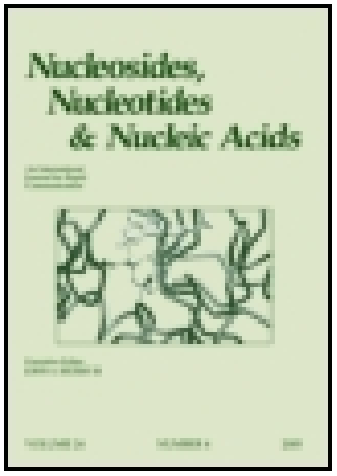

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Synthesis of Substituted-Benzyl and Sugar-Modified Analogues of 6-N-(4-Nitrobenzyl)adenosine and Their Interactions with "ES" Nucleoside Transport Systems

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NUCLEOSIDES & NUCLEOTIDES, 13(6&7), 1627-1646 (1994)

SYNTHESIS OF SUBSTITUTED-BENZYL AND SUGAR-MODIFIED ANALOGUES OF 6-*N*-(4-NITROBENZYL)ADENOSINE AND THEIR INTERACTIONS WITH "*ES*" NUCLEOSIDE TRANSPORT SYSTEMS¶

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Abstract: Four classes of 6-*X*-benzylated purine nucleosides, (i) 6-*N*-(substituted-benzyl)adenosines, (ii) 6-*N*-(4-nitrobenzyl)adenine nucleosides with modified sugars, (iii) 6-*N(S)*-(4-azidobenzyl) derivatives of adenosine, 6-thioinosine, and 6-thioguanosine, and (iv) 6-*N*-{4-*N*-[acyl(sulfonyl)amino]benzyl}adenosines, were synthesized and their binding interactions with "*es*-NT" (***e***quilibrative, inhibitor-***s***ensitive nucleoside transport) systems were studied. Several tight-binding analogues were found.

Introduction

Physiological nucleosides and various analogues enter animal cells primarily by nucleoside transport (NT) processes that include two facilitated diffusion processes,[1-4] *es* and *ei*.[5-7] Both are expressed in L1210 mouse leukemia cells[7,8] and both are sensitive to the NT inhibitor dipyridamole,[9-12] but *ei* processes are distinguished by their relative insensitivity to 1 μM NBMPR [6-*S*-(4-nitrobenzyl)-6-thioinosine], a potent, tightly-bound inhibitor of *es*-NT.[7,8,11a,12] Analogues of NBMPR also have been found to bind tightly to the *es* transporter.[13-15] Recent studies have demonstrated the expression of sodium (Na^+)-

¶ This paper is dedicated to Dr. Morio Ikehara on the occasion of his 70th birthday.

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linked NT activity in several cultured cell lines[16] including L1210 mouse leukemias,[7,10,11] and in epithelial and lymphoid tissues from several species.[17]

Site-bound analogues with 6-*N(S)*-(4-azidobenzyl) moieties were found to undergo photoaffinity coupling upon irradiation with UV light, and photolabeling of the putative NT glycoprotein was effected with [^{3}H]NBMPR.[18] Recent studies on the photochemistry of NBMPR and analogues in the presence of model compounds bearing functional groups which are found in amino acids have indicated possible photolabeling processes.[19]

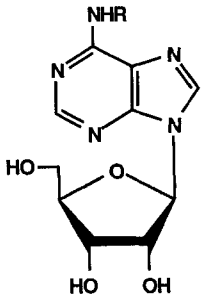
Interest in nucleoside transport has been stimulated by recent studies with anti-HIV nucleoside analogues.[20-22] The present investigation was initiated to: (1) probe the *es*-NT binding potential of 6-*N*-(substituted-benzyl)adenosine analogues of NBMPR (which would release adenosine rather than toxic 6-thioinosine upon benzylic cleavage) in search of superior NT inhibitors; (2) prepare 6-*N*- and 6-*S*-(4-azido- or 4-nitrobenzyl) analogues for *es*-NT binding evaluation (and possible photolabeling); and (3) probe *es*-NT binding of acyl-type derivatives of 6-*N*-(4-aminobenzyl)adenosine and sugar-modified analogues of 6-*N*-(4-nitrobenzyl)adenosine to evaluate promising site(s) for attachment of hydrocarbon tethers for affinity chromatographic isolation of NT glycoproteins.

Results and Discussion

Structures of the compounds prepared for this study are shown in FIGURES 1 and 2. Alkylation of adenosine and modified-sugar analogues with substituted-benzyl halides in dimethylformamide gave N1-benzylated salts which underwent Dimroth rearrangement to give[23-28] the 6-*N*-(substituted-benzyl)adenine nucleosides **1**, **3-11**, and **29-34**. Hydrogenation[26,28] of nitrobenzyl analogue **1** gave 6-*N*-(4-aminobenzyl)adenosine[26] (**2**).

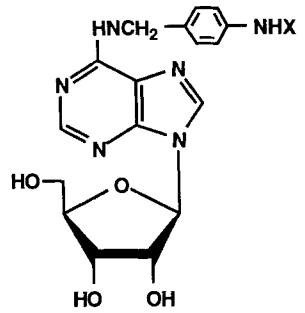
Acetylation[26] or chloroacetylation of **2** with the respective anhydrides in pyridine gave tetraacyl derivatives which provided 6-*N*-[4-(acetamido or chloroacetamido)benzyl]-adenosine (**12** or **13**) upon treatment with methanolic ammonia. Compounds **14-25** were prepared by "acylation" of the (4-amino)benzyl moiety of **2** using the transient protection methodology of Jones.[29] Protection of **2** with chlorotrimethylsilane in pyridine, treatment of the intermediate(s) with acyl, sulfonyl, or carbamyl chlorides, or phenylisothiocyanate, and deprotection with aqueous ammonia gave **14-25**.

