J. Med. Chem. 1992, 35, 2363-2368 2363

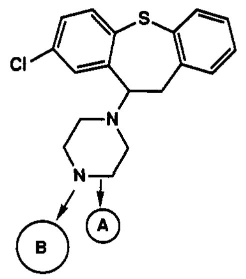

Figure 12. Schematic illustration showing the proposed *N*-alkyl interaction sites, A and B in relation to the structure of the template molecule (*S*)-octoclothepin. A is the site proposed to be used by the alkyl group in compounds with an *N*-ethyl substituent, while B is the proposed site for an *N*-benzyl substituent.

interaction model proposed by Liljefors and Bøgesø, extending this model to include the important benzamide class of DA D-2 receptor antagonists. For benzamides with an acyclic amide side chain, the most probable receptor-bound conformation is the one with an extended alkyl substituent. The enantioselectivity of the chiral benzamide of type **3** may be rationalized in terms of conformational energy differences for the receptor bound enantiomers. The *N*-alkyl substituent of the benzamides is proposed to be able to interact with two different sites for the *N*-alkyl substituent. For the benzamides studied in this work, the *N*-benzyl groups of compounds **6** and **12** are proposed to interact with one receptor site, while the alkyl group in benzamides with a *N*-ethyl group (compounds **9**, **10**, and **11**) may interact with the other site.

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Registry No. **1a**, 1141-58-8; **1b**, 971-34-6; **2**, 63224-18-0; **3**, 140660-08-8; **4a**, 140660-09-9; **4b**, 140676-32-0; **5**, 140660-10-2; **6**, 55905-53-8; **7**, 140849-89-4; **8**, 364-62-5; (*R*)-**9**, 98527-07-2; (*S*)-**9**, 84225-95-6; **10**, 23672-07-3; **11**, 110319-89-6; **12**, 75272-39-8; dopamine, 51-61-6.

2-Alkynyl Derivatives of Adenosine and Adenosine-5′-*N*-ethyluronamide as Selective Agonists at A_2 Adenosine Receptors[1]

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In the search for more selective A_2-receptor agonists and on the basis that appropriate substitution at C2 is known to impart selectivity for A_2 receptors, 2-alkynyladenosines **2a–d** were resynthesized and evaluated in radioligand binding, adenylate cyclase, and platelet aggregation studies. Binding of [^{3}H]NECA to A_2 receptors of rat striatal membranes was inhibited by compounds **2a–d** with K_i values ranging from 2.8 to 16.4 nM. 2-Alkynyladenosines also exhibited high-affinity binding at solubilized A_2 receptors from human platelet membranes. Competition of 2-alkynyladenosines **2a–d** for the antagonist radioligand [^{3}H]DPCPX and for the agonist [^{3}H]CCPA gave K_i values in the nanomolar range, and the compounds showed moderate A_2 selectivity. In order to improve this selectivity, the corresponding 2-alkynyl derivatives of adenosine-5′-*N*-ethyluronamide **8a–d** were synthesized and tested. As expected, the 5′-*N*-ethyluronamide derivatives retained the A_2 affinity whereas the A_1 affinity was attenuated, resulting in an up to 10-fold increase in A_2 selectivity. A similar pattern was observed in adenylate cyclase assays and in platelet aggregation studies. A 30- to 45-fold selectivity for platelet A_2 receptors compared to A_1 receptors was found for compounds **8a–c** in adenylate cyclase studies.

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 of 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.[2] Many of its effects can be attributed to the action at receptors located on the cell surface, which are mediated by at least two extracellular receptors divided into two major subtypes, called A_1 and A_2.[3]

At A_1 receptors the most active analogues are N^6-substituted adenosines, whereas at A_2 receptors the most active compounds are adenosine-5′-*N*-alkyluronamides. We recently reported the synthesis of N^6-substituted 1-deazaadenosines,[4] and of 2-chloro-*N*^{6}-cyclopentyladenosine (CCPA) which proved to be an agonist with high affinity and approximately 10 000-fold selectivity for A_1 adenosine receptors.[5,6]

