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THE TRIPHENYLMETHYL (TRITYL) GROUP AND ITS USES IN NUCLEOTIDE CHEMISTRY

V. Kohli , H.Blöcker and H. Köster

Institute of Organic Chemistry and Biochemistry
University of Hamburg, Martin-Luther-King-Platz 6,
D-2000 Hamburg 13, FRG

Summary: The triphenylmethyl (trityl) group can be removed from 5'-trityl 2'-deoxynucleosides (and their N-acyl derivatives) under aprotic neutral conditions without causing any side reactions. An efficient method for tritylation of N-acyl 2'-deoxynucleosides is described. Potential use of such derivatives for stepwise synthesis of deoxyoligonucleotides is discussed.

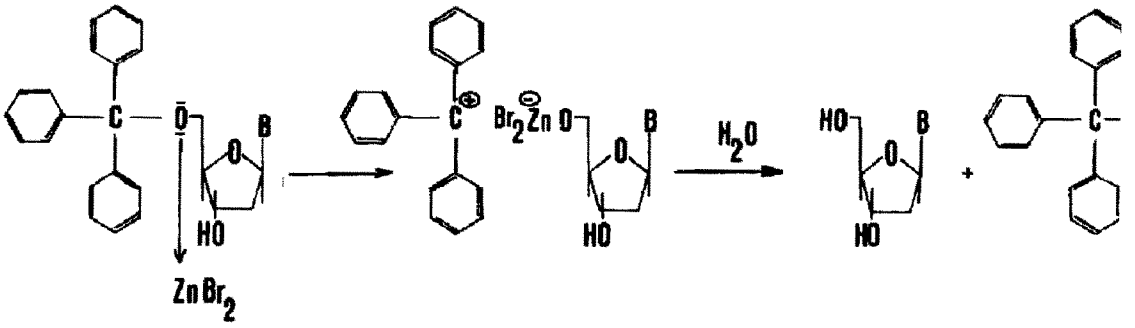
The triphenylmethyl (trityl) group has been extensively employed to protect the hydroxy functions in nucleoside chemistry. Tritylation has been described by treating the substrate alcohol with chlorotriphenylmethane in pyridine solution which shows a high degree of selectivity for the primary hydroxy groups of the polyols[1]. However, the acid hydrolytic conditions (80% aqueous acetic acid for 48 hrs) required to remove the trityl group restricted its use as a suitable protecting group for acid sensitive alcohols such as 2'-deoxynucleosides[1]. To overcome this problem Khorana and his coworkers introduced p-anisyldiphenylmethyl (monomethoxytrityl) and di-p-anisylphenylmethyl (dimethoxytrityl) groups for the protection of 5'-hydroxy functions of 2'-deoxynucleosides and their N-acyl derivatives. These protecting groups which can be removed with relatively mild conditions using 80% acetic acid within 2 hrs and 15 min respectively have found much use in deoxyoligonucleotide synthesis[2,3]. Recently, Narang and his coworkers[3] reported that the use of 80% acetic acid for removal of dimethoxytrityl group from 5-dimethoxytrityl N-benzoyl deoxyadenosine is accompanied by cleavage of the glycosidic bond upto 36% within 30 minutes. These workers also discovered that 2% benzene sulfonic acid in chloroform-methanol (7:3, v/v) at 0°C for 3 minutes removes dimethoxytrityl group from the above protected nucleoside causing depurination only to an extent of 4%.

During the course of our studies on the synthesis of deoxyoligonucleotides by phosphotriester methods we observed that the dimethoxytrityl ether bonds can be cleaved under aprotic neutral conditions under the influence of a mild Lewis acid such as Zinc bromide in dichloromethane within 5 minutes at room temperature. Indeed, scission of monomethoxytrityl

as well as the most stable trityl ether bonds can be achieved without causing any damage to the glycosidic bond from all the 5'-trityl N-acyl 2'-deoxynucleosides in dichloromethane (ca. 2 ml/mmole of the ether) with 5-10 equivalents of finely powdered anhydrous Zinc bromide[4] while stirring within 5 minutes at 20°.

