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# Cyclodextrin-based Class I Aldolase Enzyme Mimics to Catalyze Crossed Aldol Condensations

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**Abstract** A variety of mono- and unsymmetrical bifunctional β-cyclodextrins have been developed as efficient mimics of class I aldolases, some of which show a large rate acceleration and substrate selectivity. 

***Keywords:*** β-cyclodextrin, enzyme mimics, aldol condensation

The aldol condensation is the most basic C-C bond forming reaction. In biological systems, two mechanistic classes of aldolase enzymes[1] are involved in catalyzing this condensation. Class I aldolases use an ε-amine group of lysine in the active site to form a Schiff base with one of the substrates. Proton abstraction from the Cα atom of the Schiff base by a histidine imidazole group leads to subsequent enamine formation. The enamine reacts with an aldehyde to form a new C-C bond. Class II aldolases utilize metal ions as Lewis acids to facilitate the enolate formation. Cyclodextrins with two imidazole groups on the primary hydroxyl side can enhance the enolate formation[2] of a simple bound ketone by bifunctional acid-base catalysis and accelerate the intramolecular aldol condensation[3,4] of bound ketoaldehyde and dialdehyde. Herein we report the assembly of β-cyclodextrin and various amino moieties as Schiff base forming aldolase mimics to catalyze crossed aldol condensations.

The catalysts involved in this research were synthesized by several methods. The 3-amino-β-CD **5** was prepared by reacting 2,3-alloepoxido-β-CD[5,6] with sodium azide and subsequent reduction with $Ph_3P$. Hydroxybenztriazole/dicyclohexylcarbodiimide mediated acylations of the amino-β-CDs with appropriate aminoacids gave the three CD-aminoacid conjugates **3**, **4** and **7**. Nucleophilic substitutions of 6-tosyl-β-CD[7] with cysteamine and ethylenediamine gave β-CD-6-Cys **1** and β-CD-6-En **2**. The alt-β-CD-3-En **8**, with a distorted hydrophobic cavity,[8] was obtained by reacting 2,3-mannoepoxido-β-CD[9] with ethylenediamine, while the non-distorted β-CD-3-En **6** was made from the ring opening of

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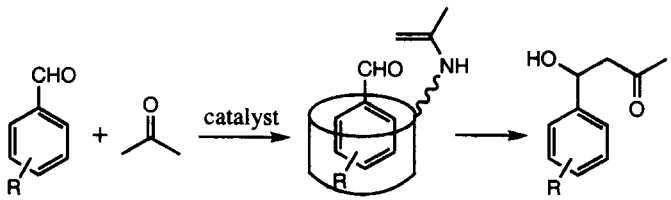
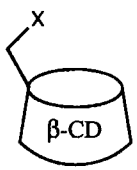
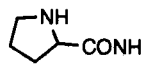
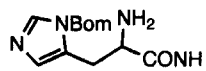
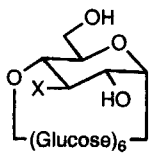


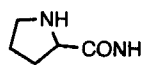

R = m-$NO_2$, H, p-Me, p-iPr, p-tBu

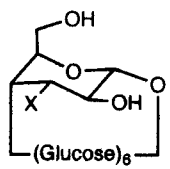



1. β-CD-6-Cys: X = $SCH_2CH_2NH_2$
2. β-CD-6-En: X = $NHCH_2CH_2NH_2$
3. β-CD-6-Proline X = D- 

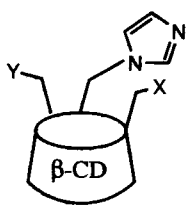
4. β-CD-6-Bom-his X = D- 

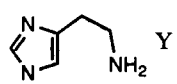




5. β-CD-3-$NH_2$: X = $NH_2$
6. β-CD-3-En: X = $NHCH_2CH_2NH_2$
7. β-CD-3-Proline X = D- 


