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 2-substituted-6-trifluoromethyl purines

ABSTRACT

A series of C-2-substituted-6-trifluoromethyl-adenosine derivatives I was prepared efficiently from OH-protected 2-nitro-6-trifluoromethyl adenosine analogues. Reactions with nucleophiles were efficiently performed at 0°C to 40°C. Removal of the ribosyl group under mildly acidic conditions yielded 2-substituted-6-trifluoromethylpurines II, which could be selectively alkylated to furnish 9-methylated-trisubstituted purines III. Bocom protected purines IV proved to perform excellently in nucleophilic substitution reactions and allowed new efficient alternative routes to biologically interesting compounds.
3.1 INTRODUCTION

In adenosine research, the introduction of substituents at the 2-position is very important to obtain biologically active compounds. By influencing the electrochemical and sterical properties of the molecule via C-2 substituents, the affinity for various receptor subtypes is changed. As discussed earlier, our goal is to introduce C-2 substituents on 6-trifluoromethylated adenosine derivatives. While very limited literature was available for the synthesis of 6-trifluoromethylpurines at all, the introduction of substituents at C-2 of these compounds was completely terra incognita.

Classically, the introduction of C-2 substituents in purines proceeds via diazotation of an amine function followed by nucleophilic aromatic displacement of the thus created halogenated purines with amines and alcohols\(^1,^2\). Other techniques like the aromatic substitution of 2-fluoro or 2-chloro substituted purine compounds often proceeds sluggishly when used for the introduction of sterically hindered amines or aromatics.\(^3\) In the 1990’s, examples have been described where lithiation and stannylation techniques are used.\(^4\) A more recent powerful technique used for functionalization of the purine skeleton is palladium catalyzed reaction with amines or boronic acids, which was applied in the synthesis of Cyclin Dependent Kinase (CDK) inhibitors e.g.\(^5,^6,^7\)

Recently, members of our group published successful attempts to make use of the enhanced electrophilicity of the 2-nitro group for C-6 substitution. The nitro group itself also has excellent properties as a leaving group. Rodenko et al. synthesized several very interesting compounds via the introduction of amines at C-2 via nitro group displacement at 80-90°C. Examples are di-substituted adenosine derivatives and conformationally restricted analogues prepared with diamines.\(^8\) Wanner et al. synthesised adenosine analogues with interesting adenosine A\(_1\) receptor affinity.\(^9\) These findings stimulated us to study nucleophilic substitution reactions of the nitro group in the newly synthesised 2-nitro-6-trifluoromethylpurines.

3.2 AMINATION AT THE 2-POSITION

Guided by the results of the docking studies from chapter 1 at the adenosine A\(_{2A}\) receptor, our interest was focused on the introduction of large amino substituents at C-2 of 6-
trifluoromethylpurines. Thus, we investigated the coupling of primary amines and cyclic secondary amines via nucleophilic aromatic substitution (SNAr). As a model reaction, 2-nitro-6-trifluoromethyl purine riboside triacetate \(\text{1}\) in scheme 3.1 was subjected to phenethylamine substitution in tetrahydrofuran. Using HPLC, the reactions were monitored at different temperatures and it turned out that substitution takes place easily already in the range of -10°C to room temperature. With the initially used base diisopropylethylamine, nucleophilic deprotection of hydroxyl groups of the ribose was detected according to HPLC analysis. When triethylamine was used instead, the acetate groups remained untouched. Moreover, the yield increased for phenethylamine (DiPEA: 75%, Et\(_3\)N: 90%) at the same reaction time. The chemistry of aminolysis of the related pyrido purines was extensively studied in our group by Odijk and Koomen.\(^{10}\) Pyridopurines were reacted with morpholine, diisopropylamine, diethylamine or n-butylamine as nucleophiles and the kinetics of aminolysis were analyzed with help of fluorescence studies. A pseudo first order constant \(k_1\) was calculated and a \(k_3\) term found. The second order reaction term \(k_2\), however, was negligible. A clear correlation was found between basicity and reaction velocity and general base catalysis was proposed.

Comparing these findings with the literature procedures for C-2 substitution of halogenated purines, which require elevated temperatures, it is striking that the 2-nitro-6-trifluoromethyl purines give fast and clean conversions already at room temperatures.

