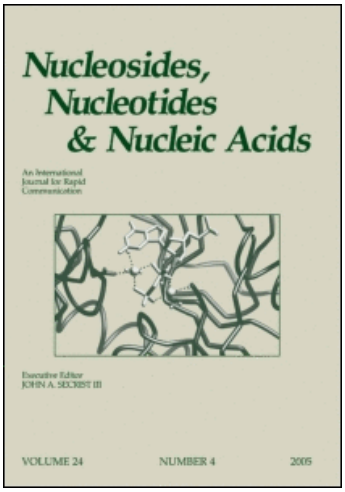

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Formation of 5- and 6-Aminocytosine Nucleosides and Nucleotides from the Corresponding 5-Bromocytosine Derivatives: Synthesis and Reaction Mechanism

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FORMATION OF 5- AND 6-AMINOCYTOSINE NUCLEOSIDES AND NUCLEOTIDES FROM THE CORRESPONDING 5-BROMOCYTOSINE DERIVATIVES: SYNTHESIS AND REACTION MECHANISM

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ABSTRACT. An improved method for the synthesis of 5-aminocytidine (3a), 5-amino-2'-deoxycytidine (3b), and their 5'-monophosphates (3c,d) from the corresponding 5-bromo pyrimidines, using liquid ammonia, is described. The respective 6-aminocytosine derivatives (4a,b,c,d), minor products of the amination reaction, were isolated and characterized. A plausible mechanism is proposed to account for the formation of both 5- and 6-substituted products.

Chemical modifications of the naturally occuring cytosine nucleosides and nucleotides have lead to a variety of useful antimetabolites and enzyme inhibitors. Substitution of the heterocyclic ring with an amino group alters the chemical properties and biological activities of the parent molecule and provides with a handle for further derivatization. The synthesis of 5-aminocytidine (3a) from 5-bromocytidine (2a) in 12% yield was reported in 1955 by Fukahara and Visser[1] employing the method previously used for the synthesis of 5-aminouridine.[2]

In this paper, we wish to report the synthesis of 3a from 2a in an improved yield of 65% by this method[1] and its extension for the preparation of other 5-aminocytosine derivatives 3b,c,d (see FIG. 1). The previously unreported 6-aminocytosine derivatives (4a,b,c,d), which form as byproducts of the amination reaction, are also described. Based on mechanistic considerations, it is proposed that the formation of 5-aminocytosines occurs by an addition-substitution-elimination reaction sequence, whereas the minor 6-aminocytosine products are formed by a

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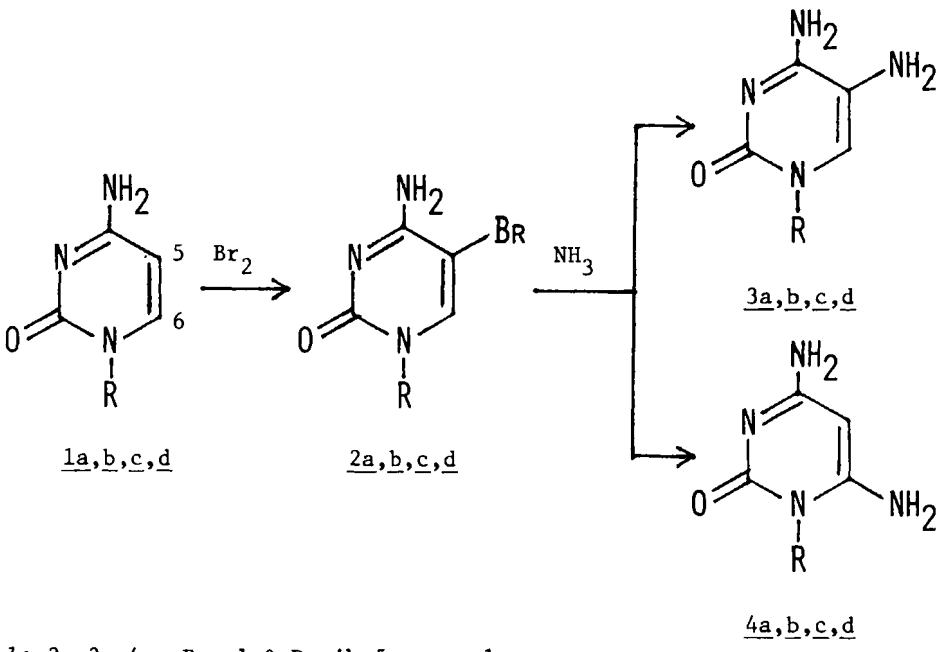
single addition-elimination reaction via formation of the less favorable cis-adduct intermediate.

