2-Substitution of N^6-Benzyladenosine-5′-uronamides Enhances Selectivity for A_3 Adenosine Receptors

Hea O. Kim,† Xiao-duo Ji,† Suhaib M. Siddiqi,† Mark E. Olah,‡ Gary L. Stiles,‡ and Kenneth A. Jacobson*,†

Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, and Departments of Medicine and Pharamacology, Duke University Medical Center, Durham, North Carolina 27710

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Adenosine derivatives bearing an N^6-(3-iodobenzyl) group, reported to enhance the affinity of adenosine-5′-uronamide analogues as agonists at A_3 adenosine receptors (*J. Med. Chem.* **1994**, *37*, 636–646), were synthesized starting from methyl β-D-ribofuranoside in 10 steps. Binding affinities at A_1 and A_{2a} receptors in rat brain membranes and at cloned rat A_3 receptors from stably transfected CHO cells were compared. N^6-(3-Iodobenzyl)adenosine was 2-fold selective for A_3 vs A_1 or A_{2a} receptors; thus it is the first monosubstituted adenosine analogue having any A_3 selectivity. The effects of 2-substitution in combination with modifications at the N^6- and 5′-positions were explored. 2-Chloro-N^6-(3-iodobenzyl)adenosine had a K_i value of 1.4 nM and moderate selectivity for A_3 receptors. 2-Chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide, which displayed a K_i value of 0.33 nM, was selective for A_3 vs A_1 and A_{2a} receptors by 2500- and 1400-fold, respectively. It was 46,000-fold selective for A_3 receptors vs the Na^+-independent adenosine transporter, as indicated in displacement of [^{3}H]N^6-(4-nitrobenzyl)-thioinosine binding in rat brain membranes. In a functional assay in CHO cells, it inhibited adenylate cyclase via rat A_3 receptors with an IC_{50} of 67 nM. 2-(Methylthio)-N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide and 2-(methylamino)-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide were less potent, but nearly as selective for A_3 receptors. Thus, 2-substitution (both small and sterically bulky) is well-tolerated at A_3 receptors, and its A_3 affinity-enhancing effects are additive with effects of uronamides at the 5′-position and a 3-iodobenzyl group at the N^6-position.

Introduction

The novel A_3 adenosine receptor[1] may be important in the regulation of CNS, cardiac, inflammatory, and reproductive functions. The expression of A_3 adenosine receptors in normal vs asthmatic lung tissue has been studied.[2] The A_3 adenosine receptor was cloned from rat brain and rat testes cDNA libraries.[1,3] Its activation stimulates phosphatidylinositol metabolism in antigen-exposed mast cells[4] and inhibits adenylate cyclase in transfected CHO cells.[1] Activation of A_3 receptors enhances the release of inflammatory mediators from mast cells,[4,5] lowers blood pressure,[6] and depresses locomotor activity.[7] A cerebroprotective effect of chronic administration of an A_3 agonist has been discovered.[8] The activation of A_3 receptors is also thought to be related to the cardioprotective preconditioning response following exposure to adenosine agonists.[9]

The structure-activity relationships of adenosine derivatives and xanthine derivatives at the rat A_3 versus A_1 and A_{2a} receptors have been explored.[10,11] The affinity of various ligands at sheep[12] and human[13] A_3 receptors has been reported to be very different from rat. At rat A_3 receptors, most xanthines known to bind to A_1 and A_2 receptors do not act as antagonists.[1,10]

We recently reported new adenosine agonist derivatives of moderate A_3 selectivity.[7,10,11] The 5′-methyluronamide modification of adenosine and the N^6-benzyl group, either alone or in combination, increases affinity in binding to A_3 receptors relative to A_1 and A_{2a} receptors.[10] Optimization of substituent groups has led to the development of the highly potent A_3 agonist N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA, **1**, Figure 1) which is 50-fold selective for A_3 vs either A_1 or A_2 receptors. A closely related, but less selective radioligand, [^{125}I]AB-MECA, **2**, was developed for characterization of A_3 receptors and found to have a K_d value of 3.6 nM in binding to rat A_3 receptors in the RBL-2H3 mast cell line.[14]

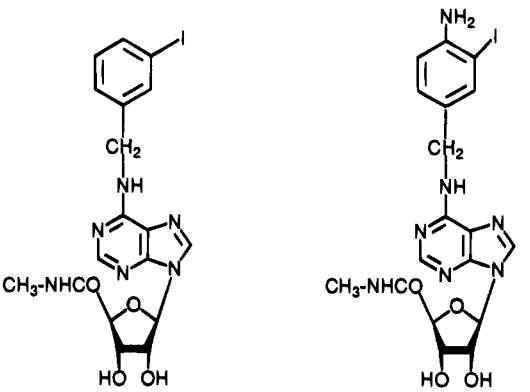

Figure 1. Structures of high-affinity A_3 receptor agonists.

In this study we have extended our previous development of A_3 selective agonists. By combining the two modifications at 5′- and N^6-positions, which were found earlier to result in moderate selectivity, with a third site of modification, the 2-position, we have dramatically

* Address correspondence to: Dr. Kenneth A. Jacobson, Bldg. 8A, Rm. B1A-17, NIDDK/NIH, Bethesda, MD 20892. Tel. (301) 496-9024. FAX (301) 402-0008.
† NIH.
‡ Duke University Medical Center.
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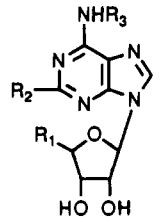
Table 1. Affinities of 5'-Uronamide Derivatives in Radioligand Binding Assays at Rat Brain A_1, A_{2a}, and A_3 Receptors[a-c]



compd	R_1	R_2	R_3	K_i (nM) A_1[a]	A_{2a}[b]	A_3[c]	A_1/A_3	A_{2a}/A_3
1[d]	CH_3NHCO	H	3-I-Bz	54	56	1.1	49	51
2[d]	CH_3NHCO	H	3-I-4-NH_2Bz	18	197	1.3	14	160
3	$HOCH_2$	H	4-$NH_2Ph(CH_2)_2$	14[f]	172 ± 50	116 ± 18	0.16	1.5
4	$HOCH_2$	H	3-I-4-$NH_2Ph(CH_2)_2$	2.1[f]		15.5[g]	0.14	
5[e]	C_2H_5NHCO	$NH(CH_2)_2$-*p*-Ph-$(CH_2)_2COOH$	H	2600	15	584	4.4	0.026
6	C_2H_5NHCO	$NH(CH_2)_2$-*p*-Ph-$(CH_2)_2CONH(CH_2)_2NH_2$	H	400	5.7	50 ± 24	8	0.11
7[e]	$HOCH_2$	Cl	H	9.3	63	1890	0.0049	0.033
8[d]	$HOCH_2$	H	cyclopentyl	0.59	462	240	0.0025	1.9
9[d]	$HOCH_2$	Cl	cyclopentyl	0.6	950	237	0.0025	4.0
10	$HOCH_2$	H	3-I-Bz	20.0 ± 8.5	17.5 ± 0.5	9.5 ± 1.4	2.1	1.8
11	$HOCH_2$	Cl	3-I-Bz	18.5 ± 4.7	38.5 ± 2.0	1.41 ± 0.17	13	27
12	$HOCH_2$	NH_2	3-I-Bz	63.8 ± 15.1	117 ± 15	181 ± 30	0.35	0.65
13	CH_3NHCO	Cl	3-I-Bz	820 ± 570	470 ± 365	0.33 ± 0.08	2500	1400
14	CH_3NHCO	CH_3NH	3-I-Bz	4890 ± 2580	4120 ± 210	3.12 ± 0.64	1600	1300
15	CH_3NHCO	CH_3S	3-I-Bz	2140 ± 100	3210 ± 1360	2.30 ± 0.96	930	1400

