Pergamon

Tetrahedron, Vol. 52, No. 13, pp. 4735-4744, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00144-5

The Synthesis of the Novel Adenosine Agonists, *exo*- and *endo*- N^6-(5,6-Epoxynorborn-2-yl)adenosine

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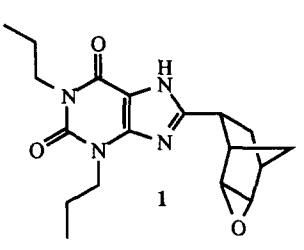
Abstract: Both racemic *exo* and *endo* isomers of N^6-(5,6-epoxynorborn-2-yl)adenosine have been synthesised and shown to be potent agonists for the A_1 adenosine receptor. Crucial to the preparation of these compounds is the synthesis of *exo* and *endo* norbornenylamines which are accessed through an optimised Curtius rearrangement.

INTRODUCTION

Adenosine is an extracellular messenger which exerts physiological effects on the cardiovascular, nervous and immune systems[1] through at least three distinct receptor subtypes that inhibit (A_1 and A_3) or stimulate (A_2) the enzyme adenylyl cyclase. The potential clinical uses of adenosine and its role in pathophysiological process has generated considerable synthetic effort towards the search for potent and receptor selective adenosine agonists and antagonists. We have recently identified[2] [(8-(5,6-epoxynorbornan-2-yl)-1,3-dipropylxanthine, ENX, **1**] as an antagonist with high affinity and selectivity for the A_1 adenosine receptor.



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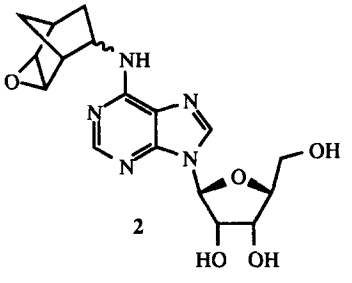

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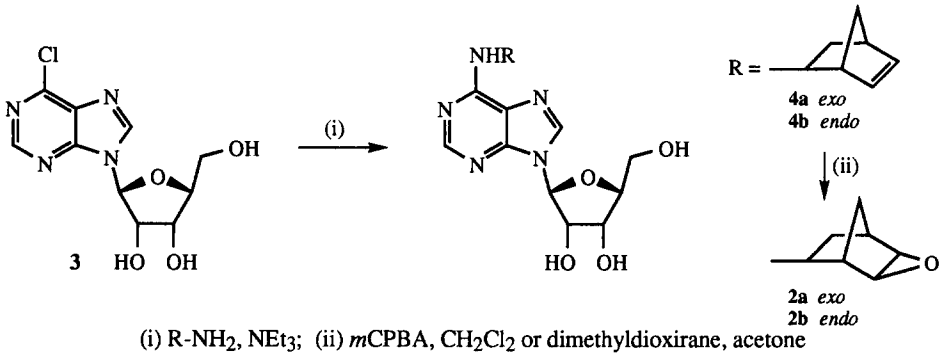
This discovery provided an encouraging lead for the development of potent A_1 agonists which have a similar pharmacological profile. Detailed structure-activity and molecular modelling studies[3,4] indicate that adenosine agonists interact with the A_1 receptor via three domains which accommodate N^6-, 2- and 9- (ribose) substituents. However, xanthine antagonists are generally believed to interact with the receptor in completely different orientation. The antagonist structure-activity profile of xanthines substituted in the 8-position closely parallels that of agonist adenosine analogs substituted in the N^6-position. This suggests that these groups interact with the same area of the receptor and that the purine ring of xanthine antagonists is flipped and rotated with respect to that of adenosine. Based on this hypothesis, we identified N^6-(5,6-epoxynorborn-2-yl)adenosine (ENAdo, **2**) as a potential A_1 receptor agonist.

RESULTS AND DISCUSSION

The typical synthesis of N^6-substituted adenosines involves the alkylation of an substituted amine with 6-chloropurine riboside (**3**, Scheme 1)[5], which in the present context required *exo*- and *endo*-5-aminonorborn-2-enes as starting materials for the target isomers of **4**.