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Scheme I

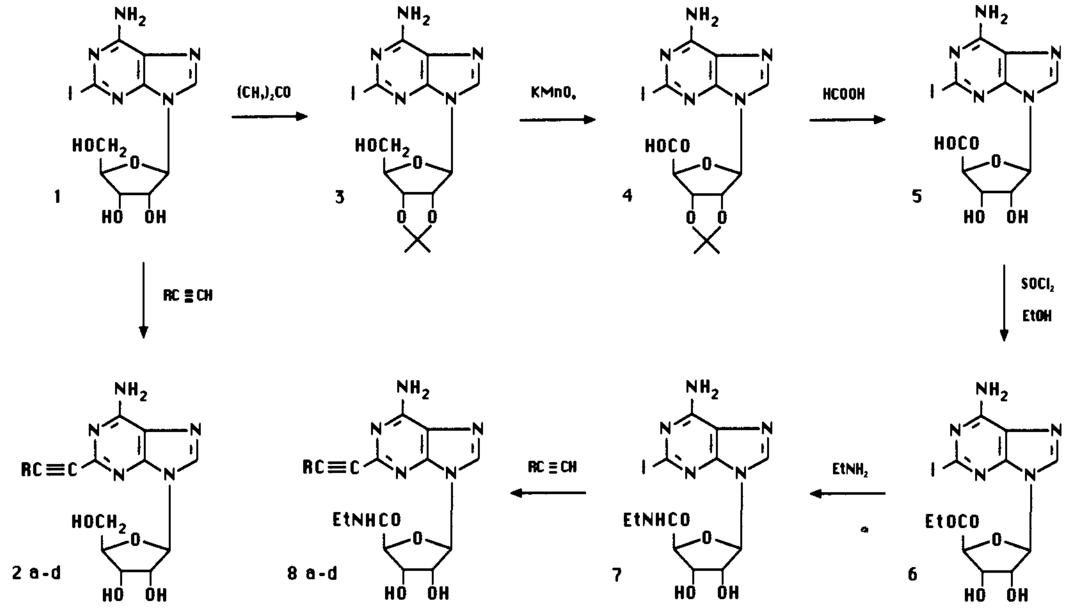

Table I. 2-Alkynyl Derivatives of Adenosine (**2a–d**) and Adenosine-5′-*N*-ethyluronamide (**8a–d**) from Scheme I

compd	R	chromatog solvent	yield, %	mp, °C[a]	formula[b]
2a	$(CH_2)_2CH_3$	$CHCl_3$-cC_6H_{12}-MeOH (78:10:12)	83	118–120[c]	$C_{15}H_{19}N_5O_4$
2b	$(CH_2)_3CH_3$	C_6H_6-AcOEt-MeOH (60:32:8)	84	115–117[c]	$C_{16}H_{21}N_5O_4$
2c	$(CH_2)_4CH_3$	$CHCl_3$-C_6H_6-MeOH (60:35:5)	70	110–112[c]	$C_{17}H_{23}N_5O_4$
2d	$(CH_2)_5CH_3$	$CHCl_3$-MeOH (90:10)	78	111–113[c]	$C_{18}H_{25}N_5O_4$
8a	$(CH_2)_2CH_3$	$CHCl_3$-C_6H_{14}-MeOH (60:28:12)	70	137–139	$C_{17}H_{22}N_6O_4$
8b	$(CH_2)_3CH_3$	$CHCl_3$-MeOH (92:8)	70	135–137	$C_{18}H_{24}N_6O_4$
8c	$(CH_2)_4CH_3$	$CHCl_3$-MeOH (90:10)	65	196–198	$C_{19}H_{26}N_6O_4$
8d	$(CH_2)_5CH_3$	$CHCl_3$-MeOH (90:10)	68	145–147	$C_{20}H_{28}N_6O_4$

[a] Uncorrected. [b] All compounds had satisfactory C, H, N, microanalyses and were within 0.4% of the theoretical value. All compounds exhibited ^1H NMR spectra consistent with the assigned structures. [c] Reference 12b.

On the other hand, the prototypical A_2 agonist adenosine-5′-*N*-ethyluronamide (NECA) showed little or no A_2 selectivity.[7] In the search for more selective A_2 receptor agonists and on the basis that appropriate substitution at C2 is known to impart selectivity for A_2 receptors,[7-11] 2-alkynyladenosines **2a–d**[12] and the corresponding 2-alkynyl derivatives of adenosine-5′-*N*-ethyluronamide **8a–d** were synthesized (Scheme I) and evaluated in radioligand binding, adenylate cyclase, and platelet aggregation studies.

