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Investigation of Stereoselectivities in the Coupling Reactions of 1-O-Methyl-3, 5-di-O-p-toluoyl-2-deoxyribofuranoside with Purines and Pyrimidines.

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Abstract: Stereoselectivities in the coupling reaction between 1-O-methyl-3, 5-di-O-p-toluoyl-2-deoxyribofuranoside and suitably protected purine and pyrimidine derivatives have been examined in acetonitrile with varying equivalents of $SnCl_4$. The best result in α-nucleoside formation was obtained when the coupling reaction was carried out in the presence of ten equivalents of $SnCl_4$.

Synthetic oligodeoxynucleotides and their analogs have found wide application in molecular biology as antisense agents in controlling gene expression.[1] The α-anomers of oligodeoxynucleotides[2] have been shown to form duplexes with complementary DNA or RNA, maintaining normal Watson-Crick base pairing specificity.[3] Further, the increased thermal stability of α/β-duplexes[4] and their resistance to nucleases[5] make them ideal candidates for antisense studies. Recently it has been shown that α-oligodeoxynucleotides can form triple helices with duplex DNA[6] similar to those formed by β-oligodeoxynucleotides,[7] serving as a diagnostic tool for the sequence specific recognition of double-helical DNA. Hence there is a growing need for large amounts of α-oligomers. The published procedures for the synthesis of α-nucleosides involving self anomerization of pyrimidine nucleosides[8] and transdeoxyribosylation of pyrimidine nucleosides with purine bases[9] not only result in a mixture of products, but also give very poor yields. Hence we began a search for new synthetic procedures for the preparation of α-purine nucleosides starting from readily available substrates. We investigated the reaction of 1-O-methyl-3, 5-di-O-p-toluoyl-2-deoxyribofuranoside with silylated purines and pyrimidines under Lewis acid catalyzed conditions.[10] In the case of purines, to achieve the required N-9 regioselectivity we chose suitably protected purine derivatives, since use of unprotected purines lead to a mixture of N-7 and N-9 regioisomers.[11]

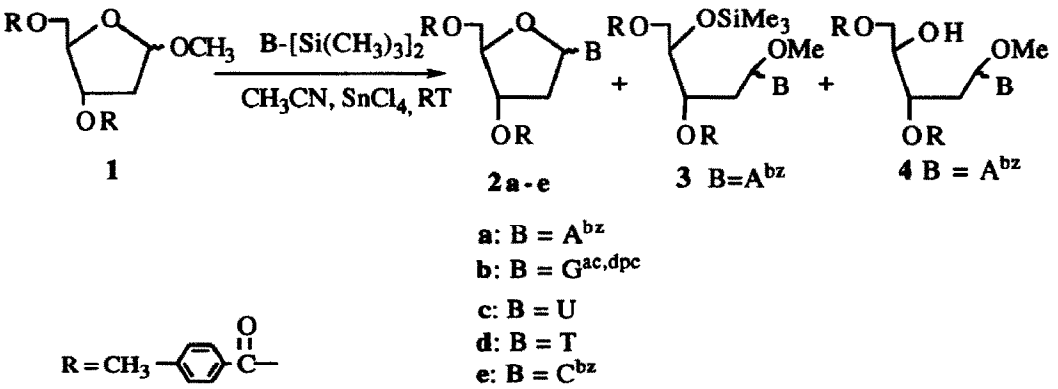

Figure 1

EXPERIMENTAL DETAILS

1-O-Methyl-3,5-di-O-p-toluoyl-2-deoxyribofuranoside was prepared from 2-deoxyribose by treatment with HCl in methanol and subsequent protection of the hydroxyl groups with p-toluoyl chloride in pyridine.[12] Initial studies were carried out using N-benzoyl adenine (1.3 equiv) and the sugar derivative **1** (1.0 equiv) in the presence of $SnCl_4$. The reaction was very sluggish. Hence N-benzoyladenine (2.2 equiv) was silylated using N, O-bistrimethylsilyl acetamide (BSA) (4.4 equiv) in acetonitrile and the silyl derivative thus obtained was condensed with the sugar derivative **1** (1.0 equiv), as described by Vorbruggen *et.al.*,[10a] in the presence of $SnCl_4$ (1.0 equiv). The reaction was monitored by TLC until the disappearance of starting material. After aqueous workup and chromatographic separation, the major fraction was isolated in 40% yield. Its ^1H NMR (300 MHz) spectrum showed it to be a mixture of the required nucleoside **2a** (α:β ratio 1:1) and the silylated acyclic nucleoside **3** (mixture of diastereomers) in the ratio 3:1. The minor fraction was the acyclic nucleoside **4** which showed only one set of signals in ^1H NMR (300 MHz) spectrum, however its ^{13}C NMR (75 MHz) showed it to be a mixture of isomers. Rigorous analysis of the major fraction by TLC revealed two close moving spots which were separated by prep TLC by multiple elution to give **2a** and **3**. Thus the silylated acyclic nucleoside was obtained for the first time in pure form as a mixture of diastereomers in the ratio of 2:1.[13] Pedersen *et.al.* recently reported a similar ring opening reaction during the formation of D-arabinofuranosyl nucleosides.[14a] They proposed that the acyclic nucleoside was arising via the silylation of the ring oxygen with trimethylsilyl trifluoromethane sulfonate used as the Lewis acid catalyst. However, the silylated intermediate was not isolated. Under our reaction conditions, we have been successful in isolating the silylated acyclic nucleoside. Trimethylsilyltrifluoromethane sulfonate being a good silylating reagent would enhance the ring cleavage if present in excess. Hence $SnCl_4$ is a superior Lewis acid for this reaction. As shown in table 1, increasing the equivalents of $SnCl_4$ not only increases the yield of the nucleoside **2a** but also favors the formation of the required α-anomer. Maximum yield and higher α to β anomeric ratio was obtained when the reaction was carried out with ten equivalents of $SnCl_4$ (entry F).

