Bioorganic & Medicinal Chemistry Letters, Vol.2, No.12, pp. 1635-1638, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

HYDROGEN BONDING EFFECTS ON THE REACTIVITY OF A PREASSOCIATING α-NUCLEOPHILE. THE SECONDARY-SIDE βCD HYDROXYLAMINE

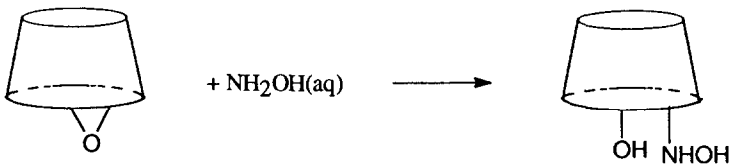
Mark A. Mortellaro and Anthony W. Czarnik*
Department of Chemistry
The Ohio State University
Columbus, Ohio 43210

(*Received* 6 *August* 1992)

Summary: The secondary-side hydroxylamine derivative of β-cyclodextrin demonstrates base-catalyzed transesterification from pH 6.5-9.5, while the primary-side derivative does not.

We have reported previously that the primary-side β-cyclodextrinyl hydroxylamine (1^{o}-βCDNHOH) binds and transacylates both activated and less activated phenyl esters.[1] However, the primary locus of phenyl ester binding is at the 2^{o}-side.[2] Furthermore, hydrogen-bonding at the βCD 2^{o}-side is anticipated to modulate the reactivity of an appended α-nucleophile just as it does the 2^{o}-OH of βCD itself.[3] We now report the synthesis and characterization of the 2^{o}-side β-cyclodextrinyl hydroxylamine (2^{o}-βCDNHOH), whose reactivity is influenced by intramolecular hydrogen bonding.

2^{o}-βCDOTs (a mixture of mono- and ditosylates) was prepared from β-cyclodextrin (βCD) according to the procedure of D'Souza[4] and was converted to the βCD-manno-2,3-epoxide (**1**) by stirring in aqueous ammonium bicarbonate solution.[5] Passage of **1** through a Dowex mixed bed ion exchange column removed unwanted salts.[6] A solution of **1** in a 50% aqueous solution of hydroxylamine[7] was stirred overnight under argon at ambient temperature. Two precipitations from ethanol removed free hydroxylamine and gave **2** as a colorless solid in 62% yield. TLC[8] showed spots at R_f 0.28 for the monohydroxylamino cyclodextrin and at R_f 0.22 for one or more (presumed) dihydroxylamino



1 **2**

cyclodextrins. Elemental analysis of the product sample likewise established a ratio of 1.4 nitrogens per CD unit, corresponding to a mixture of 60% mono- and 40% di-hydroxylamino cyclodextrin.[9] ^{1}H and ^{13}C NMR spectra, while complicated, are likewise consistent with the structure asignment. Diaxial ring opening of the epoxide by hydroxylamine is predicted by the known reactivity of **1**[5] and of related glucose derivatives.[10]

Rate constants for the reaction of p-nitrophenylacetate (PNPA) with 2^{o}-βCDNHOH, 1^{o}-βCDNHOH, CH_3NHOH, and βCD were measured at several pH values (Figure 1). As is apparent from Figure 1, 2^{o}-βCDNHOH is less reactive towards PNPA than is 1^{o}-βCDNHOH under identical conditions.

