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NONAQUEOUS OXIDATION OF NUCLEOSIDE PHOSPHITES TO THE PHOSPHATES [§]

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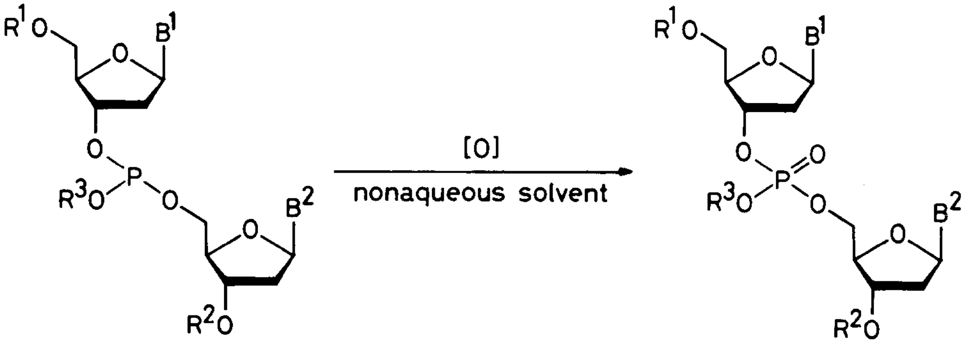
Summary : Bis (trimethylsilyl) peroxide, *t*-butyl hydroperoxide, cumene hydroperoxide, and *N*-methylmorpholine *N*-oxide serve as excellent agents for anhydrous oxidation of nucleoside phosphites to the phosphates. The reaction using bis (trimethylsilyl) peroxide is conspicuously accelerated by added trimethylsilyl triflate or Nafion-TMS.

The phosphite method[1] has been widely employed as a valuable tool for the oligonucleotide synthesis in both solution and solid phases.[2] The synthesis is generally accomplished via two key steps, namely, condensation of two nucleosides and appropriate phospho (III) esterifying agents and oxidation of the phosphite intermediates to the phosphates. Extensive investigations have been made on the phosphite forming reactions to offer a variety of versatile coupling reagents.[2] On the other hand, only a few effective methods are known for oxidation of the phosphites. At present, aqueous iodine is most conventionally employed for this purpose.[2] This procedure, however, is not quite suitable, particularly, for the automated, solid-phase synthesis, because the subsequent reaction must be carried out under anhydrous conditions, therefore requiring a superfluous drying step. Here we report new procedures for nonaqueous oxidation of dinucleoside phosphites to the corresponding phosphates,[3] which are useful for the oligonucleotide synthesis.



1*H*-Tetrazole-promoted condensation of bis (dimethylamino) methoxyphosphine[4] (1.0 equiv) and the nucleoside **1** (1.0 equiv) (18 ℃, 1 h) followed by the second nucleoside **2** (0.95 equiv) (18 ℃, 3 h) in a 1:2 THF—acetonitrile mixture gave the dinucleoside phosphite **3**[5] in 82% yield after chromatographic purification. Subsequent oxidation of **3** with TMSOOTMS[6] (4.0 equiv) in dichloromethane at ambient temperature for 3 h followed

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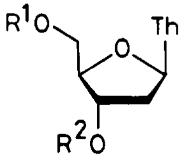
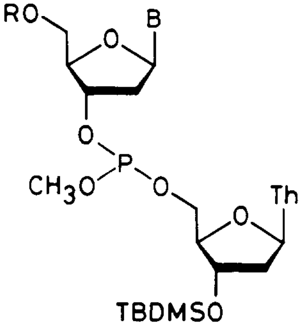
[§] This paper is dedicated to Professor Morio Ikehara on the occasion of his retirement from Osaka University in March, 1986.

by direct evaporative concentration without aqueous workup and silica-gel chromatography afforded the dinucleoside phosphate **7** in 85% isolated yield (ca. 95% yield by HPLC assay of the crude product). The oxidation was remarkably facilitated by adding a catalytic amount of trimethylsilyl triflate (TMSOTf)[7] or its resin-supported version, Nafion-TMS.[8] For example, when the phosphite **3** was treated with 2 equiv of TMSOOTMS in dichloromethane containing TMSOTf and triethylamine[9] (each 5 mol%) at −20°C, the oxidation was completed within 5 min to produce the phosphate **7** in 87% yield (see procedure A). The Nafion-TMS-assisted oxidation was accomplished at the same temperature within 30 min, giving **7** in 85% yield. In this case, the polymer-bound catalyst was removed by filtration before concentration (see procedure B). In a similar way, the phosphites **4**, **5**, and **6** could be oxidized to the corresponding phosphates. Some other oxidizing agents such as *t*-butyl hydroperoxide,[10] cumene hydroperoxide,[10] and *N*-methylmorpholine *N*-oxide[10] are also effective for the nonaqueous reaction. Several examples are listed in Table I. Such reagents are usable to oxidation of a variety of dinucleoside phosphates. The UV, ^{1}H-NMR, and HPLC analyses indicated that the heterocyclic moieties and all protecting groups (DMTr, TBDMS, and acyls) were not affected under these oxidation conditions. Among these oxidizing agents, we recommend use of TMSOOTMS with added TMSOTf or Nafion-TMS and *t*-butyl hydroperoxide because of operational simplicity.

