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Studies on Nucleosides and Nucleotides. LXXXIX. Purine Cyclonucleosides. (43). Synthesis and Properties of 2′-Halogeno-2′-deoxyguanosines[1)]

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The reaction of N^2-isobutyryl-9-(2′-*O*-trifluoromethanesulfonyl-3′,5′-di-*O*-tetrahydrofuranyl-β-D-arabinofuranosyl)guanine with tetra-*n*-butylammonium fluoride or an appropriate metal halide in dimethylformamide afforded N^2-isobutyryl-3′,5′-di-*O*-tetrahydrofuranyl-2′-halogeno-2′-deoxyguanosines. The deprotection of these products led to 2′-halogeno-2′-deoxyguanosines. The ultraviolet absorption properties, ^1H and ^{13}C nuclear magnetic resonance spectral properties of the products were recorded.

Keywords——2′-fluoro-2′-deoxyguanosine; 2′-chloro-2′-deoxyguanosine; 2′-bromo-2′-deoxyguanosine; 2′-iodo-2′-deoxyguanosine; nucleophilic reaction; TLC; UV; ^1H NMR; ^{13}C NMR

Nucleoside analogs with modifications in the sugar moieties have considerable potential for various physicochemical and biological studies.[2)] A method for synthesizing 2′-substituted-2′-deoxyadenosines and -inosines starting from 8,2′-*O*-cycloadenosine has been developed in this laboratory.[3,4)] An antibiotic, 2′-amino-2′-deoxyguanosine, was also synthesized by application of this method to guanosine derivatives.[5)] From the results of ^1H nuclear magnetic resonance (NMR) studies of 2′-substituted 2′-deoxyadenosine, a linear relationship between the electronegativity of substituents and the conformation of the ribose moiety was found.[6)] This result led us to consider that a series of 2′-halogeno-2′-deoxyguanosines might have an analogous tendency. In this paper we describe the synthesis of 2′-halogeno-2′-deoxyguanosines and their properties. The protected 2′-fluoro-2′-deoxyguanosine (III-a), which has recently been described,[7)] was contaminated with a minor product formed by nucleophilic replacement of the trifluoromethanesulfate (II) with chloride ion. We describe here the isolation and identification of the minor product. The trifluoromethanesulfonate (II) of N^2-isobutyryl-9-(2′,5′-di-*O*-tetrahydrofuranyl-β-D-arabinofuranosyl) guanine (I) was synthesized as described before[7)] and then treated with tetrabutylammonium fluoride in tetrahydrofuran at room temperature for 15 h. At the end of this time, thin-layer chromatography (TLC) (chloroform–methanol, 10: 1) of the reaction mixture indicated that the desired product (III-a) (*Rf* 0.77) along with another minor product (*Rf* 0.79) had been formed. After appropriate work-up, the mixture was applied to a column of silica gel and separated into two fractions: (1) protected 2′-fluoro-2′-deoxyguanosine (227 mg, 32.0%) and (2) a mixture of two products (145 mg). N^2-Isobutyryl-3′,5′-di-*O*-tetrahydrofuranyl-2′-fluoro-2′-deoxyguanosine (III-a) was deblocked and purified by passage through an HP-20 column to give 2′-fluoro-2′-deoxyguanosine (IV-a). The mixture of two products was also deblocked and separated by column chromatography on HP-20. Ultraviolet (UV) absorption properties, the ^1H-NMR spectrum and elemental analysis indicated that the compound obtained by deblocking the minor product was 2′-chloro-2′-deoxyguanosine. This compound was identical with 2′-chloro-2′-deoxyguanosine (IV-b) synthesized by an unambigous route as described below. The source of nucleophilic chloride might be excess trifluoromethanesulfonyl chloride. If a small amount of chloride ion is present in the reaction mixture, chloride ion would attacks the 2′-carbon predominantly because chloride ion is a much better nucleophile than fluoride ion.