However, the reactivity of 2^{o}-βCDNHOH is pH dependent (a 19-fold increase from pH 6.5-9.5) *while that of 1^{o}-βCDNHOH is virtually pH independent over the same range*

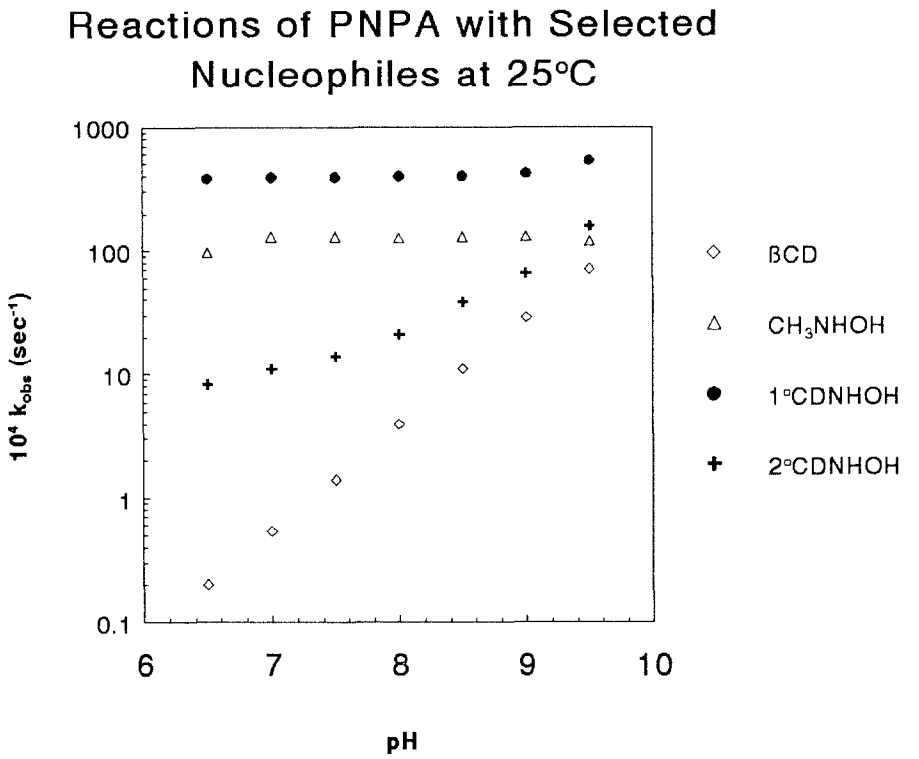


Figure 1. Observed rate constants for the reactions of the nucleophiles shown (10 mM) with PNPA (50 μM) in 0.10 M bis-tris-propane buffer at 25°C. In each case, the formation of p-nitrophenol was followed at 398 nm.

(a 1.6-fold increase). Assuming that substrate binding to the cyclodextrins is largely unaffected by pH in this region (likely given the pK_a's involved), then the contrasting reactivities must be explained in terms of how the hydroxylamine units can interact with the cyclodextrin framework. The pH-independent rate profile of 1^o-βCDNHOH parallels that of CH_3NHOH, reasonable if the 1^o-side of the cyclodextrinyl ring does not influence the acid-base microenvironment of the attatched NHOH group. The rate acceleration of the 1^o-βCDNHOH over CH_3NHOH is thus due entirely to preassociation of the substrate.

Alternatively, the pH-rate profile of the 2^o-βCDNHOH is indicative of catalysis by base. We interpret this pH effect on **2** as evidence that the 2^o-side of the cyclodextrinyl ring influences the reactivity of the attatched NHOH group. While the pK_a of a 2^o-OH is about 15-16, the first pK_a of βCD's vicinal diol is 12.1 as a result of intramolecular hydrogen bonding.[3] βCD itself is well-known to be inert at neutral pH (the βCD rate in Figure 1 is attributable entirely to buffer catalysis); rather, it demonstrates base-catalysis only above pH 10, when its 2^o-hydroxyl groups have begun to deprotonate. CPK models suggest hydrogen bonding is similarly possible between the pseudoequatorial C-2 hydroxyl and the C-3 hydroxylamine groups of **2**. Several intramolecular hydrogen bonding models involving 5 or 6 membered rings can be invoked that could rationalize the apparent base catalysis. In one such model (Figure 2), the C-2 hydroxy group hydrogen bonds to the nitrogen of the C-3 hydroxylamino group, effectively increasing the acidity of the attatched hydroxyl group. The enhanced acidity of trimethylamine oxide (pK_a 4.65)[11] relative to that of N,N-dimethylhydroxylamine (pK_a ca. 12-13)[12] is due to the addition of a full positive charge on nitrogen. Jencks has suggested that the high reactivity of hydroxylamine towards activated esters is due to intramolecular proton transfer from the hydroxylamine oxygen to the nitrogen during attack on a carbonyl.[13] In our model, intramolecular proton transfer to nitrogen at

