[Chem. Pharm. Bull.
29(4)1034—1038(1981)]

Studies on Nucleosides and Nucleotides. LXXXVII.[1] Purine Cyclonucleosides. XLII. Synthesis of 2′-Deoxy-2′-fluoroguanosine

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(Received October 8, 1980)

2′-Deoxy-2′-fluoroguanosine (VII) was synthesized starting from 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosylguanine (8,2′-O-cycloguanosine) (I). Compound I was protected at 2-NH_2 with an isobutyryl group and at 3′- and 5′-OH with tetrahydrofuranyl groups. The protected compound III was derivatized to the arabino nucleoside V and thence converted to VII by treatment with trifluoromethanesulfonyl chloride and tetra-*n*-butylammonium fluoride. The resulting 2′-deoxy-2′-fluoroguanosine showed a 3′-*endo* favored conformation.

Keywords——8,2′-O-cycloguanosine; tetrahydrofuranyl protection; inversion of arabino- to 2′-fluoro-2′-deoxyribofuranose; NMR; UV; PPC; TLC

Recently we have developed a method to introduce various substituents into the 2′-position of adenosine.[2] We found that the puckering conformation of these adenosine analogs varied continously from 2′-*endo* (S) to 3′-*endo* (N) form according to the electronegativity of the substituents.[3] Furthermore, these nucleosides were also led to 5′-diphosphates and polymerized by polynucleotide phosphorylase to polynucleotides having properties very similar to those of ribopolynucleotides.[4–9] In particular, poly(2′-deoxy-2′-azido- and fluoroinosinic acid) showed strong interferon-inducing activity when hybridized with poly(cytidylic acid),[10,11] showing that total polynucleotide conformation rather than the 2′-OH group was important for the interferon induction. Quite recently, 2′-deoxy-2′-azido- and fluoro-ATP were found to be inhibitors of RNA synthesis by virus RNA polymerase.[12]

We now wish to report on the synthesis of a guanosine analog, 2′-deoxy-2′-fluoroguanosine, which should be useful in various physical and biological studies, as has been the case with adenosine analogs.

The starting material for 2′-deoxy-2′-fluoroguanosine was 8,2′-anhydro-8-oxy-9β-D-arabinofuranosylguanine (I),[13] which has been utilized to synthesize an antibiotic, 2′-amino-2′-deoxyguanosine.[14] However, it was found that compound I itself was poorly soluble in organic solvents. Compound I was first acylated to an isobutyryl derivative at the 2-NH_2, 3′- and 5′-OH groups and then treated with 1 N NaOH at 0° to give the N^2-isobutyryl derivative (II), mp 195° in a yield of 79%. The structure of compound II was confirmed by its ultraviolet (UV) absorption maxima at 260 and 294 nm, as well as by elemental analysis data and the molecular peak at *m/e* 351 in the mass spectrum. N^2-Isobutyrylcycloguanosine (II) was then protected at 3′- and 5′-OH. For this purpose the tetrahydrofuranyl group, which has been successfully used in the case of 2′-deoxy-2′-fluoroadenosine,[2] was used. With *p*-toluenesulfonic acid as an acid catalyst, compound II was stirred with 10 equivalents of dihydrofuran in tetrahydrofuran (THF) for 1 hr at room temperature. The 3′,5′-bis-(tetrahydrofuranyl) compound III was obtained by silica gel column chromatography in a yield of 68.6%. The structure of III was confirmed by elemental analysis, and UV and nuclear magnetic resonance (NMR) spectroscopy. The NMR spectra showed H-1′ signals as two sets of doublets at around 6.48 ppm, which may indicate that four isomers due to introduction of the tetrahydrofuranyl groups fell into two groups with different glycosyl torsion angles. Similar signals also appeared in the case of the 3′-O,5′-O-tetrahydropyranyl compound.2) As in the synthesis of arabinosyladenine from 8,2'-O-cycloadenosine,15) the fully protected compound III was treated successively with hydrogen sulfide and Raney nickel. Thus, IV was dissolved in pyridine and heated with liquid hydrogen sulfide at 90° for 15 hrs in a sealed tube. Conversion of the cyclonucleoside to the 8-mercapto compound IV was confirmed by a UV peak change from 260 nm to 299 nm and a different *Rf* value in thin-layer chromatography (TLC). The glassy mercapto compound was then refluxed with Raney nickel in a dioxane-water mixture to give N^2-isobutyryl-3'-O,5'-O-bis(tetrahydrofuranyl)-D-arabinofuranosylguanine (V), mp 115—120° in a yield of 69.0%. Elemental analysis and UV absorption spectra clearly showed that the indicated structure of the compound V was correct.

