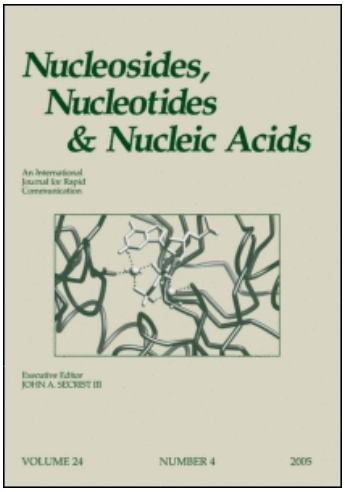

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Structure Assignment of 1-(2-Acetoxyethoxy)methyl Derivatives of 5-Chloro-6-azauracil and 5-Bromo-6-azaisocytosine

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To cite this Article Hwang, L. -C. , Wei, D. -C. , Cheng, M. -C. , Wang, Y. and Tzeng, C. -C.(1994) 'Synthesis and Structure Assignment of 1-(2-Acetoxyethoxy)methyl Derivatives of 5-Chloro-6-azauracil and 5-Bromo-6-azaisocytosine', Nucleosides, Nucleotides and Nucleic Acids, 13: 10, 2185 — 2193
To link to this Article: DOI: 10.1080/15257779408013216
URL: http://dx.doi.org/10.1080/15257779408013216

NUCLEOSIDES & NUCLEOTIDES, 13(10), 2185-2193 (1994)

SYNTHESIS AND STRUCTURE ASSIGNMENT OF 1-(2-ACETOXYETHOXY) METHYL DERIVATIVES OF 5-CHLORO-6-AZAURACIL AND 5-BROMO-6-AZAISOCYTOSINE

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Abstract

1-[(2-Acetoxyethoxy)methyl]-5-chloro-6-azauracil has been prepared and its unambiguous assignment of ^{1}H and ^{13}C peaks through the ^{1}H-^{13}C heteronuclear correlation (HETCOR) NMR experiments is described. The isosteric 1-[(2-acetoxyethoxy)methyl]-5-bromo-6-azaisocytosine has also been synthesized. The X-Ray crystallographic analysis reveals unambiguously the site of glycosylation at N^{1}. Deacetylation of both acyclonucleosides provided 5-chloro-1-[(2-hydroxyethoxy)methyl]-6-azauracil and 5-bromo-1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine respectively. Their structures have been well established by the NMR spectra and the elemental analyses.

Introduction

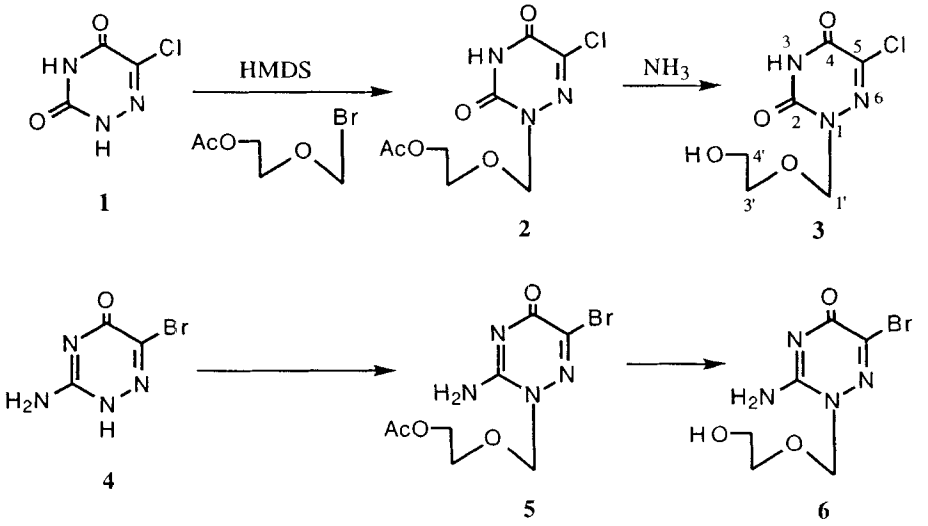
as-Triazines such as 6-azauracil and 6-azaisocytosine (bioisosteric isomers of uracil), have been proved to display a range of biological effects which include antiviral,[1-2] antitumor,[3-4] and antifungal[5] activities. 6-Azauridine, the ribonucleoside of 6-azauracil, exerts carcinostatic activity against a number of experimental tumors.[6] The mechanism of action has been ascribed to the *in vivo* formation of 6-azauridine-5'-phosphate, a competitive inhibitor of orotidine-5'-phosphate (OMP) decarboxylase with an inhibition constant, K_i, of 0.03 μM.[7-8] OMP decarboxylase catalyzes the decarboxylation of orotidine-5'-phosphate to uridine-5'-phosphate, an essential step in the *de novo* biosynthesis of pyrimidine nucleoside. To potentiate the antiviral potency of *as*-triazines,

