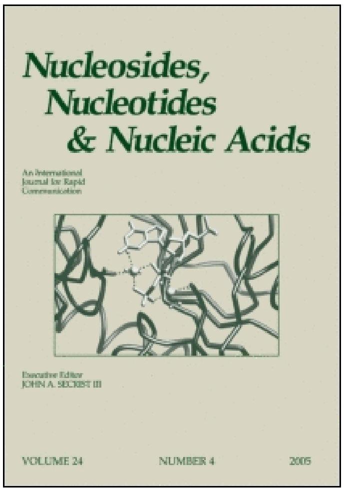

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New Trityl-Based Protecting Groups with a Mild Two-Step Removal

Erwin Happ[a]; Claudia Scalfi Happ[a]
[a] Max-Planck-Institut für Biochemie, Martinsried, FRG

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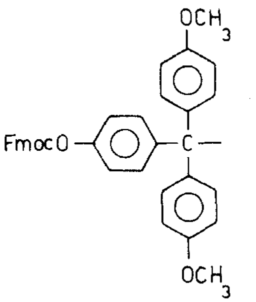
NEW TRITYL-BASED PROTECTING GROUPS WITH A MILD TWO-STEP REMOVAL

Erwin Happ* and Claudia Scalfi Happ
Max-Planck-Institut für Biochemie, D-8033 Martinsried (FRG)

Abstract Two specifically designed protecting groups 4-(9-fluorenylmethoxycarbonyl)oxy-4',4"-dimethoxytrityl (**1**) and 4-(9-fluorenylmethoxycarbonyl)amino-4',4"-dimethoxytrityl (**2**) have been investigated for possible use in oligonucleotide synthesis.

Proper protection of ribose hydroxy functions is crucial for the successful chemical synthesis of RNA. Application of the recently reported combination of two acid-labile blocking groups - tetrahydropyranyl (Thp) for 2'-OH and dimethoxytrityl (DMTr) for 5'-OH - is limited due to a concomitant loss of tetrahydropyranyls during release of the dimethoxytrityl cation under the acidic conditions employed[1]. To overcome this problem Sekine and Hata[2] introduced 4,4',4"-tris(4,5-dichlorophthalimido)trityl (CPTr) as 5'-protecting group, which is removed in a two-step fashion. After treatment with hydrazine the resulting pararosaniline ether is cleaved by a weakly acidic pyridine-acetic acid mixture. Although we have successfully employed this blocking group in the chemical synthesis of the RNA hexamer 5'r(GCAUGC)$_2$[3], in this paper we report on novel trityl-based protecting groups, where the hydrazine treatment is replaced by a ß-elimination step.



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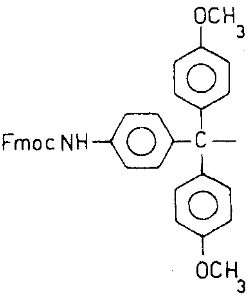

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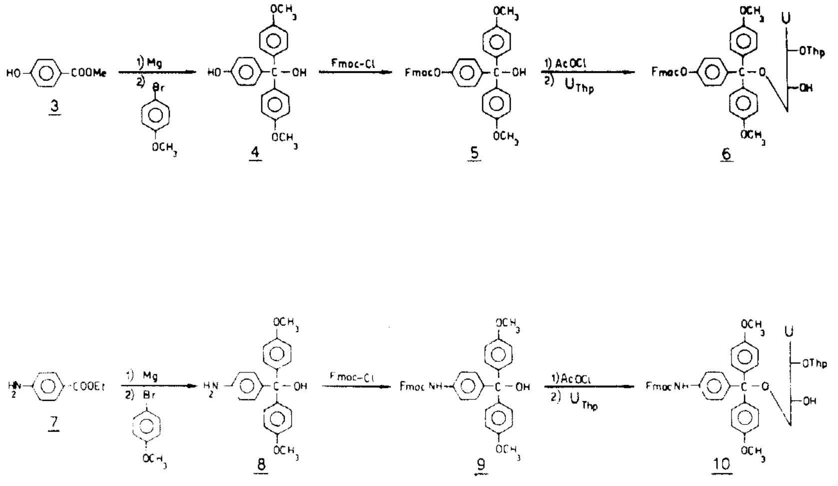

