A Facile Method for the Oxidation of Nucleoside *H*-Phosphonates to Phosphates with Bis(trimethylsilyl) Peroxide

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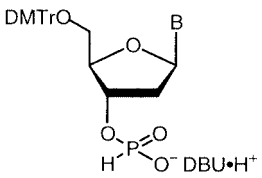
Abstract: We have developed a convenient method for achieving the oxidation of nucleoside *H*-phosphonic acid monoesters and diesters with bis(trimethylsilyl) peroxide and *N*,*O*-bis(trimethylsilyl)acetamide in the presence of trimethylsilyl triflate as a catalyst.

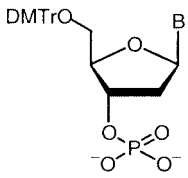
Key words: *H*-phosphonate method, nucleoside *H*-phosphonate, bis(trimethylsilyl) peroxide, nucleotide synthesis

The *H*-phosphonate method is a useful tool for the synthesis of oligonucleotides.[1] In this approach, an important subject is the development of an efficient method for oxidation of nucleoside *H*-phosphonates to the phosphates.[1a] Aqueous iodine in pyridine[2] is conventionally used for the oxidation. This strategy, however, is problematic, because the aqueous conditions bring about partial hydrolysis of the internucleotidic *H*-phosphonate linkages.[1e] Some modified methods using aqueous iodine[1a,1e,2] have been disclosed, but these also cannot completely suppress the hydrolysis. Van Boom has developed an alternative method using *tert*-butyl hydroperoxide (TBHP) in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA).[3] According to reported research,[4] BSA acts as not only a reagent to convert a nucleoside *H*-phosphonate to the reactive trimethylsilyl phosphite, but it is also a scavenger of all traces of water in this oxidation. Thus, BSA is usually employed in an excess amount toward TBHP for obtaining good results. The excess use of BSA, however, converts TBHP to a less reactive trimethylsilyl *tert*-butyl hydroperoxide and consequently causes undesired retarding of the oxidation. In addition, this method is not effective for the oxidation of nucleoside *H*-phosphonomonoesters. Recently Sekine and Wada also reported more reliable and efficient methods using (1*S*)-(+)-(10-camphorsulfonyl)oxaziridine (CSO) in the presence of *N*,*O*-bis(trimethylsilyl)benzamide for oxidation of the monoesters[5] and 2-benzenesulfonyl-3-(3-nitrophenyl)oxaziridine (PNO) in the presence of BSA[4] for oxidation of the diesters. CSO, however, is very expensive, and PNO requires the use of expensive and toxic *m*-chloroperoxybenzoic acid.[6] Thus developing alternative methods with cheap and nontoxic reagents is desirable. This paper describes such an oxidation method with bis(trimethylsilyl) peroxide (TMSOOTMS)[7] and BSA in the presence of trimethylsilyl triflate (TMSOTf) as a catalyst.[8]

First, we investigated the utility of the new method in the oxidation of the nucleoside 3'-*H*-phosphonate monoesters, **1–4**, which are prepared according to the known method via condensation of the 5'-*O*-dimethoxytritylated parent nucleosides[9] and diphenyl *H*-phosphonate.[10] The oxidation was carried out by treatment of the monoester (1.0 equiv) with TMSOOTMS (2.0 equiv) and *N*,*O*-bis(trimethylsilyl)benzamide (BSB) (5.0 equiv) in the presence of TMSOTf (0.05 equiv) at 25 °C for 4 h.[11] Isolated yields of the phosphate products were 97% for **5**, 97% for **6**, 97% for **7**, and 98% for **8**. These products were converted to the corresponding deoxyribonucleoside 3'-monophosphates by treatment with dichloroacetic acid.



1, B = Ade
2, B = Cyt
3, B = Gua
4, B = Thy



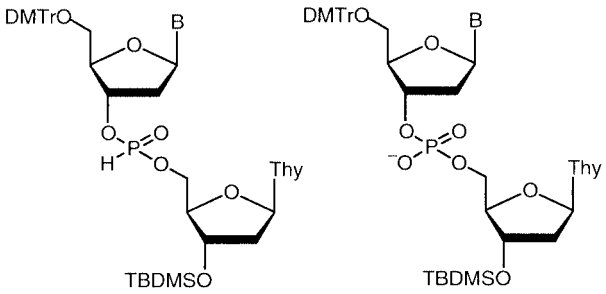
5, B = Ade
6, B = Cyt
7, B = Gua
8, B = Thy

This technique is also effective for the oxidation of dinucleoside *H*-phosphonate diesters, **9–12**, to their phosphates, **13–16**. We accomplished the conversion by exposing the *H*-phosphonates to a mixture of TMSOOTMS (2.0 equiv), BSA (1.5 equiv), and TMSOTf (0.05 equiv) in dichloromethane (25 °C, 60 min).[12] As summarized in Table 1, the desired products were isolated in excellent yields. Removal of the dimethoxytrityl and *tert*-butyldimethylsilyl protectors from **13–16** gave the corresponding dinucleoside phosphates.

