A new entry to adenosine analogues via purine nitration - Combinatorial synthesis of antiprotozoal agents and adenosine receptor ligands
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The mechanism of purine nitrination

The results described in this chapter were obtained in a concerted action with Melle Koch and will also be discussed in his thesis.

ABSTRACT

The mechanism of purine nitrination with a mixture of tetrabutylammonium nitrate and trifluoroacetic anhydride was elucidated by using NMR spectroscopy. Extensive monitoring of the nitrination of I excluded direct nitrination of the highly electrophilic C2 position and demonstrated that this reaction occurred in a three step process. Electrophilic attack by trifluoroacetyl nitrate (TFAN) on the purine N7 position results in a nitrammonium species that is trapped by a trifluoroacetate anion furnishing nitramine intermediate II. A subsequent nitramine rearrangement generates C2-nitro species III that immediately eliminates TFA to give 2-nitro-6-chloro purine IV. The involvement of radicals during the nitramine rearrangement was unequivocally established by $^{15}$N-CIDNP NMR. A radical trapping experiment disclosed that 65-70% of the nitramine rearrangement takes place intermolecularly.
6.1 INTRODUCTION

The mechanism of aromatic nitration reactions depends on the nature of the nitrating system, the actual reaction conditions, and is continuously under discussion.\(^1\)\(^2\) The direct attack of a nitronium ion on the aromatic species followed by a fast proton loss\(^3\) is still recognised as a valid model of nitration over a wide range of conditions (Scheme 6.1). For benzene or

\[
\text{path B} \quad \begin{array}{c}
\text{Ar}^+ \quad \text{NO}_2^-
\end{array}
\]

\[
\text{ArH} + \text{NO}_2^+ \quad \xrightarrow{\text{path A}} \quad +\text{Ar}_2\text{NO}_2 + \text{H}^+ \quad \text{fast} \quad \text{ArNO}_2
\]

Scheme 6.1. Classical nitration pathways.

compounds less reactive than benzene path A has always been accepted. For compounds more reactive than toluene a possible role for the electron transfer mechanism (path B) has been proposed.\(^4\) The initial reaction in path B has a high activation energy due to the difference in geometry of the nitronium ion (linear) and the nitrogen dioxide molecule (bent). Although direct nitration (path A) also involves a major change in geometry of the O-N-O group, the energy terms involved in bond formation stabilise the transition state. It has been shown by both CIDNP investigations and theoretical considerations that electron transfer plays, if any, only a minor role.\(^1\)\(^5\)

Both of the nitration pathways discussed in Scheme 6.1 are unlikely to be involved in the nitration of electron deficient substrates, like the purine system. Few examples are known of nitration of deactivated systems at room temperature. One, reported by Moodie, comprises

\[
\begin{align*}
\text{Cl} & \quad \text{NO}_2 \\
\text{Cl} & \quad \text{NO}_3^+ \\
\text{Cl} & \quad \text{O} \quad \text{NO}_2 \quad \text{Cl} \\
\text{Cl} & \quad \text{H} \quad \text{O} \quad \text{NO}_2 \quad \text{Cl} \\
\text{Cl} & \quad \text{H} \quad \text{O} \quad \text{NO}_2 \\
\text{Cl} & \quad \text{H} \quad \text{O} \quad \text{NO}_2 \\
\text{Cl} & \quad \text{H} \quad \text{O} \quad \text{NO}_2 \\
\text{Cl} & \quad \text{H} \quad \text{O} \quad \text{NO}_2
\end{align*}
\]

Scheme 6.2. Nitration of deactivated aromatic systems.
the nitration of chloro-nitrobenzenes using \( \text{N}_2\text{O}_5-\text{HNO}_3 \).\textsuperscript{6} The nitro-nitrate-addition products like 1, formed as intermediates, were observed by \( ^{15}\text{N} \) CIDNP NMR and support a radical addition mechanism (path B in Scheme 6.2), although electrophilic processes catalysed by \( \text{HNO}_3 \) seem to dominate the formation of the end products (path A). Nitrogen dioxide is not reactive enough for addition to the aromatic ring and therefore a more reactive species such as \( \text{NO}_3 \), which is formed in an equilibrium from \( \text{N}_2\text{O}_5 \), initiates the substitution reaction. Comparable mechanisms were suggested to explain unusual selectivity during Kyodai nitration with \( \text{NO}_2\text{O}_3 \), although electron transfer from electron rich substrates to \( \text{NO}_3 \) was preferred as the initiating step.\textsuperscript{7}

### 6.2 Trifluoroacetyl Nitrate in Purine Nitration Reactions

The use of the TBAN-TFAA mixture for electrophilic aromatic nitration was reported by Masci\textsuperscript{8} as an adaptation of Crivello’s nitration system, consisting of TFAA and heterogeneous metal or ammonium nitrates in inert solvents.\textsuperscript{9} The active species in both methods is the trifluoroacetyl nitrate, TFAN, formed in situ (Scheme 6.3). For benzenes high regioselectivity

\[
\begin{align*}
\text{Bu}_4\text{N}^+\text{NO}_3^- + \text{F}_3\text{C}-\overset{\cdot}{\text{O}}\overset{\cdot}{\text{C}}\text{F}_3 & \rightleftharpoons \text{Bu}_4\text{N}^+\text{O}_2\text{CCF}_3^- + \text{F}_3\text{C}=\overset{\cdot}{\text{O}}\text{NO}_2^+ \\
\text{TBAN} & \quad \text{TFAA} \\
\text{TBATFA} & \quad \text{TFAN}
\end{align*}
\]

**Scheme 6.3.** Trifluoroacetyl nitrate (TFAN) equilibria.

was obtained with this nitrating reagent. Reaction of substituted pyridines with TBAN-TFAA was reported to introduce the nitro group selectively on the 3 position.\textsuperscript{10} Apart from electrophilic aromatic nitraions, trifluoroacetyl nitrate has also been used for the nitration of enolacetates,\textsuperscript{11,12} silylenolethers,\textsuperscript{13} and the N-nitration of amides.\textsuperscript{14,15} uridines and inosines.\textsuperscript{16,17} Although generally the heterolysis of TFAN, thereby releasing nitronium ions, is considered to be the nitrating mode of action, also concerted pathways involving covalent TFAN\textsuperscript{15} and radical pathways via homolysis are believed to be operative.\textsuperscript{10,13}

The mild TBAN-TFAA nitration of purines proved to be a strongly substrate dependent process (Scheme 6.4). While (1-deaza) purines with protected hydroxyl and doubly protected amino functionalities are readily nitrated, (mono protected) adenosine triacetate and nebularin triacetate did not give any of the expected nitration products.\textsuperscript{18,19,20}
6.3 PROPOSED MECHANISMS

Both direct electrophilic nitrination of the highly electron deficient purine C2 position and the alternative mechanism via electron transfer, taking the high oxidation potential of purines into account, seem unlikely. Moreover, upon TBAN-TFAA nitrination of solid supported purines, described in Chapter 2, no concurrent nitrination of the polystyrene matrix was detected, indicating the negligible amount of strongly electrophilic nitronium ions.\(^\text{20}\) Therefore, in an earlier publication from our group a radical nitration pathway was proposed (Scheme 6.5).\(^\text{19}\) Homolytic cleavage of trifluoroacetyl nitrate as proposed by Evans et al. generates the trifluoroacetoxyl and nitrogen dioxide radicals (Scheme 6.3).\(^\text{13}\) Addition of the reactive trifluoroacetoxyl radical to the purine C-8 gives a highly delocalised radical that is stabilised by the substituent at C-6. Subsequent combination of this radical with NO\(_2\) at C-2 is followed by elimination of trifluoroacetic acid from the intermediate, which affords the product. Although in principle NO\(_2\) addition on C-4 or C-6 is also possible, this would not lead to stable products.