[a] Displacement of specific [^{3}H]PIA binding, unless noted, in rat brain membranes expressed as K_i ± SEM in nM (n = 3–6). [b] Displacement of specific [^{3}H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as K_i ± SEM in nM (n = 3–6). [c] Displacement of specific binding of N^6-[[^{125}I]-4-amino-3-iodobenzyl]adenosine-5'-*N*-methyluronamide[14] from membranes of CHO cells stably transfected with the rat A_3–cDNA, expressed as K_i ± SEM in nM (n = 3–7). [d] Values are from Gallo-Rodriguez et al.[11] [e] Values are from van Galen et al.[10] A_3 affinity measured by displacement of specific binding of [^{125}I]APNEA in membranes of CHO cells stably transfected with the rat A_3–cDNA.[1] K_i values at A_1 receptors are vs specific binding of [^{3}H]–N^6–cyclohexyladenosine or [^{3}H]R-PIA. K_i values at A_{2a} receptors are vs specific binding of [^{3}H]NECA in the presence of 50 nM N^6-cyclopentyladenosine or vs specific binding of [^{3}H]CGS 21680 in rat striatal membranes. [f] IC_{50} values (nM) vs displacement of specific binding of [^{125}I]APNEA in rat brain membranes.[23] [g] K_d value (nM) from saturation of binding of [^{125}I]APNEA in membranes of CHO cells stably transfected with the rat A_3–cDNA.[1]

increased selectivity. This study presents the first compounds that combine very high potency and selectivity, which should make them very useful as pharmacological tools and potential therapeutic agents.

Results

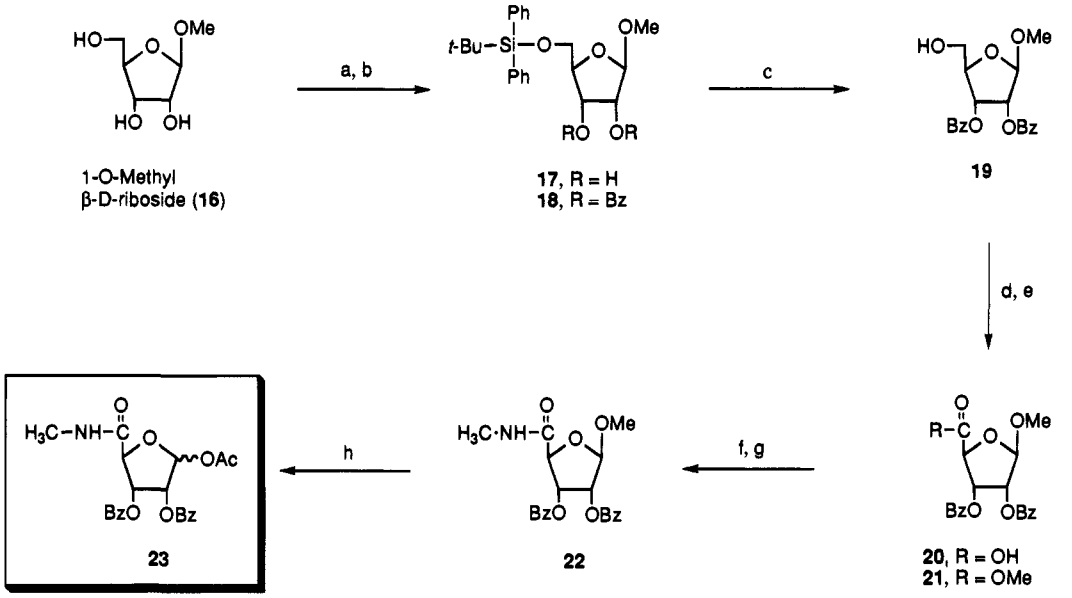
There remains a need for the development of highly selective A_3 agonists. Although the new A_3 radioligand [^{125}I]AB-MECA, **2**,[14] is of nanomolar potency at A_3 receptors and more potent than the previously used [^{125}I]APNEA, **4**,[1] it is not very selective for A_3 vs A_1 or A_{2a} receptors (Table 1). The presence of the 4-amino group of **2** decreases selectivity in comparison to moderately A_3 selective agonist, IB-MECA, **1**.[7,11] The N^6-derivative of adenosine, APNEA, **3**, has been used recently in pharmacological studies[6] to stimulate A_3 receptors, although it is actually A_1-selective. Until present, no monosubstituted adenosine derivatives have been reported to be selective for A_3 receptors.[10] In our previous study of high-affinity 5',N^6-disubstituted adenosine derivatives,[11] only 50–70-fold selectivity for A_3 vs A_1 receptors had been achieved.

New adenosine analogues (compounds **10-15**, Table 1) were synthesized according to Schemes 1 and 2 and characterized (Table 2) and tested in radioligand binding assays[14-16] for affinity at rat brain A_1, A_{2a}, and A_3 adenosine receptors. The compounds were assayed as follows: at A_1 receptors in rat cortical membranes using [^{3}H]-N^6-[(*R*)-phenylisopropyl]adenosine[15]; at A_{2a} receptors in rat striatal membranes using [^{3}H]-CGS 21680[16]; at A_3 receptors using [^{125}I]AB-MECA, **2**,[14] in membranes of CHO cells stably transfected with cDNA for rat brain A_3 receptors.[1]

Substitution at the 2-position is often associated with selectivity of adenosine agonists for A_{2a} vs A_1 receptors. For example, CGS 21680, **5**, and APEC, **6**, both having sterically bulky 2-substituents, were reported to be highly selective for A_{2a} receptors in models of adenylate cyclase.[22] Compound **6** was more potent at all three adenosine receptor subtypes than **5**, and both compounds displayed a potency order of A_{2a} > A_3 > A_1, consistent with the findings of van Galen et al.[10] that 2-substitution of adenosine is well-tolerated in binding to A_3 receptors. Among monosubstituted derivatives of adenosine, 2-(phenylamino)- and 2-chloroadenosine, **7**, have K_i values for inhibition of binding of [^{125}I]APNEA (N^6-[2-(4-aminophenyl)ethyl]adenosine) at rat A_3 receptors of 4.4 and 1.9 μM, respectively.[10] Substitution at the 2-position is also compatible with N^6-substitution for affinity at A_3 receptors. For example, 2-chloro-N^6-cyclopentyladenosine, **9** (Table 1), is nearly identical in its receptor binding profile to N^6-cyclopentyladenosine, **8**.

N^6-(3-Iodobenzyl)adenosine, **10**, was prepared from 6-chloropurine riboside and 3-iodobenzylamine hydrochloride in the presence of triethylamine in ethanol at 80 °C. Compound **10** was 2-fold selective for A_3 vs A_1 or A_{2a} receptors, making it is the first monosubstituted adenosine analogue with any selectivity for A_3 receptors. 2-Chloro-N^6-(3-iodobenzyl)adenosine, **11**, was 7-fold more potent than **10** at A_3 receptors and of moderate selectivity. 2-Amino substitution of adenosine analogues is also compatible with N^6-substitution in A_3 receptor binding but is not as favorable as 2-chloro for potency and selectivity. For example, 2-amino-N^6-(3-iodobenzyl)-adenosine, **12**, was less potent than the 2-H analogue,

Scheme 1[a]



[a] Reagents: (a) TBDPSiCl, DMAP, DMF, room temperature; (b) Bz_2O, py; (c) *n*-Bu_4NF, THF; (d) RuO_2, $NaIO_4$, $CHCl_3$–CH_3CN–H_2O (2:2:3); (e) EDAC, DMAP, MeOH; (f) $MeNH_2$, THF, 75 °C; (g) BzCl, py–CH_2Cl_2; (h) Ac_2O, H_2SO_4, AcOH.

