Novel antagonists for the human adenosine A2A and A3 receptor via purine nitration: synthesis and biological evaluation of C2-substituted 6-trifluoromethylpurines and 1-deazapurines
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Synthesis of 8-substituted 9-methyl-2-phenethylamino-6-trifluoromethylpurines

ABSTRACT

In this chapter the successful synthesis of tetra substituted purines is presented, including one of the target compounds with selected substituents at C-2, C-6, C-8 and N-9.

2-Phenethylamino-substituted 9-methyl-6-trifluoromethylpurine was converted into the corresponding 8-halopurine. The 8-halopurine reacted via nucleophilic aromatic substitution with amines to give 8-alkylamino-substituted analogs. In addition, the use of palladium catalysed reactions was very successful to introduce carbon-carbon bonds at C-8. (Hetero)-aryl groups and styryl derivatives were introduced using palladium chemistry via Suzuki and Stille coupling reactions. Sonogashira coupling procedures were applied to introduce alkynes at C-8.
4.1 INTRODUCTION

The introduction of substituents at position C-8 of adenosine derivatives and purines is commonly used to influence base pairing properties in DNA/DNA recognition, or for improvement of compounds as building blocks for synthetic oligonucleotides. Other applications are found in cytostatic or antiviral agents. Gudmundsson published a series of 8 substituted carbocyclic analogs with activity against hepatitis B virus.1 C-8 substituted adenosine derivatives are used to enhance affinity for specific adenosine receptors. Depending on the purpose, clear focus should be on activity and selectivity for subtypes. Big differences in affinity for receptor subtypes will increase selectivity, and thus functional agonism/antagonism. This paradox leads to the idea that to design highly selective and active ligands on a specific subtype, in general the activity on other receptor subtypes should be low. A large amount of research was allocated to adenosine receptors. Previously Bruns2, Jacobson3 and Olsson4 concluded that substitution at C8 carbon often leads to inactive compounds on adenosine receptor subtypes. It was suggested that such substituents force the nucleoside into the syn conformation, whereas the anti conformation is thought to be essential for receptor binding. Van der Wenden et al. described the synthesis of some C-8 substituted adenosines with modest activity on adenosine receptors5. Later, IJzerman et al. reported the synthesis of N-6 - C-8-disubstituted adenosines, in anti conformation, with enhanced affinity for the A1 receptor as exemplified in figure 4.1.6 Recently Cristalli reported a new class of 8-substituted 9-ethyladenines with high activity on the adenosine A2A receptor.7 Starting from 8-bromo-9-ethyladenine8 the A2A binding affinity and selectivity against other adenosine subtypes was improved by replacing the 8-bromine atom by an ethoxy or a furyl substituent, to form the compounds 8-ethoxy-9-ethyladenine and 8-furyl-9-ethyladenine III.9 These two compounds showed higher selectivity and affinity for the adenosine A2A receptor than the original 8-bromo-compound II.
Synthesis of 8-substituted 2-phenethylamino-6-trifluoromethyl-9-methylpurines

Figure 4.1 8-substituted adenosines and adenines

The classical synthetic schemes to achieve preparation of 8-substituted purines are based on heterocyclisation, where 5,6-diaminopyrimidines are reacted with carboxylic acid derivatives (see Scheme 4.1).10,11

Scheme 4.1. Classical retro synthetic synthesis of 8-substituted purines

The cyclisation strategies usually involve multistep procedures, resulting in moderate overall yields. When multiple functionalisations (C2/C-6/C-8) are desired, the introduction of new substituents often requires changes in the whole reaction scheme from the start.

Barton et al. reported the alkylation on 8-lithiated adenosines via hydrogen-lithium exchange,12 a procedure which was later improved by Miyasaka.13 Recent approaches are based on reactions with halopurines. In this way several aminoalkyl, aryl, or alkyl substituted purines have been prepared. The advances in organometallic chemistry and its applications in organic synthesis are enormous. Low temperature lithiation (LDA or BuLi) of purines is a general approach to 8-substituted purines. 8-lithiopurines nucleosides can be transformed into 8-carbon-substituted nucleosides on treatment with electrophiles.14 Cross coupling reactions are also widely used for the construction of C-C bonds. Treatment of purines bearing suitable leaving groups like halogens or tosylates with diverse types of organometallic compounds based on Mg, Cu, Al, Zn, Sn and B are
extensively examined for purine functionalization. Palladium catalyzed cross-coupling are powerful methods for modifications of nucleosides and will be applied in the next paragraphs. Several efficient approaches are described for the substitution of C-2 and C-6 halopurines. However, a limited number of examples of palladium-catalyzed syntheses for obtaining 8-aryl purine nucleosides are reported.

This chapter describes the introduction of halogens at C-8 on selected substrates and strategies towards various 8-functionalized 2-amino-6-trifluoromethylpurines according to Scheme 4.2.

**Scheme 4.2** Retro synthetic scheme for 8-substituted 6-trifluoromethyl-purines via halogenation

### 4.2 8-HALOPURINE VARIANTS OF 6-TRIFLUOROMETHYLPURINES

8-Iodo- and 8-bromopurines and adenosines can be prepared in various ways. Several procedures have been reported. However, the results strongly depend on the substituents present at the purine skeleton. The 8-bromo adenine compound in Figure 4.1 was prepared via bromination with N-bromosuccinimide. This reagent is commonly used for free-radical bromination by slowly releasing Br₂. In analogy, N-iodosuccinimide is used in iodination reactions.

