during the drug delivery was calculated and divided by the mean of measurements made before and after the drug pipette was brought next to the cell.

Acknowledgment. We thank Dr. A. J. Thomas for supplying the initial sample of 1 and Dr. C. F. Bigge and Mr. T. Bobovski for the preparation of pyrrole-3-carboxylic acid. We also acknowledge the assistance of Ms. Barbara Welbaum, Dr. David Dudley, and Ms. Gina Lu in carrying out the binding studies and Ms. Diane Omecinsky for performing the reported NOE experiments.

Registry No. 1, 72519-12-1; 2, 137569-65-4; 3·HCl, 137569-66-5; 3 (free base), 137569-80-3; 4·HCl, 137569-67-6; 4 (free base), 137569-81-4; 5·HCl, 137569-68-7; 5 (free base), 137569-82-5; 6·HCl, 137569-69-8; 6 (free base), 137569-83-6; 7·HCl, 137569-70-1; 7 (free base), 137569-84-7; 8, 59378-87-9; 9, 931-03-3; 10, 36476-78-5; 11, 498-94-2; 12, 3395-35-5; 13, 4043-88-3; 14, 2133-34-8; 15, 64603-90-3; 16, 137569-71-2; 17, 137569-72-3; 18, 137569-73-4; 19, 137569-74-5; 20, 137569-75-6; 21, 137569-76-7; 22, 35516-94-0; 23, 68384-73-6; 24, 137569-77-8; 25, 137569-78-9; 26, 137569-79-0; H-Sar-OH, 107-97-1; H-Gly-OH, 56-40-6; Me-Ala-OH, 3913-67-5; MeC≡CCOOMe, 23326-27-4; PrC≡CCOOEt, 16205-90-6; PhC≡CCOOEt, 2216-94-6; HC≡CCOOEt, 623-47-2; strychnine, 57-24-9.

Nucleosides and Nucleotides. 103. 2-Alkynyladenosines: A Novel Class of Selective Adenosine A_2 Receptor Agonists with Potent Antihypertensive Effects[†,1]

Akira Matsuda,*[,‡] Misao Shinozaki,[§] Toyofumi Yamaguchi,[∥] Hiroshi Homma,[‡] Rie Nomoto,[§] Tadashi Miyasaka,[§] Yohko Watanabe,[∥] and Toichi Abiru[∥]

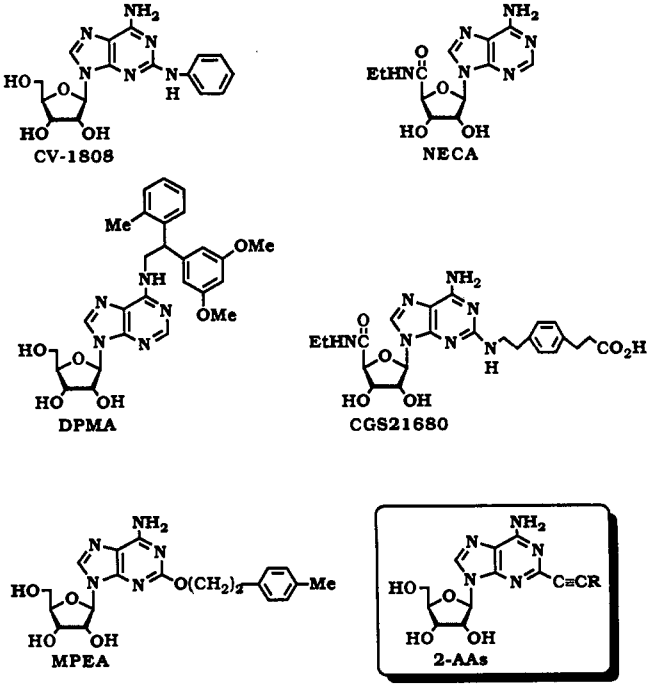
Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan, School of Pharmaceutical Sciences, Showa University, Hatanodai 1-5-8, Shinagawa-ku, Tokyo 142, Japan, and Research & Development Division, Yamasa Shoyu Co., Ltd., Araoicho 2-10-1, Choshi-shi, Chiba 288, Japan. *Received June 11, 1991*

The synthesis and receptor-binding activities at A_1 and A_2 adenosine receptors for a series of 2-alkynyladenosines are described. The palladium-catalyzed cross-coupling reaction of 2-iodoadenosine (**4a**) with various terminal alkynes in the presence of bis(triphenylphosphine)palladium dichloride and cuprous iodide in *N,N*-dimethylformamide containing triethylamine gives 2-alkynyladenosines (**5a-r**). An economical synthetic method for the preparation of 9-(2,3,5-tri-*O*-acetyl-1-β-D-ribofuranosyl)-6-chloro-2-iodopurine (**2**), which is a precursor of **4a**, is also included. Several transformation reactions of 2-(1-octyn-1-yl)adenosine (**5e**) and 2-(1-ethyn-1-yl)adenosine (**9**) and a similar cross-coupling reaction of 6-chloropurine derivative **11** and 8-bromoadenosine (**13**) with 1-octyne are also reported. Many of these 2-alkynyladenosines tested for A_1 and A_2 adenosine receptor binding activities in rat brain are selective for the A_2 adenosine receptor. Among them, 2-(1-hexyn-1-yl)adenosine (**5c**) has the highest affinity for both A_1 and A_2 receptors with K_i values of 126.5 and 2.8 nM, respectively. The structure–activity relationship of this series of compounds including 6- or 8-alkynylpurine nucleosides and 2-alkyl- and 2-alkenyladenosines is discussed in terms of potency at both receptor subtypes. Additionally, we describe how hypotensive activity and heart rate decrease brought on by **5** and some other compounds with spontaneously hypertensive rats are proportional to the order of the potency to both A_1 and A_2 binding affinities. Thus, 2-alkynyladenosines are interesting and promising as antihypertensive agents that should be considered for further detailed preclinical evaluation.

Adenosine has long been recognized as a physiologically important material to mediate a wide variety of physiological effects including decreased locomotor activity,[2] inhibition of neurotransmitter release,[3] hypothermia,[4] negative inotropic and chronotropic action,[5] inhibition of lipolysis,[6] inhibition of renin release,[7] inhibition of platelet aggregation,[8] and peripheral and cerebral vasodilation.[9] However, the therapeutic potential of adenosine is considered to be low, since extracellular adenosine is known to be unstable due to its rapid uptake into red blood cells,[10] deamination to inosine by adenosine deaminase,[11] and phosphorylation to 5′-AMP by adenosine kinase. Moreover, such a wide variety of actions is thought to have some detrimental effects in therapeutic use.

Adenosine-induced effects can be attributed to the action via interactions with two cell-surface adenosine receptor subtypes, A_1 and A_2, that either inhibit (A_1 receptor) or stimulate (A_2 receptor) the activity of adenylate cyclase. On the basis of the receptor binding–pharmacological relationship of several adenosine analogues, adenosine analogues that have preferential affinity for A_1 receptors produce depression in heart rate and cardiac contractility,

Chart I

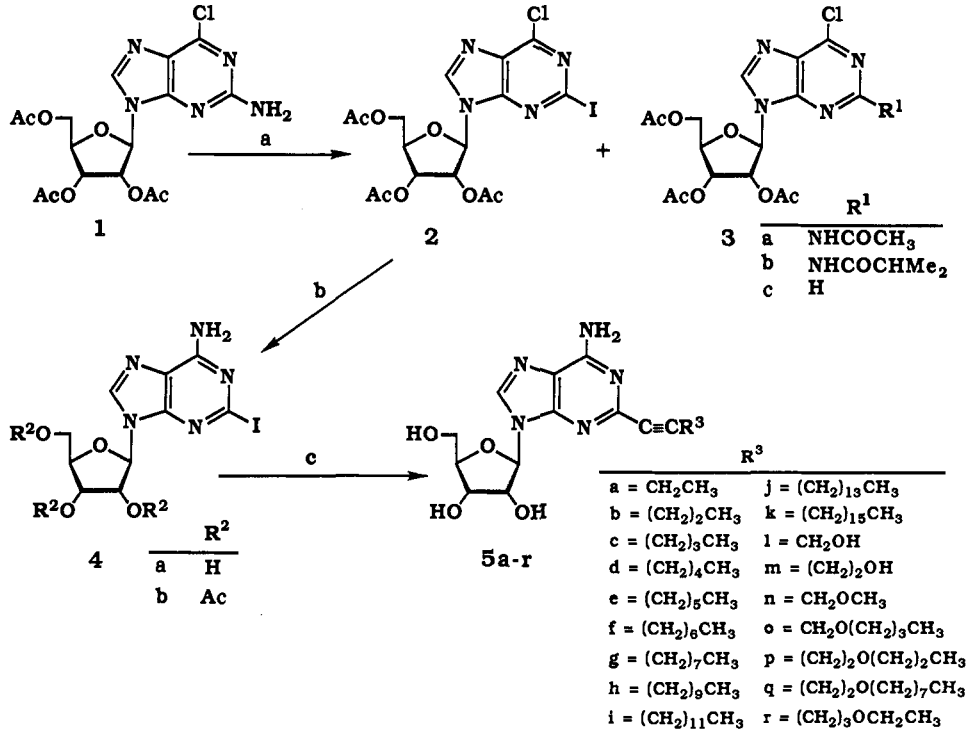


on the other hand those analogues having high affinity for A_2 receptors produce vasodilation. Adenosine agonists that

*Author to whom correspondence should be addressed.
[†] This paper is dedicated to the memory of Professor Tohru Ueda, deceased Sept 19, 1990.
[‡] Hokkaido University.
[§] Showa University.
[∥] Yamasa Shoyu Co. Ltd.

0022-2623/92/1835-0241$03.00/0

Scheme I[a]



[a] (a) CuI, I_2, CH_2I_2, isoamyl nitrite in THF, 80 °C; (b) NH_3/MeOH; (c) $HC{\equiv}C(CH_2)_nCH_3$, $(PPh_3)_2PdCl_2$, CuI, Et_3N in DMF, 80 °C.

have a selective affinity for either the A_1 or A_2 receptor will enable us to separate the actions of adenosine and contribute to the study of adenosine receptors.

To improve and separate the activities, a large number of adenosine derivatives have been synthesized. Of these analogues, N^6-cyclohexyladenosine (CHA) and N^6-cyclopentyladenosine (CPA) are reported as being highly selective A_1 agonists.[12] However, only a few potent and/or selective A_2 adenosine receptor agonists have been reported. 2-(Phenylamino)adenosine (CV-1808) was originally reported as a potent coronary vasodilator[13] and confirmed as a selective A_2 agonist.[14] 1-(6-Amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide (NECA) was also found to be a coronary vasodilator[15] and a highly potent A_2 agonist but not selective to the A_2 receptor.[14] Recently, more selective adenosine A_2 agonists such as DPMA,[16] CGS 21680,[17] and MPEA[18] shown in Chart I have been synthesized and have shown certain pharmacological activities.

(1) Part 102: Yoshimura, Y.; Iino, T.; Matsuda, A. Stereoselective radical deoxygenation of tert-propargyl alcohols in sugar moiety of pyrimidine nucleosides: Synthesis of 2'-deoxy-2'-C-alkynyl-1-β-D-arabinofuranosylpyrimidines. *Tetrahedron Lett.* **1991**, *42*, 6003–6006.
(2) Phillis, J. W.; Barraco, R. A.; DeLong, R. E. Behavioral characteristics of centrally administered analogs. *Pharmacol. Biochem. Behav.* **1986**, *24*, 261–270.
(3) Dunwiddie, T. V. The physiological role of adenosine in the central nervous system. *Int Rev. Neurobiol.* **1985**, *27*, 63–139.
(4) Wagar-Srdar, S. A.; Oken, M. M.; Morley, J. E.; Levine, A. S. A_1 and A_2 adenosine receptors modulate acetylcholine release from brain slices. *Life Sci.* **1983**, *33*, 341–343.
(5) Fredholm, B. B.; Sollevi, A. Cardiovascular effects of adenosine. *Clin. Physiol.* **1986**, *6*, 1–21.
(6) Londos, C.; Cooper, D. M. F.; Schlegel, W.; Rodbell, M. Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: Basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5362–5366.
(7) Itoh, S.; Carretero, O. A.; Murray, R. D. Possible role of adenosine in macula densa mechanism of renin release in the rabbit. *J. Clin. Invest.* **1985**, *76*, 1412–1417.
(8) Gordon, J. L. Extracellular ATP: effects, sources and fate. *Biochem. J.* **1986**, *233*, 309–319.
(9) Berne, R. M.; Winn, H. R.; Knabb, R. M.; Ely, S. W.; Rubio, R. Blood flow regulation in heart, brain and skeletal muscle. *Regul. Funct. Adenosine* **1983**, 293–317.
(10) Pfleger, E.; Seifen, E.; Schondorf, H. *Biochem. Pharmacol.* **1969**, *18*, 43–51.
(11) Baer, H. P.; Drummond, G. I.; Duncan, E. L. Formation and deamination of adenosine by cardiac muscle enzymes. *Mol. Pharmacol.* **1966**, *2*, 67–76.
(12) Angus, J. A.; Cobbin, L. B.; Einstein, R.; Maguire, H. M. Cardiovascular actions of substituted adenosine analogues. *Br. J. Pharmacol.* **1971**, *41*, 592–599.
(13) Kawazoe, K.; Matsumoto, N.; Tanabe, M.; Fujiwara, S.; Yanagimoto, M.; Hirata, M.; Kikuchi, K. Coronary and cardiohemodynamic effects of 2-phenylamino-adenosine (CV-1808) in anesthetized dogs and cats. *Arzneim.-Forsch.* **1980**, *30*, 1083–1087.
(14) Burns, R. F.;Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
(15) Prasad, R. N.; Bariana, D. S.; Fung, A.; Savic, M.; Tietje, K.; Stein, H. H.; Brondyk, H.; Egan, R. S. Modification of the 5'-position of purine nucleosides. 2. Synthesis and some cardiovascular properties of adenosine-5'-(N-substituted)carboxamides. *J. Med. Chem.* **1980**, *23*, 313–319.
(16) Bridges, A. J.; Bruns, R. F.; Ortwine, D. F.; Priebe, S. R.; Szotek, D. L.; Trivedi, B. K. N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine and its uronamide derivatives. Novel adenosine agonists with both high affinity and high selectivity for the adenosine A_2 receptor. *J. Med. Chem.* **1988**, *31*, 1282–1285.
(17) Hutchison, A. J.; Williams, M.; Dejesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.; Webb, R. L.; Zoganas, H. C.; Stone, G. A.; Jarvis, M. F. 2-(Arylalkylamino)adenosin-5'-uronamides: A new class of highly selective adenosine A_2 receptor ligands. *J. Med. Chem.* **1990**, *33*, 1919–1924.
(18) Ueeda, A.; Thompson, R. D.; Arroyo, L. H.; Olsson, R. A. 2-Aralkoxyadenosines: potent and selective agonists at the coronary artery A_2 adenosine receptor. *J. Med. Chem.* **1991**, *34*, 1340–1344.

