JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by American Chemical Society

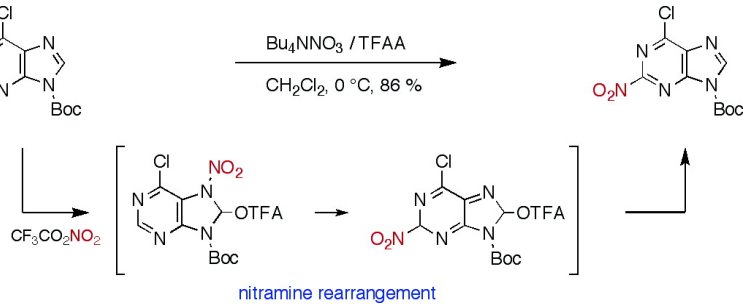
Article

The Mechanism of Selective Purine C-Nitration Revealed: NMR Studies Demonstrate Formation and Radical Rearrangement of an N7-Nitramine Intermediate

Boris Rodenko, Melle Koch, Alida M. van der Burg, Martin J. Wanner, and Gerrit-Jan Koomen

J. Am. Chem. Soc., **2005**, 127 (16), 5957-5963• DOI: 10.1021/ja042590x • Publication Date (Web): 30 March 2005

Downloaded from http://pubs.acs.org on March 25, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
ARTICLES
Published on Web 03/30/2005

The Mechanism of Selective Purine C-Nitration Revealed: NMR Studies Demonstrate Formation and Radical Rearrangement of an N7-Nitramine Intermediate

Boris Rodenko, Melle Koch, Alida M. van der Burg, Martin J. Wanner, and Gerrit-Jan Koomen*

Contribution from the Van't Hoff Institute for Molecular Sciences, Universiteit van Amsterdam, Nieuwe Achtergracht 129, NL-1018 WS Amsterdam, The Netherlands

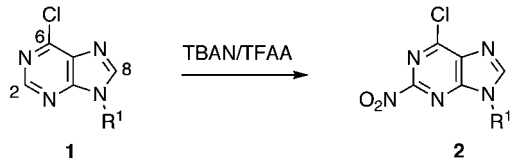
Received December 9, 2004; E-mail: gjk@science.uva.nl

Abstract: Modified purine derivatives are of great importance in biomedical sciences, and substitution reactions on the purine skeleton are intensively studied. In our laboratory, an efficient and selective purine C2-nitration reaction was developed using a mixture of tetrabutylammonium nitrate and trifluoroacetic anhydride. The resulting 2-nitro moiety appeared to be a versatile handle to introduce a variety of pharmacophores onto the purine skeleton. Since the mechanism of this selective purine C2-nitration reaction has remained unclear, we now present an extensive NMR study leading to its elucidation, using N9-Boc-protected 6-chloropurine as a model compound. Direct electrophilic aromatic nitration of the highly electron-deficient C2 position was excluded, and we demonstrate that this reaction occurs in a three-step process. Electrophilic attack by trifluoroacetyl nitrate on the purine N7 position results in a nitrammonium species that is trapped by a trifluoroacetate anion furnishing N7-nitramine intermediate **11**. This intermediate was characterized at −50 °C by ^{1}H, ^{13}C, ^{15}N, and ^{19}F NMR. At $T > -40$ °C, the N7-nitramine intermediate undergoes a nitramine rearrangement, which generates a C2-nitro species that immediately eliminates TFA to give 2-nitro-6-chloro-9-Boc purine **10**. The involvement of radicals during the nitramine rearrangement was unequivocally established by ^{15}N-CIDNP. Moreover, the emission signal observed for the rearranged product, 2-nitropurine **10**, showed that it is primarily formed in an intermolecular process. A quantitative radical trapping experiment finally disclosed that 65−70% of the nitramine rearrangement takes place intermolecularly.

Introduction

Given the pivotal role of purines in the regulation of many biological processes and as constituents of DNA and RNA, the interest in modified purine derivatives is immense in biomedical sciences. For instance, these compounds have found application as adenosine receptor ligands,[1] antiparasitic agents,[2,3] and cyclin-dependent kinase inhibitors,[4] antitumor,[4−6] and antiviral compounds.[7,8] Substitutions on the purine skeleton are thus subject to extensive study, and in pursuing this line of research, we described the selective introduction of a C2-nitro group by application of the mild tetrabutylammonium nitrate−trifluoroacetic anhydride (TBAN−TFAA)[9] system to purines of type **1** (Scheme 1).[10,11]

Scheme 1



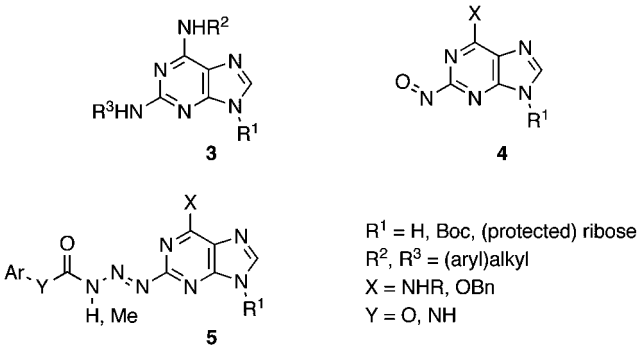
1 **2**

R^1 = H, Boc, (protected) ribose

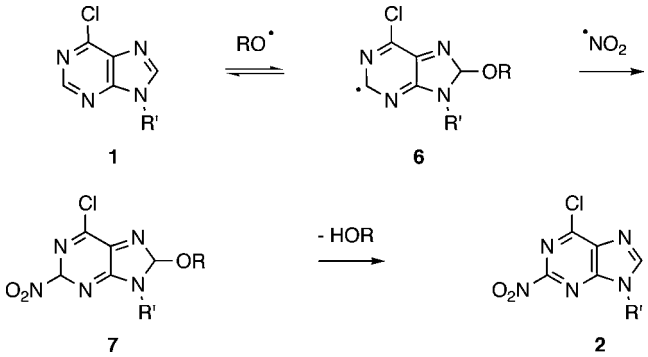
The C2-nitro group proved to be a versatile synthetic functionality. The electrophilicity of the purine C6 position is greatly enhanced by the nitro substituent, and introduction of nucleophiles on the C6 position by displacement of the 6-chloro moiety of **2** can be achieved at temperatures below 0 °C. By using various amines, 2-nitroadenosine derivatives were obtained

