SYNTHESIS AND STRUCTURE ASSIGNMENT OF 1-[(2-HYDROXY-ETHOXY)METHYL]- AND 1-[(1,3-DIHYDROXY-2-PROPOXY)METHYL]- 6-AZAISOCYTOSINE

Long-Chih Hwang,[a] Chyi-Jia Wang,[a] Gene-Hsiang Lee,[b] Yu Wang,[b] and Cherng-Chyi Tzeng[a,*]

[a] School of Chemistry, Kaohsiung Medical College, Kaohsiung City 807, Taiwan, Republic of China

[b] Department of Chemistry, National Taiwan University, Taipei City 107, Taiwan, Republic of China

Abstract- 1-[(2-Acetoxyethoxy)methyl]-6-azaisocytosine and 1-[(1,3-dibenzyloxy-2-propoxy)methyl]-6-azaisocytosine have been prepared, and their unambiguous assignment of ^{1}H and ^{13}C peaks through the ^{1}H-^{13}C heteronuclear correlation (HETCOR) nmr experiments is described. The X-ray crystallographic analysis reveals unambiguously the site of glycosylation at N_1. Deprotection of both acyclonucleosides provided 1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine and 1-[(1,3-dihydroxy-2-propoxy)methyl]-6-azaisocytosine, respectively.

Introduction

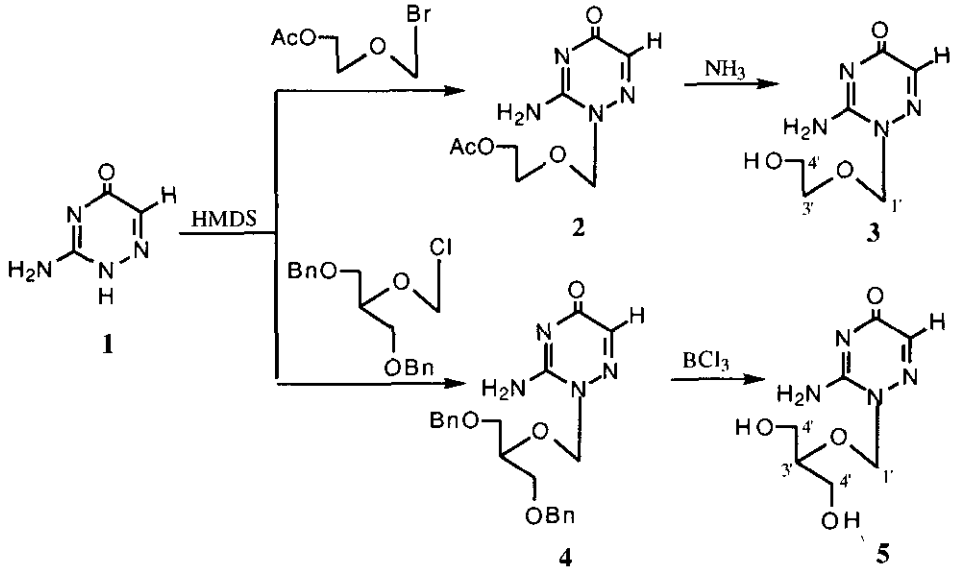
Previous studies revealed that 6-azapyrimidines (6-azauracil and 6-azacytosine) display a range of biological effects which include antiviral,[1,2] antitumor,[3,4] and antifungal[5] activities. 6-Azauridine and 6-azacytidine exhibit carcinostatic activity against a number of experimental tumors.[6] It was also found that 6-azacytidine which possesses a pronounced cancerostatic activity is deaminated *in vivo* to give 6-azauridine[7] and that cytosine arabinoside (Cytarabine) which is highly active against both mouse and human leukemias, is rather readily deaminated to the inactive uracil arabinoside by cytidine deaminase.[8] 6-Azaisocytosine, the isosteric isomer of 6-azauracil and 6-azacytosine, is of interest in both chemical and biological aspects due to its resistance to the deaminase. Synthesis of 6-azaisocytosine riboside (6-azaisocytidine)[9] and 6-azauracil acyclonucleosides[10-14] had

been previously described. Recently, we described the preparation of 1-[(2-acetoxyethoxy)methyl]-5-chloro-6-azauracil and its unambiguous assignment of ^{1}H and ^{13}C peaks through the ^{1}H-^{13}C heteronuclear correlation (HETCOR) nmr experiments.[15] The isosteric 1-[(2-acetoxyethoxy)methyl]-5-bromo-6-azaisocytosine was also prepared for the X-ray crystallographic analysis to determine the site of N-glycosylation. The present study describes the synthesis, the antiviral evaluation, and the structure assignment of 1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine and 1-[(1,3-dihydroxy-2-propoxy)methyl]-6-azaisocytosine.