Table I. Synthesis of 9-(2,3,5-Tri-O-acetyl-1-β-D-ribofuranosyl)-6-chloro-2-iodopurine (2)[a]

solvent	additives			reaction time, min	% isolated yield	
	CH_2I_2	I_2	CuI		2	3a or 3c[b]
CH_3CN	+	-	-	15	57	27
CH_3CN	-	+	-	15	39	42
CH_3CN	-	-	+	15	42	c
CH_3CN	+	-	+	15	66	c
CH_3CN	-	+	+	15	74	c
CH_3CN	+	+	+	15	82 (76)	5
THF	+	-	-	60	64	21
THF	-	+	-	30	57	c
THF	-	+	+	45	80 (77)	11
THF	+	-	+	45	81 (82)	c
THF	+	+	+	45	89 (85)	(2.7)

[a] The reaction of 2 (2 mmol) with isoamyl nitrite (1 mL) in acetonitrile (20 mL) or THF (20 mL) was heated at 80 °C (acetonitrile) or refluxing temperature (THF) in the presence of the additives (CH_2I_2, 2 mL; I_2, 2 mmol; CuI, 2.1 mmol; +, with addition; -, without addition). The yield in the parentheses is for a 12 mmol scale reaction. The workup procedure was described in the experimental section. [b] When using acetonitrile, the byproduct is 3a, and 3c was obtained from the reaction in THF. [c] The byproduct was detected on TLC analysis but not isolated.

We have described how 2-alkynyladenosines (2-AAs) were potent inhibitors of the passive cutaneous anaphylaxis reaction[19] and had cardiovascular effects in normotensive rats.[20] Our recent report has shown that 2-(1-hexyn-1-yl)adenosine (5c) and 2-(1-octyn-1-yl)adenosine (5e) are potent and selective agonists of the A_2 adenosine receptor and have a potent and long-lasting antihypertensive effect.[21] Thus, synthesis of other 2-AA derivatives and investigation of their pharmacological activities are necessary to develop a more selective A_2 agonist and understand the mechanisms of agonist–receptor coupling. In this report, we describe the further structure–activity relationship of 2-AA's (5a–r) having various lengths of alkyl, hydroxyalkyl, or alkoxyalkyl groups in terms of potency at the A_2 receptor as well as receptor subtype selectivity of rat brain homogenates and effects of these agonists on blood pressure (BP) and heart rate (HR) using spontaneous hypertensive rats (SHRs). An improved procedure for the synthesis of 6-chloro-2-iodopurine nucleoside 2, which is a precursor of 2-iodoadenosine (4a), and full accounts of the detailed synthesis of 2-AA's (5a–r) are also included. Additionally, the synthesis of 1-β-D-ribofuranosyl-6-(1-octyn-1-yl)purine (12) and 8-(1-octyn-1-yl)adenosine (14) and conversion of the 2-alkynyl group in 2-(1-octyn-1-yl)adenosine (5e) and 2-(1-ethyn-1-yl)-adenosine (9) into other functional groups are also described to examine the requirement of the acetylenic bond and the site of the alkynyl group for the activity.

Results and Discussion

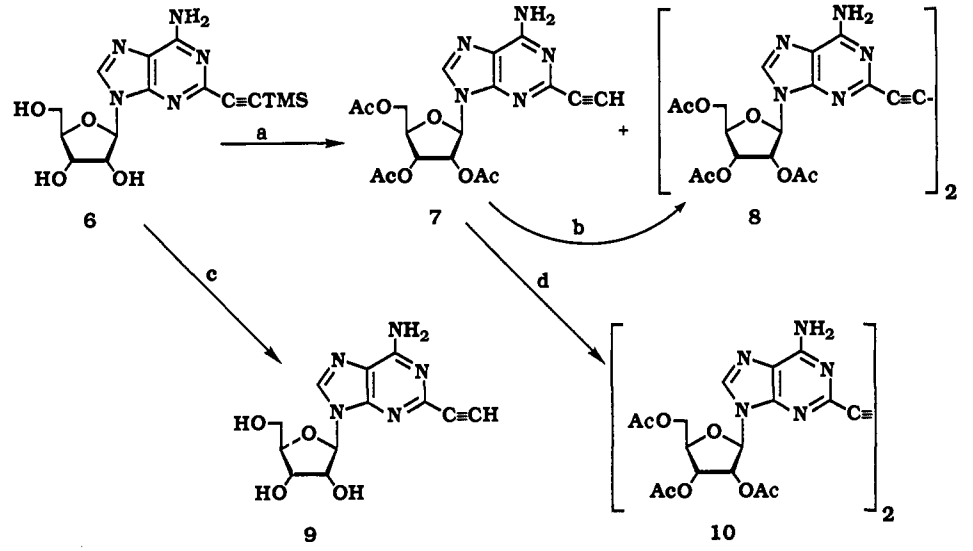
Chemistry. The original synthetic method for 6-chloro-2-iodopurine nucleoside 2, which is a precursor in the synthesis of 2-iodoadenosine (4a), has been reported by Nair and Richardson by diazotization–substitution of 2-amino-6-chloropurine derivative 1[22] with *n*-pentyl nitrite in diiodomethane (CH_2I_2) in small quantities.[23] This method, however, required a large amount of expensive CH_2I_2 as an iodide source of the reaction because of the low solubility of 1. Since we required an inexpensive method for the preparation of 2, solvents (acetonitrile or tetrahydrofuran) and some additives [CH_2I_2, I_2, and/or cuprous iodide (CuI)] as iodide sources in the reaction of 1 with isoamyl nitrite were examined, and the results are shown in Table I. In acetonitrile, the iodine substitution of 1 was completed within 15 min at 80 °C and the best yield of 2 was obtained when all of the additives were combined in the reaction mixture. When the reaction was done with one or two of the additives, the yield of 2 was moderate and 9-(2,3,5-tri-O-acetyl-1-β-D-ribofuranosyl)-2-acetamido-6-chloropurine (3a) was obtained as a byproduct. This compound (3a) could be obtained by the reaction of a purin-2-yl radical,[23] which is proposed as an intermediate of the reaction, with acetonitrile, followed by H_2O addition, being produced from the diazotization reaction. To confirm this mechanism, 1 was diazotized with isoamyl nitrite in isobutyronitrile with CH_2I_2. As would be expected, a small quantity of 2-isopropylamide derivative 3b was obtained as a minor component along with 2 in 63% yield (Scheme I). This implies that the intermediate purin-2-yl radical actually competes to react with the iodide sources and the nitrogen atom of isobutyronitrile or acetonitrile.

In tetrahydrofuran, completion of the reaction to give 2 required a rather longer period of time than in acetonitrile. Addition of two or three of the additives showed good results to afford 2. However a small amount of 6-chloropurine derivative 3c was concomitantly formed. Since a 6-amino group of adenosine derivatives has been reported to be reductively deaminated to nebularine derivatives in THF with *n*-pentyl nitrite,[24] the reductive deamination of the 2-amino group of 1 can be explained by a similar mechanism. Thus, using THF as a solvent and the combination of CH_2I_2, I_2, and CuI as iodide sources of the diazotization–iodine substitution reaction of 1 is the choice for an economical way to prepare 2.

Treatment of 2 with methanolic ammonia in a sealed tube at 60 °C for 17 h furnished the desired 2-iodoadenosine (4a)[25] as crystals from water in 90% yield.

(19) Matsuda, A.; Shinozaki, M.; Miyasaka, T.; Machida, H.; Abiru, T. Palladium-catalyzed cross-coupling of 2-iodoadenosine with terminal alkynes: synthesis and biological activities of 2-alkynyladenosines. *Chem. Pharm. Bull.* **1985**, *33*, 1766–1769.
(20) Matsuda, A.; Ueda, T. The synthesis, mutagenic and pharmacological activities of 2-carbon-substituted adenosines. *Nucleosides Nucleotides* **1987**, *6*, 85–94.
(21) Abiru, T.; Yamaguchi, T.; Watanabe, Y.; Kogi, K.; Aihara, K.; Matsuda, A. The antihypertensive effect of 2-alkynyladenosines and their selective affinity for adenosine A_2 receptors. *Eur. J. Pharmacol.* **1991**, *196*, 69–76.
(22) Robins, M. J.; Uznanski, B. Conversion of adenosine and guanosine to 2,6-dichloro, 2-amino-6-chloro, and derived purine nucleosides. *Can. J. Chem.* **1981**, *59*, 2601–2607.
(23) Nair, V.; Richardson, S. G. Modification of nucleic acid gases via radical intermediates: Synthesis of dihalogenated purine nucleosides. *Synthesis* **1982**, 670–673.
(24) Nair, V.; Chamberlain, S. D. Reductive deamination of aminopurine nucleosides. *Synthesis* **1984**, 401–403.
(25) Nair, V.; Young, D. A. A new synthesis of isoguanosine. *J. Org. Chem.* **1985**, *50*, 406–408.

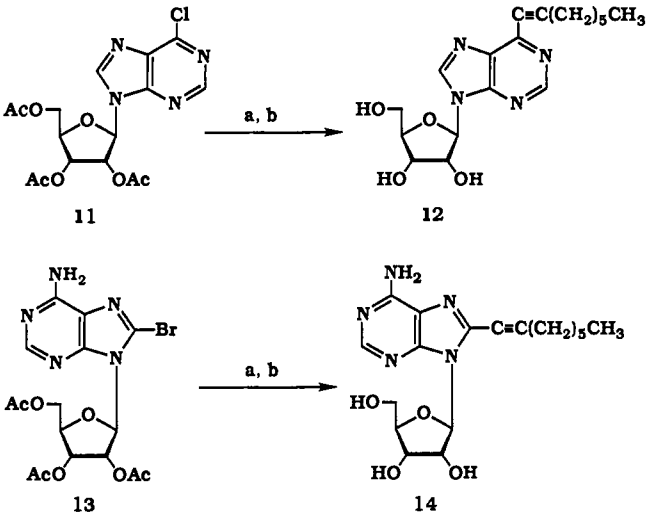
Scheme II[a]



[a] (a) 1 N NaOH, MeOH, then Ac_2O, pyridine; (b) O_2, $Cu(OAc)_2$, pyridine; (c) NH_3/MeOH; (d) compound **4b**, $(PPh_3)_2PdCl_2$, CuI, Et_3N in DMF, 80 °C.

For the palladium-catalyzed cross-coupling reaction of **4a** with terminal alkynes, a modification by Sonogashira[26] of a method originally developed by Heck[27] was used with a slight modification in this study. When **4a** was treated with a slight molar excess of 1-pentyne in the presence of catalytic amounts of bis(triphenylphosphine)palladium dichloride and CuI in *N,N*-dimethylformamide (DMF) and triethylamine at 80 °C for 1 h under an argon atmosphere, 2-(1-pentyn-1-yl)adenosine (**5b**, Scheme I) was isolated in crystals as a hemihydrate in 90% yield, after treatment with H_2S and subsequent purification by silica gel column chromatography. The structure of **5b** was confirmed by its IR, ^1H NMR, and UV spectroscopies and elemental analysis. The UV spectrum of **5b** showed an absorption maximum at 270 nm with a shoulder at 287 nm whose pattern is akin to that of 2-cyanoadenosine.[28,29] The pattern of the ^1H NMR spectrum of **5b** is similar to that of 2-iodoadenosine (**4a**). It should be noted that H_2S treatment of the reaction mixture before further purifications is necessary since without the treatment the cross-coupled product is found to be contaminated with metals. In such a case, the absorption corresponding to the H-8 (δ 8.39 in DMSO-d_6) of **5b** showed a broad singlet with a half-width of ~15 Hz, presumably due to the chelation of metals between 6-amino and N^7-groups. When crude 2-[2-(trimethylsilyl)-1-ethyn-1-yl]adenosine (**6**, Scheme II), which was obtained as a crystalline form without treatment with H_2S, was treated with 1 N NaOH in MeOH, followed by acetylation of the sugar hydroxyls, it showed two spots on TLC. After column chromatographic purification the desired **7** in 77% yield, along with a fluorescent nucleoside **8** in 3% yield, was obtained.

Scheme III[a]



[a] (a) $HC{\equiv}C(CH_2)_5CH_3$, $(PPh_3)_2PdCl_2$, Et_3N in DMF, 70 °C; (b) NH_3/MeOH.

Although both the ^1H NMR spectral patterns were quite similar, except for the absence of the acetylenic proton in **8**, the UV spectrum of **8** (λ_{max} at 331 and 263 nm) is different from that of **7** (λ_{max} 290 and 267 nm). To confirm the structure of **8**, we prepared 1,4-disubstituted 1,3-butadiyne derivative **8** and 1,2-disubstituted acetylene derivative **10**. When **7** was treated with copper(II) acetate under an oxygen atmosphere in pyridine, it gave the oxidative-coupled diyne **8** in 86% yield.[30] Compound **10** was prepared from a similar cross-coupling reaction of tri-*O*-acetyl-2-iodoadenosine **4b** with **7**. Comparison of the UV spectra of both derivatives identifies **8** as 1,4-bis(2,3,5-tri-*O*-acetyladenosin-2-yl)buta-1,3-diyne. These also suggest that the cross-coupled nucleosides without treatment with H_2S contain metals, which catalyzed such oxidative coupling of **9** under these reaction conditions.