Compounds **26** and **27** were prepared by S-alkylation[25,27] of 6-thioinosine or 6-thioguanosine, respectively, with 4-azidobenzyl bromide[30] in DMF in the presence of



1 - 11

Compound	R (phenyl ring position)
1	$CH_2C_6H_4NO_2(4)$
2	$CH_2C_6H_4NH_2(4)$
3	$CH_2C_6H_4N_3(4)$
4	$CH_2C_6H_4OCH_3(3)$
5	$CH_2C_6H_3NO_2(2)CH_3(5)$
6	$CH_2C_6H_3NO_2(2)Cl(4)$
7	$CH_2C_6H_3NO_2(3)Cl(4)$
8	$CH_2C_6H_3CH_3(3)NO_2(4)$
9	$CH_2C_6H_3CH_3(2)NO_2(3)$
10	$CH_2C_6H_3NO_2(3)CH_3(4)$
11	$CH_2C_6H_3Cl(2)NO_2(4)$



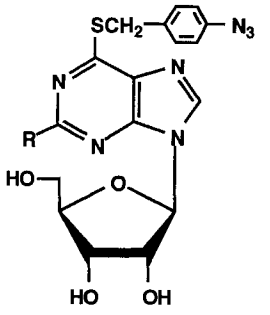
12 - 25

Compound	X
12	$COCH_3$
13	$COCH_2Cl$
14	$CO(CH_2)_3CH_3$
15	$CO(CH_2)_4CH_3$
16	$CO(CH_2)_5CH_3$
17	$CO(CH_2)_6CH_3$
18	$COC(CH_3)_3$
19	$COOCH_2CH_3$
20	COC_6H_5
21	$CON(CH_3)_2$
22	$CON(C_6H_5)_2$
23	SO_2CH_3
24	$SO_2C_6H_4CH_3(4)$
25	$CSNHC_6H_5$

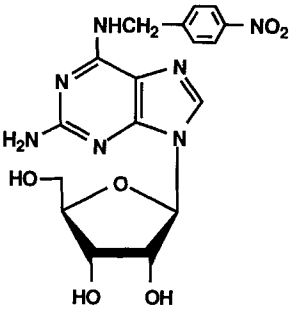
FIG. 1. Structures of Compounds **1** - **25**

potassium carbonate. Treatment of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine[31] with 4-nitrobenzylamine in DMF in the presence of molecular sieves gave **28**.[26] Spectral data were in harmony with the structures, and purities were confirmed by elemental analyses.

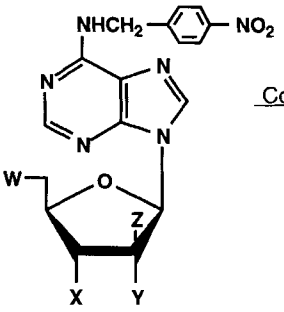
Wild-type mouse leukemia L1210 cell lines coexpress at least three nucleoside transport activities.[7,8,32,33] The present study employed the clonal line L1210/B23.1, cells of which express only the *es*-NT system.[33] Wild-type mouse lymphoma S49 cells used in this study also express *es*-NT activity. Prior mutation-selection studies yielded a new clonal cell line (AE_1) which lacks both the *es*-NT and NBMPR binding activities.[34] That result, combined with extensive data which correlate occupancy of NBMPR sites with



26, R = H
27, R = NH_2



28



Compound	W	X	Y	Z
29	OH	OH	H	H
30	OH	H	OH	H
31	OH	OH	H	OH
32	Cl	OH	OH	H
33	H	OH	OH	H
34	OH	$-OC(CH_3)_2O-$		H

29 - 34

FIG. 2. Structures of Compounds **26** - **34**

inhibition of nucleoside fluxes in erythrocytes by NBMPR, supports the hypothesis of direct association of NBMPR binding sites with the *es*-NT glycoprotein.[1,4,14,15,35]

Interaction of our analogues with the NBMPR binding sites of S49 (TABLE 1) and L1210/B23.1 cells (TABLE 2) was demonstrated and certain trends are evident in the data. (a) Ribosyl (**1**), 2'-deoxy (**29**), arabinosyl (**31**), and 5'-deoxyribosyl (**33**) sugar moieties were accepted readily at binding sites, but the 3'-deoxy analogue **30** was bound weakly in harmony with prior conclusions regarding the 3'-hydroxyl group and *es* NT activity.[36] (b) Important contributions to binding are made by the [4-(nitro, azido, and acetamido)benzyl] substituents. Reduction of **1** (4-nitro) gave weakly bound **2** (4-amino), but its acetylation gave the strongly bound (4-acetamido)benzyl analogue **12**. (c) The 6-*S*-(4-azidobenzyl)-

Table 1. Apparent Dissociation Constants at NBMPR Binding Sites on S49 Cells[a]

compound	name	K_d (nM)	method
29	2'-deoxy-6-*N*-(4-nitrobenzyl)adenosine	2.4 ± 0.2[b]	I
33	5'-deoxy-6-*N*-(4-nitrobenzyl)adenosine	3.3	I
31	9-(β-D-arabinofuranosyl)-6-*N*-(4-nitrobenzyl)adenine	4.7 ± 1.6	I
1	6-*N*-(4-nitrobenzyl)adenosine	8.7 ± 0.7[b]	I
8	6-*N*-(-3-methyl-4-nitrobenzyl)adenosine	13	I,J
32	5'-chloro-5'-deoxy-6-*N*-(4-nitrobenzyl)adenosine	16 ± 2	I
12	6-*N*-(4-acetamidobenzyl)adenosine	27	I
3	6-*N*-(4-azidobenzyl)adenosine	37 ± 6[b]	I
11	6-*N*-(2-chloro-4-nitrobenzyl)adenosine	52.0	J
9	6-*N*-(2-methyl-3-nitrobenzyl)adenosine	64.0	J
13	6-*N*-[4-(chloroacetamido)benzyl]adenosine	88	I
7	6-*N*-(3-nitro-4-chlorobenzyl)adenosine	105	J
30	3'-deoxy-6-*N*-(4-nitrobenzyl)adenosine	146 ± 32	I
10	6-*N*-(3-nitro-4-methylbenzyl)adenosine	175	I,J
5	6-*N*-(2-nitro-5-methylbenzyl)adenosine	192	I
34	2',3'-*O*-isopropylidene-6-*N*-(4-nitrobenzyl)adenosine	273 ± 26[b]	I
2	6-*N*-(4-aminobenzyl)adenosine	631 ± 63[b]	I
6	6-*N*-(2-nitro-4-chlorobenzyl)adenosine	7070	J
4	6-*N*-(3-methoxybenzyl)adenosine	17200	J