several series of nucleosides in which acyclic residues were attached to 6-azauracil and its 5-substituted derivatives by a glycosidic linkage have been prepared.[9-13] The site of glycosylation was established as N^1 but not N^3 based only on the comparison of UV spectra with their reported ribosylated counterparts.[14] The ^1H and ^{13}C assignments of these 6-azauracil acyclonucleosides and other purine acyclonucleosides[15-16] are not unambiguous because no definitive two-dimensional NMR have been applied to confirm the assignments especially the proton resonances at C-3' and C-4' are usually represented as a multiplicity (A_2B_2 type). This paper now describes the preparation of 1-[(2-acetoxyethoxy)methyl]-5-chloro-6-azauracil and its unambiguous assignment of ^1H and ^{13}C peaks through the ^1H-^{13}C heteronuclear correlation (HETCOR) NMR experiments. The isosteric 1-[(2-acetoxyethoxy)methyl]-5-bromo-6-azaisocytosine is also prepared for the X-Ray crystallographic analysis to determine unambiguously the site of *N*-glycosylation. Deacetylation of both acyclonucleosides provided 5-chloro-1-[(2-hydroxyethoxy)methyl]-6-azauracil and 5-bromo-1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine respectively. Their structures have been well established by the NMR spectra and the elemental analyses.

Results and Discussion

5-Chloro-6-azauracils (**1**)[17-18] was persilylated with hexamethyldisilazane (HMDS) and then alkylated with (2-acetoxyethoxy)methyl bromide[15] in dry acetonitrile to furnish 1-[(2-acetoxyethoxy)methyl]-5-chloro-6-azauracil (**2**) as described in Scheme 1.

