Stereo- and Regiochemistries of the Oxidations of 2-Methoxy-5-*tert*-butyl-1,3,2-dioxaphosphorinanes and the Cyclic Methyl 3′,5′-Phosphite of Thymidine by H_2O/I_2 and O_2/AIBN to P-Chiral Phosphates. ^{17}O NMR Assignment of Phosphorus Configuration to the Diastereomeric Thymidine Cyclic Methyl 3′,5′-Monophosphates

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Abstract: The stereo- and regiochemistries of oxidation of six-membered ring trialkyl phosphites under nonaqueous conditions with O_2, initiated thermally or photochemically by azobis(isobutyronitrile) (AIBN), and with the well-known reagent H_2O/I_2 have been investigated. Yields of product phosphates are high, and both reactions occur regio- and stereospecifically with retention of configuration at phosphorus as shown for the diastereomeric 2-methoxy-5-*tert*-butyl-1,3,2-dioxaphosphorinanes and cyclic methyl 3′,5′-phosphite triesters derived from thymidine. Mechanistic rationales are proposed for both processes. These oxidations are useful for the facile introduction of ^{17}O or ^{18}O label into the phosphoryl oxygen of the product cyclic phosphate. Demethylation of the cyclic thymidine methyl 3′,5′-monophosphate so-labeled yields the individual cyclic 3′,5′-monophosphate diastereomers with ^{18}O or ^{17}O specifically axial or equatorial. The O_2/AIBN oxidation can be proposed as a *nonaqueous* method for the oxidation of dinucleoside phosphite triesters and perhaps in the synthesis of oligonucleotides by the phosphite intermediate route as well. ^{17}O NMR is shown to be a convenient method to assign the absolute configuration at phosphorus to the individual oxygen-labeled diastereomers of the cyclic thymidine methyl 3′,5′-monophosphate triester on the basis of their P=O^{17} resonances, which are well-separated at 54.2 MHz ($\Delta\delta$ = 9.5, 515 Hz) and relatively narrow in CD_3CN at 80 °C. One-bond oxygen phosphorus couplings are well-resolved as well. Application of the ^{17}O results in the determination of the configurations of P-chiral thymidine 3′- and 5′-monophosphate diesters is proposed.

In recent years the phosphoramidite method of di- and oligonucleotide synthesis has moved into prominence.[1] The phosphite triesters, formed in the condensation sequence, require oxidation to the phosphate triester stage prior to dealkylation to the final phosphate diester functionality of the nucleotide product. This oxidation needs to be facile, clean, and have a high yield. Of special desirability is the ability to introduce ^{17}O or ^{18}O label regio- and stereospecifically into the phosphoryl oxygen of the phosphate triester, which after separation of the triester diastereomers, can be dealkylated to the isotopically chiral phosphate diester of specific configuration.[2]

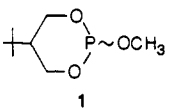
The most widely used reagent for these purposes is H_2O/I_2,[3] which does allow for ^{17}O and ^{18}O label incorporation. Recently, several papers have reported nonaqueous reagents that are said to provide an advantage, because they avoid the subsequent drying step in oligonucleotide synthesis.[4] However, these nonaqueous techniques do not allow the ready introduction of oxygen label.

Table I. Stereochemistry of the Oxidation of *cis*- and *trans*-**1** to **2**

method	*cis*/*trans*-**1**[a]	*cis*/*trans*-**2**[b]
O_2/AIBN, benzene, 75 °C	96/4	95/5
	92/8	93/7
	59/41	53/47
	16/84	20/80
I_2/H_2O, THF, −45 °C	96/4	95/5
	14/86	15/85
N_2O_2/CH_2Cl_2, −10 °C	92/8	94/6
	41/59	44/56

[a] By ^{31}P NMR. [b] By GLC analysis.

We report here an azobis(isobutyronitrile)- (AIBN-) initiated O_2 oxidation of the cyclic methyl 3′,5′-phosphite of thymidine, **3**, to the corresponding cyclic 3′,5′-phosphate triester, **4**, cleanly,



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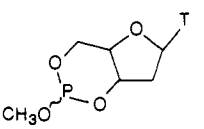

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in high yield, and with regio- and stereospecificity.[5] One may use $^{16}O_2$, $^{18}O_2$, or the isotopic mixture containing $^{17}O_2$ to introduce oxygen into the phosphoryl oxygen *regiospecifically* with *retention* of configuration at phosphorus. The potential application of this method to di- and perhaps oligonucleotide synthesis by the phosphite triester route can be recommended. We also find that the oxidation of **3** with H_2O/I_2 occurs with *retention* of configuration at phosphorus and is *regiospecific* for the introduction of label ($H_2{}^{18}O$ or $H_2{}^{17}O/I_2$) into the phosphoryl oxygen. The

(1) Caruthers, M. H.; Brill, W.; Dellinger, D. J. *Phosphorus Sulfur* **1987**, *30*, 549. Caruthers, M. H. *Science* **1985**, *230*, 281.

(2) (a) For reviews of the use of (^{16}O, ^{17}O, ^{18}O) P-chiral phosphates in enzymology, see: Gerlt, J. A. In *Phosphorus 31-NMR Principles and Applications*; Gorenstein, D. G., Ed.; Academic Press: Orlando, FL, 1984; pp 199–232. Lowe, G. *Acc. Chem. Res.* **1983**, *16*, 244. Gerlt, J. A.; Coderre, J. A.; Mehdi, S. *Adv. Enzymol. Rel. Areas Mol. Biol.* **1983**, *55*, 291. Frey, P. A. *Tetrahedron* **1982**, *11*, 1541. Buchwald, S. L.; Hansen, D. E.; Hassett, A.; Knowles, J. R. *Methods Enzymol.* **1982**, *87*, 279. Tsai, M. D. *Ibid.* **1982**, *87*, 235. Frey, P. A. In *New Comprehensive Biochemistry*; Tanner, C., Ed.; Elsevier: Amsterdam, 1982; Vol. 3, p 210. Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877. (b) $H_2{}^{18}O/I_2$ has been used recently to introduce label stereo- and regiospecifically into the P=O of dinucleosides triphosphates. Dealkylation yields the P-chiral dinucleoside diphosphate, of use in itself and for incorporation in oligonucleotides. See, for example: Potter, B. V. L.; Eckstein, F. *Nucleic Acids Res.* **1983**, *11*, 7087. Seela, F.; Ott, J.; Potter, B. V. L. *J. Am. Chem. Soc.* **1983**, *105*, 5879. Herdering, W.; Kehne, A.; Seela, F. *Helv. Chim. Acta* **1985**, *68*, 2119. Herdering, H.; Seela, F. *J. Org. Chem.* **1985**, *50*, 5314. Seela, F.; Ott, J.; Potter, B. V. L.; Herdering, W. *Nucleosides Nucleotides* **1985**, *4*, 131. For a review, see: Koziockiewic, M.; Uznanski, B.; Stec, W. J.; Zon, G. *Chem. Scr.* **1986**, *26*, 251. The stereochemistry at phosphorus has been determined in many of the above cases. For use of $H_2{}^{17}O/I_2$ to introduce ^{17}O into oligonucleotides, see: Shah, D. O.; Lai, K.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 4302.

(3) Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.

(4) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191. Fourrey, J.-L.; Varenne, J. *Ibid.* **1985**, *26*, 1217. Ogilvie, K. K.; Nemer, M. J. *Ibid.* **1981**, *22*, 2531.

(5) A preliminary account of the O_2/AIBN work was published: Gajda, T. M.; Sopchik, A. E.; Bentrude, W. G. *Tetrahedron Lett.* **1981**, *22*, 4167.