(1) Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, *J. Pharmacol. Rev.* **2001**, *53*, 527−552.
(2) Bressi, J. C.; Verlinde, C. L. M. J.; Aronov, A. M.; Shaw, M. L.; Shin, S. S.; Nguyen, L. N.; Suresh, S.; Buckner, F. S.; Van Voorhis, W. C.; Kuntz, I. D.; Hol, W. G. J.; Gelb, M. H. *J. Med. Chem.* **2001**, *44*, 2080−2093.
(3) Harmse, L.; van Zyl, R.; Gray, N.; Schultz, P.; Leclerc, S.; Meijer, L.; Doerig, C.; Havlik, I. *Biochem. Pharmacol.* **2001**, *62*, 341−348.
(4) Meijer, L.; Raymond, E. *Acc. Chem. Res.* **2003**, *36*, 417−425.
(5) Galmarini, C. M.; Mackey, J. R.; Dumontet, C. *Lancet Oncology* **2002**, *3*, 415−424.
(6) Several recent examples include: (a) Prekupec, S.; Svedrui, D.; Gazivoda, T.; Mrvo-Sermek, D.; Nagl, A.; Grdia, M.; Paveli, K.; Balzarini, J.; De Clercq, E.; Folkers, G.; Scapozza, L.; Mintas, M.; Rai-Mali, S. *J. Med. Chem.* **2003**, *46*, 5763−5772. (b) Hocek, M.; Votruba, I.; Dvorakova, H. *Tetrahedron* **2003**, *59*, 607−611.
(7) De Clercq, E.; Neyts, J. *Rev. Med. Virol.* **2004**, *14*, 289−300.
(8) Several recent examples include: (a) Diwan, P.; Lacasse, J. J.; Schang, L. M. *J. Virol.* **2004**, *78*, 4431−4434. (b) Chen, X.; Kern, E. R.; Drach, J. C.; Gullen, E.; Cheng, Y.-C.; Zemlicka, J. *J. Med. Chem.* **2003**, *46*, 1531−1537.
(9) Abbreviations used in the text: Boc (*tert*-butoxycarbonyl), DMAP (4-*N*,*N*-(dimethylamino)pyridine), TBAN (tetrabutylammonium nitrate), TFA (trifluoroacetic acid), TFAA (trifluoroacetic anhydride), TFAN (trifluoroacetyl nitrate), OTFA (trifluoroacetoxy).
(10) Deghati, P. Y. F.; Wanner, M. J.; Koomen, G.-J. *Tetrahedron Lett.* **2000**, *41*, 1291−1295.
(11) Deghati, P. Y. F.; Bieräugel, H.; Wanner, M. J.; Koomen, G.-J. *Tetrahedron Lett.* **2000**, *41*, 569−573.

Chart 1



Scheme 2



that appeared to be potent and selective agonists for the adenosine receptors.[12] In addition, the remaining C2-nitro group, with a leaving group ability comparable to that of a fluoro moiety, can simply be displaced by nucleophilic species, which was applied in the solid-phase synthesis of C2,N^6-disubstituted adenosine analogues **3** (Chart 1, R^1 = ribose).[13] Also, the nitro group itself can be modified in several ways. Raney−Nickel-catalyzed hydrogenation led to 2-amino−adenosine derivatives.[10] More importantly, hydrogenation of the nitro group with Pd or Pt on carbon completely stops in the NHOH stage, and by oxidation with sodium periodate, 2-nitroso derivatives **4** are obtained, which were useful in hetero-Diels−Alder reactions.[14] The effective conversion of C2-nitroso purines into triazenes **5** by condensation with hydrazino-functionalized pharmacophores offered purine derivatives with high selectivity for the adenosine A_1 receptor.[15] Introduction of a methyl group in the triazene side chain gave O^6-benzyl-substituted purines **5** with promising cytotoxic properties.[16]

The mild TBAN−TFAA nitration that introduces this versatile nitro group on the purine skeleton proved to be substrate dependent. Purines and 1-deazapurines with 6-chloro, 6-nitro, protected 6-hydroxyl, and doubly protected 6-amino functionalities are readily nitrated. Unprotected purines (mono-N^6-acylated), adenosine triacetate, and nebularine (6-H-purine riboside) triacetate did not give any of the expected nitration products.[10,11,17]

The use of the TBAN−TFAA mixture for electrophilic aromatic nitration was reported by Masci[18] as an adaptation of Crivello's nitration system, consisting of TFAA and heterogeneous metal or ammonium nitrates in inert solvents.[19] The active species in both methods is trifluoroacetyl nitrate, TFAN, formed in situ (eq 1).

$$Bu_4NNO_3 + (CF_3CO)_2O \rightleftharpoons Bu_4N^+CF_3CO_2^- + CF_3CO_2NO_2 \quad (1)$$

$$CF_3CO_2NO_2 \rightleftharpoons CF_3CO_2^- + NO_2^+ \quad (2)$$

$$CF_3CO_2NO_2 \rightleftharpoons CF_3CO_2^\bullet + NO_2^\bullet \quad (3)$$

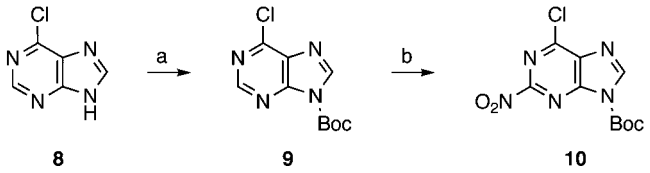
For benzenes, high regioselectivity was obtained with this nitrating reagent. More recently, reaction of substituted pyridines with TBAN−TFAA was reported to introduce the nitro group selectively on the 3 position.[20] Apart from aromatic nitrations, trifluoroacetyl nitrate has also been used for the nitration of enolacetates,[21,22] silylenolethers,[23] and the N-nitration of amides,[24,25] uridines, and inosines.[26,27] Although generally the heterolysis of TFAN (eq 2), thereby releasing nitronium ions, is considered to be the nitrating mode of action, concerted pathways involving covalent TFAN[25] and radical pathways via homolysis (eq 3) are also believed to be operative.[20,23]

