Continued elution with mixtures of ether and EtOAc gave 59 mg (51%) of diol **3** as a pale yellow oil: ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G; MS, m/e 230.176 (calcd for $C_{12}H_{24}NO_3$, 230.175).

The mixture of **1** and **2** was resubjected to hydrolysis, affording an additional 10 mg (8%) of **3**.

***trans*-2,5-Dimethyl-2,5-bis[3-(methanesulfonyloxy)-propyl]pyrrolidinyl-1-oxy (4).** To a solution of **3** (59 mg, 0.257 mmol) in 5 mL of dry CH_2Cl_2 at −20 °C was added Et_3N (0.091 g, 0.90 mmol) followed by methanesulfonyl chloride (71 mg, 0.62 mmol). After 2.5 h, the reaction was allowed to warm to 0 °C and then brine and CH_2Cl_2 were added. The organic layer was washed with brine and dried ($MgSO_4$). This was passed through silica gel (0.7 g) which was flushed with ether–EtOAc. The combined eluent was evaporated to give 96 mg (96%) of **4** as a waxy solid suitable for the next reaction: TLC (EtOAc), 1 spot, R_f 0.7; IR no OH, 1170, 1335 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G. **4** was stable when stored at 0 °C, but slowly decomposed at 25 °C.

***trans*-2,5-Dimethyl-2,5-bis(3-azidopropyl)pyrrolidinyl-1-oxy (6).** A mixture of 170 mg (0.440 mmol) of **4**, 272 mg (4.18 mmol) of NaN_3, 4.4 mL of DMF, and 0.3 mL of water was stirred at 70 °C for 3.5 h and then cooled. The solvent was removed in vacuo and the residue was extracted with CH_2Cl_2. The extract was washed with brine and water and then dried ($MgSO_4$). This was concentrated and then passed through silica gel (0.8 g), giving 86 mg (70%) of **6** as a pale yellow oil suitable for use in the next experiment: IR 2090 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G.

***trans*-2,5-Dimethyl-2,5-bis(3-aminopropyl)pyrrolidinyl-1-oxy (7) and Oxalate Salt 8.** To a solution of **6** (86 mg, 0.307 mmol) in 20 mL of dry ether was added triphenylphosphine (177 mg, 0.676 mmol). An immediate liberation of gas was observed which slowed over 30 min. The mixture was refluxed for 12 h and then the solvent was removed, affording 256 mg of the waxy phosphinimine which resisted attempts at crystallization: IR no azide peak, 1200, 1100 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G. The entire sample was dissolved in 20 mL of ethanol–water, 1:1, and refluxed for 20 h. The solvent was removed in vacuo and the residue was treated with 5 mL of cold water (resulting pH was >10). HCl (2 N) was added to the chilled solution until pH 3–4. The white precipitate that had formed was extracted into ether (50 mL) followed by CH_2Cl_2 (25 mL). The chilled aqueous phase was then basified to pH 10–12 by addition of 0.5 mL of cold 15% NaOH. Brine (4 mL) was added and then the mixture was extracted with CH_2Cl_2. The extract was dried (K_2CO_3) and concentrated to dryness, giving 66 mg (65%) of bis amino nitroxide **7** as a yellow oil: ESR (CH_2Cl_2) 3 lines, a_N = 14.75 G.

To a 40-mg (0.175 mmol) sample of **7** dissolved in 1 mL of CH_2Cl_2 was added dropwise over 5 min a solution of 36.3 mg (0.404 mmol) of dry oxalic acid dissolved in 4 mL of ether. The resulting yellowish precipitate was collected and washed with ether. It was then dissolved in 0.4 mL of water and reprecipitated by addition of cold acetone. The precipitate was washed with ether and dried, giving 61 mg (84%) of oxalate salt **8**, mp 170–175 °C dec. Recyrstallization from EtOH–water, 2:1, gave the analytical specimen: mp 180–182 °C dec; ESR (MeOH–water, 1:1), 3 lines, a_N = 16.00 G. Anal. Calcd for $C_{16}H_{30}N_3O_9$: C, 47.04; H, 7.41; N, 10.29. Found: C, 46.74; H, 7.34; N, 10.12.

