SYNTHESIS AND CARBON-13 MAGNETIC RESONANCE SPECTRA OF PYRIDINIUM SALTS DERIVED FROM NUCLEOSIDES AND NUCLEOBASES •

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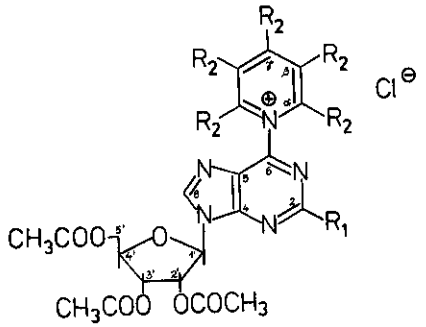
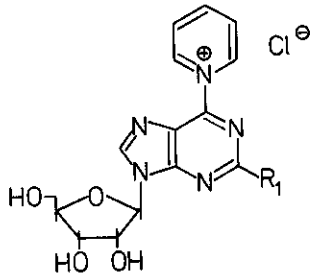
Abstract - Synthesis of N-[9-(β-D-ribofuranosyl)purin-6-yl]-pyridinium chloride 9 and N-[9-(β-D-ribofuranosyl)-2-amino-purin-6-yl]pyridinium chloride 10 is described. Carbon-13 nmr spectra of those and other nucleoside- and nucleobase-derived pyridinium salts are presented.

We have recently reported that the nucleobases and O-protected derivatives of inosine, guanosine, uridine, and thymidine undergo quantitative transformation into the water-soluble, fluorescent pyridinium salts 1-8,11-13 during pyridine-assisted phosphorylations[1-4] of their lactam systems. Analogous structures have been considered[2-5] as potential ionic side-products in oligonucleotide synthesis by the phosphotriester method. These new representatives of N-aryl-pyridinium salts, due to their interesting chemical[2-4,6-8] and photochemical[1,9] properties, have already been proposed as novel, highly reactive synthetic intermediates in the nucleoside field. Photophysical properties of a series of N-(purin-6-yl)pyridinium salts[10] and a crystallographic structure in the case of N-(2-aminopurin-6-yl)pyridinium chloride 7[11] have also been reported. In the present report we would like to describe synthetic routes to fully deprotected, labile salts N-[9-(β-D-ribofuranosyl)purin-6-yl]pyridinium chloride 9 and N-[9-(β-D-ribofuranosyl)-2-aminopurin-6-yl]pyridinium chloride 10.

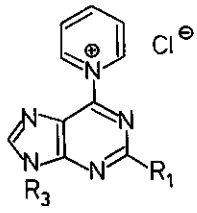
• Dedicated to Prof. Maciej Wiewiorowski on the occasion of his 70th birthday.



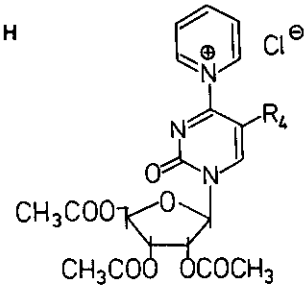
1 $R_1 = R_2 = H$
2 $R_1 = H$, $R_2 = D$
3 $R_1 = NH_2$, $R_2 = H$
4 $R_1 = NHCOCH(CH_3)_2$, $R_2 = H$
5 $R_1 = NHCOC_6H_5$, $R_2 = H$



