Transformation of 1-methyluracils and uridine to respective, 4-substituted pyrimdin-2(1H)-ones via pyridinium salts

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l-Methyluracil and l-methylthymine are converted into N-(l-methylpyrimidin-2-one-4-yl)- and N-(l,5-dimethylpyrimidin-2-one-4-yl)-pyridinium chlorides, respectively. The pyrimidinyl-pyridinium salts were then reacted with various nucleophiles under very mild conditions to give high yields of the desired 4-substituted pyrimidinones. In a similar manner 2',3',5'-tri-O-acetyluridine is efficiently converted into new 4-substituted pyrimidinone nucleosides.

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On a transformé respectivement le méthyl-1-uracile et la méthyl-1-thymine en chlorure de *N*-(méthyl-1-pyrimidinone-2-yl-4-)-pyrimidinium et en chlorure de *N*-(diméthyl-1,5-pyrimidinone-2-yl-4)-pyrimidinium. Les sels de pyrimidinyl-pyrimidinium, par réaction subséquente avec divers nucléophiles, dans des conditions très douces, donnent avec des rendements élevés, les pyrimidinones substituées en position 4 attendues. D'une façon analogue, on a transformé efficacement la tri-*O*-acétyl-2',3',5'-uridine en des nouveaux nucléosides contenant la pyrimidinone substituée en position 4.

[Traduit par la rédaction]

Introduction

The conversion of 1-alkyluracils and uridine to various 4substituted 2-pyrimidinone derivatives requires activation of the C-4 position and is often achieved with 4-chloro or thio derivatives (1). The C-4 position can also be activated by trimethylsilylation (2) or by treatment with various phosphorylating or condensing agents in the presence of 1-methylimidazole (3), 1,2,4-triazole (4), or 3-nitro-1,2,4-triazole (5) to give appropriate, reactive 4-(3-methyl-imidazolium), 4-(1,2,4-triazol-1-yl), and 4-(3-nitro-1,2,4-triazol-1-yl) derivatives. The latter method was developed in recent studies of oligonucleotide synthesis by Reese and Ubasawa (6) and is now the most frequently used method in the conversion of uridines to cytidines (4, 7, 8). Recently, the synthesis of 4substituted pyrimidine nucleosides involving a displacement of the 4-O-triisopropylphenylsulfonyl group in uridine and thymidine with malonate-type nucleophiles was reported (9)

Adamiak *et al.* showed previously (10, 11) that nucleosides having per-O-acetylated sugar residues, such as uridine, thymidine, guanosine, and inosine, undergo quantitative transformation into the corresponding water-soluble, fluorescent pyrimidin-4-yl and purin-6-yl pyridinium salts, when treated with 4-chlorophenylphosphorodichloridate in the presence of pyridine. The purinyl-pyridinium salts have already proven very useful as intermediates in purine nucleoside chemistry (12, 13). A preliminary study in the case of pyrimidinyl-pyridinium salts has demonstrated that they can serve as intermediates in various side processes during oligonucleotide synthesis using the phosphotriester method, including formation of the 4-triazolyl derivatives mentioned above (11).

In this work the reactivity of pyrimidyn-4-yl pyridinium



salts towards various O-, N-, or S-nucleophiles was tested by using 1-methyl derivatives 2a,b and tri-O-acetyl riboside 2c. Based on the results of these experiments the synthetic procedures yielding new 4-substituted pyrimidin-2(1H)-ones from 1-methyluracils (1a,b) and uridine (1c) have been developed.

Results and discussion

1-Methyluracil (1*a*), 1-methylthymine (1*b*), and 2', 3', 5'tri-O-acetyluridine (1*c*) were converted to the respective N-(pyrimidin-2-one-4-yl)-pyridinium chlorides 2*a*, 2*b*, and 2*c*, respectively, by treatment with 4-chlorophenylphosphorodichloridate in the presence of pyridine (Scheme 1), i.e., under conditions used previously for similar transformations in purine and pyrimidine nucleosides (10, 11). Pure salts were obtained either as concentrated aqueous solutions (2*a* and 2*c*) or as lyophilizates (2*b*). The samples were stable when stored at 0°C for several weeks.

The salts 2a,b were subjected to reaction with imidazole, histidine, 1-methylimidazole, 4,5-diphenylimidazole, in-

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TABLE 1. Reactivity of pyridinium salts 2a-c towards nucleophiles.

	Pyridinium salt	Nucleophile	Solvent ^a	Time (h)	% Conversion of 2	% Product ^b
- 1	2 <i>a</i>	Imidazole	а	22	100	3 <i>a</i> (81)
2	2 b	Imidazole	а	24	96	3 b (93)
3	2 <i>c</i>	Imidazole	а	18	98	3c(82)
4	2 <i>a</i>	Histidine	а	22	66	4 a (45)
5	2 b	Histidine	а	24	38	4b (38)
6	2 <i>a</i>	1-Methylimidazole	а	18	100	5 <i>a</i> (51), 6 <i>a</i> (34)
7	2 b	1-Methylimidazole	а	20	93	5b (75), 6b(15)
8	2 a	3-Chlorophenol	b	1.5	95	7 a (94)
9	2 b	3-Chlorophenol	b	1.5	95	7b (92)
10	2 <i>c</i>	3-Chlorophenol	b	1.5	100	7c (85)
11	2 a	7-Hydroxycoumarin	с	24	80	8 a (69)
12	2 b	7-Hydroxycoumarin	с	24	78	8 b (61)
13	2 <i>a</i>	Mercaptoethanol	а	0.5	100	9 a (72)
14	2 b	Mercaptoethanol	а	0.5	100	9 b (60)
15	2 <i>c</i>	Mercaptoethanol	а	0.5	100	9 c (87)
16	2 <i>a</i>	Sodium azide	d	0.25	100	10 <i>a</i> (90)
17	2 b	Sodium azide	d	0.25	100	10 b (93)
18	2 <i>c</i>	Sodium azide	а	0.25	100	10 c (82)