Scheme 2

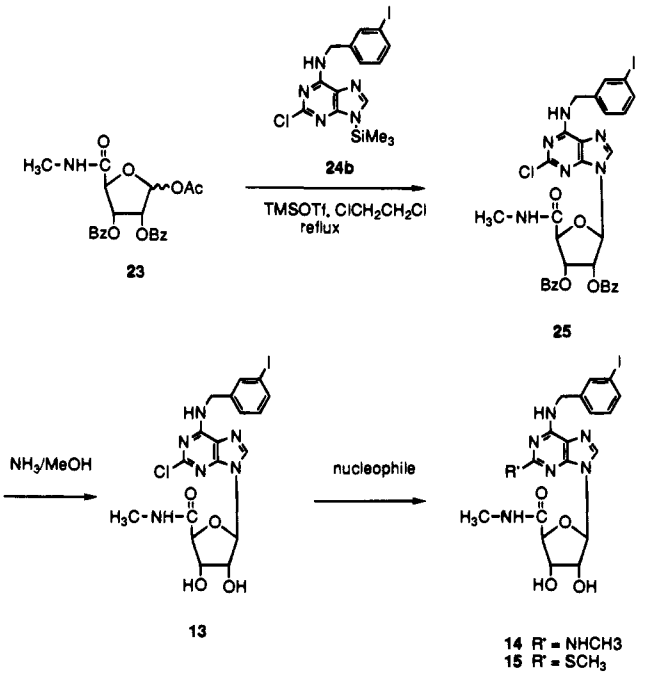


Table 2. Characterization of Intermediates and 2-Substituted-N^6-(3-iodobenzyl)adenosine Derivatives

compd	mp (°C)	formula	analysis
10	172	$C_{17}H_{18}N_5O_4I$	C, H, N
11	foam	$C_{17}H_{17}N_5O_4ClI$·0.3MeOH	C, H, N
12	152–154	$C_{17}H_{19}N_6O_4I$·1.2MeOH	C, H, N
13	206–207	$C_{18}H_{18}N_6O_4ClI$·0.5MeOH	C, H, N
14	190	$C_{19}H_{23}N_7O_4I$	*a*
15	179	$C_{19}H_{21}N_6O_4IS$	*a*
17	syrup	$C_{22}H_{30}O_5Si$	C, H
18	syrup	$C_{36}H_{38}N_7Si$	C, H
19	syrup	$C_{20}H_{20}N_7$	C, H, N
20	syrup	$C_{20}H_{18}N_8$·$0.63H_2O$	C, H
21	92.2–93.7	$C_{21}H_{20}N_8$	C, H
22	syrup	$C_{21}H_{21}NO_7$·$0.5H_2O$	C, H, N
23a,b	foam	$C_{22}H_{21}NO_8$·$0.3H_2O$	C, H, N
24a	222–224	$C_{12}H_{19}N_5ClI$	C, H, N
25	foam	$C_{32}H_{26}N_6O_6ClI$·$1.0C_6H_{14}$	C, H, N
26	foam	$C_{38}H_{29}N_5O_7ClI$$0.2C_6H_{14}$	C, H, N

[a] High-resolution MS (*m/z*) measured in FAB^+ mode. **14**: calcd for $C_{19}H_{23}N_7O_4I$ 540.0856, found 540.0867. **15**: calcd for $C_{19}H_{21}N_6O_4I_1S_1$ 557.0468, found 557.0482.

10, by factors of 3.2 (A_1 receptors), 6.7 (A_{2a} receptors), and 19 (A_3 receptors).

To evaluate the effects of triple substitution of adenosine, i.e. at 5′-, 2-, and N^6-positions on the affinity at A_3 receptors, we developed a general synthetic strategy in which a 5′-uronamide sugar moiety (Scheme **1**) was condensed with a purine moiety, such as a substituted adenosine derivative. The key sugar intermediate **23** was synthesized starting from methyl β-D-ribofuranoside (**16**), which was commercially available or could be synthesized[17] from D-ribose, in eight steps. The primary alcohol of **16** was selectively protected with *tert*-butyldiphenylsilyl chloride[18] to provide **17**, and the remaining alcohols were followed with benzoyl protection to provide **18**. Desilylation of **18** with TBAF/THF gave compound **19**. The 5′-position of **19** was oxidized using ruthenium tetroxide[19] to give compound **20** which was purified after methylation by silica gel column chromatography. The methylamide at 5-position was introduced by nucleophilic displacement of **21** with methylamine in THF and benzoyl reprotection of resulting 2,3-diol to give the sugar intermediate **23**.

In order to synthesize N^6-(3-iodobenzyl)-2-substituted-adenosine derivatives, it was necessary to prepare the corresponding adenine derivative (Schemes 2 and 3). 2,6-Dichloropurine reacted with 3-iodobenzylamine hydrochloride in the presence of triethylamine in ethanol at room temperature to provide N^6-(3-iodobenzyl)-2-chloroadenine, **24a**, which was silylated before coupling to give **24b**. The glycosidic bond was formed upon treatment of the 1′-*O*-acetyl riboside derivative **23** with the 9-silylated adenine derivative **24b** in the presence of TMSOTf as a Lewis acid catalyst (Scheme 2). Condensation of **22** with **24b** produced ribose ring opened product. Benzoyl groups of **25** were deprotected with NH_3/MeOH to produce **13**, which reacted with various nucleophiles such as methylamine/THF and sodium

Table 3. Inhibition by Various N^6-Benzyladenosine Derivatives of the Specific Binding of [^{3}H]-*S*-(4-Nitrobenzyl)-6-thioinosine at Adenosine Uptake Sites in Rat Brain Membranes and the Selectivity Ratio for Affinity at Cloned Rat A_3 Receptors (K_i values from Table 1)

compd	K_i (NBTI)[a]	K_i (NBTI)/K_i ([^{125}I]AB-MECA
N^6-benzyladenosine	203 ± 93	1.69
1	28200 ± 10700	22000
13	15200 ± 5200	46000
15	49500 ± 633	22000

[a] Expressed in nanomolar as K_i ± SEM for three or four determinations, each done in triplicate. Rat striatal membranes were incubated for 30 min at 23 °C with 0.3 nM [^{3}H]NBTI and varying concentrations of the nucleoside derivative in Tris buffer, pH 7.4 in a total of 0.5 mL. Nonspecific binding was determined in the presence of 5 μM *S*-(*p*-nitrobenzyl)-6-thioguanosine.

thiomethoxide/DME to yield compounds **14** and **15**. The benzoyl groups of **26** (Scheme 3) were similarly deprotected to produce the riboside derivative **11**.

The assignments of the anomeric structure of compounds **25** and **26** were performed based on the comparison of the coupling pattern of anomeric proton of compounds **11** and **13** with compound **10**, which is of known anomeric structure being derived from 6-chloropurine riboside.

The combination of 2-substitution with the substituent groups of compound **1** resulted in very high potency and selectivity for A_3 receptors. The A_3 affinity of the 2-chloro analogue, 2-chloro-N^6-(3-iodobenzyl)adenosine-5'-N-methyluronamide, **13**, was 3-fold greater than for IB-MECA, **1**. The affinity at A_1 and A_{2a} receptors was diminshed relative to **1**, by 15- and 9-fold, respectively. Thus, selectivities of approximately 2500-fold vs A_1 receptors and 1400-fold vs A_{2a} receptors were achieved. 2-(Methylamino)-N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide, **14**, was less potent (K_i value 3 nM), but still highly selective for A_3 receptors. 2-(Methylthio)-N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide, **15**, was also highly selective for A_3 receptors.

