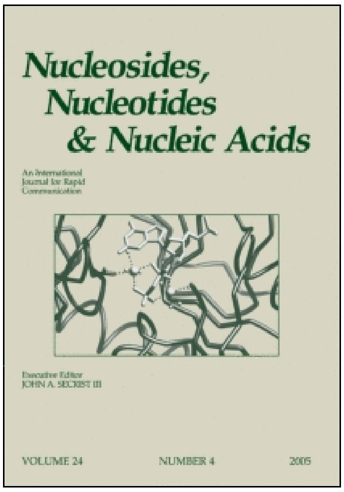

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SYNTHESIS OF N-AMINOPYRAZINIUM ANALOGS OF CYTIDINE AND 2'-DEOXYCYTIDINE

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Abstract

N-Aminopyrazine analogues of cytidine and 2'-deoxycytidine were prepared from 1-(β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine and 1-(2-deoxy-β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine, respectively, by amination with O-mesitylenesulfonylhydroxylamine.

Introduction

Bioisosteric pyrazine analogs of uridine[1], 2'-deoxyuridine[2], thymidine[3], and inosine[4] have previously been synthesized and shown to exhibit growth inhibitory activity in various microbial and tumor cell systems. It, therefore, appeared of interest to extend the replacement of the pyrimidine with the pyrazine ring to include pyrazine analogs of cytidine and 2'-deoxycytidine. An important characteristic of such pyrazine analogs might be resistance to enzymatic deamination as a result of the substitution of an N-amino group for the amidine amino group. A N-aminopyrazinium analog of cytidine has been prepared[5] and shown to be resistant to the action of cytidine deaminase. However, because of the presence of a functional group at the position corresponding to the N-3 of cytidine, which may have interfered with binding of the analog to the enzyme, this finding was considered inconclusive and the need for a closer structural analogue of the pyrimidine moiety was suggested.

Chemistry

N-Amino heterocyclic compounds have been prepared by amination[5-8] of the parent heterocycles with several reagents. Among them, O-mesitylenesulfonylhydroxylamine[8] (MSHA) appears to be a general and convenient aminating reagent. The reaction of MSHA with 1,2-dihydro-1-methyl-2-oxopyrazine (1) afforded the mesitylate salt of 4-amino-1,2-dihydro-1-methyl-2-oxopyrazine (2) in 48% yield (Scheme 1). Similarly, amination of 1-(β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine (3) gave the N-amino derivative 4 in 50% yield.

For the synthesis of the 2'-deoxycytidine analogs (Scheme 2), 2-(trimethylsilyloxy)pyrazine (5), prepared by silylation of the sodium salt of 1,2-dihydro-2-oxopyrazine, was reacted with 3,5-di-O-p-chlorobenzoyl-2-deoxy-α-D-ribofuranosyl chloride in the presence of molecular sieves and $TiCl_4$ to give a mixture of anomers 6a and 7a in 86% yield. After separation of 6a and 7a by fractional crystallization from toluene and toluene-petroleum ether, the protecting groups were removed by treatment with methanolic ammonia to give nucleosides 6b and 7b. The anomeric configuration was assigned to 6b and 7b on the basis of the splitting patterns[9] of the anomeric protons signals in the NMR spectra. Thus, the signal of H-1' appeared as a characteristic triplet for the β-anomer (6b) while that for the α-anomer (7b) appeared as a quartet. Amination of 6b in methylene chloride-methanol gave N-amino nucleoside 8 in 46% yield while similar amination of the α-anomer 7b afforded 9 in 61% yield

