New nucleoside analogs, synthesis, and biological properties*

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Abstract: In view of the importance of nucleoside analogs as enzyme inhibitors and adenosine receptor (ant)agonists, new adenosine analogs were prepared and their activities studied on adenosine deaminase *in vitro* and on A_1, A_{2A}, and A_3 receptors. Particularly useful for the synthesis of new analogs were the applications of the Pd-catalyzed Buchwald reaction and a radical nitration reaction of purine- and 1-deazapurine nucleosides.

Since several decennia, the Bioorganic group in Amsterdam has been interested in the properties of nucleoside analogs [1–3]. The synthesis and biological studies have resulted in the development of new clinically active anti-Herper compounds such as Penciclovir [4] and the application of strongly fluorescent analogs as cell-growth probes [5,6].

The clinical application of inhibitors of adenosine deaminase for the treatment of leukemia stimulated our interest in the development of new inhibitors, also for theoretical studies. With this goal in mind, we were interested in the properties of the substrate- and product-like transition-state inhibitors **1** and **2** (Fig. 1).

Even more interesting are the inhibitors **3** and **4**, that mimic the transition state so well, also geometrically, that it can be easily envisaged that the molecules will never be stable in the purine series, since the free electrons of N-1 will trigger the ring opening of the oxirane ring, restoring purine aromaticity. To obtain these systems, 1-deazapurine chemistry is required (X = C) [7,8].
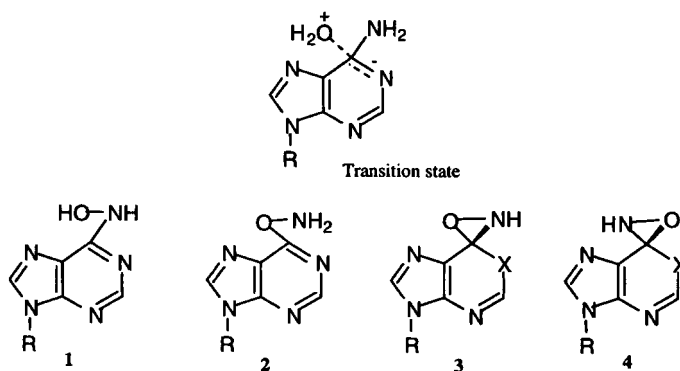

Fig. 1

For introduction of substituents in the 6-position of the purine system, the usually applied method consists of nucleophilic aromatic substitution of suitably substituted purine systems. The reaction is, of course, facilitated by the presence of the electron-withdrawing nitrogen atoms in the system, which implies that comparable reactions with deaza systems will be much more retarded.

We found that the Pd-catalyzed Buchwald reaction was also quite effective for the preparation of several amino-substituted purine derivatives, using 2,2′-bis(diphenylphosphino)-1,1′-binaphtyl (binap) as ligand and potassium t-butoxide or cesium carbonate as bases [9]. In particular, reactions with secondary amines and anilines were more effective than those without catalyst (Scheme 1).
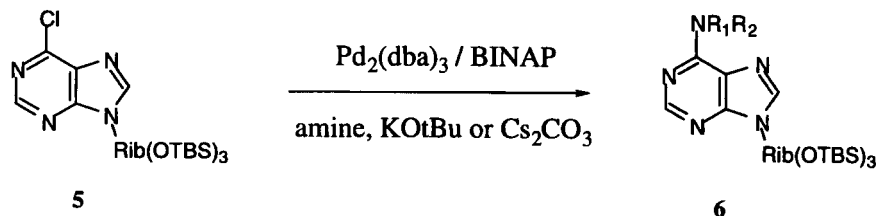
Scheme 1 Amines: piperidine (95% yield), morpholine (80%), benzylamine (78%), dimethylamine (91%), aniline (10%), and 2-aminopyridine (0%).

Particularly useful for the introduction of substituents was a radical nitration which we applied for the first time on purine systems. The reaction is carried out with tetrabutylammonium nitrate and trifluoroacetic anhydride (TBAN/TFAA) in methylene chloride at 0 °C. The resulting nitro-group could be substituted itself and also activated, as expected, other leaving groups in the system. In this way, 2-nitro adenosine **9a** was obtained and reduced to 2,6-diaminopurine riboside (Scheme 2). Also a variety of 6-substituted 2-nitro-adenosines **9b–9e** were prepared an tested both on adenosine deaminase and on adenosine receptors.
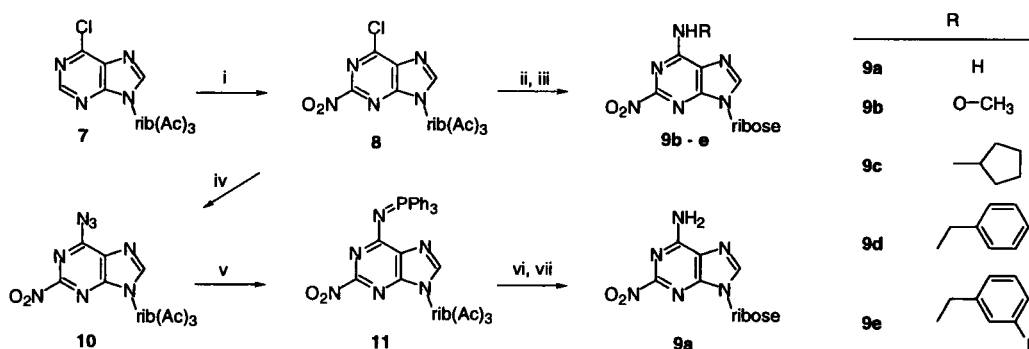
Scheme 2 (i) 1.7 eq TBAN/TFAA, DCM, 0 °C, 65–71% (3 steps); (ii) 1.2 eq RNH2, TEA, DMF, 0 °C; (iii) KCN, MeOH, 2h, rt (**9b**: 42%; **9c**: 53%; **9d**: 46%; **9e**: 56%, 2 steps); (iv) 1.0 eq NaN3, DMF, -18 °C; (v) PPh3, DCM, rt; (vi) HOAc/H2O 3/1, 45 °C, 64%, 3 steps; (vii) KCN, methanol, 2 h, rt, 80%.

