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Artificial metallooxidases from cyclodextrin diacids

Bo Wang and Mikael Bols*[a]

Abstract: Unprecedented simple artificial metalloenzymes were made from cyclodextrin diacids. Six α- and β-cyclodextrin diacids were prepared and their metalbinding and acid properties were investigated. The diacids made fairly stable complexes with copper, zinc and iron with dissociation constants of 0.4-8 x 10^{-4} M. The iron complexes were found to catalyse a Fenton like oxidation reaction of benzylic alcohols which displayed Michealis-Menten catalysis and rate accelerations up to 2700.

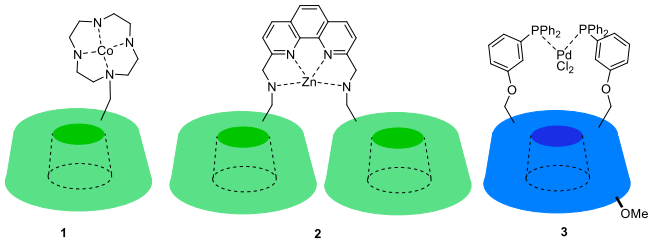

Figure 1. Cyclodextrin based artificial enzymes using a metal to assist catalysis

Introduction

Enzymes are protein macromolecules that can accelerate the rate of specific reactions tremendously.[1] The key to enzyme function is the binding of substrates in an active site – a cavity in the protein three dimensional structure. There the substrate is binding in a reactive conformation while functional groups in the enzyme facilitate reaction through proximity effects.[2] The efficiency, mildness and selectivity of enzyme catalysis makes it interesting to emulate and since most of the enzyme structure appears superfluous or a necessity associated with creating a cavity in the protein macrostructure enzyme models concentrate on the active site.[3] Such active site models (artificial enzymes) have been created from binders such as cyclodextrins,[4] calixarenes,[5] cucurbiturils and metal ligand clusters[6] and can become unique catalysts[7] or lead to a molecular understanding of enzyme catalysis.

Enzymes frequently use metal ions as part of their catalysis, where the metal ion can act as a supercharge or a reducing/oxidizing agent.[8] Consequently enzyme models containing metals have also been devised,[9] with cyclodextrin based artificial enzymes being most prominent among classical enzyme mimics.[10] Czarnik made the cobalt(III) complex **1** based on a cyclodextrin host (Figure 1) which could catalyze ester hydrolysis at neutral pH with a k_{cat}/k_{uncat} of 2900.[11] Cyclodextrin amines or heteroamines complexed with Copper,[12] Nickel[13] or Zinc[14] have similarly been shown to have esterase activity. Metallocyclodextrins with two cyclodextrins have been found excellent esterases. Recently Mao and collaborators made zinc-complexes such as **2** (Figure 1) that gave rate accelerations over 30.000[15] and were enatioselective.[16]

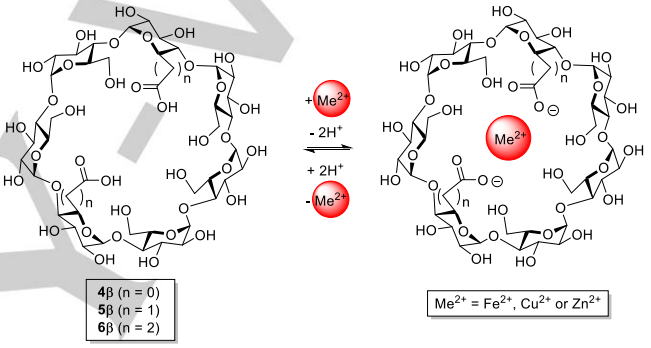

Figure 2. Cyclodextrin diacids and their proposed binding to divalent metalions.

Breslow's group have similarly demonstrated high esterase activity from bipyridine-copper cyclodextrins.[17] More organometallic type catalysts have also been made as Armspach and collaborators have made cyclodextrin phosphines such as **3** that can complex palladium and catalyse hydroformylations (Figure 1),[18] while Monflier's group have made cyclodextrin-phophine rhodium and palladium complexes that catalyze hydrogenation, hydroformylation and Suzuki couplings.[19] Reinaud, Jabin and collaborators have studied a series of metal-calixarene complexes with a higher structural sophistication than has been seen in the above mentioned cyclodextrin work and shown that the copper-complexes can act as oxidases[5] and chatecholases in organic solvents.[20a] Also palladium and nickel calixarene complexes have been reported to catalyse crosscoupling reactions.[20b,20c]

From the above work it is clear that relatively few supramolecular metal catalysts have had two or more ligand attachment sites especially when cyclodextrins are used as supramolecular hosts. Also almost all supramolecular hosts have had amine or phosphine attachment sites. Carboxylates are however also fine metal ligands and this led us to the idea that cyclodextrin diacids, some of which (**4α** and **4β**) are known,[21] might work as unprecedented simple metalloenzymes (Figure 2). We have therefore in this work synthesized the diacids with variant chain length **4**-**6** and studied their binding to divalent metalions of copper, zinc and iron. Secondly we have

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studied the catalytic properties of these complexes aiming at establishing a Fenton oxidation like system as shown in Figure 3. We find that the iron complexes function as artificial enzymes for oxidation of alcohols giving rate accelerations of up to 2600 over the uncatalyzed reaction.

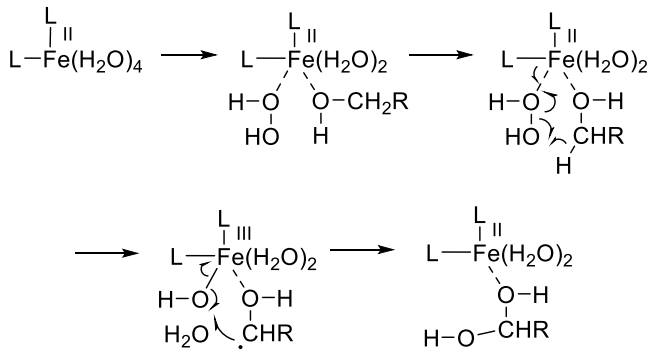
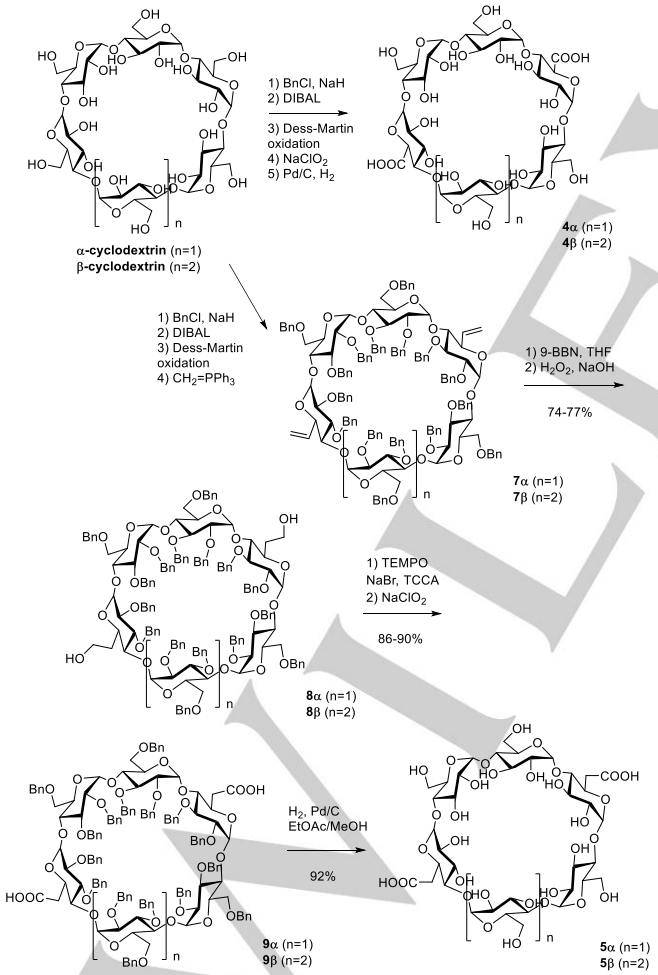


Figure 3. Aimed mode of Fenton type catalysis of cyclodextrin diacids (L) complexed to iron.

