Adenosine Receptor Agonists: Synthesis and Biological Evaluation of 1-Deaza Analogues of Adenosine Derivatives

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In a search for more selective A_1 adenosine receptor agonists, N^6-[(*R*)-(−)-1-methyl-2-phenethyl]-1-deazaadenosine (1-deaza-*R*-PIA, **3a**), N^6-cyclopentyl-1-deazaadenosine (1-deazaCPA, **3b**), N^6-cyclohexyl-1-deazaadenosine (1-deazaCHA, **3c**), and the corresponding 2-chloro derivatives **2a–c** were synthesized from 5,7-dichloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**1**). On the other hand, *N*-ethyl-1′-deoxy-1′-(1-deaza-6-amino-9*H*-purin-9-yl)-β-D-ribofuranuronamide (1-deazaNECA, **10**) was prepared from 7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**4**), in an attempt to find a more selective A_2 agonist. The activity of all deaza analogues at adenosine receptors has been determined in adenylate cyclase and in radioligand binding studies. 1-DeazaNECA (**10**) proved to be a nonselective agonist at both subtypes of the adenosine receptor. It is about 10-fold less active than NECA but clearly more active than the parent compound 1-deazaadenosine as an inhibitor of platelet aggregation and as a stimulator of cyclic AMP accumulation. The N^6-substituted 1-deazaadenosines largely retain the A_1 agonist activity of their parent compounds, but lose some of their A_2 agonist activity. This results in A_1-selective compounds, of which N^6-cyclopentyl-2-chloro-1-deazaadenosine (1-deaza-2-Cl-CPA, **2b**) was identified as the most selective agonist at A_1 adenosine receptors so far known. The activity of all 1-deaza analogues confirms that the presence of the nitrogen atom at position 1 of the purine ring is not critical for A_1 receptor mediated adenosine actions.

Adenosine appears to mediate a wide variety of physiological functions including vasodilatation, vasoconstriction in the kidney, cardiac depression, inhibition of lipolysis, inhibition of platelet aggregation, inhibition on lymphocyte functions, inhibition of insulin release and potentiation of glucagon release in the pancreas, inhibition of neurotransmitter release from nerve endings, stimulation of steroidogenesis, and potentiation of histamine release from mast cells.[1-5] Many of its effects can be attributed to the action at receptors located on the cell surface. Two classes of adenosine receptors that either inhibit (A_1 or R_i) or stimulate (A_2 or R_a) the activity of adenylate cyclase have been identified both on the basis of pharmacological profiles of sets of adenosine analogues and of the intracellular events coupled to receptor occupation.[1]

This interaction of adenosine with the receptors is very specific and requires the presence of both the adenine and the ribose moieties. The most prominent determinants for adenosine actions seem to be its 6-amino group, even if monosubstituted, the β-anomeric configuration of the ribose, and the ribofuranose C2′ and C3′ hydroxylic groups.[6,7]

At A_1 receptors the most active analogues are N6-substituted adenosines, whereas at A_2 receptors the most active compounds are *N*-alkyladenosin-5′-uronamides. Two adenosine analogues, N^6-[(*R*)-(−)1-methyl-2-phenethyl]-adenosine (*R*-PIA) and ethyladenosin-5′-uronamide (NECA), have been developed as important reagents for identifying A_1 and A_2 receptors, respectively. NECA is not, however, A_2 selective.[8] A_1 receptors exhibit a potency order *R*-PIA > NECA, whereas A_2 receptors exhibit a reverse order.[1]

Recently, in order to investigate the role of the purine nitrogens, we have examined the effects of several deaza analogues of adenosine on rat brain membranes and on human platelets.[9] It was found that 1-deazaadenosine, though less active than adenosine, displays the highest affinity for adenosine receptors whereas 3-deazaadenosine is very little active; 7-deaza- and 1,3-dideazaadenosine are inactive.

