246. Nucleosides and Nucleotides. Part 19. On Detritylation with Zinc Bromide in Oligonucleotide Synthesis[1])

by **Felix Waldmeier, Silvio De Bernardini, Colin A. Leach** and **Christoph Tamm**[2])

Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH-4056 Basel

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Summary

Zinc bromide has been shown by several groups of workers to be a useful reagent in the removal of trityl protecting groups from nucleotides. An attempt is made here to establish optimum conditions for the reaction, a strange observation is reported, and a novel workup *via* a soluble, lipophilic zinc complex is described.

Introduction. – *Kohli et al.* [2] and *Matteucci & Caruthers* [3] have recently shown that dimethoxytrityl (bis(*p*-methoxyphenyl)phenylmethyl) groups in phosphotriester intermediates can be removed by anhydrous zinc bromide. Varying and not very detailed information about the reaction conditions was given in later reports [4] [5]. The authors discussed the problem of deacylation of A^{bz} and C^{an} derivatives by $ZnBr_2$ in CH_3OH or $CH_3OH/CHCl_3$ [5], and the use of the reagent in polymer-supported syntheses [4] [5]. Very little information was made available on workup procedures or the required excess of $ZnBr_2$. So far, the most suitable procedure appears to be the use of 1 M $ZnBr_2$ in CH_2Cl_2/2-propanol 85:15, followed by neutralization, or extraction of $ZnBr_2$ with an aqueous buffer. The reaction appeared less successful in CH_2Cl_2, CH_3OH or CH_3NO_2. The occurrence of organic zincates has been reported [4]. Very recently, *Köster & Sinha* [6] have suggested replacing $ZnBr_2$ by dialkyl aluminium chloride, which is soluble in aprotic solvents.

In connection with the triester synthesis of oligonucleotides, we were interested in finding a simple and general experimental procedure for detritylation by the $ZnBr_2$-method. Therefore a series of reactions were carried out in which the concentrations of $ZnBr_2$ and CH_3OH or 2-propanol in $CHCl_3$ or CH_2Cl_2 were varied systematically. The subsequent neutralization and workup was also studied. Reactions were carried out on fully protected 3′-mononucleotides and monitored by TLC. at each stage.

Results. – A common and striking observation under various reaction conditions was that TLC. at first showed clean, total detritylation, but after neutralization or

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[2]) Author to whom correspondance should be addressed.

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hydrolysis dimethoxytrityl-containing product was again present, often in substantial amounts. We presume that although the trityl cation is readily formed, under some conditions it does not separate from the nucleotide/zinc complex; thus rapid recombination to the starting material can occur on neutralization.

We found that this phenomenon was practically independant of the method of workup. Conversely, there was a clear correlation between the tendency to recombine and the ratio of concentration of alcohol/$ZnBr_2$. Recombination could best be avoided by keeping this ratio as small as possible. Since the alcohol is required as solvent for $ZnBr_2$ in CH_2Cl_2 or $CHCl_3$, this implies the use of saturated solutions. In practice, any concentration from 0.2 M up to 1 M seems favorable; this requires *ca.* 15% 2-propanol content for a 1 M solution [5], the percentage declining roughly proportionally for lower $ZnBr_2$-concentrations. Even slightly higher alcohol concentrations increased the tendency to recombine, so the use of saturated solutions seems essential.

We also investigated the excess of $ZnBr_2$ required for complete detritylation without recombination. Although with the *Itakura* reagent only a relatively small excess seems to suffice for complete reaction (TLC.), at least 30–40 mol-equiv. of $ZnBr_2$, and preferably 50, should be added to avoid recombination. If lower concentrations of $ZnBr_2$ are used, with correspondingly less 2-propanol, slightly larger excesses appear necessary, but 50 mol-equiv. proved adequate in all cases.

In agreement with the observed trends, recombination was also found to occur when a reaction solution after complete conversion of starting material was diluted with an alcohol or with large amounts of CH_2Cl_2.