[a]See the Experimental Section for assay methods. [b]Reported previously.[15]

6-thioinosine (**26**) and 6-*S*-(4-azidobenzyl)-6-thioguanosine (**27**) compounds inhibited *es*-mediated adenosine transport to about twice the extent of 6-*N*-(4-azidobenzyl)adenosine (**12**) in L1210/B23.1 cells (TABLE 2) in general agreement with the finding that NBMPR is bound about 90-fold more tightly than **1** by the *es* transporter of S49 cells.[15]

The data in TABLE 3[37] demonstrate weak interference by 6-*N*-[4-(acetamido)-benzyl]adenosine (**12**) and analogues with the site-specific binding of [^{3}H]NBMPR to pig erythrocyte ghosts. The longer chain fatty acid amides and the benzamide **20** are bound more tightly, whereas the ethyl carbamate **19** and the methanesulfonamide **23** are bound more strongly than comparable amides and the *p*-toluenesulfonamide **24**, respectively.

Table 2. Inhibition of *es*-Mediated Adenosine Transport in L1210/B23.1 Cells[a]

compound	name	IC_{50} (nM) ± SEM[b]
31	9-(β-D-arabinofuranosyl)-6-*N*-(4-nitrobenzyl)adenine	2.5 ± 0.34
27	6-*S*-(4-azidobenzyl)-6-thioguanosine	5.0 ± 0.7[c]
29	2'-deoxy-6-*N*-(4-nitrobenzyl)adenosine	5.6 ± 1.1
26	6-*S*-(4-azidobenzyl)-6-thioinosine	6.3 ± 1.0
33	5'-deoxy-6-*N*-(4-nitrobenzyl)adenosine	6.3 ± 1.2
8	6-*N*-(3-methyl-4-nitrobenzyl)adenosine	7.3 ± 0.7[c]
9	6-*N*-(2-methyl-3-nitrobenzyl)adenosine	7.3 ± 2.0[c]
11	6-*N*-(2-chloro-4-nitrobenzyl)adenosine	9.0 ± 2.7[c]
3	6-*N*-(4-azidobenzyl)adenosine	12.8 ± 2.7
7	6-*N*-(3-nitro-4-chlorobenzyl)adenosine	16.5 ± 2.5
10	6-*N*-(3-nitro-4-methylbenzyl)adenosine	26.3 ± 5.8
25	6-*N*-(4-{[(*N*-phenyl)thiocarbamoyl]amino}benzyl)adenosine	355 ± 60.1
6	6-*N*-(2-nitro-4-chlorobenzyl)adenosine	493 ± 73.6
4	6-*N*-(3-methoxybenzyl)adenosine	1200 ± 240
5	6-*N*-(2-nitro-5-methylbenzyl)adenosine	4760 ± 1550[c]

[a]See the Experimental Section for assay methods. [b]Concentrations which reduced inward flux of adenosine (10-s assay) to 50% of that in the absence of inhibitors; means ± SEM of at least 3 assays except as noted. [c]Means of four determinations ± SEM.

Affinities of these compounds for the NBMPR binding site appeared to be inadequate for affinity purification of the transporter, so the benzyl substituent was abandoned as a site at which to tether a nucleoside derivative to a chromatographic support. The tight binding of the 5'-chloro-5'-deoxy **32** and 5'-deoxy **33** derivatives of **1** (TABLE 1) indicated that attachment of a tether to C5' of a derivative of **1** might be promising. The 5'-*S*-(2-aminoethyl)-6-*N*-(4-nitrobenzyl)-5'-thioadenosine (SAENTA) analogue was synthesized, attached to agarose beads, and utilized in partial purification of the *es* NT glycoprotein from pig erythrocyte ghosts by affinity chromatography.[37]

In summary, our 6-*N*-[4-(substituted)benzyl])adenosine derivatives with 4-(azido, nitro, and acetamido) substituents and other variations bind strongly to the *es*-NT system of different cells. Arabino and deoxy variations at C2' and chloro and deoxy modifications at

Table 3. Inhibition of NBMPR Binding to Pig Erythrocyte Ghosts[a]

compound	NH-substituent	IC_{50} (μM)
20	$-COC_6H_5$	0.21
23	$-SO_2CH_3$	0.94
22	$-CON(C_6H_5)_2$	1
17	$-CO(CH_2)_6CH_3$	3
19	$-COOCH_2CH_3$	3
24	$-SO_2C_6H_4CH_3(4)$	9
16	$-CO(CH_2)_5CH_3$	16
15	$-CO(CH_2)_4CH_3$	21
21	$-CON(CH_3)_2$	41
12	$-COCH_3$	59
14	$-CO(CH_2)_3CH_3$	64
18	$-COC(CH_3)_3$	140

[a]See ref. 37 for assay methods for these previously reported data.

C5' are well tolerated, but deoxygenation at C3' causes marked loss of binding potency with the 4-nitrobenzyl analogues. Studies are in progress to further define substituent effects on binding of nucleoside derivatives to the transporter systems of selected cell lines.

