Tetrahedron Letters 53 (2012) 5023–5026

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Artificial enzymes based on cyclodextrin with phenol as the catalytic group

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ARTICLE INFO

Article history:
Received 13 May 2012
Revised 21 June 2012
Accepted 11 July 2012
Available online 17 July 2012

Keywords:
Chemzymes
Glycosidase
Cyclodextrin
Catalysis

ABSTRACT

β-Cyclodextrin containing one or two N-linked *ortho-N*-acetylaminophenols has been synthesized and tested for their properties as artificial glycosidases. Four different *para*-nitrophenyl glycosides were used as substrates and k_{cat}/k_{uncat} values of up to 381 were found for the di-functionalized products, whereas the mono derivative showed up to 47 times rate enhancement. The optimum pH for the catalysis was found to be approximately pH 8 and the influence of phosphate concentration was investigated.

Enzymes are outstanding in their ability to catalyze reactions with rate enhancements[1] of up to 10^{19}. Significant interest has been devoted to understanding and copying nature's machines, and 'artificial enzymes' have become an important research area.[2] One of the model systems for artificial enzymes is based on cyclodextrins, which are cyclic oligomers consisting of 1,4-α-linked d-glucopyranosides, which results in an apolar cavity and a polar exterior, making it water soluble. The development in this specific area has moved from the unmodified (or randomly functionalized) cyclodextrin which can be used as an additive to dissolve organic compounds, to specifically modified artificial enzymes, chemzymes.[3] With improvements in methods for selective protecting group manipulations it has been possible to design structures which resemble the active site in an enzyme and to synthesize better and more sophisticated artificial enzymes. One group of enzymes of particular interest to us is the glycosidases, which are responsible for cleavage of the anomeric bond in various substrates. It is important for the improvements in biomass degradation as well as medicinal chemistry to understand these processes and the scope of chemzymes in this area should be investigated.

Our group has been interested in cyclodextrin-based glycosidases.[4] One advantage of these chemzymes is their broader substrate recognition compared with very specific natural enzymes.[5] It has been demonstrated that not only carboxylates, but also various other functional groups are able to function as glycosidases.[6] Bis-functionalized chemzymes normally exceed mono-functionalized chemzymes in rate acceleration, which can be explained by a higher probability that the substrate is bound in a manner where the functional group is close to the reaction site. The importance of the catalytical group orientation[7] for catalysis and pH tolerance[8] has earlier been investigated, and the need for a system which can be fine-tuned to a certain pK_a would greatly improve the future development of chemzymes. In this communication, we present the first cyclodextrin based artificial enzyme based on amido phenols.

2-Acetaminophenol was chosen as the catalytic group due to its ready introduction in the cyclodextrin scaffold by reductive amination of the known aldehyde **1**[9] to give the N-linked amino phenol, which upon acetylation would give the target chemzymes **4** and **8** in a comparatively few steps (Fig. 1). The perbenzylated β-cyclodextrin aldehyde **1** was therefore treated with 2-aminophenol in 1,2-dichloroethane (DCE) and sodium triacetoxyborane as the reductant and acetic acid as the catalyst (Scheme 1). The reaction mixture was degassed and kept in the dark to avoid oxidation of the aminophenol. When TLC showed complete conversion of the starting material, pyridine and acetic anhydride (Ac_2O) were added immediately, which gave **2** in 72% yield (2 steps). Selective *O*-deacetylation was conducted using LiOH in aqueous THF followed by neutralization using HCl (1 M in water) to give amide **3**. This product was hydrogenolyzed using Pd/C/H_2(g) in 2-methoxyethanol and TFA as the acid catalyst to give the desired mono-functionalized cyclodextrin **4** in 90% yield from **3** (Scheme 1).