Having established suitable reaction conditions, a diverse set of primary and secondary amines was selected to test the scope and limitations of the reaction and to set up a small library for biological screening. The amines were chosen with the structure activity relations (SAR) for adenosine receptors in mind, as described in chapter 1. We selected benzyl and phenethyl amines, bearing a spacer and a hydrophobic aromatic system. They reacted in high yields of 82 and 92% after deprotection respectively, as shown in table 3.1. The influence of a polar group on substitution is investigated with para hydroxy phenethyl amine which led to the product in 40% yield. In addition, a series of cyclic secondary amines with diverse substituents was selected. The hydroxyl substituted piperazines and piperidines reacted reasonably well. Also, the product of the reaction with 4-hydroxyl substituted piperidine \(3f\)
was isolated in good yield (65%). The morpholine substituted product 3g was isolated in 37% yield (table 3.1).

**Scheme 3.1** Nucleophilic attack of amines at the purine 2-position of 2-nitro-6-trifluoromethyl-9-(triacetyl-ribofuranosyl)-purine) amine. a) amine (R-NH₂), DIPEA or Et₃N, THF, 0 - 20°C b) excess amine or NH₃/MeOH treatment, rt

**Table 3.1: Nucleophilic amination of 2-nitro-6-trifluoromethylpurines**

<table>
<thead>
<tr>
<th>Product</th>
<th>R</th>
<th>Isolated yield over 2 steps (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>82%</td>
</tr>
<tr>
<td>3b</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>90%</td>
</tr>
<tr>
<td>3c</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>40%</td>
</tr>
<tr>
<td>3d</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>60%</td>
</tr>
<tr>
<td>3e</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>40%</td>
</tr>
<tr>
<td>3f</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>65%</td>
</tr>
<tr>
<td>3g</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>37%</td>
</tr>
</tbody>
</table>
### 3.3 2-ALKOXYGROUPS AT THE 2-POSITION

Alkoxy groups are well known to contribute to pharmacophoric interactions in adenosine receptor research. In the early 1990’s Ueeda and Olsson recognised the alkoxy substituent as a valuable substituent to synthesise potent and selective agonists for the coronary artery (now A2 receptor). More recently, Cristalli and coworkers described alkoxygroups at C-2 in their study on adenosine receptor antagonists. Analyzing the different substituents of large series of compounds, it appeared that phenethoxy groups at the 2-position gave products with higher affinity than the corresponding phenethylamino compounds. In addition the highest A2A versus A2B selectivity (near 400-fold) was found for the phenethoxy substituted system.

In analogy to the amination, the introduction of alkoxygroups at C-2 was initially performed with phenethylalcohol and triethylamine. However this attempt was not successful. In addition, raising the temperature to reflux temperature and adding excess of reagent did not lead to the desired product either. Literature reports describe the requirement for preactivation of alcohols to start the substitution reaction. Marumoto et al. described the preparation of 2-alkoxy adenosines by displacing the chloro group of 2',3'-O-(ethoxymethylidine)-2-chloroadenosine with an alkoxide. “Metallic Na” was reacted with an excess of an alcohol to generate the alkoxide. The chloro nucleoside was added and the excess of alcohol served as a solvent for the subsequent reaction. Because of the costs involved with expensive alcohols using them as solvents is unpractical. Olsson et al. developed a more general route, generating the alkoxides by adding n-buthyllithium to a solution of a 5% molar excess of the alcohol in dry 1,2-diethoxyethane. Refluxing the reaction mixture with the protected 2-chloroadenosine for 5 -7 days, generated the 2-alkoxyadenosines in reasonable yields. This method was also successfully applied to arylalkoxyadenosines.

We decided to follow a more practical protocol described by Cristalli, which was used to functionalise 9-ethyladenines via the corresponding 2-iodides. Starting from 2-amino-6-chloropurines, the purine system was alkylated on N9 and the 2-amino group was converted into an iodide. This was done by the classical diazotization using iso-pentyl nitrite as the nitrosating agent, followed by a halogenation procedure. The alkoxy derivatives were subsequently obtained by reaction of the synthon with 2-phenethyl alcohol in dry acetonitrile in the presence of K2CO3 or NaOH at 85°C for 18 h. We choose to perform the reaction
starting from the available iodide in an excess of alcohol by using the phenethylalcohol as a solvent too. By adding potassium carbonate, elevating the temperature to only 40°C and stirring for 18 h we succeeded in introducing the phenethoxy group in a rather mild way in satisfactory yield (4, 40%).