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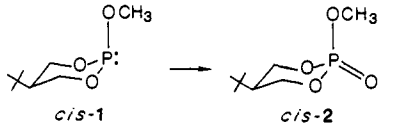
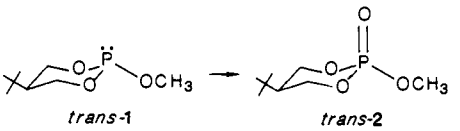
P=^{18}O or P=^{17}O triester from either oxidation is readily separated to the individual diastereomers, which are dealkylated to the diastereomeric O-labeled diesters, e.g., *cis*-**10**. Furthermore, we show ^{17}O NMR to be useful for the assignment of configuration at phosphorus in cyclic 3′,5′-monophosphate triesters, **4**, and propose the application of this technique to stereochemical studies of enzymic reactions of phosphates.

Results

Stereochemistry and Yields. The oxidation systems were first applied to the simple cyclic phosphites, the 2-methoxy-5-*tert*-butyl-1,3,2-dioxaphosphoranes, **1**, which we have used previously in stereochemical investigations.[6] The chair conformation actually populated by the cis diastereomer of **1** in solution is shown in eq 1. For *trans*-**1**, the diequatorially substituted chair conformation



(1)

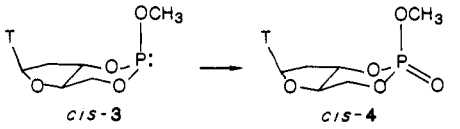


(2)

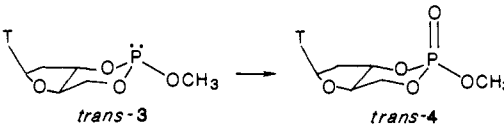
is given, eq 2, although the diaxial chair and/or twist form is known to be populated in solution.[7] Results of the oxidation of several cis/trans ratios of **1** with H_2O/I_2 are recorded in Table I. H_2O/I_2 oxidations were carried out at −20 °C in THF/pyridine), essentially according to the method of Letsinger.[3] Reactions were monitored by ^{31}P NMR and GLC. The isolated yield of isomeric phosphates **2** was 89%. Oxidations are seen to be *stereospecific with retention of configuration at phosphorus* (Table I), as indicated by reactions 1 and 2.

$^{16}O_2$/AIBN oxidations of phosphite **1** were carried out on dilute solutions in benzene to which also was added 5–10 wt. % of AIBN. Decomposition of the AIBN to the corresponding radicals, $Me_2\dot{C}CN$, responsible for initiation of oxidation, was accomplished *thermally* by heating the solutions at 70–75 °C. A slow oxygen stream was introduced over the surface of the solvent. A 2–3-h reaction time gave product phosphate triester in 96% yield (GLC). Table I shows the reaction to be highly *stereospecific with retention of configuration at phosphorus*.

The O_2/AIBN and H_2O/I_2 oxidations of cis/trans isomeric mixtures of the thymidine-based 3′,5′-cyclic phosphite, **3**, on a 100–200-mg scale similarly occurred in 80 and 84% isolated yields, respectively, and with *retentive* stereochemistry (Table II), eq 3 and 4. The O_2/AIBN reaction of **3** also was readily carried out



(3)



(4)

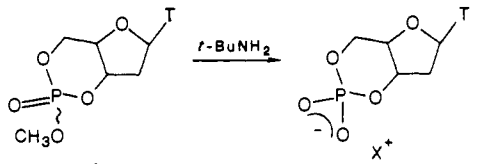
by *photochemical decomposition* of the AIBN by irradiation through Pyrex with a standard 450-W medium-pressure mercury lamp at room temperature (Table I) in a shorter reaction time (1 h) than required for the thermal reactions. In fact, AIBN could be omitted from the O_2/AIBN photoreaction (Table I) with the reaction time only being extended to 2.5 h, the time required for the thermal reactions. No oxidation occurs without AIBN at 70–75 °C or at room temperature without ultraviolet light. ^{31}P NMR proved the reactions to be very clean. The retentive stereochemistries at phosphorus of the oxidations of both **1** and **3** were confirmed by use of N_2O_4, which is known to oxidize trialkyl phosphites with retention of configuration.[8] The oxidations of isomeric **1** and **3** with N_2O_4 had been reported previously from this laboratory to be free of side products and nearly stereospecific.[9] *Indeed the utility of N_2O_4 as a nonaqueous reagent for nucleoside phosphite oxidation (^{16}O introduction) has not been realized by nucleotide chemists.*

Table II. Stereochemistry of Oxidation of *cis*- and *trans*-**3** to **4**

method	*cis*/*trans*-**3**[a]	*cis*/*trans*-**4**[a]
O_2/AIBN, benzene, 75 °C[b]	86/14	87/13
	83/17	83/17
	80/20	80/20
	56/44	55/45
	39/61	42/58
	30/70	29/71
O_2, AIBN, benzene, *hν*, 25 °C[c]	29/71	27/73
O_2, benzene, *hν*, 25 °C[b]	29/71	30/70
I_2/H_2O, THF, −45 °C	90/10	94/6
	56/44	62/38
	44/56	46/54
	33/67	34/66
N_2O_4/CH_2Cl_2, −10 °C	60/40	62/38
	24/76	27/73

[a] By ^{31}P NMR. [b] Consumption of **3** complete in 2.5 h. [c] Consumption of **3** complete in 1 h.

Dealkylation of *cis*- and *trans*-4. The thymidine-based cyclic phosphates, *cis*- and *trans*-**4**, were readily dealkylated in refluxing *tert*-butylamine, **4** → **5**.[10] Chromatography on a DEAE Seph-



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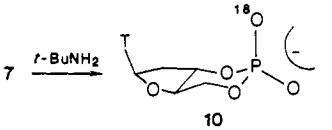
5, X = *t*-BuN$^+$H$_2$Me
6, X = NH$_4^+$

adex/NH_4HCO_3-form column yielded the ammonium salt of the cyclic diester **6** in 90% isolated yield based on phosphate **4**. The procedure thus potentially yields the diastereomerically pure ^{18}O-chiral diastereomeric cyclic phosphates, e.g., *cis*-**10**, via **7** → **10** (eq 5).

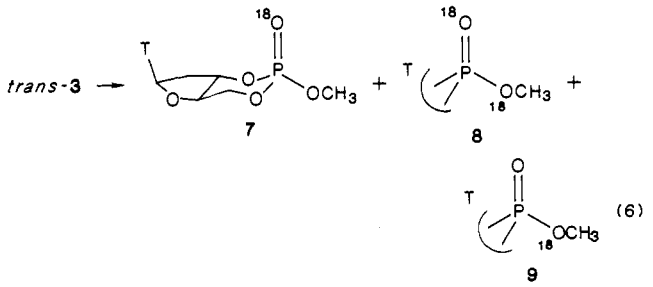


10 (5)

Regiochemistry. Once the stereospecificity of introduction of the phosphoryl oxygen by these methods was established, it was essential to determine whether the reactions are regiospecific as well. Otherwise the position of label in the desired diester, e.g., *cis*-**10** (eq 5), will not be certain. Potential nonregiospecificity is illustrated for *trans*-**3** by eq 6.

