Structure-Activity Relationships of N^6-Benzyladenosine-5'-uronamides as A_3-Selective Adenosine Agonists†

Carola Gallo-Rodriguez,‡ Xiao-duo Ji,‡ Neli Melman,‡ Barry D. Siegman,‡ Lawrence H. Sanders,‡ Jeraldine Orlina,‡ Bilha Fischer,‡ Quanlong Pu,‡ Mark E. Olah,§ Philip J. M. van Galen,‡ Gary L. Stiles,§ and Kenneth A. Jacobson*,‡

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

Received September 16, 1993®

Adenosine analogues modified at the 5'-position as uronamides and/or as N^6-benzyl derivatives were synthesized. These derivatives were examined for affinity in radioligand binding assays at the newly discovered rat brain A_3 adenosine receptor and at rat brain A_1 and A_{2a} receptors. 5'-Uronamide substituents favored A_3 selectivity in the order *N*-methyl > *N*-ethyl ≈ unsubstituted carboxamide > *N*-cyclopropyl. 5'-(*N*-Methylcarboxamido)-N^6-benzyladenosine was 37-56-fold more selective for A_3 receptors. Potency at A_3 receptors was enhanced upon substitution of the benzyl substituent with nitro and other groups. 5'-*N*-Methyluronamides and N^6-(3-substituted-benzyl)adenosines are optimal for potency and selectivity at A_3 receptors. A series of 3-(halobenzyl)-5'-*N*-ethyluronamide derivatives showed the order of potency at A_1 and A_{2a} receptors of I ~ Br > Cl > F. At A_3 receptors the 3-F derivative was weaker than the other halo derivatives. 5'-*N*-Methyl-N^6-(3-iodobenzyl)adenosine displayed a K_i value of 1.1 nM at A_3 receptors and selectivity versus A_1 and A_{2a} receptors of 50-fold. A series of methoxybenzyl derivatives showed that a 4-methoxy group best favored A_3 selectivity. A 4-sulfobenzyl derivative was a specific ligand at A_3 receptors of moderate potency. An aryl amino derivative was prepared as a probe for radioiodination and receptor cross-linking.

Introduction

Two major subclasses of adenosine receptors, A_1 and A_2, have been defined, initially based on pharmacological distinctions[1] and more recently by cloning.[2,3] A_1 receptors may couple to a variety of second messenger systems,[4,5] including inhibition of adenylate cyclase, inhibition or stimulation of phosphoinositol turnover, and activation of ion channels.[1,4] A_2 receptors stimulate adenylate cyclase and can be further subdivided into high affinity A_{2a} and low-affinity A_{2b} subtypes. A novel rat A_3 adenosine receptor subtype was defined as a result of cloning of cDNA coding for sequences resembling G-protein-coupled receptors.[4,5] In the putative transmembrane domains (as defined by molecular modeling[8,9] by analogy to bacteriorhodopsin), it showed 56% identity with the rat A_1 receptor. Curiously, xanthines, which are the classical A_1 and A_2 antagonists, do not bind to rat A_3 receptors.[6,8] A_3 receptors inhibit adenylate cyclase[6] like A_1 receptors and also stimulate phosphoinositide metabolism.[10] This receptor is unrelated to the "A_3 receptor" proposed by Ribeiro and Sebastiao.[11]

Activation of A_3 receptors is associated with the immunosuppressive[10] actions of adenosine. Fozard and Carruthers[12,13] have attributed a xanthine-insensitive component of the vasodilatory effects of adenosine agonists to A_3 receptor activation. The occurrence of A_3 receptors in the brain[6] and testes[7] also suggests that it may be important in regulation of central nervous system (CNS) function and reproduction.

We recently reported that an N^6-substituted adenosine derivative, N^6-benzyl-5'-(*N*-ethylcarboxamido)adenosine, is the first selective agonist for the A_3 receptor.[8] In the same study a computer-based molecular model of the binding site based on a previous model of A_1 receptors[14] was proposed. Binding of the ribose moiety to the A_3 receptor was proposed to closely resemble that of A_1 receptors. In this study the structure of adenosine was modified synthetically on both the ribose and purine moieties in an effort to enhance potency and selectivity in A_3 receptor binding.

Results and Discussion

5'-Uronamido and 5'-uronamido-N^6-benzyl derivatives of adenosine (Figures 1-3) were synthesized (Table 4) and tested in radioligand binding assays for affinity at rat brain A_1, A_{2a}, and A_3 adenosine receptors. The compounds were assayed for A_1 affinity in rat cortical membranes using [^{3}H]-N^6-((*R*)-phenylisopropyl)adenosine[15] and for A_{2a} affinity in rat striatal membranes using [^{3}H]CGS 21680.[16] At A_3 receptors it was necessary to use a cell line (CHO) in which rat brain A_3 receptors were stably transfected.[6] The radioligand used for binding to A_3 receptors was the nonselective [^{125}I]iodoAPNEA, as previously reported.[6,8]

Ribose Modifications. The SAR for adenosine derivatives with modifications in the ribose moiety has been found to be similar for A_3 receptors to that for A_1 and A_{2a} receptors.[8] There is little toleration of substitution or deletion of the 2'- and 3'-hydroxyl groups, but there is considerable freedom of substitution at the 5'-position. 5'-Deoxy, 5'-thio ether, and 5'-uronamide substitutions are tolerated at A_3 receptors.[8]

NECA, **3**, was reported to be among the most potent known agonists at A_3 receptors,[6] although not A_3 selective.

† Abbreviations: CGS 21680, [[2-[4-(2-carboxyethyl)phenyl]ethyl]-amino]-5'-(*N*-ethylcarboxamido)adenosine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDAC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; NECA, 5'-(*N*-ethylcarboxamido)adenosine; PIA, (*R*)-N^6-(phenylisopropyl)adenosine; Tris, tris(hydroxymethyl)aminomethane.

* 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.

® Abstract published in *Advance ACS Abstracts*, January 15, 1994.

0022-2623/94/1837-0636$04.50/0 © 1994 American Chemical Society

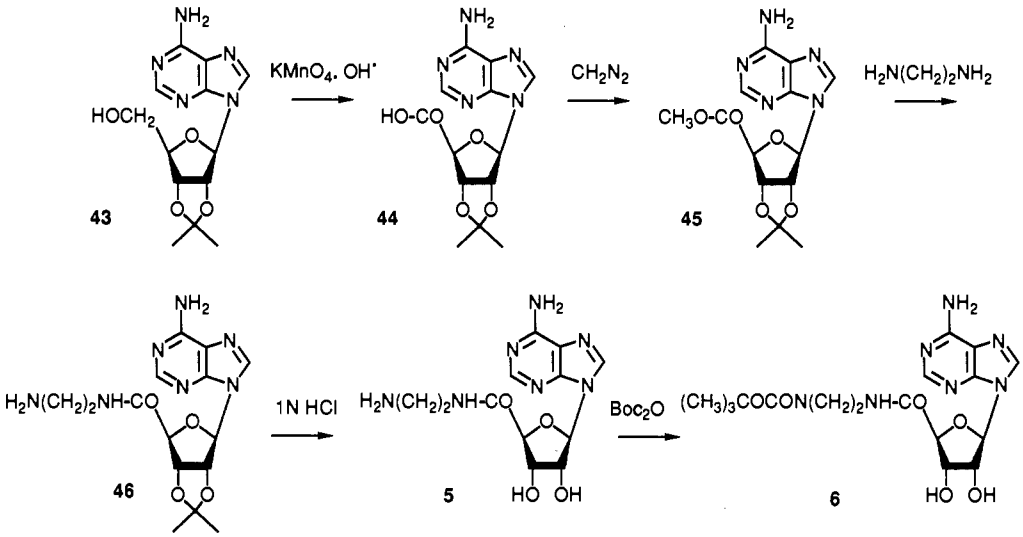


Figure 1. Synthesis of an adenosine aminoalkyl functionalized congener, which was found to bind weakly but selectively at A_3 receptors.

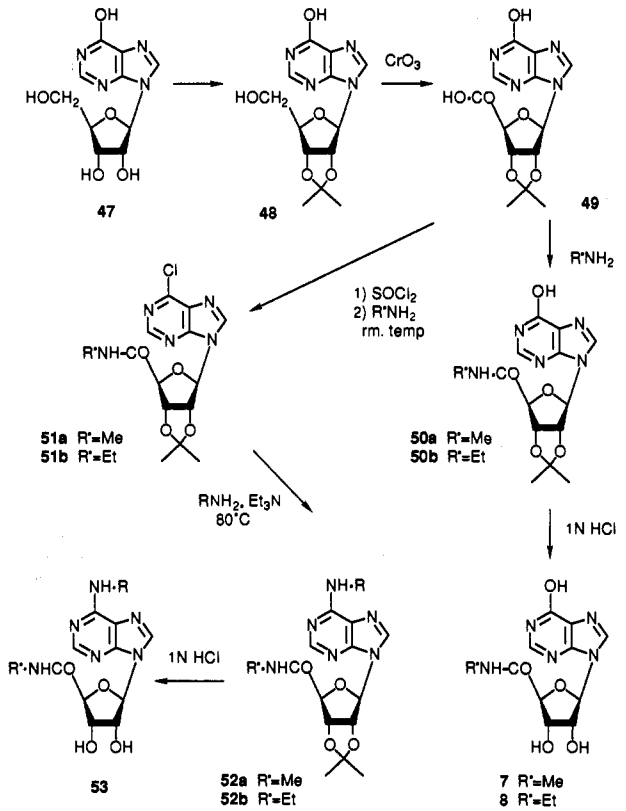


Figure 2. Synthesis of 5′-*N*-alkyluronamide derivatives of inosine and of N^6-substituted adenosine derivatives.

NECA favors both A_1 and A_{2a} receptors vs A_3 receptors by 1 order of magnitude in affinity. The 5′-uronamido group of NECA was varied (Table 1). The primary carboxamide, **1**, was weaker than NECA at A_3 receptors, with roughly the same selectivity ratios. The *N*-methylamide, **2**, was the most favored 5′-uronamide in binding to A_3 receptors. It was found to be nonselective but moderately potent (K_i 75 nM) at A_3 receptors.

Two 5′-uronamide derivatives of ethylenediamine, **5** and **6**, were synthesized (Figure 1) as functionalized congeners.[17] They were found to be much less potent than NECA in receptor affinity, despite a relatively small added substituent (an amino group) in the case of **5**. Although weak in receptor affinity, the derivatives were found to be A_3 selective, indicating the possibility of achieving greater

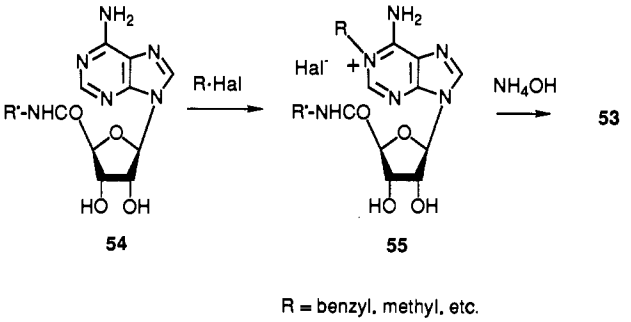


Figure 3. Synthesis of N^6-benzyl-5′-*N*-alkyluronamides and related derivatives via the Dimroth rearrangement of N1-alkylated adenosine derivatives.

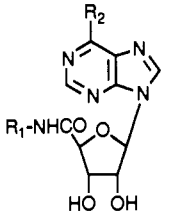
potency and selectivity by combining the 5′-*N*-(2-aminoethyl)carboxamido modification with other modifications.

Inosine (9-β-D-ribofuranosylhypoxanthine) binds weakly to adenosine receptors, with K_i values at A_1, A_{2a}, and A_3 receptors in the 20–50 μM range.[8] The 5′-*N*-ethyluronamide derivative of inosine, **8**, was also found to be a weak ligand with >20-fold selectivity for A_3 receptors.[8] The *N*-methyl analogue, **7**, was synthesized (Figure 2) and provided an apparent improvement in selectivity, with approximately the same A_3 affinity as the *N*-ethyl analogue, **8**.

