VILNIUS UNIVERSITY

GRAŽINA PETRAITYTĖ

SYNTHESIS OF 5-SUBSTITUTED AND 5,6-DISUBSTITUTED FURO[2,3-*d*]PYRIMIDINES FROM PYRIMIDIN-4(3*H*)-ONE AND BIFUNCTIONAL ELECTROPHILES. SYNTHESIS OF 5-(ARYLAMINOMETHYL)FURO[2,3-*d*]PYRIMIDINES *via* MITSUNOBU REACTION

SUMMARY OF DOCTORAL DISSERTATION

Physical sciences, chemistry (03P)

Vilnius, 2014

The research was accomplished in Vilnius University in the period of 2009-2013.

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The thesis defense will take place on April 18, 2014, at 12 h. at the Auditorium of Inorganic Chemistry at the Faculty of Chemistry of Vilnius University.
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The summary of the thesis was distributed on March 18, 2014.
The thesis is available at the the Library of Vilnius University. and Library of FTMC Institute of Chemistry.

VILNIAUS UNIVERSITETAS

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5-PAKEISTŲ IR 5,6-DIPAKEISTŲ FURO[2,3-*d*]PIRIMIDINŲ SINTEZĖ IŠ PIRIMIDIN-4(3*H*)-ONŲ IR BIFUNKCINIŲ ELEKTROFILŲ. MITSUNOBU REAKCIJOS TAIKYMAS 5-(ARILAMINOMETIL)FURO[2,3-*d*]PIRIMIDINŲ SINTEZĖJE

Daktaro disertacijos santrauka

Fiziniai mokslai, chemija (03P)

Vilnius, 2014 metai

Disertacija rengta 2009-2013 metais Vilniaus universiteto Chemijos fakulteto Organinės chemijos katedroje.

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Disertacija bus ginama viešame Chemijos mokslo krypties tarybos posėdyje 2014 m. balandžio mėn.18 d. 12 val. VU Chemijos fakulteto Neorganinės chemijos auditorijoje. Adresas: Naugarduko 24, LT-03225 Vilnius, Lietuva.

Disertacijos santrauka išsiuntinėta 2014 m. kovo mėn. 18 d.

Disertaciją galima peržiūrėti Vilniaus universiteto ir FTMC Chemijos instituto bibliotekose.

CONTENT

LIST OF ABBREVIATIONS

DEAD	diethyl azodicarboxylate;
DHFR	dihydrofolate reductase;
DMF	dimethylformamide;
EGFR	epidermal growth factor receptor;
HMPA	hexamethylphosphoramide;
m-CPBA	*meta*-chloroperoxybenzoic acid;
PDGFR	platelet-derived growth factor receptor;
THF	tetrahydrofuran;
TPP	triphenylphosphine;
TS	thymidylate synthase;
VEGFR	vascular endothelial growth factor receptor.

INTRODUCTION

The fused pyrimidines are a class of compounds with wide diversity of biological activity on account of the relationship to natural substrates of enzymes and similarity to purine bases of nucleic acids. Among these heterocycles are compounds containing furo[2,3-*d*]pyrimidine moiety.

Derivatives of furo[2,3-*d*]pyrimidine as oxygen 7-deaza analogue of biogenic purine are of particular interest as research object in organic chemistry and medicine. A. Gangjee *et al*. synthesize and investigate various furo[2,3-*d*]pyrimidines as potential inhibitors of folic acid cycle enzymes such as DHFR and TS. They also investigate furo[2,3-*d*]pyrimidines as multireceptor tyrosine kinase (EGFR, VEGFR-2, PDGFR-β) inhibitors. Y. Maeda *et al*. synthesize compounds containing a moiety of furo[2,3-*d*]pyrimidines as potent and selective glycogen synthase kinase-3 inhibitors, compounds with anti-angiogenic activities. Belgian scientists (E. D. Clercq, J. Balzarini, C. McGuigan *et al*.) synthesize and investigate bicyclic furopyrimidine nucleosides as potent ant selective varicella-zoster virus inhibitors. FV-100 is a leading drug candidate for the treatment of herpes zoster, already in phase II clinical trials. Furo[2,3-*d*]pyrimidine derivatives also have shown serine/threonine protein kinase (Akt1), non-receptor tyrosine kinase (ACK1) or the lymphocyte-specific kinase (Lck) inhibition. Some data indicate that this heterocycle can also be used to construct oligoarylenes with potential application in optoelectronic devices. So, the study on new methodologies for the construction and functionalization of fused heterocycles, including furopyrimidines, is important field of research for the synthesis of new functional compounds as well as development of heterocyclic chemistry itself.

The main goals of the present work were:

- Elaboration of the methods for the synthesis of 5-substituted and 5,6-disubstituted furo[2,3-*d*]pyrimidines from pyrimidin-4(3*H*)-one and bifunctional electrophiles;
- Evaluation of the synthesis of secondary amines *via* Mitsunobu reaction. Application of this reaction for the synthesis of (5-arylaminomethyl)furo[2,3-*d*]pyrimidines.

The tasks of the research were the following:

- to investigate the reaction of pyrimidin-4(3*H*)-one with various bifunctional electrophiles;
- to study synthetic routes of *O*-alkylation with bifunctional electrophiles and optimization of intramolecular cyclization reaction of obtained compounds.
- to investigate and optimize the Mitsunobu reaction of various *N*-sulfonylanilines with (4-amino-2-methylthiofuro[2,3-*d*]pyrimidin-5-yl)methanol.
- to evaluate biological activity of the synthesized 5-arylaminomethylthiofuro[2,3-*d*]pyrimidine derivatives.

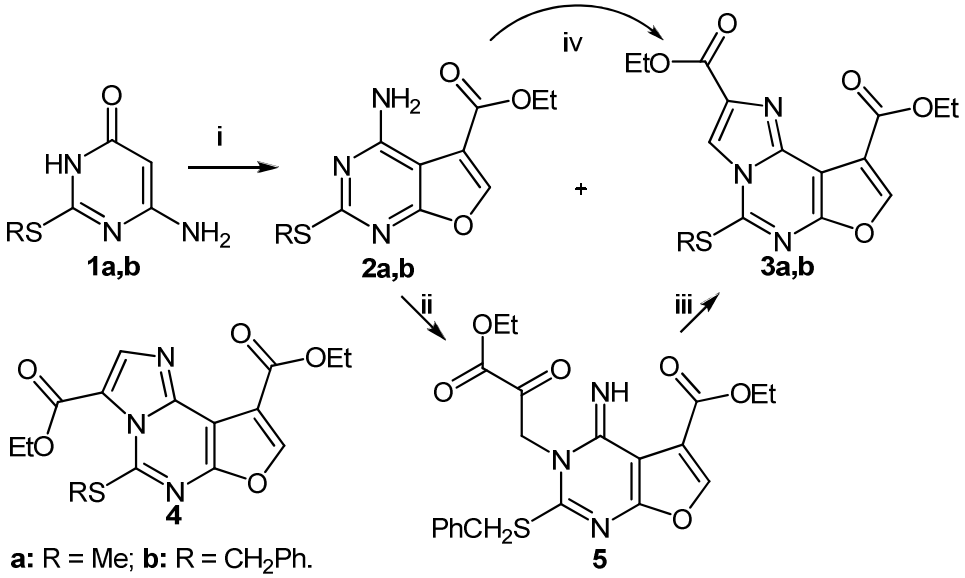
Scientific novelty: it has been shown that reaction of 2-substituted ethyl-4-aminofuro[2,3-*d*]pyrimidine-5-carboxylate with ethyl bromopyruvate gave furo[3,2-*e*]imidazo[1,2-*c*]pyrimidine-2,9-dicarboxylate derivatives. Study on the reaction of pyrimidin-4(3*H*)-one with less reactive electrophiles than ethyl bromopyruvate was performed. New method for the synthesis of furo[2,3-*d*]pyrimidin-4-one has been proposed. The proposed reaction sequence for the synthesis of 5-(arylaminomethyl)furo[2,3-*d*]pyrimidines expanded the limits of application of the Mitsunobu reaction in the synthesis of secondary amines. It has been shown that reaction of 2-methylthio-5-(phenylaminomethyl)furo[2,3-*d*]pyrimidin-4-amine with an excess of *m*-chloroperoxybenzoic acid gave a mixture of furo[2,3-*d*]pyrimidine-5-carbaldehydes. It has been found, that reaction of methylsulfonyl group of *N*-((4-amino-2-(methylsulfonyl)furo[2,3-*d*]pyrimidin-5-yl)methyl)-4-nitro-*N*-phenylbenzensulfonamide with sodium methoxide in HMPA at room temperature, gives substitution of a 4-nitro group with methoxy instead of 2-methylsulfonyl group.

