hexadecamer yielded a single band on a denaturing electropherogram (10% acrylamide/7 M urea, 60 °C) and a single Gaussian peak on an analytical HPLC chromatogram (not shown). The final yield of material was about 180 OD_{260} units (20%).

Methodology. 5-Iodo-2′-deoxyuridine (IdU) and 4,4′-dimethoxytrityl chloride were purchased from Sigma Chemical Co., St. Louis, MO, and were used without further purification. Chloro(diisopropylamino)methoxyphosphine and all other phosphoramidites were purchased from Applied Bionetics, Emeryville, CA. TLCs were run on Kodak precoated silica gel plates with fluorescent indicator. Elemental analyses were carried out by Atlantic Microlabs, Atlanta, GA. NMR spectra were determined on either a Varian EM360L or Varian 300 XL spectrometer. HPLC chromatograms were performed with a Varian 5000 HPLC system using a Whatman Partisil-10 ODS-3 reverse-phase column. All HPLC solutions were filtered through 0.45-μm filters from Milipore, Inc., Bedford, MA. Electrophoresis reagents were purchased from Bio-Rad, Inc., Rockville Centre, NY.

5′-(4,4′-Dimethoxytrityl)-5-iodo-2′-deoxyuridine. 5-Iodo-2′-deoxyuridine (1.0 g, 2.8 mmol) was treated with 4,4′-dimethoxytrityl chloride (1.4 g, 4.2 mmol), according to the literature procedures[6] to yield two major products as determined by TLC (R_f values of 0.45 and 0.62, respectively, in ethyl acetate (ETOAC)). Separation of the two compounds was effected by preparative-scale reverse-phase HPLC using the Whatman Partisil-10 ODS-3 column: The material obtained above was dissolved in acetonitrile and injected into the HPLC system in small aliquots. The first elution solvent was acetonitrile/water (60/40), which eluted a compound having an R_f = 0.44 in the above TLC system. A linear gradient of 60% to 85% acetonitrile was then run to elute a second compound, which had an R_f = 0.63. The foams obtained from the evaporation of the solvent from the eluted peaks were analyzed by ^1H NMR and elemental analysis. Peak 1 was identified as 5′-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine (1.2 g, 65%), and peak 2 was identified as 3′,5′-bis-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine (0.8 g, 30%).

[5′-(4,4′-Dimethoxytrityl)-5-iodo-2′-deoxy-3′-uridinyl](*N*,*N*-diisopropylamino)methoxyphosphine (DMTr-5-IdU Phosphoramidite). The 5′-DMTr-IdU (1.0 g, 1.4 mmol), obtained above, was treated with chloro(diisopropylamino)methoxyphosphine (300 μL, 2.1 mmol) as per McBride and Caruthers.[7] Subsequent workup of the reaction mixture yielded a single product which was pure by TLC (R_f = 0.65 in EtOAC). This product was identified as the DMTr-5-IdU phosphoramidite (0.95 g, 76%) by ^1H NMR, ^{31}P NMR (see Figure 2), and elemental analysis (Theory: C, 54.31; H, 5.56. Found: C, 54.01; H, 5.45).

Synthesis of Tritylated Oligodeoxynucleotide. The synthesis of the tritylated oligomer was performed on a 10-μmol scale by using DMTr-*N*-benzoylcytidine CPG or silica resin and 12-fold excesses of the suitably protected phosphoramidites (i.e., DMTr-thymidine, DMTr-*N*-benzoyladenosine, DMTr-*N*-benzoylcytidine, DMTr-N-isobutyrylguanosine, and DMTr-5-I-deoxyuridine-*N*,*N*-diisopropylaminomethoxyphosphines), with coupling being effected by tetrazole. The detritylation (using 3% TCA), coupling, capping, hydrolytic wash, and oxidizing steps were carried out according to the literature procedures.[8-11] The trityl moiety of the last nucleoside was left on the oligodeoxynucleotide to allow purification via trityl-selection reverse-phase HPLC. As dictated by the sensitivity of IdU to hot NH_4OH, the resin was stirred for 48 h at *room temperature* in concentrated NH_4OH (29%) to effect the phosphate deprotection, desupportylation, and base deprotection of the tritylated oligomer.

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Registry No. (5′→3′)-CG-IdU-GCTCACCGAA-IdU-GC, 104438-40-6; DMTr-5-IdU phosphoramidite, 104393-15-9; 5-iodo-2′-deoxyuridine, 54-42-2; 5′-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine, 104375-88-4; 3′,5′-bis(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine, 104375-89-5; chloro(diisopropylamino)-methoxyphosphine, 86030-43-5.

