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SYNTHESES AND PROPERTIES OF N-AMINOPYRIMIDINES

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Abstract: Syntheses of the positional isomers of N-aminopyrimidine bases (1- and 3-amino derivatives of uracil, thymine, and cytosine), 3-aminopyrimidine deoxyribonucleosides (3-amino derivatives of deoxyuridine, thymidine, deoxycytidine), and related compounds are reported. Physicochemical properties of these N-aminopyrimidines are described.

In order to examine the reaction mechanism involved in arylamination of cellular DNA by carcinogenic arylamines, we studied the chemical reactivity of DNA components toward simple electrophilic aminating agents such as hydroxylamine-O-sulfonic acid (HAOS) and 2,4-dinitrophenoxyamine (DNPA).[1-4] With regard to purine derivatives, we reported noticeable reactivities of 1-aminoadenosine[1,2] and 7-aminoguanosine[3] and also chemical characteristics of the positional isomers of mono-N-aminated guanines.[4,5] As part of an ongoing study, the present paper describes syntheses of N-aminated pyrimidine bases and nucleosides. The physicochemical properties of the N-amino group and its substituent effects on UV absorption and basicity of the pyrimidine nucleus are reported.

Syntheses of N-Aminopyrimidines

Reaction of uracil or thymine with 10 equimolar amount of HAOS under alkaline conditions gave a mixture of corresponding 1-amino (**1a** or **1b**) and 3-amino (**2a** or **2b**) derivatives at a ratio of about 3 to 2 (Scheme 1). Compound 1a was identified as 1-aminouracil by comparing its spectral data with those of an authentic specimen obtained by acid hydrolysis of 1-amino-O^4-ethyluracil (**5**), which was prepared by the selective amination on the N1 position of O^4-ethyluracil with HAOS under alkaline conditions. Amination of 1-substituted (methyl, β-D-ribofuranosyl, 2-deoxy-β-D-ribofuranosyl) uracils and thymines with HAOS under alkaline conditions yielded 3-amino derivatives (**3a-e**). Amination of the sodium salt of these 1-substituted uracils and thymines with DNPA in N, N-dimethylformamide (DMF) also proceeded, yielding 3a-e. Acid hydrolysis of 3d and 3e gave 2a and 2b, respectively. Reaction of cytosine with HAOS under alkaline conditions yielded 1-aminocytosine (**4**) as the major product, accompanied by a trace amount of 3-aminocytosine (7 free form). On the other hand, reaction of cytosine with DNPA in DMF

gave 3-aminocytosinium salt (7) selectively. Compound 4 was also obtained quantitatively by the ammonolysis of 5. 1-Substituted-3-aminocytosine derivatives (6a-c) were obtained by the reaction of 1-substituted (methyl, β-D-ribofuranosyl, 2-deoxy-β-D-ribofuranosyl) cytosine with DNPA in DMF. Acid hydrolysis of 6c afforded 7. In all amination reactions examined, yields ranged from 30 to 50 % with HAOS and from 50 to 70% with DNPA. In amination reaction with HAOS, the N-aminated compound formed was deaminated slowly by HAOS. Therefore, the optimal concentration of HAOS to get maximum yield of the N-amino compound is about 10 equimolar amount (data not shown). When HAOS was used, it appears that the difficulty in separation of the products from the inorganic salt lowered the obtainable yield of the products.

