Synthesis and antiviral studies of a novel isodideoxynucleoside: an analogue of the antiviral compound, tiazofurin

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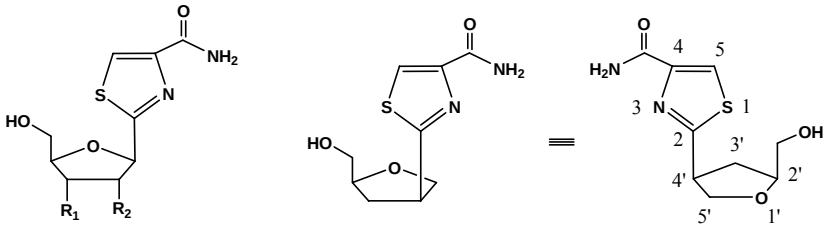
Abstract

The synthesis and antiviral studies of an isomeric dideoxynucleoside analogue of tiazofurin is described. ^{1}H and ^{13}C NMR studies, including ^{1}H-NOE, 2D-NOESY and [^{1}H, ^{13}C] gated-decoupling methods, were used to provide unequivocal evidence for the structure of the target compound.

Keywords: Tiazofurin, isodideoxynucleoside, synthesis, NMR, antiviral

Introduction

The discovery, that tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, **1** possesses antitumor activity[1] and is also endowed with broad-spectrum antiviral activity,[1,2] led to the synthesis of several tiazofurin derivatives, including analogs **2** and **3**.[3-5] In more recent studies, a series of isomeric dideoxynucleosides was synthesized in our laboratory.[6-8]



1. $R_1 = R_2 = OH$

2. $R_1 = OH, R_2 = H$

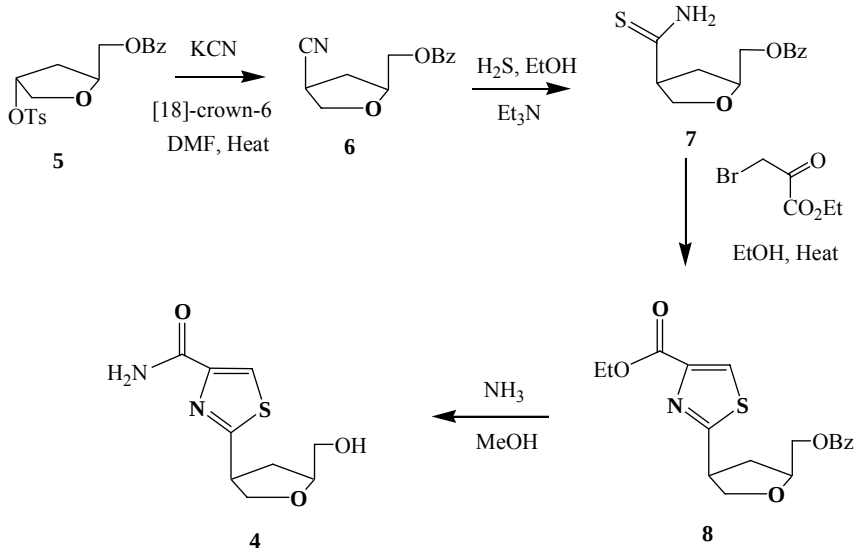
3. $R_1 = R_2 = H$

4

Among them was (-) isodideoxyadenosine, a compound which exhibited potent antiviral activity against HIV-1 and HIV-2.[9] In our continuing search for new anti-HIV agents, we synthesized and studied the unusual isomeric C-dideoxynucleoside, 4(S)-(4-carbamoyl-thiazol-2-yl)-tetrahydro-2(S)-furan-methanol **4** and this paper describes the results of our synthesis and antiviral studies.

Results and Discussion

Of the several synthetic procedures for obtaining thiazole derivatives, the reaction of thioamides with α-halocarbonyl derivatives is the best known.[10] Thus, the most appropriate starting material in the synthesis of **4** (Scheme 1) was the dideoxyribose **5**.[8] Reaction of **5** with potassium cyanide and [18]-crown-6 in dry DMF for 24h at 95° C yielded two nitriles **6** (67%) and its α-isomer (5%). Nitrile **6** was converted into thioamide **7** (58%) by treatment with hydrogen sulfide in the presence of triethylamine for 8h. Conversion of thioamide **7** into thiazole ester **8** was performed with freshly distilled ethyl bromopyruvate in refluxing ethanol. Surprisingly, two esters, **8** (48%) and its trans-isomer with the base in the α-configuration (18%), were formed. The inversion of configuration, while unexpected, may be attributed to an acid-catalyzed isomerization. Treatment of **8** with methanolic ammonia led to the tiazofurin analogue **4** in 79% yield.



