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SELECTIVE N-DEACYLATION OF N,O-PROTECTED NUCLEOSIDES BY ZINC BROMIDE

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SUMMARY N-Acyl protecting group in nucleoside derivatives can be selectively removed by treatment with zinc bromide in the presence of alcohols to give O-protected nucleosides.

The di-p-anisylphenylmethyl (DMT) group has been extensively used for the protection of the 5'-hydroxyl function in polynucleoside synthesis. Very recently, two laboratories[2,3] have independently reported a relatively mild condition for removal of the DMT group in order to overcome a side reaction, cleavage of the glycosidic bond, observed in the syntheses of polydeoxynucleotides. Under the improved conditions, the deprotection of the DMT group is performed by treatment with zinc bromide ($ZnBr_2$) suspended or solubilized in an aprotic organic solvent, such as dichloromethane[2] (CH_2Cl_2) and nitromethane[3]. However, we have observed some difficulty in the application of this condition to the synthesis of polynucleotides by the solid-phase method because of the low solubility of $ZnBr_2$ in aprotic solvents. Virtually no reaction took place on the polystyrene support[5] using a 0.1 Molar solution of $ZnBr_2$ in nitromethane[6]. Although other aprotic solvents such as acetone, dimethylformamide (DMF), dimethylsulfoxide (DMSO) and ethyl acetate, readily solubilize $ZnBr_2$ to give a 1 Molar solution, the detritylation reaction was very slow. In order to increase the rate of the reaction, we have examined the conditions using protic solvents[7].

In this paper, we report a side reaction, selective N-deacylation, observed while deblocking the DMT group from nucleoside derivatives using $ZnBr_2$ in protic solvents and an improved condition under which the DMT group can be removed rapidly without any significant side reactions. The first example of N-deacylation was observed in the reaction of N-benzoyl-5'-O-dimethoxytrityldeoxyadenosine (I) with $ZnBr_2$ (0.7 Molar) in a $CHCl_3$-MeOH (9:1 v/v) solution. After 1 minute at room temperature, the starting material (I) had completely reacted to give only one product, N-benzoyldeoxyadenosine (II), which was identical with an authentic sample.

In order to examine the stability of the glycosidic bond, the reaction mixture was allowed to stand overnight. In addition to product II, a slow moving material (30% isolated) on silica

gel thin layer chromatography (TLC) (solvent: $CHCl_3$-MeOH, 9:1 v/v) was detected and identified as deoxyadenosine III. It should be noted that MeOH has an effect of depressing the depurination reaction since a trace of N-benzoyl adenine was not detected on TLC, even after the overnight treatment of $ZnBr_2$.[8] The selective removal of the N-acyl group from nucleoside derivatives was demonstrated by the following series of reactions.

A mixture of tribenzoyldeoxyadenosine[9] (IV, 1 mmole, R = benzoyl and B = adenine) and 1 Molar solution of zinc bromide (40 mmole) in $CHCl_3$-MeOH (1:4 v/v) was allowed to stand at room temperature. After 6 hours, the starting material (IV) had completely disappeared on TLC (silica gel). The product 3',5'-O,O-dibenzoyldeoxyadenosine (V) was isolated by column chromatography on silica gel with a yield of 93.6%. This product (mp 114-117) was characterized by its elementary analysis (found: C, 62.33; H, 4.83; N, 14.84; calculated for $C_{24}H_{21}N_5O_5$: C, 62.74; H, 4.61; N, 15.24) and by its ultraviolet spectrum. When treated with concentrated NH_4OH, the compound V yielded deoxyadenosine (III). In a similar manner, tribenzoyldeoxycytidine (IV, R = benzoyl and B = cytosine) gave 3',5'-O,O-dibenzoyldeoxycytidine (V) with a yield of 93.6%, but after treatment with $ZnBr_2$ for 16 hours. The product was identified by its elementary analysis, ultraviolet spectrum and by treatment with ammonia.

TABLE I.

REACTION OF TRIBENZOYLATED DEOXYADENOSINE WITH ZINC BROMIDE (1M)

Alcohol[a]	Reaction Time (hrs)	Yield (%)[b]
MeOH	6	100
EtOH	16	100
n-PrOH	24	64
iso-PrOH	24	10
iso-BuOH	24	40

a $CHCl_3$-alcohol (1:4 v/v) was used as solvents.
b Yields were estimated by the ratio of the starting material and the product. The reaction mixture was chromatographed on TLC plates (silica gel) using a solvent ($CHCl_3$-MeOH, 9:1 v/v). The nucleosides were eluted by $CHCl_3$-MeOH (8:2 v/v) and analyzed by UV spectroscopic absorption.

Acetyl derivatives of IV (B = adenine and cytosine, and R = acetyl) reacted with $ZnBr_2$ 100-200 times faster than the corresponding benzoyl derivatives to afford V with similar yields. However, N-acetyldeoxyguanosine was completely stable under the same conditions for at least 24 hours. Similar results have been reported for the selective N-deacylation of nucleoside derivatives using hydrazine hydrate.[10] The mechanism of the selective N-deacylation could probably be explained by the postulation of the intermediate VI. The bidentate chelated intermediate[11] could be attacked by MeOH to give the N-deacylated product. Indeed, in the absence of MeOH, N-deacylation could not be detected and the rate of the reaction was dependent upon the size of the alcohols (Table I).

