Structure-Activity Relationships of 9-Alkyladenine and Ribose-Modified Adenosine Derivatives at Rat A_3 Adenosine Receptors†

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9-Alkyladenine derivatives and ribose-modified N^6-benzyladenosine derivatives were synthesized in an effort to identify selective ligands for the rat A_3 adenosine receptor and leads for the development of antagonists. The derivatives contained structural features previously determined to be important for A_3 selectivity in adenosine derivatives, such as an N^6-(3-iodobenzyl) moiety, and were further substituted at the 2-position with halo, amino, or thio groups. Affinity was determined in radioligand binding assays at rat brain A_3 receptors stably expressed in Chinese hamster ovary (CHO) cells, using [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)adenosine-5'-(*N*-methyluronamide)), and at rat brain A_1 and A_{2a} receptors using [^{3}H]-N^6-PIA ((*R*)-N^6-phenylisopropyladenosine) and [^{3}H]CGS 21680 (2-[[[4-(2-carboxyethyl)-phenyl]ethyl]amino]-5'-(*N*-ethylcarbamoyl)adenosine), respectively. A series of N^6-(3-iodobenzyl) 2-amino derivatives indicated that a small 2-alkylamino group, e.g., methylamino, was favored at A_3 receptors. N^6-(3-Iodobenzyl)-9-methyl-2-(methylthio)adenine was 61-fold more potent than the corresponding 2-methoxy ether at A_3 receptors and of comparable affinity at A_1 and A_{2a} receptors, resulting in a 3-6-fold selectivity for A_3 receptors. A pair of chiral N^6-(3-iodobenzyl) 9-(2,3-dihydroxypropyl) derivatives showed stereoselectivity, with the *R*-enantiomer favored at A_3 receptors by 5.7-fold. 2-Chloro-9-(β-D-erythrofuranosyl)-N^6-(3-iodobenzyl)adenine had a K_i value at A_3 receptors of 0.28 μM. 2-Chloro-9-[2-amino-2,3-dideoxy-β-D-5-(methylcarbamoyl)-arabinofuranosyl]-N^6-(3-iodobenzyl)adenine was moderately selective for A_1 and A_3 vs A_{2a} receptors. A 3'-deoxy analogue of a highly A_3-selective adenosine derivative retained selectivity in binding and was a full agonist in the inhibition of adenylyl cyclase mediated via cloned rat A_3 receptors expressed in CHO cells. The 3'-OH and 4'-CH_2OH groups of adenosine are not required for activation at A_3 receptors. A number of 2',3'-dideoxyadenosines and 9-acyclic-substituted adenines appear to inhibit adenylyl cyclase at the allosteric "P" site.

Introduction

Adenosine is a ubiquitous chemical messenger or "local hormone" involved in regulation of many physiological functions.[1] There are three classes of adenosine receptors: A_1, A_2, and A_3. Tremendous advances have been made in recent years in the synthesis of selective agents acting at subtypes of adenosine receptors.[2] Selective adenosine antagonists are under development for use in cognitive diseases (A_1),[3,4] renal failure (A_1),[5] Parkinson's and Huntington's diseases (A_2),[6] and cardiac arrhythmias (A_1).[7] Adenosine agonists (A_1 and A_3) are likewise of potential therapeutic interest as cerebroprotective agents, antiepileptic drugs, etc.[4]

The A_3 receptor was only recently discovered,[8] with its cloning from a rat brain library. When expressed in Chinese hamster ovary (CHO) cells, rat A_3 receptors were found to inhibit adenylyl cyclase. A_3 receptors are also present in the RBL-2H3 (rat basophilic leukemia) cell line, where adenosine activates phospholipase C.[9] Fozard and Carruthers[10] have attributed to A_3 receptor activation a component of the hypotensive effects of adenosine agonists in rats that is not antagonized by xanthines. Activation of A_3 receptors has been suggested by Downey and colleagues[11] to be involved in the cardioprotective effects of preconditioning by adenosine agonists in rabbits. The occurrence of A_3 receptors in the testes and brain[12-14] also suggests that it may be important in regulation of reproduction and CNS function. It has been suggested that A_3-selective antagonists might have anti-inflammatory properties.[15] Recently, the A_3 receptor was found to be localized on eosinophils in the human lung, and tissue from patients with pulmonary disease showed differential occurrence of A_3 receptor expression.[16] MacKenzie et al.[29] reported evidence that A_3 receptor activation inhibits the adhesion of killer lymphocytes to adenocarcinoma cells.

We have studied in detail the structure-activity relationships (SAR) for N^6- and 5'-substituted adenosine derivatives[17,18] as agonists at rat A_3 receptors and for alkylxanthines as antagonists.[19] We recently reported that an adenosine derivative, N^6-(3-iodobenzyl)-5'-(*N*-

† Abbreviations: [^{125}I]AB-MECA, N^6-(4-amino-3-iodobenzyl)adenosine-5'-(*N*-methyluronamide); ADA, adenosine deaminase; AIBN, 2,2'-azobis(2-methylpropionitrile); CGS 21680, 2-[[[4-(2-carboxyethyl)-phenyl]ethyl]amino]-5'-(*N*-ethylcarbamoyl)adenosine; CHO, Chinese hamster ovary; CNS, central nervous system; DAST, (diethylamino)-sulfur trifluoride; DMAP, 4-(dimethylamino)pyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; HMDS, 1,1,1,3,3,3-hexamethyldisilazane; IB-MECA, N^6-(3-iodobenzyl)adenosine-5'-(*N*-methyluronamide); K_i, inhibition constant; NECA, 5'-(*N*-ethylcarbamoyl)adenosine; PIA, (*R*)-N^6-phenylisopropyladenosine; THF, tetrahydrofuran; Tris, tris(hydroxymethyl)aminomethane; XAC, 8-[4-[[[[(2-aminoethyl)amino]carbonyl]-methyl]oxy]phenyl]-1,3-dipropylxanthine.

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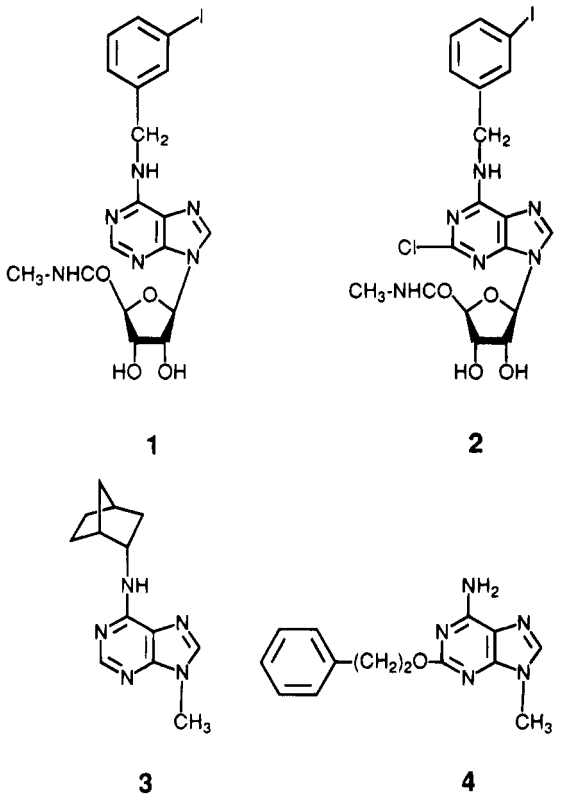


Figure 1. Structures of adenosine (**1** and **2**) and adenine (**3** and **4**) derivatives studied as adenosine receptor A_3 agonists and A_1/A_2 antagonists, respectively.

methylcarbamoyl)adenosine (IB-MECA, **1**; Figure 1), is a 50-fold selective agonist for rat brain A_3 vs A_1 or A_{2a} receptors and is selective in *in vivo* behavioral experiments.[14] Additional structure–activity probing led us to the highly A_3-selective agonist N^6-(3-iodobenzyl)-2-chloro-5′-(*N*-methylcarbamoyl)adenosine (Cl-IB-MECA, **2**).

Rat A_3 receptors are unlike A_1 and A_2 receptors in lack of antagonism by the usual high-affinity xanthine ligands, such as the xanthine amine congener, XAC. An A_3 antagonist that is selective in rodents is lacking. Many xanthines that are potent antagonists at A_1 and A_2 receptors in the rat, rabbit, and human only weakly displaced the binding of radioligand from cloned rat A_3 receptors.[20] Linden et al.[12] found that certain xanthines do bind appreciably to cloned sheep A_3 receptors but generally with less affinity than at A_1 and A_2 receptors in a variety of species. The human A_3 receptor was recently cloned,[13] and its phamacological profile was found to resemble that of the sheep A_3 receptor, i.e., many potent xanthines bind in the submicromolar range. We have studied the unusually large species dependence of affinity at A_3 receptors.[21] Xanthines that are generally A_3-selective across species are needed as pharmacological and biochemical probes, in order to define more clearly the physiological role, distribution, and regulation of A_3 adenosine receptors.

A class of 9-alkyladenine derivatives was reported to act as antagonists at A_1 or A_{2a} receptors.[22–24] There are parallels in the structural determinants of affinity among adenine derivatives (antagonists) and those of the corresponding 9-ribosides (agonists) at A_1 receptors. These structural features include cycloalkyl groups, such as the N^6-cyclopentyl group, leading to selectivity for A_1 receptors.[23] The N^6-cycloalkyladenine derivative (*R,S*)-N-0861, **3** (Figure 1), is 610-fold selective for A_1 receptors.[7] A similar attempt to introduce parallel A_{2a} selectivity in 9-methyladenine derivatives, using 2-substitution known to favor that subtype when present in adenosine analogues, was less successful.[24] 2-[(Phenylethyl)oxy]-9-methyladenine, **4**, for example, distinguishes between subtypes of A_2 receptors and appears to be selective for the A_{2a} subtype in the coronary vasculature but is nonselective between A_{2a} and A_1 receptors.[24] In the present study we have applied to the 9-alkyladenines the structural features we have determined to be important for A_3 selectivity when occurring in adenosine derivatives, including both N^6- and 2-substituents.[17,18]

Results

Chemical Synthesis. Adenine analogues modified with the N^6-(3-iodobenzyl) group were synthesized (chemical characterization in Table 1, structures and biological properties in Tables 2 and 3). Scheme I outlines the synthesis of 9-methyl derivatives of adenine. The synthesis of analogues with non-methyl substitution at the 9-position is shown in Scheme 2. The N^6-(3-iodobenzyl) substituent in adenine derivatives is likely to be well suited for A_3 affinity, on the basis of an assumed parallel in structure–activity relationship with adenosine derivatives. N^6-(3-Iodobenzyl)adenosine is the only singly substituted adenosine derivative reported to be selective for A_3 receptors in rat brain.[18] Additional modifications were made at the 9-position, using groups other than methyl, and by substituting at the 2-position. Compounds **8–24** contain acyclic substituents at the 9-position of adenine, and compounds **25–40** contain cyclic substituents. Adenine nucleoside analogues, containing erythrose (Scheme 3), modified 3′-deoxy- (**35**) or 2′,3′-dideoxyribose (**29**), 2′-substituted 2′,3′-dideoxyarabinose (**30–32**), arabinose (**39**), or talose (**40**) sugars, were included. Procedures for synthesis of 3′-deoxy and 2′,3′-dideoxy analogues are outlined in Schemes 4–6.

Scheme 1 shows the route used to synthesize 9-methyladenine derivatives. The synthesis of the 2-unsubstituted adenine derivative was carried out by substitution of 6-chloropurine, **41**, using 3-iodobenzylamine, to provide N^6-(3-iodobenzyl)adenine, **44**. This was followed by alkylation at the 9-position, resulting in the 9-methyl analogue **8**. Alternately, 2-substitution was introduced at the first synthetic stage with 2,6-dichloropurine, **42**, or 2-amino-6-chloropurine, **43**, carried through the same sequence, leading to compound **14** or **15**, respectively. 2-Chloro-N^6-(iodobenzyl)adenine, **45a**, was prepared as reported,[18] except that the reaction condition used was at 50 °C for 3 h followed by stirring overnight at room temperature, resulting in an improved yield (70%). The 2-chloro group was readily replaced at elevated temperature by various nucleophiles, such as amines (leading to compounds **16–20**) or alkoxides (leading to compounds **21** and **22**). Compound **23** was the unanticipated product of the reaction of **14** with sodium hydrosulfide in the presence of pyridine. The expected product, the corresponding 2-thiol, was not detected.