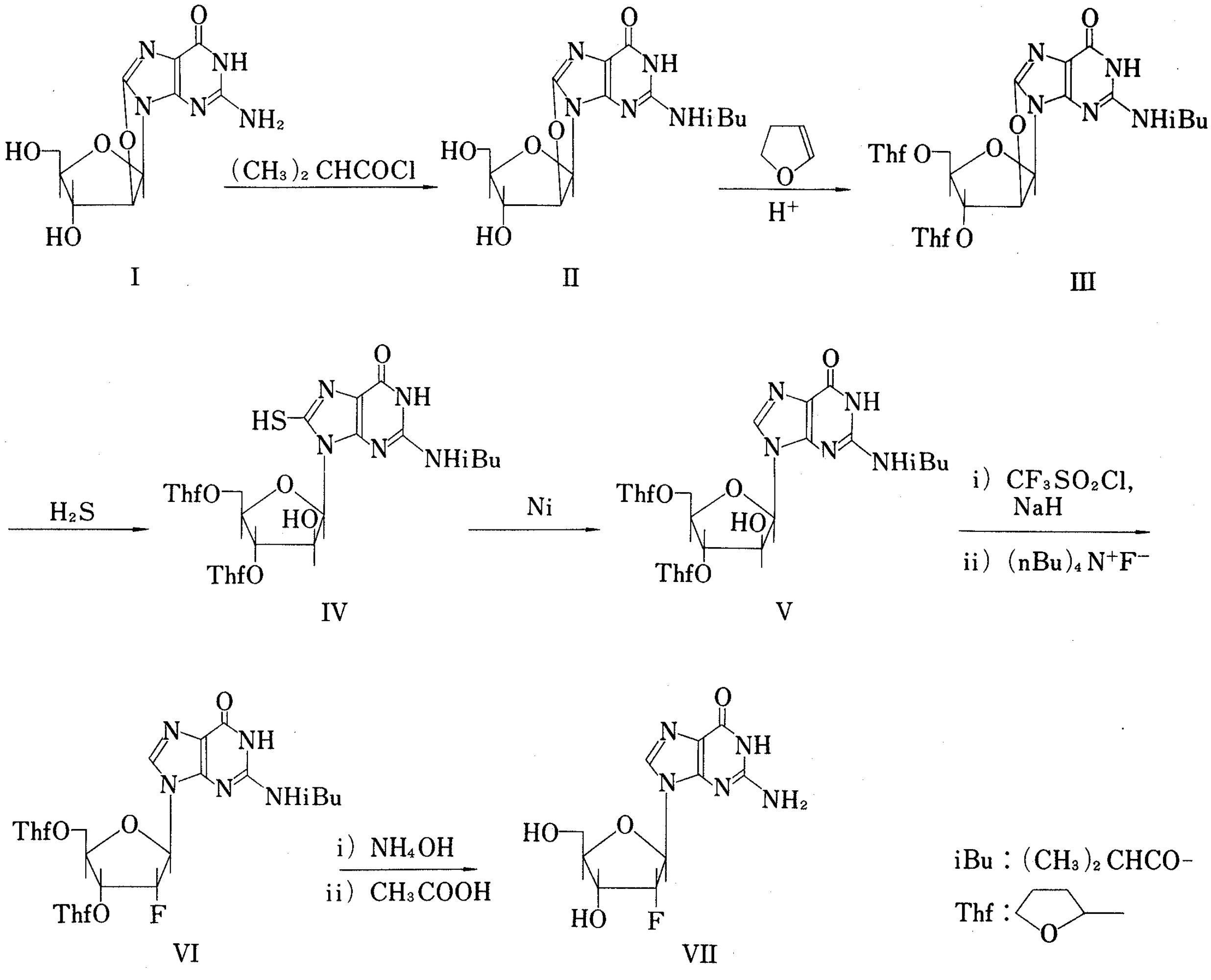

Fig. 1

Inversion of the arabino 2'-OH group to a ribo-type fluoro moiety was achieved by the introduction of a trifluoromethanesulfonyl group and reaction with tetra-*n*-butylammonium fluoride, as in the case of adenosine.2) Thus, compound V was first refluxed in tetrahydrofuran in the presence of 3 equivalents of sodium hydride for 2 hr. The completion of OH dissociation to ONa was confirmed by taking 1 drop of the reaction mixture, cooling to 0°, adding one drop of trifluoromethanesulfonyl chloride, and checking on TLC. The reaction mixture was cooled to 0° and 4 equivalents of trifluoromethanesulfonyl chloride were added with stirring for 10 min at 0°. The reaction proceeded very rapidly and its progress could be easily followed by TLC as above. After appropriate work-up, the product was reacted with 5 equivalents of tetra-*n*-butylammonium fluoride in THF solution at room temperature for 15 hr. N^2-Isobutyryl-

2′-deoxy-2′-fluoro-3′-O,5′-O-tetrahydrofuranylguanosine (VI) was obtained by silica gel column chromatography in a yield of 40.4%.