In order to obtain 2′-chloro-2′-deoxyguanosine, compound (II) was treated with lithium chloride in dimethylformamide at 50°C. Though the starting material and the product have

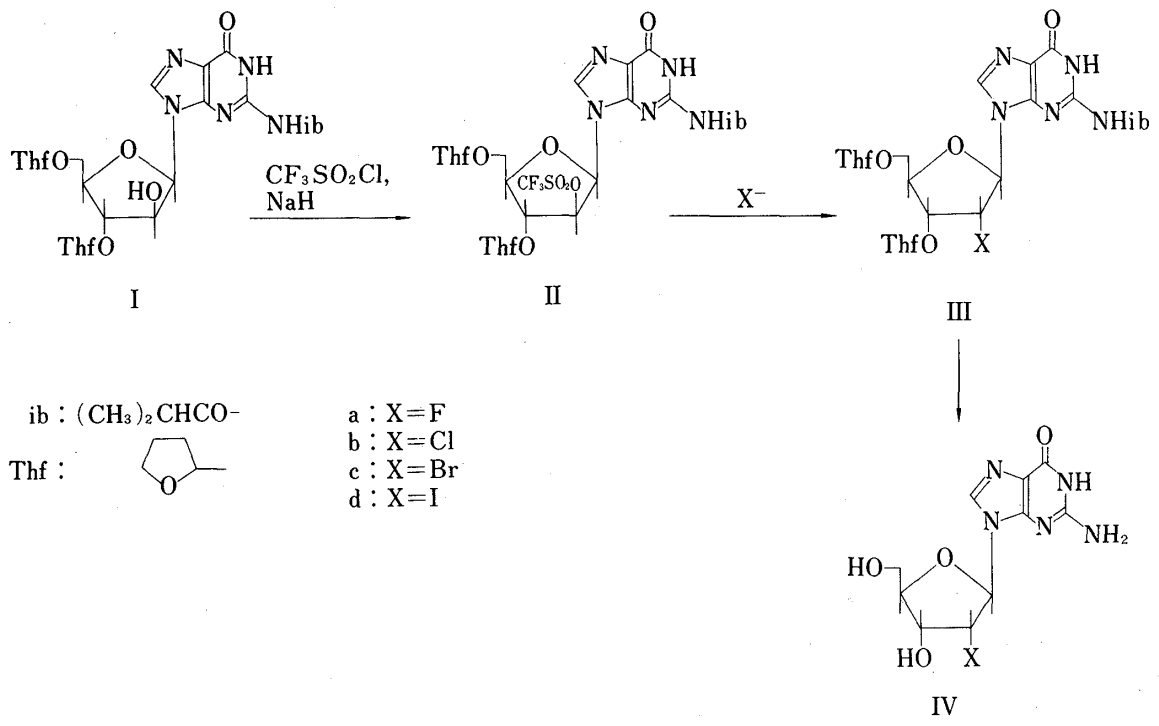

Chart 1

the same *Rf* value in usual TLC, they can be distinguished in reverse-phase TLC using acetone–H_2O (7: 3). Thirty minutes later, reverse-phase TLC analysis showed that (II) had been consumed and a product had been formed. The solvent was evaporated off *in vacuo*, and TLC (10: 1, chloroform–methanol) of the residue showed new spots of deblocked products besides that of the desired product. Thus, the mixture was deblocked without isolation and 2′-chloro-2′-deoxyguanosine (IV-b) was then isolated by chromatography on HP-20 in a yield of 35.6% based on (I). Analogously, 2′-bromo-2′-deoxyguanosine (IV-c) was obtained by the reaction of compound II with lithium bromide in 25.3% yield based on (I). We next inves-

Table I. Decomposition Temperatures, Elemental Analysis Data, and Paper Chromatographic Properties of 2′-Halogeno-2′-deoxyguanosines (IV-a,b,c, and d)