RESULTS AND DISCUSSION

1. Study on pyrimidin-4(3*H*)-one reaction with bifunctional electrophiles

1.1. Pyrimidin-4(3*H*)-one reaction with ethyl bromopyruvate

Derivatives of furo[2,3-*d*]pyrimidine can be synthesized using furan or pyrimidine derivatives as starting materials. In this work on the synthesis of furo[2,3-*d*]pyrimidines 2,6-disubstituted pyrimidin-4(3*H*)-ones were chosen as starting materials. 2-Alkylthio-6-aminopyrimidin-4(3*H*)-ones (**1a**,**b**) reacted with ethyl bromopyruvate in DMF to obtain corresponding 2-alkylthio-4-aminofuro[2,3-*d*]pyrimidine-5-carboxylates (**2a**,**b**). There are only a few examples of such an approach to furo[2,3-*d*]pyrimidine-5-carboxylates in the literature and they show that such a cyclocondensation reaction can be performed under basic conditions. However, attempts to apply the described procedures for the synthesis of **2a**,**b**, using triethylamine or K_2CO_3 as basic reagents, failed. Nevertheless, it was found that the cyclocondensation reaction of **1a**,**b** with an equivalent amount of ethyl bromopyruvate proceeds under neutral or acidic conditions to give furopyrimidines **2a**,**b** in good yields.



Scheme 1 *Reagents and conditions*: (i) $BrCH_2COCO_2Et$, DMF, r.t.; (ii) $BrCH_2COCO_2Et$, CH_2Cl_2, r.t.; (iii) 2-propanol, Δ; (iv) $BrCH_2COCO_2Et$, toluene or *o*-xylene, Δ.

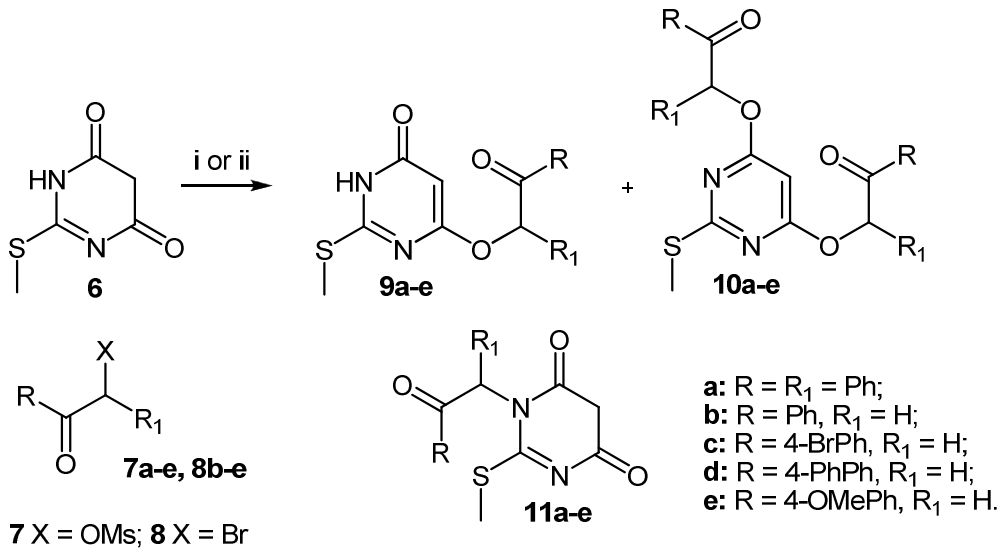
Formation of the corresponding pyrrolo[2,3-*d*]pyrimidines was not observed. When an excess of ethyl bromopyruvate was used and the reaction was carried out at room temperature together with furopyrimidines **2**, formation of furo[3,2-*e*]imidazo[1,2-

c]pyrimidines **3** was also observed. For example, **3b** was isolated as a side product (0.4%) from the reaction of **1b** with 2 eq. of ethyl bromopyruvate.

Compounds **3a**,**b** in reasonable yields were obtained by heating **2a**,**b** with ethyl bromopyruvate in toluene or *o*-xylene. Performing reaction of **2b** with ethyl bromopyruvate at room temperature in dichloromethane allowed us to isolate a small amount of intermediate **5** which during crystallization from 2-propanol quantitatively underwent conversion to furo[3,2-*e*]imidazo[1,2-*c*]pyrimidine **3b**. Reaction of **2a**,**b** with ethyl bromopyruvate can give either compounds **3a**,**b** or their isomers **4a**,**b**. Noticeable difference in the NMR spectra between these compounds must be chemical shifts of imidazole carbon atoms with attached proton (C-3 in compounds **3a**,**b** and C-2 in compounds **4a**,**b**). The signal for this carbon in the ^{13}C NMR spectra of the synthesized compounds was clearly identified from ^{13}C NMR DEPT (45°) spectra. To decide which product of the possible ones was obtained in this reaction, calculations of the NMR spectra were performed with Gausian 98 program using the DFT B3LYP method with 6-31G(d,p) basis set. Chemical shift of the calculated ^{13}C NMR spectrum for C-3 of compound **3a** was found to be 117.7 ppm. This value is in good match with δ_{exp} (C-3) = 115.2 ppm of the synthesized furo[3,2-*e*]imidazo[1,2-*c*]pyrimidine-2,9-dicarboxylate **3a**, whereas the calculated value of chemical shift for C-2 of compound **4a** is 146.7 ppm. Analogous results were also obtained for compound **3b**: δ_{exp} (C-3) = 115.3 ppm, δ_{calc} (C-3) = 118.4 ppm, while δ_{calc} (C-2) for compound **4b** is 145.9 ppm. Thus, it can be unambiguously concluded that diethyl furo[3,2-*e*]imidazo[1,2-*c*]pyrimidine-2,9-dicarboxylates **3a**,**b** are formed in the reaction of 2-alkylthio-4-aminofuro[2,3-*d*]pyrimidine-5-carboxylates **2a**,**b** with ethyl bromopyruvate.

