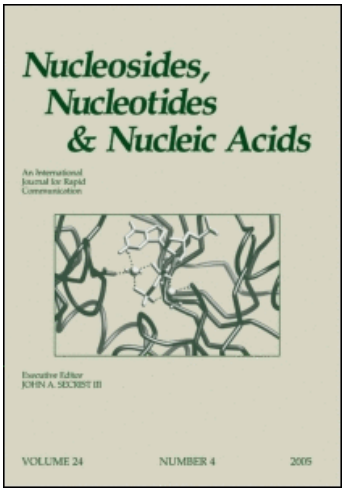

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NUCLEOSIDE PEPTIDES. 8. SYNTHESIS OF CERTAIN PEPTIDE DERIVATIVES OF RIBAVIRIN AND TIAZOFURIN

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ABSTRACT: The synthesis of certain peptide derivatives of ribavirin and tiazofurin in which the peptide linkage is on the carbamoyl group of the aglycon moiety has been accomplished. Condensation of 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic acid (3) with glycine ethyl ester, L-aspartic acid dimethyl ester, L-glutamic acid diethyl ester or L-phenylalanine methyl ester in the presence of HOBT/EDC gave the corresponding protected nucleoside peptide esters (4, 6, 8 and 10) respectively, which on ammonolysis furnished the glycineamide (5), aspartic acid diamide (7), glutamic acid diamide (9) and phenylalanineamide (11) derivatives of ribavirin. A similar treatment of 2-β-D-ribofuranosylthiazole-4-carboxylic acid with L-glutamic acid diethyl ester or L-aspartic acid dimethyl ester, followed by ammonolysis gave glutamic acid diamide (17) and aspartic acid diamide (13) derivatives of tiazofurin. None of these compounds exhibited any significant antitumor or antiviral activity in cell culture.

INTRODUCTION: The interactions between proteins and nucleic acids and binding of nucleotides to enzymes are among the most vital events in life processes.[1] Not only are electrostatic, Van der Waals, and hydrogen-bond interactions involved in these processes, but also there is considerable evidence[2] that covalent bonds other than those of the substrate may be formed and later broken in order to facilitate these enzymatic processes. Although there is considerable knowledge about the end products of these reactions, the mystery as to how these processes occur is yet to be fully solved.[3]

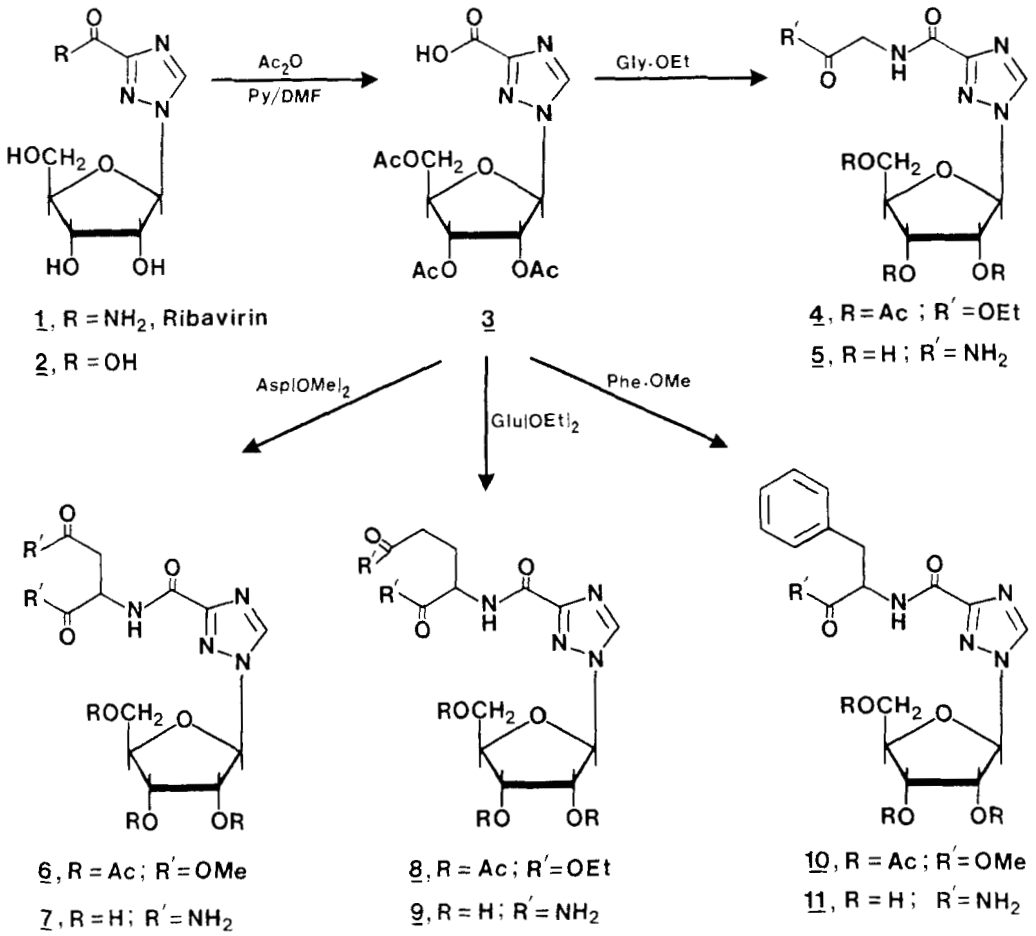
An approach to understanding and studying such inter- and intramolecular interactions through relatively simple models of nucleoside peptides was delineated in our previous publication.[4] The importance of such models serving as a link between major fields in the study of pro-