The selective introduction of the nitro group in the deactivated purine system at the highly electron-deficient 2 position and not at the 8 position is remarkable; it makes both direct electrophilic nitration[28] and the alternative mechanism via electron transfer[29] unlikely, taking the high oxidation potential of purines into account. Moreover, upon TBAN−TFAA nitration of solid-supported purine nucleosides, no substantial nitration of phenyl rings in the polystyrene matrix was detected, indicating the presence of negligible amounts of strongly electrophilic nitronium ions.[13] Therefore, in an earlier publication from our group, a radical nitration pathway was proposed (Scheme 2).[10]

Homolytic cleavage of trifluoroacetyl nitrate (eq 3) as proposed by Evans et al. generates the trifluoroacetoxyl and

(12) Wanner, M. J.; Von Freitag Drabbe Künzel, J. K.; IJzerman, A. P.; Koomen, G.-J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2141−2144.
(13) Rodenko, B.; Wanner, M. J.; Koomen, G.-J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1247−1252.
(14) Wanner, M. J.; Koomen, G.-J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1908−1915.
(15) Beukers, M. W.; Wanner, M. J.; Von Frijtag Drabbe Künzel, J. K.; Klaasse, E. C.; IJzerman, A. P.; Koomen G.-J. *J. Med. Chem.* **2003**, *46*, 1492−1503.
(16) Wanner, M. J.; Koch, M.; Koomen G.-J. *J. Med. Chem.* **2004**, *47*, 6875−6883.
(17) The 2-nitration of N^6-acetyl-2′-deoxyadenosine with $Cu(II)NO_3/Ac_2O$ was published by Kaiya, T.; Tanaka, H.; Kohda, K. *Nucleosides Nucleotides* **2002**, *21*, 427−433.
(18) Masci, B. *J. Org. Chem.* **1985**, *50*, 4081−4087.
(19) Crivello, J. V. *J. Org. Chem.* **1981**, *46*, 3056−3063.
(20) (a) Njoroge, G. F.; Vibulbhan, B.; Pinto, P.; Chan, T.-M.; Osterman, R.; Remiszewski, S.; Del Rosario, J.; Doll, R.; Girijavallabhan, V.; Ganguly, A. K. *J. Org. Chem.* **1998**, *63*, 445−451. (b) Adriano, A.; Weinstein, J.; Kelly, J.; Wolin, R.; Rosenblum, S. B.; Connolly, M.; Guzi, T.; James, L.; Carr, D.; Patton, R.; Bishop, W. R.; Kirshmeier, P.; Liu, M.; Heimark, L.; Chen, K. J.; Nomeir, A. A. *Bioorg. Med. Chem.* **1999**, *7*, 1845−1855. (c) Njoroge, G. F.; Vibulbhan, B.; Wong, J. K.; White, S. K.; Wong, S.-C.; Carruthers, N. I.; Kaminski, J. J.; Doll, R. J.; Girijavallabhan, V.; Ganguly, A. K. *Org. Lett.* **1999**, *1*, 1371−1373.
(21) Dampawan, P.; Zajac, W. W., Jr. *Synthesis* **1983**, 545−546.
(22) Rank, W. *Tetrahedron Lett.* **1991**, *32*, 5353−5356.
(23) Evans, P. A.; Longmire, J. M. *Tetrahedron Lett.* **1994**, *35*, 8345−8348.
(24) Carvalho, E.; Iley, J.; Norberto, F.; Rosa, E. *J. Chem. Res., Synop.* **1989**, 260−261.
(25) Romea, P.; Aragonès, M.; Garcia, J.; Vilarrasa, J. *J. Org. Chem.* **1991**, *56*, 7038−7042.
(26) Ariza, X.; Bou, V.; Vilarrasa, J. *J. Am. Chem. Soc.* **1995**, *117*, 3665−3673.
(27) Ariza, X.; Farràs, J.; Serra, C.; Vilarrasa, J. *J. Org. Chem.* **1997**, *62*, 1547−1549.
(28) Moodie, R. B. *Aromatic Nitration*; Schofield, K., Ed.; Cambridge University Press: Cambridge, 1980; Chapter 15.
(29) Esteves, P. M.; Walkimar de M. Carneiro, J.; Cardoso, S. P.; Barbosa, J. G. H.; Laali, K. K.; Rasul, G.; Prakash, G. K. S.; Olah, G. A. *J. Am. Chem. Soc.* **2003**, *125*, 4836−4849.

Scheme 3



nitrogen dioxide radicals.[23] Addition of the reactive trifluoroacetoxyl radical to the purine C-8 of **1** gives a highly delocalized radical that is stabilized by the substituent at C-6. Subsequent combination of **6** with NO_2 at C-2 forms intermediate **7** and is followed by elimination of trifluoroacetic acid, which affords 2-nitro product **2**.

A drawback in the mechanism proposed in Scheme 2 is the generation of the very unstable trifluoroacetoxyl radical, which rapidly decomposes to CO_2 and the trifluoromethyl radical with a reported dissociation constant of $k > 5 \times 10^4$ s^{-1}.[30] During TBAN−TFAA purine nitrations, we did not observe formation of species such as CO_2 or CF_3H that might indicate homolytic cleavage of trifluoroacetyl nitrate as the initiating step. Puzzled by these facts, we further investigated the mechanism of purine nitration and looked for reaction intermediates with NMR, and we were able to identify and characterize an N7-nitropurine intermediate; its rearrangement into the C2-nitro product accompanied by ^{15}N-CIDNP effects will be discussed in this paper as evidence for a new three-step purine nitration mechanism.