3.4 CONVERSION TO 2-AMINOSUBSTITUTED 6-TRIFLUOROMETHYL-PURINE BASES

One of our biological objectives is the search for new antagonists for the adenosine receptors. Until now, most agonists for the adenosine receptors are mimics of adenosine. The ribose moiety at N-9 usually leads to agonistic activity on the adenosine receptors via ribose interactions. As discussed in chapter 1, substituted adenine derivatives are generally correlated with antagonistic activity.

Now, having in hand a series of 2-substituted 6-trifluoromethyladenosine derivatives, ways could be explored to convert these to the corresponding purine bases. Usually, cleavage of the ribose from the purine occurs as a side-reaction during deprotection steps of OH-protected ribosides. Mostly, acid labile groups like isopropylidene or acetate groups are chosen to protect the ribose hydroxyl groups. Refluxing in 50% acetic acid or solutions with formic acid 2% and/or trifluoroacetic acid is used to deblock the hydroxyl groups. As a side-reaction, often cleavage of the N-9 - C-1’glycosidic bond is observed. We observed in our reactions, that when pure trifluoroacetic acid was used, this side reaction is promoted to be the main reaction.

When compounds 3a and 3b, obtained after mild deprotection of ribose protective groups, were stirred in pure trifluoroacetic acid at 45°C, the ribose moiety was cleaved off selectively.
(Scheme 3.2). Other parts of the purine system (amine function, or trifluoromethyl groups) were not affected and satisfactory yields of \(5a\) and \(5b\) were obtained (79-84%).

![Chemical structure](image)

**Scheme 3.2** Deribosylation  a) TFA, 45°C, 18h

Nevertheless, better yields could be realised by performing the TFA treatment directly after amination. So, after substitution by amines, the solution was evaporated to dryness and the residue was instantaneously dissolved in TFA and stirred at 45°C. After total deribosylation, normal workup procedures gave compounds \(5a\) and \(5b\) in high yield (2 steps: 89%).

In this strategy, the ribose group is in fact used as an effective protective group for purine bases. Since the starting material 6-chloropurine riboside is synthesized from the relatively cheap inosine in high overall yield, this route was readily scaled up in our laboratory to multiple grams of material, thus giving access to a range of functionalised purine bases.

### 3.5 Methylation at the Purine N9-position

For biological studies, the synthesis of a 2,6,9-trisubstituted purine derivatives was an interesting objective. The synthesis of N-9 alkylated purine substrates is of great interest in many areas of biological research (antivirals like penciclovir,\(^{15,16}\) anti cytostatic drugs,\(^{17}\) Cyclin Dependant Kinase inhibitors\(^{18}\) and receptor research). Classically, introduction of alkyl groups is carried out via strong base treatment (sodium hydride or potassium carbonate) to deprotonate N-9, followed by the addition of a variety of alkyl and arylalkyl halides.\(^{19}\) This method was used for the N9 alkylation of 6-chloropurine with methyl iodide as described in
chapter 2. However, because of the presence of an additional amine function at C2, milder conditions were evaluated to avoid regioselectivity problems. Very recently, it was reported that tetrabutylammonium fluoride (TBAF) remarkably accelerates the N9-alkylation of the purine ring with a variety of organic halides at room temperature. TBAF acts as an activator of the halide which, in combination with its basic properties, gives excellent yields. This was easily adapted to multi titer plate synthesis. Another very mild and effective method is the Mitsunobu reaction. In the early 1990’s it was also adapted to purines for the synthesis of carbocyclic guanosine analogs. Successful application has led to carba analogs of nucleosides, nucleosides with furanyl scaffolds for antiviral application and for use as Cyclin Dependent Kinase inhibitors.

We applied Mitsunobu conditions for our substrates. Condensation of methanol and the purine N-9 proton upon treatment with triphenylphosphine and diisopropyl azodicarboxylate resulted in the formation of 6a and 6b via attack of the N-9 anion on the methyl oxyphosphonium salt (Scheme 3.3).

![Scheme 3.3 Methylation of N-9: (a) PPh$_3$, diisopropyl azodicarboxylate (DIAD), MeOH, 30min or b) potassium carbonate and MeI in DMF, 30 min.](image)

The product was obtained in high yield. However, silica gel column chromatography did not totally separate the product from the triphenylphosphine oxide formed during the reaction. This was successfully circumvented by using solid phase/polymer bound triphenylphosphine, giving pure product.

