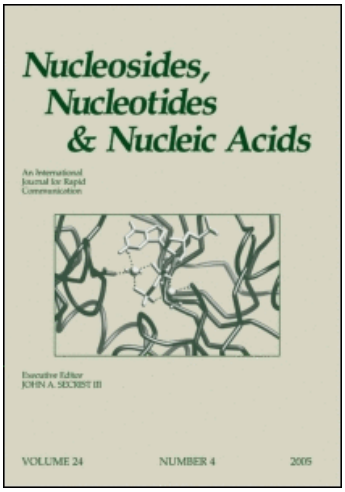

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THE SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-N-ACYL-2'-DEOXYNUCLEOSIDES. IMPROVED "TRANSIENT PROTECTION" APPROACH[1]

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ABSTRACT

The economical large-scale and high yield synthesis of 5'-O-dimethoxytrityl-N-acyl-2'-deoxynucleosides by the improved "transient protection" approach is presented. The structure of side-products and circumstances of their formation during synthesis of N-acyl-2'-deoxynucleosides according to the original "transient protection" method are also described.

INTRODUCTION

The chemical synthesis of oligodeoxynucleotides in either solution or solid-phase includes several steps. Usually the first is large-scale synthesis of pure 5'-O-dimethoxytrityl-N-acyl-2'deoxynucleosides. The "transient protection" method for synthesis of N-acyl-2'-deoxynucleosides as described by G. S. Ti et al.[2] essentially shortens and simplifies the original acylation procedures developed by Khorana.[3] Modifications for synthesizing 2-N-isobutyryl-2'-deoxyguanosine as reported by D. P. C. McGee et al.[4] made it more effective. Nevertheless, during the preparation of N-acyl-2'-deoxynucleosides using the Ti and McGee procedures, precise analysis of reaction mixtures demonstrated that considerable quantities of side-products were formed. After analyzing the structures of these side-products and the circumstances leading to their formation, an improved "transient protection" method for synthesizing 5'-O-dimethoxytrityl-N-acylated derivatives of 2'-deoxyadenosine, 2'-deoxycytidine, and 2'-deoxyguanosine was developed.

RESULTS AND DISCUSSION

The synthesis of N-acyl-2'deoxynucleosides by Ti's method is accompanied by the formation of a few side-products which decrease the

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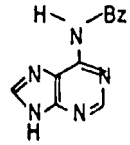
usefulness and economy of this approach especially in large-scale synthesis. The most complicated reaction mixture was the one formed during the synthesis of 6-N-benzoyl-2'-deoxyadenosine. In this case the major side products were:

i. 6-N-Benzoyladenine (**1**) - the product of N-glycosidic bond cleavage. 6-N-Benzoyladenine is formed during the hydrolysis of trimethylsilyl groups which follows the silylation and benzoylation reactions. Ca. 1.7 M solutions of pyridinium chloride, a result of using 10 equivalents of trimethylchlorosilane and benzoyl chloride, in 20% aqueous pyridine appears to be an acid strong enough to cleave the N-glycosidic bonds of **5a** and **6a** (see Figure 2). Under these conditions, complete disilylation and ca. 10% depurination of **5a** and **6a** was observed after 5 min. The same degree of depurination was observed in the control experiment when 6-N-benzoyl-2'-deoxyadenosine was treated with 10 equivalents of pyridinium chloride (1.7 M) in 20% aqueous pyridine.

ii. 5'-O,6-N-Dibenzoyl-2'-deoxyadenosine (**2b**) - formed during the benzoylation of **4a**. Compounds **5a** and **6a**, the products of benzoylation, appear to be unstable to benzoyl chloride which leads to partial deprotection of the 5'-O-trimethylsilyl group. The deblocking reagent appears to be pyridinium chloride. 5'-O-silyl groups are known to be more acid labile



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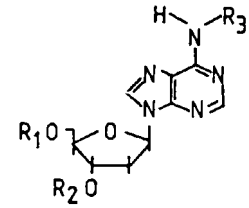