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Chemistry

The synthesis of 2-alkynyladenosines **2a–d**[12a] and of 2-alkynyl derivatives of adenosine-5′-*N*-ethyluronamide **8a–d** was accomplished by the reactions described in Scheme I. The synthesis of compounds **2a–d** was carried out by a modification of the palladium-catalyzed cross-coupling reaction reported by Matsuda et al.[12a] Treatment of a solution of 2-iodoadenosine (**1**)[13] in dry acetonitrile and triethylamine with cuprous iodide, $PdCl_2$, triphenylphosphine, and the appropriate terminal alkyne, at room temperature for 24 h under an atmosphere of N_2, effected complete conversion of the iodonucleoside to the alkynyl derivatives **2a–d** (Table I).

The synthesis of the NECA derivatives **8a–d** was accomplished by a similar cross-coupling reaction between the appropriate terminal alkynes and the new nucleoside *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**7**).

The synthesis of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**7**) is reported in Scheme I. Reaction at room temperature of 2-iodoadenosine (**1**)[13] with acetone in the presence of *p*-toluenesulfonic acid as a catalyst gave 6-amino-2-iodo-9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-9*H*-purine (**3**). This compound was oxidized with $KMnO_4$ in aqueous base to afford the carboxylic acid **4** in 76% yield. Cleavage of the acetonide of **4** with 50% formic acid at 80 °C gave 1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronic acid (**5**) in 85% yield.

Treatment of the carboxylic acid **5** with $SOCl_2$ in absolute ethanol at room temperature overnight afforded the ester **6**, which reacted with dry ethylamine at −20 °C to give the desired *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**7**).

Treatment of a solution of compound **7** in dry acetonitrile and triethylamine with cuprous iodide, $PdCl_2$, triphenylphosphine, and the appropriate terminal alkyne gave the 2-alkynyladenosine-5′-*N*-ethyluronamide derivatives **8a–d** in good yield.

Biological Evaluation and Discussion

The effects of alkynyladenosines on adenosine receptors were tested using both radioligand binding techniques and functional assays. Affinities for A_2 receptors were determined in radioligand competition assays for the receptors of rat striatum and human platelets using [^{3}H]NECA as the radioligand and N^6-cyclopentyladenosine (CPA) in order to saturate A_1 receptors. To allow determination of affinities for platelet A_2 receptors, the receptors were separated from nonreceptor binding sites by chromatographic procedures as described.[14] Affinities for A_1 receptors were determined in radioligand competition assays for the receptors of rat brain using the agonist [^{3}H]CCPA and the antagonist [^{3}H]DPCPX as radioligands. NECA, CPA, and N^6-[(*R*)-(−)-1-methyl-2-phenethyl]adenosine (*R*-PIA) were used as reference compounds. Functional activity at adenosine receptors was determined in adenylate cyclase assays by measuring A_1 receptor-mediated inhibition in rat fat cell membranes and A_2 receptor-mediated stimulation in human platelet membranes.[15]

Affinities of 2-alkynyladenosines for the A_2 receptors (Table II) were in the range of 2–40 nM for both receptor preparations. In the case of the rat striatal receptors highest affinities were found for chain lengths of six and seven (compounds **2b** and **2c**), whereas for the human platelet receptors the highest affinities were found for the shortest chain lengths (compounds **2a** and **2b**). All compounds had an A_1 receptor affinity (high-affinity component) of about 20 nM, regardless of the radioligand used. As a consequence, most compounds were moderately A_2-selective (Table II). The A_2 selectivity was highest for a chain length of seven in the case of the rat striatum receptor (~10-fold), and a chain length of five in the case of the human platelet receptor (2–3-fold). Shortly after a preliminary presentation of our results,[1] Abiru et al.[11c] reported binding data for compounds **2b** and **2d**. Their data agree reasonably with our data: there is a trend toward lower A_1 affinities and higher A_2 affinities, so that in their report A_2 selectivity is somewhat higher. We think these are just variations between laboratories, since also the reference compounds showed the same trend, including their K_D values for the radioligand.

