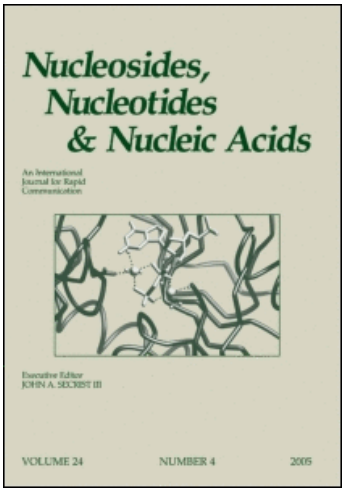

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Improved Synthesis of 2'-Fluoro-2'-Deoxyadenosine and Synthesis and Carbon-13 NMR Spectrum of Its 3',5'-Cyclic Phosphate Derivative[1]

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IMPROVED SYNTHESIS OF 2'-FLUORO-2'-DEOXYADENOSINE AND SYNTHESIS AND CARBON-13 NMR SPECTRUM[1] OF ITS 3',5'-CYCLIC PHOSPHATE DERIVATIVE

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Abstract: Some improvements were made on synthetic method for 2'-fluoro-2'-deoxyadenosine (11). Thus 11 was obtained in an overall yield of 9.3% starting from adenosine. 2'-Fluoro-2'-deoxyadenosine 3',5'-cyclic phosphate (13), an analogue of cAMP, was synthesized from 11. The carbon-13 NMR spectrum was measured. The sugar carbon signals can be unambiguously assigned since the C1', C2' and C3' have different ^{13}C-^{19}F coupling constants. Comparison of the data with those of other 3',5'-cyclic phosphate derivatives confirms the assignments of C3' and C4' signals previously proposed by us.

INTRODUCTION

2'-Fluoro-2'-deoxyadenosine (11, 2'-F-dAdo) is a unique analogue of adenosine. It takes a high population of C3'-endo furanose puckering form in C2'-endo-C3'-endo equilibrium[2] because of the highly electronegative 2'-substituent.[3] The C3'-endo puckering is a form adopted by a nucleoside residue in RNA. 2'-F-dAdo derivatives show interesting biological activities as nucleoside,[4] nucleoside triphosphate,[5] and homopolynucleotide.[6] The poly(I)·poly(C) analogue