Organoiodinane Oxyanions as Reagents for the Cleavage of a Reactive Phosphate

Robert A. Moss,* Swati Chatterjee, and Bogusława Wilk

Wright and Rieman Laboratories, Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903

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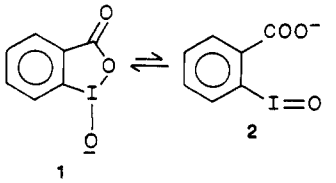
In its preferred, valence tautomeric 1-oxido-1,2-benziodoxol-3(1*H*)-one from (**1**), *o*-iodosobenzoate (**2**) is a remarkable catalyst for the cleavage of reactive phosphates in dilute cationic surfactant solution.[1] The para (to iodoso) octyloxy derivative of **1**/**2**[2] and a related functional surfactant,[3] are even more powerful reagents for the degradation of these toxic[4] compounds.



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There are several analogues of **1**/**2** where the benzo ring is substituted or the heterocyclic ring is of different size or formed by interaction of a different functionality with the iodoso moiety.[5] The continuing need for efficient catalytic reagents to decontaminate areas affected by toxic phosphates led us to determine kinetic parameters for the cleavage of the test substrate, *p*-nitrophenyl diphenylphosphate (PNPDPP), by aqueous micellar cetyltrimethylammonium chloride (CTACl) solutions of these other organoiodinane oxyanions. The new results have been obtained under conditions comparable to those previously employed with **1**/**2**.

Results and Discussion

Synthesis. The five reagents examined in this study are shown in their heterocyclic, oxyanionic forms in structures **3**–**7**. Catalyst **3**, the valence tautomer of 5-methoxy-2-iodosobenzoate, was prepared from 4-iodo-3-

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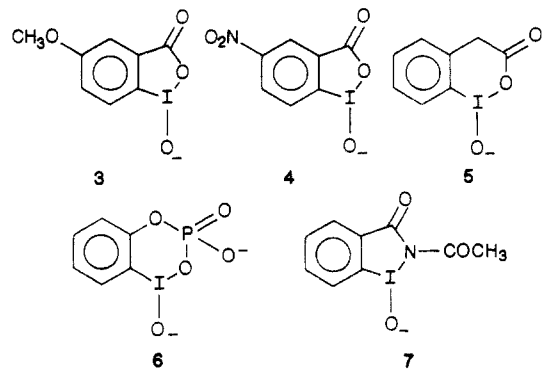

carboethoxyphenol[3] by methylation of the phenoxide (NaOEt/MeI), saponification of the ester (NaOH/aqueous MeOH), and standard oxidation of the iodo moiety by chlorination/hydrolysis.[6]

Catalyst **4**, the valence tautomer of 5-nitro-2-iodosobenzoate, was prepared by simultaneous nitration and iodo to iodoso oxidation of 2-iodobenzoic acid.[7] Catalyst **5**, the valence tautomer of *o*-iodosophenylacetate was prepared from *o*-iodobenzyl chloride by conversion to *o*-iodophenylacetonitrile (NaCN, H_2O, CH_2Cl_2, $Bu_4N^+Br^-$, phase-transfer catalysis), transformation of the cyanide moiety to the carboxylic acid (NaOH/EtOH, reflux, and then HCl), and chlorination/hydrolysis of the iodo to the iodoso group.[6,8,9]

Catalyst **6**, the valence tautomer of *o*-iodophenylphosphate, was prepared from *o*-iodophenol by the method of Leffler and Jaffe.[10] Compound **7**, the anion of 2-acetyl-1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodazole, was prepared from *o*-iodobenzamide by peracetic acid oxidation.[11]

Reagents **3–7** were either fully characterized (if new) or were comparable to the compounds described in the literature. Each gave >98% of iodoso activity in the standard $KI/Na_2S_2O_3$ iodometric titration.[6]

pK_a Determinations. The reactive forms of catalysts **1** and **3–7** are the oxyanions. Therefore, the pK_a for the IOH → IO^- ionization is an important datum in each case. A pH–rate constant profile for the cleavage of 1×10^{-5} M *p*-nitrophenyl acetate by 1×10^{-4} M **1** in 1×10^{-2} M CTACl gave pK_a = 7.25 for **1** under micellar reaction conditions.[1] In the present instance, we determined pH–rate profiles for the cleavages of PNPDPP[1-3] by **4**, **5**, or **7** under related conditions: 1×10^{-5} M PNPDPP, 1×10^{-4} M iodoso reagent, and 1×10^{-3} M CTACl in 0.02 M phosphate buffers adjusted to the appropriate pH. The solutions also contained 1.0 vol % of DMF and 0.33 vol % of CH_3CN as consequences of reagent and substrate introduction.

The case of catalyst **4** is illustrative. Pseudo-first-order rate constants for PNPDPP cleavages at 25 °C were spectrophotometrically determined by following the release of *p*-nitrophenolate ion at 400 nm at seven pH's between 6.5 and 8.0. A plot of log k_ψ vs. pH (Figure 1) gave a sharp discontinuity at pH 6.73 which was taken as the systemic pK_a of **4** under the micellar reaction conditions. This pK_a implies that **4** is ~95% in the anionic form at pH 8, where our micellar phosphate cleavage reactions are normally conducted.

