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IMPROVED, EFFICIENT SYNTHESIS FOR MULTIGRAM-SCALE PRODUCTION OF PSB-10, A POTENT ANTAGONIST AT HUMAN A_3 ADENOSINE RECEPTORS

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Abstract – The reported synthesis of PSB-10 (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), a potent A_3-selective adenosine receptor antagonist, gives only moderate yields and is not suitable for the production of PSB-10 on a multi-gram scale. Attempts to develop alternative routes and an improved procedure suitable for preparing large quantities of PSB-10, required for pharmacological studies, are described.

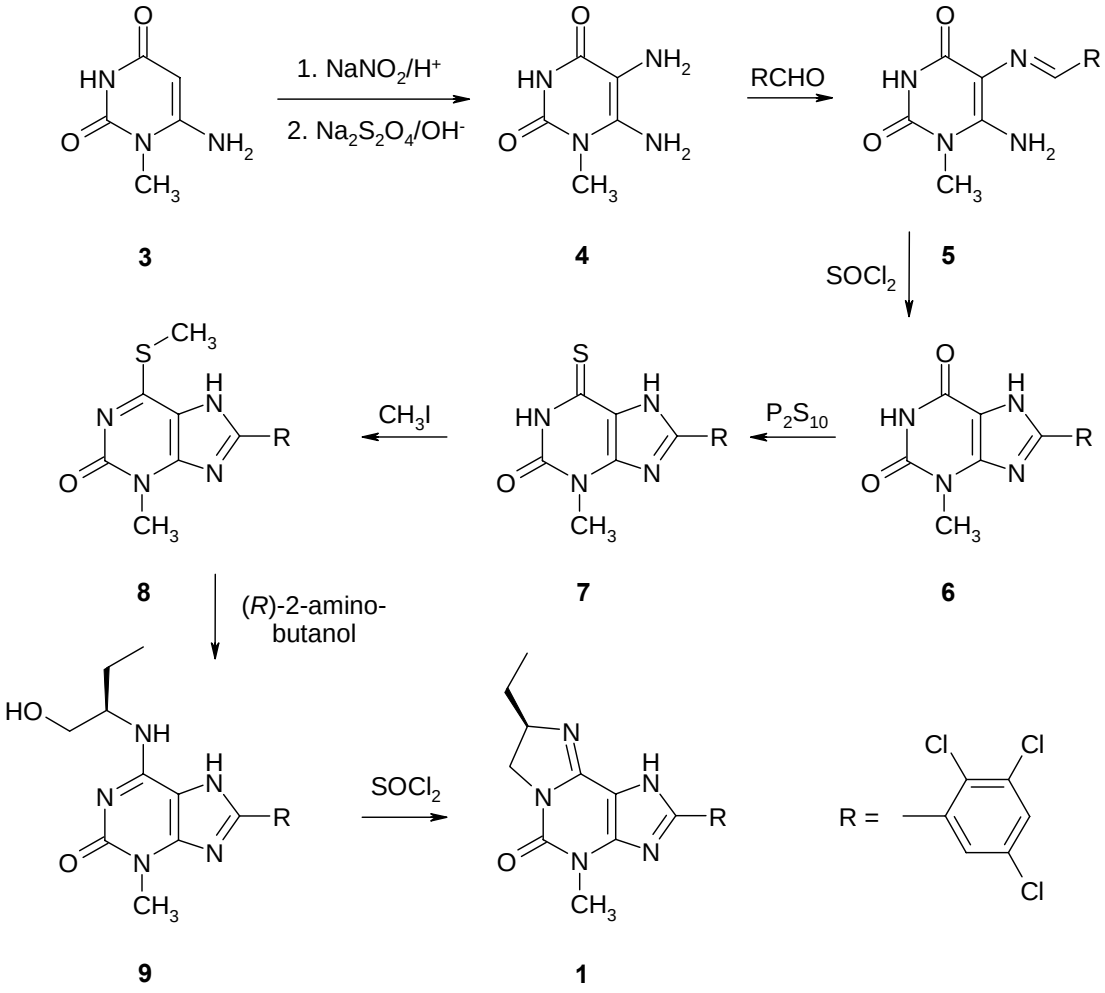
INTRODUCTION

A_3 adenosine receptors belong to the adenosine receptor family of G-protein coupled receptors, which consists of four distinct subtypes, A_1, A_{2A}, A_{2B} and A_3.[1] A_3 receptors are of considerable interest as novel drug targets.[2,3] A_3 antagonists have been proposed as novel therapeutics for the acute treatment of cerebral ischemia and stroke, and, due to their role in inflammation, as antiasthmatics, antiallergics and for the treatment of rhinitis.[4]

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 upscaling and optimization of syntheses in order to provide valuable tools for extended *in vitro* assays and particularly for *in vivo* studies.