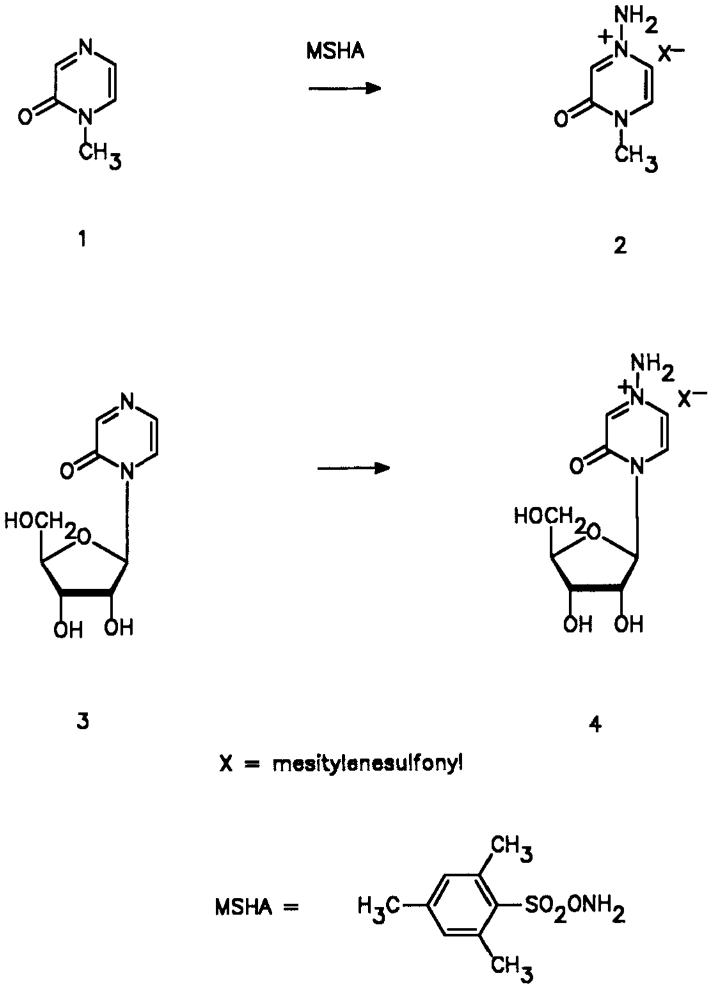
Amino nucleosides 4, 8 and 9 were found to be very hygroscopic but they appeared to be stable in aqueous solution. The UV spectrum and pH value (3.9) of an aqueous solution of 8 showed no change when stored at room temperature for three days. Because quarternary N-aminated heterocycles have been shown unstable[5,10] in weak base, we did not attempt to free the nucleosides from their salts. Alternatively, we attempted to selectively reduce the C=N bond of the pyrazine nucleoside 9 . Followed by amination, the resulting N-amino tetrahydropyrazine derivative might be expected to be more stable in the free form. However, reduction of 9 with reagents including sodium hydrosufite[11], sodium borohydride[12], sodium cyanoborohydride[13], catalytic hydrogenation[14] and lithium-chlorotrimethylsilane[15] led to complex mixtures of products.

Compounds 4, 8,and 9 showed no in vitro antitumor cytotoxicity (L1210) or antiviral (Herpes simplex I and II) and antibacterial (S. faecium) activity at 10^{-4} M.

Experimental

All melting points, taken in open capillary tubes in a Mel-Temp apparatus, and boiling points are uncorrected. Infrared spectra were recorded on a Beckman IR-8 spectrophotometer using KBr pellets. NMR spectra were recorded on a Varian A-60 spectrometer and are reported in ppm downfield from a TMS internal standard. UV spectra were

