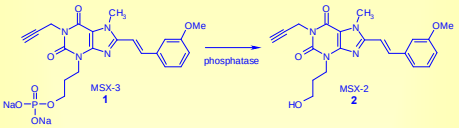
New strategies toward the gram scale production of adenosine receptor ligands

Joachim Burbiel, Jörg Hockemeyer, Mark Thorand, and Christa E. Müller

Universität Bonn, Pharmazeutisches Institut Poppelsdorf, Kreuzbergweg 26, D-53115 Bonn, Germany

MSX-3, a water-soluble A_{2A} selective adenosine receptor antagonist prodrug

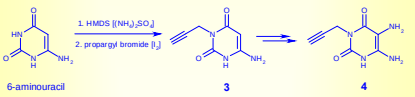
A major problem of all high-affinity A_{2A} antagonists has been their low water solubility, which limits their usefulness especially for *in vivo* studies. The introduction of highly polar substituents, e.g. sulfonic or carboxylic acid groups, into potent, A_{2A} selective antagonists resulted in a loss of receptor affinity. An alternative approach to increase water-solubility is the preparation of prodrugs, in which a polar moiety is attached to the drug but cleaved off *in vivo*.



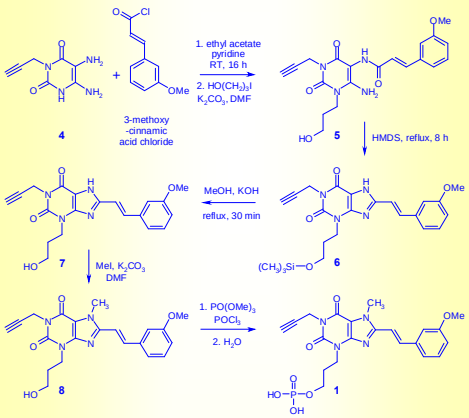
8-(*m*-Methoxystyryl)- 7-methyl- 3-(3-*O*-phosphatylpropyl)- 1-propargylxanthine disodium salt (MSX-3, **1**) has been developed as a highly water-soluble phosphate prodrug of 3-(3-hydroxypropyl)-8-(*m*-methoxystyryl)-7-methyl-1-propargylxanthine (MSX-2, **2**), a potent and selective A_{2A} antagonist. MSX-2 (**2**) is rapidly released from MSX-3 (**1**) *in vivo* by enzymatic hydrolysis of the phosphoric acid ester.[1]

The synthesis of MSX-3 (**1**) has now been improved and upscaled to obtain multigram amounts required for *in vivo* studies.

It starts from 6-aminouracil, which is silylated with hexamethyldisilazane (HMDS) and then reacted with propargyl bromide to give 6-amino-3-propargyluracil (**3**) in 75% yield.[2] This compound is nitrosated and reduced to give 5,6-diamino-3-propargyluracil (**4**) in 50% yield.



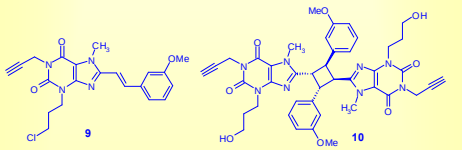
The coupling between 5,6-diamino-3-propargyluracil (**4**) and 3-methoxycinnamic acid is classically performed with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in methanol. Although the procedure described gives high yields, EDC is a very expensive reagent and thus a drawback when scaling up. Alternatively, 3-methoxycinnamic acid chloride was prepared by treatment of 3-methoxycinnamic acid with an excess of oxalyl dichloride and then coupled with 5,6-diamino-3-propargyluracil (**4**) by stirring in a mixture of ethyl acetate and pyridine at room temperature. The amide (**5**) precipitated as a colorless solid in 80% yield. This reaction can be performed in 30 g batches.



Hydroxypropylation has previously been performed with 3-bromopropanol at elevated temperatures. Higher yields have now been obtained by replacing 3-bromopropanol with 3-iodopropanol and by lowering the reaction temperature to 20°C. The product could be isolated by extraction with dichloromethane and subsequently purified by recristallisation from methanol and diethyl ether.

If ring closure was performed with HMDS, as described in the literature, a colorless by-product could be isolated. It was now identified as 8-(*m*-methoxystyryl) - propargyl - 3 - (3 - trimethylsilanyloxypropyl)xanthine (**6**). Compound **6** could easily be transformed to the desired product (**7**) by refluxing it in a methanolic potassium hydroxide solution for 30 min, a step now introduced routinely in the workup of the ring closure reaction.

After methylation at the N7-position, the phosphate group was introduced by stirring the methylated xanthine **8** with phosphoric acid trimethyl ester and phosphoryl chloride. A large excess of phosphoric acid trimethyl ester is necessary to prevent the reaction from stopping at the stage of the chloride **9**.