A major drawback in the mechanism proposed in Scheme 6.5 is the generation of the very unstable trifluoroacetoxyl radical, which rapidly decomposes to CO\(_2\) and the trifluoromethyl radical with a reported dissociation constant \(k > 5 \times 10^4\ \text{s}^{-1}\).\(^\text{21}\) During TBAN-TFAA nitrations formation of CO\(_2\) or CF\(_3\)H is not observed, which makes homolytic cleavage of trifluoroacetyl nitrate unlikely.

In the original publication it was noted, however, that in theory N\(_2\)O\(_5\) and consequently NO\(_3\) can be formed during the TBAN-TFAA nitrations as is depicted in Scheme 6.6. In the solid state dinitrogen pentoxide is composed of crystalline ionic nitronium nitrate,\(^\text{22}\) but in the
vapour phase and dissolved in organic solvents it is covalent. The covalent molecule will also
dissociate into nitrogen trioxide and nitrogen dioxide.\textsuperscript{23} In this case the highly reactive NO$_3$
radical adds to the purine C-8 position and the pathway in Scheme 6.5 is followed under
liberation of the strong acid HNO$_3$, which is buffered by tetrabutylammonium trifluoro-
acetate, TBATFA.

Although nitronium ions may be present in solution as indicated by the equilibria
mentioned above, direct electrophilic attack by the nitronium ion on the purine substrate was
excluded by a control experiment with nitronium tetrafluoroborate.\textsuperscript{19} No nitration was
observed under these conditions and starting material was recovered completely. However,
upon addition of TBAN to this mixture purine nitration did occur, albeit in moderate yields.
This points to the in situ formation of N$_2$O$_5$ as the nitrating species.

In order to test the viability of N$_2$O$_5$ intermediacy purine nitration was carried out with
preformed N$_2$O$_5$.\textsuperscript{24} Nitration with an excess of N$_2$O$_5$ of 6-chloropurine riboside triacetate 2
furnished the 2-nitro product 3 in 18\% yield, 21\% recovery of starting material, while a
considerable amount of 8-oxo product 4 was formed (Scheme 6.7). The addition of
difluoroacetate ions in the form of TBATFA led to a remarkably improved 2-nitro-product
yield. As depicted in Scheme 6.6 TFAN can be generated by reaction of TBATFA with N$_2$O$_5$.
Apparently, TFAN is a better purine nitrating species than N$_2$O$_5$, indicating an important role
for the trifluoroacetate ion.

\begin{equation}
\begin{align*}
\text{Bu}_4\text{N}^+\text{NO}_3^- + \text{F}_3\text{C}-\text{O}^-\cdot\text{NO}_2^- & \rightleftharpoons \text{Bu}_4\text{N}^+\text{O}_2\text{CCF}_3^- + \text{O}_2\text{N}^-\cdot\text{NO}_2^- \\
\text{TBAN} & \rightleftharpoons \text{TBATFA}
\end{align*}
\end{equation}

\textbf{Scheme 6.6.} N$_2$O$_5$ equilibria.

\begin{equation}
\begin{align*}
\text{Cl} & \quad \text{Rib(Ac)$_3$} \\
\text{Rib(Ac)$_3$} & \quad \text{N$_2$O$_5$} \\
\text{2} & \quad \text{3} \\
\text{O$_2$N} & \quad \text{Rib(Ac)$_3$} \\
\text{N$_2$O$_5$} & \quad \text{N$_2$O$_5$} \\
\text{3} & \quad \text{4} \\
\text{Rib(Ac)$_3$} & \quad \text{Rib(Ac)$_3$} \\
\text{18\%} & \quad \text{30\%} \\
\text{N$_2$O$_5$} & \quad \text{N$_2$O$_5$} \\
\text{21\%} & \quad \text{N$_2$O$_5$} \\
\text{with TBATFA} & \quad \text{recovered} \\
\text{38\%} & \quad \text{starting material} \\
\text{36\%} & \quad \text{21\%}
\end{align*}
\end{equation}

\textbf{Scheme 6.7.} Nitration of 6-chloro purine riboside triacetate 2 with N$_2$O$_5$. 

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6.4 DETECTION OF A 7-NITRO-8-TRIFLUOROACETOXY PURINE INTERMEDIATE

In order to obtain more insight into the purine nitrination mechanism an attempt was made to detect nitrination intermediates with NMR spectroscopy. Initially the nitrination of 6-chloropurine riboside triacetate 2 was studied. Interpretation of the spectra was hampered because the intermediates present were mixtures of diastereomers. Therefore, another substrate had to be selected that offered a clean and efficient nitrination and showed a simple NMR spectrum; 6-chloro-9-Boc purine 5 met with these requirements. This crystalline compound was easily synthesised in high yield by reaction of 6-chloro purine with Boc₂O and catalytic DMAP (Scheme 6.8). On preparative scale the nitrination of 5 was carried out in dichloromethane at 0°C and furnished 2-nitro-6-chloro-9-Boc purine 6 in 86% isolated yield.

![Scheme 6.8. Synthesis of 2-nitro-6-chloro-9-Boc purine.](image)

Monitoring the nitrination of 6-chloro-9-Boc-purine 5 with ¹H-NMR at -10°C clearly revealed the formation and decrease of a purine intermediate. Figure 6.1a shows part of the ¹H-spectrum of a mixture of 6-chloro-9-Boc purine 5 (0.13 M) and TBAN (0.26 M) in CD₂Cl₂ at -10°C. When TFAA (2 equivalents) was added to this mixture the appearance of the H8 (8.92 ppm) of the product 6 and the disappearance of the H2 (8.86 ppm) and H8 (8.63 ppm) signals of starting material 5 were observed as expected (Figure 6.1b). In addition, the fast formation and gradual decrease of a set of signals was seen that contained a singlet at 8.82 ppm (integrated as 1H), a singlet at 8.78 ppm (integrated as 1H) and a singlet at 1.48 ppm (integrated as 9H). These signals were assigned to purine intermediate 7. Some small additional peaks were detected which were ascribed to side products. After allowing the sample to warm up to room temperature, all of the starting material and intermediary peaks had disappeared and in the aromatic region only the product H8 signal remained (Figure 6.1c). The following t-butyl signals (not displayed) were observed: starting material 5 (1.65 ppm), intermediate 7 (1.48 ppm) and product 6 (1.72). The progress of the nitrination is represented graphically in Figure 6.2 as a plot of the normalised integral values against time.