A very similar pattern was observed in adenylate cyclase assays comparing A_1 receptors in rat fat cells with A_2 receptors in human platelets (Table III). Again, all compounds had identical affinities for A_1 receptors (IC_{50} values of 2–3 μM), but compounds with shorter chain lengths had higher affinities for the platelet A_2 receptors. Remarkably, all 2-alkynyladenosines **2a–d** were only 60–70% effective (E_{max}, Table III), resulting in partial agonists at A_2 receptors. The A_2 selectivities calculated from adenylate cyclase studies (Table III) were about 10-fold higher than those determined by radioligand binding. This phenomenon has been observed previously by other groups as well as ourselves.[5,7] It appears to be due firstly to the previously reported discrepancy between the high-affinity state of A_1 receptors for agonists and the corresponding IC_{50} values in inhibiting adenylate cyclase[16]—a difference much less pronounced for A_2 receptors—and secondly to complexities in functional assays as a consequence of receptor reserves.[17] The A_2 selectivity was again highest for the shortest chain length.

In order to improve this A_2 selectivity, the corresponding 2-alkynyl derivatives of NECA **8a–d** were synthesized and tested (Tables II and III). The overall pattern of results are similar to the one obtained above, but the A_2 selectivity was indeed improved. This resulted in compounds **8b** and **8c** that showed an almost 40-fold selectivity for the A_2 receptors of rat striatum compared to A_1 receptors of rat brain. Compound **8b** also had a 10–20-fold selectivity for the A_2 receptors of human platelets. A 30–45-fold selectivity for platelet A_2 receptors compared to A_1 receptors was found for compounds **8a–c** in adenylate cyclase studies (Table III).

Platelet aggregation studies confirmed the previous results. 2-Hexynyl-NECA (**8b**) was the most active inhibitor of ADP-induced platelet aggregation with an IC_{50} value of 50 nM, compared to the parent compound NECA with

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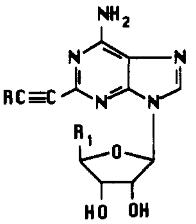
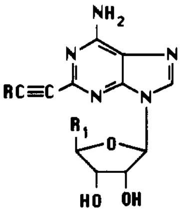
Table II. Competition of the 2-Alkynyl Derivatives of Adenosine and NECA for Radioligands at A1 and A2 Receptors



compd	R	R1	A1 [^{3}H]DPCPX high affinity	A1 [^{3}H]DPCPX low affinity	A1 [^{3}H]CCPA K_i (nM)[a]	A2 [^{3}H]NECA striatum	A2 [^{3}H]NECA platelet	A2 selectivity (K_iA1/K_iA2) DPCPX/striatum	A2 selectivity (K_iA1/K_iA2) CCPA/striatum
2a	$(CH_2)_2CH_3$	CH_2OH	16.0	849	21.5	16.4	7.3	1	1
			10.0–25.5	523–1380	11.2–41.6	8.0–33.8	2.6–20.6		
2b	$(CH_2)_3CH_3$	CH_2OH	12.5	633	15.9	3.6	8.1	3	4
			5.6–27.9	253–1580	4.7–53.2	2.2–5.9	3.8–17.4		
2c	$(CH_2)_4CH_3$	CH_2OH	32.2	1216	28.4	2.8	20.8	11	10
			13.4–77.5	777–1910	17.9–45.2	1.9–4.2	17.4–24.9		
2d	$(CH_2)_5CH_3$	CH_2OH	19.6	823	25.2	6.3	39.9	3	4
			6.5–59.1	484–1400	11.3–56.6	4.2–9.5	10.9–145		
8a	$(CH_2)_2CH_3$	CONHEt	33.0	1620	40.8	19.5	12.6	2	2
			18.0–60.0	1150–2260	27.2–61.1	4.1–93.3	4.2–37.9		
8b	$(CH_2)_3CH_3$	CONHEt	136	3160	43.9	3.8	5.7	36	12
			95–195	1420–7030	38.1–50.6	1.5–9.9	4.3–7.5		
8c	$(CH_2)_4CH_3$	CONHEt	171	2910	89	4.7	37.1	36	19
			146–201	1060–7990	69–114	2.4–9.3	17.3–79.5		
8d	$(CH_2)_5CH_3$	CONHEt	67	2940	64.4	14.8	159	5	4
			27.0–164	2040–4240	40.5–102.3	8.2–26.7	59–429		
NECA			11	650	8.2	22	70	0.5	0.4
			7.0–17	420–1000	6.2–10.9	20–25	55–89		
R-PIA			1.0	200	1.3	730	1700	0.001	0.002
			0.8–1.3	160–250	1.1–1.6	690–770	1100–2600		
CPA			0.8	130	0.8	2000	2400	0.0004	0.0004
			0.5–1.3	90–190	0.6–1.1	1400–2900	1300–4400		