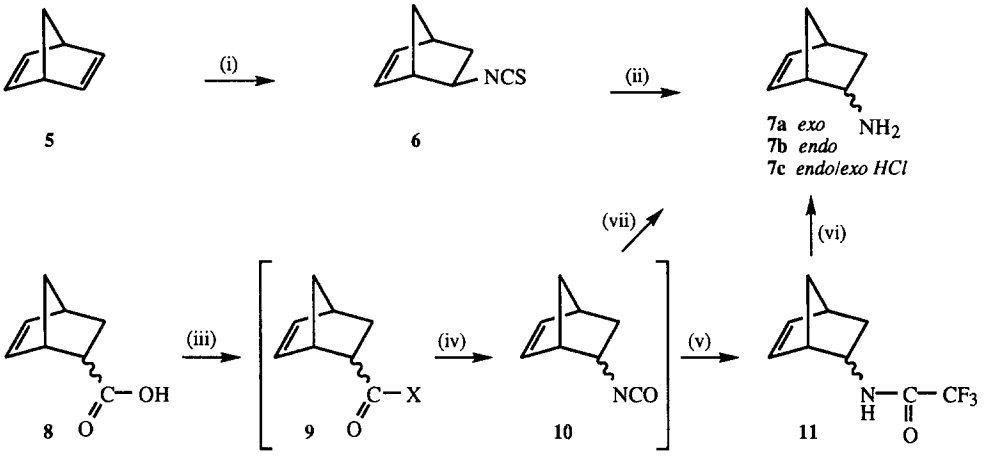


(i) R-NH_2, NEt_3; (ii) *m*CPBA, CH_2Cl_2 or dimethyldioxirane, acetone

Scheme 1

Whilst *exo*-5-aminonorborn-2-ene was readily obtained in 2 steps from norbornadiene using literature procedures[6,7] (Scheme 2, **5** → **7a**), the *endo*-isomer proved more challenging. A synthesis of **7b** from *endo*-5-norbornene-2-carboxylic acid would have the added scope of being amenable to the synthesis of the 2*R* and 2*S*-*endo* isomers since the necessary carboxylic acids are accessable by asymmetric procedures[8,9]. One such synthesis has been reported[10], but yields were very low (13%). Since the starting material is itself somewhat tedious to obtain we opted to base our synthesis upon the readily available mixture of *exo* and *endo* acids (see Scheme 2) and focus on the task of optimising the Curtius rearrangement. It is well known that the Curtius rearrangement involves the initial formation of an acyl azide via nucleophilic acyl substitution. This rearranges upon heating to form an isocyanate that can be intercepted with an appropriate nucleophile. Our initial attempts to trap the intermediate (**10**) with *t*-butanol proved unrewarding and we directed our attention to the use of trifluoroacetic acid. Trifluoroacetic acid has proved to be a useful reagent for converting intermediate isocyanates to trifluoroacetamides in a modified version of the Curtius reaction[11]. In this case TFA proved to be

an efficient trapping reagent and formation of the acyl azide from either an acid chloride (**9**, X = Cl) or a mixed anhydride (**9**, X = C(O)OEt) lead ultimately to similar yields of the isomeric trifluoroacetamides (**11**) irrespective of whether the reaction was conducted in acetone or under phase transfer conditions (see Table 1). Large scale separation of the *exo* and *endo* isomers proved difficult due to their similar polarity. However, a small quantity of the *endo* isomer was successfully purified by column chromatography and subsequent alkaline hydrolysis afforded the corresponding amine (**7b**). Although this separation was not amenable to scale up, no improvements were attempted since optically pure *endo*-norbornene-2-carboxylic acid is readily accessible via literature methodology[8,9] and its use as a starting material would avoid the need to separate isomers.



(i) KNCS, H_2SO_4; (ii) NaOH; (iii) $SOCl_2$/ClC(O)OEt, NEt_3; (iv) NaN_3, H_2O then Δ; (v) TFA; (vi) K_2CO_3; (vii) NaN_3, H_2O then 2M HCl/CCl_4

Scheme 2

A more direct approach involved acid hydrolysis of the intermediate isocyanate in a carefully adjusted biphasic mixture. Thus after refluxing the acyl azide/isocyanate in carbon tetrachloride with an equimolar amount of aqueous 2M HCl, conversion to the amine hydrochloride was achieved without affecting the norbornene double bond. This reaction proceeded in very high yield and represents the most efficient synthesis of 5-aminonorborn-2-ene hydrochloride (**7c**) yet reported. Attempts to liberate 5-aminonorborn-2-ene from the hydrochloride salt by raising the pH and extracting with organic solvents gave low yields of the free amine. This posed no difficulty in this synthesis as the hydrochloride salt was able to be used directly to alkylate 6-chloropurine riboside.

6-Chloropurine riboside reacted with *exo* and *endo*-5-aminonorborn-2-ene (**7a** and **b**, respectively) to yield the expected N^6-substituted adenosines, **4a** and **b**. The final step, conversion of these alkenes to epoxides, was initially attempted by treating N^6-(norborn-5-en-2-yl)adenosine with *m*-chloroperbenzoic acid in dichloromethane. After the addition of 1 molar equivalent of peracid two compounds were observed by t.l.c. (the first with the same R_f as starting material). Addition of *m*-chloroperbenzoic acid (a further 2 equivalents) was continued until only the second compound was observed. On workup, removal of the acid byproduct via a base wash proved unsuccessful since the product was also soluble in the aqueous layer. When the reaction

