Synthesis and Biological Activity of New Nucleoside Analogs as Inhibitors of Adenosine Deaminase.
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Chapter 3

Adenosine Analogs

3.1 Introduction

Transition state inhibitors frequently exhibit extraordinarily high binding affinities and enzyme specificities. High affinity is achieved because TS inhibitors engage in full complement of interactions used by the enzyme during catalysis to preferentially stabilize the TS and thereby lower the reaction energy barrier. Another approach involves the use of substrate analogs, which upon action of the enzyme are converted to TS inhibitors. Purine riboside (1, nebularine) is a good example in this series, which upon action of ADA is converted to enzyme-bound 6-hydroxy-1,6-dihydro-purine riboside (2).1

![Structure of purine riboside 1 and the transition state analog of purine riboside 2 from action of ADA](image)

We tried to influence this inhibitory character by introduction of several electron withdrawing and/or electron donating groups in different positions of the purine ring. Also we studied the interaction of these modified nucleosides with ADA to determine the requirements and limits for interaction of these compounds as inhibitors or substrates of ADA. In this chapter the synthesis of substituted purine ribosides is described. The structure-activity relationships of these adenosine analogs with ADA will be discussed in chapter 5.

In this chapter we will first introduce a regioselective nitration method to substitute C2 in purine ribonucleosides and deoxyribonucleosides and discuss the advantages and limitations of
this method. Conversion of this nitro substituent to a nitroso group is extensively discussed in paragraph 3.3. The mechanism of the nitration reaction is discussed in § 3.5, and structural determination of the products is shown in § 3.6. At the end of this chapter the substitution at C6 with different amines and substitution at N1 with hydroxyl and/or amine is discussed.

### 3.2 Functionalization of C2 in the purine ring

Adenosine analogs substituted at the 2-position show interesting activity in several biological systems. Apart from activity of these nucleosides with ADA, these compounds have shown interesting properties in different studies. The apoptosis inducing properties of some 2-halo-substituted adenosine analogs have been described. In addition, introduction of carbon, amino or oxygen substituents at the adenosine 2-position increases the selectivity of binding of these molecules to the different adenosine receptors. Most of the procedures towards the synthesis of 2-substituted purines are based on 2,6-dichloropurine or use guanosine as starting material.

Only a few nitration reactions of nucleosides are known in the literature due to the instability of the glycosidic linkage towards acidic conditions and/or high temperatures. Treatment of uridine triacetate with copper(II) nitrate/acetic anhydride gives 3-nitro-uridine triacetate. Nitrination of protected inosine (3) using a reagent prepared from TFAA and ammonium nitrate is described in the literature, which gives the N1 nitrated nucleoside 4.

![Diagram of nitrination reaction](image)

The TBAN/TFAA nitrating agent improved the availability of disubstituted purine systems starting from 6-substituted purine nucleosides. Nitrations with this reagent are in general performed at 0 °C in DCM, and one equivalent of TFA is formed during the substitution reaction. Nitration of 6-chloropurine riboside (5) with TBAN/TFAA is an excellent example of this method Scheme 3.3. We tried to extend this method to several other purine nucleosides. But
as will be discussed there are some limitations when functional groups like OH or NH$_2$ are present in the molecule.

![Chemical structure](image)

**Scheme 3.3**

### 3.2.1 2-Nitroadenosine

Direct nitration with TBAN/TFAA nitrating agent of adenosine triacetate (7) was not successful and protection of the amine group seemed crucial. Protection of the amine with one acetate was not enough for nitration, but with the use of penta-acetylated adenosine 8 the nitration gave the desired product (9) in 55% yield. Removal of the acetate protecting groups from 9 was not successful and already under mild conditions such as KCN in methanol, replacement of the nitro group occurred as a side reaction, affording 2-methoxyadenosine 10 in 62% yield and only 10% of the desired compound. In particular removal of the second N-acetyl group was rather slow, so substitution of the nitro group dominated to give 2-methoxyadenosine.

![Chemical structure](image)

**Scheme 3.4**

An alternative synthetic procedure starts with 6-chloropurine riboside 5, which is readily available from inosine (Scheme 3.5). First it was nitrated to give 6 then it was converted to 2-nitroadenosine triacetate 13 by replacement of the chloro substituent with sodium azide followed by conversion of the azide to the corresponding amine.
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Conditions; a) POCI₅, dimethylaniline, b) TBAN/TFAA (1.7 eq.), DCM, 65-71% over three steps; c) 1.0 eq. NaN₃, DMF, -18 °C; d) PPh₃, 1.1 eq., DCM, rt; e) AcOH/H₂O 3/1, 45 °C, 1.5 h, 64%, over three steps; f) KCN, MeOH, 2 h, rt, 80%.

Scheme 3.5

2-Nitroadenosine could be used as a precursor for several 2-aminated purines; for instance reduction of 14 with Pd/C gives 2-hydroxylamino adenosine (15) whereas reduction with Raney Ni results in complete reduction of the nitro group to yield diamino purine riboside (16).

Conditions: a) Pd/C, ethanol, 60%, b) RaNi, ethanol, 55%.

Scheme 3.6

3.2.2 2-Nitroinosine

The TBAN/TFAA nitrating reagent proved to be very sensitive to the presence of hydroxyl or amino groups in our system. Therefore direct nitration of inosine triacetate did not give the desired nucleoside. But starting from 6 and replacement of chloride under controlled conditions gave 2-nitro inosine 18 in good yield. The key step is the choice of nucleophile in the substitution of chloride, since hard nucleophiles result in decomposition of the nucleoside.
Adenosine Analogs

Scheme 3.7

\[ \text{Conditions: a) Sodium acetate (10 eq.), ethanol, reflux 8h, 84%. b) NH}_3/\text{MeOH, 0°C, 24 h, 46%}. \]

3.2.3 2-Nitropurine riboside

Nebularine, 9-(2,3,5-tri-O-acetyl-\(\beta\)-D-ribofuranosyl)-9H-purine 19 was prepared from chloride 5 by a literature method (Scheme 3.8).\(^{10}\) Direct nitration of 19 was not successful. In our previous experience on nitration of pyridine systems TBAN/TFAA nitrating agent was effective when pyridine was oxidized to the corresponding N-oxide (see chapter 2, § 2.5).\(^{11}\) So we checked if oxidation of 19 improved the nitration reaction. This oxidation was performed with several oxidants. \textit{mCPBA}, which usually is applied in this type of reactions, did not work. On the other hand, DMDO oxidation gave 1-oxo-9-(2,3,5-tri-O-acetyl-\(\beta\)-D-ribofuranosyl)-9H-purine 20 in 60%. The nitration reaction with TBAN/TFAA was performed with different amounts of the reagent, different reaction times and different temperatures. But in all cases either the starting material was recovered or it was decomposed. It seems likely that no suitable position for C-nitration in 20 is available. The unprotected nucleoside 21 was used for enzyme studies (chapter 5).

\[ \text{Conditions: a) Pd/C, H}_2, \text{ b) DMDO, 1 h, 60%, c) KCN/MeOH}. \]

Scheme 3.8

The site of oxidation was established by comparison of \(^1\text{H} \text{NMR spectra of 21 with imidazo}[4,5-b]\text{pyridine (22) and imidazo}[4,5-b]\text{pyridine-4-oxide (23). As is shown in Figure 3.1 oxidation at N3, which is close to the sugar, shifts the absorption of H1' to lower field. In N-oxide 21 the H1' has an absorption at 6.26 ppm.} \]
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Since direct nitration was not successful, a different approach was used. Reductive diazotization of 13 succeeded but problems arose during removal of the acetate protecting groups, and instead of 2-nitropurine riboside (26), 2-methoxypurine (25) was obtained as the main product.

To overcome this problem compound 27 was used in which the tert-butyldimethylsilyl group (TBS) was used as protecting group. This compound was prepared from 5 by protection with TBS. Conversion of 27 to the protected nitro adenosine 30 was carried out via the same route as is shown the Scheme 3.5. After reductive diazotization to 31 the TBS-groups were removed with fluoride ion under mild conditions to form the desired riboside.
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Conditions: a) KCN/MeOH, b) TBDMSCl, imidazole, c) TBAN/TFAA, 85 %, d) NaN₃, e) TPP, f) HOAc/H₂O 59% over three steps, g) isoamyl nitrite, 80 °C, 20 h, 68 %, h) TBAF/HOAc/THF, 38 %.

Scheme 3.10

3.3 2-Nitrosoadenosine

Aromatic C-nitroso compounds can be formed as reactive intermediates in biological systems. The intercellular lifetime of C-nitroso drugs is short, estimated to be about 30 min. At low concentrations their cytotoxic effects are negligible and they are converted to non-toxic species.¹²

3.3.1 Synthesis of 2-nitrosoadenosine

For ADA inhibition studies, 2-nitrosoadenosine 32 seems to be an interesting target. The first attempt for the synthesis of this compound was direct oxidation of hydroxylamine 15, using sodium periodate as oxidant. Oxidation with excess amount of this reagent resulted in cleavage of the C3’-C2’ bond (glycol cleavage). Under milder conditions and using less than one equivalent of oxidant the desired oxidation of the hydroxylamine function to nitroso occurred. It is known in the literature that the nitroso compound undergoes reaction with the starting material to give two isomeric azoxy dimers. The same reaction was observed in this case to produce compound 33 (E and Z isomer). The next reagent for this oxidation was t-butyl hypochlorite (t-C₄H₉OCl) but this reagent also oxidized the NH₂ at C6.
After these attempts, a different approach using acetate protected 2-nitro adenosine 36 was used. Reduction of 2-nitro-6-azidopurine 11 gave 34 in one step. The oxidation to 36 occurred smoothly but deprotection of the acetate protecting groups using several reagents resulted in decomposition before all the acetate groups were removed. Using TBS as protecting group and repeating the same sequence gave the TBS-protected nitroso compound 37 in good yield, but the problem of deprotection of the hydroxyl groups by using fluoride could not be solved, since decomposition occurred before the last TBS was removed.