Purine Modifications. The 8-position of the purine moiety is not amenable to substitution in A_3 receptor binding.[8] The 2-position may be substituted without eliminating recognition at A_3 receptors; however, among the few analogues examined there was no indication of enhancement of A_3 selectivity.[8]

Modification of the N1-position (either oxidation to the *N*-oxide or 1-deaza analogues) is tolerated at A_3 receptors.[8] The 5′-*N*-ethyluronamide modification was combined with various changes at the N1-position. NECA N1-oxide, **9**, was nonselective (Table 2), but the loss of potency versus NECA was greater for A_1 and A_{2a} receptors than for A_3 receptors. To introduce larger groups, such as 2- and 4-nitrobenzyl, NECA was alkylated at the N1-position (general reaction shown in Figure 3) resulting in **10** and **11**. The resulting potency at A_1 and A_{2a} receptors was not substantially different from NECA N1-oxide, but the potency at A_3 receptors was increased by 1 order of magnitude. Thus, **10** and **11** displayed intermediate potency and some selectivity for A_3 receptors.

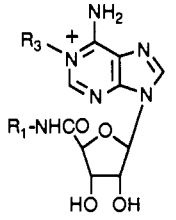
Table 1. Affinities of 5′-Uronamide Derivatives in Radioligand Binding Assays at Rat Brain A_1, A_2, and A_3 Receptors[a-c]



compd	R_1	R_2	K_i (nM) or % inhibition $K_i(A_1)$[a]	$K_i(A_2)$[b]	$K_i(A_3)$[c]	A_1/A_3	A_2/A_3
1[d]	H	NH_2	72.6	120	1410 ± 60	0.051	0.085
2[d]	Me	NH_2	83.6	66.8	72 ± 16	1.2	0.93
3[d]	Et	NH_2	6.3	10.3	113	0.071	0.091
4[d]	cyclopropyl	NH_2	6.4	13.4	1600 ± 70	0.0040	0.0084
5	H_2NEt	NH_2	0% (10^{-5})	12.5% (10^{-5})	14700 ± 2540	>1	>1
6	t-BocNHEt	NH_2	0% (10^{-5})	0% (10^{-5})	18000 ± 4270	>1	>1
7	Me	OH	0% (10^{-4})	6.2 ± 4.2% (10^{-4})	6220 ± 1220[f]	>10	>10
8[e]	Et	OH	44% (10^{-4})	31% (10^{-4})	5000	>1	>1

[a] Displacement of specific [^{3}H]PIA binding, unless noted, in rat brain membranes expressed as K_i ± SEM in nM (n = 3). [b] Displacement of specific [^{3}H]CGS 21680 binding, unless noted, in rat striatal membranes, expressed as K_i ± SEM in nM (n = 3). [c] Displacement of specific binding of [^{125}I]APNEA[10] or [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-N-methyluronamide[28] from membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as K_i ± SEM in nM (n = 3–5). [d] Values at A_1 and A_2 receptors are taken from Bruns et al.[29] K_i values at A_1 receptors are vs specific binding of [^{3}H]-N^6-cyclohexyladenosine. K_i values at A_{2a} receptors are vs specific binding of [^{3}H]NECA in the presence of 50 nM CPA in rat striatal membranes. [e] Values from ref 8. [f] Vs[^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-N-methyluronamide.

Table 2. Affinities of N^1-Derivatives of Adenosine and Other Purines in Radioligand Binding Assays at Rat Brain A_1, A_2, and A_3 Receptors[a-c]



compd	R_1	R_3	K_i (nM) or % inhibition $K_i(A_1)$[a]	$K_i(A_2)$[b]	$K_i(A_3)$[c]	A_1/A_3	A_2/A_3
9[d]	Et	O⁻	154	101	468	0.33	0.22
10[e]	Et	$CH_3C_6H_4$-4-NO_2	341 ± 52	190 ± 52	60 ± 3	5.7	3.2
11[e]	Et	$CH_2C_6H_4$-2-NO_2	196 ± 52	83.5 ± 9.5	27 ± 5	7.3	3.1

[a] Displacement of specific [^{3}H]PIA binding in rat brain membranes expressed as K_i ± SEM in nM (n = 3–4). [b] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as K_i ± SEM in nM (n = 3). [c] Displacement of specific binding of [^{125}I]APNEA[10] from membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as K_i ± SEM in nM (n = 3). [d] Values from ref 8. [e] Elemental analysis consistent with neutral imino form.

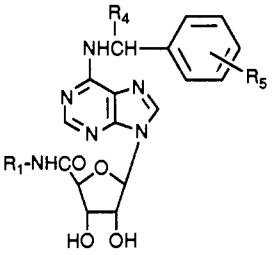
The position of substitution causing the greatest selective enhancement of potency at A_3 receptors was found to be the N^6-position. We previously reported that among N^6-aralkyl derivatives of adenosine, the benzyl group favored A_3 receptor selectivity and that this modification was compatible with the 5′-N-ethylcarboxamide.[8] Indeed, N^6-benzylNECA, **29** (Table 3), was 14-fold selective for A_3 receptors versus either A_1 or A_{2a} receptors. In this study we have modified both 5′- and N^6-positions of **29** to enhance selectivity and potency.

Disubstituted N^6- and 5′-uronamide derivatives of adenosine were prepared by two main routes (Figures 2 and 3).[18,19] The best overall yields were obtained by the route introduced by Olsson et al.[18] (Figure 2). This route consisted of oxidation of 2′,3′-isopropylideneinosine, **48**, followed by treatment with thionyl chloride to form at once the acid chloride and 6-chloro derivative. The acid chloride was displaced with an alkylamine, and the stable intermediate **51** was isolated, for further reaction with substituted benzyl bromides. An alternate method for preparing N^6-benzyladenosines (Figure 3) consisted of the Dimroth rearrangement[19] of N1-alkylated 5′-uronamidoadenosine derivatives, **55**.

As with simple 5′-uronamides, the 5′-N-methylcarboxamide analogue of N^6-benzyladenosine were most selective for A_3 receptors (compare **12**, **13**, **29**, and **42**). **13** was 37–56-fold more selective for A_3 receptors, whereas the 5′-N-cyclopropyl analogue, **42**, was nonselective and much less potent. Although the 5′-N-methyl substitution was generally favored over the 5′-N-ethyl substitution, there were several exceptions, such as the 3-nitro and 3-methyl analogues, in which the potency was more favorable in the N-ethyl series (**36** and **38** vs **17** and **20**, respectively).

Comparisons of benzyl group substitutions were made for both the N-ethyl- and the N-methyluronamide series. The stereoselectivity of binding at A_3 receptors for enantiomers at the carbon α to N^6 has been demonstrated for the R- and S-isomers of N^6-(phenylisopropyl)adenosine.[8] As for A_1 and A_{2a} receptors,[20,21] the R-isomer is favored. For the doubly modified N^6-(1-phenylethyl)-5′-uronamidoadenosine analogues, **30a** and **30b**, the R-configuration is also favored by a factor of 27 at A_3 receptors. At A_1 and A_{2a} receptors the stereoselectivity factors for R- vs S-configurations were 20-fold and 6.4-fold, respectively. Thus, A_3 receptors resembles both A_1 and A_{2a} receptors in stereoselectivity at the position α to N^6.

Table 3. Affinities of N^6-Benzyladenosine-5′-uronamide Derivatives in Radioligand Binding Assays at Rat Brain A_1, A_2, and A_3 Receptors[a-c]



R_4 = H, unless noted

			K_i (nM) or % inhibition				
compd	R_1	R_5	$K_i(A_1)$[a]	$K_i(A_2)$[b]	$K_i(A_3)$[c]	A_1/A_3	A_2/A_3
12	H	H	580 ± 99	423 ± 46	246 ± 35	2.6	1.7
13	Me	H	898 ± 124	597 ± 42	16 ± 1	56	37
14	Me	3-Cl	916 ± 121	559 ± 78	21.9 ± 0.3	42	25
15	Me	3-Br	65 ± 2	64 ± 9	1.9 ± 0.3[d]	34	34
16	Me	3-I	54 ± 5	56 ± 8	1.1 ± 0.3[d]	49	51
17	Me	3-NO_2	735 ± 5	441 ± 45	19 ± 1[d]	39	23
18	Me	3-NH_2	1000 ± 60	794 ± 118	28 ± 13[d]	36	28
19	Me	3-$NHCOCH_3$	2970 ± 330	988 ± 185	41.4 ± 0.7[d]	72	24
20	Me	3-CH_3	322 ± 23	415 ± 33	10 ± 3[d]	32	42
21	Me	3-CF_3	496 ± 50	574 ± 49	31 ± 6[d]	16	19
22	Me	4-Cl	478 ± 38	2730 ± 210	17 ± 10[d]	28	160
23	Me	4-Br	516 ± 38	2460 ± 380	12 ± 5[d]	43	200
25	Me	4-NH_2	431 ± 45	1590 ± 180	14 ± 3[d]	31	110
26	Me	4-NH_2-3-I	18.0 ± 5.0	197 ± 84	1.27 ± 0.18[d]	14	160
27	Me	3-SO_3H·Et_3N	19600 ± 2000	9070 ± 1150	1310 ± 170[d]	15	6.9
28	Me	4-SO_3H·Et_3N	0% (10^{-4})	0% (10^{-4})	4500 ± 480[d]	>100	>100
29	Et	H	87	95	6.8	14	14
30a	Et	H (R, R_4 = CH_3)	3.2 ± 0.1	259 ± 15	18 ± 4[d]	0.18	14
30b	Et	H (S, R_4 = CH_3)	65 ± 4	1650 ± 120	494 ± 91[d]	0.13	3.3
31	Et	3-F	51 ± 7	32 ± 4	10.7 ± 1.2	4.8	3.0
32	Et	3-Cl	22 ± 3	19.0 ± 0.3	1.1 ± 0.3	20	17
33	Et	3-Br	10.9 ± 1.5	6.2 ± 0.7	2.8 ± 1.9[d]	3.9	2.2
34	Et	3-I	7.7 ± 0.9	7.2 ± 0.6	0.88 ± 0.21	8.8	8.2
35	Et	2-NO_2	31 ± 4	24 ± 3	2.8 ± 0.5	11	8.6
36	Et	3-NO_2	78 ± 10	35 ± 7	8.7 ± 1.2	11	4.0
37	Et	4-NO_2	49 ± 9	574 ± 64	9.0 ± 1.3	5.4	64
38	Et	3-CH_3	36.5 ± 1.2	17.7 ± 1.8	1.2 ± 0.1	30	15
39	Et	2-OMe	52 ± 5	21 ± 3	7.1 ± 0.3	7.3	3.0
40	Et	3-OMe	69 ± 8	38 ± 6	4.3 ± 0.6	16	8.8
41	Et	4-OMe	209 ± 30	609 ± 34	11 ± 3	19	55
42	cyclopropyl	H	112 ± 13	55 ± 6	103 ± 22	1.1	0.53

[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 [^{125}I]APNEA[10] or [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-N-methyluronamide[28] from membranes of CHO cells stably transfected with the rat A_3-cDNA, expressed as K_i ± SEM in nM (n = 3–5). [d] Vs[^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-N-methyluronamide.

Substituents such as an electron-withdrawing group (e.g. nitro) or an electron-donating group (e.g. methoxy) were placed at each position of the benzene ring to probe electronic and positional effects of substitution. Among N^6-benzyl-5′-N-ethyluronamides, selectivity for A_3 vs A_1 receptors ranged from 4- to 30-fold and was greatest for the 3-methyl derivative, **38**. 3-Chloro-, **32**, 3-methoxy-, **40**, and 3-nitro-, **41**, derivatives were also very selective for A_3 vs A_1 receptors. Selectivity for A_3 vs A_{2a} receptors ranged from 2- to 64-fold and was greatest for the 4-nitro derivative, **37**, and the 4-methoxy derivative, **41**. In general, positional and/or steric effects appeared to be more important than electronic effects. For example, 4-position substitution provided high selectivity for A_3 vs A_{2a} receptors in both the N-methyl (e.g. **22**, **23**, and **25**) and N-ethyl (e.g. **37** and **41**) series.

