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ADENOSINE RECEPTOR LIGANDS WITH OXYGENATED N^6-SUBSTITUENTS

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Abstract: A number of novel adenosine analogs bearing oxygenated substituents in the N^6-position have been prepared and evaluated as A_1 adenosine agonists. Improved conditions for the synthesis of N^6-substituted adenosines and a new one pot procedure for the synthesis of 2-amino-7-oxabicyclo[2.2.1]hept-5-ene are also reported.

N^6-(5,6-Epoxynorborn-2-yl)adenosine (ENAdo) has recently been identified as a potent A_1 adenosine agonist with potential applications in the treatment of cardiac arryhthmias.[1] Unfortunately the more potent 2*S*-*endo* isomer degrades to a polar by-product upon standing, thus limiting its therapeutic potential.[1] This process is thought to be an intramolecular cyclisation involving N1 of the purine and the epoxide moiety. An analogous cyclisation was observed when 1,3-dipropyl-8-[5,6-epoxy-(1*S*,2*S*)-norborn-2-yl)xanthine was exposed to mildly acidic conditions.[2] In order to overcome this problem we have targeted analogs of ENAdo in which the epoxide in the N^6-position has been replaced by more stable alcohol, ketone and ether functionality.

The synthesis of N^6-substituted adenosines generally involves the alkylation of a substituted amine by 6-chloropurine riboside in the presence of triethylamine.[3] In this case, the purine riboside component was first protected as the *t*-butyldimethylsilyl ether derivative (**1**) to allow selective manipulation of the alcohol of the N^6-substituent later in the synthesis (Scheme 1). The amine required for this synthesis, hydroxynorborn-2-yl amine, was prepared in three steps from 5-*exo*-aminonorborn-2-ene. This involved BOC protection of the amine, hydroboration-oxidation of the alkene (borane:THF then hydrogen peroxide under basic conditions) and finally deprotection. As expected, a mixture of 5- and 6-hydroxynorborn-2-yl amine was obtained. This mixture was reacted directly with *t*-butyldimethylsilyl protected 6-chloropurine riboside (**1**) to afford N^6-(hydroxynorborn-2-yl)adenosine (**2**) in 64% yield. Column chromatography allowed isolation of pure isomers **2a** and **2b** (29 and 30%, respectively). The position of the hydroxyl group was determined from the COSY NMR spectrum. These compounds were deprotected using tetrabutylammonium fluoride to afford the corresponding adenosines **3a** and **3b** in good yield (83 and 73%, respectively). Both silica and reverse phase chromatography were required to purify these products as the tetrabutylammonium species proved to be difficult to remove. Protected N^6-(6-hydroxynorborn-2-yl)adenosine (**2b**) was also treated with pyridinium chlorochromate and then deprotected (tetrabutylammonium fluoride) to yield the ketone **4**. The oxidation step proceeded in relatively poor yield (53%) due to problems with partial deprotection and other side reactions. However, as ample material was obtained for testing, no attempt was made to optimise this process.

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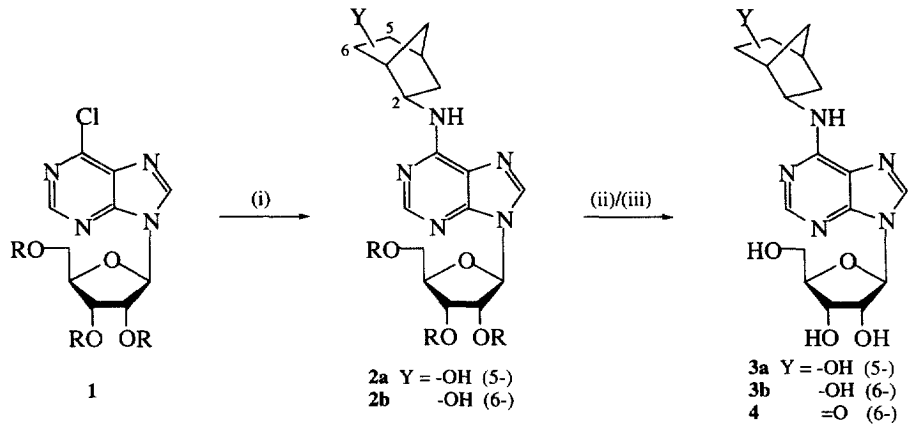
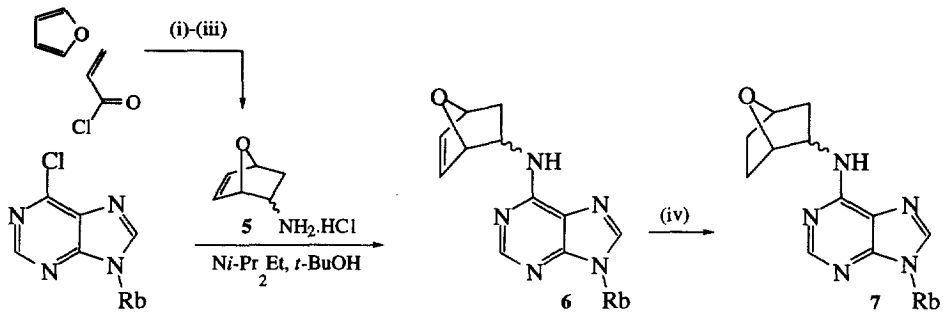


