Supporting information

for

Substrate control through per-*O*-methylation of cyclodextrin acids.

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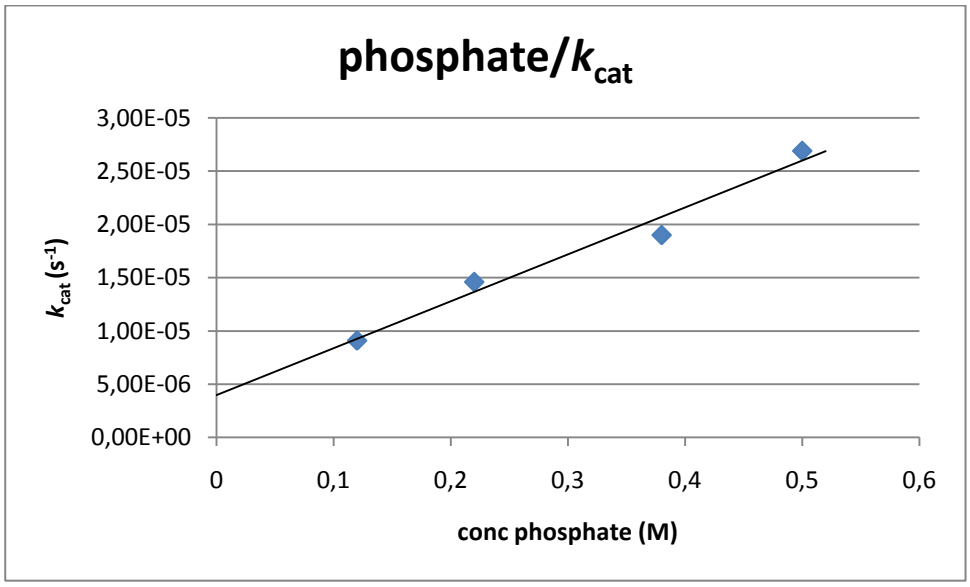
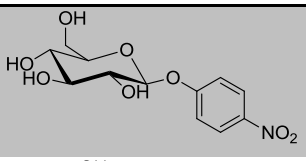
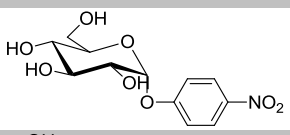
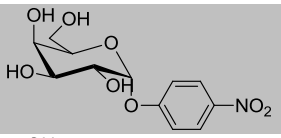
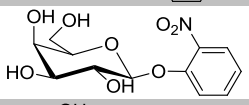
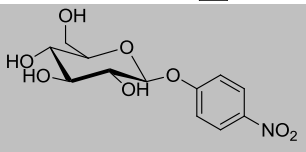
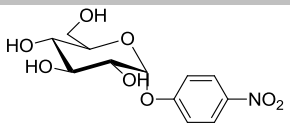
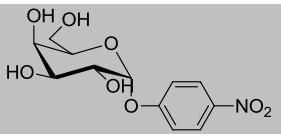
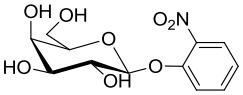
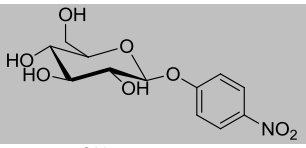
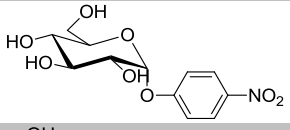
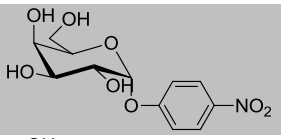
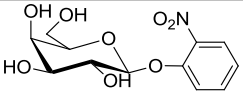
Contents:

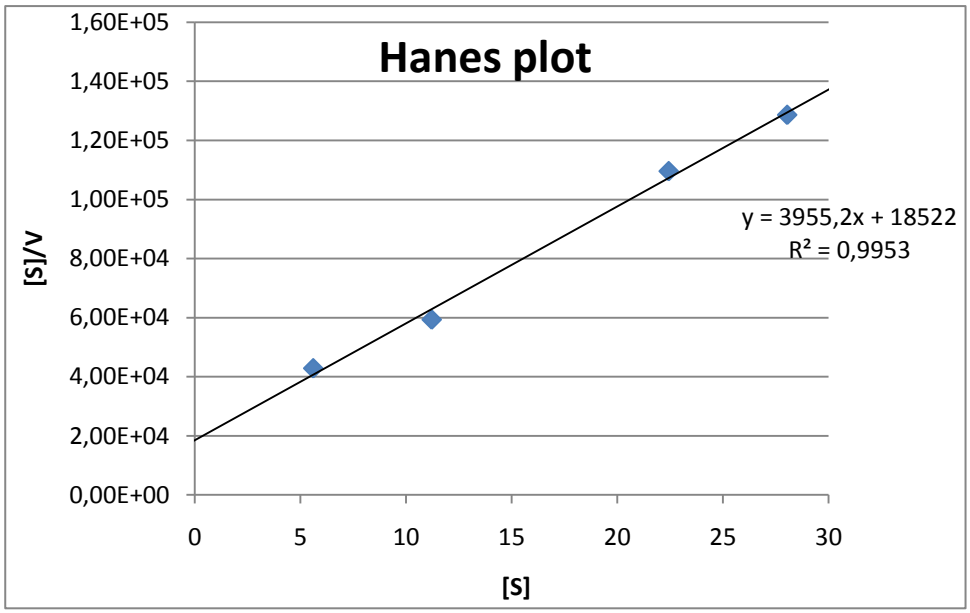
1. Figure 1S.



2. **Table 1S.** 500 mM Phosphate buffer, pH 8, 59°C

Catalyst	Substrate	k_{cat} (s^{-1})	K_M	$k_{cat/uncat}$
3 (c = 1 mM)		151 ± 1.83 x 10^{-9}	-	14 ± 9
		No catalysis	-	-
		No catalysis	-	-
		No catalysis	-	-
4		26.9 ± 1.28 x 10^{-6}	4.68 ± 0.95	974.9 ± 84.51
		21.7 ± 1.16 x 10^{-6}	4.88 ± 0.77	538.3 ± 29.14
		289 ± 6.99 x 10^{-7}	3.02 ± 0.47	763.7 ± 22.02
		55.5 ± 4.29 x 10^{-7}	0.59 ± 1.44	56.45 ± 5.34
5		173 ± 8.72 x 10^{-7}	5.53 ± 1.24	517.0 ± 50.5
		27.3 ± 2.10 x 10^{-6}	6.37 ± 1.39	646.0 ± 52.31
		131 ± 2.96 x 10^{-7}	1.97 ± 0.58	325.6 ± 38.4
		30.6 ± 5.49 x 10^{-7}	-	18.7 ± 4.11

3. Hanes plot for hydrolysis



Hanes plot for the hydrolysis of 4-nitrophenyl-β-D-glucoside, with **4** in 0.5 M Phosphate buffer, pH 8, 59°C

4. Enzyme assay:

The hydrolysis was carried out on a spectrophotometer Spetronic Genesys 5 by Milton Roy. The artificial glycosidase cyclodextrins were dissolved in phosphate buffer 0.5 M, pH 8. Substrates were dissolved in Phosphate buffer, 0.5M, pH 8. Each assay was performed on 8, 1 ml samples, with increasing substrate concentration, 5-25 mM. The enzyme concentration in each assay was constant 0.17 mM. As control, phosphate buffer 0.5 M, pH 8, was added instead of enzyme. The hydrolysis was monitored for 5 h, at 59 °C at 400 nm.

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 a Hanes-plot ([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 V_{max} vs. [S] curve. k_{cat} was calculated as V_{max} /[enzyme]. k_{uncat} was determined as the slope from a plot of V_{uncat} vs. [S].

5. Experimental:

General procedure for oxidation of aldehydes to carboxylic acids.