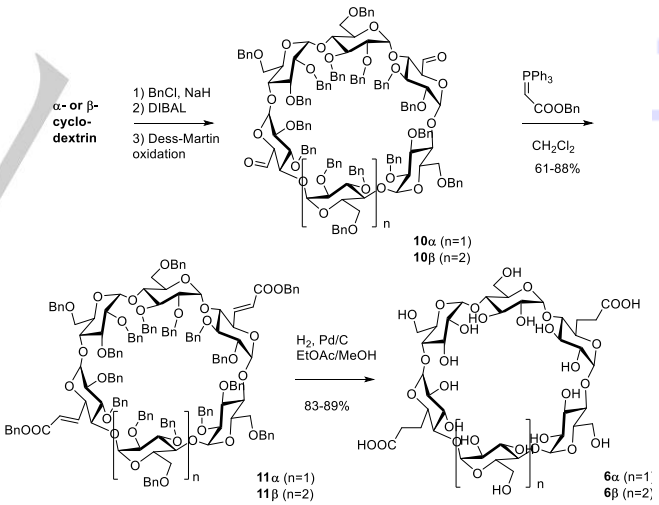


Scheme 1. Synthesis of diacids **4α**, **4β**, **5α** and **5β**.

Results and Discussion

Synthesis. α- and β-cyclodextrins with two acid groups attached to C-6 at the A and D sugar residues were desired: Compounds with a spacer of no (**4α** and **4β**), one (**5α** and **5β**) or two CH_2 groups (**6α** and **6β**) between C-5 and the carboxylate were made (Figure 2). The short diacids **4α** and **4β** were made from the native cyclodextrins by benzylation, DIBAL promoted debenzylation, oxidation and deprotection using published methods.[21] The intermediate length acids **5α** and **5β** were made as outlined in scheme 2: Native α- or β-cyclodextrin was converted to the divinyl derivatives **7α** or **7β**, respectively by benzylation, regioselective debenzylation, oxidation and wittig olefination as has previously been reported.[22] Hydroboration using 9-borabicyclo[3.3.1]nonane (9-BBN) followed by hydrogen peroxide oxidation have the diol **8α** or **8β** in 77% and 74% yields respectively. Tempo oxidation followed by Pinnick oxidation gave the diacids **9α** or **9β** in 86% and 90% yields, respectively. Finally debenzylation by catalytic hydrogenolysis with Pd/C at normal pressure gave **5α** or **5β** in 92% and 60% yield, respectively.

The longest diacids **6α** and **6β** were made from the dialdehydes **10α** or **10β** that are intermediates in the preparation of the other derivatives above (Scheme 2). Wittig reaction with benzyl (triphenylphosphoranylidene)acetate in dichloromethane gave the transalkenes **11α** or **11β** in 88% and 61% yield, respectively. Hydrogenation and hydrogenolysis with Pd/C at normal pressure gave **6α** or **6β** in 83% and 89% yield.



Scheme 2. Synthesis of long length diacids **6α** and **6β**.

pK_a determination. The six cyclodextrin diacids **4**-**6** were subjected to potentiometric titration in order to determine acidity constants. The pK_a values cannot directly be seen from a titration curve but they can be elucidated by fitting a simulated titration curve to the experimental curve.[23] From analysis of titration curves for compounds **4**-**6** (Figure S1) we obtain the

acidity constants shown in Table 1. We see three general trends in these pK_a values:

1. Compound **4α** and **β** have stronger acidic groups than **5α** and **β** that have stronger acids than **6α** and **β**
2. The difference between the pK_a values of the two acids in a compound grows in the series **4** to **5** to **6**, but there is essentially no difference between **α** and **β**.
3. The β-cyclodextrins are slightly stronger acids than α

Trend 1 is readily explained from the structure of the attached acid groups by inductive effects. Compound **4** has the acid directly attached to the glucose ring with all its electronwithdrawing oxygen. In **5** one CH_2 and in **6** two CH_2 groups have been placed between the electronwithdrawing sugar-ring resulting in less electronwithdrawing effect and a less acidic carboxylate group.

Table 1. Acidity constants of cyclodextrin diacids

	4α	**5α**	**6α**	**4β**	**5β**	**6β**
pK_a(1)	2.85	3.70	4.08	2.55	3.55	3.69
pK_a(2)	3.75	4.95	5.66	3.65	4.75	5.20

Trend 2 is actually the reverse of what one would expect based on charge separation: For simple diacids the separation of pK_a values decrease as the distance between the acids increase because it is more difficult to deprotonate a carboxylic acid when another negative charge is nearby. The ΔpK_a for **4α** and **4β** is very similar to that of a long diacid, while ΔpK_a for **5** and **6** is more similar to succinic or even malonic acids. The likely explanation is that the acid-groups in **5** and **6** is closer to each other than in **4** which also structurally makes sense.

The most likely reason for trend 3 is that there are a greater number of electronwithdrawing oxygen atoms in β-cyclodextrin than in α-cyclodextrin which to a slightly higher degree pulls electrons away from the carboxylic acids making them more acidic.

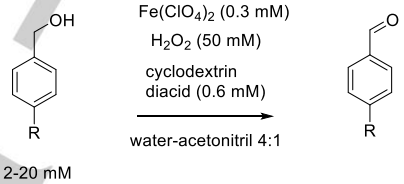
Binding studies. The binding of Cu^{2+} and Zn^{2+} ions to **4**-**6** were determined using potentiometric titration: The voltage difference between a copper or zinc electrode and a reference electrode with a fixed amount of the corresponding metal sulfate (62 μM) was measured while the ion was titrated by addition of cyclodextrin diacid. Metal concentration was calculated using the

Table 2. Dissociation constants of diacids with copper,zinc and iron (x10^{-4} M). The values in brackets were obtained by ITC; otherwise they were determined electrochemically. – means not determined

	4α	**5α**	**6α**	**4β**	**5β**	**6β**
Cu^{2+}	1.07	1.35	1.07	0.79	1.26	1.07
	[0.39]			[0.79]		
Zn^{2+}	2.14	2.95	2.29	3.72	7.94	2.51
	[0.53]			[0.69]		
Fe^{2+}	[0.60]	-	-	[0.72]	-	-

Nernst equation and was found to decrease upon cyclodextrin addition. Plots of metal versus cyclodextrin concentration showed 1:1 binding stoichiometry, and the K_d could directly be determined as the cyclodextrin concentration at the half point of titration. This gave dissociation constants to copper of ca 1 x 10^{-4} M and ca 2-8 x 10^{-4} M for Zn (Table 2). This means that **4**-**6** binds copper or zinc better than succinic acid (K_d(Cu): 2.5 x 10^{-3} M; K_d(Zn): 2.5 x 10^{-2} M) or simple organic acids.[24] There is very little variation in the size of the binding constants which suggests a certain flexibility in the interaction.

Binding was also determined using isothermal calorimetry (ITC) on compounds **4α** and **4β** (Table 2, Figure S2-S4). These results which had to be carried out with corresponding metal acetates rather than sulfates confirmed the stoichiometry and gave dissociation constants of same magnitude or somewhat lower presumably due to the different conditions of the ITC. Iron binding was also investigated and Fe^{2+} was found to bind to **4α** and **4β** well. The 1:1 stoichiometry found in these studies is consistent with the binding mode suggested in Figure 2. It was also confirmed by Electrospray MS of the diacid **4α** with Cu^{2+}, Zn^{2+} or Fe^{2+} showing in all three cases the monoprotonated complex of metal ion and cyclodextrindicarboxylate.



Scheme 3. Oxidation reaction.

Catalysis. With comparatively strong metal complexation established we examined the catalytic potential of the complexes in oxidation. The basic reaction shown in Scheme 3 was performed: A substituted benzyl alcohol (2-20 mM) was oxidized to benzaldehyde with the latter being monitored spectrophotometrically. Hydrogen peroxide was the stoichiometric oxidant and cyclodextrin and metal perchlorate were present in catalytic amounts. The solvent was water with 20% acetonitrile to keep the substrate in solution. This reaction has a slow background rate which is increased slightly if copper, zinc and iron perchlorate is added (Figure S5). However for $Fe(ClO_4)_2$ the oxidation rate increased 100-1000 times when a cyclodextrin diacid was added - this was not the case for $Cu(ClO_4)_2$ and $Zn(ClO_4)_2$. If succinic acid was added to the $Fe(ClO_4)_2$ catalysed background reaction only a small rate increase was seen (Figure S5), which clearly indicate that the cyclodextrin cavity is important.