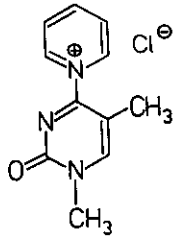
9 $R_1 = H$
10 $R_1 = NH_2$



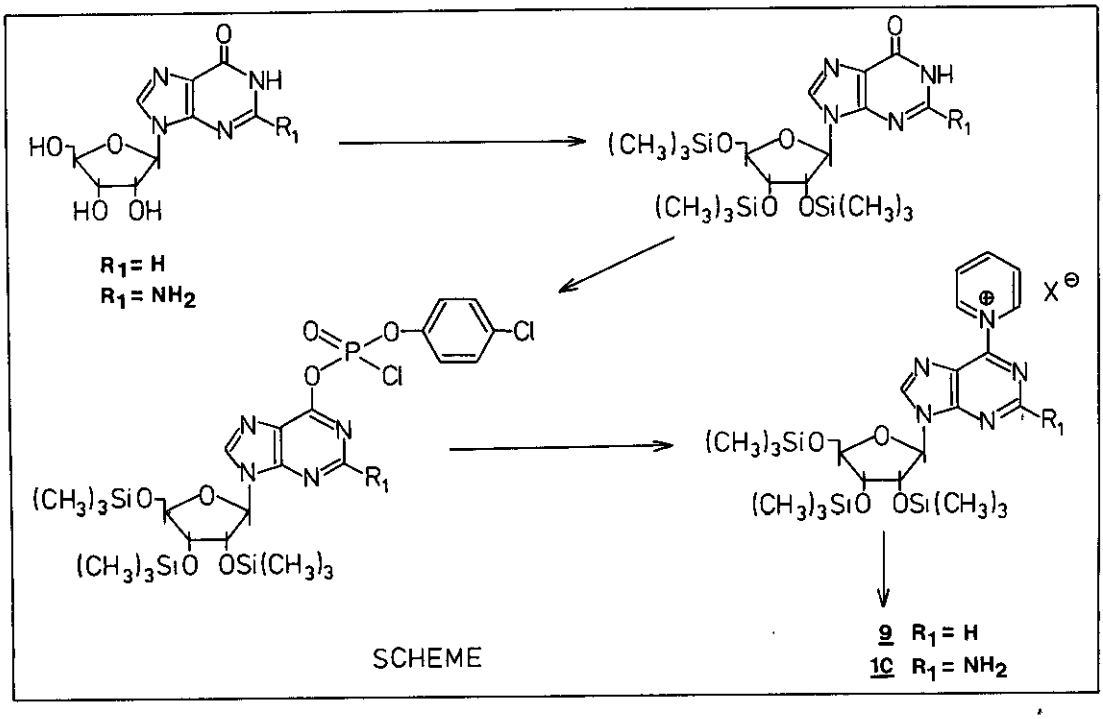
6 $R_1 = R_3 = H$
7 $R_1 = NH_2$, $R_3 = H$
8 $R_1 = H$, $R_3 = CH_3$



11 $R_4 = H$
12 $R_4 = CH_3$



13



^{13}C Nmr spectra of 9, 10 and previously synthesized pyridinium salts: 1,2[2]; 3-5[3]; 6[10]; 7[11]; 8[2]; 11,12[3] and 13[12] will also be characterized to provide a basis for further conformational studies of these novel nucleoside analogues.

"One pot" synthesis of 9 and 10 was performed utilizing the transient protection concept[13] (scheme). Under strictly anhydrous conditions inosine or guanosine was treated with trimethylchlorosilane (5 eqv.) in pyridine at 5-10°C until complete O-trimethylsilylation was detected by tlc. Subsequently, 4-chlorophenylphosphorodichloridate (1.5 eqv.) was added at 5°C and the reaction mixture kept overnight to induce O-6 phosphorylation following by displacement reaction at C-6 site. This led to O-silylated pyridinium salts. The reaction course was monitored by ^{31}P nmr and a spectrophotometrical test as described before[3]. Afterwards, the reaction mixture was treated with water (10 eqv.) to hydrolyze silyl ether protecting groups. Dilution with water and extraction with chloroform to remove lipophylic by-products led to an aqueous layer which was concentrated and subjected to chromatography on reverse-phase silica gel column using acetone gradient in 0.005N HCl. Desired fractions were collected, neutralized to pH 6.5 with Dowex 1 HCO_3^- beads, concentrated to remove pyridine and passed through Dowex 1 Cl^- column to give pure, as checked by ^1H nmr, aqueous solutions of 9 or 10 in 25 and 30% yields respectively.

Alternatively, acid promoted de-O-acetylation of 1[2] and 3[3] appeared to be useful for preparation of 9 and 10 respectively in an analytical scale e.g. for nmr measurements. Thus, solution of 1 or 3 in D_2O was acidified with DCl to pD 2.0 at 5°C and progress of the reaction was monitored by ^1H nmr. To obtain complete de-O-acetylation the solution was concentrated in vacuo at 5°C and then diluted with DCl/D_2O to maintain initial conditions. When repeated 2 or 3 times, this procedure allows to remove an excess of acetic acid and prevents the possible depurination of the pyridinium salts. Finally, the mixture was passed through Sephadex G-10, the desired fluorescent fractions collected and concentrated to give pure solutions of 9 or 10 (95% yield in both cases).