SCHEME 1

In their classical investigation on the use of several substituted trityl groups in oligonucleotide synthesis Taunton-Rigby et al.[4] reported that in 80% acetic acid 5'-O-(4-acetyloxytrityl)adenosine is cleaved in one week, whereas it takes only one hour to fully detritylate the corresponding hydroxytrityl-protected adenosine. This dramatic effect on acid-stability, due to an acyloxy->hydroxy conversion, led us to design a new concept based on the well-known dimethoxytrityl group. Introduction of a hydroxy or amino group into the 4-position of the unsubstituted phenyl ring should facilitate the acid-promoted cleavage of such new trityl groups. Protection of the hydroxy or amino function by 9-fluorenylmethoxycarbonyl (Fmoc)[5] should on the other hand enhance their acid-stability. Furthermore, the Fmoc group is easily removed by ß-elimination. Synthetic routes leading to 5'-protected nucleosides are shown in Scheme 1.

Grignard-reaction of methyl 4-hydroxybenzoate or ethyl 4-aminobenzoate with 4-bromoanisole gave the substituted tritanols (**4**) and (**8**). Protection of the phenolic hydroxy group of (**3**) and the amino function of (**7**) was not necessary, although a larger excess of Grignard-reagent had to be used.

TABLE 1. Acid-Stabilities of 5'-protected 2'-O-Thp-uridines.

5'-protecting group	pyridine/AcOH (1:3)		pyridine/HCOOH (2:3)		pyridine/HCOOH (4:7)	
	$t_{0.5}$[a] (min)	$t_{0.99}$[b] (min)	$t_{0.5}$[a] (min)	$t_{0.99}$[b] (min)	$t_{0.5}$[a] (min)	$t_{0.99}$[b] (min)
DMTr	stable		very slow deblocking			
(**1**)[c]	14	93	1.9	13	0.9	6
(**2**)[c]	25	166	2.5	17	1.7	11

[a] Pseudo first order kinetics were observed for all reactions ($t_{0.5}$=half-time); straight lines were obtained when logarithms of the percentage of substrates remaining were plotted against time.

[b] $t_{0.99}$ represents the calculated time for 99% removal of the 5'-protecting group.

[c] Substrates (**6**) and (**10**) were first treated at room temperature for 10 minutes with 0.5M DBU in CH_2Cl_2.

Fmoc-derivatives (**5**) and (**9**) were obtained by treating (**4**) or (**8**) with 9-fluorenylmethyl chloroformate in anhydrous pyridine. Conversion to the corresponding substituted trityl chlorides was achieved with acetyl chloride in dry cyclohexane. After removal of excess acetyl chloride they were found to react selectively at ambient temperature in anhydrous pyridine with substrates bearing primary hydroxy functions.

Reaction of 2'-O-Thp-uridine resulted in the two model compounds (**6**) and (**10**), which were used in deblocking studies. It should be noted that the newly synthesized 5'-protected uridine derivatives (**6**) and (**10**) are sufficiently stable, allowing their purification by conventional silica gel column chromatography[6]. Brief treatment with 0.5M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 completely removed the Fmoc group; this is in agreement with an estimated half-time of ca. 6s under similar conditions[7]. Loss of the protecting Fmoc group renders as expected the resulting 4-hydroxy or 4-amino-substituted trityl-nucleoside ethers highly acid-sensitive and allows an extremely mild deblocking in a reasonably short time .

In a kinetic study (shown in Table 1) their acid-stabilities were directly compared with the parent unsubtituted nucleoside

5'-O-DMTr-2'-O-Thp-uridine. The preliminary kinetic data indicate that the newly introduced protecting groups are compatible with the 2'-protecting tetrahydropyranyl group for a successful chemical synthesis of RNA. Their application in the preparation of synthetic long-chain DNA molecules could also avoid undesirable depurination.

The favorable properties of (**1**) and (**2**) therefore suggest their use in synthetic procedures for oligonucleotides and other related natural products.

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