The compound VI was then deprotected as follows. When VI was dissolved in pyridine and treated with conc. ammonia at 50° for 3 hr, a spot migrating more slowly than the starting material appeared on TLC. Ammonia was evaporated off and the residue was dissolved in 40% acetic acid. Heating the solution at 30° for 2 hr gave the totally deprotected nucleoside, 2′-deoxy-2′-fluoroguanosine (VII), as a glass which was purified by passage through an HP-20 column to give a pure material, mp 240° (dec.), in a yield of 43.6%.

The structure of VII was confirmed by its UV absorption properties (similar to those of guanosine) and elemental analysis. The ^{1}H NMR spectrum showed a signal of H-2′ shifted to 5.24 ppm due to the 2′-fluorine, and the H-1′,2′ and -3′ signals showed typical coupling with fluorine of 16.5, 53.0 and 18.2 Hz to give a quartet, an octet and a multiplet, respectively. Moreover, the coupling constant $J_{1'-2'}$ was 2.8 Hz, The percentage of N conformer for 2′-deoxy-2′-fluoroguanosine (69.2% in DMSO) is larger than in the case of 2′-deoxy-2′-fluoroadenosine, as $J_{1'-2'}$ of the latter nucleoside was 3.1 Hz and the percentage of N conformer was calculated to be 67%. Again, the large electronegativity of the fluoro atom has a strong torsional effect on the 2′-3′ bond and results in a strongly N-type favored sugar conformation.