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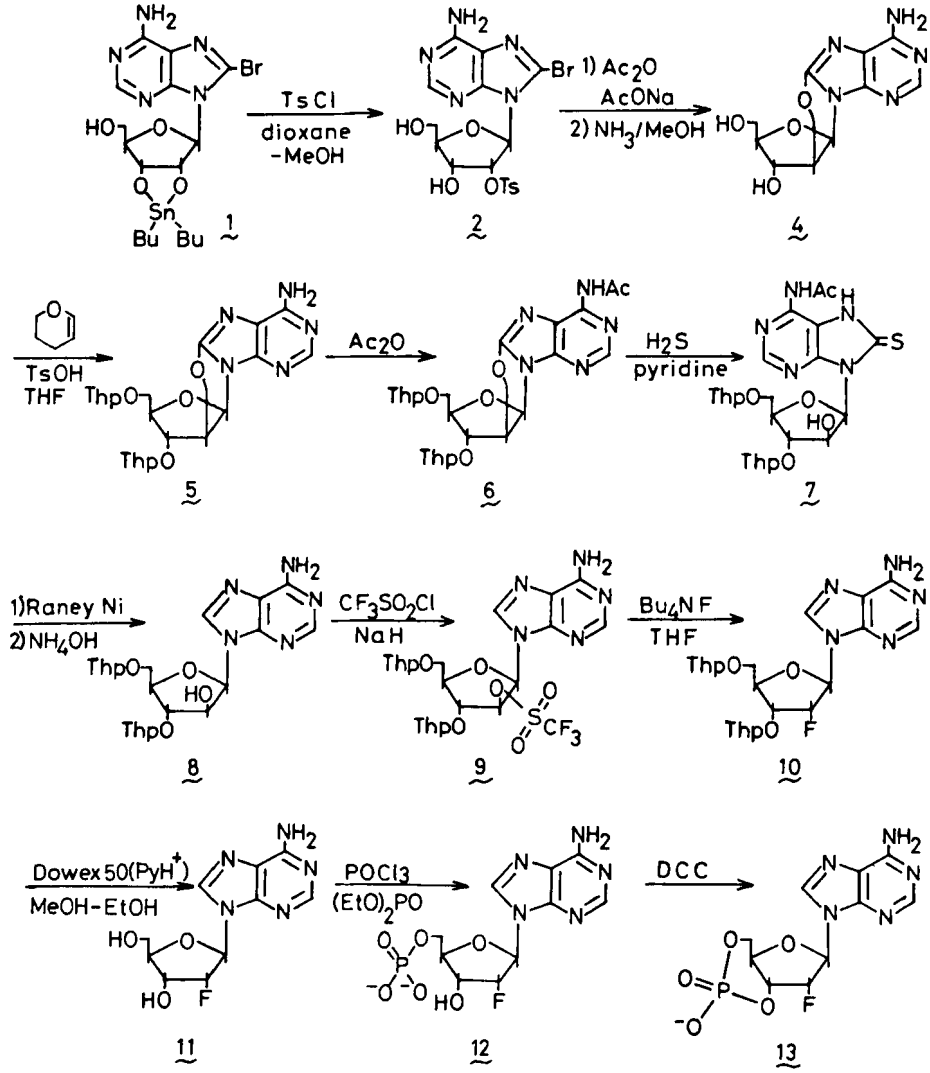
containing 2'-fluoro-2'-deoxyinosine residues shows an interferon-inducer activity as effective as the parent complex.[7] Recently it has been shown that deoxyribooligonucleotide duplexes containing 2'-fluoro-2'-deoxypurinenucleoside residues can be cleaved by restriction endonuclease EcoRI.[8] It is also shown that a 2'-F-dAdo residue in ribodinucleoside monophosphates greatly stabilizes its stacking conformation.[9,10] Therefore it is of interest to examine the biological activity of 2'-F-dAdo 3',5'-cyclic phosphate (13, 2'-F-dAdo-3',5'-P), an analogue of adenosine 3',5'-cyclic phosphate (cAMP) which is a ubiquitous regulatory molecule controlling diverse metabolic processes.[11] Moreover, it is expected that sugar carbon signals in the ^{13}C NMR spectrum of 13 can be unambiguously assigned since the C1', C2' and C3' signals have different ^{13}C-^{19}F coupling constants. Comparison of the data with those of other nucleoside 3',5'-cyclic phosphates will give a definitive solution to the disputed assignments of C3' and C4' signals for the 3',5'-cyclic phosphate derivatives.[12] In this paper, we report an improved method for the synthesis of 2'-F-dAdo and the synthesis and ^{13}C NMR spectrum of 2'-F-dAdo-3',5'-P.

RESULTS AND DISCUSSION

Improved Method for Synthesis of 2'-Fluoro-2'-deoxyadenosine

Synthesis of 2'-F-dAdo involves a rather lengthy route similar to that shown in Scheme 1 starting from adenosine.[13-15] For rapid and large scale synthesis, some improvements in yield and simplification of the procedures are desirable. In the step of 2'-O-p-toluenesulfonyl-8-bromoadenosine (2) synthesis,[16] the solvent was changed from methanol to dioxane-methanol (95:5) to reduce the amount of p-toluenesulfonyl chloride required. In the step of tetrahydropyranylation of 8,2'-O-cycloadenosine (4),[17] tetrahydrofuran (THF) was used as a solvent instead of dimethylformamide (DMF) which can produce



SCHEME 1

a N^6-dimethylaminomethylene derivative in the presence of strong acid (p-toluenesulfonic acid) and is much less volatile. In the next step for synthesis of arabinosyl-8-thioadenine derivative (7), the 6-NH_2 group of 5 was acetylated in order to activate 5 toward cleavage of the cyclo bond by liquid H_2S. This procedure reduced the reaction time from 10 h to 6 h and the reaction temperature from 110°C to 70°C. The reactions from 5 to 8 were carried out without purification of the intermediates and the overall

yield was 97%. The trifluoromethanesulfonylation of 8 was carried out under N_2 atomosphere at -50°C (iso-butanol-dry ice mixture) rather than at -60°C[13,14] in order to accelerate the reaction within the limited conditions to avoid side reactions. The yield of 9 could be improved up to 79%. In the final deprotection step, the tetrahydropyranyl group was removed by treatment with pyridinium Dowex 50 resin in an ethanol-methanol mixture (1:1) at 40°C for 4 h. The yield of this step was greatly improved (37% with 80% acetic acid[13] to 93% with the cation exchange resin). Thus 2'-F-dAdo was synthesized from adenosine in an overall yield of 9.3%.