1.2 Study on the reaction of 2-methylthiopirimidin-4,6(1*H*,5*H*)-dione with less reactive bifunctional electrophiles

In the previous chapter it was shown that some 2-disubstituted 6-amino pyrimidin-4(3*H*)-ones react with ethylbromopyruvate to furnish furo[2,3-*d*]pyrimidine derivatives **2a**,**b**. Analogous reaction of pyrimidinone **6** with less reactive bifunctional electrophiles did not give the target furo[2,3-*d*]pyrimidines outright.

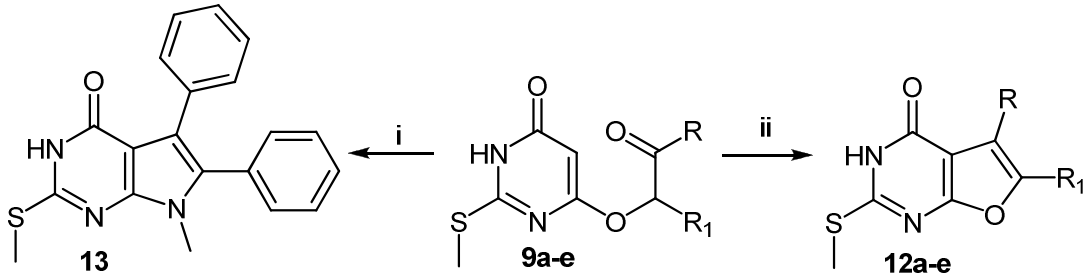


Scheme 2 *Reagents and conditions*: (i) KF or NaH, H_2O, 2-substituted or 1,2-disubstituted 2-oxoethyl methanesulfonate **7a-e**, DMF, r.t.; (ii) KF or NaH, H_2O, 4-substituted 2-bromo-1-phenylethanone **8a-e**, DMF, r.t.

2-Methylthiopyrimidin-4,6(1*H*,5*H*)-dione reacts with 2-oxo-1,2-diphenylethyl or 2-oxo-2-phenylethyl methanesulfonates (**7a-e**) or 2-bromo-1-phenylethanones (**8a-e**) in the presence of base (potassium *tert*-butoxide, Et_3N, DBU, DIPEA) in DMF to form 2-methyltio-6-(2-oxo-1,2-diphenylethoxy)-pyrimidin-4(3*H*)-one (**9a**) or 2-methyltio-6-(2-oxo-2-diphenylethoxy)pyrimidin-4(3*H*)-ones (**9b-e**) in low yields. As the main problem of this reaction – formation of di-*O*-alkylated products (**10a-e**) was identified while no *N*-alkylation products (**11a-e**) were formed. Best results were obtained when potassium fluoride or sodium hydride was used as a base then water was added for solubility of pyrimidinone salt in DMF. The structure assignment of **9a-e** and **10a-e** were based on spectral data. In the ^{1}H NMR spectrum of **9a-e** a singlet of 5-position of the pyrimidine ring at 5.55-5.99 ppm and a broad singlet of NH group at 12.38-12.42 ppm were observed. The signal of CH_2 group of compounds **9b-e** was observed at 5.64-5.75 ppm. Structure of compounds **10a-e** was established on the basis of ^{1}H NMR spectra – no signal of NH proton is observed and integral of OCH_2 signal compared to C5H is four times bigger.

The intramolecular cyclization of *O*-alkylated pyrimidines **9a-e** to furo[2,3-*d*]pyrimidines was investigated. Reactions were carried out in different solvents at elevated temperatures (toluene at reflux with catalytic amounts of conc. sulfuric acid or

PTSA; acetonitrile at reflux with $BF_3 \cdot Et_2O$ as a catalyst; at reflux nitrobenzene or *N*-methyl-2-pyrrolidone) – only starting materials were detected.



a: R = R_1 = Ph; **b:** R = Ph, R_1 = H; **c:** R = 4-BrPh, R_1 = H; **d:** R = 4-PhPh, R_1 = H; **e:** R = 4-OMePh, R_1 = H.

Scheme 2 *Reagents and conditions*: (i) HMPA, 170 °C, Ar; (ii) Silica gel 210-240 °C.

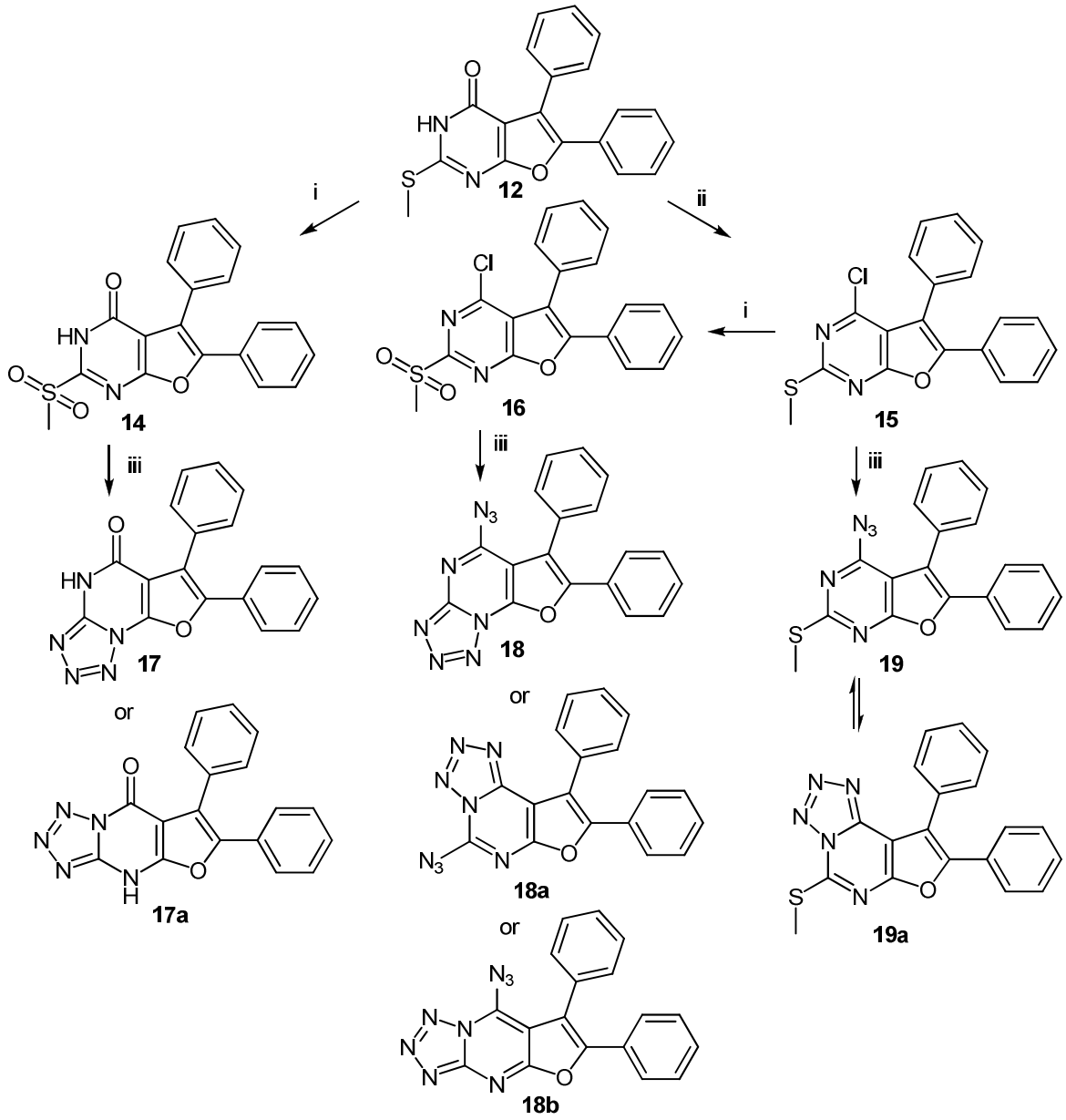
Interesting result was obtained when HMPA was used as a solvent. After NMR [^{1}H NMR spectrum of **13** give two singlets of methyl group 2.61 ppm (SMe) and 3.57 ppm (NMe); the ^{13}C NMR spectrum of this compound has signal at 30.4 ppm, which can be attributed to the carbon of NMe group] and HRMS data analysis the structure was attributed to pyrrolo[2,3-*d*]pyrimidine **13**. However, target compounds **12a-e** were obtained when reaction was carried out on silica gel at elevated temperature (210-240 °C). ^{1}H NMR spectrum of **12b-e** shows a singlet of furan ring proton downfield compared to pyrimidine ring OCH_2 of *O*-alkylated compounds **9b-e**. The yields of this cyclization reaction are around 63% (Table 1).