/2/

a. $R_1 = R_2 = H$ $R_3 = Bz$
b. $R_1 = R_3 = Bz$ $R_2 = H$
c. $R_2 = R_3 = Bz$ $R_1 = H$
d. $R_1 = R_2 = R_3 = Bz$

Figure 1. The Structure of Side Products

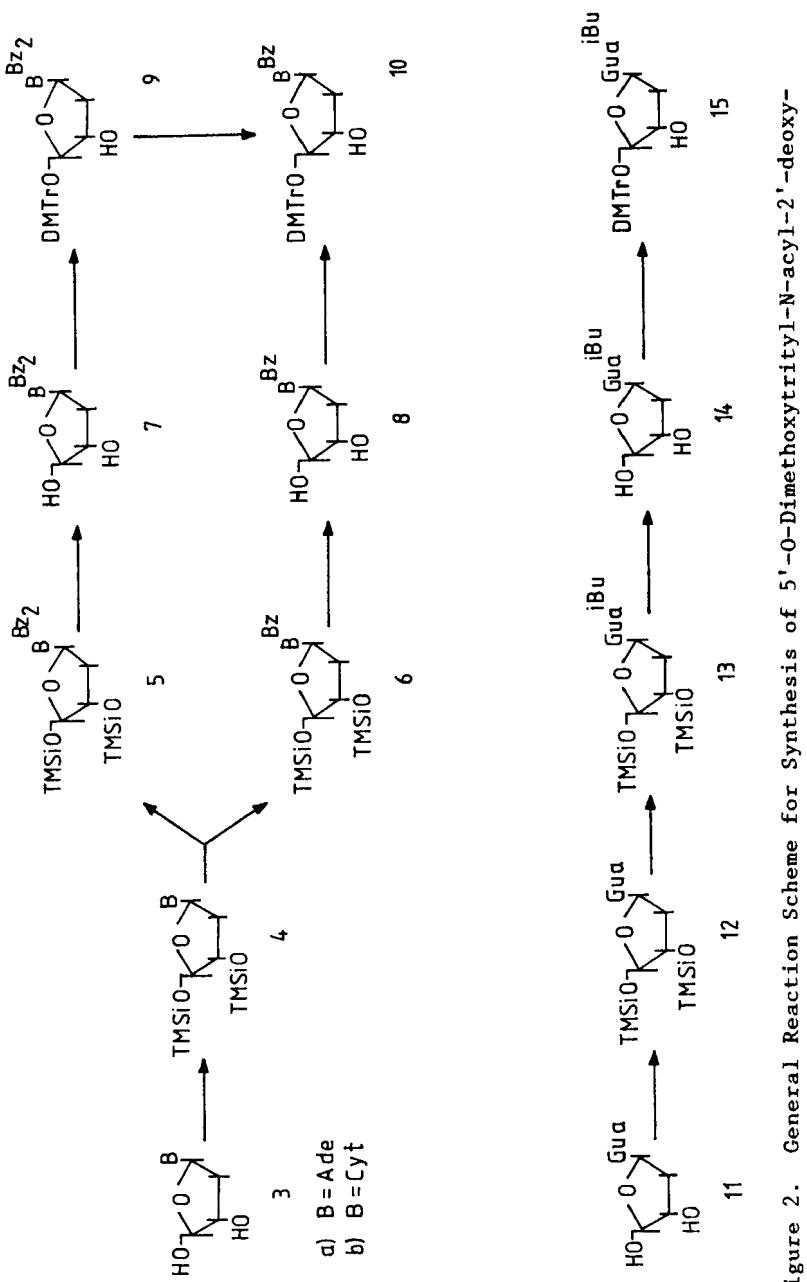

Figure 2. General Reaction Scheme for Synthesis of 5'-O-Dimethoxytrityl-N-acyl-2'-deoxynucleosides

than 3'-O-silyl groups.[5,6] This fact explains the structure of **2b**.

iii. 2'-Deoxyadenosine - not the result of incomplete benzoylation of **4a**. 2'-Deoxyadenosine is formed during treatment of the reaction mixture with 29% aqueous ammonia as used in the original procedure.[2] Transformations of 6-N,N-dibenzoyl-2'-deoxyadenosine into 6-N-benzoyl-2'-deoxyadenosine followed by partial cleavage of all N-benzoyl groups were observed using these conditions.