"Solvents were a: water, b: water/chloroform (1:1, v:v); c: water/dioxane (1:4; v:v); d: dimethylformamide. "Yields of isolated products.

dole, glycine, 3-chlorophenol, 7-hydroxycoumarin, mercaptoethanol, and azide ions. The reactions were carried out at room temperature in water or in a mixture of water and organic solvent (1,4-dioxane or chloroform) at pH ca. 6, i.e., the natural pH of the solutions. In the case of 7-hydroxycoumarin the pH of the reaction medium had to be adjusted to 7.5 in order to make the reaction go to completion (Table 1; entries 11, 12). The extent of the reactions was monitored by measuring the loss of pyridinium salts using a sensitive, spectrophotometric Zincke reaction test (11, 14). Among the nucleophiles listed above, 4,5-diphenylimidazole, indole, and glycine appeared to be unreactive towards 2a,b, whereas all the others reacted with 2a,b to give the appropriate C-4 substituted pyrimidine derivatives 3a,b-10*a*,*b*. The products 3a,b-10a,b were isolated by a combination of extraction and (or) chromatography on silica gel and identified by means of ¹H and ¹³C NMR and mass spectral techniques. The synthetic procedures developed for the preparation of 3a,b-10a,b were then applied to similar transformations of uridine. Thus, the pyridinium salt 2c derived from uridine was treated with some of the above-listed nucleophiles, imidazole, 3-chlorophenol, mercaptoethanol, and sodium azide, to give 4-substituted nucleoside derivatives 2c, 7c, 9c, and 10c, respectively. The results are summarized in Table 1, and the structures of the products are shown in Scheme 2. Some of the products are fluorescent. In these cases the emission parameters (λ_{max} and fluorescence quantum yield) are included in the experimental section.

In the case of the reaction of 2a with imidazole a quantitative conversion into 3a was also achieved with dimethylformamide or 1,4-dioxane as solvents instead of water or a water – organic solvent mixture, indicating that a broad range of solvents can be used in these transformations.

Attempts were also made to introduce the imidazol-l-yl substituent into the C-4 position of uridine as well as the synthesis of 3a, 4a, and 8a according to the method de-

scribed by Matsuda *et al.* (3) as a possible alternative to our method. However, all these attempts were unsuccessful. The HPLC analyses of the reaction mixtures after 3 h revealed almost exclusive presence of the methylimidazolium salts instead of the expected substitution products. Prolongation of the reaction time (18 h) had no effect on the progress of the reactions, except for significant deacetylation in the case of the per-O-acetylated nucleoside salt.

Reactions of 2a, b with histidine (Table 1; entries 4, 5) were less efficient than those with other nucleophiles. They are of interest, however, since they may offer the possibility of selective, chemical modification of histidine residues in proteins.

In the case of the reaction of 2a, b with 1-methylimidazole (Table 1; entries 6, 7) products derived from the imidazole ring opening, 6a,b, were isolated in addition to the expected 1-methylimidazolium salts 5a,b. This observation and the fact that 1-methylimidazolium salts derived from pyrimidine nucleosides were previously identified as potential side products in oligonucleotide synthesis when the 2,4, 6-triisopropylbenzenesulfonyl chloride/1-methylimidazole system was used as condensing reagent (11) prompted us to examine the chemical stability of salts 5a,b in acidic and basic conditions. They appeared to be stable in acidic media (0.05)N HCl, room temperature, 24 h) whereas, at pH > 7 a partial transformation into ring-opened products 6a, b occurred as a result of hydrolytic opening of the imidazolium ring of 5a,b (cf. Scheme 3). In the case of 5a the ring opening reaction occurred to a small extent (4%, HPLC) at pH 7.1 (TRIS buffer, room temperature, 24 h). When more basic conditions (TRIS, pH = 9.0, 24 h) were applied, the HPLC analysis revealed the presence of 5a (19%), 6a (75%), and 1a (6%). The ratio of 6a/1a (14:1) remained unchanged after almost all of 5a disappeared (3 days), indicating that 5a also undergoes a nucleophilic attack of hydroxide ion at C-4 under these conditions to give 1a. 5-Methyl derivative 5b appeared to be less susceptible towards hydrolytic opening of





the imidazolium ring, reflecting its diminished electron deficiency compared with 5*a*. Under analogous conditions of pH and temperature only 49% of 5*b* disappeared, after 24 h, to give 6*b* (27%) and 1*b* (22%). Both 6*a* and 6*b* were stable within the pH range 1 < pH < 9. Thus, one can conclude that formation of imidazolium salts during oligonucleotide synthesis and their further transformation into relatively stable analogs of 6*a*,*b* may result in a point mutation in the synthetic oligomer.

The structures of the compounds 6a,b were unequivocally determined with the aid of ¹H and ¹³C NMR spectrometry. The signal assignments based on the conventional one-dimensional technique (chemical shifts and decoupling) were confirmed by two-dimensional ¹H–¹H COSY spectra. In the case of 6a,b all the resonances except for N-CHO and pyrimidine C6-H and N1-CH₃ are doubled indicating the existence of an amide *cis-trans* equilibrium due to restricted N-C=O bond rotation (15). As could be expected, increasing temperature resulted in significant broadening of these signals. However, coalescence did not occur within the temperature range studied (298–373 K).

