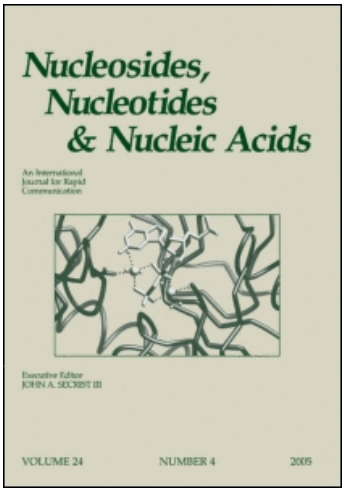

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SYNTHESIS AND ANTIVIRAL (RNA) EVALUATION OF NUCLEOSIDE ANALOGS OF TIAZOFURIN MODIFIED AT THE CARBOXAMIDE MOIETY

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Abstract: The carboxamide functionality of tiazofurin **1a** has been modified to produce the following analogs: carboximidates **5a,b**, carboxamidines **6**, **10**, tetrahydropyrimidine **7**, N-glycine **8** and N-glutamine **9**. These structural modifications abolished the *in vitro* antiviral (RNA) activity exhibited by tiazofurin against the flaviviruses (yellow fever and Japanese encephalitis viruses), bunyavirus (Punta Toro virus) and togavirus (Venezuelan equine encephalomyelitis virus). Only carboximidates **5a,b** retained marginal activity against bunyaviruses.

INTRODUCTION

The C-nucleoside, tiazofurin[1] [2-(β-D-ribofuranosyl)thiazole-4-carboxamide, **1a**] and its phosphate esters[2] exhibit antitumor activity against murine tumors including leukemias and the Lewis lung carcinoma. Antiviral activity is also observed.[1] Tiazofurin, and its selenium analog, selenazofurin **1b**, are metabolized to nicotinamide adenine

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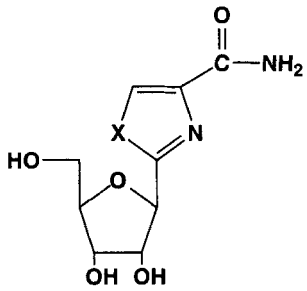
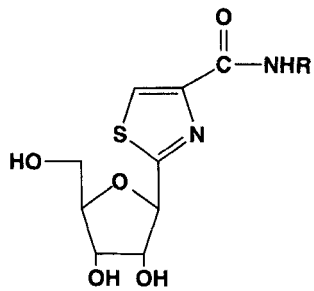
Dedicated to the spirits and memories of Dr. Michael J. Phelan, for his efforts and collegiality, and to Dr. Roland K. Robins, for his inspiration and pioneering nucleoside research.

dinucleotide[3] (NAD) analogs TAD and SAD, respectively, which are strong competitive inhibitors of inosine monophosphate dehydrogenase (IMPDH).[4] Resulting decreases in GTP and dGTP biosynthesis produce the inhibition of tumor cell proliferation observed in model systems. While tiazofurin produced significant antitumor activity in patients with myeloid blast crisis of chronic myeloid leukemia, phase I trials have revealed considerable neurotoxicity.[5] As an antiviral agent, tiazofurin exhibits a similar spectrum of *in vitro* activity to ribavirin but is much less potent.[6,7]



1a: X = S
1b: X = Se
1c: X = O



2a: R = – CH_2– $CONH_2$

2b: R = – CH – CH_2CONH_2
|
$CONH_2$

2c: R = – CH – $(CH_2)_2CONH_2$
|
$CONH_2$

Crystallographic studies of **1a** and derivatives generally reveal close attractive S-O interactions between the thiazole sulfur and the furanose ring oxygen O-1'.[8,9c] Structural modifications within the ribofuranosyl moiety of tiazofurin have been reported including preparation of deoxygenated,[9a] ara- and xylofuranosyl,[9b,9c] acyclic,[9d] pyranosyl,[9e] carbocyclic,[9f] and 5'-substituted[9g] analogs. Insertion of a sulfur atom between C-2 (thiazole) and C-1' (ribose)[9h] and replacement of the thiazole sulfur by oxygen (oxazole) to produce oxazofurin[9i] **1c** yielded minimal biological activity. Nucleoside peptides **2a-2c** have been prepared containing glycineamide[10a] **2a**, aspartic **2b** and glutamic acid diamide[10b] **2c** substituents at the C-4 carboxamide group of the thiazole ring. Despite the retention of the carboxamide functionality in these compounds, biological activity was abolished. A recent study[11] of ribavirin analogs showed that some degree of antiviral activity could be retained upon conversion of the triazole carboxamide moiety to

carboximidate and carboxamidine analogs. The carboxamidine analog of tiazofurin retains some biological activity.[2] Therefore, carboximidates **5a,b** and carboxamidines **6-10** have been synthesized and evaluated.