Table 1. Yields of *O*-alkylated derivatives **9a-e** and 5-substituted or 5,6-disubstituted 2-methylthiofuro[2,3-*d*]pyrimidin-4(3*H*)-ones **12a-e**.

Entry	R, R_1	Yield (%) of **9**	Yield (%) of **12**
1	R = R_1 = Ph	**9a** (56)	**12a** (62)
2	R = Ph, R_1 = H	**9b** (52)	**12b** (61)
3	R = 4-BrPh, R_1 = H	**9c** (46)	**12c** (51)
4	R = 4-PhPh, R_1 = H	**9d** (48)	**12d** (54)
5	R = 4-OMePh, R_1 = H	**9e** (52)	**12e** (48)

The proposed new two-step protocol for the synthesis of 5-substituted or 5,6-disubstituted furo[2,3-*d*]pyrimidines from pyrimidin-4,6(1*H*,5*H*)-ones and bifunctional electrophiles can be utilized as a tool in heterocyclic chemistry.

As one of the strategies to access *per*-arylfuro[2,3-*d*]pyrimidines could be Click-chemistry. It was decided to functionalize 2- and 4-positions of furo[2,3-*d*]pyrimidines with azido group. The reactions of furopyrimidines **12** and **15** with an excess of *m*-chloroperoxybenzoic acid gave corresponding furopyrimidine derivatives **14** and **16**. Meanwhile the reaction of compound **12** with phosphoryl chloride gave 4-chloro-2-methylthiofuro[2,3-*d*]pyrimidine (**15**). The reaction of compounds **14**-**16** with excess of sodium azide furnished the corresponding furopyrimidine derivatives **17**, **18**, **19** bearing azido group or tetrazole moiety.



Scheme 3 *Reagents and conditions*: (i) *m*-CPBA, CH_2Cl_2, 0 °C to r.t.; (ii) $POCl_3$, Δ; (iii) NaN_3, DMSO, r.t.

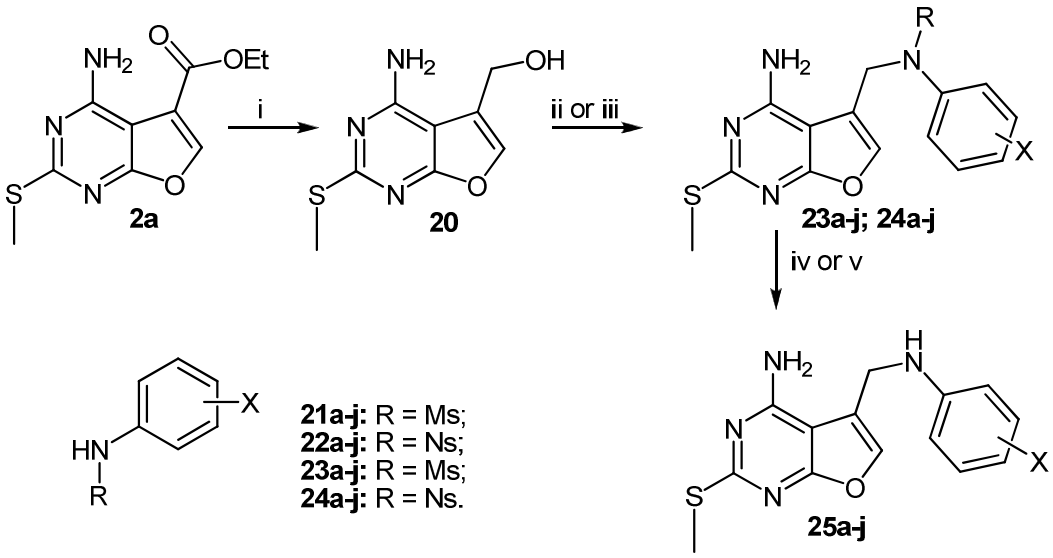
Based on NMR and IR spectra, compound **17** exists in solution and solid state only in a tetrazolo form. According to the IR spectrum compound **18a** contain only one azido group (C absorption band at 2133 cm^{-1}) and we speculated that this compound is 5-azido-6,7-diphenylfuro[3,2-*e*]tetrazolo[1,5-*a*]pyrimidine or its analogues **18a** or **18b**. ^1H NMR spectra of the compound **19** shows two signals of methylthio group and in different solvent integrals of these signals changes significantly. So, it can be claimed that compound **19** is in the dynamic equilibrium with tetrazolo form **19a**. For example, according to the ^1H NMR spectrum of compound **19** in $CDCl_3$ (60%) and C_6D_6 (53%) solutions − dominates as azide form. In solution of DMSO-d_6 dominates as tetrazolo form (88%).

2. Synthesis of 5-arylaminomethylfuro[2,3-*d*]pyrimidines

The Mitsunobu reaction allows the conversion of primary and secondary alcohols into esters, phenyl ethers, thioethers and various other compounds. For a successful course of the Mitsunobu reaction, the pK_a value of an acidic component, when diethyl azodicarboxylate (DEAD) is used, must be around 11 or lower. For this reason, anilines are out of the application range of the Mitsunobu reaction. On the other hand, the pK_a of *N*-sulfonylanilines is around this critical value and, therefore, they are suitable acidic components in the Mitsunobu reaction. We present herein the synthesis of 4-amino-5-(arylaminomethyl)-2-methylthiofuro[2,3-*d*]pyrimidines *via* the Mitsunobu reaction of the corresponding 5-(hydroxymethyl)furo[2,3-*d*]pyrimidine with various *N*-mesyl-and *N*-nosylarylamines. In order to study the scope and limitation of the reaction, a series of *N*-mesylarylamines **21a-j** and *N*-nosylarylamines **22a-j** bearing various substituents in different positions of the arylamine were employed in the Mitsunobu reaction.

