FACILE STEREOSPECIFIC SYNTHESIS OF α-ANOMERIC 2'-DEOXYNUCLEOSIDES

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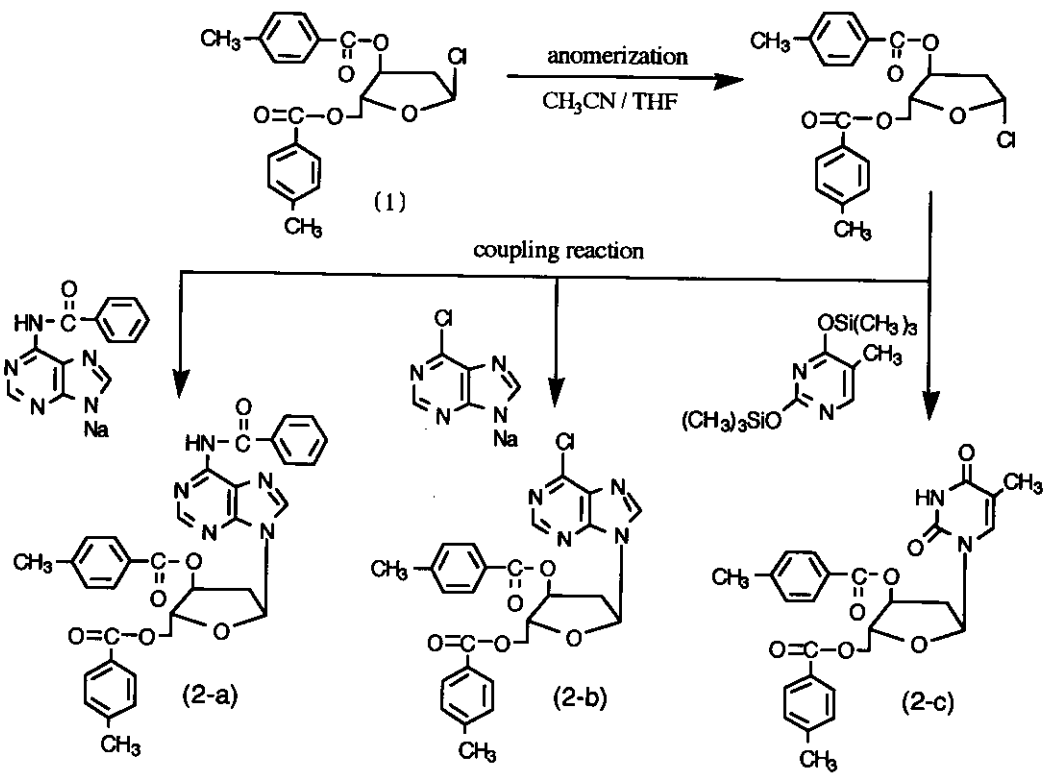
Abstract-The coupling reaction of activated nucleobases, such as the sodium salts of *N*-benzoyladenine and 6-chloropurine and 2,4-bis-*O*-trimethylsilylthymine, with 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranose (1) in a mixture of acetonitrile and tetrahydrofuran leads to the stereospecific formation of α-anomeric 2'-deoxynucleosides with satisfactory yields. The ratio of the distribution between the resulted α- and β-stereoisomers was about 3:1 in each case. The method is simple and applicable to the preparation of both purine and pyrimidine α-2'-deoxynucleosides.

INTRODUCTION

Chemically synthesized oligodeoxynucleotide analogs exclusively consisting of α-deoxynucleotides have been found to possess nuclease resistant property.[1,2] These new oligoDNA analogs preferentially bind to their complementary RNA strand rather than DNA strand.[3] Such unique characters will make α-oligoDNA a sequence specific blocker of RNA function, namely, an antisense agent which binds to complementary RNA strand and prevents a normal expression of gene information through the blocking of translation process.[4] α-OligoDNA analogs have been prepared by either phosphotriester approach[1,3,5] or more efficient phosphoramidite approach.[6] In both cases, a-anomeric deoxynucleosides were utilized as starting materials. For the stereospecific preparation of the key starting α-anomeric deoxynucleosides, however, not so many feasible methods were found in the literature.[5,7-9] Recently, we reported a limited account for the stereospecific preparation of a-anomeric deoxythymidine.[10] Subsequent to this initial study, we have found that the method is applicable to the preparation of both purine and pyrimidine α-deoxynucleosides. In this paper we describe the details of this simple synthetic procedure utilizing activated nucleobases and easily prepared 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranose.[7]

