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A convenient and efficient approach to polyfluorosalicylic acids and their tuberculostatic activity

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Abstract. We have developed the practical method for polyfluorosalicylic acids synthesis via nucleophilic *ortho-mono*-substitution of fluorine atom with magnesium methoxide. We have managed to increase the yield of targeted polyfluorosalicylic acids from good to quantitative. We have studied the tuberculostatic activity of polyfluorosalicylic acids. It has been found that minimum inhibitory concertation (MIC) of compounds is from 0.7 to 6.5 μg/mL depending on the structure.

Keywords: Polyfluorosalicylic acid, Nucleophilic substitution, Anti-mycobacterial activity, Tuberculosis, Toxicity

Tuberculosis (TB) still remains a serious disease in the world. In accordance with Global Tuberculosis Report for 2015 (World Health Organization)[1] 9.6 million people fell ill with TB and 1.5 million people died of TB. 27% from died people were HIV-infected persons as a result of the high risk of developing TB among them. Besides, the 480000 cases of multi-drug resistant (MDR) TB were recorded and 10 % of them were extensively drug-resistant TB.

One of the widely used drugs for TB treatment is para-aminosalicylic acid (PAS), which is applied in MDR therapy. In one hand, PAS is the metabolic precursor that incorporated into the folate pathway by dihydropteroate synthase – dihydrofolate synthase, generating a toxic dihydrofolate analog that subsequently inhibits dihydrofolate reductase activity.[2] On the other hand, PAS reveals inhibitory effect on mycobactin biosynthesis. PAS is structural analog of salicylic acid, which responsible for the biosynthesis of mycobactin and carboxymycobactin participating in the cell membrane construction.[3]

We envisioned the progress in anti-TB treatment by use of fluorinated salicylic acids structural analogs as possible inhibitors of mycobactin biosynthesis. The fluorine atom has been effectively and successfully used in medicinal chemistry for modulating the physiochemical and biological properties of lead compounds in drug discovery programs. High electronegativity of fluorine atom combined with extremely low polarization make these organic compounds quite unique.[4] In fact, about 20 per cent of used pharmaceutical drugs have at least one fluorine atom in structure, and it is an aromatic fluorine.[5]

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It should be noted that polyfluorosalicylic derivatives remain hardly accessible and therefore the little known class of compounds until recently. However, based on the scarce information on their biological effects one can suggest the future development in this direction. So, a number of polyfluorosalicylic amides is patented as anticoagulant agents,[6] polyfluorosalicylic N-alkohenalkylsulfone amides can be used as agents against obesity and diabetes.[7] Polyfluorinated N-{[4-oxo-6-fluoro-7-(cyclohexylamine)-1-cyclopentyl-1,4-dihydroquinolin-3-yl]methyl}-2-hydroxybenzamides are claimed as inhibitors of platelet aggregation and P2Y12 receptors.[8]

The use of Kolbe-Schmitt[9] reaction for the preparation of hardly available polyfluorosalicylic acids cannot be applied because of possible nucleophilic substitution of fluorine in fluorinated phenols.

There are a few known methods for polyfluorosalicylic acid synthesis but all of them have no preparative value due to their disadvantages. So, 2,3,4,5-tetrafluorosalicylic acid was obtained with yield of 85% from 2,3,4,5-tetrafluorophenol in the presence of *n*-butyllithium at -75 °C under CO_2 atmosphere.[10,11] The following variations of the reaction conditions and organolithium reagents allowed the regioisomeric polyfluorosalicylic acids to be obtained with yields of 61-93%.[12] In spite of high yields of target acids, the method *of fluoro-containing phenol carboxylation* has some significant disadvantages such as the use of hardly accessible and rather expensive polyfluorophenols as well as complex instrumentation for low-temperature synthesis process.

