Preassociating α-Nucleophiles Based on β-Cyclodextrin. Their Synthesis and Reactivity

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Abstract: Methods are reported for the attachment of α-nucleophiles to the primary and secondary sides of the cyclodextrin cavity. Six new materials have been prepared in which βCD has been modified by hydrazine, hydroxylamine, oxime, and hydroperoxide functionalities. Transacylating studies with *p*-NPA have demonstrated that the primary-side hydroxylamine shows the highest reactivity with a 1900-fold increase in rate over βCD at pH 6.5. Other α-nucleophiles show less remarkable rate increases in this system but, in some cases, demonstrate hydrogen-bonding to the cyclodextrin rim and inhibition kinetics.

Introduction

Because biological reactions always occur in an aqueous environment, often at pH 7.4, an important goal of enzyme mimicry research is to increase significantly the rate of a reaction under these conditions. Two essential features both enzymes and enzyme mimics must possess are a binding pocket and a reactive functional group. β-Cyclodextrin is water soluble, itself unreactive near neutral pH, and possesses a cavity capable of binding aromatic compounds. For these reasons, β-cyclodextrins that are functionalized with reactive nucleophiles have been studied widely as hydrolysis reagents and catalysts.[1]

In this paper, we report the use of α-nucleophiles as reactive moieties positioned on both the primary (1°) and secondary (2°) sides of β-cyclodextrin for the transacylation of esters. α-Nucleophiles offer several advantages as transacylating species. First, α-nucleophiles are well-known to exhibit enhanced reactivity toward acyl transfer as compared to isosteric alcohols or amines.[2] In addition, α-nucleophiles are typically less basic than isosteric compounds and therefore often exist in an unprotonated state near neutral pH.

The binding cavity of cyclodextrin allows for the pre-association of potential substrates. As the distance between the reactive group and the bound substrate is relevant to the reaction rate, α-nucleophiles potentially have the advantage of being positioned proximal to the cyclodextrin binding cavity due to their small physical size. α-Nucleophiles attached on the secondary side have the added property of being rigidly directed either toward or away from the bound substrate, whereas the primary side derivatives have an additional degree of rotational freedom. We now provide full details of the syntheses and varying transacylation reactivities of the preassociating α-nucleophiles of β-cyclodextrin derivatized with hydrazine,[3a] hydroxylamine,[3a,b] oxime,[3c] and hydroperoxide groups (Figure 1).

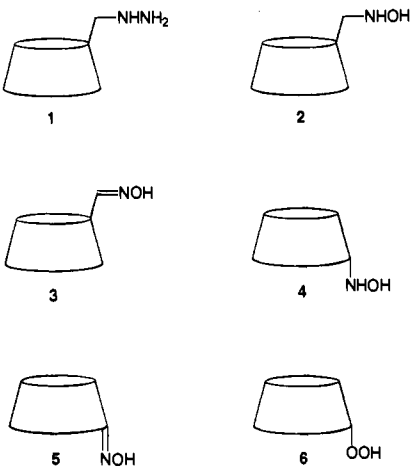

Figure 1.

Experimental Section

General Procedure. All buffer solutions were 0.1 M except where noted and were adjusted to the appropriate pH with either dilute NaOH or HCl. Buffers used were Bis-tris−propane (BTP, pH 6.5−9.5) and $NaHCO_3$ (pH 10.0), except where noted otherwise. Thin layer chromatography was carried out on aluminum-backed silica gel plates eluting with solvent system **A** (*n*-butanol/ethanol/water, 5:4:3 v/v) or **B** (ethyl acetate/isopropyl alcohol/concentrated NH_4OH/water, 7:7:4:4 v/v) and visualizing with MeOH/AcOH/H_2SO_4/*p*-anisaldehyde (200:20:10:1 v/v) spray followed by charring. FT-NMR spectra were obtained on either a Bruker AM-200, 250, or 300 spectrometer. Mass spectra were obtained on a Kratos-30 mass spectrometer. UV data were obtained on a Hewlett-Packard 8452A or 8451A diode array spectrophotometer. Elemental analyses were carried out at Galbraith Laboratories, Inc. (Norcross, GA). Melting points were obtained on an electrothermal melting point apparatus and are uncorrected.

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6-Deoxy-6-hydrazinyl-β-cyclodextrin (1). Anhydrous hydrazine (4.0 mL, 128 mmol) was added to 6-*O*-tosyl-β-cyclodextrin[4] (**7**; 1.00 g, 0.776 mmol) with stirring. In approximately 15 min, a clear brown solution was obtained which was stirred for 4 h. The solution was concentrated *in vacuo* with low heat (50 °C) to give a clear, light brown, viscous oil which was dissolved in water (2 mL). The solution was added dropwise to stirred ethanol (60 mL), producing a precipitate that was filtered to give a colorless powder. The powder was reprecipitated from ethanol (3−4×) until no signs of free hydrazine remained as evidenced by ^{1}H NMR in D_2O upon the addition of acetone. Compound **1** was obtained as a colorless powder (580 mg, 60%): mp 180−210 °C (dec); R_f 0.68 (solvent system B); ^{1}H NMR (D_2O) δ 4.95 (m, 7, H-1), 3.47−3.83 (m, 32, H-2, H-3, H-4, H-5, H-6), 3.40 (t, 2, H-4′), 3.08 (dd, 1, C*H*$_2$NHNH$_2$), 2.86 (dd, 1, C*H*$_2$NHNH$_2$); ^{13}C NMR (D_2O) δ 102.7, 102.4, 84.1, 81.9, 81.7, 73.9, 73.9, 73.0, 72.9, 72.7, 70.6, 58.3, 54.7; FAB mass spectrum *m/e* 1150 (M^+ + 1). Anal. Calcd for $C_{42}H_{72}N_2O_{34}$·7H_2O: C, 39.56; H, 6.80; N, 2.20. Found: C, 39.88; H, 6.78; N, 2.24.