mixture was evaporated and applied directly to a reverse phase MPLC column, streaking of these acid byproducts prevented purification. Purification was eventually achieved via column chromatography using a mixture of ethyl acetate, chloroform and ammonia (85/15/1) as an eluent. Replacement of the olefinic signals at δ 6.17 and 6.20 by a 2 proton singlet at δ 3.23 in the ^{1}H NMR spectrum was consistent with oxidation of the alkene moiety. However, the mass spectrum showed a molecular ion at 392 (16 mass units higher than expected) suggesting that oxidation also occurred at N1. This result was not entirely unexpected since purines commonly undergo oxidation at this position when exposed to peroxides and peracids[12,13]. The reaction was repeated with less peracid and close monitoring by t.l.c. and NMR. Since the exact activity of the *m*-CPBA was unknown (supplied as a 50-60% mixture with *m*-chlorobenzoic acid), small portions of approximately 0.1 equivalent were added over a 48 hour period. The formation of N^6-(*exo*-5,6-epoxynorborn-2-yl)adenosine-1-oxide (**12**) was detected immediately and after all of the starting material was exhausted a 1.5 : 1 ratio of **12** : **2a** was observed by NMR. The problem of N1 oxidation was eventually overcome through the use of dimethyldioxirane[14] which selectively epoxidized the alkene and afforded **2a** in high yield. The volatile nature of the principle byproduct (acetone), greatly facilitated the isolation of the product and obviated extensive chromatography. Dimethyldioxirane was the oxidant of choice due to its selectivity for the alkene moiety and the ease of purification of the oxidized product and was employed for the conversion of **4b** to **2b**.

Table 1 Curtius Rearrangement Reaction Conditions

R-COOH → R-NCO (**8** → **10**)	Trapping/Hydrolysis	Product	Yield (%)
(i) $SOCl_2$ (ii) NaN_3, Δ	*t*-BuOH	---	---
(i) $SOCl_2$ (ii) NaN_3, H_2O (NBu_4Br, CCl_4)	TFA	**11**	56
(i) ClC(O)OEt, NEt_3 (acetone) (ii) NaN_3, H_2O	TFA	**11**	57
(i) $SOCl_2$ (ii) NaN_3, H_2O (acetone)	TFA	**11**	57
(i) $SOCl_2$ (ii) NaN_3, H_2O (NBu_4Br, CCl_4)	2N HCl	**7c**	96
(i) ClC(O)OEt, NEt_3 (acetone) (ii) NaN_3, H_2O	2N HCl	**7c**	94

The compounds were tested for their potency to inhibit (-) isoproterenol stimulated cAMP accumulation in DDT_1 MF-2 (DDT) cells. This effect is mediated through the action of the A_1 adenosine receptor (A_1 AR). For comparison purposes, the epoxides **2a**, **2b** and **12** were compared to the well established and potent A_1 agonist, N^6-cyclopentyladenosine (CPA). (-) Isoproterenol (1 μM) alone stimulated cAMP accumulation in DDT cells 57 fold above the basal level. CPA and the epoxide derivatives inhibited the (-) isoproterenol stimulated cAMP accumulation in a concentration dependant manner with the EC_{50} values shown in Table 2. CPA and the racemic *exo* and *endo* isomers (**2a** and **b**) inhibited cAMP accumulation with similar EC_{50} values of about 1-2 nM. This suggests that the spatial position of the epoxynorbornyl moiety did not have a significant impact on the agonist potency for these compounds. In contrast, the N-oxide derivative of the *exo* isomer (**12**)

was much less potent than CPA or the *exo* and *endo* isomers of N^6-(5,6-epoxynorborn-2-yl)adenosine (**2a** and **b**) with an EC_{50} value of 403 nM.

Table 2. Agonist Concentration which inhibited (-)isoproperenol stimulated cAMP accumulation by 50%

Compound	EC_{50} (nM)[a]
CPA	1.7 ± 0.4
2a	1.1 ± 0.2
2b	1.0 ± 0.3
12	403 ± 46

[a] DDT cells were incubated with 1 μM (-) isoproterenol and various concentrations of the compounds for 10 min at 37 °C. The cAMP accumulated and the EC_{50} values were determined as described in the Experimental Section. Basal and (-) isoproterenol stimulated cAMP accumulated was 8 ± 3 and 458 ± 41 pmol cAMP formed per 10 min, respectively. Each value is the mean ± of 3 separate determinations performed in tripicate.

EXPERIMENTAL

Melting points (mp) were determined on an Electrothermal melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Jeol JNM-EX270 spectrometer. Unless otherwise stated d^6-DMSO was used as a solvent and TMS as an internal standard. FAB mass spectra were measured on a Jeol JMS-DX300 mass spectrometer and processed on an MSS data system. Merck Kieselgel 60 and 60 F_{254} were used for column and thin layer chromatography, respectively. Solvents were either AR grade or distilled prior to use. Acetone was dried by distillation over potassium carbonate and was stored over 4 Å sieves, while dry methanol was distilled from magnesium methoxide and stored over 3 Å sieves.

