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5-Ethynyl-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (ETCAR) and its analogues: Synthesis and cytotoxic properties

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ABSTRACT

Efficient Pd(0)-catalysed synthesis of 5-alkynyl-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide depends on the presence of different protecting groups of the ribose moiety. Peracetylated 5-iodo substrate (**15**) couples with terminal alkynes or trimethyl-[(tributylstannyl)ethynyl]silane in 50–71% and 72% yield (ETCAR), respectively, although its hydrodehalogenation to **19** is noticeable. On the other hand, hydrodehalogenation of acetonide (**16**) predominates over coupling with terminal alkyne and slightly decreases a yield of cross-coupling reaction with trimethyl[(tributylstannyl)ethynyl]silane. Alternative conditions of reaction with terminal alkynes, to exclude so far identified hydride sources to produce hydridopalladium species, have been established for acetonide **16** and allowed to achieve 72% of coupling. Fluoromethyl derivative (**42**) was prepared from its 5-hydroxymethyl precursor by fluorination with DAST. Additionally, X-ray structural analysis of **42** was performed. All 1,2,3-triazolonucleosides and two synthesized *cyclo*Sal-pronucleotides were evaluated for cytotoxic activity against K562, HeLa and HUVEC cells.

1. Introduction

Nucleoside analogues possessing five-membered heterocyclic bases have become interesting objects to design and synthesize since the discovery of antiviral potency of ribavirin (1-β-D-ribofuranosyl-1*H*-[1,2,4]triazole-3-carboxylic acid amide, **1**, Fig. 1).[1] Ribavirin has been licensed for treatment of RSV infection in children and, in combination with interferon α, in therapy of HCV infections.[2] Significant antileukemic activity in vivo of tiazofurin (2-β-D-ribofuranosyl-thiazole-4-carboxylic acid amide, **2**) guaranteed its further evaluation and finally the *C*-nucleoside **2** has been granted an orphan-drug status for treatment of chronic myelogenous leukemia in accelerated phase of blast crisis.[3] Antiviral potency of **2** and its close congener selenazofurin (**3**) have also been documented.[4] The presence of carbamoyl group attached to heterocycles of **1**–**3** is indispensible for biological activity of the compounds. It assures they structurally resemble AICA riboside (5-aminoimidazo-4-carboxamide riboside, **4**), whose 5′-monophosphate is a key intermediate in de novo biosynthesis of purine nucleotides.

Additionally, 4,5-disubstituted imidazole ribosides have drawn much attention – naturally occurring mizoribine (bredinin, 1-β-D-ribofuranosylimidazolium-5-olate-4-carboxylic acid amide, **5**) and EICAR (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxylic acid amide, **6**).[5,6] While the immunosuppressant **5** has been licensed as a drug in Japan, **6** has been known for antileukemic activity in vivo and antiviral potency against a broad spectrum of DNA and RNA viruses. It is worthy to note that EICAR exceeds **1** 10- to 100-fold in respect of the level of antiviral activity.[7] The compounds **1**, **5** and **6** are metabolized to 5′-monophosphates (RMP, mizoribine-MP, EICAR-MP) and in this form act as powerful inhibitors of inosine 5′-monophosphate dehydrogenase (IMPDH) of different organisms, occupying a substrate binding site. However, each of the inhibitors binds to the enzyme in its specific way: competitively (RMP), or as an analogue of transient-state (mizoribine-MP), or by forming a covalent bond (EICAR-MP).[2,8,9] Interestingly, *C*-nucleosides **2** and **3**, after being metabolized to the enzyme cofactor NAD^+ analogues – tiazofurin adenine dinucleotide (TAD) and selenazofurin adenine dinucleotide (SAD), respectively, inhibit IMPDH binding into the cofactor domain of the enzyme.[4b,3] Inhibition of IMPDH leads to a depletion of guanine nucleotides pools and in consequence is responsible for antiviral or antileukemic, or immunosuppressant activities demonstrated by AICAR analogues. Another synthesized triazolonucleoside – the isomer of **1** (1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide, **7**) has been generally inactive, though its 5′-monophosphate matched ribavirin monophosphate in potency to inhibit IMPDH. [10]

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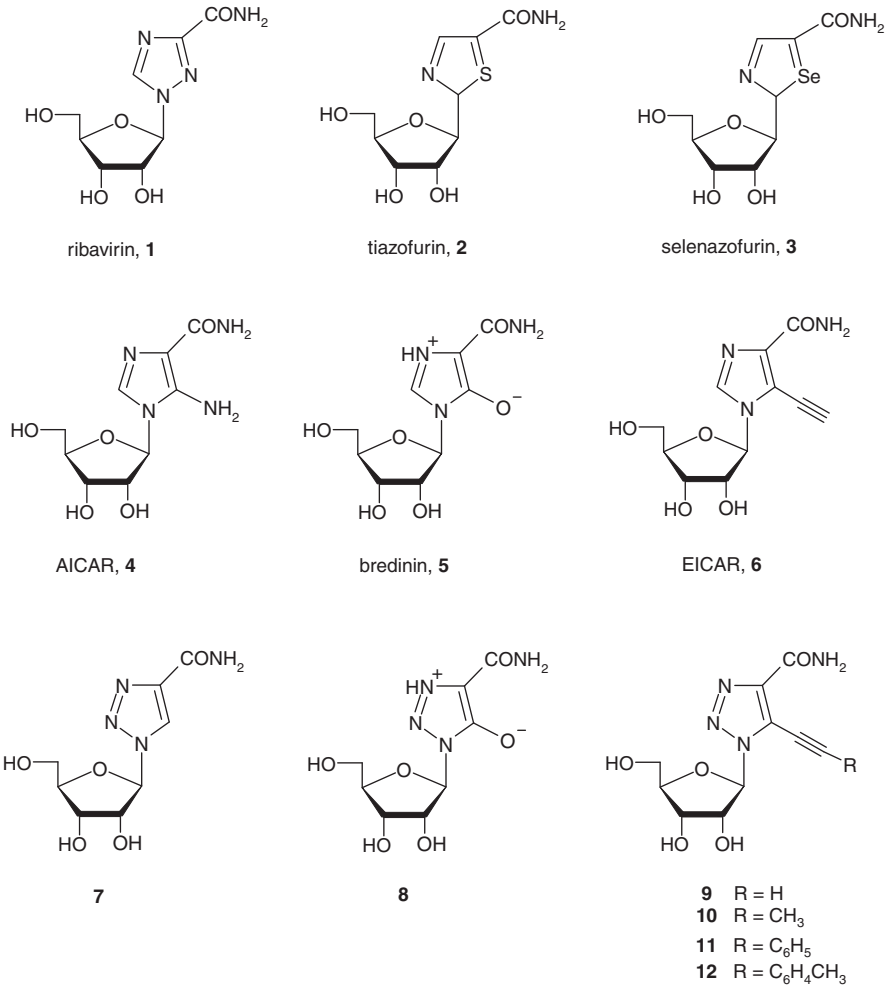


Figure 1.

Not many 4,5-disubstituted-1,2,3-triazole nucleosides analogous to AICA riboside have been synthesized so far and, in particular, evaluated for their biological activity. For example, in the case of 1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-5-olate-4-carboxylic acid amide (**8**), isosteric to **5**, accessible data are scarce and relate to the antiviral activity of **8** against measles and herpes simplex viruses.[10] It seems, that 5-substituted 4-carbamoyl-1,2,3-triazole ribosides constitute the next subgroup of AICA riboside analogues, biological activity of which should be thoroughly evaluated. It makes them also interesting objects for synthesis, especially the ones with attached alkynyl groups in position 5.

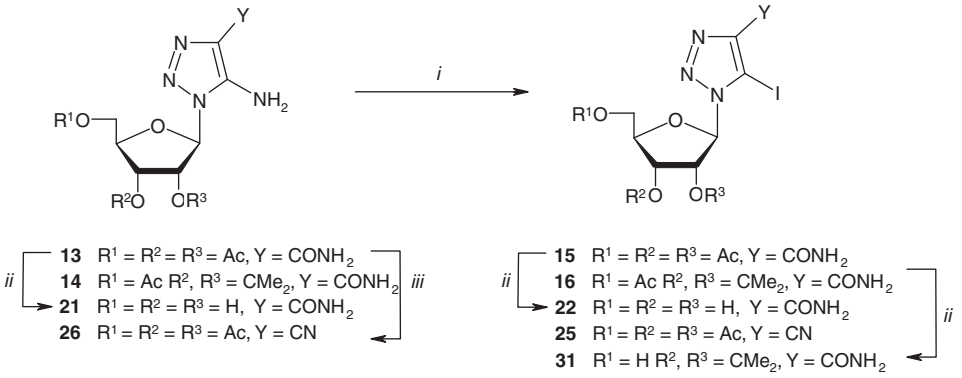
Mechanism-based inhibition of IMPDH by EICAR-MP is a consequence of irreversible addition of S^- nucleophile of cysteine residue (e.g., Cys-331 in human IMPDH) to the ethynyl group of nucleotide. We designed 5-ethynyl-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (ETCAR, **9**), which after being transformed into 5′-monophosphate, should form readily a covalent adduct with IMPDH.

2. Chemistry

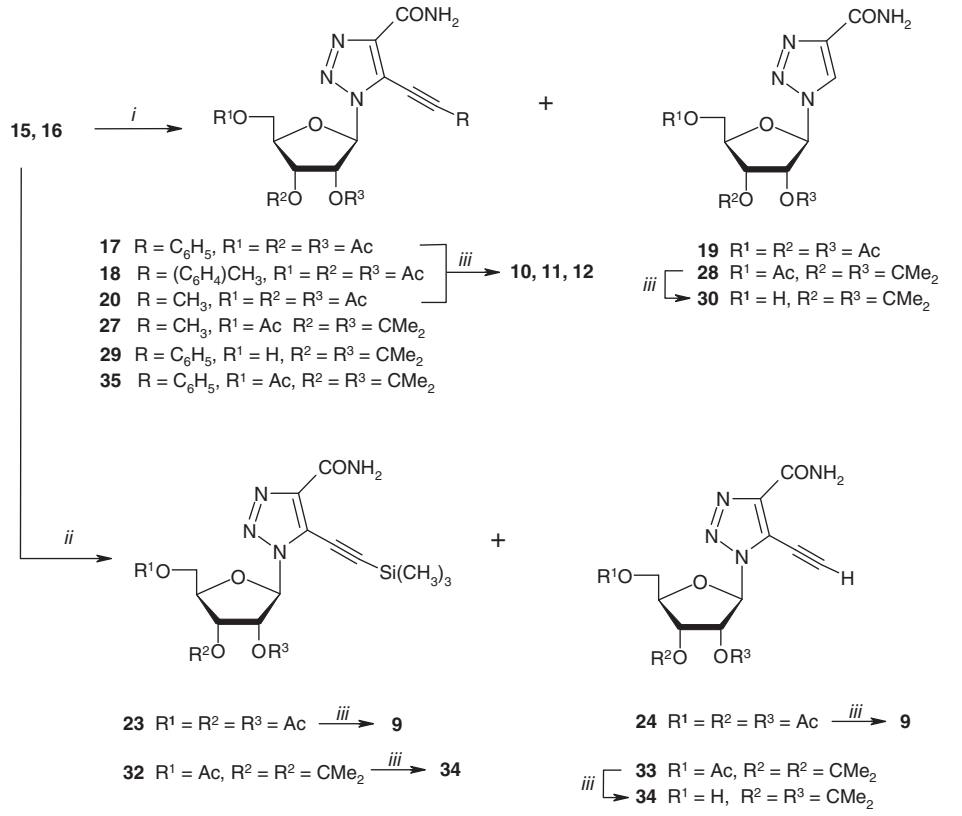
Organopalladium chemistry was exploited for the synthesis of **9** and its congeners such as 5-propynyl (**10**), 5-phenylethynyl (**11**) and toluenethynyl (**12**), but we encountered a serious problem – thermal instability of a 5-iodo precursor, while performing the introductory experiments.[11] It narrowed the scope of reaction conditions we intended to investigate, since the significant retardation of coupling reactions of 5-iodo-1,2,3-triazole could be observed in comparison with 5-iodoimidazole, considering that both heterocycles possessed the same substituent (carbamoyl) in position 4.[6] However, it did not pose a difficulty when a carbocyclic moiety was attached to 1,2,3-triazole and a coupling reaction could be carried out at 155 °C.[12]

Although a multicomponent one-pot method to efficiently synthesize 5-iodo-1,4-disubstituted triazole has been newly established on a small scale,[13] we exploited the diazotization-iodination reaction to transform 5-aminotriazolonucleoside. It had been prepared earlier by 1,3-dipolar cycloaddition of cyanoacetamide and 1-β-D-ribofuranosyl azide, carrying 2,3-*O*-isopropylidene blockade to avoid any side reaction in basic conditions.[14] Appropriately protected 5-aminonucleosides **13**[15] and **14** upon diazotization with organic nitrite in diiodomethane[6] were iodinated to the pivotal compounds **15** and **16** in 50–60% yield (Scheme 1), similarly to the respective 1,2,3-triazolo-carbanucleoside.[12]

We investigated coupling reactions of 5-iodo-1-β-D-ribofuranosyl-1,2,3-triazole-4-carboxylic acid amide in triacetate form (**15**) with phenylacetylene and tolueneacetylene according to copper-free Sonogashira protocol, using bis(benzonitrile)palladium dichloride as a catalyst, triethylamine as a base and dimethylformamide (Scheme 2). The reactions had to be performed at 100 °C and for 20 h to achieve couplings, the 5-alkynyl products **17** and **18** were isolated in a good yield (70% and 71%, respectively), regardless of a minor reaction course – hydrodehalogenation of the substrate **15** to the known triazole derivative **19.** The conditions were subse-



Scheme 1. Reagents and conditions: (i) isoamyl nitrite, CH_2I_2, 100 °C; (ii) MeONa/MeOH, Dowex H^+; (iii) Py, TsCl, rt.