3,5-Difluorosalicylic acid can be obtained from 2,4-difluorophenol and hemathylenetetramine in trifluoroacetic acid *via* Duff reaction with subsequent oxidation of intermediate aldehyde.[13]

Tetrafluorosalicylic acid was obtained by *oxidation* of 3-formyl- or 2-methyl-3-ethoxycarbonyl-5,6,7,8-tetrafluorochromones with yield of 30%.[14] The yield up to 64% was achieved by continuous extraction for 15 h with diethyl ether.[15] Obvious disadvantages of this method are difficulty of target acid isolation accompanied by the low or moderate yields. In addition, the preparation of starting polyfluorochromones is required and it is a particular non-trivial task.

The direct fluorination of salicylic acid with elemental fluorine in acetonitrile or hydrogen fluoride led to a mixture of mono 5-fluorosalicylic and 3,5-difluorosalicylic acids in ratio 2…7 to 1 correspondingly and was accompanied by incomplete conversion of initial reagents.[16]

The synthesis of polyfluorosalicylic acid by *nucleophilic ortho-substitution of fluorine atom in polyfluorobenzoic acids* with hydroxyl groups under heating with NaOH in 1,3-dimethyl-2-imidazoline has been described.[17] However, this way is characterized by the low selectivity process as a result of the regioisomeric products mixture formation. The use of alkali metals

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alkoxides in reactions with pentafluorobenzoic acid leads to selective formation of *para*-substituted products.[18]

Previously, we have described the selective *ortho*-substitution of fluorine atom in pentafluorobenzoic acid **1a** under the action of magnesium methoxide.[19] To obtain 2-methoxy-3,4,5,6-tetrafluorobenzoic acid **2a** the most suitable condition was heating the acid **1a** with magnesium methoxide in diglyme at 100 ºC for 2 h. The further temperature and the reaction time increase led to formation of disubstituted by product - 2,6-dimethoxy-3,4,5-trifluorobenzoic acid **3**, which when being heated at 130 ºC for 4 h became the main product.[19] Subsequent hydrolysis of acid **2a** *in situ* resulted in tetrafluorosalicylic acid **4a** formation. However, one significant disadvantage of this quite convenient and simple method is low yield of the desired compound **4a** (42%).

In this research, the method of polyfluorosalicylic acid synthesis by selective nucleophilic *ortho*-substitution of fluorine in polyfluorobenzoic acid under magnesium methoxide action has been improved.

For this purpose, we attempted to carry out *ortho-mono*-methoxylation of pentafluorobenzoic acid **1a** using various ratios of magnesium methoxide in weak polar solvents (Table 1).The choice of solvents can be explained by the need of fluorine nucleofugicity decreasing to prevent *para*-substituted isomers formation. At the same time, the use of weak polar solvent brings about the requirement of carrying out the process at high temperatures. Besides, to easy isolation and purification of target product, it is preferable for solvent to have not good miscibility with water unlike diglyme. In our view, toluene satisfies this set of requirements well because it has a sufficiently high boiling point (110 ºC), weak polarity (ε 2.4) and low miscibility with water (0.52 g/L).[20] In addition, this solvent can be easily regenerated for reuse in the reaction of methoxylation.

In this regard, we have focused on the use of toluene as a solvent for *ortho-mono*-methoxylation of acid **1a**. The ratio of magnesium methoxide to acid **1a** was ranged from 1 to 12 molar equivalents. The optimization results are presented in Table 1.

The optimal conditions for *ortho-mono*-substitution of fluorine in pentafluorobenzoic acid **1a** with more selective formation of 2-methoxy-3,4,5,6-tetrafluorobenzoic acid **2a** were refluxing in toluene with ratio of 1 eq. of acid **1a** and 2.5 eq. of magnesium methoxide (Scheme 1). However, in these conditions it was not possible to obtain the product **2a** in pure form as a result of incomplete conversion of parent acid **1a**. All attempts to separate compounds **1a** and **2a** were failed because of their co-crystallization since acids **1a** and **2a** have similar nature. Efforts to achieve a full conversion of pentafluorobenzoic acid **1a** by adding of more magnesium methoxide equivalents (from 3 to 5) were unsuccessful. In this case, the yield of compound **2a** is