Another classical method is subjecting the nucleoside to molecular bromine with sodium acetate dissolved in glacial acetic acid as described by Holmes and Robins in 1963. Optimization of this procedure resulted in procedures using aqueous bromine.

Miyasaka et al. reported the use of lithium bases to introduce carbon electrophiles. By generating an anion at C-8 of protected 6-chloropurine ribosides, electrophiles could be introduced in the purine ring. They used lithium diisopropylamide (LDA) to lithiate
exclusively the 8-position by carrying out the reaction at -70°C. Quenching the anion with iodine yielded the 8-iodo derivative in excellent yields.\textsuperscript{18}

We employed these procedures to attempt introduction of halogens in trisubstituted purine \textbf{1}. The results are presented in Table 4.1 and 4.2.

**Scheme 4.3** Halogenation of C-8  a) conditions: see Table 4.1 and 4.2

**Table 4.1:** Halogenating procedures to obtain 8-bromopurine \textbf{2}

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS (1.6 equiv.) DCE, 19h</td>
<td>80</td>
<td>No reaction</td>
</tr>
<tr>
<td>NBS (1.6 equiv.) THF, 60h</td>
<td>20</td>
<td>No reaction</td>
</tr>
<tr>
<td>NaOOCCH\textsubscript{3} (32), Br\textsubscript{2} (1.2 equiv.), acetic acid</td>
<td>25</td>
<td>No reaction</td>
</tr>
<tr>
<td>1) BuLi , THF 30min 2) CBr\textsubscript{4} (2.0 equiv.) 30m</td>
<td>-72</td>
<td>36</td>
</tr>
<tr>
<td>1) BuLi , THF 20 m 2) Br\textsubscript{2} (1.6 equiv.) 20 m</td>
<td>-72</td>
<td>88</td>
</tr>
</tbody>
</table>

**Table 4.2:** Halogenating procedures to obtain 8-iodopurine \textbf{3}

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIS (1.6) DCE, 19h</td>
<td>80</td>
<td>No reaction</td>
</tr>
<tr>
<td>1) LiHMDS THF 30 m, 2) I\textsubscript{2} (1.2 equiv.) 10 m</td>
<td>-72</td>
<td>No reaction</td>
</tr>
<tr>
<td>1) LDA THF 45 m, 2) I\textsubscript{2} (1.2 equiv.) 1h</td>
<td>-72</td>
<td>43</td>
</tr>
<tr>
<td>1) BuLi THF 30 m 2) I\textsubscript{2} (1.2 equiv.) 10 m</td>
<td>-72</td>
<td>72</td>
</tr>
<tr>
<td>1) BuLi THF 10 m 2) I\textsubscript{2} (1.6 equiv.) 10 m</td>
<td>-72</td>
<td>91</td>
</tr>
</tbody>
</table>
In our compound, no reaction took place when treating purine 1 with NBS or NIS at elevated temperatures. It was not clear whether the reaction was not initiated by NBS or the 8-position of the substituted purine was not available for a radical reaction or the lifetime of the purinyl radical was too short. Also the treatment of 1 with bromine in acetic acid did not result in product formation. Only starting material was recovered. Inspired by the results of Miyasaka, we tried to generate the di-anion at N-H and C-8 of 1 with LDA to induce a nucleophilic attack of the purine anion on bromine. However, again no reaction was observed. It is well known that this type of reaction is very moist sensitive. Purine 1 was previously purified by crystallisation from water and thoroughly dried at 60°C in vacuo. To check if formation of the di-anion was still obstructed by traces of moist we applied a quenching experiment using NMR. After lithiation we quenched the compound by adding CH₃OD as a deuterium source to deuterate the purine di-anion at the 8-position. NMR experiments demonstrated that no C-8 deuterium was incorporated. This implied that the desired di-anion was not formed. The drying step was clearly insufficient, so we dried the flask and the compound 1 thoroughly at 100°C in vacuo for at least 2.5 hours prior to lithiation. Indeed, now halogenation was successful. We studied the effect of butyllithium on the formation of the di anion. A clear change in colour (colourless → green → yellow) indicated that the formation of the di-anion proceeded within one minute. Quenching the di-anion with bromine or iodine gave product 2 and 3 respectively in high yields (91% and 88%). The deprotonated amine NH was re-protonated during workup procedures. To avoid the use of bromine we tried to quench the lithiated purine with CBr₄. Tetrabromomethane is reported by Boga and co-workers to be a useful “positive halogen ion” donor for performing simple, efficient and regiospecific bromination of heterocycles. Product 2 was actually formed, but the yield (36%) was significantly lower than in the procedure via bromine quenching.

Finally, we prepared 2 and 3 in good yield (88-91%) and high purity as fluffy white powders after lyophilisation. These compounds were easy to handle and good starting material for various substitution reactions at the 8-position.