Similar reactions starting from **4a** with several terminal alkynes, hydroxyalkynes, or alkoxyalkynes proceeded analogously with the formation of 2-alkynyladenosines

(26) Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: Catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. *Tetrahedron Lett.* **1975**, 4467–4470.

(27) Heck, R. F. Palladium-catalyzed reactions of organic halides with olefins. *Acc. Chem. Res.* **1979**, *12*, 146–151 and references cited therein.

(28) Matsuda, A.; Nomoto, Y.; Ueda, T. Synthesis of 2- and 8-cyanoadenosines and their derivatives. *Chem. Pharm. Bull.* **1979**, *27*, 183–192.

(29) Murakami, T.; Otsuka, M.; Kobayashi, S.; Ohno, M. Synthesis of 2-formyladenosine using diethoxyacetonitrile as a synthon. *Heterocycles* **1981**, *16*, 1315–1319.

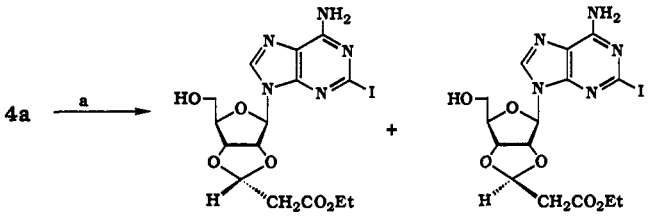
(30) Cameron, M. D.; Benett, G. E. Use of amines in the Glaser coupling reaction. *J. Org. Chem.* **1957**, *22*, 557–558.

Table II. A_1 and A_2 Receptor Binding Activities of Adenosine Analogues in Rat Brain Tissues and Their Cardiovascular Effects in SHRs

no.	K_i,[a] nM A_1	K_i,[a] nM A_2	selectivity A_1/A_2	BP ED_{30},[b] μg/kg	HR ED_{10},[c] μg/kg
5a	186 ± 9.7	21.2 ± 2.3	8.8	0.48 ± 0.13	18.4 ± 8.4
5b	154 ± 17	7.5 ± 2.1	20.5	0.18 ± 0.02	41.5 ± 1.5
5c	126 ± 8.8	2.8 ± 0.3	45.0	0.11 ± 0.03	66.9 ± 6.8
5d	170 ± 21	5.4 ± 1.7	31.5	0.14 ± 0.01	>100
5e	202 ± 20	12.1 ± 1.3	16.7	0.34 ± 0.02	>100
5f	257 ± 15	33.7 ± 5.6	7.6	0.53 ± 0.09	>100
5g	232 ± 23	32.8 ± 6.1	7.1	0.46 ± 0.05	>100
5h	267 ± 14	44.6 ± 5.7	6.0	2.05 ± 0.25	>100
5l	10.1 ± 1.4	20.5 ± 2.3	0.49	0.95 ± 0.14	1.19 ± 0.1
5m	81.4 ± 11	40.1 ± 6.4	2.0	2.53 ± 0.18	15.3 ± 2.7
5n	38.1 ± 5.1	88.1 ± 9.8	0.43	23.8 ± 2.9	44.2 ± 3.6
5o	18.5 ± 1.9	10.6 ± 1.4	1.8	1.06 ± 0.02	9.59 ± 0.1
5p	128 ± 15	10.2 ± 1.8	12.6	1.10 ± 0.09	79.9 ± 6.4
5q	1498 ± 189	307 ± 53	4.9	9.16 ± 1.50	>100
5r	244 ± 17	12.8 ± 2.1	19.1	1.42 ± 0.05	>100
6	69.8 ± 7.8	630 ± 91	0.11	1.15 ± 0.01	>100
9	272 ± 30	37.6 ± 4.8	7.3	5.91 ± 1.10	5.76 ± 0.8
12	>567	>567		>100	>100
14	>567	>567		>100	>100
17	>567	>567		>100	>100
18	>567	>567		>100	>100
19	>567	150 ± 20		5.00 ± 1.10	>100
21	>567	>567		46.7 ± 2.4	50.3 ± 5.1
22	>567	>567		>100	>100
23	>567	>567		>100	>100
CPA	1.21 ± 0.1	421 ± 68	0.003	0.50 ± 0.05	0.14 ± 0.04
NECA	13.3 ± 2.1	12.4 ± 2.3	1.1	0.19 ± 0.05	0.35 ± 0.02
CV1808	780 ± 72	107 ± 8.2	7.3	5.82 ± 1.8	>100
CG21680	1232 ± 95	8.8 ± 1.2	140	0.83 ± 0.2	>100
DPMA	103 ± 10	6.9 ± 1.3	14.9	12.1 ± 1.6	>100

[a] Inhibition constant for A_1 (rat brain membranes, [^{3}H]CHA) or A_2 (rat striatal membranes, [^{3}H]NECA) receptor binding activities of agonists. Affinities for A_1 and A_2 receptors were the mean of three separate experiments in triplicate. The K_d values for the binding of [^{3}H]CHA and [^{3}H]NECA were 2.18 ± 0.05 and 3.60 ± 0.90 nM, respectively. [b] Dose of compound which produced a 30% decrease in blood pressure of anesthetized SHRs. [c] Dose of compound which produced a 10% decrease in heart rate of anesthetized SHRs. ED_{30} and ED_{10} values were the mean of four animals.

Scheme IV[a]



15 16

[a] (a) $HC{\equiv}CCO_2Et$, $(PPh_3)_2PdCl_2$, CuI, Et_3N in DMF, 70 °C.

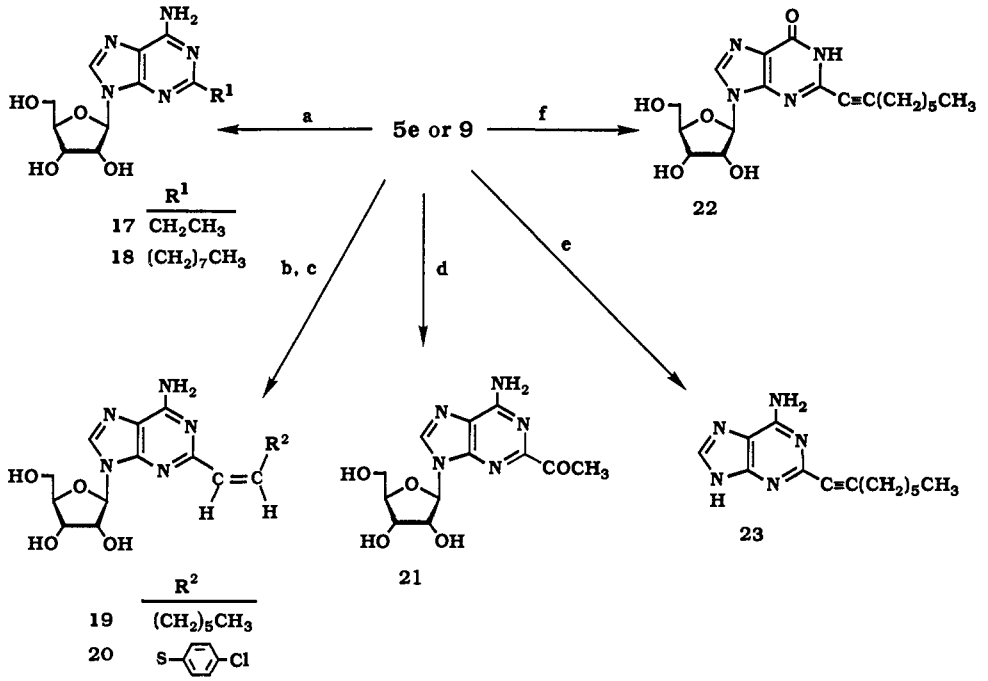
(**5a–k**), 2-(hydroxyalkynyl)adenosines (**5l,m**), or 2-(alkoxyalkynyl)adenosines (**5n–r**) in satisfactory to high isolated yields. Almost all the 2-alkynyladenosines have water of crystallization which was removed with difficulty under reduced pressure over P_2O_5. We also synthesized 1-β-D-ribofuranosyl-6-(1-octyn-1-yl)purine (**12**) and 8-(1-octyn-1-yl)adenosine (**14**) from the corresponding halogeno derivatives **11** and **13** in good yields, respectively (Scheme III). To extend these cross-coupling reactions to functionalized acetylene derivatives, an attempt to react **4a** with ethyl propiolate was made (Scheme IV). However, the products obtained from the reaction mixture were not the desired 2-substituted adenosine derivative, but were identified as stereoisomers of 2′,3′-*O*-[2-(ethoxycarbonyl)ethylidene] derivatives **15** and **16** in 37% and 23% yields, respectively. The stereochemistry at the acetal moiety was assigned from the comparison of chemical shifts of the methine protons because the *exo*-methine proton of **16** was more shielded from the sugar ring oxygen than the *endo*-methine proton of **15** in their ^{1}H NMR spectra. When **4b** was treated similarly, again the 2-substituted derivative was not obtained due to polymerization of ethyl propiolate.

Although several methods for modification at the C-2 position by carbon substituents have been reported,[19,28,31,32] this method provides a general and versatile route to the C-2 carbon substituted adenosines since an acetylene group appears to be easily functionalizable. As such examples for studying a further structure–activity relationship of binding affinities to adenosine receptors, we prepared some analogues of **9** and **5e** as outlined in Scheme V. Compound **9** was catalytically reduced in the presence of 5% Pd on charcoal under hydrogen to furnish 2-ethyladenosine (**17**).[29] Similar reduction of the acetylenic group in **5e** was unsuccessful. However, the acetylenic group of 2′,3′,5′-tri-*O*-acetyl-2-(1-octyn-1-yl)adenosine (see Experimental Section) was smoothly reduced in the presence of 5% Pd on charcoal under an H_2 atmosphere to give 2-octyladenosine (**18**) after deblocking. Partial reduction of tri-*O*-acetate of **5e** was done using a Lindler catalyst with quinoline to give (*Z*)-2-octynyladenosine (**19**) after removal of the protecting groups. Treatment of **9** with sodium *p*-chlorothiophenoxide in MeOH gave (*Z*)-2-[2-[(*p*-chlorophenyl)thio]vinyl]adenosine (**20**) in 97% yield. The *Z* geometry of these nucleosides was identified by the

(31) Marumoto, R.; Yoshioka, Y.; Miyashita, O.; Shima, S.; Imai, K.; Kawazoe, K.; Honjo, M. Synthesis and coronary vasodilating activity of 2-substituted adenosines. *Chem. Pharm. Bull.* **1975**, *23*, 759–774.

(32) Nair, V. Development of methodologies for the strategic modification of purine ribonucleoside systems. *Nucleosides Nucleotides* **1989**, *8*, 699–708 and references cited therein.

Scheme V[a]

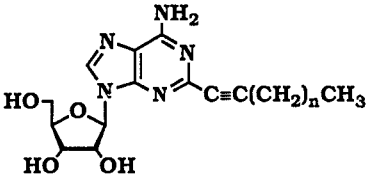


[a] (a) H_2, Pd/C, EtOH; (b) (i) Ac_2O, Et_3N, DMAP in CH_3CN, (ii) H_2, Lindlar catalyst, quinoline, in EtOAc and MeOH, (iii) NH_3/MeOH; (c) NaSPh-*p*-Cl, MeOH; (d) $Hg(OAc)_2$, aqueous AcOH, then H_2S; (e) 0.2 N HCl in dioxane, 100 °C; (*f*) $NaNO_2$, aqueous AcOH.

vicinal coupling constants of the olefin protons, 12.2 and 10.2 Hz, respectively. Treatment of **9** with mercuric(II) acetate in aqueous AcOH, followed by H_2S, furnished 2-acetyladenosine (**21**). Furthermore, **5e** was deaminated by nitrous acid to give 2-(1-octyn-1-yl)inosine (**22**), and acid hydrolysis of the glycosyl linkage of **5e** afforded 2-(1-octyn-1-yl)adenine (**23**).

Adenosine Receptor Binding Affinity and Cardiovascular Activity. The affinity of the 2-AAs for adenosine A_1 and A_2 receptors was measured in the presence of adenosine deaminase by standard radioligand binding methods. Adenosine A_1 receptor binding assays were done in rat brain (without using cerebellum and brain stem) homogenates with tritiated CHA binding by the procedure described previously.[33] Adenosine A_2 receptor binding assays were done in rat striatum with tritiated NECA by the previous methods.[34] The results are summarized in Table II.