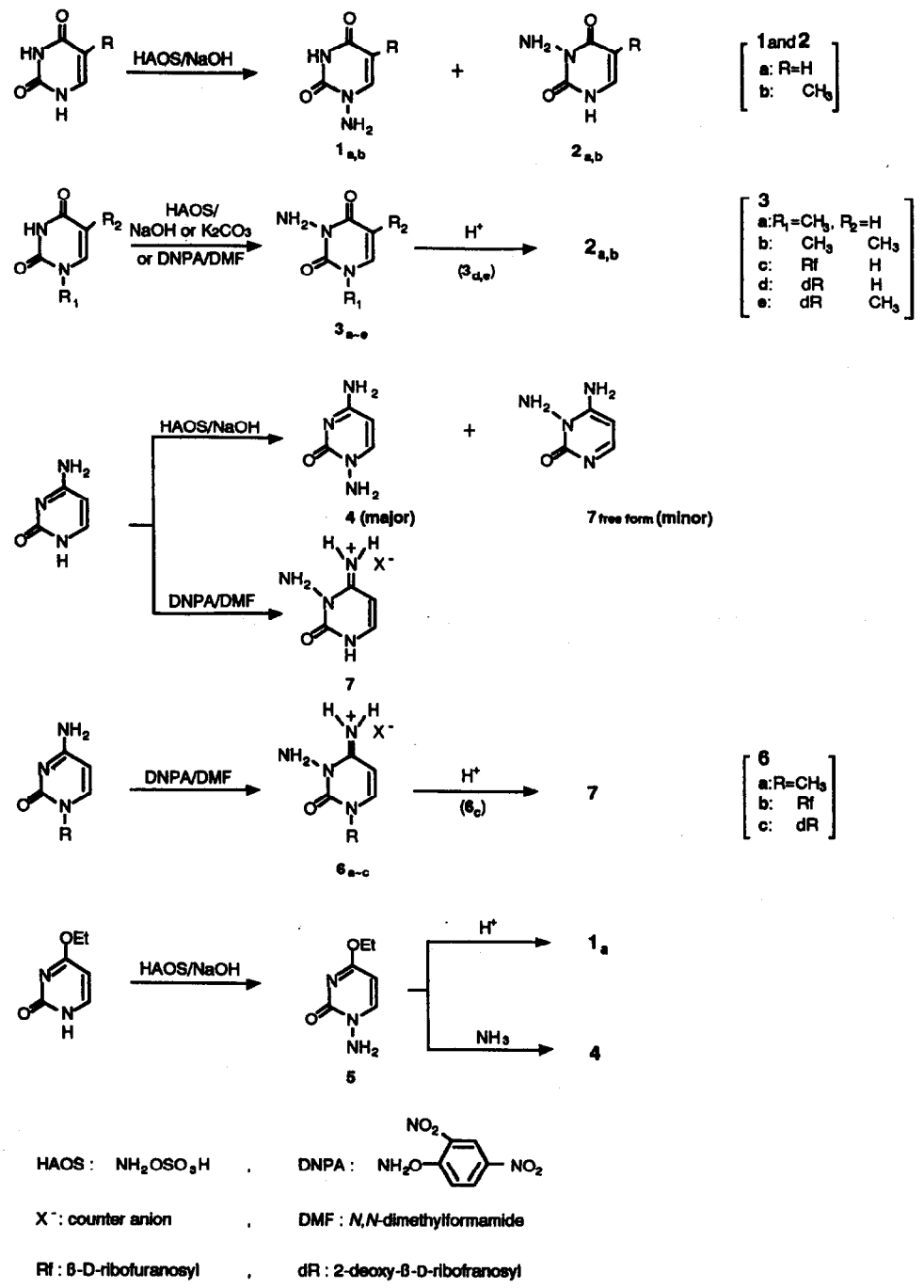
Treatment of N-aminopyrimidines with a 1.2 equimolar amount of $NaNO_2$ in aqueous AcOH at room temperature resulted in rapid deamination as previously reported for N-amino adenines[1,2] and guanines.[3] Deamination of the more basic N-NH_2 (pKa of the conjugated acid, ≥ -0.36) in 2a proceeded more rapidly than that of the less basic one (pKa -1.7) in 1a (data not shown), suggesting that an electophilic nitrosation toward the N-amino group is the rate-determining step in the deamination. Details will be published elsewhere.

Acid Dissociation Constants of N-Aminopyrimidines

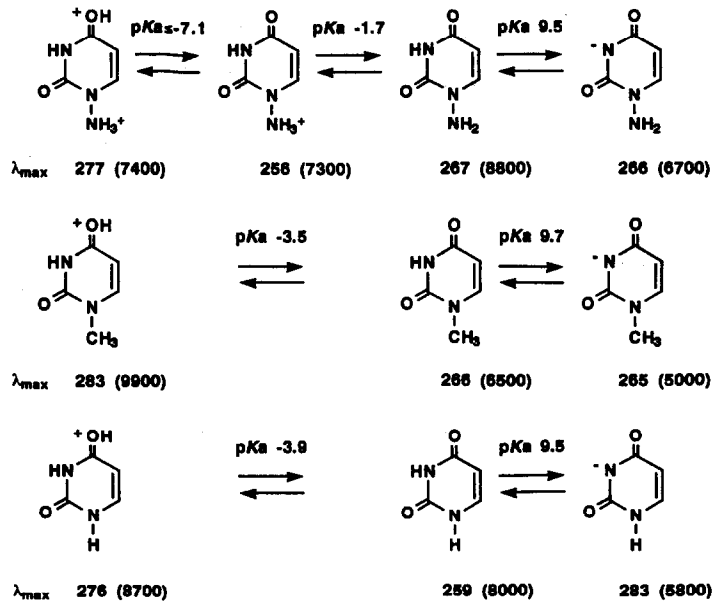
The UV spectrum of N-aminopyrimidines shifted as the acidity of the medium was changed. 1-Aminouracil (1a), for example, showed four distinct absorption curves with three isosbestic points over a range from the pH region to 80% H_2SO_4. The structure corresponding to each absorption was deduced by comparison with acidity-dependent UV spectral changes of 1-methyluracil and uracil. Scheme 2 shows the structures, λ_{max} values for each structure, and the pKa values. Another example shown in Scheme 3 is that of 1-aminocytosine (4). The pKa values of the aminopyrimidines synthesized in the present study are listed in Table 1.