Experimental[16)]

N^2-Isobutyryl-8,2′-anhydro-8-oxy-9β-D-arabinofuranosylguanine (II)——8,2′-O-Cycloguanosine (I) (2.28 g, 8.11 mmol) was dissolved in dry pyridine (80 ml) and isobutyryl chloride (3.4 g, 32.4 mmol) was added at 0°. The mixture was stirred at 0° for 1 hr. On TLC (chloroform–methanol, 20: 1), a spot appear at *Rf* 0.56. The reaction was stopped by addition of H_2O. The mixture was extracted with $CHCl_3$, then extract was washed with sat. $NaHCO_3$ aq. and water, and evaporated to dryness to give a glass. This material was dissolved in a mixture of ethanol–pyridine (4: 1, vol/vol) (35 ml) and 2 N NaOH (35 ml), which had previously been cooled to 0°, was added with stirring. After 15 min, Dowex 50 (pyridinium form, 200 ml) was added and the nucleoside was eluted with 20% aq pyridine. Removal of the pyridine by evaporation and recrystallization of the residue from H_2O gave white needles, mp 195°, in a yield of 2.25 g (6.40 mmol, 79.0%). *Anal.* Calcd for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.88; N, 19.94. Found: C, 47.63; H, 4.93; N, 19.81. UV: $\lambda_{max}^{H_2O}$ 259 nm (ε 17200), 293 (11200); $\lambda_{max}^{0.1N\ HCl}$ 258 (17500), 292 (11300); $\lambda_{max}^{0.1N\ NaOH}$ 263 (13700); $\lambda_{max}^{95\%\ EtOH}$ 257 (16200), 296 (11100).

N^2-Isobutyryl-3′-O,5′-O-(tetrahydrofuranyl)-8,2′-anhydro-8-oxy-9-β-D-arabinofuranosylguanine (III)——Isobutyrylcycloguanosine (II) (4.02 g, 11.5 mmol) was dissolved in THF (100 ml) and *p*-toluenesulfonic acid (3.95 g, 22.9 mmol) dissolved in THF (10 ml) was added at 0° with stirring. After 10 min, dihydrofuran (8.7 ml, 115 mmol) and THF (10 ml) were added dropwide. The mixture was stirred for 40 min, by which time TLC (chloroform–methanol, 10: 1) showed a spot at *Rf* 0.68 in addition to spots of starting material and monotetrahydrofuranylated compound. After 1 hr, the reaction was stopped by adding conc. ammonia and the solvent was evaporated off *in vacuo*. The residue was taken up in $CHCl_3$, and the solution was washed with $NaHCO_3$ aq. and water. The $CHCl_3$ was evaporated off, and the residual material was applied to a silica gel 60 column (3.5 × 28 cm) and eluted with $CHCl_3$–MeOH (40: 1). Fractions showing *Rf* 0.68 on TLC ($CHCl_3$–MeOH, 10: 1) were pooled and evaporated to dryness. The residue was dissolved in $CHCl_3$ and precipitated with *n*-hexane. The melting point of this material was 115° and the yield was 3.88 g, (7.86 mmol, 68.6%). *Anal.* Calcd for $C_{22}H_{29}N_5O_8 \cdot 1/3H_2O$; 53.11; H, 6.01; N, 14.08. Found: C, 53.08; H, 6.01; N, 14.02. UV: $\lambda_{max}^{50\%\ EtOH}$ 260 nm (ε15700), 298 (11000). NMR: (δ, ppm) 11.97 (br, 1H, $-\mathrm{NH}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-$), 11.70 (br, 1H, $-\mathrm{NH}\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}-$), 6.48 (d-d, 1H, H-1′, $J_{1'-2'}=6$ Hz, $J_{\mathrm{isomer}}=2.5$ Hz), 5.80 (t, 1H, H-2), 5.37 (t, 1H, H-3′), 4.86 (m, 1H, H-4′), 4.44, 4.32 (m, 2H, tetrahydrofuranyl 1-H), 3.92—3.63 (m, 6H, H-5′ and tetrahydrofuranyl 5-H_2), 2.7 (t, 1H, $J=6.9$ Hz, Isp-CH), 1.86 (m, 8H, tetrahydrofuranyl 3,4-H_2), (d, 6H, $J=6.9$ Hz, Isp-$(CH_3)_2$).

