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Synthesis of Nucleoside 5'-Deoxy-5'-Difluoromethylphosphonates

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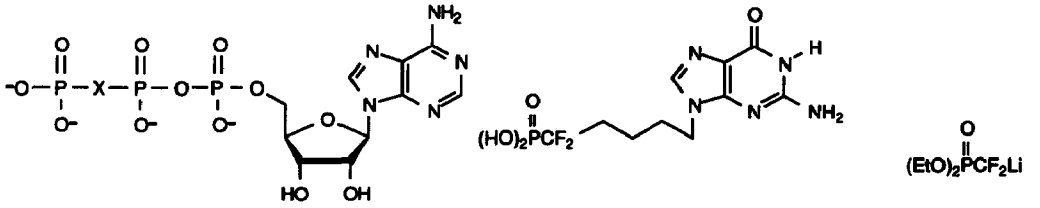
Abstract: 1-*O*-Acetyl-2,3-di-*O*-benzoyl-D-ribofuranose 5-deoxy-5-difluoromethylphosphonate was synthesized in three steps from 1-*O*-methyl-2,3-*O*-isopropylidene-β-D-ribofuranose 5-deoxy-5-difluoromethylphosphonate. Condensation of this suitably derivatized sugar with silylated pyrimidines and purines afforded novel nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Phosphonic acids often exhibit important biological properties because of their similarity to phosphates.[1] It has been suggested by Blackburn and Kent[2] that α-fluoro and α,α-difluoromethylphosphonates should mimic phosphate esters better than the corresponding phosphonates. This assumption was based both on electronic and steric considerations. Analogues of pyro- and triphosphates 1 where the bridging oxygen atoms were replaced by a difluoromethylene group have been successfully employed as substrates in enzymatic processes.[3] 9-(5,5-Difluoro-5-phosphonopentyl)guanine 2 has been utilized as a multisubstrate analogue inhibitor of purine nucleoside phosphorylase.[4] Oligonucleotides containing methylene groups in place of phosphodiester 5'-oxygens are resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5'-oxygen,[5] but can still form stable complexes with complementary sequences. Heinemann *et al.*[6] found that a single 3'-methylenephosphonate linkage had only a minor influence on the conformation of a DNA octamer double helix. Encouraged by these studies we undertook the synthesis of difluoromethylphosphonate nucleosides for incorporation into oligonucleotides.



1 **2** **3**

X = O, CH_2, CHF, CF_2, CCl_2

One common synthetic approach to α,α-difluoroalkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate 3.[7] However, our attempts to synthesize nucleoside

5'-deoxy-5'-difluoromethylphosphonates from 5'-deoxy-5'-iodonucleosides using 3 were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with 3, according to the procedure of Martin *et al.*,[8] led to a complex mixture of products. Recently, the synthesis of sugar α,α-difluoroalkylphosphonates from primary sugar triflates using 3 was described.[9] Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.

Based on the above experiments we synthesized a suitable glycosylating agent from the known D-ribose α,α-difluoromethylphosphonate 4[8] that served as a key intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