Results and Discussion

6-Azaisocytosine (**1**) was persilylated with hexamethyldisilazane (HMDS) and then alkylated with (2-acetoxyethoxy)methyl bromide[4] in dry acetonitrile to furnish 1-[(2-acetoxyethoxy)methyl]-6-azaisocytosine (**2**), as shown in Scheme 1. The ^{1}H nmr spectrum of **2** showed four singlets at δ 7.41, 7.30, 5.29, and 1.99 ppm

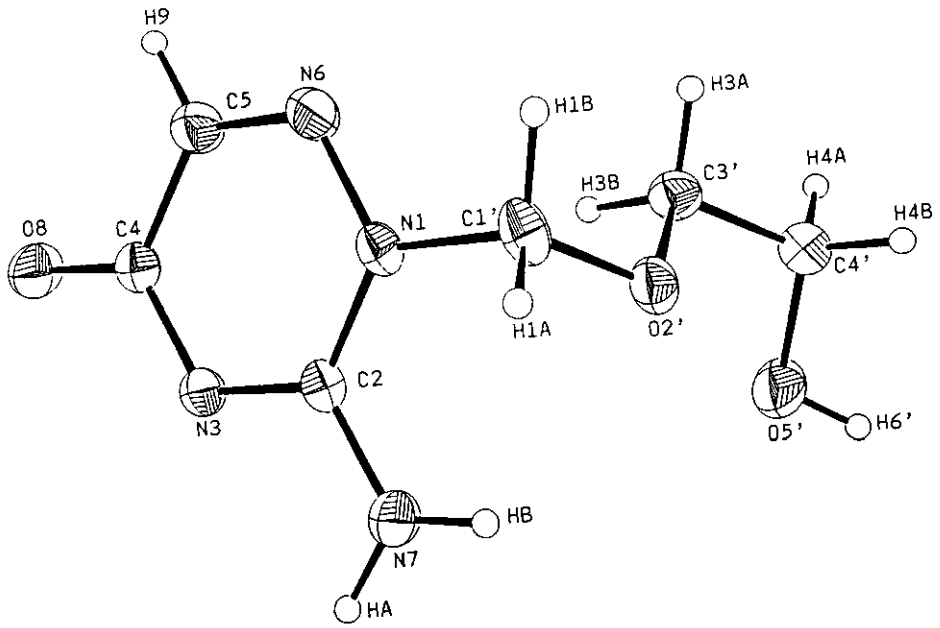
Scheme 1



corresponding to NH_2, H-5, 1'-CH_2, and CH_3, respectively. The remaining two triplets which couple to each other (A_2B_2 type, J = 2.9 Hz) at δ 4.11 and 3.73 ppm were attributed to the resonances of two methylene protons. The proton-decoupled ^{13}C-nmr and DEPT spectra of **2** indicated eight resonances which include one methyl (δ at 20.25 ppm), three methylene (δ at 81.49, 66.18 and 62.61 ppm), and four quaternary (δ at 170.42, 162.88, 155.37 and 139.34 ppm) carbons. In order to assign specific resonances within each carbon type, standard and long-range ^{1}H-^{13}C heteronuclear correlation (HETCOR) nmr experiments were carried out.