Compound	dec. (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	X	PPC (*Rf*) A	B	C
						X=F			
IV-a	240	$C_{10}H_{20}FN_5O_4 \cdot 5/6H_2O$	40.00 (40.27	4.59 4.57	23.32 23.48	6.33 6.08)	38	50	64
						X=Cl			
IV-b	230	$C_{10}H_{20}ClN_5O_4$	39.81 (39.65	4.01 3.91	23.21 23.00	11.75 11.71)	52	60	72
						X=Br			
IV-c	195	$C_{10}H_{20}BrN_5O_4$	34.70 (35.06	3.49 3.45	20.23 20.06	23.08 23.36)	53	63	71
						X=I			
IV-d	167 168 169	$C_{10}H_{20}IN_5O_4$	30.55 (30.88	3.08 2.88	17.81 17.87	32.28 32.02)	56	65	71
2′-Deoxyguanosine							39	51	63
Guanosine							25	39	54

tigated the synthesis of 2′-iodo-2′-deoxyguanosine (IV-d). However, treatment of 2′-iodo derivatives as described for 2′-chloroderivatives resulted in undesirably complex product mixtures. The cause was thought to be as follows: (1) iodide ion might be oxidized to iodine and then iodine might attack the base moiety; (2) elimination with iodide ion as the leaving group or substitution might occur during the deacylation in conc. ammonia at 50°C for 3 h. Therefore, after the substitution reaction of (II) with iodide ion, the excess iodide ion was eliminated and the product (III-a) was isolated. The yield was 33.7% from (I). Deacylation of the protected 2′-iodo-2′-deoxyguanosine (III-a) with methanolic ammonia at 30°C overnight and treatment in 80% acetic acid gave 2′-iodo-2′-deoxyguanosine (IV-d) in 69.8% yield. The physical properties of 2′-halogeno-2′-deoxyguanosines prepared in this study are summarized in Tables I—V. The structures of IV-a, b, c, d were confirmed by elemental analysis (Table I) and the UV absorption properties, which resembled those of guanosine.

In the ^{1}H NMR spectra (Table III, IV), a relationship between the electronegativity of substituents and the conformation of the sugar moiety was observed, as expected from results of ^{1}H-NMR studies of 2′-substituted 2′-deoxyadenosines.[6] In Table IV, proton spin-spin coupling constants are given together with the percentage of N conformer as calculated by the

Table II. UV Absorption Properties of 2′-Halogeno-2′-deoxyguanosines (IV-a,b,c and d)

Compound	λ_{max} nm (ε)			
	H_2O	0.1 N HCl	0.1 N NaOH	95% ethanol
IV-a	252 (13400) 270 (sh. 9200)	256 (13100) 275 (sh. 9000)	258—263 (12300)	253 (14200) 270 (sh. 9800)
IV-b	252.5 (14200) 270 (sh. 9900)	256 (13400) 275 (sh. 9300)	257—263 (12300)	253 (14900) 270 (sh. 10300)
IV-c	253 (14500) 270 (sh. 10200)	257 (13400) 275 (sh. 9300)	263—266 (12100)	254 (14800) 270 (sh. 10400)
IV-d	253 (14000) 270 (sh. 10300)	257.5 (12900) 275 (sh. 9300)	264—266 (12200)	254 (14400) 270 (sh. 10300)

Table III. ^{1}H Chemical Shifts[a] of 2′-Halogeno-2′-deoxyguanosines in DMSO-d_6

Compound	8-H	1-NH	2-NH_2	1′-H	2′-H	3′-H	4′-H	5′-H	3′-OH	5′-OH
IV-a	7.93	10.60	6.79	6.00	5.24	4.36	3.90	3.65	5.61	5.08
IV-b	7.97	10.63	6.48	5.94	4.93	4.31	3.98	3.62	5.88	5.11
IV-c	7.96	10.62	6.48	6.03	4.97	4.22	4.00	3.64	5.93	5.09
IV-d	7.93	10.63	6.47	6.07	4.93	4.00[b]	4.00[b]	3.57	5.99	5.05

a) Shifts are given in ppm (δ) relative to internal TMS.
b) These signals were not resolved.

