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 C6-Trifluoromethyl substituted Purines via C-2 Nitration

ABSTRACT

A series of 6-trifluoromethylated purine and adenosine analogs has been prepared. The TBAN/TFAA nitration of N-9 protected 6-chloropurine and of the N-9 tri-acetyl riboside of 6-chloropurine furnished highly reactive 2-nitro-6-chloropurine derivatives. The activating properties of the nitro group facilitated the subsequent introduction of a trifluoromethyl moiety at low temperatures (-20°C → room temperature). The Boc group was recognised as an excellent protective group in purine nitration. Using N-9 Boc protected 6-chloropurine, we elucidated the mechanism of purine nitration using NMR spectroscopy and different isotope labels. During the further search for optimal protective groups, the BocOM group was serendipitously found as a new promising, stable protecting group for purines, which could be used for large scale synthesis of the substituted 6-trifluoromethyl purines.

The studies towards the elucidation of the mechanism of purine nitration were performed in cooperation with Boris Rodenko, and are also discussed in his thesis. The information in this chapter is partly published as a patent application and in three papers:

2-Substituted-6-trifluoromethyl purine derivatives with adenosine-A3 antagonist activity

Rodenko, B., Koch, M., Van Der Burg, A.M., Wanner, M.J., Koomen, G.-J.
The mechanism of selective purine C-nitration revealed: NMR studies demonstrate formation and radical rearrangement of an N7-nitramine intermediate
Journal of the American Chemical Society 2005 127 (16) 5957-5963

Wanner, M.J.; Koch, M., Koomen, G.J.
Synthesis and Antitumor Activity of Methyltriazene Prodrugs Simultaneously Releasing DNA-Methylating Agents and the Antiresistance Drug O6-Benzylguanine

Wanner, M.J., Rodenko, B., Koch, M., Koomen, G.J.
New (1-deaza)purine derivatives via efficient C-2 nitration of the (1-deaza)purine ring
Nucleosides, Nucleotides and Nucleic Acids 2004 23 (8-9) 1313-1320
2.1 INTRODUCTION
Purine bases play central roles in many biological processes. Structural modification of the purine bases, and their nucleosides and nucleotides has resulted in the discovery of thousands of biologically active compounds, including many clinically used drugs. Purine analogs are used as central nervous system (CNS) drugs, as antivirals (Zovirax, penciclovir), as cytostatics (5-fluorouracil) etc. Substitutions at position 2,6,8 and 9 lead to a variety of products. Several procedures describe the introduction of substituents via nucleophilic displacement of halogenated purines. Usually, elevated temperatures (25-80°C) are applied for substitution at position C-6. Classical aromatic substitution at the 2-position is even more challenging and requires harsh conditions (120-150°C).¹

In our search for new ligands for adenosine receptors, we wanted to exploit the recently developed TBAN/TFAA purine nitration reaction.² 3⁶ A nitro group at C-2 was expected to greatly enhance the electrophilicity of the purine C-6, and at the same time allowed subsequent substitution at C-2. Recently, with nitro-chemistry, nucleoside libraries have been developed for biological evaluation as adenosine receptor agonists, as inhibitors of adenosine deaminase (ADA),³ and as therapeutics against malaria via inhibition of Trypanosoma brucei phosphoglycerate kinase.⁴

As discussed in the previous chapter, among others, we aim to synthesize substituted adenine and adenosine analogs where the 6-amino group has been replaced by a trifluoromethyl group.

It is known that substitutions with fluorine can have great influence on biological activity and for instance, has also effect on bioavailability and passage of drugs through the blood-brain barrier. Fluorinated analogues of well known drugs exert interesting biological activities (Figure 2.1).⁵
Several fluoropyrimidines such as 5-deoxy-5-fluorouridine and trifluorothymidine can act as substrates for thymidine phosphorylase, which is an enzyme correlated with angiogenic properties. The first series were originally applied in cancer chemotherapy. Later, the synthesis of prodrugs of 5-fluorouracil resulted in potent and less toxic analogues. Also in anti-inflammatory agents, fluorinated compounds are available as prostaglandin synthesis inhibitors, like flurbiprofen. Mefloquine and halofantrine, which are common medicines used against malaria, bear aromatic trifluoromethyl groups. One of the most serious side effects of halofantrine is that it can cause severe cardiac arrhythmias at normal doses. The effect of enantiomers of mefloquine/Lariam in adenosine receptor research was discussed in Chapter 1. Also tolrestat, an aldose reductase inhibitor useful in prophylaxis of diabetic neuropathy and fluoxetine (Prozac), approved for major depressive disorders, contain trifluoromethyl groups.

The substitution of hydrogen with fluorine has proven to be an effective modification in drug research. Its atomic properties explain its characteristic substituent effects. It is the smallest halogen with a van der Waals radius of 1.47 Angstrom versus 1.20 for a hydrogen atom. Fluorine has a high ionization potential (401.8 kcal/mol versus 313.6 for hydrogen) and low polarizability, which implies weak inter- and intramolecular interactions. Its strong...
electron negativity (3.98 for F and 2.2 for hydrogen) however, ensures strong inductive electron withdrawal properties and polarized bonds.

The strength of the C-F and C-CF₃ dipoles has strong consequences for lipophilicity. Fluorination, and especially aromatic fluorination, usually increases lipophilicity, compared to the corresponding hydrogen compounds. However, monofluorination or trifluoromethylation of saturated alkyl groups decreases lipophilicity. A quantitative hydrophobic substituent parameter is $\pi$, (Hansch-Leo parameter) derived from octanol-water partition coefficients. For example $\pi_{\text{CF}_3} = 0.88$ in aromatic systems and increases lipophilicity substantially, compared to $\pi_{\text{CH}_3} = 0.56$ or $\pi_{\text{NO}_2} = -0.27$ and $\pi_{\text{F}} = 0.14$.⁹

Electronically, fluorine has always inductively electron withdrawing properties, but it can be electron donating via resonance, whereas perfluoroalkyl groups are always withdrawing. The Hammet inductive and resonance substituent parameters for trifluoromethyl are $\sigma_I = 0.42$ and $\sigma_R = 0.10$

Controversy is found for the effect of fluorine on hydrogen bonding. Hydrogen bond to F-C are much weaker than the conventional type with hetero atoms (O,N), but are unambiguously observed in structures which lack other heteroatoms that would compete for the hydrogen bond.¹⁰ Also, the trifluoromethyl group has been observed to accept hydrogen bonds.

For steric effects it was for a long time incorrectly claimed, that fluorine has nearly the same size as hydrogen (Pauling van der Waals radius). Data of Bondi¹¹ and Williams and Houpt¹² in Table 2.1 show that the size of fluorine is probably closer to oxygen than to hydrogen. Fluorination thus increases the steric size of alkyl groups. The trifluoromethyl group is indeed considerably larger than the methyl group and the trifluoromethyl group is sterically at least as large as the isopropyl group. The steric van der Waals radius r_v for CH₃, (CH₃)₂CH and CF₃ are 1.80, 2.2 and 2.2, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Pauling</th>
<th>Bondi</th>
<th>Williams and Houpt</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1.20</td>
<td>1.20</td>
<td>1.15</td>
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<tr>
<td>F</td>
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<td>1.44</td>
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<tr>
<td>Cl</td>
<td>1.80</td>
<td>1.75</td>
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</tr>
<tr>
<td>O</td>
<td>1.40</td>
<td>1.52</td>
<td>1.44</td>
</tr>
</tbody>
</table>
2.2 INTRODUCTION OF TRIFLUOROMETHYL GROUPS AT C-6

2-trifluoromethyl- and 8-trifluoromethyl purine derivatives were prepared before by skeleton synthesis via heterocyclization of 5,6-diaminopyrimidines or 4-aminoimidazole-5-carboxylic acid derivatives and trifluoroacetates.\textsuperscript{13} 2-(Trifluoromethyl)-adenosine analogues were also prepared by substitution of 2-iodoadenosines with trifluoromethylzinc bromide and CuBr in DMF/HMPA.\textsuperscript{14, 15}