Substitution at the 3-position generally favored A_3 potency and selectivity. Halo substituents were varied at the 3- and 4-positions of the benzyl substituent. 3-Halo derivatives were particularly potent. The 3- and 4-bromo derivatives in the 5′-N-methyl series, **15** and **23**, respectively, were both relatively selective, but the affinity at A_3 receptors was 6-fold greater with substitution at the 3-position. There was a major dependence of the affinity at all three adenosine receptors on the halo atom, and affinities of ca. 1 nM were achieved. In the 5′-N-ethyl series, the order of potency at A_1 and A_{2a} receptors was I ~ Br > Cl > F. At A_3 receptors, the order was I ~ Cl > Br > F. In the 5′-N-ethyl series, the 3-iodobenzyl analogue, **34**, and to a lesser degree the 3-bromo analogue, **33**, were very highly potent and selective for A_3 receptors. N^6-(3-Iodobenzyl)adenosine-5′-N-methyluronamide, **16**, displayed a K_i value of 1.0 nM at A_3 receptors and selectivity versus A_1 and A_{2a} receptors of 49- and 51-fold, respectively.

Since compound **16** was both potent and selective and as such is a candidate for further pharmacological characterization, the selectivity for adenosine receptors versus other receptors was examined in a battery of radioreceptor binding assays (NovaScreen, Adheron Corporation, Hanover, MD).[22] At a concentration of 10^{-5} M, the displacement of radioligand from α-adrenergic, β-adrenergic, dopamine (D_1 and D_2), serotonin 5-HT_1, central benzodiazepine, amino acid (γ-aminobutyric acid; N-methyl-D-aspartate; kainate; quisqualate; and glycine, strychnine sensitive and insensitive, MK-801), peptide (angiotensin II, vasopressin, bombesin, neurokinin, central CCK, neu-

Table 4. Characterization of Adenosine Derivatives

compd	method[a]	% yield	mp (°C)	MS	formula	analysis
5	C	72	155–172	CI: 324	$C_{17}H_{25}N_7O_6 \cdot 1^1/_4H_2O$	C,H;N[f]
6	C	42	130–140	FAB+ 424, 368[b]	$C_{17}H_{25}N_7O_6 \cdot {}^1/_2t$-BuOH + $^1/_2H_2O$	C,H,N
7	A	57	225 dec	FAB+ 296, 160[b]	$C_{11}H_{13}N_5O_6 \cdot 1^1/_2$MeOH	C,H,N
8	A	55	198 dec	FAB+ 310 (MH^+),[b] 174	$C_{12}H_{15}N_5O_5 \cdot 1^1/_2H_2O$	C,H,N
9	D	28	158 dec	CI: 325, 309, 273		
10	B	60	140 dec	FAB+ 444[b]	$C_{19}H_{21}N_7O_8 \cdot {}^3/_4H_2O$	C,H,N
11	B	45	198 dec	FAB+ 444[b]	$C_{19}H_{21}N_7O_8 \cdot 1^1/_2H_2O$	C,H,N
12	B	40	207–208	370, 268, 254, 225	$C_{17}H_{18}N_8O_4 \cdot {}^3/_4$MeOH	C,H,N
13	A	46	114–116	384, 268, 254, 225	$C_{18}H_{20}N_8O_4 \cdot H_2O$	C,H,N
14	B	67	171–173	CI: 419/421	$C_{18}H_{19}N_6O_4Cl \cdot {}^1/_4H_2O$	C,H,N
15	A	78	170–172	462/464, 346/348, 303/305	$C_{18}H_{19}N_8O_4Br \cdot {}^3/_4H_2O$	C,H,N
16	A	55	174–177	510 (M^+), 394, 380, 351	$C_{18}H_{19}N_8O_4I \cdot {}^1/_2H_2O$	C,H,N
17	A	39	~125 dec	429, 313, 299, 271, 253	$C_{18}H_{19}N_7O_8 \cdot H_2O$	C,H,N
18	A	50	133–137	FAB+ 400, 241[b]	$C_{18}H_{21}N_7O_4 \cdot H_2O$	C,H,N
19	D	93	132 dec	FAB+ 442, 400 (MH – CH_2=C=O)[b]	$C_{20}H_{23}N_7O_5 \cdot 0.9$MeOH + $^1/_4CHCl_3$	C,H,N
20	A	76	128–129	FAB+ 399, 240[b]	$C_{19}H_{22}N_8O_4 \cdot 1^1/_4H_2O$	C,H,N
21	A	60	~110–120	452, 433, 336, 322, 293	$C_{19}H_{19}N_8O_4F_3 \cdot H_2O$	C,H,N
22	A	83	123–124	418, 302, 288, 259	$C_{18}H_{19}N_8O_4Cl \cdot 1^1/_4H_2O$	C,H,N
23	A	65	111–112	462/464, 346/348, 332/334, 303/305	$C_{18}H_{19}N_6O_4Br \cdot 2H_2O$	C,H,N
25	A	60	135 dec	399, 269, 240	$C_{18}H_{21}N_7O_4 \cdot {}^3/_4H_2O$	C,H,N
26	A	51	132 dec	CI: 526	$C_{18}H_{20}N_7O_4I \cdot {}^1/_{10}CHCl_3$ + $^3/_4$MeOH	C,H,N
27	A	59	125 dec	FAB+ 465[b]	Et_3N salt of $C_{18}H_{20}N_8O_7S \cdot 2^1/_4H_2O$	C,H,N
28	A	29	230 dec	FAB+ 465[c]	$C_{18}H_{20}N_8O_7S \cdot 3^1/_2H_2O$	C,H,N
29	B	42	170–173	CI: 399 (MH^+)	$C_{19}H_{22}N_8O_4 \cdot {}^1/_4H_2O$	C,H,N
30a	A	60	135–138	413, 282, 268, 239	$C_{20}H_{24}N_8O_4 \cdot {}^1/_4H_2O$ + $^1/_2$MeOH	C,H,N
30b	A	66	130 dec	412, 282, 268, 239	$C_{20}H_{24}N_8O_4 \cdot {}^1/_2$MeOH	C,H,N
31	A	68	192–196	416, 286, 272, 243	$C_{19}H_{21}N_8O_4F \cdot {}^1/_4H_2O$	C,H,N
32	B	62	199–200	CI: 433 (MH^+)	$C_{19}H_{21}N_8O_4Cl$	C,H,N
33	A	42	196–197	476/478, 346/348, 332/334, 303/305	$C_{19}H_{21}N_8O_4Br$	C,H,N
34	A	52	194.5–195	524, 394, 380, 351	$C_{19}H_{21}N_6O_4I$	C,H,N
35	B	34	181 dec	FAB+ 444[b]	$C_{19}H_{21}N_7O_8 \cdot {}^3/_4H_2O$	C,H,N
36	B	55	190–192	FAB+ 444[b]	$C_{19}H_{21}N_7O_8 \cdot {}^1/_5H_2O$	C,H,N
37	B	49	196 dec	FAB+ 444[b]	$C_{19}H_{21}N_7O_8 \cdot {}^1/_2H_2O$ + $^1/_2$MeOH	C,H,N
38	A	47	167–168.5	412, 282, 268, 239	$C_{19}H_{21}N_7O_6 \cdot {}^1/_4H_2O$	C,H,N
39	A	72	106–108	428, 298, 284, 255	$C_{20}H_{24}N_8O_5$	C,H,N
40	B	27	167–169	FAB+ 429, 256[b]	$C_{20}H_{24}N_6O_5 \cdot H_2O$	C,H,N
41	B	14	250 dec	428, 284, 255	$C_{20}H_{24}N_8O_5 \cdot {}^3/_4$MeOH	C,H,N
42	B	59	178–180	410, 268, 254, 225	$C_{20}H_{22}N_8O_4 \cdot {}^1/_2H_2O$	C,H,N
51a	D	53	87–94	CI: 354/356, 320	$C_{14}H_{15}N_5O_4Cl \cdot {}^3/_4H_2O$	C,H,N
51b	D	69	88–92	CI: 368–370	$C_{15}H_{17}N_5O_4Cl$	C,H,N
52a	D	96	146–148	CI: 353 (M + NH_4^+), 336, 150	$C_{14}H_{17}N_5O_5 \cdot {}^3/_4H_2O$	C,H,N
52b	D	94	240–243	CI: 350, 188	$C_{15}H_{19}N_5O_5$	C,H,N
57	D	99	135–137	CI: 140 (M + NH_4^+), 123	$C_7H_{11}N_2Cl \cdot {}^1/_4H_2O$	C,H,N
63	D	40	>280	FAB+ 188[e]	$C_7H_9NO_3S$	C,H,N
65	D	79	>280	FAB+ 266/268[e]	$C_7H_8NO_3BrS$	C,H,N,S;Br[g]
66	D	63	>280	FAB+ 188, 171, 108[e]	$C_7H_9NO_3S$	C,H,N,S

[a] Methods: A: Synthesis as in Figure 2. If from chloropurine riboside and benzylamine derivative, percent yield is for both displacement and deprotection steps. For 7 and 8 percent yield refers to condensation and deprotection steps. B: Via Dimroth rearrangement, as in Figure 3. Percent yield calculated for both alkylation and rearrangement steps or for alkylation alone for compounds 10 and 11. C: Refer to Figure 1. D: Refer to text. [b] m-bullet matrix. [c] Noba (3-nitrobenzyl alcohol) matrix. [d] Accurate mass, measure (ppm from calculated) in FAB+ mode unless noted: 18, 442.1829 (–2.3); 27, 463.1016 (3.0) (FAB–); 10, 444.1646 (3.1); 11, 444.1630 (–0.3). [e] Thioglycerol matrix. [f] N: calcd, 28.35; found, 27.18. [g] Br: calcd, 30.03; found, 29.56.

ropeptide Y, neurotensin, somatostatin, vasoactive intestinal peptide, growth factors) receptors was insignificant (0 ± 20%). The displacement of binding of radioligand from ion channels (Ca^{2+}, Cl^-, and K^+) and from second messenger sites (forskolin, phorbol ester, and inositol triphosphate) and inhibition of monoamine oxidase was also insignificant. Curiously, the displacement of radioligand at serotonin 5-HT_2, phencyclidene, peripepheral cholecystokinin receptors was 50–70% at 10^{-5} M. The observation that high affinity was not observed at any of these sites emphasizes the selectivity of compound **16** for A_3 receptors.

