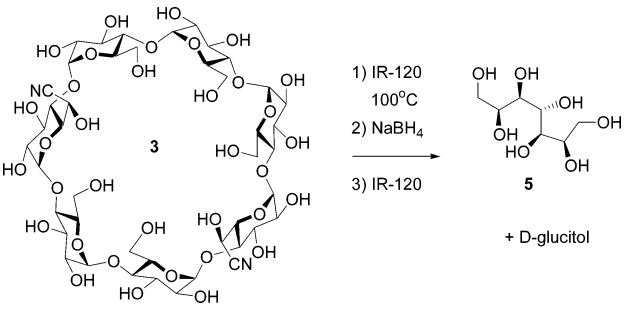

Figure 3. Determination of the stereochemistry of **3** by degradation to D-*glycero*-D-*gluco*-heptitol (**5**).

$NaBH_4$ reduction gave a 5:2 mixture of D-glucitol and a heptitol **5** that by comparison with ^{13}C NMR data[5] was shown to have the D-*glycero*-D-*gluco* configuration. This stereochemistry is consistent with attack by cyanide from the outside of the cyclodextrin **4** and the cyanohydrin hydroxyl groups in **3** pointing toward the inner face.

Compound **3** was found by NMR to catalyze the conversion of **2** into glucose (**6**) and nitrophenol (**7**). By following the formation of nitrophenol in this reaction by UV spectroscopy, the kinetics of the process was studied. The reaction follows Michaelis−Menten kinetics.[6] With increasing concentration of **2** a maximum velocity of the reaction is reached (V_{max}). A Hanes plot (see Supporting Information) of these data gives an excellent correlation, and the enzyme kinetic parameters of **3** was determined. At pH 7.4 and 25 °C, the reaction gives a K_m = 15 mM,[7] a $k_{cat} = 8.2 \times 10^{-6}$ s^{-1}, and a k_{cat}/k_{uncat} of 1217 (in the presence of 0.42 mM **3**). The catalysis can be inhibited by addition of cyclopropanol, confirming that the cyclodextrin cavity is involved in the process. No catalysis is observed with neither β-cyclodextrin nor mandelonitrile, showing that the supramolecular positioning of binding cavity and cyanohydrin group is essential for catalysis. The kinetic experiments were carried out with substrate (**2**) in concentrations of 1−25 mM and **3** in concentrations from 0.01 to 0.42 mM (Figure 4). At these conditions ($k_{cat} = 3.0 \times 10^{-5}$ s^{-1}, turnover time = 33 000 s) it is seen that even after two catalytic turnovers the catalytic rate is unchanged, which confirms true catalysis.

The catalysis by **3** of the hydrolysis of other nitrophenyl glycosides was also studied (Table 1). The catalysis of the α-glucoside has essentially the same rate as the β-glucoside, while the α-*manno* and α-*galacto* configured substrates are hydrolyzed with a slightly slower rate. The 2-nitrophenyl-β-galactopyranoside is hydrolyzed with the highest rate, but the variation in rate is nevertheless small and slightly smaller that the variation in background hydrolysis rate of the substrates.

To get insight into the nature of the catalysis the cyclodextrin analogues **8**−**12** (Figure 1) were prepared and their catalytic ability was studied. The di-6A,6D-*C*-propyl-substituted derivates **8**−**10**

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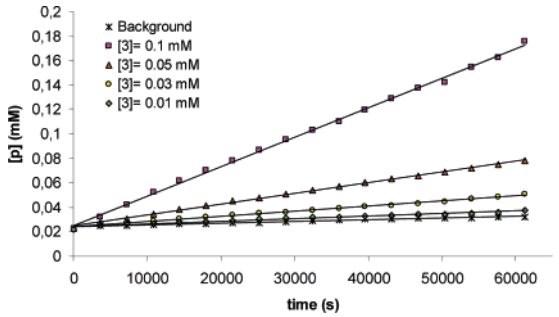

Figure 4. Progress curve for the hydrolysis of **2** (10 mM) at pH 7.4, 59 °C in the presence of different concentration of **3** (0.01−0.1 mM).

were prepared by hydrogenolysis of the known stereoisomeric benzyl-protected allyl derivatives,[4] while dialdehyde hydrate **11** was obtained by hydrogenolysis of **4**. Dinitrile **12** was obtained by conversion of the corresponding known benzyl-protected A,D diol[8] into a diiodide with Ph_3P, I_2, and imidazole (91%) followed by substitution with KCN (85%) and hydrogenolysis (100%, see Supporting Information).