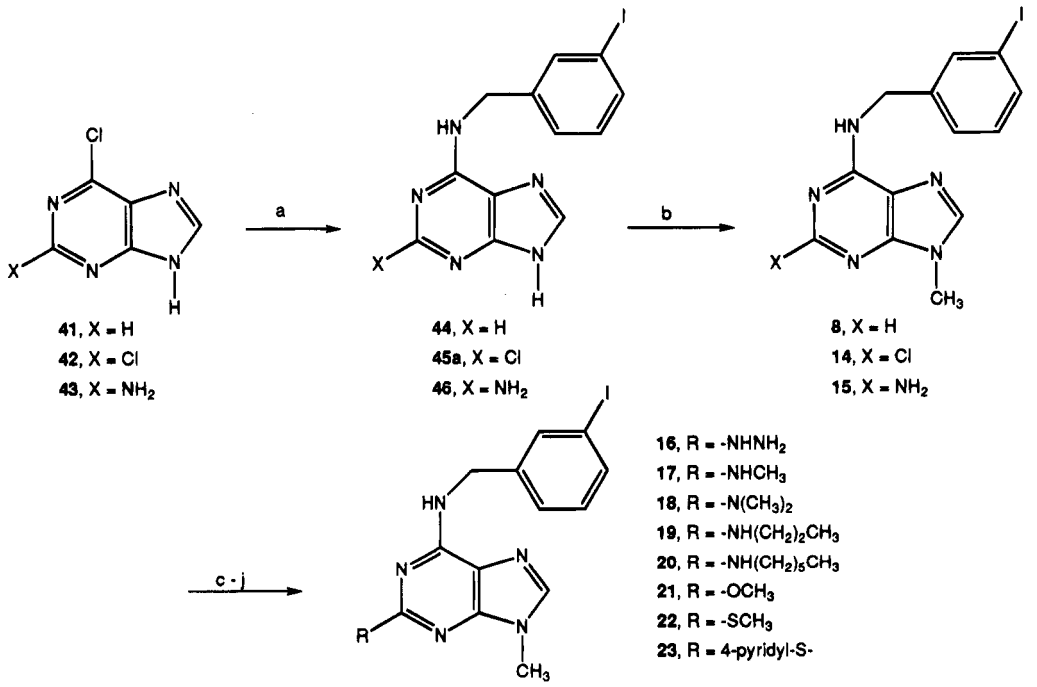
Combinations of 2- and 6-modifications with 9-substituents larger than methyl were made according to Scheme 2. A 9-(2,3-dihydroxypropyl) substituent was introduced as the isopropylidene-protected form, and the protecting group was later cleaved in acid. Replacement

Table 1. Characterization of 9-Alkyladenine and Ribose-Modified Adenosine Derivatives

compd no.	mp (°C)	MS	formula	anal.
8	159–161	366 (CI)	$C_{13}H_{12}N_5I_1 \cdot 0.3EtOAc$	C, H, N
9	185–187		$C_{14}H_{14}IN_5O$	C, H, N
10	125–128		$C_{15}H_{16}IN_5O_2 \cdot 1H_2O$	C, H, N
11	126–127		$C_{15}H_{16}IN_5O_2$	C, H, N
12	160 dec		$C_{14}H_{12}IN_5O_2 \cdot 0.5H_2O$	C, H, N
13	oil	418 (EI)	$C_{16}H_{15}IN_6 \cdot 1.5H_2O$	C, H; N[b]
14	192–193	400 (CI)	$C_{13}H_{11}N_5Cl_1I_1$	C, H, N
15	203–205	381 (CI)	$C_{13}H_{13}N_6I_1$	*a*
16	202–203	396 (CI)	$C_{13}H_{14}N_7I_1 \cdot 0.2C_6H_{14}$	C, H, N
17	185–186	395 (CI)	$C_{14}H_{15}N_6I_1$	*a*
18	190–191	409 (CI)	$C_{15}H_{17}N_6I_1 \cdot 0.6MeOH$	C, H, N
19	134–135	423 (CI)	$C_{16}H_{19}N_6I_1$	C, H, N
20	138	465 (CI)	$C_{19}H_{25}N_6I_1 \cdot 0.35C_6H_{14}$	C, H, N
21	159	396 (CI)	$C_{14}H_{14}N_5O_1I_1 \cdot 0.2C_6H_{14} \cdot 0.5MeOH$	C, H, N
22	160–161	412 (CI)	$C_{14}H_{13}N_5S_1I_1 \cdot 0.35C_6H_{14}$	C, H, N
23	199 dec	474 (CI)	$C_{18}H_{15}N_6S_1I_1$	*a*
24	130		$C_{16}H_{18}IN_5O_2S_1$	*a*
25	145–147		$C_{16}H_{15}N_5O_3Cl_1I_1$	C, H, N
26	158–161		$C_{17}H_{19}N_6O_3I_1$	*a*
27	180–182	456 (CI)	$C_{16}H_{15}N_5O_1Cl_1I_1$	*a*
29	130	387(CI)	$C_{18}H_{19}N_6O_2C_1$	*a*
30	184	553 (EI)	$C_{18}H_{17}N_9O_2Cl_1I_1$	*a*
31	98	528 (CI)	$C_{18}H_{19}N_7O_2Cl_1I_1$	*a*
32	119–129		$C_{18}H_{17}N_6O_2Cl_1I_1F_1 \cdot 2H_2O$	C, H, N
33	foam	571 (CI)	$C_{20}H_{20}N_6O_4Cl_1I_1$	*a*
34	foam	607 (CI)	$C_{19}H_{20}N_6O_5Cl_1I_1S_1$	C, H, N
35	162	528 (EI)	$C_{18}H_{18}N_6O_3Cl_1I_1$	C, H, N
36	foam	759 (EI)	$C_{29}H_{43}N_5O_5Cl_1I_1Si_1$	*a*
37	120 dec		$C_{19}H_{16}N_6O_4Cl_1I_1S_1$	C, H, N
38	foam	753 (CI)	$C_{32}H_{26}N_6O_6Cl_1I_1$	*a*

[a] High-resolution mass in FAB^+ mode m/z determined to be within acceptable limits. **15**: calcd, 381.0325; found, 381.0335. **17**: calcd, 395.0481; found, 395.0463. **23**: calcd, 475.0202; found, 475.0201. **27**: calcd, 456.0078; found, 456.0077. **29**: calcd, 386.1258; found, 386.1249. **30**: calcd, 553.0239; found, 553.0226. **31**: calcd, 527.0333; found, 527.0318. **33**: calcd, 571.0358; found, 571.0361. **36**: calcd, 760.1615; found, 760.1614. **38**: calcd, 753.0725; found, 753.0745. [b] N: calcd, 18.87; found, 17.65.

Scheme 1[a]



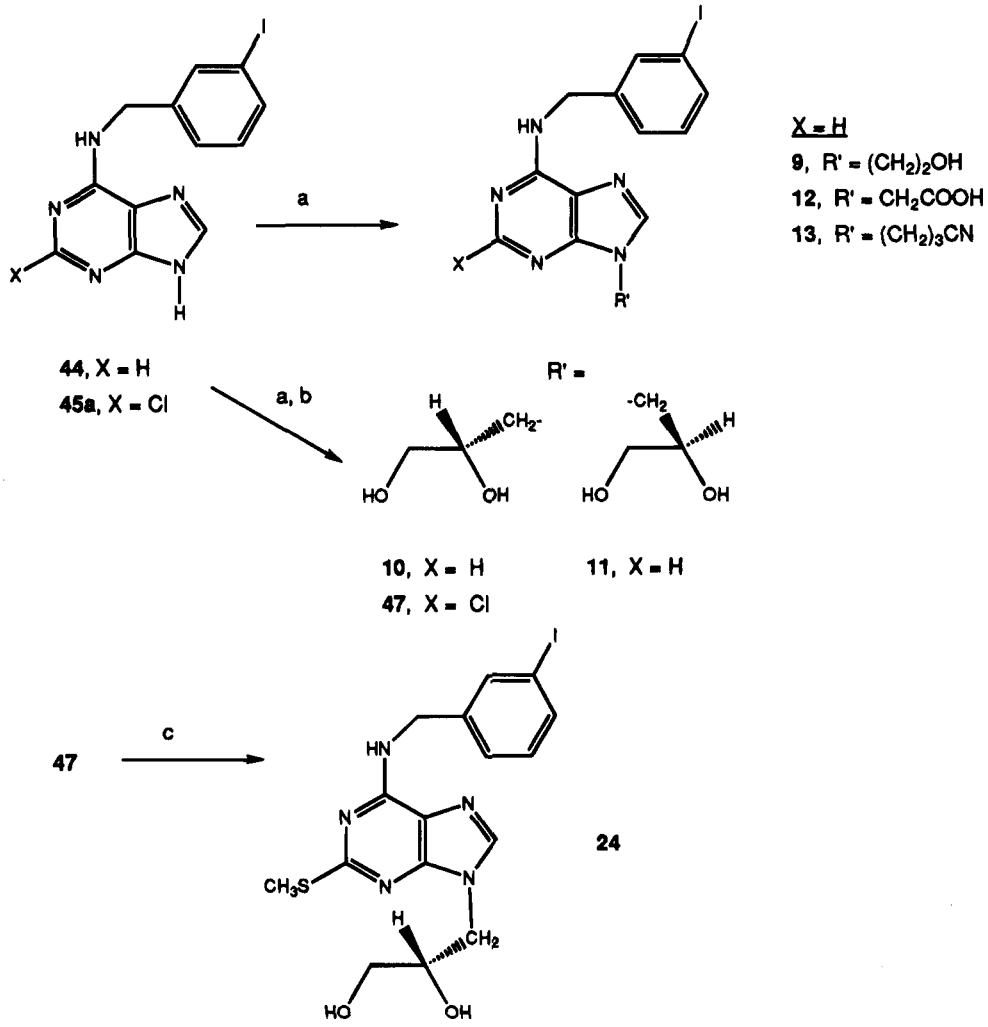
[a] Reagents: (a) 3-iodobenzylamine-HCl, triethylamine, EtOH, rt; (b) CH_3I, K_2CO_3, DMF; (c) NH_2NH_2; (d) NH_2CH_3/THF; (e) DMF, triethylamine, $CH_3O_2CCH_2NH_2$-HCl; (f) $CH_3(CH_2)_2NH_2$; (g) $CH_3(CH_2)_5NH_2$; (h) $NaOCH_3$, MeOH; (i) $NaSCH_3$, DMF–DME; (j) NaSH, pyridine.

of 2-chloro with the methylthio group was carried out as the final step, leading to compound **24**.

The synthesis of a 9-erythrose derivative, **25**, is shown in Scheme 3. Only the β-isomer was isolated from the condensation of N^6-(3-iodobenzyl)-2-chloroadenine, **45b**, with triacetylerythrose, **49**. The synthesis of compound **27**, a tetrahydrofuran derivative, was based on a similar procedure by Olsson and co-workers.[23]

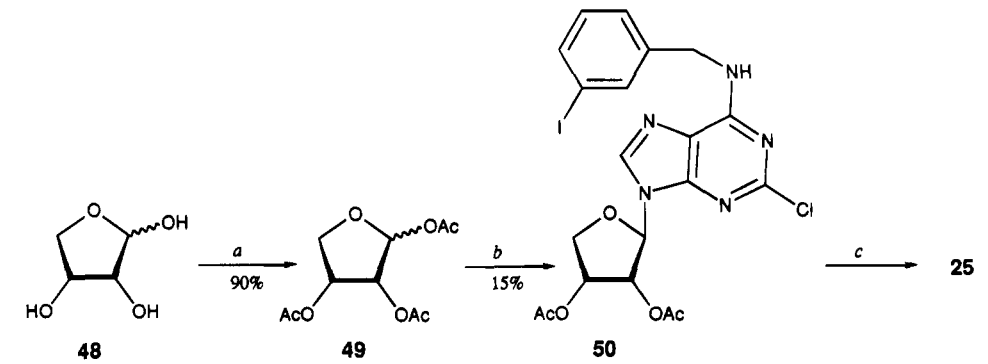
Synthesis of ribose- and arabinose-modified analogues began with 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranoside, **51** (Scheme 4). Following conversion of the 3-hydroxyl group to the 3-xanthate *in situ*, the material

Scheme 2[a]



[a] Reagents: (a) R'I (**9**, **12**), R'Br (**13**), or (2,2-dimethyl-1,3-dioxolan-4-yl)methyl *p*-toluenesulfonate, *R* (**10**, **47**) or *S* (**11**), and K_2CO_3, DMF; (b) 1 N HCl, 90 °C, 1 h; (c) $NaSCH_3$, DMF.

Scheme 3[a]

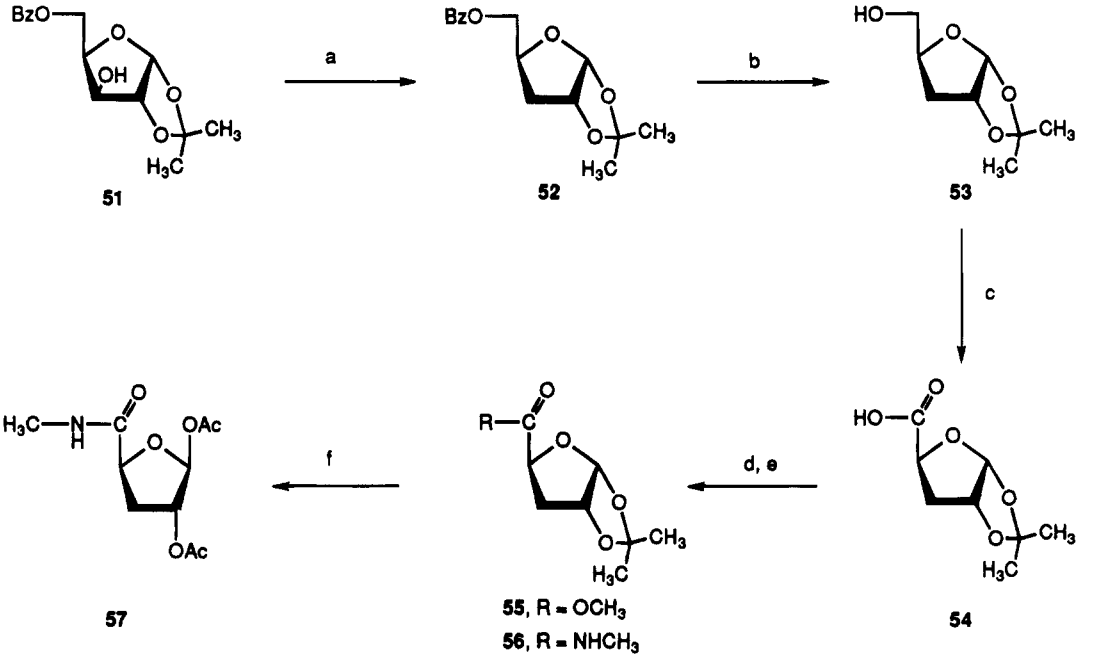


[a] Reaction conditions: (a) Ac_2O, pyridine, rt, 24 h; (b) $SnCl_4$, MeCN, N^6-(3-iodobenzyl)-2-chloroadenine, 50 °C; (c) conc NH_4OH, reflux.

was deoxygenated by the action of tributyltin hydride and triethylborane, to give compound **52**. Debenzoylation of the 5-position and oxidation of resulting alcohol **53** yielded acid **54** in good yields. The methylamide at the 5-position of compound **56** was introduced by esterification of the carboxylic acid to yield compound **55** followed by displacement with methylamine in a sealed bottle. The 1,2-isopropylidene group of compound **56** was cleaved, and the diol was acetylated in one pot by conventional methods to give compound **57**. This sugar intermediate was condensed with the silylated adenine base **45b** by a modified Vorbrüggen method[37] to produce compound **33**, which was deprotected in methanolic ammonia to yield 3'-deoxy-2-chloro-IB-MECA, **35**. Deoxygenation of compound **35** via intermediate **61** produced the deiodinated 2',3'-dideoxy compound **29**. The β-2'-azide of **30** was introduced by displacement of the mesylate group of **34** with sodium azide. Furthermore, the 2'-azide could be reduced using triphenylphosphine/ammonium hydroxide in THF–methanol[38] to give the β-2'-amino derivative **31**. The β-2'-fluoro compound **32** was synthesized by reaction of compound **35** with DAST ((diethylamino)sulfur trifluoride).