Conditions: a) \( \text{H}_2, \text{Pd/C, EtOAc/EtOH, 45}^\circ \text{C} \), b) \( \text{NaIO}_4, \text{H}_2\text{O, EtOAc} \), c) \( \text{NH}_2/\text{MeOH} \), or \( \text{KCN, MeOH} \), or \( \text{MeONa/MeOH} \), d) \( \text{TBAF or HOAc/H}_2\text{O or NH}_4\text{F/MeOH} \).

Scheme 3.12
It is well documented that the nitroso group can participate in cycloaddition reactions.\textsuperscript{13} We took advantage of this cycloaddition for the synthesis of 2-nitroso adenosine 32. Diels-Alder reaction of 36 with cyclopentadiene at the room temperature gave the cycloadduct 38 (2 diastereomers in a 1:1 ratio) in 100% yield. Deprotection of the acetate protecting groups with a catalytic amount of potassium cyanide in methanol gave a mixture of the deprotected ribosides 39 in 75% yield. The major isomer was crystallised and thermal retro Diels-Alder reaction with this compound gave the 2-nitrosoadenosine 32 in approximately 50% yield.

\[
\begin{align*}
36 & \xrightarrow{a} 38 \\
& \xrightarrow{b} 39 \\
& \xrightarrow{c} 32
\end{align*}
\]

*Conditions:* a) cyclopentadiene, 100%, b) MeOH/KCN, 75%, c) DMF, N\textsubscript{2}, 100 °C, 50%.

Scheme 3.13

Aromatic nitroso compounds show a strong tendency to form dimers and the amount of dimerization in solutions strongly depends on concentration, temperature, solvent and the presence of substituents on the aromatic system.\textsuperscript{14} Although there is a lot of information about aromatic C-nitroso compounds\textsuperscript{16} nitroso-purines are not known in the literature. Nitrosobenzene, however, exist solely as the *cis* dimer, whereas 2,6-dimethylnitrosobenzene is exclusively in the *trans*-azodioxy form.\textsuperscript{15, 16} In case of 2-nitrosoadenosine the detected dimer in solution has been assumed to be in the *cis* form by analogy with the data from nitrosobenzene.

\[
\begin{align*}
32 & \xrightleftharpoons{} 40
\end{align*}
\]

*The dimerization of nitrosoadenosine.*

Scheme 3.14
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3.3.2 UV studies of the nitroso compounds

The UV studies on 2-nitroso adenosine 32 were carried out in water at different concentrations. Three major absorption bands (namely 202, 247 and 311 nm), and a shoulder at 400 nm were observed. The ratio of molar absorptions at 311 over 400 nm is dependent of the concentration of the nitroso compound. Therefore it can be concluded that absorption at 311 nm is mainly from the monomer and absorption at 400 nm belongs to dimer 40, as shown in Figure 3.2.

One could conclude that the amount of monomer is increasing by dilution but a quantitative amount of monomer or dimer could not be detected. These results are rather different from C-nitroso aromatic compounds like nitroso-benzene, since in low concentrations the only species is monomer. This could be explained by the steric effect of the two hydrogens in the ortho positions to the nitroso group, which makes the dimerization processes more difficult, compared with 2-nitroso adenosine. The absence of hydrogen atoms in the ortho positions facilitates dimerization.

<table>
<thead>
<tr>
<th>Concentration in D2O</th>
<th>Abs (311 nm)</th>
<th>Abs (400 nm)</th>
<th>A311/A400</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.1 µM</td>
<td>1.22</td>
<td>0.34</td>
<td>3.62</td>
</tr>
<tr>
<td>22.2 µM</td>
<td>0.78</td>
<td>0.26</td>
<td>3.0</td>
</tr>
<tr>
<td>52.0 µM</td>
<td>0.41</td>
<td>0.18</td>
<td>2.3</td>
</tr>
<tr>
<td>111 µM</td>
<td>0.21</td>
<td>0.12</td>
<td>1.75</td>
</tr>
<tr>
<td>222 µM</td>
<td>0.10</td>
<td>0.07</td>
<td>1.31</td>
</tr>
<tr>
<td>333 µM</td>
<td>0.08</td>
<td>0.06</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Absorption at 311 and 400 nm in different concentrations of 32 in water.

Figure 3.2

The same study for 2-nitroso adenosine triacetate was performed. At concentrations suitable for UV measurements, many aromatic nitroso compounds exist predominantly in monomer form. Figure 3.3 shows the UV absorption of 36 in chloroform at different concentrations.
There are two absorption bands, at 248 and 371 nm in chloroform and 259 and 400 nm in DMSO, of comparable molar absorptivity. In a given solvent and in solutions containing 50 μM to 150 μM, the ratio of molar absorptivities at these two wavelengths is independent of concentration of the nitroso compound.

These results show that 2-nitroso adenosine triacetate 36 in these two solvent is present mostly in the monomeric form. Since a satisfactory conclusion could not be obtained from the UV studies on 32 and 36, ¹H NMR experiments were carried out to find a clear answer to the monomer/dimer distribution.

Table 3.2

<table>
<thead>
<tr>
<th>Concentration(CHCl₃)</th>
<th>Abs(248 nm)</th>
<th>Abs(371 nm)</th>
<th>A_{248}/A_{371}</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μM</td>
<td>0.74</td>
<td>0.20</td>
<td>3.6</td>
</tr>
<tr>
<td>100 μM</td>
<td>1.53</td>
<td>0.42</td>
<td>3.6</td>
</tr>
<tr>
<td>150 μM</td>
<td>2.18</td>
<td>0.62</td>
<td>3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration(DMSO)</th>
<th>Abs(259 nm)</th>
<th>Abs(400 nm)</th>
<th>A_{259}/A_{400}</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μM</td>
<td>0.68</td>
<td>0.22</td>
<td>3.1</td>
</tr>
<tr>
<td>100 μM</td>
<td>1.32</td>
<td>0.45</td>
<td>3.0</td>
</tr>
<tr>
<td>150 μM</td>
<td>1.76</td>
<td>0.58</td>
<td>3.0</td>
</tr>
</tbody>
</table>
3.3.3 1H NMR studies of 2-nitrosoadenosine

1H NMR spectra at different temperature and concentration of 2-nitrosoadenosine triacetate (36) were recorded. At concentrations of the order of 10 M, typically used in NMR studies, the dimer of 2-nitroso adenosine is found in amounts comparable to those of the monomer; whereas at 253 K in CD$_2$Cl$_2$ the major species of 36 is the dimer. A complete characterization of the dimer is present in the experimental. The equilibrium of monomer/dimer is shifted to the formation of the monomer at high temperatures so we tried to characterize the monomer by elevating the temperature. 1H NMR spectra of the monomer were recorded at higher temperature (340 K), but since high concentrations are necessary for 13C NMR studies, the carbon spectra of the monomer under these conditions could not be detected, also 36 is moderately stable at higher temperatures.

The following table shows the ratio of dimer and monomer of 36 at different temperatures and concentrations, in CDCl$_3$. The integral of H8 in both cases is used to determine the monomer/dimer ratio. As it is shown at room temperature and low concentration, the ratio of monomer/dimer is more than 1 and at higher concentration probably the predominate species is the Z-dimer.

<table>
<thead>
<tr>
<th>entry</th>
<th>Concentration(temperature)</th>
<th>Monomer (8.77 ppm)</th>
<th>Dimer (8.51 ppm)</th>
<th>Monomer/dimer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>700 µM (300 K)</td>
<td>1</td>
<td>0.8</td>
<td>1.25</td>
</tr>
<tr>
<td>2</td>
<td>3500 µM (300 K)</td>
<td>1</td>
<td>3</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>3500 µM (340 K)</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>700 µM (253 K)</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

* These ratio's are based on the integral of H8, not the molar ratio's. In entries 1-3 the solvent is CDCl$_3$, but in entry 4 the solvent is CD$_2$Cl$_2$.

1H NMR studies on 2-nitrosoadenosine 32 at room temperature showed that the major species is the dimer even at very low concentrations.
Table 3.3 Dimer/Monomer of 32 ratio at different concentrations in D$_2$O

<table>
<thead>
<tr>
<th>Concentration D$_2$O</th>
<th>Monomer (8.65 ppm)</th>
<th>Dimer (8.33 ppm)</th>
<th>Idimer/Imonomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µM</td>
<td>1</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>50 µM</td>
<td>1</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>110 µM</td>
<td>1</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>440 µM</td>
<td>1</td>
<td>3.6</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Both tables show that at higher concentrations as expected the equilibrium is shifted to dimer formation. In case of 2-nitroso adenosine even at very low concentration dimerization is in favor although at this concentrations the known nitroso compounds such as nitroso-benzene are in monomeric form. Probably the solvent (D$_2$O) plays a role in shifting the equilibrium towards the dimer.

