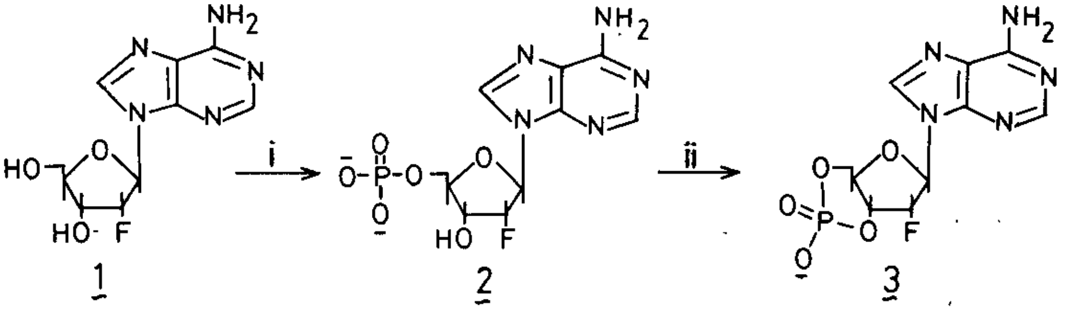
CARBON-13 NMR SPECTRUM OF 2'-FLUORO-2'-DEOXYADENOSINE 3',5'-CYCLIC PHOSPHATE

Seiichi Uesugi, Jitsuhiro Matsugi, Toshinori Kaneyasu, and
Morio Ikehara*
Faculty of Pharmaceutical Sciences, Osaka University, Suita,
Osaka 565, Japan

Abstract —— 2'-Fluoro-2'-deoxyadenosine 3',5'-cyclic phosphate (3) was synthesized and its ^{13}C nmr spectrum was measured. The sugar carbon signals can be unambiguously assigned because the C(1'), C(2') and C(3') have different ^{13}C-^{19}F coupling constants. Comparison of the present data with those of other nucleoside 3',5'-cyclic phosphates gives a definitive solution to the disputed assignment of the C(3') and C(4') signals for the 3',5'-cyclic phosphate derivatives.

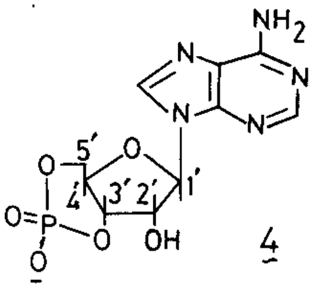
Nucleoside 3',5'-cyclic phosphates are known to play important roles in living organisms. Adenosine 3',5'-cyclic phosphate (Ado-3',5'-P, 4)[1] and guanosine 3',5'-cyclic phosphate[2] are extensively studied, and are recognized as ubiquitous regulatory molecules controlling diverse metabolic process. The 3',5'-cyclic phosphate derivatives of cytidine and uridine are also found in animal cells, and are considered to regulate cell growth.[3] ^{13}C nmr spectra of 3',5'-cyclic phosphate derivatives of four ribonucleosides and thymidine were first published by Lapper et al. in 1973.[4] During ^{13}C nmr studies on phosphate derivatives of modified nucleosides,[5,6] we found that the published assignment of the C(3') and C(4') signals of the 3',5'-cyclic phosphates should be reversed. This proposition was based on analysis of chemical shift changes upon introduction of a 3',5'-cyclic phosphate group.[7] The definitive answer to this problem can be obtained by measuring ^{13}C spectrum of a 3',5'-cyclic phosphate derivative with some kind of label on the C(3') or C(4'). For this purpose, the 3',5'-cyclic phosphate derivative of 2'-fluoro-2'-deoxyadenosine (2'-F-dAdo-3',5'-P, 3) was chosen by us since its C(3') has ^{13}C-^{19}F coupling constant which is different from those of the C(1') and C(2').[8] Moreover, it is very interesting to examine the biological activity of

