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 C-2 Substituted 1-deazapurines via nitro- and nitroso chemistry

ABSTRACT

This chapter describes new routes to 2-substituted-1-deaza purine derivatives as adenosine receptor modulating agents. The synthesis of the 1-deazapurine skeleton was optimised. The introduction of N-9 protecting groups is discussed in relation to the C-2-nitration. Nitration of 1-deazapurines furnished 2-nitro-1-deazapurines, which are converted to highly reactive nitroso species. Methods are presented towards the introduction of functional groups via nitroso condensation reactions, ene reactions, addition reactions, Mills coupling and Diels Alder reactions at low temperatures.

Contents of this chapter are partly based upon:

2-Substituted-1-deaza purine derivatives with adenosine receptor modulating activity
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Wanner, M. J., Rodenko, B., Koch, M. and 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, 1313 - 1320
5.1 INTRODUCTION

As mentioned in Chapter 1, removing nitrogen atoms from the purine ring system influences affinity for adenosine receptors. The removal of a nitrogen atom potentially involved in electrostatic interactions and/or H-bonding, significantly changes the interaction with biological targets. In addition, replacement of nitrogen by carbon atoms in the purine ring increases electron density in the remaining part of the aromatic system which may influence stacking interactions.

Upon removal of nitrogen atoms from the purine skeleton, IUPAC dictates a change in nomenclature and numbering. For example, 1-deazaadenosine (purine numbering), depicted in Figure 5.1, corresponds to 7-amino-3-β-D-ribofuranosyl-3H-imidazo[4,5-b]-pyridine following to IUPAC nomenclature. Throughout this chapter the commonly used purine numbering is applied for simplicity and easy comparison with the purine derivatives described in previous chapters.

![Image](image)

**Figure 5.1** Purine numbering for 1-deazaadenosine and IUPAC systematic numbering for the same structure (7-amino-3-β-D-ribofuranosyl-3H-imidazo[4,5-b]-pyridine)

Several examples of purine systems with N to C modifications in the five membered ring have been reported to have biological activity. For example, Imai reported in the early 1960’s that 2,6-diamino-9-deazapurines show antibacterial and antiprotozoal activity. In the 1980’s a series of 9-phenyl-7-deazapurines and 9-deazaxanthines was reported to have antagonistic activity on the A₁ and A₂ adenosine receptors. Several 2,6-diamino-9-arylmethyl-9-
deazapurines were prepared in the Holy laboratory in search for new cytostatics, however having low activity.

Other deazapurines have been modified in the 6-membered pyrimidine ring. The Koomen group has ample experience with the synthesis of deazaadenosine derivatives: 1- and 3-deazaadenosine derivatives have been developed as inhibitors of adenosine deaminase. Particularly, the 1-deazaadenosine skeleton was under more recent investigation for its adenosine receptor modulating activity not only by our group but also by the laboratory of Cristalli. In our search for $A_{2\alpha}$ receptor antagonists the class of 1-deazapurines appeared to be free from patent restrictions. Combined with our group’s synthetic experience with 1-deazaadenosines and functionalization of the purine skeleton by purine nitration, we set out to generate a new class of functionalised 1-deaza purine bases as adenosine $A_{2\alpha}$ receptor antagonists.

![Figure 5.2 Structures of 1-deazapurine 1, 2-nitro-1-deazapurine 2 and 2-nitroso-1-deazapurine 3](image)

Previous work in this compound class was focussing on $A_1$ receptor ligands. During a quest for potent and selective adenosine receptor agonists by our laboratory, it became apparent that the introduction of a small nitro-substituent at the C-2 position in 1-deazaadenosine resulted in a minor reduction in $A_1$ receptor affinity, but in a considerably increased selectivity for this receptor subtype compared to 2-nitroadenosine. Until the years 2000-2004 no further reports were published about the effect of 1-deazapurine bases on adenosine receptors. After we had finished our 1-deazapurine work, Chang and IJzerman described trisubstituted-1-deazapurines in the search for new $A_1$ receptor antagonists which they designed by modelling structural
similarities between various A\(_1\) receptor antagonists.\(^5\) Incorporation of aromatic substituents on the purine 2, 6 and 8 positions led to A\(_1\) receptor affinities in the nanomolar range.

Lately, our achievements on nitration and functionalization of 1-deazapurines and their therapeutic applications, which are discussed in the next paragraphs, are getting attention in (patent-)literature and pharmaceutical applications. Meijer et al. describe the use of our regioselective TBAN/TFAA nitration procedure in the development of 1-deazapurine CDK-inhibitors.\(^6\) Pharmacopeia Drug Discovery, in line with our strategy, targets the A\(_{2A}\) adenosine receptor with functionalized 1-deazapurines. Their 2- and 8-substituted compounds show low affinity (K\(_i\) 10µM) for the A\(_{2A}\) adenosine receptor.\(^7\)

**Approach**

The ligand based studies with a set of relevant A\(_{2A}\) ligands, described in Chapter 1, reveal that the introduction of bulky aromatic substituents at C2 leads to enhanced selectivity and antagonist activity on the adenosine A\(_{2A}\) receptor. Therefore, our final goal was to introduce such bulky moieties at the 2 position in 1-deazapurines to obtain A\(_{2A}\) selective antagonists, as illustrated in Figure 5.3. As schematically shown, an aromatic moiety is attached at C2, via an alkyl spacer group. The hypothesis is that the aromatic group can interact with a hydrophobic cavity in the adenosine receptor, while the spacer could give enough flexibility.

![Figure 5.3 Model 1-deazapurine compounds fitting in a hydrophobic pocket (X = C\(_n\))](image)

Synthetically however, deazapurines represent deactivated aromatic systems as compared to the original purine structures and preparation of derivates with substituents at the 2-position are difficult to realise. Especially, our target molecules with large substituents at C-2 had no precedence.
In the following paragraphs, an optimized synthesis of the 1-deazapurine skeleton is described. The use of various N9-protecting groups is studied in a regioselective C2-nitration. The reduction of the nitro group at the 2-position and subsequent conversion to 2-nitroso-1-deazapurine systems is discussed, followed by further functionalization and transformation of the versatile nitroso group.

5.2 SYNTHESIS OF THE 1-DEAZAPURINE SYSTEM

Until now, two main routes towards 1-deazapurines are known: one via ring closure of (optionally substituted) 2,3-diaminopyridines with orthoesters or carboxylic acids (route A in Scheme 5.1) and the other via aromatic nucleophilic displacement of halogens (C-2 or C-6 substitution, route B, Scheme 5.1). The most straightforward example of the first route, is the formation of the 1-deazapurine core via ring closure by refluxing 2,3-diaminopyridine in pyridine with triethyloorthoformate. In this way, functionality at the deazapurine 8-position can be easily introduced using other carboxylic acid derivatives at elevated temperatures. However, the introduction of desired substituents at the 1-deazapurine C-2 and C-6 position is synthetically more laborious. Following route A, those functionalities must already be present as substituents 4 and/or 6 in 2,3-diaminopyridines. The required pyridine functionalization has historically been proven difficult. The electronegative nature of the nitrogen atom in the pyridine ring changes the electron density by inductive and resonance effects, resulting in an electron poor ring system. Electrophilic substitution is therefore hampered, requiring harsh conditions. Several substituted aminopyridines were presented in the 1970’s by Schelling and Salemink, who used stannous chloride for the subsequent synthesis of 1-deazapurines, yielding small amounts of the desired diamino derivative. Recently, Oguchi et al. explored a route to introduce aromatic substituents at the C-2 deazapurine position starting from 2,6-dichloro-3-nitropyridine under Suzuki conditions. These convergent methodologies do not leave room for flexible functionalization of the 1-deazapurine skeleton as substituents are already introduced early in the synthetic route. A more divergent approach consists of route B in Scheme 5.1, where desired substituents are introduced not in the pyridine building blocks, but directly in the 1-deazapurine system. This route allows for decoration of the purine skeleton in a combinatorial fashion.
However, most of the known 2-substituted 1-deazapurines and all of the known 2-nitro substituted 1-deazapurines have a ribose moiety at the nitrogen atom at position 9. For several reasons this feature was felt to be undesirable. Firstly, purine ribosides are generally known as agonists for adenosine receptors. A notable and unique exception is the 2-substituted 6-CF3 adenosine analogs which were found to be A3 receptor antagonists in the course of our work. With the intended focus on antagonistic ligands, small N-alkyl substituents were preferred. An additional argument was the negative impact on pharmacokinetic properties of highly hydrophilic moieties like ribose. Impaired uptake in the colon and inability to pass the blood-brain barrier contribute to this issue.