Scheme 1



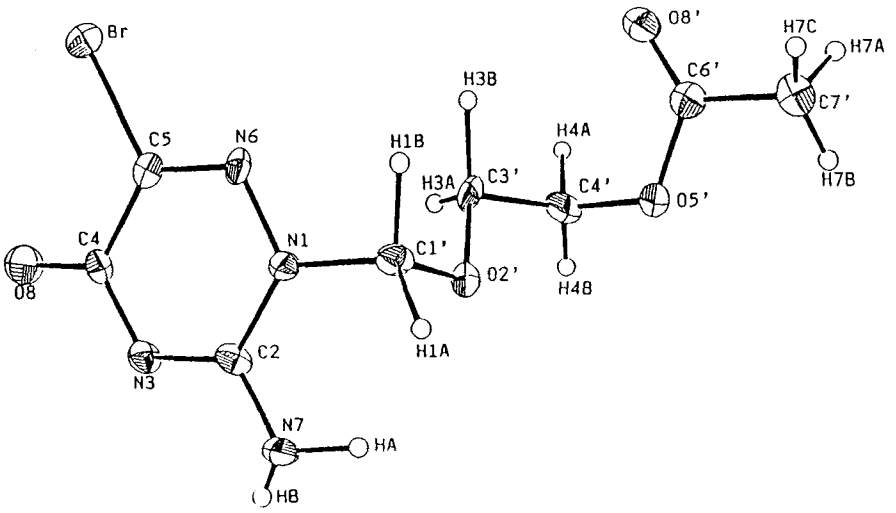


Figure 1 ORTEP drawing of **5**

The ^1H NMR spectrum of **2** showed three singlets at δ 12.64, 5.18 and 2.00 ppm corresponding to 3-NH, 1'-CH_2 and CH_3, respectively. The remaining two triplets which couple to each other (A_2B_2 type, J = 2.8 Hz) at δ 4.11 and 3.75 ppm were attributed to the resonances of ethoxy protons. However, to our knowledge, no definitive NMR experiments have been used to distinguish these two methylene protons of 3'-CH_2 and 4'-CH_2. The proton-decoupled ^{13}C-NMR and DEPT spectra of **2** indicated eight resonances which include one methyl (δ at 20.65 ppm), three methylene (δ at 79.01, 66.98 and 62.92 ppm), and four quarternary (δ at 170.32, 153.13, 148.75 and 136.62 ppm) carbons. In order to assign specific resonances within each carbon type, standard and long-range ^1H-^{13}C heteronuclear correlation (HETCOR) NMR experiments were employed. Through the standard HETCOR experiment, which was performed to reveal the direct attachment between protons and carbons, it is clear that C-1' (δ 79.01) is coupled to H-1' (δ 5.18) and the more downfield methylene carbon (δ 66.98) is coupled to the more upfield methylene protons (δ 3.75) while the the more upfield methylene carbon (δ 62.92) is coupled to the more downfield methylene protons (δ 4.11). Through the long-range HETCOR experiment, which reveals two-, three- and four-bond ^1H-^{13}C connectivities, the H-1' methylene protons were clearly coupled to carbons with resonances of δ 148.75, 136.62, 79.01 and 66.98 ppm corresponding to C-2, C-5, C-1' and C-3' respectively. The remaining carbon resonances at δ 153.13 and 62.92 ppm which were not coupled to H-1' can unambiguously assigned to C-4 and C-4' respectively. Therefore, the two triplets

Table 1 Crystal data of 1-[(2-Acetoxyethoxy)methyl]-5-bromo-6-azaisocytosine (5)

Formula	$C_8N_4O_4H_{11}Br$
Formula weight	307.10
Diffractometer used	CAD4
Space group	P na 2_1
a, Å	7.262(1)
b, Å	13.317(3)
c, Å	12.076(5)
V, $Å^3$	1167.9(6)
Z	4
D(calc), $g \cdot cm^{-3}$	1.747
λ(Mo Kα), Å	0.71069
F(000)	615
unit cell detn;#;2θ range	25, (17.28-24.50)
scan type	θ/2θ
2θ scan width, deg	2(0.7+0.35 tanθ)
2θ max, deg	50°
μ(Mo Kα), cm^{-1}	34.924
Transmission range	0.64-1.0
Crystal size,mm	0.05x0.35x0.40
Temperature, K	298
No. of unique reflns	1077
No. of obs reflns($I > 2\sigma(I)$)	789
R, Rw*	0.031, 0.027
GoF	1.25
Minimized function	$\Sigma w \mid Fo-Fc \mid^2$
Weighting scheme	$1/[\sigma^2(Fo)+0.0001Fo^2]$
g(second.ext.coeff.)x10^4	1.13(5)
$(\Delta/\sigma)_{max}$	0.0482
$(\Delta\rho)_{max,min}$ $eÅ^{-3}$	0.320,-0.290
Computation program	NRCVAX[19]

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

$R_W=[\Sigma w(\mid Fo-Fc \mid^2/\Sigma w(\mid Fo \mid^2)]^{1/2}$; $\sigma^2(Fo)$ from counting statistics

