Artificial Enzymes: Synthesis of Imidazole Substituted at C(2) of β-Cyclodextrin as an Efficient Enzyme Model of Chymotrypsin†

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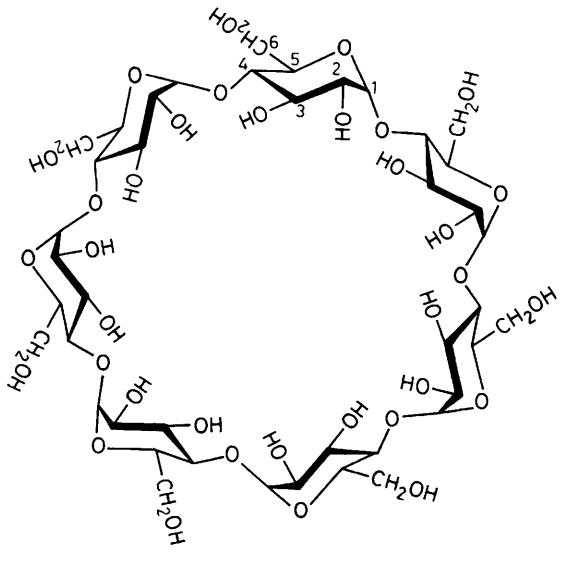
Imidazole has been attached at C(2) on the more open face of β-cyclodextrin to mimic the enzyme chymotrypsin; this chemical model is shown to be catalytically far superior to that with an imidazole on the primary side [C(6)] of cyclodextrin.

The design and synthesis of chemical models of enzymes is one of the most challenging and stimulating problems of organic chemistry. In this endeavour, cyclodextrins have emerged as important enzyme models in biomimetic chemistry due to their ability to provide hydrophobic recognition sites in aqueous solution. However, preparation of more refined and sophisticated enzyme models requires the introduction of efficient catalytic groups.[1–4]

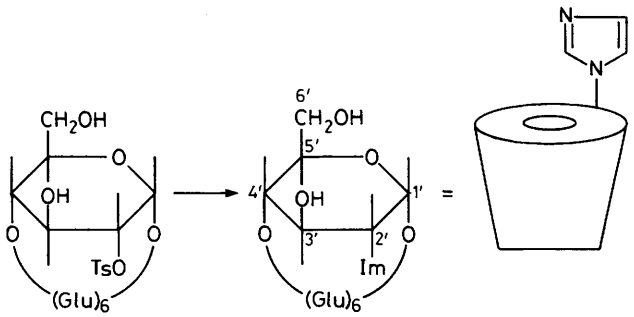
In such an attempt, Cramer and Mackensen[5] have introduced an imidazole group at C(6) of cyclodextrin (**1**) to mimic the enzymatic activity of chymotrypsin, since this enzyme has a marked optimum pH of 7 indicating the participation of a catalytic group with pK ~7 in the rate determining step. This has shown only a slight rate enhancement compared to β-cyclodextrin since the catalytic group is attached to a primary side [C(6)] on the essentially closed face of the toroidal cyclodextrin. Later efforts to attach an imidazole group to a secondary side at C(2) or C(3) which is on the more open face of cyclodextrin (**1**), in order to improve catalysis, have not been successful, especially due to difficulty in selectively making the required secondary tosylate. The availability of the authentic C(2) tosylate of β-cyclodextrin (**2**), *via* an ingeneous procedure developed by Ueno and Breslow involving a tosyl transfer reaction,[6] prompted us to attempt the reaction of (**2**) with imidazole to synthesise a catalytically efficient enzyme model of chymotrypsin. We report here the synthesis of the β-cyclodextrin imidazole compound (**3**) by the reaction of the C(2) tosylate (**2**) with imidazole. The β-cyclodextrin-2-tosylate (644.0 mg) (**2**) is reacted with imidazole (34.0 mg) in dimethylformamide (12 ml) using carbonate buffer (0.2 M, pH 9.9, 3.8 ml) for 20 h at 60 °C, then neutralised and concentrated to dryness. The product is purified by Sephadex chromatography in 12% yield.

