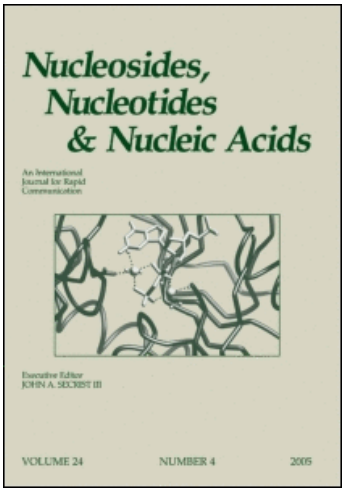


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

New (1-Deaza)Purine Derivatives via Efficient C-2 Nitration of the (1-Deaza)Purine Ring

M. J. Wanner[a]; B. Rodenko[a]; M. Koch[a]; G. J. Koomen[a]
[a] Bioorganic Chemistry, Institute of Molecular Chemistry, University of Amsterdam, Amsterdam, The Netherlands

Online publication date: 27 October 2004

To cite this Article Wanner, M. J. , Rodenko, B. , Koch, M. and Koomen, G. J.(2004) 'New (1-Deaza)Purine Derivatives via Efficient C-2 Nitration of the (1-Deaza)Purine Ring', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1313 — 1320
To link to this Article: DOI: 10.1081/NCN-200027566
URL: http://dx.doi.org/10.1081/NCN-200027566

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1313–1320, 2004

New (1-Deaza)Purine Derivatives via Efficient C-2 Nitration of the (1-Deaza)Purine Ring

M. J. Wanner, B. Rodenko, M. Koch, and G. J. Koomen*

Bioorganic Chemistry, Institute of Molecular Chemistry, University of Amsterdam, Amsterdam, The Netherlands

ABSTRACT

Nitration of substituted (1-deaza)purines using a mixture of tetrabutylammonium nitrate (TBAN) and trifluoracetic acid anhydride (TFAA) was applied to prepare nitrosubstituted (1-deaza)purines at low temperature. The nitro group influences the system twofold: 1) it activates other substituents towards nucleophilic aromatic substitution and 2) it can be substituted itself leading to a variety of di-substituted (1-deaza)purines, also via solid phase syntheses. Several of the molecules obtained were studied for their antiprotozoal activity and for interactions with the different human adenosine receptors.

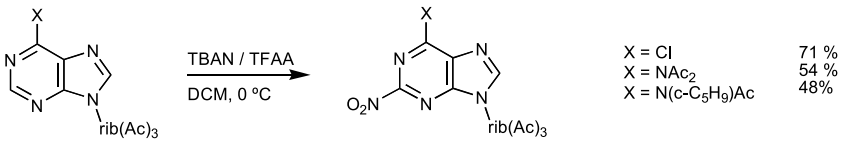
Key Words: Antiprotozoal activity; Temozolomide.

INTRODUCTION

Nucleoside and nucleotide analogues have been of interest since many years. Initially mainly as antiviral and anticancer agents, which has resulted in development of many clinically useful drugs. In view of the instability of adenosine and its many

*Correspondence: G. J. Koomen, Bioorganic Chemistry, Institute of Molecular Chemistry, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands; E-mail: gjk@science.uva.nl.

DOI: 10.1081/NCN-200027566 1525-7770 (Print); 1532-2335 (Online)



Scheme 1. Nitration of subsituted purines.

biological functions, interest in inhibitors of the metabolising enzyme adenosine deaminase is also still very lively. In addition to that, the increasing knowledge of describing processes in the CNS in molecular terms has stimulated the research in the field of the different adenosine receptors. For the development of selective agonists and (anta)gonists of the human adenosine receptors and for basic receptor studies, many more adenosine analogues with well defined structures are required and consequently also new synthetic approaches, in particular at solid phase should be developed.

A couple of years ago, a new selective nitration reaction of substituted purines and deazapurines at the 2-position was developed in our lab.[1,2] The reaction, using a mixture of tetrabutyl ammonium nitrate (TBAN) and trifluoroacetic acid anhydride (TFAA) is simple and proceeds at low temperature if the purine system is substituted at the six position (purine numbering) with a functional group without acidic protons and containing a free electron pair (Scheme 1).

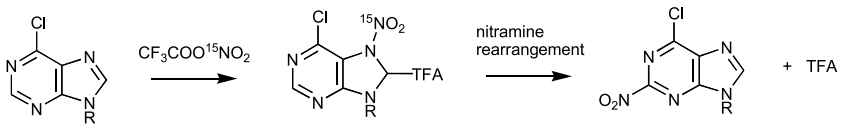
This nitration reaction turned out to be a combination of an electrophilic and a radical process, as could be established using ^{1}H, ^{13}C and ^{15}N NMR spectra.