The catalytic power of these compounds toward **2** hydrolysis is shown in Table 2. The propyl analogues **8**, **9**, and **10** afford no catalysis, similar 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 **12** is catalytic, but with a 250 times lower catalytic power, showing that the two cyanohydrin OH groups are very important for the catalysis. Finally, the dialdehyde **11**, which NMR shows is exclusively on dihydrate form in aqueous solution, is a catalyst with a catalytic efficacy of 20 times lower than **3**.

Table 1. Kinetic Parameters for the **3**-Catalyzed Hydrolysis of Various Glycosides in the Presence of 0.42 mM **3** at pH 7.4 and 59 °C

substrate	K_m (mM)	k_{cat} ($\times 10^5$ s^{-1})	k_{cat}/k_{uncat}
4-nitrophenyl-β-D-glucoside (**2**)	5.4	3.0	1047
4-nitrophenyl-α-D-glucoside	12	2.9	2147
4-nitrophenyl-α-D-mannoside	2.8	1.8	283
4-nitrophenyl-α-D-galactoside	1.0	2.3	486
2-nitrophenyl-β-D-galactoside	4.2	6.7	755

On the basis of these results, the following can be elucidated about the catalysis. Previous work on 6-*C*-substituted cyclodextrins concluded that, on the basis of modeling and the highly variant polarity of the 6*S* and 6*R* isomers, 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 **3** is that the cyanohydrin 6-OH groups are fixed in the *gt* conformation pointing toward the binding site. These OHs are essential. However, their fixed conformation in itself is not enough to promote catalysis as **9** is as uncatalytic as β-cyclodextrin itself. For this reason, nucleophilic catalysis is unlikely. The role of the cyano groups must be to draw electrons away from the OH groups, making them more acidic. This is supported by the observation that **11** is the second best catalyst. In this compound, the cyano groups have been replaced by OH groups, which are electron-withdrawing though less so. Thus, an increased acidity of these OH groups appears a crucial factor. This fits a role of the OH groups acting as general acids. We therefore propose a mechanism for the catalysis as outlined in Figure 5. A cyanohydrin OH group donates a proton to the exocyclic oxygen facilitating cleavage.

Table 2. Kinetic Parameters for the Catalysis by Different Cyclodextrin Derivatives of the Hydrolysis of 4-Nitrophenyl-β-D-glucoside (**2**) at pH 7.4 and 59 °C

catalyst	K_m (mM)	k_{cat} ($\times 10^5$ s^{-1})	k_{cat}/k_{uncat}
3 (0.42 mM)	5.4	3.0	1047
8−**10** (2.1 mM)	–	–	–
11 (0.44 mM)	7.6	0.14	48
12 (2.2 mM)	6.3	0.011	4

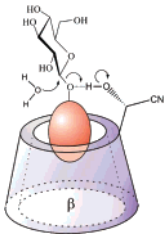

Figure 5. Proposed mechanism for the catalysis.

The cyclodextrin cyanohydrin **3** is an encouragingly potent catalyst and appears to mimic part of the mechanistic apparatus of natural glycosidases though with an entirely different functionality, the cyanohydrin. Including other parts of this apparatus such as nucleophilic catalysis is likely to improve the catalysis further.

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Supporting Information Available: Synthetic procedures for synthesis of **3** and **8**−**12**, procedures for kinetic analysis, and Hanes plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Normally an enzyme concentration that is significantly lower than substrate concentration is assumed for the Michaelis−Menten equation to be valid. However, the equation is also valid when $K_M \approx K_s$ (K_s is the substrate dissociation constant), which is the case here. Then $d[ES]/dt \approx 0$ is also fulfilled (steady-state conditions) over shorter time periods.
(7) Since k_{cat} is comparatively small, K_M is essentially identical to the dissociation constant for the enzyme−substrate complex (K_s), and the found value (15 mM) is close to the reported dissociation constant of 28 mM for this substrate to β-cyclodextrin.
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