teins and nucleic acids has recently become much more apparent. Moreover, it seemed reasonable that these nucleoside peptides might well possess unique biological properties, which would prove useful as medicinal agents.[5] Thus, in continuation of a general program for the synthesis of nucleoside peptides[6], we now report the synthesis of certain peptide derivatives of ribavirin (4-11) and tiazofurin (12,13,16 and 17) in which the peptide linkage is on the carbamoyl group of the aglycon moiety.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, 1) prepared and reported from our laboratory[7] in 1972, has shown significant broad spectrum antiviral activity against both DNA and RNA viruses in vitro and in vivo.[8] The antiviral activity of 1 has been reviewed.[9-11] In 1986, FDA (of USA and Canada) approved the use of ribavirin aerosol for treating severe infections of respiratory syncytial virus (RSV), a disease often fatal to infants and children. However, ribavirin has low lipid solubility, which has been ascribed to its failure to adequately pass the blood-brain barrier. The ribavirin peptides (4-11) should offer a range of solubility, transport characteristics and lipophilic nature differing from ribavirin itself. Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, 14) is an oncolytic C-nucleoside synthesized and reported simultaneously from our laboratory[12] and by Fuertes et al.,[13] is a promising antitumor agent[14-17] currently undergoing Phase II clinical trials. Tiazofurin exhibits potent antitumor activity against murine tumors including leukemias and the Lewis lung carcinoma.[18-20] Tiazofurin was particularly chosen in this study in an effort to determine the effects of modification of the carbamoyl group, while still maintaining an amide function at the 4-position in the thiazole ring.

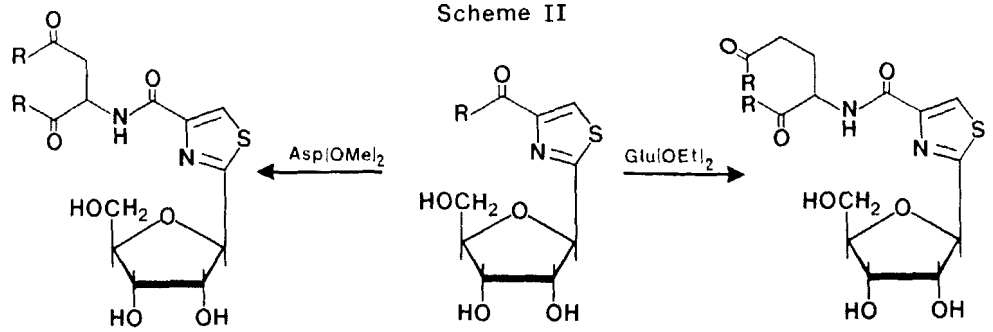
CHEMISTRY: The synthesis of these nucleoside peptides was accomplished in excellent yields via a two-step procedure involving the coupling of either 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid[7] (2) or 2-β-D-ribofuranosylthiazole-4-carboxylic acid (15) with an appropriate amino acid ester. Since the purification of the coupling product of 2 with an amino acid ester was found to be difficult, due primarily to the coelution of unreacted nucleosides, use of acetylated 2 in this re-