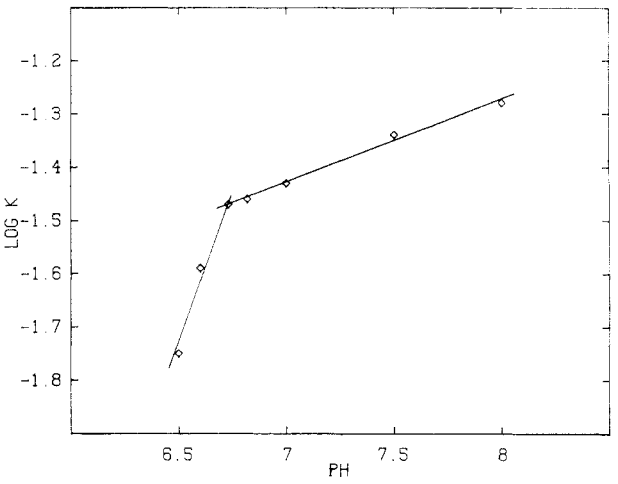

Figure 1. pH–rate profile for the cleavage of 1×10^{-5} M PNPDPP by 1×10^{-4} M **4** in 1×10^{-3} M CTACl; log $k\psi$ (s^{-1}) vs. pH. The discontinuity at pH 6.7 is taken as the systemic pK_a of **4**.

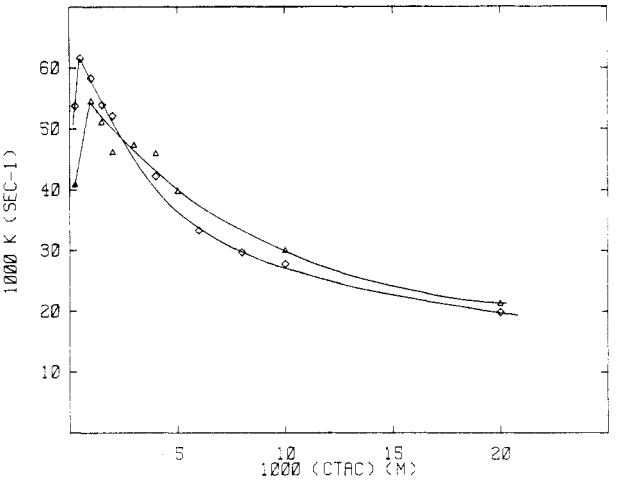

Figure 2. Pseudo-first-order rate constants ($k\psi$, s^{-1}) for the cleavages of 1×10^{-5} M PNPDPP by 1.0×10^{-4} M **3** (△) or **4** (◇) as a function of [CTACl] at pH 8.0. See text for reaction conditions and Table I for $k\psi^{max}$ values.

pH rate profiles (not shown) were similarly determined for **5** and **7** under analogous micellar conditions, affording pK_a values of 7.44 and 7.62, respectively, corresponding to ionizations of ~78% and ~71% in pH 8 micellar CTACl solutions.

pK_a values were not determined for **3** or **6**. The pK_a for **3** was taken as 7.2, that of its *n*-octyloxy analogue under CTACl micellar conditions.[2] For **6**, the systemic pK_a should be <7.86, the potentiometric pK_a of **6** in water.[10] Cationic micelles are well-known to lower the pK_a's of bound neutral acids. For example, phenol and thiophenol experience pK_a depressions of ~0.6 and 0.4–0.5 units, respectively, in micellar CTA bromide solutions.[12] If **6** experiences a similar pK_a depression in micellar CTACl, then we estimate its pK_a as ~7.4 and its extent of ionization as ~80% at pH 8.

Kinetic Studies. The catalytic properties of **3–7** were assessed from full rate constant–[surfactant] profiles for the cleavage of PNPDPP in micellar CTACl. All reactions were carried out under identical conditions: 0.02 M pH 8.0 phosphate buffer, μ = 0.08 (NaCl), 25 ± 0.5 °C, [PNPDPP] = 1.0×10^{-5} M, [catalyst] = 1.0×10^{-4} M. Except in the case of **7**, the buffer solutions also contained 1.0 vol

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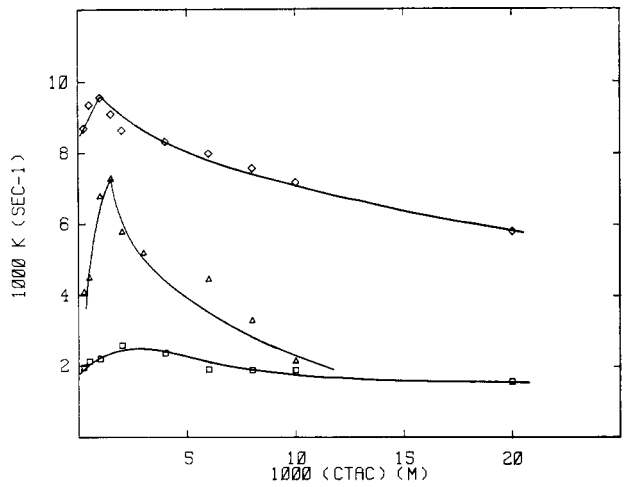


Figure 3. Pseudo-first-order rate constants ($k\psi$, s^{-1}) for the cleavages of 1×10^{-5} M PNPDPP by 1.0×10^{-4} M **5** (◇), **6** (△), or **7** (□) as a function of [CTACl] at pH 8.0. The $k\psi$ values for **7** have been arbitrarily multiplied by 10 to bring them on scale. See text for reaction conditions and Table I for k_ψ^{max} values.