Synthesis of 2'-Fluoro-2'-deoxyadenosine 3',5'-Cyclic Phosphate

2'-Fluoro-2'-deoxyadenosine 5'-phosphate (12, 2'-F-dAdo-5'-P) was synthesized by phosphorylation of 2'-F-dAdo with $POCl_3$ in triethyl phosphate.[18] After desalting, 12 was isolated by Dowex 1 anion exchange column chromatography in 80% yield. 2'-F-dAdo-3',5'-P (13) was synthesized by intramolecular condensation of the 3'-OH and the phosphate groups using dicyclohexylcarbodiimide in a relatively large volume of pyridine.[19] 2'-F-dAdo-3',5'-P was isolated by chromatography on a column of DEAE-cellulose in 73 % yield. These compounds were characterized and identified by paper electrophoresis and ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum of 2'-F- dAdo-3',5'-P shows that the coupling constant between H1' and H2' ($J_{1',2'}$) is close to 0 Hz while the $J_{1',2'}$ of 2'-F-dAdo-5'-P is 2.2 Hz. The small $J_{1',2'}$ (< 1 Hz) is characteristic of β-nucleoside 3',5'-cyclic phosphates.[20]

^{13}C NMR Spectrum of the 3',5'-Cyclic Phosphate Derivative

The ^{13}C NMR data of the 2'-F-dAdo derivatives are shown in Table 1. The resonances of C1', C2' and C3' can be unambiguously assigned since they have different ^{13}C-^{19}F coupling constants. The remaining C4' and C5' resonances can be easily distinguished because their

TABLE 1

^{13}C Chemical Shifts (ppm) and ^{13}C-^{31}P Coupling Constants (Hz, in parentheses) for 2'-F-dAdo Derivatives

Compound	C2	C4	C5	C6	C8
2'-F-dAdo	153.41	149.55	119.87	156.93	140.12
2'-F-dAdo-5'-P	152.83	148.33	118.75	155.44	140.03
2'-F-dAdo-3',5'-P	153.40	148.49	118.91	155.77	140.30

Compound	C1'	C2'	C3'	C4'	C5'
2'-F-dAdo[a]	86.58	94.05	69.17	85.00	61.30
2'-F-dAdo-5'-P[b]	86.55	94.17	68.83	82.59 (8.1)	63.64 (4.7)
2'-F-dAdo-3',5'-P[c]	89.42	91.65 (8.5)	76.72 (4.5)	71.87 (4.1)	67.61 (6.8)

a. $J_{1'F}$ = 33.9 Hz, $J_{2'F}$ = 187.8 Hz, $J_{3'F}$ = 15.9 Hz.
b. $J_{1'F}$ = 33.9 Hz, $J_{2'F}$ = 187.9 Hz, $J_{3'F}$ = 15.5 Hz.
c. $J_{1'F}$ = 34.6 Hz, $J_{2'F}$ = 191.0 Hz, $J_{3'F}$ = 16.0 Hz.

TABLE 2

Chemical Shift Changes (ppm)[a] in Sugar Carbon Signals by the Introduction of a 3',5'-Cyclic Phosphate Group

Compound	C1'	C2'	C3'	C4'	C5'
8,2'-S-cyclo-Ado-3',5'-P[b]	0.93	-6.23	7.96	-14.79	5.46
dThd-3',5'-P[c]	1.41	-5.24	5.72	-12.59	5.69
Urd-3',5'-P[c]	6.53	-1.90	6.89	-13.36	6.04
Cyd-3',5'-P[c]	5.49	-1.98	7.42	-12.63	6.39
Guo-3',5'-P[c]	5.32	-1.84	6.49	-13.59	5.72
Ado-3',5'-P[d]	3.30	-1.31	6.39	-14.35	5.37
2'-F-dAdo-3',5'-P	2.84	-2.40	7.55	-13.13	6.31
8-Br-Ado-3',5'-P[d]	2.38	-0.27	5.76	-15.01	4.77

a. δ(3',5'-cyclic phosphate) - δ(nucleoside). The positive value represents a downfield shift.

b. Data were taken from ref.23.

c. Nucleotide data were taken from ref.21 with the revised assignments.

d. Data were taken from ref.22.

chemical shift difference is usually large. Therefore, all the sugar carbon signals of 2'-F-dAdo-3',5'-P are safely assigned. The chemical shifts of the base carbon signals are very similar to those of adenosine.