The iron catalyzed reaction was optimally performed with 0.3 mM $Fe(ClO_4)_2$ and 0.6 mM of **4**, **5** or **6** (Scheme 3). Under these conditions most of the iron was complexed to cyclodextrin and the oxidation of the substrate (2 to 20 mM) displayed Michaelis-Menten kinetics giving the kinetic values shown in Tables 3-5. The cyclodextrins display no catalysis when no iron is present. Under these condition substrate conversion is kept low (<10%), but it can readily be increased by increasing time and concentration. Thus in an experiment of benzyl alcohol (0.33 M), **4β** and $Fe(ClO_4)_2$ (5 mol%) 95% conversion was

obtained in 15 minutes, but at the expense of most of the benzaldehyde being converted to benzoic acid and other unidentified products.

Table 3. Kinetic parameters for oxidation of alcohols by cyclodextrin diacids (CD). The substrates are substituted benzyl alcohols or 2-hydroxymethylnaphtalene. Solvent: MeCN:water 1:4, [Substrate] = 2-20 mM; [CD] = 0.6 mM, [$Fe(ClO_4)_2$] = 0.3 mM, [H_2O_2] = 50 mM.

CD	Substrate	k_{cat}[a]	K_m[b]	k_{cat}/k_{uncat}
4α (Fe^{2+})	*p*-OMe	650±10	11.5±0.1	481
4α (Fe^{2+})	*o*-OMe	191±3	6.4±0.3	428
4α (Fe^{2+})	*p*-Me	61±7	2.2±0.4	203
4α (Fe^{2+})	*p*-Br	1210±27	49±3.1	2689
4α (Fe^{2+})	*p*-Cl	200±12	8.1±0.4	573
4α (Fe^{2+})	*p*-CN	117±2	6.9±0.2	254
4α (Fe^{2+})	2-naphtyl	578±80	21.1±7	2408

[a] μs^{-1} [b] mM

In Table 3 we see the effect of the short α-cyclodextrin diacid **4α** on the oxidation of various benzyl alcohols. The k_{cat} is the first order rate constant of reaction of substrate bound to artificial enzyme and k_{cat}/k_{uncat} is how much higher this rate is compared to the uncatalyzed reaction i.e. the rate increase of the enzymatic reaction. The rate increase was 200-2700 over the uncatalyzed reaction with K_m values between 0.05 and 0.002 M. Since the K_m value in a case like this essentially is equal to the K_d value of the enzyme-substrate complex we see that these substrates binds with affinity which is typical for binding of aromatics to cyclodextrins but nevertheless with an affinity that is 10 to 1000 times weaker than the binding of iron. The best substrates were the *p*-bromobenzyl alcohol and 2-hydroxymethylnaphtalene which gave the highest rate accelerations within the cavity. The β-cyclodextrin version of this compound, **4β**, displayed similar efficient catalysis and substrate preference (Table 4). The highest rates were those of *p*-methoxybenzyl and *p*-bromobenzyl alcohol, while the best rate accelerations were, similarly to **4α**, those of *p*-bromobenzyl alcohol and 2-hydroxymethylnaphtalene.

To confirm whether the cyclodextrin-iron complex was in fact behaving as a metalloenzyme, inhibition experiments were performed with cyclopentanol as an inhibitor. In the presence of 5 mM cyclopentanol the rate of oxidation catalyzed by **4α** or **4β** was clearly impeded and a Hanes plot of the data showed that cyclopentanol was a competitive inhibitor with K_i values of 2.6 and 4.3 mM, respectively (Figure 4, Figure S6).

The iron complexes of the longer cyclodextrin diacids **5** and **6** also catalyzed the reaction though in general somewhat less efficient (Table 5). The catalysis displayed Michaelis-Menten saturation kinetics in accordance with a behavior of **5** and **6** as artificial enzymes and with some of the same specificity

Table 4. Kinetic parameters for oxidation of alcohols by cyclodextrin diacids (CD). The substrates are substituted benzyl alcohols or 2-hydroxymethylnaphtalene. Solvent: MeCN:water 1:4, [Substrate] = 2-20 mM; [CD] = 0.6 mM, [$Fe(ClO_4)_2$] = 0.3 mM, [H_2O_2] = 50 mM.

CD	Substrate	k_{cat}[a]	K_m[b]	k_{cat}/k_{uncat}
4β (Fe^{2+})	*p*-OMe	1090±20	22.7±0.2	807
4β (Fe^{2+})	*o*-OMe	398±15	16.5±1.1	892
4β (Fe^{2+})	*p*-Me	103±13	1.9±0.3	343
4β (Fe^{2+})	*p*-Br	1183±37	41.9±1.5	2622
4β (Fe^{2+})	*p*-Cl	340±1	10.6±0.5	974
4β (Fe^{2+})	*p*-CN	110±3	2.2±0.3	239
4β (Fe^{2+})	2-naphtyl	503±80	16.0±1.5	2117

[a] μs^{-1} [b] mM

trends such as *p*-bromobenzyl alcohol being a superior substrate. With this substrate the increase in oxidation rate was about 1700. The reason for the lower rate of catalysis is probably because of the greater distance from the metal center to the site of oxidation.

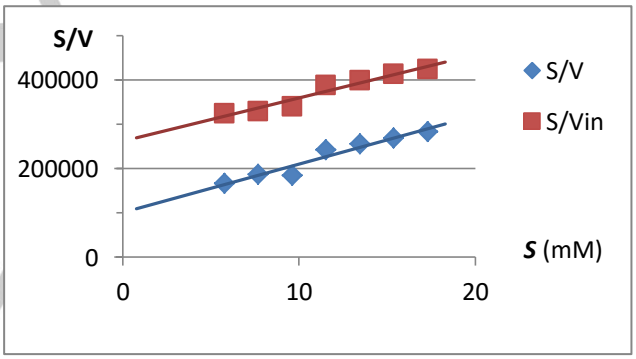


Figure 4. Hanes plot for the oxidation of 4-chlorobenzyl alcohol by **4α** (0.6 mM) in water-MeCN (4:1) with (■) and without (◆) the presence of cyclopentanol (5 mM). $Fe(ClO_4)_2$ (0.3 mM) and H_2O_2 (50 mM) are also present [S] is 4-chlorobenzyl alcohol concentration. V is starting velocity. The plot shows that cyclopentanol is a competitive inhibitor with K_i = 2.6 mM.

Table 5. Kinetic parameters for oxidation of alcohols by cyclodextrin diacids (CD). The substrates are substituted benzyl alcohols or 2-hydroxymethylnaphtalene. Solvent: MeCN:water 1:4, [Substrate] = 2-20 mM; [CD] = 0.6 mM, [$Fe(ClO_4)_2$] = 0.3 mM, [H_2O_2] = 50 mM.

CD	Substrate	k_{cat}[a]	K_m[b]	k_{cat}/k_{uncat}
5α (Fe^{2+})	*p*-OMe	310±20	37.1±4.0	230
5α (Fe^{2+})	*p*-Cl	101±9	18±1.2	289
5α (Fe^{2+})	2-naphtyl	70±0.7	4.5±0.3	292
5β (Fe^{2+})	*o*-OMe	90±15	12.7±0.5	202

5β (Fe^{2+})	2-naphtyl	83±0.5	12.0±0.4	346
6β (Fe^{2+})	*p*-OMe	290±10	15.7±0.3	215
6β (Fe^{2+})	*o*-OMe	133±2	8.4±0.2	298
6β (Fe^{2+})	*p*-Br	751±14	47±2.4	1669
6β (Fe^{2+})	*p*-CN	30±1.4	7.16±0.2	65
6β (Fe^{2+})	2-naphtyl	116±6	6.3±0.4	479

[a] μs^{-1} [b] mM

This hypothesis is confirmed by inspection of models. A model constructed of **4α** binding an octahedral iron atom with 3 water molecules and a benzyl alcohol molecule as ligand (Figure 5) show that the substrate is well accommodated in the α-cyclodextrin cativity with the iron atom situated at the narrow end. According to this model there is plenty of space for access of hydrogen peroxide to replace a water molecule ligand and undergo reaction with the alcohol. Longer acids places the iron atom further from the cavity which could be the reason for the lower rate of oxidation of **5** and **6**.