^{13}C Nmr chemical shift values and coupling constants of pyridinium salts are presented in Tables 1 and 2 respectively. Within the purine group the resonances at 151.21, 144.12 and 129.27 ppm were assigned to pyridinium cation carbons

Table 1. ^{13}C Chemical shifts of pyridinium salts 1-11 and 13[a,b]

	Aglycone								Ribose					Acetyl groups					
No	C-2	C-4	C-5	C-6	C-8	C-α	C-β	C-γ	C-1'	C-2'	C-3'	C-4'	C-5'	C=O			CH_3		
1	153.06	155.71	126.29	147.37	149.32	144.12	129.27	151.21	88.04	74.06	71.19	81.10	63.77	174.24	173.32	173.10	21.02	20.75	20.58
2	153.06	155.71	126.29	147.37	149.32	143.74[c]	128.78[c]	[d]	88.04	74.06	71.19	81.10	63.77	174.24	173.37	173.10	21.02	20.72	20.58
3	160.37	157.71	118.65	147.52	145.63	143.41	129.16	151.05	87.66	73.95	71.13	80.56	63.77	174.29	173.37	173.21	20.96	20.80	20.69
4[e]	152.73	156.74	122.88	147.26	148.72	143.79	129.21	151.37	88.30	74.17	71.35	80.89	63.93	174.18	173.32	173.16	20.91	20.75	20.64
5[f]	152.79	156.74	122.66	146.55	148.72	143.52	129.43	151.64	88.20	74.12	71.35	80.89	64.09	173.81	172.88	172.88	20.97	20.69	20.69
6	152.62	157.17	124.99	146.93	149.54	144.12	129.16	150.89	-	-	-	-	-	-	-	-	-	-	-
7	160.10	158.90	117.08	146.72	146.07	143.25	129.06	150.77	-	-	-	-	-	-	-	-	-	-	-
8[g]	152.36	156.74	125.43	146.61	151.98	144.06	129.38	151.16	-	-	-	-	-	-	-	-	-	-	-
9	152.84	155.87	126.40	147.37	149.15	144.12	129.21	151.10	89.83	74.77	70.97	86.36	61.98	-	-	-	-	-	-
10	160.21	157.75	118.60	147.37	145.52	143.41	129.11	150.99	89.73	74.33	71.08	86.14	62.09	-	-	-	-	-	-
11	155.39	164.78	98.98	152.36	-	142.60	129.32	152.14	92.48	74.49	70.38	81.05	63.39	173.91	172.78	172.78	21.13	20.69	20.69
13[h]	157.34	163.68	109.71	157.88	-	143.96	129.28	150.46	-	-	-	-	-	-	-	-	-	-	-

a) internally referenced to dioxane; δ_{diox}=67.4 ppm

b) see scheme for numbering system

c) low intensity triplet

d) signal not detected

e) isobutyryl signals:C=O,CH,CH_3 at 180.09,36.84 and 19.28 ppm

f) benzoyl signals: C=O; C-phenyl at 167.79; 133.77,133.54,129.43 and 128.64 ppm

g) CH_3 signal at 31.59 ppm

h) N1-CH_3 signal at 40.36 ppm and C5-CH_3 at 13.17 ppm

after comparison of spectra of salt 1 and its deuterated congener 2. These signals were assigned to Cγ, Cα and Cβ carbons, respectively, basing on $^{1}J_{CH}$ values (Table 2). Spectra of deuterated analogue 2 facilitated also the assignment of C-8 and C-2 carbon resonances of 1 and 9. For guanosine and guanine derived salts 3-5, 7 and 10 the C-2 signal is the only one which appears as a singlet in a proton-coupled spectra. A technique of long range selective ^{1}H decoupling (LSPD) with low power ^{1}H irradiation[14] has been applied to assign the signals of C-4 and C-6. Resonances of C-5 appear at a much higher field and can be distinguished on the basis of their chemical shifts. For pyrimidine nucleoside 11 an analysis of the coupling constants was the major source of information during peak assignment. The signal at 164.78 ppm was attributed[15] to C-4 on the basis of vicinal coupling constant value $^{3}J_{CH}$=11.0 Hz. Because of a high lability of thymidine derived salt 12[3,4] the analysis of thymine analogue 13 is presented. For the latter case, the LSPD technique allowed to assign signals at 153.34 and 163.68 ppm to carbons C-2 and C-4, respectively.