Therefore, in analogy with the 6-CF3 purines described in previous chapters, we focused on 1-deazapurines with small alkyl groups at N9. With the final aim to generate a class of A2A selective antagonistic ligands, we chose to explore a strategy based on route B combined with small N-alkyl substitution on N9.

The best entry to such structures was a route described by Cristalli et al. Our synthetic approach to the 1-deazapurine skeleton is depicted in Scheme 5.2 and involves an optimization of the procedure reported by Cristalli12. We started the synthesis of the 1-deazapurine via the condensation of 2,3 diaminopyridine with triethyl orthoformate followed by oxidation to the N-oxide with hydrogen peroxide9, 2. Subsequent nitration with nitric acid of the imidazo[4,5-b]pyridine-4-oxide and deoxygenation with phosphorous trichloride yields 6-nitro-1-deazapurine 8. This procedure was optimised by us to give a synthetic scheme with
high overall yield. The replacement of 2 or 3 equivalents of hydrogen peroxide by \textit{meta}-chloroperbenzoic acid (1.3 equivalents) resulted in an improvement to over 91% of a previously moderate step. The nitration step could be optimized by using less acid (6 equivalents instead of over 14 equivalents). In the original protocol, neutralizing the large amount of acid with ammonium hydroxide solution led to diluted solutions, which hampered precipitation of N-oxide 7. Because the modified conditions require less base for acid neutralization, the yield was significantly improved to 75%. Reduction of the N-oxide was performed by stirring the solid residue with PCl\textsubscript{3} in chloroform instead of dry acetonitrile, as reported. Compounds 7 and 8 seem to have a low solubility in chloroform, which required longer reaction times (reflux, 20h). Nevertheless, this change of solvent improved the yield from 50% to 91%. Overall, we have improved the yield from 4 to 9 from 15% to 62 % over five steps.

\begin{center}
\textbf{Scheme 5.2} Conditions: a) triethyl orthoformate, reflux 100%, b) m-Chloroperbenzoic acid, acetic acid, 91% c) HNO\textsubscript{3}, TFA, 75% d) PCl\textsubscript{3}, CHCl\textsubscript{3} , 90% e) idem, longer reaction time
\end{center}

Having an optimised route to the desired backbone, next steps are to protect N9 with a small alkyl group followed by C2 –nitration. The 2-nitro group should serve as a reactive handle for further modifications.
5.3 **DIRECT ALKYLATION OF 1-DEAZAPURINES**

The first idea was to directly methylate the N-9 group of 6-nitro-1-deazapurine 8 (Scheme 5.2) with methyl iodide under influence of sodium hydride, often applied in purine methylation. We found these conditions to be too harsh and it led to several side products. Camaioni et al. used the milder base potassium carbonate in combination with alkyl iodides for the alkylation of 2-amino-6-chloropurines.\textsuperscript{13} Using these conditions on compound 8, we were able to isolate methylated products: NMR studies identified 67% of the desired N-9 methylated, 25% N-7 methylated product 11 and 12 respectively, and 8% of the N-3 methylated product 10, depicted in Figure 5.4.

![Figure 5.4](image)

**Figure 5.4** N-3 methylated 10, N-9 methylated 11 and N-7 methylated 1-deazapurine 12

When we followed the reaction in time with HPLC, we found interesting information about the mechanism of methylation. During the first minutes of the reaction, 6-nitro-1-deazapurine was converted into 6-nitro-3-methyl-1-deazapurine 10 for more than 50%. This 3-substituted product is converted into the 9- and 7- substituted products over time. The kinetically controlled 6-nitro-3-methyl-1-deazapurine 10 thus probably acts as an intermediate in the reaction, resulting in a thermodynamically favourable distribution of methylated products. Similar results have been observed in ribosylation experiments of imidazo[4,5]pyridine by Itoh and Mizuno\textsuperscript{14}. They found that after short reaction times the 3-substituted product was formed and after prolonged reaction times a mixture of N-9 and N-7 substituted products was gradually obtained. Apparently, the N-3 substituted product is kinetically controlled and the formation of N-7 and N-9 is thermodynamically driven.
Synthesis of C2-substituted 1-deaza-9-alkylpurines

Figure 5.5 Methylation of 6-nitro-1-deazapurine 8

Table 5.1: HPLC data showing conversion to N3/N7/N9 in time

<table>
<thead>
<tr>
<th>Compound</th>
<th>t = 5 min</th>
<th>t = 45 min</th>
<th>t = 2h</th>
<th>t = 3h</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-nitro-1-deazapurine 8</td>
<td>11%</td>
<td>4%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>9-methyl-6-nitro-1-deazapurine 11</td>
<td>26%</td>
<td>47%</td>
<td>60%</td>
<td>62%</td>
</tr>
<tr>
<td>7-methyl-6-nitro-1-deazapurine 12</td>
<td>13%</td>
<td>24%</td>
<td>27%</td>
<td>29%</td>
</tr>
<tr>
<td>3-methyl-6-nitro-1-deazapurine 10</td>
<td>50%</td>
<td>25%</td>
<td>12%</td>
<td>9%</td>
</tr>
</tbody>
</table>

Very recently, a computational study was performed on isomers and tautomers referring to our work, indicating that the N3-H tautomers are indeed relatively instable in the gas and water phase which is due to significant decrease in aromaticity of the N3-H forms. In the gas phase, the equilibrium between the N7-H and N9-H tautomers was studied, showing a preference of the N9-H tautomer over the N7-H by ca. 3.5 kcal/mol. The increase in dipole moment in a water media is the crucial effect influencing the N7H/N9H tautomeric equilibrium of nitro-1-deazapurines. For the three isomers dissolved in water, the two tautomers, N7-H and N9-H, are predicted to be observed and the former should dominate slightly for the 2-nitro isomer whereas the latter for the 6-nitro and 1-nitro isomers, confirming our laboratory observations. They revealed also that the NO₂ group destabilizes the N3-H-1-deazapurine system, whereas it stabilizes the N7-H- and N9-H-1-deazapurine systems.
5.4 NITRATION OF ALKYLATED 1-DEAZAPURINES

Nitration of the both N7 and N9 alkylated 1-deazapurines was carried out with the method described for purines in chapter 2 with TBAN/TFAA in dichloromethane. However, the yields were disappointing. Only 25 – 30% formation of product could be achieved. Mostly starting material was collected, some minor side products were partly identified as 8-oxo compounds.

Scheme 5.3 Nitration of N-9 alkylated 6-nitro-1-deazapurine  a) TBAN, TFAA, DCM, 30%

This is in agreement with what we observed for the TBAN/TFAA nitration for methylated purines. The electron donating methyl group is not favourable for the nitration. In addition and in accordance with the nitration mechanism presented in Chapter 2 (i.e. electrophilic addition, followed by a nitramine rearrangement), it can be envisaged that the absence of one nitrogen atom in the ring further influences the stability of the radicals during the rearrangement and product forming step.