The 3-methyl and 3-trifluoromethyl analogues, **20** and **21**, respectively, were prepared in the *N*-methyl series. The trifluoromethyl analogue was weaker at the A_3 receptors. Thus an electron-withdrawing group at the 3-position is not favorable for potency, further supported by the relatively modest potency of the 3-nitro derivative, **17**. In the *N*-methyl series, 3- and 4-amino derivatives, **18** and **25**, respectively, were prepared by treatment of the 6-chloropurine intermediate (R′ = CH_3), **51a**, with 3- or 4-aminobenzylamine. The intermediate 3-aminobenzylamine, **57**, was prepared via catalytic reduction of the 3-nitro derivative, **56** (Figure 4A). The nucleophilic attack of the purine ring occurred selectively at the arylamine. Since the arylamino derivative **25** was prepared as a precursor for radioiodination, the expected major product of direct iodination, (3-iodo-4-aminobenzyl)adenosine-5′-*N*-methyluronamide, **26**, was prepared as a standard for purification and pharmacology. This compound proved to be less selective for A_3 versus A_1 receptors than the corresponding 3-iodo derivative, **16**. The disubstituted benzylamine intermediate **61** (Figure 4B) was prepared via protection of the alkylamine as the *tert*-butyloxycarbonyl derivative. The 3-aminobenzyl derivative, **25**, was also N-acetylated at the 2′,3′-isopropylidene-protected stage using acetic anhydride to yield after deprotection compound **19**. This acetamido derivative demonstrated that there is an unfavorable interaction at this site on the receptor. The 3-acetamido group is unfavorable at A_1 and

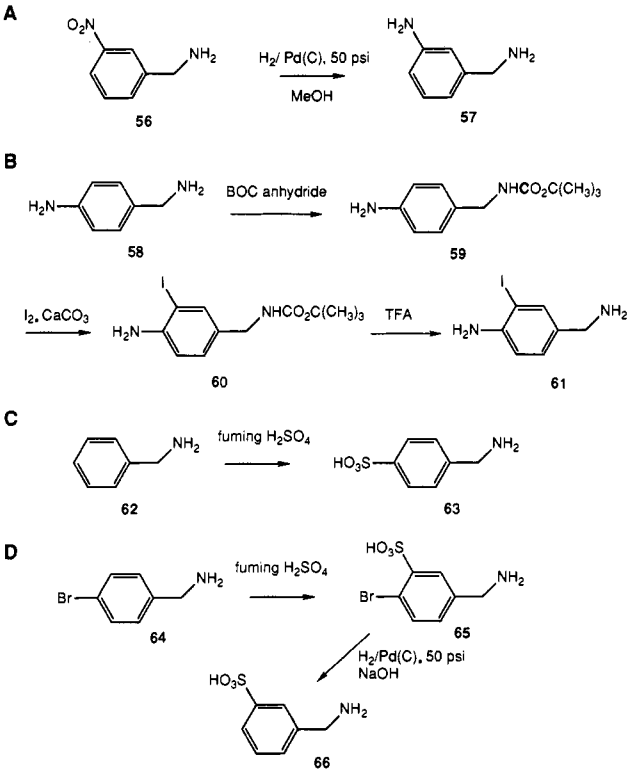


Figure 4. Synthesis of various amino- (A and B) and sulfo- (C and D) substituted benzylamine intermediates used in preparing A_3 adenosine agonists (Figure 2).

to a lesser extent at A_{2a} and A_3 receptors. Thus, compound **19** is 72-fold and 24-fold selective for A_3 vs A_1 and A_{2a} receptors, respectively.

Two sulfo analogues were prepared as a putative peripherally selective agonist, by analogy to our previous study of A_1-selective sulfoadenosine derivatives.[23] Synthesis of the 4-sulfo derivative, **28**, required intermediate **63**, which was prepared directly from benzylamine (Figure 4C). A 3-sulfo intermediate, **66**, leading to adenosine derivative **27**, was prepared via sulfonation of 4-bromobenzylamine followed by catalytic hydrogenation in basic medium (Figure 4D). Compound **28** weakly displaced radioligand from A_3 but not A_1 or A_{2a} receptors; thus, it is a highly selective ligand. It is evident that a negative charge at the 4-benzyl position is poorly tolerated at all three receptor subtypes. The 3-sulfo derivative, **27**, was slightly more potent at A_3 receptors, but considerably less selective than the 4-isomer.

Conclusions

The discovery of a novel adenosine receptor has raised uncertainty concerning previous pharmacological studies using high doses of agonists thought to be selective for the A_1 or A_{2a} receptors. N^6-Cyclopentyladenosine, which is ~400-fold selective for A_1 versus A_{2a} receptors is only 130-fold selective versus A_3 receptors.[8] CGS21680, which is ~140-fold selective for A_{2a} versus A_1 receptors, is only 39-fold selective versus A_3 receptors.[8] Careful use of these agents will be required in future pharmacological studies.

The SAR of N^6-substituted adenosine derivatives, including N^6-benzyl derivatives, has been explored at A_1 and A_{2a} receptors.[24] We have found that the low potency at A_1 and A_{2a} receptors of N^6-benzyladenosines is compatible with the A_3-potency enhancing effects of *N*-alkyluronamides. 5'-*N*-Methyluronamides and N^6-(3-substituted-benzyl)adenosines are optimal for potency and selectivity at A_3 receptors.

In order to adequately define the physiological role of A_3 receptors, selective agents are needed. In terms of therapeutic potential, a principal deficiency of A_1- and A_{2a}-selective agents has been their propensity for side effects,[25] due to the widespread tissue distribution of these receptors. A_3 receptors occur in a more limited distribution (primarily brain, heart, immune system, and testes), suggesting that A_3-selective compounds may be more useful as potential therapeutic agents than agonists of other selectivities. The presence of a sulfo group on the benzyl substituent[14] is expected to result in selectivity for peripheral vs central A_3 receptors when compound **28** is administered *in vivo*.

In this study adenosine derivatives of very high affinity and/or intermediate selectivity for A_3 receptors have been introduced. Compounds such as 5'-*N*-methyl-N^6-(3-halobenzyl)adenosines and the iodinatable[26] arylamines, **18** and **25**, are potentially useful as pharmacological and biochemical probes for A_3 receptors, to define more clearly the physiological role, distribution, and regulation of A_3 adenosine receptors.

Experimental Section

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. The following benzylamine derivatives were purchased from Aldrich (St. Louis, MO): 4-amino, 2-methoxy, 3-methyl, 3-trifluoromethyl, 3-fluoro, 3-bromo, 3-iodo, 4-chloro. 4-Nitrobenzyl bromide was purchased from Fluka (Ronkonoma, NY). 3-Nitrobenzyl bromide was purchased from Lancaster (Windham, NH). 2-Chloroadenosine and 5'-carboxamido derivatives of adenosine (compounds **3** and **4**) were purchased from Research Biochemicals International (Natick, MA). Compound **1** was the gift of Dr. John W. Daly (NIH). Analytical TLC plates and silica gel (230–400 mesh) were purchased from VWR (Bridgeport, NJ).

Inosine-5'-*N*-methyluronamide (7). 2',3'-Isopropylideneinosine-5'-carboxylic acid[18] (**51**, 50 mg, 0.155 mmol), EDAC (59 mg, 0.31 mmol), and *N*-hydroxysuccinimide (36 mg, 0.31 mmol) were dissolved in DMF (1 mL). Methylamine (40% in water, 50 μL) was added, and the mixture was stirred for 90 min. Water was added, and the solid residue (compound **50a**) was separated and dried in vacuo. Compound **50a** was purified by column chromatography (silica, eluted with chloroform–methanol–25% ammonium hydroxide, 80:20:1) to yield 49.8 mg (96%) of the pure product. The isopropylidene group was removed using 0.1 N HCl, warming at 70 °C for 1.5 h, and neutralizing with $NaHCO_3$. The title compound was purified by column chromatography (silica gel, chloroform–methanol–ammonium hydroxide, 80:20:1) to obtain 26 mg of the pure compound (57% yield). ^1H NMR (DMSO-d_6): δ 3.29 (d, J = 7.9 Hz, 3H, CH_3), 4.13–4.20 (m, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.49–4.58 (m, 1H, H-2'), 5.58 (d, J = 6.4 Hz, 1H, OH-2'), 5.68 (d, J = 4.7 Hz, 1H, OH-3'), 5.94 (d, J = 6.9 Hz, 1H, H-1'), 8.13 (s, 1H, H-2), 8.26–8.34 (m, 1H, N*H*-Me), 8.41 (s, 1H, H-8).

N^6-Benzyladenosine-5'-*N*-methyluronamide (13). 2',3'-*O*-Isopropylidene-6-chloropurine-5'-*N*-methyluronamide (**51a**, 30 mg, 85 μmol), benzylamine (9.7 μL, 89 μmol), and triethylamine

(23.7 μL, 0.70 mmol) were dissolved in absolute ethanol (1 mL). The solution was stirred at 65 °C for 16 h in a sealed vessel. The solvent was evaporated under a stream of nitrogen, and water was added to remove the triethylammonium salt. The supernatant was removed and discarded, and the insoluble residue containing intermediate was used without further purification.

Hydrochloric acid (1 N, 1 mL) was added, and the resulting solution was heated to 60 °C for 40 min. After cooling in an ice bath, sodium bicarbonate solution was added to neutralize. The suspension was extracted with ethyl acetate, and the solvent was removed under vacuum to obtain 15 mg of the title compound (R_f = 0.69, silica TLC plate, chloroform–methanol–ammonium hydroxide 80:20:1, 46% yield overall). ^{1}H NMR (DMSO-d_6): δ 2.70 (d, J = 4.3 Hz, 3H, CH_3), 4.14 (m, 1H, H-3′), 4.31 (s, 1H, H-4′), 4.59 (dd, J = 4.6 Hz, J = 7.5 Hz, 1H, H-2′), 4.71 (br s, 2H, N^6-CH_2Ph), 5.96 (d, J = 7.4 Hz, 1H, H-1′), 7.30 (m, 5H, phenyl), 8.29 (s, 1H, H-2), 8.43 (s, 1H, H-8), 8.56 (br s, 1H, N^6H-CH_2Ph), 8.86 (m, 1H, NH-Me).

N^6-(3-Iodobenzyl)adenosine-5′-N-methyluronamide (16). 2′,3′-O-Isopropylidene-N-methyl-6-chloropurine-5′-uronamide (**51a**, 35 mg, 99 μmol), 3-iodobenzylamine hydrochloride (28 mg, 104 μmol), and triethylamine (41 μL, 0.30 mmol) were dissolved in absolute ethanol (1 mL). The solution was stirred at 75 °C for 16 h in a sealed vessel. The solvent was evaporated under a stream of nitrogen, and water was added to remove the triethylammonium salt. The supernatant was removed and discarded, and the insoluble residue containing intermediate **52a** (R = 3-iodobenzyl) was used without further purification.

Hydrochloric acid (1 N, 1 mL) was added, and the resulting solution was heated to 60 °C for 4 h. After cooling in an ice bath, sodium bicarbonate solution was added to neutralize. A white solid formed and was filtered, washed with water, and dried to give 28 mg of the title compound (R_f = 0.74, silica TLC plate, chloroform–methanol–ammonium hydroxide, 80:20:1, 55% yield overall). ^{1}H NMR (DMSO-d_6): δ 2.70 (d, J = 4.6 Hz, 3H, CH_3), 4.11–4.18 (m, 1H, H-3′), 4.30 (s, 1H, H-4′), 4.54–4.63 (m, 2H, H-2′), 4.67 (br s, 2H, N^6-CH_2Ph), 4.96 (br s, 2H, NH_2), 5.53 (d, J = 6.4 Hz, 1H, OH-2′), 5.71 (d, J = 4.1 Hz, 1H, OH-3′), 5.97 (d, J = 7.6 Hz, 1H, H-1′), 7.10 (t , J = 7.7 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.72 (s, 1H), 8.29 (s, 1H, H-2), 8.44 (s, 2H, H-8), 8.56 (br s, 1H, N^6H-CH_2Ph), 8.80–8.89 (m, 1H, NH-Me).

N^6-(3-Aminobenzyl)adenosine-5′-N-methyluronamide (18). Compound **51a** (100 mg, 0.28 mmol) was dissolved in ethanol (1.5 mL) and treated with 3-aminobenzylamine hydrochloride (**57**, 47 mg, 0.30 mmol) and triethylamine (117 μL, 0.84 mmol). The solution was heated at 80 °C for 12 h in an oil bath. The solvent was evaporated, leaving a solid residue. The residue was treated with 1 N HCl (1.0 mL) and the mixture heated at 80 °C for 45 min. Sodium bicarbonate solution was added until pH 7, and the mixture was extracted three times with ethyl acetate. The solvent was removed under vacuum, and the residue was recrystallized from methanol–water to provide 56 mg of the pure product (50% yield). ^{1}H NMR (DMSO-d_6): δ 2.70 (d, J = 4.5 Hz, 3H, CH_3), 4.11–4.14 (m, 1H, H-3′), 4.30 (s, 1H, H-4′), 4.54–4.64 (m, 3H, H-2′, N^6-CH_2Ph), 4.96 (br s, 2H, NH_2), 5.53 (d, J = 6.7 Hz, 1H, OH-2′), 5.72 (d, J = 3.9 Hz, 1H, OH-3′), 5.96 (d, J = 7.7 Hz, 1H, H-1′), 6.38 (d, J = 7.9 Hz, 1H), 6.46 (d, J = 7.7 Hz, 1H), 6.50 (s, 1H), 6.91 (t, J = 7.8 Hz), 8.28 (s, 1H, H-2), 8.40 (s, 2H, H-8, N^6H-CH_2Ph), 8.87–8.97 (m, 1H, NH-Me).