The aldehyde (0.10 mmol) was dissolved in a mixture of t-BuOH (12 ml), THF (4 ml) and 2-methylbut-2-ene (4 ml). $NaClO_2$ (80%, 81 mg, 0.60 mmol, 6 eq.) and $NaH_2PO_4 \cdot H_2O$ (41 mg, 0.30 mmol, 3 eq.) were dissolved in water (4 ml) and added to the solution. The reaction mixture was stirred overnight, then quenched with HCl (1M, 25 ml) and extracted with EtOAc (4 × 25 ml). The organic extracts were dried with $MgSO_4$ and concentrated. The remaining oil was purified by flash chromatography H_2O:Isopropanol:EtOAc, 1:2:3 → 1:2:2.

Compound 3 Me-β-CD 6-acid

96 mg, clear solid, 65% yield.

$[\alpha]_D^{20}$= +140,74 (c = 1, $CDCl_3$). IR (KBr) *v*: 3436, 2978, 2928, 2834, 1716, 1653, 1562, 1457, 1162, 1113, 1109, 1040 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 5.26 (d, 1H, *J*=3.6 Hz, H-1), 5.11 (d, 1H, *J*= 3.8 Hz, H-1), 5.07 (d, 1H, *J*=3.3 Hz, H-1), 5.03 (d, 1H, *J*=3.5 Hz, H-1), 5.00 (d, 1H, *J*=3.0 Hz, H-1), 4.97 (d, 1H, *J*=3.8 Hz, H-1), 3.92-3.03 (m, 79H), 3.20-3.13 (m, 6H). ^{13}C-NMR ($CDCl_3$, 100MHz) δ_C: 171.1 (C=O), 100.0 (C-1), 99.9 (C-1),99.8 (C-1), 99.6 (C-1), 99.4 (C-1), 99.3(C-1), 82.4, 82.1, 81.9, 81.8, 81.6, 81.4, 81.3, 72.0, 71.8, 71.4, 71.2, 71.1, 71.1, 70.8, 70.6, 61.8, 61.7, 61.6, 61.5, 61.3, 61.1, 59.0, 58.9, 58.1, 57.9, 57.7. MALDI-TOF, m/z calcd. $C_{53}H_{92}NaO_{31}$: 1247.552 found: 1247.362

Compound 4 Me-β-CD 2^A -acetic acid

50 mg, clear solid, 50% yield.

IR (KBr) *v*: 3436, 2928, 2833, 1733, 1614, 1458, 1366, 1231, 1192, 1141, 1038 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ_H: 5.17-5.03 (m, 7H, H-1), 3.93-3.72(m, 15H), 3.68-3.42 (m, 59H), 3.40-3.29 (23m, H), 3.24-3.11 (m, 7H). ^{13}C-NMR ($CDCl_3$, 75MHz) δ_C: 173.1 (C=O), 99.7 (C-1), 99.6 (C-1), 99.4(C-1), 99.3 (C-1), 99.2(C-1), 99.1(C-1), 99.0(C-1), 82.5, 82.4, 82.1, 82.0, 81.0 80.9, 80.8, 80.7, 80.6, 71.9, 71.8, 71.7, 71.5, 71.2, 71.1, 71.1, 61.8, 61.7, 61.5, 61.4, 59.3, 59.1, 59.1, 59.0, 58.9, 58.8, 58.5, 58.5. MALDI-TOF, m/z calcd. $C_{64}H_{112}NaO_{37}$: 1495.678 found: 1495.332

Compound 5 Me-β-CD 2^A -propanoic acid

42 mg, clear solid, 68% yield.

