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# AN EFFECTIVE METHOD FOR REMOVAL OF THE INTERNUCLEOTIDIC PHENYLTHIO GROUP FROM FULLY PROTECTED OLIGONUCLEOTIDES BY USE OF BIS(TRIBUTYLTIN) OXIDE

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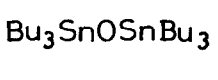
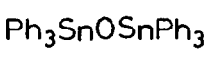
Summary: Several kinds of organotin compounds involving stannyl oximates have been examined as reagents for deblocking the internucleotidic phenylthio group from an appropriately protected S-phenyl diuridine phosphorothioate (1). Among them, bis(tributyltin) oxide was found to be very effective.

In our strategy for the chemical synthesis of DNA and RNA, internucleotidic phosphate groups have been protected with the phenylthio group. This protecting group can be removed by dilute alkali,[1-3] tetramethylguanidium 2-pyridine-syn-carboxaldoximate,[4,5] or silver acetate[6-9] in aqueous media. However, the former two methods are somewhat dangerous since a strong nucleophile, benzenethiolate ion, may be generated under such basic conditions.[10] The last method is sufficiently neutral but requires long periods of time and bothersome post-treatment. In order to improve the removal conditions, our interest was focused on the strong affinity of tin for sulfur.[11] Watanabe and Mukaiyama reported that alkoxytrialkylstannanes reacted with S,S-diphenyl alkyl phosphorodithioates to give S-phenyl dialkyl phosphorothioates.[12] On the other hand, Reese has recommended the use of tetramethylguanidium oximates for removal of internal phosphate protecting groups in oligonucleotide synthesis.[13-15] These facts led us to examine the use of organotin compounds as possible deblocking reagents for the phenylthio group.

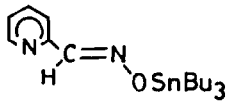
Commercially available reagents, bis(tributyltin) oxide (TBT) and bis(triphenyltin) oxide (TPT), and newly prepared reagents, tributylstannyl 2-pyridine-syn-carboxaldoximate (TBS-PAO) and tributylstannyl 2-nitrobenzaldoximate (TBS-NBAO), were chosen for this study. The latter two reagents were synthesized by



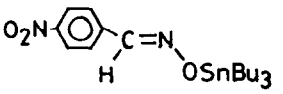

TBT




TPT




TBS-PAO




TBS-NBAO

refluxing a mixture of TBT and the corresponding oxime in toluene for 30 min followed by azeotropic removal of water with toluene, and characterized by their NMR spectra and elemental analyses.[16,17]

To test the effectiveness of these reagents, a fully protected uridylyl(3'-5')uridine (1) bearing the phenylthio group was synthesized in 80% yield by condensation of triethylammonium S-phenyl $N^4$-anisoyl-2'-O-(tetrahydropyran-2-yl)-5'-O-(4,4'-dimethoxytrityl)uridine 3'-phosphorothioate[18] (2, 0.6 mmol) with $N^4$-anisoyl-2',3'-O-(methoxymethylene)uridine[19] (3, 0.5 mmol) in the presence of mesitylenedisulfonyl chloride[2] (MDS, 1 mmol) and 3-nitro-1,2,4-triazole[20] (NT, 1.5 mmol) in pyridine (2.5 mL) for 70 min followed by silica gel column chromatography.

The reactions of 1 with the above organotin reagents were monitored by thin layer chromatography. It was found that TBS-NBAO underwent rather slow elimination of the phenylthio group from 1 in pyridine compared with the corresponding tetramethylguanidium oximate as shown in Table I. TBS-PAO was six times more effective than TBS-NBAO as in the case of the tetramethylguanidium oximates reported by Reese.[15] The reaction of 1 with TBS-PAO in pyridine proceeded 2-3 times faster than in dimethylformamide and dioxane.