SCHEME 1

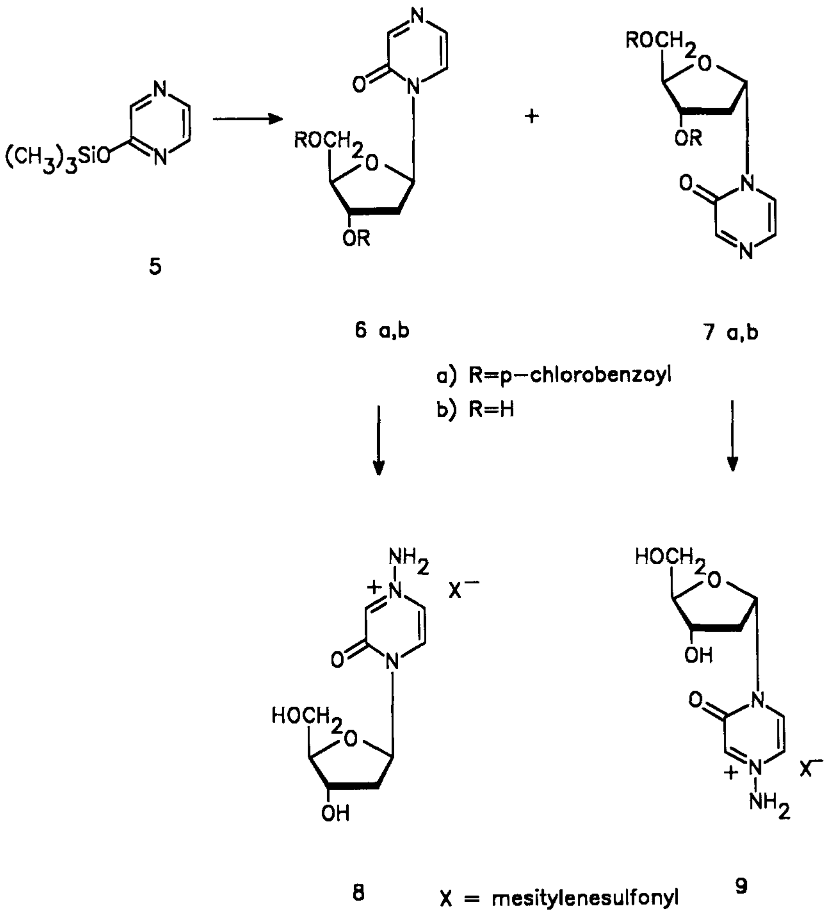


recorded on a Beckman DBG. Optical rotations were measured in a 10 cm tube with a Perkin-Elmer 141 polarimeter at 589 nm. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia.

4-Amino-1,2-dihydro-1-methyl-2-oxopyrazinium mesitylenesulfonate (2)

A solution of O-mesitylenesulfonylhydroxylamine (0.50 g, 0.002 mole) in CH_2Cl_2 (10 ml) was added with stirring to a solution of 1,2-dihydro-1-methyl-2-oxopyrazine[16] (1) (0.23 g, 0.002 mole) in

SCHEME 2



a) R=p-chlorobenzoyl
b) R=H

X = mesitylenesulfonyl

CH_2Cl_2 (10 ml). The mixture was heated at reflux temperature for 15 min and cooled to room temperature. Dry ether (80 ml) was added to precipitate the product. The hygroscopic precipitate was washed with dry ether (3 x 20 ml) and dried *in vacuo* to yield 0.35 g (48% yield) of 2, m.p. 145-147°; ir(KBr)ν_{max} 2900-3250 (broad), 1685, 1650, 1450, 1160, 845, 673 cm^{-1}; uv (MeOH)λ_{max} (log ε), 235 nm (3.88), 280 nm (3.46), 345 nm (3.33); NMR (D_2O) δ 2.19 (s, 3H), 2.50 (s, 6H), 3.53 (s, 3H), 6.92 (s, 2H), 7.47 (m, 1H), 8.00 (m, 2H).

Anal. Calcd for $C_{14}H_{21}N_3SO_5 \cdot H_2O$: C, 48.97; H, 6.16; N, 12.24; S, 9.34.

Found: C, 49.04; H, 6.23; N, 12.26; S, 9.41.

4-Amino-1-(β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazinium mesitylenesulfonate (4)

Dry CH_2Cl_2 (4.5 ml) was added with stirring to a solution of 1-(β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine[1] (3) (0.10 g, 0.0002 mole) in dry methanol (1.5 ml). A solution of O-mesitylenesulfonylhydroxylamine (0.10 g, 0.002 mole) in dry CH_2Cl_2 (5 ml) was added to the reaction solution. The mixture was refluxed for 15 min, cooled to room temperature, and dry ether (50 ml) was slowly added to precipitate the product. The suspension was cooled (0°C) for 1 hr; the light yellow precipitate was filtered, washed with dry ether (3x50 ml), and dried *in vacuo* to give 0.10 g (50%) of (4). The precipitate was recrystallized from MeOH-ether to give a light yellow powder, mp 161-162°; uv(MeOH)λ_{max} (log ε), 225 nm (4.08), 280 nm (3.34), 335 nm (3.07; ir(KBr)υ_{max} 2800-3200 (broad), 1680, 1635, 1430, 1160, 840, 670 cm^{-1}; $[\alpha]_D^{25}$ = +28.06° (c 0.36, MeOH); nmr(D_2O) δ 5.95 (1H, $H_{1'}$), 7.52 (m, 1H), 8.07 (s, 1H), 8.46 (m, 1H).