3.4 Functionalization of C2 in 2'-deoxyadenosine

Adenosines containing a halogen atom at the 2-position display cytotoxic activity, and especially 2-chloro-2'-deoxyadenosine (cladribine), which is a potent inhibitor of DNA synthesis, is currently used against leukemia and in the treatment of chronic lymphoid
malignancies. More importantly, 2-substituted-2'-deoxypurines are good inhibitors of ADA. Therefore the synthesis of 2-nitro-2'-deoxy-adenosine was undertaken. Selective protection of 2-nitroadenosine as its 3',5'-O-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl) derivative (41) proceeded satisfactory.

Functionalization of 41 at C2' with phenoxythiocarbonyl chloride, employing 4-(N,N-dimethylamino)pyridine (DMAP) as the catalyst, afforded the 3',5'-O-protected 2'-O-(phenoxythiocarbonyl) ester (42). Free radical-mediated deoxygenation with tributyltin hydride, using α,α'-azobis(isobutyronitrile) (AIBN) as the initiator, did not give the deoxygenated product, although usually under these conditions good results are obtained with adenosine and other nucleosides. This could be caused by the nitro group in the purine ring, which probably disturbs the radical reaction.

The desired compound 48 could be prepared using an alternative route starting from 2'-deoxyadenosine. As was described for the nitration of adenosine triacetate (Scheme 3.4), direct nitration of these systems without protection is not possible. First the sugar has to be protected with acetate groups and the amino group was converted into a chloride by diazotization of acetate protected amino compound. Nitration on 43 by TBAN/TFAA gave 44 in 72% yield. Afterwards the chloride was converted back into the amine 48 using the same route as described for compound 13 (Scheme 3.5).
3.5  Mechanism of the nitration

Nitration with TBAN/TFAA, which is mild nitration method, was applied in several systems. The TBAN/TFAA nitration proved to be a strongly substrate dependent process, since nucleosides such as adenosine triacetate 7 or nebularine triacetate 19 did not give any of the expected nitrated products. Formation of polar side products as a result of N-nitration and/or glycosidic bond cleavage was observed in some cases.

Since the conventional “nitronium-ion” nitration mechanism was introduced\textsuperscript{21} numerous alternative processes have been studied, all as a result of the many forms NO\textsubscript{3} can adapt. In a recent publication, Ridd reviewed a group of unconventional nitration pathways; most of them based on radical species and/or electron transfer processes.\textsuperscript{22} Only a few examples are known in which electron deficient substrates are nitrated at room temperature and from these, the nitration reactions of chloro-nitrobenzenes using N\textsubscript{2}O\textsubscript{5}/HNO\textsubscript{3} give a clear indication of a radical addition.\textsuperscript{23}

The nitro-nitrate-addition products, formed as intermediates, were observed by \textsuperscript{15}N CIDNP-NMR and support a radical addition mechanism, although electrophilic processes catalyzed by HNO\textsubscript{3}
seem to dominate the formation of the end products. NO$_2^*$ itself is not reactive enough for substituting the aromatic ring and therefore a more reactive species such as NO$_3^-$, which is formed in equilibrium from N$_2$O$_5$, initiates the substitution reaction. Comparable mechanisms were suggested to explain the unusual selectivity during Kyodai nitration with NO$_3$/O$_3$, although electron transfer from electron-rich substrates to NO$_3$ was postulated as the initiating step.$^{24}$

In the TBAN/TFAA system presumably trifluoroacetyl nitrate splits homolytically into NO$_2^*$ and the trifluoroacetate radical (Scheme 3.17).$^{25}$

$$\text{Bu}_4\text{NNO}_3 + (\text{CF}_3\text{CO})_2 \rightarrow \text{CF}_3\text{COONO}_2 \equiv \text{CF}_3\text{COO}^* + \text{NO}_2^*$$

*Generation of NO$_2^*$ from TBAN/TFAA*

Scheme 3.17

It should be noted that in theory N$_2$O$_5$ and consequently NO$_3^-$ can be formed during the TBAN/TFAA nitrations. Addition of the reactive trifluoroacetoxy radical to the imidazole C8 in 5 gives a highly delocalized radical that can be stabilised by a substituent at C6 (Scheme 3.18). In the next step combination of the radical with NO$_2^*$ takes place at C2. Elimination of trifluoroacetic acid from the unstable intermediate affords the product. In view of the high oxidation potential of purines, an alternative mechanism *via* electron transfer to NO$_3^*$ seems unlikely.

```
5 \rightarrow RO^* \rightarrow \text{NO}_2^* \rightarrow \text{H} \rightarrow \text{OR} \rightarrow \text{Cl}
```

*Proposed mechanism for nitrination of 6-chloropurine riboside*

Scheme 3.18

Any concurrent electrophilic processes during TBAN/TFAA nitration were excluded by a control experiment using nitronium tetrafluoroborate. No nitration was observed under these conditions and the starting material was completely recovered. In addition, Evans$^{25}$ and Nkoroge have already shown that radical capture by adding 4 equivalents of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) to this nitrating mixture (TBAN/TFAA) almost completely inhibited the reaction with benzocycloheptane as substrate.$^{25,26}$

Although additional studies are necessary to clarify the exact mechanism, similarities between N$_2$O$_5$ and the TBAN/TFAA nitrating mixture are obvious. The ease of handling the TBAN/TFAA mixture, in combination with the relatively mild acidic reaction conditions makes this reagent preferable over several other nitrating agents.
To summarize, for successful nitration the following substrate requirements can be deduced from these studies:

- Acidic protons (e.g. NH or OH) are not tolerated.
- Nucleophilic nitrogen atoms (as in pyridine) are not tolerated.
- Radical stabilizing substituent (Cl, NR₂, N-oxide) is required.

### 3.6 Structure determination by 1H NMR studies

The structure of 2-nitro-6-chloropurine (6) was proven by gradient accelerated HMBC spectroscopy (heteronuclear multiple bond correlation) optimized for 10 Hz coupling constants. As an illustrative example, a part of the gradient accelerated HMBC spectrum of 6 is shown in table 3.5. These data exclude the possibility of C8 nitration.

![Diagram of 2-nitro-6-chloropurine (6)]

#### Table 3-5

<table>
<thead>
<tr>
<th>Selected 3-bonds interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>H8</td>
</tr>
<tr>
<td>C4, C5, C1'</td>
</tr>
<tr>
<td>H1'</td>
</tr>
<tr>
<td>C8, C4, C3', C4'</td>
</tr>
</tbody>
</table>

### 3.7 ¹³C NMR assignment of 2-nitroadenosine

Carbon-13 magnetic resonance spectroscopy affords a possibility to study in detail the structure of the molecular framework. To assign the chemical shifts of C2, C6 and C8 in 2-nitroadenosine the proton-coupled ¹³C spectrum of this compound was recorded in d₆-DMSO. The peak from C8 is split into a multiplet, due to coupling with H8.

Literature information about the position of the C6 absorption is not unequivocal. A gross correlation of carbon-13 shift data with theoretical estimations of charge density is mostly used to determine the position of this carbon.²⁷

In the case of 2-nitroadenosine the ¹³C NMR distinction between C2 and C6 was rather difficult. The presence of the amine group at C6 was helpful. The proton decoupled ¹³C of this compound in d₆-DMSO before and after addition of a drop of D₂O was recorded. By addition of D₂O the amine proton is exchanged slowly and this exchange effects the chemical shifts of C6 in the ¹³C NMR. This exchange made a clear distinction between C2 and C6, since C6 was divided into a couple of peaks. This process was followed for 20 h and the selective ¹³C NMR is shown in Figure 3.7.
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The $^{13}$C NMR (125 MHz) of 2-nitroadenosine a) solution in $d_6$-DMSO, b) after addition of 20 μL $D_2$O and 10 min., c) the same mixture after 18 h.

Figure 3.5
3.8 Functionalization of the purine nucleoside at C6

As mentioned in chapter 1 substituents like methylamino, methoxyamino, hydroxylamino and halogens are converted into a hydroxy group by the action of ADA. We studied the effect of the size of the substituent on N-6. Starting from 6-chloropurine these compounds could easily be prepared. The procedure was to couple a small number of different amines to 6-chloro-purine riboside by a reaction in which the amine displaces the chloride. The amines chosen for these reactions were N-methoxy-N-methylamine, N-methoxyamine, N-methyl-N-hydroxylamine and N-hydroxylamine.

\[
\begin{align*}
\text{Cl} & \quad \text{ribose} + \text{RR'NH} \quad \text{EtOH, reflux} \quad \text{NRR' ribose} \\
& \text{Scheme 3.19}
\end{align*}
\]

6-N-hydroxylamine-purine riboside (53) is a substrate for ADA, but its isomer 6-O-hydroxylamine-purine riboside (54) is not known in literature. The synthesis of 54 would give another interesting C6-substituted purine with a product like structure.

\[
\begin{align*}
53 & \quad \text{Structure of N-hydroxylaminopurine and its isomer O-hydroxylaminopurineriboside.} \\
54 & \quad \text{Scheme 3.20}
\end{align*}
\]

First we tried a model reaction with 9-THP-6-chloropurine 57. The reagent N-hydroxy-phtalimide 55, was prepared by a literature method.
Deprotection of 57 gave 60. But the compound was so reactive under these conditions that we could only detect hydroxyderivative 58 in the 'H NMR spectra. The same observation has been made for the synthesis of 6-O-hydroxylamine-purine. The compound 59 was characterized by making the oxime 60 in situ with acetone. These results lead to the conclusion that the aminoxy group on carbons adjacent to electron-withdrawing heterocyclic nitrogen atoms, is too reactive to allow its isolation. This makes ADA inhibition studies impossible.

