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New 2,N^6-Disubstituted Adenosines: Potent and Selective A_1 Adenosine Receptor Agonists

Sally A. Hutchinson,[a] Stephen P. Baker[b] and Peter J. Scammells[a,*]

[a]*Centre for Chiral & Molecular Technologies, Deakin University, Geelong, VIC 3217, Australia*
[b]*Department of Pharmacology, University of Florida College of Medicine, Box 100267, Gainesville, FL 32610, USA*

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Abstract—A number of adenosine analogues substituted in the 2- and N^6-positions were synthesized and evaluated for affinity, functional potency and intrinsic activity at the A_1 and A_{2A} adenosine receptors (AR). Three classes of N^6-substituents were tested; norbornen-2-yl (series 1), norborn-2-yl (series 2) and 5,6-epoxynorborn-2-yl (series 3). The halogens; fluoro, bromo, and iodo were evaluated as C-2 substituents. All compounds showed relatively high affinity (nanomolar) for the A_1AR and high potency for inhibiting (−)isoproterenol-stimulated cAMP accumulation in hamster smooth muscle DDT_1 MF-2 cells with the 2-fluoro derivatives from each series having the highest affinity. All of the derivatives showed the same intrinsic activity as CPA. At the A_{2A}AR, all of the derivatives showed relatively low affinity and potency (micromolar) for stimulating cAMP accumulation in rat pheochromocytoma PC-12 cells. The intrinsic activity of the derivatives compared to CGS 21680 was dependent upon the halogen substituent in the C-2 position with most showing partial agonist activity. Of particular interest is 2-iodo-N^6-(2*S-endo*-norborn-2-yl)adenosine (**5e**), which is over 100-fold selective for the A_1AR, is a full agonist at this receptor subtype and has no detectable agonist activity at the A_{2A}AR.

Introduction

Adenosine is a ubiquitous autocoid that mediates a wide range of physiological effects in the cardiac, nervous and immune systems.[1] Adenosine acts via extracellular receptors, of which there are four subtypes (termed A_1, A_{2A}, A_{2B} and A_3AR), that are coupled by G-proteins to secondary messenger systems such as the enzyme, adenylate cyclase, and ion channels. The agonist/antagonist pharmacological profile and G-protein coupled effector systems associated with these receptors have been studied in detail. In the cardiovascular system, A_1 and A_2AR play important and complimentary roles in regulating the supply and demand of oxygen.[2] Activation of A_1AR in the heart produces negative chromotropy and dromotrophy which reduces oxygen demand, while stimulation of A_2AR in the vascular smooth muscle and endothelial cells results in vasodilation and an increase in oxygen supply. A_1AR agonists have potential as antiarryhthmic agents in the therapy of ventricular tachycardias, such as paroxysmal supraventricular tachycardias (PSVT). At present adenosine, marketed as Adenocard™, is the only agent that has been approved for this application. However, due to its short duration of action and modest AR subtype selectivity, there is continued interest in the development of additional AR agonists.[3] Adenosine and adenosine agonists are also thought to be involved in a cardioprotective phenomenon known as ischemic preconditioning (IPC).[4] IPC is the resistance to ischemia-induced infaction produced by exposure to a brief period of ischemia prior to a prolonged ischemic period. Although the exact mechanism is not fully elucidated, a number of studies have presented evidence to suggest that IPC is receptor mediated. As a result there has been increased interest in developing adenosine agonists as cardioprotective agents.[5,6]

We initially designed the adenosine derivative, N^6-(5,6-epoxynorborn-2-yl)adenosine (ENAdo), with these applications in mind.[7,8] While ENAdo proved to be a potent A_1AR agonist, the more efficous 2*S-endo* isomer was found to degrade upon standing, thus limiting its therapeutic potential. This process is thought to involve an intramolecular cyclisation between N1 of the purine ring system and the epoxide moiety of the N^6-substituent. An analogous cyclisation involving a 5,6-epoxynorborn-2-yl group was observed when 1,3-dipropyl-8-

*Corresponding author at current address. Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia. Tel.: +61-3-9903-9542; fax: +61-3-9903-9582; e-mail: peter.scammels@vcp.monash.edu.au

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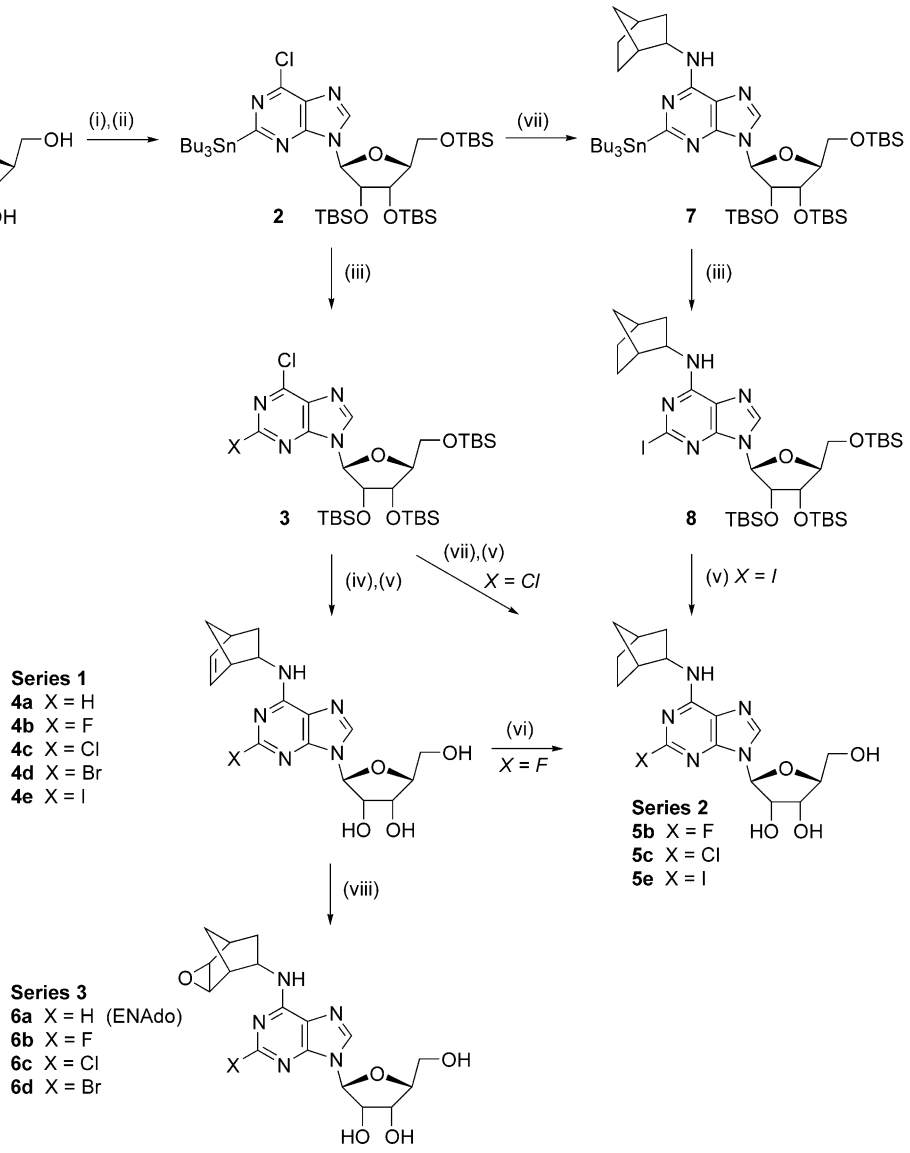
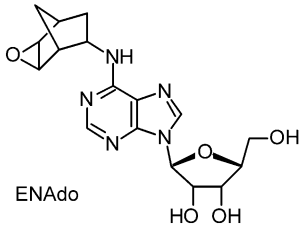