N^2-Isobutyryl-3′-O,5′-O-bis(tetrahydrofuranyl)-9β-D-arabinofuranosylguanine (V)——Compound III (4.88 g, 9.33 mmol) was dissolved in pyridine (40 ml) and N_2 gas was bubbled through the solution for 20 min. The solution was cooled in an ethanol-dry ice bath, and H_2S gas was passed for 20 min.

Volume of the solution became *ca.* 100 ml. This mixture was heated at 90° for 15 hr in a sealed tube. It was allowed to cool, then H_2S was carefully evaporated off, and flushed out completely with N_2 gas for 20 min. Pyridine was evaporated off *in vacuo* and the residue was co-evaporated with toluene. UV: $\lambda_{max}^{80\%\ EtOH}$ 235 nm (sh); 299; $\lambda_{max}^{0.1N\ HCl}$ 235 (sh), 299; $\lambda_{max}^{0.1N\ NaOH}$ 299; $\lambda_{max}^{0.1N\ NaOH}$ 299. TLC: $CHCl_3$–MeOH (10: 1) *Rf* 0.83. The resulting glassy residue was dissolved in dioxane (60 ml) and water (20 ml), and Raney Ni (8 ml) was added. The mixture was refluxed for 40 min with vigorous stirring. UV λ_{max} at 299 nm disappeared. The Raney Ni was filtered off and washed with hot dioxane. The filtrate and washings were combined, and evaporated to dryness, and the residue was dissolved in $CHCl_3$. The solution was washed with H_2O. $CHCl_3$ was evaporated off and the residue was applied to a column (3.5 × 23 cm) of silica gel 60 (120 g). Fractions containing V were collected and precipitated with $CHCl_3$–*n*-hexane. This material gave mp 115—120°. Yield, 3.38 g (6.85 mmol, 69.0%). *Anal.* Calcd for $C_{22}H_{31}N_5O_8 \cdot 1/2H_2O$: C, 52.58; H, 6.42; N, 13.94. Found: C, 52.55; H, 6.20; N, 13.80. UV: $\lambda_{max}^{50\%\ EtOH}$ 258 nm (ε 16600), 280 (12500); $\lambda_{max}^{0.1N\ HCl}$ 262 (17900), 272 (sh, 15600); $\lambda_{max}^{0.1N\ NaOH}$ 265 (13900); $\lambda_{max}^{95\%\ EtOH}$ 258 (16300), 283 (12200).

N^2-Isobutyryl-3′-O,5′-O-bis(tetrahydrofuranyl)-2′-deoxy-2′-fluoroguanosine (VI)——Compound V (1.05 g, 2.13 mmol) was dissolved in pyridine and the solution was evaporated to dryness. Traces of pyridine were removed by coevaporation with toluene from the residue, which was then dissolved in THF (40 ml). Sodium hydride (407 mg of 50% oil suspension, 8.52 mmol) dissolved in THF (10 ml) was added. After being refluxed for 2 hr, the solution was cooled to 0° and trifluoromethanesulfonyl chloride (1.13 ml, 10.7 ml) was added. The mixture was stirred for 10 min and checked by TLC (10: 1). A spot appeared at *Rf* 0.69. The solution was added dropwise to saturated $NaHCO_3$ aq. which was then extracted with $CHCl_3$. The extract was washed with $NaHCO_3$ aq. and H_2O, then evaporated to dryness. The residue was dried by coevaporation with pyridine and toluene. Tetrabutylammonium fluoride dissolved in THF (1 mmol/ml, 10.7 ml, 10.7 mmol) was added to a THF (100 ml) solution of the above residue. The mixture was stirred for 15 hr at room temperature. TLC showed a spot at *Rf* 0.31 (starting compound V *Rf* 0.14, trifluoromesyl derivative 0.48). THF was evaporated off *in vacuo* and the residue was taken up in $CHCl_3$. The solution was washed with H_2O, and $CHCl_3$ was evaporated off. The residue was dried by coevaporation with pyridine and toluene and applied to a column of silica gel H (6.8 × 5.5 cm, 80 g). Appropriate fractions (TLC *Rf* 0.31) were pooled and evaporated to dryness to give a glassy residue, which was precipitated from $CHCl_3$ and *n*-hexane. Yield was 426 mg (0.86 mmol, 40.4%).