Results and Discussion

Detection of a 7-Nitro-8-trifluoroacetoxy Purine Intermediate. To facilitate the interpretation of NMR data, a simple purine substrate was required which would offer a clean and complete nitration with the TBAN−TFAA mixture. In our hands, 6-chloro-9-Boc-purine **9**, a compound not reported before in literature, appeared to be the most suitable candidate. This crystalline compound was easily synthesized in 89% yield by reaction of 6-chloropurine **8** with Boc_2O (1.4 equiv) and catalytic DMAP (Scheme 3, step a).[31] On preparative scale, the nitration of **9** with TBAN−TFAA (1.6 equiv) was carried out in CH_2Cl_2 at 0 °C and furnished 2-nitro-6-chloro-9-Boc purine **10** in 86% isolated yield. Here, further indication that the rate-determining step is not electrophilic is obtained from the observation that an electron-withdrawing N9-Boc group increases both the rate and yield of the nitration reaction compared to an N9-ribosyl or N9-methyl substituent.

Monitoring the nitration of 6-chloro-9-Boc-purine **9** with ^{1}H NMR at −10 °C revealed, besides product formation, the presence of a purine intermediate. Figure 1 (t_0) shows part of the ^{1}H spectrum of a mixture of 6-chloro-9-Boc purine **9** (0.13 M) and TBAN (0.26 M) in CD_2Cl_2 at −10 °C. When TFAA (2 equiv) was added to this mixture, the appearance of the H8 signal (8.92 ppm) of the product **10** and the disappearance of the H2 (8.86 ppm) and H8 (8.63 ppm) signals of starting material **9** were observed as expected (Figure 1, $t_{10\ min}$). 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

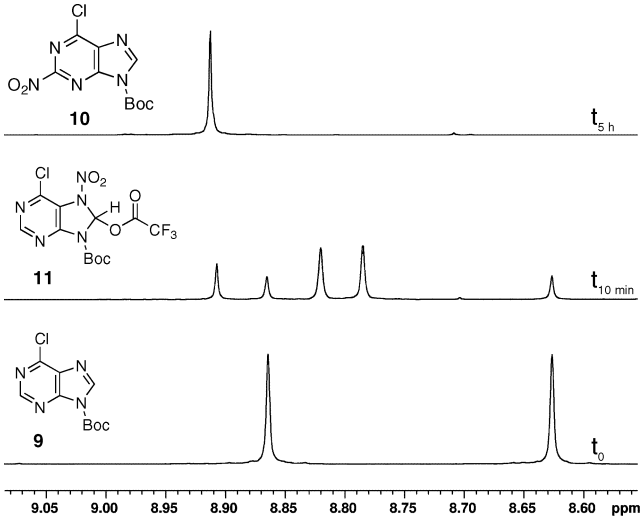


Figure 1. Aromatic region of ^{1}H NMR spectra of the nitration of 6-chloro-9-Boc-purine **9** at −10 °C in CD_2Cl_2.

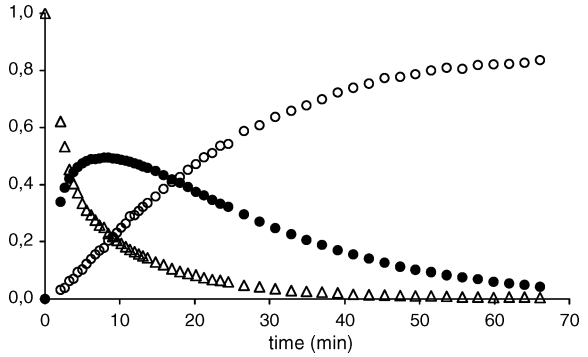


Figure 2. Progress of the nitration by plotting the ^{1}H integral values against time ($T = -10$ °C). Triangles, H2 of starting material **9**; closed circles, (H2 + H8)/2 of intermediate **11**; open circles, H8 of product **10**. During the reaction, the signals of H2 and H8 of intermediate **11** partly overlap; hence, they were jointly integrated as 2H for this graphical representation.

(integrated as 9H). These signals were assigned to intermediate **11**. Additional minor peaks were detected, which were ascribed to side products (see below and Supporting Information). After 5 h, all of the starting material and intermediary peaks had disappeared, while in the aromatic region, only the H8 signal of the product remained (Figure 1, $t_{5\ h}$). Three corresponding *tert*-butyl signals (see Supporting Information) were observed: starting material **9** (1.65 ppm), intermediate **11** (1.48 ppm), and product **10** (1.72). The progress of the nitration is represented graphically in Figure 2 as a plot of the normalized integral values against time.

A successful attempt to "freeze" the nitration reaction in the intermediate stage allowed extensive spectroscopic characterization of **11**. The progress of intermediate formation was monitored at −50 °C, and complete conversion of 6-chloropurine **9** into intermediate **11** over an 8 h period was observed, while formation of 2-nitro product **10** was suppressed to less than 3%.[32]

The ^{15}N NMR spectrum of intermediate **11** formed from 98% ^{15}N-labeled TBAN revealed a doublet at 339.6 ppm with $J_{NH} = 2.7$ Hz (Table 1). In the corresponding ^{1}H spectrum, a doublet

(30) (a) Francisco, J. S. *Chem. Phys. Lett.* **1992**, *191*, 7−12. (b) Maricq, M. M.; Szente, J. J.; Khitrov, G. A.; Francisco, J. S. *J. Phys. Chem.* **1996**, *100*, 4514−4520.

(31) For the reaction of adenine and guanine with Boc_2O, see: Dey, S.; Garner, P. *J. Org. Chem.* **2000**, *65*, 7697−7699.

(32) 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.

Table 1. Selected NMR Data from the Nitration of 6-Cl-9-Boc-purine **9** with ^{15}N-Labeled TBAN[a]

	6-Cl-9-Boc-purine **9**		Intermediate **11**		2-NO_2–6-Cl-9-Boc-purine **10**	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
^{1}H-2	8.86 (s)		8.78 (s)			
^{1}H-8	8.63 (s)		8.82 (d)	$^3J_{HN} = 2.7$		
^{13}C-2	153.5 (d)	$^1J_{CH} = 211$	157.7 (d)	$^1J_{CH} = 212$	154.2 (d)	$^1J_{CN} = 29.4$
^{13}C-8	144.3 (d)	$^1J_{CH} = 222$	93.0 (dd)	$^1J_{CH} = 194$	148.3 (d)	$^1J_{CH} = 223$
				$^2J_{CN} = 1.8$		
$^{15}NO_2$			339.6 (d)	$^3J_{NH} = 2.7$	365.0 (s)	
^{19}F			−75.86 (s)			