RESULTS AND DISCUSSION

The basic strategy for the preparation of a-nucleosides is to promote the coupling reaction of an activated nucleobase and a β-anomeric 1-halogenodeoxyribose derivative through an S_N2-type reaction. Thus, easily prepared 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranose (1)[7] was dissolved in a mixture of dry acetonitrile



Scheme 1. Preparation of α-2'-deoxyribonucleosides

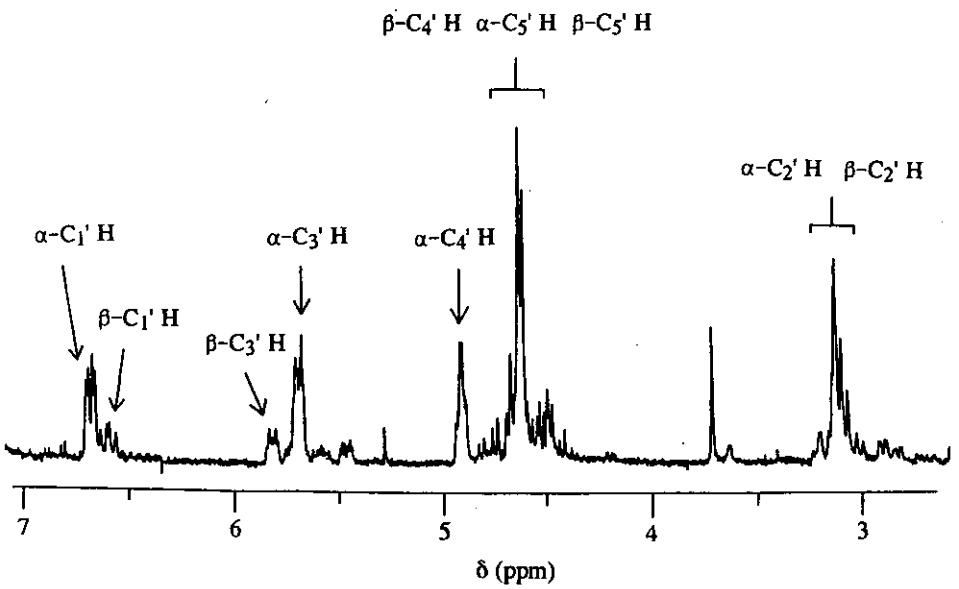


Figure 1. Proton nmr spectrum of fully protected crude 2'-deoxyadenosine in $CDCL_3$

and dry tetrahydrofuran(THF) (5 : 1), and the solution was stirred for 30 to 40 min at 10°C in an atmosphere of nitrogen. This process presumably facilitates the anomerization of the chlorine atom from α-configuration to β-configuration.[11] To the above solution was added a solution of the sodium salt of N^6-benzoyladenine[12] in the same solvent system at low temperature (5-10°C) in an atmosphere of nitrogen, and the resulting mixture was stirred overnight at low temperature. After evaporation of the reaction mixture dryness, the residue was triturated with water and the insoluble crude product was collected by filtration. Based on the proton nmr peak intensity analysis of this crude product, we estimated that the a-nucleoside formation ratio relative to the β-counterpart was approximately 3 : 1 (Figure 1). The proton nmr study also revealed that under these conditions the formation of 7-glycosylated by-product was minimal.

The crude product was then subjected to silica gel column chromatography to separate the anomeric isomers. Isolated yields of the fully protected α-anomeric deoxynucleoside (2-a) and its β-anomeric isomer were 51.3% and 16.4%, respectively. Complete deblocking of 2-a with concentrated ammonium hydroxide[13] gave α-anomeric 2'-deoxyadenosine whose physical properties were the same as in the literature.[9]

It should be noted that when this reaction was carried out in the absence of THF, the reaction rate was very slow and the formation of α-nucleoside as well as the total yield of the nucleosididic materials decreased. In addition, the coupling reaction which carried out without the pretreatment of the chlorosugar in the mixture of acetonitrile and THF led to decrease the ratio of α-anomeric nucleoside formation. Several polar and nonpolar solvents other than THF, such as dimethylformamide, dichloromethane, and chloroform, were found to promote the degradation of the chlorosugar or the formation of the undesirable β-anomeric product.