6-Deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin (2). To a solution of 6-*O*-tosyl-β-cyclodextrin (**7**; 4.51 g, 3.50 mmol) in water (150 mL) was added a 50% aqueous solution of hydroxylamine (4.4 mL) over a 10 min period with constant stirring. The reaction was stirred for 17 h and the solution then concentrated to 40 mL. A colorless precipitate was collected by filtration after addition of ethanol (200 mL) to the solution. The sample was dissolved in a minimal volume of water, concentrated to remove residual ethanol, and lyophilized to yield **2** as a fluffy colorless solid (2.13 g, 67%). Free hydroxylamine was removed by repeated precipitation from ethanol. Compound **2** yields colorless plates after recrystallization from water: R_f 0.25 (solvent system A); ^{1}H NMR (DMSO-d_6) δ 7.14 (s, 1), 5.86 (m, 14, 2° OH), 4.82 (d, 7, H-1), 4.05 (s, 6, 1° OH), 3.55−3.79 (m, H-3, H-5, H-6), 3.17−3.43 (m, H-2, H-4, C*H*$_2$NHOH), 2.83 (dd, 1, C*H*$_2$NHOH); ^{13}C NMR (DMSO-d_6) δ 102.0, 84.4, 81.6, 81.1, 73.0, 72.3, 72.6, 71.8, 68.3, 60.0, 54.7; FAB mass spectrum *m/e* 1173 (100%, M^+ + Na), 1151 (95%, M^+ + 1). Anal. Calcd for $C_{42}H_{71}N_1O_{35}$·6H_2O: C, 40.10; H, 6.65; N, 1.11. Found: C, 40.15; H, 6.71; N, 0.78.

Mixture of Mono- and Di-3-deoxy-3-(*N*-hydroxylamino)-β-cyclodextrin (4). β-Cyclodextrin *manno*-2,3-epoxide[5] (**11**) was prepared from a mixture of the 2°-side mono- and ditosylates.[6] A solution of **11** (1.48 g, 1.32 mmol) in a 50% aqueous solution of hydroxylamine (3 mL) was stirred overnight under Ar. The clear solution was added dropwise into absolute ethanol (100 mL) with stirring. The resulting colorless precipitate was collected by vacuum filtration through a glass frit, dissolved in water (25 mL), and concentrated *in vacuo* to 15 mL. The solution was lyophilized to afford a fluffy colorless solid **4**, confirmed by elemental analysis to be a 60:40 mixture of mono- and dihydroxylamines. Two precipitations from ethanol removed free hydroxylamine (0.94 g, 62%): R_f 0.28, 0.22 (solvent system A); ^{1}H NMR (DMSO-d_6) δ 7.35 (s, NH), 5.00−6.05 (m, 14, 2° OH), 4.80−5.00 (s, 7, H-1), 4.35−4.65 (m, 1° OH), 2.95−4.00 (m, H-2, H-3, H-4, H-5, H-6); ^{13}C NMR (DMSO-d_6) δ 128.2, 104.0, 101.9, 81.1, 72.4, 68.8, 63.4, 60.0, 56.2; FAB mass spectrum *m/e* 1150.33 (M^+ for $C_{42}H_{71}N_1O_{35}$), 1165.33 (M^+ for $C_{42}H_{72}N_2O_{35}$). Anal. Calcd for 0.6 $C_{42}H_{71}N_1O_{35}$·0.4$C_{42}H_{72}N_2O_{35}$·6H_2O: C, 39.91; H, 6.65; N, 1.55. Found: C, 39.79; H, 6.68; N, 1.62.

3-Deoxy-3-(*N*-hydroxylamino)-β-cyclodextrin (4). β-Cyclodextrin *manno*-2,3-epoxide[5] (**11**) was prepared from the 2°-side monotosylate.[7] A solution of **11** (1.48 g, 1.32 mmol) in a 50% aqueous solution of hydroxylamine (3 mL) was stirred overnight under Ar. The clear solution was added dropwise into absolute ethanol (100 mL) with stirring. The resulting colorless precipitate was collected by vacuum filtration through a glass frit, dissolved in water (25 mL), and concentrated *in vacuo* to 15 mL. The solution was lyophilized to afford **4** as a fluffy colorless solid containing no dihydroxylamine detectable by TLC.