The selectivity of several of the adenosine derivatives vs a nucleoside transporter previously characterized in brain[24] was probed. These experiments were carried out because of the structural similarity of the present adenosine derivatives to various 6-benzyl ethers or thioether derivatives of purine ribosides, known to be high affnity antagonists of adenosine uptake via this transporter.[24] The simple N^6-benzyl derivative of adenosine was not selective for the receptors vs the adenosine transporter. In contrast, 2-chloro-N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide, **13**, was 46 000-fold selective for A_3 receptors vs the Na^+-independent adenosine transporter, as indicated in displacement of [^{3}H]-*S*-(4-nitrobenzyl)-6-thioinosine binding in rat brain membranes. Thus, in this series of 2,6,5'-trisubstituted adenosine derivatives there was a high degree of selectivity for A_3 receptors vs potential antagonism of adenosine uptake.

The agonist properties of the selective ligands were also examined (Figure 2). In a functional assay using membranes from CHO cells stably transfected with rat A_3 receptors, compounds **1** and **13** inhibited adenylate cyclase with IC_{50} values of 90.0 ± 22.5 and 66.8 ± 9.0 nM (n = 4), respectively. Both derivatives were full agonists, with a maximal 41% inhibition of forskolin-stimulated adenylate cyclase. These two derivatives were considerably more potent in the A_3 receptor functional assay than were either N^6-benzylNECA (IC_{50} of 1.61 μM) or NECA (IC_{50} of 5.6 ± 1.9 μM, n = 3). These findings establish a rank order of potency similar to that observed in binding assays, but at higher concentrations. The K_i values for N^6-benzylNECA and NECA at rat A_3 receptors in CHO cells vs [^{125}I]APNEA were 6.8 and 113 nM, respectively.[10] Thus, the IC_{50} values for these four agonists to inhibit adenylate cyclase were 50–240-fold higher than the respective K_i values at A_3 receptors.

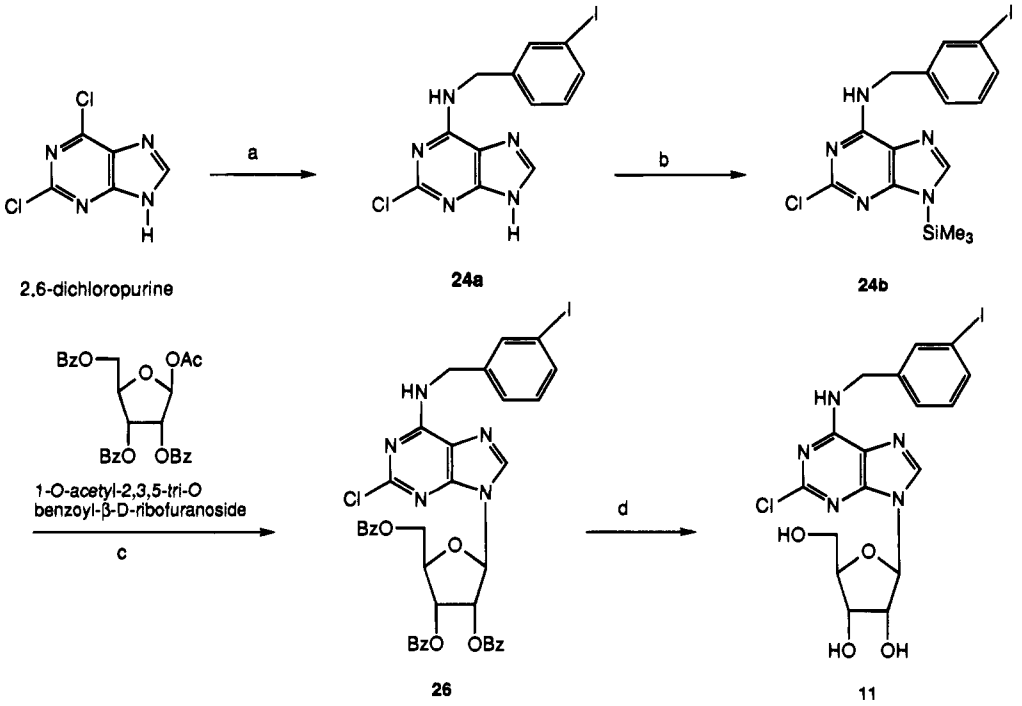
Discussion

In two earlier studies[10,11] we demonstrated that combined modification of adenosine at 5'- and at N^6-positions with groups that enhanced A_3 potency resulted in moderate A_3 selectivity. We previously showed N^6-benzyladenosine-5'-*N*-ethyluronamide (N^6-benzylNECA) to be a full agonist in inhibiting adenylate cyclase via rat A_3 receptors.[10] However, that derivative was only **1** order of magnitude selective for rat A_3 receptors vs either A_1 or A_{2a} receptors in binding assays. In this study we have introduced triple substitution of adenosine as a means of enhancing the degree of A_3 selectivity, and selectivity in binding assays of 3 orders of magnitude has now been achieved. 2-Chloro-N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide, the most potent and selective agent in binding assays, was also shown to be a full agonist in the inhibition of adenylate cyclase, with an IC_{50} of 67 nM. The agonist potency was also greater than that of other agonists, indicating a parallel between binding affinities and *relative* potencies in this functional assay. The agonist properties in another relevant functional assay, stimulation of A_3-mediated phosphoinositide metabolism, are currently being examined.

Adenosine agonists of high selectivity, such as **13**, **14**, and **15**, are needed for defining the role of A_3 receptors *in vivo*. We have demonstrated that selective agonists may have therapeutic potential as cerebroprotective agents.[8,20] Recently Downey and colleagues[9] have demonstrated the cardioprotective potential of A_3 receptor activation, based on use of APNEA coadministered with a xanthine antagonist that does not act at A_3 receptors. In this study we have shown that APNEA is 8-fold A_1 selective, and its pharmacological use is limited to such combination with antagonists of both A_1 and A_{2a} receptors. Clearly, the availablility of ligands such as **13** could be critical in pharmacological studies of A_3 receptors. A highly selective A_3 ligand would be useful as a radioligand, since the currently used high affinity ligand [^{125}I]AB-MECA, is not sufficiently selective for general application in tissue.[14]

It will be necessary to establish the selectivities of these novel A_3 agonists in different species, due to the unusually large species dependence in ligand affinity at this subtype, although differences appear to be more pronounced for antagonists than for agonists.[12,13,25] It is to be noted that 2-chloroadenosine is 17-fold less potent than NECA at rat A_3 receptors,[10] whereas at sheep A_3 receptors 2-chloroadenosine is only 1.7-fold less potent than NECA.[12] Thus, since the most selective compound in the present series, **13**, contains the 2-chloro substitution, it is likely that the selectivity will not be substantially diminshed in other species, such as sheep and human. We have shown a high degree of correlation in the relative affinities of adenosine derivatives at rat vs human A_3 receptors.[25]

Scheme 3[a]



[a] Reagents: (a) 3-iodobenzylamine–HCl, triethylamine, EtOH; (b) HMDS, $(NH_4)_2SO_4$; (c) TMSOTf, $ClCH_2CH_2Cl$; (d) NH_3/MeOH.

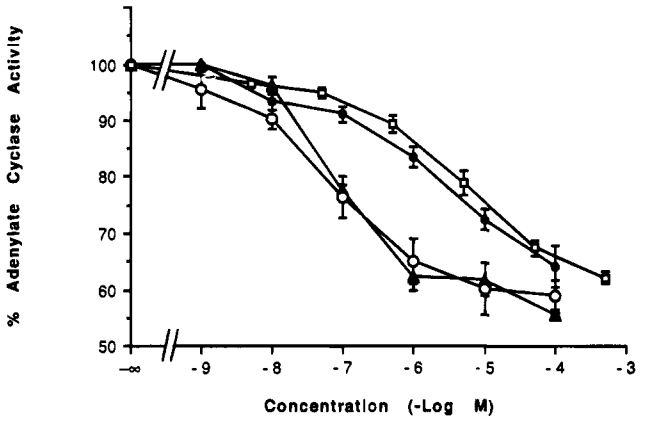


Figure 2. Inhibition of adenylate cyclase in membranes from CHO cell stably transfected with rat A_3 receptors. The assay was carried out as described in the Experimental Procedures in the presence of 1 μM forskolin. Each data point is shown as mean ± SEM for four to seven determinations. Adenosine derivatives were (number of separate experiments in parentheses): solid triangles, 1, N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (4); open circles, **13**, 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (4); solid circles, N^6-benzylNECA (7); and open squares, NECA (3). IC_{50} values were 1, 90.0 ± 22.5 nM; **13**, 66.8 ± 9.0 nM; N^6-benzylNECA, 1.61 μM; NECA, 5.6 ± 1.9 μM.