3.9 Functionalization of purine riboside at N1

Cleavage of the heterocyclic ring of the bases of nucleic acids (and their derivatives) can occur through the action of a number of reagents. Sometimes this opening of the ring is an intermediate stage of the reaction (Scheme 3.22) and is followed by its closure by groups of atoms, which differ from those in the original ring. In our group this ring opening of the purine systems at C2 was studied intensively.32,33
When a strongly electron-withdrawing group such as the 4-nitrophenyl is attached to the N1 atom of the hypoxanthine ring as in 62, the C2 becomes electrophilic enough to react with nitrogen-nucleophiles (Scheme 3.22). This leads to a fast ring reclosure of the formamidine intermediate, favoured by the loss of 2,4-dinitroaniline as the leaving group, to give the inosine derivative. Following a literature route 1-(2,4-dinitrophenyl)inosine triacetate (62) was obtained by treatment of inosine triacetate with 2,4-dinitrochlorobenzene and potassium carbonate in dimethylformamide at 80 °C. In 1H NMR this compound was present as a mixture of rotamers.

Scheme 3.22

By treatment of compound 62 with hydrazine (50% aq) compound 63 was obtained. Recrystallization from methanol gave pure crystals of 1-amino-inosine 63 in 18% yield. When compound 62 was treated with hydroxylamine product 64 was obtained in 40% yield.
3.10 Conclusions

A new and mild nitration procedure using TBAN/TFAA for functionalization of C2 in the purine ring is introduced. This nitro group was converted to a new series of 2-substituted purines. In the known synthetic procedures for 2,6 disubstituted purines 2,6-dichloropurine ribosides is used. This nitration method make an easy access to 2-nitro-6-chloropurine riboside 6 which is a cheaper alternative for the preparation of 2,6-disubstituted purines. 2-Nitrosoadenosine was prepared from the same precursor and a detailed study was carried out on the monomer/dimerization of this compound.

3.11 Acknowledgements

Martin Wanner is gratefully acknowledged for performing part of the syntheses described in this chapter. Hester van Lingen is acknowledged for the syntheses of N1-substituted inosine derivatives in §3.9.

3.12 Experimental

**General methods.** For general details see section 2.9, on page 34. For the NMR data assignments of the compounds in this chapter the following numbering has been used:

![Chemical structure](image)

2-Nitro-6-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-9H-purine (6):

A nitration mixture was prepared at 0 °C by adding of TFAA (6.34 mL, 45 mmol) to a solution of TBAN (13.7 g, 45 mmol) in dry DCM (75 mL) in ca 2 min. After 10 min this solution was added via syringe to an ice-cold solution of 6-chloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-purine (5) (12.39 g, 30 mmol) in DCM (75 mL). The reaction was quenched after 3 h at 0 °C by pouring the reaction mixture into a stirred mixture of sat. NaHCO₃ (200 mL), water (200 mL) and ether (ca 250 mL). The water layer was extracted with a 2/1 mixture of ether and DCM, the combined organic layers were washed successively with dilute NaHCO₃ (2 x 30 mL) and with water (30 mL) and dried over Na₂SO₄ (sometimes crystallisation of the product occurs during the extraction procedure). The pale yellow product was obtained by trituration with methanol (9.75 g, 21.3 mmol, 71%). An analytical sample was obtained by recrystallization from EtOAc. Mp 170 - 172 °C; ¹H NMR: δ 8.58 (s, 1H, H8), 6.30 (d 1H, J = 5.3 Hz, H1'), 5.76 (dd, 1H, J = 5.3 and 5.3 Hz, H2'), 5.57 (m, 1H, H3'), 4.52 (m, 1H, H4'), 4.44 (m, 2H, H5'), 2.16, 2.09, 2.06 (3x s, 9H, COCH₃); ¹³C NMR: δ 170.0 and 169.4 and 169.4 (C₃OCH₃), 153.1(C2), 152.7 (C6), 151.3 (C4), 68
6-N,N-Diacetylaminol9(2,3,5-tri-O-acetyl)-β-D-ribofuranosyl-9H-purine (8):
A solution of adenosine (2.67 g, 10 mmol) and DMAP (0.050 g) in acetic anhydride (25 mL) was heated in an oil bath, and the acetic acid was distilled off during the reaction together with Ac₂O (Bp 122-126°C). According to TLC a 1 / 1 mixture of tetra- and pentaacetate was formed. Additional amounts of DMAP (0.050 g) and acetic anhydride (10 mL) were added and distillation was continued for 2 h. Evaporation of the volatiles and chromatography over silica with EtOAc as eluent gave 8 as a glass (2.23 g, 7.88 mmol, 79%). 1H NMR: δ 8.89 (s, 1H, H2), 8.30 (d, 1H, J = 5.1 Hz, H1'), 5.95 (d, 1H, J = 5.3 Hz, H2'), 5.58 (m, 1H, H3'), 4.48 (m, 1H, H4'), 4.06 (m, 2H, H5'), 2.37 (s, 6H, NCOCH3), 2.15, 2.12, 2.11 (3 x s, 9H, COCH3). IR (KBr): 1748, 1602, 1577, 1368, 1221.

2-Nitro-6-diacetylamino-9(2,3,5-tri-O-acetyl)-β-D-ribofuranosyl-9H-purine (8):
Compound 8 (0.238 g, 0.50 mmol) was nitrated using 1.5 eq. nitrating agent. A clean reaction occurred, giving only product and starting material. The reaction was quenched after 1 h at 0 °C by pouring the reaction mixture into a stirred mixture of sat. NaHCO₃ (10 mL), water (10 mL). The water layer was extracted with a 2/1 mixture of ether and water. The combined organic layers were washed over Na₂SO₄. Flash chromatography and ethyl acetate as eluent gave 9 (0.082 g, 157 mmol) in 55% yield. 1H NMR: δ 8.55 (s, 1H, H2), 6.30 (d, 1H, J = 5.1 Hz, H1'), 5.73 (dd, 1H, J = 5.1 and 5.3 Hz, H2'), 5.58 (m, 1H, H3'), 4.48 (m, 1H, H4'), 4.06 (m, 2H, H5'), 2.37 (s, 6H, NCOCH3), 2.13, 2.05 (3 x s, 9H, COCH3). 13C NMR (d6-DMSO): δ 162.5, 157.2, 151.5, 139.8, 116.0, 88.4, 86.2, 73.7, 71.2, 62.3. HRMS (FAB+): obs. mass 523.1426, calc. mass C₃₇H₂₈N₄O₁₄ (M+H) 523.1425.

2-Methoxy-adenosine (10):
Compound 9 (0.070 g, 0.13 mmol) was dissolved in 5 mL of methanol. A catalytic amount of KCN was added and the mixture was stirred for 48 h. Evaporation of the solvent and recrystallization from methanol gave the product in 62% yield (26 mg, 0.08 mmol). Mp 206 - 209 °C. 1H NMR (D₂O): δ 8.12 (s, 1H, H8), 5.95 (d, 1H, J = 6.8 Hz, H1'), 4.60 (m, 1H, H2'), 4.20 (m, 1H, H3'), 3.98 (m, 1H, H4'), 3.81 (s, 3H, CH3), 3.6 (m, 2H, H5'), 2.91, 2.12, 2.09 (3 x s, 3H, COCH3). 13C NMR (d6-DMSO): δ 162.5, 157.2, 151.5, 139.8, 116.0, 88.4, 86.2, 73.7, 71.2, 62.3. HRMS (FAB+): obs. mass 330.1050, calc. mass C₂₇H₂₂N₄O₃ (M+H) 330.1059.

2-Nitro-6-azido-9(2,3,5-tri-O-acetyl)-β-D-ribofuranosyl-9H-purine (11):
Sodium azide (0.325 g, 5 mmol) was added to a solution of 6 (2.29 g, 5 mmol) in DMF (20 mL) at -18 °C (bath temperature). After 1 h at this temperature, stirring was continued at 0 °C for 2 h. Water (20 mL) was slowly added, resulting in crystallization of the product. The mixture was kept for 2 h at 0 °C, filtered and the azide was washed with water (3 x) and with 1/1 water/methanol, and dried in vacuo (2.06 g, 89%). A pure sample was obtained by recrystallization from EtOAc. Mp 164 - 166 °C. 1H NMR: δ 8.39 (s, 1H, H2), 6.28 (d, 1H, J = 5.5 Hz, H1'), 5.76 (dd, 1H, J = 5.3 and 5.3 Hz, H2'), 5.58 (m, 1H, H3'), 4.52 (m, 1H, H4'), 4.46 (m, 2H, H5'), 2.16, 2.12, 2.09 (3 x s, 3H, COCH3). 13C NMR (d6-DMSO): δ 169.8, 153.1, 152.7, 155.0, 153.9, 152.2, 145.0, 127.1, 87.3, 81.7, 73.5, 71.4, 20.6, 20.4, 20.2. IR (KBr): 2162, 1746, 1429, 1348, 1495; HRMS (EI): obs. mass for 456.1089, calc. mass C₃₉H₂₁N₇O₅ (M+H) 456.1088.

