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High Yield Protection of Purine Ribonucleosides for H-Phosphonate RNA Synthesis

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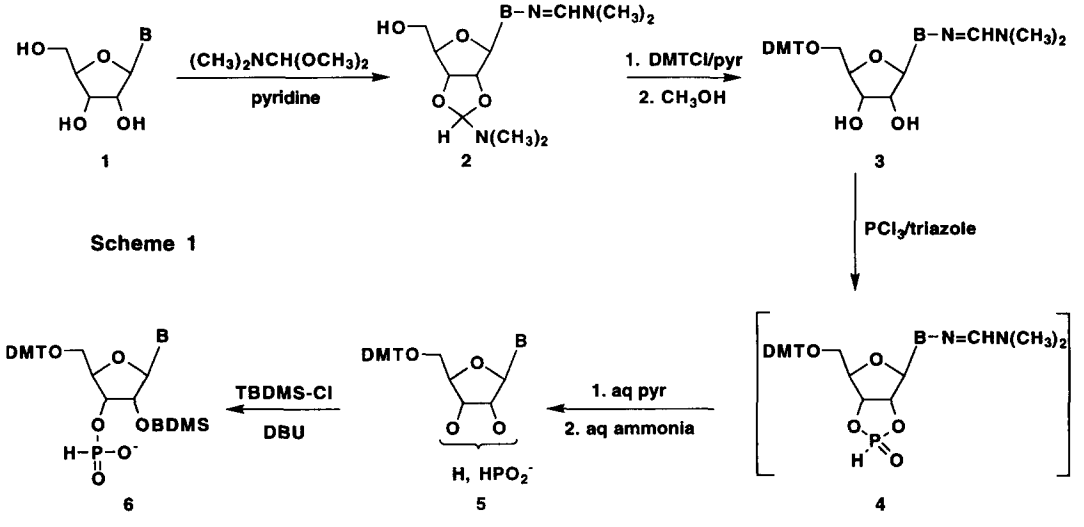
Abstract: We report a new method for protecting nucleosides for hydrogen phosphonate RNA synthesis that gives very high yields for the purines. This method is based on our finding that, for adenosine and guanosine, silylation of a mixture of 2' and 3' H-phosphonates in the presence of DBU is highly selective for the 2' hydroxyl. © 1997 Elsevier Science Ltd.

The most commonly employed procedures for protection of ribonucleosides for RNA synthesis follow the sequence: amino protection, tritylation, silylation, chromatography, phosphitylation, and another chromatography.[1] In this scheme the modest selectivity for reaction of the 2'-OH over the 3'-OH in the silylation reaction, even with a Ag^+ catalyst,[2] is a serious limitation on the overall yield obtained. Moreover, chromatographic separation of the silyl isomers can be problematic, particularly for guanosine, and silyl migration before or during phosphitylation also can occur.[3] As a result, overall yields for the preparation of fully protected ribonucleoside phosphoramidites and hydrogen phosphonates are generally in the area of 20% for guanosine and 40% for adenosine.

Low yields for nucleoside protection are an obstacle to chemical synthesis of RNA fragments which contain valuable monomers. Our interest in synthesis of specifically $^{15}N/^{13}C$ labeled RNA led us to explore alternatives to the standard protection methodology. The facile formation and hydrolysis of H-phosphonate cyclic diesters[4] offers several possible alternatives. We have found that reaction of a nucleoside having both the 2' and 3' hydroxyl groups free with a phosphitylating reagent like PCl_3/triazole, gives exclusively a mixture of the 2'- and 3'-H-phosphonates, not the 2',3'-bis H-phosphonate. This presumably occus because of the rapid formation and subsequent rapid hydrolysis of the 2',3'-cyclic H-phosphonate. The route we now report is based on our recent finding that this mixture of H-phosphonates can be silylated with *tert*-butyldimethylchlorosilane when the strong base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is present. In the absence of DBU no hydroxyl silylation occurs. In addition, although the mixture of isomeric H-phosphonates is approximately in the ratio of 2:1 (by HPLC, Figure 1), silylation in the presence of DBU proceeds so as to favor the 2' silyl isomer by as much as 9:1 for purines (Figure 2).

H-phosphonate oligonucleotide synthesis does not require amino protection for adenosine or guanosine,[5] but the tritylation reaction does need amino protection. The protection strategy shown in the Scheme below

makes use of treatment with *N,N*-dimethylformamide dimethylacetal to protect the amino groups, and also to protect the 2',3' diol functionality. The reaction of nucleosides with *N,N*-dialkylformamide dialkyl acetals was first reported by Zemlicka more than thirty years ago.[6] In that original paper both the formation of ribonucleoside *N*-dimethylaminomethylene derivatives as well as the formation of the 2',3'-*O*-dimethylaminomethylene derivatives were noted. Attempts to use the latter derivatives to effect selective 5'-acetylation, sulfonylation, or phosphorylation proved unsuccessful,[7] while the *N*-dimethylaminomethylene derivatives have been used for amino protection for oligonucleotide synthesis.[8-15] Nevertheless, we have found that the dimethylaminomethylene derivatives can be used to avoid the small amount of 2' tritylation that otherwise accompanies 5' tritylation. The 2'-DMT derivatives in some cases can be difficult to remove and their formation is easily avoided by this procedure.