Substituent Effect of the N-Amino Group on UV Absorption

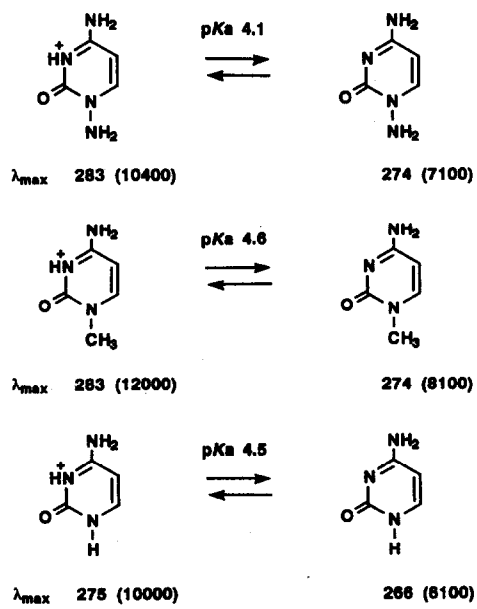
UV spectra of the neutral species of N1-NH_2 and N1-CH_3 derivatives of uracil (λ_{max} 267 and 266 nm, respectively, as shown in Scheme 2) are almost superimposable, both shifted bathochromically by 7 to 8 nm from that of uracil (λ_{max} 259 nm). This suggests that electronic perturbation by the NH_2 group toward the aromatic π-electron system of uracil is nearly equivalent to the hyperconjugative contribution of the CH_3 group. It is worth noting that differences in the λ_{max} values of N-NH_2 and the corresponding N-CH_3 derivatives fall within 0 to 3 nm for all other N-aminpopyrimidines examined here and for N-aminopurines previously reported.[2,4] In contrast to an NH_2 substituent on aromatic carbons such as in aniline, lone-paired electrons of the N-NH_2 group may not freely interact with the aromatic π-electron system, probably because these lone-paired electrons are firmly held on the nitrogen bonded to the adjacent electronegative nitrogen. The bathochromic UV change (7-8 nm) by N1-NH_2 or N1-CH_3 substitution was similar for cytosine in both neutral and monocationic forms (Scheme 3).



Scheme 1. Syntheses of *N*-aminopyrimidines.



Scheme 2. Comparison of UV spectra and p*Ka* values of 1-aminouracil with those of 1-methyluracil and uracil.



Scheme 3. Comparison of UV spectra and p*Ka* values of 1-aminocytosine with those of 1-methylcytosine and cytosine.

Table 1. p*Ka* values of *N*-aminopyrimidines and related compounds.

Compound	$>C=OH^+$	$>N-NH_3^+$	$=NH^+$	$>C=NH_2^+$	$>NH$
uracil	-3.9	—	—	—	9.5,>13
1-methyluracil	-3.5	—	—	—	9.7
1-aminouracil (1a)	≤-7.1	-1.7	—	—	9.5
1-aminothymine (1b)	≤-7.8	-1.2	—	—	9.8
3-methyluracil	nt[*a]	—	—	—	10.0
3-aminouracil (2a)	≤-6.6	≥-0.36	—	—	9.3
3-amino-1-methyluracil (3a)	<-4.4	≥-0.16	—	—	—
cytosine	—	—	4.5	—	12.2
1-methylcytosine	—	—	4.6	—	—
1-aminocytosine (4)	*b	*b	4.1	—	—
3-methylcytosine	—	—	—	7.4	—
3-aminocytosine (7)	*c	*c	—	7.2	—
1,3-dimethylcytosine	—	—	—	9.3	—
3-amino-1-methylcytosine (6a)	nt	-1.4	—	10.3	—

[*a] Not tested.

[*b] 1-Aminocytosine decomposed to cytosine gradually in 30% H_2SO_4 and rapidly in 50% H_2SO_4 at room temperature.

[*c] No UV spectral changes were observed in sulfuric acid solution between *Ho* 0 to -9.5.

With regard to the substituent effect of the $\underline{N}$-NH_3^+ group, the finding that the monocationic species of 1-aminouracil and the neutral species of uracil showed superimposable UV spectra (λ_{max} 256 and 259 nm, respectively as shown in Scheme 2) suggests that the electronic perturbation by $\underline{N}$-NH_3^+ toward the aromatic π-electron system is not greater than that by $\underline{N}$-H. Further support for this was obtained by comparing UV spectra of the dicationic species of 1-aminouracil and the monocationic species of uracil (λ_{max} 277 and 276 nm, respectively, as shown in Scheme 2). Thus, electronic perturbation by $\underline{N}$-NH_3^+ is nearly equivalent to that of $\underline{N}$-H toward the π-electron system of both the protonated and neutral nucleus of uracil. The UV spectrum of the anilinium ion is superimposable over that of benzene suggests the equivalence of NH_3^+ and H in perturbing the aromatic π-electron system.