N^6-(3-Acetamidobenzyl)adenosine-5′-N-methyluronamide (19). 2′,3′-O-Isopropylidene-6-chloropurine-5′-N-methyluronamide (**51a**, 25 mg, 71 μmol), 3-aminobenzylamine hydrochloride (**57**, 11.3 mg, 71 μmol), and triethylamine (29 μL, 0.21 mmol) were dissolved in absolute ethanol (1 mL). The solution was stirred at 85 °C overnight in a sealed vessel. The solvent was evaporated under a stream of nitrogen and dried in vacuo. DMF (0.5 mL) was added followed by triethylamine (25 μL, 0.34 mmol) and acetic anhydride (32 μL, 0.34 mmol). After 30 min the reaction was complete. The solvent was removed under nitrogen, and a preparative TLC was performed (silica, $CHCl_3$–MeOH, 90:10) to recover 22 mg (46 mmol, 64% yield for both steps) of the isopropylidene derivative. ^{1}H NMR ($CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 2.13 (s, 3H, $COCH_3$), 2.61 (d, J = 4.9 Hz, 3H, NHCH_3), 4.71 (s, 1H, H-4′), 4.83 (br s, 2H, N^6-CH_2Ph), 5.33 (s, 2H, H-2′ H-3′), 6.00 (s, 1H, H-1′), 6.75 (br s, 1H, NH-$COCH_3$), 7.09 (d, J = 7.6 Hz, 1H), 7.25 (t, J = 7.8 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.55 (s, 1H), 7.70 (s, 1H, H-2), 7.75 (s, 1H, H-8), 8.32 (br s, 1H, $NHCH_3$).

A 13-mg (27-μmol) sample of the latter compound was dissolved in 1 N HCl (0.5 mL) and warmed for 1 h at 70 °C. After cooling in an ice bath, sodium bicarbonate was added to neutralize. A solid crystallized after standing overnight at 4 °C, filtered, washed with water, and dried to give 11 mg (93% yield) of the title compound. A sample was purified by preparative TLC (silica gel, chloroform–methanol–ammonium hydroxide, 80:20:1) for microanalysis. ^{1}H NMR (DMSO-d_6): δ (s, 3H, $COCH_3$), 2.71 (d, J = 4.3 Hz, 3H, NHCH_3) , 4.13 (m, 1H, H-3′), 4.31 (s, 1H, H-4′), 4.58 (m, 1H, H-2′), 4.67 (m, 2H, N^6-CH_2Ph), 5.53 (d, J = 6.2 Hz, 1H, OH-2′), 5.72 (d, J = 4.2 Hz, 1H, OH-3′), 5.97 (d, J = 7.4 Hz, 1H, H-1′), 7.00 (d, J = 7.3 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 7.45 (s, 1H), 7.49 (d, J = 7.9 Hz, 1H), 8.28 (s, 1H, H-2), 8.42 (s, 1H, H-8), 8.52 (m, 1H, N^6H-CH_2Ph), 8.90 (m, 1H, NHCH$_3$), 9.85 (s, NHCOCH$_3$).

N^6-(3-(Trifluoromethyl)benzyl)adenosine-5′-N-methyluronamide (21). The compound was prepared as described above for **16** using 3-(trifluoromethyl)benzylamine in 60% yield, except that due to aqueous insolubility, the hydrolysis on the isopropylidene protecting group was carried out in a 1:1 mixture of 1 N HCl and methanol. ^{1}H NMR (DMSO-d_6): δ 2.70 (d, J = 4.5 Hz, 3H, CH_3), 4.12–4.17 (m, 1H, H-3′), 4.30 (d, J = 1.2 Hz, 1H, H-4′), 4.54–4.64 (m, 1H, H-2′), 4.78 (br s, 2H, N^6-CH_2Ph), 5.53 (d, J = 6.4 Hz, 1H, OH-2′), 5.72 (d, J = 4.3 Hz, 1H, OH-3′), 5.97 (d, J = 7.5 Hz, 1H, H-1′), 7.49–7.61 (m, 2H), 6.65 (d, J = 7.2 Hz, 1H), 7.71 (s, 1H), 8.29 (s, 1H, H-2), 8.45 (s, 1H, H-8), 8.64 (br s, 1H, N^6H-CH_2Ph), 8.80–8.88 (m, 1H, NH-Me).

N^6-(4-Amino-3-iodobenzyl)adenosine-5′-N-methyluronamide (26). 2′,3′-O-Isopropylidene-N-methyl-6-chloropurine-5′-uronamide (**51a**, 22.2 mg, 63 μmol) and 4-amino-3-iodobenzylamine trifluoroacetate (**61**, 63 μmol) were dissolved in absolute ethanol (1 mL). Triethylamine was added (80 mL), and the solution was stirred at 70 °C overnight. Ethanol was removed under nitrogen. Water (1 mL) was added and then removed from the resulting solid using a Pasteur pipette. The amorphous solid was dissolved in 1 N HCl and stirred at 70 °C for 1 h. After cooling the solution was neutralized with $NaHCO_3$ and then extracted with ethyl acetate (7 × 2 mL). After removal of the solvent in vacuo, the product was purified by column chromatography (silica, $CHCl_3$–MeOH–NH_4OH, 85:10:0.5) to obtain 17 mg (32 μmol, 51% yield overall). ^{1}H NMR (DMSO-d_6): δ 2.71 (d, J = 4.6 Hz, 3H, NHCH_3), 4.14 (m, 1H, H-3′), 4.30 (s, 1H, H-4′), 4.51 (br s, 2H, N^6-CH_2Ph), 4.58 (m, 1H, H-2′), 5.07 (s, 2H, NH_2), 5.52 (d, J = 6.4 Hz, 1H, OH-2′), 5.71 (d, J = 4.2 Hz, 1H, OH-3′), 5.95 (d, J = 7.6 Hz, 1H, H-1′), 6.67 (d, J = 8.1 Hz, 1H), 7.08 (dd, J = 8.3 Hz, J = 1.7 Hz, 1H), 7.56 (d, J = 1.5 Hz, 1H), 8.30 (s, 1H, H-2), 8.36–8.41 (m, 2H, H-8, N^6H-CH_2Ph), 8.90 (m, 1H, NHCH$_3$).

N^6-(3-Sulfobenzyl)adenosine-5′-N-methyluronamide Triethylammonium Salt (27). A suspension of 2′,3′-O-isopropylidene-6-chloropurine-5′-N-methyluronamide (**51a**, 40 mg, 113 μmol), 3-sulfobenzylamine (**66**, 21.2 mg, 113 μmol), and triethylamine (47 μL, 0.339 mmol) in absolute ethanol (1 mL) was stirred at 90 °C overnight in a sealed vessel. The solvent was removed under nitrogen, and the isopropylidene derivative was purified by column chromatography (silica, $CHCl_3$–MeOH–NH_4OH, 80:20:1). The isolated homogeneous band was dissolved in 1 N HCl and warmed at 65 °C for 30 min. The solvent was removed under nitrogen. The title compound was purified using reverse phase cartridges (Alltech Maxiclean, 900 mg) to obtain 38 mg (59% overall yield) as the triethylammonium salt. ^{1}H NMR (DMSO-d_6): δ 1.16 (t, J = 7.1 Hz, 9H, ($CH_3CH_2)_3NH^+$), 2.70 (d, J = 4.6 Hz, 3H, NHCH_3), 3.09 (m, 6H, ($CH_3CH_2)_3$NH), 4.13 (d, J = 4.3 Hz, 1H, H-3′), 4.29 (s, 1H, H-4′), 4.59 (dd, J = 4.6 Hz, J = 7.6 Hz, 1H, H-2′), 4.73 (m, 2H, N^6-CH_2Ph), 5.62 (br s, 2H, OH), 5.95 (d, J = 7.6 Hz, 1H, H-1′), 7.25 (m, 2H), 7.43 (d, J = 7.6 Hz, 1H), 7.60 (s, 1H), 8.29 (s, 1H, H-2), 8.42 (s, 1H, H-8), 8.63 (br s, 1H, N^6H-CH_2Ph), 8.93 (m, 2H, NHCH$_3$, (CH_3-$CH_2)_3NH^+$).

N^6-(4-Sulfobenzyl)adenosine-5′-N-methyluronamide (28). Compound **51a** (30 mg, 85 μmol), p-sulfobenzylamine (**63**, 17 mg, 90 μmol), and triethylamine (34 μL, 0.27 mmol) were combined in absolute ethanol (1 mL) and heated to 90 °C for 3 days. The mixture was filtered, and the filtrate was reduced in volume by

evaporation, leaving a viscous syrup. The isopropylidene-protected intermediate was purified (23.5 mg, recovered) on a TLC plate run in chloroform–methanol–acetic acid (85:10:5). Hydrochloric acid (1 mL, 1 N) was added, and the solution was warmed at 60 °C for 40 min. After cooling and evaporation of the solvent, methanol and ethyl acetate were added. The resulting preciptate was separated and dried to provide 14.3 mg (29% yield) of the title compound. A sample was purified by preparative TLC (chloroform–methanol–ammonium hydroxide, 70:30:1) for microanalysis. ^{1}H NMR (DMSO-d_6): δ 2.69 (d, J = 43.5 Hz, 3H, NHCH_3), 4.15 (d, J = 4.5 Hz, 1H, H-3'), 4.32 (s, 1H, H-4'), 4.58 (dd, J = 4.6 Hz, J = 7.2 Hz, 1H, H-2'), 4.71 (br s, 2H, N^6-CH_2Ph), 5.98 (d, J = 7.4 Hz, 1H, H-1'), 7.27 (d, J = 7.8 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 8.33 (s, 1H, H-2), 8.50 (br s, 2H, H-8, N^6H-CH_2Ph), 8.82 (br s, 1H, NHCH$_3$).

(*R*)-N^6-(1-Phenylethyl)adenosine-5'-*N*-ethyluronamide (30a). 2',3'-*O*-Isopropylidene-6-chloropurine-5'-*N*-ethyluronamide (**51b**, 30 mg, 81 μmol), (*R*)-(+)-α-methylbenzylamine (11.1 μL, 86 μmol), and triethylamine (22 μL, 162 μmol) were dissolved in absolute ethanol (1 mL). The solution was stirred at 80 °C for 24 h in a sealed vessel. The solvent was removed under nitrogen. HCl (1 N) (0.5 mL) was added and the solution stirred at 60 °C for 30 min. After cooling, $NaHCO_3$ was added until pH 7, and the solution was extracted with ethyl acetate (4 × 2 mL). The organic layers were combined, and the solvent was evaporated, leaving 20 mg (48 μmol, 60% yield overall) of the title compound. A sample was purified by preparative TLC for microanalysis, as described for compound **19**. ^{1}H NMR (DMSO-d_6): δ 1.05 (t, J = 7.1 Hz, 3H, CH_3), 1.53 (d, J = 6.8 Hz, 3H, CHCH_3), 3.15–3.28 (m, 2H, CH_2), 4.08–4.17 (m, 1H, H-3'), 4.28 (s, 1H, H-4'), 4.54–4.63 (m, 1H, H-2'), 5.46–5.57 (m, 2H, OH-2' N^6-CHPh), 5.72 (d, J = 4.3 Hz, 1H, OH-3'), 5.94 (d, J = 7.3 Hz, 1H, H-1'), 7.13–7.22 (m, 1H) , 7.28 (t, J = 7 Hz, 2H), 7.44 (d, J = 7.7 Hz, 2H), 8.21 (s, 1H, H-2), 8.42 (br s, 2H, H-8, N^6H-CH_2Ph), 8.83 (m, NH-Et).