However, 6-fluoroalkylated purines are quite rare in literature. 6-Trifluoromethyl-purine and 2-amino-6-trifluoromethylpurine were prepared long ago in low overall yields (3 and 6\% respectively) by multistep cyclization procedures, starting from ethyl 4,4,4-trifluoro-3-oxobutanoate.\textsuperscript{16} Direct introduction of the trifluoromethyl group in protected 6-iodopurine riboside with trifluoromethyl iodide and Cu(I) in HMPA gave moderate yields. Recently, Hocek and Holy reported the introduction of trifluoromethyl groups via a copper mediated reaction with trimethyl(trifluoromethyl)silane (CF\textsubscript{3}-TMS, Rupperts reagent) and mono and 6-di-fluoro-alkylpurines.\textsuperscript{17,18} They describe that a series of chloropurine derivatives (6-chloropurine, 6-chloro-9-(tetrahydropyran-2'-yl)purine, triacetyl-ribofuranosyl-6-chloropurine), and the corresponding 2-amino derivatives and both 6-iodopurine and 2-amino-6-iodopurine were completely unreactive in this reaction. In contrast, the THP protected 6-iodopurine afforded the 6-trifluoromethyl derivative in 85 \% yield. The 6-trifluoromethyl, 6-fluoroalkyl and 6-difluoroalkyl purines, obtained via these route showed very low cytostatic activity.\textsuperscript{19}

During our investigation, 6-bromopurines were described as starting material for trifluoromethylation using methylfluorosulfonyldifluoroacetate (FSO\textsubscript{2}CF\textsubscript{2}CO\textsubscript{2}Me, MFSDA)/CuI/HMPA/DMF\textsuperscript{20,22} and CF\textsubscript{3}I/Zn/CuI/DMF.\textsuperscript{21} The first use of FSO\textsubscript{2}CF\textsubscript{2}CO\textsubscript{2}Me for trifluoromethylation of nucleosides was described by Beal and co-workers.\textsuperscript{22} They reported the synthesis of a protected phosphoramidite of 6-trifluoromethylpurine ribonucleoside for its use in site-specific incorporation of 6-trifluoromethylpurine into RNA, to study RNA structure and RNA- modifying enzymes, particularly the RNA-editing adenosine deaminases. The elegance of liquid MFSDA or CF\textsubscript{3}-TMS compared to the gaseous and toxic CF\textsubscript{3}I makes the first reagents more appropriate for synthesis of these type of compounds.
Holy did not describe reactions with 6-bromopurines, which prompted us to include in our investigation the reaction on 6-bromo-9-tetrahydropyran-2'-yl purine. In addition, we applied the conditions to 6-halopurines with small alkyl group at position N-9. As substrates for the intended trifluoromethylation we required molecules as depicted in scheme 2.2.

Alkylation of 6-chloropurine and 6-bromopurine was accomplished with sodium hydride and methyl iodide or ethyl iodide in DMF. As expected from literature, the N-7/N-9 ratio in the product is about 40/60. Extensive column chromatography yielded the N-9 compound in good purity.

Scheme 2.1. Alkylation of 6-halopurines a) NaH, alkyl iodide, DMF, 0°C

Using Holy’s conditions (CF₃TMS) we were not able to isolate the 6-trifluoromethylated products, only trace amounts of possible trifluoromethylated products were found. Many unidentified degradative side reactions seemed to occur. In S_NAr reactions an approximate order of leaving group ability is F ≥NO₂>Cl≥Br≥I. However, during our experiments with bromo, iodo and chloro substituents we were not able to get high yields of the trifluoromethyl substituted purines(around 10-20% conversion). Even the reported successful combination (X=I, R=THP) did not work in our hands. The use of MFSDA, CuI in HMPA and DMF was also not successful for our set of substrates.
Scheme 2.2. Unsuccessful trifluoromethylation of 6-halopurines: a) CF$_3$-TMS, KF, CuI, DMF/NMP, 60°C, 20h (Holy) or FSO$_2$CF$_2$CO$_2$Me, CuI, HMPA, DMF, 70°C (Veliz/Beal).

Therefore we considered alternative strategies based on potentially more activated substrates bearing 2-NO$_2$ substitution.

2.3 NITRATION OF PURINE BASES

Purine nitration chemistry was studied in our group during several years. As we discovered that the introduction of the nitro substituent at the 2-position resulted in activation of the C-6 substituents, allowing that nucleophilic substitutions proceed at lower temperatures, this strategy was an intriguing option for trifluoroalkylation. Therefore, purine nitration was investigated for N-9-alkyl and N-9-THP-6-chloropurines and the corresponding N-9 triacetate protected riboside of 6-chloropurine (Scheme 2.3).

Scheme 2.3. Nitration of protected 6-chloropurine analogs a) TBAN/TFAA, DCM

Nitration of purines with tetrabutylammonium nitrate/ trifluoroacetic anhydride (TBAN/TFAA) works well with the slightly electron withdrawing triacetylribose moiety at N-9. 6-
Chloropurine riboside triacetate was nitrated in good yield. In contrast, the nitration of tetrahydropyranyl protected purines gave moderate yields, despite the analogies between the electronic properties of ribosides and the THP group. The nitration of N-9 methyl, ethyl substituted purines also proceeds in moderate yields (46 and 30%).

2.4 TRIFLUOROMETHYLATION OF NITRATED PURINES

New conditions were developed to introduce the trifluoromethyl group in 2-nitropurines. The original conditions applying CF$_3$TMS at high temperature with addition of CuI and KF described by Holy did not work for these compounds. The compounds degraded very rapidly, probably as a result of the high reactivity of the nitro activated system. This prompted us to critically evaluate the reagents and lower the temperature.

CuI is often used as an accelerator in substitution reactions on halogenated aromatic systems. Via a tetrahedral transition state between the copper, the halogen atoms and the purine system, substitution is facilitated. Since we found degradative side products, which indicated too high reactivity, we abolished copper iodide for activation. Omitting CuI and lowering the temperature we found small amounts of trifluoromethylated product (Scheme 2.4). However, the reaction results were variable until we further changed parameters and looked further into the properties of the reagents. We used other fluoride donors (KF, CsF and tetramethylammonium fluoride (TMAF)) and varied the stoichiometry of reagents used, the solvent (THF, DMF, acetonitril) and the reaction temperature (-70°C->20°C). It is envisaged that an attack of the fluoride at the Si atom generates the CF$_3$⁻ nucleophile in situ. The driving force is the high bond strength of the SiF bond formed.

![Scheme 2.4. Trifluoromethylation a) CF$_3$-TMS, CsF, THF, 0°C](image-url)
We observed that it was critical to pre-dry the fluoride donor \textit{in vacuo} at 300°C and that all other reaction components should be extremely dry. Reactions should be performed under pre-dried nitrogen or an argon atmosphere. The optimal reaction temperature is 0°C! At higher temperatures we observe degradation of the product. The activating fluoride donor obviously plays a role in this, for instance via stimulation of nucleophilic attack at C-8.

In this way we were able to optimise the yield of trifluoroalkylation up to 40% for the best performing triacetylfuranosyl protected purines.