All the 2-AA's, except **6**, had very potent binding activity for A_2 receptor and A_2 selectivity. The activity of compounds **5i–k** could not be measured due to their low solubilities. Thus, incorporation of an alkynyl group at the C-2 position of adenosine have an advantageous effect especially on A_2-receptor affinities. Among the series, 2-(1-hexyn-1-yl)adenosine (**5c**) showed the highest binding activity for both A_1 and A_2 receptors with K_i values of 126.5 and 2.8 nM, respectively. This compound has a 45-fold A_2 selectivity that was about 41- and 6-fold more selective than the classical A_2 agonists NECA and CV 1808, respectively. Since more selective A_2 agonists such as CGS 21680[17] and DPMA[16] have recently been reported, we compared the potency of the affinity and the subtype



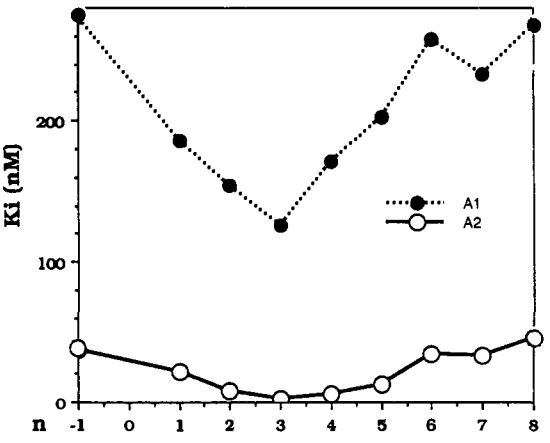


Figure 1. A_1 and A_2 receptor binding affinities of 2-alkynyladenosines (**5a–h**, **9**) in rat brain tissue.

selectivity to those of **5c**. Compound **5c** was 3-fold more potent for the A_2 receptor but about 3-fold less selective for the A_2 receptor than CGS 21680, described as the most selective A_2 agonist so far known, and **5c** has about 3-fold higher affinity as well as selectivity at the A_2 receptor than DPMA. However, increase or decrease in the number of carbon atoms in the side chain resulted in a reduced affinity at both A_1 and A_2 receptors. It is interesting to note that the degree of the decrease of affinities at both receptors is somewhat different between the A_1 and A_2 receptors as shown in Figure 1. An optimum length of the alkyl side chain to affect the affinity requires six carbon

(33) Williams, M.; Braunwalde, A.; Erickson, T. J. Evaluation of the binding of the A-1 selective adenosine radioligand, cyclopentyladenosine (CPA), to rat brain tissue. *Naunyn-Schmiedeb. Arch. Pharmacol.* **1986**, *332*, 179–183.

(34) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. *Mol. Pharmacol.* **1986**, *29*, 331–346.

atoms (hexynyl), while the A_1/A_2 affinity ratios of 2-(1-butyn-1-yl)adenosine (**5a**) vs **5c** and 2-(1-dodecyn-1-yl)-adenosine (**5h**) vs **5c** are 1.5 and 2.1 for the A_1 receptor, and 7.6 and 16 for the A_2 receptor, respectively. The affinities for both receptors of these analogues increased similarly as a function of side-chain length up to the 1-hexyn-1-yl analogue and then decreased in slightly different ways. Therefore, the nature of the C-2 binding domain of the A_2 receptor seems rather sensitive to the length of the side chains and consequently the size of a hydrophobic C-2 pocket[18,35,36] is limited, but the binding region of the A_1 receptor would not be present or poorly recognized by such alkyl side chains near the C-2 position. It is also interesting that 2-[2-(trimethylsilyl)-1-ethyn-1-yl]adenosine (**6**), with a bulky trimethylsilyl substituent, showed inversed selectivity: it is 2-fold more selective than **5c** at the A_1 receptor and 215-fold less selective at the A_2 receptor. These probes show that bulk tolerance is limited next to the acetylenic bond at the C-2 pocket of the A_2 receptor, but such bulkiness is inversely favorable for the A_1 receptor.

To investigate for further structural requirements for the affinity and the subtype selectivity, we prepared 2-(hydroxyalkyl)- and 2-(alkoxyalkynyl)adenosines (**5l–r**). 1-Hydroxypropyn-1-yl derivative **5l**, having a similar length of the alkyl side chain including a hydroxyl group as **5a**, was rather selective for the A_1 receptors with an 18-fold greater affinity than that of **5a**, while these had almost the same affinities for the A_2 receptor. One more methylene-elongated derivative, **5m**, whose side-chain length is similar to that of **5b**, again showed increased A_2 selectivity but was less potent than **5b** at the A_2 receptor. 3-Methoxypropyn-1-yl derivative **5n** was still A_1 selective, but 3-butoxypropyn-1-yl derivative **5o** became A_2 selective. Although 4-butoxybutyn-1-yl and 5-ethoxypentyn-1-yl derivatives **5p** and **5r** have a similar size in length of the side chain of **5o**, they indicated greatly reduced affinities at the A_1 receptor compared to **5o** and similar A_2 selectivities to **5e**. However, 4-(octyloxy)butyn-1-yl derivative **5q** had greatly reduced affinities to both receptors. Thus, the site of the oxygen atom in the alkyl side chains seems to affect the affinity for A_1, and the propargyl alcohol or ether systems could be important in binding to the A_1 receptors. We next examined the requirements of the site of the alkynyl group using **12** and **14** and the requirements of the acetylene function using **17–23** in terms for affinities at both receptors. However, compounds **12** and **14** showed no affinities at both receptors up to 567 nM. Reduction of the alkynyl group to alkyl or alkenyl groups had a deleterious effect on both A_1 and A_2 affinities. Inosine derivative **22** and adenine derivative **23** did not have any affinities to the receptors. These suggest that the acetylenic bond should link at the C-2 position of adenosine for the activities, due to its compact shape, electronic structure, and rigidity, which orients the position of the distal alkyl substituents.

These data together with previous observations[17,18,35,36] constitute a more precise model of the A_2 adenosine receptors around the C-2 pocket. Adenosines having —NH—, —O—, —S—, and —C≡C— groups at the C-2 position show A_2 adenosine receptor agonist activity. Especially, the phenethylamino, the phenethoxy, and the acetylenic groups profoundly increase the A_2 selectivity. In addition to these results, 1-deazaadenosines[37] and 3-deazaadenosines[38] show only weak or no affinity at the A_2 receptor in which recognition through hydrogen bonding at the N-1 and N-3 positions of adenosine is important for the A_2 affinity. Therefore, there must be a recognition site, which would correspond to the X subregion described by Olsson,[18,35] for polar substituents near the C-2 position, together with the region of the N-1 and N-3 positions. In a series of 2-alkoxyadenosines, the coronary vasodilator activity increases proportionally with chain length from methyl to hexyl. This study comes to a similar conclusion, but the total number of the atom constituted side chains for the maximum activity at A_2 receptor is 6 for the 2-AA and 7 for 2-(*n*-hexyloxy)adenosine. The side chain attached to the oxygen atom could be bent at the heteroatom in the 2-alkoxyadenosines, while the shape of the side chain of the alkynyl groups may be linear, and consequently the position of the distal group is somewhat different. This "alkyl" subregion[18,35] could recognize a linear alkyl chain since a bulky substituent introduced next to the acetylenic bond, the oxygen atom at the interposition between the acetylenic bond, and the terminal methyl group, or branched alkyl chains next to the oxygen in 2-alkoxyadenosines, uniformly decrease the activity. On the hydrophobic subregion neighboring the alkyl subregion, further studies will be necessary.

We have described how the 2-alkynyladenosines showed a potent hypotensive activity and concomitant decrease in heart rate following iv administration in normotensive rats.[20] The structure–activity relationship of these nucleosides depends on the length of the alkyl side chain, and the compounds having more than seven carbon atoms including a triple bond in the side chain showed significant hypotensive activity without a heart rate decrease. More recently, we reported that 2-(1-hexyn-1-yl)adenosine (**5c**) and 2-(1-octyn-1-yl)adenosine (**5e**) have potent BP lowering effects without showing a marked decrease in HR at a dose eliciting a decrease in BP of SHR.[21] It has been described how a selective adenosine A_2 agonist showed preferential vasodilator activity.[18,35,39] We also indicated that hypotensive effects reflect A_2 receptor activation and bradycardia reflects A_1 receptor activation.[21] The order of magnitude of hypotensive activity and bradycardia of 2-alkynyladenosines listed in Table II is similar to the order of the potency for both A_1 and A_2 binding affinities. Again **5c** had the most potent hypotensive activity with $ED_{30} = 0.11$ μg/kg, which is 7.5- and 110-fold more potent than that of CGS21680 and DPMA, respectively. We have also shown that **5c** and **5e** had markedly potent and long-lasting antihypertensive activities in oral administration to SHR.[21]

Extracellular adenosine is known to be inactivated by adenosine deaminase and this is one of the reasons for the short duration of its action.[11] However, **5a–e** were found to be totally resistant to hydrolytic deamination by adenosine deaminase from calf intestine. This observation

(35) Ueeda, A.; Thompson, R. D.; Arroyo, L. H.; Olsson, R. A. 2-Alkoxyadenosines: potent and selective agonists at the coronary artery A_2 adenosine receptor. *J. Med. Chem.* **1991**, *34*, 1334–1339.

(36) Trivedi, B.; Bruns, R. F. C2,N^6-Disubstituted adenosines: Synthesis and structure–activity relationships. *J. Med. Chem.* **1989**, *32*, 1667–1673.

(37) Cristalli, G.; Franchetti, P.; Grifantini, M.; Vittori, S.; Klotz, K.-N.; Lohse, M. J. Adenosine receptor agonists: Synthesis and biological evaluation of 1-deaza analogues of adenosine derivatives. *J. Med. Chem.* **1988**, *31*, 1179–1183.

(38) Cristalli, G.; Grifantini, M.; Vittori, S.; Balduini, W.; Cattabeni, F. Adenosine and 2-chloroadenosine deaza-analogues as adenosine receptor agonists. *Nucleosides Nucleotides* **1985**, *4*, 625–639.

(39) Haleen, S. J.; Evans, D. B. Selective effects of adenosine receptor agonists upon coronary resistance and heart rate in isolated working rabbit heart. *Life Sci.* **1985**, *36*, 127–135.

is consistent with the outcome of previous reports that C-2 functionalized adenosines are either very poor substrates or resistant to deamination by the enzyme.[32] Thus, 2-alkynyladenosines are metabolically stable analogues of adenosine and promising as antihypertensive agents which should be considered for further detailed preclinical evaluation.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a JEOL JNM-FX 100 (100 MHz) or JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. IR spectra were recorded with a JASCO IRA-I spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. The silica gel used for column chromatography was YMC gel 60A (70–230 mesh).

9-(2,3,5-Tri-*O*-acetyl-1-β-D-ribofuranosyl)-6-chloro-2-iodopurine (2). (a). Isoamyl nitrite (5 mL, 37 mmol) was added to a mixture of 9-(2,3,5-tri-*O*-acetyl-1-β-D-ribofuranosyl)-2-amino-6-chloropurine[22] (1, 5.12 g, 12 mmol), I_2 (3.04 g, 12 mmol), CH_2I_2 (10 mL, 124 mmol), and CuI (2.4 g, 12.6 mmol) in THF (60 mL). The mixture was heated under reflux for 45 min and was cooled to room temperature. Insoluble materials were removed by filtration, and the filtrate was concentrated to dryness in vacuo. The residue was purified by a silica gel column, which was washed with $CHCl_3$ until the iodine color disappeared, then eluted with 1% EtOH in $CHCl_3$. Compound **2** was obtained in 85% yield (5.49 g, crystallized from EtOH): mp 182–183 °C (lit.[23] mp 181–183 °C); MS *m/z* 538 (M^+). Continuous elution of the column with 2% EtOH in $CHCl_3$ gave 9-(2,3,5-tri-*O*-acetyl-1-β-D-ribofuranosyl)-6-chloropurine (**3c**, 134 mg, 2.7%, as a foam): MS *m/z* 412 (M^+); NMR ($CDCl_3$) δ 2.09 (3 H, s, Ac), 2.13 (3 H, s, Ac), 2.16 (3 H, s, Ac), 4.44 (3 H, br s, 4',5'-H), 5.64 (1 H, dd, 3'-H), 5.95 (1 H, t, 2'-H), 6.24 (1 H, d, 1'-H, $J_{1',2'}$ = 4.9 Hz), 8.30, 8.78 (each 1 H, s, 2- or 8-H).

(b). Isoamyl nitrite (1 mL) was added to a mixture of **1** (854 mg), iodine (508 mg), diiodomethane (2 mL), and CuI (400 mg) in acetonitrile (20 mL) at 80 °C with stirring. After evolution of nitrogen ceased (about 15 min), the mixture was cooled to room temperature and the solvent was removed in vacuo. The residue was suspended in benzene (about 30 mL) and the suspension was filtered through a Celite pad and the filtrate was concentrated to dryness. The residue was purified over a silica gel column, which was washed with $CHCl_3$ until removal of the color of iodine and then eluted with 1% EtOH in $CHCl_3$. Compound **2** was obtained (877 mg, 82%, crystallized from EtOH). Further elution of the column with 4% EtOH in $CHCl_3$ gave **3a** (47 mg, 5%, as a foam): MS *m/z* 469 (M^+); NMR ($CDCl_3$) δ 2.10 (3 H, s, Ac), 2.11 (3 H, s, Ac), 2.16 (3 H, s, Ac), 2.46 (3 H, s, NHAc), 4.46 (3 H, br s, 4',5'-H), 5.75 (1 H, m, 3'-H), 5.90 (1 H, t, 2'-H), 6.10 (1 H, d, 1'-H, $J_{1',2'}$ = 5.4 Hz), 8.12 (1 H, s, 8-H).

(c). Isoamyl nitrite (1 mL) was added to a mixture of **1** (854 mg) and CH_2I_2 (2 mL) in isobutyronitrile (20 mL). The mixture was heated at 80 °C for 25 min and the solvent was removed in vacuo. The residue was purified by a silica gel column with 1% EtOH in $CHCl_3$ to give **2** (673 mg, 63%, crystallized from EtOH). Further elution of the column with 4% EtOH in $CHCl_3$ afforded **3b** (62 mg, 6%, as a foam): MS *m/z* 485 (M^+); NMR ($CDCl_3$) δ 0.94 (3 H, d, $CHMe_2$), 1.28 (3 H, d, $CHMe_2$), 2.09 (3 H, s, Ac), 2.10 (3 H, s, Ac), 2.17 (3 H, s, Ac), 2.70–3.22 (1 H, m, $CHMe_2$), 4.47 (3 H, br s, 4',5'-H), 5.85 (2 H, m, 2',3'-H), 6.14 (1 H, d, 1'-H, $J_{1',2'}$ = 3.9 Hz), 8.11 (1 H, s, 8-H).