(*S*)-N^6-(1-Phenylethyl)adenosine-5'-*N*-ethyluronamide (30b). Same procedure as above using (*S*)-(−)-α-methylbenzylamine. ^{1}H NMR (DMSO-d_6): δ 1.05 (t, J = 7.0 Hz, 3H, CH_3), 1.54 (d, J = 7.2 Hz, 3H, CHCH_3), 3.15–3.26 (m, 2H, CH_2), 4.08–4.15 (m, 1H, H-3'), 4.28 (s, 1H, H-4'), 4.54–4.63 (m, 1H, H-2'), 5.46–5.58 (m, 2H, OH-2', N^6-CHPh), 5.72 (d, J = 4.0 Hz, 1H, OH-3'), 5.94 (d, J = 7.8 Hz, 1H, H-1'), 7.13–7.22 (m, 1H), 7.28 (t, J = 7 Hz, 2H), 7.43 (d, J = 7.7 Hz, 2H), 8.21 (s, 1H, H-2), 8.41 (br s, 2H, H-8, N^6H-CH_2Ph), 8.87 (m, NH-Et).

N^6-(3-Fluorobenzyl)adenosine-5'-*N*-ethyluronamide (31). 2',3'-*O*-Isopropylidene-6-chloropurine-5'-*N*-ethyluronamide (**51b**, 30 mg, 81 μmol) and 3-fluorobenzylamine (10.8 mg, 86 μmol) were dissolved in absolute ethanol (1 mL). Triethylamine (17 μL, 0.12 mmol) was added, and the solution was warmed at 80 °C for 16 h in a sealed vessel. No starting material (R_f = 0.41, silica TLC plates, chloroform–methanol, 95:5) remained in the mixture. The solvent was evaporated under a stream of nitrogen, and water was added to remove the triethylammonium salt. The supernatant was removed and discarded, and the insoluble residue containing intermediate **52b** (R = 3-fluorobenzyl) was used without further purification.

For removal of the isopropylidene group, hydrochloric acid (1 N, 1 mL) was added, and the resulting solution was heated to 60 °C for 45 min or until complete as judged by TLC. The intermediate **52b** and product displayed R_f values of 0.51 and 0.10, respectively (silica, chloroform–methanol, 95:5). After cooling, sodium bicarbonate solution was added to neutralize. A white solid precipitated and was filtered, washed with water, and dried to give 23 mg of the title compound (68% yield overall). ^{1}H NMR (DMSO-d_6): δ 1.06 (t, J = 7.2 Hz, 3H, CH_3), 3.20 (m, 2H, CH_2), 4.00–4.16 (m, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.56–4.64 (m, 1H, H-2'), 4.71 (br s, 2H, N^6-CH_2Ph), 5.53 (d, J = 6.4 Hz, 1H, OH-2'), 5.73 (d, J = 4.3 Hz, 1H, OH-3'), 5.97 (d, J = 7.4 Hz, 1H, H-1'), 7.03 (dt, J = 8.6 Hz, J = 2.0 Hz, H_{benzyl}-5), 7.14 (d, J = 12.7 Hz, H_{benzyl}-2), 7.17 (d, J = 8.3 Hz, H_{benzyl}-6), 7.33 (dd, J = 14.0 Hz, J = 7.9 Hz, H_{benzyl}-4), 8.26 (s, 1H, H-2), 8.44 (s, 1H, H-8), 8.9 (br s, 1H, N^6H-CH_2Ph), 8.83 (t, J = 5.6 Hz, 1H, NH-Et).

N^6-(3-Chlorobenzyl)adenosine-5'-*N*-ethyluronamide (32). To a solution of NECA (50 mg, 0.162 mmol) in DMF (1 mL) was added 3-chlorobenzyl bromide (61 μL, 0.47 mmol), and the solution was stirred in a closed vessel for 2 days at 40 °C. DMF was evaporated under a stream of nitrogen. The residue was treated with acetone (1 mL), and ether (2 mL) was added. The solvent was removed using a Pasteur pipette, and the residue, an amorphous solid, was again extracted with chloroform to remove traces of the benzyl bromide. The dried residue was treated with methanol (2.0 mL), and concentrated NH_4OH (4.0 mL) was added. The mixture was warmed in a closed tube at 90 °C for 2 h with stirring. The mixture was reduced in volume by evaporation and cooled in an ice bath, resulting in precipitation of the chromatographically pure product. The white solid was isolated by filtration to give 43 mg (62% yield) of product which melted at 199–200 °C. ^{1}H NMR (DMSO-d_6): δ 1.06 (t, J = 7.1 Hz, 3H, CH_3), 3.14–3.25 (m, 2H, CH_2), 4.11–4.16 (m, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.56–4.64 (m, 1H, H-2'), 4.71 (br s, 2H, N^6-CH_2Ph), 5.53 (d, J = 6.4 Hz, 1H, OH-2'), 5.72 (d, J = 4.2 Hz, 1H, OH-3'), 5.97 (d, J = 7.6 Hz, 1H, H-1'), 7.2–7.4 (m, 4H, phenyl), 8.26 (s, 1H, H-2), 8.44 (s, 1H, H-8), 8.60 (br s, 1H, N^6H-CH_2Ph), 8.82 (t, J = 5.8 Hz, 1H, NH-Et). Mass spectrum (CI-NH_3): m/e 433 (MH^+, base).

N^6-(2-Nitrobenzyl)adenosine-5'-*N*-ethyluronamide (35). To a solution of NECA (51 mg, 0.165 mmol) in DMF (1 mL) was added 2-nitrobenzyl bromide (107 mg, 0.496 mmol), and the solution was stirred for 2 days at 40 °C. DMF was removed under vacuum giving a syrup that crystallized when acetone and ether were added. The solvent was removed using a Pasteur pipette, and the solid was dried in vacuo and purified by preparative TLC (silica gel, ethyl acetate–2-propanol–water, 4:1:2, upper phase, R_f = 0.25) to give compound 11 in 45% yield. ^{1}H NMR (DMSO-d_6): δ 1.04 (t, J = 6.7 Hz, 3H, CH_3), 3.11–3.27 (m, 2H, CH_2), 4.12–4.18 (m, 1H, H-3'), 4.30 (d, J = 2.0 Hz, 1H, H-4'), 4.49–4.57 (m, 1H, H-2'), 5.55 (s, 2H, N^1-CH_2Ph), 5.61 (br s, 1H, OH-2'), 5.74 (br s, 1H, OH-4'), 5.88 (d, J = 7.1 Hz, 1H, H-1'), 7.06 (s, 1H, N^6H), 7.15 (d, J = 7.8 Hz, 1H, arom), 7.54 (t, J = 7.8 Hz, 1H, arom), 7.68 (t, J = 8.0 Hz, 1H, arom), 8.10 (d, J = 7.5 Hz, 1H, arom), 8.26 (s, 1H, H-2), 8.29 (s, 1H, H-8), 8.43 (t, J = 5.6 Hz, 1H, NH-Et).

Methanol (2.0 mL) and concentrated NH_4OH (2.0 mL) were added to compound 11, and the mixture was warmed in a closed vessel at 90 °C for 45 min. The solvent was evaporated, and the product was purified by preparative TLC (silica gel, ethyl acetate–2-propanol–water, 4:1:2, upper phase, R_f = 0.77) to give 25 mg of the title compound (34% yield overall). Mp: 181 °C dec. A sample was recrystallized from 2-propanol–ethyl ether for microanalysis. ^{1}H NMR (DMSO-d_6): δ 1.05 (t, J = 7.4 Hz, 3H, CH_3), 3.13–3.24 (m, 2H, CH_2), 4.09–4.16 (m, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.56–4.63 (m, 1H, H-2'), 4.94–5.13 (m, 2H, N^6-CH_2Ph), 5.54 (d, J = 7.0 Hz , 1H, OH-2'), 5.73 (d, J = 4.5 Hz , 1H, OH-4'), 5.97 (d, J = 7.7 Hz, 1H, H-1'), 7.46–7.59 (m, 2H, arom), 7.67 (t, J = 7.1 Hz, 1H, arom), 8.04 (d, J = 7.6 Hz, 1H, arom), 8.21 (s, 1H, H-2), 8.47 (s, 1H, H-8), 8.62 (m, 1H, N^6H-CH_2Ph), 8.80 (t, J = 5.5 Hz, 1H, NH-Et).

N^6-(3-Nitrobenzyl)adenosine-5'-*N*-ethyluronamide (36). The title compound was synthesized in 55% yield, according the procedure for compound **35**, using instead 3-nitrobenzyl bromide as starting material. ^{1}H NMR (DMSO-d_6): δ 1.06 (t, J = 7.1 Hz, 3H, CH_3), 3.11–3.24 (m, 2H, CH_2), 4.11–4.14 (m, 1H, H-3'), 4.30 (s, 1H, H-4'), 4.56–4.62 (m, 1H, H-2'), 4.82 (br s, 2H, N^6-CH_2Ph), 5.54 (d, J = 6.5 Hz, 1H, OH-2'), 5.73 (d, J = 4.3 Hz, 1H, OH-3'), 5.97 (d, J = 7.3 Hz, 1H, H-1'), 7.61 (t, J = 8.0 Hz, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.95 (d, J = 7.6 Hz, 1H), 8.22 (br s, 1H), 8.26 (s, 1H, H-2), 8.46 (s, 1H, H-8), 8.68–8.76 (m, 1H, N^6H-CH_2Ph), 8.82 (t, J = 5.5 Hz, 1H, NH-Et).

N^6-(4-Nitrobenzyl)adenosine-5'-*N*-ethyluronamide (37). To a solution of NECA (50 mg, 0.162 mmol) in DMF (0.5 mL) was added 4-nitrobenzyl bromide (53 mg, 0.245 mmol), and the solution was stirred for 2 days at 40 °C. DMF was removed in vacuo giving a syrup that crystallized when acetone and ether were added. The solvent was removed using a Pasteur pipette, and the solid was dried in vacuo and purified by preparative TLC (silica gel, ethyl acetate–2-propanol–water, 4:1:2, upper phase) to give compound **10** in 60% yield. ^{1}H NMR (DMSO-d_6): δ 1.06 (t, J = 7 Hz, 3H, CH_3), 3.20 (q, J = 7.2 Hz, 2H, CH_2), 4.12–4.18 (br s, 1H, H-3'), 4.29 (s, 1H, H-4'), 4.47–4.56 (m, 1H, H-2'), 5.39 (s , 2H, N^1-CH_2Ph), 5.57 (d, J = 6.3 Hz, 1H, OH-2'), 5.69 (d, J = 4.5 Hz, 1H, OH-3'), 5.87 (d, J = 7.3 Hz, 1H, H-1'),

7.58 (d, 2H, J = 8.7 Hz, arom), 8.19 (d, 2H, J = 8.8 Hz, arom), 8.27 (s, 1H, H-2), 8.37 (s, 1H, H-8). FAB$^+$ (m-bullit): m/e 444 (MH^+).

Methanol (1.0 mL) and concentrated NH_4OH (2.0 mL) were added to compound **10**, and the mixture was warmed in a closed vessel at 90 °C for 45 min. The solvent was evaporated, and the product was purified by preparative TLC (silica gel, ethyl acetate–2-propanol–water, 4:1:2, upper phase) to give 21 mg of the pure product (R_f = 0.77, 49% yield). Mp: 196 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.06 (t, J = 7.3 Hz, 3H, CH_3), 3.14–3.24 (m, 2H, CH_2), 4.11–4.17 (m , 1H, H-3′), 4.30 (s, 1H, H-4′), 4.56–4.65 (m, 1H, H-2′), 4.82 (br s, 2H, N^6-CH_2Ph), 5.55 (d, J = 6.3 Hz, 1H, OH-2′), 5.73 (d, J = 4.2 Hz, 1H, OH-3′), 5.97 (d, J = 7.5 Hz, 1H, H-1′), 7.58 (d, 2H, J = 8.6 Hz, arom), 8.17 (d, 2H, J = 8.7 Hz, arom), 8.25 (s, 1H, H-2), 8.46 (s, 1H, H-8), 8.70 (br s, 1H, N^{6}H-CH_2Ph), 8.81 (t, J = 5.6 Hz, 1H, NH-Et). Mass spectrum (CI-NH_3): m/e 444 (MH^+, base).

