Synthesis and Antiviral Activity of 1,2,4-Triazole-3-thiocarboxamide and 1,2,4-Triazole-3-carboxamidine Ribonucleosides

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The syntheses of ribonucleosides structurally related to 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**1**) are described and their antiviral activity is compared with that of **1**. Both the acid-catalyzed fusion procedure and glycosylation of the appropriate trimethylsilyl derivative provided 3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (**4**) in 80% yield. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-thiocarboxamide (**2**) and 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (**3**) and related nucleosides were prepared from **4**. In tissue culture, **3** exhibited activity against herpes simplex, rhino, and parainfluenza viruses, similar to that shown by **1**, while **2** was effective only against herpes simplex virus. Against lethal influenza A_2 virus infections in mice, **3** was effective at 75 mg/kg/day compared with **1** which showed significant activity at 37.5 mg/kg/day.

The synthetic nucleoside 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[1,2] (virazole) (**1**) has been shown by our laboratory to exhibit a broad spectrum of activity against both DNA and RNA viruses *in vitro* and *in vivo*.[2] Our interest in nucleosides structurally related to **1** has prompted us to investigate[3,4] the synthesis and antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-thiocarboxamide (**2**) and 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine (**3**). These nucleosides, while retaining steric requirements very similar to those of the corresponding carboxamide nucleoside **1**, differ in hydrogen bonding properties (S and N *vs.* O) of the 3 substituent on the triazole.

As a route to both the thiocarboxamide (**2**) and carboxamidine (**3**) triazole nucleosides, the synthesis of 3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (**4**) was investigated as follows.

The acid-catalyzed fusion procedure[5] with 3-cyano-1,2,4-triazole[6] and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose provided crystalline 3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (**4**) in 80% yield. This fusion reaction also proceeded readily in the absence of an acidic catalyst, as in the case of other 1,2,4-triazoles,[7] 1,2,3-triazoles,[8] and purines[9] with electronegative substituents, but gave a somewhat lower yield (66%) of **4**. The structure of **4** was established by conversion of this product to the corresponding carboxamide nucleoside (**1**) on treatment with NH_4OH. A second approach to the synthesis of **4** was by treatment of the trimethylsilyl derivatives of 3-cyano-1,2,4-triazole with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide at room temperature. This route afforded the cyanotriazole nucleoside **4** in the same yield (80%) as from the acid-catalyzed fusion procedure.

A minor product was observed in each of the above procedures and, after chromatography, 5-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (**5**) was isolated in 6% yield. The structure of **5** is based on the pmr spectrum (see Experimental Section) of 1-β-D-ribofuranosyl-1,2,4-triazole-5-thiocarboxamide (**7**) obtained from **5**. The positions of the signals for H-3 and H-1′ are in close agreement with those reported[10] for the corresponding carboxamide triazole nucleoside.[1]

A route to 3-cyano-1,2,4-triazole in low yield by dehydration of 1,2,4-triazole-3-carboxamide has been reported.[6] A preferred synthesis of 3-cyano-1,2,4-triazole was accomplished by ring closure of 1-cyanoformimidic acid hydrazide[11] with ethyl orthoformate.

The two cyano-1,2,4-triazole nucleosides **4** and **5** on treatment with H_2S and Et_3N provided, after deacylation, 1-β-D-ribofuranosyl-1,2,4-triazole-3 thiocarboxamide (**2**) and 1-β-D-ribofuranosyl-1,2,4-triazole-5-thiocarboxamide (**7**), respectively.

Attempts to obtain 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine (**3**) by treatment of 3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (**4**) with NH_3 in a variety of solvents resulted in mixtures of products. However, an essentially quantitative yield of **3**, isolated as the hydrochloride salt, was obtained by heating **4** with excess liquid NH_3 and 1 molar equiv of NH_4Cl.[12]

The addition of NH_2OH and N_2H_4 to the cyano moiety of **4** and deacylation afforded 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidoxime (**8**) and 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidrazone (**9**), respectively (Scheme I).

These nucleosides were evaluated for antiviral activity in tissue culture against type 3 adeno, type 1 herpes simplex, type 13 rhino, and type 3 parainfluenza viruses. In these experiments, a 24-hr monolayer of human carcinoma of nasopharynx (KB) cells in disposable plastic microplates was exposed to 320 cell culture 50% infectious doses per milliliter of virus, and concentrations of each nucleoside ranging from 1000 to 1 μg/ml were then added 15 min later. Antiviral activity was determined by observing inhibition of viral cytopathogenic effect (CPE) after a 72-hr incubation at 37°. Antiviral activity was evaluated using a previously described[13] numerical virus rating (VR) which takes into account cytotoxicity and the degree of CPE in treated and untreated cells. In this system, any VR of 0.1–0.4 was considered to indicate only slight antiviral activity, whereas a VR of 0.5 or greater signified definite antiviral activity. The results of these experiments, in which the carboxamide nucleoside **1** was used as standard, are given in Table I.