RESULTS AND DISCUSSION

Synthesis of 5- and 6-Aminocytosine Derivatives

The synthetic scheme leading to the 5- and 6-aminocytosine derivatives is outlined in FIG. 1. Treatment of the nucleosides cytidine (1a) and 2'-deoxycytidine (1b) with bromine in a mixture of pyridine and carbon tetrachloride at room temperature for 4 hours yielded the corresponding 5-bromocytidine (2a) and 5-bromo-2'-deoxycytidine (2b), which after removal of residual bromine and solvents, were isolated via anion exchange column chromatography. The 5-bromo derivatives 2a and 2b were treated with liquid ammonia at 55°C for 2 days to yield a mixture of the corresponding 5- and 6-amino substituted derivatives.



1a,2a,3a,4a, R = 1-β-D-ribofuranosyl

1b,2b,3b,4b, R = 1-(2-deoxy-β-D-ribofuranosyl)

1c,2c,3c,4c, R = 1-(5-O-phosphoryl-β-D-ribofuranosyl)

1d,2d,3d,4d, R = 1-(2-deoxy-5-O-phosphoryl-β-D-ribofuranosyl)

FIGURE 1. Synthetic Scheme.

Separation and isolation of the products was accomplished using ion exchange column chromatography followed by crystallization from aqueous ethanol. The 5- and 6-amino cytidines (3a and 4a) were obtained in yields of 65% and 9%, respectively. Compound 3b was obtained as the hydrochloride salt in 47% yield. Compound 4b was isolated and characterized, but could not be crystallized.

A similar sequence of reactions was employed for the synthesis of the nucleotide derivatives. Cytidine 5'-dihydrogen phosphate (1c) and 2'-deoxycytidine 5'-disodium phosphate (1d) were treated with bromine in a mixture of dimethylformamide and carbon tetrachloride. The reaction was monitored by UV and TLC and after completion, compounds 2c and 2d were isolated at their barium salts. After exchange of the barium to sodium, aqueous solutions of the products were lyophilized. The reaction of the freeze-dried powders of compounds 2c and 2d in liquid ammonia yielded the corresponding 5- and 6-amino substituted derivatives. The separation of the products was accomplished using cation exchange column chromatography. The isolated overall yield of 3c from 1c was 29%; that of 4c, 2.4%. The separation of 3d and 4d was performed similarly. Compound 3d was obtained in 40% yield. All attempts to crystallize 4d (homogeneous by TLC and TLE) were unsuccessful.

The 5-aminocytosine derivatives (3a,b,c,d) were found to have UV spectral properties very similar to 5-amino-1-methylcytosine[3] which has maxima at 296, 312 and 290 nm, for the neutral, cationic and dicationic species, respectively. The spectrophotometrically determined ionization constants of 3a,b,c,d (pK_a = 4.0-4.1) were in good agreement with the reported values for 5-aminocytosine (pK_a = 4.37)[4] and 5-dimethylaminocytidine 5'-phosphate (pK_a = 4.14).[5] The NMR spectra of 3a,b,c,d lacked the H_5-resonance, which is consistent with a substituted C-atom at the 5-position.

