

Efficient preparation of 2-deoxy-3, 5-di-O-*p*-toluoyl- α -D-ribofuranosyl chloride

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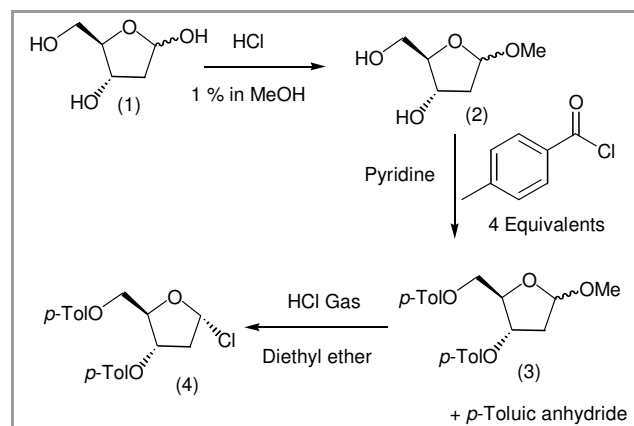
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Abstract: Two efficient methods for the synthesis of 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -D-ribose are described. Upon treatment with hydrogen chloride, 2-deoxy-3, 5-di-O-*p*-toluoyl- α -D-ribofuranosyl chloride, previously unstable, was produced as a white solid, stable in air indefinitely.

Key words: 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -D-ribose, 2-deoxy-3, 5-di-O-*p*-toluoyl- α -D-ribofuranosyl chloride, 1-O-methyl-2-deoxy- α/β -D-ribose, dry hydrogen chloride, *p*-toluic anhydride

2-Deoxy-3, 5, -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride is widely used in the synthesis of nucleotide analogs.¹ The potential of nucleotide analogs to help understand the structure and function of RNA and DNA has been well documented.² Additionally, many nucleotide analogs exhibit antiviral, antibiotic and anticancer activity.³ Therefore, the synthesis of 2-deoxy-3, 5 -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride is of paramount importance. With few modifications, the method developed by Hoffer in the late fifties has been used widely.⁴ Studies dealing with the anomeric preference of 2-deoxy-3, 5 -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride have appeared frequently, but there have been few studies on optimizing the preparation.^{5,7} We recognized the need for such a study considering that the apparent instability of this product affects the outcome of a nucleotide analog synthesis especially if performed in large scale (Scheme 1). In a typical Hoffer procedure, 2-deoxy- α/β -D-ribose (1) is methylated to give 1-O-methyl-2-deoxy- α/β -D-ribose (2). According to early studies, 1 % methanolic solution of dry hydrogen chloride is required to affect the desired furanose versus pyranose ring formation.⁶ Next, classic esterification using 2 equivalents of *p*-toluoyl chloride per hydroxyl group in dry pyridine, gives the 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -ribose (3). This compound is converted into the product, 2-Deoxy-3, 5, -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride (4), by bubbling dry hydrogen chloride gas through a solution of (3) in diethyl ether, acetic acid or adding 4 M HCl in acetic acid-dioxane solution.^{4, 7, 8} The solid product is then filtered and kept under high vacuum. Even under vacuum the product decomposes after two weeks. The overall yield of the synthesis is 70-80 %. In literature applications of the Hoffer procedure, two key purification steps were sometimes omitted in order to obtain higher yields. In the first step, product (2) was not purified by distillation (Scheme 1).⁶ In the second step, the

product (3) is not typically purified by column chromatography. We, and others have, found that even with the purification of product (3) the end product (4) is still not stable. We performed experiments to determine whether these two purification steps have any influence in the outcome of the synthesis. Product (2) was distilled under high vacuum and temperature as described.⁶



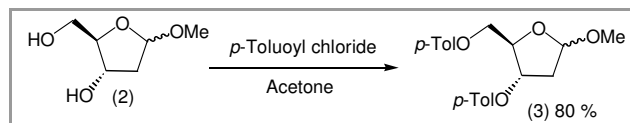
Scheme 1 The Hoffer synthesis

We repeated the Hoffer procedure using distilled 1-O-methyl-2-deoxy- α/β -D-ribose (2) (Scheme 1). The end product (4) was still unstable. MS and NMR analysis revealed the presence of *p*-toluic anhydride in the purified 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -ribose (3). Apparently, the traditional solvent systems for column chromatography such as benzene-diethyl ether and methanol-chloroform do not give a clean purification. Using hexanes to remove the *p*-toluic anhydride, and then using hexanes-ethyl acetate, we separated out the pure 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -ribose (3). This product was converted upon treatment with dry hydrochloric acid into 2-deoxy-3, 5, -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride (4).⁸ The product obtained was a white solid stable in air indefinitely. Since the esterification step was performed using freshly distilled reagent and solvents it was logical to assume that *p*-toluic anhydride was produced during aqueous work-up from the reaction of water with *p*-toluoyl chloride in the presence of pyridine. We hypothesized the apparent instability of 2-deoxy-3, 5, -di-O-*p*-toluoyl- α -D-ribofuranosyl chlo-

ride (4) is due to the presence of *p*-toluic anhydride, which carries through to the final product (4). The formation of the anhydride during the esterification was quite surprising. It has been commonly assumed that the excess *p*-toluoyl chloride would be converted into acid upon aqueous work-up. The conversion of the aromatic acid chlorides into anhydrides upon treatment with excess water will be described elsewhere.⁹

