Studies on the Alkylation of 5'-CMP under Alkaline Conditions

Khurshid Alam Khan* and M. Qudrat-e-Khuda

Pakistan Council of Scientific and Industrial Research Laboratories Complex,
Karachi-75280, Pakistan

Viqar Uddin Ahmad

H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

Z. Naturforsch. **47b**, 1307–1313 (1992); received February 27, 1992

Nucleotides, Ribose Alkylated Products, ^1H NMR Spectra

The reaction of 5'-CMP with methyl, ethyl, *n*-propyl and isopropyl iodides gave predominantly ribose alkylated products. Various alkylated derivatives have been characterized using UV spectroscopy, paper electrophoresis, ^1H NMR and FAB (negative) mass spectroscopic techniques. Under strongly alkaline conditions the 2'-O-alkylated derivatives are synthesized in maximal yields. Isopropylation reaction gave only a small yield of the 3'-O-isopropyl derivative, this could be explained on the basis of steric hindrance offered to the bulky isopropyl group at the 2'-OH position by the heterocyclic base of the nucleotide.

Introduction

Nucleotides methylated at the 2'-hydroxyl of ribose are of widespread occurrence as minor constituents of transfer and ribosomal RNAs of a variety of living organisms [1]. Various methylating agents are cytotoxic due to the alkylation of DNA bases produced by them [2]. The 2'-O-ethylated nucleotides were detected in the *t*-RNA of L-ethionine induced carcinoma [3, 4].

Many 2'-O-methylated and ethylated homopolynucleotides have been synthesized [5–7] and their properties reported; recently 2'-O-methylated oligonucleotides of defined sequences have also been prepared for use as probes in RNA hybridization studies [8]. In the above mentioned studies, generally the unprotected nucleoside has been alkylated, the various alkylated isomers were separated and the 2'-O-alkylated nucleoside was subsequently converted to the nucleotide by conventional phosphorylation methods. The studies on the direct alkylation of unprotected nucleotides leading to ribose alkylated derivatives are few; the ready availability of alkyl iodides and the reports [10] that under strongly alkaline conditions the alkylation of heterocyclic ring is minimized while the vicinal hydroxyls of ribose are slightly ionized prompted us to study the alkylation of 5'-CMP

with methyl, ethyl, normal and iso-propyl iodides under strongly alkaline conditions. The results obtained from these studies are being presented in this publication. It is hoped that the availability of nucleotides containing a wide range of alkyl groups at the ribose will lead to further studies on the effect of these groups on polynucleotide conformations, for preparation of oligonucleotides of defined sequences and for evaluation as inhibitors of enzymes responsible for nucleic acid metabolism.

Results and Discussion

A literature survey indicated that reaction conditions play a decisive role on the site of alkylation in nucleotides. Thus, Haines [11] *et al.* carried out a comparative study of the methylation of unprotected nucleotides using diazomethane and dimethyl sulphate in aqueous medium and at pH 5–7 reported, in case of 5'-CMP, formation of phosphate ester together with products containing 1-methyl-5'-CMP and its corresponding ester.

The reaction of 5'-CMP **1** was carried out for three hours using methyl iodide in a mixture of dioxane and 1 N sodium hydroxide at room temperature. Paper chromatography of the crude reaction mixture revealed the formation of new products of higher mobilities as compared to the starting material (Table I). The products were separated by preparative paper chromatography on 3 MM Whatman chromatography sheets by using solvent A. Starting from known nucleotides it

* Reprint requests to Dr. K. A. Khan.