In order to get further information about the factors governing the binding of the purine domain to adenosine receptors and in an attempt to find more active and/or more selective derivatives, we have synthesized and biologically evaluated a series of 1-deaza analogues of *R*-PIA, CHA (N^6-cyclohexyladenosine), and CPA (N^6-cyclopentyladenosine) (**2a–d** and **3a–d**). We report also the synthesis and the biological activity of the *N*-ethyl-1′-

Scheme I

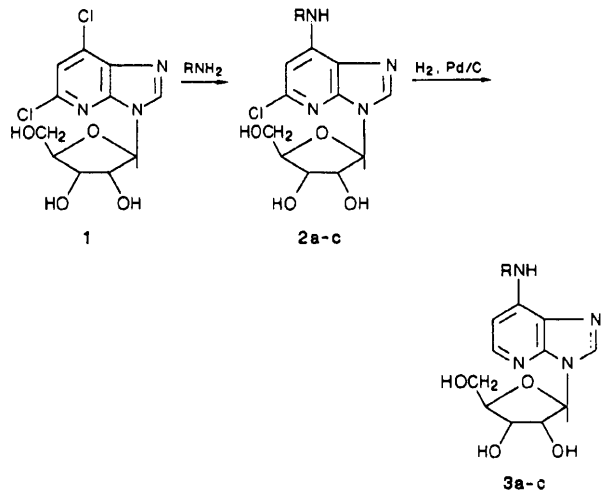

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Table I. N^6-Substituted 1-Deazaadenosines from Scheme I

compd	R	X	rectn time, h	chromat solvent	yield, %	mp,[a] °C	formula[b]
2a	(*R*)-$CH(CH_3)CH_2C_6H_5$	Cl	48	$CHCl_3$–c-C_6H_{12}–MeOH (70:17:13)	70	99–102	$C_{20}H_{23}ClN_4O_4$
2b	c-C_5H_9	Cl	24	AcOEt–MeOH–NH_3 (94:5:1)	50	166–168	$C_{16}H_{21}ClN_4O_4$
2c	c-C_6H_{11}	Cl	24	$CHCl_3$–MeOH–NH_3 (85:14:1)	50	188–190	$C_{17}H_{23}ClN_4O_4$
3a	(*R*)-$CH(CH_3)CH_2C_6H_5$	H	5	$CHCl_3$–MeOH (90:10)	80	162–165	$C_{20}H_{24}N_4O_4$
3b	c-C_5H_9	H	8	AcOEt–MeOH (95:5)	70	94–96	$C_{16}H_{22}N_4O_4$
3c	c-C_6H_{11}	H	8	$CHCl_3$–MeOH–NH_3 (80:19:1)	60	134–137	$C_{17}H_{24}N_4O_4$

[a] Uncorrected. [b] All compounds had satisfactory C, H, N, microanalyses and were within 0.4% of theoretical value. All compounds exhibited ^1H NMR spectra consistent with the assigned structures.

Table II. Effects of 1-Deazaadenosine Analogues, NECA, CPA, and *R*-PIA on Adenylate Cyclase

	A_1 receptor		A_2 receptor		A_1 selectivity,
compd	IC_{50}, nM	inhibn (rel)[a]	EC_{50}, nM	stimul (rel)[a]	EC_{50}/IC_{50}
1-deazaadenosine	6700 (4100–11000)	0.97 ± 0.05	18000 (11000–28000)	0.60 ± 0.04	2.7
1-deaza-2-Cl-PIA (**2a**)	960 (380–2400)	1.08 ± 0.09	3500 (2600–4700)	0.40 ± 0.13	3.6
1-deazaPIA (**3a**)	630 (530–760)	1.16 ± 0.26	6600 (4800–9000)	0.32 ± 0.05	10.5
1-deaza-2-Cl-CPA (**2b**)	360 (220–660)	1.07 ± 0.12	28000 (20000–39000)	0.43 ± 0.03	78
1-deazaCPA (**3b**)	970 (610–1500)	1.07 ± 019	26000 (20000–34000)	0.39 ± 0.02	27
1-deaza-2-Cl-CHA (**2c**)	2400 (2100–7000)	1.14 ± 0.21	4800 (3200–7200)	0.34 ± 0.09	2.0
1-deazaCHA (**3c**)	1600 (1000–2500)	1.09 ± 0.12	20000 (17000–23000)	0.38 ± 0.02	12.4
1-deazaNECA (**10**)	1500 (1200–1900)	1.04 ± 0.13	2800 (2100–3900)	0.57 ± 0.13	1.9
R-PIA	40 (22–72)	1	950 (650–1400)	0.53 ± 0.06	24
CPA	58 (24–140)	0.99 ± 0.07	2200 (1500–3200)	0.51 ± 0.09	38
NECA	200 (160–250)	0.93 ± 0.12	260 (190–360)	1	1.3