^{13}C NMR spectra of 3',5'-cyclic phosphate derivatives of four ribonucleosides and deoxythymidine were first published by Lapper et al.[21] During ^{13}C NMR studies on phosphate derivatives of modified nucleosides,[22,23] we found that the published assignments of C3' and C4' signals of the 3',5'-cyclic phosphates should be reversed. This proposition was based on analysis of chemical shift changes upon introduction of the 3',5'-cyclic phosphate group.[12] According to the revised assignments, it turns out that the C4' signal generally shows an unusually large upfield shift (-12 to -15 ppm) and the signals of C3' and C5', which are attached to the phosphate group, generally shows a considerable downfield shift (6-8 ppm and 5-6 ppm, respectively) when compared with those for the corresponding nucleoside. This was confirmed by selective ^{1}H decoupling experiments on the 3',5'-cyclic phosphates of adenosine and deoxythymidine.[24] The present results for 2'-F-dAdo-3',5'-P provide a further support for the assignments as shown in Table 2 where the chemical shift changes in sugar carbon signals upon introduction of a 3',5'-cyclic phosphate group are presented. The large upfield shift of C4' resonance may be caused by the close contact of the C4' with the diesterified phosphate group.

In conclusion, the improved method for synthesis of 2'-F-dAdo described here will be a great help for studying various compounds containing 2'-F-dAdo as an adenosine or deoxyadenosine analogue. The fluorine atom can be a probe for studying structure and dynamics of the molecules. The present study on 2'-F-dAdo-3',5'-P is one example of such application.

EXPERIMENTAL

General Procedures. Activated charcoal for chromatography (code no. 031-02135) was supplied by Wako

Pure Chemical Industries, Ltd., Osaka, Japan. UV absorption spectra were recorded on a Hitachi 200-10 spectrophotometer. ^{1}H NMR (90 MHz) and ^{13}C NMR (22.63 MHz) spectra were recorded with a Hitachi R-900 spectrometer operating in the FT mode. ^{1}H chemical shifts were measured downfield from DSS and ^{13}C chemical shifts were determined relative to external TMS in H_2O (for nucleotides) or internal TMS in DMSO-d_6 (for nucleosides). Mass spectra were recorded with a JEOL JMS-D300 spectrometer.

2'-O-p-Toluenesulfonyl-8-bromoadenosine(2) To a suspension of 8-bromoadenosine[25] (15.1 g, 43.6 mmol) in methanol (900 ml), di-n-butyltin oxide (11.4 g, 48 mmol) was added and the mixture was heated under reflux for 1.5 h. Methanol was removed in vacuo. The white powder of the 2',3'-di-n-butylstannylene derivative (1) thus obtained was used in the next reaction without purification. To a suspension of 1 in dioxane (414 ml)-methanol(21.8 ml), p-toluenesulfonyl chloride (24.9 g, 3eq.) and triethylamine (18.2 ml, 3 eq.) were added and the mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure. The residue was treated with methanol, filtered and washed with methanol to give 20.4 g of 2 (40.8 mmol, 93.5%) as a white solid: m.p. 232°C (decomp); UV λ_{max}^{MeOH} 266, 230(shoulder); MS (m/e) 501(M+1), 499(M-1), 471, 469, 412.

2'-O-p-Toluenesulfonyl -N^6,3',5'-O-triacetyl-8-oxyadenosine (3). 2 (35.2 g, 70.4 mmol) was dissolved in acetic acid(705 ml)-acetic anhydride(300 ml) and sodium acetate (86.6 g) was added. The mixture was heated under reflux for 1 h and evaporated to dryness in vacuo. The residue was shaken with water-$CHCl_3$. The $CHCl_3$ extracts were washed with saturated aq $NaHCO_3$ and saturated aq NaCl and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a pale yellow syrup. Crystallization from ethanol gave 26.7 g of 3 (47.2 mmol, 67%) as colorless prisms: UV λ_{max}^{EtOH} 289 nm; MS(FD, m/e) 565(M).