Scheme 2. Reagents and conditions: (i) alkyne, Et_3N, $(PhCN)_2PdCl_2$, DMF, 100 °C or phenylacetylene, K_2CO_3, $(PhCN)_2PdCl_2$, 1,4-dioxane, 100 °C; (ii) TMSC≡CSnBu_3, $(PhCN)_2PdCl_2$, CH_3CN, 90 °C; (iii) MeONa/MeOH, Dowex H^+, 0 °C.

quently applied to a more demanding reaction with propyne, it was necessary to maintain the temperature at 100 °C and to use approximately 60 equiv of alkyne to achieve efficient coupling. Standard purification followed and we obtained 5-propynyl derivative **20** in 51% yield and the deiodinated **19** (15%) as well. Triazolonucleosides **13**, **15**, **17**, **18**, and **20** were then subjected to methanolysis to afford **21**,[15] **22**, **10–12**, respectively. Regarding the synthesis of ETCAR we could easily presume the formation of dimeric product upon coupling of **15** and TMS–acetylene in the presence of a base, due to an anticipated lability of TMS group attached to a preformed product. Afterwards, we turned to the methods offering neutral conditions and attempted a Kosugi–Migita–Stille reaction, bearing in mind that the coupling with trimethyl[(tributylstannyl)ethynyl]silane (TMSE-*n*-Bu_3Sn) had been used previously for the synthesis of EICAR (77% of the protected product).[6] First, we carried out coupling experiments for the iodonucleoside **15** with TMSE-*n*-Bu_3Sn in DMF at elevated temperature using either conventional heating or a microwave oven. The results were generally unsatisfactory and we replaced DMF by acetonitrile. Despite the fact that iodonucleoside **15** was more prone to hydrodehalogenation than expected and, that we could not elaborate conditions to prevent this side reaction (22% loss of **15**), we achieved the coupling conventionally, maintaining the temperature at 90 °C for several hours. Purification of fully protected **23** by chromatography could not be omitted and resulted in a partial loss of TMS group – the oily products **23** and **24** were isolated in the total 72% yield, comparable to that one of EICAR. Methanolysis of both products afforded ETCAR (**9**), in a high yield. Coupling experiments performed next were aimed to minimize the loss of substrate **15** being dehalogenated to **19**. The synthesis of ETCAR

could be attempted with 4-cyano-5-iodo-precursor **25**, to reduce steric congestion near the reaction centre, accordingly to the successful alternative approach to EICAR synthesis (76% yield).[6] For this purpose we transformed the 5-amino derivative **13** into the nitrile **26**, which was further iodinated to **25**. Contrary to our expectations, we did not succeed in replacing the carboxamide **15** by the carbonitrile **25**, due to an instability of the latter under conditions required to achieve the coupling.

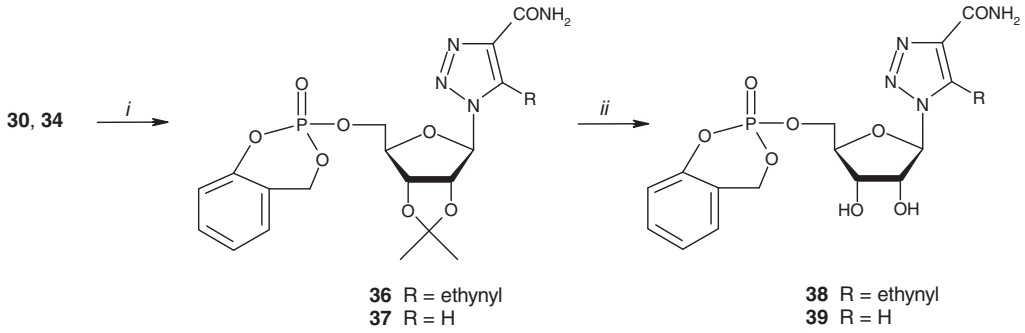
Commonly applied in coupling reactions soluble palladium(0) complexes – $dba_2Pd(0)$ and $[PPh_3]_4Pd(0)$ catalysts appeared to be useless in our case, as expected. The presence of phosphine was a driving force of hydrodehalogenation of **15** (63% of the only product **19**). Our further experiments performed with the more rigid substrate **16**, revealed substantially different reactivity of this compound while coupled with terminal alkynes or trimethyl-[(tributylstannyl)ethynyl]silane (TMSE-*n*-Bu_3Sn). Coupling of **16** with terminal alkynes generally gave unsatisfactory results, for example, 32% yield of the propynylated product **27**, and proceeded in favor of hydrodehalogenation to the compound **28** (50% yield). In turn we found almost equal amounts of both anticipated products **29** and **30** (ca. 30% each) resulting from a reaction of acetonide **31** with phenylacetylene. Fortunately, the cross-coupling of **16** with TMSE-*n*-Bu_3Sn could be achieved in 62% total yield (7% of fully protected **32**, 55% of the desilylated product **33**), but the minor course of reaction should not be disregarded (18% of the byproduct **28**). Nevertheless, after conventional deprotection of 5′-hydroxyl group acetonides **30** and **34** could be transformed further into masked nucleoside 5′-monophosphates.

Recently, many accounts have presented studies on hydrido complexes of palladium and their significance as intermediates in reactions performed with, for example, aryl halides.[16–18] For example, Zawisza and Muzart investigated a particular role of a commonly used solvent for Pd-catalysed coupling reactions (DMF), as a hydride source, and proposed a mechanism of this reaction. In accordance with the literature we assumed that Pd-catalysed hydrodehalogenation of iodosubstrates **15**, **16** or **31** was initiated by oxidative addition of a Pd(0) catalyst to produce an organopalladium(II) complex, which instead of reacting with a terminal alkyne, could coordinate to an amine present in the reaction milieu. A further hydride transfer from the already bound amine to the palladium atom would lead to the formation of hydridoorganopalladium complex. This unstable intermediate could undergo easily reductive elimination to yield triazolonucleosides **19**, **28** or **30** and a regenerated Pd(0) catalyst. Regarding possible hydride sources present in this case, both dimethylformamide (precursor of dimethylamine)[17] and triethylamine[18] should be taken into consideration. Although others have proved that H-transfer from a tertiary amine occurs even in a presence of DMF,[19] we decided to circumvent the problem by elaborating alternative reaction conditions: Et_3N and DMF were changed for potassium carbonate and 1,4-dioxane, respectively. According to this modified protocol, we achieved a successful coupling of the acetonide **16** with phenylacetylene – 72% of the desired product **35**, while hydrodehalogenation produced only 6% of the triazolonucleoside **28**. However, as far as cross-coupling reactions of **15** and **16** with organostannane are concerned, a source of the hydridopalladium complex remains unknown. In the course of our investigations we demonstrated that hydrodehalogenation could be a drawback in the synthesis of 1,2,3-triazolonucleosides via organopalladium chemistry. Particularly it should not be disregarded for substrates of increased steric congestion, which slows down dramatically a reaction of a preformed organopalladium(II) complex with a terminal alkyne.

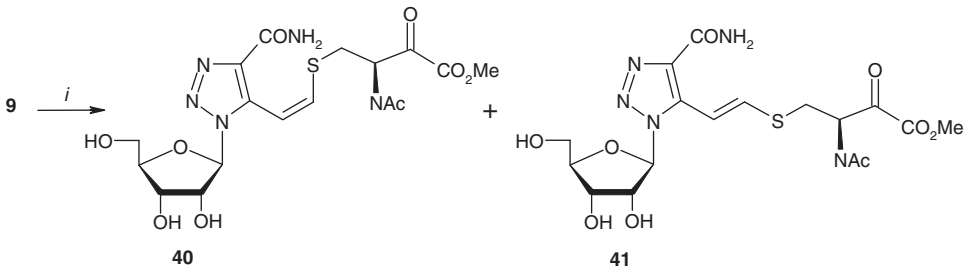
Nucleoside 5′-monophosphates (NMPs) can be converted into nonpolar derivatives (pronucleotides), usually phosphotriesters, which after entering cells, can be enzymatically and/or chemically converted into NMPs. It allows to avoid enzymatic phosphorylation of nucleoside drugs by nucleoside kinases, which does not proceed efficiently in some cases and in consequence, it lowers the efficacy of a drug. *Cyclo*Sal-pronucleotide concept introduced originally by Meier[20] has been an example of the kinase by-pass approach. This interesting proposition has been based upon nonenzymatic, stepwise pH driven hydrolysis of cyclic saligenyl esters of nucleoside 5′-monophosphates within the cell. It has been particularly advantageous, when applied to anti-HIV drugs and anticancer FdUMP.[21] However, as ribonucleosides are concerned, only two applications of the discussed pronucleotide approach have been reported so far.[22] The first one deals with the above-mentioned tiazofurin **2** and has been realized to prevent toxic effects of nonphosphorylated **2** on the central nervous system. The second has been carried out for cytostatic 6-(het)aryl-7-deazapurine ribosides to improve their pharmacological properties. Exactly for that reason we decided to prepare *cyclo*Sal-pronucleotides of inactive 5-unsubstituted derivative **7** and ETCAR **9** (Scheme 3). The latter, according to our assumption, could be the most active compound of 1,2,3-triazole series. Phosphitylation of **30** and **34** was performed with salicylchlorophosphane in the presence of triethylamine and followed by in situ oxidation to phosphotriesters **36** and **37**, which we isolated as diastereomeric mixtures in 45% and 48% yield, respectively. Subsequently, we applied the standard method (80% aq TFA) to remove isopropylidene groups and the final *cyclo*Sal-pronucleotides **38** and **39** were purified by chromatography and characterized as mixtures (1:1) of diastereoisomers (R_P, S_P).