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, **1**) is both a potent A_3 selective adenosine receptor antagonist with inverse agonistic properties,[5] and a synthetic precursor for the preparation of the [^{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 ([^{3}H]PSB-11, **2**).[6] [^{3}H]PSB-11 has proven to be a very useful pharmacological tool due to its high affinity and selectivity for human A_3 adenosine receptors, and its low degree of non-specific binding.[7~11]



Scheme 1

The published strategy for the synthesis of **1** and analogs[5,12] followed the protocol described by Shimada, Kuroda and Suzuki:[13] 1-Methyl-6-aminouracil (**3**) was nitrosated, followed by reduction to 1-methyl5,6-diaminouracil (**4**). Subsequently, imine (**5**) was obtained by reaction of **4** with 2,3,5-trichlorobenz-aldehyde, followed by cyclization to xanthine (**6**) using thionyl chloride. Compound (**6**) was thionated with phosphorus pentasulfide in pyridine (yielding **7**), followed by methylation to produce **8**. Nucleophilic substitution with 2-aminobutanol and subsequent ring closure yielded the desired imidazo[2,1-*i*]purin-5-one (**1**).[5] A similar procedure has been described for the synthesis of the structurally related A_3 adenosine receptor antagonist 2-(4-bromophenyl)-7,8-dihydro-4-propyl-1*H*-imidazo[2,1-*i*]purin-5-one (KF26777)

and derivatives thereof.[14]

The major drawbacks of this synthesis lie in the steps after the ring closure to xanthine (**6**). The thionation step involves toxic reagents (phosphorus pentasulfide, pyridine), tedious workup (large quantities of sulfur have to be separated from the product) and tends to produce moderate yields combined with poor reproducibility (20 to 60% of theoretical yield). While the methylation of thioxanthine (**7**) to yield the methylthiopurine derivative (**8**) presents no problems, the nucleophilic substitution of **8** by a sterically hindered amine (2-aminobutanol) to yield amidine (**9**) is difficult, especially with regard to product isolation since a large excess of 2-aminobutanol has to be employed and the solvent (DMSO) is difficult to remove. After ring closure of **9**, PSB-10 (**1**) is obtained, and purification of the final product (**1**) is accomplished by column chromatography, again, leading to reduced isolated yields.

RESULTS AND DISCUSSION

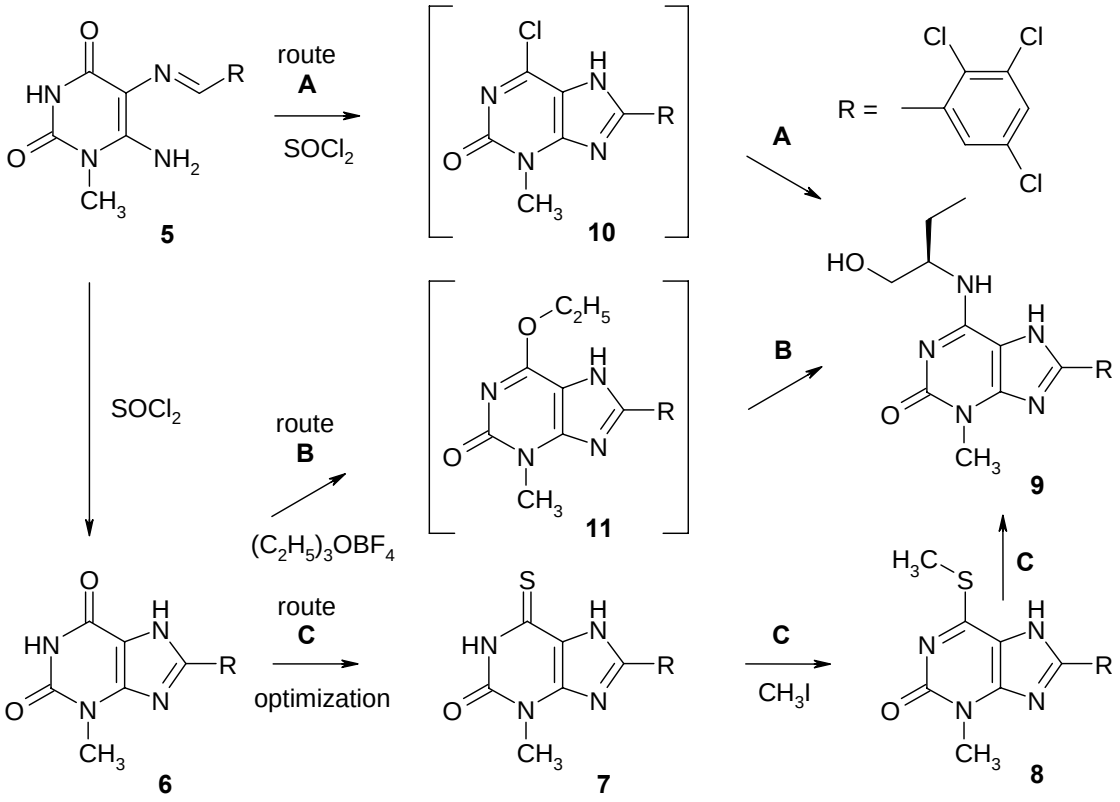
Three possible ways to overcome the described problems were examined: A) It was tried to introduce a chlorine atom at the 6-position (intermediate (**10**), Scheme 2) and to substitute it directly; B) Alternatively, an ethoxy group was to be examined as a leaving group for nucleophilic substitution (intermediate (**11**)); C) If all else failed, the classical synthesis was to be optimized.