The selectivity of compound **1** for adenosine receptors vs other neurotransmitter/modulator receptors was shown.[11] In this study, we have shown a high degree of selectivity of the doubly-substituted derivative, **1**, and the present triply-substituted adenosine derivatives for A_3 receptors vs the NBTI-sensitive adenosine uptake site. We have not tested the adenosine derivatives at the normally low-affinity A_{2b} receptor, but substitution at the 2-position of adenosine has been shown not to be well-tolerated at the mouse fibroblast A_{2b} receptor.[26]

In conclusion, 2-substitution is well-tolerated at A_3 receptors, whether it be with a small group (e.g., **11**) or a large group (e.g., **6**). The potency-enhancing effects of 2-substituents appeared to follow the order: chloro > thioether > amine. The effects of 2-substitution to enhance A_3 affinity are also additive with effects of uronamides at the 5′-position and a 3-iodobenzyl group at the N^6-position. The A_3 affinity-enhancing effect of a 2-chloro group was not additive with an N^6-cyclopentyl group. The combination of most favorable modifications at three positions has led to very potent and highly selective agonist ligands, compounds **13**–**15**.

Experimental Procedures

Chemistry. New compounds were characterized (and resonances assigned) by 300 MHz proton nuclear magnetic resonance mass spectroscopy using a Varian GEMINI-300 FT-NMR spectrometer. Unless noted, chemical shifts are expressed as ppm downfield from tetramethylsilane. Synthetic intermediates were characterized by chemical ionization mass spectrometry (NH_3) and adenosine derivatives by fast atom bombardment mass spectrometry (positive ions in a noba or m-bullet matrix) on a JEOL SX102 mass spectrometer. In the EI mode accurate mass was determined using a VG7070F mass spectrometer. C, H, and N analyses were carried out by Atlantic Microlabs (Norcross, GA), and ±0.4% was acceptable. All adenosine derivatives were judged to be homogeneous using thin-layer chromatography (silica, 0.25 mm, glass backed, Alltech Assoc., Deerfield, IL) following final purification. 2-Chloroadenosine, NECA, CGS 21680, N^6-cyclopentyladenosine, and 2-chloro-N^6-cyclopentyladenosine were obtained from Research Biochemicals International (Natick, MA). IB-MECA and APEC were synthesized as reported.[11,27] AP-NEA and iodo-APNEA were the gift of Prof. Ray A. Olsson (University of South Florida, Tampa, FL).

***N*⁶-(3-Iodobenzyl)-9-β-D-ribofuranosyladenine (10).** A mixture of 6-chloropurine riboside (purchased from Aldrich Chemical Co., 100 mg, 0.35 mmol), triethylamine (0.146 mL, 1.05 mmol), and 3-iodobenzylamine hydrochloride (103 mg, 0.38 mmol) in ethanol (2 mL) was heated for 18 h at 85 °C in a sealed bottle. After the reaction mixture was concentrated to dryness, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 10:1) to give compound **10** (148 mg, 88%) as a colorless solid: ^1H NMR (DMSO-d_6) δ 3.54 (m, 1 H, H-5′a), 3.67 (m, 1 H, H-5′b), 3.96 (d, J = 3.3 Hz, 1 H,

H-4'), 4.14 (m, 1 H, H-3'), 4.60 (m, 1 H, H-2'), 4.66 (br s, 2 H, CH_2), 5.16 (d, J = 4.4 Hz, 1 H, exchangeable with D_2O, 3'-OH), 5.34 (br s, 1 H, exchangeable with D_2O, 5'-OH), 5.43 (d, J = 6.1 Hz, 1 H, exchangeable with D_2O, 2'-OH), 5.89 (d, J = 6.0 Hz, 1 H, H-1'), 7.11 (pseudo t, J = 8.0 and 7.8 Hz, 1 H, H-5''), 7.36 (d, J = 7.6 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.72 (s, 1 H, H-2''), 8.21 (s, 1 H, H-2 or -8), 8.40 (s, 1 H, H-2 or -8), 8.48 (br s, 1 H, exchangeable with D_2O, N^6-H).

2-Chloro-N^6-(3-iodobenzyl)-9-β-D-ribofuranosyladenine (11). A mixture of compound **26** (760 mg, 0.916 mmol) and NH_3/MeOH (15 mL) was stirred for 66.5 h at room temperature. After the reaction mixture was concentrated to dryness, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 20:1) to yield compound **11** (445 mg, 94%) as a foam: ^1H NMR (DMSO-d_6) δ 3.55 (m, 1 H, H-5'a), 3.65 (m, 1 H, H-5'b), 3.94 (d, J = 3.6 Hz, 1 H, H-4'), 4.12 (m, 1 H, H-3'), 4.51 (q, J = 5.5 Hz, 1 H, H-2'), 4.60 (br d, J = 5.7 Hz, 2 H, CH_2), 5.04 (pseudo t, J = 5.7 and 5.5 Hz, 1 H, exchangeable with D_2O, 5'-OH), 5.19 (d, J = 4.9 Hz, 1 H, exchangeable with D_2O, OH), 5.47 (d, J = 6.0 Hz, 1 H, exchangeable with D_2O, OH), 5.83 (d, J = 5.5 Hz, 1 H, H-1'), 7.13 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-5''), 7.36 (d, J = 7.5 Hz, 1 H, H-4'' or -6''), 7.60 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.74 (s, 1 H, H-2''), 8.43 (s, 1 H, H-8), 8.94 (br t, J = 6.0 Hz, 1 H, exchangeable with D_2O, NH).

2-Amino-N^6-(3-iodobenzyl)-9-β-D-ribofuranosyladenine (12). A mixture of 2-amino-6-chloropurine riboside (purchased from Aldrich Chemical Co., 80 mg, 0.26 mmol), 3-iodobenzylamine hydrochloride (71.5 mg, 0.265 mmol), and triethylamine (0.11 mL, 0.79 mmol) in ethanol (1.6 mL) was heated for 24 h at 80 °C. After the reaction mixture was concentrated to dryness the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 20:1 → 10:1) to yield compound **12** (99 mg, 75%) as a colorless solid: ^1H NMR (DMSO-d_6) δ 3.52 (m, 1 H, H-5'a), 3.63 (m, 1 H, H-5'b), 3.89 (m, 1 H, H-4'), 4.10 (m, 1 H, H-3'), 4.50 (m, 1 H, H-2'), 4.60 (br s, 2 H, CH_2), 5.08 (d, J = 4.6 Hz, 1 H, exchangeable with D_2O, 3'-OH), 5.35 (m, 2 H, exchangeable with D_2O, 5'- and 2'-OH), 5.73 (d, J = 6.2 Hz, 1 H, H-1'), 5.83 (br s, 2 H, exchangeable with D_2O, NH_2), 7.11 (pseudo t, J = 7.9 and 7.8 Hz, 1 H, H-5''), 7.36 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.70 (s, 1 H, H-2''), 7.94 (s, 1 H, H-8).

