and to Dr. Robert Hershfield for his valuable assistance in the analysis of Scheme II.

References and Notes

(1) This work was supported by a Public Health Service Research Career Development Award (1-K4-GM-10010-02) from the National Institute for General Medical Sciences and by a grant (DE-03246) from the National Institutes of Health.

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(5) Abbreviations used are HCO-Phe-NH_2, *N*-formyl-L-phenylalanine amide; HCO-D-Phe-NH_2, *N*-formyl-D-phenylalanine amide; HCO-Phe-FH, *N*-formyl-L-phenylalanine formylhydrazide; HCO-D-Phe-FH, *N*-formyl-D-phenylalanine formylhydrazide; HCO-MePhe-FH, *N*-formyl-*N*-methyl-L-phenylalanine formylhydrazide; Ac-Tyr-NHPhCl, *N*-acetyl-L-tyrosine *p*-chloroanilide; Ac-D-Tyr-NHPhCl, *N*-acetyl-D-tyrosine *p*-chloroanilide; Ac-MeTyr-NHPhCl, *N*-acetyl-*N*-methyl-L-tyrosine *p*-chloroanilide.

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	N-Acyl amino	Subsite on enzyme: Side chain	Leaving group	α-H
N-Acetyl	3.67	0.727	0.788	0.400
p-Hydroxybenzyl	1.08	63.3	0.649	0.301
p-Chlorocarboxanilide	1.08	*x*	*y*	0.301
α-H	0.826	0.826	0.826	0.826

For example, 0.727 refers to placement of the *N*-acetyl group into the aromatic side-chain site. After expansion by minors and equating the positive and negative terms with the association constants for the L and D enantiomers, two linear simultaneous equations are obtained.

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(22) With an enzyme with two hydrophobic sites the dependence of the log of the binding constant on π will be biphasic unless nonhydrophobic binding forces greatly favor binding at one or another of the two sites.

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Reactions of Osmium Ligand Complexes with Nucleosides

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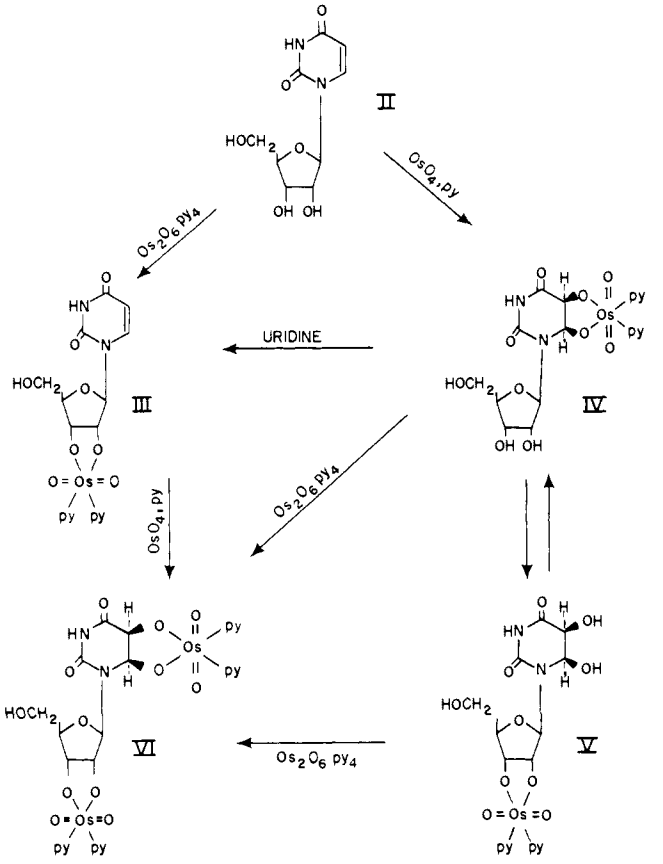
Abstract: We have synthesized bis(pyridine)oxoosmium(VI) and 2,2′-bipyridyloxoosmium(VI) esters of the common nucleosides in which osmium is bonded through the 2′- and 3′-hydroxyl groups of the sugar residue. We have also prepared the 2,2′-bipyridyloxoosmium(VI) esters of uridine, cytidine, and thymidine which result from addition of OsO_4 to the 5,6 double bond of the pyrimidine ring. Kinetic studies of the formation of the sugar esters from the nucleoside and the Os(VI) dimer, $Os_2O_6py_4$, give the apparent rate law, $v = k[S][Os(VI)][py]^{-1}[OH^-]^{0.6-0.8}$, in which the hydroxyl ion term reflects hydroxyl ion promoted dissociation of the Os(VI) dimer to monomeric species. The true rate law probably involves three terms, one zero-order, one half-order, and one first-order in hydroxyl ion. The bis(pyridine) esters undergo relatively rapid transesterification reactions with free glycols whereas the 2,2′-bipyridyl esters are much more inert. Kinetic studies of the transesterification reactions give the rate law, $v = k$[osmate ester][glycol][OH^-][py]$^{-1}$.