[a] For A1 receptors K_i values were determined from competition for [^{3}H]DPCPX (antagonist) and [^{3}H]CCPA (agonist) binding at rat brain membranes. K_i values for A2 receptors were determined at rat striatal membranes in the presence of 50 nM CPA and at solubilized receptors from human platelet membranes. Data are means from three to six independent experiments with 95% confidence limits. [b] A2 selectivity ratios were calculated with high-affinity K_i values from competition for [^{3}H]DPCPX binding at rat brain membranes and K_i values from competition for [^{3}H]NECA binding at rat striatal membranes. For comparison A2 selectivity was also determined with K_i values from competition for [^{3}H]CCPA binding and [^{3}H]NECA binding.

Table III. Effects of the 2-Alkynyl Derivatives of Adenosine and NECA on Adenylate Cyclase[a]



compd	R	R1	A1 IC_{50}, nM	A2 EC_{50}, nM	A2 select (IC_{50}/EC_{50})	E_{max}, %
2a	$(CH_2)_2CH_3$	CH_2OH	3000	127	24	65
			1150–7840	95–171		
2b	$(CH_2)_3CH_3$	CH_2OH	2300	128	19	65
			1320–4000	90–182		
2c	$(CH_2)_4CH_3$	CH_2OH	3380	414	8	65
			1200–9520	289–592		
2d	$(CH_2)_5CH_3$	CH_2OH	2910	681	4	65
			1030–8200	240–1930		
8a	$(CH_2)_2CH_3$	CONHEt	2700	62	44	100
			2010–3630	38–100		
8b	$(CH_2)_3CH_3$	CONHEt	4380	105	42	100
			1990–9640	48–229		
8c	$(CH_2)_4CH_3$	CONHEt	13090	388	34	100
			6750–25400	277–543		
8d	$(CH_2)_5CH_3$	CONHEt	13500	585	23	100
			7900–23100	479–714		

[a] All compounds inhibited adenylate cyclase via A1 receptors in rat fat cell membranes to the same degree as the full agonist CCPA, and the maximal inhibition was 45 ± 4.3% ($n = 9$, ±SEM). The maximal NECA stimulation of adenylate cyclase via A2 receptors in human platelet membranes amounted to 319 ± 16% ($n = 7$, ±SEM). Compounds **2a–d** are only partial agonists ($E_{max} = 65\%$), whereas **8a–d** are full agonists ($E_{max} = 100\%$). Values are means and SEM's or 95% confidence limits of three to four independent determinations.

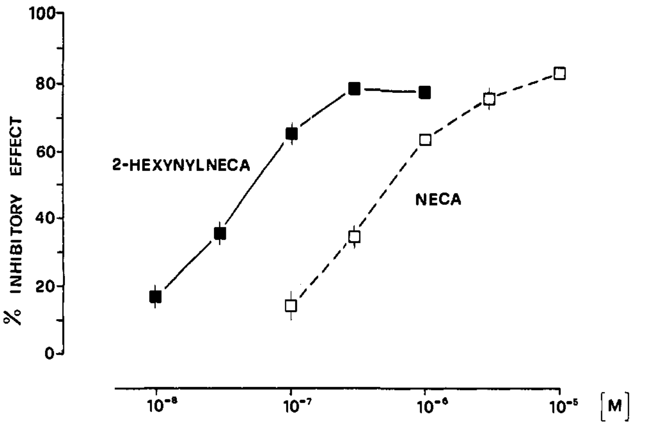


Figure 1. Dose–response curves of inhibitory effect of 2-hexynyl-NECA (■) and NECA (□) on human platelet aggregation induced by ADP. Data represent means of at least three independent determinations.

