alkene, allylic amines inaccessible by the tandem ene/[2,3]-sigmatropic reaction. We also believe that the conditions to transform the protected hydrazines into amines could be applied to other cases.[8]

Experimental Section

NMR spectra were recorded on a Bruker AM 250 (250 MHz) and on a Bruker 300 (300 MHz) spectrometer. Bis(2,2,2-trichloroethyl) azodicarboxylate was purchased from Aldrich Chemical Co.

Typical Procedure for the Ene Reaction of Olefins with BTCEAD. Preparation of 1-(2-Cyclopenten-1-yl)-1,2-hydrazinedicarboxylic Acid Bis(2,2,2-trichloroethyl) Ester (10). To a solution of cyclopentene (4) (250 mg, 3.57 mmol) in benzene (17 mL) (sealed tube) was added BTCEAD (1.70 g, 4.50 mmol). The resulting yellow-orange solution was heated at 80 °C for 12 h. The crude mixture was then purified by flash chromatography (10% ethyl acetate in hexane) to give after crystallization 1.20 g (73%) of the ene adduct **10** as a white solid: mp 132 °C; ^{1}H NMR (300 MHz, CD_3CN, 353 K) δ 2.10–2.50 (m, 4 H), 4.72 and 4.80 (2 s, 4 H), 5.30 (m, 1 H), 5.65 (bs, 1 H), 6.00 (m, 1 H), 7.70 (bs, 1 H). Anal. Calcd for $C_{11}H_{12}Cl_6N_2O_4$: C, 29.59; H, 2.69; Cl, 47.08; N, 6.28. Found: C, 29.48; H, 2.65; Cl, 46.93; N, 6.14.

1-(2-Cyclohexen-1-yl)-1,2-hydrazinedicarboxylic acid bis(2,2,2-trichloroethyl) ester (11): mp 155 °C (ethyl acetate–hexane); ^{1}H NMR (300 MHz, toluene-d_8, 380 K) δ 1.30–1.85 (m, 6 H), 4.45 and 4.55 (2 s, 4 H), 4.75 (m, 1 H), 5.50 (m, 1 H), 5.65 (m, 1 H), 6.00 (bs, 1 H). Anal. Calcd for $C_{12}H_{14}Cl_6N_2O_4$: C, 31.30; H, 3.04; N, 6.08. Found: C, 31.21; H, 3.06; N, 6.04.

1-(3-Methyl-2-cyclopenten-1-yl)-1,2-hydrazinedicarboxylic acid bis(2,2,2-trichloroethyl) ester (13): mp 114–115 °C (ethyl acetate–hexane); ^{1}H NMR (300 MHz, CD_3CN, 353 K) δ 1.75 (s, 3 H), 2.10–2.45 (m, 4 H), 4.75 (m, 5 H), 5.25 (m, 1 H), 7.70 (bs, 1 H). Anal. Calcd for $C_{12}H_{14}Cl_6N_2O_4$: C, 31.30; H, 3.04; Cl, 45.45; N, 6.01. Found: C, 31.21; H, 3.04; Cl, 45.36; N, 6.02.

1-[1-(1-Cyclohexen-1-yl)ethyl]-1,2-hydrazinedicarboxylic acid bis(2,2,2-trichloroethyl) ester (14): ^{1}H NMR (300 MHz, CD_3CN, 353 K) δ 1.30 (d, 3 H), 1.50–1.67 (m, 4 H), 1.95–2.20 (m, 4 H), 4.70 (q, 1 H), 4.80 (s, 4 H), 5.70 (m, 1 H), 7.65 (bs, 1 H). Anal. Calcd for $C_{14}H_{18}Cl_6N_2O_4$: C, 34.26; H, 3.66; N, 5.71. Found: C, 34.67; H, 3.88; N, 5.60.

1-(6-Acetoxy-2-hexen-1-yl)-1,2-hydrazinedicarboxylic acid bis(2,2,2-trichloroethyl) ester (15): ^{1}H NMR (300 MHz, CD_3CN, 348 K) δ 1.68 (quintet, 2 H), 1.95 (s, 3 H), 2.10 (q, 2 H), 4.05 (t, 2 H), 4.10 (d, 2 H), 4.79 and 4.81 (2 s, 4 H), 5.58 (m, 1 H), 5.73 (m, 1 H), 7.90 (bs, 1 H). Anal. Calcd for $C_{14}H_{18}Cl_6N_2O_6$: C, 32.30; H, 3.46; N, 5.38. Found: C, 32.13; H, 3.06; N, 5.76.

