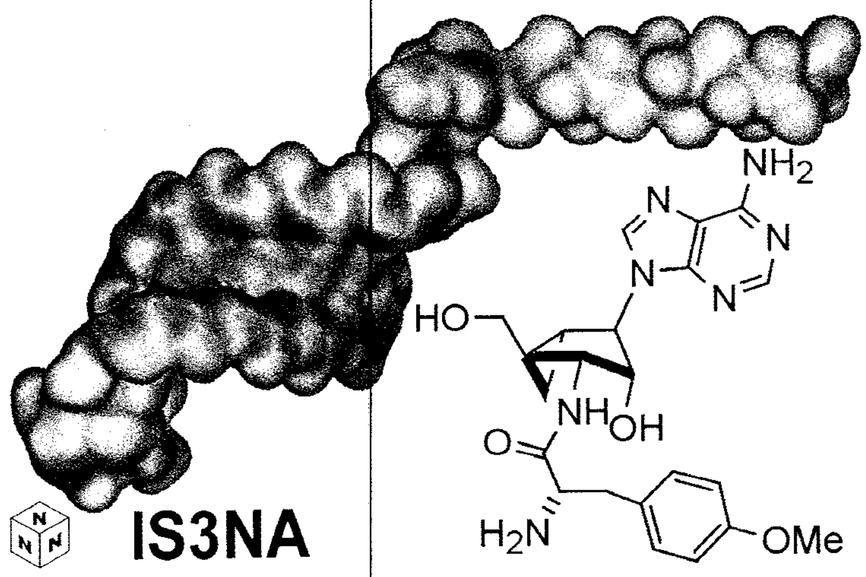
INTERNATIONAL ROUNDTABLE ON NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACIDS



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SYNTHESIS OF 2,6-BIS-(1,2,3-TRIAZOLYL)-SUBSTITUTED PURINE NUCLEOSIDES

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ABSTRACT

***O*-Acetyl protected 9-(β-D-arabinofuranosyl)-2,6-diazidopurine, obtained in 3 steps from tetraacetyl-D-arabinose and 2,6-dichloropurine, undergoes copper catalyzed 1,3-dipolar cycloaddition reactions with various terminal alkynes. After deprotection of arabinose moiety, novel 2,6-bis-(1,2,3-triazolyl)-functionalised purine arabinonucleosides were obtained. Similar purine nucleosides within *ribo* series were synthesised in a similar way.**

INTRODUCTION

One of the most powerful reactions for developing of numerous new structures of nucleoside and oligonucleotide analogs and bioconjugates is Cu(I) catalysed Huisgen 1,3-dipolar cycloaddition [1]. The reaction leads to formation of 1,4-disubstituted 1,2,3-triazoles from corresponding azides and alkynes with high yields.

Application of azide-alkyne 1,3-dipolar cycloaddition reaction in nucleoside, nucleotide and oligonucleotide chemistry was recently reviewed by F. Amblard and co-authors [2]. A large number of 1,2,3-triazolyl-functionalized nucleosides have been synthesized and their biological activity investigated in recent years, including sugar modified and base modified nucleosides, nucleoside bioconjugates, oligonucleotides with 1,2,3-triazole internucleoside linkages. Additionally, *click chemistry* was also used for DNA modifications. Within these triazole modified scaffolds substances with antiviral and anticancer activity, glycosyl transferase inhibitors, chitin synthase inhibitors, adenosine receptors agonists and antagonists were discovered.

However, only few examples of synthesis and biological activity of nucleoside analogues containing 1,2,3-triazolyl-modified purine bases are described so far (Figure 1).