Initially, 4-amino-5-(hydroxymethyl)-2-methylthiofuro[2,3-*d*]pyrimidine (**20**) was obtained in 72% yield by reduction of ethyl 4-amino-2-(methylthio)furo[2,3-*d*]pyrimidine-5-carboxylate (**2a**) with lithium aluminum hydride in tetrahydrofuran (Scheme 4). Problems with the isolation of 5-(hydroxymethyl)furo[2,3-*d*]pyrimidine **20** from the reaction mixture were solved by using saturated ammonium chloride solution for neutralization of the reaction mixture. Compound **20** reacted with *N*-mesylarylamines **21a–j** and *N*-nosylarylamines **22a–j** under Mitsunobu reaction conditions to form the

corresponding furo[2,3-*d*]pyrimidines **21a–j** and **22a–j** (Scheme 1). The reaction was found to be dependent on the reagent addition order and the concentrations of solutions used. For example, when alcohol **20** (0.1 g) was added to the reaction mixture as a solution in tetrahydrofuran (8 mL) instead of as a suspension in tetrahydrofuran (4 mL), the reaction was 2–3 times slower, and full conversion of **20** was not achieved.



Scheme 4 *Reagents and conditions*: (i) 1. $LiAlH_4$, THF, -50 °C to r.t.; 2. NH_4Cl; (ii) DEAD, Ph_3P, *N*-mesylarylamine **21a-j**, THF, 0 °C to r.t.; (iii) DEAD, Ph_3P, *N*-nosylarylamine **22a-j**, THF, 0 °C to r.t.; (iv) HBr, AcOH, PhOH, r.t., 10 d (method A); (v) $HSCH_2COOH$, $LiOH \cdot H_2O$, DMF, r.t., (method B). For X see Table 2.

Full conversion of alcohol **20** was reached only when the addition order of reagents was as follows: to a cooled solution (0 °C) of triphenylphosphine in tetrahydrofuran, DEAD was introduced and, after 10 minutes, the corresponding *N*-sulfonylarylamine was added to the solution. Finally, a suspension of alcohol **20** in tetrahydrofuran was added. These reactions require tetrahydrofuran that has been freshly distilled from $LiAlH_4$. The reactions of alcohol **20** with sulfonamides **21a–i** and **22a–i** furnished the corresponding products **23a–i** and **24a–i** in good yields (Table 1). The reaction time was up to 1 hour. The *N*-nosyl derivatives **24a–j** were generally obtained in higher yields than the corresponding *N*-mesyl derivatives **23a–j**. The results presented in Table 1 for compounds **5** and **6** also show that the Mitsunobu reaction of alcohol **2** with the *N*-mesyl- and *N*-nosylarylamines does not depend very much on the electronic nature of the substituent in the arylamine moiety; however, steric hindrance of the reaction site seems to be important. Lower yields of the 2,6-diisopropyl derivatives **23j** and **24j** were obtained, presumably because of steric effects of the adjacent isopropyl groups in the sulfonamide moiety (Table 1, entry 10). Slightly lower yields were also obtained when

other *ortho*-substituted sulfonamides were employed in the reaction with alcohol **20** (Table 2, entries 2, 5, 8; compounds **23b**, **23e**, **23h**, respectively). ^{1}H and ^{13}C NMR spectra as well as elemental analysis data of the synthesized compounds are consistent with the structures presented. In the ^{1}H NMR spectra of compounds **23e**, **23h** and **24e**, **24g**, **24h** geminal-type spin–spin coupling of the methylene protons with *J* from 11.4 to 15.3 Hz is observed.

Table 2 Yields of mesyl derivatives, nosyl derivatives and 4-amino-5-(arylaminomethyl)-2-methylthiofuro[2,3-*d*]pyrimidines

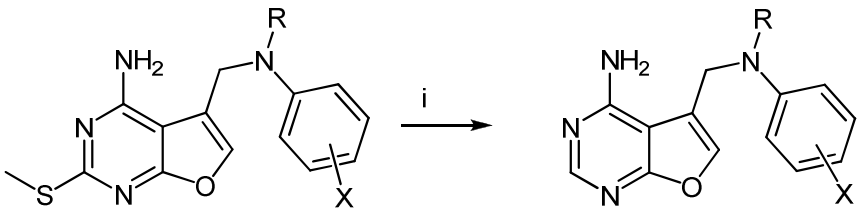
Entry	X	Yield (%) of **23**	Yield (%) of **24**	Yield* (%) of **25**
1	H	**23a:** 73	**24a:** 82	**25a:** 75
2	2,5-$(MeO)_2$	**23b:** 60	**24b:** 83	**25b:** 77
3	4-Ac	**23c:** 78	**24c:** 78	**25c:** 78
4	4-Cl	**23d:** 58	**24d:** 74	**25d:** 74
5	2,5-Cl_2	**23e:** 44	**24e:** 73	**25e:** 58
6	3,4,5-$(OMe)_3$	**23f:** 79	**24f:** 84	**25f:** 70
7	2-NO_2	**23g:** 72	**24g:** 61	**25g:** 63
8	2,3-(-CH=CH-)$_2$	**23h:** 46	**24h:** 79	**25h:** 63
9	4-Ph	**23i:** 62	**24i:** 77	**25i:** 61
10	2,6-(*i*-Pr)$_2$	**23j:** 29	**24j:** 53	**25j:** 34

* Yields are for the deprotection reaction of **24a**-**j** with mercaptoacetic acid (method B).

Removal of the *N*-mesyl group in **23a** to give compound **25a** was achieved with hydrogen bromide in acetic acid (Scheme 4). Phenol was used as a trap for the methylsulfonyl group. In general, the use of hydrogen bromide in acetic acid is a good, but time-consuming method. Full conversion of **23a** and **23i** was achieved only in 10 days; the yields of **25a** and **25i** were 76% and 68% (method A), respectively. Moreover, this method cannot be applied for removing the *N*-mesyl group from acid-sensitive substrates (e.g., **23b** or **23f**). Attempts to remove the mesyl group in compounds **5** with Red-Al® as a deprotection reagent failed. Although the starting mesyl derivatives were consumed in 10–20 minutes, the target compounds were not obtained due to the formation of a complex mixture of products. In contrast, the nosyl derivatives **24a–j** can be deprotected under mild conditions *via* the Meisenheimer complex using sulfur nucleophiles. Using mercaptoacetic acid and lithium hydroxide monohydrate as a base, deprotected products **25a–i** were obtained in good yields (58–78%) (Scheme 4, method

B; Table 2). It should be noted that sodium hydroxide as a base in this reaction did not work well.