6-Deoxy-6-oxo-β-cyclodextrin Oxime (3). 6-Deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin (**2**; 1.0 g, 0.87 mmol) was dissolved in water (100 mL), and 2 drops of concentrated aqueous ammonia was added. The solution was stirred overnight, concentrated *in vacuo* to three-fourths of the original volume, and lyophilized to give *cis/trans*-**3** as a colorless powder that was recrystallized from hot water (410 mg, 42%): mp 212−228 °C; R_f 0.35 (solvent system A); ^{1}H NMR (DMSO-d_6) δ 7.23 (d, 0.7, C*H*NOH), 6.67 (d, 0.3, C*H*NOH), 5.57−5.97 (m, 14, 2° OH), 5.54 (br s, 1, NO*H*), 4.73−5.02 (m, 7, H-1), 4.42−4.62 (m, 6, 1° OH), 4.10 (t, 1, C*H*CHNOH), 3.13−3.97 (m, H-2, H-3, H-4, H-5, H-6); ^{13}C NMR (DMSO-d_6) δ 146.7, 146.4, 102.2, 101.7, 82.8, 80.9, 80.2, 72.4, 72.2, 72.0, 69.1, 60.8, 60.3; FAB mass spectrum, *m/e* 1148.41 (M^+). Anal. Calcd for $C_{42}H_{69}NO_{35}$·6H_2O: C, 40.16; H, 6.50; N, 1.12. Found: C, 40.29; H, 6.43; N, 1.10.

6-Deoxy-6-oxo-β-cyclodextrin Oxime (3). 6-Deoxy-6-formyl-β-cyclodextrin[8] (**8**; 107 mg, 0.0944 mmol) was dissolved in a 50% aqueous solution of H_2NOH (10 mL) and stirred at room temperature for 4 h. The solution was added dropwise to ethanol (95%, 200 mL) and refrigerated for 5 h. The colorless precipitate was collected under vacuum to yield **3** (46 mg, 43%), identical in all respects to material prepared by air oxidation of **2**.

Mixture of Mono- and Di-3-deoxy-3-oxo-β-cyclodextrin Oxime (5). The mixture of mono- and di-3-deoxy-3-(*N*-hydroxylamino)-β-cyclodextrin (**4**, 133 mg, 0.116 mmol) was dissolved in water (5 mL), and concentrated aqueous ammonia was added until pH > 11 (pH paper). The solution was stirred overnight and the water removed *in vacuo*. The residue was dissolved in water (10 mL) and evaporated a second time. The residue was dissolved in water (20 mL) and lyophilized to give a fluffy colorless solid confirmed by elemental analysis to be a 60:40 mixture of mono- and dioximes (130 mg, 98%): R_f 0.39 (solvent system A); ^{1}H NMR (D_2O) δ 5.17−5.23 (s, H-1′), 4.80−5.00 (s, H-1), 3.15−4.20 (m, H-2, H-3, H-4, H-5, H-6); ^{13}C NMR (DMSO-d_6) δ 155.5, 103.5, 103.0, 102.0, 80.0, 79.5, 78.5, 73.1, 72.1, 71.8, 60.9; FAB mass spectrum, *m/e* 1148.14 (M^+ for $C_{42}H_{69}NO_{35}$), 1162.05 (M^+ for $C_{42}H_{68}N_2O_{35}$). Anal. Calcd for 0.6$C_{42}H_{69}N_1O_{35}$·0.4$C_{42}H_{68}N_2O_{35}$·6H_2O: C, 40.00; H, 6.44; N, 1.55. Found: C, 40.19; H, 6.26; N, 1.69.

Material Containing 2-Deoxy-2-hydroperoxy-β-cyclodextrin (6). β-Cyclodextrin (βCD) (10.0 g, 8.80 mmol) was dissolved in neat 30% H_2O_2 (200 mL). The resulting solution was stirred at 60 °C for 3 days, then concentrated *in vacuo* to approximately one-fourth of the original volume. The solution was added dropwise to isopropyl alcohol (1 L) and the precipitate collected under vacuum. The precipitate was dissolved in warm water (20 mL) and added dropwise to isopropyl alcohol (1 L, 2×) to yield a colorless solid containing ca. 25% **6** and 75% β-cyclodextrin (8.7 g, 86% yield): R_f 0.12 (βCD), 0.05 (**6**) (solvent system B); ^{1}H NMR (D_2O) δ 5.21 (m, 2, H-1′), 4.90 (d, 5, H-1), 3.08−4.03 (m, 42, H-2, H-3, H-4, H-5, H-6); ^{13}C NMR (D_2O) δ 104.7, 102.6, 102.5, 84.0, 79.8, 76.2, 76.0, 75.8, 74.9, 74.7, 74.4, 74.1, 72.2, 63.1; FAB mass spectrum *m/e* 1135.45 (M^+ for $C_{42}H_{70}O_{35}$), 1157.38 (M^+ + Na for $C_{42}H_{70}O_{35}$), 1173.42 (M^+ + Na for $C_{42}H_{70}O_{36}$).

Catalytic Hydrogenation of 6-Deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin. 6-Deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin (**2**; 1.5 g, 1.3 mmol) was dissolved in H_2O (100 mL) and warmed until homogeneous. Pd/C (10%, 0.25 g) was added, and the solution was hydrogenated (Parr Hydrogenator) at 25 psi for 20 h. The solution was then filtered through Celite and the solvent removed *in vacuo* to yield a colorless powder, which was identical by TLC and ^{1}H NMR to an authentic sample of 6-deoxy-6-amino-β-cyclodextrin.