***exo*-Norborn-5-ene-2-yl isothiocyanate (6)** Concentrated H_2SO_4 (38.4 g) in water (12 mL) was added dropwise over a 2 h period to a mixture of bicyclo[2.2.1]hepta-2,5-diene (60 mL), benzene (150 mL) and KSCN (57.5 g) at 35-40 °C. After 3 h the reaction mixture was cooled and water was added. The reaction mixture was filtered through a glass fritted filter funnel under vacuum and rinsed with ether (200 mL). The organic layer was separated, washed with water and dried over magnesium sulphate. Filtration and evaporation of the solvent afforded an orange liquid. Distillation under reduced pressure (70-74 °C, 1 mbar, lit.[6] 76-78 °C, 1 mmHg) yielded pure **7** (40%); *^1H NMR*: δ 1.61-1.77 (m, 4H, H3, H7), 2.92 (br s, 1H, H1/H4), 3.10 (br s, 1H, H1/H4), 3.53 (t, 1H, H2), 5.98 (dd, 1H, H5/H6), 6.21 (dd, 1H, H5/H6); *^{13}C NMR*: δ 35.6, 41.0, 46.1, 49.8, 55.3, 132.7, 140.2.

***exo*-5-Aminonorborn-2-ene (7a)** To a stirred solution of the norbornyl isothiocyanate (56.7 g, 0.375 mol) in ethylene glycol held at 100 °C, solid NaOH (45.0 g) was added over a 5 min period. The temperature was increased to 165 °C and the reaction was stirred for 3 h. After cooling, the reaction mixture was then cooled and poured into a solution of saturated potassium carbonate (1 L) and then extracted with dichloromethane (3 x 400 mL). The organic layer was separated and then extracted with HCl (2N, 3 x 200 mL), made basic with 2N NaOH and saturated with potassium carbonate. Filtration and evaporation of the solvent afforded a liquid which was distilled under vacuum (54-54.5 °C, 30 mmHg, lit.[7] 70 °C, 40-41 mmHg) to yield a tan product (14.3 g, 35 %). *^1H NMR*: δ 0.88 (dt, 1H, H3/H7), 1.19-1.48 (m, 3H, H3, H7), 2.31 (br s, 1H, H1/H4), 2.61 (br s,

1H, H1/H4), 2.72 (dd, 1, H2), 5.92, (dd, 1H, H5/H6), 5.96 (dd, 1H, H5/H6); *^{13}C NMR* ($CDCl_3$): δ 37.0, 41.2, 44.8, 50.9, 51.9, 135.0, 138.0.

***exo/endo*-Norborn-5-ene-2-carbonyl chloride (9)** A solution of **8** (3.5 g, 28.9 mmol) in thionyl chloride (2.7 mL, 35.2 mmol) under a nitrogen atmosphere was stirred overnight at room temperature. Excess thionyl chloride was evaporated under reduced pressure and the resultant oil was distilled (65 °C, 7.5 mmHg); *^{1}H NMR* ($CDCl_3$): δ 1.32-1.56 (m, H3/H7 *exo/endo*), 1.95 (m, H3/H7 *exo/endo*), 2.98 (br s, H1/H4 *exo/endo*), 3.42 (br s, H1/H4 *exo/endo*), 3.45 (m, H2 *exo/endo*), 6.03 (dd, 1H, H5/H6 *endo*), 6.12 (dd, 1H, H5/H6 *exo*), 6.21 (dd, 1H, H5/H6 *exo*), 6.26 (dd, 1H, H5/H6 *endo*); *^{13}C NMR* ($CDCl_3$): δ 30.0, 31.1, 41.7, 42.8, 42.9, 46.2, 46.8, 47.0, 49.1, 56.3, 131 5, 134.8, 138.6, 138.9, 174.8, 176.6.

2-Trifluoroacetylaminonorborn-5-ene (11)

Method A (via mixed anhydride) A solution of norborn-5-ene-2-carboxylic acid (1.75 g, 12.8 mmol) and triethylamine (1.95 mL, 1.43 g, 14.1 mmol) in dry acetone (25 mL) was cooled in an ice bath. Freshly distilled ethyl chloroformate (1.41 mL, 1.60 g, 14.7 mmol) was added and the reaction mixture was stirred at 0 °C. After this period, a solution of sodium azide (1.04 g, 16.0 mmol) in distilled water (5 mL) was added and reaction mixture was stirred for 2 hours at 0 °C. The reaction mixture was poured onto ice (~10 mL) and the aqueous phase was extracted with dichloromethane (2 x 20 mL). All organic portions were combined, dried over magnesium sulphate for 14 hours, filtered and evaporated to afford a colourless oil. This oil was taken up in dichloromethane and refluxed with trifluoroacetic acid (1.28 mL, 1.90 g, 16.7 mmol) for 14 hours. After cooling the reaction mixture was washed with saturated sodium bicarbonate (2 x 25 mL), dried over magnesium sulphate, filtered and evaporated to afford the crude product. Purification was achieved by column chromatography using chloroform/hexane (1:1) as an eluent. The final product was obtained as a white crystalline solid (1.48 g, 57%).