Another option was to perform the reaction with methyl iodide in the presence of potassium carbonate. With this procedure, products 6a and 6b were obtained in high yield and
more importantly, in higher purity after simpler purification. The reaction time was the same
for both reactions (0.5 h).

As a side reaction, methylation of the N-7-position occurred in both reactions. The
formation of the purine N-7-methyl derivative was detected with both HPLC and $^1$H-NMR
(N7-CH$_3$/N9-CH$_3$ = 1:10). However, crystallisation from water gave the pure N9-methyl
purines 6a and b in good yield. So, initially crude mixtures of 6a and 6b with 10% N7-CH$_3$
product were isolated in 96% yield. After crystallisation pure N9-CH$_3$ was isolated in 80%
yield.

### 3.6 BOCOM PROTECTIVE GROUPS AND C-2 AMINATION

In the preceding chapter, the excellent stability of the Bocom protective group was
successfully used for the introduction of the trifluoromethyl moiety and the reaction could be
scaled up easily. Therefore we studied its behaviour in further reaction sequences. We could
demonstrate that the Bocom group is also stable when subjected to mild nucleophilic
conditions. Thus, when 2-nitro-6-trifluoromethyl-9-Bocom purine was stirred in
dichloromethane at reflux temperature with selected amines, the Bocom protected 2-
substituted products were obtained. The reaction mixture could be easily purified using flash
chromatography and trituration with petroleum ether. This produced 7a and 7b in 80- 85%
yield (Scheme 3.4).

![Scheme 3.4 Amination of Bocom purines](image-url)

The unmasked purine bases were easily prepared by adding 7a and 7b to a sodium
methoxide solution in MeOH at room temperature. After 45 minutes, solid CO$_2$ was added
followed by slow addition of water. The resulting solid was filtered and washed with water. After drying at 50 °C in vacuo, products 5a and 5b were collected as pure solids in >90% yield. Overall it is observed that the N9-Bocom route is more efficient than the preparation via the ribosylated or methylated purines.

3.7 6-AMINO-2-PHENETHYL-SUBSTITUTED PURINES AND BOC CHEMISTRY

To allow direct biological comparison of CF₃ and NH₂ moieties in purines, we searched for data on the direct 6-NH₂ analog of 6b. Although the compound was known from literature, no biological data for the adenosine A₂A receptor were available. So we had to synthesize the compound ourselves. In addition, we could check the scope of our developed 2-nitropurine chemistry. A synthetic route was envisaged using the excellent selective substitution properties of the 6-chloro and 2-NO₂ groups. In a first step, 2-Nitro-6-chloro-9-methylpurine 8 (Scheme 3.5) was selectively substituted at C-6 with benzyl amine at room temperature, followed by reaction with phenethylamine at 40 °C to yield 10. Selective debenzylation with H₂/Pd-C should yield the 6-amino derivative. Unfortunately, this step failed in our hands.

![Scheme 3.5](image)

**Scheme 3.5** di-amino substituted purines of Bocom purines a) benzylamine, dipea b) phenethylamine, dipea

In the previous chapter, we showed the very interesting properties of the Boc group in purine nitration. Challenged by the disappointing reduction step of 10 to 15, we tried to use Boc chemistry to obtain the desired deprotected amine. Starting from 2-nitro-N6-N6-N9-tri-Boc protected adenine 11 (see Scheme 3.6), the N-9 Boc group was selectively removed under nucleophilic conditions in acetonitrile. Exactly 1 equivalent of piperidine was added dropwise at 0 °C to obtain 12. Compound 13 was prepared via methylation with potassium carbonate and methyl iodide. The N-9 / N-7 Me isomer ratio was less favourable than in
previous cases: 64 / 36. After chromatography, the yield over two steps is 51% \( \text{13} \) (and 29% of the corresponding N-7 isomer.)

\[
\begin{align*}
\text{Scheme 3.6 Boc route to 6-amino variant, methylation} & \\
& \text{a) piperidine (1 eq.), acetonitrile} \\
& \text{b) potassium carbonate, Mel, acetonitrile}
\end{align*}
\]

This methylated compound was treated with 1.8 equivalents of phenethylamine in DMF and stirred at room temperature overnight to yield 80% of \( \text{14} \) (Scheme 3.7). This is another example of the very mild and efficient introduction of nucleophiles at C-2. Subsequent treatment with trifluoroacetic acid removed the final Boc protective group and furnished the target product \( \text{15} \) in 81% yield. Biological data of the compounds are discussed in chapter 6.