RESULTS AND DISCUSSION

Chemistry: The reaction sequences for the preparation of tiazofurin analogs **3-10** are outlined in Figure 1. Customary alcoholic sodium alkoxide treatment of acetylated carbonitrile **4**, 2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)thiazole-4-carbonitrile,[1] followed by acidification gave methyl- and ethyl-carboximidates **5a,b** with concurrent cleavage of the ribosyl acetates. In the course of this work, it was discovered that the preparation of unesterified carbonitrile **3** was unreported in the literature.[8] Cleavage of the acetates of **4** under mildly basic conditions (40% aq. dimethylamine in methanol, 25 °C) gave **3** in 20 h without accompanying carboximidate (**5a**) formation. Treatment of **4** with sodium methoxide/methanol gave **3** in 20 min at 25 °C, however formation of **5a** is a possible by-product. Methyl carboximidate **5a** served as the precursor of analogs **6, 8-10.** Carboxamidine **6** had previously been prepared[2] in 40% yield by treatment of acetylated carbonitrile **4** with one molar equivalent of dry ammonium chloride in liquid ammonia for 16 h at 80-85 °C. Treatment of **3** with 0.1 equiv. sodium methoxide in dry methanol at 25 °C for 24 h gave carboximidate **5a** (not isolated); subsequent refluxing (2-12 h) with excess ammonium chloride gave nearly pure **6** in 85% yield.[11] Incorporation of the amidine functionality within the 1,4,5,6-tetrahydropyrimidine ring **7** was achieved in 83% yield by condensation with 1,3-diaminopropane in refluxing absolute ethanol for 3 d. N-substituted amidines **8-10** were prepared by the condensation of primary amines with carboximidate **5a.**[12] In this manner, the amino acids glycine and glutamine were conjugated to the amidine to give **8** and **9** respectively, by heating (1-2 d) in dry methanol. Similarly, N-methylation was achieved by treatment of **5a** with methylamine in dry methanol at room temperature for 3-4 h.

Tiazofurin **1a** and analogs **3, 5a-10** were evaluated to determine their *in vitro* inhibitory properties against the following RNA viruses: Japanese encephalitis (JE), yellow fever (YF), and dengue type-4 viruses (flaviviruses), Punta Toro (PT), sandfly fever (SF) and Rift Valley fever[13] (RVF) viruses (bunyaviruses), Venezuelan equine encephalomyelitis (VEE) virus (togavirus), human immunodeficiency virus type-1[14]