In contrast to **1**, which exhibits significant activity against all of the above viruses, the thiocarboxamide nucleoside (**2**) was effective only against herpes simplex virus. However, the carboxamidine derivative **3** was active against both the DNA virus, herpes simplex, and the two RNA viruses tested (rhino and parainfluenza). The other nucleosides tested did not exhibit antiviral activity in these systems.

Compounds **1** and **3** were tested against lethal influenza A_2 virus infections in mice (Table II). In this study, 14–16-g Swiss mice were exposed to an aerosol[14] of the virus in a concentration sufficient to kill 75% of the placebo-treated control animals. Intraperitoneal treatment, with each compound dissolved or suspended in saline, began 4 hr previrus exposure and continued twice daily for 9 days. The dosages utilized for each compound were determined in preliminary

Scheme I

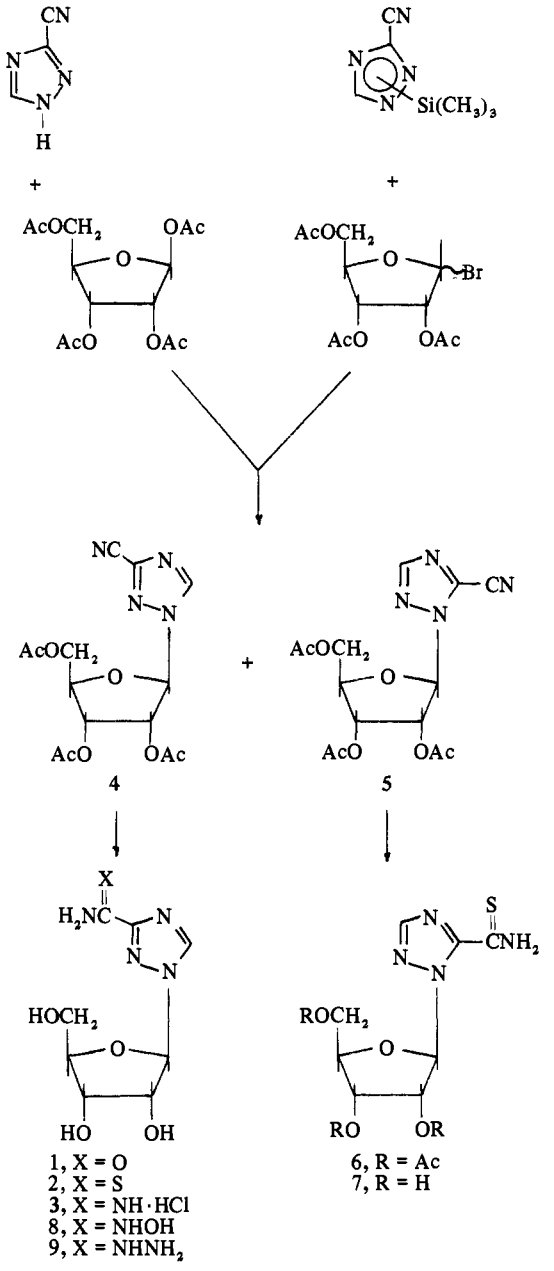

Table I. *In Vitro* Antiviral Activity (Virus Rating) against Type 3 Adeno (AV/3), Type 1 Herpes Simplex (HSV/1), Type 13 Rhino (RV/13), and Type 3 Parainfluenza (PIV/3) Viruses

	Virus rating			
Compd	AV/3	HSV/1	RV/13	PIV/3
1	0.7	1.1	0.7	0.8
2	0.0	0.8	0.1	0.0
3	0.0	1.0	0.6	0.8
7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0
9	0.0	0.3	0.0	0.0

toxicity trials to be equally nontoxic. Ten mice were used at each level of compound, and 20 animals, treated with saline only, were utilized as virus controls. All animals were observed for 21 days.

As previously reported,[2] **1** was significantly active down at least to a level of 37.5 mg/kg/day. Compound **3** was also active but only at the 75 mg/kg/day level.

The superior antiviral activity exhibited by the carboxamide nucleoside **1** as compared with the thiocarboxamide **2**, carboxamidine **3**, and related nucleosides[4] (Table I) suggests that both steric and hydrogen bonding properties of the 3 substituent on the triazole nucleoside are critical factors in the design of these antiviral nucleosides.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Evaporations were accomplished with a rotating evaporator under reduced pressure with a bath temperature of <35°. The pmr spectra were recorded at 60 MHz on a Perkin-Elmer Hitachi R20A spectrometer and chemical shifts are reported in parts per million (δ) with DSS or TMS as an internal reference. Analytical results are within ±0.4% of the theoretical values.