In view of the observation by *Matteucci & Caruthers* [3] that $ZnBr_2$ in CH_3NO_2 will cleave monomethoxytrityl ((*p*-methoxyphenyl)diphenylmethyl) ethers at the same rate as the corresponding dimethoxy compound, we investigated the time required to detritylate various mono- and dimethoxytrityl nucleotides with $ZnBr_2$ as a 1 M solution in $CHCl_3$/2-propanol. As noted by *Itakura* [5], pyrimidine nucleotides were detritylated much more slowly than purine nucleotides. However, in contrast to the results in CH_3NO_2-solution, we also found a major rate difference between mono- and dimethoxytrityl groups, the latter being removed at least 5 times faster than the former. This implies that in alcohol-containing solutions, there is no rate-limiting formation of a nucleotide-zinc chelate (*cf.* [3]). In addition it was found that detritylation in the presence of alcohols is not selective for 5′-trityl groups; 3′,5′-bis(dimethoxytrityl)thymidine lost both trityl groups at comparable rates, and only small amounts of monotrityl compound could be isolated, again in contrast to CH_3NO_2-solutions.

Neutralization of $ZnBr_2$. – Various methods of neutralizing or hydrolyzing the reaction solution were investigated. Phosphate buffer pH 7 [2] and 1 M $AcONH_4$ [3] have been proposed in the literature (two-phase reactions). We found the latter gave a clean reaction and was simple and convenient to use.

We also tried hydrolysis with H_2O/CH_3OH and H_2O/pyridine. In both cases a polar, apparently ionic compound was formed as the major product, presumably a zincate (*cf.* [4]). Addition of pyridine/CH_3OH caused the usual recombination.

We then attempted to quench the $ZnBr_2$ by complex formation, without

hydrolysis. A simple complexation reaction occurred, for example, with pyridine: $ZnBr_2$ reacted readily with 2 mol-equiv. of pyridine to give $ZnBr_2(py)_2$ [7], which has a low solubility in alcohols and is moderately soluble in other organic solvents, including CH_2Cl_2/2-propanol mixtures. The polarity and chromatographic properties of this compound are quite similar to those of nucleotide triester compounds. This is clearly inconvenient if the triester synthesis is carried out in solution with subsequent silica gel chromatography. We therefore tested other pyridine derivatives as ligands, to obtain a complex with different polarity. We found that both 2,2'-bipyridyl and 2,4,6-collidine (2,4,6-trimethylpyridine) formed slower-running complexes with low solubility in CH_2Cl_2. The bipyridyl complex precipitated virtually quantitatively from CH_2Cl_2/2-propanol mixtures and could be filtered off. Surprisingly, the collidine complex is more polar than the pyridine complex; we presume that formation of a bis-collidine complex is hindered by the 2,6-disubstitution, but this was not investigated further. A suitable non-polar complex was, however, readily obtained with commercially available 4-*t*-butylpyridine. The bis(4-*t*-butylpyridine) complex was formed rapidly and quantitatively; it is very soluble in CH_2Cl_2, $CHCl_3$, acetone and THF, but only slightly soluble in alcohols. On TLC., this compound runs faster than pyridine or nucleotide triester compounds, and it can be eluted from silica gel columns with 2% CH_3OH in CH_2Cl_2. We presume that this complex, like related zinc complexes, is tetrahedral [7].

For neutralization, 2 to 2.1 mol-equiv. of 4-*t*-butylpyridine were added to the reaction mixture, rapidly discharging the color in a slightly exothermic reaction. With 1 M $ZnBr_2$ in CH_2Cl_2/2-propanol 85:15, this yielded a saturated solution of the complex, which may crystallize. This can be avoided by dilution with CH_2Cl_2 or by use of a less concentrated $ZnBr_2$/2-propanol reagent for the detritylation.