[a] The maximal inhibition or stimulation of adenylate cyclase activity by the compounds are given relative to the reference compounds *R*-PIA for the A_1 receptor and NECA for the A_2 receptor. The inhibition by *R*-PIA was 53 ± 10% and the stimulation by NECA 250 ± 30% (means and SEMs of nine independent experiments). Values are means and SEMS or 95% confidence intervals of three independent determinations.

deoxy-1′-(1-deaza-6-amino-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**10**), the 1-deaza analogue of NECA, the most potent adenosine analogue at A_2 receptors so far known.[1]

Chemistry. The synthetic pathways for the preparation of compounds **2a–c** and **3a–c** listed in Table I, are shown in Scheme I. Reaction of 5,7-dichloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**1**)[9] with the appropriate amine gave the N^6-substituted 2-chloro-1-deazaadenosines **2a–c**. Catalytic hydrogenolysis of the chlorine atom in **2a–c** with 10% Pd/C in ethanol and 2 N NaOH afforded the corresponding derivatives **3a–c**.

The synthesis of *N*-ethyl-1′-deoxy-1′-(1-deaza-6-amino-9*H*-purin-9-yl)-β-D-ribofuranuronamide (1-deazaNECA, **10**) was accomplished by the method outlined in Scheme II. Protection of 7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**4**)[10] gave the 2′,3′-*O*-isopropylidene derivative **5**, which was oxidized with pyridinium dichromate (PDC) to yield the uronic acid **6**.

The 2′,3′-O-protected *N*-ethylribofuranuronamide derivative **7** was formed by first converting the acid **6** to its acid chloride with $SOCl_2$ under mild conditions followed by reaction of the acid chloride with dry ethylamine in chloroform at 0 °C. Deblocking of the isopropylidene protective group by 1 N HCl at 45 °C gave *N*-ethyl-1′-deoxy-1′-(1-deaza-6-nitro-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**8**) in 85% yield.

Attempts to convert **8** directly to 1-deazaNECA (**10**) by means of several reduction systems (H_2 and Pd/C or Pt or Raney nickel, also in the presence of acids or bases, Zn and acids) were unsuccessful, resulting in the denitrated purine derivative **9**. The structure of compound **9** was assigned by ^1H NMR analysis. Its ^1H NMR spectrum showed the presence of a doublet of doublet at δ 7.40 (H-1), two doublets at 8.22 (H-6) and 8.45 (H-2), respectively, and a singlet at 8.78 (H-8).

This lability of the nitro group prompted us to attempt its substitution with hydrazine hydrate at reflux and then reduction of the crude reaction mixture with Raney nickel. The structure of 1-deazaNECA (**10**) thus obtained was clearly confirmed by ^1H NMR and elemental analyses.

Biological Evaluation. The activity of the deazaadenosine analogues was determined in adenylate cyclase studies with rat fat cell membranes as an A_1 receptor selective model and human platelet membranes as an A_2 receptor selective model. The results are given in Table II. The extent of maximal inhibition by all compounds is comparable to that of the most widely used A_1 receptor

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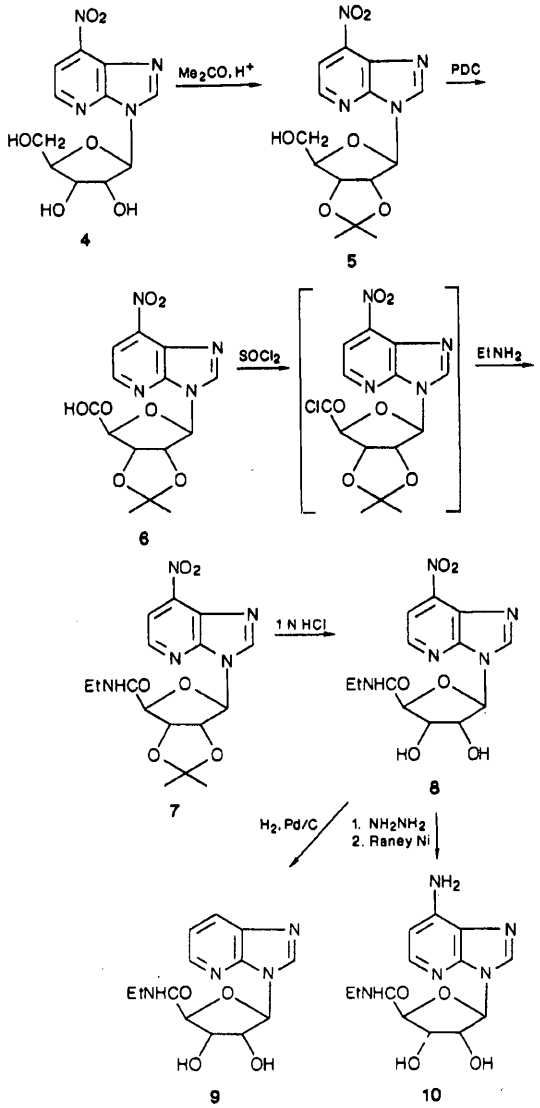
Scheme II



