Tetrahedron Vol. 49, No. 26, pp. 5859-5868, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Pyridine Assisted Phosphorylations of Nucleobase Bis-Lactam Systems. Formation and Reactivity of Dipyridinium Species.

Bohdan Skalski and Grażyna Wenska

Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

Zofia Gdaniec and Ryszard W. Adamiak *

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Noskowskiego 12, Poland

(*Received in UK* 19 *April* 1993)

Abstract: *Heteroaromatic bis-lactam systems of uracil, thymine, xanthine and O-acetylated xanthosine undergo pyridine assisted phosphorylations with formation of respective dipyridinium species* ***5-8****. Xanthosine appeared to be most reactive. Structure of those new representatives of N-aryl-pyridinium species was established on the basis of* ^{1}H *and* ^{13}C *NMR spectra. Under alkaline conditions dipyridinium species undergo transformation into stable fluorescent monosubstituted pyridinium derivatives* ***10-13****.*

INTRODUCTION

Tri-*O*-acetylinosine treated with various phosphorylating agents in the presence of pyridine forms the water-soluble, fluorescent *N*-[9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]pyridinium salt[1]. *O*(6)-Phosphorylated species are formed as reactive intermediates. Heteroaromatic lactam systems of *O*-protected nucleosides, namely guanosine, thymidine, uridine[2] and nucleobases such as hypoxanthine[1], guanine[3] and 1-methylthymine[4] undergo analogous transformations with formation of respective pyridinium salts. Some of those structures have been considered as ionic side-products in the chemical synthesis of oligonucleotides both *via* phosphortriester approach in solution[1,5] and solid support-aided phosphoramidite method[6].

On the other hand these new representatives of *N*-arylpyridinium salts, due to their interesting chemical[7-9] and photochemical[10,11] properties, have been already proposed as a novel synthetic intermediates in a nucleoside field. Most of nucleoside- and nucleobase-derived pyridinium mono salts have been subjected to extensive structural studies[4,5,12,13]

Above cited results prompted us to study reactions of uracil (**1**), thymine (**2**), xanthine (**3**) and *O*-protected xanthosine (**4**), all bearing two potentially reactive heteroaromatic lactam systems, with 4-chlorophenyl phosphorodichloridate in the presence of pyridine.

Here we report on results reflecting both preparative and spectral aspects of dipyridinium species (**5-8**) formation, their analysis by means of the ^{1}H and ^{13}C NMR, and reactivity under conditions of elevated pH. We present here a novel route to various pyrimidinyl- and purinyl- pyridinium salts and betaines, which we found superior over earlier described reactions of pyridines with corresponding chloroderivatives[14,15].

RESULTS AND DISCUSSION

Formation and structural features of dipyridinium species **5-8**.

As observed for mono heteroaromatic lactam systems[1,2] formation of pyridinium derivatives is a two step process involving *O*-phosphorylation and subsequent pyridine displacement reactions. ^{31}P NMR spectroscopy proved to be a very useful tool to observe those reactions[1,2]. To monitor the first step separately, the reactions of uracil (**1**), thymine (**2**), xanthine (**3**) and *O*-acetylated-xanthosine (**4**) with 4-chlorophenyl phosphorodichloridate (3 eqv.) were carried out in dioxane suspensions and in the presence of triethylamine (4 eqv.). After 2 h reaction mixtures were filtered into nmr tubes. Spectra showed the presence of a signal from unreacted 4-$ClC_6H_4OPOCl_2$ at +1.71 ppm and four signals assigned to diastereoisomers of bis-*O*-hosphorylated species. Their chemical shifts are collected in Table 1.

Table 1. ^{31}P Chemical Shifts (external H_3PO_4) of *O*-Phosphorylated Derivatives in Dioxane/Triethylamine

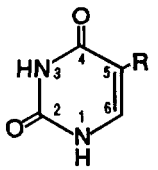
Uracil	-8.97	-9.23	-9.26	-9.34
Thymine	-9.11	-9.22	-9.34	-9.46
Xanthine	-7.61	-7.71	-8.60	-8.72
O-Ac-xanthosine	-8.88	-	-	-

The addition of pyridine at this stage to the reaction led to the disappearance of these signals and to the formation of new ones at -11.30 ppm and -6.44 ppm assigned to 4-$ClC_6H_4OPOCl/O^-/C_5H_5N^+$ and 4-$ClC_6H_4OPOCl/O^-/$ respectively[1]. Signals of bis-*O*-phosphorylated species could not be detected when reactions were performed in pyridine right from the beginning. This is in contrast to our previous observations concerning phosphorylation of mono-lactam systems[1,2] and reflects higher reactivity of bis-*O*-phosphorylated species toward pyridine.

