Kinetics of the Addition of OsO_4 to Furans: Mono(osmate ester) vs. Bis(osmate ester) Formation

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The addition of OsO_4/bipyridine and OsO_4/pyridine to various furans has been studied. The latter reagent added across C(2) and C(5) of furan and some of its derivatives to produce a mono(osmate ester) that was unreactive toward further addition of OsO_4. A variety of other furans, however, added the reagent across C(2) and C(3) of the furyl ring. This produced a highly reactive enol ether that rapidly added a second OsO_4 across C(4) and C(5). The product, a bis(osmate ester), bears the substituent osmium atoms trans to each other. Kinetics of the addition of OsO_4/bipyridine to the furyl ring of N^6-furfuryladenosine revealed the existence of bipyridine-independent and bipyridine-promoted reaction pathways. Other furans differed widely in their reactivity toward OsO_4/bipyridine. Furans may prove to be versatile reagents for the site-specific attachment of osmium atoms to macromolecules for high-resolution electron microscopic studies.

High-resolution electron microscopy of biological macromolecules requires the use of heavy-atom markers for distinguishing different components of a complex structure. Osmium tetraoxide has proved to be a valuable reagent for both conventional and high-resolution electron microscopy because the electron-dense Os atom generates contrast in the micrographs, and the reagent displays histochemical selectivity.[1] Among the endogenous cellular elements that undergo reaction with OsO_4 are unsaturated compounds such as membrane components (lipids and proteins)[2] and nucleic acids.

In the presence of a stabilizing ligand such as pyridine or 2,2'-bipyridine (bpy), OsO_4 adds to carbon-carbon double bonds to produce an osmate ester (Figure 1). In the high-resolution scanning transmission electron microscope the individual Os atoms can be visualized as electron-dense regions 2–3 Å across.[3,4]

The reactivity of OsO_4 toward carbon–carbon double bonds and the stability of the resulting osmate ester are dependent on the identity of the ligand present. Tertiary amine ligands offer considerable flexibility in the reactivity of the OsO_4/ligand mixture. Behrman and colleagues, in studies of the reaction of OsO_4 with nucleic acid and protein components, have shown that OsO_4/bpy is a highly reactive and relatively nonselective reagent, whereas OsO_4/pyridine is a less reactive and more discriminating reagent.[5,6] Osmate esters with coordinated bpy are stabler than the bis(pyridine) analogues.[7,8] Thus, manipulations such as gel filtration are more easily carried out on the OsO_4/bpy products. The lower stability of the bis(pyridine) osmates has, however, been useful for carrying out ligand-exchange reactions. In this way mercurated ligands were substituted for osmium-bound pyridine to introduce an additional heavy atom at the osmium-labeled site.[9]

The ligand also influences the rate of the transesterification of osmates [i.e., transfer of OsO_2(ligand)$_2$ groups from one glycol to another]. For electron microscopy a highly mobile marker is generally not desirable, so ligands must be selected with this in mind. Since bpy retards transesterification (relative to pyridine),[7] it is a more suitable ligand from this standpoint, but others are better.[10]

In an effort to deliver two electron-dense atoms to particular locations in macromolecules, we have used furan derivatives as OsO_4 attachment sites.[11] The furan ring is electron-rich and undergoes both electrophilic substitution and addition reactions. It is oxidized by OsO_4/H_2O_2 in the absence of ligands to produce malealdehyde and *meso*-tartaraldehyde.[12] With two double bonds, however, it has the potential to form a bis(osmate ester) upon treatment with OsO_4/ligand mixtures. This potential was realized in a study of nucleic acids selectively modified at cytosine nucleotides with a furan derivative.[11] Chemical analysis verified the 2:1 (Os/furan) ratio, and the Os atoms were visualized and enumerated by high-resolution microscopy.[3,4]

To assess the potential utility of furans as artificially introduced sites for heavy-atom attachment in macromolecules, we have determined the structure of these osmate esters and have examined the reactivity of various furans toward OsO_4/bpy and OsO_4/pyridine.

Results

NMR Studies of the Addition of OsO_4/Pyridine to Furans. Addition of OsO_4 to a furan can be envisioned to produce four isomeric products. If the first OsO_4 adds across the C(2)–C(3) double bond, the osmate ester $\alpha\beta$ shown in Figure 2 results. Addition of the second OsO_4 to this enol ether can produce the trans product ($\alpha\beta$-trans) or the cis product ($\alpha\beta$-cis). If, on the other hand, the first OsO_4 adds across C(2) and C(5) to produce $\alpha\alpha$, then the two products $\alpha\alpha$-trans and $\alpha\alpha$-cis can result from addition of the second OsO_4.