Basicity of the N-Amino Group of N-Aminopyrimidines

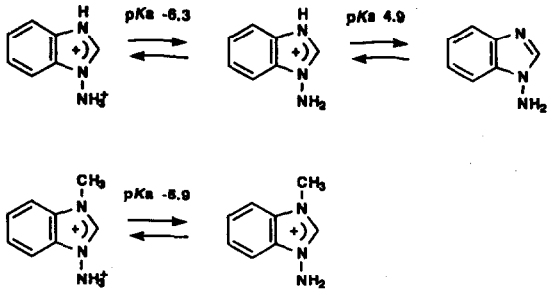
The N-Amino groups of pyrimidine bases were so weakly basic that they were not protonated over the pH region, but only in aqueous H_2SO_4. Therefore, the pKa of the N-NH_3^+ group was estimated from the acidity function (Ho)[6] of the H_2SO_4 solution employed for the measurement, in which the concentration of the protonated N-NH_3^+ species equaled that of the N-NH_2 species. The pKa was thus determined from acidity-dependent UV spectral changes. As seen in Table 1, the pKa's of 1-aminouracil (1a) and 1-aminothymine (1b) are -1.7 and -1.2, respectively. The 5-methyl substituent on the uracil nucleus slightly strengthened the basicity of the N1-NH_2 group. 3-Aminouracil (2a) and 3-amino-1-methyluracil (3a) gave pKa's of $\geq$-0.36 and $\geq$-0.16, respectively, hence the N3-NH_2 group is slightly more basic than the N1-NH_2 group.

With regard to N3-aminocytosines, the first protonation took place at the 4-amino or imino group, and not at the N-NH_2 group. The pKa -1.4 of 3-amino-1-methylcytosine (6a) is similar to those of N1-aminouracils, although protonation at the N3-NH_2 group occurred following protonation of the 4-imino group of 3-amino-1-methylcytosine. The electron-donating effect of the 4-amino group may compensate for the electron-deficient nature of the cationic pyrimidine nucleus (one of the tautomeric structure). This is in sharp contrast to the protonation profile of N-aminobenzimidazoles shown in Scheme 4, where the pKa's of the N-NH_2 group on the cationic imidazole nucleus are found to be around -6.

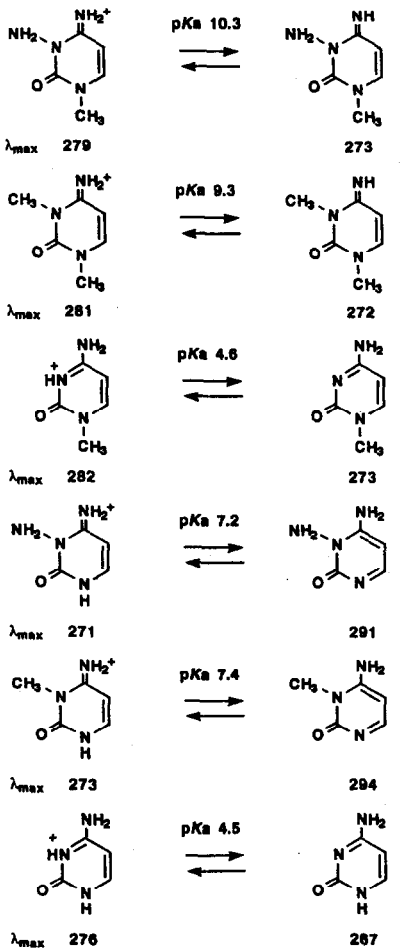
Substituent Effect of the N-Amino Group on Protonation of the Pyrimidine Nucleus

As seen in Table 1 and Scheme 2, deprotonation of the uracil nucleus became slightly easier in the N1-NH_2 derivative (pKa 9.5) than in the N1-CH_3 derivative (pKa 9.7), and easier as well in the N3-NH_2 derivative (pKa 9.3) than in the N3-CH_3 derivative (pKa 10.0). In other words, an NH_2 group is slightly more electron-withdrawing than a CH_3 group. The same tendency is seen in the protonation of N1-substituted cytosine (Scheme 3). That is, protonation of 1-aminocytosine (pKa 4.1) was slightly more difficult than that of 1-methylcytosine (pKa 4.6) or cytosine (pKa 4.5), indicating that the N1-NH_2 group is more electron-withdrawing than the corresponding CH_3 group. On the other hand, 3-amino-1-methylcytosine (pKa 10.3 as shown in Scheme 5) is slightly more basic than 1,3-dimethylcytosine (pKa 9.3), indicating that the N3-NH_2 group made protonation of the 4-imino group easier than did the N3-CH_3 group. This may be due to stabilization of the cationic structure of the pyrimidine ring (one of the tautomeric structure) through an interaction between two amino groups that are in the ortho position to each other in the cationic 3-amino-1-methylcytosine molecule.

With regard to the substituent effect of the NH_3^+ group, as seen in Scheme 2, protonation of the carbonyl oxygen of uracil was much more difficult in the presence of the N1-NH_3^+ group (pKa $\leq$-7.1) compared with N1-methyluracil (pKa -3.5) and uracil (pKa -3.9), suggesting that NH_3^+ is much more electron-withdrawing than the CH_3 and H groups.



Scheme 4. p*Ka* values of *N*-aminobenzimidazoles.



Scheme 5. Comparison of UV spectra and p*Ka* values of 3-amino-cytosines with those of 3-methylcytosines and cytosine.