Substitution of ^{16}O by ^{18}O in the P=O or OR groups attached to phosphorus in such cyclic triesters results in an *upfield* shift of the ^{31}P NMR resonance [$\Delta\delta(^{31}P)$] proportional to the P–O bond order.[11] I.e., $\Delta\delta$(P=O) > $\Delta\delta$(POR). If the introduction of ^{18}O

(6) (a) Nakaniski, A.; Nishikida, K.; Bentrude, W. G. *J. Am. Chem. Soc.* **1978**, *100*, 6398. (b) Bentrude, W. G.; Hargis, J. H.; Johnson, N. A.; Min, T. B.; Rusek, P. R., Jr.; Tan, H. W.; Wielesek, R. A. *Ibid.* **1976**, *98*, 5348.
(7) Bentrude, W. G.; Hargis, J. H. *J. Am. Chem. Soc.* **1970**, *92*, 7136. Bentrude, W. G.; Tan, H. W.; Yee, K. C. *Ibid.* **1975**, *97*, 573.
(8) Mosbo, J. A.; Verkade, J. G. *J. Am. Chem. Soc.* **1973**, *95*, 4659. Michalski, J.; Okruszek, A.; Stec, W. J. *J. Chem. Soc. D* **1970**, 1495. Denney, D. Z.; Chen, G. Y.; Denney, D. B. *J. Am. Chem. Soc.* **1969**, *91*, 6838.
(9) Bajwa, G. S.; Bentrude, W. G. *Tetrahedron Lett.* **1978**, *19*, 421.
(10) Smith, D. J. H.; Ogilvie, K. K.; Gillen, M. F. *Tetrahedron Lett.* **1980**, *21*, 861.
(11) (a) Cohn, M.; Hu, A. *J. Am. Chem. Soc.* **1980**, *102*, 913. (b) Jarvest, R. L.; Lowe, G.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1980**, 1142. (c) Gerlt, J. A.; Coderre, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 4531. (d) Gorenstein, D. G.; Rowell, R. *J. Am. Chem. Soc.* **1980**, *102*, 6165.

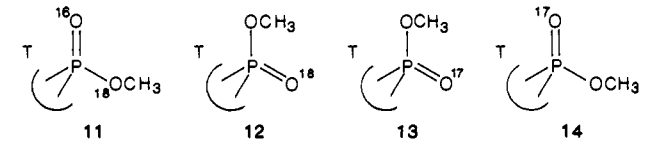


is not regiochemically clean, then from a single, e.g., *trans*, diastereomer of **3**, up to three ^{31}P NMR peaks downfield from 85% H_3PO_4 in the order (δ) **9** > **7** > **8** could be encountered (eq 6).

In Figure 1 is shown the ^{31}P NMR spectrum taken at 121 MHz for purified *trans*-**4** which had been labeled in the P=O group by the $H_2{}^{18}O/I_2$ technique using 98.3% isotopically pure $H_2{}^{18}O$. The three real or potential absorptions assignable to **7**–**9** are marked. Since the $H_2{}^{18}O$ (98.3%) is not 100% isotopically pure, the peak for *trans*-**4** incorporating only ^{16}O (**11**) also is seen and constitutes the largest of the minor peaks. Its size is close to the proportion of $H_2{}^{16}O$ in the $H_2{}^{18}o$ sample (~2%). Most notable is the extremely high *regiospecificity* of the oxidation as shown by the presence of only very tiny peaks corresponding to what *could be* **8** and **9**.

The unimportance of possible **8** and **9** is emphasized by their diminutive size relative to the ^{13}C satellites which reflect $^2J_{CP}$ and $^3J_{CP}$ from ^{13}C at the 1.1% natural abundance level of ^{13}C at C3', C4', and C5'. The same J_{CP} values were seen in the ^{13}C spectra of *trans*-**4**. The ^{13}C satellite doublets also were present in the ^{31}P spectra of **11**.

Nearly identical ^{31}P NMR results were obtained for *trans*-**4** labeled with ^{18}O by the O_2/AIBN method. ^{18}O (or ^{17}O) is easily introduced from a commercial gas bulb attached to the flask containing **3** in refluxing benzene in a simple procedure described in the Experimental Section. Thus, oxidation of **3** using 99.6% $^{18}O_2$/AIBN also gave *cis*- and *trans*-**4** with *regiospecificity of* ^{18}O *introduction essentially complete.* Mass spectrometry demonstrated that the cis and trans methyl phosphates (**4**) contained 96.3 and 97.8% ^{18}O, respectively (m/f = 319 and 321). The O_2/AIBN and H_2O/I_2 oxidations are thus reliable techniques for the preparation of diastereomerically pure P chiral diesters, illustrated here by *cis*- and *trans*-**10**. The ^{31}P resonance for **7** is moved upfield by 4.3 Hz from that of **11** [$\delta(^{31}P)$ = −6.51, acetone-d_6]. For **12** the shift ($\Delta\delta$) is 4.2 Hz [$\delta(^{31}P)$ = −4.74].



^{17}O NMR Studies of *cis*- and *trans*-4. Triesters *cis*- and *trans*-**4**, labeled at the phosphoryl oxygen with ^{17}O (**13** and **14**), proved to be easily distinguishable by ^{17}O NMR. (See Figure 2.) Indeed, they show distinctly different ^{17}O chemical shifts [δ 78.2, cis (**13**) (CD_3CN, 80 °C); δ 87.7, trans (**14**) (CD_3CN, 80 °C)], which are well-resolved at 54.2 MHz. Couplings ($^1J_{OP}$) are well-defined as well: cis (**13**) CD_3CN, 80 °C, 156 Hz; CD_3CN, 20 °C, 127 Hz. Trans (**14**), CD_3CN, 80 °C, 160 Hz; CD_3CN, 20 °C, 156 Hz. Line widths at 80 °C in CD_3CN (P-decoupled) were relatively narrow for an ^{17}O resonance: trans (**14**), 40 Hz; cis (**13**), 72 Hz. Small differences in ^{17}O NMR parameters for axial and equatorial ^{17}O have been noted previously for cyclic 3',5'-monophosphate, *diesters* like **5**.[12] The usefulness of ^{17}O NMR to assign configuration at phosphorus in simple six-membered-ring phosphate triesters has been reported,[13] but such effects in neutral cyclic nucleotide *triester* derivatives are unknown.

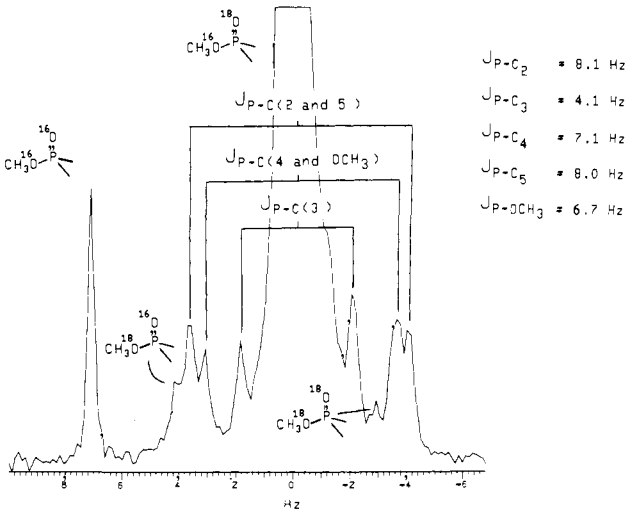


Figure 1. ^{31}P NMR spectrum of *trans*-**4** at 121 MHz. Coupling constants are from a high-resolution $^{13}C\{^1H\}$ spectrum.