Method B (via acid chloride, single phase) A solution of norborn-5-ene-2-carbonyl chloride (1.90 g, 12.5 mmol) in acetone (25 mL) was cooled in an ice bath. A solution of sodium azide (0.98 g, 15.1 mmol) in distilled water (5 mL) was added and reaction mixture was stirred for 2 hours at 0 °C. The reaction mixture was poured onto ice (~10 mL) and the aqueous phase was extracted with dichloromethane (3 x 40 mL). All organic portions were combined, dried over magnesium sulphate for 14 hours and then filtered. Trifluoroacetic acid (1.25 mL, 1.85 g, 16.2 mmol) was added to the filtrate which was then refluxed for 24 h. After cooling the reaction mixture was washed with saturated sodium bicarbonate (2 x 25 mL), dried over magnesium sulphate, filtered and evaporated to afford the crude product. Purification was achieved by column chromatography using chloroform/hexane (1:1) as an eluent. The final product was obtained as a white crystalline solid (1.41 g, 57%).

Method C (via acid chloride, phase transfer conditions) A solution of norborn-5-ene-2-carbonyl chloride (2.0 g, 13.4 mmol) in dichloromethane (25 mL) containing tetrabutylammonium bromide (50-100 mg) was cooled in an ice bath. A solution of sodium azide (1.05 g, 16.2 mmol) in distilled water (5 mL) was added and reaction mixture was stirred vigourously for 2 hours at 0 °C. The reaction mixture was poured onto ice (~10 mL) and the aqueous phase was extracted with dichloromethane (2 x 20 mL). All organic portions were combined, dried over magnesium sulphate for 14 hours and then filtered. Trifluoroacetic acid (1.14 mL, 1.69 g, 14.8 mmol) was added to the filtrate which was then refluxed for 14 hours. After cooling the reaction mixture was washed with saturated sodium bicarbonate (2 x 50 mL), dried over magnesium sulphate, filtered and evaporated to afford the crude product. Purification was achieved by column chromatography using chloroform/hexane (1:1) as an eluent. Evaporation of selected fractions afforded pure *endo*-2-trifluoroacetylaminonorborn-5-ene (0.77 g,

28%), though the overall yield of both *exo* and *endo* isomers was 56% (1.46 g); mp. 44-46 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (dt, 2H, H3/H7), 1.37-1.57 (m, 2H, H3/H7), 2.28 (m, 1H, H3/H7), 2.93 (br s, 1H, H1/H4), 3.13 (br s, 1H, H1/H4), 4.53 (m, 1H, H2), 6.04 (dd, 1H, H5/6), 6.45 (dd, 1H, H5/H6); ^{13}C NMR ($CDCl_3$) δ 35.2, 42.5, 45.8, 48.8, 50.0, 115.8 (q, J = 288.1 Hz, -CF_3), 130.7, 141.0, 156.7 (q, J = 36.6 Hz, C=O).

***endo*-5-Aminonorborn-2-ene (7b)** A mixture of 2-trifluoroacetylaminonorborn-5-ene (0.54 g, 2.6 mmol) and potassium carbonate (0.61 g, 4.4 mmol) in methanol (5 mL) and water (20 mL) were stirred at ambient temperature under an atmosphere of nitrogen for 25 hours. After concentration on a rotary evaporator, the reaction mixture was extracted with diethyl ether (3 x 20 mL). The organic phase was dried over magnesium sulphate, filtered and evaporated to yield a tan oil (0.23 g, 80%). Distillation afforded pure **7b** (152-160 °C, lit.[10] 150-160 °C). *^{1}H NMR*: δ 0.43 (dt, 1H, H3/H7), 1.20-1.30 (m, 2H, H3/H7), 1.95 (m, 1H, H3/H7), 2.72 (br s, 1H, H1/H4), 2.75 (br s, 1H, H1/H4), 3.28 (m, 1, H2), 5.97, (dd, 1H, H5/H6), 6.30 (dd, 1H, H5/H6); *^{13}C NMR* ($CDCl_3$): δ 33.6, 42.6, 47.9, 48.5, 51.1, 131.6, 138.9.