agonist *R*-PIA, but the IC_{50} values were on the whole somewhat higher for the 1-deaza compounds compared to their nondeaza counterparts.

In contrast, maximal stimulation of adenylate cyclase activity of human platelet membranes as stimulated by the deaza compounds was somewhat lower than by *R*-PIA (about 130%), but clearly less than by NECA (250%). Thus, the deaza compounds may be regarded as partial agonists at the A_2 receptor. Again, the EC_{50} values of the deaza analogues were always higher than those of their parent compounds.

All compounds showed some degree of A_1 selectivity on the basis of a comparison of IC_{50} and EC_{50} values. In the case of 1-deazaPIA (**3a**) and 1-deazaCHA (**3c**) the 2-Cl-substituted derivatives had a lower A_1 selectivity than the unsubstituted compounds (**2a** and **2c**). The contrary was true for 1-deazaCPA (**3b**): here the 2-Cl-substitution resulted in an increase of the A_1 selectivity, and in fact N^6-cyclopentyl-2-chloro-1-deazaadenosine (1-deaza-2-Cl-CPA, **2b**) had a more than twofold higher selectivity than N^6-cyclopentyladenosine (CPA), which so far has been the most selective agonist at A_1 receptor.[11]

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Table III. Effects of 1-Deazaadenosine Analogues, NECA, CPA, and *R*-PIA on Radioligand Binding to Adenosine Receptors

compd	K_i,[a] nm: A_1 receptor	K_i,[a] nm: A_2 receptor	A_1 selectivity, $K_i(A_2)/K_i(A_1)$
1-deazaadenosine	115 (91–144)	2900 (2400–3500)	25
1-deaza-2-Cl-PIA (**2a**)	18 (15–23)	3700 (3500–4000)	210
1-deazaPIA (**3a**)	7.7 (7.3–8.1)	4900 (3300–7300)	640
1-deaza-2-Cl-CPA (**2b**)	1.6 (1.1–2.5)	13200 (10900–15900)	8200
1-deazaCPA (**3b**)	7.5 (5.7–9.7)	17600 (12300–25000)	2300
1-deaza-2-Cl-CHA (**2c**)	47 (42–54)	8200 (5100–13200)	170
1-deazaCHA (**3c**)	16 (11–24)	6800 (4100–11200)	420
1-deazaNECA (**10**)	51 (41–63)	580 (540–630)	12
R-PIA	1.3 (1.1–2.4)	730 (690–770)	560
CPA	0.8 (0.6–1.0)	2000 (1400–2900)	2500
NECA	8.2 (6.2–10.9)	22 (20–25)	2.7

[a] K_i values were calculated from competition for [^{3}H]PIA binding to rat brain membranes (A_1 receptor) and for [^{3}H]NECA binding to rat striatal membranes in the presence of 50 nM CPA (A_2 receptor). Data represent means and 95% confidence intervals of three independent determinations.