Typical Procedure for the Conversion of Ene Adducts to Acetamides. Preparation of 3-Acetamido-1-cyclopentene (16). To a solution of the hydrazide **10** (500 mg, 1.12 mmol) in glacial acetic acid (3 mL) was added zinc dust portionwise over 5 min. The resulting mixture was stirred for 15 min at room temperature, and acetone (≈250 μL) was then added. After 1 h, CH_2Cl_2 was then added, and the resulting mixture filtered through a pad of Celite. The solvents were removed on rotavapor and on high vacuum pump for 1 min. CH_2Cl_2 (5 mL) was then added to the crude mixture followed by pyridine (few drops) and an excess of acetic anhydride. After the mixture stood overnight at room temperature, the solvents were removed under reduced pressure and the mixture was purified by flash chromatography (50–80% ethyl acetate in hexane) to provide 120 mg (90%) of the title compound. The acetamide was crystallized in ether–hexane to give 115 mg (85%) of white needles: mp 73–74 °C; ^{1}H NMR (250 MHz, acetone-d_6) δ 1.54 (m, 1 H), 1.82, (s, 3 H), 2.24 (m, 2 H), 2.36 (m, 1 H), 4.87 (m, 1 H), 5.63 (m, 1 H), 5.85 (m, 1 H), 7.00 (bs, 1 H); high-resolution mass spectrum, m/z for $C_7H_{12}NO$ $(M + H)^+$ calcd 126.0919, found 126.0918.

3-Acetamido-1-cyclohexene (17): mp 85 °C (ether–hexane); ^{1}H NMR (300 MHz, acetone-d_6) δ 1.43–1.80 (m, 4 H), 1.83 (s, 3 H), 1.95 (m, 2 H), 4.35 (m, 1 H), 5.53 (m, 1 H), 5.75 (m, 1 H), 6.95 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_8H_{14}NO$ $(M + H)^+$ 140.1076, found 140.1075.

1-Acetamido-2-methylenecyclohexane (18): mp 107 °C (ether–hexane); ^{1}H NMR (250 MHz, acetone-d_6) δ 1.20–1.35 (m, 2 H), 1.45–1.60 (m, 1 H), 1.70–1.95 (m, 3 H), 1.92 (s, 3 H), 2.00–2.10 (m, 1 H), 2.40 (td, 1 H), 4.30 (m, 1 H), 4.65 (d, 1 H), 4.73 (d, 1 H), 7.10 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_9H_{16}NO$ $(M + H)^+$ 154.1231, found 154.1196.

3-Acetamido-3-methyl-1-cyclohexene (19): mp 75 °C (ether–hexane); ^{1}H NMR (250 MHz, acetone-d_6) δ 1.40 (s, 3 H), 1.42–1.70 (m, 3 H), 1.80 (s, 3 H), 1.85–1.95 (m, 2 H), 2.05–2.20 (m, 1 H), 5.10 (td, 1 H), 5.80 (bd, 1 H), 6.70 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_9H_{16}NO$ $(M + H)^+$ 154.1231, found 154.1198.

3-Acetamido-1-methyl-1-cyclopentene (21): mp 65 °C (ether–hexane); ^{1}H NMR (250 MHz, acetone-d_6) δ 1.55 (m, 1 H), 1.70 (s, 3 H), 1.80 (s, 3 H), 2.10–2.40 (m, 3 H), 4.80 (m, 1 H), 5.25 (m, 1 H), 7.00 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_8H_{14}NO$ $(M + H)^+$ 140.1076, found 140.1074.

1-(1-Acetamidoethyl)-1-cyclohexene (22): ^{1}H NMR (250 MHz, acetone-d_6) δ 1.10 (d, 3 H), 1.50 (m, 4 H), 1.80 (s, 3 H), 1.95 (m, 4 H), 4.30 (quintet, 1 H), 5.50 (m, 1 H), 6.95 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_{10}H_{18}NO$ $(M + H)^+$ 168.1388, found 168.1388.