Spectral and analytical data have shown the incorporation of one imidazole moiety, but the position of attachment of imidazole either at C(2) or C(3) of cyclodextrin needs to be ascertained, as the reaction of imidazole with β-cyclodextrin-2-tosylate (**2**) can also take place by opening of the 2,3-epoxide formed *in situ* from (**2**) in the presence of a base.[7]

The ^{1}H NMR spectrum (300 MHz) shows the expected signals from imidazole and cyclodextrin protons. It could be convincingly shown by ^{13}C NMR (75.47 MHz) alone that the substitution has taken place at the C(2) position on a glucose moiety of cyclodextrin. The ^{13}C NMR in [$^2H_6$]dimethyl sulphoxide (DMSO) (Figure 1) shows in the sugar region, apart from β-cyclodextrin carbons, some single shifted carbons, denoted as prime numbers in Table 1 (imidazole carbons



(**1**)



Glu = glucose
Ts = tosyl
Im = imidazole

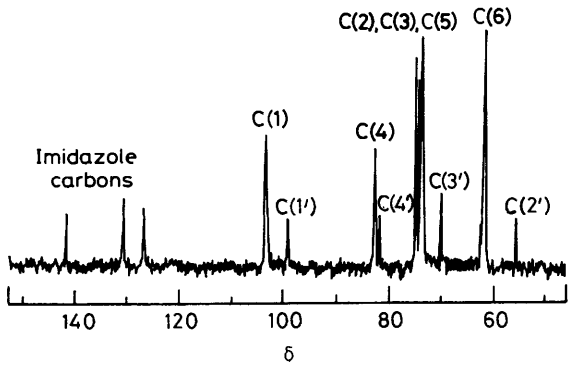


Figure 1. ^{13}C NMR spectrum of (**3**).

† IICT Communication no. 2362.

Table 1. ^{13}C NMR chemical shifts.

δ of (3) in the carbohydrate region	δ of β-CD[a]	Assignment
103.14	102.97	C(1)
99.20		C(1′)
82.47	82.59	C(4)
81.52		C(4′)
74.61	74.07	C(2)
57.38		C(2′)
73.75	73.45	C(3)
69.95		C(3′)
73.39	73.07	C(5)
61.48	60.95	C(6)

[a] Data cited from I. Tabushi and T. Nabeshima, *J. Org. Chem.*, 1985, **50**, 2638. CD = cyclodextrin.

Table 2. Pseudo-first-order rate constants[a] for the hydrolysis of *p*-nitrophenylacetate.

No.	Catalyst[b]	$10^4k(s^{-1})$[c]
1	β-CD	0.62 ± 0.01
2	β-CD with imidazole on C(6) primary side[d]	12.20 ± 0.2
3	β-CD with an imidazole on C(2) secondary side (**3**)	859.0 ± 2.5

[a] Rate of release of *p*-nitrophenol determined spectrometrically at 400 nm in Tris-HCl buffer (0.02 M, pH 7.5), catalyst (0.30×10^{-2} M), *p*-nitrophenylacetate (0.30×10^{-4} M) with 0.50% (v/v) added acetonitrile at 25 °C. [b] CD = cyclodextrin. [c] Average of three runs. [d] Prepared according to ref. 5.

are seen at δ 141.42, 130.61, and 126.73; not shown in Table 1).

The position of substitution at C(2) or C(3) on one of the glucose rings of β-cyclodextrin has been assigned by comparing the chemical shifts observed on the substituted glucose moiety with that of β-cyclodextrin, since it is known from the model nitrogen substituted sugar compounds that the carbon carrying the nitrogen substituent (α-carbon) shows a large upfield shift, whereas a small upfield shift is observed for the β-carbon and a still smaller upfield shift for the γ-carbon as compared to the parent sugar.[8—11] In compound (**3**), the C(1′) resonance (δ 99.20) is shifted upfield, the magnitude of shift (3.94 p.p.m.) corresponding to a β-carbon,[11] which shows that the imidazole is located at C(2′) (α-carbon), the latter having a large upfield shift of 17.23 p.p.m. A significant upfield shift (3.80 p.p.m.) is observed for C(3′), another β-carbon, and a very small upfield shift (0.95 p.p.m.) for C(4′) (γ-carbon). In contrast, if the substitution were at the C(3′) carbon of the glucose moiety of β-cyclodextrin, the upfield shift of C(1′) (as a γ-carbon) will be negligible, whereas a significant shift will be observed for C(4′), since it then becomes a β-carbon. It is noteworthy that ^{13}C NMR shows significant shifts only for carbons of the imidazole substituted glucose moiety, whereas the carbons of other glucose moieties remain unaffected. This observation is in accordance with the report by Breslow for β-cyclodextrin-2-tosylate.[6]

Preliminary kinetic results for the hydrolysis of *p*-nitrophenylacetate (Table 2) have shown that the chemical model (**3**) of the enzyme chymotrypsin with an imidazole on the secondary side of β-cyclodextrin has a rate constant 70 times that of β-cyclodextrin with an imidazole on the primary [C(6)] side. Thus, this is the first successful synthesis of β-cyclodextrin with an imidazole on the secondary side, which has been shown to be an improved enzyme model for mimicking chymotrypsin catalysis due to the correct stereochemical disposition of imidazole and hydroxy groups of cyclodextrin as seen in chymotrypsin. This simple model with various substituted imidazoles is being investigated further for its catalytic activity.

Received, 6th March 1989; Com. 9/00979E

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