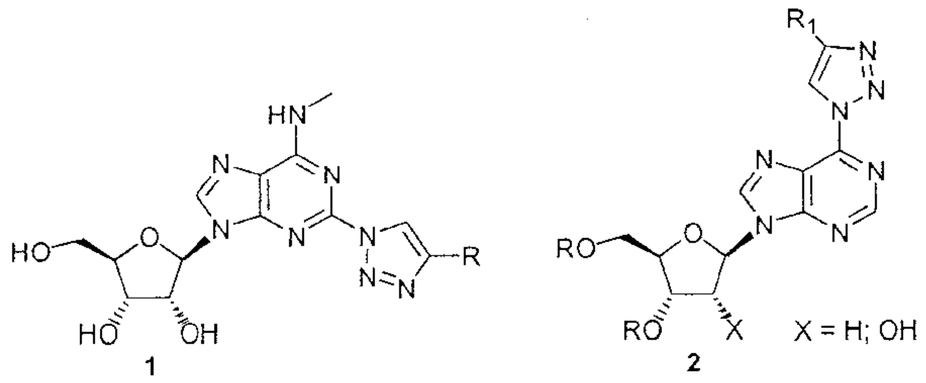


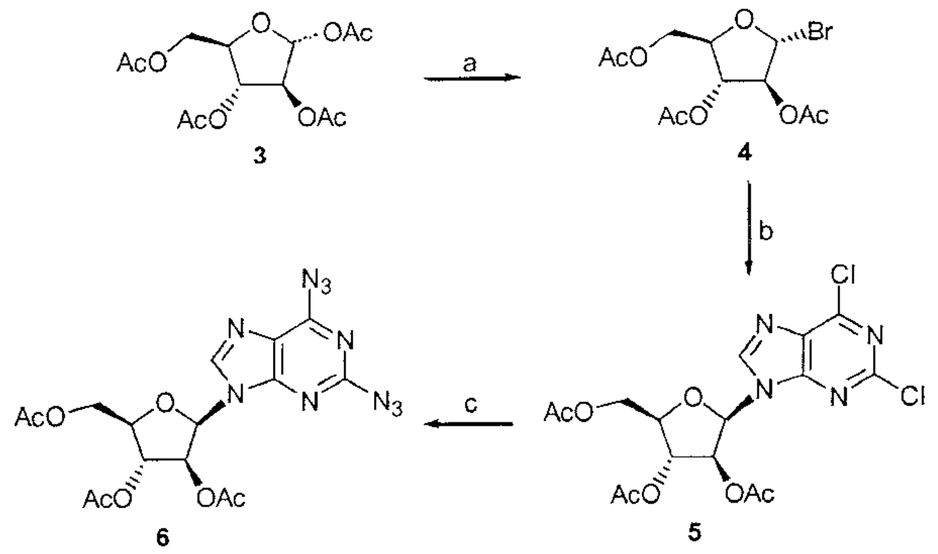
Figure 1. Recent examples of 1,2,3-triazolyl-purine nucleosides.

The synthesis of 2-(1,2,3-triazolyl)adenosine derivatives **1** was reported by K.A. Jacobson and co-workers [3]. Some of these analogues showed high binding affinity to A_3 adenosine receptors. Recently the synthesis of 6-(1,2,3-triazolyl)adenosine and deoxyadenosine derivatives **2** were also reported [4].

The aim of this investigation is to develop method for synthesis of novel 2,6-bis-(1,2,3-triazolyl)-substituted purine nucleosides within *arabino* and *ribo* series and to evaluate their biological activity. Especially we are interested in the development of new structures of arabinonucleosides, since among them are well known anticancer and antiviral pharmaceuticals, such as fludarabine, cytarabine, vidarabine, zonavir, BVaraU and others [5,6].

RESULTS AND DISCUSSION

We started the synthesis of target compounds with preparation of key intermediate, 9-(tri-*O*-acetyl-β-D-arabinofuranosyl)-2,6-diazidopurine **6** (Scheme 1).



Scheme 1. Reagents and conditions: a) HBr/AcOH; b) 2,6-dichloropurine, NaH, MeCN, r.t.; c) NaN_3, EtOH/H_2O, reflux, 28% of β-anomer over 3 steps.