1-Acetamido-2-vinylcyclohexane (23): ^{1}H NMR (250 MHz, acetone-d_6) δ 1.40–1.65 (m, 8 H), 1.85 (s, 3 H), 2.15 (m, 2 H), 4.90 (dd, 1 H), 5.05 (dd, 1 H), 6.00 (dd, 1 H), 6.60 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_{10}H_{18}NO$ $(M + H)^+$ 168.1388, found 168.1388.

1-Acetamido-6-acetoxy-2(*E*)-hexene (24): ^{1}H NMR (300 MHz, acetone-d_6) δ 1.65 (quintet, 2 H), 1.85 (s, 3 H), 1.95 (s, 3 H), 2.05 (m, 2 H), 3.70 (t, 2 H), 4.00 (t, 2 H), 5.45–5.65 (m, 2 H), 7.10 (bs, 1 H); high-resolution mass spectrum, m/z calcd for $C_{10}H_{18}NO_3$ $(M + H)^+$ 200.1286, found 200.1286.

Supplementary Material Available: ^{1}H NMR spectra for **16–19** and **21–24** (8 pages). Ordering information is given on any current masthead page.

Mechanism of Phosphodiester Cleavage with β-Cyclodextrin

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Cyclodextrins are cyclic oligomers of glucose with a hydrophobic pocket that form complexes with a range of organic compounds.[1] The secondary hydroxyl groups are clustered around one rim of the cavity and have been found to act as catalysts in the hydrolysis of esters, lactams, amides, carbonates, and other compounds.[1] These hydroxyls have a pK_a of 12 and act in some instances as nucleophiles and in some instances as a general base to promote nucleophilic attack by water.

We have examined the reaction of cyclodextrin with phosphodiesters as part of an ongoing project to investigate the transition states of phosphoryl-transfer reactions using heavy-atom isotope effects.[2] Before doing the isotope effect studies on the cyclodextrin reaction, we needed to understand the overall chemical mechanism. This report covers our elucidation of the overall mechanism.

The reactions of cyclodextrins with several different types of phosphorus compounds have been previously studied. The fission of diaryl pyrophosphates in the

(1) For reviews see: (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*, Springer-Verlag: New York, 1978. (b) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. (c) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.

(2) Weiss, P. M.; Knight, W. B.; Cleland, W. W. *J. Am. Chem. Soc.* **1986**, *108*, 2761. Weiss, P. M.; Cleland, W. W. *J. Am. Chem. Soc.* **1989**, *111*, 1928.

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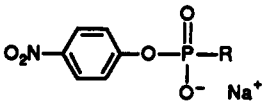
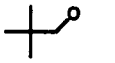
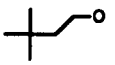
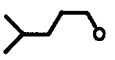
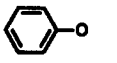
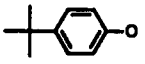
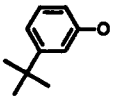
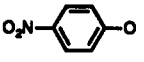
Table I. Rate Constants for Nitrophenol Release from Phosphodiester Substrates at pH 13 ($\times 10^6$ s^{-1})[a]



	R	background hydrolysis	cyclodextrin reaction	k_{cdxtrn}/k_{hydrol}[b]
1	CH_3O	0.19	0.19	1
2		0.07	2.78	40
3		0.11	10.13	92
4		0.11	2.87	26
5		0.44	7.98	18
6		0.43	0.43	1
7		0.39	5.22	13
8		4.70	42.4	9

[a] All reactions were carried out at 45 °C with concentrations of 100 mM buffer, 1 mM substrate, and, for the β-cyclodextrin reactions, 4 mM cyclodextrin. Further details appear in the Experimental Section. [b] $k_{cyclodextrin}/k_{hydrolysis}$.

presence of cyclodextrin and calcium ions was found to occur by a nucleophilic mechanism resulting in a phosphorylated cyclodextrin.[3] Cyclodextrin cleaves diaryl methylphosphonates by competing nucleophilic and general-base mechanisms.[4] Cyclodextrin catalyzes the attack of water preferentially on the P–O(2′) bond of ribonucleotide 2′–3′ cyclic phosphates.[5] The latter reaction does not proceed via a cyclodextrin inclusion complex but by a face-to-face hydrogen-bonded encounter complex between the substrate and the secondary hydroxyl side of the cyclodextrin.