N^6-(4-Methoxybenzyl)adenosine-5′-*N*-ethyluronamide (41). To a solution of NECA (50 mg, 0.162 mmol) in DMF (0.5 mL) was added 4-methoxybenzyl chloride (33 μL, 0.24 mmol), and the solution was stirred for 3 days at 40 °C. DMF was removed in vacuo, giving a syrup that crystallized when acetone and ether were added. The solvent was removed using a Pasteur pipette. Methanol (1.0 mL) and concentrated NH_4OH (2.0 mL) were added, and the mixture was warmed in a closed vessel at 90 °C for 45 min. The solvent was evaporated, and the product was purified by preparative TLC (silica gel, ethyl acetate–2-propanol–water, 4:1:2) to give 4.9 mg of the pure product which crystallized from methanol (R_f = 0.82, 14% yield). ^{1}H NMR (DMSO-d_6): δ 1.07 (t, J = 7.5 Hz, 3H, CH_3), 3.2 (m, 2H, CH_2), 3.69 (s, OCH_3), 4.12 (d, J = 5.1 Hz, 1H, H-3′), 4.29 (s, 1H, H-4′), 4.59 (dd, J = 7.6 Hz, J = 4.7 Hz, 1H, H-2′), 4.63 (br s, 2H, N^6-CH_2Ph), 5.96 (d, J = 7.7 Hz, 1H, H-1′), 6.84 (d, J = 8.5 Hz, 2H, arom), 7.27 (d, J = 8.7 Hz, 2H, arom), 8.25 (s, 1H, H-2), 8.40 (s, 1H, H-8), 8.45 (br s, 1H, N^{6}H-CH_2Ph), 8.87 (t, J = 4.9 Hz, 1H, NH-Et). Mass spectrum (CI-NH_3): m/e 429 (MH^+, base).

N^6-Benzyladenosine-5′-*N*-cyclopropyluronamide (42). To a solution of adenosine-5′-*N*-cyclopropyluronamide (20 mg, 0.062 mmol) in anhydrous DMF (1 mL) was added benzyl bromide (22 μL, 0.19 mmol), and the solution was stirred for 60 h at 40 °C. DMF was removed in vacuo, giving a syrup that crystallized when acetone and ether were added. The solvent was removed by decantation. Methanol (0.5 mL) and concentrated NH_4OH (2.0 mL) were added, and the mixture was warmed in a closed vessel at 90 °C for 2 h. The mixture was reduced in volume by evaporation and cooled in an ice bath, resulting in precipitation of the chromatographically pure product. The white solid was isolated by filtration, washed with water, and dried to give 15 mg (59% yield) of product which melted at 178–180 °C. ^{1}H NMR (DMSO-d_6): δ 0.46 (m, 2H, CH_2), 0.69 (m, 2H, CH_2), 2.70 (m, 1H, CH_3), 4.13 (m, 1H, H-3′), 4.27 (s, 1H, H-4′), 4.57 (m, 1H, H-2′), 4.71 (br s, 2H, N^6-CH_2Ph), 5.53 (d, J = 6.5 Hz, 1H, OH-2′), 5.73 (d, J = 4.1 Hz, 1H, OH-3′), 5.95 (d, J = 7.5 Hz, 1H, H-1′), 7.15–7.43 (m, 5H, phenyl), 8.22 (s, 1H, H-2), 8.42 (s, 1H, H-8), 8.56 (br s, 1H, N^{6}H-CH_2Ph), 8.88 (d, J = 3.8 Hz, 1H, NH-Me).

2′,3′-Isopropylideneadenosine-5′-carboxylic Acid (44). 2′,3′-Isopropylideneadenosine (**43**, Aldrich Chemical Co., St. Louis, MO, 0.5 g, 1.6 mmol) was dissolved in glacial acetic acid (11 mL), and chromium trioxide (0.222 g, 2.22 mmol) was added. A brown suspension formed, and the color changed gradually to dark green. The suspension was stirred at room temperature for 4 days. The dark solid formed was filtered, washed with water, and crystallized from MeOH to afford a white solid (0.28 g, 54% yield): mp 257 °C; ^{1}H NMR (DMSO-d_6): δ 8.24 (s, 1H, H-8), 8.08 (s, 1H, H-2), 7.28 (br s, 2H, NH_2), 6.33 (s, 1H, H-1′), 5.50 (ABq, 2H, H-2′, H-3′), 4.68 (s, 1H, H-4′), 1.52, 1.35 (s, 3H, Me) ppm. High-resolution MS calcd for $C_{13}H_{15}N_5O_5$ 321.1058, found 321.1073. IR (KBr): ν 3000, 1706 cm^{-1}.

Methyl 2′,3′-Isopropylideneadenosine-5′-carboxylate (45). An excess of diazomethane in ether (0.75 M) was added dropwise over 0.5 h to a suspension of compound **44** (0.22 g, 0.68 mmol) in dioxane–MeOH (1:1, 50 mL) until a clear yellowish solution was obtained. The reaction mixture was stirred at room temprature for 1.5 h. Nitrogen was bubbled through the solution until the yellow color disappeared. The solvent was removed, and the product was dried under high vacuum to produce a white solid (0.211 g, 92% yield): mp 221–222 °C; ^{1}H NMR (DMSO d_6) δ 8.25 (s, 1H, H-8), 8.05 (s, 1H, H-2), 7.32 (s, 2H, NH_2), 6.38 (s, 1H, H-1′), 5.61 (d, J = 6 Hz, 1H, H-2′), 5.43 (d, J = 6 Hz, 1H, H-3′), 4.85 (s, 1H, H-4′), 3.29 (s, 3H, CO_2Me), 1.51, 1.34 (s, 3H, Me) ppm. High-resolution MS calcd for $C_{14}H_{17}N_5O_5$ 335.1238, found 335.1230.

2′,3′-Isopropylidene-6-chloropurine-5′-methyluronamide (51a). 2′,3′-Isopropylideneinosine-5′-carboxylic acid[18] (1.1 g, 3.4 mmol) was added to a solution of thionyl chloride (0.51 mL, 0.68 mmol) and dimethylformamide (0.26 mL) in anhydrous chloroform (43 mL, dried over Al_2O_3). The mixture was heated to reflux with the exclusion of moisture for 6 h. After the mixture was cooled, the solvent was removed in vacuo leaving a syrup that was dissolved in chloroform (12 mL). The solution was cooled to 0 °C, and 2 mL of methylamine dissolved in 20 mL of chloroform was added. After being stirred for 15 min at <10 °C, the solution was extracted successively with HCl (0.1 N, 3 × 60 mL), sodium bicarbonate (0.5 M, 100 mL), and water (2 × 50 mL). The organic layer was dried ($MgSO_4$) and the sovent evaporated leaving 632 mg of the homogeneous (R_f = 0.75, chloroform–methanol–ammonium hydroxide, 80:20:1) title compound (53% yield). ^{1}H NMR (DMSO-d_6): δ 8.80 (s, 1H, purine), 8.73 (s, 1H, purine), 6.50 (s, 1H, H-1′), 5.4–5.5 (m, 2H, H-2′ and H-3′), 4.63 (s, 1H, H-4′), 2.16 (d, J = 4.7 Hz, 3H, NCH_3), 1.53 (s, 3H, i-Pr), 1.34 (s, 3H, i-Pr).

3-Aminobenzylamine Hydrochloride (57). A mixture of 3-nitrobenzylamine hydrochloride (1.0 g, 5.3 mmol) and 5% Pd/C (0.25 g) in methanol (30 mL) was shaken under hydrogen gas (50 psi) for 45 min. After filtration through Celite, the solvent was removed under vacuum to obtain the product in quantitative yield. ^{1}H NMR (MeOD): δ 3.96 (s, 2H, CH_2), 6.67–6.76 (m, 3H, H-2, H-4, H-6), 7.10–7.18 (m, 1H, H-5).

4-Amino-3-iodobenzylamine (61). Compound **59** was prepared by treatment of 4-aminobenzylamine (0.30 g, 2.45 mmol) dissolved in methanol (10 mL) with di-*tert*-butyl dicarbonate (0.36 g, 1.65 mmol), added in portions with stirring over 0.5 h. The solvent was evaporated, and the residue was suspended in ethyl acetate and filtered. The solution was extracted with 0.5 M NaH_2PO_4 (3×) and dried (Na_2SO_4). Removal of solvent in vacuo left a clear oil, 4-((*tert*-butyloxycarbonyl)amino)benzylamine (**59**, 79% yield), which solidified upon standing to form a solid that melted at 72–75 °C.

Compound **59** (29.6 mg, 0.133 mmol) was treated with I_2 (33.8 mg, 0.133 mmol), $CaCO_3$ (16.6 mg), and MeOH (0.4 mL), and the dark mixture was stirred at 70 °C for 6 h. After the mixture was cooled, the solvent was removed under vacuum. Saturated aqueous sodium bisulfite was added and extracted with ethyl acetate (3 × 3 mL). The organic layer was dried under vacuum, and the product was purified by preparative TLC (silica, $CHCl_3$–MeOH, 75:1) to give **60**, 22 mg of 4-((*tert*-butyloxycarbonyl)-amino)-3-iodobenzylamine (47% yield). ^{1}H NMR (MeOD-d_3): δ 1.43 (s, 9H, *t*-Bu), 4.03 (s, 2H CH_2Ph), 6.74 (d, 1H, J = 8.3 Hz), 7.02 (dd, 1H, J = 8.3, 1.9 Hz), 7.5 (br s, 1H).

Compound **60** was deprotected treating with trifluoroacetic acid (0.4 mL) for 10 min at room temperature. The excess of TFA was removed under nitrogen, and the residue, 4-amino-3-iodobenzylamine, was dried under vacuum and used without further purification.

4-Sulfobenzylamine (63). Benzylamine (10.2 mL, 93 mmol) was added dropwise at 0 °C to 11% fuming H_2SO_4 (30 mL). After being stirred at room temperature for 30 min, the solution was warmed to 75 °C for 1 h. After cooling, the solution was poured into cold dioxane (400 mL). The solid obtained was filtered through glass and washed several times with dioxane. The compound was purified by dissolving in a minimum volume of aqueous NH_4OH and then precipitating upon addition of HCl until pH 1 while cooling, to obtain 7.0 g of the homogeneous product (40% yield). ^{1}H NMR (DMSO-d_6): δ 4.02 (br s, 2H, CH_2), 7.38 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 8.11 (br s, 3H, NH_3^+). FAB$^+$ (glycerol): 188 (MH^+).

4-Bromo-3-sulfobenzylamine (65). 4-Bromobenzylamine hydrochloride (2.0 g, 9.0 mmol) was added portionwise to 18–24% fuming H_2SO_4 (4 mL) while cooling at 0 °C. After being stirred at room temperature for 1 h, the wine-colored solution was warmed to 100 °C for another hour. After cooling, the solution was poured into dioxane (40 mL), and the resulting precipitate

was filtered and washed several times with dioxane. The amorphous solid was dissolved in a minimum volume of 4 N NaOH and then reprecipitated following acidification with 6 N HCl. The solid was washed with water and dried to obtain 1.9 g of product (79% yield). ^{1}H NMR (DMSO-d_6): δ 3.96–4.93 (m, 2H, CH_2), 7.26 (dd, J = 8.1 Hz, J = 2.3 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 2.2 Hz, 1H), 8.13 (br s, 3H, NH_3^+). FAB$^+$ (thioglycerol): 266/268 (MH^+).

