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Ethyl(methyl)dioxirane as an Efficient Reagent for the Oxidation of Nucleoside Phosphites into Phosphates under Nonbasic Anhydrous Conditions

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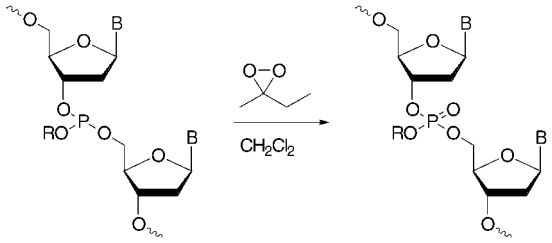
ABSTRACT



A convenient method for the oxidation of nucleoside phosphites into phosphates under nonbasic and nonaqueous conditions using commercially available ethyl(methyl)dioxirane has been developed. This oxidation is effective with both N-protected and N-unprotected strategies.

In the synthesis of nucleotides via the phosphoramidite method,[1] one of the main research subjects is the development of an efficient method for oxidation of intermediately formed nucleoside phosphites. Conventionally, iodine in aqueous pyridine has been most frequently used for the oxidation, but this method is not quite suitable for the synthesis of derivatives sensitive to water and/or a base. Therefore, oxidation under nonbasic and nonaquesous conditions has been demanded, and several strategies, including nitrogen dioxide in dichloromethane,[2] *m*-chloroperbenzoic acid (MCPBA) in dichloromethane,[3] a *tert*-butyl hydroperoxide (TBHP)/toluene solution in acetonitrile,[4,5] bis(trimethylsilyl) peroxide in dichloromethane[4,6,7] or an acetonitrile−dichloromethane mixture,[8] dimethyldioxirane in dichloromethane,[9] and (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine (CSO) in acetonitrile,[10] have been developed. These nonbasic, nonaqueous methods have some drawbacks. For example, nitrogen dioxide is highly toxic; MCPBA is also toxic. Further, MCPBA generates *m*-chlorobenzoic acid with the progress of oxidation, which frequently brings about undesired cleavage of 5′-*O*-dimethoxytrityl protection. The TBHP/toluene solution is not commercially available, and accordingly we have to prepare it by hand through somewhat tedious operations.[5] Although TBHP was commercially

(1) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123−6194 and references therein.
(2) Bajwa, G. S.; Bentrude, W. G. *Tetrahedron Lett.* **1978**, 421−424.
(3) (a) Ogilvie, K. K.; Nemer, M. J. *Tetrahedron Lett.* **1981**, *22*, 2531−2532. See also: (b) Sekine, M.; Iimura, S.; Nakanishi, T. *Tetrahedron Lett.* **1991**, *32*, 395−398.
(4) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191−4194.
(5) Preparation of a solution of TBHP in toluene: Hill, J. G.; Rossiter, B. E.; Sharpless, K. B. *J. Org. Chem.* **1983**, *48*, 3607−3608.
(6) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4195−4196.
(7) Preparation of bis(trimethylsilyl) peroxide: Dembech, P.; Ricci, A.; Seconi, G.; Taddei, M. *Org. Synth.* **1996**, *74*, 84−90.
(8) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1997**, *119*, 11758−11762.
(9) Chappell, M. D.; Halcomb, R. L. *Tetrahedron Lett.* **1999**, *40*, 1−4.
(10) (a) Manoharan, M.; Lu, Y.; Casper, M. D.; Just, G. *Org. Lett.* **2000**, *2*, 243−246. See also: (b) Wada, T.; Mochizuki, A.; Sato, Y.; Sekine, M. *Tetrahedron Lett.* **1998**, *39*, 7123−7126.

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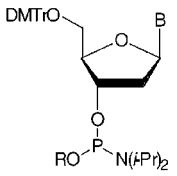
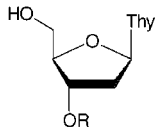
supplied as a decane solution,[11] it is very expensive. Bis(trimethylsilyl) peroxide[11] and CSO[11] are commercially available but also expensive. Further, the silylated peroxide is rather explosive. Dimethyldioxirane is rather explosive and not commercially supplied. Moreover, this reagent causes undesired oxidative modification of thymine,[12] uracil,[12] and adenine bases.[13] Therefore, development of an oxidizing agent without any of these disadvantages has been strongly demanded. We present here ethyl(methyl)dioxirane (2-butanone peroxide) as such a reagent. This peroxide is commercially available as a dimethyl phthalate solution at a lower price than MCPBA, bis(trimethylsilyl) peroxide, or TBHP/decane solution. Oxidation with this reagent can be efficiently applied to the nucleotide synthesis both in a solution phase and in a solid phase.