A successful attempt to ‘freeze’ the nitrination reaction in the intermediate stage allowed for extensive spectroscopic characterisation of this species. The progress of the intermediate formation was monitored at -50°C and complete conversion of the starting material into the
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Figure 6.1. Aromatic region of $^1$H-NMR spectra of the nitration of 6-chloro-9-Boc-purine 5 at -10 °C in CD$_2$Cl$_2$. a) $t_0$, b) $t_{10}$ min, c) $t_\infty$.

A purine intermediate 7 over an 8 hour period was observed, while formation of 2-nitro product 6 was suppressed to less than 3%.\(^{27}\)

The $^{15}$N-NMR spectrum of the intermediate formed from 98% $^{15}$N-labelled TBAN revealed a doublet at 339.6 ppm with $J_{NH} = 2.7$ Hz (Table 6.1). In the corresponding $^1$H-spectrum a doublet at 8.82 ppm with $J_{NH} = 2.7$ Hz was observed instead of a singlet. A $^1$H-decoupling

Figure 6.2. Progress of the nitration by plotting the $^1$H-integral values against time ($T = -10$ °C). Triangles: starting material 5; Closed circles: purine intermediate 7; open circles: product 6.
Table 6.1. Selected NMR data from nitration with $^{15}$N labelled TBAN.a

<table>
<thead>
<tr>
<th></th>
<th>6-Cl-9-Boc-purine 5</th>
<th>purine intermediate 7</th>
<th>2-NO$_2$-6-Cl-purine 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>δ (ppm)</strong></td>
<td><strong>J (Hz)</strong></td>
<td><strong>δ (ppm)</strong></td>
<td><strong>J (Hz)</strong></td>
</tr>
<tr>
<td>$^1$H-H</td>
<td>8.86</td>
<td>8.78</td>
<td></td>
</tr>
<tr>
<td>$^1$H-H</td>
<td>8.63</td>
<td>8.82 (d)</td>
<td>$^3$J$_{HN}$ = 2.7</td>
</tr>
<tr>
<td>$^{13}$C-2</td>
<td>153.5 (d)</td>
<td>157.7 (d)</td>
<td>$^1$J$_{CH}$ = 212</td>
</tr>
<tr>
<td>$^{13}$C-8</td>
<td>144.3 (d)</td>
<td>93.0 (d)</td>
<td>$^1$J$_{CH}$ = 194</td>
</tr>
<tr>
<td>$^{15}$NO$_2$</td>
<td>339.6 (d)</td>
<td>$^3$J$_{NH}$ = 2.7</td>
<td>365.0</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>.75.86 (s)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

The experiment confirmed $^1$H-$^{15}$N-coupling. The size of the coupling constant points toward a $^3$J$_{NH}$ coupling. Purine intermediate 7 contains a N-nitro group and no C-nitro or nitrato moiety. The $^{15}$N-chemical shift of the doublet of the purine intermediate typically lies within the range of N-nitro compounds, which is generally shifted about 20 ppm upfield relative to that of C-nitro compounds. Peaks derived from covalently bound nitrates, which appear at even higher field values relative to N-nitro derivatives, were not detected. The signal of the 2-nitro product 6, a singlet at 365.0 ppm, displays a chemical shift value characteristic for aromatic C-NO$_2$ compounds.

In the $^{13}$C spectrum of the starting material 5 C2 was found at 153.5 ppm with $^1$J$_{CH}$ = 211 Hz and C8 at 144.3 ppm with $^1$J$_{CH}$ = 222 Hz (see Table 6.1). In the spectrum of purine intermediate 7 the values for C2 ($δ = 157.7$ ppm, $^1$J$_{CH}$ = 212 Hz) were similar to those of the starting material, but C8 showed a remarkable upfield shift to 93.0 ppm and a decreased $^1$J$_{CH}$ value of 194 Hz. Moreover, C-N-coupling with $^2$J$_{CN}$ = 1.8 Hz was observed for C8 in experiments with $^{15}$N-labelled TBAN. These results indicated that aromaticity was contained in the pyrimidine part, but not in the imidazole ring. In addition, the presence of a trifluoroacetoxyl group in purine intermediate 7 was observed with $^{13}$C-NMR as indicated by a quartet at 114.2 ppm ($^1$J$_{CF}$ = 290 Hz) and a doublet at 153.8 ppm ($^2$J$_{CF}$ = 44 Hz, $^3$J$_{CH}$ = 2.9 Hz). The $^{19}$F-NMR spectrum verified the presence of the trifluoroacetoxyl group, which appeared as a singlet at -75.86 ppm. With the help of CH correlation spectra and the observed long range couplings the intermediary singlet in $^1$H-NMR at 8.78 ppm could be assigned to H2 and the doublet at 8.82 ppm to H8. The extremely high chemical shift of 8.82 ppm for H8, a proton attached to a sp$^3$ carbon atom, can be explained by the three hetero
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atoms attached to C8, the electron-withdrawing effect of the nitro, Boc and trifluoroacetoxy groups and the anisotropic effect of the carbonyl and nitrosyl moieties. During the reaction the concentration of TFA increases and H8 showed a gradual up-field shift of about 0.2 ppm as a consequence of partial protonation of the intermediate, which disturbs the anisotropic effect.

The NMR data above led us to the structure assignment for the N-nitropurine intermediate 7 as shown in Figure 6.1.

6.5 The N-Nitration-Nitramine Rearrangement Mechanism

The radical addition mechanism proposed in Scheme 6.5 appeared to be inconsistent with the obtained NMR results and a new three-step nitrination pathway was suggested as depicted in Scheme 6.9. The purine ring system is N-nitrated on nitrogen atom 7 in the imidazole ring by electrophilic attack of TFAN. The highly electrophilic imidazolium cation is trapped by a present nucleophile, in this case trifluoroacetate, furnishing the observed nitropurine intermediate 7. A subsequent nitramine rearrangement follows in which the nitro group moves to the 'para' position (i.e. C2), affording 8. Fast TFA elimination finally leads to the nitrated product 6.