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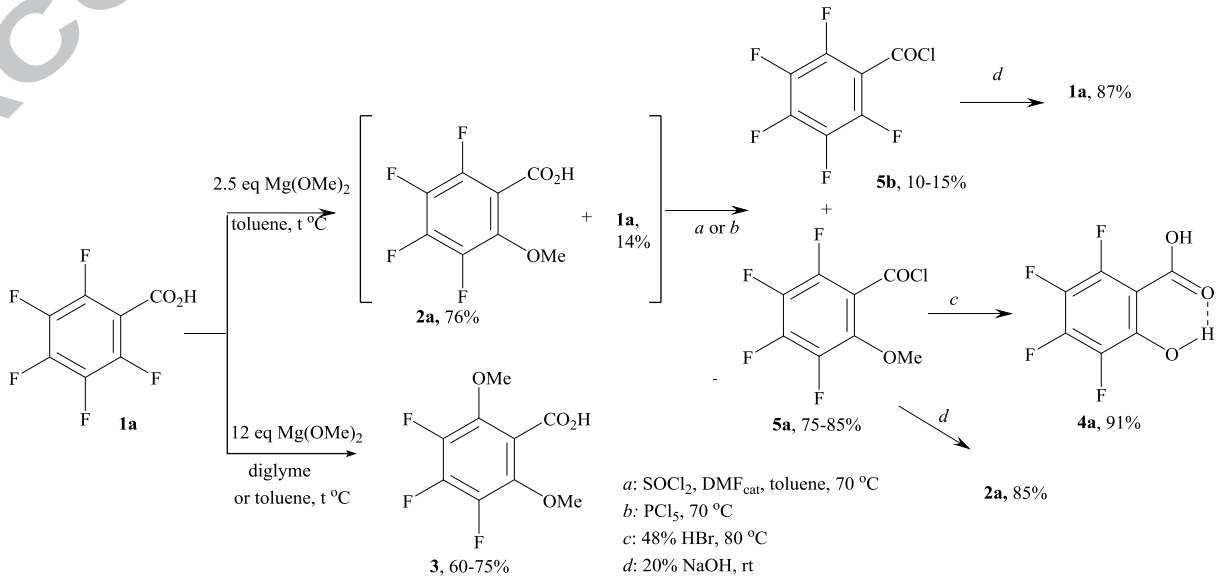
decreased owing to disubstituted product **3** formation via nucleophilic substitution of both *ortho*-fluoine atoms in acid **1a** (see Table 1).

For effective isolation and purification of acid **2a** we suggested to treat the reaction mixture with thionylchloride or phosphorus pentachloride followed by fractional distillation and separation of the resulting chlorides of 2-methoxy-3,4,5,6-tetrafluorobenzoic acid **5a** and pentafluorobenzoic acid **5b** (Scheme 1). Hydrolysis of chloroanhydride **5a** in 48% hydrobromic acid leads to tetrafluorosalicylic acid **4a**. So, we managed to increase the total yield of tetrafluorosalicylic acid **4a** by this optimization from 42% to 65% in comparison with the previous results[19] and return ~10-12% of the initial acid **1a** by hydrolysis of the pentafluorobenzoyl chloride **5b**. The analytically pure sample of 2-methoxy-3,4,5,6-tetrafluorobenzoic acid **2a** was obtained by hydrolysis of chloroanhydride **5a** with 20% NaOH in water.

Table 1.Optimization of *orto-mono*methoxylation of acid **1a**.