Through the standard HETCOR experiment, which was performed to reveal the direct attachment between protons and carbons, it is clear that C-1' (δ 81.49) is coupled to H-1' (δ 5.29), C-5 (δ 139.34) is coupled to H-5 (δ 7.30) and the more downfield methylene carbon (δ 66.18) is coupled to the more upfield methylene protons (δ 3.73) while the the more upfield methylene carbon (δ 62.61) is coupled to the more downfield methylene protons (δ 4.11). Through the long-range HETCOR experiment, which reveals two-, and three- bond ^{1}H-^{13}C connectivities, the methyl protons (δ 1.99) were found to couple to the most downfield carbonyl carbon (δ 170.42), and the H-1' methylene protons were coupled to carbons with resonances of δ 155.37 and 66.18 ppm corresponding to C-2 and C-3', respectively. The remaining carbon resonances at δ 162.88 and 62.61 ppm which did not couple to H-1' can be unambiguously assigned to C-4 and C-4', respectively. Therefore, the two triplets of proton resonances at δ 4.11 and 3.73 ppm were attributed to the 4'-CH_2 and 3'-CH_2, respectively. Deacetylation of **2** with methanolic ammonia afforded 1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine (**3**) in good overall yield. A view of a single molecule of **3** is given in Figure 1. As can be seen in the Figure, the glycosylation occurs at N1. The crystal data and the atomic parameters of all non-hydrogen atoms are listed in

Figure 1 ORTEP drawing of **3**



Tables 1 and 2, respectively. Bond lengths and bond angles are presented in Table 3. Glycosylation of persilylated derivatives of **1** with one molarequivalent of 1,3-dibenzyloxy-2-chloromethoxypropane in a non-polar solvent (dry toluene or dry dichloromethane) gave the desired 1-[(1,3-dibenzyloxy-2-propoxy)methyl]-6-azaisocytosine (**4**) in 53% yield. Debenzylation of **4** with either boron trichloride in dichloromethane or by hydrogenation in the presence of palladium oxide in the mixed solvent of absolute alcohol and cyclohexene afforded 1-[(1,3-dihydroxy-2-propoxy)methyl]-6-azaisocytosine (**5**).

Table 1 Crystal data of 1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine (**3**)

Formula	$C_6H_{10}N_4O_3$
Molecular weight	186.17
Diffractometer used	CAD4
Space group	$P\bar{1}$
a, Å	5.738 (2)
b, Å	8.1943 (6)
c, Å	9.272 (3)
α, °	72.72 (2)
β, °	77.32 (3)
γ, °	85.52 (2)
V, Å^3	406.1 (2)
Z	2
D (calc), g·cm^{-3}	1.523
λ (Mo *K*α), Å	0.71069
F (000)	196
unit cell detn; #; 2θ range	25, (20.80-28.54)
scan type	ω/2θ
2θ scan width, deg	2 (0.7 + 0.35 tan θ)
2θ max, deg	50°
μ (Mo *K*α), cm^{-1}	1.158
Crystal size, mm	0.45 x 0.50 x 0.70
Temperature, K	298
No. of unique reflns	1426
No. of obs reflns (I > 2σ (I))	1228
R, *R*w*	0.031, 0.029
GoF	1.37
Minimized function	$\Sigma w \|Fo-Fc\|^2$

Weighting scheme	$1/\sigma^2$ (*Fo*)
g (second.ext.coeff.) x 10^4	0.524 (7)
(Δ/σ)max	0.0197
$(\Delta\rho)$max, mın e$\mathring{A}^{-3}$	0.15, -0.16
Computation program	NRCVAX[16]

*$R = [\Sigma\ |Fo-Fc\ |/Fo]$

$Rw = [\Sigma w(\ |Fo-Fc\ |^2/\Sigma w(\ |Fo\ |^2)]^{1/2}$; σ^2 (*Fo*) from counting statistics