Compound	R_f in solvent systems			Paper electrophoresis (migration in cm)	
	A	B	C	Buffer I	Buffer II
5'-CMP 1	0.08	0.26	0.49	9.0	4.0
N ³ -Methyl-5'-CMP 2	0.21	0.59	0.65	4.8	4.0
2',3'-Di-O-methyl-5'-CMP 3	0.26	0.69	0.72	9.5	4.0
2'-O-Methyl-5'-CMP 4	0.15	0.49	0.62	9.0	4.1
3'-O-Methyl-5'-CMP 5	0.12	0.45	0.58	9.0	4.0
2',3'-Di-O-ethyl-5'-CMP 6	0.71	0.85	0.88	9.1	4.0
2'-O-Ethyl-5'-CMP 7	0.13	0.51	0.47	9.0	4.0
3'-O-Ethyl-5'-CMP 8	0.22	0.61	0.71	9.1	4.2
N ³ ,2'-O-Di- <i>n</i> -propyl-5'-CMP 9	0.48	0.78	0.70	5.0	4.2
2',3'-Di-O- <i>n</i> -propyl-5'-CMP 10	0.45	0.69	0.45	9.2	4.1
2'-O- <i>n</i> -Propyl-5'-CMP 11	0.12	0.34	0.31	9.0	4.0
3'-O-Isopropyl-5'-CMP 12	0.23	0.66	0.65	9.2	4.2

Table I. Paper electrophoresis and paper chromatography of alkylated nucleotides.

is possible to assign the structure of nucleotide derivatives by a combination of UV spectroscopy, paper electrophoresis, ¹H NMR spectroscopy and FAB (negative) mass spectroscopy. Any perturbation of UV spectrum from that of the unsubstituted nucleotide is suggestive of a substitution at the base moiety [13]. The shift of the anomeric proton of ribose to a lower field as compared to that of unsubstituted nucleoside or nucleotide is indicative of ribose substitution, the 2'-O-substitution being at a lower field as compared to 3'-O-substitution [14]. The presence of phosphate group can be ascertained by the deshielding of H-6 proton of cytidine by phosphate group while the phosphate alkylation can be determined by paper electrophoresis [15] at pH 8.5 and pH 4.7; at the former pH the migration of monoanion is half that of dianion. Nucleotides are generally not amenable

to mass spectrometry, unless derivatized, due to their highly polar nature. However, with the introduction of FAB mass spectrometry especially in the negative mode [17], it is now possible to determine the molecular composition of nucleotides routinely. In the present work this technique has been successfully applied to determine the molecular composition of the alkylated nucleotides, the details of mass spectral measurements will be published elsewhere.

From the reaction of 5'-CMP with methyl iodide the product corresponding to R_f 0.21 in solvent A (Table I) was obtained in 18.8% yield and showed a shift of $\lambda_{\max}$ to lower wave length at both acidic and basic pH (Table II), this shift corresponded in magnitude to that of an N³ substituted cytidine moiety [11, 12]. The electrophoretic mobility of the compound at pH 8.0 was also less than

Compound	$\lambda_{\max}$ (nm)		$\lambda_{\min}$ (nm)		Extinction coefficient ($\epsilon \times 10^{-3}$)	
	pH 2	pH 12	pH 2	pH 12	pH 2	pH 12
5'-CMP 1	280	271	240	250	13.0	8.0
N ³ -Methyl-5'-CMP 2	276	266	240	250	13.3	10.5
2',3'-Di-O-methyl-5'-CMP 3	280	270	240	250	13.4	12.6
2'-O-Methyl-5'-CMP 4	280	270	240	250	11.4	7.0
3'-O-Methyl-5'-CMP 5	280	270	242	250	10.9	7.0
2',3'-Di-O-ethyl-5'-CMP 6	279	269	240	250	10.8	9.1
2'-O-Ethyl-5'-CMP 7	279	269	240	250	12.4	11.2
3'-O-Ethyl-5'-CMP 8	279	269	240	250	10.5	10.1
N ³ ,2'-Di-O- <i>n</i> -propyl-5'-CMP 9	276	266	240	250	14.2	13.3
2',3'-Di-O- <i>n</i> -propyl-5'-CMP 10	278	270	240	250	14.1	11.1
2'-O- <i>n</i> -Propyl-5'-CMP 11	279	270	240	250	15.9	12.3
3'-O-Isopropyl-5'-CMP 12	278	269	240	250	13.8	12.1

Table II. Ultra-violet spectral data of alkylated 5'-CMP derivatives.

that of 5'-CMP [12] (Table I) indicating an N³ alkylation. The ¹H NMR spectrum exhibited a singlet at δ 3.32 integrating for three protons. The UV spectral shift as well as the location of methyl signals in the ¹H NMR spectrum indicated an N³ substitution and therefore the product was assigned as N³-methyl-5'-CMP **2**.

