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SELECTIVE REMOVAL OF TERMINAL DIMETHOXYTRITYL GROUPS

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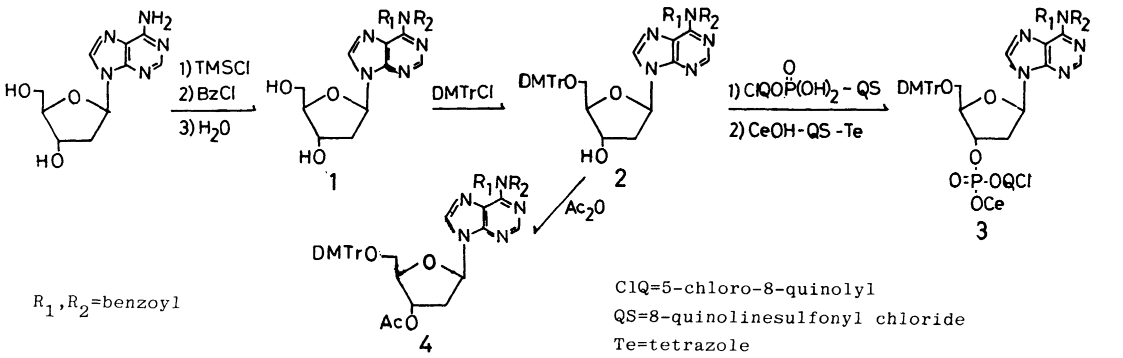
N^6,N^6,3',5'-O-Protected deoxyadenosine derivatives were found to be key intermediate for synthesis of oligodeoxyribonucleotides. Treatment of protected deoxyadenosine derivatives with 3% trichloroacetic acid in nitromethane-methanol (95:5) gave the corresponding de-dimethoxytritylated deoxyadenosine derivatives without causing any damage to the glycosidic bond.

Dimethoxytrityl group has been extensively used to the protection of 5'-hydroxyl functions in the synthesis of oligodeoxyribonucleotides by the liquid phase or solid phase methods. Recently, many workers have reported[1] a mild condition for removal of the dimethoxytrityl group in order to overcome a side reaction such as a cleavage of the glycosidic bond on N^6-benzoyladenosine in oligodeoxyribonucleotide synthesis. More recently, Hata has reported[2] that N^6-phthaloyldeoxyadenosine derivative is a key intermediate for the synthesis of oligodeoxyribonucleotides containing the adenosine unit.

In this paper, we wish to propose the N^6,N^6,3',5'-O-protected deoxyadenosine derivatives without causing any damage to the glycosidic bond in the synthesis of oligodeoxyribonucleotides.

Starting N^6,N^6,3',5'-O-protected deoxyadenosine derivatives (**3** and **4**) were prepared as follows: N^6,N^6-Dinenzoyldeoxyadenosine (**1**) was prepared according to the procedure reported by Jones.[3] However, we observed the formation of by-products (such as N^6-benzoyldeoxyadenosine) during the benzoylation of deoxyadenosine by Jones method. Consequently, the pure **1** was obtained in 85% yield after separation by silica gel coulmn chromatography.[4] N^6-Phthaloyldeoxyadenosine[2] was unstable in a weakly basic aqueous solution (pyridine-water), whereas, **1** was found to be stable under same condition. The nucleoside **1** (2.3 g, 5 mmol) was tritylated with dimethoxytrityl chloride (2.02 g, 6 mmol) in dry pyridine at room temperature for 2.5 h. The tritylated product **3** was obtained by chromatography on silica gel in 87% (3.31 g).[5] The compound **2** (3.05 g, 5 mmol) was treated with 5-chloro-8-quinolyl phosphate[6] (0.83 g, 4.8 mmol) in the presence of 8-quinolinesulfonyl chloride (QS)[7] (2.21 g, 9.6 mmol) in dry pyridine. After 1 h, the reaction was quenched with ice-water and the phosphodiester formed was extracted with CH_2Cl_2 which was washed with water. The extract CH_2Cl_2 was evaporated <u>in vacuo</u> and the residue was dissolved in dry pyridine and then 2-cyanoethanol (0.98 g, 12 mmol), QS (1.83 g, 8 mmol) and 1H-tetrazole (0.84 g, 12 mmol) were added. The reaction mixture was stirred at room temperature for 10 h. After the usual workup, chromatography on silica gel afforded the phosphodiester derivative **3**[8] (3.75 g, 89%). On the other hand, treatment of **2** with acetic anhydride in dry pyridine gave the expected product **4**[9] in 85% yield.