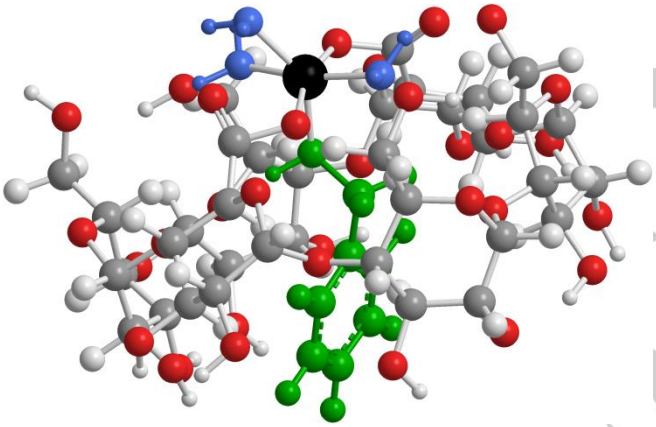


Figure 5. Model of a 1:1 complex of **4α**, iron (black), with benzyl alcohol (green) and three water-molecules (blue) coordinating to iron.

Conclusions

The cyclodextrin diacids **4**-**6** are comparatively strong acids with lower pK_a values when the acid is close to the cyclodextrin rim due to the electron withdrawing effect from the oxygen atoms in the saccharide rings. The difference in pK_a values increase with longer acid suggesting that hydrogen bonding between occur more efficiently in monoprotonated **5** and **6** than in **4**.

The cyclodextrin diacid form **4**-**6** relatively strong 1:1 complexes with Cu^{2+}, Zn^{2+} and Fe^{2+} ions which are roughly of the same strength irrespectively of the size or chainlenght of the cyclodextrin diacids.

The iron complexes were found to function as artificial oxidases for the oxidation of benzyl alcohols to aldehydes with a clear enzyme-like rate enhancing effect of the cyclodextrin cavity. Future work should focus on exploiting this effect possibly with new metals or new ligands.

Experimental Section

General information

Dry solvents were tapped from a solvent purification system. (PE is petroleum ether with boiling point 40-65C). Reactants were purchased from commercial sources and used without further purification. Potentiometric titration and pH titration were recorded by PHM210 Standard pH Meter. HRMS were recorded on a Bruker Solarix XR mass spectrometer analyzing TOF. NMR spectra were recorded on a Brüker 500 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to the residue solvent signals ($CDCl_3$: δ = 7.26 for ^{1}H-NMR and 77.16 for ^{13}C-NMR. D_2O: δ = 4.79 for ^{1}H-NMR). Assignments were aided by COSY and HSQC experiments. The oxidation experiments were monitored by Epoche 2 Microplate spectrophotometer with a 96-well quarts microplate.

6A,6D-dideoxy-6A,6D-di(hydroxymethyl)-hexadeca-*O*-benzyl-α-cyclodextrin (8α):

To a solution of 6A,6D-dideoxy-6A,6D-di-vinyl-hexadeca-O-benzyl-α-cyclodextrin **7α**[22] (1.1 g, 0.457 mmol, 1 equiv.) in THF (25 mL) was added 9-BBN (7.3 mL, 4.570 mmol, 10 equiv., 0.5 M in THF). The reaction mixture was stirred at room temperature for 4 h, then the reaction mixture was cooled down to 0°C, water (1 mL) was added to decompose the excess 9-BBN. 3 M NaOH (2 mL) and 35% H_2O_2 (2 mL) were added and the mixture was stirred overnight. EtOAc was added and the aqueous layer was extracted with EtOAc (3×25mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. Silica gel flash chromatography of the residue (PE/EtOAc: 4/1, the 3/1) afforded α-cyclodextrin **8α** (850 mg, 77%) as a white foam. $[\alpha]_D^{25}$= +25.8 (c 1.0, $CHCl_3$). R_f = 0.67 (PE/EtOAc: 2/1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34-7.06 (m, 40 H, H-Ar), 5.38 (d, 1 H, ^2J = 10.5 Hz CHPh), 5.29 (d, 1 H, ^2J = 10.9 Hz, CHPh), 5.28 (d, 1 H, $^3J_{1,2}$ = 3.0 Hz, H-1), 4.96 (d, 1 H, 2J = 10.1 Hz, CHPh), 4.94 (d, 1 H, ^2J = 11.5 Hz, CHPh), 4.86-4.84 (m, 3 H, H-1, 2×CHPh), 4.72 (d, 1 H, $^3J_{1,2}$ = 3.1 Hz, H-1), 4.63 (d, 1 H, ^2J = 11.1 Hz, CHPh), 4.52-4.38 (m, 8 H, 8×CHPh), 4.31 (d, 1 H, ^2J = 12.2 Hz, CHPh), 4.27-4.02 (m, 7 H, 3×H-3, 3×H-5, H-6), 3.96 (dd, 1H, $^3J_{5,6}$ = 4.6 Hz, $^2J_{6,6}$ = 9.0 Hz, H-6), 3.88-3.75 (m, 4 H, 2×H-4, 2×H-6), 3.52 (dd, 1H, $^3J_{1,2}$ = 3.5 Hz, $^3J_{2,3}$ = 9.9 Hz, H-2), 3.44-3.36 (m, 4 H, 2×H-2 H-4, CH2CHOH), 3.19 (m, 1 H, CH_2CHOH), 2.16 (m, 1 H, $CHCH_2OH$), 1.40 (m, 1 H, $CHCH_2OH$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.88, 139.70, 139.57, 138.86, 138.56, 138.31, 138.16, 137.90 (8×C-$Ar^{quat.}$), 128.41-126.40 (40×C-$Ar^{tert.}$), 100.34, 99.97, 98.57 (3×C-1), 84.96, 82.36, 81.64 (3×C-4), 80.74, 80.67, 80.53 (3×C-3), 80.21, 78.61, 78.34 (3×C-2), 76.28, 75.79, 74.43, 73.67, 73.54, 72.98, 72.69, 72.23 (8×CH_2Ph), 71.41, 71.23 (2×C-5), 70.10, 69.53 (2×C-6), 67.65 (C-5), 57.54 (CH_2CH_2OH), 35.60 (CH_2CH_2OH), HRMS (ESP): Calcd. For $C_{150}H_{160}NaO_{30}$ $[M+Na]^{1+}$: 2465.8898; Found: 2465.0880

6A,6D-dideoxy-hexadeca-*O*-benzyl-α-cyclodextrin-6A,6D-dimethylenecarboxylic Acid (9α):

To a solution of cyclodextrin **8α** (2.6 g, 1.065 mmol, 1 equiv.) in acetone (50 mL) was added aq. $NaHCO_3$ (6 mL), then NaBr (55 mg, 0.533 mmol, 0.5 equiv.) and TEMPO (17 mg, 0.107 mmol, 0.1 equiv.) were added at 0°C. Following slow addition of TCCA (990 mg, 4.26 mmol, 4 equiv.) at 0°C, the reaction mixture was stirred at room temperature overnight. EtOAc was added and the aqueous layer was extracted with EtOAc (3×25mL). The combined organic layers were dried over $MgSO_4$, filtered