Osmium derivatives of tRNA have of late assumed considerable importance in the x-ray crystallographic analysis of these species.[1-3] A second application, less developed in a practical way but of considerable potential, is the direct determination of the sequence of bases in nucleic acids by visualization of single heavy atoms.[4-7] In addition to these specific areas where oxoosmium species have already proven to be of importance, there are other interesting consequences and applications of the interactions of metal ions with nucleic acids such as their use in cancer chemotherapy,[8] alterations of the enzymatic specificity of nucleases,[9] and other areas summarized by Clarke and Taube.[10] It is important for these purposes that the chemistry of the reactions used to introduce osmium species into the polymers be understood. This is particularly true of the factors governing specificity and stability. We have to this end undertaken a detailed study of the reactions of oxoosmium complexes with nucleic acid components.[11-14] We report here studies on the stability and exchange reactions of nucleosides with oxoosmium ligand complexes. Preliminary reports of some

Scheme I

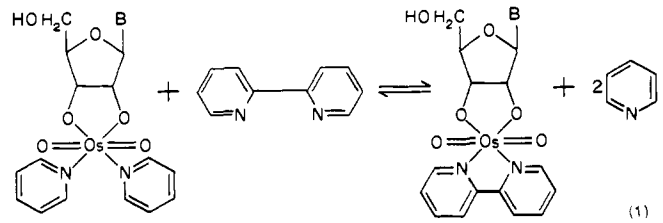


of this work have been published.[15]

Results and Discussion

Synthesis of Oxoosmium(VI) Nucleoside Sugar Esters. Scheme I summarizes the reactions of uridine with oxoosmium-pyridine complexes. Four types of oxoosmium(VI) esters can be formed. Only compounds of type III (nucleoside sugar esters) are produced when any of the common ribonucleosides is allowed to react with $Os_2O_6py_4$ or with potassium osmate, $K_2OsO_2(OH)_4$, in the presence of pyridine. These compounds are brown solids, slightly soluble in water and more soluble in dimethyl sulfoxide. Their electronic absorption spectra all show a weak maximum around 440 nm ($\epsilon \sim 100$), a minimum around 415 nm, and an analytically useful region of gradually rising molar absorptivity from 400 to 300 nm ($\epsilon_{350\,nm} \sim 500$). The ir spectra show bands characteristic of complexed pyridine[16] and a diagnostic strong band due to the asymmetric *trans*-osmyl stretch[17] at ~835 cm^{-1}. The ^1H NMR spectra were measured in Me_2SO-d_6. All proton resonances were shifted downfield relative to the parent nucleoside. The pyridine shifts were similar to those recorded previously for some related compounds.[11] The C(1′), C(2′), C(3′), and C(4′) proton resonances were shifted downfield between 0.3 and 0.7 ppm. The other protons showed smaller shifts.

An analogous series of esters was formed in which 2,2′-bipyridyl replaced the pyridine ligands. These esters were



(1)

Table I. Chromatographic Data[a]

Nucleoside	Ligand	Ester type	R_f
Adenosine	py	S	0.31
Adenosine	bipy	S	0.21
Guanosine	py	S	0.39
Guanosine	bipy	S	0.32
Uridine	py	S	0.55
Uridine	bipy	S	0.48
Uridine	py	H	0.62
Uridine	bipy	H	0.62
Uridine	py	D	0.30
Cytidine	py	S	0.49
Cytidine	bipy	S	0.40
Cytidine	py	H	0.68
Cytidine	bipy	H	0.55
Cytidine	py	D	0.36
Thymidine	py	H	0.80
Thymidine	bipy	H	0.61
Ribothymidine	py	H	0.77
Ribothymidine	py	S	0.55
Ribothymidine	py	D	0.40

[a] Chromatography was carried out on Eastman silica TLC plates using 0.05 *M* sodium phosphate buffer in water (pH 7)–pyridine (20:1 v/v). The spots were revealed by a spray containing 2% thiourea in 2 *N* HCl. S, sugar ester; H, heterocyclic ester; D, diester corresponding to compound types III, IV, and VI, respectively, in Scheme I.

prepared in best yield for uridine, cytidine, and guanosine by a ligand exchange reaction between the bis(pyridine) ester and 2,2′-bipyridyl. This method failed for adenosine. The corresponding 2,2′-bipyridyl adenosine ester was formed by reaction of adenosine with potassium osmate in the presence of 2,2′-bipyridyl. Considerable quantities of the insoluble complex $Os_2O_6(bipy)_2$[18] are formed as well by this latter route. The 2,2′-bipyridyl esters are similar in their spectral properties to the bis(pyridine) esters except that their uv spectra show an additional band in the 310-nm region ($\epsilon \sim 13000$) and lack a distinct maximum in the visible. They are readily distinguished, however, by their chromatographic mobilities and color reaction with thiourea in HCl (Table I); the bis(pyridine) esters form pink spots with this reagent while the 2,2′-bipyridyl esters become yellow. Table II gives representative spectroscopic and analytical data for some of these esters. The structure assigned to the sugar esters is supported by the fact that no reaction was observed with $Os_2O_6py_4$ when either thymidine, deoxycytidine, deoxyuridine, or cytidine arabinoside was used in place of a ribonucleoside.

