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USE OF THE TERT-BUTYLDIMETHYLSILYL GROUP FOR PROTECTING THE HYDROXYL FUNCTIONS OF NUCLEOSIDES

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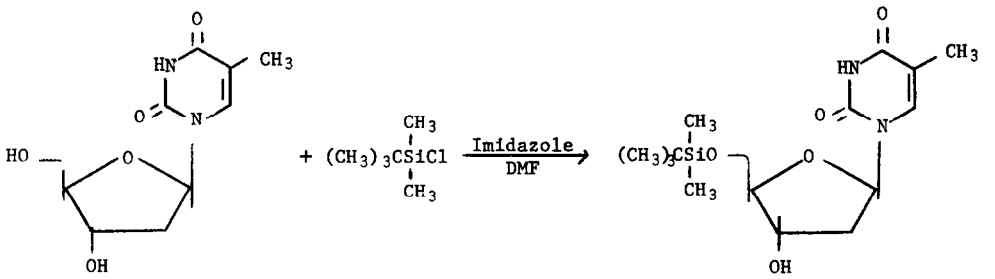
During our investigations into the synthesis and identification of nucleosides and nucleotides we have been seeking versatile hydroxyl-blocking groups for use in the synthesis of oligonucleotides (1) and which may also be useful for characterizing isomeric substituted nucleosides (2) and even the sequences of nucleotides We wish to report some initial observations of the tert-butyldimethylsilyl (TBDMS) group which, being compatible with acyl and triphenylmethyl groups in general and the benzoylpropionyl group in particular, adds great versatility to the available protecting groups for oligonucleotide synthesis. Further the TBDMS group lends itself well to the characterization of isomeric substituted nucleosides by mass spectrometry, a feature we hope to extend to the sequencing of oligonucleotides

The TBDMS ethers of nucleosides are easily prepared and are very stable compounds with sharp melting points. Further the tert-butyldimethylchlorosilane (TBDMSCl) reagent (3) is selective for the 5'-position. For example thymidine (1 mmole) was reacted with TBDMSCl (1.1 mmole) in DMF (1 ml)



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containing imidazole (2.5 mmole) at 37°C for 20 hr. and the products were separated by silica gel thick layer chromatography in ether Four compounds* were apparent with the fastest moving compound being the 3',5'-di-(tert-butyldimethylsilyl) thymidine, m.p. 105-106°C, $R_f^{Et_2O}_{TLC}$ 0.60 (16% yield). The

*Satisfactory elemental analyses were obtained for all new compounds reported.

3'-tert-butyldimethysilylthymidine (3'-TBDMST,II) was obtained in only 3% yield, m p 83-84°C, $Rf_{TLC}^{Et_2O}$ 0.40 (see below) while the 5'-isomer, I, $Rf_{TLC}^{Et_2O}$ 0.24, m p. 193-194°C, λ_{max}^{EtOH} 267 nm, λ_{min}^{EtOH} 235 nm was obtained in 60% yield. The NMR-spectrum of I showed singlets at δ(ppm. $CDCl_3$) 0.14 ($(CH_3)_2Si$), 0.92 ($(CH_3)_3CSi$) and 1.91 (5-CH_3). The mass spectrum† of I showed diagnostically important fragmentation at m/e 299 (100 relative intensity), 281 (400), 263 (135), 231 (40), 213 (170) and 201 (46) The fourth compound on the TLC plate which had remained at the origin in the ether development was eluted with ethanol and identified as thymidine (11%)

For comparison purposes 3'-TBDMST was synthesized by the following route 5'-pivaloylthymidine was treated with TBDMSCl as above and the intermediate product was hydrolyzed directly with 0 5N NaOH (EtOH:H_2O, 1·1) at room temperature for 12 hr. The solution was neutralized with Dowex 50W-X8 (pyridinium form) and the product isolated by TLC in ether. The 3'-TBDMST so obtained (58%) had $Rf_{TLC}^{Et_2O}$ 0.40, m.p. 83-84°C, λ_{max}^{EtOH} 267 nm, λ_{min}^{EtOH} 235 nm. The NMR spectrum showed singlets at δ(ppm, $CDCl_3$) 0.094 ($(CH_3)_2Si$), 0.89 ($(CH_3)_3CSi$) and 1.87 (5-CH_3). Diagnostically important fragments in the mass spectrum† occurred at 325 (2 7 relative intensity), 299 (100), 281 (9 3), 263 (3 3), 231 (500), 213 (63) and 201 (650).

The TBDMS group is stable to the normal conditions of phosphorylation. For example 5'-TBDMST (I) was phosphorylated with β-cyanoethylphosphate using triisopropylbenzenesulfonyl chloride (4) in one case and dicyclohexylcarbodiimide (5) in another as condensing agents. On work-up the cyanoethyl group was removed with 9M NH_4OH at 60°C for 1 hr and removal of the TBDMS group was completed using 80% HOAc on a steam bath for 15 min. Yields of Tp from these two reactions were 98.2% and 100% respectively

The stability of the TBDMS group was tested under a variety of conditions as shown in the following table

Hydrolysis of the TBDMS group from I

Reagent Used	Temperature	Time of Reaction	% Hydrolysis
0.5N NaOH (EtOH H_2O, 1 1)	22°C	24 hr.	80
9M NH_4OH	60°C	1 hr	6
15% NH_4OH in EtOH	22°C	1-1/2 hr.	0
H_2NNH_2:HOAc pyridine (1 2 8)	22°C	24 hr.	0
80% HOAc	steam bath	15 min.	100
$(nBu)_4NF$ in THF	22°C	30 min.	100

The advantages of this silyl group include its stability under basic conditions which remove most commonly used acyl groups, yet it can be removed using strong base or mild acid (eg. 80% HOAc). The TBDMS group is unaffected by hydrazine hydrate which selectively removes β-benzoylpropionyl groups (6) and N-acyl groups (7). However, the $^{n}Bu)_4NF$ reagent (8) which rapidly hydrolyses the TBDMS group has no affect on acyl or trityl groups. Thus the tert-butyldimethylsilyl group adds real versatility to oligonucleotide synthesis when used in combination with other protecting groups now available

The large differences in fragmentation pattern intensities in the mass spectra of the 5'- and 3'-isomers (I and II) make this a suitable means of identifying the isomers (2). The mechanisms of the fragmentations of silylated nucleosides in general and the use of the TBDMS group in sequencing oligonucleotides will be reported shortly.

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†Mass spectra were recorded on a Finnigan 1015 mass spectrometer

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