The Nitramine Rearrangement

Arylnitramine rearrangements are intensively studied ever since the discovery of the rearrangement of N-nitroaniline to o- and p-nitroaniline by Bamberger in 1893 (Scheme 6.10). He reported this rearrangement both acid-catalysed in cold hydrochloric acid, and uncatalysed
upon heating to give primarily o-nitroaniline and some p-nitroaniline, nitrosobenzene, carbon dioxide and nitrous fumes. The thermal rearrangement of N-methyl-N,p-dinitroaniline (Scheme 6.11, X=NO$_2$), neat or in dichloromethane, was reported by Barnes and Hickinbottom to afford N-methyl-2,4-dinitroaniline in high yield.$^{30}$ The rearrangement was effectively blocked in the presence of dialkylphenols, yielding mostly N-methyl-p-nitroaniline. From these observations it was inferred that free radicals were involved. This conclusion was later supported by the large positive activation volume of the decomposition of the N-methyl-N,p-dinitroaniline in a mixture of toluene and piperidine as found by Naud and Brower.$^{31}$ The positive change in activation volume is due to the increase in free space between the solvent molecules and the radical pairs generated. Homolytic reactions have positive activation volumes, while reactions which undergo polarisation either by bond breaking or bond formation have negative activation volumes.$^{32}$ After studying the thermal rearrangement of various rings-substituted N-methyl-N-nitroanilines a mechanism was proposed in which reversible homolysis leads to a radical pair (Scheme 6.11).$^{33}$ Recombination of the paired

\[ 
\text{Scheme 6.11. Homolysis of the N-NO}_2\text{ bond followed by recombination of the paired radicals.} 
\]
radicals generates the C-nitro compounds intramolecularly. The separation and escape of the radical fragments provides the ‘free’ N-methyl-anilinyl radical and NO₂. Recombination of these free radicals allows for the intermolecular formation of the C-NO₂ product. Separative diffusion and hydrogen abstraction renders N-methylaniline.

This radical pair rearrangement mechanism was originally put forward by White. He described the intermediacy of the N-methyl-anilinium radical cation and NO₂ radical pair during the acid-catalysed rearrangement of N-nitro anilines. The involvement of radicals was supported by the detection of CIDNP effects (see Section 6.6) by Ridd and coworkers during the acid-catalysed rearrangement of 2,6-dichloro-N-nitroaniline and 2,6-dibromo-N-nitroaniline. It was recognised that for nitramine rearrangements of nitroanilines with strongly electron withdrawing substituents the boundary between heterolysis (path a) and homolysis (path b) is less well defined (Scheme 6.12).

\[
\text{Scheme 6.12. Heterolysis vs. homolysis.}
\]

Another mechanism by which the nitro group can be transferred was suggested and is known as the ‘cartwheel’ mechanism shown in Scheme 6.13 for the acid catalysed nitramine rearrangement. The mechanism consists of the migration of the nitro group to the ortho position to give the ortho-nitrite. Further migrations occur rapidly as [3,3] sigmatropic shifts. Proton expulsion probably precedes the transformation of the mobile nitrito into the stable nitro form. A homolysis-recombination pathway of the ONO to NO₂ rearrangement has been suggested.

\[
\text{Scheme 6.13. Cartwheel mechanism of the nitramine rearrangement.}
\]
Nitro group rearrangements have also been observed in the nitration of arylanilides. Iley and coworkers reported the reaction of N-aryl-benzimidoyl chlorides with silver nitrate at room temperature to yield not the expected N-nitroamides, but N-(nitroaryl)benzamides with the nitro group rearranged to the ortho and para positions (Scheme 6.14).\textsuperscript{38} When the ortho and para positions were blocked, the reaction of silver nitrate with N-(nitroaryl)benzimidoyl chlorides afforded the expected N-nitroamides. A mechanism was proposed in which the intermediary O-nitro imidate rearranges via homolytic cleavage of the O-NO\textsubscript{2} bond. The rate of rearrangement appeared to be independent of the substituent in the Caryl ring, but increased with the electron withdrawing ability of the substituents in the N-aryl ring. In another study towards the rearrangement of ring substituted N-methyl-N-nitroanilines it was concluded that, in the absence of acid, substrates containing electron withdrawing substituents are more prone to rearrangement than those with electron donating groups.\textsuperscript{39}

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\includegraphics[width=\textwidth]{scheme_6.14.png}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 6.14.} Reaction pathway of N-arylimidoyl chlorides with AgNO\textsubscript{3}.

\textit{NITRAMINE REARRANGEMENT OF PURINES}

If the radical nitramine rearrangement mechanisms mentioned above are applied to the purine system a pathway as depicted in Scheme 6.15 is obtained. The N-NO\textsubscript{2} bond of the nitramine intermediate 7 splits homolytically and the paired radical fragments recombine under formation of a C2-NO\textsubscript{2} bond. The C2-NO\textsubscript{2} intermediate 8 was not detected because the subsequent TFA elimination leading to 6 is too fast on the NMR time scale.
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To validate that formation of 2-nitro product 6 occurred by rearrangement of the N7-nitro intermediate and not by other processes, the conversion of the nitramine intermediate 7, which was preformed at -50 °C in CDCl₃, into the 2-nitro product 6 was followed with ¹H-NMR at -10 °C. In Figure 6.3 the progress of the normalised integrals of H8 of nitramine intermediate 7 and the product 6 is represented graphically, demonstrating the excellent first order kinetics that indicate the expected unimolecular process. First order rate coefficients $k_N = 1.5 \times 10^{-3} \text{s}^{-1}$ (nitramine decrease) and $k_p = 1.9 \times 10^{-3} \text{s}^{-1}$ (product increase) were determined over about 4 half-lives with a high R²-value and good reproducibility. A similar discrepancy in the values of $k_N$ and $k_p$ was found by Ridd and coworkers during the acid catalysed nitramine rearrangement of 2,6-dichloro-N-nitroaniline and 2,6-dibromo-N-nitroaniline. They explained this by the occurrence of side reactions: a subsequent reaction would decrease the magnitude of the signal for the product at the end of the reaction and thus cause the extent of reaction at earlier times to be overestimated. Indeed side reactions took place as the final amount of product 6 did not reach the amount of nitramine 7 that was initially present (see also Section 6.7).

Figure 6.3. Conversion of $\text{MNNO}_2$ intermediate to the 2-nitro product followed with $^1\text{H-NMR}$ at -10°C. Closed circles: nitramine intermediate 7; open circles: product 6. $k_N$: nitramine decrease; $k_p$: product formation.
In order to prove that the rearrangement was not acid catalysed, it was conducted in the presence of DIPEA and identical reaction rates were found. Moreover, if the rearrangement would be acid catalysed, the reaction rate would increase during the course of the reaction, since TFA is generated upon product formation. Deviation from a first order correlation was not observed.

With the unimolecularity of the reaction now being established the involvement of radicals during the rearrangement was studied with $^{15}$N CIDNP NMR.