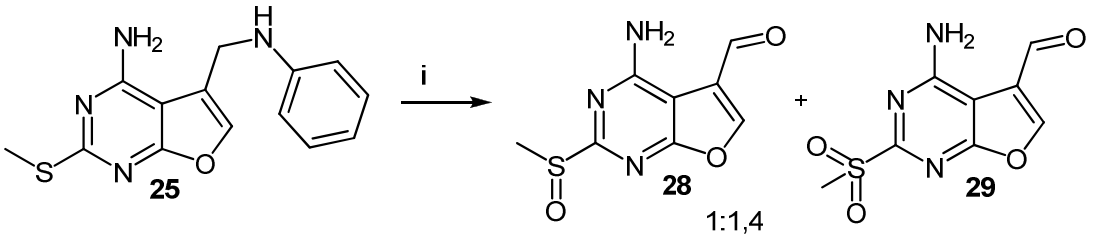
Although the 2,4-diaminopyrimidine moiety is considered obligatory for dihydrofolate reductase inhibitors, other enzymes of the folic acid cycle are not strictly bound to this fragment. We decided to remove the methylthio group of some furo[2,3-*d*]pyrimidine derivatives and investigate these compounds for anticancer activity against some solid tumor cell lines. The methylthio group was removed with Raney nickel in boiling methanol. The structure assignment of **26a**,**f**,**h**,**i** and **27a**,**f**,**h**,**i** was based on its spectral data.



Scheme 5 *Reagents and conditions*: (i) Raney nickel, MeOH, Δ.

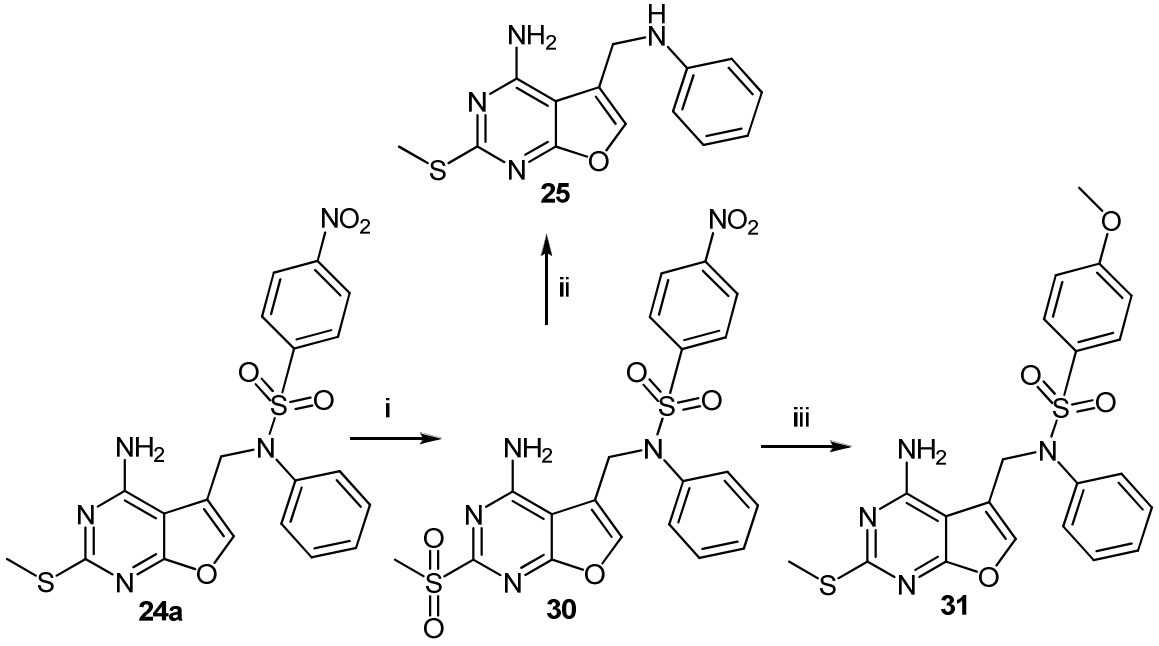
Anticancer activity of synthesized compounds was studied in Spain, La Laguna University, under the guidance of Dr. José Padron, thanks to Dr. Inga Čikotienė. 5-Arylaminomethylthio[2,3-*d*]pyrimidine derivatives (**23a**,**f**,**h**,**i**; **25a**,**f**,**h**,**i**; **26a**,**f**,**h**,**i**; **27a**,**f**,**h**,**i**) were evaluated in *vitro* against a panel of six human solid tumor cell lines: A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW (non-small cell lung), T47D (breast) and WiDr (colon). Unfortunately, these compounds do not inhibit evaluated solid tumor cell lines.

Thus, we also thought it would be useful to have a procedure for the introduction of various groups into 2 position of furo[2,3-*d*]pyrimidines. This could be achieved by oxidation of the methylthio group in compound **25a**, and subsequent nucleophilic substitution of the obtained 2-methylsulfonyl derivatives; however, the reaction of compound **25a** with an excess of *m*-chloroperoxybenzoic acid (*m*-CPBA, >3 equiv) gave an inseparable mixture of aldehydes **28** and **29** (Scheme 6).



Scheme 6 *Reagents and conditions*: (i) *m*-CPBA, CH_2Cl_2, r.t.

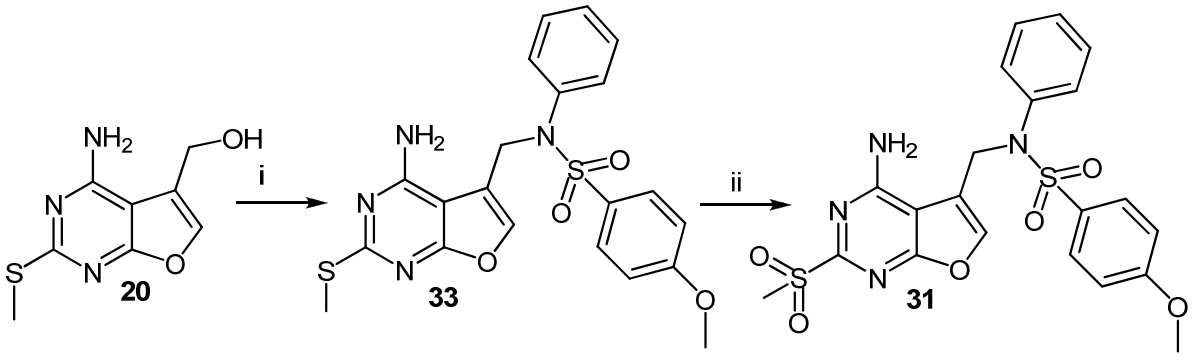
Therefore, another synthetic pathway to 2-substituted furo[2,3-*d*]pyrimidines was attempted. Oxidation of nosyl derivative **24a** with *m*-CPBA proceeded unambiguously and compound **30** was obtained in 89% yield (Scheme 7). However, the deprotection of **30** with mercaptoacetic acid gave a mixture, from which furo[2,3-*d*]pyrimidine **7a** instead of the desired deprotection product was isolated. The methylsulfonyl group underwent reduction to the methylthio group, probably either by mercaptoacetic acid or by the deprotection byproduct (*p*-nitrophenylthio)acetic acid. In this connection, nucleophilic substitution of furo[2,3-*d*]pyrimidine **30** prior to its deprotection was investigated. It was found that substitution reactions with various nucleophiles (ammonia, *n*-butylamine, sodium phthalimide, urotropine and sodium methoxide) did not take place in solvents like methanol or tetrahydrofuran.