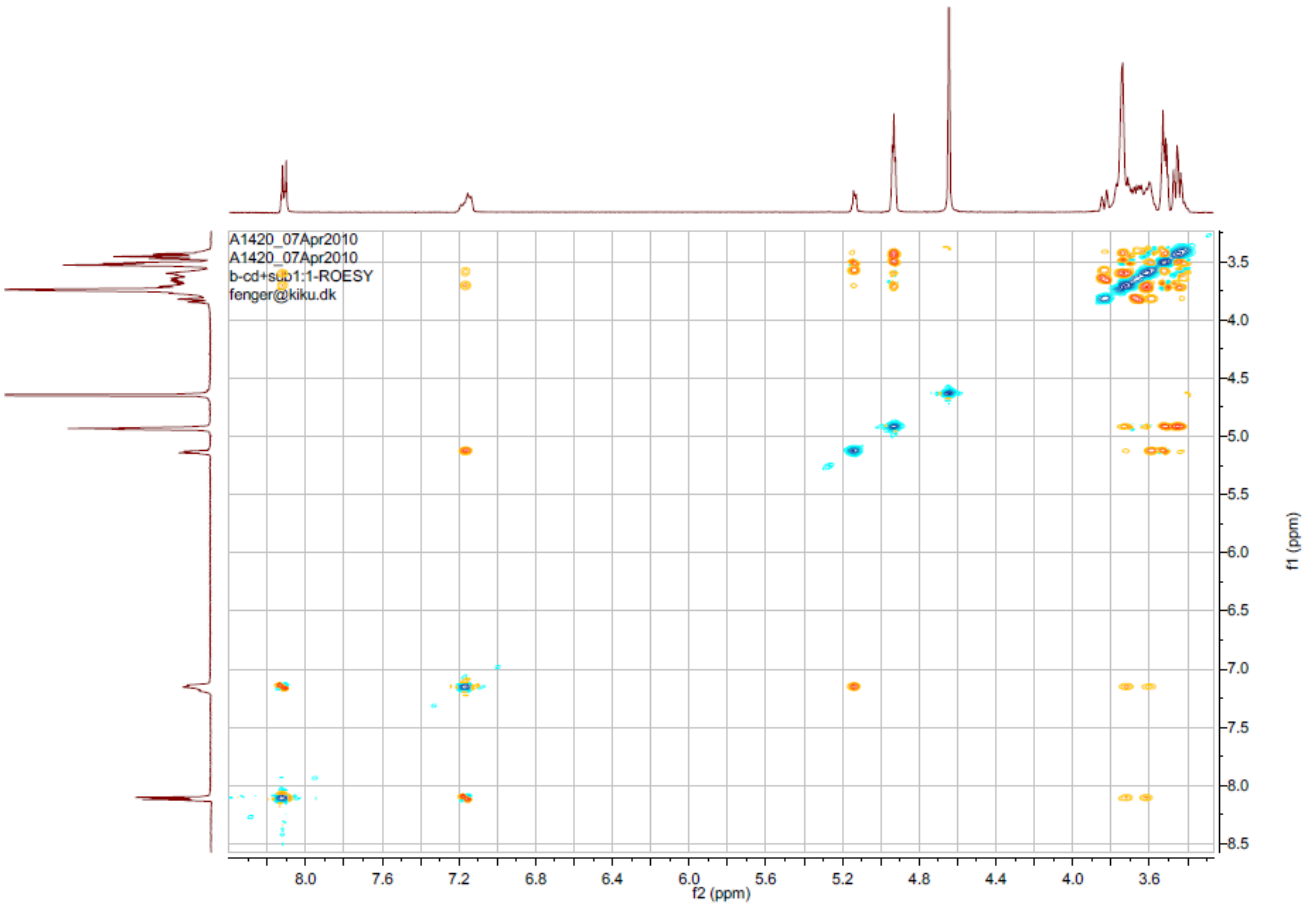
IR (KBr) *v*: 3437, 2979, 2929, 1730, 1651, 1558, 1457, 1194, 1161, 1143, 1109, 1067, 1039 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ_H: 5.19 (d, 1H, *J*=3.7 Hz, H-1), 5.17-5.14 (m, 2H, H-1), 5.13-5.09 (m, 3H, H-1), 5.04 (d, 1H, *J*=3.2 Hz, H-1), 3.94-3.71(m, 15H), 3.69-3.47 (m, 60H), 3.41-3.36 (m, 22H), 3.24-3.15 (m, 7H), (t, 2H, *J*=6,2 Hz, CH_2). ^{13}C-NMR ($CDCl_3$, 75MHz) δ_C: 175.1 (C=O), 99.4 (C-1), 99.3 (C-1), 99.2 (C-1), 99.2 (C-1), 99.1(C-1), 98.9(C-1), 98.8 (C-1), 82.4, 82.3, 82.2, 82.1, 82.0, 81.9, 81.8, 81.7, 80.8, 80.7, 80.6, 80.2, 80.0, 79.9, 79.8, 79.7, 71.7, 71.6, 71.4, 71.3, 71.2, 71.1, 71.0, 70.9, 67.4 (CH_2), 61.8, 61.6, 61.5, 61.2, 59.1, 59.0, 58.8, 58.7, 58.6, 58.5, 36.2 (CH_2). MALDI-TOF, m/z calcd. $C_{65}H_{114}NaO_{37}$: 1509.694 found: 1509.315

