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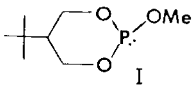
O_2/AIBN, FREE-RADICAL METHOD FOR THE STEREOSPECIFIC OXIDATION OF NUCLEOSIDE TRIALKYL PHOSPHITES: NEW PREPARATION OF THE INDIVIDUAL, DIASTEREOMERIC, ^{18}O-LABELED THYMIDINE 3',5'-CYCLIC MONOPHOSPHATES.

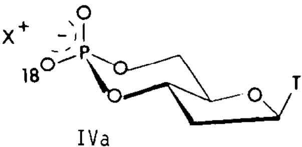
Tadeusz M. Gajda, Alan E. Sopchik and Wesley G. Bentrude*
Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Abstract: An experimentally easy method for regio- and stereospecific introduction of isotopic (^{18}O or ^{17}O) oxygen into phosphorus di- and triesters of biological interest is described and applied to ^{18}O-labeling of cTMP's.

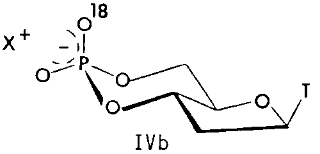
Nucleoside 3',5'-cyclic monophosphates stereospecifically labeled with ^{18}O or ^{17}O in the exocyclic oxygen atoms attached to phosphorus[1] are most valuable substrates for the study of enzyme catalyzed reactions of cyclic nucleotides as shown by recent stereochemical investigations.[2] We report here a new method for the preparation of the diastereomerically pure ^{18}O-labeled 3',5'-cyclic monophosphates of thymidine (cTMP's), IVa and IVb, via a free-radical chain process using $^{18}O_2$. The approach has the advantages of being simple and straightforward and also utilizing the oxygen isotope in its most economical form. Furthermore, the high stereo- and regiospecificities of the introduction of labeled oxygen and lack of side products suggest its application to the oxidation and ^{18}O labeling of the trialkyl phosphite intermediates frequently used in oligonucleotide synthesis.[3]



I



IVa



IVb

$X^+ = \underline{t}\text{-Bu}\overset{+}{N}H_2Me$

The use of azobisisobutyronitrile (AIBN) to initiate the O_2 oxidation of a wide structural variety of triesters of phosphorous acid to the corresponding phosphates in high yields was demonstrated earlier.[4] In the present work we showed first of all that the reaction is nearly stereospecific by use of the cyclic methyl phosphites, cis-I and trans-I. [The cis and trans geometries (Me and t-Bu relationship) of the individual phosphites and their phosphate oxidation products had been assigned previously.[5]] Thus, a stirred 0.2 M benzene solution of cis-I and trans-I [cis/trans ratio 92/8 (PMR, t-Bu peaks)] containing ~5% AIBN, over which was maintained an atmosphere of O_2, was heated at 70°. Oxidation was complete in 3h, as shown by GLC, to give the product phosphates in cis/trans ratio 93/7 (GLC) in greater than 90% yield (^{31}P NMR). Similarly, a 41/59 cis/trans ratio of phosphites yielded the phosphates in a 47/53 cis/trans ratio.

Results of the application of the method to the diastereomeric thymidine 3',5'-cyclic methyl phosphites[6] (II, Scheme I) are compiled in Table I. The stereospecific, retentive nature of the oxidation at phosphorus is again evident.[7] ^{31}P NMR showed the oxidation to be nearly quantitative. The cis and trans designations refer to the relationship of the MeO and thymyl groups.

SCHEME I:

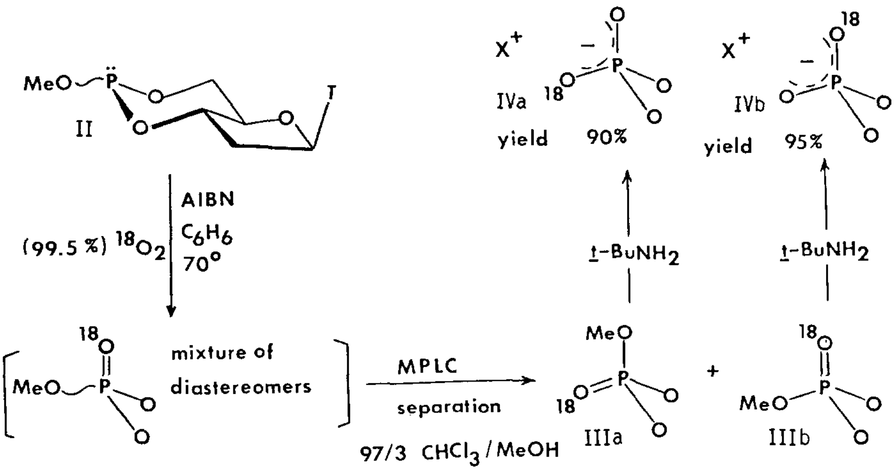


Table I. O_2/AIBN Oxidation of Thymidine Nucleoside 3',5'-Cyclic Methyl Phosphite