Prior to any biological assays we conducted a model addition of thiolate nucleophile to ETCAR with protected L-cysteine (*N*-acetyl-L-cysteine methyl ester) in the presence of catalytic amount of triethylamine in 0–5 °C (Scheme 4). The reaction proceeded readily and we observed the formation of both isomeric products *Z* (**40**) and *E* (**41**), isolated in 44% and 12% yield. On the other hand, the reaction of triacetate **24** with sodium methanethiolate, conducted in aqueous methanol at ambient temperature, afforded products of intra and intermolecular addition, respectively. Occurrence of the former reaction has been a measure of enhanced acidity of 2′-hydroxyl group of ETCAR (**9**), if compared to that of EICAR (**6**). It should be mentioned here, that the formation of an analogous product, namely 1-β-D-ribofuranosyl-5,2′-*O*-cycloethenoimidazole-4-carbonitrile has been reported to proceed under drastic conditions – with methanolic ammonia at 120 °C.[23]

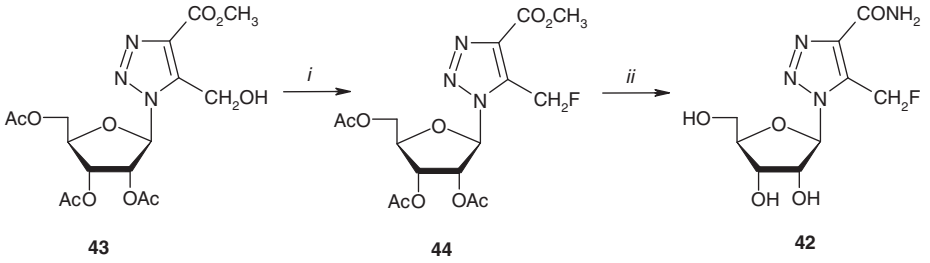
Additionally, we aimed to synthesize the triazole derivative carrying 5-fluoromethyl substituent (**42**) bearing in mind that some inosine or AICA riboside analogues possessing an electron-withdrawing group proximal to C-2 or C-5, respectively, exhibited noticeable IMPDH inhibitory activity, provided that they had been earlier efficiently 5′-phosphorylated.[24] The synthesis could be accomplished by a Heck reaction of **15** to introduce a 5-alkenyl substituent to be further transformed into the 5-hydroxymethyl product (**43**) via a multistep procedure. We abandoned this approach due to a discouraging yield of coupling with methyl acrylate. The alternative method has been already described[25] and according to it we synthesized compound **43** in Huisgen reaction of 1-β-D-ribofuranosyl azide triacetate with methyl 4-hydroxy-2-butynoate, followed by deblocking of the triazole 5-hydroxymethyl group. Subsequently, we approached fluorination of **43** with DAST (Scheme 5), and settled the conditions required to obtain the ester (**44**) in a satisfactory yield (62%). Upon methanolysis, compound **44** was transformed into the desired amide (**42**), which was then purified by crystallization to afford material suitable for X-ray analysis. Thus, we envisioned a possibility to compare compound **42** and antiviral nucleoside **1** in respect of hydrogen bonding properties, which for the latter compound have been of great importance for its biological activity. The antiviral potency of ribavirin (**1**) on RNA viruses may be explained by several mechanisms.[2] One of



Scheme 3. Reagents and conditions: (i) salicylchlorophosphane, Et_3N, CH_3CN, 0 °C, then *t*-BuOOH, 0 °C to rt; (ii) 80% aq CF_3CO_2H, 0 °C.



Scheme 4. Reagents and conditions: (i) *N*-acetyl-L-cysteine methyl ester, Et_3N, CH_3CN, 0–5 °C.



Scheme 5. Reagents and conditions: (i) DAST, CH_2Cl_2, −35 °C; (ii) MeOH sat NH_3, rt, 48 h.

them is based on mutagenic effect of the compound on viral genomic RNA that leads to depletion of the virus. The phenomenon has been known as lethal mutagenesis or 'error catastrophe'.[26] It is considered to be a consequence of base-pairing of ribavirin 5′-triphosphate with both cytosine (C) and uracil (U) due to rotation of the carboxamide group.[27]

3. Crystal and molecular structure of 42

The investigated compound (**42**) crystallizes in tetragonal $P4_32_12$ space group with one molecule of 5-fluoromethyl-1-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide in the asymmetric unit. The molecular structure and atom numbering of **42** is presented in Figure 2. The geometry of the ribose fragment generally agrees with the data found by Prusiner & Sundaralingam in two polymorphic forms, V1 and V2, of ribavirin (**1**).[28] In the present structure the conformation of the ribose ring is an envelope C3′-endo (^3E). In terms of pseudorotation the ribose conformation is characterized by $P = 20.2(1)°$ and $\tau_m = 40.9(1)°$. The glycosidic torsion angle, O(4′)–C(1′)–N(1)–C(5), which describes the relative orientation of the base with respect to the sugar is in the high anti region ($\chi = -87.2(2)°$). The torsion angle O(5′)–C(5′)–C(4′)–C(3′), $\gamma = 46.5(2)°$, indicates that the side chain has the preferred *gauche*$^+$ conformation. For comparison, in V1 the glycosidic torsion angle,

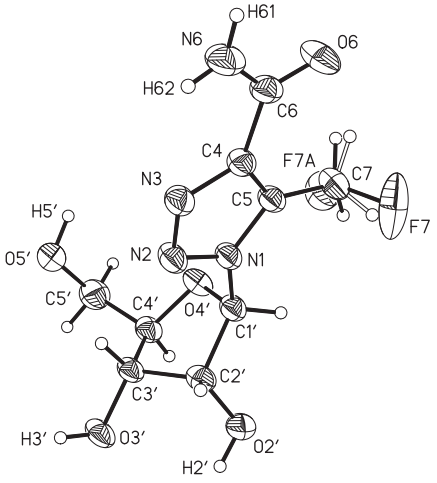


Figure 2. An ORTEP plot of the asymmetric unit contents of the crystal **42**. The atomic displacement ellipsoids have been drawn at 40% probability level. H atoms are represented as spheres of arbitrary radius.

pseudorotation phase angle P and γ torsion angle are, respectively, anti ($\chi = 10.4°$), $^3T_2$ ($P = 11.7°$) and *gauche*$^+$ while in V2 they are high anti ($\chi = 119.0°$), $_2T^1$ ($P = 335.8°$) and *trans*.[28] In the present struc-

ture the five-membered ring of the base is flat. The angle between the planes through the 1,2,3-triazole ring and the amide group is 2.7(2)°. The bond lengths of the 1,2,3-triazole ring show a delocalization of electrons and a prevalent double character of the N(2)–N(3) and C(4)–C(5) bonds. The heterocyclic N(3) and amide N(6) atom are on the same side of the triazole moiety (torsion angle N(3)–C(4)–C(6)–N(3) = 2.8(2)°). The geometry of the CH_2F group is perturbed due to disorder.

The crystal and molecular structure of 1,2,3-triazolonucleoside **42** is stabilized by classical hydrogen bonds, C–H···O and C–H···F weak interactions and stacking forces between aromatic moieties of nitrogen bases (Fig. 3). One structural motif, a pair of hydrogen-bonded bases, is particularly interesting for us to observe (Fig. 4). The N(6) atom of the carboxamide group of **42** donates hydrogen to the O(6) oxygen atom of a neighboring molecule and a short intermolecular N–H...O hydrogen bond is formed with the length of 2.911(2) Å. Two hydrogen-bonded bases are related by a two-fold axis along the diagonal [1 1 0] direction and they form an eight-membered ring with two donors and two acceptors, $R_2^2(8)$. A second motif, $R_2^2(16)$ ring, is a result of sugar...base interactions via two O(3′)–H···N(3) hydrogen bonds, related by another two-fold axis (Fig. 4). These two C_2-symmetric motifs form a ribbon-like chain of rings running along the *c* axis. The O(5′) and O(3′) atoms act as donors and acceptors in a system of homodromic cooperative O(2′)–H···O(5′)–H···O(3′)–H···N(3) hydrogen bonds. A co-operative effect combined with π–π stacking interactions stabilize additionally the structure of a 'double-ribbon'. The 'double-ribbon' propagated by a left-handed four-fold screw axis is a building block of the crystal architecture (Fig. 3).

Comparison of hydrogen bonding systems in **42**, and **1** (V1,V2) revealed differences between these structures, with two exceptions: (i) only one of the amino hydrogen atoms is engaged in classical hydrogen bonding in all compared crystals and (ii) the O(3′)–H···N(3) hydrogen bond found in **42** is present in V1. Although, in the crystals of ribavirin (V1 and V2) base pairing does not occur at all, in the crystal structure of the investigated 1,2,3-triazole-4-carboxamide derivative **42** only one rotamer is present with a *syn* conformation which is assumed to base-pair with uracil.

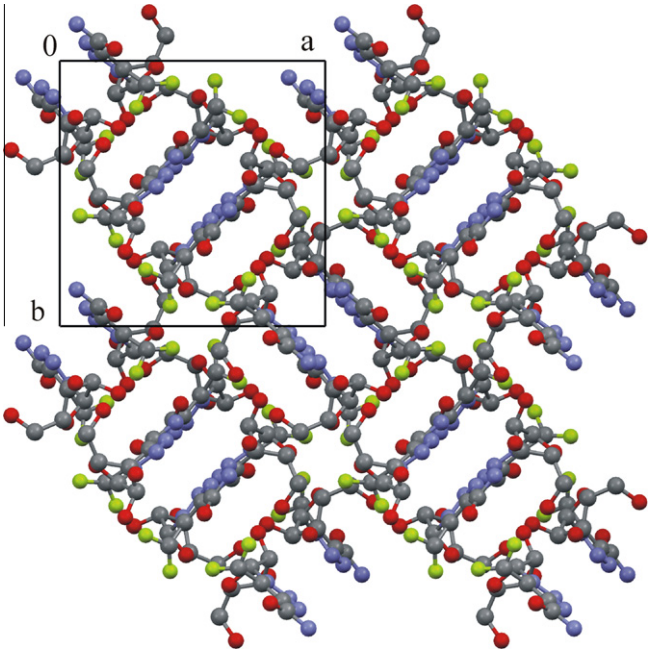


Figure 3. Crystal packing of **42**. Four unit-cells are viewed along *c* axis. Hydrogen atoms are omitted for clarity.

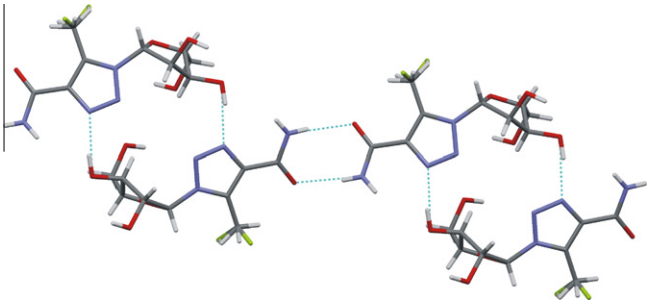


Figure 4. Base pairing in the crystal of **42**. Hydrogen bonds are indicated by the dotted lines.

4. Cytotoxicity assays

At the beginning of systematic screening of their biological activity, several 1,2,3-triazolonucleosides (**7**, **9**–**12**, **21**, **22**, **42**) and two pronucleotides (**38**, **39**) were evaluated for their cytotoxic properties against K562 (leukemia), HeLa (cervix carcinoma) and HUVEC (normal) cells. As the control (100% viability in the MTT assay) cells treated with DMSO (1%) were used. The viability of cells was determined at four different drug concentrations: 1, 1×10^{-2}, 1×10^{-4} and 1×10^{-6} mM.

Cytotoxic activities of EICAR and its congeners have been demonstrated toward a broad panel of human cancer cell lines.[6] In that study EICAR has been selected as the most promising anticancer agent comparable with 5-fluorouracil. Contrary to our expectations, 1,2,3-triazolonucleosides exhibited marginal to low levels of cytotoxic activity against the used cell lines, or were inactive (Table 1). Results of the assay in human chronic myelogenous leukemia cells (K 562): IC_{50} 90, 400 and 300 μM for ETCAR, **10** and **12**, respectively, led us to general conclusion that an ethynyl group was the optimal substituent in position 5 of triazole (IC_{50} 0.64 μM for EICAR[6]). When it was replaced by a larger alkynyl group we observed decrease of activity. Surprisingly, pronucleotide **38** only matched parent nucleoside **9** in cytotoxicity against K 562 cells, and its final toxicity toward HUVEC cells was four times higher than that of **9.** Transformation of inactive nucleoside **7** (IC_{50} >1 mM), into the form of *cyclo*-Sal-pronucleotide (**39**) assured its cytotoxicity, albeit at a low level, against HeLa cells. Cytotoxic effect of hydrophobic compound **22** seemed to be time-dependent, but the above-mentioned compounds did not display any selectivity of their cytotoxic effect against cancer versus normal cells. Compounds **21** and **42** were inactive toward cells used in the study (IC_{50} >1 mM). Interestingly, a series of 4-substituted 1,2,3-triazolonucleosides, and compound

Table 1
Inhibitory effects of 1,2,3-triazolonucleosides **7**, **9**–**12**, **21**, **22**, **42** and pronucleotides **38**, **39** on K562, HeLa and HUVEC cells

Compd	K562 IC_{50}[a] (μM)		HeLa IC_{50} (μM)		HUVEC IC_{50} (μM)	
	24 h	48 h	24 h	48 h	24 h	48 h
7	>1000	>1000	>1000	>1000	>1000	>1000
9	90	90	200	150	70	200
10	>1000	400	>1000	>1000	>1000	>1000
11	nd[b]	nd	>1000	>1000	>1000	>1000
12	800	300	>1000	700	>1000	>1000
21	>1000	>1000	>1000	>1000	>1000	>1000
22	600	80	500	250	700	70
38	100	90	1000	100	40	50
39	200	200	100	90	700	200
42	>1000	>1000	>1000	>1000	>1000	>1000

[a] The concentration of test compound required to reduce the cell survival fraction to 50% of the control.
[b] Not determined.