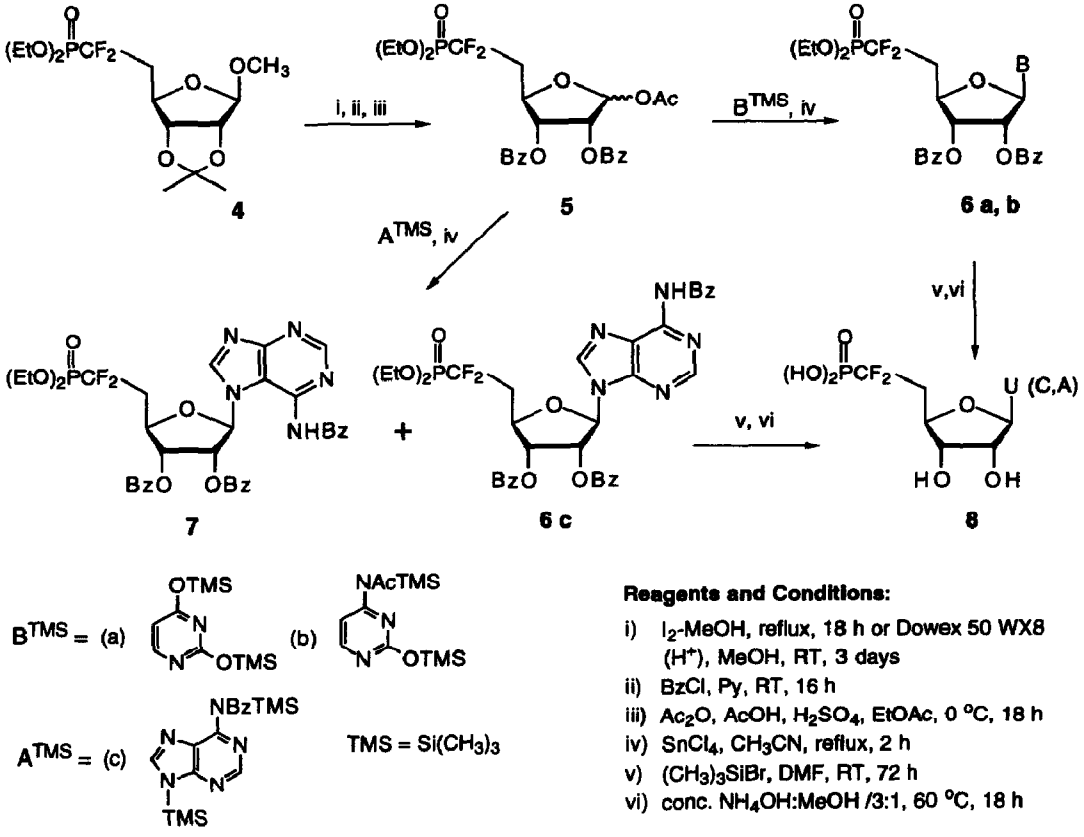


Figure 1. Synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates[15,16]

Methyl 2,3-*O*-isopropylidene-β-D-ribofuranose α,α-difluoromethylphosphonate 4 was synthesized from the 5-aldehyde according to the procedure of Martin *et al.*[8] (Figure 1). Removal of the isopropylidene group was accomplished under mild conditions (I_2-

MeOH, reflux, 18 h[10] or Dowex 50 WX8 (H^+), MeOH, RT, 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-di-*O*-benzoyl derivative, which was subjected to mild acetolysis conditions[11,12] (Ac_2O, AcOH, H_2SO_4, EtOAc, 0 °C). The desired 1-*O*-acetyl-2,3-di-*O*-benzoyl-D-ribofuranose difluoromethylphosphonate **5** was obtained in quantitative yield as an anomeric mixture. These derivatives[13] were used for selective glycosylation of silylated uracil and N^4-acetylcytosine under Vorbrüggen conditions[14] ($SnCl_4$ as a catalyst, boiling acetonitrile) to yield β-nucleosides (62% **6a**, 75% **6b**).[15] Glycosylation of silylated N^6-benzoyladenine yielded a mixture of N-9 isomer **6c** and N-7 isomer **7**[16] in 34% and 15% yield, respectively. The above nucleotides were successfully deprotected[17] using bromotrimethylsilane for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates **8** were finally purified on a DEAE Sephadex A-25 (HCO_3^- form) column using a 0.01-0.25 M TEAB gradient for elution and obtained as their sodium salts (82% **8a**; 87% **8b**; 82% **8c**).

Efforts to introduce 5'-deoxy-5'-difluoromethylphosphonate linkages into oligonucleotides, as well as the studies of biological activities of nucleoside 5'-deoxy-5'-difluoromethylphosphonates and their respective 5',3'-cyclic difluoromethylphosphonates are in progress.