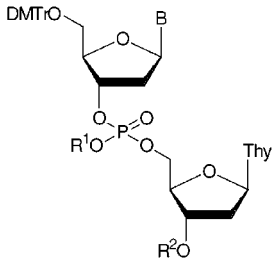
A 55% (7.3 M) solution of ethyl(methyl)dioxirane in dimethyl phthalate[14] was diluted with dichloromethane to obtain a desired concentration of the solution. The use of a 0.1 or 0.01 M solution is suitable for the reaction in a solution phase or on solid supports, respectively. These low-concentration solutions are quite stable under ordinary conditions and can be kept for a long period without any decomposition. For example, solution stored at ambient temperature for 1 month was effectively used in the following reactions. First, the utility of this peroxide was demonstrated in the solution-phase synthesis of a dinucleoside phosphate, which was conducted via the condensation of a nucleoside 3′-phosphoramidite (1 equiv) and a 5′-*O*-free nucleoside (1 equiv) by the aid of benzimidazolium triflate[15] (1 equiv) in the presence of 3Å molecular sieves and the subsequent oxidation with ethyl(methyl)dioxirane (1 equiv). In this process, the condensation and the oxidation were generally completed at 25 °C for 1 and 5 min, respectively, to give the target product as a mixture of two diastereomers in almost quantitative (>98%) yield.[16] Noteworthy here is that the oxidation was rapidly and quantitatively achieved by the use of 1 equiv of the oxidizing agent. This method is useful for the synthesis with both N-unprotected and N-protected building blocks. Actually, the dinucleoside phosphates, **17**−**29**, were prepared by the use of suitable building blocks among **1**−**15**. HPLC and ^{31}P NMR analyses showed that, in some cases, the crude product is contaminated by a small amount (<2%) of the nucleoside *H*-phosphonate, which is resulting from hydrolysis of the starting nucleoside phosphoramidite.[16] However, other undesired products were not



1, B = Ade; R = $NCCH_2CH_2$
2, B = Ade^{AOC}; R = CH_2=$CHCH_2$
3, B = Ade^{Bz}; R = $NCCH_2CH_2$
4, B = Cyt; R = $NCCH_2CH_2$
5, B = Cyt^{Ac}; R = $NCCH_2CH_2$
6, B = Cyt^{AOC}; R = CH_2=$CHCH_2$
7, B = Cyt^{Bz}; R = $NCCH_2CH_2$
8, B = Gua; R = $NCCH_2CH_2$
9, B = $Gua^{All,AOC}$; R = CH_2=$CHCH_2$
10, B = Gua^{dmf}; R = $NCCH_2CH_2$
11, B = Gua^{ibu}; R = $NCCH_2CH_2$
12, B = Thy; R = CH_2=$CHCH_2$
13, B = Thy; R = $NCCH_2CH_2$



14, R = AOC
15, R = TBDMS
16, R = $CO(CH_2)_2CONH$ ~ polystyrene



17, B = Ade; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
18, B = Ade^{AOC}; R^1 = CH_2=$CHCH_2$; R^2 = AOC
19, B = Ade^{Bz}; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
20, B = Cyt; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
21, B = Cyt^{Ac}; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
22, B = Cyt^{AOC}; R^1 = CH_2=$CHCH_2$; R^2 = AOC
23, B = Cyt^{Bz}; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
24, B = Gua; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
25, B = $Gua^{All,AOC}$; R^1 = CH_2=$CHCH_2$; R^2 = AOC
26, B = Gua^{dmf}; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
27, B = Gua^{ibu}; R^1 = $NCCH_2CH_2$; R^2 = TBDMS
28, B = Thy; R^1 = CH_2=$CHCH_2$; R^2 = AOC
29, B = Thy; R^1 = $NCCH_2CH_2$; R^2 = TBDMS

All = allyl; AOC = allyloxycarbonyl; dmf = (dimethylamino)methylene

(11) A TBHP/decane solution was supplied by Aldrich. Bis(trimethylsilyl) peroxide was supplied by United Chemical Technologies, Gelest, etc. CSO was supplied by Aldrich or another company.

(12) (a) Luppattelli, P.; Saladino, R.; Mincione, E. *Tetrahedron Lett*. **1993**, *34*, 6313−6316. (b) Saladino, R.; Bernini, R.; Crestini, C.; Mincione, E.; Bergamini, A.; Marini, S.; Palamara, A. T. *Tetrahedron* **1995**, *51*, 7561−7578.

(13) Saladino, R.; Crestini, C.; Bernini, R.; Mincione, E.; Ciafrino, R. *Tetrahedron Lett*. **1995**, *36*, 2665−2668.

(14) The ethyl(methyl)dioxirane (2-butanone peroxide)/dimethyl phthalate solution is available from Aldrich or Kishida (Japan).

(15) Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996−7997.