Trace amounts of 3'-O-6-N-dibenzoyl-2'-deoxyadenosine (**2c**) and 5',3'-O,6-N-tribenzoyl-2'-deoxyadenosine (**2d**) were also observed. These derivatives as well as those described in points i-iii significantly reduce the yield. Consequently, 6-N-benzoyl-2'-deoxyadenosine (**2a**) constitutes only ca. 80% of the reaction mixture.

Similar side-reactions, except N-glycosidic bond cleavage, were observed for 2'-deoxycytidine and 2'-deoxyguanosine. Synthesis of 2-N-isobutyryl-2'-deoxyguanosine (**14**) with trimethylchlorosilane led to an additional trimethylsilyl derivative (ca. 20%) which was probably 5',3'-O-2-N-trimethylsilyl-2'-deoxyguanosine. The use of hexamethyldisilazane as silylating reagent by D. P. C. McGee et al. solved this problem. However in the presence of hexamethyldisilazane isobutyryl chloride cannot be used as an acylating reagent.

The structure of all side-products was determined by ^{1}H NMR and UV spectra and also by comparison with the authentic samples, tlc, and chemical transformation to other derivatives.

To overcome these difficulties and simplify the procedure the following main changes were introduced:

- Conditions of silylation and acylation of 2'-deoxynucleosides were altered. Structures of side-products indicate that the side-reactions take place mainly due to the presence of pyridinium chloride which is acidic enough to cleave the N-glycosidic bond of 2'-deoxyadenosine derivatives. Therefore triethylamine, a more effective proton scavenger, was chosen instead of pyridine.
- Triethylammonium fluoride[7] was used for deprotection of trimethylsilyl groups.

- Morpholine[8] was used for selective transformation of 6-N,N-dibenzoyl into 6-N-benzoyl-2'-deoxyadenosine.

For the silylation reaction, 2'-deoxyadenosine (**3a**) or 2'-deoxycytidine (**3b**) was suspended in a mixture of chloroform and triethylamine. All products of each reaction were soluble in the reaction mixtures. Following silylation, the benzoylation reaction was then completed in 60 min using 4-dimethylaminopyridine (DMAP)[9] as catalyst. During the addition of benzoyl chloride, the reaction mixture must be cooled at -50°C to -40°C. Using these conditions, the 5'-O-trimethylsilyl group is more stable which leads to less 5'-O,N-dibenzoyl derivatives such as **2b**. Excess trimethylchlorosilane and benzoyl chloride were neutralized by adding absolute methanol. After silylation and benzoylation, the reaction mixtures contained ca. 90% and 5% of the N,N-dibenzoylated derivatives of 2'-deoxyadenosine (**5a**) and 2'-deoxycytidine (**5b**), respectively. Triethylammonium chloride was removed by extraction with saturated aqueous solution of sodium bicarbonate or, in the case of large-scale reactions, by evaporation of chloroform and filtration of the triethylammonium chloride suspended in dioxane. During the first work-up procedure some desilylation of **5** and **6** was observed. The trimethylsilyl groups were deprotected by triethylammonium fluoride in pyridine (20 min). However the reaction mixture can be safely left overnight. Trimethylfluorosilane (bp 16.4°C) generated in the reaction was gradually removed under reduced pressure. Dimethoxytritylation was carried out by treating mixtures of N-benzoyl-2'-deoxynucleosides and N,N-dibenzoyl-2'-deoxynucleosides with equimolar amounts of 4,4-dimethoxytrityl chloride in dry pyridine for 60 min. After dimethoxytritylation, N,N-dibenzoylated derivatives (**9a**, **9b**) were selectively transformed into N-monobenzoylated derivatives (**10a**, **10b**) by reaction with morpholine.