It should be noted that the UV spectral data of 5-aminocytidine (3a) determined in this work are not in complete agreement with those previously reported.[1,3] In the first report[1] describing 5-aminocytidine only one maximum was given at 304 nm (pH 4.3, too close to the pK_a), which is similar to that found in the present work. In a more recent report[3], the λ_{max} corresponding to the neutral species of 3a is 298 nm, identical with that found in this work. However, in strongly acidic solution (pH 0) the λ_{max} was reported to be at 304 nm, in contrast to

the 290 nm found in this work. One possible explanation for this discrepency is that under the strongly acidic conditions the nucleoside was hydrolyzed to 5-aminocytosine, which has spectral properties[4] very similar to those reported[3] for 3a.

The structural assignments of the newly described 6-aminocytosine derivatives 4a and 4c were based on their characteristic UV and NMR spectral properties. The prominent UV spectral characteristics of 2,4,6-trisubstituted pyrimidines containing oxo- and amino-groups are: λ_{max} at 270-280 nm and relatively large molar absorption coefficients of about 2×10^4 or more.[4] A comparison of the UV spectral properties of compounds 4a,b,c,d with a few closely related 2,4,6-trisubstituted pyrimidines is shown in TABLE 1.

The structural relationship is quite apparent. In addition, it is shown that the pK_a determined for 4c is close to the pK_a reported[5] for 6-aminocytosine (more than 2 pH units higher than that of 5-aminocytosine). A more extensive listing of UV spectral data for related compounds is available.[4]

The proton NMR spectra of the 6-aminocytosine derivatives showed a single vinylic proton singlet resonance near δ4.8, which was assign-

TABLE 1. Similarities between the 6-Aminocytosine Derivatives 4a,b,c,d and Some Related 6-Substituted Pyrimidines.

Compound	UV SPectral Data			pK_a	Ref.
	λ_{max} (nm)	ε_{max}	pH		
4a	280	27,000	3.2	-	a
	280	18,800	9.2	-	
4b	277	-	2	-	a
	277	-	8.5	-	
4c	280	25,000	3.2	6.82	a
	280	16,800	10	-	
4d	277	-	1	-	a
	277	-	9	-	
6-Aminouridine	272	24,800	7	-	8
6-Hydroxycytidine	267	19,800	7	-	15
6-Aminocytosine	272	-	2	6.56	2,6,19
	270	17,400	7.2		

[a]This work

ed to the H_5 proton. The anomeric proton doublet of 4a showed a significantly larger coupling constant ($J_{1'-2'}$ = 6.5 Hz) than that of 3a ($J_{1'-2'}$ = 4 Hz). This is in agreement with the NMR data reported for the related 6-aminouridine[7] and its 4-deoxy-4-methylthio derivative[8] ($J_{1'-2'}$ = 6.7 Hz and $J_{1'-2'}$ = 7.5 Hz, respectively).

Mechanistic Aspects of the Amination Reaction

Many nucleophilic substitution reactions at the 5-position of pyrimidines are known to occur via the addition of a nucleophile at the 6-position of the pyrimidine ring, displacement of a leaving group at the 5-position followed by regeneration of the heteroaromatic pyrimidine ring system upon elimination of the nucleophile from the 6-position.[9] The corresponding cine substituted[10] product may arise from elimination of the leaving group at the 5-position after formation of the initial adduct. Similar mechanistic pathways have been considered to account for substitution reactions of 5-bromouridine[11,12] and 5-bromocytidine[13] derivatives. In agreement with the above, we propose a plausible mechanistic pathway for the formation of 5-amino and 6-aminocytosine compounds, which is outlined in FIG. 2. In the first step a molecule of ammonia adds across the pyrimidine 5,6-double bond generating a pair of diastereomeric adducts (I and II). Adduct I, which has the H_6 proton trans to the bromine atom may undergo rapid base catalyzed elimination of HBr forming the corresponding 6-aminocytosine derivatives. The other adduct (II) may react with another molecule of ammonia resulting in the formation of intermediate III via S_N2 displacement of bromide. Upon abstraction of the more acidic proton at position 5 the trans elimination of ammonia would generate the 5-amino substituted product.