Tautomeric Structure of N3-Aminocytosines

NMR spectra of the HCl salts of 3-aminocytosine derivatives (6a-c and 7) showed a single peak for 3-amino protons (δ 5.69) and two peaks for C4-imino protons (δ 9.20) and 9.86). This feature was the same as those for 3-methylcytosines. UV spectra of 3-amino and 3-methyl derivatives of 1-methylcytosine were superimposable and pKa values measured from pH-dependent UV spectral changes were similar in magnitude, 10.3 and 9.3, respectively. These data suggest that 3-amino-1-methylcytosine and its protonated forms are the same tautomeric structures as those of 1,3-dimethylcytosine, as shown in Scheme 5. On the other hand, 1-methycytosine which lacks a 3-substituent, has a much smaller pKa value of 4.6 than its 1,3-disubstituted derivative. This might be due to a difference in tautomeric structure between 3-substituted and 3-nonsubstitited 1-methylcytosines, as shown in Scheme 5.

With regard to 3-amino and 3-methylcytosines, the non-protonated structures showed λ_{max} of 291 and 294 nm, respectively, which are much longer than those of 1,3-disubstituted cytosines. It is therefore suggested that the tautomeric structure must be different from those of 1,3-disubstituted derivatives, as shown in Scheme 5. Cytosine itself, which lacks both 1- and 3-substituents, may consistently be formulated as shown in Scheme 5.

Biological Activity of N-Aminopyrimidine Derivatives

Many modified nucleic acid derivatives are known as effective antiviral and anticancer agents. Little is known about the biological activities of the N-amino nucleic acid derivatives. With regard to the antiviral and anticancer effects of N-NH_2 derivatives, it has been reported that N-aminopyrimidine and purine bases have been derivatized to several bioactive nucleoside analogues utilizing the N-NH_2 group as a junction.[10] We are currently assessing antiviral and anticancer effects of our synthesized N-aminopyrimidine deoxynucleosides and bases and have found cytocidal activity in some derivatives. Results on those findings will be published elsewhere. As for biological activities of other derivatives, 1-amino-5-halogenouracil is reported to be a drug which acts on the central nervous system.[9]

EXPERIMENTAL

^1H NMR spectra were recorded with a JEOL GSX 400 or EX 270 spectrometer and chemical shifts are expressed in parts per million down field from internal tetramethylsilane. Mass spectra were determined on a JEOL DX-300 spectrometer. UV spectra were obtained with a Shimadzu UV-2100 spectrophotometer and pKa values were estimated from UV changes reflecting the pH of the medium. Melting points were measured with a Yanagimoto micro-melting point apparatus and are uncorrected.

1-Aminouracil (1a), 3-aminouracil (2a), 1-aminothymine (1b) and 3-aminothymine (2b) HAOS powder (5.7 g, 50 mmol) was added gradually at room temperature with stirring to a solution of uracil (550 mg, 4.9 mmol) that was dissolved in 200 mL of 0.5 N NaOH. After addition

of HAOS over 30 min, the mixture was left standing for another hour. TLC (silica gel plate, $CHCl_3$/MeOH = 9/1) of the reaction mixture showed the formation of 1a (Rf 0.35) and 2a (Rf 0.11) as well as the presence of unreacted starting material (Rf 0.21). The total yield of products 1a and 2a was about 40%. The pH of the reaction mixture was then adjusted to neutral with conc. HCl and the solvent was reduced in volume by evaporation until some salt was observed. MeOH (100 mL) was added to the residues and the precipitated salt was removed by filtration. The mother liquor was reduced again and MeOH was added to remove the salt. These procedures were repeated 2 to 3 times. Finally, the mother liquor was reduced to dryness and the products were extracted from the residue with hot MeOH (20 mL x 5). These products were purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1). Products 1a and 2a were recrystallized from EtOH-ether. Yields of 1a and 2a were 123 mg (20%) and 81 mg (13%), respectively. Compound 1a was also prepared quantitatively by acid hydrolysis of 5 in 1 N HCl at 100°C for 5 h. **1a**: mp 245-247°C(dec) [lit.[9,11] mp 247-248°C, mp 244-245°C]. ^{1}H NMR (DMSO-d_6): δ 11.2 (br s, 1H, 3-H), 7.60 (d, 1H, $J_{5,6}$ = 8.1 Hz, 6-H), 5.46 (br s, 2H, NH_2), 5.39 (d, 1H, 5-H). UV λ_{max}nm (ε): pH 1 and H_2O 267 (8800), pH 12 266 (6700). pKa ≤-7.1, -1.7, 9.5. MS m/z 127(M^+). Anal. Calcd for $C_4H_5N_3O_2$: C, 37.80; H, 3.97; N, 33.06. Found: C, 37.62, H, 3.93; N, 32.90. **2a**: mp 212-214°C [lit.[12] mp 209°C]. ^{1}H NMR (DMSO-d_6): δ 11.3 (br s, 1H, 3-H), 7.39 (d, 1H, $J_{5,6}$ = 7.6 Hz, 6-H), 5.64 (d, 1H, 5-H), 5.40 (br s, 2H, NH_2). UV λ_{max}nm (ε): pH 1 257 (7500), H_2O 257 (7200), pH 12 279 (10000). pKa ≤-6.6, ≥-0.36, 9.3. MS m/z 127(M^+). Anal. Calcd for $C_4H_5N_3O_2$: C, 37.80; H, 3.97; N, 33.06. Found: C, 37.81; H, 3.83; N, 33.60.