2'-F-dAdo-3',5'-P because the homopolynucleotides containing 2'-fluoro-2'-deoxypurinenucleoside residues have shown similar biological activities to those of the corresponding natural nucleoside derivatives.[9,10] We report here the synthesis of 2'-F-dAdo-3',5'-P and its ^{13}C nmr spectrum which confirms our previous proposition.[7]



i : $POCl_3$, $(EtO)_3PO$

ii : DCC, Pyridine



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2'-Fluoro-2'-deoxyadenosine (2'-F-dAdo, **1**) was synthesized by a modified procedure of Ikehara *et al.*[11] *via* cycloadenosine and arabinofuranosyladenine derivatives. The overall yield of **1** from adenosine was 6 %.[12] **1** was phosphorylated with $POCl_3$ in triethyl phosphate[13] to give the 5'-phosphate of **1** (2'-F-dAdo-5'-P, **2**). **2** was isolated by chromatography on a column of Dowex 1 resin in 80 % yield. 2'-F-dAdo-3',5'-P (**3**) was synthesized by intramolecular condensation of the 3'-OH and phosphate groups using dicyclohexylcarbodiimide in relatively large volume of pyridine.[14] **3** was isolated by chromatography on a column of DEAE-cellulose in 73 % yield. This compound was characterized and identified by various techniques such as paper chromatography, paper electrophoresis, ^{1}H nmr and ^{13}C nmr. The ^{1}H nmr spectrum shows that the coupling constant between H1' (δ, 6.45 ppm) and H2' (δ, 5.59 ppm) ($J_{1',2'}$) 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.[15] The ^{13}C nmr data for the sugar carbons are shown in Table I. The data of

2'-F-dAdo and its 5'-phosphate are also included. The resonances of C(1'), C(2') and C(3') are easily assigned because they have ^{13}C-^{19}F coupling constants of about 34, 190 and 16 Hz, respectively. The remaining C(4') and C(5') resonances can be easily distinguished because their chemical shift difference is usually large, and the ^{13}C-^{31}P coupling constant data of 2'-F-dAdo-5'-P confirm the assignment. Therefore, all the sugar carbon signals of 2'-F-dAdo-3',5'-P are safely assigned. The chemical shift changes in sugar carbons upon introduction of a 3',5'-cyclic phosphate group, δ(3',5'-cyclic phosphate) - δ(nucleoside), are shown in Table II. The data for Ado-3',5'-P are also included for the purpose of comparison. The both 3',5'-cyclic phosphates show about the same changes in each carbon. These results unambiguously confirm the unusually large upfield shift of C(4') resonance of Ado-3',5'-P which has been suggested by us.[7] This large upfield shift (-12 - -15 ppm), which is generally observed in 3',5'-cyclic phosphate derivatives,[7] may be caused by the close contact of C(4') with the diesterified phosphate group. Since 3',5'-cyclic phosphates have a quite rigid sugar-phosphate structure, these compounds may provide a good model system for theoretical calcu-

Table I. ^{13}C Chemical shifts (ppm, from external TMS) and ^{13}C-^{31}P coupling constants (Hz, in parentheses)*

Compound	C(1')	C(2')	C(3')	C(4')	C(5')
2'-F-dAdo	86.58	94.05	69.17	85.00	61.30
2'-F-dAdo-5'-P	86.55	94.17	68.83	82.59 (8.1)	63.64 (4.7)
2'-F-dAdo-3',5'-P	89.42	91.65 (8.5)	76.72 (4.5)	71.87 (4.1)	67.61 (6.8)

* ^{13}C nmr spectra were recorded on a Hitachi R-900 (22.63 MHz) spectrometer operating in the Fourier transform mode. The nucleotides were measured in D_2O (pD 7.5) and the nucleoside in DMSO-d_6.

Table II. Chemical shift changes (ppm)* in sugar carbons by the introduction of a 3',5'-cyclic phosphate group

Compound	C(1')	C(2')	C(3')	C(4')	C(5')
Ado-3',5'-P	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

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

lation of ^{13}C shielding effects.

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Received, 29th August, 1981