An alternative mechanism accounting for the formation of 6-amino cytosine nucleosides may involve the cyclic $O^{5'}$-6-anhydrocytidine and $O^{5'}$-6-anhydro-2'-deoxycytidine intermediates which can undergo substitution in ammonia to yield 4a or 4b. Formation of cyclic anhydronucleosides could occur via nucleophilic attack of the ionized 5'-hydroxyl group at the 6-position of the pyrimidine rings. Although the involvement of cyclic anhydronucleosides has precedence in the literature for related pyrimidine nucleoside substitution reactions[14,15] it does not appear to be important in the formation of 6-aminocytosines. This conclusion is based on the finding that the

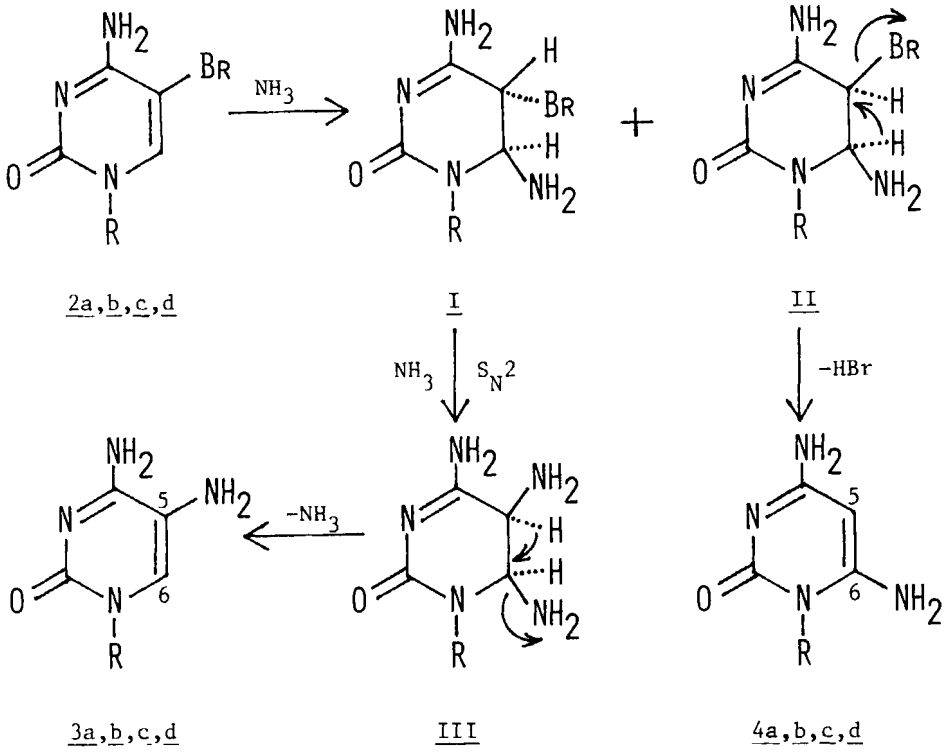


FIGURE 2. Postulated Reaction Mechanism for the Formation of 5- and 6-Aminocytosine Derivatives (the absolute stereochemistry of I, II and III is chosen arbitrarily for the sake of clarity).

5-bromonucleotides (2c and 2d), which cannot form cyclonucleosides, gave the corresponding 6-aminocytosine products 4c and 4d in relative yields similar to those of the nucleosides for 4a and 4b.

The predominance of the 5-aminocytosine product may be rationalized on the basis of the difference between the stabilities of the two diastereomeric adducts, I and II. Since the addition of ammonia across the 5,6-double bond is reversible, the relative concentrations of adduct I and II will be governed by their respective equilibrium constants, which in turn would reflect their relative stabilities. The trans adduct (I) should be more stable than adduct II, which has the bulky 5-bromo and 6-amino groups in the cis orientation. Since the 5-aminocytosine product is derived from adduct I, it should predominate over the 6-aminocytosine product, which arises from adduct II.