For the studies towards the structure elucidation of possible intermediates of the reaction via ^{15}N NMR, the corresponding fully labeled ^{15}N TBAN was prepared from TBACl and $Na^{15}NO_3$. Recently, the proposed mechanism was unequivocally established in our lab by observing CIDNP effects during the reaction at low temperature (Scheme 2).

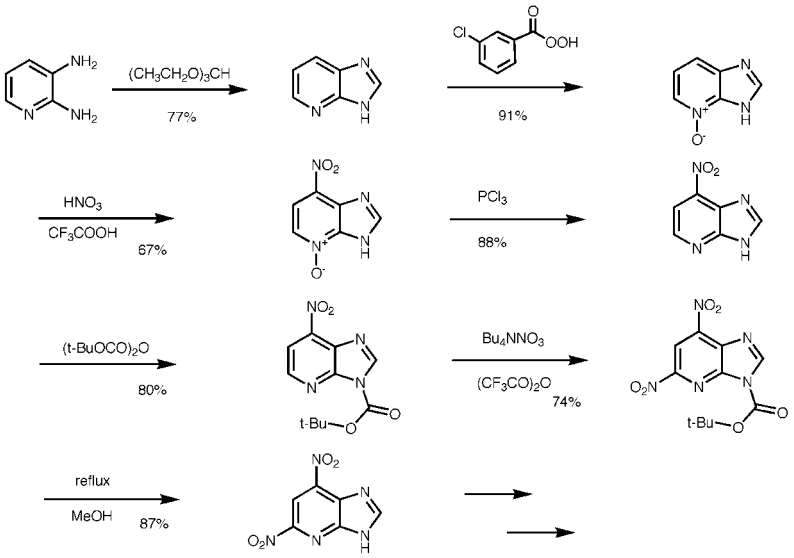
After initial nitration at N-7 of N-9 protected 6-chloropurine, addition of trifluoroacetate at C-8 takes place, leading to an intermediate which can be observed in NMR at low temperature. Slowly warming up triggers a radical rearrangement of the nitro group to carbon-2 under restoration of aromaticity via elimination of trifluoroacetic acid.

The reaction could also be carried out with 6-nitro-1-deazapurine[3] leading to 2,6-dinitro-1-deazapurine, which could also be easily substituted to difunctionalised 1-deaza systems (Scheme 3). The nitration of 1-deaza purines was improved considerably via protection of N-9 with a BOC group.

The resulting nitro groups introduced in the (deaza) purine systems could be easily substituted with different nucleophiles in aromatic nucleophilic substitution reactions.



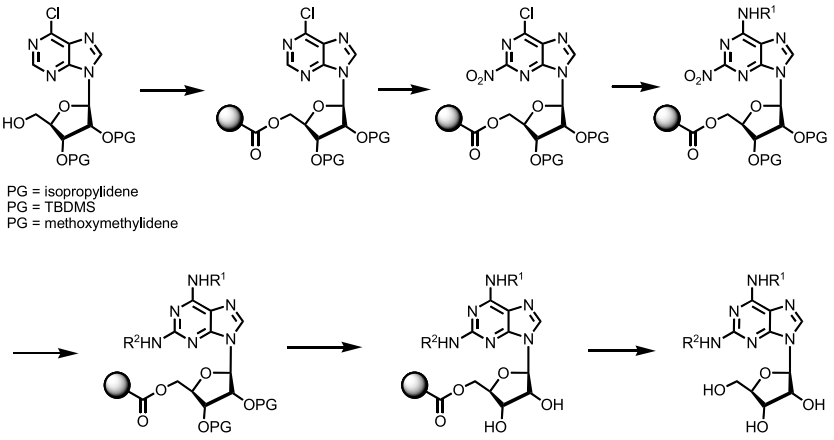
Scheme 2. Intermediate in the radical nitration.