### 4.3 8-ALKYLAMINO PURINES VIA NUCLEOPHILIC SUBSTITUTION

The 8-iodo derivative 3 gives access to a variety of 8-alkylaminopurines, which can be synthesised by nucleophilic displacement of the halogen with amines (Scheme 4.4). We chose
a small set of secondary cyclic (hetero-)alkyl amines and one primary amine. The aromatic nucleophilic substitution was performed by heating the substrates with (liquid) amines at 90°C without other solvents. The results are given in Table 4.3.

Scheme 4.4  Synthesis of 8-aminoalkylpurines via direct S_NAr. a) conditions: see Table 4.3

Table 4.3: Conditions to obtain 8-amino substituted trifluoromethylpurine derivatives

<table>
<thead>
<tr>
<th>Product</th>
<th>Amine</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Purity (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td>17</td>
<td>90</td>
<td>99</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>17</td>
<td>90</td>
<td>&gt;95</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>17</td>
<td>90</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>42</td>
<td>90</td>
<td>95</td>
<td>75</td>
</tr>
</tbody>
</table>

As expected, the more nucleophilic secondary cyclic amines reacted fast compared to the primary cyclopentylamine. The yield of the amination with pyrrolidine was lower because problems arose during work-up. We observed that the product was contaminated with the starting amine applied and with impurities in the amine which were eluted along with the product. Since we experienced some difficulties in separating the amines from the product, all the products were eluted over a solid phase extraction (SPE) column resulting in higher purity.
4.4 8-ALKYLPURINES VIA PALLADIUM CATALYSIS

As discussed earlier, palladium catalysis is one of the most powerful methods to form C-C bonds. We applied several types of palladium catalysed reactions to form new 8-alkylsubstituted products: Sonogashira, Stille and Suzuki-Miyaura coupling reactions.

4.4.1 SONOGASHIRA REACTION

The Sonogashira reaction is extensively used to prepare 2, 6 or 8-alkynyladenosines. Haloadenosines react with terminal alkynes under Pd catalysis to give the corresponding alkynyl derivatives in good yields and high functional group tolerance.20

We prepared compound 8 in excellent yield (96%) by stirring the starting material with phenylacetylene, CuI and triethylamine as a base under an argon atmosphere at room temperature for 2 hours. Recently, acetylene moieties are getting attention in click chemistry via functionalized azides to yield adenosine A₃ receptor ligands.21

\[ 
\begin{align*}
\text{Scheme 4.5} & \quad \text{Sonogashira coupling a) phenylacetylene, Pd(PPh₃)₄, CuI, DMF, Et₃N} \\
\end{align*}
\]

4.4.2 STILLE COUPLING

The Stille coupling is a very efficient C-C bond forming reaction between organostannanes and halogenated substrates. This reaction has been widely applied to purines. Mostly used is the tributyl stannane derivative in combination with tetrakis(triphenylphosphine)palladium(0) and/or an inorganic salt like CuI for better conversions. A general drawback of this reaction is the toxicity and environmental pollution of the organostannanes, which is however not a serious problem on laboratory scale. Our conversions to compounds 9, 10 and 11 as shown in Scheme 4.6 were achieved in 95%, 95%, 98% yields, respectively.
4.4.3 SUZUKI-MIYaura CROSS COUPLING

Unlike the Stille reaction, the Suzuki-Miyaura reaction has found very limited application to substitute purines, until, in the late 90’s, Cristalli and coworkers presented the synthesis of 2-alkenyladenosines from alkenylboronates. Then Hocek showed the cross coupling between boronic acids and 6-halopurines using potassium carbonate and tetrakis(triphenylphosphine)palladium(0) as a catalyst. Later Lakshman reported a modified procedure using palladium acetate and the ligand 2-(dicyclohexylphosphanyl)biphenyl, which broadened the scope even further. Boronic acids present several advantages over all the other organometallic derivatives. First of all, they have low toxicity, especially compared to stannous-containing compounds, and tolerate the presence of some unprotected functional groups. Moreover, boronic derivatives are easy to handle and nowadays a variety of boronic acids with different substituents is commercially available and inexpensive. Recently, we published a RuPhos-ligand-mediated Suzuki cross-coupling between (hetero)aryl bromides and secondary alkyltrifluoroborates which may be used in production of secondary-alkylated (hetero)aryl derivatives.

The mechanism of the Suzuki-Miyaura reaction is commonly depicted as a general catalytic cycle involving oxidative addition-transmetalation-reductive elimination sequences (Scheme
4.7). The oxidative addition is often the rate-limiting step and the relative reactivity of aryl-halides decreases in the order $I > Br > Cl$.

![Catalytic cycle of the Suzuki-Miyaura cross-coupling reaction](image)

Scheme 4.7 Catalytic cycle of the Suzuki-Miyaura cross-coupling reaction

In Scheme 4.7 the boronic acid is depicted as a neutral compound. However, it is assumed that not the neutral boronic acid, but the hydroxylated anion $RB(OH)_3^-$ is the reactive species. The neutral boronic acid is apparently activated by water and base.