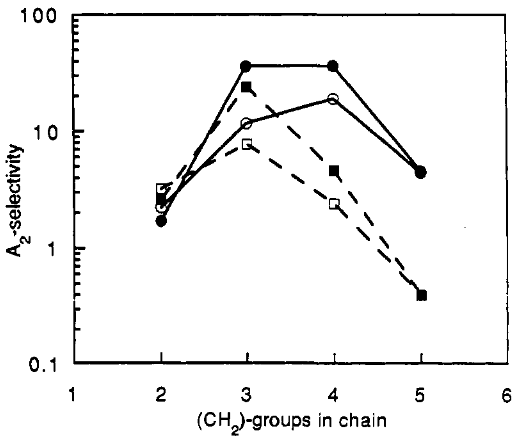


Figure 2. Influence of methylene groups in the chain of compounds **8a–d** on A_2 selectivity. Comparison of A_2 selectivity in the binding of DPCPX/striatum (●), CCPA/striatum (○), DPCPX/platelet (■), and CCPA/platelet (□).

an IC_{50} of 500 nM (Figure 1).

The compounds described here seem to be capable not only of distinguishing between A_1 and A_2 receptors, but also between the A_2 receptors of rat brain and human platelets (Figure 2). 2-Heptynyl-NECA (**8c**) and 2-octynyl-NECA (**8d**) have about a 10-fold higher affinity for the rat striatal A_2 receptor. Further studies will have to address the question whether these are only species differences, or whether they represent true A_2 receptor subtypes.

Agonists with high affinity for A_2 receptors have already been described by Bridges et al.[18a] These are adenosine and NECA derivatives with bulky N^6-substituents, in particular diphenylethyl substituents.[18b] In a direct comparison in our laboratory, these compounds had only little if any A_2 receptor selectivity (data not shown). Attempts to increase A_2 selectivity by the combination of appropriate modifications at N^6 and C5′ were not very successful.[19] Williams and co-workers[11] have recently described a series of substituted 2-amino derivatives of NECA that show even higher selectivity for A_2 receptor, but have at least 2-fold lower affinities than compounds **8b** and **8c** described here. The high affinities of the compounds described here together with reasonable selectivities should make them useful tools for the characterization of adenosine receptors.

Experimental Section

Chemistry. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VX 300 MHz spectrometer. IR spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are within ±0.4% of theoretical values.

Preparation of 2-Alkynyladenosines (2a–d). To a solution of 2-iodoadenosine[13] (**1**) (1.27 mmol) in 10 mL of dry acetonitrile and 10 mL of triethylamine under an atmosphere of N_2 were added 18.6 mg (0.0976 mmol) of cuprous iodide, 12 mg (0.0672 mmol) of $PdCl_2$, and 39 mg (0.149 mmol) of triphenylphosphine. To the mixture was added the appropriate terminal alkyne (6.3 mmol), and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for 24 h. 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–d** as chromatographically pure solids. All of the spectral data for the compounds were compatible with the structures.

Preparation of 2-Alkynyladenosine-5′-*N*-ethyluronamides (8a–d). To a solution of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**7**) (0.58 mmol) in 10 mL of dry acetonitrile and 5 mL of triethylamine under an atmosphere of N_2 were added 8.5 mg (0.0446 mmol) of cuprous iodide, 5.5 mg (0.0308 mmol) of $PdCl_2$, and 17.8 mg (0.069 mmol) of triphenylphosphine. To the mixture was added the appropriate terminal alkyne (2.9 mmol), and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for 16 h. 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 **8a–d** as chromatographically pure solids. All of the spectral data for the compounds were compatible with the structures.

6-Amino-2-iodo-9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-9*H*-purine (3). To a solution of 2 g (5.08 mmol) of 2-iodoadenosine (**1**)[13] in 100 mL of acetone was added 9.6 g of *p*-toluenesulfonic acid. The reaction mixture was stirred at room temperature for 1 h and then, after the addition of 15 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 flash chromatographed on a silica gel column, eluting with $CHCl_3$–MeOH (99:1) to give 1.62 g (74%) of **3** as a solid: mp 185–187 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.33 and 1.54 (s, 3 H each, $C(CH_3)_2$), 3.53 (m, 2 H, CH_2-5′), 4.19 (m, 1 H, H-4′), 5.07 (t, 1 H, OH), 4.93 (m, 1 H, H-3′), 5.27 (m, 1 H, H-2′), 6.05 (d, J = 2.5 Hz, 1 H, H-1′), 7.76 (s, 2 H, NH_2), 8.28 (s, 1 H, H-8). Anal. ($C_{13}H_{16}IN_5O_4$) C, H. N.