Scheme 7 *Reagents and conditions*: (i) *m*-CPBA, CH_2Cl_2, r.t.; (ii) $HSCH_2COOH$, $LiOH \cdot H_2O$, DMF, r.t.; (iii) NaOMe, HMPA, r.t.

An unexpected result was obtained when performing the reaction of **10** with sodium methoxide in HMPA at room temperature (Scheme 7). In the ^1H NMR spectrum

of the isolated compound **31** signals for a methoxy group at 3.90 ppm and a methylsulfonyl group at 3.32 ppm were observed. Significant changes were also observed in the region of the *para*-substituted aromatic system. Thus, in the ^{1}H NMR spectrum of nosyl derivative **30** two doublets at 7.98 and 8.50 ppm were observed, while the ^{1}H NMR spectrum of the obtained product **31** had two doublets at 7.20 and 7.63 ppm. The reason for such a change in the ^{1}H NMR spectrum could be due to the presence of an electron-donating methoxy substituent at the *para* position instead of a nitro group. While NMR, IR spectra and elemental analysis data are consisted with proposed structure **31**, performed alternative synthesis (scheme 8) of compound **31** confirms this structure without doubts. Although displacement of a nitro group with methoxy nucleophile is known in heterocyclic chemistry such a reaction has, to the best of our knowledge, not been reported in carbocyclic aromatics.



Scheme 8 *Reagents and conditions*: (i) TPP, DEAD, 4-methoxy-*N*-phenylbenzenesulfonamide **32**, THF, 0 °C to r.t.; (ii) *m*-CPBA, CH_2Cl_2, r.t.

In summary, the proposed reaction sequence for the synthesis of 5-(arylaminomethyl)furo[2,3-*d*]pyrimidine expands the limits of application of the Mitsunobu reaction and this reaction can be applied not only in functionalization of alcohol with arylamino moiety, but also and for the synthesis of secondary amines in general.

CONCLUSIONS AND RESULTS

1. The reaction of 2-substituted 6-aminopyrimidin-4(3*H*)-ones with ethyl bromopyruvate gave not only alkylated products but also cyclization reaction to pyrimidine-fused heterocycles. In this case formation of corresponding ethyl-4-amino-2-methylthiofuro[2,3-*d*]pyrimidin-5-carboxylates instead of pyrrolo[2,3-*d*]pyrimidine heterocycle took place.

2. It has been shown, that reaction of 2-substituted ethyl-4-aminofuro [2,3-*d*]pyrimidin-5-carboxylates with ethyl bromopyruvate afforded furo[3,2-*e*]imidazo[1,2-*c*]pyrimidine heterocycle.

3. Reaction of 4,6-dioxo-2-methylthiopyrimidine with less reactive bifunctional electrophiles compared to ethyl bromopyruvate under basic conditions gave mono- and di-*O*-alkylation products. *N*,*O*-dialkylated or *N*-alkylated products under investigated reaction conditions are not formed.

4. Two step protocol for the synthesis of 5-substituted and 5,6-disubstituted 2-methylthiofuro[2,3-*d*]pirimidin-4(3*H*)-ones from 4,6-pyrimidindiones and 1-phenyl- and/or 1,2-diphenyl-2-oxyethyl methanesulfonates or 2-bromo-1-phenylethanones has been proposed. The intramolecular cyclization of *O*-alkylated pyrimidines gives target products on silica gel at 210-240 °C temperatures.

5. Reactions of 2-methylsulfonyl-5,6-diphenylfuro[2,3-*d*]pyrimidin-4(3*H*)-one, 4-chloro-2-methylthio-5,6-diphenylfuro[2,3-*d*]pyrimidine and 4-chloro-2-methylsulfonyl-5,6-diphenylfuro[2,3-*d*]pyrimidine with sodium azide afford the corresponding derivatives of furo[2,3-*d*]pyrimidines bearing azido group or/and tetrazolo moiety. 6,7-Diphenylfuro[3,2-*e*]tetrazolo[1,5-*a*]pyrimidin-5(4*H*)-one or other isomer 6,7-diphenylfuro[2,3-*d*]tetrazolo[1,5-*a*]pyrimidin-8(4*H*)-one in solutions and in solid state exists only in the tetrazolo form. Also, no dynamic equilibrium with other forms occurs in the case of 5-azido-6,7-diphenylfuro[3,2-*e*]tetrazolo[1,5-*a*]pyrimidine. Meanwhile 4-azido-2-methylthio-5,6-diphenylfuro[2,3-*d*]pyrimidine in solutions of DMSO, $CHCl_3$, C_6H_6 is in the dynamic equilibrium with corresponding 5-methyl-8,9-diphenylfuro[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine.

6. Mitsunobu reaction can be applied for the synthesis of secondary amines, having furo[2,3-*d*]pyrimidine moiety. This was demonstrated performing reactions between (4-amino-2-methylthiofuro[2,3-*d*]pyrimidine-5-yl)methanol and various *N*-sulfonylanilines (Ms, Ns).

7. 5-Arylaminomethylfuro[2,3-*d*]pyrimidine derivatives **23a**,**f**,**h**,**i**; **25a**,**f**,**h**,**i**; **26a**,**f**,**h**,**i** and **27a**,**f**,**h**,**i** does not inhibit the growth of the following solid tumor cell line A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T47D (breast) and WiDr (colon).

8. It was found that oxidation of 4-amino-5-(phenylaminomethyl)-2-methylthiofuro[2,3-*d*]pyrimidines with excess of *m*-chloroperoxybenzoic acid removes aniline moiety and gives a mixture of 4-amino-2-(methylsulfinyl)- and 4-amino-2-(methylsulfonyl)furo[2,3-*d*]pyrimidine-5-carbaldehyde.

9. It is shown for the first time that displacement of a nitro group with methoxy nucleophile occurs not only in heterocycles, but also in carbocyclic aromatics bearing electron-withdrawing group in *para*-positions. This was a result of the reaction of *N*-{(4-amino-2-(methylsulfonyl)furo[2,3-*d*]pyrimidin-5-yl)methyl}-4-nitro-*N*-phenylbenzenesulfonamide with sodium methoxide in HMPA – nitro group was displaced instead of expected displacement of methylsulfonyl group.

SUMMARY IN LITHUANIAN

5-PAKEISTŲ BEI 5,6-DIPAKEISTŲ FURO[2,3-*d*]PIRIMIDINŲ SINTEZĖ IŠ 4-PIRIMIDINONŲ IR BIFUNKCINIŲ ELEKTROFILŲ. MITSUNOBU REAKCIJOS TAIKYMAS 5-(ARILAMINOMETIL)FURO[2,3-*d*]PIRIMIDINŲ SINTEZĖJE

Kondensuoti pirimidino heterociklai, kaip fermentų gamtinių substratų bei nukleorūgščių bazės – purino – analogai, pasižymi įvairiu biologiniu aktyvumu: antibakteriniu, antiparazitiniu, antivirusiniu, o taip pat ir priešvėžiniu aktyvumu. Tarp tokių heterociklų sutinkami ir furo[2,3-*d*]pirimidino fragmentą turintys junginiai.