7 as well, were evaluated by others, for cytostatic activity in vitro against leukemia cell lines (L1210, Molt4/C8, CEM).[29] The best antiproliferative effect (IC_{50} 36–152 μM) was established for the 4-(*p*-methoxyphenyl) derivative.

5. Experimental

5.1. General methods

Melting points were determined on MEL-TEMP II capillary melting point apparatus and are uncorrected. Elemental analyses were performed with a microanalyser Elemental Vario ELIII by Microanalytical Laboratories of the A. Mickiewicz University, Poznan. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer operating at 400 MHz and 100.6 MHz, respectively, or on Unity 300 Varian spectrometer operating at 300 and 75.4 MHz, respectively (tetramethylsilane or DMSO as internal standard). ^{31}P NMR and ^{19}F spectra were recorded on a Bruker 400 spectrometer at 162 and 376 MHz, respectively (H_3PO_4 or trifluoroacetic acid as external standard). The chemical shifts are reported in ppm (δ scale), spectra are described. Mass spectra were recorded using Bruker microTOF-q mass spectrometer in positive (ESI^+) or negative (ESI^-) mode. UV spectra were taken with Perkin Elmer Lambda EZ201 spectrophotometer. LC/UV analyses were performed on Merck Hitachi HPLC system: pump model L-7100, diode array detector model L-7450, autosampler model L-7200 and Superspher 100 (TM) RP-18 column (250 × 2 mm; Merck), gradient% MeOH in H_2O: 10% MeOH (0–2 min), 10–30% MeOH (2.1–8 min), 30–80% MeOH (8.1–12 min), 80% MeOH (12.1–18 min), 80–100% MeOH (18.1–20 min), the flow rate was 0.2 ml min^{-1}. Thin-layer chromatography (TLC) was performed on Merck precoated 60 F_{254} silica gel plates. Column chromatography was carried out on Merck silica gel 60H (40–63 μm). Anhydrous dimethylformamide (DMF), tetrahydrofuran (THF) and diethyl ether were supplied in Sure/Seal bottles (Aldrich). Methanol HPLC grade was purchased from J.T. Baker, acetone and alkynes were supplied from Aldrich. Other anhydrous solvents were prepared as follows: CH_3CN and pyridine were distilled with P_2O_5 and stored over 3 and 4 Å molecular sieves, respectively, MeOH and EtOH by treatment with magnesium turnings/iodine. Triethylamine was freshly distilled with calcium hydride. Anhydrous 1,4-dioxane was prepared by distillation with calcium hydride first, followed by distillation with sodium/benzophenone. Trimethyl[(tributylstannyl)ethynyl]silane (bp 96 °C, 0.01 Torr) was prepared as described.[30]

5.2. 1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-amino-1*H*-[1,2,3]triazole-4-carboxylic acid amide (14)

To a cooled (0 °C) solution of KOH (1.753 g, 31.25 mmol) in DMF–H_2O (7:1; 50 ml) was added cyanoacetamide (2.628 g, 31.25 mmol). The mixture was stirred at 0 °C until all of the solid material had dissolved. Next, a solution of 2,3-*O*-isopropylidene-β-D-ribofuranosyl azide[14] (5.38 g, 25 mmol) in DMF (24 ml) was added and the whole was stirred at room temperature for 5 h. The cooled (0 °C) mixture was neutralized with 80% aq AcOH and the volatiles were evaporated. The residue was co-evaporated with anhyd pyridine, then treated with anhyd pyridine (70 ml) and acetic anhydride (7.657 g, 75 mmol). The resulting mixture was stirred at room temperature overnight, then the reaction was quenched by addition of anhyd MeOH (1 ml). After evaporation the residue was taken up into CH_2Cl_2 and extracted with H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The material obtained was treated with EtOAc (40 ml), the insoluble crystalline product was separated by filtration, washed with CH_2Cl_2 and dried under vacuum to give 7.68 g of **14** (mp 153 °C, 90% yield). ^{1}H NMR (DMSO-d_6) δ 7.48 and 7.13 (2× s, 2H, CO–NH_2), 6.65 (s, 2H, NH_2), 6.26 (s, 1H, 1′-H), 5.46 (d, 1H, 2′-H), 4.97 (dd, 1H, 3′-H), 4.35 (dt, 1H, 4′-H), 4.08 and 3.85 (2× dd, 2H, 5′-H), 1.98 (s, 3H, Ac), 1.51 and 1.35 (2× s, 6H, 2× CH_3).

5.3. 5-Iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (15)

To a suspension of **13**[15] (1.255 g, 3.26 mmol, dried in vacuo over P_2O_5, at 90 °C, for 4 h) in diiodomethane (52.2 g, 195 mmol) was added isoamyl nitrite (1.335 g, 11.4 mmol). The mixture was stirred at 110 °C for 1.5 h, then it was applied onto a silica gel column. The column was eluted with CH_2Cl_2 (in order to collect diiodomethane) followed by CH_2Cl_2/MeOH (95:5) to give crude product. It was next rechromatographed using toluene/MeOH (95:5 → 9:1) to afford homogenous **15** as a solid (960 mg, 59% yield). ^{1}H NMR (DMSO-d_6) δ 7.95 and 7.60 (2× s, 2H, CO–NH_2), 6.22 (d, 1H, 1′-H), 6.05 (dd, 1H, 2′-H), 5.67 (dd, 1H, 3′-H), 4.50 (m, 1H, 4′-H), 4.32 and 4.02 (2× dd, 2H, 5′-H), 2.13, 2.10 and 1.88 (3× s, 9H, 3× Ac). ^{13}C NMR (DMSO-d_6) δ 169.87, 169.56 and 169.39 (3× *C*O–CH_3), 160.98 (CO–NH_2), 143.72 (C-4), 89.58 (C-1′), 87.45 (C-5), 80.23 (C-4′), 73.23 (C-2′), 70.20 (C-3′), 62.04 (C-5′), 20.37 and 20.30 (3× CO–*C*H_3).

5.4. 1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-iodo-1*H*-[1,2,3]triazole-4-carboxylic acid amide (16)

Prepared from **14** by the reaction procedure described for **15**. For chromatography was used CH_2Cl_2 followed by CH_2Cl_2/MeOH (97:3 → 95:5) to give the product which was next rechromatographed with EtOAc/hexane (1.5:1 → 2:1) to afford **16** as solid foam (53% yield). ^{1}H NMR (DMSO-d_6) δ 7.98 and 7.64 (2× s, 2H, CO–NH_2), 6.29 (s, 1H, 1′-H), 5.67 (d, 1H, 2′-H), 5.12 (dd, 1H, 3′-H), 4.52 (dt, 1H, 4′-H), 4.07 and 3.87 (2× dd, 2H, 5′-H), 2.00 (s, 3H, Ac), 1.60 and 1.43 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{13}H_{17}IN_4O_6Na$: 475.0085; found: 475.0095.

5.5. General procedure for the preparation of compounds 17, 18 and 20

A solution of **15** (dried in vacuo over P_2O_5, at 60 °C, for 3 h) and bis(benzonitrile)palladium dichloride (0.1 equiv) in anhyd DMF (12 ml/1 mmol of **15**) was vigorously deoxygenated with argon. Anhyd triethylamine (6 equiv) was added, followed by phenylacetylene (5 equiv), 4-methylphenylacetylene (3 equiv) or a solution of propyne (60 equiv) in anhyd DMF, respectively. The reaction mixture was heated at 100 °C overnight in a Parr reactor, then evaporated. The residue was chromatographed on silica gel column using EtOAc/hexane (1:1 → 2:1) for **17** and **18** or (2:1 → 5:1) for **20**. Further elution with EtOAc/hexane (6:1) led to the isolation of deiodinated product **19**.

5.6. 5-Phenylethynyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (17)

Oily product, 70% yield. ^{1}H NMR ($CDCl_3$) δ 7.64–7.67 and 7.37–7.48 (2× m, 5H, Ph), 6.98 and 5.65 (2× s, 2H, CO–NH_2), 6.29 (d, 1H, 1′-H), 6.07 (dd, 1H, 2′-H), 5.80 (t, 1H, 3′-H), 4.51 (m, 1H, 4′-H), 4.44 and 4.18 (2× dd, 2H, 5′-H), 2.15, 2.14 and 2.05 (3× s, 9H, 3× Ac). ^{13}C NMR ($CDCl_3$) δ 170.51, 169.41 and 169.20 (3× *C*O–CH_3), 160.58 (CO–NH_2), 142.21 (C-4), 132.24, 130.28, 128.56 and 120.77 (Ph), 123.82 (C-5), 104.73 (C≡C–Ph), 88.58 (C-1′), 81.12 (C-4′), 74.00 (C-2′), 72.45 (C≡C–Ph), 70.86 (C-3′), 62.75 (C-5′), 20.61, 20.47 and 20.43 (3× CO–*C*H_3). HRMS $[M+Na]^+$ calcd for $C_{22}H_{22}N_4O_8Na$: 493.1330; found 493.1340.

5.7. 5-(4-Methylphenylethynyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (18)

Oily material, 71% yield. ^{1}H NMR ($CDCl_3$) δ 7.54 and 7.21 (2× d, 4H, Ph), 6.98 and 5.69 (2× s, 2H, CO–NH_2), 6.29 (d, 1H, 1′-H), 6.06 (dd, 1H, 2′-H), 5.80 (t, 1H, 3′-H), 4.51 (m, 1H, 4′-H), 4.44 and 4.18 (2× dd, 2H, 5′-H), 2.40 (s, 3H, CH_3), 2.15, 2.14 and 2.05 (3× s, 9H, 3× Ac). ^{13}C NMR ($CDCl_3$) δ 170.50, 169.39 and 169.17 (3× *C*O–*C*H_3), 160.70 (CO–NH_2), 142.00 (C-4), 140.81, 132.14, 129.31 and 117.66 (Ph), 123.97 (C-5), 105.18 (C≡*C*–Ph), 88.51 (C-1′), 81.06 (C-4′), 73.98 (C-2′), 71.97 (*C*≡C–Ph), 70.85 (C-3′), 62.76 (C-5′), 21.69 (Ph–*C*H_3), 20.63, 20.48 and 20.43 (3× CO–*C*H_3). HRMS [M+Na]$^+$ calcd for $C_{23}H_{24}N_4O_8Na$: 507.1486; found 507.1487.

5.8. 5-Propynyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (20)

Oily product, 51% yield. ^{1}H NMR ($CDCl_3$) δ 6.96 and 5.73 (2× s, 2H, CO–NH_2), 6.21 (d, 1H, 1′-H), 6.03 (dd, 1H, 2′-H), 5.78 (t, 1H, 3′-H), 4.49 (m, 1H, 4′-H), 4.42 and 4.15 (2× dd, 2H, 5′-H), 2.25 (≡C–CH_3), 2.14, 2.13 and 2.05 (3× s, 9H, 3× Ac). ^{13}C NMR ($CDCl_3$) δ 170.49, 169.39 and 169.17 (3× *C*O–*C*H_3), 160.85 (CO–NH_2), 141.77 (C-4), 124.08 (C-5), 103.53 (C≡*C*–CH_3), 88.24 (C-1′), 80.97 (C-4′), 73.85 (C-2′), 70.85 (C-3′), 63.68 (C≡C–Ph), 62.72 (C-5′), 20.62, 20.48 and 20.44 (3× CO–*C*H_3), 5.42 (≡C–*C*H_3). HRMS [M+Na]$^+$ calcd for $C_{17}H_{20}N_4O_8Na$: 431.1173; found 431.1175.