Literature showed that $Pd(PPh_3)_4$ was a superior catalyst compared to $Pd(dba)_2/P(o-tol)_3$, $Pd(dba)_2/AsPh_3$ or $PdCl_2(PPh_3)_2$. ($dba =$ dibenzylidene acetone). Furthermore, $K_2CO_3$ turned out to be superior to other bases; $Na_2CO_3$, $Cs_2CO_3$. DiPEA and NaOMe did not give any reaction at all.
Scheme 4.8  Literature example of a selective Suzuki-Miyaura cross-coupling reaction with a dihalopurine derivative a) PhB(OH)$_2$, 1 equiv., Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene 100°C, 81%

If the oxidative addition is the rate limiting step, the 8-iodo purine 3 is probably the most reactive halopurine, as the relative reactivity of aryl-halides in insertion reactions decreases in the order I > Br > Cl. Therefore we examined different reaction conditions with compound 3 and 2-furanboronic acid as reactant aiming at biological interesting molecules. Using the optimal conditions from literature (with potassium carbonate in toluene), disappointing results were obtained. We studied effects of solvents (THF, DME/water, toluene), bases (potassium carbonate, dipea, potassium fluoride and sodium carbonate) and we varied the reaction temperature. 2-Furanboronic acid appeared to be quite unreactive and even if treated with K$_2$CO$_3$ in DME/H$_2$O at 90°C no product was formed. This is probably due to the fact that the leaving iodide anion is strongly complexed to palladium, thus activation of the 2-furanboronic acid is really necessary to induce transmetallation.

Formation of a purine dimer during the reaction (see Figure 4.2), proved that oxidative insertion took place, but transmetallation failed. This suggests a competition between the iodopurine 3 (Scheme 4.3) and the boronic acid towards the iodo-Pd-purine complex. The purine dimer is formed when the boronic acid is not sufficiently activated to compete with the 8-iodo-6-trifluoromethylpurine.
In Scheme 4.9, a very preliminary formation mechanism is proposed. Two oxidative additions are followed by elimination of the two purines resulting in the formation of the dimer and the remaining PdI₂. By the elimination of the iodide ligands the catalytic cycle is completed and the Pd is ready for another cycle. This is an assumption based on a comparable mechanism, described by Moreno-Mañas et al.²⁶

Because of the problems observed in attempts to introduce the 2-furanyl moiety at the C₈ position, we examined analogous reaction conditions with other boronic acids, like phenylboronic acid. Fortunately, we could isolate 8-phenyl substituted purine after treating the purine 3 with phenylboronic acid and Na₂CO₃ in THF. After some optimisation we obtained 11 in high yield (Scheme 4.10 and Table 4.4). It proved to be necessary to add at least 3 equivalents of base and 1.5 equivalents of boronic acid and to carry out the reaction under completely oxygen-free atmosphere. DME/H₂O (8:1) was used as solvent. These
conditions were successful to obtain compounds 9 - 14 in high yields. Both aryl-substituted and vinylogous (E) styryl products were synthesized. The 8-furyl derivative was eventually obtained similarly with double amounts of boronic acid in a twice diluted solution to prevent dimerisation.

Scheme 4.10  Suzuki coupling a) boronic acid, Pd(PPh₃)₄, Na₂CO₃, DME/water, 90°C

Table 4.4: Optimised reaction conditions to introduce C8-substituents at 6-trifluoromethylpurines

<table>
<thead>
<tr>
<th>Product</th>
<th>Substituent R</th>
<th>Na₂CO₃ (equiv.)</th>
<th>Time (h)</th>
<th>T (°C)</th>
<th>Solvent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>O</td>
<td>10.0</td>
<td>20</td>
<td>90</td>
<td>DME/H₂O (8:1)</td>
<td>87</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>4.0</td>
<td>22</td>
<td>90</td>
<td>DME/H₂O (8:1)</td>
<td>55</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>4.0</td>
<td>17</td>
<td>90</td>
<td>DME/H₂O (8:1)</td>
<td>93</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>4.0</td>
<td>17</td>
<td>90</td>
<td>DME/H₂O (8:1)</td>
<td>88</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>3.0</td>
<td>17</td>
<td>90</td>
<td>DME/H₂O (8:1)</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>CF₃</td>
<td>3.0</td>
<td>17</td>
<td>90</td>
<td>DME/H₂O (8:1)</td>
<td>100</td>
</tr>
</tbody>
</table>
LIGHT INDUCED ISOMERISATION OF STYRYL DERIVATIVES

Some styryl derivatives are known to be light sensitive. This makes them also interesting for use in photovoltaic cells. The two synthesised purine E styryl derivatives 13 and 14 likewise proved to be photo-labile. After 6 hours, 13 was isomerised to its Z isomer 15 by TL-light in the hood >80%, as calculated by $^1$H-NMR measurements. Finally, all E-isomer was converted to Z-isomer (Scheme 4.11). The 4-CF$_3$ analogue 14 isomerised even faster to its Z isomer 16 (~1 h), probably enhanced by trifluoromethyl electron resonance modulating effect.

![Scheme 4.11 Photo-isomerisation of purine 8-styryl derivatives 13 and 14](image)

This isomerisation process may cause problems in tests for biological activity. Without specific precautions compounds are subjected to light and therefore partial isomerisation of E-styryl derivatives takes place. In this way the activity of a mixture of Z and E isomers may be observed. The thermodynamic stable Z-isomers can be tested as such.

