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A Convenient Method for Removal of the t-Butyl Group from Nucleoside Bis(t-butyl) Phosphates under Non-acidic Conditions

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Abstract: A new method for selective removal of the t-butyl group from 3'-bis(t-butyl) phosphate esters of 5'-O-(4,4'-dimethoxytrityl)deoxyribonucleosides was developed by use of Me_3SiCl and Et_3N. Several unexpected properties of the t-butyl group were described.

Quite recently, Johns[1,2] has reported di-t-butyl N,N-diethylphosphoramidite (**BEPA**) as a new phosphitylating agent for phosphorylation of alcohols. This reaction gave cleanly bis(t-butyl) alkyl phosphates in high yields. However, its removal for conversion to unesterified monoalkyl phosphates required rather drastic acidic conditions such as 1 M HCl/dioxane, r.t., 4 h[1] and 6-90 % trifluoroacetic acid/benzene or CH_2Cl_2, r.t., 24 h.[2-4] This is only a drawback when the t-butyl group is considered to be used as an phosphate protecting group in the synthesis of acid-sensitive natural products containing phosphates. Sterically hindered alkyl groups like the t-butyl group would be available as a new type of phosphate protecting groups for the synthesis of branched RNAs or inositol polyphosphates, namely, for introduction of a protected phosphate group to 1,2-diol derivatives.[5,6]

In this paper, we wish to describe a new effective method for removal of the t-butyl group of bis(t-butyl) esters of nucleotides under non-acidic conditions.

We expected that the oxygen of the O=P double bond in bis(t-butyl) esters of monoalkyl phosphates (**1**) should be more nucleophilic than that of the corresponding primary or secondary alkyl esters (**2**) because of the inductive effect of the t-butyl group. Based upon this expectation and the inherent property of the t-butyl cation feasibly generated, we studied t-butyl/trimethylsilyl exchange reactions according to a modification of Rabinowitz-Makenna's method.[7,8]

To see if highly selective removal of the t-butyl group from alkyl bis(t-butyl) phosphates could be achieved, we have chosen appropriately N-protected 5'-O-(4,4'-dimethoxytrityl)-3'-O-[bis(t-butoxy)phosphinoyl]-

deoxyribonucleosides (**2a-d**) as substrates, which had an acid sensitive 4,4'-dimethoxytrityl and a base labile N-acyl group. Compounds **2a-d** were synthesized by reaction of the corresponding 3'-unprotected deoxyribonucleoside derivatives (**1a-d**) with **BEPA** followed by oxidation with mCPBA. In these reactions, dichloromethane was superior to acetonitrile as the solvent. In the former solvent, all the two-step phosphorylations proceeded cleanly to give essentially single spots on TLC after the mCPBA oxidation. It was observed that the bis(t-butyl) esters **2a-d** were considerably decomposed during silica gel column chromatography. However, this silica gel catalyzed de-t-butylation was avoided by addition of 0.5% pyridine to the eluant (CH_2Cl_2-MeOH) during the chromatographic separation. Thus, compounds **2a-d** were obtained as pure materials in high yields as shown in Table I.

Next, de-t-butylation of **2a** was studied under various conditions. As a result, we found that **2a** underwent facile and selective de-t-butylation with 7.5 equiv (relative to one t-Bu group) of trimethylsilyl chloride in the presence of 10 equiv of triethylamine in acetonitrile at 75 °C for 2 h to give quantitatively 5'-O-(4,4'-dimethoxytrityl)thymidine 3'-phosphate (**3a**).

Table 1. Introduction of Bis(t-buthoxy)phosphinyl Group into the 3'-Hydroxyl Group of N-Protected 5'-O-(4,4'-Dimethoxytrityl)deoxyribonucleosides (**1**)