***exo/endo*-5-Aminonorborn-2-ene hydrochloride (7c)**

Method A (via acid chloride) A solution of norborn-5-ene-2-carbonyl chloride (2.66 g, 17.0 mmol) in CCl_4 (25 mL) containing tetrabutylammonium bromide (~50 mg) was cooled in an ice bath. A solution of sodium azide (1.33 g, 20.5 mmol) in distilled water (5 mL) was added and reaction mixture was stirred vigourously for 2 hours at 0 °C. The reaction mixture was poured onto ice (~10 mL) and the aqueous phase was extracted with CCl_4 (2 x 25 mL). All organic portions were combined and refluxed with 2M HCl (8.5 mL) for 17 hours. After cooling, the aqueous phase was collected and the CCl_4 washed with 0.5 M HCl (10 mL). Evaporation of the combined aqueous layers afforded a white solid (2.37 g, 96 %). This solid was either purified by trituration with ethyl acetate or used directly for the synthesis of **4b**.

Method B (via mixed anhydride) A solution of norborn-5-ene-2-carboxylic acid (3.74 g, 27.1 mmol) and triethylamine (4.40 mL, 3.21 g, 31.7 mmol) in dry acetone (40 mL) was cooled in an ice bath. Freshly distilled ethyl chloroformate (2.98 mL, 3.38 g, 31.2 mmol) in acetone (15 mL) was added and the reaction mixture was stirred for 30 min at 0 °C. After this period, a solution of sodium azide (2.21 g, 34.0 mmol) in distilled water (10 mL) was added and reaction mixture was stirred for 2 hours at 0°C. The reaction mixture was poured onto ice (~10 mL) and the aqueous phase was extracted with CCl_4 (2 x 25 mL). All organic portions were combined and refluxed with 2M HCl (13.6 mL) for 20 hours. After cooling, the aqueous phase was collected and the CCl_4 washed with 0.5 M HCl (10 mL). Evaporation of the combined aqueous layers afforded an oily solid which was triturated with ethyl acetate (3.72 g, 94 %); mp. 256-265 °C (dec.); *^{1}H NMR*: δ 0.85-2.09 (m, H3 *exo, endo*, H7 *exo, endo*), 2.82 (br s, H1/H4 *endo*), 2.99 (br s, H1/H4 *exo*), 3.02 (br s, H1/H4 *exo*), 3.08 (br s, H1/H4 *endo*), 3.38 (m, H2 *exo, endo*), 5.96 (dd, H5/H6 *endo*), 6.05 (dd, H5/H6 *exo*), 6.20 (dd, H5/H6 *exo*), 6.37 (dd, H5/H6 *endo*), 8.03 (br s, NH_2), 8.50 (br s, HCl); *^{13}C NMR*: δ 31.6, 40.8, 41.9, 44.7, 44.8, 45.2, 45.4. 47.9, 49.3, 50.2, 130.3, 133.9, 139.3, 140.4. Integration of the ^{1}H NMR signals indicated that the mixture contained ~ 85% of the *endo*-isomer.

N^6-(*exo*-Norborn-5-en-2-yl)adenosine (4a) A solution of *exo*-5-aminonorborn-2-ene (2.02 g, 18.5 mmol) in dry methanol (20 mL) was added to a solution of 6-chloropurine riboside (5.0 g, 17.4 mmol) and triethylamine (3.53 g, 34.9 mmol) in dry methanol (40 mL). After 24 h reflux another molar equivalent of *exo*-5-aminonorborn-2-ene was added and refluxing was continued for a further 40 h. After evaporation of the solvent and excess triethylamine, the crude product was purified by column chromatography using

$CHCl_3$/MeOH/NH_3 (80/20/1) as an eluent. Pure **4a** was isolated in 98% yield; mp. 108-113 °C; *^{1}H NMR* δ 1.37-1.73 (m, 4H, H3", H7"), 2.78 (br s, 1H, H1"/H4"), 2.83 (br s, 1H, H1"/H4"), 3.20 (m, 1H, H2"), 3.62 (m, 2H, H5a',b'), 3.96 (d, 1H, H4'), 4.14 (d, 1H, H3'), 4.60 (m, 1H, H2'), 5.19 (d, 1H, OH), 5.44 (d, 2H, 2x OH), 5.89 (d, 1H, H1'), 6.16 (dd, 1H, H5"/H6"), 6.20 (dd, 1H, H5"/H6"), 7.97 (br s, 1H, NH), 8.23 (br s, 1H, H2/8), 8.35 (s, 1H, H2/8); *^{13}C NMR* δ 34.5, 41.5, 46.5, 48.3, 51.7, 62.4, 71.4, 74.5, 86.8, 89.0, 120.2, 135.4, 140.1, 140.7, 148.7, 153.4, 154.9.