Scheme 1

Assignment of the configuration at C-4 of compounds **6** and **7** was made on the basis of ^{1}H-NOE difference spectroscopy. Upon irradiation of H-4, compounds **6** and **7** showed marked NOE effect on H-2α, whereas the α-isomer did not show enhancement of the resonance of H-2. In addition, for compound **6**, irradiation of H-2 showed an NOE enhancement of 3.4% in H-4, much larger compared to the *trans*-isomer with the α-cyano group. These and other NOE results

were confirmed by assignments made on the basis of 2D-NOESY NMR data. For example, for the *trans*-diastereoisomer of **4** (with α-base), 2D-NOESY NMR spectrum showed a cross-peak between H-4' and –$\underline{CH_2}$OH, whereas this cross-peak was absent from the 2D-NOESY spectrum of **4**. Assignment of the ^{13}C data was made on the basis of [^{1}H, ^{13}C] gated-decoupled NMR spectra.

In vitro anti-HIV studies on the isodideoxytiazofurin **4** in HIV-1 infected cell lines revealed that it was inactive but relatively non-toxic. Antiviral studies against other viruses are planned.

Experimental Section

General Procedures. Melting points reported are uncorrected and were determined on an Electrothermal Engineering Ltd. Melting point apparatus. Ultraviolet (UV) spectra were recorded on a Cary 3 UV-Visible spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on a AC-300 and WM-360 instruments. Column chromatographic separations were carried out using 230-400 mesh silica gel. Purities of intermediates and final products were determined by a combination of ^{1}H and ^{13}C NMR spectra, quantitative UV data and chromatographic analysis.

2(S)-(Benzoyloxymethyl)-4(*R*)-cyano-tetrahydrofuran (6). To a solution of compound **5** (500 mg, 1.33 mmol) in dry DMF (10 ml) was added potassium cyanide (135 mg, 2.07 mmol) and [18]-crown-6 (360 mg, 1.36 mmol). The solution was stirred at 95°C for 24h and the solvent was evaporated under reduced pressure. The oily residue was chromatographed on silica gel (column: 20 x 3 cm, solvent: $CHCl_3$/EtOAc 9:1). The slower migrating zone yielded **6** as colorless oil (190 mg, 62%). TLC ($CHCl_3$/EtOAc 9:1): R_f = 0.7. ^{1}H-NMR ($CDCl_3$): δ 2.11 (m, *1H*, H_β-3); 2.50 (m, *1H*, H_α-3); 3.15 (m, *1H*, H-4); 4.01 (dd, *1H*, J = 7.3, 9.0 Hz, C*H*$_2$OBz); 4.10 (dd, *1H*, J=6.1, 8.8 Hz, C*H*$_2$OBz); 4.31 (m, *1H*, H-2); 4.37 (dd, *1H*, J = 4.5, 11.7 Hz, H_α-5); 4.47 (dd, *1H*, J = 3.7, 11.7 H_β-5); 7.42 (t, *2H*, J = 7.7 Hz, arom. H); 7.52 (t, *1H*, J = 7.5 Hz, arom. H); 8.04 (d, *2H*, J = 7.7 Hz, arom. H). From the faster migrating zone, the α-isomer was obtained as a colorless oil (15 mg, 5%). TLC ($CHCl_3$/EtOAc 9:1): R_f = 0.75. ^{1}H-NMR ($CDCl_3$): δ 2.19 (m, *1H*, H_β-3); 2.46 (m, *1H*, H_α-3); 3.16 (m, 1H, H-4); 3.94 (dd, *1H*, J=7.0, 8.8 Hz, C*H*$_2$OBz); 4.03 (dd, *1H*, J=7.4, 8.9 Hz, C*H*$_2$OBz); 4.21 (dd, 1H, J=7.2, 11.7 Hz, H_β-5); 4.32 (dd, *1H*, J=5.4, 11.7 Hz, H_α-5); 4.46 (m, *1H*, H-2); 7.43 (t, *2H*, J=7.8 Hz, arom. H); 7.56 (t, *1H*, J=7.5 Hz, arom. H); 8.05 (d, *2H*, J=7.8 Hz, arom. H). ^{13}C-NMR ($CDCl_3$): δ 28.6, 32.7, 65.2, 70.3, 77.0, 120.1, 128.4, 129.5, 129.7, 133.2, 166.2.

2(*S*)-(Benzoyloxymethyl)-4(*S*)-thiocarbamoyl-tetrahydrofuran (7). A solution of **6** (250 mg, 1.08 mmol) in ethanol (1.8 ml) and triethylamine (200 µl) was maintained at room temperature while hydrogen sulfide was bubbled into the solution for 6h. The solvent was removed under reduced pressure and the oily residue was purified by flash chromatography (column: 15 x 3 cm; solvent: $CHCl_3$/MeOH 95:5). Isolation of the main zone furnished **7** as a light yellow oil (150 mg, 52%). TLC ($CHCl_3$/MeOH 95:5):R_f = 0.4. ^{1}H-NMR ($CDCl_3$): δ 2.16 (m, *1H*, H_β-3); 2.48 (m,