Synthesis of **6** was realized in 3 steps without extensive purification of intermediates. Reaction of tetra-*O*-acetyl-D-arabinose (**3**) with HBr/AcOH in standard conditions afforded **4**. Coupling of **4** with 2,6-dichloropurine in acetonitrile in the presence of sodium hydride gave 9-(tri-*O*-acetyl-β-D-arabinofuranosyl)-2,6-dichloropurine (**5**) as a mixture of anomers (β/α ≈ 7/1). We found that separation of anomers is more efficient in the next stage. Therefore, after workup of the reaction mixture and evaporation of solvent, **5** was reacted with sodium azide in boiling diluted (80%) ethanol to afford crude diazide **6**. Purification of the latter by silica gel column chromatography gave pure β-anomer as white foam with 28% yield in a three step sequence from **3**. Diazide **6** is unstable in the daylight and at elevated tem-

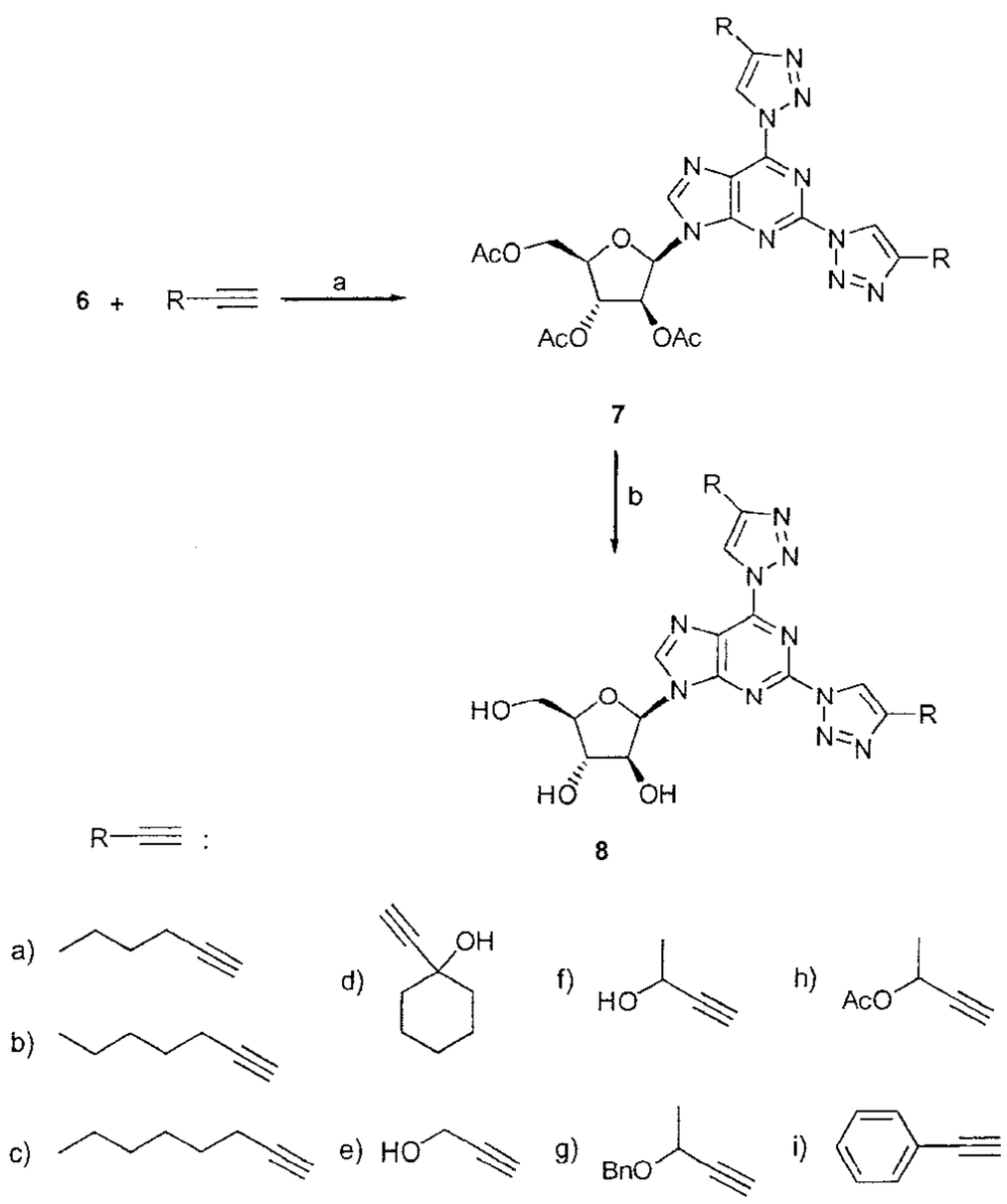
peratures, but it is quite stable for a long period of time when stored in darkness below 5 °C.