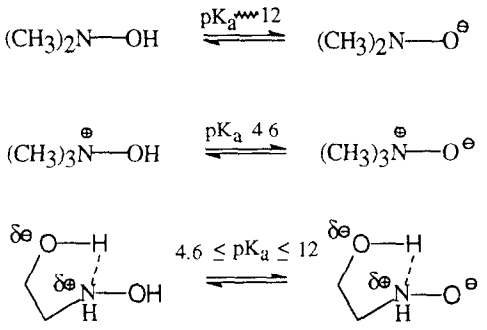


Figure 2.

neutral pH is not likely and thus consistent with the observed slower reactivity of the 2^{o}-βCDNHOH compared to CH_3NHOH. This would suggest, but not prove, O-acylation, as we have observed previously using the 1^{o}-side derivative.[1] To the best of our knowledge, this is the first time secondary-side hydrogen bonding has been used to modulate the pK_a of a cyclodextrin derivative. Of course, such pK_a effects on nucleophilic groups are observed commonly in enzymes themselves.

Acknowledgment. This work was support by a grant from The Office of Naval Research. FT-NMR spectra were obtained with equipment funded in part by NIH grant 1 S10 RR01458-01A1. A.W.C. thanks the A.P. Sloan and Dreyfus Foundations for support in the form of fellowships and Eli Lilly and Co. for support in the form of a granteeship.

Notes and References

1) Fikes, L. E.; Winn, D. T.; Sweger, R. W.; Johnson, M. P. and Czarnik, A. W. *J. Amer. Chem. Soc.* **1992**, *114*, 1493.

2) Bergeron, R.; Rowan, R. *Bioorg. Chem.* **1976**, *5*, 425.

3) VanEtten, R. L.; Clowes, G. A.; Sebastian, J. F.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3253.

4) Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275. The authors report a 70:30 mixture of mono- and ditosylated products.

5) Breslow, R. ; Czarnik, A. W. *J. Amer. Chem. Soc.* **1983**, *105*, 1390.

6) Akkaya, E. U.; Czarnik, A. W. *J. Phys. Org. Chem.* **1992**, *5*, 0000.

7) Obtained from Sachem, Austin, TX.

8) TLC was carried out on aluminum-backed silica plates, eluting with n-butanol/ethanol/water (5:4:3 v/v) and visualizing with MeOH/AcOH/H_2SO_4/p-anisaldehyde (200:20:10:1) followed by charring.

9) While working with a 60:40 mixture of mono- and disubstituted derivatives is not ideal, this method to obtain secondary side samples offers much higher yields than the previous method (Ueno, A. and Breslow, R. *Tetrahedron Lett.* **1982**, *23*, 3451), which yields almost exclusively mono-. To confirm that there is not a qualitative difference between these samples, we prepared 2 using the "old" method. Rate measurements made using this sample of 2 at pH's 7 and 9 gave rates only $\leq$ 15% slower than reported in Figure 1.

10) Williams, N. R. *Adv. Carbohyd. Chem.* **1970**, *25*, 109.

11) Nylen, P. *Chem. Abstr.* **1938**, *32*, 8888[9].

12) Roberts, J. S. in *Comp. Organic Chem.*, Vol. 2, Barton, D. Ed.; Pergamon Press: Oxford, 1979; p 185.

13) Jencks, W. P.; Carriulo, J *J. Amer. Chem. Soc.* **1960**, *82*, 1778.