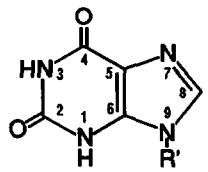
In parallel, aliquots of the reaction mixtures in pyridine, all of standard stoichiometry, were analysed by UV in order to find an overall rate of pyridinium product formation. Opening of pyridinium ring in a 0.1M NaOH giving rise to characteristic band of λ_{max} ca. 460 nm (Zincke reaction[16,17]) was applied as the basis of analytical tests. The quantitative conversion of tri-*O*-acetyl-xanthosine (conc. 0.01 M, above stoichiometry) into **4** was observed within 30 min and further prolongation of the reaction time caused the gradual degradation of the product. Low solubility of both the substrates (**1-3**) and products (**5-7**) do not allow to accomplish the UV test for all compounds in question in a quantitative manner. However the following rate of dipyridinium product formation was clearly observed: *tri-O-acetylxanthosine >> uracil ~ xanthine > thymine*. Since results of ^{31}P NMR studies indicated high reactivity of bis-*O*-phosphorylated species it might be concluded that above given order reflects reactivity of corresponding lactam systems toward phosphorylation agent .

When performed on a preparative scale reactions in pyridine led to dipyridinium products in a quantitative manner as checked by TLC and HPLC (see experimental). Their work-up based on modification of the procedure described previously for monopyridinium salts[2,5] gave **5-8**. Dipyridinium species **5** and **6** were obtained as stable colorless crystals in 90% yield upon addition of isopropanol to the respective concentrated

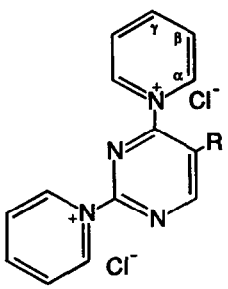
aqueous solutions. Xanthine derived dipyridinium product 7 did not crystallize under these conditions. It was obtained as a yellowish solid in 68-75% yield upon lyophylization or evaporation (< 30°C) of aqueous solution. Dication **8**, derived from 2',3',5'-tri-O-acetyl-xanthosine, appeared to be highly labile and its preparation in a pure, solid state was virtually impossible. Finally the procedure based on multiple treatment of highly colored reaction mixture with charcoal, pH kept rigorously below 6.1, gave **8** as concentrated (0.1M), chromatographically homogenous, aqueous solution. The UV spectra measured in sequence during work-up showed gradual changes - notably new absorption band at 350 nm appeared. Concomitantly the formation of an ionic degradation product with an intense yellow fluorescence was detected by TLC and HPLC.



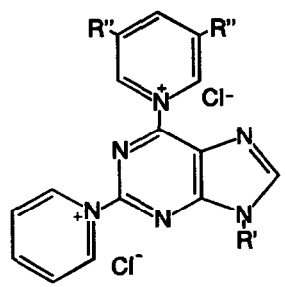
1. R = H
2. R = CH_3



3. R' = H
4. R' = 2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl



5. R = H
6. R = CH_3



7. R' = H
8. R' = 2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl
9. R' = H; R'' = CH_3

For **7**, the pH dependent UV spectra indicate the occurrence of a prototropic equilibrium similar to those reported previously for some *N*-(purin-6-yl)mono-pyridinium salts[13,18]. The estimated pK_a value of 7, pK_a 4.9, is approximately two orders of magnitude lower than that for the *N*-purin-6-yl pyridinium chloride[18] reflecting the electron withdrawing effect of the second pyridinium ring . The pH dependence of the chemical shift values was also observed in the ^1H NMR spectrum of **7** (see below).

Among all the dications **5-8** only purine derivatives **7** and **8** exhibit intense fluorescence in water at room temperature with the emission maximum at 500 and 416 nm, respectively. Red shift, ca. 80 nm of λ_{max} on going from **8** to **7** is due to the existence of the latter compound in a deprotonated form in water.