In addition, the affinity for adenosine receptors was examined in radioligand binding studies. All compounds competed for the binding of [^{3}H]PIA to A_1 receptors with Hill factors of about 1. With the exception of NECA itself, all compounds also competed for the binding of [^{3}H]NECA to bovine striatal membranes with Hill factors of about 1, and the competition curves were adequately fitted, assuming a one-site model; this suggests that these compounds did not compete for the low-affinity nonreceptor binding of [^{3}H]NECA.[8] In analogy to the data of Bruns et al.,[8] the high affinity component of the competition of NECA for [^{3}H]NECA binding was regarded as A_2 receptor binding. The K_i values of the competition are given in Table III. The same order of potency and selectivity was obtained with bovine striatal membranes as the source of A_2 receptors, although the affinities for the bovine receptor were somewhat higher.[12] This suggests that at least for the compounds studied in this report there are no major differences between the bovine and the rat striatal A_2 receptor.

In agreement with other studies,[11] the binding experiments suggest a much higher A_1 selectivity of the compounds than adenylate cyclase experiments. This is due to the higher affinity of the agonists for A_1 receptors in binding studies, which may reflect the formation of the high affinity state in the absence of guanine nucleotides.[13] Differences between the A_2 receptors of human platelets and rat and bovine striatum may also be the reason for this discrepancy. However, the order of selectivity remains essentially the same: 1-deaza-2-Cl-CPA (**2b**) has a threefold higher selectivity than CPA, although the value of 2500 for CPA is higher than the value of 783 originally

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Table IV. Activity of 1-DeazaNECA as an Inhibitor of Platelet Aggregation Induced by ADP[a]

compd	IC_{50},[b] μM
1-deazaNECA	5.96 (5.18–6.85)
1-deazaadenosine	65.5 (49.5–86.7)
NECA	0.60 (0.52–0.70)
adenosine	5.94 (3.25–10.85)

[a] ADP concentration was 7 μM. [b] Concentration that inhibits platelet aggregation by 50%. Data represent means and 95% confidence intervals of three independent determinations.

reported by Moss et al.[11] Several deaza compounds have a higher A_1 selectivity than *R*-PIA in the binding experiments.

1-DeazaNECA (10) has been also studied as an inhibitor of platelet aggregation induced by ADP. The results, given in Table IV, showed that 1-deazaNECA (10) is about 10-fold more active than 1-deazaadenosine with an IC_{50} value of 5.96 μM. NECA is also 10-fold more active than adenosine.[14] This finding confirms that in the series of 1-deazaadenosine derivatives the presence of a 5'-[(ethylamino)carbonyl] group increases the affinity for A_2 receptors.

In conclusion, 1-deazaNECA (10) can be regarded as nonselective agonist at both subtypes of the adenosine receptor, whereas N^6-substituted 1-deazaadenosines largely retain the A_1 agonist activity of their parent compounds, but lose some of their A_2 agonist activity. This results in A_1-selective compounds, of which 1-deaza-2-Cl-CPA (**2b**) was identified as the most selective agonist at A_1 adenosine receptors so far known. In addition, the activity of all 1-deaza analogues points out once more that the presence of the nitrogen atom at position 1 of the purine ring is not critical for the adenosine actions.[15]

Experimental Section

Chemistry. Melting points were determined with a Büchi apparatus and are uncorrected. ^1H NMR spectra were obtained with a Varian EM-390 90-MHz spectrometer, with tetramethylsilane as an internal standard. IR spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer. TLC were carried out on precoated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel (Merck) was used. Microanalytical results are indicated by atomic symbols and are within ±0.4% of theoretical values.

Preparation of N^6-Substituted 2-Chloro-1-deazaadenosines (2a–c). To a solution of 5,7-dichloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine[9] (1.1 mmol) in ethanol was added the appropriate amine (4 mmol). The reaction mixture was heated at 135 °C in a sealed tube (Table I). The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column, eluting with a suitable mixture of solvents (Table I) to give **2a–c** as chromatographically pure solids. All of the spectral data for the compounds were compatible with the structures.

Preparation of N^6-Substituted 1-Deazaadenosines (3a–c). To a solution of **2a–c** (1 mmol) in 80 mL of ethanol and 2 mL of 2 N NaOH was added 0.3 g of 10% Pd/C, and the mixture was shaken with hydrogen at 50 psi for the time reported in Table I. The catalyst was removed, and the filtrate was concentrated to dryness. The residue was chromatographed on a silica gel column, eluting with the solvent mixtures reported in Table I to give **3a–c** as pure solids. All of the spectra data for the compounds were compatible with the structures.