The product corresponding to R_f 0.26 in solvent A was obtained in 19.1% yield and did not show alkylation at the base or phosphate groups as judged by the similarity of UV and electrophoretic data with the parent compound (Table I, II). The ¹H NMR spectrum in D₂O exhibited the H-5, H-6 and H-1' signals as doublets at expected δ values (see experimental part). Two singlets integrating for three protons each were located at δ 3.52 and 3.41, and were assigned to 2'-O- and 3'-O-methyl groups respectively. These values are in agreement with those reported for 2'-O- and 3'-O-methyl signals of cytidine [16]. The structure of this product was therefore assigned as 2',3'-Di-O-methyl-5'-CMP **3**. The structure was further confirmed by FAB (negative) mass spectrum which exhibited molecular ion [M-H] at m/z 350 corresponding to the correct molecular formula C₁₁H₁₇N₃O₈P.

The product corresponding to R_f 0.15 was obtained in 38.3% yield, its UV spectrum was similar to 5'-CMP (Table II) and so was its electrophoretic mobility at pH 8.5 indicating the absence of alkylation at the base and phosphate moieties. The anomeric proton of ribose was located at δ 6.03 and was shifted downfield as compared to the anomeric proton of 5'-CMP which was located at δ 5.98 at identical concentration; this downfield displacement suggested alkylation of 2'-OH of ribose. The singlet of methyl protons was located at δ 3.52 substantiating the assignment. FAB (negative) mass spectrum exhibited molecular ion [M-H] at m/z 336 corresponding to correct molecular formula C₁₀H₁₅N₃O₈P. This compound was therefore characterized as 2'-O-methyl-5'-CMP **4**.

The product corresponding to R_f 0.12 in solvent A was obtained in 11.5% yield, its UV spectrum and electrophoretic mobility were similar to 5'-CMP, excluding substitution at base and phosphate moieties. The ¹H NMR spectrum of the product exhibited the anomeric proton at δ 5.72 which is shifted upfield as compared to the H-1' of 5'-CMP indicating alkylation at 3'-OH group ribose; furthermore a singlet integrating for three

methyl protons was observed at δ 3.41. The FAB (negative) mass exhibited molecular ion [M-H] at m/z 380 corresponding to molecular formula C₁₀H₁₃N₃O₈PNa₂, the product was therefore characterized as 3'-O-methyl-5'-CMP disodium salt **5**.

The reaction of 5'-CMP with ethyl iodide was carried out similar to methylation except that the reaction was done at 60 °C for optimum yields. The product of R_f 0.71 in solvent A (Table I), was obtained in 21.1% yield; its UV spectrum (Table II) and electrophoretic mobility were similar to 5'-CMP, therefore alkylation at base and phosphate moieties was excluded. The ¹H NMR spectrum of the compound exhibited the protons H-5, H-6 and H-1' at expected δ values (experimental part). The ethyl signals were located at δ 1.15 as two triplets, each integrating for three protons, and were ascribed to two methyl groups. A multiplet at δ 3.65 integrating for four protons, was ascribable to two methylenes of the ethyl groups; this indicated the presence of two ethyl groups at the 2'-O- and 3'-O-hydroxyls of ribose. The compound was therefore assigned as 2',3'-Di-O-ethyl-5'-CMP **6**. The structure is supported by FAB mass in the negative mode which exhibited [M⁻] at m/z 379 corresponding to molecular formula C₁₃H₂₂N₃O₈P.

The product corresponding to R_f 0.13 in solvent A was obtained in 51.7% yield and from UV and electrophoresis data no evidence of base or phosphate alkylation was obtained. The ¹H NMR spectrum, however, exhibited a downfield shift of the anomeric proton, which was located at δ 6.00, as compared to the anomeric proton of 5'-CMP at δ 5.98; indicating a substitution at 2'-OH of ribose. The ethyl signals were located at δ 1.18, a triplet integrating for three protons ascribable for the methyl group and the methylene signal at δ 3.65, a quartet, integrating for two protons. The product was therefore characterized as 2'-O-ethyl-5'-CMP as the disodium salt **7**. FAB (negative) mass exhibited the molecular ion [M-H] at m/z 394 corresponding to molecular formula C₁₁H₁₅N₃O₈PNa₂.