4.5 CONCLUDING REMARKS

In summary, it was possible to introduce various substituents at C-8. Iodination and bromination were achieved via lithiation. Further functionalization was efficiently achieved with amines by nucleophilic substitution. Palladium catalysis proved to be very efficient in C-C bond formation. A small series of C-8 substituted 6-trifluoroalkyl-2-phenethylamino purines was prepared. We demonstrated that the synthesis of the 8-furyl target compound could be realised. Overall, new classes of tetrasubstituted purine compounds are formed with a trifluoromethyl group at C-6 and freely chosen substituents at C-2, C-8 and N-9.
4.6 ACKNOWLEDGEMENTS

Remko Detz is gratefully acknowledged for his work at the Suzuki reaction. Adri van den Hoogenband of Solvay Pharmaceuticals is kindly acknowledged for encouraging conversations in homogeneous cross coupling chemistry.

4.7 EXPERIMENTAL

General information.

For experimental details see Chapter 2.

8-Bromo-9-methyl-2-phenethylamino-6-trifluoromethylpurine 2

The procedure similar described for the preparation of 3 (HPLC: $R_{f,\text{product}} = 4.6$). After workup of the reaction mixture, silica gel column chromatography (eluent: 30 mL MTBE, 20 mL 2% MeOH in MTBE) gave, after evaporation, compound 2 (110 mg, 88%) as a white solid (after freeze-drying: fluffy white crystals), mp 131-139°C. $^1$H-NMR (400MHz, CDCl$_3$), $\delta$ [ppm]: 7.33-7.21 (m, 5H, H$_{ar}$), 5.91 (br s, 1H, NH), 3.93 (br m, 2H, NCH$_2$), 3.80 (s, 3H, CH$_3$), 2.98 (t, $J = 7.1$ Hz, 2H, CH$_2$). $^{19}$F-NMR (500MHz, CDCl$_3$), $\delta$ [ppm]: -70.18 (6-CF$_3$).

8-Iodo-9-methyl-2-phenethylamino-6-trifluoromethylpurine 3

Compound 1 (0.500 g, 1.56 mmol) was dried in vacuo at 100°C for 2.5 h. After cooling under a positive pressure of dry N$_2$, dry THF (18 mL) was added at room temperature and the solution was cooled further to $-72^\circ$C (ethanol/dry ice). After addition of n-BuLi (2.4 mL of a 1.6 M solution in hexane, 3.9 mmol), while stirring at $-72^\circ$C, the colourless solution turned yellow. After 15 minutes I$_2$ (0.633 g, 2.49 mmol) was added and after 5 minutes the reaction was completed (HPLC: $R_{f,\text{product}} = 4.7$). To the red solution silica gel (~4 g) was added and the whole was evaporated to dryness. Silica gel column chromatography ($\Phi = 4$ cm, eluent: 200 mL MTBE, 200 mL 1% MeOH in MTBE) gave, after freeze-drying (CH$_3$CN/H$_2$O 6:1), product 3 (0.637 g, 91%) as fluffy white crystals, mp 139-142°C. $^1$H-NMR (400MHz, CDCl$_3$), $\delta$ [ppm]: 7.33-7.21 (m, 5H, H$_{ar}$), 5.96 (br s, 1H, NH), 3.93 (br m, 2H, NHCH$_2$), 3.77 (s, 3H, CH$_3$), 2.98 (t, $J = 7.1$ Hz, 2H, CH$_2$). $^{19}$F-NMR (500MHz, CDCl$_3$), $\delta$ [ppm]: -70.12 (6-CF$_3$).
9-methyl-2-phenethylamino-8-N-pyrrolidinyl-6-trifluoromethylpurine 4

Compound 3 (10 mg, 0.022 mmol) was dissolved in pyrrolidine (2 ml, 24 mmol) and stirred at 90°C under dry N₂. The progress of the reaction was followed with HPLC (Rₜ,product = 4.3) and TLC (Rₕ,product = 0.55 in MTBE with 2% MeOH). After 17 hours the solution was diluted with DCM (4 ml) and evaporated to dryness with silica gel. Flash chromatography (Ø = 1.2 cm, EA/PE 1:2, gradient EA) gave, after evaporation, compound 4 (4.8 mg, 56%) as a white solid.

\[ ^1\text{H-NMR} (400 MHz, CDCl₃), \delta [ppm]: 7.32-7.19 (m, 5H, Hₐr), ~5.6 (br s, 1H, NH), 3.90 (q, J = 6.9 Hz, 2H, NCH₂), 3.77 (s, 3H, CH₃), 3.69 (br m, 4H), 2.99 (t, J = 7.4 Hz, 2H, CH₂), 2.04 (br m, 4H). \]

9-methyl-8-N-morpholinyl-2-phenethylamino-6-trifluoromethylpurine 5

This compound was prepared from compound 3 (10 mg, 0.022 mmol) and morpholine (2 mL, 23 mmol) by the procedure described for the preparation of compound 6. HPLC: Rₕ,product = 4.4 and TLC: Rₕ,product = 0.49 (MTBE with 2% MeOH). Flash chromatography (Ø = 1.2 cm, EA/PE 2:1) gave, after evaporation, compound 17 (8.3 mg, 90%) as an orange solid.

\[ ^1\text{H-NMR} (400 MHz, CDCl₃), \delta [ppm]: 7.33-7.21 (m, 5H, Hₐr), ~5.8 (br s, 1H, NH), 3.94 (br m, 2H, NHCH₂), 3.88 (t, J = 4.7 Hz, 4H, H-11 and H-15), 3.67 (s, 3H, CH₃), 3.30 (t, J = 4.7 Hz, 4H), 2.99 (t, J = 7.3 Hz, 2H, CH₂). \]