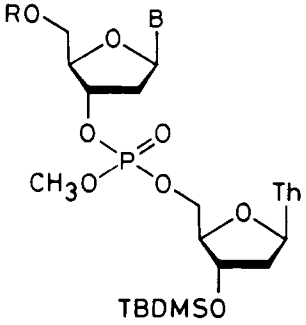
TMSOOTMS oxidation with TMSOTf as the catalyst (procedure A): To a mixture of the phosphite 8 (232 mg, 0.25 mmol) and triethylamine (14.5 mg, 0.014 mmol) were added successively a 1.27 *M* solution of TMSOOTMS (0.39 mL, 0.50 mmol) in dichloromethane (5.5 mL) and 0.11 *M* dichloromethane solution of TMSOTf (0.11 mL, 0.012 mmol) at −20°C. The mixture was stirred at −20°C for 20 min and then concentrated under vacuum (ca. 60 mmHg) to a small volume (ca. 1 mL). Column chromatography of the liquid on silica gel using a 1:60:60 mixture



1, R^1 = MMTr; R^2 = H
2, R^1 = H; R^2 = TBDMS



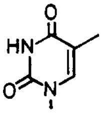
3, B = Th; R = MMTr
4, B = Cy^{bz}; R = DMTr
5, B = Ad^{bz}; R = DMTr
6, B = Gu^{ib}; R = DMTr



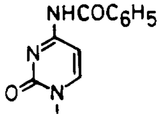
7, B = Th; R = MMTr
8, B = Cy^{bz}; R = DMTr
9, B = Ad^{bz}; R = DMTr
10, B = Gu^{ib}; R = DMTr

MMTr = p-$CH_3OC_6H_4(C_6H_5)_2C$; DMTr = $C_6H_5(p$-$CH_3OC_6H_4)_2C$; TBDMS = t-$C_4H_9(CH_3)_2Si$

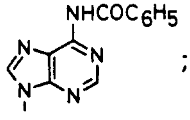
Th =



; Cy^{bz} =



; Ad^{bz} =



; Gu^{ib} =

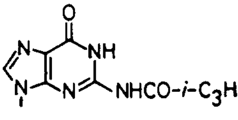


Table I. Nonaqueous Oxidation of Dinucleoside Phosphites[a]

phosphite	oxidizing agent (equiv), catalyst	conditions		phosphate, % yield[b]
		temp/℃	time	
3	TMSOOTMS (4)	20	3 h	**7**, 85[c]
3	TMSOOTMS (2), TMSOTf[d,e]	−20	5 min	**7**, 95, 87[c]
3	TMSOOTMS (2), Nafion-TMS[f]	−20	30 min	**7**, 85[c]
3	hydrogen peroxide[g] (2)	0	5 min	**7**, 94
3	*t*-butyl hydroperoxide (2)	0	5 min	**7**, 91
3	cumene hydroperoxide (2)	0	40 min	**7**, 92
3	di-*t*-butyl peroxide (4)	20	36 h	**7**, 45
3	trimethylamine *N*-oxide (4)[h]	25	24 h	**7**, 5
3	*N*-methylmorpholine *N*-oxide (4)[h]	25	7 h	**7**, 80[c]
3	pyridine *N*-oxide (4)	20	40 h	**7**, 1
3	dimethyl sulfoxide[i]	100	5 h	**7**, 17
4	TMSOOTMS (2), Nafion-TMS[f]	−20	1 h	**8**, 89[c]
4	*t*-butyl hydroperoxide (2)	0	10 min	**8**, 93[c]
5	TMSOOTMS (2), Nafion-TMS[f]	−20	1 h	**9**, 88[c]
5	*t*-butyl hydroperoxide (2)	0	10 min	**9**, 93[c]
6	TMSOOTMS (2), Nafion-TMS[f]	−20	1 h	**10**, 85[c]
6	*t*-butyl hydroperoxide (2)	0	5 min	**10**, 84[c]

[a] The reaction was carried out in dichloromethane unless otherwise stated. [b] Yield was determined by HPLC analysis unless otherwise noted. [c] Isolated yield. [d] Five mol% of TMSOTf was used. [e] Five mol% of triethylamine was added. [f] Five mol% of Nafion-TMS (0.84 mmol/g)[8] was employed. [g] Anhydrous hydrogen peroxide in ether prepared according to the literature method [I. Saito, R. Nagata, K. Yuba, and T. Matsuura, *Tetrahedron Lett.*, **24**, 1737 (1983)]. [h] The reaction was carried out in acetone. [i] Dimethyl sulfoxide was used as solvent.