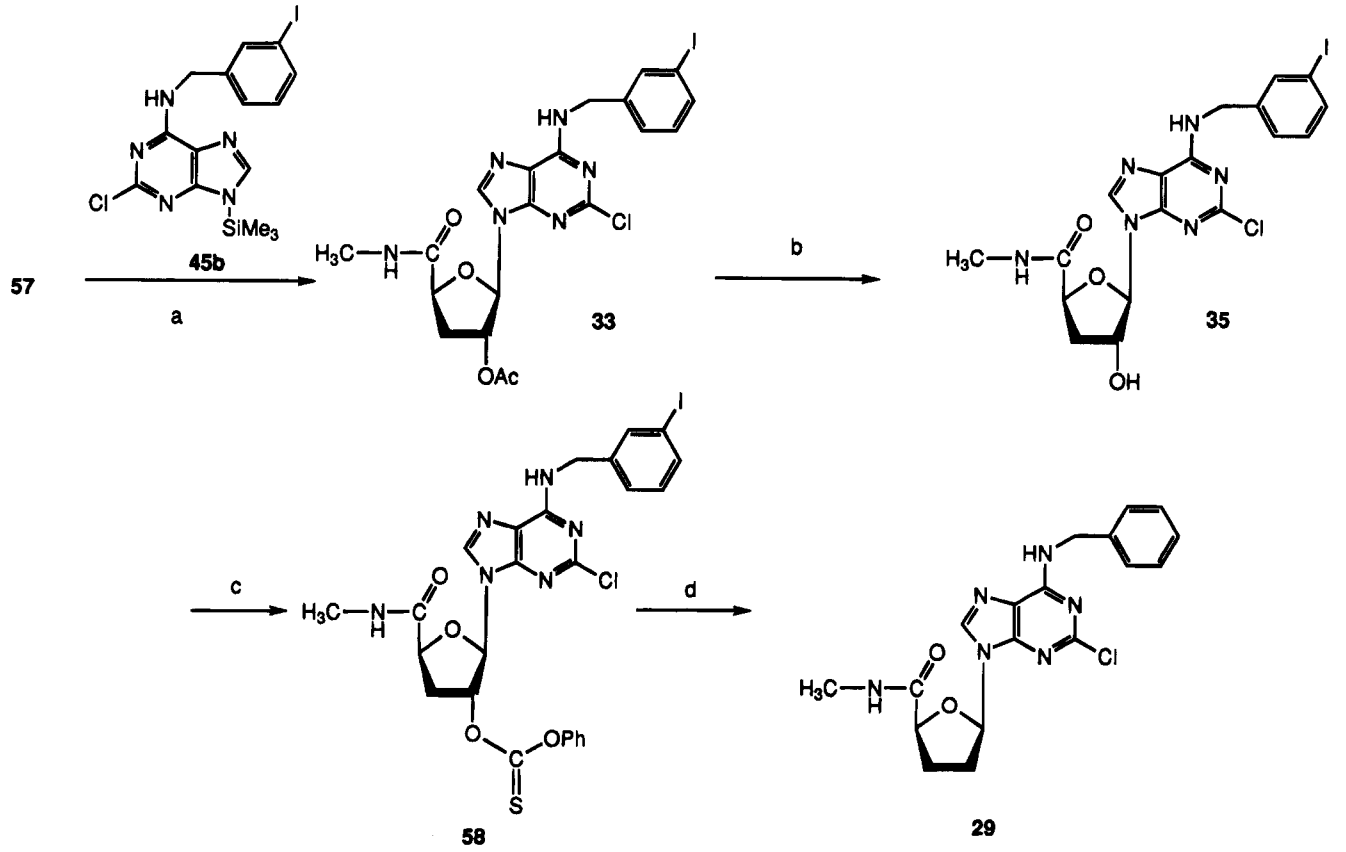
In an attempt to synthesize 2-chloro-2'-deoxy-N^6-(3-iodobenzyl)adenosine, the 3'- and 5'-hydroxyl groups of

Scheme 4[a]



[a] Reagents: (a) i. CS_2, NaH, MeI, THF, ii. Bu_3SnH, Et_3B, benzene; (b) NH_3/MeOH; (c) RuO_2, $NaIO_4$, $CHCl_3$:CH_3CN:H_2O (2:2:3); (d) MeOH, EDAC, DMAP; (e) CH_3NH_2/THF; (f) H_2SO_4, Ac_2O, AcOH.

Scheme 5[a]

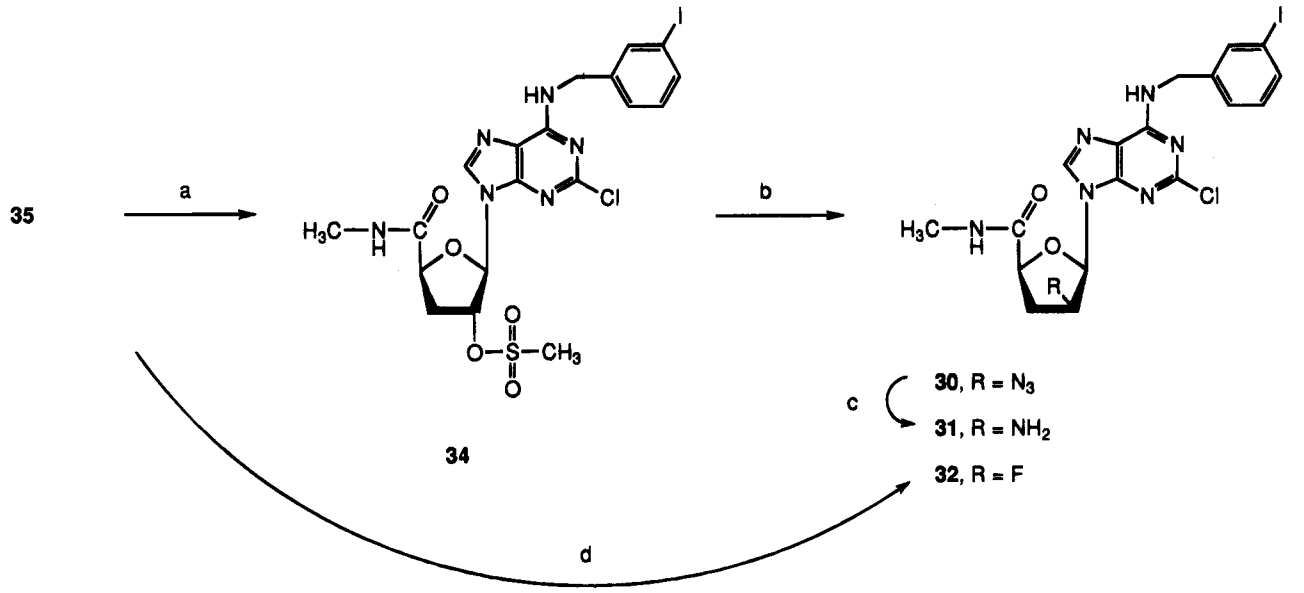


[a] Reagents: (a) TMSOTf, $Cl(CH_2)_2Cl$; (b) NH_3MeOH; (c) PhOC(S)Cl, DMAP, AcCN; (d) *n*-Bu_3SnH, Et_3B, benzene.

2-chloro-N^6-(3-iodobenzyl)adenosine[18] were protected with 1,1,3,3,-tetraisopropyldisiloxyl protective group to yield compound **36**. However, attempted deoxygenation of **36** using tributyltin hydride and AIBN (2,2′-azobis-(2-methylpropionitrile)) in toluene[39] was sluggish and did not give the desired product.

Biological Activity. The analogues were tested in radioligand binding assays (Table 2) using rat cortical A_1 receptors or striatal A_{2a} receptors or in CHO cells stably transfected with rat brain A_3 receptors.[8,20] Radioligands for A_1 and A_{2a} receptors were the selective agonists [^{3}H]-N^6-(*R*)-phenylisopropyladenosine[26] and [^{3}H]CGS 21680, respectively.[27] The radioligand used for binding to A_3 receptors was the recently reported high-affinity agonist [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide)).[25]

Scheme 6[a]



[a] Reagents: (a) methanesulfonyl chloride, pyridine, CH_2Cl_2; (b) NaN_3, DMF, 100 °C; (c) PPh_3, NH_4OH, THF–MeOH; (d) DAST.

Compound **5** (N-0840)[23] and the corresponding 9-ethyl derivative **6** were similar in their binding profiles at adenosine receptors. The previously reported high selectivity of **5** for A_1 vs A_{2a} receptors was even greater vs A_3 receptors. The structure of EHNA, **7**, an inhibitor of adenosine deaminase, corresponded to removal of the cyclopentyl group of **5** and lengthening and hydroxylation of the 9-alkyl chain. The affinity of EHNA was comparable to that of **5** and **6**; thus it bound well at A_1 receptors and only weakly at A_{2a} and A_3 receptors.

The inclusion of the 3-iodobenzyl group at the N^6-amine position of 9-methyladenine resulted in compound **8**. The K_i value of this analogue at A_3 receptors was 48 μM: very weak, yet more potent than the cyclopentyl analogues **5** and **6**. At A_1 receptors compound **8** was 1 order of magnitude less potent than the corresponding N^6-cyclopentyl analogue **5**. Thus, although perhaps not optimized for A_3 selectivity in the adenine series, the 3-iodobenzyl group had properties favorable toward such selectivity. Consequently, it was included in additional analogues.

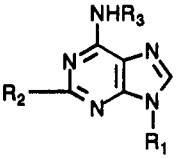
With N^6-substitution constant, the 9-alkyl substituent was varied in compounds **9**–**13**. An anionic alkyl group, as in the carboxylic acid derivative **12**, led to diminished affinity at all receptor subtypes. Hydroxylic alkyl groups at the 9-position (compounds **9**–**11**) offered no advantage in affinity at A_3 receptors vs 9-Me. The hydroxyethyl derivative **9** was nearly identical in A_3 affinity to the corresponding methyl analogue **4** yet was 4–6-fold less potent at both A_1 and A_{2a} receptors. A pair of chiral dihydroxy analogues, **10** and **11**, demonstrated moderate stereoselectivity of binding favoring the *R*-configuration β to the 9-nitrogen. The *R*-isomer **10** was 5.7-fold more potent at A_3 receptors than the corresponding *S*-isomer **11**. No selectivity was observed at A_1 receptors, and at A_{2a} receptors the enantiomers differed in affinity by only 2-fold. Compound **10** was slightly more potent at A_1 and A_3 receptors than the monohydroxy derivative **9**. The 9-(2,3-dihydroxypropyl)-adenines also appeared to have favorable water solubility. The maximum aqueous solubility of compound **10** was found to be 0.6 mM.

Substitution at the 2-position was probed in N^6-(iodobenzyl)-9-methyladenine derivatives **14**–**24**. Such substitutions had major effects on the affinity at A_3, and to a lesser degree A_1 and A_{2a}, receptors. Chloro (**14**), amino (**15**), alkylamino (**16**–**20**), methyl ether (**21**), and methylthio ether (**22**–**24**) groups were included at this position. The 2-chloro analogue **14** was moderately A_1-selective, by 110-fold vs A_3 receptors but only by 6-fold vs A_{2a} receptors. Affinity of **15** at A_1 receptors was 13-fold greater than that found for the corresponding 2-unsubstituted derivative **8**, while affinities at A_{2a} and A_3 receptors were unchanged.

Among 2-amino derivatives (**15**–**20**), K_i values at A_3 receptors ranged from 1 to roughly 100 μM. At A_1 receptors the range was more narrow, with the most potent displaying a K_i value of 0.33 μM (2-*n*-propylamino, **19**) and the least potent 8.6 μM (2-*n*-hexylamino, **20**). The primary amine **15** was identical in A_1 affinity to the 2-unsubstituted derivative **8**. Substitution on the 2-amino group indicated that a small alkyl group, as in the 2-methylamino analogue **17**, was favored at rat A_3 receptors. Lengthening of the chain (compounds **19** and **20**) or formation of the corresponding hydrazine derivative **16** greatly diminished affinity at A_3 receptors while maintaining affinity at A_1 receptors. Thus, the 2-hydrazino compound **16** was 20-fold selective for A_1 vs A_3 receptors. In addition to having diminished potency at A_3 receptors, the longer chain 2-amino analogues **19** and **20** proved to be of low water solubility, which interfered during the binding assay. 2-Dialkylamino, **18**, vs monoalkylamino, **17**, substitution was less well tolerated at rat A_3 receptors than at A_1 and A_{2a} receptors.