Structure of dipyridinium products **5-8** was proved by ^1H and ^{13}C NMR spectroscopy. The data are collected in Tables 2.1., 2.2 and 3, respectively.

The assignment of the proton signals of both pyridinium substituents in **5** and **6** was based on H,H COSY spectra. To discriminate between positions C-2 and C-4 of the pyrimidine ring 1D NOE experiment was performed. After irradiating the thymine methyl group of **6** the NOE was observed for signals at 9.38 ppm (H-α4, 12%) and 9.50 ppm (H-6, 32%). In case of xanthine derived dipyridinium product 7 signals of H-α2 and H-α6 strongly overlap. To assign signals of each of the two pyridinium cations we have taken the advantage of their different reactivity. The C-6 pyridinium was found to be the faster leaving group in the nucleophilic displacement reaction (see below). Treatment of aqueous solution of **7** with 3,5-lutidine at pH 8 led to the formation of analog **9** with the simultaneous appearance of pyridine. Comparison of the ^1H NMR spectra of **7** and **9** enabled the identification of the C-2 and C-6 pyridinium proton signals.

Table 2.1. Proton Chemical Shifts of Compounds **5-9** in D_2O[a].

Proton	5	6	7	8[b]	9
H-α2	10.24	10.16	10.18	10.19	10.18
H-α4	9.85	9.38	-	-	-
H-α6	-	-	10.20	10.25	9.73
H-β2	8.47	8.41	8.36	8.42	8.36
H-β4	8.51	8.49	-	-	-
H-β6	-	-	8.46	8.48	-
H-γ2	9.03	8.97	8.89	8.96	8.89
H-γ4	9.06	9.02	-	-	-
H-γ6	-	-	8.97	8.99	8.61
H-5	8.70	-	-	-	-
H-6	9.66	9.50	-	-	-
H-8	-	-	8.96	9.17	8.96
CH_3	-	-	2.63	-	2.68

[a] internally referenced to dioxane: diox = 3.71 ppm.
[b] ribose : 6.71 (H-1'), 6.21 (H-2'), 5.78 (H-3'), 4.46 (H-4', H-5'); acetyls: 2.19, 2.16, 1.97.

The chemical shifts of both purine derivatives **7** and **9** depend strongly on pH. Very good conformity of chemical shifts was obtained when pH of solutions was adjusted to 5.5 and 6.5, respectively.

Solution of **5** in D_2O was subjected to exchange process with pyridine-d_5 in order to achieve unambiguous assignments for both pyridinium substituents in the ^{13}C NMR spectrum. Changes in the pyridinium part of the molecule were monitored by means of ^1H NMR spectra. The downfield signal in ^1H NMR spectrum assigned previously to H-α4 protons has decreased in time and simultaneously new signals from released pyridine have appeared. After one hour 70% of pyridinium cation at C-4 and 40% at C-2 have exchanged with pyridine-d_5. To stop exchange process at this stage the mixture was acidified with DCl to pD 3. Finally, the mixture was passed through Sephadex G-10, the desired fractions collected and concentrated to give solution of partly deuterated **5**. Analogous process of exchange took place also for thymine-derived di-

pyridinium product **6** but it was somewhat slower. After 3 days 70% of pyridinium cation at C-4 and 10% at C-2 have exchanged with pyridine-d_5. This makes distinguishing between both cations straightforward.

Table 2.2 Coupling constants $J_{H,H}$ (± 0.1 Hz)

J	5	6	7	8	9
α2 β2	7.2	7.2	7.2	7.2	7.2
α2 γ2	1.4	1.4	1.4	1.4	1.4
β2 γ2	7.8	7.8	7.8	7.7	7.8
α4 β4	7.2	7.2	-	-	-
α4 γ4	1.4	1.4	-	-	-
β4 γ4	7.8	7.9	-	-	-
α6 β6	-	-	7.1	7.1	-
α6 γ6	-	-	1.4	1.4	1.7
β6 γ6	-	-	7.8	7.8	-
H-5 H-6	5.5	-	-	-	-
CH_3 H-6	-	0.7	-	-	-
α6 CH_3	-	-	-	0.6	
γ6 CH_3	-	-	-	-	1.4

Table 3. Carbon Chemical Shifts of Compounds **5-9** in D_2O[a].