Table 2 Atomic Parameters x,y,z and Beq of **5**

	x	y	z	Beq
Br	0.12134(10)	0.15646(4)	0.25000	3.16(4)
N1	0.0845(8)	0.4001(3)	0.0564(5)	2.38(25)
C2	0.0645(9)	0.4779(5)	0.1306(6)	2.3(3)
N3	0.0691(7)	0.4640(3)	0.2376(6)	2.62(24)
C4	0.0904(9)	0.3705(4)	0.2804(5)	2.3(3)
C5	0.1050(10)	0.2907(5)	0.1976(5)	2.8(3)
N6	0.1090(8)	0.3034(4)	0.0932(5)	2.42(24)
N7	0.0337(9)	0.5677(4)	0.0885(5)	3.5(3)
O8	0.0965(8)	0.3554(3)	0.3799(4)	4.3(3)
O8'	0.5719(8)	0.2501(3)	-0.2825(4)	4.2(3)
C1'	0.1016(10)	0.4122(4)	-0.0630(6)	2.6(3)
O2'	0.2761(7)	0.4494(3)	-0.0934(4)	3.16(23)
C3'	0.4169(10)	0.3751(4)	-0.0850(6)	2.8(3)
C4'	0.5806(11)	0.4082(5)	-0.1525(6)	3.3(4)
O5'	0.5337(6)	0.4155(3)	-0.2662(4)	3.25(23)
C6'	0.5361(11)	0.3299(5)	-0.3221(6)	3.3(3)
C7'	0.4792(12)	0.3440(5)	-0.4415(7)	4.3(4)
HA	0.065(9)	0.576(4)	0.009(5)	4.5(17)
HB	0.020(6)	0.607(3)	0.116(4)	0.8(11)
H1A	0.029(8)	0.473(4)	-0.097(5)	3.7(16)
H1B	0.062(11)	0.329(5)	-0.100(7)	7.1(22)
H3A	0.455(7)	0.378(3)	-0.030(4)	1.4(12)
H3B	0.362(11)	0.288(5)	-0.116(7)	6.8(21)
H4A	0.716(8)	0.356(3)	-0.137(5)	2.5(14)
H4B	0.608(8)	0.468(3)	-0.136(5)	2.0(12)
H7A	0.573(12)	0.313(5)	-0.480(7)	8.5(26)
H7B	0.525(9)	0.409(5)	-0.457(7)	6.3(20)
H7C	0.370(11)	0.302(5)	-0.464(7)	7.9(24)

Estimated standard errors refer to the last digit printed

Beq = $8/3\ \pi^2\ {}_{i,j}U_{ij}a_ia_ja_i{}^*a_j{}^*$

Table 3 Bond Lengths (A) and Bond Angles (degree) of **5**

Br-C5	1.899(6)	N7-Ha	0.99(7)	C4'-O5'	1.418(9)
N1-C2	1.378(8)	N7-Hb	0.63(4)	C4'-H4a	1.22(5)
N1-N6	1.374(7)	O8'-C6'	1.194(8)	C4'-H4b	0.84(5)
N1-C1'	1.457(9)	C1'-O2'	1.410(9)	O5'-C6'	1.325(8)
C2-N3	1.305(10)	C1'-H1a	1.05(6)	C6'-C7'	1.512(11)
C2-N7	1.318(8)	C1'-H1b	1.23(7)	C7'-H7a	0.92(8)
N3-C4	1.357(8)	O2'-C3'	1.427(8)	C7'-H7b	0.94(6)
C4-C5	1.463(9)	C3'-C4'	1.507(10)	C7'-H7c	1.01(8)
C4-O8	1.219(8)	C3'-H3a	0.72(5)		
C5-N6	1.273(9)	C3'-H3b	1.29(7)		