Diazide **6** was reacted with various terminal alkynes in copper-catalyzed 1,3-dipolar cycloaddition reaction, using $CuSO_4 \cdot 5H_2O$ and sodium ascorbate as a catalyst generating system. We investigated various reaction conditions; thus the reaction temperature was varied from 15-20 °C to 80 °C and suitability of various solvents (THF, acetone/water, DCM/water, *tert*-butanol/water) was evaluated.

At ambient temperature reactions are rather slow and they take at least 24...48 and even more hours to go to completion. On the other hand, at elevated temperatures amount of side products increases, possibly due to degradation of diazidonucleoside. From solvents used highest yields of cycloaddition products were obtained in *tert*-butanol/water.

Therefore, we proceeded with reaction conditions that included mixing of diazide **6** with alkynes in *tert*-butanol/water solution in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate at room temperature.

Reactions were controlled by TLC and/or HPLC. After work-up bis-triazolyl-nucleosides **7** were isolated by silica gel column chromatography. The isolated yields of products **7a-i** ranged from 40% to 65%.



Scheme 2. Reagents and conditions: i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, *t*-BuOH/H_2O, r.t., 40-65% isolated yield; ii) NH_3/EtOH/H_2O, r.t., 70-80%.

The compounds **7**, containing OH group in the substituent R are suitable for further transformation and derivatization. After deacetylation of **7** with NH_3/EtOH/H_2O solution target compounds 9-(β-D-arabinofuranosyl)-2,6-di-(1,2,3-triazolyl)purine derivatives **8** were obtained in 70-80% yields.

2,6-Bis-triazolyl-substituted nucleosides within *ribo* series were synthesized in a similar way. Thus, reaction of 9-(tri-*O*-acetyl-β-D-furanosyl)-2,6-dichloropurine with sodium azide afforded 9-(tri-*O*-acetyl-β-D-furanosyl)-2,6-diazidopurine, which was reacted with alkynes to give corresponding triazole derivatives in moderate yields. After deprotection a range of 2,6-bis-(1,2,3-triazolyl)-substituted purine ribonucleoside analogues was obtained.

We suppose that moderate yields of bis-triazolyl-nucleoside derivatives are obtained partially due to thermal instability of diazidopurine derivatives. This might lead to the extensive formation of side products.

Newly synthesised products were analysed by their ^1H and ^{13}C NMR spectra and elemental analysis/HRMS. Thermal and/or photochemical stability of key intermediate **6** and its *ribo* analogue will be studied in details. Recent investigations have revealed and re-actualized the long standing interest about azide-tetrazole equilibrium of azidopurines [4]. Fully deprotected nucleosides **8** will be tested for their biological activity and these data will be reported in due course.

CONCLUSION

We have demonstrated that 2,6-diazidopurine nucleosides undergo Cu(I) catalysed Huisgen 1,3-dipolar azide-alkyne cycloaddition reactions with various terminal alkynes. In this transformation several novel 2,6-bis-triazolyl-functionalised purine nucleosides within *arabino* and *ribo* series have been obtained in moderate to good yields. The above mentioned nucleoside analogues are interesting from the viewpoint of their potential biological activity. On the other hand, the developed sequence rises an interest about stability and further synthetic applications of 2,6-diazidopurine nucleosides.

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