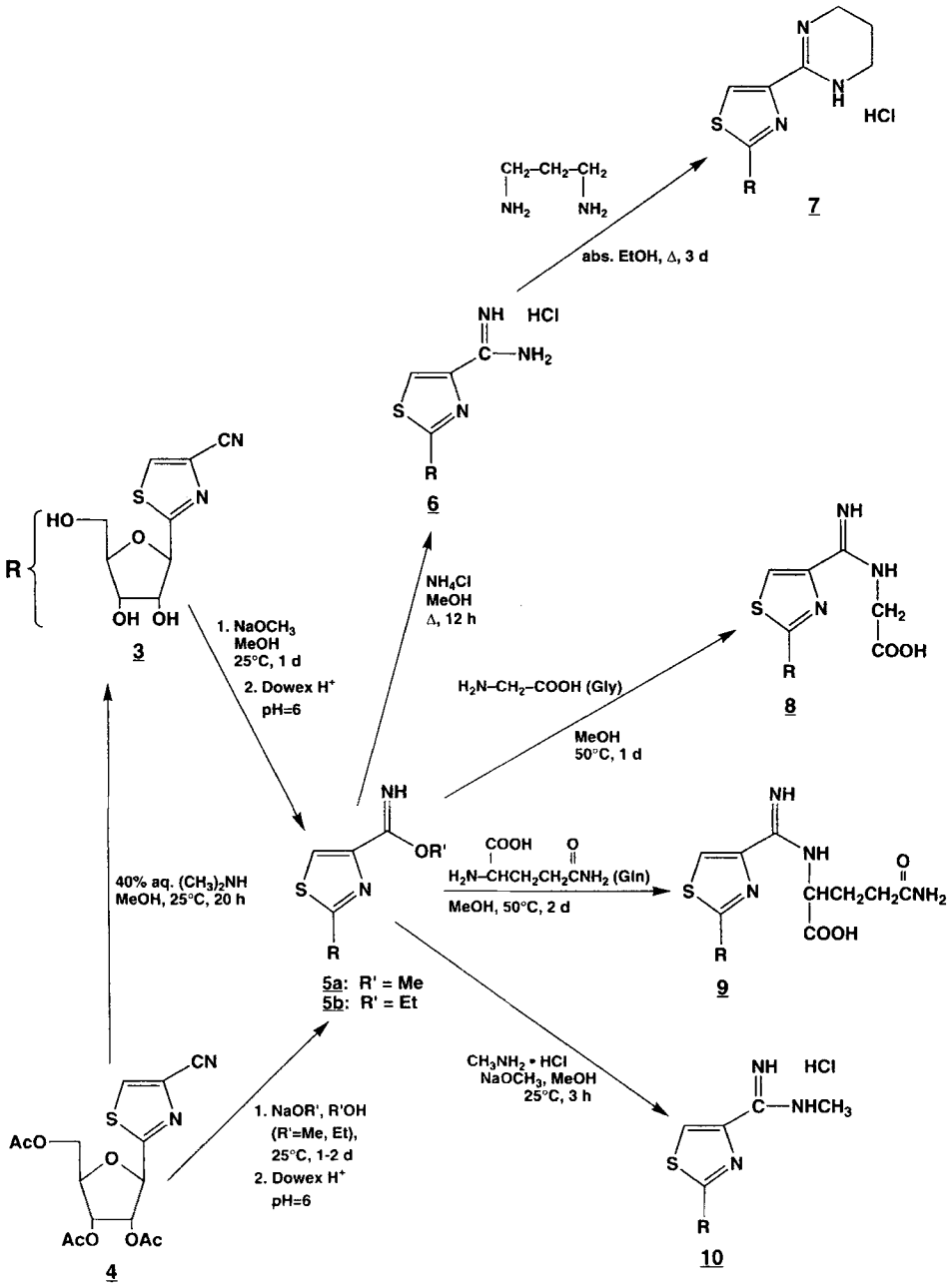


FIG. 1. Synthetic Routes for Tiazofurin Analogs

(HIV-1, retrovirus), vesicular stomatitis virus[6] (VSV, rhabdovirus) and the DNA-containing vaccinia virus (VV, poxvirus) and adenovirus type 2 (Ad2). Methodologies for evaluation of antiviral activity have been described elsewhere.[6,11,13,14] The antiviral assays used determined the 50% inhibition (IC_{50}) of virus-induced cytopathic effect (CPE) by an MTT assay; efficacy against dengue-4[11] and RVF viruses[13] was determined by plaque reduction assays. The concentration of test compound which was cytotoxic to 50% of uninfected cells (TC_{50}) was also determined. Activity is expressed as a therapeutic index, TI, a ratio of these values, (TC_{50}/IC_{50}).

In vitro antiviral activity of tiazofurin was originally observed[1] against type 3 parainfluenza and type 13 rhinoviruses (RNA) and type 1 herpes simplex virus (DNA). Subsequent evaluation[6] has demonstrated activity against JE, YF and vaccinia viruses. *In vitro* activity[7a] has also been noted against the bunyavirus, PT virus, (an RNA virus related to RVF and SF viruses). However, when administered to PTV-infected mice, tiazofurin is not as active, potent or selective as ribavirin or its carboxamidine analog.[7b] Activity of **1a** was absent or marginal against VSV, SF and RVF viruses. In order to provide a comparison for antiviral evaluation of tiazofurin analogs, **1a** was evaluated using MTT methodology. Marginal or no activity was observed against AD2, VSV and the bunyaviruses RVF and SF; while Punta Toro virus-induced CPE was reduced by only 30-50%. Activity was noted against the togavirus, VEE (average IC_{50} = 16.7 μg/mL, TI = 6). Activity against flaviviruses was less dramatic; reductions of JE- and YF virus-induced CPE were only 25-50%. Corresponding IC_{25} values of 57-77 μg/mL against YF virus and an average IC_{50} value of 74 μg/mL (TI = 1.4) against JE virus were observed. At 2.5 μg/mL, tiazofurin produced 96% plaque reduction in the dengue type 4 assay, however this concentration was also toxic to the MK-2 cells.