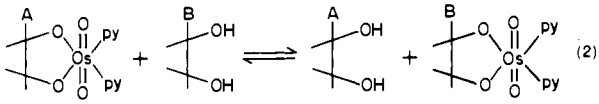
Reactions of Uridine with OsO_4–Pyridine. In contrast to the reaction of uridine with Os(VI) complexes which gives only a single product as described above, chromatography of the reaction mixture of uridine with osmium tetraoxide in the presence of pyridine reveals at least three osmium-containing products whose relative quantities change with time. One of these cochromatographs with uridine sugar ester (III, Scheme I); a second cochromatographs with the product formed by reaction of III with OsO_4 and pyridine and is therefore presumably the diosmate ester VI. The third is the first one which appears in time and thus appears to be IV, the uridine heterocyclic ester. Compound V has not yet been detected. It is unlikely that the sugar esters result from direct reaction of OsO_4 with the sugar residue since, although in the case of uridine the sugar ester appears within minutes, in the case of the purines, detectable traces of sugar ester appear only after several hours. We attribute the rapid appearance of the sugar ester during the reaction of osmium tetraoxide with the pyrimidine nucleosides to a transesterification reaction and the much slower appearance of the sugar esters for the purine nucleosides to

Table II. Spectrometric and Analytical Data

Nucleoside	Ligand	Ester type[a]	λ max, nm (ε)[b]	OsO_2 ν_{asym}, cm^{-1}[c]	δ, HC(1′)[d]	Formula	Found (calcd)[e] C	H	N	Os
Adenosine	py	S	256 (22900), 440 (83)	839	6.47 (5.97)	$C_{20}H_{21}O_6N_7Os$	36.9 (37.2)	3.25 (3.25)	14.8 (15.2)	29.9 (29.5)
Adenosine	bipy	S	256 (22600), 312 (13600)	834	6.33 (5.97)	$C_{20}H_{19}O_6N_7Os{\cdot}2H_2O$	35.0 (35.4)	3.36 (3.38)	14.2 (14.4)	
Guanosine	py	S	256 (20850), 430 (sh, 111)	834	6.10 (5.68)	$C_{20}H_{21}O_7N_7Os$	35.8 (36.4)	3.05 (3.18)	14.8 (14.8)	28.75 (28.75)
Guanosine	bipy	S	248 (25600), 312 (13700)	836	6.23 (5.68)	$C_{20}H_{19}O_7N_7Os{\cdot}2H_2O$	34.1 (34.5)	3.49 (3.31)	14.2 (14.1)	
Uridine	py	S	256 (19750), 440 (83)	828	6.23 (5.78)	$C_{19}H_{20}O_8N_4Os$	36.7 (36.7)	3.31 (3.21)	9.1 (9.0)	31.0 (30.6)
Uridine	bipy	S	257 (17800), 312 (12200)	828	6.25 (5.78)	$C_{19}H_{18}O_8N_4Os{\cdot}3H_2O$	33.2 (33.9)	3.52 (3.56)	7.5 (8.0)	
Cytidine	py	S	263 (15300), 448 (89)	835	6.25 (5.83)	$C_{19}H_{21}O_7N_5Os{\cdot}H_2O$	35.6 (35.9)	3.30 (3.62)	11.3 (11.0)	28.6 (29.9)
Cytidine	bipy	S	272 (13850), 312 (12650)	830	6.27 (5.83)	$C_{19}H_{19}O_7N_5Os{\cdot}2H_2O$	34.2 (34.8)	3.52 (3.51)	10.6 (10.7)	
Thymidine	bipy	H	240 (sh, 11650), 312 (12300), 435 (141)	832	6.23 (6.30)	$C_{20}H_{23}O_9N_4Os{\cdot}2H_2O$	35.0 (35.0)	3.88 (3.34)	7.9 (8.1)	

[a] S represents a sugar ester; H, a heterocyclic ester corresponding to compound types III and IV, respectively, in Scheme I. [b] Measured in aqueous phosphate buffer, pH 7. [c] In KBr. [d] In Me_2SO-d_6, Me_4Si internal standard, 35°. The values for the parent nucleosides are given in parentheses. [e] Percent calculated values are given in parentheses.

a prior reduction of osmium tetraoxide to an Os(VI) species by the glycol. Very slow reactions of OsO_4 with glycols have been previously observed.[11,17b] The transesterification mechanism has been confirmed by showing that when guanosine, cytidine, or uridine is incubated with the adenosine sugar ester, adenosine and the nonadenosine sugar ester are formed according to eq 2. The kinetics of these exchange reactions are discussed in the next section.



(2)

Reactions of Pyrimidine Nucleosides with OsO_4–2,2′-Bipyridyl. The 2,2′-bipyridyl heterocyclic esters of both cytidine and uridine were easily isolatable in contrast to their bis(pyridine) esters because rapid ester interchange does not occur. Prolonged incubation of these esters in pyridine solutions resulted in decomposition but no evident formation of the bis(pyridine) esters. These esters undergo ester interchange reactions but at much slower rates than is the case for the bis(pyridine) esters.

Ligand Dissociation from the Esters and the Complexes. Ligand dissociation from oxoosmium(VI) species is pH-dependent and, for the complexes (as opposed to the esters), further complicated by a monomer–dimer equilibrium[13]. It is useful to treat the general case for pH-dependent dissociation as follows.

$$\mathrm{Os(VI)py}_x + \alpha\mathrm{HO}^- \underset{K}{\rightleftharpoons} \mathrm{Os(VI)py}_{x-n} + n\mathrm{py} \qquad (3)$$

Since pyridine concentrations were determined by distribution between aqueous and organic phases (represented by py_a and py_o), the equilibrium may be expressed by eq 4.[19]

$$K = \frac{((\mathrm{py_a} + \mathrm{py_o})/n)\mathrm{py_a}^n}{([\mathrm{Os(VI)py}_x]_i - (\mathrm{py_a} + \mathrm{py_o})/n)[\mathrm{OH}^-]^\alpha} \qquad (4)$$