Table IV. Coupling Constants (Hz) and Conformation of the Sugar Moiety of 2′-Halogeno-2′-deoxyguanosine (IV-a,b,c and d)

Compound	$J_{3'-3'OH}$	$J_{5'-5'OH}$	$J_{1'-2'}$	$J_{2'-3'}$	$J_{3'-4'}$	$J_{1'-2'}+J_{3'-4'}$	Population of conformer[c]	N (%)
IV-a	5.8	5.4	2.8	4.1	6.3	9.1	69	
IV-b	4.8	5.5	6.9	4.8	3.0[a]	9.9	30	
IV-c	5.1	5.6	7.6	4.8	2.2	9.8	22	
IV-d	5.6	5.4	8.7	4.6	[b]	[b]	13	

a) Observed after addition of D_2O.
b) The 3′ and 4′ signals of IV-d were not resolved.
c) $J_{1'-2'}+J_{3'-4'}$ was assumed to be 9.8 Hz.

Table V. ^{13}C Chemical Shifts[a] of 2′-Halogeno-2′-deoxyguanosines (IV-a,b,c and d) and Related Compounds in DMSO-d_6[b]

Compound	C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C-3′	C-4′	C-5′
IV-a	87.43	84.31	50.34	90.30	68.76	18.60	27.26	1.78	17.42	−6.14
						$J_{1'F=32.5Hz}$	$J_{2'F=187.2Hz}$	$J_{3'F=15.6Hz}$	$J_{4'F=2.1Hz}$	
IV-b	87.49	84.88	50.27	90.28	68.82	20.04	−5.00	3.68	19.41	−5.42
IV-c	87.46	84.88	50.27	90.25	68.88	20.28	−13.12	3.65	19.59	−5.33
IV-d	87.37	85.00	50.27	90.19	68.85	21.84	−34.75	4.67	19.68	−4.85
2′-Deoxy-guanosine	87.58	84.52	50.41	90.82	68.76	16.36	−26.80	4.44	21.22	−4.56
Guanosine	87.30	84.96	50.37	90.42	69.25	20.14	7.37	4.07	18.88	−4.86

a) Shifts are given in ppm (δ) relative to internal dioxane.
b) Concentration, 50 mg in 0.7 ml of DMSO-d_6.

method of Altona and Sundaralingam.[8] Again, the population of the N conformer decreases in the order of F>Cl>Br>I.

The results of ^{13}C NMR studies show that the base carbons are almost unaffected, but that sugar carbon signals, particularly the C-2′ signals, are very much influenced by substituents, suggesting substitution at the 2′-carbon. A linear relationship between the electronegativity of substituents and the difference of the chemical shifts of each compound from those of 2′-deoxyguanosine was observed, as was the case with adenosine analogs.[9]

Synthesis of oligomers containing 2′-substituted 2′-deoxyguanosines is in progress in our laboratory.

Experimental[10]

N²-Isobutyl-3′,5′-di-*O*-tetrahydrofuranyl-2′-fluoro-2′-deoxyguanosine (III-a)——A solution of 706mg (1.43 mmol) of I and 206 mg (4.29 mmol) of sodium hydride (about 50% oil suspension) in 30 ml of tetrahydrofuran (THF) was refluxed for 2 h. The mixture was cooled to 0°C and treated with 0.61 ml (5.7 mmol) of trifluoromethanesulfonyl chloride. After a few minutes, the reaction was complete as indicated by TLC in chloroform–methanol (10: 1): *Rf* 0.54, 0.60 (I): *Rf* 0.69 (II). The mixture was added dropwise to saturated sodium bicarbonate, which was then extracted with chloroform. The extract was washed with saturated sodium bicarbonate, and then with water. The residue obtained by evaporation of the chloroform solution was dried by coevaporation with pyridine and traces of pyridine were removed by coevaporation with toluene. The residue was dissolved in 60 ml of THF and treated with 7.15 ml of a solution of tetra-*n*-butylammonium fluoride in THF (1 mmol/ml). The mixture was kept at room temperature for 15 h. TLC in benzene–acetone (1: 1) showed a spot at *Rf* 0.31 (II, *Rf* 0.48). The mixture was concentrated. The residue was taken up in chloroform and the solution was washed with water. The chloroform solution was concentrated and the residue was chromatographed on 80 g of silica gel. Elution with chloroform–methanol (50: 1) gave two fractions: (1) the protected 2′-fluoro-2′-deoxyguanosine (IIIa) (227 mg, 32.0%) and (2) a mixture of IIIa and a by-product (145 mg). Fractions were evaporated to dryness.