and concentrated in vacuum. The residue was dissolved in a mixture of ^{t}BuOH (25 mL), THF (25 mL) and 2-methyl-2-butene (2.26 mL, 21.3 mmol, 20 equiv.), and $NaClO_2$ (1.44 g, 15.975 mmol, 15 equiv.) and NaH_2PO_4 (2.49 g, 15.975 mmol, 15 equiv.) in water (6 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 × 20 mL). The organic phase was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuum. The residue was purified by chromatography (DCM/MeOH: 98/2 containing 1% HCOOH) afforded dicarboxylic acid-α-cyclodextrin **9α** (2.26 g, 86%) as a white foam. $[\alpha]_D^{25}$= +35.6 (*c* 0.55, $CHCl_3$), R_f = 0.55 (DCM/MeOH: 95/5), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.25-6.69(m, 40 H, H-Ar), 5.25 (d, 1 H, ^2J = 10.4 Hz, CHPh), 5.13 (d, 1 H, ^2J = 11.3 Hz, CHPh), 4.94 (d, 1 H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.90 (d, 1 H, ^2J = 11.4 Hz, CHPh), 4.84 (d, 1 H, ^2J = 11.4 Hz, CHPh), 4.80 (d, 1 H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.75 (d, 1 H, ^2J = 11.5 Hz, CHPh), 4.72 (d, 1 H, ^2J = 11.6 Hz, CHPh), 4.61 (d, 1 H, $^3J_{1,2}$ = 3.6 Hz, H-1), 4.51-4.21 (m, 11 H, 10×CHPh, H-5), 4.09-4.04 (m, 3 H, 2×H-3, H-5), 3.94-3.85 (m, 4 H, H-3, H-4, 2×H-5), 3.72 (d, 1 H, $^2J_{6,6}$ = 11.1 Hz, H-6), 3.57 (dd, 1H, $^3J_{5,6}$ = 6.4 Hz, $^2J_{6,6}$ = 11.5 Hz, H-6), 3.45 (t, 1 H, $^3J_{3,4}$ = $^3J_{4,5}$ = 9.3 Hz, H-4), 3.35 (dd, ^{1}H, $^3J_{1,2}$ = 3.4 Hz, $^3J_{2,3}$ = 10.6 Hz, H-2), 3.33 (dd, 1H, $^3J_{1,2}$ = 3.2 Hz, $^3J_{2,3}$ = 9.5 Hz, H-2), 3.28-3.18 (m, 4 H, H-2, H-6, H-4, C<u>H</u>COOH), 2.23 (m, 1 H, C<u>H</u>COOH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.42 (CH_2<u>C</u>OOH), 139.72, 139.68, 139.51, 138.86, 138.32, 138.15(2C), 137.48 (8×C-Ar$^{quat.}$), 128.67-126.77 (40×C-Ar$^{tert.}$), 101.12, 100.98, 97.73 (3×C-1), 85.80, 82.42, 81.14 (3×C-4), 80.94, 80.58, 80.04 (3×C-3), 79.87, 78.27, 78.06 (3×C-2), 76.53, 75.26(2C), 73.85, 73.37, 73.01, 72.53, 72.39 (8×CH_2Ph), 72.03, 71.32 (2×C-5), 69.92 (C-6), 68.87 (C-5), 68.76 (C-6), 38.15 (<u>C</u>H_2COOH). HRMS (ESP): Calcd. For $C_{150}H_{157}O_{32}$ $[M+H]^{1+}$: 2471.8740; Found: 2471.0381

6A,6D-dideoxy-α-cyclodextrin-6A,6D-dimethylenecarboxylic Acid (5α):

To a solution of cyclodextrin **9α** (2.6 g, 1.065 mmol, 1 equiv.) in a mixture of MeOH/EtOAc (50 mL) were added Pd/C (2.4 g) and TFA (cat.). The reaction mixture was stirred at room temperature overnight under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave the cyclodextrin **5α** (1.0 g, 92%) as a white foam. $[\alpha]_D^{25}$= +101.0 (*c* 0.6, H_2O), ^{1}H NMR (D_2O, 500 MHz): δ 5.12 (d, 1 H, $^3J_{1,2}$ = 3.0 Hz, H-1), 5.08 (d, 1 H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.01 (d, 1 H, $^3J_{1,2}$ = 2.9 Hz, H-1), 4.23 (t, 1 H, H-5), 4.03-3.98 (m, 3 H, 3×H-3), 3.91-3.79 (m, 9 H, 2×H-5, 4×H-6), 3.69-3.63 (m, 4 H, 3×H-2, H-4), 3.59-3.47 (m, 2 H, 2×H-4), 3.22 (d, 1 H, $^2J_{6,6}$ = 14.7 Hz, C<u>H</u>COOH), 2.56 (dd, 1 H, C<u>H</u>COOH), ^{13}C NMR (D_2O, 125 MHz): δ 175.67 (CH2<u>C</u>OOH), 101.64, 101.43, 100.56 (3×C-1), 84.80, 81.45, 80.58 (3×C-4),73.28, 73.01, 72.88 (3×C-3), 71.88, 71.81 (2×C-5), 71.63, 71.58, 71.48 (3×C-2), 68.98 (C-5), 60.39, 60.05 (2×C-6), 37.21 (<u>C</u>H_2COOH), HRMS (ESP): Calcd. For $C_{38}H_{61}O_{32}$ $[M+H]^{1+}$: 1029.3145; Found: 1029.3167, Calcd. For $C_{38}H_{60}O_{32}Na$ $[M+Na]^{1+}$: 1051.2965; Found: 1051.2988.

6A,6D-dideoxy-hexadeca-*O*-benzyl-α-cyclodextrin-6A,6D-di(ethylidenecarboxylic acid benzylester) (11α):

To a solution of 6A,6D-dialdehydo-hexadeca-*O*-benzyl-α-cyclodextrin **10α** [ref] (1.0 g, 0.414 mmol, 1 equiv.) in dichloromethane (20 mL) was added $Ph_3PCHCOOBn$ (876 mg, 2.070 mmol, 5 equiv.). The reaction mixture was stirred at room temperature overnight, then water was added and the aqueous phase was extracted with EtOAc (3×25mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was purified by chromatography (PE/EtOAc: 4/1) afforded α-cyclodextrin **11α** (960 mg, 88%) as a white foam. $[\alpha]_D^{25}$= +47.5 (c 0.8, $CHCl_3$), R_f = 0.66 (PE/EtOAc: 2/1), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44-7.18 (m, 46 H, 45×H-Ar, CH=CHCOOBn), 6.09 (d, 1 H, $^3J_{trans}$ = 15.3 Hz, CH=C<u>H</u>COOBn), 5.38 (d, 1 H, $^3J_{1,2}$ = 3.2 Hz, H-1), 5.37 (d, 1 H, ^{2}J = 11.5 Hz, CHPh), 5.35 (d, 1 H, ^2J =10.9 Hz, CHPh), 5.19 (d, 1 H, ^2J = 12.4 Hz, CH=CHCOOC<u>H</u>Ph), 5.18 (d, 1 H, $^3J_{1,2}$ =3.5 Hz, H-1), 5.05 (d, 1 H, ^2J = 11.4 Hz, CHPh), 5.01 (d, 1 H, ^2J =11.4 Hz, CHPh), 4.96 (d, 1 H, ^2J = 12.4 Hz, CH=CHCOOC<u>H</u>Ph), 4.95-4.92 (m, 3 H, H-1, 2×CHPh), 4.72 (d, 1 H, ^{2}J = 11.6 Hz, CHPh), 4.67 (dd, 1 H, $^3J_{5,6}$ =4.1 Hz, $^2J_{6,6}$ = 9.7 Hz, H-6), 4.61 (d, 1 H, ^{2}J = 12.0 Hz, CHPh), 4.57-4.47 (m, 6 H, 6×CHPh), 4.42 (d, 1 H, ^2J = 11.6 Hz, CHPh), 4.36 (d, 1 H, ^2J = 12.1 Hz, CHPh), 4.29-4.12 (m, 6 H, 3×H-3, 2×H-4, H-5), 4.06-3.98 (m, 2 H, 2×H-5), 3.67 (dd, 1 H, $^3J_{1,2}$ = 3.7 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 3.63-3.53 (m, 5 H, H-2, H-4, 3×H-6), 3.67 (dd, 1 H, $^3J_{1,2}$ =3.1 Hz, $^3J_{2,3}$ = 9.9 Hz, H-2), ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.80 (CH=CH<u>C</u>OOBn), 145.62 (CH=<u>C</u>HCOOBn), 139.57, 139.49, 139.39, 138.82, 138.53, 138.36, 138.25, 138.22, 136.20 (9×C-Ar$^{quat.}$), 128.64-126.72 (45×C-Ar$^{tert.}$), 121.58 (<u>C</u>H=CHCOOBn), 99.25, 99.19, 97.81 (3×C-1), 83.26 (C-4), 81.34, 81.07, 80.92 (3×C-3), 80.35 (C-4), 79.62 (C-2), 79.38 (C-4), 78.98, 78.85 (2×C-2), 76.18, 75.70, 74.93, 73.48, 73.43, 73.32, 72.88, 72.63 (8×CH_2Ph), 71.84, 71.77, 69.71 (3×C-5), 69.10, 69.01 (2×C-6), 66.06 (CH=CHCOO<u>C</u>H_2Ph), HRMS (ESP): Calcd. For $C_{166}H_{168}NaO_{32}$ $[M+Na]^{1+}$: 2698.1278; Found: 2698.1386

