Chem. Pharm. Bull.
29(9)2408—2412(1981)

Studies on Nucleosides and Nucleotides. LXXXVIII.[1] Synthesis of a Non-hydrolyzable Substrate Analog of Ribonuclease Tl, 2′-Deoxy-2′-fluoroguanylyl-(3′—5′)-uridine

MORIO IKEHARA* and JUNKO IMURA

Faculty of Sciences, Osaka University, Suita, Osaka 565, Japan

(Received January 5, 1981)

2′-Deoxy-2′-fluoroguanylyl-(3′—5′)-uridine (GfpU) was synthesized as a non-hydrolyzable substrate analog of ribonuclease T1. 2′-Deoxy-2′-fluoroguanosine was derivatized to the N^2-isobutyryl-5′-monomethoxytrityl compound and condensed with 2′,3′-di-*O*-acetyluridine 5′-monophosphate by the use of dicyclohexylcarbodiimide to obtain GfpU. For structural elucidation of the dinucleoside monophosphate, 2′-deoxy-2′-fluoroguanosine 3′-monophosphate was also synthesized.

The conformation of GfpU was found to take a right-handed, weakly stacked form as deduced by ultraviolet, circular dichroism, and nuclear magnetic resonance (NMR) spectroscopy.

Keywords——N^2-isobutyryl-2′-deoxy-2′-fluoroguanosine; N^2-isobutyltyl-5′-monomethoxytrityl-2′-deoxy-2′-fluoroguanosine; UV, CD, NMR, TLC; PPC

Recently we have reported on the synthesis of 2′-substituted 2′-deoxyadenosines *via* protected arabinosyl nucleosides obtained from 8,2′-*O*-cyclonucleosides.[2] From the coupling constants $J_{1'-2'}$ in the ^{1}H-nuclear magnetic resonance (NMR) spectra of these compounds,[3] we deduced that 2′-fluoro-2′-deoxyadenosine (Af) had a strongly 3′-endo (N type) favored conformation in contrast to normal ribonucleosides, which usually have 2′-endo conformation.[4] We synthesized four dinucleoside monophosphates containing Af and adenosine and confirmed that the stacking tendency of adenine bases in these dimers was related to the proportion of the N type conformers.[5] We therefore synthesized 2′-deoxy-2′-fluoroguanosine(Gf) starting from 8,2′-*O*-cycloguanosine[6] with the tetrahydrofuranyl group[7] for protection of 3′- and 5′-OH. Gf was also found to be in the N-type conformation by ^{1}H NMR spectroscopy.[8]

In this paper we wish to report the synthesis of 2′-deoxyguanylyl-(3′—5′)-uridine (GfpU) (I). Ribonuclease Tl[9] is known to hydrolyze the G3′p5′X bond on the 5′ side in ribonucleic acid (RNA) and many physicochemical studies have been done to elucidate the mechanism of hydrolysis. GpU itself is hydrolyzed by the enzyme and guanosine 3′-phosphate has been used as a substrate analog. As found in the case of adenosine, GfpU might be very suitable substrate analog because it must have a conformation similar to that of GpU and it is not hydrolyzed at all because of the absence of a 2′-OH group.

We first used N^2-isobutyryl-2′-deoxy-2′-fluoro-3′,5′-bis(tetrahydrofuranyl)-guanosine (II), which has been synthesized as an intermediate leading to 2′-deoxy-2′-fluoroguanosine,[8] as the starting material. Compound II was treated with 40% acetic acid at room temperature for 12 h to give N^2-isobutyryl-2′-deoxy-2′-fluoroguanosine (III), mp 245°C (dec.), in a yield of 58%. The structure of III was confirmed by elemental analysis, ultraviolet (UV) absorption measurements and paper chromatography.

Compound III was then protected at 5′-OH with a monomethoxytrityl (mmTr) group by using mmTr chloride at room temperature overnight. Purification of the product N^2-isobutyryl-5′-mmTr-2′-deoxy-2′-fluoroguanosine (IV) through a short silica gel column gave only a glassy material in quantitative yield. The yellow color developed by heating with acid and the thin layer chromatography (TLC) behavior supported the structure IV.