2-Chloro-N^6-(3-iodobenzyl)-9-[5-(methylcarbamoyl)-β-D-ribofuranosyl]adenine (13). A mixture of compound **25** (27 mg, 0.036 mmol) and NH_3/MeOH (15 mL) was stirred for 16 h at room temperature. After rotary evaporation of the volatiles, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 20:1 → 10:1) to give compound **13** (13.4 mg, 68.7%) as a colorless solid: ^1H NMR (DMSO-d_6) δ 2.72 (d, J = 4.3 Hz, 3 H, $NHCH_3$), 4.17 (br s, 1 H, H-3'), 4.32 (s, 1 H, H-4'), 5.55 (m, 1 H, H-2'), 4.61 (br d, J = 5.5 Hz, 2 Hz, CH_2), 5.56 (d, J = 6.4 Hz, 1 H, exchangeable with D_2O, 2'-OH), 5.72 (d, J = 4.3 Hz, 1 H, exchangeable with D_2O, 3'-OH), 5.92 (d, J = 7.2 Hz, 1 H, H-1'), 7.13 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-5''), 7.36 (d, J = 7.5 Hz, 1 H, H-4'' or -6''), 7.61 (d, J = 7.8 Hz, 1 H, H-4'' or -6''), 7.75 (s, 1 H, H-2''), 8.27 (br d, J = 4.3 Hz, 1 H, exchangeable with D_2O, NH), 8.49 (s, 1 H, H-8), 9.02 (br t, J = 6.2 and 5.7 Hz, 1 H, exchangeable with D_2O, N^6H).

N^6-(3-Iodobenzyl)-2-(methylamino)-9-[5-(methylcarbamoyl)-β-D-ribofuranosyl]adenine (14). A solution of **13** (10 mg, 0.018 mmol) in 2 N CH_3NH_2/THF (1.5 mL) was heated for 3 days at 90 °C. After the reaction mixture was concentrated to dryness, the residue was purified on a preparative TLC ($CHCl_3$–MeOH, 10:1) to give **14** (7 mg, 70%) as a colorless solid: ^1H NMR (DMSO-d_6) δ 2.66 (d, J = 4.7 Hz, 3 H, -$NHCH_3$), 2.76 (d, J = 4.3 Hz, 3 H, $NHCH_3$), 4.18 (m, 1 H, H-3'), 4.25 (s, 1 H, H-4'), 4.57 (br s, 2 H, CH_2), 4.69 (m, 1 H, H-2'), 5.47 (d, J = 6.5 Hz, 1 H, exchangeable with D_2O, 2'-OH), 5.59 (d, J = 4.6 Hz, 1 H, exchangeable with D_2O, 3'-OH), 5.84 (d, J = 7.2 Hz, 1 H, H-1'), 6.28 (br d, J = 4.4 Hz, exchangeable with D_2O, NH), 7.11 (pseudo t, J = 8.0 and 7.8 Hz, 1 H, H-5''), 7.38 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.70 (m, 1 H, exchangeable with D_2O, NH), 7.76 (s, 1 H, H-2''), 8.02 (s, 1 H, H-8), 8.05 (br s, 1 H, exchangeable with D_2O, NH).

N^6-(3-Iodobenzyl)-2-(methylthio)-9-[5-(methylcarbamoyl)-β-D-ribofuranosyl]adenine (15). A solution of **13** (15 mg, 0.029 mmol) and sodium thiomethoxide (4.0 mg, 0.057 mmol) in anhydrous ethylene glycol dimethyl ether (2 mL) was heated at 80 °C, under nitrogen atmosphere, for 3 days. After cooling to room temperature, the reaction mixture was neutralized with glacial acetic acid and evaporated to dryness. The residue was purified on a preparative TLC (CH_2Cl_2–MeOH, 9.5:0.5) to give **15** (5.6 mg, 36.5%) as a yellow solid: ^1H NMR (DMSO-d_6) δ 2.43 (s, 3 H, SH_3), 2.74 (d, J = 4.3Hz, 3 H, $NHCH_3$), 3.48 (br s, 2 H, 2 × OH), 4.19 (m, 1H, H-3'), 4.31 (s, 1 H, H-4'), 4.62 (br s, 3 H, CH_2 & H-2'), 5.87 (d, J = 7.9 Hz, 1 H, H-1'), 7.11 (pseudo t, J = 8.0 and 7.8 Hz, 1 H, H-5''), 7.90 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.58 (d, J = 7.9 Hz, 1 H, H-4'' or -6''), 7.76 (s, 1H, H-2''), 8.24 (br s, 1 H, NH), 8.35 (s, 1 H, H-8), 8.68 (br s, 1 H, NH).

Methyl 5-(*tert*-Butyldiphenylsilyl)-β-D-ribofuranoside (17). To a mixture of methyl β-D-ribofuranoside (**16**, purchased from Sigma Chemical Co., 460 mg, 2.8 mmol) and anhydrous methylene chloride (20 mL) were added triethylamine (0.468 mL, 3.36 mmol), *tert*-butyldiphenylchlorosilane (0.9 mL, 3.46 mmol), and DMAP (13.7 mg, 0.112 mmol) successively at room temperature. The reaction mixture was stirred for 18 h at room temperature under nitrogen. The reaction mixture was washed with water (20 mL), saturated ammonium chloride (20 mL), and brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was separated by silica gel column chromatography ($CHCl_3$–MeOH, 50:1) to yield compound **17** [R_f = 0.48 ($CHCl_3$–MeOH, 10:1), 618 mg, 54.8%] as a thick syrup: ^1H NMR (DMSO-d_6) δ 0.96 (s, 9 H, *t*-Bu), 3.22 (s, 3 H, OCH_3), 3.61 (dd, J = 11.0 and 5.3 Hz, 1 H), 3.74 (d, J = 4.4 Hz, 1 H), 3.81 (dd, J = 11.0 and 2.7 Hz, 1 H), 3.90 (m, 1 H), 4.00 (m, 1 H), 4.68 (s, 1 H, H-1'), 4.84 (br s, 1 H, exchangeable with D_2O, OH), 5.05 (br s, 1 H, exchangeable with D_2O, OH), 7.45 and 7.67 (m, 10 H, Ph_2).

Methyl 5-(*tert*-Butyldiphenylsilyl)-2,3-dibenzoyl-β-D-ribofuranoside (18). To a solution of compound **17** (579 mg, 1.44 mmol) in methylene chloride–pyridine (4:1, 12.5 mL) was added dropwise benzoyl chloride (0.367 mL, 3.16 mmol) at 0 °C. The reaction mixture was stirred for 2.5 h at 0 °C and for 14.5 h at room temperature. Ice was added to quench the reaction, and the mixture was stirred for 1 h. Methylene chloride (100 mL) was added, and two phases were separated. Organic layer was washed with water, saturated ammonium chloride, and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness to give crude compound **18**, which was then purified by silica gel column chromatography (Hx–EtOAc, 5:1 → 1:1) to yield compound **18** [R_f = 0.75 ($CHCl_3$–MeOH, 10:1), 869 mg, 99%] as a thick syrup: ^1H NMR ($CDCl_3$) δ 1.05 (s, 9 H, *t*-Bu), 3.42 (s, 3 H, OCH_3), 3.86 (dd, J = 11.1 and 4.8 Hz, 1 H, H-5a), 3.92 (dd, J = 11.1 and 4.7 Hz, 1 H, H-5b), 4.48 (dd, J = 10.6 and 4.6 Hz, 1 H), 5.15 (s, 1 H), 5.63 (d, J = 5.0 Hz, 1 H), 5.82 (pseudo t, J = 5.8 and 5.4 Hz, 1 H), 7.29–8.18 (m, 20 H, Ar).