Scheme 1. (i) *t*-BDMSCl, imidazole, DMF; (ii) LTMP, Bu_3SnCl, THF; (iii) XeF_2, AgOTf, 2,6-di-*tert*-butyl-4-methylpyridine (for X = F), *N*-chlorosuccinimide, THF (for X = Cl), *N*-bromosuccinimide, THF (for X = Br) and I_2, THF (for X = I); (iv) 2*S*-*endo*-aminonorborn-2-ene hydrochloride (**9**), N*i*-Pr_2Et, *t*-BuOH; (v) NH_4F, MeOH; (vi) H_2, Pd/C, EtOH; (vii) 2*S*-*endo*-aminonorbornane hydrochloride (**10**), N*i*-Pr_2Et, *t*-BuOH; (viii) dimethyldioxirane, MeOH.

[5,6-epoxy-(1*S*,2*S*)-norborn-2-yl)xanthine was exposed to mildly acidic conditions.[9] In order to overcome this problem a range of ENAdo analogues were targeted which were devoid of the offending epoxide. These compounds possessed norborn-5-en-2-yl (series 1) or norborn-2-yl groups (series 2) in the N^6-position. Alternatively, adenosine analogues which still possessed a N^6-(5,6-epoxynorborn-2-yl) group, but were also substituted in the 2-position (series 3) were investigated. It was thought that the electron withdrawing effects and/or the steric bulk of a 2-halo group might reduce the reactivity of N1, thus limiting the degradative processes observed for ENAdo. The incorporation of small, polar substituents in the 2-position has also been shown to increase A_1 affinity and potency, though this effect appears to be somewhat dependant on the substituent pattern of the agonist.[10] In the present study, the affinity, functional potency and intrinsic activity of the new adenosine derivatives were determined for the A_1 and A_{2A}AR's, the most widely studied AR's in the cardiovascular system.



Results and Discussion

A range of adenosine agonists with halogens in the 2-position (fluoro, chloro, bromo and iodo) and norbornyl substituents in the N^6-position (norborn-5-en-2-yl, norborn-2-yl, 5,6-epoxynorborn-2-yl) were synthesised (Scheme 1). Adenosine analogues that bear substituents

in the 2- and N^6-positions can be prepared from 6-chloropurine riboside. 6-Chloropurine riboside (**1**) was either purchased (Sigma-Aldrich) or prepared in three steps from inosine.[11] Once obtained, the ribose hydroxyl groups of 6-chloropurine riboside were protected as *tert*-butyldimethylsilyl (TBS) ethers[12] and the 2-position of the purine was lithiated and stannylated using known procedures to afford compound **2**.[13] Substituents were then introduced into the 2-position by reacting TBS-protected 2-tributylstannylpurine riboside (**2**) with the appropriate electrophile; xenon difluoride for 2-fluoro, *N*-chlorosuccinimide for 2-chloro, *N*-bromosuccinimide for 2-bromo and iodine for 2-iodo (Scheme 1, compounds **3b**–**e**, respectively). Target compounds possessing an N^6-norborn-5-en-2-yl substituent (series 1) were then prepared from these synthetic intermediates in two steps. The N^6-substituent was introduced by reacting the TBS-protected 2-halo-6-chloropurine ribosine with 2*S*-*endo*-aminonorborn-2-ene hydrochloride (**9**) in the presence of *N,N*-diisopropylethylamine (Hünig's base). Deprotection using an excess of ammonium fluoride afforded the desired target compounds. Target compounds possessing an N^6-norborn-2-yl substituent (series 2) were prepared by one of three methods. 2-Fluoro-N^6-norborn-2-yladenosine (**5b**) was prepared simply by hydrogenating the alkene moiety of 2-fluoro-N^6-norborn-5-en-2-yl adenosine (**4b**). This approach proved unsuccesssful in the case of the 2-chloro due to concomitant reduction of the 2-halo moiety. Hydrogenation of the alkene moiety prior to incorporation of the N^6-substitutent overcame this problem and afforded 2-chloro-N^6-norborn-2-yladenosine (**5c**) in good yield. An alternative synthetic sequence was employed for 2-iodo-N^6-norborn-2-yladenosine (**5e**) in which the iodine was introduced later in the synthesis (just prior to deprotection). This modification was made to obtain a route suitable for the preparation of a ^{123}I labelled analogue for subsequent single photon emission computer tomography (SPECT) studies. This research is in progress and will be reported in due course. Finally, target compounds possessing an N^6-(5,6-epoxynorborn-2-yl) substituent (series 3) were prepared from the corresponding N^6-(norbor-5-en-2-yl)adenosines by treating them with dimethyldioxirane. This oxidant selectively forms the desired epoxide without oxidising N1 to the corresponding N1-oxide.[7] The order of the epoxidation and deprotection steps was not critical. The *tert*-butyldimethylsilyl protected 2-fluoro and 2-chloro-N^6-(norbor-5-en-2-yl)adenosines were first deprotected with ammonium fluoride prior to epoxidation with dimethyldioxirane, while this order was reversed in the synthesis of 2-bromo analogue.