As the nucleotide component, uridine 5′-monophosphate (pU) was transformed to the 2′,3′-di-*O*-acetyl derivative (V) by the usual acetylation with acetic anhydride and pyridine

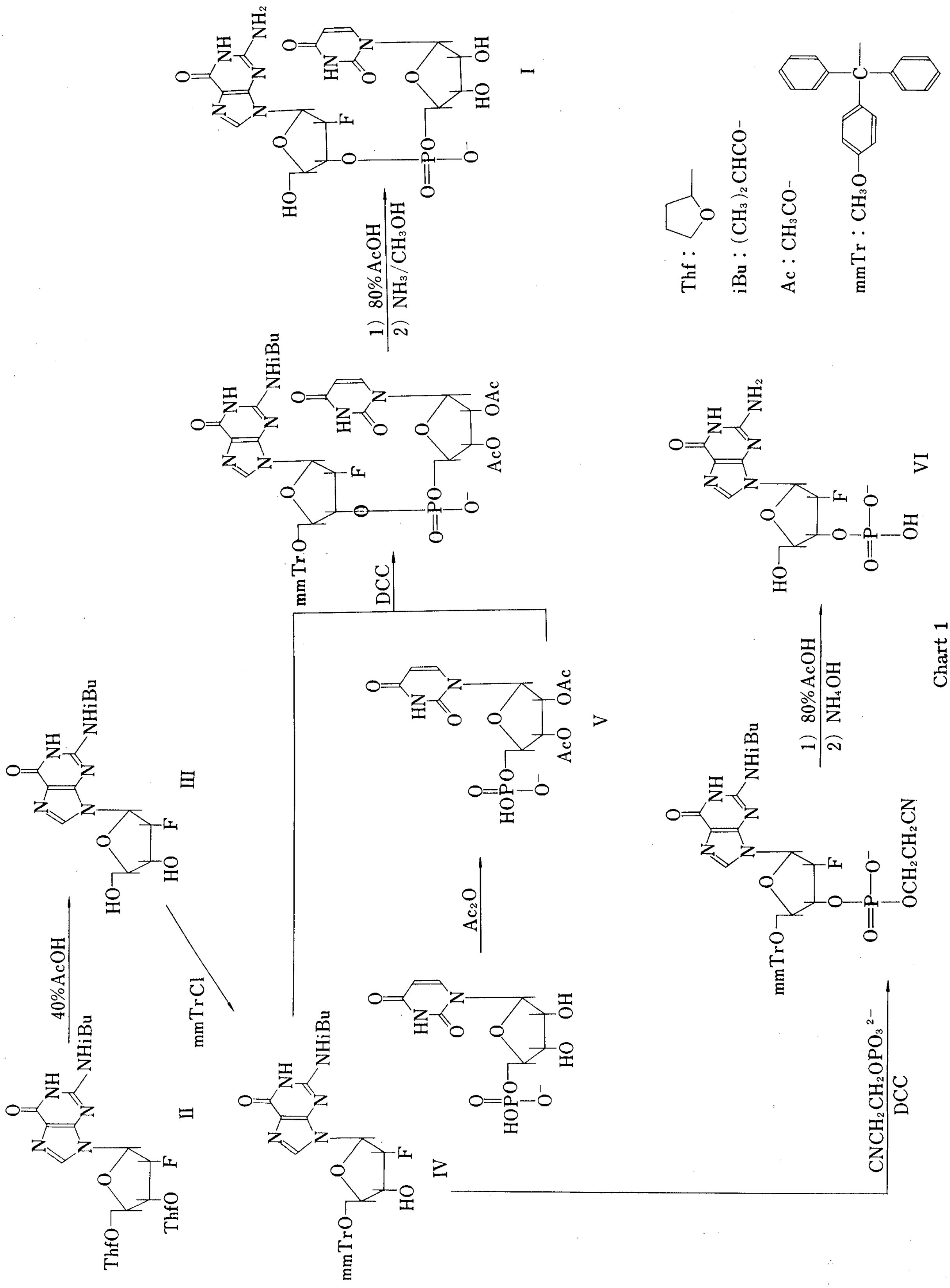
I
II
III
IV
V
VI
40%AcOH
mmTrCl
Ac_2O
DCC
1) 80%AcOH
2) NH_3/CH_3OH
1) 80%AcOH
2) NH_4OH
$CNCH_2CH_2OPO_3^{2-}$
DCC
Thf :
iBu : $(CH_3)_2CHCO-$
Ac : CH_3CO-
mmTr : CH_3O
ThfO
mmTrO
HO
F
NHiBu
NH_2
AcO
OAc
HOPO
OCH_2CH_2CN