After we published our novel routes to 6-trifluoromethyl substituted purines, very recently the laboratory of Iaroshenko described several alternative routes of preparing mono- or 2,6-di-substituted trifluoromethylpurines or fused pyridines.\textsuperscript{23} One method describes the reaction of 2-(dialkylamino)-1,3-thiazol-4-amines and 1,2-dimethyl-1H-imidazol-5-amine with aryl isocyanates and 2,4,6-tris(trifluoromethyl)-1,3,5-triazine leading to thiapurines and purines containing trifluoromethyl groups in the 2- and 6-positions of the pyrimidine ring.\textsuperscript{24} The other route acts via inverse electron-demand Diels-Alder reaction between trifluoromethyltriazines and electron-enriched amino heterocycles, finally coupled to a ribose moiety to yield a 2,6-di-trifluoromethyl substituted adenosine analog.\textsuperscript{25}

\textbf{2.5 BOC PROTECTION OF PURINES AND PURINE NITRATION}

However, meanwhile we were looking at ways to optimise synthetic routes towards nitrated purine bases. Recently, the \textit{tert}-butoxycarbonyl group (Boc) was published as a protective group for purines by Dey and Garner.\textsuperscript{26} They used it as an acid-labile group to protect exocyclic amines in purine-containing \(\alpha\)-helical peptide nucleic acids (\(\alpha\)PNAs). In our opinion, the Boc group could also have interesting properties for purine core nitration: after nitration and/or substitution, the Boc group can be cleaved off via mild acidolysis an replaced by other functional groups. An overview of the reactions of amines and alcohols with di-\textit{tert}-butyl dicarbonate and 4-(dimethylamino)pyridine, including mechanistic details was reported by Hassner and Basel.\textsuperscript{27}
While Garner focused on the synthetic aspects of di-amino adenines and guanines, we were also interested in the mechanistic aspects. Therefore a number of 6-substituted purines such as adenine and 6-chloropurine were protected with Boc₂O and DMAP to give, after equilibration of the initially formed mixtures of N-7 and N-9 isomers, good yields of the thermodynamically favoured N-9 Boc-substituted products (Scheme 2.5 and Table 2.2). For the unsubstituted purine (R=H) the thermodynamically favoured isomers turned out to be N-7.

![Scheme 2.5. Boc protection of purines]

**Table 2.2 Boc protection of purine derivatives**

<table>
<thead>
<tr>
<th>Starting compound R</th>
<th>Product R</th>
<th>equiv. Boc₂O</th>
<th>time</th>
<th>ratio N-9/N-7</th>
<th>isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₂</td>
<td>NBoc₂</td>
<td>4</td>
<td>1 h</td>
<td>68 / 32</td>
<td>79</td>
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<tr>
<td>NH₂</td>
<td>NBoc₂</td>
<td>4</td>
<td>18 h</td>
<td>73 / 27</td>
<td>78</td>
</tr>
<tr>
<td>NH₂</td>
<td>NBoc₂</td>
<td>4</td>
<td>96 h</td>
<td>92 / 8</td>
<td>82</td>
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<tr>
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<td>3</td>
<td>3 h</td>
<td>100 / 0</td>
<td>90</td>
</tr>
<tr>
<td>Cl</td>
<td>Cl</td>
<td>3</td>
<td>6 h</td>
<td>100/0</td>
<td>95</td>
</tr>
<tr>
<td>OH</td>
<td>OBoc</td>
<td>3</td>
<td>5 h</td>
<td>100 / 0</td>
<td>81</td>
</tr>
<tr>
<td>OBn</td>
<td>OBn</td>
<td>1.5</td>
<td>1 h</td>
<td>80 / 20</td>
<td>87</td>
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<td>OBn</td>
<td>OBn</td>
<td>1.5</td>
<td>18 h</td>
<td>98 / 2</td>
<td>89</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>1.5</td>
<td>1 h</td>
<td>14 / 86</td>
<td>96</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>1.5</td>
<td>18 h</td>
<td>4/96</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>1.5</td>
<td>44 h</td>
<td>0 / 100</td>
<td></td>
</tr>
</tbody>
</table>

We found that the Boc group at N-9 is not only acid labile, but is in particular sensitive for nucleophilic attack. Reflux in methanol or in aqueous acetonitril is sufficient to remove the
Boc group. A further example of this sensitivity is observed during the DMAP-catalyzed formation of the Boc purines: the kinetically controlled mixture of N7 / N9 isomers that is initially formed, equilibrates in favour of the more stable N-9 isomer after prolonged reaction times (see Table 2.2). Obviously DMAP reacts reversibly with the Boc substituent in the product via an intermediate salt, depicted in Scheme 2.5 (R = H).

Recently, in a similar way, N-6 Boc carbamate protection of purines was described for N6 alkylation via Mitsunobu conditions offering a milder alternative to the traditional nucleophilic aromatic substitution of the corresponding 6-chloropurines.²⁸

The electron withdrawing and hydrolytically cleavable Boc substituent displayed excellent properties during nitration of the purine C-2 position by TBAN/TFAA. The nitration of Boc protected purines showed to be superior to the reaction with N9-alkyl and THP protected purines.

As can be seen in Scheme 2.6, nitration of N-9 Boc analogs of 6-chloropurine, 6-N-diboc-aminopurine and 6-O-benzylpurine with tetrabutylammonium nitrate/ trifluoroacetic anhydride (TBAN/ TFAA) at 0 °C was fast and high yielding. On the other hand, N-9 Boc-protected hypoxanthine (R = OH) and both N-7 and N-9 Boc purine derivatives (R = H in scheme 2.6) gave no 2-nitro product, similarly to earlier findings in purine nitration. Interestingly, in contrast to the N-9 isomer, the N-7 isomer of Boc protected adenine could not be nitrated under these conditions.
Scheme 2.6. nitration of Boc protected purines, followed by deprotection a) TBAN/TFAA, DCM b) TFA, rt c) MeOH, reflux

Removal of the Boc-group from the N-9 position of 6-chloro-2-nitropurine was readily accomplished under acidic conditions (TFA, room temperature) or neutral conditions (methanol, room temperature) to give 2-nitroadenine, 2-nitrohypoxanthine and 6-chloro-2-nitropurine (Scheme 2.6).

With neat TFA, tri-Boc-2-nitro-adenine was completely deprotected to 2-nitroadenine. Under the same conditions, 6-O-benzyl-2-nitro-purine directly produced 2-nitrohypoxantine. Reflux of the tri-Boc protected adenine in methanol was sufficient to remove the Boc substituent from N9 and one of the N6-Boc substituents. Treatment of the C-6 O-benzyl substituted purine with methanol left the O-benzyl group intact.

Apart from general interest in the reaction mechanism, our mechanistic studies were also aimed at elucidating the cause for the observed general pattern of substrate properties of nitration. All non 6-non-substituted (R=H) purine analogs and N7 and N-9 Boc protected derivatives (nebularine) failed to give the expected nitration. Non- or partly protected 6-NH$_2$ and 6-OH purine analogs (adenosine triacetate, mono-N-acylated, 6-OH, N9 Boc-purine) were likewise unsuccessful as substrates in substitution.

This may be explained in the next paragraphs and Chapter 5, where focus is on the mechanism of (deaza-)purine nitration.
Anti tumour drug development using nitro and Boc chemistry

In parallel to our efforts in synthesizing adenosine receptor ligands, the Boc group and nitration chemistry could be successfully applied to the synthesis of antitumor drugs: We described the synthesis of methyltriazene prodrugs simultaneously releasing DNA-methylating agents diazomethane, methylhydrazine and the inhibitor of the DNA repair protein alkylguanine-DNA alkyltransferase (AGT), antiresistance drug $O^6$-benzylguanine.\textsuperscript{29} AGT is a suicide enzyme that is degraded after transferring a single methyl group.

![Figure 2.2](image)

**Figure 2.2** Antitumor agent temozolomide (left) and the methyltriazene prodrug and its conversion to cytostatic compound diazomethane and the AGT enzyme inhibitor

Hydrolytic pathways of several substrates were studied, revealing that the p-nitrophenyl substituted triazene had an optimal hydrolysis rate with a half life of 23 minutes, closely resembling that of existing antitumor agent temozolomide and has a 100% selectivity for the desired fragmentation route yielding $O^6$-benzylguanine and diazomethane. In vitro antitumour studies in the 60 human tumour cell line panel of the National Cancer Institute show a tenfold higher activity (IC\textsubscript{50} value of 10 µM) compared to temozolomide (IC\textsubscript{50} value of 100µM).