C2-N1-N6	120.6(5)	C1'-O2'-C3'	112.4(5)
C2-N1-C1'	124.8(5)	O2'-C3'-C4'	108.9(5)
N6-N1-C1'	114.4(5)	O2'-C3'-H3a	107(4)
N1-C2-N3	122.2(6)	O2'-C3'-H3b	112(3)
N1-C2-N7	116.7(6)	C4'-C3'-H3a	100(4)
N3-C2-N7	121.0(6)	C4'-C3'-H3b	110(4)
C2-N3-C4	120.7(6)	H3a-C3'-H3b	115(5)
N3-C4-C5	114.5(6)	C3'-C4'-O5'	110.7(6)
N3-C4-O8	122.1(6)	C3'-C4'-H4a	112(3)
C5-C4-O8	123.4(5)	C3'-C4'-H4b	109(4)
Br-C5-C4	117.4(4)	O5'-C4'-H4a	112(3)
Br-C5-N6	117.0(5)	O5'-C4'-H4b	102(4)
C4-C5-N6	125.6(6)	H4a-C4'-H4b	108(4)
N1-N6-C5	116.3(5)	C4'-O5'-C6'	115.6(5)
C2-N7-Ha	115 (3)	O8'-C6'-O5'	124.3(7)
C2-N7-Hb	125 (4)	O8'-C6'-C7'	123.5(6)
Ha-N7-Hb	116 (5)	O5'-C6'-C7'	112.1(6)
N1-C1'-O2'	111.9(5)	C6'-C7'-H7a	102(5)
N1-C1'-H1a	115 (3)	C6'-C7'-H7b	101(5)
N1-C1'-H1b	104 (4)	C6'-C7'-H7c	113(5)
O2'-C1'-H1a	94 (3)	H7a-C7'-H7b	92(6)
O2'-C1'-H1b	115 (4)	H7a-C7'-H7c	101(7)
H1a-C1'-H1b	115 (5)	H7b-C7'-H7c	137(6)

of proton resonances at δ 4.11 and 3.75 ppm were attributed to the 4'-CH_2 and 3'-CH_2 respectively. Deacetylation of **2** with methanolic ammonia afforded 5-chloro-1-[(2-hydroxyethoxy)methyl]-6-azauracil (**3**) in a good overall yield. Glycosylation of the persilylated derivative from 5-bromo-6-azaisocytosine (**4**) with one molar equivalent of (2-acetoxyethoxy)methyl bromide gave the desired 1-[(2-acetoxyethoxy)methyl]-5-bromo-6-azaisocytosine (**5**). A view of a single molecule of **5** is given in Figure 1. As can be seen in the figure, the glycosylation occurs at N^1. The crystal data and the atomic parameters of all non-hydrogenic atoms are listed in Table 1 and Table 2 respectively. Bond lengths and bond angles are presented in Tables 3. Deacetylation of **5** with methanolic ammonia afforded 5-bromo-1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine (**6**) in a good overall yield.

Experimental

Melting points were determined on a YANACO micromelting point apparatus and are uncorrected. The ultraviolet absorption spectra were obtained on a Beckman UV-Visible spectrophotometer. Infrared spectra were recorded on a Hitachi 260-30 spectrophotometer. Nuclear magnetic resonance (^{1}H and ^{13}C)spectra were obtained with a Varian Gemini-200 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane as an internal standard. Thin-layer chromatography was run on precoated (0.2 mm) silica gel 60 F-254 plates manufactured by EM Laboratories, Inc., and short-wave ultraviolet 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]-5-chloro-6-azauracil **2**

5-Chloro-6-azauracil (**1**, 1.48 g, 10 mmol) was suspended in hexamethyldisilazane (HMDS; 25 ml) and then a catalytic amount of chlorotrimethylsilane (ca. 2 ml) was added. The mixture was heated under reflux with the exclusion of moisture until a clear solution was obtained (ca. 4 h). 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 (60:1) and the proper fractions were combined and evaporated to give **2** (1.91g, 73% yield) which is pure enough for the next reaction. ^{1}H-NMR ($CDCl_3$): δ 2.00(s, 3, CH_3), 3.75 (t, 2, 3'-CH_2, J = 2.8 Hz), 4.11(t, 2, 4'-CH_2, J = 2.8 Hz), 5.18(s, 2, 1'-CH_2), 12.64(br s, 1, NH); ^{13}C-NMR ($CDCl_3$): δ 20.65(CH_3), 62.92(C-4'), 66.98(C-3'), 79.01(C-1'), 136.62(C-5), 148.75(C-2), 153.13(C-4), 170.32(CO); UV λ_{max}(log ε): 273(3.84) (0.1 N HCl), 259(3.86) (H_2O), 259(3.85) (0.1 N NaOH). Anal. Calcd for $C_8H_{10}ClN_3O_5$: C, 36.45; H, 3.82; N, 15.94. Found: C, 36.08; H, 3.91; N, 15.80.