The phosphodiesters in Table I were synthesized,[6] and their cleavage rates were measured in the absence and presence of β-cyclodextrin. On the basis of known dissociation constants,[1] it is expected that for diesters 2–7 the preferred complex will have the more hydrophobic R group inside and the *p*-nitrophenol leaving group outside the cyclodextrin cavity. Where R is methyl (1), the preference will be reversed and the *p*-nitrophenyl ring will be complexed. This series of substrates allows us to test the effect of the length of the complexing hydrophobic group and to test for leaving group preference between the complexed or the uncomplexed ester group. We also wanted to determine whether the cyclodextrin cleaved the diester substrates by a nucleophilic or a general-base mechanism.

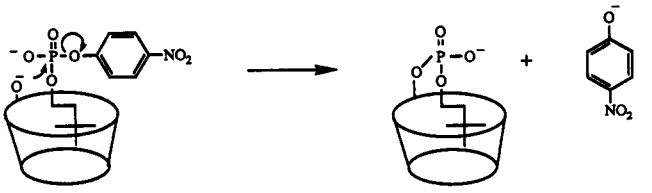
Results

The rate constant for the cleavage of each of the phosphodiesters was measured in buffer at pH 13 with and without β-cyclodextrin added. The results are listed in Table I. The accelerations in cleavage caused by the cyclodextrin ranged from a high of 92 for **3** to a low of 1 (no cyclodextrin reaction) for **1** and **6**. The differences in the acceleration of *p*-nitrophenol release from the different diesters by cyclodextrin most likely reflect varying proximities of the phosphate group to the secondary hydroxyls of the cyclodextrin as the length of the hydrophobic group varies. The lack of any cyclodextrin reaction with the *p-tert*-butylphenyl ester **6** indicates the phosphoryl group is placed out of reach of the cyclodextrin hydroxyl groups when the *p-tert*-butyl group is in the cavity but is brought into closer proximity when the *tert*-butyl group is in the meta position in **7**. This same behavior has been noted in reactions of cyclodextrins with *tert*-butylphenyl esters.[7]

Substrates **1** and **8** will both form cyclodextrin complexes with the nitrophenol group in the cavity, but only **8** exhibited a reaction. The lack of any measurable cyclodextrin reaction with the methyl ester **1** indicates a strong preference for phosphodiester cleavage to occur in complexes with the leaving group outside the cyclodextrin cavity. This same preference is found in the cyclodextrin-catalyzed cleavage of diaryl pyrophosphates.[3]

The pH profile from pH 9 to 13 of the cyclodextrin reaction with substrate **3** is essentially flat below pH 12 and then shows an increase in rate of 1 order of magnitude between pH 12 and 13. This is consistent with a mechanism involving the secondary hydroxyl groups of the cyclodextrin. A reaction at pH 13 using a 3-fold excess of phosphodiester over cyclodextrin ceased after one-third of the substrate was consumed. This rules out a mechanism where the cyclodextrin acts as a general-base catalyst for the addition of water to the phosphodiester. Analytical TLC of this reaction mixture indicated that all of the cyclodextrin in the reaction mixture had been altered. A similar experiment was run on a larger scale, and the cyclodextrin product was isolated by preparative thick-layer chromatography on silica gel and subsequent reversed-phase HPLC. The purified cyclodextrin derivative showed a single proton-decoupled ^{31}P NMR resonance at 3.2 ppm. Proton NMR showed that the 3,3-dimethylbutyl group was still attached, indicating the product to be a cyclodextrin 3,3-dimethylbutyl phosphodiester.