3-Cyano-1,2,4-triazole. A mixture of triethyl orthoformate (150 ml) and 1-cyanoformimidic acid hydrazide[11] (25.2 g, 0.30 mol) was cooled to 0° and a solution (4.0 ml) of dioxane saturated with anhydrous HCl was added with stirring. The mixture was stirred with cooling in an ice bath for 5 hr and stirring at 25° was continued for 15 hr. The mixture was evaporated to dryness and Et_2O (500 ml) was added to the residue. The solution was filtered and washed with water and the organic layer was dried over $MgSO_4$. The solution was filtered and the Et_2O was removed. Crystallization of the product from EtOAc–C_6H_6 provided 16.0 g (56.8%) of 3-cyano-1,2,4-triazole with mp 185–187°. All properties of the compound were identical with those of an authentic sample prepared by the method of Cipens and Grinsteins.[6] *Anal.* ($C_3H_2N_4$) C, H, N.

3-Cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (4) and 5-Cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (5). Method 1. A mixture of 3-cyano-1,2,4-triazole (9.41 g, 0.10 mol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (31.8 g, 0.10 mol) was heated in an oil bath maintained at 150°. Bis(*p*-nitrophenyl) phosphate (100 mg) was added with stirring; heating at 150° under diminished pressure was continued for 15 min. The residue was dissolved in $CHCl_3$, the solution was filtered, and the solvent removed. Crystallization of the product from Et_2O provided 28.2 g (80%) of **4** with mp 96–97°: nmr ($CDCl_3$) δ 6.03 (d, 1, $J_{1',2'}$ = 2.5 Hz, 1'-H), 8.41 (s, 1, 5-H). *Anal.* ($C_{14}H_{16}N_4O_7$) C, H, N.

Method 2. A solution of 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl bromide (from 15.9 g, 50.0 mmol, of the 1-*O*-Ac derivative) and the trimethylsilyl derivative of 3-cyano-1,2,4-triazole (prepared by treatment of 5.17 g, 55.0 mmol, of the triazole with hexamethyldisilazane at reflux) in dry MeCN (150 ml) was kept at room temperature for 3 days. The solvent was removed and addition of Et_2O to the residue gave crystalline **4** (10.8 g). Chromatography of the filtrate over silica gel with $CHCl_3$–Me_2CO (20:1) provided a mixture of products (2.9 g) in the first fractions and an additional 3.2 g of **4** (total yield 80%) in subsequent fractions.

Chromatography of the mixture (2.9 g), which contained sugar degradation products, over silica gel with $CHCl_3$ gave **5** (1.0 g, 6%) as a syrup: nmr ($CDCl_3$) δ 6.21 (d, 1, $J_{1',2'}$ = 3.0 Hz, 1'-H), 8.16 (s, 1, 3-H). *Anal.* ($C_{14}H_{16}N_4O_7$) C, H, N.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (1). A mixture of **4** (3.52 g, 10.0 mmol) and concentrated NH_4OH (25 ml) was heated on the steam bath for 1 hr. The solvent was removed and the product was crystallized from EtOH to give 2.0 g (82%) of **1** which was identical with an authentic sample.[1]

Table II. Effect on Influenza A_2 Virus-Induced Infections

Compd	Dose, mg/kg/day	Toxicity controls, surv/total	Surv/total[a]	Surv increase, P[b]	Mean surv time,[c] days	Mean surv time increase, P[d]
1	75	5/5	7/10	<0.02	10.7	>0.05
	37.5	5/5	9/10	<0.001	9.0	
3	75	5/5	6/10	<0.1	10.8	>0.05
	37.5	5/5	0/10		9.8	
	0		5/20		10.1	

[a] Determined on day 21 postinfection. [b] P = probability (χ^2 analysis). [c] Animals dying on or before day 21. [d] P = probability (t test).

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-thiocarboxamide (2).** A mixture of **4** (8.0 g, 22.7 mmol), Et_3N (14.0 ml), and EtOH (200 ml) was stirred at 25° while H_2S gas was passed into the solution for 2 hr. The solvent was removed and the residue was treated with a solution of NaOMe (600 mg) in MeOH (150 ml) for 3 hr at 25°. After the solution was neutralized with Bio-Rad AG 50W-X2(H), it was filtered, and the solvent was removed. Crystallization of the product from aqueous EtOH provided 4.5 g (76%) of **2** with mp 173–175°: nmr (DMSO-d_6) δ 5.86 (d, 1, $J_{1',2'}$ = 3.5 Hz, 1'-H), 8.92 (s, 1, 5-H), 9.50 and 9.95 (s, 2, NH_2); λ_{max} (H_2O) 233 nm (ε 7600), 297 (8650). *Anal.* ($C_8H_{12}N_4O_4S$) C, H, N, S.

