STEREOSPECIFIC SYNTHESIS OF NOVEL 1,3-THIAZOLIDIN-2-YL ANALOGUES OF PSEUDOURIDINE

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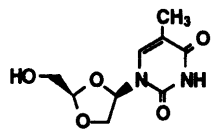
The stereospecific synthesis is described of the first member of a new class of C-nucleoside analogues in which the sugar ring is replaced with a 1,3-thiazolidine ring. The 1,3-thiazolidin-2-yl analogues (**1**) and (**2**) of pseudouridine were prepared stereospecifically *via* condensation of L or D-cysteine with a formylated nucleobase.

KEY WORDS pseudouridine; 1,3-thiazolidine; stereospecific synthesis

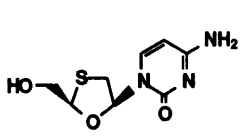
Many nucleoside analogues have been synthesized and evaluated as antiviral or anticancer agents. In a wide variety of the methodologies of their modifications, we have been interested in the nucleoside analogues which contain more than one heteroatoms within the carbohydrate framework.[1] For example, Dioxolane-T[2] is a thymidine analogue in which the deoxyribose is replaced by a 1,3-dioxolane ring, and BCH-189[3] is a cytidine analogue in which the ribose is replaced by a 1,3-oxathiolane ring. Both nucleoside analogues exhibit remarkable antiviral activity. Interestingly, of all stereoisomers of BCH-189, 3TC,[4] the (2*R*,5*S*) isomer, having the antipodal configuration to natural nucleosides, has the most excellent anti-HIV activity (highest activity and lowest cytotoxicity), and is currently undergoing clinical evaluation for anti-HIV and -HBV activities.[4]

On the other hand, since the discovery of pseudouridine from *t*-RNA by Cohn *et al.* in 1959,[5] many natural and synthetic C-nucleoside analogues have been reported, and some of them have been shown to exhibit interesting bioactivity including antiviral or anticancer activity.[6] However, few C-nucleoside analogues having a modified sugar unit have ever been reported.[7]

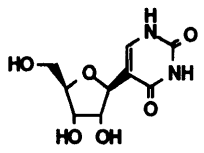
Therefore, we planned the design and synthesis of a new class of C-nucleoside analogues in which the carbohydrate ring is replaced by another saturated heterocycle, which is of potential medicinal interest. In this paper, we report the stereospecific synthesis of novel pseudouridine analogues (**1**) and its enantiomer (**2**), in which the ribose is replaced by a 1,3-thiazolidine ring.



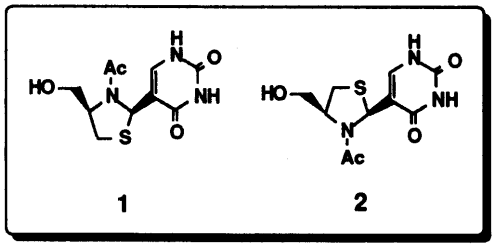
(-)-dioxolane-T



(-)-BCH-189 (3TC)



pseudouridine



1 **2**

The key compound for the synthesis of **1** is compound **6**, where two chiral centers (C-2, C-4) are in the 1,3-thiazolidine ring (Chart 1). The C-4 asymmetric carbon of **6** would be introduced from L-cysteine by condensation with a nucleobase having a formyl group, because it has been well known that the condensation of cysteine with formaldehyde gives 4-thiazolidinecarboxylic acid.[8] The second problem, the stereostructure at C-2, would be solved by N-acylation at 3-NH of the 1,3-thiazolidine ring, because it is known that acylation of the diastereomeric mixture of 2,4-disubstituted 1,3-thiazolidines under adequate conditions affords N-acylated 2,4-*cis* isomer exclusively by

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epimerization at C-2.[9]