A previously proposed mechanism[2] for the formation of 5-aminouridine from 5-bromouridine involves the direct nucleophilic displace-

ment of bromide by ammonia. The validity of this mechanism has recently been questioned[9] and the more likely addition-displacement-elimination reaction sequence was suggested. The demonstration of the formation of 6-aminocytosine derivatives from the corresponding 5-bromopyrimidines in the present work provides evidence against a direct displacement mechanism, and strongly favors the proposed addition-displacement-elimination mechanism for the formation of 5-aminopyrimidine nucleosides and nucleotides in the reaction of ammonia with the corresponding 5-bromopyrimidine derivatives.

EXPERIMENTAL SECTION

Melting points were determined with a Mel-Temp capillary apparatus, and are uncorrected. Ultraviolet spectra were taken with a Varian Cary 118 or Beckman Model 25 recording spectrophotometer. NMR spectra were obtained from Varian T-60 spectrometer using tetramethylsilane in DMSO-d_6 or sodium 3-trimethylsilylpropionate in D_2O, as internal standards. Optical rotations were measured in a Perkin-Elmer 141 polarimeter with a sodium lamp (wavelength of 589 nm). TLC was performed using Eastman Chromagram sheets (#13254 cellulose with fluorescent indicator) with the following solvent systems: system A, 1-butanol, acetic acid, water (5:2:3, v/v); system B, 2-propanol, conc. NH_4OH, water (7:1:2, v/v); system C, ethanol, 1 M ammonium acetate (1:1, v/v). A CAMAG apparatus powered by an ISCO source (Model 490) was used for the thin layer electrophoresis measurements with 0.05 M Na-acetate (pH 6.5) as the electrolyte. Spectrophotometric pK_a determinations were done in buffered solutions of the test compound. The pH was adjusted with 3 N HCl or 2 N KOH solutions and measured directly in the cuvette using a combination microelectrode with a Metrohm Model 103 pH meter. Elemental analysis were performed by Atlantic Microlab, Inc., Atlanta, Georgia.

5-Amino-1(β-D-ribofuranosyl)cytosine (3a) and 6-Amino-1(β-D-ribofuranosyl)cytosine (4a).

To a suspension of cytidine (1a, 23 g, 95 mmol) in pyridine (175 mL, anhydrous) was added a solution of bromine in carbon tetrachloride (7.2 mL in 75 mL) over a period of 30 min. The stoppered reaction mixture was stirred for an additional 3 h at room temperature, the solvent was then removed in vacuo and the residual syrup was dissolved

in aqueous sodium carbonate (5 g Na_2CO_3 in 100 mL water). The mixture was repeatedly coevaporated with water until the residual pyridine was removed. The resulting crude 2a was dissolved in water and applied to a column of BioRad AG 50W 1x8 (200-400 mesh, H^+, 4 equivalents). The resin was washed with water (ca. 2.0 L), and then eluted with 1 M ammonium hydroxide. The fractions containing 2a were evaporated to dryness in vacuo. The residue was dissolved in hot water and allowed to cool. The crystalline product was collected and dried to give 2a: yield 15 g (49%); NMR (D_2O) δ 5.9 (d, 1, J = 2 Hz, H-1'), 8.2 (s, 1, H-6); UV (0.1 N HCl) λ_{max} 300 nm (lit.[15] λ_{max} 299 nm), (pH 7) λ_{max} 289 nm (lit.[16] λ_{max} = 289 nm).

5-Bromocytidine (2a, 6.5 g, 20 mmol) and liquid ammonia (50 mL) were placed in a 100 mL steel bomb and heated for 50 h at 55°C. After opening of the bomb the ammonia was allowed to evaporate at room temperature in a fume hood. The light brown residue remaining was taken up in water and partially decolorized with cellulose powder. The combined washings from the cellulose were evaporated in vacuo to approximately 15 mL and applied to a 60x2.4 cm column of BioRad AG 1x8 (formate, 200-400 mesh) and eluted with distilled water. Compound 4a was eluted first, followed by 3a. Overlapping fractions were rechromatographed. The appropriate fractions were evaporated in vacuo or lyophilized.