2-Nitroadenosine triacetate (13):
Triphenylphosphine (1.32 g, 5 mmol) was added to portions of a solution of 11 (2.06 g, 4.44 mmol) in DCM (25 mL). After the nitrogen evolution stopped, the solvent was removed by evaporation to give crude iminophosphorane 12, which was used without purification in the next step.
Compound 12 was dissolved in acetic acid (12 mL), diluted with water (4 mL) and stirred during 1.5 h at 45 - 50 °C.
The acid was neutralized using aqueous Na₂CO₃ and the product was extracted with EtOAc. Crystallization of the residue, obtained after drying and evaporation, yielded 13 as pale yellow crystals (1.40 g, 3.20 mmol, 72%).

Data for 12: 1H NMR: δ 8.09 (s, 1H, H2), 7.90 (m, 5H, Ar), 7.54 (m, 10H, Ar), 6.18 (d, 1H, J = 5.2 Hz, H1'), 5.78 (dd, 1H, J = 5.2 and 5.3 Hz, H2'), 5.65 (m, 1H, H3'), 4.47 (m, 1H, H4'), 4.42 (m, 2H, H5'). 2.13, 2.09, 2.05 (3x s, 9H, COCH₃).

Data for 13: Mp 145 - 146 °C; 1H NMR: δ 8.16 (s, 1H, H2), 6.23 (d, 1H, J = 5.5 Hz, H1'). 6.18 (s, b, NH₂), 5.76 (dd, 1H, J = 5.3 and 5.3 Hz, H2'), 5.63 (m, 1H, H3'), 4.48 (m, 1H, H4'), 4.46 (m, 2H, H5'). 2.17, 2.12, 2.09 (3x s, 9H, COCH₃); 13C NMR: δ 170.2 and 169.8 and 169.6 (COCH₃), 156.3 (C2), 155.2 (C6), 148.8 (C4), 142.3 (C8), 121.7 (C5), 86.7 (C1'), 80.6 (C4'), 73.5 (C2'), 63.2 (C3'), 60.2 (C5'); IR (KBr): 1345, 2831, 1430, 1369, 1325; HRMS (FAB+): obs. mass 313.0895, calc. mass C₆H₁₄N₂O₄ (M+H) 313.0897.

2-Nitroadenosine (14):
Compound 13 (864 mg, 2.0 mmol) was dissolved in methanol (10 mL) and 20 mL THF, and catalytic amount of KCN (0.065 g, 1.0 mmol) was added. Stirring at room temperature for 2 h followed by addition of TFA (77 µL, 1mmol). CAUTION: HCN is formed. Crystallization over night at 4 'C, gave the product in 80% yield (0.498 g). Mp 218 - 220 °C; 1H NMR (d₆-DMSO): δ 8.67 (s, 1H, H2), 8.31 (s, b, NH₂), 5.92 (d, 1H, J = 6.8 Hz, H1'), 5.53 (d, 1H, J = 6.1 Hz, OH), 5.27 (d, 1H, J = 5.0 Hz, OH), 5.02 (d, 1H, J = 5.6 Hz, OH), 4.59 (dd, 1H, J = 6.8 and 5.3 Hz, H2'), 4.18 (m, 1H, H3'), 3.98 (m, 1H, H4'), 3.60 (m, 2H, H5'); 13C NMR (d₆-DMSO): δ 158.9, 157.6, 151.4, 146.8, 124.5, 91.6, 88.8, 76.8, 73.4, 64.4; HRMS (FAB+): obs. mass 313.0895, calc. mass C₆H₁₄N₂O₄ (M+H) 313.0897.

2-N-Hydroxylamino-adenosine (15):
A mixture of 14 (50 mg, 0.16 mmol) and palladium on carbon (10 mg, 10%) in ethanol (5 mL) was hydroxylated at 1 atm for 15 minutes. The mixture was filtered over hydroxyl washed with 20 mL of ethanol, then concentrated in vacuo. Trituration with ethanol gave the product in 60% yield (28 mg, 0.096 mmol). Mp 185 - 195 °C; 1H NMR (d₆-DMSO): δ 8.56 and 8.27 (s, b, NHOH), 8.03 (s, 1H, H2), 6.96 (s, b, NH₂), 5.80 (d, 1H, J = 6.8 Hz, H1'), 5.39 (d, 1H, J = 6.1 Hz, OH), 5.20 (d, 1H, J = 5.0 Hz, OH), 5.14 (d, 1H, J = 5.6 Hz, OH), 4.59 (dd, 1H, J = 6.8 and 5.3 Hz, H2'), 4.18 (m, 1H, H3'), 3.98 (m, 1H, H4'), 3.60 (m, 2H, H5'); 13C NMR (d₆-DMSO): δ 162.8, 156.2, 155.9, 151.0, 137.1, 114.8, 86.9, 85.7, 73.2, 70.8, 64.9; HRMS (FAB+): obs. mass 299.1103, calc. mass C₆H₁₄N₂O₅ (M+H) 299.1104.

2-Aminoadenosine (16):
To a solution of 14 (50 mg, 0.16 mmol) in ethanol (5 mL), excess Raney nickel was added, and it was hydrogenated at 1 atm for 2 h. The mixture was filtered over hydroxyl and it was washed with 20 mL of ethanol, then concentrated in vacuo. The product was obtained by filtration after trituration with ethanol in 55% yield (24 mg, 0.088 mmol). Mp 235 - 239 °C; 1H NMR (d₆-DMSO): δ 7.93 (s, 1H, H2), 6.77 (s, b, NH₂), 5.72 (s, b, NH₂), 5.72 (d, 1H, J = 6.8 Hz, H1'), 4.59 (dd, 1H, J = 6.8 and 5.3 Hz, H2'), 4.18 (m, 1H, H3'), 3.98 (m, 1H, H4'), 3.60 (m, 2H, H5'); 13C NMR (d₆-DMSO): δ 158.9, 157.6, 151.4, 146.8, 141.2, 124.5, 91.6, 88.8, 76.8, 73.4, 64.4; HRMS (FAB+): obs. Mass 283.1154, calc. mass C₆H₁₄N₂O₅ (M+H) 283.1155.

2-Nitrosine triacetate (17):
Compound 6 (0.458 g, 1.0 mmol) and sodium acetate (0.82 g, 10 mmol) were refluxed in ethanol (10 mL) for 8 h. The mixture was diluted with methanol (10 mL) and oxalic acid (0.81 g, 9 mmol) was added. Silica (10 g) was added and after removal of the solvents the residue was applied to a column of silica, packed with 2% MeOH in EtOAc. Elution with 15% MeOH in EtOAc gave the product as a yellow glass (0.370 g, 84% yield). 1H NMR: δ 7.26 (s, 1H, H8), 6.16 (d, 1H, J = 5.4 Hz, H1'), 5.72 (t, J = 5.4 Hz, 1H, H2'), 5.61 (t, J = 5.4 Hz 1H, H3'), 5.2 (m, 1H, H4'), 4.42 (m, 2H, H5'); IR (KBr): 1631, 1430, 1369, 1325; HRMS (FAB+): obs. mass 440.1055, calc. mass C₆H₁₄N₂O₅ (M+H) 440.1053.
2-Nitroinosine (ammonium salt) (18):
Compound 17 (0.370 g, 0.84 mmol) was stirred in a mixture of methanol (5 mL) and aqueous ammonia (25%, 15 mL) at rt during 24 h. Evaporation of the solvents, co-evaporation with ethanol and trituration with hot ethanol gave, after cooling in ice 2-nitroinosine as an amorphous, yellow ammonium salt (0.127 g, 0.39 mmol, 46%). Mp dec. above 250 °C; 1H NMR (D2O): δ 8.34 (s, 1H, H6), 6.08 (d, 1H, J = 6.1 Hz, H1'), 4.84 (t, J = 6.1 Hz, 1H, H2'), 4.75 (m, 1H, H3'), 4.45 (m, 1H, H4'), 3.96 (m, 2H, H5'); 13C NMR (D2O): δ 169.6, 158.3, 151.1, 129.4, 91.6, 88.6, 76.4, 73.7, 64.4. HRMS: no spectrum could be obtained with El and FAB.

9-(2,3,5-tri-O-acetyl)-L-D-ribofuranosyl-9//-purine (19):
6-Chloro-9-(2,3,5-tri-O-acetyl)-L-D-ribofuranosyl-purine 5 (1.60 g, 3.87 mmol) and sodium acetate (0.80 g) were dissolved in 50 mL ethanol and 15 mL EtOAc and hydrogenated using 10% palladium on carbon (0.20 g) at 50 psi during 6 h. The catalyst was removed by filtration (Hyflo) and the filtrate concentrated in vacuo. Dissolved in 50 mL ethanol and 15 mL EtOAc and hydrogenated using 10% palladium on carbon (0.20 g) at 50 psi during 24 h. Evaporation of the solvents, co-evaporation with ethanol and trituration with hot ethanol gave, after stirring at 0 °C for 5 h gave the compound in 56% (22 mg, 0.056 mmol) yield. The reaction mixture was purified by flash chromatography on silica (10% methanol in DCM); the compound showed blue fluorescence on TLC. 1H NMR: δ 9.18 (s, 1H, H6), 9.02 (s, 1H, H2), 6.26 (d, 1H, J = 5.2 Hz, H1'), 5.99 (t, J = 5.4 Hz 1H, H2'), 5.69 (t, J = 5.4 Hz 1H, H3'), 4.45 (m, 3H, H4', H5'), 2.16 and 2.12 and 2.09 (s, 3H, COCH3).