Contrary to these results, TBT and TPT resulted in considerable rapid dephenylthiolation. Particularly, 15 fold excess of the former resulted in complete deprotection of the phenylthio group in 1 h. Since the tin oxide behaves as almost neutral speacies in pyridine, large excess amounts of TBT can be used without damage of other functional groups. In this point TBT was advantageous over tetramethylguanidium oximates. Much acceleration was achieved by use of 150 fold excess of TBT so that the phenylthio group could be very rapidly

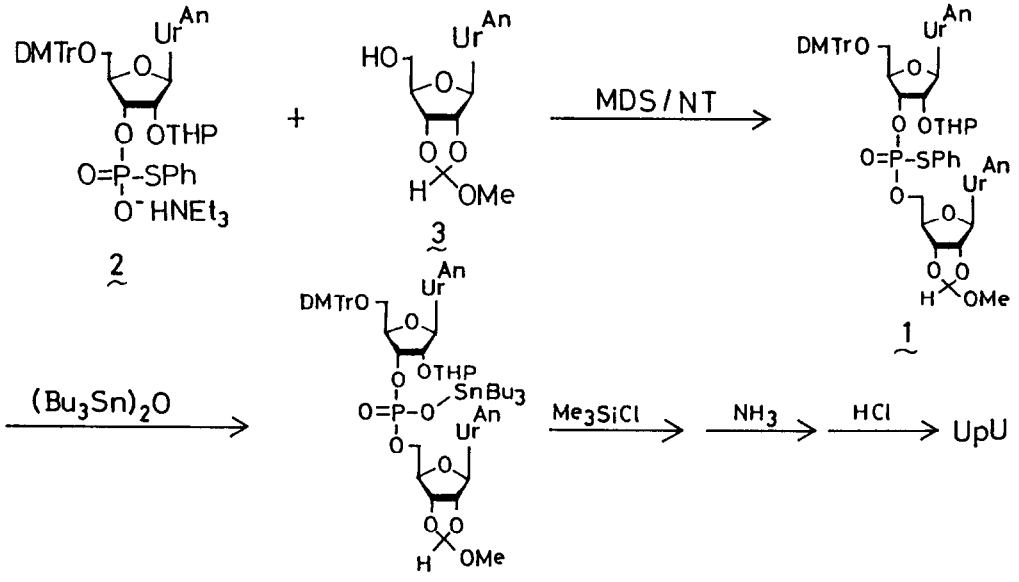


Table I. Conditions and results of removal of the internal phenylthio group from 1.

| reagent | equiv. | concentration (M) | solvent | half time | time (comp) | isolated yield of UpU (%) |
|---|---|---|---|---|---|---|
| TMG-PAO | 15 | 0.5 | Py | 15 min | 60 min | 81 |
| TMG-NBAO | 15 | 0.5 | Py | 20 min | 60 min | 93 |
| TBS-PAO | 15 | 0.5 | Py | 30 min | 5 h | 84 |
| TBS-MBAO | 15 | 0.5 | Py | 3 h | 29 h | 85 |
| TBS-PAO | 15 | 0.5 | dioxane | 2 h | 22 h | 80 |
| TBS-PAO | 15 | 0.5 | DMF | 4 h | 48 h | 90 |
| TBS-PAO +TBT (1:1) | 15 | 0.5 | Py | 10 min | 80 min | 86 |
| TBS-NBAO +TBT (1:1) | 15 | 0.5 | Py | 20 min | 90 min | 85 |
| TBT | 1.5 | 0.05 | Py | 40 min | 7 h | 79 |
| TBT | 15 | 0.5 | Py | 10 min | 60 min | 83 |
| TBT | 150 | 5 | Py | 1 min | 20 min | 81 |
| TPT | 15 | 0.5 | Py | 1 h | 6 h | 86 |
| TPT | 50 | 1 | Py | 15 min | 4 h | 91 |

TMG-PAO: tetramethyguanidium 2-pyridine-syn-carboxaldoximate; TMG-NBAO: tetramethylguanidium 4-nitrobenzaldoximate; Py: pyridine; DMF: dimethylformamide

removed at room temperature in 20 min. The excess tin reagent was easily removed by addition of trimethylsilyl chloride where upon the tin reagent was converted to tributylstannyl chloride which could be extracted with ether.[21] The mixture was treated successively with concentrated ammonia-pyridine (5:1, v/v) at 60 °C for 3 h, extracted three times with ether, and evaporated under reduced pressure. The residue was treated with 0.01 M HCl (pH 2.0) at 20 °C for 24 h, neutralized with ammonia, and finally chromatographed on Whatman 3 MM papers developed with 2-propanol-conc. ammonia-water (7:1:2, v/v/v) to give UpU in 87% isolated yield as shown in Table I. In these successive treatments, no byproducts were detected. TPT was also useful as a nonvolatile tin reagent although this reagent can not be used in large excess because of its poorer solubility in pyridine so that the dephenylthiolation required somewhat longer periods of time.