The product corresponding to R_f 0.22 in solvent A was obtained in 5.70% yield, and indicated an unsubstituted base and phosphate groups. The ¹H NMR spectrum exhibited a shift of anomeric proton at δ 5.95, which indicated a substitution of 3'-OH group. Therefore the compound was assigned as 3'-O-ethyl-5'-CMP **8**. FAB (negative) mass ex-

hibited the $[M-H]$ at m/z 350, corresponding to molecular formula $C_{11}H_{17}N_3O_8P$.

The reaction of *n*-propyl iodide with 5'-CMP was carried out at 70–75 °C, paper chromatography of the crude reaction mixture showed the formation of three new products of higher R_f (Table I) as compared to the starting material. The product of R_f 0.48 on paper electrophoresis exhibited slower mobility as compared to that of 5'-CMP at pH 8 and its UV spectrum showed a shift of λ_{max} at both acid and basic pH (Table II) which was of the same order as that of a N^3 substituted CMP derivative [11, 12]. The FAB (negative) mass spectrum exhibited the molecular ion $[M^-]$ at m/z 429 corresponding to molecular formula $C_{15}H_{25}N_3O_8PNa$; indicating the presence of two *n*-propyl groups. On the basis of 1H NMR the second propyl group was assigned on 2'-OH as the anomeric proton was shifted downfield at δ 6.01 as compared to 5'-CMP at δ 5.98. The signals for two CH_3 groups of the propyl were located at δ 0.88 as two triplets integrating for six protons, a multiplet at δ 1.58 integrating for four protons ascribable to two CH_2 groups and a multiplet at δ 3.63 integrating for four protons ascribable to two OCH_2 groups. The UV, NMR and FAB spectral evidence led this compound to be characterized as $N^3,2'$ -O-*n*-propyl-5'-CMP as the monosodium salt **9**.

The product corresponding to R_f 0.45 in solvent A was obtained in 13.7% yield, its UV spectrum and electrophoresis showed no substitution at the base or phosphate moieties (Table I, II) while the 1H NMR spectrum indicated the presence of two *n*-propyl groups. Two triplets at δ 0.87 integrating for 6H and ascribable to two CH_3 protons were present. A multiplet at δ 1.56 for 4H ascribable to protons of two CH_2 groups and two triplets at

δ 3.64 for 4H ascribable to the protons of two O- CH_2 groups were also present. The FAB (negative) mass spectrum exhibited the molecular ion $[M^-]$ at m/z 429 which corresponded to the molecular formula $C_{15}H_{25}N_3O_8PNa$; the product was therefore assigned as 2',3'-Di-O-*n*-propyl-5'-CMP monosodium salt **10**. The compound of R_f 0.12 in solvent A was obtained in 38.1% yield. Its UV spectrum as well as electrophoretic mobility was similar to that of 5'-CMP indicating unsubstituted base and phosphate moieties. The 1H NMR spectrum exhibited the anomeric proton at δ 6.00, a downfield shift as compared to 5'-CMP indicating a 2'-OH substitution. A triplet at δ 0.84 is ascribable to CH_3 protons of *n*-propyl, a sextet at δ 1.57 for CH_2 protons and triplet at δ 3.61 for OCH_2 protons of the propyl group. FAB (negative) mass spectrum exhibited m/z 387 $[M]^-$ corresponding to molecular formula $C_{12}H_{19}N_3O_8PNa$, the compound was therefore assigned as 2'-O-*n*-propyl-5'-CMP as the monosodium salt, **11**. The reaction of 5'-CMP with isopropyl iodide was carried out at 70 °C, the faster moving product of R_f 0.23 in solvent A was obtained in 11.2% yield. The UV and electrophoresis (Table I, II) indicated no substitution at base or phosphate portions. The 1H NMR spectrum showed anomeric H-1' signal at δ 5.89, a shift indicating isopropylation at 3'-OH group. The isopropyl protons were located at the expected values. A double doublet for 6H was located at δ 1.06 and was ascribed to two methyls, a multiplet 1H was located at δ 3.64 and was ascribed to OCH group. The FAB mass spectrum showed the molecular ion peak $[M]^-$ at m/z 409 corresponding to molecular formula $C_{12}H_{18}N_3O_8PNa_2$ the structure was therefore assigned as 3'-O-isopropyl-5'-CMP disodium salt **12**.