Modification of the tiazofurin carboxamide functionality to produce the nitrile **3**, carboxamidine **6** and N-methylcarboxamidine **10** virtually abolished all *in vitro* antiviral activity observed for **1a** against VEE, PT, JE, and YF viruses. In addition, **3**, **6**, and **10** were inactive against dengue-4, SF, RVF, HIV-1 (in both CEM-6 and MT-2 cells), VSV, VV and Ad2 viruses. Uninfected Vero cell toxicity was lowered to TC_{50} >1000 μg/mL, for **3** and **10** and 270-320 μg/mL for **6**. Conversion of the carboxamide group to the methyl (**5a**) and ethyl (**5b**) carboximidates produced only marginal activity for the methyl analog against Ad2 virus (IC_{50} = 180 μg/mL, TI = 1.8) and the bunyaviruses, PT, SF and

RVF viruses. Reductions of only 25-49% were observed in SF- and PT virus-induced CPE, IC_{25} = 70, 53 μg/mL, respectively. In the RVF virus assay, 49% plaque reduction was observed at a non-toxic concentration of 100 μg/mL however **5a** became toxic to Vero cells at 250 μg/mL. Incorporation of the carboxamidine functionality within a tetrahydropyrimidine ring (**7**) also produced no activity, with greater cell toxicity (TC_{50} = 100-200 μg/mL). Conjugation of the amino acids glycine and glutamine at the carboxamidine nitrogen produced **8** and **9**; both were devoid of antiviral activity and cellular toxicity was lowered (TC_{50} of >1000 and >3200 μg/mL, respectively).

As a point of comparison, tiazofurin **1a** was evaluated for *in vitro* antitumor activity in the National Cancer Institute 60 human tumor cell line panel.[15] Inhibitory activity was observed in the leukemia cell lines, including the P388 and the adriamycin-resistant P388 cell lines. Analogues evaluated included methylcarboximidate **5a**, carboxamidine **6**, tetrahydropyrimidine **7** and N-glutamine carboxamidine **9**. All were devoid of antitumor activity except for **9** which was inhibitory only to the growth of the HOP-18 cells in the non-small cell lung tumor panel; no activity was observed against the other seven lung cell lines. A possible explanation is that **9** may be acting as an anti-metabolite and blocking glutamine uptake by the HOP-18 cells, since no effect was observed in any other tumor cell line. In general, a portion of the observed variations in both antitumor and antiviral properties (e.g. potency) of these tiazofurin analogs appear to be cell-line dependent.

An antiviral (RNA) structure/activity study has been reported[11] for ribavirin, 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide. Modification of its carboxamide moiety to the carboxamidine and conjugation of the latter with amino acids (glycine, asparagine, glutamine) and with alkyl groups, led to some retention of antiviral activity (especially against bunyaviruses) with general decreased toxicity and potency.[11] In contrast, in the case of tiazofurin, any modification of the carboxamide functional group virtually eliminates all biological activity. However, amino acid-conjugated tiazofurin analogs similarly showed decreased cellular toxicity. Ribavirin inhibits *de novo* GTP biosynthesis by inhibiting IMPDH, blocking the enzyme at the IMP attachment site; whereas, tiazofurin blocks at the cofactor NAD/NADH site as its TAD analog.[3,16] This and other studies[10a,b] therefore strongly suggest that an unsubstituted carboxamide moiety appears to be a stringent requirement for the preservation of the biological activity of tiazofurin.