Furo[2,3-*d*]pirimidino heterociklas, biogeninio purino deguoninis 7-deazaanalogas, yra svarbus tyrimo objektas organinėje chemijoje bei medicinoje. Furo[2,3-*d*]pirimidino dariniai sintetinami ir tyrinėjami kaip potencialūs folio rūgšties ciklo fermentų dihidrofolatreduktazės (DHFR), timidinsintazės (TS), tirozinkinazės receptorių (EGFR, VEGFR-2, PDGFR-β), veiksnūs ir/ar selektyvūs glikogensintazės kinazės-3 (GKS-3), Varicella-zoster viruso inhibitoriai. Vienas perspektyviausių VZV inhibitorių, kandidatas juostinės pūslelinės gydymui, yra FV-100 – furo[2,3-*d*]pirimidino fragmentą turintis nukleozidas - šiuo metu esantis II klinikinių tyrimų stadijoje. Taip pat furo[2,3-*d*]pirimidino dariniai sutinkami ir kaip serino/treonino baltymų kinazę (Akt1), nereceptorinę tirozino kinazę (ACK1) ar limfocitams būdingą baltymų kinazę (Lck) inhibuojantys junginiai. Be jau minėto furo[2,3-*d*]pirimidino darinių biologinio aktyvumo, šie junginiai gali būti panaudoti ir konstruojant oligoarilenines sistemas, kurios pasižymi OLED technologijose pritaikomomis optoelektroninėmis savybėmis. Taigi, naujų heterociklinių junginių, tame tarpe ir furo[2,3-*d*]pirimidinų sintezės bei funkcionalizavimo naujų metodų paieška ir esamų tobulinimas įneša indėlį ne tik į heterociklų chemijos plėtojimą, bet ir suteikia įrankius sintetinantiems heterociklinius junginius praktiniam taikymui.

Šio darbo tikslai:

- 5-pakeistų bei 5,6-dipakeistų furo[2,3-*d*]pirimidinų sintezės metodo sukūrimas pradiniais junginiais pasitelkiant pirimidin-4(3*H*)-onus ir bifunkcinius elektrofilus;

- Antrinių aminų sintezės galimybės pasitelkiant Mitsunobu reakciją įvertinimas. Šios reakcijos pritaikymas (5-arilaminometil)furo[2,3-*d*]pirimidinų sintezėje.

Šiame darbe parodyta, kad 2-pakeisti etil-4-aminofuro[2,3-*d*]pirimidin-5-karboksilatai reaguodami su etilbrompiruvatu sudaro atitinkamus furo[3,2-*e*]imidazo[1,2-*c*]pirimidino darinius. Ištirta pirimidin-4(3*H*)-onų reakcija su mažiau reaktingais nei etilbrompiruvatas bifunkciniais elektrofilais ir sukurtas naujas furo[2,3-*d*]pirimidin-4(3*H*)-onų sintezės metodas. Pasiūlyta 5-(arilaminometil)furo[2,3-*d*]pirimidinų sintezės schema, išplečianti Mitsunobu reakcijos taikymą aminų sintezėje. Parodyta, kad oksiduojant 4-amino-5-(fenilaminometil)-2-metiltiofuro[2,3-*d*]pirimidiną *meta*-chlorbenzenperoksikarboksirūgšties pertekliumi susidarė atitinkamų furo[2,3-*d*]pirimidin-5-karbaldehidų mišinys. Nustatyta, kad veikiant *N*-{(4-amino-2-(metilsulfonil)furo[2,3-*d*]pirimidin-5-il)metil}-*N*-fenil-4-nitrobenzensulfonamidą natrio metoksidu HMPA, vyksta ne 2-metilsulfonil-, bet nitrogrupės, esančios nozilfragmente, nukleofilinis pakeitimas metoksigrupe.

LIST OF PUBLICATIONS

Publications in the journals inscribed into the list approved by Information Scientific Institute (ISI):

1. Masevicius, V., **Petraityte**, **G**., Tumkevicius, S. Synthesis of 4-amino-5-(arylaminomethyl)-2-methylthiofuro[2,3-*d*]pyrimidines *via* Mitsunobu reaction of 4-amino-5-(hydroxymethyl)-2-methylthiofuro[2,3-*d*]pyrimidine with *N*-mesyl- and *N*-nosylarylamines. *Synthesis* **2012**, 44, 1329-1338.
2. Masevicius, V., **Petraityte**, **G**., Tumkevicius, S. Reaction of 2-alkylthio-6-aminopyrimidin-4(3*H*)-ones with ethyl bromopyruvate. Synthesis of furo[2,3-*d*]pyrimidine and furo[3,2-*e*]imidazo[1,2-*c*]pyrimidine carboxylates. *Chem. Heterocycl. Comp*. **2009**, 45, 357-360.

Publications in International and Lithuanian conference proceedings:

1. **Petraityte**, **G**., Masevicius, V., Tumkevicius, S. Study on the Reaction of Pyrimidin-4,6-diones with some Bifunctional Electrophiles. Two Step Synthesis Protocol to Furo[2,3-*d*]pyrimidines. *11th National Lithuanian Conference: Chemistry 2013*. Vilnius, **2013**, September 27, 92.
2. **Petraityte**, **G**., Masevicius, V., Tumkevicius, S. Study on the reactions of 6-substituted-2-methylthiopyrimidin-4-ones with some bifunctional electrophiles. Synthesis of *per*-arylfuro[2,3-*d*]pyrimidines. International Conference on Organic Synthesis "*Balticum Organicum Syntheticum 2012*" Talinn, Estonia, July 1-4, **2012**, 158.
3. **Petraitytė**, **G**., Prievelytė, G., Masevičius, V. 5-Formilfuro[2,3-*d*]pirimidinų susidarymas 4-amino-5-(fenilaminometil)-2-metiltiofuro[2,3-*d*]pirimidiną veikiant *m*-CPBR. *Mokslinė konferencija* "*Organinė chemija*". Kaunas, Lietuva, pranešimų medžiaga, balandžio 25 d., **2012**, 48.
4. **Petraitytė**, **G**., Masevičius, V., Tumkevicius, S. 2,6-Dipakeistų pirimidin-4-onų reakcijų su dvicentriais elektrofilais tyrimas. Mokslinė konferencija "*Organinė chemija*". Kaunas, Lietuva, pranešimų medžiaga, balandžio 27 d., **2011**, 38.

5. **Petraityte**, **G**., Masevicius, V., Tumkevicius, S. Sulfonanilides – acid components of Mitsunobu reaction in the synthesis of 5-(arylaminomethyl)furo[2,3-*d*]pyrimidines. Conference on Organic Synthesis "*Balticum Organicum Syntheticum 2010*". Riga, Latvia, June 27-30, **2010**, 162.

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ACKNOWLEDGEMENTS

I am very sincerely grateful to my supervisor assoc. prof. Viktoras Masevičius for his huge input to this work: for patience, support, helping to plan experiments, to interpret the results and to write thesis. Thanks and for giving an opportunity to work in his research group.

I am grateful to all colleagues who worked with me at laboratory for patience, useful advices, support and goodwill.

I am also grateful to all colleagues for Faculty of Chemistry for useful advice, discussion and goodwill.

Finally my warmest appreciation goes to my parents, brothers and sister for full support, helping and understanding.