1-N-Oxo-9-(2,3,5-tri-O-acetyl)-L-D-ribofuranosyl-9//-purine (20):
Freshly prepared dimethyldioxirane (DMDO, 8 mL in acetone) was added at 0 °C to 19 (37.8 mg, 0.10 mmol). Stirring at 0 °C for 5 h gave the compound in 56% (22 mg, 0.056 mmol) yield. The reaction mixture was purified by flash chromatography on silica (10% methanol in DCM); the compound showed blue fluorescence on TLC. 1H NMR: δ 8.92 (d, J = 1.7 Hz, 1H, H6), 8.90 (d, J = 1.7 Hz, 1H, H8), 8.32 (s, 1H, H2), 6.26 (d, 1H, J = 5.2 Hz, H1'), 6.17 (t, J = 5.2 Hz, 1H, H2'), 5.87 (t, J = 5.4 Hz 1H, H3'), 5.56 (m, 3H, H4', H5'), 2.16 and 2.13 and 2.09 (s, 3H, COCH3); 13C NMR: δ 169.7, 169.1, 145.4, 142.3, 134.2, 86.7, 85, 73.6, 71.6, 61.7, 20.5, 20.2, 20.1. APT and J-resolved C-H correlation shows an extraordinary large coupling constant between H8 and C8.

2-Methoxy-9-L-D-ribofuranosyl-9//-purine (25):
Removal of the acetates of compound 24 was carried out with a catalytic amount of KCN in methanol. During this reaction the nitro group was substituted by methoxy group. This substitution is complete even before the last acetate has been removed. 1H NMR (D2O): δ 9.44 (s, 1H, H-6), 9.16 (s, 1H, H8), 6.28 (d, 1H, J = 6.3 Hz, H1'), 5.70 (m, 1H, H5').
2-Nitro-6-chloro-9-(2,3,5-tri-O-tert-butyldimethylsilyl)-β-D-ribofuranosyl-9H-purine (28):

A nitrating mixture was prepared at 0 ºC by adding TFAA (0.21 mL, 1.5 mmol) to a solution of TBAN (0.457 g, 1.5 mmol) in dry DCM (3 mL). This mixture was added via syringe to an ice-cold solution of 6-chloro-9-(2,3,5-tri-O-tert-butyldimethylsilyl)-β-D-ribofuranosyl-purine 27 (0.62 g, 1.0 mmol) in DCM (2 mL). The reaction was quenched after 3 h at 0 ºC by pouring the reaction mixture into a mixture of saturated NaHCO₃ (2 x 10 mL) and water (10 mL) and ether (15 mL). The water layer was extracted with a 2/1 mixture of ether and DCM, the combined organic layers were washed successively with dilute NaHCO₃ (2 x 10 mL) and with water (10 mL) and dried over Na₂SO₄. After chromatography on neutral Al₂O₃ (PE/EtOAc 10/1) 0.561 g (0.85 mmol, purity 95% according to NMR) was obtained. "H NMR: δ 8.86 (s, 1H, H8), 6.12 (d, 1H, J = 4.2 Hz, H1'), 4.59 (dd, 1H, J = 4.2 and 4.3 Hz, H2'), 4.20 (m, 1H, H3'), 4.18 (m, 1H, H4'), 3.83 (m, 2H, H5'), 0.96, 0.92, 0.81 (3x s, t-Bu); 0.17, 0.16, 0.11 (3x s, SiCH₃); 13C NMR: δ 152.4 (C2), 152.1 (C6), 151.1 (C4), 148.0 (C8), 134.8 (C5), 89.6 (C1'), 85.6 (C4'), 76.2 (C2'), 71.1 (C3'), 61.8 (C5'), 25.7 and 25.4 (2x CCH₃), 18.4 and 17.9 (2x CCH₃), -4.5, -4.9, -4.9, -5.1 (4x SiCH₃); IR (KBr): 1345, 1490; HRMS (FAB): obs. mas. 674.2992, calc. mas. for C₂₅H₂₆N₂O₃Si₁₅ (M+H) 674.2992.

2-Nitro-6-amino-9-(2,3,5-tri-O-tert-butyldimethylsilyl)-β-D-ribofuranosyl-9H-purine (30):

A solution of 2-nitro-2,3,5-tri-O-TBS-adenosine (0.300 g, 0.46 mmol) in a mixture of THF (4 mL) and isoamylic nitrate (4 mL) was refluxed at 80 ºC during 20 h. Concentration of the solution, chromatography (PE/EtOAc 4/1) gave pure 30 as a glass (0.300 g, 0.47 mmol, 59% over 3 steps). "H NMR: δ 8.45 (s, 1H, H8), 7.39 (s, b, NH), 6.14 (d, 1H, J = 4.2 Hz, H1'), 4.66 (dd, 1H, J = 4.2 and 4.3 Hz, H2'), 4.33 (m, 1H, H3'), 4.15 (m, 1H, H4'), 3.83 (m, 2H, H5'), 0.94, 0.91, 0.821 (3x s, t-Bu); 0.15, 0.13, 0.09, 0.07, 0.00, -0.01 (4x s, SiCH₃); "C NMR: δ 156.6, 155.7, 148.9, 142.9, 121.7, 89.9, 85.5, 75.9, 71.4, 26.3, 26.0, 26.0, 25.9, 21.2, 18.8, 18.3, 18.1, -4.1, -4.6, -4.5, -4.7, -5.1, -5.3.

2-Nitro-9-(2,3,5-tri-O-tert-butyldimethylsilyl)-β-D-ribofuranosyl-9H-purine (31):

A solution of 2-nitro-2,3,5-tri-O-TBS-adenosine (0.300 g, 0.46 mmol) in a mixture of THF (4 mL) and isoamylic nitrite (4 mL) was refluxed at 80 ºC during 20 h. Concentration of the solution, chromatography (PE/EtOAc 4/1) gave the deaminated compound (0.200 g, 0.32 mmol, 68%) as a glass. "H NMR (500 MHz): δ 9.22 (s, 1H, H6), 8.88 (s, 1H, H8), 6.14 (d, 1H, J = 4.2 Hz, H1'), 4.63 (dd, 1H, J = 4.2 and 4.3 Hz, H2'), 4.33 (m, 1H, H3'), 4.22 (m, 1H, H4'), 3.83 (m, 2H, H5'), 0.97, 0.92, 0.82 (3x s, t-Bu); 0.17, 0.16, 0.10, 0.09, 0.09 (4x s, SiCH₃); "C NMR: δ 156.6, 155.7, 148.9, 142.9, 121.7, 89.9, 85.5, 75.9, 71.4, 26.3, 26.0, 26.0, 25.9, 21.2, 18.8, 18.3, 18.1, -4.1, -4.6, -4.5, -4.7, -5.1, -5.3.

2-Nitropurine riboside (26):

Tetraethylammonium fluoride 3H₂O (0.38 g, 1.20 mmol) was added to a solution of compound 31 (0.160 g, 0.26 mmol) and acetic acid (0.084 mL, 1.4 mmol) in THF (5 mL). After stirring at rt during 20 h the solution was diluted with some PE and directly applied to a column of silica in EtOAc. Elution with 8% MeOH in EtOAc and crystallization from methanol gave 2-nitro-nehuraline 26 (28.7 mg, 0.097 mmol, 38%). Mp 172 - 176 ºC; "H NMR (D₂O): δ 9.24 (s, 1H, H6), 9.10 (s, 1H, H2), 6.22 (d, 1H, J = 4.9 Hz, H1'), 4.75 (dd, 1H, J = 6.8 and 5.3 Hz, H2'), 4.43 (m, 1H, H3'), 4.12 (m, 1H, H4'), 3.85 (m, 2H, H5'); "C NMR (d₅-DMSO): δ 154.0, 151.6, 149.5, 148.9, 136.9, 87.9, 85.8, 73.7, 69.9, 60.8; HRMS (FAB): Obs. mass 298.0779, calcd mass for C₁₀H₁₇N₃O₃ (M+H) 298.0788.

Azo-ox formation from 15 with NaIO₄ (33):
2-Hydroxylamino adenosine (15) (0.5 g, 1.67 mmol) in water (2 mL) was added sodium periodate (0.171 g, 0.8 mmol). After a few minutes solid product was formed. After addition of water the azoxy compound was obtained in 30%. Since this compound is a mixture of E and Z isomers (60:40) the 1H NMR of the mixture is reported here. 1H NMR (d6-DSMO): δ 8.60, 8.41 (2x s, H2), 8.15, 7.66 (s, 2H, NH2), 5.91 (d, 1H, J = 4.5 Hz, H1'), 4.52 (m, 2H, H2'), 4.13 (m, 1H, H3' in E or Z), 3.99 (m, 1H, H3' in E or Z), 3.61-3.25 (m, 6H, H4', H5').