To protect and solubilize the pyrimidine base, the *t*-butyl group which had been used in the synthesis of pseudouridine was selected.[10] Lithiation and following formylation of **4**, obtained from 5-bromouracil (**3**) in two steps,[10] gave the aldehyde **5** in 80% yield.[11] Then, the thiazolidine ring was constructed by condensation of L-cysteine with **5** in 50% ethanol, and subsequent acetylation with excess acetyl chloride *in situ* gave **6** quantitatively. The complete purification of **6** was impossible because its carboxyl group deprotected *t*-butyl groups of the pyrimidine base during the purification procedures, but it was confirmed by the ^{1}H-NMR spectrum that **6** was obtained as the only diastereomer.[12] After the conversion of **6** to the mixed anhydride, it was reduced to **7** with $NaBH_4$ in THF. The alcohol **7** was isolated by flash column chromatography on silica gel (34% yield from **5**).[13] Subsequent removal of *t*-butyl protecting groups of **7** under mild acidic conditions (AcOH:MeOH = 2:1, at room temperature) gave the desired pseudouridine analogue **1** as colorless needles in 69% yield.[14, 15] Its enantiomer **2** was synthesized by the same procedure except for utilizing D-cysteine.[16,17]

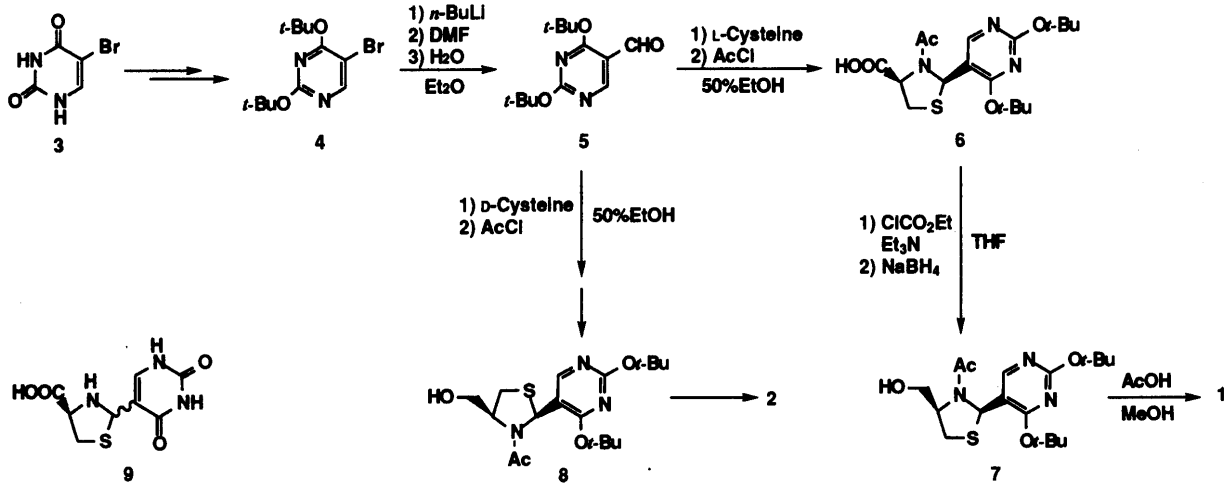

Chart 1

The absolute configuration of **1** was established to be *cis* (2*R*,4*R*) by X-ray crystallographic analysis of **7** (Fig. 1).[18] The enantiomeric purities of **7** and its enantiomer **8** were determined as >99.9% ee by the HPLC method on a chiral stationary phase (CHIRALCEL OD).

The ^{1}H-NMR spectrum of **1** in DMSO-d_6 exhibited hindered internal rotation about the N-acetyl bond. Because the rate of interconversion between two rotational conformers is sufficiently slow on the NMR time-scale at room temperature, one set of signals arising from the two conformers is observable. These signals were coalesced completely at 363K.[9,19]

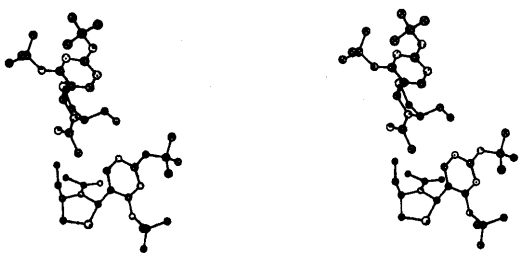

Fig. 1. X-ray Structure of **7** (Stereoview)

In summary, we report the first stereospecific synthesis of optically active 1,3-thiazolidin-2-yl C-nucleoside analogues (**1**) and (**2**) *via* condensation of L- or D-cysteine with a formylated nucleobase.