EXPERIMENTAL SECTION

All solvents were distilled before use and dried when necessary. All chemicals were reagent grade. Evaporations were conducted at bath temperatures $\leq$30 °C with a Buchi rotary evaporator under water aspirator or mechanical oil pump vacuum. Melting points were determined with a Mel-Temp capillary apparatus and are uncorrected. Elemental analysis data were obtained from Atlantic Microlab, Inc., Atlanta, GA. IR spectra were recorded using a Beckman AccuLab 2 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded with a Nicolet NMC NT-300 NB spectrometer operating at 300.65 MHz. Chemical shifts are expressed in parts per million referenced to tetramethylsilane (^{1}H NMR) or dimethylsulfoxide at 39.5 ppm (^{13}C NMR). Chemical shifts (δ) for multiples were measured from the appropriate centers. Coupling constants (J) are recorded in Hz. Thin layer chromatography (TLC) was performed on Woelm F silica gel sheets (254/366) with detection of products under a short wavelength UV lamp and/or spraying with 40% methanolic H_2SO_4 and charring. Preparative TLC was performed on prescored silica gel plates GHLF, 250 microns (Analtech Corp., Newark, DE).

2-(β-D-Ribofuranosyl)thiazole-4-carbonitrile (3). Method A: A methanolic (100 mL) solution of 2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)thiazole-4-carbonitrile[1] **4** (1 g, 2.4 mmol) and aqueous 40% dimethylamine (0.5 mL) was stirred at room temperature for 20 h at which time TLC (4:2:1 ethyl acetate / n-propanol / water) indicated the absence of **4.** The solvent was removed *in vacuo*, the residue dissolved in water (2 mL), placed on preparative TLC plates, eluted with the above solvent (R_f = 0.78), and removed from the support with ethanol. Removal of solvent *in vacuo* gave a semi-crystalline residue which was recrystallized from methanol providing **3** (0.50 g, 2.09 mmol, 87%) mp 129-130 °C. ^{1}H NMR (90 Mz, DMSO-d_6) δ 3.7 (m, 2H, H-5'), 3.92 (m, 3H, H-2', H-3', H-4'), 4.0-4.2 (m, 3H, OH), 5.03 (d, 1H, H-1'), 8.80 (s, 1H, H-5); IR (KBr) γ 3400, 3340, 3120, 2960, 2880, 2230, 1485, 1425, 1290, 1180, 1110, 1040, 950, 930, 880, 860, 770, 700 cm^{-1}; *Anal.* Calcd for $C_9H_{10}N_2O_4S$: C, 44.62; H, 4.13; N, 11.56; S, 13.23. Found: C, 44.71; H, 3.98; N, 11.46; S, 13.26. CA Registry #: 144660-78-6. **Method B:** Sodium methoxide (2.0 mg) was added to a solution of **4**[1] (0.5 g, 1.36 mmol) in methanol (6 mL) to bring the pH to 9.5. The solution was stirred for 20 min at 25 °C while monitoring by TLC to avoid formation of the methyl carboximidate. After completion, the solution was

neutralized with Dowex-H^+ resin, filtered, and evaporated to dryness. The resulting crystalline solid was recrystallized from methanol / ethyl acetate to yield **3** (0. 235 g, 70%), mp 129-131 °C.

Methyl 2-(β-D-ribofuranosyl)thiazole-4-carboximidate (5a). Compound **5a** was prepared as described for the ethyl analog **5b** except that sodium methoxide was used. The reaction was allowed to proceed at 25 °C for 1-2 days and was monitored by TLC (CH_2Cl_2 / MeOH, 6:1, R_f = 0.4 for **5a**). Workup as described gave a white solid (67% yield) mp 126-7 °C. ^{1}H NMR (DMSO-d_6) δ 3.57 (m, 2H, H-5'), 3.83 (s, 3H, OCH_3), 3.91 (m, 2H, H-2', H-3'), 4.05 (t, J = 3.9, 4.26 Hz, 1H, H-4'), 4.95 (d, 1H, J = 5.0 Hz, H-1'), 4.87, 5.04, 5.42 (3m, 3H, OH), 8.03 (s, 1H, H-5), 8.55 (brs, 1H, NH). ^{13}C NMR[17] (DMSO-d_6) δ 172.75 (C-2), 162.14 (C-4), 146.47 (C=NH), 121.39 (C-5), 84.98 (C-4'), 81.91 (C-1'), 76.94 (C-3'), 71.25 (C-2'), 61.81 (C-5'), 52.96 (OCH_3). *Anal.* Calcd for $C_{10}H_{14}O_5N_2S$: C, 43.79; H, 5.14; N, 10.21; S, 11.69. Found: C, 43.85; H, 5.15; N, 10.18; S, 11.62.