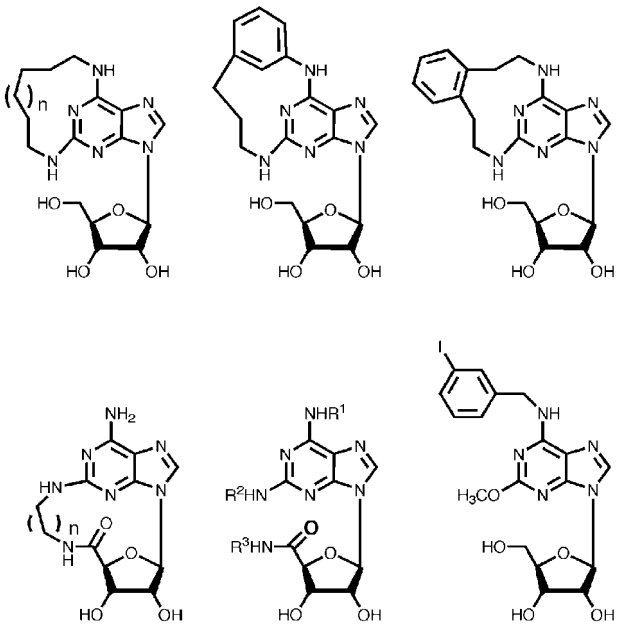
Scheme 3. Synthesis of 1-deazapurines.

In the purine series even aromatic amines showed sufficient nucleophilicity. An additional advantage of introducing the nitro group is the enhancement of the reactivity of other functional groups toward nucleophilic substitution. For instance, a 6-chloro group in a purine ring system can be replaced in presence of a 2-nitro substituent at temperatures below 0°C.

This nitration reaction could also be carried out on solid support at room temperature (Scheme 4). The protected ribonucleosides were coupled to carboxystyrene. As expected from a mechanistic point of view, nitration of the polystyrene support



Scheme 4. Solid phase synthesis of disubstituted adenosine analogues.

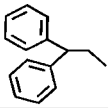
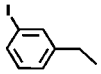
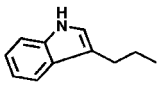
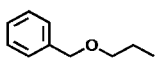
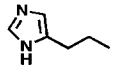


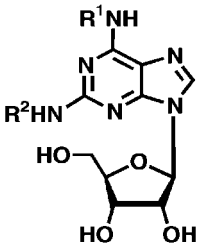
Scheme 5. Cyclophane type analogues.

did not take place under the reaction conditions. After cleavage, the products were purified via semi preparative HPLC. The procedure gave access to a variety of disubstituted purine analogues.[4]

Thus, both in solution as well as on solid support, replacement of both 6-substituents and 2-nitro groups resulted in the preparation of a wide variety of

Table 1. Antiprotozoal activity-IC50's in μM.

R^2 \ R^1					
	*T.b.r*	0.40	8.0	5.4	2.1
	P.f.	> 7	3.3	> 7	6.1
	*T.b.r*	21	25	20	19
	P.f.	> 7	1.8	> 7	5.6
	*T.b.r*	4.2	28	27	8.4
	P.f.	> 7	3.1	> 7	> 7
	*T.b.r*	38	72	43	76
	P.f.	> 7	> 7	> 7	> 7



disubstituted purine and 1-deazapurine systems both for biological screening and for studies of the interactions with adenosine receptors.

To block the syn/anti rotations around the glycosidic bond, syntheses of 5′-2 cyclonucleosides were also realised (Scheme 5).

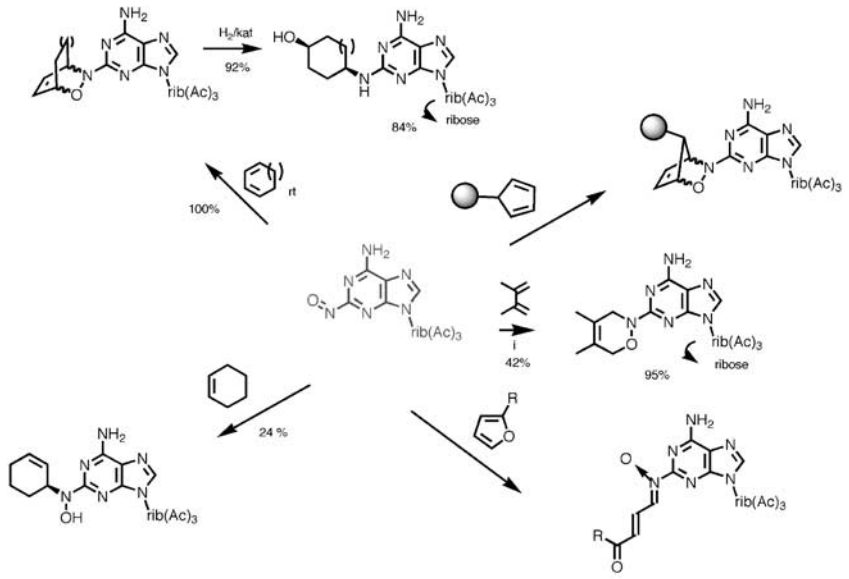
Since Plasmodium Falciparum, the causative parasite of Malaria is not capable to carry out its own de novo purine syntheses, it is strongly dependent on supply of nucleosides and nucleobases from its host. Via its transportmechanism, uncommon and reactive nucleoside analogues can be administered to the parasite. Many of our di- and trisubstituted analogues were tested in cooperation with R. Brun, Tropical Institute, Basel on Plasmodium falciparum (P.f.) and Trypanosoma Brucei (T.b.r.) (see Table 1).