5.9. 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (19)

White solid, 5–15% yield. ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H, 5-H), 7.07 and 5.89 (2× s, 2H, CO–NH_2), 6.20 (d, 1H, 1′-H), 5.84 (dd, 1H, 2′-H), 5.56 (t, 1H, 3′-H), 4.51 (m, 1H, 4′-H), 4.41 and 4.25 (2× dd, 2H, 5′-H), 2.13, 2.12 and 2.10 (3× s, 9H, 3× Ac). ^{13}C NMR ($CDCl_3$) δ 170.34, 169.42 and 169.16 (3× *C*O–*C*H_3), 161.38 (CO–NH_2), 142.93 (C-4), 125.27 (C-5), 90.45 (C-1′), 81.05 (C-4′), 74.38 (C-2′), 70.30 (C-3′), 62.51 (C-5′), 20.65, 20.42 and 20.34 (3× CO–*C*H_3).

5.10. General procedure for the preparation of compounds 10–12 and 22

A solution of compound **17**, **18**, **20** or **15** in anhyd MeOH (10 ml/1 mmol of substrate) was cooled to 0 °C and 0.2 M MeONa in anhyd MeOH (0.2–0.5 equiv) was added. The mixture was allowed to react at +5 °C overnight. It was cooled again to 0 °C and neutralized with Dowex 50WX8-100 ion exchange resin, which was then separated by filtration. The solution was evaporated and the resulting oil was chromatographed on silica gel column with CH_2Cl_2/MeOH (9:1 → 6:1) for **10** and **22** or (95:5 → 9:1) for **11** and **12**.

5.11. 5-Propynyl-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (10)

Crude solid product (90% yield) was subjected to crystallization from iPrOH to give white crystalline material: mp 180 °C. ^{1}H NMR (DMSO-d_6) δ 7.83 and 7.57 (2× s, 2H, CO–NH_2), 5.91 (d, 1H, 1′-H), 5.65 (d, 1H, 2′-OH), 5.29 (d, 1H, 3′-OH), 4.79 (t, 1H, 5′-OH), 4.67 (dd, 1H, 2′-H), 4.24 (dd, 1H, 3′-H), 3.98 (dd, 1H, 4′-H), 3.38–3.57 (m, 2H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 160.54 (CO–NH_2), 142.43 (C-4), 122.89 (C-5), 102.07 (C≡C–Ph), 90.18 (C-1′), 86.16 (C-4′), 73.68 (C-2′), 70.59 (C-3′), 64.31 (*C*≡C–Ph), 61.72 (C-5′), 4.60 (≡C–*C*H_3). Anal. Calcd for $C_{11}H_{14}N_4O_5$: C, 46.81; H, 5.00; N, 19.85. Found: C, 46.72; H, 5.10; N, 19.68.

5.12. 5-Phenylethynyl-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (11)

Chromatographically purified solid product (68% yield) was next crystallized from iPrOH to give white needles: mp 166–169 °C. ^{1}H NMR (DMSO-d_6) δ 7.98 and 7.67 (2× s, 2H, CO–NH_2), 7.62–7.66 and 7.48–7.55 (2× m, 5H, Ph), 6.03 (d, 1H, 1′-H), 5.71 (d, 1H, 2′-OH), 5.34 (d, 1H, 3′-OH), 4.82 (t, 1H, 5′-OH), 4.72 (dd, 1H, 2′-H), 4.27 (dd, 1H, 3′-H), 4.01 (dd, 1H, 4′-H), 3.40–3.61 (m, 2H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 160.45 (CO–NH_2), 143.25 (C-4), 131.70, 130.40, 129.03 and 120.36 (Ph), 122.16 (C-5), 102.25 (C≡*C*–Ph), 90.77 (C-1′), 86.31 (C-4′), 73.78 (C-2′), 73.70 (*C*≡C–Ph), 70.54 (C-3′), 61.66 (C-5′). Anal. Calcd for $C_{16}H_{16}N_4O_5$: C, 55.81; H, 4.68; N, 16.27. Found: C, 55.73; H, 4.77; N, 16.17.

5.13. 5-(4-Methylphenylethynyl)-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (12)

Crude solid product (83% yield) was subsequently crystallized from iPrOH to result in white crystalline material: mp 195–196 °C. ^{1}H NMR (DMSO-d_6) δ 7.96 and 7.65 (2× s, 2H, CO–NH_2), 7.53 and 7.33 (2× d, 4H, Ph), 6.02 (d, 1H, 1′-H), 5.70 (d, 1H, 2′-OH), 5.33 (d, 1H, 3′-OH), 4.82 (t, 1H, 5′-OH), 4.71 (dd, 1H, 2′-H), 4.27 (dd, 1H, 3′-H), 4.01 (dd, 1H, 4′-H), 3.40–3.61 (m, 2H, 5′-H), 2.37 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 160.50 (CO–NH_2), 143.07 (C-4), 140.52, 131.65, 129.66 and 117.36 (Ph), 122.34 (C-5), 102.61 (C≡*C*–Ph), 90.71 (C-1′), 86.28 (C-4′), 73.78 (C-2′), 73.22 (*C*≡C–Ph), 70.56 (C-3′), 61.69 (C-5′), 21.18 (Ph–*C*H_3). Anal. Calcd for $C_{17}H_{18}N_4O_5$: C, 56.98; H, 5.06; N, 15.63. Found: C, 56.90; H, 5.34; N, 15.34.

5.14. 5-Iodo-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (22)

Crude product (74% yield) was crystallized from MeOH to give crystalline material: mp >132 °C (dec). ^{1}H NMR (DMSO-d_6) δ 7.89 and 7.55 (2× s, 2H, CO–NH_2), 5.91 (d, 1H, 1′-H), 5.69 (d, 1H, 2′-OH), 5.37 (d, 1H, 3′-OH), 4.80 (t, 1H, 5′-OH), 4.75 (dd, 1H, 2′-H), 4.26 (dd, 1H, 3′-H), 3.98 (dd, 1H, 4′-H), 3.40 and 3.54 (2× m, 2H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 161.09 (CO–NH_2), 143.15 (C-4), 91.66 (C-1′), 87.54 (C-5), 86.20 (C-4′), 73.73 (C-2′), 70.58 (C-3′), 61.67 (C-5′). Anal. Calcd for $C_8H_{11}IN_4O_5$: C, 25.96; H, 3.00; N, 15.14. Found: C, 25.74; H, 3.14; N, 15.13.

5.15. 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-5-trimethylsilyle thynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (23) and 5-ethynyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3] triazole-4-carboxylic acid amide (24)

A solution of **15** (685 mg, 1.38 mmol, dried in vacuo over P_2O_5, at 60 °C, for 3h) and bis(benzonitrile)palladium dichloride (53 mg, 0.14 mmol) in anhyd CH_3CN (18 ml) was vigorously deoxygenated with argon. Trimethylsilylethynyltributylstannane (802 mg, 2.07 mmol) was added and the mixture was heated at 90 °C for 18 h in a glass pressure tube. It was next diluted with CH_3CN (30 ml), extracted with hexane (3 × 50 ml) and evaporated. The residue was chromatographed on silica gel column with EtOAc/hexane (1.5:1 → 3:1) as an eluent. Product **23** was isolated as an oil (325 mg, 51% yield). ^{1}H NMR ($CDCl_3$) δ 6.93 and 5.72 (2× s, 2H, CO–NH_2), 6.21 (d, 1H, 1′-H), 5.94 (dd, 1H, 2′-H), 5.77 (t, 1H, 3′-H), 4.41–4.50 (m, 2H, 4′,5′-H), 4.18 (dd, 1H, 5′-H), 2.14, 2.13 and 2.06 (3× s, 9H, 3× Ac), 0.31 (s, 9H, TMS). HRMS [M+Na]$^+$ calcd for $C_{19}H_{26}N_4O_8SiNa$: 489.1412; found: 489.1434. Desilylated product **24** was obtained as an oil (114 mg, 21% yield). ^{1}H NMR ($CDCl_3$) δ 6.98 and 5.84 (2× s, 2H, CO–NH_2), 6.23 (d, 1H, 1′-H), 6.04 (dd,

1H, 2′-H), 5.76 (t, 1H, 3′-H), 4.41–4.50 (m, 2H, 4′,5′-H), 4.18 (dd, 1H, 5′-H), 4.00 (s, 1H, C≡CH), 2.14, 2.13 and 2.06 (3× s, 9H, 3× Ac). HRMS $[M+Na]^+$ calcd for $C_{16}H_{18}N_4O_8Na$: 417.1017; found: 417.1033. Further elution with EtOAc/hexane (6:1) gave **19** (113 mg, 22% yield).

5.16. 5-Ethynyl-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (ETCAR, 9)

A solution of **23** or **24** in anhyd MeOH (8 ml/1 mmol of substrate) was cooled to 0 °C and 0.2 M MeONa in anhyd MeOH (0.5 equiv) was added under argon. The mixture was allowed to react at +5 °C under an argon atmosphere overnight. It was cooled again to 0 °C and neutralized with Dowex 50WX8-100 ion exchange resin, which was then separated by filtration. The solution was evaporated and the resulting oil was chromatographed on silica gel column with CH_2Cl_2/MeOH (6:1) to afford **9** as solid foam in 57–80% yield. It was dissolved in EtOAc/MeOH, concentrated and kept at +5 °C to give white crystalline material: mp 106–108 °C. ^{1}H NMR (DMSO-d_6) δ 7.93 and 7.66 (2× s, 2H, CO–NH_2), 5.92 (d, 1H, 1′-H), 5.67 (d, 1H, 2′-OH), 5.33 (s, 1H, C≡CH), 5.31 (d, 1H, 3′-OH), 4.79 (t, 1H, 5′-OH), 4.69 (dd, 1H, 2′-H), 4.24 (dd, 1H, 3′-H), 3.98 (dd, 1H, 4′-H), 3.39–3.58 (m, 2H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 160.24 (CO–NH_2), 143.62 (C-4), 121.84 (C-5), 95.66 (C≡*C*H), 90.40 (C-1′), 86.33 (C-4′), 73.64 (C-2′), 70.54 (C-3′), 68.04 (*C*≡CH), 61.59 (C-5′). Anal. Calcd for $C_{10}H_{12}N_4O_5{\cdot}1H_2O$: C, 41.96; H, 4.93; N, 19.57. Found: C, 42.01; H, 5.05; N, 19.31.

5.17. 5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carbonitrile (26)

4-Toluenesulfonyl chloride (534 mg, 2.8 mmol) was added to a solution of **13** (771 mg, 2.0 mmol) in dry pyridine (20 ml). After being stirred at room temperature for 24 h, the reaction was quenched by addition of anhyd EtOH (2 ml) at 0 °C and the volatiles were removed by evaporation. The residue was treated with EtOAc/H_2O (1:1), the separated organic layer was washed with brine, dried with Na_2SO_4 and evaporated. The residue was chromatographed on a silica gel column with CH_2Cl_2/MeOH (99:1 → 97:3) as an eluent to afford the product which was purified on a next silica gel column using toluene-EtOH (9:1). Isolated oily **26** turned into solid foam on drying in vacuo, 437 mg (59% yield). ^{1}H NMR ($CDCl_3$) δ 5.98 (d, 1H, 1′-H), 5.95 (dd, 1H, 2′-H), 5.50 (t, 1H, 3′-H), 5.12 (s, 2H, NH_2), 4.49 (m, 1H, 4′-H), 4.34 (m, 2H, 5′-H), 2.14 and 2.04 (2× s, 9H, 3× Ac). ^{13}C NMR ($CDCl_3$) δ 170.32, 169.56 and 169.50 (3× *C*O–CH_3), 147.44 (C-5), 111.88 (CN), 105.22 (C-4), 88.42 (C-1′), 81.64 (C-4′), 72.42 (C-2′), 70.22 (C-3′), 62.46 (C-5′), 20.52, 20.43 and 20.39 (3× CO–*C*H_3). HRMS $[M+Na]^+$ calcd for $C_{14}H_{17}N_5O_7Na$: 390.1026; found: 390.1076.