- 1 $R_1 = R_2 = R_3 = H; R_4 = R_5 = Na$
- 2 $R_1 = CH_3; R_2 = R_3 = R_4 = R_5 = H$
- 3 $R_1 = H; R_2 = R_3 = CH_3; R_4 = R_5 = H$
- 4 $R_1 = H; R_2 = CH_3; R_3 = R_4 = R_5 = H$
- 5 $R_1 = R_2 = H; R_3 = CH_3; R_4 = R_5 = Na$
- 6 $R_1 = H; R_2 = R_3 = C_2H_5; R_4 = R_5 = H$
- 7 $R_1 = H; R_2 = C_2H_5; R_3 = H; R_4 = R_5 = Na$
- 8 $R_1 = R_2 = H; R_3 = C_2H_5; R_4 = R_5 = H$
- 9 $R_1 = R_2 = n-C_3H_7; R_3 = R_4 = H; R_5 = Na$
- 10 $R_1 = H; R_2 = R_3 = n-C_3H_7; R_4 = H; R_5 = Na$
- 11 $R_1 = H; R_2 = n-C_3H_7; R_3 = R_4 = H; R_5 = Na$
- 12 $R_1 = R_2 = H; R_3 = iso-C_3H_7; R_4 = R_5 = Na$

The studies reported above indicate that under strongly alkaline conditions 5'-CMP can be alkylated with alkyl iodides predominantly at the ribose moiety with the 2'-O-alkylated isomers being synthesized in maximal yields. The reaction of isopropyl iodide is interesting as only the 3'-O-isomer was obtained in small yield under our reaction conditions. Molecular models show that the heterocyclic ring will offer substantial steric hindrance to the incoming bulkier isopropyl group at the 2'-OH group; the 3'-OH group is freely available for the reaction to occur. The smaller yield of the 3'-O-isopropyl derivative may be explained by difficult ionisation of the 3'-OH group in the presence of a neighbouring negatively charged phosphate.

Experimental

Cytidine-5'-monophosphate (5'-CMP) was purchased from BDH, England. Paper chromatography was performed in all glass apparatus in a descending manner using solvent systems, A, Isopropanol: $\text{NH}_4\text{OH}:\text{H}_2\text{O}$ (7:1:2 v/v); B, Ethanol: 1 M Ammonium acetate, pH 7.4 (7:3 v/v) and C, *n*-propanol: $\text{NH}_4\text{OH}:\text{H}_2\text{O}$ (55:10:35). Preparative paper chromatography was carried out on 3 MM Whatman filter sheets. Paper electrophoresis was done on Whatman No.1. paper strips 43.5×8.8 cm at 400 volts and 18 Amperes for two hours, using buffer I 0.05 M phosphate pH 8.0 and buffer II, 0.025 M sodium acetate pH 4.5.

UV spectral data were obtained on UNICAM SP 500 spectrophotometer and ^1H NMR spectra on a BRUKER AM-300 spectrometer. FAB mass spectra in the negative mode were recorded on a Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 computer system. For ^1H NMR the concentration of samples was maintained constant as 5 mg/0.5 ml solutions in D_2O .

General alkylation procedure

5'-CMP (500 mg) was dissolved in a mixture of 1 N sodium hydroxide (5 ml) and 1,4-dioxane (5 ml) and while stirring magnetically the desired alkyl halide (2 ml) was added. Depending upon the alkyl halide the reaction mixture was stirred at room temperature or at elevated temperatures for 3 h and paper chromatography in solvent A, B and C was carried out to monitor the formation of products.