Compound 3a was crystallized from aqueous ethanol: yield 3.4 g (65%); mp 220°C dec (lit.[3] mp 211-212°C dec); $[\alpha]_D^{25}$ +6.1° (c 1.17, H_2O), [lit.[3] $[\alpha]_D^{25}$ +4° (c 2.7, H_2O)]; R_f 0.48 (system A), 0.31 (system B); NMR (D_2O) δ 3.9 (m, 2, H-5'), 6.92 (d, 1, J = 4 Hz, H-1'), 7.53 (s, 1, H-6); UV (0.5 N HCl) λ_{max} 215 nm (ε 10,000), 290 nm (ε 8,100), (pH 2.3) λ_{max} 220 nm (ε 11,500), 310 nm (ε 7,300), (pH 7) λ_{max} 222 nm (ε 14,000), 298 nm (ε 6,200).

Anal. Calcd for $C_9H_{14}N_4O_5$: C, 41.86; H, 5.46; N, 21.70. Found: C, 42.07; H, 5.51; N, 22.02.

The hydrochloride salt of 3a was prepared by dissolving 3a in a small amount of boiling water, and to this absolute methanol that contained 1.5 equivalents of HCl (from conc. HCl) was added. After cooling 3a·HCl slowly crystallized out from solution: mp>300°C (lit.[3] mp>320°C).

Anal. Calcd for $C_9H_{15}N_4O_5Cl$: C, 36.68; H, 5.13; N, 19.01. Found: C, 36.40; H, 5.04; N, 18.76.

Compound 4a was obtained in a yield of 0.5 g (9%). A sample was crystallized from a small amount of water: mp 159-163°C; R_f 0.63 (system A), 0.49 (system B); NMR (D_2O) δ 3.87 (d, 1, J = 3 Hz, H-5'), 4.8 (s, 1, H-5), 6.33 (d, 1, J = 6.5 Hz, H-1'); UV (pH 3.2) λ_{max} 280 nm (ε 25,000), (pH 9.2) λ_{max} 280 nm (ε 16,800).

Anal. Calcd for $C_9H_{14}N_4O_5 \cdot 0.5H_2O$: C, 40.44; H, 5.65; N, 20.96. Found: C, 40.00; H, 5.23; N, 21.08.

5-Amino-1(2-deoxy-β-D-ribofuranosyl)cytosine (3b) and 6-amino-1(2-deoxy-β-D-ribofuranosyl)cytosine (4b).

Dry 2'-deoxycytidine·HCl (1b, 2.9 g, 1.1 mmol) was suspended in 100 mL of anhydrous pyridine and a solution of bromine (0.6 mL) in 75 mL of carbon tetrachloride was added in portions over a period of 30 min. The reaction was stirred for 3 h, then the solvent was evaporated in vacuo. The resulting syrup was neutralized by the addition of 0.6 g sodium carbonate in 50 mL water. Residual pyridine was removed via coevaporation with water (3x).

Separation of 2b from starting material 1b was accomplished by anion exchange chromatography on a column of BioRad AG 1x8 (formate, 200-400 mesh, 2.4x60 cm). Unreacted 1b was eluted with water from the column, followed by 2b. The fractions containing the product were combined and lyophilized to give 2b as a dry powder: yield 75%; UV (0.1 N HCl) λ_{max} 300 nm (lit.[15] λ_{max} 300 nm), (H_2O) λ_{max} 287 nm (lit.[15] λ_{max} 287 nm).

5-Bromo-2'-deoxycytidine (2b, 6.6 mmol) and liquid ammonia (50 mL) were added to a bomb (100 mL) and heated at 57°C for 48 h. The ammonia was evaporated, and the reaction product decolorized with cellulose powder. A concentrated solution of the mixture was chromatographed as described for 3a. Fractions of 10 mL were collected and fractions 18-20 and 22-41, containing 4b and 3b, respectively, were lyophilized.