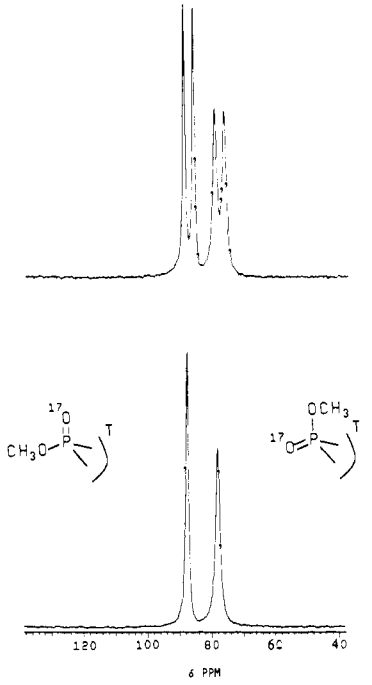


Figure 2. ^{17}O NMR spectra at 54.2 MHz of P=^{17}O labeled *cis*- and *trans*-**4** in CD_3CN at 80 °C. Chemical shifts are relative to external H_2O. Top, phosphorus-coupled. Bottom, phosphorus-decoupled.

Efficiency of O_2 in ^{18}O Labeling. The introduction of ^{18}O label by use of $^{18}O_2$, contained in a bulb attached to the flask in which the phosphite to be oxidized is dissolved in benzene (see Experimental Section), relies on the solubility of oxygen in benzene. Since the system is closed, a partial vacuum is created as $^{18}O_2$ is consumed. Introduction of nitrogen to equalize pressure still leaves the partial pressure of $^{18}O_2$ reduced as the reaction progresses, and eventually the reaction becomes too slow. Fortunately, $^1/_2$ mol of O_2 delivers 1 mol worth of oxygen. (See discussion of mechanism below.) In a reaction in which an excess of $(MeO)_3P$ was dissolved in C_6H_6 at the 1 M concentration level, an amount of $(MeO_3)PO$ was formed corresponding to 60% consumption of the available O_2 in 12 h and 95% in 2 days. Of course, efficiency of O_2 consumption is only a consideration when the expense of isotopically enriched O_2 is involved.

Discussion

The O_2/AIBN oxidation of phosphites **1** and **3** is indeed highly regio- and stereospecific, proceeding with retention of configuration at phosphorus and in yields equivalent to those we obtained with

(12) Gerli, J. A.; Demou, P. C.; Mehdi, S. *J. Am. Chem. Soc.* **1982**, *104*, 2848.

(13) Eliel, E. L.; Chandrasekaran, S.; Carpenter, L. E., II; Verkade, J. G. *J. Am. Chem. Soc.* **1986**, *108*, 6651.

the classical H_2O/I_2 method. Its success with nucleoside-based cyclic phosphite **3** suggests its potential use in di- and perhaps oligonucleotide synthesis via phosphite intermediates. The photoinitiated AIBN/O_2 oxidation avoids reflux temperatures, and photoirradiation at $\lambda > 300$ nm (Pyrex) precludes base dimerization which potentially can compete at shorter wavelengths. Introduction of isotopically labeled oxygen is readily accomplished with fairly efficient utilization of $^{17}O_2$ or $^{18}O_2$. An apparatus that circulates labeled oxygen through the solution would likely increase the efficiency and rate of oxygen consumption. Of course as a nonaqueous method for oxidation of nucleoside-based phosphites with unlabeled oxygen, the efficiency of oxygen utilization is not important. Reasonably rapid, extremely clean oxidations result. The minor amounts of AIBN decomposition products are removed on workup or can be avoided altogether by omitting AIBN in the photoreactions and irradiating for the same amount of time required for the thermal AIBN-initiated oxidations.

The radical-chain oxidation of phosphites by O_2 is known[14] but has not previously been applied to nucleotides. Moreover, its stereo- and regiospecificities have not been determined. Mechanistically, a chain reaction featuring propagation steps **9** and 10

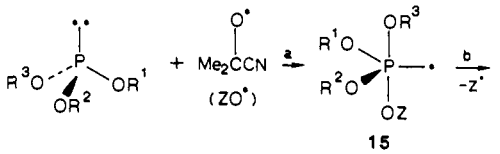
$$\text{AIBN} \rightarrow 2\ Me_2\dot{C}CN \quad (7)$$

$$Me_2\dot{C}CN + O_2 \rightarrow Me_2C(CN)OO^{\bullet} \quad (8)$$

$$Me_2(CN)OO^{\bullet} + P(OR)_3 \rightarrow Me_2C(CN)O^{\bullet} + OP(OR)_3 \quad (9)$$

$$Me_2C(CN)O^{\bullet} + P(OR)_3 \rightarrow Me_2\dot{C}CN + OP(OR)_3 \quad (10)$$

is quite reasonable.[14] We showed earlier[15] that alkoxy radicals transfer oxygen to phosphorus (step 9) with retention of configuration, sequence 11. A phosphoranyl radical, **15**, doubtless is



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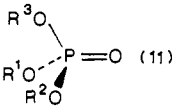
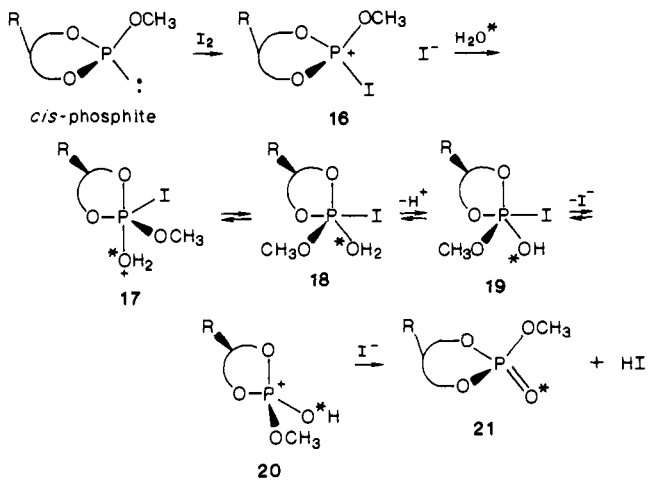


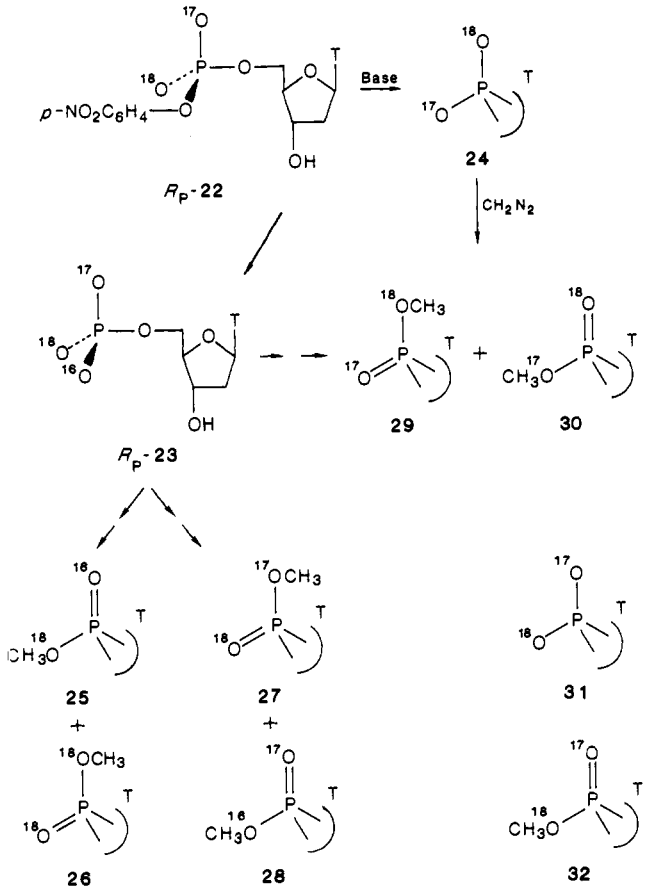
involved. Reaction 9 may involve a peroxy phosphoranyl radical or oxygen transfer may occur concertedly. Step 11b with Z = $Me_2C(CN)^{\bullet}$ is particularly favored. AIBN/O_2 oxidations of phenyl phosphites occur readily, whereas with *t*-BuOOBu-*t* displacement of phenoxy radical by *tert*-butoxy radical results.[16]