Final remarks. – The results show that various concentrations of $ZnBr_2$ in CH_2Cl_2/2-propanol mixtures can cleave the dimethoxytrityl group from nucleotide triester compounds. It is, however, necessary to ensure that saturated solutions and a sufficient excess of reagent are used, to avoid recombination during neutralization. In general, an excess of *ca.* 50 mol-equiv. is recommended. In addition to 1 M $ZnBr_2$ in CH_2Cl_2/2-propanol 85:15, less concentrated solutions with a correspondingly smaller 2-propanol content appear practical, *e.g.* 0.5 M $ZnBr_2$ in CH_2Cl_2/2-propanol 92.5:7.5. After *ca.* 10–15 min at RT., $ZnBr_2$ can be extracted with an excess of 1 M $AcONH_4$ or be neutralized in solution by addition of 2.1 mol-equiv. of 4-*t*-butylpyridine. We assume that the latter method should also be applicable to solid-phase synthesis: $ZnBr_2$ could be washed from the polymeric carrier in the form of a soluble complex.

The observed recombination reaction is not understood and has not been discussed or even been mentioned in the literature so far. Possibly it is the source of unsatisfactory results obtained occasionally with $ZnBr_2$.

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Experimental Part

General. Nucleotides were prepared as described in [1]. Anhydrous $ZnBr_2$ and 4-*t*-butylpyridine were purchased from *Fluka AG*, Buchs. TLC. was carried out on precoated silica gel plates from *Merck*, eluted with CH_2Cl_2/MeOH 9:1.

Detritylation studies. The $ZnBr_2$-concentration was varied in the range 0.2–1.0 M, the CH_3OH- or 2-propanol concentration in the range 3–50%, and the excess of $ZnBr_2$ in the range 5–100 mol-equiv. with respect to the dimethoxytrityl compound. Reactions were carried out at RT. and monitored by TLC. Detritylation was generally complete within 10–15 min, but after neutralization a spot corresponding to starting material was again visible on TLC., often representing a major component. For example 100 mol-equiv. of $ZnBr_2$ in CH_2Cl_2/2-propanol 80:20 achieved complete detritylation at first, but all methods of neutralization caused at least 50% recombination. With higher percentages of alcohol, *e.g.* 1 M $ZnBr_2$ in MeOH/$CHCl_3$ 1:1, 70–100 mol-equiv. $ZnBr_2$ were needed to achieve complete detritylation, and subsequent neutralization caused 80–90% recombination.

Successful detritylation was achieved with a range of saturated $ZnBr_2$ solutions, including 1 M $ZnBr_2$ in CH_2Cl_2/2-propanol 85:15, 0.7 M $ZnBr_2$ in CH_2Cl_2/2-propanol 89:11, and 0.5 M $ZnBr_2$ in CH_2Cl_2/2-propanol 92.5:7.5. In each case, use of *ca.* 20 mol-equiv. of $ZnBr_2$ led to complete detritylation followed by partial recombination during workup, but 50 mol-equiv. of $ZnBr_2$ was sufficient to prevent recombination.

4-t-Butylpyridine/$ZnBr_2$ complex. A 1 M solution of $ZnBr_2$ in CH_2Cl_2/2-propanol 85:15 was treated with 2.1 mol-equiv. of 4-*t*-butylpyridine. Subsequent addition of CH_3OH precipitated the product as a white powder in essentially quantitative yield, m.p. 205–210°. – UV. ($CHCl_3$): 255 nm (ε 5250). – IR. (KBr): 1610, 1500, 1420, 1225, 1070, 1025, 835, 730 cm^{-1}. – ^{1}H-NMR. (60 MHz, $CDCl_3$): 8.75 (*m*, 4 H); 7.55 (*m*, 4 H); 1.35 (*s*, 18 H).

It was found that 1 g of complex dissolved in 1.7 ml of CH_2Cl_2; addition of alcohols reduced the solubility. On TLC., the complex runs faster than nucleotide triester compounds or pyridine, and it can be eluted from silica gel columns with *ca.* 2% CH_3OH in CH_2Cl_2.

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