Anal. Calc. for $C_{18}H_{25}N_3SO_8$. 1/2 H_2O: C, 47.78; H, 5.57; N, 9.29; S, 7.08.

Found: C, 47.96; H, 5.86; N, 9.78; S, 6.87.

1-(3,5-Di-O-p-chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine 6a and its α-anomer 7a.

A mixture of the sodium salt of 1,2-dihydro-2-oxopyrazine (11.8 g, 0.10 mole) and trimethylsilyl chloride (13.8 g, 0.12 mole) in dry benzene (200 ml) was heated at reflux temperature with stirring for 3 h, cooled to room temperature and filtered. The precipitate was washed with dry benzene and the combined filtrate was evaporated. The liquid residue was distilled at 74°/5.2 mm Hg to give 2-(trimethylsilyloxy)pyrazine (5) (12.3 g, 0.073 mole, 73%) which was dissolved in dry 1,2-dichloroethane (500 ml). Molecular sieves (4A 103 g, Linde 1/16" pellets; Ventron #87956), were added to this solution followed by, after 10 min stirring at room temperature, $TiCl_4$ (1.69 g, 0.009 mole) and 3,5-di-O-p-chlorobenzoyl-2-deoxy-α-D-ribofuranosyl chloride (14.75 g, 0.034 mole). The reaction mixture was stirred under nitrogen at room temperature for 24 h and filtered. The filtrate was added to a saturated aqueous $NaHCO_3$ solution (800 ml) and the mixture was filtered through Celite which was washed with $CHCl_3$ (150 ml). The aqueous phase was washed with $CHCl_3$ (2 x 100 ml) and the combined organic phase was washed with dilute NaCl solution (800 ml) and dried (Na_2SO_4). The solution was evaporated and the residue (16.0 g) was purified by dry column chromatography on silica gel, using $CHCl_3$-EtOAc (2:1) as the eluent. The fraction corresponding to the product was evaporated and the residue was taken up into CH_2Cl_2. The solution was filtered and evaporated to give 14.5 g (86%) of white crystals. Recrystallization of this product from toluene (550 ml) gave slightly impure 1-(3,5-di-O-p-chlorobenzoyl-2-deoxy-α-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine 7a (7.15 g). Repeated recrystallization of this product from toluene (500 ml) gave pure α-anomer 7a (6.42 g), mp 168-169°; $[\alpha]_D^{25}$ = -160.7° (c 0.415, $CHCl_3$); uv (MeOH)λ_{max} (log ε), 245 nm (4.50), 320 nm (3.80); ir(KBr)ν_{max} 1720 (ester, C=O), 1650 cm^{-1} (amide, C=O); nmr ($CDCl_3$) δ 6.36 (pseudo-quartet, J = 1.5 and 6.0 Hz, $W_{H1'}$ = 13 Hz, $H_{1'}$).

The combined mother liquid from the crystallization of 7a was evaporated *in vacuo* and the residue was dissolved in hot toluene (150 ml). Petroleum ether (150 ml) was added slowly to this solution and the mixture was kept at room temperature overnight. The white crystals were collected by filtration and dried to give 2.95 g of impure 1-(3,5-di-O-p-chlorobenzoyl-2-deoxy-β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine (6a). Petroleum ether (200 ml) was slowly added to the refluxing filtrate to give 3.0 g of the pure β-anomer 6a mp 163-164°; $[\alpha]_D^{25}$ = +68.1° (c 0.43, $CHCl_3$); uv(MeOH)λ_{max} log ε), 245 nm (4.60), 320 nm (3.80); ir(KBr)ν_{max} 1720 (ester, C=O), 1660 cm^{-1} (amide, C=O); nmr ($CDCl_3$) δ 6.43 (t, J = 6.5 Hz, $W_{H1'}$ = 17.0 Hz, $H_{1'}$.

1-(2-Deoxy-β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine (6b)

The blocked nucleoside 6a (1.0 g, 0.002 mole) was treated with methanolic ammonia (150 ml) as described above for the α-anomer. Removal of the solvent gave a yellow residue. The residue was washed with dry ether (3 x 30 ml) and dissolved in dry methanol (15 ml). Dry ether (15 ml) was added to this solution and the mixture was kept at -20°C overnight to give 0.34 g (77%) of 6b as light yellow crystals, mp 162-164°; uv (95% EtOH)λ_{max} (log ε), 325 nm (3.76); $[\alpha]_D^{25}$ = +180.8° (c 0.34, MeOH); ir(KBr)ν_{max} 3370 (broad, OH), 1660 cm^{-1} (amide, C=O); nmr (DMSO-d_6-D_2O) δ 6.24 (t, J = 6.5 Hz, $W_{H1'}$ = 17.0 Hz, $H_{1'}$).