For the preparation of 1b and 2b, the procedure employed for the preparation of 1a and 2a was followed. Compounds 1b and 2b were recrystallized from EtOH as white needles in 15% and 12% yields, respectively. **1b**: mp 233-236°C(dec) [lit.[10] mp 234-235°C]. ^{1}H NMR (DMSO-d_6): δ 11.3 (br s, 1H, 3-H), 7.50 (s, 1H, 6-H), 5.41 (br s, 2H, NH_2), 1.74 (s, 3H, CH_3). UV λ_{max}nm (ε): pH 1 and H_2O 272 (10000), pH 12 271 (7600). pKa ≤-7.8, -1.2, 9.8. MS m/z 141(M^+). Anal. Calcd for $C_5H_7N_3O_2$: C, 42.90; H, 5.00; N, 29.77. Found: C, 42.52; H, 4.93; N, 30.19. **2b**: mp 206-207°C(dec) [lit.[10] mp 205-207°C]. ^{1}H NMR (DMSO-d_6): δ 11.0 (br s, 1H, NH), 7.26 (s, 1H, 6-H), 5.41 (br s, 2H, N-NH_2), 1.81 (s, 3H, CH_3). UV λ_{max}nm (ε): pH 1 263 (8900), H_2O 263 (8600), pH 12 271 (7600). MS m/z 141(M^+). Anal. Calcd for $C_5H_7N_3O_2$: C, 42.90; H, 5.00; N, 29.77. Found: C, 42.81; H, 5.03; N, 29.89.

3-Amino-1-methylthymine (3b) 1-Methylthymine[7] (41.6 mg, 0.30 mmol) was dissolved in 5 mL of water containing 0.30 mmol of NaOH. After the solvent was removed by evaporation, the sodium salt of 1-methylthymine thus obtained was dried in vacuo. DNPA (123 mg, 0.62 mmol) in 2 mL DMF was then added. The mixture was left at 37°C for 6 days. TLC (silica gel plate, $CHCl_3$/MeOH = 3/1) of the reaction mixture showed the formation of 3b (Rf 0.64, about 30% yield) and the presence of unreacted starting material (Rf 0.68). After the solvent was removed by evaporation, 10 mL of H_2O were added and

the pH of the solution was adjusted to neutral with diluted HCl. The solution was washed with ether and the product was extracted with $CHCl_3$. The $CHCl_3$ layer was dried with anhydrous magnesium sulfate and the product was purified by alumina column chromatography ($CHCl_3$/MeOH = 9/1). Recrystallization of the product from EtOH produced white needles in a 5.8 mg (12.6%) yield. **3b**: mp 194-197°C. ^{1}H NMR (DMSO-d_6): δ 7.54 (s, 1H, 6-H), 5.49 (s, 2H, NH_2), 3.31 (s, 3H, 1-CH_3), 1.83 (s, 3H, 5-CH_3). UV λ_{max}nm (ε): pH 1 271 (8000), H_2O and pH 12 270 (7700). MS m/z 155(M^+). Anal. Calcd for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.13. Found: C, 46.34; H, 6.14; N, 27.13. Compounds 3a,c were prepared as described previously.[8]

3-Aminodeoxyuridine (3d) and 3-aminothymidine (3e) 2'-Deoxyuridine (228 mg, 1 mmol) and K_2CO_3 (2.76 g, 20 mmol) were dissolved in 45 mL of H_2O and the mixture was kept at 70°C. HAOS powder (1.1 g, 10 mmol) was added gradually to this solution with stirring. After 1h, the reaction mixture was cooled and the pH of the solution was adjusted to neutral with conc. HCl. The yield of 3d estimated by TLC and UV was about 50%. The solvent was reduced to 10 mL by evaporation and after the resulting precipitates were removed by filtration, the mother liquor was evaporated to dryness. The product was extracted several times from the residue with hot MeOH and purified using silica gel ($CHCl_3$/MeOH = 6/1) and Sephadex LH20 (MeOH) column chromatography. Recrystallization of the product from EtOH-ether resulted in white needles in a 30 mg (25.7%) yield. **3d**: mp 165-169°C. ^{1}H NMR (DMSO-d_6): δ 7.88 (d, 1H, $J_{5,6}$ = 8.3 Hz, 6-H), 6.18 (t, 1H, $J_{1',2'}$ = 6.6 Hz, 1'-H), 5.82 (d, 1H, 5-H), 5.46 (br s, 2H, NH_2), 5.30 (br d, 1H, J = 4.0 Hz, 3'-OH), 5.05 (br t, 1H, J = 4.8 Hz, 5'-OH), 4.24 (m, 1H, 3'-H), 3.81 (q, 1H, 4'-H), 3.59 (q, 2H, 5'-H), 2.24-2.06 (m, 2H, 2'-H). UV λ_{max}nm (ε): pH 1 260 (9000), H_2O and pH 12 260 (8800). FAB-MS m/z 244($M + H)^+$. Anal. Calcd for $C_9H_{13}N_3O_5$: C, 44.45; H, 5.39; N, 17.28. Found: C, 44.26; H, 5.29; N,17.26.

