INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

CLICK REACTIONS WITH AZIDES DERIVED FROM 5-METHYL URIDINE

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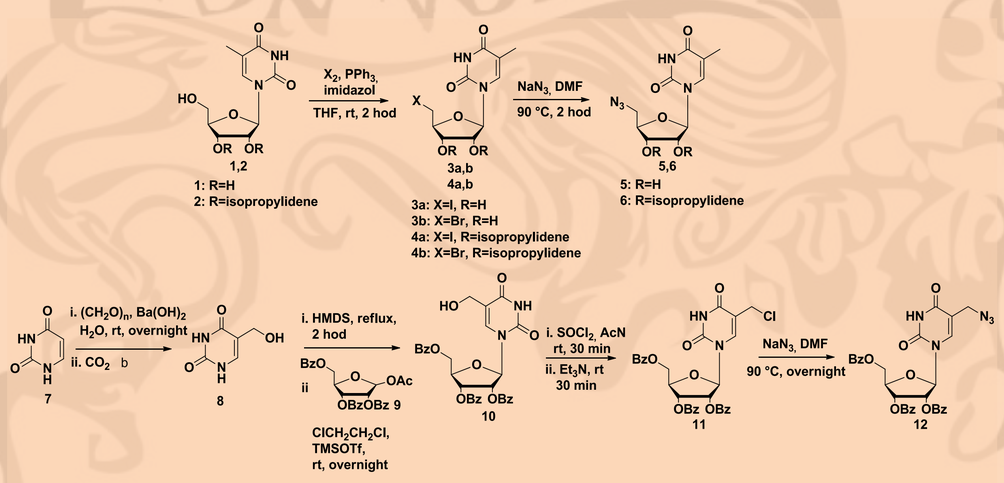
INTRODUCTION

Formation of triazoles by catalyzed click reactions is often used for bimolecular ligation and *in vivo* tagging[1-4]. Unfortunately this use limited due to the toxicity of metal catalyst. For these reason new bioorthogonal system for copper-free click chemistry are being developed[1]. The application of azide cycloaddition in pyrimidine nucleobase chemistry is very limited. In our study we studied reactivity of known azides derived from 5-methyluridine toward catalysed and copper free click reactions. Using of simple nucleosides led to formation of triazoles in which we had possibility to perform experiments for defining their structures.

EXPERIMENTAL

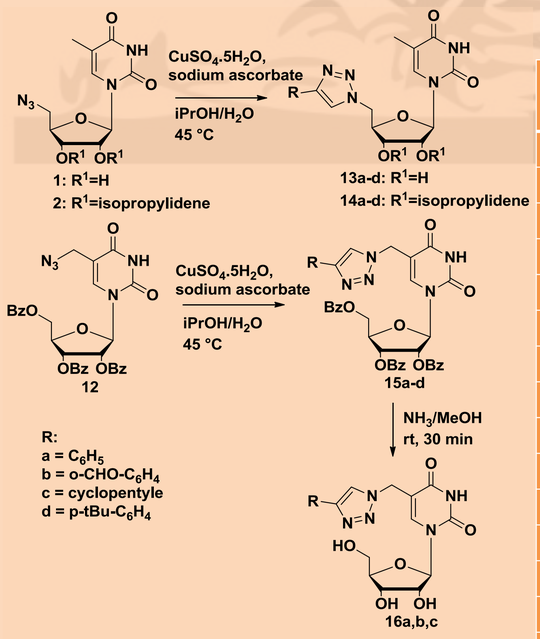
Firstly we focused on developement of synthetic approach to azidouridines **5**, **6** and **12**. Our strategy in synthesis of 5´-azidoderivatives **5** and **6** is based on two-step synthesis starting from 5-methyluridine (Scheme 1). For synthesis of compound **12** we used ribosylation of hydroxymethylene uracil **7** under standard Vorbrüggen conditions (Scheme 1). Although treatment of derivatives **1** and **2** with iodine or bromine in the presence of PPh_3 and imidazole was successfully used for preparation of halogenderivatives **3a,b** and **4a,b** (Scheme 1), application of this method to derivative **10** completely failed. Therefore we focused on finding of other synthetic method. Simple substitution by thionylchloride proved to be the best way because product **11** was formed quantitatively (Scheme 1).



Scheme 1.: Preparation of azides 5,6,12.

CuAAC

Azides **5,6,12** were used for studying of catalysed click reactions with four commercial available acetylenes. Copper sulphate pentahydrate was used as a source of Cu(I) ions, which were generated by exposure of sodium ascorbate. All triazoles **13a-d – 15a-d** were formed quantitatively, regioselectively and rapidly (Scheme 3). By removal of benzoyl group from triazoles **15a-d** we obtained products **17a-d**. Structures of each triazoles were determined by ^1H – ^1H COSY, ^{13}C – ^1H HSQC and ^{13}C – ^1H HMBC experiments.



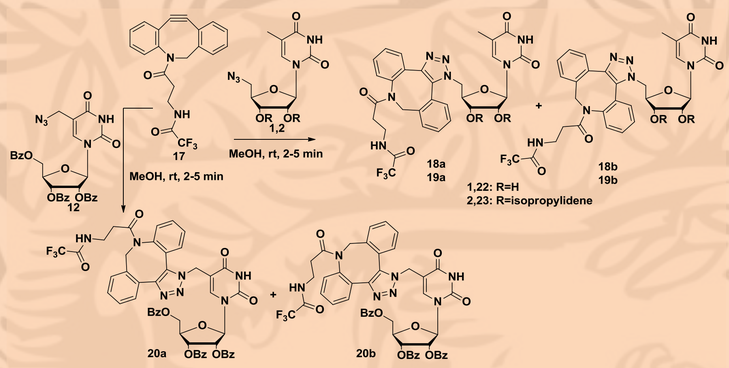
Scheme 2.: CuAAC with azides 5,6,12.