Experimental Section

Chemistry. General Methods. Uncorrected melting points were determined on a hot stage apparatus. UV spectra (neutral solutions in H_2O and/or MeOH) were recorded on a Hewlett Packard 8451A spectrometer. ^1H NMR spectra (Me_2SO-d_6, Me_4Si internal) were recorded with Bruker WH-(200 or 400) spectrometers. Elemental analyses and high-resolution mass spectra were determined at the University of Alberta. TLC plates and silica gel (kieselgel 60, 230-400 mesh) were purchased from Merck. Solvents were dried and distilled by usual methods. Nucleoside products were purified by recrystallization and/or chromatography on silica gel columns. TABLES 4 and 5 list the synthetic methods used and spectral and characterization data. Typical synthetic methods are illustrated with the following compounds.

Table 4. Synthetic Methods, Properties, and Spectral and Elemental Analysis Data

Compound	Method	Yield %	mp °C (a)	Formula	Elemental Analyses Found (Calcd.) C	H	N	UV max nm (ε)	MS *m/z* (Calcd.)[b]
1	A	64	174-176 (c)	$C_{17}H_{18}N_6O_6$	50.74 (50.58	4.51 4.52	20.88 20.59)	272 (26 600)	402 (402)
2	E	77	192-194 (c)	$C_{17}H_{20}N_6O_4$ ($\bullet 0.5H_2O$)	53.53 (53.68	5.54 5.32	22.03 21.71)	269 (23 300)	372.1548 (372.1548)
3	B	30	185-186 (d)	$C_{17}H_{18}N_8O_4$	51.25 (50.98	4.56 4.68	28.13 28.09)	266 (29 000)	370.1379 (370.1392)[e]
4	C	32	153-154 (c)	$C_{18}H_{21}N_5O_5$	55.81 (55.82	5.46 5.54	18.08 17.97)	270 (24 100)	387.1541 (387.1544)
5	C	24	144-145 (d)	$C_{18}H_{20}N_6O_6$	51.92 (51.88	4.84 4.93	20.18 20.05)	267 (27 800)	416.1459 (416.1446)
6	C	30	218-219 (c)	$C_{17}H_{17}ClN_6O_6$	46.75 (46.70	3.92 4.02	19.24 19.10)	267 (28 900)	438.0873 (438.0870)[f]
7	C	67	182-183 (c)	$C_{17}H_{17}ClN_6O_6$	46.75 (46.61	3.92 3.97	19.24 19.32)	268 (27 600)	438.0866 (438.0870)[g]
8	C	52	152-153 (c)	$C_{18}H_{20}N_6O_6$	51.92 (51.96	4.84 4.88	20.18 20.21)	270 (28 400)	416.1445 (416.1446)
9	C	61	144-145 (c)	$C_{18}H_{20}N_6O_6$	51.92 (52.10	4.84 4.98	20.18 20.17)	269 (26 000)	416.1445 (416.1446)
10	C[h]	67	170-172 (c)	$C_{18}H_{20}N_6O_6$	51.92 (51.91	4.84 4.89	20.18 19.82)	268 (27 000)	416.1449 (416.1446)

11	C	21	166-168 (c)	$C_{17}H_{17}ClN_6O_6$	46.75 (46.56	3.92 3.86	19.24 19.12)	270 (29 600)	438.0866 (438.0870)[i]
12	F	86	166-168 (c)	$C_{19}H_{22}N_6O_5$	55.07 (55.15	5.35 5.42	20.28 19.95)	267 (30 200)	414.1660 (414.1652)
13	F	22	175-177 dec. (c)	$C_{19}H_{21}ClN_6O_5$ ($\bullet 0.5H_2O$)	49.84 (49.98	4.84 4.62	18.35 18.20)	269 (34 000)	j
14	G	64	197-198 (k)	$C_{22}H_{28}N_6O_5$	57.88 (57.76	6.18 6.12	18.41 18.41)	267 (22 100)	456.2120 (456.2121)
15	G	48	187-188 (k)	$C_{23}H_{30}N_6O_5$	58.71 (58.78	6.43 6.28	17.86 17.61)	267 (23 700)	470.2285 (470.2278)
16	G	72	183-185 (k)	$C_{24}H_{32}N_6O_5$	59.49 (59.91	6.66 6.72	17.34 17.35)	267 (22 000)	484.2422 (484.2434)
17	G	56	181-183 (k)	$C_{25}H_{34}N_6O_5$	60.23 (60.25	6.87 6.83	16.86 16.53)	267 (24 500)	498.2564 (498.2591)
18	G	66	142-144 (k)	$C_{22}H_{28}N_6O_5$	57.88[l] (56.69	6.18 6.07	18.41 18.07)	267 (21 400)	456.2128 (456.2121)
19	G	85	175-177 (c)	$C_{20}H_{24}N_6O_6$	54.05 (54.09	5.44 5.45	18.91 18.50)	242 (19 200) 269 (23 100)	444.1770 (444.1757)
20	G	71	254-255 (c)	$C_{24}H_{24}N_6O_5$	60.50 (60.21	5.08 5.12	17.64 17.42)	273 (28 600)	476.1794 (476.1808)
21	G	61	149-151 (c)	$C_{20}H_{25}N_7O_5$	54.17 (53.69	5.68 5.64	22.11 21.80)	268 (30 900)	398.1341 (398.1339)[m]
22	G	56	182-183 (c)	$C_{30}H_{29}N_7O_5$	63.48 (63.16	5.15 5.16	17.27 17.16)	270 (37 100)	398.1331 (398.1339)[n]
23	G	60	132-133 (k)	$C_{18}H_{22}N_6O_6S$	47.99[o] (47.41	4.92 4.80	18.66 18.61)	268 (19 400)	450.1277 (450.1322)

(continued)