1-(2-Deoxy-α-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazine (7b)

The blocked nucleoside 7a (0.5 g, 0.001 mole) was suspended in dry methanol (75 ml) which was presaturated with ammonia at 0° for 20 min. The suspension was stirred at 5° overnight. The resulting clear solution was evaporated to give a slightly yellow syrup. The syrup was separated by preparative tlc on silica gel using $CHCl_3$-MeOH (4:1) as the developing solvent. The band corresponding to the product was extracted with CH_2Cl_2-MeOH (1:1) to afford a colorless syrup which was dissolved in a small volumn of MeOH-CH_2Cl_2 (1:3) and filtered. Removal of the solvent gave 0.21 g (95%) of 7b as a colorless syrup.

4-Amino-1-(2-deoxy-β-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazinium mesitylenesulfonate (8)

Dry CH_2Cl_2 (2 ml) was added to a stirred solution of the β-nucleoside 6b (0.15 g, 0.0007 mole) in dry methanol (3 ml). After stirring for a few minutes, a solution of O-mesitylenesulfonylhydroxylamine (0.15 g, 0.0007 mole) in dry CH_2Cl_2 (5 ml) was added. The mixture was then refluxed for 15 min and cooled to room temperature. Dry ether (80 ml) was added to the reaction mixture to precipitate the product. The suspension was further cooled (0°) for 1 hr. The light yellow precipitate was filtered, washed with dry ether (3x20 ml) and dried *in vacuo* to give 0.16 g (46%) of compound 8, mp 73-75°; uv(MeOH)λ_{max} (log ε), 235 nm (4.06), 280 nm (3.66), 345 nm (3.56); $[\alpha]_D^{25}$ = +43.9° (c 0.22, MeOH); ir(KBr)υ_{max} 3260-3430 (broad), 2900-3200 (broad), 1680, 1640, 1430, 1160, 845, 670 cm^{-1}; nmr (D_2O)δ 6.16 (t, J = 6.5 Hz, $W_{H1'}$ = 18.0 Hz, $H_{1'}$).

Anal. Calcd. for: $C_{18}H_{25}N_3SO_7.H_2O$: C, 48.53; H, 6.11; N, 9.43; S, 7.20.

Found: C, 48.67; H, 6.16; N, 9.50; S, 7.14.

4-Amino-1-(2-deoxy-α-D-ribofuranosyl)-1,2-dihydro-2-oxopyrazinium mesitylenesulfonate (9)

Dry CH_2Cl_2 (5 ml) was added to a stirred solution of the α-nucleoside 7b (0.21 g, 0.0009 mole) in dry methanol (1 ml) followed by a solution of O-mesitylenesulfonylhydroxylamine (0.22 g, 0.001 mole) in dry CH_2 Cl_2 (5 ml). The mixture was refluxed for 15 min, cooled to room temperature, and dry ether (50 ml) was added to precipitate the product. The mixture was cooled further (0°C) and the yellow precipitate was collected by filtration. The precipitate was washed with dry ether (3x20 ml) to yield 0.26 g (61%) of 9, mp 84-86°; uv(MeOH)λ_{max} (log ε), 234 nm (4.00), 280 nm (3.61), 345 nm (3.56); $[\alpha]_D^{25}$ = -50.8° (c 0.51, MeOH); ir(KBr)υ_{max} 3240-3420 (broad), 2900-3200 (broad), 1680, 1640, 1430, 1160, 840, 670 cm^{-1}; nmr (D_2O) δ 6.02 (pseudoquartet, J = 1.5 and 6.0 Hz, $W_{H1'}$ = 14.0 Hz, $H_{1'}$).

Anal. Calcd for: $C_{18}H_{25}N_3SO_2.1/2\ H_2O$: C, 49.53; H, 6.00; N, 9.62; S, 7.34.

Found: C, 49.35; H, 6.25; N, 9.66; S, 7.35.

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