6. ROESY experiments and binding.

β-CD, TRIMEB and 4-nitrophenyl-β-D-glucopyranoside were dissolved in D_2O, evaporated, and redissolved in D2O. 1.7:1 complexes were made of both 4-nitrophenyl-β-D-glucopyranoside:β-CD and 4-nitrophenyl-β-D-glucopyranoside:TRIMEB.

The parameters used for the 2D ROESY experiment were the same as used in:

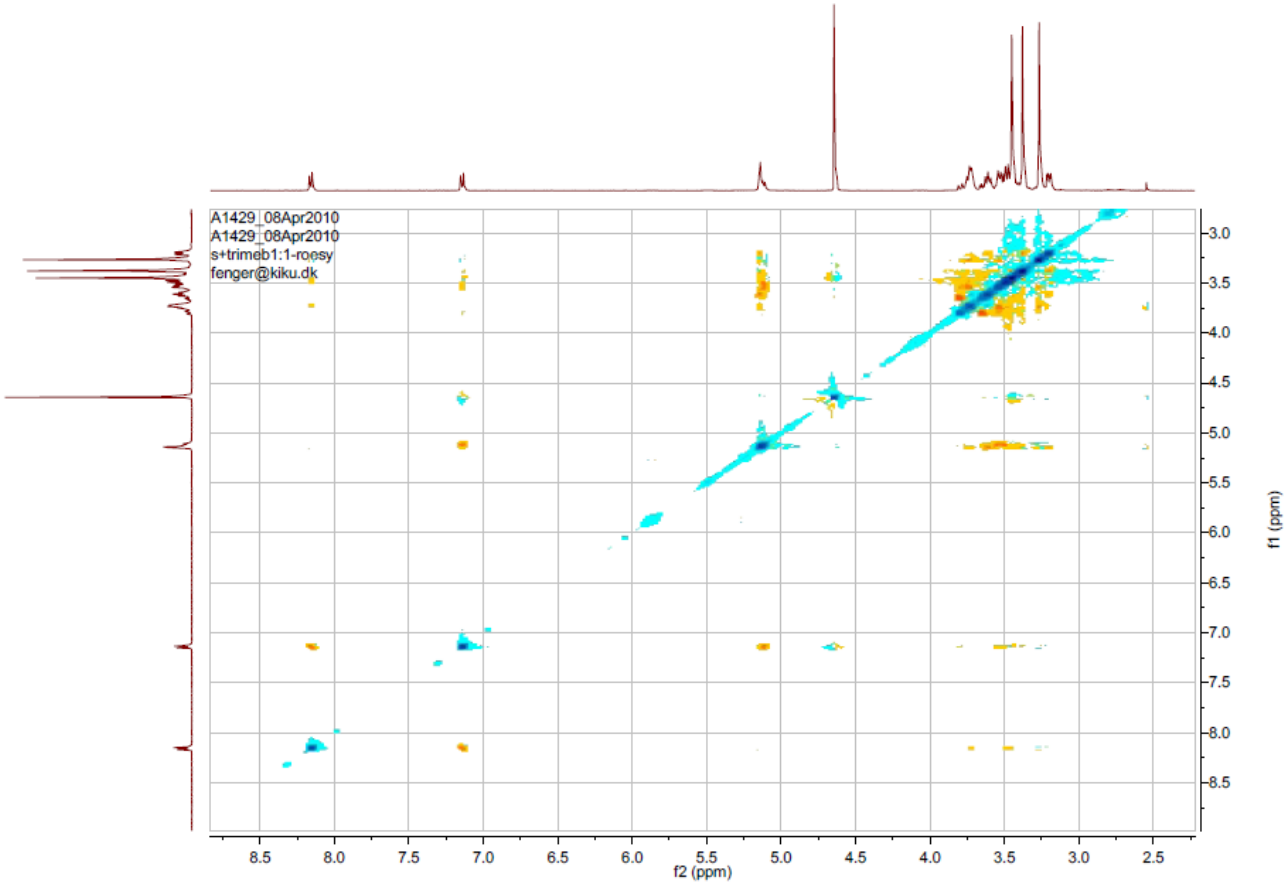
Salvatierra, D.; Jaime, C.; Virgili, A.; Sánchez-Ferrando, F. *J. Org. Chem.* **1996**, *61*, 9578-9581