The 2,N^6-substituted adenosine derivatives were tested for affinity by displacement of radioligand binding at the A_1AR in DDT cell membranes and the A_{2A}AR in PC-12 cell membranes. These assays were performed in the presence of 5′-guanylyl-imdodiphosphate to maintain the receptors in the agonist low affinity state, a condition which more closely emulates the environment of intact cells. In addition, the potency and intrinsic activity for each derivative was determined with respect to A_1AR mediated inhibition of (−)isoproterenol-stimulated cAMP accumulation in DDT cells and A_{2A}AR mediated stimulation of cAMP accumulation in PC-12 cells. As reported in Table 1, the affinity of the classical A_1AR agonist CPA is 4.7 nM. The 2-fluoro substituted N^6-norborn-5-en-2-yl (**4b**), N^6-norborn-2-yl (**5b**) and N^6-(5,6-epoxynorborn-2-yl) (**6b**) derivatives have 3.6- to 7.8-fold greater affinity for the A_1AR as compared to CPA. The 2-chloro and 2-bromo compounds from the N^6-norborn-5-en-2-yl series (**4c** and **4d**, respectively) and the 2-chloro derivative from the N^6-norborn-2-yl (**5c**) and N^6-(5,6-epoxynorborn-2-yl) (**6c**) series have affinities similar that that for CPA. However, the 2-unsubstituted N^6-norborn-5-en-2-yladenosine (**4a**), 2-bromo N^6-(5,6-epoxynorborn-2-yl) derivative (**6d**), 2-iodo N^6-norborn-5-en-2-yl (**4e**) and N^6-norborn-2-yl derivatives (**5e**) have affinities that are 3.2- to 10.3-fold lower as compared to CPA. The potency (IC_{50}) for derivatives **4a**, **4d**, **4e**, **5c**, and **6d** to inhibit (−)isoproterenol-stimulated cAMP accumulation was lower whereas the potency of **4b**, **5b**, **5c**, and **6b** was higher as compared to CPA. The IC_{50} values ranged from 1.7- (**4a**) to 21- (**5e**) fold lower as compared to their respective K_i values. All of the derivatives from each series showed the same intrinsic activity as CPA for mediating the inhibition of (−)isoproterenol-stimulated cAMP accumulation in DDT cells (Table 1).

The selective A_{2A}AR agonist CGS 21680 was used for comparison with the 2,N^6-disubstituted adenosine derivatives (Table 2). The affinity of CGS 21680 for the A_{2A}AR in PC-12 cell membranes is 0.17 μM whereas the potency (EC_{50}) for this agonist to stimulate cAMP accumulation in PC-12 cells is 0.02 μM. All of the derivatives from each N^6 series have lower affinity for the A_{2A}AR and lower potency (EC_{50}) for stimulating cAMP accumulation as compared to CGS 21680. The

Table 1. K_i, IC_{50} and intrinsic activity (IA) for adenosine derivatives at the A_1AR from DDT_1 MF-2 cells

Compd	$K_{i\ (nM)}$	$IC_{50\ (nM)}$	IA
CPA	4.7±1.0 (7)	0.99±0.11 (10)	1.00 (10)
4a	19.7±3.2 (3) *	11.6±2.7 (5)*	0.99±0.02 (5)
4b	0.9±0.3 (5)†	0.13±0.04(9)*	1.03±0.03 (9)
4c	3.9±0.9 (5)	0.62±0.23(9)	1.06±0.03 (9)
4d	7.0±0.7 (4)	3.6±1.1 (6)*	1.03±0.02 (6)
4e	21.8±3.1 (4) *	12.4±5.6 (7) *	1.07±0.05 (7)
5b	0.6±0.1 (3)‡	0.23±0.09 (3)†	0.99±0.04 (4)
5c	2.2±0.2 (3)	0.21±0.06 (5) *	1.05±0.05 (5)
5e	48.8±0.9 (3)*	2.3±1.0 (3)†	1.05±0.05 (4)
6b	1.3±0.2 (4)‡	0.4±0.05 (3) **	1.09±0.08 (4)
6c	4.4±0.6 (4)	0.56±0.28 (3)	1.08±0.08 (4)
6d	14.9±3.6 (4)†	2.9±0.13 (3) *	1.09±0.04 (4)

K_i values were determined from the concentration of the compounds that inhibited specific [^{3}H]CPX binding by 50% in DDT cell membranes. IC_{50} values are the concentration of compounds that produced half-maximal inhibition of 1 μM (−)isoproterenol-stimulated cAMP accumulation. Intrinsic activity (IA) is the maximal inhibition of (−)isoproterenol-stimulated cAMP accumulation as compared to the maximum effect of CPA which is set at 1.00. The inhibition of cAMP accumulation by 1 μM CPA and the other compounds was used for the IA calculation. This concentration produced the maximal inhibition as determined by the concentration-response curves. Numbers in parentheses are the N. The maximal CPA-mediated inhibition of (−)isoproterenol-stimulated cAMP accumulation was 84±2% ($n=10$). *$p<0.005$, †$p<0.01$, ‡$p<0.025$ and **$p<0.05$.

Table 2. K_i, EC_{50} and intrinsic activity (IA) for adenosine derivatives at the $A_{2A}AR$ from PC-12 cells

Compd	K_i (μM)	EC_{50} (μM)	IA
CGS 21680	0.17±0.03 (9)	0.02±.002 (10)	1.00 (10)
4a	65.5±11 (4)*	6.7±3.4 (4)*	0.89±0.1 (4)
4b	71.9±3.9(4) *	2.3±0.5 (3) *	0.98±0.06 (3)
4c	49.3±8.5 (6) *	2.7±0.7 (4) *	0.55±0.03 (4)*
4d	26.9±3.7(3)*	2.4±0.4 (5) *	0.24±0.03 (5)*
4e	11.1±0.5(4)*	4.0±1.3 (4) *	0.03±0.02 (4)*
5b	10.1±3.3 (4) *	2.2±0.4 (6) *	0.84±0.05 (6)†
5c	10.7±1.5 (5)*	1.3±0.2 (6) *	0.33±0.04 (6)*
5e	6.4±1.5 (4)*	ND (4)	ND (4)
6b	19.4±6.8 (5) *	3.9±0.9 (5)*	0.71±0.07 (5)‡
6c	101.5±11.6 (4)*	6.9±1.3 (4)*	0.55±0.03 (4)*
6d	129.3±19.3 (4)*	18.7±3.9 (5)*	0.34±0.04 (5)*

K_i values were determined from the concentration of the compounds that inhibited specific [^{3}H]ZM241385 binding by 50%. EC_{50} values are the concentration of compounds that stimulated cAMP accumulation at 50% of the maximum. Intrinsic activity (IA) is the maximal stimulation of cAMP accumulation as compared to the maximum for CGS 21680 which is set at 1.00. The stimulation of cAMP accumulation by 10 μM CGS 21680 and by 100 μM for the other compounds was used for the IA calculation. These concentrations produced the maximal stimulation as determined by the concentration–response curves. Numbers in parentheses are the N. The maximal CGS 21680-stimulated cAMP accumulation was 10,796±854 pmol/mg protein/10 min. *$p<0.005$, †$p<0.025$ and ‡$p<0.01$ compared to CGS 21680. ND, no detectable stimulation of cAMP accumulaion at concentrations up to 200 μM.

EC_{50} values ranged from 2.8- (**4e**) to 31- (**4b**) fold lower than their respective K_i values. The intrinsic activity of the 2-substituted N^6 derivatives was dependent upon the substituent in the 2 position. In the norbornene series, the 2-unsubstituted (**4a**) and 2-fluoro (**4b**) derivatives have intrinsic activity similar to CGS 21680 whereas the intrinsic activity was lower for the 2-chloro (**4c**), 2-bromo (**4d**) and 2-iodo (**4e**) derivatives. The intrinsic activity of the 2-fluoro (**5b**) and 2-chloro (**5c**) derivatives of the norbornane series were reduced compared to CGS 21680 and no agonist activity was detectable for the 2-iodo (**5e**) compound. All three of the 2-halogen substituted derivatives in the epoxynorbornane series had lower intrinsic activities than CGP 21680.