The use of $H_2{}^{17}O$ of $H_2{}^{18}O$ with I_2 is the classical method of labeled-oxygen introduction during the phosphitylation method of nucleotide and oligonucleotide synthesis. The above results establish the retentive stereochemistry and the total regiospecificity of the I_2/H_2O reaction. While our work was in progress, a preliminary account of the retentive stereochemistry of the H_2O/I_2 oxidation of 5′-*O*-thyminyl 3′-*O*-thymidine methyl phosphite was reported[17] as well as a preliminary study of the stereochemistry of H_2O/I_2 oxidation of phosphites **1** and **3**, which our work confirms.[17] However, in the earlier work no data regarding the degree of stereospecificity with **1** and **3** were given, as only a *single* diastereomer (cis) of **1** and **3** was used. A number of $H_2{}^{18}O/I_2$ oxidations of dinucleoside alkyl phosphites have been shown to be regiospecific and stereospecific,[2b] although the retentive nature

Scheme I



Scheme II



of oxidation with respect to phosphorus configuration was not established for this extremely useful reaction.

The retentive stereochemistry of this reaction is readily understood in terms of plausible sequence **16–21**, Scheme I. The ring in **17–19** is attached equatorial/apical to phosphorus, but diequatorial attachment would yield the same stereochemistry. Here, and in the oxidation of acyclic phosphites, the stereochemistry[18] is controlled by the apical introduction of H_2O and equatorial position of iodine in the initial pentacovalent intermediate, in this case **17**.

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(15) Reference 6b. Radical oxidations of the type exemplified by eq 8–10 have been studied recently in the context of polymer oxidation: Schwetlick, K.; Koenig, T.; Rueger, C.; Pointeck, J.; Habicher, W. J. *Polym. Degrad. Stab.* **1986**, *15*, 97. Schwetlick, K.; Koenig, T.; Rueger, K.; Pointeck, J. *Ibid.* **1986**, *16*, 360.

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(17) Cullis, P. M. *J. Chem. Soc., Chem. Commun.* **1984**, 1510.

(18) Holmes, R. R. *Pentacoordinated Phosphorus*; ACS Monograph 176; American Chemical Society: Washington, DC; 1980; Vol. 2, Chapter 2. For earlier reviews, see: (a) Ramirez, F. *Acc. Chem. Res.* **1968**, *1*, 168. (b) Westheimer, F. H. *Ibid.* **1968**, *1*, 70. (c) Mislow, K. *Ibid.* **1970**, *3*, 321. (d) Muetterties, E. L. *Ibid.* **1970**, *3*, 266. (e) Holmes, R. R. *Ibid.* **1972**, *5*, 296. (f) Ramierz, F; Ugi, I. *Bull. Soc. Chim. Fr.* **1974**, 453.

^{17}O NMR Correlation. The potential use of ^{17}O chemical shifts in studies of enzymic phosphoryl transfer is illustrated by Scheme II. Thymidine diester **22** has been a useful substrate in investigation of the stereochemistry of its phosphodiesterase-catalyzed hydrolysis to the 5'-monophosphate.[19] (Analogous research with the 3'-nucleotide phosphodiesterases and the analogous 3'-diester can be cited.[20]) Typically, the absolute configuration of the starting diester, e.g., (R_P)-**22**, has been determined by hydrogenolysis to (R_P)-**23** followed by the classical cyclization methylation sequence[11,20c,21] to generate **25**–**30**. The ^{31}P NMR chemical shift method [based on incremental ^{18}O effects on $\delta(^{31}P)$] confirms the R_P configuration by indicating that the ^{18}O of the cis triester is in the P=^{18}O (**26**) rather than the $^{18}OCH_3$.[11] (Analogous conclusions are drawn from the ^{18}O location in the trans isomer.) Since base-catalyzed cyclization of **22** directly to **24** and **31** is straightforward (inversion at phosphorus),[22] the direct formation of **24** and not **31**, if confirmable by ^{17}O NMR, would establish the R_P configuration of **22** in simple fashion. While the ^{17}O resonances of diesters **24** and **31** can be observed, the difference in chemical shifts between the axial and equatorial ^{17}O nuclei for (^{17}O,^{18}O)cdAMP and -cTMP is only 1.6–1.9 ppm.[20d,23] Because of the extreme quadrupolar broadening of these lines, even with phosphorus decoupling only a crude estimation of the ratio **24**/**31** as an approximate[20b] measure of configurational purity of (R_P)-**22** could be made at 36.6 MHz;[20d] only slight improvement would result at 54.2 MHz.

However, the ratio **29**/**32**, can be readily determined at the 54.2-MHz ^{17}O frequence of a 400-MHz instrument with phosphorus decoupling ($\Delta\delta$ = 9.5 ppm, 515 Hz; Figure 2). The methylation of **24** and **31** is quantitative.[11b,20b] The ratio of **29**/**32**, normalized for the cis/trans ratio of diastereomers formed, then gives directly the ratio (R_P)-**22**/(S_P)-**22**. The cis/trans diastereomer ratio is easily obtained on the same sample from the ^1H spectrum of the $CH_3OP(O)$ or H_1' regions at 300 MHz or from the ^{31}P spectrum (^{16}O,^{18}O- and ^{18}O,^{18}O-containing phosphate triesters).

It must be emphasized that this method cannot be used to determine the configuration of **23**. Thus **28**, will be formed from (R_P)-**23** but at the same time **32** will result from (S_P)-**23**, and these will be seen as a single peak in the ^{17}O NMR spectrum.

Summary. Both the new O_2/AIBN and standard H_2O/I_2 methods of oxidation of trialkyl phosphites have been investigated with the simple system **1** and the thymidine-based methyl cyclic 3',5'-monophosphite **3**. The reactions occur readily, in high yields, and with essentially complete regio- and stereospecificities. The stereochemistry at phosphorus is retained in both cases. These features combine to make both oxidations ideal for the preparation of P-chiral phosphate diesters through the introduction of the P=^{18}O or P=^{17}O functionality and subsequent dealkylation to the desired diester of known configuration. This was demonstrated by the ready dealkylation of **4** to the cyclic 3',5'-monophosphate **5** in high yields, including the P-chiral diesters (eq 5). Although the regio- and stereospecificity of the H_2O/I_2 oxidation has been previously established, only preliminary evidence for the retentive nature of the oxidation had been available. That retention should be observed is by no means obvious a priori. The nonaqueous *O_2/AIBN method should be useful for* di- and *possibly* oligonucleotide synthesis by the phosphite triester route, even for introduction of ^{16}O, as it avoids the necessary drying step which follows H_2O/I_2 oxidation.

The ^{17}O NMR spectrum of the methyl phosphate triesters **4** allows them to be readily characterized configurationally without resort to assistance from ^{31}P NMR. Indeed, the axial or equatorial position of the P=^{17}O is readily assigned on the basis of relative ^{17}O chemical shifts and P-decoupled line widths. A use for this technique to characterize the configuration of certain P-chiral phosphate *diesters* of use in enzymic studies can be proposed (Scheme II). This method promises to be excellent for more quantitative evaluation of configurational purity.