For the preparation of 3-aminothymidine (3e), thymidine (242 mg, 1 mmol) was treated with HAOS and purified as described for the preparation of 3d. Recrystallization of the product from EtOH-ether resulted in white crystals in a 52.5 mg (20.4%) yield. **3e**: mp 139-141°C. ^{1}H NMR (DMSO-d_6): δ 7.75 (s, 1H, 6-H), 6.21 (t, 1H, $J_{1',2'}$ = 6.8 Hz, 1'-H), 5.48 (br s, 2H, NH_2), 5.26 (d, 1H, J = 4.3 Hz, 3'-OH), 5.05 (t, 1H, J = 5.1 Hz, 5'-OH), 4.25(m, 1H, 3'-H), 3.79 (m, 1H, 4'-H), 3.60 (m, 2H, 5'-H), 2.14-2.10 (m, 2H, 2'-H), 1.86 (s, 3H, CH_3). UV λ_{max}nm (ε): pH 1 265 (8700), H_2O and pH 12 265 (8300). FAB-MS m/z 258($M + H)^+$. Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.31; H, 5.84; N, 16.07. Amination of the sodium salt of 2'-deoxyuridine or thymidine with DNPA in DMF following a procedure reported previously[8] also yielded 3d or 3e, respectively. Acid hydrolysis of 3d or 3e in 1 N HCl at 100°C for 1 h yielded 2d or 2e quantitatively.

1-Aminocytosine (4) Method 1). 1-Amino-O^4-ethyluracil (5) (14 mg, 0.1 mmol) was dissolved in a small amount of MeOH in a stainless cylinder. After an excess amount of NH_3 was added to the solution which was kept cool with dry ice-acetone, the mixture was heated at 100°C, 50 atmospheres for 20 h. After the reaction, the solvent was removed and the

product obtained was recrystallized from MeOH. Yield 9.4 mg (58 %). Method 2). HAOS powder (5.7 g, 50 mmol) was added gradually at room temperature with stirring to a solution of cytosine (555 mg, 5 mmol) that was dissolved in 200 mL of 0.5 N NaOH. After 3 h, the pH of the reaction mixture was adjusted to neutral with conc. HCl. Then, the crude products were separated using as described for the preparation of 1a and finally purified using both silica gel ($CHCl_3$/MeOH = 7/3) and Sephadex LH20 (H_2O) column chromatography. Recrystallization of the product from H_2O-MeOH yielded 4 in a 25 mg (20%) yield. Under these reaction conditions, a trace amount of 3-aminocytosine was also obtained. **4**: mp 257-260°C. ^{1}H NMR (DMSO-d_6): δ 7.56 (d, 1H, $J_{5,6}$ = 7.1 Hz, 6-H), 6.91 (br s, 2H, 4-NH_2), 5.56 (d, 1H, 5-H), 5.40 (br s, 2H, 1-NH_2). UV λ_{max}nm (ε): pH 1 283 (10400), H_2O and pH 12 274 (7200). p*K*a 4.1. MS *m*/*z* 126(M^+). Anal. Calcd for $C_4H_6N_4O$: C, 38.09; H, 4.80; N, 44.42. Found: C, 37.80; H, 4.75; N, 43.98.

1-Amino-O^4-ethyluracil (5) HAOS powder (351 mg, 3.1 mmol) was added gradually at room temperature with stirring to a solution of O^4-ethyluracil[7] (43.5 mg, 0.31 mmol) that was dissolved in 12 mL of 0.5 N NaOH, and the product obtained was separated by the procedure as described for 1a. Recrystallization of the product from AcOEt gave 5 in an 18 mg (37%) yield. **5**: mp 100-101°C. ^{1}H NMR (DMSO-d_6): δ 7.94 (d, 1H, $J_{5,6}$ = 7.2 Hz, 6-H), 5.85 (d, 1H, 5-H), 5.74 (br s, 2H, NH_2), 4.26 (q, 2H, *J* = 6.9 Hz, CH_2), 1.28 (t, 3H, CH_3). UV λ_{max}nm (ε): pH 1 279 (5700), H_2O and pH 12 276 (5700). p*K*a 0.82. MS *m*/*z* 155(M^+). Anal. Calcd for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.36; H, 5.73; N, 27.37.