5.18. 5-Iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carbonitrile (25)

To a suspension of **26** (423 mg, 1.15 mmol) in diiodomethane (18.48 g, 69 mmol) isoamyl nitrite (472 mg, 4.03 mmol) was added. The resulting mixture was stirred at 100 °C for 1 h, then it was applied onto a silica gel column. The column was eluted with CH_2Cl_2 (in order to collect diiodomethane) followed by CH_2Cl_2/MeOH (99:1 → 98:2) to give the title product which was rechromatographed using toluene-EtOH (98:2 → 96:4) for elution to isolate **25** as an oil (254 mg, 46% yield). ^{1}H NMR ($CDCl_3$) δ 6.06 (d, 1H, 1′-H), 6.08 (dd, 1H, 2′-H), 5.74 (dd, 1H, 3′-H), 4.52 (m, 1H, 4′-H), 4.39 and 4.16 (2× dd, 2H, 5′-H), 2.16, 2.14 and 2.03 (3× s, 9H, 3× Ac). ^{13}C NMR ($CDCl_3$) δ 170.37, 169.37 and 169.26 (3× *C*O–CH_3), 129.95 (C-4), 110.74 (CN), 90.80 (C-1′), 88.01 (C-5), 81.81 (C-4′), 74.04 (C-2′), 70.62 (C-3′), 62.25 (C-5′), 20.54, 20.41 and 20.39 (3× CO–*C*H_3). HRMS $[M+Na]^+$ calcd for $C_{14}H_{15}IN_4O_7Na$: 500.9883; found: 500.9925.

5.19. 1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-propynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (27) and 1-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (28)

Prepared from **16** by the procedure described for **20**. After column chromatography with EtOAc/hexane (2:1 → 3:1) as an eluent, the oily product **27** was isolated in 32% yield. ^{1}H NMR (DMSO-d_6) δ 7.88 and 7.62 (2× s, 2H, CO–NH_2), 6.24 (s, 1H, 1′-H), 5.50 (d, 1H, 2′-H), 5.03 (dd, 1H, 3′-H), 4.45 (dt, 1H, 4′-H), 4.08 and 3.87 (2× dd, 2H, 5′-H), 2.23 (s, 3H, ≡C–CH_3), 1.96 (s, 3H, Ac), 1.53 and 1.35 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{16}H_{20}N_4O_6Na$: 387.1275; found 387.1284. Further elution with EtOAc/hexane (6:1) gave deiodinated product **28** in 50% yield. ^{1}H NMR (DMSO-d_6) δ 8.68 (s, 1H, 5-H), 7.93 and 7.53 (2× s, 2H, CO–NH_2), 6.38 (s, 1H, 1′-H), 5.43 (d, 1H, 2′-H), 5.00 (dd, 1H, 3′-H), 4.50 (dt, 1H, 4′-H), 4.10 and 3.97 (2× dd, 2H, 5′-H), 1.91 (s, 3H, Ac), 1.52 and 1.34 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{13}H_{19}N_4O_6Na$: 349.1119; found 349.1132.

5.20. 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-5-iodo-1*H*-[1,2,3]triazole-4-carboxylic acid amide (31)

Prepared by methanolysis of **16** according to the standard procedure. ^{1}H NMR (DMSO-d_6) δ 7.89 and 7.57 (2× s, 2H, CO–NH_2), 6.17 (s, 1H, 1′-H), 5.59 (d, 1H, 2′-H), 4.98 (dd, 1H, 3′-H), 4.90 (t, 1H, 5′-OH), 4.20 (m, 1H, 4′-H), 3.18 (m, 2H, 5′-H), 1.52 and 1.37 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{11}H_{15}IN_4O_5Na$: 432.9979; found 432.9986.

5.21. 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-5-phenylethynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (29)

Prepared from **31** by the procedure described for **17.** After column chromatography with ethanol in toluene (5–10%) as an eluent, the oily product **29** was isolated in 27% yield. ^{1}H NMR (DMSO-d_6) δ 7.98 and 7.68 (2× s, 2H, CO–NH_2), 7.66–7.63 and 7.55–7.49 (2× m, 5H, Ph), 6.33 (s, 1H, 1′-H), 5.58 (d, 1H, 2′-H), 4.99–4.93 (dd overlap. t, 2H, 3′-H, 5′-OH), 4.24 (m, 1H, 4′-H), 3.27 (m, 2H, 5′-H) 1.52 and 1.36 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{19}H_{20}N_4O_5Na$: 407.1326; found 407.1332. Further elution with ethanol in toluene (20%) afforded **30** as a solid in 33% yield.

5.22. 1-(5-*O*-Acetyl-2,3-isopropylidene-β-D-ribofuranosyl)-5-trimethylsilylethynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (32) and 1-(5-*O*-acetyl-2,3-isopropylidene-β-D-ribofuranosyl)-5-ethynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (33)

Prepared from **16** by the procedure described for **23** and **24**. After column chromatography with EtOAc/hexane (1:1 → 1.5:1) as an eluent, oily products **32** and **33** were isolated in 7% and 55% yield, respectively. **32**: ^{1}H NMR ($CDCl_3$) δ 6.89 and 5.57 (2× s, 2H, CO–NH_2), 6.32 (s, 1H, 1′-H), 5.45 (d, 1H, 2′-H), 5.00 (dd, 1H, 3′-H), 4.55 (dt, 1H, 4′-H), 4.01–4.15 (m, 2H, 5′-H), 2.03 (s, 3H, Ac), 1.61 and 1.41 (2× s, 6H, 2× CH_3), 0.31 (s, 9H, TMS). HRMS $[M+Na]^+$ calcd for $C_{18}H_{26}N_4O_6SiNa$: 445.1514; found: 445.1521. **33**: ^{1}H NMR ($CDCl_3$) δ 6.94 and 5.68 (2× s, 2H, CO–NH_2), 6.34 (s, 1H, 1′-H), 5.52 (d, 1H, 2′-H), 5.02 (dd, 1H, 3′-H), 4.55 (dt, 1H, 4′-H), 4.01–4.15 (m, 2H, 5′-H), 4.00 (s, 1H, C≡CH), 2.03 (s, 3H, Ac), 1.61 and 1.41 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{15}H_{18}N_4O_6Na$: 373.1119; found 373.1122. Further elution with EtOAc/hexane (6:1) gave **28** in 18% yield.

5.23. 1-(5-*O*-Acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-phenylethynyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (35)

A solution of **16** (90 mg, 0.2 mmol, dried in vacuo over P_2O_5, at 60 °C, for 3 h) and bis(benzonitrile)palladium dichloride (8 mg, 0.02 mmol) in anhyd 1,4-dioxane (3 ml) was vigorously deoxygenated with argon. To the solution was added K_2CO_3 (28 mg, 0.2 mmol, dried as described for **16**), followed by phenylacetylene (102 mg, 1.0 mmol). The reaction mixture was heated at 100 °C overnight, then evaporated. The residue was chromatographed on silica gel column using EtOAc/hexane (1.5:1 → 2:1) to give **35** (61 mg, 72% yield). Further elution with EtOAc/hexane (8:1) led to the isolation of deiodinated product **28** (4 mg, 6% yield). **35**: ^{1}H NMR (DMSO-d_6) δ 7.99 and 7.68 (2× s, 2H, CO–NH_2), 7.64–7.68 and 7.49–7.55 (2× m, 5H, Ph), 6.39 (s, 1H, 1′-H), 5.59 (d, 1H, 2′-H), 5.04 (dd, 1H, 3′-H), 4.50 (m, 1H, 4′-H), 3.88–4.10 (m, 2H, 5′-H), 1.94 (s, 3H, Ac), 1.53 and 1.37 (2× s, 6H, 2× CH_3). HRMS $[M+Na]^+$ calcd for $C_{21}H_{22}N_4O_6Na$: 449.1432; found 449.1450.

5.24. 1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (30) and 5-ethynyl-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (34)

Methanolysis of compounds **28**, **32** and **33** was carried out according to general procedure described for preparation of **10–12** and **22.** The resulting crude products were chromatographed on silica gel with EtOAc/hexane (3:1) to afford **30** and **34** as solid foam (90–95% yield). **30**: ^{1}H NMR (DMSO-d_6) δ 8.70 (s, 1H, 5-H), 7.93, 7.54 (2× s, 2H, CO–NH_2) 6.31 (d, 1H, 1′-H), 5.31 (dd, 1H, 2′-H), 5.06 (b s, 1H, 5′-OH), 4.93 (dd, 1H, 3′-H), 4.28 (dt, 1H, 4′-H), 3.39 (d, 2H, 5′-H), 1.52, 1.34 (2× s, 6H, 2× CH_3); ^{13}C NMR (DMSO-d_6) δ 161.35 (CO–NH_2), 143.20 (C-4), 125.87 (C-5), 112.85 (CH_3)$_2$*C*, 93.68 (C-1′), 88.14 (C-4′), 84.28 (C-2′), 81.67 (C-3′), 61.22 (C-5′), 26.78, 25.02 (*C*H_3)$_2$C. HRMS $[M+Na]^+$ calcd for $C_{11}H_{16}N_4O_5$Na: 207.1013; found: 307.1003. **34**: ^{1}H NMR (DMSO-d_6) δ 7.95, 7.68 (2× s, 2H, CO–NH_2), 6.19 (s, 1H, 1′-H), 5.54 (d, 1H, 2′-H), 5.33 (s, 1H, C≡CH), 4.97 (dd, 1H, 3′-H), 4.92 (t, 1H, 5′-OH), 4.21 (td, 1H, 4′-H), 3.25 (m, 2H, 5′-H), 1.51, 1.35 (2× s, 6H, 2× CH_3); ^{13}C NMR (DMSO-d_6) δ 160.13 (CO–NH_2), 143.78 (C-4), 121.74 (C-5), 112.73 (CH_3)$_2$*C*, 95.97 (C≡*C*H), 91.52 (C-1′), 88.98 (C-4′), 83.28 (C-2′), 81.75 (C-3′), 67.95 (*C*≡CH), 60.88 (C-5′), 26.65, 24.93 (*C*H_3)$_2$C. HRMS $[M+Na]^+$ calcd for $C_{13}H_{16}N_4O_5$Na: 331.1013; found: 331.1021.

5.25. General procedure for the preparation of compounds 36 and 37

The reactions were carried out in an argon atmosphere under strictly anhydrous conditions.

To a cooled (0 °C) and stirred solution of nucleoside **30** or **34** (0.63 mmol) in CH_3CN (20 ml) was injected triethylamine (2.0 equiv, 1.26 mmol).). Next, a solution of salicylchlorophosphane (2.0 equiv, 1.26 mmol) in CH_3CN (2 ml) was added dropwise within 5 min. The reaction was complete after stirring for further 15 min, as determined by TLC (CH_2Cl_2/MeOH 9:1). Oxidation of intermediate phosphites was performed immediately by adding *tert*-butylhydroperoxide (2 equiv of 5.5 M solution in decane) to the reaction mixture at 0 °C. It was stirred for 30 min and allowed to warm up to room temperature. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel short column with 5% MeOH in CH_2Cl_2 to yield the pronucleotides **36** and **37**.