5.5 BOC PROTECTION OF 1-DEAZAPURINES

We therefore chose for an additional strategy to nitrated N9-alkyl-1-deazapurines and selected Boc as temporary protective group. Boc protection of adenine and adenosine bases proved to be useful. In Chapter 2, the positive effect of Boc protection on the yield of purine nitration is shown. The desired N-alkyl substitution will be attempted in a later stage. In Scheme 5.4, the synthesis of Boc-protected 1-deazapurines is described. 4-(Dimethylamino)pyridine (DMAP) en di-tert-butyl dicarbonate (BOC₂O) are used to protect N-9 in compound 8 and 9 in analogy to the purine derivatives in Chapter 2.
Synthesis of C2-substituted 1-deaza-9-alkylpurines

Scheme 5.4  Boc protection of 1-deazapurines a) (Boc)₂O, DMAP, dcm, rt, 30 minutes

A slight excess of (Boc)₂O (1.4 equiv.) and a catalytic amount of DMAP (5%) was used (to protect compound 8 and 9). Via stirring in petroleum ether, the compounds were isolated. The use of methanol in combination with heating had to be avoided, because the Boc group easily splits off at reflux temperature by nucleophilic attack of MeOH. The thermodynamically most favourable N-9 isomer is formed in nice excess. The N-7 Boc product was found only in an amount of 10%.

5.6  TBAN/TFAA NITRATION OF BOC PROTECTED 1-DEAZAPURINES

Nitration of the N9 Boc protected deazapurine is performed in the range of 0 to -18 °C leading to far better yields than starting from the N9 alkylated 1-deazapurines. The general yield was good, resulting in about 78% yield of 6-chloro-2-nitro derivative 16 and 70% of 2,6-dinitro-1-deazapurine 15. Starting a nitration from di-nitro product 15, some formation of 8-oxo side product 17 is observed which is confirmed via NMR studies. Following to the mechanism of purine nitration, 8-oxo formation can take place when the nitrated product 15 forms again a N7-nitramino-8-trifluoroacetoxy intermediate. Since nitramine rearrangement is not possible anymore, a 8-trifluoroacetoxy dinitro-1-deazapurine compound will form. During aqueous work-up, hydrolysis occurs, leading to 8-oxo product 17.
Nitration at the C-1 position on the 1-deazapurine system has never been observed. The introduction of the nitro group on the 2-position is electronically favoured. Probably, the electronic properties of the 1-deazapurine system force the radical pairs to combine at the 2-position in the product forming step of the nitramine rearrangement. These o,p rearrangements are in agreement with the observed pattern in Bamberger nitramine rearrangements. In paragraph 5.13 the mechanistic aspects of 1-deazapurine and pyridine nitration are further studied.

Similar results have been recently found in our lab in the nitration of region isomeric Boc protected 3-deazapurines (Scheme 5.6). These nitrations also led to 2-NO₂-substituted nitrated products, which were confirmed by NMR experiments using nuclear Overhauser effects (NOE). The methyl protons of the Boc group show clear NOE effects with H₈ and H₃, confirming C-2 nitration.
5.7 BOC DEPROTECTION OF NITRATED 1-DEAZAPURINES

The Boc groups were removed via solvolysis at reflux temperature in methanol for 15 hours. After cooling, the resulting products slowly precipitated and could be filtered off and washed with cold methanol. The products were obtained in excellent yields, as indicated in Scheme 5.7. When the 2,6-dinitro-9-Boc-1-deazapurine \( 15 \) was deprotected, a minor side product \( 22 \) was found, which was not observed during deprotection of the 6-chloro-2-nitro analog \( 16 \). A possible explanation is, that the nitro group has stronger electron withdrawing properties than the chloro-substituent, thus making the C-8 position more sensitive to nucleophilic addition of methanol.

After completion of the nitration reaction, it is also possible to add MeOH directly and reflux the mixture. In this way the product is easily prepared in a one pot procedure without an extra purification step and in higher overall yield.

5.8 AZIDE INTRODUCTION AT NITRATED 1-DEAZAPURINES

A generally used way to introduce the amino moiety in adenine analogs is via the introduction of an azide group, which can be readily reduced with hydrogen to the desired amine substituent. In an analogous way we envisaged to get the 2-nitro-6-amino-1-deazapurine in hands. The 2-nitro group would then provide us a new synthetic handle for further functionalization.
An excess of NaN₃ (3 equivalents) in dry DMF was used to convert derivatives 20 and 21 into the identical corresponding azide 23. The reaction was monitored with HPLC. After completion, water was added dropwise (over 4 times the volume of DMF) with gentle stirring to precipitate the product and facilitate workup.

Route A has advantages over route B. Azide 23 obtained from dinitro compound 20 had a white colour, while compound 23 formed from the 6-chloro-2-nitro derivative 21 had a red colour, due to a minor contamination. The reaction with 6-chloro-2-nitro-1-deazapurine 21 was also very slow compared to the dinitro analog (70 hours at 60 °C versus 3 hours at room temperature!).

This seems to be in contrast with the general observation that in a SₐAr mechanism the chlorine atom is a better leaving group than a NO₂ group. This is further evidence that changes occur in the electronic property of the nitro group in the 1-deazapurine system, and that breaking the carbon-halogen bond is not the rate determining step in this reaction, which is also observed in the high reactivity of a fluorine moiety in a nucleophilic aromatic substitution (leaving group order F>NO₂>OTs>Cl).

5.9 N-9 METHYLATION OF 6-AZIDO-2-NITRO-1-DEAZAPURINES

The introduction of the azide substituent allowed very selective N9 methylation over N7 methylation. With the regularly used excess of 4 equivalents of methyl iodide a good yield (79%) of the desired region isomer 24 was obtained. A smaller excess (2 equiv.) further raised the yield to 88%.

Scheme 5.8  Azide introduction on nitrated 1-deazapurines Route A) NaN₃ (3 eq.), dry DMF, rt, 3h, Route B) NaN₃ (3 eq.), dry DMF, 60 °C, 70h

![Scheme 5.8](image-url)
We observed great differences in methylation of 6-nitro or 6-azido substituted 1-deazapurines, using different reagents and conditions, as depicted in Table 5.2. The azide is a narrow but straight substituent and can rotate around the C6-N bond (sp hybridised) of the 1-deazapurine system. The length of the azide substituent may sterically hinder attack on the N-7-position, thus favouring the N-9-position. Another option for the regioselectivity is mesomeric interaction of the azide group (R-N=N+=N-) in 1-deazapurines. The electron donating properties of the azide substituent (R-N=N+=N- $\leftrightarrow$ R$'\equiv$N-N) on the deazapurine system may additionally contribute to N9 regioselectivity.

**Table 5.2 Methylation of 6-N$_3$- or 6-NO$_2$-1-deazapurines**

<table>
<thead>
<tr>
<th>Substituent at C-6 position</th>
<th>Conditions</th>
<th>N-7 : N-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_3$</td>
<td>K$_2$CO$_3$, MeI</td>
<td>1 : 9</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>PPh$_3$, MeOH</td>
<td>1:2</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>K$_2$CO$_3$, MeI</td>
<td>1 : 1.5</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>PBu$_3$, MeOH</td>
<td>1:1.5</td>
</tr>
</tbody>
</table>

Methylation of the 1-deazapurine system with the NO$_2$ substituent at the C-6-position results in a less selective methylation 1:1.5 / N-7:N-9. The application of Mitsunobu conditions for the introduction of the methyl group gives comparable(1:1.5) or slightly (1:2) better ratios. However, afterwards, phosphineoxide side products have to be removed or polymer supported reagents have to be used. From the results it can be observed that the role
of the azide substituent is far more important than variation of conditions applied in the methylation of the 6-nitro derivative. The highly favourable influence of the 6-azido moiety on regioselective N-9 alkylation, made intermediate 23 an ideal stage to perform this desired step in the overall route to the target functionalized deazapurines.