5-Chloro-1-[(2-hydroxyethoxy)methyl]-6-azauracil **3**

A solution of **2** (1.32 g, 5 mmol) in methanolic ammonia (previously saturated at 0°C; 150 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 (8:1) as an eluent. The homogenous fractions were pooled and evaporated. The residue was crystallized from ethanol to provide **3** (0.81 g, 81% yield). mp 144 - 145°C; ^{1}H-NMR (DMSO-d_6): δ 3.47 & 3.51(m, 4, OCH_2CH_2O),

5.11(s, 2, 1'-CH_2); ^{13}C-NMR (DMSO-d_6): δ 61.41(C-4'), 71.88(C-3'), 80.32(C-1'), 138.13 (C-5), 155.51(C-2), 159.88(C-4). MS, *m/z* 222(M^+); UV λ_{max}(log ε): 272(3.75) (0.1 N HCl), 260(3.89) (H_2O), 259(3.69) (0.1 N NaOH). Anal. Calcd for $C_6H_8ClN_3O_4$: C, 32.52; H, 3.64; N, 18.96. Found: C, 32.08; H, 3.69; N, 18.66.

1-[(2-Acetoxyethoxy)methyl]-5-bromo-6-azaisocytosine **5**

5-bromo-6-azaisocytosine (1.91 g, 10 mmol) was treated as described above for the preparation of **2** to yield the desired pure product **5** (1.53g, 50%). mp 164-165°C; ^{1}H NMR(DMSO-d6): δ 1.99(s, 3, CH_3), 3.76 (t, 2, 3'-CH_2, *J* = 2.8 Hz), 4.12(t, 2, 4'-CH_2, *J* = 2.8 Hz), 5.29(s, 2, 1'-CH_2), 7.62(s, 2, NH_2). ^{13}C-NMR (DMSO-d6): δ 20.74 (CH_3), 63.35(C-4'), 67.14(C-3'), 82.63(C-1'), 136.39(C-5), 156.40(C-2), 159.25(C-4), 171.69(CO). MS, m/z 307, 309 (M^++1); UV λ_{max}(log ε): 259(3.78) (0.1 N HCl), 260(3.97) (H_2O), 229(4.14) (0.1 N NaOH). Anal. Calcd for $C_8H_{11}BrN_4O_4$: C, 31.29; H, 3.61; N, 18.24. Found: C, 31.26; H, 3.65; N, 18.25.

5-Bromo-1-[(2-hydroxyethoxy)methyl]-6-azaisocytosine **6**

Compound **5** (0.77 g, 2.5 mmol) was deacetylated by the same manner as described for the preparation of **3**. The crude product obtained was crystallized from EtOH to give **6** (0.56 g, 85%). mp 194-195°C; ^{1}H-NMR(DMSO-d6): δ 3.52(t, 2, 3'-CH_2), 3.56(t, 2, 4'-CH_2), 4.73 (br s, 1, 4'-OH), 5.28(s, 2, 1'-CH_2), 7.59(s, 2, NH_2); ^{13}C-NMR (DMSO-d6): δ 60.17(C-4'), 70.64(C-3'), 82.57(C-1'), 135.42(C-5), 155.46(C-2), 158.21(C-4); MS, m/z 265, 267 (M^++1); UV λ_{max}(log ε): 261(3.82) (0.1 N HCl), 259(3.76) (H_2O), 229(4.21) (0.1 N NaOH). Anal. Calcd. for $C_6H_9BrN_4O_3$: C, 27.19; H, 3.42; N, 21.14. Found: C, 27.93; H, 3.59; N, 21.26.

Acknowledgments

We gratefully acknowledge financial support from the National Science Council of the Republic of China (NSC 75-0412-B037-12)

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Received May 30, 1994
Accepted July 4, 1994