The crude reaction mixture was concentrated to 2 ml at 37 °C and was separated by preparative paper chromatography on 3 MM Whatman sheets, using solvent A. The bands corresponding to the products were excised and eluted with distilled water (80 ml). The eluate was evaporated under vacuum and the products were thoroughly dried before making spectral and chromatographic measurements.

The reaction with methyl iodide was carried out at room temperature and resulted into the formation of four new products.

*N*³-Methylcytidine-5'-monophosphate (2)

The product corresponding to R_f 0.21 in solvent A (Table I) was obtained as an amorphous powder (98 mg; 18.8%). – ^1H NMR (D_2O) exhibited δ 8.05 (d, J = 6.9 Hz; 1H, H-6); δ 6.25 (d, J = 6.9 Hz, 1H, H-5); δ 6.0 (d, J = 3.0 Hz, 1H, H-1') and δ 3.32 (s, 3H, N-CH₃).

2',3'-Di-O-methylcytidine-5'-monophosphate (3)

The product corresponding to R_f 0.26 solvent A was an amorphous solid (104 mg; 19.1%). – ^1H NMR (D_2O), exhibited signals at δ 8.0 (d, J = 8.0 Hz, 1H, H-6); δ 6.27 (d, J = 8.0 Hz, 1H, H-5); δ 5.98 (d, J = 2.5 Hz, 1H, H-1'); δ 3.52 (3H, s, 2'-OCH₃); δ 3.41 (s, 3H, 3'-OCH₃).

FAB (negative ion) mass spectrum exhibited m/z 350 [M-H] corresponding to molecular formula $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8\text{P}$, m/z 442 [M-H+glycerol], m/z 321 [M-2×CH₃].

2'-O-Methylcytidine-5'-monophosphate (4)

The product corresponding to R_f 0.15, solvent A was an amorphous powder (200 mg; 38.3%). – ^1H NMR (D_2O) exhibited δ 8.05 (d, J = 8.0 Hz, 1H, H-6); δ 6.20 (d, J = 8.0 Hz, 1H, H-5); δ 6.03 (d, J = 2.5 Hz, 1H, H-1'; for CMP H-1' at δ 5.98); δ 3.52 (s, 3H, 2'-OCH₃).

FAB (negative ion) exhibited m/z 336 [M-H] corresponding to molecular formula $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_8\text{P}$; m/z 428 [M-H+glycerol], m/z 322 [M-H-CH₃].

3'-O-Methylcytidine-5'-monophosphate disodium salt (5)

The product of R_f 0.12 in solvent A was an amorphous powder (60 mg; 11.5%). – ^1H NMR (D_2O) exhibited signals at δ 8.07 (d, J = 7.5 Hz, 1H, H-6); δ 5.98 (d, J = 7.5 Hz, 1H, H-5); δ 5.72 (d, J = 2.6 Hz, 1H, H-1'); δ 3.42 (s, 3H, 3'-OCH₃).

FAB (negative ion) m/z 380 [M-H] corresponding to molecular formula $\text{C}_{10}\text{H}_{13}\text{N}_3\text{O}_8\text{PNa}_2$.

2',3'-Di-O-ethylcytidine-5'-monophosphate (6)

The product of R_f 0.71 in solvent A was an amorphous solid (124 mg; 21.1%). – ^1H NMR (D_2O) exhibited signals at δ 7.98 (d, J = 8.0 Hz, 1H, H-6), δ 6.15 (d, J = 8.0 Hz, 1H, H-5); δ 6.02 (d, J = 3.0 Hz, 1H, H-1'); δ 1.15 (2×t, 6H, 2×CH₃); δ 3.65 (m, 4H, 2×OCH₂).

FAB (negative ion) mass spectrum exhibited m/z 379 $[\text{M}]^-$ corresponding to molecular formula $\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_8\text{P}$; m/z 470 $[\text{M}-\text{H}+\text{glycerol}]$.