MSX-2 (**2**) and MSX-3 (**1**) have to be handled with great care since MSX-2 (**2**) has been found to photodimerise. The dimerisation product (1α, 2α, 3β, 4β)- 1,3-bis[3-(3-hydroxypropyl)- 7-methyl- 1-prop- 2-inyl- 3,7-dihydro-purin-2,6-dion-8-yl]-2,4-bis(3-methoxy)phenylcyclobutane (**10**) was isolated and characterised. Although the dimerisation products of the xanthine derivatives synthesised from MSX-2 (**2**) have not yet been isolated, we expect them to be photosensitive as well. Apart of the dimerisation styrylxanthine derivatives are known to undergo an E/Z-photoisomerisation in dilute solutions.[3]

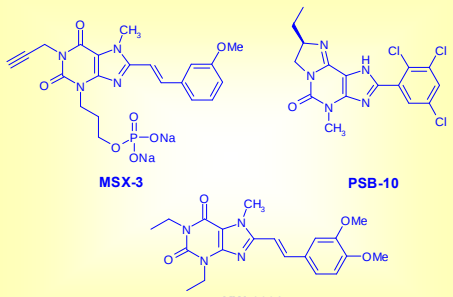
The synthesis of MSX-3 (**1**) has been refined and optimised. Three by-products have been identified and characterised (**7**, **9** and **10**). A total of 10 g of MSX-3 (free acid) have been synthesised in an overall yield of 19% (calculated from 6-aminouracil).

Introduction

Adenosine receptors are a class of G-protein-coupled receptors that comprise four subtypes, designated A_1, A_{2A}, A_{2B} and A_3. Selective antagonists at adenosine receptor subtypes are of considerable interest as novel therapeutics for a number of indications, including M. Parkinson (A_{2A}), and asthma (A_{2B}, A_3).

Although many potent and/or selective adenosine receptor ligands are known today, the syntheses described frequently allow the preparation of only milligram amounts of the compounds. In most animal models however, gram quantities of pure compounds are required.

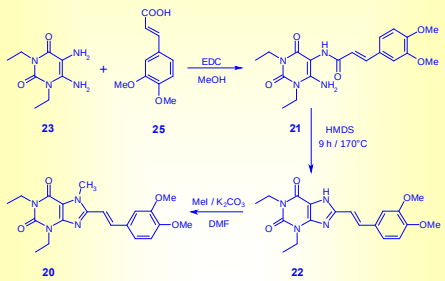
Our research focuses on the upscaling and optimisation of syntheses, in order to provide valuable tools for pharmacological *in vivo* studies.



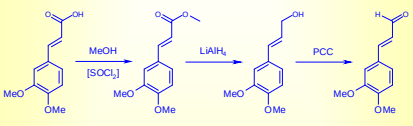
KW-6002, an A_{2A} selective adenosine receptor antagonist

8-[2(*E*)-(3,4-Dimethoxyphenyl)vinyl]-1,3-diethyl-7-methylxanthine (KW-6002, **20**) is an A_{2A} selective adenosine receptor antagonist currently undergoing phase IIb clinical trial for Parkinson´s disease and depression in the U.S. and Japan.[8]

The major drawback of the published synthesis[9] is the poor yield of the cyclisation of amide **21** to nor-KW-6002 (**22**) (13% of theoretical yield).



At first we tried to avoid amide **21** by condensing 5,6-diamino-1,3-diethyluracil (**23**) with 3,4-dimethoxycinnamic aldehyde (**24**) and then performing oxidative cyclisation with thionyl chloride (see PSB-10 section). As 3,4-dimethoxycinnamic aldehyde (**24**) is not commercially available several methods of synthesis were examined. The highest yield (57% of theoretical) was archieved by methylation of 3,4-dimethoxycinnamic acid (**25**) to the corresponding methyl ester **26**, reducing the ester to alcohol **27** with lithium aluminium hydride and subsequently oxidizing it with pyridinium chlorochromate (PCC) to the desired aldehyde **24**.



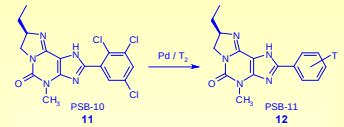
Although this synthesis could be established on a gram scale, the considerable efforts and the relatively poor yield forced us to look for other solutions.

The ring closure of amide **21** is usually performed in aqueous sodium hydroxide solution.[9] *Sauer* et al. descibed cyclisations of similar systems using HMDS at 110-120°C.[1] As under these conditions no reaction took place in our system, even after several days, harsher conditions were employed: Amide **21** was heated to 170°C with HMDS in a sealed pressure tube for 9 hours. After hydrolysis with methanol KW-6002 (**20**) could be isolated in 93% yield.

3,9 g of KW-6002 were synthesised by this method in an overall yield of 55% (40%) (calculated from 3,4-dimethoxycinnamic acid (diethyluracil)).

