

## Rapid Microwave-Assisted Synthesis of Modified Pyrimidine and Purine Pyranonucleosides as Novel Cytotoxic, Antiviral Agents and Glycogen Phosphorylase B Inhibitors

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### Abstract

Nucleosides take an important place in medicinal chemistry as the structural basis for the development of therapeutic agents. The chemistry of substituted base-modified nucleosides has been an interesting field of study over the last two decades owing to their biological properties. This mini-review summarizes recent efforts on the synthesis of C5- and C8-alkynyl base-modified pyranonucleoside analogues using Sonogashira cross-coupling reaction under microwave irradiation.

**Keywords:** Sonogashira reaction; Base-modified; Pyranonucleosides; Microwave irradiation; Cytotoxic/antiviral activity

### Introduction

Several purine- and pyrimidine-substituted nucleosides exhibit activity in both solid tumors and hematological malignancies, behaving as antimetabolites, competing with physiological nucleosides, and consequently, interacting with a large number of intracellular targets to induce cytotoxicity [1]. Among them, alkynyl-modified uridines exhibited significant antiviral [2-4] and anticancer activities [4]. e.g., the internal aromatic-substituted alkyne *p*-tolylethynyl-2'-deoxyuridine showed high potency against MCF-7 (IC<sub>50</sub> 0.9 ± 0.2 μM), comparable to 5-fluorouracil and Cisplatin [5] while 5-ethynyl-2'-deoxyuridine, was the most potent inhibitor against MCF-7 and MDA-MB-231 human breast cancer cells (IC<sub>50</sub> 0.4 ± 0.3 and 4.4 ± 0.4 μM, respectively), the same compound also inhibited the replication of HSV-1, HSV-2 (herpes simplex virus type 1, 2) and VV (vaccinia virus) at concentrations of 0.1-1 μg/mL [6,7]. Little effort has been made towards the synthesis of C8-modified purine nucleosides, nevertheless, some interesting biological properties have been reported: e.g., selected 8-alkynyl adenosines were selective ligands for the A<sub>3</sub> adenosine receptor subtype behaving as adenosine antagonists [8], and various C8-modified 2'-deoxy adenosines induced delayed chain termination *in vitro* and showed moderate anti HIV-1 activity in cell culture [9]. Considering the progress made in this direction, the present mini review presents an update of recent developments on pyrimidine- and purine-modified pyranonucleosides that possess interesting biological properties. In particular, the molecular design, synthesis and biological activity of C5-alkynyl pyrimidines and C8-alkynyl purine pyranonucleosides is presented. Aiming at a more detailed structure-activity relationship studies, a variety of alkyne substituents R are reported, such as linear alkyl chains and arenes substituted with linear and branched alkyl groups.

### Results and Discussion

In 2013, numerous novel C5-alkynyl uracil and cytosine glucopyranonucleosides were first synthesized and biologically evaluated [10]. Analogues **3a,b-9a,b**, **11a** and **12a** were prepared *via* their iodinated precursors **2a** and **2b** using Pd(0)-catalyzed Sonogashira cross-coupling reactions under microwave irradiation (Figure 1). When compared to conventional heating [11], the MW technology completed the synthesis much faster, while the yields of the products were slightly increased (by 3-13%). All the newly

synthesized compounds were evaluated for their cytostatic activity against human cervix carcinoma (HeLa), human lymphocytes (CEM) as well as murine leukemia (L1210) cells. The 5-substituted uracil pyranonucleosides showed superior antiproliferative activity to their cytosine counterparts. Among the nucleosides tested, the phenylethynyl uracil pyranonucleoside derivative **5a**, effectively inhibited tumor cell proliferation (IC<sub>50</sub> 5.2-6.2 μM) similar to that of 5-fluorouracil (IC<sub>50</sub> 0.33-18 μM), whereas its cytosine congener showed no appreciable cytostatic action (IC<sub>50</sub> 201-250 μM). The cyclization of **3a** and **4a** to the target bicyclic nucleosides **10a** and **11a** achieved through extended reaction time (irradiation for 8 min) and it was based on a 5-*endo-dig* electrophilic cyclization *via* an O-hetero-annulation process. Kinetic studies also showed that 1-(β-D-glucopyranosyl)-5-ethynyluracil **9a** was the best glycogen phosphorylase b (GPb) inhibitor (Ki 4.7 μM). Crystallography revealed that inhibitors with a long C5-alkynyl group exploited interactions with the b-pocket of the active site and induced significant conformational changes of the 280s loop compared to GPb complex with compounds hosting a short C5-alkynyl group. The results highlight the importance in the length of the aliphatic groups used to enhance inhibitory potency for the exploitation of the hydrophobic b-pocket. The most active inhibitor also had a moderate effect on glycogenolysis at the cellular lever with an IC<sub>50</sub> value of 291.4 μM.