Table 1.: Summarizing of yields for CuAAC.

cmpd	R	Reaction time	Yield [%]
13a	C_6H_5	2 h	62
13b	o-CHO-C_6H_4	2 h	58
13c	cyclopentylene	2 h	63
13d	p-tBu-C_6H_4	2 h	45
14a	C_6H_5	30 min	50
14b	o-CHO-C_6H_4	30 min	70
14c	cyclopentylene	30 min	60
14d	p-tBu-C_6H_4	30 min	66
15a	C_6H_5	2 h	73
15b	o-CHO-C_6H_4	2 h	74
15c	cyclopentylene	2 h	44
15d	p-tBu-C_6H_4	2 h	49
16a	C_6H_5	-	48
16c	cyclopentylene	-	29
16d	p-tBu-C_6H_4	-	25

Copper free click reactions

Copper free click reactions of azides **5,6,12** were studied with azocine derivative **17** (Scheme 3). All reactions which were made in methanol proceed very fast almost immediately. All products were formed as a mixture of two regioisomers in ratio 1:1. Isomers **18a,b** and **19a,b** were successfully separated by semipreparative HPLC in excellent purity. Surprisingly, 1H NMR spectra show presence of two componds in case of **19a** and even four compounds in case of **18a,b** and **19b**. The number of these isomers and their ration wasn´t changed with temperature (Figure 1). But surprisingly the change of solvents caused shifting of signals and also their ratio (Figure 2).



Scheme 3.: Copper free click reactions with azocine 17.

Table 2.: Ratio of two isomers.

Cmpd	Solvent	Ratio*
18a	Methanol	3:1
	$CDCl_3$	2.5:1
	DMFA	1.6:1
	D_2O	8.3:1
	DMSO	1.5:1
19a	Acetone	4:1
	$CDCl_3$	8:1
	DMFA	2.5:1
	DMSO	2.2:1
	Methanol	10:1

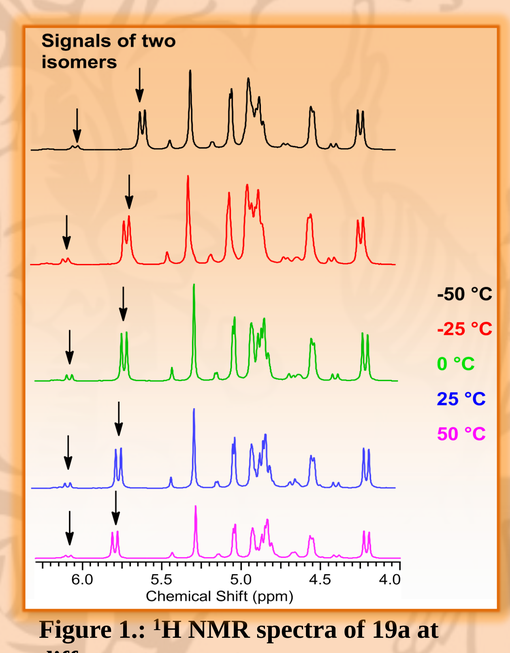


Figure 1.: ^1H NMR spectra of 19a at different temperatures.

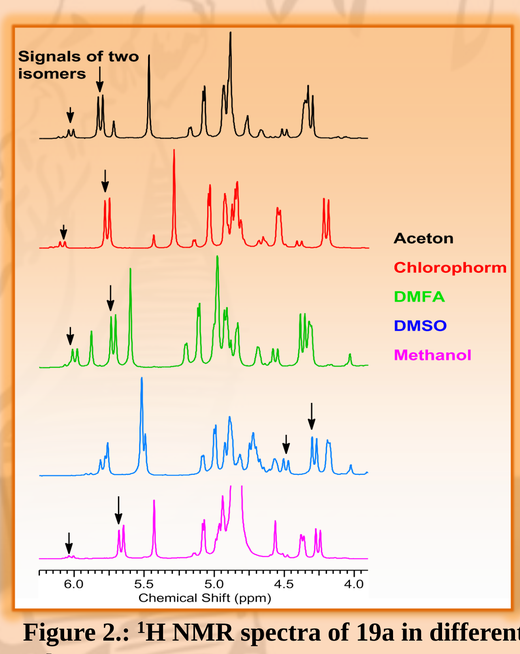


Figure 2.: ^1H NMR spectra of 19a in different solvents.

Structure of **18a** and **19a** was identified by identification of ^1H, ^{13}C and ^{15}N signals by 2D experiments, especially ^1H – ^1H gROESY a ^1H – ^{15}N gHMBC. Derivatives **20a,b** weren´t isolated, because of their very bad separation.

CONCLUSION

Route for synthesis of 5 and 5´- azido derivative of thymidine riboside was developed. These derivatives were successfully applied in conversion to range of structurally new 5 and 5´–(4-substituted-1*H*-1,2,3-triazol-1-yl) derivatives via copper catalysed azide-alkyne cycloadition reaction. On the other hand we studied copper-free click reactions on 5- and 5´-azidomethyl uridines with azocine derivative **17**. Reactions proceeded very fast and triazoles are formed as a mixture of two regioisomers in ratio 1:1. The structure of triazoles 22a and 23a were determinated by heteronuclear 2D correlation. All derivatives showed presence of unknown isomers in NMR spectra. Their ratio is dependent on the type of solvent, but not on the temperature.

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