1-Deaza-6-nitro-9-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)-9*H*-purine (5). To a solution of 5.4 g (18 mmol) of 7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**4**)[10] in 400 mL of acetone was added 34.5 g of *p*-toluenesulfonic acid. The reaction mixture was stirred at room temperature for 1 h and then, after the addition of 54 g of $NaHCO_3$, stirred again for 3 h. The solid was removed and washed two times with EtOAc, and the filtrate was concentrated to dryness. The residue was chromatographed on a silica gel column, eluting with EtOAc to give 4.34 g (71%) of **5** as a solid: mp 109–112 °C; ^1H NMR (Me_2SO-d_6) δ 1.34 and 1.56 (s, 6 H, $C(CH_3)_2$), 3.58 (d, J = 5 Hz, 2 H, CH_2-5'), 4.32 (m, 1 H, H-4'), 4.59 (s, 1 H, OH), 5.02 (m, 1 H, H-3'), 5.43 (m, 1 H, H-2'), 6.39 (d, J = 2.5 Hz, 1 H, H-1'), 8.05 (d, $J_{2,1}$ = 7 Hz, 1 H, H-2), 8.74 (d, $J_{1,2}$ = 7 Hz, 1 H, H-1), 9.03 (s, 1 H, H-8). Anal. ($C_{14}H_{16}N_4O_6$) C, H, N.

1'-Deoxy-1'-(1-deaza-6-nitro-9*H*-purin-9-yl)-2',3'-*O*-isopropylidene-β-D-ribofuranuronic Acid (6). To a solution of 4 g (11.9 mmol) of **5** in 30 mL of dry *N*,*N*-dimethylformamide was added 13.4 g of pyridinium dichromate (PDC), and the mixture was set aside in the dark at room temperature for 30 h. The reaction mixture was extracted several times with EtOAc, and the organic layers were collected, dried (Na_2SO_4), and evaporated. The residue was triturated with a mixture of $CHCl_3$–MeOH (95:5), and the resulting solid was filtered and washed with EtOAc to give 1.67 g (40%) of **6** as a white solid: mp 206–208 °C dec; IR ν_{max} 1728 (COOH); ^1H NMR (Me_2SO-d_6) δ 1.41 and 1.58 (s, 3 H, $C(CH_3)_2$), 4.81 (s, 1 H, H-4'), 5.61 (s, 2 H, H-3' and H-2'), 6.60 (s, 1 H, H-1'), 8.04 (d, $J_{2,1}$ = 5.3 Hz, 1 H, H-2), 8.65 (d, $J_{1,2}$ = 5.3 Hz, 1 H, H-1), 8.98 (s, 1 H, H-8), 13.00 (s, 1 H, COOH). Anal. ($C_{14}H_{14}N_4O_7$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-(1-deaza-6-nitro-9*H*-purin-9-yl)-2',3'-*O*-isopropylidene-β-D-ribofuranuronamide (7).** A solution of 1 g (2.85 mmol) of **6** in 4.5 mL of dry DMF was added dropwise to 9 mL of ice-cooled $SOCl_2$. The mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature and stirred for 2 h. After evaporation in vacuo at room temperature (oil pump), the crude acid chloride was dissolved in 12 mL of dry $CHCl_3$, and the solution was added dropwise to an ice-cooled mixture of 2 mL of dry ethylamine in 23 mL of dry $CHCl_3$. After 10 min, the