R_1,R_2=benzoyl

ClQ=5-chloro-8-quinolyl
QS=8-quinolinesulfonyl chloride
Te=tetrazole

Results of depurination of the protected adenosine derivatives by treatment with various acidic reagents are presented in Figs. 1 and 2. From these results, the followings are concluded: (i) The fully protected deoxyadenosine derivatives (**4** and **5**) are more stable to acid than other deoxyadenosine derivatives. (ii) The rates of depurination are much faster than those after detritylation. (iii) Trichloroacetic acid (3%) in a mixture of CH_3NO_2 and MeOH (95:5) is an effective acidic reagent for the de-dimethoxytritylation step. Using this reagent, de-dimethoxytritylation is completed in less than 5 min, and within this time, depurination was not detected. When comparing $ZnBr_2$ in CH_3NO_2 to our reagent, 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5), we found that the depurination rates of $ZnBr_2$ and

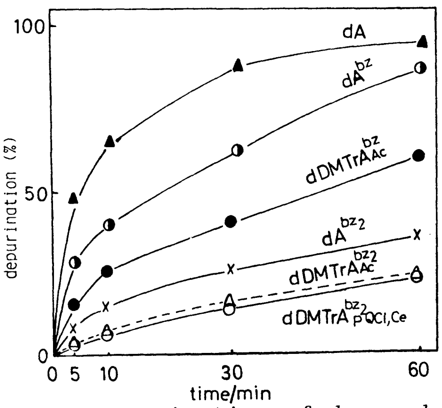


Fig. 1. Depurination of deoxyadenosine derivatives with 80% AcOH.

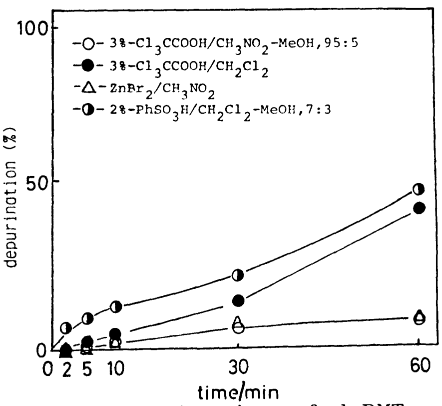


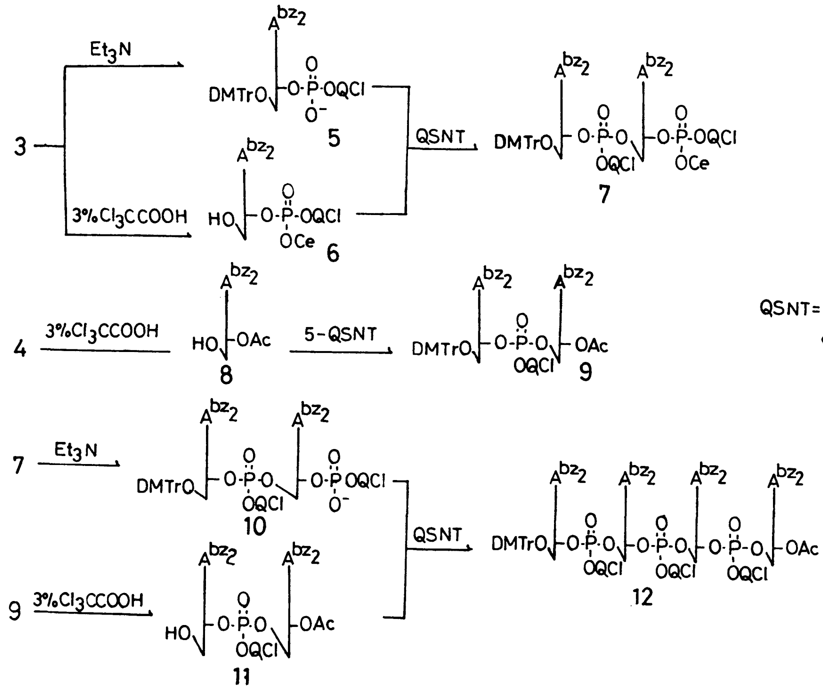
Fig. 2. Depurination of d-DMTr-bz_2AOAc with various acidic reagents.

In each case, the substrate (0.01 mmol) was treated with various acidic reagents (2 ml) at 25 °C and the rates of depurination were estimated by thin layer chromatography.

3% Cl_3CCOOH were comparable, but the rate of detritylation was much slower than with 3% Cl_3CCOOH. The results obtained indicate that the fully protected deoxyadenosine 3 in combination with 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5) is a highly effective unit for the oligodeoxyribonucleotide synthesis containing the deoxyadenosine.

Next, we examined the synthesis of dimers (7 and 9), and tetramer (12) by using the deoxyadenosine units 3 and 4. Removal of dimethoxytrityl group from 3 was performed by treatment with 3% Cl_3CCOOH in CH_3NO_2-MeOH (95:5) (0.007 mmol of 3/ ml) at room temperature for 3 min. The mixture was quenched with pyridine and extracted with CH_2Cl_2. The extract CH_2Cl_2 was washed with water, dried over Na_2SO_4 and evaporated in vacuo. The residue was chromatographed on silica gel to give the corresponding detritylated product (6) in 95% yield. In this reaction, depurination was not detected. On the other hand, the phosphotriester 3 (1.503 g, 1.5 mmol) was treated with Et_3N (12 ml) in CH_3CN (12 ml) at room temperature for 2 h. Following removal of most of the solvent in vacuo, the residue and 6 (0.699 g, 1.0 mmol) were coevaporated with dry pyridine. The residue was dissolved in dry pyridine (7 ml) and then 8-quinolinesulfonyl-3-nitro-1,2,4-triazole (QSNT)[10] (1.14 g, 3.75 mmol) was added. After 3 h, the reaction was completed and the usual work-up gave the corresponding dinucleotide derivative (7) in 90% (1.474 g) yield. Similarly, treatment of 4 (0.546 g, 1.0 mmol) with 3% Cl_3CCOOH afforded the detritylated product (8) in 95% (0.249 g) yield, whereas mild treatment of 3 (1.052 g, 1.05 mmol) with Et_3N gave the phosphodiester (5). A solution of both compounds 8 (0.184 g, 0.7 mmol) and 5 in dry pyridine (6 ml) was treated with QSNT (0.875 g, 2.88 mmol) at room temperature for 3 h. The fully protected dinucleotide (9) was obtained in 92% (1.318 g) yield after separation by silica gel column chromatography.