All of the N^6-norbornene, N^6-norborane and N^6-epoxynorborane derivatives showed high affinity and potency at the A_1AR with K_i and IC_{50} values in the sub to mid nanomolar range. Within this range, modest affinity changes were dependent upon the 2-halogen substitution. In the three N^6 series, the 2-fluoro derivatives showed the highest affinity, which was decreased by substitution with chloro, bromo or iodo. In contrast to the affinity changes, all of the derivatives showed the same intrinsic activity as CPA for the inhibition of (−)isoproterenol-stimulated cAMP accumulation in DDT cells. This suggests they are full agonists at the A_1AR. The affinity and potency of the derivatives were relatively weak for the $A_{2A}AR$ with K_i and EC_{50} values in the low to mid micromolar range. The IC_{50} and EC_{50} values for the derivatives at the A_1 and $A_{2A}AR$, respectively were lower than their K_i values. This discrepancy between the IC_{50} or EC_{50} and K_i values is common for agonists at guanine nucleotide binding protein coupled receptors and indicates that a significant receptor reserve exists for the compounds in altering cAMP accumulation in both cell types. Based upon binding affinity, all of the derivatives show high selectivity for the A_1AR over the $A_{2A}AR$. This A_1AR selectivity ranged from 131-fold for **5e** to 79,888-fold for **4b**. In contrast to the constant and high intrinsic activity at the A_1AR, the intrinsic activity of the derivatives at the $A_{2A}AR$ decreased (compared to CGS 21680) as a function of the 2-substituted halogen with the order of I > Br > Cl > F. Therefore, those derivatives with a reduced intrinsic activity appear to act as partial agonists at the $A_{2A}AR$. Previously, it was shown that 2-fluoroadenosine acted as a partial agonist at the $A_{2A}AR$ in PC-12 cells[14] and our data with the 2-fluoro and the other 2-halo substituted N^6-derivatives is consistent with and extends that report. Of particular interest was the observation that the iodo derivative **5e** had no detectable agonist activity at the $A_{2A}AR$. Thus, in terms of receptor subtype selectivity, this derivative has relatively high affinity and full agonist activity for the A_1AR while being devoid of agonist activity at the $A_{2A}AR$. These observations may provide leads for the development of additional selective and partial agonists for adenosine receptors. Although the focus of the present work was to characterize the interaction of the compounds with the A_1 and $A_{2A}AR$'s, additional studies will be needed to determine if the compounds have affinity for and functional effects mediated by the A_{2B} and A_3AR subtypes.

Experimental

Melting points were determined on a Reichert Hot-Stage microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian 300 Unity Plus spectrometer and chemical shifts are given in ppm (δ) relative to $CDCl_3$. High resolution ES-MS data (Bruker BioApex 47e FTMS with an Analytica Electrospray Source) was obtained for all novel compounds. Thin layer chromatography was carried out using 0.2 mm plates using Merck silica gel 60 F254. Column chromatography was achieved using Merck silica gel 60 (particle size 0.063–0.200 mm, 70–230 mesh). 6-Chloropurine riboside (**1**) was either purchased (Sigma-Aldrich) or prepared in three steps from inosine.[11] Once obtained, the ribose hydroxyl groups of 6-chloropurine riboside were protected as *tert*-butyldimethylsilyl ethers[12] and the 2-position of the purine was lithiated and stannylated[13]

using known procedures. 2*S*-*Endo*-norborn-5-en-2-yl amine (**9**) was also prepared by literature methods.[7]

General procedure for N^6-substitution

A mixture of 9-(2,3,5-tris-*O*-TBS-β-D-ribofuranosyl)-6-chloro-2-halopurine (1 molar equiv), *2S-endo*-aminonorborn-5-ene (1.2 equiv) and N(*i*-Pr)$_2$Et (3 equiv) were heated to reflux for 12–48 h in *t*-BuOH (4 mL). The reaction mixture was reduced in vacuo on SiO_2 and purified by column chromatography (petroleum ether/EtOAc, 10:1).

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-2-fluoro-N^6-(2*S*-*endo*-norborn-5-en-2-yl)adenosine (3b). The reaction of compound **2b** (115 mg, 0.18 mmol), *2S-endo*-aminonorborn-5-ene (37 mg, 0.25 mmol) and *N*(*i*-Pr)$_2$Et (0.15 mL, 0.86 mmol) afforded **3b** as a transparent solid (114 mg, 88%). Mp 51–53 °C. ^{1}H NMR ($CDCl_3$) δ −0.18, −0.02, 0.10, 0.11, 0.12, 0.13 (s, 3H, SiMe), 0.82, 0.93, 0.94 (s, 9H, *t*-Bu), 0.86–0.91 (m, 1H, H-3n″), 1.40 (d, 1H, H-7s″/H-7a″), 1.46 (d, 1H, H-7s″/H-7a″), 2.23–2.37 (m, 1H, H-3x″), 2.91 (br s, 1H, H-1″/H-4″), 3.22 (br s, 1H, H-1″/H-4″), 3.77 (dd, 1H, H-5a′/H-5b′), 4.03 (dd, 1H, H-5a′/H-5b′), 4.26 (t, 1H, H-4′), 4.34 (t, 1H, H-3′), 4.66 (t, 1H, H-2′), 4.84–4.90 (br m, 1H, H-2′), 5.79 (br d, 1H, NH), 5.89 (d, 1H, H-1′), 6.08–6.09 (m, 1H, H-5′/H-6′), 6.40–6.42 (m, 1H, H-5″/H-6″), 7.99 (br s, 1H, H-8).

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-2-chloro-N^6-(2*S*-*endo*-norborn-5-en-2-yl)adenosine (3c). The reaction of compound **2c** (175 mg, 0.26 mmol), *2S-endo*-aminonorborn-5-ene (58 mg, 0.40 mmol) and N(*i*-Pr)$_2$Et (0.2 mL, 1.1 mmol) afforded **3c** as a transparent viscous oil (205 mg, 95%). ^{1}H NMR ($CDCl_3$) δ −0.15, −0.01, 0.10, 0.11, 0.13, 0.14 (s, 3H, SiMe), 0.83, 0.93, 0.94 (s, 9H, *t*-Bu), 0.91–0.95 (m, 1H, H-3n″), 1.45 (d, 1H, H-7s″/H-7a″), 1.54 (d, 1H, H-7s″/H-7a″), 2.29–2.37 (m, 1H, H-3x″), 2.91 (br s, 1H, H-1″/H-4″;), 3.22 (br s, 1H, H-1″/H-4″), 3.77 (dd, 1H, H-5a′/H-5b′), 4.06 (dd 1H, H-5a′/H-5b′), 4.11 (t, 1H, H-4′), 4.31 (t, 1H, H-3′), 4.71 (t, 1H, H-2′), 4.92–4.98 (br m, 1H, H-2″), 5.88 (d, 1H, H-1′), 6.06–6.09 (m, 1H, H-5″/H-6″), 6.40–6.43 (m, 1H, H-5″/H-6″), 7.98 (br s, 1H, H-8).