Recently, C5-aryl ethynyl uracil glucopyranonucleosides **13,15** and C5-arylethyl uracil pyranonucleosides **17a,e,i,j,k** were also prepared [12] and evaluated for their cytostatic and antiviral activities. The protected analogues **12a,e,i,j** (Figure 2) showed better cytostatic activity against human lymphocyte CEM tumor cells (IC<sub>50</sub> 18-42 μM) compared to their unprotected counterparts **13a,e,i,j** which did not enhance growth inhibition of CEM cells (IC<sub>50</sub> >250 μM), while derivatives **17a,e,i,j,k** were devoid of significant cytostatic activity (IC<sub>50</sub> 94-250 μM). Their antiviral activities were measured against a large

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Received April 27, 2017; Accepted May 05, 2017; Published May 10, 2017

**Citation:** Manta S, Dimopoulou A, Kollatos N, Kyrkou G, Spanos S, et al. (2017) Rapid Microwave-Assisted Synthesis of Modified Pyrimidine and Purine Pyranonucleosides as Novel Cytotoxic, Antiviral Agents and Glycogen Phosphorylase B Inhibitors. Med Chem (Los Angeles) 7: 865-868. doi: [10.4172/2161-0444.1000443](https://doi.org/10.4172/2161-0444.1000443)

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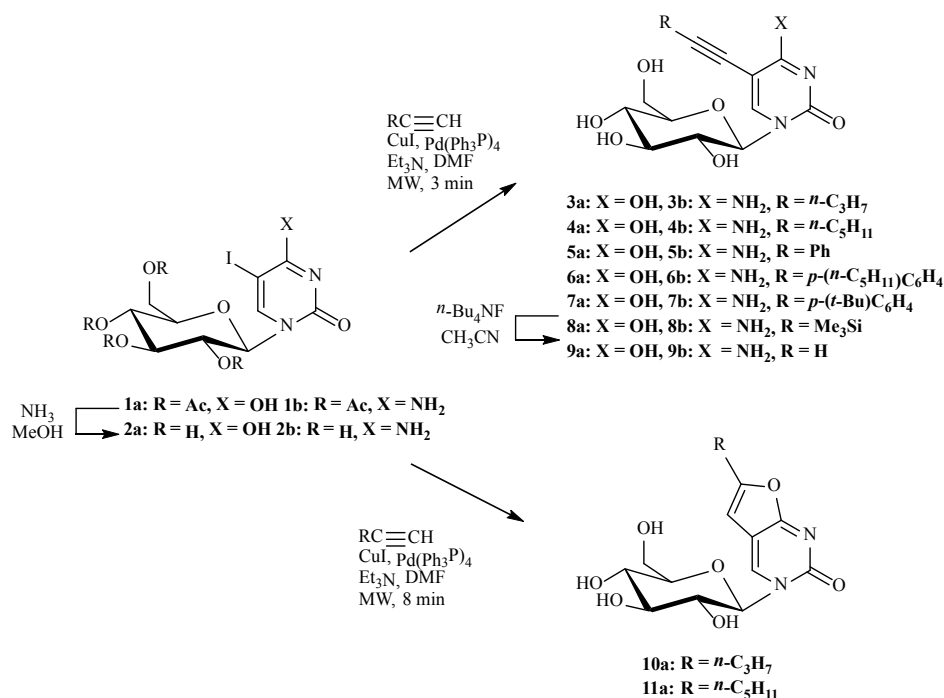


Figure 1: Synthesis of C5-alkynyl pyrimidine glucopyranonucleosides.