2-Hydroxyamin-6-amino-9-[(2,3,5-tri-0-acetyl)-β-D-ribofuranosyl]-9H-purine (34):
To 93 mg of 2-nitro-6-azidopurine (11) (0.09 g, 0.2 mmol) in a mixture of EtOAc and EtOH (2/0.5 mL) was added 5 mg of Pd/C and 1 atm of H2. After 3 h at 35 °C the mixture was filtered over hyflo and the solvent was evaporated to a glass (quantitative). 1H NMR: δ 8.42 (s, 1H, NHOH), 7.71 (s, 1H, H2), 6.45 (s, 1H, NH2), 6.06 (d, 1H, J = 4.1 Hz, H1'), 5.98 (dd, 1H, J = 4.1 and 5.3 Hz, H2'). 5.78 (m, 1H, H3'), 4.39 (m, 2H, H4', H5'), 2.10, 2.05, 2.02 (3x s, 9H, COCH3).

2-Nitroso-6-amino-9-[(2,3,5-tri-0-acetyl)-β-D-ribofuranosyl]-9H-purine (36):
To 22 mg (20.5 mg, 0.05mmol) of compound 34 in EtOAc (1 mL), was added sodium periodate (12.8 mg, 0.06 mmol) in 0.5 mL water. After 1 h at room temperature the mixture was extracted with EtOAc (2 x 10 mL), dried and the solvent evaporated to give the product in 54% (12 mg, 0.028 mmol). As mentioned before this compound in NMR studies at low temperature (-20 °C) consist of only the dimer.

1H NMR (CDCl3) at 253 K (dimer): δ 8.29 (s, 1H, H2), 6.45 (s, 1H, NH2), 6.27 (d, 1H, J = 6.0 Hz, H1'), 5.58 (m, 1H, H2'), 5.45 (m, 1H, H3'), 4.42 (m, 3H, H4', H5'), 2.10, 2.05, 2.02 (3x s, 9H, COCH3).

Data for monomer: 1H NMR (CD3OD, 330 K) only monomer: 8.64 (s, 1H, H2), 6.42, 6.35 (2x d, 2H, J = 5.8 Hz, H4'', H5''), 5.77 (d, J = 5.2, 1H, H1'), 5.45, 5.43 (2x m, 1H, H3'), 4.64 (s, 1H, H2', for one isomer), 4.50 (s, 1H, H2', for the other isomer), 4.50-4.41 (m, 2x 3H, H4', H5').

2-Nitroso adenosine (32):
Compound 38 (40 mg, 0.11 mmol) was heated in DMF at 90 °C for 15 min, under a dry nitrogen flow to remove the resulting cyclopentadiene. Evaporation of the solvent and recrystallization from water gave the product in 51% yield (0.166 mg, 0.056 mmol).

1H NMR (CD3OD, 330 K) only monomer: 8.59 (s, 1H, H8), 6.62 (d, 1H, J = 5.4 Hz, H1'), 4.80 (m, 1H, H2'), 4.42 (m, 1H, H3', H4'), 3.79-3.96 (m, 2H, H5').

1H NMR (d6-DSMO, 330 K) of the monomer: (8.68 (s, H2), 7.70 (b, 2H, NH2), 6.05 (d, 1H, J = 5.4 Hz, H1'), 4.99 (t, J = 4.6 Hz, H2'), 4.87(m, 1H, H3'), 4.67 (t, 1H, J = 5.4 Hz, H4'), 4.24 (d, 1H, J = 4.2 Hz, OH), 4.02 (d, 1H, J = 3.9 Hz, OH) 3.62-3.72 (m, 2H, H5').
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2-Nitro-3',5'-O-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)adenosine (41):
To a suspension of 2-nitroadenosine 14 (0.125 g, 0.39 mmol) in dried pyridine (2.5 mL) was added 1.3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.158 mL, 158 mg, 0.5 mmol) and the mixture was stirred for 3 h at ambient temperature while protected from moisture. Volatile materials were evaporated in vacuo, and the residue was partitioned between EtOAc and water. The organic phase was successively washed with ice-cold HCl (2 x 5 mL), water, saturated NaHCO₃, and saturated brine. Then it was dried and evaporated. The resulting compound 41 (0.33 mol, 0.184 g, 83%) was pure enough to be used in the next step. 'H NMR: δ 8.16 (s, 1H, H8), 6.10 (s, b, NH₂), 6.00 (d, 1H, J = 1.1 Hz, H1'), 5.00 (dd, 1H, J = 4.2 and 1.1 Hz, H2'), 4.61 (m, 1H, H3'), 4.05 (m, 3H, H4', H5'), 3.33 (s, b, OH), 1.12, 1.11, 1.10, 1.08, 1.07, 1.06, 1.05, 1.04 (8x s, CH₃).

2-Nitro-2'-O-(phenoxycarbonyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)adenosine (42):
To 0.184 g (0.33 mmol) of 41 were added dried acetonitrile (2.3 mL), DMAP (0.085 g, 0.7 mmol), and propylchlorothionocarbonate (60 µL, 75.8 mg, 0.44 mmol). The solution was stirred for 16 h at room temperature, evaporated to dryness in vacuo, and worked up as described for compound 41. 'H NMR: δ 8.22 (s, 1H, H8), 7.41-7.13 (m, 5H, Ar), 6.50 (s, b, NH₂), 6.31 (d, 1H, J = 5.3 Hz, H2'), 6.24 (d, 1H, J = 1.1 Hz, H1'), 5.13 (m, 1H, H3'), 4.21-4.01 (m, 3H, H4', H5'), 1.11, 1.10, 1.06, 1.05, 1.02 (8x s, CH₃).

2-Nitro-6-chloro-9-(2-deoxy-3,5-di-O-acetyl-β-D-ribofuranosyl)-9H-purine (44):
Sodium azide (0.163 g, 2.5 mmol) was added to a solution of 44 (1.038 g, 2.5 mmol) in DMF (20 mL) at -18 °C bath temperature. After 1.5 h at this temperature stirring was continued at 0 °C for 2 h. Water (10 mL) was slowly added. The mixture was kept for 2 h at 0 °C, whereas compound 45 precipitated. It was filtered and the azide was washed with water (3 x) and with 1/1 water/methanol, and dried in vacuo (0.895 g, 85%). Triphenylphosphine (0.66 g, 2.5 mmol) was added in portions to a solution of 45 (0.9 g, 4.25 mmol) in DCM (15 mL). After the nitrogen evolution stopped, the solvent was removed by evaporation to give crude iminophosphorane 46, which was used without purification in the next step.

Compound 46 was dissolved in acetic acid (5 mL), diluted with water (2 mL) and stirred during 1 h at 45 - 50 °C. The acid was neutralized using aqueous Na₂CO₃, and the product was extracted with EtOAc. Crystallization of the residue, obtained after drying and evaporation, yielded the amine as pale yellow crystals (0.633 g, 1.60 mmol, 72%). To 120 mg of 47 (0.303 mmol) dissolved in 1:1 mixture of methanol and THF, and 3.3 mg of KCN (0.05 mmol) was added. After 4 h at room temperature, it was concentrated in vacuo and the residue recrystallized from ethanol to give 39.5 mg of 48 (0.14 mmol, 64%, needle). Mp 240 °C; 'H NMR (D₂O): δ 8.57 (s, 1H, H8), 6.33 (t, 1H, J = 7.0 Hz, H1'), 4.43 (m, 1H, H3'), 4.38 (m, 3H, H4', H5'), 3.63-3.54 (m, 2H, H2'); 13C NMR (D₂O): δ 156.8, 156.1.

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1H NMR (CD₂OD, 300 K) dimer/monomer in 4:1 ratio (0.8 mg of 32 in 0.5 mL of solvent): 8.63, 8.53 (2x s, 1H, H8), 6.13 (dd, 1H, J = 5.8 Hz, H1', monomer), 5.78 (d, 1H, J = 5.2 Hz, H1', dimer), 3.48-4.82 (rest of sugar hydrogens for both species). No mass spectra could be obtained.
6-(N-Methoxy,N-methyl)aminoo-9-β-D-ribofuranosyl-9H-purine (50):

O,N-Dimethyldihydroxylamine hydrochloride (0.682 g, 7 mmol) was neutralized by dissolving in a solution of KOH (0.396 g, 0.7 mmol) in 10 mL dry ethanol. The resulting solution was stirred at room temperature for 30 min and then filtered to remove KCl. To 5 mL of this solution 0.203 mg of 6-chloropurine riboside (0.7 mmol) was added and the reaction mixture was stirred for 3.5 h at 79 °C. Solvent was evaporated in vacuo. The resulting oil was crystallized from ethanol. ¹H NMR (200 MHz, d6-DMSO): Δ 8.59 and 8.38 (s, 2H, H2 and H8), 5.96 (d, 1H, J = 5.6 Hz, H1') 4.53 (m, 1H, H2'), 4.16 (m, 1H, H3'), 3.83 (m, 1H, H4'), 3.77 (m, 2H, H5'), 2.82 (s, 3H, CH3); ¹³C NMR (d6-DMSO) Δ 61.2, 70.2, 74.0, 85.6, 87.3, 124.2, 139.7, 145.6, 146.0, 154.0; HRMS (FAB®): obs. mass 297.0947, calc. mass for C16H16N4O4 (M+H) 297.0948.