\[
\begin{align*}
\text{Scheme 3.7 Boc route to 6-amino variant, amination} & \\
& \text{a) phenethylamine, DMF, rt} \\
& \text{b) TFA, DCM}
\end{align*}
\]

To study the order of reaction steps in the conversion from \( \text{13} \) to \( \text{14} \), the reaction was monitored with proton NMR. Two potential intermediate products \( \text{16} \) and \( \text{17} \), as depicted in Scheme 3.8 were both observed. Initially, a fast reaction of the first amine occurs, giving two options: C-2 substitution or Boc mono-deprotection. When the 6-diBoc is intact, C2 substitution proceeds fast, leading to intermediate \( \text{16} \). Probably, the second Boc group further lowers the electron density at C2, thus enhancing aromatic nucleophilic substitution. The alternative option, initial Boc deprotection to \( \text{17} \), takes place via activation by the C-2 nitro group, which we also use for our 6-trifluoromethylpurine syntheses and deprotection at N6. After the first substitution, the next step to \( \text{14} \) appears to be very slow. The C-2 amine
substituent does not accelerate attack at C-6 the same way as the nitro group. On the other hand, in intermediate 17 with one of the N protecting Boc groups removed, the C-2 position is also less activated, resulting in slow conversion of 17 to product 14.

Scheme 3.8 Proposed routes for amine substitution and mono Boc deprotection of di-Boc protected N9-methylpurines

3.8 CONCLUDING REMARKS

We successfully introduced substituents at the C-2 position of 6 trifluoromethylpurine derivatives via amination with a diverse set of amines and ether formation with 2-phenylethanol. After removal of the N-9 protecting ribose group, methylation produced 2-substituted-6-trifluoromethyl-9-methylpurines. The Bocom group proved to be stable in nucleophilic environment and was applied to generate a new alternative route to our target molecules. Boc protected 2-nitro-9-methyladenines were successfully used to synthesize the natural amino bioisosteres of our target molecules. NMR analysis of the formed products gave insight in the different steps of the deprotection/substitution mechanism intermediates.
3.9 ACKNOWLEDGEMENTS

We wish to thank Remko Detz for his work at the phenethyl substituted 6-trifluoromethyl ribose substrates. Martin Wanner is acknowledged for his experiments with Boc protected purines.

3.10 Experimental

General information.

For experimental details see Chapter 2.

2-phenethylamino-6-trifluoromethyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-purine 2b

A THF (10 ml) solution containing 6-Trifluoromethyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-nitropurine (2.6 g, 5.3 mmol), Et₃N (0.97 ml, 6.9 mmol) and phenylethylamine (0.66 ml, 5.3 mmol) was stirred at 0°C for 22 h followed by warming up till room temperature. The reaction was followed by HPLC. After completion the reaction was quenched by adding silica gel. The suspension was concentrated to dryness. The resulting powder was purified by column chromatography using a gradient mixture of MTBE - MTBE with 1% MeOH – MTBE with 4% MeOH and concentrated under reduced pressure. The resulting pure product was obtained as a yellow/white foam in 90% yield.

₁H-NMR (400MHz, CDCl₃), δ [ppm]: 7.96 (s, 1H, H-8), 7.34-7.21 (m, 5H, Ar), 6.15 (d, J = 4.9 Hz, 1H, H-1’), 6.13 (br s, 1H, NH), 5.83 (t, J = 5.3 Hz, 1H, H-2’), 5.71 (t, J = 5.1 Hz, 1H, H-3’), 4.45 (m, 2H, 5’-CH₂), 4.38 (m, 1H, H-4’), 3.93 (br m, 2H, NHCH₂), 3.00 (t, J = 7.1 Hz, 2H, CH₂), 2.16 (s, 3H, acetyl), 2.10 (s, 3H, acetyl), 2.09 (s, 3H, acetyl).

