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Novel 5-alkyl(aryl)-substituted ribavirine analogues: synthesis and antiviral evaluation

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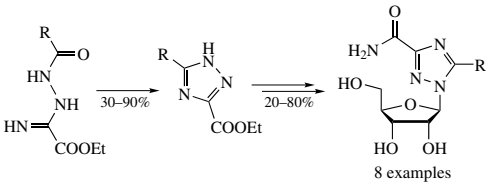
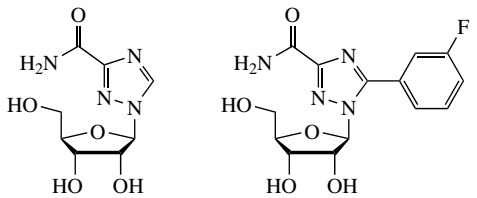
Novel 5-alkyl(aryl)-substituted 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamides were prepared in three steps. Their antiviral activity was evaluated.



Ribavirin (Virazole, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a nucleoside antiviral agent with a wide antiviral spectrum used against both RNA and DNA viruses.[1,2] Several hypotheses are existing concerning targets and activity mechanism of ribavirin,[3–5] which is more of a mystery. The ribavirin analogues bearing bulky substituents at the 5-position of the triazole ring were first mentioned quite recently.[6] Later, some syntheses of similar analogues **1–3** as well as antiviral and anticancer activities were reported.[7–12] Generally authors modified the molecule of ribavirin itself or its nucleoside precursors using Pd-catalyzed cross-coupling reactions. Thus, the anomeric configuration of the product was predetermined since the modification occurred at the 5-position of the triazole ring. Unfortunately, this approach is limited by the substrate diversity and the substituent type containing multiple bonds. The classical method includes the heterocyclic base synthesis with subsequent ribosylation, chemical or chemoenzymatic, and allows one to expand the variety of substituents. However, only a small number of the ribavirin



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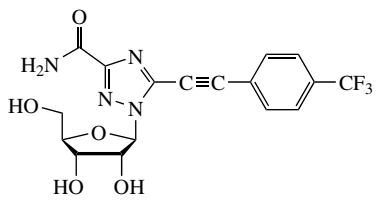

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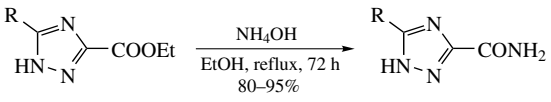


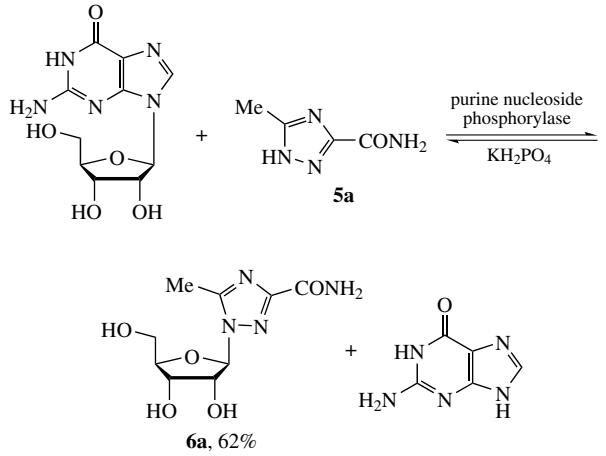
4a–h **5a–h**

a R = Me **e** R = cyclopropyl
b R = Et **f** R = Bu^i
c R = Pr **g** R = Bu^t
d R = Pr^i **h** R = Ph

Scheme 1

analogues were obtained by this route, possibly because of low synthetic availability of respective substituted heterocyclic bases.

Previously reported by our group synthetic pathways of various 5-substituted 1-*H*-1,2,4-triazole-3-carboxylic acids[13] provided a set of heterocyclic bases for the ribavirin analogue synthesis (Scheme 1). 5-Substituted ethyl 1-*H*-1,2,4-triazole-3-carboxylates **4a–h** were obtained from β-*N*-acylamidrazones and corresponding acid chlorides with subsequent amide preparation by treatment with ammonia. Nucleoside **6a** was synthesized from amide **5a** by the described procedure[14,15] (Scheme 2). The activity of this ribavirin analogue against reproduction of viruses that cause hazardous human infections including human/avian influenza A virus was investigated.[14] Amides **5b–h** appeared to be unsuitable substrates for recombinant purine nucleoside phosphorylase (EC 2.4.2.1) from *E. Coli*, and this was in agreement with our previous conclusions.[16] Since this limitation made impossible the use of stereoselective chemoenzymatic synthesis as for **5a**, the protected nucleosides **7b–h** were obtained by condensation of ethyl esters **4b–h** with ribose tetraacetate at 160 °C in the presence of acid catalyst by the modified method[17] (Scheme 3). Originally, this method includes melting of D-ribofuranose tetraacetate with subsequent addition of triazole and catalyst, at reduced pressure. The yield of the nucleoside with protecting groups (for instance **7b**) was only 28% by this route. We succeeded in improving the yield to 54% by dissolving all reaction com-