Substituting for $[HO^-]^\alpha$ and expressing py_o as $m py_a$, we have

$$K = \frac{(m/n)\mathrm{py_a}^{n+1}[\mathrm{H}^+]^\alpha}{([\mathrm{Os(VI)py}_x]_i - (m/n)\mathrm{py_a})K_w{}^\alpha} \qquad (5)$$

or

$$\mathrm{py_a}^{n+1} = \frac{(n/m)KK_w{}^\alpha([\mathrm{Os(VI)py}_x]_i - (m/n)\mathrm{py_a})}{[\mathrm{H}^+]^\alpha} \qquad (6)$$

If the degree of dissociation is small with respect to the initial concentration of the Os(VI) species, then at constant Os(VI) concentration, log py_a will be a linear function of pH with slope = $\alpha/n + 1$. Figure 1 shows plots of log py_a vs. pH for $Os_2O_6py_4$ and for the thymine bis(pyridine) oxoosmium(VI) ester. The plots are reasonably linear over the pH range 7.5–9 with slopes of 0.31 and 0.48, respectively, implying dissociations of two and one pyridine ligands, respectively ($\alpha = 1$).

We attribute the smaller slope in the pH 6–7.5 region to a pH-independent dissociation, i.e., $\alpha \rightarrow 0$. The leveling in the high pH region represents complete dissociation to form the osmate ion, $OsO_2(OH)_4{}^{2-}$. Figure 2 shows pyridine dissociation from the oxoosmium(VI)–pyridine complex as a function of Os(VI) concentration. These data show that at the Os(VI) concentration used for the determinations given in Figure 1 that the degree of pyridine dissociation is indeed small. We do not report equilibrium constants for the dissociation because of our incomplete knowledge of the monomer–dimer equilibrium. Our previously published data supported an equilibrium scheme for the complexes involving dissociation of only one pyridine. Reexamination of these experiments by Shaffer[20] showed a possible complication in

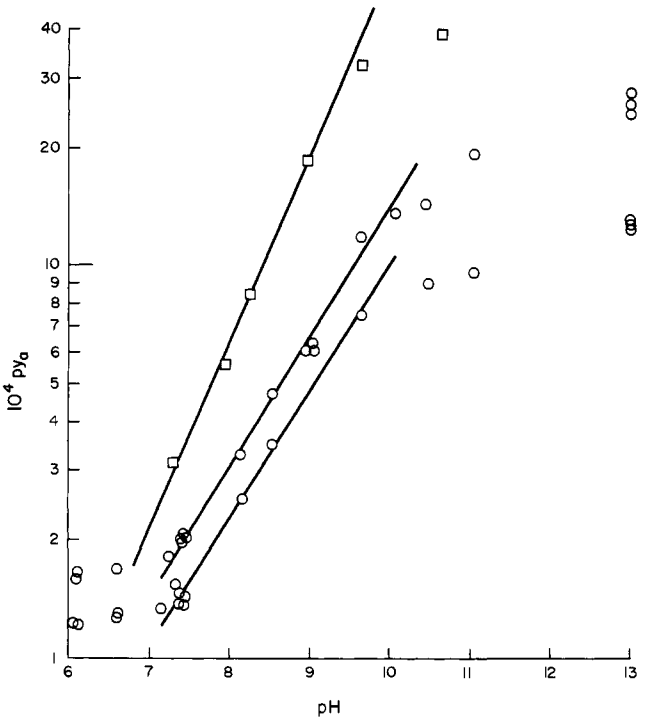


Figure 1. Plots of log py_a vs. pH for $Os_2O_6py_4$ and for the thymine bis(pyridine) oxoosmium(VI) heterocyclic ester. Py_a represents pyridine concentration in the aqueous phase after distribution between equal volumes of the aqueous phase at the pH specified and CCl_4. T = 23-25°. O—O, $Os_2O_6py_4$ at initial concentrations of 1×10^{-3} and 2×10^{-3} M. Py_a values at pH 13 are the calculated concentrations for complete dissociation. □—□, the thymine ester at 1.12×10^{-2} M initial concentration.

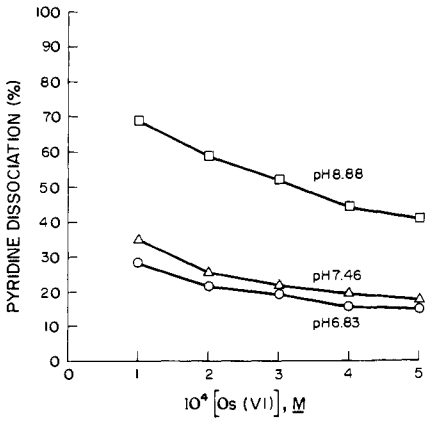


Figure 2. Plots of % pyridine dissociation from $Os_2O_6py_4$ vs. initial concentration of the complex at several pH values. Pyridine dissociation was measured by distribution between the aqueous phase and CCl_4 at 23-25°. Os(VI) concentration is calculated as the monomer.

these data due to oxidation of Os(VI) species by peroxides in the ether used for the distribution measurements. We regard our newer measurements using carbon tetrachloride as the more reliable. We have not considered dissociation of both pyridines from a monomeric complex Mpy_2 because, as we have previously noted,[13] these solutions undergo no observable decomposition after several days in air at room temperature and since it has been shown[12] that pyridine-free oxoosmium(VI) species are not stable under these conditions.