of methanol, ethyl acetate, and hexane as eluent gave the phosphate **7** (207 mg, 87% yield) as a ca. 1:1 mixture of the diastereomers bearing the following spectral characteristics: IR ($CHCl_3$) 3380, 1720, 1690, 1460, 1280 cm^{-1}; UV (CH_3OH) λ_{max} 266 nm (ε 17800); ^1H NMR ($CDCl_3$) δ 0.05 (s, $Si(CH_3)_2$), 0.88 (s, $SiC(CH_3)_3$), 1.43, 1.90 (two s's, C(5)-CH_3), 2.0—2.7 (m, 2 C(2')H_2), 3.4 (br s, MMTrOC(5')H_2), 3.68 (d, J = 9 Hz, P(O)OCH_3), 3.8 (s, p-$CH_3OC_6H_4$), 3.9—4.5 (m, SiOC(3')H, 2 C(4')H, 2 P(O)OC(5')H), 5.2 (m, P(O)OC(3')H), 6.24, 6.43 (two t's, J = 6 Hz, 2 C(1')H), 6.8—6.9, 7.1—7.5 (two m's, 2 C_6H_5, p-$CH_3OC_6H_4$), 7.58 (br s, 2 C(6)H), 9.97 (br s, 2 NH); ^{31}P NMR (a 1 : 1 mixture of $CDCl_3$ and C_6H_6) 0.18 and 0.15 ppm (1 : 1) downfield from H_3PO_4.

TMSOOTMS oxidation with Nafion-TMS catalyst (procedure B): A mixture of the phosphite **8** (103 mg, 0.11 mmol), a 1.27 *M* dichloromethane solution of TMSOOTMS (0.18 mL, 0.22 mmol), and Nafion-TMS (0.84 mmol/g, 6.6 mg, 0.0056 mmol) in dichloromethane (1.7 mL) was stirred at −20℃. After 30 min, the resin was removed by filtration and washed with dichloromethane. The filtrate was evaporated under aspirator-reduced pressure to give an oil, which was subjected to a silica-gel column chromatography with the above-mentioned eluent to afford **7** (89.4 mg, 85% yield).

REFERENCES AND NOTES

1. R. L. Letsinger, J. L. Finnan, G. A. Heavner, and W. B. Lunsford, *J. Am. Chem. Soc.*, **97**, 3278 (1975).
2. M. A. Dorman, S. A. Noble, L. J. McBride, and M. H. Caruthers, *Tetrahedron*, **40**, 95 (1984), and references cited therein.
3. The following reagents have been used for the nonaqueous oxidation of nucleoside phosphites. Nitrogen oxide : G. S. Bajwa and W. G. Bentrude, *Tetrahedron Lett.*, **1978**, 421. *m*-Chloroperbenzoic acid : K. K. Ogilvie and M. J. Nemer, *ibid.*, **22**, 2631 (1981). Oxygen—azobis (isobutyronitrile) : T. M. Gaida, A. E. Sopchik, and W. G. Bentrude, *ibid.*, **22**, 4167 (1981). Iodosobenzene diacetate and tetrabutylammonium periodate : J.-L. Fourrey and J. Varenne, *ibid.*, **26**, 1217 (1985).
4. H.-J. Lee and S.-H. Moon, *Chem. Lett.*, **1984**, 1229.
5. A ca. 1 : 1 mixture of the diastereomers showing ^{31}P-NMR signals at 140.12 and 140.03 ppm downfield from H_3PO_4 standard in $CDCl_3$.
6. Prepared according to the method reported in P. G. Cookson, A. G. Davies, and N. Fazal, *J. Organometal. Chem.*, **99**, C31 (1975). This reagent is thermally rather stable which can be collected by distillation, bp ca. 40 ℃/30 mmHg.
7. The driving force of the facile oxidation is the high stability of phosphoryl function and hexamethylsiloxane [R. Noyori, S. Murata, and M. Suzuki, *Tetrahedron*, **37**, 3899 (1981)] . The acceleration by TMSOTf catalyst is understandable by assuming the following mechanism involving ion-pair intermediates. Alternatively, since silicon atom bearing an electron-withdrawing substituent (s) can accommodate the fifth ligand, TMSOTf may act a simple Lewis acid catalyst without breakage of the Si—O bond.

$$\mathrm{TMSOTf} + \mathrm{TMSOOTMS} \longrightarrow \mathrm{TMSO\overset{+}{O}(TMS)_2\ OTf^-}$$

$$\mathrm{TMSO\overset{+}{O}(TMS)_2\ OTf^-} + \mathrm{(RO)_3P} \longrightarrow \mathrm{TMSO(RO)_3P^+\ OTf^-} + \mathrm{TMSOTMS}$$

$$\mathrm{TMSO(RO)_3P^+\ OTf^-} \longrightarrow \mathrm{(RO)_3P{=}O} + \mathrm{TMSOTf}$$

8. Nafion ® is supplied from Aldrich Chemicals Co. as perfluorinated ion-exchange powder. For the preparation and synthetic application of Nafion-TMS, see S. Murata and R. Noyori, *Tetrahedron Lett.*, **21**, 767 (1980).
9. In the reaction using TMSOTf stored for a long period, addition of triethylamine is necessary for neutralization of a trace of free TfOH resulting from decomposition of TMSOTf, which brings about undesired detritylation. By contrast, when freshly prepared TMSOTf was employed, no detritylation was observed even in the absence of the amine.
10. In the reaction with these reagents, addition of TMSOTf was not helpful.

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