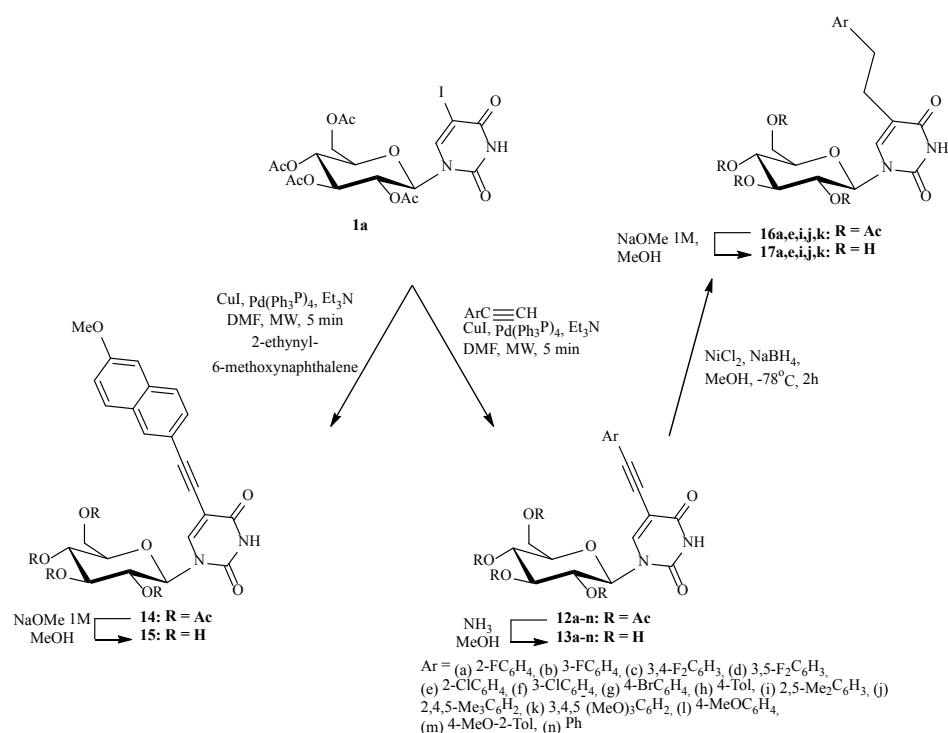
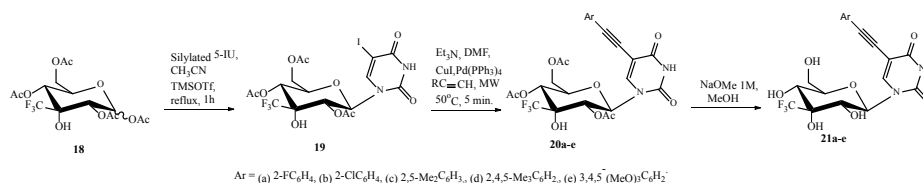


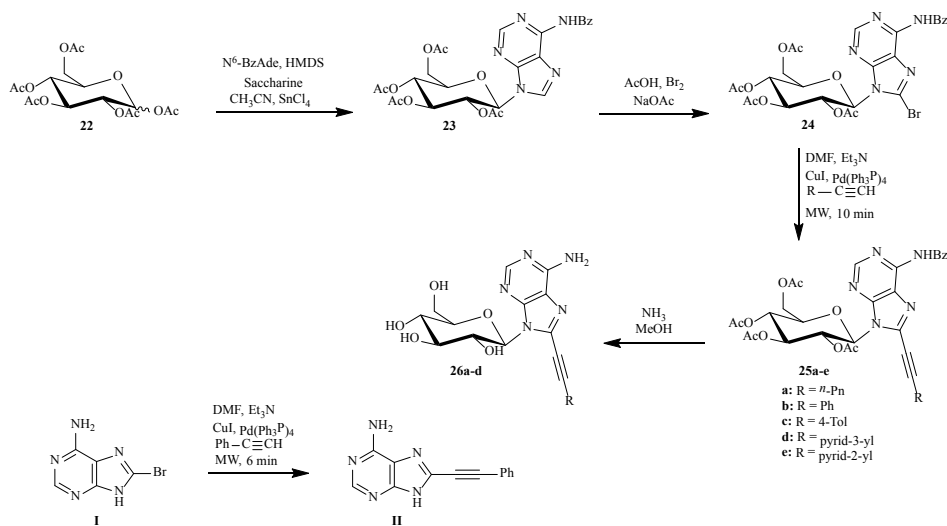
Figure 2: Synthesis of C5-arylalkynyl uracil glucopyranonucleosides and of 1-(β-D-glucopyranosyl)-5-[arylethyl]uracil.