8. Alt-β-CD-3-En X = $NHCH_2CH_2NH_2$




9. β-CD-6-His-Im: X = 

Y = OH

10. β-CD-6-Cys-Im: X = OH Y = $SCH_2CH_2NH_2$

Table 1 Kinetic Parameters for the Aldolisation of m-Nitro benzaldehyde and p-t-Butylbenzaldehyde with Acetone[a]

| Catalyst | m-nitrobenzaldehyde | | p-t-butylbenzaldehyde | |
|---|---|---|---|---|
| | $k_{cat}(x10^{-6}\ s^{-1})$ | $k_{cat}/k_{uncat}$ | $k_{cat}(x10^{-7}\ s^{-1})$ | $k_{cat}/k_{uncat}$ |
| none | 2.22 | 1.0 | 2.50 (0.286[b]) | 1.0 |
| **1** | 4.73 | 2.1 | 18.3 | 7.3 |
| **2** | 4.34 | 2.0 | 3.39 | 1.4 |
| **3** | 6.77 | 3.0 | 2.71 | 1.1 |
| **4** | 5.00 | 2.2 | 5.94 | 2.4 |
| **5** | 3.83 | 1.7 | | |
| **6** | 10.7 | 4.8 | 169 (43.9[b]) | 68 (153[b]) |
| **7** | 6.67 | 3.0 | 4.87 | 1.9 |
| **8** | 4.50 | 2.0 | 6.13 | 2.5 |
| **9** | 3.73 | 1.7 | 54.0 | 21.6 |
| **10** | 5.99 | 2.7 | 201 (77.8[b]) | 80 (272[b]) |

[a]Measured at 20 °C in phosphate buffer (40 mM, pH 7.25 for nitrobenzaldehyde and 36 mM, pH 8.00 for t-butylbenzaldehyde), 1.0 mM arylaldehyde, 20% v/v acetone, 0.20 mM catalyst. The k's are pseudo-first-order rate constants with and without catalyst at the specified concentration, not values from a Michaelis-Menten treatment.

[b]The data in parentheses are obtained at pH 7.00.

2,3-alloepoxido-β-CD.[5,6] The two bifunctional catalysts β-CD-6B-His-6A-Im **9** and β-CD-6A-Cys-6B-Im **10** were synthesized from 6A,6B-diiodo-β-CD[10] by successive substitutions with imidazole and the second nucleophiles.[11] All the CD catalysts were characterised by MS and NMR.

Kinetic studies of the aldol condensation were conducted in aqueous phosphate buffer with 20% (v/v) acetone, 1.0 mM aryl aldehyde, and 0.20 mM catalyst. Under these conditions, β-hydroxyketone formed as the sole detectable condensation product. The initial rates of aldol formation were measured by analytical HPLC on a RP-18 column.

Table 1 shows that the condensation of m-nitrobenzaldehyde with acetone was not affected strongly by the CD catalysts. In the case of *p-t*-butylbenzaldehyde, which binds in the CD cavity more strongly than *m*-nitrobenzaldehyde and with a different geometry, a larger rate acceleration was observed. Among the catalysts carrying the ethylenediamine unit the non-distorted secondary face β-CD-3-En **6** was very effective as was the primary face catalyst **2**, but the distortion of the binding cavity (catalyst **8**) caused a dramatic loss of rate. β-CD-3-Proline **7** is functionalized at the same position of a non-distorted hydrophobic cavity as that of β-CD-3-En **6** but with its secondary cyclic amino group **7** is a much poorer catalyst. Obviously both the hydrophobic binding and primary amino group are important for the catalysis. The pH-rate profile for the aldol condensation of p-t-butylbenzaldehyde with acetone catalyzed by β-CD-3-En **6** rises to a plateau above pH 8.2. Thus the active catalyst is the monoprotonated ethylenediamine unit, presumably forming a Schiff base with the ketone as in Class I aldolase enzymes.

Table 2. Relative Rate Constants[a] for the Aldol Condensation of Alkylbenzaldehydes with Acetone and with Cyclopentanone

| Substrate | Aldehyde, R = | H | p-Me | p-iPr | p-tBu | p-tBu |
|---|---|---|---|---|---|---|
| | Ketone | acetone | | | | cyclopentanone |
| Catalyst | **2** β-CD-6-En | 2.8 | 3.7 | 19 | 67 | 12 |
| | **6** β-CD-3-En | 2.5 | 3.4 | 37 | 68 | 3.0 |
| | **8** Alt-β-CD-3-En | 1.5 | 1.4 | 2.5 | 2.5 | 1.4 |
| | **10** β-CD-6-Cys-Im | 2.5 | 2.8 | 18 | 80 | 4.0 |