(16) **General procedure** for the preparation of a dinucleoside phosphate via the phosphoramidite approach with ethyl(methyl)dioxirane oxidation. A mixture of a nucleoside phosphoramidite (0.1 mmol), a nucleoside (0.1 mmol), and benzimidazolium triflate (0.1 mmol) in acetonitrile (0.2 mL) containing powdery molecular sieves 3Å (30 mg) was stirred at 25 °C for 1 min. To this mixture was added a 0.1 M ethyl(methyl)dioxirane solution in dichloromethane (1.0 mL), and stirring was continued at 25 °C for an additional 5 min. The reaction mixture was diluted with dichloromethane (10 mL). The molecular sieves were removed by filtration. The filtrate was diluted with petroleum ether (300 mL) to give the target product as precipitates. The ^{31}P NMR analysis using H_3PO_4 as the standard indicated that the yield of the desired product was generally >98%, and in some cases a small amount (<2%) of a nucleoside *H*-phosphonate, which results from hydrolysis of the starting nucleoside phosphoramidite, was formed as a byproduct. HPLC analysis also gave a similar result.

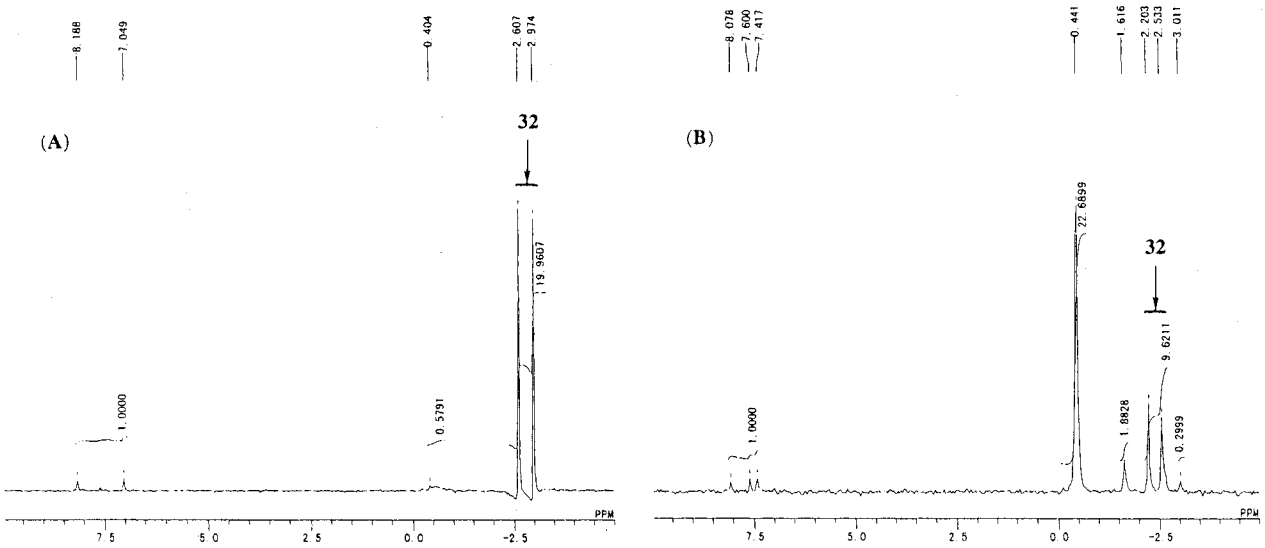
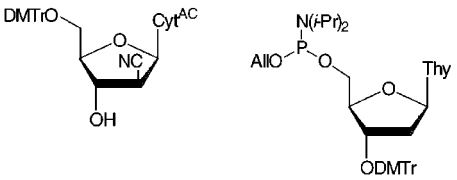


Figure 1. The ^{31}P NMR spectra of the crude products of **32**. (A) Prepared by the method using ethyl(methyl)dioxirane oxidation. (B) Prepared by the method using I_2/H_2O/pyridine oxidation.

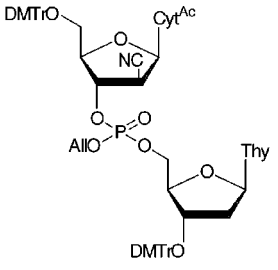
detected at all in these analyses. These results indicated that nucleoside bases with and without protection, the protecting groups on the bases, carbohydrates, and internucleotide linkages underwent no damages in the preparation.