Robins and his co-workers reported that the reaction of the sodium salt of 6-chloropurine with the chlorosugar in acetonitrile alone afforded the β-anomer of the corresponding 6-chloropurine deoxynucleoside as the major product.[14] When we tried the same reaction in the presence of THF as described above, the major product was the α-anomeric nucleoside. Thus, the reaction of 6-chloropurine sodium salt with (1) produced an anomeric mixture of 6-chloro-α- and 6-chloro-β-2'-deoxy-3',5'-di-*O*-*p*-toluoylribofuranosylpurine.[8] Upon separation, 6-chloro-α- and 6-chloro-β-2'-deoxy-3',5'-di-*O*-*p*-toluoylribofuranosylpurines were isolated in the yields of 52.5% and 18.3%, respectively.[15]

The method was also applicable to the preparation of a pyrimidine nucleoside. When bis-*O*-trimethylsilylthymine[16] was allowed to react with the chlorosugar (1) under the same conditions as above, α-anomeic 2'-deoxythymidine[7] was obtained as a major product. However, the yield was slightly higher (61%) than the case of 2'-deoxyadenosine and 6-chloro-2'-deoxypurine. The ratio of α-anomeric thymidine relative to the β-counterpart was found to be approximately 3 : 1 based on proton nmr intensity analysis.[10]

The present method provides an easy synthetic route to the unnatural type of both purine and pyrimidine α-deoxynucleosides with satisfactory yields.

Synthesis of α-oligodeoxynucleotide using these α-deoxynucleosides is now proceeding and the results will be reported elsewhere.

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EXPERIMENTAL

^{1}H nmr spectra were measured on a Varian Gemini-200 spectrometer. All chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane. Chromatographic purification using columns were performed with Merck Kiesel gel 60H by flash technique. Melting points were determined on a Yanagimoto MP-500D electrothermal apparatus and uncorrected. All reaction solvents were dried in the usual manner and all reactions were carried in an atmosphere of nitrogen.

N^6-Benzoyl-α-2'-deoxy-3',5'-di-*O*-*p*-toluoyladenosine (2-a).

1-α-Chloro-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranose (210 mg, 0.54 mol) was dissolved in a mixture (20 ml) of acetonitrile and tetrahydrofuran (5 : 1) and the solution was stirred for 30 to 40 min at 10°C in a sealed flask. To this solution was added a solution of *N*^{6}-benzoyladenine sodium salt (130 mg, 0.5 mmol) in the same solvent. The resulting mixture was stirred at 10°C for 10 h. After evaporation of the reaction mixture to dryness, the residue was triturated with water. The insoluble material was collected by filtration, washed with water, then subjected to silica gel column chromatography using 5% methanol in dichloromethane as the eluent. Fractions containing *N*^{6}-benzoyl-α-2'-deoxy-3',5'-di-*O*-*p*-toluoyladenosine(2-a) and its β-anomer were collected and concentrated. Each residue was triturated with hexane and recrystallized from ethanol. The yields of (2-a) and its β-anomer were 51.3% (166 mg) and 16.4% (53 mg), respectively. An analytical sample of 2-a was dried at the room temperature under vacuum for 1 h. (2-a) : mp 98.3-99.8°C. Anal Calcd for. $C_{33}H_{29}O_6N_5$ C_2H_5OH 0.5 H_2O: C, 65.00; H, 5.61; N, 10.83; Found: C, 65.27; H, 5.44; N, 10.66. ^{1}HNmr ($CDCl_3$) δ 9.09 (1H, br, s, NH), 8.79 (1H, s, H-2 or H-8), 8.37 (1H, s, H-2 or H-8), 8.09-7.88 (5H, m, benzoyl), 7.92 and7.60 (4H, H-2,6 of *p*-toluoyl), 7.29 and 7.19 (4H, H-3,5 of *p*-toluoyl) 6.68 (1H, dd, H-1', J = 1.9 Hz and 7.6 Hz), 5.75-5.67 (1H, m, H-3'), 4.97-4.93 (1H, m, H-4'), 4.69-4.63 (2H, m, H-5' and H-5"), 3.15-3.02 (2H, m, H-2' and H-2"), 2.43 (3H, s, CH_3 of *p*-toluoyl), 2.37 (3H, s, CH_3 of *p*-toluoyl).

