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(12) (a) The optimum yield of **4a** was obtained with 1.5 equiv of the Meerwein salt. (b) **4a**: for IR (KBr), see Figure 2; NMR ($CDCl_3$) δ 1.95 (s, 3 H, SMe), 4.08 (m, 2 H, ring methylenes), 4.22 (m, 2 H, ring methylenes), 7.27–7.55 (m, 5 H, C_6H_5); mp 42–43 °C.
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(14) The other contaminants are presumably Na_2S and $NaBF_4$.
(15) Attempts at making the lithium salt using LiH were unsuccessful; also, in view of anticipated overlap of ^1H NMR signals, crown ethers could not be used.
(16) One observes no acyclic O-alkylated product (viz., $MeOCH_2CH_2OC(=S)C_6H_5$) in these methylation reactions of solid **2a**; this was confirmed by comparison of TLC, IR, and NMR data of authentic material prepared by the reaction of 2-methoxyethanol and methyl thionobenzoate in the presence of NaH/dimethoxyethane. However, when, instead of solid **2a**, the entire heterogeneous reaction mixture (i.e., **3a** + NaH → **2a**) was trapped (after 15-min reaction time) with 1 equiv of $Me_3O^+ {}^-BF_4$, one did observe (TLC, NMR) a small amount (<3%) of 2-methoxyethyl thionobenzoate.
(17) In the IR spectra of thiono compounds, one generally finds strong bands in the region 1200–1000 cm^{-1} attributable to C=S vibration; unfortunately, certain single-bond vibrations also appear in this region (cf. Janssen, M. J. In "The Chemistry of Carboxylic Acids and Esters", Patai, S., Ed.; Interscience: New York, 1969; p 715). Nevertheless, in our hands, the spectra of methyl thionobenzoate and ethyl thionobenzoate reveal characteristic strong C=S bands at 1235 and 1245 cm^{-1}, respectively; these bands are absent in the spectra of methyl and ethyl benzoate. In the spectrum of **2a**, the sharp medium-intensity band at 1210 cm^{-1} has a counterpart in the spectrum of **4a** at 1225 cm^{-1}; these bands are in all likelihood due to C—O vibrations and *not* to C=S vibration.
(18) To the extent that **2a** has to dissolve in CH_2Cl_2 or in MeCN in order to be alkylated (with $Me_3O^+ {}^-BF_4$ or MeI), it is quite likely that this structural assignment also holds for the dominant solution structure of **2a**.[16] To rule out a kinetic preference of S- over O-methylation, a mixture of $CH_3(CH_2)_4CH_2O^- {}^+Na$ and $CH_3(CH_2)_4CH_2S^- {}^+Na$ (0.77:1.00 molar ratio) was treated with 0.5 equiv of $Me_3O^+ {}^-BF_4/CH_2Cl_2$; the ^1H NMR spectrum of the resulting solution revealed a MeO/MeS ^1H NMR signal ratio of 1.6:1.0, thereby proving that there is no overwhelming preference for S- over O-methylation.
(19) The order of reactivities toward water appears to be **2a** < **2c** < **2b, 2d.**
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(21) For an excellent discussion, see Jencks, W. P. *Adv. Enzymol.* **1975,** *43*, 268–281.
(22) Interestingly, the reaction of NaH and $HSCH_2CH_2OC(=O)C_6H_5$ in MeCN, followed by treatment with MeI, gave only $MeSCH_2CH_2OC(=O)C_6H_5$ and no 2-methoxy-2-phenyl-1,3-oxathiolane.
(23) The reaction of NaH and $HOCH_2CH_2OC(=O)C_6H_5$ in MeCN, followed by treatment with $Me_3O^+ {}^-BF_4/CH_2Cl_2$, did not lead to any 2-methoxy-2-phenyl-1,3-dioxolane; instead, the predominant products appeared to be dimethoxyethane and 1,2-dibenzoyloxyethane.

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A Pair-Specific Osmium Reagent for Polynucleotides

Sir:

Osmium(VIII) reagents show kinetic specificity for thymine residues among the common bases in polynucleotides.[1] An osmium(VI) ester is formed by addition to the 5,6 double bond. Ligands alter the nature of the reaction of osmium(VIII) species with olefins profoundly. The structures,[2a] the kinetics of formation,[2b] and the hydrolytic stability[2b] of the products are all changed. We have used the effects exerted by ligands to design a pair-specific osmium reagent. Scheme I outlines our strategy. The ligand, instead of being free in solution, is specifically attached to a cytosine residue. This specifically localized ligand then affects the kinetics of formation and stability of an osmate ester formed at thymine residues in its vicinity. Thymine residues not in the vicinity of the ligand would be attacked by osmium(VII) reagents very slowly and, if formed, would be hydrolyzed rapidly.[2b]

Table I reports kinetic data for the formation of the osmate ester of a modified thymine–cytosine dinucleoside monophosphate[3] together with some relevant comparative data.

The rate law for the reaction of osmium tetroxide with olefins contains two terms, one for the reaction without and one for the reaction with ligand:[7] rate = $(k_0 + k)[L][OsO_4][S]$ where L is ligand and S is the olefin. When the ligand is attached to the substrate (SL), the rate law contains only one term: rate = $k_0'[OsO_4][SL]$. The k_0 and k_0' terms can be compared directly by their rate constants. Our results show that k_0' is ~1600 times larger than k_0. This shows that, in the absence of added external ligand, a polynucleotide so modified could probably be labeled with an osmium atom with excellent selectivity at those thymine residues adjacent to modified cytosine residues.