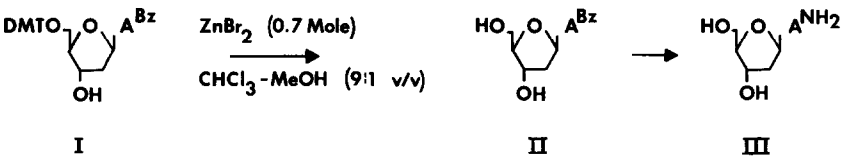
Using the same concentration of $ZnBr_2$ (1 Molar), complete removal of the N-benzoyl group from IV (B = adenine and R = benzoyl) in MeOH was effected in 6 hours. However, when ethanol was used, it took 16 hours to complete the reaction and only 10% of IV reacted with $ZnBr_2$ after

TABLE II.

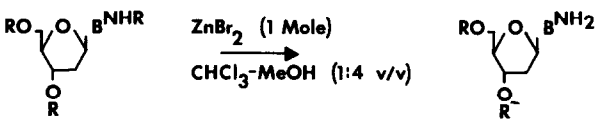
REACTION TIME TO COMPLETE THE DETRITYLATION OF FOUR KINDS OF THE FULLY PROTECTED NUCLEOTIDES BY ZINC BROMIDE AND 2% BENZENE SULFONIC ACID (BSA).

Fully Protected Nucleotides I Base	Reaction Time (Minutes) $ZnBr_2$	2% BSA
Adenine (X = A^{Bz})	1	1
Cytidine (X = C^{Bz})	7	6
Guanine (X = G^{iBu})	1	1
Thymine (X = T)	3	10

The fully protected nucleotides (10 mg) were treated with:
a) Zinc bromide (1 Molar solution) in ethylacetate (0.5 ml) in the presence of triazole (0.1 Molar equivalent to zinc bromide) and molecular sieves 4A (10 mg) at room temperature.
b) Zinc bromide (1 Molar solution) in CH_2Cl_2-isopropanol (85:15 v/v, 0.5 ml) at room temperature and 2% BSA solution (0.5 ml) in $CHCl_3$-MeOH (85:15 v/v) at 0°C. The reaction mixutre was analyzed by silica gel TLC plates develped in $CHCl_3$-MeOH (9:1 v/v).

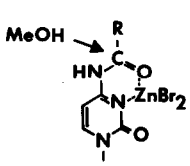


I II III

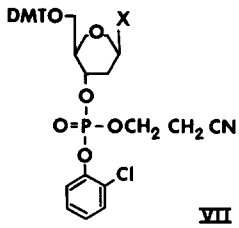


IV V

B = adenine or cytosine
R = acetyl or benzoyl
X = T, A^{Bz}, C^{Bz}, G^{iBu}



VI



VII

24 hours when isopropanol was used. Furthermore, the fact that benzanilide was completely stable when treated with zinc bromide supports the hypothetical bidentate chelated structure VI as a reaction intermediate.

In order to overcome this side reaction, various combinations of organic solvents with sterically hindered alcohols, such as iso-propanol and *tert*-butanol, were examined for the solvent for $ZnBr_2$. A mixed solvent of CH_2Cl_2 and iso-propanol (85:15 v/v) easily dissolved $ZnBr_2$ to give a 1 Molar solution and the detritylation went to completion in a very short time (Table II). Furthermore, no side reactions (N-deacylation or depurination) were observed when the fully protected nucleotide VII (X = A^{BZ}) was treated with this solution overnight at room temperature. The applicaton of this detritylation condition to the polynucleotide syntheses on solid supports is under investigation.

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REFERENCES AND NOTES

1. Present address: Institute of Organic Chemistry, Polish Academy of Sciences, Poznan, Poland.
2. Kohli, V.; Blocker, H. and Koster, H. (1980) Tet. Lett. 21, 2683.
3. Matteucci, M.D. and Caruthers, M.H. (1980) Tet. Lett. 21, 3243.
4. Miyoshi, K.; Miyake, T.; Hozumi, T. and Itakura, K. (1980) Nucl. Acids Res. 8, 5473.
5. Miyoshi, K.; Arentzen, P.; Huang, T. and Itakura, K. (1980) Nucl. Acids Res. 8, 5507.
6. Another factor to be considered is low swelling of the polystyrene support in nitromethane and, therefore, the DMT group may be hindered by the styrene matrix.
7. Koster and his co-workers mentioned (Pef. 2) that a very concentrated solution of anhydrous $ZnBr_2$ in dry methanol can be used for deblocking of the trityl group without any side reactions.
8. It was reported (Pef. 3) that the treatment of I with $ZnBr_2$ (0.1 Mole) in CH_3NO_2 for 10 hours gave 50% of the depurinated product, N-benzoyl adenine. Under our conditions, $ZnBr_2$ (0.5 Mole) in CH_3NO_2-MeOH (9:1 v/v) overnight, no depurinated product was detected.
9. Tribenzoylated product was made by treatment of II with 2.2 equivalents of benzoic anhydride in the presence of N,N-dimethylaminopyridine.
10. Letsinger, R.L.; Miller, P.S. and Grams, G.W. (1968) Tet. Lett. 22, 2621.
N-Benzoyl group can be removed from the protected deoxyadenosine and deoxycytidine using hydrazine hydrate, but N-acetyldeoxyguanosine is completely stable under these conditions.
11. Corey, E.J.; Gras, J-L. and Ulrich, P. (1976) Tet. Let. 809. The bidentate chelation mechanism was proposed for the cleavage of β-methoxyethoxymethyl ether protecting group by $ZnBr_2$.

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