Our proposed hydrolytic routes and active methylating species were acknowledged in a recent report from the Food and Drug administration on the drug approval of temozolomide in combination with radiotherapy\textsuperscript{30} and in several studies on carbamate hydrolysis\textsuperscript{31} and antitumor drug reviews\textsuperscript{32, 33}. 
2.6 MECHANISM OF PURINE NITRATION

2.6.1 INTRODUCTION

The Boc protected purines could not be used for subsequent trifluoromethylation experiments, because the protective group is too labile under the reaction conditions. However, the good nitrating properties and high yields prompted us to use this purine derivative for further studies towards the mechanism of purine nitration. In addition, in the $^1$H NMR spectrum the N9-Boc group gives only one singlet in the alkyl region, while ribose protected purines give a range of NMR signals and interactions. The methyl protected purine also fulfills this prerequisite, but is hampered by low nitration yields. To unravel the reaction mechanism, the 6-Cl-N9-Boc purine seemed to have the optimal properties to start our quest.

We also observed surprising properties of the nitration reaction: Purines and 1-deazapurines with 6-chloro, 6-nitro, protected 6-hydroxyl, and doubly protected 6-amino functionalities are readily nitrated. Unprotected purines (mono-N6-acylated), adenosine triacetate, and nebularine (6-H-purine-riboside) triacetate did not give any of the expected nitration. $^{34,35}$ We observed the same trend in the nitration of Boc protected purines.

The mechanistic studies have been published and are presented in paragraphs 2.6.2-2.6.6. $^{36}$

2.6.2 TRIFLUORACETYL NITRATE AS NITRATING SPECIES

The use of the TBAN-TFAA mixture for electrophilic aromatic nitration was reported by Masci as an adaptation of Crivello’s nitration system, consisting of TFAA and heterogeneous metal or ammonium nitrates in inert solvents. $^{37}$ The active species in both methods is trifluoroacetyl nitrate, TFAN, formed in situ, as shown in Scheme 2.7. Generally nitronium ions formed from the heterolysis of TFAN, are predicted to be the nitrating species. However, mechanisms involving covalent TFAN$^{38}$ and radical pathways via homolysis are also viable. $^{39}$
The selective introduction of the nitro group in the deactivated purine system at the highly electron-deficient C-2 position and not at the C-8 position is remarkable; it makes both direct electrophilic nitration and the alternative mechanism via electron transfer unlikely, taking the high oxidation potential of purines into account. Moreover, upon TBAN-TFAA nitration of solid-supported purine nucleosides, no substantial nitration of phenyl rings in the polystyrene matrix was detected, indicating the presence of negligible amounts of strongly electrophilic nitronium ions. Therefore, in an earlier publication from our group, a radical nitration pathway was proposed (Scheme 2.8).

Homolytic cleavage of trifluoroacetyl nitrate generates the trifluoroacetoxy and nitrogen dioxide radicals, presented in scheme 2.7. Addition of the reactive trifluoroacetoxy radical to the purine C-8 (Scheme 2.8) gives a highly delocalized radical that is stabilized by the lone pair of the substituent at C-6. Subsequent combination of this intermediate with NO$_2^\cdot$ at C-2 and elimination of trifluoroacetic acid, yields the 2-nitro product. A weak point in the mechanism proposed in Scheme 2.8 is the putative generation of the very unstable trifluoroacetoxy radical, which is known to rapidly decompose to CO$_2$ and the trifluoromethyl radical with a reported dissociation constant of $k > 5\cdot10^4$s$^{-1}$. During TBAN-TFAA purine nitration, we did not observe formation of species such as CO$_2$ or CF$_3$H that...
might indicate homolytic cleavage of trifluoroacetyl nitrate as the initiating step. These findings urged us to take a deeper look into the nitration mechanism.

### 2.6.3 DETECTING A 7-NITRAMINO PURINE INTERMEDIATE

In our search to clarify the mechanism of purine nitration we tried to extensively follow a nitration reaction with NMR. As discussed in paragraph 2.6.1 the Boc protected 6-chloropurine seemed to be an excellent candidate for *in situ* NMR research, with good substrate properties for nitration.

When we monitored the nitration reaction mixture at -10°C, we were able to detect signals for an intermediate with increasing concentration in time, followed by a decrease and complete product formation in five hours as depicted in Figure 2.3.

![Scheme 2.9. TBAN/TFAA nitration in NMR tube a) TBAN / TFAA 1.6 equiv., -10°C, 1h](image.png)
Figure 2.3 Aromatic region of $^1$H-NMR spectra of the nitration of 6-chloro-9-Boc-purine at -10 °C in CD$_2$Cl$_2$.

The progress of the nitration is represented graphically in Figure 2.4 as a plot of the normalized integral values against time.
A successful attempt to “freeze” the nitration reaction in the intermediate stage allowed extensive spectroscopic characterization of the intermediate. The progress of intermediate formation was monitored at -50 °C, and complete conversion of 6-chloropurine 1 into intermediate 3 over an 8 h period was observed, while formation of 2-nitro product 2 was suppressed to less than 3%. By using 98% 15N-labelled TBAN, prepared from TBACl and 15N-NaNO₃, we were able to identify a doublet at 339.6 ppm with $J_{NH} = 2.7$ Hz (table 2.3). In the corresponding $^1$H-spectrum a doublet at 8.82 ppm with $J_{NH} = 2.7$ Hz was found instead of a singlet. A $^1$H decoupling experiment confirmed $^1$H - $^{15}$N coupling. The value of the coupling constant points toward a $^3$J$_{NH}$ coupling. Also, the $^{15}$N NMR spectrum revealed that intermediate 3 contains an N-nitro group and no C-nitro or -nitrato moiety. The $^{15}$N chemical shift of the doublet of intermediate 3 typically lies within the range of N-nitro compounds, which is generally shifted about 20 ppm upfield relative to that of C-nitro compounds.⁴¹ Peaks derived from covalently bound nitrates, which appear at even higher field values relative to N-nitro derivatives, were not detected.⁴² The $^{15}$N signal of the nitro group in product 2, a singlet at 365.0 ppm, displays a chemical shift value characteristic for aromatic C-NO₂ compounds.
In the $^{13}$C spectrum of starting material 1, C2 was found at 153.5 ppm with $^1J_{CH} = 211$ Hz and C8 at 144.3 ppm with $^1J_{CH} = 222$ Hz. In the spectrum of intermediate 3, the values for C2 ($\delta = 157.7$ ppm, $^1J_{CH} = 212$ Hz) were similar to those of the starting material, but C8 showed a remarkable upfield shift to 93.0 ppm and a decreased $^1J_{CH}$ value of 194 Hz. Moreover, C-N coupling with $^2J_{CN} = 1.8$ Hz was observed for C8 in experiments with $^{15}$N-labelled TBAN.

Table 2.3. NMR data from the nitration of 6-Cl-9-Boc-purine with $^{15}$N-labelled TBAN$^a$

<table>
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<tr>
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<th>6-Cl-9-Boc-purine 1</th>
<th>intermediate 3</th>
<th>2-NO$_2$-6-Cl-9-Boc-purine 2</th>
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<td>$^1$H-2</td>
<td>8.86 (s)</td>
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<tr>
<td>$^1$H-8</td>
<td>8.63 (s)</td>
<td>8.82 (d)</td>
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<td>$^1J_{CH} = 212$</td>
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<td>93.0 (dd)</td>
<td>148.3 (d)</td>
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<tr>
<td></td>
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<td></td>
<td>$^2J_{CN} = 1.8$</td>
</tr>
<tr>
<td>$^{15}$NO$_2$</td>
<td>339.6 (d)</td>
<td>$^5J_{NH} = 2.7$</td>
<td>365.0 (s)</td>
</tr>
<tr>
<td>C-$^{19}$F</td>
<td>-75.86 (s)</td>
<td>$^3J_{NH} = 2.7$</td>
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</table>

$^a$ Recorded in CD$_2$Cl$_2$ at $-50$ °C.