Experimental Section

Methods and Materials. 5-*tert*-Butyl-2-methoxy-1,3,2-dioxaphosphorinane (**1**) was prepared in a manner previously described.[6,7] Isomerization of the phosphite **1** to various trans/cis ratios other than that originally formed during synthesis was done by heating a chloroform solution of **1** at ~60 °C for prolonged periods. For example, an initial 85/15 trans/cis mixture isomerized to 75/25 in ~10 h. Final equilibrated trans/cis ratio for **1** is about 10/90. Solvents used for chromatography were HPLC grade. Reagent-grade ethyl acetate and *tert*-butylamine were distilled prior to use. Methylene chloride, pyridine, and tetrahydrofuran were predried with calcium hydride, then distilled, and stored under argon. Anhydrous ethyl ether was used as received. Methanol for the preparation of phosphites was distilled from magnesium. Pyridine hydrochloride (hygroscopic) was purified by recrystallization from ethyl acetate/ethyl ether, sublimed, stored in a dessicator, and weighed out under argon. 2,2'-Azobis(2-methylpropionitrile) (AIBN) was purchased from Aldrich Chemical Co. and used as received. N_2O_4 was obtained from Matheson Gas Products and oxygen from Linde Gas. Deuterated NMR solvents (chloroform, dimethyl sulfoxide, and pyridine), also used occasionally as a reaction solvents for NMR-scale reactions, were obtained from Stohler Isotope Co. and used without further purification. $H_2{}^{17}O$ (20% enriched) was obtained from KOR Isotopes. $H_2{}^{18}O$ (98.3 atom %) was B.O.C. Ltd material. $^{18}O_2$ was from Stohler Isotope Chemicals, and the $^{17}O_2$ was obtained from Cambridge Isotope Laboratories.

Silica gels for medium-pressure absorption chromatography (silica gel 60, 230–400 mesh) and gravity flow chromatography (60–200 mesh) were the products of Merck (Darmstadt) and Baker Chemical Co., respectively. HPLC purification and/or separations of nucleotide phosphates **4** were performed with a Rainin Dynamax 21.4 × 250 mm silica column eluted with 95/5 chloroform/methanol. Elution order was trans and then cis.

Trans/cis ratios of phosphites **1** and **3** and phosphates **4** were determined by ^{31}P NMR using either peak heights or peak integration. (Peak widths at half-height for the trans/cis pair were the same.) The NMR phosphorus relaxation times (T_1) for the diastereomeric phosphite or phosphate pairs were assumed close enough so as not to introduce any significant error in the reported ratios. When comparing phosphite to phosphate in the same mixture by the above method, a relaxation delay on the order of 45 s (>5T_1) was used. ^{31}P NMR spectra were obtained with a Varian FT-80A (32-MHz), SC-300 (121-MHz), or XL-300 (121-MHz) spectrometer. Positive chemical shifts (ppm) are downfield from external 85% H_3PO_4. ^1H NMR spectra were taken on Varian XL300 or XL400 instruments. ^{17}O spectra (54.2 MHz, XL400) were referenced to external H_2O. ^{17}O spectra were obtained with a Varian probe with P-decoupling capabilities. The trans/cis ratios of the phosphates **2** were obtained by the ^{31}P NMR method or by integration of gas chromatography peak traces with nearly identical results. Gas chromatography was performed on a Hewlett–Packard 5830A gas chromatograph with thermal conductivity detection. Equivalent detector responses for cis and trans isomers of a diastereomeric pair were assumed. (Glass column, 6 mm × 2 m, packed with 3% QF-1 on Gas Chrom Q and temperature programmed from 100 to 220 °C at 15 °C/min or isothermal at 175 °C, helium flow 30 mL/min.)

Thymidine Cyclic Methyl 3',5'-Phosphite (3). In a dry round-bottom flask equipped with stir bar, vacuum adapter (Ace Glass type 9175), and rubber septum were combined, under an argon atmosphere, dimethylamino 3',5'-cyclic thymidine phosphoramidite[9] (1.0 g, 3.2 mmol), anhydrous pyridine hydrochloride (370 mg, 3.2 mmol), and 40 mL of dry methylene chloride. The mixture was stirred to homogeneity. From a microliter syringe was added 129 μL (3.2 mmol) of dry methanol at a rate of 1 drop every 5 s, and the reaction was stirred for an additional 5 min. Approximately half the methylene chloride was then removed under vacuum and replaced with a 50/50 mixture of ethylene acetate and ethyl ether. The contents were again reduced in half, and more EtOAc/Et_2O was added. This solvent replacement was repeated four to five times, leaving a final volume of ~25 mL. Pyridine hydrochloride usually began precipitating out as a flocculent white solid during the second cycle.

The reaction mixture was then quickly filtration chromatographed under nitrogen on a 1 × 25 cm glass column packed with ~2 g of silica gel and eluted with 50/50 ethyl acetate/ethyl ether. The first 50–60 mL was collected. Removal of solvents in vacuo yielded typically 800–900

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(20) (a) Mehdi, S.; Gerlt, J. A. *J. Am. Chem. Soc.* **1982**, *104*, 3223. (b) Jarvest, R. L.; Lowe, G. *Biochem. J.* **1981**, *199*, 447. (c) Mehdi, S.; Gerlt, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 7018. (d) For a summary paper using 3',5'-diesters, see: Mehdi, S.; Gerlt, J. A. *Biochemistry* **1984**, *23*, 4844.
(21) The details of this approach are given in ref 2.
(22) This reaction is reported for the 3'-diester in ref 19d.
(23) Coderre, J. A.; Mehdi, S.; Gerlt, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 1872.

mg (85–95% yield) of phosphite **3**. ^{1}H NMR analysis at 300 MHz showed such preparations to be completely pure within the limits of the NMR observation (>90–95%). ^{31}P NMR analysis gave the same conclusion. Phosphite isomer ratios were usually about 60/40, trans/cis, from ambient-temperature preparations but occasionally higher. Higher trans/cis ratios were obtained by adding the methanol at lower temperatures. *trans*-**3**: ^{31}P NMR δ 129.5 (acetone-d_6), 130.0 (CD_2Cl_2), 129.4 (C_6D_6), 131.4 ($CDCl_3$). *cis*-**3**: ^{31}P NMR δ 123.2 (acetone-d_6), 123.1, (CD_2Cl_2), 123.3, (C_6D_6), 124.5 ($CDCl_3$). *On examination of* ^{1}*H* NMR spectra of samples containing predominately *cis*- or *trans*-**3**, it was possible to assign the resonances observed to the individual diastereomers. *trans*-**3**: ^{1}H NMR (C_6D_6) δ 6.40 (br s, 1 H, H_6), 5.90 (dd, 1 H, $H_{1'}$), 4.18 (ddd, 1 H, $H_{5'b}$), 3.89 (ddd, 1 H, $H_{4'}$), 3.76 (ddd, 1 H, $H_{5'a}$), 3.70 (apparent q, 1 H, $H_{3'}$), 3.21 (d, 3 H, POMe, J_{HP} = 9.0 Hz), 1.70–1.90 (m, 2 H, $H_{2'a}$, $H_{2'b}$), 1.70 (d, 3 H, 5-Me, J_{HH} = 1.3 Hz). *cis*-**3**: ^{1}H NMR (acetone-d_6) δ 7.48 (d, 1 H, H_6), 6.22 (d, 1 H, $H_{1'}$), 4.67 (dddd, 1 H, $H_{3'}$), 4.45 (ddd, 1 H, $H_{5'a}$), 4.26 (ddd, 1 H, $H_{5'b}$), 3.65 (ddd, 1 H, $H_{4'}$), 3.5 (d, 6 H, Me_2N), 2.43–2.46 (m, 2 H, $H_{2'a}$, $H_{2'b}$), 1.83 (d, 3 H, 5-CH_3).