2′-Fluoro-2′-deoxyguanosine (IV-a)——A solution of 227 mg (0.458 mmol) of (IIIa) in 3 ml of pyridine was treated with 50 ml of 28% ammonium hydroxide. The mixture was kept at 50°C for 3 h, then evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml of acetic acid, and 30 ml of water was then added. The mixture was kept at 30°C for 2 h. The solution was concentrated and the last traces of acid were removed by coevaporation with water. The residue was dissolved in water, then the solution was washed with chloroform and evaporated to dryness. The residue (*ca.* 5000 A_{253}) was chromatographed on a column of HP-20 (ϕ 2.8 cm × 24.5 cm, 150 ml) and eluted with 20% methanol (2 l) and water (2 l) in a linear gradient; 3570 A_{253} of IV-a was eluted at 4.5% methanol. The solvent was removed and the residue was crystallized from water to afford 57 mg (44%) of IVa as colorless needles.

Deprotection of the Mixture of By-product and III-a——The procedure for the deprotection of 145 mg of the mixture was similar to that for the pure III-a. The final product (3060 A_{253}) was chromatographed on a column of HP-20 as before and separated into two fractions: (a) IVa which was eluted at 5% methanol (1430 A_{253}) [crystallization from water gave 33 mg (0.116 mmol) of IVa]; (b) a by-product (IVb) which was eluted at 11% methanol (730 A_{253}) (crystallization gave 10 mg of IVb). *Anal.* Calcd for $C_{10}H_{12}ClN_5O_4 \cdot 1/3H_2O$: C, 39.04; H, 4.15; Cl, 11.52; N, 22.76. Found: C, 38.99; H, 3.82; Cl, 11.28; N, 22.55. This was identical with IVb prepared by the treatment of II with lithium chloride (*vide infra*). The total yield of IVa was 90 mg (23.5%) based on I and that of the by-product (IVb) was 10 mg (2.3%).

2′-Chloro-2′-deoxyguanosine (IV-b)——The trifluoromethanesulfonate (II) of I, [obtained from 1.00 g (2.00 mmol) of I] and 848 mg (20.0 mmol) of lithium chloride were dissolved in 30 ml of dimethylformamide (DMF) and the solution was stirred at 50°C. After 30 min, the reaction was complete as indicated by silanized silica gel TLC in acetone–water (7: 3): II, *Rf* 0.52, IIIb, *Rf* 0.60. After concentration of the DMF solution, the residue was dissolved in 120 mmol of acetic acid–water (2: 3). The solution was kept at room temperature for 18 h. The solvent was evaporated off and traces of acid were removed by coevaporation with ethanol. The residue was treated with 80 ml of 28% ammonium hydroxide. The mixture was kept at 50°C for 3 h and worked up as in the preparation of IVa. The crude product (21000 A_{253}) was applied to a column of HP-20 (ϕ 3.5 cm × 42 cm, 400 ml) and eluted with a linear gradient (3 l × 3 l) formed from water and 20% methanol. IVb was eluted at 16.5% methanol and the yield was 11600 A_{253}. The solvent was evaporated off and crystallization of the residue from water gave 215 mg (0.713 mmol, 35.6% based on I) of IVb.

2′-Bromo-2′-deoxyguanosine (IV-c)——II [obtained from 1.00 g (2.00 mmol) of I] and 1.0 g (10 mmol) of lithium bromide were dissolved in 30 ml of DMF and the solution was stirred at 50°C for 30 min. After concentration of the solution, the residue was deprotected just as described for IVb. The crude product (24840 A_{252}) was applied to a column of HP-20 (ϕ 3.5cm × 42 cm, 400 ml) and eluted with a linear gradient (total 6 l) formed from 10% methanol and 30% methanol. IVc was eluted at 18% methanol and the yield was 10600 A_{252}. The solvent was evaporated off and the residue was crystallized from water to give 175 mg (0.506 mmol, 25.3% based on I) of IVc.