**6.6 CHEMICALLY INDUCED DYNAMIC NUCLEAR POLARISATION (CIDNP)**

Chemically Induced Dynamic Nuclear Polarisation refers to the perturbation of the nuclear spins away from the expected Boltzmann distribution.\(^{41,42}\) The effect is observed in NMR spectra as an abnormal intensity of the NMR signals; the signals display either enhanced absorption or emission. In other words, when dealing with $I = 1/2$ nuclei, allowing for only two nuclear spin states ($\alpha$ and $\beta$) in a high magnetic field, this means that one of the two states is either over- or underpopulated as compared to the Boltzmann distribution (Figure 6.4). For simplicity only the case of $I = 1/2$ nuclei will be discussed, since they reflect the spin quantum number of common nuclei like $^1$H, $^{13}$C, $^{15}$N and $^{19}$F. The discussion applies of course to all magnetic nuclei.

Reactions occurring through radical pairs are able to generate nuclear polarisation, when the products resulting from a radical pair depend on the lifetime of the singlet (antiparallel

![Energy Diagram](image)

**Figure 6.4.** Populations of nuclei with $\alpha$ or $\beta$ spins.
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Figure 6.5. Energy vs. separation (r) of singlet and triplet states of a radical pair in a magnetic field.

electron spins) and triplet (parallel electron spins) states. In a high magnetic field the triplet (T₀) and singlet (S) states of the radical pair become degenerate, when the two radical components are greater than 5 Å apart and then S-T₀ mixing can occur (Figure 6.5). Because S-T₀ mixing involves hyperfine interactions (hfi) due to coupling of the nuclear spins with the unpaired electron spins, it is faster for one nuclear spin state, say β, than for the other, ergo α.

Scheme 6.16 outlines the radical pair mechanism (though oversimplified), which accounts for the CIDNP effect. Consider for example the case of an initial singlet radical pair generated by thermal homolytic cleavage of AB, a singlet precursor. Spin selection rules state that product formation can only be the result of the encounter of two radicals paired in a singlet state. The nuclear spin state, that gives the fastest S-T₀ mixing, say β, will be most rapidly converted to the unreactive triplet state. The radical fragments A with β nuclear spin (represented as Aβ) will live

Scheme 6.16. Radical pair mechanism.
longer and some will ultimately escape from the geminate pair to become free radicals. The geminate product $A^aB$ generated by primary recombination of the radical pair in the singlet state will then display an overpopulation of the $\alpha$ nuclear spin state compared to the normal Boltzmann distribution and net polarisation is the consequence. The escaped, free radicals can give rise to side reactions or to random encounter to form a free radical pair. Now the nuclear spin state, that gives the fastest $S-T_0$ mixing ($\beta$) will be most rapidly converted to the reactive singlet state and the secondary recombination product $A^\beta B$ will be overpopulated in $\beta$ nuclear spins. If all the radicals eventually yield the same product, either during recombination within the original geminate pair or after separation, then the product signal will be unpolarised, unless the lifetime of the free radicals is long enough for loss of some of the polarisation via relaxation processes in the radicals.

Kaptein has developed a set of equations, known as the Kaptein rules, that allows one to predict the phase, i.e. enhanced absorption or emission, of the polarisation. It is written as follows for the net polarisation of atom $i$ derived from the radical $a$ in the pair of radicals $A,B$:

$$\Gamma_{ne} = \mu \epsilon a\nu (g_A - g_B)$$

Here $\Gamma_{ne}$ is the phase of the net polarisation observed (positive for enhanced absorption signals, negative for emission signals), $\mu$ is derived from how the radical pair is formed (positive when formed from a triplet precursor or by the encounter of free radicals, negative when formed from a singlet precursor), $\epsilon$ is derived from how the products are formed (positive for geminate products, negative for secondary recombination products), $a\nu$ is the sign of the hyperfine coupling constant for the nucleus $i$ in radical $A$, and $(g_A - g_B)$ is the difference between the $g$ values (obtained from epr data) of radicals $A$ and $B$.

$^{15}$N-CIDNP NMR has been a valuable tool in elucidating reaction pathways in nitration reactions. When the equation is applied to the polarisation of $^{15}$N nuclei, an additional negative sign must be added because of the negative magnetogyric ratio of the $^{15}$N nucleus. In the NO$_2$ radical the negative magnetogyric ratio of the $^{15}$N nucleus also causes $a_N$ to be negative and Kaptein's rule then takes the following form:

$$\Gamma_{ne} = \mu \epsilon (g_{nitr} - g_B)$$

The analysis can be further simplified if the reasonable assumption is made that the $g$ value of the organic purinyl radical is larger than the $g$ value of NO$_2$ (2.0000). The conclusions of Kaptein's rule can then simply be related to how the radicals are formed and how they react to form the product.

In a thermal radical rearrangement the radical pair is generated from a singlet precursor, so $\mu$ is negative. From the phase of the polarised NMR signals one can then conclude whether the
rearrangement occurs intra- or intermolecularly. If the product is formed in an intramolecular fashion, it is a geminate product, the result of a recombination reaction within the radical pair (ε is positive) and enhanced absorption is observed. If the product is formed in an intermolecular fashion, it is a secondary recombination product, the result of recombination after separation from the original pair (ε is negative) and emission is observed. Conclusions are less obvious if the rearrangement both has an intra- and an intermolecular counterpart. Since the observed NMR signal matches the sum of the unpolarised and polarised (positive and/or negative) material formed, even no net polarisation at all can be the consequence.

**15N-CIDNP NMR IN PURINE NITRATION**

When the rearrangement of nitramine 7 at 0 °C is followed with 15N NMR indeed CIDNP effects are observed (Figure 6.6). During several half lives the nitramine intermediate signal (N) shows enhanced absorption. In the early stage of the reaction at $t = 2$ min the 2-NO$_2$-product signal (P) shows emission, while during the remainder of the rearrangement a reduced absorption signal is observed until after about 4 half lives (reaction nearly complete) no CIDNP effects are observed any more. The observed CIDNP effects are graphically represented in Figure 6.7. These results can be explained with the mechanism shown in Scheme 6.17. The

![Figure 6.6. 15N CINDP NMR spectra of rearrangement of 0.45 M nitramine. S: nitrobenzene standard; N: nitramine intermediate; P: 2-nitro product.](image)
enhanced absorption of the nitramine intermediate signal (N) obviously indicates the reverse reaction of the radical pair to reform nitramine 7 by primary recombination within the original pair. The emission signal for the product (P) points to an intermolecular process. Free radicals

\[ \text{Scheme 6.17. Purine nitration mechanism obtained from NMR studies.} \]
re-encounter to form the secondary recombination product 6. In the beginning of the reaction its contribution to the NMR signal is larger, since the concentration of the escaped (or free) radicals is higher as is the chance of random free radical encounters.