Table I. Kinetic Parameters for Micellar Cleavages of PNPDPP[a]

reagent	10^3[CTACl], M[b]	$10^2 k_\psi^{max}$, s^{-1}	k_{cat}, L/M·s[c]
none[d]	1.00	0.018	
1[e]	1.00	6.45	759
3	1.00	5.46	635
4	0.50	6.16	648
5	1.00	0.956	123
6	1.50	0.73	91[f]
7	2.00	0.026	3.6

[a] Conditions: 0.02 M pH 8.0 phosphate buffer, μ = 0.08 (NaCl), 25 ± 0.5 °C, [PNPDPP] = 1.0×10^{-5} M, [reagent] = 1.0×10^{-4} M, 1.0 vol % DMF, 0.33 vol % CH_3CN. [b] Concentration of CTACl at which k_ψ^{max} was observed; see Figures 2 and 3. [c] $k_{cat} = k_\psi^{max}$/[reagent], corrected for 100% ionization of the catalytic reagent. See text for discussion of pK_a's and extents of ionization. [d] No catalyst present. This value may be taken as "k_0" in micellar CTACl alone. [e] From ref 2. [f] This value is estimated based on a pK_a of 7.4; see text.

% of DMF and 0.33 vol % of CH_3CN. Solubilization of **7** required sonication (80 W, 15 min, 25 °C) in the CTACl buffer solution. Pseudo-first-order rate constants, k_ψ, were determined for PNPDPP cleavage at each [CTACl], for each catalyst, by spectroscopically following the release of *p*-nitrophenoxide ion at 400 nm. The reproducibility (duplicate runs) of $k\psi$ was better than ±3.3% with all catalysts, save in one instance with **4** at 8×10^{-3} M, where the average deviation in $k\psi$ was ±5.8%.

The resulting $k\psi$/[CTACl] profiles appear in Figure 2 (for **3** and **4**) and Figure 3 (**5**–**7**). In Table I, we collect values of $k_\psi$max for each catalyst from the appropriate profile, as well as the CTACl concentration necessary to obtain $k_\psi$max. Also included in Table I are calculated second-order "catalytic" rate constants ($k_{cat} = k_\psi^{max}$/[catalyst]). These are corrected for 100% ionization of the IOH reagents to IO^- (see above). The last column of Table I therefore provides a qualitative comparison of the cleavage power of the various reagents.

Turnover Experiments. In order to examine the catalytic efficiency of the organoiodinane oxyanion reagents **3**–**6**, kinetic runs were carried out in the presence of *excess* PNPDPP. Two regimes were studied: a substrate/catalyst ratio of 2:1 in 1×10^{-3} M CTACl and a substrate/catalyst ratio of 5:1 in 1×10^{-2} M CTACl. The results appear in Table II, where the observed pseudo-first-order rate constants are compared with $k\psi$ for 1:10 substrate/catalyst ratios under the two surfactant concentration regimes. "Burst" kinetics[3] were not observed in the excess substrate experiments.

Table II. Cleavage of Excess PNPDPP by Micellar Catalysts[a]

		1×10^{-3} M CTACl		1×10^{-2} M CTACl[c]	
cat.[b]	10^5 [PNPDPP], M	[PNPDPP]/[cat.]	k_ψ, s^{-1}	[PNPDPP]/[cat.]	k_ψ, s^{-1}
none[d]			0.00018		0.00018
1[e]	1.0			1:10	0.026
1[e]	50.0			5:1	0.024
3	1.0	1:10	0.055	1:10	0.023
3	20.0 or 50.0	2:1	0.038	5:1	0.021
4	1.0	1:10[f]	0.062	1:10	0.024
4	20.0 or 50.0	2:1	0.035	5:1	0.020
5	1.0	1:10	0.0096	1:10	0.0070
5	20.0 or 50.0	2:1	0.0058	5:1	0.0064
6	1.0	1:10[g]	0.0073	1:10	0.0020
6	20.0 or 50.0	2:1	0.0042	5:1	0.0014

[a] Conditions: as in Table I, note *a*; [catalyst] = 1.0×10^{-4} M in all cases, other concentrations as indicated. *p*-Nitrophenoxide ion was followed at 440 nm when [PNPDPP] = 5×10^{-4} M and at 400 nm elsewhere. [b] See text for structures of catalysts. [c] 1.67 vol % of acetonitrile and 1.0 vol % of DMF were present in these runs. [d] No catalyst present, in micellar CTACl alone. [e] From ref 1. [f] [CTACl] = 0.5×10^{-3} M. [g] [CTACl] = 1.5×10^{-3} M.