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12. Commonly used acetolysis conditions (Ac_2O, AcOH, H_2SO_4, RT) led to the destruction of **5**.

13. During the preparation of this manuscript Levy, S.G.; Watson, D.B.; Buckley, K.; Carson, D.A.; Cottam, H.B. reported the use of intermediate **5** in a similar context in abstract ORGN #328 to be presented at the 207th Meeting of the American Chemical Society, San Diego, March 13-18, 1994.

14. (a) Vorbrüggen, H. *Nucleoside Analogs. Chemistry, Biology and Medical Applications*, NATO ASI Series A *26*, Plenum Press, New York, London, **1980**; pp. 35-69. (b) The use of $F_3CSO_2OSi(CH_3)_3$ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 1-ethyluracil or 9-ethyladenine byproducts: Podyukova, N.S.; Karpeisky, M.Y.; Kolobushkina, L.I.; Mikhailov, S.N. *Tetrahedron Lett.* **1987** *28*, 3623-3626 and references cited therein.

15. In a typical glycosylation experiment 1-*O*-acetyl-2,3-di-*O*-benzoyl-D-ribofuranose difluoromethylphosphonate **5** (1 mmol) was dissolved in dry CH_3CN (17 mL) and added, under argon, to a solution of the silylated nucleobase (2 mmol). The latter was prepared by refluxing the nucleobase with 1,1,1,3,3,3-hexamethyldisilazane:pyridine/1:1 (4 mL) until complete dissolution occurred followed by the removal of volatiles under reduced pressure and coevaporation with dry toluene (2 x 10 mL). Tin (IV) chloride was added (1.1 mmol) and the mixture was heated under reflux for 2 h. After cooling to RT, the mixture was diluted with dichloromethane (100 mL) and extracted with aq. $NaHCO_3$ (50 mL) and H_2O (30 mL). The organic layer was dried (Na_2SO_4) and concentrated to a syrup. The product was purified by flash chromatography using a 1-5% methanol in dichloromethane gradient. Selected analytical data: ^{31}P-NMR (^{31}P) and ^{1}H-NMR (^{1}H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was $CDCl_3$ unless otherwise noted. **5**: ^{1}H δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, αH1), 6.42 (s, βH1), 5.74 (d, $J_{2,3}$ 4.9, βH2), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, βH3), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, αH3), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, αH2), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{P,F}$ 105.2), 7.67 (t, $J_{P,F}$ 106.5). **6a**: ^{1}H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',F}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{H,F}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{P,F}$ 104.0). **8c**: ^{31}P (*vs* DSS) (D_2O) δ 5.71 (t, $J_{P,F}$ 87.9).

16. Compound **7** was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

17. In a typical deprotection experiment bromotrimethylsilane (2.64 mL, 20 mmol) was added dropwise under argon to a stirred solution of fully protected nucleoside 5'-difluoromethylphosphonate (1 mmol) in dry DMF (7 mL). The reaction mixture was set aside at RT for 72 h or heated at 65 °C for 2 h. TLC in 2-propanol:NH_4OH:H_2O/7:1:2 showed complete cleavage of the ethyl ester groups. The solution was concentrated to a syrup under reduced pressure and the residue coevaporated twice with dry toluene. NH_4OH (15 mL) and methanol (5 mL) were added and the solution kept at 60 °C for 18 h. Volatiles were removed *in vacuo* and the residue dissolved in 0.01M TEAB and applied to a column of DEAE Sephadex A-25 (HCO_3^-) (2.5 x 30 cm). Elution using 0.01-0.25 M TEAB gradient and removal of buffer by evaporation and then multiple coevaporations with methanol yielded a syrup that was dissolved in H_2O (10 mL) and passed through a column of Dowex 50 WX8 (Na^+). Evaporation of the eluate to dryness yielded the nucleoside 5'-difluoromethylphosphonate sodium salt as a white powder.

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