The mode of methylimidazolium ring opening, determining the site of attachment of the formyl group to the methylamino nitrogen in 6a,b, was deduced from the following observations. In the long-range ${}^{1}\text{H}{-}^{1}\text{H}$ COSY spectrum of 6a in DMSO the formyl proton signal (δ 8.06 ppm) is coupled with N-CH₃ proton signals (δ 3.26 and 3.10 ppm). Moreover, the N-H signals (δ 9.33 and 8.77 ppm) were correlated with C=C-H proton resonances whereas no correlation with the N-CH₃ signal was observed. The resonance of the N-CH₃ group of the opened imidazolium ring appears as a singlet in ${}^{1}\text{H}$ spectra measured in aprotic solvents (CDCl₃, CD₃CN, DMSO- d_6) in which H/D exchange does not occur. These observations are consistent with the proposed structures of 6a,b.

Aryl groups were proposed by Reese and Skone for protection of uracil on O-4 in oligoribonucleotide synthesis and several 4-O-phenyl derivatives were synthesized via 4-triazolyl derivatives (16). We found that similar derivatives can be obtained via pyrimidinyl-pyridinium salts. Treatment of 2a,b,c with 3-chlorophenol in a chloroform-water two-phase system gave the desired 4-O-(3-chlorophenyl) derivatives 7a,b,c with 94, 92, and 85% yields, respectively.

Mercaptoethanol and azide ion appeared to be the most reactive among all the nucleophiles used. Addition of mercaptoethanol to aqueous solutions of 2a,b, and 2c caused complete loss of pyridinium salts after ca. 30 min, whereas in the case of sodium azide the reaction was completed within 15 min to give, respectively, 4-(2-hydroxythioethyl) derivatives 9a,b,c and 4-azido derivatives 10a,b,c with excellent yields (cf. Table 1; entries 13–18). The 4-(2-hydroxyethylthio) derivative 9c was also obtained directly from O-protected uridine in a one-pot manner with an overall yield 78% (vide infra).

It has been observed previously that 4-azido substituted pyrimidine (3, 17) and 6-azido substituted purine (18) nucleosides tend to form tetrazolo structures in solution. In the purine series the azidoazomethine-tetrazole equilibrium was shown to be influenced by the nature of the solvent (18). In the case of 10a,b,c the infrared spectra in DMSO exhibit the presence of an absorption at ca. 2230 cm⁻¹ due to the azido group while no such absorption could be observed in chloroform solutions. The ¹H and ¹³C NMR spectra, on the other

hand, show the existence of a single tautomeric form in each of these solvents. Thus, one can conclude that in DMSO 10a,b,c exist in the azido form whereas in chloroform the tautomeric tetrazolo form exists, exclusively.

In conclusion, we have demonstrated that pyrimidin-4-yl pyridinium salts are highly reactive towards various nucleophiles to give the appropriate, 4-substituted derivatives. The very mild conditions required for both the activation and substitution steps and the high yields of the products make these compounds attractive intermediates for modification of uracils.

Experimental

Pyridine and dioxane were purified and dried as described previously (10). 1-Methyluracil and 1-methylthymine were synthesized according to the reported procedure (19). Synthesis of 2c was described previously (11). All the other reagents (Merck, Aldrich, and Fluka) were used as received. Thin-layer chromatography was performed on Merck F_{254} and Merck RP silica gel plates. Merck PSC 60- F_{254} silica gel plates were used for preparative separations. Column chromatography was performed on H60, MN 100– 200 mesh, and RP silica gels (Merck) and NM 300 cellulose (Macherey Nagel). The following solvent systems were used for TLC: chloroform/methanol, 30:1 (A); chloroform/methanol, 20:1 (B); chloroform/methanol, 10:1 (C); ethyl acetate (D); ethyl acetate/methanol, 15:1 (E); water (F); ethanol (G); ethanol/30% aqueous acetic acid, 2:1 (H); ethanol/water, 3:1 (I); all v/v.

HPLC separations in the case of the stability studies of 5a,b were performed on a Waters 600E instrument using an ODS column eluted isocratically with a mixture of CH₃CN/H₂O/CH₃COOH, 25:74.9:0.1 (v:v:v). Melting points were taken on a Boetius apparatus and are uncorrected. UV-absorption and fluorescence emission spectra were measured on a Zeiss UV–VIS M-40 spectrophotometer and a Perkin Elmer MPF 44 spectrofluorometer. Fluorescence quantum yields were determined relative to quinine bisulfate ($\phi = 0.55$, 1 N H₂SO₄) (20). Mass spectra were obtained by using a Jeol JMS-D-100 mass spectrometer. A Varian Gemini 300 VT instrument was used to obtain ¹H and ¹³C NMR spectra. The chemical shifts $\delta_{\rm H}$ and $\delta_{\rm C}$ are reported in ppm relative to TMS and dioxane as internal standards. Microanalyses were performed on a Perkin Elmer 2400 CHN analyser.

N-[1-Methylpyrimidin-2(1H)-one-4-yl]pyridinium chloride, 2a and N-[1,5-dimethylpyrimidin-2(1H)-one-4-yl]pyridinium chloride, 2b

A dry sample of 1-methyluracil or 1-methylthymine (3 mmol) was dissolved in anhydrous pyridine (50 mL) and treated with 4chlorophenylphosphorodichloridate (0.73 mL, 4.5 mmol). The mixture was stirred for 18 h. The precipitate formed was filtered, dissolved in ice-cold water, and neutralized to pH ca. 6.2–6.5 with Dowex-1 resin (HCO₃⁻). The resin was filtered, and the filtrate concentrated under vacuum to ca. 10 mL and passed through a Dowex-1 (Cl⁻) column. The eluates were concentrated (<30°C) to give ca. 0.1 M aqueous solutions of 2a and 2b. In the case of the more stable 2b the solution was lyophilized to give a yellow solid (86% yield). Some selected spectral data for 2a,b are presented below.