5.10 ATTEMPTED DIRECT NUCLEOPHILIC SUBSTITUTION WITH AMINES

To obtain amino substituted 1-deazapurines, direct aminations on the 1-deazapurine system in the di nitro stage were attempted. In Chapter 3 the efficient introduction of amines in 2-nitro-6-CF₃ compounds was described. Unfortunately, the analogous introduction of amino substituents on 2,6-dinitro-1-deazapurines was unsuccessful in reactions with benzylamine, or phenethylamine, due to the absence of the ring nitrogen atom. When compound 20 was warmed to 50°C or at reflux temperature in THF (Scheme 5.10), ring opening occurred and product 25 was isolated according to NMR studies. The reaction apparently occurs via an addition reaction of the amine to the N7-C8 double bond. Amination of azide intermediates 23 or 24 also did not lead to amino substituted 1-deazapurines. In this case formation of addition products was not observed, most likely due to lower electron withdrawing properties of the azide substituent, compared to the nitro group.

Scheme 5.10 Direct amination of dinitro-1-deazapurine a) benzylamine, THF, reflux 60%

While this route using direct substitution on the nitro group was not successful, an alternative route was explored using nitroso chemistry.

5.11 REDUCTION AND OXIDATION OF THE 6-AZIDO-2-NITRO 1-DEAZAPURINE SYSTEM

Aromatic nitroso compounds are useful as reactive organic species. The introduction of nitroso functions may provide new handles to introduce functional groups. Recently, members of our group presented the synthesis of the hitherto unknown, but stable, 2-
nitrosoadenosine from the corresponding 2-nitropurine ribosides. Several examples of functional group introductions were given. This challenged us to investigate the possibilities of nitroso chemistry for the more deactivated 1-deazapurines. In Scheme 5.11 the introduction of the nitroso function in the deazapurine skeleton is presented. It appeared that side reactions can occur easily in our 1-deazapurine system.

Scheme 5.11 Formation of 2-nitroso-1-deazapurine 28 and accompanying side reactions

\[ \begin{align*}
24 & \xrightarrow{a} 26 & \quad \text{a) } \text{H}_2/\text{Pt} (20 \text{ mol\%}) , \text{EtOAc} , \text{ for } 26 \ 1h / \text{ for } 27 \ 18h / \text{ for } 29 > 18h \ (\text{condition } a^* ) \quad \text{b) } \text{NaIO}_4(\text{aq.}) \quad 0^\circ\text{C}
\end{align*} \]

During hydrogenation, compound 26 is formed first in 1 hour. This compound could be isolated in a purified yield of 88%. By extending the reaction time, further conversion to hydroxyl-amino derivative 27 was observed. By exceeding reaction times (condition a*), diamino compound 29 was formed. The reaction was carefully monitored with TLC and HPLC until compounds 26 : 27 : 29 were present in a ratio of 5 : 90 : 5. Then, hydrogenation was stopped and the mixture was oxidised \textit{in situ} to nitroso compound 28. The synthesis of nitroso compound 28 was accomplished in an overall yield of 65% (based on intermediate 26).

Aromatic nitroso compounds like 28 show a strong tendency to form azooxy-dimers in a reversible process under influence of oxidation with sodium periodate.\textsuperscript{21, 22} This was also
observed for compound 28. Another side reaction that may occur is dimerisation of nitroso-products to azodioxy compound 30. The amount of dimerisation in solution is dependent on the presence of substituents at the aromatic system, but probably also on concentration, temperature and solvent. As to the double bond geometry, nitrosobenzene exists only as the cis dimer, whereas 2,6-dimethylnitrosobenzene is exclusively in the trans-azodioxy form. In analogy with the data on nitroso benzene we assume that our observed 2-nitroso-1-deazapurine dimer is in the cis form, too. Although, there is a lot of information about C-nitroso compounds in general, little information is yet available for nitroso-purines or deazapurines. Our group published the first introduction of nitroso moieties at adenosine analogs. This is the first example of nitroso group introduction at 1-deazapurines.

![Scheme 5.12](image)

**Scheme 5.12** First introduction of the nitroso function in 1-deazapurines a) \( \text{H}_2/\text{Pt (20 mol\%)} \), EtOAc, b) \( \text{NaIO}_4 \text{ (aq.) } 0^\circ\text{C} \)

### 5.12 Functionalisation of the 2-Nitroso System

As indicated in the introduction of this chapter, it was envisaged to introduce substituents at C-2 to enhance selectivity for the \( A_{2A} \) receptor. With the nitroso moiety as a handle, it would be possible to try cycloadditions and condensations reactions to obtain new classes of compounds in the 1-deazapurine series. Wanner et al. described that Diels-Alder condensations were successful with purine nitroso species, to yield new substituted purine structures. In the next paragraphs our efforts to new C-2-substituted 1-deazapurines starting from nitroso-1-deazapurines are presented.
5.13 Diels-Alder Reactions with Dienes

Compound 28 was reacted with dienes in MeOH at reflux temperature. With cyclohexadiene, compound 31 is formed easily within one hour, according to TLC. However, the first attempts to isolate and purify the product resulted in low yields, (35%). The properties of 31 gave difficulties upon flash chromatography. The slightly acidic silica resulted in extreme tailing from the column. A pre-treated basic column (slurry method), eluens EA/ MeOH (15%)/ NH₄OH (1.5%), gave better results (~50%). Highest isolated yield was obtained by concentrating the reaction mixture in vacuo followed by stirring in cold methanol at 0 ºC. Filtration gave 75% of pure 31.

![Scheme 5.13 Diels Alder reactions with 2-nitroso-1-deazapurine 28 a) methanol, reflux](image)

Product 31 is presumed to be a mixture of endo and exo stereo isomers. The endo product will probably be formed in majority, because syn addition is energetically favoured.

It was difficult to isolate the cyclopentadiene addition product 32. In this case easy reversal of the equilibrium type addition reaction, which is not uncommon in Diels Alder type reactions, hampers product isolation. This could be shown via NMR studies. The crude product was concentrated in vacuo in a NMR tube and an NMR spectrum was recorded, which showed starting compound 28, 5% of product 32 and no cyclopentadiene (probably removed during concentration in vacuo). When an excess of freshly distilled cyclopentadiene
was added, in five minutes the reaction changed colour from red to yellow and NMR showed the equilibrium to be forced to the desired product 32.

5.14 HYDROGENATION OF DIELS ALDER PRODUCTS

By hydrogenation of 31, the cis amino-cyclohexanol derivative 33 is formed. The compound was obtained in pure form by stirring in cold methanol and filtration (47%).

Scheme 5.14 Ring opening of Diels Alder products 31 and 32: a) H$_2$./Pd/C (10%), MeOH reflux, 1.5 h

Reduction and ring opening of compound 32 was attempted in a similar way. Because the Diels Alder reaction (Scheme 5.13) was quite sensitive and work up was complicated, direct hydrogenation of the in situ formed bicycle [2.2.1.]hept-5-en-3-yl derivative 32 was tried. Unfortunately, isolation of product 34 remained difficult and we decided to explore other coupling reactions.

5.15 ATTEMPTED MILLS COUPLING WITH AMINES

Mills coupling between nitrosoaromatics and anilines is a method for the preparation of diazo compounds. The resulting diazo compounds are usually sensitive to light. The kinetic E isomer can be converted to a thermodynamic mixture of Z and E isomers. Recently, the nucleophilic reactivity of aniline derivatives towards nitroso phenyl compounds was studied. Acetic acid-catalyzed condensation of 28 with 2 eq. of aniline at 70°C should have produced
the desired diazo compounds 35. However this was not observed. Also the more activated amine \( p \)-anisidine, with the 4-OCH\(_3\) moiety activating the amino group for nucleophilic attack, did not lead to formation of the desired product 36.

![Scheme 5.15 Attempted Mills coupling using 2-nitroso-1-deazapurine and anilines a) amine (5 equiv.), CH\(_3\)COOH (2 equiv.), MeOH, 3h](image)

Raising the temperature or addition of trifluoroacetic acid did not improve the reaction. For adenosine derivatives successful Mills couplings have been reported. Apparently, this is another example of the deactivated character of the 1-deazapurine system and new alternative approaches would be necessary to obtain the target molecules.