The bis-functionalized cyclodextrin **8** was synthesized in a similar way using the known[8] perbenzylated β-cyclodextrin dialdehyde **5** as the starting material (Scheme 2). Reductive amination followed by acetylation gave **6** in 34% yield. Selective cleavage of the phenol acetates gave **7** in 86% yield, which was subjected to Pd/C-mediated hydrogenolysis of the benzyl protective groups to give the bis-functionalized product **8** in 91% yield.[10]

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http://dx.doi.org/10.1016/j.tetlet.2012.07.050

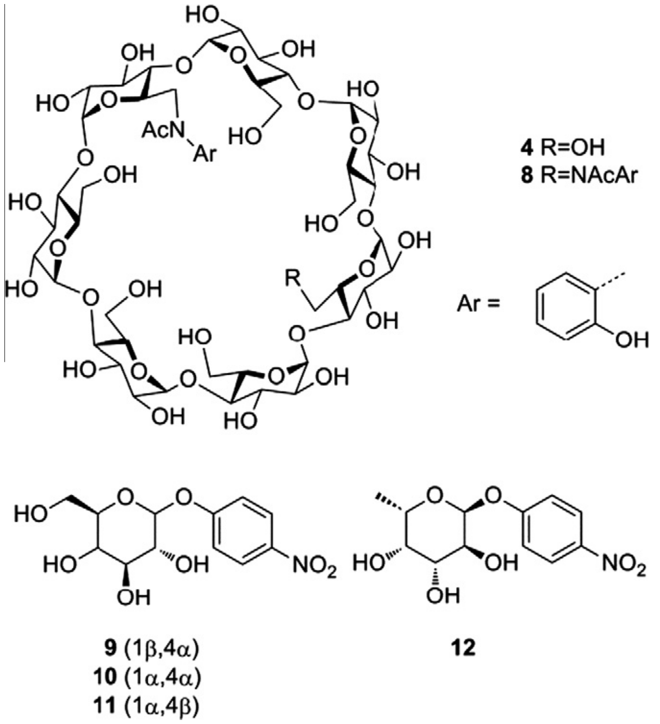
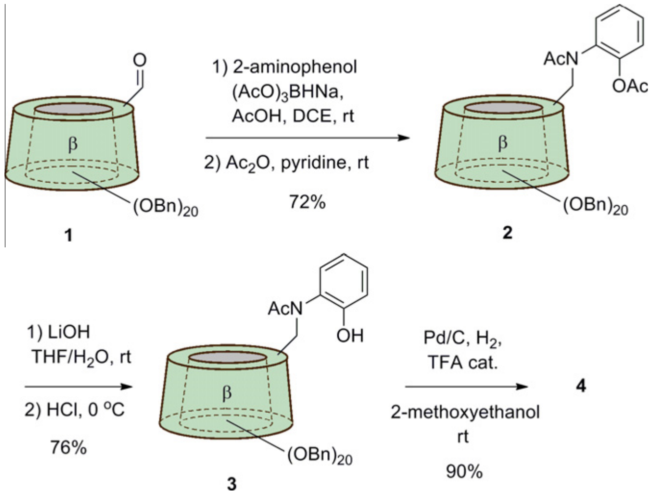


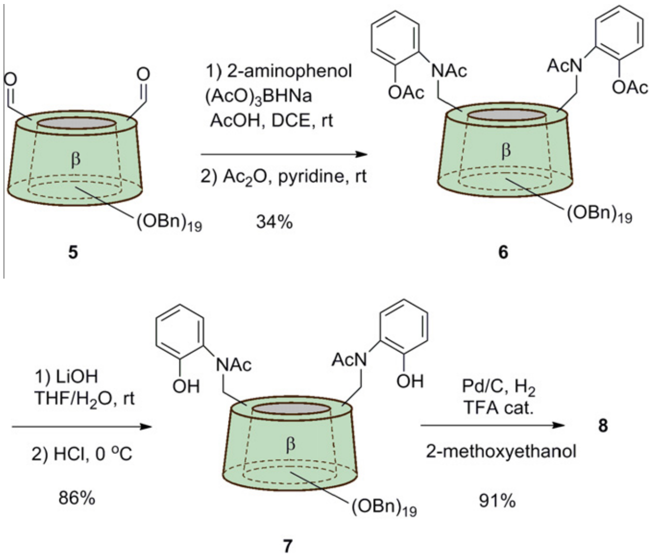
Figure 1. Artificial glycosidases **4** and **8** and substrates **9**–**12**.