3',5'-Di-O-tetrahydropyranyl-8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (5). A solution of 3 (26.6 g, 47.1 mmol) in methanol (200 ml) was saturated with NH_3 gas under cooling with ice-salt mixture. The mixture was heated at 70°C for 7 h in a sealed tube. After spontaneous evaporation of NH_3, 8,2'-anhydro-8-oxy-9-β-D-arabinofuranosyladenine (4) crystallized out as prisms. After filtration and washing with methanol, 11.9 g of 4 (44.7 mmol, 94.7%) was obtained: m.p. 225°C (decomp). This sample was used in the next reaction without purification. To a suspension of 4 (44.7 mmol) in tetrahydrofuran (THF, 44.7 ml), p-toluenesulfonic acid (8.5 g) was added at room temperature and the mixture was stirred for 10 min. To the mixture cooled with ice-salt mixture, dihydropyran (40.7 ml) was added dropwise. An additional amount of p-toluenesulfonic acid (8.5 g) was added and the mixture was stirred for 3 h under cooling. Concd NH_4OH (10 ml) was added and the mixture was evaporated to dryness in vacuo. The residue was treated with water and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with water, dried over anhydrous $MgSO_4$ and evaporated to dryness. Crystallization from ethanol gave 17.4 g of 5 (40.2 mmol, 90.5%): m.p. 246-249°C; UV λ_{max}^{EtOH} 256 nm; MS (m/e) 433(M), 349, 265, 151. Anal. Calcd for $C_{20}H_{27}N_5O_6$: C, 55.42; H, 6.28, N, 16.16. Found: C, 55.44; H, 6.24; N, 16.21.

3',5'-Di-O-tetrahydropyranyl-9-β-D-arabinofuranosyladenine (8). 5 (4.77 g, 11 mmol) was treated with actic anhydride (14.2 ml) in pyridine at 80°C for 3 h. The solvent was removed in vacuo. The residue was treated with pyridine-water (1:1) and the mixture was evaporated to dryness. The residue was dried by repeated evaporation with anhyd pyridine. The yellow syrup containing the N^6-acetyl derivative (6) was dissolved in pyridine. The mixture was cooled with dry ice-ethanol mixture and H_2S gas was introduced for 30 min. The mixture was heated at 70°C for 6 h in a sealed tube.

H_2S was expelled by spontaneous evaporation and bubbling with N_2 gas. The solvent was evaporated and the resulting yellow syrup containing 8-thioadenine arabinoside derivative (7) was used for further reaction. The syrup was dissolved in dioxane (66 ml) and treated with Raney Ni (28 ml) suspended in water-ethanol (1:1) at 60°C for 30 min. The catalyst was removed by filtration and the filtrate was treated with ethanol(110 ml)-concd NH_4OH(110 ml) at room temperature for 1 h. The volatile materials were evaporated. The residue was added to water and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with water and saturated aq NaCl, dried over anhyd $MgSO_4$ and evaporated. A solution of the resulting syrup in $CHCl_3$ was added dropwise to n-pentane and precipitates of 8 (4.63 g, 10.6 mmol, 96.6%) were collected. Recrystallization from methanol-water (3:1) gave colorless prisms: m.p. 193-198°C; UV λ_{max}^{EtOH} 260 nm; MS (70 eV, m/e) 435(M), 356, 277. Anal. Calcd for $C_{20}H_{29}N_5O_6$: C, 55.16, H, 6.71; N, 16.08. Found: C, 55.10, H, 6.72; N, 15.91.

3',5'-Di-O-tetrahydropyranyl-2'-fluoro-2'-deoxyadenosine (10). 8 (1.31 g, 3 mmol) was dissolved in THF (30 ml) and NaH (126 mg) was added. The mixture was stirred under N_2 atomosphere at 0°C for 2 h. Trifluoromethanesulfonyl chloride (0.4 ml) was added dropwise to the mixture cooled with dry ice-iso-butanol mixture with stirring. The reaction mixture was stirred at the same temperature for 30 min and added dropwise into saturated aq $NaHCO_3$(150 ml)-ice(150 g) mixture. The precipitates of the sulfonylated derivative (9) (1.16 g, 2 mmol, 67%) were collected and used in the following step without purification. To a solution of 9 (2.27 g, 4 mmol) in THF (30 ml), 1.5 M tetra-n-butylammonium fluoride in THF (10 ml) was added dropwise with stirring at 0°C and the mixture was stirred for 5 h at the same temperature. The solvent was evaporated in vacuo. The residue was treated with water and

extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with water and saturated aq NaCl, dried over anhyd $MgSO_4$ and evaporated to dryness. The residue was treated with 5% aq acetic acid (50 ml) at room temperature for 10 h with stirring to destroy an elimination byproduct.[14] The solvent was removed under reduced pressure to give a yellow syrup. Crystallization from methanol and recrystallization from methanol-water (1:1) gave 629 mg of 10 (1.44 mmol, 36%) as colorless needles: m.p. 176-179°C; UV λ_{max}^{EtOH} 260 nm; MS (20 eV, m/e) 438(M+1), 437 (M), 408, 352, 268. Anal. Calcd for $C_{20}H_{28}N_5O_5F$: C, 54.91, H, 6.45; N, 16.01. Found: C, 54.86; H, 6.49; N, 15.92.