6-(N-Methoxy,N-methyl)amino-9-β-D-ribofuranosyl-9H-purine (51):

Methoxylamine hydrochloride (2.9 g, 34.7 mmol) was dissolved in a solution of 1.96 g of KOH (34.9 mmol) in 35 mL of dry EtOH. The resulting solution was stirred at room temperature for 30 minutes and then filtered. To this solution, 6-chloropurine riboside (1.01 g, 3.51 mmol) was added and the reaction mixture was refluxed for 8 h. After 18 h at 0 °C the product crystallised. Crystals were separated, washed with water. Recrystallization from ethanol gave the product in 52% yield (0.543 g, 1.83 mmol). Mp 199 - 201°C; ¹H NMR (200 MHz, d6-DMSO): Δ 8.25 and 7.79 (s, 2H, H2 and H8), 5.81 (d, 1H, J = 5.6 Hz, H1'), 4.84 (m, 1H, H2'), 4.12 (m, 1H, H3'), 3.94 (m, 1H, H4'), 3.78 (s, 3H, CH3), 3.45 (m, 2H, H5'); HRMS (FAB®): obs. mass 298.1150, calc. mass for C16H16N4O4 (M+H) 298.1151.

6-(N-Methyl,N-hydroxyl)amino-9-β-D-ribofuranosyl-9H-purine (52):

The amine was liberated by dissolving 2.90 g of MeNH2HCl (34.7 mmol) in a solution of 1.96 g of KOH (34.9 mmol) in 35 mL of dry EtOH. The resulting solution was stirred at room temperature for 30 min and then filtered. To this solution 6-chloropurine riboside (1.03 g, 3.59 mmol) was added and the reaction mixture was refluxed for 2 h. After allowed to cooling down to room temperature, the precipitate was recrystallized from ethanol to give 0.616 g of product (2.07 mmol, 58%). ¹H NMR (200 MHz, d6-DMSO): Δ 8.245 and 8.29 (s, 2H, H2 and H8), 5.94 (d, 1H, J = 5.6 Hz, H1'), 5.80-4.85 (s, b, NOH), 4.60 (m, 1H, H2'), 4.16 (m, 1H, H3'), 3.98 (m, 1H, H4'), 3.66 (m, 3H, CH3), 3.62 (m, 2H, H5'); HRMS (FAB®): obs. mass 299.1150, calc. mass for C16H16N4O4 (M+H) 299.1151.

6-(N-Hydroxyamino-9-β-D-ribofuranosyl-9H-purine (53):

The amine was liberated by dissolving 2.45 g of hydroxylamine hydrochloride (35.3 mmol) in a solution of 1.98 g of KOH (35.3 mmol) in 18 mL of dry EtOH. The resulting solution was stirred at room temperature for 30 min. and filtered. To the filtrate 6-chloropurine riboside (1.03 g, 3.59 mmol) was added and the reaction mixture was refluxed at 79 °C for 3.5 h. After cooling down to room temperature, sediment was recrystallized from ethanol to give 0.613 g of product (2.16 mmol, 60%); Mp 210 - 212 °C; ¹H NMR (200 MHz, d6-DMSO): Δ 8.15 and 7.83 (s, 2H, H2 and H8), 5.79 (d, 1H, J = 5.6 Hz, H1'), 4.48 (m, 1H, H2'), 3.96 (m, 1H, H3'), 3.59 (m, 1H, H4'), 3.43 (m, 2H, H5'); HRMS (FAB®): obs. mass 289.1151, calc. mass for C16H16N4O4 (M+H) 289.1152.

6-Phtalmidino-9-(tetrahydro-2-pyranyl)purine (57):

N-hydroxyphthalimide 55 (0.057 g, 0.3 mmol) was dissolved in 8 mL of dry DMSO at room temperature. Then sodium hydride (NaH, 0.02 g, 8mol) was added. The solution turned red-brown due to formation of the anion. After adding compound 56 (0.086 g, 0.29 mmol) the solution was stirred at room temperature for 36 h. After addition of 50 mL of ethyl acetate and extraction with water (2x 50mL) the organic layer was dried. Crystallization from ethyl acetate/petroleum ether gave the product in 75% yield (90 mg, 0.22 mmol). ¹H NMR (200 MHz): Δ 8.46 (2× s, 2H, H2, H8),
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7.94 (m, Ar), 7.83 (m, Ar), 5.78 (m, 1H, THP), 4.18 (m, 1H, THP), 3.7 (m, 1H, THP), 2.03-2.16 (m, 3H, THP), 1.74-1.78 (m, 3H, THP).

6-O-Hydroxamino-9-(tetrahydro-2-pyranyl)purine (59):
50 mg (0.12 mmol) of 57 was dissolved in dry DCM. Methyl hydrazine (6 µL, 0.12 mmol) was added at -20 °C, and the reaction mixture was stirred for 3 h. Since it was not possible to purify the sample, an oxime derivative was made in situ by addition of acetone. Evaporation of solvent gave a mixture of 60 and 59. Chromatography with EtOAc/11% MeOH gave 60 in 34% yield (0.04 mmol, 0.01 g).

Data for oxime 60: ¹H NMR: δ 10.22, 9.72 (2 x s, 2H, H2, H8), 5.78 (m, 1H, THP), 4.18 (m, 1H, THP), 3.51 (m, 1H, THP), 2.10, 2.13 (2 x s, 6H, CH3), 2.15-2.20 (m, 3H, THP), 1.84-1.88 (m, 3H, THP); HRMS (FAB+) obs. mass 246.1106, calcld mass for C₁₂H₁₄NO₂N (M+H) 246.1117.

1-(2,4-Dinitrophenyl)inosine triacetate 61.
A mixture of 3 (1.97 g, 5.0 mmol), 2 eq of 2,4-dinitrochlorobenzene (2.59 g, 12.8 mmol) and 2 eq of potassium carbonate (1.74 g, 12.6 mmol) in anhydrous dimethylformamide (25 mL) was heated at 80 °C for 25 h. After cooling, the mixture was kept at 4 °C for 18 h. The precipitate was filtered and washed with chloroform. The filtrates and washing were concentrated in vacuo. Purification with flash column chromatography with gradient methanol/chloroform (0 to 4%) gave 61 in 87% yield.

¹H NMR: δ 8.9 (1H, m, H2), 8.57 (1H, m, H8), 8.2 (1H, Ar), 7.5 (1H, Ar), 7.78 (1H, m, Ar), 6.10 (d, 1H, J = 4.2 Hz, H1'), 5.81 (m, 1H, H2'), 5.51 (m, 1H, H3'), 4.42 - 4.23 (m, 3H, H4' and H5').

1-Amino-inosine 62.
Compound 61 (520 mg, 0.93 mmol) was treated with 13.5 mL of hydrazine (1:1 water) and stirred for 18 h. A mixture of 62 and the ring open compound was formed. The brown crystals which were obtained from methanol/chloroform appeared to be the desired product (46 mg, 18%). Evaporation of the rest gave 135 mg of the open ring compound. Data for 63. Mp 215-218 °C; ¹H NMR (d₆-DMSO): δ 8.39, 8.43 (2H, s, H2 and H8), 5.89 (d, 1H, J = 4.2Hz, H1'), 5.51 (1H, OH), 5.22 (1H, OH), 5.07 (t, 1H, OH), 4.5 (m, 1H, H2'), 4.15 (m, 1H, H3'), 3.96 (m, 1H, H4'), 3.60 (m, 2H, H5'), 3.00 (m, 4H, H6), 2.10 (m, 2H, H7'). ¹³C NMR (d₆-DMSO): δ 153.9, 146.0, 146.6, 139.7, 124.2, 87.3, 85.6, 74.0, 70.2, 61.2; HRMS (FAB+): obs. mass 284.1010, calcld mass for C₁₀H₁₄NO₄ (M+H) 284.0995.

1-Hydroxy-inosine 63.
Hydroxylamine hydrochloride (697 mg, 10 mmol) was dissolved in ethanol (12.5 mL) and the mixture was refluxed for 3 h. A solution of potassium hydroxide (560 mg, 10 mmol) in ethanol (5mL) was added to it. After 10 minutes a solution of compound 61 (436 mg, 0.78 mmol) in DMF (11 mL) was added and the mixture was heated at 80 °C for 4.5 h. The reaction mixture was dried in vacuo, treated with ammonium hydroxide (10 mL) stirred for 18 h and concentrated in vacuo. The residue was crystallized from CH₃Cl/MeOH (1:1) and further purified by flash chromatography on silica (CH₃Cl/MeOH, 30%). Recrystallisation from methanol gave the product 63 in 40% yield. Mp 215-218 °C; ¹H NMR (d₆-DMSO): δ 8.59, 8.39 (2x s, 1H, H2, H8), 5.87 (d, 1H, J= 5.6 Hz, H1'), 5.52 (s, 2H, OH), 5.22 (s, 2H, OH), 5.06 (s, 2H, OH), 4.49 (m, 1H, H2'), 4.14 (m, 1H, H3'), 3.95 (m, 1H, H4'), 3.58 (m, 2H, H4', H5'); ¹³C NMR: δ 61.2, 70.2, 74.0, 85.6, 87.3, 124.2, 139.7, 145.6, 146.0, 154.0; HRMS (FAB+): obs. mass 285.0861, calcld mass for C₁₀H₁₃NO₅(M+H) 285.0835.

3.13 References and notes.


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