Table 2. $^{1}J_{CH}$ Coupling constants [± 0.6 Hz].

C-H	Compound No										
	1	3	4	5	6	7	8	9	10	11	13
C2-H2	214.8	-	-	-	213.6	-	213.4	213.6	-	-	-
C8-H8	218.5	217.3	218.5	219.4	215.5	215.4	215.9	218.5	217.3	-	-
Cα-Hα	195.3	195.3	195.9	195.6	195.3	195.3	195.3	195.3	195.3	194.7	195.3
Cβ-Hβ	175.8	175.2	175.8	-	175.8	177.3	177.3	177.9	178.2	177.6	178.2
Cγ-Hγ	174.6	174.6	174.6	174.6	174.6	174.6	174.6	174.6	173.9	174.6	174.6
C1'-H1'	170.9	169.7	170.3	172.2	-	-	-	168.4	167.2	175.2	-
C2'-H2'	161.1	160.5	161.1	160.2	-	-	-	151.4	150.5	161.7	-
C3'-H3'	161.1	160.5	162.0	160.2	-	-	-	153.6	152.6	159.6	-
C4'-H4'	152.6	151.4	155.6	155.9	-	-	-	150.8	150.8	154.4	-
C5'-H5'	152.6	148.9	151.4	150.8	-	-	-	142.2	142.5	150.7	-
C5-H5	-	-	-	-	-	-	-	-	-	183.7	-
C6-H6	-	-	-	-	-	-	-	-	-	189.2	186.8

The conformation of N-glycosidic bond of purine nucleosides in solution in terms of preferred "syn" and "anti" conformations may be qualitatively expressed[16] by the magnitude of the difference of ^{13}C nmr chemical shifts Δ=6C2'-6C3'. For inosine- and guanosine-derived salts 9 and 10 this value equals 3.88 and 3.25 ppm, respectively, indicating the preferred "anti" conformation for those nucleosides.

EXPERIMENTAL

Nucleosides were from Waldhof. Exchange resins and molecular sieves from Serva and Sephadex G-10 from Pharmacia were used. Trimethylchlorosilane (Fluka) and 4-chlorophenylphosphorodichloridate (Aldrich) were freshly distilled before use. Pyridine was refluxed over calcium hydride, distilled and stored over molecular sieves 4A. Pre-coated silica gel plates (Merck $60F_{254}$) were used for tlc analysis in the following solvent systems: A) chloroform-methanol 9:1 v/v; B) ethanol-1M ammonium acetate aq. 7:3 v/v. Reverse-phase silica gel 60 (Merck, Art. No.7719) was used for column chromatography.

Spectra were recorded on the following spectrometers: uv - Carl Zeiss Jena M-40; *fluorescence - Perkin Elmer MPF3; ^1H and ^{31}P nmr - Jeol FX90Q at 90 and 34.6* MHz respectively with use of dioxane (internal) and 85% H_3PO_4 (external) standards. ^{13}C Nmr spectra were measured at 22.5 MHz using the same instrument with a digital resolution of 1.2 Hz per point. Typical acquisition parameters were as follows: spectral width 5000 Hz; 8 K; 40°flip angle; 1.2 s pulse repetition time. The proton coupled spectra were performed with zero filling giving a digital resolution of 0.6 Hz. The magnitudes of J were determined by line separation. Samples were measured in D_2O with dioxane as an internal reference. Chemical shifts are converted to the δ_{TMS} scale (δ_{diox}=67.4 ppm).

Synthesis of N-[9-(β-D-ribofuranosyl)purin-6-yl]pyridinium chloride 9 and its 2-amino congener 10.