Scheme I

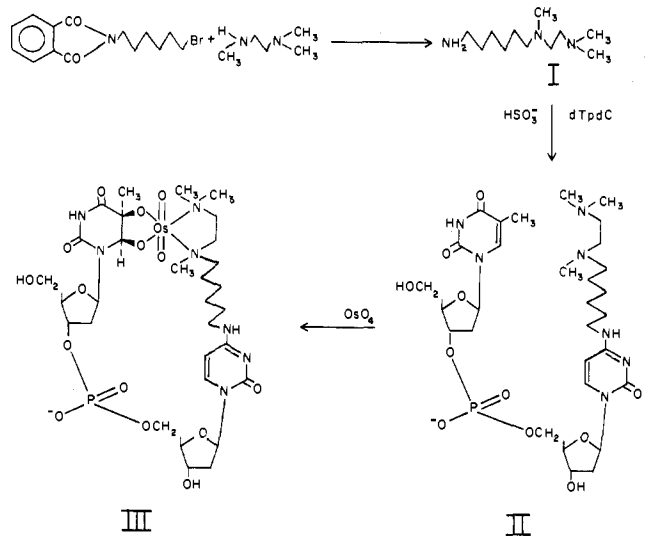

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Table I. Rate Data[a]

substrate	ligand	k_0, M^{-1} min^{-1}	k, M^{-2} min^{-1}	10^{10} rate, M min^{-1}	rel rate
thymine		0.25[b]		22.5	1
thymine	TMEN[c]	0.25[b]	6400 ± 700[d]	40	1.8
thymine	I	0.25[b]	10 000 ± 800[e]	49.5	2.2
dTpdC	I	0.25[b]	10 700 ± 800[f]	53	2.4
II		386 ± 24[g]		36 000	1600

[a] General conditions: 25 °C, 0.1 M sodium phosphate buffer, pH 7. Rates were calculated for [L] = [S] = 3×10^{-5} M; [OsO_4] = 3×10^{-4} M using the rate laws given in the text. All values are the average of at least three determinations. Infinity values were checked after 10 half-lives. Log plots were linear for at least 3 half-lives. [b] Extrapolated from the value at 8 °C (Ragazzo and Behrman[7]) using E_a = 9.5 kcal mol^{-1} (Subbaraman et al.[2b]). [c] *N,N,N′,N′*-Tetramethylethylenediamine. [d] Measured by the increase in absorbance at 360 nm under pseudo-first-order conditions: [OsO_4] = 4×10^{-4}; [thymine] = 5×10^{-3}; [TMEN] = $0.5–1.0 \times 10^{-2}$ M. [e] Measured as in footnote *d* but with [I] = 1.1×10^{-2} M. [f] Measured by the decrease in absorption at 270 nm under pseudo-first-order conditions: [dTpdC] = 3×10^{-5}; [OsO_4] = 3.2×10^{-4}; [I] = 4.85×10^{-2} M. [g] Measured as in footnote *f*: [II] = 3×10^{-5}; [OsO_4] = 3.1×10^{-4} M.

It is also interesting to make a comparison of the rate of reaction of this system with the rate in the presence of free added ligand. Since these rate laws are of different forms, we must compare rates under specified conditions rather than rate constants. This is also shown in Table I. One can calculate that the concentration of free added ligand necessary to bring the rate of reaction in the presence of free added ligand up to the rate in the modified case is 0.04 M. That is, the ligand in the modified case is at a kinetically effective concentration 1300 times that in the free case.

We are extending this work to examine the effects of chain length in the attached ligand and also to the design of a reagent specific for triplets.[8]

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(3) Synthesis of III: the ligand, I, was made according to Scheme I in 20% yield by following the general procedures of ref 4. The modified dinucleoside monophosphate, II, was made in 65% yield by reacting dTpdC[5] with I using the cytosine-specific, bisulfite-catalyzed transamination reaction described by Shapiro and Weisgras.[6] The transaminated product, II, isolated by gel filtration and preparative paper chromatography, showed a UV maximum at 272 nm (pH 7). Its concentration was estimated by assuming the same extinction coefficient for it as for dTpdC (21 500, pH 7, 272 nm). Its ^1H NMR spectrum (90 MHz) was consistent with the structure given. Upon reaction of II with 1 molar equiv of osmium tetroxide, the NMR spectrum changed in a way consistent with the formation of the osmate ester, III: the thymine H-6 singlet at δ 7.64 shifts to a pair of singlets at 5.47 and 5.16; the thymine methyl singlet at 1.88 shifts to a multiplet centered at 1.64. In addition, the N-methyl resonances of the ligand residue appear as three pairs of doublets at δ 2.97, 2.84, and 2.70. The upfield shifts of the thymine protons upon saturation of the 5,6 double bond are consistent with literature values (Subbaraman et al.[1]); the multiplicity observed is accounted for by the fact that four diastereomers can be formed. The UV spectrum of the osmate ester, III, showed quantitatively the expected decrease in absorbance at 272 nm due to saturation of the olefinic bond. The observed ratio of absorptivity at 272 nm for II and III was 1.44. The calculated ratio based on cytosine and the osmate ester of thymine with the ligand TMEN is 1.38.

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