**THE QUESTION OF INTRAMOLECULARITY**

White has shown that the nitramine rearrangement (Scheme 6.11) has both an intra- and intermolecular counterpart.\(^{48}\) By diverting the free NO\(_2\) radicals that have escaped from the original radical pair through reduction with hydroquinone he was able to block the intermolecular part of the rearrangement as shown in Scheme 6.18. It appeared that the intermolecular part of the rearrangement of N-methyl-N-nitroaniline was about 40\%.\(^{49}\) He found that increasing the hydroquinone concentration above a certain point had no further effect on the product yield. This indicated that the hydroquinone only reacted with the ‘escaped’ radicals and not with the paired radicals. The difference in o/p ratio with (68:32) or without (31:69) diverting agent implied that the ‘structure’ or average configuration of the radical pair varies depending on its source (nitramine homolysis or secondary recombination of free radicals). Since the radical reassociation step is an equilibrium process, the resulting radical pair probably possesses the lowest energy, most stable average configuration, resulting in C-NO\(_2\) product formation. That explains why in our case N-NO\(_2\) formation (high energy conformation, kinetically favoured) is in competition with C-NO\(_2\) formation during the intramolecular recombination. This is expressed in the enhanced absorption for N-NO\(_2\) intermediate 7. Intermolecular recombination on the other hand primarily leads to the thermodynamically more stable C-NO\(_2\) compound accounting for the observed emission signal of 6.

The rearrangement of nitramine intermediate 7 in the presence of 3 equivalents of hydroquinone revealed that only 30-35\% of 2-nitro product 6 was generated. This indicated that 65-70 \% of the purine nitramine rearrangement occurred intermolecularly. As expected, a greatly enhanced absorption was observed for product 6 as a consequence of removal of the emittive contribution to the NMR signal, indicating an exclusive intramolecular process. In
agreement, the signal of nitramine intermediate 7 still displayed enhanced absorption as can be seen in Figure 6.8.

When the rearrangement was performed in the presence of the radical scavenger TEMPO (3 equivalents) only 10% of product formation was observed, while the remainder was denitratred to starting material. Apparently the TEMPO radical is so reactive that it intervenes with the paired radicals, thus ‘kidnapping’ some of the radical partners responsible for intramolecular rearrangement. While in the TEMPO experiment the product signal displayed a strong enhanced absorption, the signal of nitramine intermediate 7 was only slightly enhanced, which is another indication for incursion of TEMPO in the paired radicals, thereby influencing the equilibria outlined in Scheme 6.17.

6.7 SIDE PRODUCTS: 8-OXO PURINE FORMATION

While monitoring the nitration reaction with NMR, two equivalents of TFAN were used and in the spectra a set of peaks was observed, derived from side product formation, that deserves more comment. The signals were observed both during direct nitration of 6-chloro-9-Boc-purine 5 and during the nitramine rearrangement after preformation of nitramine intermediate 7. The peaks disappeared after completion of the reaction by warming the sample
The mechanism of purine nitration

up to room temperature. In the \( ^{15}\text{N}-\text{NMR} \) spectra a set of signals composed of a singlet at 363.4 ppm and a doublet at 335.9 with \( J_{\text{NH}} = 2.7 \text{ Hz} \) corresponded to a doublet at 9.10 ppm with \( J_{\text{NH}} = 2.7 \text{ Hz} \) (integrated as 1H) and singlet at 1.54 ppm (integrated as 9H) in the \( ^1\text{H}-\text{NMR} \) spectra and a singlet at -75.95 ppm in \( ^{19}\text{F}-\text{NMR} \) spectra. These NMR-signals were interpreted as the consequence of an equilibrium between 2-nitro product 6 and a secondary nitramine intermediate due to a consecutive addition of TFAN leading to the observed dinitro intermediate 9 as depicted in Scheme 6.19. Since the para position is already substituted, nitramine rearrangement is not possible and either conversion back to the 2-nitro-6-chloro purine 6 occurs or, alternatively, hydrogen transfer gives rise to 2-nitro-6-chloro-8-trifluoroacetoxy purine 10. Hydrolysis of this latter compound upon aqueous work-up explains the formation of 8-oxo purines like 11 as incidentally isolated after nitration reactions, especially with a large excess of nitrating agent.

\[
\text{Nitramine rearrangement}
\]

\[
\text{Hydrolysis}
\]

**Scheme 6.19.** A dinitro intermediate leads to 8-oxo-purine formation.

6.8 **Concluding Remarks**

In summary, extensive monitoring of the TBAN-TFAA purine nitration reaction with NMR spectroscopy excludes direct nitration of the highly electrophilic C2 position and demonstrates that this reaction occurs in a three step process. Electrophilic attack by TFAN on the purine N7 position results in a nitrammonium species that is trapped by the trifluoroacetate anion furnishing nitramine intermediate 7. Subsequent nitramine rearrangement generates a C2-nitro species 8 that eliminates TFA to give 2-nitro-6-chloro purine 6. Moreover, the involvement
of radicals during the nitramine rearrangement is unequivocally established by $^{15}$N-CIDNP NMR. A radical trapping experiment discloses that 65-70% of the rearrangement takes place intermolecularly.

6.9 ACKNOWLEDGEMENTS

Melle Koch and the author wish to thank Martin Wanner for encouraging us to carry out this investigation and for the nitration involving dinitrogen pentoxide. The assistance of Jan Geenevasen, Jan Meine Ernsting and especially Lidy van der Burg with operating the 500 MHz NMR spectrometer is gratefully acknowledged.

6.10 EXPERIMENTAL

**General information.** All nitration monitoring experiments ($^1$H, $^{13}$C, $^{15}$N, $^{19}$F) were carried out on a Varian Inova 500 spectrometer operating at 11.74 T (499.9 MHz for $^1$H; 125.7 MHz for $^{13}$C; 50.7 MHz for $^{15}$N, 470.4 MHz for $^{19}$F) using a 5 mm SW probe or a 10 mm broadband tunable probe. The spectra were determined in deuterated chloroform or dichloromethane obtained from Cambridge Isotope Laboratories Ltd. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane ($^1$H, $^{13}$C) or liquid NH$_3$ ($^{15}$N) or CCl$_3$F ($^{19}$F). Coupling constants J are given in Hz. For the nitration experiments the solvents were run over a neutral alumina plug prior to use. Na$^{15}$NO$_3$ (98% $^{15}$N) was purchased from Aldrich. $^{15}$N-nitrobenzene was obtained according to Shackelford’s method for the nitration of benzene by replacing tetramethylammonium nitrate with $^{15}$N-labelled TBAN.$^{50}$ Dichloromethane was distilled freshly from prior to use subsequently from phosphorous pentoxide and calciumhydride. All other commercially available chemicals were used without further purification.

**N$_2$O$_5$ nitration of 6-chloro pyrurine riboside triacetate 2.** N$_2$O$_5$ (0.108 g; 1.0 mmol) was added to a solution of 6-chloropurine riboside triacetate 2 (0.124 g; 0.30 mmol) in dry CH$_2$Cl$_2$ (1.5 mL) at 0 °C under a nitrogen atmosphere. After stirring for one hour the solution was diluted with light petroleum. This solution was placed directly on a silica column and flash chromatography with EtOAc offered 2-nitro-6-chloropurine riboside triacetate 3 (24.7 mg; 18%), 2-nitro-6-chloro-8-oxo-purine riboside triacetate 4 (42.8 mg; 30%) and starting material 2 (26.0 mg; 21%).