When OsO_4 was allowed to react with various furans in D_2O/pyridine-d_5 and the NMR spectrum of the solution was recorded, the Os/furan ratio of the product was obtained by integration of reactant and product signals. The results are shown in Table I. It was found that furan itself, as well as furans bearing methyl substituents, react to

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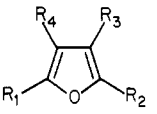
Table I. Stoichiometry of Addition of OsO_4/Pyridine to Selected Furans



compd	R_1	R_2	R_3	R_4	Os/furan ratio
1	H	H	H	H	0.9 [a,b]
2	CH_3	H	H	H	1.1 [b]
3	CH_3	CH_3	H	H	1.1 [b]
4	$COCH_3$	H	H	H	2.1 [c]
5	CHO	H	H	H	2.2 [a]
6	CO_2H	H	H	H	2.3 [b]
7	H	H	$CO_2CH_2CH_3$	H	1.9 [a]
8	N^6-adenylmethyl [d]	H	H	H	1.8 [a]
9	N^6-adenosylmethyl [e]	H	H	H	2.2 [a]
10	CO_2H	CO_2H	H	H	2.1 [b]
11	H	H	CO_2H	CO_2H	1.8 [a]
12	$CH_3CO_2CH_2$	$CH_3CO_2CH_2$	H	H	1.9 [b,c]
13	H	H	$CH_3CO_2CH_2$	$CH_3CO_2CH_2$	2.3 [a]
14	CH_2OH	CHO	H	H	2.2 [a]
15	H	H	$CO_2CH_2CH_3$	$CO_2CH_2CH_3$	2.0 [a]
16	CH_2OH	CH_2OH	H	H	2.1 [b]

[a] Integration of α-hydrogen signals. [b] Integration of β-hydrogen signals. [c] Integration of methyl signals. [d] Kinetin, N^6-furfuryladenine. [e] Kinetin riboside, N^6-furfuryladenosine.

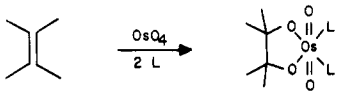


Figure 1. Reaction of OsO_4 with an alkene in the presence of tertiary amine ligands (L) produces an osmate ester.

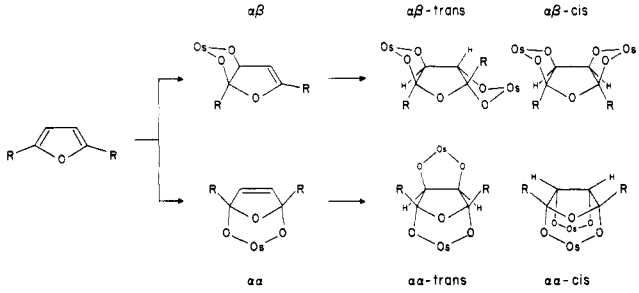


Figure 2. Possible modes of addition of OsO_4 to a disubstituted furan are shown. Two of the oxygen atoms and the ligands on osmium have been omitted for clarity. Actual conformations of the products are not implied.

produce a 1:1 (Os/furan) adduct. Examination of the spectra revealed that the adducts had structure $\alpha\alpha$. For furan–OsO_4py_2: δ 5.32 [d, C(3)H and C(4)H], 6.29 [d, C(2)H and C(5)H]. For dimethylfuran–OsO_4py_2: δ 2.31 [s, C(2)CH_3 and C(5)CH_3], 5.43 [s, C(3)H and C(4)H]. These $\alpha\alpha$ products failed to take up another OsO_4, even on prolonged standing. To assess the possibility that steric effects might hamper OsO_4 addition to the C(3)–C(4) double bond of the $\alpha\alpha$ product, we carried out a study of *cis*- and *trans*-2,5-dimethoxy-2,5-dihydrofuran. When OsO_4/pyridine-d_5 addition was studied by NMR, it was found that the cis isomer underwent OsO_4 addition more rapidly than the trans isomer. This was evidenced by the more rapid disappearance of signals due to the cis isomer (δ 3.60, 5.78 and 6.30) than those due to the trans isomer (δ 3.56, 6.08, and 6.32) and concomitant appearance of the corresponding product signals (principally δ 3.68, 5.02, and 5.56).

The furans that added OsO_4 twice are also shown in Table I. In no case that was studied could OsO_4 addition be held to one for elucidation of whether formation of $\alpha\alpha$

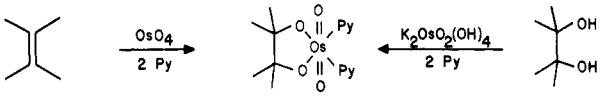


Figure 3. Two pathways for formation of an osmate ester (center) are shown, OsO_4 plus alkene (left) and Os(VI) plus glycol (right).