Methyl 2,3-Dibenzoyl-β-D-ribofuranoside (19). A solution of compound **18** (849 mg, 1.39 mmol) and 1.0 M tetrabutylammonium fluoride in THF (1.53 mL, 1.53 mmol) was stirred for 2 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography (Hx–EtOAc, 1:1) to yield compound **19** [R_f = 0.50 (Hx-EtOAc, 1:1), 461 mg, 89%] as a thick syrup: ^1H NMR (DMSO-d_6) δ 3.38 (s, 3 H, OCH_3), 3.62 (m, 2 H, H-5), 4.37 (q, J = 5.2 Hz, 1 H, H-4), 5.02 (pseudo t, J = 6.0 and 5.3 Hz, 1 H, exchangeable with D_2O, 5-OH), 5.18 (s, 1 H, H-1), 5.45 (m, 1 H, H-2), 5.53 (t, J = 5.2 Hz, 1 H, H-3), 7.42–7.88 (m, 10 H, Ar).

1-*O*-Methyl 2,3-Dibenzoyl-β-D-ribofuronic Acid (20). A mixture of compound **19** (374.8 mg, 1.01 mmol), ruthenium(IV) oxide (10 mg), and sodium periodate (1161 mg, 5.43 mmol) in $CHCl_3$–CH_3CN–H_2O (2:2:3, 14 mL) was stirred vigorously for 2.5 h at room temperature. Chloroform (20 mL) was added, and semisolid was removed by filtration. The two layers of filtrate were separated, and aqueous layer was extracted with

chloroform (2 × 40 mL). Combined organic layer and extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. After drying *in vacuo* overnight, 1-*O*-methyl-2,3-dibenzoyl-β-D-ribofuranuronic acid (**20**, 340 mg, 89.5%) was obtained as a thick syrup: ^{1}H NMR ($CDCl_3$) δ 3.56 (s, 3 H, OCH_3), 4.90 (d, J = 6.1 Hz, 1 H, H-4), 5.25 (s, 1 H, H-1), 5.66 (d, J = 5.0 Hz, 1 H, H-2), 6.00 (pseudo t, J = 5.7 and 5.4 Hz, 1 H, H-3), 7.32–7.99 (m, 10 H, Ar).

Methyl 1-*O*-Methyl-2,3-dibenzoyl-β-D-ribofuranuronate (21). *N*-Ethyl-*N*′-(diaminopropyl)carbodiimide (EDAC, 198 mg, 1.04 mmol) was added to a solution of acid (**20**, 0.16 g, 0.414 mmol) in MeOH (3 mL), and the reaction mixture was stirred for 3 h at room temperature. After the solvent was removed by rotary evaporation, the residue was dissolved in chloroform (50 mL), washed with water (30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was purified on a preparative TLC (Hx–EtOAc, 1:1) to yield methyl 1-*O*-methyl-2,3-dibenzoyl-β-D-ribofuronate [**21**, R_f = 0.77 (Hx–EtOAc, 1:1), 120 mg, 72.3%] as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 3.52 (s, 3 H, 1-OCH_3), 3.82 (2, 3 H, 5-OCH_3), 4.84 (d, J = 6.2 Hz, 1 H, H-4), 5.21 (s, 1 H, H-1), 5.62 (d, J = 4.8 Hz, 1 H, H-2), 6.01 (pseudo t, J = 5.7 and 5.5 Hz, 1 H, H-3), 7.32–7.99 (m, 10 H, Ar).

***N*,1-*O*-Dimethyl-2,3-dibenzoyl-β-D-ribofuranuronamide (22).** A mixture of methyl ester **21** (35 mg, 0.087 mmol) and 2.0 M methylamine in THF (3 mL) was heated for 15 h at 50 °C in a sealed tube. The volatiles were removed by evaporation, and the residue was reacted with benzoyl chloride (0.15 mL, 1.29 mmol) in methylene chloride–pyridine (2:1, 6 mL) for 3 h at room temperature. After workup as procedure for compound **18**, the residue was separated by preparative TLC (Hx–EtOAc, 1:1) to yield compound **22** [R_f = 0.28 (Hx–EtOAc, 1:1) or 0.77 ($CHCl_3$–MeOH, 10:1), 25 mg, 72%] as a syrup: ^{1}H NMR ($CDCl_3$) δ 2.92 (d, J = 5.0 Hz, 3 H, NHCH_3), 3.58 (s, 3 H, 1-OCH_3), 4.82 (d, J = 5.5 Hz, 1 H, H-4), 5.24 (s, 1 H, H-1), 5.60 (m, 1 H, H-2), 5.87 (t, J = 5.1 Hz, 1 H, H-3), 6.68 (br m, 1 H, NH), 7.33–7.99 (m, 10 H, Ar).

***N*-Methyl-1-*O*-acetyl-2,3-dibenzoyl-α-D-ribofuronamide (23a) and *N*-methyl-1-*O*-acetyl-2,3-dibenzoyl-β-D-ribofuranuronamide (23b).** To a solution of **22** (1.533 g, 3.84 mmol) and acetic anhydride (3.8 mL, 40.3 mmol) in glacial acetic acid (19 mL) was added dropwise concentrated H_2SO_4 (1.125 mL, 21.1 mmol), and the reaction mixture was stirred for 15 h at room temperature. After water (30 mL) was added slowly, the mixture was extracted with methylene chloride (150 mL × 3) and the organic layer was washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and connectrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 20:1) to give a mixture of **23a** and **23b** [R_f = 0.71 and 0.76 ($CHCl_3$–MeOH, 20:1), respectively, 0.55 g, 33.5%] as a foam. Analytical samples were separated by preparative TLC ($CHCl_3$–MeOH, 20:1): ^{1}H NMR ($CDCl_3$) (compound **23a**) δ 2.10 (s, 3 H, OAc), 2.91 (d, J = 4.9 Hz, 3 H, -NHCH_3), 4.96 (s, 1 H, H-4), 5.45 (pseudo t, J = 5.5 and 5.0 Hz, 1 H, H-2), 6.08 (d, J = 5.9 Hz, 1 H, H-3), 6.71 (d, J = 4.7 Hz, 1 H, H-1), 6.72 (br s, 1 H, NH), 7.28 (pseudo t, J = 7.8 and 7.7 Hz, 2 H, Ar), 7.48 (q, J = 7.8 Hz, 3 H, Ar), 7.62 (pseuso t, J = 7.7 and 6.9 Hz, 1 H, Ar), 7.79 (d, J = 7.4 Hz, 2 H, Ar), 8.13 (d, J = 7.8 Hz, 2 H, Ar); (compound **23b**) δ 2.17 (s, 3 H, OAc), 2.90 (d, J = 4.9 Hz, 3 H, NHCH_3), 4.89 (d, J = 6.2 Hz, 1 H, H-4), 5.73 (d J = 4.9 Hz, 1 H, H-2), 5.96 (t, J = 5.9 Hz, 1 H, H-3), 6.43 (s, 1 H, H-1), 6.50 (br s, 1 H, NH), 7.37 (pseudo t, J = 7.8 and 7.6 Hz, 4 H, Ar), 7.48–7.58 (m, 2 H, Ar), 7.93 (d, J = 8.1 Hz, 2 H, Ar), 7.98 (d, J = 7.3 Hz, 2 H, Ar).