1′-Deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-*O*-isopropylidene-β-D-ribofuranuronic Acid (4). To a stirred solution of 1.6 g (3.7 mmol) of **3** in 200 mL of H_2O were added 0.60 g of KOH and, dropwise, a solution of 1.70 g (10.8 mmol) of $KMnO_4$ in 50 mL of H_2O. The mixture was set aside in the dark at room temperature for 20 h. The reaction mixture was cooled to 5–10 °C and then decolorized by a solution of 4 mL of 30% H_2O_2 in 16 mL of water, while the temperature was maintained under 10 °C using an ice–salt bath. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo to about 15 mL and then acidified to pH 4 with 2 N HCl. The resulting precipitate was filtered off and successively washed with water,

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acetone, and ether to give 1.25 g (76%) of 4 as a white solid: mp 187–190 °C; IR $\nu_{\max}$ 1590, 1640 cm^{-1} (COOH); ^{1}H NMR (Me$_2$SO-d_6) δ 1.33 and 1.49 (s, 3 H each, C(CH_3)$_2$), 4.64 (s, 1 H, H-4′), 5.35 (d, $J_{3',2'}$ = 5.6 Hz, 1 H, H-3′), 5.41 (d, $J_{2',3'}$ = 5.6 Hz, 1 H, H-2′), 6.23 (s, 1 H, H-1′), 7.53 (s, 1 H, COOH), 7.67 (s, 2 H, NH$_2$), 8.17 (s, 1 H, H-8). Anal. ($C_{13}H_{14}IN_5O_5$) C, H, N.

1′-Deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-*β*-D-ribofuranuronic Acid (5). A solution of 1.72 g (3.85 mmol) of 4 in 80 mL of 50% HCOOH was stirred at 80 °C for 1.5 h. The reaction mixture was evaporated in vacuo, the residue was dissolved in water, and the solution was evaporated. This process was repeated several times until there was no odor of formic acid in the residue. Recrystallization from water yielded 1.33 g (85%) of 5 as a white solid: mp 217–220 °C dec; ^{1}H NMR (Me$_2$SO-d_6) δ 4.28 (m, 1 H, H-3′), 4.41 (d, J = 2.1 Hz, 1 H, H-4′), 4.81 (m, 1 H, H-2′), 5.95 (d, J = 6.7 Hz, 1 H, H-1′), 7.78 (s, 2 H, NH$_2$), 8.38 (s, 1 H, H-8), 12.98 (br s, 1 H, COOH). Anal. ($C_{10}H_{10}IN_5O_5$) C, H, N.

Ethyl 1′-Deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-*β*-D-ribofuranuronate (6). To a cooled (5 °C) and stirred solution of 1.29 g (3.17 mmol) of 5 in 150 mL of absolute ethanol was added dropwise 1.15 mL of ice-cooled SOCl$_2$. The mixture was stirred at room temperature overnight and then brought to pH 8 with saturated aqueous NaHCO$_3$. The mixture was filtered, and the filtrate was concentrated in vacuo. Recrystallization of the residue from water–ethanol (1:1) gave 900 mg (65%) of 6 as a white solid: mp 221–223 °C dec; IR $\nu_{\max}$ 1728 cm^{-1} (COOEt); ^{1}H NMR (Me$_2$SO-d_6) δ 1.21 (t, 3 H, CH$_2$$CH_3$), 4.18 (q, 2 H, CH_2CH$_3$), 4.34 (m, 1 H, H-3′), 4.47 (s, 1 H, H-4′), 4.58 (m, 1 H, H-2′), 5.96 (d, J = 6.7 Hz, 1 H, H-1′), 7.74 (s, 2 H, NH$_2$), 8.33 (s, 1 H, H-8). Anal. ($C_{12}H_{14}IN_5O_5$) C, H, N.

