Directed Arbuzov-Type Reactions of 2-Cyano-1,1-dimethylethyl Deoxynucleoside Phosphites

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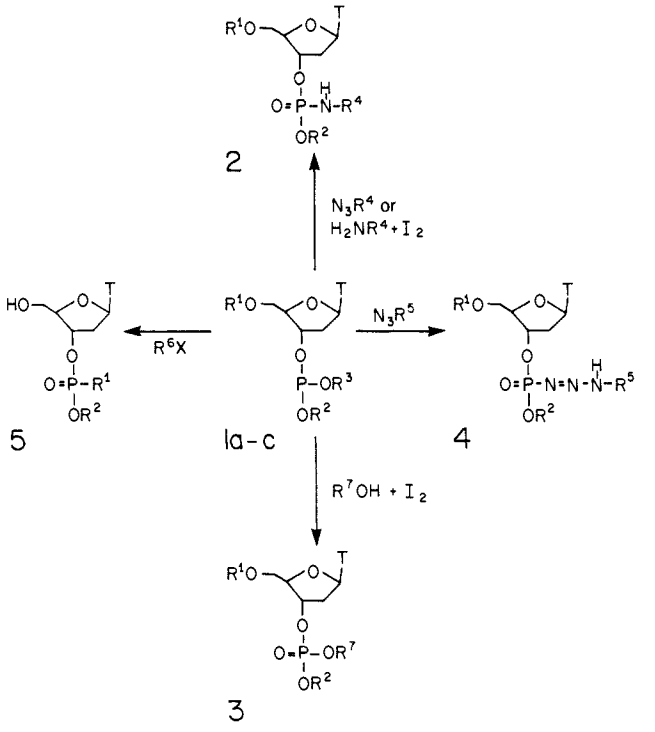
The second step of the general Arbuzov reaction is believed to have S_N2 character.[1] Recently however, this concept has been questioned,[2] and it has been shown that the reaction mechanism varies in character with the reagent and the solvent. Here we report that alkyl deoxynucleoside phosphites having alkyl groups which stabilize the S_N1 character of the second stage of the Arbuzov reaction react selectively with most electrophiles and eliminate only the appropriate tertiary alkyl-protecting group. Upon the basis of this concept, we demonstrate that various modified phosphates of general interest for biochemical purposes can be prepared from the same synthon, 2-cyano-1,1-dimethylethyldeoxynucleoside phosphite (**1a**).

Since the introduction of the phosphite method for the synthesis of deoxyoligonucleotides,[3] the oxidation of the intermediate phosphite triester to the corresponding phosphate triester has generally been accomplished with aqueous iodine. Recently however, both 2-cyano-1,1-dimethylethyldeoxydinucleoside phosphite (**1a**)[4] and *o*-methylbenzyldeoxydinucleoside phosphite (**1b**)[5] have been shown to yield the corresponding phosphate diester when oxidized under these conditions.[5,6] This oxidation and accompanying selective loss of the phosphorus protecting group appears to proceed cleanly and in the absence of side products as a very high yield of a deoxyicosanucleotide has been reported with the latter compound. These observations have led us to assume that an iodophosphonium ion is the intermediate during this oxidation which, in the case of **1a**, eliminates the 2-cyano-1,1-dimethylethyl group.

We have exploited this selective elimination process for the purpose of synthesizing several different internucleotide phosphate analogues. When **1a** reacts under anhydrous conditions with iodine (1.0 equiv) in the presence of *n*-butylamine (250 equiv) in THF, the corresponding phosphoramidate (**2**) forms quantitatively (^{31}P NMR).[7] Similarly, **1c** with iodine (1.0 equiv) and methanol forms the methyl phosphate[8] **3**. Both reactions are spontaneous at room temperature as judged by the ^{31}P NMR analysis and the immediate decolorization of the iodine solution. When **1a** reacts with iodine in anhydrous THF without other nucleophiles, upfield resonances (δ −44.3 and −44.5 ppm) in ^{31}P NMR are observed. These signals indicate the presence of the corresponding deoxydinucleoside phosphoryl iodide. Under aqueous conditions, the symmetric deoxydinucleoside pyrophosphate (δ −13.9 ppm) is always seen before formation of the diester. However, it is still not clear from these observations whether the reactions proceed via the phosphoryl iodide or the iodophosphonium ion intermediate.

The reactivity of **1a** toward other electrophiles has also been investigated. At 55 °C, **1a** and *n*-butyl azide (33 equiv) yield **2** quantitatively (^{31}P NMR) after 8 h.[9,10] With 3-azido-*N*-ethylcarbazole (1.4 equiv), **1a** reacts quantitatively (^{31}P NMR) after 72 h to form the deoxydinucleoside phosphoroazoamidate (**4**)[11] rather than the monophosphazene as usually derived from Staudinger conditions[12] (analysis by fast atom bombardment, FAB-MS).