Scheme 1. Synthesis of the artificial glycosidase **4**.

With the two model enzymes **4** and **8** in hand their catalytic properties were studied in the hydrolysis of 4-nitrophenyl glycosides **9**–**12** in phosphate buffer at 59 °C and pH 5.9–8.0. The experiments were based on monitoring the formation of 4-nitrophenol spectrophotometrically.[11]

Under these conditions, native β-cyclodextrin has no effect on the hydrolysis, but there is a spontaneous background reaction, which must be subtracted. Thus experiments were performed at different substrate concentrations (1–8 mM) with and without the presence of **4** or **8** (0.2–0.3 mM). After subtraction of the uncatalyzed (i.e., background) reactions from the catalyzed (i.e., cyclodextrin containing reactions), the net catalyzed rate was obtained for each substrate concentration. These data were used to create a Hanes plot (Fig. 2) from which K_M and V_{max} were determined.[12] The values shown in Table 1 are for many different conditions. We see that **4** and **8** displayed enzyme-like catalysis with substrates **9**–**12** with k_{cat} between 10^{-6} s^{-1} and 10^{-5} s^{-1}, and K_M from 0.5 to 6.6 mM. We also found that the rate acceleration (k_{cat}/k_{uncat}) performed by **4** or **8** varied from 29 to 381. Compared to previous artificial glycosidase catalysts this means that phenols **4** and **8** are less potent than cyanohydrins[8] have about the same effectiveness as the carboxylates[6a] and are more effective than aldehyde hydrates,[8] fluorinated alcohols,[6b] sulfates,[6a] and phosphates.[13]



Scheme 2. Synthesis of the artificial glycosidase **8**.

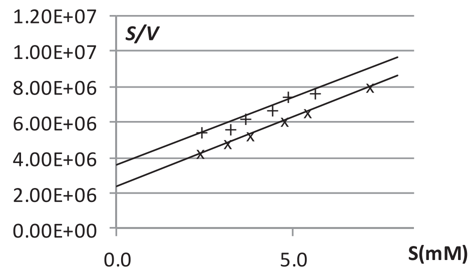


Figure 2. Hanes plot (S/V in mM/Unit vs substrate concentration in mM) for the hydrolysis of **10** catalyzed by **8** in the presence of no (×) or the competitive inhibitor *c*-pentanol (+). The K_i for *c*-pentanol is 14.3 mM.

Table 1
Kinetic constants for the conversion of substrates in phosphate buffer at 59 °C. The catalyst concentration was varied from 0.2 to 0.3 mM. *E* means enzyme and *S* means substrate

E	*S*	[PO$_4^-$] (mM)	pH	k_{cat} (10^7 s^{-1})	K_M(mM)	k_{cat}/k_{uncat}
8	9	500	5.9	28.9	0.59	29
8	9	500	6.8	25.8	2.86	90
8	9	500	7.1	30.7	2.53	45
8	9	500	7.4	88.0	6.57	268
8	9	50	8.0	18.0	2.25	96
8	9	100	8.0	23.2	3.08	76
8	9	250	8.0	46.5	3.86	202
8	9	500	8.0	83.4	5.91	381
8	9	500	8.9	47.7	4.30	112
8	10	500	8.0	64.2	3.04	358
8	11	500	8.0	78.5	2.04	290
8	12	500	8.0	69.6	3.86	121
4	9	500	7.4	10.3	4.58	31
4	9	500	8.0	10.4	1.62	47
4	10	500	8.0	10.1	4.42	56
4	11	500	8.0	12.6	0.93	47