For the synthesis of 5'-O-dimethoxytrityl-2-N-isobutyryl-2'-deoxyguanosine (**15**), hexamethyldisilazane was used for silylation.[4] This reagent selectively protected hydroxyl groups. As mentioned previously, removal of excess silylating reagent before the next reaction is very important. Hexamethyldisilazane and isobutyryl chloride probably react to generate trimethylchlorosilane.[10] Presence of trimethylchlorosilane can then lead to the formation of additional silylated 2'-deoxyguanosine derivatives. The excess hexamethyldisilazane was

removed by co-evaporation, first with dimethylformamide and then with absolute methanol to remove traces of the disilazane. In contrast to the 5', 3'-di-O-trimethylsilyl-2'-deoxyadenosine and 2'-deoxycytidine derivatives, the analogous derivative of 2'-deoxyguanosine was only slightly soluble in many solvents. In pyridine, this 2'-deoxyguanosine derivative was partially soluble but nevertheless reacts quantitatively using a 1.3-fold excess of isobutyryl chloride. In other solvents such as dichloromethane and chloroform, acylation was much slower and incomplete even with 4-dimethylaminopyridine[9] (DMAP) as a catalyst. The final steps in the synthesis of 5'-O-dimethoxytrityl-2-N-isobutyryl-2'-deoxyguanosine (desilylation and dimethoxytritylation) were completed as outlined for 2'-deoxyadenosine and 2'-deoxycytidine except morpholine was not used since the disubstituted derivative of 2'-deoxyguanosine does not form.

Optimization of reaction conditions following these changes led to the synthesis of the 5'-O-dimethoxytrityl-N-acyl-2'-deoxynucleosides in 83-88% yield as one flask syntheses. The main side-products (5'-O,N-diacyl-2'-deoxynucleosides) were isolated in ca. 5-7% yield. By this approach the synthesis of very pure 5'-O-dimethoxytrityl-N-acyl-2'-deoxynucleosides in high yield is possible in a short time, 1-2 days, depending on the scale of the reaction.

EXPERIMENTAL SECTION

General Procedure

Deoxynucleosides were purchased from Pharma Waldhof. TLC Kieselgel type H (Merck) was used for separation of final products. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) and Kieselgel 60 F_{254} siliconized (Merck) plates. The ^1H NMR spectra were recorded on Jeol FX 90Q with Me_4Si as internal reference. The UV spectra were recorded on Specord UV Vis.

Synthesis of 5'-O-dimethoxytrityl-6-N-benzoyl-2'-deoxyadenosine (**10a**) and 5'-O-dimethoxytrityl-4-N-benxoyl-2'-deoxycytidine (**10b**)

2'-Deoxyadenosine monohydrate (**3a**, 26.92 g, 100 mM) or 2'-deoxycytidine hydrochloride (**3b**, 26.27 g, 100 mM) was dried by evaporation with dry pyridine. In the case of 2'-deoxyadenosine, the substrate was dissolved in hot pyridine (200 mL) and evaporate. The 2'-deoxynucleoside was suspended in a mixture of 500 mL of chloroform (methanol free)

and triethylamine (108.5 mL, 750 mM) and stirred at room temperature. Trimethylchlorosilane (38.0 mL, 300 mM) in 100 mL of chloroform was added in 15 min. After the next 15 min the reaction mixture was cooled at -50°C to -40°C and 4-dimethylaminopyridine (1.26 g, 10 mM) was added. Benzoyl chloride (34.8 mL, 300 mM and 17.4 mL, 150 mM, for **3a** and **3b**, respectively) was diluted in 100 mL of chloroform and added dropwise during 15 min. The reaction was maintained at room temperature for 45 min. After this time 24.0 mL (600 mM) of absolute methanol was added and after 10 min the reaction mixture was evaporated to near dryness. 100 mL of dioxane were then added to the residue, and the mixture again evaporated to near dryness. The residue was suspended in 200 mL dioxane, and the suspension filtered to remove insoluble by-products. The solution was evaporated to a gum and 300 mL of 1 M pyridine solution of triethylammonium fluoride (300 mM) was added and kept for 20 min under the pressure of 700-600 mm Hg. Next 120 mL of saturated aqueous solution of sodium bicarbonate was added. The reaction mixture was evaporated and dried by co-evaporation twice with 50 mL of dry pyridine. The residue was suspended in 100 mL of pyridine and filtered. The filtrate was co-evaporated twice with dry pryidine to leave a gum. 200 mL of dry pyridine and dimethoxytrityl chloride (33.8 g, 100 mM) were then added. After 60 min 21.8 mL (250 mM) and 16.1 mL (150 mM) of morpholine (for **3a** and **3b**, respectively) was added and left for 30 min. A saturated aqueous solution of sodium bicarbonate (250 mL) was then added to the reaction mixture and the resulting mixture was extracted with chloroform. The separated organic layer was washed with 600 mL of 0.5 M aqueous solution of sodium dihydrogen phosphate in order to remove the remaining morpholine as the phosphate salt. The organic layer was evaporated and purified by chromatography on silica gel column. Yield of synthesis for all steps: 58.0 g (88%) or 53.3 g (86%) for 2'-deoxyadenosine and 2'-deoxycytidine, respectively. The products were chromatographically and spectroscopically (UV, ^{1}H NMR) identical with authentic materials.[2,4]