Comments. With the exception of the rather unreactive benziodazole **7**, Table I reveals reagents **3**–**6** to be good to excellent catalysts for the cleavage of the test phosphate substrate PNPDPP in mildly basic, dilute aqueous micellar CTACl solutions. For example, a 300-fold enhancement in $k\psi$ is provided by 10^{-4} M **3** in 10^{-3} M CTACl, relative to the CTACl solution alone.

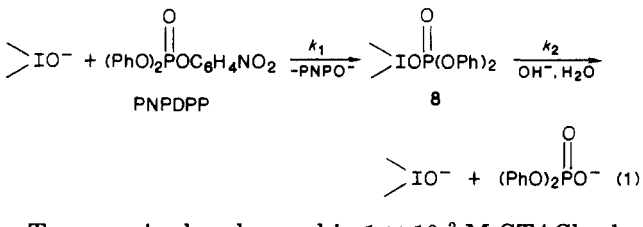
When corrected for differing extents of ionization at pH 8, the potency of the various IO^- reagents in the micellar cleavage of PNPDPP stands in the order **1** > **3** ~ **4** ≫ **5** > **6** ≫ **7**. It is perhaps ironic that the originally studied *o*-iodosobenzoate (**1**/**2**)[1] is superior to any of its newly examined relatives. Larger or differently constructed heterocyclic rings, as in **5**–**7**, afford kinetically inferior IO^- cleavage reagents. Substitution of electron-donating (CH_3O) or withdrawing (NO_2) substituents para to the IO^- function of **1** (leading to **3** or **4**, respectively) gives reagents that are slightly inferior to **1**.

Two points of inteerest are connected with the kinetic similarity of reagents **1**, **3**, and **4**. (a) The ~20-fold kinetic advantage of the *n*-octyloxy analogue of **3**[2] over either **3** or **1** is most likely connected with improved binding to the CTACl micelle rather than with an electronic effect on IO^- due to the RO substituent. (b) It is initially surprising that the wide substituent variation between reagents **1**, **3**, and **4** causes so little alteration in their kinetic behavior toward PNPDPP; i.e., the IO^- substituent (specifically the negative charge density on oxygen) is little affected by CH_3O to H to NO_2 manipulation at the para carbon atom. This suggests that the iodine atom does not very effectively transmit electronic changes between the benzo ring and the oxyanion, perhaps as a result of the 5p–2p orbital mismatch between I and O or C.[13,14]

The efficient catalytic turnover previously noted for the *o*-iodosobenzoate/CTACl system[1,2] persists with reagents **3**–**6** (Table II). In 10^{-2} M CTACl, reagents **1**, **3**, and **4** are comparably reactive toward PNPDPP, and $k\psi$ is nearly identical whether [substrate]/[catalyst] is 1:10 or 5:1.

(13) See, however, ref 5, p 752.
(14) For example, the NO_2 to CH_3O substituent change accounts for ~1.1 log unit enhancement of ionization in the para-substituted benzoic acids, but only ~0.5 log unit enhancement in the ionization of **4** vs. **3** (see above).

These reagents exhibit true catalytic turnover and afford rate enhancements of ~110–130 (relative to CTACl alone) even in the presence of 5-fold excess substrate. Although less reactive toward PNPDPP, reagents **5** and **6** also exhibit turnover kinetics. If we take eq 1 as mechanistically representative of the overall phosphate cleavage, then, at least at 1×10^{-2} M CTACl and pH 8, both the k_1 and k_2 processes occur at similar rates; the absence of burst kinetics[3] in the presence of excess substrate clearly shows that k_1 cannot be substantially larger than k_2.



Turnover is also observed in 1×10^{-3} M CTACl solutions. Here, however, $k\psi$ is reduced ~30–40% in the presence of 2-fold excess substrate, relative to the case where the catalyst is in 10-fold excess. Partly, this is due to the circumstance that 10^{-3} M is close to the optimal CTACl concentration for the k_1 step (see Table I). Even so, rate enhancements of ~200 are brought about at 10^{-3} M CTACl by **3** or **4**, in the presence of *excess* substrate. These reagents are therefore efficient catalysts for the degradation of active phosphates under micellar conditions.

Experimental Section

General Methods. Melting points are uncorrected. NMR spectra were measured with a Varian T-60 spectrometer and chemical shifts are reported relative to internal Me_4Si. IR spectra were obtained on a Perkin-Elmer Model 727B spectrometer. Microanalyses were preformed by Robertson Laboratory, Florham Park, NJ.

Materials. PNPDPP was prepared and purified by literature methods.[15] CTACl was obtained from Eastman and recrystallized several times from methanol/ether.