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-carboxamidine Hydrochloride (3).** A mixture of **4** (7.04 g, 20.0 mmol), NH_4Cl (1.07 g, 20.0 mmol), and anhydrous NH_3 (150 ml) was heated in a bomb at 85° for 18 hr. After removal of excess NH_3, the residue was crystallized from MeCN–EtOH to provide 5.30 g (95%) of **3** with mp 177–179° dec: nmr (DMSO-d_6) δ 5.97 (d, 1, $J_{1',2'}$ = 3.5 Hz, 1'-H), 9.26 (s, 1, 5-H). *Anal.* ($C_8H_{14}ClN_5O_4$) C, H, Cl, N.

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-carboxamidoxine (8).** A solution of **4** (3.0 g, 8.52 mmol) and excess NH_2OH in EtOH (100 ml) was refluxed with stirring for 2 hr. The solvent was removed and the product was crystallized from aqueous EtOH to provide 2.0 g (91%) of **8** with mp 212–214° dec. *Anal.* ($C_8H_{13}N_5O_5$) C, H, N.

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-carboxamidrazone (9).** A solution of **4** (1.76 g, 5.0 mmol) in EtOH (50 ml) was treated with N_2H_4 (97%, 1.0 ml) and the solution was stirred at room temperature for 48 hr. The solid material was collected and recrystallized from aqueous EtOH to give 1.2 g (93%) of **9** with mp 179–180° dec. *Anal.* ($C_8H_{14}N_6O_4$) C, H, N.

1-(**2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-thiocarboxamide** (6). Treatment of **5** with H_2S and Et_3N in EtOH as in the preparation of **2** followed by chromatography of the crude product over silica gel with $CHCl_3$ and crystallization from Et_2O–CH_2Cl_2 afforded **6** with mp 109–111°: nmr (DMSO-d_6) δ 7.23 (d, 1, $J_{1',2'}$ = 1.5 Hz, 1'-H), 8.26 (s, 1, 3-H), 10.15 and 10.55 (s, 2, NH_2). *Anal.* ($C_{14}H_{18}N_4O_7S$) C, H, N, S.

1-β-D-**Ribofuranosyl-1,2,4-triazole-5-thiocarboxamide (7).** Deacetylation of **6** with NaOMe as in the preparation of **2** and crystallization from EtOH provided **7** with mp 168–169°: nmr (DMSO-d_6) δ 6.86 (d, 1, $J_{1',2'}$ = 2.5 Hz, 1'-H), 8.11 (s, 1, 3-H), 10.10 and 10.50 (br s, 2, NH_2); λ_{max} (H_2O) 230 nm (ε 6000), 287 (6800). *Anal.* ($C_8H_{12}N_4O_4S$) C, H, N, S.

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Notes

Structure–Activity Relationships in Reactivators of Organophosphorus-Inhibited Acetylcholinesterase. 6. 2-Hydroxyiminomethylimidazolium Iodides†

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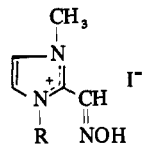
Recently we found[1] that methiodides of *syn*-2-hydroxyiminomethyl-1-methylimidazole (**1**) and of *syn*-2-hydroxyiminomethyl-1-benzylimidazole (**2**) are effective reactivators *in vitro* of acetylcholinesterase (AChE) inhibited by diisopropylphosphorofluoridate (DFP). Compound **2** is about twice as active as compound **1**. The increased reactivating capacity of **2** may be rationalized as due to greater bond strength with the enzyme surface resulting from van der Waals attractions and hydrophobic bonds.



1, R = CH_3
2, R = $CH_2C_6H_5$

We suspected that replacement of the methyl group by bulkier groups might produce agents giving stronger hydrophobic interactions with the enzyme and therefore yielding greater reactivating capacity. Therefore, we have prepared two homologous series of quaternary salts by treating 1-methyl- and 1-benzyl-2-hydroxyiminomethylimidazole with *n*-alkyl iodides, where the alkyl group varies from ethyl to *n*-octyl (Table I).

The reactivating capacity of the new products was determined *in vitro* following the method of Ashani, *et al.*,[2] on bovine erythrocyte AChE inhibited by DFP. Since the ef-

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