N^6-(*endo*-Norborn-5-en-2-yl)adenosine (4b) 6-Chloropurine riboside (0.70 g, 2.44 mmol) and *endo*-5-aminonorborn-2-ene (0.32 g, 2.93 mmol) were dissolved in dry methanol (20 mL) under an atmosphere of nitrogen. Triethylamine (0.51 mL, 0.37 g, 3.66 mmol) was added and the reaction mixture was refluxed for 48 hours. After this period, HPLC monitoring indicated no further change was occurring so the solvent was evaporated to afford a tan oily solid. Pure **4b** was obtained as a white foam (0.69 g, 79%) after column chromatogaphy with ethyl acetate/methanol/ammonium hydroxide (90/10/1); mp. 108-112 °C; *^{1}H NMR* δ 1.11-2.14 (m, 4H, H3", H7"), 2.79 (br s, 1H, H1"/H4"), 2.83 (br s, 1H, H1"/H4"), 3.22 (br s, 1H, H2"), 3.61 (m, 2H, H5a',b'), 3.97 (d, 1H, H4'), 4.15 (d, 1H, H3'), 4.61 (m, 1H, H2'), 5.20 (d, 1H, OH), 5.45 (d, 2H, 2x OH), 5.89 (d, 1H, H1'), 5.97 (dd, 1H, H5"/H6"), 6.35 (dd, 1H, H5"/H6"), 6.93 (br s, 1H, NH), 8.25 (br.s, 1H, H2/8), 8.36 (s, 1H, H2/8); *^{13}C NMR* δ 33.5, 42.1, 45.5, 47.9, 50.4, 61.6, 70.6, 73.5, 85.8, 87.9, 119.6, 131.6, 138.8, 139.6, 148.2, 152.2, 154.4.

N^6-(*exo*-5,6-Epoxynorborn-2-yl)adenosine (2a) Dimethyldioxirane in acetone (40.0 mL, ~0.1 M, ~4.0 mmol) was added dropwise to a solution of N^6-(*exo*-norborn-5-en-2-yl)adenosine (0.5 g, 1.39 mmol) in dry methanol (100 mL) at 0-5 °C. The reaction mixture was stirred for 8 h at 0-5 °C and then 12 h (overnight) at room temperature. The solvent was evaporated under reduced pressure and the crude product purified by column chromatography ($CHCl_3$/MeOH/NH_3, 80:20:1) to yield a white crystalline product (0.42 g, 80%); mp. 240-242 °C; *^{1}H NMR*: δ 1.15 (m, 2H, H3"/H7"), 1.72 (m, 2H, H3"/H7"), 2.41 (br s, 1H, H1"/H4"), 2.51 (br s, 1H, H1"/H4"), 3.18 (m, 2H, H5", H6"), 3.64 (m, 2H, H5a',b'), 4.00 (d, 1H, H4'), 4.10 (br s, 1H, H2"), 4.18 (d, 1H, H3'), 4.64 (m, 1H, H2'), 5.24 (br s, 1H, OH), 5.50 (br s, 2H, 2xOH), 5.92 (d, 1H, H1'), 7.88 (d, 1H, NH), 8.26 (s, 1H, H2/8), 8.38 (s, 1H, H2/8); *^{13}C NMR*: δ 23.0, 34.3, 36.3, 42.7, 49.1, 49.6, 51.0, 61.8, 70.8, 73.7, 86.0, 88.1, 119.8, 139.9, 148.6, 152.4, 154.1; *HR MS* ($C_{17}H_{22}N_5O_5$) calc. 376.16208, found 376.16109).

N^6-(*endo*-5,6-Epoxynorborn-2-yl)adenosine (2b) Dimethyldioxirane in acetone (27.8 mL, ~0.1 M, ~2.78 mmol) was added dropwise to a solution of N^6-(*endo*-norborn-5-en-2-yl)adenosine (0.5 g, 1.39 mmol) in dry methanol (40 mL) at 0-5 °C. The reaction mixture was stirred for 4 h at 0-5 °C and then 2 hours at room temperature. The solvent was evaporated under reduced pressure and the crude product purified by column chromatography (EtOAc/MeOH/NH_3, 90:10:1) to yield a white crystalline product (0.36 g, 68%); mp. 207-214 °C (dec.); *^{1}H NMR*: δ 0.93-2.08 (m, 4H, H3", H7"), 2.42 (br s, 1H, H1"/H4"), 2.56 (br s, 1H, H1"/H4"), 2.87 (br s, 1H, H1"/H4"), 3.23-3.41 (m, 3H, H2", H5", H6"), 3.68 (dd, 2H, H5'), 4.03 (d, 1H, H4'), 4.22 (br s, 1H, H3'), 4.67 (br s, 1H, H2'), 5.22 (br s, 1H, OH), 5.44 (br s, 2H, 2xOH), 5.97 (d, 1H, H1'), 7.84 (br s, 1H, NH), 8.25 (s, 1H, H2/8), 8.38 (s, 1H, H2/8); *^{13}C NMR*: δ 25.2, 31.1, 36.4, 38.5, 48.3, 50.7, 61.6, 70.6, 73.4, 85.8, 87.9, 120.0, 139.7, 148.3, 152.2, 154.7.; *HR MS* ($C_{17}H_{22}N_5O_5$) calc. 376.16208, found 376.16228.