Scheme I



action was found to be beneficial. Thus, acetylation of 2 with acetic anhydride in pyridine/DMF solution gave a 64% yield of 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic acid (3, Scheme I). Compound 3 was coupled to glycine ethyl ester hydrochloride in DMF using 1-hydroxybenzotriazole (HOBT) and the water soluble carbodiimide 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC). The resulting 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxyglycine ethyl ester (4) was separated by flash silica gel column chromatography. Treatment of 4 with methanolic ammonia resulted in the ammonolysis of the ester function with concomitant deacetylation to give

Scheme II



12, R = OMe
13, R = NH_2

14, R = NH_2, Tiazofurin
15, R = OH

16, R = OEt
17, R = NH_2

1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxyglycineamide (5). A similar condensation of 3 with L-aspartic acid dimethyl ester, L-glutamic acid diethyl ester or L-phenylalanine methyl ester in the presence of HOBT and EDC gave the corresponding protected nucleoside peptide esters (6, 8 and 10, respectively) in excellent yields. Further ammonolysis of 6, 8 and 10 with MeOH/NH_3 readily gave the desired aspartic acid diamide (7), glutamic acid diamide (9) and phenylalanineamide (11) derivatives of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid.

These coupling reactions were further extended to 2-β-D-ribofuranosylthiazole-4-carboxylic acid (15). Treatment of tiazofurin[12] (14) with 1N NaOH at 60°C gave 15 (Scheme II), which was isolated in over 88% yield. Coupling of 15 with L-glutamic acid diethyl ester in DMF in the presence of EDC resulted in the formation of the blocked nucleoside peptide 16 in 95% yield. Ammonolysis of 16 with MeOH/NH_3 gave 2-β-D-ribofuranosylthiazole-4-carboxyglutamic acid diamide (17). In a similar manner, coupling of 15 with L-aspartic acid dimethyl ester led to the formation of 12, which on subsequent ammonolysis furnished 2-β-D-ribofuranosylthiazole-4-carboxyaspartic acid diamide (13) in near quantitative yield.

Thus, the synthesis of certain peptide derivatives of ribavirin and tiazofurin in which the peptide linkage is on the carbamoyl group of the aglycon moiety has been accomplished by the conventional procedures.

The amino acid conjugates of ribavirin (5, 7, 9, and 11) and tiazofurin (13 and 17) synthesized during this study were tested (for experimental details , see ref. 21) in parallel with tiazofurin for their inhibitory effects on the growth of L1210 murine lymphocytic leukemia, WIL2 human B lymphoblastic leukemia, and CCRF-CEM human T lymphoblastic leukemia in cell culture. None of these compounds exhibited any inhibitory effects on these cell lines. Similarly, the nucleoside peptides were tested (for experimental details, see ref. 22) against parainfluenza type 3, measles, vaccinia and herpes simplex type 2 viruses in cell culture in parallel with ribavirin and pyrazofurin. These compounds were devoid of any significant antiviral activity in cell culture.