Scheme 1. (i) 5- and 6-Hydroxynorborn-2-yl amine, NEt_3, MeOH; (ii) NBu_4F, THF; (iii) for **2b** → **4**: PCC, CH_2Cl_2 then NBu_4F, THF *(R = t-BDMSi)*.

The introduction of an oxa-bridged bicyclic substituent into the N^6-position of adenosine using this synthetic approach required the use of 2-amino-7-oxa-bicyclo[2.2.1]hept-5-ene. 2-Amino-7-oxa-bicyclo[2.2.1]heptane has been prepared by the Diels–Alder reaction of furan with nitroethylene followed by catalytic hydrogenation.[4] This approach suffers from the disadvantage that the Diels–Alder adduct is prone to rearrangement due to the inductively withdrawn electronic charge from the allylic sigma bond. In fact 2-(β-nitroethyl)furan was reported to be the main product (71%) when furan was reacted with nitroethlyene at ambient temperature for 48 h.[5] An additional disadvantage is the availability of nitroethylene which cannot be purchased from the major chemical suppliers and can be difficult to prepare and purify. Nitroethylene is generally prepared from nitroethanol which is in turn prepared from nitromethane and paraformaldehyde in a reaction which is both inefficient and potentially explosive.[6] The small scale purification of nitroethylene is plagued by polymerisation problems. A nitroethylene transfer reagent (2-nitroethylphenyl sulphoxide) has been developed, though its preparation required five steps and proceeded in low overall yield (13% from ethylene glycol).[6] In order to bypass these problems we have developed a new synthesis of 2-amino-7-oxa-bicyclo[2.2.1]hept-5-ene hydrochloride (**5**)[7] which involved a Diels–Alder reaction between furan and acryolyl chloride followed by a mild variant of the Curtius rearrangement (Scheme 2). Acryolyl chloride and furan were allowed to react until an equilibrium had been reached (determined by ^1H NMR) and the crude mixture was used for the Curtius rearrangement. Briefly, this involved generation of an acyl azide, rearrangement to the corresponding isocyanate and hydrolysis to **5**. The hydrolysis was effected by refluxing the isocyanate in carbon tetrachloride with one equivalent of dilute acid in a carefully controlled biphasic reaction (to avoid reaction of the alkene). The resultant amine hydrochloride was used in the next synthetic step without purification.



Scheme 2. (i) 25 °C, 18 h; (ii) NaN_3, NBu_4Br, CCl_4/H_2O; (iii) 2 M HCl/CCl_4; (iv) H_2, Pd/C, AcOH/MeOH *(Rb = ribose)*.

The reaction of 2-amino-7-oxabicyclohept-5-ene hydrochloride with 6-chloropurine riboside under the standard conditions (triethylamine, methanol, reflux[3]) failed to give complete conversion, even after long reaction times. After 16 h a 17% yield of the desired N^6-substituted adenosine was isolated. The use of a sterically hindered base in conjunction with a hindered, higher boiling solvent (Hünig's base and *t*-butanol) improved this conversion dramatically without causing any appreciable side reactions between the solvent or base and 6-chloropurine riboside. A 71% yield of N^6-(7-oxabicyclo[2.2.1]hept-5-en-2-yl)adenosine (**6**) was achieved using these reaction conditions. An *exo*:*endo* ratio of approximately 1:1.5 was apparent by ^1H NMR. Hydrogenation of the alkene moiety (H_2, 10% Pd/C, AcOH/MeOH) afforded **7** in 85% yield.

