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Regioselective nitration of purine nucleosides: synthesis of 2-nitroadenosine and 2-nitroinosine

Paymaneh Y. F. Deghati, Martin J. Wanner and Gerrit-Jan Koomen *

Laboratory of Organic Chemistry, Institute of Molecular Chemistry, University of Amsterdam, Nieuwe Achtergracht 129, NL-1018 WS Amsterdam, The Netherlands

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Abstract

Nitration reactions of 6-substituted purine nucleosides with tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA) have been studied. This nitrating mixture selectively nitrates C-6 substituted purines at the 2-position, but the method is limited to substrates without NH or OH substituents. Acetylated 6-chloropurine riboside was cleanly nitrated (DCM, 0°C, 71%) and converted to nitro substituted adenosine and inosine in a few simple steps.

Keywords: nitration; adenosine; inosine.

Adenosine analogs substituted at the 2-position show interesting activity in several biological systems. For instance adenosines containing a halogen atom at the 2-position display cytotoxic activity, and especially 2-chloro-2′-deoxyadenosine (cladribine), which is a potent inhibitor of DNA synthesis, is currently used against leukaemia and in the treatment of chronic lymphoid malignancies.[1] The apoptosis inducing properties of some 2-halo-substituted adenosine analogs were recently published.[2] In addition, introduction of carbon, amino or oxygen substituents at the adenosine 2-position increases the selectivity of binding to adenosine receptors.[3,4]

Most of the procedures towards the synthesis of 2-substituted adenosines are based on 2,6-dichloropurine or use guanosine as starting material.[5] To improve the availability of disubstituted purine systems we describe here our studies to functionalize 6-substituted nucleosides at the 2-position.

During our investigations towards selective derivatization of 1-deazapurine[6] and 1-deaza-2-azapurine ribosides,[7] we introduced the tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA) system[8] for the nitration of electron deficient and acid labile nucleosides. The surprising mildness and selectivity of this reagent stimulated us to apply this nitration to the purine series using adenosine and inosine as starting materials.

* Corresponding author.

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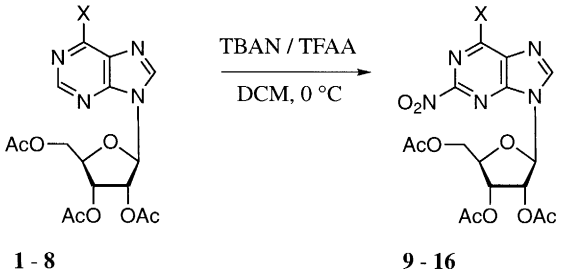
Nitration of protected inosine using a reagent prepared from TFAA and ammonium nitrate is described in the literature and gives the N^1-nitrated nucleoside (Scheme 1).[9] Application of the TBAN/TFAA nitration on triacetyl protected inosine **3** or adenosine **4** was, in our hands, also ineffective for *C*-nitration. We were however pleased to find that triacetyl protected 6-chloropurine riboside **2** was cleanly nitrated using TBAN/TFAA. Of the two available carbon positions in the purine ring only the 2-position was reactive, and crystalline 2-nitro-6-chloropurine riboside **10** was readily obtained in good yield.[10] To investigate the scope of this reaction we returned to triacetyl adenosine, which was completely acetylated using acetic anhydride and DMAP at 130°C to remove all the acidic hydrogen atoms. Nitration of *N*,*N*-diacetyladenosine triacetate **6** was now possible to give 55% of the corresponding 2-nitro derivative **14**. Extension of the nitration method was accomplished using N^6-cyclopentyladenosine (CPA) as a substrate. Peracetylation (Ac_2O/DMAP, 120°C) gave **7** which was subsequently nitrated to give **15** as the only product.