Kinetics. Reaction of $Os_2O_6py_4$ with the Ribonucleosides. The pseudo-first-order rate constant, k_ψ, was unaffected by changing the initial concentration of the limiting component, $Os_2O_6py_4$, in the range 1.6×10^{-4}-1.6×10^{-3} M. k_ψ

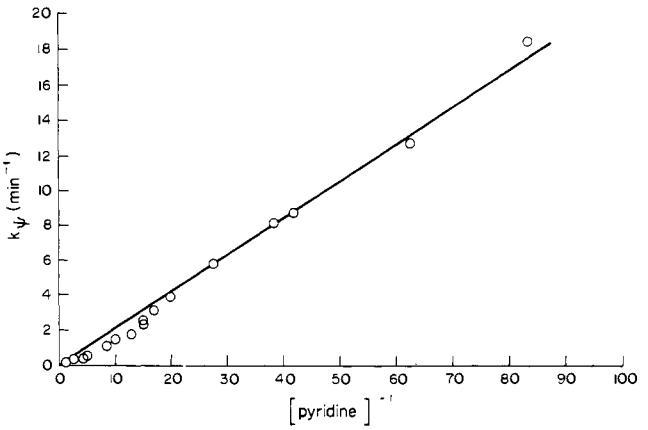


Figure 3. k_ψ vs. [pyridine]$^{-1}$ for the reaction between uridine and $Os_2O_6py_4$. [Uridine] = 2×10^{-2} M; [$Os_2O_6py_4$] = 1×10^{-4} M; pH 10, sodium carbonate buffer 0.15 M; μ = 0.255 M with NaCl; T = 18°, water.

varied linearly with nucleoside concentration in the range 1-20 $\times 10^{-3}$ M. Neither buffer concentration nor ionic strength affected the rate significantly in the range 0.1-0.2 M. k_ψ varied linearly with the reciprocal of pyridine concentration at pH 7.26. At pH 10, a small nonlinear region was observed in the high pyridine concentration range (Figure 3). Plots using pyridine activity[14] did not improve the linearity. The rate of reaction increased with pH such that the apparent dependence on hydroxyl ion concentration was between 0.6 and 0.8 in the pH range 8-10 (Figure 4). In the pH range 7-8, the rate approaches zero-order dependence on hydroxyl ion concentration.

Thus the rate law in the pH range 8-10 is given by

$$v = k[\text{Os(VI)}][\text{S}][\text{OH}^-]^{0.6\text{-}0.8}[\text{py}]^{-1}$$

Observed rate constants are collected in Table III. The kinetic dependencies correspond essentially to those previously observed.[13] In our previous work, however, the half-order dependence on hydroxyl ion concentration was entirely attributed to cis-trans isomerization of the glycol substrate. Since that explanation is not tenable with our present substrates, we conclude that the cis-trans isomerization could not have been the entire reason for the half-order dependence although it may have contributed to it in part. The mechanism previously proposed[13] predicted first-order dependence on the hydroxyl ion concentration in the absence of any complications such as cis-trans isomerization of the substrate. To account for our present findings we must add to that proposal a pH-independent mechanism for the low pH region and a pH-dependent mechanism with a hydroxyl ion dependence less than one. We propose the following steps in which D represents the Os(VI) dimer, M the Os(VI) monomer, and S the substrate. We have not specified ligands, other than pyridine, complexed with the monomeric species due to our uncertainty about their structures.

I. the previously proposed dissociation[13]

$$\text{Dpy}_4 \overset{K_1}{\rightleftharpoons} 2\text{Mpy}_2$$

$$2\text{Mpy}_2 + 2\text{OH}^- \overset{K_2}{\rightleftharpoons} 2\text{Mpy} + 2\text{py}$$

