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THE USE OF ZINC BROMIDE FOR REMOVAL OF DIMETHOXYTRITYL ETHERS FROM DEOXYNUCLEOSIDES

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Zinc Bromide has been observed to detritylate specifically 5'-trityldeoxydeoxynucleosides whereas 3'-trityl ethers are not cleaved. Of additional practical consideration and in contrast to protic acids, no depurination was observed with this reagent.

Corey reports the use of β-methoxyethoxymethyl ether (MEM) as a hydroxyl protecting group (1). Deprotection is achieved by exploiting the bidentate chelating ability of the MEM group with a suitable Lewis acid such as $ZnBr_2$ or $TiCl_4$. 5'-trityl nucleosides have a similar potential bidentate chelation site (Figure 1) and therefore detritylation should occur rapidly using these Lewis acids. This mechanism also predicts that 3'-trityl ethers should be relatively

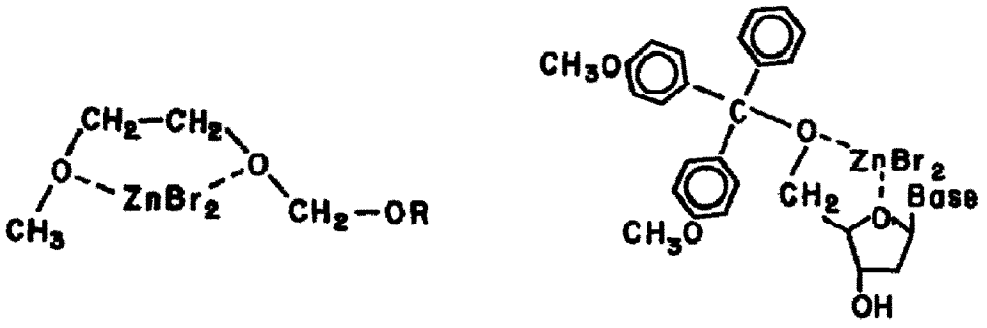

Figure 1. Possible chelation mechanism for $ZnBr_2$ with MEM ethers and tritydeoxynucleosides.

stable toward $ZnBr_2$ or $TiCl_4$ and that the rate of depurination (glycosidic bond cleavage) should be significantly different. Results outlined in this communication show that $ZnBr_2$ is superior to protic acids for removal of the trityl ether since detritylation is extremely fast and proceeds without concomitant depurination. Additionally the specific removal of 5'-trityl ethers with $ZnBr_2$ provides a simple pathway to the synthesis of 3'-trityldeosynucleosides.

Previous work with MEM ethers has involved a heterogeneous system consisting of $ZnBr_2$ powder and methylenechloride. When 5'-dimethoxytritylthymidine was added to a suspension of $ZnBr_2$ in methylenechloride and the reaction was quenched after 30 minutes with 1 M ammonium acetate, quantitative detritylation of the nucleoside had occurred. This heterogeneous system is not applicable for detritylation of synthetic oligonucleotides attached covalently to an insoluble polymer support (2). The reactivity of $ZnBr_2$ in various homogeneous

solutions was therefore examined and the results are reported in Table 1. Tnese results suggest that nitromethane is superior among the solvents tested

Table 1. The Rate of Detritylation of 5'-dimethoxytritylthymidine in a $ZnBr_2$ Saturated Solution at Room Temperature.

Solvent	% Detritylation	Time
CH_2Cl_2	trace	24 hrs
THF	trace	24 hrs
Acetone	10%	24 hrs
Dioxane	100%	2 hrs
CH_3NO_2	100%	1 min

since detritylation was complete within one minute. A related important question concerns the extent of depurination during the time required for complete detritylation. As can be seen from the results reported in Table 2, $ZnBr_2$ is a far more selective reagent than the protic acid in removal of the trityl group without depurination. Depurination has been a problem in the multistep synthesis of purine containing deoxyoligonucleotides involving several detritylation

Table 2. The Rate of Detritylation and Depurination of 5'-Dimethoxytrityl-N-benzoyldeoxyadenosine Using Various Solutions.*

Solution	Temp.	Detritylation Time	Detritylation %	Depurination Time	Depurination %
satd. $ZnBr_2/CH_3NO_2$**	18°C	<1 min	100	10 hrs	50
satd. $ZnBr_2/CH_3NO_2$	0°C	10 min	100	21 hrs	<5
2% toluenesulfonic acid/$CHCl_3$:CH_3OH (7:3)	18°C	<1 min	100	5 min	50
0.5% toluenesulfonic acid/$CHCl_3$:CH_3OH (7:3)	0°C	10 min	100	8 hrs	50

*All depurination results summarized in this communication were obtained by analyzing reaction mixtures using reverse phase high pressure liquid chromotography. Estimates of detritylation times were by thin layer chromotography.
**A nitromethane solution saturated with $ZnBr_2$ is approximately 0.1 M in $ZnBr_2$.

steps. We have also observed that added selectivity can be achieved if the nitromethane is carefully distilled from CaH_2 and treated with molecular sieves (distilled nitromethane was used in the experiments reported in this communication). Presumably trace amounts of water in reagent grade nitromethane react with $ZnBr_2$ to form protic acids which enhance the depurination rate. The remaining base protected deoxynucleosides were also checked for stability with $ZnBr_2$. The results as reported in Table 3 show that all are more stable toward degradation with $ZnBr_2$ than was N-benzoyldeoxyadenosine and that all bases rapidly undergo detritylation without forming degradation products.