2'-O-Ethylcytidine-5'-monophosphate disodium salt (7)

The product had R_f 0.13 in solvent A (281 mg; 51.7%). – ^1H NMR (D_2O) exhibited signals at δ 8.15 (d, J = 8.0 Hz, 1H, H-6), δ 6.15 (d, J = 8.0 Hz, 1H, H-5); δ 6.00 (d, J = 3.0 Hz, 1H, H-1'); δ 1.15 (t, 3H, CH₃); δ 3.65 (q, 2H, –OCH₂).

FAB (negative ion) mass spectrum exhibited m/z 394 $[\text{M}-\text{H}]^-$ corresponding to molecular formula $\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_8\text{PNa}_2$, m/z 367 $[\text{M}+\text{H}-\text{C}_2\text{H}_5]$, m/z 459 $[\text{M}+\text{H}-\text{C}_2\text{H}_5+\text{glycerol}]$.

3'-O-Ethylcytidine-5'-monophosphate (8)

The compound showed an R_f of 0.22 in solvent A, (31 mg; 5.70%). – ^1H NMR (D_2O) exhibited signals at δ 7.93 (d, J = 8.0 Hz, 1H, H-6); δ 6.35 (d, J = 8.0 Hz, 1H, H-5); δ 5.95 (d, J = 3.0 Hz, 1H, H-1'); δ 1.15 (t, 3H, CH₃); δ 3.65 (q, 2H, OCH₂).

FAB (negative ion) mass spectrum exhibited m/z 350 $[\text{M}-\text{H}]^-$, corresponding to molecular formula $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8\text{P}$, m/z 442 $[\text{M}-\text{H}+\text{glycerol}]$.

***N*³-2'-O-Di-*n*-propylcytidine-5'-monophosphate monosodium salt (9)**

The reaction of 5'-CMP with *n*-propyliodide was carried out at 70 °C for 3 h. The product corresponding to R_f 0.48 in solvent A was obtained as an amorphous hygroscopic solid (51 mg; 7.6%). – ^1H NMR (D_2O) exhibited signals at δ 8.11 (d, J = 7.8 Hz, 1H, H-6); δ 6.21 (d, J = 7.8 Hz, 1H, H-5); δ 6.01 (d, J = 3.8 Hz, 1H, H-1'); δ 0.88 (2×t, 6H, 2×CH₃); δ 1.58 (m, 4H, 2×CH₂); δ 3.63 (m, 4H, 2×OCH₂).

FAB (negative ion) mass spectrum exhibited m/z 429 $[\text{M}]^-$ corresponding to molecular formula

$\text{C}_{15}\text{H}_{25}\text{N}_3\text{O}_8\text{PNa}$, m/z 407 $[\text{M}+\text{H}-\text{Na}]$, m/z 499 $[\text{M}+\text{H}-\text{Na}+\text{glycerol}]$, m/z 521 $[\text{M}+\text{glycerol}]$.

2',3'-Di-O-*n*-propylcytidine-5'-monophosphate monosodium salt (10)

The product had R_f 0.45 in solvent A and was obtained as amorphous powder (91 mg; 13.7%). – ^1H NMR (D_2O) exhibited δ 8.10 (d, J = 8.0 Hz, 1H, H-6); δ 6.16 (d, J = 8.0 Hz, 1H, H-5); δ 5.99 (d, J = 3.4 Hz, 1H, H-1'); δ 0.87 (2×t, 6H, 2×CH₃); δ 1.56 (m, 4H, 2×CH₂); δ 3.64 (2×t, 4H, O–CH₂).

FAB (negative ion) mass spectrum showed $[\text{M}]^-$ at m/z 429, corresponding to molecular formula $\text{C}_{15}\text{H}_{25}\text{N}_3\text{O}_8\text{PNa}$; m/z 407 $[\text{M}+\text{H}-\text{Na}]$, m/z 386 $[\text{M}-n\text{-propyl}]$, m/z 343 $[\text{M}-2n\text{-propyl}]$, m/z 521 $[\text{M}+\text{glycerol}]$.

2'-O-*n*-propylcytidine-5'-monophosphate monosodium salt (11)

The product showed R_f 0.12 in solvent A, (229 mg; 38.1%). – ^1H NMR (D_2O) exhibited δ 8.11 (d, J = 7.0 Hz, 1H, H-6); δ 6.00 (d, J = 7.0 Hz, 1H, H-5); δ 6.00 (d, J = 3.0 Hz, 1H, H-1'); δ 0.84 (3H, t, CH₃); δ 1.57 (sextet, 2H, CH₂); δ 3.61 (t, 2H, OCH₂).