EXPERIMENTAL SECTION: Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 300 MHz with IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to Me_4Si as an internal standard. The presence of water as indicated by elemental analyses was verified by ^{1}H NMR. Infrared spectra (IR in KBr) were obtained on a Beckman Acculab 2 spectrophotometer. Elemental analyses were performed by Robertson Laboratory Inc., Madison, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). E. Merck silica gel (230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°C.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic acid (3). A solution of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid[7] (2, 5.8 g, 23.7 mmol) in anhydrous pyridine (30 mL) and dry DMF (30 mL) was treated with acetic anhydride (12.1 g, 118.5 mmol) and the mixture was stirred at room temperature overnight, with the exclusion of moisture. The solution was evaporated to dryness and the residue was suspended in water (50 mL). The aqueous mixture was extracted with EtOAc (2 x 75 mL) and the combined organic phase was washed with sat-

urated brine solution (50 mL). After drying (anhydrous Na_2SO_4), EtOAc was evaporated to dryness to give a crystalline solid, 5.6 g (64%); mp 163-164°C; IR: ν 1690 (C=O)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 2.01-2.08 (3s, 9, $3COCH_3$), 6.34 (d, 1, J=3.0 Hz, $C_1'H$), 8.88 (s, 1, C_5H) and 13.61 (br s, 1, COOH). Anal. Calcd for $C_{14}H_{17}N_3O_9$ (371.27): C, 45.28; H, 4.62; N, 11.31. Found: C, 45.47; H, 4.62; N, 11.27.

General Procedure of Coupling Involving an Amino Acid Ester and 3. To a solution of 3 (0.93 g, 2.5 mmol) in N,N-dimethylformamide (DMF, 50 mL) were added the requisite amino acid ester monohydrochloride (2.5 mmol) and 1-hydroxybenzotriazole monohydrate (HOBT, 0.38 g, 2.5 mmol), and the mixture was cooled to 0°C in an ice-salt bath. To this cooled, stirred solution was added 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 0.57 g, 3 mmol) and N-methylmorpholine (0.33 mL, 3 mmol). The reaction mixture was stirred at 0°C for 3 h and at room temperature overnight. The solvent was evaporated and the oily residue was suspended in water (50 mL). The aqueous mixture was extracted with EtOAc (2 x 50 mL) and the combined organic phase was washed with saturated brine solution (50 mL). After drying (anhydrous Na_2SO_4), EtOAc was evaporated and the residue was purified on a flash silica gel column (5 x 25 cm) using dichloromethane:acetone (8:2, v/v) as the eluent.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxyglycine ethyl ester (4). The amino acid used: glycine ethyl ester hydrochloride (0.35 g, 2.5 mmol). The title compound was obtained as hygroscopic foam; yield, 1.05g (92%). IR: ν 1660 and 1720 (C=O), 3330 (NH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 1.19 (t, 3, CH_2CH_3), 2.00-2.09 (3s, 9, $3COCH_3$), 4.10 (q, 2, CH_2CH_3), 4.38 (m, 2, gly CH_2), 6.34 (d, 1, J=3.0 Hz, $C_1'H$), 8.91 (s, 1, C_5H) and 8.92 (d, 1, NH). Anal. Calcd for $C_{18}H_{24}N_4O_{10}$ (456.37): C, 47.36; H, 5.30; N, 12.27. Found: C, 47.30; H, 5.07; N, 12.10.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxyaspartic acid dimethyl ester (6). The amino acid used: L-aspartic acid dimethyl ester hydrochloride (0.49 g, 2.5 mmol). Compound 6 was obtained as foam; yield, 1.26 g (98%). IR: ν 1640 and 1700 (C=O), 3300 (NH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 2.02-2.09 (3s, 9, $3COCH_3$), 2.92 (m, 2, asp CH_2), 3.60 and 3.64 (2s, 6, $COOCH_3$), 4.88 (q, 1, asp CH), 6.34 (d, 1, J=3.0 Hz, $C_1'H$), 8.92 (s, 1, C_5H) and 8.96 (d, 1, NH). Anal. Calcd for $C_{20}H_{26}N_4O_{12}$ (514.41): C, 46.69; H, 5.09; N, 10.88. Found: C, 46.73; H, 5.00; N, 10.68.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxyglutamic acid diethyl ester (8). The amino acid used: L-glutamic acid diethyl ester hydrochloride (0.59 g, 2.5 mmol). Compound 8 was obtained as oil; yield, 1.35 g (97%). IR: ν 1685 and 1740 (C=O), 3360 (NH)cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23-1.35 (m, 6, $2CH_2CH_3$), 1.90-2.50 (m, 13, $3COCH_3$ + glu $2CH_2$), 4.12 (q, 2, CH_2CH_3), 4.26 (q, 2, CH_2CH_3), 4.84 (m, 1, glu CH), 6.05 (d, 1, J=3.5 Hz, C_1,H), 7.77 (d, 1, NH) and 8.37 (s, 1, C_5H). Anal. Calcd for $C_{23}H_{32}N_4O_{12}$ (556.49): C, 49.63; H, 5.79; N, 10.06. Found: C, 49.89: H, 5.75; N, 9.81.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxyphenylalanine methyl ester (10). The amino acid used: L-phenylalanine methyl ester hydrochloride (0.53 g, 2.5 mmol). The title compound was obtained as foam; yield, 1.25 g (94%). IR: ν 1680 and 1750 (C=O), 3400 (NH)cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.10-2.15 (3s, 9, $3COCH_3$), 3.75 (s, 3, $COOCH_3$), 4.47 (m, 2, ala CH_2), 5.10 (m, 1, ala CH), 6.04 (d, 1, J=3.0 Hz, C_1,H), 7.17-7.34 (m, 5, phenyl), 7.59 (d, 1, NH) and 8.33 (s, 1, C_5H). Anal. Calcd for $C_{24}H_{28}N_4O_{10}$ (532.46): C, 54.13; H, 5.30; N, 10.52. Found: C, 53.96; H, 5.22; N, 10.29.