6-Deoxy-6-*N*-((*O*-acetyl)hydroxylamino)-β-cyclodextrin (13). To a solution of 6-deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin (**2**; 130 mg, 0.111 mmol) in bis-tris−propane buffer (0.050 M, pH 7.0, 50 mL) was added a solution of *m-tert*-butylphenyl acetate[9] (**12**; 21 mg, 0.11 mmol) in acetonitrile (2 mL). Acetonitrile (13 mL) was added and the homogeneous solution stirred for 43 h. The solution was concentrated *in vacuo* and passed through a microcolumn of Dowex MR-12 resin to remove the buffer salts. The appropriate fractions were pooled and lyophilized to yield a colorless solid (9.2 mg, 7%): R_f 0.33 (solvent system B); ^{1}H NMR (DMSO-d_6) δ 9.15 (s, PhOH), 7.64−

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

(7) Ueno, A.; Breslow, R. *Tetrahedron Lett.* **1982**, *23*, 3451. The tosylate prepared in this manner is homogeneous but isolated in very low yield.

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7.75 (t, NH), 7.02–7.12 (dd, PhH), 6.75–6.85 (dd, PhH), 6.52–6.59 (dd, PhH), 5.59–5.86 (m, 2° OH), 5.42–5.52 (m), 4.76–4.97 (d, H-1), 4.63–4.75 (m), 4.31–4.59 (m, 1° OH), 3.49–4.00 (m, H-3, H-5, H-6), 3.17–3.48 (m, CH_2NH, H-2, H-4), 2.98–3.15 (m, CH_2NH), 1.98 (s, CH_3CO), 1.22 (s, *t*-butyl). Irradiation centered at δ 3.07 caused the triplet (δ 7.64–7.75) to coalesce to a doublet.

Active Oxygen Assay.[10] A 0.1 N As_2O_3 solution was prepared by dissolving As_2O_3 (2.5 g) in water (100 mL). NaOH was added until the solution was homogeneous. The pH was adjusted to 7.0 by the addition of 1 N HCl. $NaHCO_3$ (6.25 g) was added, and the volume was adjusted to 250 mL with water. Then to the As_2O_3 solution (20 mL) was added the mixture to be tested (0.6 g). H_2SO_4 (1 N, 25 drops) was added followed by $NaHCO_3$ (0.5 g). The solution was then titrated against a 0.1 N I_2 solution. From the titration volume, the amount of As^{+3} remaining in the solution could be calculated and hence the amount of active oxygen determined.

Transacylations of *p*-Nitrophenyl Acetate and *m*-Nitrophenyl Acetate. The reactions of *p*-NPA and *m*-NPA were carried out in the appropriate buffer at 23 °C. Reaction was initiated by the addition of 10 μL of *p*-NPA or *m*-NPA solution (5.0×10^{-3} M in CH_3CN) to 990 μL of the cyclodextrin solution (10 mM in buffer, except where noted). For reactions with CH_3NHOH, CH_3CHNOH, and *t*-butyl hydroperoxide, appropriate solutions (10 mM in buffer) were substituted for the cyclodextrin solution. The formation of *p*-nitrophenol or *m*-nitrophenol was followed spectroscopically at 398 or 390 nm, respectively. Pseudo-first-order rate constants were calculated using the ENZFITTER program (Elsevier-BIOSOFT, Cambridge, U.K.) and are reported to within ±10%.

Inhibition Kinetics. Transacylation reactions of *m*-nitrophenyl acetate with **6** were monitored at pH 8.5 in Bis-tris–propane buffer as stated previously with the appropriate amount of *n*-butanol added to the cyclodextrin solution, yielding solutions containing 0, 55, and 110 mM *n*-butanol.

Transacylation of *m-tert*-Butylphenyl Acetate (12). 6-Deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin (**2**; 255 mg, 0.222 mmol) was dissolved in Bis-tris–propane buffer (50 mM, 9 mL), and the pH was adjusted to 7.0. To this solution was added a solution of **12** (4.5 mM, 2 mL) in acetonitrile. At timed intervals, aliquots (500 μL) were removed and added to a solution of Bis-tris–propane (50 mM, pH 9.5, 500 μL). The absorbance of the resulting solution (pH 9.3) was measured immediately, and the formation of *m-tert*-butylphenol was followed spectroscopically at 286 nm. The reaction rate using βCD and CH_3NHOH was determined using an analogous procedure with minimal DMSO added for solubility.

Results and Discussion

Synthesis. The known 1°-side monotosylate of β-cyclodextrin (**7**)[4] was the starting material for preparation of our primary-side derivatives (Scheme 1). Compound **7** was recrystallized three times from hot water and used without further purification. 6-Deoxy-6-hydrazinyl-β-cyclodextrin (**1**) was synthesized by reaction of **7** with anhydrous hydrazine for 4 h at room temperature.[3a] Isolation and purification of **1** was accomplished by repeated precipitations from ethanol. All physically entrained NH_2NH_2 was determined to be removed from the sample by examining the ^1H NMR (D_2O) of **1** with added acetone. The reaction of acetone with free hydrazine results in the equilibrium formation of the acetone hydrazone, which exhibits methyl singlets at δ 1.72 and 1.81. In contrast, the hydrazone formed by reaction of acetone and **1** (**9**; Scheme 2) exhibits singlets at δ 1.74 and 1.83.