2-Iodoadenosine (4a). A solution of compound **2** (6.0 g, 11.1 mmol) in methanolic ammonia (60 mL, saturated at 0 °C) in a steel sealed tube was heated to 60 °C for 17 h. After the tube was cooled to room temperature then degassed, the solvent was removed in vacuo. The residue was crystallized from hot water to give **4** (3.94 g, 90%): mp 145–147 °C (lit.[25] mp 142–144 °C); MS *m/z* 393 (M^+); UV (H_2O) λ_{max} 267 nm (ε 13 200); NMR (DMSO-d_6 + D_2O) δ 3.59 (2 H, m, 5'-Ha,b), 3.95 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.52 (1 H, t, 2'-H), 5.18 (1 H, d, 1'-H, $J_{1',2'}$ = 6.4 Hz), 8.29 (1 H, s, H-8). Anal. ($C_{10}H_{12}IN_5O_4$) C, H, N.

2',3',5'-Tri-*O*-acetyl-2-iodoadenosine (4b). Triethylamine (1.8 mL) was added to a suspension of **4a** (580 mg, 1.48 mmol), DMAP (15 mg), and Ac_2O (1.5 mL) in CH_3CN (30 mL).[40] The mixture was stirred for 1 h at room temperature then MeOH (1 mL) was added. The whole was concentrated to dryness and the residue was partitioned between EtOAc and H_2O. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was crystallized from MeOH to give **4b** (690 mg, 90%): mp 145–147 °C; MS *m/z* 519 (M^+); NMR ($CDCl_3$) δ 2.10 (3 H, s, Ac), 2.14 (3 H, s, Ac), 2.17 (3 H, s, Ac), 4.41 (3 H, br s, 4',5'-H), 5.66 (1 H, m, 3'-H), 5.78 (1 H, t, 2'-H), 5.99 (2 H, br s, 6-NH_2), 6.13 (1 H, d, 1'-H, $J_{1',2'}$ = 4.9 Hz), 7.84 (1 H, s, 8-H). Anal. ($C_{16}H_{18}IN_5O_7$) C, H, N.

General Method for the Preparation of 2-AA's (5a–k). Compound **4a** (393 mg, 1 mmol), CuI (9.5 mg, 0.05 mmol), bis(triphenylphosphine)palladium dichloride (36 mg, 10 mol %), triethylamine (0.16 mL, 1.2 mmol), and a terminal alkyne (1.2 equiv) in DMF (7 mL) were heated at 80 °C for several hours under an argon atmosphere. After the starting material was completely consumed, as judged by TLC ($CHCl_3$/EtOH = 10:1, v/v), the reaction mixture was concentrated to dryness under reduced pressure. The residue was dissolved in $CHCl_3$, and H_2S gas (30 s) and then N_2 gas were introduced to the solution. The suspension was filtered through a Celite pad and washed with $CHCl_3$. The combined filtrate and washings were concentrated to dryness in vacuo, and the residue was purified by a siilca gel column using appropriately mixed EtOH/$CHCl_3$ solvent system.

2-(1-Pentyn-1-yl)adenosine (5b). The reaction of **4a** with 1-pentyne for 1 h gave **5b** (307 mg as a hemihydrate, 90%, crystallized from MeOH/H_2O): mp 129–132 °C; MS *m/z* 333 (M^+), 201 (B^+ + 1); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 287 (sh) (ε 9300), 270 nm (ε 13 600); UV λ_{max} (0.5 N HCl) 290 (sh) (ε 12 700), 272 nm (ε 18 600); NMR (DMSO-d_6 + D_2O) δ 1.01 (3 H, t, Me), 1.58 (2 H, m, CH_2CH_3), 2.39 (2 H, m, C≡CCH_2), 3.62 (2 H, m, 5'-Ha,b), 4.00 (1 H, m, 4'-H), 4.16 (1 H, dd, 3'-H), 4.54 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{15}H_{19}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(1-Hexyn-1-yl)adenosine (5c). The reaction of **4a** with 1-hexyne for 1 h gave **5c** (302 mg as a hydrate, 85%, crystallized from MeOH/H_2O): mp 121–125 °C; MS *m/z* 347 (M^+), 215 (B^+ + 1); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 286 (sh) (ε 12 000), 270 nm (ε 17 000); UV λ_{max} (0.5 N HCl) 286 (sh) (ε 14 000), 270 nm (ε 19 600); NMR (DMSO-d_6 + D_2O) δ 0.92 (3 H, t, Me), 1.51 (4 H, m, $CH_2CH_2CH_3$), 2.42 (2 H, m, C≡CCH_2), 3.63 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.16 (1 H, dd, 3'-H), 4.54 (1 H, t, 2'-H), 5.88 (1 H, d, 1'-H), 8.44 (1 H, s, H-8). Anal. ($C_{16}H_{21}N_5O_4 \cdot H_2O$) C, H, N.

2-(1-Heptyn-1-yl)adenosine (5d). The reaction of **4a** with 1-heptyne for 1 h gave **5d** (344 mg as a hemihydrate, 93%, crystallized from MeOH/H_2O): mp 113–115 °C; MS *m/z* 361 (M^+), 229 (B^+ + 1); IR (KBr) δC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 286 (sh) (ε 9800), 270 nm (ε 14 500); UV λ_{max} (0.5 N HCl) 288 (sh) (ε 13 200), 272 nm (ε 19 300); NMR (DMSO-d_6 + D_2O) δ 0.89 (3 H, t, Me), 1.42 (6 H, m, $(CH_2)_3$), 2.52 (2 H, m, C≡CCH_2), 3.60 (2 H, m, 5'-Ha,b), 4.05 (1 H, m, 4'-H), 4.14 (1 H, dd, 3'-H), 4.53 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{17}H_{23}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(1-Octyn-1-yl)adenosine (5e). The reaction of **4a** with 1-octyne for 1 h gave **5e** (330 mg as a hydrate, 84%, crystallized from MeOH/H_2O): mp 101–103 °C; MS *m/z* 375 (M^+), 243 (B^+

(40) Matsuda, A.; Shinozaki, M.; Suzuki, M.; Watanabe, K.; Miyasaka, T. A convenient method for the selective acylation of guanine nucleosides. *Synthesis* **1986**, 385–386.
(41) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (Ki) and the concentration of the inhibitor which causes 50 percent inhibition (IC_{50}) of an enzynic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(42) Fleming, W. W.; Westfall, D. P.; De La Lande, I. S.; Jellett, L. B. Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmacol. Exp. Ther.* **1972**, *181*, 339–345.

+ 1); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 287 (sh) (ϵ 10 200), 269 nm (ϵ 15 300); UV λ_{max} (0.5 N HCl) 294 (ϵ 9900), 271 nm (ϵ 14 800); NMR (DMSO-d_6 + D_2O) δ 0.88 (3 H, t, Me), 1.34 (8 H, m, $(CH_2)_4$), 2.48 (2 H, m, C≡CCH_2), 3.61 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.53 (1 H, t, 2'-H), 5.85 (1 H, d, 1'-H), 8.38 (1 H, s, H-8). Anal. ($C_{18}H_{25}N_5O_4 \cdot H_2O$) C, H, N.

2-(1-Nonyn-1-yl)adenosine (5f). The reaction of **4a** with 1-nonyne for 1 h gave **5f** (342 mg as a hemihydrate, 86%, crystallized from MeOH/H_2O): mp 89–94 °C dec; IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 284 (sh) (ϵ 13 000), 270 nm (ϵ 17 400); UV λ_{max} (0.5 N HCl) 292 (ϵ 14 000), 271 nm (ϵ 18 800); NMR (DMSO-d_6 + D_2O) δ 0.87 (3 H, t Me), 1.28–1.56 (10 H, m, $(CH_2)_5$), 2.40 (2 H, m, C≡CCH_2), 3.61 (2 H, m, 5'-Ha,b), 3.95 (1 H, m, 4'-H), 4.12 (1 H, dd, 3'-H), 4.53 (1 H, t, 2'-H), 5.89 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{19}H_{27}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(1-Decyn-1-yl)adenosine (5g). The reaction of **4a** with 1-decyne for 1 h gave **5g** (370 mg as a hydrate, 88%, crystallized from MeOH/H_2O): mp 123 °C; MS m/z 403 (M^+), 272 (B^+ + 1); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (MeOH) 287 (sh) (ϵ 11 000), 269 nm (ϵ 15 900); NMR (DMSO-d_6 + D_2O) δ 0.86 (3 H, t, Me), 1.28 (12 H, m, $(CH_2)_6$), 2.41 (2 H, m, C≡CCH_2), 3.67 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.18 (1 H, dd, 3'-H), 4.57 (1 H, t, 2'-H), 5.89 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{20}H_{29}H_5O_4 \cdot H_2O$) C, H, N.

2-(1-Dodecyn-1-yl)adenosine (5h). Compound **5h** (435 mg as a hydrate, 97%, crystallized from MeOH) was obtained from **4a** with 1-dodecyne for 1 h: mp 128–130 °C; MS m/z 431 (M^+), 300 (B^+ + 2); IR (KBr) νC≡C 2220 cm^{-1}; UV λ_{max} (MeOH) 287 (sh) (ϵ 11 000), 268 nm (ϵ 15 700); NMR (DMSO-d_6 + D_2O) δ 0.84 (3 H, t, Me), 1.25 (16 H, m, $(CH_2)_8$), 2.40 (2 H, m, C≡CCH_2), 3.64 (2 H, m, 5'-Ha,b), 4.00 (1 H, m, 4'-H), 4.17 (1 H, dd, 3'-H), 4.55 (1 H, t, 2'-H), 5.87 (1 H, d, 1'-H), 8.38 (1 H, s, H-8). Anal. ($C_{22}H_{33}N_5O_4 \cdot H_2O$) C, H, N.

2-(1-Tetradecyn-1-yl)adenosine (5i). Compound **5i** (427 mg, 93%, crystallized from MeOH) was obtained from **4a** with 1-tetradecyne for 1 h: mp 131–134 °C; MS m/z 459 (M^+), 328 (B^+ + 1); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (MeOH) 285 (sh) (ϵ 11 000), 269 nm (ϵ 15 900); NMR (DMSO-d_6 + D_2O) δ 0.86 (3 H, t, Me), 1.28 (20 H, m, $(CH_2)_{10}$), 2.41 (2 H, m, C≡CCH_2), 3.67 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.18 (1 H, dd, 3'-H), 4.57 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{24}H_{37}N_5O_4$) C, H, N.

2-(1-Hexadecyn-1-yl)adenosine (5j). Compound **5j** (486 mg as a hemihydrate, 98%, crystallized from MeOH) was obtained from **4a** with 1-hexadecyne for 3 h: mp 134–136 °C; MS m/z 487 (M^+), 356 (B^+ + 2); IR (KBr νC≡C 2220 cm^{-1}; UV λ_{max} (MeOH) 284 (sh) (ϵ 11 000), 269 nm (ϵ 15 800); NMR (DMSO-d_6 + D_2O) δ 0.84 (3 H, t, Me), 1.25 (24 H, m, $(CH_2)_{12}$), 2.40 (2 H, m, C≡CCH_2), 3.64 (2 H, m, 5'-Ha,b), 4.00 (1 H, m, 4'-H), 4.17 (1 H, dd, 3'-H), 4.55 (1 H, t, 2'-H), 5.87 (1 H, d, 1'-H), 8.38 (1 H, s, H-8). Anal. ($C_{26}H_{41}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(1-Octadecyn-1-yl)adenosine (5k). Compound **5k** (377 mg as a hemihydrate, 72%, crystallized from MeOH) was obtained from **4a** with 1-octadecyne for 24 h: mp 131–134 °C; MS m/z 515 (M^+), 384 (B^+ + 2); IR (KBr) νC≡C 2220 cm^{-1}; UV λ_{max} (MeOH) 287 (sh) (ϵ 11 000), 270 nm (ϵ 15 800); NMR (DMSO-d_6 + D_2O) δ 0.86 (3 H, t, Me), 1.27 (28 H, m, $(CH_2)_{14}$), 2.39 (2 H, m, C≡CCH_2), 3.61 (2 H, m, 5'-Ha,b), 3.95 (1 H, m, 4'-H), 4.12 (1 H, dd, 3'-H), 4.52 (1 H, t, 2'-H), 5.85 (1 H, d, 1'-H), 8.40 (1 H, s, H-8). Anal. ($C_{28}H_{45}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(3-Hydroxy-1-propyn-1-yl)adenosine (5l). Compound **5l** (308 mg, 96%, crystallized from EtOH) was obtained from **4a** with propargyl alcohol for 1 h: mp 212–215 °C; MS m/z 321 (M^+); UV λ_{max} (H_2O) 289 (ϵ 8900), 270 (ϵ 13 300), 267 (sh) nm (ϵ 13 000); UV λ_{max} (0.5 N HCl) 294 (ϵ 11 900), 271 (ϵ 17 800), 282 (sh) nm (ϵ 17 300); NMR (DMSO-d_6 + D_2O) δ 3.66 (2 H, m, 5'-Ha,b), 4.01 (1 H, m, 4'-H), 4.18 (1 H, dd, 3'-H), 4.32 (2 H, s, CH_2OH), 4.55 (1 H, t, 2'-H), 5.88 (1 H, d, 1'-H), 8.42 (1 H, s, H-8). Anal. ($C_{13}H_{15}N_5O_5$) C, H, N.

2-(4-Hydroxy-1-butyn-1-yl)adenosine (5m). Compound **5m** (300 mg, 85%, crystallized from EtOH) was obtained from **4a** with 3-butyn-1-ol for 1 h: mp 151–153 °C; MS m/z 335 (M^+); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 286 (sh) (ϵ 10 100), 270 nm (ϵ 14 800); UV λ_{max} (0.5 N HCl) 291 (sh) (ϵ 13 300), 271 nm (ϵ 19 600); NMR (DMSO-d_6 + D_2O) δ 3.59 (2 H, m, CH_2CH_2OH), 3.59 (2 H, m, 5'-Ha,b), 4.04 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.52 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{14}H_{17}N_5O_5 \cdot H_2O$) C, H, N.