2-benzylamino-6-trifluoromethyl-adenosine 3a

2-benzylamino-6-trifluoromethyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl) purine was prepared according to the procedure for 2b. This product (0.090 g, 0.16 mmol) was stirred in a 1:1 solution of NH₃ in MeOH for 4 h. Solvent evaporation and sublimation afforded compound 3a (0.057 g, 82%).
\(^{1}\text{H-NMR}\) (400MHz, DMSO), \(\delta\) [ppm]: 8.55 (s, 1H, H-8), 7.37-7.23 (m, 5H, H\_ar), 6.5 (br s, 1H, NH), 5.94 (d, 1H, H-1’), 5.49 (d, 1H, OH), 5.27 (bs, 1H, OH), 5.25 (bs, 1H, OH), 4.72 (t, 1H, H-2’), 4.61 (t, 1H, H-3’), 4.38 (m, 1H, H-4’), 4.13 (br m, 2H, NHCH\_2), 3.97 (2H, H5’)

\(^{19}\text{F-NMR}\) (500MHz, DMSO), \(\delta\) [ppm]: -68.64 (6-CF\_3).

2-phenethylamino-6-trifluoromethyl-adenosine 3b

2-phenethylamino-6-CF\_3-9-(2,3,5-tri-O-acetyl-\(\beta\)-D-ribofuranosyl)-purine 2b was stirred in a 1:1 solution w/w of NH\_3 in methanol for 18 h. The product was purified by flash chromatography (MTBE with 5% MeOH) to yield, after sublimation, compound 3b (0.033g, 60%)

\(^{1}\text{H-NMR}\) (400MHz, DMSO, D\_2O), \(\delta\) [ppm]: 7.99 (s, 1H, H-8), 7.34-7.21 (m, 5H, H\_ar), 6.12 (br s, 1H, NH), 5.93 (bs, 1H, H-1’), 4.64 (m, 1H, H-2’), 4.27(m, 1H, H-3’), 4.19 (m, 1H, H-4’), 4.16 (m, 2H, 5’), , 3.27 (br m, 2H, NHCH\_2), 2.7 (br m, 2H, CH\_2).

\(^{19}\text{F-NMR}\) (500MHz, DMSO), \(\delta\) [ppm]: -63.95 (6-CF\_3).

2-(4-hydroxy-phenethylamino)-6-trifluoromethyl-adenosine 3c

2-nitro-6-CF\_3-9-(2,3,5-tri-O-acetyl-\(\beta\)-D-ribofuranosyl)-purine (0.125g, 0.25 mmol,) was treated with 5 equivalents of tyramine in 5 ml THF. The product was purified by flash chromatography (EA) to yield compound 3c (0.046g, 40%)

\(^{1}\text{H-NMR}\) (400MHz, DMSO, D\_2O), \(\delta\) [ppm]: 8.52 (s, 1H, H-8), 7.39-7.0 (m, 5H, H\_ar), 5.92 (bs, 1H, H-1’), 4.59 (m, 1H, H-2’), 4.16(m, 1H, H-3’), 3.93 (m, 1H, H-4’), 3.43 (br m, 2H, NHCH\_2), 2.81 (br m, 2H, CH\_2).

2-(N-hydroxy-ethyl-piperazinyl)-6-trifluoromethyl-adenosine 3d

A THF (5 ml) solution containing 6-Trifluoromethyl-9-(2,3,5-tri-O-acetyl-\(\beta\)-D-ribofuranosyl)-2-nitropurine (0.374 g, 0.8 mmol) and hydroxy-ethylpiperazine (1.0 ml, 8.1 mmol) was stirred at room temperature for 18 h. The reaction was followed by HPLC. After completion the reaction was quenched by adding silica gel. The suspension was concentrated to dryness. The resulting powder was purified by column chromatography using a gradient
mixture of MTBE: MTBE with 1% MeOH – MTBE with 4% MeOH and concentrated under reduced pressure. The resulting pure product was obtained in 60 % yield.

$^1$H-NMR (400MHz, DMSO, D$_2$O), $\delta$ [ppm]: 8.57 (s, 1H, H-8), 5.99 (d, 1H, H-1’), 4.6 (t, 1H, H-2’), 4.4 (d, 1H, H-3’), 4.29 (m, 1H, 4’), 4.1 (d, 2H, H5’), 3.56 (t, 2H), 2.59 (m, 8H), 2.48 (t, 2H)

2-piperidinyl-6-trifluoromethyl-adenosine 3e

2-piperidinyl-6-trifluoromethyl-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl) purine was prepared following the procedure for 3b. This product (0.038 g) was treated with 1:1 NH$_3$ solution and MeOH. Flash chromatography (MTBE +2.5% MeOH) yields 3e (0.030 g, 40%)

$^1$H-NMR (400MHz, CDCl3), $\delta$ [ppm]: 7.89 (s, 1H, H-8), 5.82 (d, 1H, H-1’), 5.0 (t, 1H, H-2’), 4.44 (d, 1H, H-3’), 4.32 (m, 1H, 4’), 4.1 (bs, 4H), 3.93 and 3.76 (d, 2H, H5’), 1.72 (m, 6H).