6-Chloro-α-2'-deoxy-3',5'-di-*O*-*p*-toluoylribofuranosylpurine (2-b).

This compound was synthesized in a manner similar to that described for the foregoing compound using 6-chloropurine sodium salt (88 mg, 0.5 mmol) and 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoylribofuranose (210 mg, 0.54 mol). The separation of the anomeric isomers was accomplished by preparative scale thin layer chromatography developed twice with 5% methanol in dichloromethane. The yields of 6-chloro-α-2'-deoxy-3',5'-di-*O*-*p*-toluoylribofuranosylpurine and its β-anomer were 52.3% (135 mg) and 18.2% (47 mg), respectively. An analytical sample was recrystallized from ethanol and dried at the room temperature under vacuum for 3 h. (2-b) : mp 110.8-112.3°C (lit.,[14] 85-88°C). Anal Calcd for $C_{26}H_{23}N_4O_5Cl$ 0.5 H_2O: C, 60.52; H, 4.69; N, 10.86; Found : C, 60.72; H, 4.62; N, 10.79. ^{1}HNmr ($CDCl_3$) δ 8.71 (1H, s, H-2 or H-8), 8.50 (1H, s, H-2 or H-8), 7.97 and 7.52 (4H, H-2,6 of *p*-toluoyl), 7.29 and 7.18 (4H, H-3,5 of *p*-toluoyl) 6.68 (1H, dd, H-1', J = 1.9 Hz and 7.6 Hz), 5.75-5.67 (1H, m, H-3'), 4.97-4.93 (1H, m, H-4'), 4.69-4.63 (2H, m, H-5' and H-5"), 3.15-3.02 (2H, m, H-2' and H-2"), 2.42 (3H, s, CH_3 of *p*-toluoyl), 2.39 (3H, s, CH_3 of *p*-toluoyl).

α-2'-Deoxy-3',5'-di-*O*-*p*-toluoylthymidine (2-c).

Bis-*O*-trimethylsilylthymine (324 mg, 1.2 mmol) was dissolved in a mixture (10 ml) of acetonitrile and tetrahydrofuran (5:1). To this solution was added a solution of 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-ribofuranose (500 mg, 1.3 mmol) prepared in the same manner as above. The whole mixture was stirred at 4°C for 15 h and the solution was poured into cold 5% aqueous $NaHCO_3$ (40 ml). The mixture was extracted with dichloromethane (30 ml X 3) and the extracts were dried over $MgSO_4$ and evaporated under reduced pressure. The residual thick gum was applied to silica gel column flash chromatography (Kiesel gel 60H) using chloroform-ethyl acetate (1:1) as the eluent. The yields of α-2'-deoxy-3',5'-di-*O*-*p*-toluoylthymidine and its β-isomer thus obtained were 60.7% (360 mg) and 19.1% (116 mg), respectively. (2-C) : mp 142.3-143.6°C (lit.,[7] 139°C). ^{1}HNmr ($CDCl_3$) δ 8.66 (1H, br, s, NH), 7.93 and 7.79 (4H, H-2,6 of *p*-toluoyl), 7.41 (1H, s, H-5), 7.28 and 7.23 (4H, H-5,6 of *p*-toluoyl), 6.37 (1H, dd, H-1', J = 1.7 Hz and 7.2 Hz), 5.63-5.59 (1H, m, H-3'), 4.91-4.86 (1H, m, H-4'), 4.54-4.50 (2H, m, H-5' and H-5"), 3.05-2.88 (2H, m, H-2' and H-2"), 2.43 (3H, s, CH_3 of *p*-toluoyl), 2.41 (3H, s, CH_3 of *p*-toluoyl), 1.87 (3H, s, CH_3).

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