### 5.16 AMINE CONDENSACTIONS WITH 2-NITROSO-1-DEAZAPURINES

Condensation on non aromatic amines with nitrosoaromatic systems form interesting approaches for the synthesis of another new class of compounds: hydrazones. In this paragraph we describe successful amine condensations in analogy to the Mills coupling between anilines en nitroso aromatic systems. Key differences in the reaction are the higher nucleophilicity of the amines and the possibility of the system to rearrange to (more) stable products. To illustrate the scope, we used a series of substituted benzyl amines, phenethylamine and cyclohexylmethylamine.
Several conditions were explored. It was found that under basic conditions a side reaction occurs whereby dimers of the nitroso species are formed, as follows: The 6-amino group of the 1-deazapurine 28 reacts with a nitroso moiety of another molecule of 1-deazapurine in Mills-coupling manner to yield an azo compound. To circumvent this side reaction, an excess of amine (5 – 10 equiv.) has to be used, with 2 equiv. of acetic acid. The use of trifluoroacetic acid did not make a difference. In this way, it was possible to perform the amine
condensations at room temperature. Because of the high amounts of amine used, the work up was complicated. By using a basic slurry method it was possible to obtain the benzyl- and phenethylamine product in 35 – 50 % yield.

Special attention was given to compound 41, an interesting azo compound which was isolated without rearrangement to the hydrazone in 48% yield. When this compound was subjected to triethylamine conditions, and refluxed in methanol overnight, the corresponding hydrazone 42 was formed in a yield of 27%.

In this way a series of new substituted 1-deazapurines was prepared with interesting activity on different adenosine receptors which will be discussed in the next chapter.\(^\text{25}\)

![Scheme 5.17 Hydrazine formation from azo compound 41](image)

**Scheme 5.17** Hydrazine formation from azo compound 41 a) EtOH, cat. Et\(_3\)N, reflux, overnight

### 5.17 STUDY ON THE MECHANISTIC ASPECTS OF 1-DEAZAPURINE AND PYRIDINE NITRATION

1-deazapurines were often considered as a special class of substituted pyridine derivatives. However, in chemical reactions pyridines and 1-deazapurine differ in reactivity and properties. In this chapter available mechanistic data on pyridine nitration are summarized and extrapolated to the related 1-deazapurine system. Pyridines are deactivated towards electrophilic aromatic substitution, so harsh conditions are usually required.

Literature describes nitration with HNO\(_3\)/H\(_2\)SO\(_4\) with only 3 % yield of 3-nitopyridine. Better conversions were only reported when pyridine-N-oxides were subjected to this reagent. Deghati et al. compared the nitration of pyridine-N-oxide using HNO\(_3\)/H\(_2\)SO\(_4\) and TBAN/TFAA (see Scheme 5.18). They found 69% conversion to para-nitropyridine-N-oxide 43 with HNO\(_3\)/H\(_2\)SO\(_4\). When a little excess of TBAN/TFAA was used as the reagents, the 3,5-dinitropyridine-N-oxide product 45 was isolated in reasonable amounts together with a
very small amount of 3-nitopyridine-N-oxide 46. Pyridines produced no C-nitrated products using the TBAN/TFAA reagent, when not converted to the N-oxide.26, 27.

These findings and our recent efforts in revealing the mechanism of purine nitration (see Chapter 2) prompted us to study the relations between pyridine nitration and 1-deazapurine nitration. We hoped to get more insight in the mechanisms involved in pyridine and 1-deazapurine nitration with TBAN/TFAA. Are electron poor pyridines nitrated and is it possible to steer regioselectivity? To start this study several electron deficient pyridines were selected with electron poor substituents at 2-, 3- or 4-position.

Scheme 5.18 Nitration of pyridine-N-oxides

Scheme 5.19 Regioselectivity in TBAN/TFAA nitration of 2-and 3-substituted pyridines TBAN/TFAA(2 equiv.), dichloromethane, 0°C
We did observe nitration at position 3 for the 2-substituted pyridine as well for the 3-
substituted pyridine in Scheme 5.19. Nitration of 2-cyano-pyridine, surprisingly only yielded
starting material.

We also selected broader series of 4-substituted pyridines as these might be most comparable
to 6-substituted 1-deazapurines (see Scheme 5.20). These experiments with different 4-
substituents show a trend towards 3-substitution where mono nitration occurs in 20-40%
consequently at the 3-position. Also considerable amounts of dinitration is observed, mainly
at 6-position. Surprisingly, it was found that when, in separate experiments, pure 3-nitro-4-
acetylpyridine was subjected to TBAN/TFAA reaction conditions, no di-nitro products are
formed.

Scheme 5.20 Regioselectivity in TBAN/TFAA nitration of 4-substituted pyridines
TBAN/TFAA(2 equiv.), dichloromethane, 0°C

An alternative approach to nitration of pyridines is described by Bakke et al. using liquid SO₂
as solvent and excess NO₂ (N₂O₄) and ozone as initiator (Scheme 5.21).²⁶,²⁷
Later, this procedure was improved by preformation the N$_2$O$_5$ and alternative workup with aqueous sulphuric acid. We followed this reaction and work up procedure to get more information about the mechanisms involved. It appeared from a $^1$H-NMR study that a N-nitro 1,2-dihydro- and 1,4-dihydropyridine intermediates were formed with the sulphuric acid. These intermediates were capable of, intramolecularly, rearranging via a 1,3 or 1,5 sigmatropic shift. After releasing dihydrogensulphite, the 3-nitropyridine as depicted in Scheme 5.22 is released.

Starting from these observations an analogous mechanism could be described for the TBAN/TFAA nitration of pyridines. It is well known from our purine nitration work that trifluoroacetyl nitrate is the active species. When we apply the TBAN/TFAA nitration conditions to 4-CN pyridine, we first observed the formation of a precipitate, which could be in equilibrium with the addition product of trifluoroacetate anion as depicted in Scheme 5.23.
Synthesis of C2-substituted 1-deaza-9-alkylpurines

Scheme 5.23 formation of a nitramine pyridine intermediate

This intermediate could lead to product formation via the Bakke type mechanism, although a radical pathway as discussed for purine nitramino TFAN intermediates cannot be excluded. The trifluoroacetate could have radical directing and stabilizing features resulting in the observed conversions.

If we compare 1-deazapurine nitration with pyridine nitration, it is clear that the 1-deazapurine system cannot be considered a simple 2,3-disubstituted pyridine. While in 4-substituted pyridine nitration, we observe reaction at the 3-position, the corresponding 6-substituted 1-deazapurines are nitrated at the 2-position with TFAN (see Scheme 5.24).

Scheme 5.24 Comparing 1-deazapurine (2 and 3 substituted pyridine) and pyridine nitration

The key difference is that the nitramino intermediate formed in 1-deazapurines can be at N-7 in the imidazole part, in the other part of the bicyclic ring system. The radical mechanism for purine nitration of Chapter 2 that has been proven with NMR studies, could eventually be applied to 1-deazapurines and pyridines as well. In Scheme 5.25 it is described that after addition of trifluoroacetyl nitrate and the formation of the nitramino intermediate at N7, a radical rearrangement can take place. Via homolytic bond cleavages, the nitro group can rearrange to position 2, leading to the end product under release of TFA. This mechanism explains that nitro substitution at C1 is not observed. Rearranging to C2 clearly is the favoured radical pathway for an intermediate with a 7-nitro group as depicted in Scheme 5.25.
In analogy, for the nitroamino intermediates of pyridines, a possible radical addition via the N-nitro intermediate should give mainly 3-substituted nitro-pyridines. This gives more evidence that a radical pathway could be involved and agrees well with our current experimental observations for the nitration of 1-deazapurines and pyridines.
5.18 CONCLUDING REMARKS

The synthesis of the skeleton of 1-deazapurine bases is highly optimised. A new protecting
group for 1-deazapurines is presented (Boc), which allowed selective nitration in very good
yields. The application of nitroso chemistry for the introduction of functional groups in 1-
deazapurines was hitherto unknown. Amine condensation reactions and cyclo-additions on
the 2-nitroso-1-deazapurine core yield new classes of C-2-substituted 1-deazapurines. Finally,
nitration of pyridines was studied to give more insight in the nitration mechanism. It was
suggested that a nitramino intermediate is present for both deazapurine and pyridine nitration.
Radical nitramino rearrangement pathways fit our observations for the mechanism of 1-
deazapurine and pyridine nitration.