These results indicated that aromaticity was retained in the pyrimidine part, but not in the imidazole ring. In addition, the presence of a trifluoroacetoxy group in intermediate 3 was observed with $^{13}$C NMR, as indicated by a quartet at 114.2 ppm ($^1J_{CF} = 290$ Hz) and a double quartet at 153.8 ppm ($^2J_{CF} = 44$ Hz, $^3J_{CH} = 2.9$ Hz). The $^{19}$F NMR spectrum established the presence of the trifluoroacetoxy group, which appeared as a singlet at -75.86 ppm. With the help of CH correlation spectra and the observed long-range couplings, the intermediary singlet in $^1$H NMR at 8.78 ppm could be assigned to H2 and the doublet at 8.82 ppm to H8. The extremely high chemical shift of 8.82 ppm for H8, a proton attached to an sp$^3$ carbon atom, can be explained by the combination of three heteroatoms attached to C8, the electron-withdrawing effect of the nitro, Boc, and trifluoroacetoxy groups and the anisotropic effect of the carbonyl and nitrosyl moieties. During the reaction, the concentration of TFA increases.
and H8 of 3 showed a gradual upfield shift of about 0.2 ppm as a consequence of partial protonation, which disturbs the anisotropic effect. These combined NMR data led us to the structure assignment for the N-nitro intermediate 3, as shown in Figure 2.4.

### 2.6.4 NITRAMINE REARRANGEMENT OF PURINES

The previously proposed radical addition mechanism in Scheme 2.8 appeared to be inconsistent with the obtained NMR results and a new three step nitration pathway was suggested as depicted in Scheme 2.10. The purine ring system is N-nitrated at nitrogen atom 7 in the imidazole ring by electrophilic attack of TFAN. The highly electrophilic imidazolium cation is rapidly trapped by a nucleophile present, in this case trifluoroacetate, furnishing the observed nitramine intermediate. Some nitramine rearrangements refer to the migration of the nitro group of N-nitroaniline to the ortho and para positions to yield a mixture of o- and p-nitroaniline. If this would go similar, then in the case of the purine nitramine intermediate, both ortho positions C4 and C6 are blocked, leaving para carbon atom 2 as the only available position for migration of the nitro group. Which makes aromatization to 2 via elimination of trifluoroacetic acid possible.

\[
\text{Scheme 2.10 TBAN/TFAA formation of intermediate 3 followed by a nitramine rearrangement}
\]
To validate that formation of 2-nitropurine 2 takes place via a monophasic process of rearrangement of the N7-nitro intermediate and not via other routes, we preformed nitramine intermediate 3 at -50 °C in CDCl₃ and monitored its conversion into 2-nitro purine 2 with ¹H NMR at -10 °C. In Figure 2.5, the progress of the normalized integral values of H8 of nitramine intermediate 3 and 2-nitro purine 2 is represented graphically.

The excellent first-order kinetics confirms the expected unimolecular process. First-order rate coefficients, \( k_N = 1.5 \cdot 10^{-3} \text{ s}^{-1} \) (nitramine 3 decrease) and \( k_P = 1.9 \cdot 10^{-3} \text{ s}^{-1} \) (product 2 increase), were determined over about 4 half-lives with a high \( R^2 \) value and good reproducibility. The minor difference in the values of \( k_N \) and \( k_P \) is explained by the occurrence of side reactions; a subsequent reaction of the product would decrease the magnitude of its signal at the end of the reaction and thus cause the extent of reaction at earlier times to be overestimated.

As both thermal⁴⁴ and acid-catalyzed⁴⁵,⁴³ nitramine rearrangements of nitroanilines have been reported, we also allowed the rearrangement to proceed in the presence of the base DIPEA, and identical reaction rates were found. This proved that the rearrangement was thermal and not acid catalyzed. Moreover, if the rearrangement would have been acid catalyzed, the reaction rate would be expected to increase during the course of the reaction, as
TFA is generated upon product formation. Deviation from first-order correlation was not observed.

### 2.6.5 $^{15}$N CIDNP NMR IN PURINE NITRATION

With the unimolecularity of the reaction now being established, we chose to further study the mechanism of the rearrangement with $^{15}$N NMR, which has been a valuable tool in elucidating reaction pathways in nitration reactions.\textsuperscript{46} Several mechanisms have been put forward for the nitramine rearrangement, both heterolytic and homolytic pathways. The observation of CIDNP effects in $^{15}$N NMR spectra during the rearrangement of nitroaniline derivatives would offer convincing evidence that radicals were involved, thus supporting a homolytic rearrangement mechanism.\textsuperscript{47} Chemically Induced Dynamic Nuclear Polarization refers to the perturbation of the nuclear spins away from the expected Boltzmann distribution.\textsuperscript{48} The effect is observed in NMR spectra as an abnormal intensity of the NMR signals; the signals display either enhanced absorption or emission.

From the phase of the polarized NMR signals, one can then conclude whether the rearrangement occurs intra- or intermolecularly. If the product is formed in an intramolecular fashion, enhanced absorption is observed. If the product is formed in an intermolecular fashion, it is the result of recombination of the radicals after separation of the original pair and emission is observed. Conclusions are less obvious if the rearrangement has both an intra- and an intermolecular component. Since the observed NMR signal matches the sum of the unpolarized and polarized (positive and/or negative) material formed, even no net polarization at all can be the consequence. When we followed the rearrangement of nitramine 3 at 0°C with $^{15}$N NMR, indeed, clean CIDNP effects were observed (Figure 2.6).
During several half-lives of the rearrangement, the doublet of nitramine intermediate 3 at 339.6 ppm showed enhanced absorption. In the early stage of the reaction at \( t = 2 \) min, the singlet of C2-NO\(_2\) product 2 at 365.0 ppm showed a short emission signal. During the remainder of the rearrangement, a reduced absorption signal was observed until, after about 4 half-lives (reaction nearly complete) no CIDNP effects were observed any longer. These results can be explained with the radical mechanism shown in Scheme 2.11.

**Figure 2.6** \(^{15}\text{N}\) CIDNP NMR spectra of the rearrangement of 0.45 M nitramine intermediate 3. S: nitrobenzene standard; N: nitramine intermediate 3; P: 2-nitro product 2.
The enhanced absorption of nitramine intermediate 3 indicates the reverse reaction of the radical pair to re-form nitramine 3 by immediate collapse of the primary radical pair. The initial emission signal for product 2 points to an intermolecular process in which the paired radicals separate and become free radicals. The latter re-encounter to re-form the radical pair and, subsequently, the secondary recombination C-2 nitro intermediate, which immediately eliminates trifluoroacetic acid to render product 2. In the early stage of the reaction, this contribution to the NMR signal is larger since the concentration of the escaped (i.e., free) radicals is higher as is the chance of random free-radical encounters.

In a radical trapping experiment with hydroquinone, which only reacts with free radicals and not with paired radicals, we examined the exact contributions of the inter- and intramolecular components of the rearrangement.
Using this method, White has shown that the nitramine rearrangement of, for example, \(N\)-methyl-\(N\)-nitroaniline, has both an intra- and intermolecular component. Monitoring the rearrangement of nitramine intermediate 3 in the presence of 3 equiv of hydroquinone with \(^{15}\)N NMR revealed that only 30-35\% of 2-nitro product 2 was still generated. This indicated that 65-70\% of the purine nitramine rearrangement occurred intermolecularly and led to reformation of 1. As expected, a greatly enhanced absorption was observed for product 2 due to the absence of the emittive contribution to the NMR signal (Figure 2.7).
In agreement with this, the signal of nitramine intermediate 3 still displayed enhanced absorption. When this experiment was repeated with the radical scavenger TEMPO, only 10% of product formation was observed. Probably TEMPO intervenes with both free and paired radicals, thus almost completely inhibiting the rearrangement.

2.6.6 MECHANISM SUMMARY

By monitoring the purine nitration with NMR, we found the nitration reaction being a three step process. Electrophilic attack by TFAN (trifluoroacetyl nitrate) on N7 results in nitramino intermediate 3. Nitramine rearrangement (Scheme 2.11 ) leads to a C-2 nitrated species that eliminates trifluoroacetic acid to yield the 2-nitro-6-chloropurine 2. The involvement of radicals has unequivocally been established via CIDNP experiments.