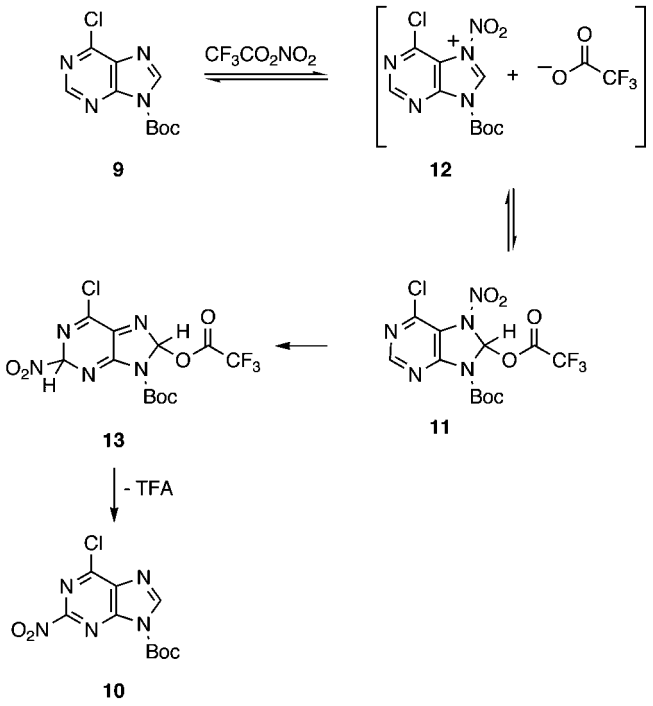
[a] Recorded in CD_2Cl_2 at −50 °C.

at 8.82 ppm with $J_{NH} = 2.7$ Hz was observed instead of a singlet. A ^{1}H decoupling experiment confirmed ^{1}H−^{15}N coupling. The value of the coupling constant points toward a $^3J_{NH}$ coupling. Also, the ^{15}N NMR spectrum revealed that intermediate **11** contains an N-nitro group and no C-nitro or -nitrato moiety. The ^{15}N chemical shift of the doublet of intermediate **11** typically lies within the range of N-nitro compounds, which is generally shifted about 20 ppm upfield relative to that of C-nitro compounds.[33] Peaks derived from covalently bound nitrates, which appear at even higher field values relative to N-nitro derivatives, were not detected.[34] The ^{15}N signal of the nitro group in product **10**, a singlet at 365.0 ppm, displays a chemical shift value characteristic for aromatic C−NO_2 compounds.

In the ^{13}C spectrum of starting material **9**, C2 was found at 153.5 ppm with $^1J_{CH} = 211$ Hz and C8 at 144.3 ppm with $^1J_{CH} = 222$ Hz (see Table 1). In the spectrum of intermediate **11**, the values for C2 ($\delta = 157.7$ ppm, $^1J_{CH} = 212$ Hz) were similar to those of the starting material, but C8 showed a remarkable upfield shift to 93.0 ppm and a decreased $^1J_{CH}$ value of 194 Hz. Moreover, C−N coupling with $^2J_{CN} = 1.8$ Hz was observed for C8 in experiments with ^{15}N-labeled TBAN. These results indicated that aromaticity was retained in the pyrimidine part, but not in the imidazole ring. In addition, the presence of a trifluoroacetoxy group in intermediate **11** was observed with ^{13}C NMR, as indicated by a quartet at 114.2 ppm ($^1J_{CF} = 290$ Hz) and a double quartet at 153.8 ppm ($^2J_{CF} = 44$ Hz, $^3J_{CH} = 2.9$ Hz). The ^{19}F NMR spectrum verified the presence of the trifluoroacetoxy group, which appeared as a singlet at −75.86 ppm. With the help of CH correlation spectra and the observed long-range couplings, the intermediary singlet in ^{1}H NMR at 8.78 ppm could be assigned to H2 and the doublet at 8.82 ppm to H8. The extremely high chemical shift of 8.82 ppm for H8, a proton attached to an sp^3 carbon atom, can be explained by the three heteroatoms attached to C8, the electron-withdrawing effect of the nitro, Boc, and trifluoroacetoxy groups and the anisotropic effect of the carbonyl and nitrosyl moieties. During the reaction, the concentration of TFA increases and H8 of **11** showed a gradual upfield shift of about 0.2 ppm as a consequence of partial protonation, which disturbs the anisotropic effect.

These combined NMR data led us to the structure assignment for the N-nitro intermediate **11**, as shown in Figure 1.

Scheme 4



The N-Nitration−Nitramine Rearrangement Mechanism. The radical addition mechanism proposed in Scheme 2 appeared to be inconsistent with the obtained NMR results, and a new N-nitration−addition−nitramine rearrangement pathway was suggested, as is depicted in Scheme 4. The purine ring system is N-nitrated in the imidazole ring on nitrogen atom 7 by electrophilic attack of TFAN. The highly electrophilic imidazolium cation **12** is rapidly trapped by a suitable nucleophile, in this case, trifluoroacetate, furnishing the observed nitramine intermediate **11**. This step emphasizes the essential requirement of a nucleophilic species, such as the trifluoroacetate anion, for C-nitration to occur; the mere reaction of 6-chloropurine **9** with nitronium ions results only in the recovery of starting material as was shown in a control experiment with nitronium tetrafluoroborate.[10] Subsequently, from **11**, a nitramine rearrangement takes place in which the nitro group moves to the "*para*" carbon atom C2.[35] The classical nitramine rearrangement refers to the migration of the nitro group of *N*-nitroaniline to the *ortho* and *para* positions to yield a mixture of *o*- and *p*-nitroaniline.[36] In the case of purine nitramine intermediate **11**, both *ortho* positions C4 and C6 are blocked, leaving *para* carbon atom 2 as the only available position for migration of the nitro group. The product of this rearrangement, intermediate **13**, was not detected because the subsequent TFA elimination leading to 2-nitropurine **10** is too fast on the NMR time scale.