Scheme 2

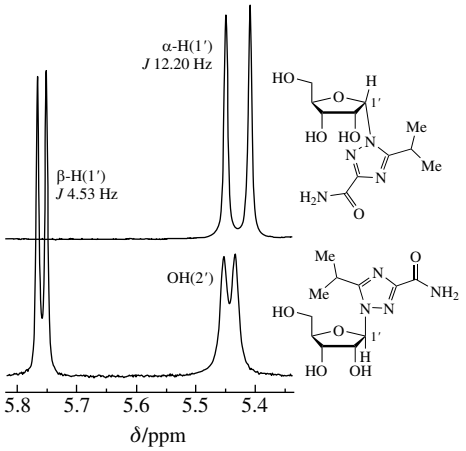
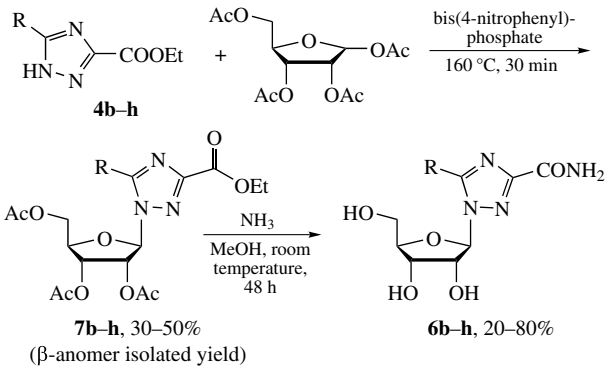


Figure 1 ^{1}H NMR signals for anomeric proton in **6d** and its α-anomer.

ponents in ethanol, which was later removed *in vacuo*. Compounds **7b–h** were isolated by chromatography on silica and further converted to the target analogues **6b–h** by treatment with ammonia (see Scheme 3).



Scheme 3

The similar chemical shifts and spin–spin coupling constants $J_{H(1')H(2')}$ of signals for H(1′)-proton in the ^{1}H NMR spectra (Table 1) clearly show the same anomeric configuration for all synthesized compounds. According to published data,[18] the obtained values for chemical shifts and $J_{H(1')H(2')}$ of signals are characteristic of β-anomer. α-Anomer of **6d** with greatly distinct chemical shift and $J_{H(1')H(2')}$ of signal (Figure 1) was isolated in minor amounts. In the ^{1}H-^{1}H NOESY spectra for nucleoside **6e** (see Online Supplementary Materials) interactions between protons H(1′) and OH(2′), H(2′) and H(3′), H(1′) and cyclopropyl ring proton $(CH_2)_2CH$ were observed, while the H(1′)–H(2′) interaction cross-peak was missing. All these data fully confirm the predicted structure and anomeric configuration for compound **6e**. The anomeric proton chemical shift and the constant $J_{H(1')H(2')}$ values significantly differ for compound **6g** (see Table 1). There is no obvious reason for this difference; probably it is defined by steric factors. Nevertheless, ^{1}H-^{1}H NOESY spectra for **6g** confirms its β-configuration. Anomeric configuration for the rest nucleosides was confirmed similarly.†

Table 1 ^{1}H NMR spectral data for nucleosides **6a–h**.

Compound	R	$\delta_{H(1')}$/ppm	$J_{H(1')H(2')}$/Hz
6a	Me	5.75	3.8
6b	Et	5.73	4.4
6c	Pr	5.74	4.4
6d	Pr^i	5.75	4.6
6e	cyclopropyl	5.92	4.2
6f	Bu^i	5.75	4.6
6g	Bu^t	6.68	2.9
6h	Ph	5.86	4.0

The antiviral activity of the compounds **6a–h** was estimated *in vitro* on the reproduction of hepatitis C, herpes simplex, and influenza A viruses in cell cultures. For hepatitis C infection human hepatoma cell line Huh7 was used, this cell line contains full-size hepatitis C virus and luciferase gene. The cytotoxicity of the test compounds for uninfected Huh7 cells was determined by the standard MTT assay. The results are summarized in Table 2.

The antiviral activity and cytotoxicity were also estimated *in vitro* on influenza A virus H1N1/USSR/77 in cells MDCK as well as in Vero E6 cells infected with herpes simplex virus (type 1, L2 strain), and the prevention of the development of the virus-induced cytopathic effect (CPE) was evaluated.[19] Cytotoxicity was determined by monolayer condition using optical methods. The ribavirin analogues **6c–f**,**h** exhibited no toxicity and antiviral activity against influenza A and herpes simplex virus even at the maximum concentration studied (1250 μM).†

The preliminary antiviral estimation of the synthesized compounds shows that alkyl or aryl substituents at the 5-position of the heterocyclic base reduce antiviral activity of ribavirin analogues. Thus, our results indirectly confirm the hypothesis that the correlation exists between antiviral activity of ribavirin analogues and the presence of rigid ethynyl group (or isosteric one) at the 5-position of the triazole ring.[8] The developed synthetic route to 5-substituted ribavirin analogues allows one to continue structure/activity studies and reveal novel challenging antiviral agents in the series.

Table 2 Anti-HCV activity of ribavirin analogues **6a–h**.

Compound	EC_{50}^a/μM	CC_{50}^b/μM	SI^c
Ribavirin	6.5±0.1	470±14	78.3±4.6
6a	40.3±1.8	>500	>12.4
6b	40.4±1.1	>500	>12.4
6c	38.2±1.7	>500	>13.1
6d	54.0±8.3	>500	>9.3
6e	>100	>500	~5
6f	99.0±9.1	>500	>5.1
6g	32.1±0.9	>500	>15.6
6h	19.0±0.7	>500	>26.3

[a] 50% effective concentration. [b] 50% cytotoxic concentration. [c] Selectivity index is a CC_{50}/EC_{50} ratio.

† For synthesis and spectral data of the compounds, as well as for methods of antiviral investigation, see Online Supplementary Materials.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2016.04.012.

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