Table 2 Atomic Parameters x,y,z and B_{eq} of **3**

	x	y	z	B_{eq}
N1	0.39381(24)	0.34386(17)	0.25658(14)	2.75(6)
C2	0.2252(3)	0.40273(19)	0.16956(17)	2.55(7)
N3	0.23101(23)	0.36404(17)	0.03947(15)	2.73(6)
C4	0.4072(3)	0.25991(20)	-0.00535(18)	2.72(7)
C5	0.5804(3)	0.19731(22)	0.09280(20)	3.29(8)
N6	0.5750(3)	0.23682(18)	0.21732(16)	3.25(6)
N7	0.0514(3)	0.50450(19)	0.21446(16)	3.47(7)
O8	0.42032(23)	0.21965(17)	-0.12575(14)	4.01(6)
C1'	0.3995(3)	0.39212(22)	0.39636(18)	3.26(8)
O2'	0.20189(21)	0.33229(13)	0.51447(12)	3.06(5)
C3'	0.1961(3)	0.14875(20)	0.57060(19)	3.01(7)
C4'	-0.0083(3)	0.09737(22)	0.70410(20)	3.41(8)
O5'	-0.22459(23)	0.15027(21)	0.65359(16)	5.01(8)
HA	-0.052(3)	0.5511(23)	0.1473(21)	4.5(4)
HB	0.030(3)	0.5248(23)	0.3016(21)	4.4(4)
H1A	0.393(3)	0.5147(21)	0.3709(19)	3.7(4)
H1B	0.551(3)	0.3424(22)	0.4277(20)	3.7(4)
H3A	0.344(3)	0.1052(22)	0.6065(20)	3.8(4)
H3B	0.172(3)	0.1016(21)	0.4856(19)	3.6(4)
H4A	-0.002(3)	-0.0277(21)	0.7460(19)	3.7(4)
H4B	0.007(3)	0.1458(21)	0.7899(19)	3.3(4)
H9	0.704(3)	0.1203(23)	0.0655(21)	4.2(4)
H6'	-0.331(4)	0.158(3)	0.730(3)	7.5(6)

Estimated standard errors refer to the last digit printed. .

$B_{eq} = 8/3\ p^2\ _{i,j}U_{ij}a_ia_ja_i^*a_j^*$

Table 3 Bond Lengths (Å) and Bond Angles (degree) in **3**

N1-C2	1.3629(20)	C5-N6	1.2822(22)	C3'-C4'	1.488(3)
N1-N6	1.3653(19)	C5-H9	0.955(18)	C3'-H3a	0.977(17)
N1-C1'	1.4706(20)	N7-Ha	0.935(18)	C3'-H3b	1.013(17)
C2-N3	1.3284(20)	N7-Hb	0.853(18)	C4'-O5'	1.4133(22)
C2-N7	1.3260(21)	C1'-O2'	1.3959(22)	C4'-H4a	0.983(17)
N3-C4	1.3471(21)	C1'-H1a	0.982(17)	C4'-H4b	1.011(16)
C4-C5	1.4553(24)	C1'-H1b	0.996(17)	O5'-H6'	0.840(23)
C4-O8	1.2406(19)	O2'-C3'	1.4384(19)		
C2-N1-N6	121.73(13)	N1-N6-C5	116.36(14)	O2'-C3'-H3b	109.0(10)
C2-N1-C1'	123.47(14)	C2-N7-Ha	116.8(11)	C4'-C3'-H3a	108.5(10)
N6-N1-C1'	114.79(13)	C2-N7-Hb	122.6(12)	C4'-C3'-H3b	109.0(10)
N1-C2-N3	122.28(14)	Ha-N7-Hb	120.6(16)	H3a-C3'-H3b	111.6(14)
N1-C2-N7	119.10(14)	N1-C1'-O2'	112.24(13)	C3'-C4'-O5'	109.31(14)
N3-C2-N7	118.61(14)	N1-C1'-H1a	108.4(10)	C3'-C4'-H4a	107.8(10)
C2-N3-C4	118.43(13)	N1-C1'-H1b	105.7(10)	C3'-C4'-H4b	111.0(9)
N3-C4-C5	117.37(14)	O2'-C1'-H1a	106.4(10)	O5-C4'-H4a	110.5(10)
N3-C4-O8	120.94(15)	O2'-C1'-H1b	111.2(10)	O5'-C4'-H4b	111.6(9)
C5-C4-O8	121.69(15)	H1a-C1'-H1b	112.9(14)	H4a-C4'-H4b	106.5(13)
C4-C5-N6	123.80(15)	C1'-O2'-C3'	112.77(12)	C4'-O5'-H6'	108.8(16)
C4-C5-H9	119.1(11)	O2'-C3'-C4'	108.92(13)		
N6-C5-H9	117.1(11)	O2'-C3'-H3a	109.7(10)		

Antiviral Studies

Antiviral and cytotoxicity assays of the new acyclic nucleosides against HSV-1 and HSV-2 in Human Foreskin Fibroblast (HFF) cells were performed by the cytopathic effect (CPE) inhibition assay.[17] None of the compounds were active against HSV-1 and HSV-2 or exhibited toxic effects in uninfected HFF cells when tested up to 100 mM.