N^2-Isobutyryl-3′,5′-di-*O*-tetrahydrofuranyl-2′-iodo-2′-deoxyguanosine (III-d)——II [obtained from 0.83 g (1.69 mmol) of I] and 2.52 g (16.9 mmol) of sodium iodide were dissolved in 25 ml of DMF and stirred at 50°C for 30 min. After concentration of the solution, the residue was dissolved in dichloromethane containing a small amount of pyridine and washed with 30% sodium thiosulfate and 5% pyridine. The dichloromethane solution was concentrated *in vacuo* and the residue was added dropwise to *n*-hexane. Chromatography of the precipitate on silica gel (50 g) with 50: 1 chloroform–methanol as a developer afforded IIIa (yield, 407 mg, 33.7%).

2′-Iodo-2′-deoxyguanosine (IV-d)——407 mg (0.674 mmol) of IIId was deacylated with 60 ml of methanolic ammonia (saturated at 0°C) for 14 h at 30°C. The mixture was evaporated to dryness and the residue was dissolved in 75 ml of 40% acetic acid. After 4.5 h at 30°C, the solvent was removed and the residue was dissolved in water. The solution was then washed with chloroform and evaporated to dryness. The residue was crystallized from water to afford 47 mg (0.121 mmol) of IVd. The mother liquors (8800 A_{252}) were chromatographed on HP-20 (ϕ 3.5 cm × 26 cm, 250 ml) and eluted with a linear gradient (total 6 l) formed from 10% methanol and 25% methanol. IVd (5000 A_{252}) was eluted at 20% methanol. The solvent was evaporated off and the residue was crystallized from water to afford IVd in a total yield 182 mg (69.8%) based on III-d.

References and Notes

1) Part LXXXVIII of this series: M. Ikehara and J. Imura, *Chem. Pharm. Bull.*, **29**, 2408 (1981).
2) R.J. Suhadolnik, "Nucleosides as Biological Probes," Wiley Interscience, New York, 1979.
3) M. Ikehara, T. Maruyama, and H. Miki, *Tetrahedron*, **34**, 1133 (1978).
4) M. Ikehara and H. Miki, *Chem. Pharm. Bull.*, **26**, 2449 (1978).
5) M. Ikehara and T. Maruyama, *Chem. Pharm. Bull.*, **26**, 240 (1978).
6) S. Uesugi, H. Miki, M. Ikehara, H. Iwahashi, and Y. Kyogoku, *Tetrahedron Lett.*, **1979**, 4073.
7) M. Ikehara and J. Imura, *Chem. Pharm. Bull.*, **29**, 1034 (1981).
8) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972); C. Altona and M. Sundaralingam, *ibid.*, **95**, 2333 (1973).
9) S. Uesugi, H. Miki, and M. Ikehara, *Chem. Pharm. Bull.*, **29**, 2199 (1981).
10) UV absorption spectra were taken with a Hitachi 200-10 spectrophotometer. NMR spectra were taken with a Hitachi R-900 spectrometer (90 MHz for ^1H and 22.63 MHz for ^{13}C) operating in the Fourier transform mode. DMSO-d_6 was used as a solvent and tetramethylsilane (for ^1H) and dioxane (for ^{13}C) were used as internal references. TLC was performed on Merck silica gel 60 F_{254} and Merck silica gel 60 F_{254} silanized. Paper chromatography (PPC) was performed on Toyo Roshi filter paper No. 51A using the following solvent systems; A, isopropanol–conc. ammonium hydroxide–water (7: 1: 2); B, *n*-butanol–acetic acid–water (5: 2: 3), C, ethanol–1 M ammonium acetate pH 7.5 (7: 3). All melting points are uncorrected.