2-Bromo-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)-N^6-(2*S*-*endo*-norborn-5-en-2-yl)-adenosine (3d). The reaction of compound **2d** (98 mg, 0.14 mmol), *2S-endo*-aminonorborn-5-ene (41 mg, 0.28 mmol) and N(*i*-Pr)$_2$Et (0.2 mL, 1.1 mmol) afforded **3d** as a transparent solid (95 mg, 87%). Mp 67–70 °C. ^{1}H NMR ($CDCl_3$) δ −0.16, −0.02, 0.10, 0.11, 0.12, 0.13 (s, 3H, SiMe), 0.83, 0.92, 0.94 (s, 9H, *t*-Bu), 0.88–0.93 (m, 1H, H-3n″), 1.44 (d, 2H, H-7s″, H-7a″), 1.53 (d, 2H, H-7s″, H-7a″), 2.30–2.36 (m, 1H, H-3x″), 2.90 (br s, 1H, H-4″), 3.21 (br s, 1H, H-1″), 3.76 (dd, 1H, H-5a′/H-5b′), 4.05 (dd, 1H, H-5a′/H-5b′), 4.10–4.13 (m, 1H, H-4′), 4.30 (t, 1H, H-3′), 4.72 (t, 1H, H-2′), 4.85–4.91 (br m, 1H, H-2″), 5.61 (br d, 1H, NH), 5.87 (d, 1H, H-1′), 6.05–6.08 (m, 1H, H-6″), 6.39–6.42 (m, 1H, H-5″), 7.97 (br s, 1H, H-8).

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-N^6-((2*S*)-*endo*-norborn-5-en-2-yl)-2-iodo-adenosine (3e). The reaction of compound **2e** (268 mg, 0.35 mmol), *2S-endo*-aminonorborn-5-ene (74 mg, 0.51 mmol) and *N*(*i*-Pr)$_2$Et (0.29 mL, 1.7 mmol) afforded **3e** as a transparent solid (246 mg, 84%). Mp 71–73 °C. ^{1}H NMR ($CDCl_3$) δ −0.13, −0.01, 0.10, 0.11, 0.12, 0.13 (s, 3H, SiMe), 0.83, 0.92, 0.94 (s, 9H, *t*-Bu), 0.89–0.93 (m, 1H, H-3n″), 1.44 (d, 1H, H-7s″/H-7a″), 1.52 (d, 1H, H-7s″/ H-7a″), 2.32–2.36 (m, 1H, H-3x″), 2.89 (br s, 1H, H-4″), 3.20 (br s, 1H, H-1″), 3.76 (dd, 1H, H-5a′/H-5b′), 4.04 (dd, 1H, H-5a′/H-5b′), 4.08–4.13 (m, 1H, H-4′), 4.30 (t, 1H, H-3′), 4.73 (t, 1H, H-2′), 4.85 (br m, 1H, H-2″), 5.41 (br d, 1H, NH), 5.84 (d, 1H, H-1′), 6.04–6.06 (m, 1H, H-6″), 6.38–6.41 (m, 1H, H-5″), 7.90 (br s, 1H, H-8).

General procedure for TBS deprotection

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-2-halo-N^6-(2*S*-*endo*-norborn-5-en-2-yl)adenosine and ammonium fluoride (10–30 equiv) were heated to ∼60 °C in dry methanol (5 mL) for 17–70 h. The reaction mixture was then packed on SiO_2 and purified by column chromatography ($CHCl_3$/MeOH, 95:5).

2-Fluoro-N^6-(2*S*-*endo*-norborn-5-en-2-yl)adenosine (4b). Compound **3b** (32 mg, 0.044 mmol) was reacted with ammonium fluoride (71 mg, 1.9 mmol) to afford the title compound **4b** as a white solid (14 mg, 83%). Mp 114–115 °C. ^{1}H NMR ($CDCl_3$) δ 0.88–0.92 (m, 1H, H-3n″), 1.40 (d, 2H, H-7a″), 1.53 (d, 2H, H-7s″), 2.28–2.32 (m, 1H, H-3x″), 2.92 (br s, 1H, H-4″), 3.18 (br s, 1H, H-1″), 3.73 (d, 1H, H-5a′/H-5b′), 3.91 (d, 1H, H-5a′/H-5b′), 4.24 (br s, 1H, H-4′), 4.42 (br d, 1H, H-3′), 4.72 (br m, 1H, H-2″), 4.84 (t, 1H, H-2′), 5.77 (d, 1H, H-1′), 6.01 (m, 1H, H-6′), 6.09 (br d, 1H, OH-3′), 6.41 (m, 1H, H-5′), 7.89 (br s, 1H, H-8). ES-MS calcd 378.1577 (M + H). Found 378.1564.

2-Chloro-N^6-(2*S*-*endo*-norborn-5-en-2-yl)adenosine (4c). Compound **3c** (122 mg, 0.17 mmol) was reacted with ammonium fluoride (165 mg, 4.45 mmol) to afford the title compound **4c** as a white solid (58 mg, 83%); mp 126–128 °C. ^{1}H NMR ($CDCl_3$) δ 0.84–0.93 (m, 1H, H-3n″), 1.41 (d, 2H, H-7a″), 1.53 (d, 2H, H-7s″), 2.07–2.34 (m, 1H, H-3x″), 2.92 (br s, 1H, H-4″), 3.20 (br s, 1H, H-1″), 3.71 (d, 1H, H-5a′/H-5b′), 3.91 (d, 1H, H-5a′/H-5b′), 4.26 (br s, 1H, H-4′), 4.37 (br d, 1H, H-3′), 4.76–4.80 (br m, 1H, H-2″), 4.93 (t, 1H, H-2′), 5.75 (d, 1H, H-1′), 5.97–6.00 (m, 1H, H-6″), 6.39–6.42 (m, 1H, H-5″), 7.81 (br s, 1H, H-8). ES-MS calcd 394.1282 (M + H). Found 394.1268.

N^6-(*endo*-Norborn-5-en-2-yl)-2-bromoadenosine (4d). Compound **3d** (192 mg, 0.25 mmol) was reacted with ammonium fluoride (92 mg, 2.5 mmol) to afford the title compound **4d** as a white solid (69 mg, 63%). Mp 130–131 °C. ^{1}H NMR ($CDCl_3$) δ 0.87–0.91 (m, 1H, H-3n″), 1.40 (d, 1H, H-7a″), 1.52 (d, 1H, H-7s″), 2.26–2.32 (m, 1H, H-3x″), 2.90 (br s, 1H, H-4″), 3.20 (br s, 1H, H-1″), 3.69 (dd, 1H, H-5a′/H-5b′), 3.89 (dd, 1H, H-5a′/H-5b′), 4.23 (br s, 1H, H-4′), 4.36 (br d, 1H, H-3′), 4.74–4.78 (br m, 1H, H-2″), 4.89 (t, 1H, H-2′), 5.74 (d, 1H, H-1′), 5.97–5.99 (m, 1H, H-6″), 6.05 (d, 1H, NH), 6.37–6.39 (m, 1H, H-5″), 7.81 (br s, 1H, H-8). ES-MS calcd 438.0777 (M + H). Found 438.0760.