The nitration reaction could also be successfully applied to the 1-deaza sytems. The reactions described in Scheme 2 were also applied to 1-deazapurine nucleosides, leading among others to 2-nitro-1-deaza adenosine **14a**, whose structure was confirmed by X-ray crystallography. Further conversions produced amongst others **14b,c** (Scheme 3).

Particularly useful was the use of N-oxide **12** as a substrate, since this allows introduction of a nitro group in the 1-position (purine numbering). The nitro group could be easily reduced to an amino group (**13**), just to restore the free pair of nitrogen electrons in a slightly different position than in the purine systems.
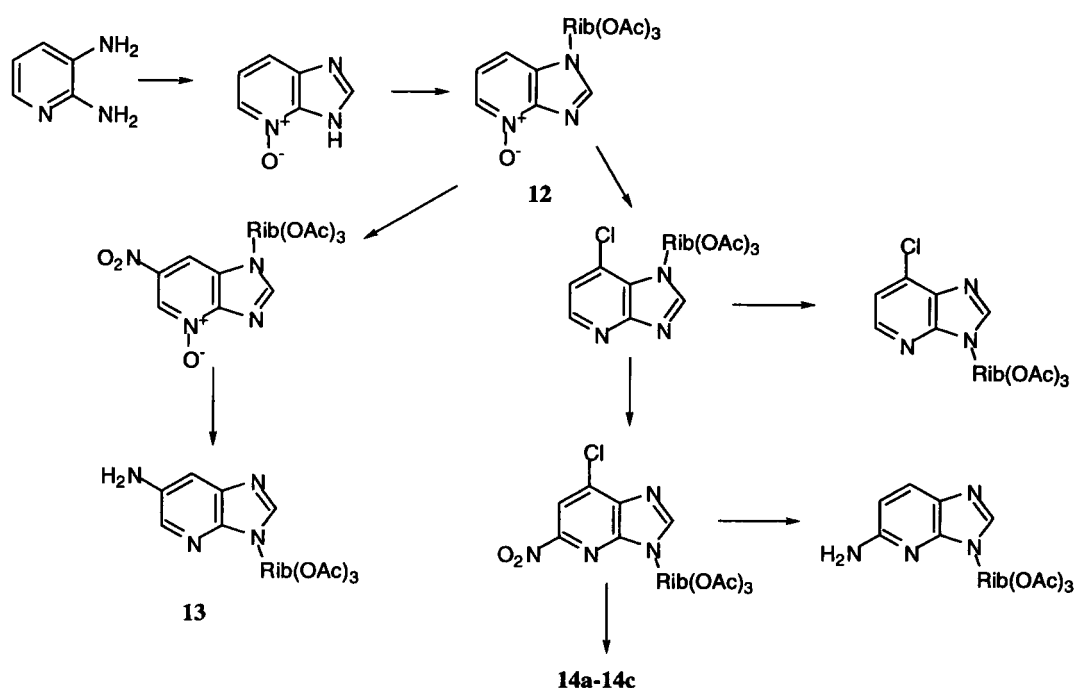
Scheme 3

Most of the compounds obtained were tested *in vitro* for their activity either as a substrate or as an inhibitor on adenosine deaminase from calf intestinal mucosa (Sigma). 2-Nitro-adenosine **9a** turned out to be a good inhibitor of the enzyme (Ki = 11 μM). In general, the enzyme tolerated a variety of substituents in the 2-position, leading to active competitive inhibitors. Also, 2-nitro1-deaza adenosine was an effective inhibitor (Ki = 8 μM), although less effective than the parent compound 1-deaza-adenosine (Ki = 0.3 μM).

Studies toward combinatorial approaches via binding of adenosine derivatives with the 5′ hydroxy group to a solid support are currently underway.

Several nitro purine and deazapurine derivatives, described in this paper, were tested in Prof. I. Jzerman's lab at Leiden University on binding to the different adenosine receptors. Preliminary results obtained are presented in Table 1 [12]:

**Table 1** Adenosine receptor affinities (K_i values ± SEM) as determined in radioligand binding studies.

Compounds	R	A_1 (K_i, nM)[a]	A_{2A} (K_i, nM)[b]	A_3(K_i, nM)[c]
9a	H	344 ± 16	286 ± 112	202 ± 103
9b	methoxy	1160 ± 570	9%[d]	35.8 ± 27.8
9c	cyclopentyl	47.1 ± 3.4	3510 ± 1940	222 ± 145
9d	benzyl	1420 ± 240	20%[d]	163 ± 43
9e	3-I-benzyl	138 ± 30	1440 ± 790	12.0 ± 3.7
14a	H	646 ± 150	437 ± 147	216 ± 89
14b	cyclopentyl	52.6 ± 6.8	52%[d]	340 ± 54
14c	3-I-benzyl	110 ± 36	208 ± 22	9.8 ± 3.2

[a] Displacement of [³H]DPCPX from rat cortical membranes
[b] Displacement of [³H]ZM241,385 from rat striatal membranes
[c] Displacement of [¹²⁵I]-ABMECA from human A_3 receptors expressed on HEK293 cells
[d] % displacement at 10 μM
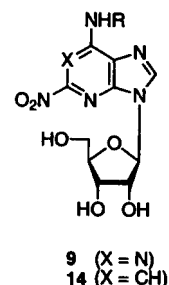
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