2D ROESY of [Sub]/[β-CD]

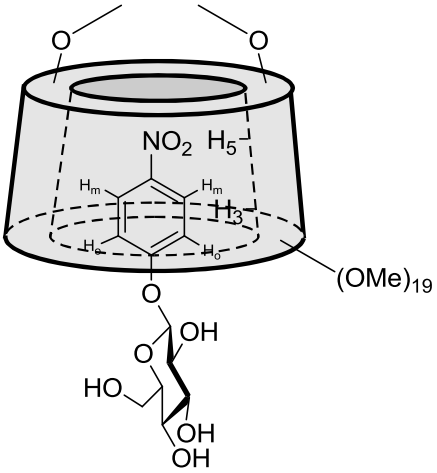
The 2D ROESY experiment gives a linear combination of the two possible binding modes.

For the complex [Sub]/[β-CD] H_m (to glycoside bond) can feel just as much H-3 as H-5, whereas H_o feels a little more H-3 than H-5. This can only be the case if both binding modes are present.



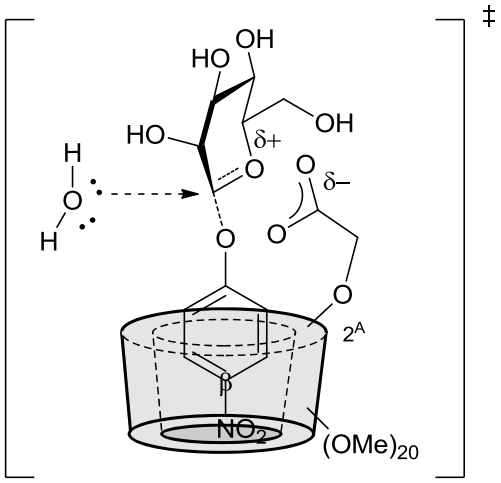
2D ROESY of [Sub]/[TRIMEB]

For the [Sub]/[TRIMEB] 2D ROESY experiments, H_m feels most H-3 an little H-5, were as H_o feels very little H-3, and no H-5, this can only be the case if just one binding mode is present, the one pictured below



Binding mode of 4-nitrophenol-β-D-glucopyranoside and TRIMEB

7. Possible transition state.



Proposed transition state for hydrolysis, the positive charge forming during substitution is stabilized by the carboxylate ion. This is also verified by the fact that no catalysis was observed at pH 3, in a 0.1 M citrate phosphate buffer, 59°C .