The isolated UpU was analyzed by HPLC and finally characterized by enzyme assay with nuclease $P_1$ which showed complete digestion to U and pU in a 1:1 ratio.

These results strongly suggest that the method described here can be applied to the solid phase synthesis. This work is now in progress.

References and Notes


1) M. Sekine and T. Hata, Tetrahedron Lett., 1711 (1975).
2) M. Sekine, J. Matsuzaki, and T. Hata, Tetrahedron Lett., 22, 3209 (1981).
3) S. Honda, K. Terada, Y. Satoh, M. Sekine, and T. Hata, Chem. Lett., 15 (1982).
4) S. Honda, K. Urakami. K. Koura, K. Terada, Y. Sato, K. Kohno, M. Sekine, T. Hata, Tetrahedron, 40, 153 (1984).
5) T. Kamimura, M. Tsuchiya, K. Urakami, K. Koura, M. Sekine, K. Shinozaki, K. Miura, and T. Hata, J. Am. Chem. Soc., 104, 4552 (1984).
6) A. Kume, M. Sekine, and T. Hata, Tetrahedron Lett., 23, 4365 (1982).
7) M. Sekine, J. Matsuzaki, and T. Hata, Tetrahedron Lett., 23, 5287 (1982).
8) M. Sekine and T. Hata, J. Am. Chem. Soc., 105, 2044 (1983).
9) M. Sekine and T. Hata, J. Org. Chem., 48, 3112 (1983).
10) C. B. Reese, R. C. Titmas, and L. Valente, J. Chem. Soc., Perkin I, 2451 (1981).
11) A. G. Davies and P. J. Smith, "Comprehensive Organometallic Chemistry" ed. by G. Wilkinson, F. G. A. Stone, and E. W. Abel, Vol. 2, pp 604-610 (1982), Pergamon Press.
12) Y. Watanabe and T. Mukaiyama, Chem. Lett., 389 (1979).
13) C. B. Reese, R. C. Titmas, and L. Yau, Tetrahedron Lett., 2727 (1978).
14) C. B. Reese and L. Zard, Tetrahedron Lett., 4443 (1978).
15) C. B. Reese and L. Zard, Nucleic Acids Res., 9, 4611 (1981).
16) TBS-PAO: Yield, 99.8%; Anal. Calcd for $C_{18}H_{32}N_2OSn$: C, 52.58; H, 7.85; N, 6.81. Found: C, 52.54; H, 7.94; N, 6.70. $^1H$ NMR ($CDCl_3$): $\delta$ 0.92 (t, 9, J=4.5 Hz, $CH_3$), 1.20 (m, 18, $CH_2$), 7.11 (t, 1, J=3 Hz, ArH), 7.58 (t, 1, J=4 Hz, ArH), 7.83 (t, 1, J=4 Hz, ArH), 8.32 (s, 1, CH=N), 8.55 (d, 1, J=2 Hz, ArH).
17) TBS-NBAO: Yield, 98.5%: Anal. Calcd for $C_{19}H_{32}N_2O_3Sn$: C, 50.14; H, 7.09; N, 6.15. Found: C, 50.03; H, 7.15; N, 6.19. $^1H$ NMR ($CDCl_3$): $\delta$ 0.94 (t, 9, J=3 Hz, $CH_3$), 1.20-1.86 (m, 18, $CH_2$), 7.71 (d, 2, J=2 Hz, ArH), 8.19 (d, 2, J=2 Hz, ArH), 8.21 (s, 1, CH=N).
18) T. Kamimura, T. Masegi, K. Urakami, K. Koura, M. Sekine, and T. Hata, Chem. Lett., 1051 (1983).
19) A. Kume, H. Tanimura, S. Nishiyama, M. Sekine, and T. Hata, Synthesis, in press.
20) J. F. M. de Rooij, G. Wille-Hazeleger, P. H. van Deursen, J. Serdijn, J. H. van Boom, Recl. Trav. Chim. Pays-Bas, 98, 537 (1979).
21) K. Yamaguchi, T. Kamimura, and T. Hata, J. Am. Chem. Soc., 102, 4534 (1980).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