6A,6D-dideoxy-α-cyclodextrin-6A,6D-diethylenecarboxylic acid (6α):

To a solution of cyclodextrin **11α** (2.21 g, 0.826 mmol, 1 equiv.) in a mixture of MeOH/EtOAc (50 mL) were added Pd/C (2.2 g) and TFA (cat.) The reaction mixture was stirred at room temperature for 2 days under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave cyclodextrin **6α** (0.72 g, 83%) as a white foam. $[\alpha]_D^{25}$= +108.8 (*c* 0.5, H_2O), ^{1}H NMR (D_2O, 500 MHz): δ 5.09 (d, 1 H, $^3J_{1,2}$ = 3.0 Hz, H-1), 5.05 (d, 1 H, $^3J_{1,2}$ = 3.1 Hz, H-1), 5.01 (d, 1 H, $^3J_{1,2}$ = 3.0 Hz, H-1), 4.00-3.80 (m, 10 H, 3×H-3, 3×H-5, 4×H-6), 3.64-3.60 (m, 5 H, 3×H-2, 2×H-4), 3.36 (t, 1 H, $^3J_{3,4}$ = $^3J_{4,5}$ = 8.8 Hz, H-4), 2.61-2.36 (m, 3 H, 2×CH_2C<u>H</u>COOH, C<u>H</u>CH_2COOH), 1.74 (m, 1 H, C<u>H</u>CH_2COOH), ^{13}C NMR (D_2O, 125 MHz): δ 177.82 (CH_2CH_2<u>C</u>OOH), 101.68, 101.55, 101.04 (3×C-1), 85.78, 81.11, 80.88 (3×C-4), 73.37, 73.32, 73.13 (3×C-3), 71.86(2C) (2×C-5), 71.75, 71.72, 71.59 (3×C-2), 70.22 (C-5), 60.19, 60.07 (2×C-6), 29.81 (CH_2<u>C</u>H_2COOH), 26.17 (<u>C</u>H_2CH_2COOH), HRMS (ESP): Calcd. For $C_{40}H_{65}O_{32}$ $[M+H]^{1+}$: 1057.3458; Found: 1057.3481, Calcd. For $C_{40}H_{64}O_{32}Na$ $[M+Na]^{1+}$: 1079.3278; Found: 1079.3297.

6A,6D-dideoxy-6A,6D-di(hydroxymethyl)-nonadeca-*O*-benzyl-β-cyclodextrin (8β):

To a solution of 6A,6D-dideoxy-6A,6D-di-vinyl-nonadeca-*O*-benzyl-β-cyclodextrin **7β**[ref] (1.6 g, 0.564 mmol, 1 equiv.) in THF (25 mL) was added 9-BBN (11.3 mL, 5.64 mmol, 10 equiv., 0.5 M in THF). The reaction mixture was stirred at room temperature for 4 h, then the reaction mixture was cooled down to 0°C, water (1 mL) was added to decompose the excess 9-BBN. 3 M NaOH (2 mL) and 35% H_2O_2 (2 mL) were added and the mixture was stirred overnight. EtOAc was added and the aqueous layer was extracted with EtOAc (3×25mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. Silica gel flash chromatography of the residue (PE/EtOAc: 4/1, the 3/1) afforded β-cyclodextrin **8β** (1.2 g, 74%) as a white foam. $[\alpha]_D^{25}$= +35.2 (c 1.0, $CHCl_3$), R_f = 0.65 (PE/EtOAc: 2/1), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.29-7.01 (m, 95 H, H-Ar), 5.63 (d, 1 H, $^3J_{1,2}$ = 3.7 Hz, H-1), 5.26-5.16 (m, 7 H, 2×H-1, 5×CHPh), 5.00 (d, 1 H, ^{2}J = 11.4 Hz, CHPh), 4.98 (d, 1 H, $^3J_{1,2}$ = 3.5 Hz, H-1), 4.93 (d, 1 H, $^3J_{1,2}$ = 3.3 Hz, H-1), 4.85 (d, 1 H, ^{2}J = 11.4 Hz, CHPh), 4.83 (d, 1 H, $^3J_{1,2}$ = 3.4 Hz, H-1), 4.80-4.70 (m, 10 H, H-1, 9×CHPh), 4.67 (d, 1 H, ^{2}J = 10.6 Hz, CHPh), 4.60 (d, 1 H, ^{2}J = 11.4 Hz, CHPh), 4.55-4.31 (m, 20 H, 20×CHPh), 4.17-3.82 (m, 19 H, 7×H-3, 5×H-4, 2×H-5, 5×H-6), 3.79-3.70 (m, 4 H, H-4, 3×H-6), 3.64 (d, $^2J_{6,6}$ = 10.2 Hz, H-6), 3.58 (d, $^2J_{6,6}$ = 10.0 Hz, H-6), 3.55-3.31 (m, 12 H, 7×H-2, H-4, 4×CH_2C<u>H</u>OH), 2.30 (m, 1 H, C<u>H</u>CH_2OH), 2.10 (m, 1 H, C<u>H</u>CH_2OH), 1.55 (m, 1 H, C<u>H</u>CH_2OH), 1.35 (m, 1 H, C<u>H</u>CH_2OH). ^{13}C

NMR ($CDCl_3$, 125 MHz): δ 139.59(2C), 139.54, 139.49, 139.47, 139.43, 139.33, 138.85, 138.80, 138.63, 138.59, 138.47, 138.42, 138.36, 138.32, 138.30, 138.08, 138.04, 137.96 (19×C-$Ar^{quat.}$), 128.49-126.53 (95×C-$Ar^{tert.}$), 99.40, 99.26, 99.18, 98.21, 98.15, 98.03, 97.73 (7×C-1), 83.06 (C-4), 81.57, 81.14, 81.10, 80.92(2C), 80.85(2C) (7×C-3), 80.81, 80.68, 80.47, 80.30, 80.20 (5×C-4), 80.12, 79.51, 78.91, 78.70, 78.59, 78.51 (6×C-2), 78.01 (C-4), 77.91 (C-2), 76.50, 76.09, 75.82, 75.60, 75.08, 74.98, 74.18, 73.76, 73.62, 73.56, 73.54, 73.52, 73.24, 73.07, 72.91, 72.82, 72.80, 72.61, 72.42 (19×CH_2Ph), 71.96, 71.91, 71.73, 71.63, 71.14 (5×C-5), 69.84, 69.81, 69.77, 69.58, 69.01 (5×C-6), 67.89, 67.79 (2×C-5), 58.47, 58.07 (2×CH2CH2OH), 35.49, 34.84 (2×CH_2CH_2OH).

6A,6D-dideoxy-nonadeca-*O*-benzyl-β-cyclodextrin-6A,6D-dimethylenecarboxylic acid (9β):