The oxidative ring closure of imine (**5**) to xanthine (**6**) is performed in thionyl chloride as a reagent and solvent. After removing excess thionyl chloride we observed that an orange solid remained in the flask. In the usual workup, water was added at this stage, leading to the formation of a gas (presumably hydrochloric acid) and liberation of xanthine (**6**) as a pale yellow solid. As 6-chloropurines are common products in reactions of 6-hydroxypurines with thionyl chloride or phosporus pentachloride,[15] we assumed that the observed solid may be the chloroxanthine (**10**). A similar intermediate has been reported by a Japanese group in an analogous synthesis.[16]

We conducted several experiments to convert the proposed chloroxanthine (**10**) to amidine (**9**). Reaction with neat (*R*)-2-aminobutanol yielded the desired product (**9**), but only in 5 to 10 % of the theoretical yield. Experiments using *N*-methylimidazole as a solvent were more promising: Up to 20% of the theoretical yield of **9** could be isolated. Although about 20 experiments were undertaken, varying reaction temperature (from –10°C to 150°C), solvent (DMSO, diethoxyethane, ethanol, *N*-methylimidazole, triethyl amine and mixtures thereof) and workup procedures, to optimize this reaction, no better yields could be obtained and reproducibility was poor.

The next approach was to prepare the imino ether (**11**) and to convert it directly to the amidine (**9**) without prior isolation. Xanthine (**6**) was reacted with triethyloxonium fluoroborate (Meerwein´s salt)[17] in 1,2-dimethoxyethane (glyme) and was then directly converted to amidine (**9**) by the addition of (*R*)-2-

aminobutanol. A yield of 11% of amidine (**9**) and 74% of xanthine (**6**) could be isolated. Although we tried to optimize this type of reaction (e.g. by using different sovents (chloroform, dichloromethane, triethylamine), or different temperature protocols) no better conversion could be obtained.



Scheme 2

Finally, we attempted the optimization of the classical synthetic route. As the cyclization of imine (**5**) to xanthine (**6**) presented no problem, even on a multi-gram scale, our endeavor started with the thionation step replacing the previously used phosphorus pentasulfide by Lawesson's reagent (2,4-bis(4-methoxy-phenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane). Lawesson's reagent has already been used for the selective monothionation of uracil derivatives in both pyridine and hexamethylphosphoric acid triamide (HMPA) as a solvent.[18] As HMPA is highly toxic, expensive and rather inconvenient to remove due to its high boiling point, we examined several other solvents. Although initial attempts with tetrahydrofurane were already encouraging, glyme und 1,2-diethoxyethane were also tried. Yields were high with all of these solvents (77%, 83% and 54% respectively). Glyme was finally selected, not only because it gave the highest achievable yield, but also because of its miscibility with water, making workup easy: After the rection was finished, water and sodium hydroxide were added and sulfur was filtered off. The filtrate was acidified with hydrochloric acid and the solid product (**7**) could be separated by filtration. Methylation of thioxanthine (**7**) was achieved with methyl iodide.[5] The second major