Phosphite ratio, cis/trans[a]	Phosphate ratio, cis/trans[a]
44/56	45/55
20/80	20/80
17/83	17/83
14/86	13/87

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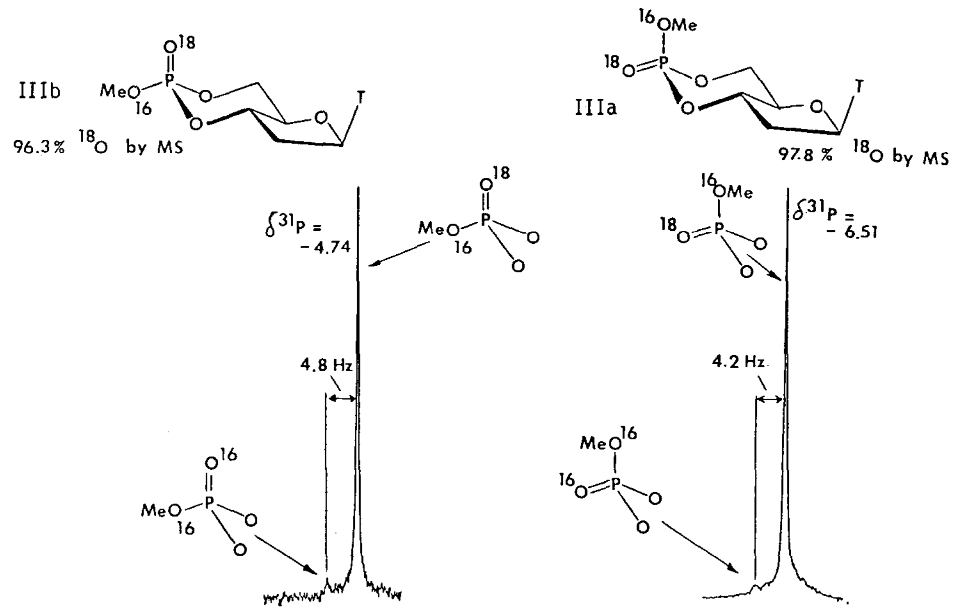
[a] ^{31}P NMR integration.

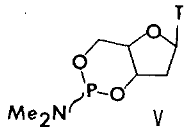
Use of 99.6% $^{18}O_2$ at one atmosphere yielded the diastereomeric ^{18}O-labeled methyl esters of thymidine 3',5'-cyclic monophosphate, III (Figure I). Mass spectroscopy demonstrated that the ci and trans methyl phosphates contained 96.3 and 97.8% ^{18}O, respectively. (From m/e = 319 and m/e 321 MS peaks in the unlabeled and labeled materials.) Figure I confirms by high-field ^{31}P NMR (109.2 MHz) the high percentage of ^{18}O incorporation in the methyl phosphates as well as the regiospecificity of the reactions. The ^{31}P peak of the cis diastereomer ($\delta^{31}P = -6.51$, acetone-d_6) is that of the compound having ^{18}O incorporated in the phosphoryl oxygen. This resonance is isotope-shifted upfield from the very weak peak assignable to unlabeled material by the expected amount (4.2 Hz).[1a,c,8] An analogous pair of peaks is noted for the trans diastereomer (Major resonance at $\delta^{31}P = -4.74$). In neither case is there seen a peak resulting from the Me^{18}OP(^{16}O) product. This compound, arising from non-regiospecific introduction of ^{18}O, would appear about 1.2 Hz upfield of the ^{16}O/^{16}O product.[8]

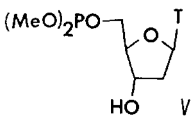
The diastereomers of III are readily separated in a single pass by medium pressure liquid chromatography on SiO_2 with 97/3 $CHCl_3$/MeOH as elutant. A 300-500 mg scale reaction is easily handled. The individual diastereomer, IIIa or IIIb (Scheme I), is quantitatively dealkylated to the t-BuNHMe salt of the ^{18}O-labeled diastereomer of cTMP, IVa or IVb, on 20 h of reflux in t-$BuNH_2$ as solvent.[9] Products IVa and IVb precipitate during reflux and are readily isolated by simple filtration in 90-95% yields based on the methyl phosphates. Only a single peak at $\delta^{31}P$ = -2.1 (D_2O) could be found in the ^{31}P NMR spectrum of each of these diesters indicating that they are at least 95% pure. The overall yield of each isotopically labeled diester from starting phosphite II is >80%. The sequence of preparation is summarized in Scheme I.