The affinities of 2-thio and 2-oxo ethers were compared. The most dramatic difference between the 2-methoxy (**21**) and 2-methylthio (**22**) ethers was found at A_3 receptors, at which the 2-methylthio analogue was 61-fold more potent. Thus, compound **22** proved to be only slightly selective (5–6-fold) for A_3 vs either A_1 or A_{2a} receptors. At A_1 receptors **21** was somewhat more potent (4-fold) than **22**, while at A_{2a} receptors there was no difference in affinity with K_i values of approximately 1 μM. The affinity of compound **23** indicates that the

Table 2. Affinities of 9-Alkyladenine and Ribose-Modified Adenosine Derivatives in Radioligand Binding Assays at Rat Brain A_1, A_{2a}, and A_3 Receptors[a-c]



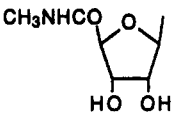
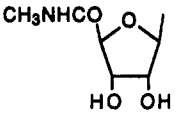
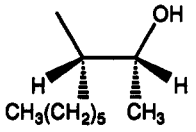
				K_i (μM) or % inhibition				
Compound	R_1	R_2	R_3	$K_i(A_1)$[a]	$K_i(A_{2a})$[b]	$K_i(A_3)$[c]	A_1/A_3	A_{2a}/A_3
1		H	3-I-Bz	0.054	0.056	0.0011	49	51
2		Cl	3-I-Bz	0.820	0.470	0.00033	2500	1400
5[d]	CH_3	H	cyclopentyl	0.54	11	1330±390	0.00041	0.0083
6[d,e]	CH_3CH_2	H	cyclopentyl	0.44	17	30% (10^{-4})	<<1	<1
7[e]		H	H	0.455	60% (10^{-4})	57% (10^{-4})	<<1	-
8	CH_3	H	3-I-Bz	5.73±1.88	2.23±1.33	48.3±6.0	0.12	0.046
9	$HO(CH_2)_2$	H	3-I-Bz	22.9±3.7	15.1±1.6	62.5±14.5	0.37	0.24
10	*R*-$HOCH_2$-CHOH-CH_2-	H	3-I-Bz	13.8±2.2	18.9±1.9	24.9±10.7	0.55	0.76
11	*S*-$HOCH_2$-CHOH-CH_2-	H	3-I-Bz	19.1±2.2	41.8±12.5	142±13	0.13	0.29
12	HO_2C-CH_2	H	3-I-Bz	17% (10^{-4})	9% (10^{-4})	225±17	-	-
13	$NC(CH_2)_3$	H	3-I-Bz	6.03±1.37	18.7±5.8	185±17	0.032	0.10
14	CH_3	Cl	3-I-Bz	0.45±0.11	2.7±0.56	51.0±10.0	0.0088	0.053
15	CH_3	NH_2	3-I-Bz	5.57±1.32	3.22±1.52	40.1±5.7	0.14	0.080
16	CH_3	NH_2NH	3-I-Bz	5.44±0.05	19.6±7.8	109±9	0.050	0.18
17	CH_3	CH_3NH	3-I-Bz	0.648± 0.102	3.56±0.84	0.974 ±0.340	0.67	3.7
18	CH_3	$(CH_3)_2N$	3-I-Bz	1.48±0.12	9.89±3.01	15.0±0.9	0.099	0.66
19[f]	CH_3	$CH_3(CH_2)_2NH$	3-I-Bz	0.33±0.08	1.72±0.70	20% (30 μM)	<<1	<<1
20[f]	CH_3	$CH_3(CH_2)_5NH$	3-I-Bz	4.48±0.82	11±4% (10^{-5})	19% (30 μM)	<1	-
21	CH_3	CH_3O	3-I-Bz	0.50±0.21	1.24±0.11	18.3±12.9	0.027	0.068
22	CH_3	CH_3S	3-I-Bz	1.89±0.59	1.64±0.39	0.299 ±0.074	6.3	5.5
23	CH_3	4-pyridyl-S	3-I-Bz	0.84±0.19	11.6±4.0	166±57	0.0051	0.070
24	*R*-$HOCH_2$-CHOH-CH_2-	CH_3S	3-I-Bz	1.34±0.09	78.9±23.5	8.59±4.29	0.16	9.2

Table 2 (Continued)

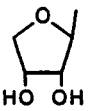
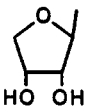
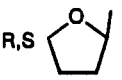
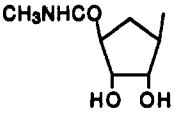
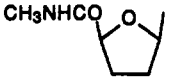
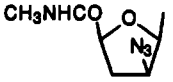
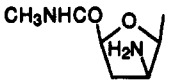
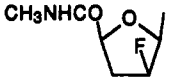
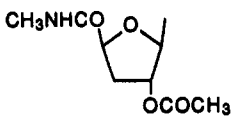
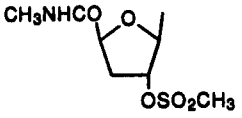
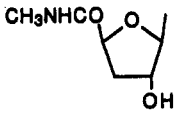
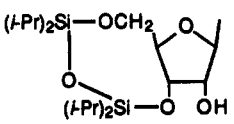
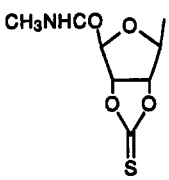
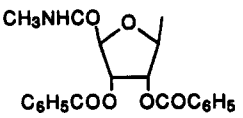
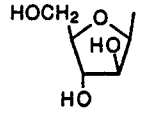
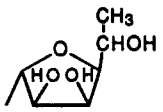
25		Cl	3-I-Bz	0.811 ±0.123	2.89±1.00	0.276 ±0.110	2.9	10
26		CH_3NH	3-I-Bz	0.660 ±0.010	3.39 ±0.29	73.1±11.3	0.0090	0.046
27[f]		Cl	3-I-Bz	0.174 ±0.017	4.12±0.18	3.47±0.58	0.050	1.2
28		H	3-I-Bz	35.9±8.3	28±5% (10^{-4})	19.5±4.7	1.8	>1
29		Cl	Bz	11.5±1.3	220±65	30.9±1.3	0.37	7.1
30		Cl	3-I-Bz	0.401 ±0.041	28.1±3.2	6.01±0.63	0.067	4.7
31		Cl	3-I-Bz	6.69±0.74	2% (10^{-4})	3.40±0.79	2.0	>50
32		Cl	3-I-Bz	1.42±0.27	98.0±9.7	17.8±2.4	0.080	5.5
33		Cl	3-I-Bz	0.778 ±0.044	15.9±3.7	0.0625 ±0.0310	12	250
34		Cl	3-I-Bz	1.29±0.08	41.9±6.2	7.27±1.19	0.18	5.8
35		Cl	3-I-Bz	1.03 ±0.15	4.66 ±0.74	0.0329 ±0.0078	31	140
36		Cl	3-I-Bz	66.3±19.8	18±2% (10^{-4})	13.1±3.5	5.1	>7
37		Cl	3-I-Bz	0.179 ±0.024	0.871 ±0.219	0.0122 ±0.0013	15	71
38		Cl	3-I-Bz	21% (10^{-4})	7% (10^{-4})	55±2% (10^{-4})	-	-

Table 2 (Continued)

39		Cl	H	24.2±7.9	90.0±12.7	14%(10^{-5})	-	-
40		H	H	150±28	54.7±3.1	6%(10^{-4})	<1	<1

[a] Displacement of specific [^{3}H]PIA binding, unless noted, in rat brain membranes expressed as $K_i \pm$ SEM (μM) or percent inhibition at the indicated molar concentration ($n = 3-6$). [b] Displacement of specific [^{3}H]CGS 21680 binding, unless noted, in rat striatal membranes expressed as $K_i \pm$ SEM (μM) or percent inhibition at the indicated molar concentration ($n = 3-6$). [c] Displacement of specific binding of [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide)[25] from membranes of CHO cells stably transfected with the rat A_3-cDNA expressed as $K_i \pm$ SEM (μM) or percent inhibition at the indicated molar concentration ($n = 3-7$). [d] Values at A_1 and A_{2a} receptors are from Thompson et al.[23] [e] Values are from van Galen et al.[20] A_3 affinity was measured by displacement of specific binding of [^{125}I]APNEA in membranes of CHO cells stably transfected with the rat A_3-cDNA.[8] 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 CPA or vs specific binding of [^{3}H]CGS 21680 in rat striatal membranes. [f] Low aqueous solubility.

bulky pyridyl ring is tolerated at the 2-position well at A_1 and poorly at A_3 receptors. The combination of selectivity enhancing features at the 2- and 9-positions in compound **24** failed to achieve an additive effect on A_3 selectivity; instead the compound was 9-fold A_1-selective.

There is evidence that at A_1 receptors 2′,3′-dideoxyadenosines and other truncated ribose analogues act as antagonists or partial agonists.[31-34] Thus, in an effort to identify leads for selective antagonists, derivatives of adenosine, i.e., based on 9-ribosides and other cyclic groups, were also included (compounds **25**–**38**). Selectivity for A_3 vs A_{2a} receptors was observed for adenosine analogues **25** and **29**–**36**. Omission of the 5′-hydroxymethyl group in the erythrose derivative **25** provided slight A_3 vs A_1 selectivity with a K_i value of 0.28 μM. Combination of favorable N^6- and 2-substitution, as in compound **26**, maintained roughly micromolar potency at A_1 and A_{2a} receptors but was not tolerated at A_3 receptors. A tetrahydrofuran derivative, compound **27**, had a K_i value of 3.5 μM at A_3 receptors. Compound **28**, the carbocyclic analogue of IB-MECA, **1**, was reported previously[31] to be slightly selective for A_3 receptors.

Compounds **29**–**35** contain 3′-deoxy or 2′,3′-dideoxy modifications of ribose-5′-(*N*-methylamide). The β-2′-azido derivative **30** was slightly more potent than the corresponding fluoro derivative **32** at all the receptor subtypes. A β-2′-amino derivative, **31**, was 2-fold selective for A_3 vs A_1 receptors and inactive at A_{2a} receptors. The 3′-deoxy analogue of IB-MECA, compound **35**, was moderately A_3-selective (31-fold vs A_1 receptors) in the binding assays.

In compounds **33**, **34**, and **36**–**38**, the ribose hydroxyl groups have been blocked by acylation or silylation. It is possible that some of the binding displacement observed resulted from lability of the blocking group, in which case these derivatives would constitute prodrugs. Although they were all found to be stable in aqueous medium, the consequences of incubation with membranes remain untested. The potential use of these relatively hydrophobic yet biologically active adenosine analogues for *in vivo* therapeutics is under investigation.

Two other adenine glycosides, i.e., compounds **39** and **40**, derivatives of arabinose and talose, respectively, having free 2′,3′-dihydroxy groups have been included in this study. These derivatives displayed only weak affinity at A_1 and A_{2a} receptors and no selectivity.

We examined the agonist and antagonist properties of 9-alkyladenine and adenosine derivatives in an adenylyl cyclase assay in A_3 receptor-transfected CHO cells (Table 3). As in previous studies,[18] adenylyl cyclase was inhibited by IB-MECA, **1**, with an IC_{50} value of $\sim 10^{-7}$ M in A_3-transfected CHO cells (Figure 2), with a maximal degree of inhibition of 40–50%. The corresponding 2-chloro-3′-deoxyadenosine derivative, compound **35**, proved to be a full agonist in the A_3-mediated inhibition of adenylyl cyclase (Figure 2). 5′-Deoxy-5′-(methylthio)adenosine (Table 3) gave a robust agonist response at A_3 receptors. This compound was reported to be an agonist at A_1 receptors, a low-efficacy agonist at A_{2a} receptors,[35] and an antagonist at A_{2b} receptors.[36]

Although the novel 9-methyladenine derivatives were designed to act as adenosine antagonists, we were unable to detect antagonism of A_3 agonist-elicited inhibition of adenylyl cyclase in the transfected CHO cells. Compound **22**, the 9-methyl 2-methylthio analogue, displaced radioligand binding with at K_i value < 1 μM (Table 2), but at concentrations as high as 50 μM it failed to reverse the agonist-induced inhibition of adenylyl cyclase. Curiously, compound **22** alone inhibited adenylyl cyclase in A_3-transfected CHO cells by 19%. Other ribose-truncated adenosine derivatives, such as the 9-acyclic compound **24**, the erythrose derivative **25**, and the 2′,3′-dideoxyadenosine derivative **29**, similarly were found to inhibit adenylyl cyclase in A_3-transfected CHO cell membranes (Table 3).

We investigated the possibility that the observed inhibition of adenylyl cyclase resulted from action at a site other than A_3 receptors. For example, certain adenosine analogues with extensively modified ribose moieties, e.g., 9-(tetrahydrofur-2-yl)adenine,[40] have been found to inhibit adenylyl cyclase by acting directly on the catalytic subunit at the allosteric "P" site. The control experiments, in which selected adenine and adenosine derivatives were tested for effects on adenylyl cyclase in untransfected CHO cells, were carried out. Several of the agents, such as **35** and **37**, showed little inhibition, relative to that observed with the transfected cells (Table 3). Thus, for **35** and **37**, activation of A_3 receptors is still the most plausible explanation for the biological activity. However, for some of the derivatives, e.g., compounds **24** and **29**, degrees of inhibition of

Table 3. Effects on Adenylyl Cyclase in CHO Cells, Either Stably Transfected with Rat A_3 Adenosine Receptors or Untransfected[a]

compd	conc (μM)	ratio conc/$K_i(A_3)$	% inhibition of a. cyclase CHO (A_3)	% inhibition of a. cyclase CHO (cntrl)	effect on IB-MECA dose–resp curve
1	100	9.1×10^4	44.4 ± 1.0	nd	*c*
	0.1	91	22.0 ± 0.9	nd	*c*
7	40		5.9	nd	*c*
22	100	330	19.5	nd	*c*
24	40	4.6	5.5 ± 2.5	7.3 ± 3.3	*c*
25	100	360	28.8 ± 4.0	14.9 ± 1.4	nd
29	40	1.3	7.5 ± 3.8	10.8 ± 4.4	*c*
30	20	3.3	25.7 ± 3.6	18.0 ± 3.5	nd
	100	17	27.8	nd	nd
32	100	5.6	11.2	nd	nd
33	20	320	12.7 ± 1.0	nd	nd
35	40	1200	35.2 ± 7.8	5.2 ± 2.6	nd
	100	3000	49.2 ± 3.7	nd	nd
36	100	7.6	8.7	nd	nd
37	100	8200	63.4 ± 5.8	4.9 ± 2.7	nd
39	40		16.1	nd	nd
40	40		0	nd	*c*
5′-MeSAdo	40	28	19.7 ± 2.7	nd	nd

[a] In the presence of 1 μM forskolin. [b] Average ± SEM for three determinations or a single value. [c] No antagonism. nd, not determined.