2-(3-Methoxy-1-propyn-1-yl)adenosine (5n). Compound **5n** (210 mg, 61%, crystallized from EtOH) was obtained from **4a** with 3-methoxy-1-propyne for 1 h: mp 118–123 °C; UV λ_{max} (H_2O) 289 (sh), 270 (ϵ 13 200), 264 (sh) nm; UV λ_{max} (0.5 N HCl) 294 (ϵ 9600), 271 (ϵ 14 100), 264 (sh) nm; NMR (DMSO-d_6 + D_2O) δ 3.35 (3 H, s, OMe), 3.67 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.32 (2 H, m, C≡CCH_2), 4.55 (1 H, t, 2'-H), 5.87 (1 H, d, 1'-H), 8.43 (1 H, s, H-8). Anal. ($C_{14}H_{17}N_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

2-(3-Butoxy-1-propyn-1-yl)adenosine (5o). Compound **5o** (198 mg, 51%, crystallized from EtOH) was obtained from **4a** with 3-butoxy-1-propyne for 1 h: mp 105–110 °C; UV λ_{max} (H_2O) 289 (ϵ 8900), 270 nm (ϵ 13 000); UV λ_{max} (0.5 N HCl) 290 (ϵ 9800), 270 nm (ϵ 14 000); NMR (DMSO-d_6 + D_2O) δ 0.90 (3 H, t, Me), 1.34, 1.52 (each 2 H, m, CH_2CH_2), 3.51 (2 H, t, CH_2O), 3.62 (2 H, m, 5'-Ha,b), 3.95 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.35 (2 H, m, C≡CCH_2), 4.54 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.43 (1 H, s, H-8). Anal. ($C_{17}H_{23}N_5O_5 \cdot {}^2/_3H_2O$) C, H, N.

2-(4-Propoxy-1-butyn-1-yl)adenosine (5p). Compound **5p** (162 mg, 42%, as a powder from $CHCl_3$/EtOAc) was obtained from **4a** with 4-propoxy-1-butyne for 1 h: IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 284 (sh), 270 nm (ϵ 14 000); UV λ_{max} (0.5 N HCl) 293 (ϵ 10 600), 270 nm (ϵ 15 200); NMR (DMSO-d_6 + D_2O) δ 0.87 (3 H, t, Me), 1.53 (2 H, m, CH_2CH_3), 2.65 (2 H, t, C≡CCH_2), 3.38–3.65 (6 H, m, 5'-Ha,b, CH_2OCH_2), 3.95 (1 H, m, 4'-H), 4.12 (1 H, dd, 3'-H), 4.52 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.42 (1 H, s, H-8). Anal. ($C_{17}H_{23}N_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

2-[4-(Octyloxy)-1-butyn-1-yl]adenosine (5q). Compound **5q** (36 mg, 8%, as a powder from MeOH/H_2O) was obtained from **4a** with 4-(octyloxy)-1-butyne for 1 h: IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (MeOH) 290 (sh), 270 nm (ϵ 14 200); NMR (DMSO-d_6 + D_2O) δ 0.84 (3 H, t, Me), 1.23–1.47 (12 H, m, $(CH_2)_6$), 2.63 (2 H, t, C≡CCH_2), 3.42 (2 H, t, CH_2O), 3.53–3.69 (4 H, m, 5'-Ha,b, CH_2O), 3.96 (1 H, m, 4'-H), 4.14 (1 H, dd, 3'-H), 4.52 (1 H, t, 2'-H), 5.87 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{22}H_{33}N_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

2-(5-Ethoxy-1-pentyn-1-yl)adenosine (5r). Compound **5r** (143 mg, 38%, as a powder from $CHCl_3$/Et_2O) was obtained from **4a** with 5-ethoxy-1-pentyne for 1 h: IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 285 (sh), 270 nm (ϵ 13 200); UV λ_{max} (0.5 N HCl) 293 (ϵ 10 000), 270 nm (ϵ 14 400); NMR (DMSO-d_6 + D_2O) δ 1.12 (3 H, t, Me), 1.76 (2 H, t, OCH_2CH_2), 2.44 (2 H, t, C≡CCH_2), 3.42–3.70 (6 H, m, 5'-Ha,b, CH_2OCH_2), 3.95 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.54 (1 H, t, 2'-H), 5.85 (1 H, d, 1'-H), 8.39 (1 H, s, H-8). Anal. ($C_{16}H_{18}N_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

2-[2-(Trimethylsilyl)-1-ethyn-1-yl]adenosine (6). Compound **6** (352 mg, 97%, crystallized from MeOH/H_2O) was obtained from **4a** with (trimethylsilyl)acetylene for 1 h: mp 168–170 °C; MS m/z 363 (M^+), 231 (B^+ + 1); IR (KBr) νC≡C 2160 cm^{-1}; UV λ_{max} (H_2O) 293 (ϵ 11 400), 271 nm (ϵ 16 200); NMR (DMSO-d_6 + D_2O) δ 0.23 (9 H, s, $SiMe_3$), 3.61 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.13 (1 H, dd, 3'-H), 4.48 (1 H, t, 2'-H), 5.87 (1 H, d, 1'-H), 8.45 (1 H, s, H-8). Anal. ($C_{15}H_{21}N_5O_4Si$) C, H, N.

2-(1-Butyn-1-yl)adenosine (5a). 1-Butyne was introduced to a mixture of **4a** (1.0 g, 2.54 mmol), CuI (100 mg), bis(triphenylphosphine)palladium dichloride (70 mg, 10 mol %), and triethylamine (0.43 mL, 3.05 mmol) in DMF (20 mL) for 10 min at −20 °C. The mixture was stirred for 16 h at 50 °C under a 1-butyne atmosphere. The solvent was removed under reduced pressure and the residue was dissolved in EtOH. H_2S gas was introduced into the solution and the mixture was filtered through a Celite pad. The filtrate was concentrated to dryness and the residue was purified over a silica gel column (3.3 × 12 cm), which was washed with 1–4% EtOH in $CHCl_3$ then eluted with 8% EtOH in $CHCl_3$. The main UV-absorbing fractions were combined and concentrated to dryness to leave a white solid which was crystallized from EtOH to give **5a** (456 mg, 56%, crystallized from EtOH): mp 127–133 °C; MS m/z 319 (M^+), 188 (B^+ + 1); IR (KBr) νC≡C 2220 cm^{-1}; UV λ_{max} (H_2O) 288 (sh) (ϵ 12 000), 270 nm (ϵ 17 000); NMR (DMSO-d_6 + D_2O) δ 1.16 (3 H, t, Me), 3.62 (2 H, m, 5'-Ha,b), 3.96 (1 H, m, 4'-H), 4.18 (1 H, dd, 3'-H), 4.54 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.43 (1 H, s, H-8). Anal. ($C_{14}H_{17}N_5O_4 \cdot {}^4/_3H_2O$) C, H, N.

2′,3′,5′-Tri-*O*-acetyl-2-(1-ethyn-1-yl)adenosine (7) and 1,4-Bis(2,3,5-tri-*O*-acetyladenosin-2-yl)buta-1,3-diyne (8). NaOH (1 N, 10 mL) was added to a solution of crude **6** (2.9 g, 7.9 mmol) in MeOH (50 mL), which was prepared from **4a** with (trimethylsilyl)acetylene without treatment with H_2S, and stirred for 1 h at room temperature. The reaction mixture was neutralized with 1 N HCl and then concentrated to dryness and coevaporated several times with EtOH/toluene. To a suspension of the residue in dry pyridine (50 mL) was added Ac_2O (5 mL) and the mixture was stirred overnight at room temperature. MeOH (10 mL) was added to the mixture and the whole was stirred for 1 h and then concentrated to dryness in vacuo. The residue was purified by a silica gel column with 4% EtOH in $CHCl_3$. From this fraction, **7** (2.6 g, 77%, crystallized from MeOH) was obtained: mp 174–176 °C; IR (KBr) νC≡C 2110 cm^{-1}; UV λ_{max} (MeOH) 290 (ϵ 8700), 267 nm (ϵ 14100); NMR ($CDCl_3$) δ 2.07 (3 H, s, Ac), 2.15 (3 H, s, Ac), 2.16 (3 H, s, Ac), 3.00 (1 H, s, C≡CH), 4.41 (3 H, br s, 4′,5′-H), 5.60 (1 H, m, 3′-H), 5.93 (1 H, t, 2′-H), 6.20 (1 H, d, 1′-H, $J_{1',2'}$ = 5.9 Hz), 7.58 (2 H, br s, 6-NH_2), 8.46 (1 H, s, 8-H). Anal. ($C_{18}N_{19}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N. Continuous elution of the column with 8% EtOH in $CHCl_3$ afforded **8** (90 mg, 3%, crystallized from EtOH): mp 239–242 °C; IR (KBr) νC≡C 2130 cm^{-1}; UV λ_{max} (MeOH) 331 (ϵ 27100), 263 nm (ϵ 29400); UV λ_{min} 285 (ϵ, 15400), 258 nm (ϵ 28900); NMR (DMSO-d_6) δ 2.04 (3 H, s, Ac), 2.05 (6 H, s, Ac), 2.13 (6 H, s, Ac), 4.34 (6 H, br s, 4′,5′-H), 5.61 (2 H, m, 3′-H), 5.93 (2 H, t, 2′-H), 6.21 (2 H, d, 1′-H, $J_{1',2'}$ = 5.4 Hz), 7.73 (4 H, br s, 6-NH_2), 8.49 (2 H, s, 8-H). Anal. ($C_{36}H_{36}N_{10}O_{14}$) C, H, N.

Synthesis of 8 from 7. Oxygen was introduced to a solution of **7** (50 mg, 0.12 mmol) and $Cu(OAc)_2 \cdot H_2O$ (200 mg) in pyridine (6 mL) for 30 min at room temperature. The solvent was removed in vacuo and the residue was purified by a silica gel column with 8% EtOH in $CHCl_3$. From this fraction, **8** (43 mg, 86%, crystallized from EtOH) was obtained. The specimen of **8** prepared from **7** was identical to the authentic sample described above.

1,2-Bis(2,3,5-tri-*O*-acetyladenosin-2-yl)acetylene (10). Compound **7** (210 mg, 0.5 mmol) was added to a mixture of **4b** (260 mg, 0.5 mmol), bis(triphenylphosphine)palladium dichloride (28 mg), CuI (8 mg), and Et_3N (1.5 mL) in DMF (3.5 mL). The mixture was heated at 80 °C for 1 h with stirring under an argon atmosphere. The solvent was removed in vacuo and the residue was dissolved in $CHCl_3$. H_2S gas was introduced to the mixture for 30 s and then N_2 gas was purged. The mixture was concentrated and purified by a silica column with 8% EtOH in $CHCl_3$. From this fraction, a hygroscopic reddish crystalline **10** (342 mg, 83%, crystallized from hot EtOH) was obtained: mp 149–155 °C; UV λ_{max} (MeOH) 313 (ϵ 19300), 266 nm (ϵ 25000): UV λ_{min} (MeOH) 285 (ϵ 13800), 257 nm (ϵ 23000); NMR (DMSO-d_6) δ 2.05 (3 H, s, Ac), 2.06 (6 H, s, Ac), 2.13 (6 H, s, Ac), 3.80 (6 H, m, 4′,5′-H), 4.35 (2 H, m, 3′-H), 5.54 (2 H, t, 2′-H), 5.99 (2 H, d, 1′-H, $J_{1',2'}$ = 5.9 Hz), 7.66 (4 H, br s, 6-NH_2), 8.53 (2 H, s, 8-H). Anal. ($C_{34}H_{36}N_{10}O_{14} \cdot H_2O$) C, H, N.

2-(1-Ethyn-1-yl)adenosine (9). Compound **6** (1.39 g, 3.8 mmol) in NH_3/MeOH (20 mL, saturated at 0 °C) in a sealed bottle was treated for 2 h at room temperature and the solvent was removed in vacuo. The residue was crystallized from EtOH to afford **9** (1.04 g, 93%): mp 205–207 °C dec; MS m/z 291 (M^+), 159 ($B^+ + 1$); IR (KBr) νC≡C 2110 cm^{-1}; UV λ_{max} (H_2O) 286 (sh) (ϵ 9200), 268 (ϵ 15000), 263 (sh) nm (ϵ 14500); UV λ_{min} (H_2O) 242 nm (ϵ 7300); NMR (DMSO-d_6 + D_2O) δ 3.68 (2 H, m, 5′-Ha,b), 3.93 (1 H, s, C≡CH), 4.01 (1 H, m, 4′-H), 4.18 (1 H, dd, 3′-H), 4.56 (1 H, t, 2′-H), 5.88 (1 H, d, 1′-H), 8.42 (1 H, s, H-8). Anal. ($C_{12}H_{13}N_5O_4$) C, H, N.