Ethyl 2-(β-D-ribofuranosyl)thiazole-4-carboximidate (5b). To a solution of 2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)thiazole-4-carbonitrile[1] **4** (5.0 g, 13.6 mmol) in dry ethanol (100 mL) was added a molar solution of freshly-prepared ethanolic sodium ethoxide (20.5 mL) and the resulting solution stirred at room temperature overnight. The solution was acidified with ethanol-washed Dowex H^+ resin. The resin was filtered off, and the filtrate concentrated to dryness *in vacuo.* The residue was chromatographed over silica gel (flash chromatography) with 10% ethanol in dichloromethane as eluent to yield an amorphous solid (2.97 g, 73%). ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, -$CH_2C\underline{H}_3$), 3.55 (m, 2H, H-5'), 3.88, 4.01 (2m, 2H, H-2', H-3'), 4.27 (q, 2H, -$C\underline{H}_2CH_3$), 4.64, 5.05, 5.39 (t, d, d, 3H, -OH), 4.93 (d, 1H, J = 5.0 Hz, H-1'), 7.99 (s, 1H, H-5), 8.48 (s, 1H, -NH). *Anal.* Calcd for $C_{11}H_{16}N_2SO_5$: C, 45.82; H, 5.59; N, 9.72; S, 11.12. Found: C, 45.81; H, 5.61; N, 9.65; S, 11.00.

2-(β-D-Ribofuranosyl)thiazole-4-carboxamidine hydrochloride (6). A solution of 2-(β-D-ribofuranosyl)thiazole-4-carbonitrile **3** (4 g, 16.4 mmol) and sodium methoxide (87 mg, 1.6 mmol) in dry methanol (25 mL) was stirred in the absence of moisture for 24 h at room temperature. TLC analysis (5:1 methylene chloride / methanol or 4:2:1 ethyl acetate / isopropanol / water, R_f of **3** = 0.4, 0.5, respectively) revealed the absence of **3.** Ammonium chloride (1 g, 1.1 equiv) was added and the solution refluxed overnight. The

absence of further evolution of ammonia and a TLC spot at the origin replacing that of **5a** indicated complete reaction. The reaction mixture, from which some product **6** had crystallized was cooled to 0° C, filtered and the crystalline material rinsed with cold methanol and ether. Drying *in vacuo* over P_2O_5 gave a white solid, mp 210-211 °C. The filtrate was evaporated *in vacuo* and the residue crystallized from isopropanol; total amount of **6**, 4.1 g (13.9 mmol, 84.6%). ^{1}H NMR[2] (DMSO-d_6) δ 3.55 (m, 2H, H-5'), 3.94 (m, 2H, H-2', H-3'), 4.12 (d, J = 3.84 Hz, 1H, H-4'), 5.00 (d, J = 5.04 Hz, 1H, H-1'), 4.92, 5.17, 5.45 (3m, 3H, OH), 9.07 (s, 1H, H-5), 9.42 (brs, 3H exchangeable, NH); ^{13}C NMR[17] (DMSO-d_6) δ 174.25 (m, C-2), 157.47 (d, J = 1.4 Hz, C-4), 141.98 (d, J = 5.5 Hz, C=N), 128.71 (d, J = 192.4 Hz, C-5), 85.17 (C-4'), 81.67 (C-1'), 76.90 (C-3'), 71.31 (C-2'), 61.61 (C-5'). *Anal.* Calcd for $C_9H_{14}O_4N_3SCl$: C, 36.55; H, 4.77; N, 14.21; S, 10.84; Cl, 11.99. Found: C, 36.60; H, 4.78; N, 14.26; S, 10.78; Cl, 12.08.