Higher utility of the present method than that of oxidation using iodine in aqueous pyridine was remarkably observed in the synthesis of nucleotides labile to a base and/or moisture. For example, the base-sensitive compound **32** (a diasteremeric mixture),[17] which is a key intermediate for the synthesis of biologically important 2′-*C*-cyano-2′-deoxy-1-*β*-D-*arabino*-pentafuranosylcytosine (CNDAC) 3′-phosphates,[18] was prepared in >90% overall yield via the condensation of the nucleoside **30**[18b] and the phosphoramidite **31**[17] by the use of 1*H*-tetrazole as the promoter and the subsequent oxidation using a 0.1 M solution of ethyl(methyl)-dioxirane in dichloromethane under similar conditions as described above;[16] little decomposition of **32** took place under the oxidation conditions (see the ^{31}P NMR spectrum of the crude product shown in Figure 1). In contrast, the use of iodine in aqueous pyridine for the oxidation caused considerable decomposition of **32** to give the target compound in <25% yield (see Figure 1).[17]

The ethyl(methyl)dioxirane oxidation can be applied to the solid-phase synthesis of oligonucleotides. The synthesis of $^{5'}$GCACACCCAATTCTGAAAAT$^{3'}$ (**33**) was representatively carried out starting from the thymidine (**16**) attached to high-cross-linked polystyrene resins. The chain elongation was achieved with **2**, **6**, **9**, and **12** as the monomer units and benzimidazolium triflate as the promoter in the manner previously reported,[19] in which the phosphite intermediates were oxidized by the use of a 0.01 M ethyl(methyl)dioxirane solution. The average yield of one-base elongation including the oxidation was 99.2%, attaining an overall yield of 86.3%. The deprotection by exposure to a mixture of $Pd_2[(C_6H_5\text{-}CH{=}CH)_2CO]_3{\cdot}CHCl_3$ and $(C_6H_5)_3P$ in the presence of diethylammonium carbonate in THF (50 °C, 60 min)[20] and the subsequent detachment of the product from the solid supports by treatment with a 28% aqueous ammonia solution (25 °C, 60 min) gave the oligomer **33**, which has high purity in a crude form, exhibited in its capillary gel electrophoresis (CGE) and MALDI-TOF mass profiles (Figure 2). The



30 **31**



32

(17) For the synthesis of a CNDAC phosphate: Hayakawa, Y.; Kawai, R.; Otsuki, K.; Kataoka, M.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2559−2562.

(18) For biological activites and properties of CNDAC and related compounds: (a) Matsuda, A.; Nakajima, N.; Azuma, A.; Tanaka, M.; Sasaki, T. *J. Med. Chem.* **1991**, *34*, 2917−2919. (b) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem.* **1993**, *36*, 4183−4189. (c) Matsuda, A.; Azuma, A. *Nucleosides Nucleotides* **1995**, *14*, 461−471. (e) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Mastuda, A. *J. Med. Chem.* **1995**, *38*, 3391−3397.

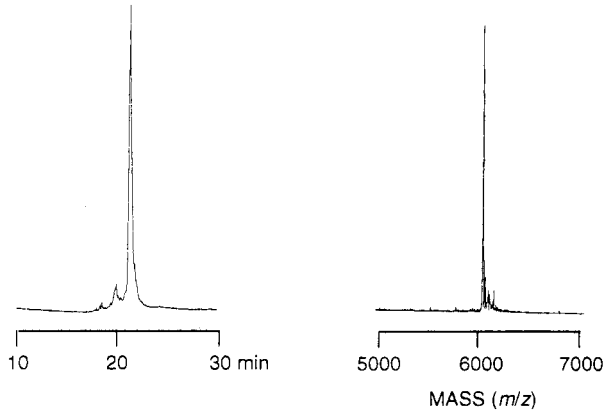


Figure 2. Capillary gel electrophoresis and MALDI-TOF mass profiles of the oligonucleotide **33** in a crude form.

average yield of one-base elongation and the purity of the target product observed here were quite similar to those obtained by the synthesis using a TBHP/toluene solution for the oxidation.[21] This result indicated that no detectable modification of nucleoside bases and carbohydrates takes place in the approach using ethyl(methyl)dioxirane for the oxidation step. Thus, the efficiency of the present method in the solid-phase synthesis compares favorably with that of the TBHP strategy.

In summary, we disclosed that ethyl(methyl)dioxirane is a useful reagent for the oxidation of nucleoside phosphites into phosphates under nonbasic and nonaqueous conditions. This method is particularly effective for the preparation of moisture- and/or base-sensitive nucleotides and can be applied to both solution-phase and solid-phase syntheses.

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Supporting Information Available: Characterization data including ^{1}H NMR, ^{31}P NMR, and/or MALDI-TOF mass spectral data for new compounds **19**, **21**, **23**, **26**, and **27**, and CGE and MALDI-TOF mass spectral data of the crude product of **33** prepared by the method using TBHP oxidation. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Hayakawa, Y.; Wakabayashi, S.; Kato, H., Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691−1696.

(20) (a) Hayakawa, Y.; Hirose, M.; Noyori, R. *Tetrahedron* **1995**, *51*, 9899−9916. See also: (b) Sakakura, A.; Hayakawa, Y. *Tetrahedron* **2000**, *56*, 4427−4435 and references therein.

(21) In the synthesis employing TBHP oxidation, one-base elongation was achieved in an average 99.5% yield.