Reduction of the nitro group in 2-nitroadenosine with H_2/Pd, followed by oxidation of the resulting hydroxyaminogroup produced nitrosoadenosine, which could also be used for a variety of structural modifications, partly via hetero Diels-Alder reactions. The possibility of reductive ringopening of the N–O bridge led to a new series of substituted adenosine derivatives[5] (Scheme 6).

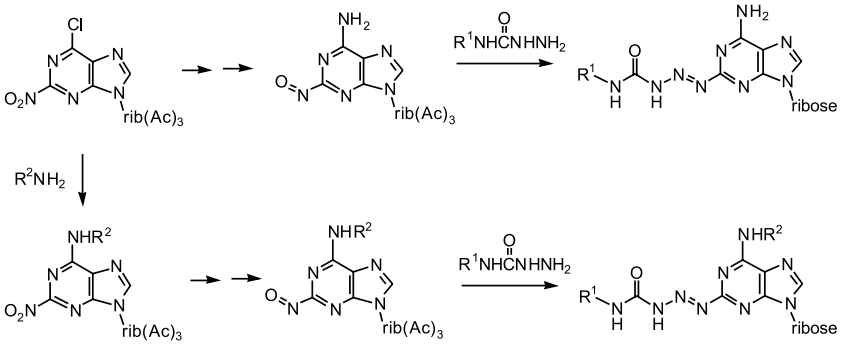
The synthetic approach developed for the anticancer agent temozolomide[6] could be used for the synthesis of a series of substituted carbamoyl triazenes (Scheme 7).

In cooperation with the group of IJzerman (University of Leiden) these compounds were tested for their activity on different Adenosine receptors. Some of the analogues, like TCPA showed remarkable selective agonist activity on the human A1 receptor[7] (see Table 2).

The clinically active cytostatic Temozolomide exerts is activity via in vivo production of the methyldiazonium ion (protonated diazomethane), a powerful DNA alkylator, which alkylates guanine bases in DNA at the O-6 position.



Scheme 6. Nitroso adenosine, a versatile synthon.



Scheme 7. Preparation of biologically active triazenes.

Resistance of cancer cells against temozolomide is developed by the production of the repair enzyme O^6-alkylguanine-DNA-alkyltransferase (AGT). By combining the drug with O^6-benzylguanine the level of the repair protein is strongly diminished.

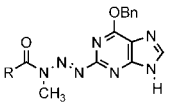
Our synthetic approach allowed for the synthesis of a series of molecules in which both the alkylating agent and the enzyme inhibitor are formed at the same time in vivo.

Table 2. Activity of Triazene derivatives on the different human Adenosine receptors.

		K_i ± S.E.M. in nM (n = 3) or % displacement at 10 μM (n = 2)			
R^1	R^2	A_1	A_{2A}	A_3	A_{2B}
H	H	140 ± 34	88 ± 12	209 ± 62	9%
Ethyl	H	45 ± 17	19 ± 10	113 ± 21	15%
Cyclohexyl	H	5.8 ± 1.8	25 ± 3	63 ± 17	6%
Phenyl	H	6.1 ± 1.3	25 ± 3	110 ± 20	54%
4-Cl-Phenyl	H	15 ± 11	4.4%	39 ± 6	21%
3-Cl-Phenyl	H	15 ± 4	29.9%	59 ± 25	64%
2-Cl-Phenyl	H	23 ± 8	45 ± 19	179 ± 33	30%
4-MeO-Phenyl	H	20 ± 10	25 ± 1	73 ± 11	15%
2-MeO-Phenyl	H	20 ± 3	125 ± 49	81 ± 13	18%
3-CF_3-Phenyl	H	insoluble	insoluble	insoluble	insoluble
Benzyl	H	42 ± 15	25 ± 11	147 ± 18	9%
O-Phenyl*	H	43%	38.9%	577 ± 138	6%
Thiazol	H	24 ± 1	25 ± 1	610 ± 340	79%
H	Phenyl	204 ± 136	17.7%	175 ± 57	10%
Phenyl	Phenyl	430 ± 132	24.3%	90 ± 12	9%
Phenyl (TCPA)	Cyclopentyl	2.8 ± 0.8	210 ± 20	600 ± 230	10%
Phenyl	Ph_2CHCH_2	3.1 ± 0.9	980 ± 110	140 ± 20	10%
CCPA		6.4 ± 1.8	639 ± 55	281 ± 56	
NECA		12 (9.6–15)	60 ± 10	11 ± 0.8	2200 ± 600

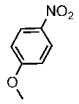
Table 3. Stability of the precursors of diazomethane and O6-benzylguanine.