Carbon	5	6	7	8 [b]	9
C-2	156.04	154.24	149.58	149.86	149.74
C-4	160.91	159.56	159.78	157.18	159.14
C-5	117.03	129.97	126.58	127.97	126.39
C-6	166.52	167.90	146.78	147.74	147.28
C-8	-	-	153.38	152.36	153.15
C-α2	142.06	141.90	142.31	142.44	142.30
C-α4	143.19	144.93	-	-	-
C-α6	-	-	144.41	144.39	140.95
C-β2	129.22	129.22	129.13	129.38	129.13
C-β4	129.65	129.54	-	-	-
C-β6	-	-	129.44	129.54	140.63
C-γ2	152.40	151.92	151.33	151.92	151.38
C-γ4	152.24	151.05	-	-	-
C-γ6	-	-	151.87	152.36	152.51
CH_3	-	14.79	-	-	18.64

[a] internally referenced to dioxane (= 67.4 ppm).
[b] ribose : 88.64 (C-1'), 80.84 (C-4'), 74.06 (C-2') 70.87 (C-3'), 63.66 (C-5');
acetyls: 173.48, 174.18, 173.21, 20.71, 20.54, 20.34, .

The assignment of C-2 and C-4 signals of **5** and **6** was based on analysis of long range coupling constants. In addition C2-(^{13}C)-labelled (10%) analog of **5** (prepared from C2-(^{13}C)-uracil) was analyzed. The two of the four quaternary carbons of **7** and **8** i.e. C-5 and C-4 can be distinguished on the basis of their chemical shifts. Those resonating at 149.58 ppm and 146.78 ppm and 149.86 and 147.74 were assigned to C-2 and C-6 of **7** and **8** respectively on the basis of the chemical reactivity of these salts[19]. ^{13}C NMR chemical shifts of **5-9** are collected in Table 3.

Structures of pyrimidine derived dipyridinium chlorides **5** and **6** have been fully confirmed by X-ray analysis[20,21] which reveals an interesting difference between both salts in a crystal state. For thymine-derived **6**, considerable rotation of 4-pyridinium ring out of pyrimidine plane (53.5°) was observed.

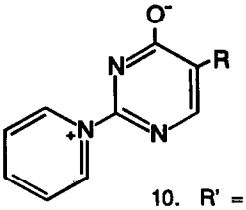
Reactivity of dipyridinium species under hydrolytic conditions.

Experiments concerning the isolation of dipyridinium species **5**, **6** and **8** clearly indicated their different stability. Under hydrolytic conditions (6.5 <pH< 8.5, room temperature) the compounds were found (HPLC) to undergo almost quantitative conversion into monopyridinium derivatives with simultaneous release of equimolar amount of pyridine. On the other hand, when more alkaline conditions were applied (0.1M aqueous NaOH) the opening of pyridinium ring due to attack of hydroxyl ion on α-carbons prevail (Zincke reaction)[1,2,7].

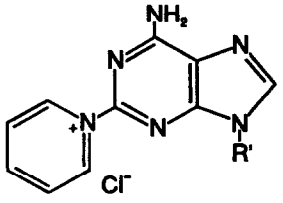
Treatment of the dipyridinium species **5**, **6**, **8** on the preparative scale with 0.1M aqueous sodium bicarbonate (pH 8.5, 22°C.) led to efficient formation of a new monopyridinium species **10**, **11** (85-90% yield) and **13** (65% yield) respectively. Lower yield of nucleoside **13** was due to a partial deacetylation. The relative reactivity within this group of dipyridinium compounds was found to be **8** > **5** > **6**. The most reactive compound **8** was transformed to **13** within 1 h at room temperature. The reaction of **5** required 24 h to go to completion, while **6** reacted in 75% in this period of time. Dipyridinium compounds **5**, **6** and **8** are more capable of displacement reaction then pyrimidin-4-yl and purin-6-yl monopyridinium species studied earlier[1,2,4,7,8]. For example *N*-[1-(methyl)pyrimidin-2(1H)-one-4-yl]-pyridinium chloride[4] kept in 0.1M sodium bicarbonate at room temperature for 24 h gave only 1-methyluracil with 20% yield.