5-Methoxy-2-iodosobenzoic Acid (5-Methoxy-1-hydroxy-1,2-benziodoxol-3(1*H*)-one, 3-OH). To a solution of NaOEt (1 g of Na in 30 mL of ethanol, 43.5 mmol) was added 1.0 g (3.4 mmol) of ethyl 5-hydroxy-2-iodobenzoate.[3] The resulting solution was added dropwise over 1 h to 3.0 g (21 mmol) of CH_3I. The reaction mixture was stirred at 25 °C for 4 days and then refluxed for 10 h. Ethanol was removed on the rotary evaporator, and the residue was extracted with chloroform. Sodium iodide was filtered; the chloroform was backwashed with water twice, dried, and concentrated on the rotary evaporator to afford crude ethyl 5-methoxy-2-iodobenzoate. This was dissolved in EtOAc and chromatographed on silica gel with 5:1 hexane/EtOAc as eluent. We obtained 700 mg (2.29 mmol, 67% yield) of the pure oily ethyl 5-methoxy-2-iodobenzoate.[16]

The ester was saponified by refluxing 700 mg (2.29 mmol) in 30 mL of methanol and 5 mL of 1 M aqueous NaOH for 5 h. The reaction mixture was cooled and acidified with 6 N HCl. Excess methanol was removed under reduced pressure. The residue was thoroughly extracted with $CHCl_3$. The extract was dried over $MgSO_4$, filtered, and stripped of solvent to give crude 5-methoxy-2-iodobenzoic acid that was recrystallized from $CHCl_3$/hexane to give 450 mg (1.62 mmol, 71%) of the pure acid, mp 135–138 °C.[17] Anal. Calcd for $C_8H_7IO_3$: C, 34.5; H, 2.54; I, 45.7. Found: C, 34.3; H, 2.64; I, 45.9.

The 5-methoxy-2-iodobenzoic acid was oxidized to the desired iodoso compound by the method of Lucas and Kennedy.[6] Thus 450 mg (1.62 mmol) of the iodide was dissolved in ~10 mL of $CHCl_3$ and cooled in an ice–salt mixture while Cl_2 gas was passed through for 3–4 h. A yellow crystalline precipitate separated, was washed with a few milliliters of $CHCl_3$, and then was mixed with 1.6 g of Na_2CO_3 and several grams of crushed ice to obtain a paste. To this was added 2.5 mL of 3 N aqueous NaOH. After 15 min of being stirred (with addition of serveral chips of ice), the solution was neutralized with 3 N H_2SO_4. The resulting white precipitate was filtered, washed with $CHCl_3$, and dried in vacuo to give 340 mg (1.16 mmol, 72%) of 5-methoxy-2-iodosobenzoic acid: mp 200–203 °C dec; IR (Nujol) 2430 (I–OH),[9] 1620 (C=O)[9] cm^{-1}. The compound showed 100 ± 5% of iodoso activity by iodometric titration.[6]

5-Nitro-2-iodosobenzoic Acid (5-Nitro-1-hydroxy-1,2-benziodoxol-3(1*H*)-one, 4-OH).[7] This compound was prepared in 48% yield from *o*-iodobenzoic acid (Aldrich) exactly as described by Morrison and Hooz:[7] mp 227–228 °C dec (lit.[7] mp 229–229.5 °C dec); IR (Nujol, cm^{-1}): 2450 (I–OH),[9] 1620 (C=O), 1480 and 1380 (NO_2) cm^{-1} (lit. IR[7] 1616, 1523, and 1348 cm^{-1}). The iodoso compound gave 100 ± 5% of activity by iodometric titration.[6]

***o*-Iodosophenylacetic Acid (5-OH).**[8] A mixture of 4.0 g (16 mmol) of *o*-iodobenzyl chloride (Aldrich) in 15 mL of CH_2Cl_2 was refluxed for 10 h with 2.5 g (51 mmol) of NaCN and 60 mg (0.18 mmol) of tetra *n*-butylammonium bromide in 6 mL of water.[18] Solvent was removed under reduced pressure, and the residue was distilled over a short Vigreux column to give 3.4 g (14 mmol, 88%) of *o*-iodophenylacetonitrile: bp 95–97 °C (0.3 mmHg) [lit.[19] bp 140 °C (4 mmHg); IR (neat) 2250 (CN) cm^{-1}.

The nitrile (3.0 g, 12 mmol) was dissolved in 15 mL of ethanol, combined with a solution of 2.7 g (48 mmol) of KOH in 5 mL of water, and the whole was refluxed for 4 h.[20] Ethanol was stripped, and the residue was dissolved in 20 mL of water and washed with benzene. The aqueous solution was acidified with concentrated HCl to afford 3 g (11 mmol, 91%) of crude *o*-iodophenylacetic acid. Recrystallization from 30% aqueous ethanol afforded a pure sample: mp 112–114 °C (lit. mp 114–116 °C,[20] 113–114 °C[8]); IR ($CHCl_3$) 1710 (C=O) cm^{-1}.