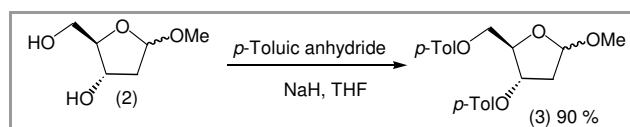
Reactions between the aromatic acid chlorides and alcohols in the presence of tertiary amines are generally slow reactions.^{10,11} Excess starting materials are routinely used to drive these reactions to completion. In light of the observations discussed, it is desirable to eliminate the excess *p*-toluoyl chloride from the reaction mixture. In order to understand better the reaction between equivalent amounts of 1-O-methyl-2-deoxy- α/β -D-ribose (2) and *p*-toluoyl chloride we conducted a model study (Table 1). Ethanol and isopropanol were used to model the primary and secondary alcohols. Equivalent amounts of all reactants were implemented. According to the data obtained, when pyridine is used as a solvent and base, the esterification of isopropanol was not complete after 10 hours (Table 1). Excess starting material is required to bring this reaction to completion within a reasonable timeframe. Using excess *p*-toluoyl chloride would result in the formation of the undesired *p*-toluic anhydride as byproduct. The only alternative in this case would be a lengthy purification procedure. 2-Deoxy- α/β -D-ribose is too expensive to use in excess.

To create a simple and economic procedure using equivalent amounts of all reactants we tested two possible methods. The main goal was to avoid byproduct formation. One method involves using a solvent that does not dissolve the tertiary amine hydrochloride byproduct but does dissolve the starting materials and our product (Equation 1). One such method is described elsewhere.⁹ Another method is to use *p*-toluic anhydride to affect the same esterification in equivalent amounts (Equation 2). 1-O-methyl-2-deoxy- α/β -D-ribose was produced according to a literature procedure.⁷ Triethyl amine was added after the reaction was complete to neutralize the hydrochloric acid and the product was distilled under high vacuum and temperature.



Equation 1 2 equiv. of *p*-toluoyl chloride were dissolved together with the starting material in dry acetone (0.03 mol/ 50ml). 2.2-equiv. of triethyl amine was added slowly and the solution was stirred overnight. After filtering triethylamine hydrochloride, the filtrate was evaporated. Flash chromatography (Hexanes first then Hexanes/Ethyl acetate 15-1) afforded the pure product in 80% yield

The process that we described in ref. 9 was adapted without any problems in this case since 1-O-methyl-2-deoxy- α/β -D-ribose is soluble in acetone (Equation 1). In this case it is necessary to distill the starting material (2). Also drying overnight under vacuum is recommended to eliminate traces of methanol.



Equation 2 4 equiv. of NaH were added to a solution of the starting material in dry THF (0.01 mol/100 ml) at 0° C. 2 equiv. of *p*-toluic anhydride were added slowly in portions. After the addition was complete the solution was stirred at 60-70° C for 4 hours. The reaction mixture was quenched with sat. sodium bicarbonate solution at 0° C, and then extracted with ethyl acetate. Flash chromatography (see equation 1) afforded the pure product in 90% yield.

Using *p*-toluic anhydride as the esterification reagent proved to be advantageous (Equation 2)¹². There was little byproduct and the crude reaction mixture was very easy to purify by column chromatography. It is necessary to make sure that there is no methanol in the starting material (2). Distilling the starting material is not required but it is recommended. The product 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -ribose (3) obtained by both methods was converted into the 2-deoxy-3, 5, -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride (4) according to a literature procedure.⁸ The final product was again stable in air indefinitely. In conclusion, two efficient preparations of 1-O-methyl-3, 5-di-O-*p*-toluoyl-2-deoxy- α/β -ribose (3) have been developed. The end product, 2-deoxy-3, 5, -di-O-*p*-toluoyl- α -D-ribofuranosyl chloride (4) was synthesized in overall 50% yield. The spectral data we obtained are in agreement with the data published previously in the literature.⁷

Table 1

Entry	Alcohol	d-Solvent	Reaction time (hours)	% Conversion
1	EtOH	CDCl ₃	10	96
2	EtOH	CDCl ₃	10	25
3	iPrOH	d5-pyridine	10	98
4	iPrOH	d5-pyridine	10	78

A 0.015 M solution, relative to *p*-toluoyl chloride and alcohol, in the appropriate deuterated solvent was prepared. When CDCl₃ was used one equiv. of TEA was added. All reagents were dried over molecular sieves prior to use. The progress of the reaction was monitored every 10 min. % conversion was monitored by ¹H NMR at room temperature.

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- (12) *p*-Toluic anhydride was prepared as follows: One equivalent of *p*-toluoyl chloride, half equivalent of water and one equivalent of triethyl amine were dissolved in acetone (0.03mol/30ml). The solution was stirred for 2 hours then filtered. Triethylamine hydrochloride was washed with another 30 ml of acetone and the combined acetone solutions evaporated to afford the product.

New efficient syntheses of 2-deoxy-D-ribose derivative