***N*-Ethyl-1′-Deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-*β*-D-ribofuranuronamide (7).** A mixture of 620 mg of 6 and 18 mL of dry ethylamine was stirred at −20 °C for 3 h and then at room temperature overnight. The reaction mixture was diluted with absolute ethanol, and the precipitated product was filtered off and washed with dry ether to give 530 mg (85%) of 7 as a pure solid: mp 232–234 °C; IR $\nu_{\max}$ 1637, 1560 cm^{-1} (C═O, amide); ^{1}H NMR (Me$_2$SO-d_6) δ 1.06 (t, 3 H, CH$_2$$CH_3$), 3.28 (m, 2 H, CH_2CH$_3$), 4.16 (m, 1 H, H-3′), 4.31 (d, J = 2.1 Hz, 1 H, H-4′), 4.58 (m, 1 H, H-2′), 5.91 (d, 1 H, J = 7.3 Hz, H-1′), 7.79 (s, 2 H, NH$_2$), 8.15 (t, 1 H, NH), 8.40 (s, 1 H, H-8). Anal. ($C_{12}H_{15}IN_6O_4$) C, H, N.

Biological Studies. Membrane Preparation. Membranes from rat brain and rat striatum were prepared as described.[20] Human platelet membranes were prepared according to the method of Hoffman et al.[21] A$_2$ receptors from platelet membranes were solubilized as described recently.[14] Rat fat cells were isolated as described by Honnor et al.,[22] and their membranes were prepared according to MeKeel and Jarett.[23]

Radioligand Binding Assays. Radioligand binding at A$_1$ receptors from rat brain membranes was measured as described in detail for the antagonist [^{3}H]DPCPX[24] and the agonist [^{3}H]CCPA.[6] [^{3}H]NECA was used to measure A$_2$ receptor binding in rat striatal membranes, according to the procedure of Bruns et al.,[7] in a total value of 250 μL containing 50 μg of protein. A$_1$ receptor was saturated with 50 nM cyclopentyladenosine. Binding to solubilized A$_2$ receptors from human platelets was performed with [^{3}]NECA as described.[14]

Adenylate Cyclase Assay. Inhibition of adenylate cyclase activity via A$_1$ receptors was measured in rat fat cell membranes in the presence of 10 μM forskolin, and stimulation of adenylate cyclase via A$_2$ receptors was determined in human platelet membranes.[15]

Data Analysis. Concentration–response curves containing at least seven different concentrations in duplicate were fitted by nonlinear regression to the Hill equation as described.[16] Binding data were analyzed by the curve-fitting program SCATFIT according to a one-site model.[25] A two-site model was assumed if the fit was significantly improved ($p < 0.01$, F test).

Platelet Aggregation Assay. Platelet aggregation was measured by modification of the method of Born and Cross[4,26] using a Platelet Aggregation Profiler Model Pap-3 (Bio Data Corp.). The aggregative agent ADP was purchased from Sigma Chemical Co. Blood was obtained by venipuncture in the forearms of apparently healthy humans and collected in polyethylene tubes containing a 1:9 volume of 3.8% sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation at 1200 rpm for 15 min, while platelet-poor plasma (PPP) was obtained by centrifugation at 4500 rpm for 20 min.

A 20-μL aliquot of the test sample, dissolved in 0.5% of DMSO in water, was added to a cuvette containing 470 μL of PRP, and a 20 μL aliquot of 0.5% of DMSO in water was added to the test control. The cuvette was placed in the aggregation meter and allowed to incubate at 37 °C for 5 min, after which 10 μL of 2.5 × 10^{-6} ADP (final concentration) was added to the PRP.

The percent inhibition of aggregation by a test compound was calculated by dividing the maximal deflection in the optical density curve in the presence of the compound by that observed in the control and then multiplying by 100. Data represent means of at least three independent determinations.

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Registry No. 1, 35109-88-7; **2a**, 99044-60-7; **2b**, 90596-73-9; **2c**, 90596-74-0; **2d**, 90596-75-1; **3**, 141018-25-9; **4**, 141018-26-0; **5**, 141018-27-1; **6**, 141018-28-2; **7**, 141018-29-3; **8a**, 141062-05-7; **8b**, 141018-30-6; **8c**, 141018-31-7; **8d**, 141018-32-8; 1-pentyne, 627-19-0; 1-hexyne, 693-02-7; 1-heptyne, 628-71-7; 1-octyne, 629-05-0.

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