N^6-(*endo*-Norborn-5-en-2-yl)-2-iodoadenosine (4e). Compound **3e** (123 mg, 0.14 mmol) was reacted with ammonium fluoride (120 mg, 3.2 mmol) to afford the title compound **4e** as a white solid (68 mg, 100%). Mp 144–146 °C. ^{1}H NMR ($CDCl_3$) δ 0.90–0.96 (m, 1H, H-3n″), 1.43 (d, 1H, H-7a″), 1.53 (d, 1H, H-7s″), 2.30–2.38 (m, 1H, H-3x″), 2.93 (br s, 1H, H-4″), 3.21 (br s, 1H, H-1″), 3.73 (d, 1H, H-5a′/H-5b′), 3.92 (d, 1H, H-5a′/H-5b′), 4.29 (br s, 1H, H-4′), 4.36 (br d, 1H, H-3′), 4.72–4.79 (br m, 1H, H-2″), 4.95 (t, 1H, H-2′), 5.73 (d, 1H, H-1′), 5.88 (d, 1H, NH), 6.00–6.04 (m, 1H, H-6″), 6.41–6.43 (m, 1H, H-5″), 7.67 (br s, 1H, H-8). ES-MS calcd 486.0638 (M + H). Found 486.0616.

2-Fluoro-N^6-(2*S*-*endo*-norborn-2-yl)adenosine (5b). Compound **4b** (9 mg, 0.024 mmol) was dissolved in MeOH (5 mL) and stirred for 22 h with 10% Pd/C under a H_2 atmosphere. Filtration through filter aid followed by evaporation of the solvent in vacuo afforded the desired product **5b** (7 mg, 77%) as a white solid. Mp 102–104 °C. ^{1}H NMR ($CDCl_3$) δ 0.86–0.99 (m, 1H, H-3n″), 1.18–1.58 (m, 6H, H-5″, H-6″, H-7s″, H-7a″), 2.10–2.19 (m, 1H, H-3x″), 2.27 (br s, 1H, H-4″), 2.58 (br s, 1H, H-1″), 3.77 (d, 1H, H-5a′/H-5b′), 3.91 (d, 1H, H-5a′/H-5b′), 4.26–4.37 (br m, 2H, H-2″, H-4′), 4.47 (br s, 1H, H-3′), 4.88 (br s, 1H, H-2′), 5.82 (br s, 1H, H-1′), 7.79 (br s, 1H, H-8). ES-MS calcd 380.1734 (M + H). Found 380.1738.

2-Chloro-N^6-(2*S*-*endo*-norborn-2-yl)adenosine (5c). The reaction of compound **3c** (124 mg, 0.19 mmol), 2*S*-*endo*-aminonorbornane hydrochloride (36 mg, 0.24 mmol) and $N(i\text{-Pr})_2$Et (0.2 mL, 1.1 mmol) afforded a transparent viscous oil (134 mg, 95%). ^{1}H NMR ($CDCl_3$) δ −0.16, −0.02, 0.09, 0.10, 0.12, 0.13 (s, 3H, SiMe), 0.82, 0.92, 0.94 (s, 9H, *t*-Bu), 1.11–1.67 (m, 7H, H-3n″, H-5″, H-6″, H-7″), 2.12–2.25 (m, 1H, H-3x″), 2.26 (br s, 1H, H-4″), 2.64 (br s, 1H, H-1″), 3.76, (dd, 1H, H-5a′/H-5b′), 4.06 (dd, 1H, H-5a′/H-5b′), 4.10 (t, 1H, H-4′), 4.32 (t, 1H, H-3′), 4.47 (br s, 1H, H-2″), 4.72 (t, 1H, H-2′), 5.89 (d, 1H, H-1′), 6.10 (br s, 1H, NH), 8.04 (br s, 1H, H-8).

2′,3′,5′-Tris-*O*-(*tert*-butyldimethylsilyl)-N^6-((2*S*)-*endo*-norborn-2-yl)-2-chloroadenosine (132 mg, 0.18 mmol) was reacted with ammonium fluoride (71 mg, 1.9 mmol) to afford a white solid (66 mg, 93%). Mp 141–142 °C. ^{1}H NMR ($CDCl_3$) δ 0.91–0.97 (m, 1H, H-3n″), 1.25–1.50 (m, 6H, H-5″, H-6″, H-7″), 2.08–2.20 (m, 1H, H-3x″), 2.26 (br s, 1H, H-4″), 2.60 (br s, 1H, H-1″), 3.72 (d, 1H, H-5a′/H-5b′), 3.91 (d, 1H, H-5a′/H-5b′), 4.26 (br s, 1H, H-4′), 4.29–4.34 (br m, 1H, H-2″), 4.34 (br s, 1H, H-3′), 4.94–4.96 (m, 1H, H-2′), 5.79 (d, 1H, H-1′), 6.57 (d, 1H, OH-3′), 7.88 (br s, 1H, H-8). ES-MS calcd 396.1439 (M + H). Found 396.1434.

2-Iodo-N^6-(*endo*-norborn-2-yl)adenosine (5d). 2′,3′,5′-Tris-*O*-TBS-N^6-((2*S*)-*endo*-norborn-2-yl)-2-iodo-adenosine (**8**, 85 mg, 0.10 mmol) and NH_4F (97 mg, 2.62 mmol) were heated to ∼60 °C in MeOH (4.5 mL) for 10 h. The reaction mixture was then packed on SiO_2 and purified by column chromatography ($CHCl_3$/MeOH, 95:5) to yield **5d** (42 mg, 84%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.94–0.98 (m, 1H, H-3n′), 1.21–2.17 (m, 6H, H-3″, H-5″, H-6″, H-7″), 2.28 (br s, 1H, H-4″), 2.62 (br s, 1H, H-1″), 3.72-3.94 (m, 3H, H-4′, H-5′), 4.30 (br m, 1H, H-2′), 4.37 (br s, 1H, H-3′), 4.92 (br s, 1H, H-2′), 5.51 (br s, 1H, NH), 5.77 (d, 1H, H-1′), 6.32 (d. 1H, OH), 6.38 (d, 1H, OH), 7.75 (br s, 1H, H-8). ES-MS calcd 488.0795 (M + H). Found 488.0790.