General Procedure of Ammonolysis of Nucleoside Peptide Esters. A solution of the nucleoside peptide ester (3.3 mmol) in methanolic ammonia (75 mL, saturated at 0°C) in a pressure bottle was stirred at room temperature for 15 h. The bottle was cooled, opened and the ammonia allowed to evaporate. Removal of the solvent and crystallization of the residue from appropriate solvent gave the nucleoside peptide derivatives.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxyglycineamide (5). Crystallization of the residue from aqueous ethanol gave 0.90 g (91%) of 5 as colorless needles; mp 144-146°C. IR: ν 1670 (C=O), 3200-3400 (NH, NH_2, OH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 3.96 (m, 2, gly CH_2), 5.81 (d, 1, J=3.5 Hz, C_1,H), 7.08 and 7.41 (2s, 2, $CONH_2$), 8.45 (d, 1, NH) and 8.89 (s, 1, C_5H). Anal. Calcd for $C_{10}H_{15}N_5O_6 \cdot 1/2H_2O$ (310.23): C, 38.71; H, 5.19; N, 22.56. Found: C, 38.75; H, 5.32; N, 22.30.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxyaspartic acid diamide (7). Crystallization of the residue from aqueous methanol gave 0.88 g (72%) of 7 as colorless crystals; mp 194-195°C. IR: ν 1620 (C=O), 3200-3400 (NH, NH_2, OH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 2.49-2.72 (m, 2, asp CH_2), 4.61 (m, 1, asp CH), 5.82 (d, 1, J=3.5 Hz, C_1,H), 6.93 and 7.12 (2s, 2,