reaction mixture was carefully concentrated in vacuo and the residue was dissolved in water and extracted several times with $CHCl_3$. The organic phase was dried (Na_2SO_4) and evaporated, and the residue was chromatographed on a silica gel column. Elution with EtOAc–c-C_6H_{12} (50:50) gave 500 mg (46%) of **7** as a solid: mp 65–67 °C; IR ν_{max} 1665 (C=O, amide); ^1H NMR (Me_2SO-d_6) δ 0.55 (t, 3 H, CH_2CH_3) 1.38 and 1.58 (s, 3 H, $C(CH_3)_2$, 2.76 (m, 2 H, CH_2CH_3), 4.61 (s, 1 H, H-4'), 5.52 (s, 2 H, H-3' and H-2'), 6.55 (s, 1 H, H-1'), 7.48 (d, $J_{2,1}$ = 5.5 Hz, 2 H, H-2 and NH), 8.31 (d, $J_{1,2}$ = 5.5 Hz, 1 H, H-1), 8.74 (s, 1 H, H-8). Anal. ($C_{16}H_{19}N_5O_6$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-(1-deaza-6-nitro-9*H*-purin-9-yl)-β-D-ribofuranuronamide (8).** A solution of 400 mg (1.06 mmol) of **7** in 10 mL of 1 N HCl was heated at 45 °C for 1 h. The reaction mixture was neutralized with solid $NaHCO_3$ and then extracted several times with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (92:8) yielded 300 mg (85%) of **8** as a solid: mp 103–106 °C; ^1H NMR (Me_2SO-d_6) δ 1.20 (t, 3 H, CH_2CH_3), 3.43 (m, 2 H, CH_2CH_3), 4.40 (m, 2 H, H-3' and H-4'), 4.81 (m, 1 H, H-2'), 6.30 (d, J = 8 Hz, 1 H, H-1'), 7.65 (d, $J_{2,1}$ = 5.5 H, H-2), 8.50 (d, $J_{1,2}$ = 5.5 Hz, 2 H, H-1 and NH), 9.02 (s, 1 H, H-8). Anal. ($C_{13}H_{15}N_5O_6$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-(1-deaza-9*H*-purin-9-yl)-β-D-ribofuranuronamide (9).** To a solution of 240 mg (1.71 mmol) of **8** in 100 mL of methanol was added 150 mg of 10% Pd/C and 1 mL of NaOH, and the mixture was shaken with hydrogen at 20 psi for 4 h. The catalyst was removed by filtration, the filtrate was evaporated to dryness, and the residue was chromatographed on a silica gel column. Elution with CH_2Cl_2–c-C_6H_{12}–EtOH (50:25:25) gave 145 mg (70%) of **9** as a solid: mp 130–133 °C; ^1H NMR (Me_2SO-d_6) δ 1.09 (t, 3 H, CH_2CH_3), 3.25 (m, 2 H, CH_2CH_3), 4.21 (m, 1 H, H-3'), 4.34 (m, 1 H, H-4'), 4.74 (m, 1 H, H-2'), 6.14 (d, 1 H, J = 7 Hz, H-1'), 7.40 (dd, 1 H, H-1), 8.22 (d, J = 7 Hz, 1 H, H-6), 8.45 (d, J = 4.5 Hz, 1 H, H-2), 8.67 (m, 1 H, NH), 8.78 (s, 1 H, H-8). Anal. ($C_{13}H_{16}N_4O_4$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-(1-deaza-6-amino-9*H*-purin-9-yl)-β-D-ribofuranuronamide (1-DeazaNECA 10).** A mixture of 240 mg (0.71 mmol) of **8** and 10 mL of 85% hydrazine hydrate was refluxed for 1.5 h under nitrogen atmosphere. After the reaction