All of the analogs of ENAdo proved to be shelf stable with no degradation being observed upon standing at room temperature over a period of several weeks. An assay measuring the inhibition of (–) isoproterenol (1 μM) stimulated cAMP accumulation in DDT_1 MF-2 cells, an effect mediated through activation of the A_1 adenosine receptor, was used to assess agonist potency.[8] Both isomers of N^6-(hydroxynorborn-2-yl)adenosine (**3a** and **b**) retained reasonable potency at the A_1 adenosine receptor (EC_{50} values of 8.0 and 9.2 nM, respectively). The potency of N^6-(6-hydroxynorborn-2-yl)adenosine was improved by oxidation of the norbornyl hydroxyl to the corresponding ketone (compound **4**: EC_{50} = 2.7 nM). N^6-(7-Oxabicyclo[2.2.1]hept-5-en-2-yl)adenosine (**6**) inhibited the cAMP accumulation in a concentration dependant manner with an EC_{50} value of 21.5 nM. Reduction of the alkene moiety increased agonist potency by a factor of three (compound **7**: EC_{50} = 7.1 nM). It is anticipated that the synthesis of pure stereoisomers of this compound (*exo*/*endo* and *R*/*S*) may produce even more potent A_1 adenosine agonists.

Compound	Purification[a,b]	EC_{50}[c] (nM)
exo-ENAdo[1]		1.1 ± 0.2 (3)
endo-ENAdo[1]		1.0 ± 0.3 (3)
3a	A	8.0 ± 2.8 (3)
3b	A	9.2 ± 2.3 (3)
4	A	2.7 ± 0.4 (4)
6	B	21.5 ± 0.7 (3)
7	C	7.1 ± 3.1 (3)

[a]Column chromatography using A) $CHCl_3$/MeOH (9:1) on silica gel 60 + MeOH/H_2O/NH_3 (60:40:0.1) on RP-8; B) $CHCl_3$/MeOH (9:1); C) $CHCl_3$ → $CHCl_3$/*i*-PrOH (6:4) gradient. [b]Accurate high-resolution ES-MS spectra were obtained for all products. [c]DDT_1 MF-2 cells were incubated with 1 μM (–) isoproterenol, 50 mM rolipram and varying concentrations of the agonists for 10 min at 37 °C. The concentration of each agonist that inhibited (–) isoproterenol-stimulated cAMP accumulation by 50% (EC_{50}) was calculated from nonlinear regression analysis. Basal cAMP accumulated was typically less than 5% of the total accumulated in the presence of 1 μM (–) isoproterenol. Data are the mean ± SE and the number in parentheses are the experimental N.

References and Notes

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7. Furan (3.6 mL, 49 mmol) and acryloyl chloride (4.0 mL, 49 mmol) were stirred for 18 h at 25 °C. The reaction mixture was dissolved in CCl_4 (35 mL) and tetrabutylammonium bromide (100 mg) was added. After cooling to 0 °C a solution of NaN_3 (3.183 g, 49 mmol) in water (12 mL) was added and the reaction was stirred vigorously for 2 h at 0 °C. The reaction was poured onto ice and the aqueous phase extracted with CCl_4. All organic portions were combined and refluxed with 2 M HCl (23 mL) for 21 h. The aqueous phase was collected and the organic phase was washed with 0.5 M HCl. Evaporation of the combined aqueous portions afforded **5** (99%). Crude **5** (312 mg, 2.1 mmol), 6-chloropurine riboside (294 mg, 1.0 mmol) and N(*i*-Pr)$_2$Et (0.73 mL, 4.2 mmol) were refluxed in dry *t*-butanol (75 mL) for 46 h. Evaporation and purification by column chromatography (chloroform/methanol, 9:1) afforded **6** (71%). mp 134–135 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 1.18–2.47 (m, 2H, H3″a,b), 3.82 (dd, 2H, H5′a,b), 4.17 (d, 1H, H4′), 4.23 (m, 1H, H2″), 4.34 (br s, 1H, H3′), 4.74 (m, 2H, H2′), 4.84–5.23 (m, 2H, H1″,H4″), 5.95 (d, 1H, H1′), 6.30–6.64 (m, 2H, H5″,H6″), 8.24 (br s, 2H, H1,H8); High-resolution ES MS calcd 362.1464, found 362.1463.
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