2*a*: UV (H₂O) λ_{max} : 268 nm, 310 nm; ¹H NMR (D₂O) δ : 9.53 (m, 2, pyridine C α -H), 8.91 (m, 1, pyridine C γ -H), 8.73 (d, *J* = 6.9 Hz, 1, C6-H), 8.34 (m, 2, pyridine C β -H), 7.34 (d, *J* = 6.9 Hz 1, C5-H), 4.68 (s, 3, N-CH₃); ¹³C NMR δ : 163.24, 157.61, 151.64, 142.60, 129.21, 98.82, 40.31.

2b: UV (H₂O) λ_{max} : 263 nm (ε 5300), 334 nm (ε 5000); ¹H NMR (D₂O) δ: 9.18 (m, 2, pyridine Cα-H), 8.93 (m, 1, pyridine Cγ-H), 8.61 (s, 1, C6-H), 8.36 (m, 2, pyridine Cβ-H), 4.75 (s, 3, N-CH₃), 2.13 (s, 3, C-CH₃); ¹³C NMR δ chemical shifts were identical with those reported previously (21).

General procedure for the syntheses of 3-10

Water was used as a solvent unless otherwise specified. 0.1 M aqueous solutions of 2a,b or 2c (aliquots corresponding to 1–5 mmol samples of the salts) were treated with the appropriate nucleophile (3 equiv.) and the reaction mixture kept at room temperature until almost complete loss of pyridinium salt was detected by the Zincke test (14). The reaction times and yields are summarized in Table 1. Isolation procedures and spectral data for the products are presented below.

1-Methyl-4-(imidazol-1-yl)pyrimidin-2(1H)-one, 3a

(Table 1; entry 1)

Water was removed under reduced pressure. The solid residue was chromatographed twice on SiO₂ (systems D and A) and the product crystallized from isopropanol: mp > 260°C; UV (H₂O) λ_{max} : 304 nm (ϵ 9400), 239 nm (ϵ 8800); fluorescence emission λ_{max} : 363 nm (ϕ = 0.002); ¹H NMR (TFA-*d*/acetone-*d*₆) δ : 9.66 (m, 1, imidazole C2-H), 8.55 (d, *J* = 7.1 Hz, 1, C6-H), 8.27, 7.72 (2m, 2, imidazole C4-H and C5-H), 7.30 (d, *J* = 7.1 Hz, 1, C5-H), 3.91 (s, 3, N-CH₃); ¹³C NMR (TFA-*d*/acetone-*d*₆) δ : 160.15, 159.31, 150.67, 136.48, 123.53, 120.93, 97.68, 41.12; MS *m/z* (relative intensity): M⁺ 176(88), 175(100), 149(10), 134(18), 107(9). Anal. calcd. for C₈H₈N₄O: C54.53, H 4.58, N 31.80; found: C 54.47, H 4.54, N 31.84.

1,5-Dimethyl-4-(imidazol-1-yl)pyrimidin-2(1H)-one, 3b (Table 1; entry 2)

The aqueous solution was extracted with chloroform. The organic layer was dried and the solvent evaporated. The crude product was chromatographed (SiO₂, system D) and then recrystallized from isopropanol; mp 151–153°C (dec.); UV (H₂O) λ_{max} : 316 nm (ϵ 8700), 238 nm (ϵ 7000); fluorescence emission λ_{max} : 376 nm (ϕ = 0.002); ¹H NMR (CDCl₃) δ : 8.27 (s, 1, imidazole C2-H), 7.73 (s, 1, C6-H), 7.65 and 7.16 (2m, 2, imidazole C4-H and C5-H), 3.60 (s, 3, N-CH₃), 2.31 (s, 1, C5-CH₃); ¹³C NMR (CDCl₃) δ : 159.34, 155.49, 151.05, 137.02, 130.41, 118.16, 104.35, 38.46; MS *m/z* (relative intensity): M⁺ 190(69), 189(100), 163(6), 148(8), 140(6). Anal. calcd. for C₆H₁₀N₄O: C 56.83, H 5.30, N 29.45; found: C 56.67, H 5.27, N 29.32.

1-(2',3',5'-Tri-O-acetyl-β-D-ribofuranosyl)-4-(imidazol-1-yl)pyrimidin-2-(1H)-one, 3c (Table 1; entry 3)

The aqueous solution was extracted with chloroform. The organic layer was dried and then concentrated under reduced pressure. The residue was chromatographed on SiO₂ (system D). Fractions containing pure product were collected and evaporated under vacuum to give **3***c* as a foam. UV (H₂O) λ_{max} : 305 nm (ϵ 7800), 242 nm (ϵ 8700); fluorescence emission λ_{max} : 374 nm (ϕ = 0.002); ¹H NMR (CDCl₃) δ : 8.41 (br s, 1, imidazole C2-H), 8.13 (d, *J* = 7.3 Hz, 1, C6-H), 7.70 and 7.19 (2s, 2, imidazole C4, C5-H), 6.58 (d, *J* = 7.3 Hz, 1, C5-H), 6.14 (d, 1, Cl'-H), 5.51–5.26 (m, 2, sugar C-H), 4.53–4.42 (m, 3, sugar C-H), 2.15, 2.13 and 2.12 (3s, 9, COCH₃); ¹³C NMR (CDCl₃) δ : 170.12, 169.53, 158.96, 154.41, 145.57, 131.82, 128.35, 116.32, 94.60, 89.72, 80.35, 73.85, 70.00, 62.85, 20.75, 20.42; high resolution MS, *m*/*z*: M⁺ 420.12781 (C₁₈H₂₀N₄O₈ requires 420.12796 amu).