2′,3′-*O*-[2-(Ethoxycarbonyl)ethylidene]-2-iodoadenosines (15, 16). Ethyl propiolate (0.12 mL, 1.18 mmol) was added to a mixture of **4a** (393 mg, 1 mmol), bis(triphenylphosphine)palladium dichloride (35 mg), CuI (9 mg), and Et_3N (3 mL) in DMF (7 mL). The reaction mixture was heated at 80 °C for 30 min with stirring under an argon atmosphere. The solvent was removed in vacuo and the residue was purified by a silica gel column with 2% EtOH in $CHCl_3$. Both compounds were separated in this system and the less polar **15** (183 mg, 37%, crystallized from EtOH) was eluted first and then **16** (114 mg, 23%, crystallized from EtOH). Data for **15**: mp 104–105 °C; MS m/z 491 (M^+); UV λ_{max} (MeOH) 266 nm (ϵ 14300); UV λ_{min} (MeOH) 239 nm (ϵ 6300); NMR ($CDCl_3$) δ 1.28 (3 H, t, CH_2CH_3), 2.74 (2 H, d, CH_2CO_2Et, J = 4.9 Hz), 3.76 (1 H, dd, H-5′a, $J_{4',5'a}$ = 2.9, $J_{5'a,5'b}$ = 12.5 Hz), 3.95 (1 H, dd, H-5′b, $J_{4',5'b}$ = 1.0 Hz), 4.19 (2 H, q, CH_2CH_3), 4.43 (1 H, br s, H-4′), 5.13 (1 H, dd, H-3′, $J_{2',3'}$ = 6.4, $J_{3',4'}$ = 2.4 Hz), 5.27 (1 H, dd, H-2′, $J_{1',2'}$ = 3.4, $J_{2',3'}$ = 6.4 Hz), 5.78 (1 H, t, end H, J = 4.9 Hz), 5.89 (1 H, d, H-1′, $J_{1',2'}$ = 3.4 Hz), 6.52 (2 H, br s, 6-NH_2), 7.76 (1 H, s, H-8). Anal. ($C_{15}H_{18}IN_5O_6$) C, H, N. Data for **16**: mp 108–110 °C; MS m/z 491 (M^+); UV λ_{max} (MeOH) 266 nm (ϵ 14000); UV λ_{min} (MeOH) 239 nm (ϵ 6300); NMR ($CDCl_3$) 1.30 (3 H, t, CH_2CH_3), 2.88 (2 H, d, CH_2CO_2Et, J = 5.4 Hz), 3.78 (1 H, dd, H-5′a, $J_{4',5'a}$ = 2.0, $J_{5'a,5'b}$ = 12.7 Hz), 3.99 (1 H, dd, H-5′b, $J_{4',5'b}$ = 1.5 Hz), 4.21 (2 H, q, CH_2CH_3), 4.57 (1 H, br s, H-4′), 5.05 (1 H, dd, H-3′, $J_{2',3'}$ = 5.9, $J_{3',4'}$ = 1.0 Hz), 5.21 (1 H, dd, H-2′, $J_{1',2'}$ = 4.9, $J_{2',3'}$ = 5.9 Hz), 5.51 (1 H, t, exo H, J = 5.4 Hz), 5.86 (1 H, d, H-1′, $J_{1',2'}$ = 4.9 Hz), 6.25 (2 H, br s, 6-NH_2), 7.75 (1 H, s, H-8). Anal. ($C_{15}H_{18}IN_5O_6$) C, H, N.

2-Ethyladenosine (17). A suspension of **9** (291 mg, 1 mmol) and 10% Pd/C (30 mg) in EtOH (30 mL) was shaken under hydrogen (atmospheric pressure) at room temperature for 2 h. The reaction mixture was filtered through a Celite pad and further washed with EtOH, and then the filtrate and washings were concentrated in vacuo. The residue was crystallized from EtOH/Et_2O to give **17** (260 mg, 88%): mp 116–118 °C. Synthesis of this compound was reported by Marumoto et al.,[31] but the melting point was not given.

2-Acetyladenosine (21). $Hg(OAc)_2$ (490 mg, 1.54 mmol) was added to a solution of **9** (204 mg, 0.7 mmol) in 50% aqueous AcOH (10 mL). The mixture was stirred for 19 h at room temperature. H_2S gas was introduced to the mixture for 2 min, and the insoluble materials were removed by filtration with a Celite pad. The filtrate was concentrated to dryness and the residue was crystallized from hot H_2O to give **21** (148 mg, 68%): mp 232–233 °C; IR (KBr) νC=O 1705 cm^{-1}; UV λ_{max} (H_2O) 312 nm (ϵ 5800), 265 (sh) (ϵ 10300), 261 nm (ϵ 10400); UV λ_{min} (H_2O) 281 (ϵ 4100), 249 nm (ϵ 7900); NMR (DMSO-d_6 + D_2O) δ 2.66 (3 H, s, $COCH_3$), 3.62 (2 H, m, H-5′a,b), 3.96 (1 H, m, H-4′), 4.24 (1 H, m, 3′-H), 4.69 (1 H, t, 2′-H), 5.99 (1 H, d, 1′-H, $J_{1',2'}$ = 5.9 Hz), 8.53 (1 H, s, H-8). Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

(*Z*)-2-(1-Octen-1-yl)adenosine (19). Triethylamine (1.5 mL) was added to a suspension of **5e** (900 mg, 2.4 mmol), DMAP (25 mg), and Ac_2O (0.95 mL) in CH_3CN (20 mL). The mixture was stirred for 15 min at room temperature and MeOH (2 mL) was added to the solution. The solvent was removed in vacuo and the residue was partitioned between EtOAc and H_2O. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was crystallized from MeOH to afford 2′,3′,5′-tri-*O*-acetyl-2-(1-octyn-1-yl)adenosine (1.1 g, 92%): mp 133–136 °C; MS m/z 501 (M^+); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (MeOH) 287 (sh) (ϵ 9800), 268 nm (ϵ 14700): UV λ_{min} (MeOH) 244 nm (ϵ 6800); NMR ($CDCl_3$) δ 0.89 (3 H, t, Me), 1.31 (4 H, m, CH_2), 1.66 (2 H, m, CH_2), 2.07 (3 H, s, Ac), 2.15 (6 H, s, Ac), 2.16 (3 H, s, Ac), 2.45 (2 H, t, C≡CCH_2), 4.39–4.42 (3 H, m, 4′,5′-H), 5.56 (1 H, dd, 3′-H), 5.77 (1 H, dd, 2′-H), 5.81 (2 H, br s, 6-NH_2), 6.32 (1 H, d, 1′-H, $J_{1',2'}$ = 5.9 Hz), 8.00 (1 H, s, 8-H). Anal. ($C_{24}H_{31}N_5O_7 \cdot {}^1/_5H_2O$) C, H, N. A suspension of the above nucleoside (2.0 g, 4.0 mmol), quinoline (1.6 mL), and Lindlar catalyst (0.8 g) in a mixture of EtOAc (40 mL) and MeOH (20 mL) was stirred for 3 h at room temperature under an H_2 atmosphere. The catalyst was removed by filtration and the filtrate was concentrated to dryness in vacuo. The residue was treated with NH_3/MeOH (80 mL, saturated at 0 °C) in a sealed bottle overnight at room temperature. The solvent was removed in vacuo and the residue was purified by a silica gel column with 10% MeOH in $CHCl_3$ to afford **19** (1.34 g, 89%, crystallized from aqueous MeOH): mp 125–126 °C; UV λ_{max} (H_2O) 268 nm (ϵ 13800); UV λ_{min} (H_2O) 251 nm (ϵ 9000); UV λ_{max} (0.5 N HCl) 273 nm (ϵ 14100); UV λ_{min} (0.5 N HCl) 252 nm (ϵ 8600); NMR (DMSO-d_6) δ 0.85 (3 H, t, Me), 1.24–1.45 (8 H, m, CH_2 × 4), 2.83 (2 H, m, CH=CHCH_2), 3.60 (2 H, m, H-5′a,b), 3.95 (1 H, m, H-4′), 4.14 (1 H, m, 3′-H), 4.65 (1 H, t, 2′-H), 5.16 (1 H, d, OH), 5.39 (2 H, m, OH × 2), 5.85–5.94 (2 H, m, H-1′, vinylic), 6.22 (1 H, d, 2-C*H*=CH, J = 12.2 Hz), 7.16 (2 H, br s, 6-NH_2), 8.31 (1 H, s, H-8). Anal. ($C_{18}H_{27}N_5O_4 \cdot {}^1/_4H_2O$) C, H, N.

2-Octyladenosine (18). A suspension of 2′,3′,5′-tri-*O*-acetate of **5e** (1.04 g, 2.1 mmol) described above and 5% Pd–charcoal (400 mg) was stirred overnight at room temperature under an H_2 atmosphere. The catalyst was removed by filtration and the

filtrate was concentrated to dryness. The residue was treated with NH_3/MeOH (50 mL) overnight at room temperature. The solvent was removed in vacuo and the residue was purified by a silica gel column with 10% MeOH in $CHCl_3$ to afford **18** (710 mg, 90%, as a foam): UV λ_{max} (H_2O) 263 nm (ϵ 13700); UV λ_{min} (H_2O) 229 nm (ϵ 3300); UV λ_{max} (0.5 N HCl) 258 nm (ϵ 14000); UV λ_{min} (0.5 N HCl) 232 nm (ϵ 4000); NMR (DMSO-d_6) δ 0.85 (3 H, t, Me), 1.24–1.76 (12 H, m, $CH_2 \times 6$), 2.61 (2 H, t, 2-C≡CCH_2), 3.61 (2 H, m, H-5′a,b), 4.00 (1 H, m, H-4′), 4.14 (1 H, m, 3′-H), 4.66 (1 H, dd, 2′-H), 5.18 (1 H, d, OH), 5.39 (1 H, m, OH), 5.66 (1 H, t, 5′-OH), 5.83 (1 H, d, H-1′, $J_{1',2'}$ = 6.6 Hz), 7.22 (2 H, br s, 6-NH_2), 8.22 (1 H, s, H-8). Anal. ($C_{18}H_{29}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(1-Octyn-1-yl)inosine (22). $NaNO_2$ (2.0 g, 29 mmol) was added to a solution of **5e** (1.88 g, 5 mmol) in aqueous 50% AcOH (200 mL). The mixture was stirred for 4 h at room temperature. The solvent was removed in vacuo and the residue taken in H_2O (100 mL) was extracted with a mixture of $CHCl_3$/MeOH (4:1, v/v), followed by washed with brine. The organic phase was concentrated to dryness and the residue was purified by a silica gel column with 10% MeOH in $CHCl_3$ to afford **22** (1.25 g, 66.5%, as a powder from EtOAc/Et_2O): IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 291 nm (ϵ 12200); UV λ_{max} (0.5 N NaOH) 264 nm (ϵ 12000); NMR (DMSO-d_6) δ 0.89 (3 H, t, Me), 1.31 (4 H, m, $CH_2 \times 4$), 1.44 (2 H, m, CH_2), 1.57 (2 H, m, CH_2), 2.49 (2 H, t, 2-C≡CCH_2), 3.62 (2 H, m, H-5′a,b), 3.95 (1 H, m, H-4′), 4.12 (1 H, m, 3′-H), 4.43 (1 H, dd, 2′-H), 5.07 (1 H, d, OH), 5.18 (1 H, m, OH), 5.47 (1 H, t, 5′-OH), 5.84 (1 H, d, H-1′, $J_{1',2'}$ = 5.5 Hz), 8.35 (1 H, s, H-8). Anal. ($C_{18}H_{24}N_4O_5 \cdot {}^1/_2H_2O$) C, H, N.

2-(1-Octyn-1-yl)adenine (23). A solution of **5e** (800 mg, 2.13 mmol) in a mixture of 0.2 N HCl (20 mL) and dioxane (20 mL) was heated at 100 °C for 7 h. The mixture was cooled to room temperature and the resulting precipitates were collected by filtration. The precipitate was suspended in aqueous MeOH, neutralized with 0.2 N NaOH, collected by filtration, and crystallized from MeOH to give **23** (310 mg, 60%): mp 263–264 °C; IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (H_2O) 285 (sh), 270 nm (ϵ 13000); UV λ_{min} (H_2O) 246 nm (ϵ 5700); UV λ_{max} (0.5 N HCl) 288 (sh), 272 nm (ϵ 16900); UV λ_{min} (0.5 N HCl) 250 nm (ϵ 7800); UV λ_{max} (0.5 N NaOH) 280 nm (ϵ 14300); UV λ_{min} (0.5 N NaOH) 254 nm (ϵ 5600); NMR (DMSO-d_6) δ 0.89 (3 H, t, Me), 1.28–1.56 (8 H, m, $CH_2 \times 4$), 2.39 (2 H, t, 2-C≡CCH_2), 7.20 (2 H, br s, 6-NH_2), 8.13 (1 H, s, H-8). Anal. ($C_{13}H_{17}N_5$) C, H, N.

(*Z*)-2-[2-[(*p*-Chlorophenyl)thio]vinyl]adenosine (20). NaOMe (1 N, 0.1 mL, MeOH solution) was added to a suspension of **9** (291 mg, 1 mmol) and *p*-chlorothiophenol (224 mg, 1.5 mmol) in anhydrous MeOH (25 mL). The mixture was stirred for 41 h at room temperature and then neutralized with AcOH. The crystalline materials were collected by filtration and washed well with MeOH and Et_2O to give **20** (427 mg, 97%): mp 146–148 °C eff; UV λ_{max} (MeOH) 322 (ϵ 24800), 267 nm (ϵ 14400); UV λ_{min} (MeOH) 282 (ϵ 10200), 248 nm (ϵ 6900); NMR (DMSO-d_6 + D_2O) δ 3.68 (2 H, m, H-5′a,b), 4.03 (1 H, m, H-4′), 4.20 (1 H, m, 3′-H), 4.75 (1 H, t, 2′-H), 5.94 (1 H, d, 1′-H, $J_{1',2'}$ = 5.9 Hz), 6.51 (1 H, d, vinyl, $J_{a,b}$ = 10.3 Hz), 7.08 (1 H, d, vinyl, $J_{a,b}$ = 10.3 Hz), 7.41–7.63 (5 H, m, Ph), 8.35 (1 H, s, H-8). Anal. ($C_{18}H_{18}ClN_5O_4S$) C, H, N.