To validate that formation of 2-nitropurine **10** takes place via the monophasic process of rearrangement of the N7-nitro intermediate and not via other routes, we preformed nitramine intermediate **11** at −50 °C in $CDCl_3$ and monitored its conversion into 2-nitro purine **10** with ^{1}H NMR at −10 °C. In Figure 3, the progress of the normalized integral values of H8 of nitramine intermediate **11** and 2-nitro purine **10** is represented graphically (for real-time spectra, see Supporting Information).

(33) Berger, S.; Braun, S.; Kalinowski, H.-O. *NMR Spectroscopy of the Non-Metallic Elements*; J. Wiley & Sons: Chichester, U.K., 1997; pp 207−212.
(34) (a) Moodie, R. B.; Stephens, R. J. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1059−1064. (b) Moodie, R. B.; Sanderson, A. J.; Willmer, R. *J. Chem. Soc., Perkin Trans. 2* **1991**, 645−650. (c) Titov, A. I. *Tetrahedron* **1963**, *19*, 557−580.
(35) Shine, H. J. *Aromatic Rearrangements*; Elsevier: Amsterdam, 1967; pp 235−249.
(36) Bamberger, E.; Landersteiner, K. *Ber.* **1893**, *26*, 482−495.

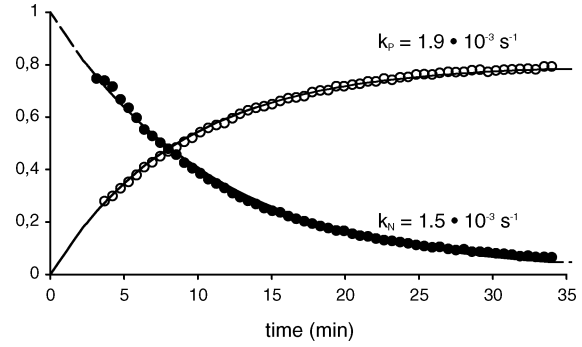


Figure 3. Conversion of N-nitro intermediate **11** to C2-nitro product **10** followed with ^{1}H NMR at −10 °C. Closed circles, nitramine intermediate **11**; open circles, product **10**. k_N, nitramine decrease; k_P, product formation.

The excellent first-order kinetics confirm the expected unimolecular process. First-order rate coefficients, $k_N = 1.5 \times 10^{-3}$ s^{-1} (nitramine **11** decrease) and $k_P = 1.9 \times 10^{-3}$ s^{-1} (product **10** increase), were determined over about 4 half-lives with a high R^2 value and good reproducibility. The slight difference in the values of k_N and k_P is explained by the occurrence of side reactions (vide infra); 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.[37]

As both thermal[38−40] and acid-catalyzed[36,41] nitramine rearrangements of nitroanilines have been reported, we also allowed the rearrangement to proceed in the presence of the base DIPEA, and identical reaction rates were found (see Supporting Information). This proved that the rearrangement was thermal and not acid catalyzed. Moreover, if the rearrangement would be acid catalyzed, the reaction rate would increase in the course of the reaction, as TFA is generated upon product formation. Deviation from a first-order correlation was not observed. To our knowledge, this is the first identified example of a nitramine rearrangement in a purine/pyrimidine system.

^{15}N-CIDNP in Purine Nitration. With the unimolecularity of the reaction now being established, we chose to study the mechanism of the rearrangement with ^{15}N NMR, which has been a valuable tool in elucidating reaction pathways in nitration reactions.[42] Several mechanisms have been put forward for the nitramine rearrangement, both heterolytic[43] and homolytic pathways.[41] The observation of CIDNP effects in ^{15}N NMR spectra during the rearrangement of nitroaniline derivatives offered convincing evidence that radicals were involved, thus supporting a homolytic rearrangement mechanism.[37,44] Chemically Induced Dynamic Nuclear Polarization refers to the perturbation of the nuclear spins away from the expected Boltzmann distribution.[45,46] The effect is observed in NMR spectra as an abnormal intensity of the NMR signals; the signals display either enhanced absorption or emission. The phase of the net polarization observed, Γ_{ne} (positive for enhanced absorption signals, negative for emission signals), can be predicted with Kaptein's rules.[47] When applied to the polarization of ^{15}N nuclei, the equation takes the following form:[48]

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

Here, μ 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); ϵ is derived from how the products are formed (positive for reaction within the radical pair, negative for reaction after separation of the radical pair); a_i is the sign of the hyperfine coupling constant for the observed 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.

In the case of the rearrangement of nitramine intermediate **11**, the above equation can be reduced to the extent that the sign of Γ_{ne} discloses whether the rearrangement takes places in an inter- or intramolecular fashion. In the NO_2 radical, the negative magnetogyric ratio of the ^{15}N nucleus also causes a_N to be negative,[49] while the analysis can be further simplified if the reasonable assumption is made that the g value of the organic purinyl radical[50] is larger than the g value of NO_2 (2.0000).[49] The conclusions of Kaptein's rules can now 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 μ is negative. Thus, from the phase of the polarized NMR signals, one can then conclude whether the rearrangement occurs intra- or intermolecularly. If the product is formed in an intramolecular fashion, it is 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 the result of recombination of the radicals after separation of the original pair (ϵ is negative) and emission is observed. Conclusions are less obvious if the rearrangement has both an intra- and an intermolecular counterpart. Since the observed NMR signal matches the sum of the unpolarized and polarized (positive and/or negative) material formed, even no net polarization at all can be the consequence.

