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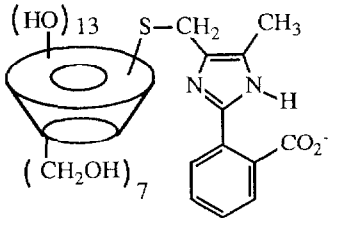
A Novel Synthesis of Substituted Imidazoles, and a Reexamination of a Purported Chymotrypsin Model

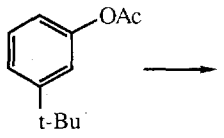
Ronald Breslow* and Shin Chung
Department of Chemistry, Columbia University
New York, New York 10027 U.S.A.

Abstract: Rearrangement of an N-hydroxyimidazole to a side-chain functionalized derivative permits easy synthesis of a purported chymotrypsin mimic that contains a "catalytic triad" as part of a cyclodextrin molecule. Contrary to the previous claims, the added functional groups do not play a role in the cyclodextrin reaction with a typical substrate, but in fact slow the reaction because of steric hindrance.

The enzyme Chymotrypsin uses an imidazole group, a serine hydroxyl, and an aspartate carboxylate to perform the hydrolysis of peptides and related substrates. This catalytic triad operates in other enzymes as well. Thus it has been of interest to incorporate such catalytic groups into enzyme mimics such as those based on the cyclodextrins, which can bind hydrophobic substrates. We synthesized the 2,3-epoxide of β-cyclodextrin (cycloheptaamylose) in order to attach such groups,[1] but found that opening the epoxide with imidazole derivatives such as 4(5)-mercaptomethylimidazole produced catalysts with no



1



2

t-Butylphenoxide

plus

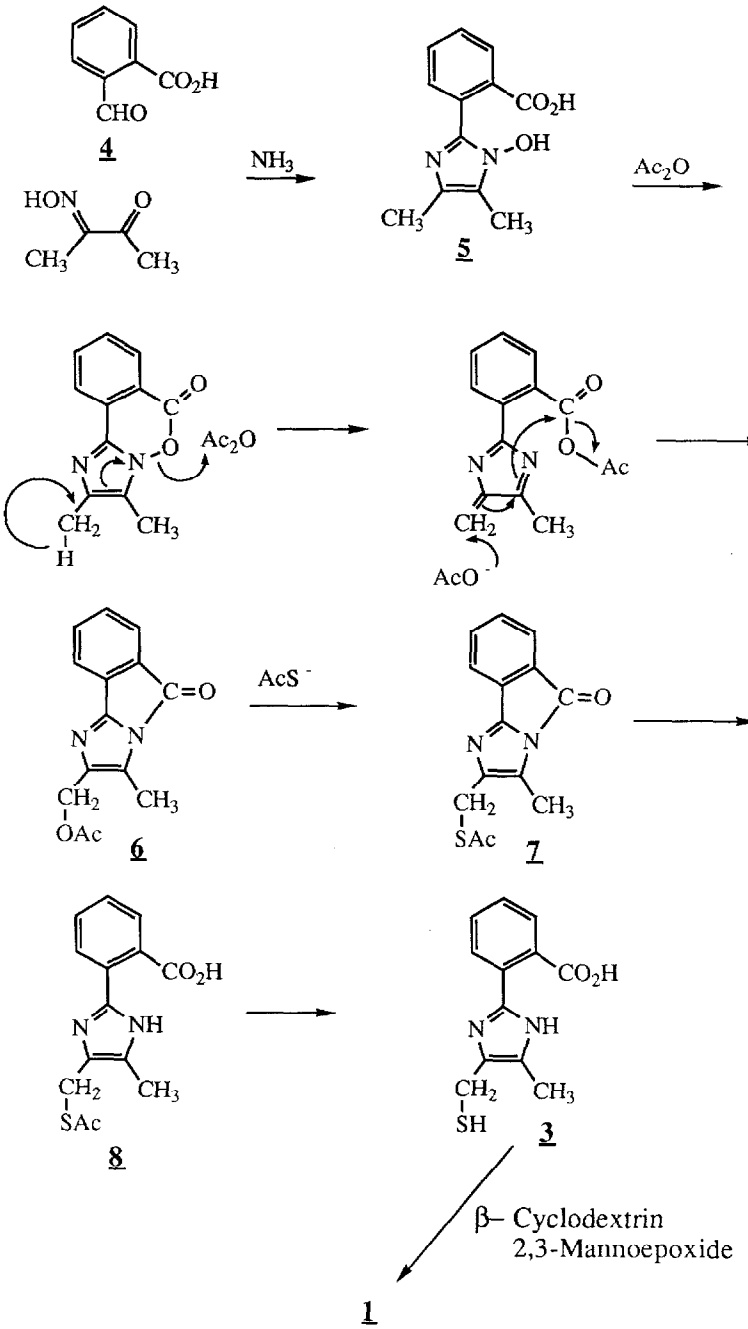
Acetylated Cyclodextrin

greater reactivity toward typical substrates than β-cyclodextrin alone.[2] Thus we were surprised to see the claim[3-6] that compound **1** was a good chymotrypsin mimic.