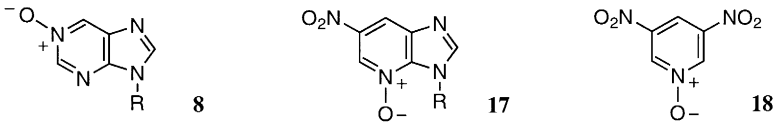


1 - 8 **9 - 16**

substrate	X	product	yield(%)
1	H	**9**	-
2	Cl	**10**	71
3	OH	**11**	-
4	NH_2	**12**	-
5	HNAc	**13**	-
6	NAc_2	**14**	55
7	N(Cp)Ac	**15**	48
8	H (*N*-1-oxide)	**16**	-

Scheme 1.

This TBAN/TFAA nitration proved to be a strongly substrate dependent process, since nucleosides such as adenosine tetraacetate **5** or nebularine triacetate **1** did not give any of the expected nitrated products. Formation of polar side products as a result of *N*-nitration and/or glycosidic bond cleavage was observed. Nebularine *N*-1-oxide **8**, prepared by DMDO oxidation of **1** was also not suitable as a substrate although the introduction of *N*-oxides in the 1-deazapurine riboside series was shown to be very successful for the synthesis of nitro compound **17** (Fig. 1).[6] It seems likely however that no suitable position for *C*-nitration in **8** is available since under these conditions *N*-oxides have strong *meta*-directing properties. We have observed before that 3,5-dinitro derivative **18** was formed from pyridine *N*-oxide. Pyridine itself produced no *C*-nitrated products under these conditions since *N*-nitration is prevailing.[11]



8 **17** **18**

Fig. 1. R=2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl

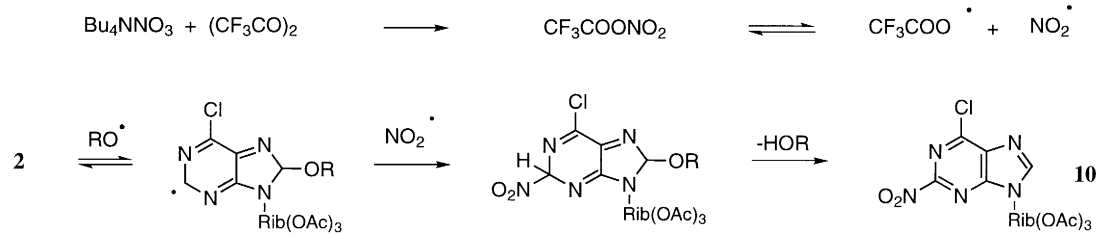
To summarize, for successful nitration the following substrate requirements can be deduced from these studies:

– no acidic protons (e.g. NH or OH);
– no nucleophilic nitrogen atoms (as in pyridine);
– radical stabilizing substituent (Cl, NR_2, *N*-oxide) is required.

1. Mechanism of the nitration

Since the conventional 'nitronium-ion' nitration mechanism was introduced[12] numerous alternative processes have been observed, all as a result of the many forms NO_x can adopt. In a recent publication, Ridd[13] reviewed a group of unconventional nitration pathways, most of them based on radical species and/or electron transfer processes. Only a few examples are known in which electron deficient substrates are nitrated at room temperature and from these, the nitration reactions of chloro-nitrobenzenes using N_2O_5/HNO_3[14] give a clear indication of radical addition. The nitro-nitrate-addition products, formed as intermediates, were observed by ^{15}N CIDNP NMR and support a radical addition mechanism, although electrophilic processes catalyzed by HNO_3 seem to dominate the formation of the end products. NO_2 is not reactive enough for addition to the aromatic ring and therefore a more reactive species such as NO_3, which is formed in an equilibrium from N_2O_5, initiates the substitution reaction. Comparable mechanisms were suggested to explain unusual selectivity during Kyodai nitration with NO_2/O_3, although electron transfer from electron rich substrates to NO_3 was preferred as the initiating step.[15]

In the TBAN/TFAA system, trifluoroacetyl nitrate presumably splits homolytically into NO_2 and the trifluoroacetate radical (Scheme 2).[16] It should be noted that in theory N_2O_5 and consequently NO_3 can be formed during the TBAN/TFAA nitrations. Addition of the reactive trifluoroacetyl radical to the imidazole C-8 in e.g. **2** gives a highly delocalized radical that is stabilized by the substituent at C-6. In the next step, combination of the radical with NO_2 takes place at C-2, which is the only available carbon atom. Elimination of trifluoroacetic acid from the unstable intermediate affords the product. In view of the high oxidation potential of purines the alternative mechanism via electron transfer to NO_3 seems unlikely.