The iodophenylacetic acid (0.60 g, 2.3 mmol) was converted to the iodo dichloride by the method of Baker et al,[9] giving 0.65 g (2.0 mmol, 87%) of yellow crystals, mp, 104–106 °C dec, after drying in vacuo (lit.[9] mp, 104 °C dec). The dichloride was then hydrolyzed to 0.25 g (0.90 mmol, 45%) of crude o-iodosophenylacetic acid by the procedure of Leffler;[8] although it was necessary to acidify with 6 N HCl to pH 6 after the $NaHCO_3$ treatment in order to obtain the crystalline product. Recrystallization[8] gave 120 mg of pure product: mp 126–128 °C dec [lit.[8] mp 130 °C (dependent on rate of heating)]; IR (Nujol) 2420 (I=O),[8] 1600 (C=O)[8] cm^{-1}. The compound showed 98% of iodoso activity by iodometric titration.[6]

***o*-Iodosophenylphosphoric Acid (1,3-Dihydroxy-1*H*-1,2,4,3-benziodadioxaphosphorin 3-Oxide, 6-OH).**[10] This compound was prepared by the three-step sequence of Leffler and Jaffe.[10] In the first step, 5.0 g (23 mmol) of *o*-iodophenol (Lancaster Synthesis) was reacted with 3.6 g (23 mmol) of phosphorus oxychloride and 2.0 g (25 mmol) of dry pyridine in 15 mL of hexane. Workup[10] and distillation gave 5.2 g (15 mmol, 67%) of *o*-iodophenoxyphosphorus oxychloride, bp 120–122 °C (0.025 mmHg) [lit.[10] bp 117 °C (0.025 mmHg). Next, 5.0 g (15 mmol) of this product was vigorously stirred for 30 min with 30 mL of water, affording, after workup,[10] crude *o*-iodophenylphosphoric acid as an hygroscopic semisolid. The latter was immediately oxidized at 0 °C by dropwise addition of 8 g (53 mmol) of 34% peracetic acid (Aldrich) over 45 min. The remainder of the procedure and purification followed the literature[10] and afforded 3.1 g (9.8 mmol, 65%) of crude *o*-iodosophenylphosphoric acid. Recrystallization from 150 mL of water (75–78 °C) gave 1.4 g of the pure material as fine white needles, mp 125–127 °C dec (lit.[10] mp 123–124 °C dec). The compound showed 100% of iodoso activity by iodometric titration.[6]

(15) Gulick, W. M., Jr.; Geske, D. H. *J. Am. Chem. Soc.* **1966**, *88*, 2928.
(16) NMR ($CDCl_3$) δ 1.42 (t, J = 7 Hz, 3 H, CH_2CH_3), 3.77 (s, 3 H, OCH_3), 4.40 (q, J = 7 Hz, 2 H, OCH_2), 6.70 (dd, J = 3, 9 Hz, 1 H, aryl H_4), 7.33 (d, J = 3 Hz, 1 H, aryl H_6), 7.80 (d, J = 9 Hz, 1 H, aryl H_3).
(17) The NMR spectrum was very similar to that of the ester[16] but lacked the OCH_2CH_3 resonances. The COO*H* resonance appeared at δ 10.75 as a broad singlet.
(18) Cf.: Starks, C. M. *J. Am. Chem. Soc.* **1971**, *93*, 195.
(19) Rapson, W. S.; Shuttleworth, R. G. *J. Chem. Soc.* **1941**, 487.
(20) Sindelar, K.; Metysova, J.; Protiva, M. *Collect. Czech. Chem. Commun.* **1972**, *37*, 1734.

o-Iodoso-N-acetylbenzamide (2-Acetyl-1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodazole, 7-OH).[11] To 50 mL of concentrated ammonium hydroxide was added with stirring 10 g (38 mmol) of o-iodobenzoyl chloride (Aldrich). The precipitate of crude o-iodobenzamide was filtered, washed with cold water, recrystallized from hot water, and dried to give 8.0 g (32 mmol, 84%) of pure o-iodobenzamide: mp 183–185 °C (lit.[21] mp 183 °C); IR (KBr) 3500, 3400 (NH_2), 1680 (C=O) cm^{-1}.

To a slurry of 7.0 g (28 mmol) of o-iodobenzamide in 50 mL of glacial acetic acid was added 15 g of Aldrich 34% peracetic acid (67 mmol peracid). The addition was carried out slowly and with stirring at 30 °C. After an additional 30 min, excess water was added, and the precipitate was filtered and dried. It was then extracted with ether to give a residual white powder (7). We obtained 6.3 g (21 mmol, 75%) of 7: R_f 0.35 [on Aldrich precoated silica gel on polyester TLC plates with fluorescent indicator, using MeOH eluent (R_f for o-iodobenzamide is 0.85 under these conditions)]; mp, 143–145 °C dec (lit.[11] mp 140 °C dec); IR (KBr) 1665, 1615 (C=O)[11] cm^{-1}. The compound showed 99% of iodoso activity by iodometric titration.[6]