$CONH_2$), 7.39 (d, 2, $CONH_2$), 8.51 (d, 1, NH) and 8.91 (s, 1, C_5H). Anal. Calcd for $C_{12}H_{18}N_6O_7$ (358.26): C, 40.23; H, 5.06; N, 23.45. Found: C, 40.15; H, 5.12; N, 23.23.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxyglutamic acid diamide (9). The residue was crystallized from aqueous methanol to yield 1.07 g (87%) of the title compound; mp >80°C (dec.). IR: ν 1640 (C=O), 3200-3400 (NH, NH_2, OH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 1.84-2.10 (m, 4, glu 2 CH_2), 4.35 (m, 1, glu CH), 5.82 (d, 1, J=3.5 Hz, C_1,H), 6.76 and 7.32 (2s, 2, $CONH_2$), 7.22 and 7.57 (2s, 2, $CONH_2$), 8.24 (d, 1, NH) and 8.92 (s, 1, C_5H). Anal. Calcd for $C_{13}H_{20}N_6O_7.1/2H_2O$ (381.34): C, 40.94; H, 5.55; N, 22.04. Found: C, 41.02; H, 5.22; N, 21.80.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxyphenylalanineamide (11). The residue was further purified on a flash silica gel column (2.5 x 15 cm) using dichloromethane:methanol (8:2, v/v) as the eluent, to yield 0.90 g (68%) of 11 as foam. IR: ν 1680 (C=O), 3300-3400 (NH, NH_2, OH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 4.01 (m, 2, ala CH_2), 4.65 (m, 1, ala CH), 5.81 (d, 1, J=3.5 Hz, C_1,H), 7.13 and 7.65 (2s, 2, $CONH_2$), 7.20-7.27 (m, 5, phenyl), 8.17 (d, 1, NH) and 8.89 (s, 1, C_5H). Anal. Calcd for $C_{17}H_{21}N_5O_6$ (391.34): C, 52.17; H, 5.41; N, 17.88. Found: C, 51.95; H, 5.33; N, 17.76.

2-β-D-Ribofuranosylthiazole-4-carboxylic acid (15). A solution of tiazofurin[12] (14, 6.0 g, 23.04 mmol) in 1N NaOH (50 mL) was warmed to 60°C for 4 h with stirring. The solution was cooled to 0°C and acidified to pH 3 with Dowex-50 (H^+) resin. The resin was removed by filtration and the aqueous solution was lyophilized to yield an analytically pure fluffy solid of 15, 5.5 g (88.2%). IR: ν 1700 (C=O), 3380 (OH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 4.91 (d, 1, J=5.0 Hz, C_1,H) and 8.36 (s, 1, C_5H); ^{13}C NMR (300 MHz, Me_2SO-d_6): δ 61.96, 71.37, 77.10, 82.14, 85.04, 128.60, 147.75, 162.50 and 172.52. Anal. Calcd for $C_9H_{11}NO_6S.1/2H_2O$ (270.25): C, 40.08; H, 4.48; N, 5.18; S, 11.86. Found: C, 39.69; H, 4.72; N, 4.75; S, 12.17.

2-β-D-Ribofuranosylthiazole-4-carboxyglutamic acid diethyl ester (16). L-Glutamic acid diethyl ester hydrochloride (1.12 g, 4.5 mmol) and triethylamine (TEA, 0.45 g, 4.5 mmol) were added to a solution of 15 (1.17 g, 4.5 mmol) in DMF (100 mL). The resulting solution was cooled to 0°C before EDC (0.95 g, 4.95 mmol) and HOBT (15 mg) were added in succession. The resulting mixture was stirred at 0°C for 1 h and at

room temperature for 15 h. The solvent was evaporated and the residue was purified on a flash silica gel column (5 x 30 cm) using 5% methanol in dichloromethane as the eluent to yield 1.9 g (95%) of 16 as a foam. IR: ν 1670 and 1730 (C=O), 3380 (NH, OH)cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.60 (t, 6, $2CH_2\underline{CH_3}$), 2.08 and 2.38 (m+t, 4, $2\underline{CH_2}$), 4.10 (2q, 4, $2\underline{CH_2}CH_3$), 4.47 (q, 1, glu C$\underline{H}$), 4.97 (d, 1, J=4.8 Hz, $C_1\underline{H}$), 8.27 (s, 1, $C_5\underline{H}$) and 8.55 (d, 1, N$\underline{H}$); ^{13}C NMR (300 MHz, Me_2SO-$\underline{d}_6$): δ 14.07, 25.80, 30.16, 51.44, 59.98, 60.79, 61.79, 71.25, 76.94, 81.94, 84.85, 124.93, 149.10, 160.71, 171.37, 172.26 and 172.67. Anal. Calcd for $C_{18}H_{26}N_2O_9S$ (446.47): C, 48.42; H, 5.87; N, 6.27; S, 7.18. Found: C, 48.33; H, 6.06; N, 5.99; S, 7.21.