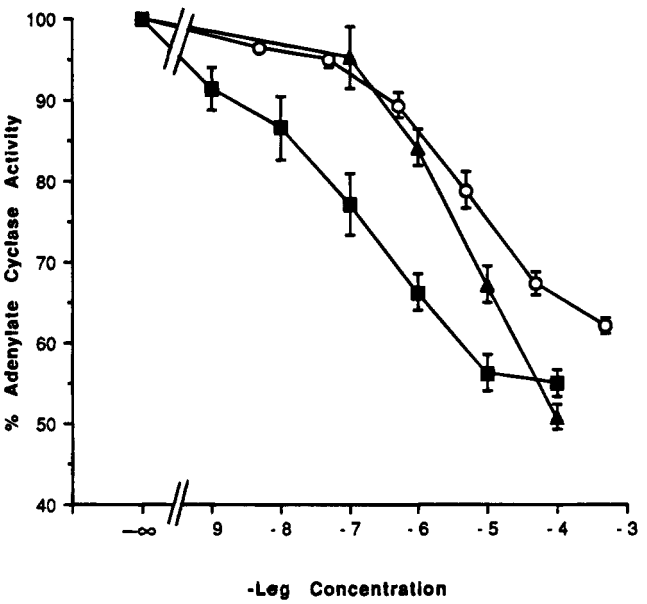


Figure 2. Agonist-elicited inhibition of adenylyl cyclase via rat A_3 receptors in transfected CHO cells: circles, NECA; squares, Cl-IB-MECA; triangles, compound **35**.

adenylyl cyclase in control and transfected CHO cells were comparable. Therefore the "P" site may account for the cyclase inhibition seen for these adenine derivatives. Compounds **24** and **29** were roughly equipotent in inhibiting adenylyl cyclase directly, and the 2′,3′-dideoxy 2′-azido derivative **30** was somewhat more potent, with 18% inhibition at a concentration of 20 μM. Compounds **25** and **30** appeared to have mixed A_3 agonist and "P" site inhibitory properties, since the inhibition in transfected CHO cells was significantly greater than in control CHO cells.

Another possible explanation for apparent inhibition of adenylyl cyclase through a non-receptor-mediated mechanism is that some of the compounds might be inhibiting adenosine deaminase and thereby raising the levels of endogenous agonist, which becomes available to activate A_3 receptors. EHNA, **7**, is known as an effective inhibitor of this enzyme and indeed at 40 μM inhibited adenylyl cyclase in the transfected CHO cells by 6%. However, with respect to most of the analogues synthesized in this study, it is known that the N^6-substitution precludes potent interaction with adenosine deaminase, either as substrate or inhibitor, at adenosine deaminase.[41]

Discussion

In this study 9-alkyladenine and truncated adenosine derivatives were examined for selectivity for rat A_3 receptors. Among the compounds studied, **22**, **25**, **28**, **31**, **33**, and **35–38** were somewhat A_3-selective in binding to rat adenosine receptors. Several of the compounds, **17**, **24**, **29**, **30**, **32**, and **34**, were A_3-selective vs A_{2a} but not A_1 receptors. K_i values determined at A_3 receptors were at best in the 10^{-7}–10^{-6} M range. Due to the well-documented large species dependence among adenosine antagonists, specifically xanthines, at A_3 receptors,[8,12,13,21] it will be essential to examine compounds from this series for affinity at A_3 receptors in other species. Even some of the nonselective adenines in this study may turn out to be selective ligands at human or sheep A_3 receptors. It is still undetermined whether these species differences represent distinct receptor subtypes.

A comparison of the structural features of A_3-selective agonists[17,18] with the present results is useful. Due to the high selectivity of N^6-(3-iodobenzyl)adenosine derivatives for A_3 receptors, the same N^6-substituent was included in most of the present adenine and adenosine derivatives. Although the N^6-iodobenzyl group was found to be preferred over the N^6-cyclopentyl group at A_3 receptors (with a 28-fold increase in affinity in **8** vs **5**), the SAR at this position must be explored in greater detail in order to draw general conclusions concerning adenosine/adenine parallels at this position. The 2-methylamino and 2-methylthio groups were favorable for affinity at A_3 receptors in both the adenosine[18] and adenine series, yet the 2-chloro group, which resulted in A_1 selectivity in this series, was present in the highly A_3 selective agonist N^6-(3-iodobenzyl)-2-chloroadenosine-5′-(methyluronamide).[18] Thus, in the series of N^6-(3-iodobenzyl)adenosine-5′-(methyluronamide)s, A_3 affinity varied in the order: 2-Cl > 2-CH_3S > 2-CH_3NH. In the current series of 9-methyladenines, the order was 2-CH_3S > 2-CH_3NH ≫ 2-Cl. Effects on affinity of substitution at 9- and 2-positions were highly interdependent; the groups were simply not additive. 2-Methylthio and 2-methylamino groups did not maintain micromolar affinity at A_3 receptors when combined with

9-substituents larger than methyl, i.e., dihydroxypropyl (**24**) and erythrose (**26**), respectively. The lack of additivity in the structure–activity relationships of the adenine derivatives possibly indicates that those analogues having large 9-substituents and those with small 9-substituents have different binding modes. Another possible explanation for the low affinity of **26** could be that the methylamino group is increasing the basicity of the 6-amino group; a positive charge on the compound at neutral pH would render it much less active.

Increasing the number of hydroxyl groups on the 9-substituent (cf. **8**–**11**) enhanced water solubility and had only minor effects on A_3 affinity. The pair of chiral N^6-(3-iodobenzyl) 9-(2,3-dihydroxypropyl) derivatives showed stereoselectivity only at A_3 receptors, with the *S*-isomer more potent. If the 2′- and 3′-hydroxyl groups of the adenine derivatives correspond spatially at the receptor binding site to the 2′- and 3′-hydroxyls of receptor-bound adenosine, then it is the *S*-isomer that would more closely resemble adenosine. Thus, this slight stereoselectivity is without explanation.

Bruns[30] reported that certain analogues of adenosine that were missing portions of the ribose moiety, such as the erythrose derivative, were low-efficacy activators or even antagonists of human A_{2b} receptors. Bruns also was the first to detect adenosine antagonism by 9-methyladenine.[42] On the basis of these and later findings,[31–34] we have prepared various deoxy and other analogues of known A_3-selective agonists, in an effort to identify antagonists. Included in this study are derivatives of 9-alkyladenine and 9-erythrose adenine.

The apparent inhibition of adenylyl cyclase by the dihydroxypropyl derivative **24** and the 2′,3′-dideoxy analogue **29** was shown to be due not to A_3 receptor activation but to action at the allosteric "P" site on adenylate cyclase. It is likely that the adenylyl cyclase inhibition exhibited by the 9-methyladenine derivative **22** and the 2′,3′-dideoxy 2′-fluoro derivative **32** was also due to "P" site inhibition. The erythrose derivative **25** inhibited adenylyl cyclase as both a "P" site ligand and an A_3 agonist in roughly equal proportions.

In the case of agonists there is a good correlation between potency in inhibiting cyclase in the rat A_3-transfected cell membranes and the relative K_i values obtained in binding experiments at A_3 receptors.[18] However, there is a discrepancy for antagonists at rat A_3 receptors. We have shown that theophylline, which has a K_i value of 85 μM at rat A_3 receptors,[21] and other xanthines lack functional antagonistic properties vs A_3 agonist-elicited adenylyl cyclase inhibition in A_3-transfected CHO cell membranes.[20] So far, none of the ligands we have examined, including xanthines in a previous study,[19] are useful antagonists at A_3 receptors (even nonselective). Although some of these adenine derivatives, such as **22**, were considerably more potent in A_3 binding than theophylline, functional antagonism in this assay was not seen, perhaps because of inhibition of adenylate cyclase through the "P" site. It will be instructive to test the adenine derivatives at A_3 receptors in other species, in which a possible gain in receptor affinity may avoid the "P" site complication.

Structural requirements for activation at A_3 receptors do not include either the 3′-hydroxyl group (see **35**) or the 4′-CH_2OH group (see **25**). The 3′-deoxy-IB-MECA derivative, **35**, elicited a full inhibitory effect on adenylyl cyclase (Figure 2), and **37**, the 2′,3′-thiocarbonyl derivative of Cl-IB-MECA, caused a >60% inhibition at high concentrations. Thus both gave full agonist responses. Similarly, it has been shown that the 3′-deoxy analogue of R-PIA was a full agonist at A_1 receptors.[33] Additional pharmacological studies are needed to clearly distinguish full and partial agonism of the other adenosine derivatives, such as the erythrose derivative **25**, the azido derivative **30**, and the 2′-acetoxy 3′-deoxy derivative **33**. At A_{2b} receptors, adenosine-9-β-D-erythrofuranoside, the parent structure related to **25**, acted as a competitive antagonist.[30]

In conclusion, we have demonstrated the feasibility of developing A_3 receptor-selective ligands based on substituted adenine derivatives, although optimization of selectivity remains a challenge. But the expectation of antagonizing rat A_3 receptors in an adenylyl cyclase functional assay by modifying the ribose sugar has not been realized. It is possible that antagonism of second-messenger effects by adenine derivatives, that in our study weakly inhibited adenylyl cyclase, or xanthines, that bind to A_3 receptors but have no effect on agonist-elicited inhibition of adenylyl cyclase, would be observed under different conditions. Such conditions might include higher concentrations of the agents, use of a different species in which higher affinity is attained,[21] or another functional assay, such as phospholipase C.[9] It is also possible that non-purine antagonists will provide leads for A_3 selectivity, although a screen of five such A_1 antagonists of diverse structure indicated negligible affinity at rat A_3 receptors.[20]

Experimental Section

Chemistry. New compounds were characterized (and resonances assigned) by 300 MHz proton nuclear magnetic resonance 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) on a JEOL SX102 mass spectrometer. In the EI mode accurate mass was determined using a VG7070F mass spectrometer. C, H, and N analyses (Table 1) were carried out by Atlantic Microlabs (Norcross, GA), and ±0.4% was acceptable. All adenine derivatives were judged to be homogeneous using thin layer chromatography (silica gel, 0.25 mm, glass backed; Alltech Assoc., Deerfield, IL) following final purification. Compound **5**, 2-chloroadenosine, and EHNA were obtained from Research Biochemicals International (Natick, MA). Analytical TLC plates and silica gel (230–400 mesh) were purchased from VWR (Bridgeport, NJ). Compound **6** was kindly provided by Prof. Ray A. Olsson (University of South Florida). The solubility of **10** was measured by boiling the solid in water and cooling followed by measurement of the concentration by UV. The ϵ_{270} (λ_{max}) value for compound **3** in methanol was found to be 19 000. IB-MECA, Cl-IB-MECA, and compound **38** were prepared as described.[17,18] Compounds **39** and **40** were obtained from Dr. John W. Daly (NIDDK).

***N*^{6}-(3-Iodobenzyl)-9-methyladenine (8).** A mixture of 6-chloropurine (**41**; 100 mg, 0.65 mmol), 3-iodobenzylamine hydrochloride (192 mg, 0.71 mmol), and triethylamine (0.27 mL, 1.94 mmol) in absolute ethanol (2 mL) was heated for 24 h at 80 °C in a sealed tube. After cooling, a solid was collected by suction filtration, washed with ethyl alcohol, and dried to give compound **44** (191.3 mg, 84.0%): ^{1}H NMR (DMSO-d_6) δ 4.67 (br s, 2 H, CH_2), 7.11 (pseudo t, J = 7.6 and 7.5 Hz, 1 H, H-16), 7.37 (d, J = 7.9 Hz, 1 H, H-17), 7.58 (d, J = 7.6 Hz, 1 H, H-15), 7.73 (s, 1 H, H-13), 8.12 and 8.17 (each s, 1 H, H-8 and H-2), 8.25 (br s, 1 H, exchangeable with D_2O, N^6H), 12.95 (br s, 1 H, exchangeable with D_2O, N_9H).

To a solution of compound **44** (100 mg, 0.28 mmol) in dry DMF (4 mL) were added anhydrous potassium carbonate (78.7 mg, 0.57 mmol) and methyl iodide (0.365 mL, 5.7 mmol). The reaction mixture was stirred for 1 h and 40 min at room

temperature. The solid was removed by suction, and the residue was purified by preparative TLC (chloroform–methanol, 10:1) to give compound **8** [R_f = 0.51 (chloroform–methanol, 10:1); 25 mg, 24.0%]: ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3 H, CH_3), 4.67 (br s, 2 H, CH_2), 7.10 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.36 (d, J = 7.5 Hz, 1 H, H-17), 7.58 (d, J = 7.7 Hz, 1 H, H-15), 7.71 (s, 1 H, H-13), 8.12 and 8.21 (each s, 1 H, H-8 and H-2), 8.29 (br s, 1 H, exchangeable with D_2O, N^6H).

9-(2-Hydroxyethyl)-N^6-(3-iodobenzyl)adenine (9). To a solution of compound **44** (20 mg, 0.056 mmol) and iodoethanol (100 μL) in dry DMF (0.5 mL) was added anhydrous K_2CO_3 (50 mg). The mixture was stirred at room temperature for 10 h and filtered to remove inorganic solids. The filtrate was evaporated to dryness and the residue purified by preparative TLC (CH_2Cl_2–MeOH, 10:1) to give compound **9** (R_f = 0.42), 27 mg (80%): ^{1}H NMR (DMSO-d_6) δ 3.20 (br s, 1 H, exchangeable with D_2O, OH), 3.75 (t, J = 7 Hz, 2 H, CH_2), 4.21 (t, J = 7 Hz, 2 H, CH_2), 4.67 (br s, 2 H, CH_2), 7.10 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.40 (d, J = 7.5 Hz, 1 H, H-17), 7.57 (d, J = 7.7 Hz, 1 H, H-15), 7.71 (s, 1 H, H-13), 8.12 and 8.20 (each s, 1 H, H-8 and H-2), 8.31 (br s, 1 H, exchangeable with D_2O, N^6H).

(*R*)-9-(2,3-Dihydroxypropyl)-N^6-(3-iodobenzyl)adenine (10). To a solution of compound **44** (60 mg, 0.267 mmol) and (*R*)-(−)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl *p*-toluenesulfonate (100 mg, 0.35 mmol) in dry DMF (2 mL) was added anhydrous K_2CO_3 (200 mg). The reaction mixture was heated at 50 °C for 20 h. After cooling to room temperature, the reaction mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in 1 N HCl (10 mL) and heated at 80 °C for 1 h. With cooling in ice, the reaction mixture was neutralized by dropwise addition of concentrated NH_4OH and evaporated to dryness. The residue was purified by preparative TLC (CH_2Cl_2–MeOH, 9:1, R_f = 0.35) to give **10**, 80 mg (70%): ^{1}H NMR (DMSO-d_6) δ 3.46 (m, 2 H, CH_2), 3.68 (m, 2 H, CH_2), 4.05 (m, 1 H, CH), 4.67 (br s, 2 H, CH_2), 4.85 (t, 1 H, exchangeable with D_2O, OH), 5.12 (d, 1 H, exchangeable with D_2O, OH), 7.13 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.36 (d, J = 7.5 Hz, 1 H, H-17), 7.52 (d, J = 7.7 Hz, 1 H, H-15), 7.64 (s, 1 H, H-13), 8.07 and 8.19 (each s, 1 H, H-8 and H-2), 8.33 (br s, 1 H, exchangeable with D_2O, N^6H).