Reaction of 2a with histidine (Table 1; entry 4)

The reaction mixture was concentrated and chromatographed on a reversed phase silica gel column (system F). The fractions containing 4*a* were collected, the solvent evaporated to dryness, and the solid residue recrystallized from a mixture of isopropanol-water: mp 233-240°C (dec.); UV (H₂O) λ_{max} : 305 nm (ϵ 8700), 238 nm (sh); fluorescence emission λ_{max} : 363 nm (ϕ = 0.0007); ¹H NMR (D₂O) δ : 7.80 (br s, 1, histidine C2-H), 7.23 (d, *J* = 7.3 Hz, 1, C6-H), 6.93 (br s, 1, histidine C5-H), 5.78 (d, *J* = 7.3 Hz, 1, C5-H), 3.57 (m, 1, CH-CO), 3.19 (s, 3, N-CH₃), 3.07 (m, 2, CH₂); ¹³C NMR (D₂O) δ : 174.99, 164.98, 159.94, 154.03, 137.07, 135.88, 118.60, 97.05, 55.69, 38.40, 29.52. Anal. calcd. for C₁₁H₁₃N₅O₃: C 50.18, H 4.97, N 26.60; found: C 49.87, H 4.85, N 26.33.

*Reaction of 2*b with histidine (Table 1; entry 5)

A work-up utilizing the same procedure as for 2a (see above) gave a crystalline sample of 4b: mp 194–197°C; UV (H₂O) λ_{max} : 318 nm (ϵ 8800), 240 nm (sh); fluorescence emission λ_{max} : 376 nm ($\phi = 0.0007$); ¹H NMR (D₂O) δ : 8.22 (s, 1, histidine C2-H), 8.15 (s, 1, C6-H), 7.55 (s, 1, histidine C5-H), 4.00 (m, 1, CH-CO), 3.55 (s, 3, N-CH₃), 3.12 (m, 2, CH₂), 2.21 (s, 3, C-CH₃); ¹³C NMR (D₂O) δ : 174.18, 159.99, 158.04, 154.68, 138.86, 137.40, 117.95, 108.46, 55.37, 39.44, 29.42, 15.38. Anal. calcd. for C₁₂H₁₅N₅O₃: C 51.98, H 5.45, N 25.25; found: C 51.67, H 5.41, N 25.11.

Reaction of pyridinium salt 2a with 1-methylimidazole

(Table 1; entry б)

The reaction mixture was extracted with CHCl₃. The organic layer was dried, concentrated under vacuum, and chromatographed on an SiO₂ column (B) to yield a pure sample of 6a: mp 200°C; UV (H₂O) λ_{max} : 301 nm (ϵ 20 700), 225 nm (sh); ¹H NMR $(CDCl_3/CD_3OD, 5:1) \delta: 8.09 (s, 1, CHO), 7.55 (d, J = 7 Hz, 1, 1)$ C6-H), 7.15 and 6.88 (2m, total 1, C=CH), 6.08 and 5.94 (2d, J = 7.1 and 7.3 Hz, total 1, C5-H), 5.50 and 5.26 (2d, J = 7.1 and 6.6 Hz, total 1, C=CHO), 3.44 (s, 3, N-CH₃), 3.19 and 3.03 (2s, total 3H, cis and trans CO-N-CH₃); ¹³C NMR (CDCl₃/CD₃OD) δ: 164.16 and 162.48 (aldelhyde C=O), 162.11 and 160.96 (C4), 157.82 (C=O), 147.09 (C6), 121.36 and 116.38 (C=C), 110.74 and 109.38 (C=C), 95.51 (C5), 37.87 (N-CH₃), 37.49 and 31.89 (N-CH₃); MS m/z (relative intensity): M⁺ 208(37), 180(18), 164(25), 151(15), 150(100), 144(20), 138(29), 136(12), 126(6). Anal. calcd. for C₉H₁₂N₄O₂: C 51.91, H 5.80, N 26.90; found: 51.84, H 5.79, N 26.85.

The aqueous layer remaining after extraction with CHCl₃ was concentrated and chromatographed on cellulose (systems G, I). The fractions containing imidazolium salt **5***a* were concentrated to remove ethanol and then passed through a Dowex (Cl⁻) column. The aqueous eluate was treated with charcoal and evaporated to dryness. The sample of **5***a* was stored at 5°C and was stable for over 2 weeks: UV (H₂O) λ_{max} : 313 nm (ϵ 4800), 242 nm (sh); fluorescence emission λ_{max} : 371 nm (ϕ = 0.14); ¹H NMR (D₂O) δ : 9.56 (s, 1, imidazole C2-H), 8.32 (d, *J* = 7.3 Hz, 1, C6-H), 8.04 and 7.53 (2d, 2, imidazole C4-H and C5-H), 6.90 (d, *J* = 7.3 Hz, 1, C5-H), 3.88 (s, 3, N-CH₃), 3.48 (s, 3, N-CH₃); ¹³C NMR (D₂O) δ : 158.20 (C4), 156.25 (imidazole C3), 137.02 (C2), 126.02 (C6), 120.60 (imidazole C4 and C5), 96.71 (C5), 40.10 (N-CH₃), 37.60 (N-CH₃). Anal. calcd. for C₉H₁₁N₄OClxH₂O: C 44.17, H 5.35, N 22.89; found: C 41.10, H 5.27, N 22.71.