Chart 1

in a yield of 79%. On paper electrophoretograms this compound migrated as expected for diAc-uridine 5′-monophosphate (UMP). The compound V was then condensed with the protected 2′-deoxy-2′-fluoroguanosine (IV) using dicyclohexylcarbodiimide (DCC) as a condensing reagent. The reaction was continued for 25 h at 30°C, then stopped by the addition of H_2O. The monomethoxytrityl group was removed by treatment with 80% acetic acid at 30°C for 22 h and acyl groups were removed with methanolic ammonia at room temperature for 19 h. The nucleotidyl material was chromatographed on a column of diethylaminoethyl (DEAE) cellulose by linear gradient elution with triethylammonium bicarbonate (TEAB) buffer. Material in the first and second peaks was rechromatographed on the same DEAE cellulose column. The product 2′-deoxy-2′-fluoroguanylyl-(3′—5′)-uridine (I) was obtained in a yield of 62%. UV absorption maxima at around 257 nm (see "Experimantal"), paper chromatography and electrophoresis supported the structure I. Digestion of I with venom phosphodiesterase gave Gf and pU in a 1: 1 ratio.

CD spectra (Fig. 1) showed that even in neutral solution, GfpU (I) had a very weakly stacked conformation, because the spectrum is almost the same as that under acidic conditions. At pH 12 guanine and uracil bases dissociate to anionic forms and totally different CD spectra appear. ^{1}H-NMR data for compound I are listed in Table I. As regards the dimerization shifts, H-8 of Gfp shifted very little, whereas H-6 and H-5 of pU shifted significantly. These results suggested that GfpU had a right-handed stacked conformation. As already reported for Gf,[8] the amount of N-conformer of Gfp was calculated to be 80% and that of GfpU was 83%. This is a rather small increase, because the N-type furanose conformation in the monomer state is already favorable for stacking in A-form RNA as previously shown in the case of 2′-deoxy-2′-fluoroadenosine dimers. Finally, 2′-deoxy-2′-fluoroguanosine 3′-phosphate was synthesized as a reference compound for NMR studies of GfpU. The protected nucleoside (IV) was treated with β-cyanoethyl phosphate[10] and DCC at 90°C for 26 h. After appropriate deprotection and workup procedures, the product Gfp (VI) was obtained in a yield of 81.8% by DEAE-cellulose column chromatography. The UV absorption properties and chromatographic behavior were consistent with the structure VI.

Studies on the complex formed between GfpU and RNase T1 will be reported elsewhere.

Experimental[11]

N^2-Isobutyryl-2′-deoxy-2′-fluoroguanosine (III)——N^2-Isobutyryl-3′,5′-bis-tetrahydrofuranyl-2′-deoxy-2′-fluoroguanosine (II) (1.31 g, 2.64 mmol) was dissolved in 40% acetic acid and kept at room temperature for 12 h. After checking the deprotection by TLC, the solvent was evaporated off *in vacuo*. The residue was recrystallized from water to give the product (III) as pinkish needles, mp 245°C. Yield, 540 mg (1.57 mmol, 57.6%). *Anal.* Calcd for $C_{14}H_{18}O_5N_5F$: C, 47.32; H, 5.11; N, 19.71; F, 5.35. Found: C, 47.47; H, 4.92; N, 19.88; F, 5.11. UV: nm (ε) $\lambda_{\max}^{50\%\ \mathrm{EtOH}}$ 260 (17300), 281 (12700); $\lambda_{\max}^{0.1\mathrm{N}\ \mathrm{HCl}}$ 257 (21300), 275 (14800, sh); $\lambda_{\max}^{0.1\mathrm{N}\ \mathrm{NaOH}}$ 265 (17100); $\lambda_{\max}^{95\%\ \mathrm{EtOH}}$ 257.5 (16000), 282 (12100). PPC: *Rf*(A) 0.80 (G, 0.53) *Rf*(B) 0.65 (G, 0.28) *Rf*(C) 0.82 (G, 0.40). TLC: ($CHCl_3$–EtOH, 5: 1) *Rf* 0.33.