Solvent	$Mg(OMe)_2$, eq	Ratio of mixture **A**, % in accordance with ^{19}F NMR spectroscopy in $CDCl_3$		
		Compound **1a**	Compound **2a**	Compound **3**
diglyme	12	0	25	75
	12	0	15	85
	5	2	35	63
toluene	3	12	63	25
	2.5	14	76	10
	2	26	65	8
	1	51	46	3



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Scheme 1

We carried out X-Ray analysis for tetrafluorosalicylic acid **4a** for the first time (Fig. 1, CCDC 1447869). It is found that the effective intramolecular hydrogen bond is realized in molecule **4a** between hydrogen atom of hydroxyl group and oxygen atom of carbonyl moiety H1…O1 1.71(4) Å. The same bond in non-fluorinated analogue is characterized by a somewhat larger value of length 1.85(4) Å.[21] Hence we can assume that the hydroxyl group in tetrafluorosalicylic acid **4a** is more acidic in comparison with a similar group in non-fluorinated salicylic acid. In general, the acidic properties of tetrafluorosalicylic acid **4a** should be higher. It confirms the difference in calculated pKa values of compound **4a** (1.53±0.28)[22] and salicylic acid (3.01±0.10).[22] The enhancement of acidic nature of substance **4a** can be caused by influence of electron-withdrawing fluorine atoms. There are planar chain dimers through intermolecular hydrogen bonds in crystal of the molecule **4a** [dimers: O1…H2*, H2…O1* 1.81(3) Å, 1.81(2) Å, chains: O2…H1** 2.44(2) Å (Fig. 1)] similarly, the non-fluorinated analogue.[21]

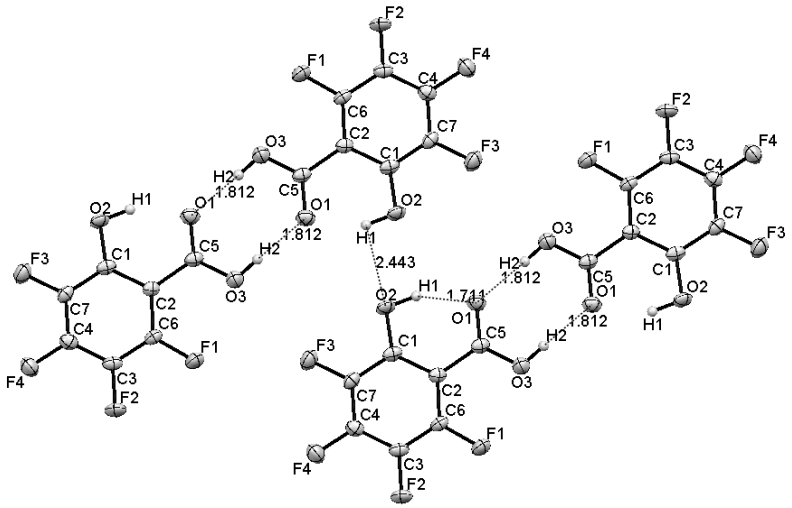

Figure 1. ORTEP view of molecule **4a.**

The developed approach has been applied for selective *ortho-mono*-methoxylation of 2,3,4,5-tetrafluoro- **1b**, 2,3,4-trifluoro- **1c** and 2,3,5-trifluoro- **1d** benzoic acids which have the only fluorine atom in the *ortho*-position as compared to pentafluorobenzoic acid **1a**. It is significantly simplifies the method for corresponding 3,4,5-trifluoro-, 3,4-difluoro- and 4,5-difluorosalicylic acid synthesis. So, acids **1b-d** interact with 4 eq. of magnesium methoxide, which is necessary to full conversion of initial reagents **1b-d**, by refluxing in toluene (or diglyme) to give 2-methoxy-3,4,5-trifluoro- **2b**, 2-methoxy-3,4-difluoro **2c**, and 2-methoxy-4,5-difluoro- **2d** benzoic acids as single products. The subsequent hydrolysis of acids **2b-d** in 48% hydrobromic acid leads to 3,4,5-trifluoro- **4b**, 3,4-diflluoro- **4c**, and 4,5-difluoro- **4d** salicylic acids (Scheme 2) with nearly quantitative yields.