The dry powder of 3b was dissolved in a minimum amount of hot water and 40 mL of ethanol containing 6 mmol of hydrochloric acid was added. The solution was kept at 4°C overnight and the solid formed collected (0.64 g). The filtrate was concentrated to give a second crop (0.15 g). An analytical sample was prepared by recrystallization from methanol. The combined yield of 3b: 47%; mp 127-130°C; R_f 0.62 (system A), 0.40 (system B); NMR (D_2O) δ 2.33 (m, 2, H-2'), 3.8 (m, 2, H-5'), 4.1 (m, 1, CH), 4.5 (m, 1, CH), 6.27 (pseudo-t, 1, J = 6 Hz,

H-1'), 7.60 (s, 1, H-6); UV (0.5 N HCl) λ_{max} 214 nm (ε 12,000), 290 nm (ε 10,000), (pH 2.0) λ_{max} 219 nm (ε 13,000), 311 nm (ε 8,000), (pH 7) λ_{max} 221 nm (ε 17,000), 298 nm (ε 7,400); pK_a 4.03.

Anal. Calcd for $C_9H_{15}N_4O_4Cl$: C, 38.79; H, 5.42; N, 20.10. Found: C, 38.85; H, 5.67; N, 19.73.

Attempts at crystallization of 4b have failed; the freeze-dried powder was homogeneous by TLC: R_f 0.67 (system A), 0.37 (system B); UV (pH 1) λ_{max} 277, (pH 9) λ_{max} 277.

5-Amino-1(β-D-ribofuranosyl)cytosine 5'-Dihydrogen Phosphate (3c) and 6-Amino-1(β-D-ribofuranosyl)cytosine 5'-Dihydrogen Phosphate (4c).

Anhydrous cytidine 5'-dihydrogen phosphate (1c, 4.8 g, 14.8 mmol) was placed in a 250 mL round bottom flask which contained dry dimethyl formamide (60 mL). To this bromine in carbon tetrachloride (0.6 M, 48 mL) was added and the flask stoppered. The mixture was magnetically stirred at 25°C for 6 h then evaporated in vacuo to a syrup, which was coevaporated with 60% ethanol until the unreacted bromine was removed. The resulting colorless solution was neutralized (pH 7.5) with saturated barium hydroxide solution, then absolute ethanol (two volumes) was added. The precipitated barium salt was separated by filtration. The barium of 2c was exchanged for sodium with BioRad Chelex 100 resin (Na^+, 100-200 mesh, 20 g) and the aqueous solution containing the sodium salt of 2c was lyophilized and used without further purification. UV (0.1 N HCl) λ_{max} 300 nm (lit.[18] λ_{max} 299 nm).

Liquid ammonia (60 mL) was added to a 100 mL stainless steel bomb which contained the lyophilized powder of 2c (6.8 g, 14 mmol), the bomb was sealed and heated for 50 h at 55°C. After the reaction the ammonia was allowed to evaporate. The resulting light brown residue was dissolved in water and evaporated in vacuo, redissolved in water (20 mL) and applied to a column (BioRad 50W 1x8, pyridinium form, 200-400 mesh, 90x2.4 cm) and eluted with water. Fractions of 15 mL were collected. Fractions 26-40 and 50-73, containing 3c and 4c, respectively, were lyophilized.

The dry powder of 3c was dissolved in a small amount of boiling water and chilled. The clear crystals of 3c formed were collected and dried: yield 1.6 g (29%); mp>300°C; R_f 0.29 (system A), 0.46 (system C); NMR (D_2O) δ 6.0 (d, 1, J = 3 Hz, H-1'), 7.75 (s, 1, H-6); UV (0.5 N

HCl) λ_{max} 213 nm (ε 9,900), 288 nm (ε 9,600), (pH 2) λ_{max} 218 nm (ε 11,200), 311 nm (ε 6,700), (pH 7) λ_{max} 222 nm (ε 14,600), 310 nm (ε 6,300); pK_a 4.09.