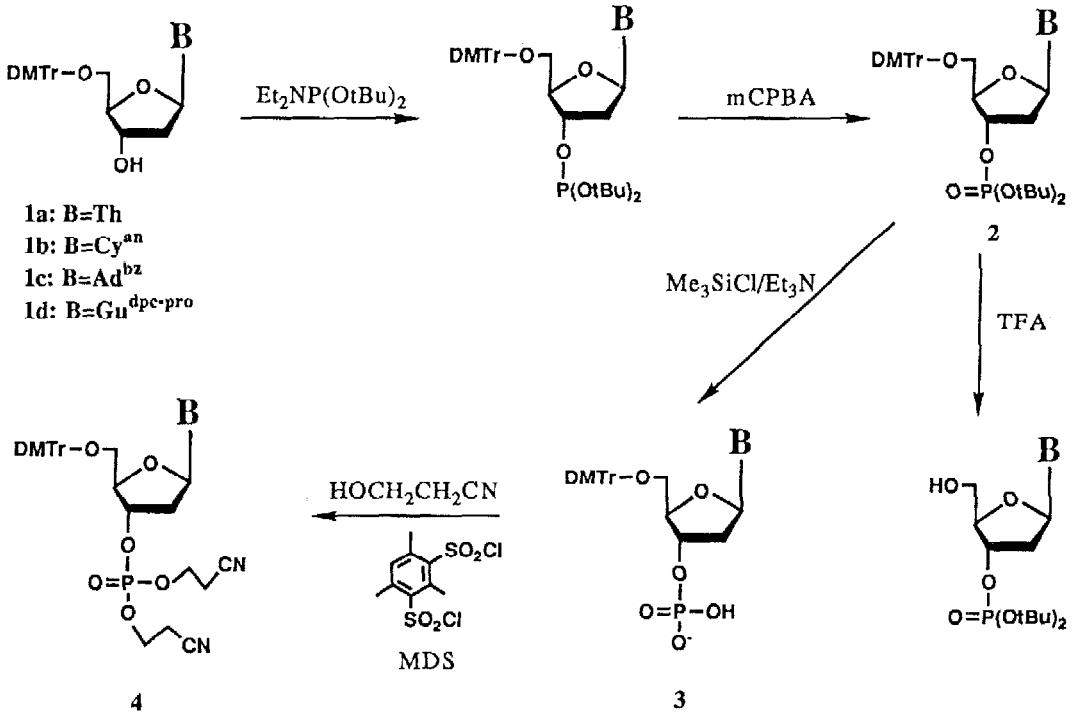
B	BEPA equiv	tetrazole equiv	time min	mCPBA equiv	time min	yield of **2** %	^{31}P NMR[a] ppm (δ)
Th	1.2	1.8	30	1.44	25	86	-10.41
Cy^{an}	1.2	1.8	45	1.44	20	87	-10.47
Ad^{bz}	1.2	1.8	30	1.44	20	86	-10.66
$Gu^{dpc\cdot pro}$	1.5	1.8	60	1.80	30	93	-10.66

[a]$CDCl_3$-pyridine (20:1, v/v) was used as the solvent.

Table 2. Selective De-t-butylation of the Bis(t-butyl) Esters (**2**) and Esterification of the 3'-Phosphates (**3**) with 2-Cyanoethanol

B	Me_3SiCl equiv[b]	Et_3N equiv[b]	temp °C	time h	$HO(CH_2)_2CN$ equiv	MDS equiv	time h	yield of **4** %	^{31}P NMR[a] ppm (δ)
Th	7.5	10	75	2	20	10	2	81	-3.29
Cy^{an}	15	20	75	1	20	10	2	44	-3.39
Ad^{bz}	10	12.5	75	1	20	10	2	63	-3.21
$Gu^{dpc\cdot pro}$	7.5	10	75	1	20	10	2	59	-3.81

[a]$CDCl_3$-pyridine (20:1, v/v) was used as the solvent.
[b]Equiv relative to one t-Butyl group.



The other 3'-bis(t-butyl) esters **2b-d** were also de-t-butylated under similar or modified conditions as summarized in Table 2. It should be emphasized that the de-t-butylation of **2a-d** proceeded quantitatively without the loss of the DMTr and N-acyl groups and the cleavage of the bond between the 3'-carbon and the phosphate oxygen.

The thymidylic acid derivative **3a** selectively deprotected could be isolated by paper chromatography (2-propanol:conc NH_3:H_2O, 7:1,2, v/v/v) in ca. 80 % yield as its aqueous pyridine solution. We observed that the 3'-phosphate derivative **3a** was decomposed when dried for isolation because of intramolecular phosphate group catalyzed detritylation. Therefore **3a-d** were in situ converted to their bis(2-cyanoethyl) esters (**4a-d**) by treatment with an excess amount of cyanoethanol (10 equiv) in the presence of mesitylenedisulfonyl dichloride (**MDS**)[10] to confirm complete removal of the t-butyl group. The results of the esterification are shown in Table 2. Unexpectedly, in the case of the compound **3c**, side reactions occurred to a unnegligible extent. Nonetheless, all the bis(cyanoethyl) esters obtainted were characterized by ^{31}P NMR which showed uniformly single peaks at ca. -3 ppm.

Compared with the instability of the bis(t-butyl) esters **2a-d** on silica

gel, it was interestingly found that compound **2a** remained intact upon treatment under mildly acidic conditions. Thus, **2a** underwent selective detritylation with 1% trifluoroacetic acid in CH_2Cl_2 at r. t. for 5 min to give the 5'-hydroxyl product (**5**) in 91% yield.

Interestingly, it was found that **2a** was thermerlly stable when heated in pyridine at a rather high temperature of 105 °C for 2.5 h. It was also confirmed that **2a** was completely stable toward concentrated ammonia-pyridine (1:1, v/v) at r.t. for 24 h and MDS (10 equiv) in pyridine for 2 h.

In conclusion, the present method would give a trigger for the extended use of the t-butyl group as a readily accessible phosphate protecting group in the realm of nucleotide chemistry as well as inositol polyphosphate chemistry since mild conditions for its removal were disclosed throughout this study.

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