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5.20 EXPERIMENTAL SECTION

General information.
For experimental details see Chapter 2.

Imidazo[4,5-b]pyridine 5

A mixture of 25.2 g (0.231 mol) of 2,3-diaminopyridine, 500 ml of triethylorthoformate and some crystals of para-toluenesulfonic acid was heated at reflux for 3 h. The solution was evaporated to dryness in vacuo and the residue was heated at reflux with 250 ml of concentrated hydrochloric acid for 1 h. The mixture was allowed to cool, neutralized with solid Na₂CO₃ and extracted with ethyl acetate. The combined extracts were dried and the solvent was removed at reduced pressure. The residue was dissolved in absolute ethanol, treated with activated charcoal, filtered and then solvent was evaporated to give 23.1 g of product as salmon colored crystals (84% yield). Mp 152-153 °C; 'H NMR (DMSO-d₆): δ 8.44 (s, H₈), 8.37 (d, J = 4.7 Hz, H₂), 8.03 (d, J = 8.0 Hz, H₆), 7.25 (q, H₁).

Imidazo [4,5-b] pyridine-4-oxide 6

To 8.5 g (0.071 mol) of imidazo[4,5-b]pyridine was added 18 ml (0.315 mol) of 100% acetic acid and 4.5 ml (0.051 mol) of 35% H₂O₂. The mixture was heated at 75 °C for 3 h after which acetic acid and water were evaporated in vacuo. To the resulting red-brown solution again 18 ml (0.315 mol) acetic acid (100) and 4.5 ml (0.051 mol) 35% H₂O₂ were added. After heating for 3 h at 75 °C, the mixture was cooled to rt and the white precipitate was filtered and washed with acetic acid and ether. The resulting white crystals were dried to obtain 9.86 g (70.8 yield) of the product. (acetic acid salt (1:1)). 'H NMR (DMSO-d₆): δ 8.43 (s, H₈), 8.21 (d, J = 6.3 Hz, H₂), 7.62 (d, J = 8.2 Hz, H₆), 7.23 (q, H₁), 1.93 (s, acetic acid).

7-Nitro-imidazo[4,5-b]pyridine-4-oxide 7

To a cold (0 °C) solution of 9.78 g ( 0.050 mol) of imidazo [4,5-b] pyridine-4-oxide in 50 ml of trifluoroacetic acid was added, drop wise, 33.5 ml of 90 fuming nitric acid. The mixture was heated at 90 °C for 3 h, cooled and poured into crushed ice. Neutralization was carried out with concentrated ammonium hydroxide to pH 8-9, while maintaining the temperature below 30 °C. The resulting yellow solid was filtered, washed with ice water and dried to give
6.26 g of the product as light yellow needles (70% yield). 'H NMR (DMSO-d₆): δ 8.11 (s, H₈), 7.98 (d, J = 7.1 Hz, H₂), 7.78 (d, J = 7.1 Hz, H₁).

7-Nitroimidazo[4,5-b]pyridine 8
To a solution of 5.0 g (0.028 mol) of 7-Nitro-imidazo[4,5-b]pyridine-4-oxide in 90 ml of dry acetonitrile was added drop wise 20.7 ml of phosphorustrichloride and the mixture was heated at 80 °C for 2 h. After cooling, a white solid precipitated, which was collected by filtration and washed with ether and sat. sodium carbonate solution. Recrystallization from water provided 2.21 g of the product as yellow needles (49% yield).
Alternative route: To a solution of 5.0 g (0.028 mol) of 7-Nitro-imidazo[4,5-b]pyridine-4-oxide in 50 ml of dry chloroform was added drop wise 12 ml of phosphorustrichloride and the mixture was heated at reflux for 16h. Cooling on ice, filtering and neutralising the suspension with saturated sodium carbonate followed by filtering and drying over P₂O₅ in vacuo yields 4.02 gram of 8 (88%) mp: 237-240°C
'H NMR (DMSO-d₆): δ 8.78 (s, H₈), 8.70 (d, J = 5.4 Hz, H₁), 8.02 (d, J = 5.4 Hz, H₂)
IR: 3108, 1541, 1504, 1383, 1336

6-chloro-1-deazapurine 9
The compound 9 is formed when reaction times to form 6-nitro-1-deazapurine are prolonged.
mp: 186°C 'H NMR (6-Cl) (d₆-DMSO) δ(H) 8.54 (1H, s, 8-H), 8.31 (1H, br m, 2-H), 7.42-7.41 (1H, d, J = 5.12 Hz, 1-H); (6-NO₂): 13.71 (1H, br, NH), 8.78 ( 1H, s, 8- H), 8.70-8.69 (1H, d, J = 5.4 Hz, 2-H), 8.02-8.01 (1H, d, J = 5.4 Hz, 1-H).

9-Boc 6-nitro-1-deazapurine 13
To a solution of 6-nitro-1-deazapurine 8 (5 g, 30 mmol,) and BOC₂O (10 g, 46 mmol) in dry dichloromethane (100 ml) was added dimethylaminopyridine (0.250 g, 5 mass%) and the mixture was stirred at room temperature for 1.5 h. The reaction was diluted with PE and quenched by adding silica and the mixture was filtered over hyflo. Evaporating the solvent yielded the crude product. Crystallization with EA/PE afforded the product (5.47 g, 80 %) as light yellow needles. mp: 223-226°C
\(^1\)H NMR (d\textsubscript{6}-DMSO) \(\delta\) 9.11 (s, 1H, H-8), 8.81 (d, \(J\) 5.3 , 1H, H-2), 8.10 (d \(J\) 5.3, 1H, H-1), 1.67 ( s, 9H t-Bu).

IR: 2986, 1777, 1752, 1530, 1492

9-Boc-6-Chloro-1-deazapurine 14
To a solution of 6-chloro-1-deazapurine 9 (10 g, 65.1 mmol,) and BOC\textsubscript{2}O (19.8 g, 91.2 mmol) in dry dichloromethane (100 ml) was added dimethylaminopyridine (0.5 g, 5 mass%) and the mixture was stirred at room temperature for 30 minutes. The reaction was quenched by adding silica and the mixture was filtered over hyflo. Evaporating the solvent yielded the crude product. Trituration with petroleum ether followed by treatment with ether yielded the product as a white solid (6.29 g, 76%), mp: 183.5\(^\circ\)C decomposition

\(^1\)H NMR (d\textsubscript{6}-DMSO) \(\delta\) 8.89 (s, 1H, H-8), 8.47-8.46 (d, \(J\) 5.3 , 1H, H-2), 7.62-7.61 (1H, d, \(J\) 5.3, H-1), 1.66 ( s, 9H t-Bu).

9-Boc-6-chloro-2-nitro-1-deazapurine 16
A solution of 6-chloro-9-Boc-1-deazapurine (1 g, 3.94 mmol) and tetrabutyl-ammoniumnitrate (1.8 g, 5.91 mmol) in dry dichloromethane (12 ml) was stirred in an ice bath. TFAA (835 \(\mu\)L, 5.91 mmol) was added dropwise. After 1.5 h the reaction was complete and MeOH (20 ml) was added. The mixture was concentrated and cooled to give yellow crystals. The crude product was washed with 3x3ml cold MeOH. Drying in vacuo at 50 \(^\circ\)C afforded the product (0.916 g, 78%) as yellow needles. mp: 288.2-290\(^\circ\)C decomposition

\(^1\)H NMR (d\textsubscript{6}-DMSO) \(\delta\) 9.22 (s, 1H, H-8), 8.58 (s, 1H, H-1), 1.68 ( s, 9H t-Bu).