5.26. *cyclo*Saligenyl-5-ethynyl-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxamide-5′-phosphate (36)

0.135 g of colorless oil, yield 45%. ^{1}H NMR (DMSO-d_6) δ 7.97, 7.70 (2× s, 2× 2H, CO–NH_2), 7.37 (t, 1H, H-4$_{aryl}$), 7.33 (t, 1H, H-4$_{aryl}$), 7.26–7.16 (m, 4H, H-5$_{aryl}$, H-6$_{aryl}$) 7.11 (d, 1H, H-3$_{aryl}$), 7.02 (d, 1H, H-3$_{aryl}$), 6.24 (s, 1H, 1′-H), 6.22 (s, 1H, 1′-H), 5.48–5.27 (m, 8H, CH_2OP, 2′H, C≡CH), 4.99 (ddd, 2H, 3′-H), 4.46 (m, 2H, 4′-H), 4.18 (m, 2H, H-5'') 4.00 (m, 2H, H-5′), 1.50, 1.33 (2× s, 2× 6H, (CH_3)$_2$C). ^{13}C NMR (DMSO-d_6) δ 160.06 (CO–NH_2), 149.32 (d, C-2$_{aryl}$), 143.76 (C-4), 129.75, 129.67 (C-6$_{aryl}$), 125.99 (C-4$_{aryl}$), 124.46, 124.39 (C-5$_{aryl}$), 121.80 (C-5), 120.83, 120.70 (2× d, C-1$_{aryl}$), 118.25, 118.13 (2× d, C-3$_{aryl}$), 113.12 (CH_3)$_2$C, 96.12 (C≡*C*H), 91.26 (C-1), 86.51, 86.41 (C-4′), 83.57 (C-2′), 80.83 (C-3′), 68.50, 68.35 (2× d, C$_{benzyl}$), 67.78 (s, *C*≡CH), 66.70 (m, C-5′), 26.59, 26.54, 24.91, 24.88 (*C*H_3)$_2$C. ^{31}P NMR (DMSO-d_6) δ -10.21. HRMS $[M+Na]^+$ calcd for $C_{20}H_{21}N_4O_8PNa$: 499.0989; found: 499.0990.

5.27. *cyclo*Saligenyl-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxamide-5′-phosphate (37)

0.136 g of colorless oil, yield 48%. ^{1}H NMR (DMSO-d_6) δ 8.69, 8.66 (2× s, 2× 1H, 5-H), 7.94, 7.56 (2× s, 2× 2H, CO–NH_2), 7.38–7.32 (m, 2H, H-4$_{aryl}$), 7.26–7.17 (m, 4H, H-5$_{aryl}$, H-6$_{aryl}$), 7,12 (d, 1H, H-3$_{aryl}$), 7.05 (d, 1H, H-3$_{aryl}$), 6.42 (d, 1H, 1′-H), 6.39 (d, 1H, 1′-H), 5.50–5.27 (m, 6H, CH_2OP, 2′-H), 4.96 (dd, 1H, 3′-H), 4.93 (dd, 1H, 3′-H), 4.45 (m, 2H, 4′-H), 4.25 (m 2H, H-5''), 4.12 (m, 2H, H-5′), 1.50 and 1.31 (2× s, 6H, 2× CH_3), 1.31 (d, 6H, (CH_3)$_2$C). ^{13}C NMR (DMSO-d_6) δ 161.09 (CO–NH_2), 149.30, 149.24 (dd, C-2$_{aryl}$), 143.19, 143,13 (C-4), 129.73 (d, C-6$_{aryl}$), 126.54, 126.46 (C-5), 126.01 (C-4$_{aryl}$), 124.44 (C-5$_{aryl}$), 120.90, 120.80 (2× d, C-1$_{aryl}$), 118.24, 118,12 (2× d, C-3$_{aryl}$), 113,16 (CH_3)$_2$*C*); 92.64, 92.58 (C-1′), 85.80, 85.69 (2× d, C-4′), 83.92 (C-2′), 80.83 (C-2′), 68.52, 68.34 (2× d, C$_{benzyl}$), 67.10, 66.86 (C-5′), 26.59, 24.92 (*C*H_3)$_2$C). ^{31}P NMR (DMSO-d_6) δ −9.72, −9.69. HRMS $[M+Na]^+$ calcd for $C_{18}H_{21}N_4O_8PNa$: 475.0989; found: 475.1006.

5.28. *cyclo*Saligenyl-5-ethynyl-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxamide-5′-*O*-phosphate (38)

A solution of **36** (0.135 g, 0.28 mmol) in 80% aq TFA (5 ml) was stirred at 0 °C for 1 h. The volatiles were removed under reduced pressure and the residue coevaporated with anh ethanol (3 × 10 ml). The product was purified by chromatography on a silica gel short column with 10% MeOH in CH_2Cl_2 as an eluent. It afforded 0.109 g of **38** as glassy oil (88% yield). An analytical sample was precipitated with methanol-hexane (mp 75 °C). ^{1}H NMR (DMSO-d_6) δ 7.92, 7.68 (2× s, 2× 2H, CO–NH_2), 7.38–7.33 (m, 2H, H-4$_{aryl}$), 7.25–7.13 (m, 4H, H-5$_{aryl}$, H-6$_{aryl}$), 7.08 (d, 1H, H-3$_{aryl}$), 7.03(d, 1H, H-3$_{aryl}$), 5.92 (d, 2H, 1′-H), 5.83 (m, 2H, 2′-OH), 5.54–5.30 (m, 8H, CH_2OP, 3′-OH, C≡*C*H), 4.59 (m, 2H, 2′-H), 4.34–4.28 (m, 4H, 3′-H, 4′-H), 4.18–4.13 (m, 4H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 160.17 (CO–NH_2), 149.34, 149.25(2× d, C-2$_{aryl}$), 143.53 (C-4), 129.66 (C-6$_{aryl}$), 125.98, 125.94 (C-4$_{aryl}$), 124.34, 124.31(C-5$_{aryl}$), 121.73, 121.69 (C-5), 120.80, 120.68 (2× d, C-1$_{aryl}$), 118.17, 118.05 (2× d, C-3$_{aryl}$), 95.76, 95.70 (C≡*C*H), 90.50 (C-1′), 82.83, 82.74 (C-4′), 73.74, 73.67 (C-2′), 70.02 (C-3′), 68.42, 68.33 (2× d, C$_{benzyl}$); 67.91, 67.87 (*C*≡CH), 67.46, 67.30 (2× d, C-5′). ^{31}P NMR (DMSO-d_6) δ -10.13, -9.97. UV (MeOH) λ_{max} (ε) = 244 nm (15100). HRMS (ESI^-) (M-1) calcd for: $C_{17}H_{16}N_4O_8P$ 435.0711, found 435.0687. HRMS (ESI^+) $[M+Na]^+$ calcd for $C_{17}H_{17}N_4O_8PNa$: 459.0676; found: 459.0687. Analytical HPLC t_R: 22.50 min.

5.29. *cyclo*Saligenyl-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxamide-5′-*O*-phosphate (39)

Compound **37** (0.13 g, 0.29 mmol) was deprotected with 80% aq TFA (5 ml) according to the procedure described for **36.** Pronucleotide **39** was purified analogously to **38.** It afforded 0.1 g of **39** as glassy oil (85% yield). ^{1}H NMR (DMSO-d_6) δ 8.69, 8.68 (2× s, 2× 1H, 5-H), 7.89, 7.53 (2× s, 2× 2H, CO–NH_2), 7.35–7.32 (m, 2H, H-4_{aryl}), 7.25–7.16 (m, 4H, H-5_{aryl}, H-6_{aryl}), 7.12 (d, 1H, H-3_{aryl}), 7.07 (d, 1H, H-3_{aryl}), 6.00 (d, 2H, 1′-H), 5.76 (m, 2H, 2′-OH), 5.50–5.35 (m, 6H, CH_2OP, 3′-OH), 4.40 (m, 2H, 2′-H), 4.37–4.16 (m, 8H, 3′-H, 4′-H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 161.24 (CO–NH_2), 149.40, 149.30 (2× d, C-2_{aryl}), 143.17 (C-4), 129.69 (C-6_{aryl}), 126.01 (C-5), 125.73, 125.67 (C-4_{aryl}), 124.37 (C-5_{aryl}), 120.86, 120.75 (2× d, C-1_{aryl}), 118.20, 118.10 (2× d, C-3_{aryl}), 92.08 (C-1′), 82.59, 82.50 (C-4′), 74.38, 74.32 (C-2′), 69.89, 69.86 (C-3′), 68.44, 68.35 (2× d, C_{benzyl}), 67.66, 67.52 (2× d, C-5′); ^{31}P NMR (DMSO-d_6) δ -9.95, -9.84. UV (MeOH) λ_{max} (ε) = 266 nm (2300). HRMS (ESI^-) (M-1) calcd for: $C_{15}H_{16}N_4O_8P$ 411.0711, found 411.0690. HRMS (ESI^+) $[M+Na]^+$ calcd for $C_{15}H_{17}N_4O_8PNa$: 435.0676; found: 435.0686. Analytical HPLC t_R: 22.44 min.

5.30. (*Z*)- and (*E*)-5-{2-[2-(Acetylamino)-2-(methoxycarbonyl) ethylthio]vinyl}-1-β-D-ribofuranosyl-1*H*-[1,2,3]triazole-4-carboxylic acid amide (40 and 41)

To a cooled (0 °C) solution of **9** (188 mg, 0.7 mmol) in anhyd CH_3CN (14 ml) was added N-acetyl-L-cysteine methyl ester (124 mg, 0.7 mmol) and anhyd triethylamine (21 mg, 0.21 mmol). The mixture was allowed to react at 5 °C overnight, next it was neutralized at 0 °C with AcOH and evaporated. The residue was chromatographed on a silica gel column with CH_2Cl_2/MeOH (9:1) to isolate oily products **41** (isomer E, 20 mg, 6% yield) and **40** (isomer *Z*, 139 mg, 45% yield). **41**: ^{1}H NMR (DMSO-d_6) δ 8.51 (d, 1H, NH), 7.95 (d, 1H, 5-C*H*=CH, *J* = 15.6 Hz), 7.88 and 7.52 (2× s, 2H, CO–NH_2), 6.66 (d, 1H, CH=C*H*, *J* = 15.6 Hz), 5.83 (d, 1H, 1′-H), 5.56 (d, 1H, 2′-OH), 5.24 (d, 1H, 3′-OH), 4.79 (m, 2H, 5′-OH and CH), 4.55 (m, 1H, 2′-H), 4.25 (dd, 1H, 3′-H), 3.99 (dd, 1H, 4′-H), 3.66 (s, 3H, OCH_3), 3.35–3.58 (m, 2H, 5′-H), 3.04–3.19 (m, 2H, CH_2), 1.86 (s, 3H, N-Ac). HRMS $[M+Na]^+$ calcd for $C_{16}H_{23}N_5O_8SNa$: 468.1165; found: 468.1151. **40**: ^{1}H NMR (DMSO-d_6) δ 8.36 (d, 1H, NH), 7.79 and 7.45 (2× s, 2H, CO–NH_2), 6.90 (d, 1H, 5-C*H*=CH, *J* = 10.4 Hz), 6.43 (d, 1H, CH=C*H*, *J* = 10.4 Hz), 5.71 (d, 1H, 1′-H), 5.55 (d, 1H, 2′-OH), 5.25 (d, 1H, 3′-OH), 4.76 (m, 1H, 5′-OH), 4.55 (m, 1H, 2′-H), 4.47 (m, 1H, CH), 4.25 (dd, 1H, 3′-H), 3.95 (dd, 1H, 4′-H), 3.63 (s, 3H, OCH_3), 3.35–3.58 (m, 2H, 5′-H), 3.04–3.19 (m, 2H, CH_2), 1.85 (s, 3H, N-Ac). ^{13}C NMR (DMSO-d_6) δ 170.72 (*C*O-OCH_3), 169.59 (*C*O–CH_3), 161.69 (CO–NH_2), 139.49 (C-4), 137.77 (5-CH=*C*H), 134.69 (C-5), 110.48 (5-*C*H=CH), 90.35 (C-1′), 85.71 (C-4′), 74.04 (C-2′), 70.55 (C-3′), 61.84 (C-5′), 52.48 (NH-CH), 52.16 (O–CH_3), 34.77 (S-CH_2), 22.22 (CO–*C*H_3). HRMS $[M+Na]^+$ calcd for $C_{16}H_{23}N_5O_8SNa$: 468.1165; found: 468.1160.