Isomerization of Thymidine Cyclic Methyl 3′,5′-Phosphite (3). The isomerization of *trans*-**3** to *cis*-**3** to obtain various desired cis/trans ratios was followed by ^{31}P NMR. In a 10-mm NMR tube were combined 100 mg of a trans/cis mixture of phosphites, ~3 mL of $CDCl_3$, and ~5 mg of anhydrous pyridine hydrochloride. The tube was flushed well with argon and then sealed with an air-tight cap. The tube was repeatedly heated (~1 min) until the chloroform just started to boil, removed from the heat source, shaken briefly, and again heated to boiling. The ^{31}P NMR was taken on the cooled solution, and the above procedure was repeated until the desired trans/cis ratio was reached. Under these conditions isomerization was complete in less than 30 min. The equilibrium cis/trans ratio is about 95/5. The pyridine hydrochloride was then removed by total evaporation of the chloroform, addition of several milliliters of 50/50 ethyl ether/ethyl acetate, and then filtration under argon through a short column of silica gel in a manner similar to that described above for the preparation of **3**. Little or no decomposition was observed by ^{31}P NMR under anhydrous conditions. The cis/trans ratios of catalyst-free phosphite solutions thus attained remained unchanged over a period of 2.5 h at 75 °C in benzene.

General Oxidation Procedure with Molecular Oxygen and AIBN. In a round-bottom flask equipped with a stir bar and short reflux condenser, which was fitted at the top with a rubber septum, were combined 100 mg of either phosphite **1** or **3** and 5–10 mg of AIBN in 10 mL of benzene. Through the septum was inserted two hypodermic needles. One reached to ~1 cm above the surface of the liquid level. To it was attached an oxygen gas source. The second needle served as an outlet and was attached to an oil bubbler to monitor gas flow. Oxygen gas was then flowed slowly over the solvent surface. The flask was heated in an oil bath kept at 70–75 °C for a period of 2.5 h with an occasional addition of benzene to keep the liquid level roughly constant. The solution was then cooled to room temperature. The solvent was removed in vacuo. In the case of phosphite **1**, a 96% yield of *cis*- and *trans*-**2** was determined by gas chromatography. Phosphite **3** gave cis and trans phosphates **4**, which comprised over 95% of the total peak area in the ^{31}P NMR of the crude reaction mixture. The combined isolated yield of the individual isomers of **4** from a single pass of the residue from solvent evaporation through an HPLC column was 80%, with individual isomer purity greater than 98% as determined by both ^{31}P and ^{1}H NMR.

Photoinitiated Oxidation of 3. In a dry 10-mm Pyrex tube sealed with a rubber septum were combined **3** (50 mg, 0.16 mmol), AIBN (20 mg, 0.12 mmol), and 3 mL of dry benzene. A needle connected to an oxygen source was then inserted such that it reached the bottom of the tube along with another only long enough to serve as an exit for excess oxygen. A slow but steady oxygen stream was passed through the solution. The mixture was then irradiated with a 450-W medium-pressure Hanovia lamp with the sample cooled in a water bath at room temperature for 1 h, during which **4** began to precipite as a white solid. Solvent was removed in vacuo. ^{31}P and ^{1}H NMR indicated that the only products present were *trans*- and *cis*-**4**. An identical procedure *without added AIBN* required 2.5 h for consumption of all **3** and gave *cis*- and *trans*-**4** of excellent purity.

Oxidation with Iodine and Water. Essentially the procedure of Letsinger[3] was used. To the phosphite (100–200 mg) in 4 mL of THF and 1 mL of pyridine, cooled to −45 °C, was added a solution of 1 mL of THF, 0.3 mL of water, and 100 mg of iodine. Addition was done dropwise with enough time between additions to allow the solution to turn from yellow to colorless and was stopped when a very slight yellow color persisted for several min after the last addition. Phosphate **4** partially precipitated as formed as a white solid. On further cooling of the reactior flask, additional **4** precipitated which was filtered off.

The above procedure was modified when using ^{18}O- or ^{17}O-labeled water by adding a 2-fold molar excess of labeled water via a microliter syringe simultaneously with the iodine/tetrahydrofuran solution. Reaction times were somewhat longer, and samples were taken periodically to check for completion of reaction. Workup was as above. Isolated yield from use of $H_2{}^{16}O$ for phosphate **2** was 89%, while that for **4** (mixture of isomers) was 84% (HPLC). ^{31}P and ^{1}H NMR confirmed the structures to be identical with those formed on N_2O_4 oxidation (see below).

Oxidation of Phosphites with N_2O_4. Thymidine Cyclic Methyl 3′,5′-Monophosphate (4). To a stirred solution of **3** (ca. 100 mg) in 10 mL of dry CH_2Cl_2 under argon and cooled to −20 °C was added dropwise a saturated solution of N_2O_4 in CH_2Cl_2. The reaction mixture was titrated to completion with the N_2O_4 solution, with intermediate colors ranging from pale green to light yellow. Addition was stopped when, in some cases, a slight yellow-brown color persisted for several minutes after the last addition indicating an excess of N_2O_4, or in others, a clear pale blue color appeared. The mixture was allowed to warm to room temperature and stirred for an additional 5 min. Removal of solvent in vacuo left a dry, off-white foam. Conversions, estimated by ^{31}P NMR, were essentially quantitative. The diastereomers of **4** were routinely separable by MPLC (SiO_2, 97/3 ethylacetate/ethanol, 500-mg quantities) or HPLC (SiO_2, 95/5 $CHCl_3/CH_3OH$, 100-mg quantity): ^{31}P NMR (acetone-d_6) δ trans, −4.7; cis, −6.4. *cis*-**4**: ^{1}H NMR (acetone-d_6) δ 10.41 (br s, 1 H, 1-NH), 7.54 (br q, 1 H, H_5), 6.40 (dd, 1 H, $H_{1'}$), 4.94 (apparent q, 1 H, $H_{3'}$), 4.61 (ddd, 1 H, $H_{5'b}$), 4.45 (ddd, 1 H, $H_{5'a}$), 3.97 (ddd, 1 H, $H_{4'}$), 3.67 (d, 3 H, POMe, J_{HP} = 12 Hz), 2.58–2,72 (m, 2 H, $H_{2'a}$ and $H_{2'b}$), 1.84 (d, 3 H, 5-Me, J_{HH} = 1.1 Hz). *trans*-**4**: ^{1}H NMR (acetone-d_6) δ 7.46 (br q, 1 H, H_5), 6.32 (dd, 1 H, $H_{1'}$), 4.91 (apparent q, 1 H, $H_{3'}$), 4.52 (ddd, 1 H, $H_{5'b}$), 4.36 (ddd, 1 H, $H_{5'a}$), 3.97 (m, 1 H, $H_{4'}$), 3.67 (d, 3 H, POMe, J_{HP} = 11.8), 2.43–2.56 (m, 2 H, $H_{2'a}$, $H_{2'b}$), 1.72 (d, 3 H, 5-Me, J_{HH} = 1 Hz). Anal. Calcd for mixture of diastereomers: C, 41.50; H, 4.75; P, 9.73. Found: C, 41.64; H, 4.84; P, 9.67.