The reactivity of xanthine derived dipyridinium compound **7** differs substantially from that of **5**, **6** and **8**. No reaction in 0.1M sodium bicarbonate took place, most probably due to stabilizing effect of negative charge formed in purine skeleton upon N(9)-H deprotonation (pK_a 4.9 for **7**). Reaction of **7** under elevated pH (0.1M NaOH) led mainly to C-6-pyridinium ring opening and eventually to the formation of monopyridinum product **12** (50% yield as determined by HPLC). The opening of C-2 pyridinium ring in **7** and subsequent formation of guanine derived monopyridinium product[13] was a minor process (<10% as determined by HPLC).

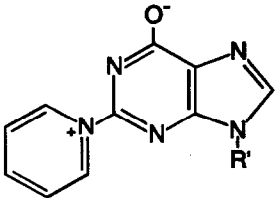
Compounds **10**, **11** and **13** are inner salts. Salt **12** was isolated as its chloride. The structures of all the compounds were proven by ^{1}H and ^{13}C NMR spectra. The site of attachment of pyridinium moiety was confirmed by the efficient (>90% yield HPLC determined) transformation of salts **10** - **13** into isocytosine, 5-methylisocytosine, 2,6-diaminopurine, and a mixture of guanine and guanosine (3:1) respectively as checked by diode-array HPLC comparison with authentic samples. These reactions require strongly basic media (3.0-5.0M NaOH, 22°C). No pyridine was found in neutralized reaction mixtures. Instead, glutaconate aldehyde was indentified as to be expected according to Zincke reaction mechanism[16,17].



10. R' = H
11. R' = CH_3



12. R' = H



13. R' = 2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl

The properties of salts **5** - **8** in alkaline media revealed the different reactivity of both pyridinium substituents attached to pyrimidine and purine moieties. The C-4 pyrimidinyl- and C-6 purinyl- pyridinium residues were found to be much more reactive both in respective displacement and addition of hydroxyl ion. The monopyridinium compounds **10** - **13** fluoresce at room temperature with emission maximum in the region 550-600 nm. They are stable from pH 1 to 12. These properties together with the advantage of their mild and efficient preparation can make them useful fluorescent derivatives of nucleobases.

EXPERIMENTAL

^{31}P NMR spectra were recorded on a JEOL FX-90Q spectrometer at 36.2 MHz. ^{31}P chemical shifts are referenced to external 85% H_3PO_4. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker MSL-300 spectrometer. All samples were measured in D_2O with dioxane as an internal reference. Chemical shifts are converted to the TMS scale (diox ^{1}H = 3.71 ppm; diox ^{13}C = 67.4 ppm). UV absorption was measured on a Perkin-Elmer Lambda-17 spectrophotometer. Fluorescence spectra were collected on a Perkin-Elmer MPF 66 spectrofluorimeter. Perkin-Elmer 2400 CHN analyzer was used for elemental analysis. pK_a values were determined by spectrophotometric titrations[22]. Thin-layer chromatography was performed on Merck F_{254} silica gel plates using solvent systems: A: EtOH - 1.0M aq. ammonium acetate (7:3 v/v), B: isopropanol-water-conc.ammonia (7:2:1 v/v/v). Column chromatography was performed on Merck RP silica gel using systems C: water, D: acetonitrile gradient in water, E: acetonitrile gradient in 0.01M HCl. HPLC was performed on a Waters 600E instrument using diode-array detector on NovaPak C-18 and DeltaPak C-4 columns applying isocratic and gradient modes (acetonitrile in 0.1M aqueous ammonium acetate).

Synthesis of dipyridinium species **5** - **7**. *General procedure.* A solution of the appropriate heterocyclic base (5 mmol) in dry pyridine (100 mmol) was treated for 18 h in dark with 4-chlorophenyl phosphorodichloridate

(17.5 mmol). Ice-cold water (100 ml) was added and the reaction mixture was concentrated under reduced pressure to 25% of the initial volume. Dilution with water and evaporation was repeated until all pyridine was removed. Aqueous solution was then neutralized (pH 6.1 - 6.5) with Dowex-1 resin HCO_3^- form. The filtrate was concentrated to a small volume (5 ml) and passed through Dowex-1 resin (Cl^- form). Crystalline dipyridinium species **5** and **6** precipitated from the concentrated aqueous solutions upon addition of isopropanol in 90% yield. To obtain dipyridinium product derived from xanthine the eluate from Dowex-1 (Cl^- form) was decolourized with charcoal (Norrit) and adjusted to pH 3 with aqueous HCl. Solid sample of the salt was obtained upon lyophilyzation with 75% yield.