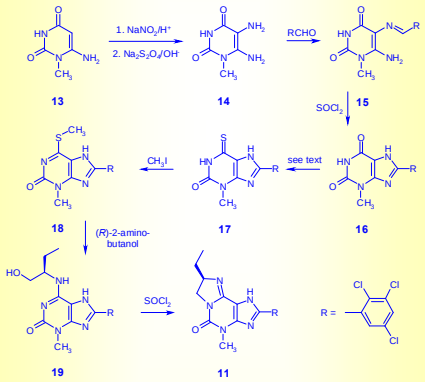
PSB-10, an A_3 selective adenosine receptor antagonist

8-Ethyl- 4-methyl- 2-(2´,3´,5´-trichlorophenyl)-(8*R*)- 4,5,7,8-tetrahydro- 1*H*-imidazo[2,1-*i*]purin-5-one (PSB-10, **11**) is both an A_3 selective adenosine receptor antagonist by itself[4] and a synthetic precursor to [^{3}H]-labelled 8-Ethyl-4-methyl-2-phenyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one (PSB-11, **12**).[5]



The classical synthesis follows the route first described by *Shimada*, *Kuroda* and *Suzuki*:[6] 1-methyl-6-aminouracil (**13**) was nitrosated and then reduced to 1-methyl-5,6-diaminouracil (**14**). Subsequently imine **15** was obtained by reaction with 2,3,5-trichlorobenzaldehyde, followed by cyclization to xanthine **16** using thionyl chloride. Compound **16** was thionated with phosphorus pentasulfide in pyridine (**17**), followed by methylation (**18**). Nucleophilic substitution with 2-aminobutanol and subsequent ring closure yielded the desired imidazo[2,1-*i*]purin-5-one (**11**).

The major drawbacks of this synthesis lie in the steps after the ring closure of the xanthine **16**. The thionation step involves toxic reagents, a tedious workup (a lot of sulfur has to be separated from the product) and tends to produce moderate yields and poor reproducibility (20 to 60% of theoretical amount). While the methylation of thioxanthine **17** presents no problems, synthesis of amidine **19** is difficult, especially separation of the product from the by-products. Purification of the final product is accomplished by column chromatography leading, again, to reduced isolated yields.



Our improved process overcomes most of these problems. In the thionation step both, reagent and solvent, were changed. Inspired by the work of *Kaneko* et al.[7] who selectivly monothionated uracil derivatives with Lawesson´s reagent both in pyridine and HMPT, we decided to try thionation of xanthine **16** with this reagent in THF, glyme (1,2-dimethoxyethane), or 1,2-diethoxyethane. Yields were high with all of these solvents (77%, 83%, and 54% respectively). Glyme was finally chosen as the best solvent, not only because of the highest archievable yield, but also because of its miscibility with water, making workup even easier: After stirring the reaction mixture (multigram scale) for 4 hours at 80°C, water and sodium hydroxide were added and sulfur was filtered off. The filtrate was acidified with hydrochloric acid and the solid product **17** was separated by filtration and dried at 110°C.

The second improvement lay in the synthesis of amidine **19** from thioiminoether **18**. Here the solvent was changed from DMSO to chloroform and the reaction temperature was lowered from 150 to 62°C. After the reaction was finished, dichloromethane was added and a solid phase extraction on silica gel was performed. This is the only chromatographic step employed in the whole synthesis. Yields typically range between 70 and 80%.

The column chromatography usually used to purify the final product after cyclization with thionyl chloride was substituted by a simple re-cristallisation from methanol / isopropanol yielding more than 80% of PSB-10 (**11**) in a very pure form.

By this procedure 6,8 g of PSB-10-HCl have been produced. The overall yield of the synthesis (determined from 2,3,5-trichlorobenzaldehyde as the most expensive reagent) was raised from 10 to 29%.

Summary

- ✓ Three selective adenosine receptor antagonists have been synthesised on a gram scale
- ✓ The syntheses have been optimised, both to increase the yields and to make them more economic
- ✓ By-products have been identified and strategies to avoid them have been developed

Literature:

[1] Sauer, R.; Maurinsh, J.; Reith, U.; Fülle, F.; Klotz, K.-N.; Müller, C. *J. Med. Chem.*, **43**, 440-448 (2000)
[2] Müller, C. *Tetrahedron Lett.*, **32**, 6539-6540 (1991)
[3] Nonaka, Y.; Shimada, J.; Nonaka, H. *J. Med. Chem.*, **36**, 3731-3733 (1993)
[4] Müller, C. *Mini Reviews in Medicinal Chemistry*, **1**, 417-427 (2001)
[5] Müller, C.; Diekmann, M.; Thorand, M.; Ozola, V. *Bioorg. & Med. Chem. Lett.*, **12**, 501-503 (2002)
[6] Shimada, K.; Kuroda, T.; Suzuki, F. *J. Heterocyclic Chem.*, **30**, 241-246 (1993)
[7] Kaneko, K.; Katayama, H.; Wakabayashi, T.; Kumonaka, T. *Synthesis*, 152-154 (1988)
[8] Kyowa Hakko, press release february 18th, 2002
[9] Rabasseda, X.; Sorbera, L.; Martín, L.; Leeson, P.; Castañer, J. *Drugs of the Future*, **26** (1), 20-24 (2001)

Pharmaceutical Sciences Bonn