***trans*-1-[Methoxy(trifluoromethyl)phenylacetoxy]-2,5-dimethyl-2,5-bis[3-(benzoyloxy)propyl]pyrrolidine (10) and Diesters 5 and 9.** A solution containing diol **3** (21 mg, 0.091 mmol), benzoyl chloride (64 mg, 0.46 mmol), and pyridine (0.8 mL) was stirred at 0 °C for 4 h and then at 25 °C for 6 h. The solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. This was washed with cold, saturated $NaHCO_3$ and brine and then dried ($MgSO_4$). Removal of the solvent gave 80 mg of residue containing some benzoyl chloride. Preparative TLC (silica gel, elution with hexane–ether, 7:3) gave 38 mg (94%) of pure **5** as a yellow oil: IR 1715 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G. This was dissolved in ether (4 mL) and hydrogenated[1] over 20 mg of 10% Pd/C at 1 atm for 30 min. The mixture was filtered and the solvent was removed. The residue (crude **9**) was dissolved in 1.6 mL of dry CCl_4 containing 0.4 mL of pyridine. To this was added (+)-methoxy(trifluoromethyl)phenylacetyl chloride[13] (65 mg, 0.26 mmol; 0.65 mL of a CCl_4 stock solution) dropwise over 5 min. After 21 h the solvent was removed and the residue was dissolved in CH_2Cl_2. This was washed with chilled 5% HCl, 5% $NaHCO_3$, and brine and then dried ($MgSO_4$). Removal of the solvent gave 41.5 mg of residue that contained some unreacted **9**. Preparative TLC over silica gel (hexane–ether, 1:1) with recovery of the upper (R_f 0.5) band gave 26 mg (65%) of **10** as a colorless oil: IR 1770, 1715 cm^{-1}; 360-MHz ^1H NMR δ 1.160 (s, 6), 1.31–2.16 (m, 12), 3.491 and 3.513 (two s, 1:1, 3) 4.10–4.44 (m, 4), 7.30–7.49 (m, 7), 7.50–7.60 (m, 4), 7.96–8.15 (m, 4); ^{19}F NMR δ −71.767 and −71.809 (two s, 1:1) (from internal hexafluorobenzene taken to be −163 ppm). Anal. Calcd for $C_{36}H_{40}NO_7F$: C, 65.93; H, 6.15; N, 2.14. Found: C, 65.49; H, 6.31; N, 2.17.

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The Synthesis of a Deoxyoligonucleotide Incorporating 5-Iododeoxyuridine

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X-ray scattering methods must be included among the major techniques for studying macromolecular structure, both in solution and in the solid state. In both phases, the presence of a heavy atom label greatly facilitates the elucidation of the geometric and dynamic structure of the molecule. Of the common covalent modifications of nucleic acids, replacement of the thymine methyl group with an iodine atom is one of the most favorable: The iodine atom has a high electron density, yet its presence introduces a minimal perturbation to the molecular structure (e.g., ref 1). Because of the recent successes in utilizing phosphoramidite-based solid-state synthesis for oligodeoxynucleotides, we decided to attempt the synthesis of 5-iodouridine-containing oligonucleotides by the phosphoramidite method.

Several preparations of oligonucleotides incorporating modified deoxyuridine have recently been reported. For example, Metzler, et al. synthesized the 16 nucleotide base pair O_R3 operator containing 5-fluorodeoxyuridine via a phosphotriester method.[2] The preparation of oligomers incorporating 5-bromodeoxyuridine via a mixed phosphotriester–phosphoramidite method has also been detailed by Delort et al.[3] We wish to report the synthesis of a 5-iododeoxyuridine phosphoramidite and its incorporation into a deoxyhexadecanucleotide via phosphoramidite methodology. The oligomer is an analogue of strand 2 of immobile nucleic acid junction J_1,[4,5] and it is expected to be useful for solution and solid-state X-ray scattering studies.

The first step in this synthesis was the preparation of [5′-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxy-3′-uridinyl](*N*,*N*-diisopropylamino)methoxyphosphine (DMTr-5-IdU phosphoramidite, shown in Figure 1) via standard procedures.[6,7] The deoxyoligonucleotide (5′→3′)-CG-IdU-

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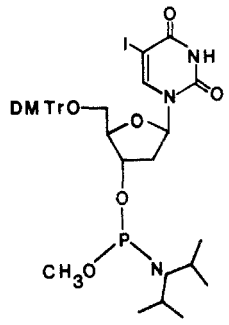

Figure 1. [5′-(4,4′-Dimethoxytrityl)-5-iodo-2′-deoxy-3′-uridinyl](*N*,*N*-diisopropylamino)methoxyphosphine (i.e., DMTr-5-IdU phosphoramidite). Note: DMTr = 4,4′-dimethoxytrityl.

GCTCACCGAA-IdU-GC was then synthesized by using phosphoramidite protocols.[8-11] The final deprotection steps were modified to ensure minimal loss of iodine. This deprotection procedure was developed from a study of the sensitivity of 5-IdU to NH_4OH. Purification and analysis of the final oligomer were accomplished by HPLC and gel electrophoretic techniques, respectively. We have also adapted the synthesis procedure to an Applied Biosystem Model 380B DNA Synthesizer.