Reaction of pyridinium salt 2b with 1-methylimidazole (Table 1; entry 7)

Extraction of the reaction mixture with chloroform and column chromatography of the organic layer over SiO₂ (C) gave **6***b*: mp 192°C; UV λ_{max} (H₂O): 308 nm (ϵ 18 700), 224 nm (sh); ¹H NMR (CDCl₃/CD₃OD 5:1) δ : 8.10 (s, 1, CHO), 7.41 (d, J = 1 Hz, 1, C6-H), 6.84 and 6.98 (2d, J = 7.5 and J = 7.2 Hz, total 1, C=CH), 5.20 and 5.70 (dd, J = 5.7 Hz and J = 1 Hz; and d, J = 7.2 Hz, total 1, C=CH), 3.41 (s, 3, N-CH₃), 3.22 and 3.09 (2s, total 3, CO-N-CH₃), 1.99 and 2.05 (2d, total 3, C-CH₃); ¹³C NMR (CDCl₃/CD₃OD 5:1) δ : 161.99, 159.9, 157.66, 144.33, 115.62, 109.60, 103.05, 38.02, 37.54, 12.40; MS m/z (relative intensity): M⁺ 222(27), 194(19), 178(16), 164(100), 163(25), 152(21), 150(11), 140(10). Anal. calcd. for C₁₀H₁₄N₄O₂: C 54.04, H 6.34, N 25.10; found: C 53.97, H 6.26, N 25.02.

The aqueous layer obtained after extraction with CHCl₃ was concentrated under vacuum and chromatographed over cellulose (systems G, I). Fractions containing *5b* were concentrated and passed through a Dowex (Cl⁻) column. The eluate was evaporated to dryness under vacuum to give *5b* as a white solid: UV (H₂O) λ_{max} : 324 nm (ϵ 4700); fluorescence emission λ_{max} : 387 nm (ϕ = 0.104); ¹H NMR (D₂O) δ : 9.27 (s, 1, imidazole C2-H), 8.24 (s, 1, C6-H), 7.86 and 7.51 (2m, 2, imidazole C4-H and C5-H), 3.87 (s, 3, N-CH₃), 3.48 (s, 3, N-CH₃), 2.11 (s, 3, C-CH₃); ¹³C NMR (D₂O) δ : 156.58, 144.93, 137.81, 125.10, 122.33, 108.90, 39.87, 37.43,

14.30. Anal. calcd. for $C_{10}H_{13}N_4OClxH_2O$: C 46.42, H 5.84, N 21.65; found: C 46.27, H 5.83, N 21.49.

1-Methyl-4-(3-chlorophenoxy)pyrimidin-2(1H)-one, 7a (Table 1; entry 8)

3-Chlorophenol (0.933 g, 7.26 mmol) and triethylamine (0.35 mL, 2.5 mmol) were added to a two-phase system composed of an aqueous solution of pyridinium salt 2a (20 mL, 2.42 mmol) and chloroform (20 mL). The mixture was stirred vigorously for 2 h in the absence of light. The chloroform layer was separated and fractionated on a silica gel column (system E) to give 7*a*: mp 146–148°C; UV (H₂O) λ_{max} : 280 nm (ϵ 7800); ¹H NMR (CDCl₃) δ : 7.61 (d, J = 7.1 Hz, 1, C6-H), 7.15 (m, 4, aromatic H), 6.04 (d, J = 7.1 Hz, 1, C5-H), 3.48 (s, 3, N-CH₃); ¹³C NMR (CDCl₃) δ : 171.04, 156.31, 152.29, 149.37, 134.80, 130.30, 126.13, 122.44, 120.28, 94.81, 38.08; high resolution MS *m/z*: 236.03518 (C₁₁H₉N₂O₂Cl requires 236.03519 amu). Anal. calcd. for C₁₁H₉N₂O₂Cl: C 55.82, H 3.83, N 11.84; found: C 55.80, H 3.85, N 11.63.

1,4-Dimethyl-4-(3-chlorophenoxy)pyrimidin-2(1 H)-one, 7b (Table 1; entry 9)

This compound was obtained according to the procedure described for 7*a*. Fractions from a silica-gel column (system E) were collected and evaporated to dryness. The residue was dissolved in methanol and decolorized with charcoal. Crystallization from ethyl acetate gave 7*b* as white needles: mp 211°C; UV (H₂O) λ_{max} : 289 nm (ϵ 7300); ¹H NMR (DMSO-*d*₆/CDCl₃) δ : 7.99 (d, J = 1.0 Hz, 1, C6-H), 7.30 (m, 4, aromatic H), 3.34 (s, 3, N-CH₃), 2.03 (d, J = 1.0 Hz, 3, C-CH₃); ¹³C NMR (DMSO-*d*₆/CDCl₃) δ : 169.31, 155.22, 152.57, 148.61, 133.28, 130.62, 125.53, 122.39, 120.87, 101.96, 36.89, 11.43; high resolution MS, *m/z*: 250.05066 (C₁₂H₁₁N₂O₂Cl requires 250.05083 amu). Anal. calcd. for C₁₂H₁₁N₂O₂Cl: C 57.49, H 4.42, H 11.18; found: C 57.46, H 4.42, N 11.21.

l-(2',3'-5'-Tri-O-acetyl-β-D-ribofuranosyl)-4-(3-chlorophenoxy)pyrimidin-2(1 H)-one, 7c (Table 1; entry 10)

The pyridinium salt 2*c* was reacted with 3-chlorophenol under conditions analogous to those used for synthesis of 7*a*. The chloroform layer was separated and chromatographed on SiO₂ (CHCl₃ and system A). Appropriate fractions were collected and the solvent evaporated to give 7*c* as an oil. UV (H₂O) λ_{max} : 278 nm (ϵ 7700); ¹H NMR (CDCl₃) δ : 7.91 (d, J = 7.4 Hz, 1, C6-H), 7.20 (m, 4, aromatic H), 6.18 (d, J = 7.4 Hz, 1, C5-H), 6.11 (d, 1, C1'-H), 5.39, 5.32, and 4.38 (3m, 5, sugar C-H), 2.15, 2.14, and 2.10 (3s, 9, COCH₃); ¹³C NMR (CDCl₃) δ : 170.88. 169.94, 169.37, 154.85, 151.51, 143.58, 134.52, 130.09, 126.18, 122.07, 120.00, 96.00, 89.00, 79.74, 73.49, 69.68, 62.75, 20.76, 20.46. High resolution MS *m*/*z*: M⁺ 480.09340 (C₂₁H₂₁N₂O₉Cl requires 480.09313 amu).