9-β-D-Ribofuranosyl-6-(1-octyn-1-yl)purine (12). A mixture of **11** (850 mg, 2 mmol), bis(triphenylphosphine)palladium dichloride (30 mg), CuI (30 mg), and 1-octyne (0.33 mL, 2.2 mmol) in dry DMF (14 mL) and Et_3N (6 mL) was treated at 70 °C for 3 h with stirring under an argon atmosphere. The solvent was removed in vacuo and the residue was dissolved in $CHCl_3$. H_2S was introduced to the solution, and the insoluble materials were removed by filtration through a Celite pad. The filtrate was concentrated to dryness and the residue was purified by a silica gel column with 1% EtOH in $CHCl_3$ to give 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-octynylpurine (549 mg, 57%, as a foam). A solution of the above compound (486 mg, 1 mmol) was treated with NH_3/MeOH (20 mL) in a sealed bottle overnight at room temperature. The solvent was concentrated in vacuo and the residue was crystallized from aqueous MeOH to afford **12** (220 mg, 77%): mp 158–160 °C; MS *m/z* 360 (M^+); UV λ_{max} (MeOH) 288 nm (ϵ 16600); UV λ_{min} (MeOH) 249 nm (ϵ 5000); NMR (DMSO-d_6 + D_2O) δ 0.89 (3 H, t, Me), 1.26–1.62 (8 H, m, CH_2-(CH_2)$_4$Me), 2.62 (2 H, m, CH_2(CH_2)$_4$Me), 3.66 (2 H, m, H-5′a,b), 4.01 (1 H, q, H-4′, *J* = 3.4, 3.6, 4.9 Hz), 4.21 (1 H, t, H-3′, $J_{2',3'}$ = 4.9, $J_{3',4'}$ = 4.9 Hz), 4.61 (1 H, q, H-2′, $J_{1',2'}$ = 5.4, $J_{2',3'}$ = 4.9 Hz), 6.03 (1 H, d, H-1′, $J_{1',2'}$ = 5.4 Hz), 8.82 (1 H, s, H-8), 8.96 (1 H, s, H-2). Anal. ($C_{18}H_{24}N_4O_4$) C, H, N.

8-(1-Octyn-1-yl)adenosine (14). A mixture of **13** (945 mg, 2 mmol), bis(triphenylphosphine)palladium dichloride (30 mg), CuI (30 mg), and 1-octyne (0.33 mL, 2.2 mmol) in dry DMF (14 mL) and Et_3N (6 mL) was heated at 70 °C for 1.5 h with stirring under an argon atmosphere. The solvent was removed in vacuo and the residue was dissolved in $CHCl_3$. H_2S gas was introduced to the solution, and the insoluble materials were removed by filtration through a Celite pad. The filtrate was concentrated to dryness and the residue was purified by a silica gel column with 0.5% EtOH in $CHCl_3$ to give 2,3,5-tri-*O*-acetyl-8-(1-octyn-1-yl)-adenosine (975 mg, 97%, crystallized from EtOH): mp 88–90 °C; MS *m/z* 501 (M^+); IR (KBr) νC≡C 2230 cm^{-1}; NMR ($CDCl_3$) δ 0.91 (3 H, t, Me), 1.26–1.74 (8 H, m, CH_2(CH_2)$_4$Me), 2.05 (3 H, s, Ac), 2.10 (3 H, s, Ac), 2.14 (3 H, s, Ac), 2.55 (2 H, m, CH_2-(CH_2)$_4$Me), 4.28–4.58 (3 H, m, H-4′, 5′a,b), 5.97 (3 H, m, H-3′, 6-NH_2), 6.22–6.30 (2 H, m, H-1′, 2′), 8.34 (1 H, s, H-2). A solution of the above compound (545 mg, 1.1 mmol) was treated with NH_3/MeOH (20 mL) in a sealed bottle overnight at room temperature. The solvent was concentrated in vacuo and the residue was crystallized from MeOH to afford 14 (319 mg, 78%): mp 179–181 °C; MS *m/z* 375 (M^+); IR (KBr) νC≡C 2230 cm^{-1}; UV λ_{max} (MeOH) 301 (sh) (ϵ 14800), 293 (ϵ 20600), 287 (sh) nm (ϵ 19000); UV λ_{min} (MeOH) 248 nm (ϵ 3300); NMR (DMSO-d_6) δ 0.88 (3 H, t, Me), 1.13–1.76 (8 H, m, CH_2(CH_2)$_4$Me), 2.57 (2 H, m, CH_2(CH_2)$_4$Me), 3.63 (2 H, m, H-5′a,b), 3.99 (1 H, m, H-4′), 4.19 (1 H, m, H-3′), 4.97 (1 H, q, H-2′), 5.94 (1 H, d, H-1′, $J_{1',2'}$ = 6.4 Hz), 7.55 (2 H, br s, 6-NH_2), 8.14 (1 H, s, H-2). Anal. ($C_{18}H_{25}N_5O_4$) C, H, N.

Adenosine Receptor Binding Assay. The A_1 receptor binding assay was done by previously described method,[33] with a slight modification, using female Wistar rat brain (without using cerebellum and brain stem) homogenates. The activity of each compound was assessed in three separate experiments. For each experiment, a dose–response curve consisting of 8–10 concentrations was made for each compound. All assays were run in triplicate in 96-well microtiter plates in a final volume of 200 μL containing 2.5 nM [^{3}H]CHA, 0.02 unit of adenosine deaminase (type VI, Sigma), and a test compound solution. Nonspecific binding was measured in the presence of 10 μM 2-chloroadenosine (CADO). Test compounds for competition studies were dissolved in DMSO to obtain stock solutions. Each stock solution was diluted with distilled water to an appropriate concentration when used. The concentration of DMSO was less than 0.01% in each incubation. The reaction was started by the addition of the A_1 receptor preparation at a final protein concentration of 200–220 μg/mL and the mixture was incubated at 23 °C for 2 h. The incubation was stopped by filtration of the reaction mixture through a 9-mm GB-140 glass-fiber filter sheet (Advantec) under reduced pressure using a Titertek cell harvester (550, Flow Laboratories). Nonbound radioactivity was removed by washing the filter with 3.5 mL of ice-cold buffer. The filter, which was isolated from the filter sheet, was put into a scintillation vial containing 6 mL of scintillation cocktail (ACS II, Amersham). The radioactivity was counted in a liquid scintillation counter at an efficiency of 40%.

The A_2 receptor binding assay was done as previously reported,[34] with a slight modification. The A_2 receptors prepared from striata of Wistar rats were used. The binding assay for A_2 receptor was as described for the A_1 receptors except that Tris-HCl buffer containing 10 mM $MgCl_2$ and 5 nM [^{3}H]NECA in place of 2.5 nM [^{3}H]CHA were used, 50 nM CPA was added to the incubation medium to eliminate A_1 receptor binding, and the concentration of CADO for measurement of nonspecific binding was 100 μM.

A computerized nonlinear, least-squares analysis program (SP-1. 2. 3., programmed by Dr. Ono, University of Tokyo, Japan) was used to derive the dissociation constant (K_d). The inhibition value (IC_{50}) was calculated from a nonlinear, log transformation of specific binding data from the ligand binding assay. The ligand–receptor binding inhibition constant (K_i) was calculated from the IC_{50} value by using the Cheng and Prusoff equation.[41]

Blood Pressure and Heart Rate. Male SHR anesthetized by an iv injection of urethane (500 mg/kg) and α-chloralose (40

mg/kg) solution. Blood pressure was measured with a pressure transducer (MPU-0.5, NEC San-ei) through a polyethylene cannula inserted into the left carotid artery. Heart rate was monitored with a cardiotachometer (NEC San-ei) triggered by the arterial pressure pulse. A femoral vein was cannulated with a polyethylene cannula for drug administration. Test compounds were dissolved in DMSO to form stock solutions, and appropriate dilutions in saline were prepared for injection. The solution of test compound (0.03-100 μg/mL) was administered iv in a volume of 1 mL/kg at 5-min intervals in a cumulative manner. The concentration of DMSO in the solution for injection was less than 3.4%, a concentration that did not affect the BP and HR in SHR.

Changes in BP and HR were expressed in terms of percent changes of their control values, which were obtained before the injection of a test compound into the rat. Agonists were compared for their potency to decrease BP and HR. The relative potency to decrease BP was estimated on the basis of the EC_{30} value, the mean dose that produced a 30% decrease in BP of SHR. Similarly, the relative potency to decrease HR was estimated on the basis of the ED_{10} value, the mean dose that produced a 10% decrease in HR of SHR. ED_{30} and ED_{10} values were calculated by the method of Fleming et al.[42]

Adenosine Deaminase Assays. Adenosine deaminase [EC.3.5.4.4] from calf intestinal mucosa (type III) was obtained from the Sigma Chemical Co. The enzyme experiments were done in 0.05 M phosphate buffer (pH 7.5). The assay solutions containing about 1×10^{-4} M of the compounds (**5a-e**) and 15 μg of the enzyme were incubated at 25 °C for 26 h. The absorbances at λ_{max} of these compounds were not changed.

Acknowledgment. This investigation was supported in part by Grants-in-Aid for Developmental Scientific Research and Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture of Japan.

Registry No. 1, 16321-99-6; 2, 5987-76-8; **3a**, 137896-02-7; **3b**, 137896-13-0; **4a**, 35109-88-7; **4b**, 94042-04-3; **5a**, 137896-03-8; **5b**, 99044-60-7; **5c**, 90596-73-9; **5d**, 90596-74-0; **5e**, 90596-75-1; **5e** tri-*O*-acetyl derivative, 133560-05-1; **5f**, 131242-48-3; **5g**, 100647-74-3; **5h**, 109232-63-5; **5i**, 109232-59-9; **5j**, 109232-60-2; **5k**, 109232-62-4; **5l**, 90596-71-7; **5m**, 99044-58-3; **5n**, 137896-14-1; **5o**, 137896-15-2; **5p**, 137896-16-3; **5q**, 137896-17-4; **5r**, 137896-18-5; **6**, 90596-72-8; **7**, 90596-76-2; **8**, 90596-77-3; **9**, 99044-57-2; **10**, 137896-04-9; 11, 5987-73-5; **12**, 137896-05-0; 12 tri-*O*-acetyl derivative, 137915-41-4; **13**, 31281-86-4; 14, 137896-06-1; 14 tri-*O*-acetyl derivative, 137896-19-6; **15**, 137915-39-0; **16**, 137915-40-3; **17**, 37151-12-5; **18**, 137896-07-2; **19**, 137896-08-3; **20**, 137896-09-4; **21**, 137896-10-7; **22**, 137896-11-8; **23**, 137896-12-9.

Substituted 4,6-Diaminoquinolines as Inhibitors of C5a Receptor Binding

Thomas J. Lanza,† Philippe L. Durette,† Thomas Rollins,‡ Salvatore Siciliano,‡ Dana N. Cianciarulo,‡ Sumire V. Kobayashi,‡ Charles G. Caldwell,† Martin S. Springer,‡ and William K. Hagmann*,†

Departments of Medicinal Chemical Research and Immunology Research, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065. *Received December 4, 1990*

The anaphylatoxin C5a is implicated in a number of inflammatory diseases. It is a highly cationic protein with 13 of 74 amino acids being either arginine or lysine. A search focusing on positively charged molecules, particularly amine-containing functionalities, led to the discovery of substituted 4,6-diaminoquinolines 1 [*N,N'*-bis(4-amino-2-methyl-6-quinolyl)urea] and 7 [6-*N*-(2-chlorocinnamoyl)-4,6-diamino-2-methylquinoline) as inhibitors of C5a receptor binding. These two compounds inhibited the binding of radiolabeled C5a to its receptor isolated from human neutrophils with IC_{50}'s = 3.3 and 12 μg/mL, respectively. Our efforts to enhance their potencies by chemical modification revealed a narrow profile of potency for effective C5a receptor binding inhibition.

Activation of the complement system in response to immunological and nonimmunological events functions to focus and amplify the inflammatory response. In addition to the assembly of the membrane attack complex, numerous complement-derived peptides are released that interact with cellular components to propagate the inflammatory process.[1-4] These peptides called anaphylatoxins include C3a, a 77 amino acid peptide derived from C3 by the action of C3-convertases; C4a, a 77 amino acid protein produced from C4 by C1s; and C5a, a 74 amino acid protein, glycosylated in humans, cleaved from C5 by C5-convertase. Their properties include the cellular release of vasoactive amines and lysosomal enzymes, enhanced vascular permeability, and the contraction of smooth muscle. C5a also causes neutrophil chemotaxis and aggregation, stimulation of leukotriene and oxidative product release, induction of interleukin-1 transcription by macrophages, and enhanced antibody production.[5-7] For these reasons, blocking the action of C5a on its receptors may represent an intriguing therapeutic target for the treatment of diseases characterized by an excessive activity and recruitment of inflammatory cells.

The rational design of a small molecular weight antagonist to mimic the potent binding of the C5a molecule to its receptor ($K_d \approx 1 \times 10^{-10}$ M) presents a challenging problem even with considerable structural information. The three-dimensional structures of human[8] and porcine[9]

* To whom correspondence and reprint requests should be sent.
† Department of Medicinal Chemistry Research.
‡ Department of Immunology Research.

(1) Hugli, T. E. The Structural Basis for Anaphylatoxin and Chemotactic Functions of C3a, C4a, and C5a. *CRC Crit. Rev. Immunol.* 1981, *1*, 321-366.
(2) Vogt, W. Activation of the Complement System. *Agts. Actions* 1983, *13*, 391-397.
(3) Greer, J. Comparative Structural Anatomy of the Complement Anaphylatoxin Proteins C3a, C4a, and C5a. *Enzyme* 1986, *36*, 150-163.
(4) Egwang, T. G.; Befus, A. D. The Role of Complement in the Induction and Regulation of Immune Responses. *Immunology* 1984, *51*, 207-224.
(5) Weigle, W. O.; Goodman, M. G.; Morgan, E. L.; Hugli, T. E. Regulation of Immune Response by Components of the Complement Cascade and Their Activated Fragments. *Springer Semin. Immunopathol.* 1983, *6*, 173-194.
(6) Yancey, K. B. Biological Properties of Human C5a: Selected *in vitro* and *in vivo* Studies. *Clin. Exp. Immunol.* 1988, *71*, 207-210.
(7) Schindler, R.; Lonnemann, G.; Stanley, S.; Koch, K.-M.; Dinarello, C. A. Transcription, not Synthesis, of Interleukin-1 and Tumor Necrosis Factor by Complement. *Kidney International* 1990, *37*, 85-93.

0022-2623/92/1835-0252$03.00/0