Cyclic Thymidine 3′,5′-Monophosphate (6). In a round-bottom flask were combined thymidine methyl cyclic 3′,5′-monophosphate (**4**; 0.151 g, 0.47 mol) and 15 mL of freshly distilled *tert*-butylamine. The mixture was stirred and refluxed overnight and then evaporated to dryness in vacuo to yield the *tert*-butyl methyl ammonium salt **5**. The entire contents were purified and converted to the ammonium salt as outlined below.

DEAE Sephadex was first swollen in 1 M ammonium bicarbonate and then loaded into a 75 × 2.5 cm glass MPLC column filled ~75% full. Approximately 1 L of 1 M ammonium bicarbonate was passed through the column, followed by 1 L of deionized water. The above nucleotide was then loaded onto the column and flushed with deionized water until no impurity peaks were observed by UV at 265 nm. The nucleotide was eluted starting with water and then a linear gradient with 1 M ammonium bicarbonate with a pump flow rate of ~2 mL/min. The fractions of the only major product that eluted were combined, and the water was removed on a rotary evaporator. Occasional addition of methanol (~20 mL) and gentle heating with a water bath at 40 °C aided the evaporation, which left a white solid that was further warmed in vacuo at 40 °C to decompose the last traces of residual buffer, giving 0.138 g (90.1%) of the ammonium salt **6** with a purity of at least 98%; ^{31}P NMR (D_2O) δ −1.9 ppm. The structure was confirmed by ^{1}H NMR spectroscopy.

O_2/AIBN Introduction of ^{18}O or ^{16}O Label. The commercial sample of labeled O_2 was contained in a glass bulb (normally 100 mL) fitted with a magnetically activated break seal. A two-way vacuum stopcock was attached below the break seal. This apparatus was joined by way of a ground-glass joint to a round-bottom flask fitted near the top with a three-way vacuum stopcock, on one end of which was a rubber septum. A solution of phosphite and AIBN in benzene was added to the flask through the rubber septum. The entire apparatus was freeze–thaw degassed several times and closed off under vacuum. The break seal to the oxygen bulb was broken, and the solution was heated to ~75 °C for a period of 2 h. Dissolution of oxygen could be aided by magnetic stirring of the benzene solution. In some cases the reaction was monitored periodically by ^{31}P NMR after removing an aliquot through the side arm via the rubber septum. At the completion of oxidation, the contents of the reaction flask were frozen to condense benzene vapors, and the stopcock to the oxygen reservoir was closed to save the labeled oxygen for subsequent oxidations.

Quantitative Assessment of O_2 Uptake in O_2/AIBN Oxidations. A 100-mL reaction flask was configured as described above. It was attached by way of a ground-glass joint to a two-way vacuum stopcock, the other end of which was joined to a 250-mL round-bottom flask which served as an oxygen reservoir. The total volume of the reservoir system was measured to be 264 mL. The entire system was evacuated and into it bled pure oxygen (four cycles). The reservoir was closed off, and the rest of the system was thoroughly flushed with nitrogen. The reaction flask was charged with trimethyl phosphite (5.25 g, 0.0424 mol) and AIBN (200 mg, 1.2 mmol) in 25 mL of dry benzene. The three-way stopcock was closed, and the stopcock to the oxygen reservoir was opened.

The contents of the magnetically stirred reaction flask were heated to 70 °C. After ~3 h, nitrogen was carefully bled in to relieve the slight vacuum within the system, and an aliquot of AIBN (100 mg in 2 mL of benzene) was added. This procedure was repeated eight times. Periodically a sample was drawn for GLC or ^{31}P NMR analysis to determine the ratio of phosphite to phosphate. (No other products were detected.) After 12 h the phosphite had consumed 60% of the available oxygen. After approximately 2 days, the consumption was nearly 95%.

Acknowledgment. Support of this work by Grant CA 11045 from the NCI of the PHS and by a grant from the NSF is gratefully acknowledged.

Registry No. *cis*-**1**, 23201-70-9; *trans*-**1**, 23201-71-0; *cis*-**2**, 26344-07-0; *trans*-**2**, 26344-06-9; *cis*-**3**, 66386-45-6; *trans*-**3**, 66386-46-7; *cis*-**4**, 120056-30-6; *trans*-**4**, 120056-31-7; **5**, 119998-99-1; **6**, 119999-00-7; dimethylamino 3',5'-cyclic thymidine phosphoramidite, 40652-74-2.

Energetics of the Singlet and Triplet States of Alkylnitrenium Ions: Ab Initio Molecular Orbital Calculations

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Abstract: The structures and energies of the nitrenium ions NH_2^+, CH_3NH^+, $(CH_3)_2N^+$, $\overline{CH_2(CH_2)_3N}^+$, and $\overline{CH_2(CH_2)_4N}^+$ were studied by using ab initio molecular orbital calculations with various basis sets and corrections for electron correlation up to the MP4(SDTQ) level. On the singlet energy surface, CH_3NH^+ and $(CH_3)_2N^+$ are predicted to be transition states for degenerate hydrogen migration in the isomeric immonium ions. CH_3NH^+ was predicted to have a triplet, and $\overline{CH_2(CH_2)_3N}^+$, a singlet ground state. The singlet-triplet energy difference in the remaining secondary ions was small, and an unambiguous determination of their ground-state multiplicities was not possible. For CH_3NH^+ and $(CH_3)_2N^+$, the calculated energies of both states are too high to be consistent with their supposed formation in the EI mass spectra of amines, even as transient intermediates. The most detailed calculations were carried out on NH_2^+. At the MP4(SDTQ)/6-311+G(3d2f,2p)//MP2/6-311+G(3d,2p) level including vibrational corrections, the singlet-triplet energy difference was calculated to be 32.4 kcal mol^{-1}, 2.3 kcal mol^{-1} greater than the experimental value.

The nitrenium ion, NH_2^+ (**1**), is an electron-deficient species isoelectronic with methylene. However, in contrast to the latter, the nitrenium ion and its simple alkyl derivatives have received relatively little attention.[1] The relative energetics of the lowest electronic states are known only for the parent. Indeed, only in this case is even the ground-state multiplicity known with certainty. Here, a recent photoionization study[2] of the corresponding neutral radical showed the lowest singlet to lie 30.1 ± 0.2 kcal mol^{-1} above the triplet ground state. Several alkyl- and dialkylnitrenium ions have been prepared in the gas phase by charge reversal collisional activation (CR CA) of the corresponding negative ions.[3] However, other evidence[4-9] for the existence of these ions in the gas phase is tentative. From the CA spectra of the $C_2H_6N^+$ and $C_3H_8N^+$ ions derived from a wide variety of compounds, Levsen and McLafferty[7] concluded that only the isomeric immonium ions had lifetimes $>10^{-5}$ s.

The pioneering work of the Gassman group clearly indicates that in solution electron-deficient divalent nitrogen species are involved in a wide variety of reactions including ring cleavages and rearrangements.[1b,11,12] However, whether these species correspond to true reaction intermediates, or simply to transitory points on the reaction pathway, remains an open question. The initially convincing evidence for the existence of certain bicyclic nitrenium ions as discrete intermediates, based on what appeared to be a heavy-atom-catalyzed conversion to the triplet,[11] now seems less clear-cut.[13-15]

In contrast to the situation for the simple aliphatic, and alicyclic nitrenium ions, the aryl derivatives have been the subject of numerous investigations,[1a,16,17] many of them stimulated by the suggestion[18] that these species are involved in aromatic amine carcinogenesis.

Quantitatively reliable ab initio molecular orbital calculations for the singlet-triplet energy differences in nitrenium ions have been reported only for the parent (**1**).[19-23] Other ab initio cal-

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