FIGURE I: Regiospecificity by ^{31}P NMR at 109.2 MHz



Labeled $^{16}O/^{17}O/^{18}O$/cTMP's can doubtless also be obtained by this route. One need simply use $CH_3{}^{18}OH$ or $CH_3{}^{17}OH$ in the alcoholysis[6] of the phosphoramidite precursor to phosphite II followed by $^{17}O_2$ or $^{18}O_2$ oxidation. We have also applied the O_2/AIBN method to the trivalent nucleoside derivatives V[10] and VI. Product oxides are formed cleanly and near-quantitatively.


V


VI

The ^{18}O introduction is experimentally very easy and straightforward. The bulb containing $^{18}O_2$ is fitted with a vacuum stopcock and connected using ground glass joints by way of a tee to a single-necked reaction flask. The benzene solution of phosphite to be oxidized and AIBN is thoroughly degassed by several conventional freeze-thaw cycles after which the reaction apparatus is isolated from the vacuum, and the stopcock to the $^{18}O_2$ bulb is opened. The solution is then heated to 70°. The solubility of O_2 in benzene even at less than 1 atm of pressure is sufficient for the oxidation to proceed efficiently.

In addition to being an experimentally simple and very high yield process, the O_2/AIBN method allows the use of isotopic oxygen in its lowest-cost, molecular form. Both oxygen atoms of the molecule are utilized. Using $^{18}O_2$ and phosphite in 10/1 molar ratio we have run easily two successive oxidations from the same $^{18}O_2$ sample. The reaction is not much affected by the reduced O_2 pressure, and clearly several more reactions could have been carried out. As evidence of this, the partial pressure of O_2 in air at one atmosphere is sufficient to effect the efficient introduction of ^{16}O. The method has the further advantage that removal of the potentially troublesome HI, byproduct from the usual I_2/H_2O oxidation,[3] is not necessary.

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References:

1. a) J.A. Gerlt, J.A. Coderre, J. Am. Chem. Soc., 102, 4531 (1980); b) J. Baraniak, K. Lesiak, M. Sochacki, and W.J. Stec, ibid., 102, 4533 (1980); c) R.L. Jarvest, G. Lowe and B.V.L. Potter, J. Chem. Soc., Chem. Commun., 1142 (1980).

2. J.A. Coderre, S. Mehdi and J.A. Gerlt, J. Am. Chem. Soc., 103, 1872 (1981); R.L. Jarvest and G. Lowe, J. Chem. Soc., Chem. Commun., 1145 (1980); J.A. Coderre and J.A. Gerlt, J. Am. Chem. Soc., 103, 6594 (1980).

3. See e.g.: a) M.D. Matteucci and M.H. Caruthers, J. Am. Chem. Soc., 103, 3185 (1981); b) R.L. Letsinger and W.B. Lunsford, J. Am. Chem. Soc., 98, 3655 (1976).

4. W.G. Bentrude, Tetrahedron Lett., 3543 (1965).

5. W.G. Bentrude and J.H. Hargis, J. Am. Chem. Soc., 92, 7136 (1970); R.W. Warrent, C.N. Caughlan, J.H. Hargis, K.C. Yee and W.G. Bentrude, J. Org. Chem., 43, 4266 (1978).

6. G.S. Bajwa and W.G. Bentrude, Tetrahedron Lett., 421 (1978). Phosphite II is made in >90% yield from V typically prepared in 20-25% yield directly from reaction of thymidine with $(Me_2N)_3P$.

7. The retentive nature of the AIBN/O_2 oxidation was further confirmed by comparison to products using N_2O_4 whose stereochemistry is well known (J.A. Mosbo and J.G. Verkade, J. Am. Chem. Soc., 95, 4659 (1973)).

8. D.G. Gorenstein and R.G. Rowell, J. Am. Chem. Soc., 102, 6165 (1980).

9. Method of D.J.H. Smith, K.K. Ogilve, and M.F. Gillen, Tetrahedron Lett., 861 (1980).

10. Dr. Katalin Komives, unpublished results from this laboratory.

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