The synthesis of 6-deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin (**2**) was accomplished in a similar manner.[3a] Reaction of **7** with a 50% aqueous solution of hydroxylamine over a period of 17 h at room temperature yields crude **2**. Removal of free hydroxylamine was again accomplished with repeated precipitations from ethanol and monitored by analyzing the ^1H NMR (D_2O) of **2** with added acetone. In this case the reaction of

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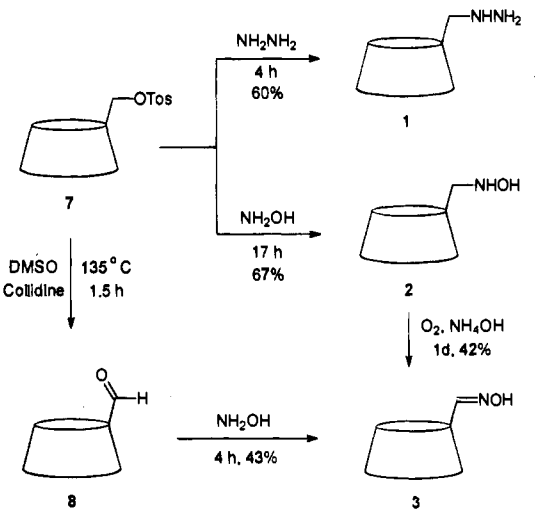
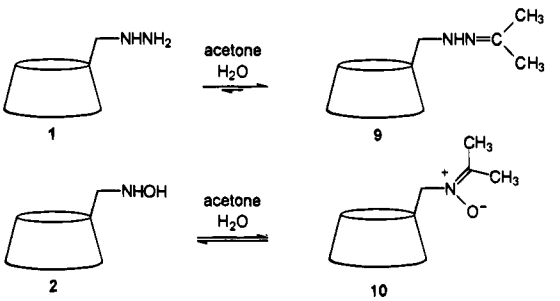
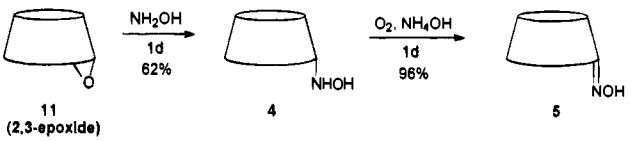
Scheme 1



Scheme 2



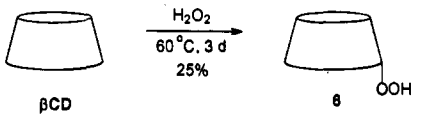
Scheme 3



acetone with hydroxylamine results in the formation of acetaldoxime having methyl singlets at δ 1.75 and 1.79. Reaction of **2** with a large excess of acetone forms the corresponding nitrone (**10**) resulting in singlets at δ 2.00 and 2.12. It was also necessary to verify N-alkylation of the tosylate as opposed to O-alkylation. Catalytic hydrogenation of **2** yielded 6-deoxy-6-amino-β-cyclodextrin, not β-cyclodextrin itself, confirming N-alkylation.

3-Deoxy-3-(*N*-hydroxylamino)-β-cyclodextrin (**4**) was prepared[3b] by stirring a 50% aqueous hydroxylamine solution with β-cyclodextrin *manno*-2,3-epoxide (**11**)[5] overnight at room temperature (Scheme 3). The epoxide used in this procedure was synthesized from a mixture of mono- and ditosylates to yield a mixture of mono- and diepoxides in a 60:40 ratio, respectively, based on NMR integrations of the tosylate starting material. This ratio of mixtures is retained in the hydroxylamine. Mass spectral analysis shows a mixture of the mono- and dihydroxylamines, while elemental analysis confirms these to be in a 60:40 ratio, respectively. Although working with a mixture is clearly not ideal, this synthetic method is the only one available that allows for large-scale preparation of the 2°-side hydroxylamine. In addition, several kinetic runs using the more precious monohydroxylamine sample (prepared from the monotosylate) revealed similarly unexceptional rate accelerations. Although in principle the hydroxylamine may attack carbon 2 or 3 of the glucose unit, the primary site of reaction is predicted to be the 3 position on the basis of the known

Scheme 4



reactivity of the β-cyclodextrin epoxide[5] and that of related glucose epoxides.[11]

6-Deoxy-6-oxo-β-cyclodextrin oxime (**3**) was prepared via the air oxidation of **2** by reaction overnight under basic conditions at room temperature.[3c] Compound **3** was isolated as a mixture of E and Z isomers as evidenced by ^{1}H NMR (DMSO-d_6), which shows two doublets at δ 7.2 and 6.8 corresponding to the two isomers of the proton signal C*H*NOH. This compound is also prepared via the addition of hydroxylamine to the 1°-side monoaldehyde (**8**), which is synthesized via Nace oxidation of **7**.[12] The secondary-side oxime, 3-deoxy-3-oxo-β-cyclodextrin oxime (**5**), was prepared[3c] via air oxidation of **4** overnight to yield a mixture of the mono- and dioxime by mass spectral data. Elemental analysis of **5** confirms a 60:40 mixture of mono- and dioximes as expected on the basis of the mixture ratio of **4**.

A material containing 25% active oxygen incorporated into β-cyclodextrin was prepared by the oxidation of β-cyclodextrin itself (βCD) in 30% H_2O_2 at 60 °C for 3 days (Scheme 4). The cyclodextrins were isolated by precipitation from isopropyl alcohol. The amount of active oxygen incorporated into the sample was assayed by the procedure of Siggia.[10] Physically entrained hydrogen peroxide was determined to be removed after three sequential precipitations as evidenced by the attainment of a constant level of active oxygen content in the isolated solid. An assay of reaction aliquots over time revealed that active oxygen incorporation increased up to but not beyond 3 days.[13] The material was found to contain a component with one hydroperoxide group as evidenced by mass spectral analysis. We have designated this component as 2-deoxy-2-hydroperoxy-β-cyclodextrin (**6**). Although the presence of other β-cyclodextrin hydroperoxide moieties is not excluded, the experimentally simple formation of a material that contains a chiral oxidizing agent is still inherently valuable.