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mixture was evaporated to dryness, 40 mL of oxygen-free water and 2 g of Raney nickel catalyst was added to the residue, and the mixture was refluxed for 1.5 h. The catalyst was removed by filtration and evaporated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH–NH_3 (80:19:1) gave 110 mg (50%) of **10** as a pure solid: mp 188–190 °C; IR ν_{max} 1660 (C=O, amide); ^1H NMR (Me_2SO-d_6) δ 1.10 (t, 3 H, CH_2CH_3), 3.23 (m, 2 H, CH_2CH_3), 4.12 (d, 1 H, H-3′), 4.32 (s, 1 H, H-4′), 4.71 (m, 1 H, H-2′), 5.98 (d, J = 8.5 Hz, 1 H, H-1′), 6.48 (d, $J_{1,2}$ = 5.5 Hz, 1 H, H-1), 6.60 (s, 2 H, NH_2), 7.91 (d, $J_{2,1}$ = 5.5 Hz, 1 H, H-2), 8.30 (s, 1 H, H-8), 9.44 (m, 1 H, NH). Anal. ($C_{13}H_{17}N_5O_4$) C, H, N.

Biological Studies. Membrane Preparation. Membranes from rat brain, rat striatum, and bovine striatum were prepared as described.[13] Rat striatum was dissected according to Glowinski and Iversen.[16] Human platelet membranes were prepared by the method of Hoffman et al.[17] Rat fat cells were isolated as described by Honnor et al.,[18] and their membranes were prepared according to McKeel and Jarett.[19]

Adenylate Cyclase Assay. The activity of adenylate cyclase of the respective membranes was determined as previously described.[20]

Radioligand Binding Assays. The binding of [^{3}H]PIA to A_1 adenosine receptors of rat brain membranes was measured at 37 °C in the presence of adenosine deaminase as described.[13] The binding of [^{3}H]-5′-*N*-ethylamino)carbonyl]adenosine ([^{3}H]NECA) to the A_2 aenosine receptors of rat and bovine striatal membranes was measured at 37 °C according to Bruns et al.,[11] with the following modifications: the incubation volume was reduced to 250 μL containing 100 μg of protein; the A_1 receptor was saturated with 50 nM cyclopentyladenosine.

Data Analysis. Concentration–response curves containing at least eight different concentrations in duplicate were fitted by nonlinear regression to the Hill equation as described.[21] Binding data were analyzed by the curve-fitting program SCTFIT.[22] With this program, the simplest model (one site) was considered to be valid unless fitting with a more complex model resulted in a significantly better fit ($p < 0.01$, F test).

Platelet Aggregation Assay. Inhibition of platelet aggregation induced by ADP was determined as previously described.[15] IC_{50} values for each inhibitor were estimated from graphical plots of present inhibition vs log molar concentration (at least three to five different concentrations) and were defined as the concentrations that produce 50% inhibition of the aggregatory response to ADP. Data represent means and 95% confidence intervals of three independent determinations.

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Registry No. 1, 56707-85-8; **2a**, 113628-01-6; **2b**, 113646-62-1; **2c**, 113628-11-8; **3a**, 113628-02-7; **3b**, 113628-03-8; **3c**, 113628-04-9; **4**, 109151-84-0; **5**, 113628-05-0; **6**, 113646-63-2; **6** (acid chloride), 113628-06-1; **7**, 113628-07-2; **8**, 113628-08-3; **9**, 113628-09-4; **10**, 113628-10-7; (*R*)-$H_2NCH(CH_3)CH_2Ph$, 156-34-3; (c-C_5H_9)NH_2, 1003-03-8; (c-C_6H_{11})NH_2, 108-91-8; $EtNH_2$, 75-04-7; adenylate cyclase, 9012-42-4.

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Synthetic Polyamine Analogues as Antineoplastics

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In this paper, we report on the synthesis and biological activity of a number of N-alkylated spermine compounds. The dialkylspermines N^1,N^{12}-dimethylspermine (DMSPM-**2**), N^1,N^{12}-diethylspermine (DESPM-**3**), and N^1,N^{12}-dipropylspermine (DPSPM-**4**) are all shown to inhibit the growth of L1210 cells in culture with IC_{50} values of less than 1 μM at 96 h. Furthermore, DESPM-**3** is shown to be similarly active against Daudi and HL-60 cells in culture. A structure–activity relationship is shown to exist between the position at which spermine is alkylated and its antiproliferative properties. The activity of 10 μM DESPM-**3** against L1210 cells was shown to be cytostatic, with greater than 90% cell viability by trypan blue exclusion, even after a 144-h exposure. When L1210 cells were treated with 10 μM DESPM-**3** over a 144-h period, their size and mitochondrial DNA content were gradually but substantially diminished. However, flow cytometric measurements of the nuclear DNA content of these treated cells at 96 h indicated only slightly reduced S and G_2 populations and significant changes only after 144 h. A cloning assay performed on the cells after 96 h of exposure to this drug (10 μM) indicated that the cells were not growing. Finally, when male DBA/2 mice, inoculated with L1210 leukemia cells, were treated with DESPM-**3**, their life span was increased in excess of 200% relative to untreated controls. Moreover, many long-term survivors were apparently tumor free at the end of the experiment (60 days).

The role of polyamines in proliferative processes has received considerable attention in recent years.[1-5] In fact, interruption of the polyamine metabolic network has been at least partially successful in controlling the growth of cancer cells.[6,7] The antineoplastic drugs, α-(difluoromethyl)ornithine (DFMO) and methylglyoxal bis(guanylhydrazone) (MGBG), are both potent inhibitors of enzymes that are critical to polyamine biosynthesis. DFMO

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