2′-Deoxy-2′-fluoroguanosine (VII)——The protected nucleoside (VI) (277 mg, 0.458 mmol) was dissolved in pyridine (5 ml) and conc. NH_4OH (50 ml) was added. The mixture was heated at 50° for 3 hr, then cooled. NH_3 and solvent were evaporated off. The residue was dissolved in acetic acid (20 ml) and water (30 ml), and the solution was kept at 30° for 2 hr. Completion of the reaction was confirmed by TLC ($CHCl_3$–MeOH, 3: 2) and the solvent was evaporated off. Traces of AcOH were removed by coevaporation with H_2O and the residue was dissolved in H_2O. The solution was washed with $CHCl_3$. The residue obtained by removal of the H_2O gave 5000 A_{253} unit (UV: $\lambda_{max}^{H_2O}$ 253, 270 nm sh).

The nucleoside VII was eluted with 20% MeOH (2 l) and water (2 l) in a linear gradient. 2′-Deoxy-2′-fluoroguanosine was eluted at 4.5% MeOH and the yield was 3570 A_{253}. Recrystallization from H_2O gave colorless needles, mp 240° (dec.), in a yield of 57 mg (0.20 mmol, 43.6%). *Anal.* Calcd for $C_{10}H_{20}N_5O_4F \cdot 5/6H_2O$: C, 40.00; H, 4.59; N, 23.32; F, 6.33. Found: C, 40.27; H, 4.57; N, 23.48; F, 6.08. UV: nm (ε) $\lambda_{max}^{H_2O}$ 252 (13400), 270 (sh, 9200); $\lambda_{max}^{0.1N\ HCl}$ 256 (13100), 275 (sh, 9000); $\lambda_{max}^{0.1N\ NaOH}$ 258—263 (12000); $\lambda_{max}^{95\%\ EtOH}$ 253 (14200), 270 (sh, 9800). Paper chromatography: *Rf* (A) 0.58 (guanosine, 0.51), *Rf*(B) 0.39 (guanosine; 0.28), *Rf*(C) 0.45 (guanosine, 0.29). ^1H NMR: (DMSO-d_6) 10.60 (s, 1H, NH-1), 7.93 (s, 1H, H-8), 6.49 (s, 2H, 2-NH_2), 6.00 (q, 1H, H-1′, $J_{1'-2'}=2.8$, $J_{1'-F}=16.5$), 5.61 (d, 1H, 3′-OH, $J_{3'-OH'}=5.8$), 5.24 (octet, 1H, H-2′, $J_{2'-3'}=4.1$, $J_{2'-F}=53.0$), 5.08 (t, 1H, 5′-OH, $J_{5'-OH_5'}=5.4$), 4.36 (m, 1H, H-3′, $J_{3',4'}=6.3$, $J_{3'-F}=18.2$ Hz), 3.90 (m, 1H, H-4′), 3.65 (m, 2H, H-5′).

References and Notes

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16) UV absorption spectra were taken with a Hitachi 200-10 spectrophotometer. ^{1}H NMR spectra were recorded on a Hitachi R-22 spectrometer (90 MHz, ambient probe temperature 34°). Chemical shifts were measured from an internal tetramethylsilane. Paper chromatography was performed on Toyo filter paper No. 51A by the ascending technique. Solvents were: A, EtOH–1 M NH_4OAc (7: 3, vol/vol); B, iso-PrOH-conc. NH_4OH–H_2O (7: 1: 2); C, *n*-BuOH–AcOH H_2O (5: 2: 3). TLC was performed on Merck Kieselgel G (type 60).