II. a pH-independent dissociation

$$\text{Dpy}_4 \overset{K_3}{\rightleftharpoons} 2\text{Mpy} + 2\text{py}$$

III. a second pH-dependent dissociation which yields 2 mol of pyridine per mole of hydroxyl ion in accordance with the

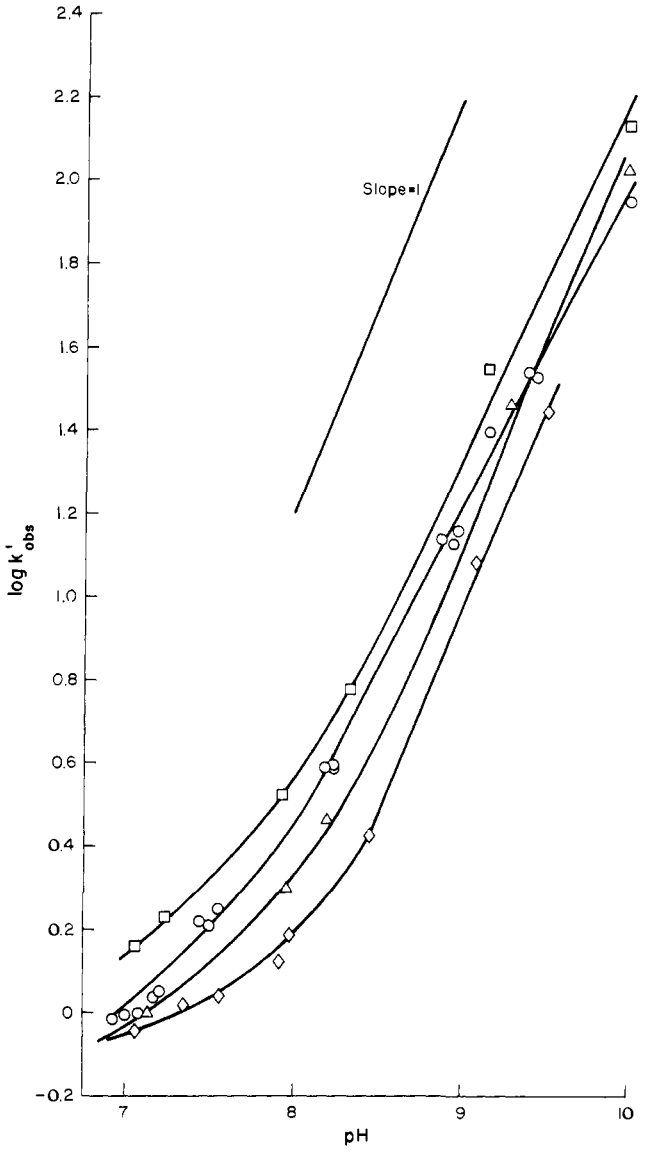


Figure 4. pH-rate profiles for the reaction of nucleosides with $Os_2O_6py_4$: [pyridine] = 8×10^{-2} *M*; □—□ [adenosine] = 1.28×10^{-2} *M*; ○—○ [uridine] = 2×10^{-2} *M*; △—△ [cytidine] = 2×10^{-2} *M*. *T* = 18°, water; $k_{obsd}' = k_\psi/[S]$; $[Os_2O_6py_4] = 1.6 \times 10^{-4}$–$1.6 \times 10^{-3}$ *M*. Data for ethylene glycol are given as well (◇—◇) although the structure of the product with this substrate is atypical.[17b]

linear portion of the dissociation data for $Os_2O_6py_4$ shown in Figure 1

$$Dpy_4 + OH^- \overset{K_4}{\rightleftharpoons} Dpy_3OH^- + py$$

$$Dpy_3OH^- \overset{K_5}{\rightleftharpoons} 2Mpy + py$$

The rate step is

$$Mpy + S \xrightarrow{k} SMpy \xrightarrow[+py]{fast} product$$

The rate law for this step is

$$v = k[Dpy_4]^{1/2}[S][py]^{-1}[OH]^n$$

where $n = 1$ for I, $n = 0$ for II, and $n = \frac{1}{2}$ for III. Our observed dependence on $[OH^-]$ is approximately zero for the pH range 7–8 (pathway II) and between one-half and one for the pH range 8–10 (pathways I + III). These mechanisms all predict a square root dependence on $[Dpy_4]$. We observe first-order dependence. We may rationalize this apparent discrepancy as follows: where $[D_i]$ = initial dimer concentration, $[D_a]$ = actual dimer concentration, and so on, [Os total] = $2[D_i] = 2[D_a] + [M_a]$. At constant [py], $[OH^-]$, $[M_a] = K[D_a]^{1/2}$. Thus $[D_i] = [D_a] + (K/2)[D_a]^{1/2}$. If dissociation is largely completely, then $[D_i] \simeq (K/2)[D_a]^{1/2}$, i.e., the rate will be proportional to $[D_i]$, as found. Average molecular weight determinations suggest that essentially complete monomerization occurs in the concentration and pH range used for the kinetic studies.[13] Nikolskii et al.[21] report complete monomerization at much higher concentration in apparent contradiction to our results.[13]

Table III. Kinetics of the Reactions of Nucleosides with $Os_2O_6py_4$[a]

Substrate	pH	k_{obsd}, min^{-1}
Uridine	7.00	0.0772
Uridine	8.18	0.309
Uridine	8.99	1.16
Uridine	9.45	2.74
Uridine	10.00	7.18
Cytidine	7.13	0.080
Cytidine	7.96	0.159
Cytidine	8.21	0.231
Cytidine	9.27	2.31
Cytidine	10.00	8.25
Adenosine	7.07	0.105
Adenosine	7.94	0.267
Adenosine	8.36	0.475
Adenosine	9.15	2.88
Adenosine	10.00	11.05
Guanosine	10.00	10.6

[a] Temperature = 18°; [pyridine] = 0.08 *M*; [uridine] = 0.02 *M*; [cytidine] = 0.020 *M*; [adenosine] = 0.0128 *M*; [guanosine] = 0.01 *M*; $[Os_2O_6 \cdot py_4] = 0.16$–$1.60$ m*M*; $k_{obsd} = k_\psi$ [py]/[S]; λ = 320–370 nm; all rate data ±5%, water.

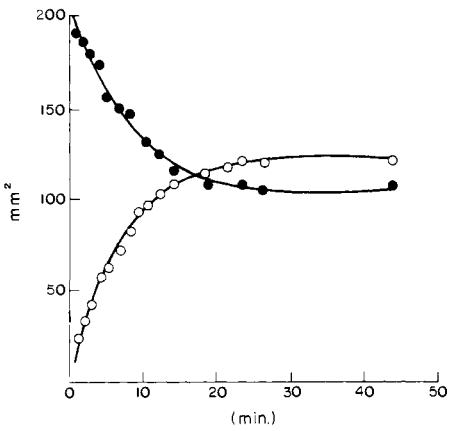


Figure 5. The approach to equilibrium of the reaction between thymine bis(pyridine) oxoosmium(VI) ester and cytidine, initial concentrations 8.3×10^{-2} *M* each in 80% Me_2SO-d_6–20% D_2O, 35°. The *y* axis gives the integrated values of the methyl proton resonances of the thymine ester (●—●) and of the product, *cis*-thymine glycol (○—○).