9-methyl-2-phenethylamino-8-N-piperidinyl-6-trifluoromethylpurine 6

Compound 3 (10 mg, 0.022 mmol) was dissolved in piperidine (2 ml, 24 mmol) and stirred at 90°C under dry N₂. The progress of the reaction was followed by HPLC (Rₜ,product = 5.1). After 17 hours the solution was diluted with DCM (4 ml) and evaporated with silica gel to dryness. Silica gel column chromatography (Ø = 1.2 cm, EA/PE 1:1) gave, after evaporation, compound 6 (9 mg, 99%) as a yellow white solid.

\[ ^1\text{H-NMR} (400 MHz, CDCl₃), \delta [ppm]: 7.33-7.20 (m, 5H, Hₐr), ~5.8 (br s, 1H, NH), 3.92 (q, J = 6.2 Hz, 2H, NHCH₂), 3.64 (s, 3H, CH₃), 3.26 (br m, 4H), 2.99 (t, J = 7.4 Hz, 2H, CH₂), 1.79-1.73 (br m, 4H), 1.69-1.67 (br m, 2H). \]
8-cyclopentylamino-9-methyl-2-phenethylamino-6-trifluoromethylpurine 7

Compound 3 (10 mg, 0.022 mmol) was dissolved in cyclopentylamine (2 ml, 24 mmol) and stirred at 90°C under dry N₂. HPLC: $R_t$ product = 4.5 and TLC: $R_f$ product = 0.29 (EA/PE 1:2). After 42 hours the reaction mixture was purified via flash chromatography ($\Theta$ = 1.2 cm, EA/PE 1:2) and SPE (Supelco, packed with 1 g silica gel) (eluent: DCM with 2% MeOH, 15 ml) to afford compound 7 (6.7 mg, 75%). ¹H-NMR (400MHz, CDCl₃), $\delta$ [ppm]: 7.32-7.20 (m, 5H, Har), ~5.7 (br s, 1H, NH), 4.30-4.25 (m, 1H), ~4.2 (br s, 1H), 3.91 (m, 2H, NHC₂H₂), 3.53 (s, 3H, CH₃), 2.99 (t, $J$ = 7.4 Hz, 2H, CH₂), 2.16-2.12 (m, 2H, cyclopentyl), 1.79-1.65 (m, 4H, cyclopentyl), 1.57-1.54 (m, 2H, cyclopentyl).

2-phenethylamino-8-phenylethynylene-9-methyl-6-trifluoromethylpurine 8

Compound 3 (60 mg, 0.134 mmol), Pd(PPh₃)₄ (15 mg, 0.013 mmol), Cu (I) (6 mg, 0.033 mmol) and phenylacetylene (0.026 ml, 0.234 mmol) were stirred in dry DMF (2 ml). Et₃N (0.023 ml, 0.168 mmol) was added and the mixture was stirred under Ar. After 2 hours the reaction mixture was diluted with ether (10 ml) and water (10 ml). The water layer was extracted three times with ether (10 ml). The organic layers were washed with water (10 ml) and a saturated NaCl solution in water (10 ml). The organic layer was dried with Na₂SO₄. Flash chromatography ($\Theta$ = 1.2 cm, EA/PE 1:3) afforded compound 8 (54 mg, 96%) as a solid. ¹H-NMR (400MHz, CDCl₃), $\delta$ [ppm]: 7.65 (d, 2H), 7.47-7.40 (m, 3H), 7.33 -7.22 (m, 5H, Har), 6.12 (br s, 1H, NH), 3.94 (br m, 2H, NHC₂H₂), 3.92 (s, 3H, CH₃), 3.01 (t, 2H, CH₂). MS: m/z 422.1581 (M⁺ + H. C₂₃H₁₈N₅F₃ requires 422.1514).

8-(2'-furyl)-9-methyl-2-phenethylamino 6-trifluoromethylpurine 9

This compound was prepared from compound 3 (20 mg, 0.045 mmol), 2-furanboronic acid (25 mg, 0.224 mmol), Pd(PPh₃)₄ (13 μg, 0.011 mmol) and Na₂CO₃ (aq, 0.447 mmol) in DME/H₂O (8:1) (4.5 ml) by the procedure described for the preparation of compound 11. After completion (HPLC: $R_t$ product = 4.8; TLC (EA/PE 1:3): $R_f$ product = 0.09) the workup of the
reaction mixture followed. Silica gel column chromatography (PE/EA (2:1), 120 ml) gave compound 9 (15 mg, 87%) after evaporation as a white solid. $^1$H-NMR (500MHz, CDCl$_3$), $\delta$ [ppm]: 7.68 (s, 1H), 7.35-7.25 (m, 5H, H$_{ar}$), 7.16 (s, 1H), 6.65 (s, 1H), 6.12 (br s, 1H, NH), 4.08 (s, 3H, CH$_3$), 3.98 (br m, 2H, NHCH$_2$), 3.03 (t, $J = 7.0$ Hz, 2H, CH$_2$). $^{19}$F-NMR (500MHz, CDCl$_3$), $\delta$ [ppm]: -69.96 (6-CF$_3$). $^{13}$C-NMR (500MHz, CDCl$_3$) $\delta$ [ppm]: 154.19, 150.64 (q, $J = 38$ Hz), 150.12, 144.75, 144.2, 143.13, 138.67, 128.83 (2C), 128.59 (2C), 126.50, 120.05 (q, $J = 275$ Hz, CF$_3$), 119.72, 113.06, 112.16, 42.15, 35.73, 30.81. MS: m/z 388.1381 (M$^+$ + H. C$_{19}$H$_{17}$ON$_5$F$_3$ requires 388.1385).