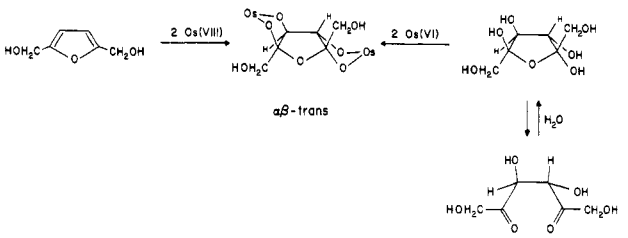


Figure 4. Formation of a bis(osmate ester) in two ways is shown for $\alpha\beta$-trans structure proof. Only the $\alpha\beta$-trans structure can form from 5-oxofructose and Os(VI) because of the fixed configurations of the chiral carbon atoms in the 5-oxofructose. Two oxygen atoms and ligands on osmium have been omitted for clarity.

or $\alpha\beta$ products had occurred. Furthermore, no unique NMR characteristics are expected for the resulting bis(osmate ester) products, and thus they might have had any of the four structures shown in Figure 2.

Structure of a 2:1 Os/Furan Adduct. The structure of one of the 2:1 Os/furan adducts was established by an unambiguous synthetic approach. Osmate esters of the type under investigation can be produced in two ways: (1) OsO_4 addition to an alkene (Figure 3, left) and (2) esterification of a glycol by the Os(VI) compound $K_2OsO_2(OH)_4$ (Figure 3, right). Of the four possible bis(osmate ester) products (Figure 2), one is unique ($\alpha\beta$-trans) in that it exhibits a trans arrangement of oxygen atoms across C(3)–C(4) of the ring. The synthesis of an $\alpha\beta$-trans adduct from both OsO_4 and Os(VI) requires that the substituted furan and the appropriate aliphatic polyhydroxy compound be available. One such combination is illustrated in Figure 4. If addition of OsO_4 to 2,5-bis(hydroxymethyl)furan produces the $\alpha\beta$-trans product, then it gives the same product that assembles from Os(VI) and the sugar derivative 5-oxofructose (D-*threo*-2,5-hexodiulose), except that the former produces racemic bis(osmate ester)

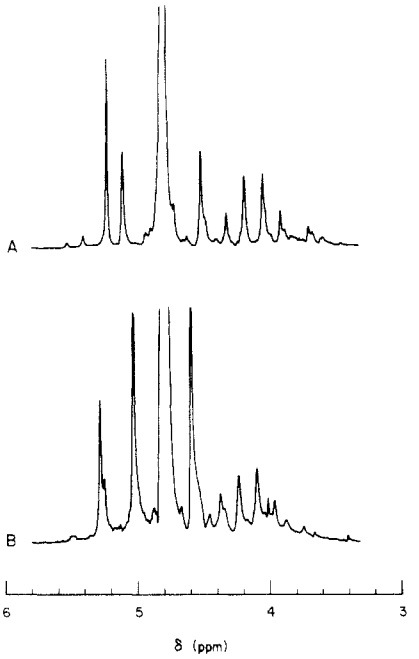


Figure 5. NMR spectra of the bis(osmate ester) produced by OsO_4 plus 2,5-bis(hydroxymethyl)furan (A) and Os(VI) plus 5-oxofructose (B). The latter combination is constrained to produce the $\alpha\beta$-trans product.

Table II. Chromatographic Characteristics of Osmate Esters [a]

compd [b]	R_f of osmate	compd [b]	R_f of osmate
1	0.40	8	0.30
3	0.48	12	0.49
5	0.36	13	0.48
6	0.26	16	0.43

[a] Silica gel thin-layer plates; solvent CH_3OH–pyridine (9:1). [b] See Table I for compound identities and Os/furan ratios.

and the latter produces only one enantiomer.

The two reactions outlined above were carried out, and the NMR spectra of the respective products were obtained (Figure 5). The spectrum of OsO_4/pyridine-d_5 plus 2,5-bis(hydroxymethyl)furan is shown in Figure 5A; the spectrum of $K_2Os^{VI}O_2(OH)_4$ plus 5-oxofructose is shown in Figure 5B. The results clearly show that both approaches yield the same bis(osmate ester). The singlet at δ 5.3 arises from C(3)H and C(4)H, and the AB quartet centered at δ 4.2 arises from the diastereotopic methylene protons of the CHH′–OD groups. (Between the singlet and the quartet of each spectrum are signals due to HDO and spinning side bands. The shoulder on the δ 5.3 singlet in Figure 5B is a spinning side band.) Small differences in chemical shifts of corresponding signals in the two spectra are probably due to slight differences in solution composition (see Experimental Section). The conclusion that these two products were identical was further verified by thin-layer chromatography. The two products were found to cochromatograph (R_f 0.4). The chromatographic characteristics of other adducts are displayed in Table II.

The ultimate product of the reactions outlined in Figure 4 may in fact be an osmate ester involving the substituent hydroxymethyl groups, but this does not alter the conclusions that the two approaches yield the same product and that the oxygen atoms on C(3) and C(4) of the ring are trans to each other (i.e., that the $\alpha\beta$-trans product formed upon OsO_4 addition to the furan).