N^2-Isobutyryl-5′-O-monomethoxytrityl-2′-deoxy-2′-fluoroguanosine (IV)——Compound III (285 mg, 0.8 mmol) was dried by coevaporation with dry pyridine and dissolved in pyridine (8 ml). Monomethoxytrityl chloride (296 mg, 0.96 mmol) was added and the mixture was kept at room temperature for 17 h. After checking the completion of the reaction, water was added. The product was extracted with $CHCl_3$, and the extract was washed with water. $CHCl_3$ was evaporated off and the residue was dried by coevaporation with pyridine and toluene. The residue was dissolved in $CHCl_3$ and tritanol was removed by chromatography through a short (3×2.5 cm) column of Silica gel (9 g). The eluted nucleoside was dissolved in $CHCl_3$ and an attempt was made to precipitate it with *n*-hexane, but no powdery material was obtained. The crude product was used as such in the following reactions.

2′,3′-Di-O-acetyluridine 5′-phosphate (V)——The disodium salt of uridine 5′-phosphate (370 mg, 1 mmol) was dissolved in 30% aq. pyridine and Dowex 50 resin (pyridinium form 5 ml) was added. This mixture was applied to a column of Dowex 50 (pyridinium form, 5 ml). Elution with 50% pyridine (40 ml) and evaporation with added pyridine gave the anhydrous pyridinium salt of UMP. This material was dissolved in pyridine (5 ml) and acetic anhydride (2 ml) was added. The mixture was kept at room temperature for ammonium bicarbonate (pH 7.5). The bands at R(pU-U) 0.64 and 0.87 showed the completion of the reaction. After 5 h, the solvent was removed *in vacuo*, and the residue was dried by coevaporation with pyridine then precipitated from ether as a white powder. The yield was 385 mg (0.79 mmol, 79%).

2′-Deoxy-2′-fluoroguanylyl-(3′—5′)-uridine (I)——N^2-Isobutyryl-5′-monomethoxytrityl-2′-deoxy-2′-fluoroguanosine (IV) (260 mg, 0.41 mmol, 6100 A_{260}) and diacetyl-UMP (V, 300 mg, 0.60 mmol) were coevaporated *in vacuo* with added pyridine, then dissolved in anhydrous pyridine (5 ml). Dicyclohexylcarbodiimide (DCC) (825 mg, 4.0 mmol) was added and the solution was kept for 25 h at 30°C. After checking the extent of reaction by pepar electrophoresis, water was added to the solution. Stirring for 13 h at room temperature gave precipitates of dicyclohexylurea, which were removed by filtration. The filtrate and washings (pyridine) were combined and evaporated to dryness *in vacuo*. The residue was dried by coevaporation with added toluene. To the residue, 80% acetic acid (10 ml) was added and the solution was kept at 30°C for 22 h. Total detritylation was confirmed by TLC, then the solvent was evaporated off and traces of acetic acid were removed by coevaporation with water several times. The residue was dried by coevaporation successively with pyridine and toluene and kept in MeOH (25 ml previously saturated with dry NH_3 gas at 0°C) at room temperature for 19 h. The solvent was carefully removed by evaporation *in vacuo*. The residue was dissolved in 50% pyridine and washed with ether. The water solution was evaporated to dryness *in vacuo*. Water was added to the residue, and it was again evaporated to dryness. The yield was 10400 A_{257}. This material was dissolved in H_2O (200 ml) and applied to a column (1.5 × 40 cm = 70 ml) of DE 23 (bicarbonate form). The column was washed with H_2O (1 1) and eluted with 0—0.15 M triethylammonium bicarbonate (TEAB) buffer (total 4 1) in a linear gradient. The flow rate was 15 ml/15 min/fraction. Peaks I and II contained 8000 A_{257} of the product, which was rechromatographed on a column (1.5 × 45 cm = 80 ml) of DE 23 (bicarbonate form). Elution was achieved with 0—0.1 M TEAB buffer (total 41) in a linear gradient. The first peak contained 5600 OD_{257} units of the product, GfpU (0.256 mmol as calculated from ε 21900). The yield from the protected Gf(III) was 62.4%. UV: nm (εp) $\lambda_{max}^{H_2O}$ 257 (21900), $\lambda_{min}^{H_2O}$ 228 (8900); $\lambda_{max}^{pH\,7}$ 257 (23100), $\lambda_{min}^{pH\,7}$ 226.5 (7700); $\lambda_{max}^{pH\,2}$ 257.5 (20700), $\lambda_{min}^{pH\,2}$ 229 (5400); $\lambda_{max}^{pH\,12}$ 262 (19600); $\lambda_{min}^{pH\,12}$ 236 (9500). Paper chromatography: *Rf*(B) 0.19, *Rf*(C) 0.45, *Rf*(A) 0.17. CD: Taken at 21°C (Fig. 1) and pH 7 (0.01 M sodium cacodylate, 0.1 N NaCl) (55.8 μmol/ml) λ_{max} 215 nm ([θ] +2400), 248 nm ([θ] +1800), 266 nm ([θ] −2700), 280 nm ([θ] +3400); crossover 260 nm, 272 nm. The spectrum was also taken at pH 2 (0.01 M HCl, 0.1 M NaCl) (44.3 μmol/ml) λ_{max} 215 nm ([θ] +1500, 250 nm ([θ] +9900), 267 nm ([θ] −7000), 287 nm ([θ] +5600); crossover 259 nm, 276 nm. At pH 12 (0.01 N NaOH, 0.1 M NaCl) (24.7 μmol/ml) λ_{max} 225 nm ([θ] −5300), 265 nm ([θ] +6600); crossover 248 nm. ¹H-NMR was taken as the sodium salt, obtained from the triethylammonium salt by passage through Dowex 50 (pyridinium form) and Dowex 50 (Na⁺ form) columns (see Table I).