Kinetic Studies. Reactions were followed on a Gilford Model 250 spectrophotometer coupled to a Gilford Model 6051 recorder. Constant-temperature circulating baths maintained reaction temperatures at 25 ± 0.5 °C. All buffers were prepared from steam-distilled water (distilled, USP, Electrified Water Co., East Orange, NJ) and were purged with nitrogen. Rate constants were obtained from computer-generated correlations of log ($A_\infty - A_t$) with time for the appearance of p-nitrophenoxide ion at 400 nm (unless otherwise indicated; cf., Table II). Conditions for all of the kinetic runs are described above. Rate constants are depicted graphically in Figures 2 and 3. Micellar reactions were generally followed to >90% completion and showed good first-order kinetics ($r > 0.999$). Values of k_ψ^{max} appear in Table I. Rate constants in the presence of excess PNPDPP are collected in Table II.

Acknowledgment. We are grateful to the U.S. Army Research Office for financial support. We thank Dr. Shanti Swarup and Professor Larry Romsted for helpful discussions.

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A Practical Synthesis of Mitomycin A and Its Analogues

D. M. Vyas,* D. Benigni, R. A. Partyka, and T. W. Doyle

Bristol-Myers Company, Pharmaceutical Research and Development Division, Wallingford, Connecticut 06492

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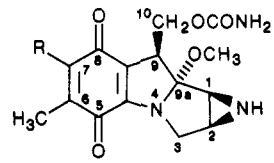
Mitomycin C (1),[1,2] an antineoplastic antibiotic isolated from fermentation broth of *Streptomyces caespetosius*, is currently used clinically in combination cancer chemotherapy against a wide variety solid tumors.[3] Analogue research targeted toward more efficacious and less myelosuppressive derivatives has proceeded at a steady pace. A useful semisynthetic approach has centered on the synthesis of 7-substituted mitosanes, namely, the 7-amino substituted (**2**)[4] and 7-alkoxy (**3**)[5] mitosanes; both of these

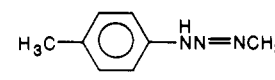


1, R = NH_2
2, R = R_1NH_2
3, R = OR_1
4, R = OCH_3
5, R = OH

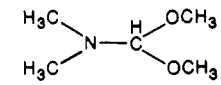
are prepared from mitomycin A (**4**).[4,6] The key source of mitomycin A, besides fermentation[2] (poor yield) is chemical synthesis[6] which involves base hydrolysis of **1** to an unstable intermediate **5** followed by methylation with diazomethane to **4**. Due to the inherent instability of **5** and the hazards involved in working with diazomethane, this process is undesirable for routine and large-scale synthesis. Moreover, with the lack of an efficient in-house fermentation source of mitomycin A (**4**), it was highly desirable for our analogue program to develop a practical and an efficient synthetic route to **4**.

In this paper we report a new synthetic process for mitomycin A (**4**), which is amenable to routine and scale-up preparations. The new methodology described herein is further extended to the preparation of 7-alkoxymitosanes **3**, directly from **5**; hitherto **3** was prepared by a simple alcoholysis process[5] involving reaction of mitomycin A (**4**) with a large excess of alcohol (ROH) in the presence of a catalytic amount of base. The serious limitations of this method are the reactivity (nucleophilicity) and the physical properties of the reacting alcohol; i.e., less nucleophilic alcohols such as 2-fluoroethanol do not react and viscous and solid alcohols are not suitable for the alcoholysis reaction.[7] Moreover, in many instances product isolation and purification is difficult.[7]

At the outset, 7-hydroxy-9a-methoxymitosane (**5**) was considered as a vinylogous acid. Consequently, known reagents which are good esterification agents were regarded as suitable alternatives to diazomethane O-alkylation of **5**. Two such commercial reagents are dimethylformamide dimethylacetal (**6**)[8] and 1-methyl-3-p-tolyltriazene (**7**).[9]



7



6

The former, although being a good methylating agent, is also an efficient formylating agent known to react with primary amines, amides, and urethanes to yield the corresponding amidines, whereas triazenes, e.g., **7** are only known to alkylate acids,[9] certain alcohols, phenols, and mercaptans.[10]

Since all our attempts to methylate 7-hydroxy-9a-methoxymitosane (**5**) with dimethylformamide dimethylacetal failed[11] we focussed our attention on the use of commercially available triazene **7**. Thus, when **5** was treated with approximately 2–3 equiv of triazene **7** in

(1) A trivial system of nomenclature which has found wide use in the mitomycin literature identifies mitomycin C (**1**) as 7-amino-9a-methoxymitosane and mitomycin A (**4**) as 7,9a-dimethoxymitosane.
(2) Remers, W. A. *Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol. 1, pp 221–276 and references cited therein.
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(10) Pochinok, V. Y. *Ukr. Khim. Zh. (Russ. Ed).* **1955**, *21*, 496, 682; **1952**, *18*, 631.
(11) Treatment of **5** with an excess of acetal in chloroform afforded only the C-10 carbamoyl amidine product of **5**. No indication (TLC) of methylation at the 7-OH functionality was observed.

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