9-methyl-2-phenethylamino-8-thienyl-6-trifluoromethylpurine 10

This compound was prepared from compound 3 (20 mg, 0.045 mmol), 2-thiopheneboronic acid (12 mg, 0.089 mmol), Pd(PPh$_3$)$_4$ (13 mg, 0.011 mmol) and Na$_2$CO$_3$ (51 mg, 0.179 mmol) in DME/H$_2$O (6:1) (2.0 mL) by the procedure described for the preparation of 11 to give compound 10 (11 mg, 55%) as a yellow solid.

$^1$H-NMR (400MHz, CDCl$_3$), $\delta$ [ppm]: 7.62 (d, 1H), 7.55 (d, $J = 0.7$ Hz, 1H), 7.31-7.20 (m, 5H$_{ar}$), 6.13 (br s, 1H, NH), 4.02 (s, 3H, CH$_3$), 3.94 (br m, 2H, NHCH$_2$), 3.02 (t, $J = 7.3$ Hz, 2H, CH$_2$).

9-methyl-2-phenethylamino-8-phenyl-6-trifluoromethylpurine 11

8-Iodo-6-trifluoromethyl-9-methyl-2-phenethylaminopurine (compound 3, 40 mg, 0.089 mmol), Pd(PPh$_3$)$_4$ (26 mg, 0.022 mmol) and phenylboronic acid (22 mg, 0.179 mmol) were dissolved in DME (4 ml) and after addition of Na$_2$CO$_3$·10H$_2$O (102 mg, 0.358 mmol) in H$_2$O (0.5 ml) the solution was stirred at 90°C under Ar. The progress of the reaction was followed by HPLC ($R_{t,product} = 5.0$). After 17 hours the mixture was diluted with EA (10 ml) and water (5 ml). The water layer was extracted two times with EA (7 ml). The organic layers were washed with water (10 ml) and a saturated NaCl solution in water (10 ml). The organic layer was dried with Na$_2$SO$_4$. Flash chromatography ($\Phi = 1.2$ cm, EA/PE 1:3) afforded compound 11 (33 mg, 93%) as a light brown solid. UV (EA) $\lambda_{max} = 304$ nm ($\varepsilon = 1.5x10^4$). $^1$H-NMR (400MHz, CDCl$_3$), $\delta$ [ppm]: 7.75 (d, $J = 3.1$ Hz, 2H), 7.56-7.53 (m, 3H), 7.32-7.22 (m, 5H, 

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Synthesis of 8-substituted 2-phenethylamino-6-trifluoromethyl-9-methylpurines

8-(3-fluorophenyl)-9-methyl-2-phenethylamino-6-trifluoromethylpurine 12

This compound was prepared from compound 3 (40 mg, 0.089 mmol) and 3-fluorophenylboronic acid (25 mg, 0.179 mmol) by the procedure described for the preparation of compound 11. The reaction was monitored by TLC and HPLC (HPLC: Rt_product = 5.0; TLC (EA/PE 1:3): R_f,product = 0.22, R_f,reactant = 0.15). After the conversion was complete, the mixture was diluted with EA (10 ml) and water (5 ml). The water layer was extracted two times with EA (7 ml). The organic layers were washed with water (10 ml) and a saturated NaCl solution in water (10 ml). The organic layer was dried with Na_2SO_4. Flash chromatography (gradient: PE/EA (4:1) - PE/EA (2:1), 120 mL) afforded, after evaporation, compound 12 (33 mg, 88%) as a white solid.

UV (EA) \( \lambda_{\text{max}} = 308 \text{ nm} \) (\( \varepsilon = 1.7 \times 10^4 \)).

\(^1\)H-NMR (400MHz, CDCl_3), \( \delta \) [ppm]: 7.54-7.48 (m, 3H, F-phenyl), 7.33-7.22 (m, 6H, 5Har + 1H F-phenyl), 6.07 (br s, 1H, NH), 3.94 (br m, 2H, NHCH_2), 3.93 (s, 3H, CH_3), 3.02 (t, J = 7.2 Hz, 2H, CH_2).

\(^19\)F-NMR (500MHz, CDCl_3), \( \delta \) [ppm]: -69.97 (3F, 6-CF_3), –111.36 (1F, 18-F).

\(^13\)C-NMR (500MHz, CDCl_3) \( \delta \) [ppm]: 162.81 (d, J = 248 Hz), 154.52, 150.73 (2), 138.66, 131.15, 130.74 (d, J = 8 Hz), 128.85 (2), 128.63 (2), 126.54 , 124.60 (d, J = 3 Hz), 120.05 (q, J = 275 Hz), 119.86 , 117.52 (d, J = 21 Hz), 116.15 (d, J = 23 Hz), 42.15, 35.72 , 31.05

MS: m/z 416.1489 (M^+ + H. C_21H_{18}N_5F_4 requires 416.1498).