5. UV (H_2O), λ_{max}, nm (ε), : 232 (19 500), 267 (17 400); (0.1M NaOH): 458 (62800); ^1H and ^{13}C NMR data in Tables 2&3; Anal.calcd. $C_{14}H_{12}N_4Cl_2 \cdot H_2O$: %C, 51.70; %H, 4.34; %N, 17.23. Found: %C, 51.40; %H, 4.29; %N, 17.05.

6. UV (H_2O), λ_{max}, nm (ε), : 264 (18 900), (0.1M NaOH): 461 (64 000); ^1H and ^{13}C NMR data in Tables 2&3; Anal. calcd. $C_{15}H_{14}N_4Cl_2 \cdot 2H_2O$: %C, 50.42; %H, 5.04; %N, 15.68. Found: %C, 50.04; %H, 5.08; %N, 15.66.

7. UV (H_2O), λ_{max}, nm (ε), : 272 (15 000), 290 (sh 10 700), (0.1M NaOH): 468 (55 100); pK_a 4.9; fluorescence (H_2O): 500nm. ^1H and ^{13}C NMR data in Tables 2&3; Anal.calcd. $C_{15}H_{12}N_6Cl_2 \cdot 2H_2O$: %C, 46.99; %H, 4.21; %N, 21.92. Found: %C, 47.38; %H, 3.97; %N, 22.06.

Synthesis of dipyridinium compound **8** *derived from 2',3',5'-tri-O-acetylxanthosine* **4.** 2',3',5'-Tri-O-acetylxanthosine (0.82 g, 2 mmol) was made anhydrous by evaporation from pyridine (20 mmol). Residue was redissolved in anhydrous pyridine (20 ml) and 4-chlorophenyl phosphorodichloridate (1.2 ml, 7.4 mmol) added. The mixture was kept overnight at 22°C in dark. Ice-cold water (150 ml) was added and the solution was concentrated (30° C) to ca. 50 ml under reduced pressure. The pH of the solution was adjusted to 5.8 - 6.1 by the addition of wet Dowex-1 resin (HCO_3^- form) under continuos flow of nitrogen. Resin was filtered off, the filtrate concentrated (10 ml) and placed on a Dowex-1 (Cl^- form) column. Charcoal (Norrit) was added to aqueous eluate and the mixture was stirred for 15 min. and then filtered. Treating with charcoal was repeated until no absorption peak at 330 nm could be seen in the UV spectrum of the sample. After final concentration under vacuum at 30° C, a stock solution of pure **4** (ca 0.1 M, 50% yield estimated from UV spectrum assuming ε value at 264 nm to be identical with that for **7** at pH 2: =18.600) was obtained. It was stored at ca. 5° C.

8. UV (H_2O), λ_{max} nm, : 232, 254, 290; (0.1M NaOH): 465; fluorescence (H_2O), λ_{max} :416 nm.; ^1H and ^{13}C NMR data in Tables 2&3.

Hydrolysis of dipyridinium species **5**, **6**, **8** *with 0.1M aqueous* $NaHCO_3$ *to form* **10**, **11**, **13**. Dipyridinium species **5**, **6**, **8** (1 mmol) were treated with 0.1M $NaHCO_3$ aq.(40 ml) for 24 h at 22°C. Reaction mixtures were neutralized with 0.1M HCl and concentrated under reduced pressure to small volume. Acetonitrile was added to precipitate inorganic salt, filtrate concentrated and purified for **10** and **11** on RP-silica gel eluted with system C and in the case of **13** on RP-silica gel eluted with system D. Concentrated aqueous solutions were desalted by passage through Sephadex G10 (3ml), appropriate fractions concentrated to the oil and treated with acetone to obtain solidified: **10** (155 mg, 74%), **11** (178 mg, 80%) and **13** (280 mg, 60%).