number of DNA and RNA viruses including herpes simplex virus type 1,2 (HSV-1,2), vaccinia virus (VV), adeno-virus 2 (Ad-2) and human coronavirus 229E (HCoV-229E). Only analogue 17i exhibited the best anti-VV activity (EC<sub>50</sub> 10 μM): 10- and 25-fold more active than

the reference drugs Ganciclovir (CMV) (EC<sub>50</sub> 100 μM) and Acyclovir (ACV) (EC<sub>50</sub> 250 μM), respectively, on HEL cell culture. Novel C5-arylalkynyl uracil pyranonucleosides bearing 3'-C-trifluoromethyl-D-allose as sugar moiety were recently synthesized *via* microwave-



**Figure 3:** Synthesis of 1-(3'-C-trifluoromethyl-β-D-glucopyranosyl)-5-[arylethynyl]uracil.



**Figure 4:** Synthesis of C8-alkynyl adenine glucopyranonucleosides and 8-phenylethynyl-adenine.

assisted Sonogashira coupling and were biologically evaluated [12]. The protected nucleosides **20c,d** proved to be cytotoxic against human lymphocyte (CEM) cells ( $IC_{50}$  19-20  $\mu$ M) similarly to 5-fluorouracil ( $IC_{50}$  0.33-18  $\mu$ M), contrary to their unprotected analogues **21c,d** which proved to be inactive ( $IC_{50}$  >178  $\mu$ M) (Figure 3).

Finally, the synthesis of the first purine glucopyranonucleosides C8-alkynyl adenines **25, 26** and 8-phenylethynyl-adenine (**II**) itself were also reported (Figure 4) [13] using the Sonogashira cross-coupling reaction. The cytostatic potential of compounds **25e** ( $IC_{50}$  2.9-5.9  $\mu$ M) and **II** ( $IC_{50}$  3.0-10.0  $\mu$ M) was an order of magnitude lower than 5-fluorouracil ( $IC_{50}$  0.33-0.54  $\mu$ M) on murine leukemia (L1210), and human cervix carcinoma (HeLa) cells, while the same compounds ( $IC_{50}$  1.2-4.2  $\mu$ M) were more active than 5-fluorouracil ( $IC_{50}$  18  $\mu$ M) on human lymphocyte (CEM) cells.

## Conclusion

In the present review, we focused our attention on the relatively new literature data, concerning the C5- and C8-alkynyl base-modified glucopyranonucleosides, as well as 8-phenylethynyl-adenine itself, *via* Sonogashira coupling conditions under microwave irradiation. With chemists becoming increasingly interested in biology, the demand for novel bioactive compounds with improved therapeutic potential is becoming high. Moreover, extensive structure-activity relationship studies of the novel pyranonucleoside analogues concerning different substituents in the aromatic ring as well as more detailed investigations on the molecular drug targets are envisaged.

## Acknowledgements

This work was supported in part by the Postgraduate Program "Toxicology", Department of Biochemistry and Biotechnology, University of Thessaly.

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**Citation:** Manta S, Dimopoulou A, Kollatos N, Kyrkou G, Spanos S, et al. (2017) Rapid Microwave-Assisted Synthesis of Modified Pyrimidine and Purine Pyranonucleosides as Novel Cytotoxic, Antiviral Agents and Glycogen Phosphorylase B Inhibitors. *Med Chem (Los Angeles)* 7: 865-868. doi: [10.4172/2161-0444.1000443](https://doi.org/10.4172/2161-0444.1000443)

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