EXPERIMENTAL

Melting points were determined on a YANACO micromelting point apparatus and are uncorrected. The uv absorption spectra were obtained on a Beckman UV-Visible spectrophotometer. Ir spectra were recorded on a Hitachi 260-30 spectrophotometer. Nmr (^{1}H and ^{13}C)spectra were obtained with a Varian Gemini-200 spectrometer. Chemical shifts are expressed in ppm (δ) with tetramethylsilane as an internal standard. Tlc was

run on precoated (0.2 mm) silica gel 60 F-254 plates manufactured by EM Laboratories, Inc., and short-wave uv light (254 nm) was used to detect the uv absorbing sports. Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer.

1-[(2-Acetoxyethoxy)methyl]-6-azaisocytosine (**2**)

6-Azaisocytosine (1.12 g, 10 mmol) was suspended in hexamethyldisilazane (HMDS; 25 ml, 118 mmol) and then a catalytic amount of ammonium sulfate (*ca.* 60 mg, 0.53 mmol) was added. The mixture was heated under reflux with the exclusion of moisture until a clear solution was obtained (*ca.* 4 h). The excess HMDS was removed under reduced pressure to give silylated intermediate as an oil, which was dissolved in dry acetonitrile (20 ml) and cooled to 0°C. To this stirred solution was added a solution of (2-acetoxyethoxy)methyl bromide (1.97g, 10 mmol) in dry acetonitrile (15 ml). The reaction mixture was stirred at room temperature for 24 h (monitored by tlc). The solvent was evaporated to afford crude product as an oil which was applied to a silica gel column. The column was eluted with a mixed solvent of $CHCl_3$ and MeOH (20:1) and the proper fractions were combined and evaporated. The residue thus obtained was crystallized from CH_2Cl_2 and MeOH (1:1) to give **2** (1.17g, 52% yield). mp 198-199°C; uv: λ_{max} (log ε) 252 (3.74) (0.1 N HCl); 248 (3.88) (H_2O); 225 (4.26) (0.1 N NaOH); ^{1}H nmr (DMSO-d_6): δ 1.99 (s, 3H, CH_3), 3.73 (t, 2H, J = 2.8 Hz, 3'-CH_2), 4.11 (t, 2H, J = 2.8 Hz, 4'-CH_2), 5.29 (s, 2H, 1'-CH_2), 7.30 (s, 1H, H-5), 7.41 (s, 2H, NH_2); ^{13}C nmr (DMSO-d_6): δ 20.25 (CH_3), 62.61 (C-4'), 66.18 (C-3'), 81.49 (C-1'), 139.34 (C-5), 155.37 (C-2), 162.88 (C-4), 170.42 (CO); Ms, *m/z* 228 (M^+). Anal. Calcd for $C_8H_{12}N_4O_4$: C, 42.11; H, 5.30; N, 24.55. Found: C, 42.08; H, 5.32; N, 24.52.

1-[(2-Hydroxyethoxy)methyl]-6-azaisocytosine (**3**)

A solution of **2** (0.57 g, 2.5 mmol) in methanolic ammonia (previously saturated at 0°C; 100 ml) was stirred at room temperature in a sealed flask for 24 h. The solvent was then evaporated to give a residual solid as the crude product, which was purified by silica gel chromatography using $CHCl_3$-MeOH (5:1) as an eluent. The proper fractions were combined and evaporated. The residue thus obtained was crystallized from ethanol to provide **3** (0.42 g, 89% yield). mp 206 - 207°C; uv: λ_{max} (log ε) 253 (3.71) (0.1 N HCl); 250 (3.79) (H_2O); 225 (4.14) (0.1 N NaOH); ^{1}H nmr (DMSO-d_6): δ 3.53 (m, 4H, OCH_2CH_2O), 4.68 (br s, 1H, 4'-OH), 5.28 (s, 2H, 1'-CH_2), 7.29 (s, 1H, 5-H), 7.38 (s, 2H, NH_2); ^{13}C nmr (DMSO-d_6): δ 59.92 (C-4'), 70.21 (C-3'), 82.06 (C-1'), 139.31 (C-5), 155.47 (C-2), 162.84 (C-4); Ms, *m/z* 186 (M^+). Anal. Calcd for $C_6H_{10}N_4O_3$: C, 38.71; H, 5.41; N, 30.09. Found: C, 38.79; H, 5.45; N, 30.04.