Table 4. Continued

24	G	72	123-137 (p)	$C_{24}H_{26}N_6O_6S$	54.74 (54.68	4.98 5.11	15.96 15.70)	268 (22 400)	394.1209 (394.1211)[q]
25	G	76	131-133 (c)	$C_{24}H_{25}N_7O_4S$	56.79 (56.46	4.96 5.04	19.32 19.30)	274 (39 100)	372.1542 (372.1546)[r]
26	D	84	133-134 (c)	$C_{17}H_{17}N_7O_4S$	49.15 (48.98	4.12 4.12	23.60 23.39)	257 (18 500) 286 (27 400) 292 (27 400)	415.1049 (415.1065)
27	D	67	93-96 (s)	$C_{17}H_{18}N_8O_4S$	47.44 (47.42	4.21 4.21	26.03 26.03)	257 (21 400) 314 (13 200)	t
28	H	36	123-126 (u)	$C_{17}H_{19}N_7O_6$ (•0.5*i*-PrOH)	49.66 (49.79	5.18 5.28	21.91 21.54)	220 (27 400) 262 (17 800)[v] 283 (22 500)	417.1386 (417.1399)
29	B	67	181-182 (c)	$C_{17}H_{18}N_6O_5$ (•MeOH)	51.67 (51.99	5.29 5.00	20.08 20.48)	270 (27 800)	t
30	B	52	209-210 (d)	$C_{17}H_{18}N_6O_5$	52.85 (52.69	4.70 4.72	21.75 21.63)	270 (26 000)	j
31	B	53	230-231 (c)	$C_{17}H_{18}N_6O_6$	50.75 (50.69	4.51 4.60	20.89 20.55)	270 (28 000)	402.1282 (402.1289)
32	A	35	108-109 dec. (d)	$C_{17}H_{17}ClN_6O_5$	48.52 (48.36	4.07 4.17	19.97 19.89)	270 (27 000)	j
33	A	51	184-185 (c)	$C_{17}H_{18}N_6O_5$	52.85 (52.71	4.70 4.67	21.75 21.94)	271 (26 700)	386.1342 (386.1340)
34	A	61	192-193 (c)	$C_{20}H_{22}N_6O_6$ (•0.5MeOH)	53.70 (53.48	5.27 5.01	18.33 18.62)	271 (29 400)	t

[a]Crystallization solvent. [b]M^+ unless otherwise noted. [c]Methanol. [d]Ethanol. [e]M - N_2. [f][^{35}Cl] *m/z* 436.0896 (436.0900). [g][^{35}Cl] *m/z* 436.0889. [h]No molecular sieves. [i][^{35}Cl] *m/z* 436.0902. [j]No characteristic ion. [k]Methanol/ether. [l]Oxygen: 17.37 (17.52). [m]M - 45. [n]M - 169. [o]Sulfur: 6.91 (7.21). [p]Solid foam. [q]M - 132. [r]M - 135. [s]Benzene. [t]Not determined. [u]Propan-2-ol/hexanes. [v]Shoulder.

Table 5. ^{1}H NMR Chemical Shift (and "Apparent" Coupling Constant) Data[a]

Compound	H8[b]	H2[b]	H1'[c] ($J_{1'-2'}$)	H2'[d] ($J_{2'-3'}$)	H3'[d] ($J_{3'-4'}$)	H4'[e] ($J_{4'-5'}$)	H5',H5''[e] ($J_{5'-5''}$)	NH/NH$_2$[b,f]	OH[g]	Other Peaks[h]
1	8.38	8.17	5.88 (6.0)	4.60[e]	4.13[e]	3.96	3.50-3.70	8.53	5.05-5.45	8.15, 7.55, 4.88
2	8.38	8.19	5.88 (6.0)	4.62[e] (5.0)	4.15[e] (3.5)	3.97 (4.8)	3.50-3.74	8.19, 4.90	5.44, 5.42, 5.18	7.00, 6.47, 4.53
3	8.40	8.20	5.91 (6.0)	4.63 (5.0)	4.16 (3.5)	3.80[d] (4.0)	3.68, 3.56 (12.0)	8.50	5.46, 5.38, 5.20	7.40, 7.17, 4.70
4	8.38	8.20	5.90 (6.0)	4.62 (5.5)	4.15 (3.5)	3.96[d] (3.5)	3.68, 3.56 (12.0)	8.42	5.45, 5.38, 5.19	7.20, 6.92, 6.88, 4.69, 3.72
5	8.45	8.20	5.92 (6.0)	4.64[e]	4.17[e]	3.98[d] (3.5)	3.67, 3.62 (12.0)	8.48	5.47, 5.35, 5.21	8.00, 7.37, 7.33, 5.00
6	8.46	8.19	5.92 (6.0)	4.64[e]	4.16[e]	3.98[d] (3.5)	3.68, 3.57 (12.0)	8.60	5.50, 5.36, 5.25	8.17, 7.77, 7.53, 4.96
7	8.42	8.22	5.91 (6.0)	4.63 (5.0)	4.16 (3.0)	3.98[d] (4.0)	3.68, 3.57 (12.5)	8.60	5.46, 5.35, 5.21	8.04, 7.71, 7.65, 4.78
8	8.43	8.22	5.91 (6.0)	4.63 (5.0)	4.16 (3.0)	3.97[d] (3.5)	3.68, 3.57 (12.5)	8.60	5.46, 5.35, 5.20	7.95, 7.45, 7.39, 4.77, 2.50
9	8.42	8.22	5.91 (6.0)	4.63 (5.5)	4.16 (3.5)	3.97[d] (3.5)	3.68, 3.57 (12.0)	8.54	5.46, 5.36, 5.20	7.70, 7.54, 7.34, 4.78, 2.44
10	8.42	8.22	5.91 (6.0)	4.62 (5.5)	4.16 (3.0)	3.97[d] (3.5)	3.68, 3.57 (12.0)	8.59	5.46, 5.36, 5.20	7.98, 7.62, 7.44, 4.77, 2.48