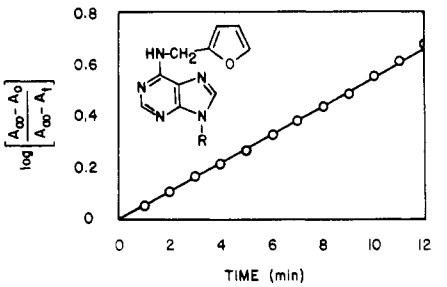


Figure 6. Kinetics of the reaction of OsO_4 (0.1 mM) with kinetin riboside (1.8 mM) in the presence of bpy (2 mM) at 25 °C.

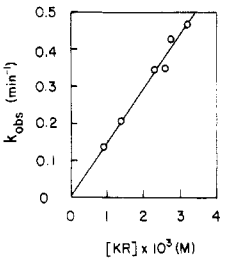


Figure 7. First-order dependence of reaction rate on kinetin riboside (KR) concentration is demonstrated for the reaction with OsO_4 in 5 mM bpy at 25 °C.

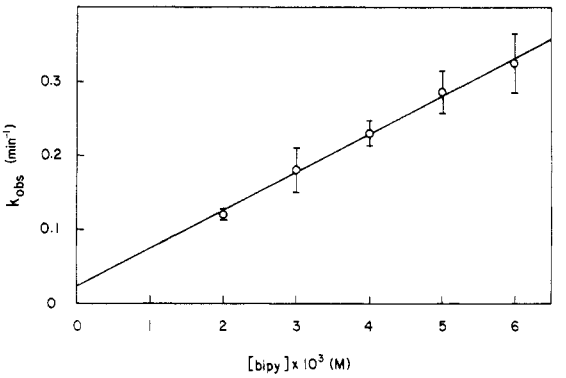


Figure 8. Dependence of reaction rate on bpy concentration is demonstrated for the reaction of OsO_4 with kinetin riboside (1.8 mM) at 25 °C. Kinetic constants for the bpy-promoted and bpy-independent pathways were obtained from the slope and intercept, respectively (see Experimental Section). Each point is the average of three determinations.

Kinetics of OsO_4/Ligand Addition to Furans. The kinetics of the reaction of OsO_4 with N^6-furfuryladenosine (kinetin riboside, KR) in aqueous bipyridine solutions were studied, and the reaction was found to obey the rate law shown in eq 1, in which $v = -d[KR]/dt$. Kinetic studies

$$v = k_0[KR][OsO_4] + k_1[KR][bpy][OsO_4] \quad (1)$$

were carried out under pseudo-first-order conditions, with KR and bpy present in excess over OsO_4.

A typical run is shown in Figure 6. First-order disappearance of OsO_4 is indicated by the observed linearity over 2–3 half-times. Other furans such as kinetin (not the riboside) showed linearity over 4 or more half-times.

First-order dependence on KR concentration was verified by the linearity of the plot of the pseudo-first-order rate constant k_{obsd} vs. [KR], as shown in Figure 7.

The existence of a bpy-promoted as well as a bpy-independent pathway was revealed by the plot of k_{obsd} vs. [bpy] shown in Figure 8. The rate constants k_0 and k_1 were obtained from this straight line (intercept and slope, respectively). For KR it was found that $k_0 = 7 \pm 2$ M^{-1} min^{-1} and $k_1 = (1.4 \pm 0.1) \times 10^4$ M^{-2} min^{-1}.

Table III. Reactivity of Furans toward OsO_4/bpy and OsO_4/Pyridine

compd	k', M^{-1} min^{-1} [a]	k_{obsd} min^{-1} [b]
1	24	
3	750	
4	1.0	
6	2.8	
8	71	
9	75	0.065
thymidine	99	0.027

[a] Reactions were carried out with 5 mM bpy at pH 7.0 and 25.0 °C; uncertainty is estimated at ±5%, except for compound 3, which is estimated at ±15%. [b] Reactions were carried out with 54 mM pyridine and 5.4 mM substrate at pH 7.4 and 25.0 °C; uncertainty is estimated at ±5%.

A detailed study of 2,5-dimethylfuran, which formed a 1:1 Os/furan adduct in the NMR experiments (in contrast with KR), revealed similar dependence on [OsO_4], [dimethylfuran], and [bpy], although experimental error in this very fast reaction precluded the detection and quantitation of a bpy-independent pathway (data not shown).

To compare the reactivities of a variety of furans, we carried out kinetic studies under pseudo-first-order conditions, as before, except that [bpy] was kept constant. The concentration of the furan was varied to give a convenient reaction rate. Assumption of a rate law of the form of eq 1 gives eq 2 and 3, in which $v = -d[\text{substrate}]/dt$.

$$v = (k_0 + k_1[\text{bpy}])[\text{substrate}][OsO_4] \quad (2)$$

$$v = k'[\text{substrate}][OsO_4] \quad (3)$$

Thus, k' can be compared for a variety of furans and is a combined measure of the reactivity of the furan toward OsO_4 in both the bpy-promoted and bpy-independent pathways. The values of k' for various furans are displayed in Table III. Since the reaction rate is first order in [OsO_4], the rate constants are for addition of the first OsO_4 in the cases that result in a 2:1 Os/furan adduct (see Experimental Section).