1-Methyl-4-(coumarin-7-oxy)pyrimidin-2(1H)-one, 8a (Table 1; entry 11)

The reaction of 2*a* with 7-hydroxycoumarin was carried out in a mixture of dioxane/water 4:1 (v/v) at pH 7.5 adjusted with NaHCO₃. Crystals of pure 8*a*, precipitated from the reaction mixture, were filtered: mp 161°C; UV (CH₃OH) λ_{max} : 305 nm (ϵ 13 100, sh), 285 nm (ϵ 16 500); fluorescence emission λ_{max} : 378 nm (ϕ = 0.002); ¹H NMR (TFA-*d*/acetone-*d*₆) δ : 8.67 (d, *J* 7.6 Hz, 1, C6-H), 8.15 (d, *J* = 9.4 Hz, 1, coumarin C3-H), 7.9 (m, 1, aromatic H), 7.42 (m, 2, aromatic H), 6.75 (d, *J* = 9.4 Hz, 1, coumarin C4-H), 6.45 (d, *J* = 7.6 Hz, 1, C5-H), 3.86 (s, 3, N-CH₃); ¹³C NMR (TFA-*d*/acetone-*d*₆) δ : 173.36, 166.06, 161.13, 156.14, 154.19, 151.27, 146.93, 132.90, 121.36, 119.63, 118.05, 111.56, 94.97, 40.41; MS *m*/*z* (relative intensity): M⁺ 270(100), 200(8), 188(19), 187(97), 159(43), 149(8), 109(6). Anal. calcd. for C₁₄H₁₀N₂O₄: C 62.22, H 3.72, N 10.37; found: C 62.07, H 3.69, N 10.18.

1,5-Dimethyl-4-(coumarin-7-oxy)pyrimidin-2(1H)one, 8b (Table 1; entry 12)

The solvent and pH were the same as for synthesis of **8***a*. The reaction mixture was concentrated under vacuum, and the crystalline product was filtered off and separated from unreacted 7-hydroxycoumarin by chromatography on SiO₂ plates (system E): mp 238°C; UV (CH₃OH) λ_{max} : 307 nm (ϵ 12 700), 288 nm (ϵ 12 600); fluorescence emission λ_{max} 376 nm (ϕ = 0.001); ¹H NMR (TFA-*d*/ acetone-*d*₆) δ : 8.34 (s, 1, C6-H), 8.13 (d, *J* = 9.5 Hz, 1, coumarin C3-H), 7.86 (m, 1 aromatic H), 7.38 (m, 2, aromatic H), 6.71 (d, *J* = 9.5 Hz, 1, coumarin C4-H), 3.89 (s, 3, N-CH₃), 2.40 (s, 3, C-CH₃); ¹³C NMR (TFA-*d*/acetone-*d*₆) δ : 171.93, 166.45, 157.38, 155.94, 155.03, 153.66, 147.19, 132.05, 120.56, 117.30, 114.29, 112.07, 40.53, 11.94; MS *m*/*z* (relative intensity): (M⁺ + 1) 285(18), M⁺ 284(100), 270(6), 214(7), 187(19), 159(15), 149(10), 146(7), 134(9). Anal. calcd. for C₁₅H₁₂N₂O₄: C 63.37, H 4.25, N 9.85. Found: C 63.14, H 4.21, N 9.73.

l-Methyl-4-(2-hydroxyethylthio)pyrimidin-2(1H)one, **9**a (*Table 1; entry 13*)

The reaction mixture was extracted with CHCl₃. The extract was chromatographed on silica gel plates (system E). Product **9***a* was obtained as an oil that solidified upon addition of ethyl acetate: mp 70–72°C; UV (H₂O) λ_{max} : 301 nm (ϵ 20 700), 225 nm (sh); ¹H NMR (CDCl₃/CH₃OD) δ : 7.46 (d, J = 6.9 Hz, 1, C6-H), 6.30 (d, J = 6.9 Hz, 1, C5-H), 3.70 (t, J = 6.5 Hz, 2, CH₂), 3.49 (s, 3, N-CH₃), 3.30 (t, J = 6.5 Hz, 2, CH₂); ¹³C NMR (CDCl₃/CH₃OD) δ : 177.87, 156.04, 145.47, 104.46, 61.11, 32.35, 32.02. MS *m/z* (relative intensity): M⁺ (186(1), 166(4), 156(18), 142(45), 141(35), 127(12), 126(100). Anal. calcd. for C₇H₁₀N₂O₂S: C 45.14, H 5.41, N 15.04; found: C 45.03, H 5.37, N 14.94.

1,5-Dimethyl-4-(2-hydroxyethylthio)pyrimidin-2(1H)one, 9b (Table 1; entry 14)

The reaction mixture was extracted with CHCl₃ and the organic layer was dried and concentrated under reduced pressure. The residue was separated on an SiO₂ column (system E): mp 110°C; UV (H₂O) λ_{max} : 308 nm (ϵ 18 700), 224 nm (sh); ¹H NMR (CDCl₃/CD₃OD) δ : 7.29 (d, J = 0.8 Hz, 1, C6-H), 3.76 (t, 2, CH₂), 3.48 (s, 3, N-CH₃), 3.33 (t, 2, CH₂), 2.04 (d, J = 0.8 Hz, 3, C-CH₃); ¹³C NMR (CDCl₃/CD₃OD) δ : 178.19, 155.92, 143.30, 113.07, 61.11, 38.08, 32.02, 13.81; MS *m*/*z* (relative intensity): M⁺ 200(5), 183(5), 170(13), 156(100), 155(64), 140(11). Anal. calcd. for C₈H₁₂N₂O₂S: C 47.98, H 6.04, N 13.99; found: C 47.76, H 5.98, N 13.87.