2'-Fluoro-2'-deoxyadenosine (11). A solution of 10 (214 mg, 0.49 mmol) in methanol(7.3 ml)-ethanol(7.3 ml) was treated with pyridinium Dowex 50 resin (2.5 g) at 40 °C for 4 h. The mixture was shaken occasionally. The resin was removed by filtration and washed with pyridine-triethylamine-water (1:3:3) mixture. The filtrate and washings were combined and evaporated to dryness. Crystallization from water gave 122 mg of 11 (0.45 mmol, 92.7%) as colorless prisms: m.p. 233°C; MS (m/e) 269.0922 (calcd for $C_{10}H_{12}N_5O_3F$: 269.0922); UV λ_{max}^{EtOH} 260 nm.

2'-Fluoro-2'-deoxyadenosine 5'-Phosphate (12). A mixture of $POCl_3$ (0.3 ml, 3.3 mmol) and triethyl phosphate (3 ml) was stirred at 0°C for 15 min. 11 (120 mg, 0.45 mmol) was added and the mixture was stirred at 0°C for 6 h. Completion of phosphorylation reaction was checked by paper electrophoresis at pH 7.5. The reaction mixture was poured into ice-water (600 ml). The resulting solution was applied on a column of activated charcoal for chromatography. The column was washed thoroughly with water and eluted with 50% aq ethanol containing 5% concd NH_4OH. The desalted product was chromatographed on a column (0.8 x 25 cm) of Dowex 1 (formate form) resin. Elution was carried out with a

linear gradient of formic acid (0-0.2 M, total 2 l) at a flow rate of 18 ml/10 min. 5,210 A_{260} units of 12 (79.8%) were eluted at around 0.11 M formic acid concentration. The formic acid was removed by coevaporation with water and lyophilization. ^{1}H NMR (D_2O, pD 7.5) δ 8.52(s,1,H8), 8.23(s,1,H2), 6.40(dd,1,$J_{1',2'}$=2.2 Hz,$J_{1',F}$ =16.2 Hz,H1'), 5.43(dq,1,$J_{2',3'}$=4.1 Hz,$J_{2',F}$=52.3 Hz,H2'). The ^{13}C NMR data are presented in Table 1.

2'-Fluoro-2'-deoxyadenosine 3',5'-Cyclic Phosphate (13). A mixture of 12 (free acid, 0.36 mmol) and 4-morpholine-N,N'-dicyclohexylcarboxamidine[19] (148 mg, 0.72 mmol) was dissolved in 80% aq pyridine. The carboxamidinium salt of 12 was dried by repeated coevaporation with anhyd pyridine and finally dissolved in anhyd pyridine (36 ml). This solution was added dropwise to a boiling solution of dicyclohexylcarbodiimide (148 mg, 0.72 mmol) in anhyd pyridine (36 ml) over a period of 2 h. The reaction mixture was heated under reflux for 6 h. Water (50 ml) was added and the mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the residue shaken with an ether-water mixture. The solid materials were removed by filtration and the aq layer was treated with concd NH_4OH (2 ml). After addition of ethanol (5 ml), the volatile materials were removed by evaporation. The residue was dissolved in water and chromatographed on a column (1.2 x 25 cm) of DEAE-cellulose (bicarbonate form). Elution was carried out with a linear gradient of triethylammonium bicarbonate buffer (pH 7.5) (0-0.15 M, total 3 l). 3,803 A_{260} units of 13 (0.26 mmol, 73%) were eluted at around 0.02 M salt concentration. The salt was removed by repeated coevaporation with water and lyophilization. ^{1}H NMR (D_2O, pD 7.5) δ 8.24(s,1,H8), 8.21 (s,1,H2), 6.45(d,1,$J_{1',F}$=22.3 Hz,H1'), 5.59(dd,1, $J_{2',3'}$= 4.0 Hz,$J_{2',F}$=53.9 Hz,H2'). The ^{13}C NMR data are presented in Table 1.

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