3-Sulfobenzylamine (66). 4-Bromo-3-sulfobenzylamine (1.0 g, 3.76 mmol) was suspended in 20 mL of water, and 4 N NaOH was added until dissolution (pH = 12.5). Next, 5% Pd (C) was added, and the mixture was hydrogenated (50 psi) overnight. It was filtered through glass wool, and 12 N HCl was added until pH = 1, but no precipitate formed. Inorganic salts precipitated following addition of ethanol. The remaining solution was partially lyophilized, and the solid was filtered and dried leaving 440 mg of the pure product (63% yield). ^{1}H NMR (DMSO-d_6): δ 4.03 (q, J = 5.8 Hz, 2H), CH_2), 7.33–7.38 (m, 2H), 7.57–7.63 (m, 1H), 7.78 (s, 1H), 8.13 (br s, 3H, NH_3^+).

Compounds **15, 17, 20, 22, 23, 25, 33, 34,** and **38** were synthesized according to the procedure for compound **16**, using as starting materials the correspondening benzylamines. Compounds **12, 14, 39,** and **40** were synthesized according with the procedure for compound **32**.

Biological Methods: Receptor Binding. Materials. F-12 (Ham's) medium, fetal bovine serum (FBS) and penicillin/streptomycin were from Gibco BRL (Gaithersburg, MD). [^{125}I]APNEA was prepared as descibed previously.[26] [^{3}H]R-PIA was from Amersham (Arlington Heights, IL), and [^{3}H]CGS 21680 was from DuPont NEN (Boston, MA). Adenosine deaminase (ADA) was from Boehringer Mannheim (Indianapolis, IN). Composition of lysis buffer: 10 mM Tris/5 mM EDTA, pH 7.4 at 5 °C. 50/10/1 buffer: 50 mM Tris; 10 mM $MgCl_2$; 1 mM EDTA, pH 8.26 at 5 °C. All other materials were from standard local sources and of the highest grade commercially available.

Cell Culture and Membrane Preparation. CHO cells stably expressing the A_3 receptor[6] were grown in F-12 medium containing 10% FBS and penicillin/streptomycin (100 U/mL and 100 μg/mL, respectively) at 37 °C in a 5% CO_2 atmosphere. When cells had reached confluency, they were washed twice with Dulbecco's phosphate buffer solution before dislodging after addition of 3 mL of trypsin-EDTA. For the final passage cells were grown in 150- × 50-mm tissue culture dishes. Cells were washed twice with 10 mL of lysis buffer. After addition of 5 mL of lysis buffer, cells were mechanically scraped and homogenized in an ice-cold Dounce homogenizer (20 strokes by hand). The suspension was centrifuged at 43000g for 10 min. The pellet was resuspended in the minimum volume of ice-cold 50/10/1 buffer required for the binding assay and homogenized in a Dounce homogenizer. Typically, six to eight 175-cm^2 flasks were used for a 48-tube assay. Adenosine deaminase was added to a final concentration of 3 units/mL, and the suspension was incubated at 37 °C for 15 min; the membrane suspension was subsequently kept on ice until use. When large batches (ca. 100 flasks) were processed, homogenization was performed with a Polytron (Brinkman, Luzern, Switzerland) and further workup was as described above. The preparation was stored at −70 °C and retained its [^{125}I]APNEA binding properties for at least 1 month.

Radioligand Binding. Binding of [^{125}I]APNEA to CHO cells stably transfected with the A_3 receptor clone was performed essentially as described.[26] Assays were performed in 50/10/1 buffer in glass tubes and contained 100 μL of the membrane suspension, 50 μL of [^{125}I]APNEA (final concentration 0.5 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]APNEA 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 40 μM R-PIA. K_i values were calculated according to Cheng–Prusoff,[27] assuming a K_d for [^{125}I]APNEA of 17 nM.[8] Alternately, [^{125}I]-4-amino-3-iodobenzyladenosine-5'-N-methyluronamide[28] was used as the radioligand (final concentration 0.1 nM, K_d = 1.6 nM) with no significant difference in K_i values compared to [^{125}I]APNEA.

Binding of [^{3}H]PIA to A_1 receptors from rat brain membranes and of [^{3}H]CGS 21680 to A_2 receptors from rat striatal membranes was performed as described previously.[8,23]

Rat cerebral cortical membranes and striatal membranes were prepared[18] and treated with adenosine deaminase (2 units/mL) for 30 min at 37 °C prior to storage at −70 °C. Solid samples of the adenosine derivatives were dissolved in DMSO and stored in the dark at −20 °C. The stock solutions were diluted with DMSO to a concentration of ≤0.1 mM prior to adding to the aqueous medium. The final concentration of DMSO in the assay medium was generally 2%.

At least six different concentrations spanning 3 orders of magnitude, adjusted appropriately for the IC_{50} of each compound, were used. IC_{50} values, computer-generated using a nonlinear regression formula on the GraphPAD program (Institute for Scientific Information), were converted to apparent K_i values using K_d values[23] of 1.0 and 14 nM for [^{3}H]PIA and [^{3}H]CGS 21680 binding, respectively, and the Cheng–Prusoff equation.[27]

Acknowledgment. C.G. and N.M. thank the Cystic Fibrosis Foundation for financial support. The technical assistance of Ms. Qitian Pu is acknowledged.

References

(1) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Perspective. Adenosine receptors: pharmacology, structure-activity relationships and therapeutic potential. *J. Med. Chem.* **1992**, *35*, 407–422.
(2) Libert, F.; Schiffmann, S. N.; Lefort, A.; Parmentier, M.; Gerard, C.; Dumont, J. E.; Vanderhaeghen, J.-J.; Vassart, G. The orphan receptor cDNA RDC7 encodes an A1 adenosine receptor. *EMBO J.* **1991**, *10*, 1677–1682.
(3) Maenhaut, C.; Sande, J. v.; Libert, F.; Abramowicz, M.; Parmentier, M.; Vanderhaegen, J. J.; Dumont, J. E.; Vassart, G.; Schiffmann, S. RDC8 codes for an adenosine A2 receptor with physiological constitutive activity. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 1169–1178.
(4) Stiles, G. L. Adenosine receptors. *J. Biol. Chem.* **1992**, *267*, 6451–6454.
(5) Arend, L.; Gusovsky, F.; Handler, J. S.; Rhim, J. S.; Daly, J. W.; Spielman, W. S. Adenosine sensitive phosphoinositide turnover in a newly established renal cell line. *Am. J. Physiol.* **1989**, *256*, F1067.
(6) Zhou, Q. Y.; Li, C. Y.; Olah, M. E.; Johnson, R. A.; Stiles, G. L.; Civelli, O. Molecular cloning and characterization of an adenosine receptor - the A3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7432–7436.
(7) Meyerhof, W.; Müller-Brechlin, R.; Richter, D. Molecular cloning of a novel putative G-protein coupled receptor expressed during rat spermiogenesis. *FEBS Lett.* **1991**, *284*, 155–160.
(8) (a) van Galen, P. J. M.; van Bergen, A. H.; Gallo-Rodriguez, C.; Melman, N.; Olah, M. E.; IJzerman, A. P.; Stiles, G. L.; Jacobson, K. A. Unpublished data. (b) van Bergen, A.; van Galen, P. J. M.; Stiles, G. L.; Jacobson, K. A. A_3 receptors: Structure activity relationships and molecular modeling. ACS 206th National Meeting, Chicago, IL, August 1993, Abstract MEDI217.
(9) Van Galen, P. J. M.; Stiles, G. L.; Michaels, G.; Jacobson, K. A. Adenosine A_1 and A_2 receptors: Structure-function relationships. *Med. Res. Rev.* **1992**, *12*, 423–471.
(10) Ramkumar, V.; Stiles, G. L.; Beaven, M. A.; Ali, H. The A_3AR is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J. Biol. Chem.* **1993**, *268*, 168871–6890.
(11) Ribeiro, J. A.; Sebastiao, A. M. Adenosine receptors and calcium: basis for proposing a third (A3) adenosine receptor. *Prog. Neurobiol.* **1986**, *26*, 179–209.
(12) Carruthers, A. M.; Fozard, J. M. Adenosine A3 receptors: two into one won't go. *Trends Pharm. Sci.* **1993**, *14*, 290–291.
(13) Fozard, J. R.; Carruthers, A. M. Adenosine A3 receptors mediate hypotension in the angiotensin II-supported circulation of the pithed rat. *Br. J. Pharmacol.* **1993**, *109*, 3–5.
(14) IJzerman, A. P.; van Galen, P. J. M.; Jacobson, K. A. Molecular modeling of adenosine receptors. I. The ligand binding site on the A_1 receptor. *Drug Design Disc.* **1992**, *9*, 49–68.
(15) Schwabe, U.; Trost, T. Characterization of adenosine receptors in rat brain by (−) [^{3}H]N^6-phenylisopropyladenosine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *313*, 179–187.
(16) Jarvis, M. F.; Schutz, R.; Hutchison, A. J.; Do, E.; Sills, M. A.; Williams, M. [^{3}H]CGS 21680, an A_2 selective adenosine receptor agonist directly labels A_2 receptors in rat brain tissue. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888–893.
(17) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. Functionalized congeners of 1,3-dialkylxanthines: preparation of analogues with high affinity for adenosine receptors. *J. Med. Chem.* **1985**, *28*, 1334–1340.

(18) Olsson, R. A.; Kusachi, S.; Thompson, R. D.; Ukena, D.; Padgett, W.; Daly, J. W. N^6-substituted N-alkyladenosine-5′-uronamides: bifunctional ligands having recognition groups for A_1 and A_2 adenosine receptors. *J. Med. Chem.* **1986**, *29*, 1683–9.
(19) *Chemistry of Nucleosides and Nucleotides*; Townsend, L., Ed.; Plenum Press: New York, 1988; Vol. 1, p 239.
(20) Ukena, D.; Olsson, R. A.; Daly, J. W. Definition of subclasses of adenosine receptors associated with adenylate cyclase: interaction of adenosine analogs with inhibitory A1 receptors and stimulatory A2 receptors. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 365–76.
(21) Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. Structure-activity relationships for N^6-substituted adenosines at a brain A_1-adenosine receptor with a comparison to an A_2-adenosine receptor regulating coronary blood flow. *Biochem. Pharmacol.* **1986**, *35*, 2467–2481.
(22) Sweetnam, P. M.; Caldwell, L.; Lancaster, J.; Bauer, C., Jr.; McMillan, B.; Kinnier, W. J.; Price, C. H. The role of receptor binding in drug discovery. *J. Nat. Prod.* **1993**, *56*, 441–455.
(23) Jacobson, K. A.; Nikodijevic, O.; Ji, X. D.; Berkich, D. A.; Eveleth, D.; Dean, R. L.; Hiramatsu, K.; Kassell, N. F.; van Galen, P. J. M.; Lee, K. S.; Bartus, R. T.; Daly, J. W.; Lanoue, K. F.; Maillard, M. Synthesis and biological activity of N^6-(p-sulfophenyl)alkyl and N^6-sulfoalkyl derivatives of adenosine. Water soluble and peripherally selective adenosine agonists. *J. Med. Chem.* **1992**, *35*, 4143–4149.
(24) Daly, J. W.; Padgett, W. L. Agonist activity of 2- and 5′-substituted adenosine analogs and their N^6-cycloalkyl derivatives at A_1-adenosine and A_2-adenosine receptors coupled to adenylate cyclase. *Biochem. Pharmacol.* **1992**, *43*, 1089–1093.
(25) Jacobson, K. A.; Trivedi, B. K.; Churchill, P. C.; Williams, M. Novel Therapeutics Acting via Purine Receptors. *Biochem. Pharmacol.* **1991**, *41*, 1399–1410.
(26) Stiles, G. L.; Daly, D. T.; Olsson, R. A. The A_1 adenosine receptor. Identification of the binding subunit by photoaffinity cross-linking. *J. Biol. Chem.* **1985**, *260*, 10806–10811.
(27) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC50) of an enzyme reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(28) Olah, M. E.; Gallo-Rodriguez, C.; Jacobson, K. A.; Stiles, G. L. *Mol. Pharmacol.*, in press.
(29) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. *Mol. Pharmacol.* **1986**, *29*, 331–346.