N^6-(*exo*-5,6-Epoxynorborn-2-yl)adenosine-1-oxide (12) *m*-Chloroperbenzoic acid (216 mg, 1.25 mmol) was added to a solution of N^6-*exo*-norborn-5-en-2-yl adenosine (359 mg, 1.0 mmol, 1.0 eq) in

dichloromethane (40 mL). The reaction was stirred at room temperature while being monitored by tlc. Additional *m*-chloroperbenzoic acid (173 mg, 1.0 mmol) was added after 24 and then 48 h. After a further 24 h the solvent was evaporated to afford the crude product. Purification was effect via column chromatography using $CHCl_3$/MeOH/NH_3 (80/20/1) as an eluent yielded pure **12** (239 mg, 61%); mp. 125-132 °C; *^1H NMR*: δ 1.15 (dd, 2H, H3/H7), 1.76-1.95 (m, H3/H7), 2.46 (br s, 1H, H1"/H4"), 2.55 (br s, 1H, H1"/H4"), 3.23 (m, 3H, H2", H5", H6"), 3.62 (m, 2H, H5a',b'), 3.95 (d, 1H, H4'), 4.15 (br s, 1H, H3'), 4.63 (s, 1H, H2'), 5.12 (br s, 1H, OH), 5.27 (d, 1H, OH), 5.72 (d, 1H, OH), 5.90 (d, 1H, H1'), 8.17 (br s, 1H, NH), 8.60 (s, 1H, H2/8), 8.65 (s, 1H, H2/8); *^{13}C NMR*: δ 22.8, 34.8, 36.4, 43.8, 48.8, 50.7, 52.1, 61.1, 70.1, 73.8, 85.5, 87.4, 118.5, 142.2, 142.7, 142.8, 146.3. *MS* ($C_{17}H_{22}N_5O_6$) m/e 392.

Cell culture. DDT_1 MF-2 cells (American Type Culture Collection) were grown as monolayers in Dulbecco's Modified Eagle's Medium containing 5% fetal bovine serum, 100 U/mL penicillin G, 0.1 mg/mL steptomycin and 2.5 μg/mL amphotericin B in a water-humidified 5% CO_2 and 95% air mixture at 37 °C. Cells were seeded at 0.2-10^4 cells/cm^2 and subcultured twice weekly after detachment using divalent cation-free phosphate buffered saline containing 1 mM ethylenediamine tetraacetic acid (EDTA). Experiments were performed on cells that were grown to 1 day preconfluent.

cAMP Determinations. The potency of the A_1 AR agonists was determined by their ability to inhibit (-) isoproterenol stimulated cAMP accumulation. DDT cells were detached by incubation in 5 mL of divalent cation-free Hand's Balanced Salt Solution containing 1 mM EDTA. The cell suspension was centrifuged at 500 g for 5 min, washed once more by gentle resuspension and centrifugation and resuspended in HBSS. The cells (0.15 - 2 mg protein) were then incubated in microfuge tubes with 0.5 mL HBSS containing 100 μM rolipram, 1 μM (-) isoproterenol and varying concentrations of the adenosine receptor agonists (0.05 - 1000 nM) for 10 min at 37 °C. At the end of the incubation, the reaction was terminated by placing the tubes in a boiling water bath for 5 min. After cooling to room temperature, the tubes were centrifuged for 2 min at 10,000 g and supernatants were saved. The protein content of the cells was determined by the method of Lowry et al.[15] using bovine serum albumin as standard.

The cAMP content of the supernatants was determined by a competitive protein binding assay as described previously[16]. Briefly, an aliquoit of the supernatant (50 μl) was incubated in a volume of 0.2 mL containing 25 mM Tris-HCl buffer (pH 7.0), 8 mM theophylline, 0.8 pmol [^{3}H]cAMP (31.4 Ci/mmol, New England Nuclear) and 20 μg of bovine heart cAMP dependent protein kinase (Sigma Chemical Co.) at 4 °C for 1 hr. At the end of the incubation, 75 μl of a 50% (v/v) hydroxyapatite-water suspension was added to each tube followed by 4 mL of ice-cold 10 mM Tris-HCl Buffer (pH 7.0). The suspension was then poured onto a Whatman GF/B glass fiber filter under reduced pressure, the filter was washed with a further 6 mL of ice buffer, placed in a liquid scintillation counter. The amount of cAMP in the samples was calculated from a standard curve using known concentrations of unlabeled cAMP. The effective concentration of compounds which give 50% inhibition of maximal cAMP accumulation were determined using a concentration effect analysis with nonlinear regression algorithum (Marquardt-Levenberg).

ACKNOWLEDGEMENTS

The support of the Australian Research Council is gratefully acknowledged.

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(*Received in UK* 14 *December* 1995; *revised* 30 *January* 1996; *accepted* 1 *February* 1996)