To a solution of cyclodextrin **8β** (1.1 g, 0.383 mmol, 1 equiv.) in acetone (40 mL) was added aq. $NaHCO_3$ (6 mL), then NaBr (20 mg, 0.192 mmol, 0.5 equiv.) and TEMPO (6.1 mg, 0.038 mmol, 0.1 equiv.) were added at 0°C. Following slow addition of TCCA (356 mg, 1.532 mmol, 4 equiv.) at 0°C, the reaction mixture was stirred at room temperature overnight. EtOAc was added and the aqueous layer was extracted with EtOAc (3×25mL). The combined organic layers were dried over MgSO4, filtered and concentrated in vacuum. The residue was dissolved in a mixture of tBuOH (20 mL), THF (20 mL) and 2-methyl-2-butene (0.81 mL, 7.66 mmol, 20 equiv.), and $NaClO_2$ (0.52 g, 5.75 mmol, 15 equiv.) and NaH_2PO_4 (0.896 g, 5.75 mmol, 15 equiv.) in water (5 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 × 20 mL). The organic phase was dried ($MgSO_4$), filtered, and the organic solvent was removed in vacuum. The residue was purified by chromatography (DCM/MeOH: 98/2 containing 1% HCOOH) afforded dicarboxylic acid-β-cyclodextrin (1.0 g, 90%) as a white foam. $[\alpha]_D^{25}$= +36.9 (*c* 0.45, $CHCl_3$), R_f = 0.55 (DCM/MeOH: 95/5). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34-6.98(m, 95 H, H-Ar), 5.64 (d, 1 H, $^3J_{1,2}$ = 4.0 Hz, H-1), 5.36 (d, 1 H, $^3J_{1,2}$ = 3.3 Hz, H-1), 5.23 (t, 2 H, ^2J = 10.4 Hz, 2×CHPh), 5.14 (d, 1 H, ^2J = 10.6 Hz, CHPh), 5.10 (d, 1 H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.09 (d, 1 H, ^2J = 10.6 Hz, CHPh), 4.92 (d, 1 H, $^3J_{1,2}$ = 3.3 Hz, H-1), 4.84-4.66 (m, 13 H, 3×H-1, 10×CHPh), 4.61-4.25 (m, 24 H, 2×H-5, 24×CHPh), 4.19 (d, 2 H, $^2J_{6,6}$ = 11.3 Hz, 2×H-6), 4.07-3.69 (m, 23 H, 7×H-3, 7×H-4, 5×H-5, 4×H-6), 3.61-3.30 (m, 11 H, 4×H-6, 7×H-2), 3.10 (d, 1 H, $^2J_{6,6}$ = 13.7 Hz, C<u>H</u>COOH), 2.97 (d, 1 H, $^2J_{6,6}$ = 16.2 Hz, C<u>H</u>COOH), 2.42 (dd, 1 H, $^3J_{5,6}$ = 8.3 Hz, $^2J_{6,6}$ = 16.2 Hz, C<u>H</u>COOH), 2.26 (m, 1 H, C<u>H</u>COOH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 193.05, 183.08 (2×CH_2<u>C</u>OOH), 139.62, 139.49, 139.40, 139.38, 139.22, 139.18, 139.00, 138.78, 138.75, 138.50, 138.49, 138.26, 138.13, 138.03, 138.02, 137.98(2C), 137.95, 137.90 (19×C-$Ar^{quat.}$), 128.37-126.40 (95×C-$Ar^{tert.}$), 100.07, 99.37(2C), 98.98, 98.05, 98.03, 97.15 (7×C-1), 81.68-78.50 (7×C-2, 7×C-3, 7×C-4), 76.36, 76.23, 75.83, 75.17, 74.92, 74.49, 74.01, 73.50, 73.40, 73.32(3C), 73.23, 73.05, 72.91(2C), 72.62, 72.25, 72.18 (19×CH_2Ph), 72.05, 71.75, 71.44, 71.39, 71.36 (5×C-5), 69.72, 69.44, 69.30, 68.74(3C, 6×C-6) 68.67, 67.25 (2×C-5), 38.24, 37.81 (2×CH_2COOH). HRMS (ESP): Calcd. for $C_{177}H_{183}NaO_{37}$ $[M+Na]^{1+}$: 2924.2414; Found: 2924.2349

6A,6D-dideoxy-β-cyclodextrin-6A,6D-dimethylenecarboxylic Acid (5β):

To a solution of cyclodextrin **9β** (2.4 g, 0.827 mmol, 1 equiv.) in a mixture of MeOH/EtOAc (50 mL) were added Pd/C (2.4 g) and TFA (cat.). The reaction mixture was stirred at room temperature for 2 days under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave cyclodextrin **5β** (0.530 g, 60%) as a white foam. $[\alpha]_D^{25}$= +119.0 (*c* 0.4, H_2O). ^{1}H NMR (D_2O, 500 MHz): δ 5.12-5.03 (m, 7 H, 7×H-1), 4.20 (br, 2 H, 2×H-5), 3.94-3.43 (m, 36 H, 7×H-2, 7×H-3, 7×H-4, 5×H-5, 10×H-6), 3.20-3.10 (m, 2 H, 2×C<u>H</u>COOH), 2.65-2.51 (m, 2 H, 2×C<u>H</u>COOH). ^{13}C NMR (D_2O, 125 MHz): δ 177.64, 177.62 (2×CH_2<u>C</u>OOH), 102.09 (3C), 102.06(2C), 101.48, 101.41 (7×C-1), 84.99, 84.89 (2×C-4), 81.43, 81.41, 81.34, 81.29, 80.83, 80.80, 80.76 (7×C-2), 73.18(2C), 73.06, 73.02, 72.94, 72.92, 72.88, 72.86, 72.07, 72.03, 71.97, 71.95, 71.89, 71.81(2C), 71.80(2C) (7×C-3, 5×C-4, 5×C-5), 69.03, 68.95 (2×C-5), 60.15(2C), 59.79, 59.76, 59.60 (5×C-6), 36.70, 36.67 (2×<u>C</u>H_2COOH). HRMS (ESP): Calcd. For $C_{44}H_{70}O_{37}Na$ $[M+Na]^{1+}$: 1213.3493; Found: 1213.3512

6A,6D-dideoxy-hexadeca-*O*-benzyl-β-cyclodextrin-6A,6D-di(ethylidenecarboxylic acid benzylester) (11β):

To a solution of dialdehydo-β-cyclodextrin **10β**^{ref} (2.0 g, 0.703 mmol, 1 equiv.) in dichloromethane (30 mL) was added $Ph_3PCHCOOBn$ (1.5 g, 3.515 mmol, 5 equiv.). The reaction mixture was stirred at room temperature overnight, then water was added and the aqueous phase was extracted with EtOAc (3×30mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was purified by chromatography (PE/EtOAc: 4/1) afforded β-cyclodextrin **11β** (1.34 g, 61%) as a white foam. $[\alpha]_D^{25}$= +58.2 (c 1.0, $CHCl_3$), R_f = 0.68 (PE/EtOAc: 2/1), ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.28-6.87 (m, 107 H, 105×H-Ar, 2×CH=CHCOOBn), 5.96 (d, 1H, $^3J_{trans}$ = 15.6 Hz, CH=C<u>H</u>COOBn), 5.91 (d, 1 H, $^3J_{trans}$ = 15.5 Hz, C<u>H</u>=CHCOOBn), 5.39 (d, 1 H, $^3J_{1,2}$ = 3.7 Hz, H-1), 5.24 (d, 1 H, ^2J = 11.4 Hz, CHPh), 5.16 (d, 1 H, ^2J = 10.3 Hz, CHPh), 5.15 (d, 1 H, $^3J_{1,2}$ = 3.7 Hz, H-1), 5.13 (d, 1 H, $^3J_{1,2}$ = 3.6 Hz, H-1), 5.10-5.02 (m, 3 H, 3×CHPh), 4.98 (d, 1 H, $^3J_{1,2}$ = 3.3 Hz, H-1), 4.94 (d, 1 H, $^3J_{1,2}$ = 3.5 Hz, H-1), 4.90 (d, 1 H, ^2J = 13.3 Hz, CH=CHCOOC<u>H</u>Ph), 4.88 (d, 1 H, $^3J_{1,2}$ = 3.7 Hz, H-1), 4.85 (d, 1 H, $^3J_{1,2}$ = 3.1 Hz, H-1), 4.81 (d, 1 H, ^2J = 12.7 Hz, CH=CHCOOC<u>H</u>Ph), 4.80 (d, 1 H ^2J = 11.2 Hz, CHPh), 4.76 (d, 1 H, ^2J = 10.8 Hz, CHPh), 4.76-4.66 (m, 7 H, 2×CH=CHCOOC<u>H</u>Ph, 5×CHPh), 4.61 (d, 1 H, ^2J = 10.7 Hz, CHPh), 4.58-4.48 (m, 4 H, 2×CHPh, 2×H-5), 4.43-4.19 (m, 24 H, 22×CHPh, 2×H-5), 4.14 (d, 1 H, ^2J = 11.7 Hz, CHPh), 4.01-3.76 (m, 20 H, 7×H-3, 5×H-4, 3×H-5, 5×H-6), 3.48-3.24 (m, 14 H, 7×H-2, 2×H-4, 5×H-6). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.63, 165.55 (2×CH=CH<u>C</u>OOBn), 145.97, 145.92 (2×CH=CH<u>C</u>OOBn), 139.63, 139.59, 139.58, 139.50, 139.35, 139.32, 139.10, 138.84, 138.73, 138.71, 138.63, 138.50, 138.43, 138.30, 138.27, 138.25, 138.21, 138.11, 138.08, 136.06, 136.01 (21×C-$Ar^{quat.}$), 128.64-126.72 (105×C-$Ar^{tert.}$), 122.01, 121.44 (2×CH=<u>C</u>HCOOBn), 99.25, 98.98, 98.88, 98.63, 98.44, 98.15, 98.07 (7×C-1), 81.90, 81.23 (2×C-4), 81.12, 81.09, 80.97(2C), 80.88 (5×C-3), 80.26 (C-4), 80.02, 79.97 (2×C-3), 79.82, 79.31, 79.19 (3×C-4), 79.12, 79.07, 78.95(2C), 78.91, 78.52(2C) (7×C-2), 77.87 (C-4), 76.30, 76.19, 76.14, 75.93, 75.38, 75.13, 74.51, 73.73, 73.57, 73.46, 73.35(2C), 73.30, 73.22, 73.12, 72.88, 72.64, 72.49, 72.46 (19×CH_2Ph), 72.02, 71.81, 71.61, 71.46, 71.43, 69.96 (6×C-5), 69.44 (C-6), 69.41 (C-5), 69.38, 68.87(2C), 68.73 (4×C-6), 66.01, 65.83 (2×CH=CHCOO<u>C</u>H_2Ph).