	R	Hydrolysis product	t1/2 (min) at pD 7.8 (NMR)
	Amides		
NA-73	Methyl	Acetate	3500
NA-74	Phenyl	Benzoate	2300
NA-111	p-nitrophenyl	p-nitrobenzoate	960
NA-79	3-pyridyl	Nicotinate	530
	urethanes		
NA-75	Ethoxy	Ethanol CO_2	3500
NA-76	Phenyloxy	Phenol CO_2	1150
NA-77	p-nitrophenyloxy	p-nitrophenol CO_2	23
NA-80	p-methoxycarbonyl phenyloxy	Methyl p-hydroxy benzoate	147
NA-78	Dimer	CO_2	430
	temozolomide	CO_2	25

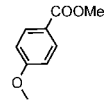


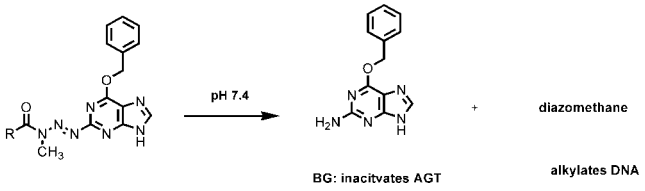
NA-76 R =

NA-77



NA-80





Introduction of different substituents could be used for the finetuning of the half lives of the molecules (Table 3).

Biological studies are currently in progress in cooperation with the group of G.J. Peters, VU University Medical Center, Amsterdam.

CONCLUSIONS

Nitration of substituted (deaza)purines using a mixture of tetrabutylammonium nitrate and trifluoracetic acid anhydride is a powerful synthetic instrument to prepare nitrosubstituted heteroyclic systems at low temperature. The nitro group influences the system twofold: 1) it activates other substituents towards nucleophilic aromatic substitution and 2) it can be substituted with different nucleophiles leading to a variety of di-substituted (deaza)purines, also via solid phase syntheses.

Several of the molecules obtained are of interest for different biological reasons, like antiprotozoal activity and for interaction studies with the different human adenosine receptors.

REFERENCES

1. Deghati, P.Y.F.; Wanner, M.J.; Koomen, G.J. Regioselective nitration of purine nucleosides: synthesis of 2-nitroadenosine and 2-nitroinosine. Tetrahedron Lett. **2000**, *41*, 1291–1295.
2. Deghati, P.Y.F.; Wanner, M.J.; Bieräugel, H.; Koomen, G.J. Mild and regioelective nitration of 1-deazapurine nucleosides using TBAN/TFAA. Tetrahedron Lett. **2000**, *41*, 569–573.
3. Cristalli, G.; Franchetti, P.; Grifantini, M.; Vittori, S.; Klotz, K.-N.; Lohse, M.J. Adenosine receptor agonists: synthesis and biological evaluation of 1-deaza analogues of adenosine derivatives. J. Med. Chem. **1988**, *31*, 1179–1183.
4. Rodenko, B.; Wanner, M.J.; Koomen, G.J. Solid phase synthesis of C-2, N^6-disubstituted adenosine analogues. J. Chem. Soc., Perkin I **2002**, 1245–1252.
5. Wanner, M.J.; Koomen, G.J. Syntheses and properties of 2-nitrosoadenosine. J. Chem. Soc., Perkin I **2001**, 1908–1915.
6. Wanner, M.J.; Koomen, G.J. A new syntheses of temozolomide. J. Chem. Soc., Perkin I **2002**, 1877–1880.
7. Beukers, M.W.; Wanner, M.J.; Von Frijtag Drabbe Künzel, J.K.; Klaasse, E.C.; IJzerman, A.P.; Koomen, G.J. N^6-Cyclopentyl-2-(3-phenylaminocarbonyl-triazene-1-yl)adenosine (TCPA) a very selective agonist with high affinity for the human adenosine A_1 receptor. J. Med. Chem. **2003**, *46*, 1492–1503.