[a]The relative rate constant is $k_{cat}/k_{uncat}$. For the reaction of acetone, it is measured under the conditions described in Table 1. HPLC conditions: R = H, elution with 20~50% *aq.* MeCN in 8 min., detection at 210 nm; R = *p*-Me, elution with 40% aq. MeCN in 11 min., detection at 210 nm; R = iPr, elution with 50~80% *aq.* MeCN in 8 min., detection at 220 nm; R = tBu, elution with 50~70% *aq.* MeCN in 13 min., detection at 220 nm. The reaction of cyclopentanone is conducted at 27 °C in phosphate buffered (36 mM, pH 8.00) 20% *aq.* MeCN solution with 1.0 mM *t*-butylbenzaldehyde, 220 mM cyclopentanone and 0.20 mM catalyst, and an eluant of 65% *aq.* MeOH is applied.

For such a bimolecular condensation, significant catalysis requires that binding and catalytic groups cooperate in promoting the reaction. Thus we examined the aldol reaction of a series of *p*-alkylbenzaldehydes that are expected to have comparable reactivity in simple aldol condensation but that have different binding affinities for CD. As is shown in Table 2, the alt-β-CD-3-En **8** with its distorted cavity is a poor catalyst for all the substrates. β-CD-6-En **2** and β-CD-3-En **6** both have the non-distorted hydrophobic cavity and show similarly increasing catalysis of the reaction when the substrate is gradually made more hydrophobic, from benzaldehyde to *p-t*-butylbenzaldehyde.

The aldol condensation of *p-t*-butylbenzaldehyde with cyclopentanone is also catalyzed. The lower values of $k_{cat}/k_{uncat}$ (observed initial rates for formation of the product divided by initial aldehyde concentration) do not simply reflect the lower concentration of cyclopentanone vs. acetone, since these concentrations were also used to determine $k_{uncat}$. With cyclopentanone, the primary side catalyst **2** was the most effective.

To imitate the function of the active His-Im group in class I aldolases, we also developed two unsymmetrical bifunctional β-CDs **9** and **10**. A good synergistic effect of the two catalytic groups is evidenced in the aldol condensation of *p-t*-butylbenzaldehyde with acetone (Table 1 and Table 2). The additional imidazole group makes the β-CD-6-Cys-Im **10** over 10 times more efficient than β-CD-6-Cys **1** (Table 1) and among the most efficient of the catalysts studied. However, this catalyst does not accelerate greatly the condensation of cyclopentanone (Table 2), but is 3 times less effective than β-CD-6-En **2** when cyclopentanone is used as aldol donor.

Thus our studies show that we can imitate the function of the amino group in class I aldolases, and have selectivity for both the receptor and donor in a crossed aldol condensation. It remains to be seen whether such enzyme mimics, or related ones, have useful applications in organic synthesis.

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**References**

1. Hoecke, B. L.; Tsolas, O.; Lai, C. Y. *The Enzymes*; Boyer, P.D. (ed); Academic Press: New York, **1972**, 3rd ed, Vol. VII, Chap. 6.
2. Breslow, R.; Graff, A. *J. Am. Chem. Soc.* **1993**, *115*, 10988.
3. Desper, J. M.; Breslow, R. *J. Am. Chem. Soc.* **1994**, *116*, 12081.
4. Breslow, R.; Desper, J.; Huang, Y. *Tetrahedron Lett.* **1996**, *37*, 2541.
5. Fujita, K.; Tahara, T.; Imoto, T.; Koga, T. *J. Am. Chem. Soc.* **1986**, *108*, 2030.
6. Yuan, D-Q.; Fujita, K.; Ohta, K. *Chem. Commun.* **1996**, 821.
7. Zhong, N.; Byun, H.-S.; Bittman, R. *Tetrahedron Lett.* **1998**, *39*, 2919, report a recent improved synthesis of this compound.
8. Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390.
9. Ueno, A.; Breslow, R. *Tetrahedron Lett.* **1982**, *23*, 3451.
10. Breslow, R.; Canary, J. W.; Varney, M.; Waddell, S. T.; Yang, D. *J. Am. Chem. Soc.* **1990**, *112*, 5212.
11. Regiochemistry established by X-ray crystallography of an intermediate.