(*S*)-9-(2,3-Dihydroxypropyl)-N^6-(3-iodobenzyl)adenine (11). Compound **11** was synthesized as described for **10** (same scale) from (*S*)-(+)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl *p*-toluenesulfonate. Yield of the purified product **11** was 69%. The ^{1}H NMR in DMSO-d_6 was similar to that of compound **10**.

2-[N^6-(3-Iodobenzyl)adenin-9-yl]acetic Acid (12). This compound was prepared by a similar procedure as described for **9**, starting with compound **44** (0.056 mmol), iodoacetic acid (100 mg), and K_2CO_3 (50 mg) in dry DMF (0.5 mL). The reaction mixture was neutralized with glacial acetic acid and evaporated to dryness. The yield of **12** after preparative TLC purification (CH_2Cl_2–MeOH, 9:1, R_f = 0.25) was 31 mg (85%): ^{1}H NMR (DMSO-d_6) δ 4.55 (s, 2 H, CH_2), 4.78 (s, 2 H, CH_2), 7.16 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.42 (d, J = 7.5 Hz, 1 H, H-17), 7.61 (d, J = 7.7 Hz, 1 H, H-15), 7.79 (s, 1 H, H-13), 8.40 and 8.45 (each s, 1 H, H-8 and H-2), 8.90 (br s, 1 H, exchangeable with D_2O, N^6H), 12.90 (br s, 1 H, CO_2H).

9-(3-Cyanopropyl)-N^6-(3-iodobenzyl)adenine (13). A solution of N^6-(3-iodobenzyl)adenine (**44**; 50 mg, 140 μmol), 4-bromobutyronitrile (300 mg, 2.0 mmol), and anhydrous potassium carbonate (150 mg, 1.1 mmol) in DMF (2 mL) was stirred for 12 h at 80 °C. Following addition of 10 mL of half-saturated sodium chloride, an oil separated. The oil was chromatographed on a preparative silica gel TLC plate (chloroform–methanol, 95:5, R_f = 0.31) to give compound **13** (40 mg, 66%): MS (EI) m/z 418 (M^+), 350, 291, 232, 187.

2-Chloro-N^6-(3-iodobenzyl)-9-methyladenine (14). A solution of 2,6-dichloropurine (**42**; 2 g, 10.6 mmol), 3-iodobenzylamine hydrochloride (3.14 g, 11.6 mmol), and triethylamine (4.42 mL, 31.7 mmol) in ethanol (20 mL) was stirred for 5 days at room temperature. A solid was collected by suction, washed with a small amount of ethanol, and dried to give compound **45a** (2.32 g, 57.0%) which was recrystallized from methanol: ^{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, H-16), 7.36 (d, J = 7.5 Hz, 1 H, H-17), 7.61 (d, J = 7.5 Hz, 1 H, H-15), 7.74 (s, 1 H, H-13), 8.14 (s, 1 H, H-8), 8.75 (br s, 1 H, exchangeable with D_2O, N^6H), 13.14 (br s, 1 H, exchangeable with D_2O, N_9H); MS (CI, NH_3) m/z 386 (M^+ + 1).

A mixture of compound **45a** (356 mg, 0.92 mmol), methyl iodide (2.08 mL, 32.4 mmol), and potassium carbonate (256 mg, 1.85 mmol) in DMF (12 mL) was stirred for 1 h and 40 min at room temperature. After filtration of potassium carbonate, the filtrate was mixed with water (100 mL) and chloroform (30 mL). During evaporation of the organic solvent, a slightly yellow solid formed. It was collected by suction and dried to yield compound **14** (303 mg, 82.0%): ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3 H, CH_3), 4.60 (br s, 2 H, CH_2), 7.13 (t, J = 7.6 Hz, 1 H, H-16), 7.36 (d, J = 7.7 Hz, 1 H, H-17), 7.60 (d, J = 7.7 Hz, 1 H, H-15), 7.73 (s, 1 H, H-13), 8.14 (s, 1 H, H-8), 8.80 (br s, 1 H, exchangeable with D_2O, N^6H).

2-Amino-N^6-(3-iodobenzyl)-9-methyladenine (15). A mixture of 6-chloroguanine (**43**; 100 mg, 0.59 mmol), 3-iodobenzylamine hydrochloride (175 mg, 0.65 mmol), and triethylamine (0.25 mL, 1.79 mmol) in ethanol (2 mL) was heated for 94 h at 80 °C. The solution was cooled and crystallized by addition of water. A colorless solid was collected by suction and dried to give compound **46** (161 mg, 75.0%): ^{1}H NMR (DMSO-d_6) δ 4.62 (br s, 2 H, CH_2), 5.70 (br s, 2 H, exchangeable with D_2O, NH_2), 7.11 (pseudo t, J = 7.9 and 7.7 Hz, 1 H, H-16), 7.37 (d, J = 7.6 Hz, 1 H, H-17), 7.57 (d, J = 7.9 Hz, 1 H, H-15), 7.71 (s, 1 H, H-13), 7.66 (s, 1 H, H-8), 12.09 (br s, 1 H, exchangeable with D_2O, N^6H).

A mixture of compound **46** (100 mg, 0.27 mmol), methyl iodide (0.35 mL, 5.46 mmol), and anhydrous potassium carbonate (75 mg, 0.54 mmol) in dry DMF (4 mL) was stirred for 1.1 h at room temperature. A solid was removed by suction filtration, and the filtrate was concentrated and purified by preparative TLC (chloroform–methanol, 10:1) to give compound **15** [R_f = 0.46 (chloroform–methanol, 10:1); 3 mg, 2.9%]: ^{1}H NMR (DMSO-d_6) δ 3.54 (s, 3 H, CH_3), 4.61 (br s, 2 H, CH_2), 5.86 (br s, 2 H, exchangeable with D_2O, NH_2), 7.10 (t, J = 7.7 and 7.6 Hz, 1 H, H-16), 7.27 (d, J = 7.3 Hz, 1 H, H-17), 7.36 (d, J = 7.5 Hz, 1 H, H-15), 7.55 and 7.58 (each s, 1 H, H-13 and H-8), 7.75 (br s, 1 H, exchangeable with D_2O, N^6H).

2-Hydrazino-N^6-(3-iodobenzyl)-9-methyladenine (16). A solution of compound **14** (25 mg, 0.06 mmol) in hydrazine hydrate (1 mL) was heated for 17 h at 82 °C in a sealed bottle. Water (3 mL) was added, and a colorless solid was separated by suction and dried to yield compound **16** (19.9 mg, 80.6%): ^{1}H NMR (DMSO-d_6) δ 3.59 (s, 3 H, 9-CH_3), 4.08 (br s, 2 H, exchangeable with D_2O, NH_2), 4.61 (br s, J = 5.3 Hz, 2 H, CH_2), 7.10 (t, J = 7.6 Hz, 1 H, H-16), 7.35 (s, 1 H, exchangeable with D_2O, NH), 7.39 (d, J = 7.6 Hz, 1 H, H-17), 7.57 (d, J = 7.6 Hz, 1 H, H-15), 7.73 (s, 2 H, H-13 and H-8), 7.92 (br s, 1 H, exchangeable with D_2O, N^6H).

N^6-(3-Iodobenzyl)-2-(methylamino)-9-methyladenine (17). A mixture of compound **14** (25 mg, 0.06 mmol), 2 M methylamine in THF (1 mL), and 40% methylamine in water (1 mL) was stirred for 14 h at 85 °C in a sealed bottle. After removal of volatiles *in vacuo*, the residue was triturated with methanol–water and a solid was collected by suction, washed with water (10 mL), and dried to give compound **17** (22 mg, 89.0%): ^{1}H NMR (DMSO-d_6) δ 2.76 (d, J = 4.6 Hz, 3 H, $NHCH_3$), 3.55 (s, 3 H, 9-CH_3), 4.59 (br s, 2 H, CH_2), 6.28 (br s, 1 H, exchangeable with D_2O, $NHCH_3$), 7.10 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.38 (d, J = 7.6 Hz, 1 H, H-17), 7.57 (d, J = 7.6 Hz, 1 H, H-15), 7.67 (s, 1 H, H-13), 7.35 (s, 1 H, H-8), 7.83 (br s, 1 H, exchangeable with D_2O, N^6H).

2-(Dimethylamino)-N^6-(3-iodobenzyl)-9-methyladenine (18). A mixture of compound **14** (40 mg, 0.1 mmol), glycine methyl ester hydrochloride (310 mg, 2.47 mmol), and triethylamine (0.7 mL, 5.0 mmol) in DMF (2 mL) was heated for 22 h at room temperature in a sealed bottle. After cooling, the mixture was concentrated to dryness and purified using silica gel column chromatography (chloroform–methanol, 20:1) to give compound **18** (25 mg, 53.5%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 3.06 (s, 6 H, $N(CH_3)_2$), 3.58 (s, 3 H, 9-CH_3), 4.55 (br s, 2 H, CH_2), 7.10 (pseudo t, J = 8.0 and 7.6 Hz, 1 H,

H-16), 7.38 (d, J = 7.7 Hz, 1 H, H-17), 7.56 (d, J = 8.0 Hz, 1 H, H-15), 7.70 (s, 1 H, H-13), 7.77 (s, 1 H, H-8), 7.92 (br s, 1 H, exchangeable with D_2O, N^6H).

N^6-(3-Iodobenzyl)-9-methyl-2-(*n*-propylamino)adenine (19). A mixture of compound **14** (22.5 mg, 0.056 mmol) and *n*-propylamine (2 mL) was stirred at 85 °C for 36 h in a sealed bottle. After evaporation of volatiles, the residue was purified on preparative TLC (chloroform–methanol, 20:1) to give compound **19** (17.3 mg, 72.8%) as a slightly yellow solid: ^1H NMR (DMSO-d_6) δ 0.85 (pseudo t, J = 7.5 and 7.3 Hz, 3 H, CH_3), 1.47 (sixtet, J = 7.2 Hz, 2 H, CH_2), 3.30 (m, 2 H, CH_2), 3.54 (s, 3 H, 9-CH_3), 4.58 (br s, 2 H, CH_2), 6.33 (br s, 1 H, exchangeable with D_2O, NH), 7.10 (pseudo t, J = 8.0 and 7.7 Hz, 1 H, H-16), 7.36 (d, J = 7.7 Hz, 1 H, H-17), 7.57 (d, J = 8.2 Hz, 1 H, H-15), 7.66 (s, 1 H, H-13), 7.72 (s, 1 H, H-8), 7.80 (br s, 1 H, exchangeable with D_2O, N^6H).

2-(*n*-Hexylamino)-N^6-(3-iodobenzyl)-9-methyladenine (20). A mixture of compound **14** (23.5 mg, 0.059 mmol) and *n*-hexylamine (1 mL) was heated for 4.5 days at 80 °C in a sealed bottle. After evaporation of volatiles, the residue was purified on preparative TLC (chloroform–methanol, 20:1) to give compound **20** (23.5 mg, 86.0%): ^1H NMR (DMSO-d_6) δ 0.84 (m, 3 H, CH_3), 1.25 (m, 6 H, CH_2), 1.45 (m, 2 H, CH_2), 3.17 (m, 2 H, CH_2), 3.54 (s, 3 H, 9-CH_3), 4.58 (br s, 2 H, CH_2), 6.32 (br s, 1 H, exchangeable with D_2O, NH), 7.09 (pseudo t, J = 7.8 and 7.6 Hz, 1 H, H-16), 7.35 (d, J = 7.8 Hz, 1 H, H-17), 7.57 (d, J = 7.7 Hz, 1 H, H-15), 7.66 (s, 1 H, H-13), 7.71 (s, 1 H, H-8), 7.82 (br s, 1 H, exchangeable with D_2O, N^6H).

N^6-(3-Iodobenzyl)-2-methoxy-9-methyladenine (21). A mixture of compound **14** (21 mg, 0.052 mmol) and sodium methoxide (1.5 mg of Na) was heated for 14 h at 85 °C in a sealed bottle. The reaction mixture was concentrated to dryness, and the residue was crystallized from methanol–water to give compound **21** (19 mg, 86.0%): ^1H NMR (DMSO-d_6) δ 3.64 (s, 3 H, 9-CH_3), 3.81 (s, 3 H, OCH_3), 4.59 (br s, 2 H, CH_2), 7.11 (t, J = 7.6 Hz, 1 H, H-16), 7.37 (d, J = 7.6 Hz, 1 H, H-17), 7.59 (d, J = 7.6 Hz, 1 H, H-15), 7.74 (s, 1 H, H-13), 7.92 (s, 1 H, H-8), 8.37 (br s, 1 H, exchangeable with D_2O, N^6H).

N^6-(3-Iodobenzyl)-9-methyl-2-(methylthio)adenine (22). A mixture of compound **14** (24.4 mg, 0.061 mmol) and sodium thiomethoxide (8 mg, 0.1 mmol) in DMF–DME (1:1, 1.5 mL) was heated for 22 h at 110 °C in a sealed bottle. After cooling, the reaction mixture was concentrated to dryness and the residue was purified using silica gel column chromatography (chloroform–methanol, 20:1) to give compound **22** (13 mg, 52.0%) as a colorless solid: ^1H NMR (DMSO-d_6) δ 2.45 (s, 3 H, SCH_3), 3.67 (s, 3 H, 9-CH_3), 4.60 (br s, 2 H, CH_2), 7.11 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.36 (d, J = 7.0 Hz, 1 H, H-17), 7.58 (d, J = 8.0 Hz, 1 H, H-15), 7.74 (s, 1 H, H-13), 7.99 (s, 1 H, H-8), 8.43 (br s, 1 H, exchangeable with D_2O, N^6H).