Scheme I[a]



[a] a: R^2 = 3′-acetylthymidylyl, R^1 = 4,4′-dimethoxytrityl, R^3 = 2-cyano-1,1-dimethylethyl, R^4 = *n*-butyl; b: R^2 = 3′-acetylthymidylyl, R^5 = 3-(*N*-ethylcarbazoyl), R^3 = *o*-methylbenzyl, R^6 = methyl; c: R^2 = ethyl, R^7 = methyl, R^3 = 2-cyano-1,1-dimethylethyl.

Several reports have discussed the possibility of utilizing Arbuzov reactions to form internucleotide alkyl phosphonates,[13] but so far this concept, as a general approach, has not proven successful. Stimulated by the above results, we investigated the reactivity of **1a** with alkyl halides by using the normal Arbuzov reaction. When **1a** is treated with a series of alkyl iodides (R = Me, Et, *n*-Pr, *i*-Pr), only methyl iodide reacts at 55 °C within a reasonable time (24 h). From ^{31}P NMR analysis, this reaction yields a heterogeneous mixture of compounds with the main product being the 4,4′-dimethoxytrityl phosphonate (**5**) rather than the expected methylphosphonate. Formation of this product presumably occurs by methyl iodide mediated detritylation followed by electrophilic attack of the 4,4′-dimethoxytrityl carbenium ion at phosphorus.[13c] However, we have also observed that reaction of **1c** with 4,4′-dimethoxytritylchloride (1.1 equiv) at 55 °C for 72 h yields various alkylphosphonates (^{31}P NMR) which indicates degradation of intermediates or reaction products.

This work therefore shows that Arbuzov-type reactions can be directed to follow an S_N1 mechanism during the second step and

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(7) 5′-*O*-Dimethoxytritylthymidine 3′-*O*-(5′-*O*-thymidylyl-3′-*O*-acetyl) *N*-butylphosphoramidate (**2**): ^{31}P NMR ($CDCl_3$) δ 9.0 and 8.7 ppm; FAB^+, 303 (DMT^+); FAB^-, 945 (M^-), 678 (DMTdT-3′-PO_2NHBu), 418 (5′-PO_2NHBu-dT-3′-OAc).
(8) 5′-Dimethoxytritylthymidine ethyl methylphosphate (**3**): ^{31}P NMR ($CDCl_3$) δ −1.0 ppm; FAB^+, 666 (M^+), 455 (DMT^+ + matrix), 303 (DMT^+); FAB^-, 650 (M − methyl), 636 (M − ethyl).
(9) A similar reaction has been reported under hydrolytic conditions: Letsinger, R. L.; Schott, M. E. *J. Am. Chem. Soc.* **1981**, *103*, 7394.
(10) Compound **2** has already been described in ref 7. The compounds obtained by the two separate pathways were identical according to ^{31}P NMR and TLC (R_f 0.47, CH_2Cl_2:MeOH, 9:1, v/v).
(11) 5′-*O*-Dimethoxytritylthymidine 3′-*O*-(5′-*O*-thymidylyl-3′-*O*-acetyl) *N*-(3-(*N*-ethylcarbazolyl))phosphoroazoamidate (**4**): ^{31}P NMR (THF, external lock) δ 3.5 and 3.2 ppm; FAB^+, 1083 (M + 1), 556 (5′-HO_2P-N_3-(*N*-Et-carbazolyl)-dT-3′-OAc-1); FAB^-, 1081 (M − 1), 815 (5′-DMTdT-PO_2-N_3-(*N*-Et-carbazolyl), 555 (5′-HO_2P-N_3-(*N*-Et-carbazolyl)-dT-3′-OAc).
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as a consequence form a variety of internucleotide linkages such as phosphoramidates, azoamidates, and esters. Moreover, by oxidation with *tert*-butylhydroperoxide or sulfur, **1a** also forms either the dinucleoside phosphate or thiophosphate triester, respectively, without loss of the 2-cyano-1,1-dimethylethyl protecting group (unpublished observation). Since phosphite triesters are intermediates in solid phase oligonucleotide synthesis via the phosphoramidite method,[14] this approach can now be extended so that one synthon, the deoxynucleoside 3′-phosphoramidite having a 2-cyano-1,1-dimethylethyl (or perhaps *o*-methylbenzyl) protection group, is used to introduce several phosphate analogues uniquely at specific sites in any combination with natural internucleotide linkages.