The highly reactive 2,3-dihydrofuran (0.3 mM) was studied at 8 °C (0.5 mM bpy), and k_{obsd} was ~1 min^{-1}. By use of k_0 and k_1 for KR, it can be calculated that 2,3-dihydrofuran at 8 °C is ~300 times more reactive than KR at 25 °C. This supports the idea that the 4,5 double bond of the 1:1 Os/furan compound $\alpha\beta$ is highly reactive and serves to explain why intermediates of this type were not detectable in the NMR experiments.

Discussion

Addition of OsO_4 to alkenes in the presence of a tertiary amine ligand produces osmate esters, as shown in Figure 1. The reactive heterocyclic furan has the potential to add OsO_4 twice to form a bis(osmate ester). Addition of the first OsO_4 can be envisioned to produce either the $\alpha\beta$ or the $\alpha\alpha$ product shown in Figure 2. Addition of the second OsO_4 cis or trans to the first produces the four possible 2:1 Os/furan products (three meso and one *dl* pair).

By NMR spectroscopic studies several furans were categorized according to whether they produced a 1:1 Os/furan or a 2:1 product. The results, displayed in Table I, suggest that furans with methyl groups as well as furan itself add only one OsO_4·py_2 group. When other substituents were present (including both those that activate and those that deactivate the ring), the furan added two OsO_4·py_2 groups.

Examination of the NMR spectra of the furan and 2,5-dimethylfuran adducts revealed that the OsO_4·py_2 had added across C(2) and C(5) to yield the $\alpha\alpha$ product. This product was unreactive toward further addition of OsO_4. The failure of the $\alpha\alpha$ product to add OsO_4 may be the result of steric effects. The sensitivity of OsO_4 addition to steric effects, well documented in other systems,[12] was also found in the case of furans. When *cis*- and *trans*-2,5-dimethoxy-2,5-dihydrofuran, which resemble the $\alpha\alpha$ product in that they have a C(3)–C(4) double bond and two oxygen atoms on C(2) and C(5), were treated with OsO_4/pyridine-d_5, the cis isomer reacted more rapidly. Since the electronic character of the C(3)–C(4) double bond in the two isomers ought to be very similar, the difference in reactivity probably results from steric factors. Thus, while subtler effects may be operative in the $\alpha\alpha$ case, a simple steric effect could easily account for the unreactivity of the $\alpha\alpha$ product toward further addition of OsO_4.

In order to learn which of the bis(osmate ester) products formed in the case of furans that formed 2:1 Os/furan adducts, we took advantage of the fact that osmate esters can be assembled from glycols and Os(VI) as shown in Figure 3. A bis(osmate ester) that could be prepared by using OsO_4 plus a furan as well as Os(VI) plus an aliphatic glycol was selected. Addition of OsO_4 to 2,5-bis(hydroxymethyl)furan to produce the $\alpha\beta$-trans product is illustrated in Figure 4. Also shown is the assembly of the same product from Os(VI) and 5-oxofructose (D-*threo*-2,5-hexodiulose), which probably exists predominantly as a bicyclic bis(hemiacetal) in solution in facile equilibrium with other forms.[13] The cyclic form shown resembles the furanose ring observed in the solid state by X-ray crystallography.[14] The $\alpha\beta$-trans product is unique in the sense that it is the only one of the four possible products shown in Figure 2 that possesses a trans arrangement of the C(3) and C(4) oxygen atoms.

The two reaction sequences shown in Figure 4 were carried out, and the NMR spectra of the products were recorded. The spectrum of the product of the OsO_4 plus furan pathway is shown in Figure 5A, and the spectrum of the product of the Os(VI) plus 5-oxofructose pathway is shown in Figure 5B. It is clear from examination of the chemical shifts and coupling constants that both products are the same, a conclusion further supported by their cochromatography on silica gel thin-layer chromatography plates (Table II).

The validity of this structure proof rests on the assumption that the configurations of the chiral carbon atoms of the osmate are preserved. That assumption is justified by the observation that osmates generally are relatively stable, and when they do undergo rearrangements, it is via Os–O bond cleavage rather than O–C bond cleavage.[10] Thus, even if rearrangements occurred in solution, they very likely would not alter the configurations of the chiral carbon atoms. Furthermore, had the two approaches not yielded the same isomer, the spectra would have been expected to be different. Conversion of furans into osmate esters resulted in large changes in the chemical shifts of the β-hydrogens (e.g., δ 7.1 to 6.0 for **10**, 6.6 to 5.7 for **12**, and 6.5 to 5.6 for **16**). This sensitivity of the β-hydrogens to the influence of the two nearby OsO_4·py_2 groups suggests that isomeric osmates would display distinguishable spectral characteristics.