Compounds **1**, **2**, **7** and **8** were tested for antiviral[20] and anticancer[21] activities, but no activity or toxicity was found for these compounds.

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11) All new compounds gave satisfactory analytical and spectroscopic characteristics.
12) ^{1}H-NMR (270 MHz, $CDCl_3$) of compound **6** shows that it is the mixture of two rotational comformers, major: δ 1.62 (9H, s, *t*-Bu), 1.67 (9H, s, *t*-Bu), 2.21 (3H, s, $COCH_3$), 3.36 (1H, dd, *J*=9.6, 12.2 Hz, C_5-H), 3.51 (1H, dd, *J*=6.9, 12.2 Hz, C_5-H), 4.78 (1H, dd, *J*=6.9, 8.3 Hz, C_4-H), 6.39 (1H, s, C_2-H), 8.95 (1H, s, C_6-H); minor: δ 1.62 (9H, s, t-Bu), 1.67 (9H, s, *t*-Bu), 2.08 (3H, s, $COCH_3$), 3.21 (2H, dd, *J*=9.6, 12.2 Hz, C_5-H), 3.38 (2H, dd, *J*=6.9, 12.2 Hz, C_5-H), 4.94 (1H, dd, *J*=6.9, 9.6 Hz, C_4-H), 6.01 (1H, s, C_2-H), 9.06 (1H, s, C_6-H). One set signals of these comformers coalesced at 343K.
13) Compound **7**: m.p. 155-157 °C, $[\alpha]_D^{19.5} = +276.14°$(c 1.00, MeOH)
14) Compound **1**: m.p. 283 °C (dec.)
15) We observed that the target compound **1** decomposed readily under deacylation conditions. It is well known that pyrrolidine-modifying nucleosides decompose by deprotection of N-acyl, -Boc and -Z groups.[22] Then the biological activities are examined without deprotection. In our work, omitting acylation after the condensation of **5** and L-cysteine gave the diastereomeric mixture (1:1)[9b] of **9**, which showed no antiviral and anticancer activities.
16) Compound **8**: m.p. 155-157 °C, $[\alpha]_D^{19.2} = -265.24°$(c 1.00, MeOH)
17) Compound **2**: m.p. 283 °C (dec.)
18) *Crystal data for* **7**: $C_{18}H_{29}N_3O_4S$, M=383.51, monoclinic, space group $P2_1$, *a*=10.907(3), *b*=11.118(2), *c*=17.274(9) Å, β=91.04(4)°, *V*=2094.4 Å^3, *Z*=4, and *Dc*= 1.216 gcm^{-3}. The reflection data of 2460 reflections with 0<θ<45° were collected on a Rigaku AFC-5 diffractometer using monochromated Mo*Kα* radiation and ω–2θ scan technique ($0 \le h \le 11$, $0 \le k \le 11$, $-18 \le l \le 18$). The structure was solved by the direct method and refined by the full-matrix least-squares method. The final *R* value was 0.0475 for 2603 observed reflections [$F > 3\sigma(F)$].
19) The same phenomenon has already been observed in the ^{1}H-NMR spectra of N-acetylated thiazolidines.
20) HIV in MT-4 cells and type A Influenza virus in MDBK cells
21) CCD-19Lu, CCRF-CEM, p388, p388/ADM, p388/CPT, B16, Lewis, Lu-65, Lu-99, A549, RERF-LC-AI and HT-29 cell lines
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