General procedure for epoxidation

Dimethyldioxirane (∼0.1 M, 2 equiv) was added dropwise to a solution of the N^6-(norborn-5-en-yl)adenosine in MeOH (4 mL) at 0 C. After 2 h an additional 0.25 equiv of dimethyldioxirane was added and stirring was continued for 10 min. The solvent was removed in vacuo and the crude product purified by column chromatography ($CHCl_3$/MeOH, 95:5).

(2*S*)-N^6-(*endo*-5,6-Epoxynorborn-2-yl)-2-fluoro-adenosine (6b). Compound **4b** (23 mg, 0.061 mmol) was treated with dimethyldioxirane (∼0.1 M, 1.2 mL + 0.4 mL) to afford a white solid (19 mg, 80%). Mp 198–201 °C (dec). ^{1}H NMR ($CDCl_3$) δ 0.88 (dd, 1H, H-7a′), 1.11–1.22 (m, 1H, H-3n″), 1.35 (dd, 1H, H-7s″), 2.12–2.22 (m, 1H, H-3x″), 2.53 (br s, 1H, H-4″), 2.93 (br s, 1H, H-1″), 3.30 (d, 1H, H-5″), 3.33 (d, 1H, H-6″), 3.69 (d, 1H, H-5a′/H-5b′), 4.18 (d, 1H, H-5a′/H-5b′), 4.26 (d, 1H, H-4′), 4.29 (dd, 1H, H-3′), 4.49–4.53 (m, 1H, H-2″), 4.64 (t, 1H, H-2′), 5.72 (d, 1H, H-1′), 7.87 (br s, 1H, H-8). ES-MS calcd 416.1346 (M + Na). Found 416.1345.

(2*S*)-N^6-(*endo*-5,6-Epoxynorborn-2-yl)-2-chloroadenosine (6c). Compound **4c** (58 mg, 0.15 mmol) was treated with dimethyldioxirane (∼ 0.1M, 3.7 mL) to afford a white solid (48 mg, 78%). Mp 199–201 °C dec. ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 2H, H-7a″), 1.07–1.19 (m, 1H, H-3n″), 1.33 (d, 1H, H-7s″), 2.12–2.21 (m, 1H, H-3x″), 2.51 (br s, 1H, H-4″), 2.88 (br s, 1H, H-1″), 3.27 (d, 1H, H-5″), 3.35 (d, 1H, H-6″), 3.68 (d, 1H, H-5a′/H-5b′), 3.89 (d, 1H, H-5a′/H-5b′), 4.17 (br s, 1H, H-4′), 4.27 (d, 1H, H-3′), 4.47–4.52 (br m, 1H, H-2″), 4.66 (t, 1H, H-2′), 5.71 (d, 1H, H-1′), 7.27 (d, 1H, NH), 7.87 (br s, 1H, H-8). ES-MS calcd 410.1231 (M + H). Found 410.1229.

(2*S*)-N^6-(*endo*-5,6-Epoxynorborn-2-yl)-2-bromoadenosine (6d). Compound **3d** (92 mg, 0.12 mmol) was dissolved in methanol (6 mL) and cooled to 0 °C before being treated with dimethyldioxirane (∼0.1 M, 2.3 mL). The reaction was stirred at this temperature for 2.5 h then a further 23 h at room temperature. The solvent was removed in vacuo and the crude product purified by column chromatography (petroleum ether/EtOAc, 4:1) to yield TBS-protected (2*S*)-N^6-(*endo*-5,6-epoxynorborn-2-yl)-2-bromoadenosine (88 mg, 92%); mp 48–51 °C. ^{1}H NMR ($CDCl_3$) δ −0.15, −0.01, 0.92, 0.10, 0.13, 0.14, (s, 3H, SiMe), 0.83, 0.92, 0.94 (s, 9H, *t*-Bu), 0.94 (d, 1H, H-7a″), 1.22–1.27 (m, 1H, H-3n″), 1.39 (d, 1H, H-7s″), 2.19-2.29 (m, 1H, H-3x"), 2.55 (br s, 1H, H-4″), 2.99 (br s, 1H, H-1″), 3.27 (d, 1H, H-5″), 3.31 (d, 1H, H-6″), 3.77 (dd, 1H, H-5a′/H-5b′), 4.04 (dd, 1H, H-5a′/H-5b′), 4.10 (d, 1H, H-4′), 4.30 (t, 1H, H-3′), 4.56–4.62 (m, 1H, H-2″), 4.69 (t, 1H, H-2′), 5.89 (d, 1H, H-1′), 8.04 (br s, 1H, H-8).

TBS-protected (2*S*)-N^6-(*endo*-5,6-epoxynorborn-2-yl)-2-bromoadenosine (83 mg, 0.10 mmol) was treated with ammonium fluoride (61 mg, 1.6 mmol) to afford the title compound **6d** (15 mg) in 33% yield. Mp 197–200 °C dec. ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 1H, H-7a″), 1.09–1.40 (m, 1H, H-3n″), 1.41 (d, 1H, H-7s″), 2.15–2.24 (m, 1H, H-3x″), 2.54 (d, 1H, H-4″), 2.93 (d, 1H, H-1″), 3.29 (d, 1H, H-5″), 3.32 (d, 1H, H-6″), 3.72 (dd, 1H, H-5a′/H-5b′), 3.94 (dd, 1H, H-5a′/H-5b′), 4.22 (d, 1H, H-4′), 4.29 (dd, 1H, H-3′), 4.51–4.55 (m, 1H, H-2″), 4.70 (t, 1H, H-2′), 5.73 (d, 1H, H-1′), 7.87 (br s, 1H, H-8). ES-MS calcd 454.0726 (M + H). Found 454.0722.

2′,3′,5′-Tris-*O*-TBS-N^6-((2*S*)-*endo*-norborn-2-yl)-2-tributyltinadenosine (7). A mixture of the 6-chloro-2-tributyltin-9-(2,3,5-tris-*O*-TBDMS-β-D-ribofuranosyl)purine (**2**, 638 mg, 0.69 mmol), (2*S*)-*endo*-norborn-2-yl amine (138 mg, 0.93 mmol) and $N(i\text{-Pr})_2Et$ (0.50 mL, 2.87 mmol) was heated to reflux in *t*-BuOH (10 mL) for 23 h. The reaction mixture was reduced in vacuo on SiO_2 and purified by column chromatography (hexane/EtOAc, 5:1) to afford 2′,3′,5′-Tris-*O*-TBS-N^6-((2*S*)-*endo*-norborn-2-yl)-2-tributyltinadenosine (280 mg, 41%) as a transparent solid (mp 47–48 °C). ^{1}H NMR ($CDCl_3$) δ −0.05, 0.01, 0.09, 0.10, 0.14, 0.15 (s, 3H, SiMe), 0.84, 0.93, 0.96 (s, 9H, *t*-Bu), 1.02–2.18 (m, H3″, 5″, 6″, 7″, Bu_3Sn), 2.26 (s, 1H, H-4″), 2.67 (s, 1H, H-1″), 3.78–3.83 (m, 1H, H5a′), 4.02–4.31 (m, 2H, H4′, 5b′), 4.33 (t, 1H, H3′), 4.51 (br s, 1H, H2″), 4.64 (t, 1H, H2′), 5.91 (br s, 1H, NH), 6.03 (d, 1H, H1′), 8.09 (br s, 1H, H-8).