It is not known whether any or all of the other 2:1 Os/furan adducts are of the $\alpha\beta$-trans type. The very fact, however, that they are 2:1 adducts supports the conclusion that they are of the $\alpha\beta$ type. If instead they were $\alpha\alpha$

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products, OsO_4 addition would likely have stopped at 1:1 Os/furan as in the case of furan itself and its methyl derivatives. Furthermore, these 2:1 Os/furan adducts are expected to be trans products, as observed in the case of 2,5-bis(hydroxymethyl)furan, because of the steric requirements of the $OsO_4 \cdot py_2$ group.

Addition of OsO_4 resembles addition of other electrophiles to furans. Addition of singlet oxygen, for example, to furan[15] and 2,5-dimethylfuran[16] also takes place across C(2) and C(5). Addition of ozone to 2,5-diarylfurans yields bond-cleavage products indicative of both 2,3- and 2,5-addition.[17]

The general behavior of furans toward OsO_4/pyridine can be summarized as follows. If the first OsO_4 adds across C(2) and C(5), then the $\alpha\alpha$ product forms. This product is unreactive toward further addition of OsO_4. If, instead, the first OsO_4 adds across C(2) and C(3), then the $\alpha\beta$ product forms. This reactive enol ether rapidly adds another OsO_4 to yield a bis(osmate ester), in which the two Os atoms are trans to each other. Thus, mono(osmate ester) vs. bis(osmate ester) formation has been rationalized in terms of the site of addition of the first OsO_4.

The reactivity of kinetin riboside toward OsO_4/bpy was examined kinetically. Kinetin riboside, which formed a 2:1 Os/furan product with OsO_4/pyridine in the NMR studies, was found to react with OsO_4/bpy according to the rate law shown in eq 4.

$$v = (k_0[KR] + k_1[KR][bpy])[OsO_4] \quad (4)$$

This behavior is typical of many substrates in their reaction with OsO_4/bpy. The pseudo-first-order disappearance of OsO_4 was cleanly followed (Figure 6). The first-order dependence on [KR] was revealed by the data shown in Figure 7. The existence of a bpy-promoted and a bpy-independent pathway can be inferred from the data displayed in Figure 8, since the rate is linearly dependent on [bpy], but extrapolation to zero bpy concentration shows that reaction still takes place. Reliable estimates of the bpy-independent rate constants cannot be obtained from the reaction carried out in the absence of bpy for reasons that have been previously discussed.[6] The rate constants k_0 and k_1 were evaluated, and kinetin riboside was found to be approximately as reactive as thymidine in the bpy-promoted OsO_4 addition reaction.

The relative reactivities of a variety of furans toward OsO_4/bpy and OsO_4/pyridine were also assessed. Examination of the values of k' displayed in Table III clearly reveals a wide range in reactivities of various furans toward OsO_4. From the kinetics it is apparent that the furyl ring of kinetin riboside is slightly more reactive than thymidine toward OsO_4/pyridine ($k_{obsd}^{KR}/k_{obsd}^{Thd} = 2.4$). Thus, a macromolecule containing both (such as a chemically modified DNA) would be expected to undergo OsO_4 attachment at both sites, assuming both are equally accessible. Furthermore, uridine is about 10 times less reactive than thymidine, so one might successfully add OsO_4 to the furyl rings in a suitably modified RNA while leaving the uracils largely unaffected. This conclusion does not hold for the OsO_4/bpy reagent because the rate differences are decreased by the high reactivity/low selectivity of this reagent.

Suitably designed furans readily undergo addition of two heavy atoms under mild conditions and may prove to be of considerable value in a wide range of electron microscopic studies of appropriately modified macromolecular assemblies.

Experimental Section

Furans and pyridine-d_5 were from Aldrich Chemical Co., except as noted below. 2-Acetylfuran and 2-furaldehyde were purified by distillation. 3,4-Furandicarboxylic acid was recrystallized from ethanol. 5-Oxo-D-fructose ("5-keto-D-fructose") was from Chemical Dynamics Corp., and its identity was verified by NMR.[13] 2,5-Bis(hydroxymethyl)furan was prepared by reduction of 5-(hydroxymethyl)-2-furaldehyde with $NaBH_4$; mp 77.5–79 °C ($CHCl_3$) (lit.[18] mp 75.5–77, 80 °C). 2,5-Bis(acetoxymethyl)furan was prepared by treating bis(hydroxymethyl)furan with acetic anhydride in pyridine; mp 63.5–65.5 °C (EtOH) (lit.[18] mp 64 °C). 2,5-Furandicarboxylic acid was synthesized and esterified (CH_3OH/H_2SO_4), and the ester was purified by column chromatography (silica/$CHCl_3$). Following saponification and acidification, the diacid was recrystallized from H_2O; neutralization equivalent 79.0 [lit.[19] 78.7–79.0 (theory 78.0)]. The furans obtained by the preceding syntheses had the expected NMR spectra. Osmium tetraoxide was from Sigma Chemical Co. and Electron Microscopy Sciences. $K_2OsO_2(OH)_4$ was from Alfa Products. Analyses were performed by Galbraith Laboratories, and melting points are uncorrected. The concentration of OsO_4 was estimated by UV spectroscopy[5] and analysis.[8]