9-methyl-2-phenethylamino-8-\( E \)-styryl-6-trifluoromethylpurine 13

This compound was prepared from compound 3 (40 mg, 0.089 mmol) and \( E \)-2-phenylvinylboronic acid (26 mg, 0.179 mmol) by the procedure described for the preparation of compound 11. The reaction was monitored by HPLC (R_t,product = 5.2) and TLC (EA/PE 1:3) (R_f,product = 0.24). Workup of the reaction mixture and flash chromatography (gradient: EA/PE (1:5) – EA/PE (1:2), 150 mL) gave, after evaporation, compound 13 (38 mg, 100%) as a red/orange oil. Yellow crystals (24 mg) were obtained after crystallization from methanol. \(^1\)H-
**NMR** (400MHz, CDCl₃), δ [ppm]: 7.85 (d, J = 15.9 Hz, 1H), 7.60 (d, J = 7.0 Hz, 2H), 7.44-7.37 (m, 3H), 7.34-7.24 (m, 5H, H_ar), 7.05 (d, J = 15.9 Hz, 1H), 6.08 (br s, 1H, NH), 3.97 (br m, 2H, NHCH₂), 3.90 (s, 3H, CH₃), 3.03 (t, J = 7.2 Hz, 2H, CH₂).

**13C-NMR** (500MHz, CDCl₃) δ [ppm]: 153.32, 151, 149.41, 140, 138.69, 134.96, 130.12, 129.03 (2C), 128.90 (2C), 128.61 (2C), 127.68 (2C), 126.52, 119.95 (q, J = 275 Hz), 118.2, 110.70, 42.39, 35.69, 29.16.

MS: m/z 424.1742 (M⁺ + H. C₂₃H₂₁N₅F₃ requires 424.1749).

**9-methyl-2-phenethylamino-8-E-(4-trifluoromethylstyryl)-6-trifluoromethylpurine 14**

This compound was prepared from compound 3 (40 mg, 0.089 mmol) and E-2-(4-trifluoromethylphenyl)vinylboronic acid (39 mg, 0.179 mmol) by the procedure described for the preparation of compound 11. The reaction was monitored by HPLC (Rᵗ_product = 5.4) and TLC (EA/PE 1:3) (Rᶠ_product = 0.22). After workup of the reaction mixture, flash chromatography (gradient: EA/PE (1:5) – EA/PE (1:2), 150 mL) furnished, after evaporation, compound 14 (44 mg, 100%) as a red/orange oil. Yellow crystals (29 mg) were obtained after crystallization from methanol. UV (EA) λ_max = 354 nm (ε = 2.1x10⁴). **¹H-NMR** (400MHz, CDCl₃), δ [ppm]: 7.87 (d, J = 16.0 Hz, 1H), 7.71-7.66 (m, 4H), 7.35-7.23 (m, 5H, H_ar), 7.14 (d, J = 15.9 Hz, 1H, H-11), 6.1 (br s, 1H, NH), 3.96 (br m, 2H, NHCH₂), 3.92 (s, 3H, CH₃), 3.04 (t, J = 7.2 Hz, 2H, CH₂).

**¹⁹F-NMR** (500MHz, CDCl₃), δ [ppm]: -63.20 (15-CF₃), -70.16 (6-CF₃).

**¹³C-NMR** (500MHz, CDCl₃) δ [ppm]: 154.03, 150.11, 149.21, 141.43, 138.68, 136.15, 131.12 (q, J = 32 Hz, C-21), 128.87 (2C), 128.64 (2C), 127.54 (2C), 126.55, 125.95 (q, J = 3.7 Hz, 2C), 123.89 (q, J = 272 Hz), 120.05 (q, J = 275 Hz), 120.1, 114.54, 42.11, 35.74, 29.04.

MS: m/z 492.1627 (M⁺ + H. C₂₄H₂₆N₅F₆ requires 492.1623).

**9-methyl-2-phenethylamino-8-Z-styryl-6-trifluoromethylpurine 15**

Compound 13 was converted into 8-Z-styryl-6-trifluoromethyl-9-methyl-2-phenethylaminopurine by exposure to daylight for 1-2 h in dichloromethane.
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$^1$H-NMR (400MHz, CDCl$_3$), δ [ppm]: characteristic doublet from 7.05 (d, E-form, $J = 15.9$ Hz, H-11) to 6.53 (d, Z-form, $J = 12.3$ Hz, 1H).

9-methyl-2-phenethylamino 8-Z-(4-trifluoromethylstyryl)-6-trifluoromethylpurine 16

Compound 14 was completely converted into 9-methyl-2-phenethylamino 8-Z-(4-trifluoromethylstyryl)-6-trifluoromethylpurine 16 by exposure to daylight for 6 – 8 h in dichloromethane. $^1$H-NMR (400MHz, CDCl$_3$), δ [ppm]: characteristic doublet from 7.14 (E-form, $J = 15.9$ Hz, H-11) to 6.65 (d, Z-form, $J = 12.4$ Hz, 1H).
4.8 REFERENCES

Synthesis of 8-substituted 2-phenethylamino-6-trifluoromethyl-9-methylpurines