6A,6D-dideoxy-β-cyclodextrin-6A,6D-diethylenecarboxylic acid (6β):

To a solution of cyclodextrin **11β** (2.28 g, 0.734 mmol, 1 equiv.) in a mixture of MeOH/EtOAc (50 mL) were added Pd/C (2.0 g) and TFA (cat.) The reaction mixture was stirred at room temperature for 2 days under hydrogen atmosphere. Filtration through Celite and evaporation of the solvent gave cyclodextrin **6β** (0.793 g, 89%) as a white foam. $[\alpha]_D^{25}$= +125.9 (*c* 0.6, H_2O). ^{1}H NMR (D_2O, 500 MHz): δ 5.05-4.95 (m, 7 H, 7×H-1), 3.92-3.66 (m, 24 H, 7×H-3, 7×H-5, 10×H-6), 3.60-3.49 (m, 12 H, 7×H-2, 5×H-4), 3.31-3.27 (m, 2 H, 2×H-4), 2.53-2.47 (m, 2 H, 2×CH_2C<u>H</u>COOH), 2.41-2.35 (m, 2 H, 2×CH_2C<u>H</u>COOH), 2.30-2.24 (m, 2 H, 2×C<u>H</u>CH_2COOH), 1.73-1.65 (m, 2 H, 2×C<u>H</u>CH_2COOH). ^{13}C NMR (D_2O, 125 MHz): δ 177.64, 177.62 (2×CH_2CH_2<u>C</u>OOH), 102.07(2C), 101.88, 101.84(2C), 101.34, 101.22 (7×C-1), 85.52(2C), 81.11, 81.00, 80.96, 80.63, 80.55 (7×C-4), 73.18(2C), 73.11(2C), 73.09, 72.88, 72.83 (7×C-3), 72.17, 72.14, 72.09(2C), 72.01, 71.96, 70.90 (7×C-2), 71.78(2C),

71.73, 71.55, 71.53, 70.34(2C) (7×C-5), 60.23, 60.07(2C), 59.98, 59.94 (5×C-6), 29.76, 29.75 (2×$CH_2\underline{C}H_2COOH$), 26.10(2C) (2×$\underline{C}H_2CH_2COOH$). HRMS (ESP): Calcd. For $C_{146}H_{74}O_{37}Na$ $[M+Na]^{1+}$: 1241.3806; Found: 1241.3830

Determination of pK_a values of dicarboxylate-cyclodextrins:

A solution of 5.0×10^{-5} M cyclodextrin in 50 mL 0.1 M KCl was prepared. Then 2 mL 0.04 M HCl was added. The solution was titrated by dropwise addition 0.04 M KOH. All solutions were prepared using boiling deionized water to avoid the errors caused by CO_2 in water. After each addition the total volume of added KOH and electrode potential (mV) were noted. The obtained data was plotted and fitted to give pK_a value of different dicarboxylate-cyclodextrins (Figure S1 and Table 1).

Determination of binding constant of different metals with cyclodextrins by means of potentiometric titration:

Different stock solution were prepared: A) $CuSO_4$ (6.2×10^{-5} M) in 0.1 M Na_2SO_4; B) $ZnSO_4$ (6.2×10^{-5} M) in 0.1 M Na_2SO_4 and C) cyclodextrins **4α**, **5α**, **6α**, **4β**, **5β**, or **6β** in concentrations 2×10^{-3} M or 1×10^{-2} M. The cell consisted of a 50-mL beaker, a saturated calomel electrode and a piece of well-polished metal sheet (copper or zinc, it was washed with diluted nitric acid to remove the oxide on the surface). 40 mL of solution A or B was taken for a measurement and stirred continuously by a magnetic stirrer. The potential was varied by dropwise addition of solution C. The obtained data were plotted and fitted with nonlinear regression to give the binding constant of the metal with cyclodextrin.

ITC measurement:

An isothermal calorimeter (ITC200, MicroCal Inc., USA) was used for simultaneously determining the binding constant and the inclusion enthalpy of the studied complexes at 298K. Degassed solutions were used in both cell (1.8 mL, 2 mM) and syringe (280 μL, 20 mM). For all protocols, experiments were implemented as follows. After the addition of an initial aliquot of 5 μL, 28 aliquots of 10 μL of the syringe solution were delivered (over 10 s for each injection). The time interval between two consecutive injections was 240 s and the agitation speed was 307 rpm for all experiments. The resulting heat flow was recorded as a function of time. In addition, the heat of dilution (for each partner) was eliminated by subtracting the raw signal obtained for the corresponding blank titrations (i.e., only one partner in cell or syringe, the other compartment being filled with buffer). The peak area following each addition was obtained by integration of the resulting signal and was expressed as the heat effect per injection. Binding constants and inclusion enthalpies were finally determined by nonlinear regression analysis of the binding isotherms.

Oxidation assay:

In a platereader 10 samples (0.25 mL each) of the appropriate substrate at different concentrations(2-20 mM) in water/CH_3CN (4/1) solution containing cyclodextrindicarboxylate (0.6 mM) and $Fe(ClO_4)_2$ (0.3 mM) or nothing (as control) was simultaneously started by addition of 50 mM H_2O_2. The reactions were followed at 37 °C using UV absorption at an appropriate wavelength[25] and typically monitored for 2 h. Velocities were determined as the slope of the progress curve of each reaction. The velocities of the uncatalyzed reactions were obtained directly from the control samples, those of the catalyzed reactions were calculated by subtracting the uncatalyzed rate from the total rate of the appropriate cyclodextrin-containing sample. The v_{cat} values were used to construct Hanes plots ([S]/v vs. [S]) to ensure that the reaction follows Michaelis–Menten kinetics. In that case K_m and v_{max} were determined using least-squares nonlinear regression fitting to the vmax vs. [S] curve. k_{cat} was calculated as v_{max}/[cyclodextrin]. k_{uncat} was determined as the slope from a plot of v_{uncat} vs. [S].

Inhibition Assay:

In a platereader 6-7 samples (0.25 mL each) of the appropriate substrate at different concentrations (5-18 mM) in water/CH_3CN (4/1) solution containing cyclodextrindicarboxylate (0.6 mM) and $Fe(ClO_4)_2$ (0.3 mM) or nothing (as control) reactions were simultaneously started by addition of H_2O_2 to a final concentration of 50 mM. The reactions were followed at 37 °C using UV absorption at 286 nm and typically monitored for 2 h. This was performed with and without cyclopentanol (5 mM). From the obtained data, K_m (without cyclopentanol) and K'_m (with cyclopentanol) could be determined by the Hanes plot, then the K_i value could be calculated (using $K_i = [I]/(K'_m/K_m-1)$).

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Keywords: artificial enzyme • oxidase • ironenzyme • cyclodextrin • enzyme kinetics

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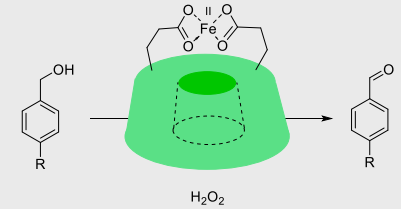

*Bo Wang and Mikael Bols**

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