Scheme I



Conclusions

Taken together, these data point to a mechanism for cleavage of complexed phosphodiesters by cyclodextrin consisting of nucleophilic attack by one of the secondary hydroxyl groups of the cyclodextrin from an inclusion complex in which the leaving group resides outside the cavity (Scheme I). The reaction is a net phosphoryl transfer from *p*-nitrophenol to the cyclodextrin, producing a cyclodextrin phosphodiester that is catalytically inactive. The phosphodiester mechanism observed in this work is thus similar to that for diaryl pyrophosphates[3,8] but differs

(3) Heinrich, N.; Cramer, F. *J. Am. Chem. Soc.* **1965**, *87*, 1121.
(4) Brass, H. J.; Bender, M. L. *J. Am. Chem. Soc.* **1973**, *95*, 5391.
(5) Komiyama, M. *J. Am. Chem. Soc.* **1984**, *111*, 3046.
(6) Turner, A. F.; Khorana, H. G. *J. Am. Chem. Soc.* **1959**, *81*, 4651.
(7) Van Etten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242.
(8) The arylcyclodextrin phosphodiester generated in the pyrophosphate study[3] hydrolyzed to the phenol and the cyclodextrin monoester. The less activated alipatic alcohol cyclodextrin diester formed in this study did not hydrolyze to a detectable degree under our reaction conditions.

from that for phosphonates[4] where cyclodextrin acts competitively as a general base for the addition of water and as a nucleophile. The nucleophilic mechanism observed in this phosphodiester work also differs from that seen for ribonucleotide 2′–3′ cyclic phosphodiesters.[5] The latter substrates do not form cyclodextrin inclusion complexes, reacting instead via a face-to-face hydrogen-bonded complex with the secondary hydroxyl side of the cyclodextrin. The ability of the phosphodiesters in this study to form inclusion complexes accounts for the difference in mechanism.

Experimental Section

β-Cyclodextrin was purchased from Aldrich and used as received. All phosphodiester substrates (except for 8, which was purchased from Sigma) were prepared from *p*-nitrophenyl phosphorodichoridate and the corresponding alcohol or phenol by the method of Turner.[6] The sodium salt of each diester obtained after ion exchange was characterized by phosphorus and proton NMR.

Kinetic reactions were carried out in sealed 2-mL vials in a thermostated water bath at 45 °C. Reactions were made up to concentrations of 100 mM buffer (NaOH/KCl, pH 13.0), 1 mM substrate, and, for the β-cyclodextrin reactions, 4 mM cyclodextrin. Rates of reaction were measured by withdrawing aliquots at fixed time intervals and assaying for nitrophenolate anion at 400 nm. A control experiment ensured that the presence of the β-cyclodextrin had no effect on the absorbance of nitrophenolate anion. The data were fitted to the single-exponential program EXPFIT of Cleland to obtain the rate constants in the table; good fits of the data were obtained, and standard errors in the rates were 3% or less in all cases.

In the experiment to recover the phosphorylated cyclodextrin, a 4-mL reaction mixture containing β-cyclodextrin (2.5 mM, 10 μmol) and phosphodiester **3** (7.5 mM, 30 μmol) was allowed to react under the same conditions for 3 days by which time *p*-nitrophenol release had slowed to the uncatalyzed background rate. Analytical TLC on silica gel eluting with 2-propanol/water/triethylamine (1:1:1) showed the disappearance of β-cyclodextrin (R_f = 0.23) and the appearance of a new spot at R_f = 0.32. Cyclodextrin spots were visualized after elution by spraying the dried TLC plate with 2 M sulfuric acid and heating the TLC plate on a hot plate; cyclodextrin compounds appear as black spots. After the reaction mixture was evaporated down to a volume of about 0.5 mL, the derivatized cyclodextrin was separated from nitrophenol and unreacted phosphodiester by preparative TLC on silica gel with the same solvent system described above. The cyclodextrin product was then further purified by reversed-phase HPLC, eluting with 25% ethanol in water. ^{1}H NMR (D_2O), in addition to the cyclodextrin signals showed δ 1.03 (s, 9 H), 1.73 (t, *J* 7.4 Hz, 2 H), and 4.14 (dd, *J* = 7.4 and 7.4 Hz, 2 H). For comparison, the signals from the 3,3-dimethylbutyl group in the starting diester **3** in D_2O were δ 0.91 (s, 9 H), 1.59 (t, *J* = 7.4 Hz, 2 H), and 4.07 (dd, *J* = 7.4 Hz, 2 H).

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