**Figure 2.7** CIDNP effects using hydroquinone as free radical scavenger. S: nitromethane standard; N=nitramine intermediate; P = 2-nitro product
2.7 SERENDIPITOUS FINDING OF A NEW PROTECTIVE GROUP FOR PURINES: BOCOM

In our search for new protective groups for efficient purine nitration, it was surprisingly found that when 6-chloro-9-Boc-purine was stirred in dichloromethane solution containing para-formaldehyde this results in a very stable analog substituted on N9 with –CH₂-O-Boc (Boc-O-Me-, Bocom).

Scheme 2.13 Synthesis of N9 Bocom-protected 6-chloropurine, followed by nitration: a) para-formaldehyde, DMAP, DCM, reflux 65%, b) TBAN/TFAA, DCM, 1,5 h. 65%

The Bocom protective group proved to be very stable and not very sensitive to nucleophilic attack, but can be efficiently removed via sodium methoxide treatment. Nitration with TBAN/TFAA furnished the nitrated derivative in reasonably good yield, 65%, (scheme 2.13).

The excellent stability of this group was shown in the presence of the reagents of the trifluoroalkylation reaction. We were able to isolate 31% of the trifluoromethylated product. This new method, gives a fast route to 2-nitro-6-trifluoromethyl substituted purines.

Scheme 2.14 Introduction of CF₃ at Bocom protected purines a) CF₃-TMS, CsF, THF, -20 - 0°C, 31%
2.8 CONCLUDING REMARKS

We reported the introduction of the C-2 nitro group via the TBAN/TFAA purine nitration on protected 6-chloropurine bases. Nitration was initially performed with medium yields on alkyl, THP and triacetyl-ribose analogs. Boc and Bocom protected derivatives proved to be particular suited substrates. The Boc group appeared to be very suitable for investigating the mechanism of purine nitration. With NMR we were able to disclose that the mechanism of purine nitration proceeds via electrophilic addition of TFAN followed by a radical rearrangement of the nitramine formed.

In summary, it was clearly shown that we developed new procedures to introduce a trifluoromethyl group at the C-6 position of purines. Literature procedures require high temperatures and metal ions, while the presently described trifluoromethylation reaction is performed at -20°C or in an ice bath using only cesium fluoride and trifluoromethyl silane reagent, starting from C-2 nitrated purines. The nitro group clearly enhances substitution of the halogen by the trifluoromethyl group. The newly found and stable Bocom group proved to be efficient in purine nitration and trifluoroalkylation. Several trifluoromethylated purine bases and ribosides have been made with a nitro group at C-2, to serve as a handle for further functionalization at the C-2 position.

2.9 ACKNOWLEDGEMENTS

Martin Wanner has been at the basis of purine nitration research in our lab. His important results with Boc and Bocom protected systems opened new perspectives in purine nitration. His fascination for the mechanism of the nitration reaction, stimulated Boris Rodenko and the author to study the reaction in full detail. Jan Geenevasen, Jan Meine Ernsting and especially Lidy van der Burg are acknowledged for their assistance with operating the 500 MHz NMR spectrometer with the advanced CIDNP experiments.

2.10 EXPERIMENTAL

General

All reagents and solvents were used as commercially available, unless indicated otherwise. Flash chromatography refers to purification using the indicated eluents and Janssen Chimica
silica gel 60 (0.030–0.075 mm). Melting points were measured with a Leitz melting point microscope. Infrared (IR) spectra were obtained from CHCl₃ solutions unless indicated otherwise, using a Bruker IFS 28 FT-spectrophotometer and wavelengths are reported in cm⁻¹. Proton nuclear magnetic resonance (¹H NMR) spectra and carbon nuclear magnetic resonance (¹³C NMR; APT) spectra were determined in CDCl₃ at 300 K using a Bruker ARX 400 spectrometer, unless indicated otherwise. All nitration monitoring experiments (¹H, ¹³C, ¹⁵N, ¹⁹F) were carried out on a Varian Inova 500 spectrometer operating at 11.74 T (499.9 MHz for ¹H; 125.7 MHz for ¹³C; 50.7 MHz for ¹⁵N, 470.4 MHz for ¹⁹F using a 5 mm SW probe or a 10 mm broadband tunable probe. The spectra were determined in deuterated chloroform or dichloromethane obtained from Cambridge Isotope Laboratories Ltd. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane (¹H, ¹³C) or liquid NH₃ (¹⁵N) or CCl₃F (¹⁹F). Coupling constants J are given in Hz. For the nitration experiments the solvents were run over a neutral alumina plug prior to use. Na¹⁵NO₃ (98%¹⁵N) was purchased from Aldrich.¹⁵N-nitrobenzene was obtained according to Shackelford’s method for the nitration of benzene by replacing tetramethylammoniumnitrate with ¹⁵N-labelled TBAN. Dichloromethane was distilled freshly prior to use subsequently from phosphorous pentaoxide and calciumhydride. All other commercially available chemicals were used without further purification. Elemental analyses were performed by Kolbe, Mülheim a.d.Ruhr, Germany.

**6-chloro-9-methylpurine**

A solution of 20 g of 6-chloropurine (0.13 mol) in 75 ml dry DMF was cooled in an ice-bath. 6.72 g (0.168 mol, 60% disp. in oil) of NaH was added in portions and the solution was stirred for ten minutes. 10.45 ml (0.168 mol) of MeI was added slowly. The reaction was followed via TLC. After 5 hours the reaction was quenched by adding water (200 ml). The solvent mixture was removed on a rotary evaporator under reduced pressure in a water bath of 60°C. The crude residue was dissolved in water and extracted with DCM + 5% MeOH. The organic fractions were collected and concentrated under reduced pressure (24 g, 9-Me/7-Me 3:2). Column chromatography (DCM + 1% MeOH) yielded 12.49 g (0.074 mol) pure product (57%) and some mixture (7/9) fractions. ¹H NMR (DMSO-d₆): 9-Me δ 8.79 (s, H₈), 8.66 (s, H₂), 3.88 (s, 9-Me) 7-Me δ 8.79 (s, H₈), 8.74 (s, H₂), 4.10 (s, 7-Me)
6-chloro-9-methyl-2-nitro-purine

TFAA (3.95 ml; 28 mmol) was added dropwise to a solution of 6-chloro-9-Me-purine (3 g; 17 mmol) and TBAN (8.65 g; 28 mmol) in dry DCM (50 ml) at 0 °C under a nitrogen atmosphere. After stirring for 1.5 h the solution was poured into 100 ml of sat. aqueous NaHCO3-ice (1:1) and Et2O (100 ml) was added. The aqueous layer was extracted with 3 portions of 60 ml Et2O-CH2Cl2 (3:1). The collected organic layers were washed with H2O (2x50 ml) and brine (1x50 ml) and dried with Na2SO4. Evaporation to dryness afforded the crude product (2 g). Stirring in MeOH and filtration yielded the pure product (1.66 g; 46%).

1H NMR (CDCl3) δ 8.37 (s, 1H, H-8), 4.06 (s, 3H, Me)

9-methyl-2-nitro-6-trifluoromethyl-purine

To a suspension of CsF (0.144 g, 0.95 mmol) and 2-NO2-6-Cl-9-Me-purine (0.100 g, 0.468 mmol) in dry THF (5 ml) CF3TMS (1.87 mmol) was added at 0°C. After 5 minutes of stirring the ice/water bath was removed and the mixture was stirred vigorously at room temperature. After 6 h the reaction was diluted with ether and water. The waterlayer was extracted 3 times with ether. The combined organic layers were dried with a saturated NaCl solution and treated with sodium sulphate. The solution was concentrated to dryness under reduced pressure. The resulting oil was purified by flash chromatography (eluens EA) and concentrated under reduced pressure. The resulting pure product (0.35 g, 30%) was obtained as a white foam.