5.31. 5-Hydroxymethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid methyl ester (43)

Compound **43** has been prepared according to a procedure of Earl and Townsend[25] by 1,3-cycloaddition of 1-β-D-ribofuranosyl azide triacetate[31] (1.77 g, 5.9 mmol) with methyl 4-hydroxy-2-butynoate[32] (0.875 g, 7.67 mmol). The reaction was conducted in toluene at 100 °C for 24 h and resulted in the formation of two regioisomers, separated by chromatography on silica gel to afford faster moving product, 5-hydroxymethyl-isomer **43**: 1.47 g as a colorless solid (62% yield) and a slower moving 4-hydroxymethyl-isomer 0.128 g as a solid (6%). **43**: ^{1}H NMR (DMSO-d_6) δ 6.47 (d, 1H, 1′-H), 5.91 (dd, 1H, 2′-H), 5.77 (t, 1H, CH_2O*H*), 5.64 (dd, 1H, 3′-H), 4.93, 4.87 (2 overlap. dd, 2H, CH_2OH), 4.47 (m, 1H, 4′-H), 4.35 and 4.07 (2× dd, 2H, 5′-H), 3.87 (s, 3H, OCH_3), 2.11, 2.07, 1.92 (3× s, 9H, 3× Ac). 1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-4-hydroxymethyl-1*H*-[1,2,3]triazole-5-carboxylic acid methyl ester: ^{1}H NMR (DMSO-d_6) δ 6.70 (s, 1H, 1′-H), 5.96 (d, 1H, 2′-H), 5.67 (t, 1H, CH_2O*H*), 5.29 (t, 1H, 3′-H), 4.69 (d, 2H, CH_2OH), 4.46 (m, 1H, 4′-H), 4.30 and 4.02 (2× m, 2H, 5′-H), 3.90 (s, 3H, OCH_3), 2.13, 2.08, 1.89 (3× s, 9H, 3× Ac).

5.32. 5-Fluoromethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid methyl ester (44)

A reaction was carried out in argon atmosphere under anhydrous conditions at −35 °C. To a cooled flask containing CH_2Cl_2 (7 ml) was injected DAST (0.280 g, 2.16 mmol). Likewise, a solution of substrate **43** (0.298 g, 0.72 mmol) in CH_2Cl_2 (7 ml) was added in 5 min. and a reaction mixture was stirred for 3 h and 30 min. According to TLC only trace amount of substrate remained and a reaction mixture was neutralized with cold 0.5 M $NaHCO_3$ adjusting temperature to −5 °C. After CO_2 evolution ceased, EtOAc was added (100 ml) to the mixture and organic layer was separated, carefully washed with water, brine and finally with water. Next, the organic solution was dried with Na_2SO_4, filtered and reduced in vacuo to yellow oil (0.330 g). Fluorinated product was purified on a silica gel short column with EtOAc/hexane (1:1). It afforded **44** as a white solid: 0.187 g (62% yield). ^{1}H NMR (DMSO-d_6) δ 6.47 (d, 1H, 1′-H), 6.00 (dd, 1H, 2′-H), 5.91, 5.78 (d, 2× 1H, $^2J_{H,F}$ = 48 Hz, CH_2F), 5.64 (m, 1H, 3′-H), 4.51 (m, 1H, 4′-H), 4.31 and 4.06 (2× dd, 2H, 5′-H), 3.90 (s, 3H, OCH_3), 2.12, 2.09, 1.88 (3× s, 9H, 3× Ac). ^{13}C NMR (DMSO-d_6) δ 169.82, 169.48, 169.22 (3× *C*OCH_3), 160.40 (4-CO_2CH_3), 137.59 (C-4), 136.30 (d, $J_{C,F}$ = 19 Hz, C-5), 88.33 C-1′), 73.28 (C-2′), 70.77 (d, $J_{C,F}$ = 164 Hz, CH_2F), 70.03 (C-3′), 52.31 (OCH_3), 20.26 (3× CO*C*H_3). HRMS $[M+Na]^+$ calcd for $C_{16}H_{20}FN_3O_9Na$: 440.1076; found: 440.1087.

5.33. 5-Fluoromethyl-1-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (42)

To a carefully dried compound **44** (0.155 g, 0.38 mmol) was added methanol saturated with ammonia (30 ml, 10 M solution) and resulted solution was stirred in a pressure tube at 20 °C for 2 days. After this time according to TLC a single product was formed and no starting material remained in the reaction mixture. The volatiles were removed under reduced pressure, and the residue was co-evaporated with ethanol to ease evacuation of residual acetamide to afford carboxamide **42**: 0.096 g as colorless oil (89%). Analytical sample (68 mg) was crystallized from MeOH/EtOH (1:3) to give 43 mg of crystals (mp 190 °C), solvated with alcohols (according to ^{1}H NMR: 8% and 6%, respectively). The filtrate afforded next portion of crystals on standing at 8 °C for several days, this material was suitable for X-ray analysis. **42**: ^{1}H NMR (DMSO-d_6) δ 8.08, 7.52 (2× s, 2H, CO–NH_2), 5.99 (2× d, 1H,, $J_{H,H}$ = 16 Hz, $J_{H,F}$ = 48 Hz CH_2F), 5.96 (d, 1H, 1′-H), 5.83 (2× d, 1H, $J_{H,H}$ = 16 Hz, $J_{H,F}$ = 48 Hz CH_2F), 5.63 (b s, 1H, 2′-OH), 5.29 (b s, 1H, 3′-OH), 4.79 (t, 1H, 5′-OH), 4.66 (b s, 1H, 2′_H), 4.25 (d, 1H, 3′-H), 3.99 (dd, 1H, 4′-H), 3.56 and 3.42 (2× m, 2H, 5′-H). ^{13}C NMR (DMSO-d_6) δ 161.69 (CO–NH_2), 140.24 (d, $J_{C,F}$ = 4 Hz, C-4), 133.93 (d, $J_{C,F}$ = 18 Hz, C-5), 90.78 (C-1′), 86.06 (C-4′), 74.06 (C-2′), 70.85 (d, $J_{C,F}$ = 162 Hz, CH_2F), 70.36 (C-3′), 61.47 (C-5′). ^{19}F NMR (DMSO-d_6) δ -135.91 (t). Anal. Calcd for $C_9H_{13}FN_4O_5$: C, 39.13; H, 4.74; N, 20.28. Found: C, 39.09; H, 4.94; N, 20.33.

5.34. Crystallographic procedure

X-ray diffraction measurements of a single crystal of **42** were carried out at room temperature using graphite-monochromated

Table 2
Crystal data and structure refinement for 5-fluoromethyl-1-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide (**42**)

Empirical formula	$C_9H_{13}FN_4O_5$
Formula weight	276.25
Temperature	294 K
Wavelength	1.54184 Å
Crystal system	tetragonal
Space group	$P\,4_32_12$
Unit cell dimensions	a = 12.55938(3) Å c = 14.62874(5) Å
Volume	2307.51(1) Å^3
Z	8
Calculated density	1.590 g/cm^3
Absorption coefficient	1.223 mm^{-1}
F(0 0 0)	1152
Crystal size	0.48 × 0.38 × 0.22 mm
θ range for data collection	9.31–74.39°
Limiting indices	$-15 \leqslant h \leqslant 15$ $-15 \leqslant k \leqslant 15$ $-18 \leqslant l \leqslant 18$
Reflections collected/unique	94350/2350 [R_{int} = 0.0389]
Completeness to = 26.37°	99.2%
Max. and min. transmission	0.7747 and 0.5913
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2350/0/234
Goodness-of-fit on F^2	1.083
Final R indices [I >2(I)]	R_1 = 0.0280, wR_2 = 0.0781
R indices (all data)	R_1 = 0.0281, wR_2 = 0.0782
Extinction coefficient	0.0021(4)
Absolute structure parameter	0.03(18)
Largest diff. peak and hole	0.154 and −0.139 e Å^{-3}

Cu $K\alpha$ radiation and a KM4-CCD diffractometer.[33] Integrated intensities were obtained using the CrysAlisPro program.[33] The structure was solved by direct methods with SHELXS-97 and refined by full-matrix least squares minimization of $\Sigma w(F_o^2 - F_c^2)^2$ using SHELXL-97.[34] All hydrogen atoms were derived from a difference Fourier map and included in the refinement with isotropic B factors. The final model contains two CH_2F units with a common C(7) centre and two sets of fluorine atoms populated with 0.71/0.29 occupancy. Two hydrogen atoms of the less populated CH_2F group were generated geometrically and during the refinement process their positions and B_{iso} were fixed. The crystal data and details of data processing are given in Table 2. Table 3 contains the hydrogen bonds geometry. Molecular illustrations were prepared using the ORTEPII[35] and the Mercury programs,[36] and the XP package.[37] Ring puckering analysis was done using WinGX (version 1.64.05) system of programs.[38] Atomic parameters in CIF format are available as Electronic Supplementary Publication from Cambridge Crystallographic Data Centre (CCDC 823702)[†].

5.35. Cells and cytotoxicity assay

Human umbilical vein endothelial cells (HUVEC) were isolated from freshly collected umbilical cords and cultured in plastic dishes coated with gelatin, in RPMI 1640 medium supplemented with 20% FBS (fetal bovine serum), 90 U/ml heparin, 150 μg/ml ECGF (Endothelial Cell Growth Factor, Roche Diagnostics, Mannheim, Germany) and antibiotics (100 μg/ml streptomycin and 100 U/ml penicillin). 10×10^3 cells were seeded on each well on 96-well plate (Nunc). The HeLa (human cervix carcinoma) and K562 (leukemia) cells were cultured in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum, in a 5% CO_2–95% air atmosphere. 7×10^3 cells were seeded on each well on 96-well plate (Nunc). 24 h later cells were exposed to the test compounds for another 24 or 48 h. Stock solutions (100 mM) of test compounds were freshly prepared in DMSO. The final concentrations of the compounds tested in the cell cultures were: 1, 1×10^{-2}, 1×10^{-4} and 1×10^{-6} mM. The concentration of DMSO in the cell culture medium was 1%.

The values of IC_{50} (the concentration of test compound required to reduce the cell survival fraction to 50% of the control) were calculated from dose-response curves and used as a measure of cellular sensitivity to a given treatment. The cytotoxicity of all compounds was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, as described previously.[39] Briefly, after 24 or 48 h of incubation with drugs, the cells were treated with the MTT reagent and incubation was continued for 2 h. MTT-formazan crystals were dissolved in 20% SDS and 50% DMF at pH 4.7 and absorbance was measured at 562 and 630 nm on an ELISA-PLATE READER (FLUOstar Omega, BMG Labtech, Germany). As a control (100% viability), we used cells grown only in the presence of vehicle (1% DMSO).

Table 3
Geometry of the hydrogen bonds (Å, °) for 5-fluoromethyl-1-β-D-ribofuranosyl)-1*H*-[1,2,3]triazole-4-carboxylic acid amide, **42**

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
O(2′)–H(2′)···O(5′)^{i}	0.87(2)	2.00(3)	2.830(2)	160(2)
O(3′)–H(3′)···N(3)^{ii}	0.89(2)	1.91(2)	2.776(2)	165(2)
O(5′)–H(5′)···O(3′)^{iii}	0.90(3)	1.94(3)	2.801(2)	160(2)
N(6)–H(61)···O(6)^{iv}	0.81(2)	2.10(2)	2.911(2)	173(2)
C(7)–H(7B)···O(6)	0.96	2.39	3.057(2)	126
C(3′)–H(31′)···O(5′)	0.93(2)	2.61(2)	2.981(2)	104(1)
C(7)–H(71)···O(6)^{v}	0.96(5)	2.50(4)	3.422(2)	161(4)
C(7)–H(72)···O(4′)^{iii}	0.94(3)	2.70(3)	3.403(2)	132(2)
C(4′)–H(4′)···F(7A)^{v}	0.95(2)	2.62(2)	3.098(5)	112(1)
C(1′)–H(H1′)···F(7)^{vi}	0.97(2)	2.60(2)	3.138(3)	115(1)

Symmetry codes: (i) $x + 0.5$, $-y + 0.5$, $-z + 0.25$; (ii) $-y$, $-x$, $-z + 0.5$; (iii) $y - 0.5$, $-x + 0.5$, $z + 0.25$; (iv) $-y$, $-x$, $-z + 0.75$; (v) $-y + 0.5$, $x + 0.5$, $z - 0.25$; (vi) y, x, $-z + 1$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.050.

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[†] CCDC 823702 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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