Thin-layer chromatography was carried out on hard-layer silica gel plates from Analtech Inc. with CH_3OH–pyridine (9:1 v/v) as the developing solvent. After development, plates were sprayed with 2% thiourea in 2 M HCl to reveal osmium-containing spots.

NMR Spectroscopy of Osmate Esters. ^1H NMR spectroscopy was carried out at 60, 90, or 100 MHz. Typically, 0.10 mmol of the furan was added to 0.10 mL of pyridine-d_5, and then 0.40 mL of 0.18 M OsO_4 dissolved in D_2O was added. After addition of 0.05 mL of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in D_2O, the spectrum was recorded. Each of the furans listed in Table I cleanly formed a single 1:1 or 2:1 Os/furan product. The lower limit of detection of a secondary product is estimated at ~3% (of total furan) on the basis of observed peak intensities and noise levels in typical spectra. All solutions examined were homogeneous, thus eliminating the possibility that a product was not detected because of insolubility. Addition of one OsO_4 was distinguished from addition of two OsO_4 groups by integration of product and reactant signals. The uncertainty in the Os/furan ratio is estimated at approximately ±5%. Comparison of the amount of osmate ester formed to the amount of OsO_4 added revealed that osmate ester formation was virtually complete under these conditions.

Structure of a 2:1 Os/Furan Adduct. In the case of 5-oxofructose (D-*threo*-2,5-hexodiulose), 0.06 mmol of $K_2OsO_2(OH)_4$ was suspended in 200 μL of D_2O plus 25 μL each of pyridine-d_5 and ~8 M DCl in D_2O. Addition of 0.03 mmol of 5-oxofructose in 200 μL of D_2O was followed by stirring for 2 h. After addition of DSS the NMR spectrum was recorded. Product signals had emerged at this point, but they were weak. Addition of 0.04 mmol of $K_2OsO_2(OH)_4$ followed by agitation for 15 min and then filtration yielded an improved spectrum (Bruker WH-90 FT NMR, 50 scans). For comparison, 2,5-bis(hydroxymethyl)furan was treated with OsO_4 in D_2O/pyridine-d_5, and DCl was added to give a solution of comparable composition. The NMR spectrum was then recorded. To verify that an AB quartet was present, the spectrum of a comparable solution was recorded at both 100 and 60 MHz, and the signal separations and relative intensities were analyzed mathematically.[20] All studies were performed on freshly prepared solutions because of the instability of this bis(osmate ester).

Kinetics of OsO_4/Ligand Addition to Furans. Kinetics of the reaction of OsO_4 and pyridine (50 mM NaH_2PO_4, final pH 7.4) or 2,2′-bipyridine (50 mM NaH_2PO_4, final pH 7.0) with various

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furans were carried out at 25.0 ± 0.1 °C under pseudo-first-order conditions with OsO_4 the limiting reagent. The progress of the reaction was monitored by ultraviolet absorption spectroscopy with a Perkin-Elmer Model 552 spectrophotometer equipped with a thermoelectric cuvette holder. Kinetics were generally followed at 315 nm for OsO_4/bpy solutions and at 450 nm for OsO_4/pyridine solutions. Plots of $\log[(A_\infty - A_0)/(A_\infty - A_t)]$ vs. time were linear over at least 4 half-times, except in the case of kinetin riboside plus OsO_4/pyridine, in which curvature became apparent after 2–3 half-times. The initial concentrations of substrate (furan) and ligand (pyridine or bpy) were varied to establish the rate law and to evaluate rate constants.

In the case of a furan that produced only a 1:1 Os/furan adduct, the rate of appearance of UV-absorbing osmate groups was exactly equal to the rate of disappearance of the furan. In the case of a furan that produced a 2:1 Os/furan adduct, the rate of formation of individual UV-absorbing osmate groups was twice the rate of disappearance of furan, so $d[OsO_4]/dt = 2(d[KR]/dt)$, assuming the intermediate monoadduct did not accumulate (i.e., d[monoadduct]/dt = 0). Since we monitored osmate appearance and not furan disappearance, the rate constants for furan disappearance were calculated by using the relationship in eq 5.

$$-d[KR]/dt = k_{obsd}[OsO_4]/2 \quad (5)$$

Combining eq 1 and 5 gives eq 6. An examination of the validity

$$k_{obsd} = 2(k_0[KR] + k_1[KR][bpy]) \quad (6)$$

of applying the steady-state approximation to the intermediate monoadduct in the case of kinetin, as described above, follows.