I-(2',3',5'-*Tri*-O-acetyl-β-*D*-ribofuranosyl)-4-(2-hydroxyethylthio)-pyrimidin-2(1 H)one, 9c (Table 1; entry 15)

The product was isolated as described above. System E was used for chromatography. UV (H₂O) λ_{max} : 301 nm (ϵ 19 600); ¹H NMR CDCl₃) δ : 7.64 (d, J = 7.0 Hz, 1, C6-H), 6.44 (d, J = 7.0 Hz, 1, C5-H), 6.19 (d, 1, C1'-H), 5.42, 5.35, and 4.38 (3m, 5, sugar C-H), 3.73 (t, J = 6.5 Hz, 2, CH₂), 3.30 (t, J = 6.5 Hz, 2, CH₂), 2.16, 2.14, and 2.10 (3s, 9, COCH₃); ¹³C NMR δ : 177.56, 169.96, 169.59, 169.50, 156.82, 146.12, 103.81, 88.89, 80.06, 73.36, 69.88, 62.77, 61.13, 32.47, 20.67, 20.36, 20.23; high resolution MS, m/z: M⁺ 430.10427 (C₁₇H₂₂N₂O₉S requires 430.10444 amu).

One-pot synthesis of 9c

The solution of 2', 3', 5'-tri-*O*-acetyluridine (1 mmol) in dry pyridine (10 mL) was treated with 4-chlorophenylphosphorodichloridate (1.5 mmol) and left overnight at room temperature followed by addition of mercaptoethanol (7 mmol). The Zincke test indicated 9% conversion of the pyridinium salt after ca. 1 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in aqueous sodium bicarbonate and then extracted with chloroform. Chromatography on SiO₂ (system E) gave 7*c* in 78% overall yield.

I-Methyl-4-azidopyrimidin-2(1H)-one, 10a (Table 1; entry 16)

The aqueous solution containing 2 mmol of 2a was evaporated (<30°C) to dryness. The solid residue was dissolved in dry di-

methylformamide (20 mL) and sodium azide (0.195 g, 3 mmol) was added. The resulting mixture was stirred for 15 min and then the solvent was evaporated under vacuum at 35°C. The residue was treated with water (30 mL) and the aqueous solution extracted with chloroform. The organic layer was dried over Na₂SO₄, concentrated, and treated with hexane to give crystals of **10***a*: mp 206–208°C; UV (H₂O) λ_{max} : 270 nm (ϵ 8500, sh), 258 nm (ϵ 10 000); ¹H NMR (DMSO-*d*₆) δ : 7.73 (d, *J* = 7.3 Hz, 1, C6-H), 6.94 (d, 1, C5-H), 3.73 (s, 3, N-CH₃); ¹³C NMR (DMSO-*d*₆) δ : 151.42, 143.62, 141.52, 92.26, 36.62; high resolution MS, *m/z*: 151.04932 (C₅H₅N₅O requires 151.04936 amu). Anal. calcd. for C₅H₅N₅O: C 39.73, H 3.33, N 46.34; found: C 39.60, H 3.28, N 46.49.

1,5-Dimethyl-4-azidopyrimidin-2(1H)-one, 10b

(Table 1; entry 17)

This compound was obtained according to the procedure described for **10***a*. The crude product was chromatographed on an SiO₂ column (system B): mp 178–180°C; UV (H₂O) λ_{max} : 273 nm (ϵ 7500, sh), 257 nm (ϵ 9500); ¹H NMR (DMSO-*d*₆) δ : 7.76 (d, *J* = 1.2 Hz, 1, C6-H), 3.56 (s, 3, N-CH₃), 2.28 (d, *J* = 1.2 Hz, 3, C-CH₃); ¹³C NMR (DMSO-*d*₆) δ : 152.19, 143.30, 138.05, 101.64, 36.46, 12.19; high resolution MS, *m/z*: 165.06494 (C₆H₇N₅O requires 165.06498 amu). Anal. calcd. for C₆H₇N₅O: C 43.63, H 4.27, N 42.40; found: C 43.41, H 4.25, N 42.27.

I-(2',3',5'-Tri-O-acety*I*-β-*D*-ribofuranosy*I*)-4-azidopyrimidin-2(*I* H)-one, *I0*c (Table 1; entry 18)

The aqueous solution of 2c was treated with sodium azide. Pure **10***c*, which precipitated from the reaction mixture, was filtered off. An additional amount of the product was isolated from the filtrate by extraction (CHCl₃) and chromatography on SiO₂ (system D). UV (H₂O/CH₃OH, 9:1) λ_{max} : 265 nm (ϵ 7000, sh), 251 nm (ϵ 7600); ¹H NMR (CDCl₃) δ : 7.71 (d, *J* = 7.8 Hz, 1, C6-H), 6.98 (d, *J* = 7.8 Hz, 1, C5-H), 6.29 (d, 1 Cl'-H), 5.45, 5.39, and 4.39 (3m, 5, sugar C-H), 2.17, 2.14, and 2.09 (3s, 9, COCH₃); ¹³C NMR (CDCl₃) δ : 169.99, 169.59, 169.50, 150.53, 142.58, 133.85, 94.70, 88.78, 80.69, 73.24, 69.95, 62.75, 20.68, 20.37, and 20.23; high resolution MS, *m/z*: M⁺ 395.10757 (C₁₅H₁₇N₅O₈ requires 395.10749 amu).

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