Transesterification Kinetics. Quantitative measurements of the transesterification reaction were made using thymine and thymidine heterocyclic osmate esters[11] as the osmium donor because of the convenience of following these components in the ^1H NMR spectrometer. The methyl group protons of thymine and its derivatives undergo a large chemical shift upon conversion to the corresponding osmate ester.[11] Figure 5 shows the approach of this system to equilibrium. Figure 6 shows plots of five runs and Table IV values for $k_{f\,obsd}$ as a function of ligand concentration. Plots of $[py]^{-1}$ vs. $k_{f\,obsd}$ are linear. The rate is also dependent on acidity. A plot of $k_{f\,obsd}$ vs. pD is linear with a slope of about 0.85. The rate law for the forward reaction is thus: $v = k_f$[osmate

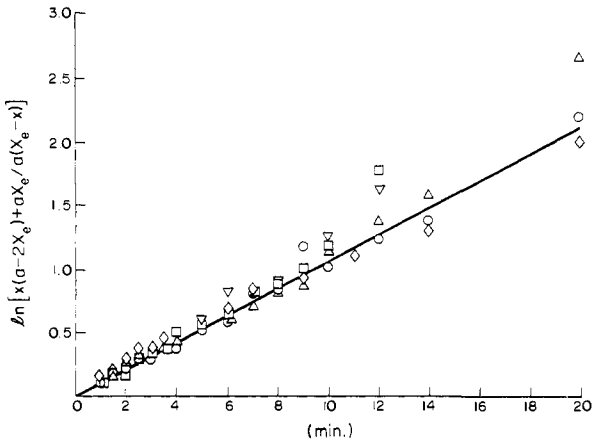


Figure 6. Evaluation of the rate constant for the transesterification reaction given in Figure 5. The points represent data from five runs under identical conditions. $k_{f\,obsd} = 0.598\ M^{-1}\ min^{-1}$ with a standard deviation of 2.8%.

ester][glycol][OH^-][py]$^{-1}$. More limited data were obtained for transesterifications involving bipyridyl esters because of the slowness of these reactions. Table IV presents some representative runs and also compares the half-time for exchange of some bis(pyridine) and 2,2′-bipyridyl esters as a function of ligand concentration.

Our results suggest some modifications in the approach to single-site labeling of polynucleotides with oxoosmium reagents. We have shown that both the rate of formation and the rate of transesterification of the bis(pyridine)oxoosmium(VI) esters are inversely dependent on ligand concentration. It is particularly important that the rate of the transesterification reaction be controlled for effective single-site labeling. The bis(pyridine) esters are quite reactive even in the presence of high concentrations of pyridine. In view of this, the use of a bidentate ligand, such as 2,2′-bipyridyl, has the advantage of forming an analogous series of esters of greatly increased stability. For example (Table IV), the half-time for exchange in the presence of 0.167 *M* ligand is increased from 56 min when the ligand is pyridine to about 8 months when the ligand is 2,2′-bipyridyl. This is a decrease in the exchange rate by a factor of about 6000.

Table IV. Kinetics of Transesterification Reactions[a]

[Ligand][b] added, *M*	$k_{f\,obsd}$, $M^{-1}\ min^{-1}$	$t_{1/2}$
I. Thymine Bis(pyridine) Ester + Cytidine		
0	0.598 ± 0.01	20 min
0.083	0.375 ± 0.2	32 min
0.110	0.275 ± 0.05	44 min
0.167	0.215 ± 0.15	56 min
0.222	0.170 ± 0.05	71 min
0.332	0.122	98 min
II. Thymidine 2,2′-Bipyridyl Ester + Cytidine		
0	1.9×10^{-2}	10 hr
0.0083	5×10^{-3}	1.67 days
0.167	3.1×10^{-5}	8 months

[a] Conditions: 80% Me_2SO-d_6–20% D_2O; $T = 35°$; [osmate ester]$_i$ = [cytidine]$_i$ = 0.083 *M*; $k_{f\,obsd} = k_f$[OH^-] [L]$^{-1}$. [b] The ligand is py for the first six entries, bipy for the last three.

Experimental Section

Reagents. Potassium osmate was made by the alkaline reduction of OsO_4 with ethanol[22] and $Os_2O_6py_4$ by the method of Subbaraman et al.[13] Other chemicals were reagent grade and were obtained from commercial sources.

Instrumentation. Ultraviolet spectra were measured using a Perkin-Elmer Model 202 instrument, visible spectra on a Cary Model 118C, ir spectra on a Perkin-Elmer Model 237B grating instrument, and NMR spectra on a Varian Associates Model T-60 (60 MHz) at 35°.