Results and Discussion

5-Iodo-2′-deoxyuridine was treated with 4,4′-dimethoxytrityl chloride in pyridine to yield the 5′-mono- and 3′,5′-bistritylated compounds.[6] Separation of the 5′-DMTr-5-IdU from the bistritylated material was effected by preparative-scale reverse-phase HPLC, using a water/acetonitrile solvent system. Identification of the eluted compounds was determined by ^1H NMR and elemental analysis. The DMTr-5-IdU thus obtained was sufficiently pure (>95%) that it could be used without further purification. Phosphitylation of this compound by chloro(diisopropylamino)methoxyphosphine was carried out according to McBride and Caruthers.[7] Purity (>99%) and authenticity of the resultant phosphoramidite were confirmed by TLC, elemental analysis, and ^1H and ^{31}P NMR. Figure 2 shows the ^{31}P NMR spectrum of the final compound. As can be seen, DMTr-5-IdU phosphoramidite is characterized by resonances at 149.5 and 148.8 ppm from internal phosphoric acid (sealed capillary tube) due to two stereoisomers. The minor peak at 140.7 ppm is unidentified.

The 10-μmol solid-state synthesis of the hexadecamer incorporating the DMTr-5-IdU phosphoramidite was carried out with reagents and conditions as per the literature.[8-11] Average step yields were greater than 98%, as determined by trityl assay.[12] Detritylation of and subsequent coupling to the IdU residues were as quantitative and rapid as for the unmodified nucleotides. It should be emphasized that a highly heterogeneous final product is obtained unless a hydrolytic wash is included before the oxidation step, once the 5-IdU has been introduced into the system.[11]

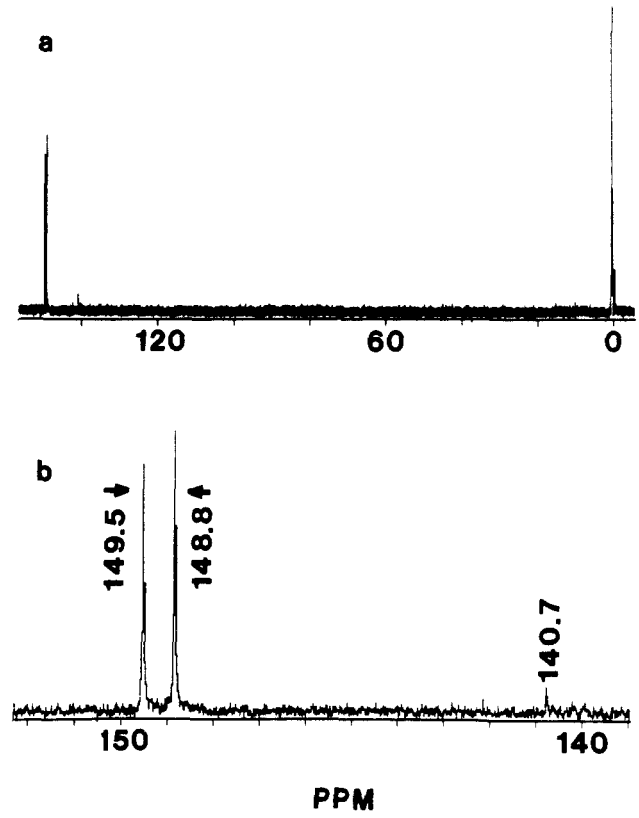

Figure 2. ^{31}P NMR spectrum of DMTr-5-IdU phosphoramidite from (a) 0 to 160 ppm (from phosphoric acid) and (b) from 139 to 152 ppm.

The sensitivity of aromatic iodides to nucleophilic substitution mandated the use of conditions milder than usually employed[8-11] for the phosphate deprotection and base deprotection steps. The usual base deprotection procedure (NH_4OH, 55 °C, overnight) led to some decomposition when applied to a control of IdU. Phosphate and base deprotection were both effected through 48-h treatment with NH_4OH at room temperature, as has been suggested.[13,14] HPLC, TLC, and elemental analysis (data not shown) confirmed that IdU was unaffected by this procedure.

The final desired oligomer was separated from the failure sequences and other impurities by trityl-selection reverse-phase HPLC.[15] The pooled fractions were lyophilized to a solid which was treated with 80% acetic acid to effect the final detritylation. The final oligomer was then desalted on a Sephadex G-50-50 column. The final

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(16) **Note Added in Proof.** Enzymatic degradation of the final oligomer by snake venom phosphodiesterase and alkaline phosphatase and subsequent analysis of the digest by reverse-phase HPLC indicated the ratio of incorporated nucleosides as 5.8:4.1:1.0:2.9:2.0 for C:G:T:A:IdU.

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

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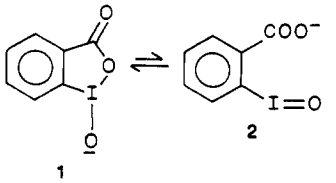
In its preferred, valence tautomeric 1-oxido-1,2-benziodoxol-3(1*H*)-one from (**1**), *o*-iodosobenzoate (**2**) is a remarkable catalyst for the cleavage of reactive phosphates in dilute cationic surfactant solution.[1] The para (to iodoso) octyloxy derivative of **1**/**2**[2] and a related functional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



1 2

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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