10. UV (H_2O), λ_{max}, nm (ε): 222 (18.000), 255 (8.300), 310 (3400), (0.1M HCl, protonated form): 268 (10.100): pK_a = 3.02; fluorescence (H_2O) λ_{max}: 570 nm; ^1H NMR (D_2O), δ ppm: 9.66 (dd, J=1.3 Hz, J=6.9 Hz, 2, H-α), 8.72 (tt, J=1.4, J=7.81 Hz, 1, H-γ), 8,17 (m, 2, H-β), 8.08 (d, J=6.1 Hz, 1, H-6), 6.41 (d, J=6.1 Hz, 1, H-5); ^{13}C NMR (D_2O), δ ppm: 176.57 (C-4), 156.78 (C-2), 156.28 (C-6), 149.77 (C-γ), 141.56 (C-α), 128.43 (C-β), 113.58 (C-5); Calcd. for $C_9H_7N_3O \cdot 2H_2O$: %C 51.67, %H 5.29, %N 20.08. Found: %C 51.48, %H 5.27, %N 20.20.

11. UV (H_2O), λ_{max}, nm (ε): 227 (14.900), 258 (7.700), 323 (3.300), (0.1M HCl, protonated form), 272 (9.500), pK_a = 3.28, fluorescence (H_2O): λ_{max} :595 nm; ^1H NMR (D_2O): δ ppm: 9.54 (d, J=6.5 Hz, H-α), 8.68 (tt, J=1.47, J=7.81 Hz, 1, H-γ, 8.12 (t, J=7.2 Hz, 2, H-β), 7.77 (s, 1, 6-H), 1.89 (s, 3, CH_3), ^{13}C NMR (D_2O) δ ppm: 175.70 (C-4), 154.85 (C-2), 153.03 (C-6), 149.3 (C-γ), 141.01 (C-α), 128.39 (C-β), 123.46 (C-5), 14.00 (CH_3); Calcd. for $C_{10}H_9N_3 \cdot 2H_2O$: %C 53.80, %H 5.87, %N 18.82. Found: %C 53.89, %H 5.84, %N 18.62.

13. UV (H_2O), λ_{max}, nm (ε): 237 (14.700), 337 (3.400), (0.1M HCl, protonated form); 225 nm (11.600), 263 (7.600), 306 (4.400); pK_a=2.19; fluorescence (H_2O): λ_{max}: 584 nm; ^1H NMR (D_2O), δ ppm: 9.77 (d, J=6.45 Hz, 2, α-H), 8.67 (t, J=7.7 Hz, 1, γ-H), 8.10 (t, J=7.3 Hz, 2, β-H), 8.07 (s, 1, 8-H), 6.18 (d, J=3.1 Hz, 1, 1'-H), 5.99 (m, 1, 2'-H), 5.80 (m, 1, 3'-H), 4.48 (m, 1, 4'-H), 4.29 (m, 2, 5',5"-H), 2.17, 2.14 and 1.75 (3s, 9, -$COCH_3$); ^{13}C NMR (D_2O) δ ppm: 173.99, 173.44, 173.13 ($COCH_3$), 167.90 (C-6), 151.81 (C-γ), 149.98 (C-2), 149.78 (C-8), 141.38 (C-4), 141.27 (C-α), 128.37 (C-β), 125.12 (C-5), 87.92 (C-1'), 79.79 (C-4'), 74.10 (C-2'), 70.73 (C-3'), 63.25 (C-5'), 20,76, 20.71 and 20.59 ($COCH_3$); Calcd. for $C_{21}H_{21}N_5O_8 \cdot 2H_2O$: %C 49.70, %H 4.96, %N 13.80. Found: %C 49.45, %H 4.81, %N 13.54.

Hydrolysis of dipyridinium compound 7 *in 0.1M NaOH to form* **12**. Xanthine derived dipyridinium compound 7 (270 mg, 0.7 mmol) was treated with 0.1M NaOH (100 ml) at 22°C. After 7 h substrate reacted completely as detected by HPLC with formation of one major product and seven others. Reaction mixture was neutralized to pH 6.2 with 1.0M hydrochloric acid, concentrated under reduced pressure and applied on RP-silica gel column. Elution with C system to remove inorganic salt and subsequently with E system gave fractions containing major product. Their evaporation under reduced pressure led to **12** as yellow solid (50 mg, 20% yield).