Synthesis of 5'-O-dimethoxytrityl-2-N-isobutyryl-2'-deoxyguanosine (**15**)

2'-Deoxyguanosine monohydrate (**11**, 28.52 g, 100 mM) was evaporated twice with dry dimethylformamide (250 mL and 150 mL). The residue was suspended in 150 mL of dimethylformamide and stirred at room tempera-

ture. Hexamethyldisilazane (41.0 mL, 400 mM) was added for 10 min. After the next 15 min the reaction mixture was evaporated to dryness and once again evaporated with 100 mL of dimethylformamide and 15 mL (250 mM) of absolute methanol. After 5 min the mixture was evaporated to dryness followed by 100 mL of dry pyridine. The residue was suspended in 300 mL of dry pyridine. To the reaction mixture cooled at -50°C to -40°C with stirring isobutyryl chloride(12.6 mL, 120 mM) in 100 mL of pyridine was added during 15 min. The mixture was left at room temperature for 60 min. The reaction mixture was poured into a saturated aqueous solution of sodium bicarbonate and extracted with chloroform. The combined organic phase was dried and evaporated. The gum was dissolved in 300 mL of 1 M pyridine solution of triethylammonium fluoride. The next steps (desilylation and dimethoxytritylation) were the same as for derivatives of 2'-deoxyadenosine and 2'-deoxycytidine except that addition of morpholine and work-up with the sodium dihydrogen phosphate solution were omitted. Yield of synthesis for all steps: 53.1 g (83%). The product was chromatographically and spectroscopically (UV, ^{1}H NMR) identical with the authentic material.[2,4]

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REFERENCES

1. A preliminary communication was presented in the poster section at the IUPAC 14th International Symposium on the Chemistry of Natural Products, July 9-14, 1984, Poznań, Poland
2. G. S. Ti, B. L. Gaffney and R. A. Jones, J. Am. Chem. Soc. 104, (1982)
3. H. Schaller, G. Weiman, B. Lerch and H. G. Khorana, J. Am. Chem. Soc. 85, 3821 (1963)
4. D. P. C. McGee, J. C. Martin and A. S. Weeb, Synthesis, 540 (1983)
5. K. K. Ogilvie, S. L. Beaucage, A. L. Schifman, N. Y. Theriault and K. L. Sadana, Can. J. Chem. 56, 2768 (1978)
6. W. T. Markiewicz, J. Chem. Research (S), 24 (1979)
   J. Chem. Research (M), 181 (1979)

7. W. T. Markiewicz, E. Biała, R. W. Adamiak, K. Grześkowiak, R. Kierzek, A. Kraszewski, J. Stawiński and M. Wiewiórowski, Nucleic Acids Res. Symposium Series No 7, 115 (1980)
8. P. A. Lyon, C. B. Reese, J. Chem. Soc. Perkin Trans. I, 2645 (1974)
9. G. Hofle, W. Steglich, H. Vorbraiggen, Angew. Inter., 17, 569 (1978)
10. C. Eaborn, "Organosilicon Compounds," Butterworths Scientific Publications, London, 1960, p. 304

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