1H-NMR (400MHz, CDCl3), δ [ppm]: 8.27 (s, 1H, H-8), 4.01 (s, 3H, N-Me)

19F-NMR (500MHz, CDCl3), δ [ppm]: -69.01 (6-CF3).

9-Boc-6-chloro purine 1

A suspension of 6-chloropurine (15.5 g; 0.10 mol), Boc2O (31 g; 0.14 mol) and DMAP (0.3 g; 2 mmol) in dry CH2Cl2 (150 ml) was stirred for 3 h until a clear solution was obtained. Light petroleum (25 ml) and silica gel (10 g) were added, the mixture was filtered over highflow and the solids were rinsed with EtOAc. Evaporating the solvent yielded the crude product (24.4 g; 96%). Recrystallisation from a mixture of EtOAc-light petroleum afforded a first batch of white needles (12.1 g; 48%). A second batch was obtained by recrystallising the concentrated filtrate (10.5 g; 41%). decomp. > 111 °C; 1H NMR (CDCl3) δ 8.90 (s, 1H, H-2), 8.55 (s, 1H, H-8), 1.71 (s, 9H, CCH3). 13C NMR (CDCl3) δ 153.46 (d, J 211.2, C-2), 151.06
(d, J 13.2, C-6) 150.84 (dd, J 12.6, J 4.7, C-4), 144.83 (CO), 144.26 (d, J 221.6, C-8), 132.02 (dd, J 12.7, J 1.2, C-5), 87.49 (-CCH₃), 27.22 (q, J 127.6, -CCH₃). H-8 was identified by a NOE-experiment (400 MHz, CDCl₃): saturation (4.5 s) of the t-Bu protons led to 0.29 % NOE on H-8 and 0 % on H-2.

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**9-Boc-6-chloro-2-nitro-purine 2**

TFAA (2.25 mL; 16 mmol) was added dropwise to a solution of 6-chloro-9-Boc-purine (2.55 g; 10 mmol) and TBAN (4.87 g; 16 mmol) in dry CH₂Cl₂ (25 ml) at 0 °C under a nitrogen atmosphere. After stirring for 1 h the solution was poured into 75 mL of sat. aqueous NaHCO₃-ice (1:1) and Et₂O (75 ml) was added. The aqueous layer was extracted with 3 portions of 50 mL Et₂O-CH₂Cl₂ (3:1). The collected organic layers were washed with H₂O (2x50 mL) and brine (1x50 mL) and dried with Na₂SO₄. Evaporation to dryness afforded the crude product (2.82 g; 94%). Trituration with cold MeOH furnished 2-nitro-6-chloro-9-Bocpurine 2 as a light yellow solid (2.58 g; 86%). decomp. > 117 °C; 1H NMR (CD₂Cl₂) δ 8.92 (s, 1H, H-8), 1.72 (s, 9H, CCH₃). 13C NMR (CD₂Cl₂) δ 154.15 (C-2), 152.97 (C-6) 151.40 (d, J=4.5,C-4), 148.33 (d, J= 223.4, C-8), 144.47 (CO), 135.49 (d, J=12.1, C-5), 89.49 (-CCH₃), 27.55 (q, J 127.6, -CCH₃).

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

To a suspension of CsF (5.3 g, 35 mmol) and 6-chloro-9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-2-nitropurine (10.0 g, 21.8 mmol) in dry THF (40 ml) CF₃TMS (5.2 ml, 35 mmol) was added at 0°C. After 5 minutes of stirring the ice/water bath was removed and the mixture was stirred vigorously at room temperature. The reaction was followed by HPLC (Rₜ,product = 3.7 and Rₜ,reactant = 3.3). After 19 h the reaction was quenched by adding silica gel. The suspension was concentrated to dryness under reduced pressure. The resulting powder was purified by column chromatography using a gradient mixture of MTBE/MeOH.
Chapter 2

(MTBE with 1% MeOH – MTBE with 4% MeOH) and concentrated under reduced pressure. The resulting pure product (2.7 g, 25%) was obtained as a light yellow foam.

\(^1\)H-NMR (400MHz, CDCl\(_3\)), \(\delta\) [ppm]: 8.45 (s, 1H, H-8’), 6.27 (d, \(J = 5.3\) Hz, 1H, H-1’), 5.81 (t, \(J = 5.5\) Hz, 1H, H-2’), 5.62 (t, \(J = 5.1\) Hz, 1H, H-3’), 4.52 (m, 1H, H-4’), 4.43 (m, 2H, 5’-CH\(_2\)), 2.18 (s, 3H, acetyl), 2.11 (s, 3H, acetyl), 2.09 (s, 3H, acetyl).

\(^19\)F-NMR (500MHz, CDCl\(_3\)), \(\delta\) [ppm]: -69.06 (6-CF\(_3\)).

9-BocOM-6-chloropurine

To a solution of 6-chloro-9-Boc-purine (5.65 g, 22mmol) in dichloromethane was added para-formaldehyde (1 g, 33 mmol) and dimethylaminopyridine (0.27 g; 2.2 mmol). The mixture was refluxed for 4h until the conversion was complete according to HPLC. Silica was added and the solvent was evaporated. Flashchromatography (EA/PE 2:3) furnished the product in 65% yield as a colorless oil, which solidified after extensive evaporation in vacuo.

\(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.82 (s, 1H, H-8), 8.43 (s, 1H), 6.18 (s, 2H, CH\(_2\)-O) 1.49 (s, 9H, CCH\(_3\)).

9-BocOM-6-chloro-2-nitropurine

TFAA (2.25 ml; 13 mmol) was added dropwise to a solution of 6-chloro-9-BocOM-purine (2.84 g; 10 mmol) and TBAN (4.87 g; 13 mmol) in dry CH\(_2\)Cl\(_2\) (25 ml) at 0 °C under a nitrogen atmosphere. After stirring for 1.5 h the solution was poured into 75 ml of sat. aqueous NaHCO\(_3\)-ice (1:1) and Et\(_2\)O (75 ml) was added. The aqueous layer was extracted with 3 portions of 50 ml Et\(_2\)O-CH\(_2\)Cl\(_2\) (3:1). The collected organic layers were washed with H\(_2\)O (2x50 ml) and brine (1x50 ml) and dried with Na\(_2\)SO\(_4\). Evaporation to dryness afforded the crude product (2.0 g; 70%). Crystallisation from ethylacetate furnished 2-nitro-6-chloro-9-BocOM-purine as a white solid (1.85 g; 65%). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.70 (s, 1H, H-8), 6.23 (s, 2H, CH\(_2\)-O) 1.49 (s, 9H, CCH\(_3\)). IR \(\nu\) 1750

9-BocOM-2-nitro-6-trifluoromethylpurine

To a suspension of CsF (1.38 g, 9 mmol) and 6-chloro-9-BocOM-2-nitropurine (2.0 g, 6 mmol) in dry THF (10 ml), CF\(_3\)TMS (2.7 ml, 18 mmol) was added at 0°C. After 5 minutes of
stirring the ice/water bath was removed and the mixture was stirred vigorously at room temperature. After 5 h the reaction was quenched by adding silica gel. The suspension was concentrated to dryness under reduced pressure. The resulting powder was purified by column chromatography (EA/PE 1:1) and concentrated under reduced pressure. The resulting pure product (0.8 g, 31%) was obtained as a light yellow solid.

\[ ^{1}H-NMR \ (400MHz, \ CDCl_3), \ \delta \ [ppm]: \ 8.59 \ (s, \ 1H, \ H-8), \ 6.20 \ (s, \ 2H, \ CH_2O), 1.48 \ (s, \ 9H). \]

\[ ^{19}F-NMR \ (500MHz, \ CDCl_3), \ \delta \ [ppm]: \ -69.08 \ (6-CF_3). \]

**Synthesis of \(^{15}\)N-labelled TBAN.**

Tetrabutylammonium chloride (2.0 g; 8.4 mmol) was added to a stirred solution of \(^{15}\)N-labelled sodium nitrate (1.1 g; 12.6 mmol) in water (4 mL). When a white solid precipitated from the solution, CH\(_2\)Cl\(_2\) (5 mL) was added and the biphasic system was stirred vigorously for 1 h. The layers were separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (3x5mL). The combined organic layers were washed with water (1x4ml) and dried with a large amount of Na\(_2\)SO\(_4\). After filtration and washing the remaining Na\(_2\)SO\(_4\) thoroughly, the solvent was evaporated and drying in vacuo at 50 °C gave \(^{15}\)N lab NMR \(\delta\) 381.3 (s).