12. UV (H_2O), λ_{max}, nm (ε): 240 (12.100), 266 (8.100), 330 (3.100); (0.1M HCl): 239 (24.400), 267 (19.000), 314 (5.300); (0.1M NaOH): 242 (21.900), 356 (6.500): pK_a=8.81; fluorescence (H_2O): λmax: 557 nm; ^1H NMR (D_2O), δ ppm: 9.83 (dd, J=1.2 Hz, J=6.9 Hz, 2, α-H), 8.73 (tt, J=1.2 Hz, J=7.7 Hz, 1, γ-H), 8.18 (m, 3, β-H, 8-H); ^{13}C NMR (D_2O) δ ppm 155.69 (C-4), 150.73 (C-2), 149.89 (C-8), 144.33 (C-6), 141.42 (C-γ), 128.57 (C-α), 119.47 (C-β), 117.16 (C-5) ; Calcd. for $C_{10}H_9N_5Cl \cdot H_2O$: %C 47.53, %H 4.38, %N 27.71. Found: %C 47.38, %H 4.31, %N 27.53.

ACKNOWLEDGMENTS

We thank Professor S. Paszyc for his interest in this work. This work was supported by the Polish Ministry of Education and Polish Academy of Sciences.

REFERENCES AND NOTES

1. Adamiak, R. W.; Biała, E.; Skalski, B. *Nucleic Acids Res.*, **1985**, *13*, 2989-3003.
2. Adamiak, R. W.; Biała, E.; Gdaniec, Z.; Mielewczyk, S; Skalski, B. *Chem.Scr.*, **1986**, *26*, 3-7 .
3. Jaskólski, M.; Skalski, B.; Adamiak, D. A.; Adamiak, R. W. *Acta Cryst.*, **1987**, *C43* , 2110 .
4. Wenska, G.; Skalski, B.; Gdaniec, Z. *Can.J.Chem.*, **1992**, *70*, 856-862 .
5. Adamiak, R. W.; Biała, E.;Gdaniec, Z.; Mielewczyk, S.; Skalski, B. *Chem. Scr.*, **1986** *26*, 7-11 .
6. Scott, E. J.; Scott, D. J. *Nucleic Acid Res.*, **1987**, *15*, 8221-8229.
7. Adamiak, R.W.; Biała E ; Skalski, B.; *Angew.Chem.Int.Ed.Engl.*,**1985**, 24, 1054-1057 .
8. Seela, F.; Herdering, W.; Kehne, A. *Helv.Chim.Acta*, **1987**, *70*, 1649-1657.
9. Mielewczyk, S.; Gdaniec, Z.; Bobrowska, G.; Adamiak, R. W. *Nucleosides & Nucleotides*, **1987**, 6 *(1-2)*, 273-277.
10. Skalski, B.; Bartoszewicz, J.; Paszyc, S.; Gdaniec, Z.; Adamiak, R. W. *Tetrahedron* , **1987**,*43*, 3955-3961.
11. Skalski, B.; Paszyc, S.; Adamiak, R. W.; Steer, R. P.; Verrall, R. F. *J.Chem.Soc.Perkin.Trans. II*, **1989**, 1691-1696.
12. Gdaniec, Z.; Mielewczyk, S.; Adamiak, R. W.; Skalski, B. *Heterocyles*, **1988**, *27*, 2807-2814.
13. Skalski, B.; Paszyc, S.; Adamiak, R. W.; Steer R. P.; Verrall, R. E. *Can.J.Chem.*,**1990**, *68*, 2164-2170.
14. Bredereck, H.; Christmann, O.; Graudumus, J.; Koser, W. *Angew. Chemie*, **1960**, *72*, 708.
15. Werner, D.; Hoffman, H. *Arch. Pharmaz. (Weinheim)*, **1974**, 307, 301-308.
16. Zincke, Th. *Liebigs Ann. Chem.*, **1903**, *330*, 361-374.
17. Krohnke, F. *Angew. Chemie*, **1963**, *75*, 317-329.
18. Skalski, B.; Steer, R.P.; Verrall, R.E. *J.Amer.Chem.Soc.*, **1988**, *110*, 2055-2061.
19. INADEQATE -type spectra giving ^{13}C-^{13}C coupling constants due to low solubility of the sample was difficult to apply.
20. Jaskólski, M.; Skalski, B.; Adamiak, D. A. *Acta Cryst.*, **1988**, *C44*, 1409-1412.
21. Surma, K.; Adamiak, D. A.; Gawron, M.; Skalski, B. *ibid*, **1992**, *C48*, 298-300.
22. Albert, A.; Sergeant, E.P., The determination of ionization constants, 3rd ed. **1984**, p.70. Chapman and Hall, New York.