3-Aminodeoxycytidine hydrochloride (6c) Deoxycytidine monohydrate (228.2 mg, 0.93 mmol) and DNPA (242.0 mg, 1.2 mmol) were dissolved in a mixture of 3 mL of DMF and 5 mL of MeOH and the solution was kept at 37°C for 4 days. After the reaction, the solvent was removed by evaporation and a small amount of 0.1 N HCl was added. After the insoluble portion was removed by filtration, the mother liquor was washed with ether several times. After the aqueous phase was reduced to a small volume, EtOH was added and cooled resulting in dark yellow plates. Yield 86 mg (33%). **6c**: mp >300°C. ^{1}H NMR (DMSO-d_6): δ 9.86 (br s, 1H, 4-NH_2), 9.20 (br s, 1H, 4-NH_2), 8.19 (d, 1H, $J_{5,6}$ = 7.9 Hz, 6-H), 6.21 (d, 1H, 5-H), 6.10 (t, 1H, $J_{1',2'}$ = 6.3 Hz, 1'-H), 5.69 (br s, 2H, 3-NH_2), 5.36 (d, 1H, *J* = 4.3 Hz, 3'-OH), 5.13 (t, 1H, *J* = 5.0 Hz, 5'-OH), 4.23 (m, 1H, 3'-H), 3.88 (m, 1H, 4'-H), 3.60 (m, 2H, 5'-H), 2.3-2.1 (m, 2H, 2'-H). UV λ_{max}nm (ε): pH 1 and H_2O 275 (11700), pH 12 265.5 (9400). FAB-MS *m*/*z* 243[M(free form) + H]$^+$. Anal. Calcd for $C_9H_{13}N_4O_4$.HCl: C, 38.79; H, 5.43; N, 20.10. Found: C, 38.58; H, 5.41; N, 19.93. Acid hydrolysis of 6c in 1 N HCl at 100°C for 1 h yielded 7 quantitatively. Compounds 6a,b were prepared as reported previously.[8]

3-Aminocytosine hydrochloride (7) Method 1). Compound 6c (30.8 mg, 0.11 mmol) was heated in 20 mL of 1 N HCl at 100°C for 2 h. After the solvent was removed, the residues were recrystallized from EtOH yielding an almost quantitative amount of white crystals. Method 2). A mixture of cytosine (222 mg, 2 mmol) and DNPA (796 mg, 4 mmol) in 600 mL of

DMF was kept at 70°C. After 3 h, TLC showed that the reaction was almost complete. After the solvent was removed, 50 mL of H_2O were added to the residue and the pH of the solution was adjusted to 4 with conc. HCl. Precipitates which appeared were removed by filtration. The mother liquor was washed with AcOEt and evaporated to dryness. The product was purified using both silica gel ($CHCl_3$/MeOH = 7/3) and Sephadex LH20 (H_2O) column chromatography. Recrystallization of the product from EtOH gave 7 in a 41 mg (13%) yield. 7: mp 256-258°C. ^{1}H NMR (DMSO-d_6): δ 12.4 (br s, 1H, NH), 9.86 (br s, 1H, 4-NH_2), 9.05 (br s, 1H, 4-NH_2), 7.73 (d, 1H, $J_{5,6}$ = 7.3 Hz, 6-H), 6.11 (d, 1H, 5-H), 5.63 (s, 2H, 3-NH_2). UV λ_{max}nm (ε): pH 1 and H_2O 271 (9500), pH 12 222(sh) (10000), 291 (12000). p*K*a 7.2. Anal. Calcd for $C_4H_6N_4O$.HCl: C, 29.55; H, 4.34; N, 34.46. Found: C, 29.95; H, 4.44; N, 34.32.

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REFERENCES

1. Huang, G.-F. (Kohda, K.); Okamoto, T.; Maeda, M.; Kawazoe, Y. Tetrahedron Lett. **1973**, 4541-4544.
2. Huang, G.-F. (Kohda, K.); Maeda, M.; Okamoto, T.; Kawazoe, Y. Tetrahedron **1975**, 31, 1363-1367.
3. Kohda, K.; Baba, K.; Kawazoe, Y. Tetrahedron, **1990**, 46, 1531-1540.
4. Kohda, K.; Yasuda, M.; Ukai, H.; Baba, K.; Yamagata, Y.; Kawazoe, Y. Tetrahedron **1989**, 45, 6367-6374.
5. Yamagata, Y.; Tomita, K.; Kohda, K.; Kawazoe, Y. Acta Cryst. **1992**, C48, 318-320.
6. Paul, M.A.; Long, F.A. Chem. Rev. **1957**, 57, 1-45.
7. Synthetic Procedures in Nucleic Acid Chemistry, ed by W.W. Zorbach and R.S. Tipson, Interscience, New York, 1968.
8. Maeda, M.; Kawazoe, Y. Chem. Pharm. Bull. **1975**, 23, 844-852.
9. Imaizumi, M.; Kano, F.; Sakata, S. Chem. Pharm. Bull. **1992**, 40, 1808-1813.
10. Harnden, M.R.; Jarvest, R.L. J. Chem. Soc. Perkin Trans.1 **1991**, 2073-2079.
11. Klotzer, W.; Herberz, M. Monatsch. Chem. **1965**, 96, 1731-1738.
12. Klotzer, W. Monatsch. Chem. **1966**, 97, 1116-1124.