FAB (negative ion) mass spectrum exhibited m/z 387 $[\text{M}]^-$, corresponding to molecular formula $\text{C}_{12}\text{H}_{19}\text{N}_3\text{O}_8\text{PNa}$; m/z 343 $[\text{M}-\text{H-alkyl}]$; m/z 365 $[\text{M}+\text{H}-\text{Na}]$ and m/z 479 $[\text{M}+\text{glycerol}]$.

3'-O-Isopropylcytidine-5'-monophosphate disodium salt (12)

The reaction with isopropyl iodide was carried out at 70 °C, the product corresponding to R_f 0.23 in solvent A was a brownish powder (71.5 mg; 14.3%). – ^1H NMR (D_2O) exhibited δ 7.98 (d, J = 7.8 Hz, 1H, H-6); δ 5.96 (d, J = 7.8 Hz, 1H, H-5); δ 5.89 (d, J = 4.2 Hz, 1H, H-1'), δ 1.06 (dd, 6H, (CH₃)₂); δ 3.64 (m, 1H, >CHO).

FAB (negative ion) mass spectrum exhibited m/z 409 $[\text{M}]^-$ corresponding to molecular formula $\text{C}_{12}\text{H}_{18}\text{N}_3\text{O}_8\text{PNa}_2$; m/z 387 $[\text{M}+\text{H}-\text{Na}]$; m/z 365 $[\text{M}+2\text{H}-2\times\text{Na}]$; m/z 344 $[\text{M}+\text{H-alkyl}-\text{Na}]$; m/z 501 $[\text{M}+\text{glycerol}]$; m/z 593 $[\text{M}+2\times\text{glycerol}]$.

- [1] R. H. Hall, *The Modified Nucleosides in Nucleic Acids*, Columbia Press, New York (1971).
- [2] G. P. Wheeler, *Cancer Res.* **22**, 651 (1962).
- [3] E. Farber, *Adv. Cancer Res.* **7**, 383 (1963).
- [4] B. J. Ortwerth and G. D. Novelli, *Cancer Res.* **29**, 381 (1969).
- [5] F. Rottman and K. Heinlein, *Biochemistry* **7**, 2634 (1968).
- [6] K. A. Khan and F. Rottman, *Fed. Europ. Biochem. Soc. FEBS Letters* **28**, 25 (1972).
- [7] F. Rottman, K. Frederici, P. Comstock, and K. A. Khan, *Biochemistry* **13**, 2762 (1974).
- [8] H. Inoue, Y. Hayase, A. Imura, S. Iwai, K. Miura, and E. Ohtsuka, *Nucl. Acid. Res.* **15**(15)6131 (1987).
- [9] I. Tazawa, S. Tazawa, J. L. Alderfer, and P. O. P. T'so, *Biochemistry* **11**, 4931 (1972).
- [10] M. Ikehara and S. Uesugi, *Tetrahedron* **28**, 3687 (1973).
- [11] J. A. Haines, C. B. Rees, and A. Todd, *J. Chem. Soc.* **1964**, 1406.
- [12] R. L. C. Brimacombe and C. B. Reese, *J. Chem. Soc. (C)*, **1966**, 588.
- [13] P. Brookes and P. D. Lawley, *J. Chem. Soc.* **1962**, 1384.
- [14] A. D. Broom, M. P. Schweizer, and P. O. P. T'so, *J. Am. Chem. Soc.* **89**, 3612 (1967).
- [15] B. E. Griffin and C. B. Reese, *Biochim. Biophys. Acta* **68**, 185 (1963).
- [16] J. T. Kusmerek, J. Giziewickz, and D. Shugar, *Biochemistry* **12**, 194 (1973).
- [17] A. M. Hogg, J. G. Kelland, J. C. Vederas, and C. Tamm, *Helv. Chim. Acta* **69**, 908 (1986).