2-(β-D-Ribofuranosyl)thiazole-4-(1,4,5,6-tetrahydropyrimidin-2-yl) hydrochloride (7). A solution of carboxamidine **6** (1.0 g, 3.4 mmol), distilled 1,3-diaminopropane (255 mg, 3.44 mmol, 0.29 mL) in absolute ethanol (110 mL) was heated under reflux in the absence of moisture for 48 h. Reaction progress was monitored by TLC in 7:3 CH_3CN / 0.1 M NH_4Cl (R_f's = 0.14 and 0.23 for **6** and **7** respectively) as well as by the observation of evolved ammonia gas. An additional portion (26 mg, 0.345 mmol) of 1,3-diaminopropane was added and the mixture refluxed for an additional 24 h at which time the reaction was complete. The solvent was removed *in vacuo* and the residue washed with diethyl ether (50 mL, pre-dried over anhydrous $CaCl_2$). Removal of the ether *in vacuo* yielded a white solid (1.09 g, mp 174-9 °C). Successive recrystallizations from 1:1 isopropanol / methanol (50 mL) gave two crops of **7**, (0.95 g total, 2.83 mmol, 83.3%), mp 178-180 °C. ^{1}H NMR (DMSO-d_6) δ 1.96 (t, J = 4.5 Hz, 2H, H-4"), 3.49 (t, J = 5.4 Hz, 4H, H-3", H-5"), 3.57 (m, 2H, H-5'), 3.93 (m, 2H, H-2', H-3'), 4.11 (dd, 1H, H-4'), 4.92 (t, J = 5.3 Hz, 1H, OH), 4.98 (d, J = 5.1 Hz, 1H, H-1'), 5.17 (d, J = 4.9 Hz, 1H, OH), 5.45 (d, J = 5.7 Hz, 1H, OH), 9.07 (s, 1H, H-5), 10.36 (brs, 2H, NH); ^{13}C NMR (DMSO-d_6) δ 174.09 (C-2), 151.35 (C-4), 141.83 (C=N), 126.49 (C-5), 85.16 (C-4'), 81.66 (C-1'), 76.90 (C-3'), 71.31 (C-2'), 61.61 (C-5'), 38.35 (C-3"), 17.73 (C-4"). *Anal.* Calcd for $C_{12}H_{18}O_4N_3SCl$: C, 42.92; H, 5.40; N, 12.51; S, 9.55; Cl, 10.56. Found: C, 42.84; H, 5.45; N, 12.47; S, 9.45; Cl, 10.62.

2-(β-D-Ribofuranosyl)thiazole-4-N-acetoxycarboxamidine (8). A slurry of glycine (150 mg, 2 mmol) in dry methanol (50 mL) was added to a dry methanolic (20 mL) solution of methylcarboximidate **5a**. The mixture was stirred with warming for 1 h to dissolve the glycine then heated at 60 °C for 5 min. at which time TLC (7:3 CH_3CN / 0.1 M NH_4Cl) revealed only one spot. (Alternatively, the mixture can be stirred at 50 °C for 24 h.) A white crystalline solid precipitated. Filtration *in vacuo* and drying gave **8** (540 mg, 85%) mp 234-235 °C. ^1H NMR (D_2O/DMSO-d_6) δ 3.82 (m, 2H, C$\underline{H}_2$), 4.13 (s, 2H), 4.22 (m, 2H), 4.39 (t, J = 4.6 Hz, 1H), 4.82 (s, exchangeable H's), 5.22 (d, J = 5.4 Hz, 1H, H-1'), 8.55 (s, 1H, H-5); ^{13}C NMR[11] (DMSO-d_6) δ 174.53, 174.03 (COOH or C-2), 156.91 (dt, C-4), 143.71 (d, J = 5 Hz, C=N), 128.54 (d, J = 192 Hz, C-5), 86.49 (C-4'), 82.96 (C-1'), 77.99 (C-3'), 72.74 (C-2'), 63.17 (C-5'), 47.13 (t, J = 141 Hz, -CH_2-). *Anal.* Calcd for $C_{11}H_{16}O_6N_3S$: C, 41.64; H, 4.76; N, 13.24; S, 10.10. Found: C, 41.74; H, 4.76; N, 13.20; S, 10.01.