The present oxidation is usable in the solid-phase synthesis of oligonucleotides. Its utility was demonstrated in the preparation of TpTpTpTpTpTpTpT (**17**) on controlled pore glass. The chain elongation was carried out by the use of **4** as the monomer units and pivaroyl chloride as an

Table 1 Oxidation of Dinucleoside *H*-Phosphonate Diesters

H-phosphonate	product	isolated yield, %
9	**13**	95
10	**14**	92
11	**15**	90
12	**16**	96

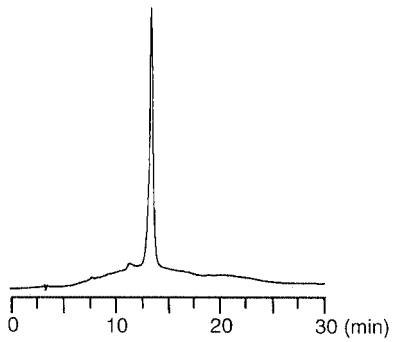


9, B = Ade^{AOC}
10, B = Cyt^{AOC}
11, B = Gua^{AOC}
12, B = Thy

13, B = Ade^{AOC}
14, B = Cyt^{AOC}
15, B = Gua^{AOC}
16, B = Thy

AOC = CH_2=$CHCH_2OCO$; DMTr = $C_6H_5(p\text{-}CH_3OC_6H_4)_2C$
TBDMS = $t\text{-}C_4H_9(CH_3)_2Si$

activator, to give the T_8-*H*-phosphonate intermediate, which was subjected to the TMSOOTMS oxidation. The average coupling yield was 91%. The resulting product was detached from the solid supports by treatment with conc. ammonia (25 °C, 60 min) to furnish **17**. The ^{31}P NMR spectrum of the crude product showed a signal at δ –0.366 ppm, arising from the phosphates, but no signals around 10 ppm due to the *H*-phosphonates. The HPLC profile (Fig. 1) indicated that **17** has high purity in a crude form.



HPLC profile of crude **17**. Conditions: COSMOSIL 5C18-MS column; buffer A: 0.1 M TEAA; buffer B: 5% CH_3CN–0.1 M TEAA; gradient: linear 0% to 70% B in 30 min; detection: 260 nm; flow rate: 1.0 mL/min; temperature: 40 °C.

Figure 1

We developed a new approach for the oxidation of nucleoside 3'-*H*-phosphonates using TMSOOTMS and BSA in the presence of TMSOTf as a catalyst. This method is advantageous over existing methods, since it can oxidize both monoesters and diesters with easily prepared, inexpensive reagents.

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(11) A representative procedure for the oxidation of a nucleoside 3'-*H*-phosphonate monoester: To a solution of thymidine 3'-*H*-phosphonate (**4**) (134 mg, 0.18 mmol) were successively added a 1.0M solution of TMSOOTMS in dichloromethane (0.35 mL, 0.35 mmol), BSB (0.23 mL, 237 mg, 0.88 mmol), and a 0.26 M solution of TMSOTf in dichloromethane (35 μL, 9.0 μmol), and the mixture was stirred at 25 °C. After 4 h, to the reaction mixture was added a 3% solution of DBU in methanol (10 mL) and stirring was continued for 10 min. The mixture was diluted with a 50% aqueous pyridine (10 mL) and washed with dichloromethane (5 mL x 3). The aqueous layer was diluted with ethanol (10 mL) and the resulting mixture

was concentrated to dryness to give 5'-*O*-(*p,p'*-dimethoxytrityl)thymidine 3'-monophosphate (**8**) (159 mg, 97% yield).

(12) A typical procedure for the oxidation of a dinucleoside *H*-phosphonate diester: A mixture of dithymidine *H*-phosphonate **12** (96.0 mg, 0.10 mmol), a 1.0M solution of TMSOOTMS in dichloromethane (0.20 mL, 0.20 mmol), BSA (38.0 μL, 31.3 mg, 152 μmol), and a 0.26 M solution of TMSOTf in dichloromethane (19 μL, 5.0 μmol) was stirred at 25 °C for 90 min. A 3% DBU solution in methanol (10 mL) was added and the mixture was stirred for additional 10 min. The resulting mixture was diluted with dioxane (5 mL) and evaporated to dryness. This operation was further repeated three times to give an oil, which was dissolved in dichloromethane (10 mL). The organic solution was washed with water (5 mL), dried, and concentrated to give **16** (109 mg, 97% yield).

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