1H, H_α-3); 3.53 (m, *1H*, H-4); 3.90 (dd, *1H*, J = 6.8, 9.2 Hz, C*H*$_2$OBz); 4.13 (dd, *1H*, J = 4.8, 9.2 Hz, C*H*$_2$OBz); 4.30 (m, *1H*, H-2); 4.42 (dd, *1H*, J = 5.9, 11.9 Hz, H_α-5); 4.52 (dd, *1H*, J = 3.3, 11.9 H_β-5); 7.44 (t, *3H*, J = 7.8 Hz, 2 arom. H, 1 N*H*$_2$); 7.57 (t, *1H*, J = 7.6 Hz, arom. H); 7.75 (s, br, 1*H*, N*H*$_2$); 8.03 (d, *2H*, J = 8.4 Hz, arom. H). ^{13}C-NMR ($CDCl_3$): δ 35.4, 53.2, 65.5, 73.1, 77.5, 128.4, 129.5, 129.7, 133.2, 166.5, 210.5.

2-[2(*S*)-(Benzoyloxymethyl)tetrahydrofuran-4(*S*)-yl]-thiazole-4-carboxylic ethyl ester (8). A mixture of **7** (100 mg, 0.38 mmol) and ethyl bromopyruvate (160 µl 223 mg, 1.15 mmol) in ethanol (1 ml) was refluxed for 6h and the solvent was evaporated and the oily residue was purified by flash chromatography (column: 20 x 3 cm; solvent: $CHCl_3$/EtOAc 9:1). From the slower migrating zone, **8** was isolated as a colorless oil (65 mg, 48%). TLC ($CHCl_3$/EtOAc 9:1): R_f = 0.2. ^{1}H-NMR ($CDCl_3$): δ 1.38 (t, *3H*, J = 7.1 Hz, C*H*$_3$); 2.11 (m, *1H*, H_β-3′); 2.68 (m, *1H*, H_α-3′); 3.98-4.08(m, *2H*, C*H*$_2$OBz); 4.24 (m, *1H*, H-4′); 4.36-4.44 (m, *4H*, H_β-5′, H-2′, OC*H*$_2$CH$_3$); 4.50 (m, 1H, H_α-5′); 7.41 (t, *2H*, J = 7.7 Hz, arom. H); 7.54 (t, *1H*, J = 7.4 Hz, arom. H); 8.02 (d, *2H*, J = 8.3 Hz, arom. H); 8.05 (s, *1H*, H-5). The faster migrating zone (α-isomer) yielded (25 mg, 18%) as slight yellow oil. TLC ($CHCl_3$/EtOAc 9:1): R_f = 0.25. ^{1}H-NMR ($CDCl_3$): δ 1.37 (t, *3H*, J = 7.1 Hz, C*H*$_3$); 2.35 (m, *1H*, H_β-3′); 2.45 (m, *1H*, H_α-3′); 4.00 (m, *2H*, C*H*$_2$OBz); 4.31-4.44 (m, *5H*, H-5′, H-2′, OC*H*$_2$CH$_3$); 4.57 (m, 1H, H-4′); 7.42 (t, *2H*, J = 7.6 Hz, arom. H); 7.54 (t, *1H*, J = 7.4 Hz, arom. H); 8.04 (d, *2H*, J = 7.9 Hz, arom. H): 8.07 (s, *1H*, H-5). ^{13}C-NMR ($CDCl_3$): δ 14.3, 35.5, 43.6, 61.5, 66.2, 73.6, 76.5, 127.1, 128.4, 129.6, 129.7, 133.2, 146.0, 161.2, 166.4, 172.2.

4(*S*)-(4-Carbamoyl-thiazol-2-yl)-tetrahydro-2(*S*)-furanmethanol (4). A solution of compound **8** (100 mg, 0.28 mmol) in methanolic ammonia (2 ml, MeOH saturated with NH_3 at 0°C) was stirred at r.t. for 60h. Then, the solvent was evaporated and the oily residue was purified by FC (column: 10 x 3 cm, solvent: $CHCl_3$/MeOH 95:5). The main zone was evaporated yielding compound 4 (50 mg, 79%) as colorless oil. TLC ($CHCl_3$/MeOH 95:5): R_f = 0.2 UV (MeOH): 233 (6900). ^{1}H-NMR ($CDCl_3$): δ 2.12 (m, *1H*, H_β-3′); 2.47 (m, *1H*, H_α-3′); 2.94 (s, br, *1H*, O*H*); 3.64 (m, *1H*, H-4′); 3.79-3.87 (m, *2H*, C*H*$_2$OH); 4.02 (m, *1H*, H_α-5′); 4.13-4.20 (m, *2H*, H_β-5′, H-2′); 6.24 (s, br, *1H*, CON*H*$_2$); 7.16 (s, br, *1H*, CON*H*$_2$); 8.02 (s, *1H*, H-5). ^{13}C-NMR ($CDCl_3$): δ 34.7, 43.5, 64.0, 73.1, 80.4, 123.9, 149.1, 163.0, 171.8. Anal. Calc. for $C_9H_{12}N_2O_3S$ 0.75H_2O: C, 44.71; H, 5.63; N, 11.59. Found: C, 44.92; H, 5.02, N, 11.40

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