Method 1. Dry inosine (537 mg, 2 mmol) or guanosine (567 mg, 2 mmol) was suspended in anhydrous pyridine (10 ml) and trimethylchlorosilane(1.27 ml, 10 mmol) was added through rubber septum under stirring at 5-10°C. After 10 min cooling bath was removed and reaction mixture was stirred for additional 3 h until complete O-trimethylsilylation was detected by tlc (R_f 0.40 and 0.32 in system A for inosine and guanosine derivatives respectively). 4-Chlorophenyl-phosphorodichloridate (0.49 ml, 3 mmol) was injected at 5°C within 2 min and

reaction mixture left under stirring at room temperature in dark for 18-22 h. During the reaction course samples were withdrawn in order to monitor the decay of O^6-phosphorylated intermediate (^{31}P nmr; -9.3 ppm) and subsequent formation of related pyridinium salt (spectrophotometry; pH 11.5 , 462 nm). The reaction mixture was concentrated to one forth of the initial volume, treated with water (0.36 ml, 20 mmol) at -10°C, left for 30 min at room temp., diluted with water (10 ml) and extracted with chloroform (5 ml). Aqueous layer was concentrated in vacuo to half of the volume and subjected to chromatography on reverse-phase silica gel short column (h=6 cm, Ø=4 cm) using acetone gradient in 0.005N HCl. Desired fluorescent fractions were collected, neutralized to pH 6.5 with freshly prepared Dowex1 HCO_3^- beads at 5°C, concentrated to 1 ml and passed through Dowex1 Cl^- column (h=10 cm, Ø=0.8 cm) to give pure aqueous solution of **9** or **10** in 25 and 30% yields respectively as checked by spectrophotometrical analysis.

9: stable for 2 months when kept frozen at -20°C; photolabile (see ref.9); tlc, system B, R_f=0.25; uv, (H_2O, pH 6.0), nm (ε): 244 (3700), 273 (8600), 299 (7300); fluorescence, Exc. 313 nm, Em. 438 nm; ^1H nmr, (D_2O) δ_{ppm}, 10.02 (d, 2H, Cα-H, J=7.1 Hz), 9.15 (s, C2-H), 8.98 (s, C8-H), 8.91 (t, Cγ-H, J=7.8 Hz), 8.40 (m, 2H, Cβ-H), 6.32 (d, C1'-H, J=5.1 Hz), 4.90 (t, C2'-H, J=5.1 Hz), 4.49 (t, C3'-H, J=4.9 Hz), 4.30 (m, C4'-H), 3.30 (m, 2H, C5'-H).

10: stable for a few weeks when kept frozen at -20°C; tlc, system B, R_f=0.37; uv, (H_2O, pH 6.0), nm (ε): 214 (14000), 231 (23600), 305 (980), 368 (4200); fluorescence, Exc. 366 nm, Em. 617 nm; ^1H nmr (D_2O) δ_{ppm}, 9.92 (d, 2H, Cα-H, J=7.1 Hz), 8.92 (t, Cγ-H, J=7.8 Hz), 8.50 (s, C8-H), 8.38 (m, 2H, Cβ-H), 6.08 (d, C1'-H, J=5.6 Hz), 4.85 (t, C2'-H, J=5.3 Hz), 4.48 (t, C3'-H, J=5.3 Hz), 4.26 (m, C4'-H), 3.89 (m, 2H, C5'-H).

Method 2. Lyophilizate of N-[9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)purin-6-yl]pyridinium chloride **1**[12] (98 mg, 0.2 mmol) or its 2-amino congener **3**[13] (101 mg, 0.2 mmol) was dissolved in D_2O (1 ml) acidified with DCl to pD 2.0 and kept at 5°C (refrigerator). Progress of the reaction was monitored by ^1H nmr. After 12 h solution was concentrated in vacuo at 5°C and then diluted with DCl/D_2O to maintain initial conditions. This step was repeated once or twice. Finally, the mixture was applied to the Sephadex G-10 column (h=10 cm, Ø=0.8 cm) prepared in D_2O. Fluorescent fractions eluted with D_2O were collected and concentrated (to 0.5 ml) to give pure solutions of **9** or **10** in 95% yield.

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