2-(4-OH-piperidinyl)-6-trifluoromethyl-adenosine 3f

2-(4-OH-piperidinyl)-6-trifluoromethyl-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl) purine was prepared following the procedure for 3b. This product (0.040 g, 0.07 mmol) was treated with 1:1 NH$_3$ solution and MeOH. Flash chromatography (MTBE +2.5% MeOH) yields 3f (0.020 g, 65%)

$^1$H-NMR (400MHz, DMSO, D$_2$O), $\delta$ [ppm]: 7.86 (s, 1H, H-8), 5.80 (d, 1H, H-1’), 5.2 (t, 1H, H-2’), 4.6 (d, 1H, H-3’), 4.29 (m, 1H, 4’), 4.1 (bs, 4H), 3.80 and 3.74 (d, 2H, H5’), 1.90 (m, 4H).

2-morpholinyl-6-trifluoromethyl-adenosine 3g

6-Trifluoromethyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-nitropurine 1 (0.100 g, 0.2 mmol) was treated with morpholine (0.175 ml, 2 mmol) and triethylamine (0.2 ml) in 1ml THF. After the reaction was complete, the product was purified bij flash chromatography (EA) to yield 3g (30 mg, 37%).

$^1$H-NMR (400MHz, DMSO), $\delta$ [ppm]: 8.6 (s, 1H, H-8), 6.00 (d, 1H, H-1’), 5.64 (d, 1H, H-2’), 5.47(d, 1H, H-3’), 4.66 (m, 2H, 5’), 4.34- 4.13 (m, 8H).
**2-phenethyloxy-6-trifluoromethyl-adenosine 4**

6-Trifluoromethyl-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-2-nitropurine 1 (0.150g, 0.4 mmol) was dissolved in phenethylalcohol (3 ml) and K₂CO₃ (0.084 g, 0.8 mmol) was added. The reaction was warmed to 40°C for 18h. After cooling to room temperature, the mixture was extracted three times with DCM/water. The organic layers were concentrated and purified by flash chromatography (gradient MTBE - MTBE+10% MeOH) to give compound 4 (0.068 g, 40%).

1H-NMR (400MHz, CDCl₃, D₂O), δ [ppm]: 8.13 (s, 1H, H-8), 7.32-7.19 (m, 5H, H₅, H₆, H₇), 5.92 (bs, 1H, H-1'), 5.0 (bs, 1H, H-2'), 4.51(m, 1H, H-3'), 4.32 (br m, 2H, NHCH₂), 3.96 (m, 1H, H-4'), 3.88 (m, 2H, 5'), 3.19 (br m, 2H, CH₂).

**2-benzylamino-6-trifluoromethylpurine 5a**

(2 available methods, see also 5b) A solution of 6-trifluoromethyl-2-benzylamino-9-BocOM purine (0.100 g, 0.23 mmol) in MeONa/MeOH (2.3 mmol) was stirred at room temperature. After 45 minutes solid CO₂ was added. Water was added slowly to the suspension. The solid was filtered and washed with water. After drying at 50°C in vacuo, it was collected as a pure solid (0.060 g, 89%)

1H-NMR (400MHz, DMSO-d₆), δ [ppm]: 13.0 (br s, 1H, 9-NH), 8.34 (br s, 1H, NH), 8.33 (s, 1H, H-8), 7.38-7.23 (m, 5H, H₅, H₆, H₇), 4.71 (br s, 2H, NHCH₂).