Material **6** was found to decompose over time to yield aldehyde functionalities by various indicative tests with 2,4-dinitrophenylhydrazine, Tollen's reagent, and NH_2OH. This compound did not correspond to the primary-side aldehyde[8] and therefore established the position of the hydroperoxide moiety to be on the secondary side. The decomposition to a dialdehyde structure was accelerated in the presence of base and is a known decomposition pathway for β-hydroxy hydroperoxides.[14]

Although we were unable to establish whether the hydroperoxide moiety is located on C-2 or C-3 of the cyclodextrin ring, we propose its formation at C-2. Although many oxygen nucleophiles are reported to be unreactive toward hydrogen peroxide,[15] the internal H-bonding between C-2 and C-3 hydroxyl groups may activate the C-2 hydroxyl toward such nucleophilic attack. Alternatively, a backside S_N2 attack of hydrogen peroxide on the C-2 position is at least conceivable, if unlikely. Both of these mechanisms intricately involve the cyclodextrin structure in making them feasible.

Table 1. Rate Constants for the Transacylation of *p*-NPA (50 μM) in 0.1 M BTP at 23 °C with Various Nucleophiles (All 10 mM)

	$10^4 k_{obsd}$ (s^{-1})			
pH	βCD	MeNHOH	**2**	**4**
6.5	0.20	97	380	8.5
7.0	0.54	130	390	12
7.5	1.4	130	390	15
8.0	4.0	130	400	25
8.5	11	140	410	49
9.0	29	160	450	95
9.5	71	190	600	230

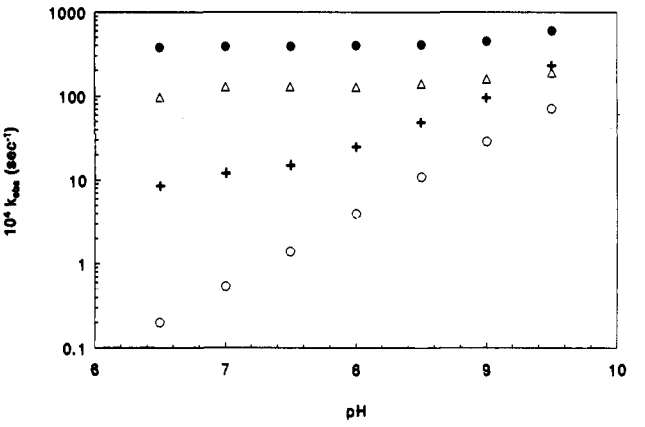


Figure 2. Rate constants for the transacylation of *p*-NPA (50 μM) with βCD (○), CH_3NHOH (△), **2** (●), and **4** (+) (all 10 mM) in 0.1 M BTP at 23 °C.

The decomposition of **6** in solution was followed at pH 8.5 by monitoring the decrease in its reactivity over time. Material **6** was found to have a half-life of about 1 h under these conditions. In the presence of EDTA, a compound capable of complexing trace metals in solution, the half-life was increased to 3 h. As a solid, **6** retains elevated activity even after 4.5 months.

Kinetics. Rates for the transacylation reactions of *p*-nitrophenyl acetate (*p*-NPA) were determined in the presence of the α-nucleophile derivatives by spectroscopically monitoring the release of *p*-nitrophenol at 398 nm. These rates were compared to the rates for βCD itself and for appropriate non-preassociating α-nucleophiles. It is important to note that βCD is unreactive toward transacylation until the 2°-side hydroxyl groups (pK_a 12.01) begin to deprotonate above pH 10; rate constants at lower pHs reflect only catalysis by buffer.

The transacylation reaction rates for **2**, **4**, βCD, and CH_3NHOH over the pH range 6.5−9.5 are shown in Table 1. As seen in Figure 2, the most striking feature is the large rate acceleration of **2** over that of βCD; at pH 6.5, a rate increase of 1900-fold is observed. This accelerated rate is maintained throughout the entire pH range. Although CH_3NHOH demonstrates a similar reactivity trend, **2** maintains a 4-fold rate increase over CH_3NHOH. This modest increase is credited to the preassociation of the substrate in the cyclodextrin cavity. As expected for a preassociation pathway, a study of the concentration of **2** versus reaction rate demonstrates saturation kinetics (Figure 3).

The enhanced reactivity of **2** encouraged the examination of the transacylation of an unactivated ester, *m-tert*-butylphenyl acetate[9] (**12**) with this compound. At pH 7.0, a k_{obsd} of 1.5×10^{-3} s^{-1} was measured for reaction with **2** resulting in a half-

(11) Williams, N. R. *Adv. Carbohydr. Chem. Biochem.* **1970**, *25*, 109.
(12) Yoon, J.; Hong, S.; Martin, K. A.; Czarnik, A. W. *J. Org. Chem.*, in press.
(13) A "spike" in the active oxygen content was observed at 7−8 days. However, this is believed to be due to an increase in the percentage of **6** precipitated at this point and not actually indicative of an increase in the amount of hydrogen peroxide incorporated into the sample.
(14) (a) Hoffman, J. *J. Am. Chem. Soc.* **1957**, *79*, 503. (b) For carbohydrate peroxides, see: Schulz, M.; Boeden, H.-F.; Berlin, P. *Liebigs Ann. Chem.* **1967**, *703*, 190.
(15) Edwards, J. O. *Peroxide Reaction Mechanisms*; Interscience Publishers: New York, 1962; pp 97−98.