(continued)

Table 5. Continued

11	8.44	8.20	5.91 (6.0)	4.62 (5.0)	4.15 (3.5)	3.97[d] (3.5)	3.18, 3.57 (12.0)	8.63	5.46, 5.31, 5.20	8.29, 8.14, 7.51, 4.82
12	8.40	8.38	5.91 (6.5)	4.63 (5.5)	4.17 (3.0)	3.99[d] (3.5)	3.68, 3.57 (12.5)	9.87, 8.40	5.47, 5.43, 5.21	7.49, 7.26, 4.69, 1.00
13	8.36	8.19	5.88 (6.0)	4.61 (5.5)	4.14 (3.5)	3.96[d] (3.0)	3.68, 3.56 (12.5)	11.24, 8.41	5.43, 5.38, 5.18	7.49, 7.28, 4.69, 4.23
14	8.37	8.20	5.90 (6.0)	4.62[e] (6.0)	4.15[e] (3.0)	3.98 (3.0)	3.68, 3.56	9.87, 8.37	5.46, 5.41, 5.21	7.50, 7.25, 4.65, 2.27, 1.54, 1.28, 0.90
15	8.39	8.22	5.91 (6.0)	4.61[e] (5.8)	4.16[e] (3.0)	3.97 (3.0)	3.69, 3.56	9.80, 8.39	5.45, 5.39, 5.18	7.51, 7.27, 4.67, 2.25, 1.57, 1.28, 0.86
16	8.36	8.20	5.89 (6.0)	4.62[e] (5.5)	4.15[e] (3.5)	3.94 (3.0)	3.67, 3.54	9.77, 8.36	5.42, 5.38, 5.16	7.40, 7.25, 4.76, 2.23, 1.51, 1.23, 0.82
17	8.40	8.23	5.92 (6.0)	4.65[e] (5.0)	4.16[e] (3.5)	4.00 (3.5)	3.69, 3.57	9.82, 8.40	5.47, 5.42, 5.21	7.52, 7.27, 4.67, 2.27, 1.55, 1.25, 0.85
18	8.40	8.23	5.92 (6.0)	4.64[e] (5.5)	4.16[e] (3.0)	3.99 (3.0)	3.57, 3.70	9.18, 8.40	5.47, 5.42, 5.21	7.57, 7.28, 4.80, 1.22
19	8.40	8.22	5.92 (6.0)	4.63[e] (6.0)	4.17[e] (3.0)	3.99 (3.0)	3.70, 3.58	9.53, 8.40	5.47, 5.41, 5.20	7.39, 7.26, 4.67, 4.11, 1.22
20	8.39	8.22	5.91 (6.0)	4.64[e] (5.0)	4.15[e] (3.5)	3.97 (3.5)	3.68, 3.56	10.21, 8.43	5.46, 5.41, 5.20	7.94, 7.69, 7.60-7.49 7.32, 4.70
21	8.34	8.20	5.90 (6.0)	4.61[e] (5.0)	4.16[e] (3.0)	3.98 (3.0)	3.69, 3.57	8.24, 8.20	5.46, 5.42, 5.20	7.36, 7.21, 4.64, 2.90
22	8.73	8.57	6.30 (6.0)	4.76[e] (5.5)	4.27[e] (3.0)	4.06 (3.0)	3.76, 3.64	8.75	5.66, 5.61, 5.38	7.47-5.55, 7.63-7.81
23	8.40	8.23	5.92 (6.0)	4.64[e] (5.0)	4.17[e] (3.0)	3.99 (3.0)	3.69, 3.57	9.64, 8.45	5.48, 5.41, 5.22	7.34, 7.17, 4.70, 2.96

24	8.34	8.16	5.90 (6.0)	4.59[e] (5.0)	4.14[e] (3.0)	3.97 (3.0)	3.68, 3.55	10.13, 8.34	5.45, 5.40, 5.20	7.60, 7.30, 7.17, 7.00, 4.62, 2.32
25	8.38	8.12	5.90 (6.0)	4.62[e] (5.5)	4.15[e] (3.0)	3.97 (3.0)	3.68, 3.55	9.74, 8.46	5.44, 5.38, 5.18	7.44, 7.36, 7.30, 7.10, 4.68
26	8.82	8.76	6.02 (6.0)	4.62 (5.5)	4.21 (4.0)	4.00[d] (4.0)	3.71, 3.68 (12.0)		5.55, 5.26, 5.13	7.54, 7.09, 4.68
27	8.18		5.79 (6.0)	4.47 (5.5)	4.11 (4.0)	3.90[d] (4.0)	3.63, 3.54 (12.0)	6.65	5.42, 5.15, 5.07	7.52, 7.04, 4.54
28	7.98		5.79 (6.0)	4.53 (5.0)	4.11 (3.6)	3.92[d] (3.5)	3.64, 3.53 (12.0)	8.10, 5.87	5.41, 5.16	8.20, 7.60, 4.77
29	8.37	8.18	6.36[d] (6.0)	2.35[e]	4.35[e]	3.89	3.45-3.70	8.56	5.28, 5.13	8.15, 7.56, 4.85
30	8.48	8.26	5.93 (2.5)	4.60[e]	2.26[e] 1.93[e]	4.40	3.72, 3.55 (12.0)	8.60	5.70, 5.16	8.22, 7.62, 4.82
31	8.25	8.22	6.29 (5.0)	4.16[e]	4.16[e]	3.79[d] (4.0)	3.68, 3.64 (12.0)	8.55	5.63, 5.54, 5.10	8.19, 7.59, 4.82
32	8.43	8.25	5.80 (6.0)	4.79 (5.5)	4.25 (4.5)	4.11[d] (5.0)	3.98, 3.86 (12.0)	8.63	5.63, 5.49	8.20, 7.89, 4.84
33	8.38	8.20	5.87 (5.0)	4.69 (5.0)	3.99[e]	3.99	1.32[c] (6.0)	8.55	5.43, 5.17	8.16, 7.57, 4.83
34	8.40	8.22	6.15 (3.2)	5.35 (6.0)	4.97 (3.0)	4.23	3.56[i] (5.4)	8.60	5.19	8.18, 7.58, 4.83, 1.56, 1.33