2-(β-D-Ribofuranosyl)thiazole-4-N-(α-carboxy-4'-butyramido)carboxamidine (9). Carboximidate **5a** (850 mg, 3.1 mmol) was added to a solution of L-glutamine (440 mg, 3.1 mmol) in dry methanol (120 mL). The resulting solution was refluxed excluding moisture for 2 d accompanied by the precipitation of a white crystalline solid. At this point, TLC (7:3 CH_3CN / 0.1 M NH_4Cl) indicated complete reaction. The solid was filtered under nitrogen, washed with cold 1:1 acetone-isopropanol and dried *in vacuo* over P_2O_5. A second crop of **9** was obtained from the chilled mother liquor after addition of an equal volume of acetone. In total, **9** was obtained as white needles (700 mg, 61%) mp 199-200 °C. (Alternatively, **9** was also obtained by evaporation of the crude reaction mixture *in vacuo* followed by trituration of the residual oil with dry ether for 24 h at 0 °C). ^1H NMR (DMSO-d_6) δ 1.95 (d, J = 5.8 Hz, 2H, -C$\underline{H}_2$$CH_2CO$), 2.20, 2.46 (2m, 2H, -$CH_2C\underline{H}_2$CO), 3.57 (dt, 2H, H-5'), 3.91 (m, 2H, H-2', H-3'), 4.08 (t, J = 4.7 Hz, 1H, H-4'), 5.00 (d, J = 4.9 Hz, 1H, H-1'), 5.45, 5.82 (2 x brs, exchangeable), 6.97, 7.61 (2s, 2 x 1H, exchangeable), 8.89 (s, 1H, H-5), 9.65 (brs, 2H, NH); ^{13}C NMR[11] (DMSO-d_6) δ 175.07, 174.61 (C-2 or C(O)NH_2), 169.99 (COOH), 152.93 (C-4), 142.32 (C=N), 126.78 (C-5), 85.15 (C-4'), 81.89 (C-1'), 77.12 (C-3'), 71.27 (C-2'), 61.68 (C-5'), 55.80 (NH$\underline{C}$HCOOH), 30.83 ($\underline{C}H_2CONH_2$), 26.59 (-CH$\underline{C}H_2CH_2$-). *Anal.* Calcd for $C_{14}H_{21}O_7N_4S$: C, 43.29; H, 5.19; N, 14.43; S, 8.25. Found: C, 43.39; H, 5.23; N, 14.37; S, 8.17.

2-(β-D-Ribofuranosyl)thiazole-4-N-methylcarboxamidine hydrochloride (10). A dry methanolic (10 mL) solution containing imidate **5a** (0.5 g, 1.82 mmol), sodium methoxide (9.4 mg, 0.174 mmol) and methylamine hydrochloride (crystalline, 136 mg, 2.01 mmol), was sealed and stirred for 3-5 h at room temperature. The reaction progress was monitored by TLC in either 6:1 CH_2Cl_2 / methanol (R_f's of **5a** and **10** = 0.36 and 0.04) or in 7:3 CH_3CN / 0.1 M NH_4Cl (R_f's 0.61 and 0.21, respectively). A white solid crystallized in part during the reaction and was filtered *in vacuo* and dried providing **10**, (0.295 g) mp 229-231 °C. The filtrate was evaporated *in vacuo* and the residue crystallized from isopropanol / methanol (as needed to solubilize) to produce additional **10** (0.248 g) in 96% overall yield. ^{1}H NMR (DMSO-d_6) δ 3.03 (s, 3H, CH_3), 3.61 (m, 2H, H-5'), 3.93 (m, 2H, H-2', H-3'), 4.12 (dd, 1H, H-4'), 4.92 (t, J = 5.3 Hz, 1H, OH), 4.99 (d, J = 5.1 Hz, 1H, H-1'), 5.16 (d, J = 4.8 Hz, 1H, OH), 5.46 (d, J = 5.6 Hz, 1H, OH), 9.09 (s, 1H, H-5), 9.77 and 10.06 (brs, 2H, NH); ^{13}C NMR (DMSO-d_6) δ 174.10 (C-2), 155.20 (C-4), 142.16 (C=N), 127.26 (C-5), 85.16 (C-4'), 81.65 (C-1'), 76.87 (C-3'), 71.31 (C-2'), 61.60 (C-5'), 29.19 (CH_3). *Anal.* Calcd for $C_{10}H_{16}O_4N_3SCl$: C, 38.77; H, 5.21; N, 13.57; S, 10.35; Cl, 11.44. Found: C, 38.85; H, 5.22; N, 13.52; S, 10.28; Cl, 11.52.

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