Entry	Equivalent of $SnCl_4$	Yield(%)*	Anomeric Ratio$ $\alpha : \beta$
A	0.05	19	46.5 : 53.5
B	1.0	30	50 : 50
C	2.0	38	53.5 : 46.5
D	4.0	70	57.5 : 42.5
E	7.0	80	68 : 32
F	10.0	92	75 : 25
G	1.00+10.0	85	74 : 26

Table 1

*All the yields reported refer to pure nucleoside **2a** isolated after chromatographic purification. $ Anomeric ratios were determined by integration of H-1', H-3' and H-8 signals in ^1H NMR (300 MHz).

TLC analysis from the beginning of the reaction showed the formation of the required nucleoside **2a** as the major product and only a minor amount (2%) of the acyclic nucleoside **4** was formed. Increasing the $SnCl_4$ equivalents up to 15 equiv neither altered the α:β ratio nor the yield. Decreasing the silylated adenine derivative from 2.2 equiv to 1.3 equiv gave similar results, but required 64h to complete the reaction.

The solvent plays a crucial role in the success of the reaction. While the reaction proceeds very well in acetonitrile, the reaction conducted in 1, 2-dichloroethane under identical conditions gave lower yield (50%).

Addition of one equivalent of $SnCl_4$ and stirring the reaction mixture at room temperature for 24h (until the disappearance of the starting material) showed three spots on TLC. Introduction of an additional 10 equiv of $SnCl_4$ and stirring for an extra 64h resulted in the formation of nucleoside **2a** as the major product (85% yield, entry G). From these results, we conclude that under the reaction conditions the acyclic nucleosides **3** and **4** formed can be cyclized to yield the required nucleoside **2a**, as proposed by Pedersen *et.al*.[14]

In the optimized procedure, N-benzoyl adenine (2.2 equiv) in freshly distilled acetonitrile under an inert atmosphere was stirred with BSA (4.4 equiv) at 80° C for 30 minutes. The reaction mixture was cooled to 0° C and methyl glycoside **1** (1.0 equiv) and $SnCl_4$ (10 equiv) were added. The reaction mixture was stirred at room temperature until the disappearance of the starting material. The reaction mixture was diluted with ethyl acetate and poured into ice cold saturated sodium bicarbonate solution. The mixture was extracted with ethyl acetate, the organic extract dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel using chloroform containing increasing proportions of ethyl acetate.(0-50%) as the eluent. The reaction sequence was extended using suitably protected guanine, uracil, thymine and cytosine. The results are shown in table 2. Eventhough the yield is slightly lower in the case of the guanosine derivative (75%), it gives a suitably protected and highly organic-soluble derivative for further elaborations. In the case of pyrimidine nucleosides, while the reaction proceeds in very high yields, the α- and β-anomers are formed in approximately equal amounts.

Nucleoside	Yield(%)*	Anomeric Ratio$ α : β
2a	92	75 : 25
2b	75	65 : 35
2c	95	46 : 54
2d	97	55 : 45
2e	95	42 : 58

Table 2.
*All the yields refer to protected nucleosides isolated after chromatographic purification. $ Anomeric ratios were determined by integration of H-1' signals in ^{1}H NMR (300 MHz).

In this report, we describe an efficient synthesis of purine and pyrimidine nucleosides in high yields starting from readily available 1-O-methyl-3, 5-di-O-p-toluoyl-2-deoxyribofuranoside, avoiding the lengthy preparation of relatively unstable 1-chloro-3, 5-di-O-acyl-2-deoxyribofuranose or 1, 3, 5-tri-O-acyl-2-deoxyribofuranose, which are the traditional intermediates in nucleoside synthesis.[10,11,15,16] The protected nucleosides can be deprotected[15] and converted into the required α-nucleoside phosphoramidites by the literature procedures.[17]

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