When we followed the rearrangement of nitramine **11** at 0 °C with ^{15}N NMR, indeed, CIDNP effects were observed (Figure 4). During several half-lives of the rearrangement, the doublet of nitramine intermediate **11** at 339.6 ppm showed enhanced absorption (see Supporting Information for real-time spectra). In the early stage of the reaction at $t = 2$ min, the singlet of 2-NO_2 product **10** at 365.0 ppm showed an emission signal, while during the remainder of the rearrangement, a reduced

(37) A similar discrepancy in the values of k_N and k_P was found by Ridd and co-workers during the acid-catalyzed nitramine rearrangement of 2,6-dichloro-*N*-nitroaniline and 2,6-dibromo-*N*-nitroaniline: Abu-Namous, A. M. A.; Ridd, J. H.; Sandall, J. P. B. *Can. J. Chem.* **1986**, *64*, 1124−1129.
(38) Barnes, T. J.; Hickinbottom, W. J. *J. Chem. Soc.* **1961**, 2616−2620.
(39) Naud, D. L.; Brower, K. R. *J. Org. Chem.* **1992**, *57*, 3303−3308.
(40) Naud, D. L. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1321−1324.
(41) (a) White, W. N.; Klink, J. R. *J. Org. Chem.* **1977**, *42*, 166. (b) White, W. N.; White, H. S.; Fentiman, A. *J. Org. Chem.* **1976**, *41*, 3166−3170 and earlier papers in this series.
(42) Ridd, J. H. *Chem. Soc. Rev.* **1991**, *20*, 149−165.
(43) (a) Banthorpe, D. V.; Hughes, E. D.; Williams, D. L. H. *J. Chem. Soc.* **1964**, 5349−5361. (b) Banthorpe, D. V.; Thomas, J. A.; Williams, D. L. H. *J. Chem. Soc.* **1965**, 6135−6140. (c) Brownstein, S.; Bunton, C. A.; Hughes, E. D. *J. Chem. Soc.* **1958**, 4354−4357.
(44) Ridd, J. H.; Sandall, J. P. B. *J. Chem. Soc., Chem. Commun.* **1982**, 261−262.
(45) Lepley, A. R.; Closs, G. L. *Chemically Induced Magnetic Polarization*; J. Wiley & Sons: New York, 1973.
(46) Muus, L. T.; Atkins, P. W.; McLaughlan, K. A.; Pedersen, J. B. *Chemically Induced Magnetic Polarization*; D. Reidel Publishing Company: Dordrecht, The Netherlands, 1977.
(47) Kaptein, R. *J. Chem. Soc., Chem. Commun.* **1971**, 732−733.
(48) (a) Clemens, A. H.; Ridd, J. H.; Sandall, J. P. B. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1659−1665. (b) The additional negative sign must be added because of the negative magnetogyric ratio of the ^{15}N nucleus. See: Porter, N. A.; Dubay, G. R.; Green, J. G. *J. Am. Chem. Soc.* **1978**, *100*, 920−925.
(49) Morton, J. R.; Preston, K. F.; Strach, S. J. *J. Phys. Chem.* **1979**, *83*, 533−536.
(50) Hellwege, K.-H.; Fischer, H. *Landolt-Börnstein: Numerical Data and Functional Relationships in Science and Technology*; Springer-Verlag: Berlin, 1977.

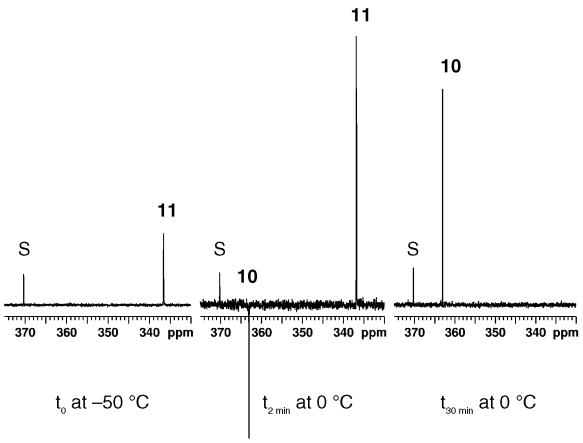
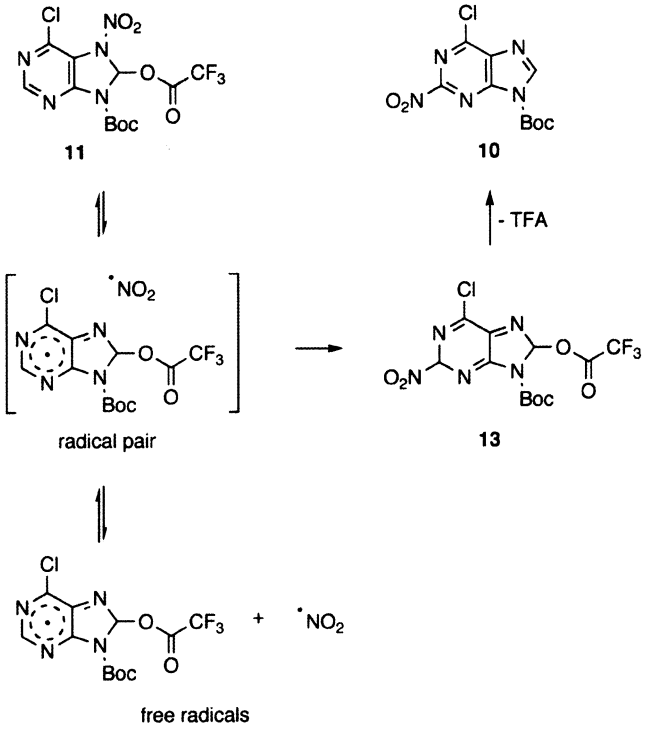


Figure 4. ^{15}N-CIDNP NMR spectra of the rearrangement of 0.45 M nitramine. S: ^{15}N nitrobenzene standard; nitramine intermediate **11** (doublet); 2-nitro product **10** (singlet).

Scheme 5



absorption signal was observed until after about 4 half-lives (reaction nearly complete) no CIDNP effects were observed any longer. These results can be explained with the radical mechanism shown in Scheme 5. The enhanced absorption of nitramine intermediate **11** indicates the reverse reaction of the radical pair to re-form nitramine **11** by immediate collapse of the primary radical pair. The emission signal for product **10** points to an intermolecular process in which the paired radicals separate and become free radicals. The latter re-encounter to re-form the radical pair and, consequently, the secondary recombination intermediate **13**, which immediately eliminates TFA to render product **10**. In the beginning of the reaction, this contribution to the NMR signal is larger since the concentration of the escaped (i.e., free) radicals is higher as is the chance of random free-radical encounters.

Since the reassociation of free radicals to a radical pair is an equilibrium process, the resulting radical pair probably possesses the lowest energy and most stable average configuration, leading to C−NO_2 product formation.[51] During the intramolecular recombination, N−NO_2 formation (high-energy conformation, kinetically favored) is in competition with C−NO_2 formation. This is expressed in the enhanced absorption for N−NO_2 intermediate **11**. Accordingly, intermolecular recombination primarily leads to the thermodynamically more stable C−NO_2 compound, accounting for the observed emission signal of **10**.