Under these mild conditions the commonly used N-acyl protecting groups namely isobutyryl, benzoyl, anisoyl to protect the amino functions of the heterocyclic bases were unaffected. Other protecting groups like the trichloroethyl and o-chlorophenyl on phosphate functions of 5'-dimethoxytrityl N-acyl 2'-deoxynucleosides 3'-phosphotriesters[5] were also stable under these mild conditions for selective removal of the trityl groups.

The mechanism of detritylation could probably follow the following course[6], with the Lewis acid $ZnBr_2$ acting as electron acceptor from the trityl ethereal oxygen whereas the inherent stability of the trityl carbenium ion as observed by concommittant colouration of the reaction mixture acts as a driving force for the forward detritylation pathway:



B = Thymine
N-benzoyladenine
N-isobutyrylguanine
N-anisoylcytosine

Other Lewis acids like $AlCl_3$, $ZnCl_2$, and $SnCl_4$ also gave similar results. Very recently Engels reported the use of a rather aggressive Le acid (BF_3/methanol) for detritylations[7].

In the recent years the rather labile dimethoxytrityl group has been used for the 5'-protection of 2'-deoxynucleosides and their N-acyl-derivatives for stepwise synthesis of deoxyoligonucleotides using phosph triester methods[8]. It has been reported that dimethoxytrityl group gets

partially removed during handelling of the condensation products[8,9], presumably owing to its high lability to acidic conditions. H.Lund estimated the ease of ionization of p-methoxy substituted triphenyl carbinols increases in the order: triphenyl carbinol, 1.0, p-anisyldiphenyl carbinol, 6.3, di-p-anisylphenyl carbinol, 34.0[10].

Having found that trityl group which is rather stable to mild acidic conditions can be quantitatively removed with $ZnBr_2$ without any side reactions, we focussed our attention to find a suitable procedure for tritylation of deoxynucleosides and their N-acyl derivatives. The classical method for the preparation of trityl ethers involves reaction of the alcohol substrates with chlorotriphenylmethane in the presence of pyridine as solvent at temperatures ranging from room temperature upto 100°. A recent publication describes the application of N-tritylpyridinium fluoroborate in DMF as a potent tritylating agent[11]. Tritylations have also been achieved with a simpler procedure using 4-N,N-dimethylaminopyridine and triethylamine for reactions between trityl chloride and alcohol in N,N-dimethylformamide solution at room temperature[12].

In our laboratory we have developed a convenient method for tritylation which involves traping the hydrogen chloride formed during reaction of chlorotriphenylmethane with N-acyl 2'-deoxynucleosides in neutral solvents like dichloromethane at room temperature. This was done using powdered molecular sieves of 4 Å pore size as neutral acid scavengers[5], thus avoiding the use of any base as an HCl captor. Usually for 1 mmole of N-acyl 2'-deoxynucleosides, 1.2 mmole of trityl chloride and 4 g of powdered molecular sieves were used in 100 ml of dichloromethane. The slurry was stirred at room temperature for 8-10 hrs. However, some 5-10% of ditritylated products along with the required product were isolated under these conditions. To avoid ditritylation about 5 ml of anhydrous pyridine were added in each case. This remarkably increased the specificity of tritylations towards the 5'-hydroxy function of N-acyl 2'-deoxynucleoside. Overall yields of 70-90% for 5'-trityl N-acyl 2'-deoxynucleosides were obtained after purification by short column chromatography on silica gel. These compounds which were isolated as crystalline or precipitated solids and characterized by ^{1}H-nmr gave satisfactory elemental analyses.

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References and footnotes:

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4. A very concentrated solution of anhydrous $ZnBr_2$ in dry methanol can instead be used to avoid powdering of the extremely hygroscopic salt. The $ZnBr_2$-methanol solution alone also gave quantitative cleavage of the stable trityl group without any side reactions. After the detritylation is over the product can be isolated quantitatively by extraction into ethyl acetate and washing with 1 M phosphate buffer pH 7.5, followed by with water.
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