2-Chloro-N^6-(3-iodobenzyl)adenine (24a). A solution of 2,6-dichloropurine (purchased from Aldrich Chemical Co., 1 g, 5.3 mmol), 3-iodobenzylamine hydrochloride (1.7 g, 5.8 mmol), and triethylamine (2.2 mL, 15.35 mmol) in ethanol (10 mL) was stirred for 5 days at room temperature. The colorless solid formed was collected by suction, washed with small amount of cold ethanol, and dried to give compound **24a** (1.16 g, 60%): mass (EI) 385 (M^+); ^{1}H NMR (DMSO-d_6) δ 4.59 (br s, 2 H, CH_2), 7.13 (pseudo t, J = 8.2 and 7.5 Hz, 1 H, Bn), 7.36 (d, J = 7.5 Hz, 1 H, Bn), 7.61 (d, J = 7.5 Hz, 1 H, Bn), 7.74 (s, 1 H, Bn), 8.14 (s, 1 H, H-8), 8.76 (br s, 1 H, exchangeable with D_2O, NH), 13.14 (br s, 1 H, exchangeable with D_2O, NH); UV (MeOH) λ_{max} 281.7, 257.5, 232.5 nm.

2-Chloro-N^6-(3-iodobenzyl)-9-[5-(methylcarbamoyl)-2,3-di-*O*-benzoyl-β-D-ribofuranosyl]adenine (25). A mixture of 2-chloro-N^6-(3-iodobenzyl)adenine (**24a**, 165 mg, 0.43 mmol), ammonium sulfate (catalytic amount), and HMDS (15 mL) was refluxed for 4 h under nitrogen to provide the silylated derivative **24b**. The clear solution was concentrated to dryness *in vacuo* with exclusion of moisture, and the residue was dissolved in dry dichloroethane (6 mL). A solution of **23** (141 mg, 0.33 mmol) in dry dichloroethane (6 mL) and TMSOTf (83 μL, 0.43 mmol) were added, and the reaction mixture was stirred for 0.5 h at room temperature and refluxed for 62 h under nitrogen. Saturated $NaHCO_3$ (10 mL) was added, and the mixture was stirred for 15 min. Two layers were separated, and the aqueous layer was extracted with methylene chloride (50 mL × 3), washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was purified on a preparative TLC ($CHCl_3$–MeOH, 20:1) to give **25** [R_f = 0.58 ($CHCl_3$–MeOH, 20:1), 83 mg, 33 %] as a foam: MS (CI NH_3) 753 (M^++1); ^{1}H NMR ($CDCl_3$) δ 3.10 (d, J = 4.6 Hz, 3 H, NHCH_3), 4.79 (br s, 2 H, CH_2), 4.97 (s, 1 H, H-4′), 6.08 (m, 1 H, H-3′), 6.15-6.25 (m, 3 H, H-2′, 1′, NH), 7.06–8.06 (m, 15 H, Ar), 8.52 (br s, 1 H, NH).

2-Chloro-N^6-(3-iodobenzyl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)adenine (26). A mixture of 2-chloro-N^6-(3-iodobenzyl)adenine (**24a**, 0.84 g, 2.18 mmol), ammonium sulfate (catalytic amount), and HMDS (20 mL) was refluxed for 5 h under nitrogen to provide the silylated derivative **24b**. The clear solution was concentrated to dryness *in vacuo* with exclusion of moisture, and the residue was dissolved in dry dichloroethane (6 mL). A solution of acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (purchased from Janssen Chimica Chemical Co., 1 g, 1.98 mmol) in dry dichloroethane (12 mL) and TMSOTf (0.42 mL, 2.18 mmol) were added, and the reaction mixture was stirred for 20 min at room temperature and refluxed for 14 h under nitrogen. After similar workup for compound **25**, the residue was purified by silica gel column chromatography (Hx–EtOAc, 2:1) to give **26** [R_f = 0.11 (Hx–EtOAc, 3:1), 1.495 g, 91%] as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 4.69–4.92 (m, 5 H, CH_2, H-4′, H-5′), 6.15 (m, 3 H, H-2′, H-3′, NH), 6.45 (d, J = 4.3 Hz, 1 H, H-1′), 7.07 (pseudo t, 1 H, Bn), 7.31–8.10 (m, 20 H, Ar).

Methods for Receptor Binding and Adenylate Cyclase Measurement. Procedures for preparation of rat brain membranes and CHO cell membranes were as reported.[10,11,14] For binding experiments, membrane homogenates were frozen and stored at −20 °C for ≤2 months. Adenosine deaminase (ADA) was from Boehringer Mannheim (Indianapolis, IN). [^{3}H]R-PIA was from Amersham (Arlington Heights, IL), and [^{3}H]CGS 21680 was from DuPont NEN (Boston, MA). [^{125}I]-AB-MECA was prepared as described by Olah et al.[14]

Binding of [^{125}I]AB-MECA to CHO cells stably transfected with the A_3 receptor clone was performed essentially as described.[11,14] Assays were performed in 50 mM Tris/10 mM $MgCl_2$/1 mM EDTA buffer (adjusted to pH 8.26 at 5 °C) in glass tubes and contained 100 μL of the membrane suspension, 50 μL of [^{125}I]AB-MECA (final concentration 0.3 nM) ,and 50 μL of inhibitor. Inhibitors were routinely dissolved in DMSO and were then diluted with buffer; final DMSO concentrations never exceeded 1%; this concentration did not influence [^{125}I]-AB-MECA binding. Incubations were carried out in duplicate for 1 h at 37 °C, and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman gamma 5500B γ-counter. Nonspecific binding was determined in the presence of 200 μM NECA. K_i values were calculated according to Cheng–Prusoff,[21] assuming a K_d for [^{125}I]AB-MECA of 1.48 nM.[6]

Binding of [^{3}H]PIA to A_1 receptors from rat cortical membranes and of [^{3}H]CGS 21680 to A_2 receptors from rat striatal membranes was performed as described previously.[8,11] Adenosine deaminase (2 units/mL) was present during the preparation of brain membranes. Additional deaminase was not added during incubation with the radioligand.

Competition for binding of [^{3}H]NBTI was carried out by a modification of the procedure of Marangos et al.[24] Rat striatal membranes, prepared as above, were subjected to incubation for 30 min at 23 °C with 0.3 nM [^{3}H]NBTI and varying concentrations of the nucleoside derivative in Tris buffer, pH 7.4, in a total of 0.5 mL. For nonspecific binding 5 μM *S*-(*p*-nitrobenzyl)-6-thioguanosine (Sigma, St. Louis, MO) was added, and specific binding was 95% of total. A K_d value of 0.15 nM was used in the calculation of K_i values.[24] Specific binding was 95% of total.

Adenylate cyclase was assayed in membranes from CHO cells stably expressing the rat A_3 receptor, prepared as above, using a previously reported method.[10] The method involved addition of [α-^{32}P]ATP to membranes in the presence of forskolin to stimulate adenylate cyclase and papaverine as a phosphodiesterase inhibitor. The reaction was terminated by addition of a stop solution containing 20 000 cpm/mL [^{3}H]cyclic AMP. The total radiolabeled cyclic AMP was isolated on columns of Dowex 50 ion-exchange resin and alumina. Maximal inhibition of adenylate cyclase activity corresponded to ~40% of total activity under conditions of stimulation (typically by 6–8-fold) in the presence of 1 μM forskolin. IC_{50} values were calculated using InPlot (GraphPad, San Diego, CA).

Abbreviations: AB-MECA, N^6-(4-amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide; APNEA, N^6-[2-(4-aminophenyl)ethyl]adenosine; CGS 21680, 2-[4-[(2-carboxyethyl)-phenyl]ethyl-amino]-5'-*N*-(ethylcarbamoyl)adenosine; CHO, Chinese hamster ovary; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDAC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; IB-MECA, N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide; NBTI, *S*-(4-nitrobenzyl)-6-thioinosine; NECA, 5'-*N*-(ethylcarbamoyl)adenosine; PIA, (*R*)-N^6-(phenylisopropyl)adenosine; TBAF, tetrabutylammonium fluoride; TBDPSiCl, *tert*-butyldiphenylsilyl chloride; THF, tetrahydrofuran; TMSOTf, trimethylsilyl trifuoromethanesulfonate; Tris, tris(hydroxymethyl)-aminomethane.

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