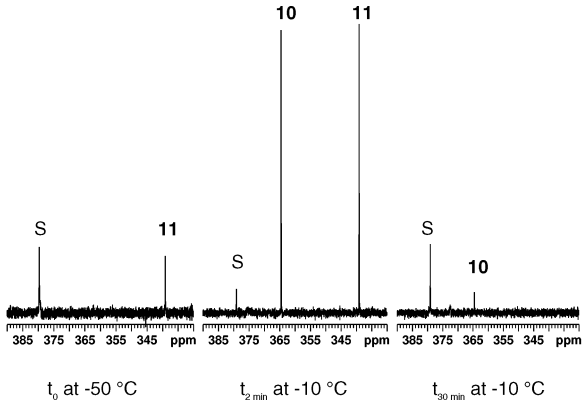


Figure 5. CIDNP effects when the intermolecular rearrangement is prevented by diverting the free radicals with hydroquinone. S: nitromethane standard; nitramine intermediate **11** (doublet); 2-nitro product **10** (singlet).

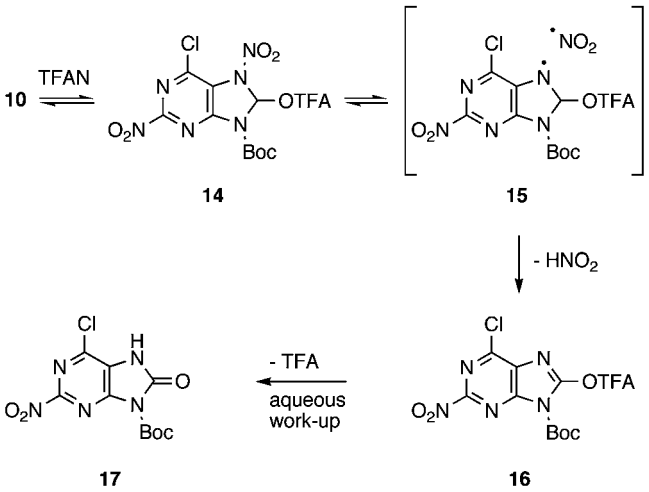
The large emittive CIDNP signal of **10** suggested a major contribution of the intermolecular route toward its formation. In a radical trapping experiment with hydroquinone, which only reacts with free radicals and not with paired radicals, we examined the exact contributions of the inter- and intramolecular counterparts of the rearrangement. By diverting the free NO_2 radicals that have escaped from the original radical pair through reduction with hydroquinone, the intermolecular route toward intermediate **13** is efficiently blocked. Using this method, White has shown that the nitramine rearrangement of, for example, *N*-methyl-*N*-nitroaniline, has both an intra- and intermolecular counterpart.[51,52]

Monitoring the rearrangement of nitramine intermediate **11** in the presence of 3 equiv of hydroquinone with ^{15}N NMR revealed that only 30−35% of 2-nitro product **10** was generated. This indicated that 65−70% of the purine nitramine rearrangement occurred intermolecularly. As expected, a greatly enhanced absorption was observed for product **10** due to the absence of the emittive contribution to the NMR signal (Figure 5). In agreement, the signal of nitramine intermediate **11** still displayed enhanced absorption.

8-Oxo Purine Formation. While monitoring the nitration reaction with NMR, a slight excess of TFAN was used, and in the spectra, a minor set of peaks was observed that deserves more comment. These signals were observed both during direct nitration of 6-chloro-9-Boc-purine **9** and during the nitramine rearrangement after preformation of nitramine intermediate **11**. The peaks disappeared after warming the sample to room temperature. In the ^{15}N NMR spectra, a set of signals composed of a singlet at 363.4 ppm and a doublet at 335.9 with J_{NH} = 2.7 Hz corresponded in the ^{1}H NMR spectra to a doublet at 9.10 ppm with J_{NH} = 2.7 Hz (integrated as 1H) and a singlet at 1.54 ppm (integrated as 9H) and in the ^{19}F NMR spectra to a

(51) White, W. N.; White, H. S. *J. Org. Chem.* **1970**, *35*, 1803−1805.
(52) White, W. N.; Golden, J. T. *J. Org. Chem.* **1970**, *35*, 2759−2762.

Scheme 6



singlet at −75.95 ppm. These NMR signals were interpreted as the consequence of a consecutive addition of TFAN to 2-nitropurine **10** leading to the observed dinitro intermediate **14**, as depicted in Scheme 6. Since the *para* position is already substituted, nitramine rearrangement is not possible and either retroreaction to 2-nitro-6-chloro purine **10** occurs or, alternatively, loss of nitrous acid gives rise to 2-nitro-6-chloro-8-trifluoroacetoxy purine **16**. Hydrolysis of this latter compound upon aqueous workup explains the formation of 8-oxo purines, such as **17**, which are incidentally isolated after purine nitration reactions, especially when a large excess of nitrating agent is used.

Conclusion

In summary, extensive monitoring of the TBAN−TFAA purine nitration reaction with NMR spectroscopy excluded direct nitration of the highly electrophilic C2 position and demonstrated that this reaction occurs via an N-nitration−addition−nitramine rearrangement pathway. Electrophilic attack by TFAN on the purine N7 position results in a nitrammonium species that is trapped by a nucleophile, in this case, the trifluoroacetate anion, furnishing nitramine intermediate **11**. A subsequent purine nitramine rearrangement generates C2-nitro species **13** that eliminates TFA to give the product 2-nitro-6-chloro-9-Boc-purine **10**. Moreover, the involvement of radicals during the nitramine rearrangement was unequivocally established by ^{15}N-CIDNP. A radical trapping experiment disclosed that 65−70% of the nitramine rearrangement takes place intermolecularly.

Acknowledgment. The authors thank Jan Geenevasen and Jan Meine Ernsting for their help and commitment during the NMR measurements.

Supporting Information Available: Experimental details and NMR spectra (^{1}H, ^{13}C, [^{13}C,^{1}H]-COSY, ^{15}N-CIDNP, ^{19}F) of nitration reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

JA042590X