2,6-dinitro-1-deazapurine 20
A solution of 6-nitro-9-Boc-1-deazapurine (4 g, 15 mmol) and tetrabutylammoniumnitrate (0.92 g, 23 mmol) in dry dichloromethane (5 ml) was stirred at -18\(^\circ\)C. TFAA (430 \(\mu\)L, 18 mmol) was added dropwise. After 1.5 h the nitration reaction was complete and MeOH (20 ml) was added. The solution was refluxed for 15h while the product precipitates. Cooling and
filtering and drying the suspension furnished the pure product (2.28g, 72%) as light yellow crystals. mp: 294°C

\(^1\)H NMR (d\textsubscript{6}-DMSO) \(\delta\) 14.45(bs, 1H, H-9), 9.16(s, 1H, H-8), 8.81 (d, 1H, H-1).

6-chloro-2-nitro-1-deazapurine 21
2-nitro-6-chloro-9-Boc-1-deazapurine (1.0 g, 3.35 mmol, intermediate B) in MeOH (20 ml) was stirred at 70°C. After 2 h the product started to precipitate. After 15 h the suspension was cooled at 0°C for 30 min and filtered. The precipitate was washed with cold MeOH and dried in vacuo at 50°C to furnish the product (0.5 g, 76%) as a yellow solid. mp: 294.5°C

\(^1\)H NMR(d\textsubscript{6}-DMSO) \(\delta\) 14.20 (bs, 1H, H-9), 8.92 (s, 1H, H-8), 8.41 (d, 1H, H-1).

6-azido-2-nitro-1-deazapurine 23
First possible route: a suspension of 2-nitro-6-chloro-1-deazapurine 21 (12 mmol,) and NaN\textsubscript{3} (2.5 g, 37 mmol) in dry DMF (25 ml) was stirred and warmed to 60°C. After 60 h the reaction was cooled to room temperature and water was added slowly (100 ml). The product precipitated and was filtered. Washing with water and ether, followed by drying in vacuo at 50°C furnished the product (2.12g, 82%) as a solid.

Alternative route: a suspension of 2,6-dinitro-1-deazapurine 20 (2.5 g, 12 mmol,) and NaN\textsubscript{3} (2.4 g, 37 mmol) in dry DMF (20 ml) was stirred at room temperature for 3h. After completion, water was added slowly (100 ml). The product precipitated and was filtered. Washing with water and ether, followed by drying in vacuo at 50°C furnished the product (2.22g, 90%) as a off white solid. mp: 220-224 °C decomposition

\(^1\)H NMR(d\textsubscript{6}-DMSO) \(\delta\) 14.02 (bs, 1H, H-9), 8.78 (s, 1H, H-8), 7.81 (s, 1H, 1-H).

6-azido-9-methyl-2-nitro-1-deazapurine 24
To a suspension of 2-nitro-6-azido-1-deazapurine 23 (6 g, 29.2 mmol) and K\textsubscript{2}CO\textsubscript{3} (8.09 g, 58.5 mmol) in dry DMF (150 ml) was added MeI (3.65 ml, 58.5 mmol). After 1 h the reaction was complete and water (300 ml) was added slowly. The product precipitated and the suspension was cooled in an ice-bath. The crude product was washed with water and ether
and dried in vacuo at 50°C to yield 5.61 g (88%) of the desired product as a white solid. mp: 172°C decomposition

$^1$H NMR (d$_6$-DMSO) $\delta$ 8.77 (s, 1H H-8), 7.80 (s, 1H H-1), 3.92 (s, 3H, CH$_3$).

6-amino-9-Me-2-nitro-1-deazapurine 26

To a solution of 2-nitro-6-azido-9-methyl-1-deazapurine (1 g, 4.5 mmol, Intermediate G) was added Pd/C 10% (0.200 g, 20 mol%). The solution was stirred at room temperature under a H$_2$ atmosphere. After 1h the reaction was complete. Flash chromatography (EA) of the crude reaction mixture afforded the pure product as a red solid (0.775 g. 88%). mp: 260 - 264°C

$^1$H NMR (d$_6$-DMSO) $\delta$  8.35 (s, 1H H-8), 7.36 (s, 1H, H-1), 7.15 (bs, 2H, NH$_2$), 3.79 (s, 3H, CH$_3$).

6-amino-2-hydroxylamino-9-methyl-1-deazapurine 27

A suspension of 2-nitro-6-azido-9-methyl-1-deazapurine 26 (1 g, 45.6 mmol) and 10% Pt/C (0.2 g, 20% m) in EA (200 ml) was refluxed under a H$_2$ atmosphere. After 18h the reaction was filtered over Hyflo (EA/MeOH). The filtrate was used immediately in the oxidation reaction to the nitroso derivative.

$^1$H NMR (d$_6$-DMSO) $\delta$  8.34 (s, 1H, H-8), 7.36 (s, 1H, H-1), 7.14 (bs, 2H, NH$_2$), 3.80 (s, 3H, CH$_3$).

6-amino-9-methyl-2-nitroso-1-deazapurine 28

The crude mixture of 2-hydroxylamino-6-amino-9-methyl-1-deazapurine (intermediate I) was cooled to 0°C. An ice-cold solution of NaIO$_4$ (1.95 g, 9.13 mmol) in H$_2$O (50 ml) was added slowly. After 1h the reaction was complete and the organic layer was separated from the water layer. The aqueous layer was extracted with EA + 5% MeOH and dried with Na$_2$SO$_4$. The yellow solution was concentrated and triturated with cold MeOH to yield 0.53 g (65%) of the desired product as a red solid. mp: 220°C.

$^1$H NMR (d$_6$-DMSO) $\delta$  8.46 (s,1H, H-8), 7.37 (s,1H, H-1), 7.00 (bs, 2H, NH$_2$), 3.92 (s, 3H, CH$_3$).

2-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-6-amino-9-methyl-1-deazapurine 31
To a suspension of 2-nitroso-6-amino-9-methyl-1-deazapurine 28 (0.1 g, 0.56 mmol,) in MeOH (15 ml) was added slowly 1,3-cyclohexadiene (105 \( \mu l \), 1.13 mmol). After 30 m the reaction was complete and the mixture was concentrated and triturated in cold MeOH. The product was washed with cold MeOH and dried at 50°C in vacuo to yield 101.9 mg (75 %) of the desired product as a white solid. mp: 169.9°C.

\[ ^1H \text{NMR (d}_6\text{-DMSO)} \delta \ 7.82 (s, 1H, H-8), 6.52 (t, 1H, CH), 6.30 (t, 1H, CH), 6.12 (bs, 2H, NH2), 5.99 (s, 1H, H-1), 5.21 (t, 1H, CH), 4.70 (t, 1H, CH), 3.63 (s, 3H, CH3), 2.08 (q, 2H, CH2), 1.55 (q, 2H, CH2), 1.33 (q, 2H, CH2);
\]

\[ m/z \ 258.1355 (M^+ + H.) C_{13}H_{16}N_5O \text{ requires } m/z \ 258.1277 \]

6-amino-2-(cis-4-hydroxycyclohexylamino)-9-methyl-1-deazapurine 33
A suspension of 2-(2-oxa-3-azabicyclo[2.2.2]oct-5-en-3-yl)-6-amino-9-methyl-1-deazapurine (50 mg, 0.194 mmol, compound 1) and 10% Pd/C in MeOH (4 ml) was stirred under 1 atm hydrogen for 1.5 h at 70 °C. Filtration, evaporation and stirring in cold MeOH afforded the pure 2-(cis-4-hydroxycyclohexylamino)-6-amino-9-methyl-1-deazapurine (23.8 mg, 47%) as white solid.

\[ ^1H \text{NMR (d}_6\text{-DMSO)} \delta \ 7.62 (s, 1H, H-8), 5.72 (s, 2H, NH2), 5.69-5.67 (m, 1H, NH), 5.57 (s, 1H, H-1), 4.34 (s, 1H, OH), 3.69 (s, 2H), 3.57 (s, 3H, CH3), 1.63 (m, 6H, cyclo), 1.53-151 (m, 2H, cyclo)
\]

\[ m/z \ 262.1668 (M^+ + H.) C_{13}H_{19}N_5O \text{ requires } m/z \ 262.1590 \]