The results reported in Table 4 are of synthestic and mechanistic interest. The general utility of $ZnBr_2$ as a detritylating reagent was tested by measuring the rate of ether cleavage for 5'-tritylthymidine, 5'-monomethoxytritylthymidine, and 5'-dimethoxytritylthymidine. Both 5'-monomethoxytritylthymidine

Table 3. The Rate of Detritylation and Degradation of 5'-Trityldeoxynucleosides Using $ZnBr_2$ at Room Temperature.

Deoxynucleoside	Detritylation Time	%	Degradation Time	%
5'-Dimethoxytrityl-N-isobutyrldeoxyguanosine	<1 min	100	50 hrs	50
5'-Dimethoxytrityl-N-benzoyldeoxycytosine	<1 min	100	24 hrs	0
5'-Dimethoxytritylthymidine	<1 min	100	24 hrs	0

and 5'-dimethoxytritylthymidine appear to be detritylated at the same rate. In contrast the 5'-dimethoxytrityl ether is cleaved approximately ten times more rapidly than the 5'-monomethoxytrityl ether using protic acids (3). Additionally 3'-monomethoxytritylthymidine is detritylated at a considerably slower rate than the 5'-isomer. These results therefore strongly suggest that detritylation

Table 4. The Rate of Detritylation of Various Tritylthymidines Using Saturated $ZnBr_2$ in Nitromethane at 0°C.

Nucleoside	Time	% Detritylation
5'-Dimethoxytritylthymidine	1 min	50
5'-Monomethoxytritylthymidine	1 min	50
5'-Tritylthymidine	10 min	50
3'-Monomethoxytritylthymidine	30 min	<10

proceeds via a bidentate chelation mechanism involving the 5'- and deoxyribose ring oxygens and $ZnBr_2$ (Figure 1). For 5'-dimethoxytritylthymidine and 5'-monomethoxythymidine, the formation of this complex appears to be the rate determining step. With 5'-tritylthymidine the rate determining step is presumably cleavage of the ether.

The large 5'-detritylation selectivity can be exploited as a convenient method for synthesizing 3'-tritylated deoxynucleosides. Initially the 3', 5'-dityrityldeoxynucleoside can be synthesized using an excess of the appropriate trityl chloride. The 3'-trityldeoxynucleoside can then be generated from this intermediate using $ZnBr_2$ in nitromethane. In order to test this possibility, 3'-dimethoxytritylthymidine was prepared. Deoxythymidine (0.10 g, 0.4 mM) and dimethoxytritylchloride (0.415 g, 1.23 mM) were allowed to react in anhydrous pyridine (6 ml) for two days at 18°C. The reaction was quenched by addition of methanol (1 ml) followed by removal of solvent *in vacuo*. The resulting crude reaction mixture as a gum was dissolved in CH_2Cl_2 (20 ml), washed with 1 M $NaHCO_3$ (20 ml) and water, reconcentrated to a gum, and reconcentrated twice more to a gum with 20 ml portions of toluene in order to remove trace amounts of pyridine. The reaction mixture was dissolved in CH_3NO_2 (10 ml), cooled to 0°C and added to a stirred slurry of $ZnBr_2$ (0.55 g, 2 mmole) in CH_3NO_2 (10 ml) at 0°C. After 10 minutes the reaction was quenched with 1 M ammonium acetate (20 ml). Methylenechloride (20 ml) was added and the organic phase was washed with water and saturated sodium chloride, dried over sodium sulfate, and

evaporated to a gum *in vacuo*. 3'-dimethoxytritylthymidine was isolated in 70% yield by preparative thin layer chromatography of the crude reaction mixture. The mobility of the isolated product was identical in two solvent systems (ethyl acetate and 10% methanol in $CHCl_3$) to 3'-dimethoxytritylthymidine that had been prepared by a published procedure (4). Additionally the 5'-hydroxyl was shown to be available for chemical reactions since acylation with acetic anhydride produced 5'-acetyl-3'-dimethoxytritylthymidine.

The results reported in this communication demonstrate that $ZnBr_2$ is superior to protic acids for removal of trityl ethers from deoxynucleosides. Furthermore the specificity of this reagent can be exploited by preparing 3'-tritylnucleosides. This $ZnBr_2$ cleavage procedure is now being incorporated with excellent preliminary results into our polymer support methodology for synthesizing oligonucleotides containing purines.

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