Repeating the experiment, but with addition of TBATFA (0.142 g; 0.40 mmol), afforded a mixture of different composition: 2-nitro-6-chloropurine riboside triacetate 3 (52.2 mg; 38%), 2-nitro-6-chloro-8-oxo-purine riboside triacetate 4 (51.8 mg; 36%) and starting material 2 (9.2 mg; 7%).

**6-Chloro-9-Boc-purine (5).** A suspension of 6-chloropurine 4 (15.5 g; 0.10 mol), Boc$_2$O (31 g; 0.14 mol) and DMAP (0.3 g; 2 mmol) in dry CH$_2$Cl$_2$ (150 mL) was stirred for 3 h until a clear solution was obtained. Light petroleum (25 mL) and silica gel (10 g) were added, the mixture was filtered over high-flow and the solids were rinsed with EtOAc. Evaporating the solvent yielded the crude product (24.4 g; 96%). Recrystallisation from a mixture of EtOAc-light petroleum afforded a first batch of white needles (12.1 g; 48%). A second batch was obtained by recrystallising the concentrated filtrate (10.5 g; 41%). decomp. > 111 °C; $^1$H NMR (CDCl$_3$) δ 8.86 (s, 1H, H-2), 8.64 (s, 1H, H-8), 1.65 (s, 9H, CCH$_3$). $^{13}$C NMR (CDCl$_3$) δ 153.46 (d, J 211.2, C-2), 151.06 (d, J 13.2, C-6) 150.84 (dd, J 12.6, J 4.7, J 4.7,
Nitratio n of 6-chloro-9-Boc-purine 5. TFAA (2.25 mL; 16 mmol) was added dropwise to a solution of 6-chloro-9-Boc-purine 5 (2.55 g; 10 mmol) and TBAN (4.87 g; 16 mmol) in dry CH₂Cl₂ (25 mL) at 0 °C under a nitrogen atmosphere. After stirring for 1 h the solution was poured into 75 mL of sat. aqueous NaHCO₃-ice (1:1) and Et₂O (75 mL) was added. The aqueous layer was extracted with 3 portions of 50 mL Et₂O-CH₂Cl₂ (3:1). The collected organic layers were washed with H₂O (2x50 mL) and brine. The solvent was removed under reduced pressure and the crude product (2.58 g; 94%) was used without further purification.

Formation of 6-chloro-7-15N-nitro-8-trifluoroacetox y purine intermediate 7. A 5 mm NMR tube containing a solution of 6-chloro-9-Boc purine 5 (20 mg; 0.10 mmol) and 15N labelled TBAN (61 mg; 0.20 mmol) in CD₂Cl₂ (0.7 mL) was placed in an acetonitrile-dry ice bath of −10 °C. The reaction was started by the addition of TFAA (28 μL; 0.20 mmol). The contents were mixed and the tube was transferred into the spectrometer probe set to 50 °C and locked and shimmed within 2 minutes. 1H spectra were then recorded every 30 seconds using single pulses during several half lives. Then the sample was brought to rt and a 'te' spectrum was recorded again at −10 °C.

Synthesis of 15N-labelled TBAN. Tetrabutylammonium chloride (2.0 g; 8.4 mmol) was added to a stirred solution of 15N-labelled sodium nitrate (1.1 g; 12.6 mmol) in water (4 mL). When a white solid precipitated from the solution, CH₂Cl₂ (5 mL) was added and the biphasic system was stirred vigorously for 1 h. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3×5 mL). The combined organic layers were washed with water (1×4 mL) and dried with a large amount of Na₂SO₄. After filtration and washing the remaining Na₂SO₄ thoroughly, the solvent was evaporated and drying in vacuo at 50 °C gave 15N labelled TBAN as white crystals (2.2 g, 85%). mp 117-119 °C; lit. 116-118 °C (unlabelled). 15N NMR δ 381.3 (s).

NMR data for 15N-labelled 6-chloro-9-Boc-purine 6. 1H NMR δ 8.92 (s, 1H, H-8), 1.72 (s, 9H, CH₂CH₃). 13C δ 154.15 (d, J₁CN 29.4, C-2), 152.97 (d, J₁CN 4.6, C-6) 151.40 (d, J₁CN 4.0, J 4.5, C-4), 148.33 (d, J₁CN 2.7).
Kinetic and CIDNP studies.

All kinetic and \(^{15}\)N CIDNP NMR studies were carried out using CDC1\(_3\) as a solvent in 10 mm NMR tubes fitted with a coaxial insert containing a 0.15 M external reference solution of \(^{15}\)N labelled nitrobenzene (370.4 ppm) or nitromethane (379.4 ppm) in CDC1\(_3\).

Purine nitramine rearrangement. A 10 mm NMR tube containing a 0.15 M solution of nitramine intermediate 7 in CDC1\(_3\) (2.0 ml) preformed by the method described above and stored at -50 °C was placed in a bath of the appropriate temperature for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to the appropriate temperature and locked and shimmed within 2 minutes. The rearrangement was followed with \(^1\)H and \(^{15}\)N NMR. \(^1\)H spectra were recorded every 30 seconds or every minute; for the \(^{15}\)N CIDNP NMR experiments spectra were recorded every 3 minutes using single pulses with a pulse angle of 90° or every 45 seconds using single pulses with a pulse angle of 45°. Complete relaxation was ensured. \(^{15}\)N NMR relaxation times \(T_1\) were determined applying \(\pi\pi/2\) pulse sequences. Nitrobenzene: \((T_1)_0\) °C = 94 ± 4.7 s. Nitromethane: \((T_1)_{-10}\) °C = 98.9 ± 18.1 s. 2-nitro purine 6: \((T_1)_{0}\) °C = 23.4 ± 0.8 s, \((T_1)_{-10}\) °C = 19.8 ± 0.7 s. CIDNP data are given in Table 8 and Table 9.

For the runs followed by \(^1\)H NMR, the first order rate coefficients (k\(N\)) obtained from the decrease of the nitramine intermediate 7 were calculated over 3-4 half-lives from the plots of ln(N\(H_2\)/I\(_8\)) against time or ln(N\(H_8\)/I\(_8\)) against time, where N\(H_2\) and N\(H_8\) are the integral values of the nitramine H-2 and H-8 respectively and I\(_8\) the integral value of the tetrabutylammonium signal at 3.18 ppm used as an internal standard. The rate coefficients (k\(P\)) obtained from the increase of 2-nitro product were calculated from a plot of ln[(P\(H_8\)/I\(_8\))\(_{t=\infty}\) - (P\(H_8\)/I\(_8\))\(_t\)] against time, where P\(H_8\) are the integral values of the product H-8. Regression coefficients were 0.998 ± 0.002. The rate coefficients mentioned were measured in duplo (see Table 7).