[a]Me_2SO-d_6 (Me_4Si) solutions at 200 or 400 MHz. [b]Singlet. [c]Doublet unless otherwise indicated. [d]Doublet of doublets unless otherwise indicated. [e]Multiplet unless otherwise indicated. [f]Broad singlet. [g]Doublets for OH2' and OH3', and triplets for OH5' were usually observed. [h]Peak multiplicities and coupling constants were usually as expected for the benzylic, phenyl, and other substituent protons. [i]Triplet.

Method A. 6-*N*-(4-Nitrobenzyl)adenosine[26] (1). To a solution of adenosine (5.34 g, 20 mmol) in freshly distilled DMF (100 mL) was added 4-nitrobenzyl bromide (12.9 g, 60 mmol) and the mixture was stirred at ambient temperature for 3 days. The mixture was evaporated in vacuo (<45 °C) and coevaporated with toluene (30 mL x 2). The gummy residue was triturated with hot acetone (200 mL x 2) and Et_2O (200 mL). The powdery precipitate was collected and dried (P_4O_{10}). This solid was dissolved in MeOH (125 mL), added to a mixture of Me_2NH/MeOH (125 mL, 1:1), and stirred at ambient temperature for 4 h. The solution was evaporated, the gum was coevaporated with EtOH (x 2), and the residue was partitioned between EtOAc/H_2O. The aqueous layer was extracted with EtOAc (x 2) and the combined organic layer was washed with H_2O, evaporated, and coevaporated with EtOH (x 3). The resulting residue was recrystallized from MeOH (x 2) to give **1** (5.14 g, 64%).

Method B. 6-*N*-(4-Azidobenzyl)adenosine (3). To a solution of adenosine (4.0 g, 15 mmol) in freshly distilled DMF (40 mL) was added dry, powdered 4 Å molecular sieves (5 g) and 4-azidobenzyl bromide[30] (5.3 g, 25 mmol). The mixture was stirred overnight at ambient temperature (protected from light and moisture), filtered, and the filtrate was evaporated to a small volume in vacuo. The resulting yellow-brown syrup was dissolved in MeOH (50 mL), Me_2NH/MeOH (20 mL, 1:1) was added, and the mixture was stirred at ambient temperature for 6 h. The resulting precipitate was collected and recrystallized from EtOH to give **3** (1.8 g, 30%).

Method C. 6-*N*-(3-Methoxybenzyl)adenosine (4). To a suspension of adenosine (3.0 g, 11 mmol) in DMF (30 mL) was added dry, powdered 4 Å molecular sieves (3 g), 3-methoxybenzyl chloride (3.5 g, 22 mmol), and NaI (3.3 g, 22 mmol). The mixture was stirred at 80 °C for 6 h, evaporated, and treated with Me_2NH/MeOH as described in methods A and B. The product was purified by silica column chromatography and crystallized from MeOH to give **4** (1.4 g, 32%).

Method D. 6-*S*-(4-Azidobenzyl)-6-thioinosine (26). K_2CO_3 (0.67 g, 4.8 mmol) and 4-azidobenzyl bromide[30] (2.0 g, 9.4 mmol) were added to a solution of 6-thioinosine (1.0 g, 3.5 mmol) in DMF (10 mL). The mixture was stirred at ambient temperature for 30 min, evaporated in vacuo, and the residue was stirred with Et_2O/H_2O (1:1, 60 mL) for 10 min. Insoluble solids were filtered and recrystallized from MeOH to give **26** (1.23 g, 84%).

Method E. 6-*N*-(4-Aminobenzyl)adenosine[26] (2). To a solution of **1** (0.8 g, 2.0 mmol) in AcOH (50 mL) was added 5% Pd/C (1.6 g). This mixture was hydrogenated (15 psi) overnight at ambient temperature. The catalyst was filtered, the filtrate was evaporated, and the residue was dissolved in MeOH (30 mL). Crystals separated from the solution a few minutes later and were recrystallized from MeOH to give **2** (0.57 g, 77%).

Method F. 6-*N*-[4-(Acetamido)benzyl]adenosine[26] (12). A solution of **2** (1.0 g, 2.6 mmol) and Ac_2O (1.4 mL, 1.5 g, 15 mmol) in pyridine (50 mL) was stirred at ambient temperature for 6 h. MeOH (2 mL) was added and the mixture was stirred at 0 °C for 10 min in an ice/H_2O bath. The solution was evaporated and the residue was partitioned between $CHCl_3$ and 5% $NaHCO_3/H_2O$. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to give 1.3 g (90%) of the tetraacetyl intermediate as a solid foam. Saturated NH_3/MeOH (8 mL) was added to a solution of this compound (1.5 g, 2.8 mmol) in MeOH (30 mL) and stirring was continued overnight at ambient temperature. The solution was evaporated, the gum was dissolved in hot MeOH, filtered, and the filtrate was cooled to give **12** (0.98 g, 86%). A sample for analysis was recrystallized (MeOH).