1-[(1,3-Dibenzyloxy-2-propoxy)methyl]-6-azaisocytosine (**4**)

6-Azaisocytosine (1.12 g, 10 mmol), HMDS (25 ml, 118 mmol), and ammonium sulfate (0.1g, 0.88 mmol) were heated under reflux with the exclusion of moisture until a clear solution was obtained. The excess HMDS was removed *in vacuo* and the residual oil dissolved in dry dichloromethane (30 ml). To this solution was added 1,3-dibenzyloxy-2-chloromethoxypropane (3.21 g, 10 mmol) in dry dichloromethane (15 ml) and the resulting mixture was allowed to stir at room temperature for 24 h (monitored by tlc). The organic solvent was evaporated to give a residual oil as a crude product, which was purified by silica gel chromatography using a mixed solvent of $CHCl_3$: MeOH (60 :1). The proper fractions were combined and evaporated to give a solid residue, which was crystallized from CH_2Cl_2 and MeOH (1:1) to afford **4** (2.1 g; 53%). mp 94-96°C; uv: λ_{max} (log ε) 254 (3.72) (0.1 N HCl); 252 (3.65) (H_2O); 218 (4.18) (0.1 N NaOH); ^{1}H nmr (DMSO-d_6): δ 3.49 (m, 4H, 4'-CH_2), 4.00 (m,1H, 3'-CH), 4.45 (s, 4H, Ar-CH_2), 5.40 (s, 2H, 1'-CH_2), 7.29 (s, 1H, 5-H), 7.30 (m, 10H, Ar), 7.36 (s, 2H, NH_2); ^{13}C nmr (DMSO-d_6): δ 69.34 (C-4'), 72.07 (Ar-C), 76.03 (C-3'), 81.41 (C-1'), 139.26 (C-5), 155.48 (C-2), 163.00 (C-4); Ms, *m/z* 397 (M^++1). Anal. Calcd for $C_{21}H_{24}N_4O_4$: C, 63.62; H, 6.10; N, 14.13. Found: C, 63.66; H, 6.12; N, 14.23.

1-[(1,3-Dihydroxy-2-propoxy)methyl]-6-azaisocytosine (**5**)

To an ice-salt cooled solution of **4** (1.19 g, 3 mmol) in dry dichloromethane (30 ml) was added boron trichloride in dichloromethane (5 ml, 1 M solution, 5 mmol). The mixture was stirred at the same temperature (-8°C) for 30 min (monitored by tlc to ensure the reaction was complete). A solution (40 ml) of methanol and dichloromethane (1:1) was then added and the resulting mixture was allowed to warm to room temperature. The solvents were evaporated under reduced pressure to give a solid residue, which was crystallized from ethanol to obtain **5** (0.53 g, 82%). mp 137-138°C; uv: λ_{max} (log ε) 254 (3.90) (0.1 N HCl); 249 (3.82) (H_2O); 225 (4.24) (0.1 N NaOH); ^{1}H nmr (DMSO-d_6): δ 3.41 (m, 4H, 4'-CH_2), 3.60 (m, 1H, 3'-CH), 4.29 (br s, 2H, OH), 5.43 (s, 2H, 1'-CH_2), 7.56 (s, 1H, 5-H), 8.21 (s, 2H, NH_2); ^{13}C nmr (DMSO-d_6): δ 61.09 (C-4'), 80.92 (C-3'), 82.55 (C-1'), 139.84 (C-5), 153.45 (C-2), 157.17 (C-4); Ms, *m/z* 217 (M^++1). Anal. Calcd for $C_7H_{12}N_4O_4$: C, 38.89 ; H, 5.59; N, 25.91. Found: C, 38.85; H, 5.65; N, 25.60.

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