Analyses. Elemental analyses for carbon, hydrogen, and nitrogen were done by the Het-Chem-Co., Harrisonville, Mo. Osmium analyses were carried out by a combination of the techniques of Criegee et al.[23] and of Goldstein et al.[24] An aliquot of an osmium(VI) compound was oxidized to OsO_4 using CrO_3-H_2SO_4 at 180–200°. The osmium tetraoxide was collected in an ice-cold CCl_4 trap. The whole system was constructed of glass and the joints were sealed with syrupy phosphoric acid. The quantity of OsO_4 in the CCl_4 was measured spectrophotometrically at 279 nm (ϵ 1706). A standard curve was made using known quantities of $Os_2O_6py_4$. A plot of osmium added vs. osmium recovered was linear over the range 1–15 mg of osmium. A small but constant (i.e., independent of the amount added) amount of osmium was lost by absorption in the phosphoric acid used for sealing the joints.[23] Pyridine was determined by Ang's method.[25]

Oxoosmium(VI) Esters. (a) Bis(pyridine) Sugar Esters (Scheme I, III). These were synthesized following the procedure described by Subbaraman et al.[13] for the esters formed from thymine glycol. The adenosine ester crystallized from the reaction mixture.[26] The guanosine, uridine, and cytidine esters were purified by column chromatography on activated alumina (guanosine) or silica (uridine and cytidine) using methanol-pyridine (9:1 v/v) as eluent. These were not obtained in crystalline form. Some of the physical properties of these esters are given in Table II. More complete data, including NMR and ir spectra, are to be found in ref 27.

(b) 2,2′-Bipyridyl Sugar Esters. The uridine, cytidine, and guanosine bipyridyl esters were made in best yield by ligand exchange with the bis(pyridine) esters. The synthesis of the guanosine ester is typical. The guanosine bis(pyridine) sugar ester (0.162 g, 0.2 mmol) and 0.045 g (0.3 mmol) of 2,2′-bipyridyl were dissolved in 20 ml of water and stirred for 2 hr at room temperature. Fine reddish-brown crystals appeared as the solvent was removed under a stream of air. These were washed with acetone and dried over KOH in vacuo: yield 0.129 g (81%). Only starting material was recovered when this route was applied to the adenosine ester. This compound was therefore made by an alternate route. Adenosine (0.267 g, 0.99 mmol) and 0.156 g (1 mmol) of 2,2′-bipyridyl were dissolved in 200 ml of water. Potassium osmate, $K_2OsO_2(OH)_4$, 0.368 g (1 mmol), dissolved in 50 ml of water was added. The pH of the solution was adjusted to 7 with 0.1 *N* HCl. The solution was filtered after 30 min, allowed to stand overnight, and filtered again. The volume of the solution was reduced to about 50 ml whereupon crystallization of the product began. The crude product (0.55 g, 87%) was recrystallized from 75 ml of water to yield 0.38 g (60%) of the dihydrate in the form of rosettes. The pyrimidine nucleoside bipyridyl sugar esters could not be crystallized.

(c) Pyrimidine Nucleoside 2,2′-Bipyridyl Heterocyclic Esters (Scheme I, IV). These were prepared by reaction of equimolar quantities of osmium tetraoxide, 2,2′-bipyridyl, and the pyrimidine nucleoside in water following procedures already published.[11,12] The uridine, cytidine, and thymidine esters were prepared in this way and gave satisfactory elemental analyses.

(d) Uridine Tetrapyridinebis[oxoosmium(VI)] Diester (Scheme I, VI). Uridine (0.122 g, 0.5 mmol) was allowed to react with 0.196 g (0.25 mmol) of $Os_2O_6py_4$ in 20% aqueous pyridine overnight. A solution of 0.130 g (0.51 mmol) of OsO_4 in 1 ml of pyridine was added with stirring. The solvent was removed in vacuo after 2 hr giving a 97% yield of crude product (0.5 g). This material contained a small amount of the bis(pyridine) sugar ester as revealed by TLC. The product (100 mg) was purified by column chromatography on silica using 10% aqueous pyridine as eluent: yield 64 mg (64%). Anal. Calcd for $C_{29}H_{30}O_{12}N_6Os_2$: C, 33.67; H, 2.89; N, 8.12; Os, 36.77; py, 30.58. Found: C, 35.17; H, 3.27; N, 8.82; Os, 35.5; py, 29.35, 30.27.

(e) Thymine bis(pyridine) heterocyclic ester was prepared as described by Subbaraman et al.[11] It crystallized readily from water as thick rhombs.

Kinetics. Reactions of $Os_2O_6py_4$ with ribonucleosides were carried out under pseudo-first-order conditions with the osmium(VI) species limiting. The progress of the reaction was followed between 320 and 370 nm. Plots of log $(A_\infty - A_0)/(A_\infty - A_t)$ were linear with time for about 70% of the reaction. Pseudo-first-order rate constants, k_ψ, were calculated from the slopes of these plots.

Transesterification kinetics were carried out in 80% Me_2SO-20% D_2O by integration of the methyl group protons of thymine and its derivatives using ^1H NMR measurements. This exchange reaction is a second-order reversible reaction of the type

$$A + B \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C + D$$

$$-dA/dt = k_f[A][B] - k_r[C][D]$$

which can be integrated[28] to the following when [A] = [B]

$$\ln\left(\frac{x(a - 2x_e) + ax_e}{a(x_e - x)}\right) = k_f\left(\frac{2a(a - x_e)}{x_e}\right)t$$

where a = initial concentration of A, x = concentration of C, x_e = equilibrium concentration of C, and t = time.

Ligand Dissociation. Measurements were made following the procedure of Subbaraman et al.[13] except that CCl_4 was used as the organic phase. The distribution coefficient, $D = [py_o]/[py_a]$, was 2.0.

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