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The Resolution of Racemic Hydroperoxides: The Preparation of Optically Pure Hydroperoxide Natural Products[1]

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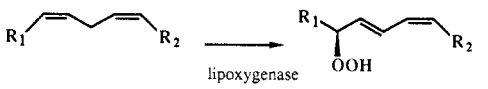
Lipoxygenase enzymes[3] catalyze the conversion of polyunsaturated fatty acids to fatty acid hydroperoxides and these hydroperoxides serve as important intermediates in the formation of diverse compounds of biological importance (Scheme I). A new stereocenter is generated in the lipoxygenase reaction, and fatty acid hydroperoxides isolated from natural sources are essentially one enantiomer if they are formed enzymatically.[3,4] Nonenzymatic autoxidation of fatty acid substrates also gives fatty acid hydroperoxides,[5] but racemic mixtures are formed in this process.[4]

Despite the importance of unsaturated hydroperoxides in chemistry, biology, and medicine, no general method for the preparation of these labile compounds has been reported. Although the natural products can be obtained from enzymatic sources, unnatural enantiomers are unavailable because all chemical syntheses described give racemic mixtures.[6-8] We report here what appears to be a general solution to this problem, the resolution of unsaturated hydroperoxide enantiomers by liquid chromatography of diastereomeric derivatives. This method allows, for the first time, the nonenzymatic preparation of optically pure allylic or dienylic hydroperoxide natural products.

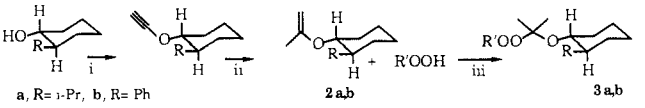
Preliminary screens of several hydroperoxide derivatives of the structure R-OO-CR′XOR″, **1**, were carried out. Peracetals **1** (R′ = alkyl or H, X = H) are readily formed[9] and stable to chromatography but require harsh conditions for deprotection,[10] while perortho esters[11] (R′ = H, aryl or alkyl and X = OR″) and peraminals[12] are unstable to chromatography. Perketals **1** with R′ = X = alkyl are readily prepared[13,14] from hydroperoxides, are stable to normal or reverse-phase chromatography, and can be deprotected under very mild acid conditions.

We have had the most success with resolution utilizing the vinyl ethers **2a** and **2b**, prepared from menthol and (−)-*trans*-2-phenylcyclohexanol[15] (Scheme II). Standard procedures for vinyl ether synthesis directly from the alcohol were unsuccessful, but the path through the acetylenic ether gave **2a** in 58% and **2b** in 84% yield for the two steps.[16] The perketals **3** could be prepared from **2** and hydroperoxide with pyridinium *p*-toluenesulfonate (PPTS) catalyst in yields of 92-100%. Hydroperoxide **4** was converted to the perketal **3a**, and **4**-**7**[17] (R = Me) were reacted with **2b** to give the corresponding perketals **3b**. In each case

Scheme I



Scheme II[a]



[a] (i) a Cl_3C_2H, KH, imidazole, b *n*-BuLi, (ii) MeMgBr/CuBr, (iii) PPTs/CH_2Cl_2.

Table I. Data for Hydroperoxide Resolution

racemic hydroperoxide	diastereomeric perketal ratio[a]	diastereomeric perketal yield[b]	resolved hydroperoxides yield[c]	resolved hydroperoxides ee[d]	resolved hydroperoxides rotation[e]
4	>99/1	77	85	97	+104 (*R*)
	1.5/98.5	76	89	95	−102 (*S*)
5 (R = Me)	>99/1	53	92	99	−1.9 (*R*)
	3.5/96.5	56	95	93	+1.6 (*S*)
6 (R = Me)	>98/2	91	90	96	−8.7 (*R*)
	>99/1	93	91	99	+9.1 (*S*)
7 (R = Me)	99/1	60	74	98	−4.3 (*R*)
	>99/1	59	64	98	+3.6 (*S*)

[a] Each pair of diasteromeric perketals is listed in order of reverse-phase chromatographic elution. [b] Yield of purified diastereomer based on 50% of racemic hydroperoxide. [c] Purified hydroperoxide isolated from hydrolysis of resolved perketals. [d] Assessed by HPLC on reformed perketal for **5**, **6**, and **7**; capillary GC of alcohol Mosher ester of **4**. Perketals derived from **4** were prepared from auxiliary of only 97% ee. [e] Measured in chloroform (c = 0.5-0.9).

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(17) In a representative procedure, 1 mmol of hydroperoxide is dissolved in 5-10 mL of dry CH_2Cl_2. Enol ether (1.2 equiv) is added along with 2-5% of PPTS. After the reaction is judged complete by TLC (usually 5-15 min), a small amount of $KHCO_3$ is added. Several volumes of CCl_4 are added, and the CH_2Cl_2 is removed (rotary evaporator or argon stream). The resulting solution is directly loaded onto a flash column of 230-400 mesh silica, and the perketal eluted with EtOAc/petroleum ether, typically in approximately 95% yield.

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