The 2-cyanoethyl and dimethoxytrityl groups were removed from 7 (0.734 g, 0.45 mmol) and 9 (1.318 g, 0.64 mmol) according to the above procedures to give 10 and 11 (96%, 0.694 g), respectively. A solution of both the components 10 and



11 (0.339 g, 0.3 mmol) in dry pyridine (3 ml) was treated with QSNT (0.342 g, 1.13 mmol) for 3 h. The fully protected tetradeoxyadenylate (12) was isolated by chromatography on silica gel in 85% (0.686 g).

Complete deblocking of the fully protected tetranucleotide (12) was performed as follows: The tetramer 12 (27 mg, 10 μmol) was treated with 0.06 M-N^1,N^1,N^3,N^3-tetramethylguanidium salt of 2-pyridine carboaldoxime[11] in dioxane-water (2:1) (1 ml) at room temperature for 18 h. The solution was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with 50% aqueous pyridine. The filtrate was evaporated in vacuo and the residue was treated with conc.ammonia at 60 °C for 5 h. The solution was concentrated and 80% AcOH was added. After 15 min, the solution was evaporated with water and then with pyridine. The residue was dissolved in water and washed with ether, and then with concentrated to an oil. The deblocked tetramer d-ApApApA was obtained in 80% (201 OD) yield after chromatographic separation using Toyo Roshi No. 514 paper with n-PrOH-conc.NH_4OH-H_2O (55:10:35, v/v). The purity of d-ApApApA was checked by PE and HPLC on μ Bondapak C_{18} as well as hydrolysis with nucleaae P1 to d-A and d-pA in the ratio of 1.00:2.98.

References

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4) Mp 98-100 °C; UV (MeOH) λmax 274, 249 nm, λmin 264 nm; ^{1}H NMR ($CDCl_3$-D_2O) δ 2.12-2.75 (2H, m, 2'-CH_2), 3.69 (2H, br. s, 5'-CH_2), 3.95 (1H, m, 4'-CH), 4.49 (1H, m, 3'-CH), 6.45 (1H, t, $J_{1',2'}$=7 Hz, 1'-CH), 6.99-7.95 (10H, m, ArH), 8.20 (1H, s, 2-CH), 8.42 (1H, s, 8H); Found: C, 62.48; H, 4.97; N, 14.95%. Calcd for $C_{24}H_{21}N_5O_5$: C, 62.74; H, 4.61; N, 15.24%.
5) ^{1}H NMR ($CDCl_3$) δ 2.50-298 (2H, m, 2'-CH_2), 3.45 (2H, br. s, 5'-CH_2), 3.75 (6H, s, OCH_3), 4.06 (1H, m, 4'-CH), 4.46 (1H, m, 3'-CH); 6.37 (1H, t, $J_{1',2'}$=7.1 Hz, 1'-CH), 6.65-7.89 (25H, m, ArH), 8.15 (1H, s, 2-CH), 8.52 (1H, s, 8-CH); Found: C, 69.93; H, 5.37; N, 8.91%. Calcd for $C_{44}H_{39}N_5O_7$.1/2: C, 69.64; H, 5.32; N, 9.23%.
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8) UV (MeOH) λmax 276, 230 nm, λ min 260 nm; Found: C, 68.51; H, 4.48; N, 9.95%. Calcd for $C_{57}H_{47}N_7O_{10}Cl$: C, 68,33; H, 4.73; N, 9.78%.
9) ^{1}H NMR ($CDCl_3$) δ 2.12 (3H, s, Ac), 2.30-2.99 (2H, m, 2'-CH_2), 3.50 (2H, br. s, 5'-CH_2), 3.70 (3H, s, OCH_3), 4.22 (1H, m, 4'-CH), 5.55 (1H, m, 3'-CH), 6.30 (1H, t, $J_{1',2'}$=7.2 Hz, 1'-CH), 6.62-8.92 (25H, m, ArH), 8.15 (1H, s, 2-CH), 8.52 (1H, s, 8-CH); Found: C, 69,91; H, 5.39 ; N, 8.46%. Calcd for $C_{46}H_{41}N_5O_8$: C, 70.22; H, 5.14; N, 8.71%.
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