N^6-(3-Iodobenzyl)-9-methyl-2-(4-pyridylthio)adenine (23). A mixture of compound **14** (20.4 mg, 0.051 mmol) and sodium hydrosulfide hydrate (11 mg, 0.2 mmol) in pyridine (1.5 mL) was heated for 5 days at 100 °C in a sealed bottle. After cooling, the reaction mixture was concentrated to dryness and the residue was purified using preparative TLC (chloroform–methanol, 20:1) to give compound **23** (6.5 mg, 27.4%) as a yellow solid: ^1H NMR (DMSO-d_6) δ 3.78 (s, 3 H, CH_3), 4.70 (br s, 2 H, CH_2), 7.13 (pseudo t, J = 7.6 and 7.5 Hz, 1 H, H-16), 7.29 (d, J = 7.2 Hz, 2 H, pyr), 7.45 (d, J = 7.2 Hz, 1 H, H-17), 7.60 (d, J = 8.2 Hz, 1 H, H-15), 7.86 (s, 1 H, H-13), 8.22 (s, 1 H, H-8), 8.73 (d, J = 7.2 Hz, 2 H, pyr), 9.03 (br s, 1 H, exchangeable with D_2O, N^6H).

2-Chloro-9-(β-D-erythrofuranosyl)-N^6-(3-iodobenzyl)-adenine (25). To a solution of D-erythrose 1,2,3-triacetate (**49**; 0.5 g, 2.03 mmol, prepared from erythrose and acetic anhydride/pyridine) in dry acetonitrile (10 mL), cooled to 0 °C, were added **45a** (0.8 g, 2.08 mmol) and $SnCl_4$ (0.8 mg, 3.07 mmol). After warming to room temperature, the reaction mixture was heated at 70 °C for 20 h. Solvent was removed *in vacuo*, and the residue was dissolved in concentrated NH_4OH. This solution was refluxed for 1 h. After evaporation of volatiles, the residue was purified using preparative TLC (eluent CH_2Cl_2–MeOH, 9.5:0.5, R_f = 0.45) to give **25** (150 mg, 15%): ^1H NMR (DMSO-d_6) δ 3.93 (m, 2 H, CH_2), 4.27 (m, 1 H, H-3′), 4.43 (m, 1 H, H-2′), 4.60 (br s, 2 H, CH_2), 5.31 (d, J = 4.5 Hz, 1 H, exchangeable with D_2O, OH), 4.50 (d, J = 4.5 Hz, exchangeable with D_2O, OH), 6.13 (d, J = 5.9 Hz, 1 H, H-1′), 7.14 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.34 (d, J = 7.5 Hz, 1 H, H-17), 7.60 (d, J = 7.8 Hz, 1 H, H-15), 7.62 (s, 1 H, H-13), 8.37 (s, 1 H, H-8), 8.85 (br s, 1 H, exchangeable with D_2O, N^6H).

9-(β-D-Erythrofuranosyl)-2-(methylamino)-N^6-(3-iodobenzyl)adenine (26). A solution of **25** (10 mg, 0.021 μmol) in MeOH (1 mL) and 40% aqueous methylamine (1 mL) was heated in a sealed vessel at 100 °C for 5 days. After cooling to room temperature, the volatiles were evaporated and the residue was purified using preparative TLC (eluent CH_2Cl_2–MeOH, 9.5:0.5) to give **26** as a white solid (9.6 mg, 98%): ^1H NMR (DMSO-d_6) δ 2.80 (s, 3 H, NH*Me*), 3.86 (m, 2 H, CH_2), 4.40 (m, 1 H, H-2′), 4.60 (s, 2 H, CH_2), 5.29 (d, J = 4.5 Hz, 1 H, exchangeable with D_2O, OH), 4.98 (d, J = 4.5 Hz, exchangeable with D_2O, OH), 6.13 (d, J = 5.9 Hz, 1 H, H-1′), 7.14 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16″), 7.34 (d, J = 7.5 Hz, 1 H, H-17), 7.59 (d, J = 7.8 Hz, 1 H, H-15), 7.59 (s, 1 H, H-8), 8.60 (br s, 1 H, exchangeable with D_2O, N^6H).

2-Chloro-6-[(3-iodobenzyl)amino]-9-(2-tetrahydrofuryl)-9*H*-purine (27). A solution of **45a** (350 mg, 0.91 mmol), 2,3-dihydrofuran (0.38 g, 5.42 mmol), and 6 drops of ethanesulfonic acid in 30 mL of dry ethyl acetate was heated for 20 h at 50 °C. After cooling to the room temperature, volatiles were removed by rotary evaporation and the residue was purified on preparative silica gel TLC plates (eluent CH_2Cl_2–MeOH, 10:1). After recrystallization from MeOH, **27** (53 mg, 13%) was obtained as a white solid: ^1H NMR (DMSO-d_6) δ 2.15 (m, 2 H, H-3′), 2.45 (q, J = 7.38 Hz, 2 H, H-2′), 3.92 (q, J = 7.38 Hz, 1 H, H-4′), 4.14 (q, J = 7.49 Hz, 1 H, H-4′), 4.60 (d, J = 5.65 Hz, 2 H, *CH_2*-Ph), 6.21 (t, J = 5.1 Hz, 1 H, H-1), 7.14 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.35 (d, J = 7.5 Hz, 1 H, H-17), 7.60 (d, J = 7.8 Hz, 1 H, H-15), 7.62 (d, J = 7.8 Hz, 1 H, H-13), 7.74 (s, 1 H, H-8), 8.87 (br s, 1 H, exchangeable with D_2O, N^6H).

N^6-Benzyl-2-chloro-9-[2,3-dideoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]adenine (29). A mixture of compound **35** (58.55 mg, 0.11 mmol), (phenoxythio)carbonyl chloride (0.027 mL, 0.19 mmol), and DMAP (35.7 mg, 0.29 mmol) in dry acetonitrile (1.5 mL) was stirred for 6.5 h at room temperature. The reaction mixture was concentrated to dryness, and the residue was purified using preparative TLC (chloroform–methanol, 20:1) to give compound **58** as a glassy solid: ^1H NMR ($CDCl_3$) δ 2.75 (m, 1 H, H-3′a), 2.89 (d, J = 4.7 Hz, 21 H, N*H*-CH_3), 3.05 (m, 1 H, H-3′b), 4.75 (m, 3 H, H-4′ and CH_2), 5.81 (m, 1 H, H-2′), 6.12 (s, 1 H, H-1′), 7.00–7.80 (m, 10 H, Ar).

A mixture of compound **58**, 1.0 M triethylborane in hexanes (0.28 mL, 0.28 mmol), and tributyltin hydride (0.074 mL, 0.28 mmol) in benzene was stirred for 2.5 h at room temperature. The reaction mixture was concentrated to dryness, and the residue was purified using preparative TLC (chloroform–methanol, 20:1) to yield compound **29** (11 mg, 23%) as a colorless solid: ^1H NMR ($CDCl_3$) δ 2.24–2.60 (m, 4 H, H-2′ and H-3′), 2.89 (d, J = 4.8 Hz, 3 H, NHC*H_3*), 4.53 (dd, J = 8.5 and 4.8 Hz, 1 H, H-4′), 4.76 (br s, 2 H, CH_2), 6.01 (pseudo t, J = 6.8 and 5.9 Hz, 1 H, H-1′), 6.24 (br s, 1 H, NH), 7.23–7.31 (m, 4 H, H-13,15,16,17), 7.66 (s, 1 H, H-8), 7.83 (br s, 1 H, N^6H).

9-[2-Azido-2,3-dideoxy-5-(methylcarbamoyl)-β-D-arabinofuranosyl]-2-chloro-N^6-(3-iodobenzyl)adenine (30). A mixture of compound **34** (56.6 mg, 0.12 mmol) and sodium azide (83 mg, 1.26 mmol) in anhydrous DMF (2.5 mL) was heated for 41 h at 100 °C. Diethyl ether (30 mL) and water (25 mL) were added, and two layers were separated after shaking. The aqueous layer was extracted with ether (3 × 30 mL), and the combined organic layer and extracts were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was purified using preparative TLC (chloroform–methanol, 20:1) to give compound **30** [R_f (chloroform–methanol, 20:1) = 0.29; 22 mg, 34%] as a colorless solid: ^1H NMR ($CDCl_3$) δ 2.61–2.87 (m, 2 H, H-3′), 2.89 (d, J = 5.0 Hz, 3 H, NHC*H_3*), 4.37 (dd, J = 11.1 and 5.2 Hz, 1 H, H-2′), 4.56 (dd, J = 8.5 and 6.1 Hz, 1 H, H-4′), 4.71 (br s, 2 H, CH_2), 6.18 (br s, 1 H, NH), 6.19 (d, J = 4.9 Hz, 1 H, H-1′), 7.05 (t, J = 7.7 Hz, 1 H, H-16), 7.30 (d, J = 6.8 Hz,

1 H, H-17), 7.43 (br s, 1 H, N^6H), 7.59 (d, J = 7.6 Hz, 1 H, H-15), 7.68 (s, 1 H, H-13), 7.81 (s, 1 H, H-8).

9-[2-Amino-2,3-dideoxy-5-(methylcarbamoyl)-β-D-arabinofuranosyl]-2-chloro-N^6-(3-iodobenzyl)adenine (31). A solution of compound **30** (15 mg, 0.027 mmol) and triphenylphosphine (78 mg, 0.3 mmol) in dry THF (2 mL) was stirred for 3 days at room temperature. Water (0.5 mL) and methanolic ammonia (5 mL) were added, and the reaction mixture was stirred for 21 h at room temperature. The reaction mixture was concentrated to dryness, and the residue was purified using preparative TLC (chloroform–methanol, 10:1) to give compound **31** (6 mg, 43%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 2.01 (m, 1 H, H-3′a), 2.45 (m, 1 H, H-3′b), 2.64 (d, J = 4.7 Hz, 3 H, NHCH_3), 3.80 (m, 1 H, H-2′), 4.40 (pseudo t, J = 8.7 and 7.5 Hz, 1 H, H-4′), 4.63 (br s, 2 H, CH_2), 6.09 (d, J = 5.7 Hz, 1 H, H-1′), 7.13 (pseudo t, J = 8.2 and 7.7 Hz, 1 H, H-16), 7.37 (d, J = 7.5 Hz, 1 H, H-17), 7.61 (d, J = 8.0 Hz, 1 H, H-15), 7.75 (s, 1 H, H-13), 8.11 (br s, 2 H, NH_2, exchangeable with D_2O), 8.52 (s, 1 H, H-8), 8.88 (br s, 1 H, exchangeable with D_2O, N^6H).

2-Chloro-9-[2,3-dideoxy-2-fluoro-5-(methylcarbamoyl)-β-D-arabinofuranosyl]-N^6-(3-iodobenzyl)adenine (32). To a −78 °C solution of 3′-deoxy-Cl-IB-MECA (**35**; 20 mg, 0.04 mmol) in dry dichloromethane (0.5 mL) was added 50 μL of DAST. After stirring at −78 °C for 2 h, the reaction mixture was warmed to room temperature over a period of 1 h and the reaction quenched by adding methanol (0.5 mL) and solid K_2CO_3 (2 mg). The solvent was removed by evaporation, and the residue was purified using preparative TLC (CH_2Cl_2–MeOH, 9.5:0.5, R_f = 0.3) to give **32**, 10 mg (50%): ^{1}H NMR (DMSO-d_6) δ 2.75 (m, 2 H, H-3′), 3.31 (s, 3 H, NH*Me*), 4.65 (br s, 2 H, CH_2), 4.75 (m, 1 H, H-2′), 5.51 (d, J = 3.6 Hz, H-4′), 6.21 (d, J = 4.0 Hz, 1 H, H-1′), 7.13 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.36 (d, J = 7.5 Hz, 1 H, H-17), 7.61 (d, J = 7.8 Hz, 1 H, H-15), 7.60 (s, 1 H, H-13), 8.35 (s, 1 H, H-8), 8.90 (br s, 1 H, exchangeable with D_2O, N^6H).

9-[2-Acetyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-2-chloro-N^6-(3-iodobenzyl)adenine (33). A mixture of 2-chloro-N^6-(3-iodobenzyl)adenine (**45a**; 163 mg, 0.42 mmol), ammonium sulfate (catalytic amount), and HMDS (10 mL) was refluxed for 2 h under N_2. The reaction mixture was concentrated to dryness *in vacuo* with exclusion of moisture. The resulting white solid **45b** was dissolved in dry 1,2-dichloroethane (1 mL), and a solution of compound **57** (75 mg, 0.3 mmol) in dry 1,2-dichloroethane (2 mL) and TMS triflate (0.082 mL, 0.42 mmol) were added. The reaction solution under N_2 was stirred for 1.5 h at room temperature and then refluxed for 17 h at 90 °C. Saturated $NaHCO_3$ (10 mL) and methylene chloride (10 mL) were added, and the mixture was stirred for 15 min. Two layers separated, and the aqueous layer was extracted with methylene chloride (3 × 30 mL). The combined organic layer and extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was separated on preparative TLC (chloroform–methanol, 20:1) to give compound **33** (71 mg, 42%): ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3 H, OAc), 2.50 and 2.75 (each m, 1 H, H-3′), 2.89 (d, J = 4.7 Hz, 3 H, NHCH_3), 4.70 (m, 3 H, H-4′ and CH_2), 5.31 (m, 1 H, H-2′), 5.85 (d, J = 3.2 Hz, 1 H, H-1′), 6.31 (br s, 1 H, NH), 7.02 (pseudo t, J = 7.8 and 7.6 Hz, 1 H, H-16), 7.29 (d, J = 7.6 Hz, 1 H, H-17), 7.58 (d, J = 7.8 Hz, 1 H, H-15), 7.67 and 7.72 (each s, 1 H, H-8 and H-13), 7.84 (br s, 1 H, exchangeable with D_2O, N^6H); UV (MeOH) λ_{max} 271.5 nm.