Scheme 1

The reaction of a nucleoside (**1**) with *N,N*-dimethylformamide dimethylacetal (4 eq) in pyridine gives **2** within 15 h at room temperature. The dimethylacetal reaction is quantitative and **2** is isolated simply by evaporation of pyridine and excess acetal. Tritylation under standard conditions (1.2 eq of dimethoxytrityl chloride in pyridine at room temperature for 4 hours) is followed by removal of the labile 2',3'-dimethylaminomethylidine group by addition of methanol to give **3**, which is isolated by evaporation of solvents. Phosphitylation of **3** is achieved by adding it slowly as a pyridine solution to a pre-equilibrated mixture of 3 eq of phosphorous trichloride, 10 eq of triazole, and 27 eq of *N*-methyl morpholine in methylene chloride maintained at about -60°C (dry ice/isopropanol). After 30 minutes at low temperature, the mixture is poured into water containing 5 eq of $DBU^+HCO_3^-$ and stirred for 10 min. Separation of the methylene chloride layer and

concentration gives a mixture of 2' and 3' H-phosphonates. For guanosine, one extraction with 70% *n*-butanol/30% ethyl acetate is required. The amidine group is removed to give **5** by dissolving the gum in 95% ethanol, adding concentrated ammonia until the solution is just cloudy, and maintaining the sealed flask at room temperature for 15 hours. After evaporation of most of the ammonia, the product is isolated by extraction with 50%-70% *n*-butanol in ethyl acetate and precipitation into diethyl ether. Silylation is accomplished by reaction with 1.3 eq of tert-butyldimethylchlorosilane and 2 eq DBU in DMF for 15 h. The product is isolated by addition of water and extraction with methylene chloride along with 3 eq of $DBU^+HCO_3^-$. The pure $DBUH^+$ salt **6** is obtained by a single normal phase chromatography using a gradient of 0 to 15% methanol in methylene chloride containing 3% pyridine. The polar H-phosphonate group enhances the chromatographic separation of the isomers, so that this chromatography is straightforward even for guanosine.

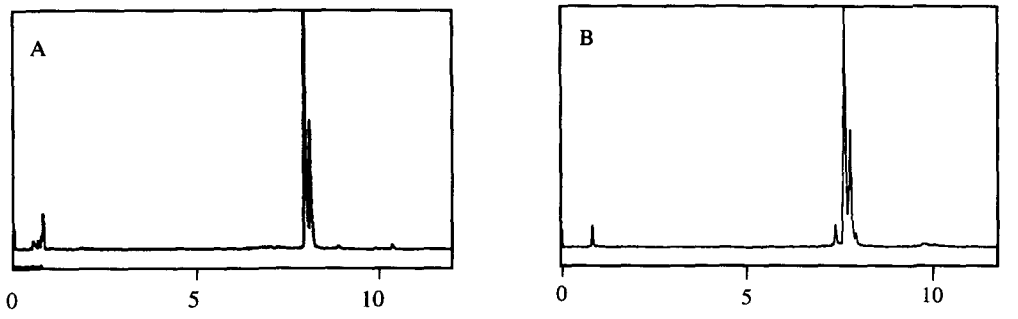


Figure 1. HPLC (2-40% acetonitrile: 0.1 M triethylammonium acetate in 5 min at 2 mL/min on a Waters 8mm x 10 cm C18 RCM column) of the mixture of 2'- and 3'-H-phosphonates (**5**) for: panel A, adenosine; panel B, guanosine.

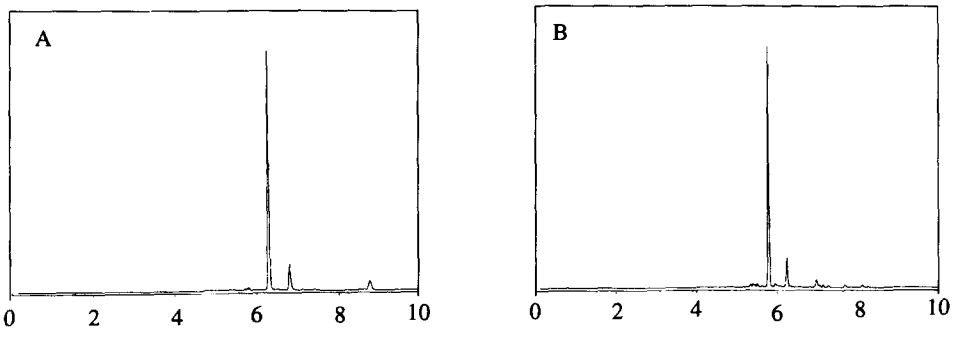


Figure 2. HPLC (2-80% acetonitrile: 0.1 M triethylammonium acetate in 5 min at 2 mL/min on a Waters 8mm x 10 cm C18 RCM column) of the crude mixture of **6** (large peak) and the 3'-silyl isomer (small peak) for: panel A, adenosine; panel B, guanosine.

The 2',3'-dimethylaminomethylidine group in **2** is sufficiently labile that partial hydrolysis occurs during reversed-phase HPLC, so that **2** cannot be shown to be homogeneous by HPLC. Figure 1 shows HPLC of the mixture of 2' and 3' H-phosphonates (**5**) obtained with adenosine and guanosine. This ratio is typical for all four nucleosides. Figure 2 shows HPLC of the crude reaction mixture after silylation for adenosine and guanosine.

The selectivity for the silylation of the 2'-OH is generally 85 to 90% with adenosine and guanosine, while under the same conditions uridine and cytidine show little selectivity. This method gives overall yields for purines of 60-70%. This is three times the overall yield that we typically obtained for guanosine using standard procedures. We have used this procedure to protect specifically $^{15}N/^{13}C$ labeled adenosine and guanosine for incorporation into RNA fragments for NMR studies.[16-18]

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