Rearrangement in the presence of DIPEA. DIPEA (3 equiv; 153 μL; 0.90 mmol) was added to a solution of the 7-nitro-8-trifluoroacetox y purine intermediate 7 (0.15 M) in CDC1\(_3\) (2.0 ml) preformed by the method described above and stored at -50 °C. The sample was placed in an acetone-ice bath of -10 °C for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to -10 °C and locked and shimmed within 2 minutes. The rearrangement was followed with \(^1\)H NMR and first order rate coefficients were determined as described above (see Table 7).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Temperature</th>
<th>Additive</th>
<th>k (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15 M</td>
<td>0 °C</td>
<td>-</td>
<td>(k_N = 6.0\times10^{-3})</td>
</tr>
<tr>
<td>0.15 M</td>
<td>0 °C</td>
<td>-</td>
<td>(k_P = 7.4\times10^{-3})</td>
</tr>
<tr>
<td>0.15 M</td>
<td>-10 °C</td>
<td>-</td>
<td>(k_N = 1.5\times10^{-3})</td>
</tr>
<tr>
<td>0.15 M</td>
<td>-10 °C</td>
<td>-</td>
<td>(k_P = 1.9\times10^{-3})</td>
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<tr>
<td>0.15 M</td>
<td>-10 °C</td>
<td>DIPEA (3 equiv)</td>
<td>(k_N = 1.8\times10^{-3})</td>
</tr>
<tr>
<td>0.15 M</td>
<td>-10 °C</td>
<td>DIPEA (3 equiv)</td>
<td>(k_P = 2.0\times10^{-3})</td>
</tr>
</tbody>
</table>

\(^a\) k's determined in duplo.
CIDNP data

**Table 8.** $^{15}$N CIDNP integral values normalised on external $^{15}$N-nitrobenzene; 0.15 M nitramine.\(^a\)

<table>
<thead>
<tr>
<th>t (min)(^b)</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>8</th>
<th>11</th>
<th>14</th>
<th>17</th>
<th>20</th>
<th>23</th>
</tr>
</thead>
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<td></td>
</tr>
<tr>
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<td>-0.48</td>
<td>0.63</td>
<td>0.89</td>
<td>0.99</td>
<td>1.04</td>
<td>1.04</td>
<td>1.03</td>
<td>1.00</td>
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</tbody>
</table>

\(^a\) 0.15 M nitramine rearranged at 0 °C in CDCl\(_3\); the reaction was followed for a longer period but no further changes were observed.\(^b\) Time measured from the moment the sample was taken out of a bath of appropriate temperature.

**Table 9.** $^{15}$N CIDNP integral values normalised on external $^{15}$N-nitrobenzene; 0.45 M nitramine.\(^a\)

<table>
<thead>
<tr>
<th>t (min)(^b)</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>8</th>
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<th>14</th>
<th>17</th>
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<td>-1.19</td>
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<td>0.90</td>
<td>0.97</td>
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</table>

\(^a\) 0.45 M nitramine rearranged at 0 °C in CDCl\(_3\); the reaction was followed for a longer period but no further changes were observed.\(^b\) Time measured from the moment the sample was taken out of a bath of appropriate temperature.

**Rearrangement in the presence of hydroquinone.** A 10 mm NMR tube containing a solution of 6-chloro-9-Boc purine 5 (59 mg; 0.30 mmol) and a substoichiometric amount of $^{15}$N labelled TBAN (85 mg; 0.28 mmol) in CDCl\(_3\) (2.0 ml) was placed in an acetonitrile-dry ice bath of −50 °C and pre-cooled TFAN (40 μL; 0.28 mmol) was added. Purine intermediate 7 was allowed to be formed during 2 days at −50 °C in order to remove all TFAN present. Hydroquinone (99 mg; 0.90 mmol) was added to this solution and the sample was placed in a bath of −10 °C for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to −10 °C and locked and shimmed within 2 minutes. As an external standard $^{15}$N-nitromethane ($T_j = 113 ± 12$ s) was used instead of $^{15}$N-nitrobenzene. The rearrangement was followed with $^{15}$N NMR, 1x45° pulse, 40 sec. delay (see Table 10). After completion of the reaction the amount of product was more accurately determined with $^1$H NMR: ≈35 % of nitramine intermediate 7 had been converted into product 6 ($T_j = 19.8 ± 0.7$ s).

**Table 10.** $^{15}$N CIDNP integral values normalised on external $^{15}$N-nitromethane; 0.15 M nitramine in the presence of hydroquinone (3 equiv).\(^a\)

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<th>6.6</th>
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<th>15.0</th>
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<tr>
<td>Product 6</td>
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<td>0.40</td>
<td>0.39</td>
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</table>

\(^a\) 0.15 M nitramine rearranged at -10 °C in CDCl\(_3\).\(^b\) Time measured from the moment the sample was taken out of a bath of appropriate temperature.
Chapter 6

Rearrangement in the presence of TEMPO. TEMPO (140 mg; 0.90 mmol) was added to a solution of the 7-nitro-8-trifluoroacetoxypyrimidine intermediate 7 (0.15 M) in CDCl₃ (2.0 ml) preformed by the method described above and stored at -50°C. The sample was placed in a bath of 0°C for 30 seconds with occasional shaking, and was subsequently transferred into the spectrometer probe set to 0°C and locked and shimmed within 2 minutes. The rearrangement was followed with ¹⁵N NMR, 1x45° pulse, 30 sec. delay (see Table 11).

Table 11. ¹⁵N CIDNP integral values normalised on external ¹⁵N-nitrobenzene; 0.15 M nitramine in the presence of TEMPO (3 equiv).a

<table>
<thead>
<tr>
<th>t (min)b</th>
<th>Intermediate 7</th>
<th>Product 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>0.59</td>
<td>2.89</td>
</tr>
<tr>
<td>2.5</td>
<td>0.42</td>
<td>1.94</td>
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<tr>
<td>3</td>
<td>0.16</td>
<td>1.19</td>
</tr>
<tr>
<td>3.5</td>
<td>0.12</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>0.76</td>
<td>0.73</td>
</tr>
<tr>
<td>4.5</td>
<td>0.66</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
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<td>5.5</td>
<td>0.34</td>
<td>0.27</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
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<tr>
<td>6.5</td>
<td>0.76</td>
<td>0.20</td>
</tr>
<tr>
<td>7</td>
<td>0.76</td>
<td>0.20</td>
</tr>
<tr>
<td>7.5</td>
<td>0.66</td>
<td>0.20</td>
</tr>
<tr>
<td>8.5</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td>13</td>
<td>0.12</td>
<td>0.20</td>
</tr>
</tbody>
</table>

a 0.15 M nitramine rearranged at 0°C in CDCl₃, b time measured from the moment the sample was taken out of bath of appropriate temperature.

6.11 REFERENCES


The mechanism of purine nitration

27. The composition of the sample did not change, when placed in a freezer at -80 °C; after 5 months still no conversion of the intermediate to the end product had occurred.