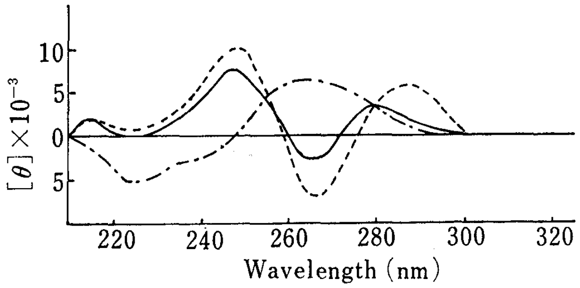

Fig. 1

---- pH 2; —— pH 7; —·— pH 12

Table I. NMR Data for 2′-Deoxy-2′-fluoroguanylyl-(3′—5′)-uridine and Related Compounds

		Chemical Shifts (ppm)				
Gfp(pD 5.5)		H-8	H-1′	H-2′	H-5′	H-5″
		7.97	6.21	5.56	3.97	3.86
			$J_{1'2'}=2.0$ Hz	$J_{2'3'}=4.8$ Hz	$J_{4'5'}=2.4$ Hz	$J_{4'5''}=4.5$ Hz
			$J_{1'F}=18.8$ Hz	$J_{2'F}=51.6$ Hz	$J_{5'5''}=-12.7$ Hz	
GfpU(pD 7.5)	Gfp-	7.95	6.15	5.53	4.02	3.87
			$J_{1'2'}=1.7$ Hz	$J_{2'3'}=4.5$ Hz	$J_{4'5'}=2.0$ Hz	$J_{4'5''}=4.1$ Hz
			$J_{1'F}=17.2$ Hz	$J_{2'F}=51.7$ Hz	$J_{5'5''}=-13.0$ Hz	
	-pU	H-6	H-5	H-1′		
		7.78	5.65	5.87		
		$J_{56}=8.2$ Hz		$J_{1'2'}=3.3$ Hz		
pU(pD 5.5)		7.95	5.93	5.96		
		$J_{56}=8.0$ Hz		$J_{1'2'}=4.1$ Hz		
Gfp[a]		H-8	H-1′	H-2′	H-5′	H-5″
		0.02	0.06	0.03	−0.05	−0.01
pU[a]		H-6	H-5	H-1′		
		0.17	0.28	0.09		

[a] Dimerization shifts (δmonomer−δdimer),

Enzymatic Hydrolysis of GfpU——GfpU (3 OD_{257}) was incubated with snake venom phosphodiesterase (1 mg/ml, 2 μl) in 50 mM TEAB buffer (pH 7.5, 98 μl) in a total volume 100 μl at 37°C for 15 h. Hypochromicity at 257 nm was 9%. The reaction mixture was subjected to paper electrophoresis in 0.5 M TEAB buffer at pH 7.5. Gf and pU were eluted from the spots: 0.575 $A_{252.5}$ and 0.405 A_{251} units. This corresponds to Gf: pU = 1.00: 0.94 as calculated with values of ε 13400 and 10000 for Gf and pU, respectively.