2′,3′,5′-Tris-*O*-TBS-N^6-((2*S*)-*endo*-norborn-2-yl)-2-iodoadenosine (8). 2′,3′,5′-Tris-*O*-TBS-N^6-((2*S*)-*endo*-norborn-2-yl)-2-tributyltinadenosine (**7**, 110 mg, 0.11 mmol) and iodine were stirred in anhydrous THF for 5 h at room temperature. The reaction mixture was diluted with 5% NaS_2O_3 until the colour disappeared and the product was extracted with CH_2Cl_2 (3×20 mL) and washed with brine (15 mL). Column chromatography using hexane/ethyl acetate (15:1) as an eluent to afforded pure 2′,3′,5′-tris-*O*-TBS-N^6-((2*S*)-*endo*-norborn-2-yl)-2-iodo-adenosine (67 mg, 73%) (mp 69–71 °C). ^{1}H NMR ($CDCl_3$) δ −0.13, −0.01, 0.10, 0.11, 0.12, 0.13 (s, 3H, SiMe), 0.83, 0.92, 0.94 (s, 9H, Bu^t), 0.89–0.93 (m, 1H, H-3n″), 1.44 (d, 1H, H-7s″/H-7a″), 1.52 (d, 1H, H-7s″/H-7a″), 2.32–2.36 (m, 1H, H-3x″), 2.89 (br s, 1H, H-4″), 3.20 (br s, 1H, H-1″), 3.76 (dd, 1H, H-5a′/H-5b′), 4.04 (dd, 1H, H-5a′/H-5b′), 4.08–4.13 (m, 1H, H-4′), 4.30 (t, 1H, H-3), 4.73 (t, 1H, H-2′), 4.85 (br m, 1H, H-2″), 5.41 (br d, 1H, NH), 5.84 (d, 1H, H-1′), 6.04–6.06 (m, 1H, H-6″), 6.38–6.41 (m, 1H, H-5″), 7.90 (br s, 1H, H-8).

2*S*-*endo*-Aminonorbornane hydrochloride (10). 2*S*-*endo*-Aminonorborn-5-ene hydrochloride (**7**, 39 mg, 0.27 mmol) was dissolved in MeOH (2 mL) and stirred for 17 h with 10% Pd/C under a H_2 atmosphere. Filtration through Celite followed by concentration in vacuo afforded the title compound **8** (36 mg, 92%) as a white solid (mp 169–172 °C). ^{1}H NMR (CD_3OD) δ 1.05–1.10 (m, 1H, H-3n″), 1.41–1.67 (m, 6H, H-5″, H-6″, H-7″), 2.02–2.10 (m, 1H, H-3x″), 2.31 (br s, 1H, H-4″), 2.49 (br s, 1H, H-1″), 3.52 (dt, 1H, H-2″). ^{13}C NMR ($CDCl_3$) δ 22.0, 30.0, 35.8, 38.0, 39.3, 41.1, 53.2.

Drug solutions

All adeonsine derivatives were dissolved in DMSO at a concentration of 10 mM and on the day of use were diluted with Hank's balanced salt solution (HBSS, for cAMP assays) or 50 mM Tris–HCl buffer at pH 7.4 (for receptor assays). Control incubations contained the same final concentration of DMSO.

Cell culture and membrane preparation. Hamster smooth muscle DDT_1 MF-2 (DDT) and rat pheochromocytoma PC-12 cells were grown as monolayers on 100 cm plastic Petri dishes as described previously.[15] Cells were subcultured twice weekly and used for experiments at one day preconfluence. For membrane preparation, attached cells were rinsed twice with 10 mL of HBSS followed by the addition of 5 mL of 50 mM Tris–HCl at pH 7.4 containing 5 mM $MgCl_2$ (Tris–Mg). The cell were scrapped free of the dish using a rubber policeman and the suspension was centrifuged for 10 min at 48,000*g*. The pellet was resuspended in ice-cold Tris–Mg and centrifuged again as above. The final pellet was resuspended in an equal volume of Tris–Mg for assays. The protein content of the membranes was determined by the Bradford method[16] using bovine serum albumin as standard.

Receptor and cAMP assays

The displacement of specific [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine (2.5 nM) binding from the A_1AR in DDT cell membranes and [^{3}H]ZM241385 (1.5 nM) binding to the A_{2A}AR in PC-12 membranes by the compounds was determined as previously reported.[17] These displacement assays were performed in the presence of 100 μM 5′-guanylyl-imdodiphosphate to keep the receptor in the agonist low affinity state. For each compound, 8–10 concentrations were used to obtain complete displacement curves for analysis.

The stimulation of cAMP accumulation content of DDT and PC-12 cells was determined by washing monolayers of DDT or PC-12 cells twice with 10 mL each of HBSS and detaching the cells by gentle scraping with a rubber scraper in 5 mL of HBSS. The cell suspension was then centrifuged at 500*g* for 5 min and the cell pellet was gently resuspended in an equal volume of HBSS. Aliquots of the cell suspension were then incubated in HBSS (0.5 mL total volume) containing 50 μM rolipram (to inhibit phosphodiesterase activity) and other drugs for 10 min at 37 °C. At the end of the incubation, the suspensions were placed in a boiling water bath for 5 min, cooled to room temperature and centrifuged at 13,000*g* for 10 min. The cAMP content of the supernatant was determined by radioimmunoassay as described in detail previously.[18] Using DDT cells, the A_1AR mediated inhibition of (−)isoproterenol (1 μM) stimulated cAMP accumulation was determined. Typically, 7–9 concentrations of each compound were employed to obtain full concentration-response relationships. In PC-12 cells, 7–9 concentrations of each compound were used to obtain complete concentration–response relationships for A_{2A}AR mediated stimulation of cAMP accumulation.

Data analysis

The concentration of compounds that inhibited specific radioligand binding by 50% (IC_{50}) was determined by nonlinear regression analysis using the GraphPad Inplot program (GraphPad Software, San Diego, CA, USA). The dissociation constant (K_i) for the compounds were calculated from the IC_{50} using the conversion described by Cheng and Prusoff.[19] The concentration of compounds that inhibited (IC_{50}) or stimulated (EC_{50}) cAMP accumulation by 50% and the maximal response (plateau region) were determined by nonlinear regression analysis of the concentration–response for cAMP accumulation using the GraphPad software. The intrinsic activity is expressed as a fraction of the maximal response for each compound compared to the maximal determined for the standard compounds CPA (A_1AR response in DDT cells) and CGS 21680 (A_{2A}AR response in PC-12 cells). Statistical analysis of the data was performed using the Student's *t*-test and differences were considered significant if $p < 0.05$.

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