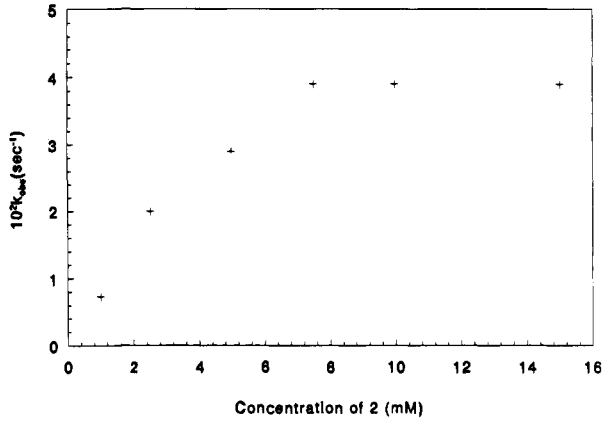
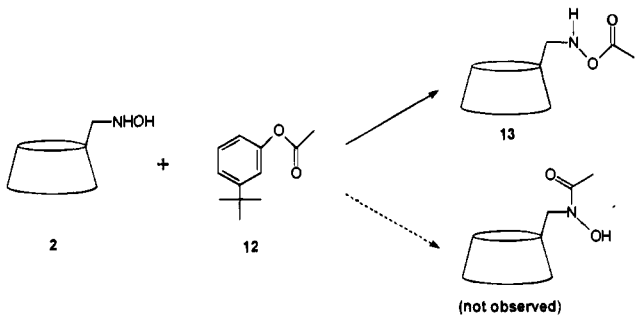


Figure 3. Rate constants for the transacylation of *p*-NPA (50 μM) with varying concentrations of **2** in 0.1 M BTP at pH 7.0 and 23 °C.

Scheme 5



life of 7.5 min. By comparison, the reaction with CH_3NHOH and βCD shows a half-life of 4.8 h. In buffer alone, hydrolysis of **12** occurs to less than 5% after 7 days. Thus, **2** exhibits high reactivity even with this less activated ester.

Since N-alkylated hydroxylamines can acylate at either the N or the O,[16] a preparative scale reaction between **12** and **2** was performed to determine the site of alkylation (Scheme 5).

^{1}H NMR analysis of the reaction product (DMSO-d_6) shows a single proton triplet at δ 7.65 that is D_2O exchangeable. Decoupling experiments demonstrate one-bond coupling to a single, diastereomeric H-6 proton (δ 3.06). This allows assignment of the signal at δ 7.65 as the NH proton and, thus, the assignment of the product of acylation as the O-acylated compound, **13**.

Compound **4** shows lower reactivity than **2** with only a 43-fold increase in rate over βCD at pH 6.5 (Figure 2).[3b] However, the rate is unexpectedly pH dependent with a 27-fold increase in rate from pH 6.5 to 9.5. In contrast, both **2** and CH_3NHOH are pH independent, increasing in rate only by a factor of 2 over the same pH range. The pH dependency of **4** is suggestive of base catalysis. Since CH_3NHOH does not show similar pH dependence, the effect can be interpreted as evidence that the 2°-side hydroxyl groups of cyclodextrin influence the reactivity of the attached NHOH group. CPK models suggest that H-bonding is possible between the C-2 hydroxyl and the C-3 hydroxylamine group. Several intramolecular H-bonding models involving five- or six-membered rings can be invoked. In one model (Figure 4), the C-2 hydroxyl H-bonds to the nitrogen of the hydroxylamine group, placing a partial positive charge on the nitrogen. The pK_a of the attached hydroxylamine group can then be estimated to be between 4.6 and 12, the pK_a's of a nonprotonated hydroxylamine (*N,N*-dimethylhydroxylamine) and a model for N-protonated hydroxylamine (trimethylamine oxide). As Jencks has proposed that the high reactivity of hydroxylamine derivatives toward esters is due to intramolecular proton transfer from the hydroxylamine oxygen to the nitrogen during attack,[2] the increased pK_a invoked by our H-bonding model may inhibit the normal reactivity of the hydroxylamine group. This can rationalize the observed slower reactivity of **4** as compared to CH_3NHOH. In addition, this would also favor an O-alkylation pathway as in the case of **2**.

(16) Roberts, J. S. *Comprehensive Organic Chemistry*; Pergamon Press: New York, 1979; Vol. 2, pp 185–217.

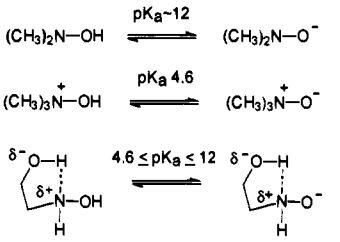


Figure 4. H-bonding model of **4**.