**Monitoring the nitration of 6-chloro-9-Boc-purine 1**

A 5 mm NMR tube containing a solution of 6-chloro-9-Boc purine 9 (20 mg; 0.10 mmol) and \(^{15}\)N labelled TBAN (61 mg; 0.20 mmol) in CD\(_2\)Cl\(_2\) (0.7 ml) was placed in an acetone-ice bath of −10 °C. The reaction was started by the addition of TFAA (28 μL; 0.20 mmol). The contents were mixed and the tube was transferred into the spectrometer probe set to -10 °C and locked and shimmed within 2 minutes. \(^1\)H spectra were then recorded every 30 seconds during several half lives using single pulses.

**Formation of 6-chloro-7-\(^{15}\)N-nitro-8-trifluoroacetoxy purine intermediate 3**

A 5 mm NMR tube containing a solution of 6-chloro-9-Boc purine 9 (20 mg; 0.10 mmol) and \(^{15}\)N labelled TBAN (61 mg; 0.20 mmol) in CD\(_2\)Cl\(_2\) (0.7 ml) was placed in an acetonitrile-dry ice bath of −50 °C and pre-cooled TFAA (28 μL; 0.20 mmol) was added. Complete conversion of the starting material to 7-nitro-8-trifluoroacetoxy purine intermediate was
observed within 8 hrs as monitored by $^1$H NMR at $-50 \, ^\circ$C. NMR data were recorded at $-50 \, ^\circ$C; $^1$H NMR $\delta$ 8.82 variable (d, $J_{HN}$ 2.7, 1H, H-8), 8.78 (s, 1H, H-2), 1.48 (s, 9H, CCH$_3$); $^{13}$C NMR $\delta$ 159.77 (bs, C-4), 157.69 (d, $J$ 212.4, C-2), 153.75 (dq, $J_{CF}$ 44, $J_{CH}$ 2.9, C$_2$H$_2$O$_2$CF$_3$) 151.09 (d, $J$ 13.1, C-6), 144.92 (bs, CO), 117.99 (C-5), 114.2 (q, $J_{CF}$ 290, C$_2$H$_2$O$_2$CF$_3$), 93.01 (dd, $J_{CH}$ 194, $J_{CN}$ 1.8, C-8), 86.73 (CCH$_3$), 27.18 (q, $J$ 127.0, CCH$_3$); $^{15}$N NMR $\delta$ 339.55 (d, $J_{NH}$ 2.7); 19F NMR $\delta$ -75.86 (s). CH-correlation data: cross peaks were found for (H-8) 8.82 - (C-8) 93.01 and (H-2) 8.78 - (C-2) 157.69.

NMR data for 2-$^{15}$N-nitro-6-chloro-9-Boc purine 2

$^1$H NMR (CD$_2$Cl$_2$) 8.92 (s, 1H, H-8), 1.72 (s, 9H, CCH$_3$). $^{13}$C NMR (CD$_2$Cl$_2$) $\delta$ 154.15 (d, $J_{CN}$ 29.4, C-2), 152.97 (d, $J_{CN}$ 4.6, C-6) 151.40 (dd, $J_{CN}$ 4.0, J 4.5, C-4), 148.33 (d, $J$ 223.4, C-8), 144.47 (CO), 135.49 (d, $J$ 12.1, C-5), 89.49 (CCH$_3$), 27.55 (q, $J$ 127.6, CCH$_3$). $^{15}$N $\delta$ 365.02 (s).

Kinetic and CIDNP studies

All kinetic and $^{15}$N CIDNP NMR studies were carried out using CDCl$_3$ as a solvent in 10 mm NMR tubes fitted with a coaxial insert containing a 0.15 M external reference solution of $^{15}$N labelled nitrobenzene (370.4 ppm) or nitromethane (379.4 ppm) in CDCl$_3$.

Purine nitramine rearrangement

A 10 mm NMR tube containing a 0.15 M solution of nitramine intermediate 3 in CDCl$_3$ (2.0 ml) preformed by the method described above and stored at $-50 \, ^\circ$C was placed in a bath of the appropriate temperature for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to the appropriate temperature and locked and shimmed within 2 minutes. The rearrangement was followed with $^1$H and $^{15}$N NMR. $^1$H spectra were recorded every 30 seconds or every minute; for the $^{15}$N CIDNP NMR experiments spectra were recorded every 3 minutes using single pulses with a pulse angle of 90° or every 45 seconds using single pulses with a pulse angle of 45°. Complete relaxation
was ensured. $^{15}$N NMR relaxation times T1 were determined applying $\pi-\pi/2$ pulse sequences. Nitrobenzene: (T1) $0 \degree C = 94 \pm 4.7$ s. Nitromethane: (T1) $-10 \degree C = 98.9 \pm 18.1$ s. 2-nitro purine 6: (T1)$0 \degree C = 23.4 \pm 0.8$ s, (T1)-10 $\degree C = 19.8 \pm 0.7$ s. For the runs followed by 1H NMR, the first order rate coefficients ($k_N$) obtained from the decrease of the nitramine intermediate 3 were calculated over 3-4 half-lives from the plots of $\ln$(NH2/IS) against time or $\ln$(NH8/IS) against time, where NH2 and NH8 are the integral values of the nitramine H-2 and H-8 respectively and IS the integral value of the tetrabutylammonium signal at 3.18 ppm used as an internal standard. The rate coefficients ($k_P$) obtained from the increase of 2-nitro product were calculated from a plot of $\ln$[$(P_{H8}/IS)_{t=\infty} - (P_{H8}/IS)_{t}$] against time, where $P_{H8}$ are the integral values of the product H-8. Regression coefficients were 0.998 $\pm$ 0.002. The rate coefficients mentioned were measured in duplo.

**Rearrangement in the presence of DIPEA**

DIPEA (3 equiv; 153 $\mu$L; 0.90 mmol) was added to a solution of the 7-nitro-8-trifluoroacetoxy purine intermediate 3 (0.15 M) in CDCl3 (2.0 ml) preformed by the method described above and stored at $-50 \degree C$. The sample was placed in an acetone-ice bath of $-10 \degree C$ for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to $-10 \degree C$ and locked and shimmed within 2 minutes. The rearrangement was followed with $^1$H NMR and first order rate coefficients were determined as described above.

**Rearrangement in the presence of hydroquinone.**

A 10 mm NMR tube containing a solution of 6-chloro-9-Boc purine 1 (59 mg; 0.30 mmol) and a substoichiometric amount of $^{15}$N labelled TBAN (85 mg; 0.28 mmol) in CDCl3 (2.0 ml) was placed in an acetonitrile-dry ice bath of $-50 \degree C$ and pre-cooled TFAA (40 $\mu$L; 0.28 mmol) was added. Purine intermediate 3 was allowed to be formed during 2 days at $-50 \degree C$ in order to remove all TFAN present. Hydroquinone (99 mg; 0.90 mmol) was added to this solution and the sample was placed in a bath of $-10 \degree C$ for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to $-10 \degree C$ and locked and shimmed within 2 minutes. As an external standard $^{15}$N-nitromethane ($T_1 = 113 \pm 12$ s) was used instead of $^{15}$N-nitrobenzene. The rearrangement was followed with $^{15}$N-NMR, 1x45°
pulse, 40 sec. delay. After completion of the reaction the amount of product was more accurately determined with 1H-NMR: 35 % of nitramine intermediate 3 had been converted into product 6 ($T_1 = 19.8 \pm 0.7$ s).
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