Anal. Calcd for $C_9H_{15}N_4O_8P \cdot 2H_2O$: C, 28.88; H, 5.12; N, 14.97. Found: C, 29.16; H, 5.01; N, 15.01.

The freeze-dried 4c was dissolved in a small quantity of hot water and let stand overnight at 4°C. The crystalline product was collected and dried to give clear needles of 4c: yield 0.12 g (2.4%); mp 206-208°C dec; R_f 0.31 (system A), 0.32 (system C); NMR (D_2O) δ 4.77 (s, 1, H-5), 6.38 (d, 1, J = 3 Hz, H-1'); UV (pH 3.2) λ_{max} 280 nm (ε 25,000), (pH 10) λ_{max} 280 nm (ε 16,800); pK_a 6.82.

Anal. Calcd for $C_9H_{15}N_4O_8P$: C, 31.96; H, 4.47; N, 16.57. Found: C, 32.10; H, 4.62; N, 16.72.

5-Amino-1(2-deoxy-β-D-ribofuranosyl)cytosine 5'-Dihydrogen Phosphate (3d) and 6-Amino-1(2-deoxy-β-D-ribofuranosyl)cytosine 5'-Dihydrogen Phosphate (4d).

Anhydrous 2'-deoxycytidine 5'-phosphate (1d disodium salt, 4.5 g, 12.6 mmol) was placed in a 250 mL round bottom flask which contained 100 mL of dry dimethylformamide, and 1.4 mL of bromine in 80 mL of carbon tetrachloride was added. The reaction mixture was stirred at room temperature for 4 h. After the reaction, the solvent was removed in vacuo and the resulting syrup coevaporated with 60% aqueous ethanol until the mixture became colorless. To the resulting syrup aqueous $Ba(OH)_2$ was added. The barium salt of 2d was precipitated from the solution with ethanol and exchanged to the sodium salt as described above for 2c, and lyophilized: UV (0.1 N HCl) λ_{max} 300 nm.

The freeze-dried powder of 2d (disodium salt, 2.8 mmole) and liquid ammonia (20 mL) were added to a 30 mL stainless steel bomb and heated at 55°C for 50 h. After the reaction, the ammonia was allowed to evaporate and the residue was taken up in water and evaporated in vacuo. The syrup was redissolved in water and the pH adjusted to 4 with 1 N HCl. Compounds 3d and 4d were isolated using the same column chromatographic system as described above for 3c and 4c. The fractions containing the corresponding products were identified via UV and lyophilized.

The freeze-dried powder of 3d was dissolved in a small amount of hot water and refrigerated overnight. The crystals (needles) formed

were collected and dried in vacuo (120 mg). Additional 247 mg of 3d was obtained from the mother liquor by precipitation with ethanol. Yield: 40%; mp 178-181°dec; R_f 0.34 (system A), 0.39 (system C); NMR (D_2O), δ 2.35 (m, 1, H-2'), 6.8 (pseudo-t, 1, J = 6 Hz, H-1'), 7.78 (s, 1, H-6); UV (0.5 N HCl) λ_{max} 214 nm (ε 12,000), 290 nm (ε 10,000), (pH 2) λ_{max} 219 nm (ε 13,000), 310 nm (ε 8,100), (pH 7) λ_{max} 221 nm (ε 16,900), 298 nm (ε 7,350).

Anal. Calcd for $C_9H_{15}N_4O_7P \cdot 0.5H_2O$: C, 32.63; H, 4.87; N, 16.92. Found: C, 32.80; H, 4.55; N, 16.78.

Attempts to crystallize 4d were unsuccessful. The freeze-dried powder of 4d was homogeneous by TLC: R_f 0.35 (system A), R_f 0.36 (system C); UV (pH 1) λ_{max} 277, (pH 9) λ_{max} 277.

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