2′-Deoxy-2′-fluoroguanosine 3′-Phosphate (VI)——N^2-Isobutyryl-5′-monomethoxytrityl-2′-fluoro-2′-deoxyguanosine (IV) (3100 A_{260}, 0.20 mmol) was dissolved in pyridine (20 ml) containing β-cyanoethylphosphate (obtained from 86 mg of the Ba salt, 0.30 mmol) and coevaporated with added pyridine. DCC (310 mg, 1.5 mmol) was added to a pyridine (5 ml) solution of the residue and the mixture was kept at 30°C for 26 h. Then 50% aqueous pyridine (20 ml) was added and the solution was kept at room temperature for 13 h. Dicyclohexylurea was removed by filtration and the filtrate and washings were evaporated to dryness. To the residue, 80% acetic acid (20 ml) was added and the whole was kept at 50°C for 1 h with stirring. The solution was evaporated to dryness and traces of AcOH were removed by coevaporation with water. The residue was dissolved in conc. NH_4OH (20 ml) and kept at 50°C for 2 h. The solution was washed with ether and evaporated to dryness *in vacuo*. The residue was dissolved in 0.01 N HCl (500 ml) and applied to a column (1.3 × 9 cm) of activated charcoal. Elution with 50% EtOH containing 5% NH_4OH (200 ml) gave Gfp in a yield of 2700 A_{253}. This material was dissolved in H_2O (100 ml) and applied to a column (1.0 × 20 cm = 16 ml) of DE 23 (bicarbonate form). After washing with water (11) the column was eluted with 0—0.15 M TEAB buffer (total 2 1) in a linear gradient. The flow rate was 15 ml/5 min/fraction. Gfp was obtained in a yield of 2160 A_{253} (0.164 mmol calculated as ε 13200, 81.8%). UV: nm (ε) $\lambda_{max}^{H_2O}$ 253 (13200), 270 (sh, 9600), $\lambda_{max}^{H_2O}$ 223.5 (3500); $\lambda_{max}^{pH\ 7}$ 252 (13700), 270 (sh, 9500), $\lambda_{min}^{pH\ 7}$ 221 (2700); $\lambda_{max}^{pH\ 2}$ 254 (12600), 270 (sh, 8900), $\lambda_{min}^{pH\ 2}$ 230 (4500); $\lambda_{max}^{pH\ 12}$ 257—263 (11600), $\lambda_{min}^{pH\ 12}$ 224 (2400). PPC: *Rf*(B) 0.09, *Rf* (C) 0.19, *Rf*(A) 0.26.

Acknowledgement This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, for which the authors are grateful.

References and Notes

1) Part 87 of this series: M. Ikehara and J. Imura, *Chem. Pharm. Bull.*, **29**, 1034 (1981).
2) M. Ikehara and H. Miki, *Chem. Pharm. Bull.*, **26**, 2449 (1978).
3) S. Uesugi, H. Miki, M. Ikehara, H. Iwahashi, and Y. Kyogoku, *Tetrahedron Lett.*, **1979**, 4073.
4) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).
5) S. Uesugi, Y. Takatsuka, M. Ikehara, D.M. Cheng, L.S. Kan and P.O.P. Ts'o, *Biochemistry*, **20**, 3056 (1981)
6) M. Ikehara and T. Maruyama, *Tetrahedron*, **31**, 1369 (1975).
7) M. Ikehara and J. Imura, *J. Carbohyd., Nucleosides. Nucleotides*, **7**, 131 (1980).
8) M. Ikehara and J. Imura, *Chem. Pharm. Bull.*, **29**, 1034 (1981).
9) F. Egami, K. Takahashi and T. Uchida, "Nucleic Acid Research Molecular Biology", J. N. Davidson and W. E. Cohn ed., vol. 3, Academic Press, New York, 1964, p. 58.
10) G.M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
11) UV absorption spectra were taken on a Hitachi 124 spectrophotometer. Papaer chromatography was performed by the descending technique in the following solvents: A, EtOH–1 M NH_4OAc (7: 3); B, iso-PrOH–conc. $NH_4OH–H_2O$ (7: 1: 2); C, *n*-BuOH–AcOH–H_2O (5: 2: 3). CD spectra were taken with a Jasco ORD/UV-5 spectropolarimeter equipped with a CD attachment. NMR spectra were taken with a Hitachi R-900 spectrometer. Chemical shifts were measured from an external tetramethylsilane capillary.