Scheme 2.

Any concurrent electrophilic processes during TBAN/TFAA nitration were excluded by a control experiment using nitronium tetrafluoroborate. No nitration was observed and the starting material was recovered. In addition, Evans[16] and Njoroge[8] have already shown that radical capture by adding up to 4 equiv. of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) to the TBAN/TFAA nitrating mixture almost completely inhibited the reaction.

Although additional studies are necessary to clarify the exact mechanism, similarities between N_2O_5 and the TBAN/TFAA combination are obvious. The ease of handling the TBAN/TFAA mixture, in combination with the relatively weakly acidic reaction conditions[17] makes this reagent preferred over several other nitrating agents.

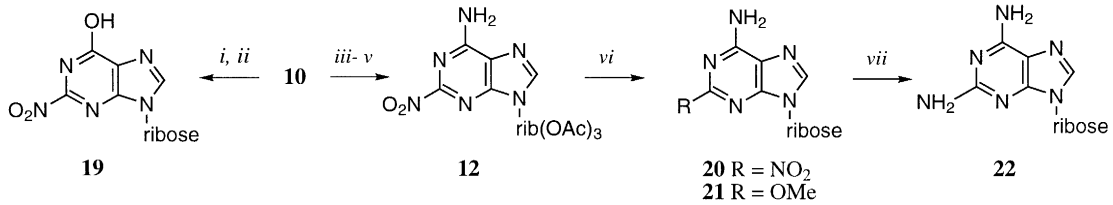
2. Synthesis of 2-nitroadenosine and 2-nitroinosine

The versatility of 2-nitro-6-chloropurine riboside **10** was demonstrated by its conversion to the biologically interesting 2-nitroadenosine **20** and 2-nitroinosine **19**. Only 2-nitroinosine is described in

the literature via formation in small amounts as a side product during deamination of guanosine with nitrous acid.[18]

In a first approach to 2-nitroadenosine, the acetate protective groups were removed from 2-nitroadenosine pentaacetate **14**. However, under mild conditions such as KCN in methanol, replacement of the nitro group occurred as a side reaction, affording 2-methoxyadenosine **21** in 47% yield and only 10% 2-nitroadenosine **20**. In particular, removal of the second *N*-acetyl group was rather slow, allowing substitution of the nitro group to give **21**.

Alternatively 2-nitro-6-chloropurine riboside **10** was converted to 2-nitroadenosine triacetate **12** (Scheme 3) by replacement of the chloro substituent with sodium azide followed by conversion of the azide to the corresponding amine. This triacetate was deacetylated to 2-nitroadenosine **20**[19] in satisfactory yield using KCN in methanol. Finally the nitro substituent in **20** was hydrogenated to give the known 2-aminoadenosine **22**.



Scheme 3. R=2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl (*i*) NaOAc, EtOH, 81%; (*ii*) NH_3, MeOH, 52%; (*iii*) NaN_3, DMF; (*iv*) PPh_3; (*v*) HOAc, H_2O, 64%, three steps; (*vi*) KCN, MeOH, 68%; (*vii*) H_2, Raney Ni, 80%

2-Nitroinosine **19** was obtained by reaction of **10** with sodium acetate followed by aminolysis of the acetates. Loss of the nitro group during the deprotection is prevented by deprotonation of the 6-OH by ammonia.

The position of the nitro group in these nucleosides was unequivocally established using NMR spectroscopy: long range correlation between C-1′ and H-8 and between H-1′ and C-8 was observed. These structural assignments were confirmed by comparison of the spectroscopic data of diamine **22** with literature values.

In conclusion, a mild and selective nitration method for purine ribosides has been developed, which has given access to a series of new purine nucleosides. The synthesis of further derivatives and studies of their biological activity are currently in progress.

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