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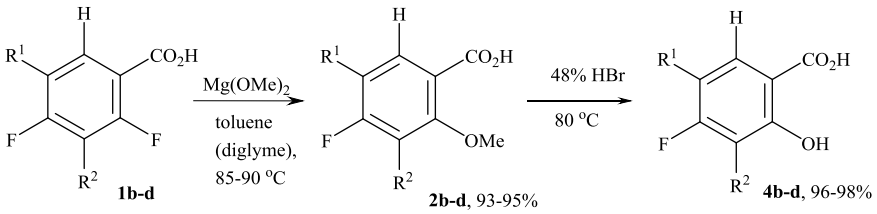

R^1, R^2 = F (**b**); R^1 = H, R^2 = F (**c**); R^1 = F, R^2 = H (**d**)

Scheme 2

This method of tri- and difluorosalicylic acids **4b-d** synthesis is an excellent alternative to carboxylation of tri- and difluorophenols.[12] Our approach is distinguished by the ease of process, high yield of the desired acids **4b-d** (total yield over 90 %) and the absence of expensive initial reagents polyfluorophenols.

Synthesized polyfluorosalicylic acids **4a-d** were screened for activity against *Mycobacterium tuberculosis* $H_{37}Rv$, *avium*, *terrae*, and MDR strains. An efficiency of compounds **4a-d** were compared to that of known drugs izoniazid, ofloxacin (Table 2) and PAS (MIC for strain $H_{37}Rv$ is 0.3-1 μg/mL and *M. avium* - 32–128 μg/mL).[23] It is noted that salicylic acid in small doses activates the process of mycobactin synthesis,[3] whereas a suppression growth of *M. tuberculosis* occurs in high concentrations (MIC > 250 μg/mL).[24] We have found that the presence of fluorine atoms in aromatic ring of salicylic acids has the significant influence on activity in relation to *Mycobacterium*. The MIC reduces to 0.7…6.5 μg/mL depending on the structure of compounds **4a-d** (Table 2). Moreover, acids **4a,c,d** have the considerable activity against *M. avium*, *M. terrae* and MDR strains.

The toxicity of polyfluorosalicylic acids **4a-c** is comparable to that of isoniazid (Table 2). However, decreasing the fluorine content in the molecule reduces a toxicity. So, 2-hydroxy-4,5-difluorobenzoic acid **4d** combines a high activity with lower LD_{50} (Table 2). Moreover, the fluorinated compounds have a great potential for further chemical transformations toward increasing of their efficiency and reducing of toxicity.

In summary, we have developed the convenient and efficient approach to polyfluorosalicylic acids **4a-d** synthesis *via* nucleophilic *ortho*-fluorine atom substitution. The MIC values 0.7 μg/mL of polyfluorosalicylic acids **4a,d** for all strains allow us to conclude about perceptivity of research area concerning chemistry of polyfluorosalicylic derivatives.

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Table 2. In vitro anti-tuberculosis activity of compounds **4a-d** and their acute in vivo toxicity in mice

Compound	Anti-mycobacterial activity against different strains of Mycobacterium tuberculosis (MIC in μg/mL)				LD_{50}, mg/kg
	$H_{37}Rv$	*M. avium*	*M. terrae*	MDR	
4a	0.7	0.7	0.7	0.7	100
4b	6.5	n.d.[a]	n.d.	n.d.	>100
4c	1.5	1.5	1.5	1.5	>150
4d	0.7	0.7	0.7	0.7	>300
Ofloxacin	0.1	0.1	0.1	0.1	5450[d]
Izoniazid	0.1	0.1	0.1	n.a.[b]	100[d]
PAS	0.3-1[c]	32-128 (n.a.)[c]	n.d.	n.d.	4000[d]

[a] n.d. – not detected, [b] n.a. – non-active, [c] Ref [23], [d] DrugBank

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Supplementary material

Supplementary data (CIF file, experimental procedures, characterization of new compounds and biological assays protocols) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl….

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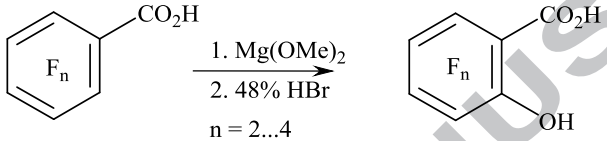

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