2-Chloro-9-[3-deoxy-2-(methylsulfonyl)-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl)adenine (34). Compound **35** (100 mg, 0.18 mmol) was dissolved in an equivolume mixture of dry pyridine and methylene chloride (4 mL), and methanesulfonyl chloride (0.05 mL, 0.65 mmol) was added. The reaction mixture was stirred for 1.5 h at room temperature, and the solvents were removed using rotary evaporation. The residue was purified using silica gel column chromatography (chloroform–methanol, 20:1) to give compound **34** (87.5 mg, 78%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 2.66 (ddd, J = 11.1, 7.5, and 3.9 Hz, 1 H, H-3′a), 2.86 (m, 1 H, H-3′b), 2.89 (d, J = 5.0 Hz, 3 H, NHCH_3), 3.03 (s, 3 H, OSO_2CH_3), 4.72 (m, 3 H, H-4′ and CH_2), 5.39 (m, 1 H, H-2′), 6.05 (d, J = 3.1 Hz, 1 H, H-1′), 6.31 (br s, 1 H, NH), 7.05 (pseudo t, J = 7.8 and 7.6 Hz, 1 H, H-16), 7.28 (d, J = 7.7 Hz, 1 H, H-17), 7.58 (d, J = 7.7 Hz, 1 H, H-15), 7.67 and 7.77 (each s, 1 H, H-8 and H-13), 8.65 (br s, 1 H, exchangeable with D_2O, N^6H).

2-Chloro-9-[3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl)adenine (35). A mixture of compound **33** (15 mg, 0.027 mmol) and NH_3/MeOH (1.5 mL) was stirred for 18 h at room temperature. The reaction mixture was concentrated to dryness, and the residue was purified using silica gel column chromatography (chloroform–methanol, 20:1) to give compound **35** (6.22 mg, 43%) as a slightly yellow solid: ^{1}H NMR (DMSO-d_6) δ 2.15–2.23 and 2.26–2.35 (each m, 1 H, H-3′), 2.65 (d, J = 4.3 Hz, 3 H, NHCH_3), 4.55–4.68 (m, 4 H, H-2′, H-4′, CH_2), 5.83 (d, J = 3.9 Hz, 1 H, OH, exchangeable with D_2O), 5.90 (s, 1 H, H-1′), 7.13 (t, J = 7.6 Hz, 1 H, H-16), 7.37 (d, J = 7.6 Hz, 1 H, H-17), 7.61 (d, J = 7.7 Hz, 1 H, H-15), 7.75 (s, 1 H, H-13), 8.14 (br s, 1 H, exchangeable with D_2O, N*H*CH_3), 8.59 (s, 1 H, H-8), 8.95 (br t, J = 5.7 Hz, 1 H, exchangeable with D_2O, N^6H).

2-Chloro-N^6-(3-iodobenzyl)-9-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxyl)-β-D-ribofuranosyl]adenine (36). To a solution of 2-chloro-N^6-(3-iodobenzyl)adenosine[18] (300 mg, 0.58 mmol) in dry pyridine (9 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.41 mL, 1.28 mmol) at room temperature, and the reaction mixture was stirred for 2.5 h at room temperature. After workup as described,[39] the residue was purified via silica gel column chromatography (chloroform–methanol, 100:1) to give compound **36** (375 mg, 91%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 0.91–1.18 (m, 28 H, isopropyl), 3.17 and 3.49 (each s, 1 H), 4.03 (m, 3 H), 4.52 (d, J = 5.3 Hz, 1 H), 4.70 (br s, 2 H), 5.01 (m, 1 H), 5.83 (s, 1 H), 6.15 (br s, 1 H), 7.01 (t, J = 7.6 Hz, 1 H, H-16), 7.28 (d, J = 7.6 Hz, 1 H, H-17), 7.55 (d, J = 7.7 Hz, 1 H, H-15), 7.66 (s, 1 H, H-13), 7.78 (s, 1 H, H-8).

2-Chloro-N^6-(3-iodobenzyl)-9-[5-(methylcarbamoyl)-2,3-*O*-(thiocarbonyl)-β-D-ribofuranosyl]adenine (37). To a solution of Cl-IB-MECA (**2**; 10 mg, 0.02 mmol) in dry DMF (0.5 mL) were added 1,1-thiocarbonyldiimidazole (30 mg, 0.17 mmol) and DMAP (2 mg). The resulting mixture was stirred overnight at room temperature. After removal of DMF using rotary evaporation under high vacuum, the residue was purified using preparative TLC (CH_2Cl_2–MeOH, 9.5:0.5, R_f = 0.6) to give **37**, 8.6 mg (80%): ^{1}H NMR (DMSO-d_6) δ 2.73 (d, J = 4.3 Hz, 3 H, NH*Me*), 4.21 (m, 1 H, H-3′), 4.62 (br s, 2 H, CH_2), 5.09 (s, 1 H, H-4′), 5.95 (m, 1 H, H-2′), 6.31 (d, J = 7.3 Hz, 1 H, H-1′), 7.14 (pseudo t, J = 7.9 and 7.6 Hz, 1 H, H-16), 7.40 (d, J = 7.6 Hz, 1 H, H-17), 7.60 (d, J = 7.8 Hz, 1 H, H-15), 7.76 (s, 1 H, H-13), 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).

5-*O*-Benzoyl-3-deoxy-1,2-isopropylidene-α-D-ribofuranose (52). A solution of 5-*O*-benzoyl-1,2-isopropylidene-α-D-xylofuranose (5.9 g, 0.02 mol) and carbon disulfide (6.03 mL, 0.1 mol) in anhydrous THF (60 mL) was immersed in an ice bath under N_2 atmosphere. Sodium hydride in mineral oil (60%, 1.6 g, 0.04 mol) was added all at once. The reaction mixture was stirred for 50 min at 0 °C, and methyl iodide (25.7 mL, 0.4 mol) was added. After stirring for 1 h at 0 °C, the reaction mixture was neutralized with glacial acetic acid until the precipitate dissolved. The mixture was concentrated to dryness *in vacuo*. The residue was dissolved in ethyl acetate and filtered through a short silica gel column (hexanes–ethyl acetate, 10:1) to give the xanthate as a brown thick syrup.

A mixture of the xanthate, tributyltin hydride (7.6 mL, 0.029 mol), and triethylborane (28.6 mL, 0.029 mol) in benzene was stirred for 4 h at room temperature. The reaction mixture was concentrated to dryness, and the residue was purified using silica gel column chromatography (hexanes–ethyl acetate, 100:1 → 10:1 → 3:1) to give compound **52** (1.67 g, 30%): ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3 H, isopropylidene), 1.47 (s, 3 H, isopropylidene), 1.69 (td, J = 13.1 and 4.8 Hz, 1 H, H-3b), 2.12 (dd, J = 13.3 and 4.2 Hz, 1 H, H-3a), 4.29 (dd, J = 12.1 and 6.0 Hz, 1 H, H-5b), 4.50 (m, 2 H, H-4 and H-5a), 4.72 (t, J = 4.2 Hz, 1 H, H-2), 5.81 (d, J = 3.7 Hz, 1 H, H-1), 7.35–8.01 (m, 5 H, Bz).

3-Deoxy-1,2-isopropylidene-α-D-ribofuranose (53). A mixture of **52** (1.67 g, 6 mmol) and methanolic ammonia (50 mL, saturated at 0 °C) was stirred for 5 days at room

temperature. The reaction mixture was concentrated to dryness, and the residue was purified using silica gel column chromatography (hexanes–ethyl acetate, 100:1 → 1:1) to give compound **53** (0.83 g, 79%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H, isopropylidene), 1.45 (s, 3 H, isopropylidene), 1.66–1.83 (m, 1 H, H-3b), 1.95 (dd, J = 13.4 and 4.6 Hz, 1 H, H-3a), 3.50 (m, 1 H, H-5b), 3.83 (1 H, H-5a), 4.28 (1 H, H-4), 4.70 (pseudo t, J = 4.2 and 4.1 Hz, 1 H, H-2), 5.76 (d, J = 3.6 Hz, 1 H, H-1).

3-Deoxy-1,2-isopropylidene-α-D-5-ribofuronic Acid (54). A mixture of compound **53** (0.503 g, 2.89 mmol), ruthenium oxide (38 mg), and sodium periodate (2.47 g, 11.6 mmol) in acetonitrile:chloroform:water (2:2:3, 14 mL) was stirred vigorously for 4 h at room temperature. After separation of the two layers, the aqueous layer was extracted with chloroform (3 × 50 mL). The combined organic layer and extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, concentrated to dryness, and dried *in vacuo* to give compound **54** (0.537 g, 98%) as a solid: ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 3 H, isopropylidene), 1.46 (s, 3 H, isopropylidene), 1.91 (td, J = 12.3 and 4.3 Hz, 1 H, H-3b), 2.48 (dd, J = 13.6 and 5.2 Hz, 1 H, H-3a), 4.70 (m, 2 H, H-2 and H-4), 5.89 (d, J = 3.3 Hz, 1 H, H-1).

Methyl 3-Deoxy-1,2-isopropylidene-α-D-ribofuronamide (56). The mixture of compound **54** (0.48 g, 2.55 mmol), EDAC (1.226 g, 6.42 mmol), and DMAP (0.031 g, 0.25 mmol) in anhydrous methanol (10 mL) was stirred for 24 h at room temperature. The reaction mixture was concentrated to dryness, and the residue was dissolved in chloroform (30 mL) and water (20 mL). Two layers separated, and the aqueous layer was extracted with chloroform (3 × 30 mL). The combined organic layer and extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was purified using silica gel column chromatography (chloroform–methanol, 20:1) to give compound **55** (0.217 g, 42%): ^{1}H NMR ($CDCl_3$) δ 1.33 and 1.55 (each s, 3 H, isopropylidene), 1.90–2.00 (m, 3 H, H-3a), 2.39–2.45 (dd, J = 13.5 and 4.9 Hz, 1 H, H-3b), 3.78 (s, 3H, OCH_3), 4.71 (dd, J = 10.9 and 5.0 Hz, 1 H, H-2 or -4), 4.77 (t, J = 4.2 Hz, 1 H, H-2 or -4), 5.95 (d, J = 3.4 Hz, 1 H, H-1).

A solution of compound **55** (217 mg, 1.07 mmol) and 2 M methylamine in THF (5 mL) was heated for 24 h at 55 °C in a sealed tube. The reaction mixture was concentrated to dryness, and the residue was dried *in vacuo* to give compound **56** (216 mg, 99%) as needles: ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3 H, isopropylidene), 1.44 (s, 3 H, isopropylidene), 1.69–1.78 (m, 1 H, H-3b), 2.53 (dd, J = 13.7 and 5.2 Hz, 1 H, H-3a), 2.77 (d, J = 4.9 Hz, 3 H, $NHCH_3$), 4.59 (dd, J = 11.1 and 5.2 Hz, 1 H, H-4), 4.69 (t, J = 4.0 Hz, 1 H, H-2), 5.81 (d, J = 3.5 Hz, 1 H, H-1), 6.42 (br s, 1 H, exchangeable with D_2O, N^6H). Anal. Calcd for $C_9H_{15}N_1O_4$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.97; H, 7.65; N, 6.93.

Methyl 3-Deoxy-1,2-diacetyl-β-D-ribofuronamide (57). A mixture of compound **56** (189 mg, 0.94 mmol), concentrated sulfuric acid (0.276 mL, 5.18 mmol), and acetic anhydride (0.93 mL, 9.86 mmol) in glacial acetic acid (4.68 mL) was stirred for 18 h at room temperature. Following cooling in an ice bath, saturated $NaHCO_3$ solution (10 mL) and methylene chloride (10 mL) were added slowly, and the mixture was stirred for 10 min. After separation of the two layers, the aqueous layer was extracted with methylene chloride (3 × 30 mL). The organic layer and extracts were combined, washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, concentrated to dryness, and dried *in vacuo* to yield crude compound **57** (184 mg, 80%) as a yellow syrup: ^{1}H NMR ($CDCl_3$) δ 2.00 and 2.03 (each s, 3 H, OAc), 2.25–2.35 (m, 1 H, H-3b), 2.40–2.47 (m, 1 H, H-3a), 2.77 (d, J = 5.0 Hz, 3 H, $NHCH_3$), 4.68 (m, 1 H, H-4), 5.12 (d, J = 4.8 Hz, 1 H, H-2), 6.12 (s, 1 H, H-1), 6.35 (br s, 1 H, exchangeable with D_2O, N^6H). Anal. Calcd for $C_{10}H_{15}N_1O_6$: C, 48.98; H, 6.17; N, 5.71. Found: C, 58.94; H, 6.06; N, 5.42.

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]-AB-MECA was prepared as described.[25] [^{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. The incubation buffer for A_3 competition experiments consisted of 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.

CHO cells stably expressing the A_3 receptor[8] were grown, and cell membranes were prepared by homogenization and centrifugation, as previously described.[17,20] The preparation was stored at −70 °C and retained its A_3 radioligand binding properties for at least 1 month.

Binding of [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide) ([^{125}I]AB-MECA) to membranes from CHO cells stably transfected with the A_3 receptor clone was performed essentially as described.[20,25] Assays were performed in 50/10/1 buffer 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 then diluted with buffer; final DMSO concentrations never exceeded 2%. Incubations were carried out in duplicate for 1 h at 37 °C and terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). Nonspecific binding was determined in the presence of 200 μM NECA.

Binding of [^{3}H]PIA to A_1 receptors from rat brain membranes and binding of [^{3}H]CGS 21680 to A_2 receptors from rat striatal membranes were performed as described previously.[20,26,27] Rat cerebral cortical membranes and striatal membranes were prepared and treated with adenosine deaminase (2 U/mL) for 30 min at 37 °C prior to storage at −70 °C. Nonspecific binding was determined in the presence of 2-chloroadenosine at a concentration of 10 μM for A_1 receptors and 200 μM for A_{2a} receptors.

For all radioligand binding assays, IC_{50} values were computer-generated using a nonlinear regression formula using the InPlot program (GraphPad Software, San Diego, CA) and converted to apparent K_i values using K_d values of 1.0 and 14 nM for [^{3}H]PIA and [^{3}H]CGS 21680 binding, respectively, and the Cheng–Prusoff equation.[28] The K_d for [^{125}I]AB-MECA was assumed to be 1.48 nM as found previously at cloned rat A_3 receptors in CHO cells.[25] Adenylyl cyclase in transfected CHO cell membranes was measured as previously described.[8,20]

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