There are three observations that suggest that the use of the steady-state approximation is valid in these kinetics experiments. First, the NMR experiments failed to reveal the existence of the monoadduct even when the furan was present in excess and OsO_4 was added slowly and with vigorous stirring. Second, the enol ether 2,3-dihydrofuran, an analogue of the $\alpha\beta$ monoadduct, is orders of magnitude more reactive than furan itself. Third, the following kinetics experiment provided an independent evaluation of the rate constants for addition of OsO_4/bpy to kinetin without use of the steady-state approximation, and the results of both methods agreed.

The kinetics of addition of OsO_4/bpy to kinetin were carried out with OsO_4/bpy in excess and kinetin as the limiting reagent. This assured that the final product would be the 2:1 Os/furan adduct, which was verified experimentally on the basis of the magnitude of the absorbance increase of the solution. In this experiment kinetin disappears via a pseudo-first-order process, as does the intermediate $\alpha\beta$-type monoadduct. The data, plotted as absorbance vs. time, were fitted by an iterative procedure based on a published computer program.[21] Reasonable values for the extinction coefficients (at 315 nm) of kinetin, the monoadduct (1.2×10^4 M^{-1} cm^{-1}), and the diadduct (2.5×10^4 M^{-1} cm^{-1}) were selected. Then the two variables, the pseudo-first-order rate constants for addition of OsO_4/bpy to kinetin and to the monoadduct, were calculated by minimizing the deviation of the calculated absorbances from the observed absorbances. With OsO_4 at 0.72 mM, bpy at 2.0 mM, and kinetin at 0.03 mM initially, we found the pseudo-first-order rate constants to be 0.021 min^{-1} and >5 min^{-1} for the first and second step, respectively. This allows k' to be estimated at 73 M^{-1} min^{-1}, in good agreement with the value 71 M^{-1} min^{-1} obtained by using the steady-state approximation (OsO_4 limiting). Thus, use of the steady-state approximation appears to be justified in the case of kinetin. Taken together, these three reasons argue strongly that the approximation is valid for the other furans that ultimately produce a 2:1 Os/furan adduct. The results obtained using the approximation, displayed in Table III, are inherently more accurate and easier to obtain than those obtained by the curve-fitting method.

Bis(osmate ester) of 2-Acetylfuran. A solid bis(osmate ester) was prepared from an aqueous solution containing 20 mM OsO_4, 20 mM bpy, and 10 mM 2-acetylfuran (freshly distilled). The solid was collected by suction filtration, washed with water and ethyl acetate, finely ground, rewashed, and dried in vacuo. Anal. Calcd for $C_{26}H_{22}Os_2N_4O_{10}$: C, 33.55; H, 2.38; N, 6.02. Found: C, 33.90; H, 2.73; N, 5.89.

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Kinetic Analysis of the Ring Opening of an *N*-Alkyloxazolidine. Hydrolysis of 2-(4-Methylphenyl)-2,3-dimethyl-1,3-oxazolidine

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Hydrolysis of the title oxazolidine, III, occurs in two separate reaction stages, reversible ring opening to a cationic Schiff base, IV, followed by a considerably slower formation of hydrolysis products. The ring opening occurs in an H^+-catalyzed reaction and in a pH-independent reaction, with the crossover between the two occurring at about pH 5. A general acid catalyzed pathway ($\alpha = 0.70$) is also observed. The equilibrium constant (pK_{I^+}) for IV ⇄ III + H^+ is 7.45, this number being obtained spectroscopically and in a kinetic analysis. The kinetic analysis also furnishes a dissociation constant (pK_{SH^+}) for the protonated oxazolidine of 6.19, the difference between pK_{SH^+} and pK_{I^+} showing that after attainment of equilibrium the conjugate acid of III is a 19:1 mixture of cationic Schiff base and protonated oxazolidine. The formation of hydrolysis products involves rate-limiting addition of water or hydroxide ion to IV, although a small percentage of a reaction via an oxocarbonium ion derived from C–N cleavage of the protonated oxazolidine cannot be ruled out. Rate constants for the water and hydroxide addition are slower than their intramolecular counterparts, this being particularly true in comparing hydroxide ion reactions. This occurs despite the fact that the ring closure is a supposedly disfavored 5-endo-trigonal process.

Oxazolidines are cyclic acetal analogues with one oxygen replaced by nitrogen. These heterocycles hydrolyze relatively rapidly, even in basic media, producing the corresponding carbonyl compound and β-amino alcohol. A feature of their hydrolysis is that a ring-opened cationic Schiff base is often observed as an intermediate in acid

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