2-β-D-Ribofuranosylthiazole-4-carboxyglutamic acid diamide (17). In a similar manner as for 5, the title compound was prepared by using 16 (0.77 g, 1.73 mmol) and MeOH/NH_3 (40 mL) to yield 0.64 g (94%) as foam. IR: ν 1655 (C=O), 3230-3450 (NH_2, OH)cm^{-1}; ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 1.80-2.30 (m, 4, $2\underline{CH_2}$), 4.38 (m, 1, glu C$\underline{H}$), 4.96 (d, 1, J=5.2 Hz, $C_1\underline{H}$), 6.76, 7.25, 7.34 and 7.63 (4s, 4, $2CON\underline{H_2}$), 8.10 (d, 1, N$\underline{H}$) and 8.25 (s, 1, $C_5\underline{H}$); ^{13}C NMR (300 MHz, Me_2SO-$\underline{d}_6$): δ 28.52, 31.20, 51.93, 61.81, 71.25, 77.01, 81.95, 84.97, 124.52, 149.32, 159.98, 172.71, 172.82 and 173.63. Anal. Calcd for $C_{14}H_{20}N_4O_7S.1/4H_2O$ (392.90): C, 42.80; H, 5.26; N, 14.26; S, 8.16. Found: C, 42.83; H, 5.61; N, 13.96; S, 8.21.

2-β-D-Ribofuranosylthiazole-4-carboxyaspartic acid dimethyl ester (12). A solution of 15 (1.04 g, 4 mmol) in DMF (100 mL) was treated with L-aspartic acid dimethyl ester hydrochloride (1.0 g, 5.1 mmol), TEA (0.71 mL, 5.1 mmol), EDC (0.97 g, 5.1 mmol) and HOBT (15 mg), and worked up as described for 16 to yield 1.33 g (82.6%) of 12. IR: ν 1670 and 1740 (C=O), 3390 (NH, OH)cm^{-1}; ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 2.93 (m, 2, asp CH_2), 3.58 and 3.61 (2s, 6, $2COOC\underline{H_3}$), 4.88 (m, 1, asp C$\underline{H}$), 4.93 (d, 1, J=5.2 Hz, $C_1\underline{H}$), 8.28 (s, 1, $C_5\underline{H}$) and 8.73 (d, 1, N$\underline{H}$); ^{13}C NMR (300 MHz, Me_2SO-$\underline{d}_6$): δ 35.55, 48.64, 51.91, 52.58, 61.93, 71.41, 77.13, 81.96, 85.06, 125.42, 148.94, 160.50, 171.12, 171.17 and 172.96. Anal. Calcd for $C_{15}H_{20}N_2O_9S$ (404.39): C, 44.55; H, 4.99; N, 6.93; S, 7.93. Found: C, 44.35; H, 5.10; N, 6.88; S, 8.19.

2-β-D-Ribofuranosylthiazole-4-carboxyaspartic acid diamide (13). A solution of 12 (1.2 g, 2.97 mmol) in MeOH/NH_3 (50 mL) was treated as described for 5 to give 1.05 g (94%) of 13. IR: ν 1660 (C=O), 3220-

3460 (NH_2, OH)cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 2.70 (m, 2, asp CH_2), 4.65 (m, 1, asp CH), 4.95 (d, 1, J=4.8 Hz, C_1,H), 6.93, 7.13, 7.40 and 7.42 (4s, 4, $2CONH_2$), 8.25 (s, 1, C_5H) and 8.34 (d, 1, NH); ^{13}C NMR (300 MHz, Me_2SO-d_6): δ 37.06, 49.53, 61.80, 71.21, 76.98, 82.0, 84.82, 124.58 149.42, 160.0, 171.82 and 172.56. Anal. Calcd for $C_{13}H_{18}N_4O_7S.1/2\ H_2O$ (383.37): C, 40.73; H, 5.00; N, 14.61; S, 8.36. Found: C, 40.53; H, 4.97; N, 14.72; S, 8.54.

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* To whom correspondence should be addressed.

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