improvement was the preparation of amidine (**9**) from the methylthiopurine (**8**). Here the solvent was changed from dimethyl sulfoxide[5] to chloroform and the reaction temperature was lowered from 150°C[5] to 62°C, reducing the appearance of side products and thus improving the yield. It has been difficult to separate the product from the required large excess of (*R*)-2-aminobutanol as their solubilities are very similar. When performing column chromatography of the raw product, (*R*)-2-aminobutanol will change the composition of the solvent and prevent reasonable separation. After many experiments we came up with the following procedure: After the reaction was finished, dichloromethane was added and a solid phase extraction on silica gel was performed. Dichloromethane eluted the by-products while product (**9**) could only be eluted by mixtures of dichloromethane and ethanol (see EXPERIMENTAL). Yields typically ranged from 70 to 80%. While the reaction conditions of the final cyclization remained unchanged, the workup procedure was also considerably improved. Two observations allowed this: Firstly, we discovered that the product isolated after evaporation of excess thionyl chloride was the monohydrochloride of PSB-10 (**1**) and, secondly, we found that this hydrochloride was well soluble in methanol but virtually insoluble in isopropanol. Thus the remaining solid after distilling off excess thionyl chloride was taken up in a small quantity of hot methanol. Then, isopropanol was added until the solution became slightly turbid. After leaving this solution for 12 h at room temperature, the product was filtered off. More isopropanol was added to the mother liquor and the procedure was repeated. By this method PSB-10-HCl could be isolated in a purity exceeding 99% and yields of about 90%.

CONCLUSION

The synthesis of PSB-10 (**1**) has been improved concerning yield and practicability. Although we did not succeed in altering the reaction pathway substantially, several critical steps were changed to ensure reproducibility on a multi-gram scale. The overall yield in this 8-step synthesis was raised from 10 to 29% (based on 2,3,5-trichlorobenzaldehyde).

EXPERIMENTAL

All commercially available reagents and solvents were used without further purification. (*R*)-2-Amino-butanol of high optical purity was obtained from Merck. Solid phase extraction was performed using silica gel H without calcium sulfate (Fluka). Melting points were determined on a Wepa “apotec” melting point apparatus (Höhr-Grenzhausen, Germany) and are uncorrected. IR spectra were measured by a Perkin Elmer “1600” fourier transformation IR spectrometer. ^{1}H- and ^{13}C-NMR data of the compounds have been published previously.[5]

6-Amino-1-methyl-5-(2,3,5-trichlorobenzylideneamino)-1,2,3,4-tetrahydro-2,4-pyrimidinedione (**5**) and 3-methyl-8-(2,3,5-trichlorophenyl)-3,7-dihydro-1*H*-purine-2,6-dione (**6**) were prepared as described.[5]
6-(1-Hydroxymethyl-(1*R*)-propylamino)-3-methyl-8-(2,3,5-trichlorophenyl)-3,7-dihydro-2*H*-purin-2-one (**9**).

Route A:

At –18°C *N*-methylimidazole (1.0 mL, 13 mmol) was mixed with 10 mL (130 mmol) of thionyl chloride. Imine (**5**)[5] (0.7 g, 2 mmol) was added to the light-yellow suspension and the mixture was refluxed for 10 min. Excess thionyl chloride was distilled off under reduced pressure. The orange-colored residue was suspended in 20 mL of *N*-methylimidazole. The dark green suspension turned to deep red upon the addition of (*R*)-2-aminobutanol (2.0 mL, 21 mmol). After heating to 80°C for 90 min, ice was added and the mixture was extracted with three 80 mL portions of dichloromethane. The combined organic phases were washed with water, dried over magnesium sulfate and concentrated under reduced pressure. The remaining oil was purified by solid phase extraction and column chromatography, as described for route C, yielding 0.17 g (20.4%) of amidine (**9**) as a colorless solid.