6-amino-2-benzylhydrazone-9-methyl-1-deazapurine 37
Condensation of 2-nitroso-6-amino-9-methyl-1-deazapurine (0.1 g, 0.565 mmol), benzylation (740 \( \mu l \), 6.77 mmol) and acetic acid CH3COOH (64.6 \( \mu l \), 1.13 mmol) was performed as described for compound 3. Flash chromatography (EA/MeOH/NH4OH 88.5:10:1.5). The product was triturated with cold methanol and dried in vacuo to yield 52.6 mg (35%) of the desired product as a yellow solid. mp: 231.7-232.8°C;

\[ ^1H \text{NMR (d}_6\text{-DMSO)} \delta \ 10.68 (s, 1H, NH), 7.95 (s, 1H, H-8), 7.77 (s, 1H, CH), 7.64-7.62 (d, 2H, o-Ph), 7.41 (t, 2H, m-Ph), 7.31 (t, 1H, p-Ph), 6.46 (s, 1H, 1-H), 6.17 (bs, 2H, NH2)
\]

\[ m/z \ 267.1358 (M^+ + H.) C_{13}H_{19}N_5O \text{ requires } m/z \ 267.1280 \]
6-amino-2-(4-methoxy-2-benzylhydrazon)-9-methyl-1-deazapurine 38
Condensation of 2-nitroso-6-amino-9-methyl-1-deazapurine (0.1 g, 0.565 mmol), 4-methoxy-benzylamine (369 µl, 2.82 mmol) and acetic acid CH₃COOH (64.6 µl, 1.13 mmol) was performed as described for compound 3. Flash chromatography (EA/MeOH/NH₄OH 94.5:5:0.5). The product was triturated with cold methanol and dried in vacuo to yield 58.5 mg (35%) of the desired product as a yellow solid.

¹H NMR (d₆-DMSO) δ 10.39 (s, 1H, NH), 7.91 (s, 1H, 8-H), 7.75 (s, 1H, CH), 7.56 (d, J 8.53 Hz, 2H, m-Ph), 6.98 (d, J 8.52 Hz, 2H, o-Ph), 6.42 (s, 1H, 1-H), 6.13 (bs, 2H, NH₂), 3.80 (s, 3H, OCH₃), 3.64 (s, 3H, CH₃)
m/z 297.1464. (M⁺ + H.) C₁₃H₁₉N₅O requires m/z 297.3271

6-amino-2-(2,4-dimethoxy-2-benzylhydrazon)-9-methyl-1-deazapurine 39
Condensation of 2-nitroso-6-amino-9-methyl-1-deazapurine (0.075 g, 0.423 mmol), 2,4-dimethoxy-benzylamine (318 µl, 2.12 mmol) and acetic acid CH₃COOH (48.4 µl, 0.847 mmol) was performed as described for compound 3. Flash chromatography (EA/MeOH/NH₄OH 94.5:5:0.5). The product was triturated with cold methanol and ether and dried in vacuo to yield 48.3 mg (35%) of the desired product as a off white solid.

¹H NMR (d₆-DMSO) δ 10.38 (s, 1H, NH), 8.17 (s, 1H, 8-H), 7.78-7.75 (s, 2H, CH and 1-H), 6.61-6.6 (m, 2H, Ph), 6.40 (s, 1H, Ph), 6.11 (bs, 2H, NH₂), 3.85 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.63 (s, 3H, CH₃)
m/z 327.1569 (M⁺ + H.) C₁₃H₁₉N₅O requires m/z 327.3531

6-amino-9-methyl-2-((2-phenylethylidene)hydrazinyl)-1-deazapurine 40
A mixture of 2-nitroso-6-amino-9-methyl-1-deazapurine (0.1 g, 0.565 mmol), 2-phenylethylamine (858 µl, 0.677 mmol) and acetic acid CH₃COOH (64.6 µl, 1.13 mmol) was stirred in MeOH. After 3 h the suspension had dissolved. The yellow solution was concentrated and diluted with PE and purified by flash chromatography (EA/MeOH/NH₄OH 88.5:10:1.5). The product was triturated with cold methanol and dried in vacuo to yield 55.4 mg (35%) of the desired product as a yellow solid. mp: 182.1°C
Synthesis of C2-substituted 1-deaza-9-alkylpurines

\[^1\text{H NMR (d}_6\text{-DMSO)} \delta\]

\[
\begin{align*}
10.07 & \text{ (s, 1H, NH),} & 7.73 & \text{ (s, 1H, 8-H),} & 7.37-7.32 & \text{ (3H, m, CH/Ph),} & 7.27-7.25-7.23 & \text{ (m, 3H, Ph),} & 6.28 & \text{ (s, 1H, 1-H),} & 6.1 & \text{ (bs, 2H, NH\textsubscript{2}),} & 3.60 & \text{ (s, 3H, CH\textsubscript{3}),} & 3.56-3.54 & \text{ (d, 2H, CH\textsubscript{2})}
\end{align*}
\]

\[m/z\; 281.1515\; (M^+ + \text{H.})\; \text{C}_{13}\text{H}_{19}\text{N}_5\text{O requires } m/z\; 281.1436\]

6-amino-2-cyclohexylmethylazo-9-methyl-1-deazapurine 41

Condensation of 2-nitroso-6-amino-9-methyl-1-deazapurine (0.1 g, 0.565 mmol), cyclohexylmethylamine (367 µl , 2.82 mmol) and acetic acid CH\textsubscript{3}COOH (64.6 µl, 1.13 mmol) was performed as described for compound 39. Flash chromatography (EA/MeOH/NH\textsubscript{4}OH 94.5:5:0.5). The product was triturated with ether and dried \textit{in vacuo} to yield 72.6 mg (48%) of the desired product as a bright yellow solid.

\[^1\text{H NMR (d}_6\text{-DMSO)} \delta\]

\[
\begin{align*}
9.81 & \text{ (s, 1H, NH),} & 7.71 & \text{ (s, 1H, 8-H),} & 7.17 & \text{ (d, J 5.31 Hz, 1H, CH),} & 6.22 & \text{ (s, 1H, 1-H),} & 6.04 & \text{ (s, 2H, NH\textsubscript{2}),} & 3.6 & \text{ (s, 3H, CH\textsubscript{3}),} & 2.18-2.17 & \text{ (m, 1H, cyclohexyl),} & 1.8-1.76-1.71 & \text{ (m, 5H, 5x CH) 1.29-1.18 & \text{ (m, 5H, CH)}
\end{align*}
\]

\[m/z\; 273.1828\; (M^+ + \text{H.})\; \text{C}_{13}\text{H}_{19}\text{N}_5\text{O requires } m/z\; 273.1749\]

6-amino-2-((cyclohexylmethyl)hydrazinyl)- 9-methyl-1-deazapurine 42

2-cyclohexylmethylazo-6-amino-9-methyl-1-deazapurine (0.03 g , 0.111 mmol, compound 41) was dissolved in EtOH (5 ml) and a catalytic amount of N(Et\textsubscript{3}. The mixture was refluxed overnight. The mixture was concentrated in vacuo, triturated with cold ether and MeOH and filtered producing 8 mg (27%) of the desired product as a yellow solid.

\[^1\text{H NMR (d}_6\text{-DMSO)} \delta\]

\[
\begin{align*}
9.81 & \text{ (s, 1H, NH),} & 7.71 & \text{ (s, 1H, 8-H),} & 7.16 & \text{ (d, J 4.91 Hz, 1H, CH),} & 6.22 & \text{ (s, 1H, 1-H),} & 6.04 & \text{ (s, 2H, NH\textsubscript{2}),} & 3.6 & \text{ (s, 3H, CH\textsubscript{3})}
\end{align*}
\]
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