Method G. (Transient Protection Sequence.) 6-*N*-[4-(Heptanamido)benzyl]adenosine (16). Chlorotrimethylsilane (1.5 mL, 1.3 g, 11.8 mmol) was added to a solution of **2** (0.3 g, 0.8 mmol, dried by evaporated with pyridine x 3) in dry pyridine (10 mL) and stirring was continued at ambient temperature until a clear solution was obtained. Heptanoyl chloride (0.5 mL, 0.5 g, 3.2 mmol) was added, the mixture was stirred at ambient temperature for 3 h, cooled in an ice bath, H_2O (2.0 mL) was added, and stirring was continued for 30 min. NH_3/H_2O (29%, 6 mL) was added, the mixture was stirred at ambient temperature for 2 h, evaporated to near dryness, and coevaporated with MeOH (x 2). The residue was triturated with hot EtOH, insoluble salts were filtered, the filtrate was evaporated, and the residue was purified by silica column chromatography. The product was crystallized from MeOH/Et_2O (x 3, diffusion[38]) to give **16** (0.28 g, 72%).

Method H. 2-Amino-6-*N*-(4-nitrobenzyl)adenosine (28). A solution of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine (1.0 g, 3.3 mmol) and 4-nitrobenzylamine hydrochloride (1.5 g, 8 mmol) was heated at 80 °C in DMF in the presence of 4 Å

molecular sieves for 6 h. The mixture was filtered, the filtrate was evaporated in vacuo, and the product was purified by column chromatography followed by "recrystallization" from *i*-PrOH/hexanes to give **28** (0.5 g, 36%) as a yellow powder.

Biology. Studies with Cultured Cells. S49 cells were maintained at 37 °C under 5% CO_2 in air in antibiotic-free Fischer's medium or in RPMI 1640 medium, both with 10% horse serum; after 30-35 subculture generations, cultures were restarted from frozen stocks. For binding and transport experiments, cells were propagated in roller bottles turned at 1.5 rpm; cell concentrations were kept below 5 x 10^5 cells/mL and cell proliferation was exponential with doubling times of about 15 h. Assays of NBMPR binding were conducted in serum-free Fischer's medium lacking bicarbonate, but supplemented with 20 mM HEPES (pH 7.4 at 20 °C). For measurement of inward fluxes of adenosine, washed L1210/B23.1 cells were suspended in Dulbecco's phosphate-buffered saline (DPBS).

NBMPR binding. Affinities of test compounds for the NBMPR binding sites of S49 cells were determined from their inhibitory effects on the equilibrium binding of [^{3}H]NBMPR to S49 cells. **Method I.** S49 cells were incubated at 22 °C with graded concentrations (0.07-1.5 nM) of [^{3}H]NBMPR in the presence of various concentrations of the unlabelled test compound. The replicate incubation mixtures (1.0 mL, 2-4 x 10^6 cells, duplicates) were contained in 1.5 mL plastic centrifuge tubes over "transport oil"[11a] (150 μL). After 30 min at 22 °C, cells were pelleted under the oil (16,000 x *g*, 30 sec) and medium samples were assayed for ^{3}H content.[33] After removal of the medium, tubes were washed above the oil with water which was removed by aspiration along with most of the oil. Cell pellets were dissolved in 0.5 mL of 5% Triton X100 for ^{3}H assay. The ^{3}H content of cell pellets was corrected for nonspecific retention of [^{3}H]NBMPR, defined as the cellular ^{3}H acquired when the equilibration procedure was conducted in the presence of unlabelled 3 μM 6-*S*-(4-nitrobenzyl)-6-thioguanosine (NBTGR). In Method I, binding data were subjected to mass law analysis by the Feldman equation.[39] **Method J.** Experimental conditions were the same as in Method I, but assays of [^{3}H]NBMPR binding were conducted with a series of test inhibitor concentrations keeping the labelled ligand at a constant concentration. The binding data were analyzed by plotting reciprocals of the cell content of specifically bound NBMPR versus inhibitor concentration in analogy with the Dixon method[40] for analysis of competitive inhibition of enzyme activity. Each test

compound was assayed at 4 or more concentrations for inhibition of the site-specific binding of [^{3}H]NBMPR to S49 cells in triplicate assay mixtures containing 0.1 nM [^{3}H]NBMPR. With each group of compounds assayed, the K_d values were calculated by a weighted, least squares algorithm adapted from Cleland.[41]

Adenosine transport. The uptake of 1 μM [2,8-^{3}H]adenosine (Moravek Biochemicals) by L1210/B23.1 cells during intervals of 10 sec at 22 °C was employed as a measure of initial rates of the adenosine permeation process (time courses of 1 μM adenosine uptake by these cells were linear under these conditions, data not shown). Replicate assay mixtures were prepared in 1.5 mL plastic centrifuge tubes that contained DPBS medium (100 μL) and 2-4 x 10^6 L1210/B23.1 cells layered over "transport oil"[11a] (100 μL). Intervals of adenosine uptake were initiated by adding 100 μL portions of 3 μM [2,8-^{3}H]adenosine and ended by addition of 100 μL of 120 μM dilazep (Hoffman LaRoche), a potent "stopper" of nucleoside transport,[15] in DPBS. Assay mixtures were immediately spun (16,000 x *g*, 30s) and supernatant fractions above the oil were aspirated. Tube surfaces above the oil were rinsed with water, and cell pellets were lysed in 5% Triton X100 for determination of the ^{3}H-content of the cells. Assays were conducted in triplicate. Determinations of pellet water space and pellet extracellular space were made using ^3H_2O and [U-^{14}C]sucrose (Du Pont Canada, NEN). Cellular concentrations of [^{3}H]adenosine were calculated in terms of cell water.

For determination of the influence of test compounds on adenosine uptake rates, cells were incubated (15 min at 22 °C, 4 x 10^7 cells/mL) in DPBS medium containing graded concentrations of test compounds. Cell batches were then assayed for the ability to take up [^{3}H]adenosine (as above), each batch from its own post-exposure medium (with a test compound). IC_{50} values for test compounds (concentrations that reduced adenosine uptake rates to 50% of that in the absence of the test compound) were determined from plots of fractional rates (test over control) versus log inhibitor concentrations.

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