**2-phenethylamino-6-trifluoromethylpurine 5b**

A solution of 6-Trifluoromethyl-9-(2,3,5-tri-O-acetyl-β-D-ribosyl)-2-phenethylaminopurine (2.65 g, 4.7 mmol) in trifluoroacetic acid (15 ml) was stirred at 40°C. The reaction progress was followed by HPLC (Rₜ,product = 3.7). After 24 h the mixture was coevaporated with toluene and methanol. Trituration with ether furnished the product as a light grey solid (1.15 g, 80%). 1H-NMR (400MHz, DMSO-d₆), δ [ppm]: 13.4 (br s, 1H, 9-NH), 8.32 (s, 1H, H-8), 8.32 (br s, 1H, NH), 7.31-7.21 (m, 5H, H₅, H₆), 4.16 & 3.72 (br m, 2H, NHCH₂), 2.96 (t, J = 7.5 Hz, 2H, CH₂).
2-benzylamino-9-methyl-6-trifluoromethylpurine 6a

6a was made via Mitsunobu conditions with polymer bound triphenylphosphine to facilitate purification. To a solution of 2-benzylamino-6-trifluoromethylpurine 5a (0.047 g, 0.16 mmol) in DCM (2 ml), was added triphenylphosphine polymer bound (0.133 g, 3 mmol/g, 0.4 mmol), MeOH (0.016 ml, 0.4 mmol) and diisopropylazodicarboxylate (DIAD) (0.078 ml, 0.4 mmol). The mixture was stirred slowly at room temperature and after 1 h the reaction was complete. Flash chromatography (EA) afforded 6a (0.035 g, 71%)

$^1$H-NMR (400MHz, CDCl₃), δ [ppm]: 7.80 (s, 1H, H-8), 7.34-7.22 (m, 5H, Har), 6.15 (br s, 1H, NH), 4.01(s, 3H), 3.95 (br s, 2H, NHCH₂)

9-methyl-2-phenethylamino-6-trifluoromethylpurine 6b

To a solution of 6- Trifluoromethyl-2-phenethylaminopurine 5b (0.600 g, 1.95 mmol) in DMF (14 ml) K₂CO₃ (0.351 g, 2.54 mmol) and MeI (0.158 ml, 2.54 mmol) were added. The mixture was stirred at room temperature and after 0.5 h the reaction was complete (HPLC: Rₜ, product = 4.1). Crystallization occurred after the slow addition of water (17 ml). After stirring for 0.5 h the mixture was filtrated and the residue was washed with four 10-ml portions of water. The filtrate was again filtrated after 1 h and the residue was washed with three 5-ml portions of water. The residue was dried in vacuo at 60°C obtaining the product as a white solid (503 mg, 80%).

$^1$H-NMR (400MHz, CDCl₃), δ [ppm]: 7.83 (s, 1H, H-8), 7.33-7.21 (m, 5H, Har), 6.17 (br s, 1H, NH), 3.95 (br m, 2H, NHCH₂), 3.87 (s, 3H, CH₃), 3.01 (t, J = 7.1 Hz, 2H, CH₂)

2-benzylamino-9-BocOM-6-trifluoromethylpurine 7a

A THF (5 ml) solution containing 6-trifluoromethyl-2-nitro-9-BocOM-purine (0.4 g, 9 mmol, chapter 2), diisopropylethylamine (0.174 ml, 10 mmol) and benzylamine (0.66 ml, 10 mmol) was stirred at room temperature for 5 h. The reaction was followed with HPLC. After completion the reaction was quenched by adding silica gel. The suspension was concentrated to dryness. The resulting powder was purified by flash chromatography (EA/PE 1:1) and concentrated under reduced pressure. The resulting product was obtained in 81 % yield (0.37 g).
$^1$H-NMR (400MHz, CDCl$_3$), $\delta$ [ppm]: 8.12 (s, 1H, H-8), 7.40-7.28 (m, 5H, H$_{ar}$), 6.30 (bs, 1H, NH), 6.10 (s, 2H, CH$_2$O), 4.86 (bs, 2H, CH$_2$NH), 1.47 (s, 9H)

9-BocOM-2-phenetylamino-6-trifluoromethylpurine 7b

A DCM (5 ml) solution containing 6-trifluoromethyl-2-nitro-9-BocOM-purine (0.365 mmol, see chapter 2), diisopropylethylamine (0.4 mmol) and phenyl-ethyamine (0.4 mmol) was stirred at reflux temperature for 4 h. After completion the reaction was purified by flashchromatography (EA/PE 1:1). The resulting solid was triturated with PE to give compound 28 in 80% yield (0.129 g).

$^1$H-NMR (400MHz, CDCl$_3$), $\delta$ [ppm]: 8.10 (s, 1H, H-8), 7.31-7.19 (m, 5H, H$_{ar}$), 6.23 (bs, 1H, NH), 6.09 (s, 2H, CH$_2$O), 3.92 (bs, 2H, CH$_2$NH), 2.99 (t, 2H), 1.48 (s, 9H)
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