The data reported did not seem to support this claim, since the reported[3-6] rate of reaction of **1** with m-t-butylphenyl acetate (**2**) is actually four times less than the known rate[7] for β-cyclodextrin alone. Furthermore, the reaction was performed at pH 10.7. This high pH is needed for the optimum rate of reaction of cyclodextrin with **2**, but it should not be needed with **1** for a proposed pathway in which the imidazole acts as a general base to deprotonate a cyclodextrin hydroxyl group. It was even reported[3-6] that the reaction with **1** was first order in hydroxide ion, which rules out participation of the imidazole. However, it seems that the purported catalytic activity of **1** is being taken seriously, since it has been discussed in a number of reviews. Thus we have independently prepared **1**, and evaluated it. With substrate **2** it behaves as a significantly less reactive cyclodextrin, with no evidence for any contribution from the "catalytic triad".

The reported synthesis involves the preparation of imidazole derivative **3**, and its reaction with our epoxide; no details of the preparation of **3** or physical data on intermediates or products were published.[8] We have devised a particularly convenient synthesis of **3** that could be of general use in imidazole chemistry. Reaction of phthaldehydic acid (**4**) with butanedione monooxime and ammonia in the standard way[9] afforded the N-hydroxyimidazole derivative **5** in quantitative yield. The compound showed the expected ^{1}H NMR signals, with aromatic protons and methyl signals at 2.14 and 2.22 ppm. On heating 10.4 g of **5** in 100 ml 2/1 acetic acid/acetic anhydride with 10 g sodium acetate and 10 g $NiCl_2 \cdot H_2O$ at reflux for 2 hrs it was converted to 6.4 g of the acetoxymethyl compound **6** (the yields were generally 60-80%). This showed the expected ^{1}H NMR aromatic signals, a methyl at 2.11, an acetyl at 2.47, and a methylene at 5.00 ppm; in the ^{13}C NMR there were signals at δ 8.68, 25.72, 30.36, 161.91, and 195.20 in addition to the aromatic signals from 122 to 152 ppm. The reaction[10] is presumably related to the well known rearrangement of 2-methylpyridine N-oxides to acetoxymethyl derivatives. We envisioned the mechanism[11] of Scheme 1 when we designed this reaction.

Reaction of 330 mg of **6** with 1 g potassium thioacetate in 30 ml DMSO under N_2 at 70° for 30 min afforded **7** in 70% yield as yellow crystals, mp 153-155°, MS M + 1 = 273, ^{1}H NMR ($CDCl_3$) δ CH_2 4.02, CH_3 2.46, acetyl 2.36. With acetone/aqueous HCl this was hydrolyzed in quantitative yield to the colorless thioester acid **8**. This was directly deacetylated with NH_4HCO_3, and the thiol reacted with pure β-cyclodextrin 2,3-mannoepoxide[1] to form **1**. The compound was purified by repeated anion exchange chromatography (DEAE Sephacel). The structure is clear from the MS (FAB) of 1365 (M + 1) and the expected ^{1}H NMR signals (D_2O) at 2.29 (methyl), 7.5-8.0 (aromatic), and 3.4-5.2 (cyclodextrin). Particularly diagnostic is the signal at 2.95 ppm (dd, J = 3.6 and 10.9) characteristic of diaxial opening of the epoxide at C-3 by a thiol.[1]


CO2H
CHO
4
HON
O
CH3
CH3
NH3
CO2H
N
N
OH
CH3
CH3
5
Ac2O
O
C
O
N
N
O
Ac2O
CH2
CH3
H
O
C
O
Ac
N
N
CH2
CH3
AcO
C=O
N
N
CH2
CH3
OAc
6
AcS
C=O
N
N
CH2
CH3
SAc
7
CO2H
N
NH
CH2
CH3
SAc
8
CO2H
N
NH
CH2
CH3
SH
3
β– Cyclodextrin
2,3-Mannoepoxide
1

Scheme 1

The rate of deacetylation of m-t-butylphenyl acetate (4.74×10^{-4} M) at $25.0 \pm 0.1°$ with compound **1** and with simple β-cyclodextrin in water at various concentrations (1.35 to 8.11×10^{-5} M) was followed at 290 nm. The substrate is in excess, so the rates showed good first order dependence on catalyst concentration. With **1** the pseudo-first-order rate constant at pH 10.57 was 2.8 times as fast as that at pH 10.12, showing that the reaction is first order in hydroxide ion. With β-cyclodextrin at pH 10.12 the rate constant was 4.4 times as large as with **1**. Thus the extra functional groups of **1** actually slow the reaction, presumably by partially blocking access to the cyclodextrin cavity, and certainly play no useful catalytic role.

It seems clear from this that the simple incorporation of a catalytic triad into cyclodextrin is itself not enough to produce a good chymotrypsin mimic. Presumably with careful attention to the problems of geometry and flexibility related catalysts will indeed be effective, both with simple ester substrates and with the peptides that are the true targets of such work.[12]

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8. After this work was substantially completed we received some experimental details from Prof. V. T. D'Souza. We thank him for his assistance in this unfortunate problem.
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10. We have also performed the same process with a phenyl substituent, or an o-bromophenyl group, in place of the o-carboxyphenyl group of **5**. The yields are not quite as good in these cases, in which an external acetyl group substitutes for the function assigned to the internal carboxyl of **5**.
11. In this mechanism there may well be an additional acetyl group on the imidazole, forming an N-acetylimidazolium ion intermediate. Since we have no direct evidence for it, it is omitted from Scheme 1.

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