Table 2. Rate Constants for the Transacylation of *p*-NPA (50 μM) in 0.1 M BTP at 23 °C with Various Nucleophiles (All 10 mM)

	10^4k_{obsd} (s^{-1})			
pH	βCD	MeCHNOH	**3**	**5**
6.5	0.20	1.2	7.1	
7.0	0.54	6.9	10	18
7.5	1.4	21	35	
8.0	4.0	71	160	78
8.5	11	160	380	370
9.0	29	420		870

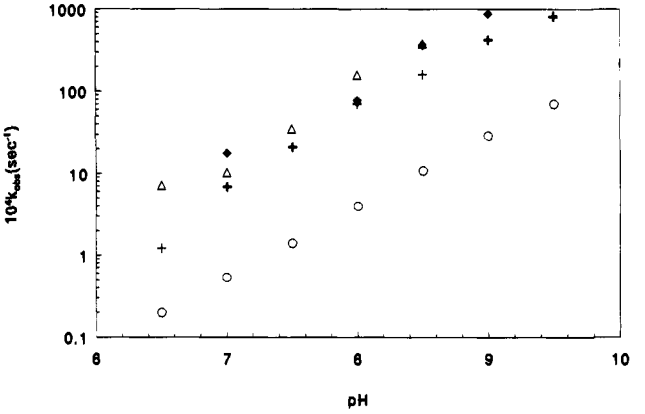


Figure 5. Rate constants for the transacylation of *p*-NPA (50 μM) with βCD (○), acetaldoxime (+), **3** (△), and **5** (◆) (all 10 mM) in 0.1 M BTP at 23 °C.

Since **4** is isolated as a mixture of mono- and dihydroxylamines, a small amount of sample was prepared which contained no observable dihydroxylamine.[7] Rate constants for the hydrolysis of *p*-NPA using this sample were measured at pH 7 and 9, $k_{obsd} = 11 \times 10^{-4}$ and 83×10^{-4} s^{-1}, respectively. A comparison with the results in Table 1 shows that the more extensive kinetic data obtained using the mixture are reflective of monosubstitution.

The transacylations of *p*-NPA with the 1°- and 2°-side oxime derivatives, **3** and **5**, are shown in Table 2 and Figure 5. Over the pH range 6.5–9.0, both compounds are more reactive than βCD with an average increase in reactivity of approximately 30-fold. Both compounds show pH dependence as seen with the non-preassociating compound, CH_3CHNOH. However, neither compound shows a significant rate enhancement over that afforded by CH_3CHNOH.

Reaction rates using **1** show a noticeable acceleration over βCD only at pH 7.0 with an 11-fold increase (Table 3). In this case, the hydrolysis of a less activated ester, *m*-nitrophenyl

Table 3. Rate Constants for the Transacylation of p-NPA and *m*-NPA (Each 50 μM) with **1** (5 mM) in 0.1 M BTP at 23 °C

	10^4k_{obsd} (s^{-1})	
pH	*p*-NPA	*m*-NPA
7.0	5.9	
8.0	7.7	35
9.0	32	64

Table 4. Rate Constants for the Transacylation of *p*-NPA (50 μM) at 23 °C with Various Nucleophiles (All 10 mM) in 0.1 M BTP (pH 7 and 9), 0.1 M Trizma (pH 8), or 0.1 M $NaHCO_3$ (pH 10)

	10^4k_{obsd} (s^{-1})		
pH	βCD	*t*-BuOOH	**6**
7.0	0.47	0.26	0.83
8.0	2.1	1.0	5.1
9.0	29	16	60
10.0	170	61	270

acetate (*m*-NPA), was also studied. Even though this ester is less reactive, its transacylation is faster in the presence of **1** than is that of the activated ester, *p*-NPA. This suggests that the geometry of the meta compound upon binding brings the two reactive groups into a more favorable position for reaction as compared to the para compound.

Table 4 shows the results for the reactions of *p*-NPA with **6** and *tert*-butyl hydroperoxide; material **6** does not show a significant acceleration over βCD or the reference non-preassociating compound, *tert*-butyl hydroperoxide. The unreactive nature of **6** was further examined by studying the hydrolysis of *m*-NPA in the presence of an inhibitor, *n*-butanol. *n*-Butanol acts as an inhibitor by binding in the cyclodextrin cavity, preventing preassociation of the substrate.[17] However, increasing concentrations of the inhibitor did not decrease the rate of reaction of **6** with *m*-NPA. Therefore, this particular derivative is reacting via a nonassociative pathway. This type of result would be expected if the reactive hydroperoxide group is positioned outside of the cavity, away from the bound substrate, as would be anticipated by nucleophilic reaction of ROH on H_2O_2.

(17) Tee, O. S.; Hoeven, J. J. *J. Am. Chem. Soc.* **1989**, *111*, 8318.

Conclusion

We have synthesized and studied six materials containing new derivatives of β-cyclodextrin appended with various α-nucleophiles. By far the most reactive of this series is 6-deoxy-6-(*N*-hydroxylamino)-β-cyclodextrin, which shows high reactivity toward both activated and unactivated esters. Although not as reactive, 3-deoxy-3-(*N*-hydroxylamino)-β-cyclodextrin demonstrates that the cyclodextrin ring can influence attached groups via hydrogen-bonding. The results from the hydrolysis of *p*-NPA and *m*-NPA with 6-deoxy-6-hydrazinyl-β-cyclodextrin further show that the geometry of the substrate binding into the cyclodextrin cavity affects the reactivity. Not only must the substrate be bound in a productive manner, but the reactive moiety must also be in a position to interact with the substrate. This is demonstrated by the fact that material containing 2-deoxy-2-hydroperoxy-β-cyclodextrin is not reacting via preassociation of the substrate. It is expected that the synthetic availability of these new derivatives will facilitate their study in other reaction systems, which may well show different relative reactivities.

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