Route B:

Xanthine (**6**)[5] (0.69 g, 2.0 mmol) was suspended in 20 mL of 1,2-dimethoxyethane. After the addition of triethyloxonium fluoroborate (0.48 g, 2.5 mmol), the mixture was stirred at rt for 16 h. The resulting yellow solution was concentrated under reduced pressure to give an orange-colored oil. (*R*)-2-Aminobutanol (5.0 mL, 52 mmol) was added and the solution was heated to 140°C for 4 h. After the addition of 20 mL of water, the suspension was acidified with 2 N aqueous HCl solution and extracted with three 50 mL portions of dichloromethane. The combined organic phases were washed with water, dried over magnesium sulfate and concentrated under reduced pressure. The resulting orange-colored solid was purified by column chromatography, as described for route C, yielding 95 mg (0.23 mmol, 11%) of amidine (**9**) and 0.51 g (1.5 mmol, 74%) of xanthine (**6**).

Route C:

Xanthine (**6**)[5] (3.46 g, 10 mmol) was suspended in 100 mL of 1,2-dimethoxyethane by sonication. After the addition of 3.24 g (8.0 mmol) of Lawesson's reagent, the mixture was stirred under nitrogen for 4 h at 80°C. After the addition of 100 ml of water the mixture was brought to pH 14 by the addition of a 10% solution of sodium hydroxide. The precipitated sulfur was filtered off the red solution. The filtrate was acidified with 2 N aqueous HCl solution to pH 2 and the precipitated product was filtered off. After drying at 110°C for 4 h, 2.99 g (82.7%) of 3-methyl-6-thioxo-8-(2,3,5-trichlorophenyl)-3,7-dihydro-1*H*-

purine-2,6-dione (**7**) was obtained as a yellow solid. Mp 261°C (decomp), IR (cm^{-1}): 3389, 3171, 3042, 2964, 1684, 1603, 1561, 1373, 1214, 1119, 1027, 862, 828, 789, 744, 688, 615, 599, 585, 540.

Thioxanthine (**7**) (1.68 g, 4.65 mmol) was suspended in a mixture of 20 mL of 2% aqueous sodium hydroxide solution and 8 mL of ethanol by sonication. At 0°C 1.0 g (7.0 mmol) of methyl iodide was added. After stirring for 45 min at rt, the mixture was neutralized with 2 N aqueous HCl solution. The precipitate was filtered off and washed with water. After drying it in a desiccator over silica gel at rt, 1.64 g (93.9%) of 3-methyl-6-methylthio-8-(2,3,5-trichlorophenyl)-3,7-dihydro-2*H*-purin-2-one (**8**) was obtained as a dark yellow solid. Mp 215°C, IR (cm^{-1}): 3415, 3069, 2925, 1700, 1609, 1552, 1515. 1412, 1375, 1354, 1313, 1235, 1150, 1125, 1084, 1003, 970, 864, 830, 774, 734, 619, 551.

Compound (**8**) (1.6 g, 4.4 mmol) was suspended in 40 mL of chloroform. After the addition of 3 mL (30 mmol) of (*R*)-2-aminobutanol the mixture was refluxed for 7 h (TLC control, **9** shows an intensive blue fluorescence at 366 nm). After cooling to rt 50 mL of dichloromethane was added and the complete mixture was poured onto a chromatographic column (40 mm diameter) filled with 20 g of silica gel H, preconditioned with dichloromethane. The column was eluted with 100 mL of dichloromethane followed by 100 mL portions containing 1, 2, 5 and 10 % respectively of ethanol in dichloromethane, until the product was eluted. After evaporation of the solvent and drying at 110°C, yellow honey-like product was obtained, containing approx. 80-90% of the amidine (**9**). This product was used for the next step without further purification. For analytical purposes, a small amount of compound (**9**) was chromatographed on silica gel 60 using a solution of 2 % of isopropanol in dichloromethane as eluent. IR (cm^{-1}): 3422, 2943, 2865, 2804, 1695, 1635, 1560, 1491, 1372, 1251, 1210, 1113, 997, 745, 564.

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, **1**).

The raw amidine (**9**) (1.5 g, route C) was dissolved in 160 mL of thionyl chloride at –18°C and subsequently refluxed for 1 h. Excess thionyl chloride was distilled off under reduced pressure. The residue was taken up in 30 mL of hot methanol. Isopropanol was added until the solution became turbid. After leaving this solution for 12 h at rt, the product was filtered off. PSB-10 (**1**, 0.64 g, 1.5 mmol) was obtained as fine colorless needles. More isopropanol was added to the mother liquor and the procedure was repeated, yielding another 0.53 g of PSB-10 (**1**). The overall yield for the last two steps was 61.4%.

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